Representación, filtrado y segmentación de imágenes I/II

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https://bni.cl/biomat.php

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(Before) We Start...

If TV Science was more like REAL Science (excerpt)
http://phdcomics.com/comics.php?f=1156
https://twitter.com/phdcomics/status/387502010955608065

With more pixels, however...

“

The immediate, obvious damage is wasted NIH funding and wasted thinking in the field because people are using these results as a starting point for their own experiments.

THOMAS SÜDHOF | STANFORD UNIVERSITY

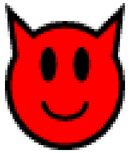
https://www.science.org/content/article/potential-fabrication-research-images-threatens-key-theory-alzheimers-disease
https://www.science.org/doi/epdf/10.1126/science.add9993
Science, Vol 377, Issue 6604, pp. 258-363, 2022

Experience

Training

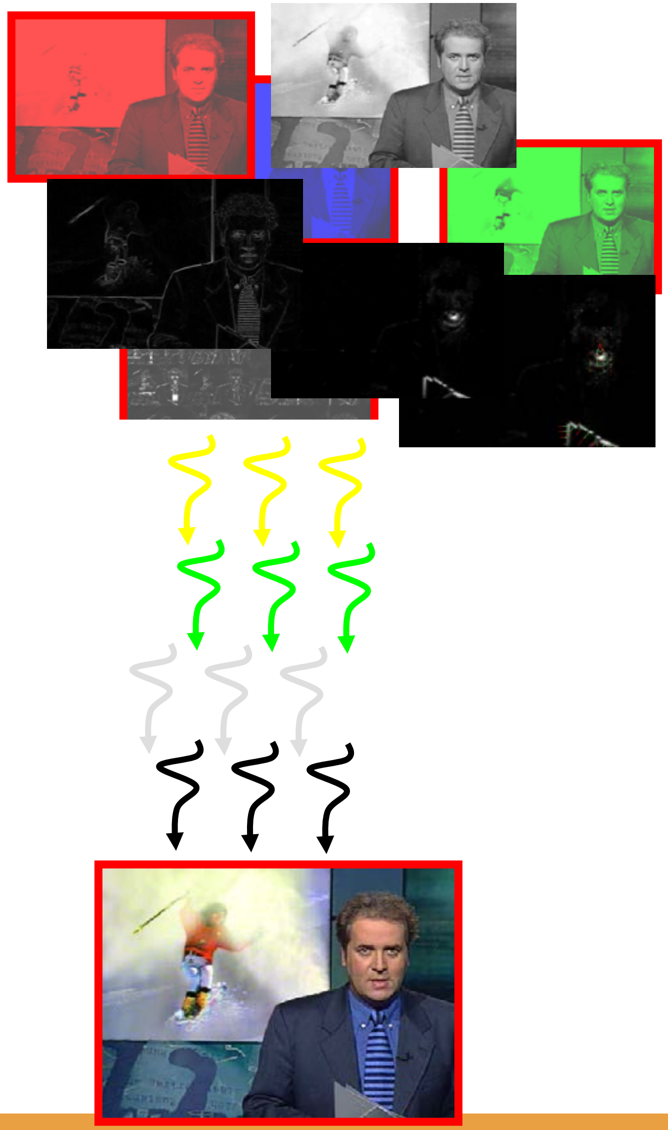
Expectation

Anticipation



Cognitive complement, ...

Symbolic representation, model



- Common criteria

Color similarity (regions)

Color transitions or gradients (boundaries)

0. Concepts recap

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

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

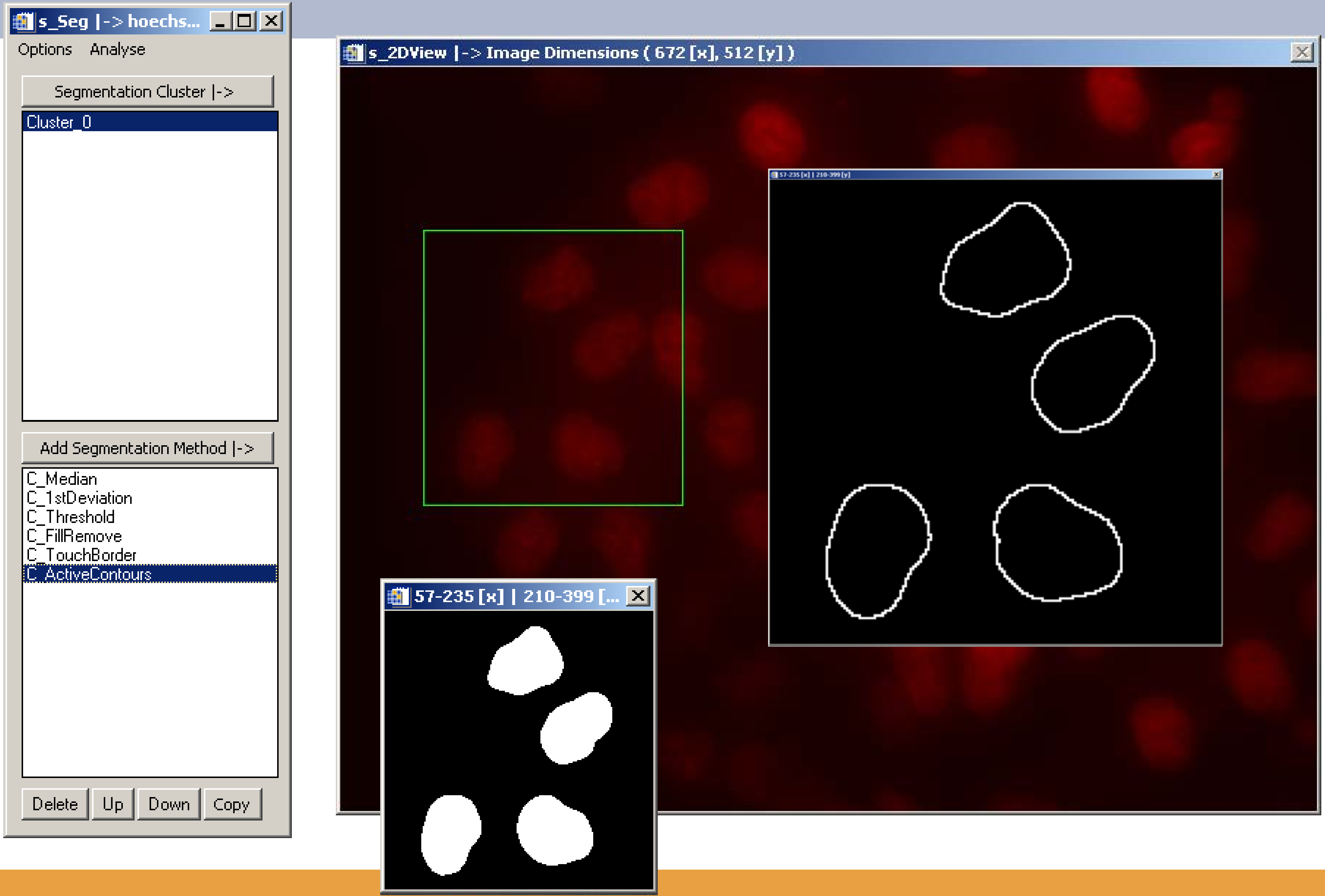
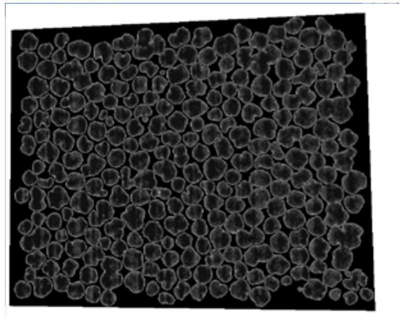
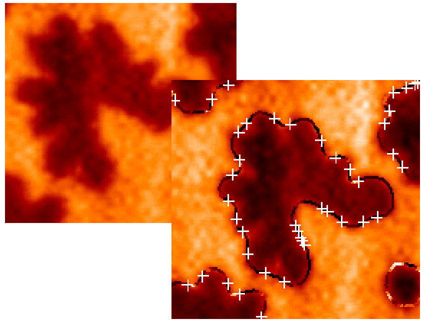
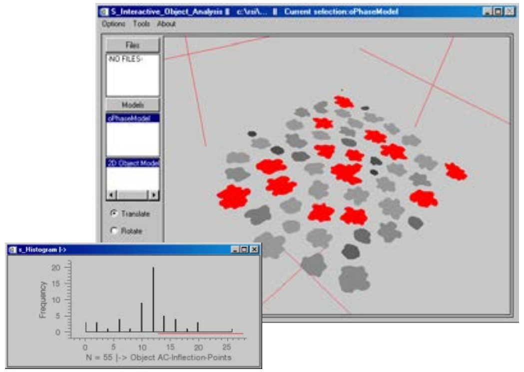

s_Seg |-> hoechs...
Options
Analyse
Segmentation Cluster |->
Cluster_0
Add Segmentation Method |->
C_Median
C_1stDeviation
C_Threshold
C_FillRemove
C_TouchBorder
C_ActiveContours
Delete
Up
Down
Copy
s_2DView |-> Image Dimensions (672 [x], 512 [y])
57-235 [x] | 210-399 [...

Image Processing







Acquisition
- Microscopes
- Cameras
- …

(pre)Treatment
- Restoring (e.g. PSF deconvolution)
- Contrast enhancement
- Artifact removal

Analysis
- ROIs / models
- Measurements

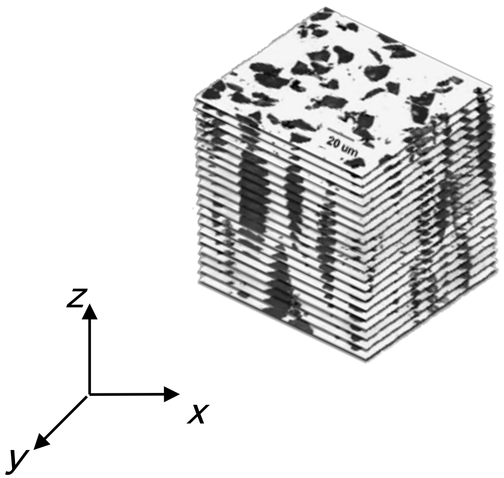
Understanding
- Higher level task
- Hypotheses support

images

images

images + descriptions

- Digital image processing means…

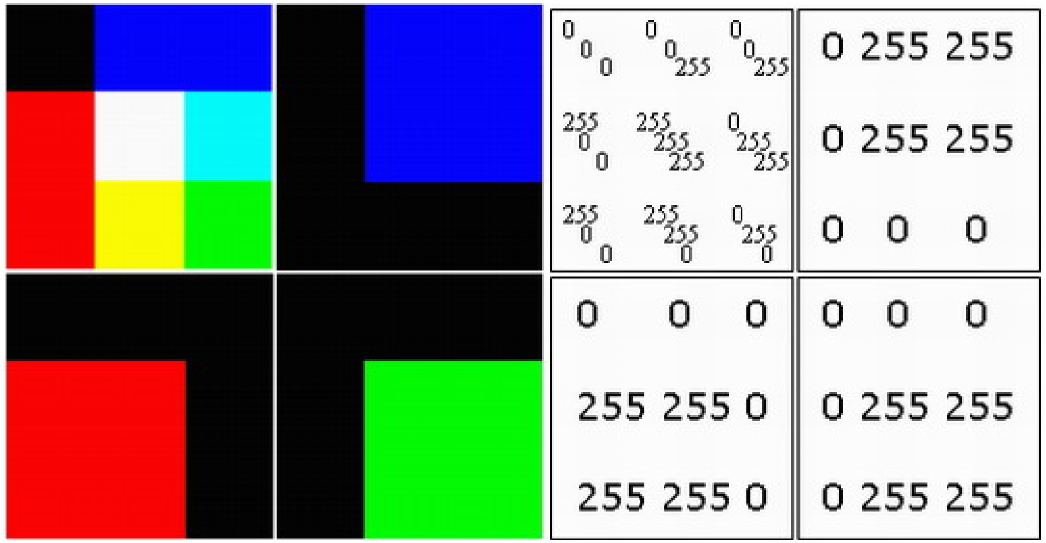


  - Digital… discrete, finite
    - A discrete set is composed by elements which are “isolated” one from another
      Examples:
      - Natural numbers {1, 2, 3, …} (infinite set)
      - Natural numbers from 1 to 10 (finite set)
    - If not discrete? Continuum
      Example: real numbers in the [0,1] interval (infinite set)

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

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

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

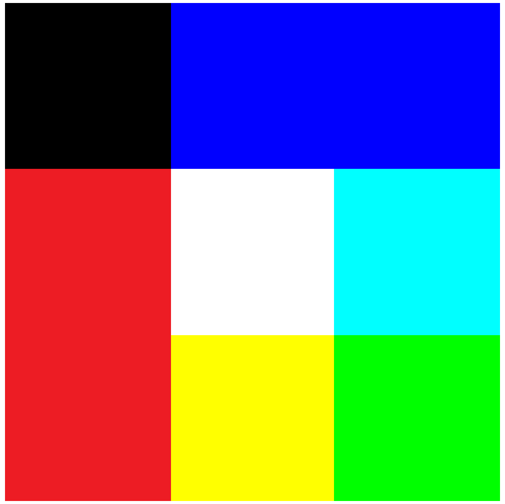


$$I = f(x, y)$$

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

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

- RGB image
  - Three channels for respective primary colors: Red, Green, Blue. 8 bits determine [0,255] range



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

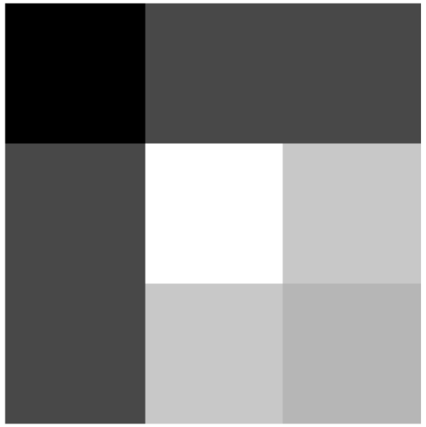
$$r[x,y] \; g[x,y] \; b[x,y]$$

  - Analogous case: CMYK (Cyan, Magenta, Yellow, Black)

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

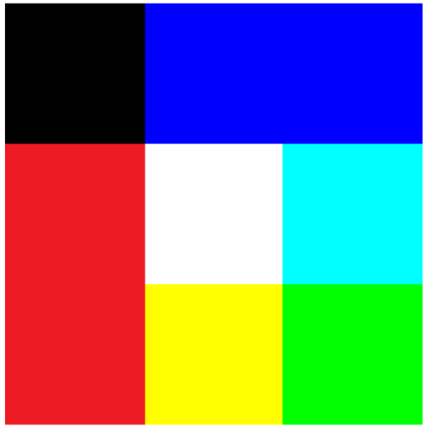
0	85	85
85	255	170
85	170	85

$I[x, y]$



How to go from an RGB to a greyscale image?

- From RGB to greyscale

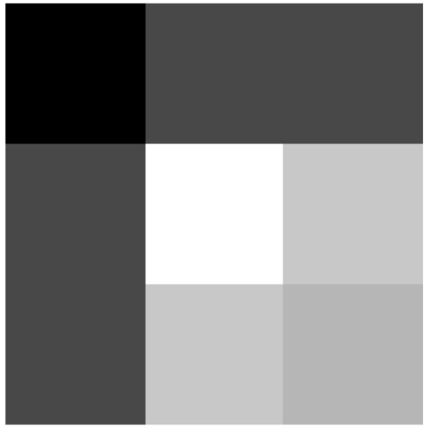


0	0	0
0	0	0
0	255	255
255	255	0
0	255	255
0	255	255
255	255	0
0	255	255
0	0	0

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

0	85	85
85	255	170
85	170	85

$I[x,y]$



How good is the human eye resolving colors in R, G or B tones?

- Other than raster images…
  - A vector image is defined by using a set of base elements (like shapes or curves), instead of explicitly give the color/intensity for each pixel
  - Example: SVG images; base functions (wavelet, splines, Fourier)

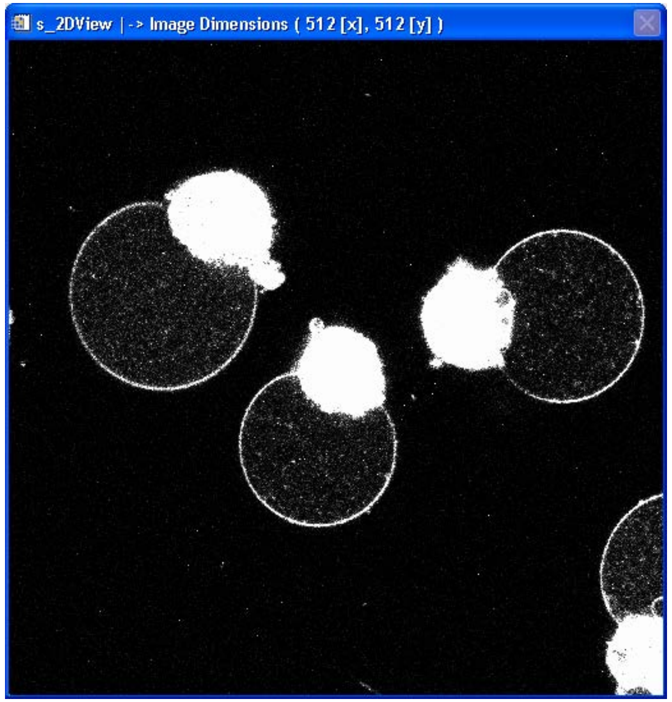
- In order to show a vector image in a common digital screen (pixel matrix) a **rasterization** algorithm is applied

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

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

Aubert & Kornprobst (2006)
Mathematical Problems In Image Processing

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



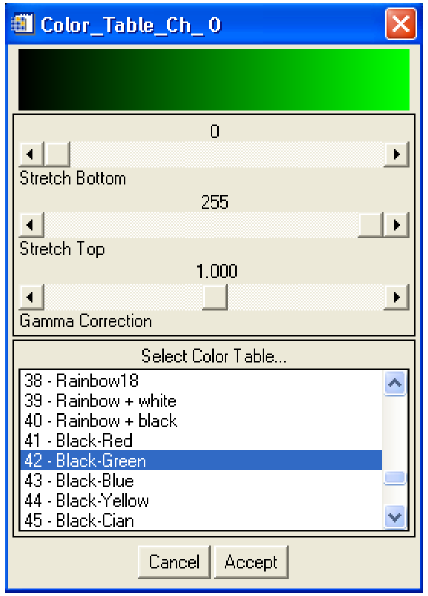
8 bit greyscale image

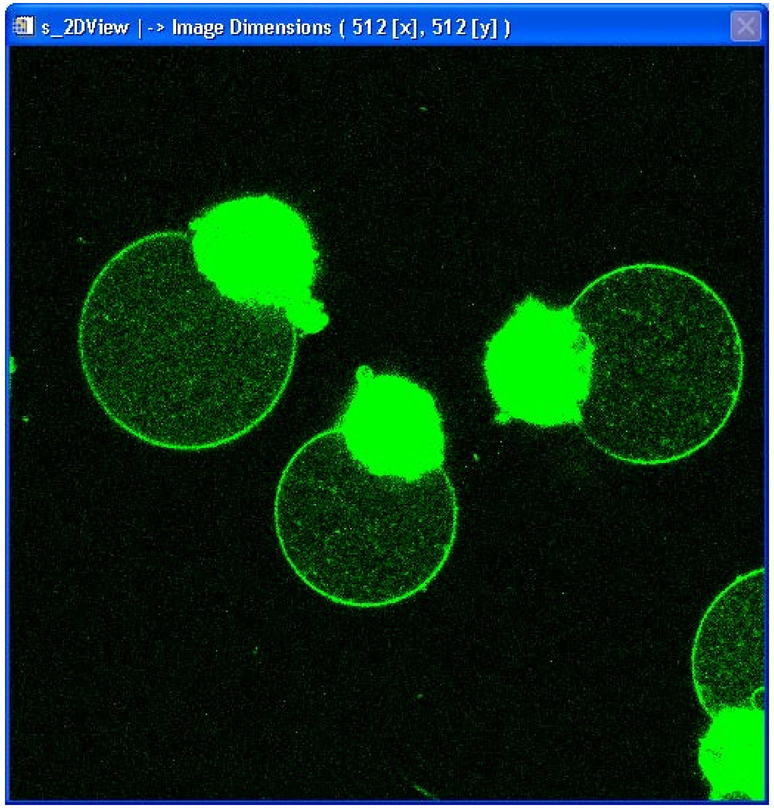
$I(290,267) = 220$

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

- It is possible to define color *lookup tables* (LUTs) for visualization purposes. For instance, a greyscale image can be displayed using a black-to-green LUT...

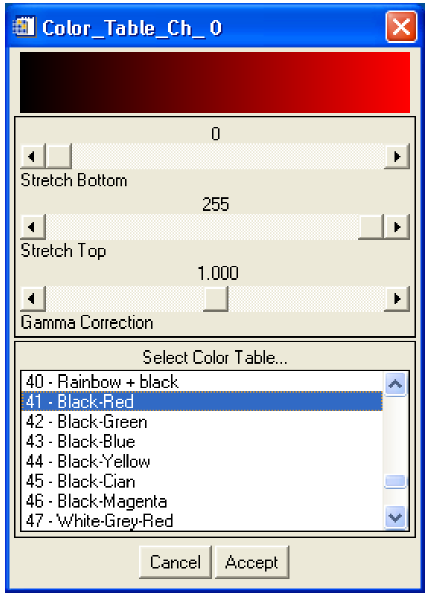
r	g	b
0	0	0
0	1	0
0	2	0
:	:	:
:	:	:
:	:	:
:	:	:
0	200	0
:	:	:
:	:	:
0	255	0





- Now with a black-to-red LUT...

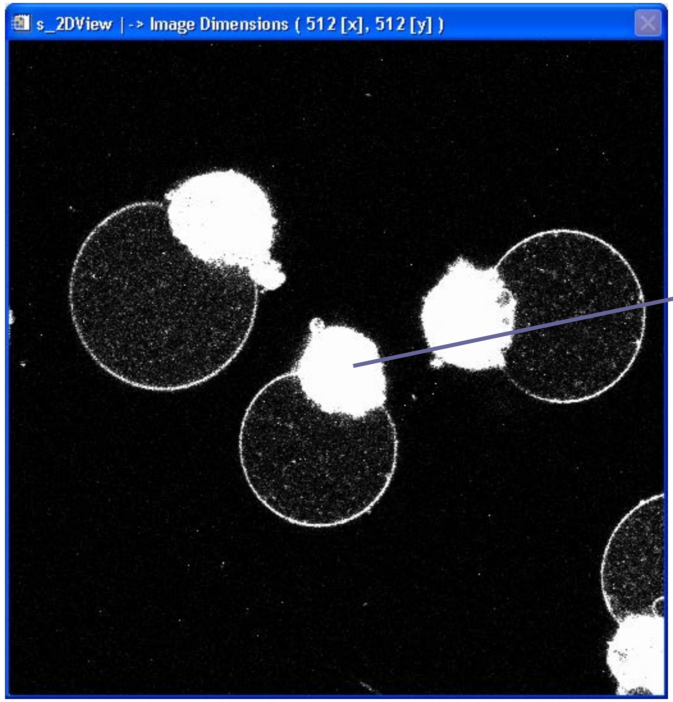
r	g	b
0	0	0
1	0	0
2	0	0
:	:	:
:	:	:
:	:	:
:	:	:
220	0	0
:	:	:
:	:	:
255	0	0



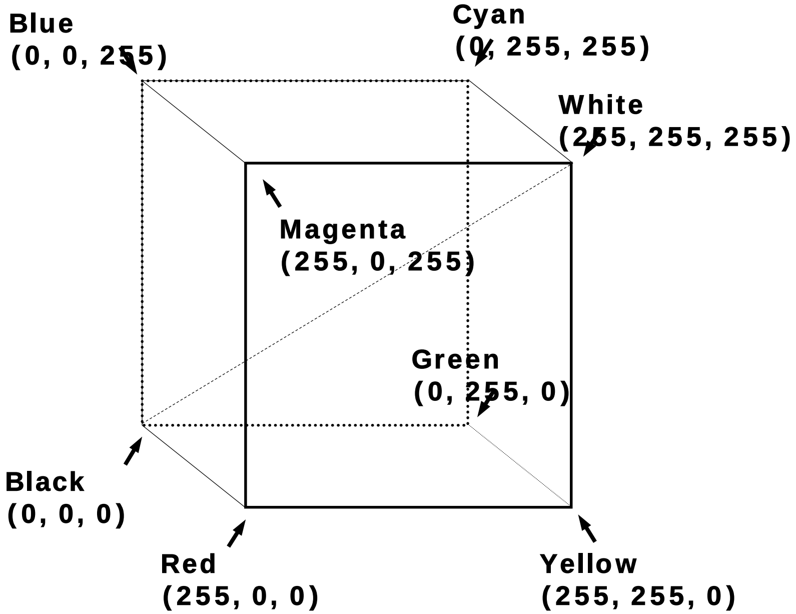
In ImageJ/FIJI: Go to the "Image"->"Lookup Tables" menu

- ...or any custom color table

r	g	b
0	0	0
:	:	:
220	220	220
:	:	:
255	255	255



$I(290,267) = 220$



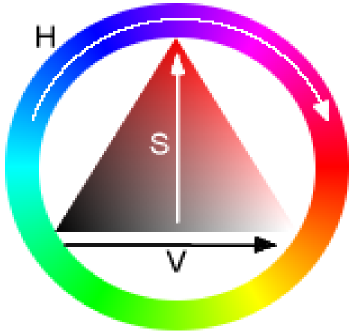
$$Red = [r_0, r_1, \ldots, r_{255}]$$
$$Green = [g_0, g_1, \ldots, g_{255}]$$
$$Blue = [b_0, b_1, \ldots, b_{255}]$$

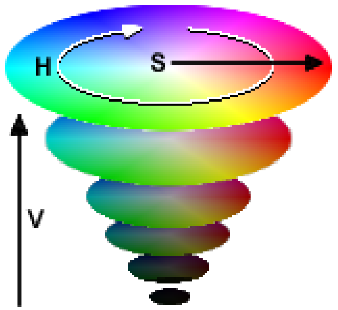
HSV (hue, saturation, value) model

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

- **Hue**
  color „type“, range 0-360° (0° red, 120° green, 240° blue)
- **Saturation**
  color „intensity“, range 0-100%.
- **Value**
  brightness, range 0-100%.

HSV is a **non linear** transformation from the RGB color space.



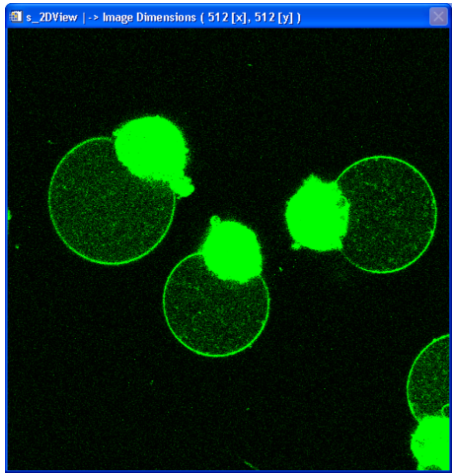


$$H = \begin{cases} \Theta & G \geq B \\ 2\pi - \Theta & G \leq B \end{cases}$$

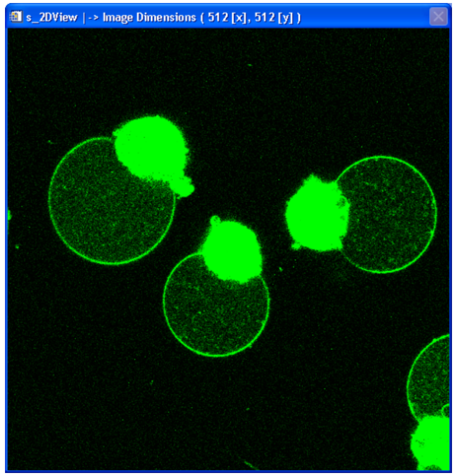
$$S = 1 - 3\frac{\min(R,G,B)}{R+G+B}$$

$$I = \frac{R+G+B}{3}$$

$$\Theta = \arccos\left[\frac{1}{2}\frac{(R-G)(R-B)}{\sqrt{(R-G)^2+(R-B)(G-B)}}\right]$$



Occlusions may occur in 3D visualization

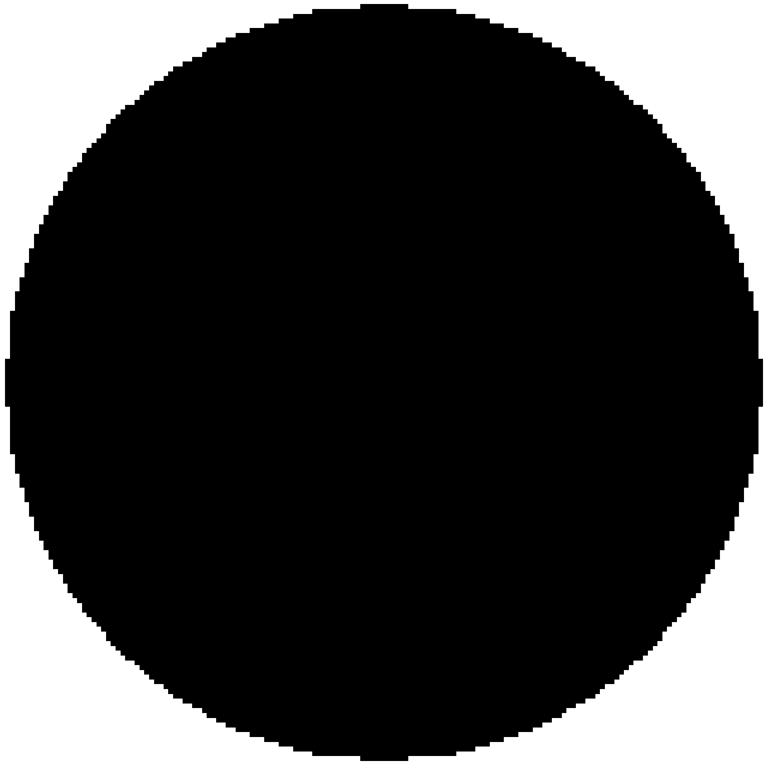


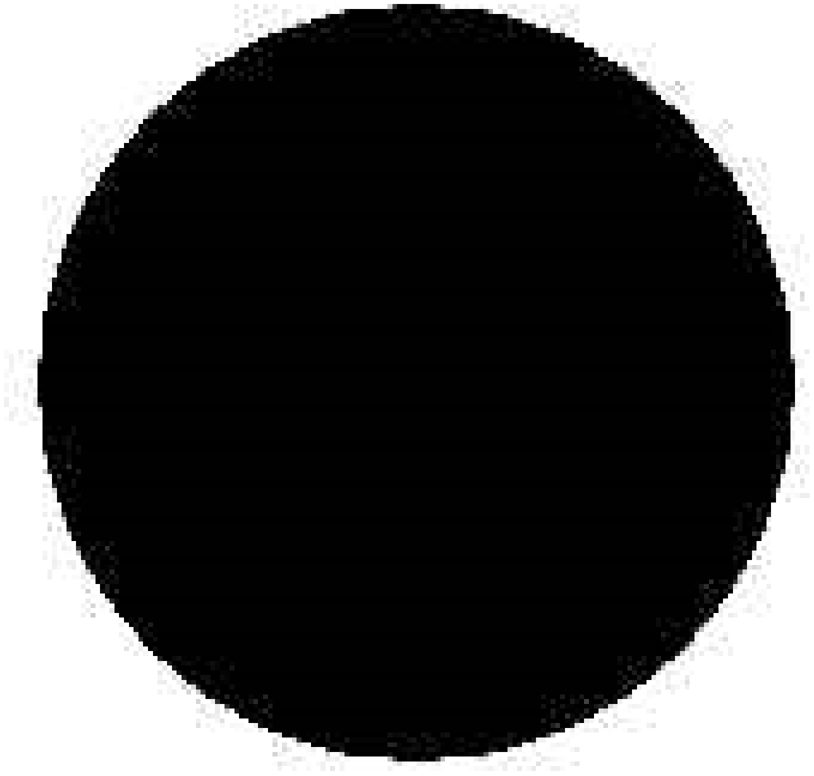
[R, G, B, α]

pacity values can
e associated to
ixels (or voxels in
D) for visualization
purposes

- Function representation
  - Raster, SVG, base functions
- Color mode
  - grayscale
  - color (RGB, CMYK, HSV, Lab, etc.)
- Color depth (bit depth)
  - How many bits for how many values (e.g. 8 bits, 32 bits)
  - Number format
    - Integer (typically unsigned, e.g. TIFF)
    - Decimal (can be signed, e.g. ICS)
- Also important: storage mode
  - "Raw": each pixel value is stored (lots of space)
  - Compressed, with or without information loss (e.g. JPEG *lossy* format, TIFF can be compressed or uncompressed)

- Lossless vs. Lossy

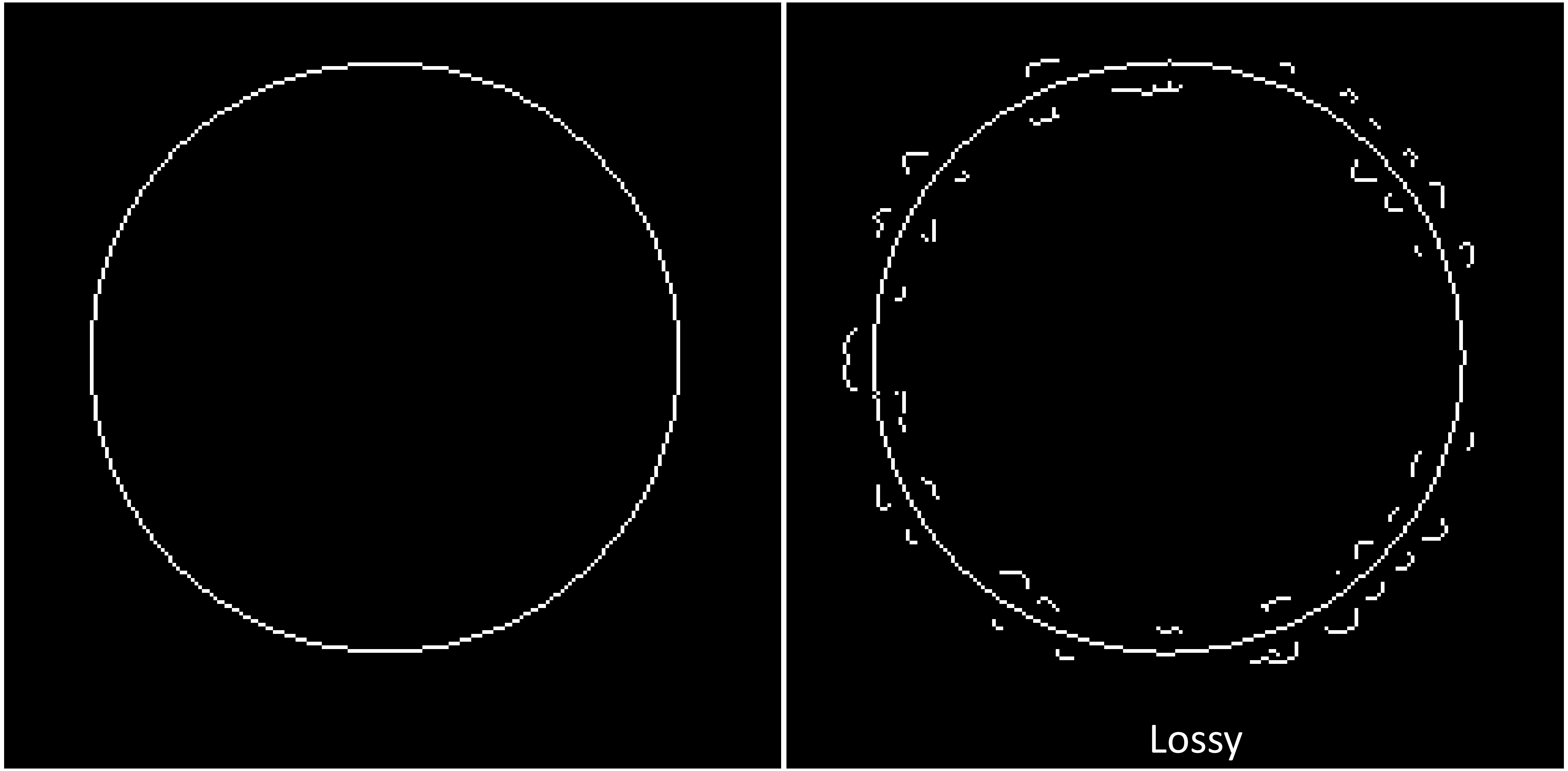




Lossy

- Lossless vs. Lossy

Canny edge dectetor



Standard formats for 2D images

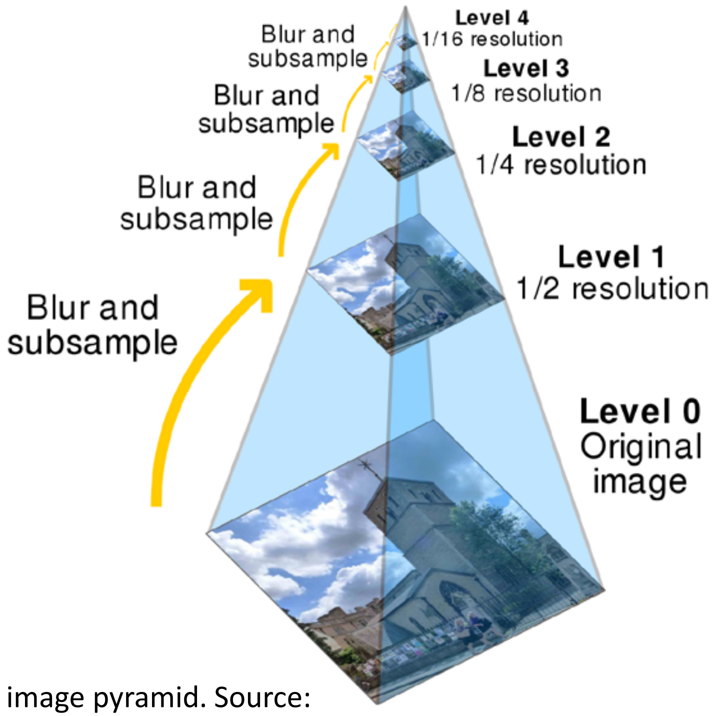
- PNG
- TIFF (2D/3D multi-channel, LUT support, some variants allow for compression)

Developing formats for large n-dimensional array containers

- N5
- Zarr

https://github.com/saalfeldlab/n5
https://imagej.net/N5
https://www.youtube.com/watch?v=InqZ_NsgB4g

https://zarr.readthedocs.io/en/stable/



A 2D image pyramid. Source: https://en.wikipedia.org/wiki/Pyramid_(image_processing)

- 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

- Some 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

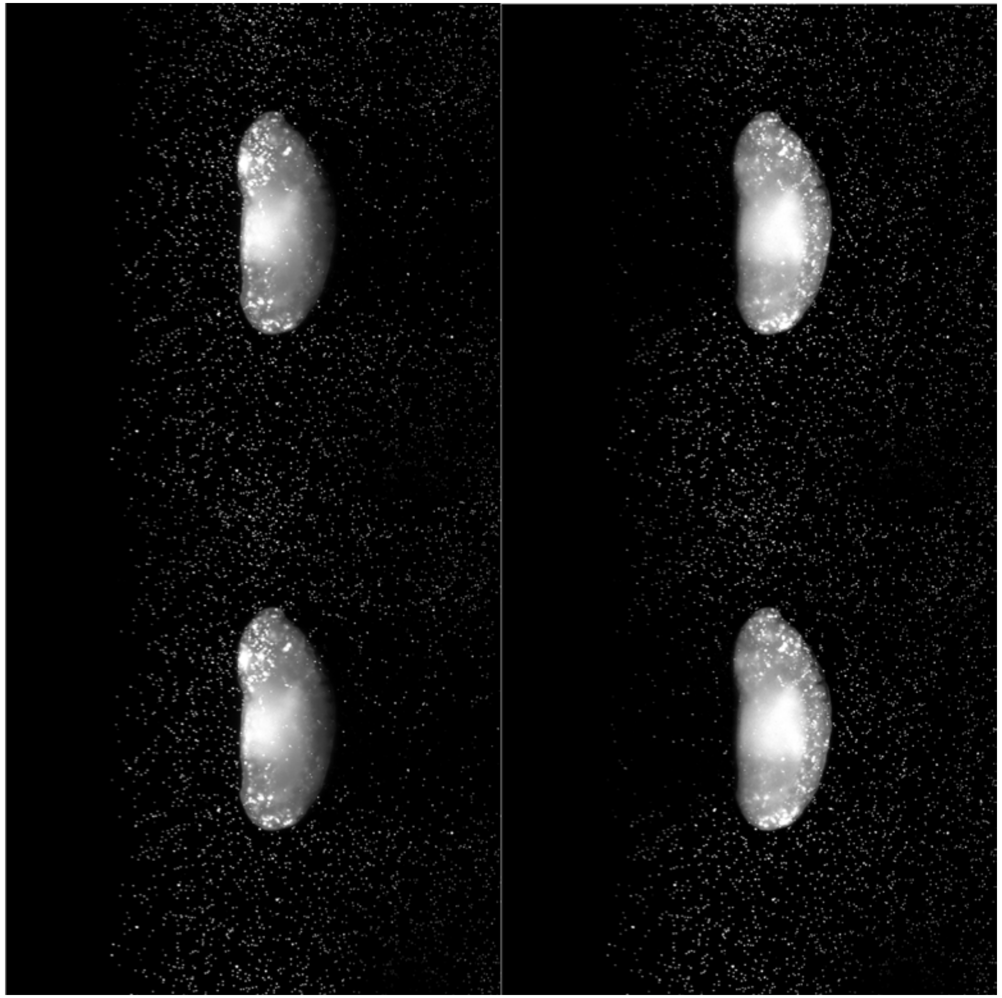
Coming soon: motion estimation & tracking, morphology… characterization

Next year(?): classification

- 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

- Registration

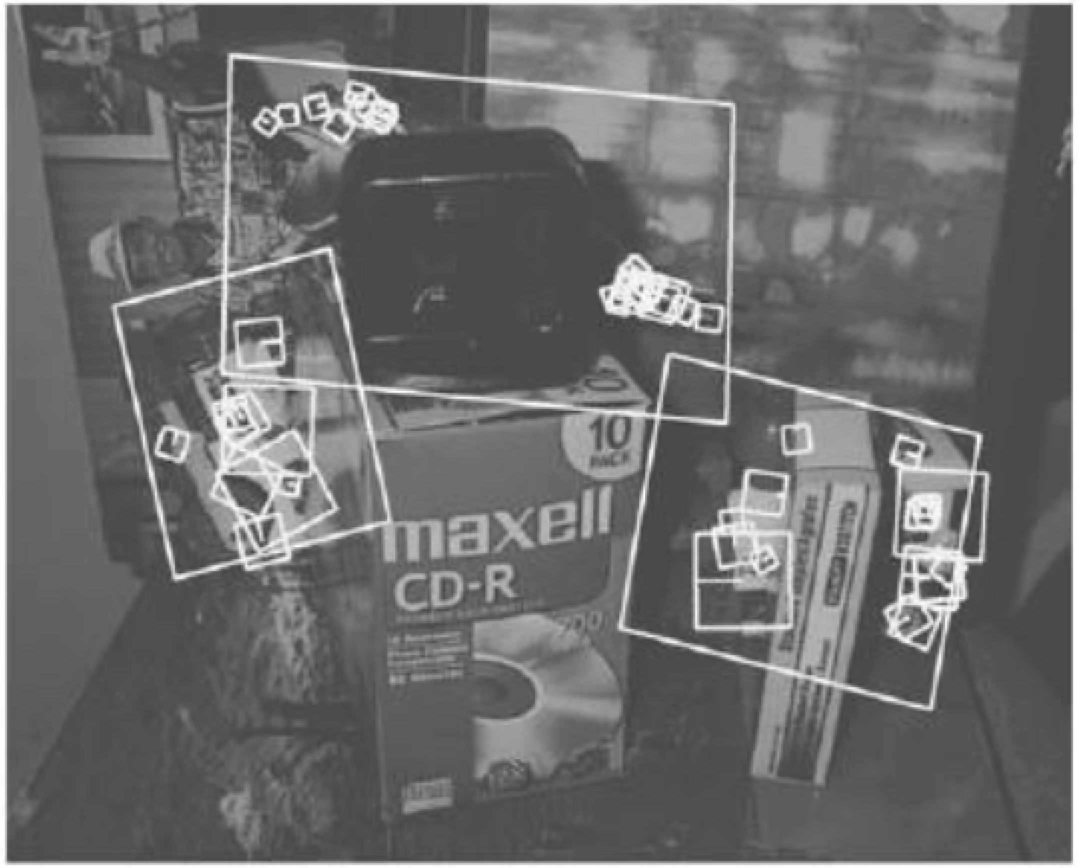


In FIJI: Plugins->Registration
Additional FIJI plugins: StackReg, TurboReg
http://bigwww.epfl.ch/algorithms.html

Applied example: 4-view LSFM image merging

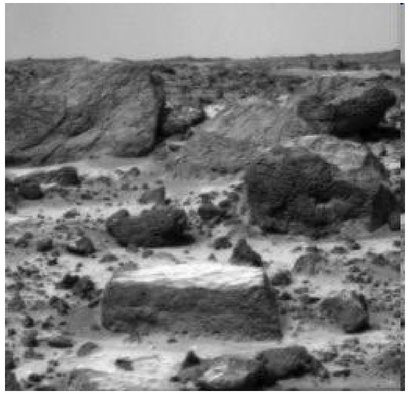
C . Parada & C. Bolatto (UdelaR, Uruguay), L. Alé (LEO/SCIAN & BNI)

- Identification (features, objects, scenes)

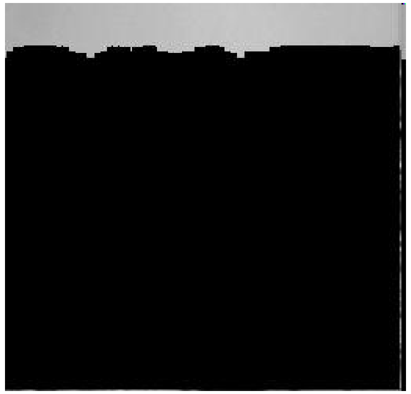


Scale Invariant **Feature** Transformation (SIFT), D Lowe (2004). Image from J Clemons (2009)

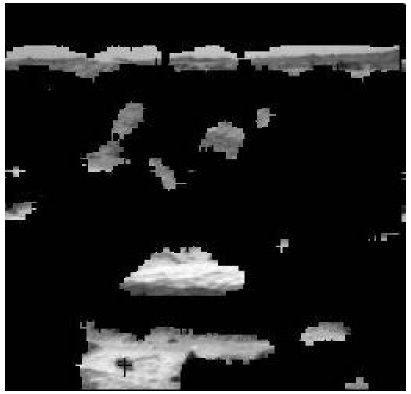
- Segmentation



Sol 3, Mars
Pathfinder Mission

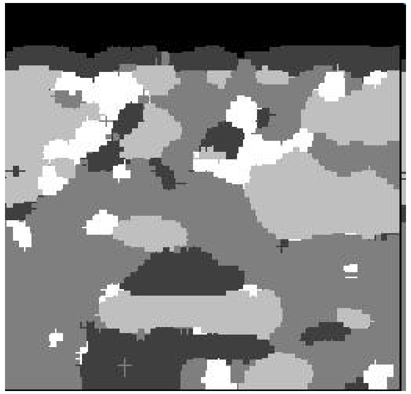


Sky / Flat



Dust / Horizon

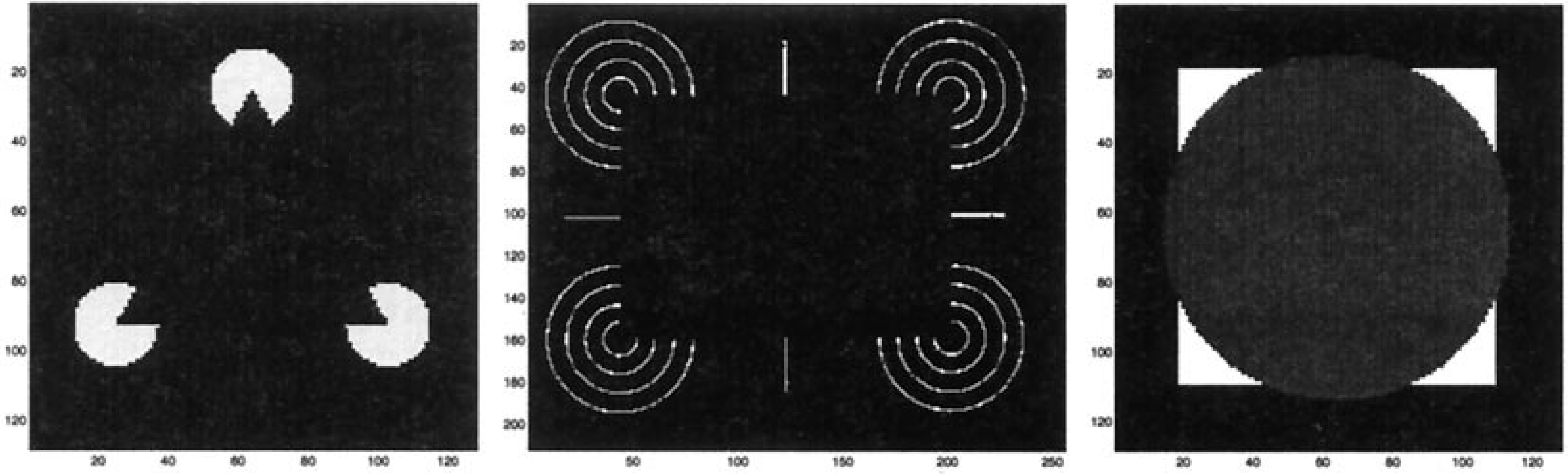
...etc...



Final segmentation

Source: NASA

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

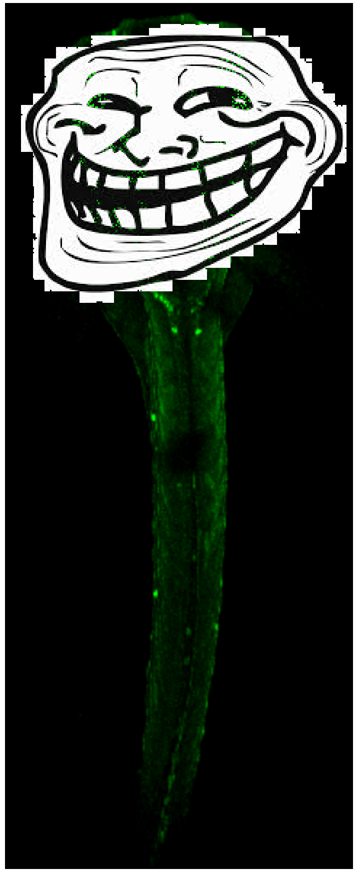


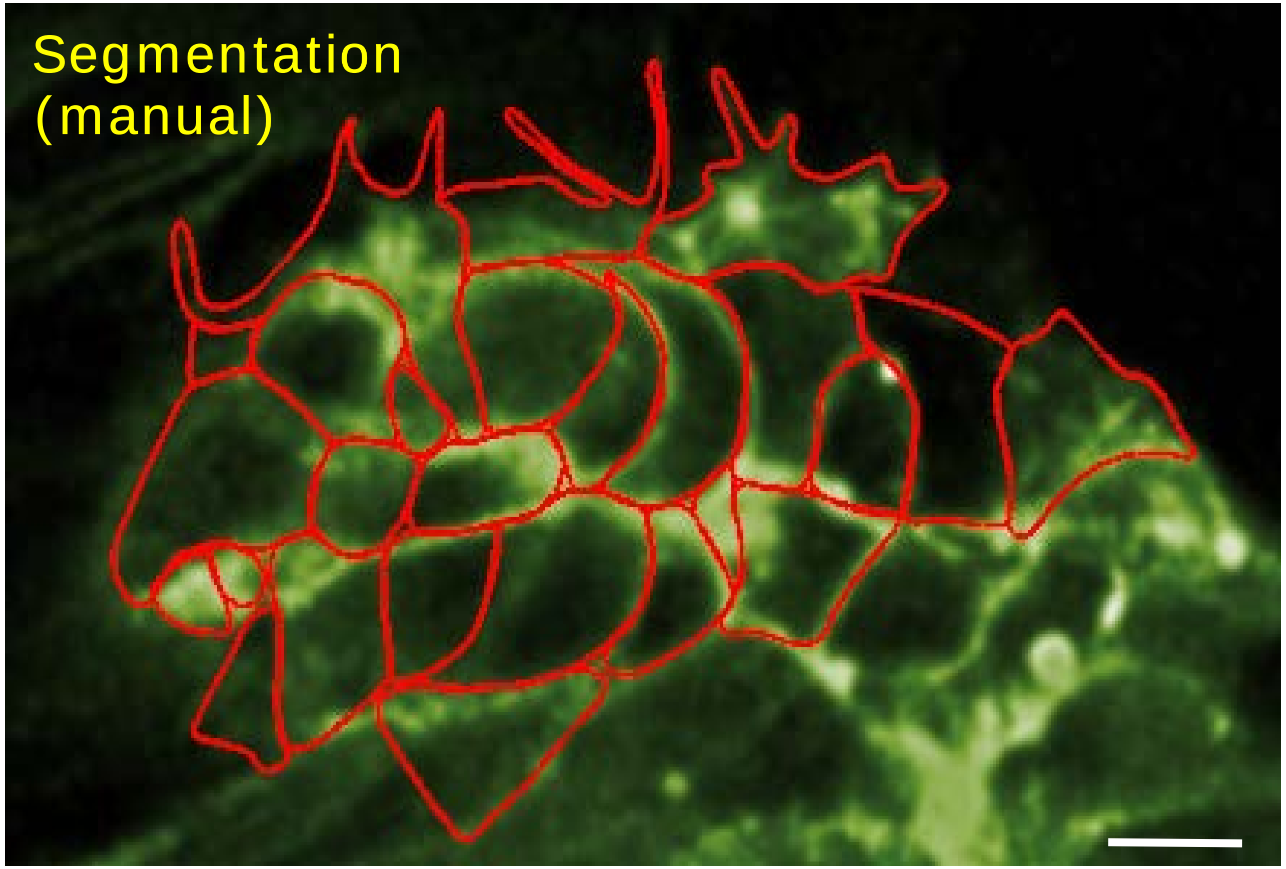
  - …not always (almost never) enough information for a 100% accurate segmentation.

Marr (1982) Vision

Sarti & Sethian (2000) Subjective surfaces. PNAS

Problem?





CG Lemus (LEO, SCIAN-Lab, BNI)

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.