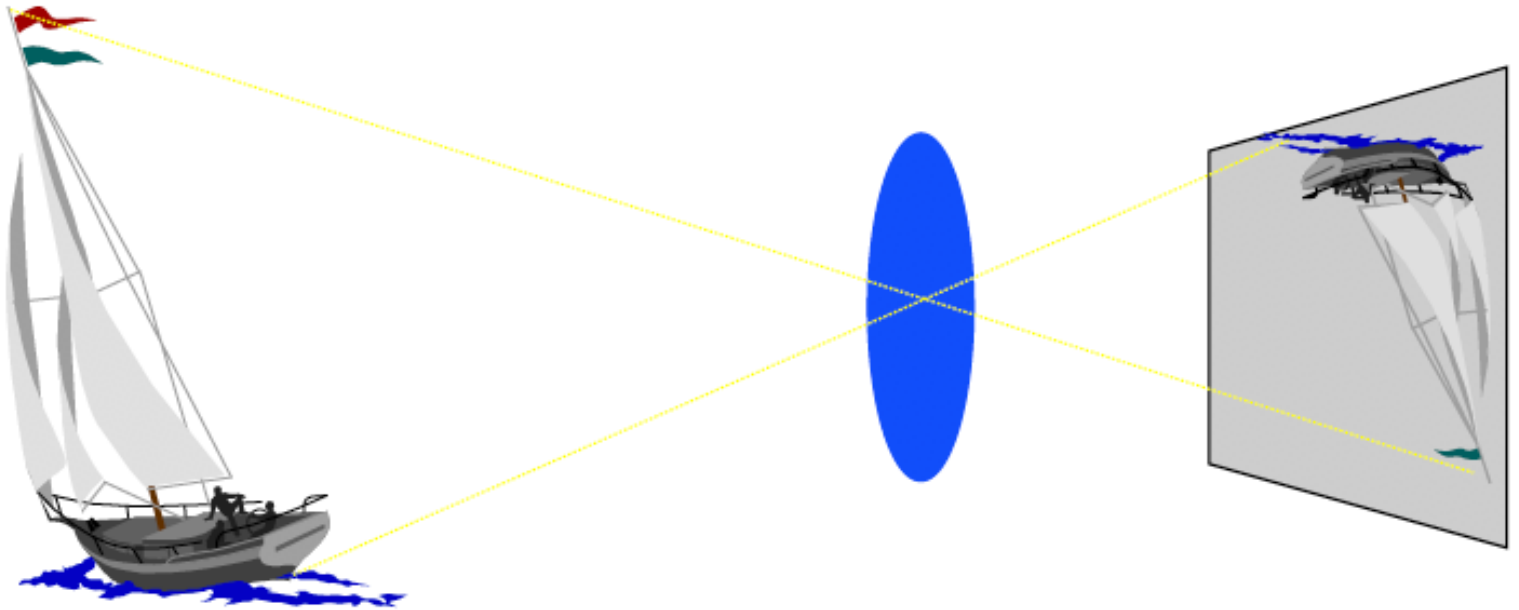
Outline

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

Imaging principles



Scene

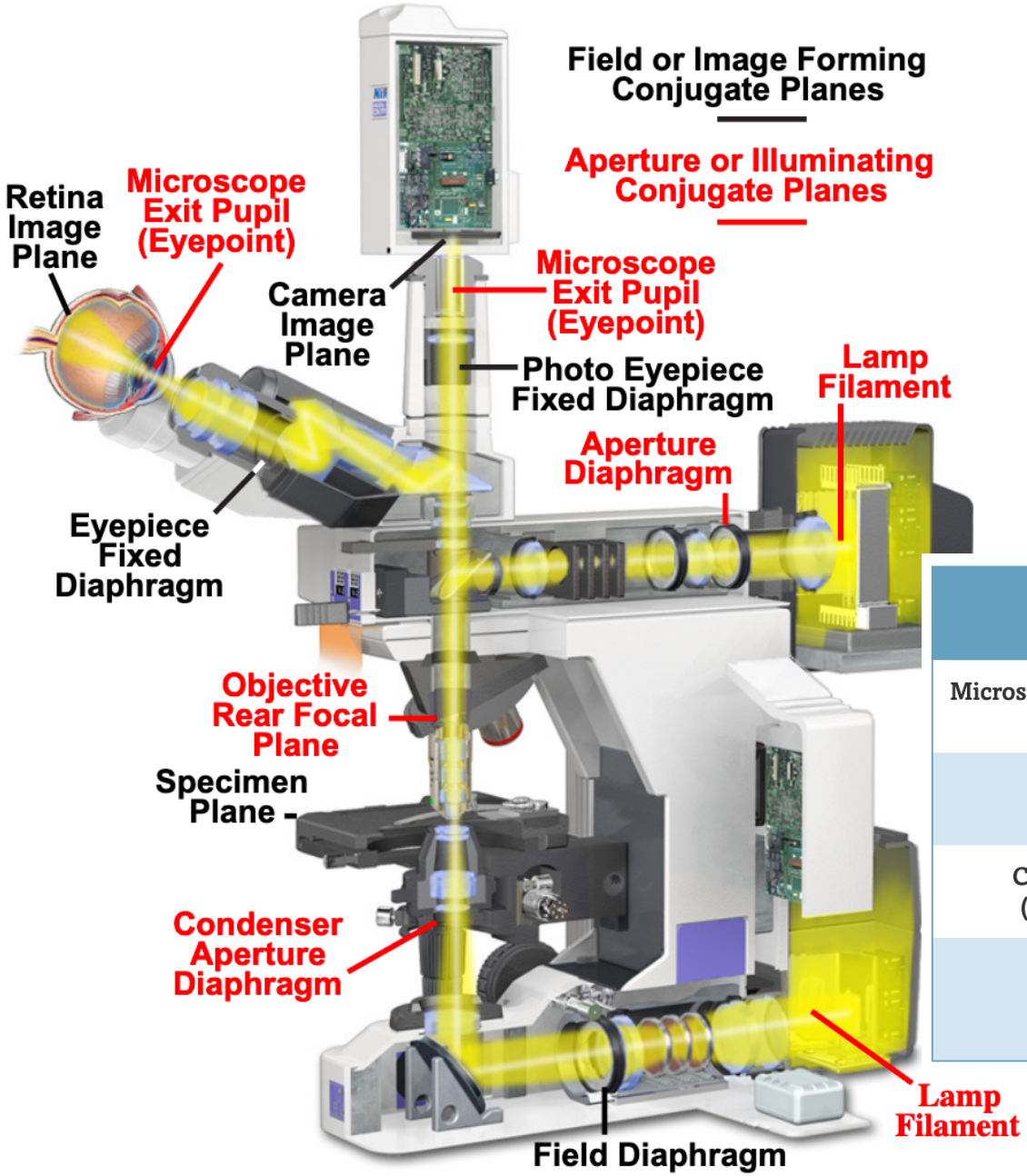
Lens

Sensor

Image on film

Microscope

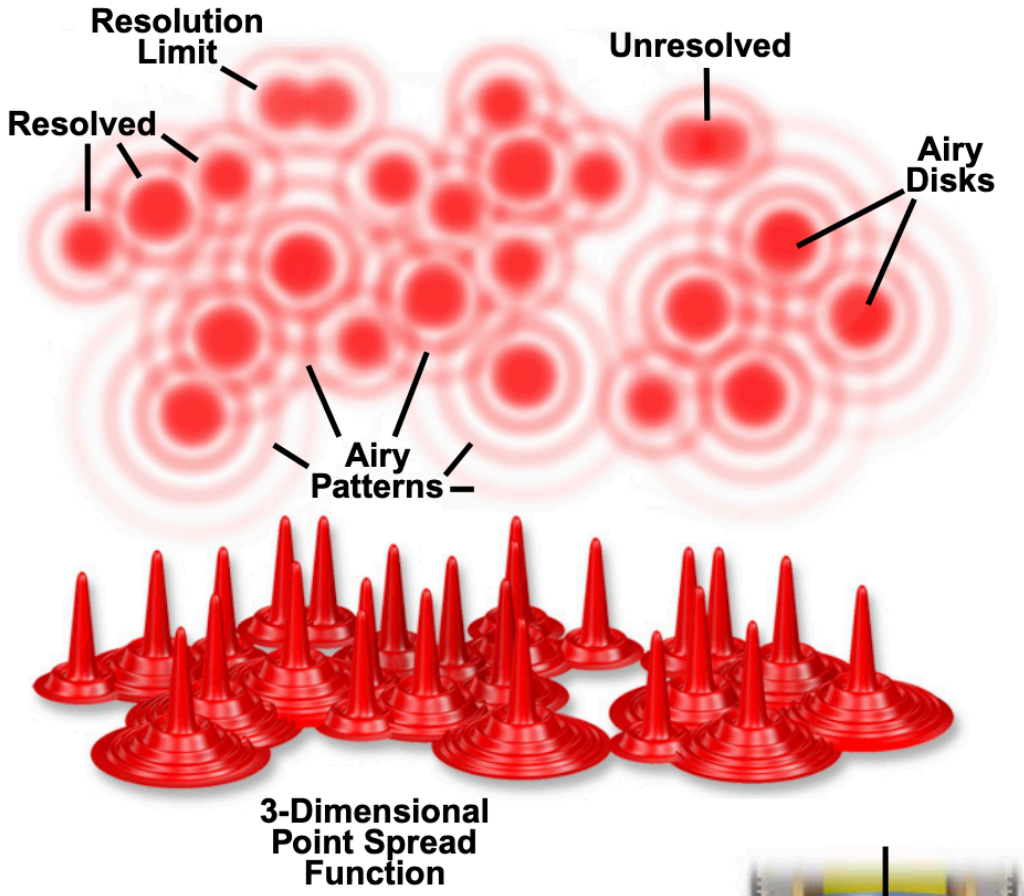
Figure 1 - Conjugate Planes in the Optical Microscope



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)

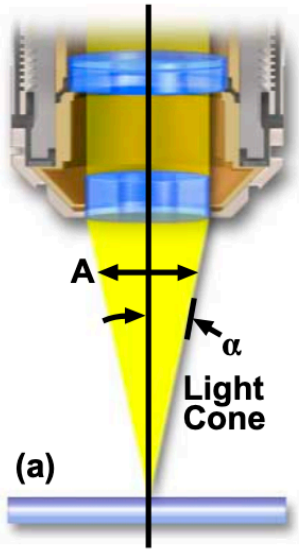
Resolution & numerical aperture

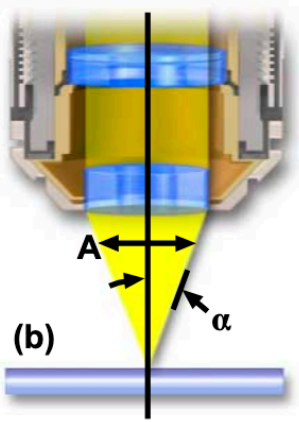
Figure 1 - Airy Patterns and the Limit of Resolution



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

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



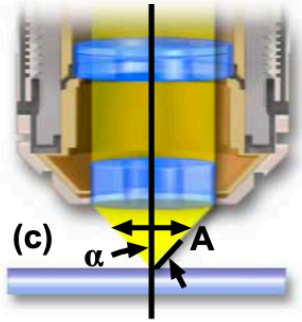


NA = n • sin(α)

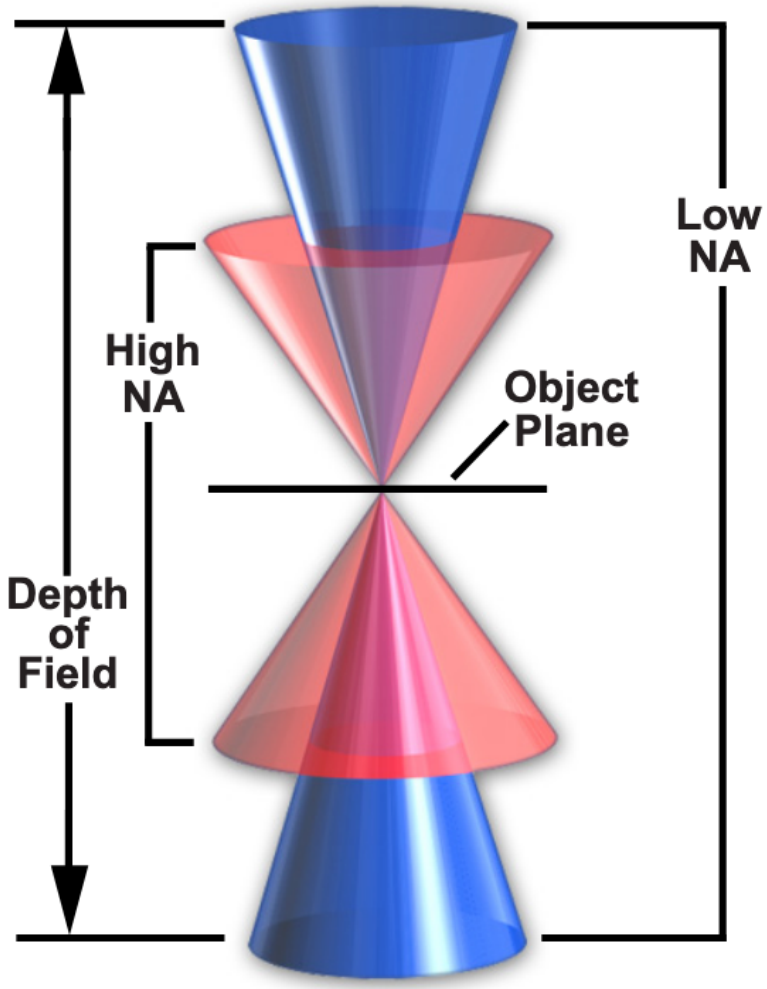
(a) α = 7° NA = 0.12

(b) α = 20° NA = 0.34

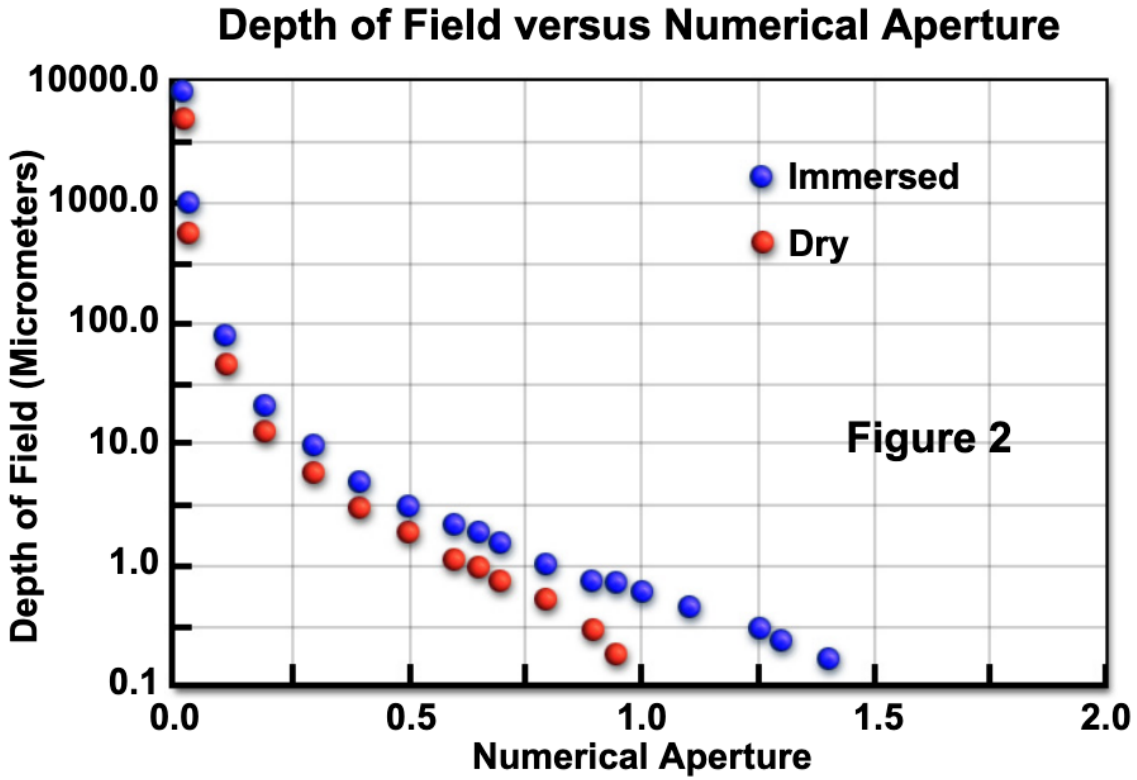
(c) α = 60° NA = 0.87



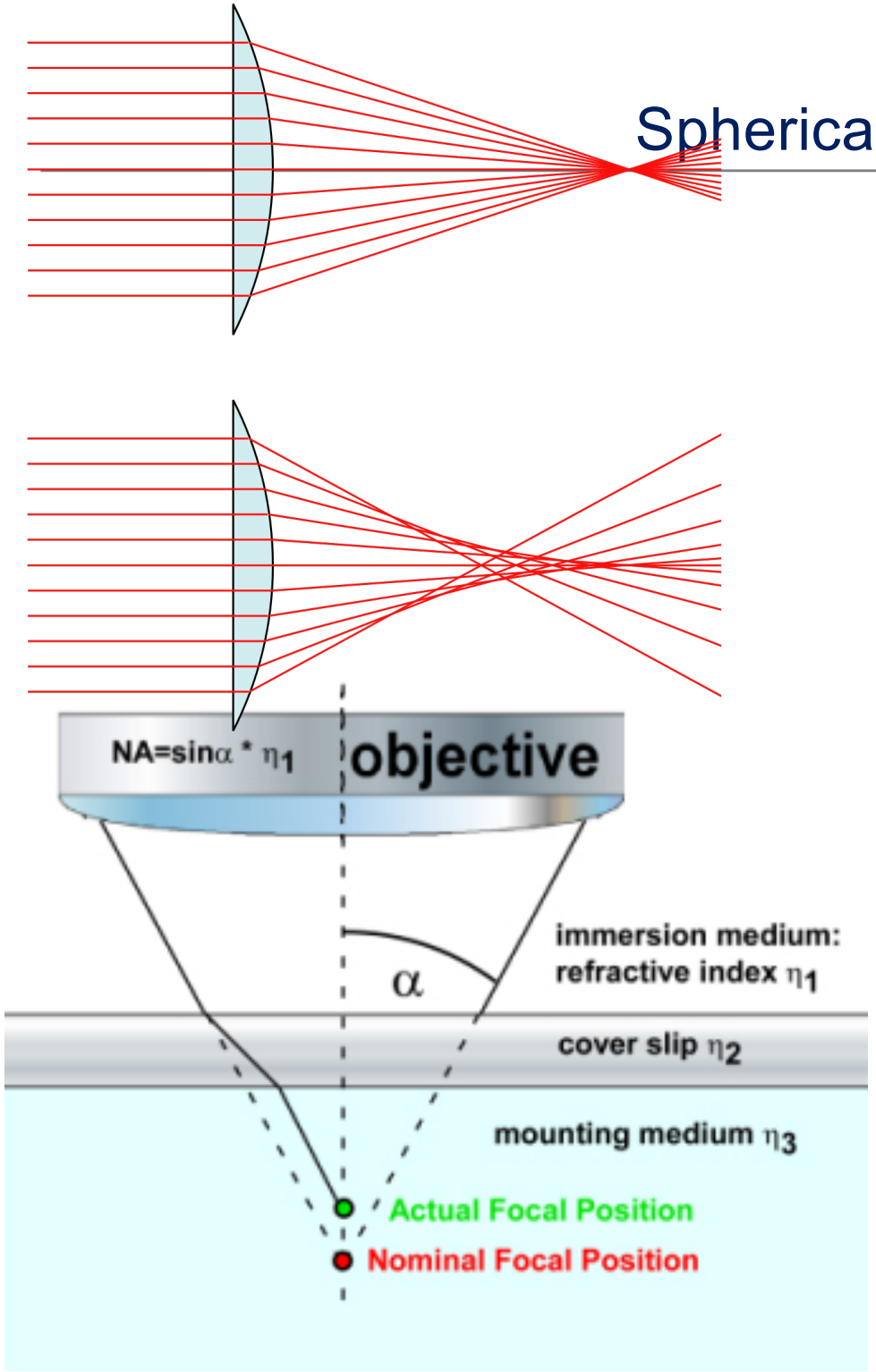
Depth of field/focus

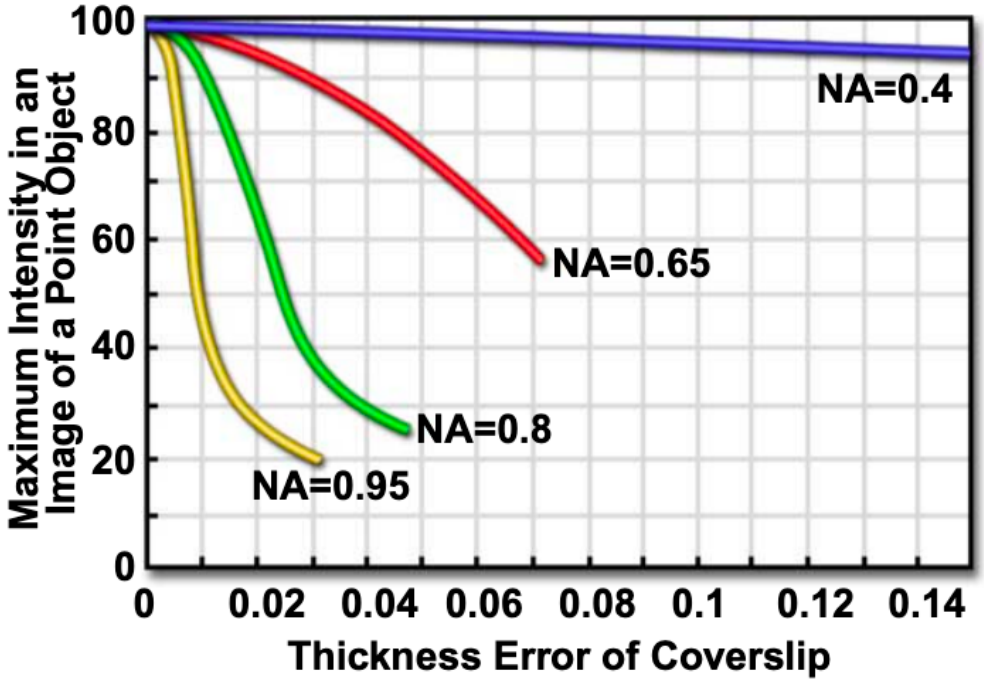


depth of field = the distance from nearest object plane in focus to farthest plane also simultaneously in focus.

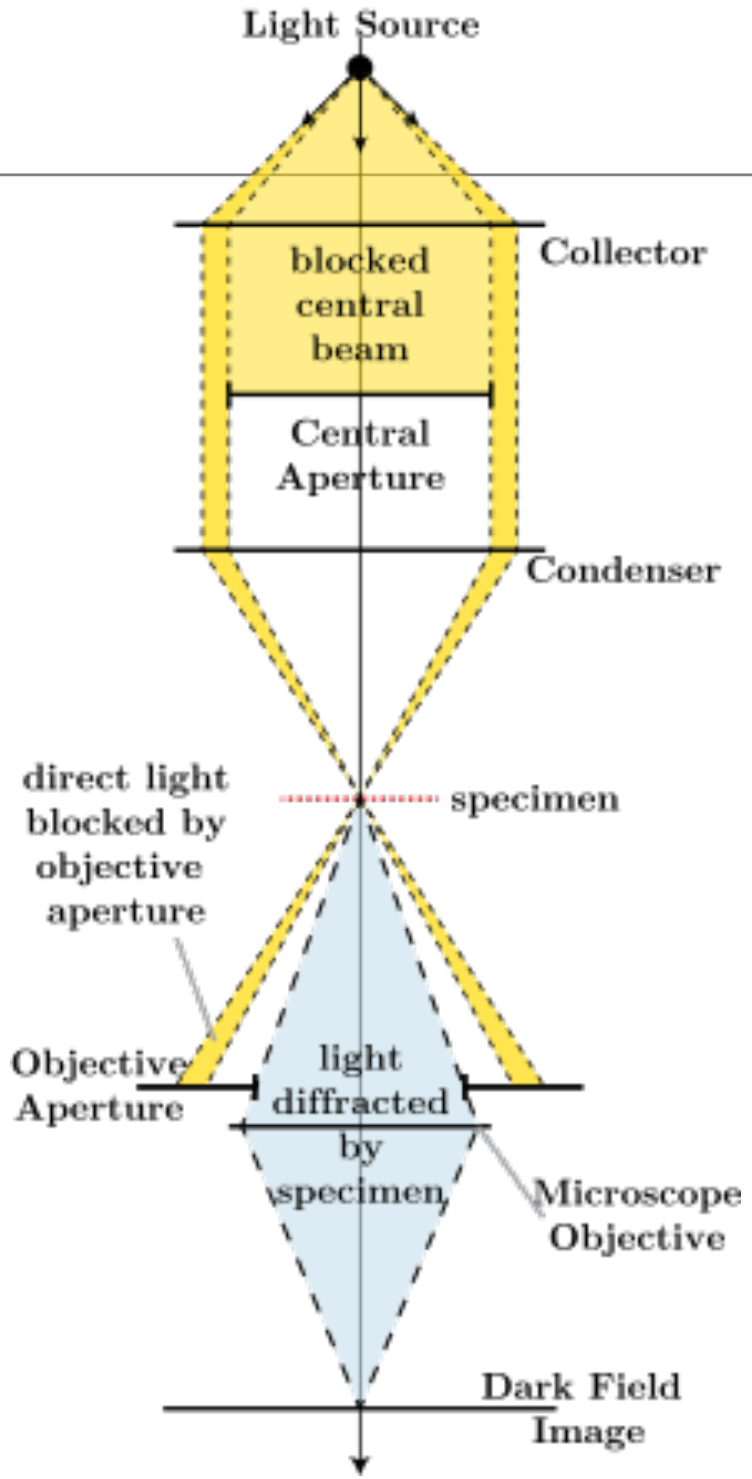


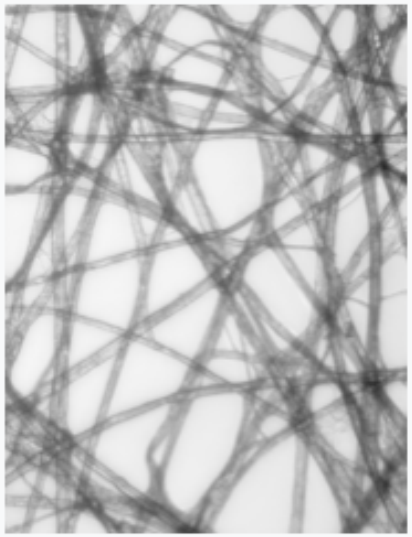
Spherical aberration & coverslip correction

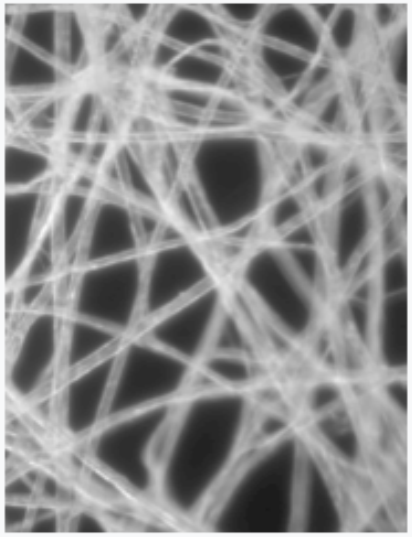




Dark field microscopy







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.

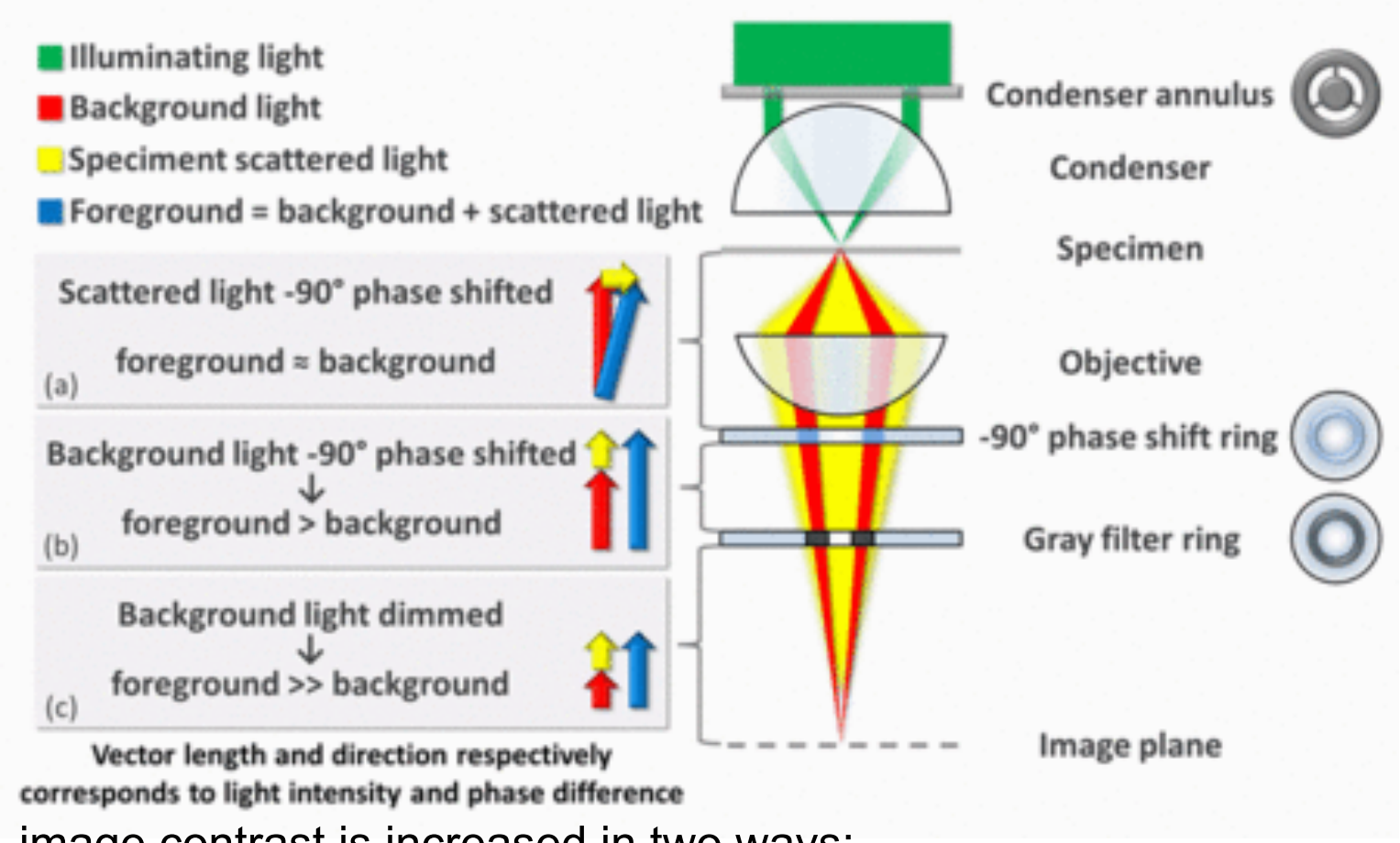
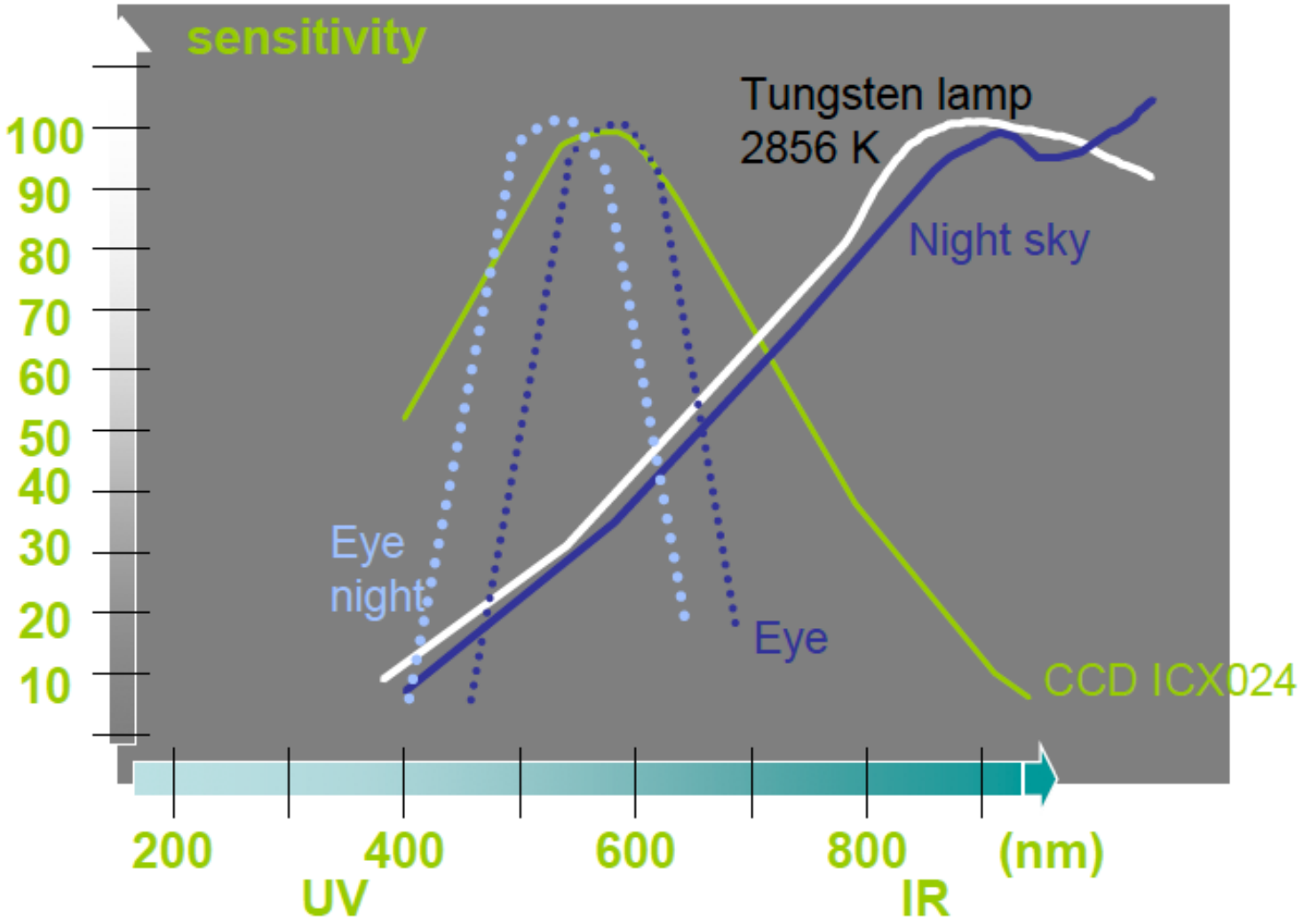


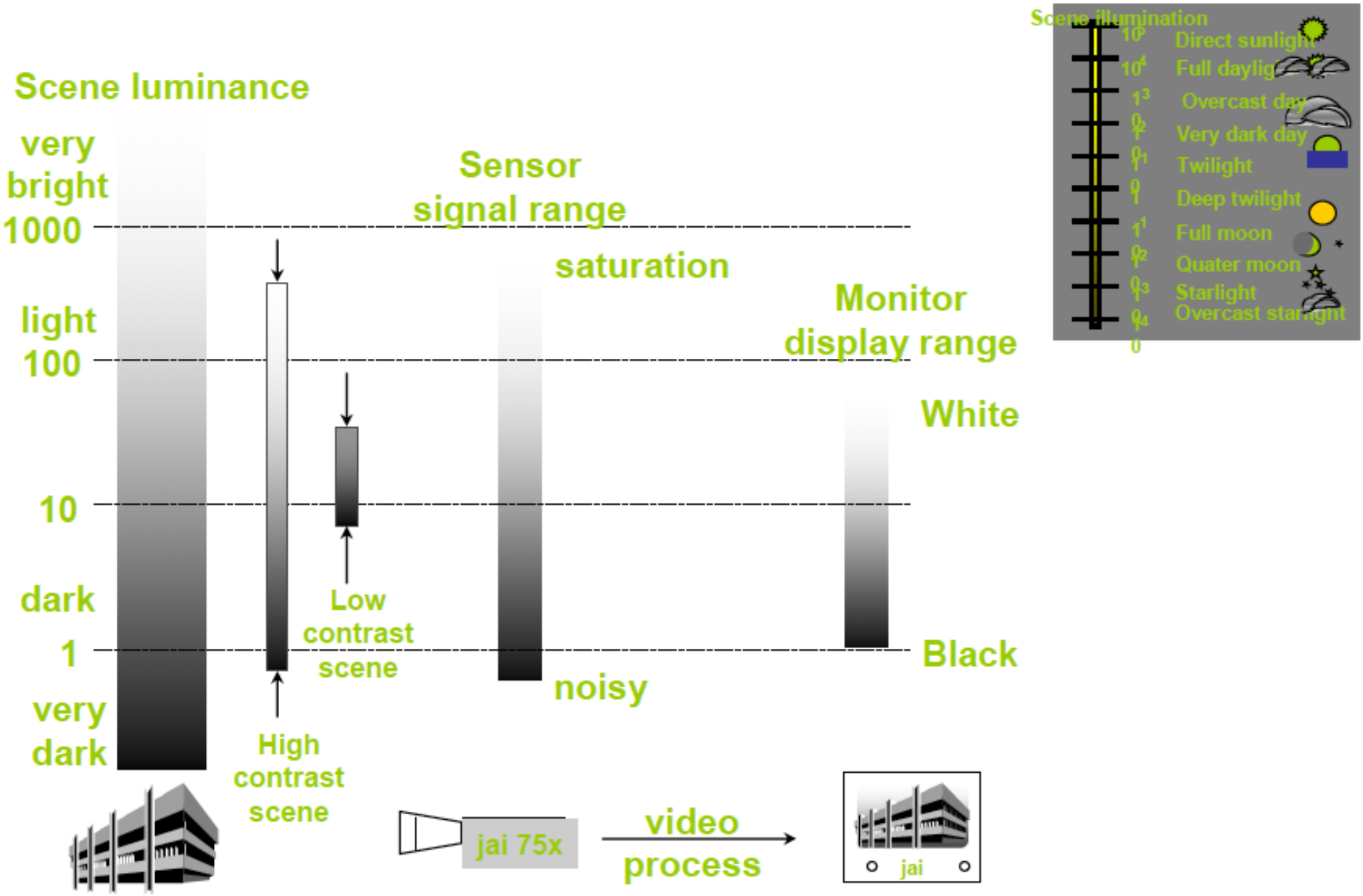
image contrast is increased in two ways:

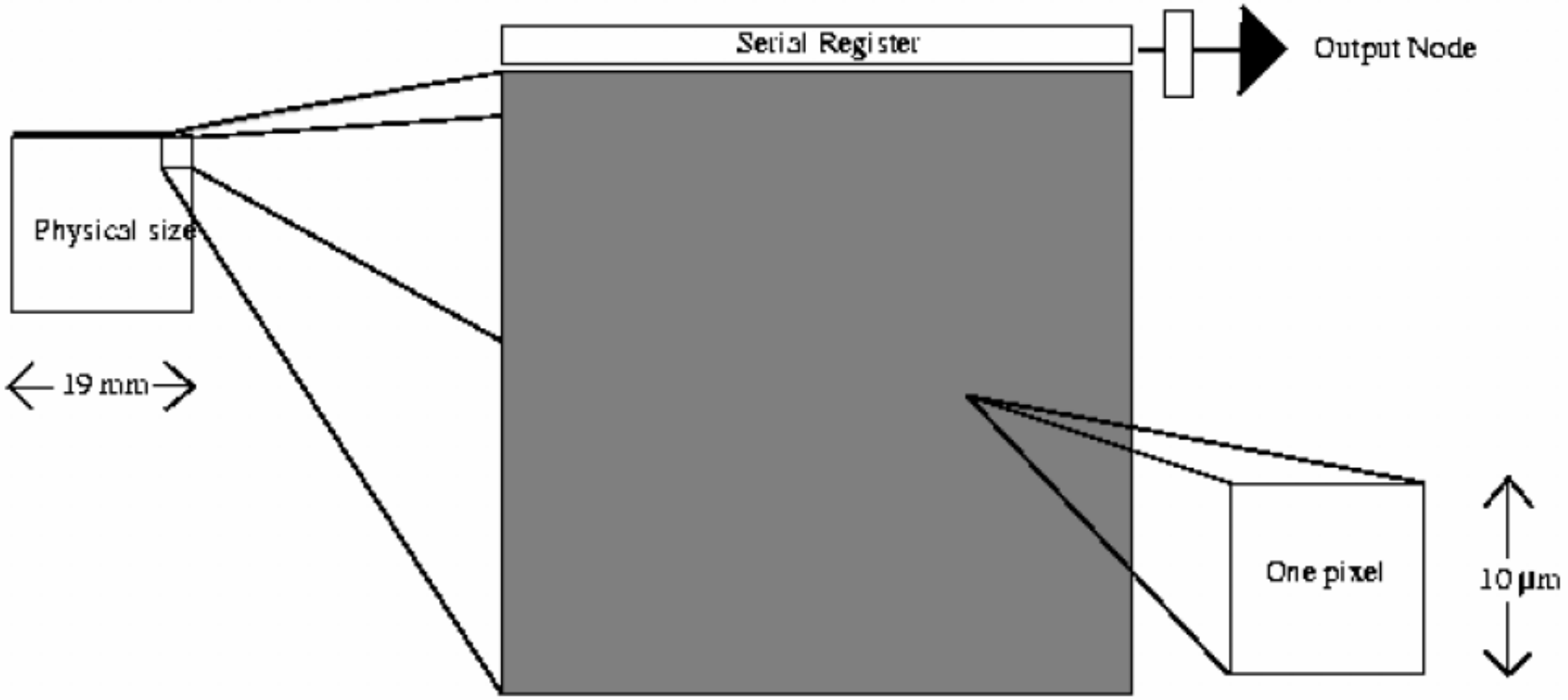
- by generating constructive interference between scattered and background light
- by reducing the amount of background light that reaches the image plane

Imaging principles

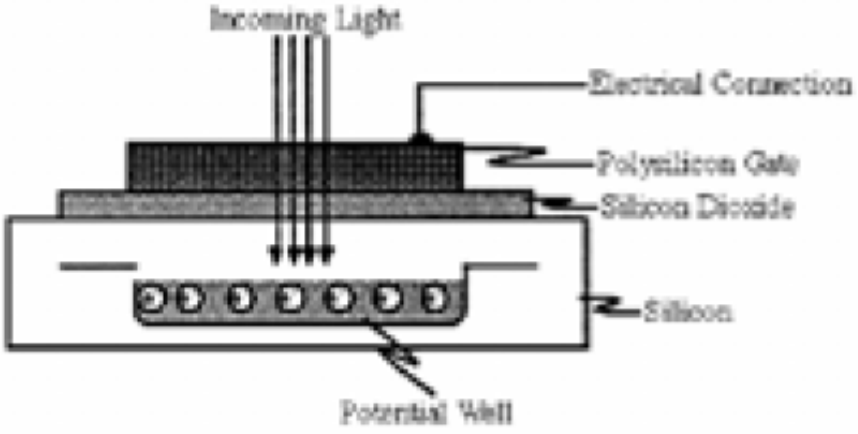


Luminance and contrast




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

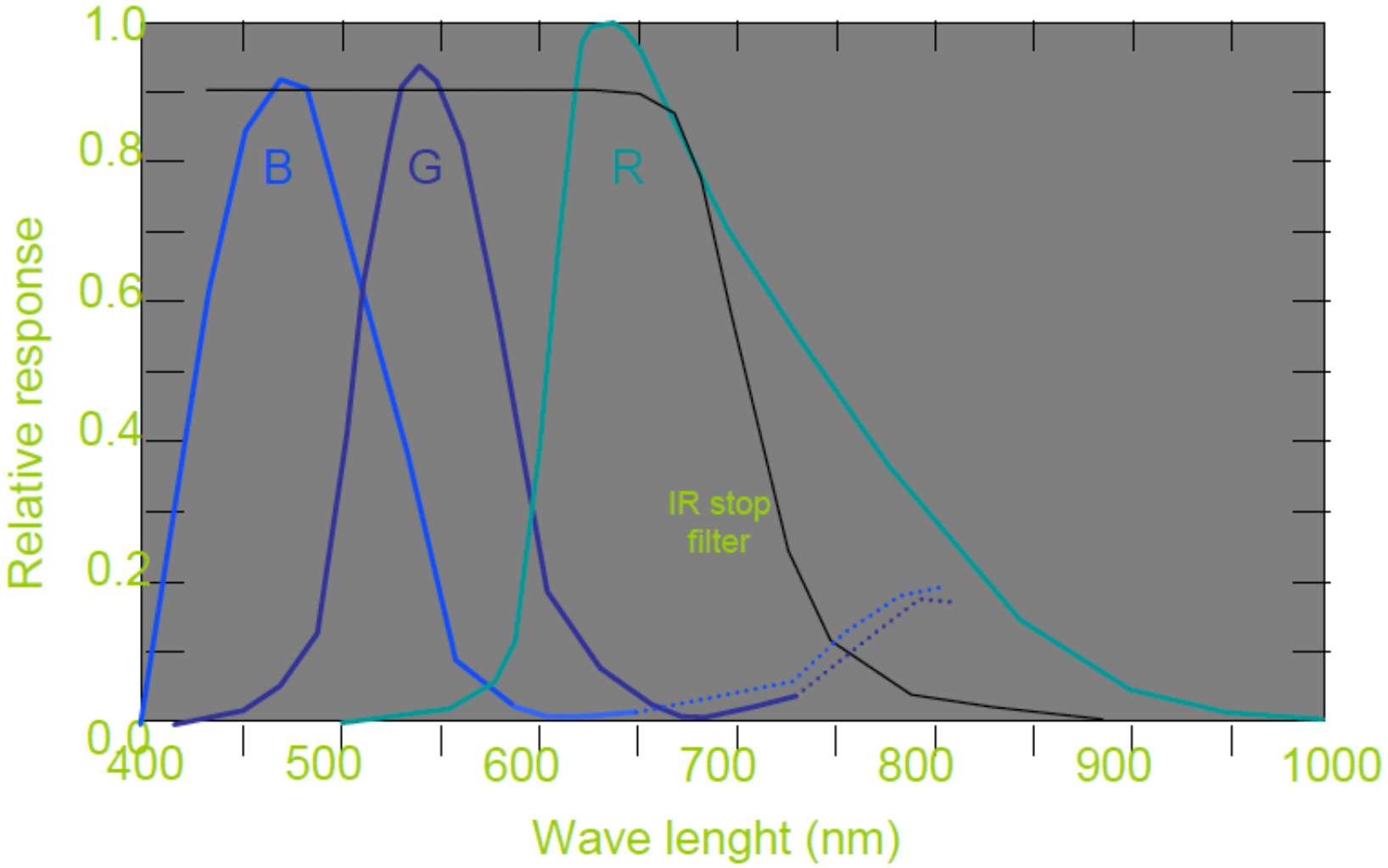
Drawbacks

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

MORE SENSITIVE THAN THE EYE

16 bit: cooled sensor

Color

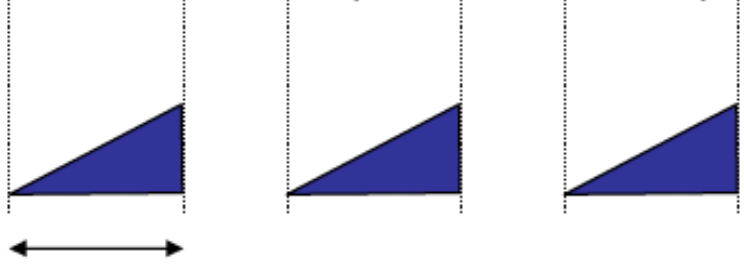

1.0
0.8
0.6
0.4
0.2
0.0
Relative response
B
G
R
IR stop
filter
400
500
600
700
800
900
1000
Wave lenght (nm)

Flicker

Lamp supply
50 Hz

Light

Photocharge
EIA camera



Shuttertime = 10 msec

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?

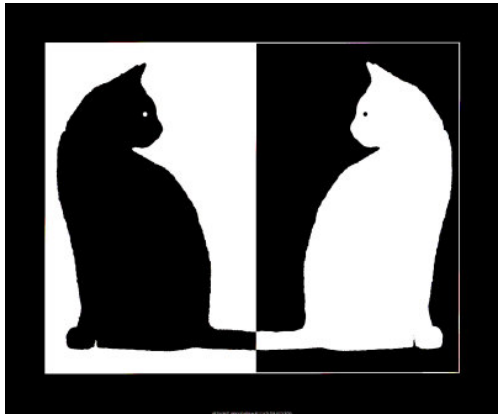
$$f = f(x, y)$$

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

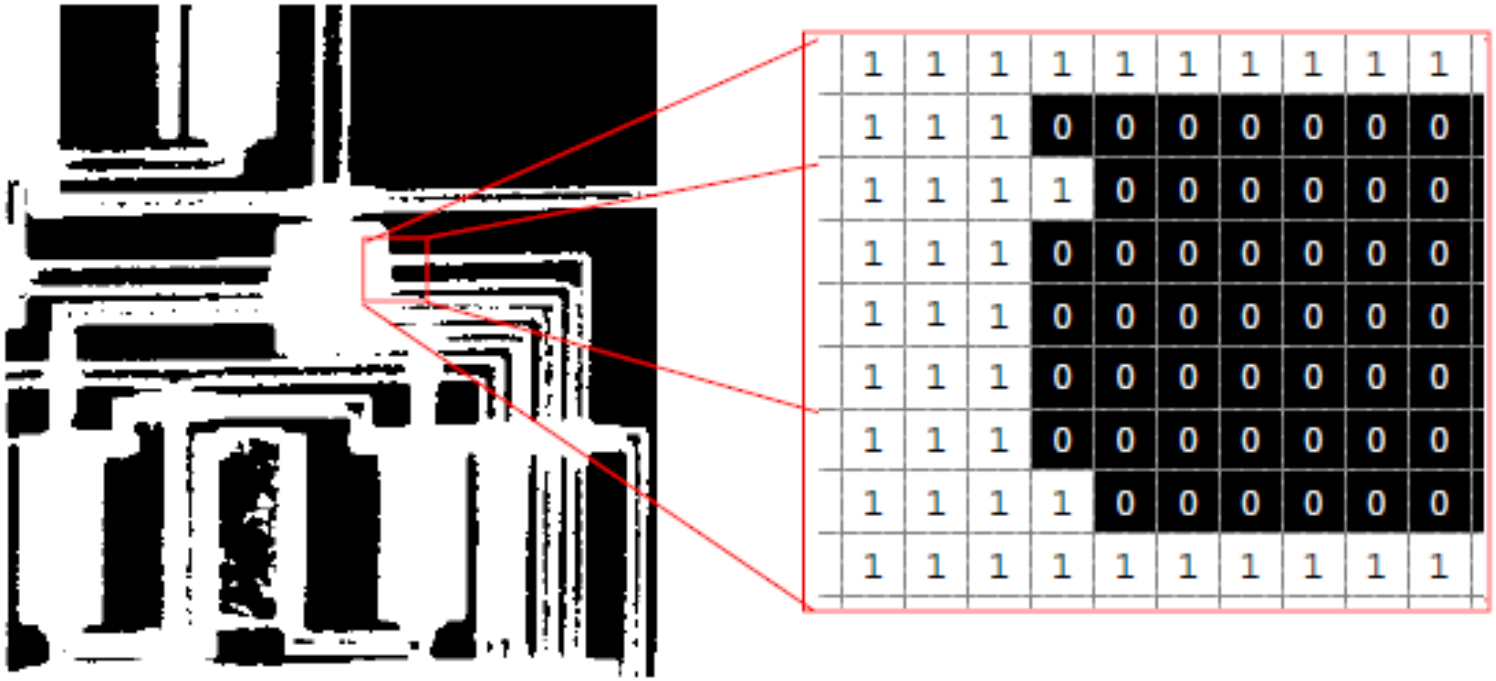
[RGB]
Red Green Blue

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 image




```
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:

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

1 bit depth ($2^1 = 2$) – black and white

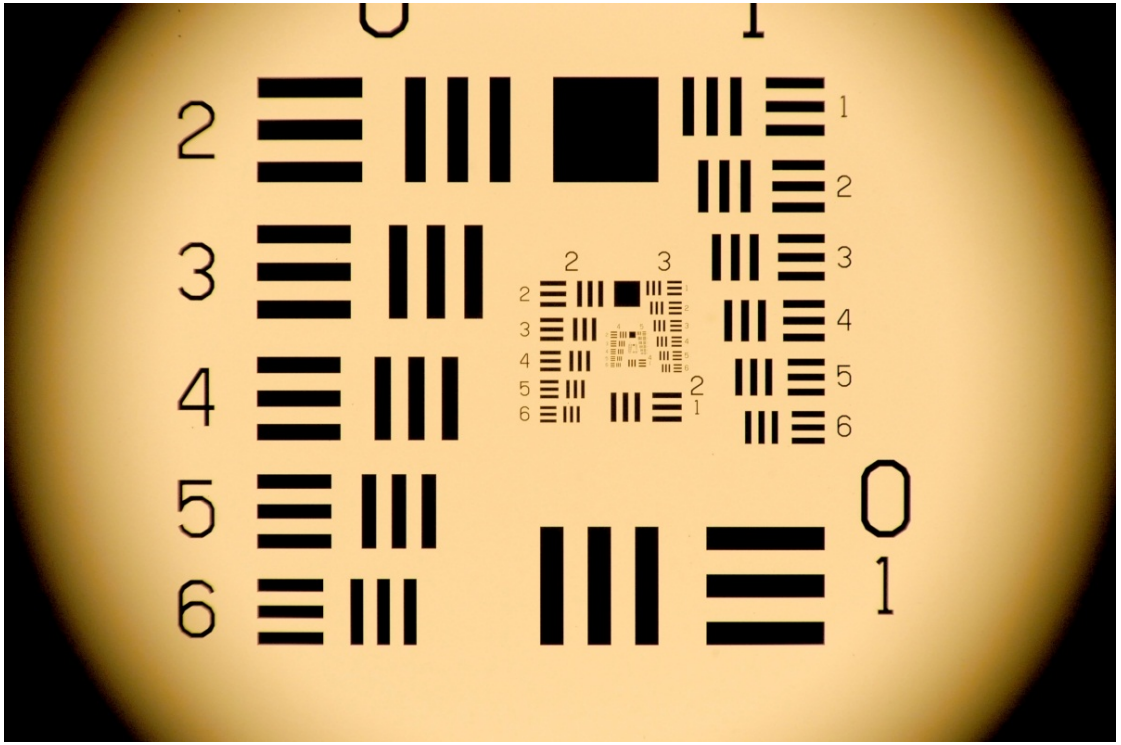
8 bit depth ($2^8 = 256$) – gray scale 0..256

12, 16 bit gray scale

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

Spatial resolution of images

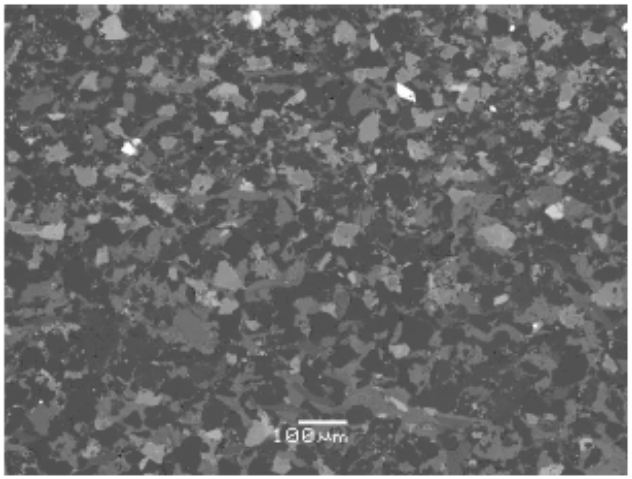
http://en.wikipedia.org/wiki/Image_resolution



Spatial resolution of optical system - Number of independent pixels per unit length

Why do we need image analysis?

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



Segmentation procedure

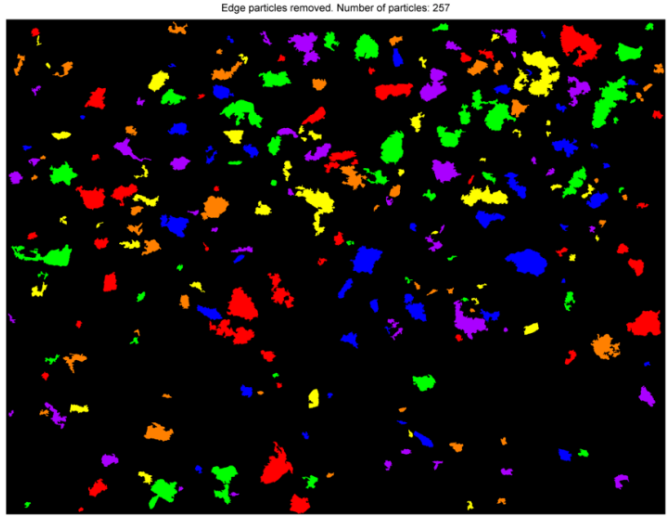


Image segmentation quick steps:

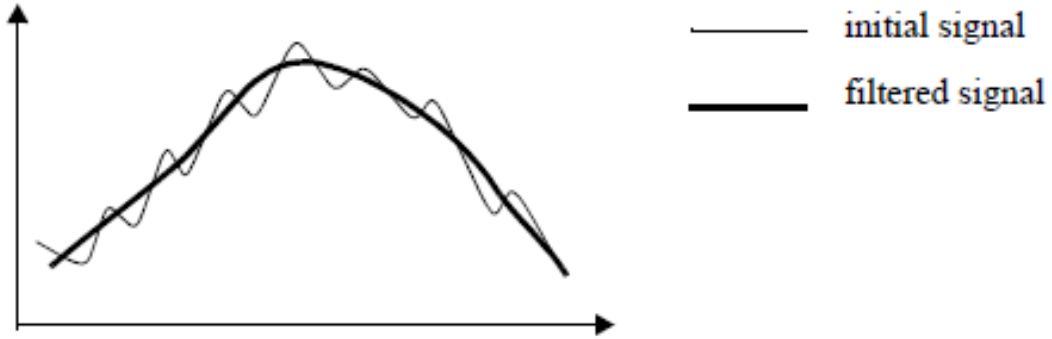
- RGB → gray
- Filter
- Thresholding → binary
- Labeling connected components
- Geometrical analysis of connected components

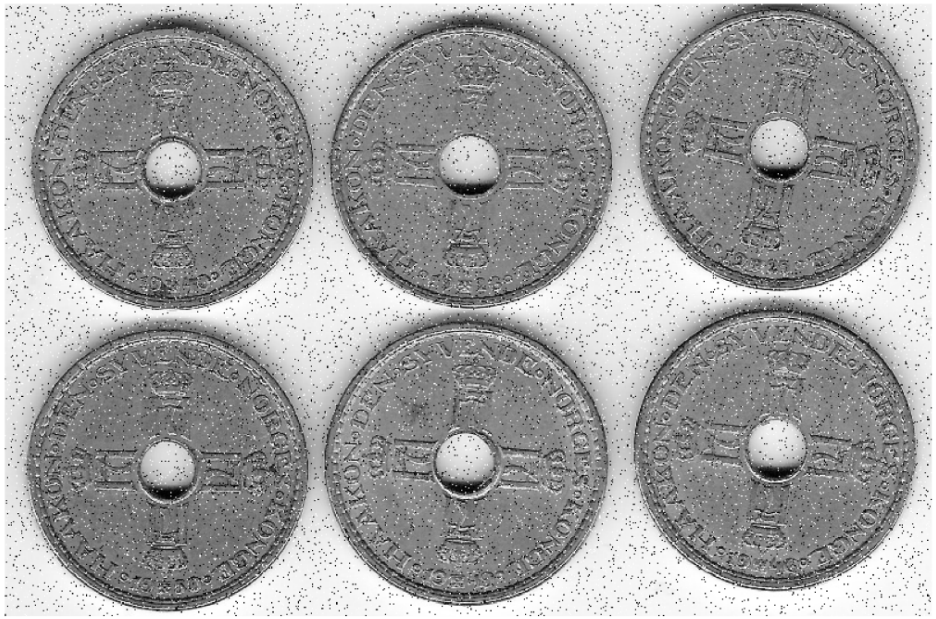
RGB to gray scale

```
im_bw = rgb2gray(im_bw);
Im_bw = im(:,:,1);
Im_bw = (im(:,:,1) + im(:,:,2) + im(:,:,3))/3;
```

Noise removal

- Filtering – smoothing
- Background correction





Convert to black and white

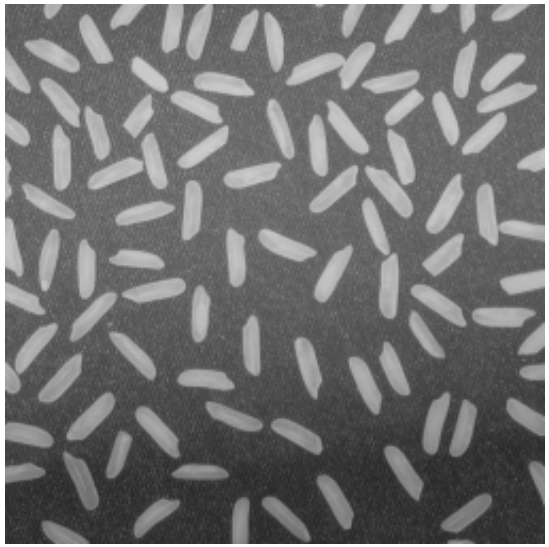
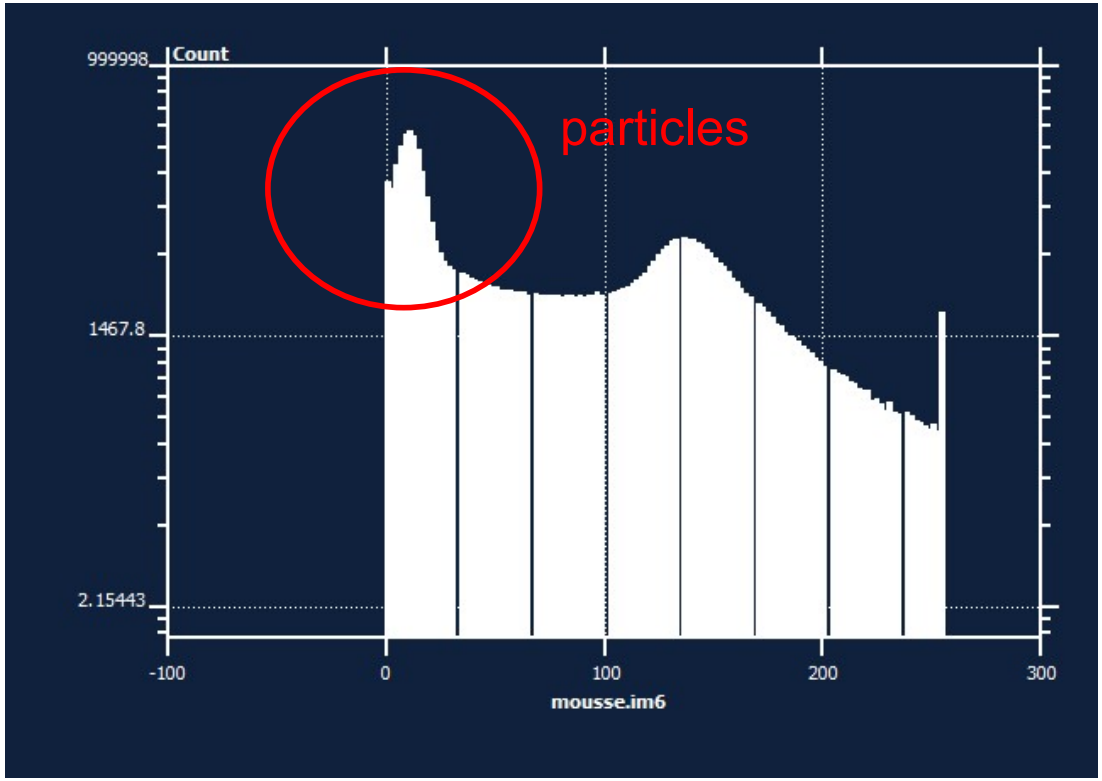


Image histogram



Thresholding intensity interval (a,b)

Labeling connected components

0	0	0	140	140	140	140	140	140	140
0	0	0	0	140	140	140	140	140	140
0	0	0	0	0	140	140	140	140	140
0	0	0	0	0	140	140	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

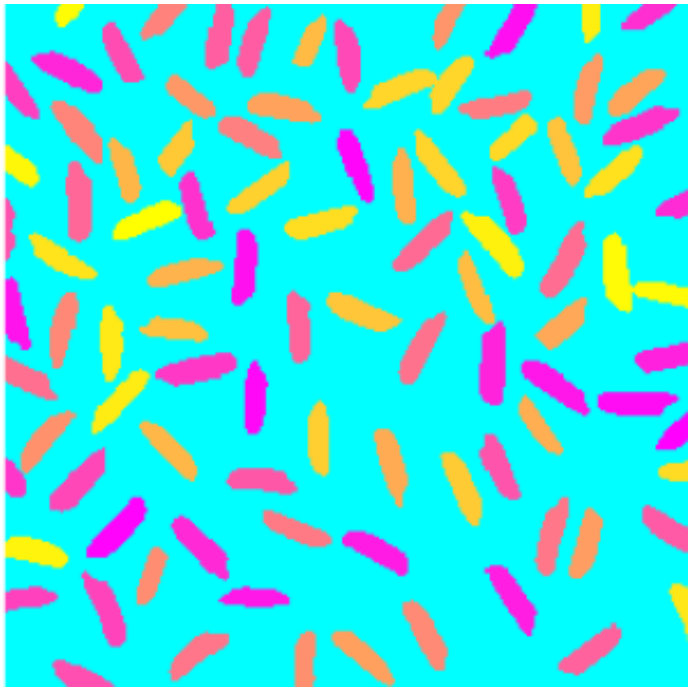
4 or 8 neighbor connectivity

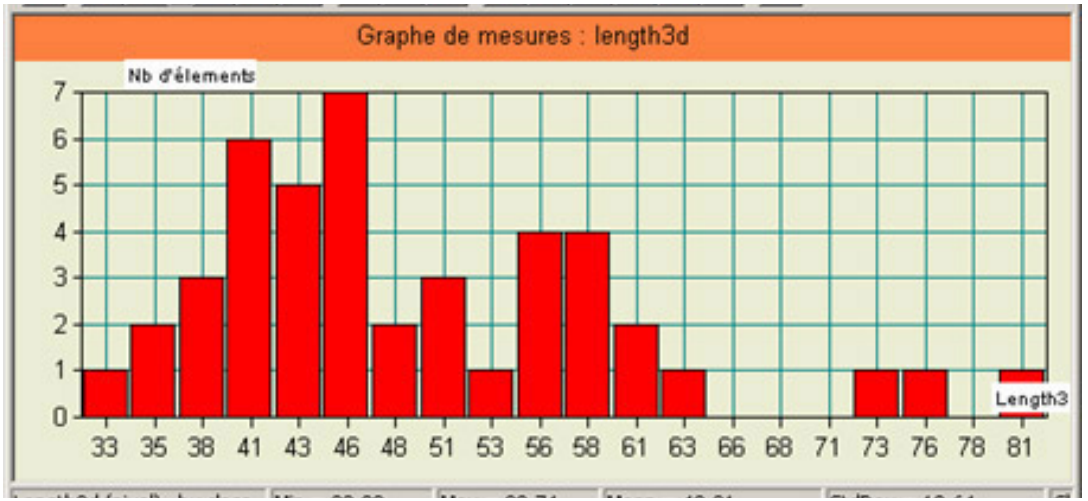
1 1 1
1 1 1
1 1 1

Figure B.9: 4-connectivity of pixels in a 3x3 pixel-environment. The center pixel (1) is connected to its nearest neighbours (1's), but not its next nearest neighbours (1's).

minutes

Particle size distribution





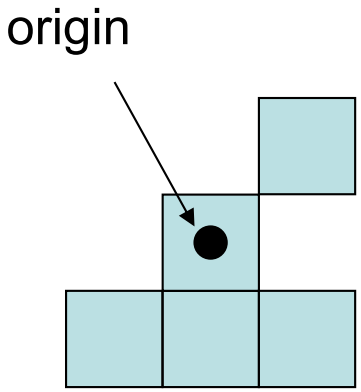
How to make segmentation

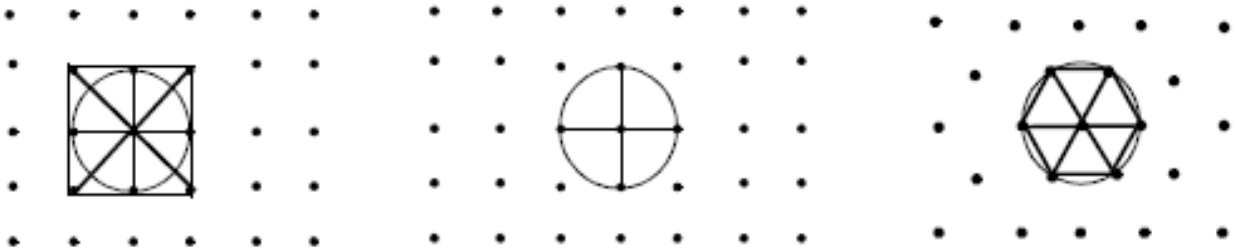
?

Morphological operators

Morphological transformation are based on a structural element

- size
- shape
- center location

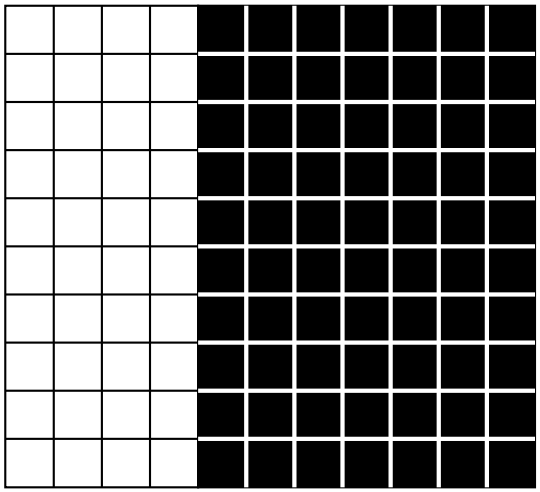


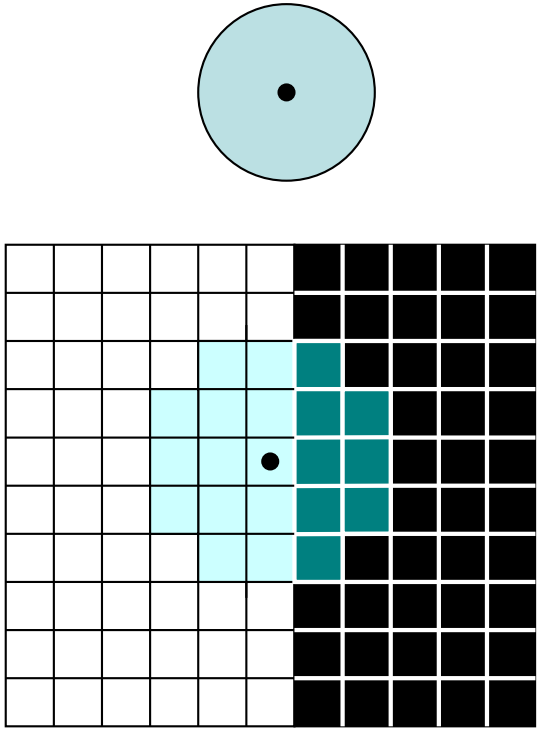


Erosion and dilation – basic operations

Erosion

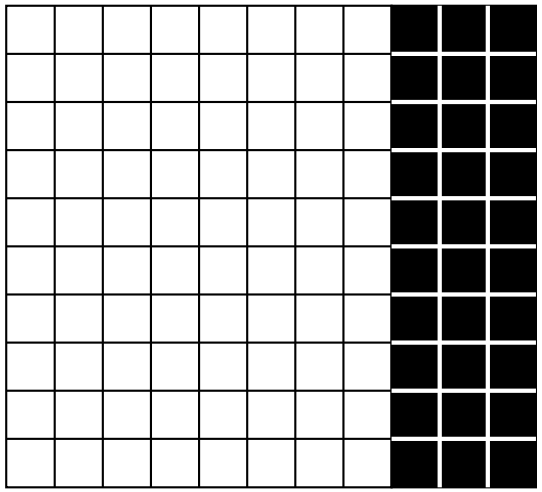
"Set the value at the origin to the minimum value of pixels in the structural element"





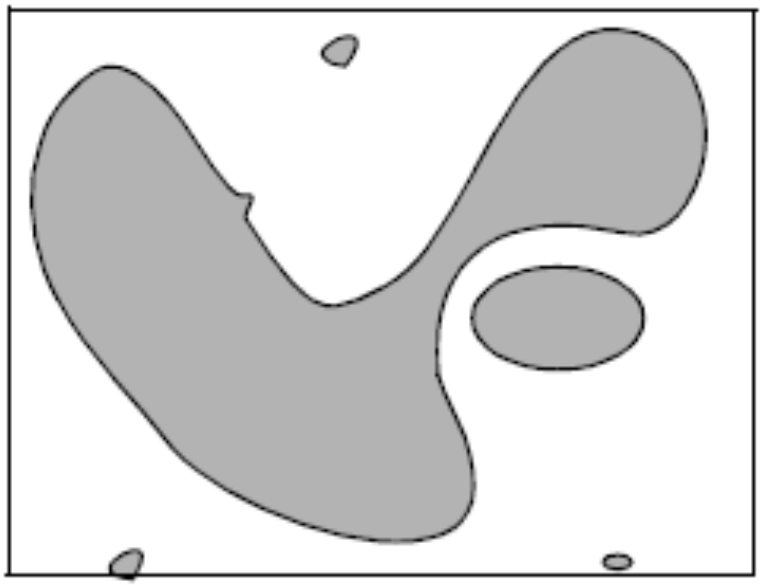
Dilation

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

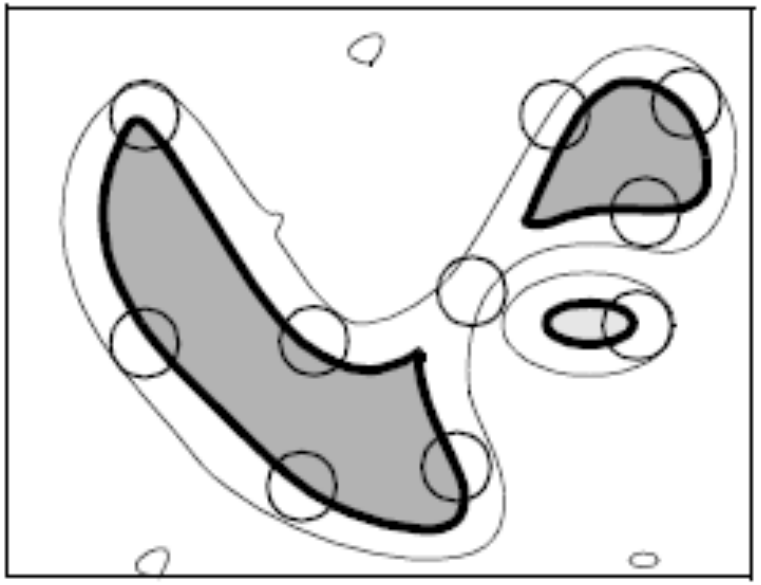


Erosion

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



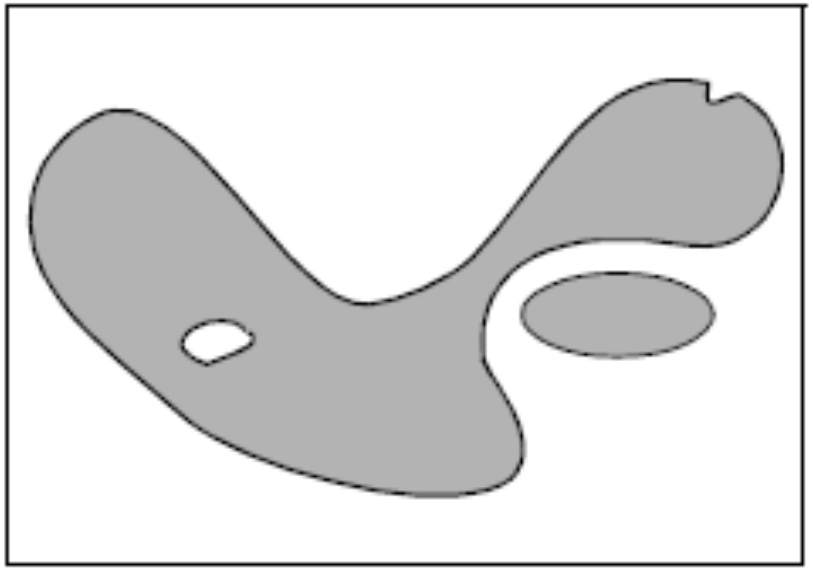
input image



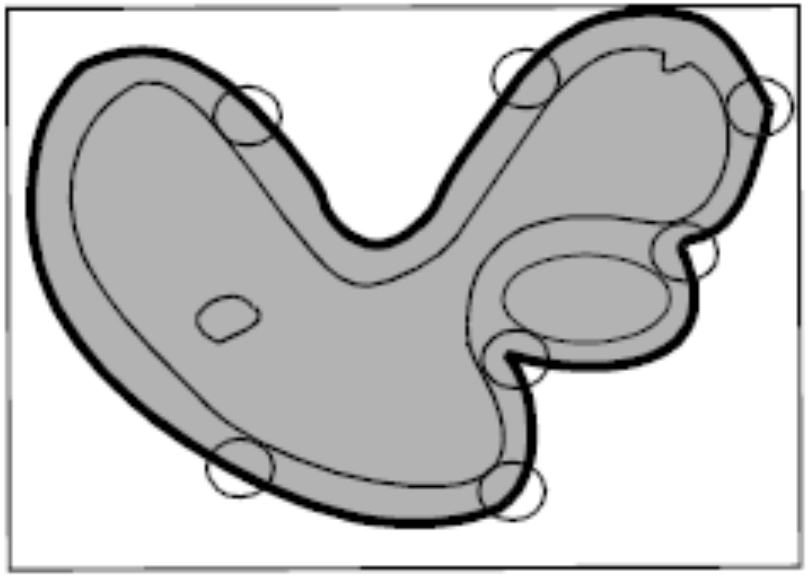
eroded image

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

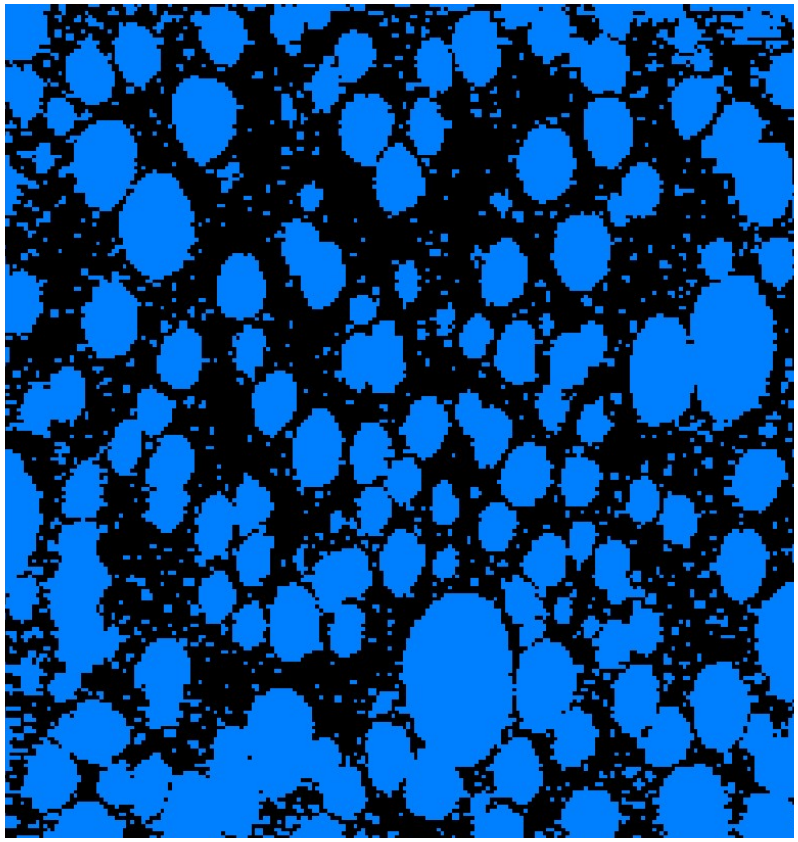
Original image ⟶ Erosion ⟶ Dilation

Closing

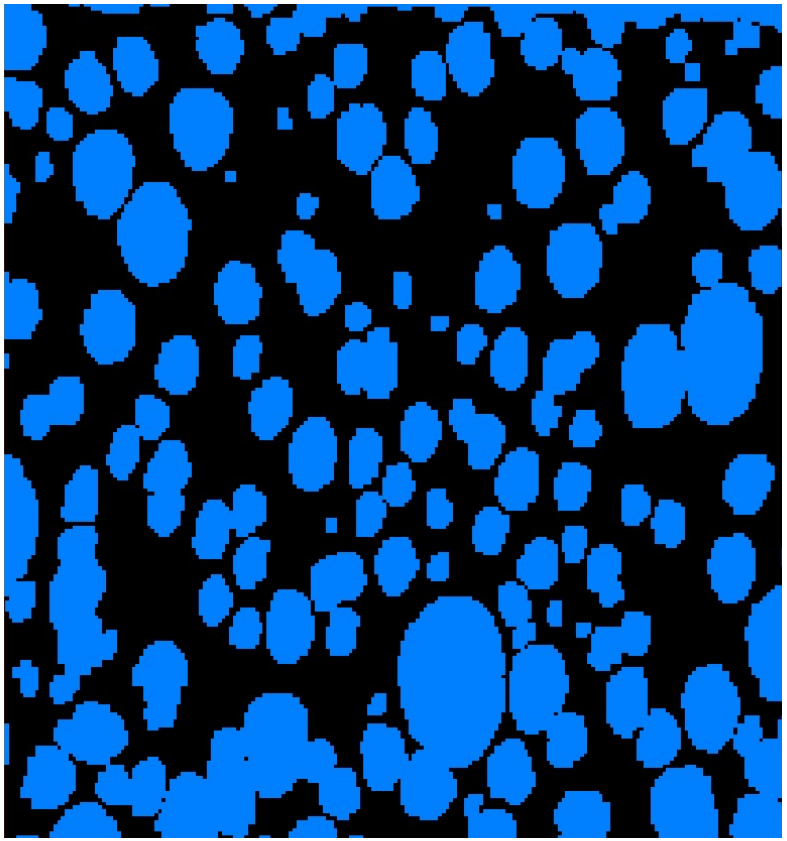
Original image → Dilation → Erosion

Noise removal

Original image

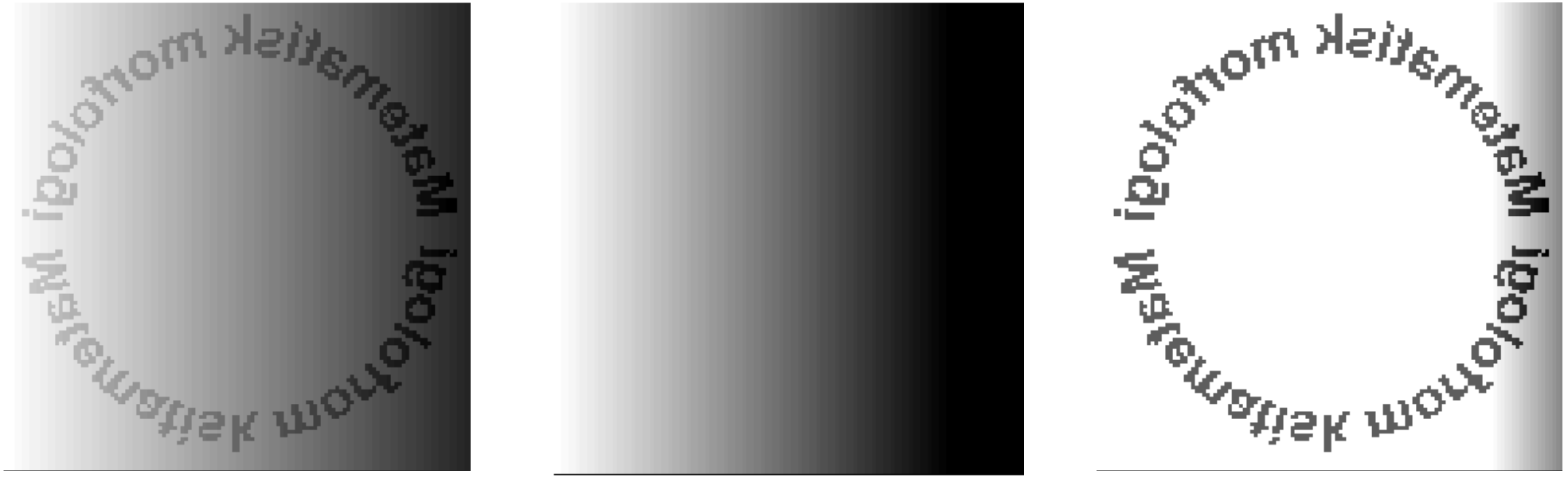


After opening

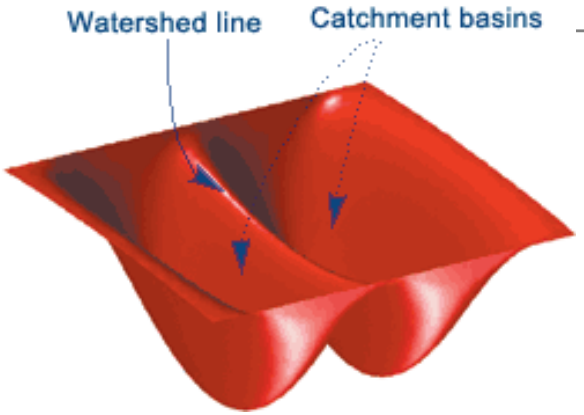


Background correction

Original image → Opening → IM ÷ Opening



Watershed



Flooding of image topography

Water rise from a set of markers

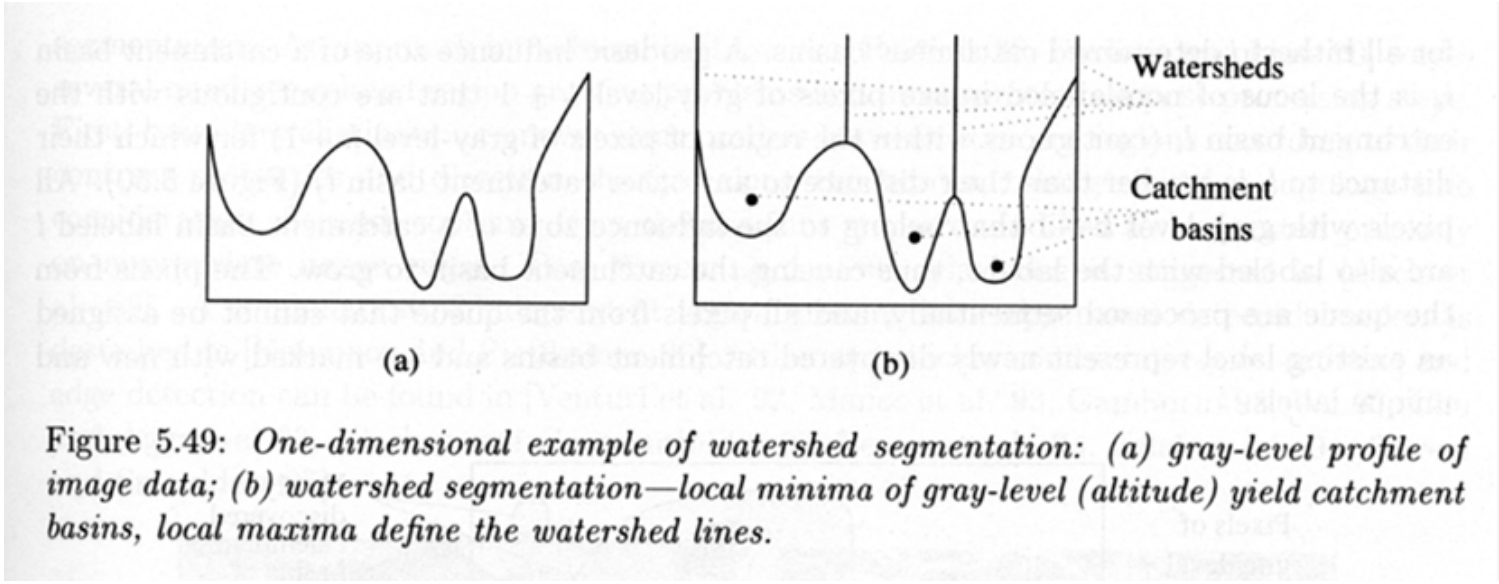
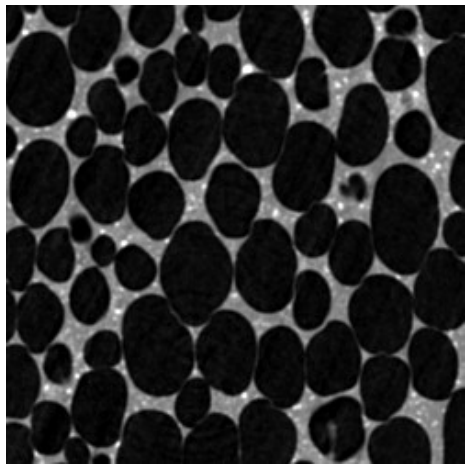


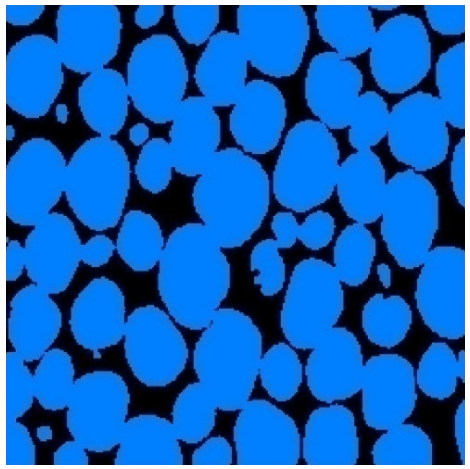
Figure 5.49: *One-dimensional example of watershed segmentation: (a) gray-level profile of image data; (b) watershed segmentation—local minima of gray-level (altitude) yield catchment basins, local maxima define the watershed lines.*

Example of workflow using watershed

Gray level image

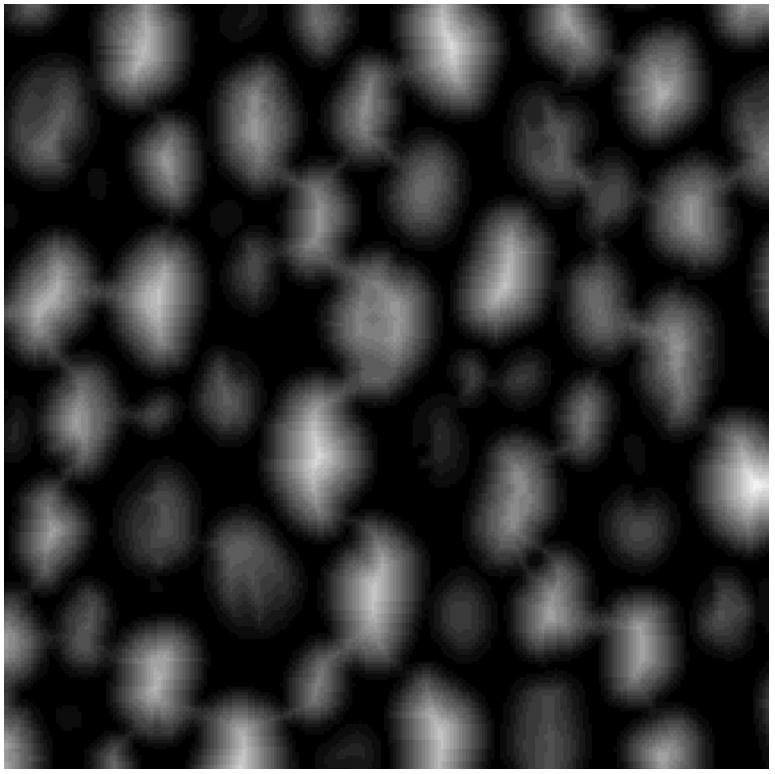


Binary image



Reconstruction of individual pores in foam

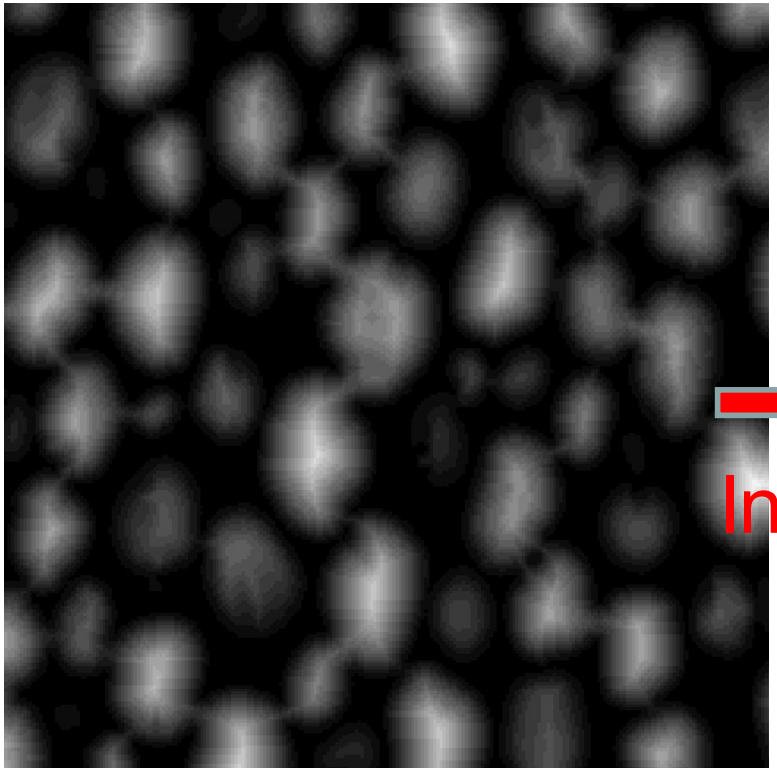
Distance map



Example of workflow using watershed

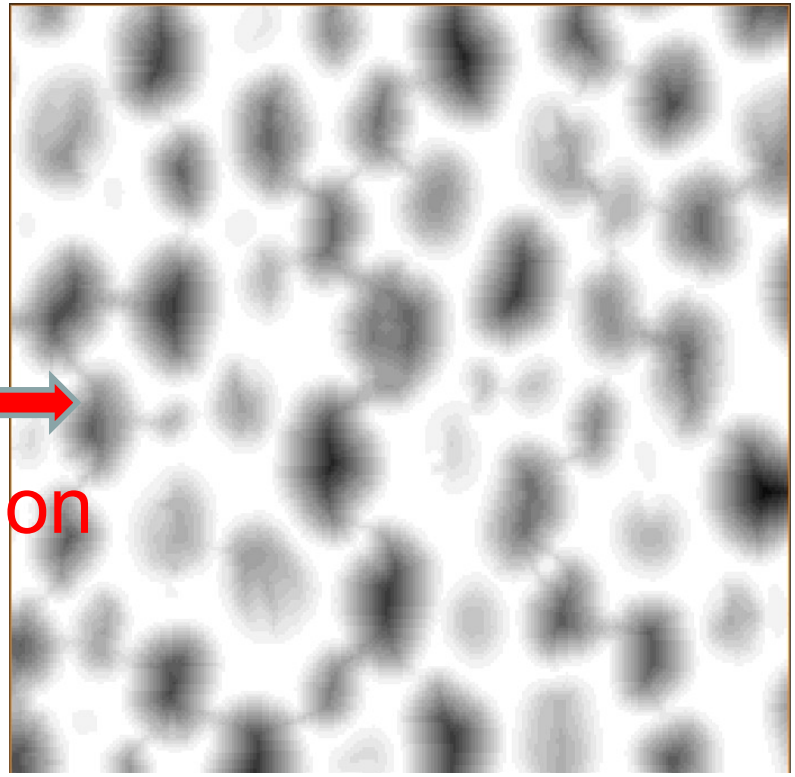
Valleys for watershed

Distance map



Inversion

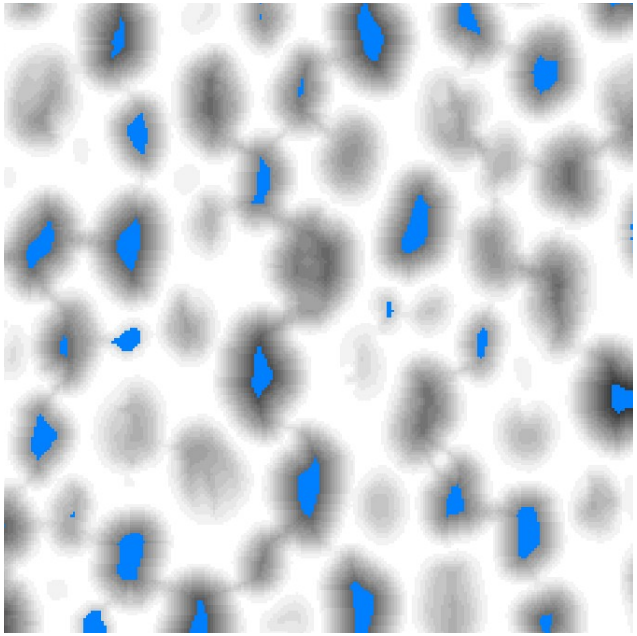
Inversed distance map



Example of workflow using watershed

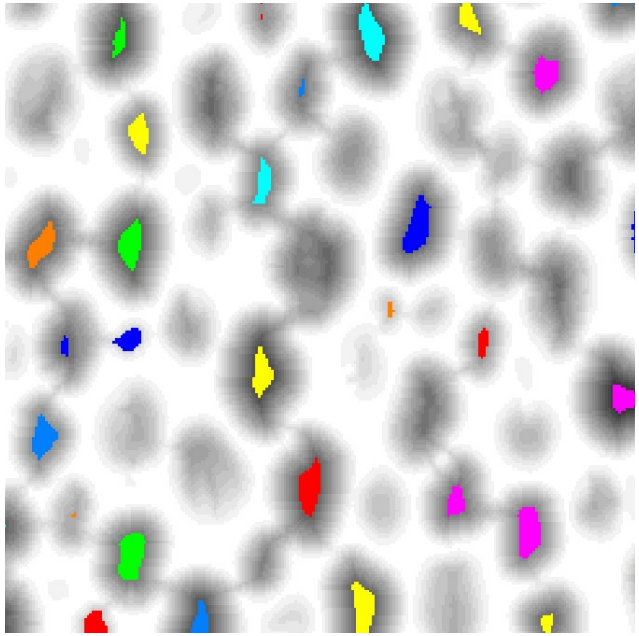
Create markers

Maxima on distance map



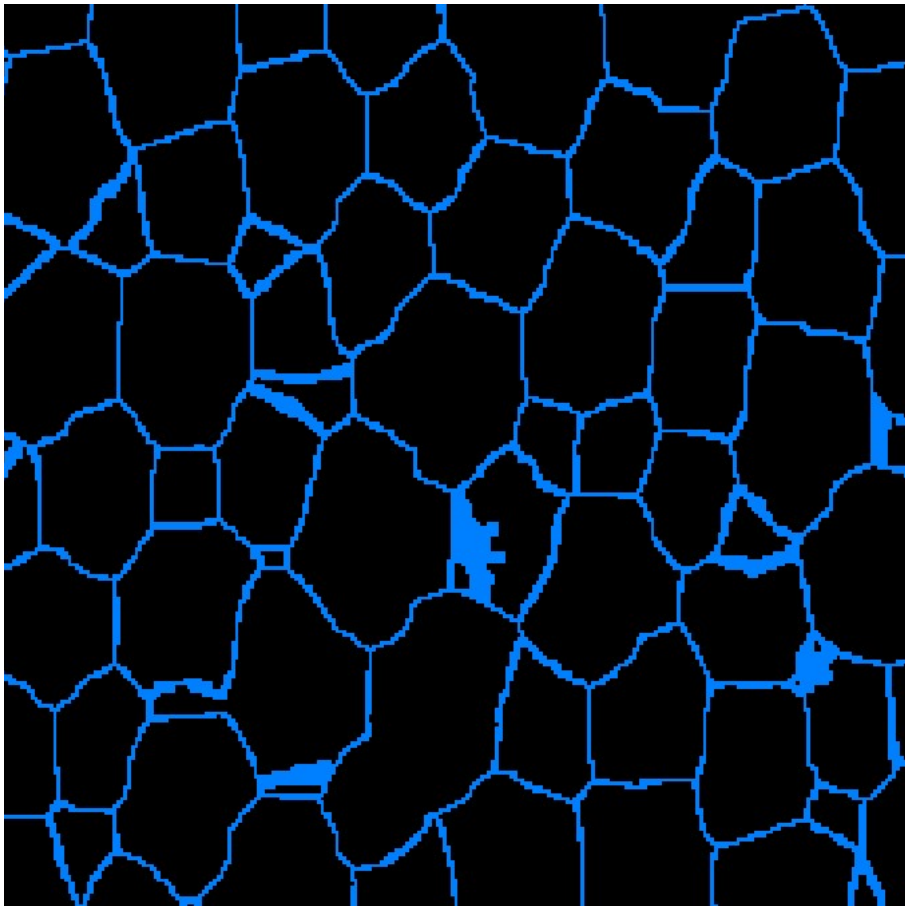
Labeling

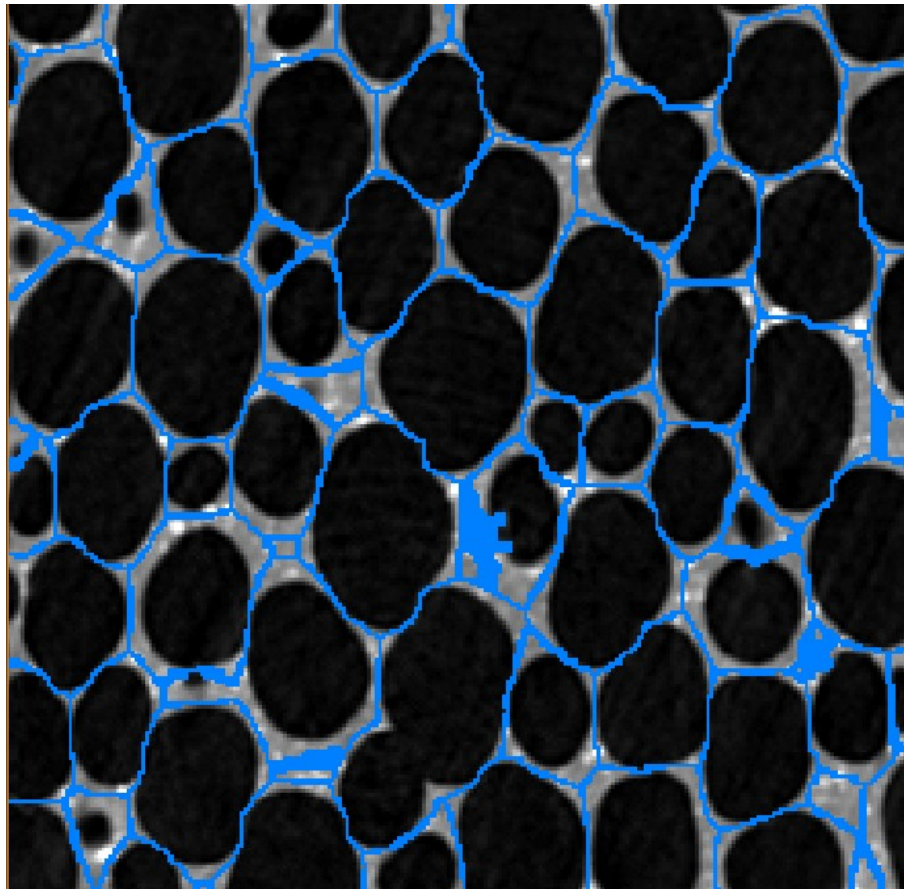
Markers



Example of workflow using watershed

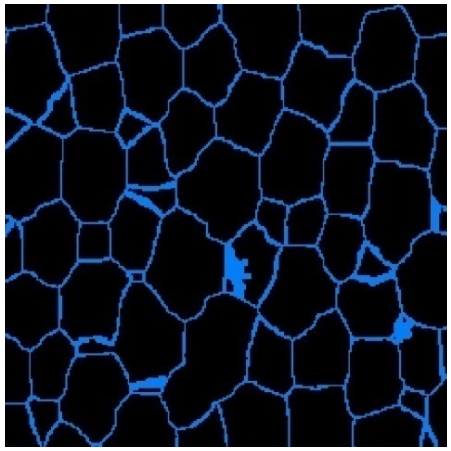
Watershed lines –
boundaries between regions

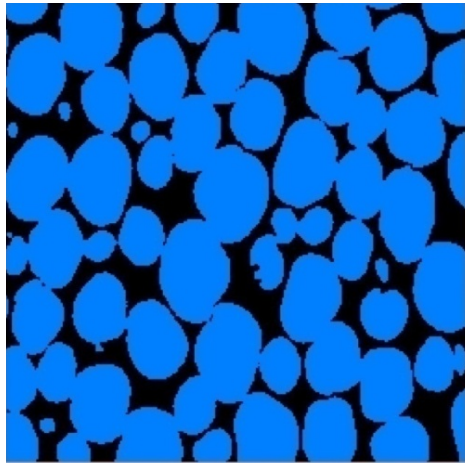


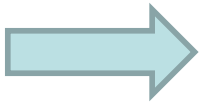


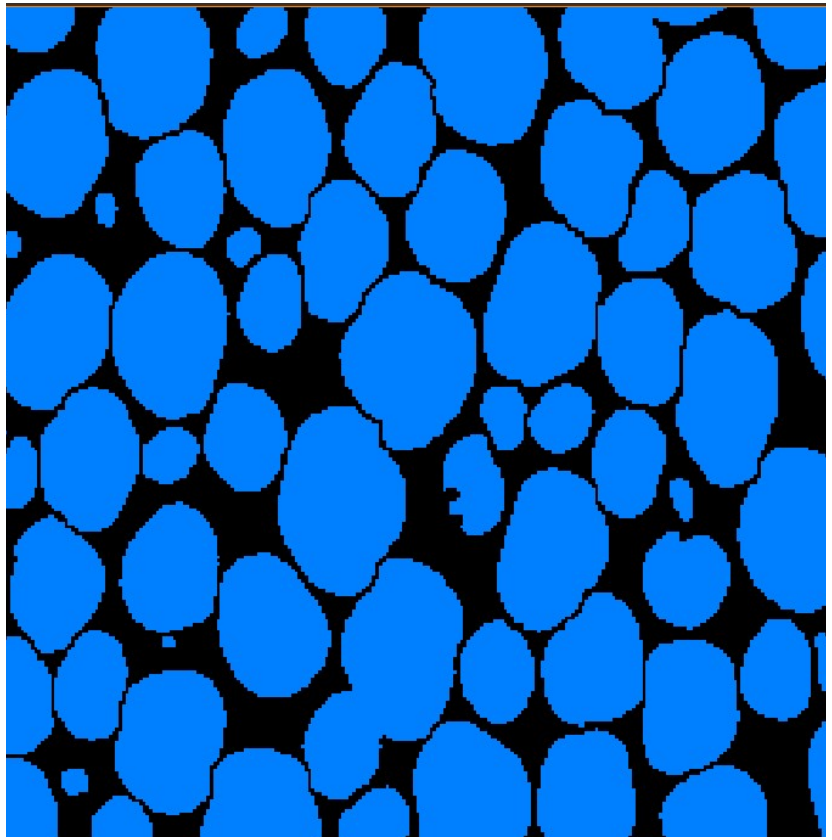
Example of workflow using watershed

Subtraction of watershed
lines gives separated pores



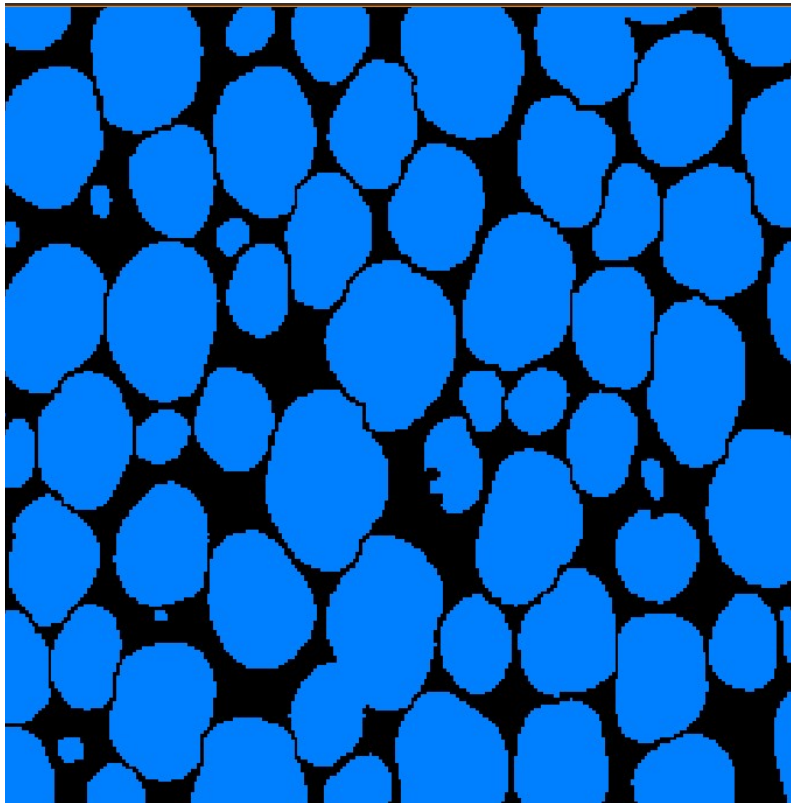


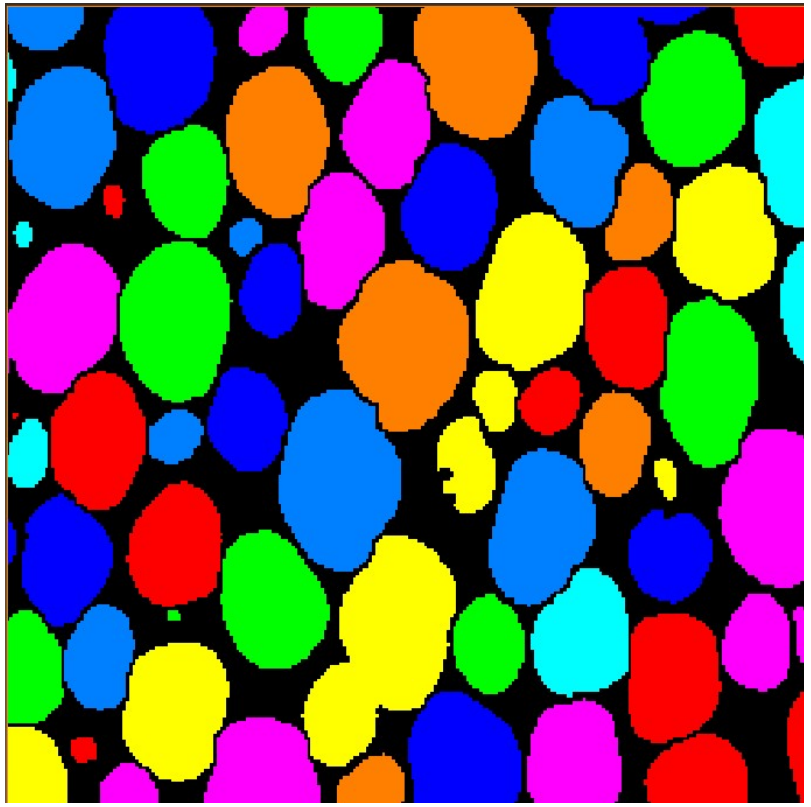




Example of workflow using watershed

Labeling of connected components





Best tools

- Fiji (imageJ)
- Matalb
- Python