



$$\left(\partial_\theta^2 + \cot \theta \partial_\theta - \frac{1}{\sin^2 \theta} + \frac{1}{2} - \frac{R^2}{l^2}\right) \frac{v_\theta}{R} = -\frac{\partial_\theta c_\theta(\theta)}{4\eta_\theta^2}$$

MARCH⁵⁻¹⁴
2024

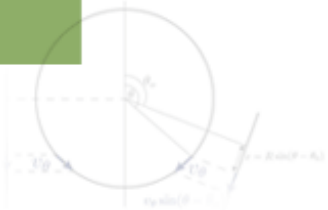


CELL MORPHODYNAMICS
LATIN AMERICA

INTERNATIONAL COURSE

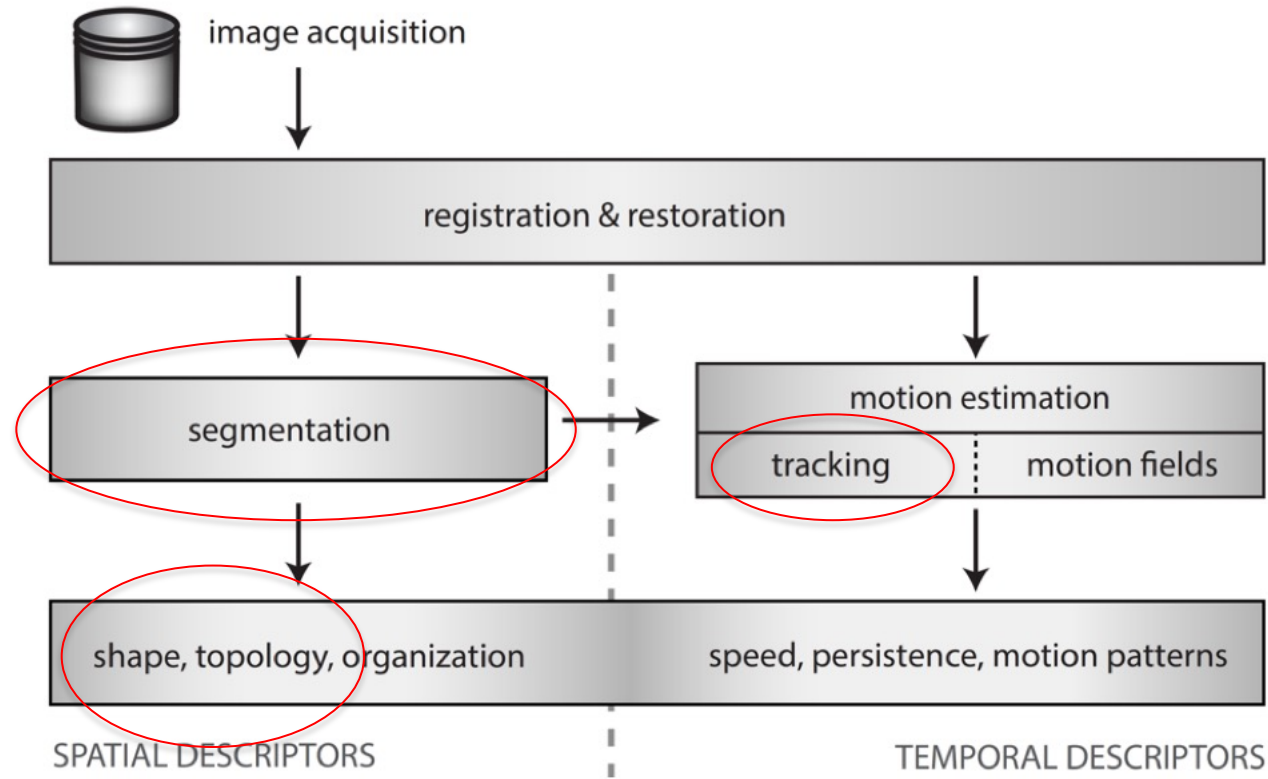
Optics, Forces & Development

SANTIAGO / CHILE



Principles of Image Processing & Quantification: image formation, segmentation

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www.scian.cl



Castañeda V, Cerda M et al, 2014

Computational Methods for Analysis of Dynamic Events In Cell Migration.
 Current Molecular Medicine, 14(2), 291-307.

1. Introduction

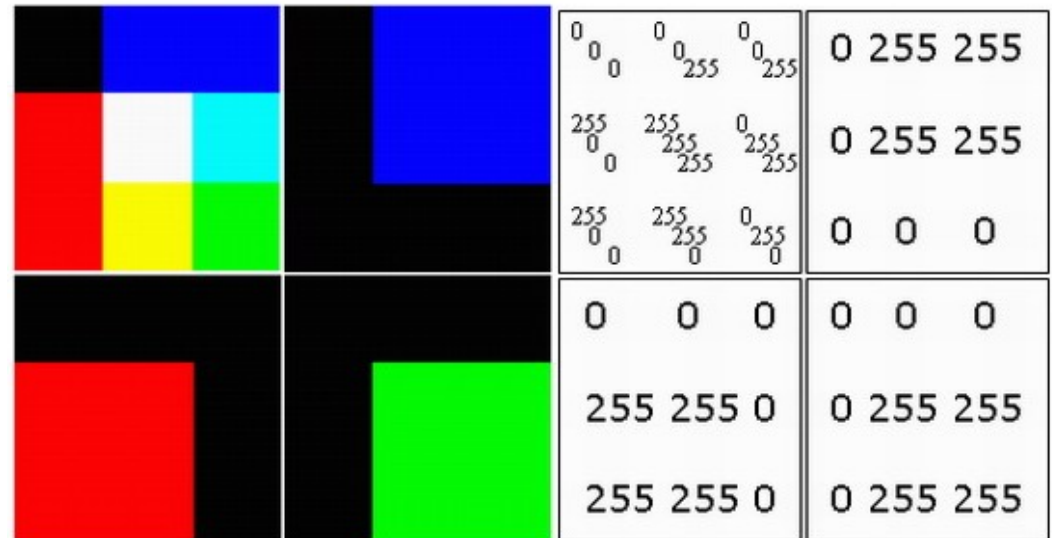
- Digital imaging
- Segmentation*

2. Descriptors

- Shape & Topology*
- *Tracking*

- 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



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

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

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

- ...so, a digital image can be treated as a **function** (in the mathematical sense)...
 - on a discrete domain
 - with numeric values associated to each elements, representing a property (such as color, brightness, depth, etc.)

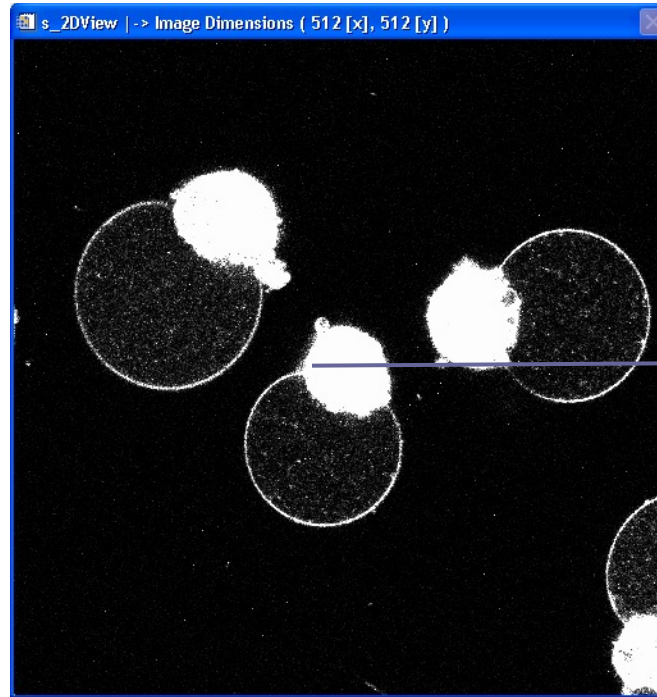
- Greyscale image
 - A brightness (intensity) level is defined for each pixel

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)

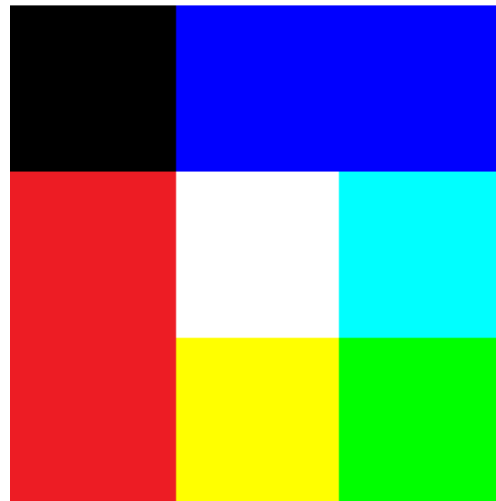


$I(290,267) = 220$

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

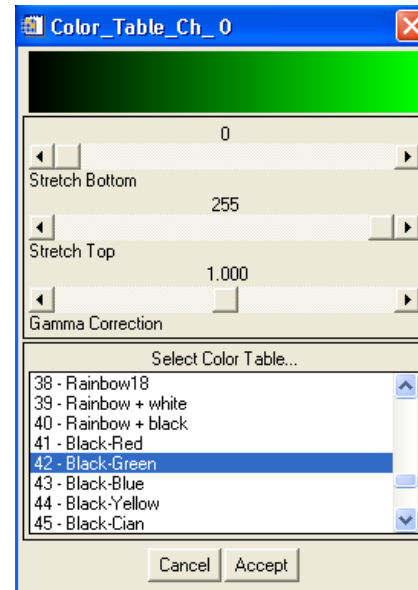
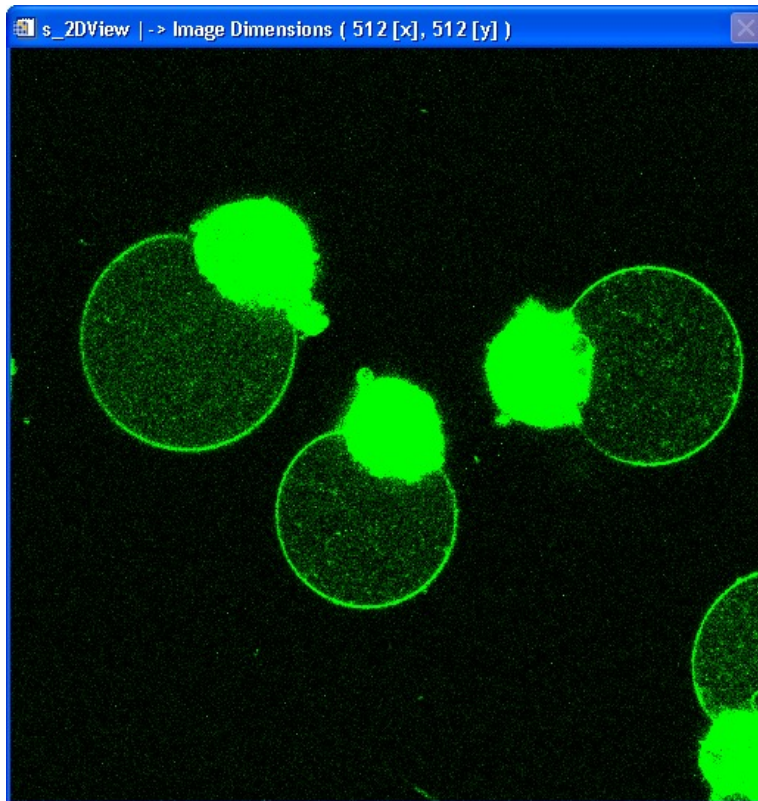


0	0	0
0	0	0
0	255	255
255	255	0
0	255	255
0	255	255
255	255	0
0	255	255
0	0	0

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

- 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.

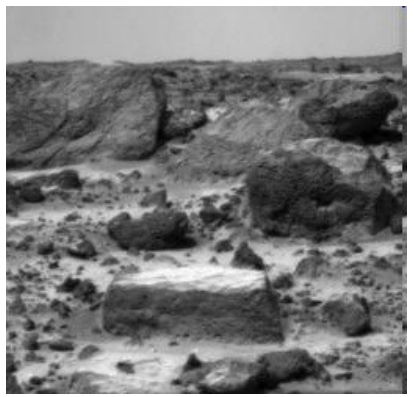


	r	g	b
0	0	0	0
0	0	1	0
0	0	2	0
:	:	:	:
:	:	:	:
:	:	:	:
:	:	:	:
0	0	200	0
:	:	:	:
:	:	:	:
0	0	255	0

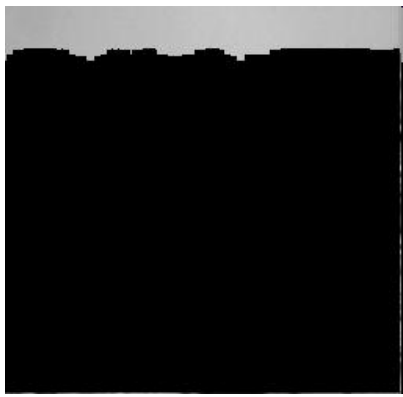
- Segmentation
 - The partitioning of a given image into regions of interest (ROIs) according to given criteria (e.g. color).
 - After segmentation, further characterizations can be performed upon the resulting ROIs.

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

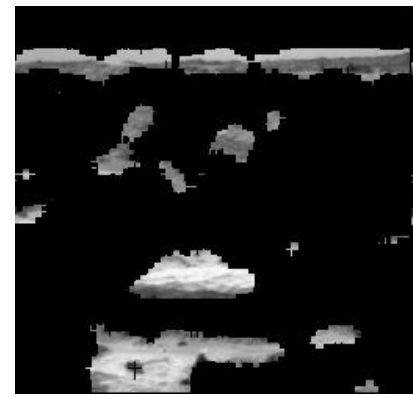
Segmentation



Sol 3, Mars
Pathfinder Mission

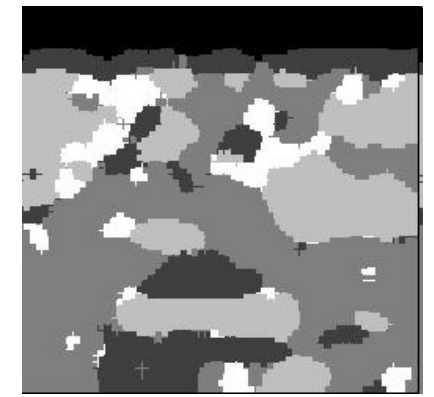


Sky / Flat



Dust / Horizon

...etc...



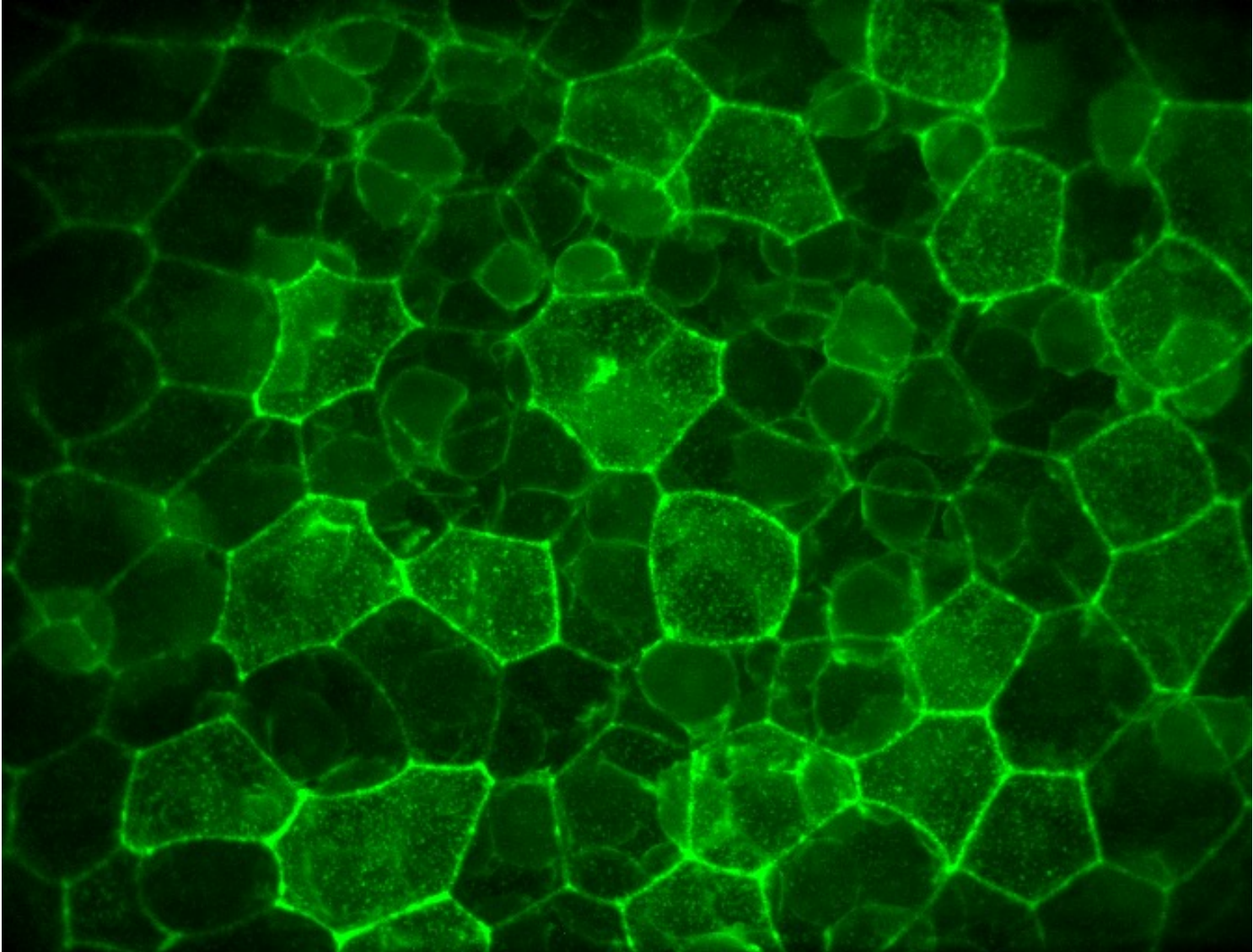
Final segmentation

- Not only objects as ROIs, but features...



Scale Invariant Feature Transformation (SIFT), D Lowe (2004). Image from J Clemons (2009)

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**

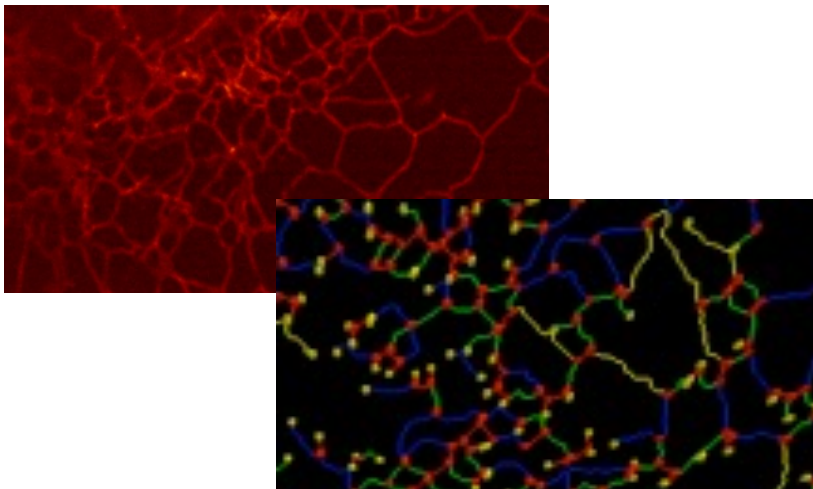
[1] Jason D. Hipp et al. Tryggo: Old Norse for truth: The real truth about ground truth. New insights into the challenges of generating ground truth maps for WSI CAD algorithm evaluation. *Pathol. Inform* 2012, 3:8

[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.

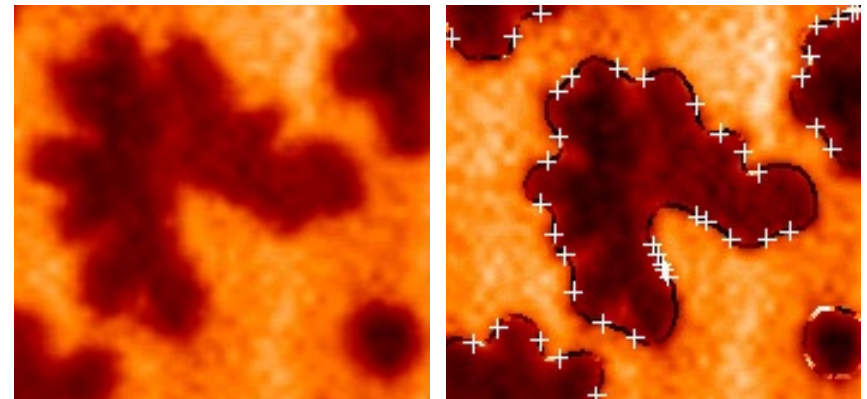
- Segmentation is the first step toward further quantifications
 - In addition to images, ROI models and data structures can suit for different types of descriptions

Parameter estimation...

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



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

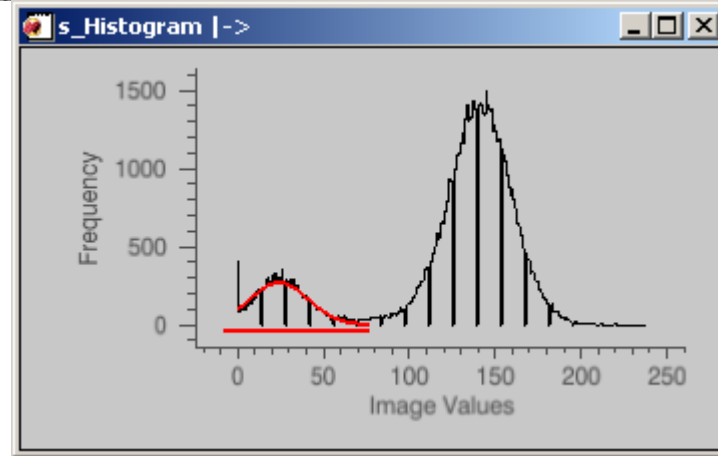
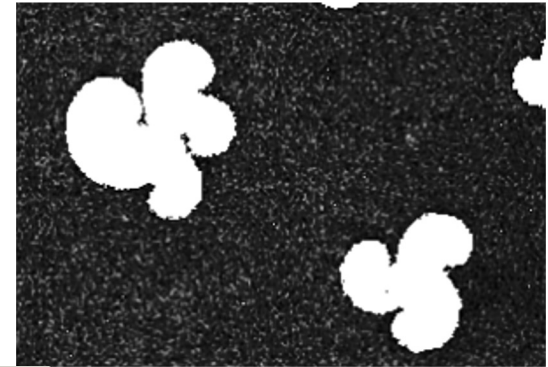
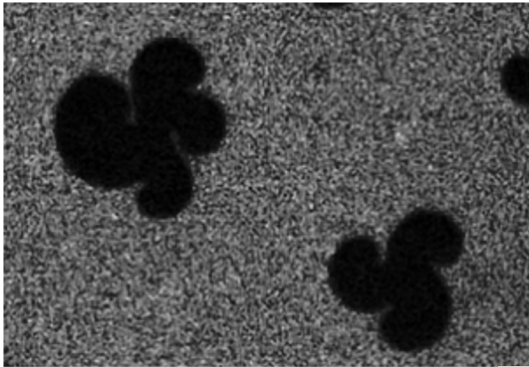


Lipid monolayers
J Jara (2006), Fanani et al (2010)

1. Classic approaches (filters)
 - Thresholding
 - + Convolution filters

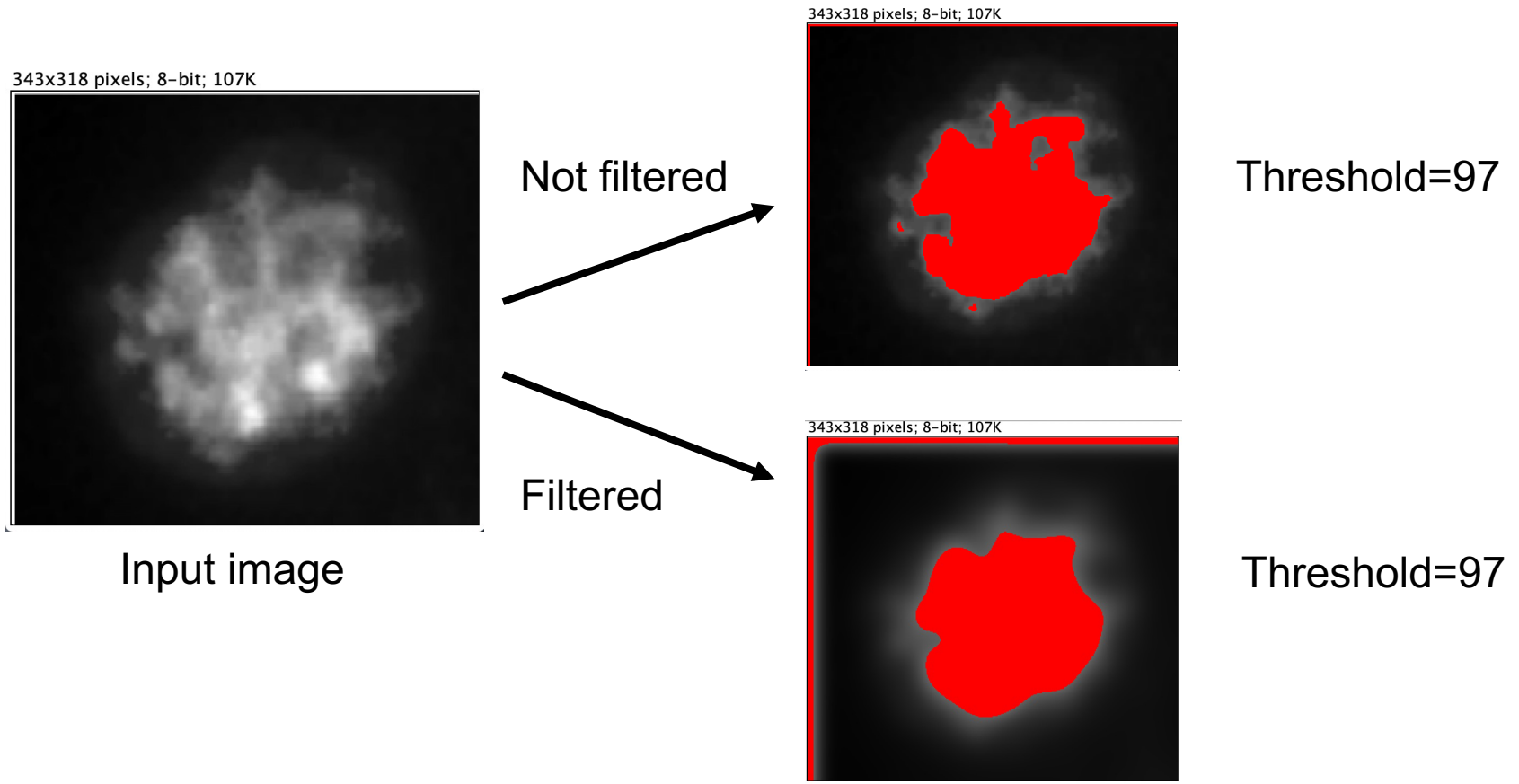
2. Advanced approaches
 - Shape priors (*pattern matching*)
 - Deformable models (active contours)
 - parametric
 - implicit

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



Intensity histograms

- But I may want to highlight a specific structure: e.g. contours only, or inside cell, before segmentation...
- The idea is to apply “filters” to do the highlight



- Convolution

- Lots of filters based on this principle

<http://en.wikipedia.org/wiki/Convolution>

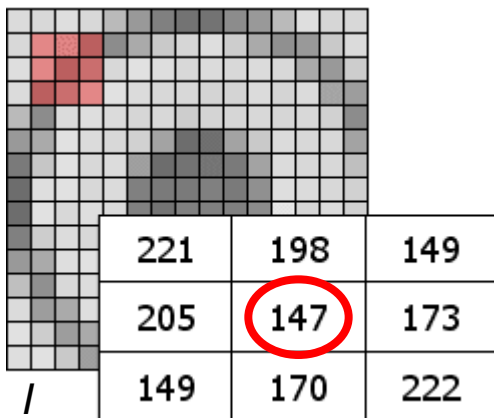
- **Matrix convolution**, in our case, is an operation between two matrices, namely...

- the input image, I

- a *kernel*, K

-1	0	1
-2	0	2
-1	0	1

K



221	198	149
205	147	173
149	170	222

I

$$\begin{aligned}
 (K \otimes I)_{i,j} = & (-1 \cdot 221) \\
 & + (0 \cdot 198) \\
 & + (1 \cdot 149) \\
 & + (0 \cdot 205) \\
 & + (0 \cdot \mathbf{147}) \\
 & + (0 \cdot 173) \\
 & + (-1 \cdot 149) \\
 & + (0 \cdot 170) \\
 & + (1 \cdot 222) = -63
 \end{aligned}$$

Adapted from James Matthews, 2002

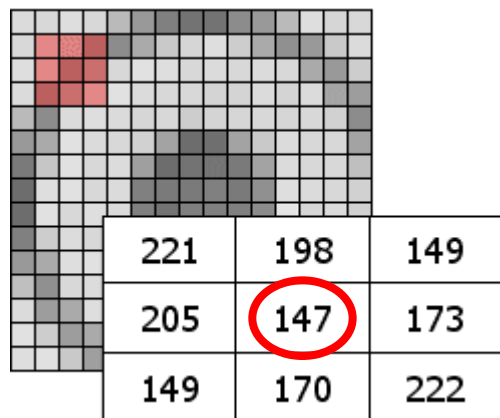
<http://www.generation5.org/content/2002/convolution.asp>

Segmentation – basics

K

-1	0	1
-2	0	2
-1	0	1

I



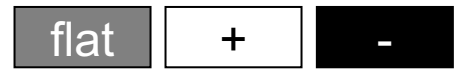
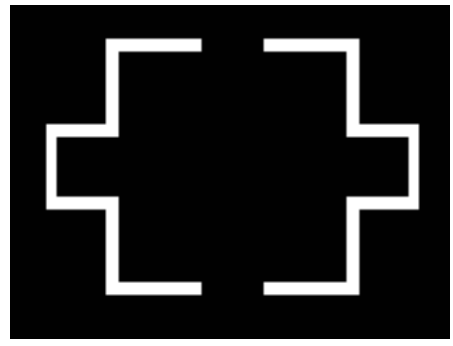
$(K \otimes I)_{i,j} =$

- $(-1 * 222)$
- $+ (0 * 170)$
- $+ (1 * 149)$
- $+ (-2 * 173)$
- $+ (0 * \mathbf{147})$
- $+ (2 * 205)$
- $+ (-1 * 149)$
- $+ (0 * 198)$
- $+ (1 * 221) = +63$

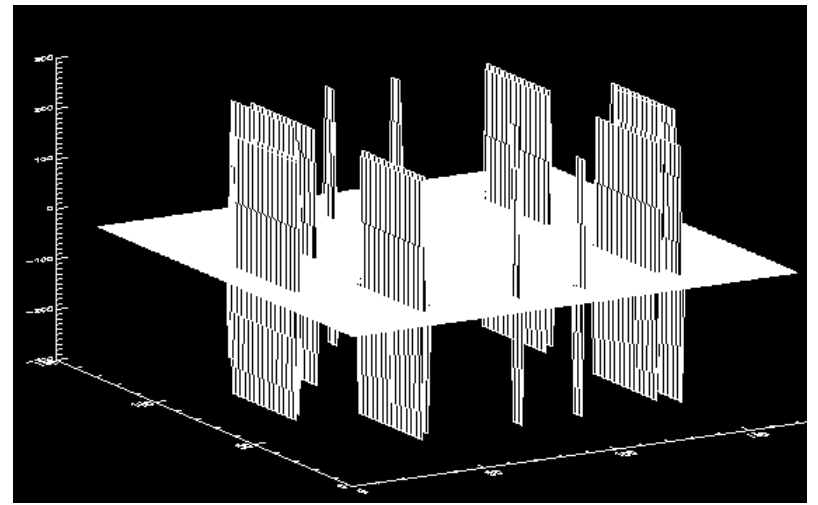
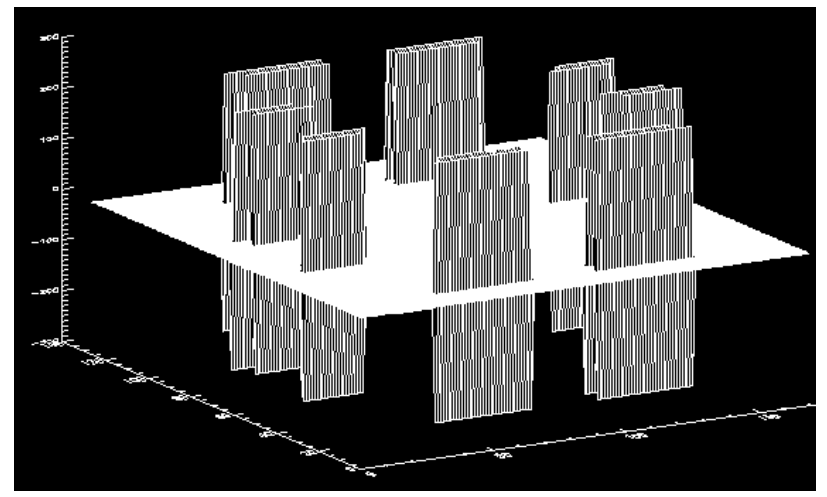
Matrix convolution can be implemented in different ways... beware of the algorithm!

- Intensity gradients (discrete approximation)

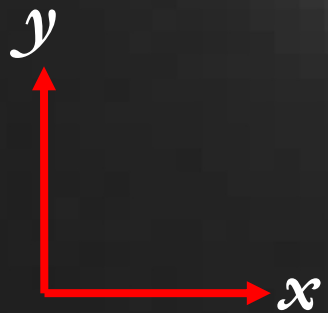
$$\frac{\partial I}{\partial x} \approx$$



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



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

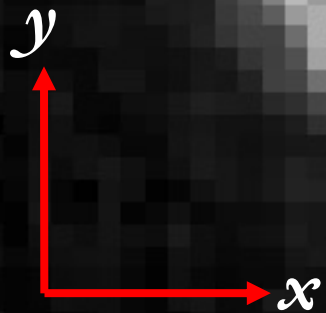


I_y



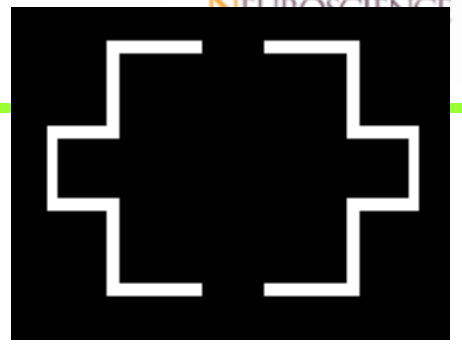
“Edgemap”

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



Segmentation – basics

- Intensity gradients (discrete approximation)

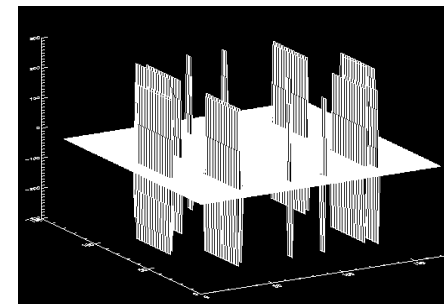


$$I = I(x, y)$$

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

$\Delta x = 1$ pixel

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

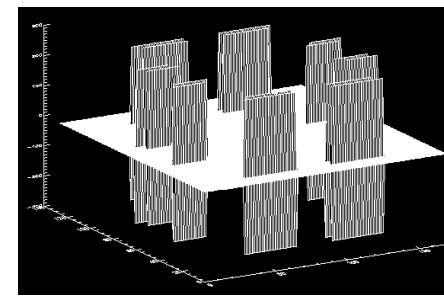
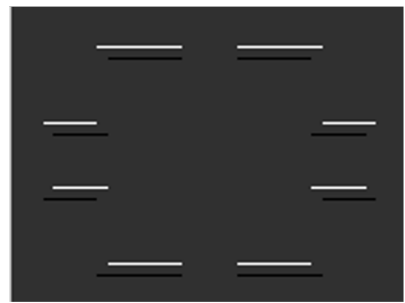


$$\frac{\partial I}{\partial y} \approx \frac{I(x, y + \Delta y) - I(x, y)}{\Delta y} = K_y \otimes I$$

$\Delta y = 1$ pixel

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

flat + -



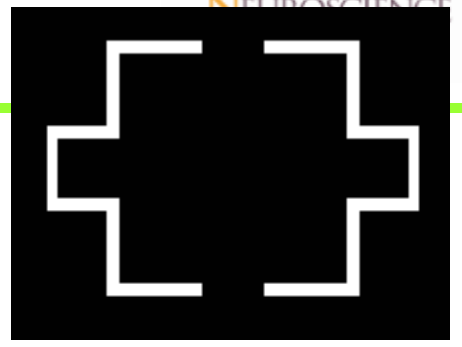
- Kernels...

Laplace

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

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

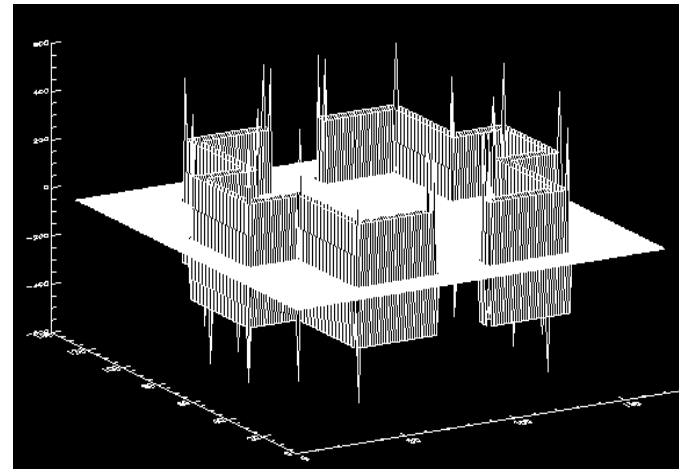
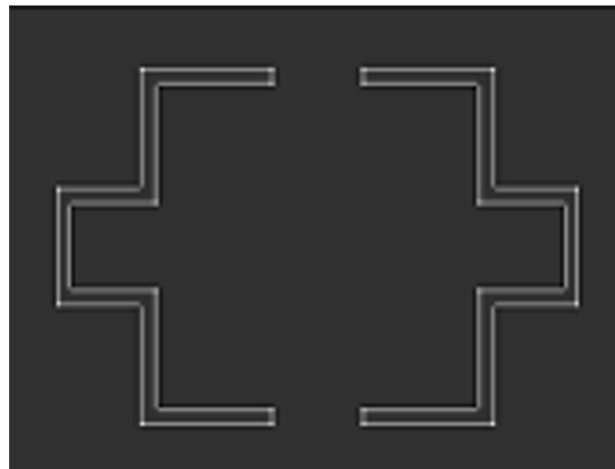
$$\nabla^2 I \approx \frac{f(x + \Delta x, y) + f(x, y + \Delta y) - 4f(x, y) + f(x - \Delta x, y) + f(x, y - \Delta y)}{(\Delta x)^2} = K_L \otimes I$$



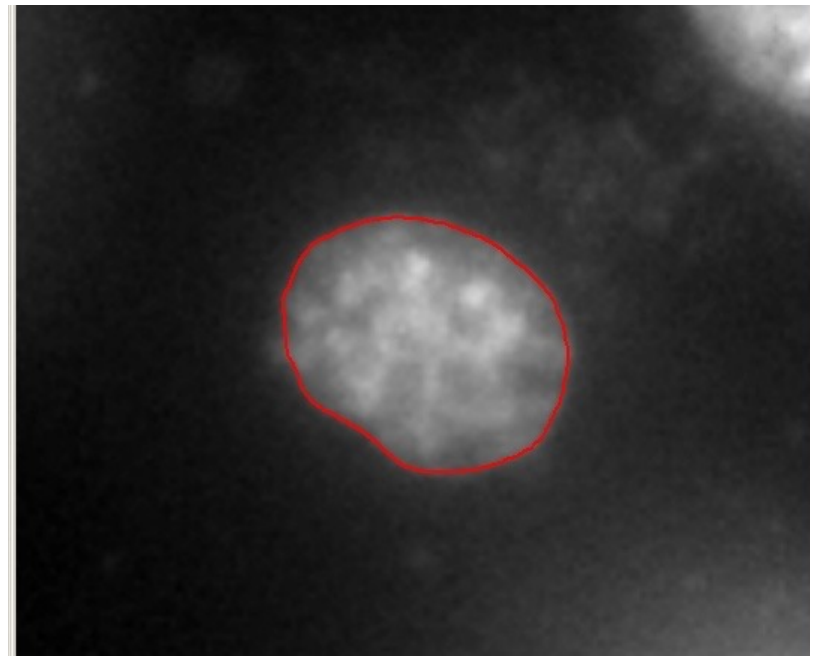
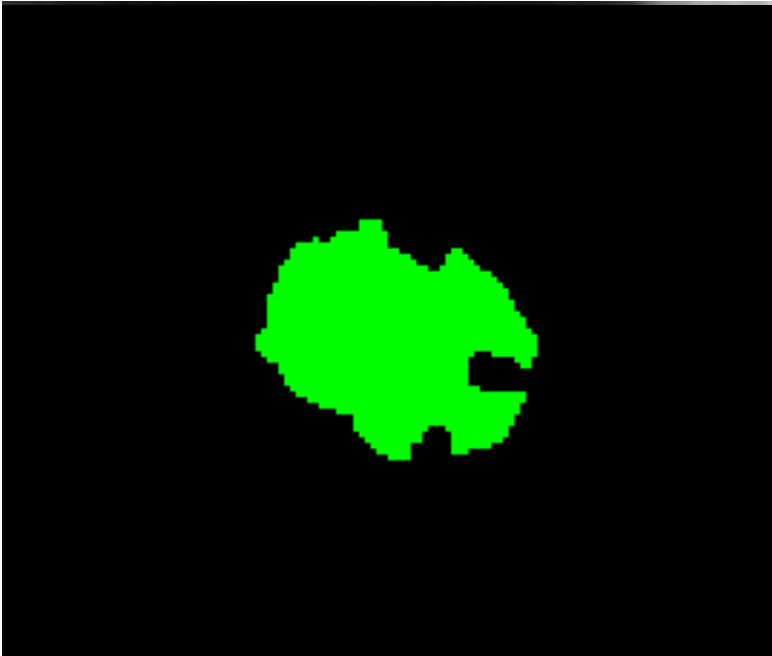
$I = I(x, y)$

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

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



- “Some” times more information is needed in order to achieve a good segmentation



- Template matching
 - “Classic model” Hough transform
 - Applies to circles, line segments and a variety of shapes

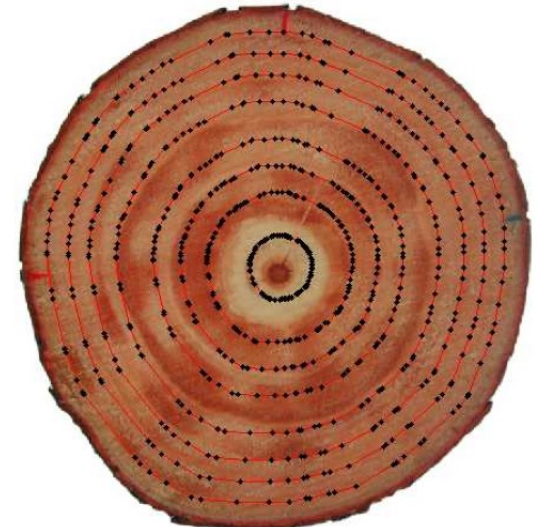
Hough P (1959)

If we can detect edges we can approximate a circle (or other shapes)

A test is performed to determine the circles with “best fit”



(c) Sample Tree 8.

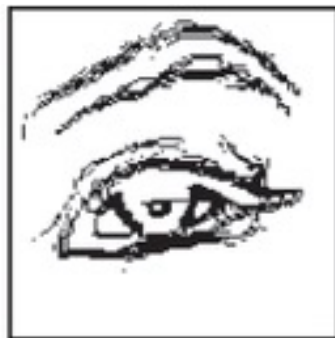


(d) Fully automatic recognition with the input figure 5(c).

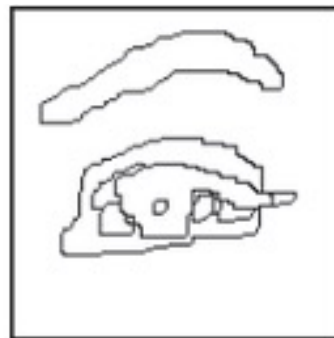
- Variational methods
 - Based on energy minimization, defining integral models
 - Idea: to include desirable features on segmented images (like homogeneous regions, short or smooth ROI boundaries)
 - Optimum solutions found by partial differential equations
 - Examples: Mumford-Shah, Ambrosio-Tortorelli, Chan-Vese (details in the book from Aubert & Kornprobst 2006)



image I



main discontinuities in I

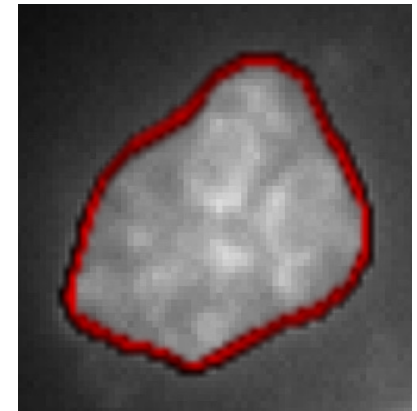
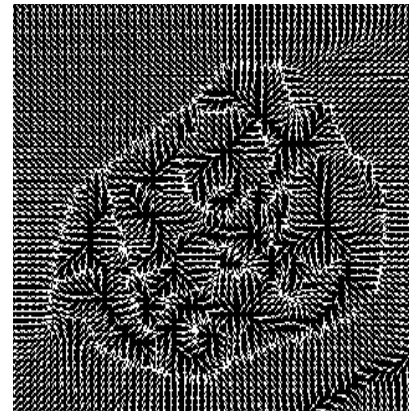
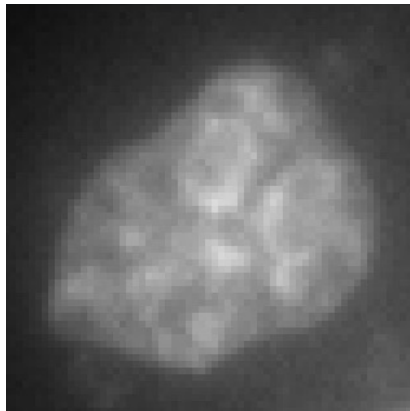


ROI boundaries B



piecewise smooth
image J

- Active contour models
 - Optimization of different properties



input
 image
 + initial guess

contour $C(s)$
 - elasticity
 (contraction)
 - rigidity
 (bending, cornering)

force field
 - repulsion
 - attraction

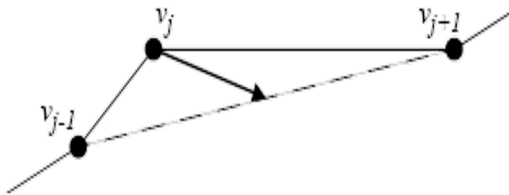
output: force balance
 minimal energy

First active contours approach:
 Kass, Witkin & Terzopoulos (1988)
 "Snakes"

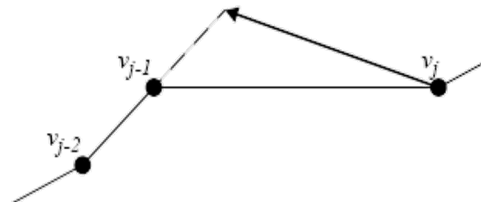
- Snakes: optimization derived from a **variational** approach
 - Minimization of an **integral functional**
 “a snake minimizes its energy”

$$E = \int_0^1 \frac{1}{2} \left[\alpha \left| \frac{\partial C(s)}{\partial s} \right|^2 + \beta \left| \frac{\partial^2 C(s)}{\partial s^2} \right|^2 \right] + E_{ext}[C(s)] ds$$

Elasticity term
(coefficient α)



**Internal energy,
 contour dependant**
 Rigidity term
 (coefficient β)



**External energy,
 image dependant**