

Image Processing I-II



2025
October, 13-24

Innovative Biotechnological Approaches for Biofilm

Curso Binacional Uruguay – Chile 2025

Instituto de Investigaciones Biológicas Clemente Estable (IIBCE)
Instituto de Neurociencia Biomédica (BNI), ICBM, F-Med, U-Chile

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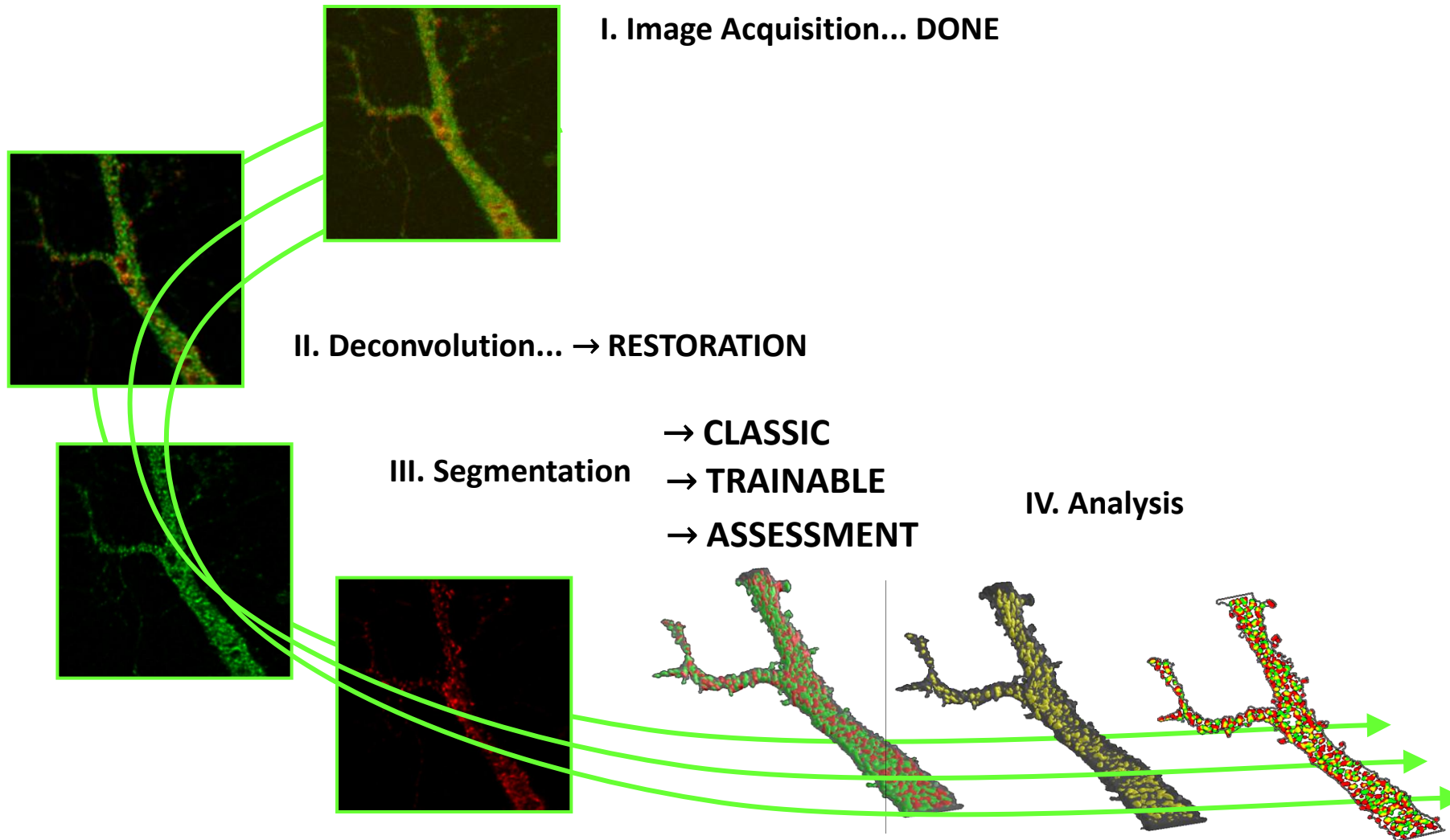
Laboratory for Scientific Image Analysis (SCIAN-Lab)
Centro de Informática Médica y Telemedicina (CIMT)
Biomedical Neuroscience Institute (BNI)
Instituto de Ciencias Biomédicas (ICBM)
www.scian.cl / www.cimt.cl



Thanks / Bedankt



Outlines



> Processing | Analysis Tasks

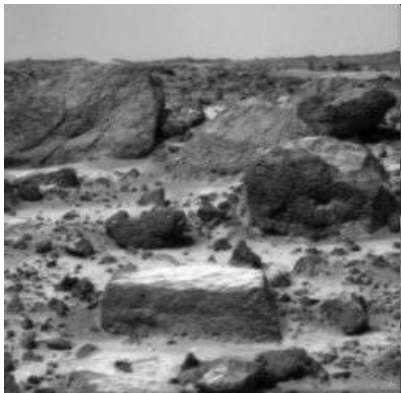
- **Segmentation**

- 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

> Processing | Analysis Tasks

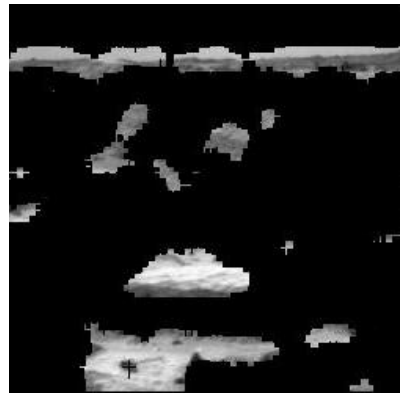
- **Segmentation** (“pre Machine Learning era”)



Sol 3, Mars
Pathfinder Mission

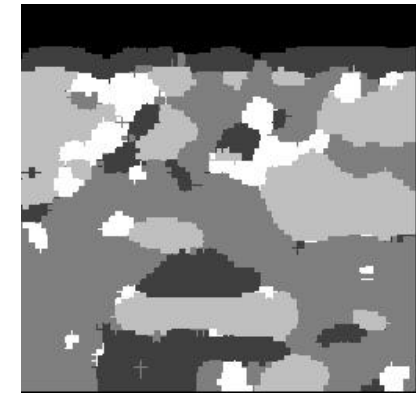


Sky / Flat



Dust / Horizon

...etc...

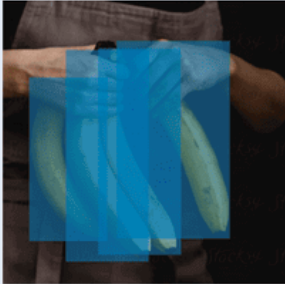
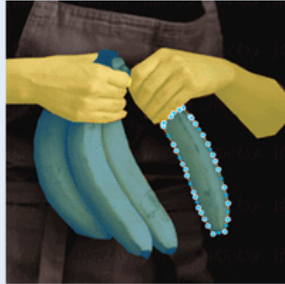


Final segmentation

Source: NASA

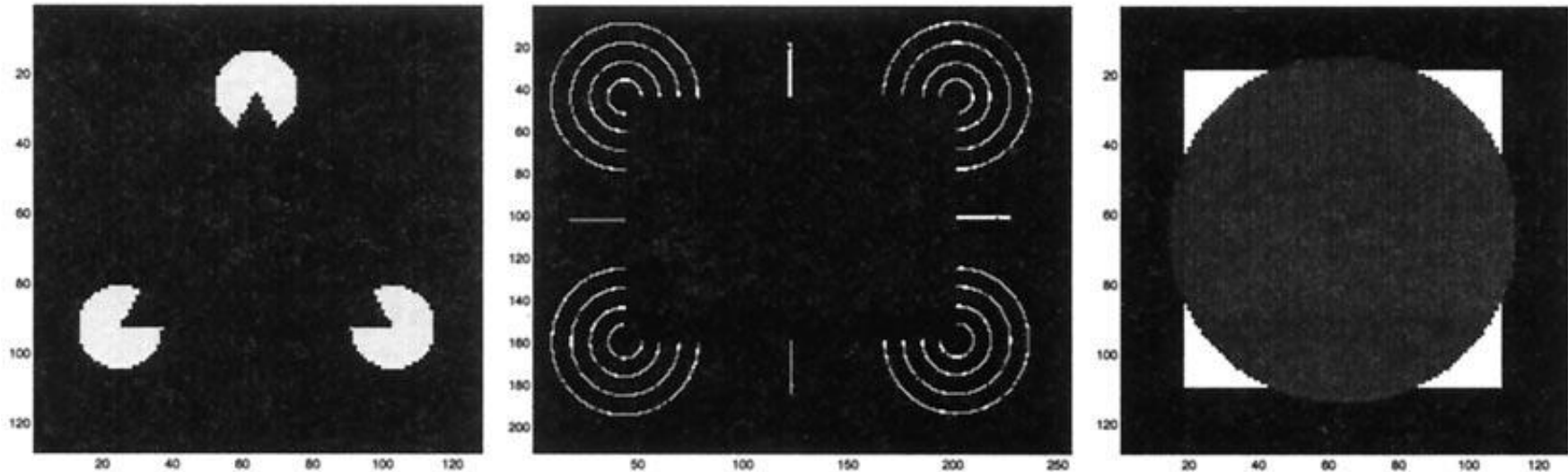
> Processing | Analysis Tasks

- **Segmentation** (“2020’s Machine Learning era”)

Classification	Object Detection	Semantic Segmentation	Instance Segmentation
			
✓ Presence ✗ Location ✗ Count ✗ Size ✗ Shape	✓ Presence ✓ Location ✓ Count ✗ Size ✗ Shape	✓ Presence ✓ Location ✗ Count ! Size ! Shape	✓ Presence ✓ Location ✓ Count ✓ Size ✓ Shape
OUTPUT Banana exists: Yes / No	OUTPUT There are 4 bananas	OUTPUT There is banana in these pixels	OUTPUT There are 4 bananas of this shape, size and grade

> Processing | Analysis Tasks

- Segmentation... ¿Which features? ¿Which criteria?
 - The segmentation problem has a subjective component



- ...not always (almost never) enough information for a 100% accurate segmentation.

D. Marr (1982) Vision

Sarti & Sethian (2000) Subjective surfaces. PNAS

> Processing | Analysis Tasks | Segmentation

Problem?



Segmentation
(manual)



> Processing | Analysis Tasks | Segmentation

Problems

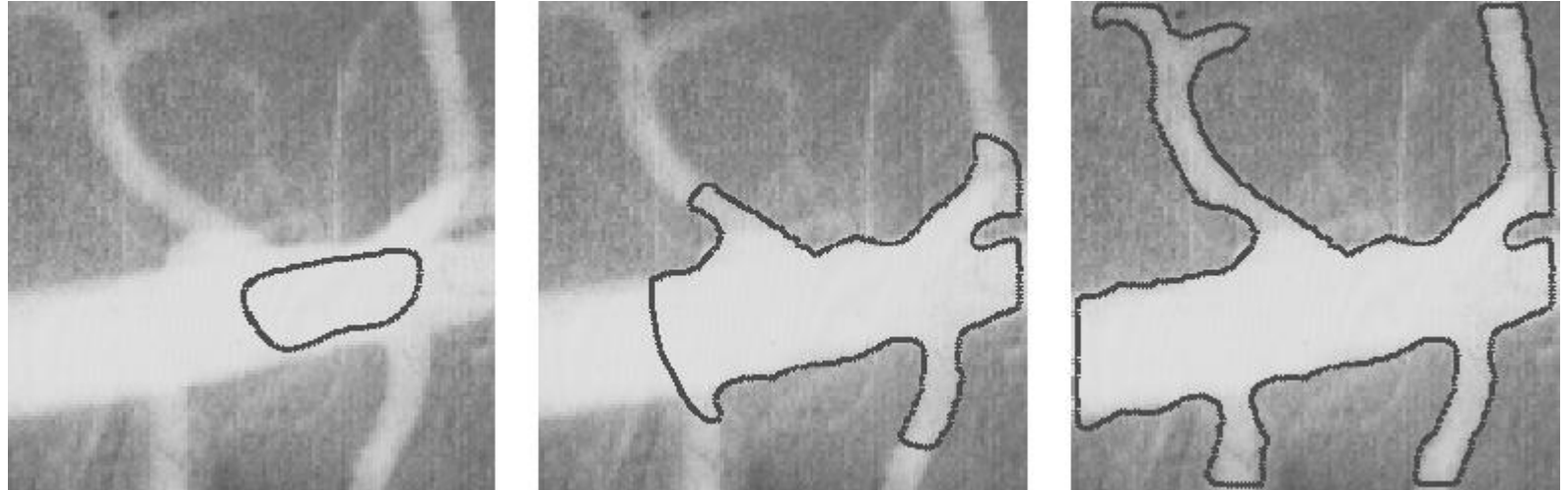
- Lack of absolute criteria or standards (**Ground Truth, Gold Standard** [1,2])
- Missing or erroneous information (e.g. non-specific markers in samples)
- **What to do?**
A “good” (i.e. carefully performed and controlled) acquisition to ease this task

[1] Jason D. Hipp et al. Tryggo: Old nose 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.

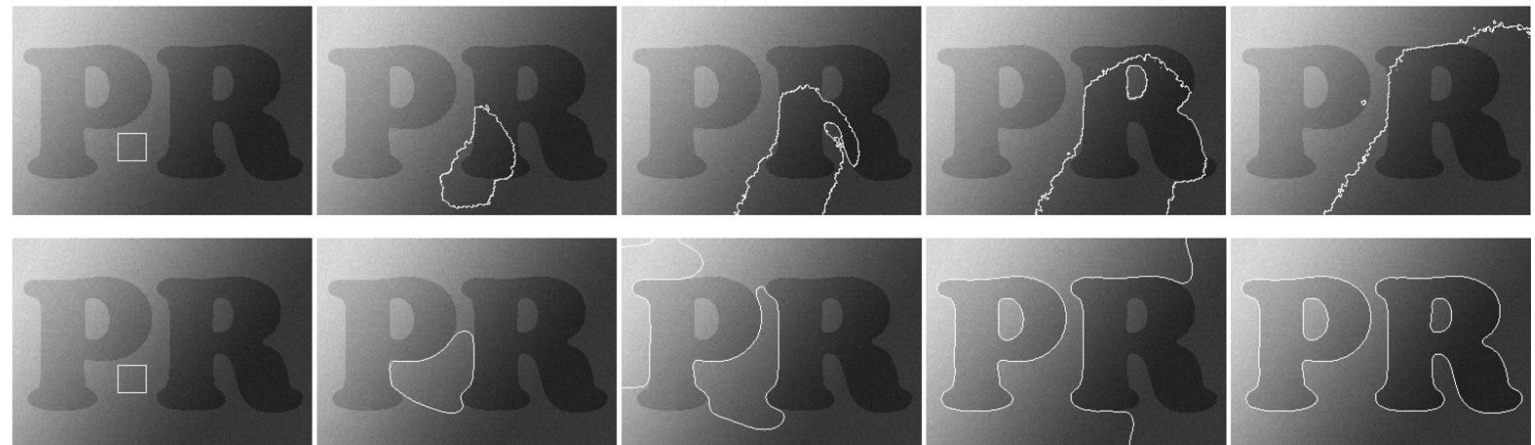
> Processing | Analysis Tasks | Segmentation

Segmentation models regarded as “good” for a given case can be “bad” for other



J A Sethian – Fast marching and level set methods

http://math.berkeley.edu/~sethian/2006/Applications/Medical_Imaging/artery.html

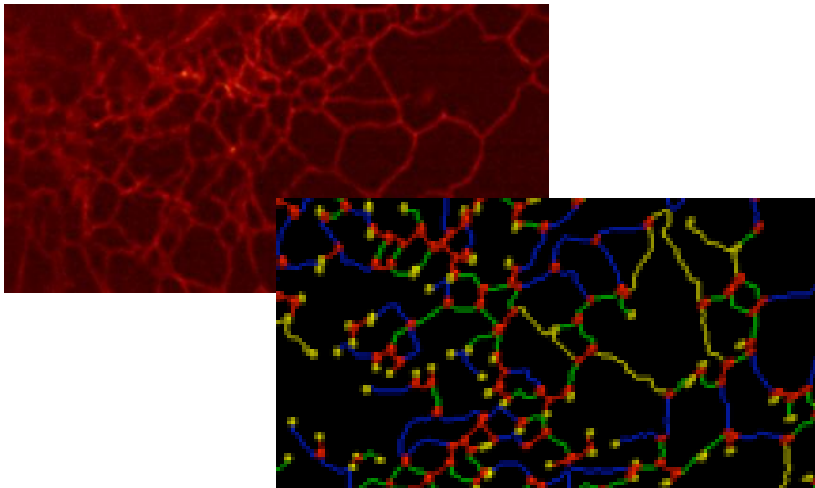


X Xie (2010) Magnetostatic Active Contours

> Processing | Analysis Tasks | Segmentation

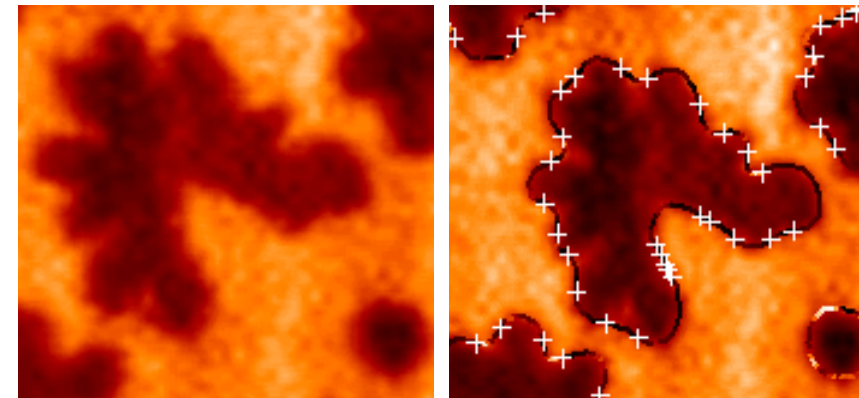
- Segmentation is the first step toward further analysis
 - In addition to images, ROI models and data structures can suit for different types of descriptions

Parameter estimation...



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

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



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

Segmentation approaches

(one posible categorization)

1. Filter-based approaches

- Thresholding
- Matrix convolution filters
- Mathematical morphology
- Fourier
- ...

2. Advanced approaches

- Shape priors (*pattern matching*)
- Clustering methods (k-means, region growing, graph cuts, entropy)
- Deformable models (active contours)
 - parametric
 - Implicit
- ...

• 3. Trainable (*machine learning*) approaches

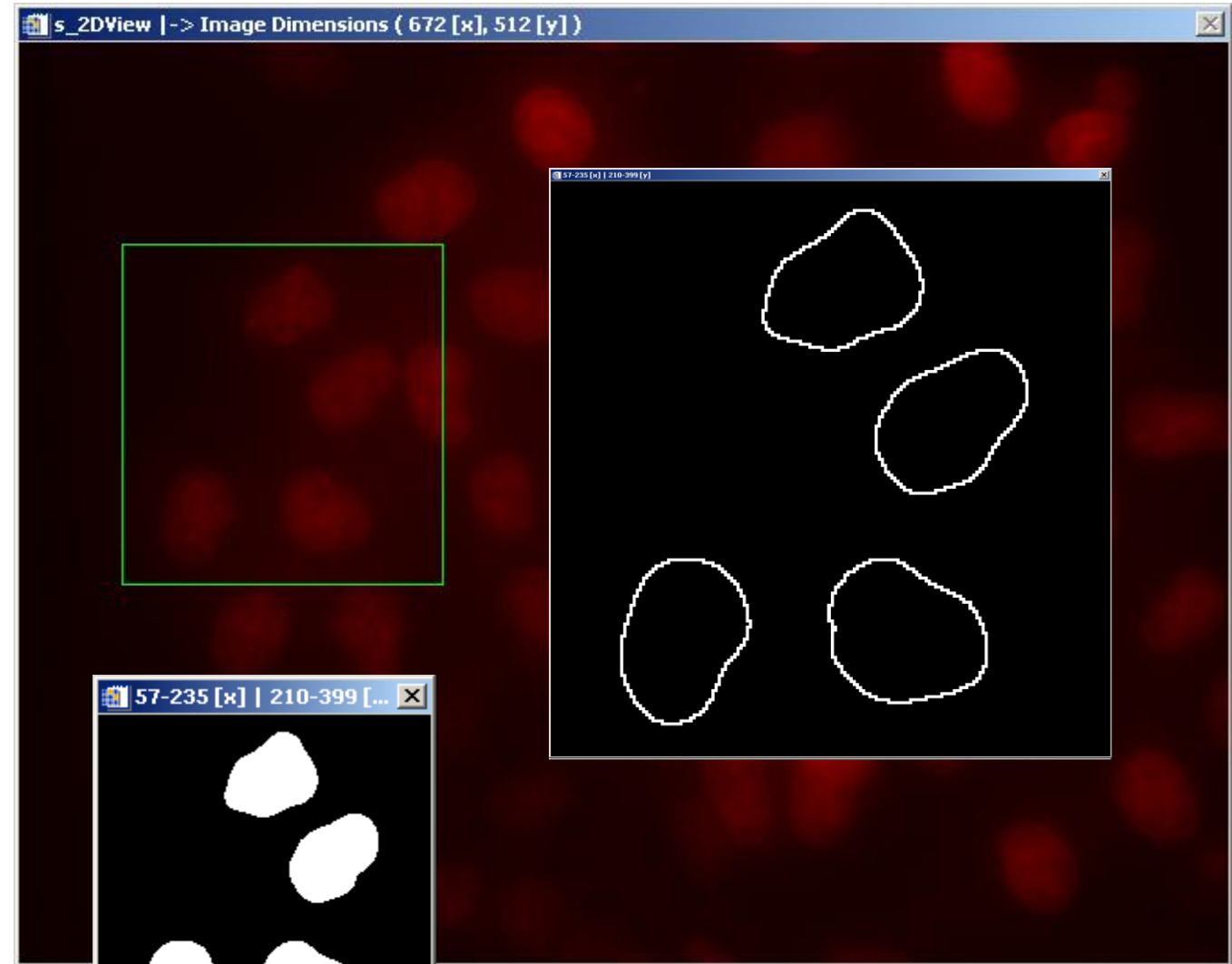
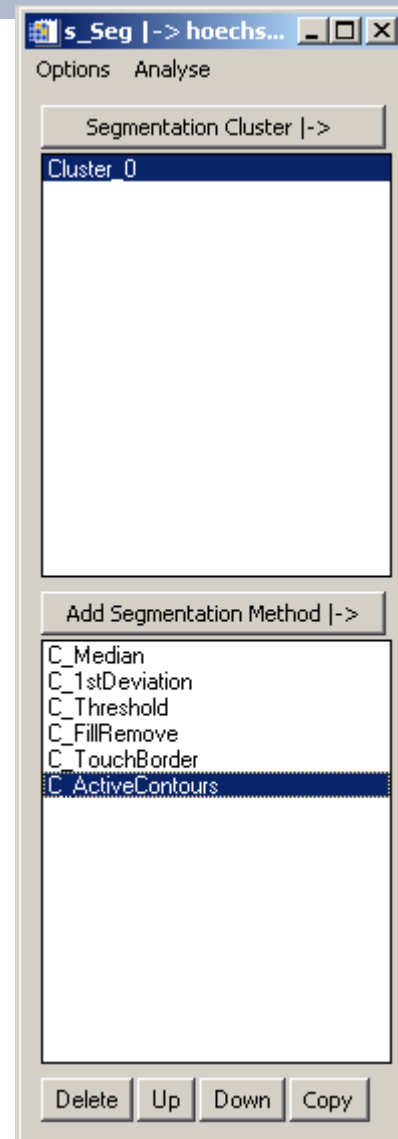
- Support Vector Machine (SVM)
- Random forest
- Neural networks (“classical NNs”)
- Deep learning (convolutional neural networks, CNNs)
- ...

Handbook in Spanish for ImageJ/FIJI (Ethics! Careful with the discussion)

[https://www.researchgate.net/publication/313768335 Analisis de Imagenes de Microscopia con ImageJ](https://www.researchgate.net/publication/313768335_Analisis_de_Imagenes_de_Microscopia_con_ImageJ)

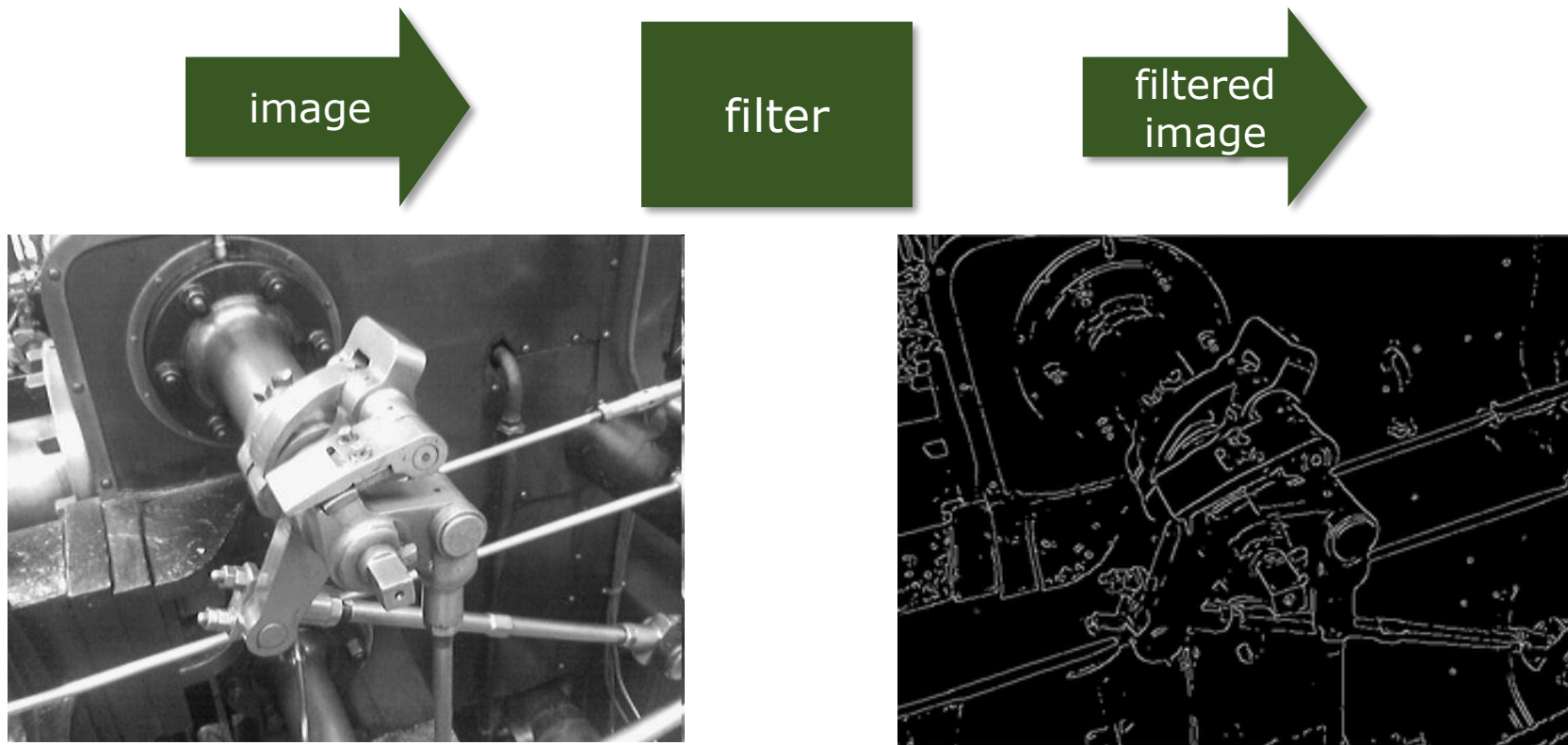
> Processing | Analysis Tasks | Segmentation

Filters can ease or improve segmentation



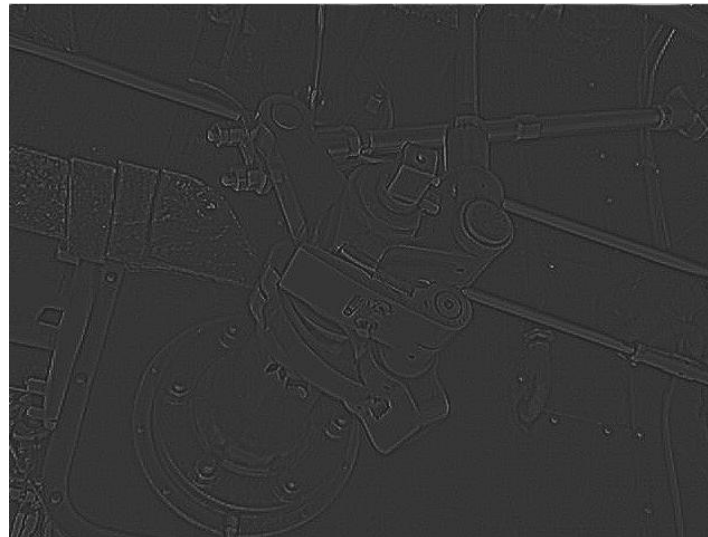
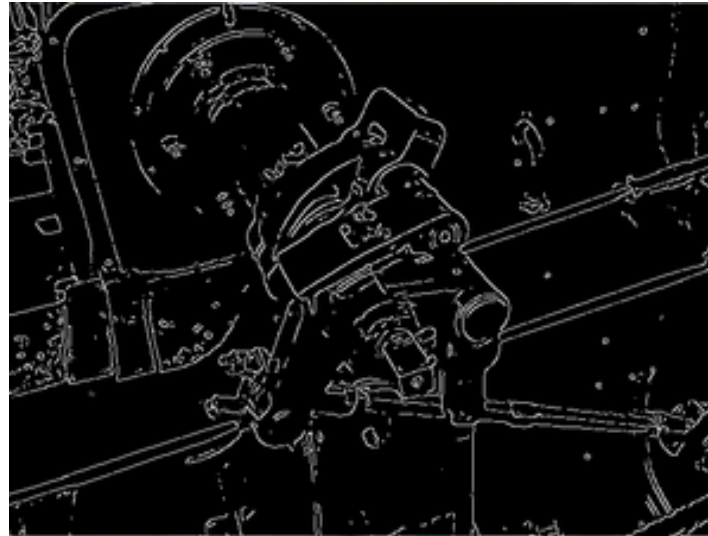
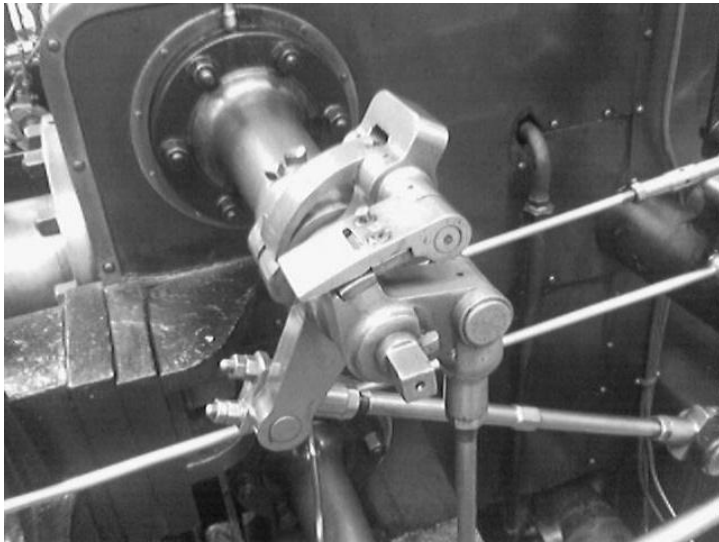
> Processing | Analysis Tasks | Segmentation

- “Filter” approach



> Processing | Analysis Tasks | Segmentation

- A sample filter...

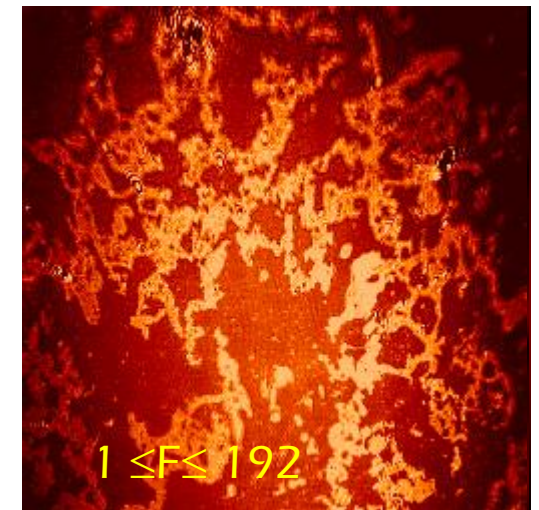
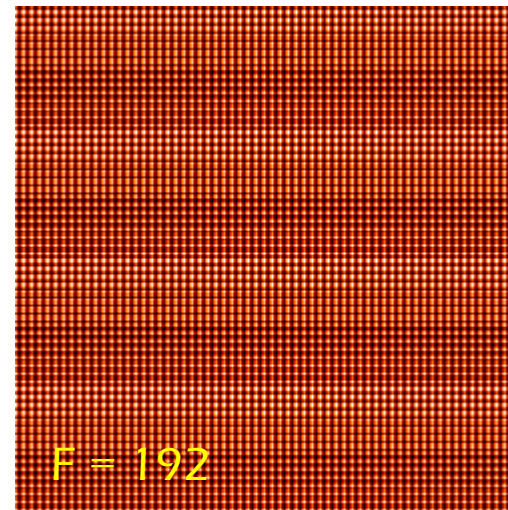
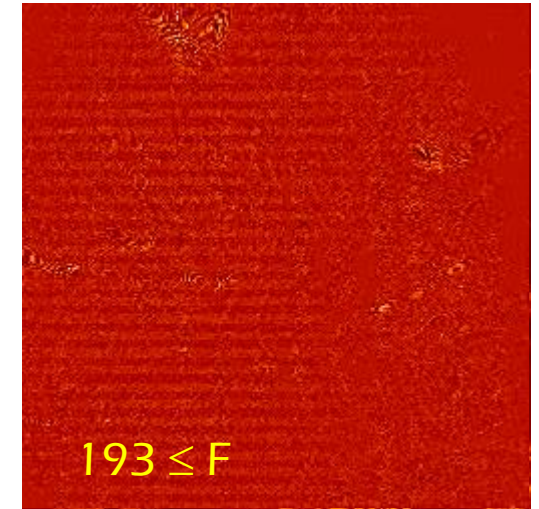
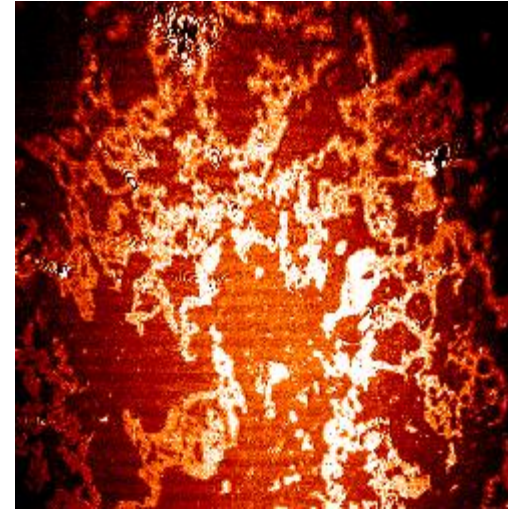


> Processing | Filters

- Fourier filtering (once more!)
- Think of a different base (e.g. polynomials)

https://imagejdocu.tudor.lu/plugin/filter/fit_polynomial/start

<https://imagej.nih.gov/ij/plugins/inserm514/>



> Processing | Analysis Tasks | Segmentation with Filters

Thresholding

Example: Otsu

Convolution based

Convolution operation

Examples: gradient, Laplace, Sobel, Gaussian

Morphological

Morphological operators

Size

Thinning / skeletonization

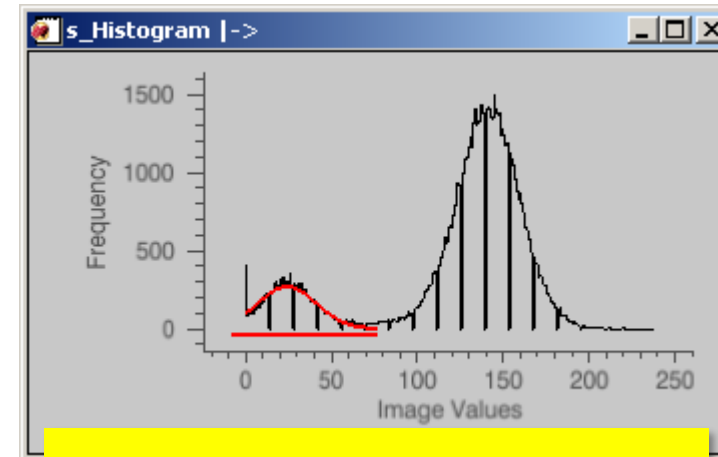
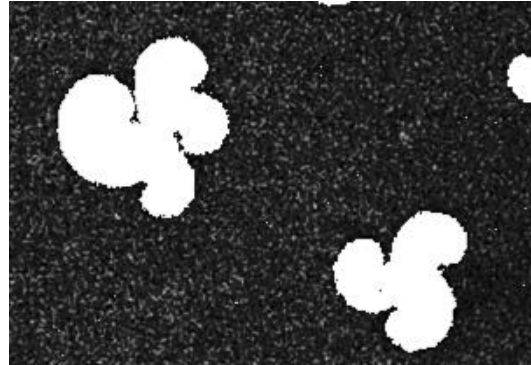
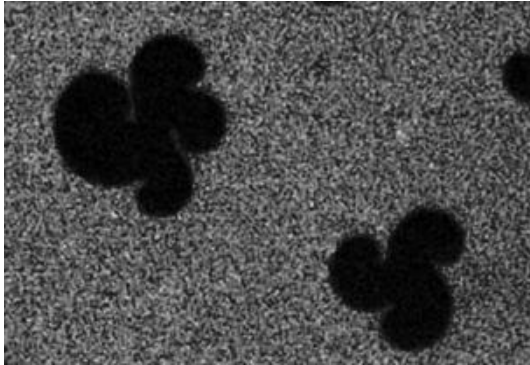
Arithmetic-logic

AND, OR, XOR

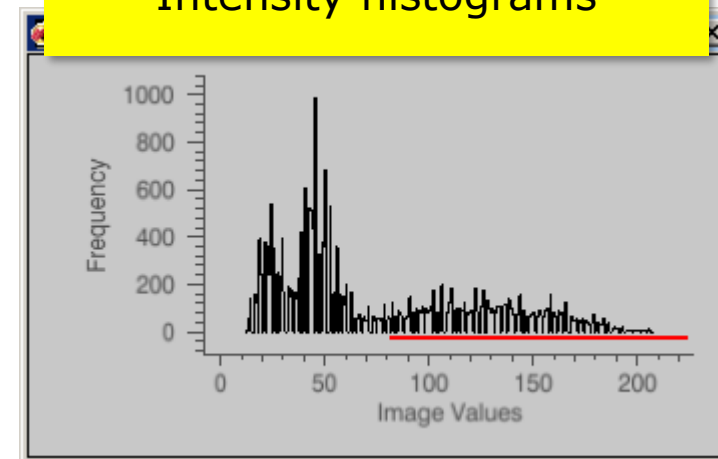
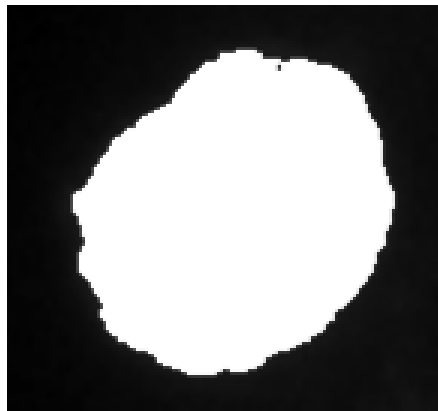
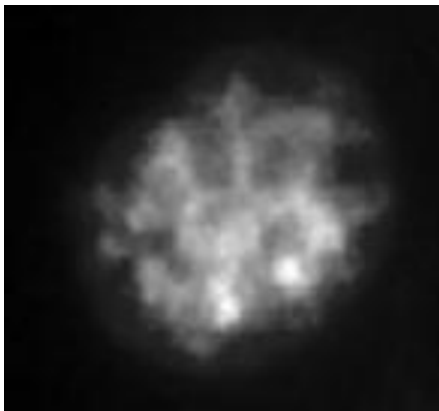
And a long etc.

> Processing | Analysis Tasks | Segmentation with Filters

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

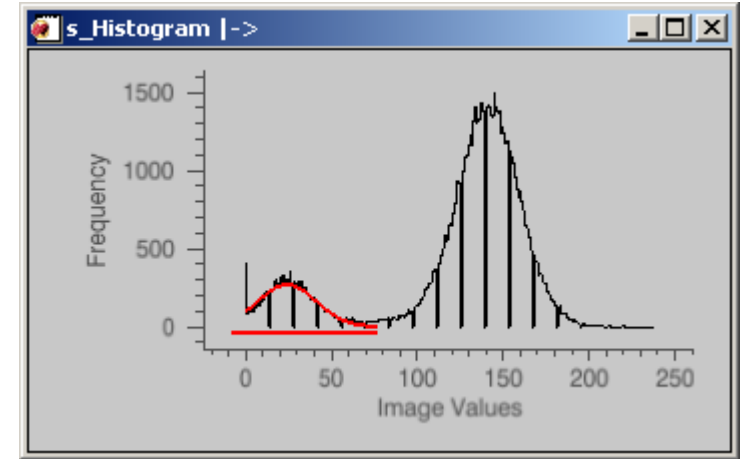
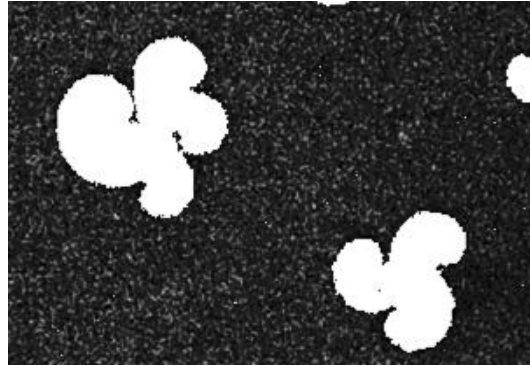
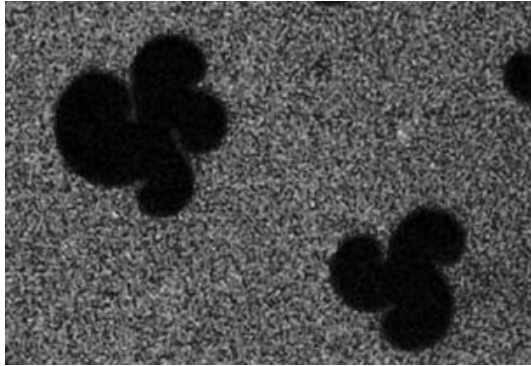


Intensity histograms



> Processing | Analysis Tasks | Segmentation with Filters

- Here comes the Intensity Histogram...



- Throwback to the acquisition...
 - Offset
 - Clipping (sometimes "Saturation"...try not to confuse with the HSV Saturation)

More “Concepts”!

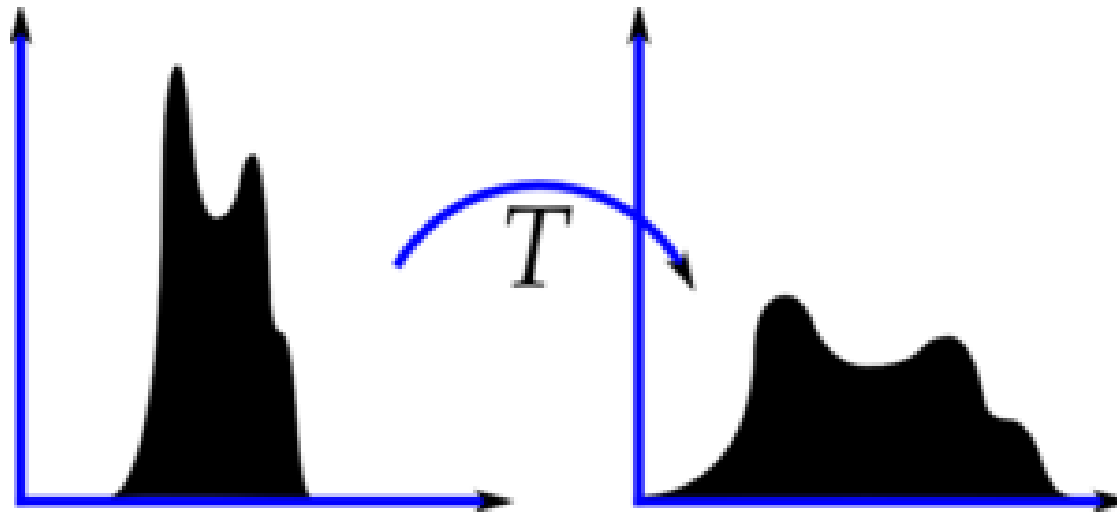
- Dynamic Range
- Clipping (in photography can be referred to as “color saturation”)

Some examples with music (“Loudness war”)

<https://www.youtube.com/watch?v=dcKDMBuGodU>

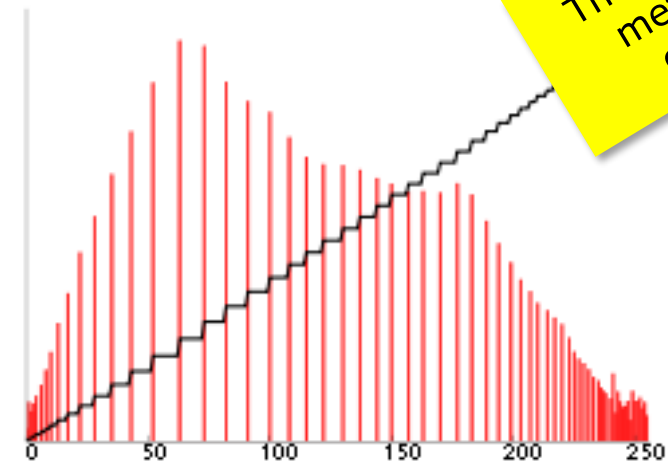
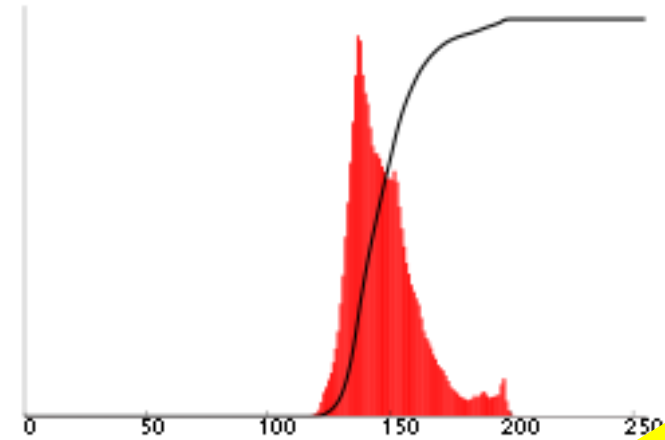
<https://www.youtube.com/watch?v=u9Fb3rWNWDA>

- Histogram equalization



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

- Histogram equalization



There are (many) more methods... adaptive, contrast-limited...

> Processing | Filters for Segmentation

N. Otsu (1979)

- Otsu threshold

- Idea: to separate the image pixel in two classes (sets), minimizing the sum of variances from both classes



$$\min \sigma_w^2(t) = \omega_1(t)\sigma_1^2(t) + \omega_2(t)\sigma_2^2(t)$$

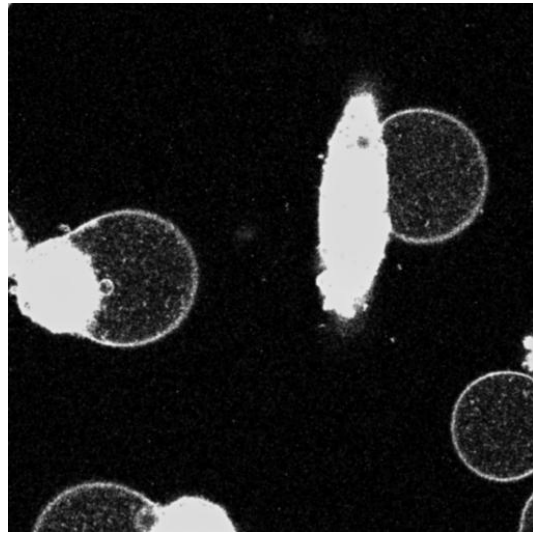
t : threshold, ω_i : probability of class i

Algorithm

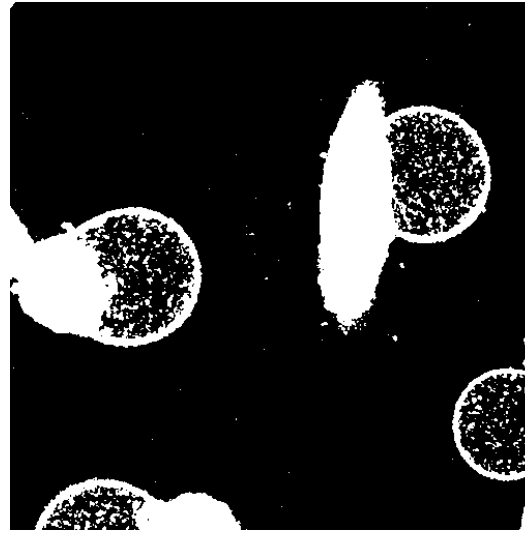
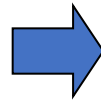
1. Compute histogram and probabilities of each intensity level
2. Set up initial $\omega_i(0)$ and $\mu_i(0)$
3. Step through all possible thresholds $t = 1 \dots \text{maximum intensity}$
 1. Update ω_i and μ_i
 2. Compute $\sigma_b^2(t)$
4. Desired threshold corresponds to the maximum $\sigma_b^2(t)$
5. You can compute two maximums (and two corresponding thresholds). $\sigma_{b1}^2(t)$ is the greater max and $\sigma_{b2}^2(t)$ is the greater or equal maximum
6. Desired threshold =
$$\frac{\text{threshold}_1 + \text{threshold}_2}{2}$$

> Processing | Filters

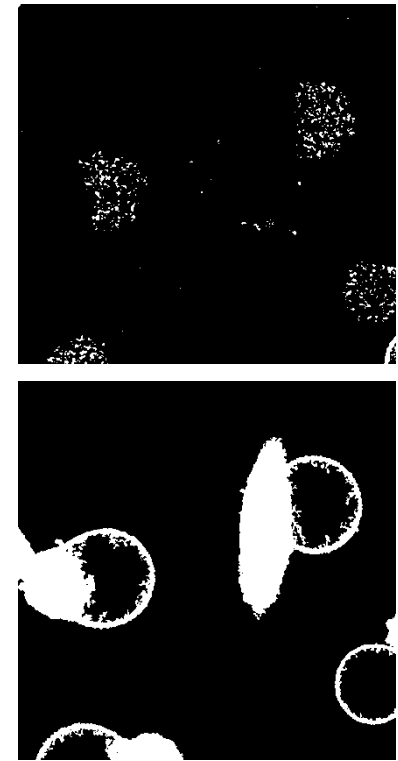
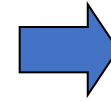
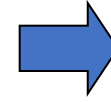
- Morphology-based filters (morphological operations on binary regions)
 - Example: size selection



Input greyscale image



After thresholding...



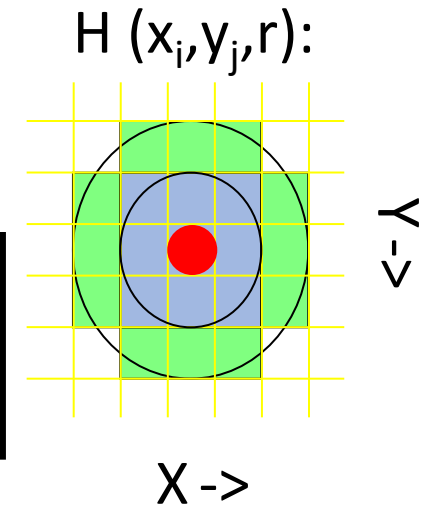
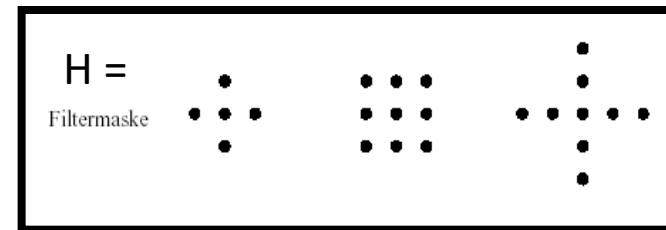
Size selection

> Processing | Filters

- Filtering with morphological operators:

Filtering with morphological operators:

- Structuring element, template or mask H ...
- Additional rules...



Polynomial filters $y(m, n) = \bar{h}_1[x(m, n)] + \bar{h}_2[x(m, n)],$

$$\bar{h}_1[x(m, n)] = \sum_{\substack{p=0 \\ (p,q) \neq (0,0)}}^{P-1} \sum_{q=0}^{Q-1} a(p, q) \cdot x(m-p, n-q)$$

$$\bar{h}_2[x(m, n)] = \sum_{\substack{p=0 \\ (p,q) \neq (0,0)}}^{P-1} \sum_{q=0}^{Q-1} \sum_{\substack{k=0 \\ (k,l) \neq (0,0)}}^{P-1} \sum_{l=0}^{Q-1} b(p, q, k, l) \cdot x(m-p, n-q) \cdot x(m-k, n-l)$$

> Processing | Filters

- Mathematical morphology

Minkowski Operations

Addition... dilation: $A \oplus S = \{(m, n) | [S + (m, n)] \cap A \neq \emptyset\}.$

Subtraction ... erosion: $A \ominus S = \{(m, n) | [S + (m, n)] \subseteq A \neq \emptyset\}$

...opening : $A \circ S = (A \ominus S) \oplus S,$

...closing : $A \bullet S = (A \oplus S) \ominus S,$

- A: image
- S: structuring element

> Processing | Filters

- Mathematical morphology

$$A \oplus S = \{(m, n) | [S + (m, n)] \cap A \neq \emptyset\}.$$

$$A \ominus S = \{(m, n) | [S + (m, n)] \subseteq A \neq \emptyset\}$$

$$A \circ S = (A \ominus S) \oplus S,$$

$$A \bullet S = (A \oplus S) \ominus S,$$

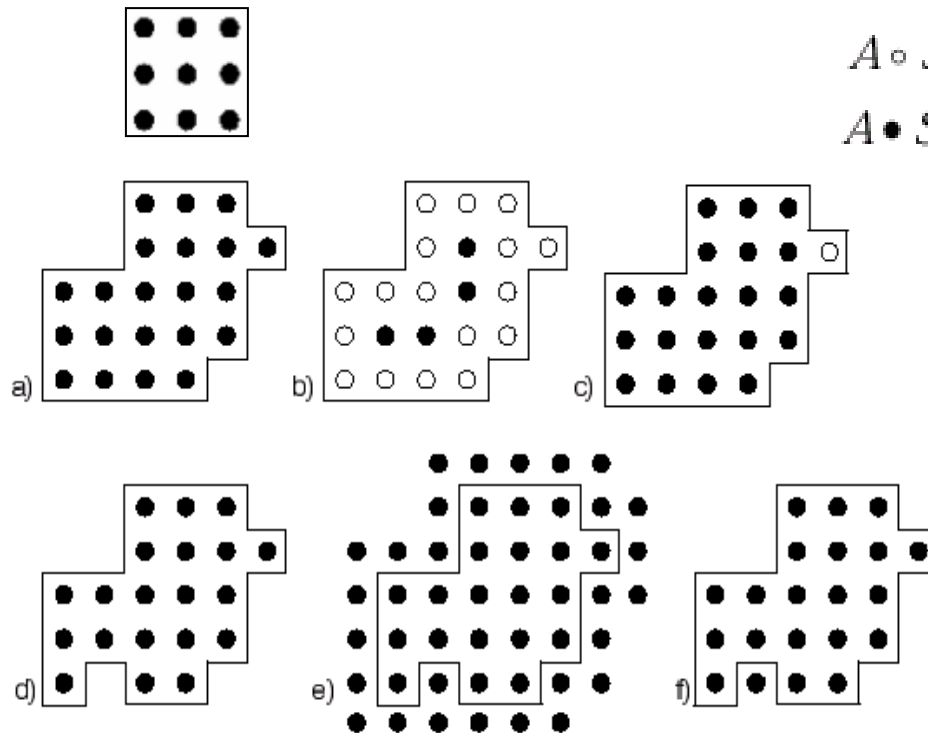
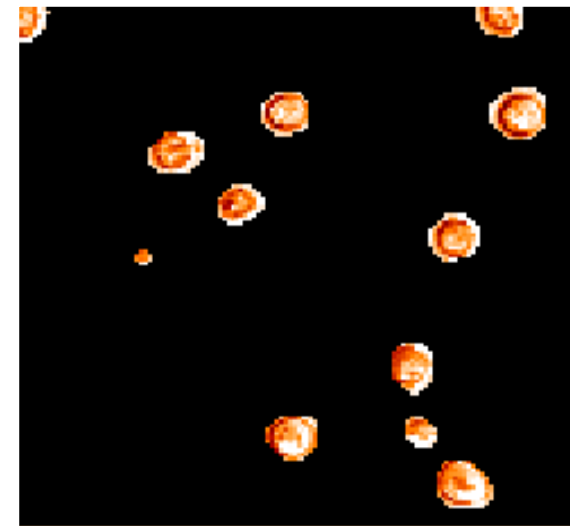


Abb. 2.5. a Originalform b erodiert c opening (Dilatation von b)
d Originalform e dilatiert f closing (Erosion von e)

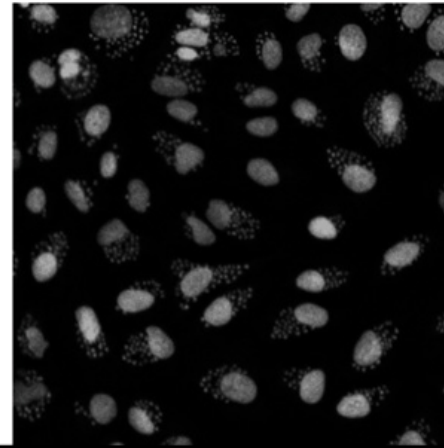


In ImageJ: “Image” > “Binary” > “Options...”

> Processing | Filters

- Watershed
(Distance transform
+ Watershed)

Procesamiento de Imágenes y Bioseñales I – Práctico Segmentación



Dato útil: el comando <Ctrl> + <Shift> + D permite duplicar la imagen activa en FIJI/ImageJ. Resulta útil para conservar el resultado del último filtro aplicado.

Figura 1. Imágenes de línea de osteoblastos humanos infectados con *Trypanosoma cruzi* (Nature 2010).

El objetivo de este práctico es realizar una segmentación de imágenes 2D usando FIJI para obtener datos básicos, responder las preguntas indicadas en esta misma hoja y entregarla al final del práctico.

1. **Segmentación 2D.** Sobre la imagen de entrada, similar a la de la Figura 1, se aplicarán distintos filtros para generar imágenes blanco/negro que representen a las regiones de interés (blanco) sobre el fondo (negro). Comience por segmentar los núcleos de osteoblastos. Algunas indicaciones para utilizar FIJI:
 - a. Para binarizar una imagen en (escala de grises) use la opción del menú "Image / Adjust / Threshold"
 - b. Los filtros binarios: erosión, dilatación, apertura están el menú "Process / Binary", y en el menú "Binary / Options" puede indicar cuantas veces realizar la operación.
 - c. Luego de obtenida una primera segmentación, puede utilizar el filtro Watershed para separar núcleos muy cercanos, disponibles desde el menú "Process / Binary / Watershed".

https://en.wikipedia.org/wiki/Watershed_image_processing

> Processing | Convolution Filtering

- **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



221	198	149
205	147	173
149	170	222

$$\begin{aligned} &= (-1 \cdot 221) \\ &+ (0 \cdot 198) \\ &+ (1 \cdot 149) \\ &+ (-2 \cdot 205) \\ &+ (0 \cdot \mathbf{147}) \\ &+ (2 \cdot 173) \\ &+ (-1 \cdot 149) \\ &+ (0 \cdot 170) \\ &+ (1 \cdot 222) = -63 \end{aligned}$$

Adapted from James Matthews, 2002

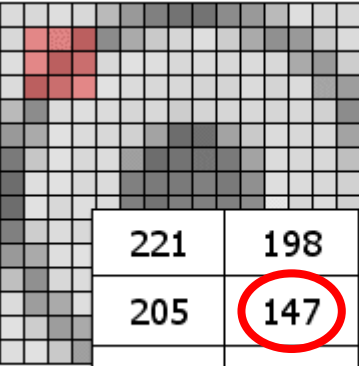
<http://www.generation5.org/content/2002/convolution.asp>

> Processing | Convolution Filtering

K

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

I

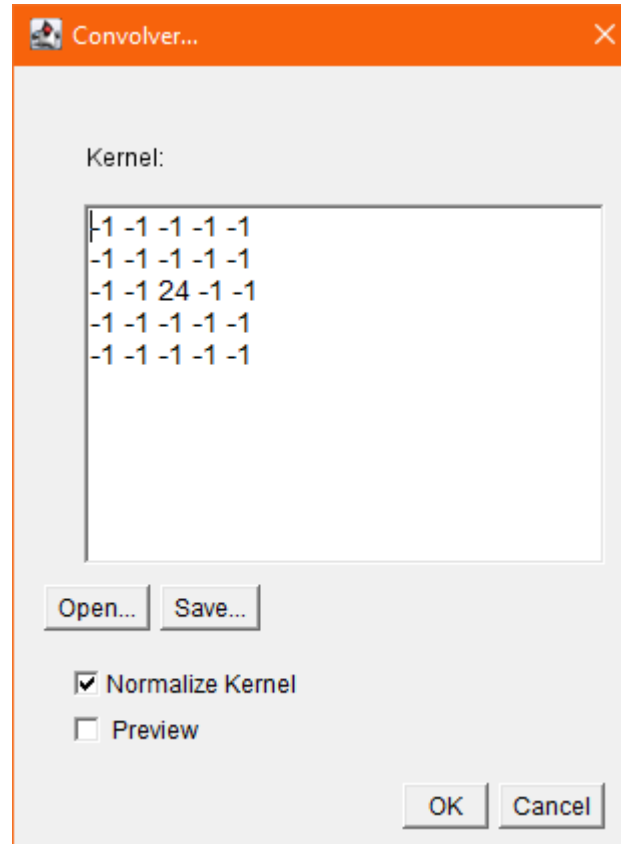


221	198	149
205	147	173
149	170	222

$(K \otimes I)_{i,j} = (-1 * 222)$
 $+ (0 * 170)$
 $+ (1 * 149)$
 $+ (-2 * 173)$
 $+ (0 * \mathbf{147})$
 $+ (2 * 205)$
 $+ (-1 * 149)$
 $+ (0 * 198)$
 $+ (1 * 221) = +63$

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

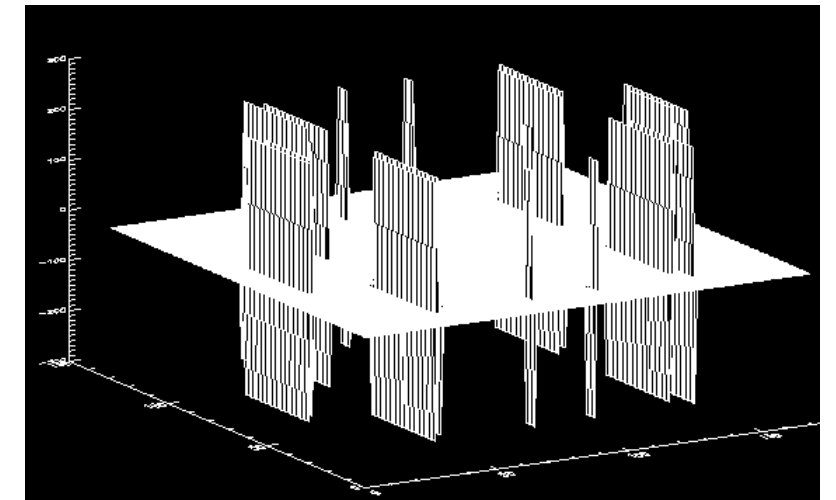
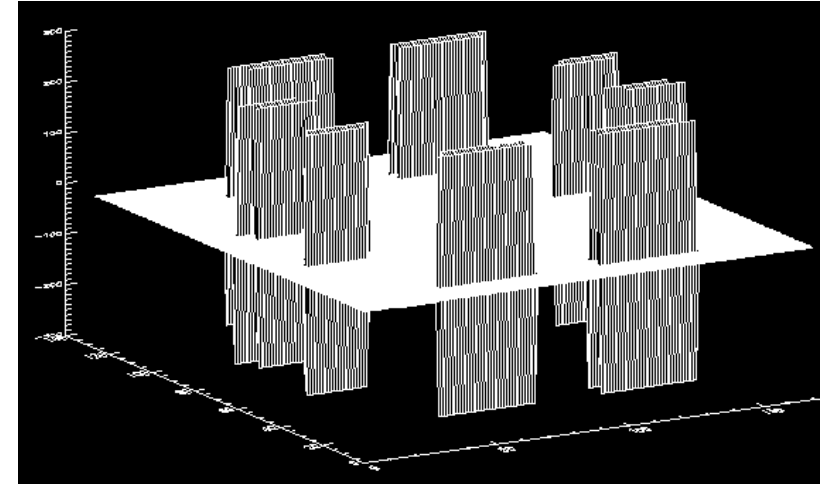
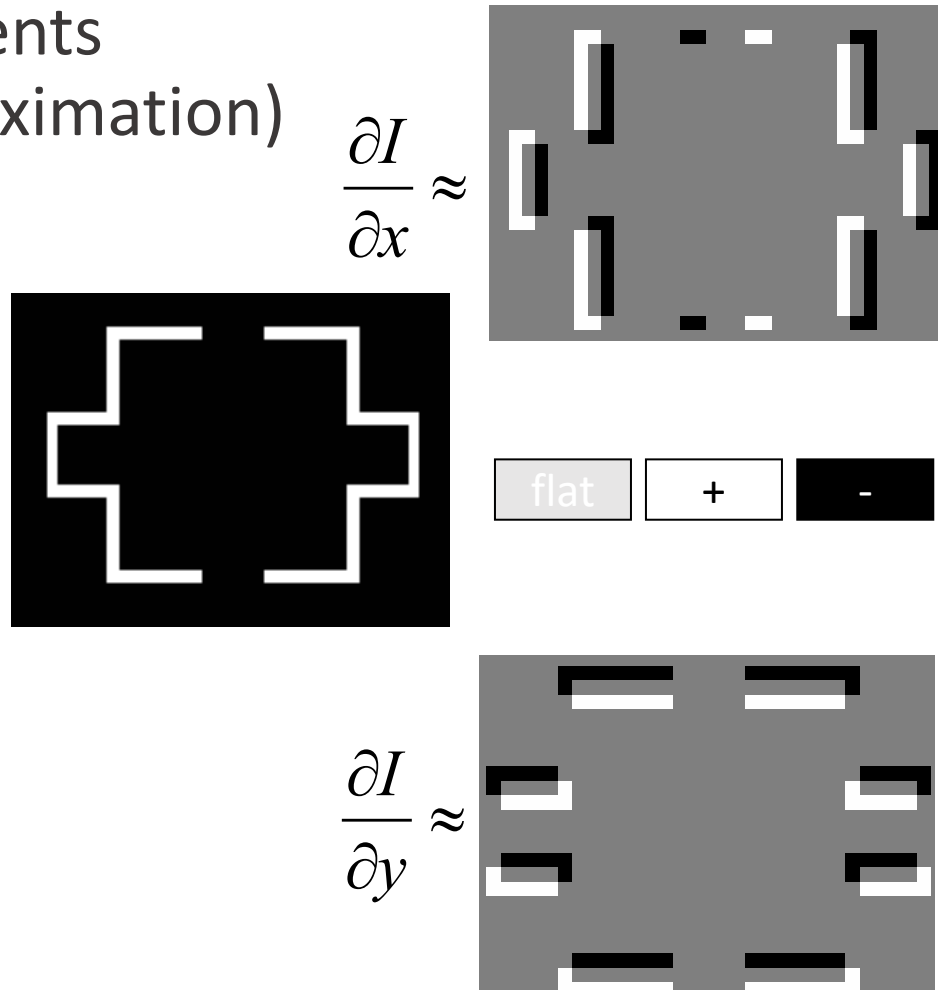
> Processing | Convolution Filtering



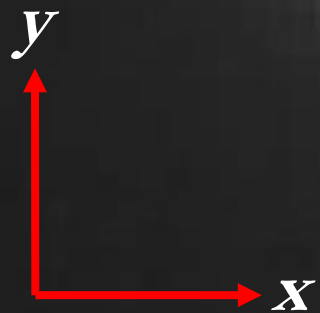
In ImageJ: “Image” > “Filters” > “Convolve...”

> Processing | Convolution Filtering

- Intensity gradients (discrete approximation)



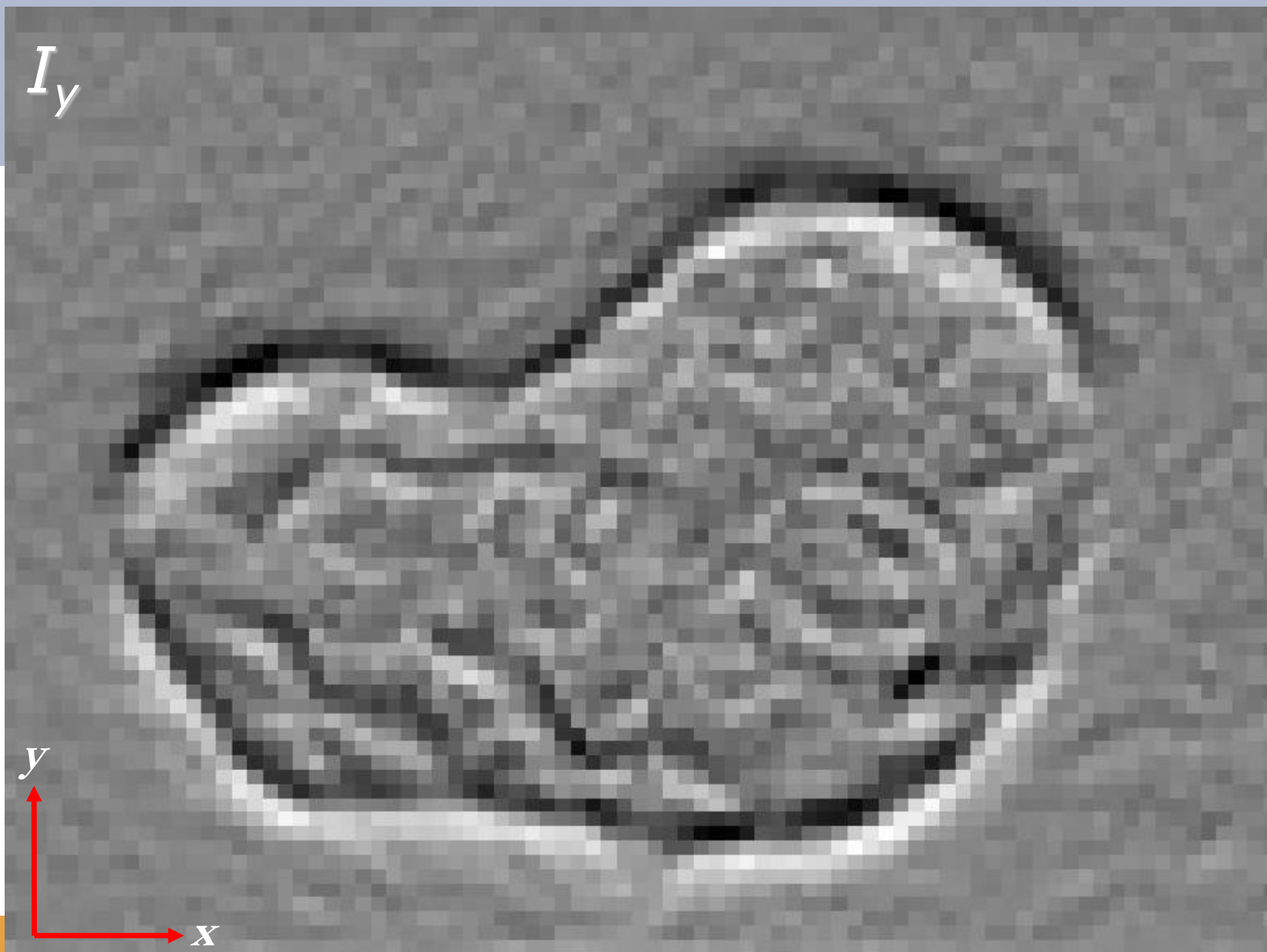
$$I = I(x, y)$$



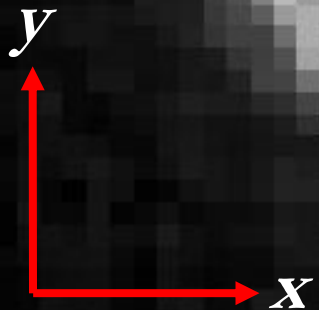
I_y

y

x

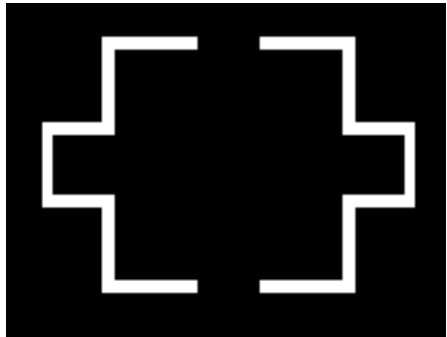


“Edgemap”
 $|\nabla I| = |I_x| + |I_y|$



> Processing | Convolution Filtering

- Intensity gradients (discrete approximation)

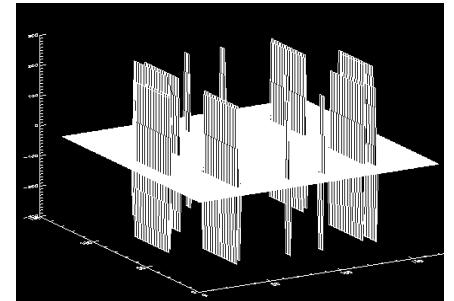


$I = I(x, y)$

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

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

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

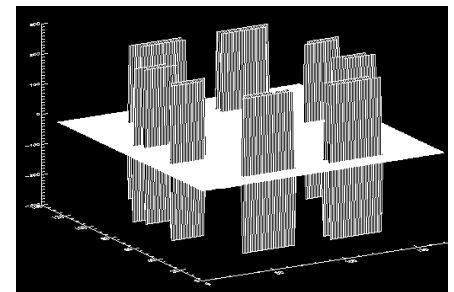


$$\frac{\partial I}{\partial y} \approx \frac{I(x, y + \Delta y) - I(x, y)}{\Delta y} = Ky \otimes I$$

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

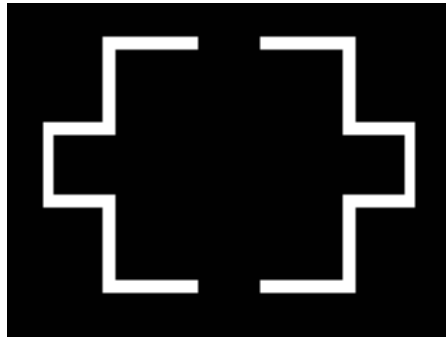
$$Ky = \begin{Bmatrix} 0 & 1 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 0 \end{Bmatrix}$$

flat + -



> Processing | Convolution Filtering

Kernels...



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

Laplace

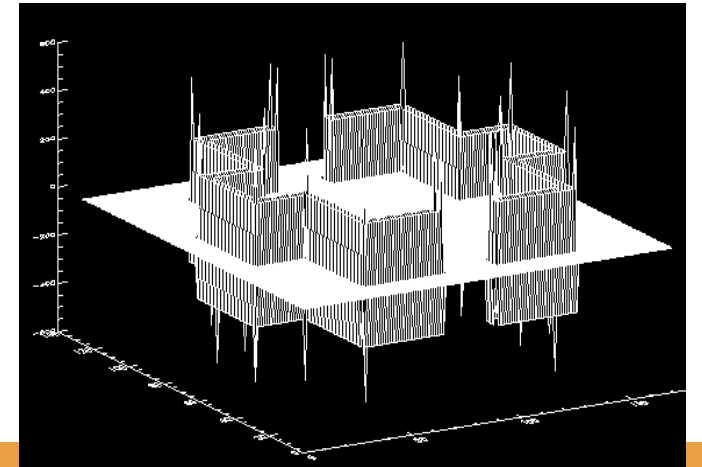
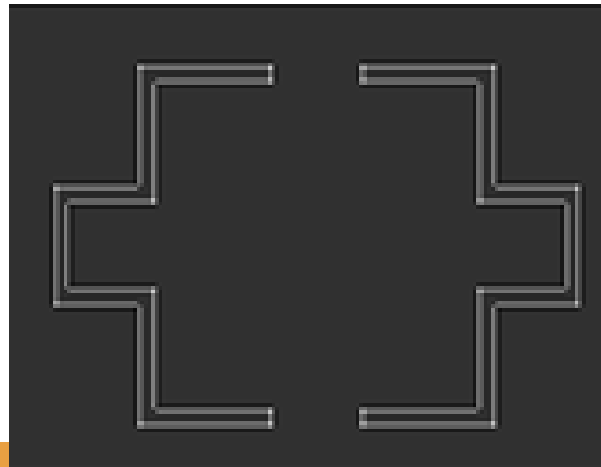
$$\nabla^2 I = \frac{\partial^2 I}{\partial x^2} + \frac{\partial^2 I}{\partial y^2}$$

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

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

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

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



> Processing | Convolution Filtering

Edgemaps

An **edgemap** filter takes intensity changes as ROI boundaries or “edges”

$$f = \sqrt{(Kx \otimes I)^2 + (Ky \otimes I)^2}$$

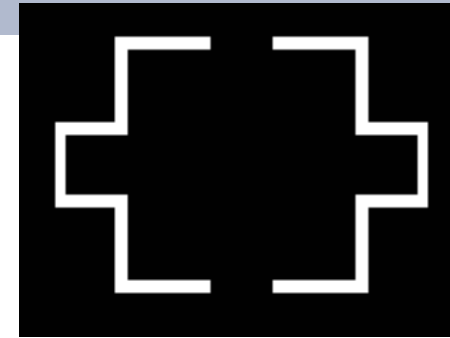
Example:Sobel filter
(notice the thick ROI edges)

$$\begin{matrix} \begin{Bmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{Bmatrix} & \begin{Bmatrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{Bmatrix} \\ Sx & Sy \end{matrix}$$

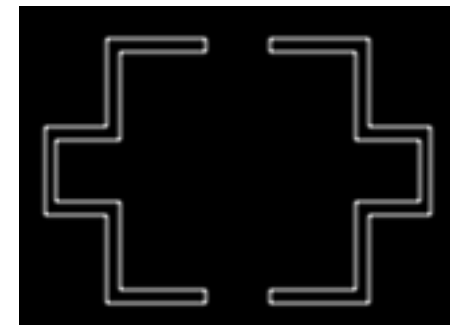
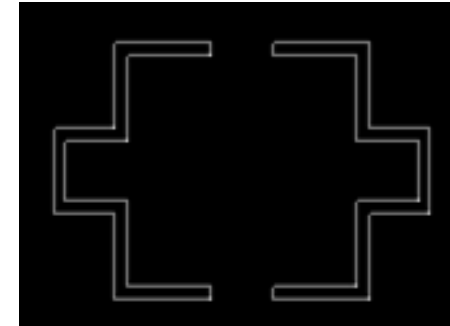
$$Sx \otimes I?$$

$$Sy \otimes I?$$

$$f_{Sobel} = \sqrt{(Sx \otimes I)^2 + (Sy \otimes I)^2}$$

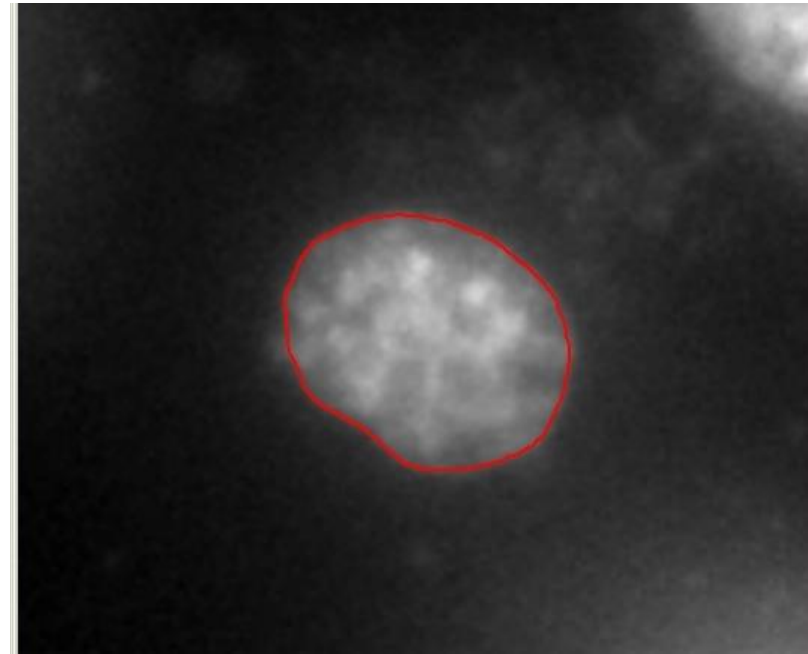
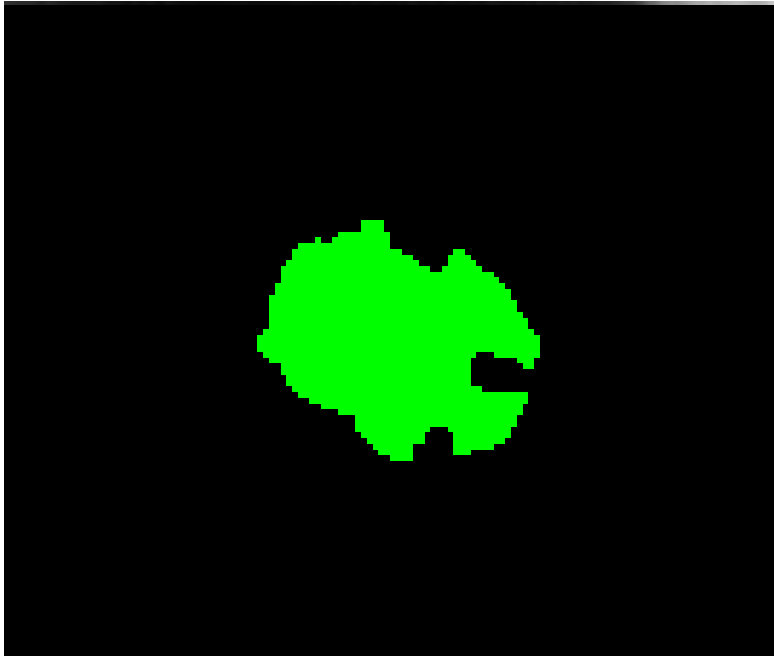


$$I = I(x, y)$$

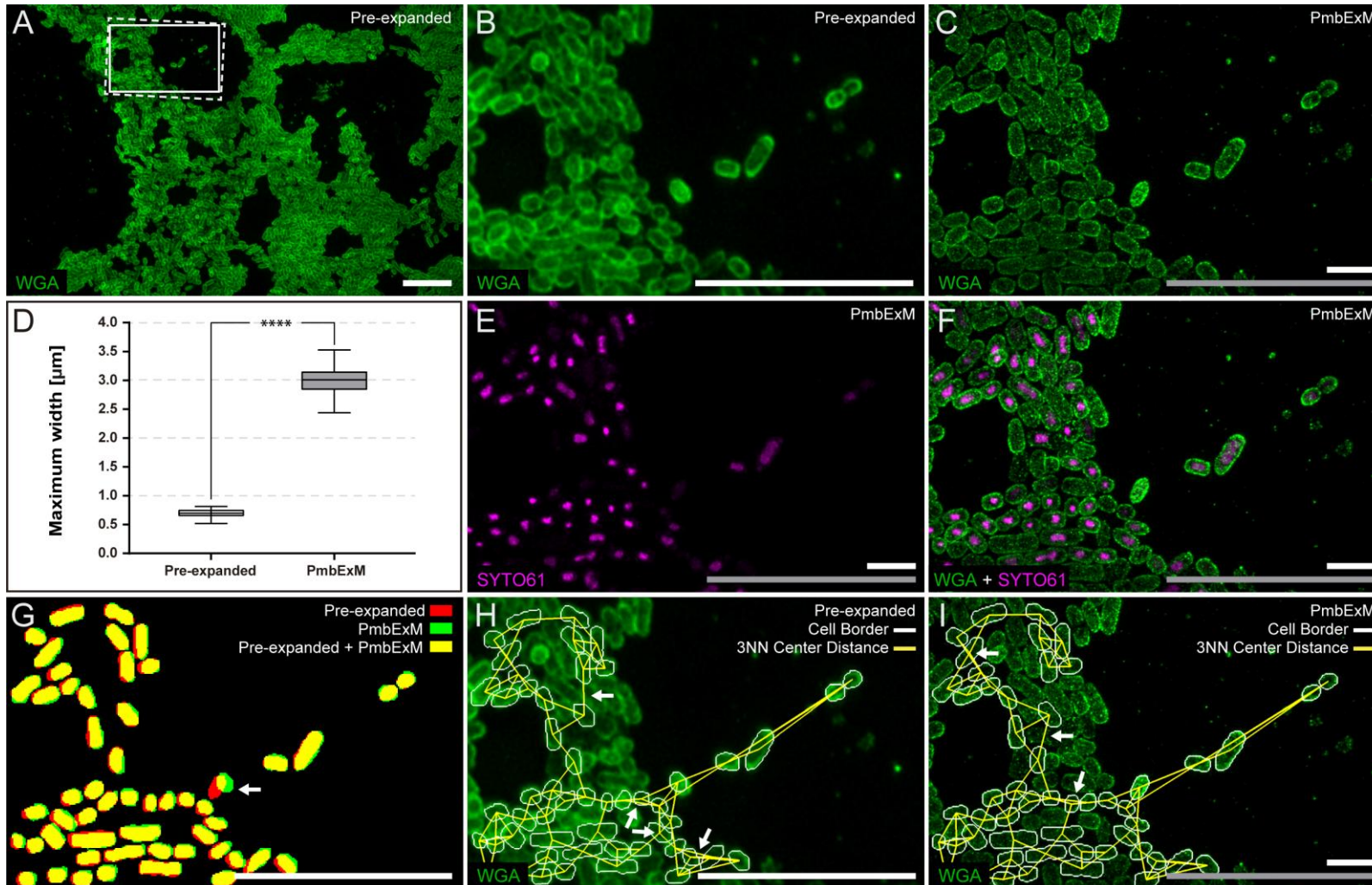


> Processing | Segmentation

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



> Processing | Segmentation



Example / Motivation: *P. mirabilis* / to assess whether an ExM protocol can preserve bacterial shape...

What to do? (What did we do?)

Segment and measure bacterial size and vicinity...

- Size differences
- Perimeter/Contour features
- Nearest Neighbors (distances)

Pre-requisite: pre- & post-ExM images aligned (registration)

> Processing | Segmentation

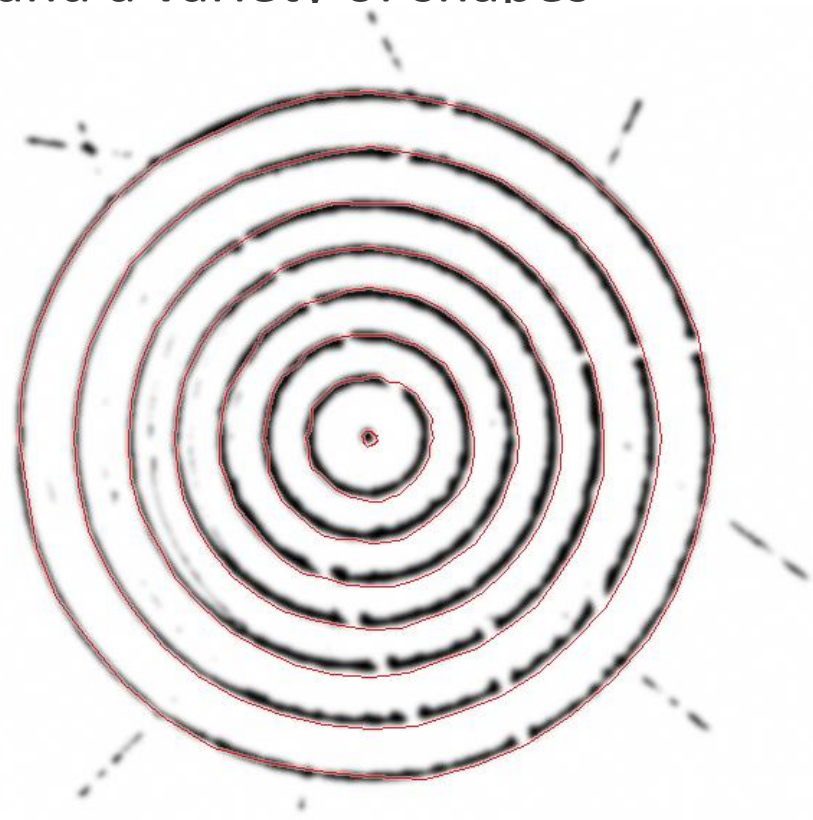
- Template matching
 - “Classic model” Hough transform
 - Applies to circles, line segments and a variety of shapes

Hough P (1959)

If we can detect edges we can
approximate a circle (center, radius)

n connected edges and m ($=n$ or $<> n$)
circles

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



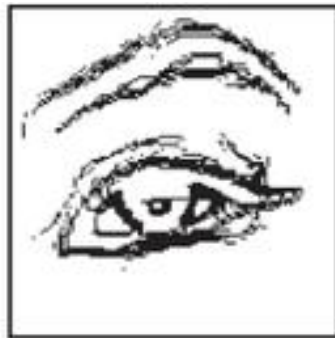
Aguilar P, Hitschfeld N (DCC, 2010)

> Processing | Segmentation

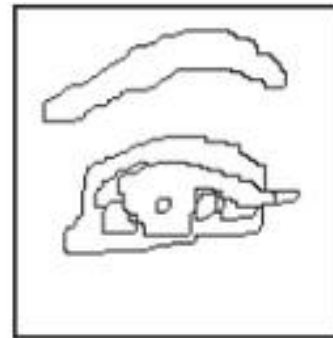
- Variational methods
 - Based on energy minimization, defining integral models
 - Idea: to include desirable features on segmented images (like homogeneous regions, short or smooth ROI boundaries)
 - Optimum solutions found by partial differential equations
 - Examples: Mumford-Shah, Ambrosio-Tortorelli,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



image I



main discontinuities in I



ROI boundaries B



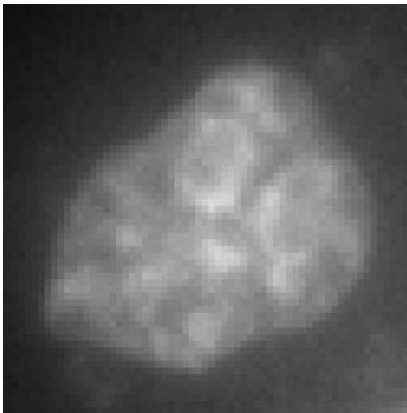
piecewise smooth
image J

$$E[J, B] = C \int d\vec{x} (I(\vec{x}) - J(\vec{x}))^2 + A \int_{D/B} \vec{\nabla} J(\vec{x}) \cdot \vec{\nabla} J(\vec{x}) d\vec{x} + B \int_B ds$$

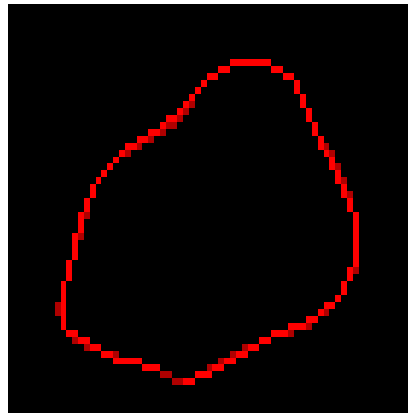
The Mumford & Shah functional (1989)

> Segmentation | Parametric Active Contours

- Active contour models
 - Optimization of different properties

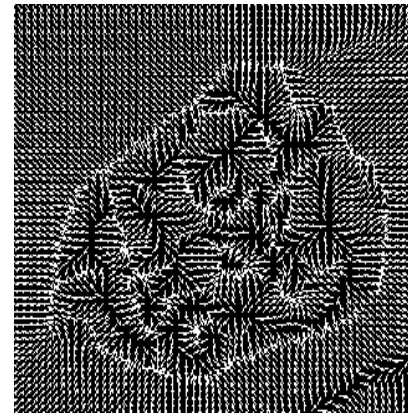


input
image
+ initial guess



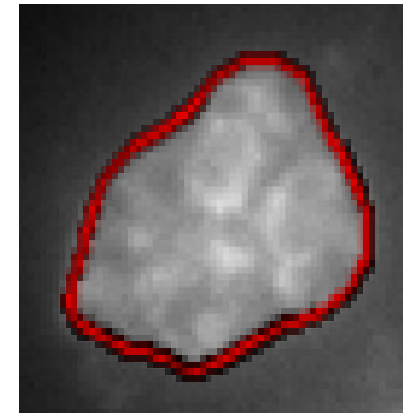
contour $C(s)$

- elasticity
(contraction)
- rigidity
(bending, cornering)



force field

- repulsion
- attraction

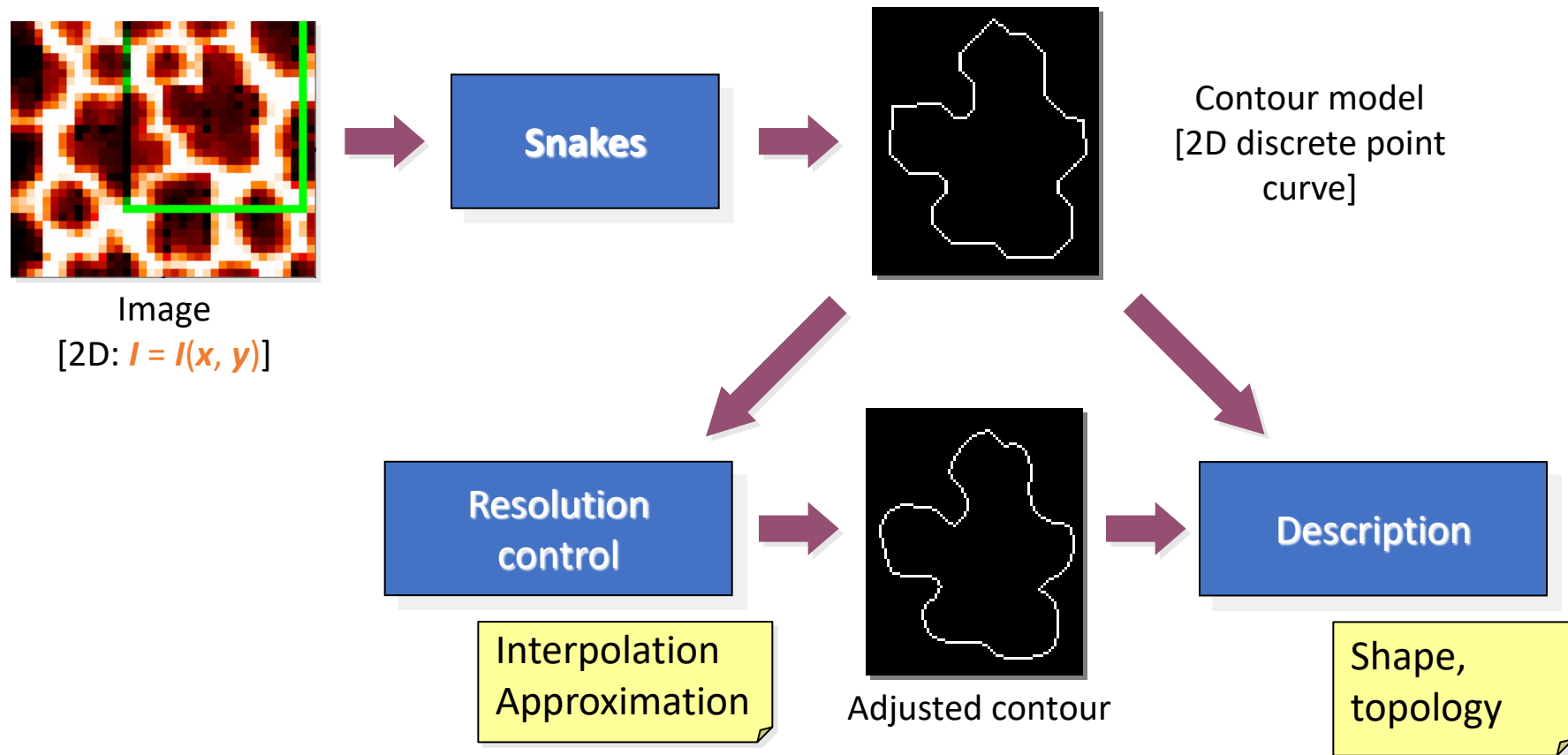


**output: force
balance**
minimal energy

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

> Segmentation | Contour Based

- 2D Parametric Active Contours a.k.a. *Snakes*



> Segmentation | Contour Based

Snakes... 1 contour function $\rightarrow$ 1 ROI
(this is called *parametric approach*)

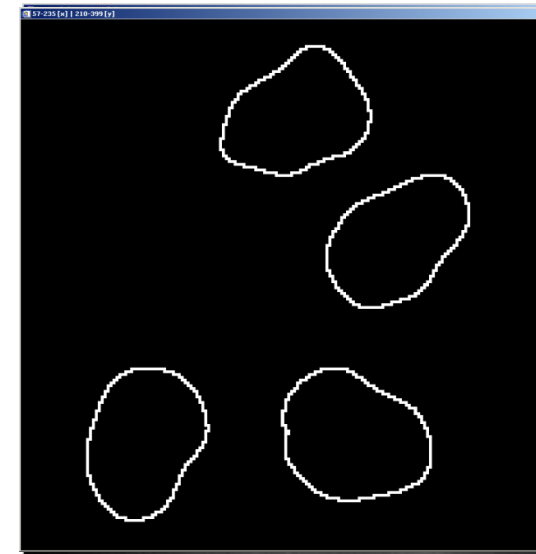
- 2D parametric curve

$$C = C(s) = [x(s), y(s)]$$

$s \in [0, 1]$ (arbitrary length)

- 2D discretization

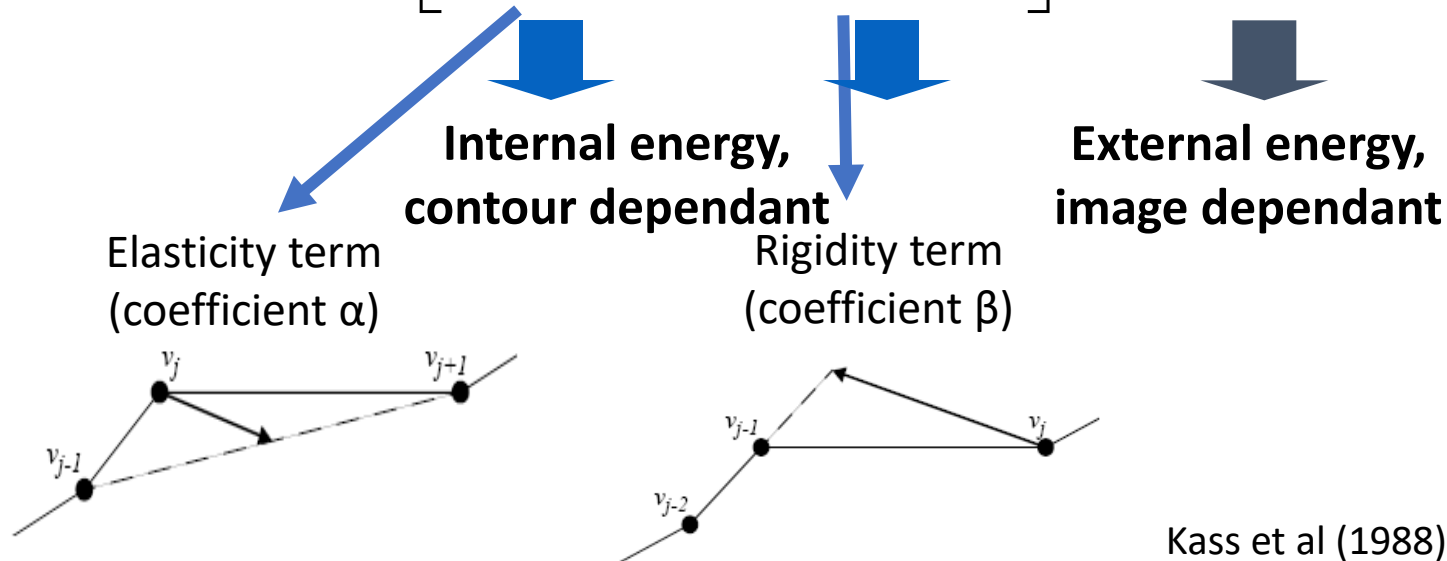
$$C = \{[x_i, y_i]; i = 0..n\}$$



> Segmentation | Contour Based

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

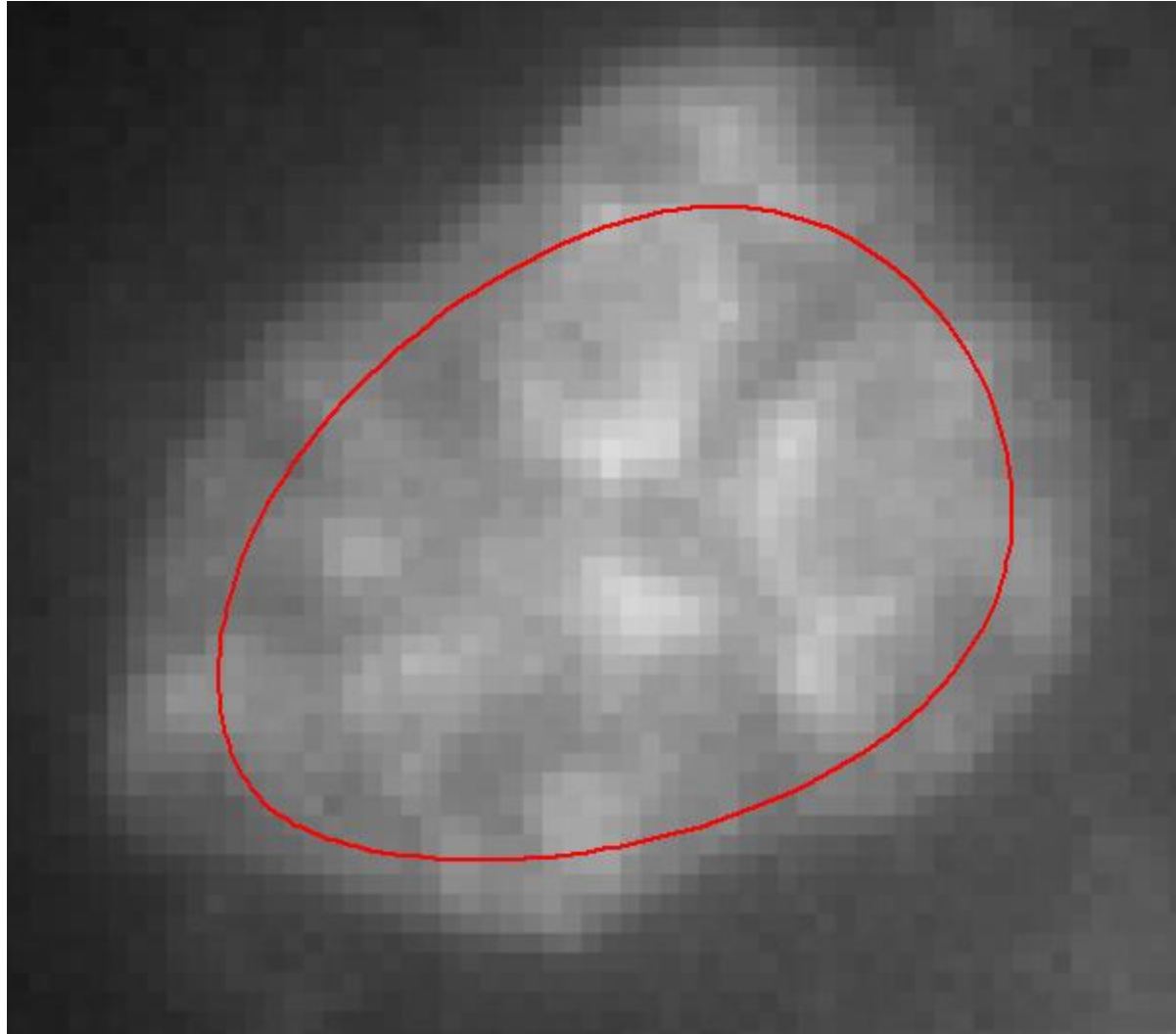
$$E = \int_0^1 \frac{1}{2} \left[\alpha \left| \frac{\partial C(s)}{\partial s} \right|^2 + \beta \left| \frac{\partial^2 C(s)}{\partial s^2} \right|^2 \right] + E_{ext}[C(s)] ds$$



Kass et al (1988) Snakes: active contour models
Int. J. of Computer Vision 1(4): 321-331

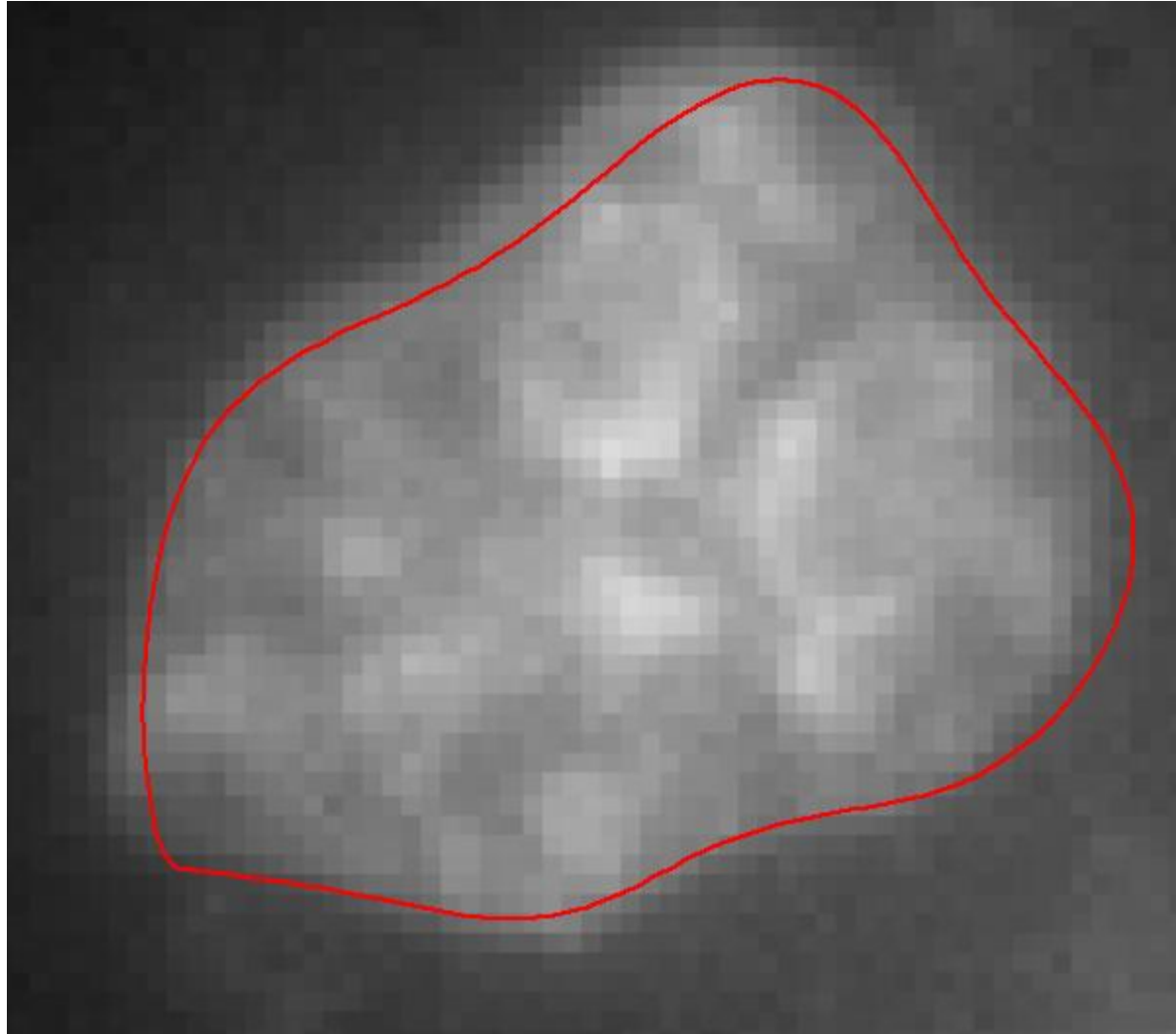
> Segmentation | Contour Based

Snake Elasticity - α



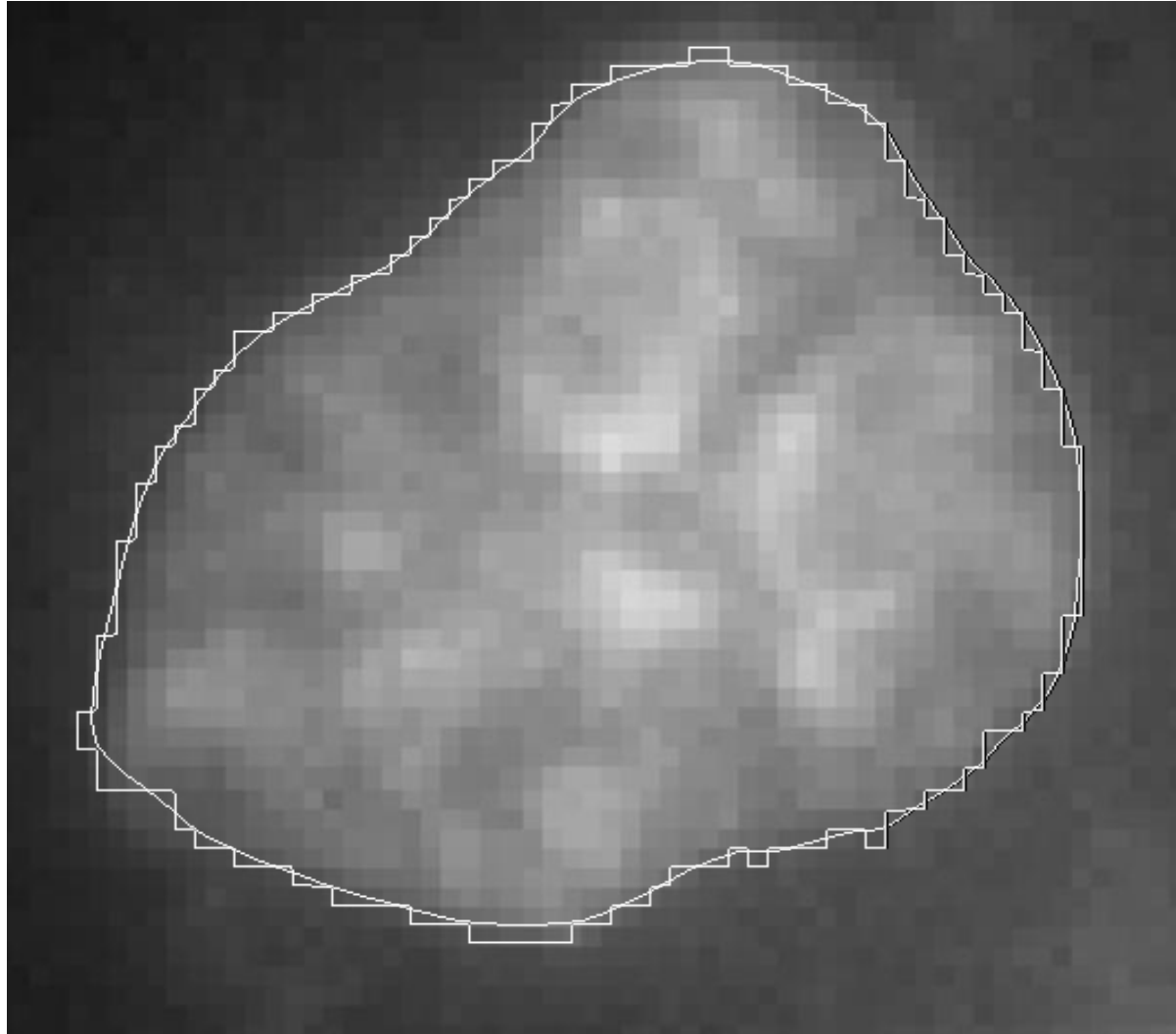
> Segmentation | Contour Based

Snake Rigidity - θ



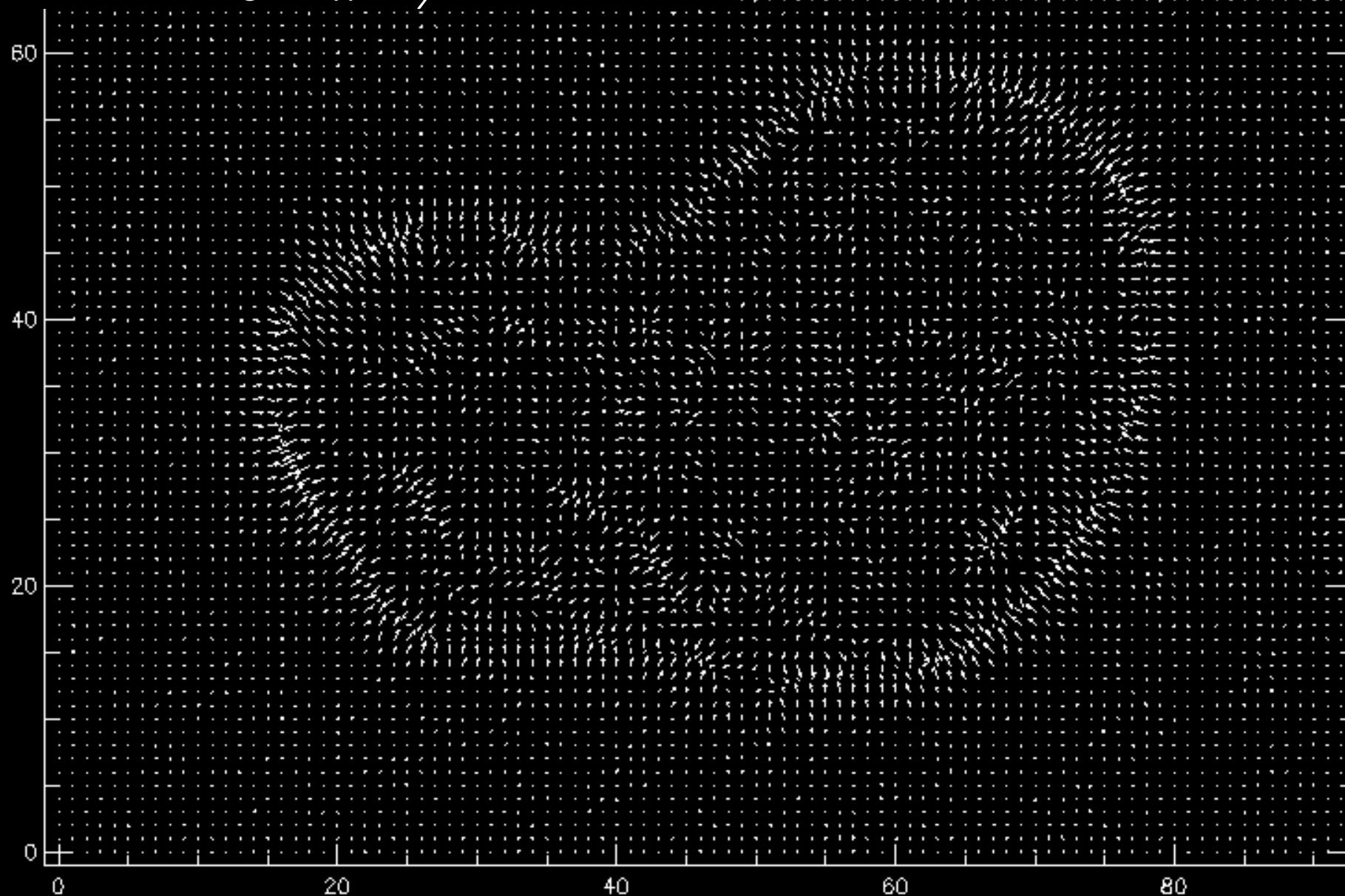
> Segmentation | Contour Based

Contour point density/sampling

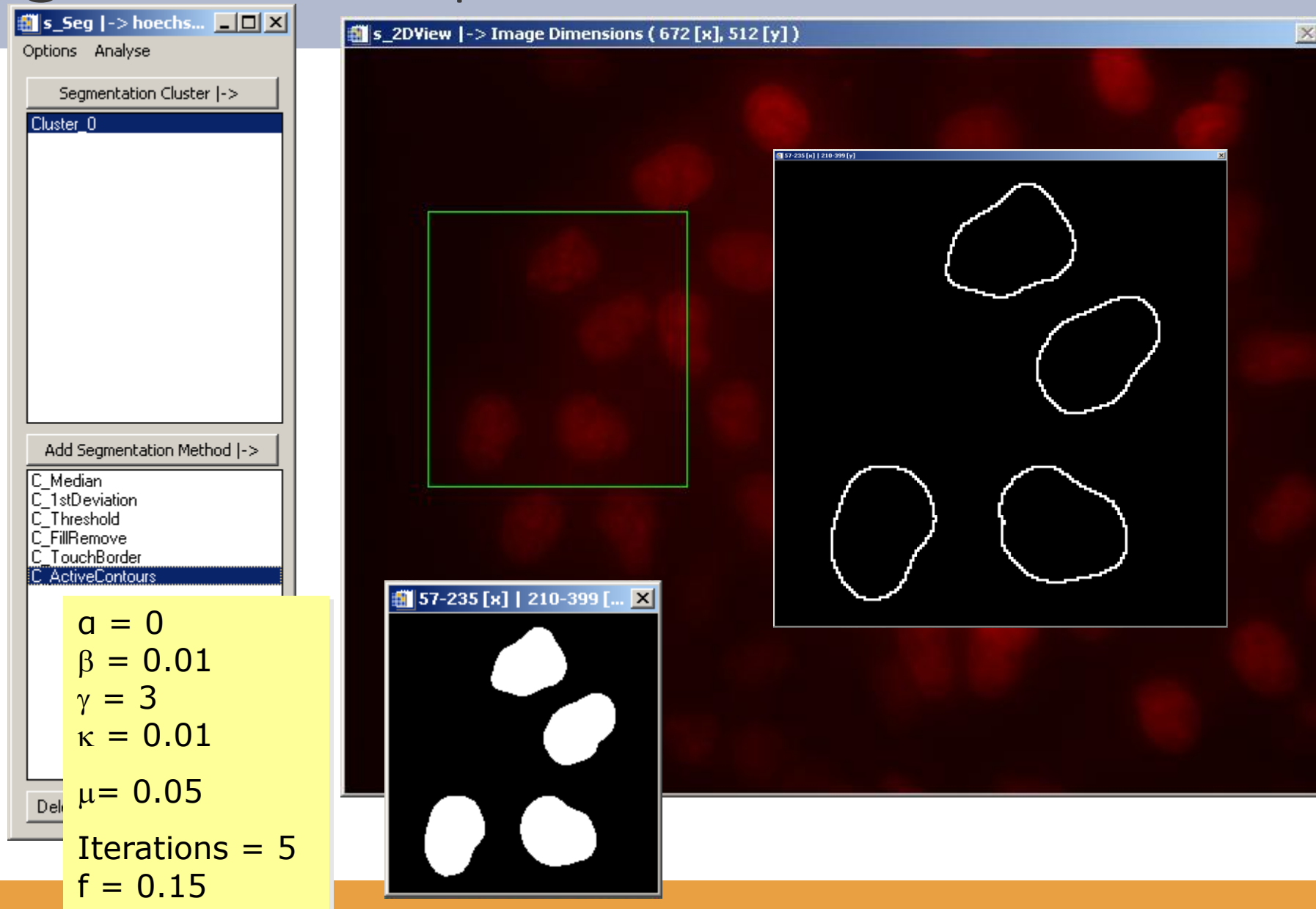


Intensity gradient vectors

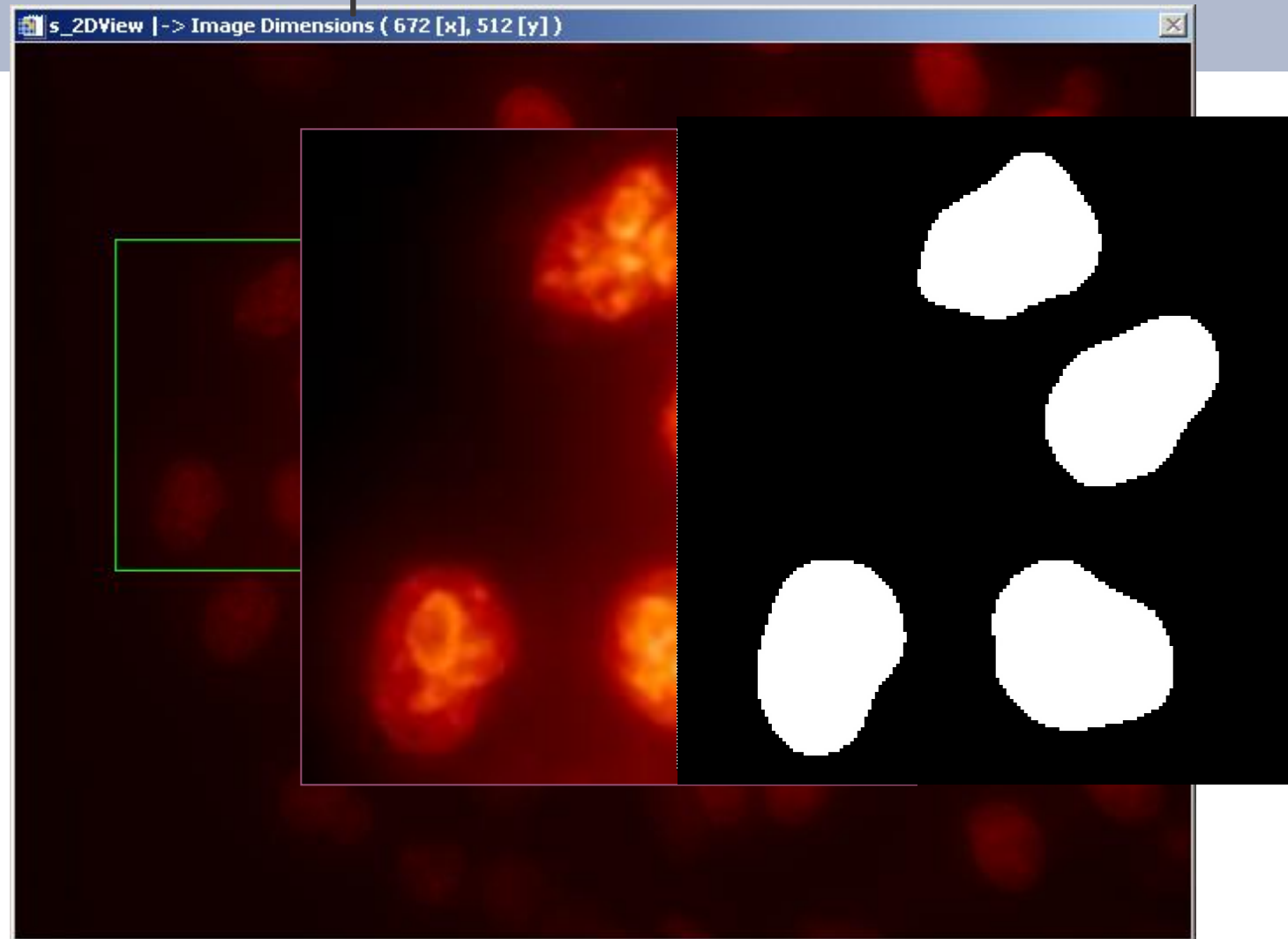
$$V_0 = [I_x, I_y]$$



> Segmentation | Active Contours

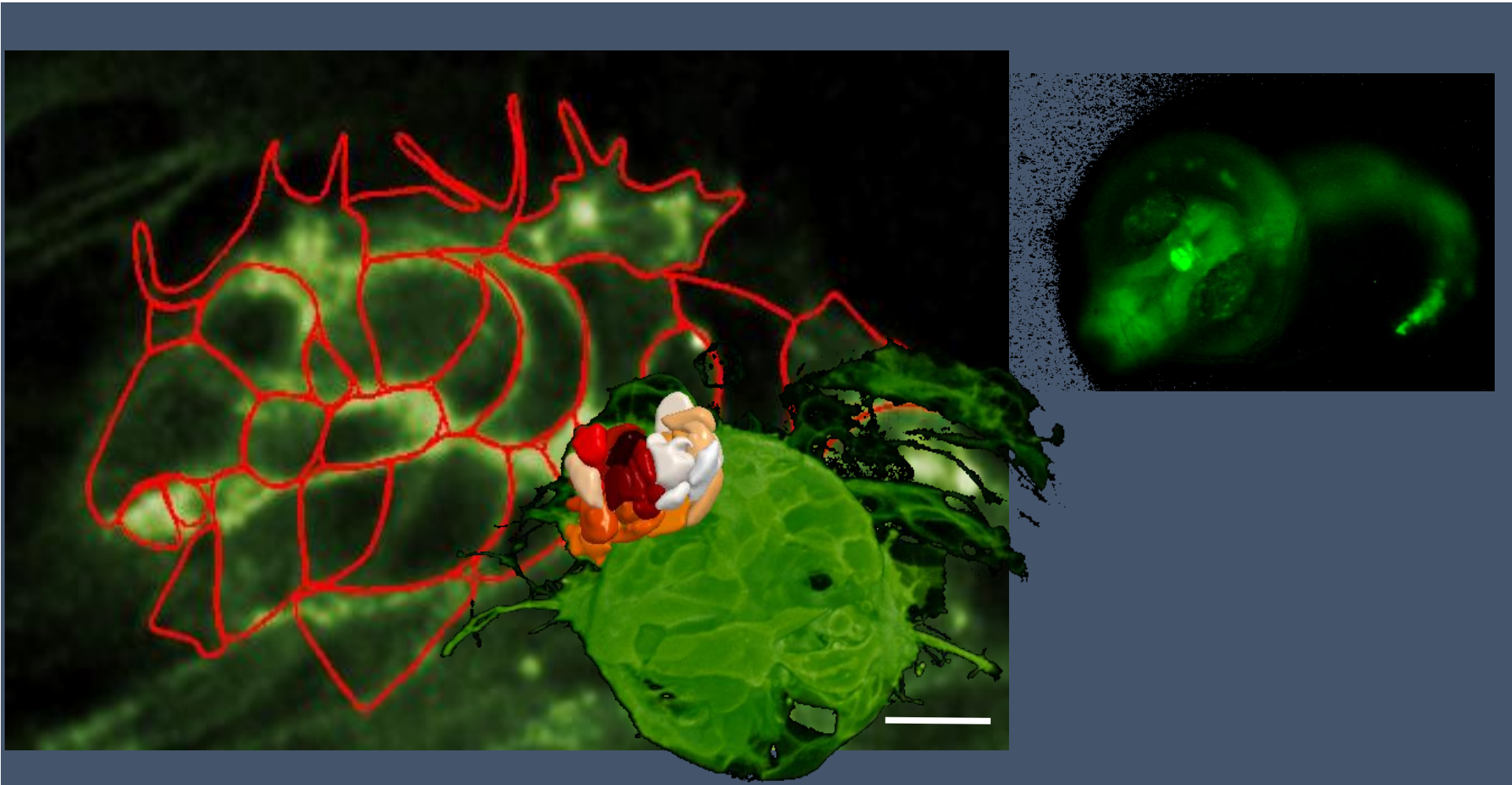


> Segmentation | Active Contours



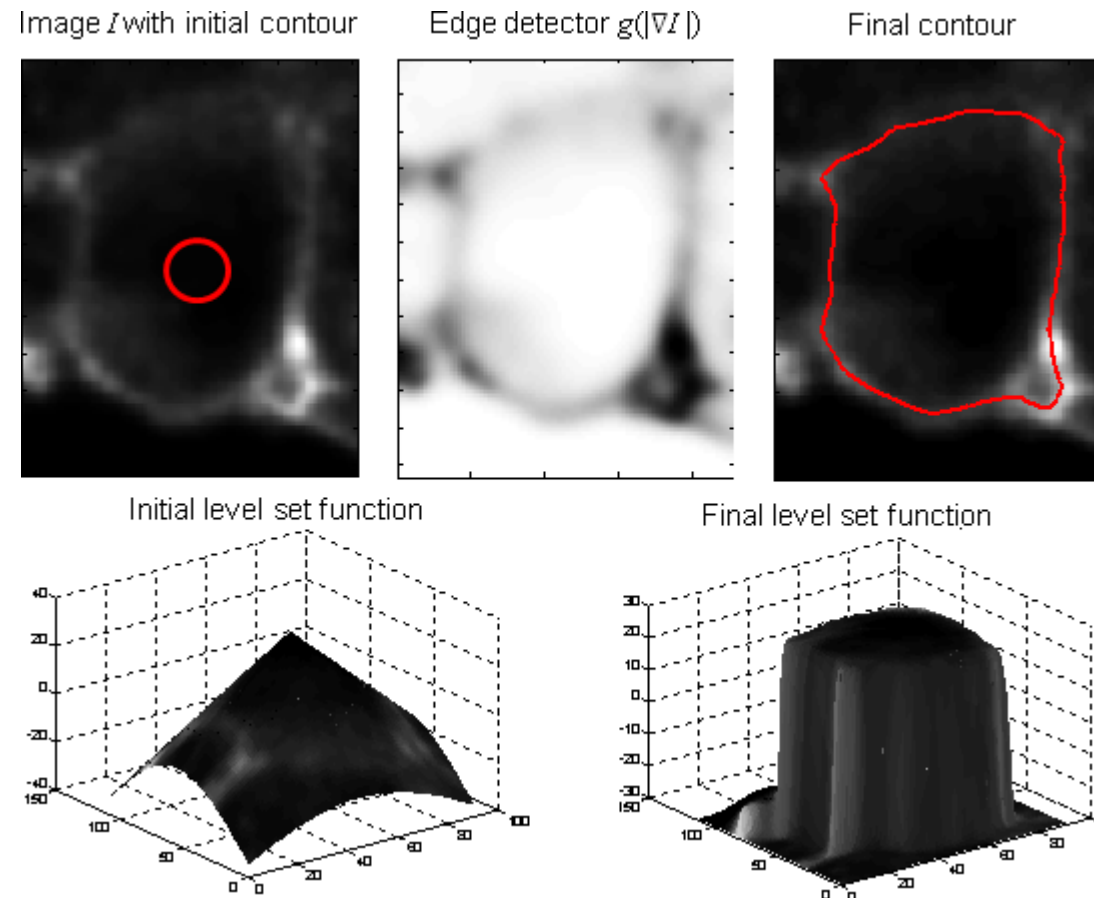
> Segmentation | Active Contours

Hardest cases: initialization by manual ROI sketching



> Segmentation | Implicit (“Geometric”) Contours

Subjective Contours (Sarti & Sethian, PNAS 2002; Zanella et al. 2010 TIP)



<http://ieeexplore.ieee.org/document/5280277/?arnumber=5280277&tag=1>

> Segmentation | Contour Models

Boundary model construction

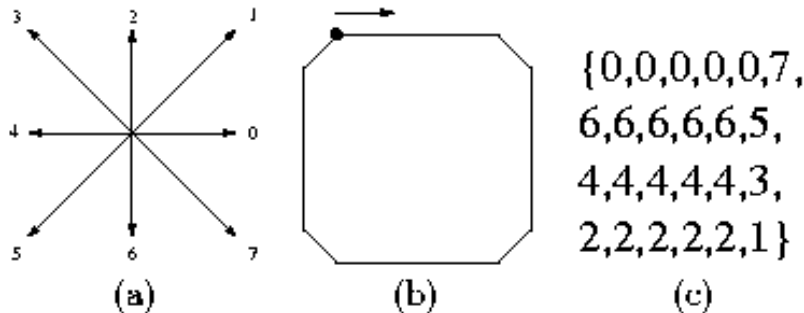
- 2D Freeman chain code

Books:

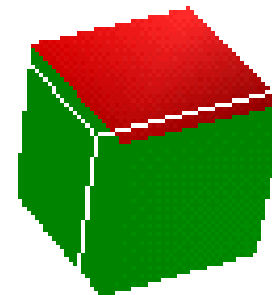
Nixon & Aguado, Feature extraction & image processing

González & Woods

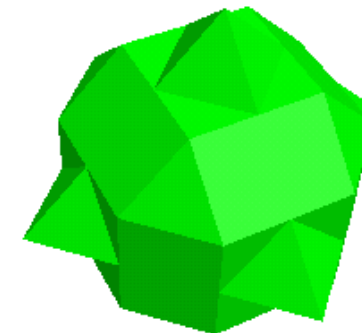
- 3D mesh models (voxel based):
marching cubes (surface meshes), *tetrahedra* (volume meshes)



2D polygon chain code



Voxel ("3D pixel") model



3D polygon mesh

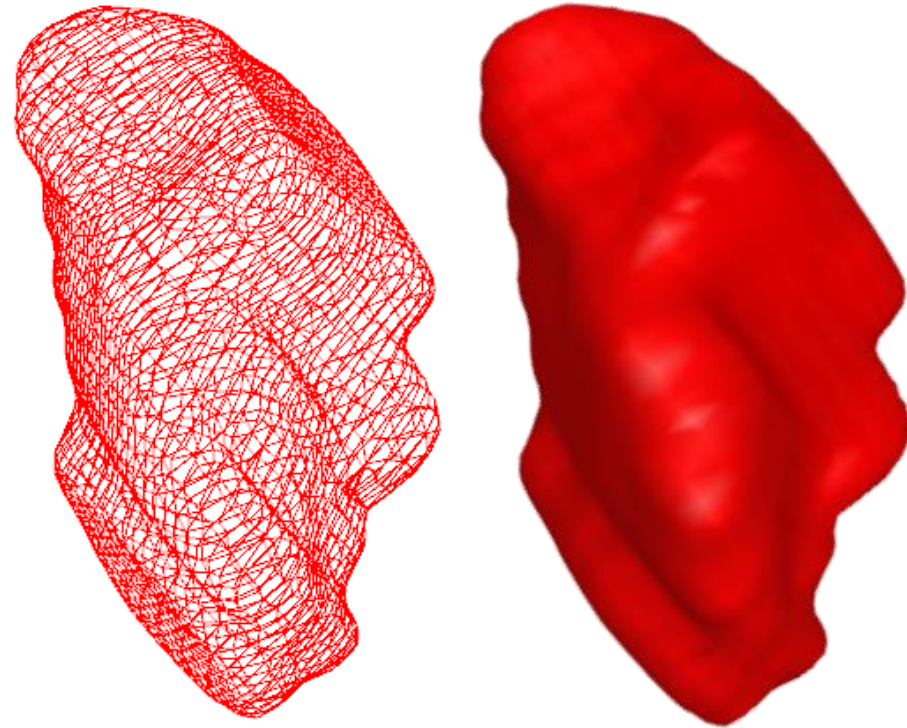
> Segmentation | Contour Models

- A typical 3D surface mesh model is formed by:

- Nodes or vertices
- Polygons

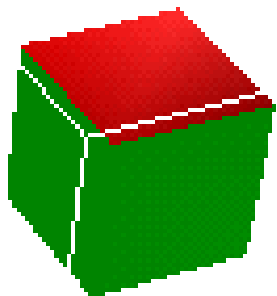
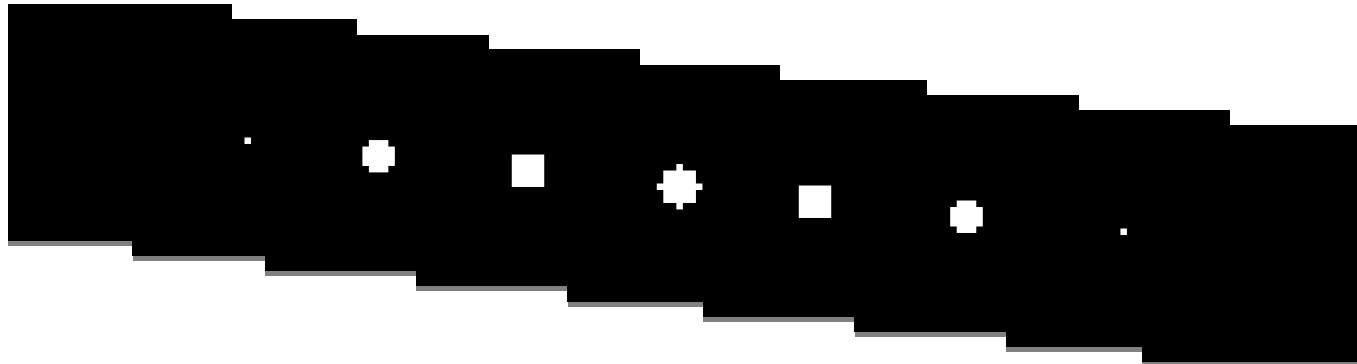
- Other models

- Polynomial surfaces
(splines, Bézier, NURBS, ...)
- Primitives composition



> Segmentation | Contour Models

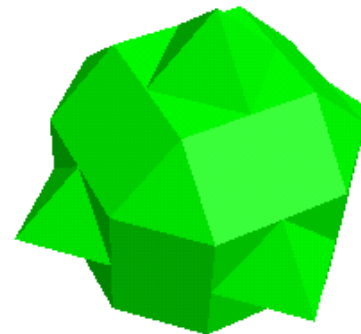
- Surface mesh model construction
 - Many approaches for construction
 - Many approaches for modeling



Voxel ("3D pixel")
model



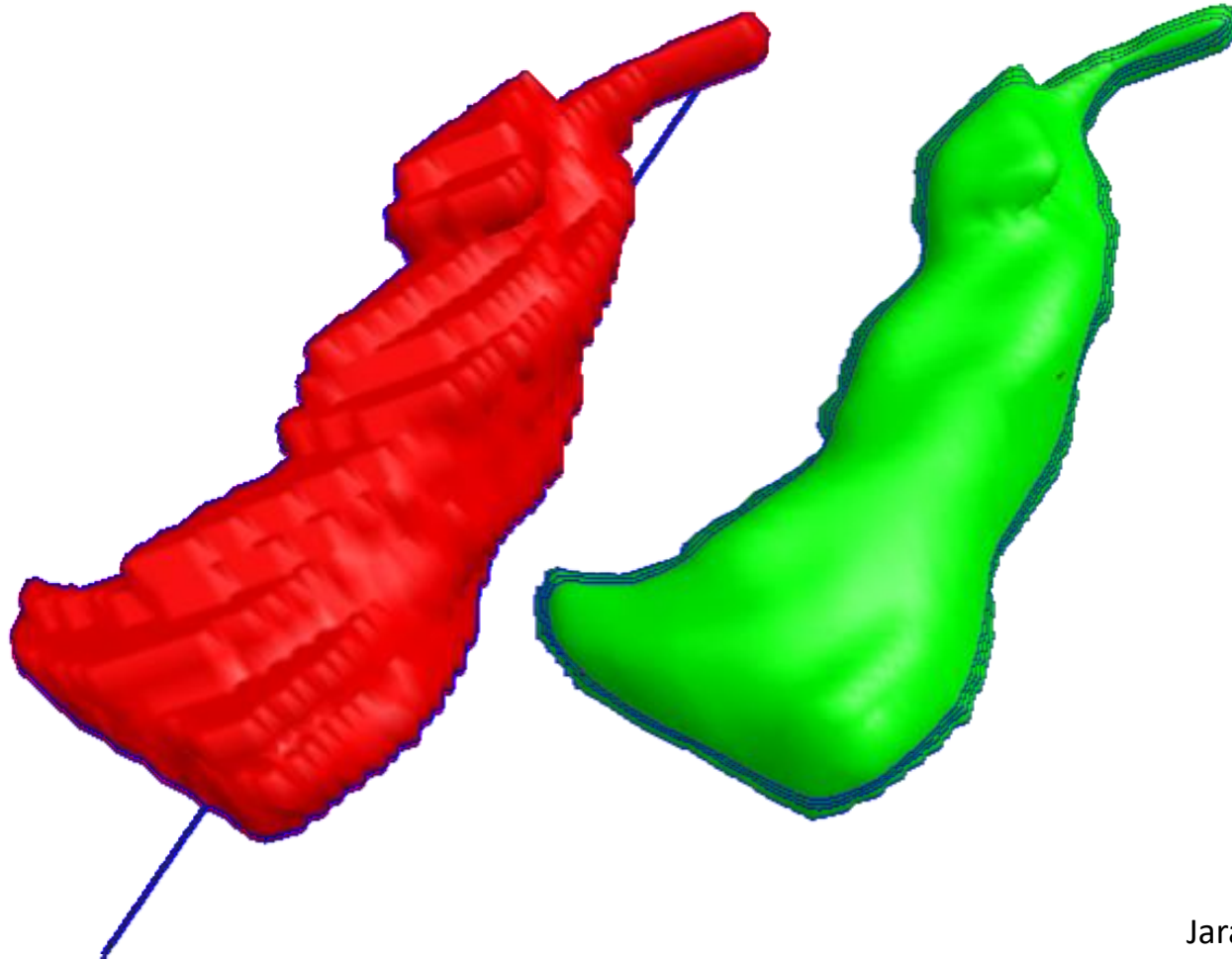
Triangle mesh



Triangular and
quarilateral mesh

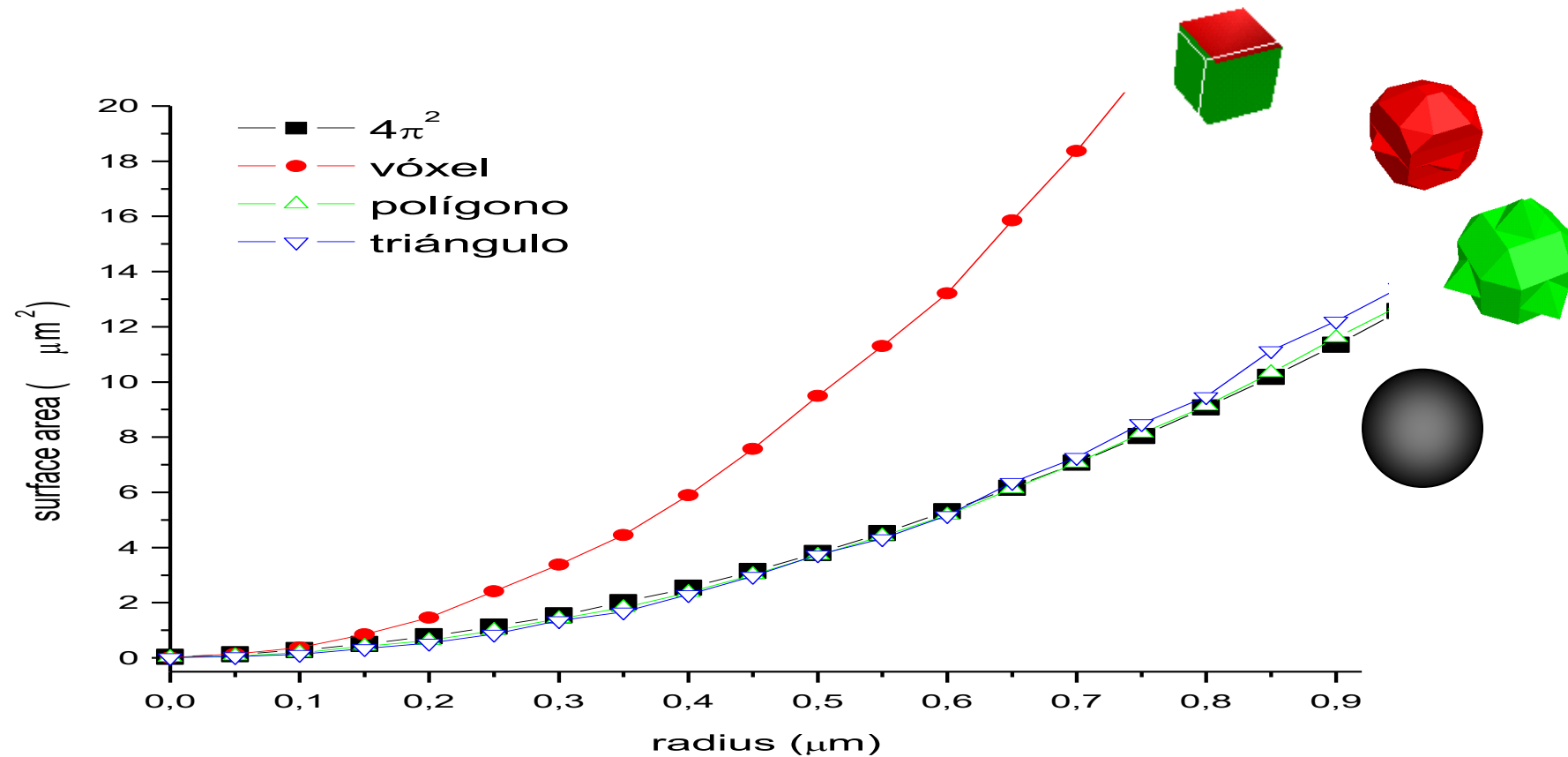
> Segmentation | Contour Models

- Sample 3D ROI



> Segmentation | Contour Models

- ROI model affects measurements!

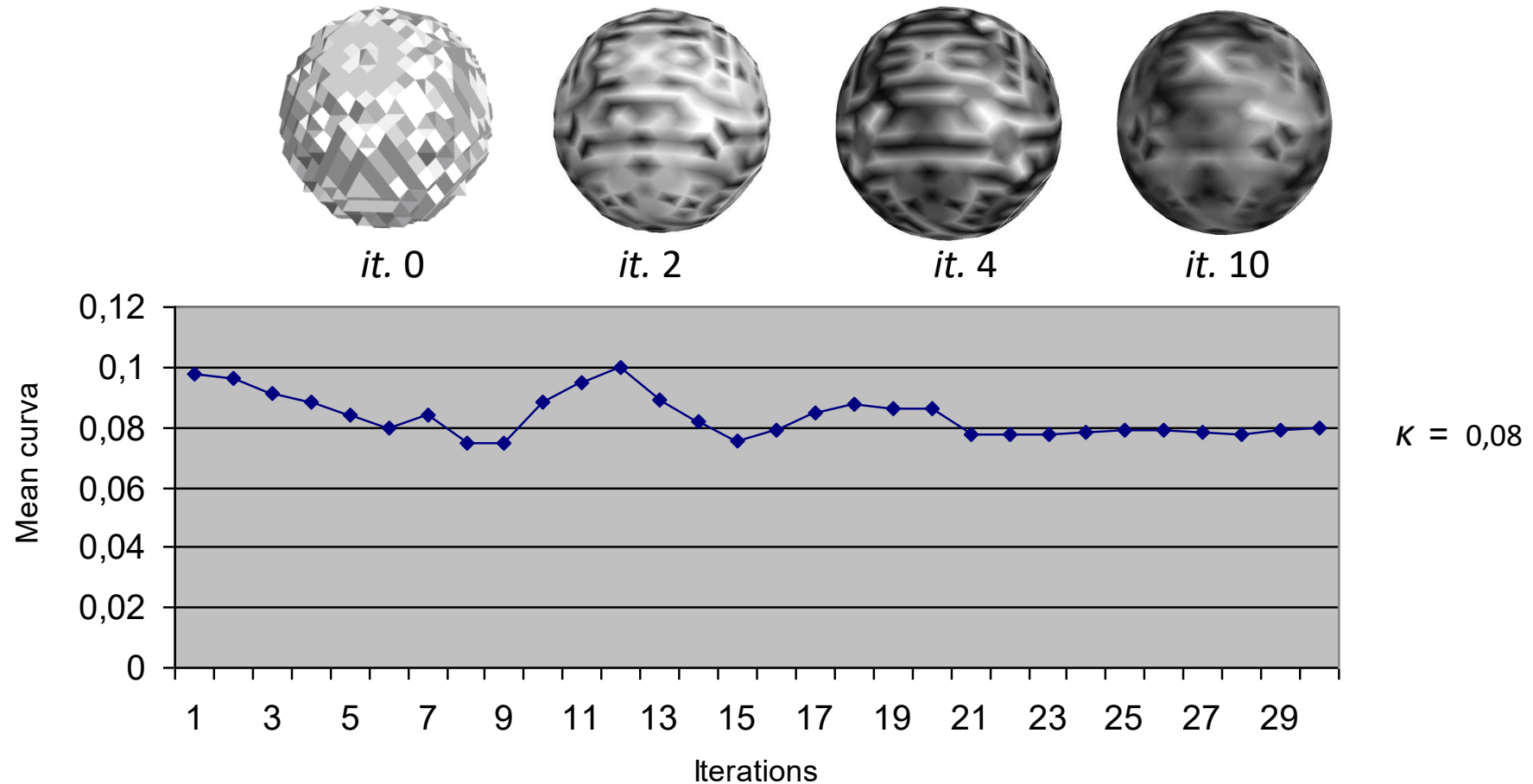


$r = 1\mu\text{m} = 20 \text{ z-slices}$

2D $\rightarrow$ perimeter
3D $\rightarrow$ surface area

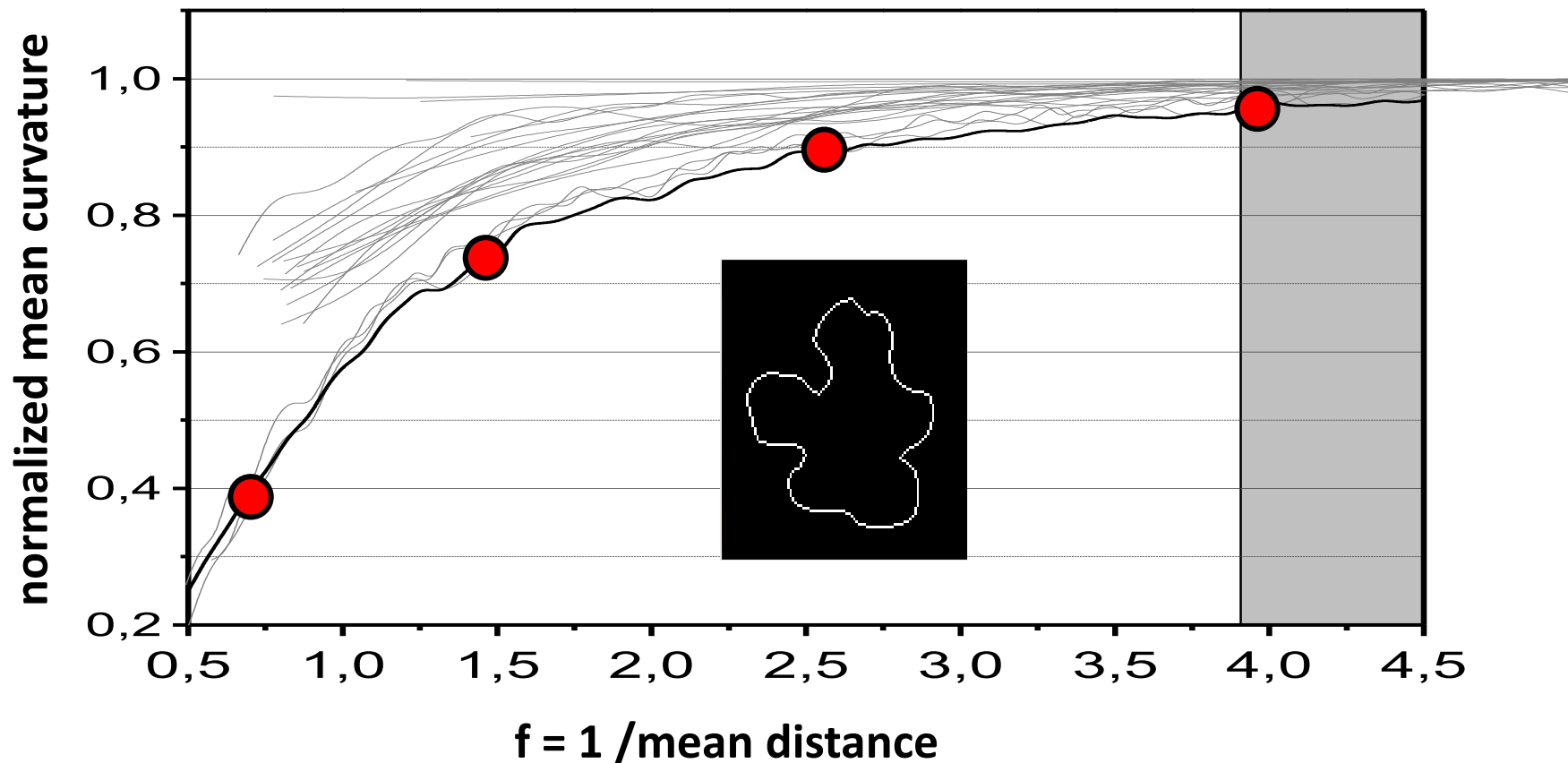
> Segmentation | Contour Models

- Calibrating contour resolution. Example:



> Segmentation | Contour Models

- Calibrating contour resolution. Example:



> Some free and/or open source tools

- Java based (Java runtime required)
 - ImageJ (<http://rsbweb.nih.gov/ij/>, public domain)
 - **FIJI** (<http://fiji.sc>; GPL license) with plugins...
 - MorpholibJ
 - MicrobeJ
 - Weka trainable segmentation
 - Icy (<http://icy.bioimageanalysis.org>; GPLv3 license)
- Others
 - CellProfiler (<http://cellprofiler.org>; GPL, BSD licenses)
 - Slicer (www.slicer.org; BSD license)
 - IPOL (Image Processing Online): open access electronic journal with peer reviewed articles + code (languages: C/Python) + examples (www.ipol.im; BSD / GPL / LGPL licenses or similar)
 - SCIAN-Soft by yours truly (www.scian.cl, github.com/scianlab)
 - Bioimage Analysis Lectures. Robert Haase (2020)
<https://www.youtube.com/playlist?list=PL5ESQNfM5lc7SAMstEu082ivW4BDMvd0U>