Procesamiento de Imágenes III-IV

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www.scian.cl / www.cimt.cl / https://bni.cl/biomat.php

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> Filtros

Visualization
(screen + print)

Inverted greyscale LUT + contrast enhancement for publication. Scale bars: 10 µm

Jara-Wilde et al. (2020) Journal of Microscopy

> Procesamiento

- **Image Analysis**

The extraction of meaningful descriptions of features of interest from images

Adapted from
Young I, Gerbrands J, van Vliet L (1995)
Fundamentals of Image Processing. Delft: PH

> Procesamiento

- Examples of analysis tasks
  - **Objects/regions identification (segmentation)**
    - Cells and/or their organelles
  - **Registration: image, region and/or feature “matching”**
    - Drift correction of the sample (from acquisition)
    - Relative speeds/displacements inside a given cell or reference system
    - Correspondence finding between images, objects or sections of these
  - **Motion estimation, object tracking**
    - Individual & collective migration
  - **Morphology, topology, texture characterization**
  - **Classification**
    - Detection of different populations, anomalies

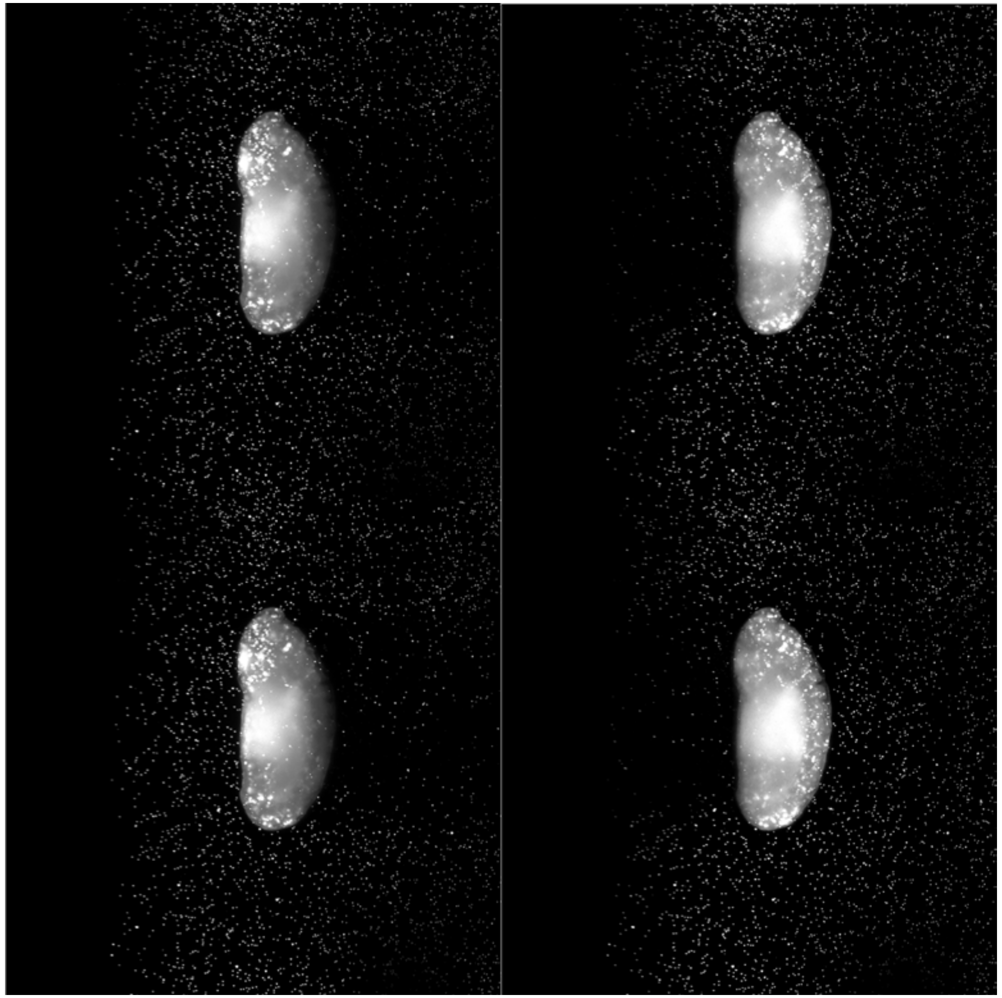
Now: segmentation

Coming soon: motion estimation & tracking, morphology… characterization

Next year(?): classification

> Procesamiento

- Registration



In FIJI: Plugins->Registration
Additional FIJI plugins: StackReg, TurboReg
http://bigwww.epfl.ch/algorithms.html

Applied example: 4-view LSFM image merging

C . Parada & C. Bolatto (UdelaR, Uruguay), L. Alé (LEO/SCIAN & BNI)

> Concepts

- Identification (features, objects, scenes)

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

> Procesamiento

- **Segmentation**

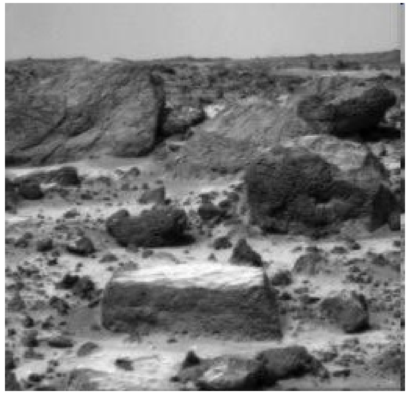
  - The partitioning of a given image into regions of interest (ROIs) according to given criteria (e.g. color).

  - After segmentation, further characterizations can be performed upon the resulting ROIs.

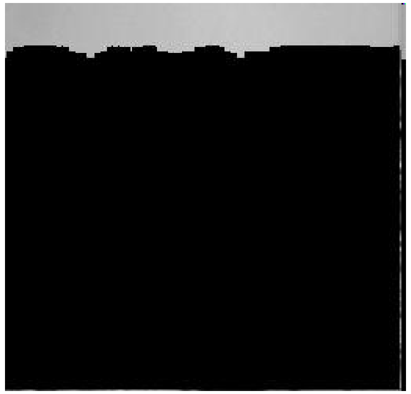
Shapiro LG and Stockman GC (2001):
“Computer Vision”, pp 279-325
New Jersey, Prentice-Hall

> Procesamiento

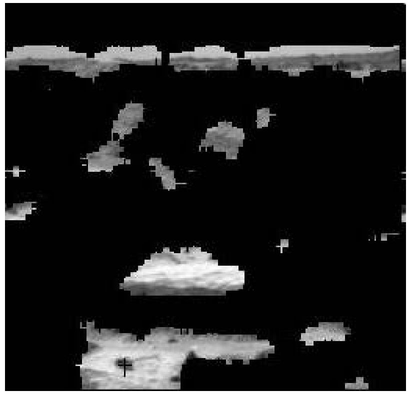
- **Segmentation** ("pre Machine Learning era")



Sol 3, Mars
Pathfinder Mission

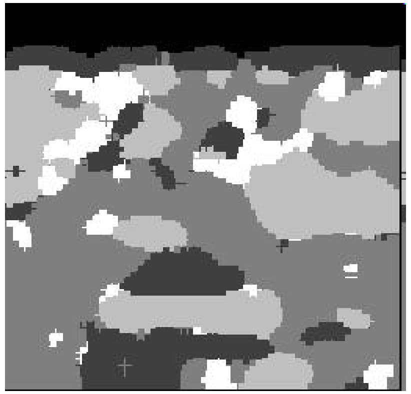


Sky / Flat



Dust / Horizon

...etc...

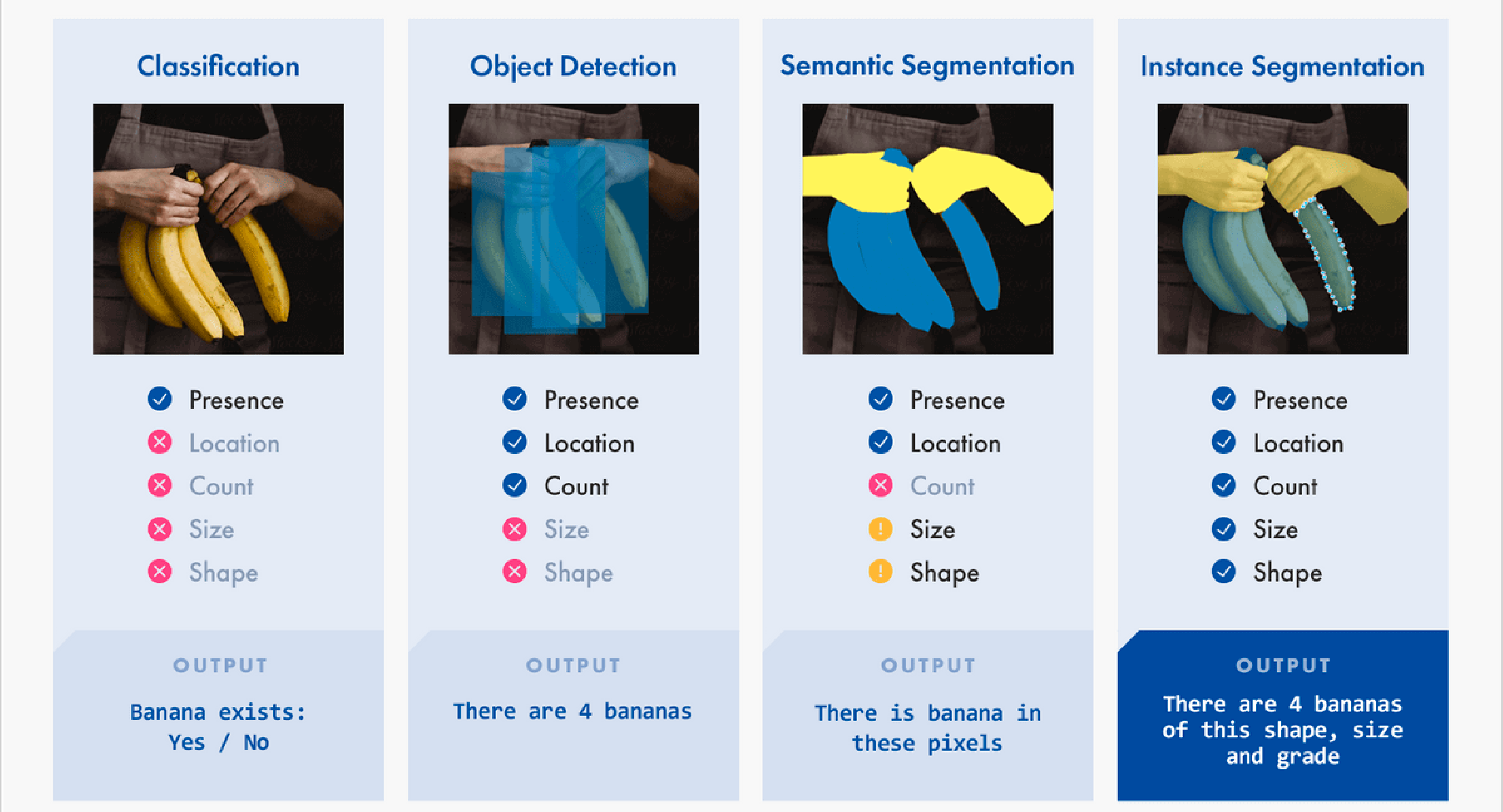


Final segmentation

Source: NASA

> Procesamiento

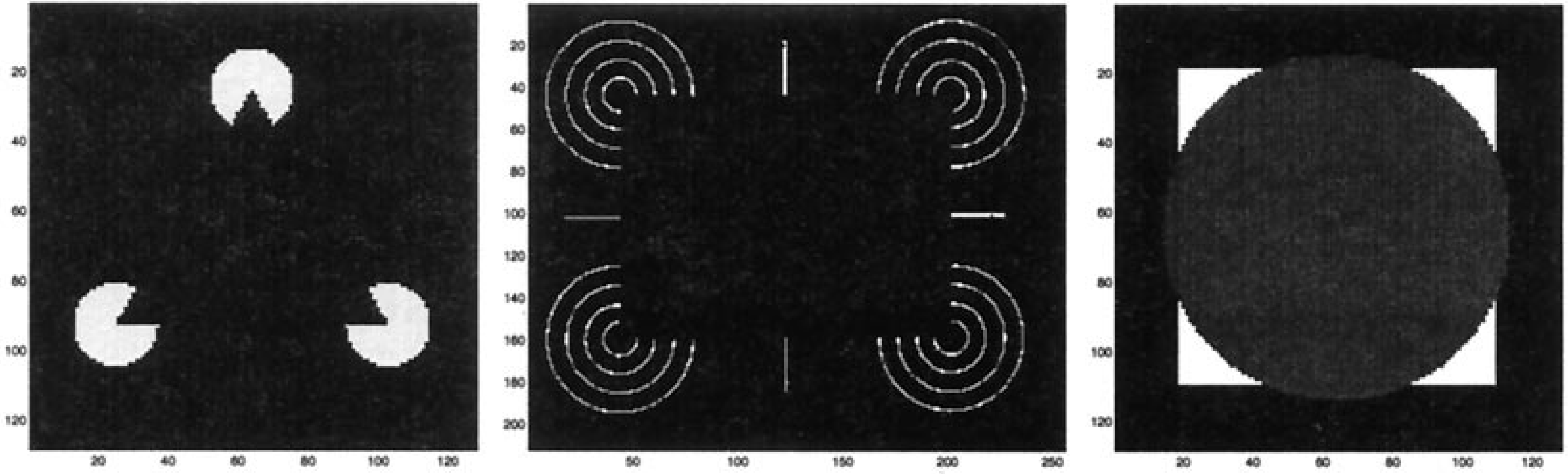
- **Segmentation** (“2020’s Machine Learning era”)



C. Vásquez (UdeC/UCh)

> Concepts

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



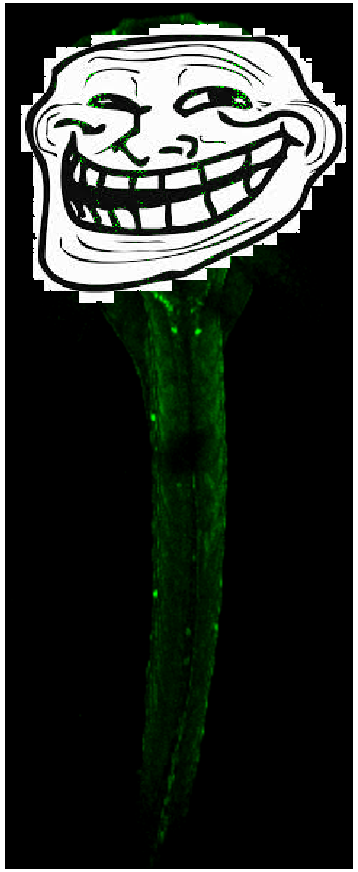
  - …not always (almost never) enough information for a 100% accurate segmentation.

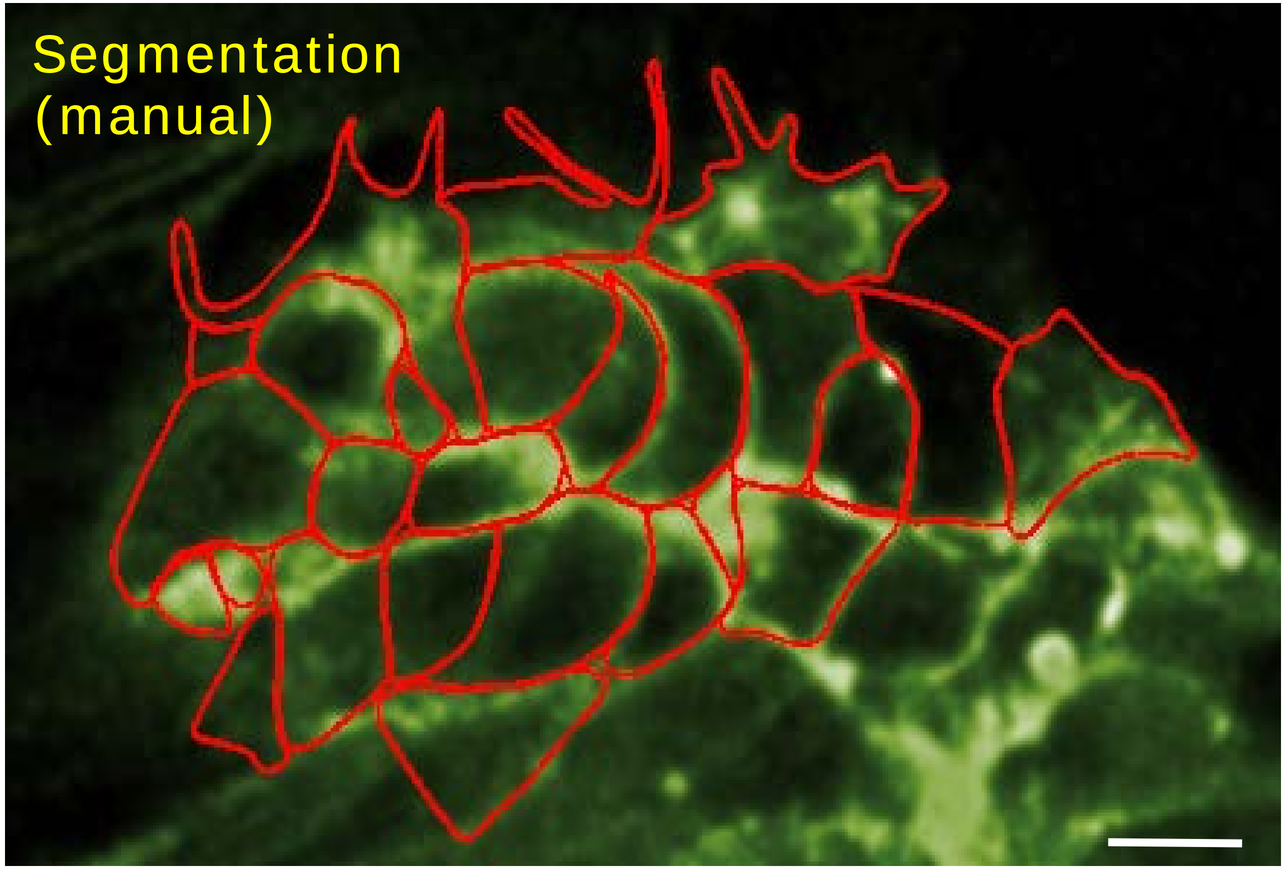
D. Marr (1982) Vision

Sarti & Sethian (2000) Subjective surfaces. PNAS

> Procesamiento

Problem?





CG Lemus, S Härtel, ML Concha (LEO, SCIAN-Lab, BNI)

> Procesamiento

Problems

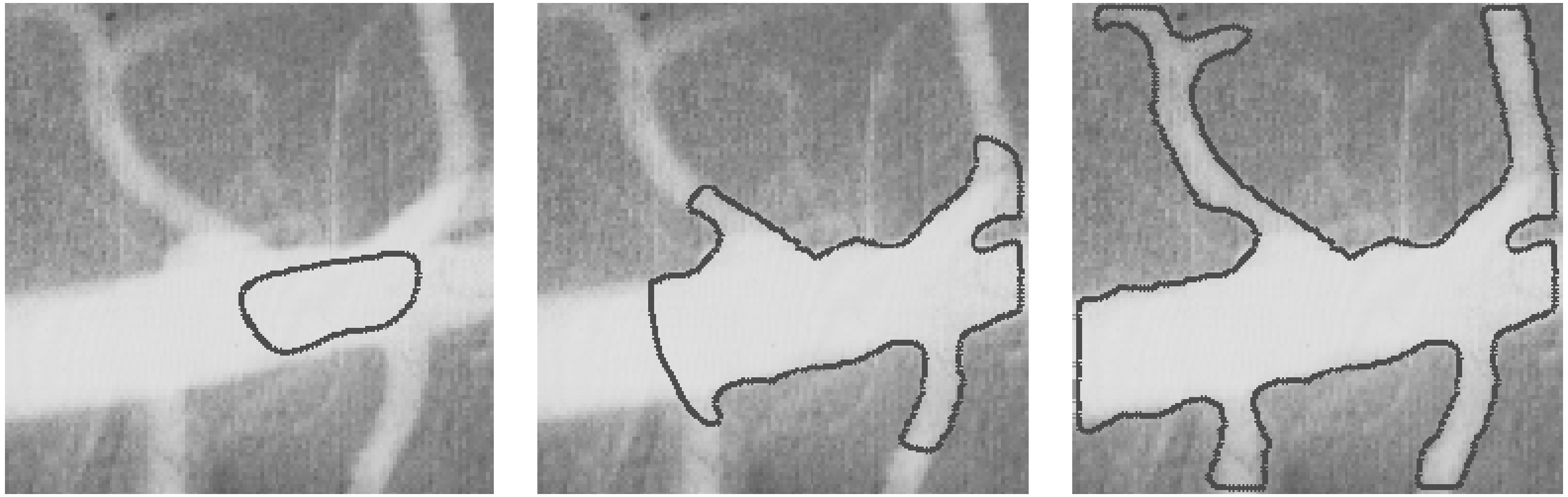
- Lack of absolute criteria or standards (**Ground Truth**, **Gold Standard** [1,2])
- Missing or erroneus information (e.g. non-specific markers in samples)
- What to do?
  A "good" (i.e. carefully performed and controlled) acquisition to ease this task

[1] Jason D. Hipp et all. 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

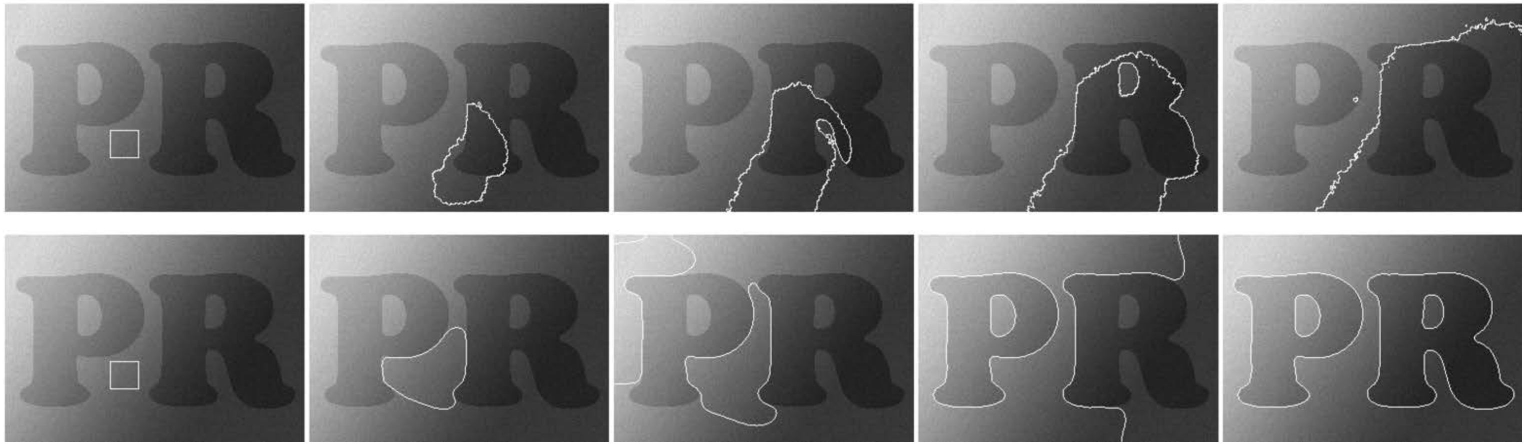
[2] Luc Bidaut, Pierre Jannin. Biomedical multimodality imaging for clinical and research applications: principles, techniques and validation. In Molecular Imaging:Computer Reconstruction and Practice (NATO Science for Peace and Security Series B: Physics and Biophysics), Springer, 2008, ISBN-13: 978-1402087516.

> Procesamiento

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

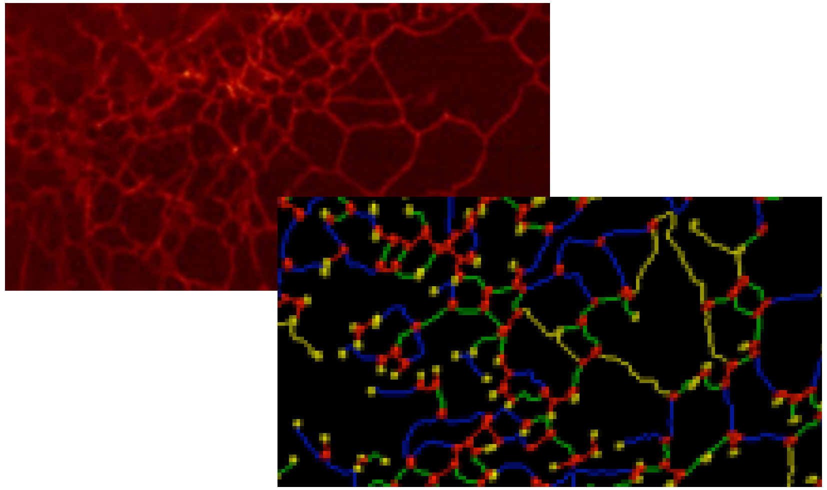


X Xie (2010) Magnetostatic Active Contours

> Procesamiento

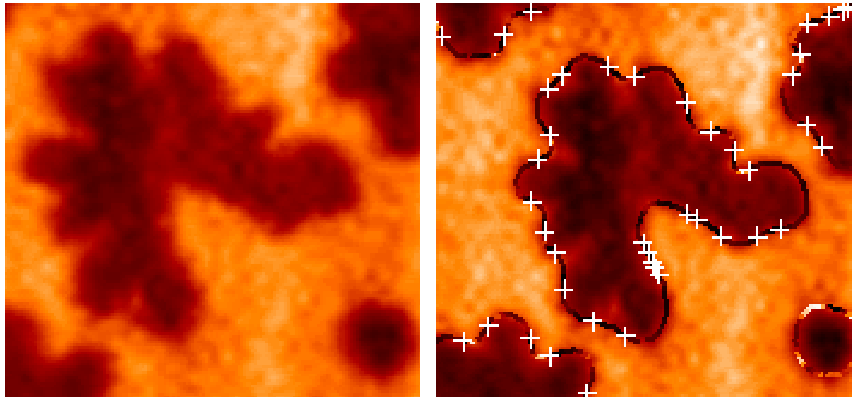
- Segmentation is the first step toward further analysis
  - In addition to images, ROI models and data structures can suit for different types of descriptions

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)

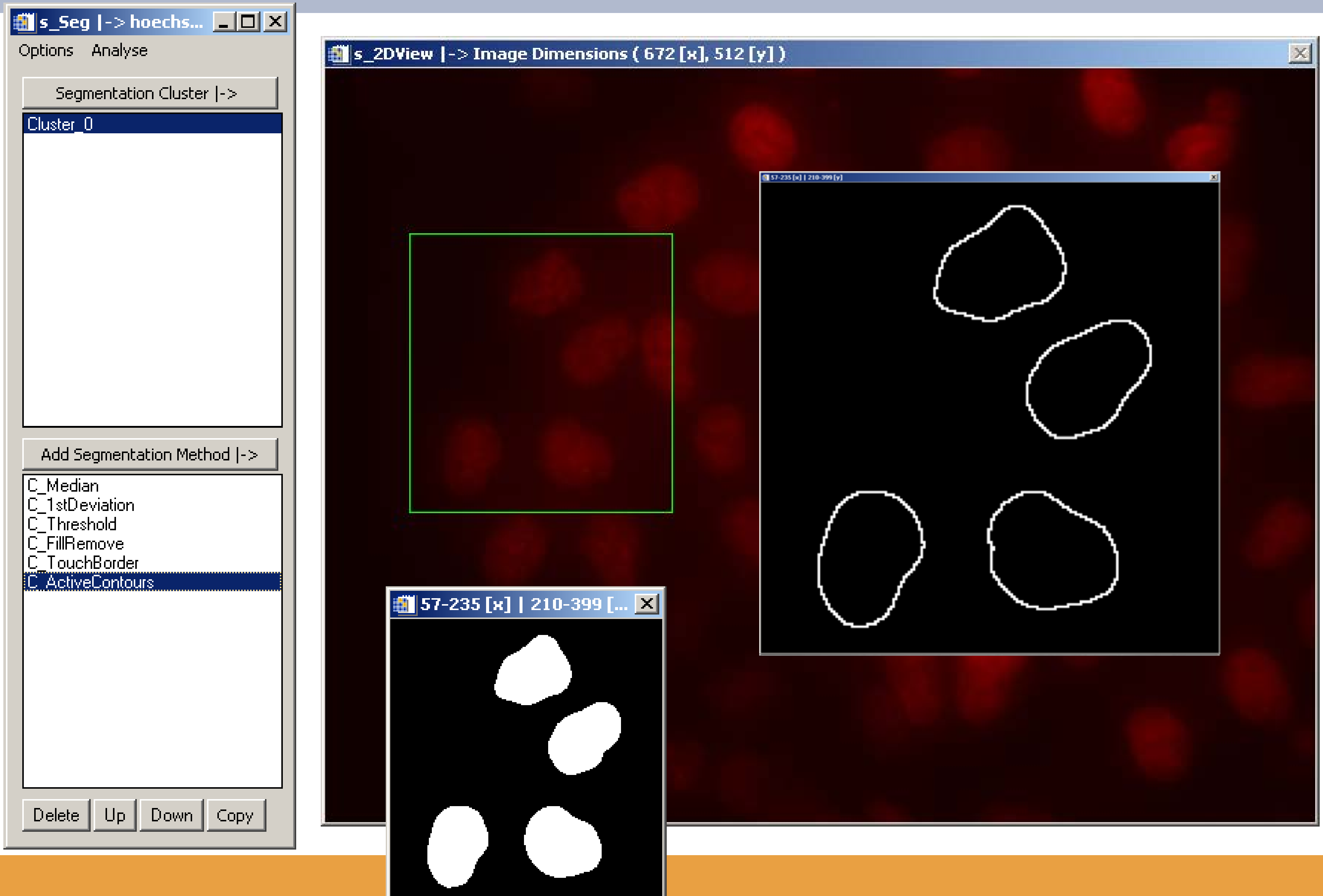
1. Filter-based approaches
   - Thresholding
   - Matrix convolution filters
   - Mathematical morphology
   - Fourier
   - …
2. Advanced approaches
   - Shape priors (*pattern matching*)
   - Clustering methods (k-means, region growing, graph cuts, entropy)
   - Deformable models (active contours)
     - parametric
     - Implicit
   - …

- 3. Trainable approaches (*machine learning*)
  - SVM
  - Random forest
  - Deep learning
  - …

Handbook in Spanish for ImageJ/FIJI (Ethics! Careful with the discussion)
https://www.researchgate.net/publication/313768335_Analisis_de_Imagenes_de_Microscopia_con_ImageJ

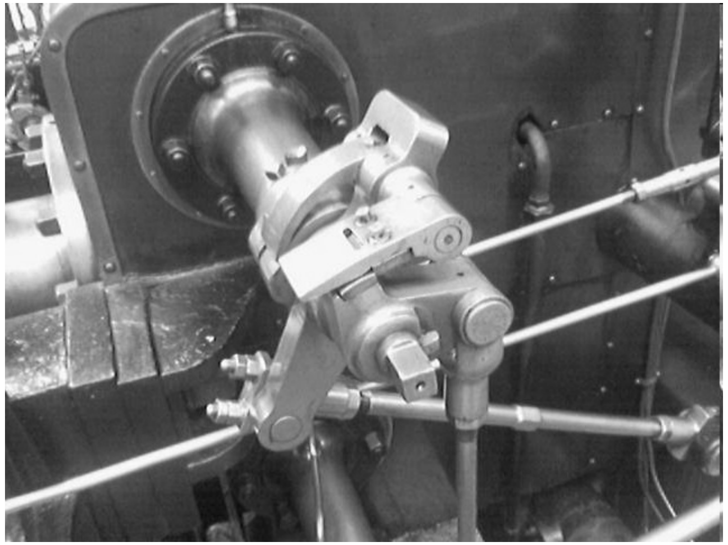
> Segmentación

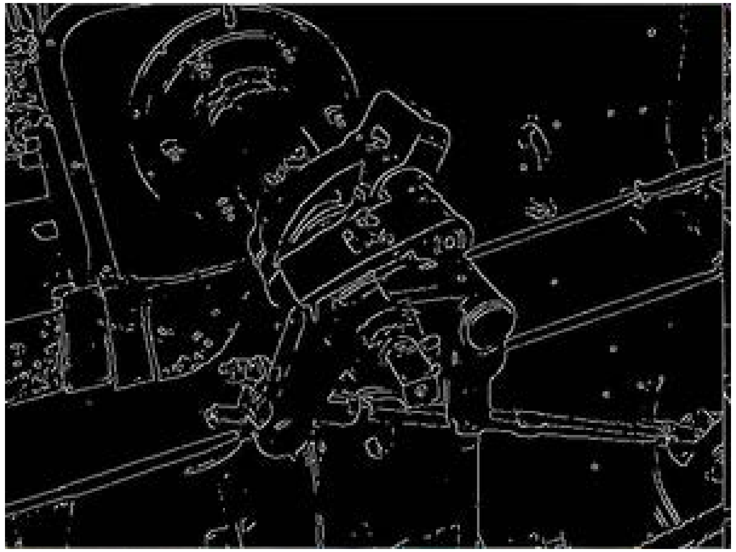
Filters can ease or improve segmentation



> Filtros

- "Filter" approach

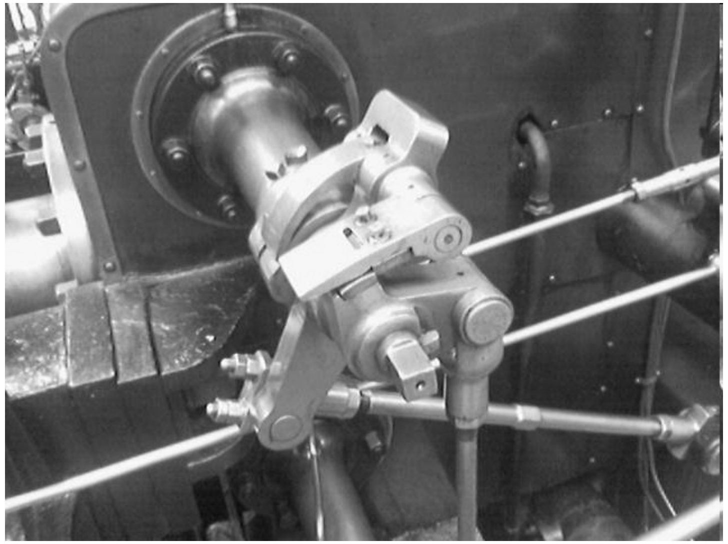


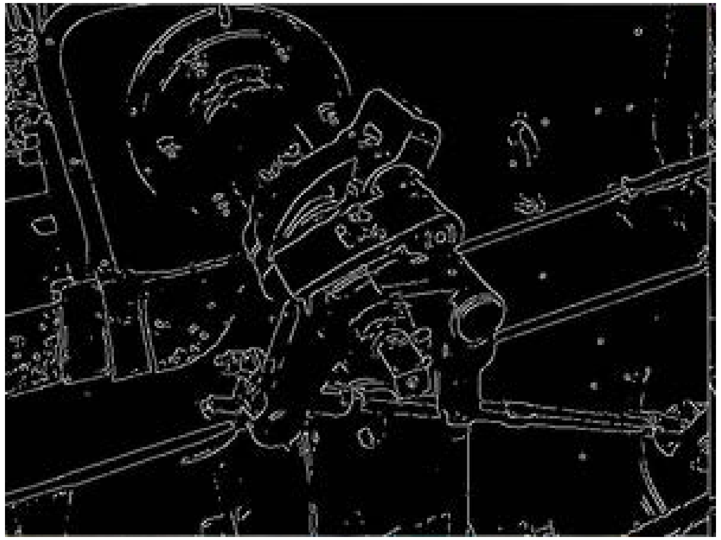


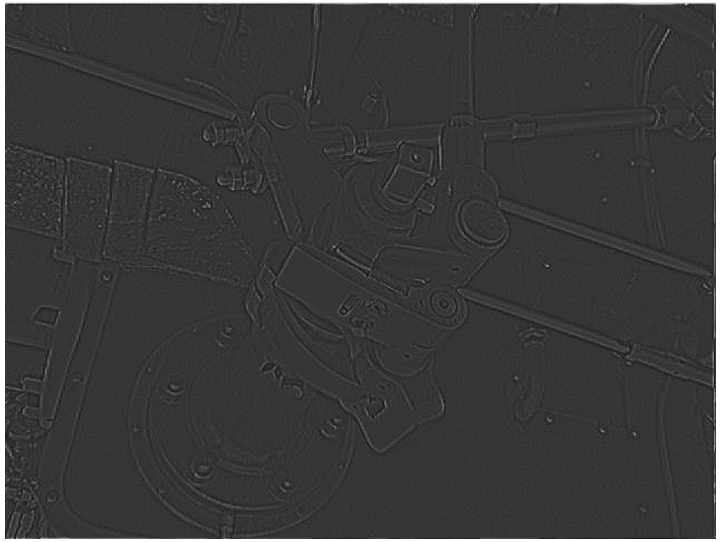
Images from Wikipedia

> Filtros

- A sample filter…





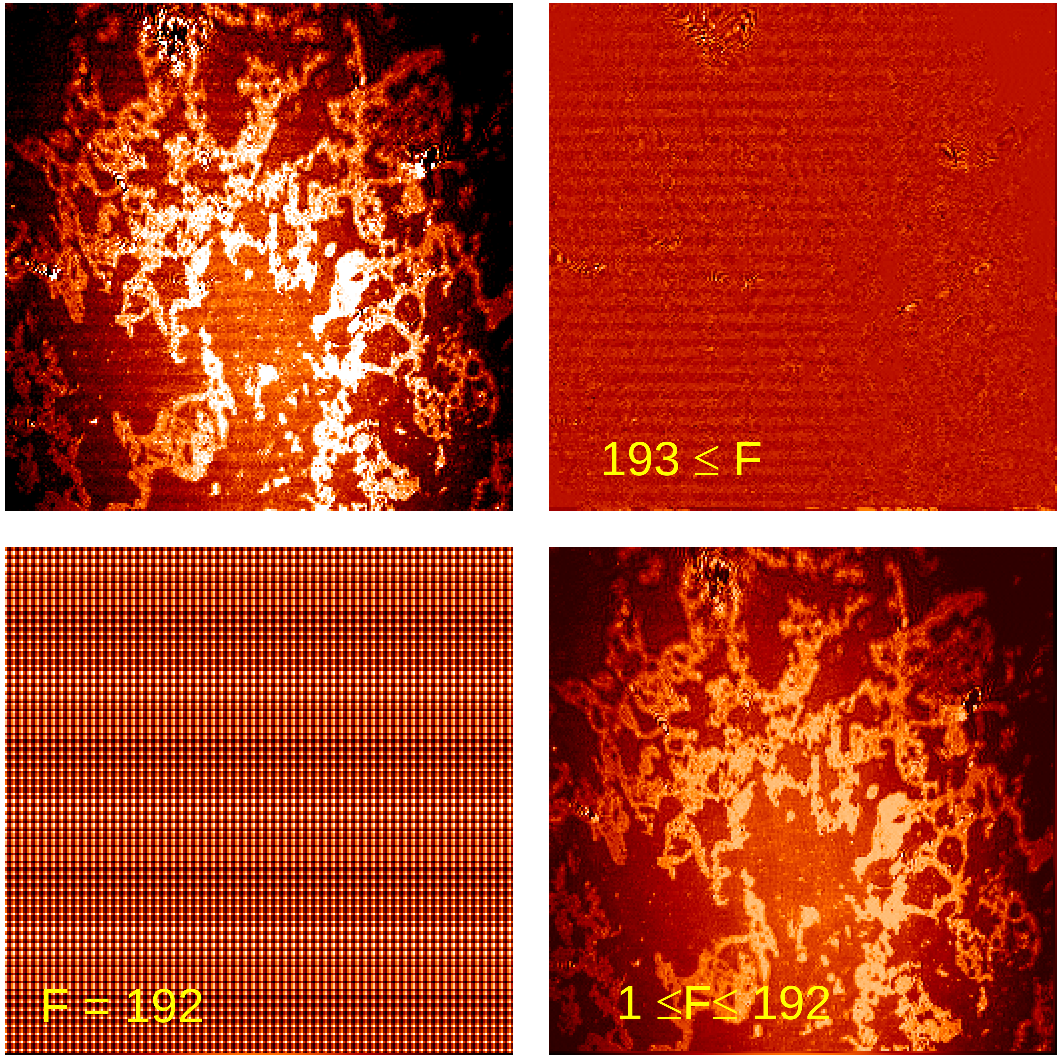


> Filtros

- 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/



> Filtros

Thresholding
- Example: Otsu

Convolution based
- Convolution operation
- Examples: gradient, Laplace, Sobel, Gaussian

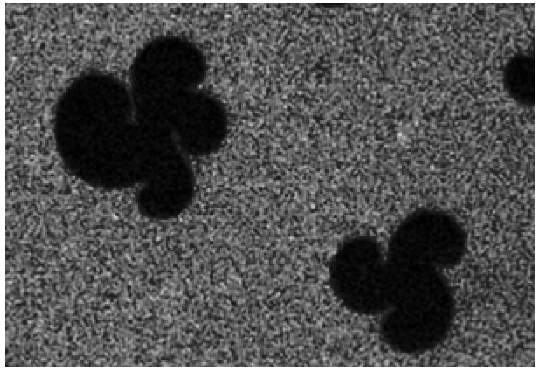
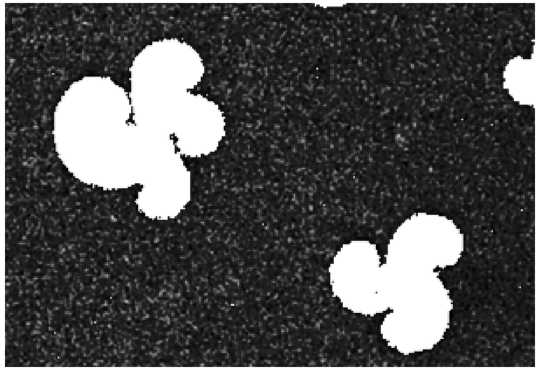
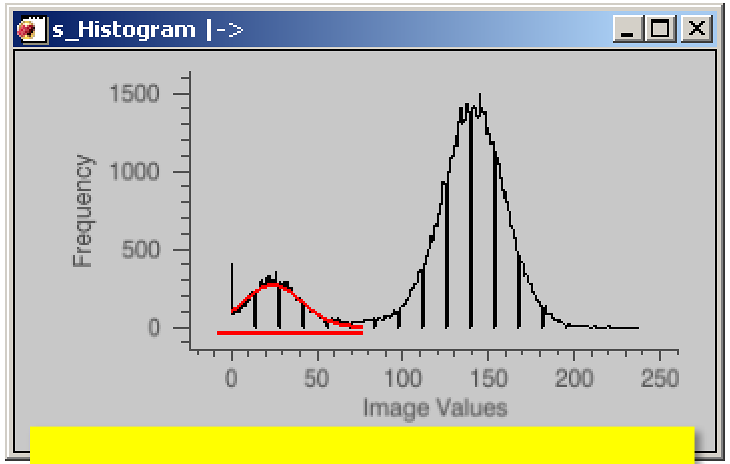
Morphological
- Morphological operators
- Size
- Thinning / skeletonization

Arithmetic-logic
- AND, OR, XOR

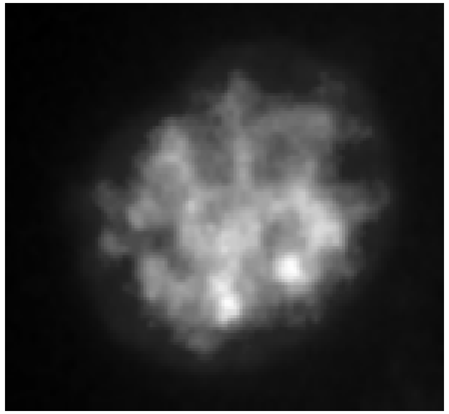
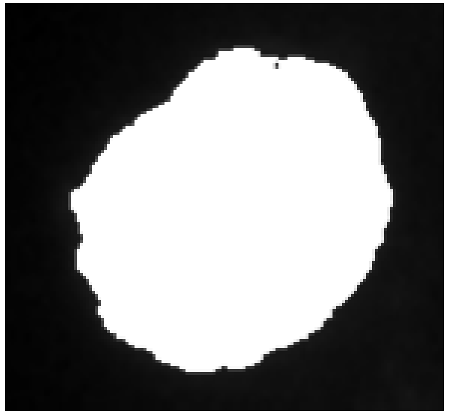
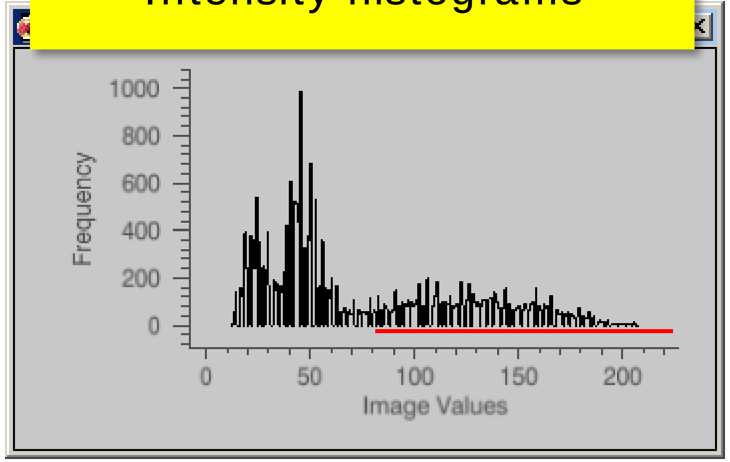
And a long etc.

> Filtros e Histogramas

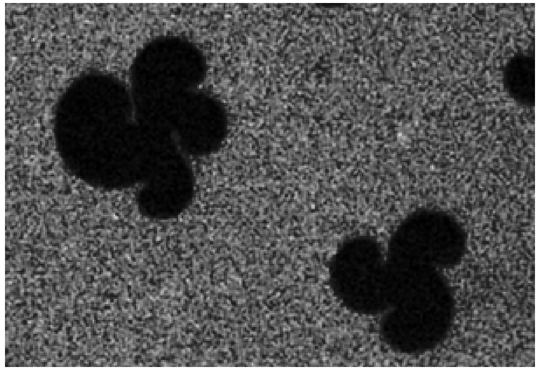
- Threshold filter segmentation: ROIs (white) / background (black)

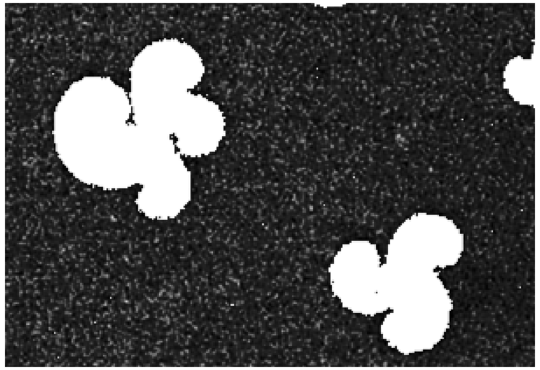




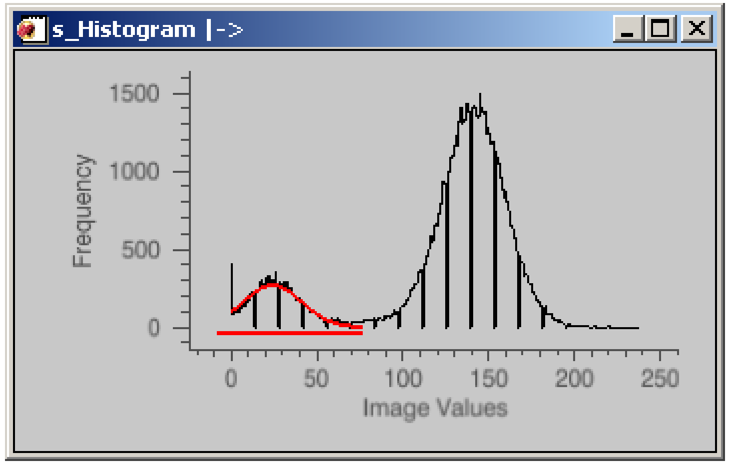
Intensity histograms

- Here comes the Intensity Histogram







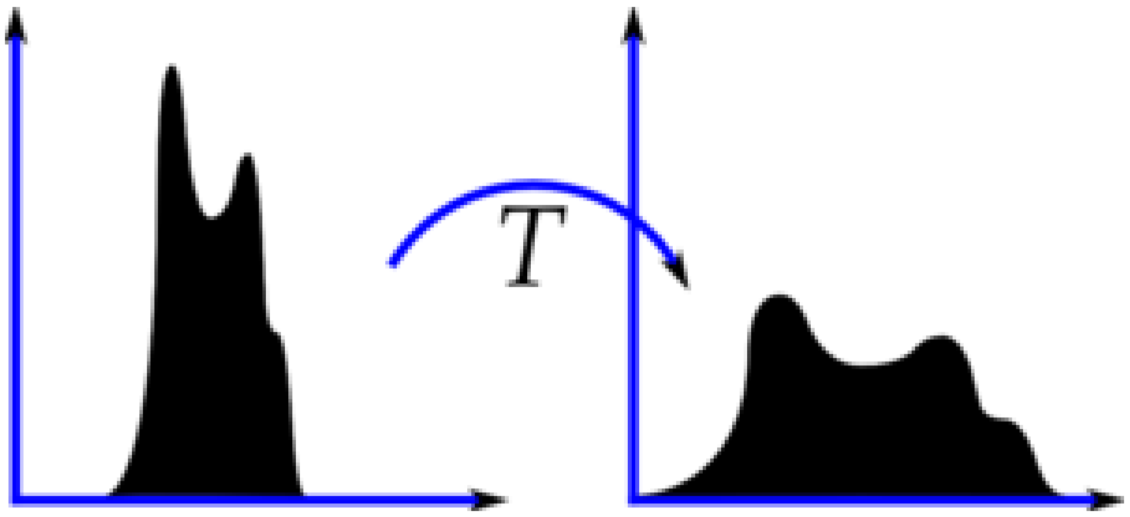
- Throwback to the acquisition…
  - Offset
  - Clipping (sometimes “Saturation”…try not to confuse with the HSV Saturation)

More “Concepts”!

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

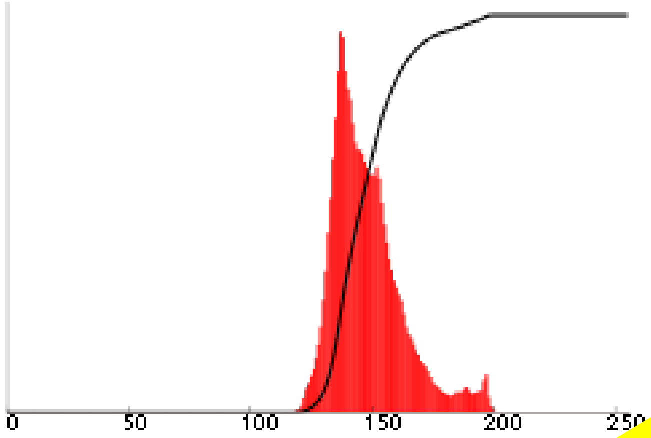
Some examples with music (“Loudness war”)
https://www.youtube.com/watch?v=dcKDMBuGodU
https://www.youtube.com/watch?v=u9Fb3rWNWDA

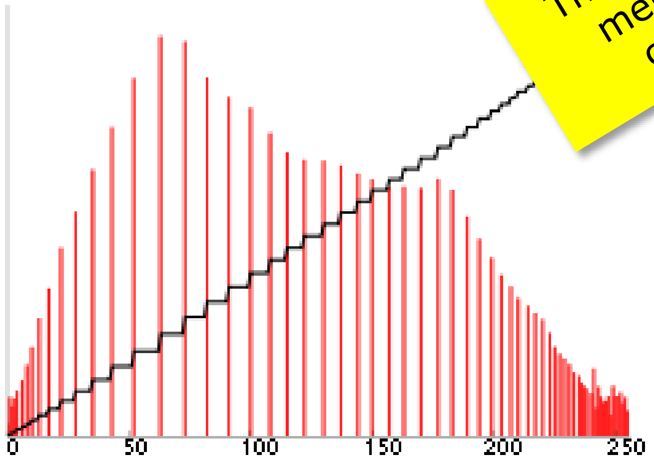
- Histogram equalization



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

- Histogram equalization





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

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

N. Otsu (1979)

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

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

$$t : \text{threshold}, \quad \omega_i : \text{probability of class } i$$

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 $t = 1 \ldots$ 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 = $\frac{threshold_1 + threshold_2}{2}$

- **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

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

Adapted from James Matthews, 2002
http://www.generation5.org/content/2002/convolution.asp

K

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

I

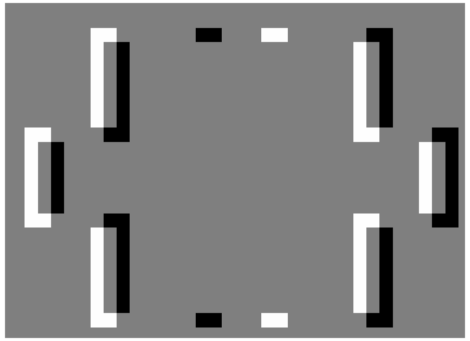
$$(K \otimes I)_{i,j}$$ = (-1 * 222)
+ (0 * 170)
+ (1 * 149)
+ (-2 * 173)
+ (0 * **147**)
+ (2 * 205)
+ (-1 * 149)
+ (0 * 198)
+ (1 * 221) = +63

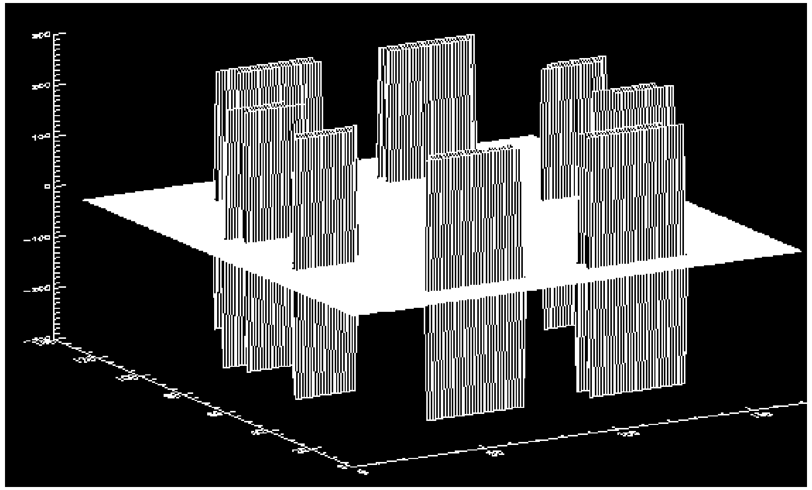
Matrix convolution can be implemented in different ways… beware of the algorithm!

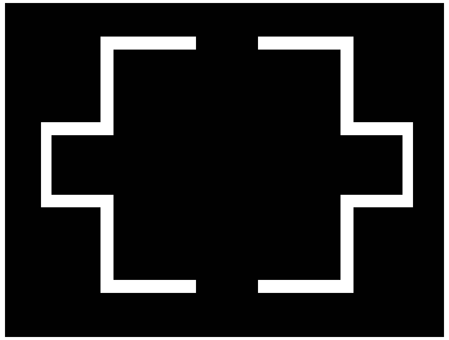
James Matthews, 2002 http://www.generation5.org/content/2002/convolution.asp

- Intensity gradients (discrete approximation)

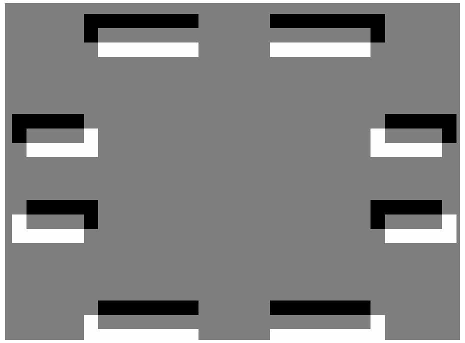
$$\frac{\partial I}{\partial x} \approx$$

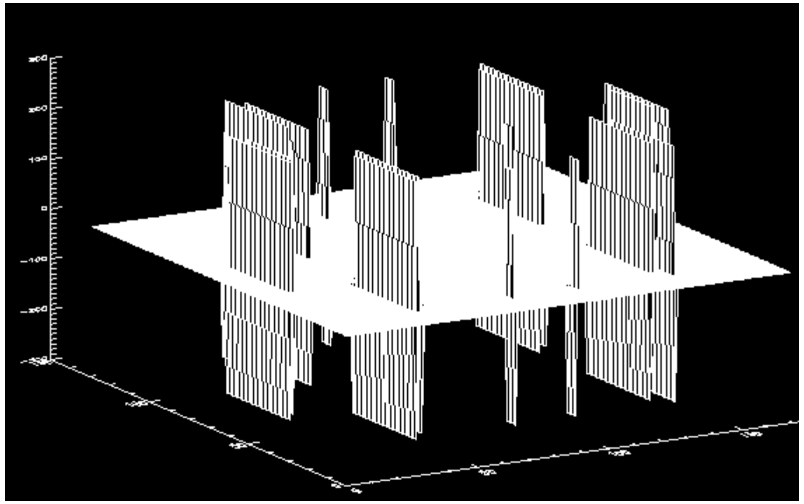


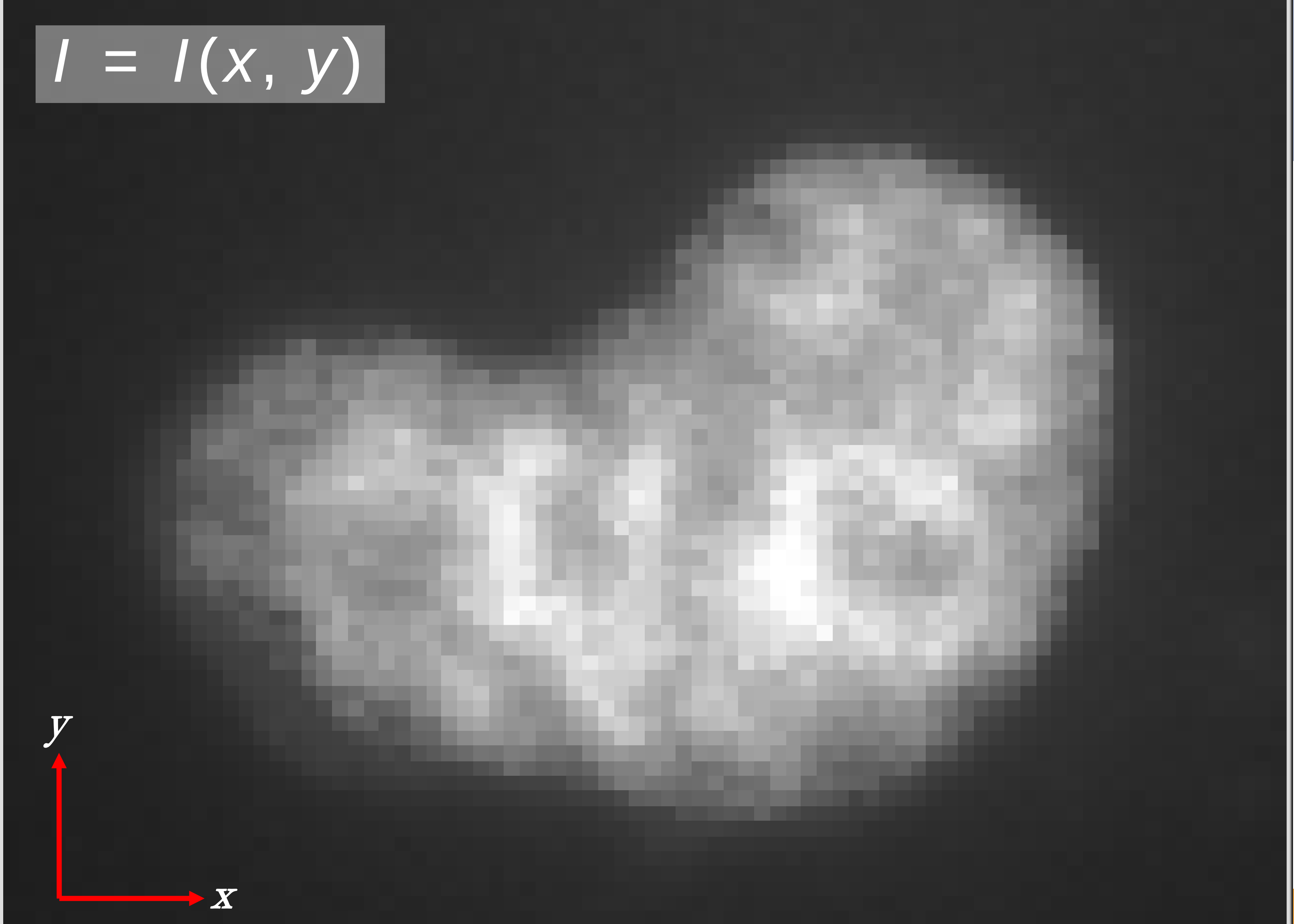


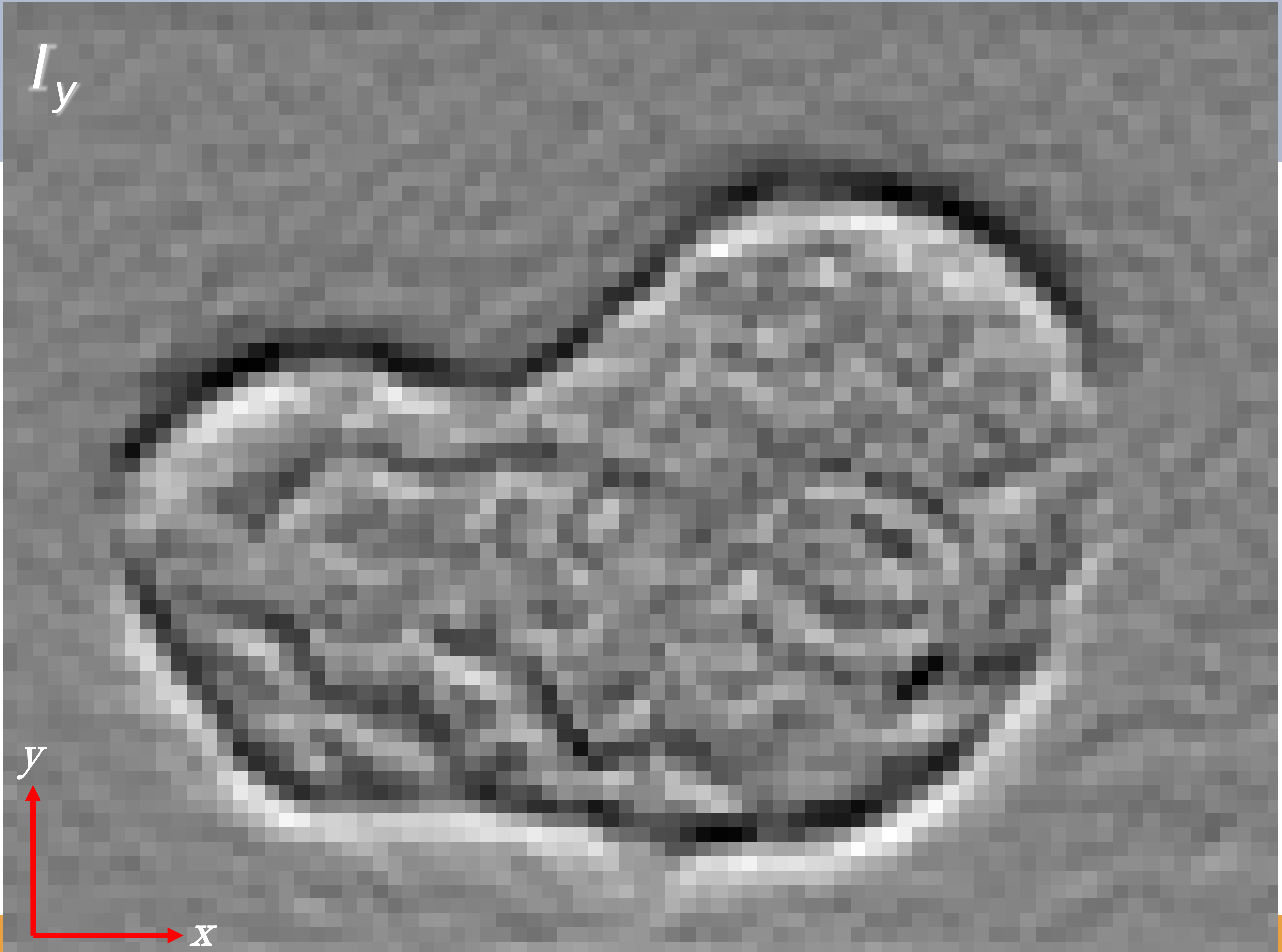


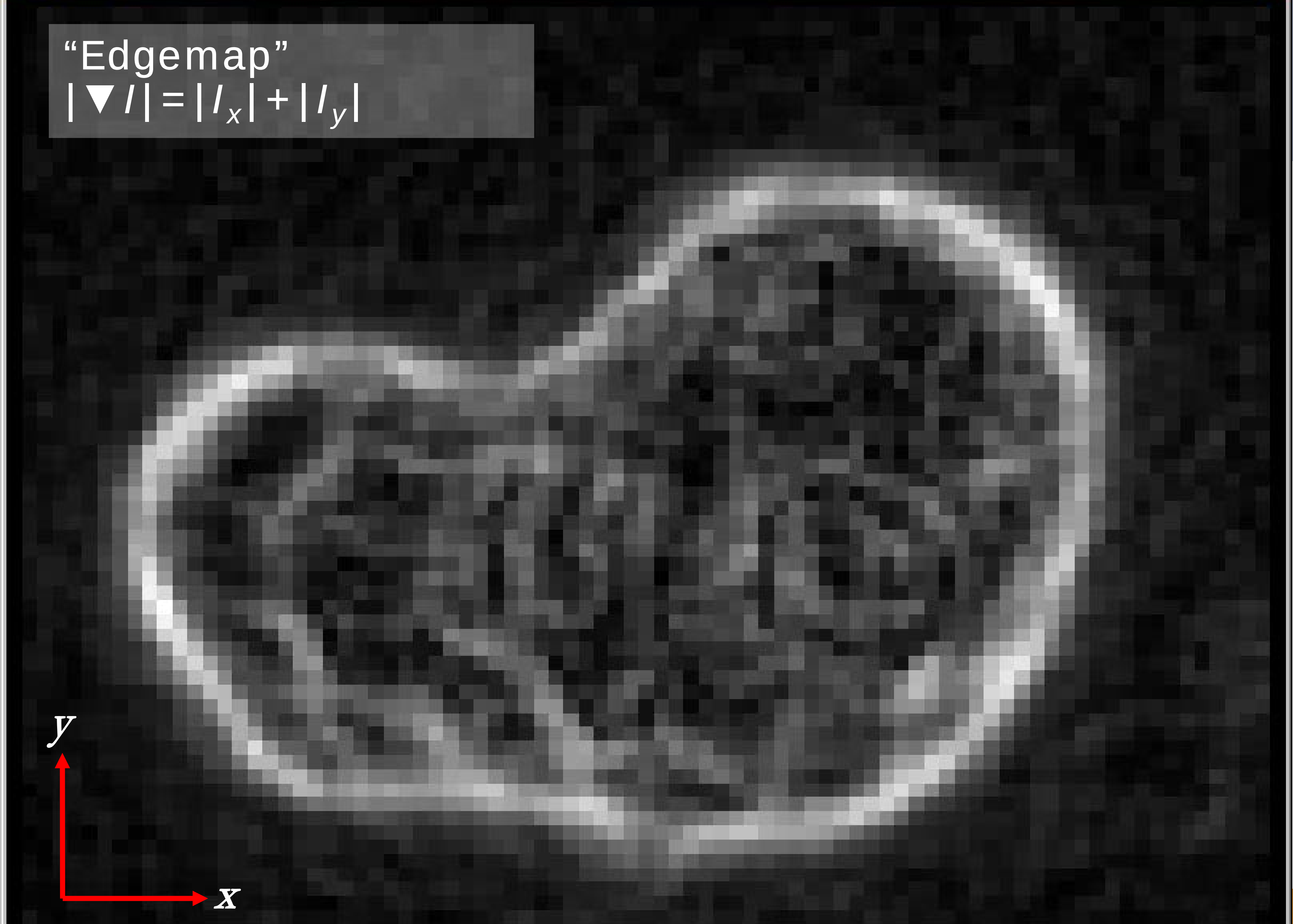
$$\frac{\partial I}{\partial y} \approx$$



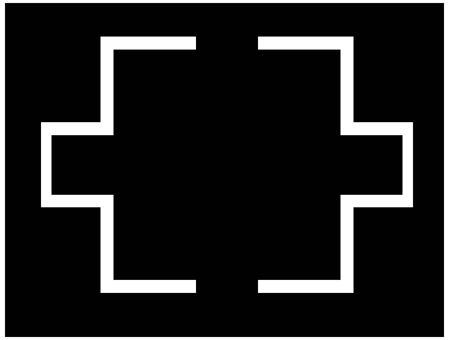



I = I(x, y)
y
x


I_y
y
x


"Edgemap"
$|\nabla I| = |I_x| + |I_y|$
y
x

- Intensity gradients (discrete approximation)

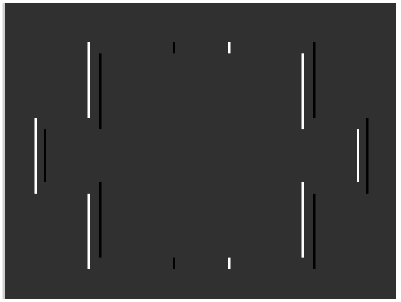


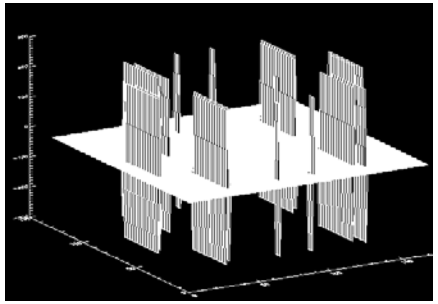
$$I = I(x, y)$$

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

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

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



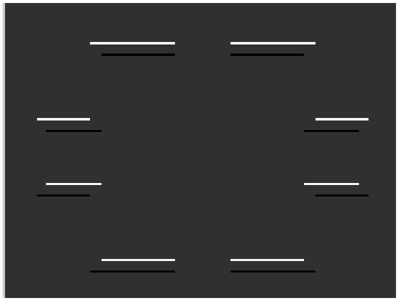


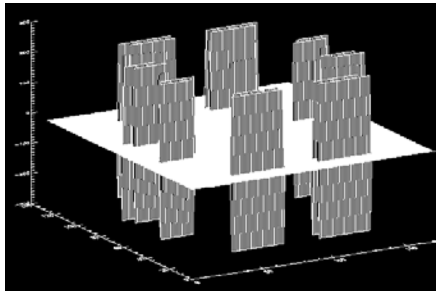
flat | + | -

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

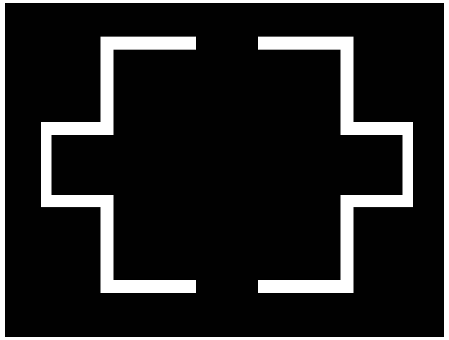
$\Delta y = 1\,\text{pixel}$

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





Kernels…



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

Laplace

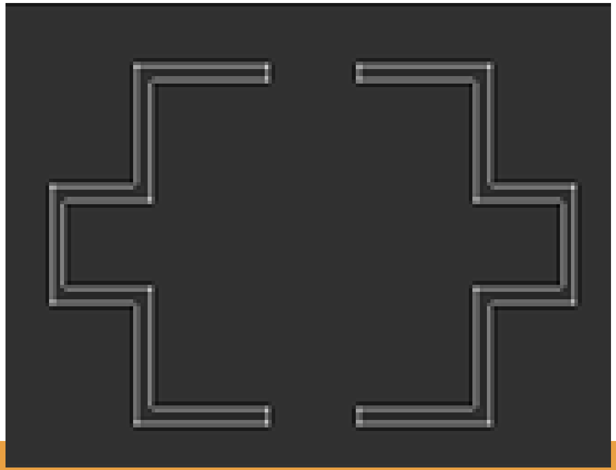
$$\nabla^2 I = \frac{\partial^2 I}{\partial x^2} + \frac{\partial^2 I}{\partial y^2}$$

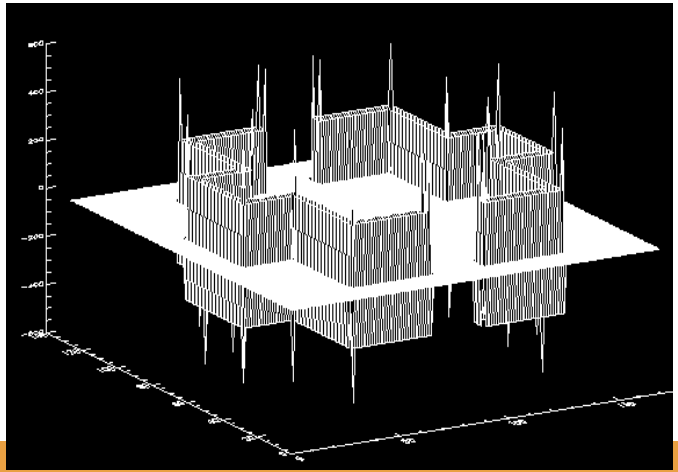
$$\nabla^2 I \approx \frac{f(x+\Delta x, y) - 2f(x,y) + f(x-\Delta x, y)}{(\Delta x)^2} + \frac{f(x, y+\Delta y) - 2f(x,y) + f(x, y-\Delta y)}{(\Delta y)^2}$$

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

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

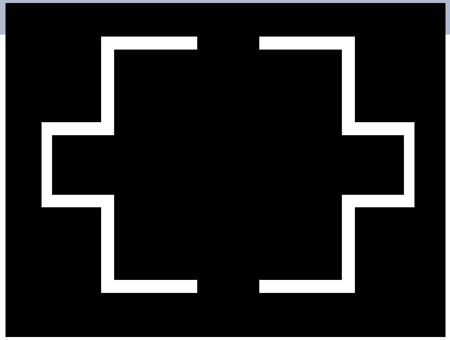
$$K_L = \begin{Bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{Bmatrix}$$





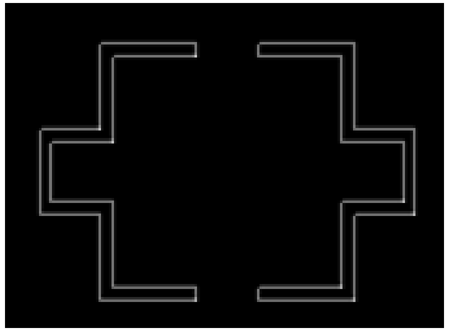
Edgemaps

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



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

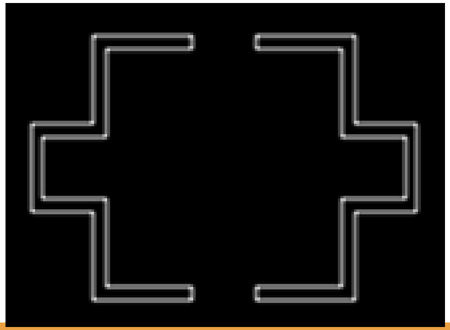
$$f = \sqrt{(Kx \otimes I)^2 + (Ky \otimes I)^2}$$



Example:Sobel filter
(notice the thick ROI edges)

$$\left\{\begin{matrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{matrix}\right\} \qquad \left\{\begin{matrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{matrix}\right\}$$

$$Sx \qquad\qquad Sy$$

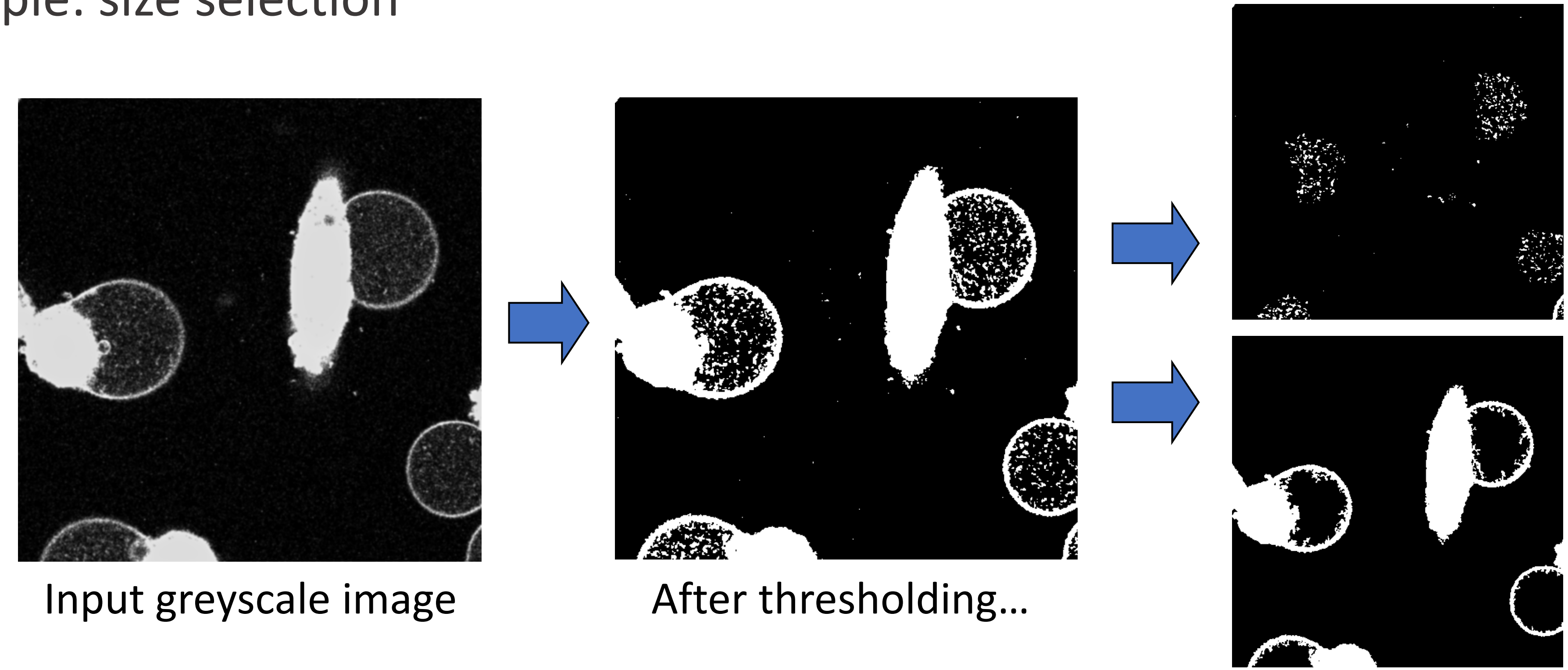


$Sx \otimes I$?

$Sy \otimes I$?

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

- Morphology based filters
  - Example: size selection



Input greyscale image

After thresholding…

Size selection

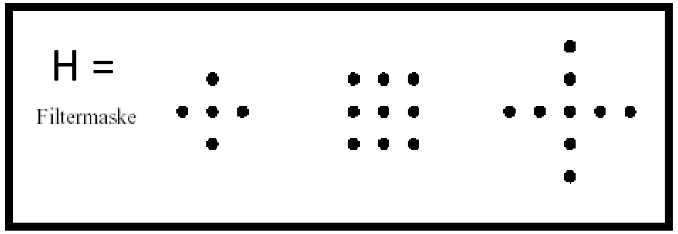
How to define a size-select algorithm?

- Filtering with morphological operators:

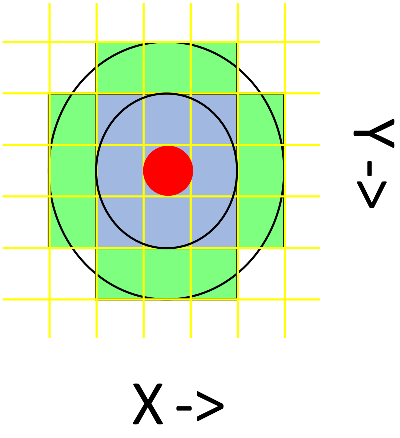
Filtering with morphological operators:

- Structuring element, template or mask H ...

- Additional rules...



H (x_i, y_j, r):



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

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

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

- Mathematical morphology

Minkowski Operations

Addition... dilation: $A \oplus S = \{(m,n) | [S + (m,n)] \cap A \neq 0\}.$

Substraction ... erosion: $A \ominus S = \{(m,n) | [S + (m,n)] \subseteq A \neq 0\}$

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

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

- A: image
- S: structuring element

- Mathematical morphology

$$A \oplus S = \{(m,n) \,|\, [S + (m,n)] \cap A \neq 0\}.$$

$$A \ominus S = \{(m,n) \,|\, [S + (m,n)] \subseteq A \neq 0\}$$

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

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

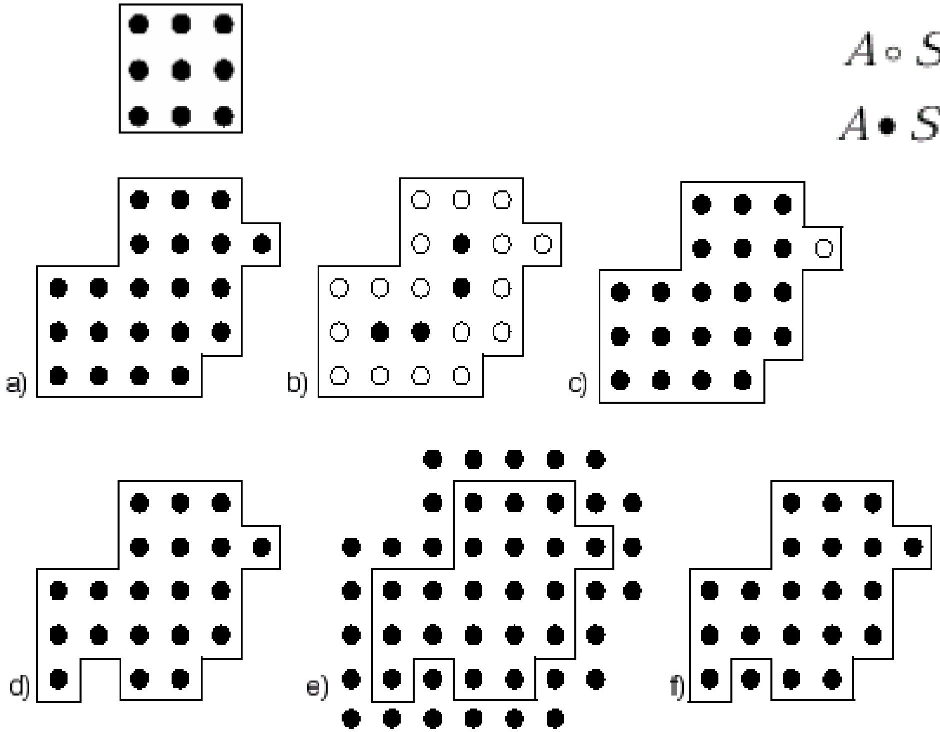
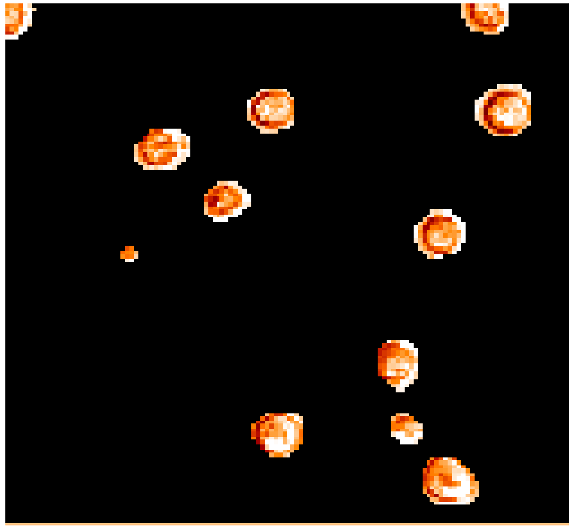
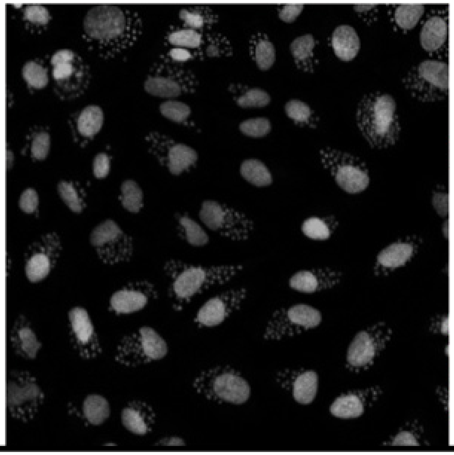


Abb. 2.5. **a** Originalform **b** erodiert **c** opening (Dilatation von b)
d Originalform **e** dilatiert **f** closing (Erosion von e)



- 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.

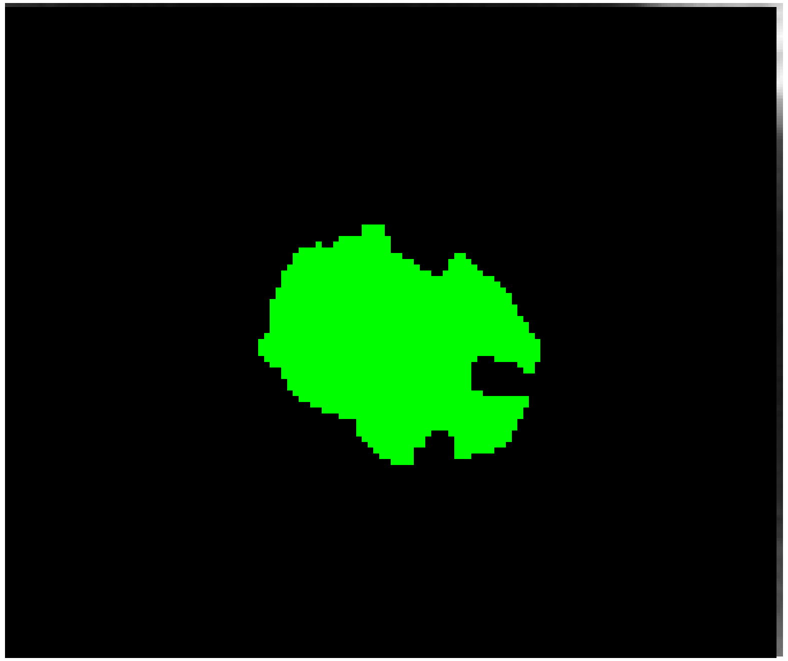
Figura 1. Imágenes de línea de osteoblastos humanos infectados con *Trypanosoma cruzi* (Nature 2010).

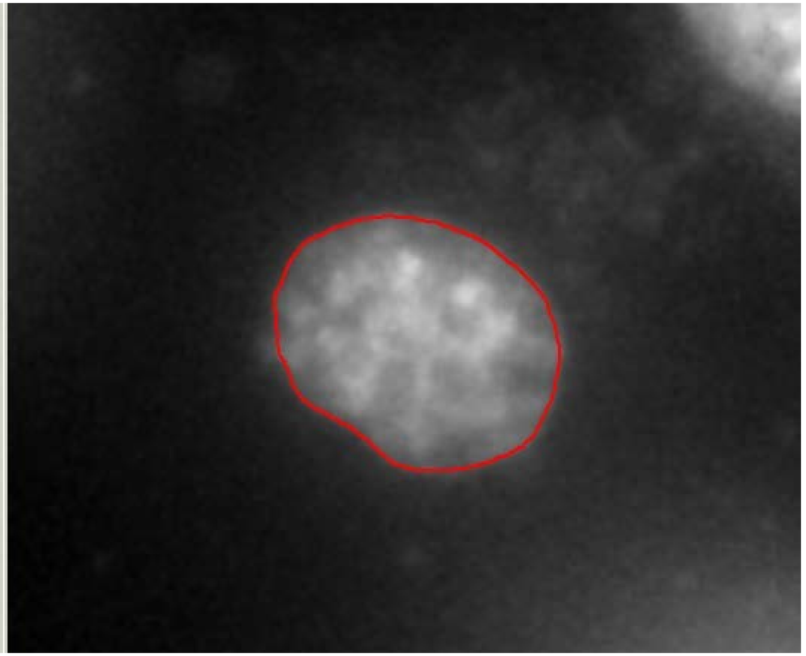
El objetivo de este práctico es realizar una segmentación de imágenes 2D usando FIJI para obtener datos básicos, responder las preguntas indicadas en esta misma hoja y entregarla al final del práctico.

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)

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





Segmentation - Advanced

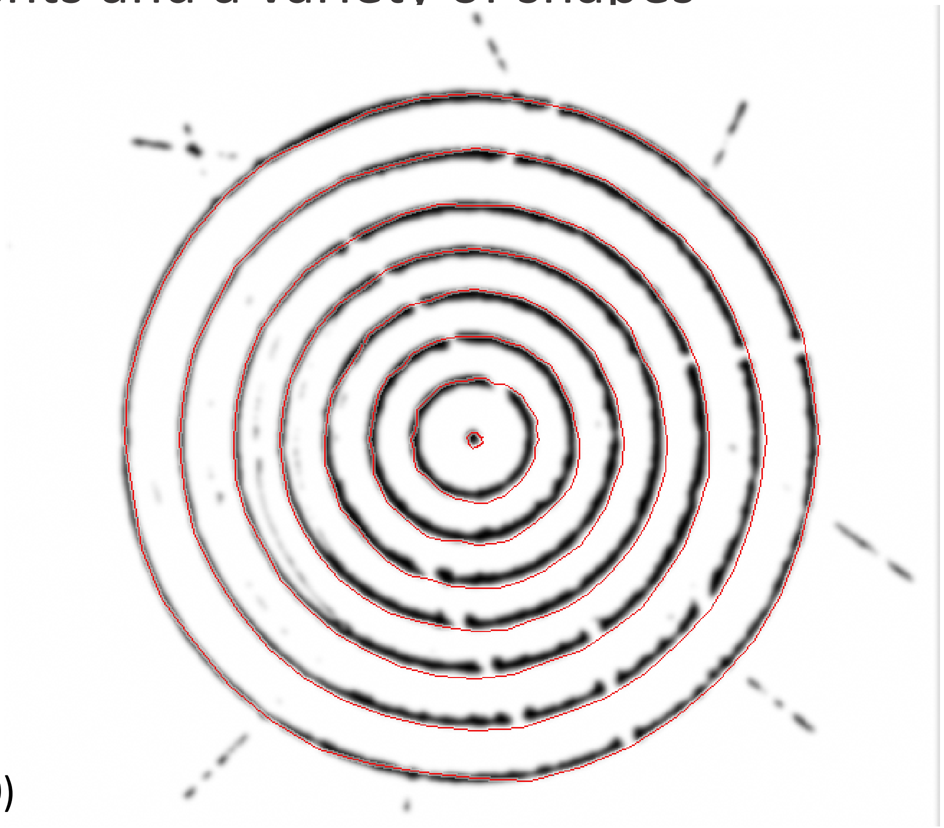
- Template matching
  - “Classic model” Hough transform
  - Applies to circles, line segments and a variety of shapes

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)

Segmentation - Advanced

- Variational methods
  - Based on energy minimization, defining integral models
  - Idea: to include desirable features on segmented images (like homogeneus 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)

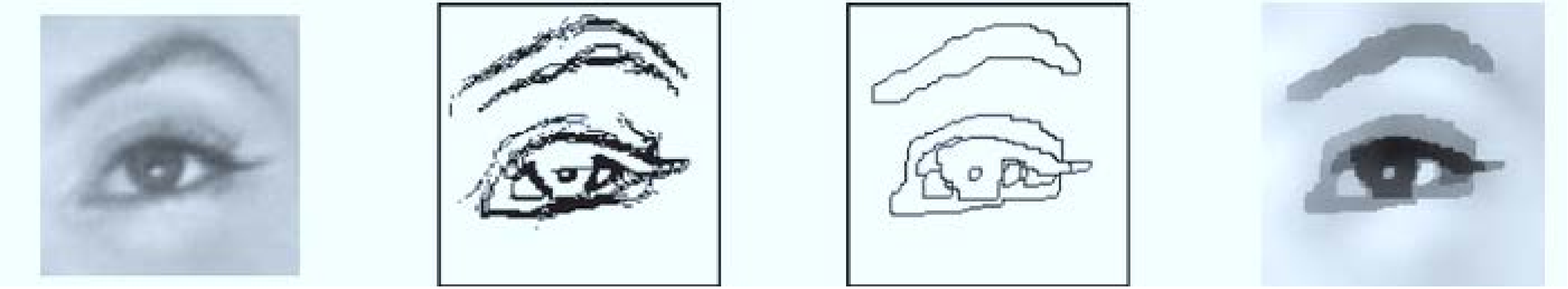


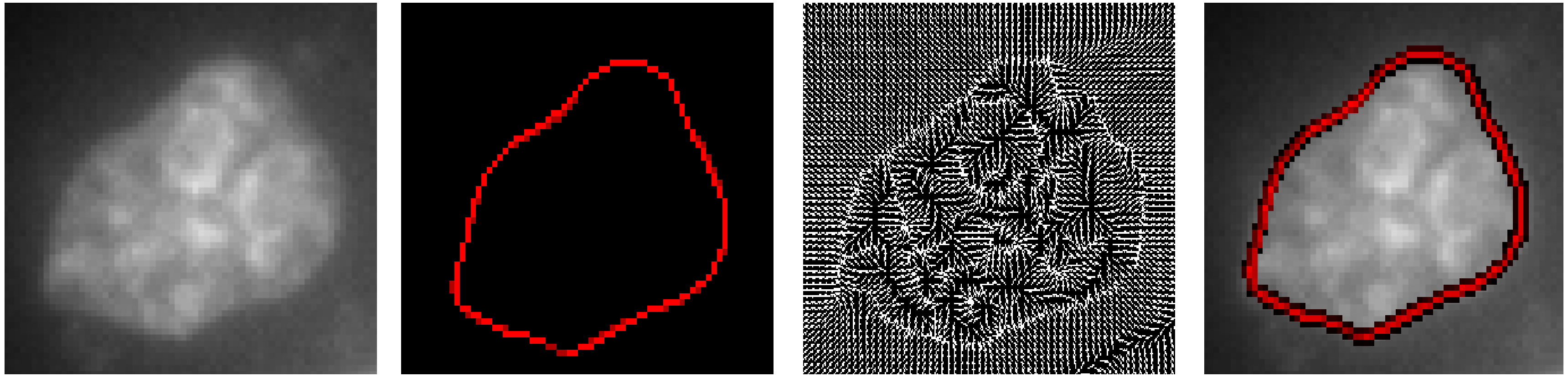
image I | main discontinuties in I | ROI boundaries B | piecewise smooth image J

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

The Mumford & Shah functional (1989)

Parametric Active Contours

- Active contour models
  - Optimization of different properties



input
image
+initial guess

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

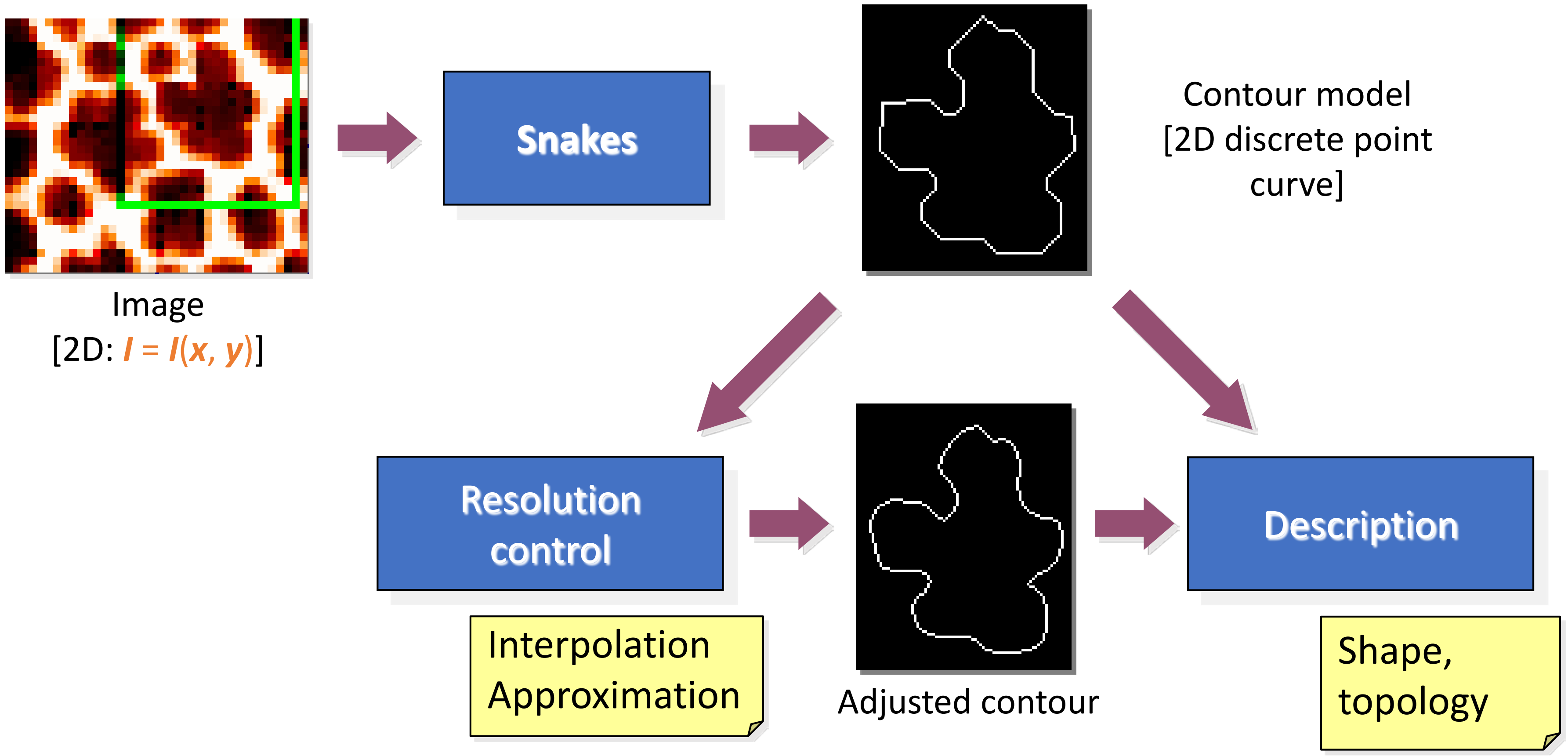
force field
- repulsion
- attraction

output: force balance
minimal energy

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

> Segmentación por contorno

- 2D active contours or *snakes*



> Segmentación por contorno

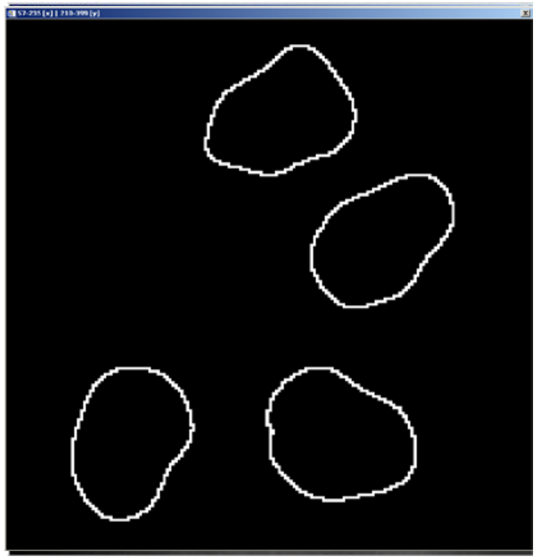
Snakes… 1 contour function → 1 ROI
(this is called *parametric approach*)

– 2D parametric curve

$C = C(s) = [x(s), y(s)]$
$s \in [0, 1]$ (arbitrary length)

– 2D discretization

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



> Segmentación por contorno

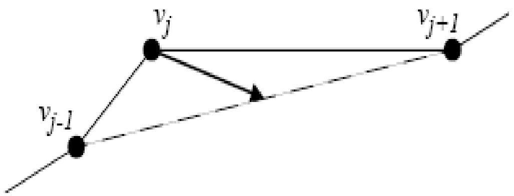
- Snakes: optimization derived from a variational approach
  - Minimization of an integral functional… "a snake minimizes its energy"

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

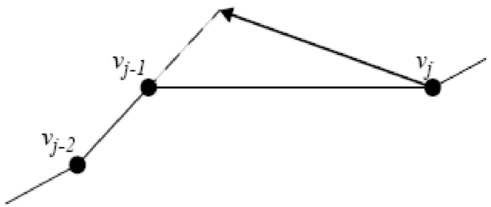
Internal energy, contour dependant

External energy, image dependant

Elasticity term (coefficient α)



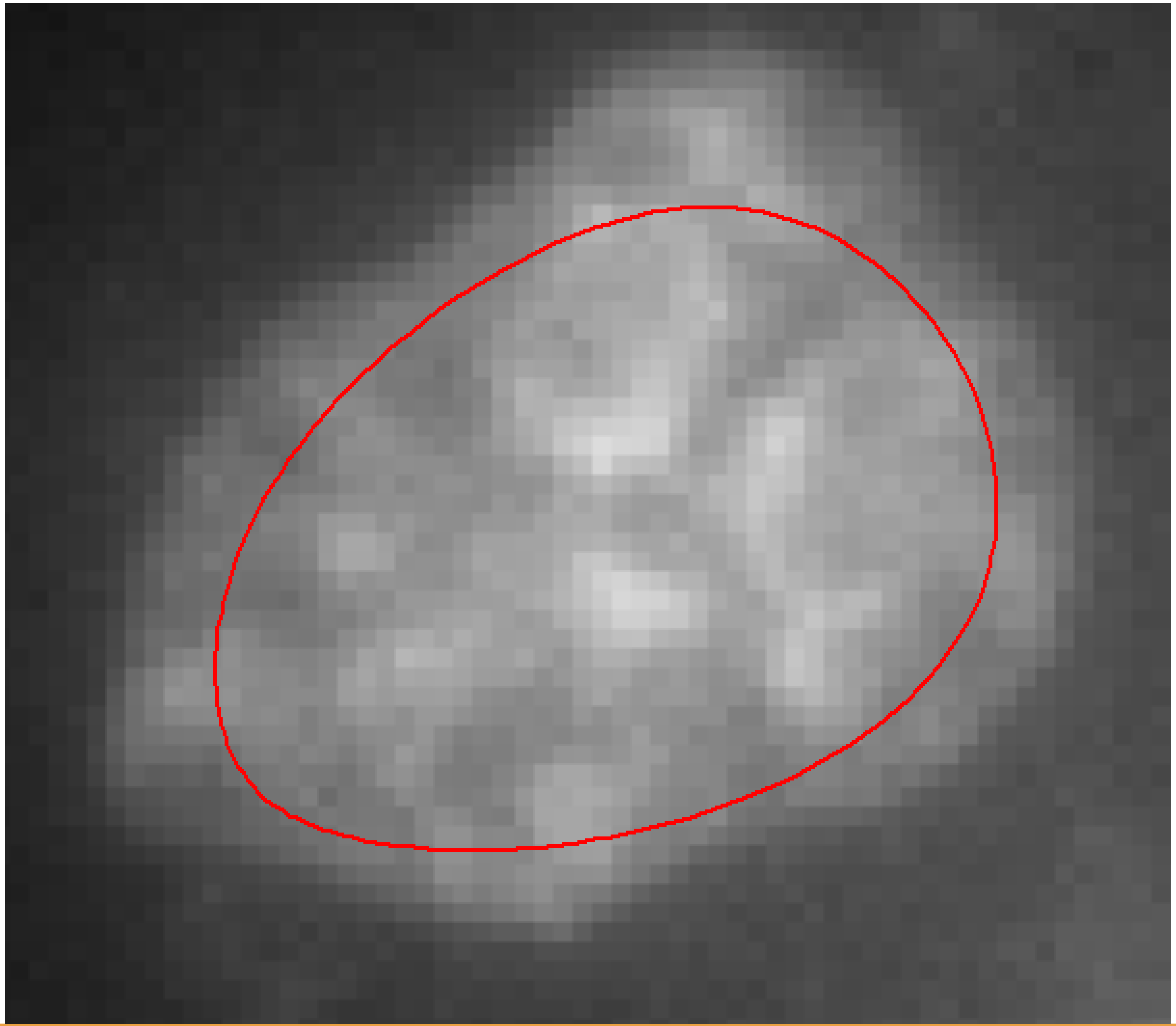
Rigidity term (coefficient β)



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

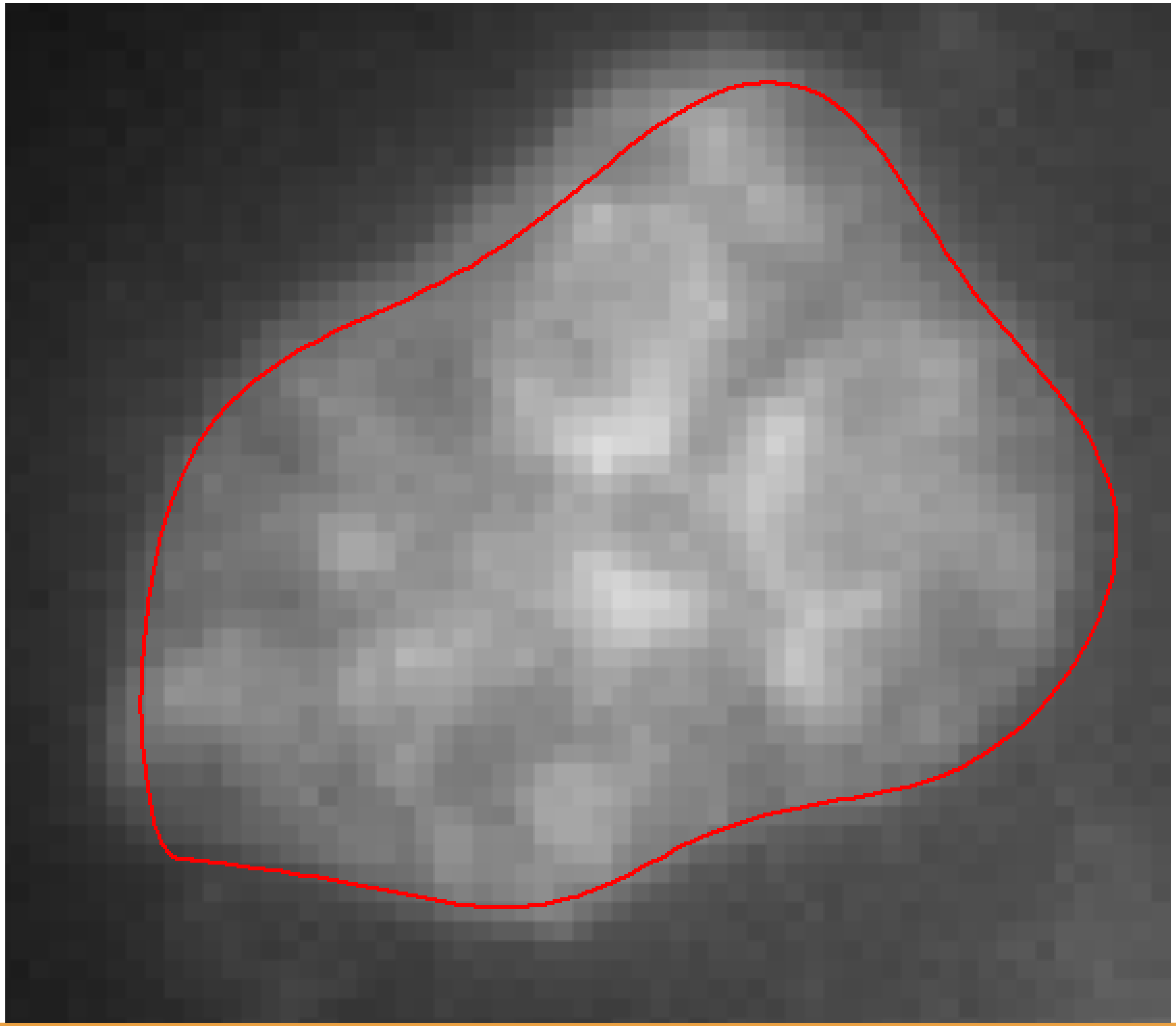
Parametric Active Contours

Elasticity - ***α***



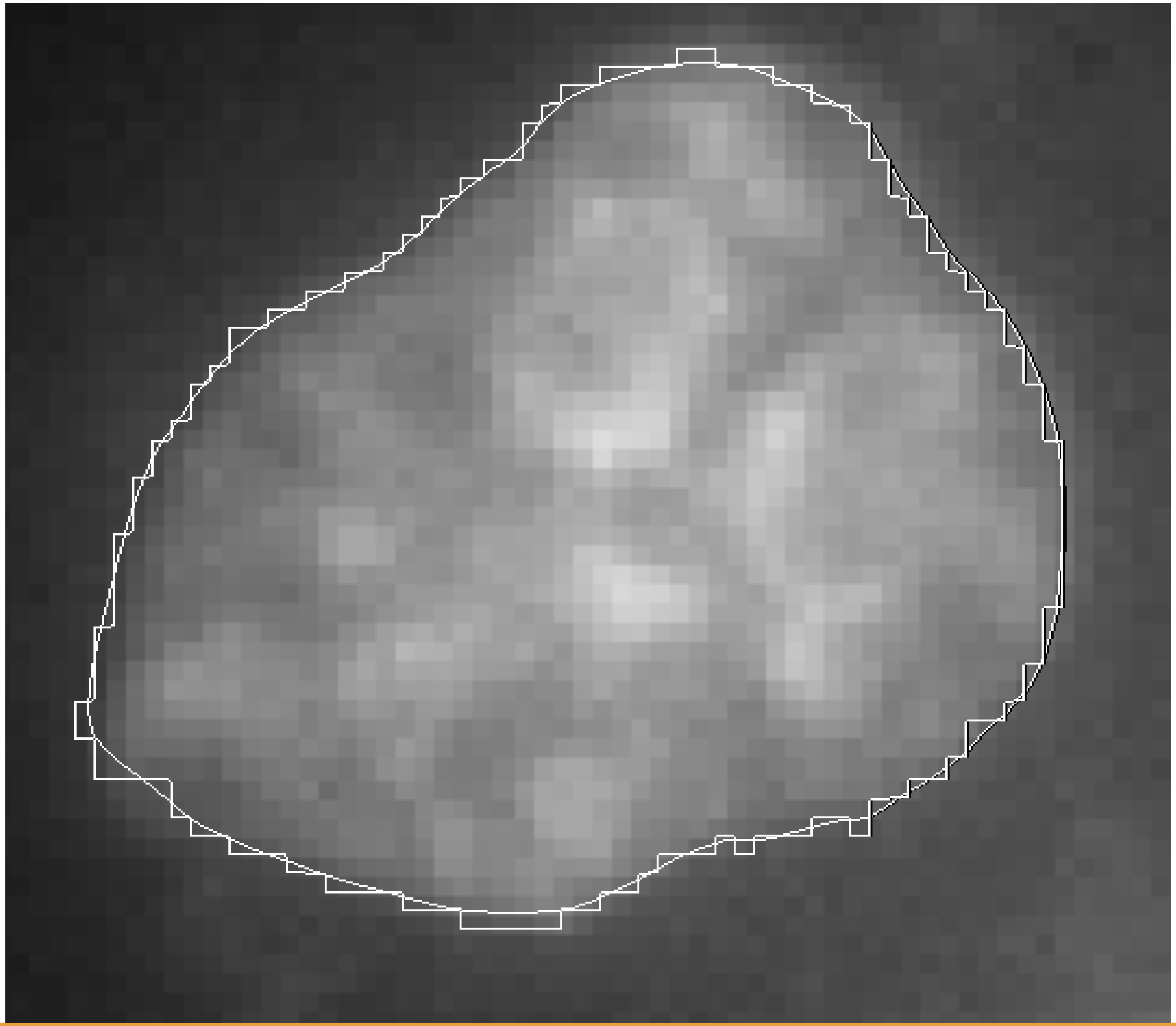
Parametric Active Contours

Rigidity - β

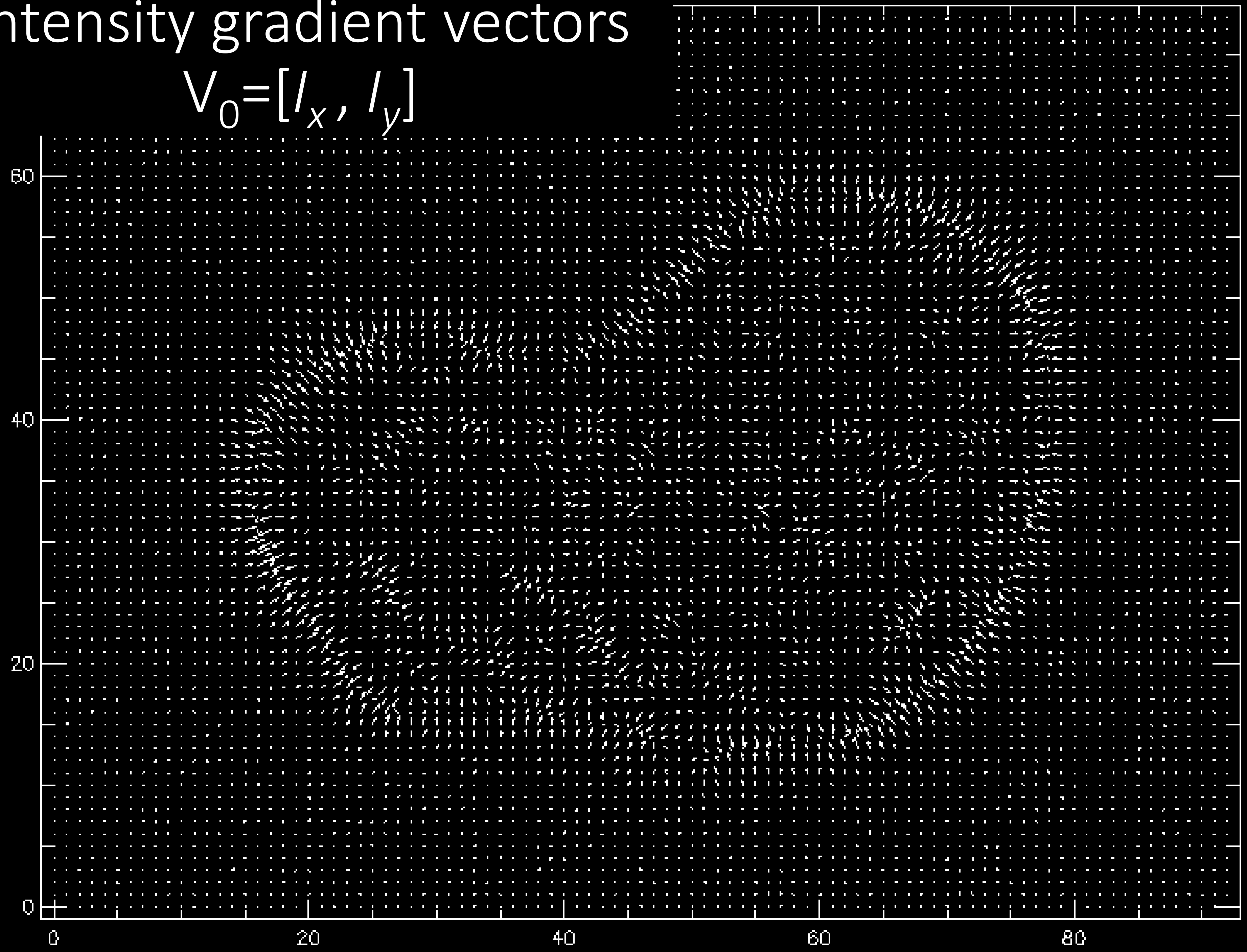


Parametric Active Contours

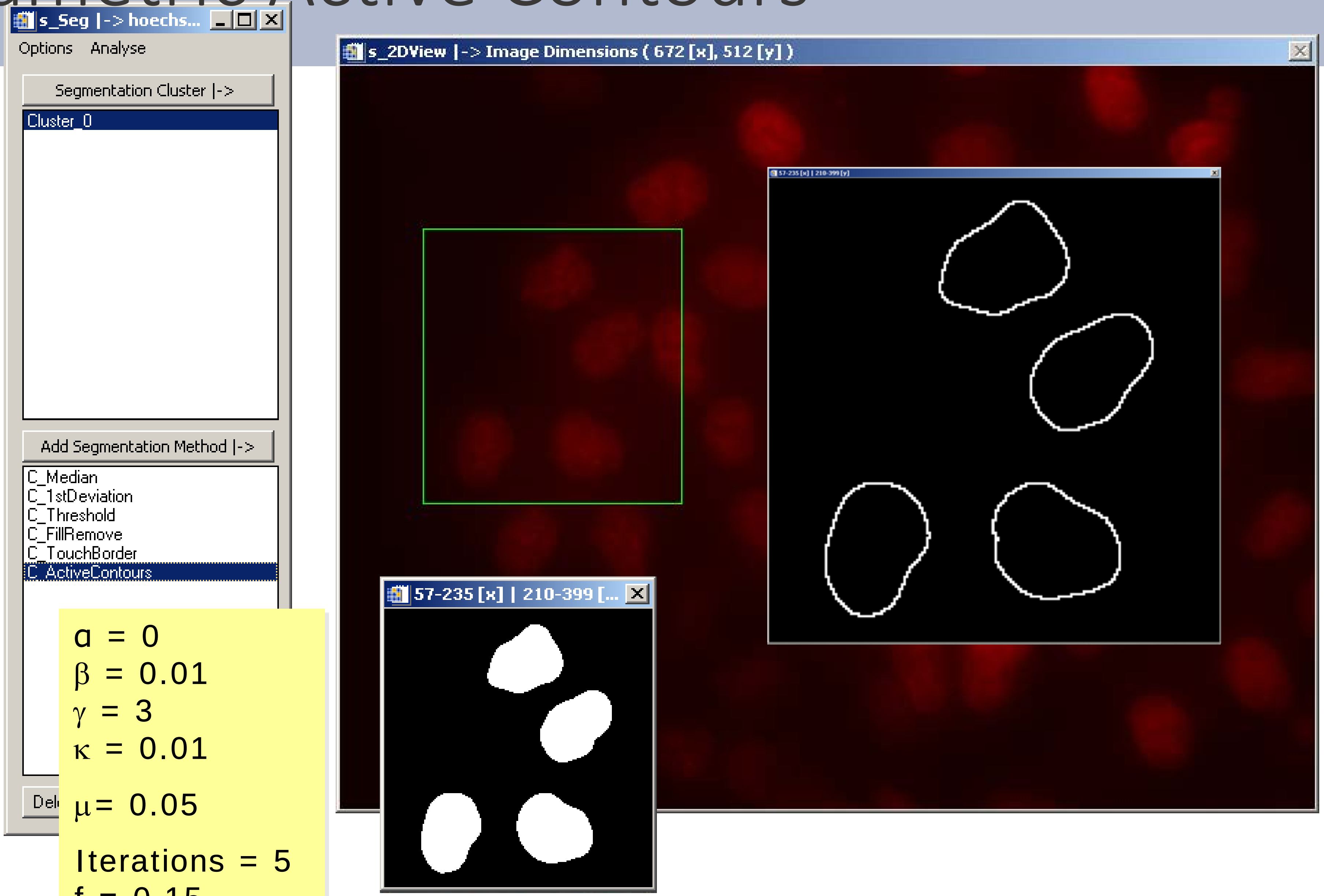
Contour point density/sampling



Intensity gradient vectors
$V_0=[I_x, I_y]$



Parametric Active Contours

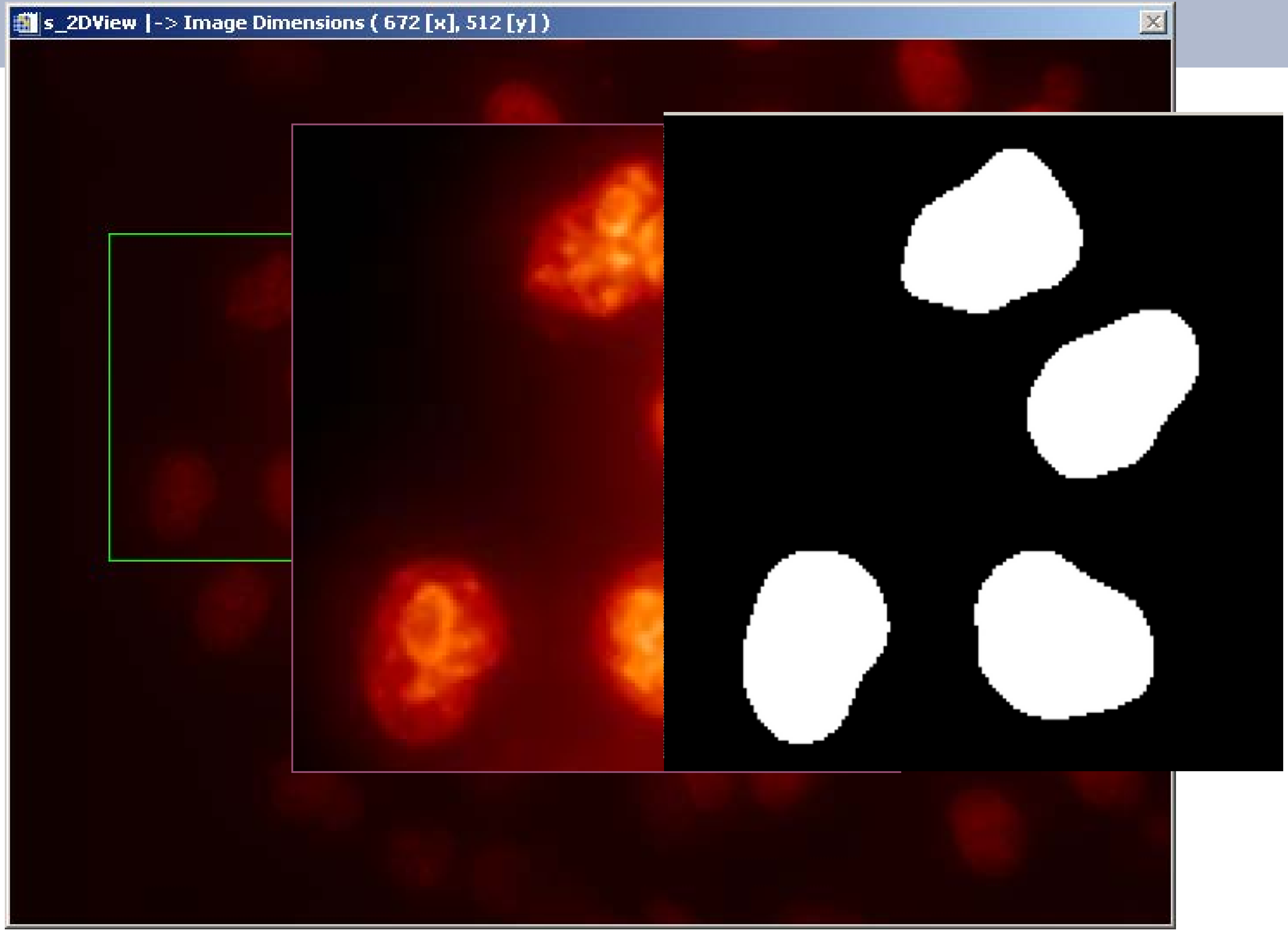


$\alpha = 0$
$\beta = 0.01$
$\gamma = 3$
$\kappa = 0.01$

$\mu = 0.05$

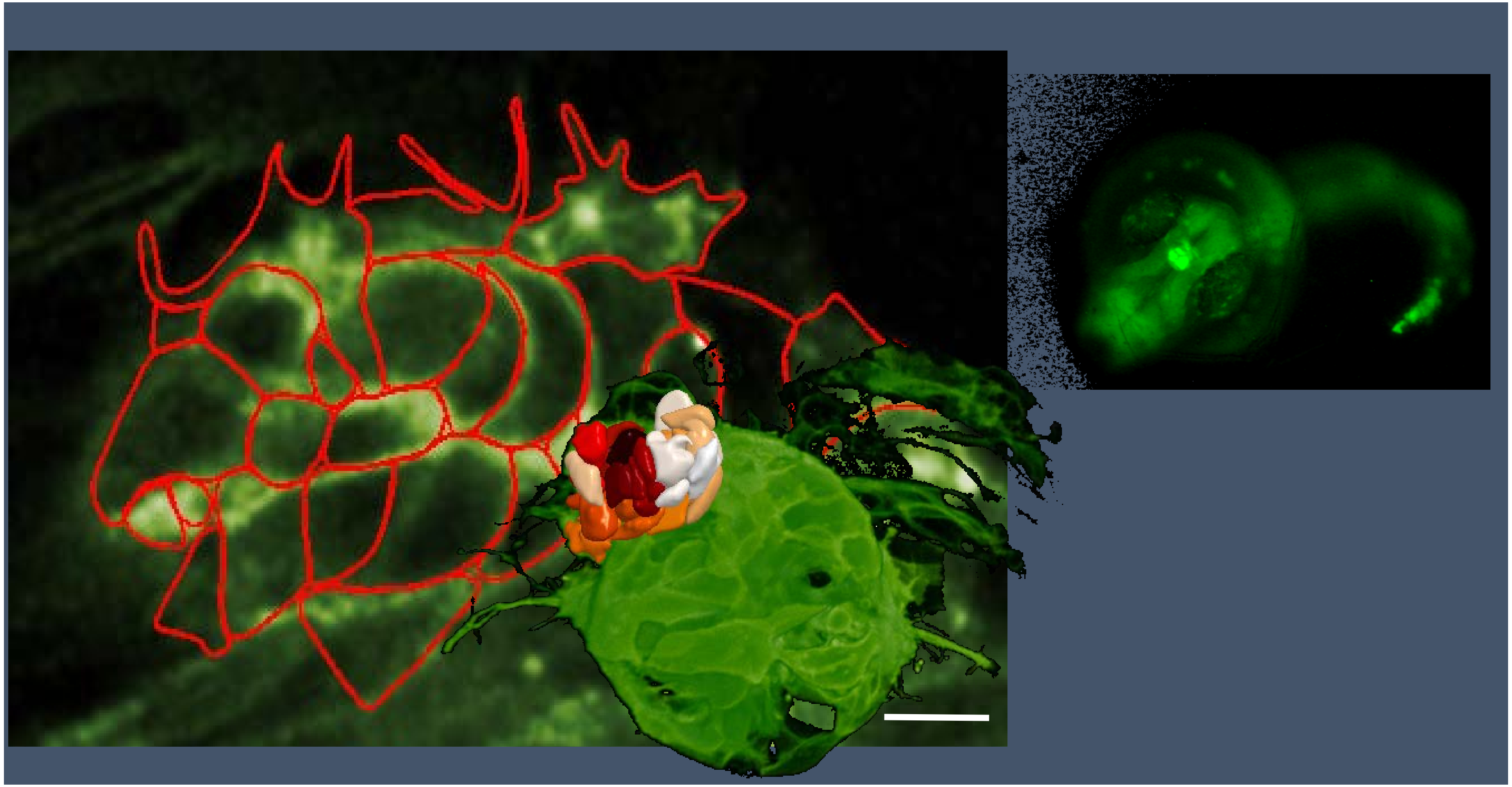
Iterations = 5
f = 0.15

Parametric Active Contours



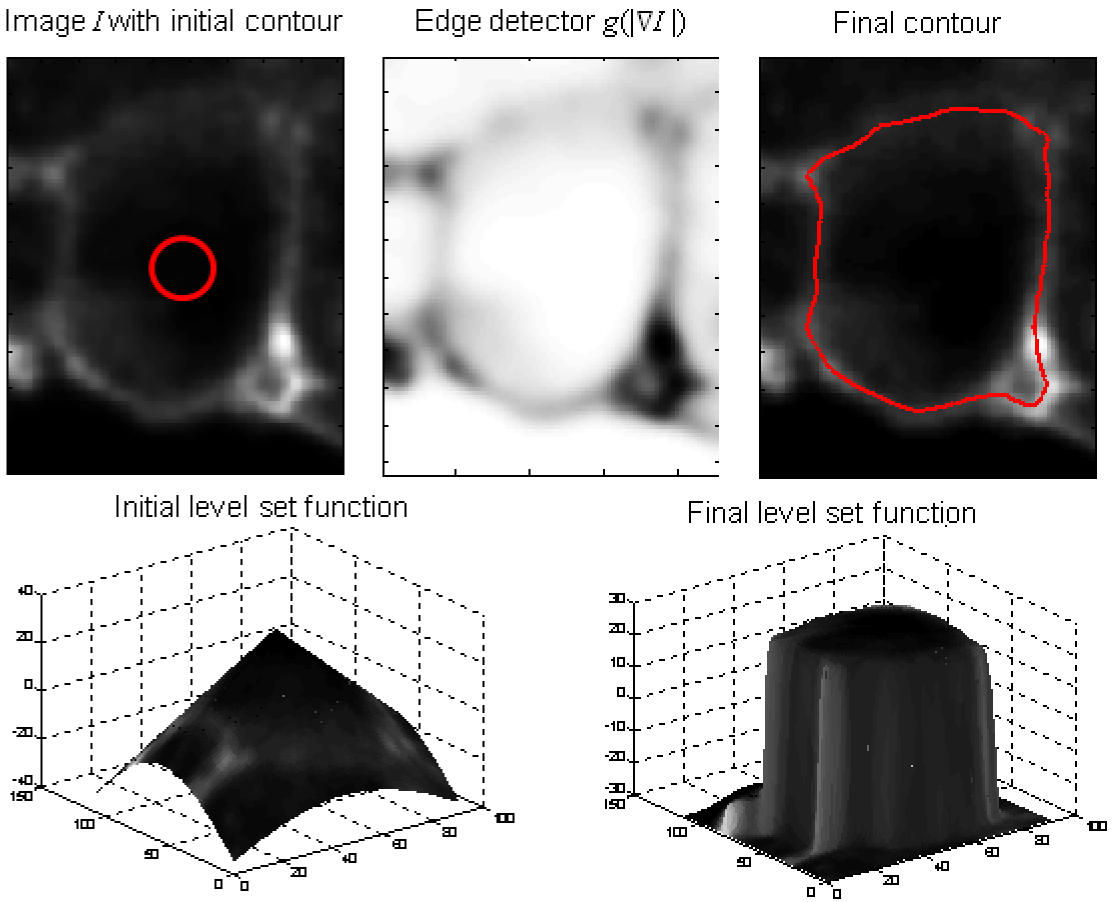
Parametric Active Contours

Hardest cases: initialization by manual ROI sketching



Implicit Active Contours

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



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

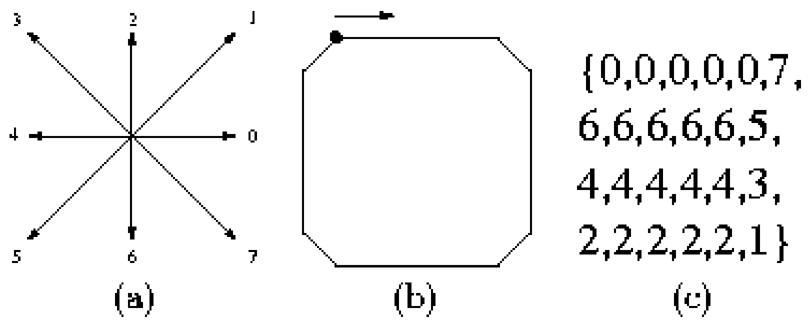
> Segmentación/análisis por contorno

Boundary model construction

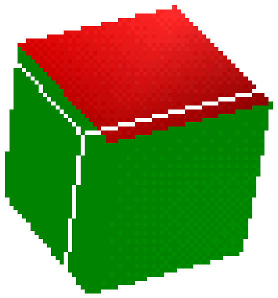
- 2D Freeman chain code

Books:
Nixon & Aguado, Feature extraction & image processing
González & Woods

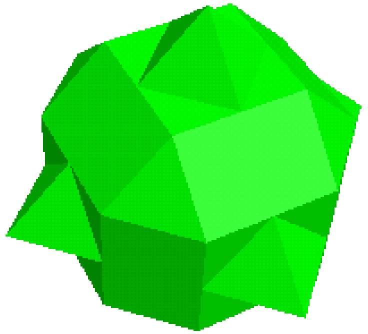
- 3D mesh models (voxel based): *marching cubes* (surface meshes), *tethraedra* (volume meshes)



2D polygon chain code



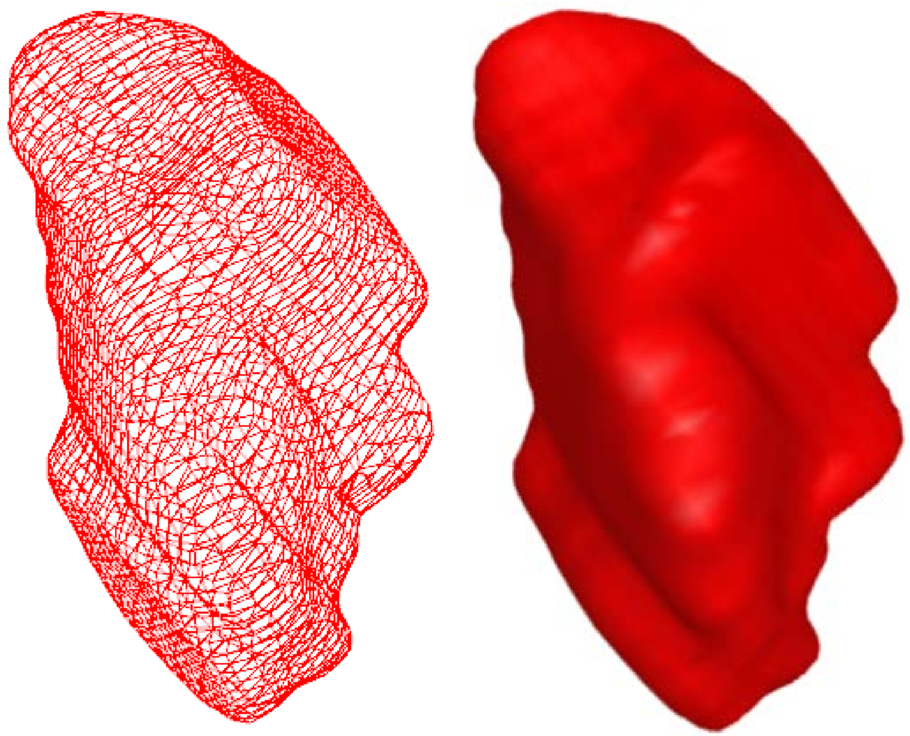
Voxel (“3D pixel”) model



3D polygon mesh

> Segmentación/análisis por contorno

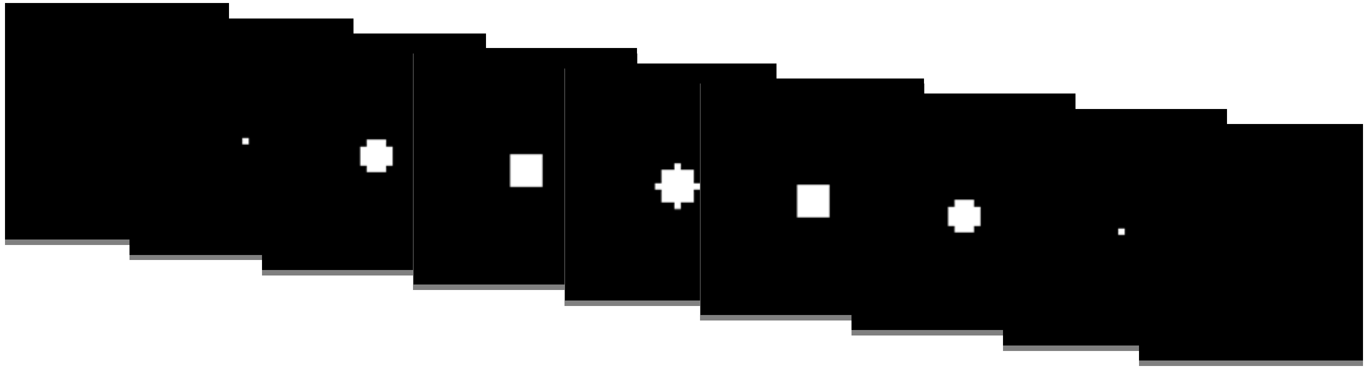
- A typical 3D surface mesh model is formed by:
  - Nodes or vertices
  - Polygons

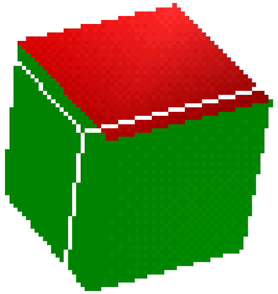


- Other models
  - Polynomial surfaces (splines, Bézier, NURBS, …)
  - Primitives composition

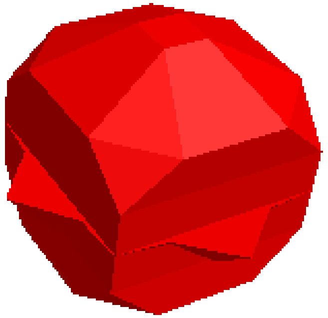
> Segmentación/análisis por contorno

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

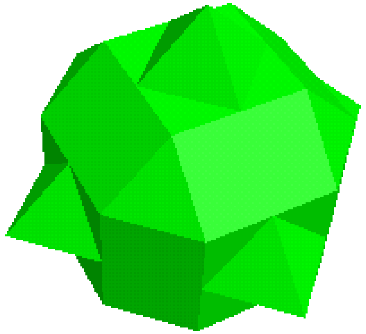




Voxel ("3D pixel") model



Triangle mesh

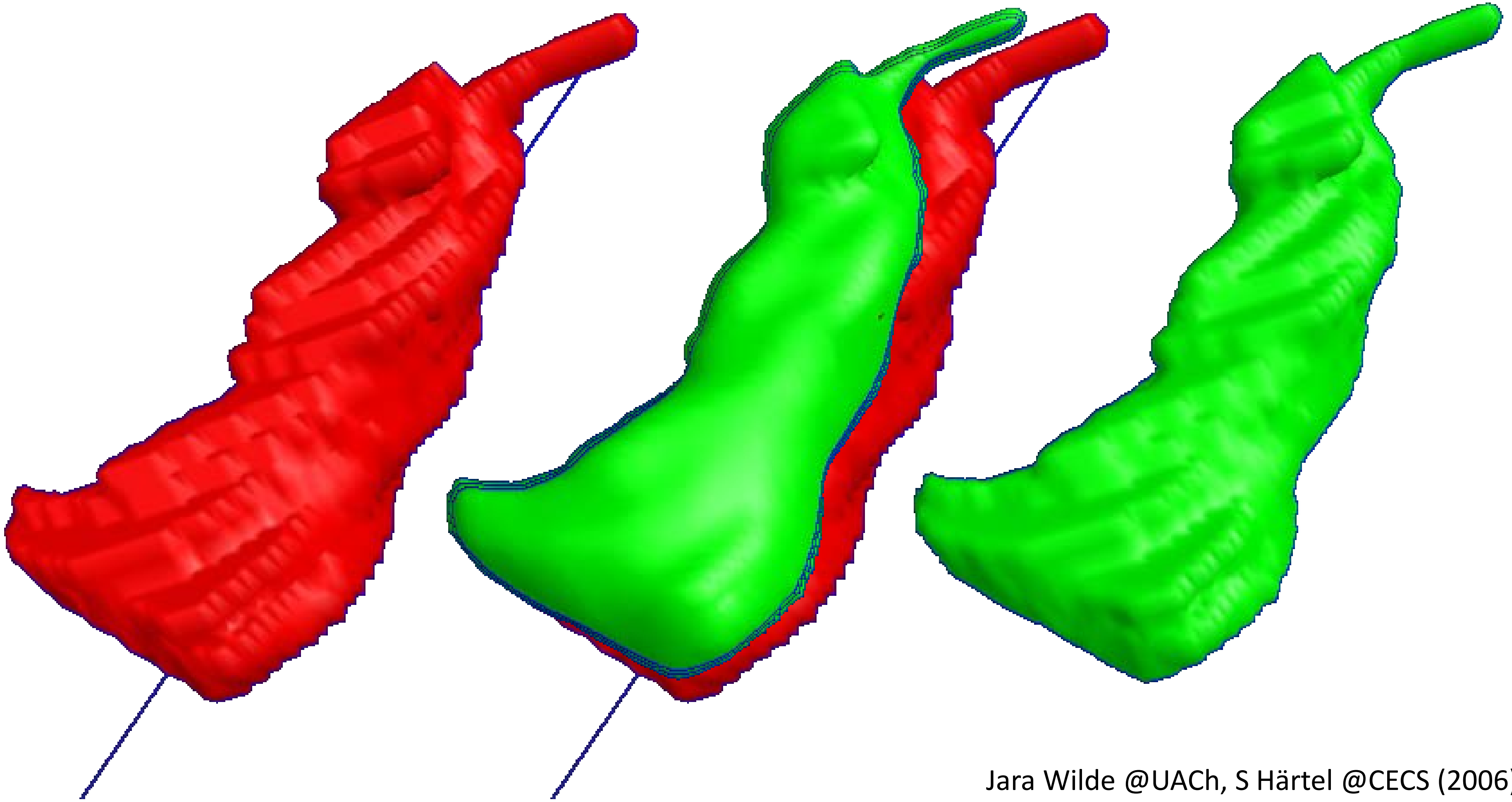


Triangular and quarilateral mesh

Jara Wilde @UACh, S Härtel @CECS (2006)

> Segmentación/análisis por contorno

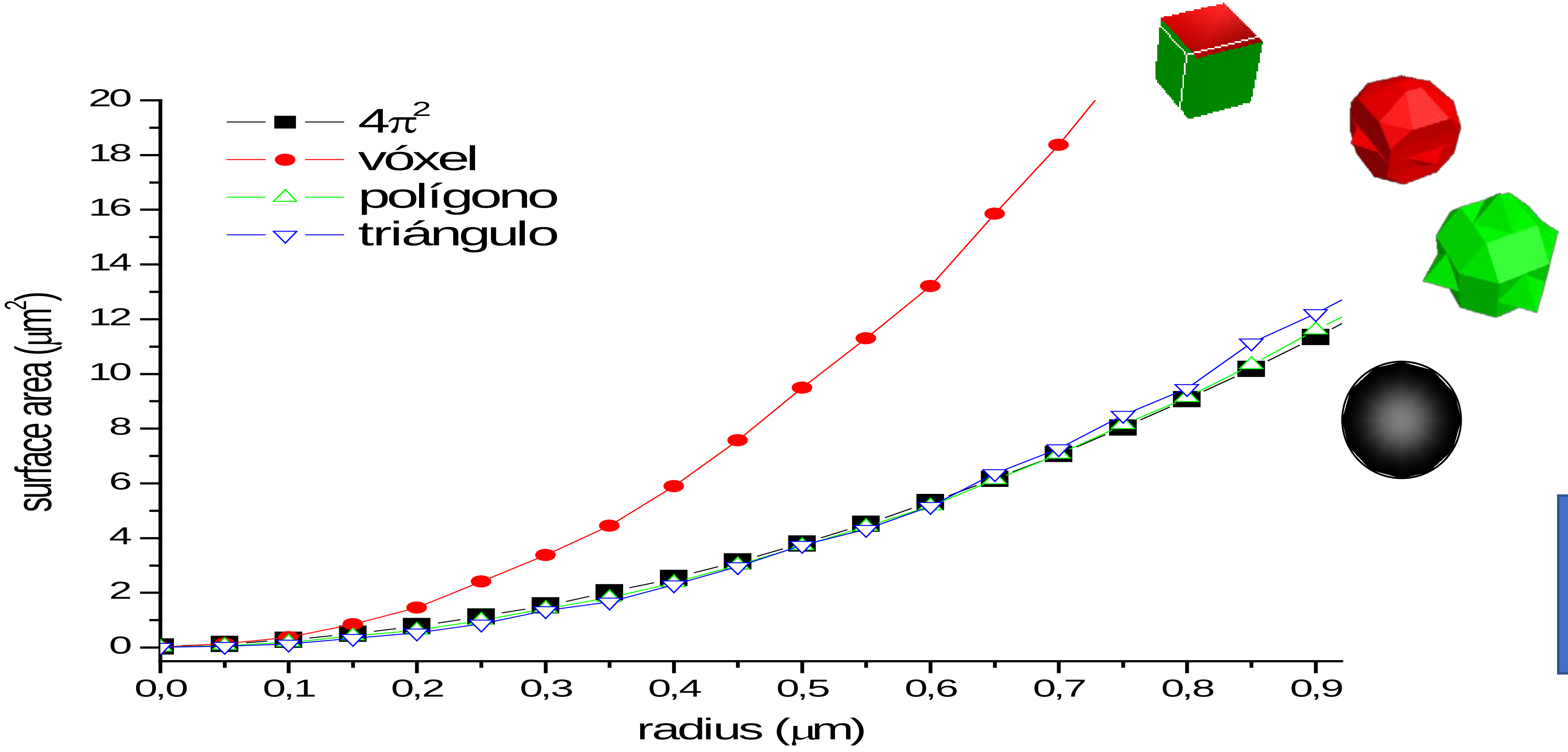
- Sample 3D ROI



Jara Wilde @UACh, S Härtel @CECS (2006)

> Segmentación/análisis por contorno

- ROI model affects measurements.



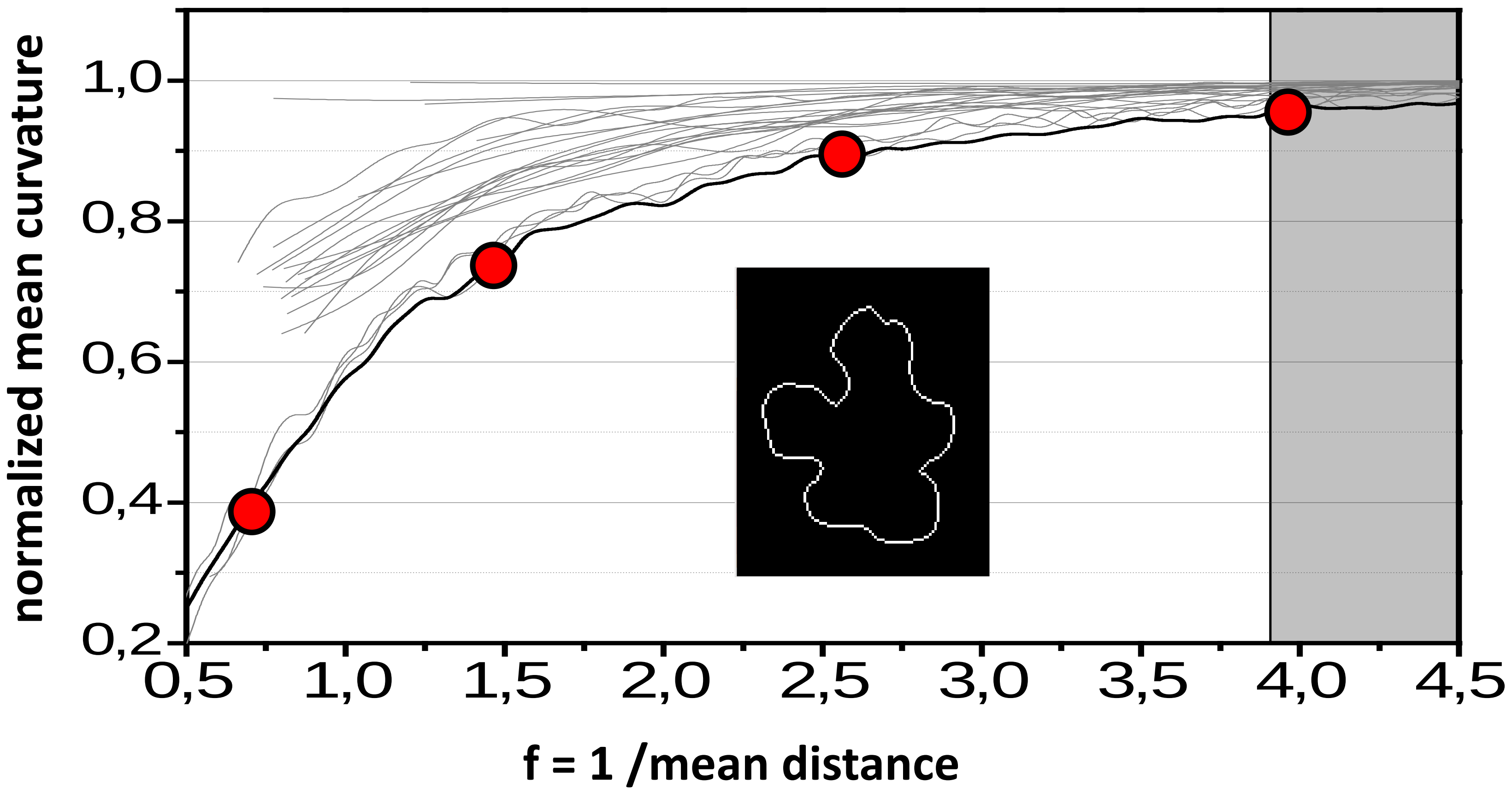
r = 1µm = 20 z-slices

2D → perimeter
3D → surface area

Jara Wilde @UACh, S Härtel @CECS (2006)

> Segmentación/análisis por contorno

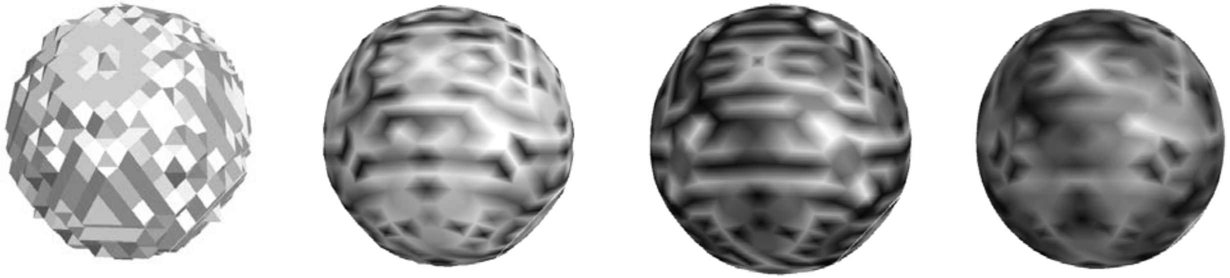
- Calibrating contour resolution. Example



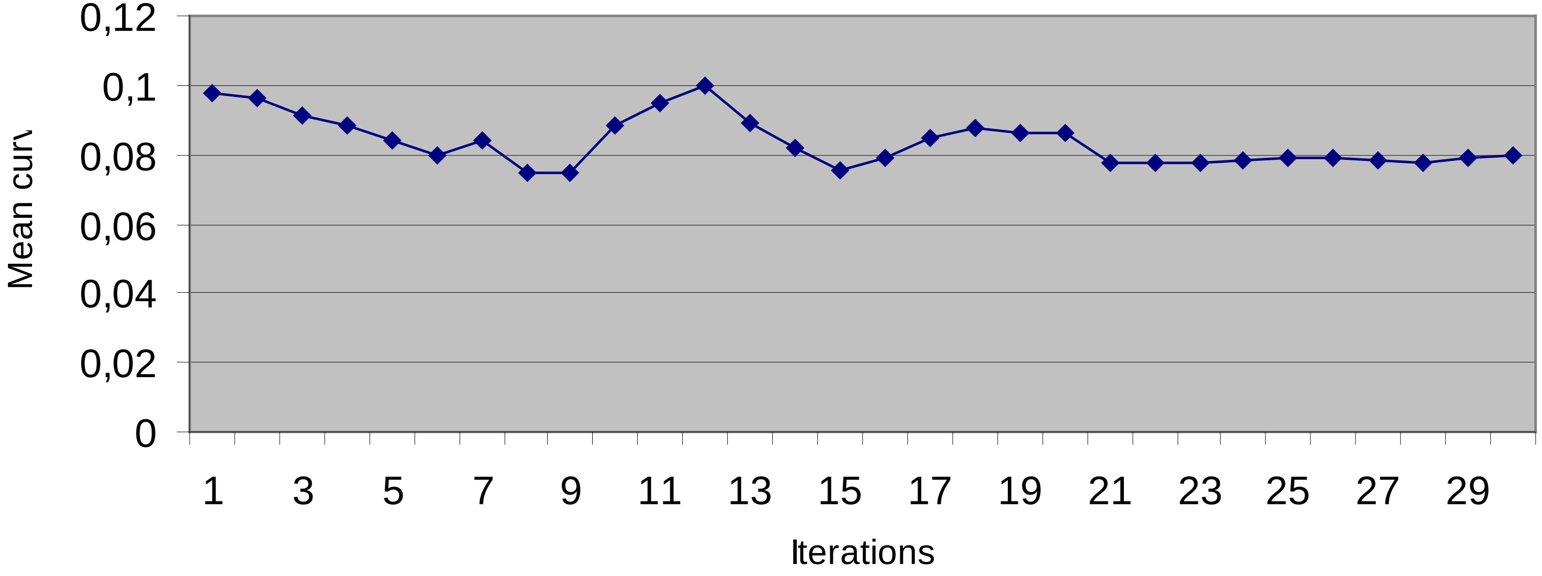
Jara Wilde @UACh, S Härtel @CECS (2006)

> Segmentación/análisis por contorno

- Calibrating contour resolution. Example



it. 0 *it.* 2 *it.* 4 *it.* 10



κ = 0,08

Jara Wilde @UACh, S Härtel @CECS (2006)

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