Bioimage Informatics

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Course

Image Acquisition & Segmentation

École polytechnique fédérale de Lausanne

BIO-410 □ BIOIMAGE INFORMATICS

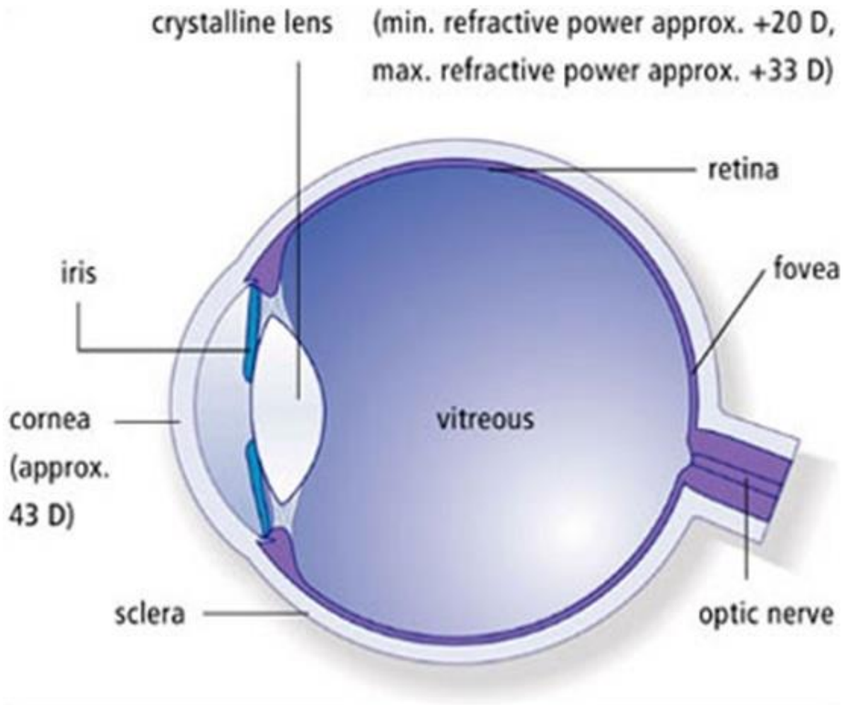
Image Acquisition

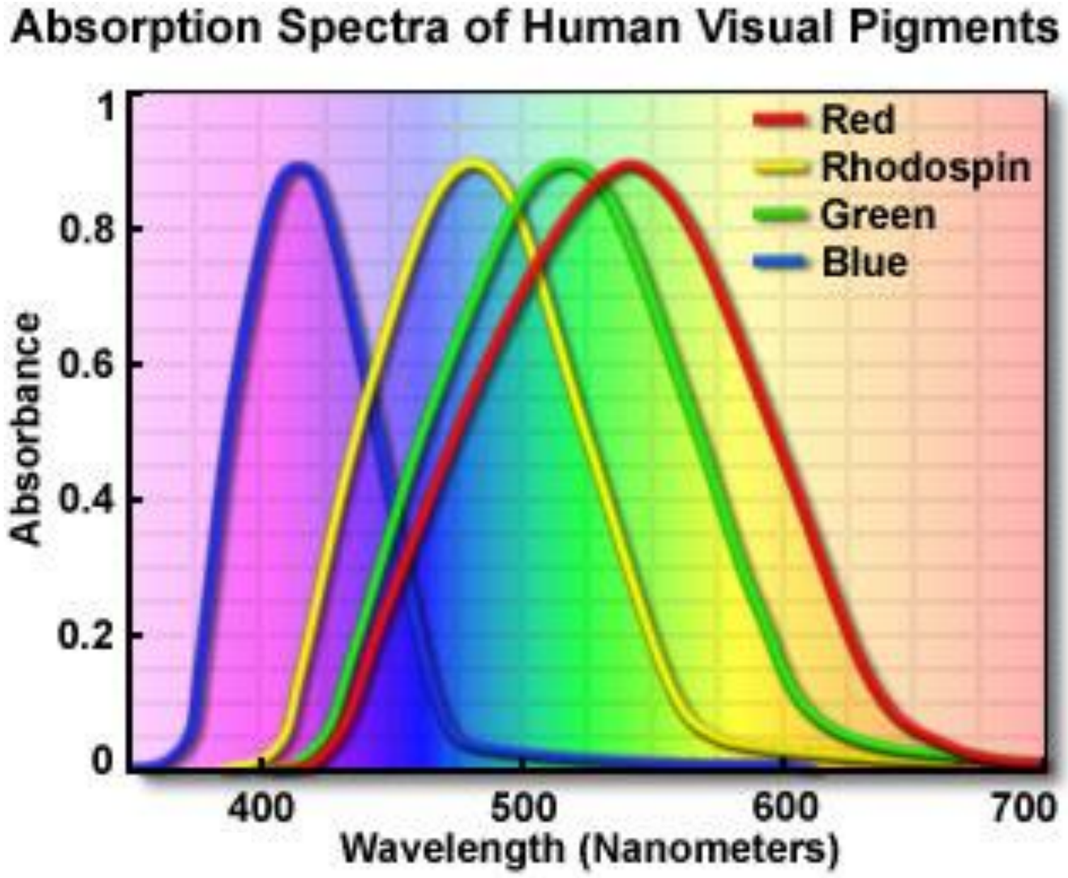
- How are images generated?
- What are images?
- What are digital images and pixels?
- Why must we know and care about it?

Image Acquisition

- Which types of image generation do you know?
- Which types of imaging devices do you know?
- Which technological developments have been transformative for imaging?
- What is the difference between an analogue and a digital image?

Human eye

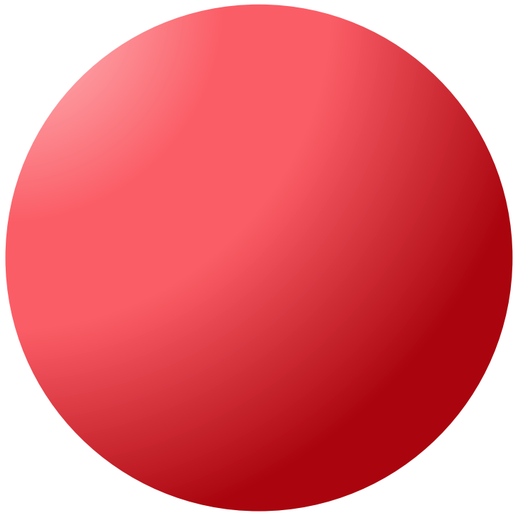


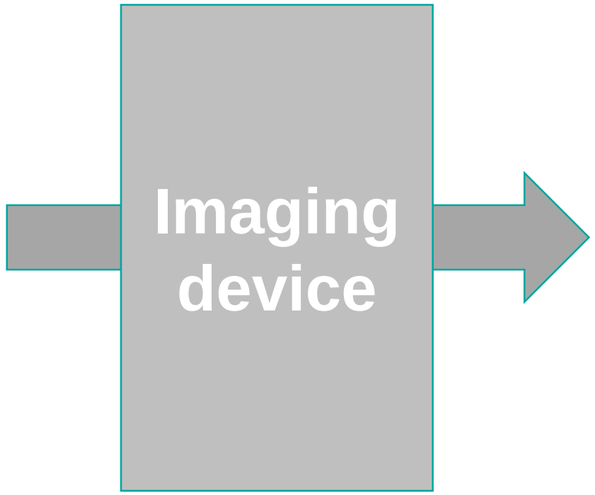


Most perfect sensor for light detection up to now

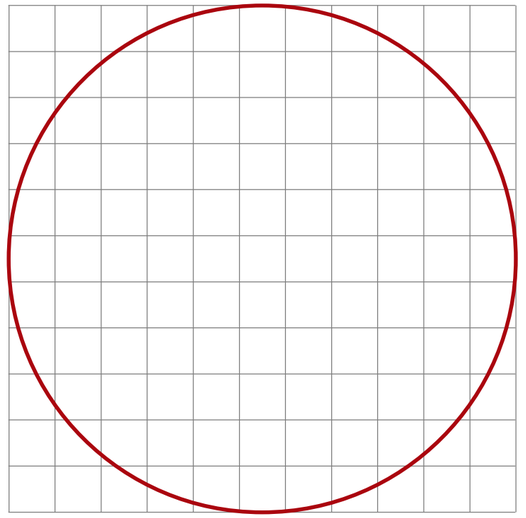
Digital Imaging

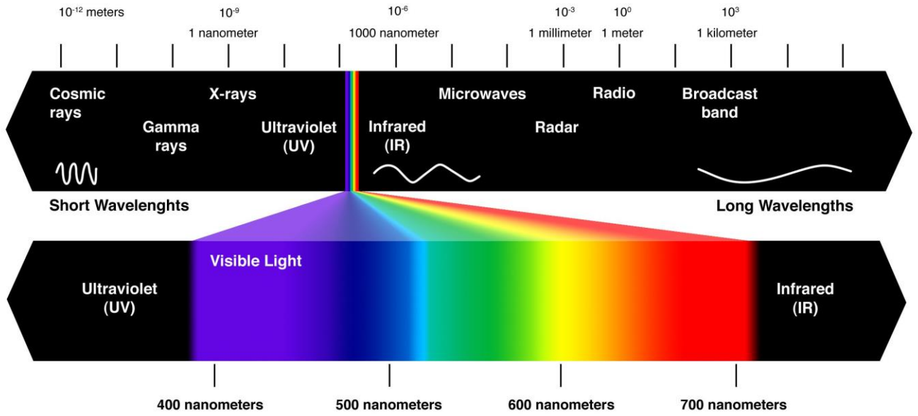
object
continuous space





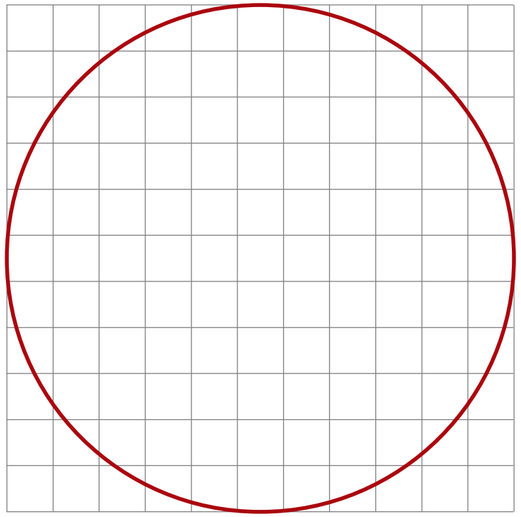
Digital image
discrete space

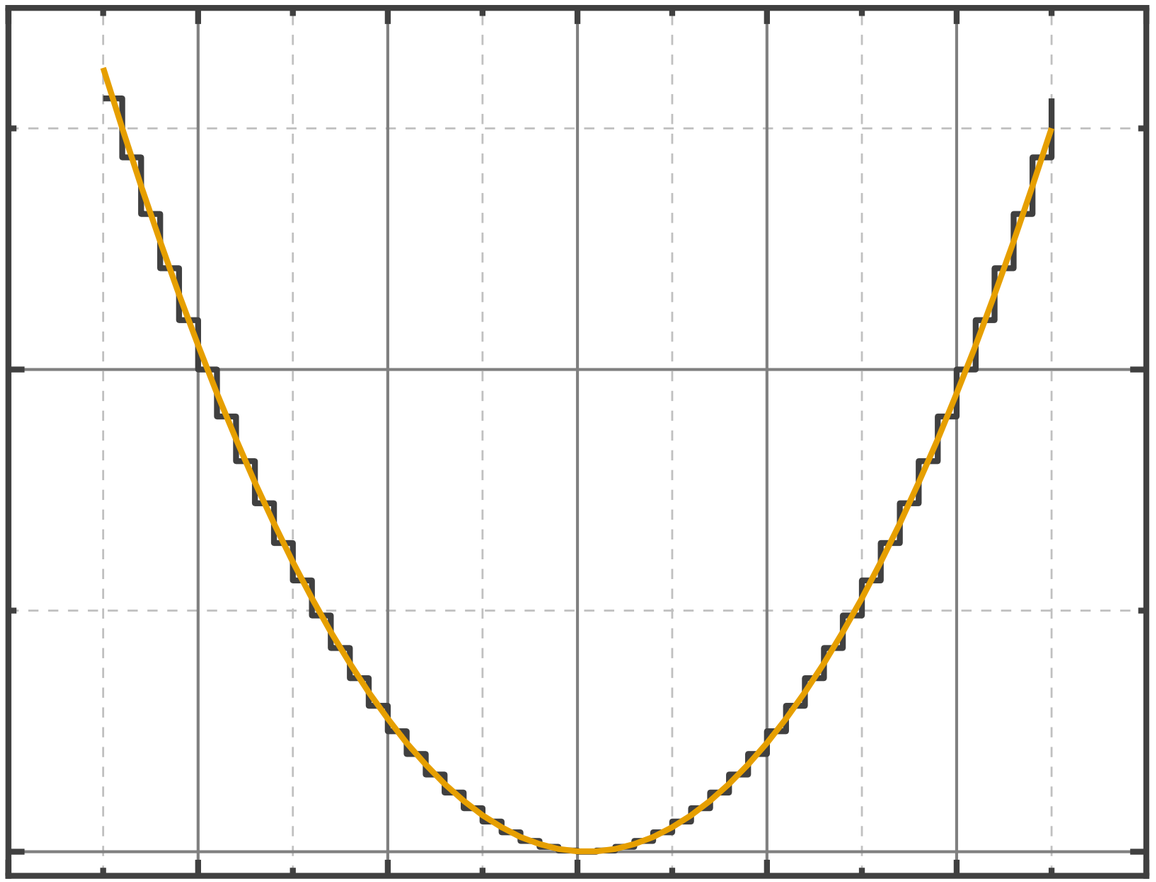




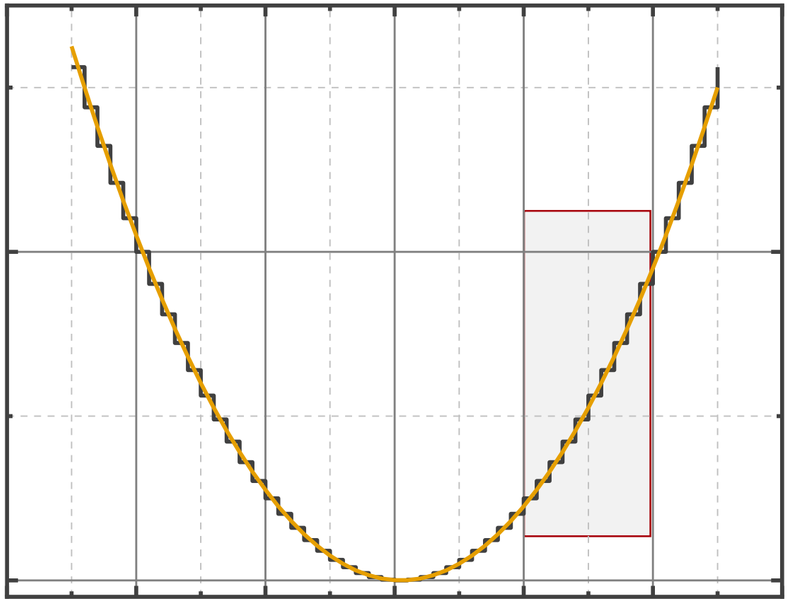
Discrete space

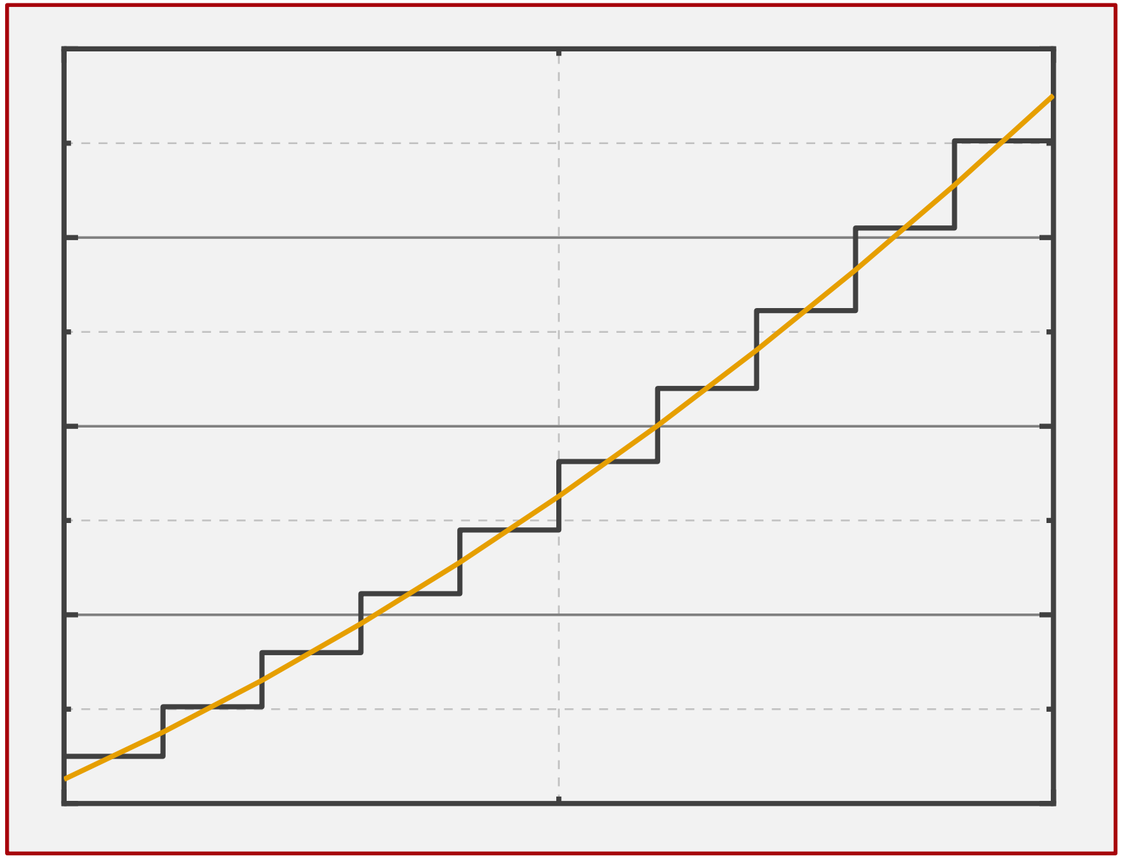
image
discrete space



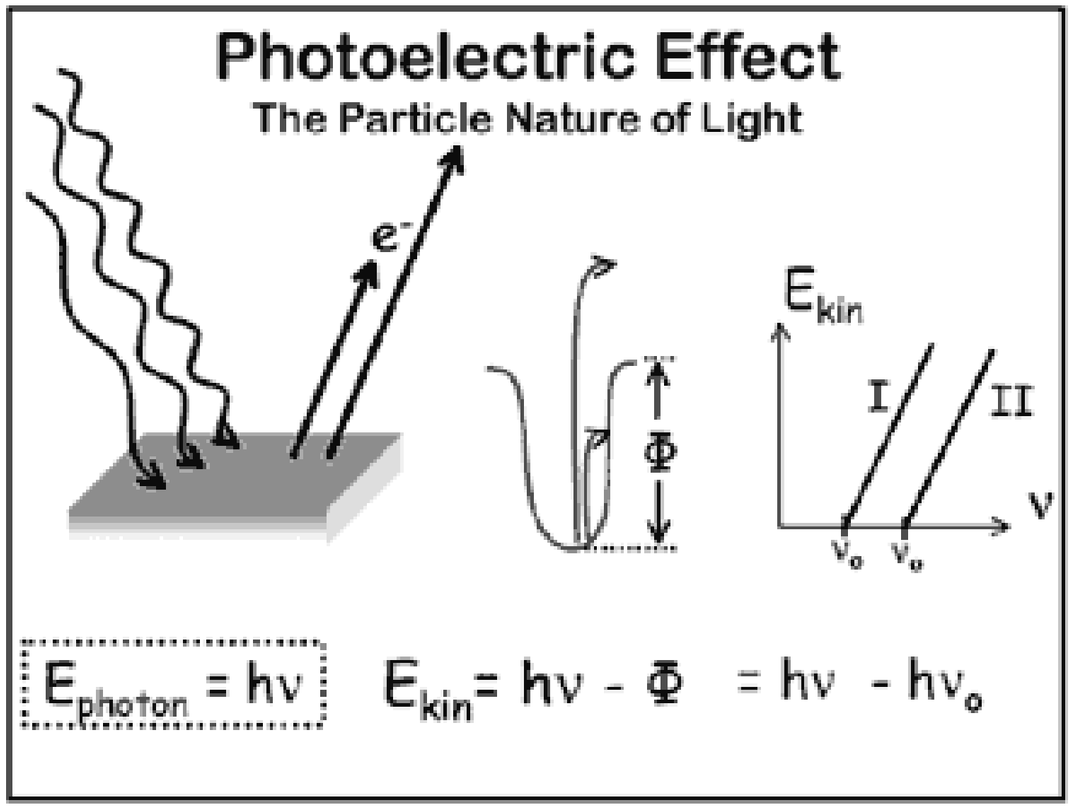


Continuous to discrete mapping





Digital detection devices



UC Berkeley's Digital Chem1A

photons

photo-electrons

Read out
AD conversion

pixel value

Array detection devices

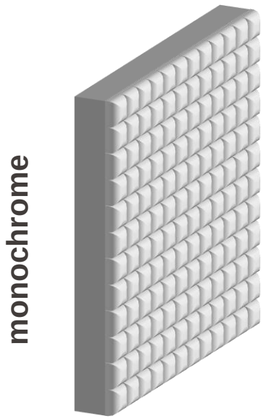
photons

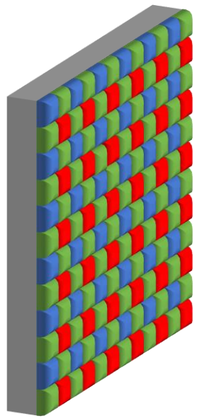
↓

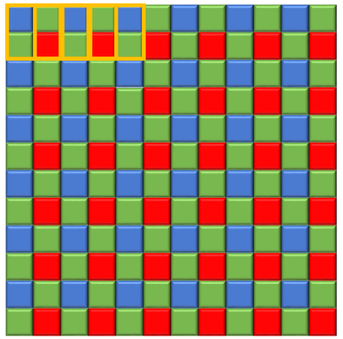
photo-electrons

↓ Read out AD conversion

pixel value







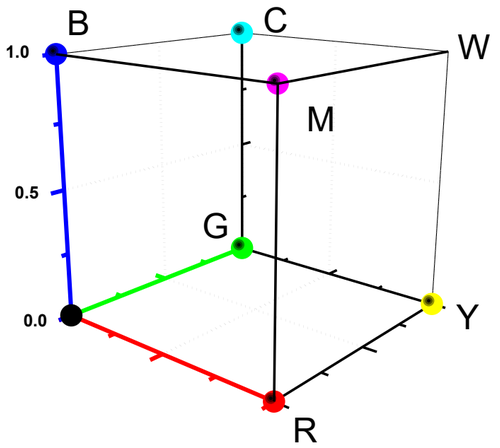
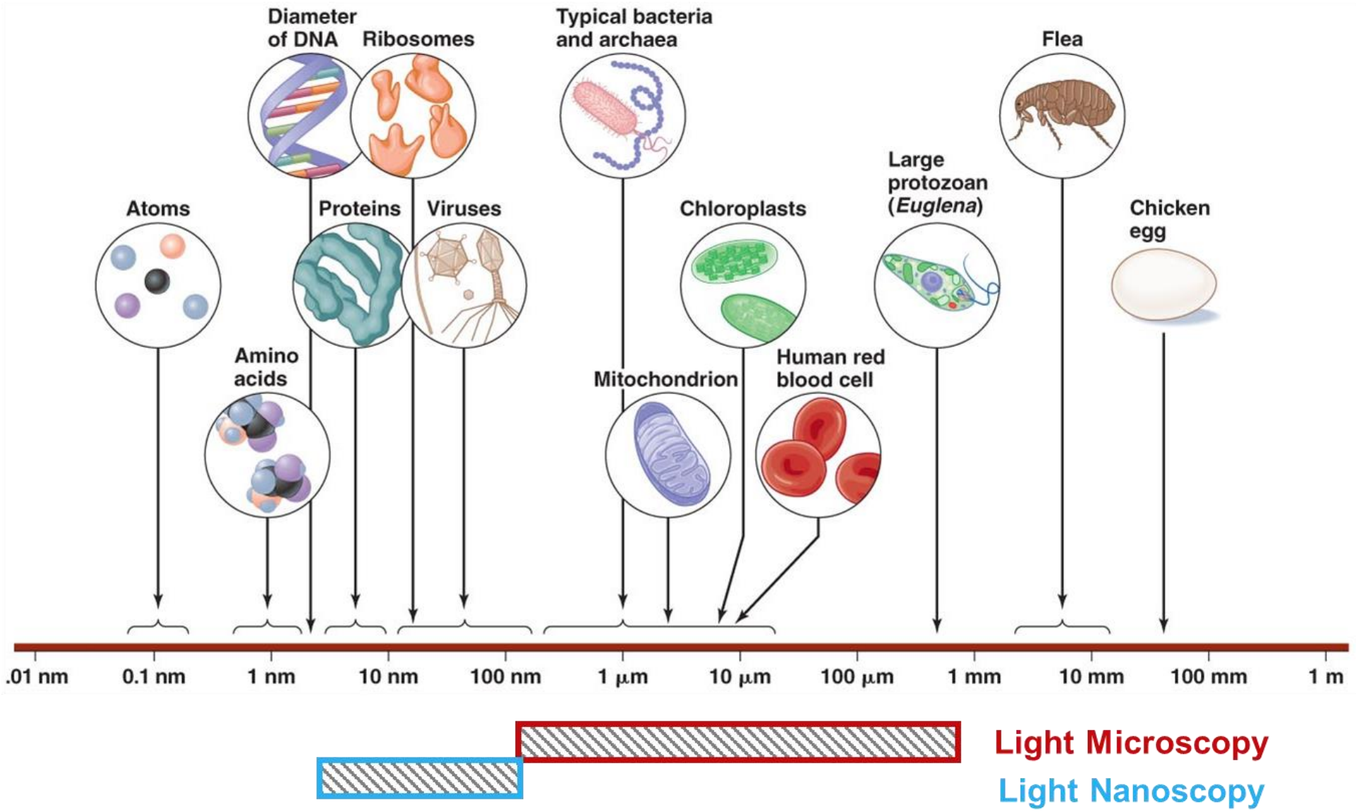


Image Acquisition in light microscopy

Light Microscopy

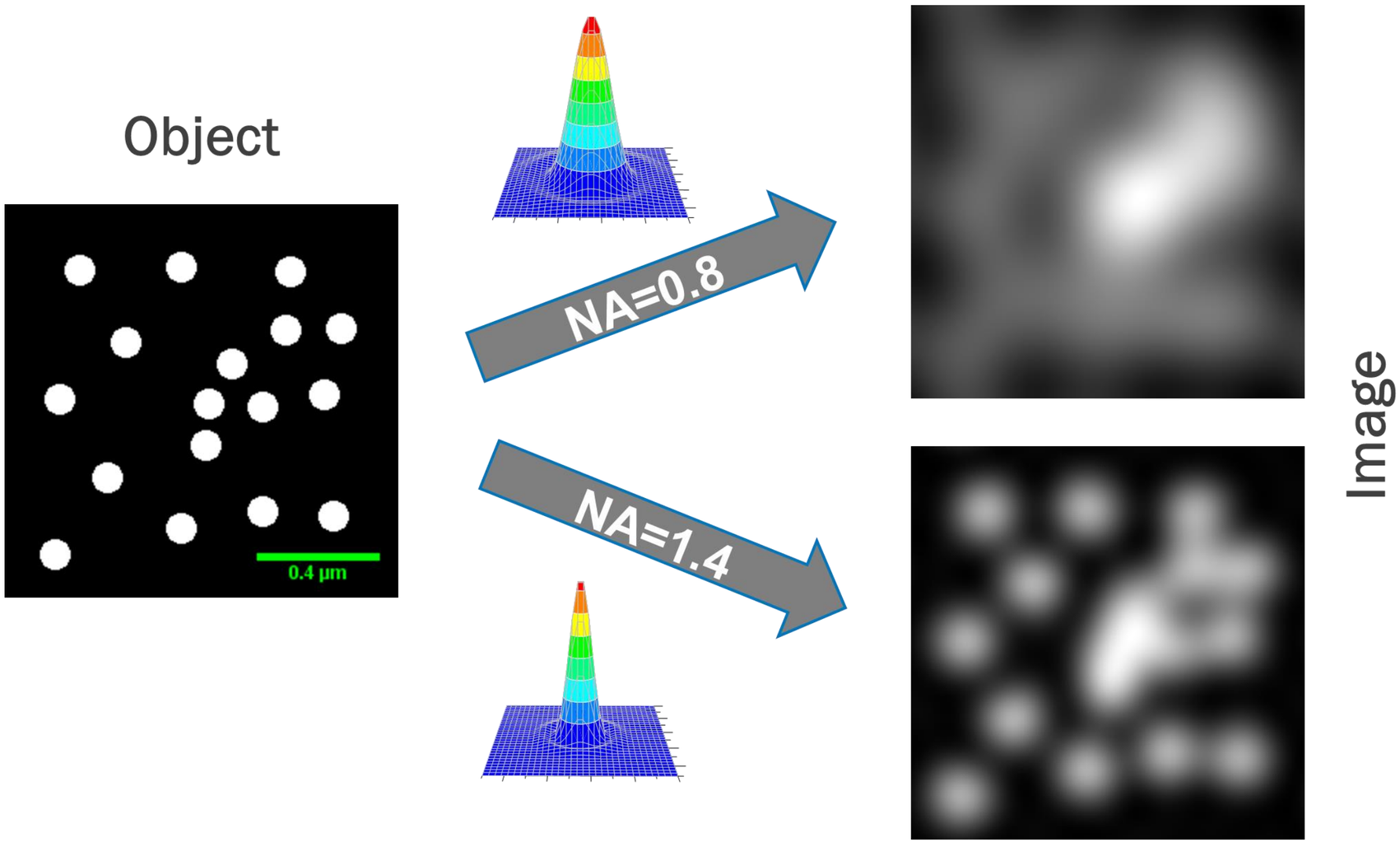
- Magnification
- Resolution
- Contrast



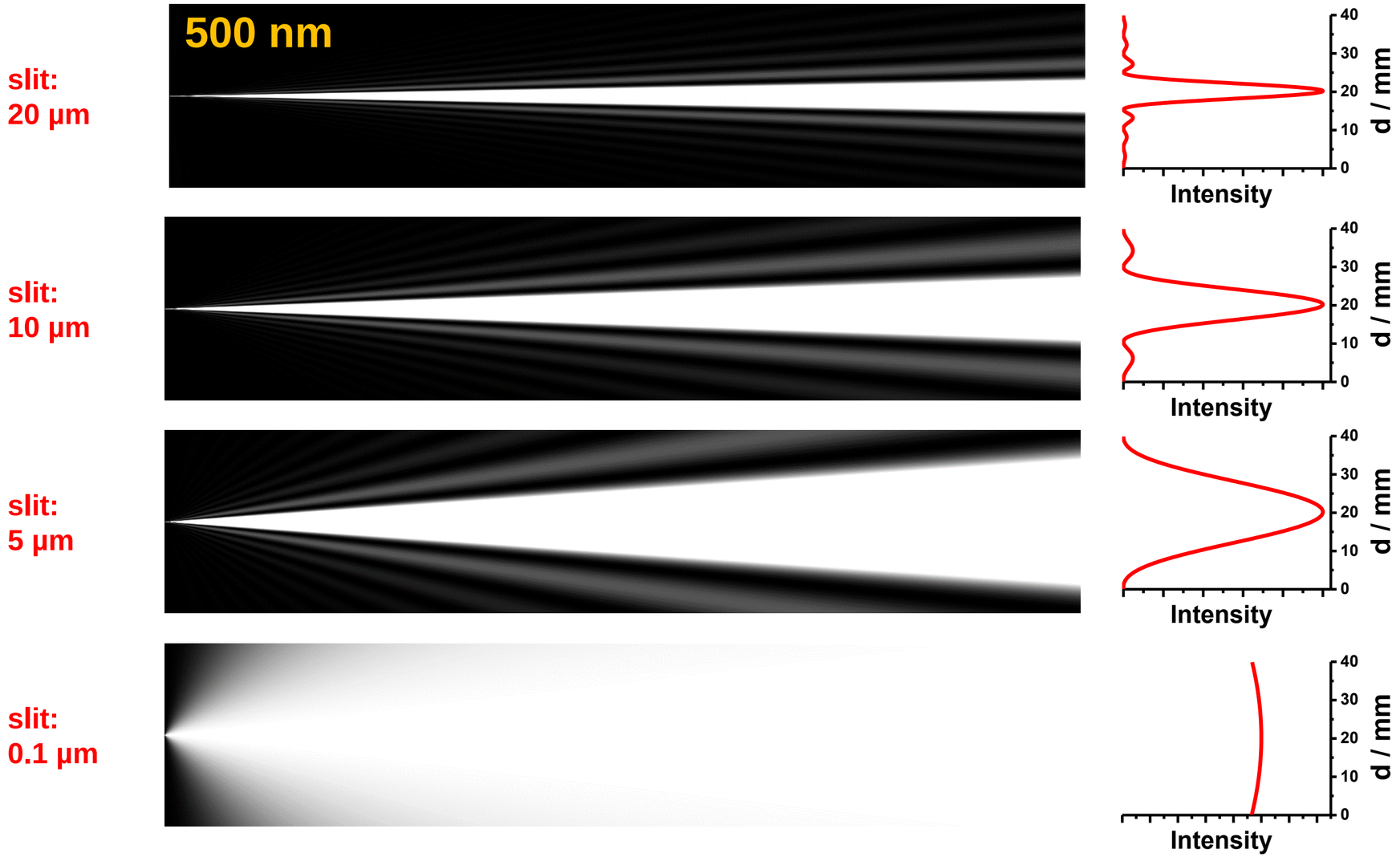
Contrast

- Light matter interaction
  - Absorption/Scattering
  - Emission
  - Transmission
  - Reflectance
- Light interacts with atoms, molecules and nanostructures
- Fluorescence microscopy
  - Dark background (high sensitivity)

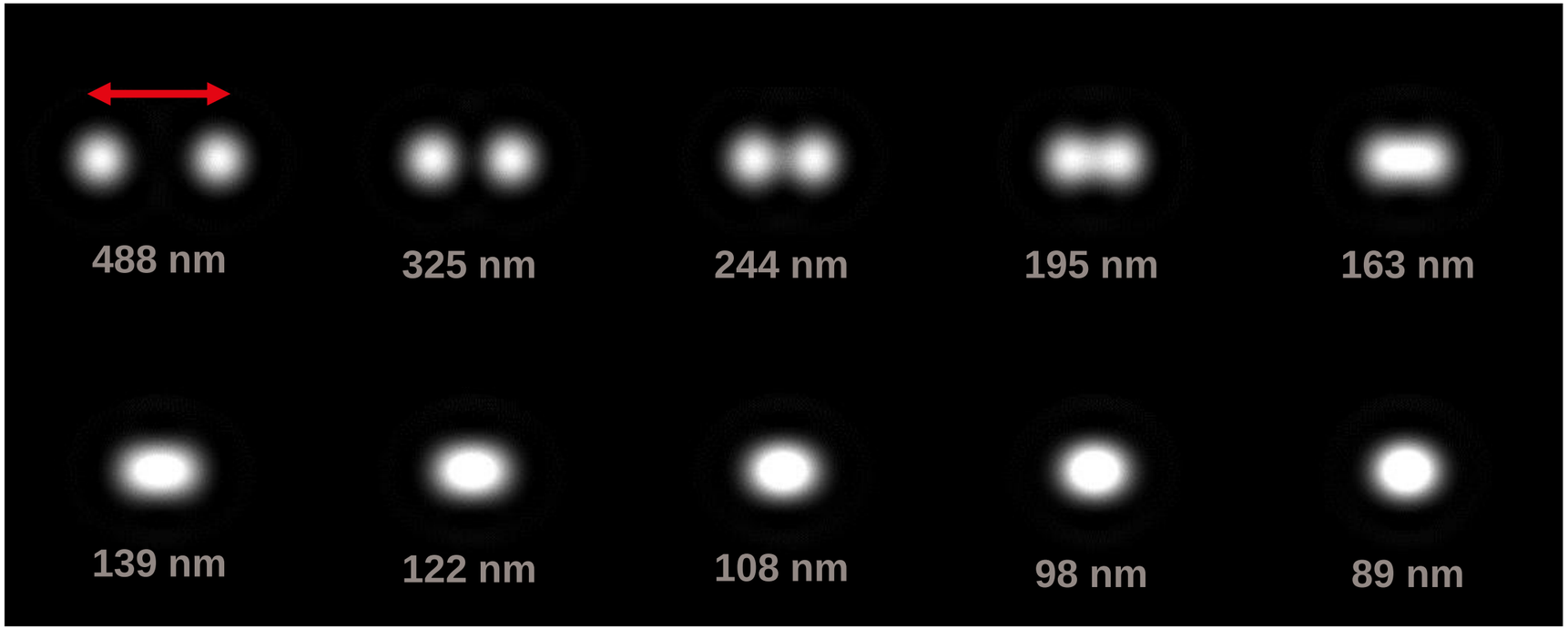
Low pass filtering



Diffraction Single Slit



Resolution

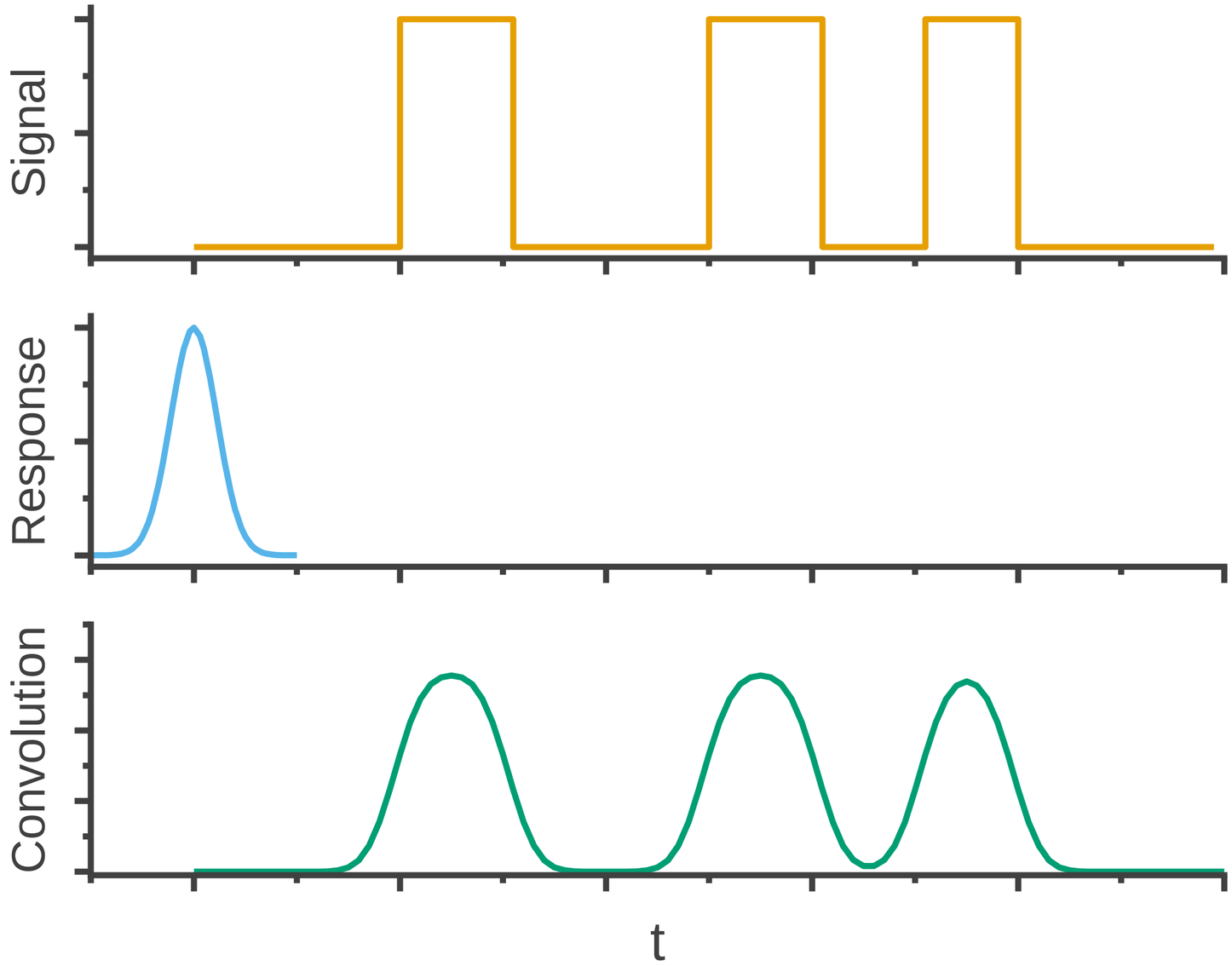


wavelength: 488 nm NA=1.4

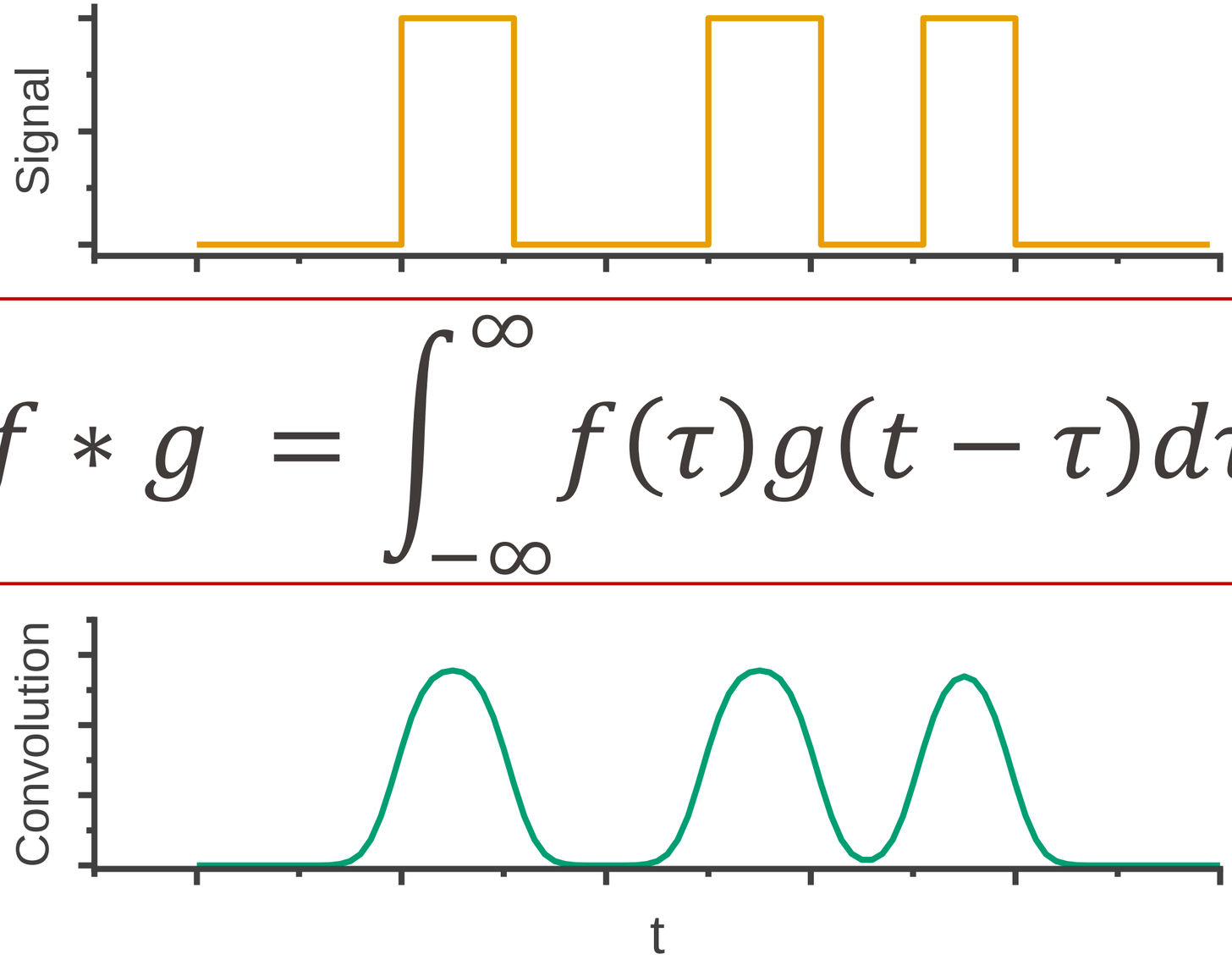
Rayleigh: 212 nm

Abbé: 174 nm

Convolution

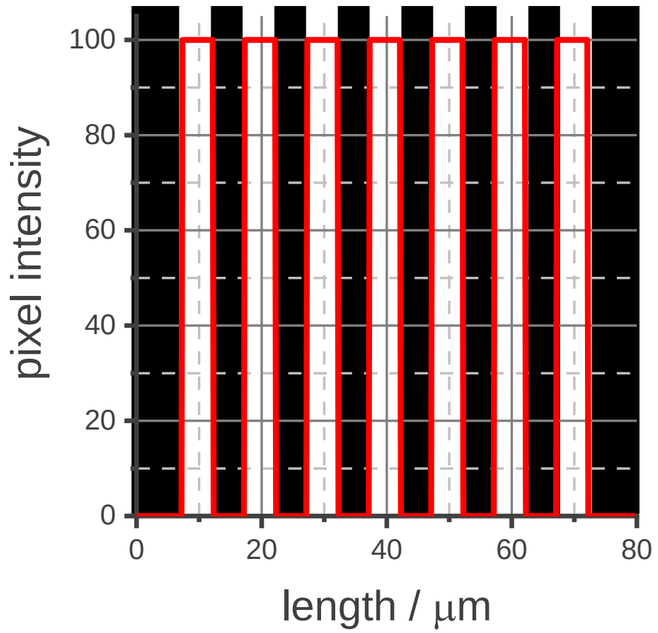


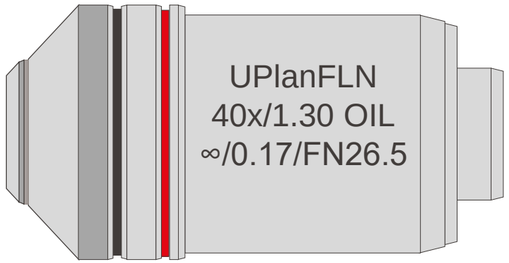
Convolution

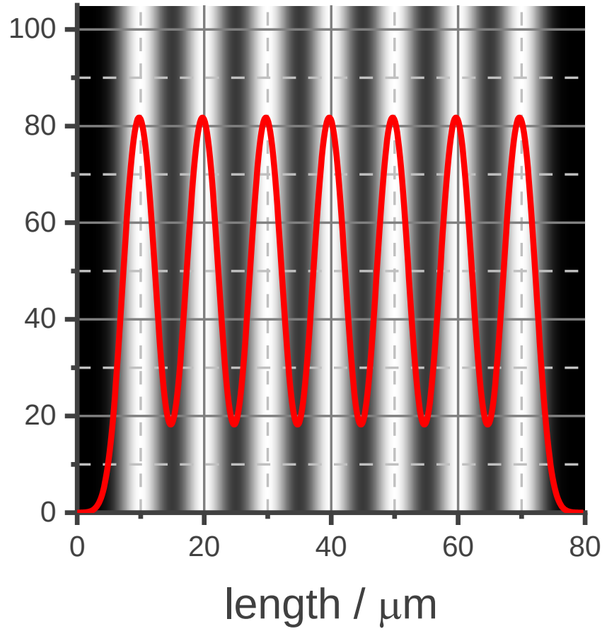


$$f * g = \int_{-\infty}^{\infty} f(\tau) g(t - \tau) d\tau$$

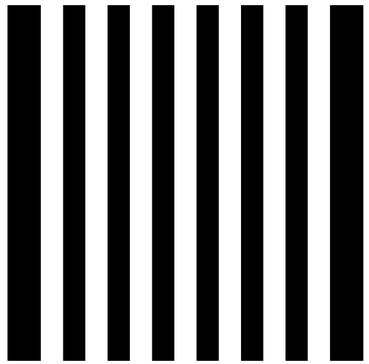
Low pass filtering

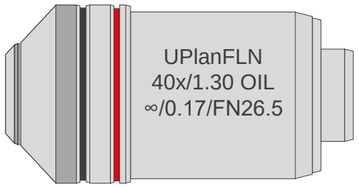


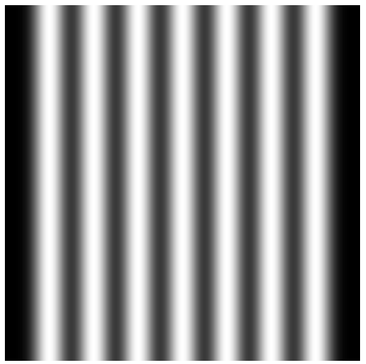




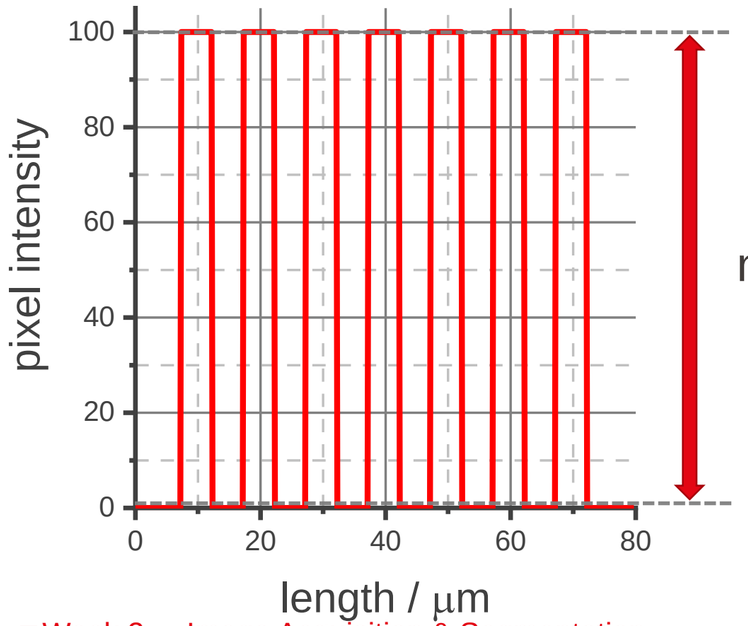
Contrast

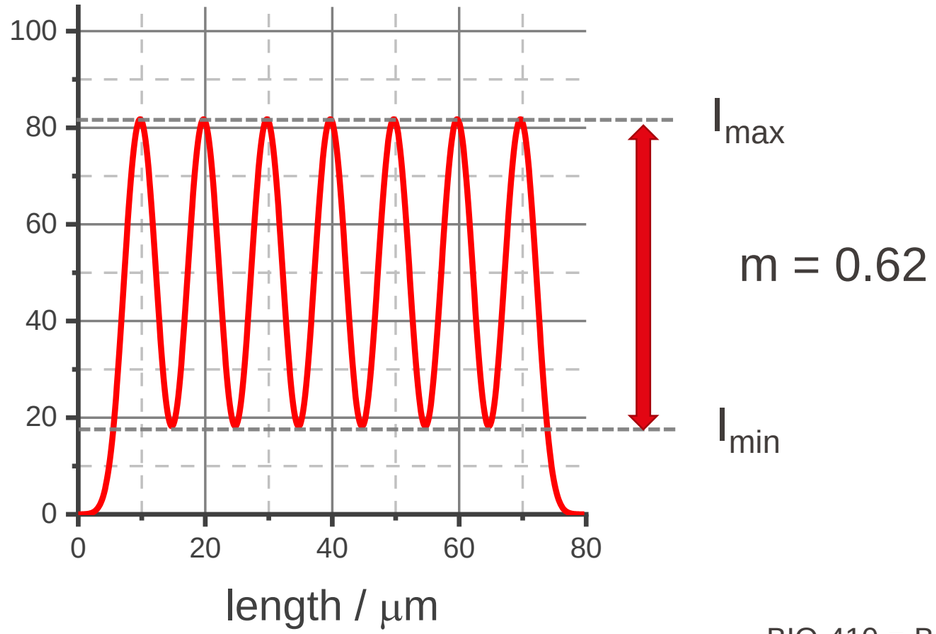




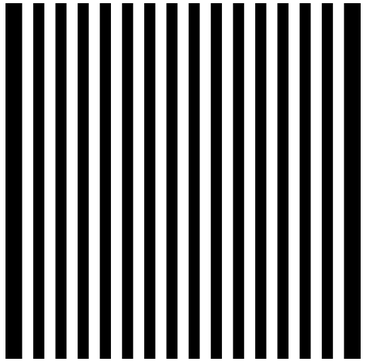


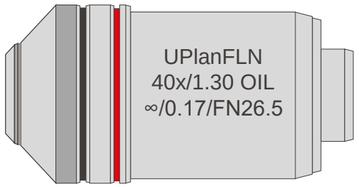
$$modulation = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

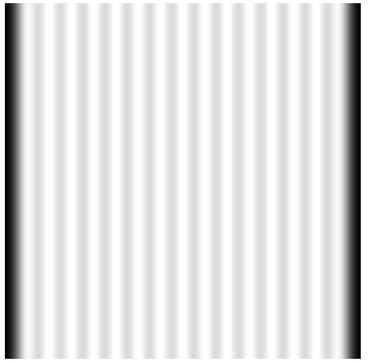




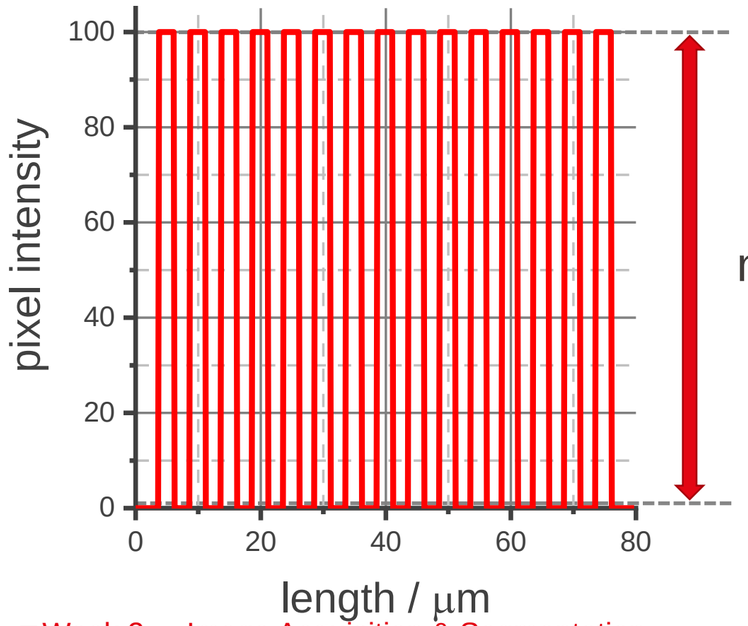
Contrast

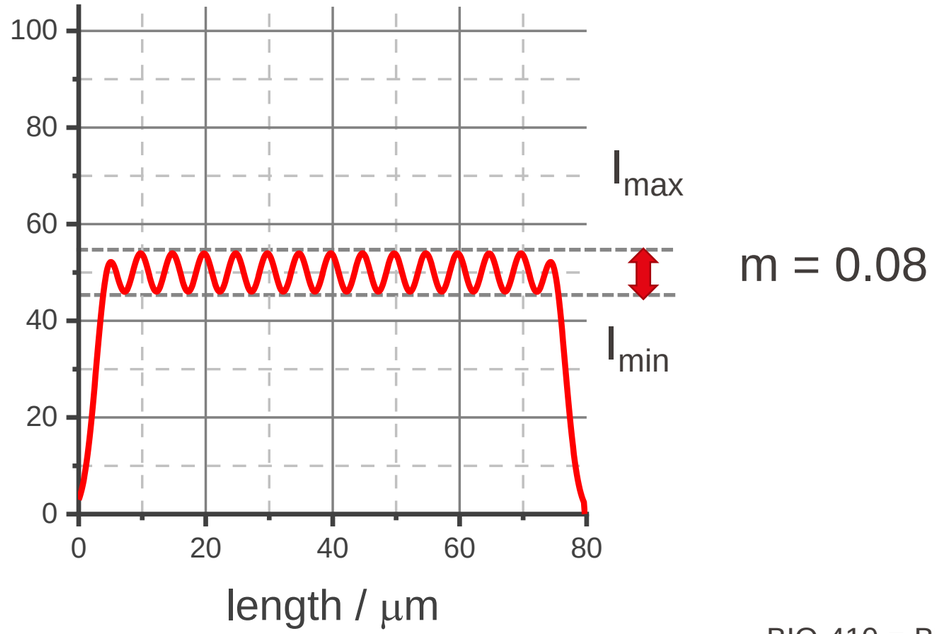






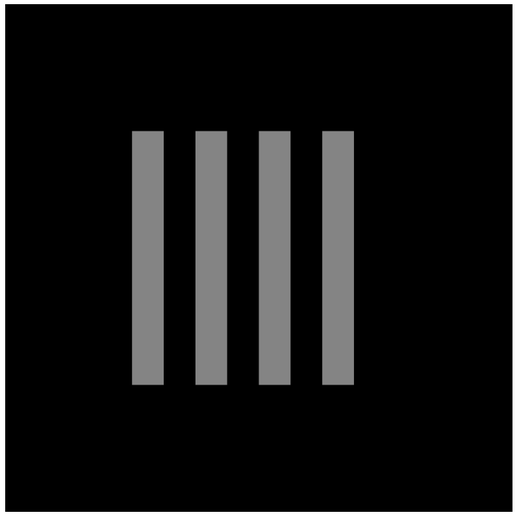
$$modulation = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

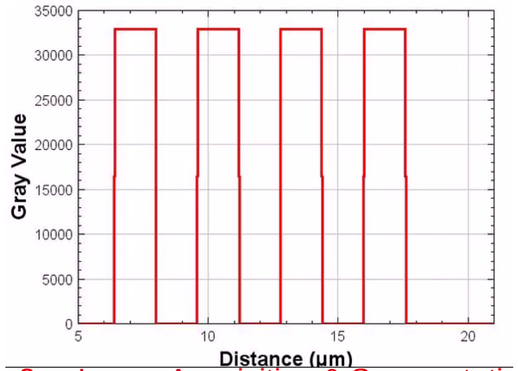




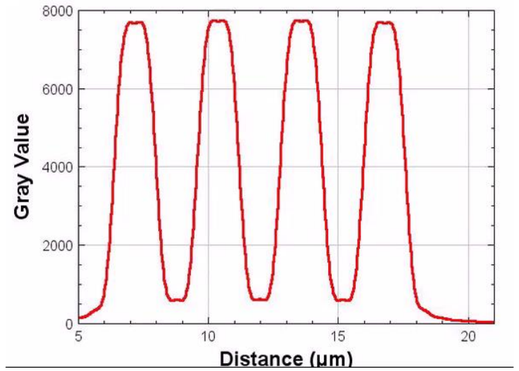
Modulation Transfer Function

Object

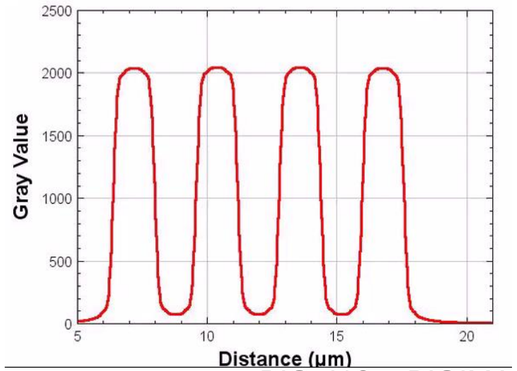




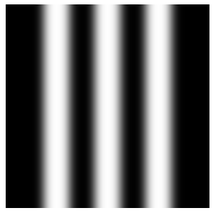
NA 0.4



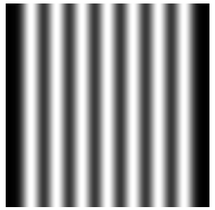
NA 0.8



Modulation Transfer Function

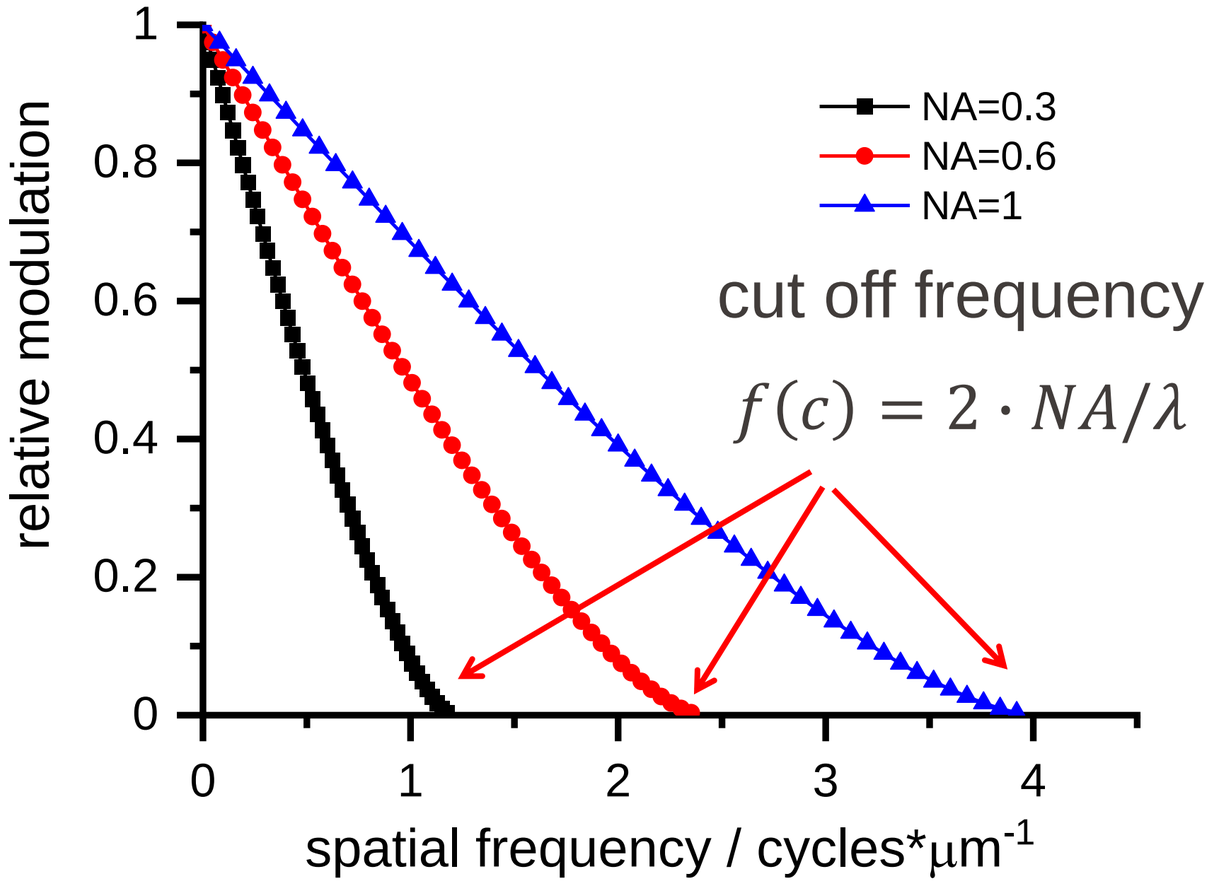


low spatial frequencies
high contrast

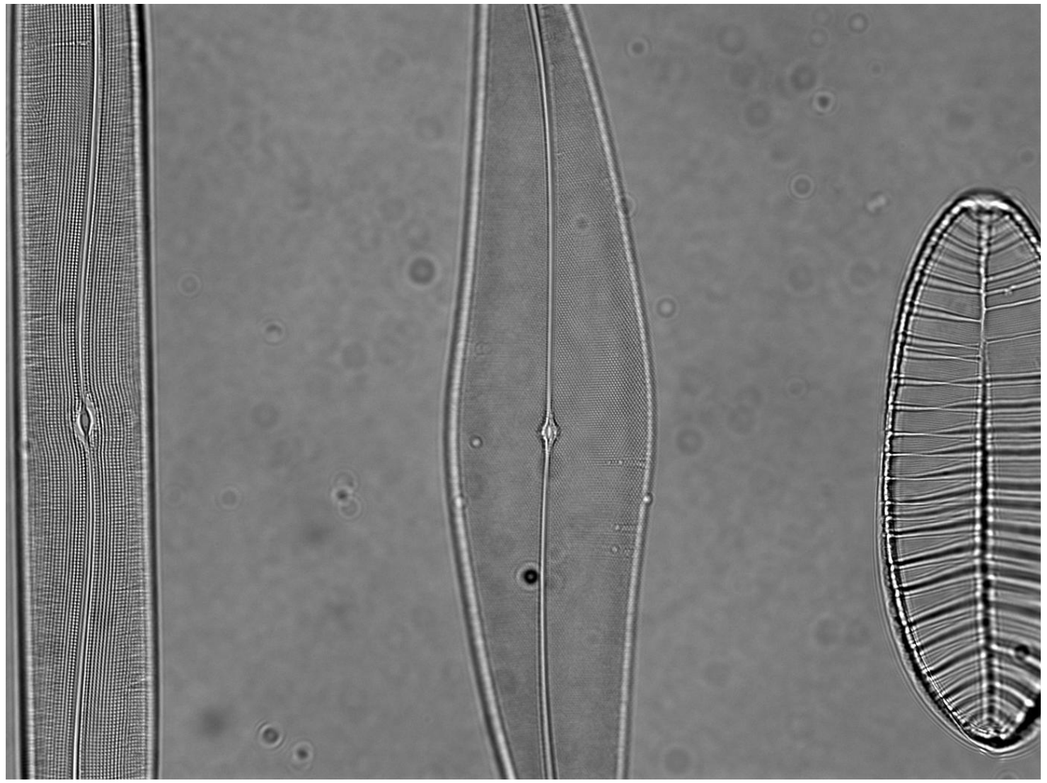


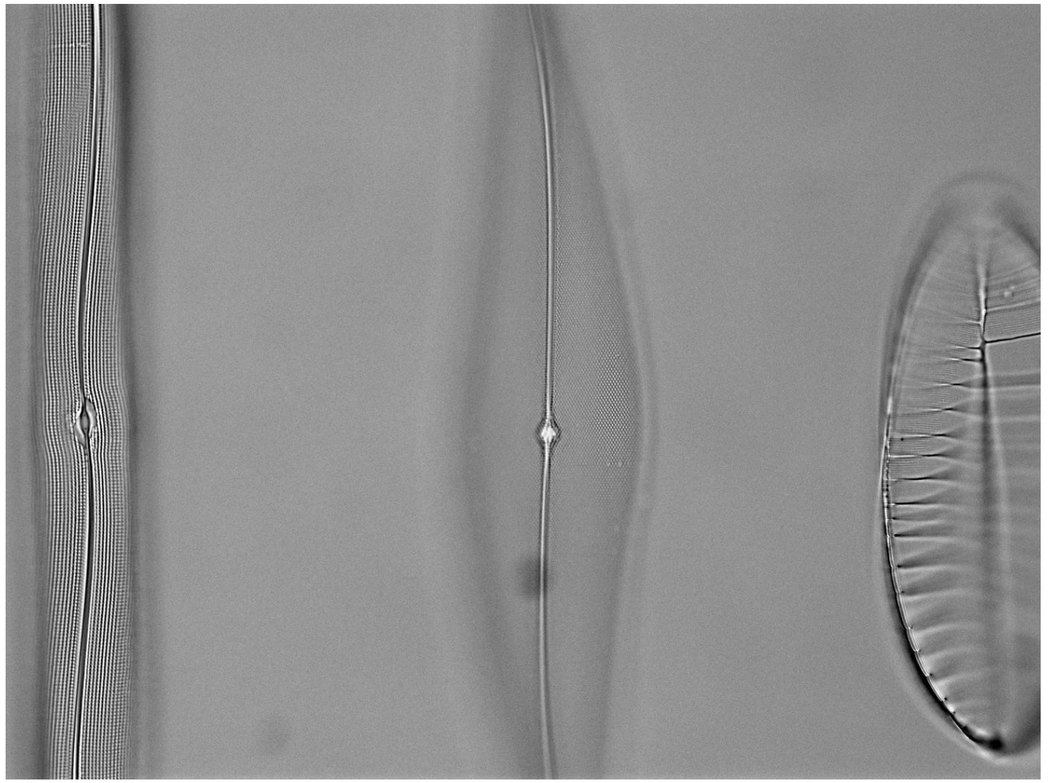
medium spatial frequencies
medium contrast

high spatial frequencies
low contrast

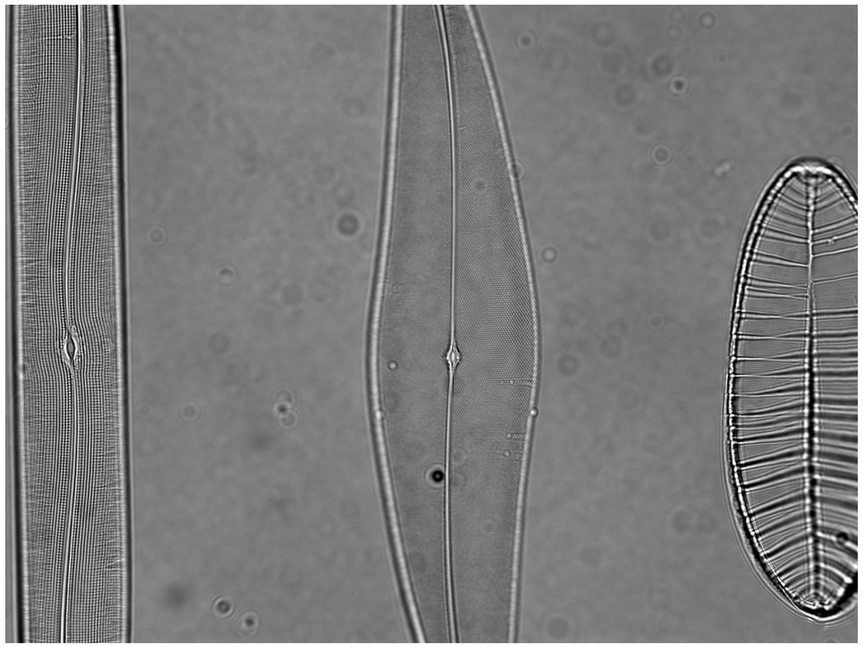


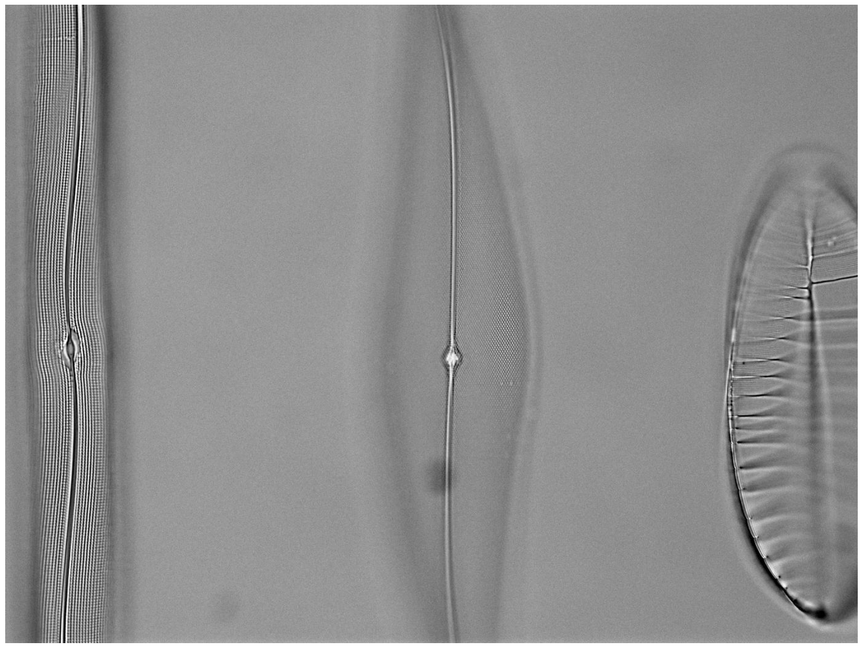
Are the images taken from the same object?





Images are artefacts

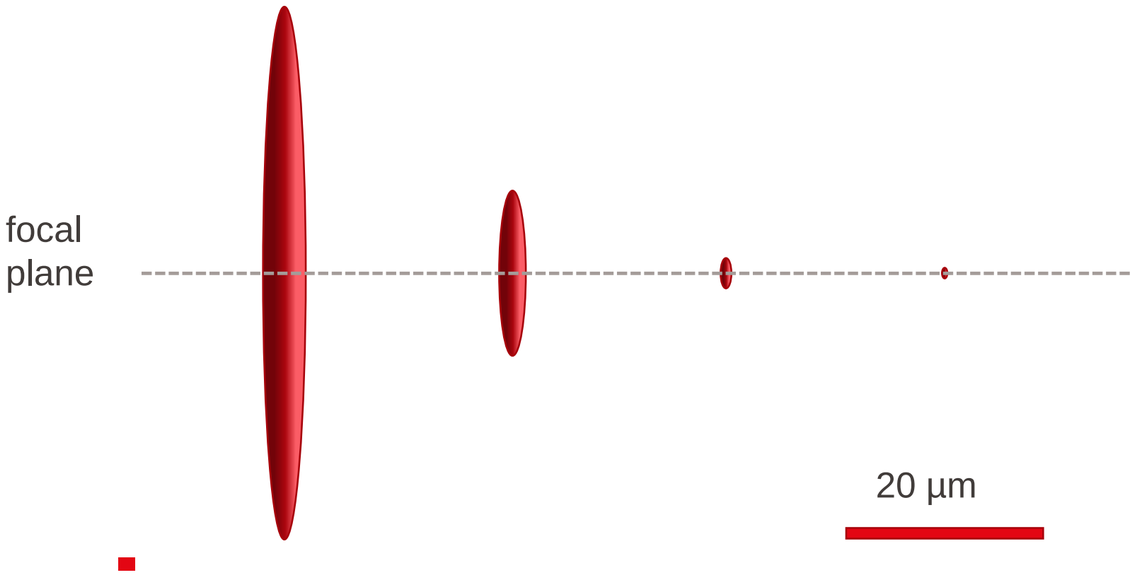




object (O) → image (I)

response function

Optical Slicing



$$\mathrm{DoF} = \frac{\lambda \cdot n}{NA^2} + \frac{n}{M \cdot NA} \cdot e$$

$$\text{Lateral resolution} = \frac{\lambda}{2 \cdot NA}$$

M	NA	n	DoF/µm	Lateral resolution/ µm
2	0.06	1	54.72	4.58
4	0.1	1	16.80	2.75
10	0.25	1	3.15	1.10
20	0.5	1	1.20	0.55
40	0.65	1	0.80	0.42
50	0.95	1.518	1.04	0.29
60	0.8	1	0.69	0.34
100	1.25	1.518	0.91	0.22
100	0.9	1	0.62	0.31

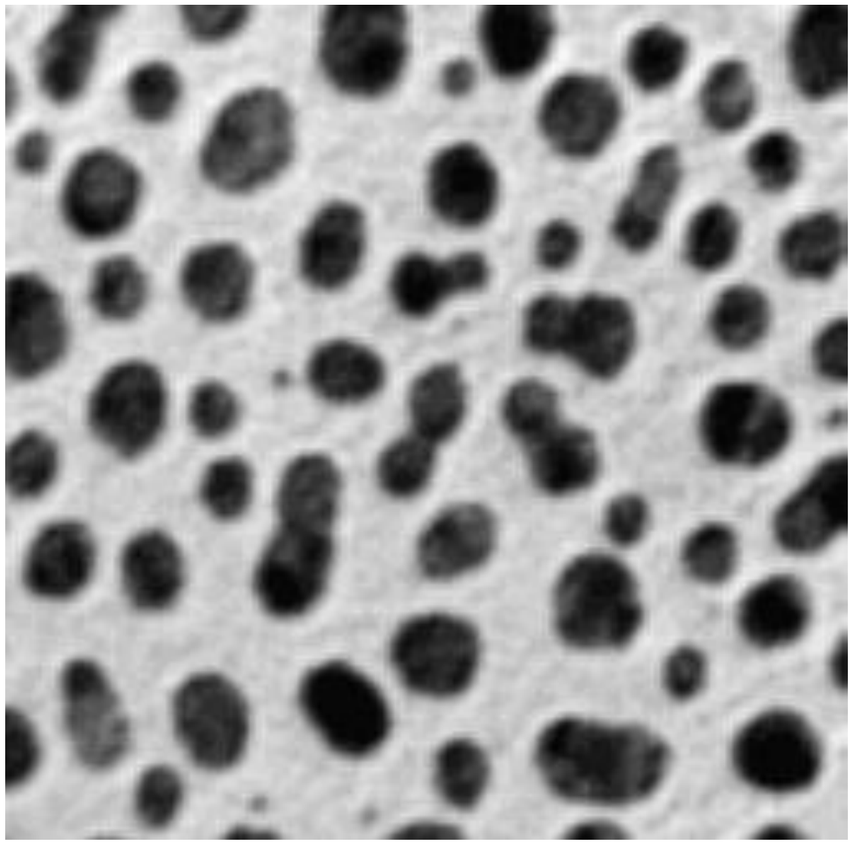
λ: wavelength
0.55 µm

e: pixel size camera
6.45 µm

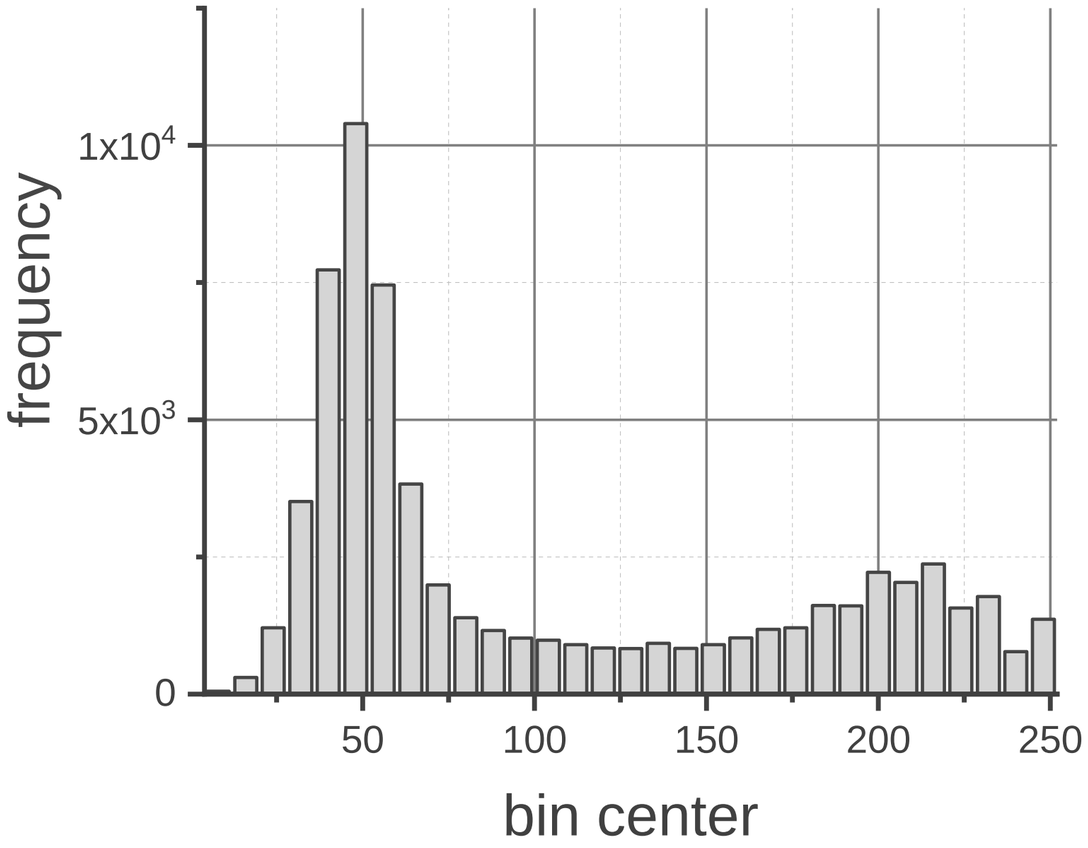
Segmentation

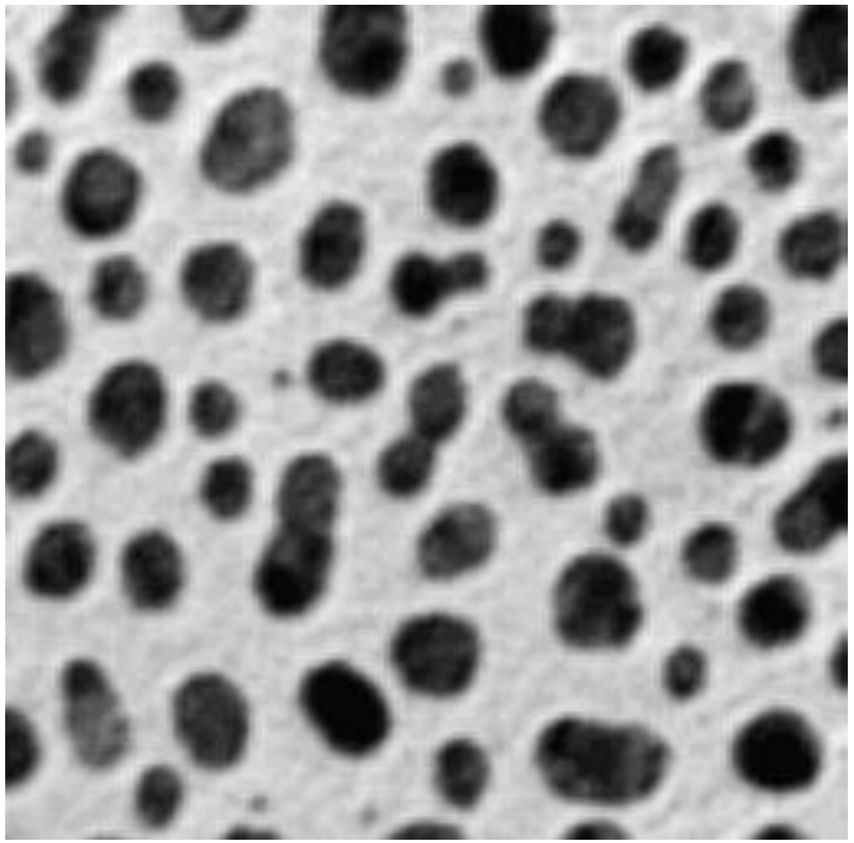
Image Segmentation

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

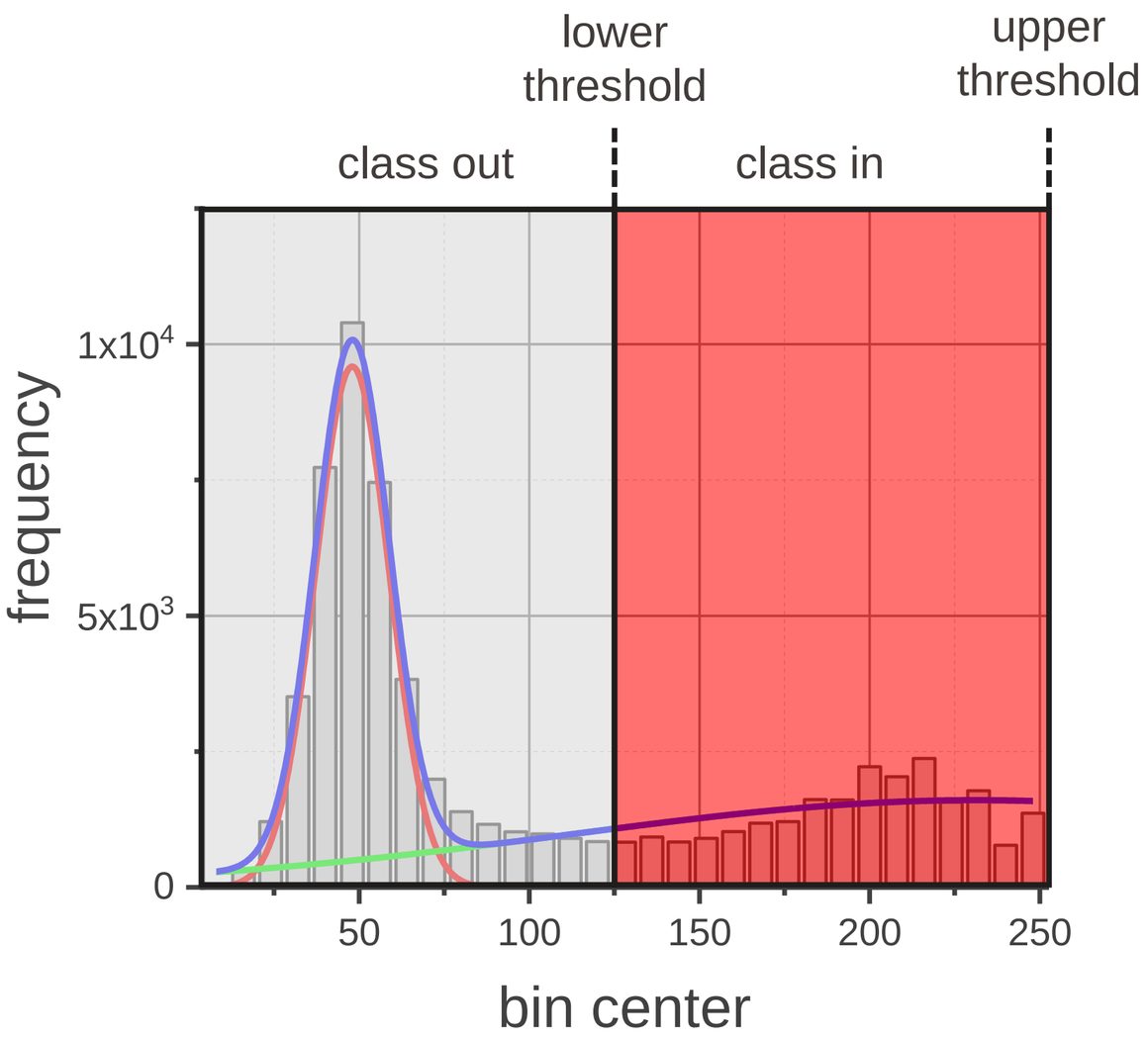


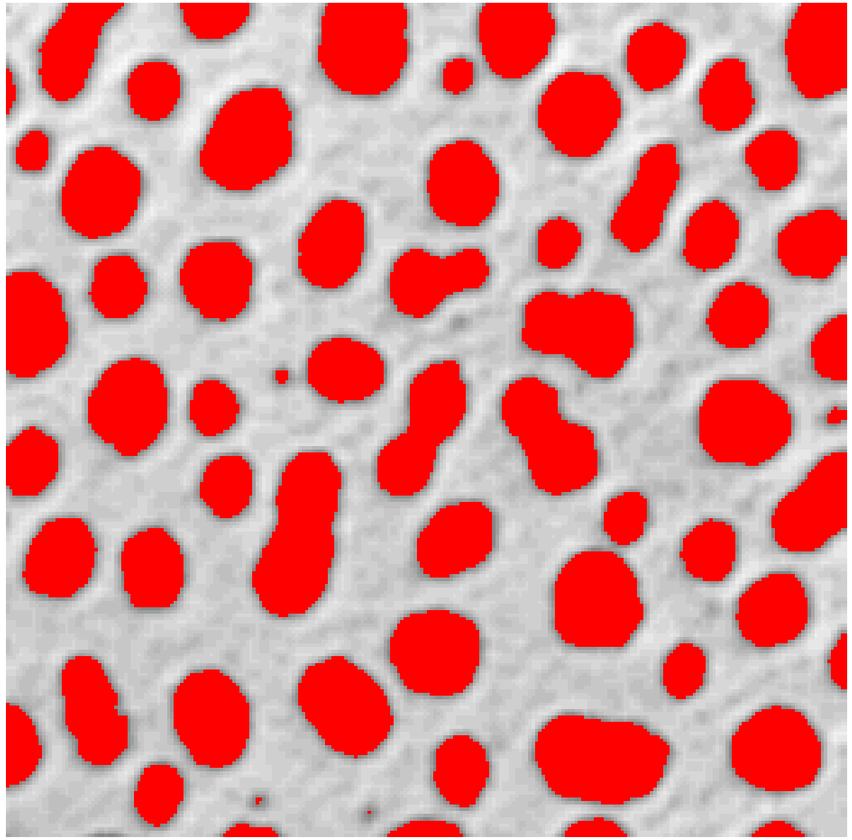
Intensity Thresholding



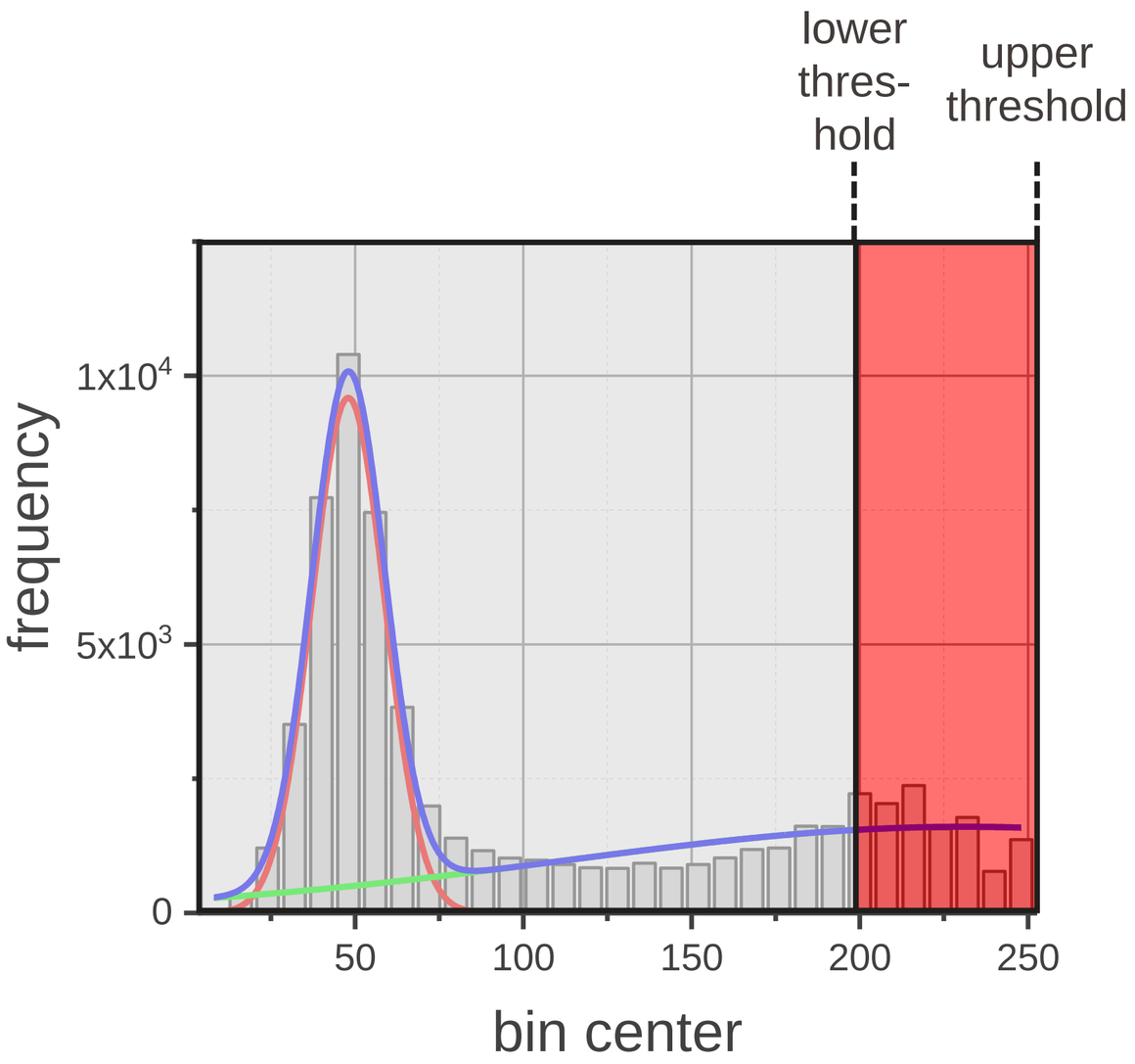


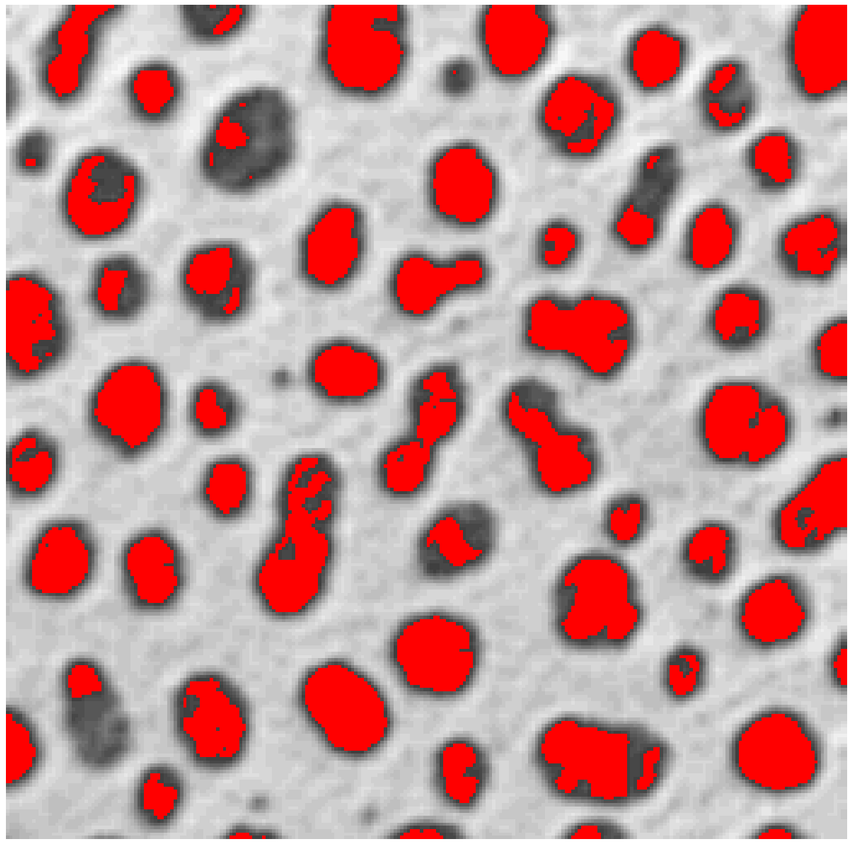
Intensity Thresholding



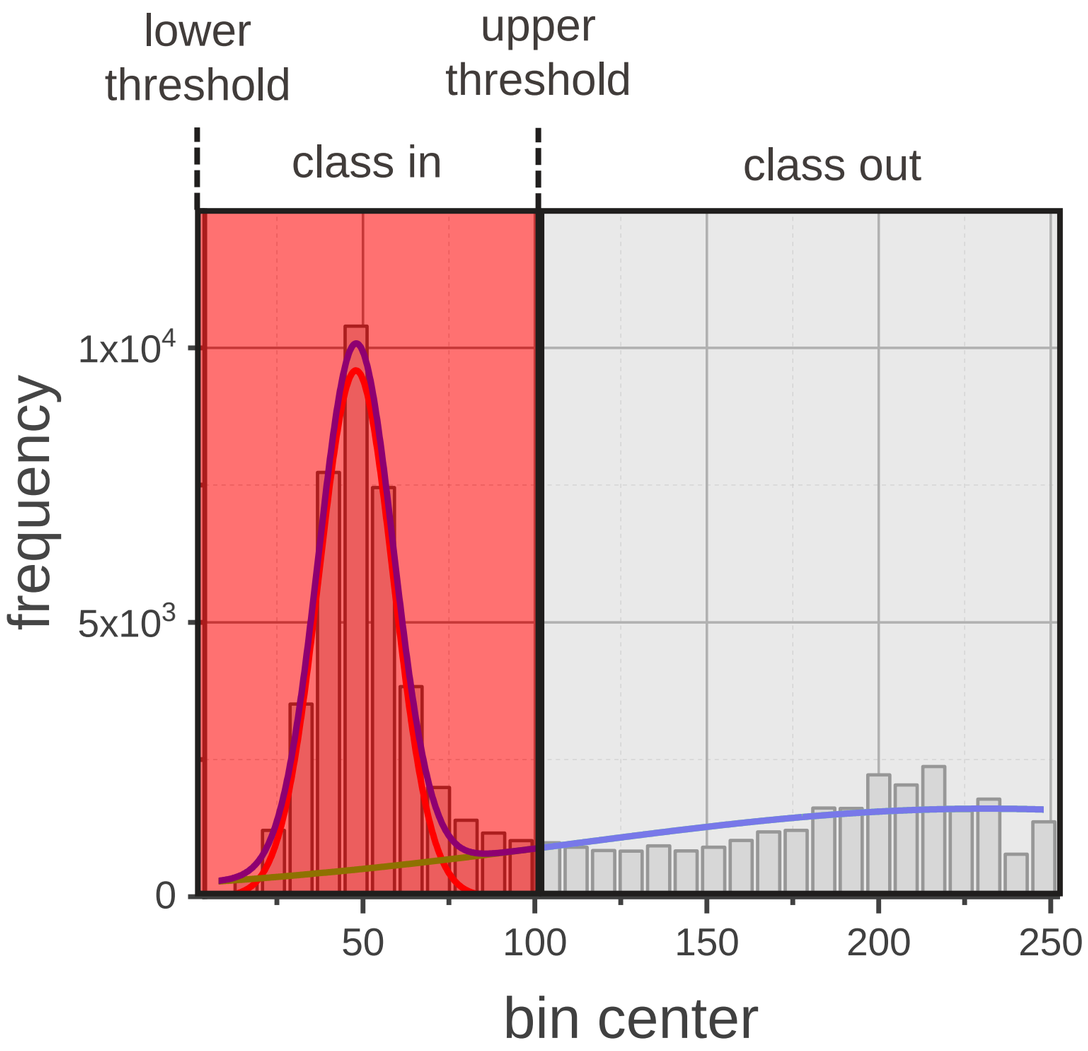


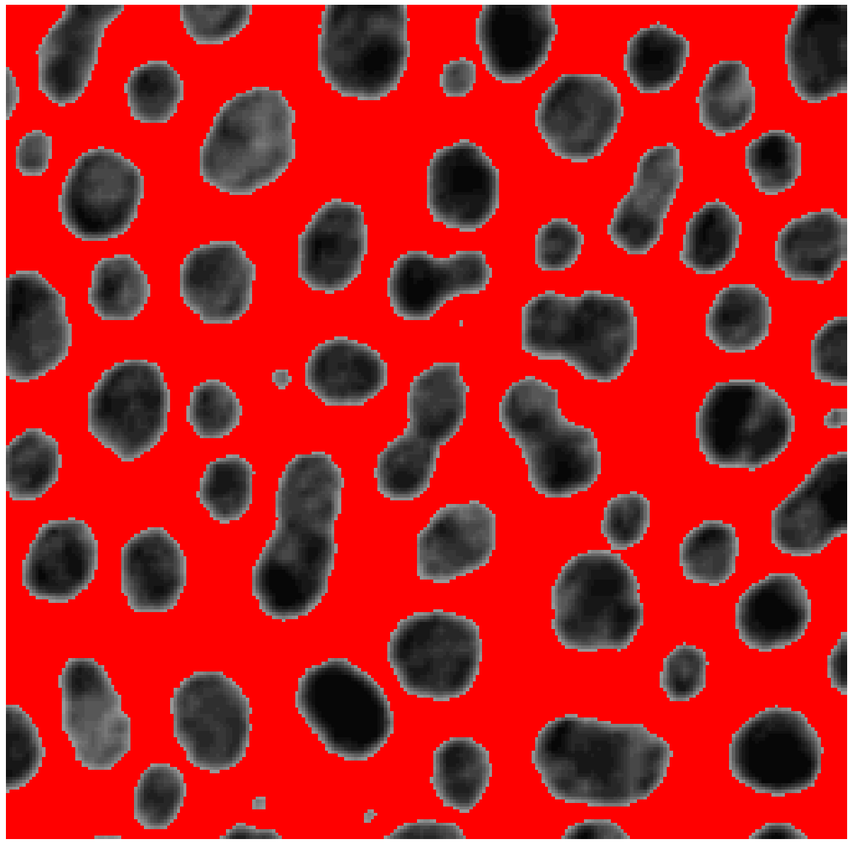
Intensity Thresholding





Intensity Thresholding



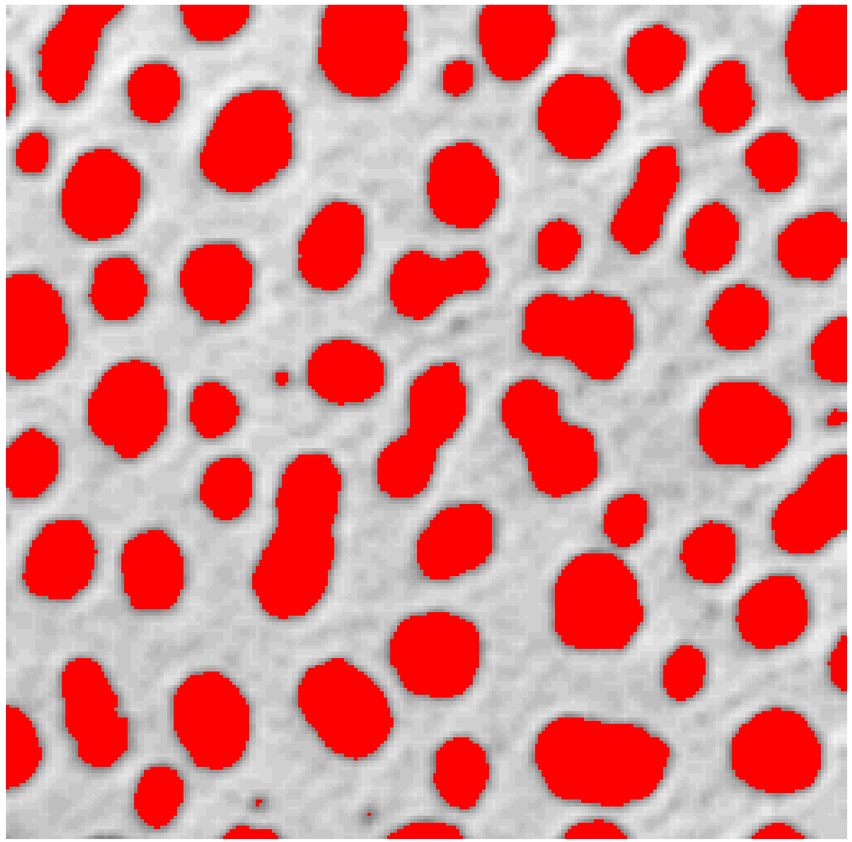


Intensity Thresholding

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

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

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

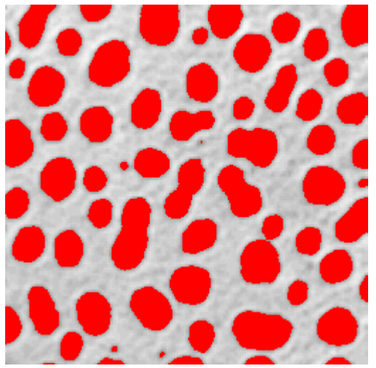


Basic global thresholding

- 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:

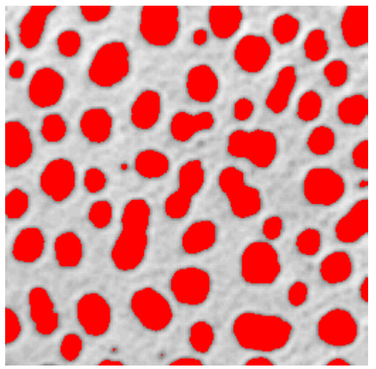
$$T = 0.5(m_1 + m_2)$$

- Repeat until ΔT is smaller then a predefined value



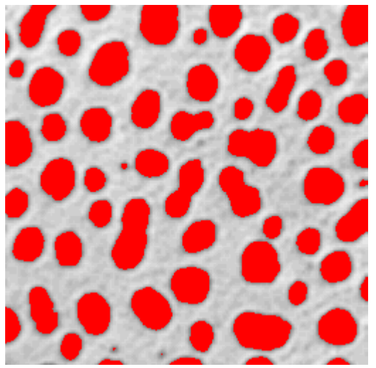
T_{start}=103

m_1=186
m_2= 52
T_1 = 121



T_{start}=121

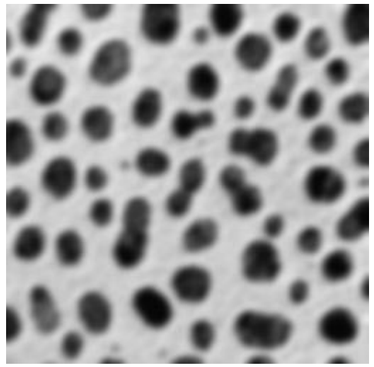
m_1=195
m_2= 56
T_2 = 125

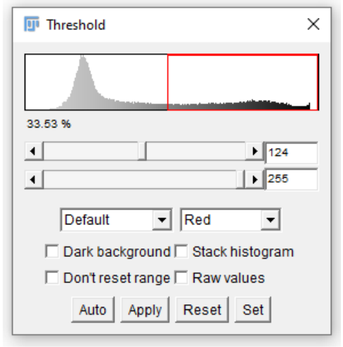


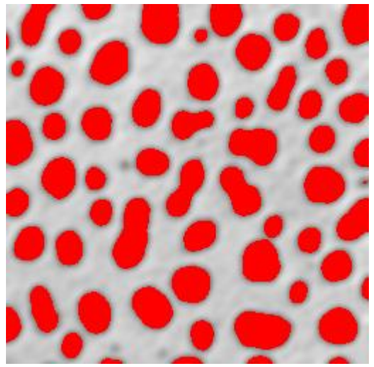
T_{start}=125

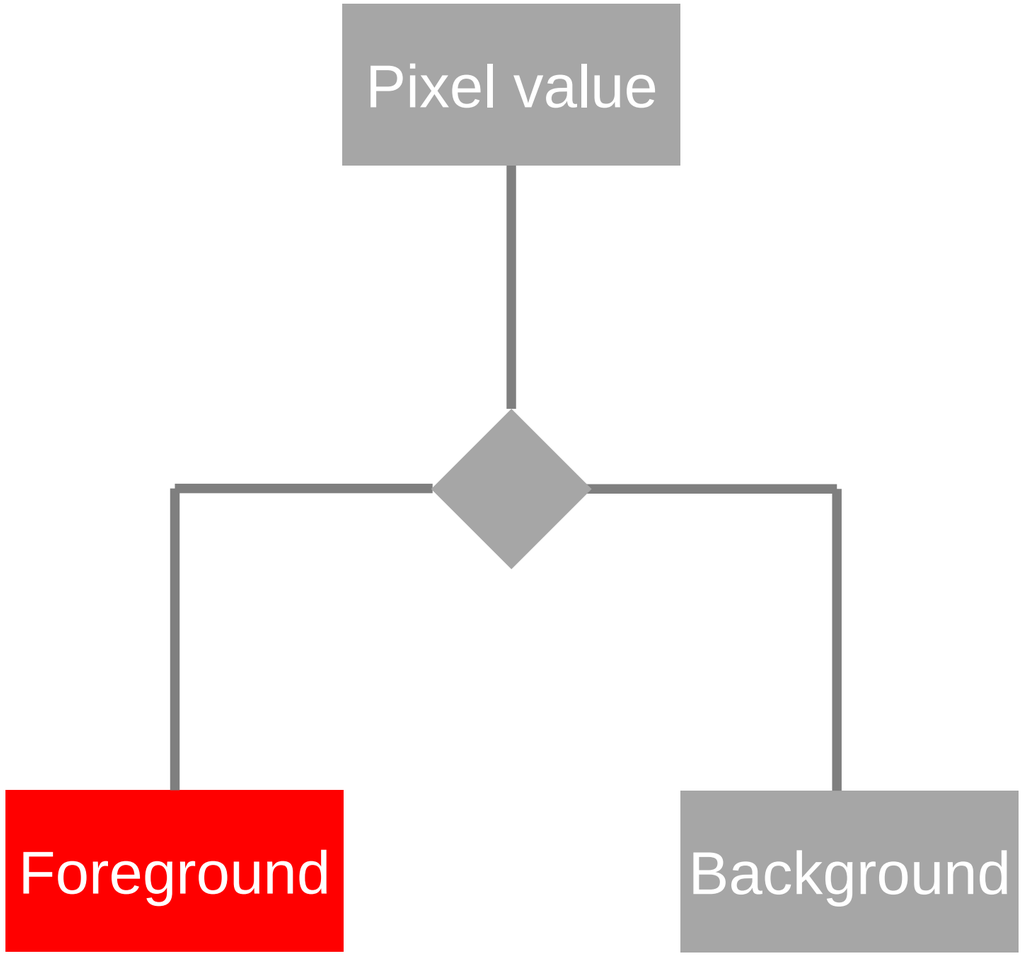
m_1=195
m_2= 56
T_3 = 125

Thresholding

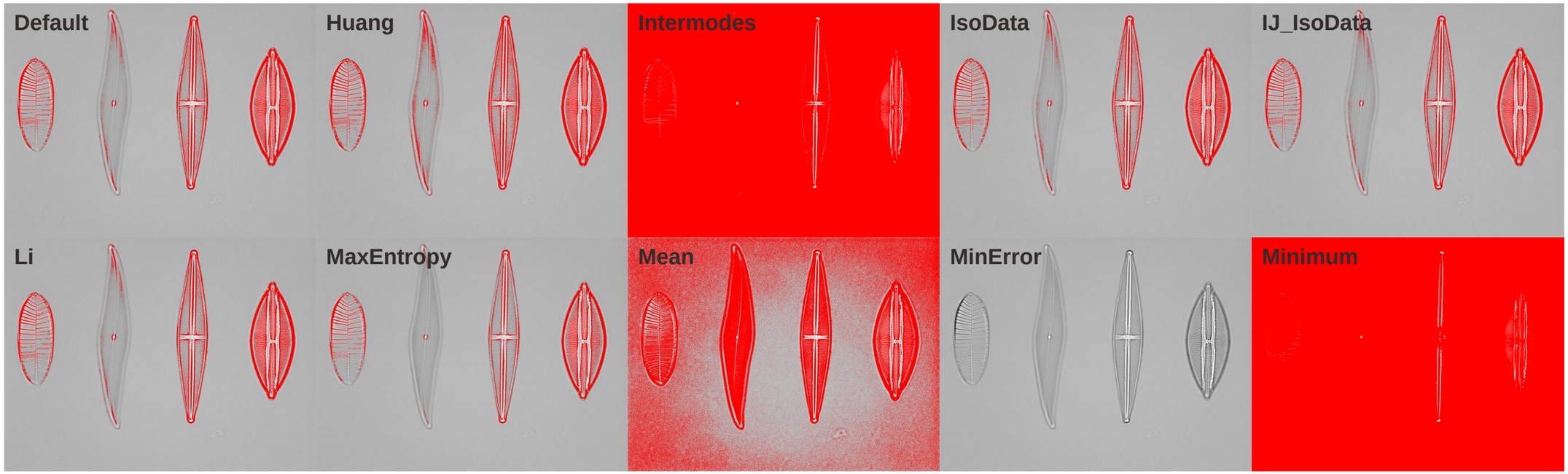




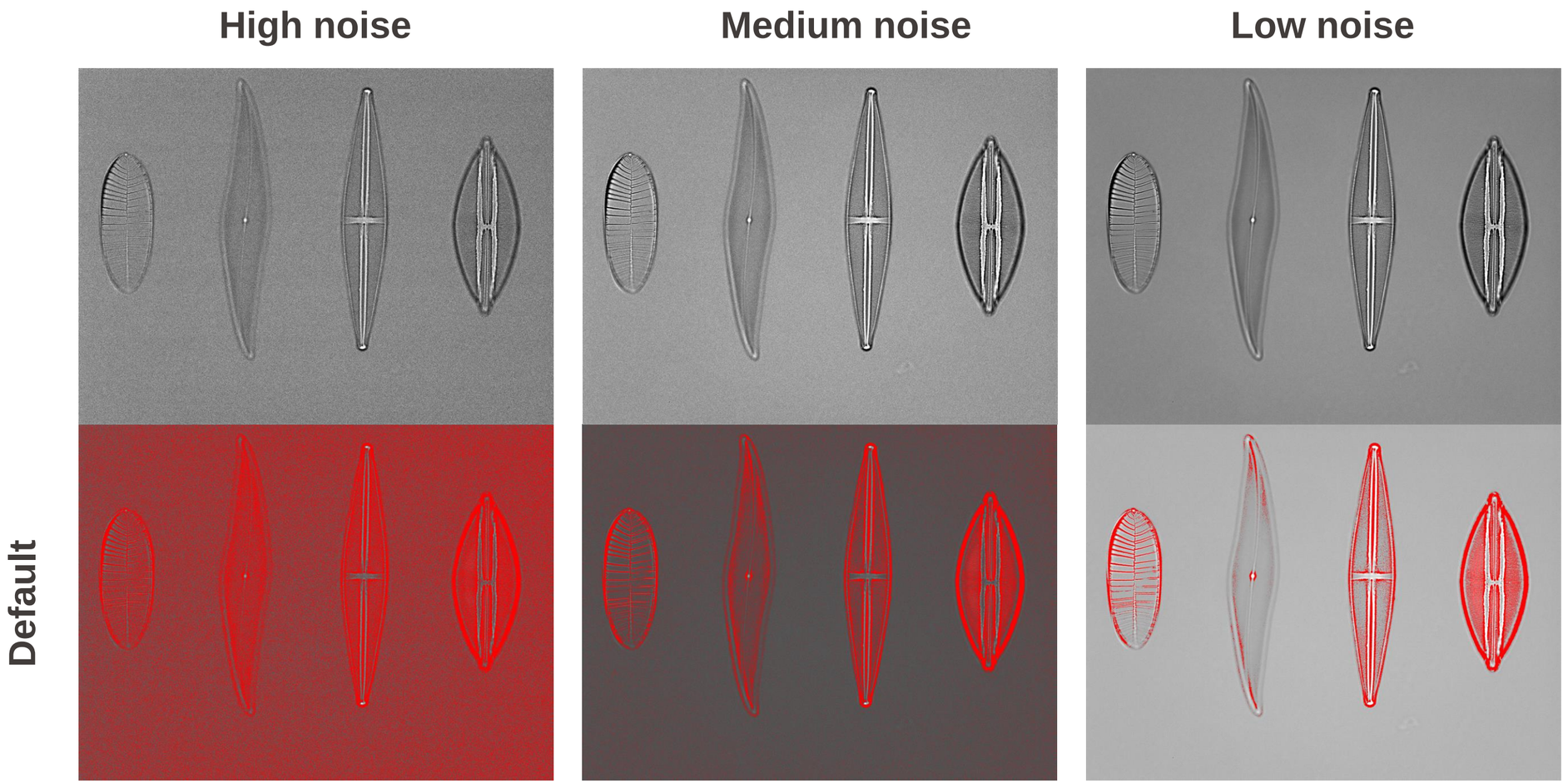




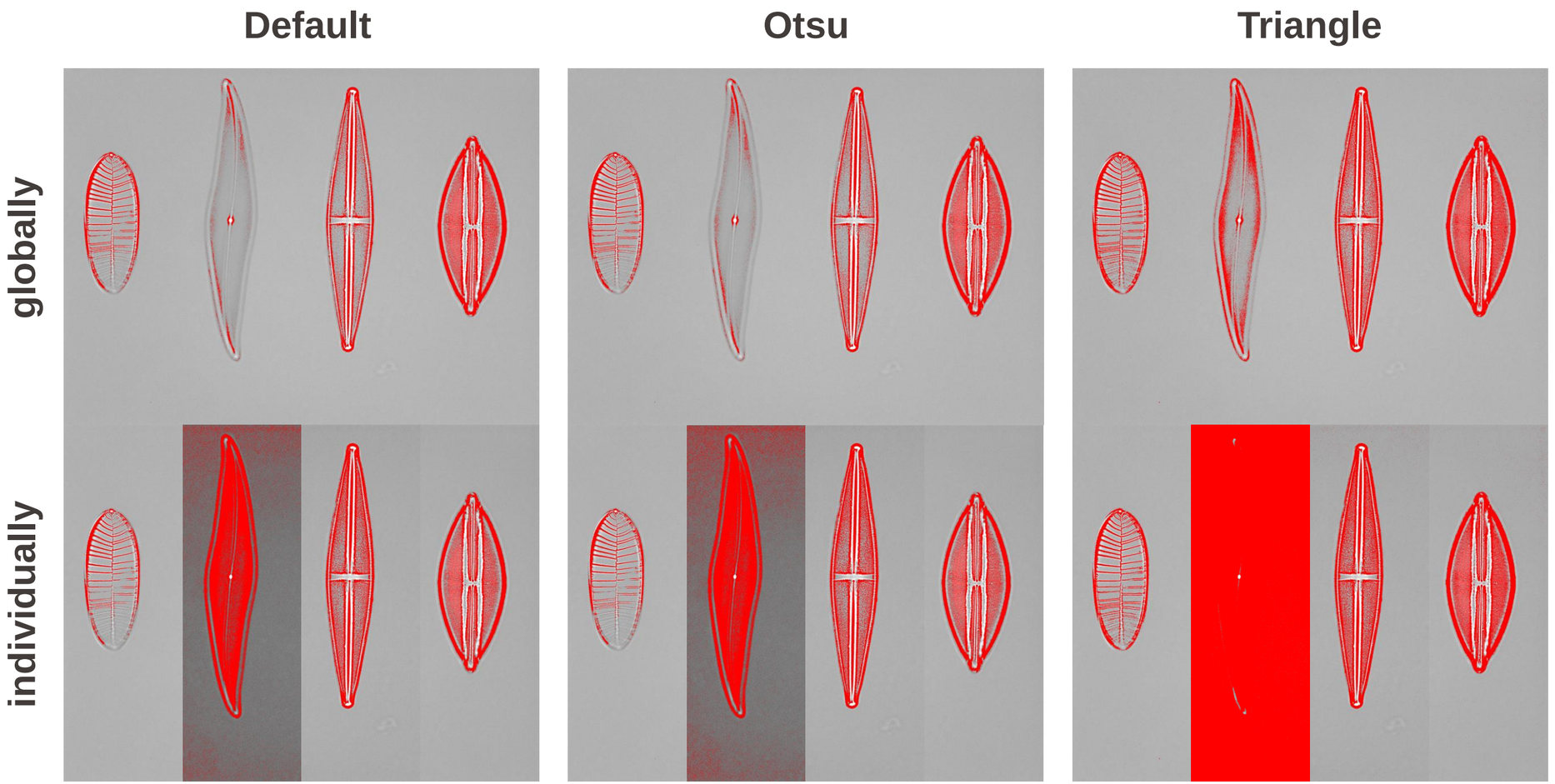
Thresholding algorithms



Thresholding
Content dependency



Thresholding
Context dependency



Thresholding

- Pros :
  - Simple
  - Automatic Threshold using Algorithm
- Cons :
  - Noise
  - Variability
  - Saturation

Simple Image Filtering

Image Filtering

- What is Filtering
  - Linear Filters
  - Nonlinear Filters
- Uses in Biological Images
  - Artifact/Noise removal
  - Background subtraction
  - Feature enhancement

(Spatial) Filtering

- Manipulations of the pixel data in order to improve an aspect of the image
  - Increase contrast
  - Remove uneven background
  - Enhance features

Summary: Point Operations

- Mapping pixel values without changing the size, geometry or local structure of the image
- *«homogeneous operation»*
- $a' \leftarrow f(a)\ or\ I'(u,v) \leftarrow f(I(u,v)$
- Modifying brightnes contrast, adjusting display settings

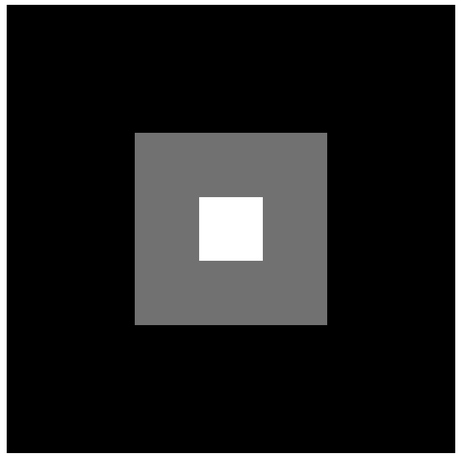
Filtering

- Sharp image: local intensities rise/drop sharply
- Blurred Image: local intensities rise/drop smoothly

Linear Filters

1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	5	5	5	1	1
1	1	5	10	5	1	1
1	1	5	5	5	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1

1	1	1
1	1	1
1	1	1



Filter **Output**

Linear Filters

1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	5	5	5	1	1
1	1	5	10	5	1	1
1	1	5	5	5	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1

1	1	1
1	1	1
1	1	1

$1 * 1 + \cdots =$

Input **Filter** **Output**

Linear Filters

1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	5	5	5	1	1
1	1	5	10	5	1	1
1	1	5	5	5	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1

1	1	1
1	1	1
1	1	1

$$1 * 1 + 1 * 1 + \cdots =$$

Input **Filter** **Output**

Linear Filters

1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	5	5	5	1	1
1	1	5	10	5	1	1
1	1	5	5	5	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1

1	1	1
1	1	1
1	1	1

$$1 * 1 + 1 * 1 + 1 * 1 + \cdots =$$

Input **Filter** **Output**

Linear Filters

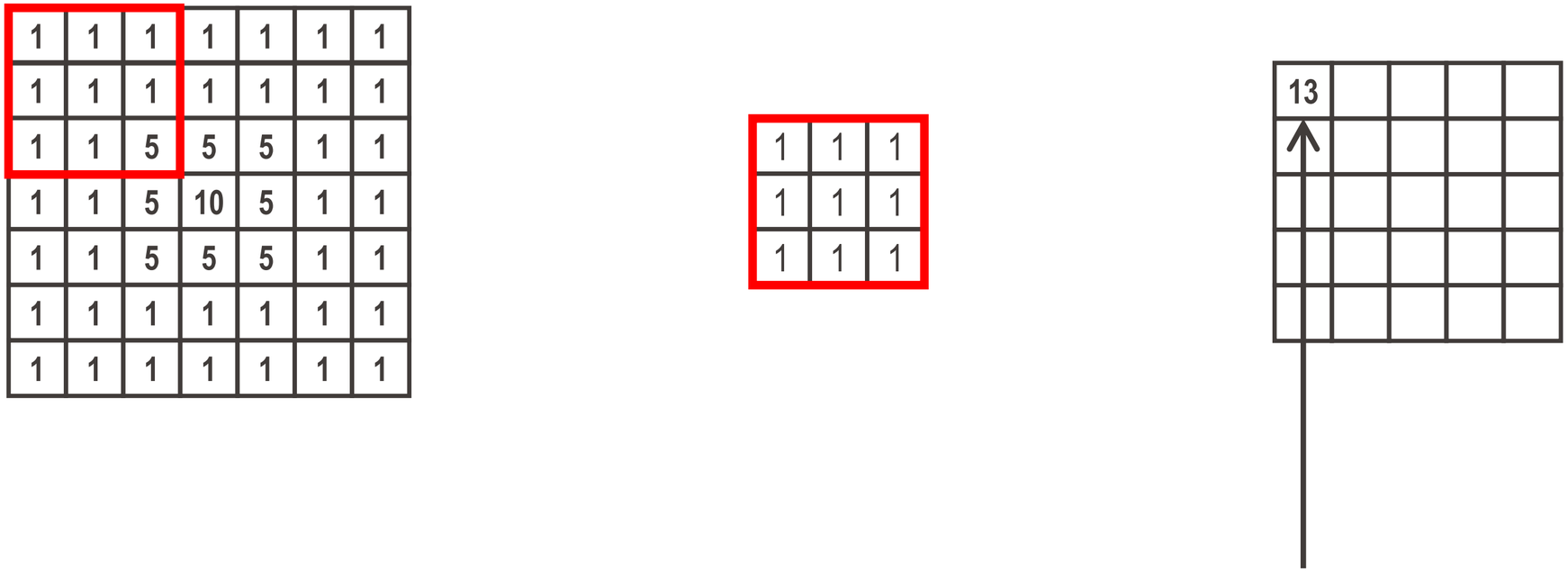
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	5	5	5	1	1
1	1	5	10	5	1	1
1	1	5	5	5	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1

1	1	1
1	1	1
1	1	1

$$1*1+1*1+1*1+1*1+1*1+1*1+1*1+1*1+5*1=$$

Input **Filter** **Output**

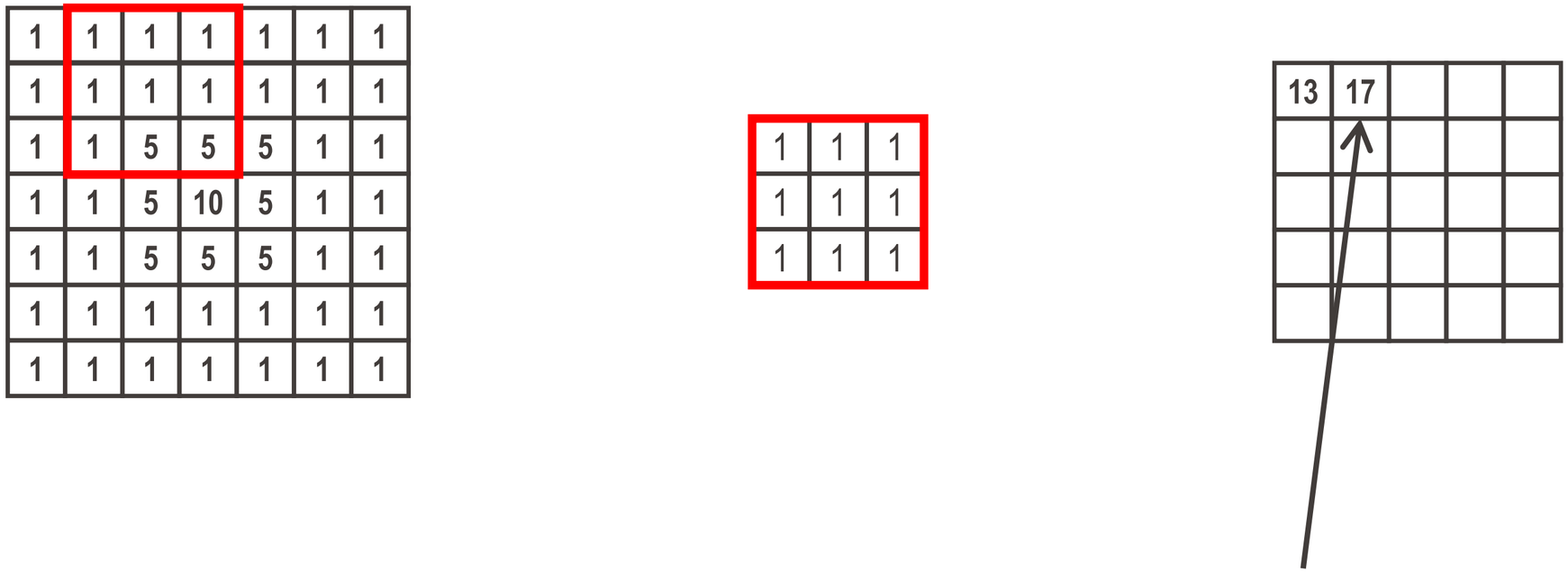
Linear Filters



$$1*1+1*1+1*1+1*1+1*1+1*1+1*1+1*1+5*1=13$$

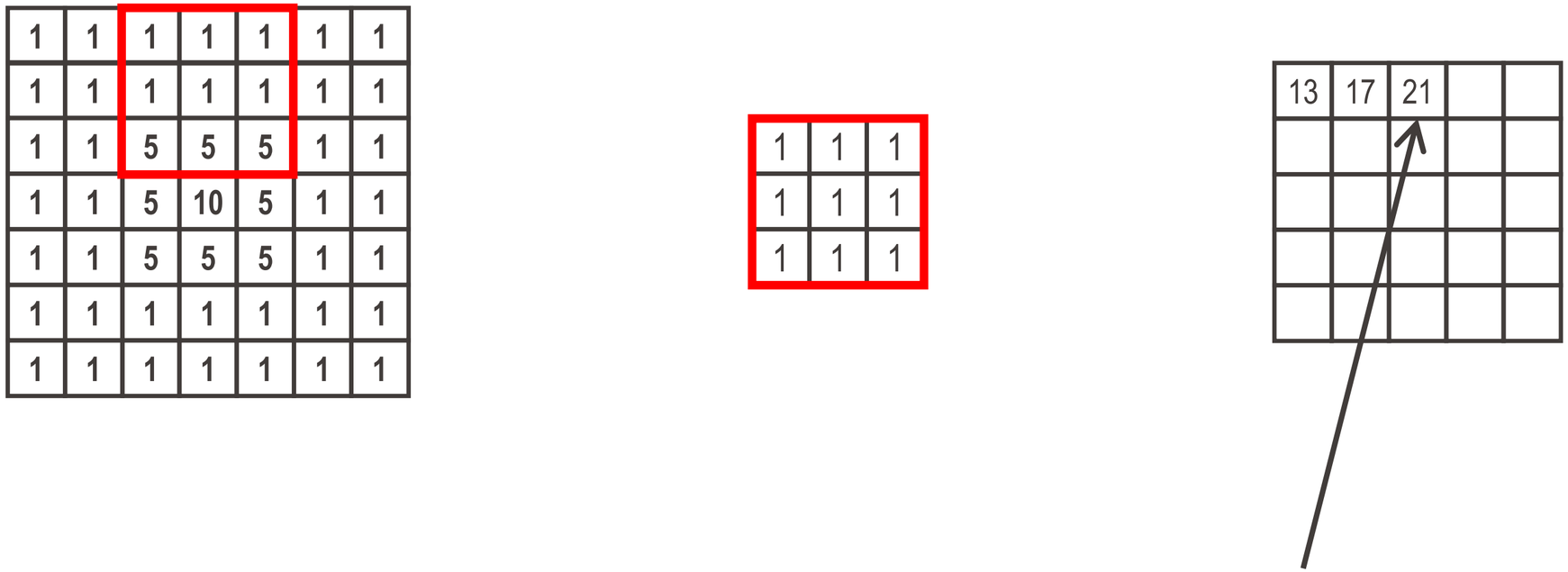
Input **Filter** **Output**

Linear Filters



$$1 * 1 + 1 * 1 + 1 * 1 + 1 * 1 + 1 * 1 + 1 * 1 + 1 * 1 + 5 * 1 + 5 * 1 = 17$$

Linear Filters



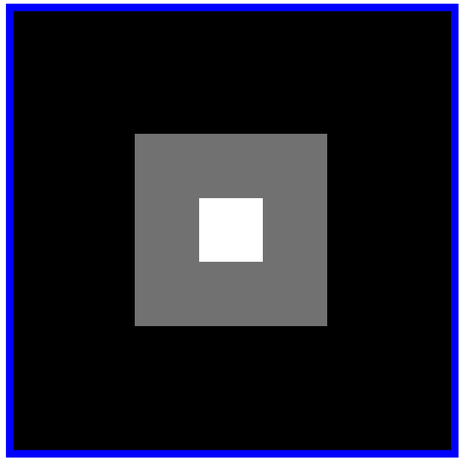
$$1 * 1 + 1 * 1 + 1 * 1 + 1 * 1 + 1 * 1 + 1 * 1 + 5 * 1 + 5 * 1 + 5 * 1 = 21$$

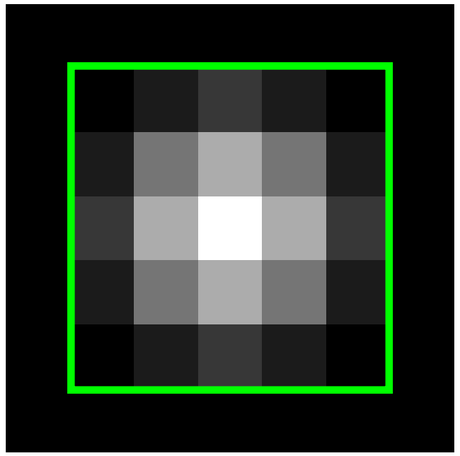
Linear Filters

1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	5	5	5	1	1
1	1	5	10	5	1	1
1	1	5	5	5	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1

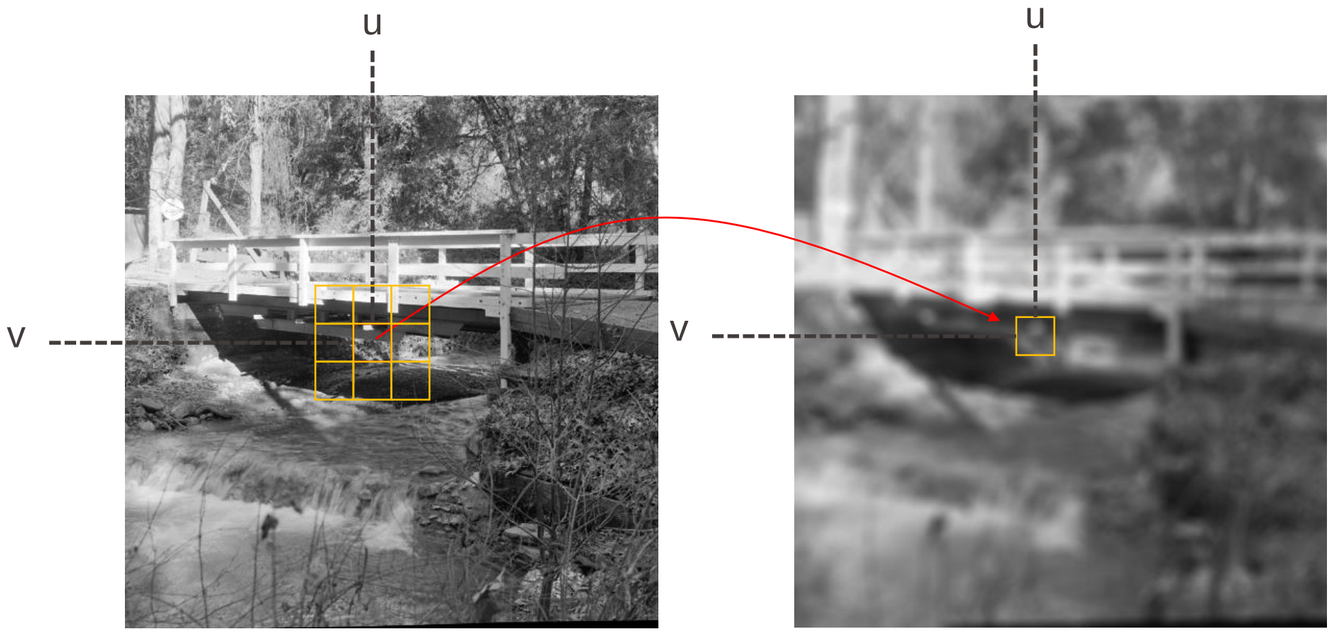
1	1	1
1	1	1
1	1	1

13	17	21	17	13
17	30	38	30	17
21	38	50	38	21
17	30	38	30	17
13	17	21	17	13





Filtering



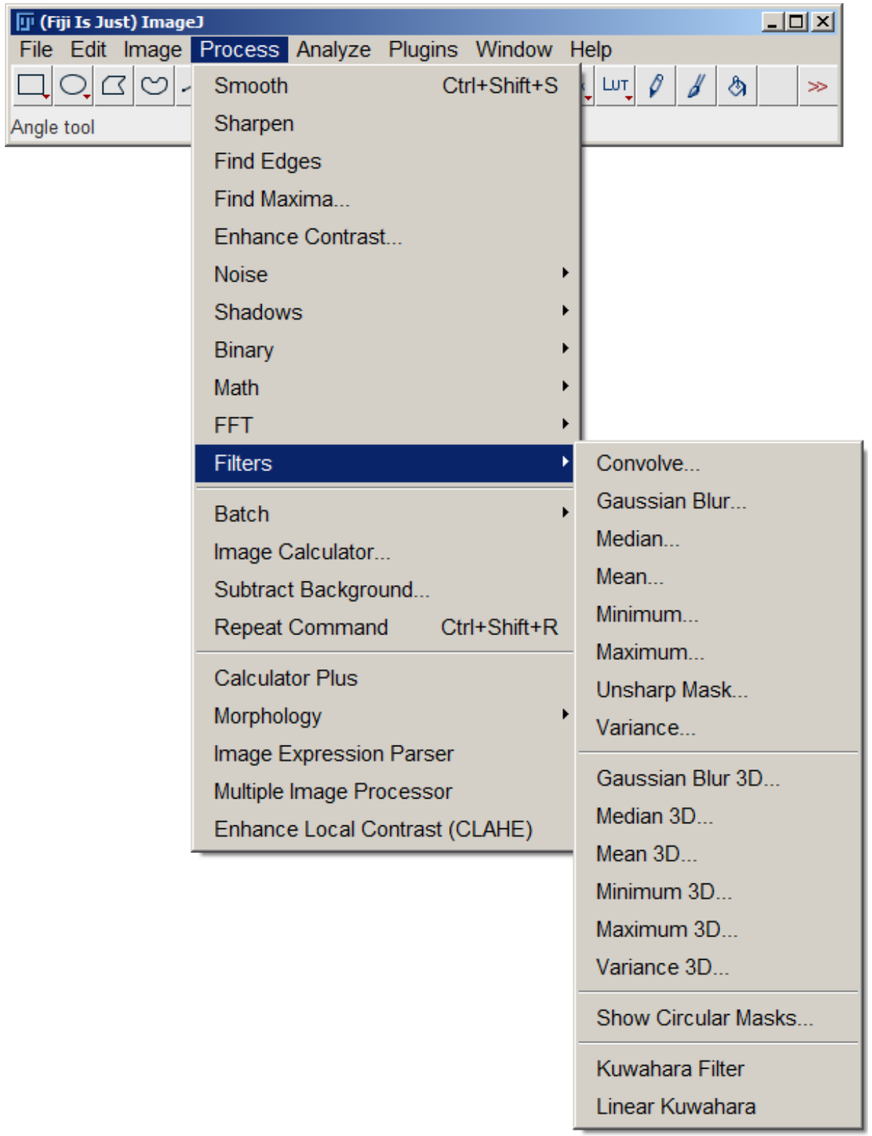
$$I'(u,v) = \frac{p_1 + p_2 + p_3 + p_4 + p_5 + p_6 + p_7 + p_8 + p_9}{9}$$

$$I'(u,v) = \frac{1}{9} \sum_{j=-1}^{1} \sum_{i=-1}^{1} I(u+i, v+j)$$

Filter Matrix:

$$H(i,j) = \frac{1}{9} \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix}$$

Image Filtering in Fiji



Summary

- Linear Filters
  - Convolution
  - Modifies pixels as a linear function of their neighbors
  - Super fast operation (Parallelizable)
- Non Linear Filters
  - High performance
  - Better tunable (parameters)

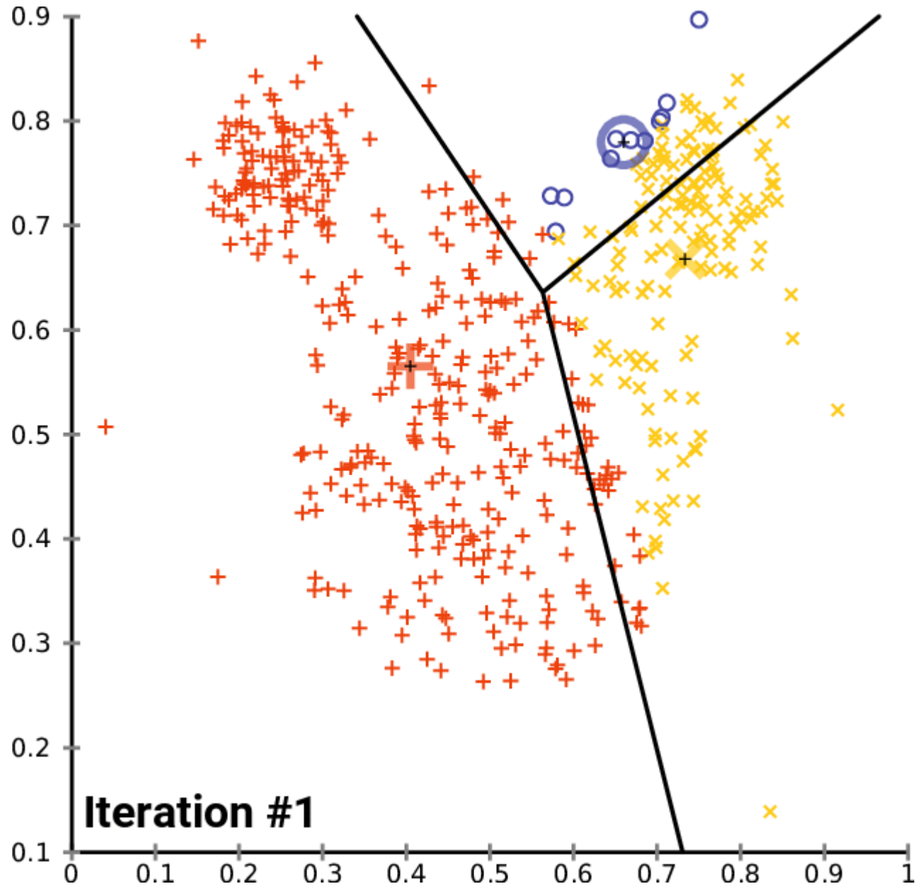
Segmentation Techniques

- Thresholding
- Clustering
- Region Growing
- Machine Learning

Clustering

- Mathematical Algorithm
  that will make Cluster of Pixels
  - k-means

Clustering



- Mathematical Algorithm that will make Cluster of Pixels
  - k-means

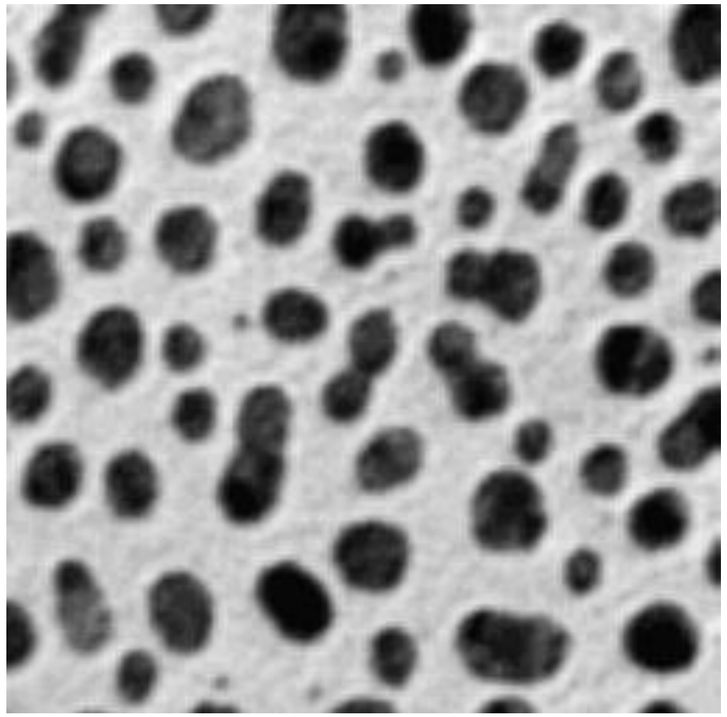
Clustering

- Mathematical Algorithm
  that will make Cluster of Pixels
  - k-means (centroid)
  - Expectation-maximization (Distribution)
  - …

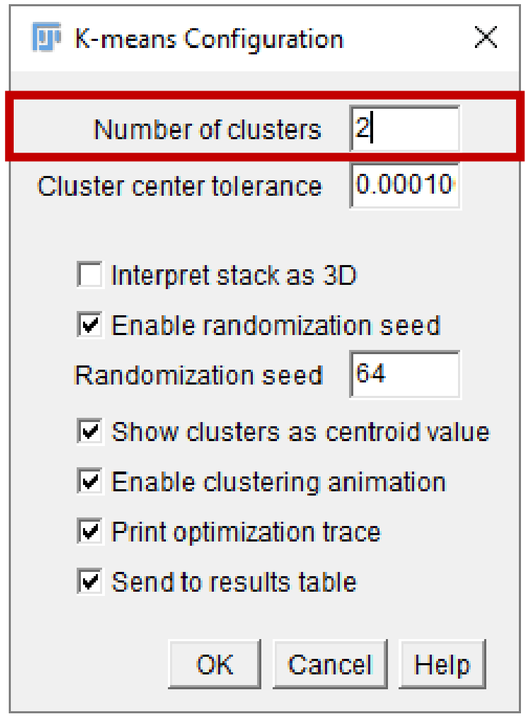
Clustering Based

- Mathematical Algorithm
  that will make Cluster of Pixels
  - k-means (centroid)
  - Expectation-maximization (Distribution)
  - …
- Implementation for ImageJ
  - k-means clustering in **IJ Plugins Toolkit**
  - Expectation-maximization in **xLib**

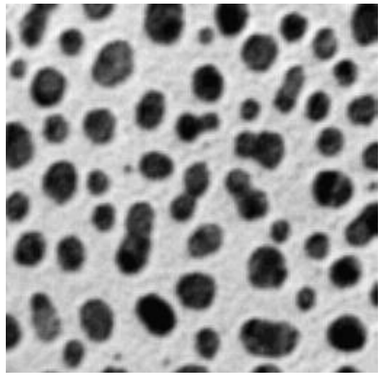
k-means clustering in IJ Plugins Toolkit



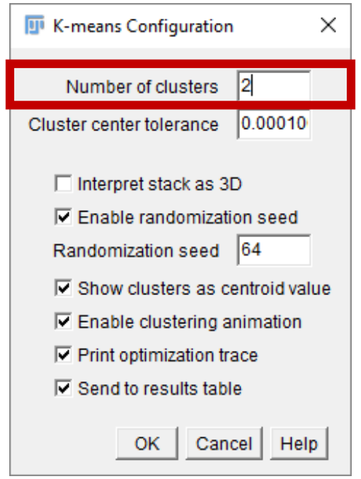
Define the number of clusters

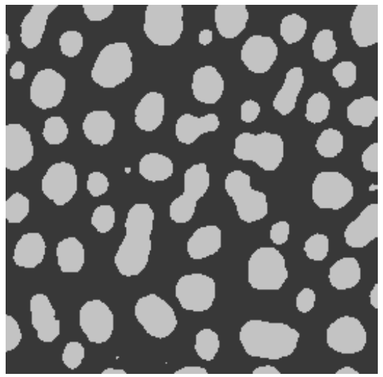


k-means clustering in IJ Plugins Toolkit



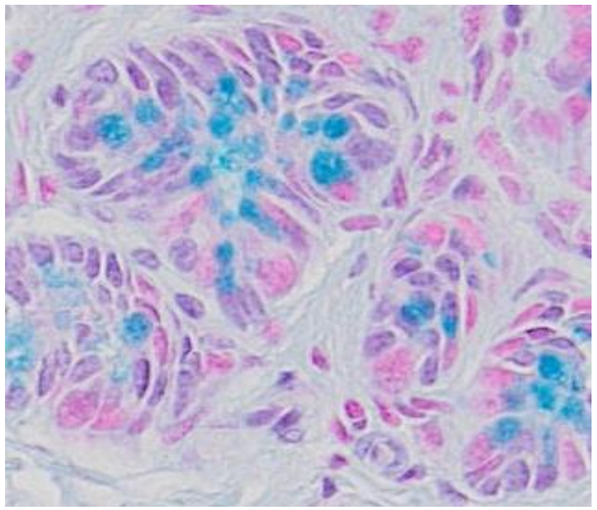
Define the number of clusters



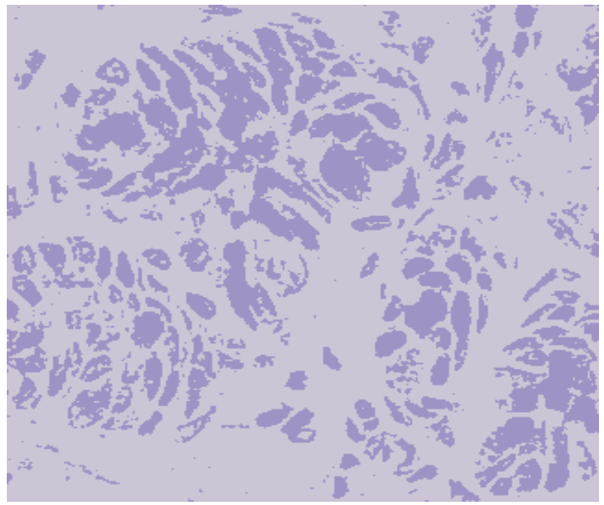


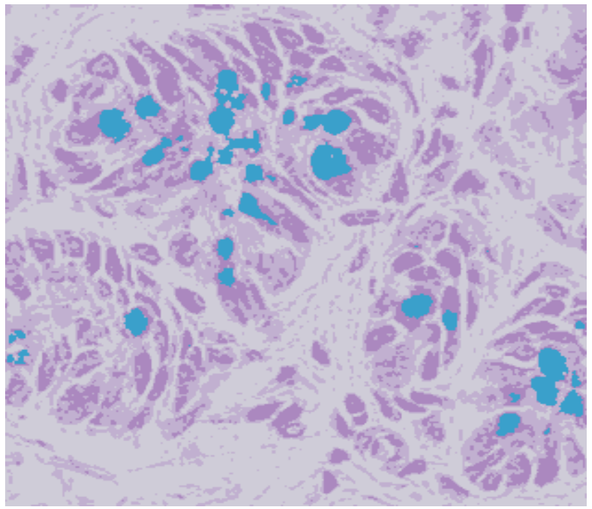
k-means clustering in IJ Plugins Toolkit

Original

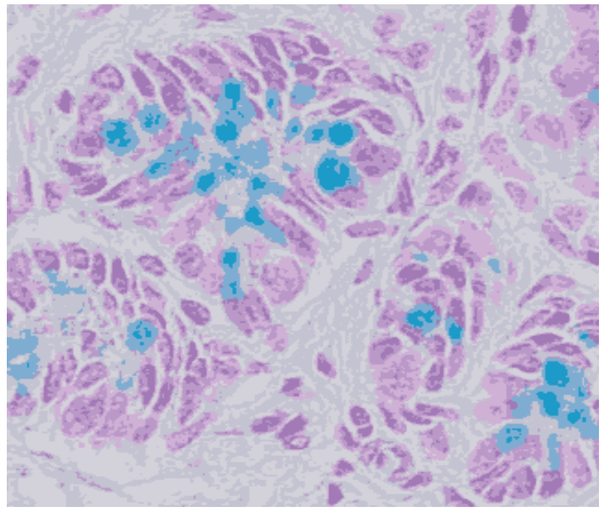


K=2





K=4



K=7

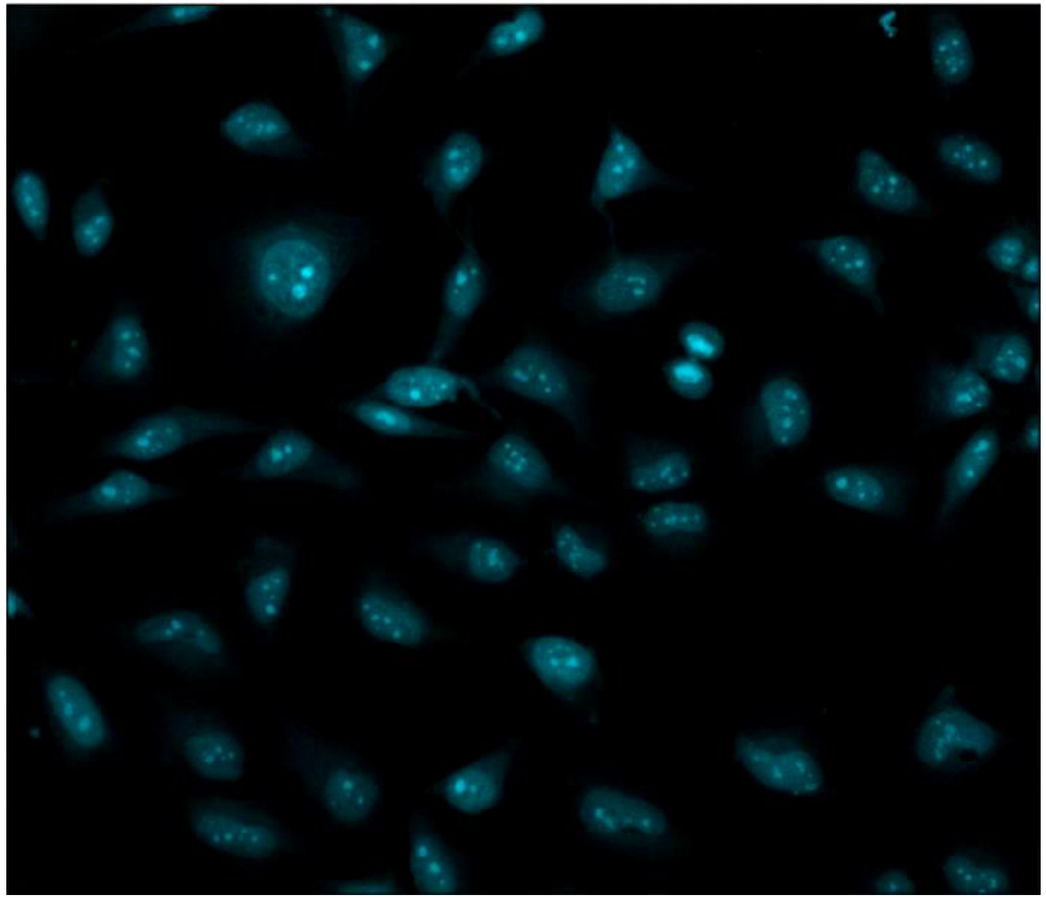
Clustering

- Pros
  - More than 2 categories
  - Works on RGB image
- Cons
  - Needs apriori knowledge (categories number)
  - Initial Centroids
  - Local Minima

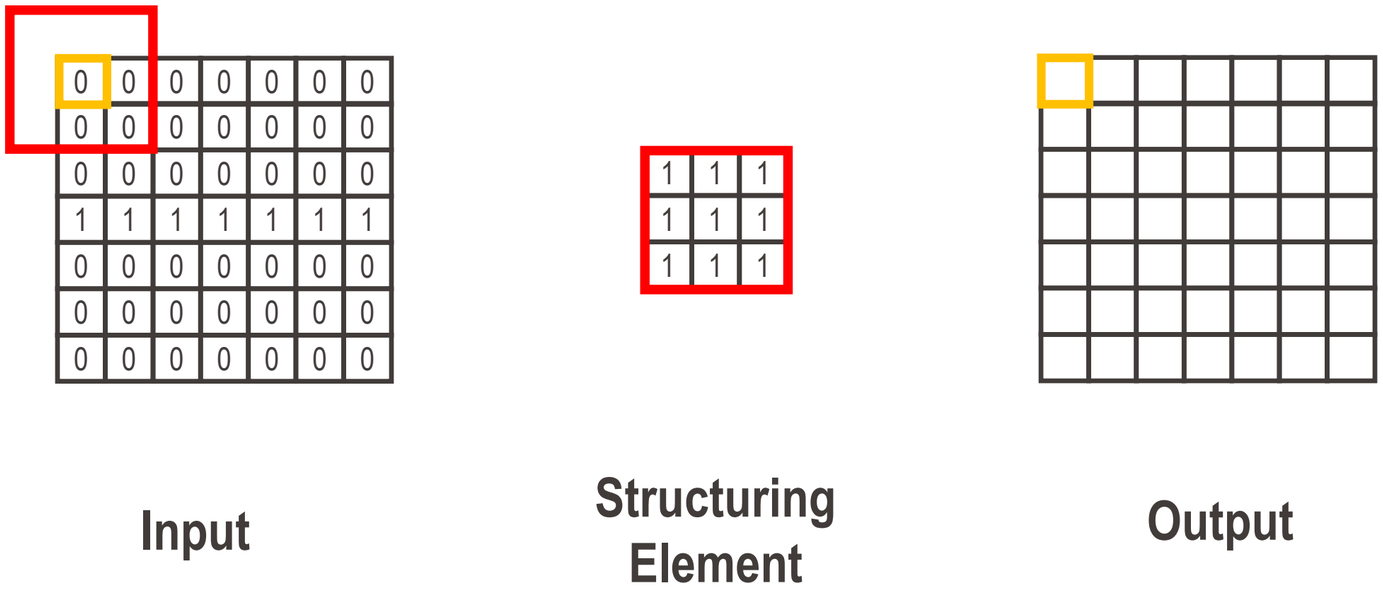
Object Shaping

Morphological Operations

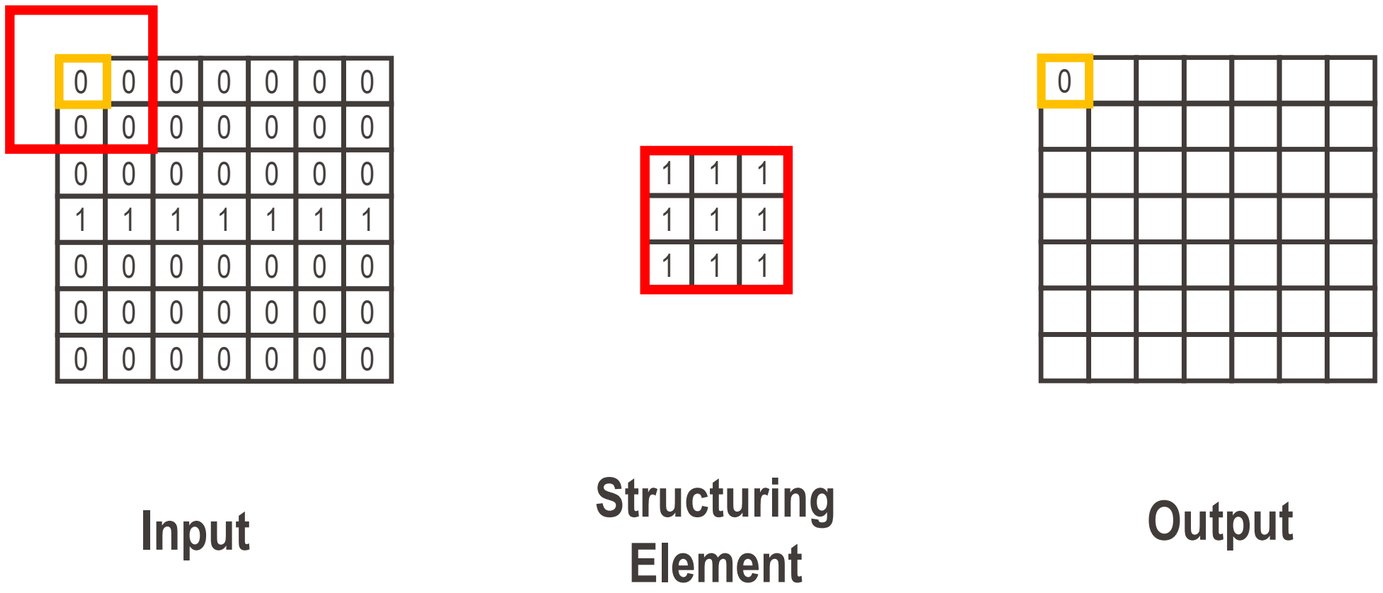
- Analysis of geometrical structures
- Basic operations:
  - Erosion
  - Dilation
  - Opening
  - Closing
- Initiallly developed for binaray images.
- Introduced by Georges Matheron and Jean Sera (Ecole de Mines de Paris)



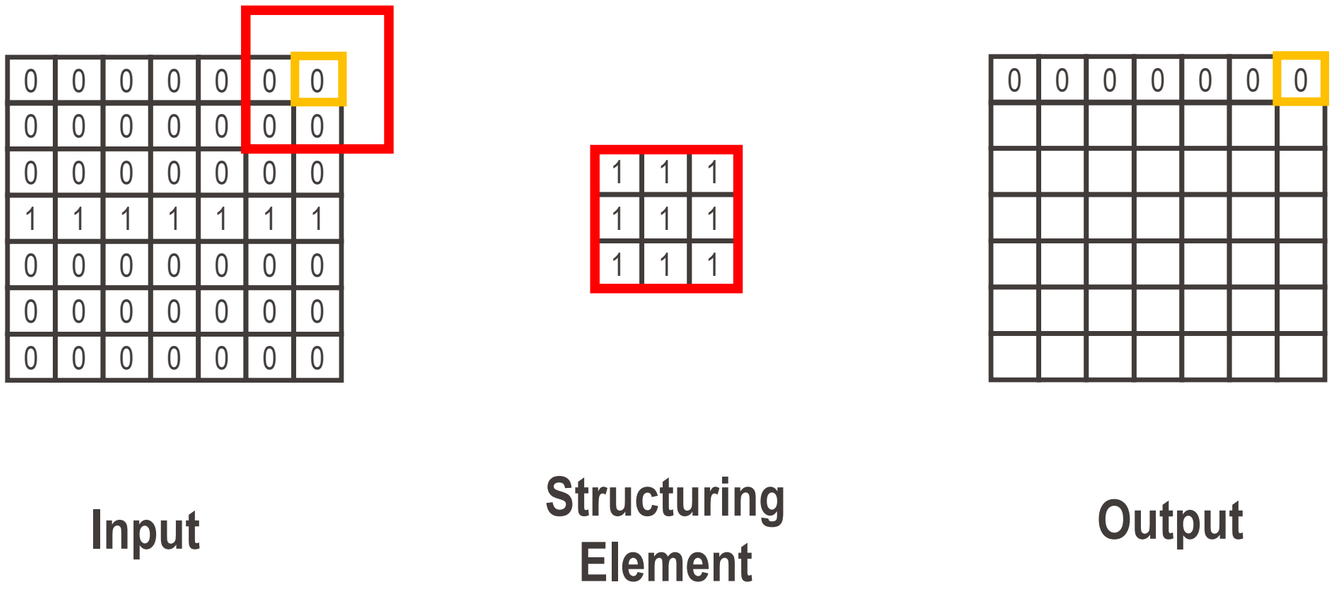
Morphological Operations



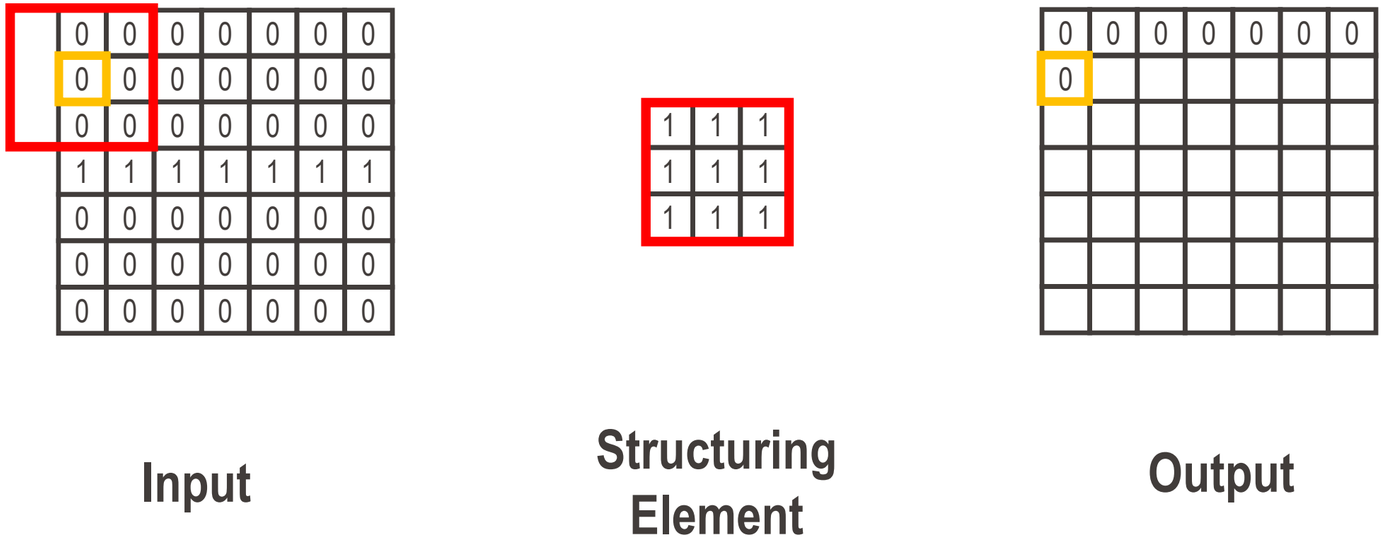
Morphological Operations



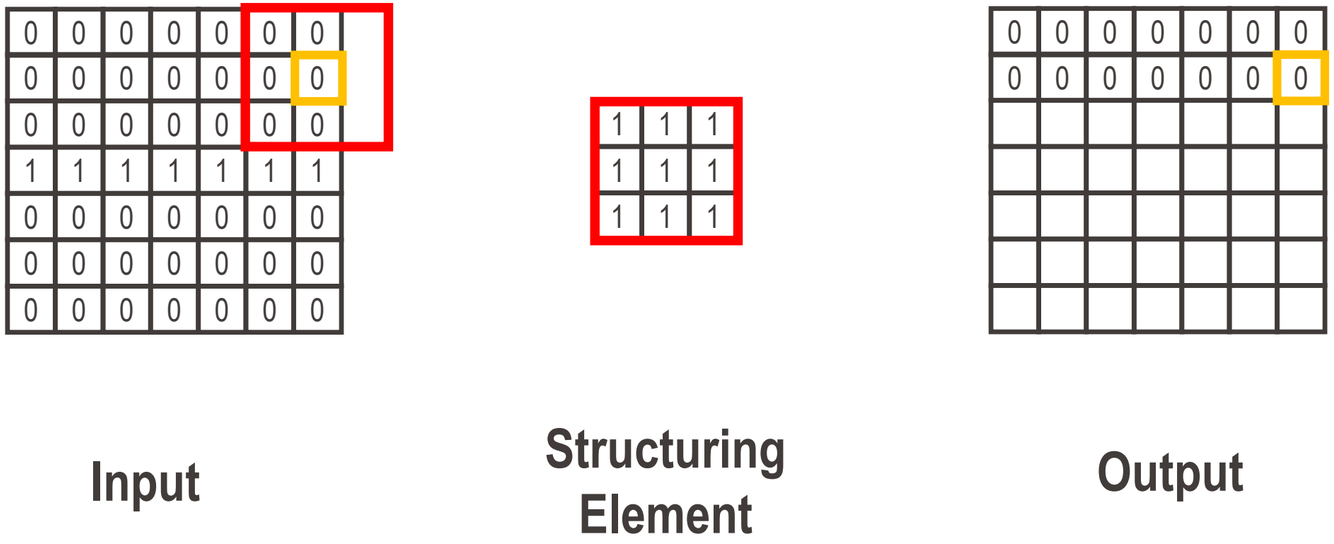
Morphological Operations



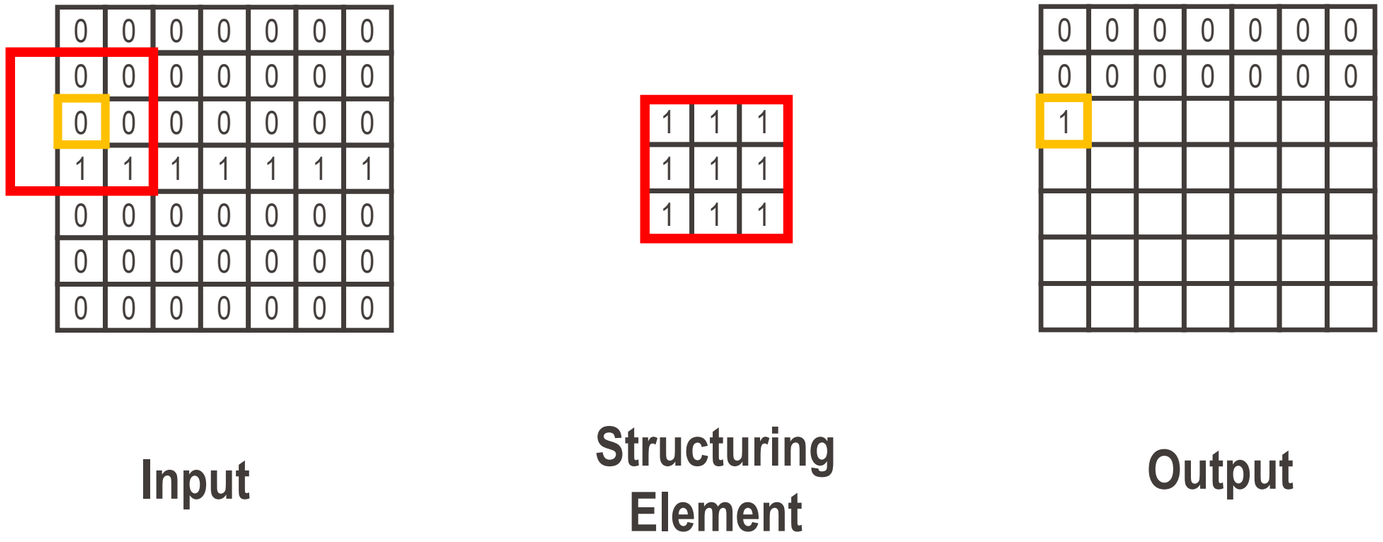
Morphological Operations



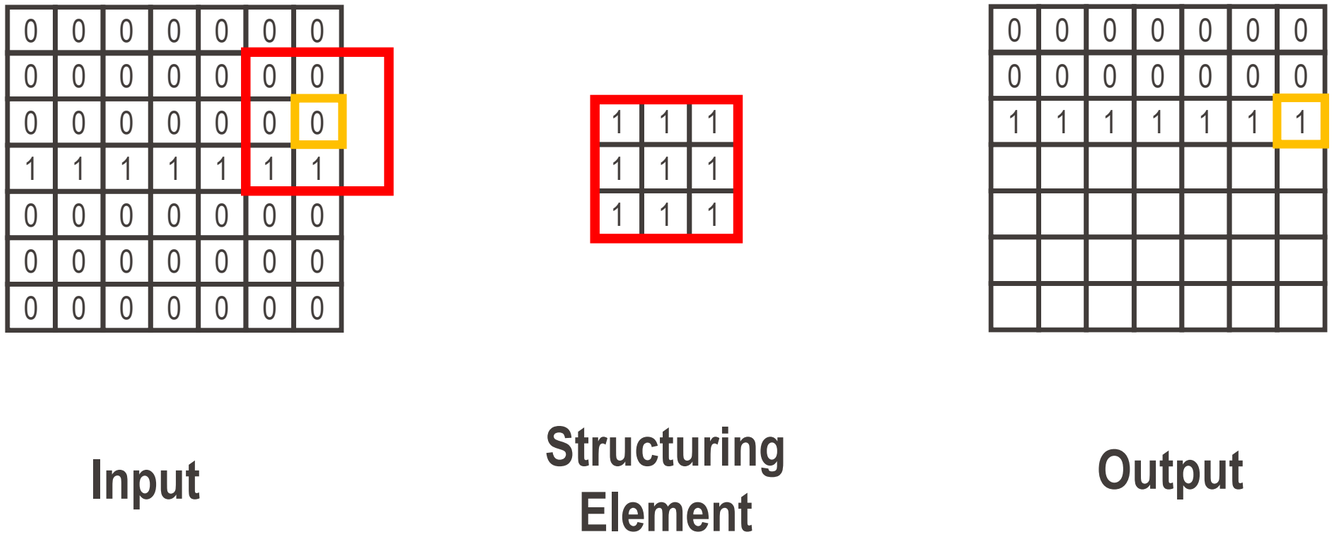
Morphological Operations



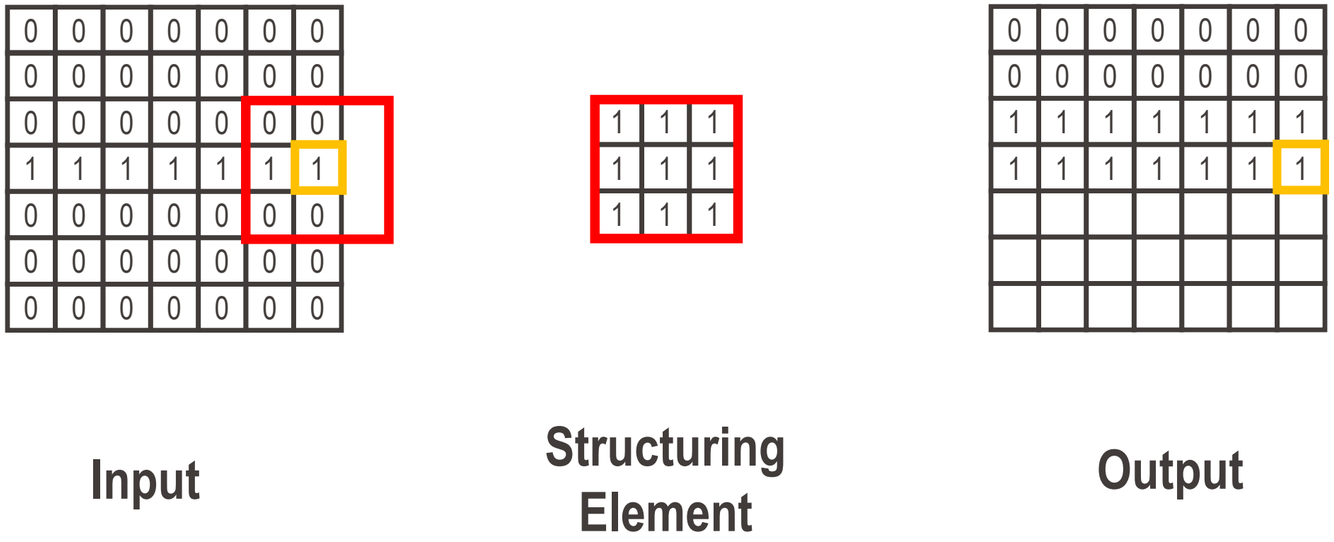
Morphological Operations



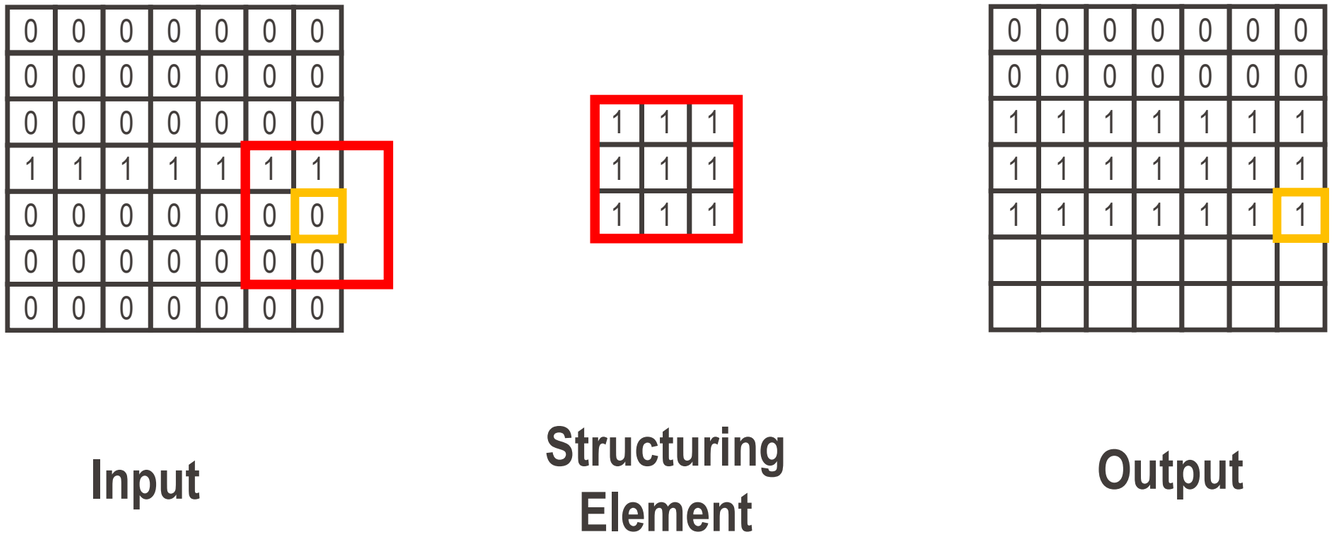
Morphological Operations



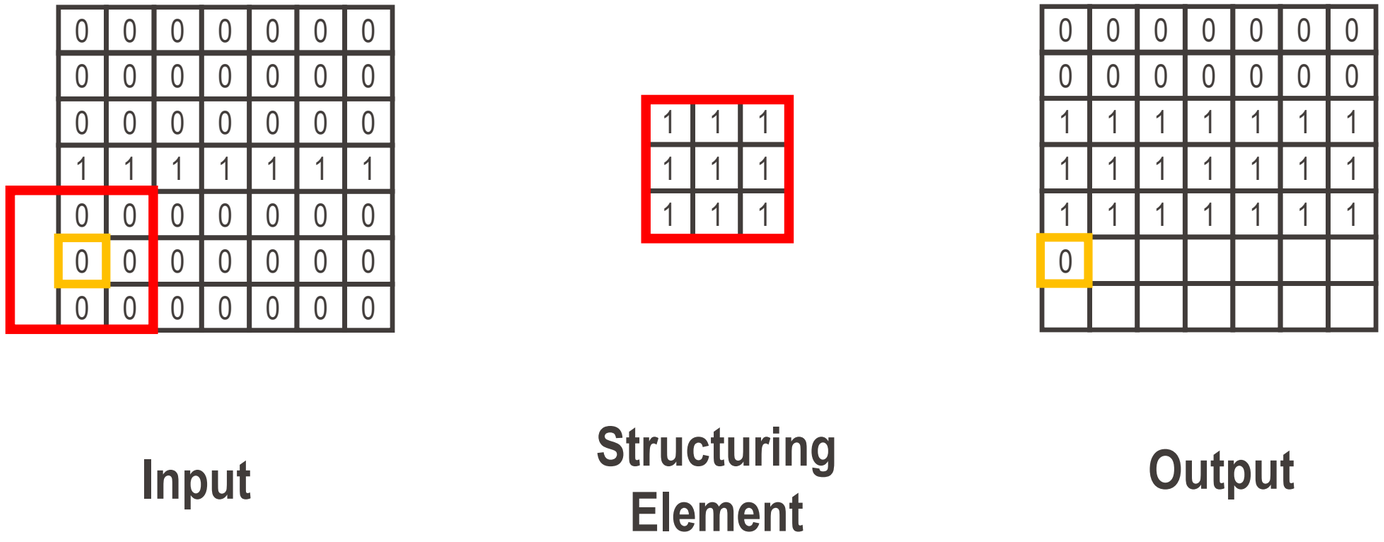
Morphological Operations



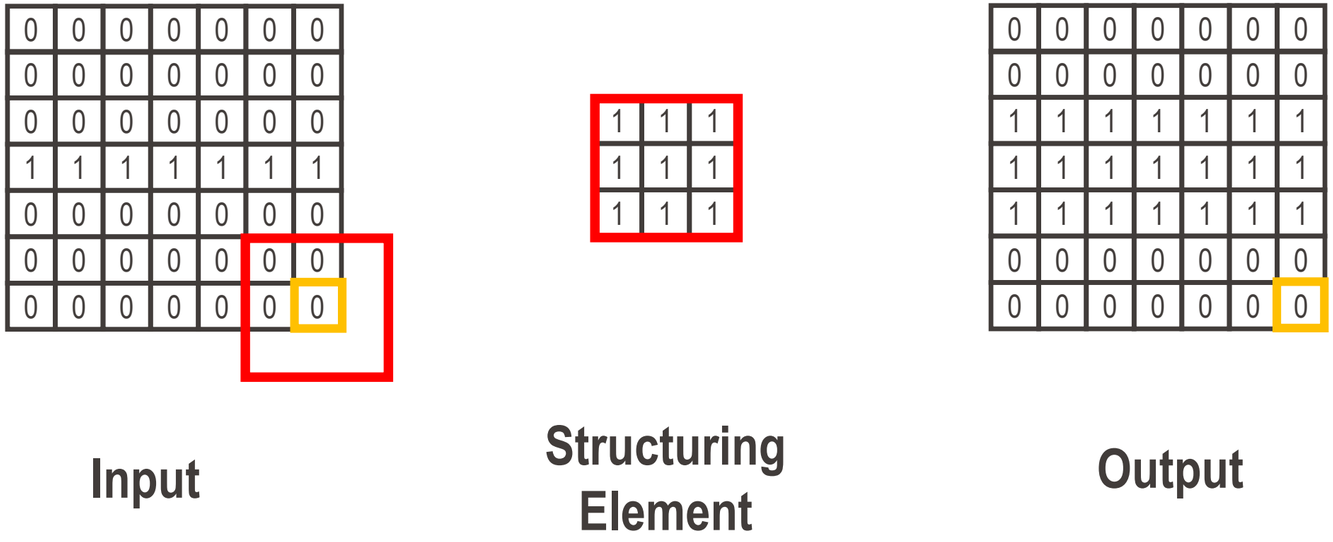
Morphological Operations



Morphological Operations

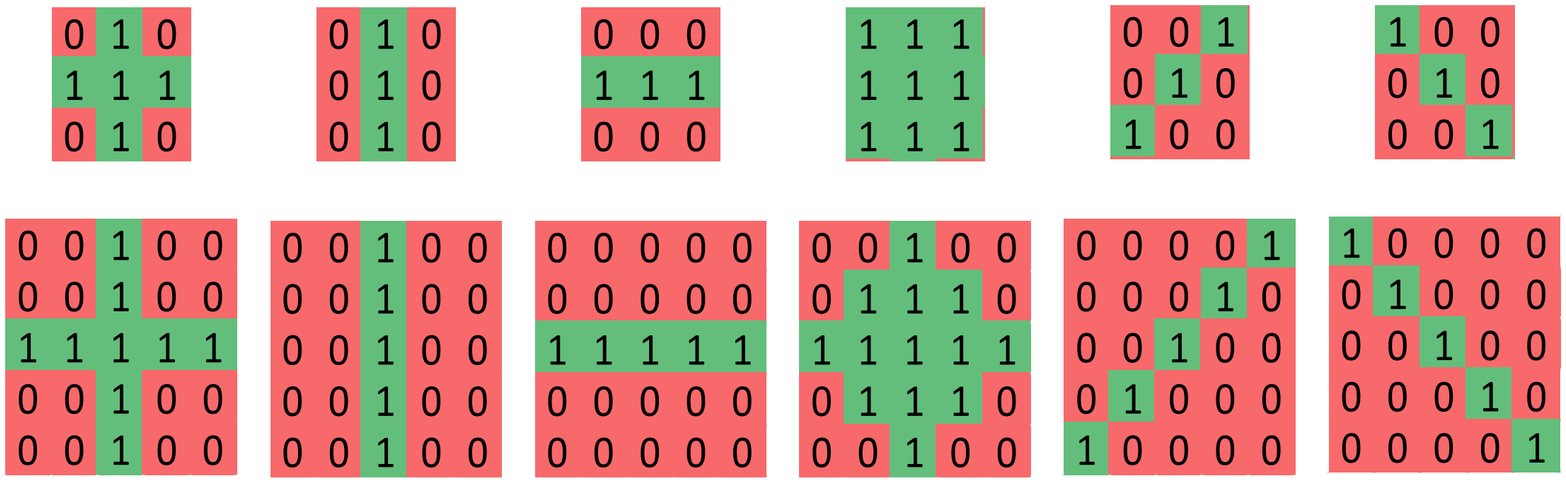


Morphological Opertaions



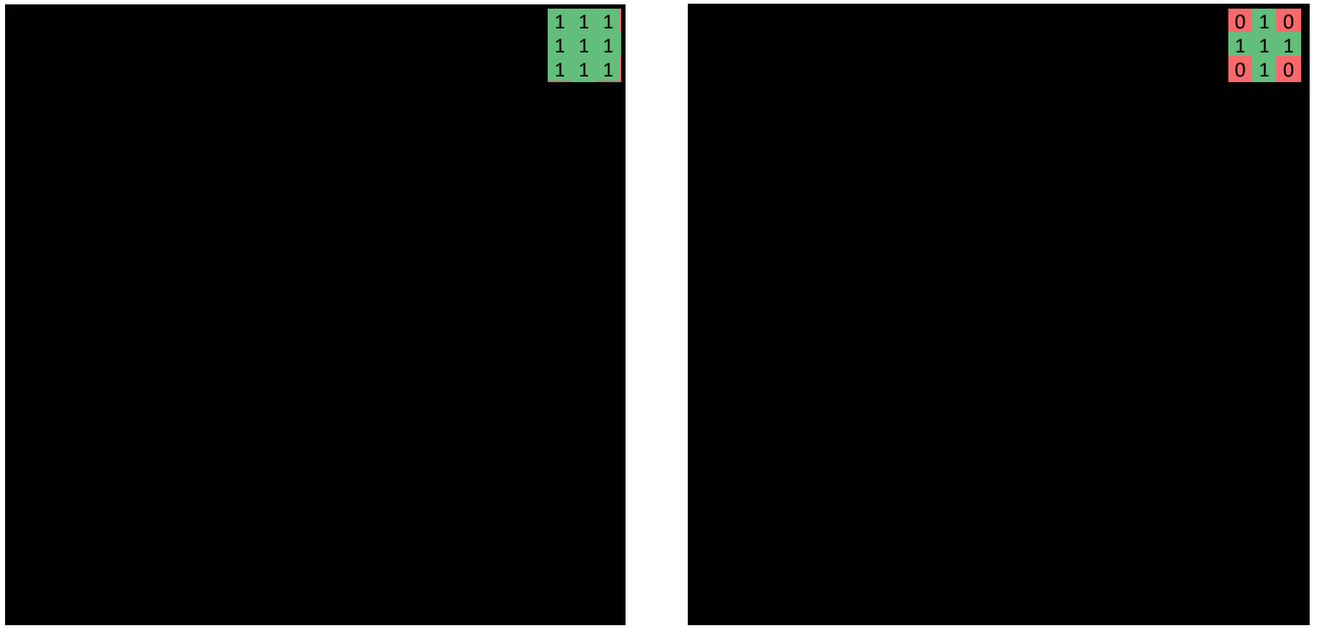
Morphological Operations

- Probe the image with a structuring element.
- Structuring element can have different shapes
- Superimpose the probe with a pixel of the image
- Keep/reject the original pixel



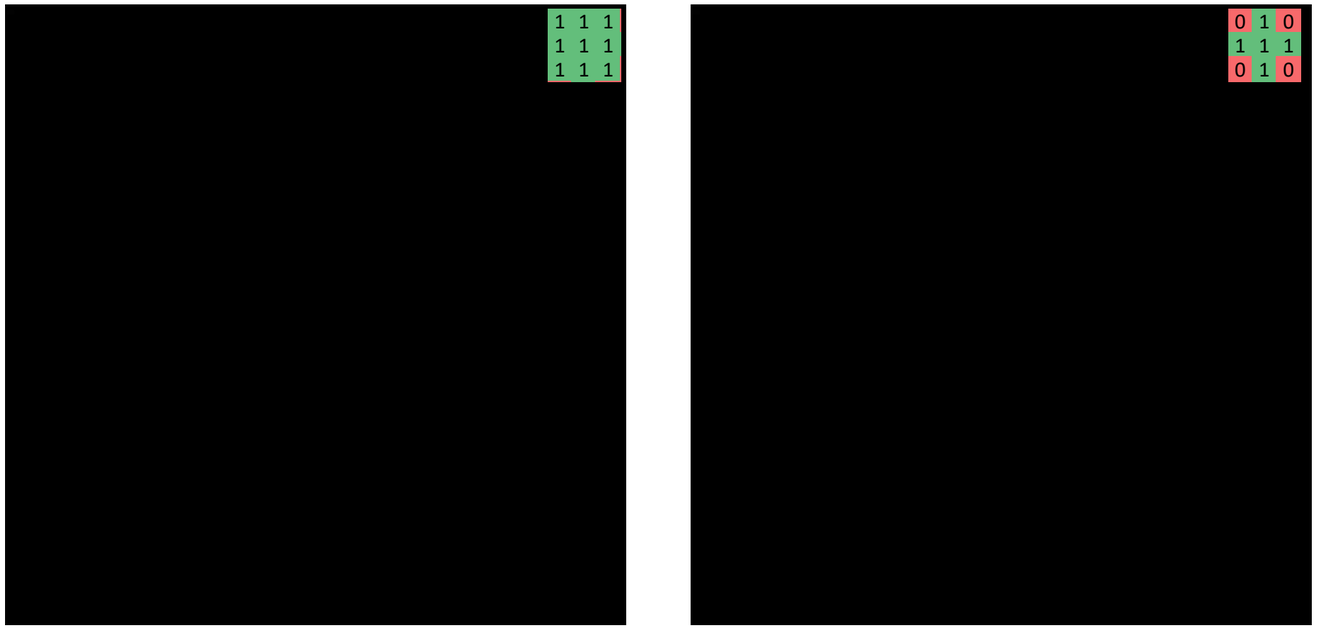
Morphological Operations

Dilation



Morphological Operations

Erosion

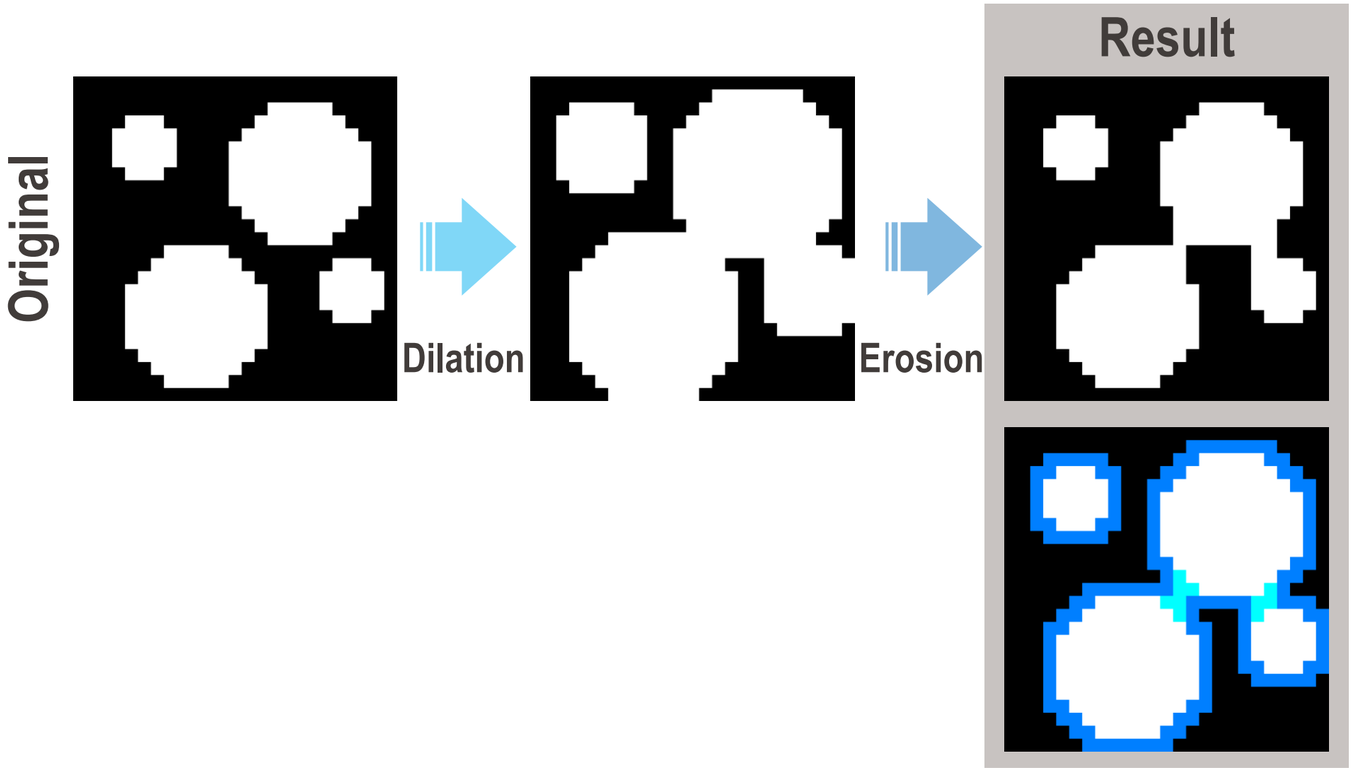


Morphological Operations

- Erosion
- Dilation

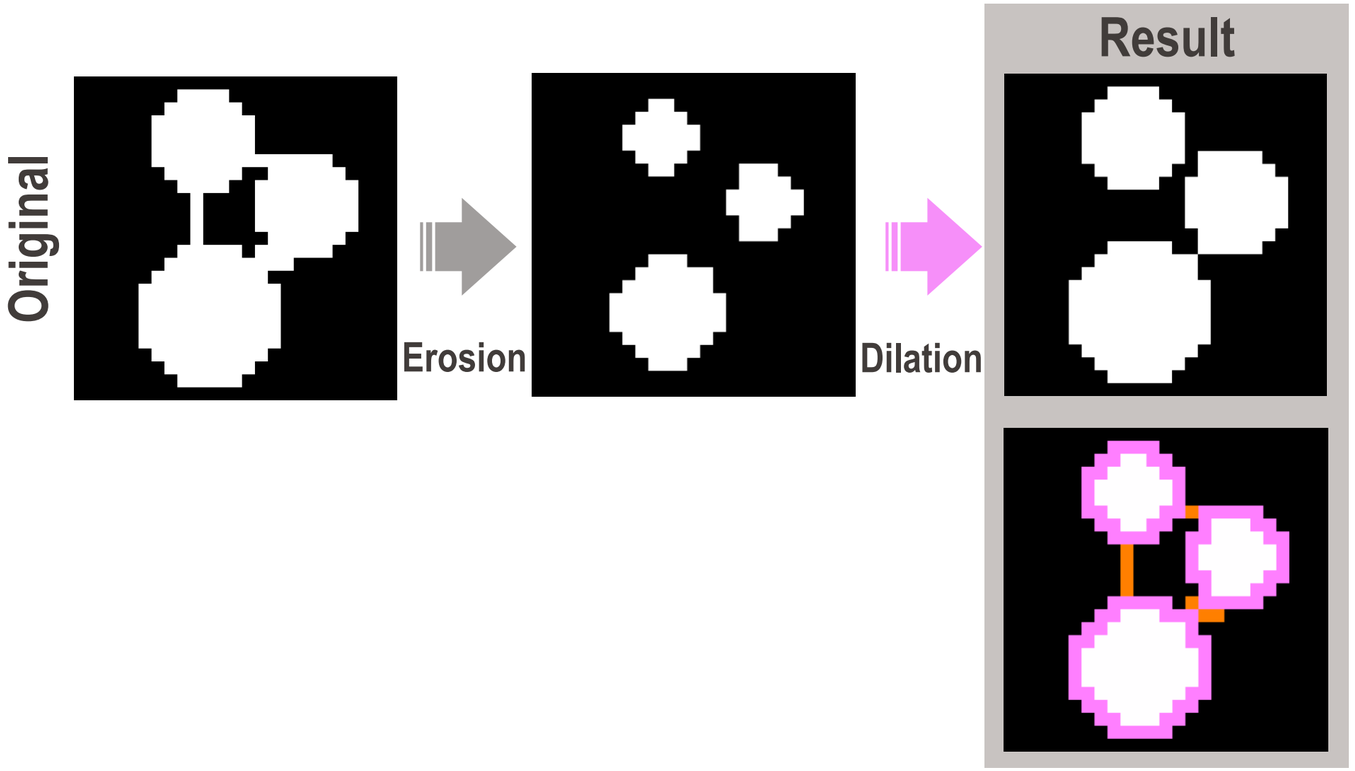
Morphological Operations

Closing: Dilation followed by Erosion



Morphological Operations

Opening: Erosion followed by Dilation

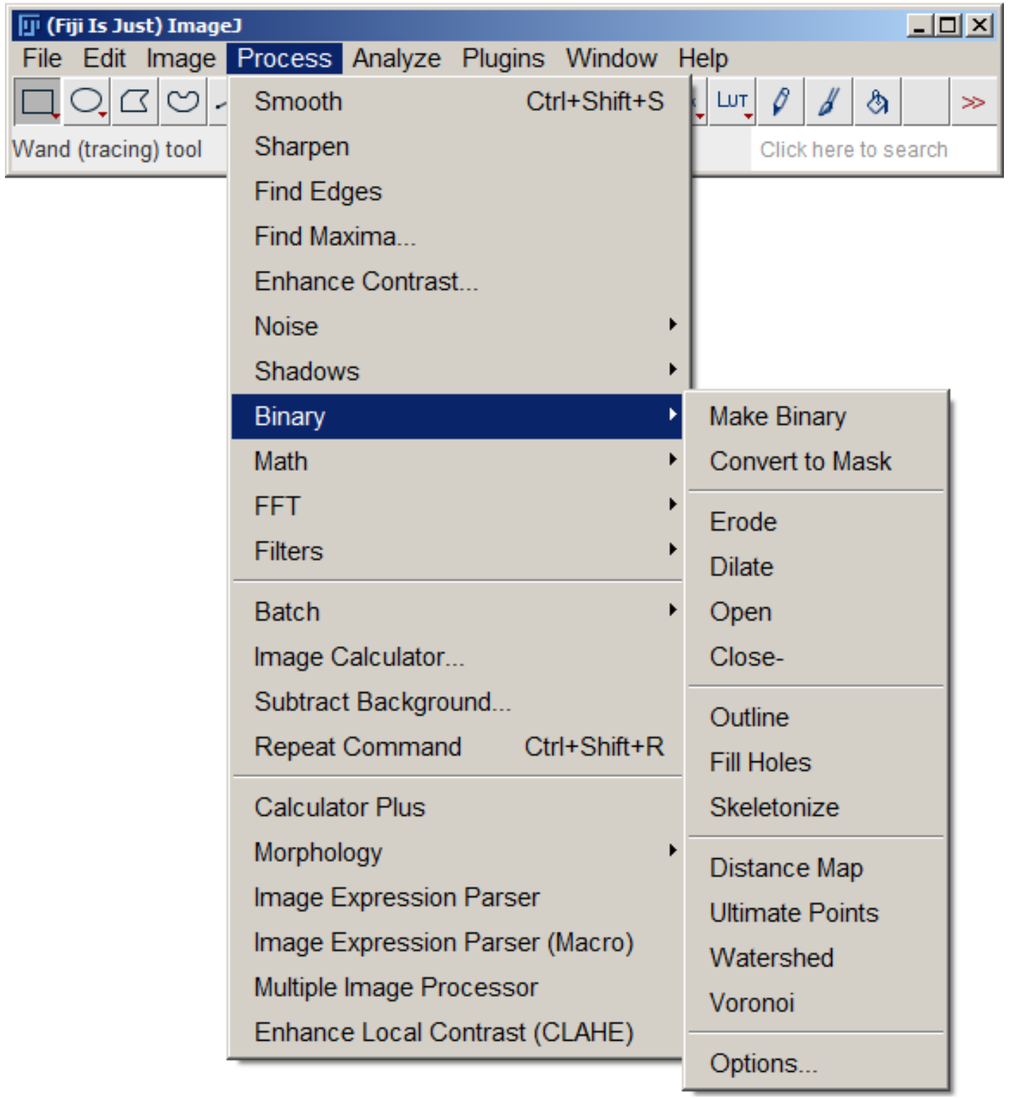


Morphological Operations

- Erosion
- Dilation

- Closing
  - Erosion+Dilation
- Opening
  - Dilation+Erosion

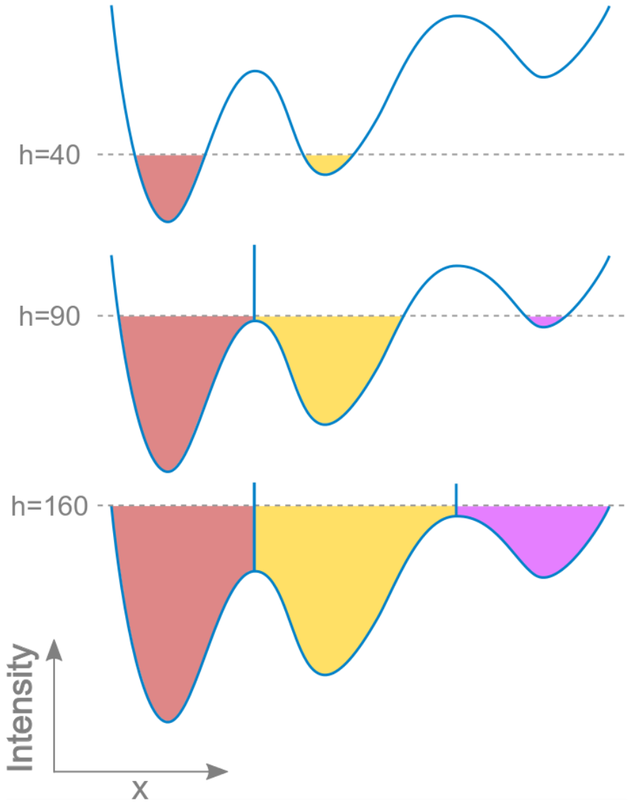
Binary-Based Operations



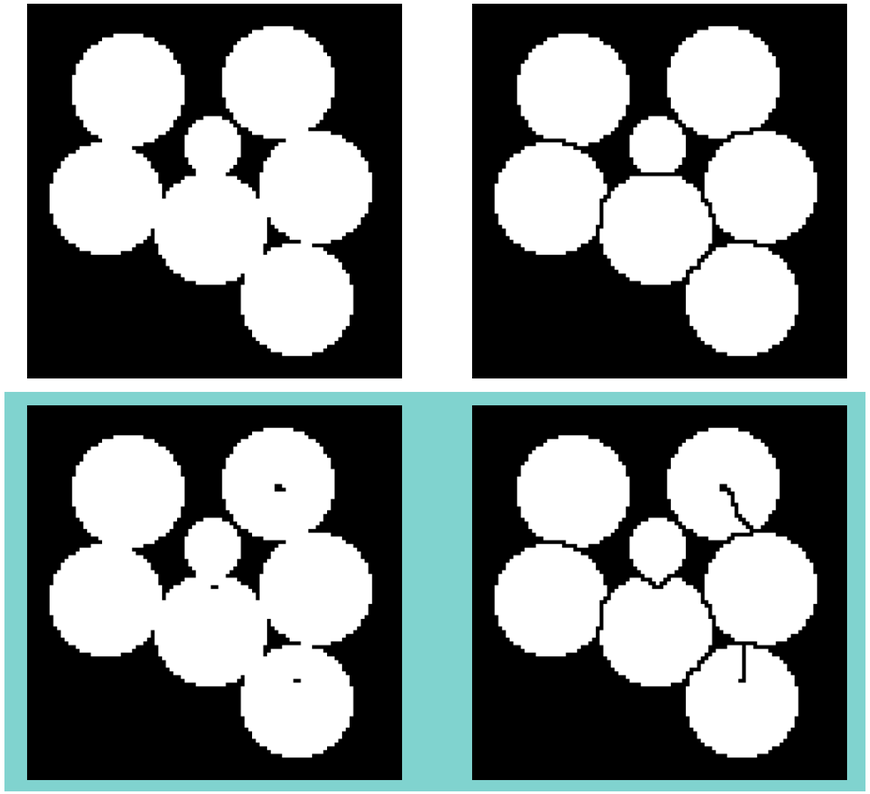
Binary-Based Operations

- Watershed
- Fill Holes
- Skeletonization
- Voronoi
- Distance Map

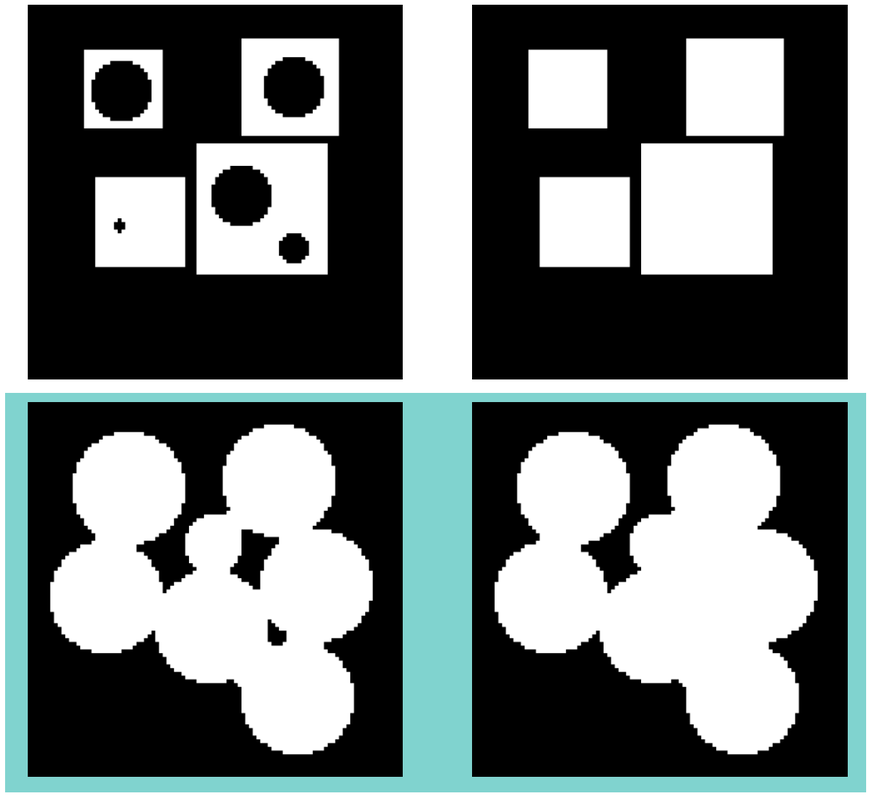
Watershed



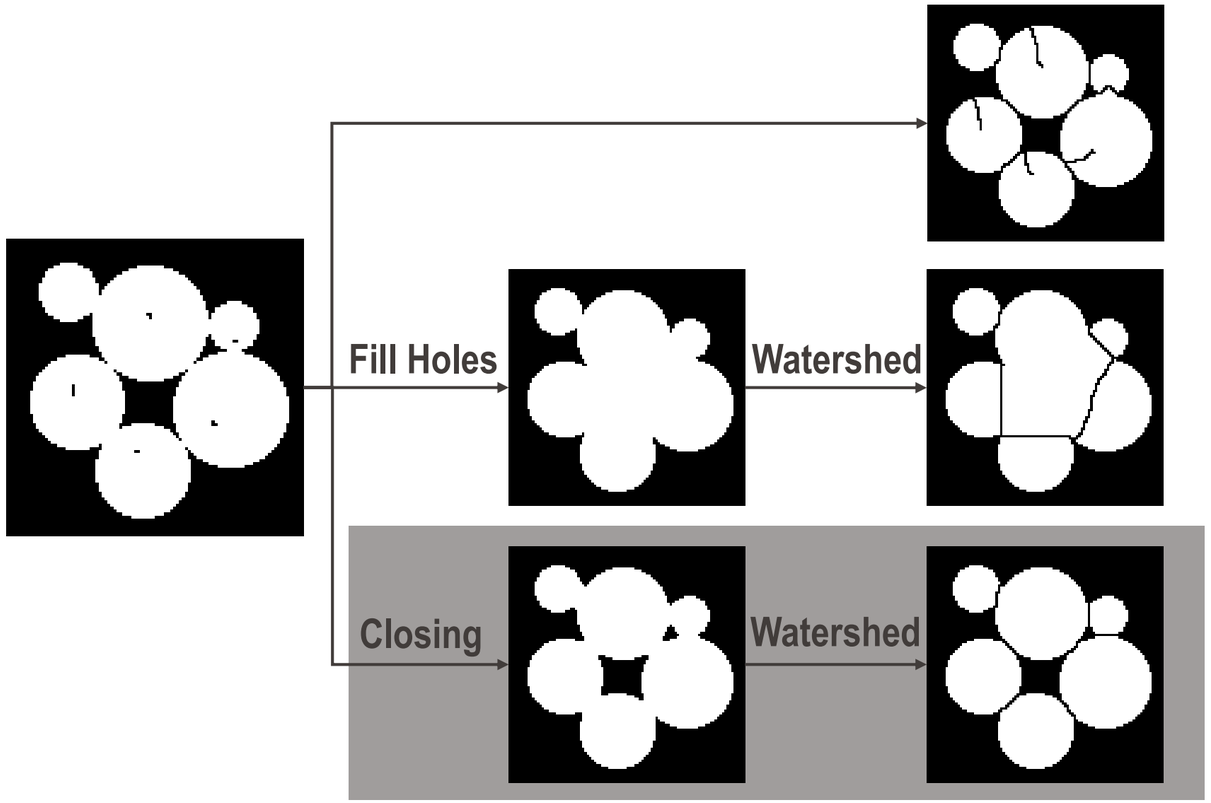
https://imagej.net/Classic_Watershed



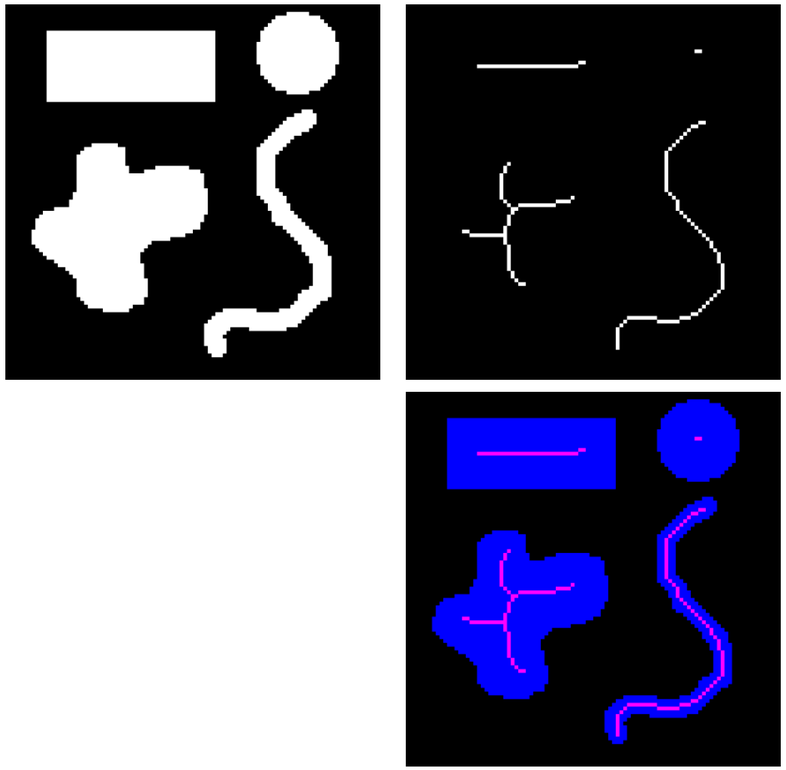
Fill Holes



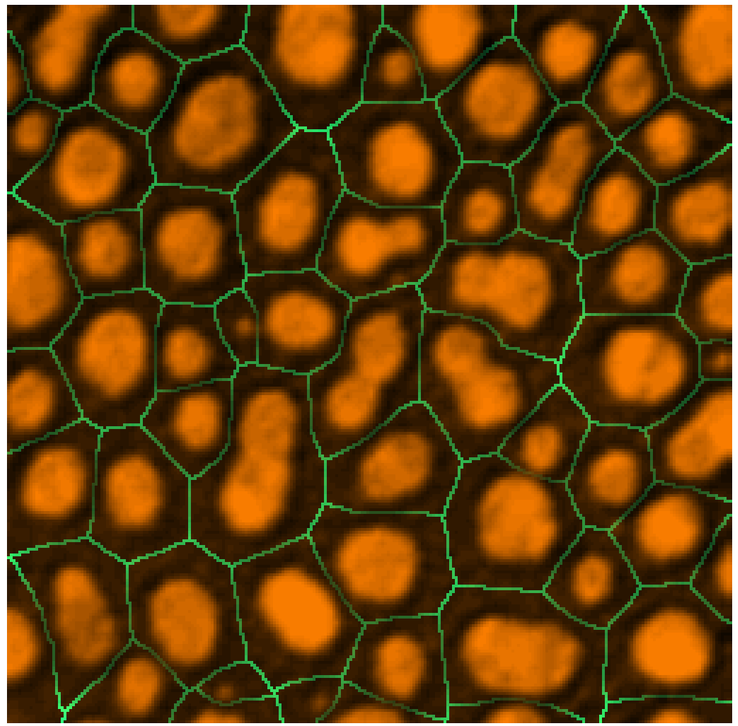
Combined Operations

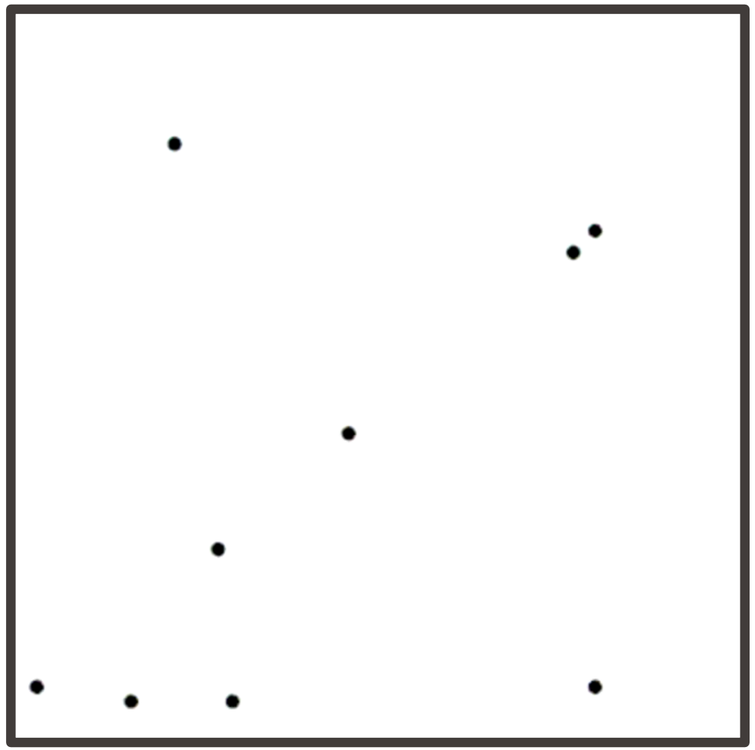


Skeletonization

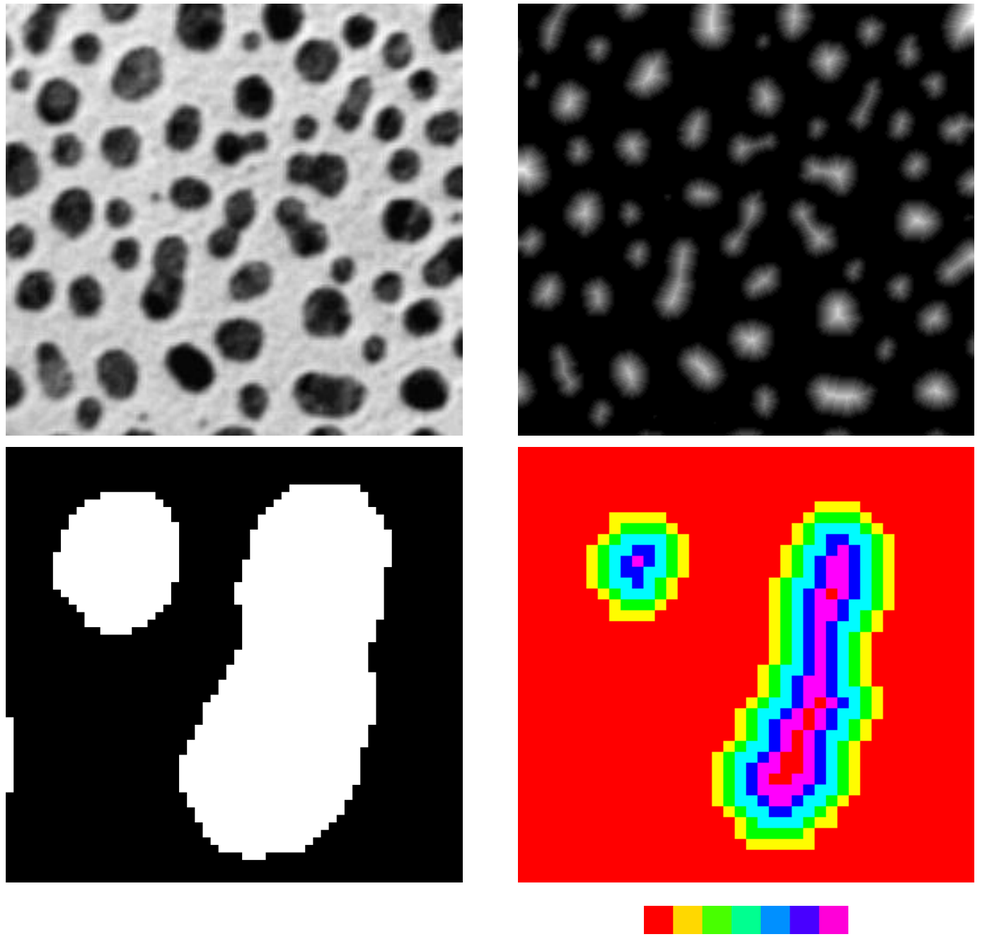


Voronoi





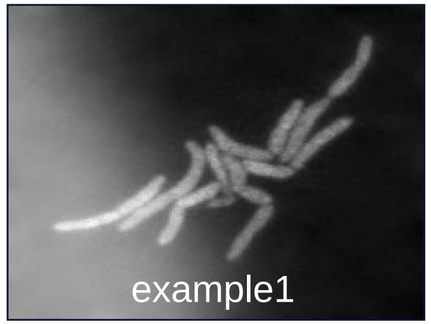
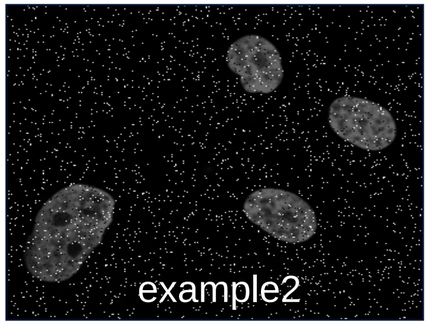
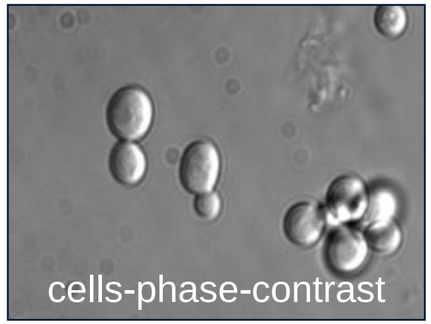
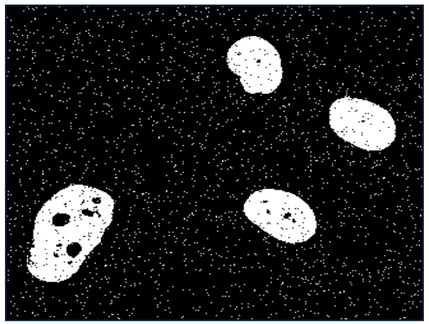
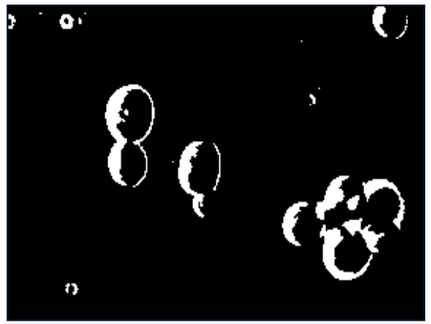
Distance Map



Summary

- Erosion
- Dilation
- Closing
  - Erosion+Dilation
- Opening
  - Dilation+Erosion
- Extension to 3D Multidimensional data (z-stack, time).
- Watershed
- Fill Holes
- Skeletonization
- Voronoi
- Distance Map

Thresholding

	Non-uniform backg.	Noise	Modality acquisition	Complex object
Source	 example1	 example2	 cells-phase-contrast	 zebra
Automatic Threshold				
	After preprocessing 	After preprocessing 	Impossible to threshold Required: physical modelling of the acquisition	Impossible to threshold Required: a prior knowledge, object modelling