Procesamiento de Imágenes II

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

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0. Concepts

- Signal & noise
- Signal amplitude
- Signal frequency/frequencies
- Signal amplitude
    - Fluorescence intensity

- ~~Pixel / Voxel~~
- ~~Bit~~
- ~~(Sample) Bit depth~~
- Sampling frequency/interval
- Filter

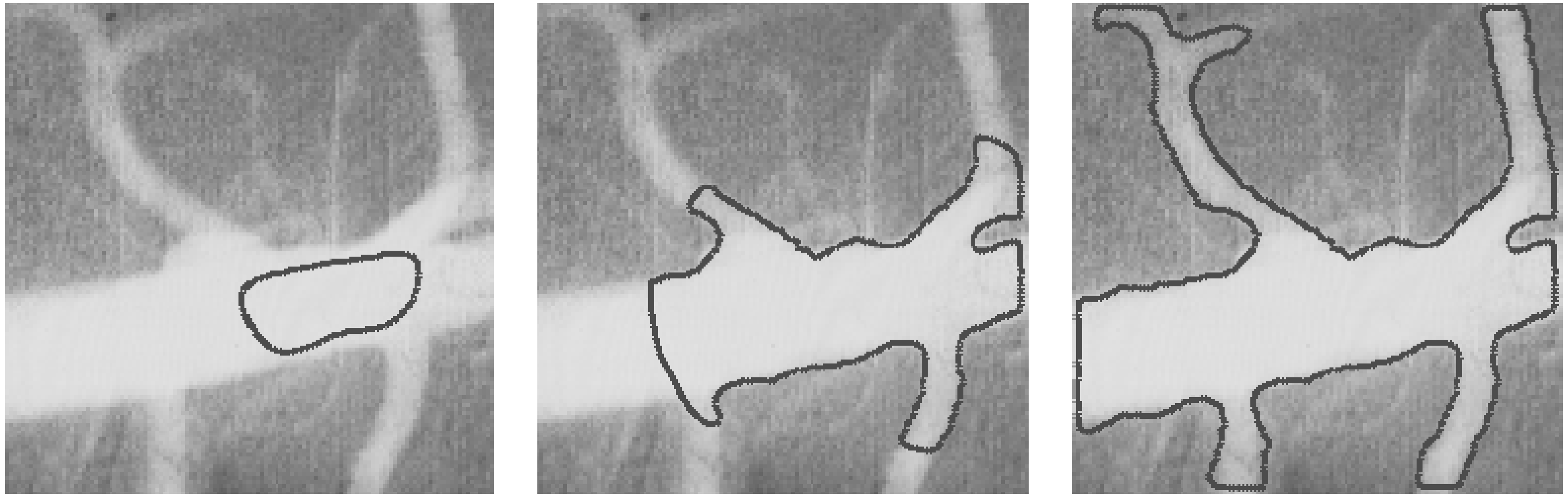
Problems

- 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

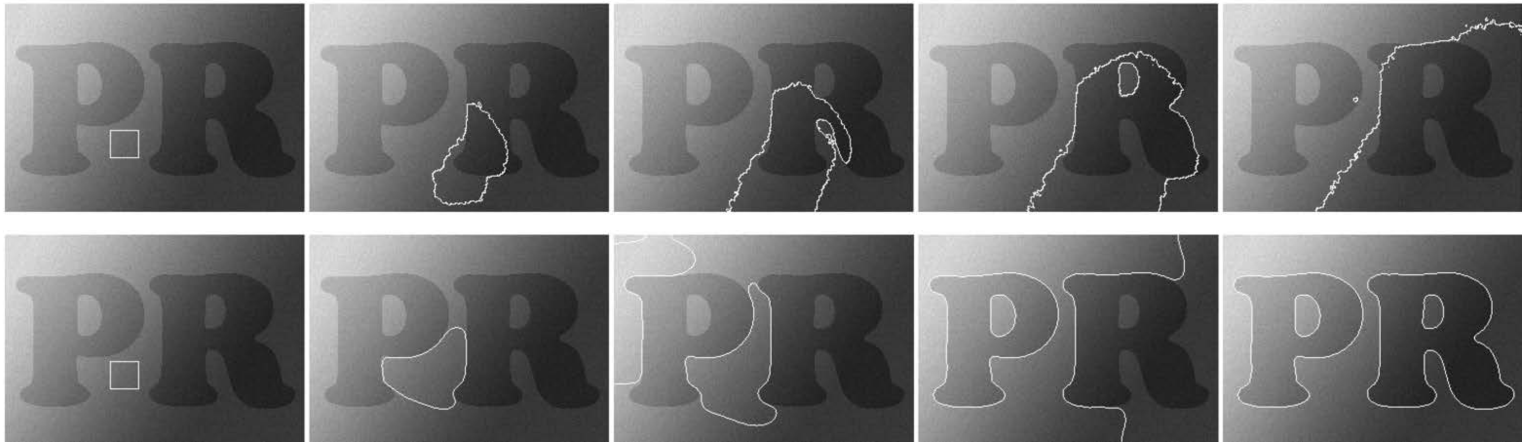
[1] Jason D. Hipp et all. Tryggo: Old norse for truth: The real truth about ground truth. New insights into the challenges of generating ground truth maps for WSI CAD algorithm evaluation. Pathol. Inform 2012, 3:8

[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.

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

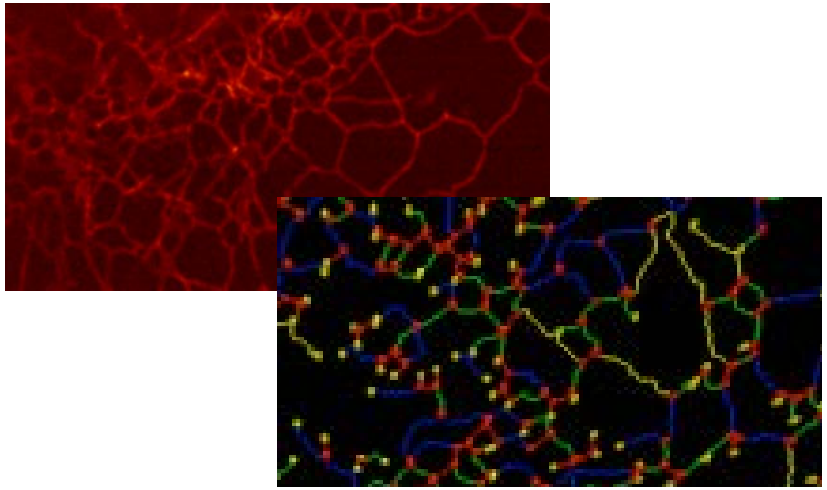


X Xie (2010) Magnetostatic Active Contours

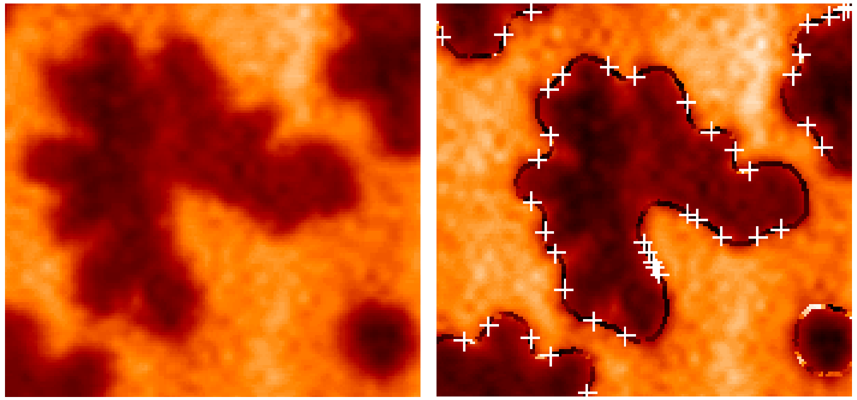
- Segmentation is the first step toward further quantifications
  - In addition to images, ROI models and data structures can suit for different types of descriptions

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)

Segmentation approaches

(One posible categorization)

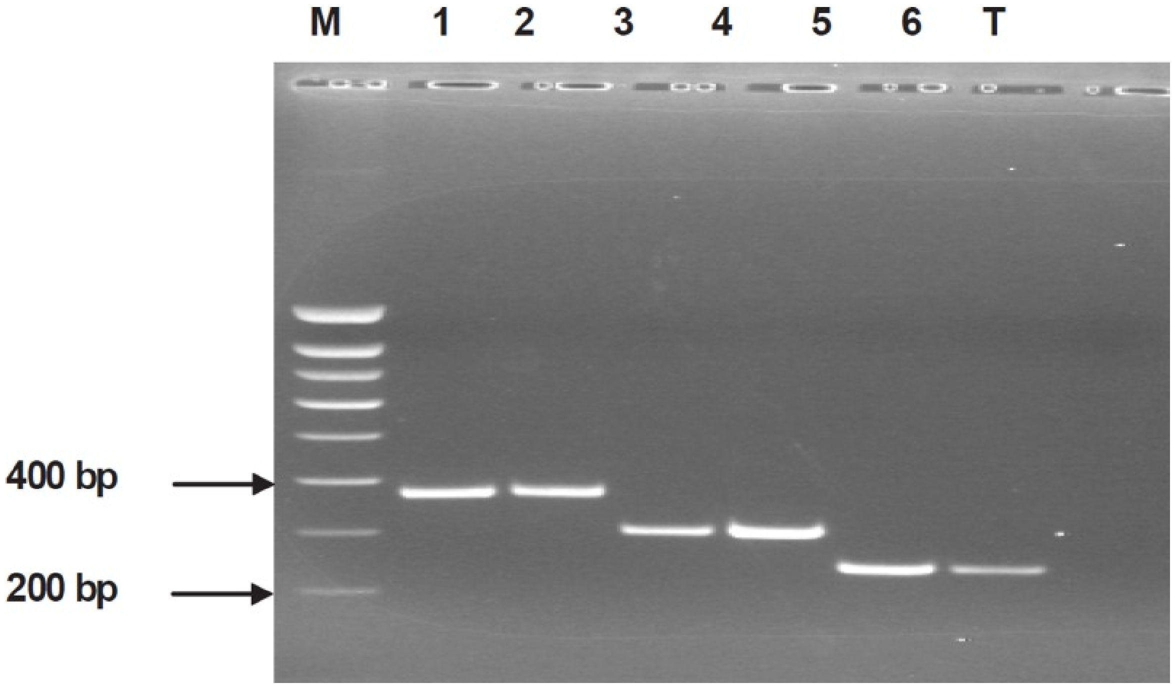
1. Classic approaches (filters)
   - Thresholding
   - Matrix convolution filters
   - Mathematical morphology
   - Fourier
   - …
2. Advanced approaches
   - Shape priors (*pattern matching*)
   - Clustering methods (k-means, region growing, graph cuts, entropy)
   - Deformable models (active contours)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

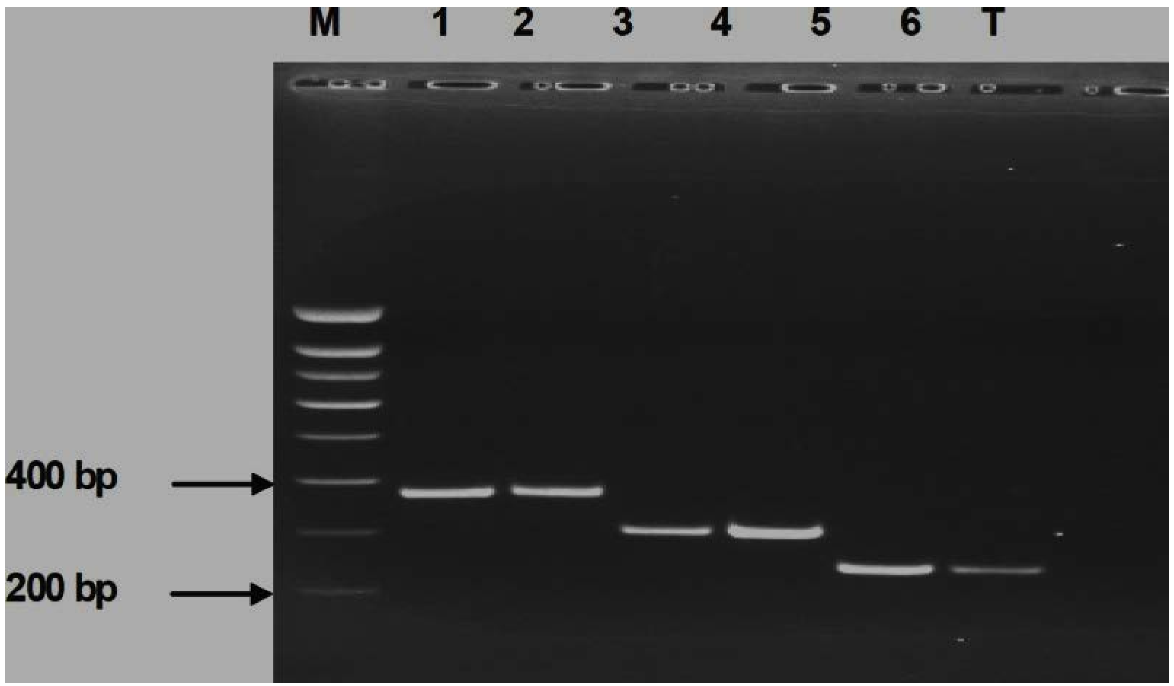
- 3. Trainable approaches (*machine learning*)
  - SVM
  - Random forest
  - Deep learning
  - …

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

- Filters… for what?

Example 1: PCR





https://www.ncbi.nlm.nih.gov/books/NBK402331/figure/wt_manguin1_ch8.F6/

Filters… for what?

- Example 2: Western blots

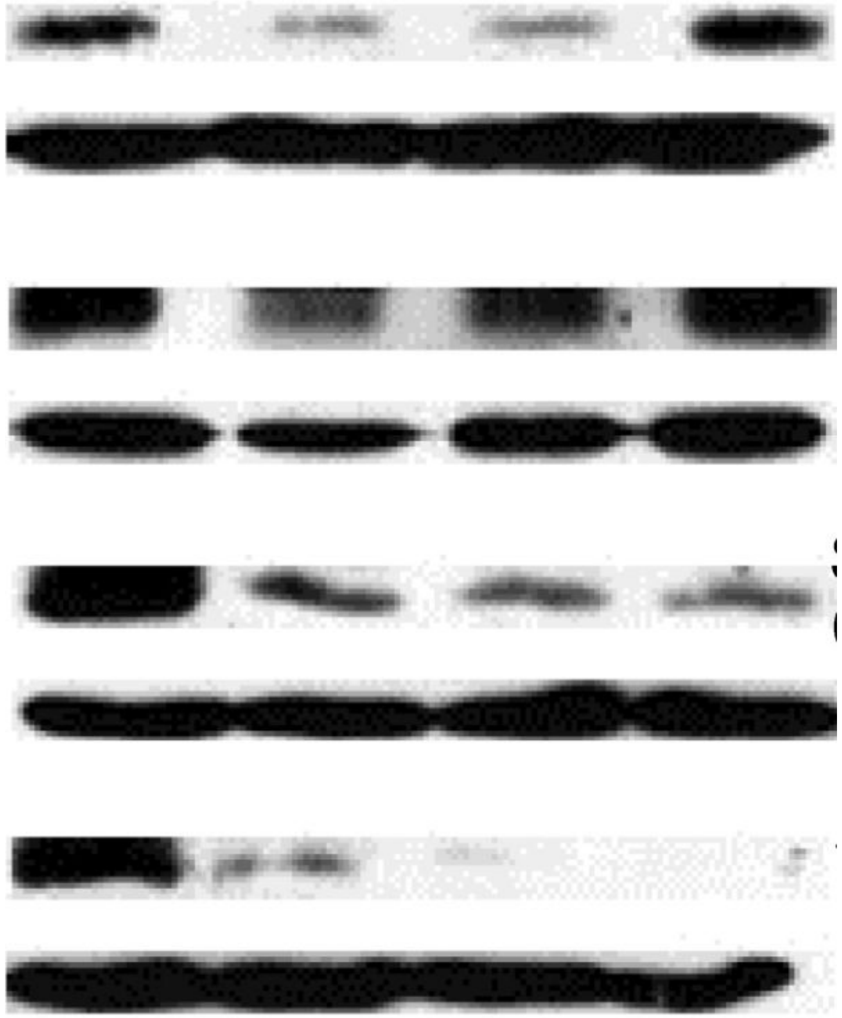
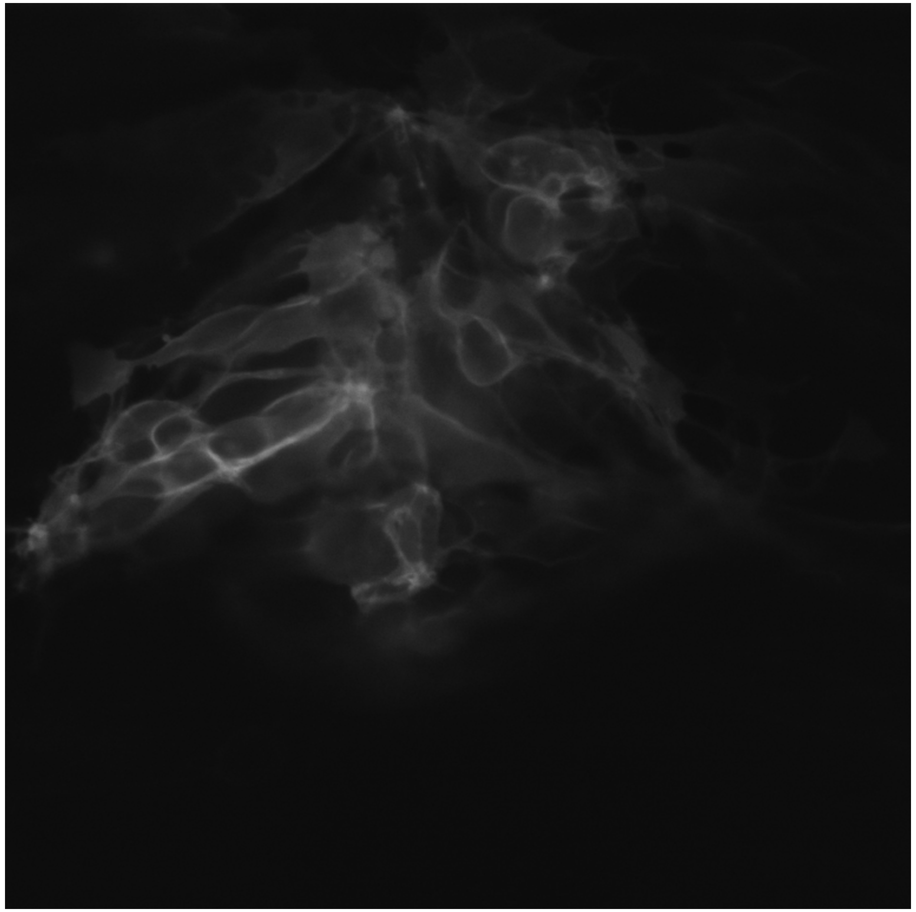


Image source: https://www.elsevier.com/editors-update/story/publishing-ethics/the-art-of-detecting-data-and-image-manipulation

Filters… for what?



Fluorescent zebrafish embryo parapineal complex

Transgenic *flh*::gap43-EGFP zebrafish embryo , 38 hpf (hours post-fertilization). Spinning disk microscopy

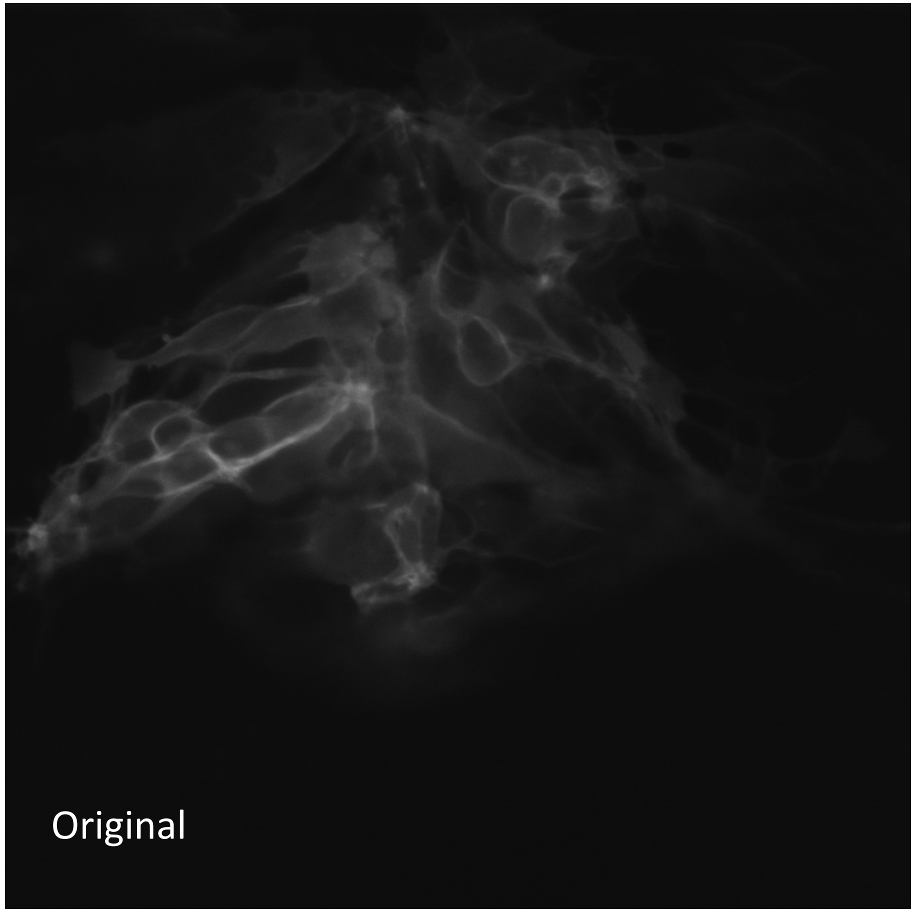
C.G. Lemus (LEO/SCIAN)

8 bits per pixel, 768x768x69 voxels

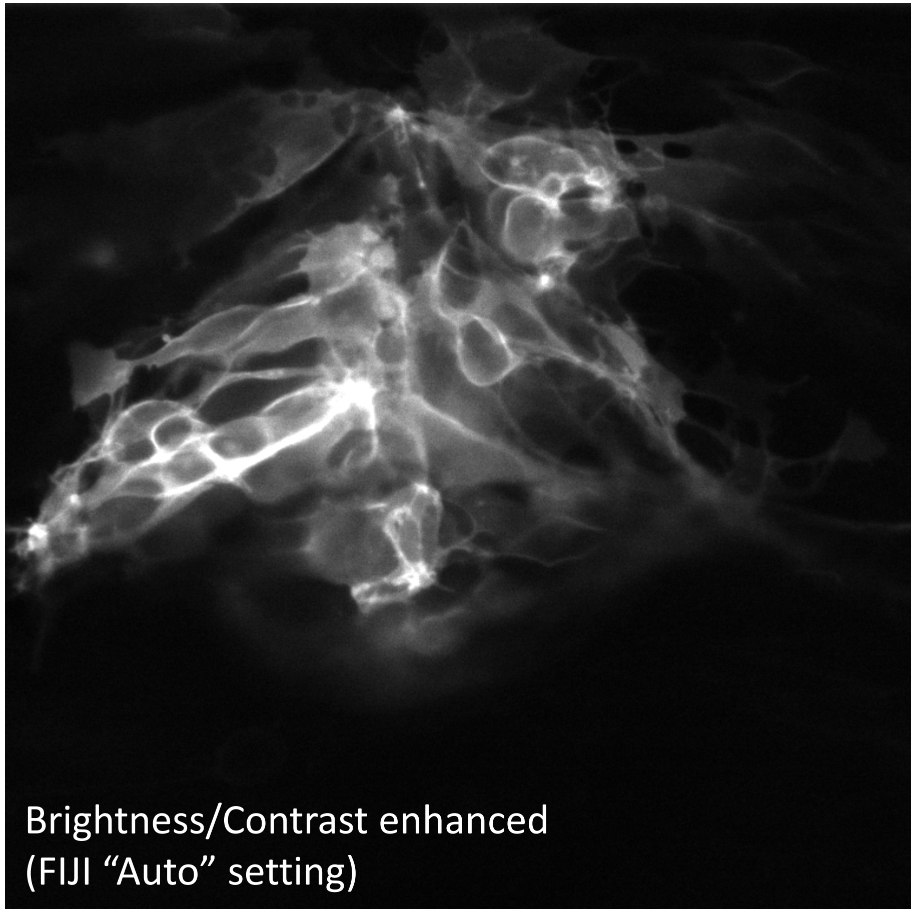
166x166x500 nm^3 voxel size

Filters… for what?

Visualization (screen)



Original



Brightness/Contrast enhanced
(FIJI “Auto” setting)

- Filters… for what?

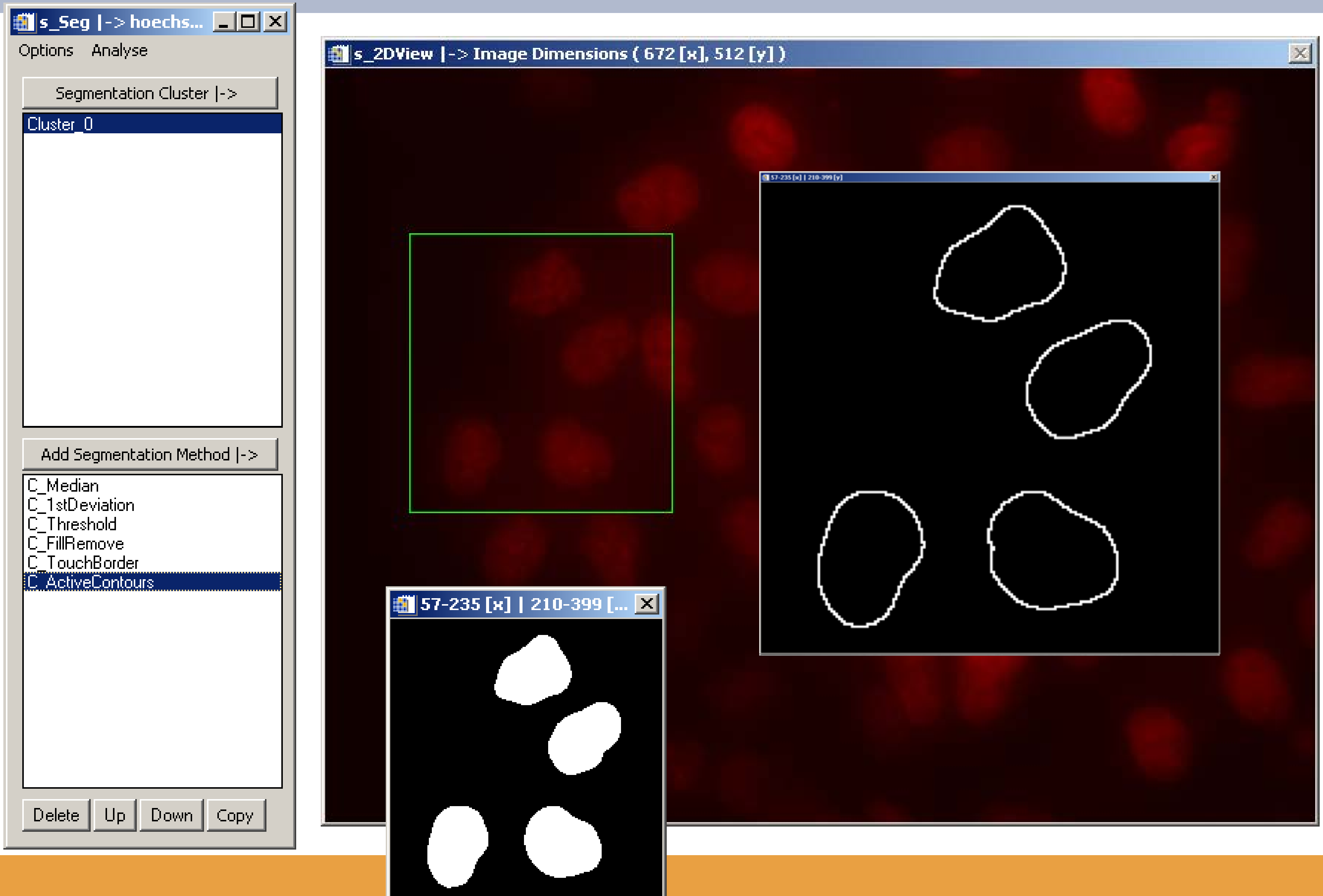
Visualization
(screen + print)

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

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

Filters… for what?

Easier segmentation

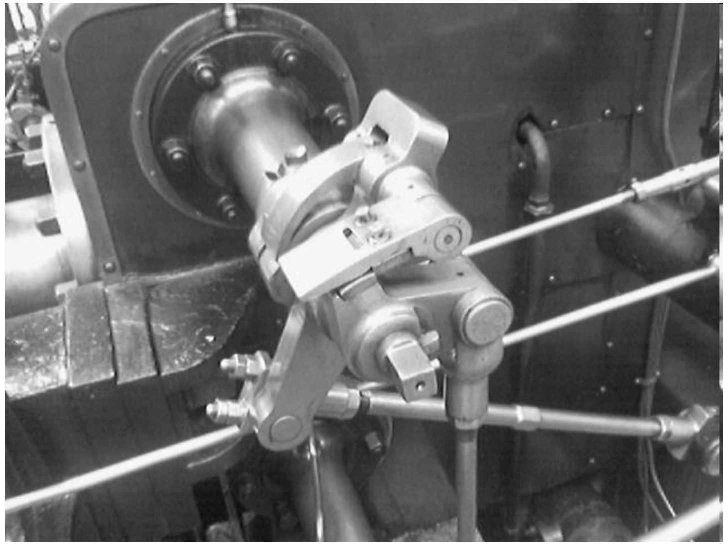


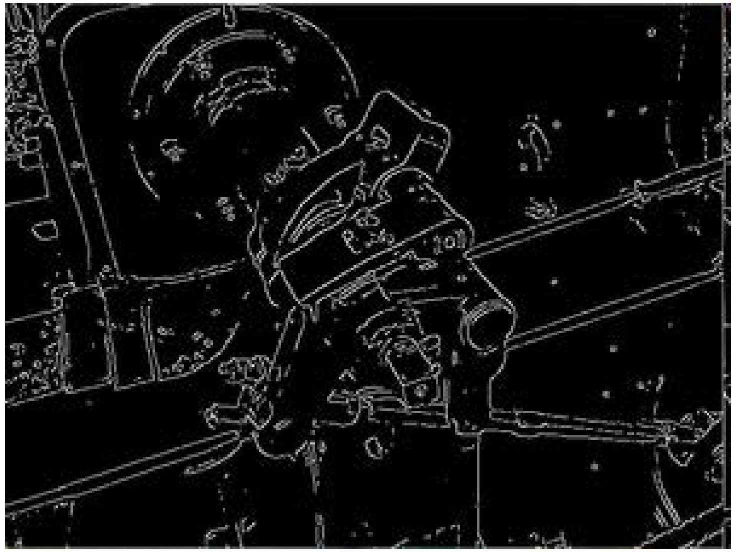
- “Filter” approach

image

filter

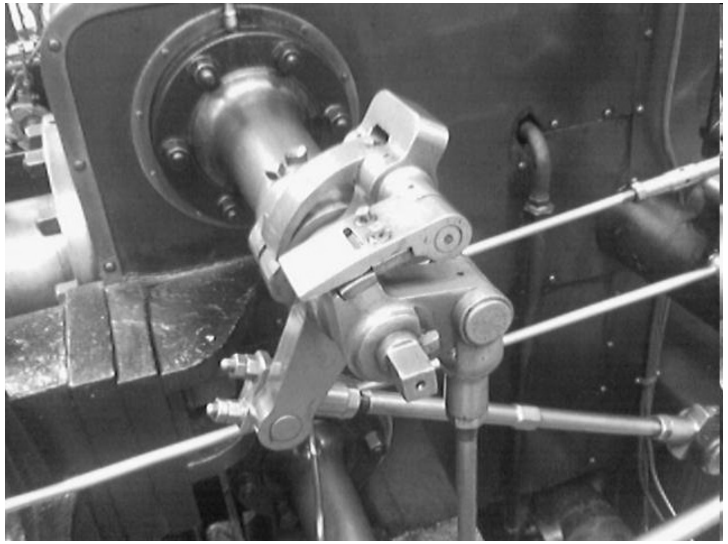
filtered image

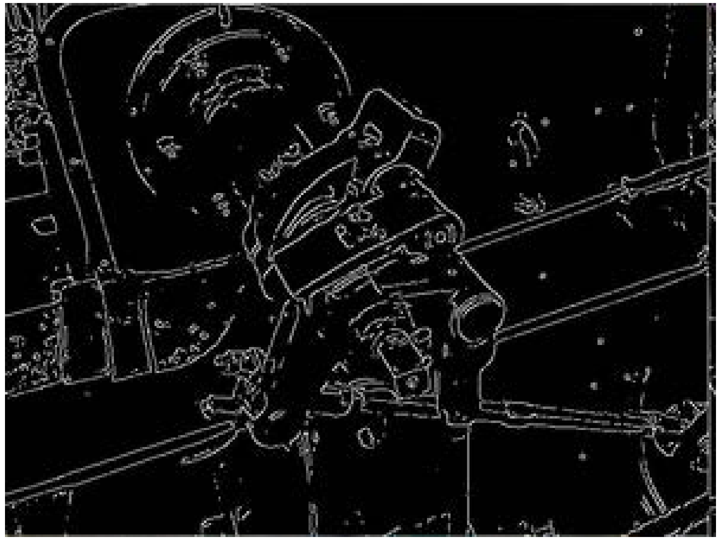


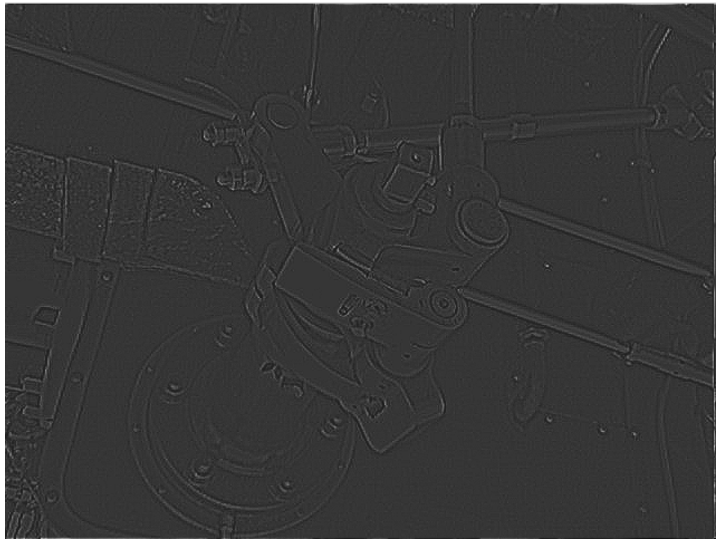


Images from Wikipedia

- A sample filter…





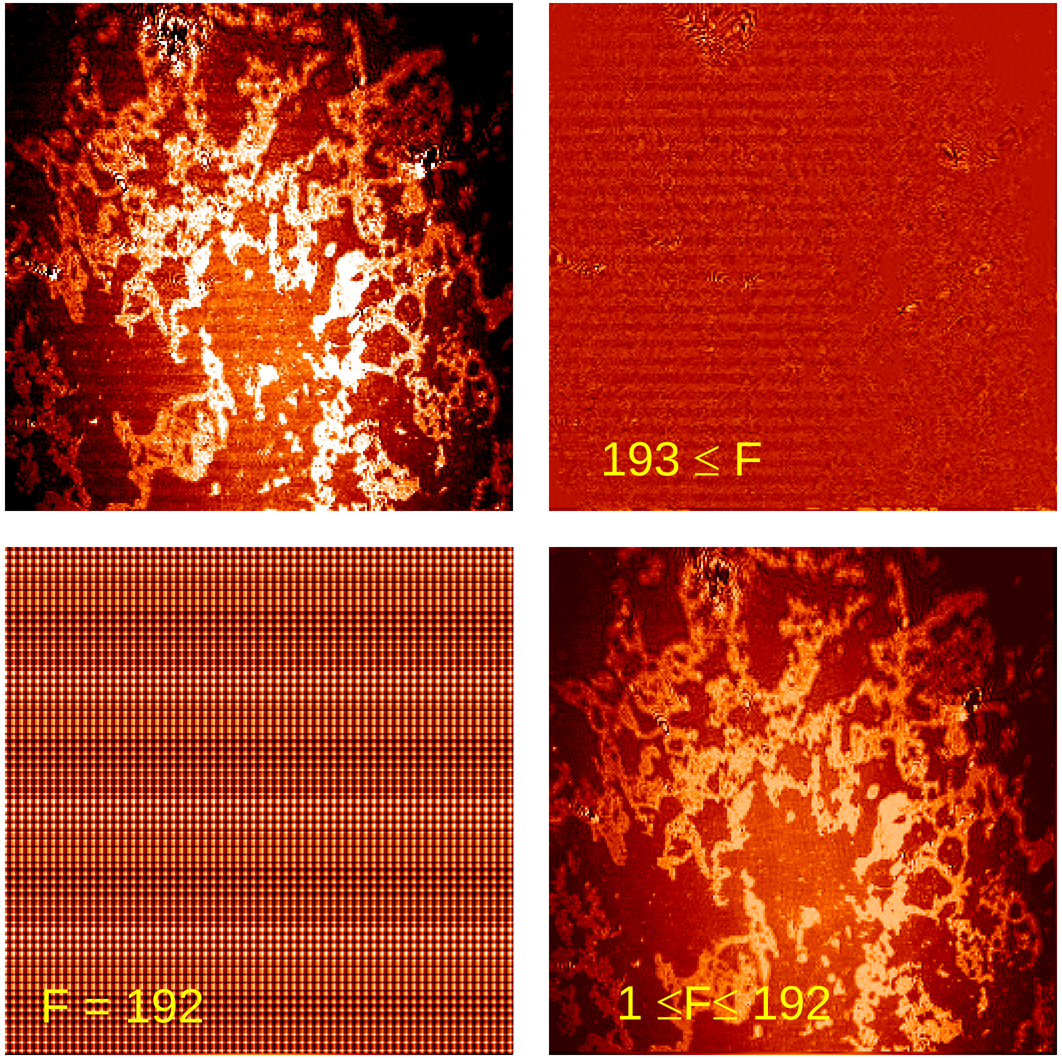


- Fourier filtering

- Think of a different base (e.g. polynomials)

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

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



Thresholding
- example: Otsu

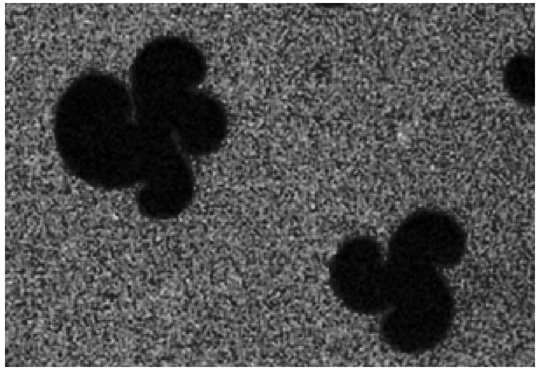
Convolution based
- Convolution operation
- Examples: gradient, Laplace, Sobel, Gaussian

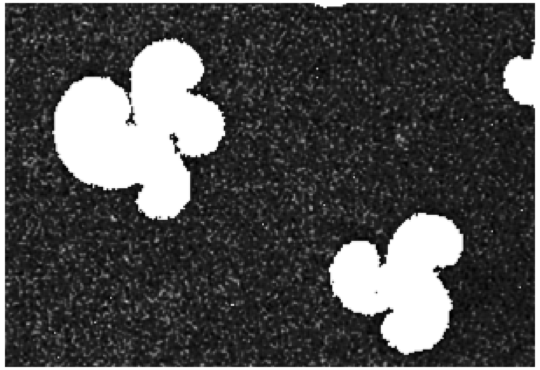
Morphological
- Morphological operators
- Size
- Thinning / skeletonization

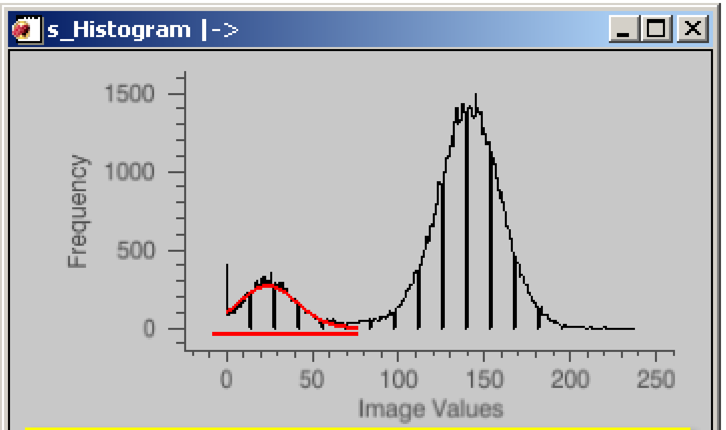
Arithmetic-logic
- AND, OR, XOR

And a long etc.

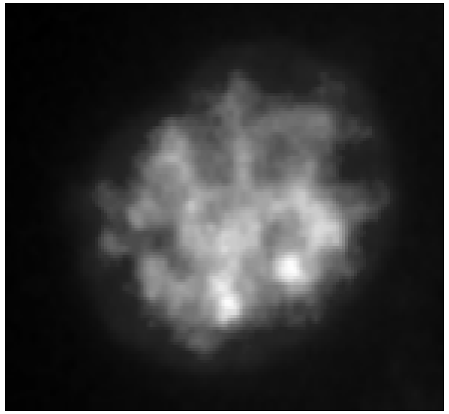
- Threshold filter segmentation: ROIs (white) / background (black)

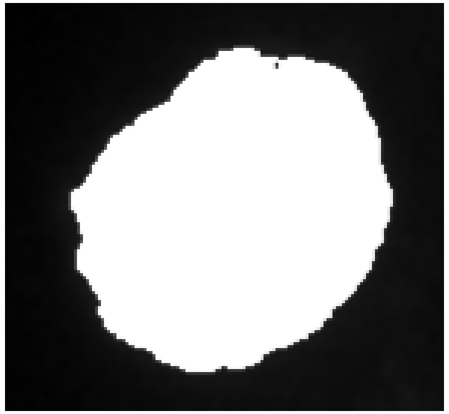


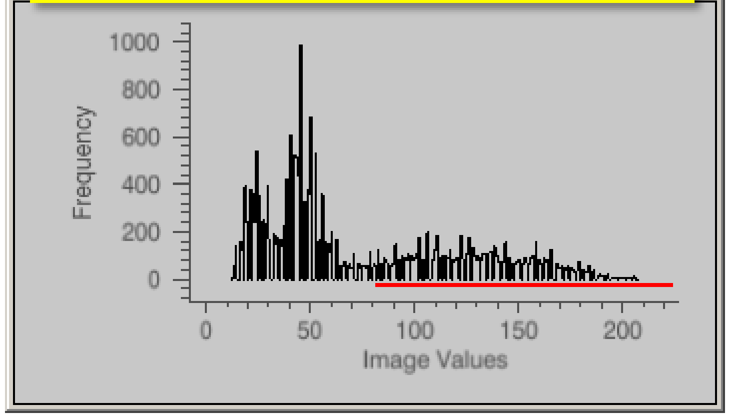




Intensity histograms

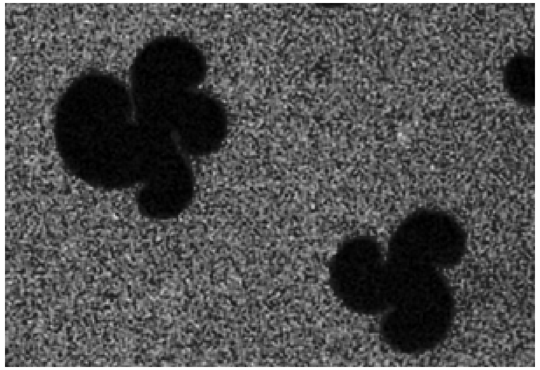


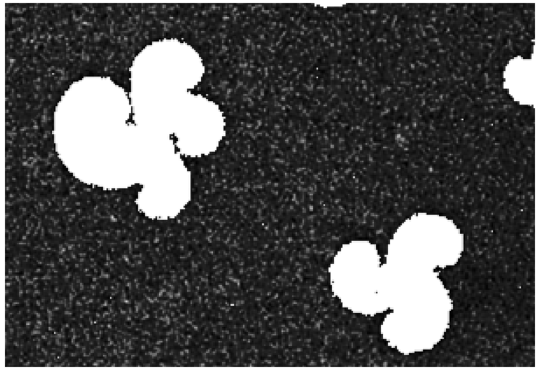


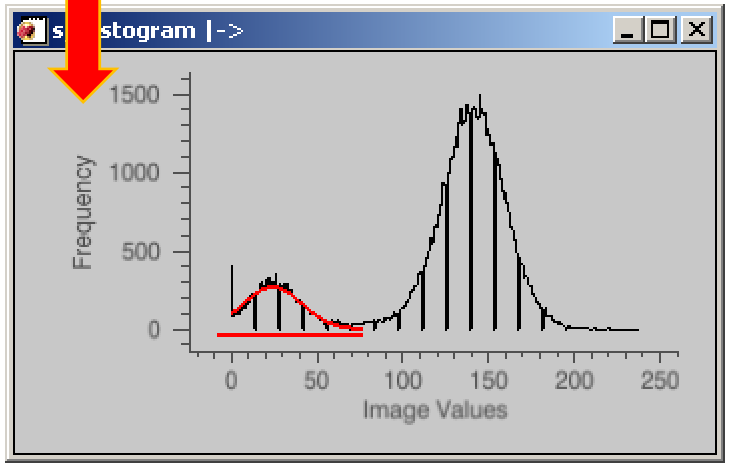


Let’s call this “statistical frequency” to avoid confusions with the Nyquist/time/space domain frequency

- Here comes the Intensity Histogram

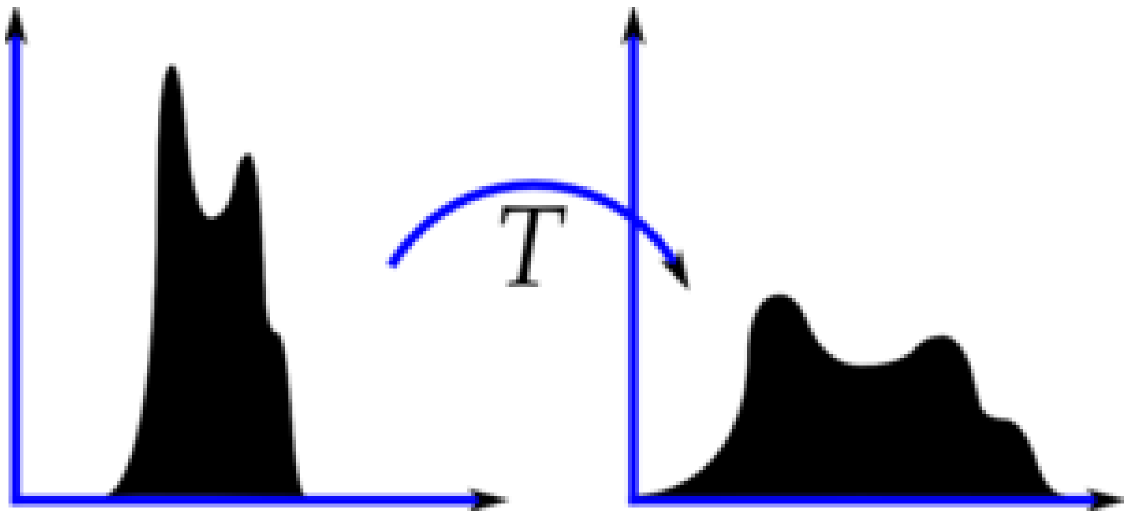






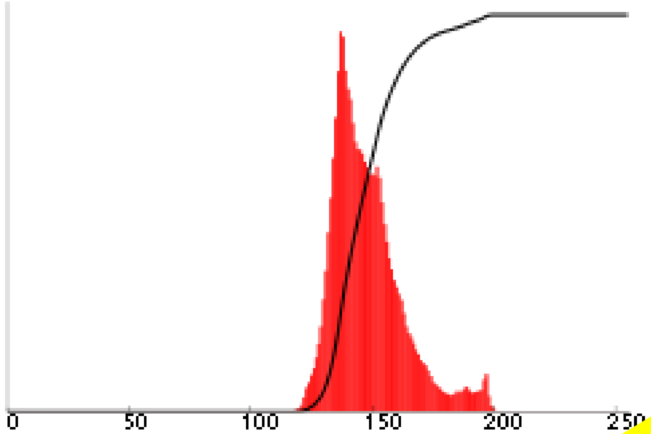
- Throwback to the acquisition…
  - Offset
  - Clipping (sometimes “Saturation”…try not to confuse with the HSV Saturation)

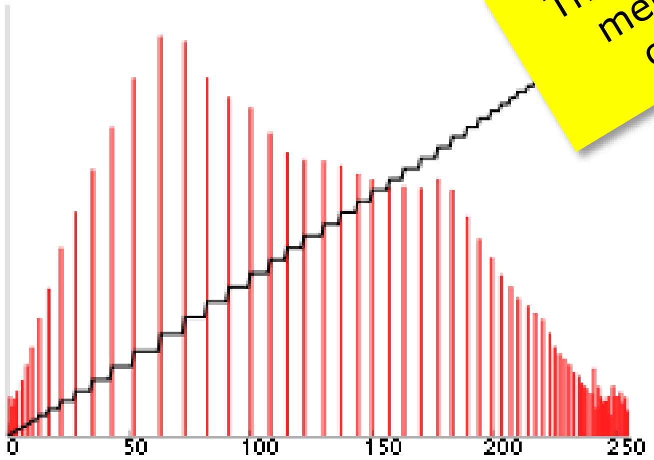
- Histogram equalization



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

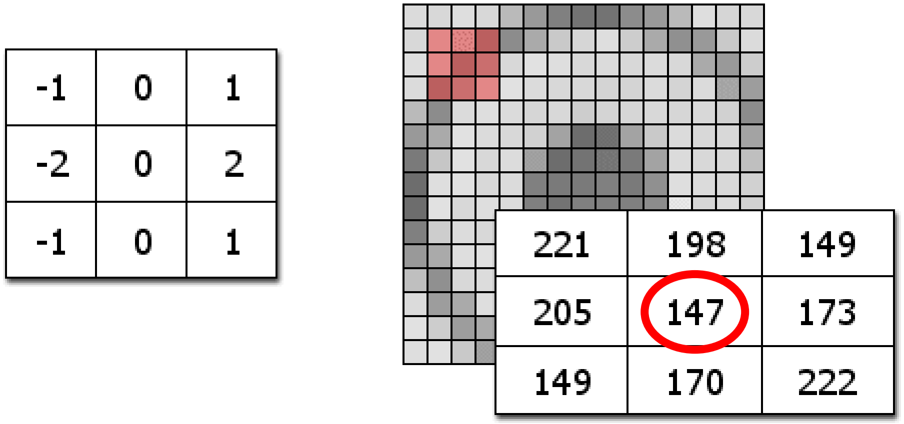
1. Compute histogram and probabilities of each intensity level
2. Set up initial $\omega_i(0)$ and $\mu_i(0)$
3. Step through all possible thresholds $t = 1 \ldots$ maximum intensity
    1. Update ω_i and μ_i
    2. Compute $\sigma_b^2(t)$
4. Desired threshold corresponds to the maximum $\sigma_b^2(t)$
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 = $\dfrac{threshold_1 + threshold_2}{2}$

A Couple 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

- 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*



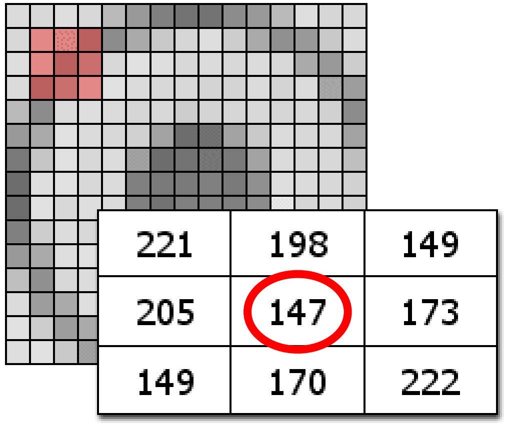
$$\begin{aligned}&= (-1 \cdot 221)\\ &+ (0 \cdot 198)\\ &+ (1 \cdot 149)\\ &+ (-2 \cdot 205)\\ &+ (0 \cdot \mathbf{147})\\ &+ (2 \cdot 173)\\ &+ (-1 \cdot 149)\\ &+ (0 \cdot 170)\\ &+ (1 \cdot 222) = -63\end{aligned}$$

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

K

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

I



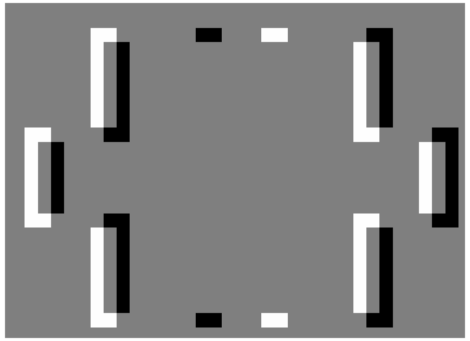
$$(K \otimes I)_{i,j} = (-1 * 222) + (0 * 170) + (1 * 149) + (-2 * 173) + (0 * \mathbf{147}) + (2 * 205) + (-1 * 149) + (0 * 198) + (1 * 221) = +63$$

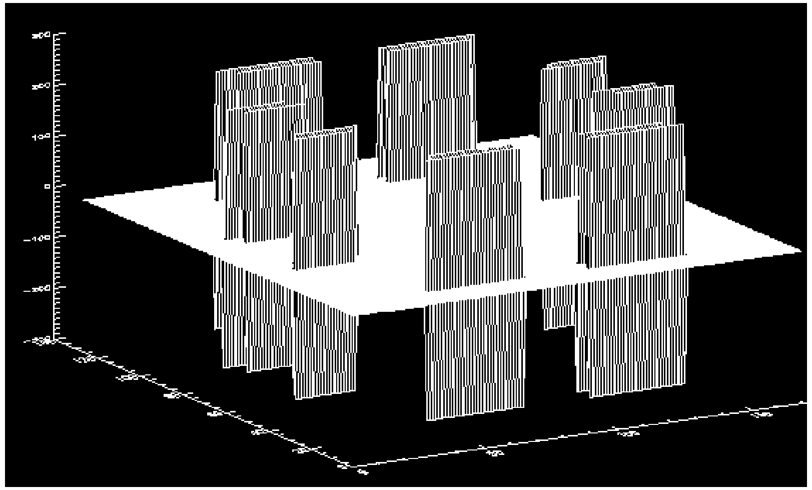
Matrix convolution can be implemented in different ways… beware of the algorithm!

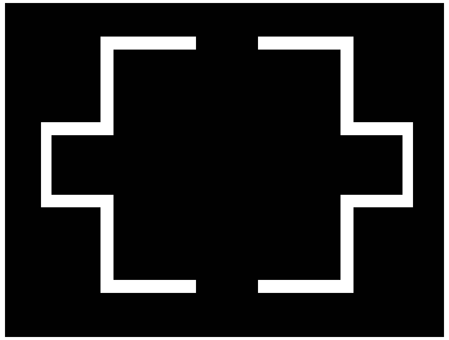
James Matthews, 2002 http://www.generation5.org/content/2002/convolution.asp

- Intensity gradients (discrete approximation)

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

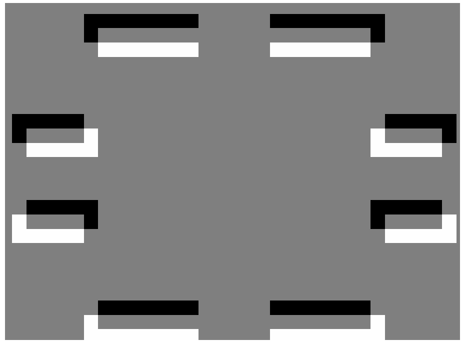


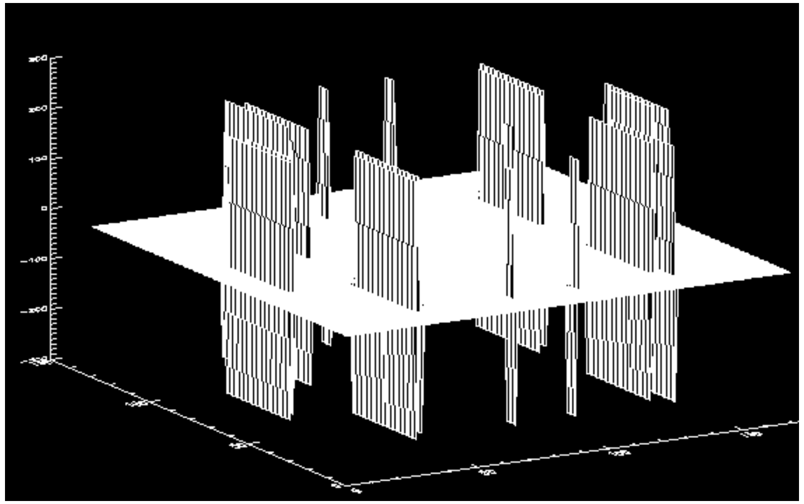


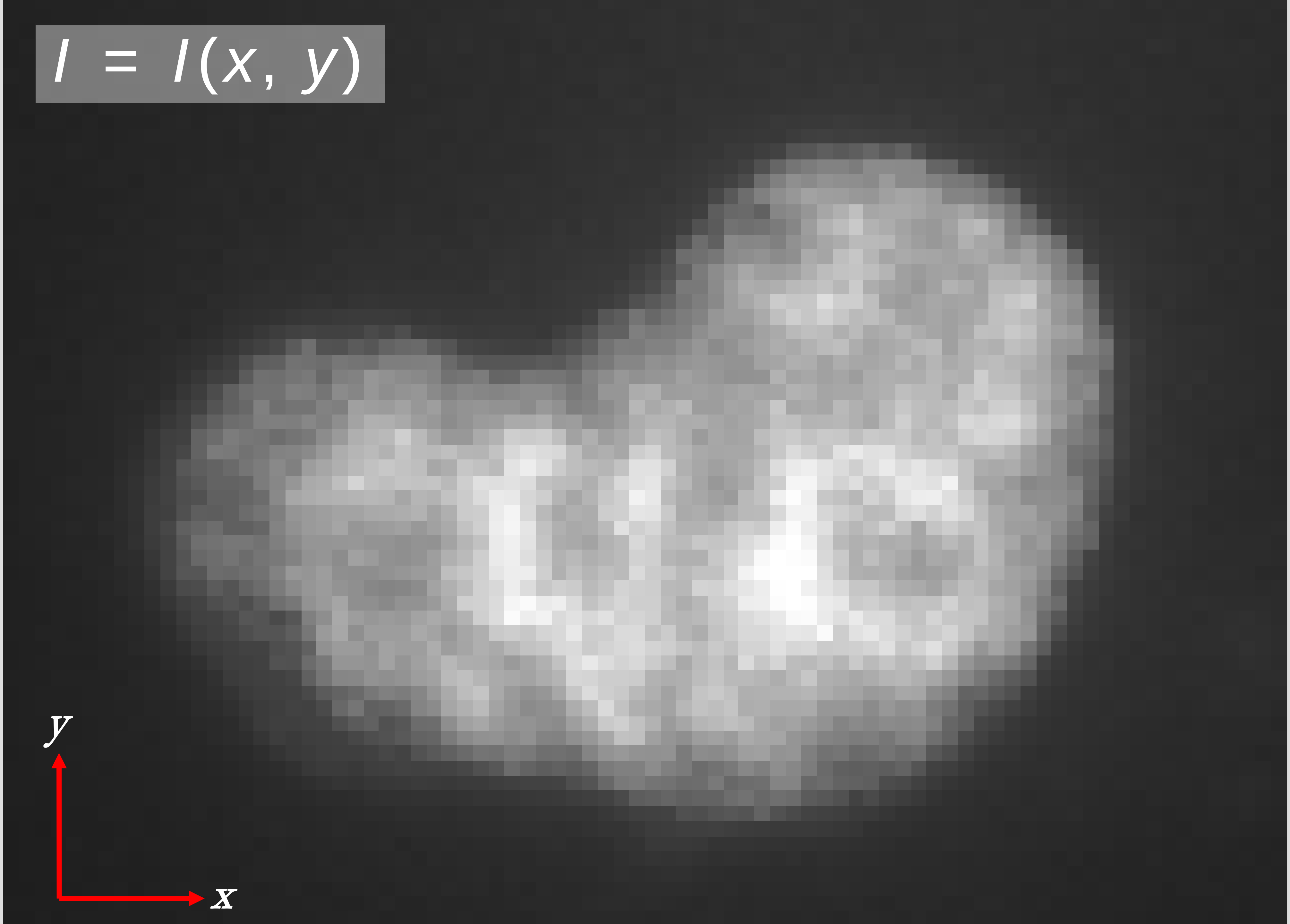


flat | + | -

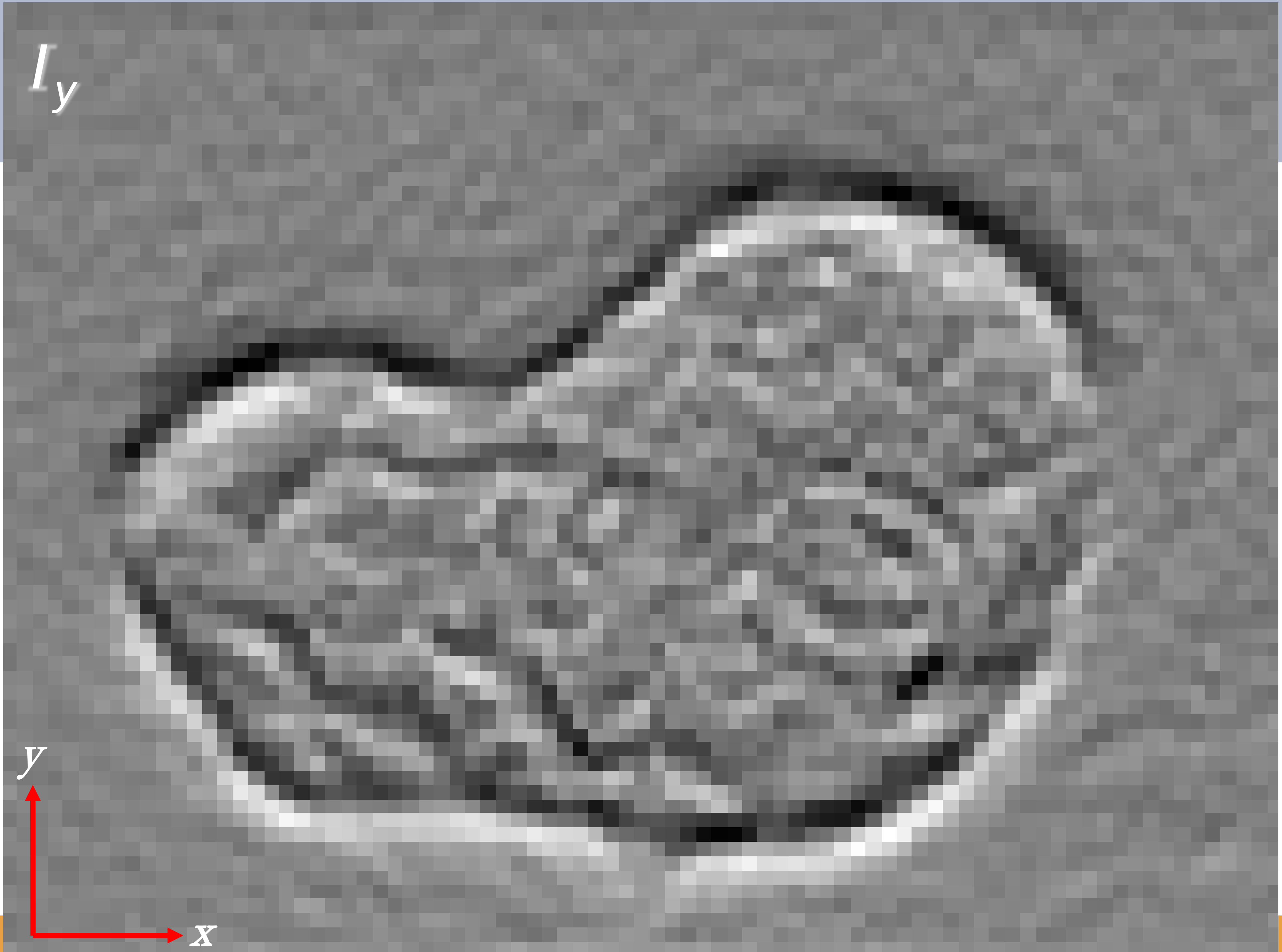
$$\frac{\partial I}{\partial y} \approx$$

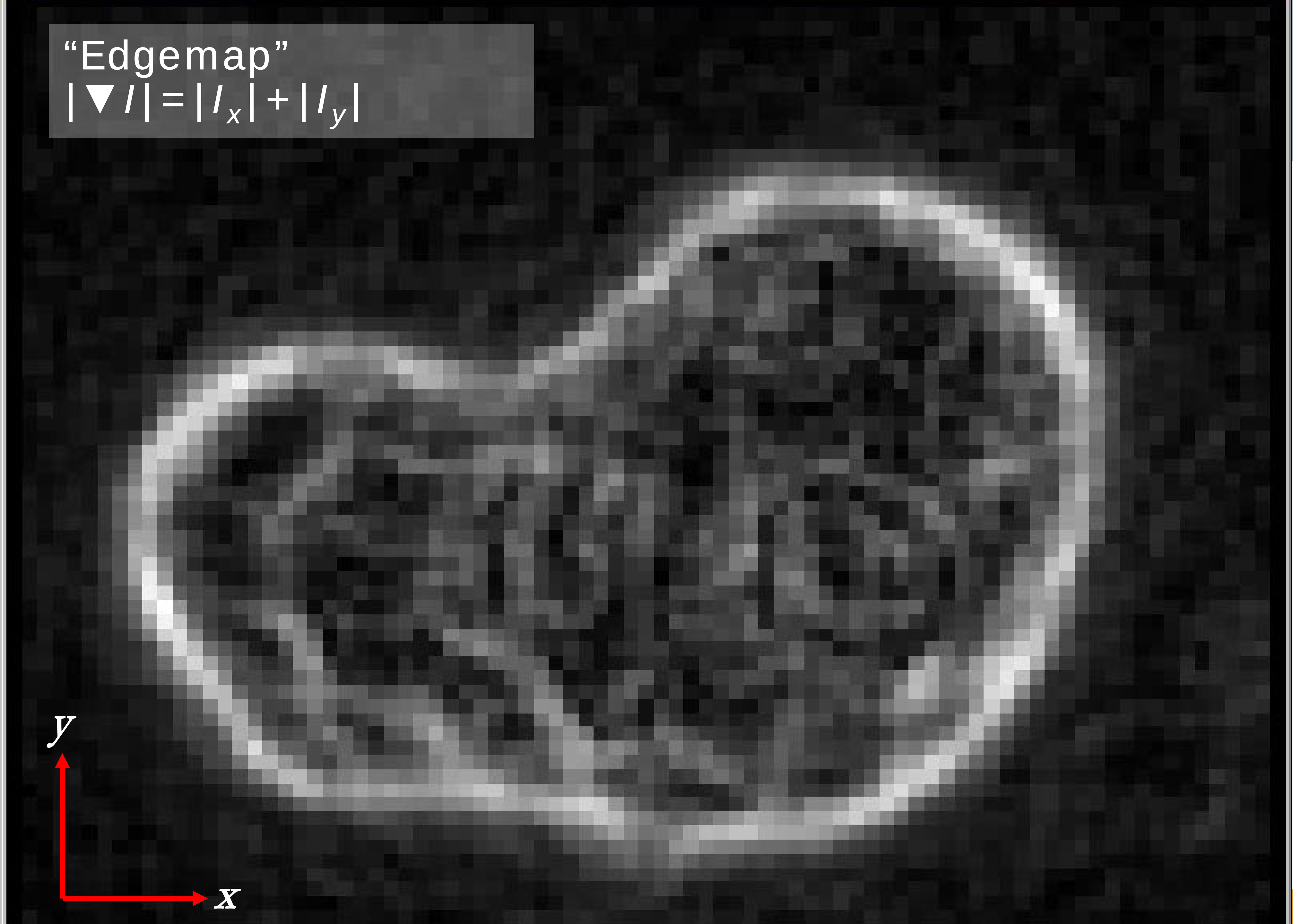






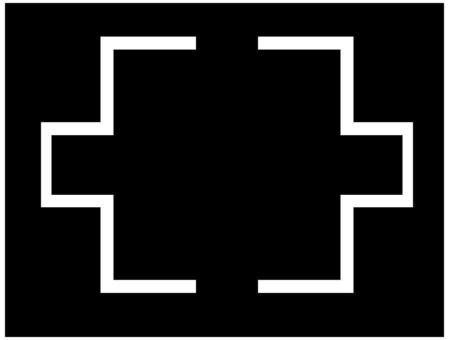
$I = I(x, y)$
y
x


I_y
y
x



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

- Intensity gradients (discrete approximation)

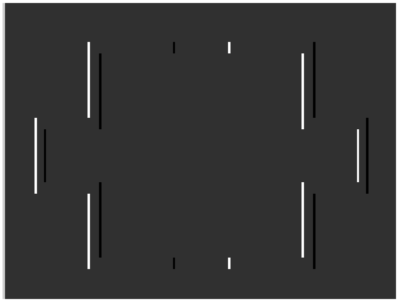


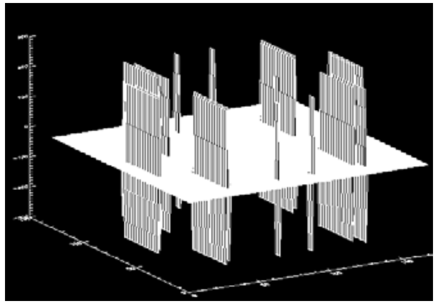
$$I = I(x,y)$$

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

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

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



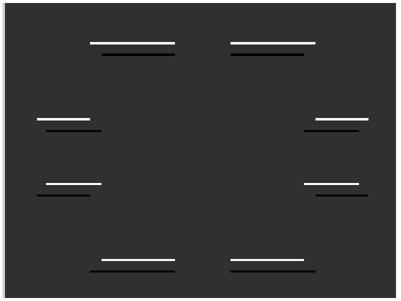


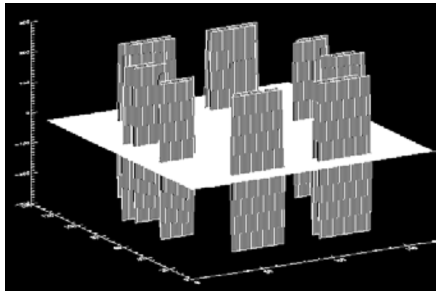
flat | + | -

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

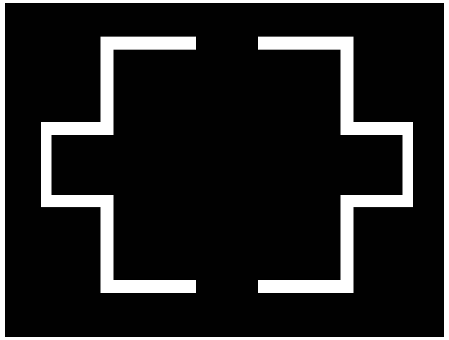
$$\Delta y = 1\,\text{pixel}$$

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





Kernels…



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

Laplace

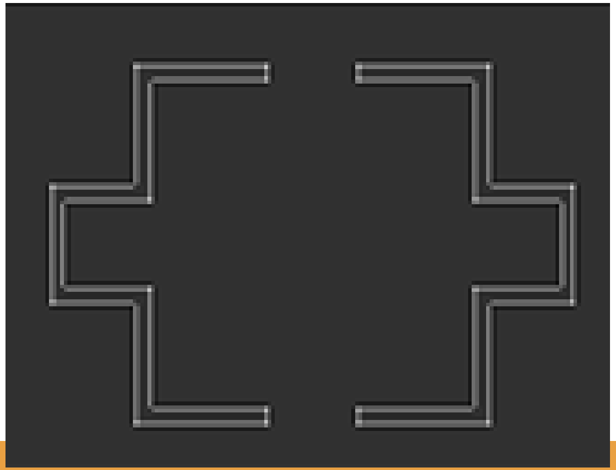
$$\nabla^2 I = \frac{\partial^2 I}{\partial x^2} + \frac{\partial^2 I}{\partial y^2}$$

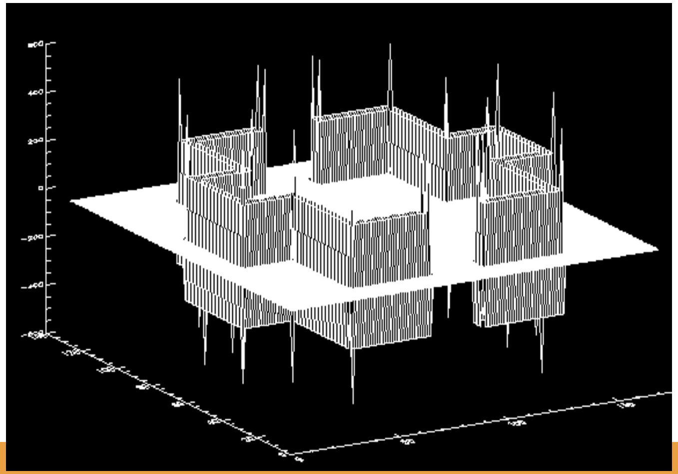
$$\nabla^2 I \approx \frac{f(x+\Delta x, y) - 2f(x,y) + f(x-\Delta x, y)}{(\Delta x)^2} + \frac{f(x, y+\Delta y) - 2f(x,y) + f(x, y-\Delta y)}{(\Delta y)^2}$$

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

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

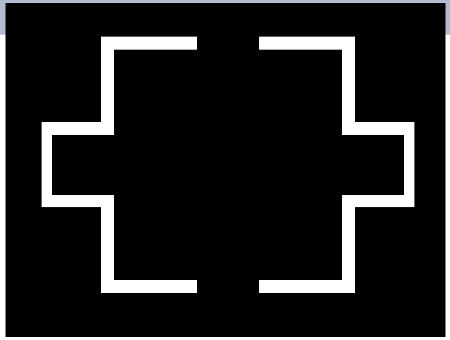
$$K_L = \begin{Bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{Bmatrix}$$





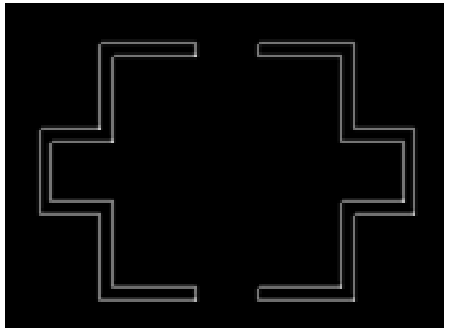
Edgemaps

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



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

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



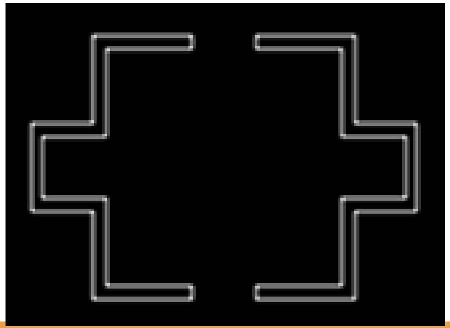
Example:Sobel filter
(notice the thick ROI edges)

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

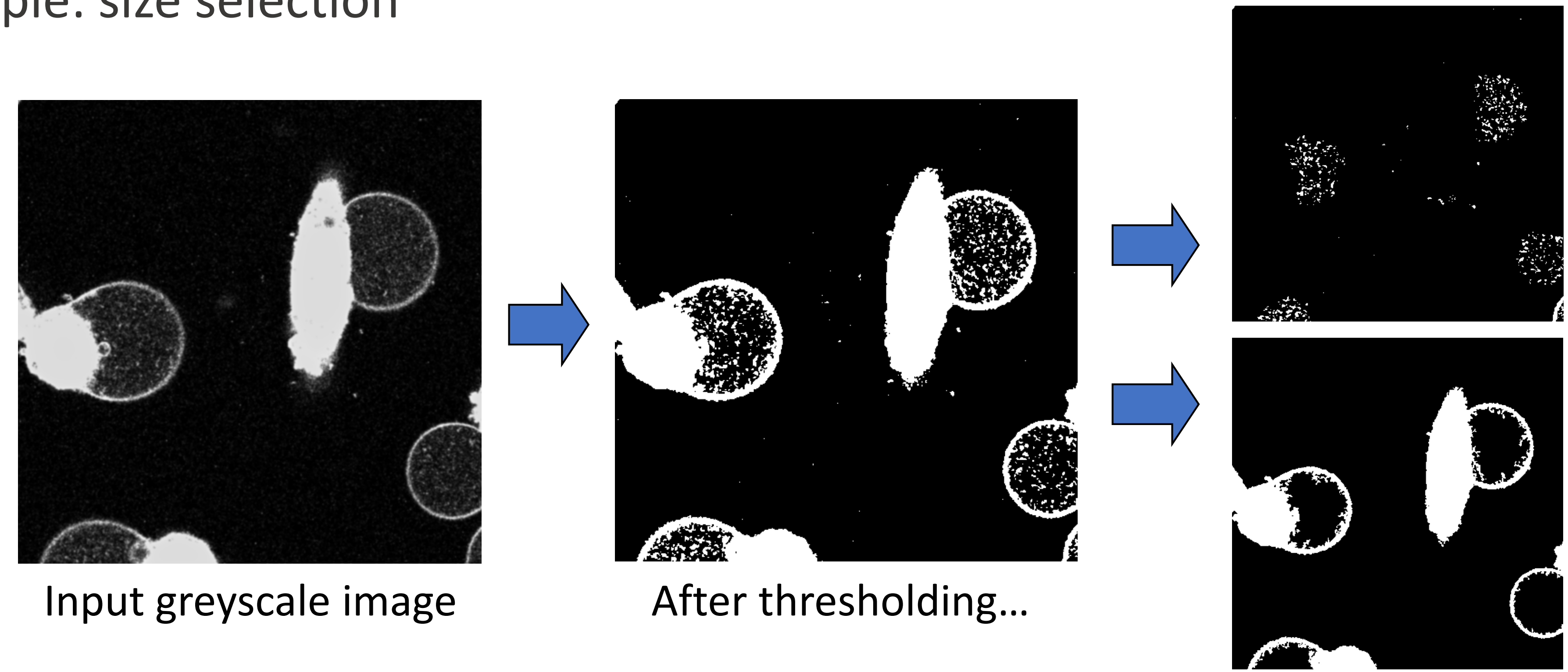
$Sx \otimes I$?

$Sy \otimes I$?

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



- Morphology based filters
  - Example: size selection



Input greyscale image

After thresholding…

Size selection

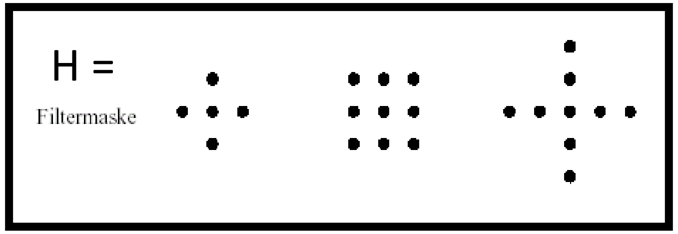
How to define a size-select algorithm?

- Filtering with morphological operators:

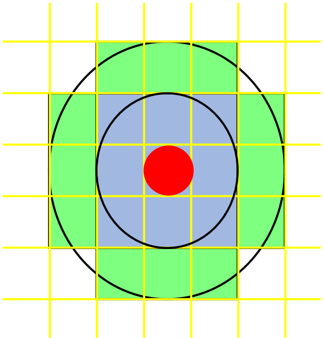
Filtering with morphological operators:

- Structuring element, template or mask H ...

- Additional rules...



H (x_i,y_j,r):



Y ->

X ->

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

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

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

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

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

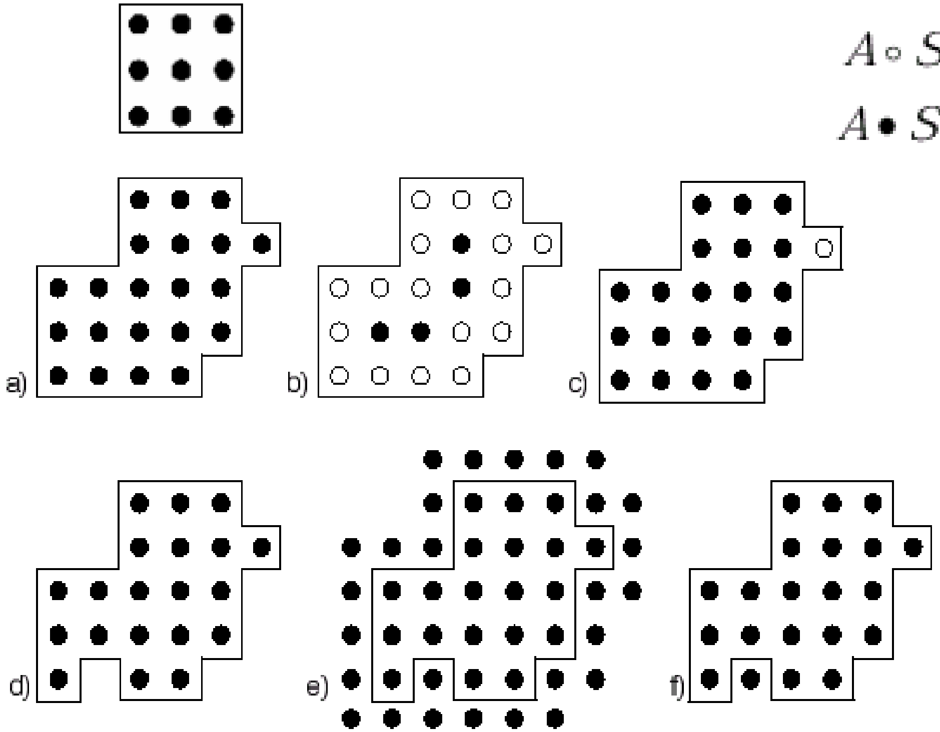
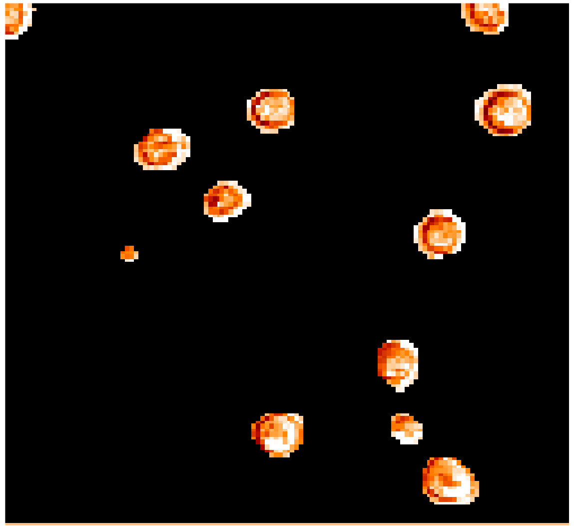


Abb. 2.5. **a** Originalform **b** erodiert **c** opening (Dilatation von b)
d Originalform **e** dilatiert **f** closing (Erosion von e)



- Watershed (Distance transform + Watershed)

Procesamiento de Imágenes y Bioseñales I – Práctico Segmentación

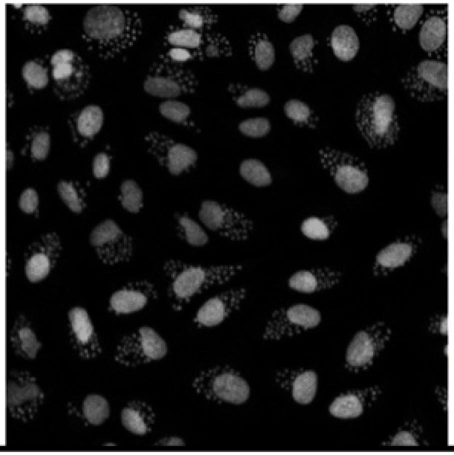


Figura 1. Imágenes de línea de osteoblastos humanos infectados con *Trypanosoma cruzi* (Nature 2010).

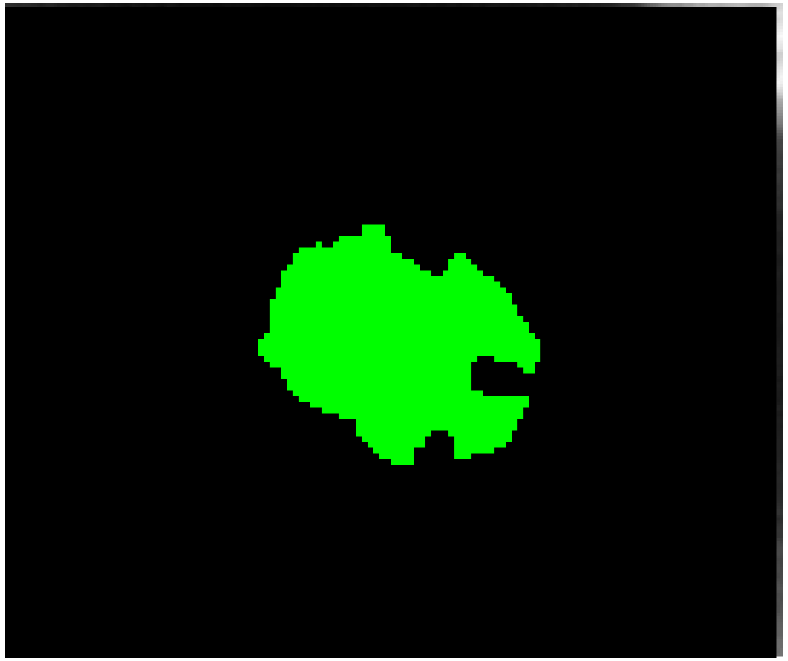
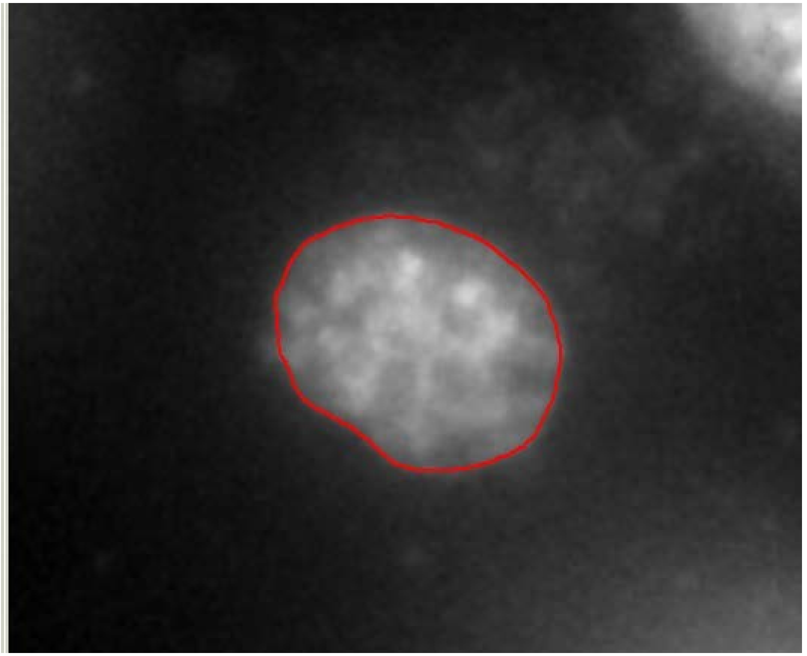
Dato útil: el comando <Ctrl> + <Shift> + D permite duplicar la imagen activa en FIJI/ImageJ. Resulta útil para conservar el resultado del último filtro aplicado.

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

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Some free and open source software tools

- Java based (Java runtime required)
  - ImageJ (http://rsbweb.nih.gov/ij/, public domain)
  - FIJI (http://fiji.sc; GPL license)
  - 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)

Literature, Links & Software

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