



FACULTAD DE MEDICINA
UNIVERSIDAD DE CHILE

ICBM
INSTITUTO
DE CIENCIAS
BIOMÉDICAS

CIMT
CENTRO DE
INFORMÁTICA MÉDICA
Y TELEMEDICINA

LA SERENA SCHOOL
FOR DATA SCIENCE
Applied Tools for Data-driven Sciences

• AURA Campus
La Serena - Chile

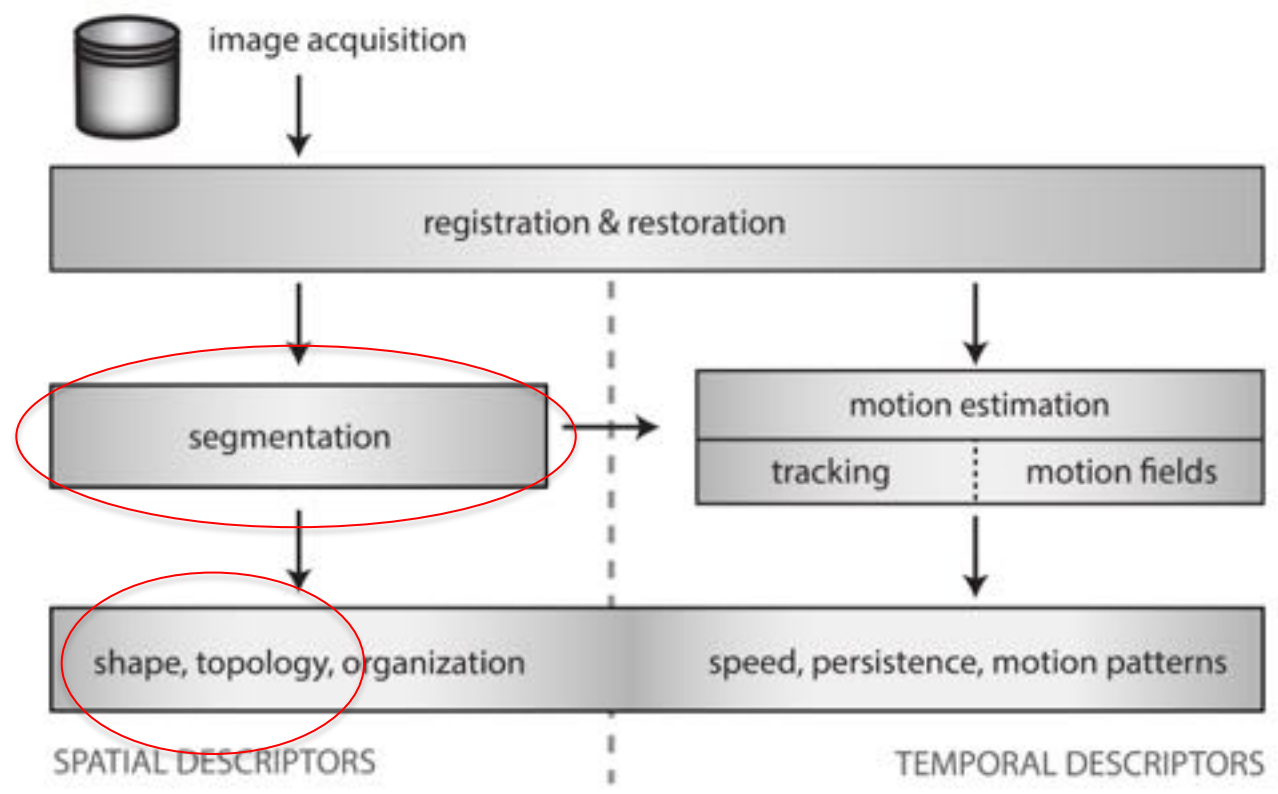
Image Processing 1

images, segmentation, shape

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1. Introduction

- Images
- Segmentation

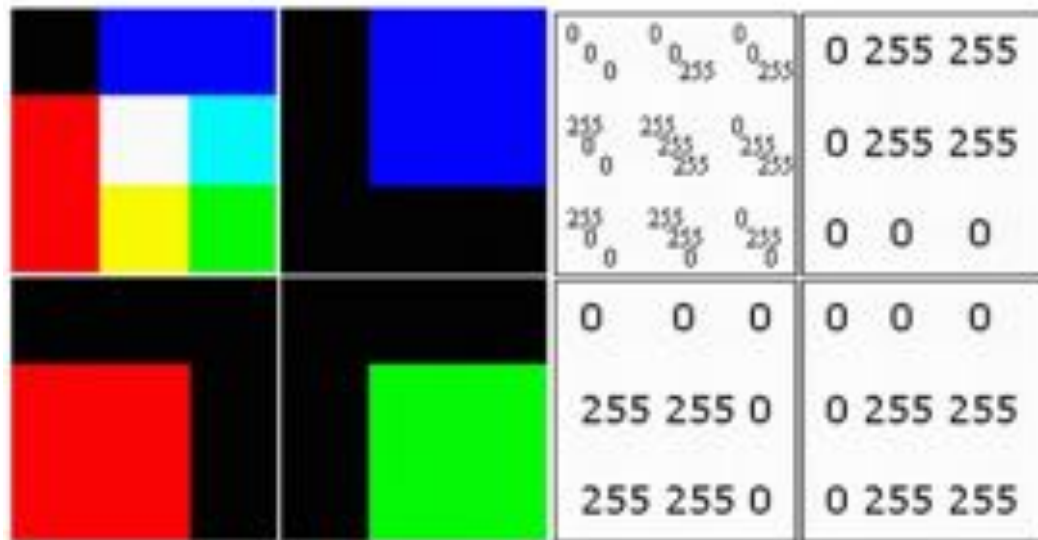
2. Descriptors

- Shape

- A **digital image** can be defined as a function over a discrete space

- A typical 2D image model is the **raster image**: array (matrix) of **pixels** in cartesian coordinates (x, y)

- A numeric value for **brightness (intensity)** or **color** is associated to each pixel



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

$$(x, y) \in [0, \dim_x - 1] \times [0, \dim_y - 1]$$

$$I[x_i, y_j] = f[x_i, y_j]$$

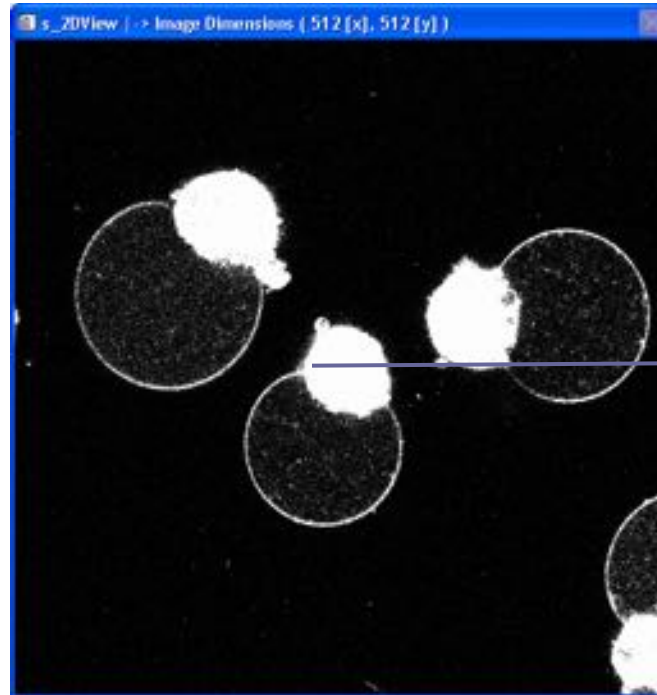
- Greyscale image
 - A brightness (intensity) level is defined for each pixel

0	85	85
85	255	170
85	170	85

$I[x,y]$



Binary value	Decimal value
0000 0000	0 (black)
0000 0001	1
0000 0010	2
0000 0011	3
0000 0100	4
0000 0101	5
0000 0110	6
0000 0111	7
0000 1000	8
...	...
1111 1011	251
1111 1100	252
1111 1101	253
1111 1110	254
1111 1111	255 (blanco)



$I(290,267) = 220$

8 bit greyscale image

A n bit greyscale image encodes up to 2^n intensity values

- RGB image
 - Three channels for respective primary colors: Red, Green, Blue

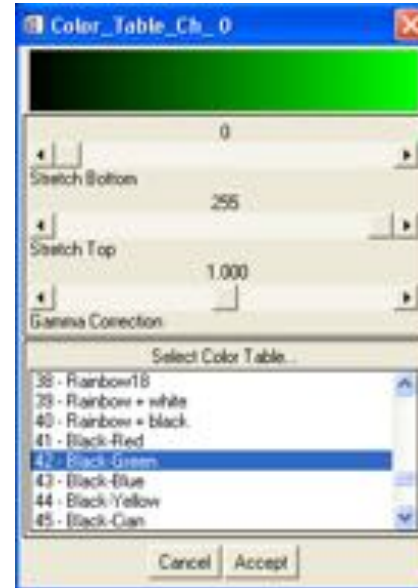
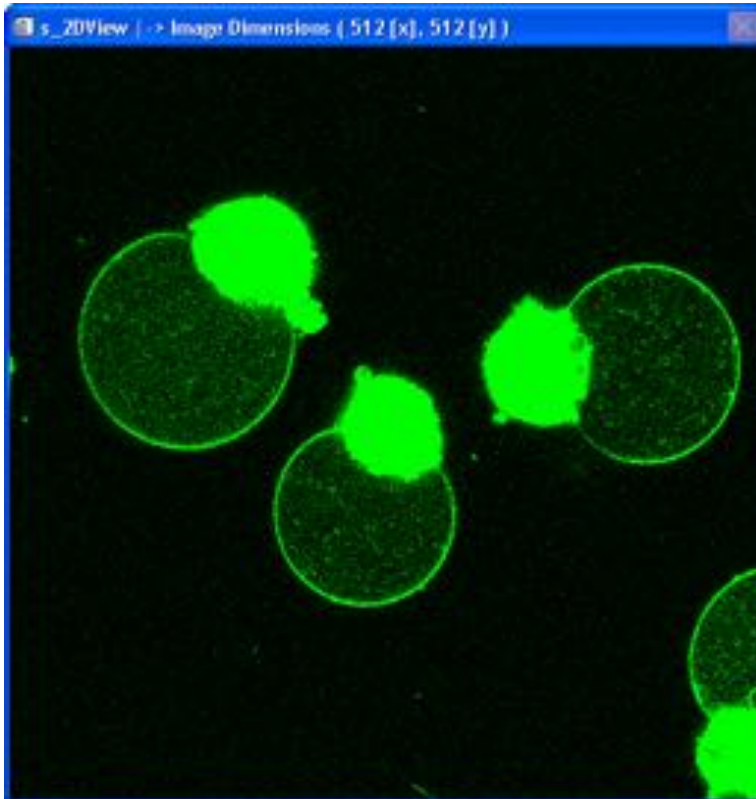


0	0	0
0	0	0
0	255	255
255	255	0
0	255	255
0	255	255
255	255	0
0	255	255
0	0	0

$$r[x, y] \quad g[x, y] \quad b[x, y]$$

- Other color spaces are HSV, LAB

- It is possible to define color tables (or lookup tables, LUTs) for visualization purposes. A grayscale image can be displayed using a green scale.

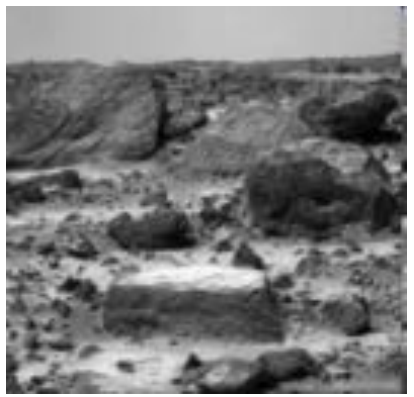


	r	g	b
0	0	0	0
0	0	1	0
0	0	2	0
:	:	:	:
:	:	:	:
:	:	:	:
:	:	:	:
0	0	200	0
:	:	:	:
:	:	:	:
0	0	255	0

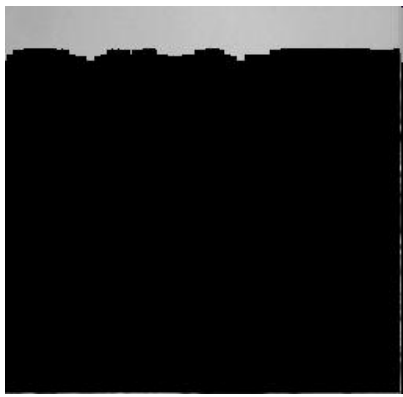
- Segmentation
 - The partitioning of a given image into regions of interest (ROIs) according to given criteria (e.g. color).
 - After segmentation, further characterizations can be performed upon the resulting ROIs.

Shapiro LG and Stockman GC (2001):
“Computer Vision”, pp 279-325
New Jersey, Prentice-Hall

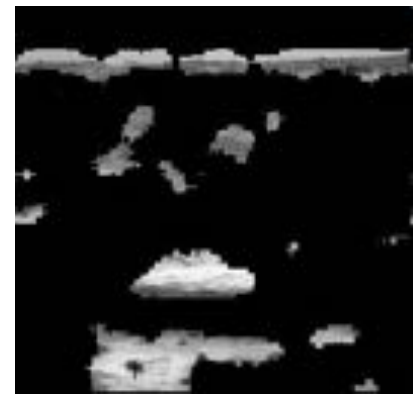
Segmentation



Sol 3, Mars
Pathfinder Mission

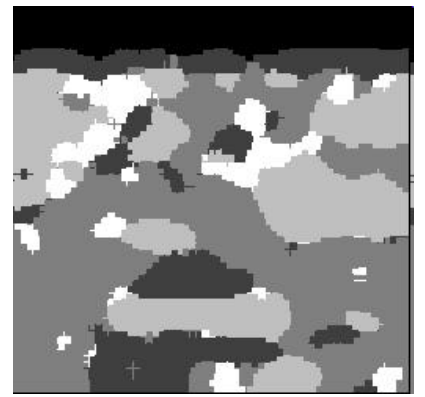


Sky / Flat



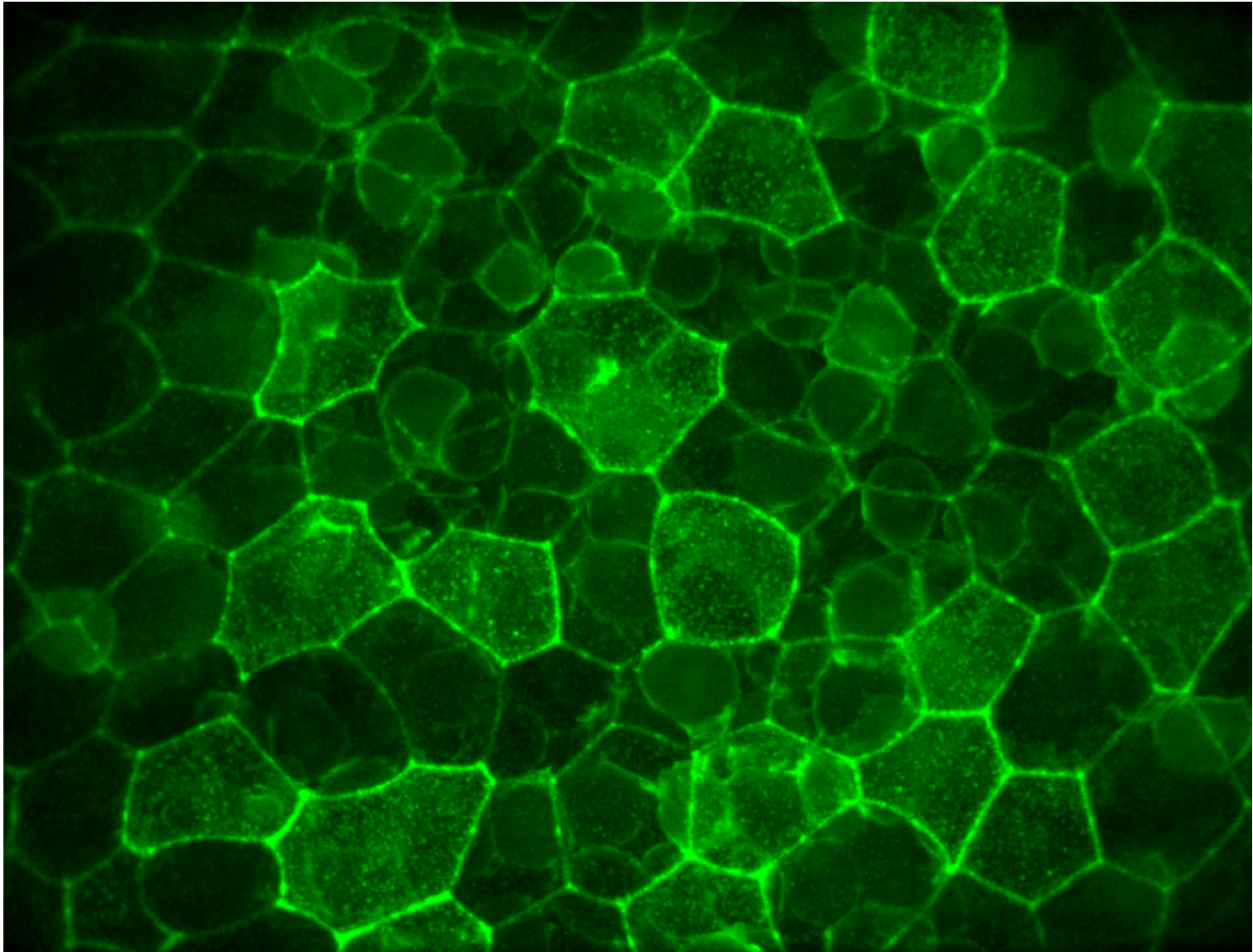
Dust / Horizon

...etc...



Final segmentation

Segmentation



Max Z-Project from confocal microscopy of Fundulus N. [data by German Reig 2015]

Problems

- Lack of absolute criteria or standards (Ground Truth, Gold Standard [1,2])
- Missing or erroneous information (e.g. non-specific markers in samples)
- **What to do? A “good” (i.e. carefully performed and controlled) acquisition ease this process**

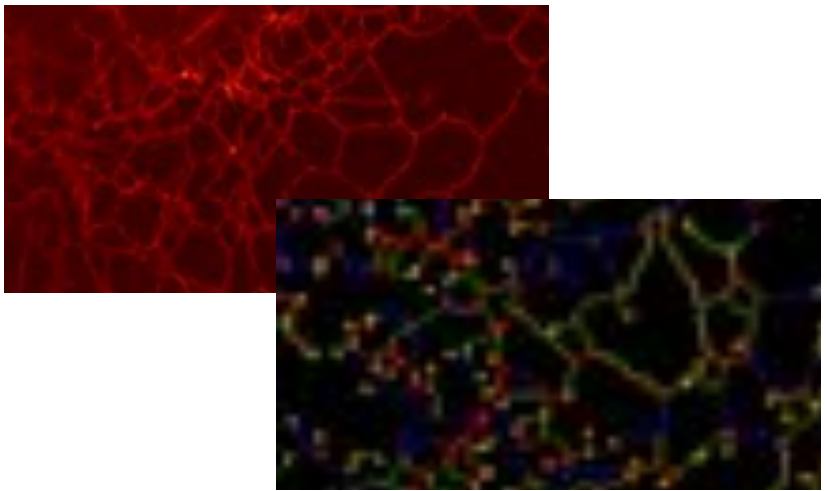
[1] Jason D. Hipp et al. Tryggo: Old Norse for truth: The real truth about ground truth. New insights into the challenges of generating ground truth maps for WSI CAD algorithm evaluation. *Pathol. Inform* 2012, 3:8

[2] Luc Bidaut, Pierre Jannin. Biomedical multimodality imaging for clinical and research applications: principles, techniques and validation. In *Molecular Imaging: Computer Reconstruction and Practice* (NATO Science for Peace and Security Series B: Physics and Biophysics), Springer, 2008, ISBN-13: 978-1402087516.

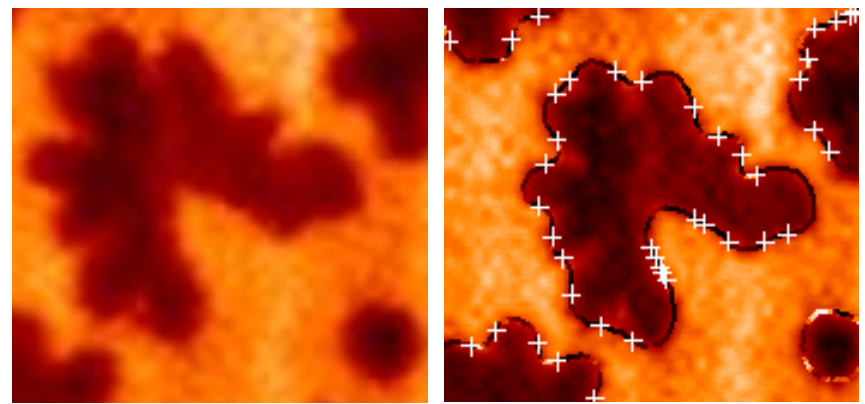
- Segmentation is the first step toward further quantifications

Parameter estimation...

- Size: area, perimeter
- Boundary: inflections, shape
- Topology: connectivity, endpoints



Endoplasmic reticulum in a COS-7 cell
O Ramirez, L Alcayaga (2012)

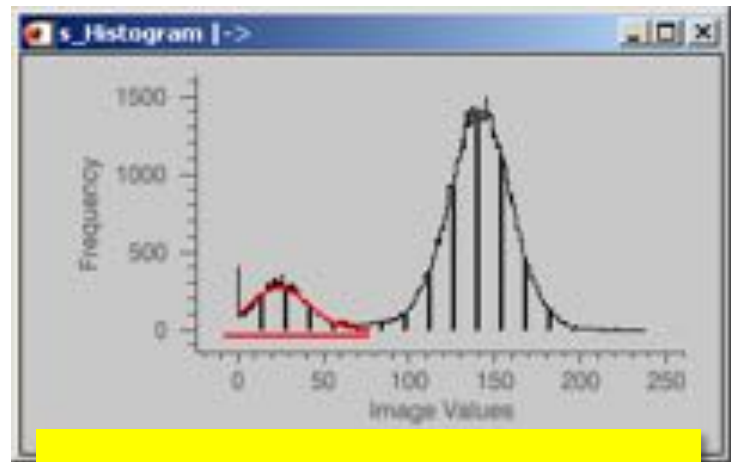
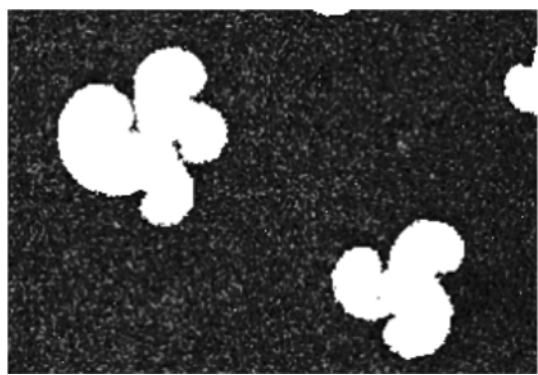
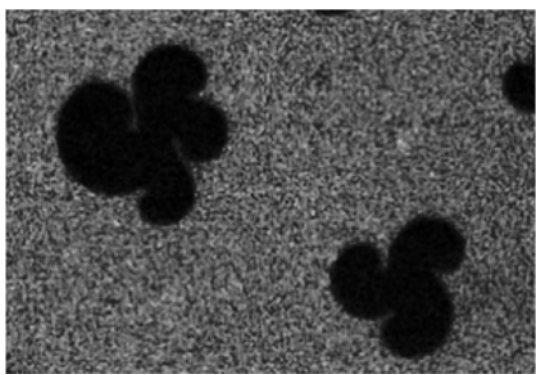


Lipid monolayers
J Jara (2006), Fanani et al (2010)

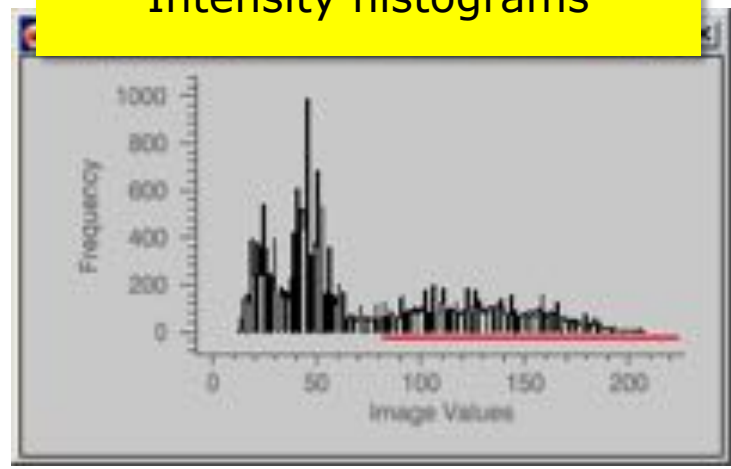
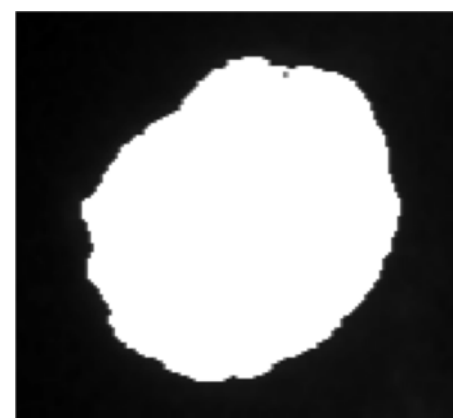
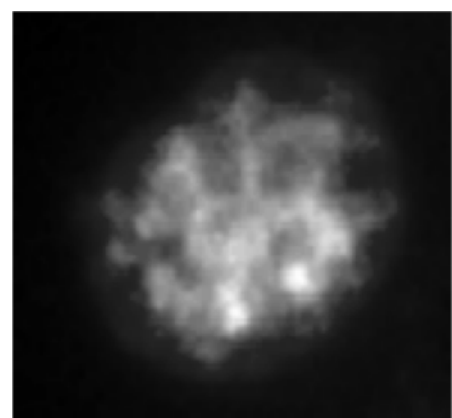
1. Classic approaches (filters)
 - Thresholding
 - Matrix convolution filters + threshold
 - Mathematical morphology

2. Advanced approaches
 - Shape priors (*pattern matching*)
 - Deformable models (active contours)
 - parametric
 - implicit

- Threshold filter segmentation: ROIs (white) / background (black)



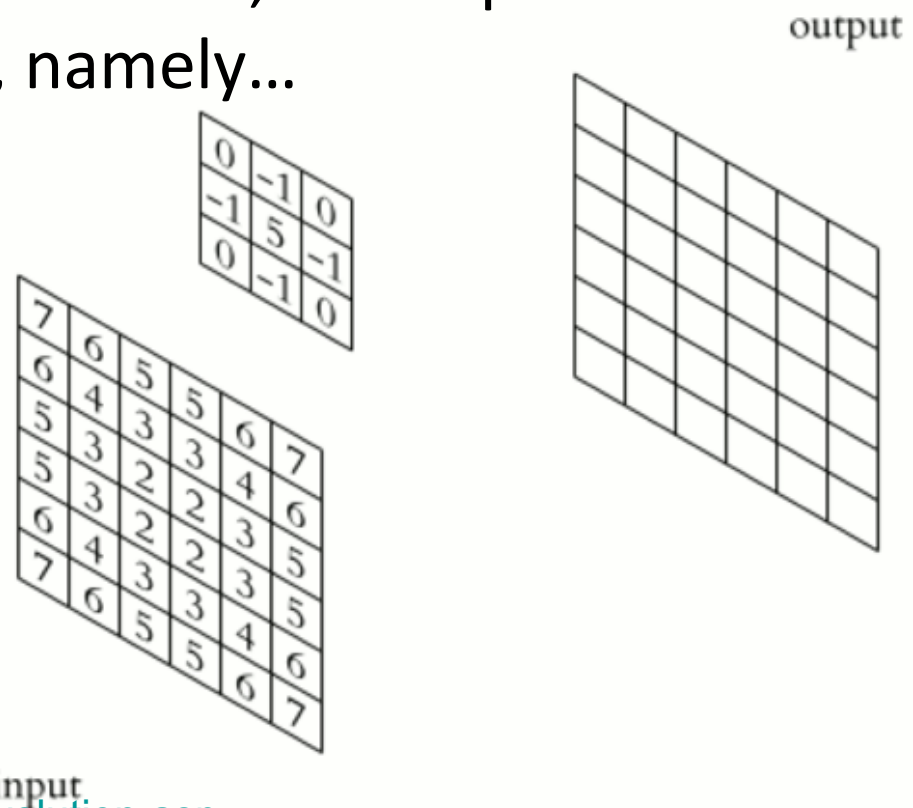
Intensity histograms



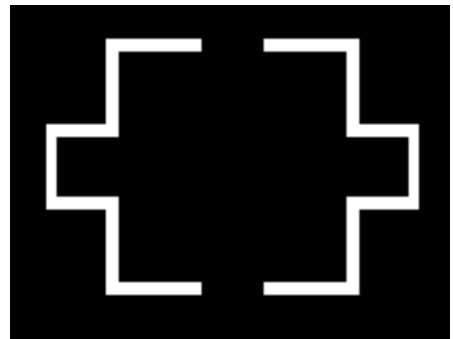
- Convolution
 - Lots of filters based on this principle
<http://en.wikipedia.org/wiki/Convolution>

- **Matrix convolution**, in our case, is an operation between two matrices, namely...

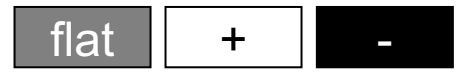
- the input image, I
- a *kernel*, K



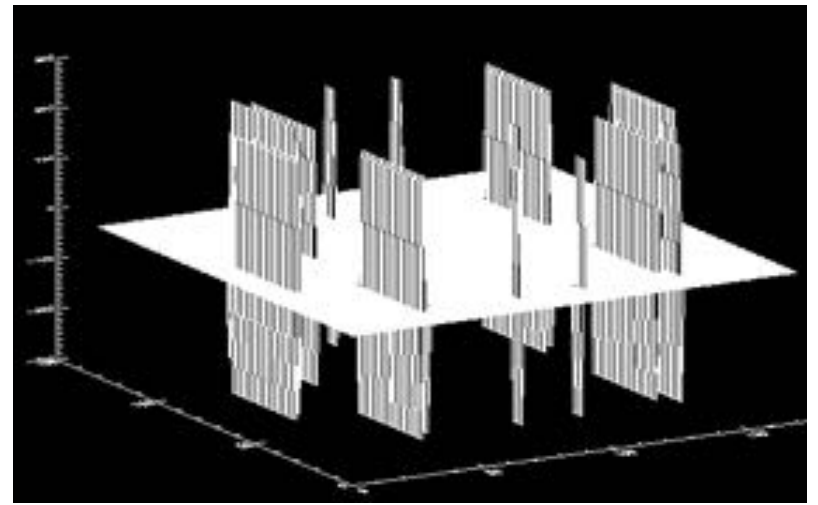
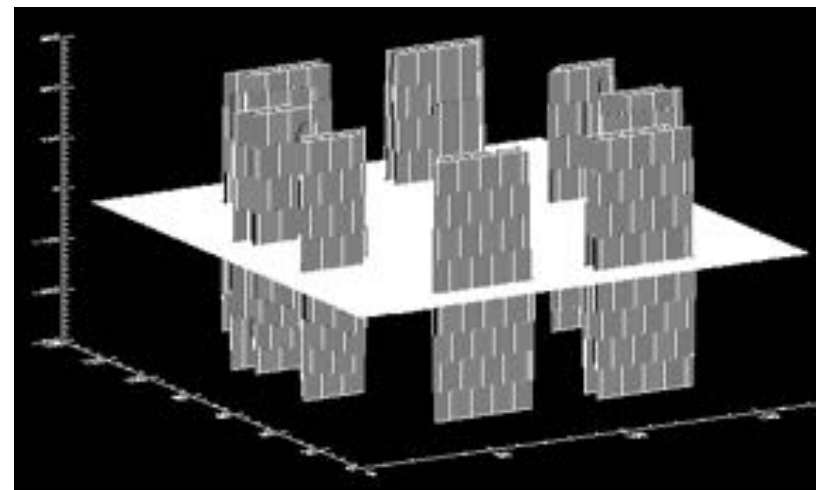
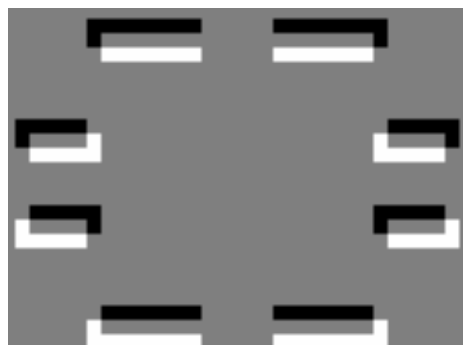
- Intensity gradients (discrete approximation)



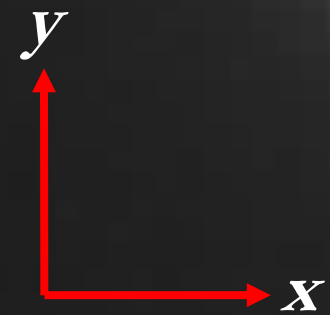
$$\frac{\partial I}{\partial x} \approx$$



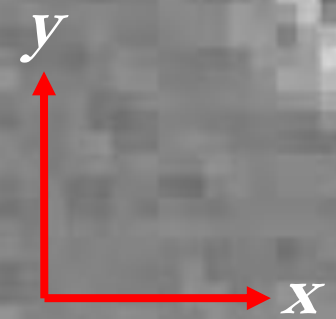
$$\frac{\partial I}{\partial y} \approx$$



$$I = I(x, y)$$

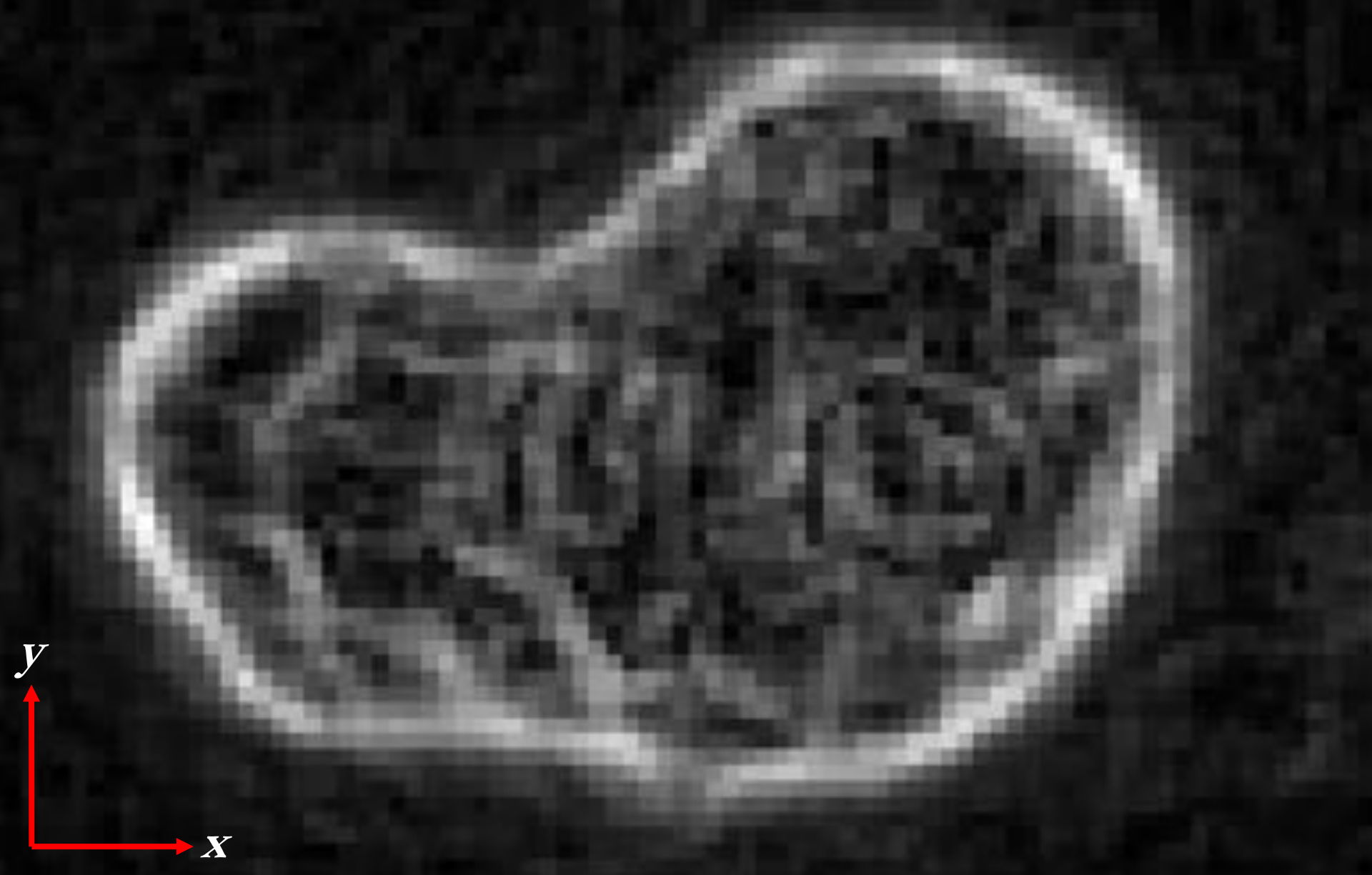


I_y



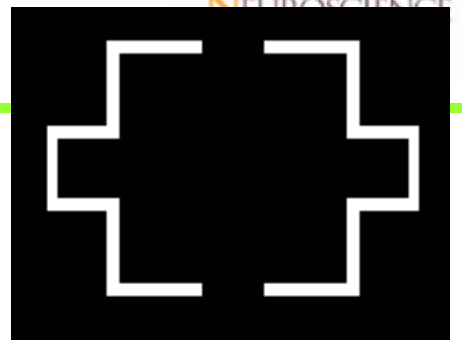
“Edgemap”

$$|\nabla I| = |I_x| + |I_y|$$



Segmentation – basics

- Intensity gradients (discrete approximation)

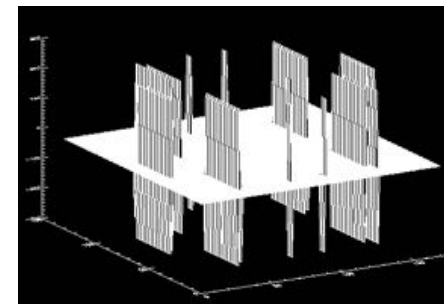


$$I = I(x, y)$$

$$\frac{\partial I}{\partial x} \approx \frac{I(x + \Delta x, y) - I(x, y)}{\Delta x} = K_x \otimes I$$

$\Delta x = 1 \text{ pixel}$

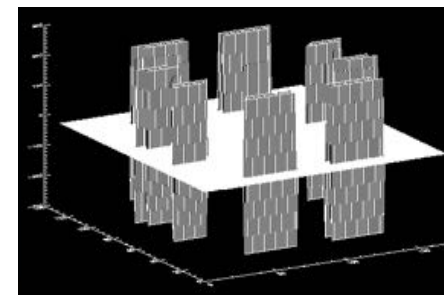
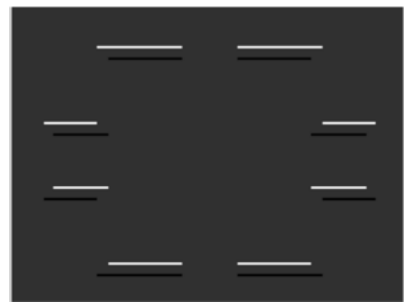
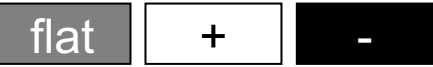
$$K_x = \begin{Bmatrix} 0 & 0 & 0 \\ 0 & -1 & 1 \\ 0 & 0 & 0 \end{Bmatrix}$$



$$\frac{\partial I}{\partial y} \approx \frac{I(x, y + \Delta y) - I(x, y)}{\Delta y} = K_y \otimes I$$

$\Delta y = 1 \text{ pixel}$

$$K_y = \begin{Bmatrix} 0 & 1 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 0 \end{Bmatrix}$$



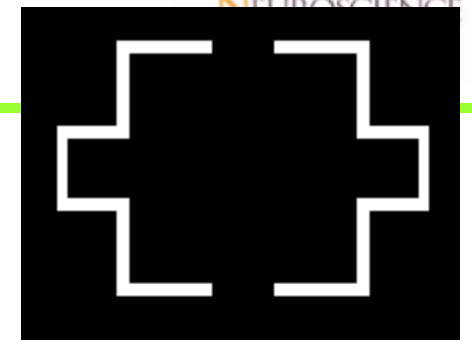
- Kernels...

Laplace

$$\nabla^2 I = \frac{\partial^2 I}{\partial x^2} + \frac{\partial^2 I}{\partial y^2}$$

$$\nabla^2 I \approx \frac{f(x + \Delta x, y) - 2f(x, y) + f(x - \Delta x, y)}{(\Delta x)^2} + \frac{f(x, y + \Delta y) - 2f(x, y) + f(x, y - \Delta y)}{(\Delta y)^2}$$

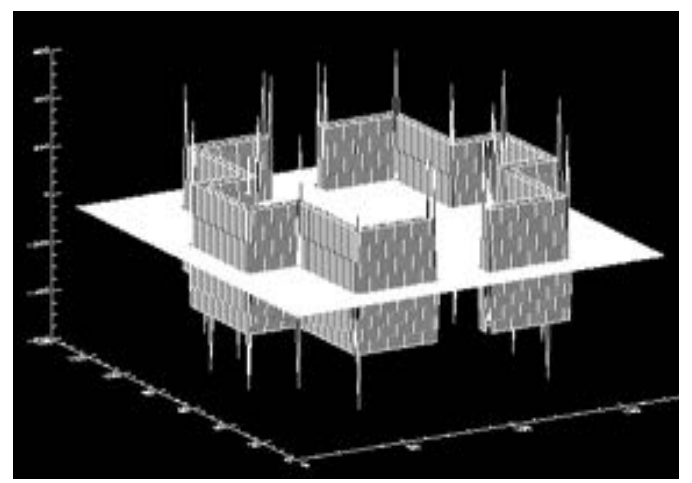
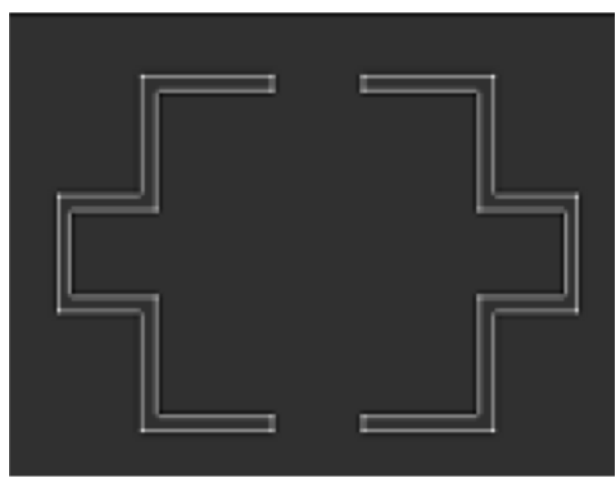
$$\nabla^2 I \approx \frac{f(x + \Delta x, y) + f(x, y + \Delta y) - 4f(x, y) + f(x - \Delta x, y) + f(x, y - \Delta y)}{(\Delta x)^2} = K_L \otimes I$$



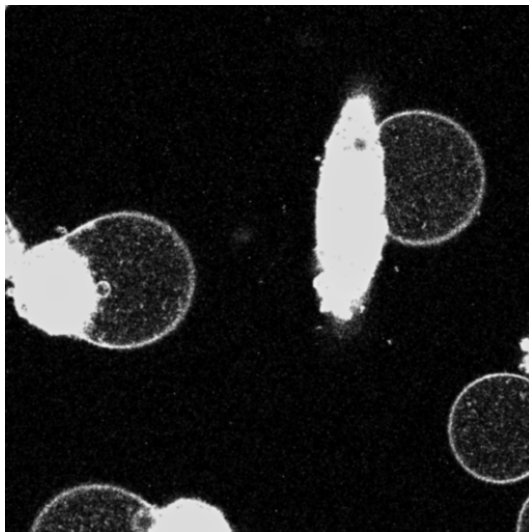
$I = I(x, y)$

$\Delta x = \Delta y = 1$ pixel

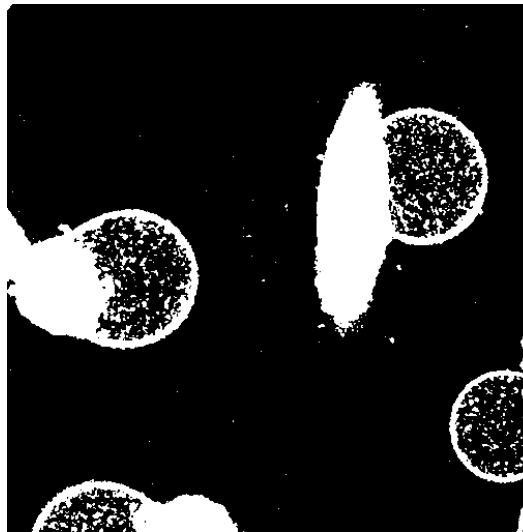
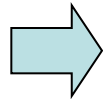
$$K_L = \begin{Bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{Bmatrix}$$



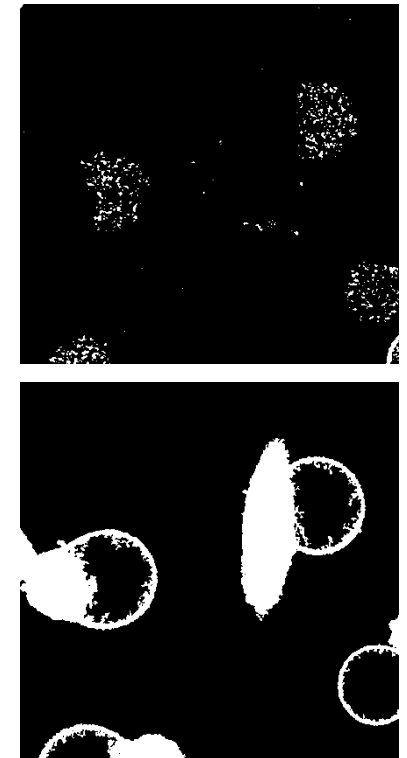
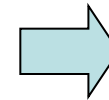
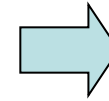
- Morphology based filters
 - Example: size selection



Input greyscale image



After thresholding...



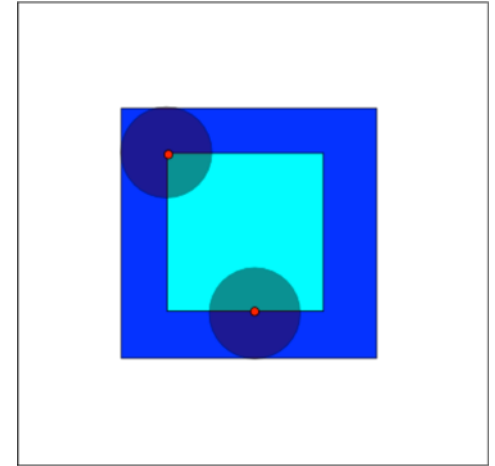
Size selection

How to define a size-select algorithm?

- Mathematical morphology

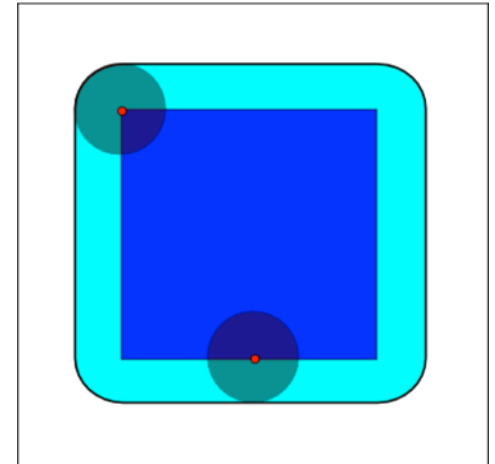
Erode

$$A \ominus B = \{z \in \mathbb{R}^2 \mid B_z \subseteq A\}$$



Dilate

$$A \oplus B = \bigcup_{b \in B} A_b$$



What is B?

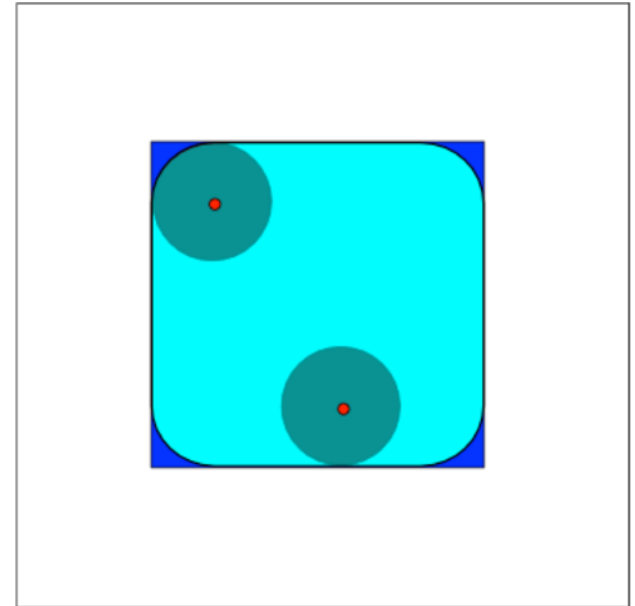
- Mathematical morphology

To open:

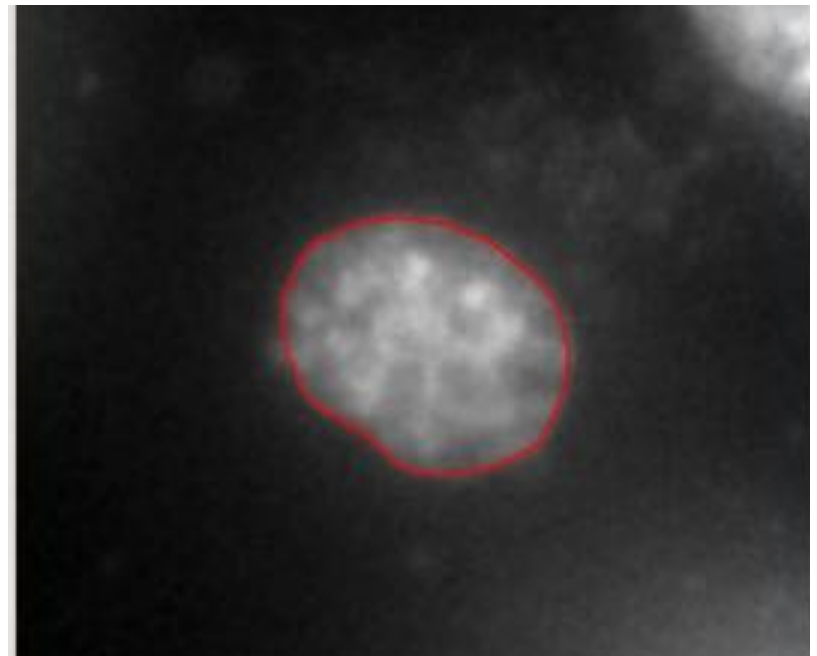
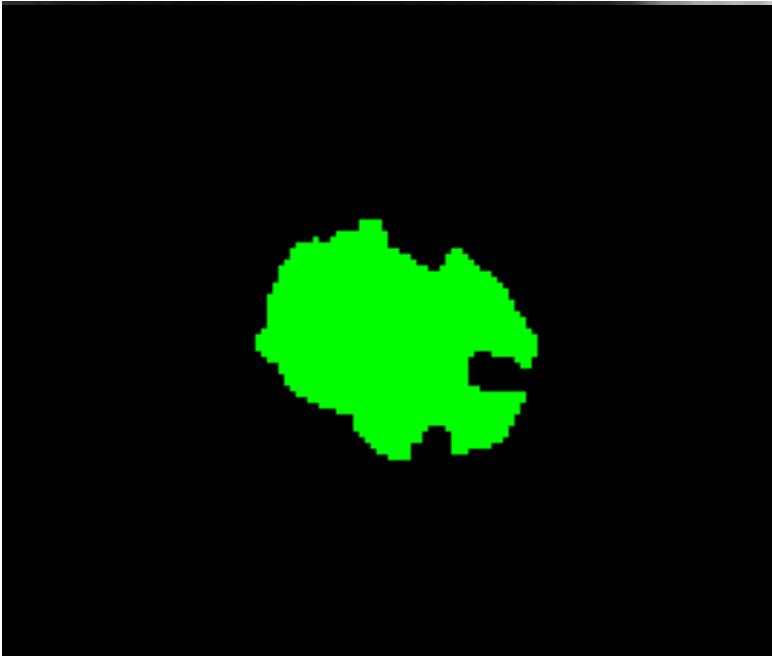
$$A \circ B = (A \ominus B) \oplus B$$

To close:

$$A \bullet B = (A \oplus B) \ominus B$$



- “Some” times more information is needed in order to achieve a good segmentation (see additional material)



1. Introduction

- Images
- Segmentation

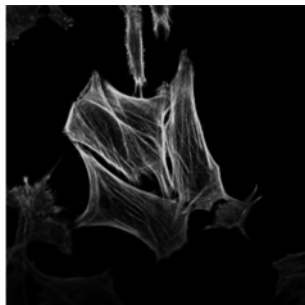
2. Descriptors

- Shape

Shape descriptors

Ex. 1

Shape and structure analysis of fibroblast actin fibers (astrocytes)



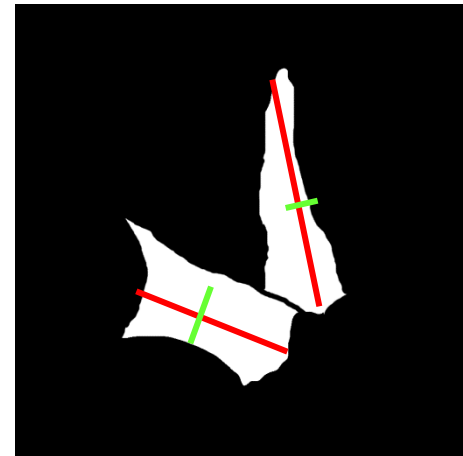
Ex. 2

Zebrafish parapineal organ neuron



1. Geometrical descriptors: location, perimeter, area, volume, curvature
2. Moments of morphology (order 0-2)
3. Topology in computer science (skeletons)

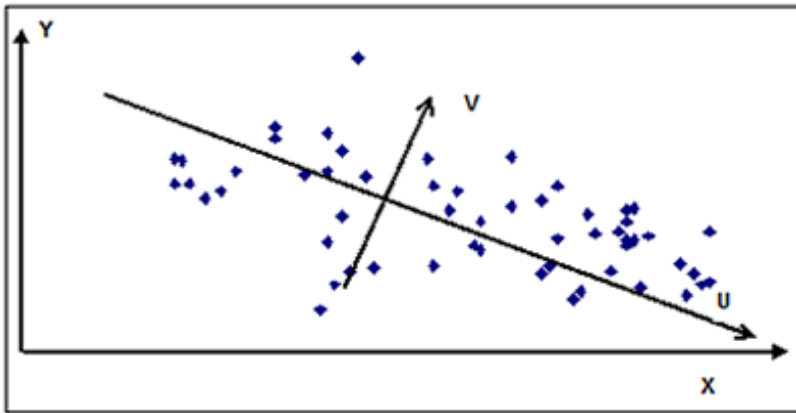
Location, perimeter, area and volume partially describe an object, but not its shape.



Original image (2D),
Prof. Lissette Leyton

How to quantify the amount of roundness of an object?

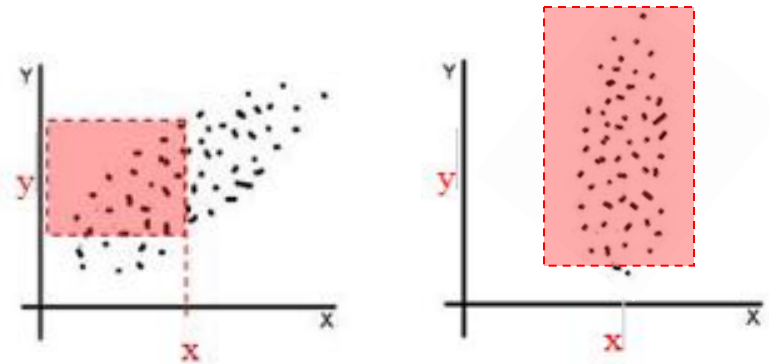
Variance



Axes U and V maximize **the variance in U**

$$\sigma_x^2 = \frac{\sum (x - \bar{x})^2}{N}$$

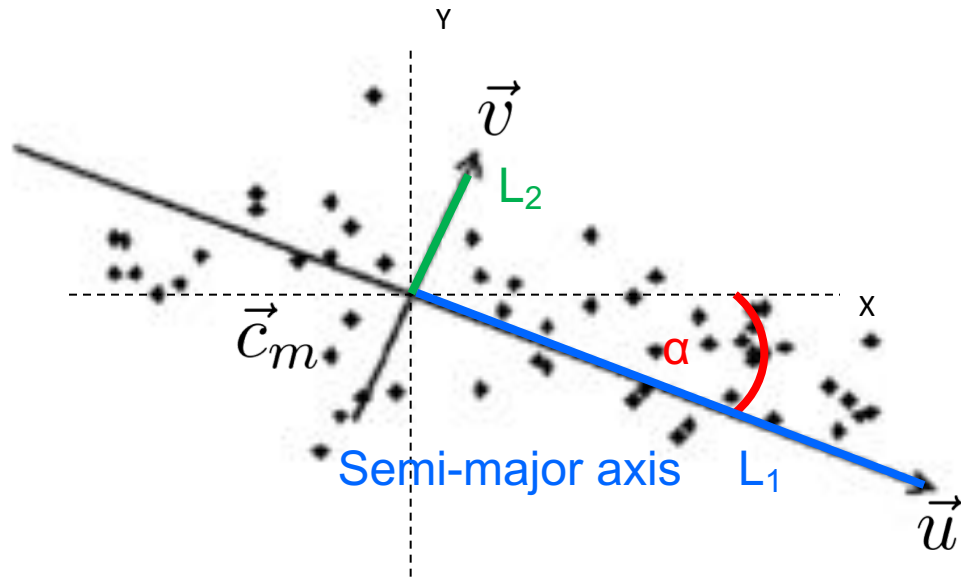
Covariance



Positive covariance Zero covariance

$$\sigma_{xy}^2 = \frac{\sum (x - \bar{x})(y - \bar{y})}{N}$$

We look for two parameters to characterize the binary ROI...

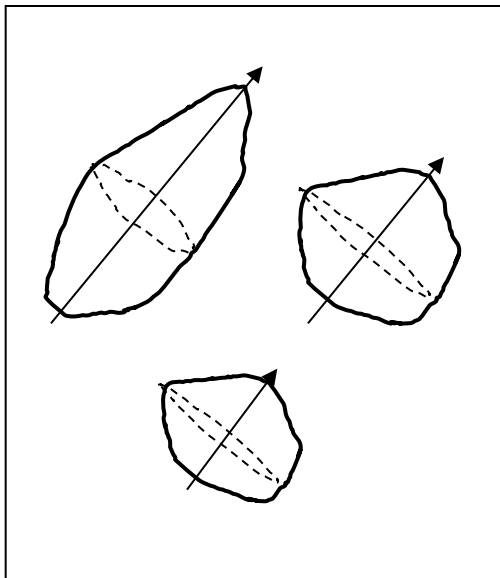


- If the variance/covariance matrix is diagonal for a certain rotation α ,
- Semi-major axis length l is a function of second-order moments

$$L_1^2 = \frac{1}{2} \left(\sigma_{xx}^2 + \sigma_{yy}^2 + \sqrt{(\sigma_{xx}^2 - \sigma_{yy}^2)^2 + 4(\sigma_{xy}^2)^2} \right)$$

$$L_2^2 = \frac{1}{2} \left(\sigma_{xx}^2 + \sigma_{yy}^2 - \sqrt{(\sigma_{xx}^2 - \sigma_{yy}^2)^2 + 4(\sigma_{xy}^2)^2} \right)$$

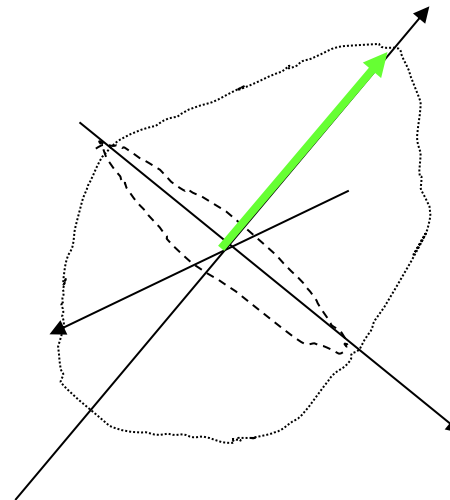
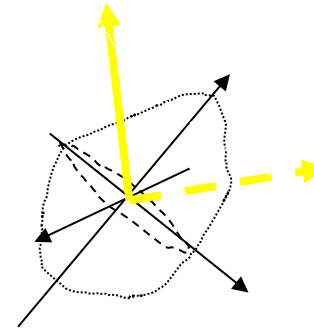
Second order moments of morphology describe a ROI main axis (principal components)



- The **major axis** corresponds to the direction with the biggest variance.
- The **secondary axis (minor in 2D)** is orthogonal to the major axis, giving in 3D the direction of the second biggest variance.
- The **third axis** (3D) is orthogonal to the major and secondary axes.

Principal axes are useful object descriptors because...

- they directly define the object *length, height, and width*
- using principal axes, similarities between objects can be found

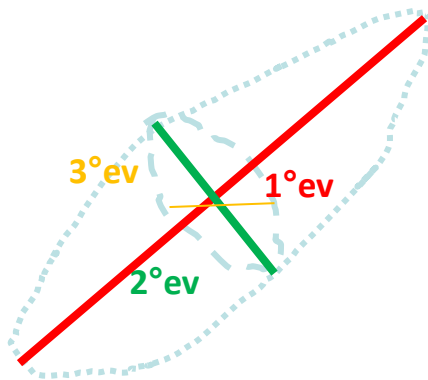


By combining the principal axes, we can define *Elongation*, *Relative Elongation*, and *Flatness*.

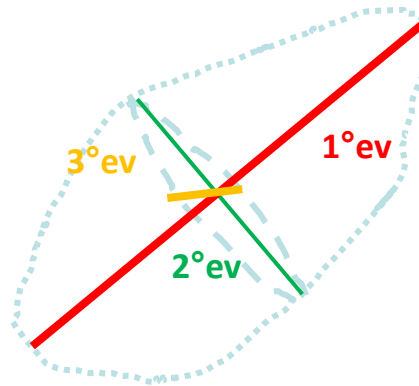
$$Elong = 1 - \frac{2^{\circ}ev}{1^{\circ}ev}$$

$$R.Elong = 1 - \frac{3^{\circ}ev}{1^{\circ}ev}$$

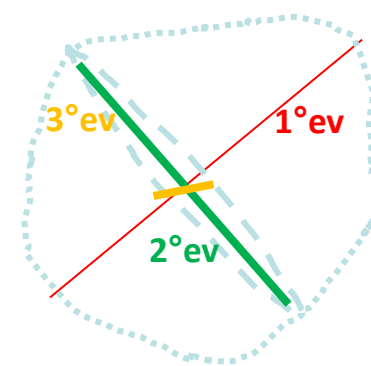
$$Flatness = 1 - \frac{3^{\circ}ev}{2^{\circ}ev}$$



$1^{\circ}ev \gg 2^{\circ}ev$
 Elong. ~ 1



$1^{\circ}ev \gg 3^{\circ}ev$
 R. Elong. ~ 1



$2^{\circ}ev \gg 3^{\circ}ev$
 Flatn. ~ 1

- The variance allows to compute principal axes (eigenvectors) and to quantify dispersion for each principal axis direction (eigenvalues)
- Higher order moments describe more detailed information like asymmetry or kurtosis
- Composed parameters between eigenvalues deliver morphological parameters like elongation or flatness
- **EXERCISE: Compute elongation [python notebook]**