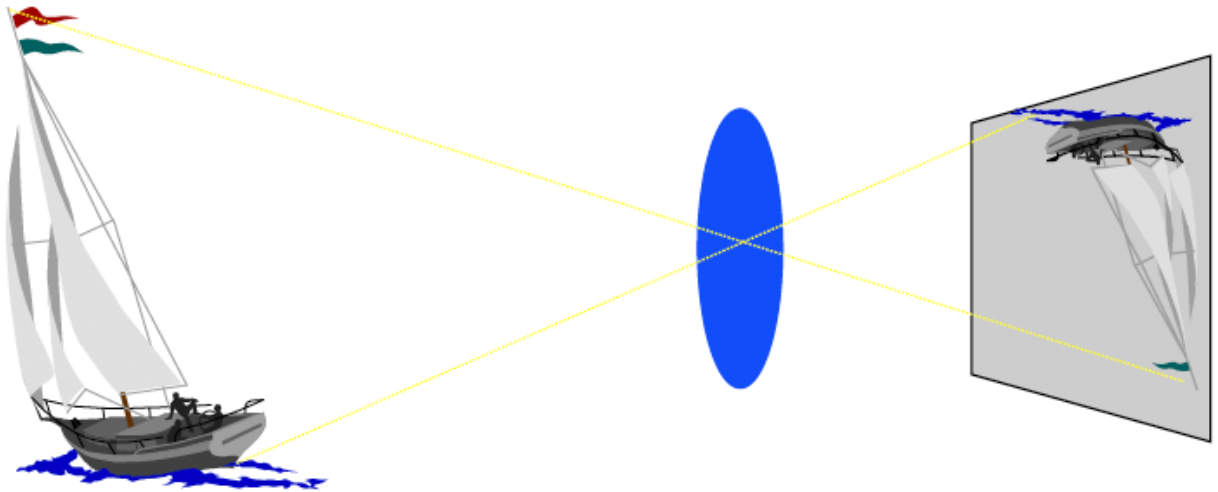
Microscopy and image analysis

Image analysis Outline

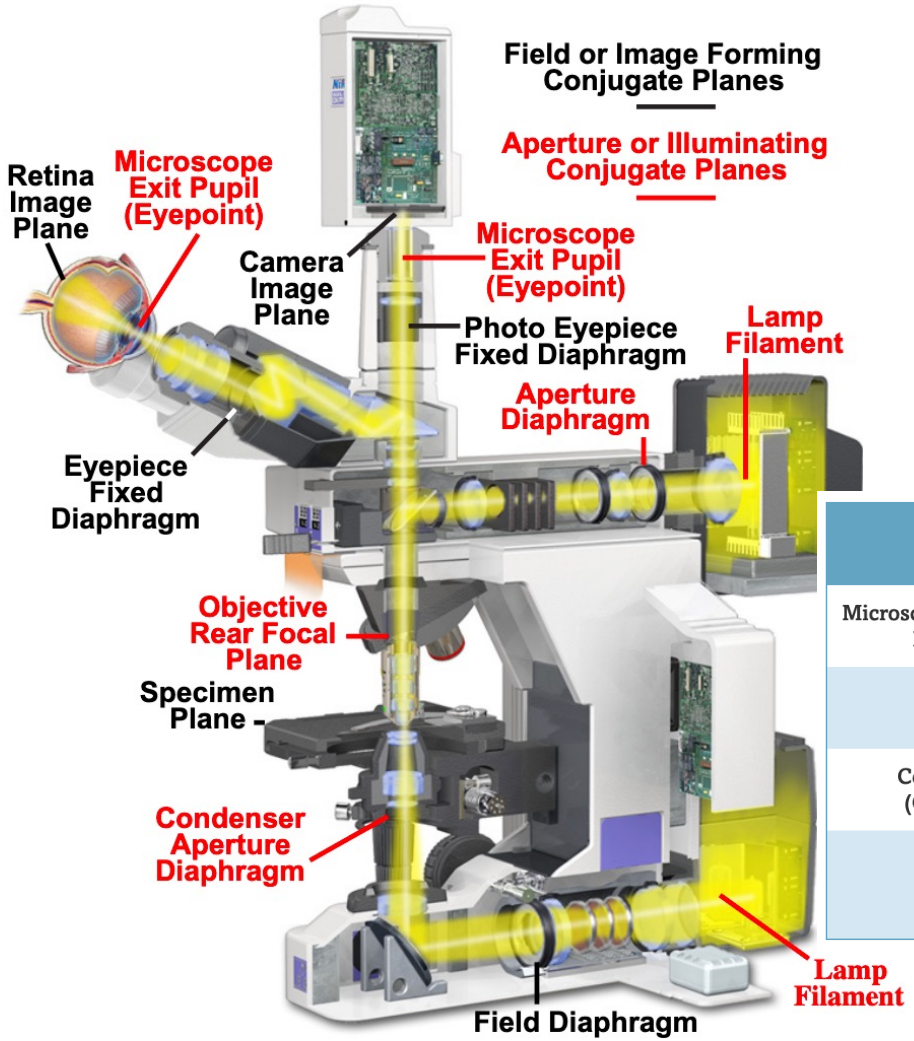
- Microscope imaging and cameras (quick repeat)
- What is a digital image? (quick repeat)
- Image types and resolution (quick repeat)
- Why do we need image analysis?
- How to do image analysis (basic steps)?
- Morphological operators
- Watershed algorithm
- Examples

Imaging principles



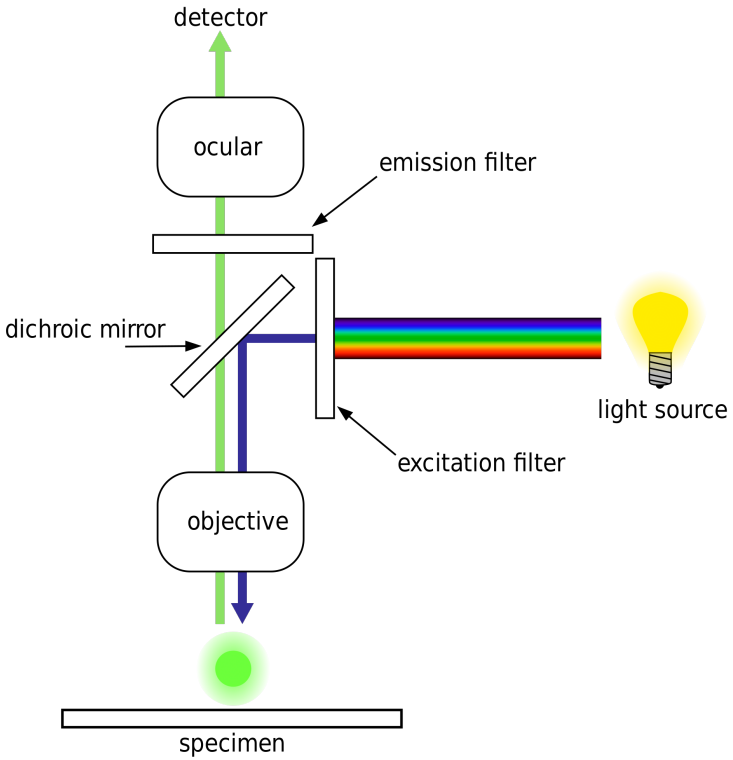
Mi

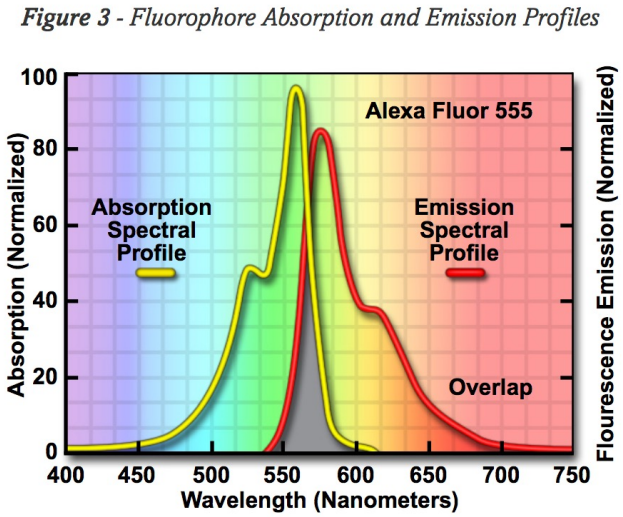
Figure 1 - *Conjugate Planes in the Optical Microscope*



Aperture or Illuminating Conjugate Plane Set	Field or Image-Forming Conjugate Plane Set
Microscope Exit Pupil: Eye Iris Diaphragm, Ramsden Disc, and Eyepoint	Retina of the Eye Camera Image Plane
Objective Rear Focal Plane (Objective Rear Aperture)	Intermediate Image Plane (Eyepiece Fixed Diaphragm)
Condenser Aperture Diaphragm (Condenser Front Focal Plane)	Specimen Plane (Object Plane)
Lamp Filament	Field Diaphragm (Field Stop) (Köhler Diaphragm)

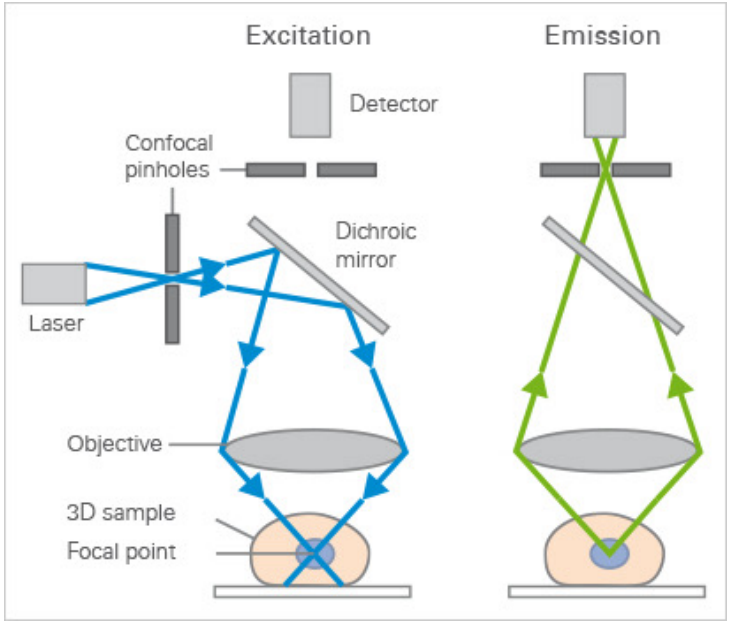
Principle of fluorescence microscopy





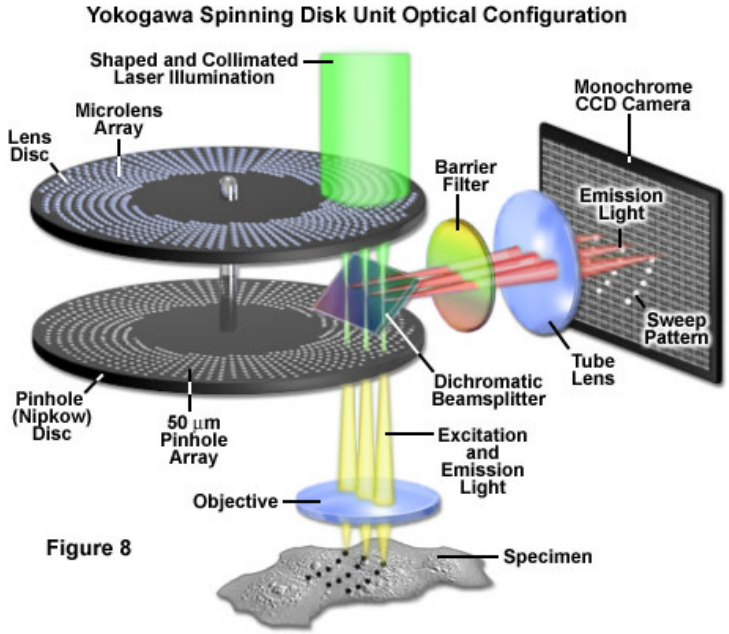
Confocal fluorescence microscopy

Confocal laser scanning microscopy



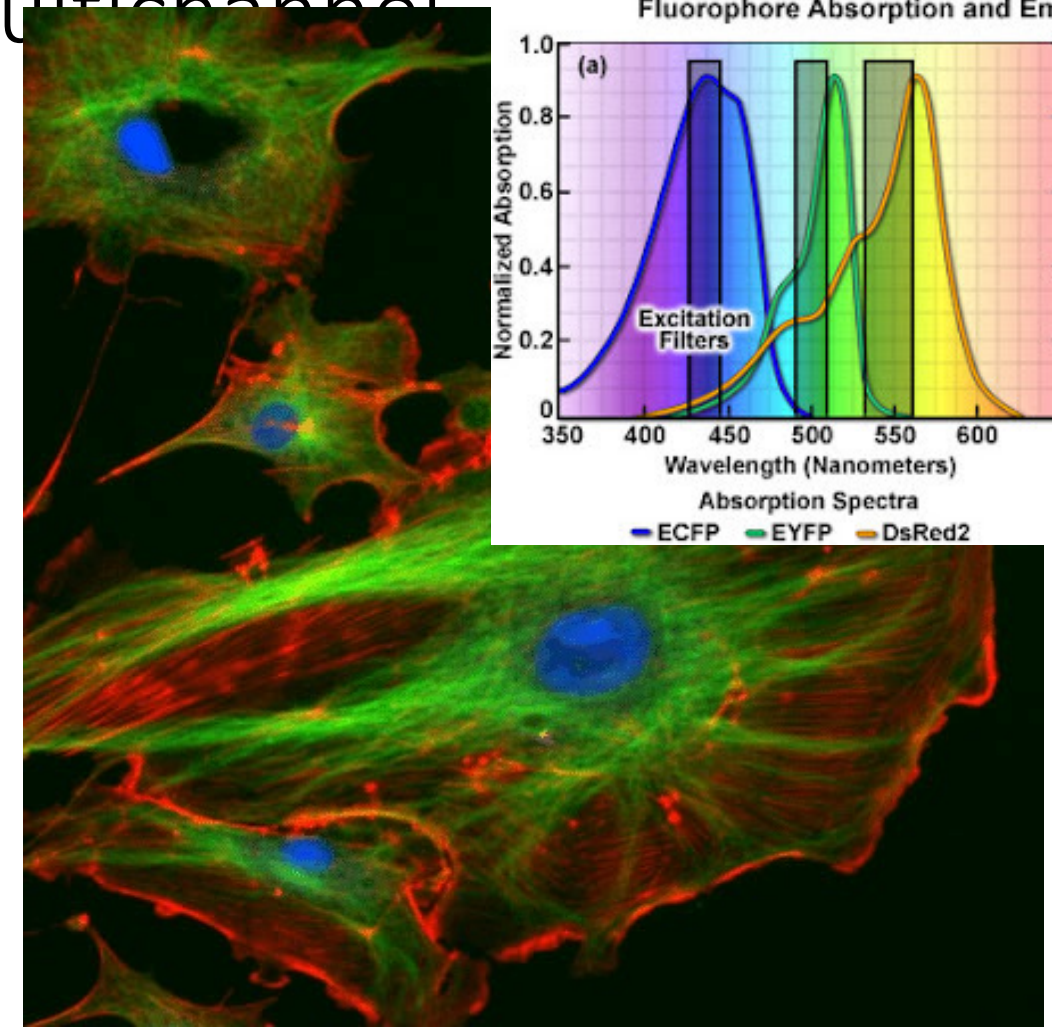
High resolution, slow

Spinning disk confocal microscopy



Lower resolution, faster

Multichannel



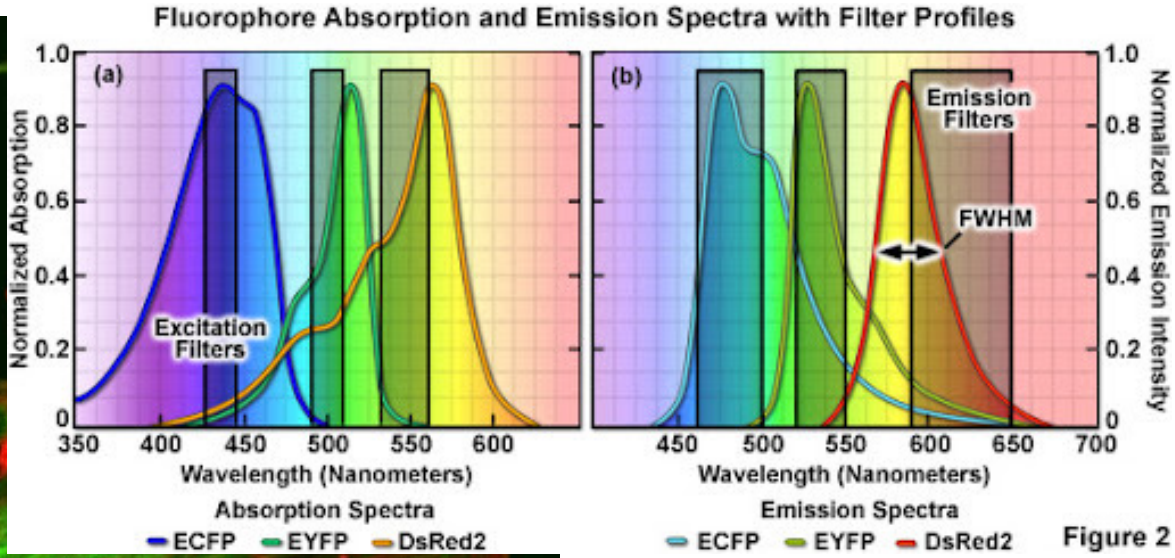
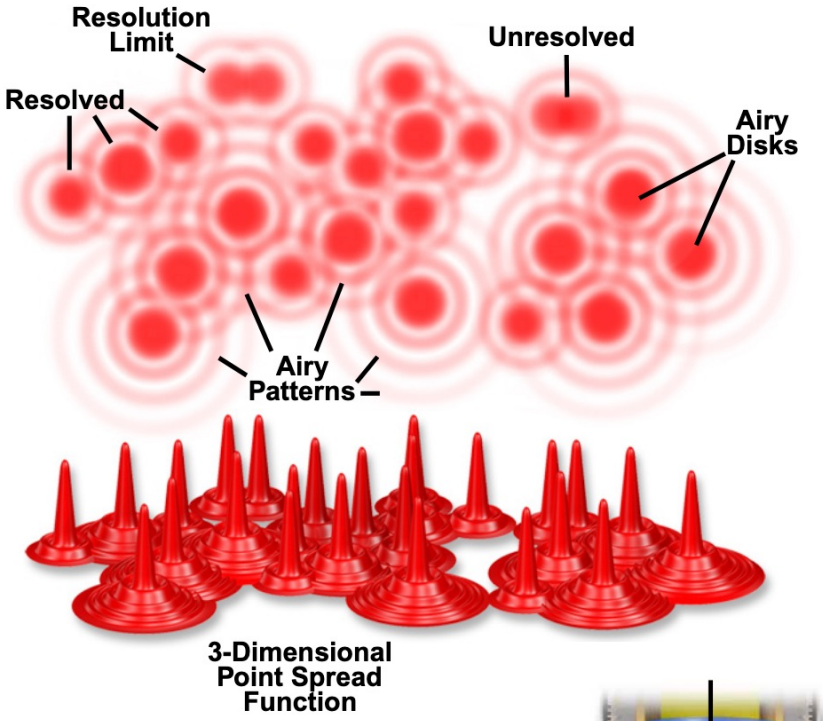


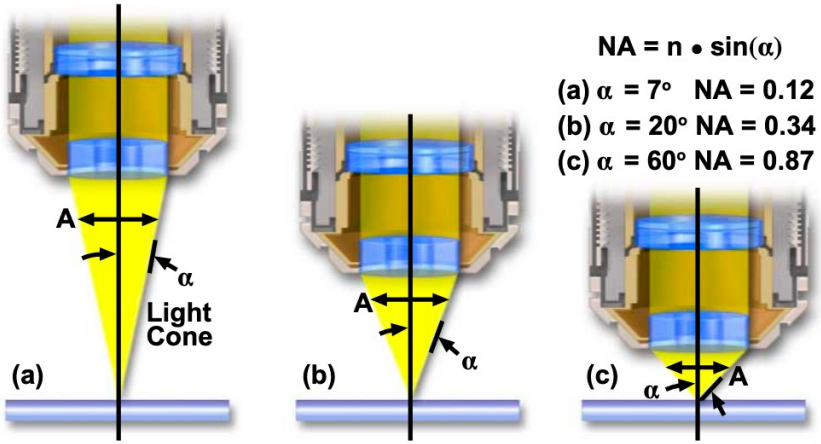
Figure 2

Figure 1 - Airy Patterns and the Limit of Resolution

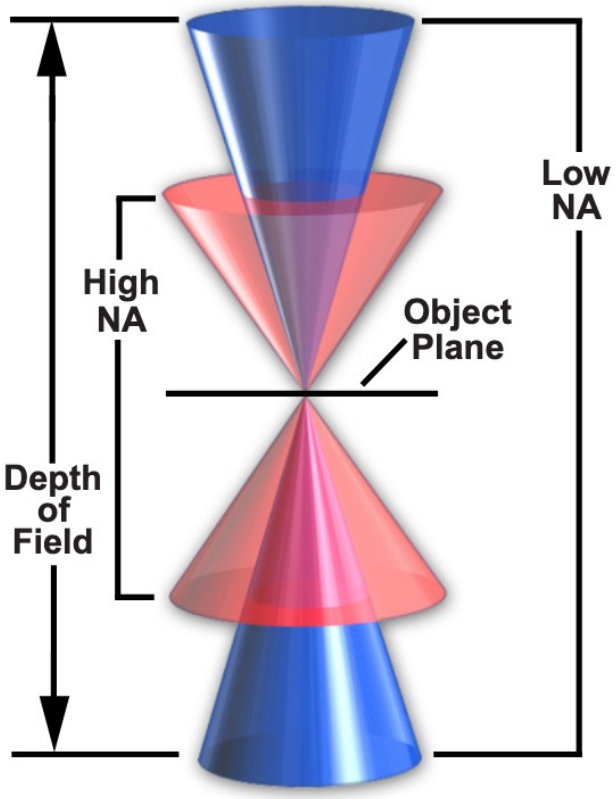


$$r = 0.61\frac{\lambda}{NA}$$ reflected light

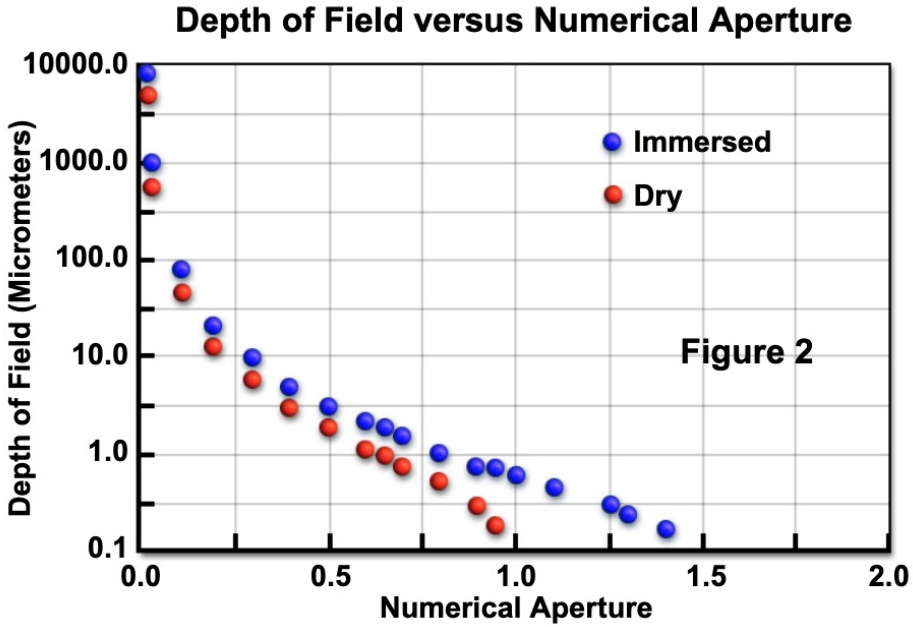
$$r = 1.22\frac{\lambda}{NA_o + NA_c}$$ transmitted light



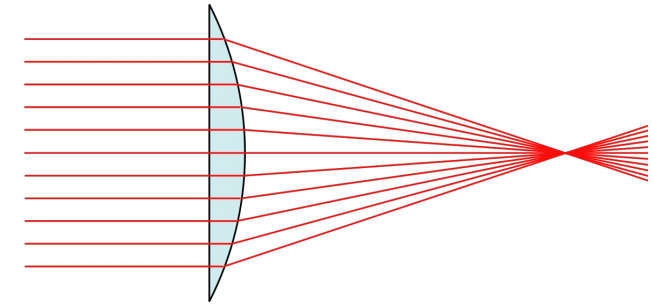
Depth of field/focus

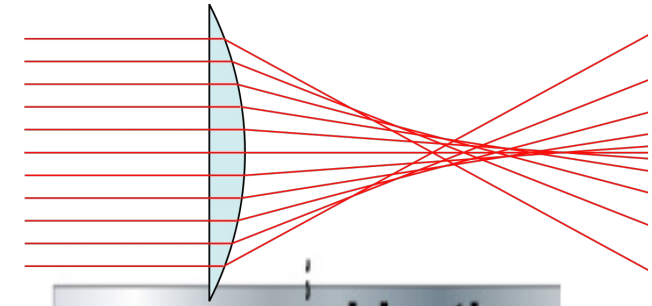


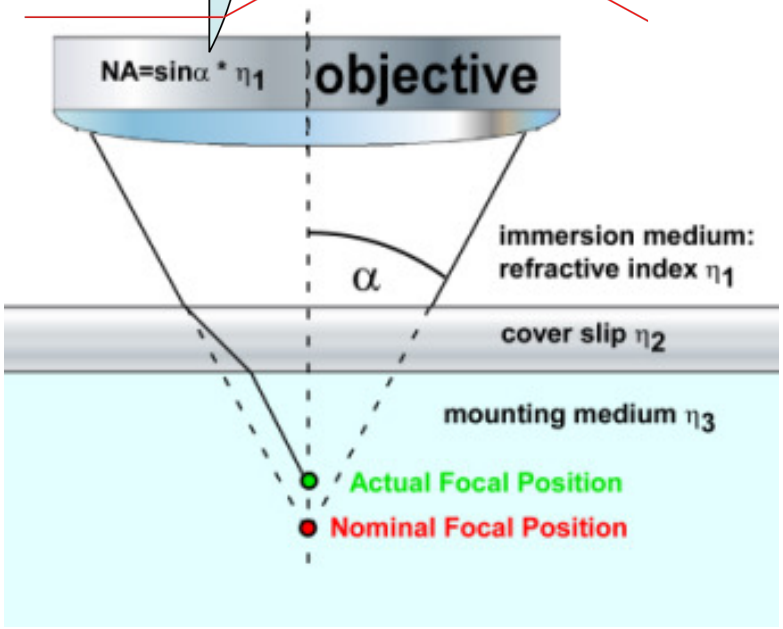
depth of field = the distance from nearest object plane in focus to farthest plane also simultaneously in focus.

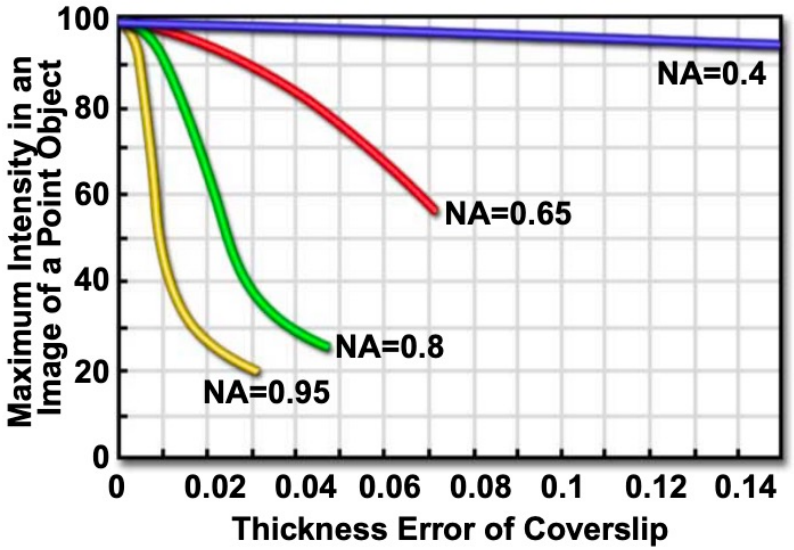


Spherical aberration & coverslip correction

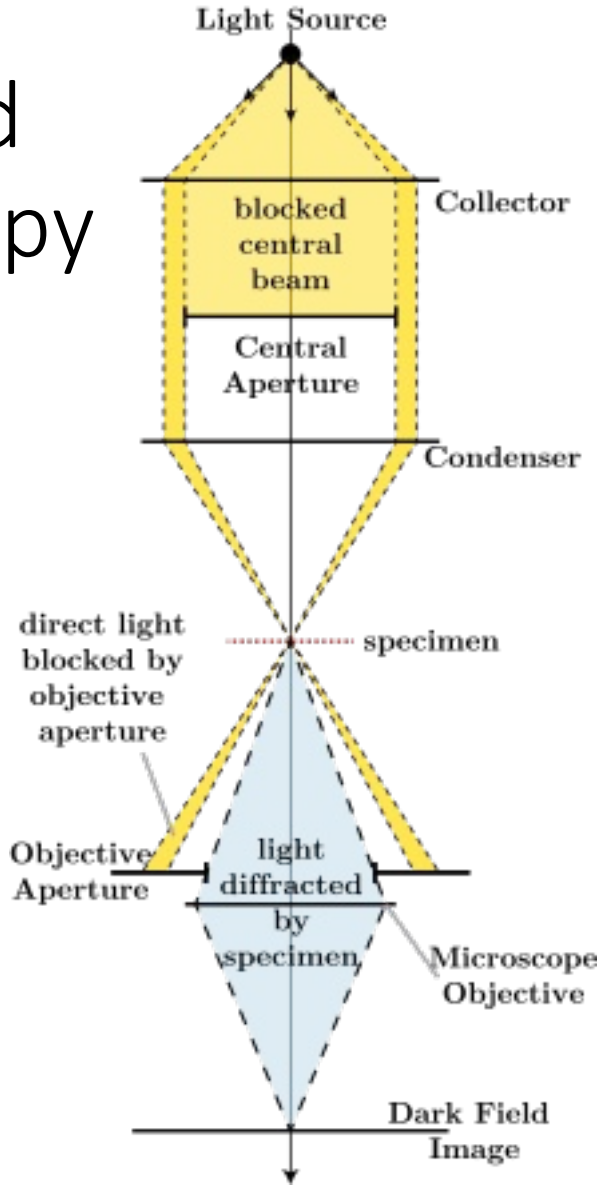


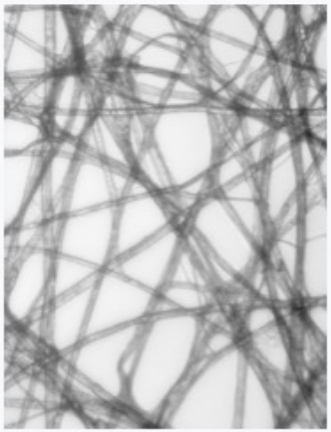


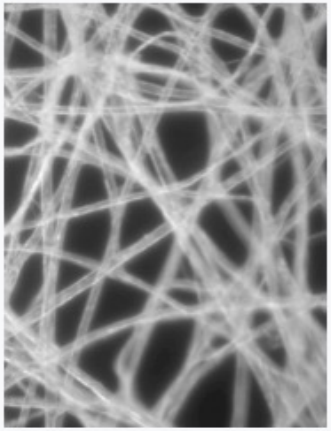




Dark field microscopy







Phase contrast

making phase changes visible in phase-contrast microscopy is

- to separate the illuminating (background) light from the specimen-scattered light (which makes up the foreground details) and
- to manipulate these differently.

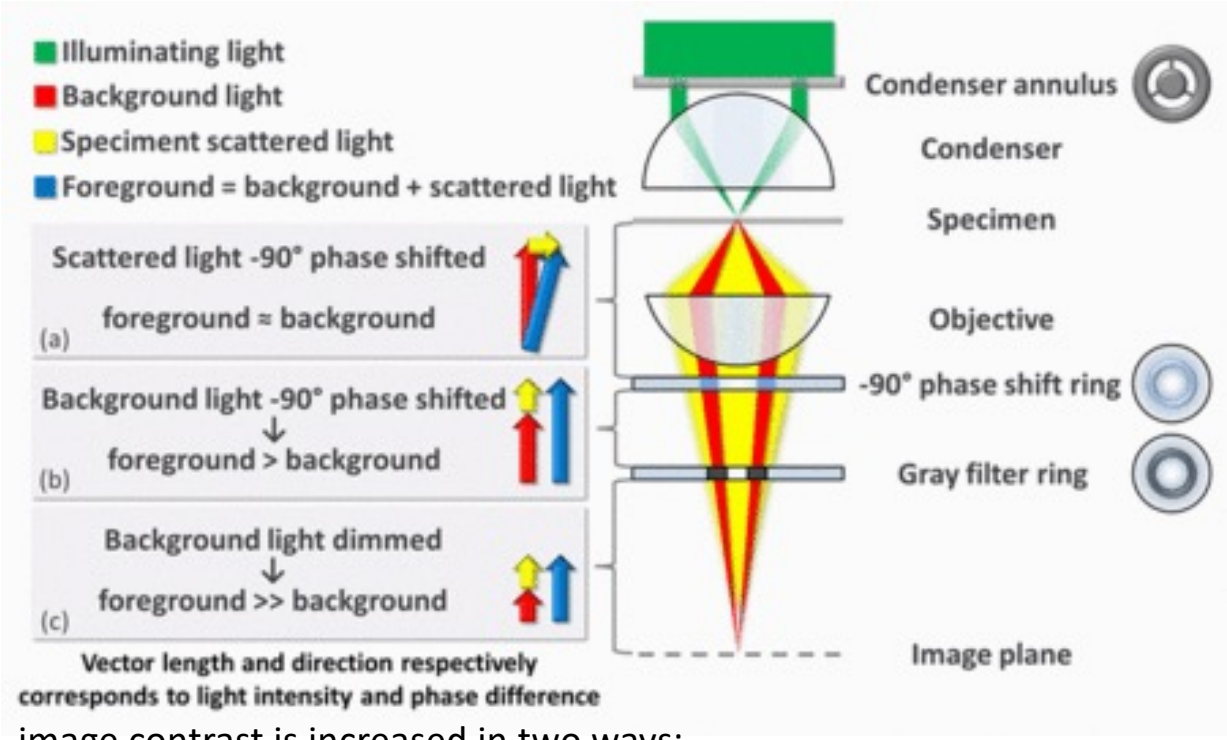
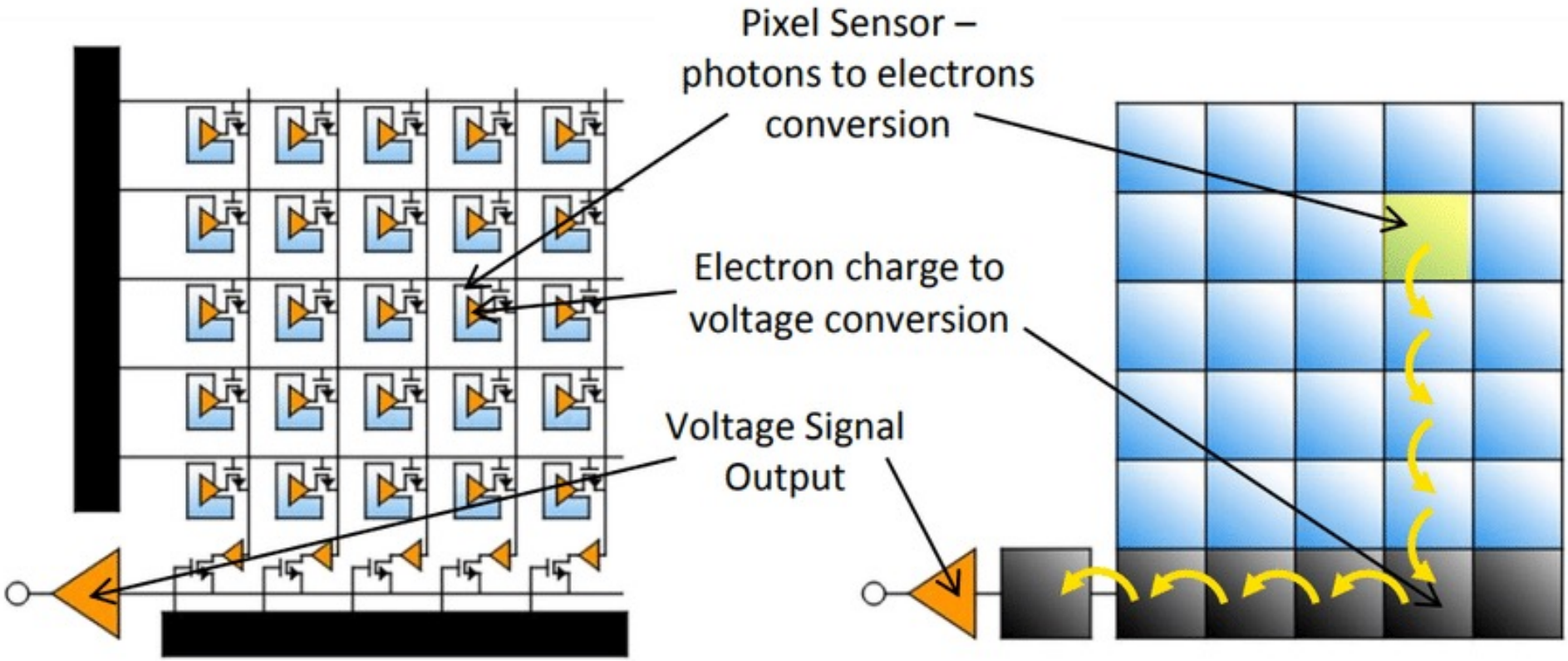


image contrast is increased in two ways:

- by generating constructive interference between scattered and background light
- by reducing the amount of background light that reaches the image plane

CMOS

CCD


Pixel Sensor –
photons to electrons
conversion
Electron charge to
voltage conversion
Voltage Signal
Output

Noise

- Shot noise / thermal / dark current
- Read-out noise
- Saturation / Glare / Blooming
- High energetic ”cosmic” rays
- ”Digital noise” / Moirè patterns

High Resolution Digital Cameras

Advantages

- Light sensitive
- High spatial and dynamical resolution
- Low noise

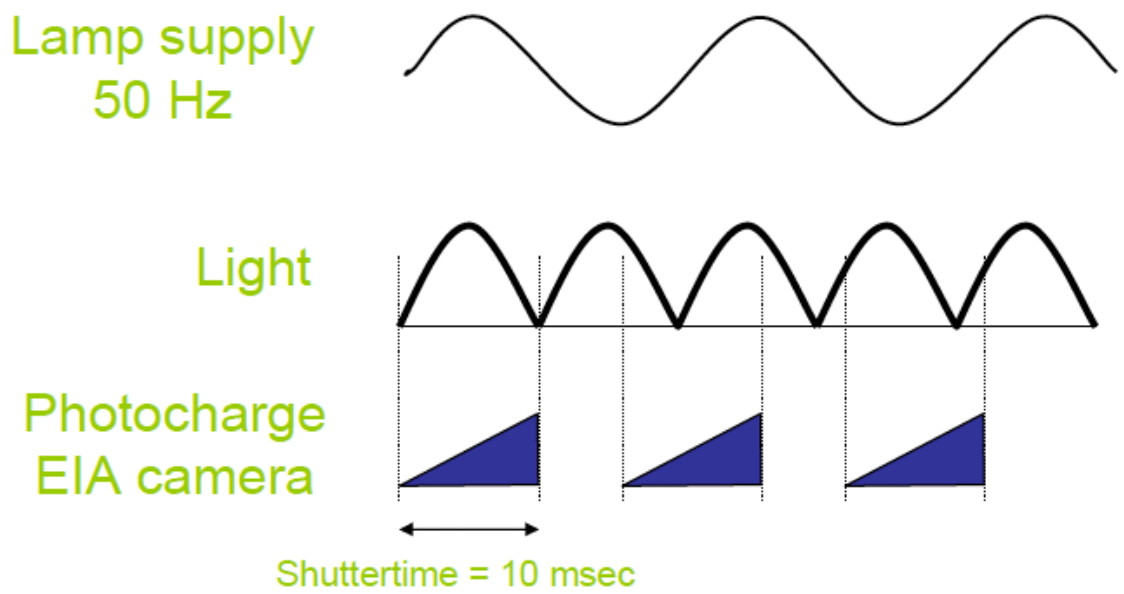
MORE SENSITIVE THAN THE EYE

Drawbacks

- Slow data transfer
- Produces much data
- Requires custom made software
- Not user friendly
- Expensive

16 bit: cooled sensor

Flicker



Shutter time = one light period, photocharge = constant

Result = no flicker and reduced sensitivity

Practical tips for adjusting video camera

- Turn off automatic adjustments
- Turn down Gain (it only adds noise)
- Adjust light intensity and shutter speed
  - until histogram covers intensity range
  - shutter speed must be short enough for desired frame rate
  - shutter speed long enough to avoid flicker

What is an image?



$$f = f(x, y)$$

12	0	234	122	54	65
78	34	215	23	23	34
109	65	30	117	54	54
140	23	111	214	65	76
11	12	245	213	235	189
155	0	78	0	0	67
178	198	201	0	12	42

Pixels MxN

Image types

- Intensity images – grey level
- Binary images – black and write
- RGB images – color images



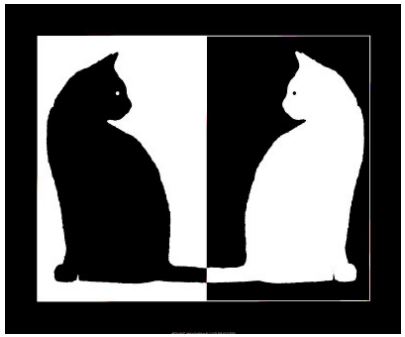




Image quality

Image quality:

- Number of pixels in the matrix – image size
- Intensity range

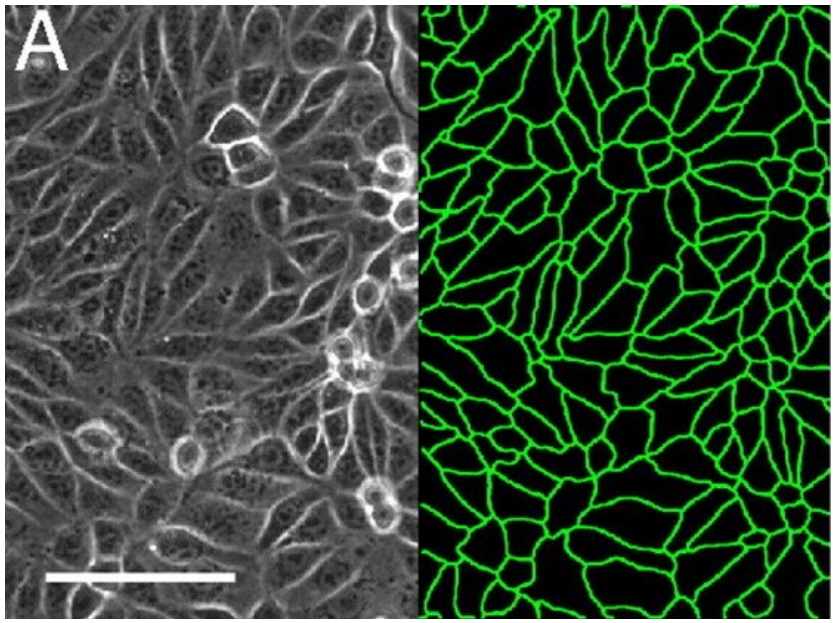
1 bit depth ($2^1 = 2$) – black and white
8 bit depth ($2^8 = 256$) – gray scale 0..256
12, 16 bit gray scale
24 bit depth (256 shades of RGB) – true color

Why do we need image analysis?

Morphological analysis – a mathematical tool to investigate geometrical structure of binary or grayscale image

Segmentation

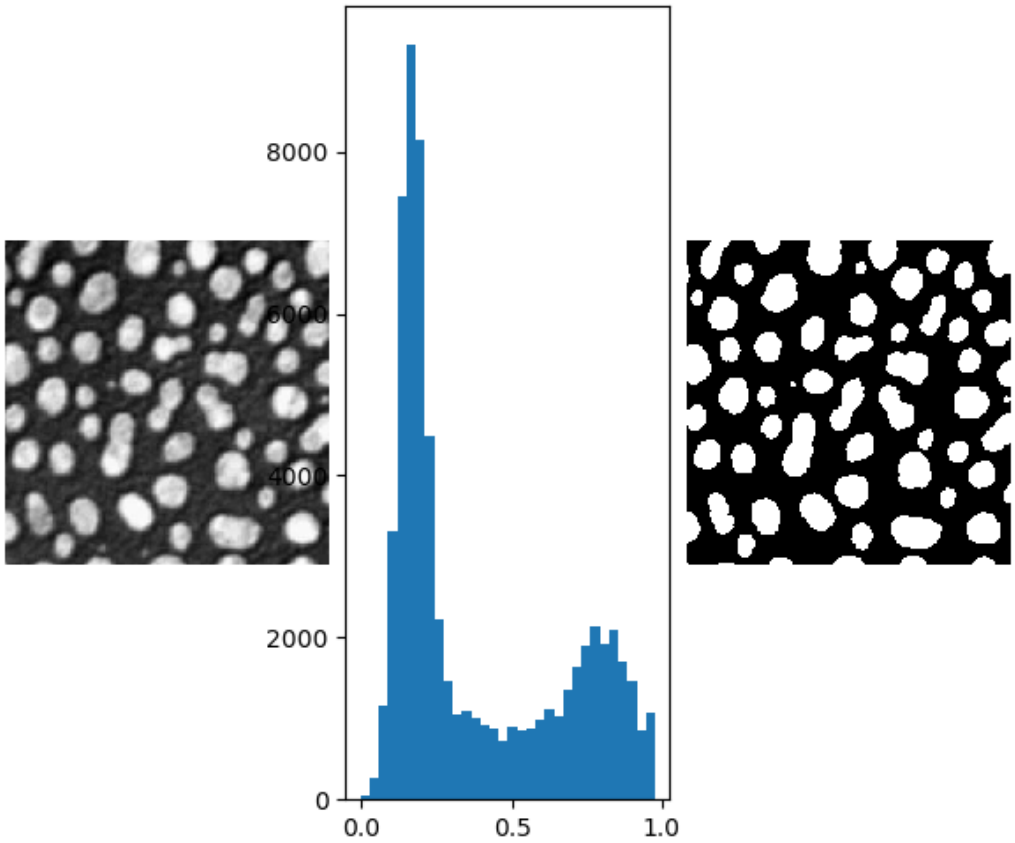
procedure



Basic steps of image analysis

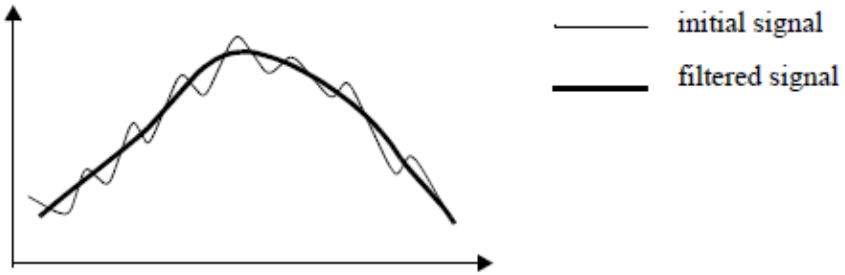
Image segmentation quick steps:

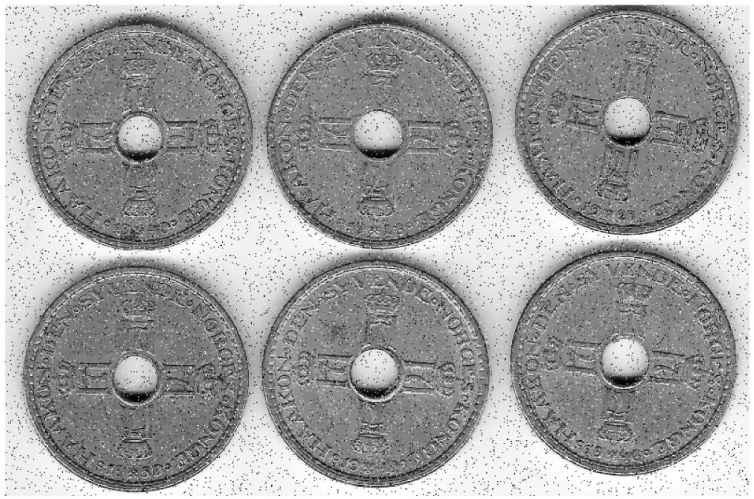
- Filter
- Thresholding → binary
- Labeling connected components
- Geometrical analysis of connected components



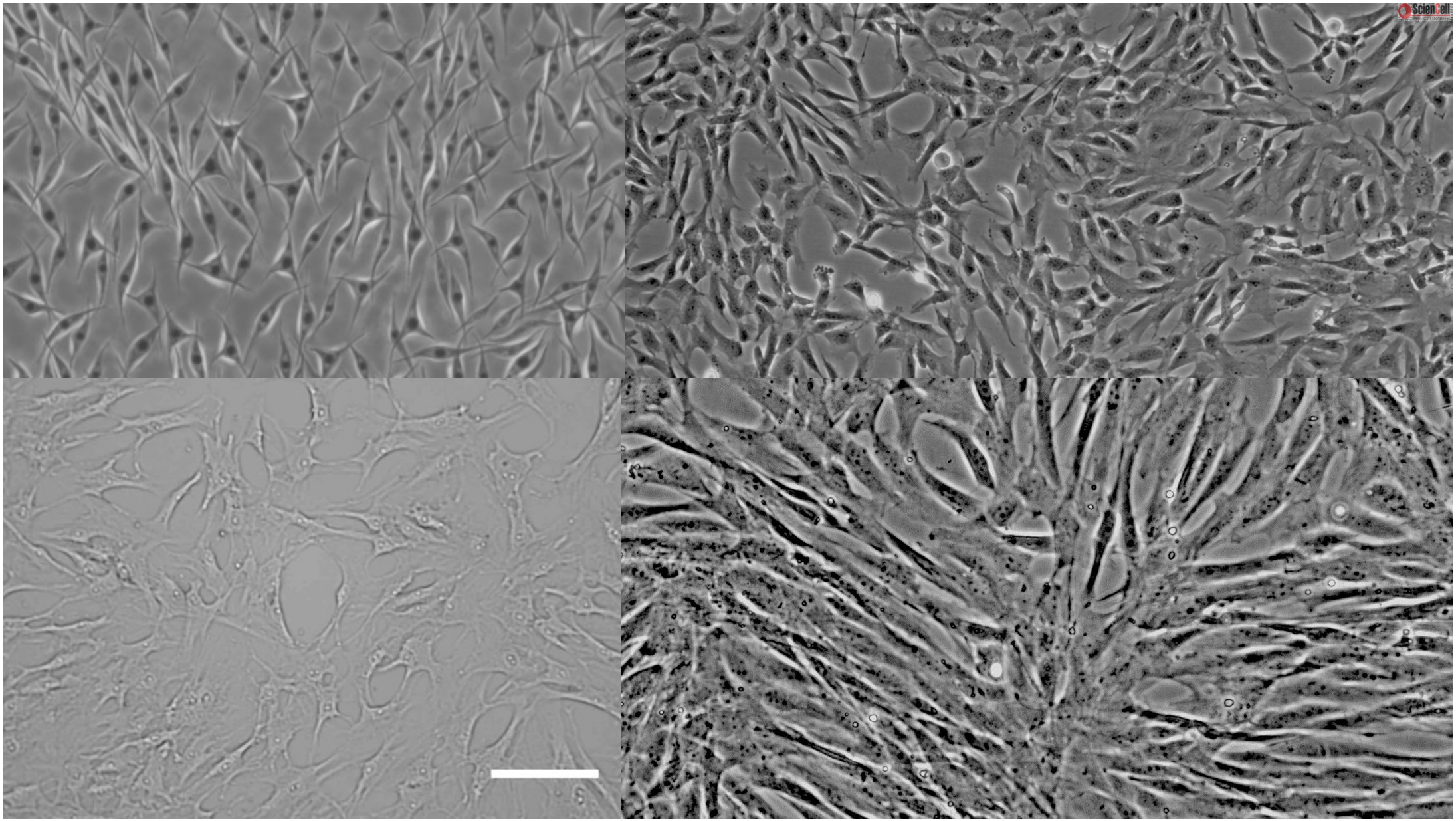
Noise removal

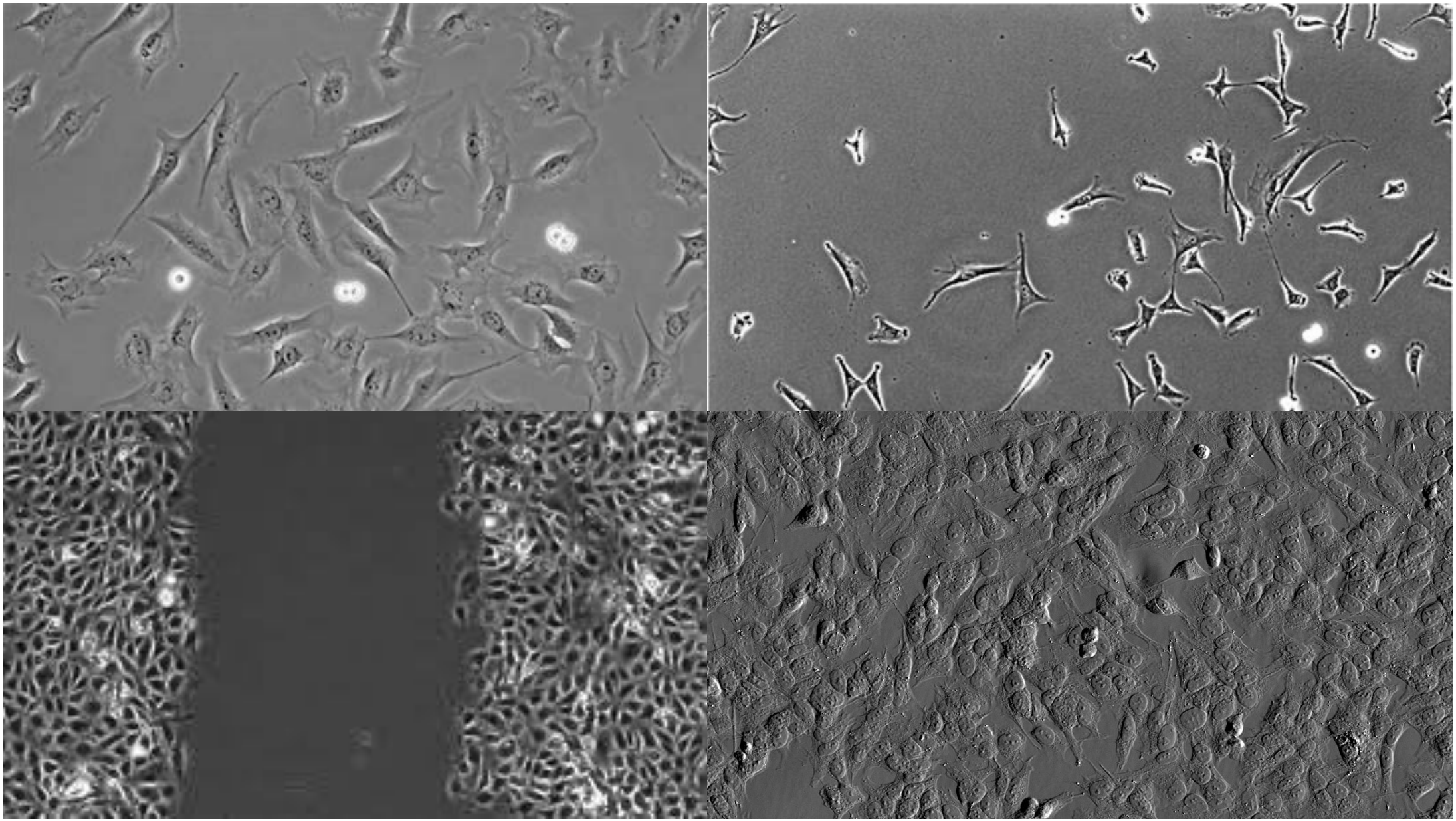
- Filtering – smoothing
- Background correction







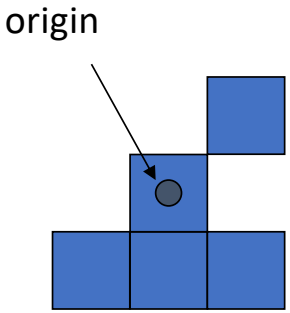


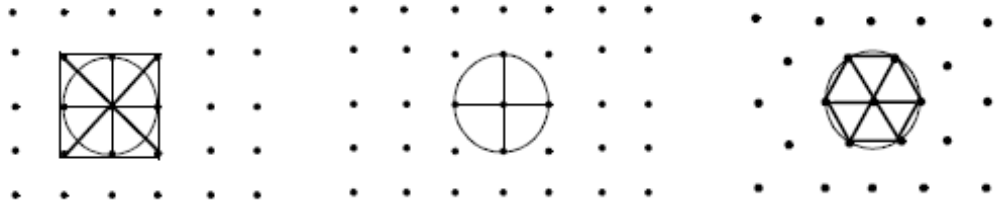


Morphological operators

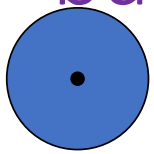
Morphological transformation are based on a structural element

- size
- shape
- center location



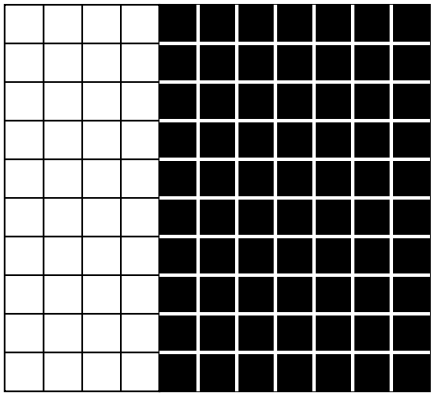


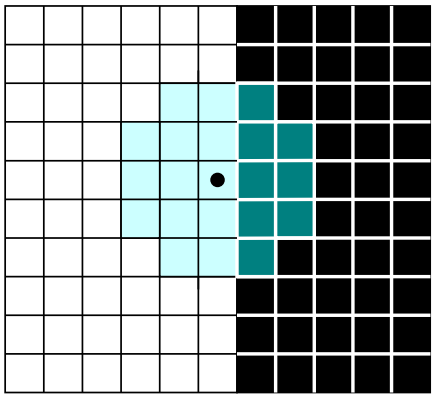
Erosion and dilation – basic operations



Erosion

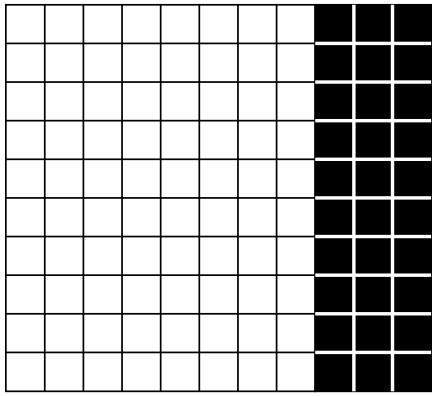
"Set the value at the origin to the minimum value of pixels in the structural element"





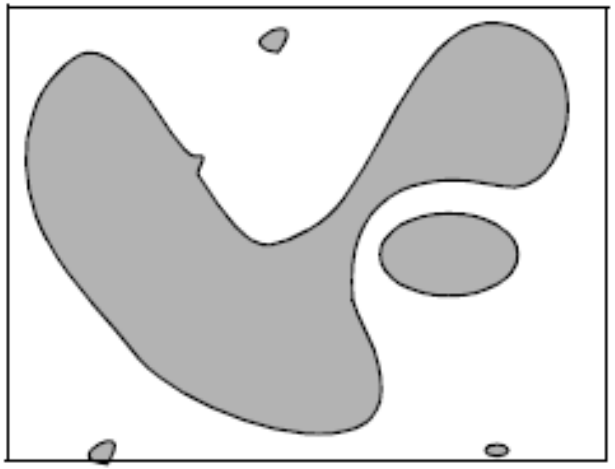
Dilation

"Set the value at the origin to the maximum value of pixels in the structural element"

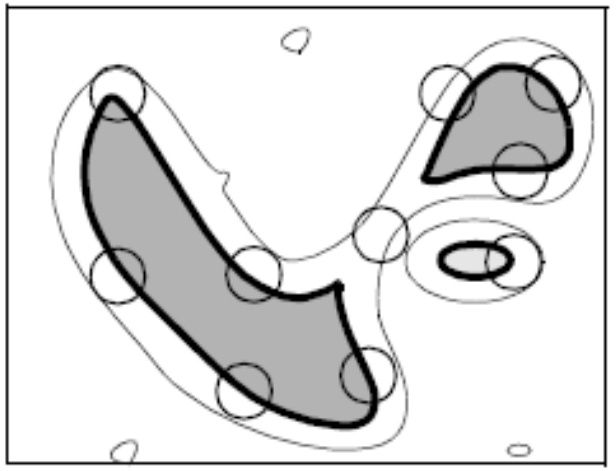


Erosion

- removes isolated points
- discards peaks on the boundaries
- disconnects some particles



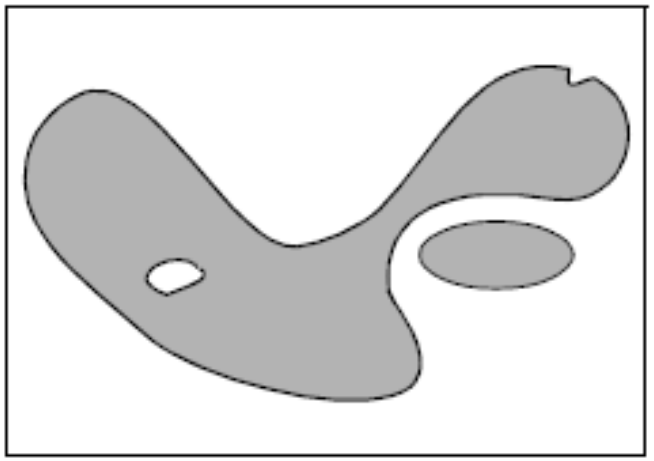
input image



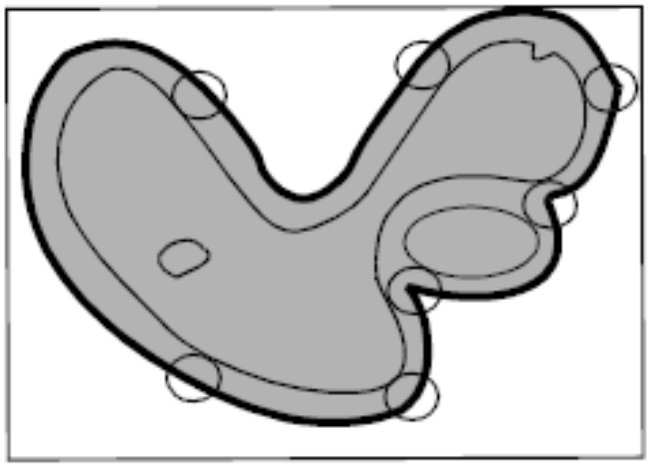
eroded image

Dilation

- fills small holes inside particles
- enlarges the size of the particles
- connects neighboring objects



input image



dilated image

Opening and closing

Opening = Erosion + Dilation

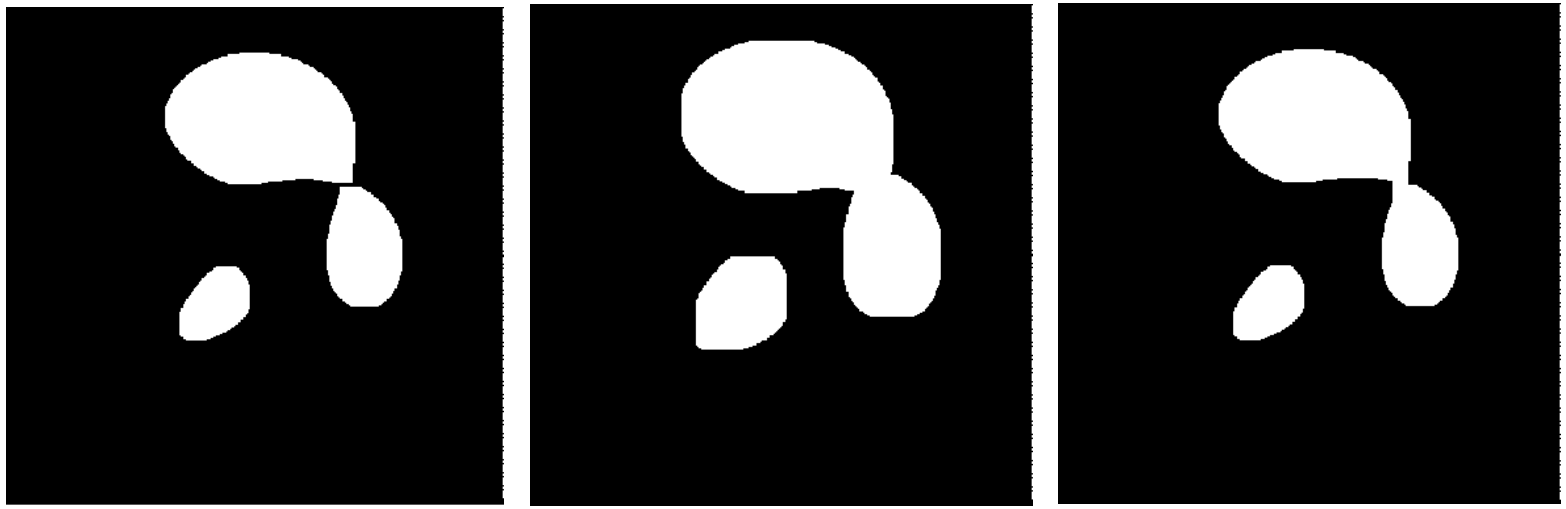
Closing = Dilation + Erosion

Original image → Erosion → Dilation



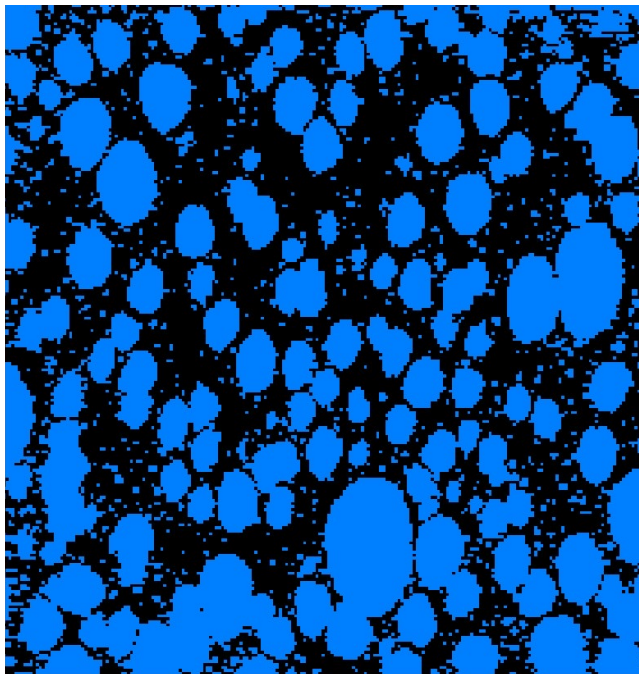
Closing

Original image → Dilation → Erosion

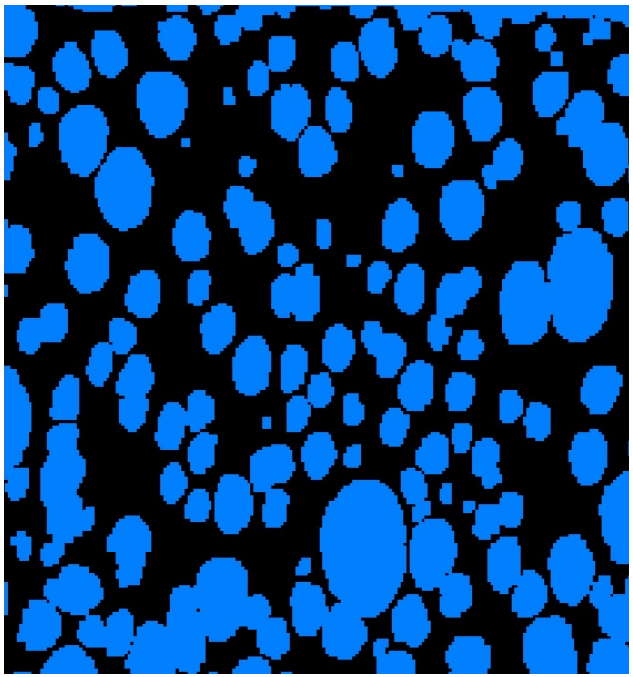


Noise removal

Original image

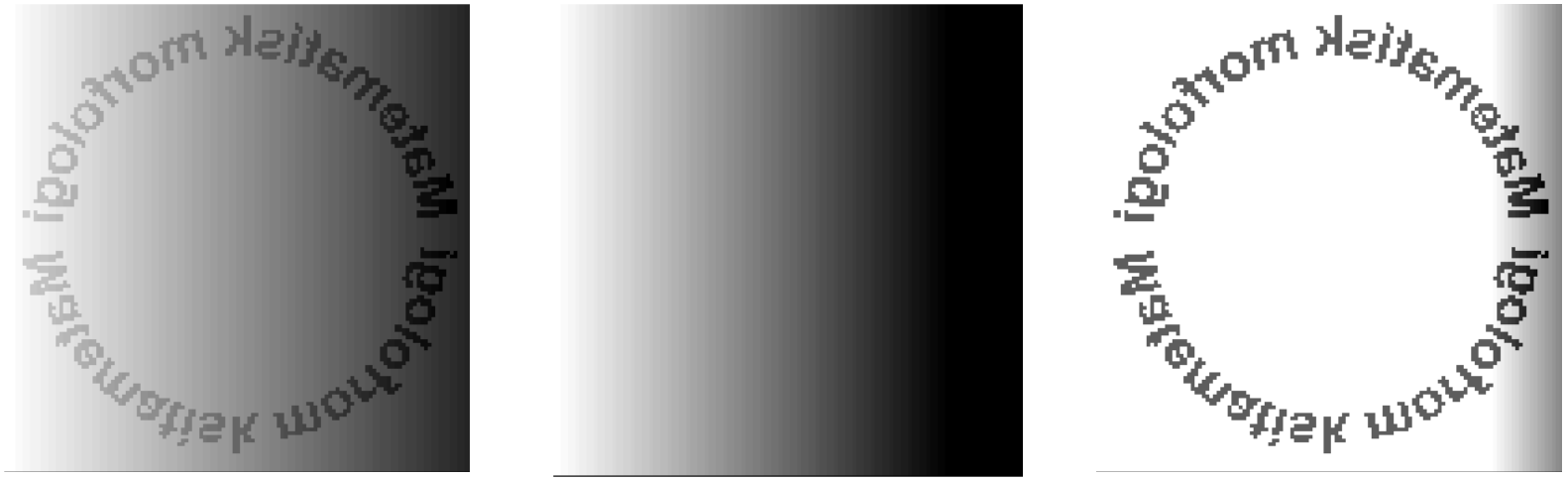


After opening



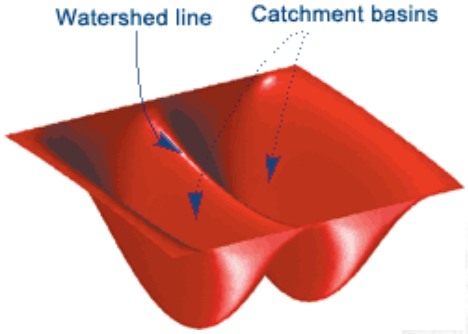
Background correction

Original image → Opening → IM ÷Opening



Watershed

Flooding of image topography



Water rise from a set of markers

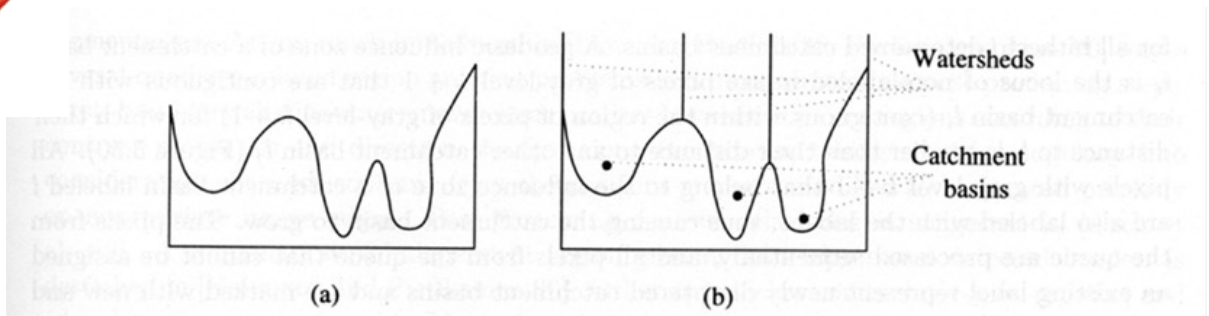
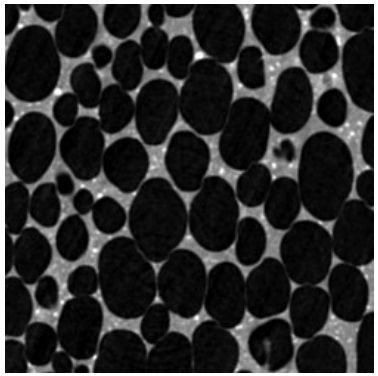


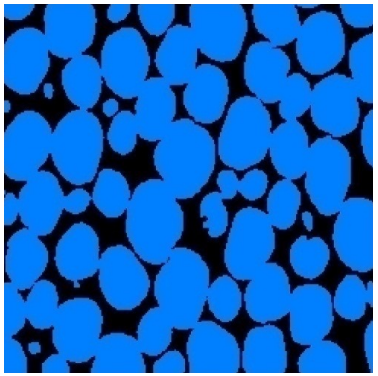
Figure 5.49: *One-dimensional example of watershed segmentation: (a) gray-level profile of image data; (b) watershed segmentation—local minima of gray-level (altitude) yield catchment basins, local maxima define the watershed lines.*

Example of workflow using watershed

Gray level image

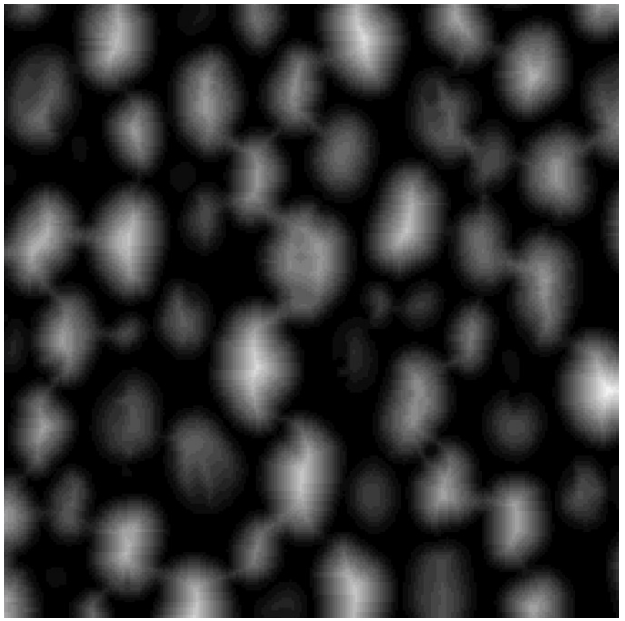


Binary image



Reconstruction of individual pores in foam

Distance map

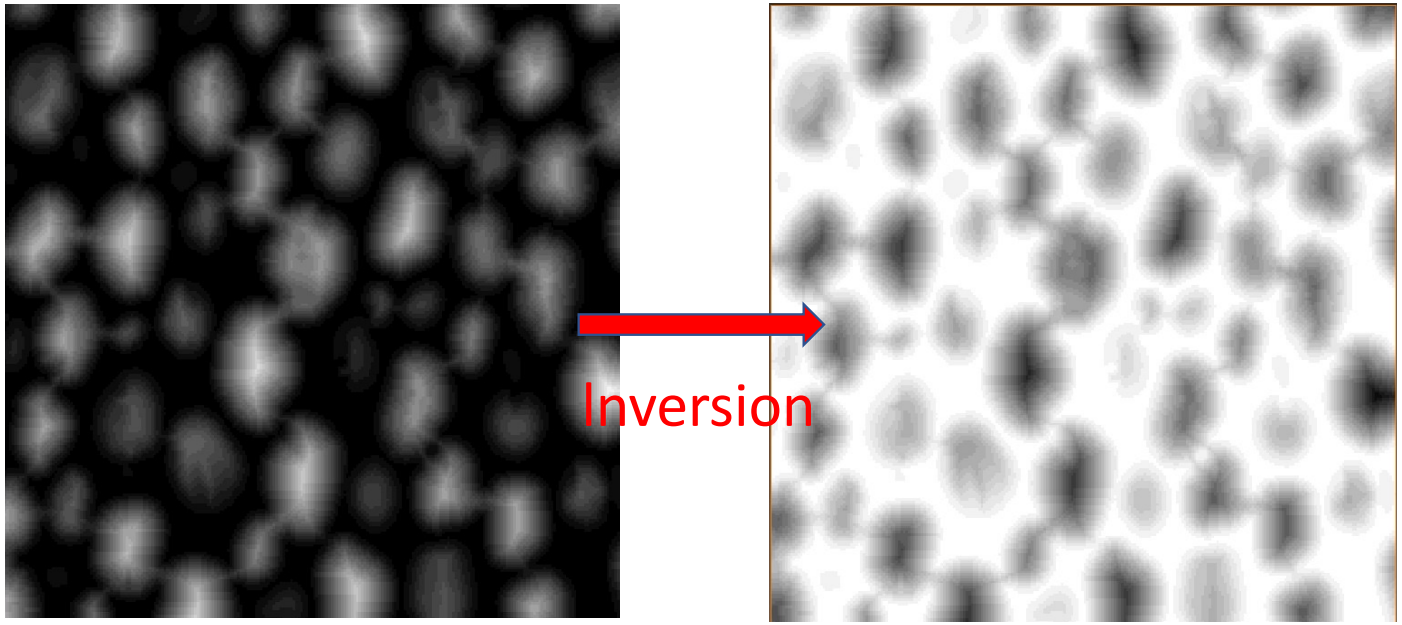


Example of workflow using watershed

Valleys for watershed

Distance map

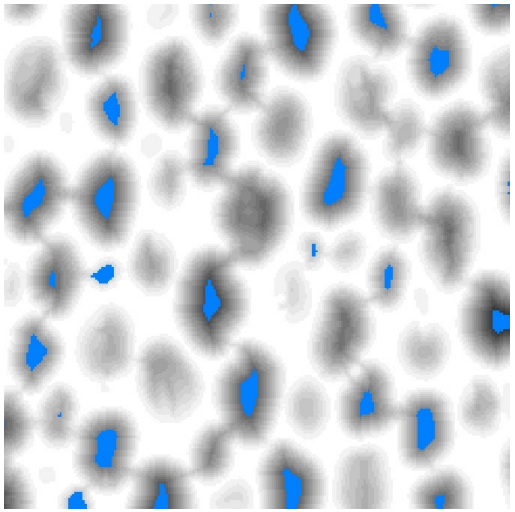
Inversed distance map



Example of workflow using watershed

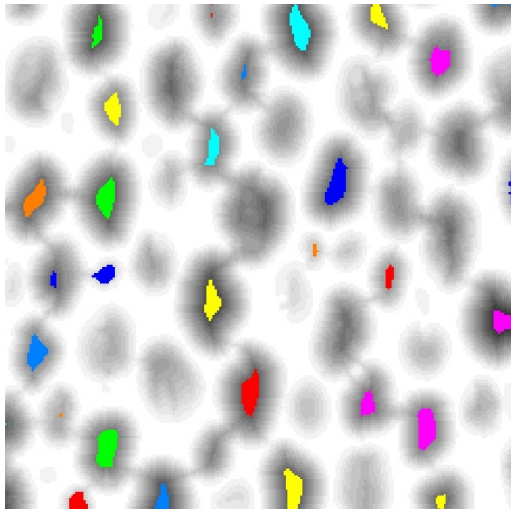
Create markers

Maxima on distance map



Labeling

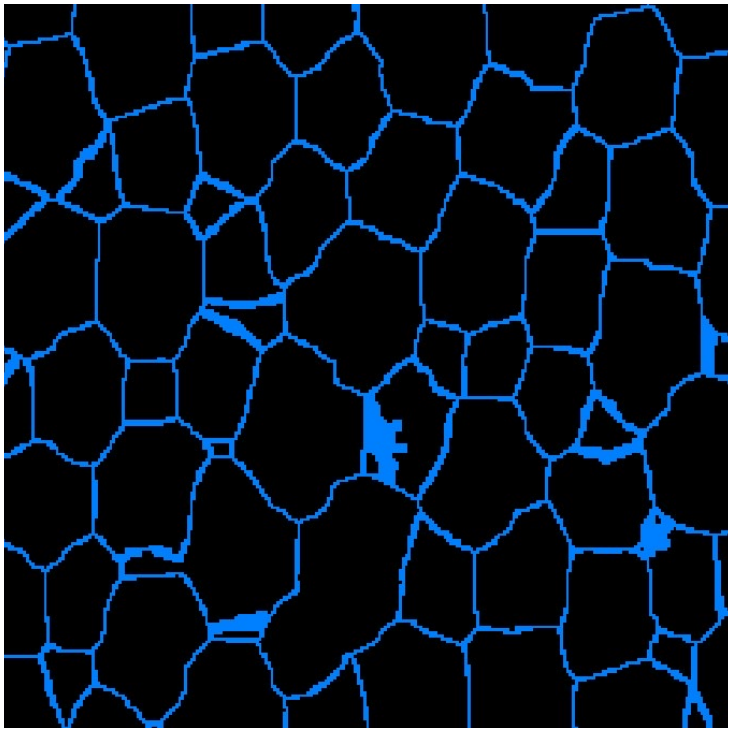
Markers

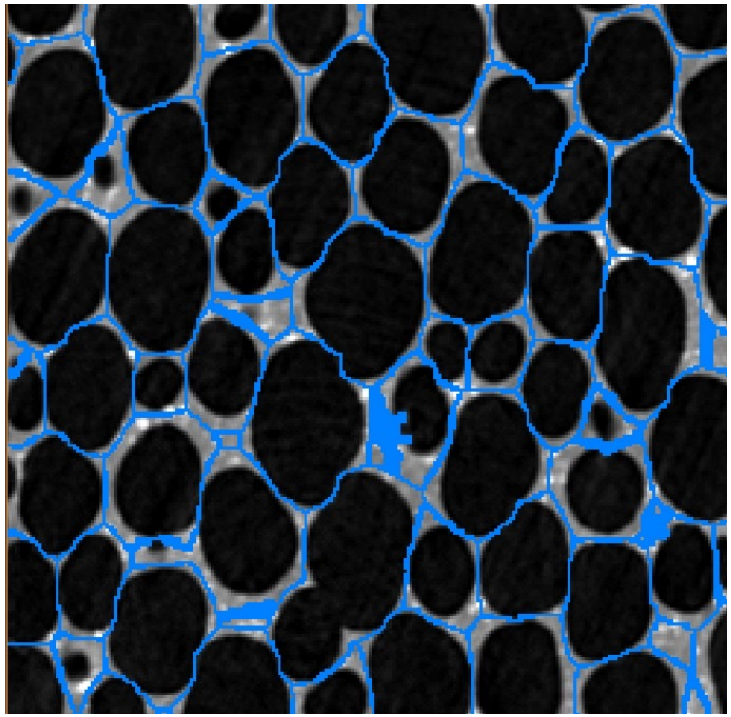


Example of workflow using watershed

Watershed lines –

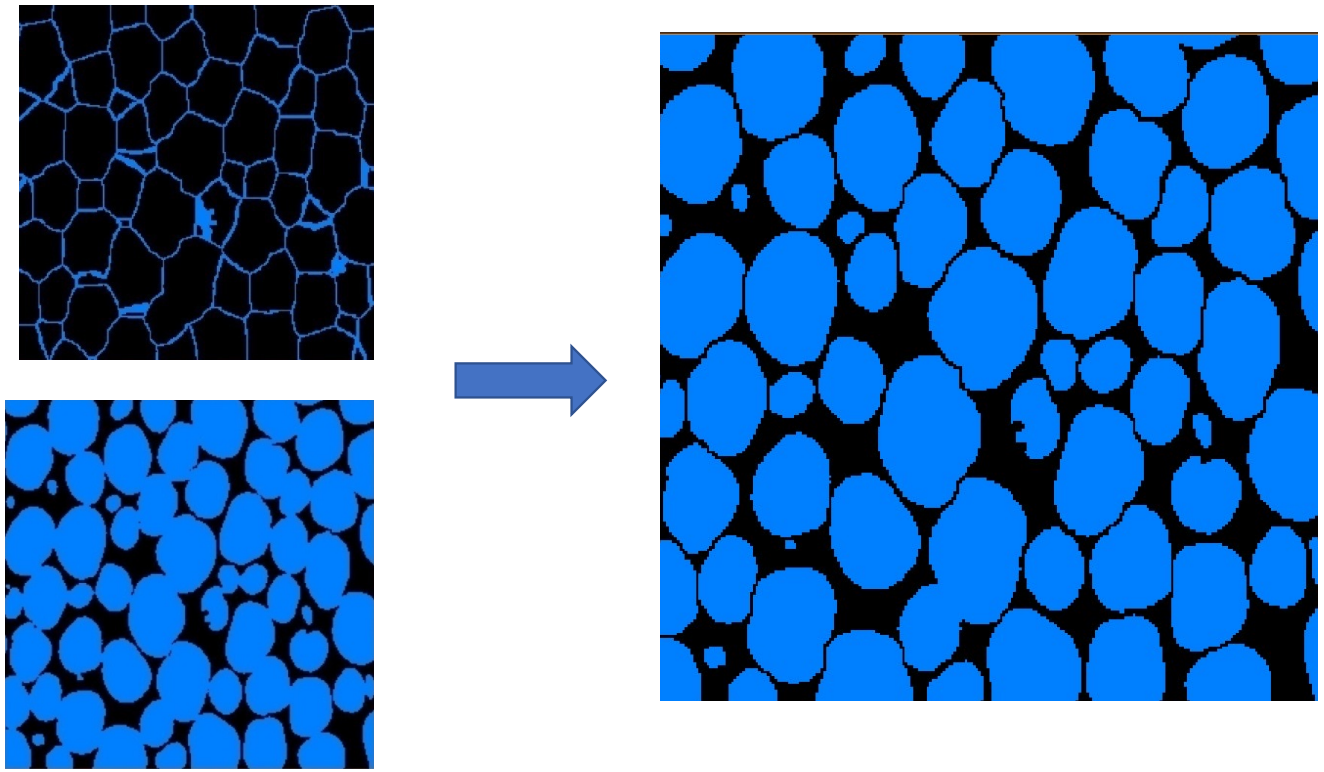
boundaries between regions





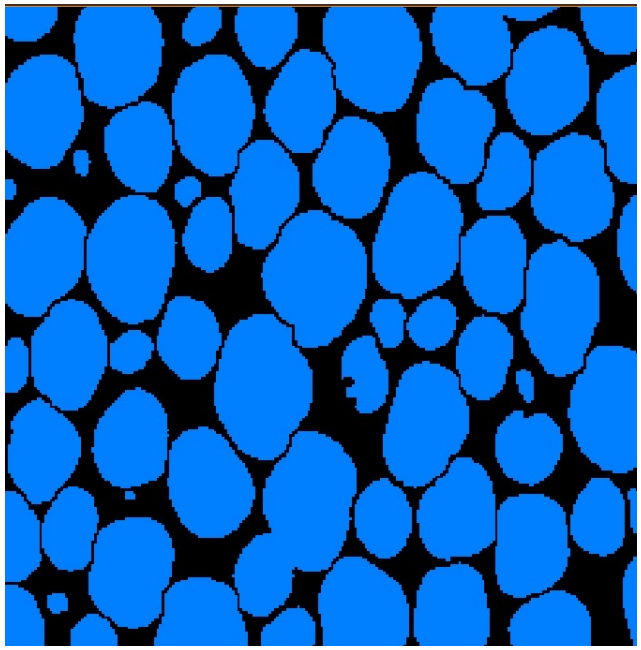
Example of workflow using watershed

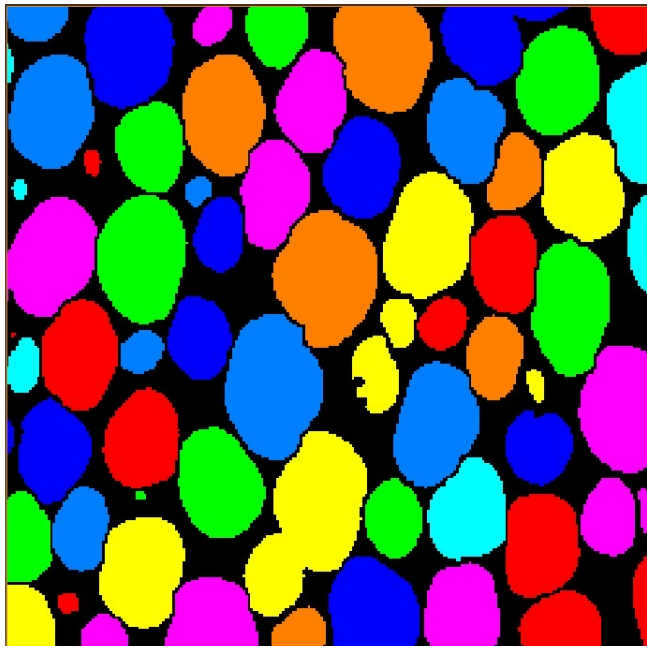
Subtraction of watershed lines gives separated pores



Example of workflow using watershed

Labeling of connected components





Best tools

- Fiji (imageJ)
- Matalb
- Python