Lecture 2

FYS4715 2021

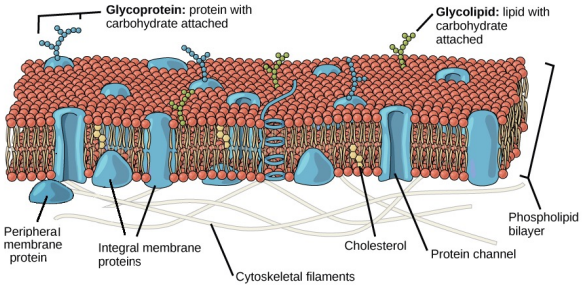
Whats inside cells, contd, statistical mechanics, diffusion, random walks

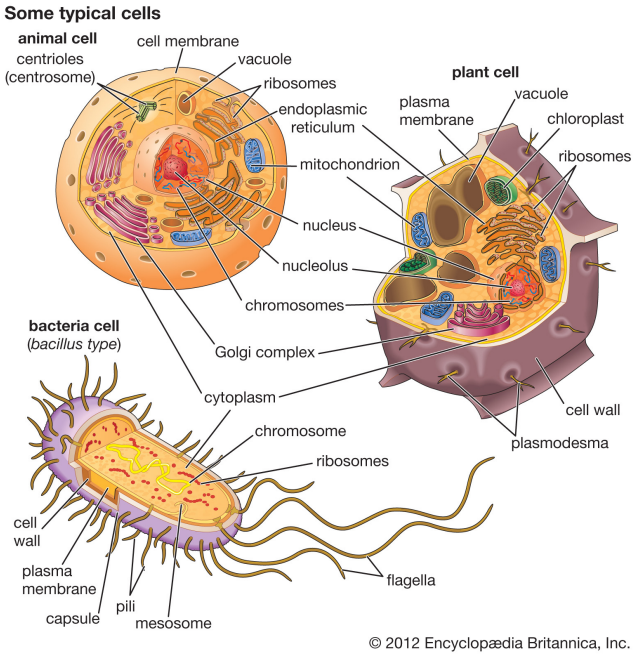
Cells – fundamental functional units of life

- enclosed by plasma membrane
- interior «soup» called cytoplasm
- organized in organelles = specialized compartments surrounded by membrane
  - nucleus: contains the genetic information necessary for cell growth and reproduction
  - mitochondria: responsible for the energy transactions necessary for cell survival
  - lysosomes: digest unwanted materials within the cell
  - endoplasmic reticulum & Golgi apparatus: organization of the cell by synthesizing selected molecules and then processing, sorting, and directing them to their proper locations

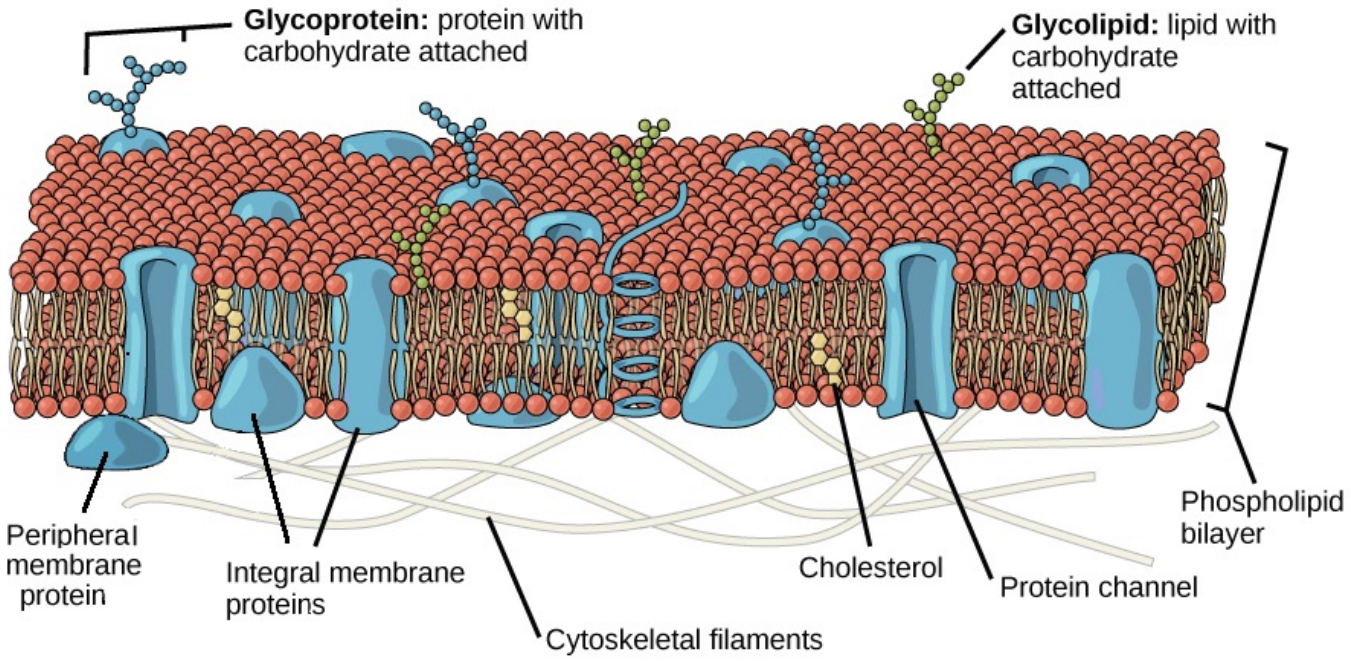
- https://www.allencell.org/

cyto- = cell

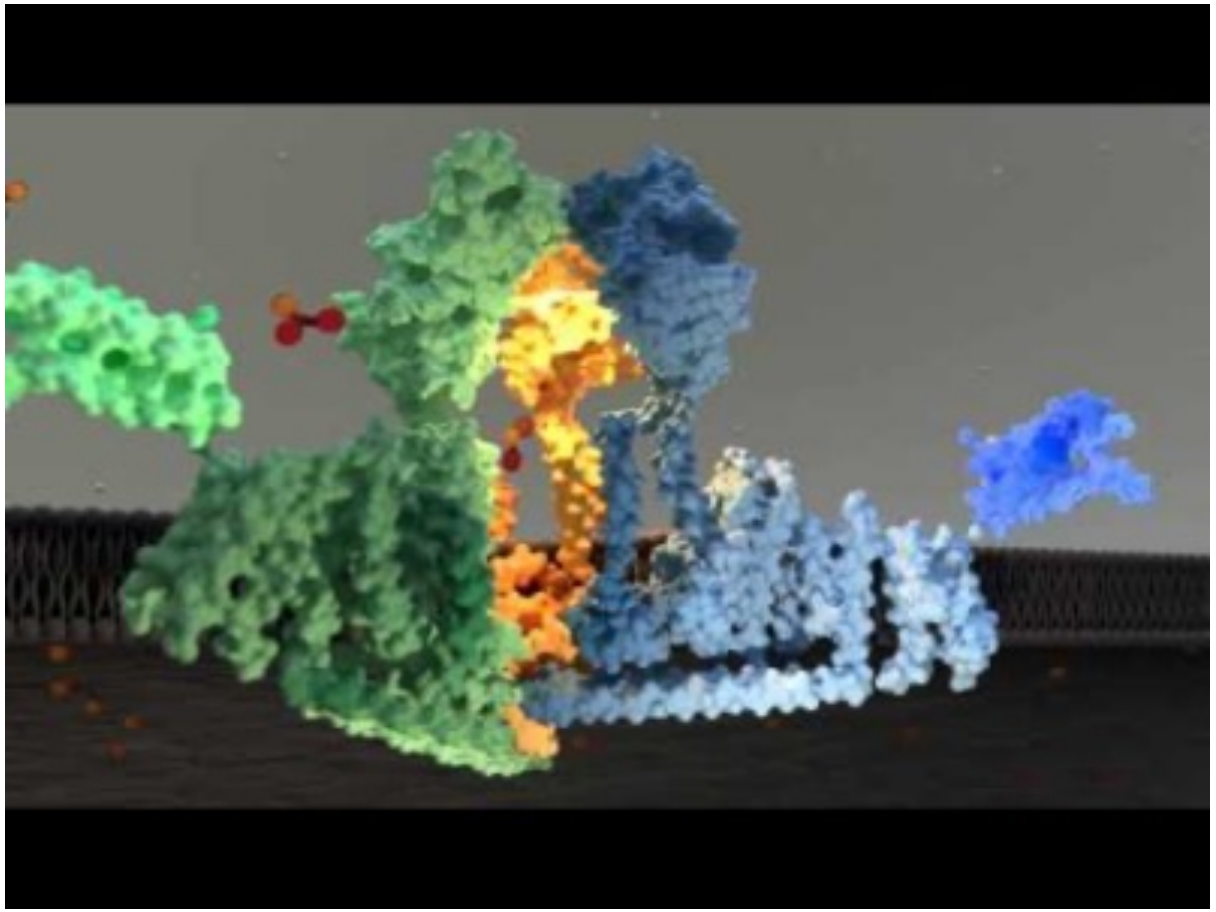




Plasma membrane

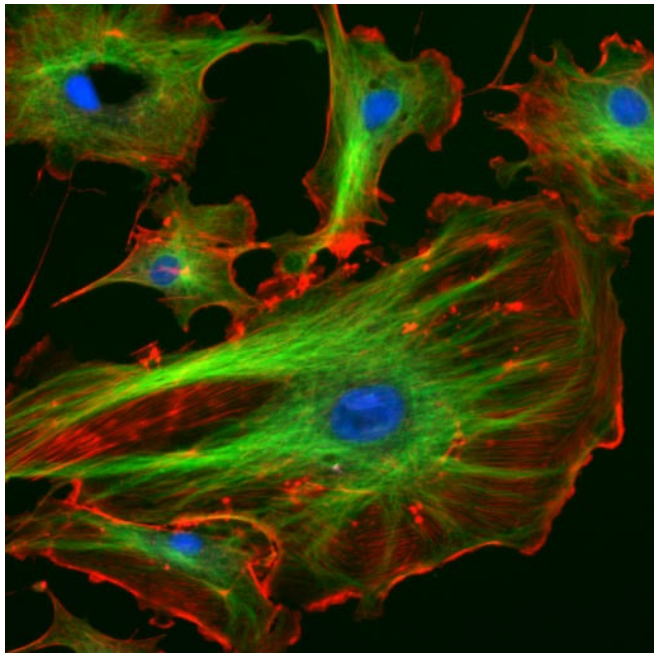


Ion channels

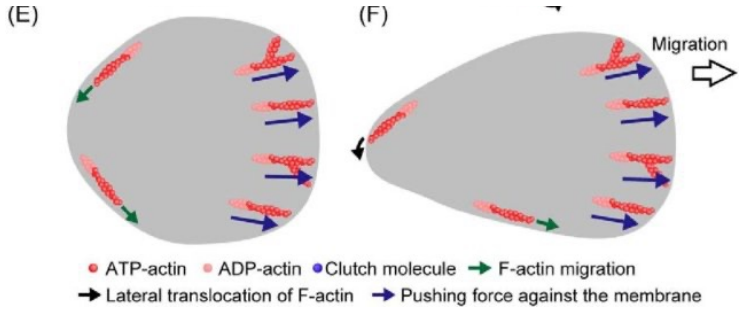


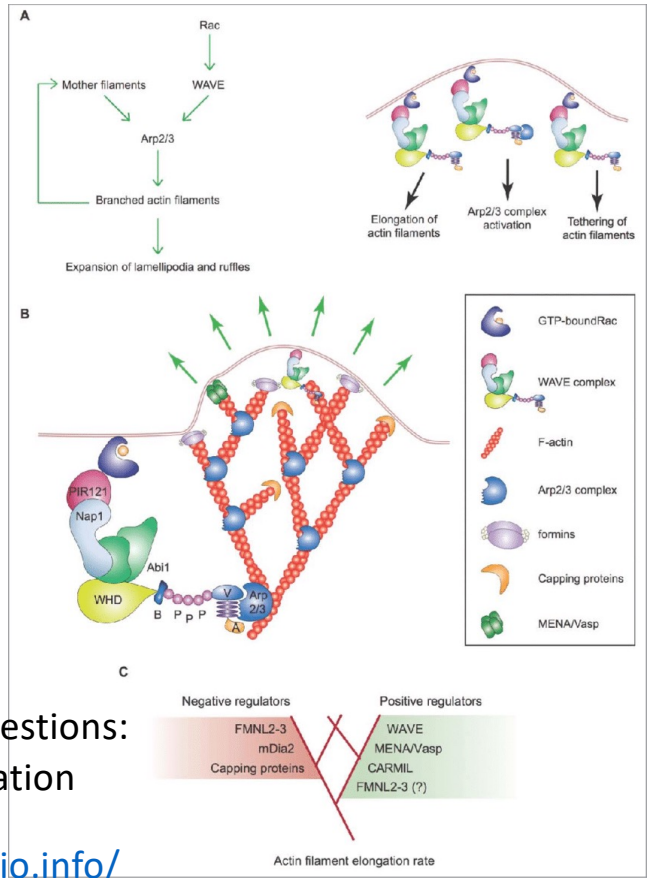
Cytosceleton

- actin filaments (7 nm Ø)
- microtubules (25 nm Ø)
- intermediate filaments (10 Ø)



The eukaryotic cytoskeleton. Actin filaments are shown in red, and microtubules composed of beta tubulin are in green.





G-actin monomer

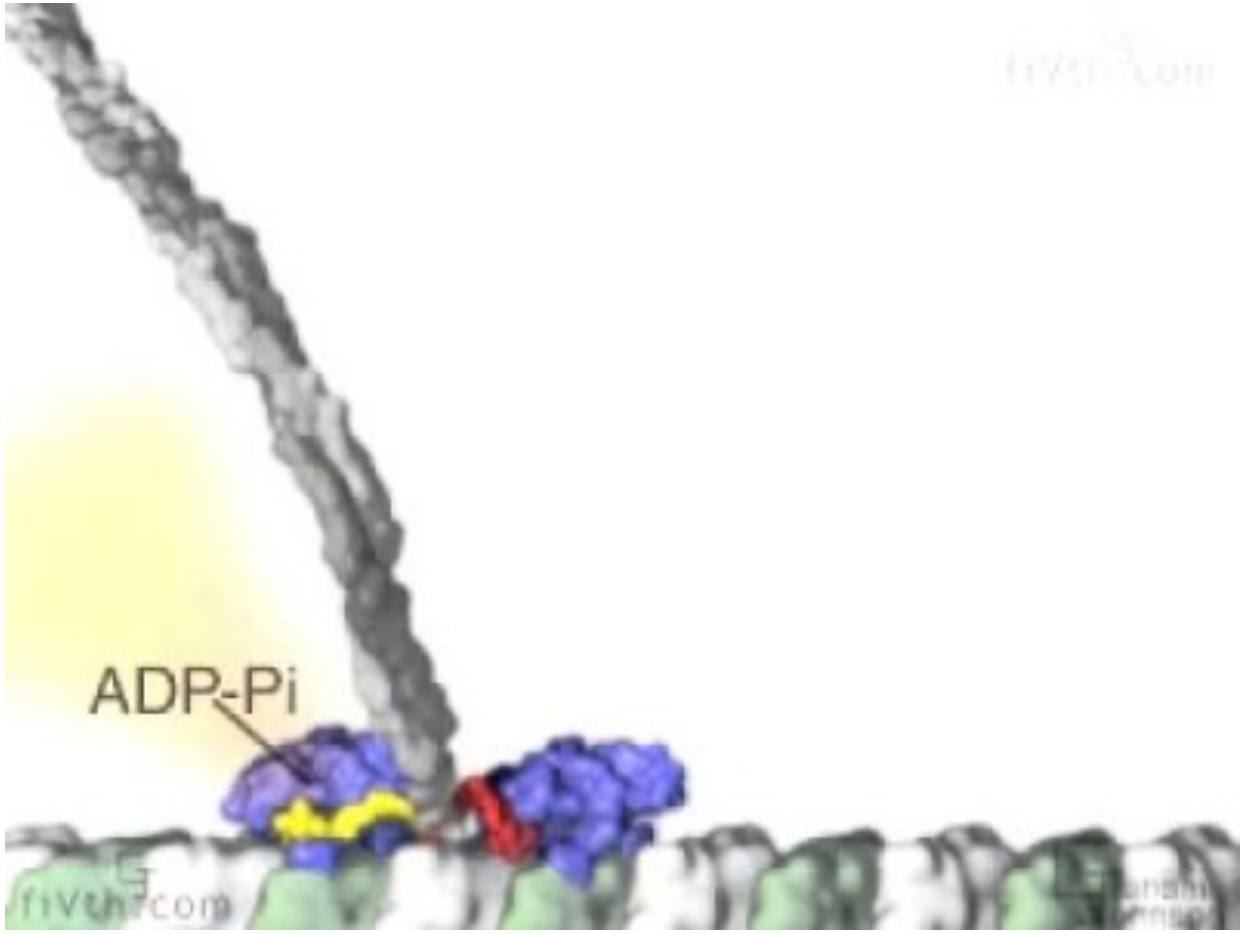
F-actin polymer

physics questions:

- polarization
- force

https://www.mechanobio.info/

Microtubules & kinesin motors



Crash course in greek and latin:

Angio-		Vessel
-atomy, -otomy		cutting
Auto-		self
Brachy		short
Cata- (katalysis)		dissolving
Carcino-		tumor (crab-like)
Centro-, -centric		centre
-ceptor, ceptive		capere, to take
Chromo-		color
Chrono-		time
-cyte, cyto-	hollow	
Diplo		double
e-, ec-		out of
Endo-		within, inside
Exo-		outside
Extra-		beyond
Erythro-		red
-gen, genous	descent	
-genic, -genous		birth, descent, origin
-genic, -genous		to produce

Angiogenesis =production of vessels

Carcinogenesis = Production (development)of cancer

Crash course in greek and latin:

Glia-	glue
Haem-	blood
Histo-	tissue
Homeo-	alike
Homo-	the same
Hyper-	above
Hypo-	under
Infero-	beneath
Infra-	below
Inter-	between
Intra-	within
Iso-	equal
-kinesis, -kinetic	kinesis=movement
Leuko-	white
Lipo-	fat
-lysis, -lysin	dissolving
Macro-	large
Medi-	middle

Crash course in greek and latin:

-mere, mero-	a part		
Meta-		after	
Metabolism	change		Centromere= middle part
Micro-		small	
Mito- (mitosis)		a tread	
Mono-		single	
Muta-		mutare=to change	telomere= end part
Necro-		dead	
Neuro-		nerve	
-nomics		law	
Oligo-		few	
Onco-		bulk, mass	
Ortho-		straight	
Para-		beside	
Per-		through	
Peri-		around	
-phage, -phagous		phagein=to eat	
-phil		to love	

Crash course in greek and latin:

-phobe	to fear
Photo-	light
Plasma-, -plasm	form
-plicate	to fold
Post-	after
Pre-	before
Pro-	before
Proto-	first
Re-	back
Retro-	backwards
Serum	whey (myse)
-some, soma-	body
Stereo-, -steric	solid
Sub-	under
Super-	over
Supra-	above
Sym-, syn-	with

Crash course in greek and latin:

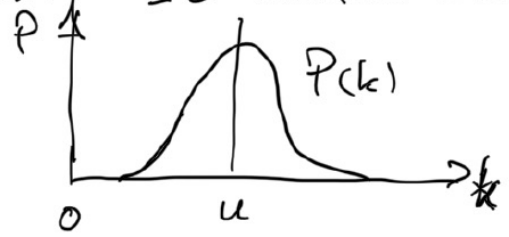
-synthesis	composition
Tauto-	the same
Tele-	far
Teleo-	complete
Telo-, telio-	end
Trans-	across
Ultra-	beyond

Diffusion and friction in fluids

- Did you do 2D RW?
- Demo 1drw?

Diffusion ~ random motion
- independent of details

4.1.3 1D random walk



steplengths kL

$u = \langle k_j \rangle = \sum_k k P_k$ - drift

$\langle x_N \rangle = N u L$

$\sigma_N^2 = \langle (x_N - \langle x_N \rangle)^2 \rangle = 2Dt$

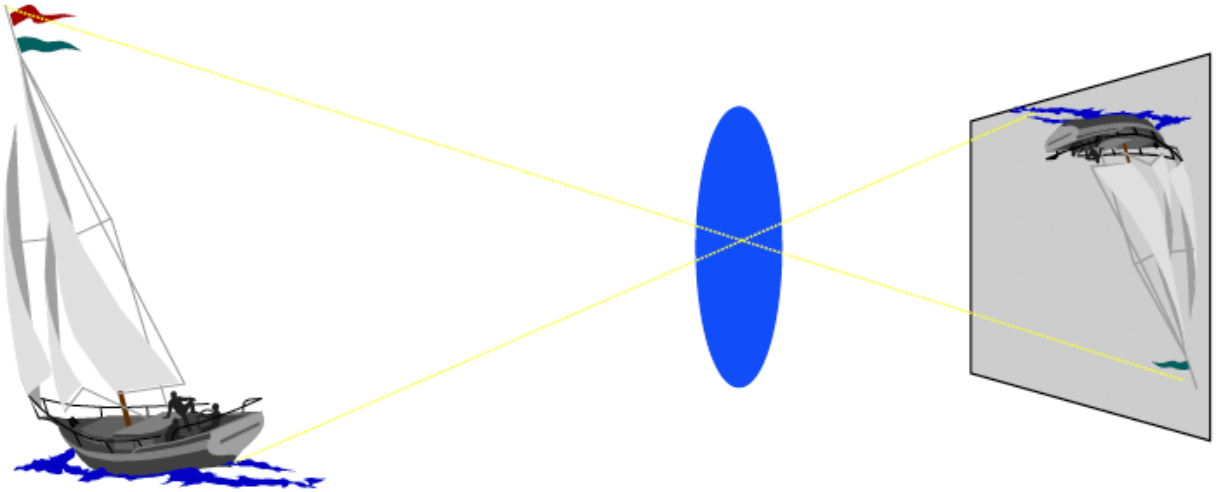
Life at low Reynolds number

- For next Tuesday: You read Purcell's paper
- Tuesday:
  - You present the paper
  - We discuss life at low Reynolds number
  - Discuss Your turn 1A, Prob 1.3, 2.2, 2.5

Image analysis Outline

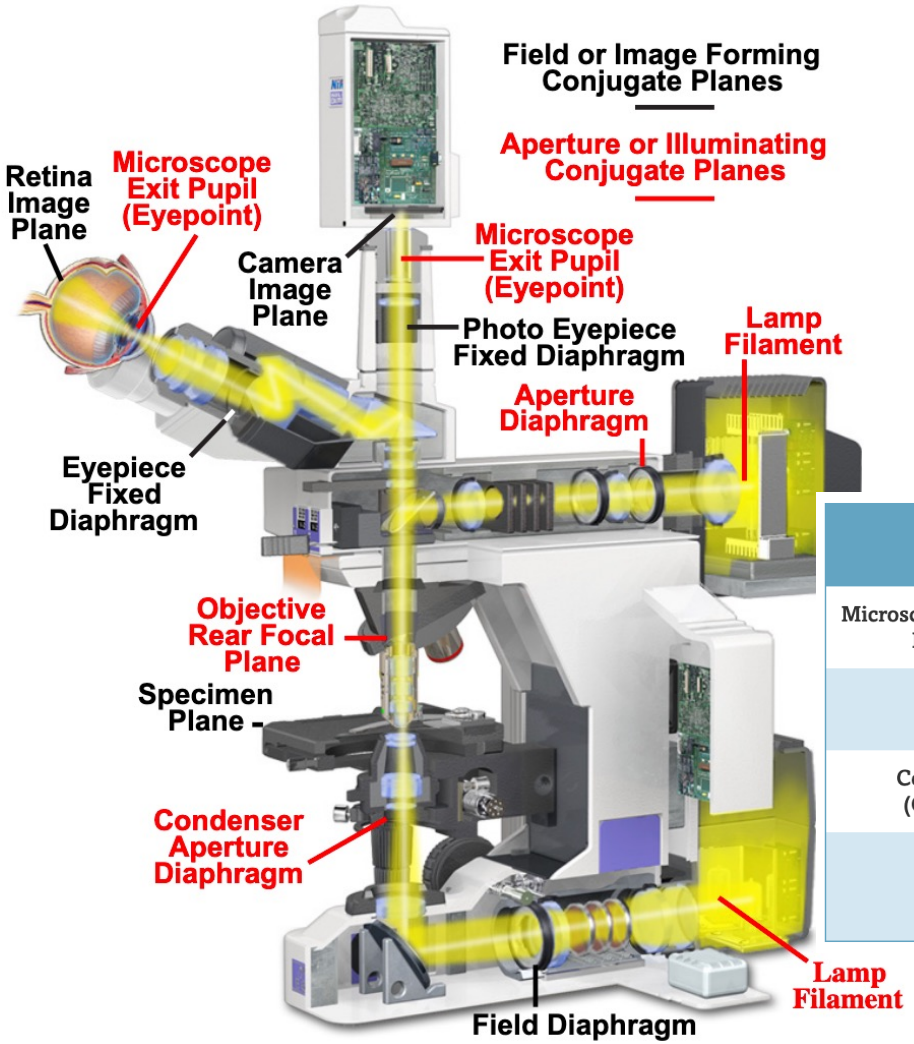
- Microscope imaging and cameras (quick repeat)
- What is a digital image? (quick repeat)
- Image types and resolution (quick repeat)
- Why do we need image analysis?
- How to do image analysis (basic steps)?
- Morphological operators
- Watershed algorithm
- Examples

Imaging principles



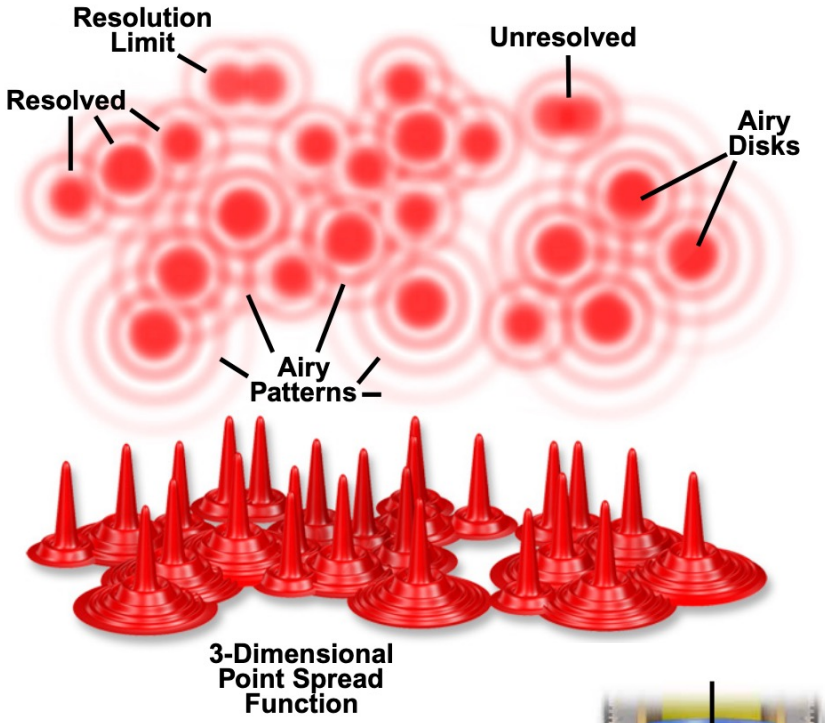
Mi

Figure 1 - *Conjugate Planes in the Optical Microscope*



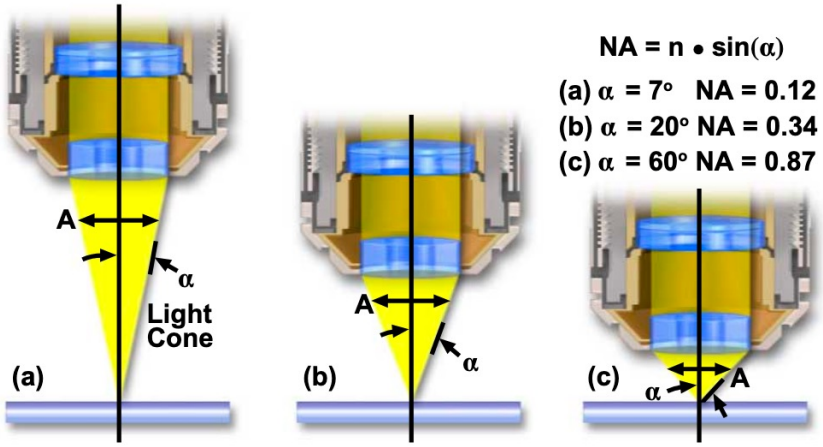
Aperture or Illuminating Conjugate Plane Set	Field or Image-Forming Conjugate Plane Set
Microscope Exit Pupil: Eye Iris Diaphragm, Ramsden Disc, and Eyepoint	Retina of the Eye Camera Image Plane
Objective Rear Focal Plane (Objective Rear Aperture)	Intermediate Image Plane (Eyepiece Fixed Diaphragm)
Condenser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

Figure 1 - Airy Patterns and the Limit of Resolution

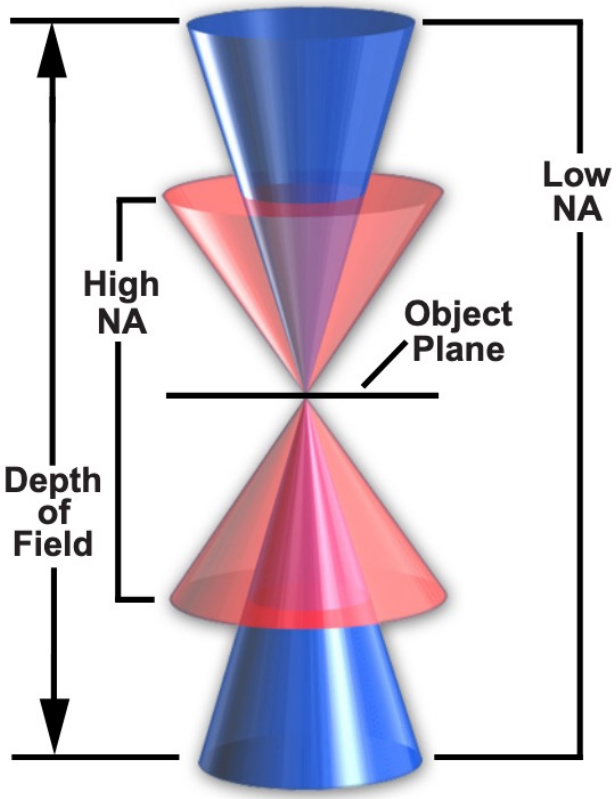


$$r = 0.61\frac{\lambda}{NA}$$ reflected light

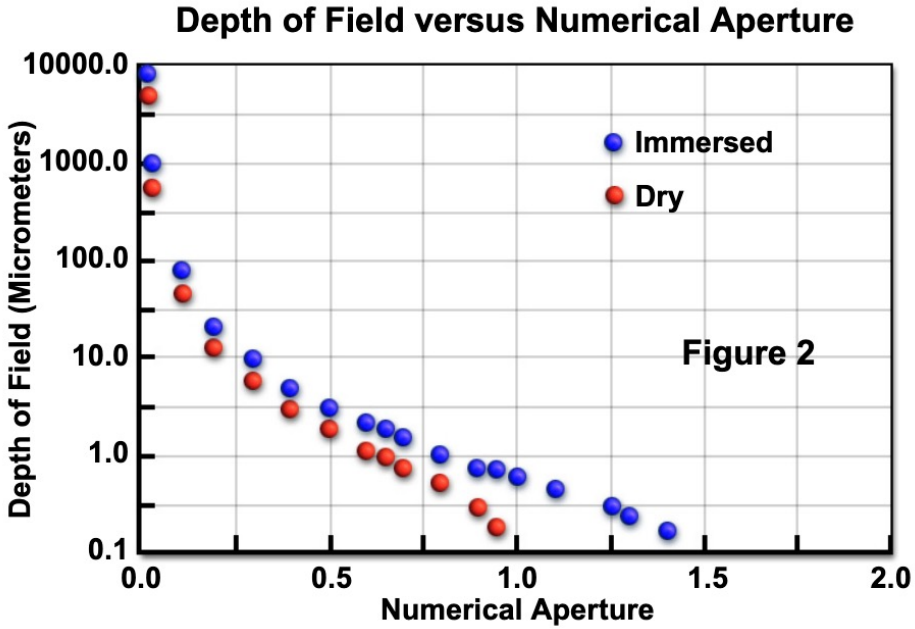
$$r = 1.22\frac{\lambda}{NA_o + NA_c}$$ transmitted light



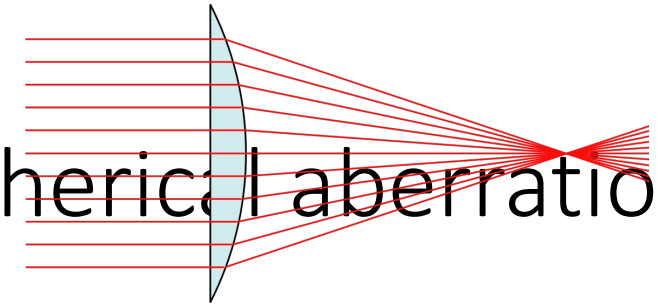
Depth of field/focus

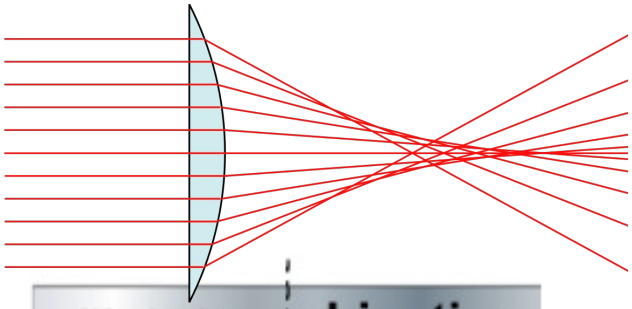


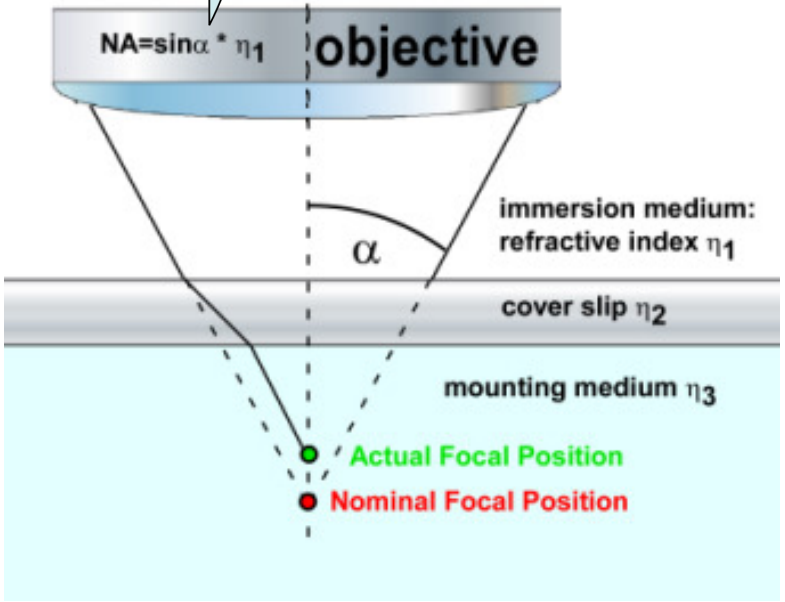
depth of field = the distance from nearest object plane in focus to farthest plane also simultaneously in focus.

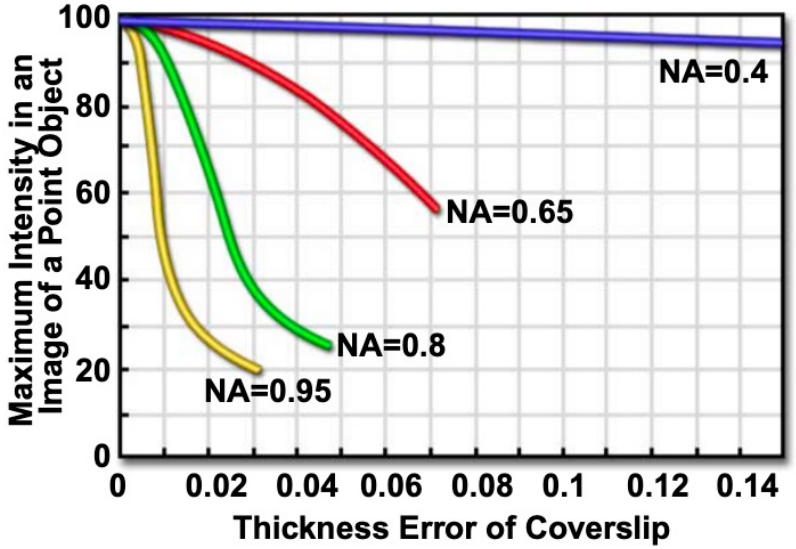


Spherical aberration & coverslip correction

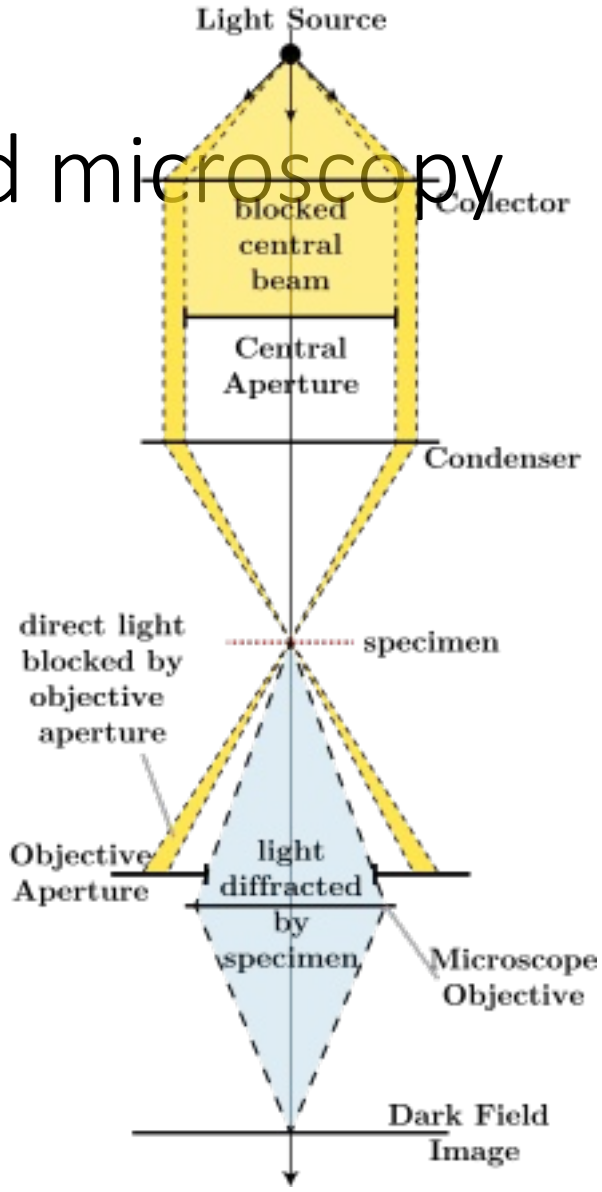


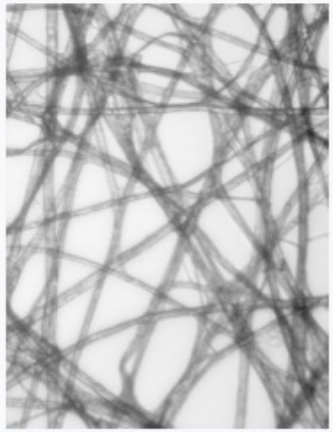


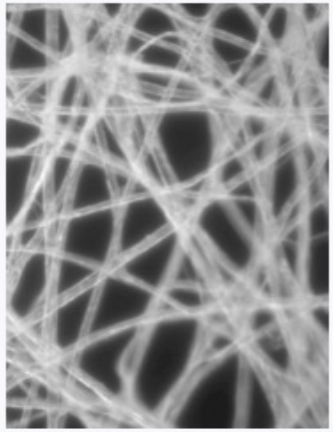




Dark field microscopy







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.

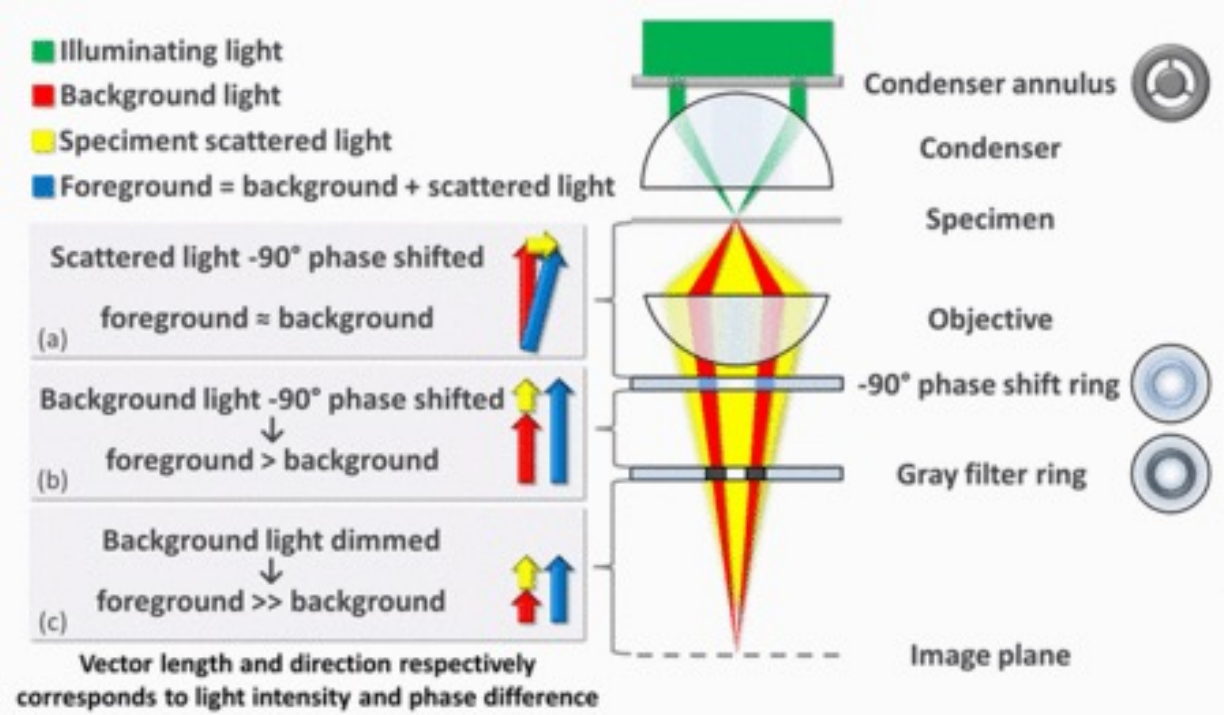
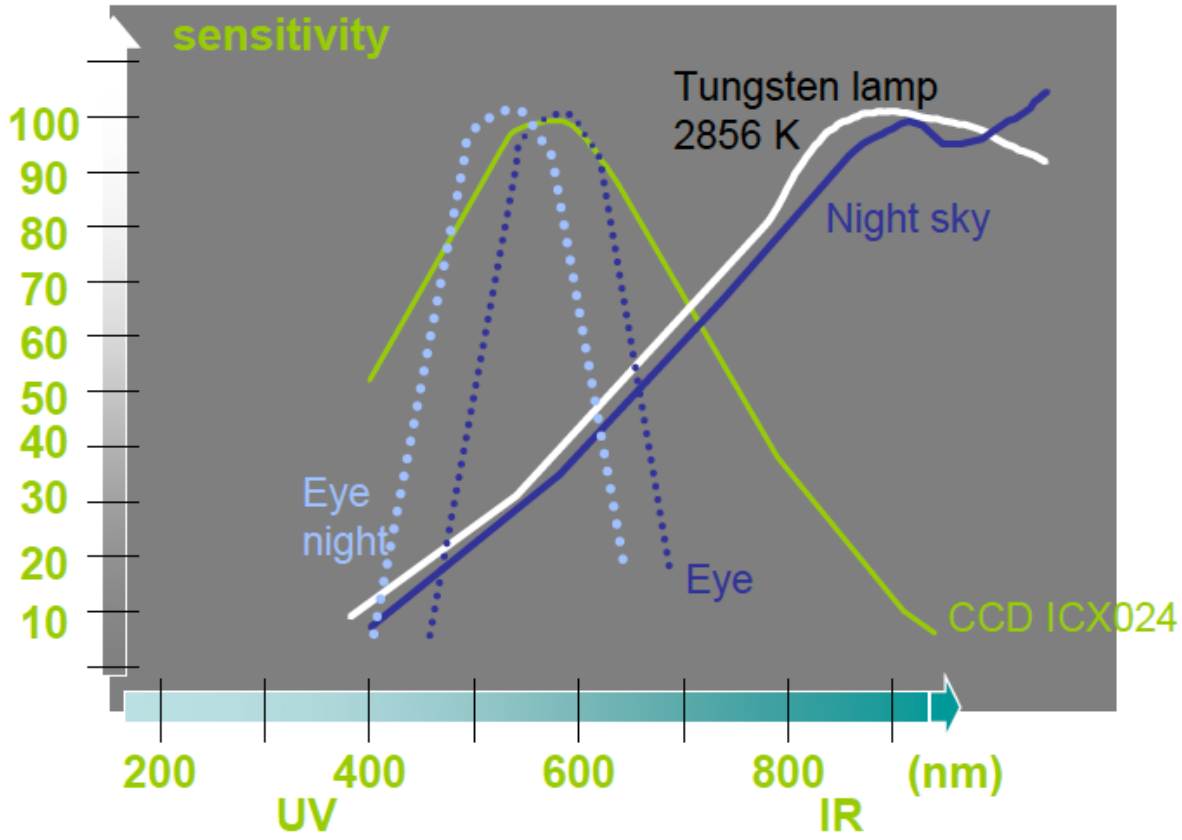


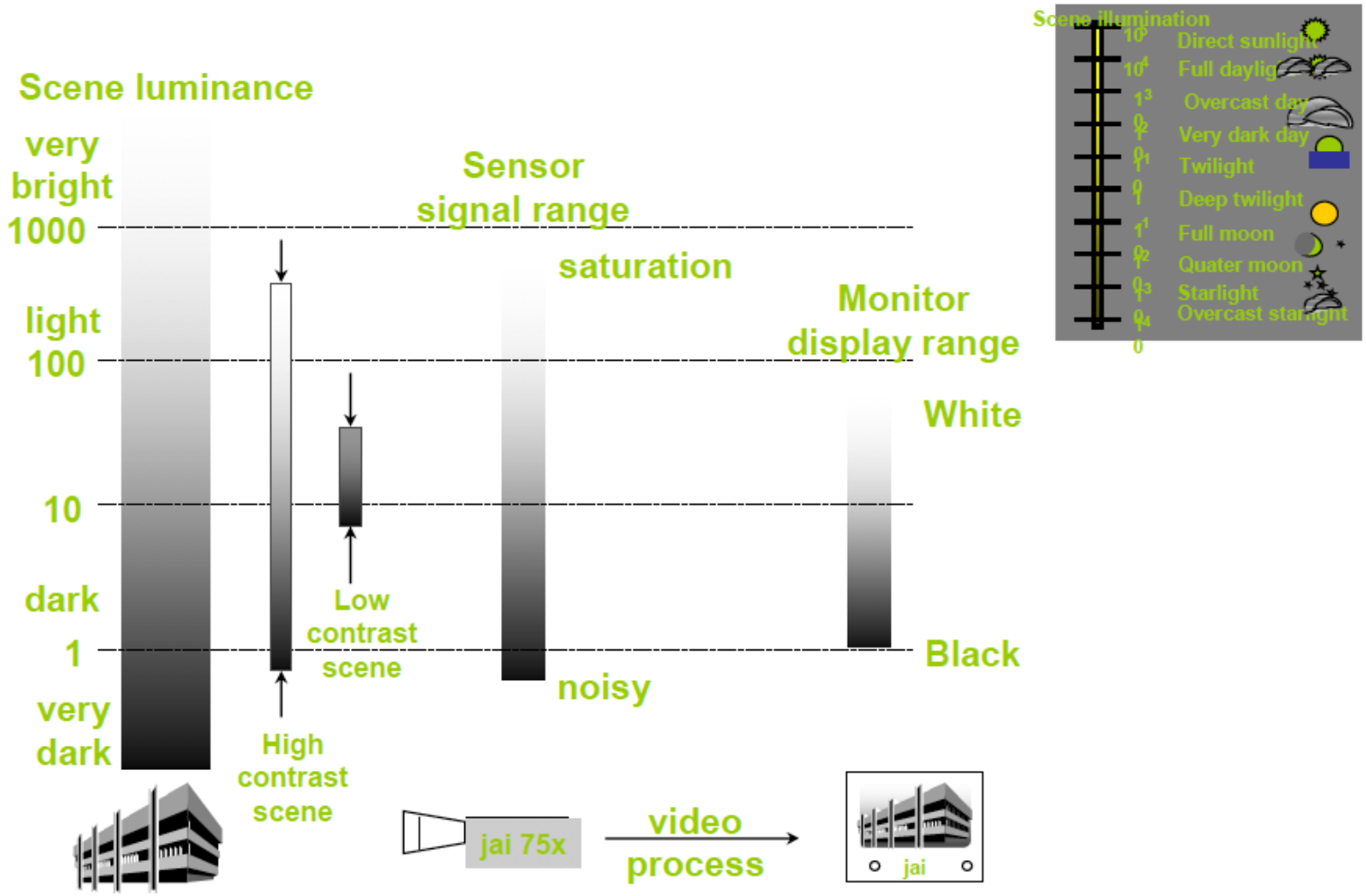
image contrast is increased in two ways:

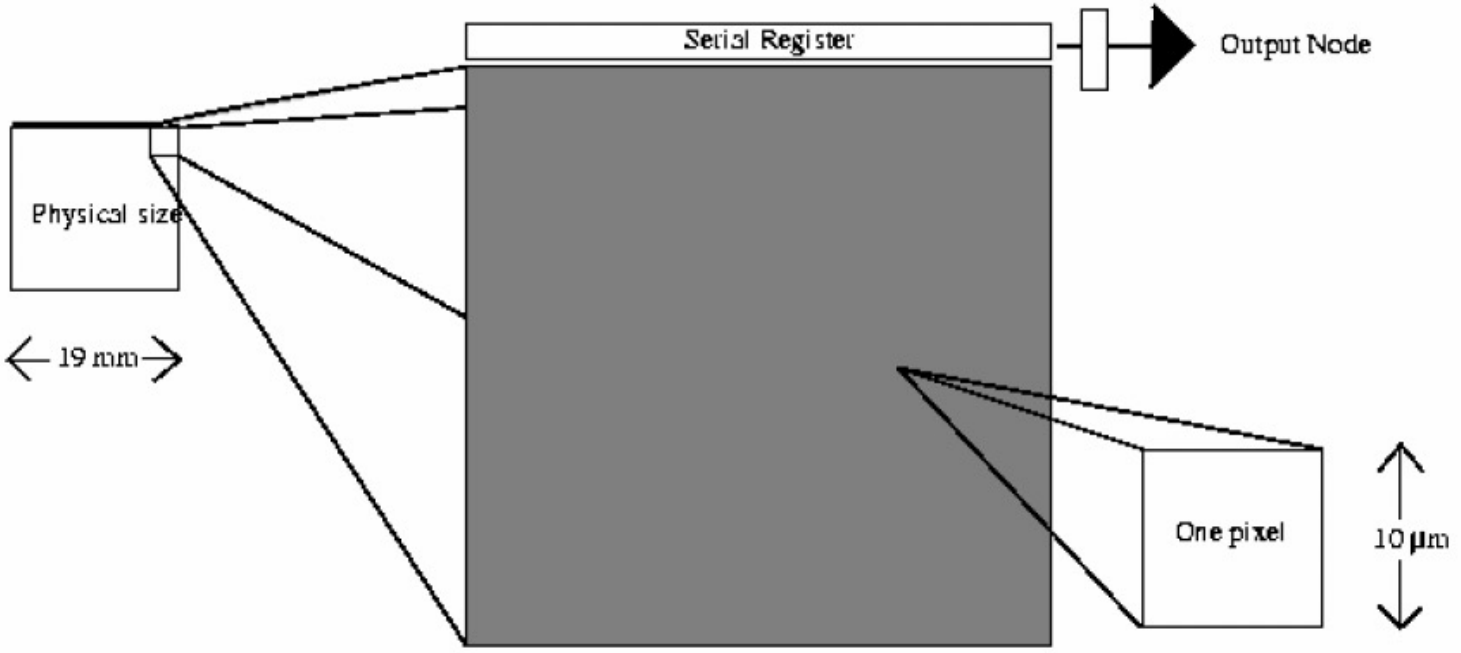
- by generating constructive interference between scattered and background light
- by reducing the amount of background light that reaches the image plane

Imaging principles

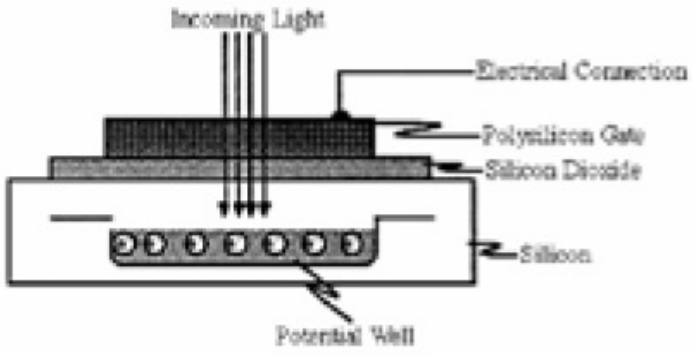


Luminance and contrast




Serial Register
Output Node
Physical size
19 mm
One pixel
10 µm

CCD


Incoming Light
Electrical Connection
Polysilicon Gate
Silicon Dioxide
Silicon
Potential Well

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

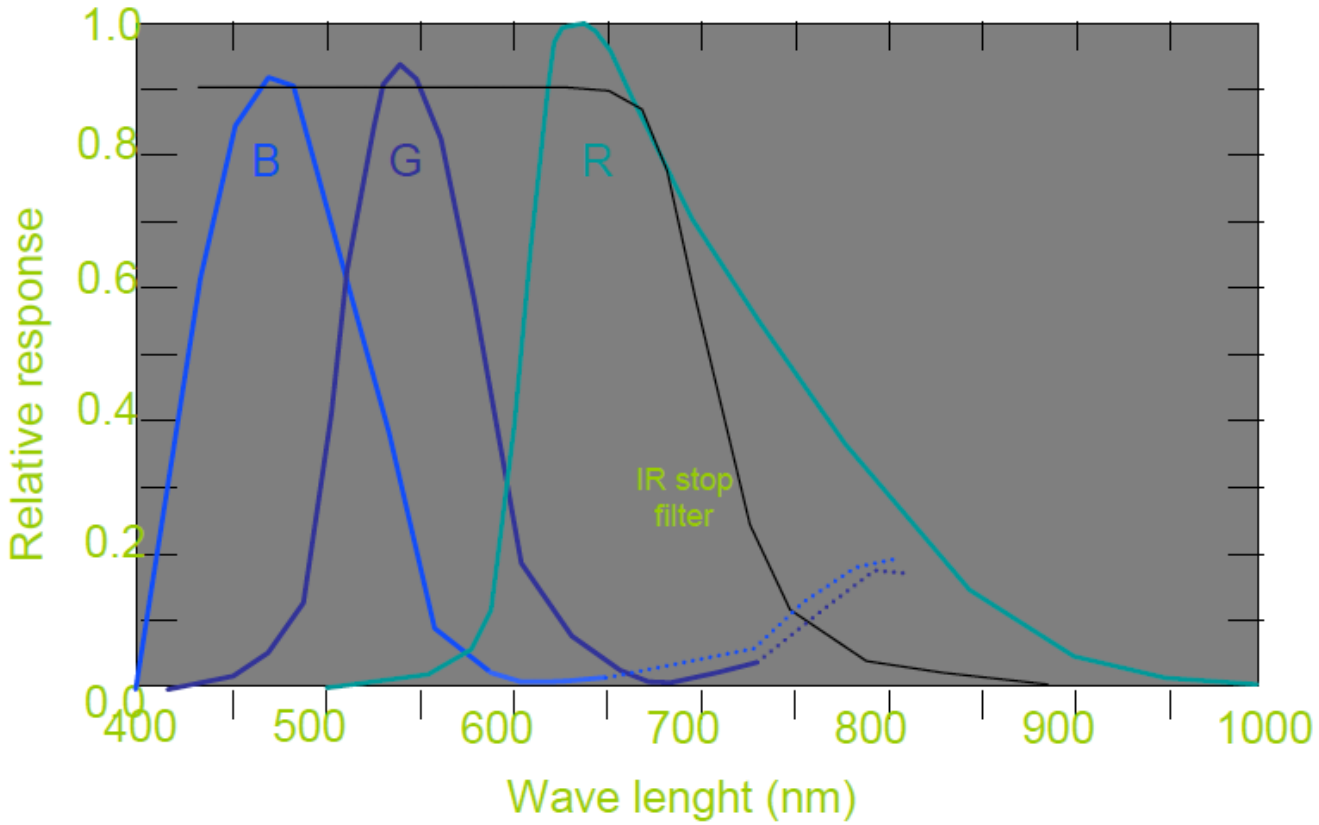
MORE SENSITIVE THAN THE EYE

Drawbacks

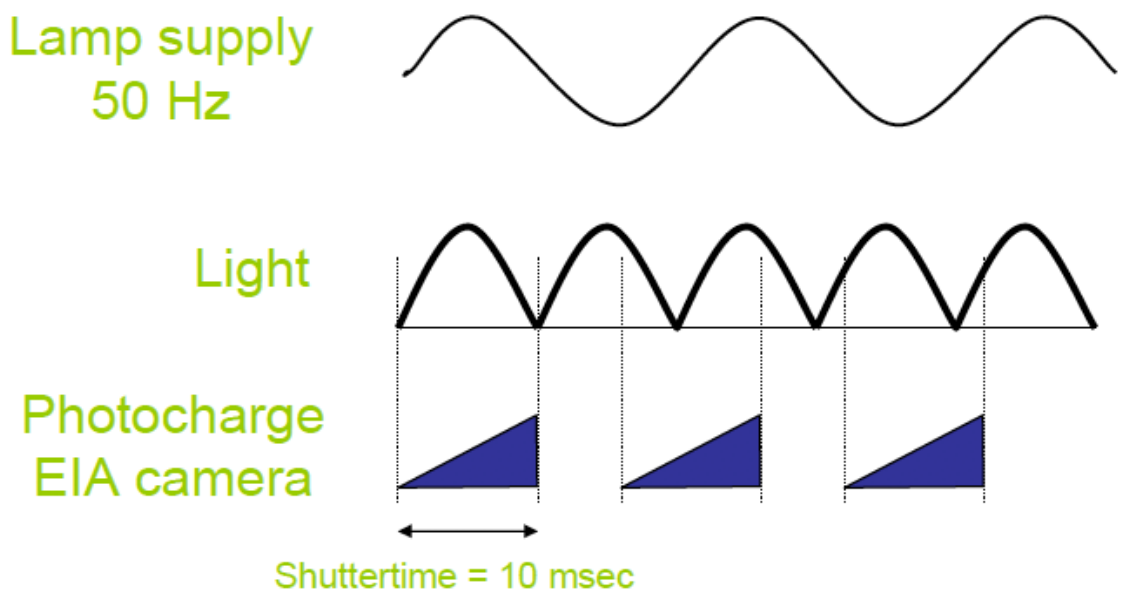
- Slow data transfer
- Produces much data
- Requires custom made software
- Not user friendly
- Expensive

16 bit: cooled sensor

Color


1.0
0.8
0.6
0.4
0.2
0.0
Relative response
B
G
R
IR stop
filter
400
500
600
700
800
900
1000
Wave lenght (nm)

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?

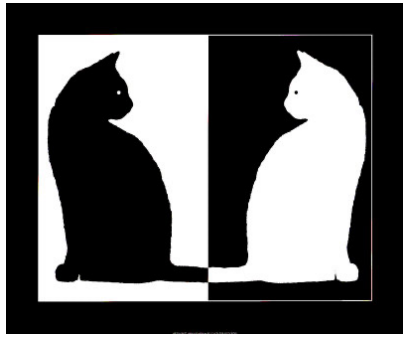
$$f = f(x, y)$$

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



Color image

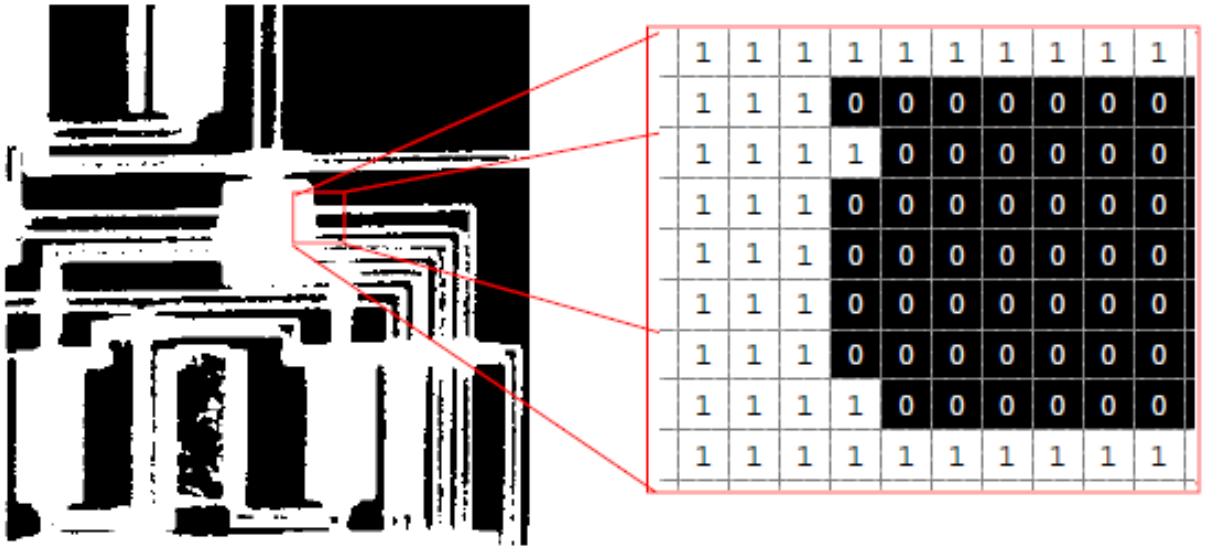
[RGB]
Red Green Blue

M x N x 3

Matlab image processing toolbox:

```
im = imread('landscape.jpg');
figure(1),imshow(im)
whos im
imfinfo('landscape.jpg')
A = im(1000:1010,1000:1010,:);
```

Binary ir




```
im_bw = imread('black_and_white_cats-1541.jpg');
im_bw = rgb2gray(im_bw);
im_bw = im2bw(im_bw);
imwrite(im_bw,'bw_cats.png');
figure,imshow(im_bw)
whos im_bw
unique(im_bw)
```

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

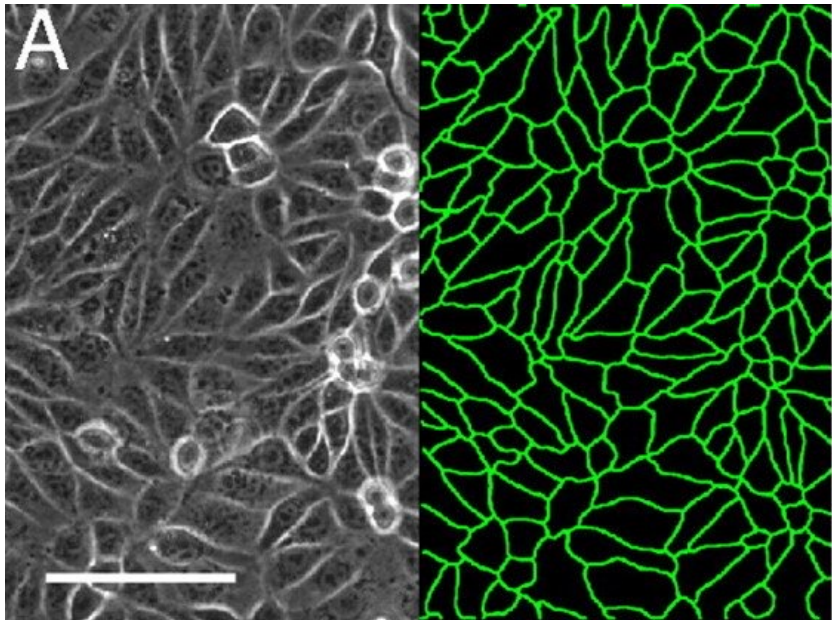
24 bit depth (256 shades of RGB) – true color

Why do we need image analysis?

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

Segmentation

procedure



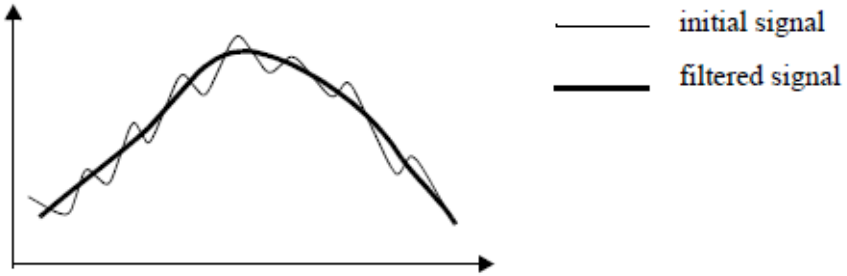
Basic steps of image analysis

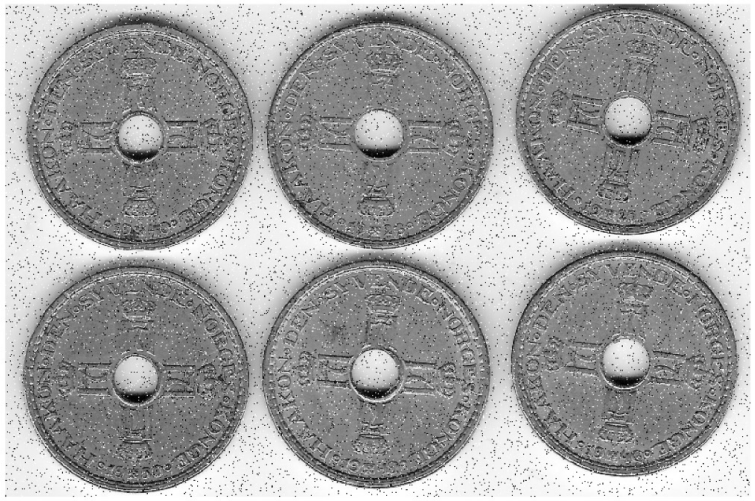
Image segmentation quick steps:

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

Noise removal

- Filtering – smoothing
- Background correction





Convert to black and white

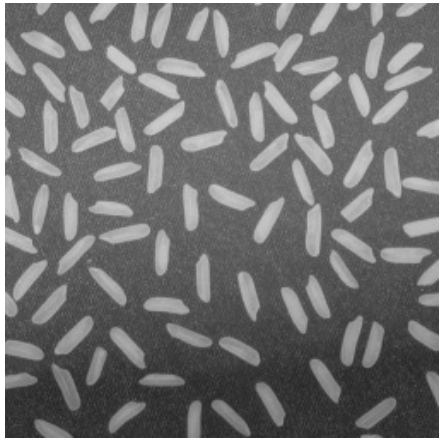
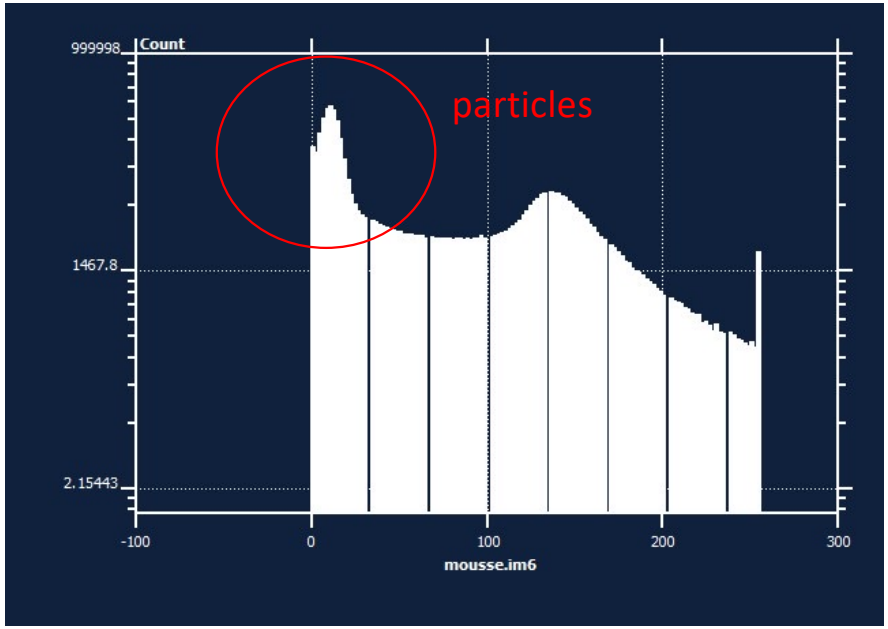
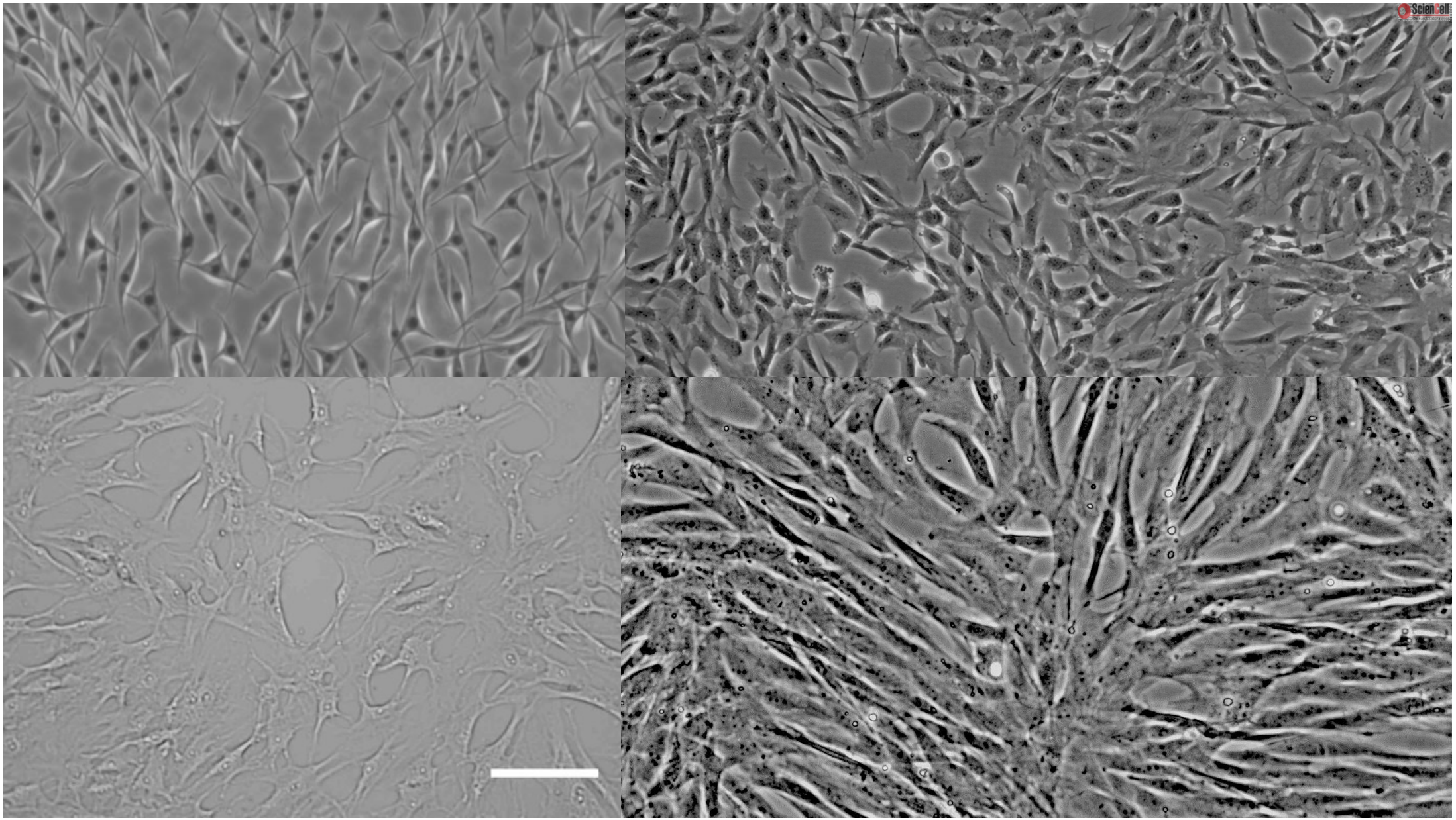
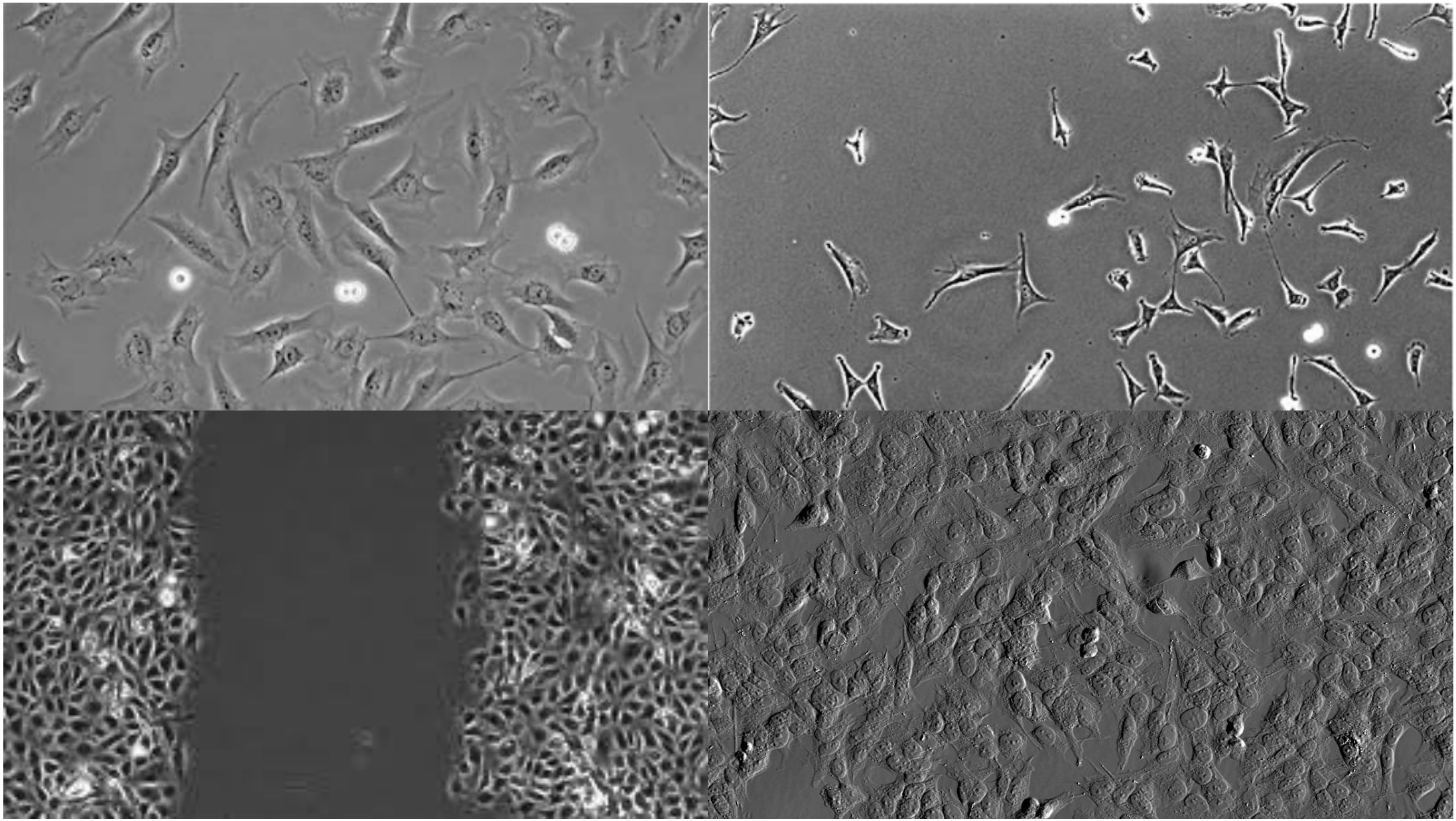


Image histogram



Thresholding intensity interval (a,b)

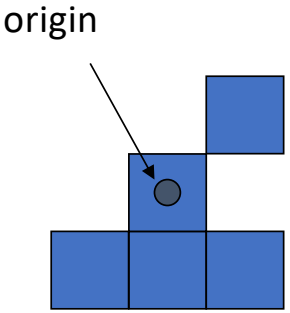


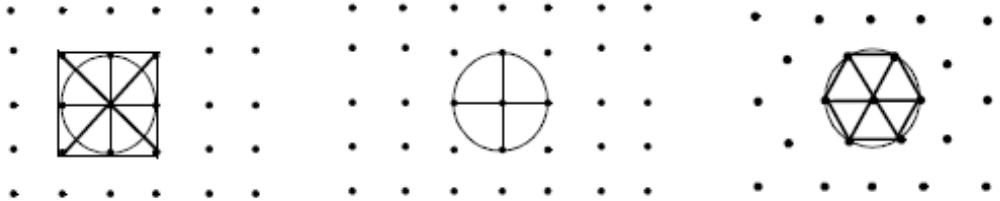


Morphological operators

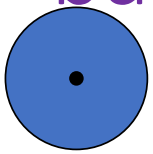
Morphological transformation are based on a structural element

- size
- shape
- center location



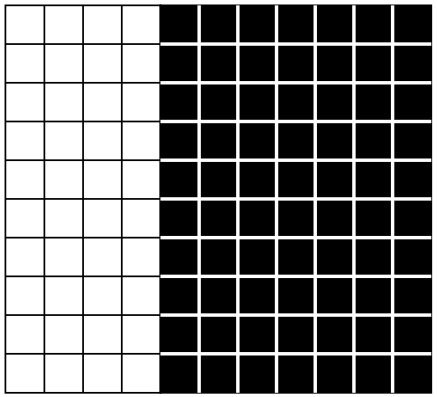


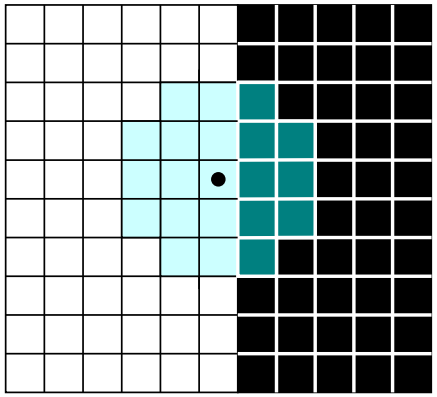
Erosion and dilation – basic operations



Erosion

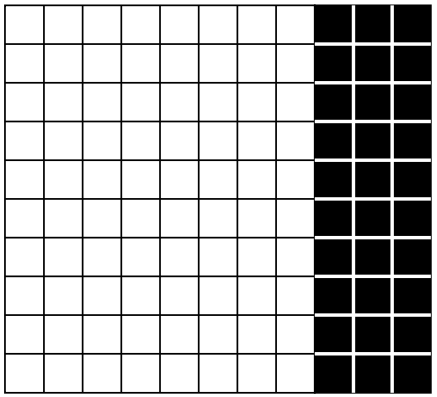
"Set the value at the origin to the minimum value of pixels in the structural element"





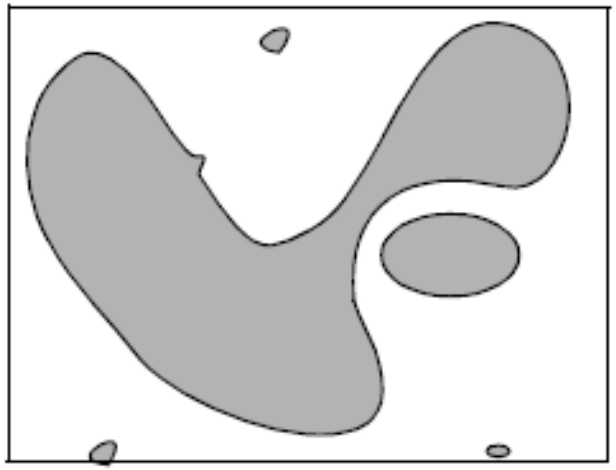
Dilation

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

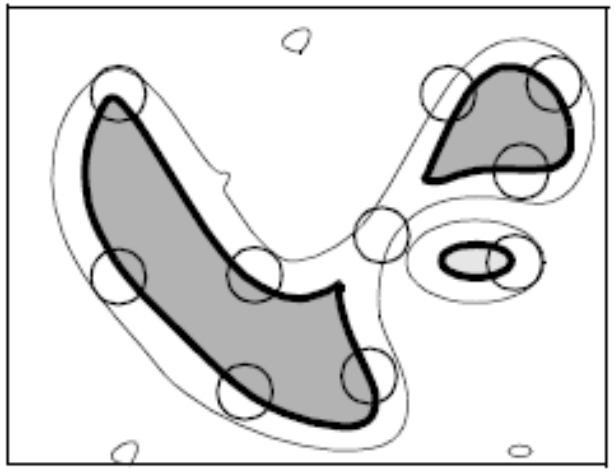


Erosion

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



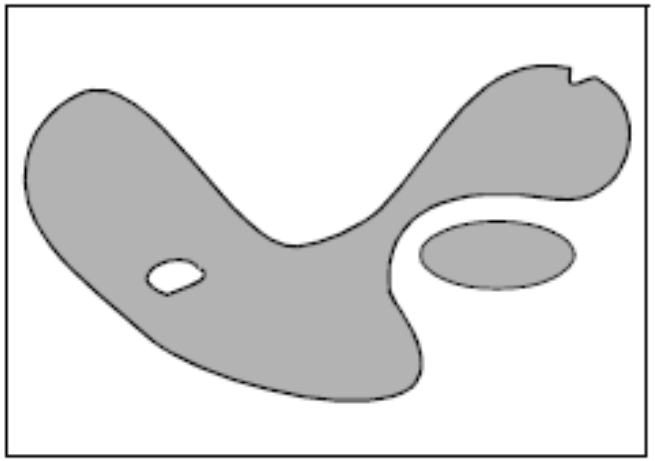
input image



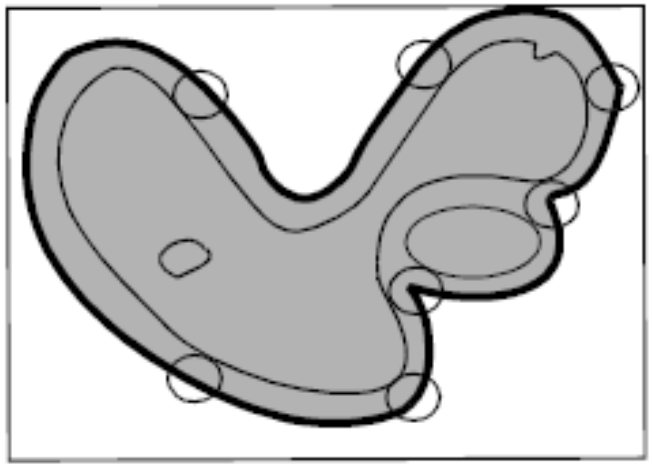
eroded image

Dilation

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



input image



dilated image

Opening and closing

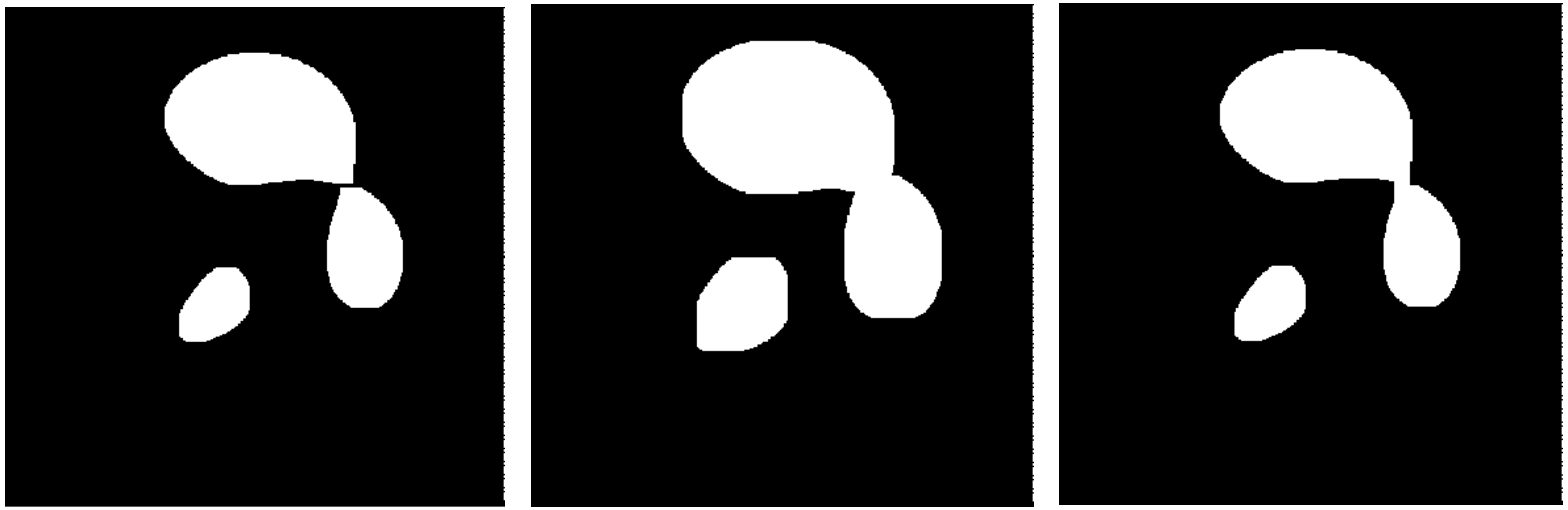
Opening = Erosion + Dilation

Closing = Dilation + Erosion

Original image → Erosion → Dilation

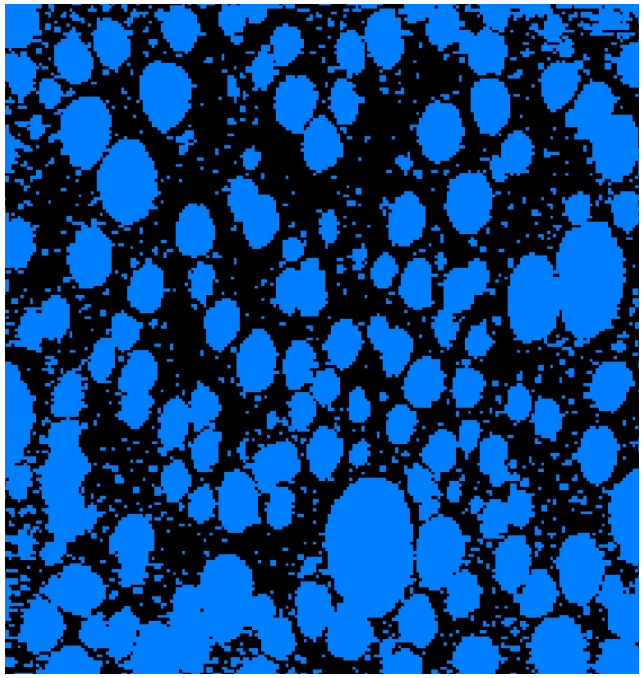
Closing

Original image → Dilation → Erosion

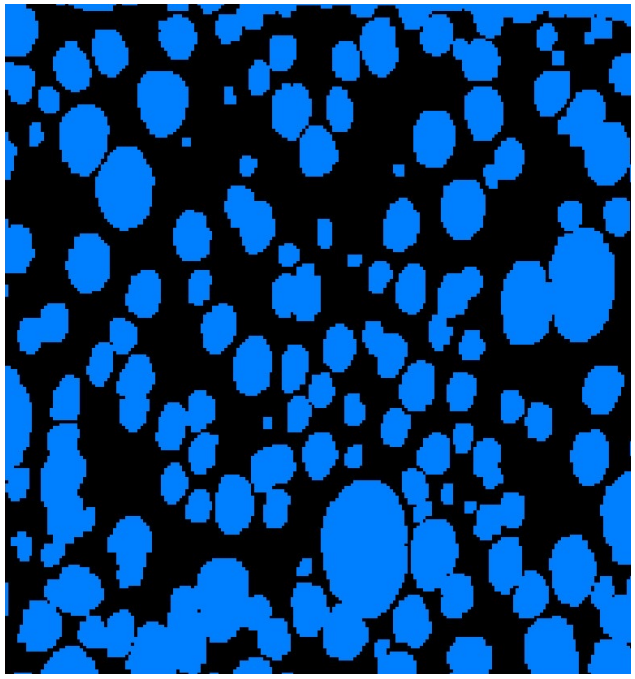


Noise removal

Original image

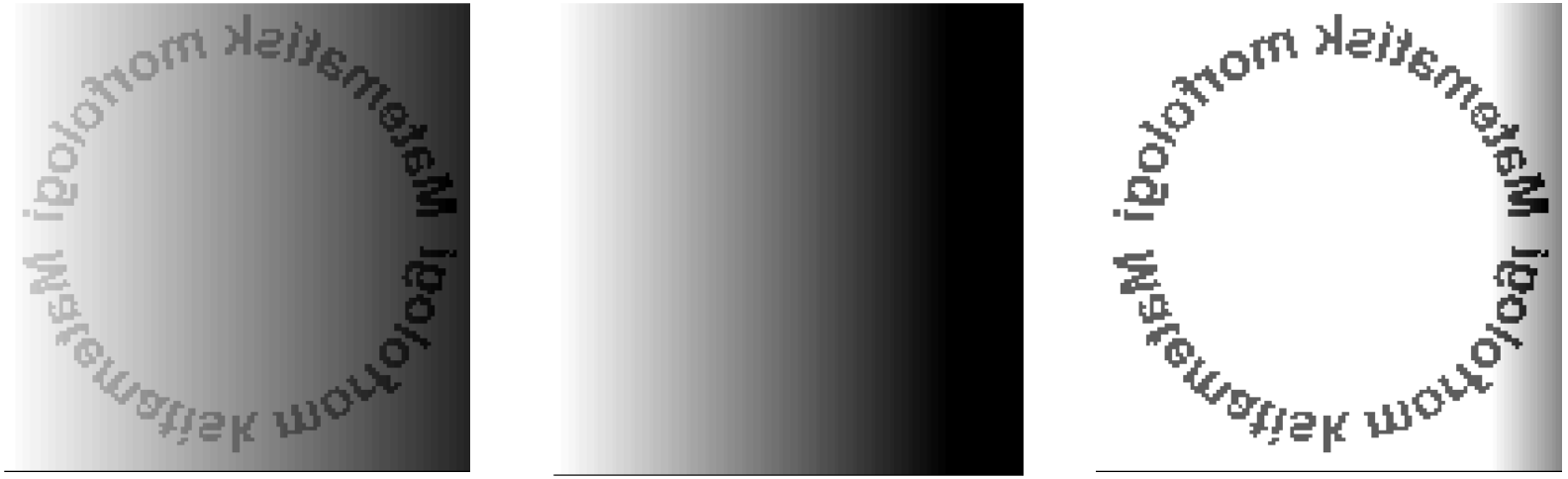


After opening

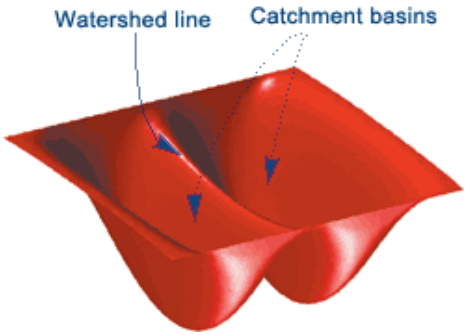


Background correction

Original image → Opening → IM ÷Opening



Wat



Flooding of image topography

Water rise from a set of markers

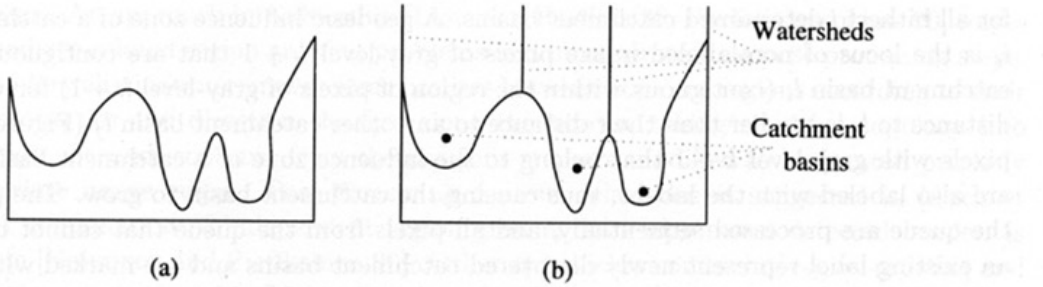
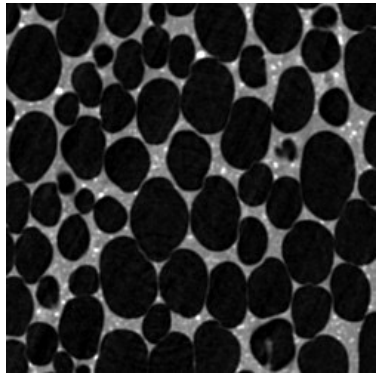


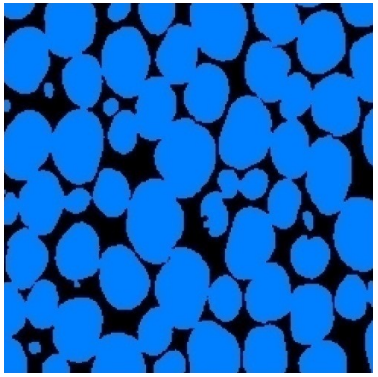
Figure 5.49: *One-dimensional example of watershed segmentation: (a) gray-level profile of image data; (b) watershed segmentation—local minima of gray-level (altitude) yield catchment basins, local maxima define the watershed lines.*

Example of workflow using watershed

Gray level image

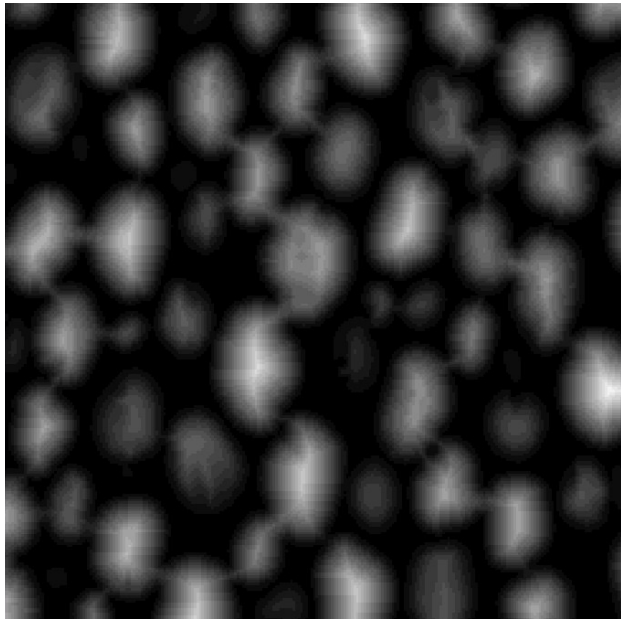


Binary image



Reconstruction of individual pores in foam

Distance map

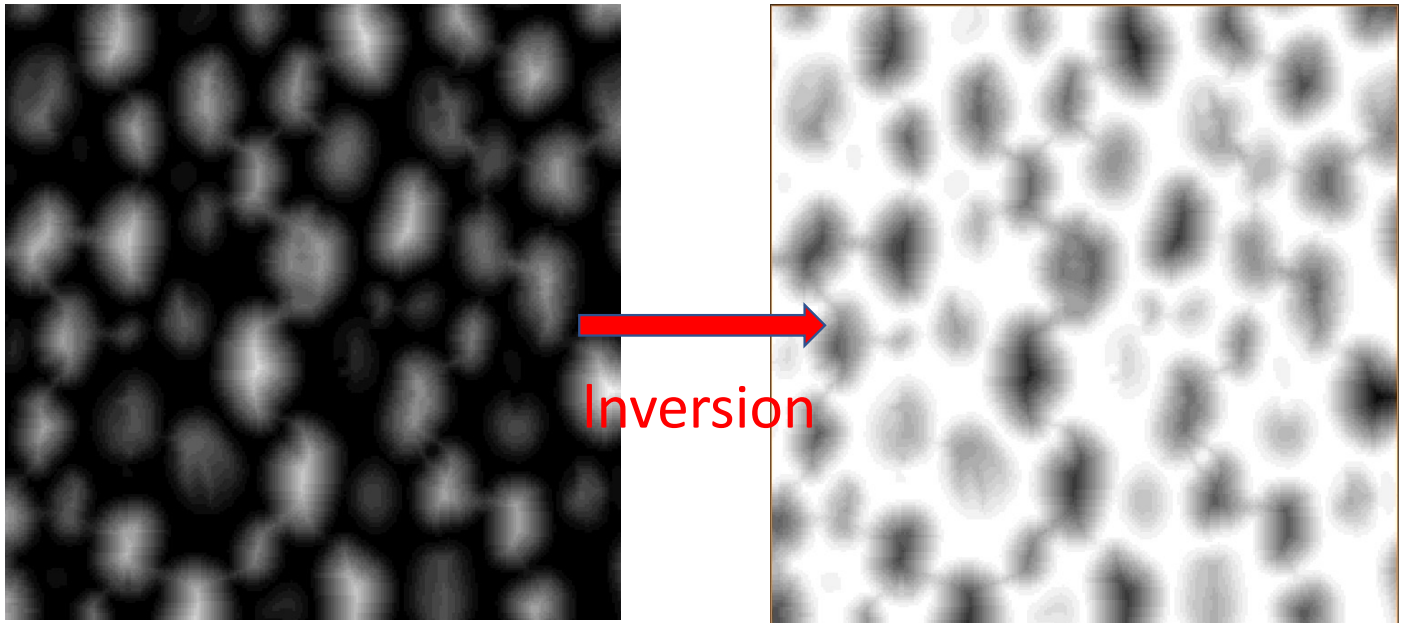


Example of workflow using watershed

Valleys for watershed

Distance map

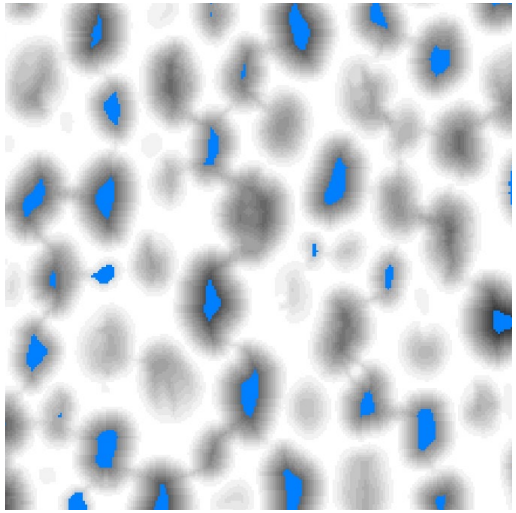
Inversed distance map



Example of workflow using watershed

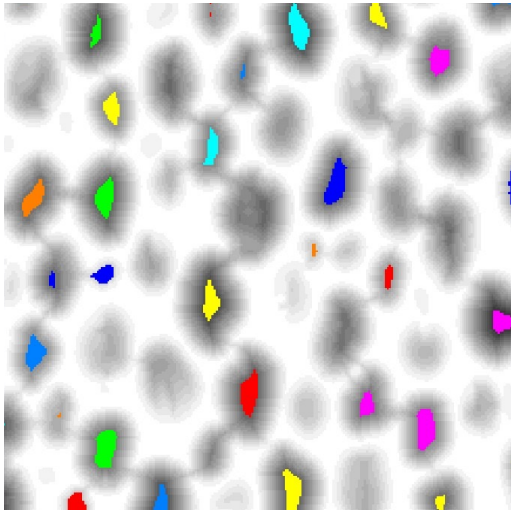
Create markers

Maxima on distance map



Labeling

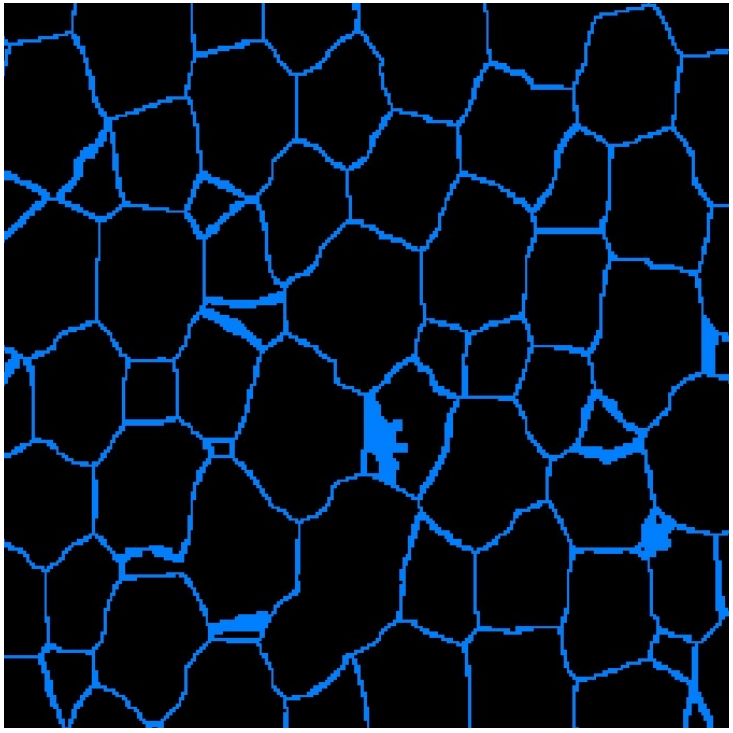
Markers

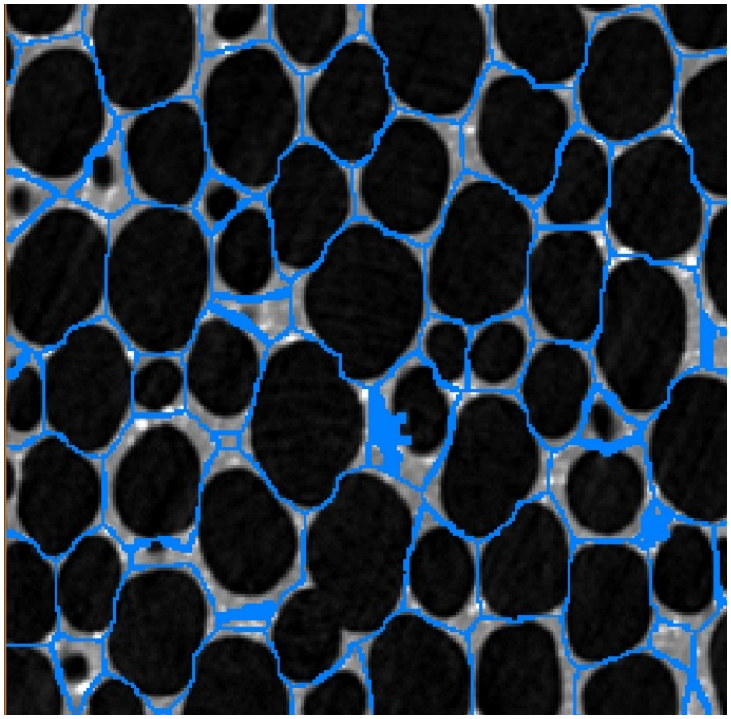


Example of workflow using watershed

Watershed lines –

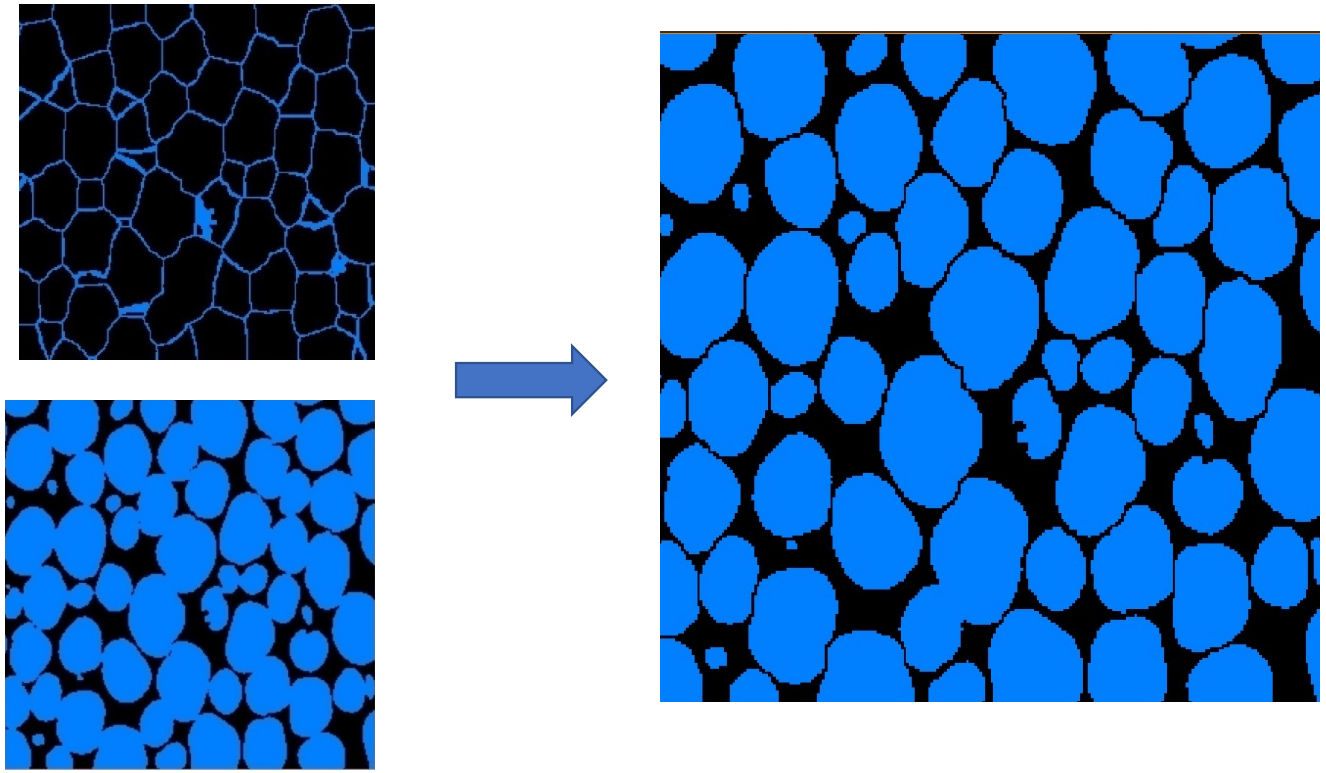
boundaries between regions





Example of workflow using watershed

Subtraction of watershed lines gives separated pores



Example of workflow using watershed

Labeling of connected components