Image Processing I-II

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www.scian.cl / www.cimt.cl

Thanks / Bedankt

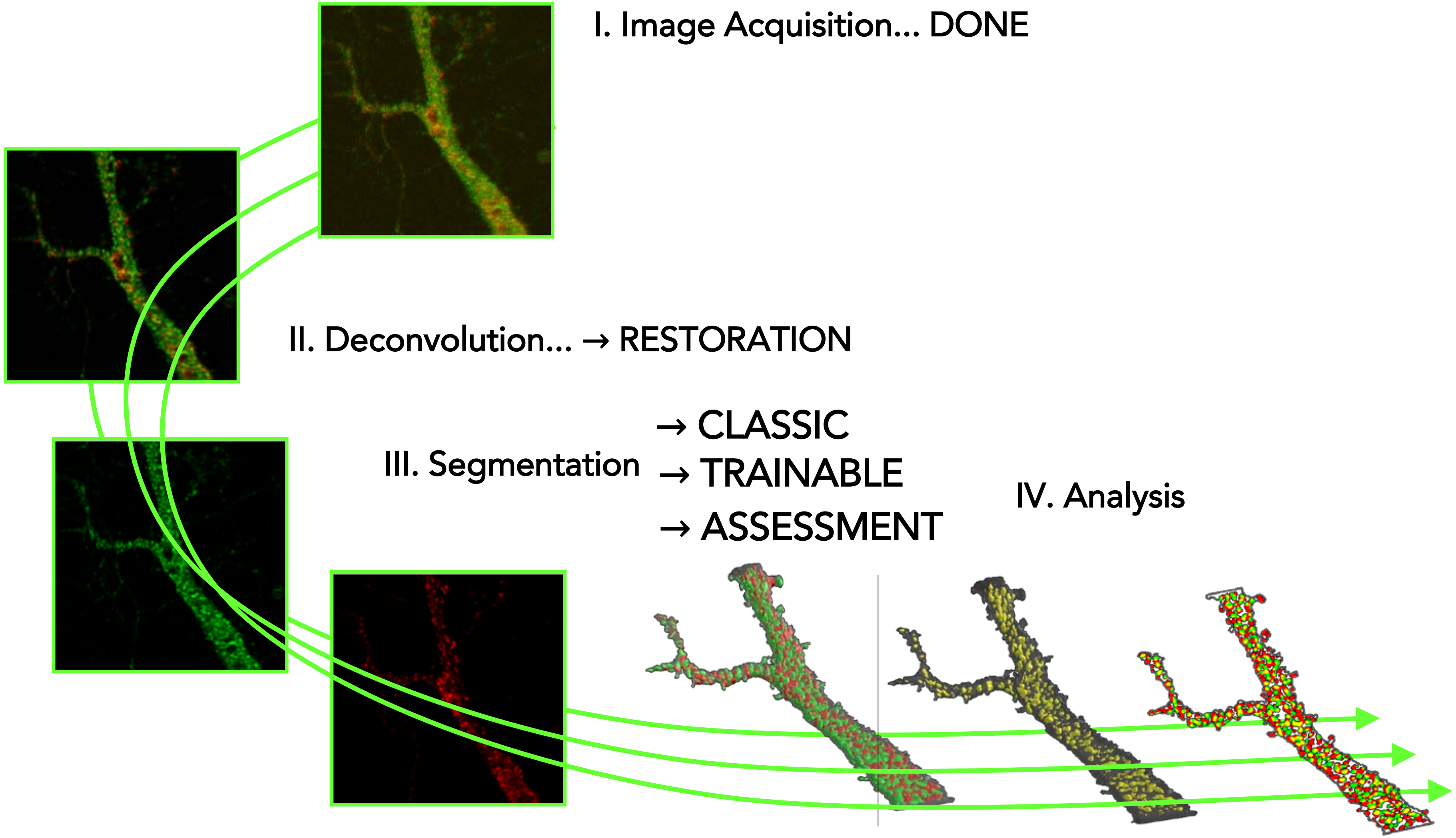
Outlines

I. Image Acquisition… DONE

II. Deconvolution… → RESTORATION

III. Segmentation
→ CLASSIC
→ TRAINABLE
→ ASSESSMENT

IV. Analysis



Outline

I. Principles & Concepts

II. Processing
- Classic Tools & Segmentation

III. Software Tools

Outline

I. Principles & Concepts

II. Processing

III. Software Tools

> Principles

What is…

- …a pixel?
- …a voxel?
- …a bit?
- …a (raw) image?
- …JPEG?

> Principles

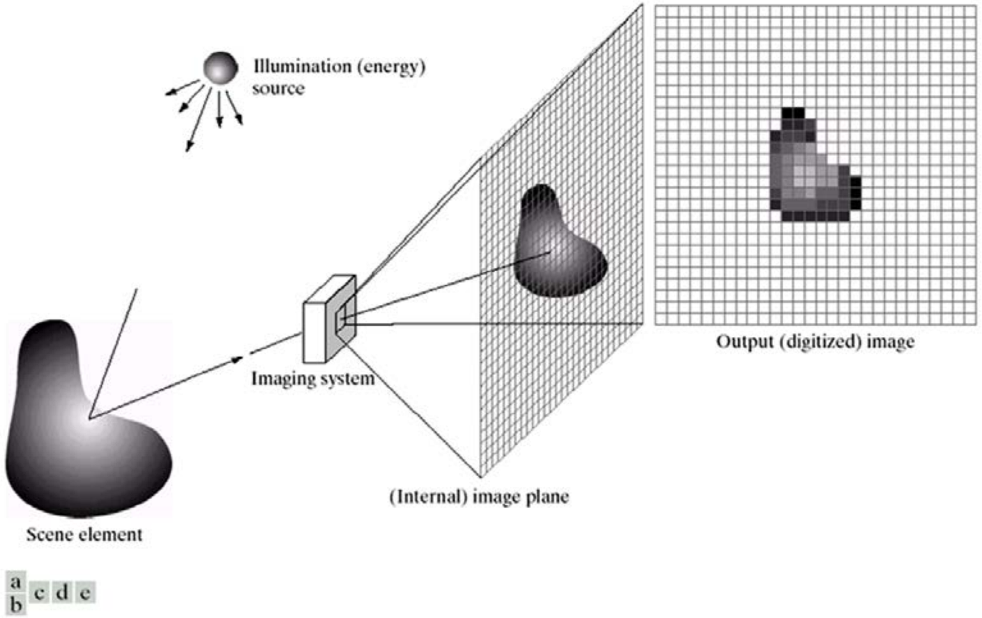


FIGURE 2.15 An example of the digital image acquisition process. (a) Energy ("illumination") source. (b) An element of a scene. (c) Imaging system. (d) Projection of the scene onto the image plane. (e) Digitized image.

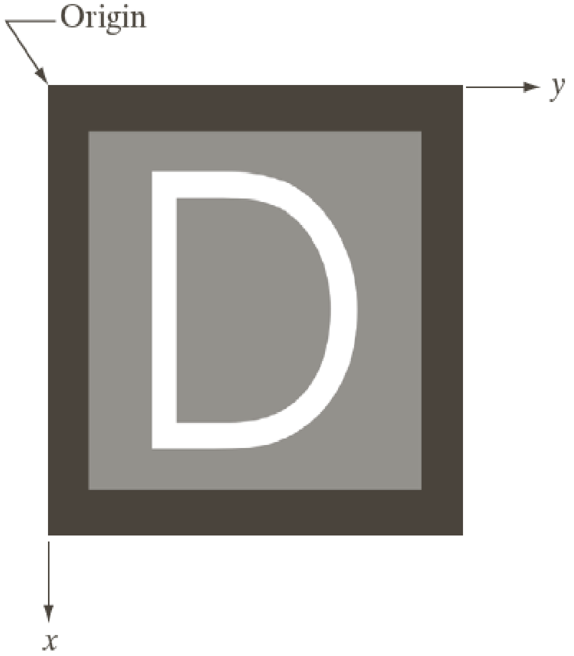
A snapshot of objects by the a camera, telescope, microscope, or any other device (often optical) that registers tone and/or light-shade variations.

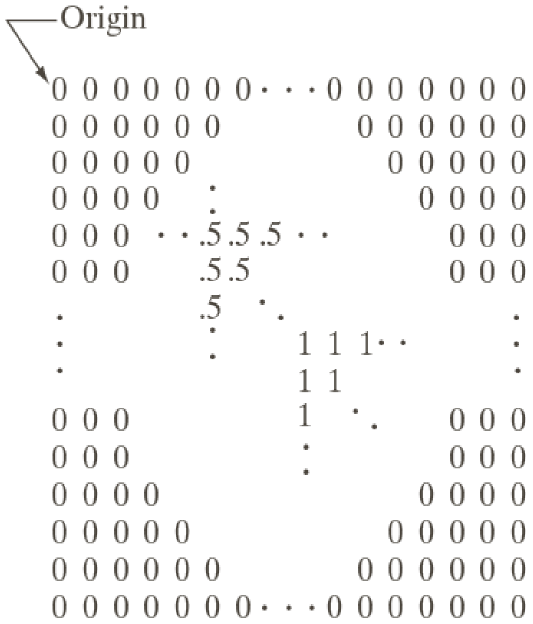
*Analog signal: a representation of a time-or-space varying quantity (*analogous* to it).

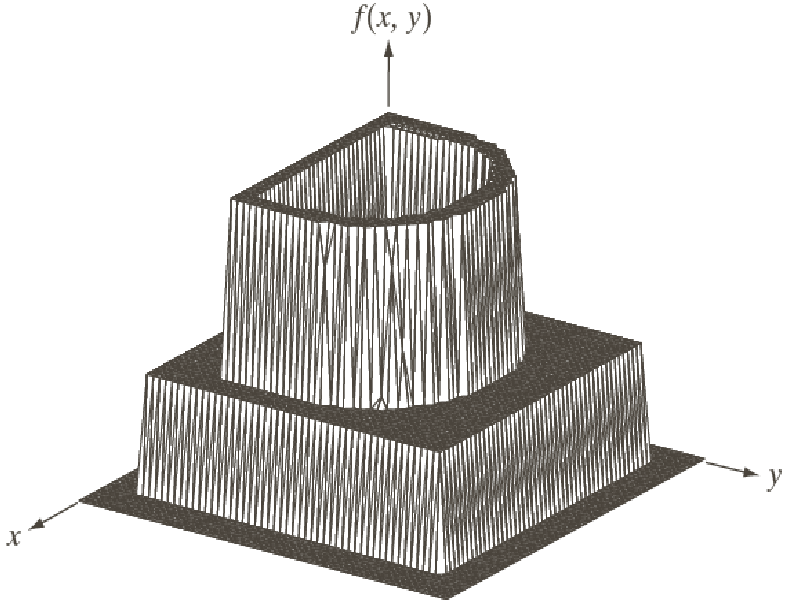
> Principles

We can think of the color or intensity of an image as a function (f) of the x, y, z coordinates.

2D Example: gray level intensity image $I = I(x, y)$

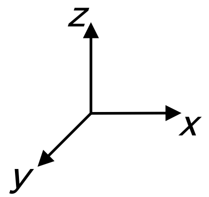


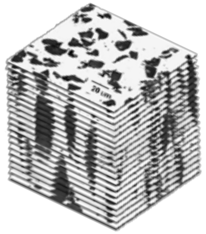


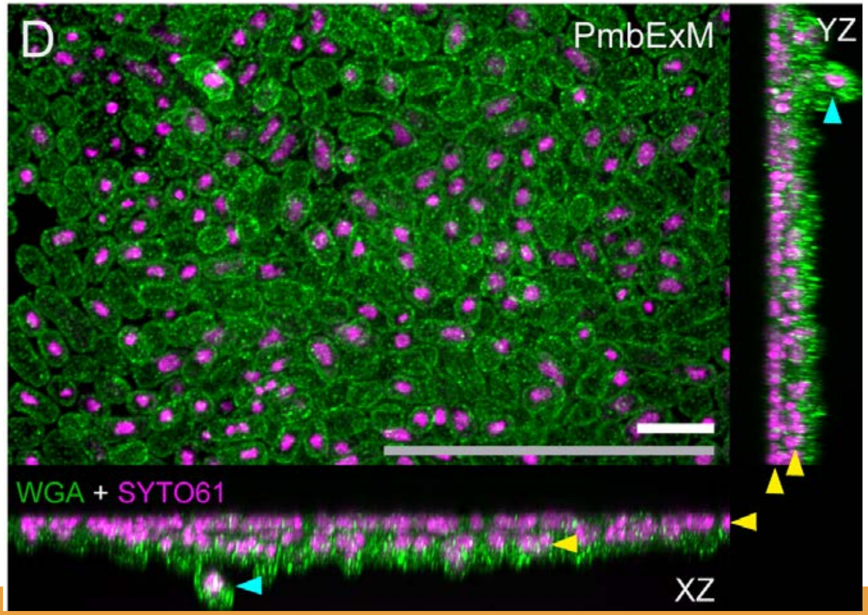


> Concepts

- **Digital image processing** involves…
  - Digital… discrete, finite







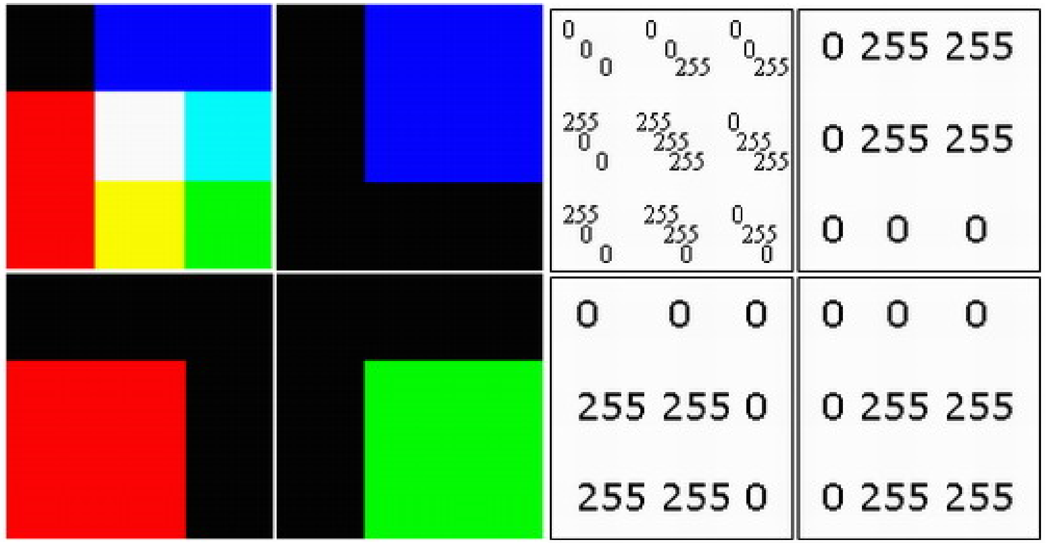
- A discrete set is composed by elements which are "isolated" one from another
  Examples:
  - Natural numbers {1, 2, 3, …} (infinite set)
  - Natural numbers from 1 to 10 (finite set)

- If not discrete? Continuum
  Example: real numbers in the [0,1] interval (infinite set)

Expanded *P. mirabilis*. Castagnini *et al.* (2024) Journal of Microbiological Methods
(In ImageJ/FIJI go to "Image" > "Stacks" > "Orthogonal Views")

> Concepts

- A **digital image** can be defined as a function over a discrete space
  - A typical 2D image model is the **raster image**: array (matrix) of **pixels** in cartesian coordinates (*x, y*)
  - A numeric value for **brightness (intensity)** or **color** is associated to each pixel



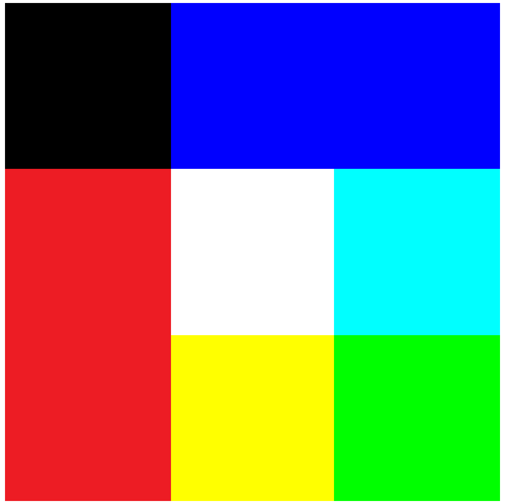
$$I = f(x, y)$$

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

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

> Concepts

- **Color space** (through examples)
  - RGB space: 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

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

  - Other spaces: CMYK (Cyan, Magenta, Yellow, Black), HSB, HSV, Lab, …

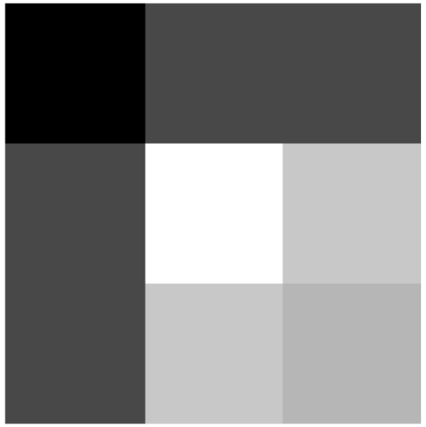
> Concepts

- **Greyscale image**

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

$$\begin{matrix} 0 & 85 & 85 \\ 85 & 255 & 170 \\ 85 & 170 & 85 \end{matrix}$$

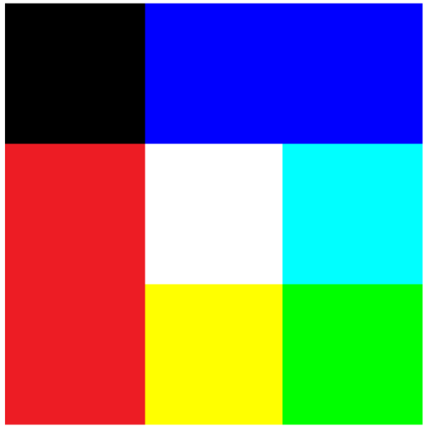
$$I[x, y]$$



How to go from an RGB to a greyscale image?

> Concepts

- From RGB to greyscale

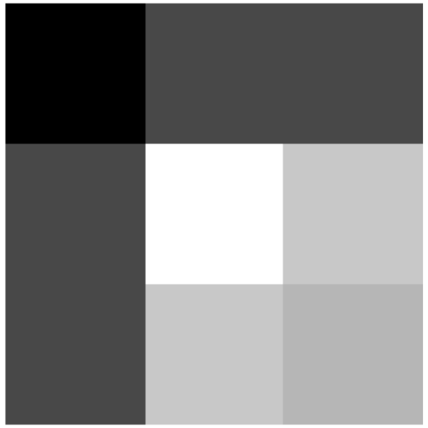


0	0	0
0	0	0
0	255	255
255	255	0
0	255	255
0	255	255
255	255	0
0	255	255
0	0	0

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

0	85	85
85	255	170
85	170	85

$I[x,y]$

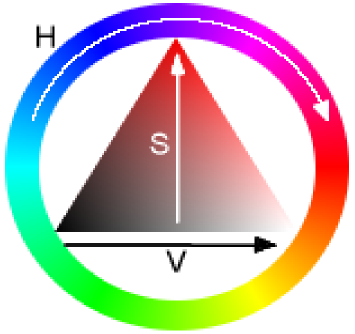


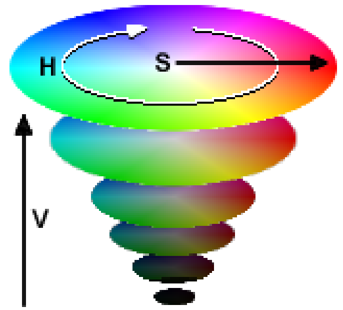
How good is the human eye resolving colors in R, G or B tones?

Concepts

- **Color space** (through examples)

  - HSV (hue, saturation, value) color space
    https://en.wikipedia.org/wiki/HSL_and_HSV
    - **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.

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

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

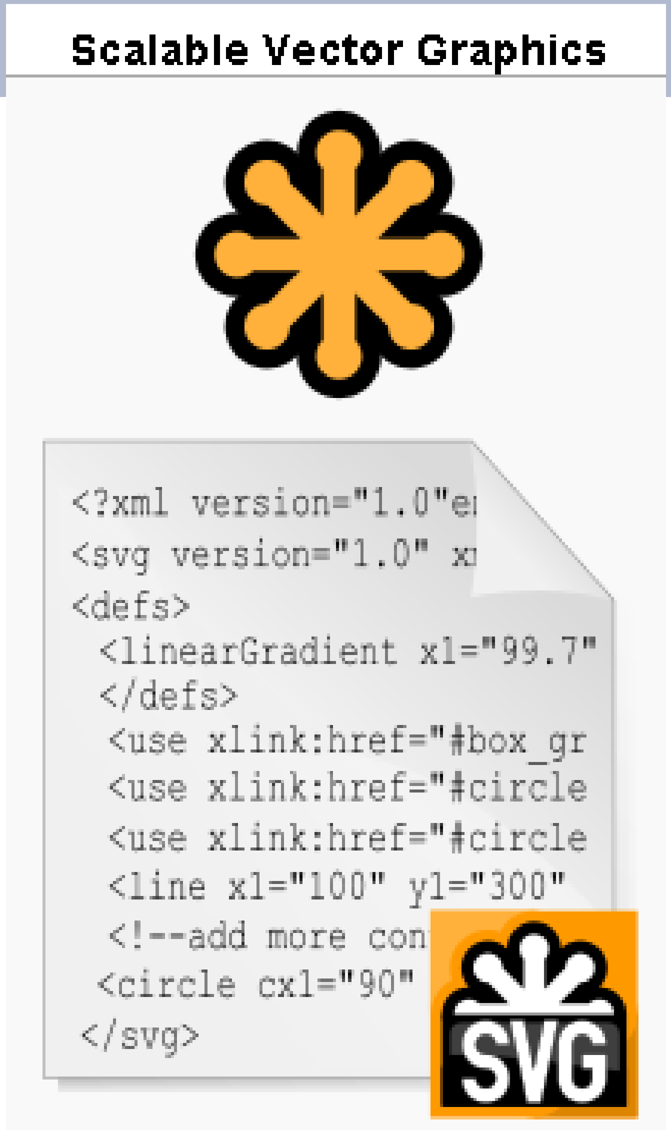
$$I = \frac{R+G+B}{3}$$

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

Concepts

- 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/method is required.



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

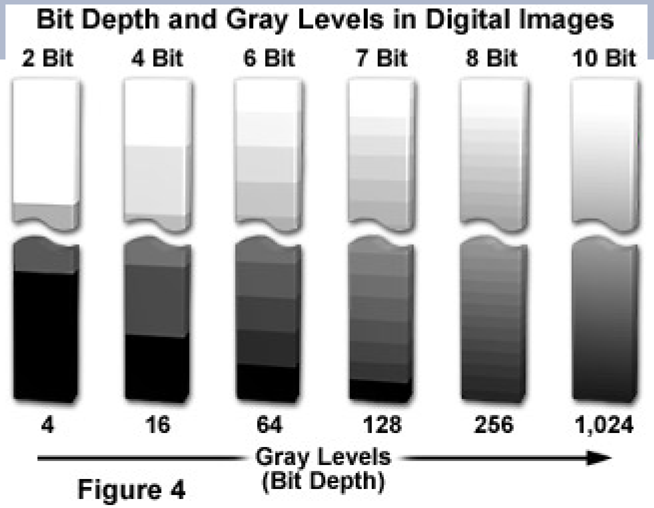
> Concepts

- …so, a digital image can be treated as a mathematical 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.)
- Nowadays, digital means binary

Aubert & Kornprobst (2006)
Mathematical Problems In Image Processing

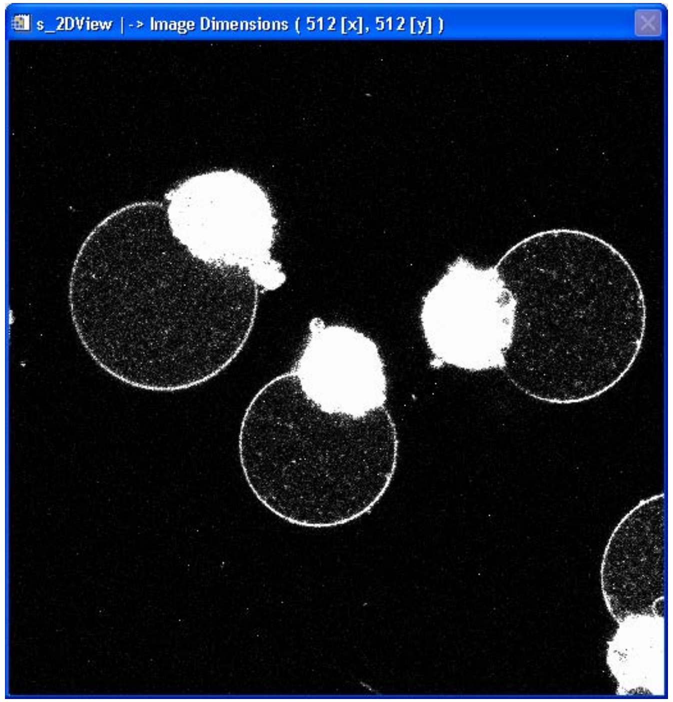
> Concepts

https://zeiss-campus.magnet.fsu.edu/articles/basics/digitalimaging.html



Bit depth

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

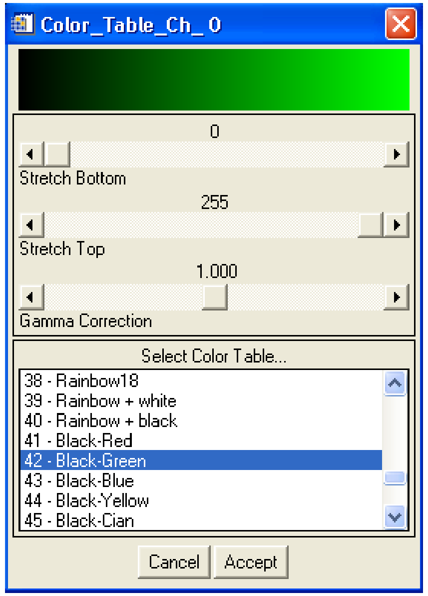
$I(290,267) = 220$

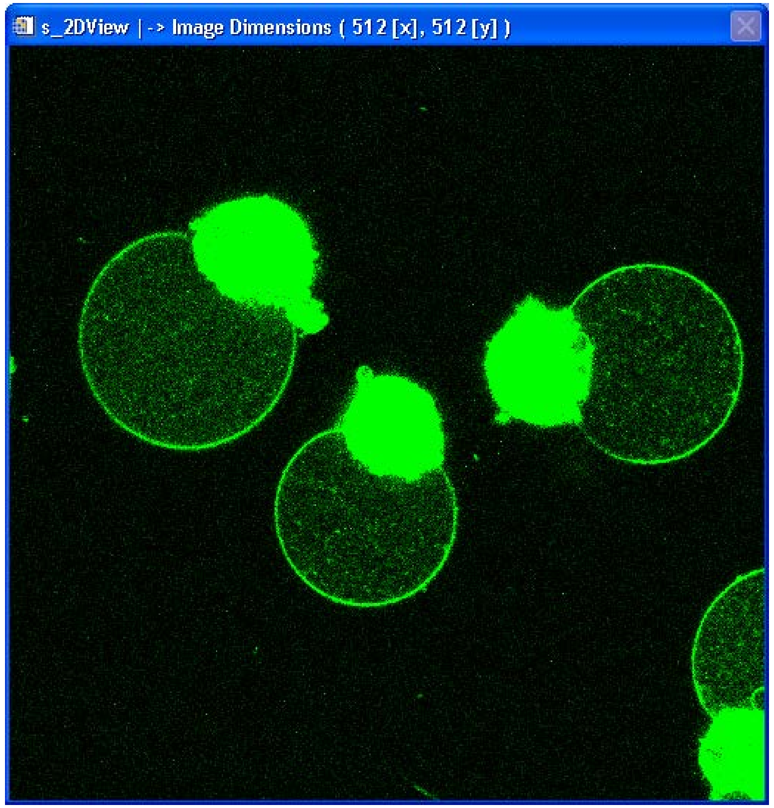
A n-bit greyscale image encodes up to 2^n intensity values

> Concepts

- Color **lookup tables** (LUTs) map intensity values to custom colors. For example, 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

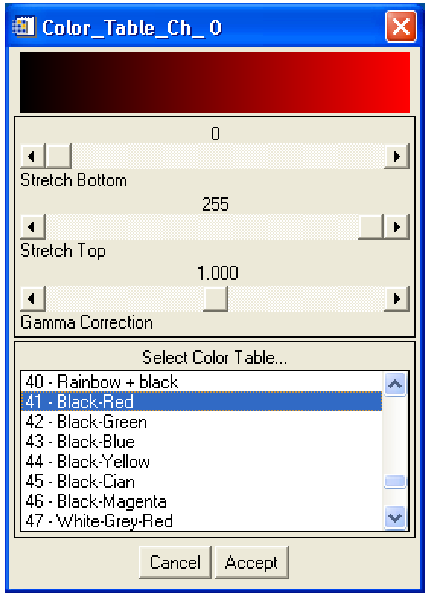




> Concepts

- Black-to-red LUT…

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

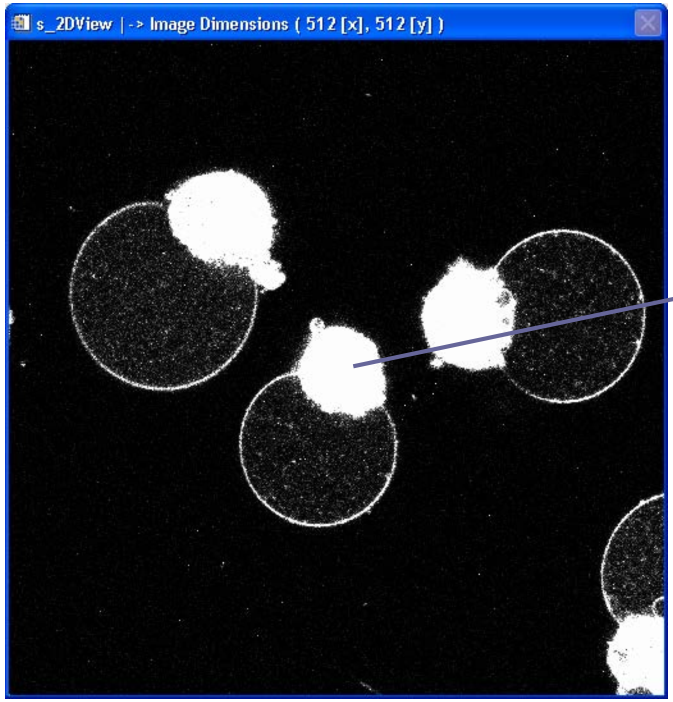


In ImageJ/FIJI: Go to the "Image"->"Lookup Tables" menu

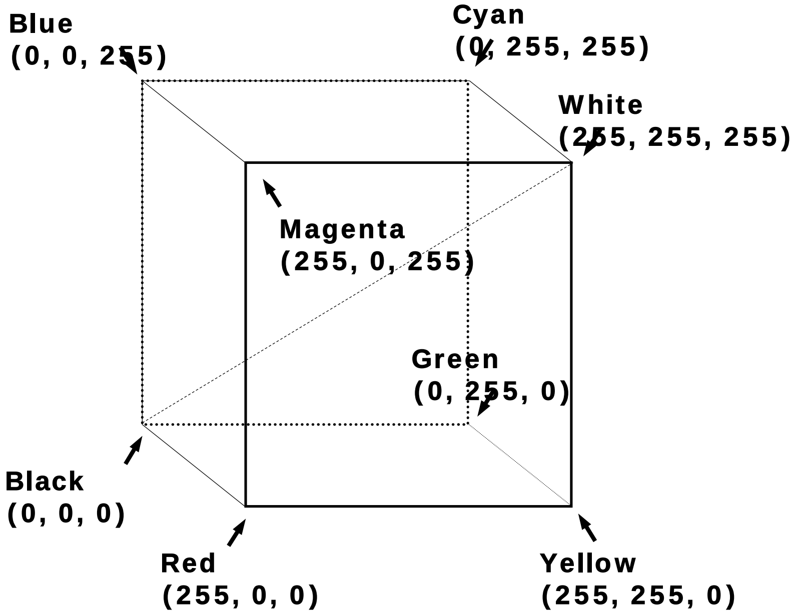
> Concepts

- ...or any custom color table

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

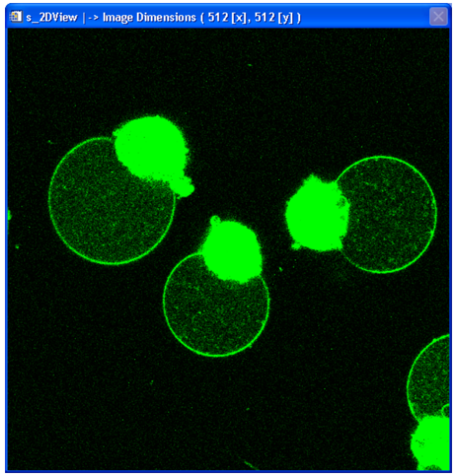


$I(290,267) = 220$



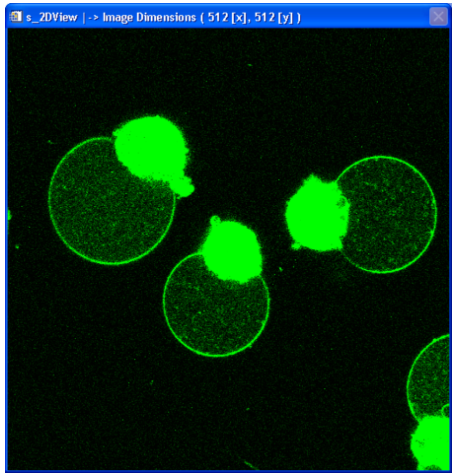
$$
\begin{aligned}
\text{Red} &= [r_0, r_1, \ldots, r_{255}] \\
\text{Green} &= [g_0, g_1, \ldots, g_{255}] \\
\text{Blue} &= [b_0, b_1, \ldots, b_{255}]
\end{aligned}
$$

> Concepts



Occlusions may occur in 3D visualization

> Concepts

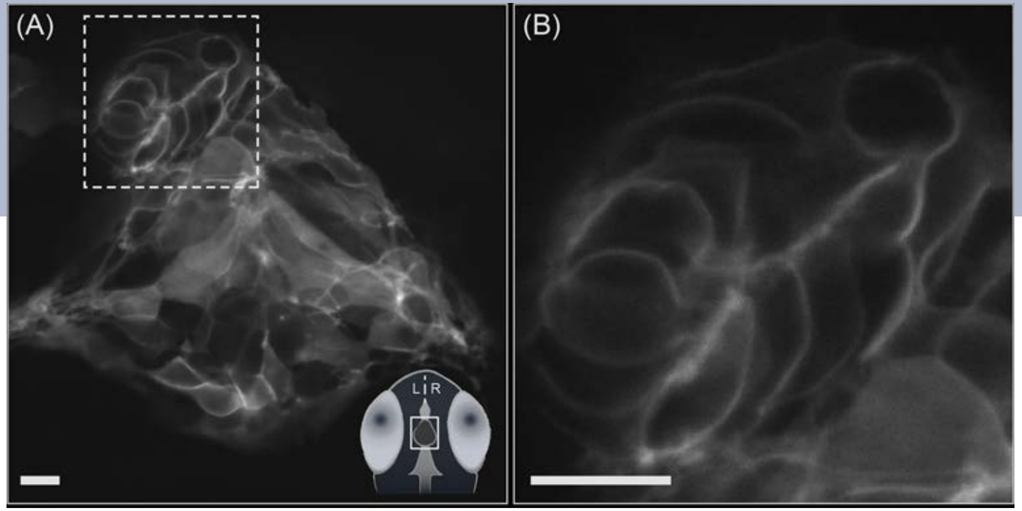


[R, G, B, α]

pacity values can
e associated to
ixels (or voxels in
3D) for visualization
purposes

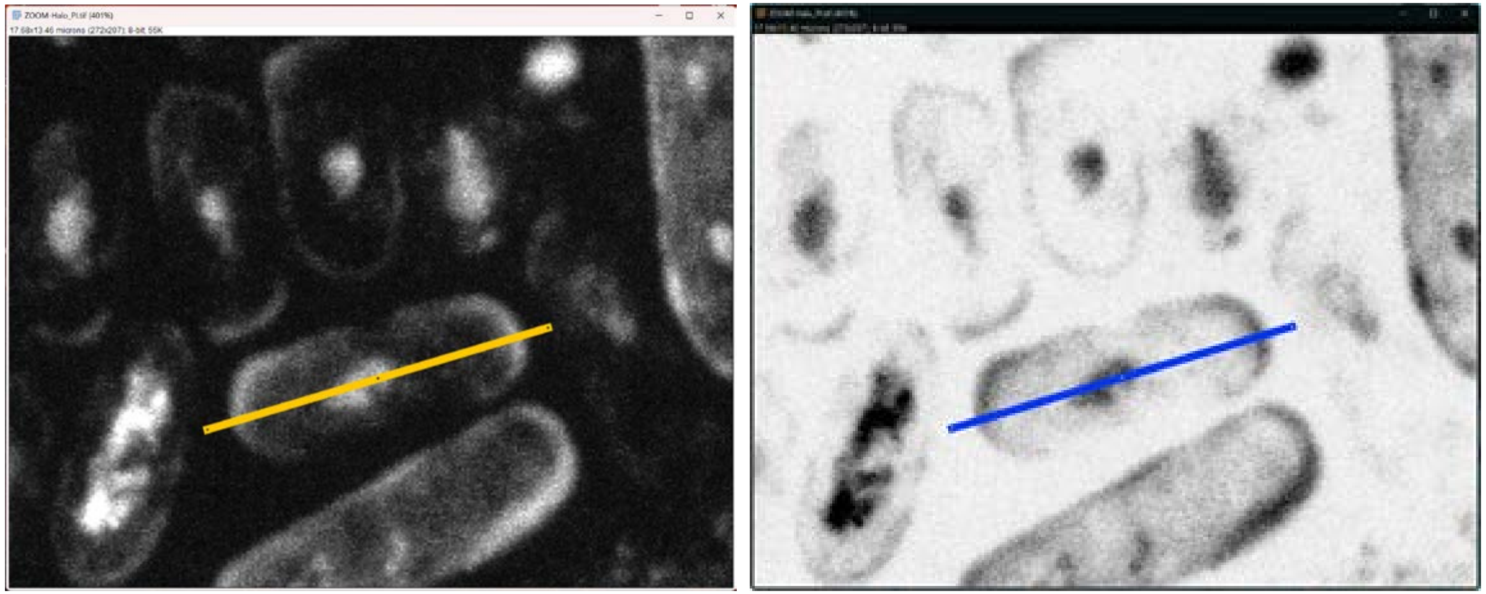
> Concepts

Visualization LUTS
(screens and/or prints)



Inverted greyscale LUT + contrast enhancement for publication. Zebrafish brain cells (eGFP membrane labeling). Scale bars: 10 µm

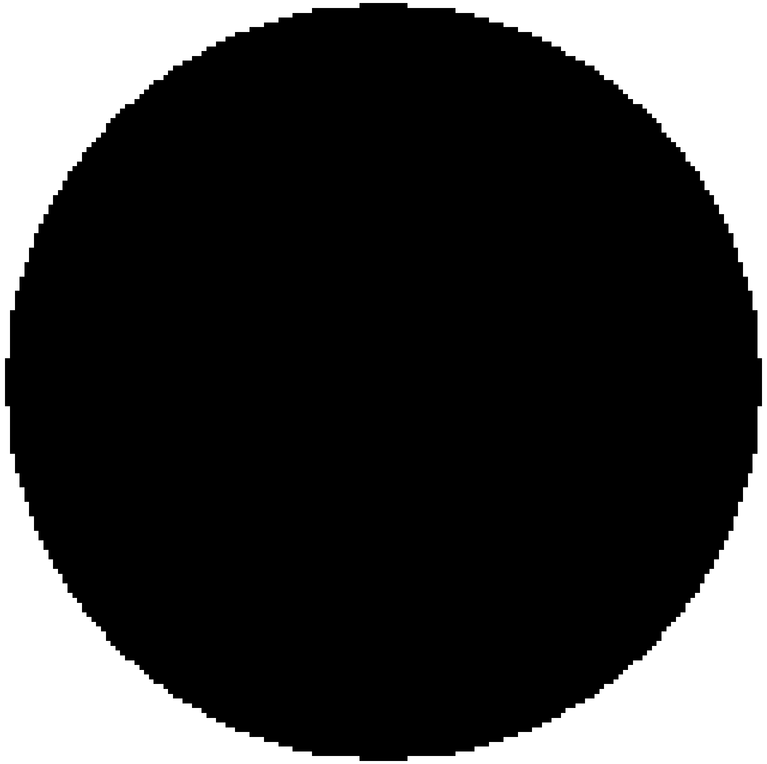
Jara-Wilde *et al.* (2020) Journal of Microscopy

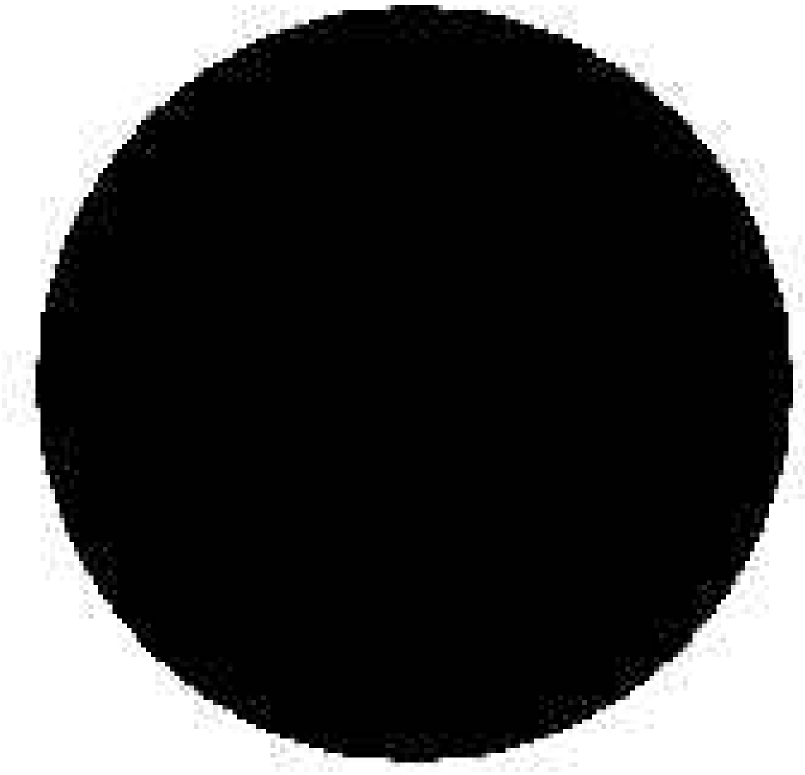


Expanded *P. mirabilis*. Castagnini *et al.* (2024) Journal of Microbiological Methods

> Concepts

- **Lossless** (left) and **Lossy** (right) file/compression

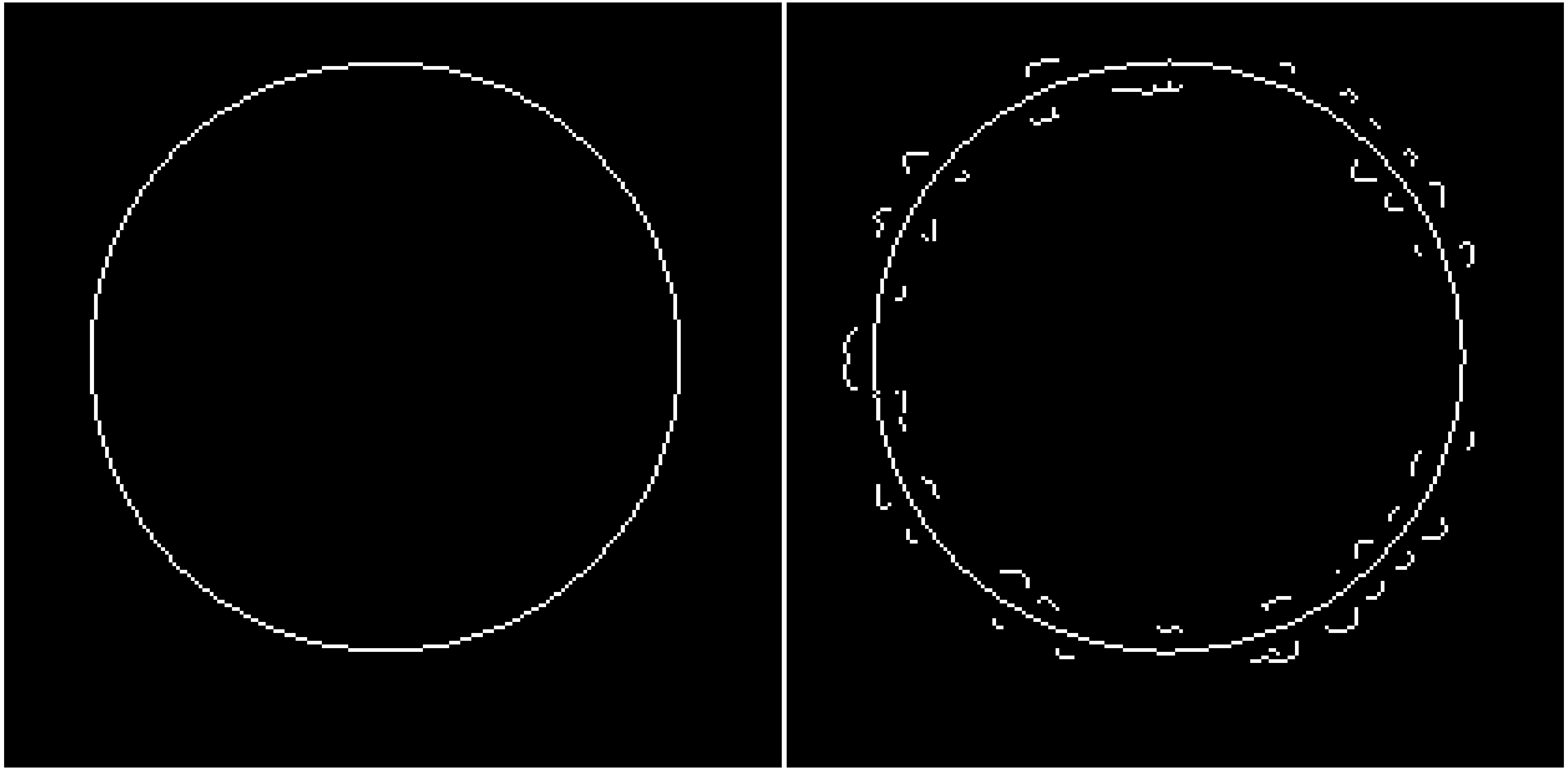




Lossy

> Concepts

- **Lossless** vs. **Lossy** (Canny edge detector filter)



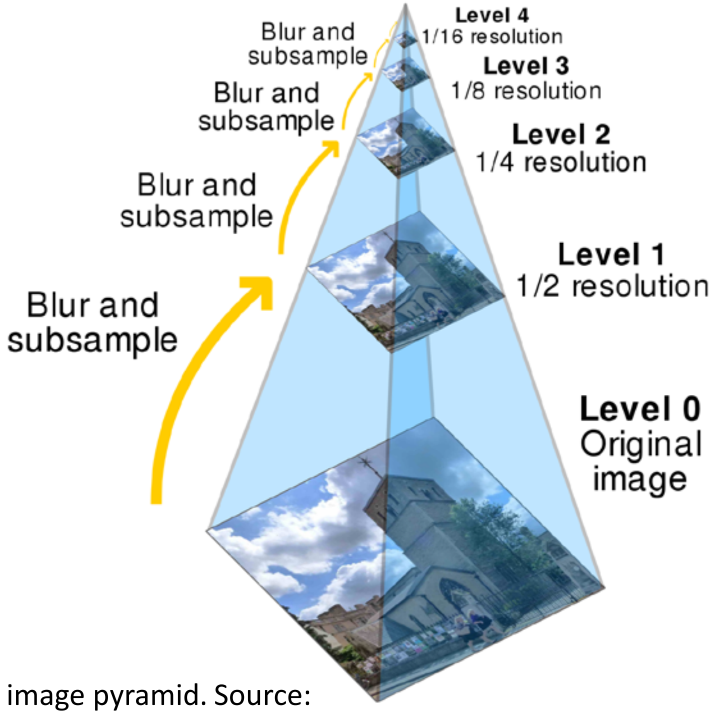
> Concepts

File formats for images
("useful" lossless formats listed here)

- PNG (2D)
- TIFF (2D/3D multi-channel, LUT support, some allow for compression)
- NGFF: Next generation file formats for large n-dimensional array containers
  - N5
  - Zarr

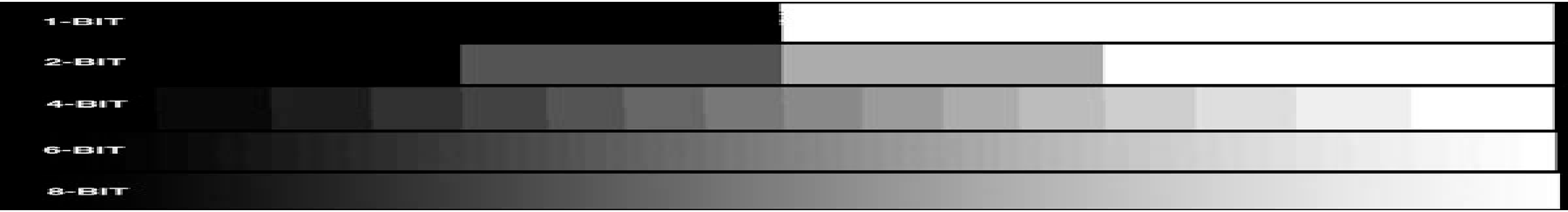
https://github.com/saalfeldlab/n5
https://imagej.net/N5
https://www.youtube.com/watch?v=InqZ_NsgB4g

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

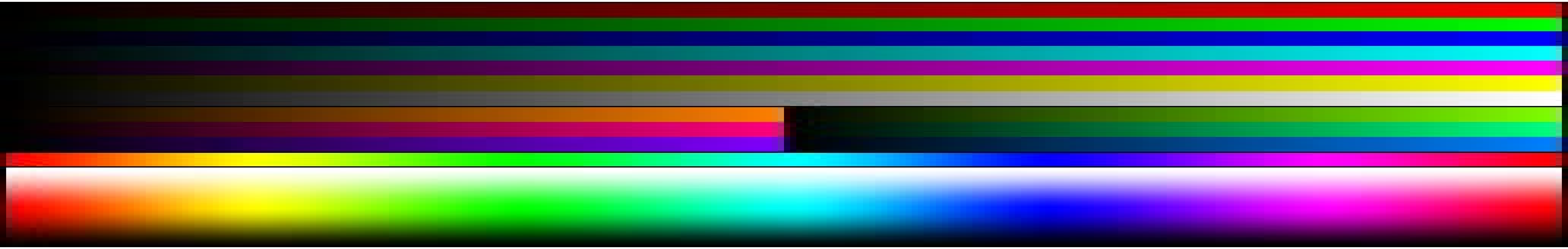


A 2D image pyramid. Source: https://en.wikipedia.org/wiki/Pyramid_(image_processing)

Concepts



https://www.projectorcentral.com/All-About-Bit-Depth.htm (8-bit rendering in most web browsers and monitors)



https://en.wikipedia.org/wiki/File:RGB_24bits_palette_color_test_chart.png

How many levels can YOU distinguish?

How many levels can YOUR DISPLAY show?

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

> Concepts (recap)

- 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)
- File format
  - “Raw”: each pixel value is stored (lots of space), native vendor format (*sigh*)
  - Compressed, with or without information loss
    (e.g. JPEG *lossy* format, TIFF can be compressed or uncompressed)
- Management…

So far…

Conversion / Transduction

Light (photons) → Voltage (tension)

Sampling

Signal capture at defined intervals (time & space)

Quantization

Numerical value mapping

Coding

Numerical value data coding
(Raw, TIFF, PNG, JPEG, N5, Zarr)

Also: *Metadata-ing*

Timestamp, Acquisition Device Data, File permissions, …

Outline

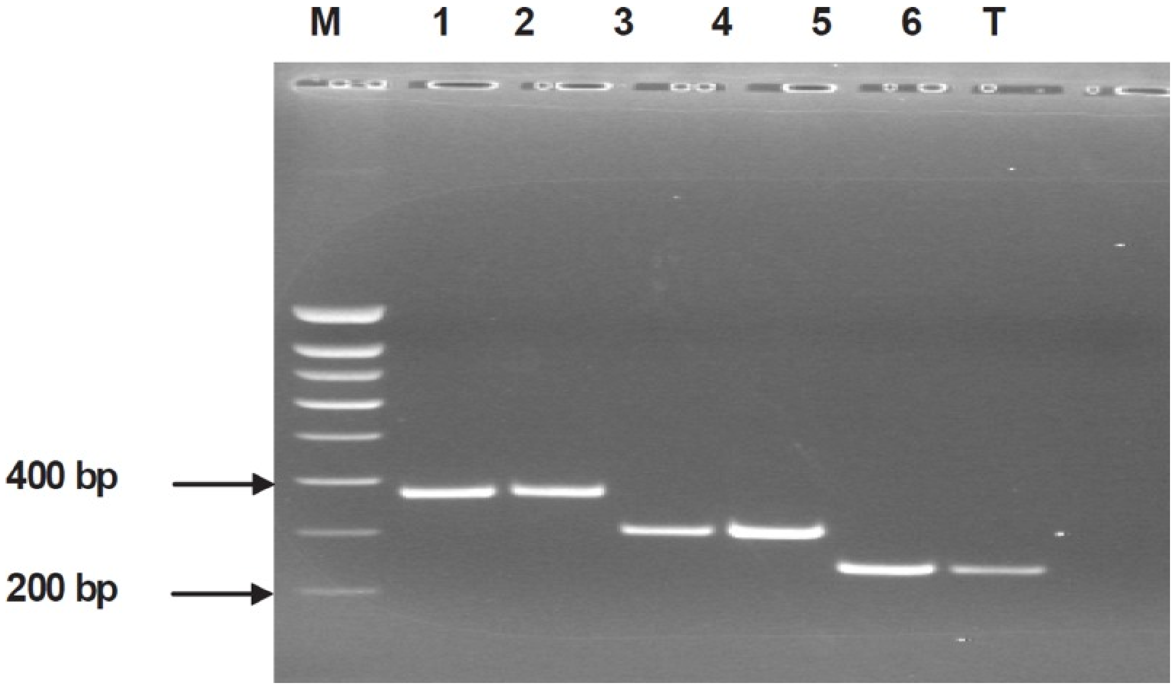
I. Principles & Concepts

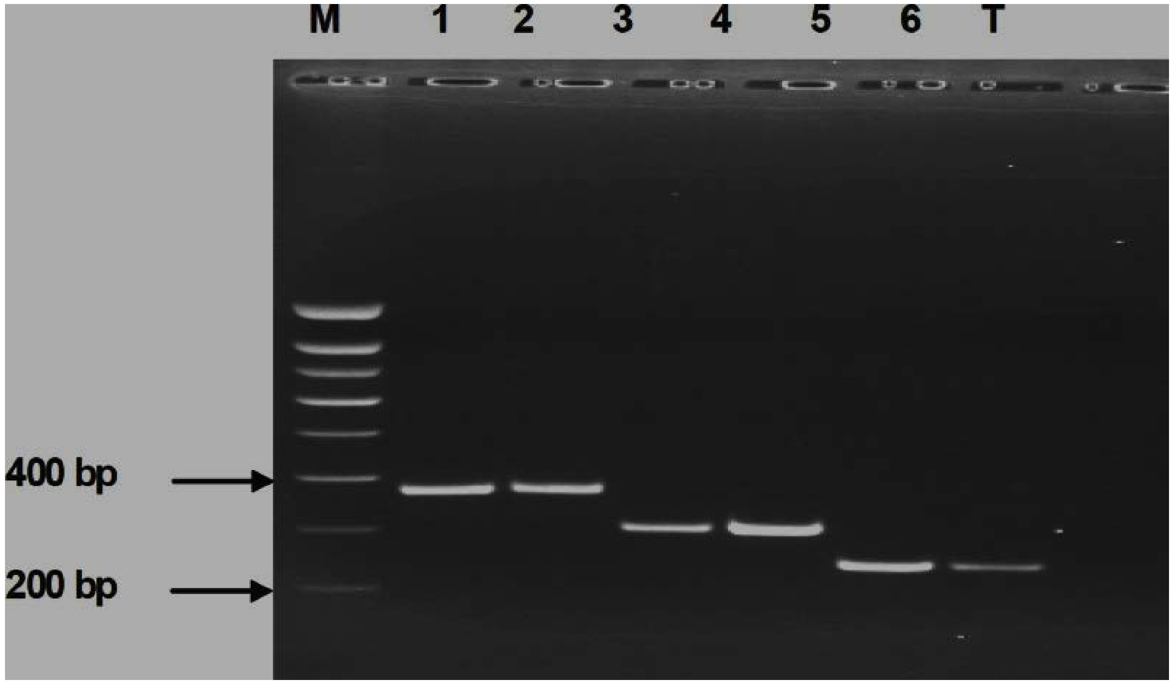
II. Processing

III. Software Tools

> Processing | Filters

Example 1: PCR

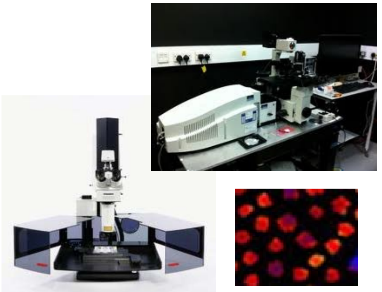


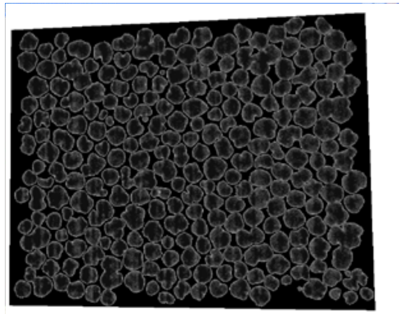


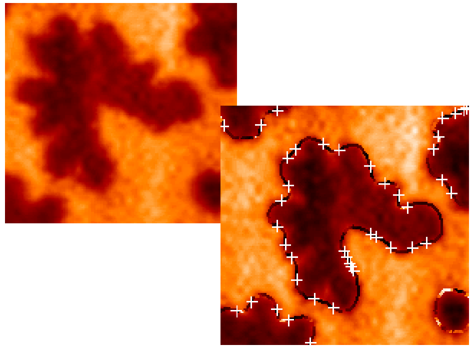
https://www.ncbi.nlm.nih.gov/books/NBK402331/figure/wt_manguin1_ch8.F6/

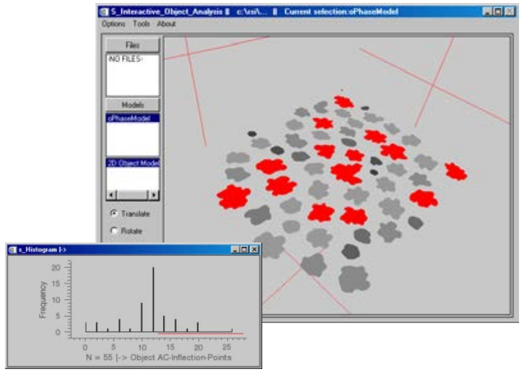
> Concepts

Image Processing









I. Acquisition

- Microscopes
- Cameras
- …

II. (pre)Treatment or Filtering

- Restoring (e.g. deconvolution)
- Contrast enhancement
- Artifact removal
- …

III. Analysis

- ROIs / models
- Measurements

IV. Understanding

- Higher level task
- Hypotheses support

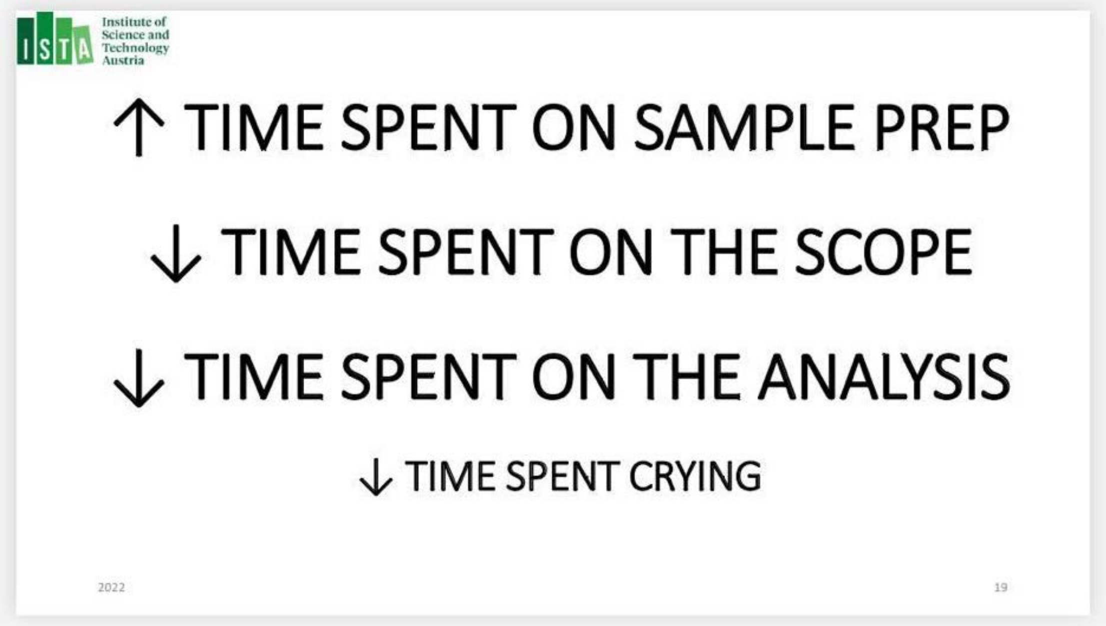
images

images

images + descriptions

> Principles

If TV science was more like real science
http://phdcomics.com/comics.php?f=1156



Source: BioImage Analysts all over the world in the 2020s

> Principles

- "As image analysts at two major imaging facilities, we are regularly asked to replicate the typically vague methods in published papers and find this task ranges from straight-forward, over pleasantly challenging, to impossible." K. Miura, S. Nørrelykke (2021)

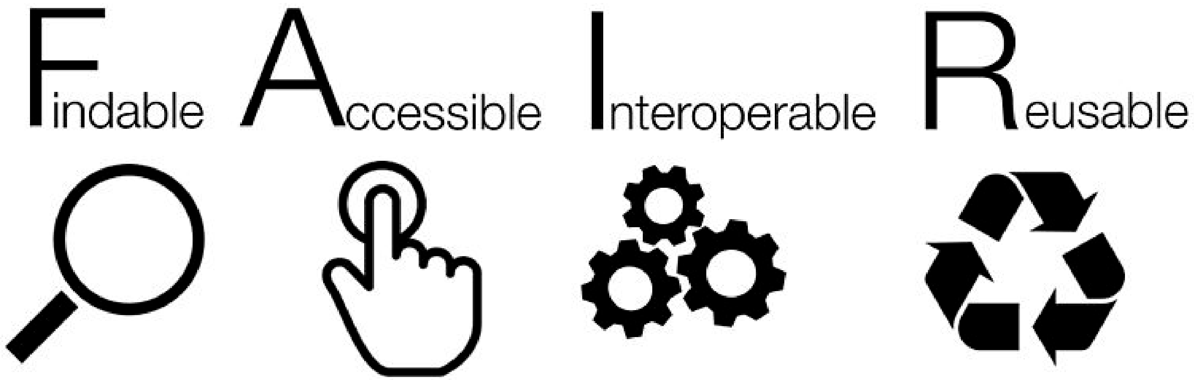
https://doi.org/10.15252/embj.2020105889

- Reproducibility
  - Methods
  - Results
  - Inferential

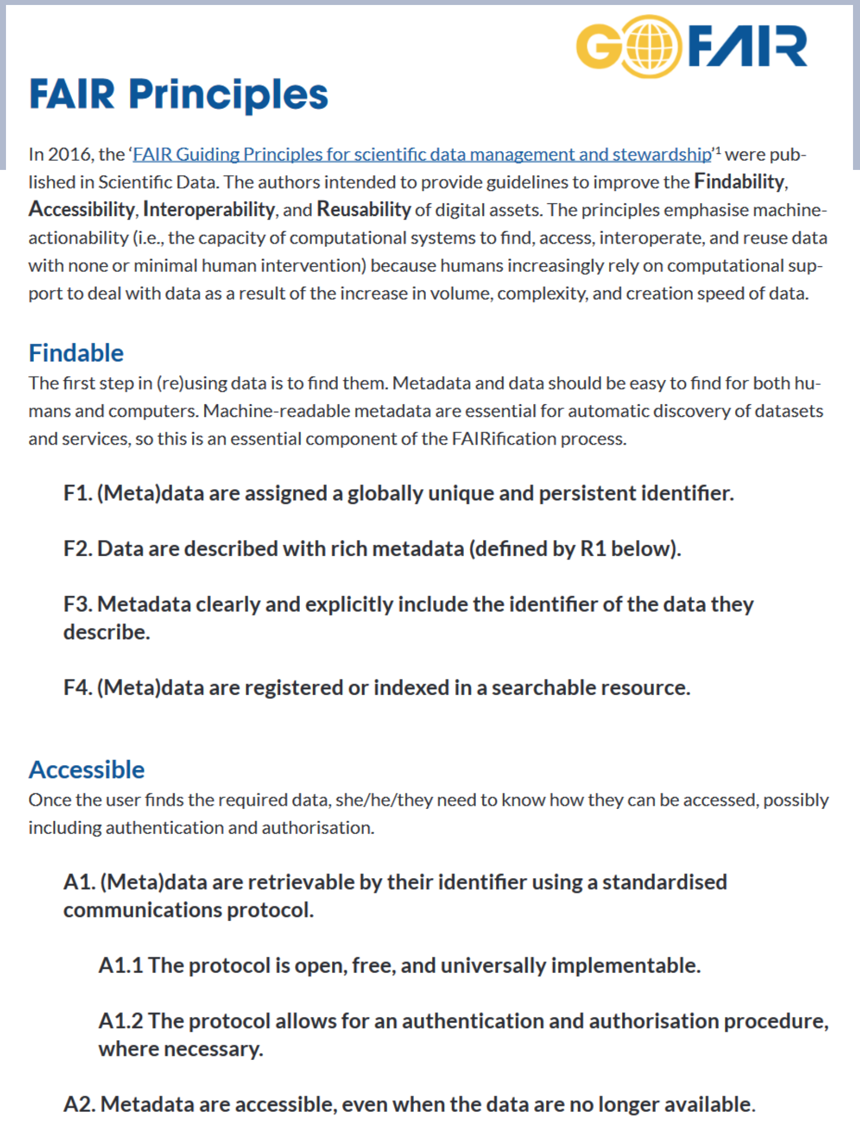
Goodman, S. N., Fanelli, D., & Ioannidis, J. P. (2016)
What does research reproducibility mean?
Science translational medicine, *8*(341), 341ps12-341ps12

> Principles

- FAIR data management
  https://www.go-fair.org/fair-principles/



- FAIR is not the same as Open



FAIR Principles

In 2016, the 'FAIR Guiding Principles for scientific data management and stewardship'[1] were published in Scientific Data. The authors intended to provide guidelines to improve the **Findability**, **Accessibility**, **Interoperability**, and **Reusability** of digital assets. The principles emphasise machine-actionability (i.e., the capacity of computational systems to find, access, interoperate, and reuse data with none or minimal human intervention) because humans increasingly rely on computational support to deal with data as a result of the increase in volume, complexity, and creation speed of data.

Findable

The first step in (re)using data is to find them. Metadata and data should be easy to find for both humans and computers. Machine-readable metadata are essential for automatic discovery of datasets and services, so this is an essential component of the FAIRification process.

F1. (Meta)data are assigned a globally unique and persistent identifier.

F2. Data are described with rich metadata (defined by R1 below).

F3. Metadata clearly and explicitly include the identifier of the data they describe.

F4. (Meta)data are registered or indexed in a searchable resource.

Accessible

Once the user finds the required data, she/he/they need to know how they can be accessed, possibly including authentication and authorisation.

A1. (Meta)data are retrievable by their identifier using a standardised communications protocol.

A1.1 The protocol is open, free, and universally implementable.

A1.2 The protocol allows for an authentication and authorisation procedure, where necessary.

A2. Metadata are accessible, even when the data are no longer available.

> Principles

nature methods

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Perspective | Published: 14 September 2023

Community-developed checklists for publishing images and image analyses

Christopher Schmied, Michael S. Nelson, Sergiy Avilov, Gert-Jan Bakker, Cristina Bertocchi, Johanna Bischof, Ulrike Boehm, Jan Brocher, Mariana T. Carvalho, Catalin Chiritescu, Jana Christopher, Beth A. Cimini, Eduardo Conde-Sousa, Michael Ebner, Rupert Ecker, Kevin Eliceiri, Julia Fernandez-Rodriguez, Nathalie Gaudreault, Laurent Gelman, David Grunwald, Tingting Gu, Nadia Halidi, Mathias Hammer, Matthew Hartley, Marie Held, Florian Jug, Varun Kapoor, Ayse Aslihan Koksoy, Judith Lacoste, Sylvia Le Dévédec, Sylvie Le Guyader, Penghuan Liu, Gabriel G. Martins, Aastha Mathur, Kota Miura, Paula Montero Llopis, Roland Nitschke, Alison North, Adam C. Parslow, Alex Payne-Dwyer, Laure Plantard, Rizwan Ali, Britta Schroth-Diez, Lucas Schütz, Ryan T. Scott, Arne Seitz, Olaf Selchow, Ved P. Sharma, Martin Spitaler, Sathya Srinivasan, Caterina Strambio-De-Castillia, Douglas Taatjes, Christian Tischer & Helena Klara Jambor

— Show fewer authors

Nature Methods (2023) | Cite this article

Abstract

Images document scientific discoveries and are prevalent in modern biomedical research. Microscopy imaging in particular is currently undergoing rapid technological advancements. However, for scientists wishing to publish obtained images and image-analysis results, there are currently no unified guidelines for best practices. Consequently, microscopy images and image data in publications may be unclear or difficult to interpret. Here, we present community-developed checklists for preparing light microscopy images and describing image analyses for publications. These checklists offer authors, readers and publishers key recommendations for image formatting and annotation, color selection, data availability and reporting image-analysis workflows. The goal of our guidelines is to increase the clarity and reproducibility of image figures and thereby to heighten the quality and explanatory power of microscopy data.

https://doi.org/10.1038/s41592-023-01987-9

Checklist for image publishing

Image format	
Focus on relevant image content (e.g., crop, rotate, resize)	Minimal
Separate individual images	
Show example image used for quantifications	
Indicate position of zoom view/inset in full-view/original image	
Show images of the range of the described phenotype	

Image colors and channels	
Annotation of channels (staining, marker, etc.) visible	Minimal
Adjust brightness/contrast, report adjustments, use uniform color scales	
Image comparison: use the same adjustments	
Channel colors: high visibility on the background Best visibility: grayscale	
Multicolors: provide grayscale for each color channel	
Multicolor: if channels are merged, make accessible to color-blind individuals	
Provide intensity scales (calibration bar) for grayscale, color, pseudocolor etc.	Recommended
Pseudocolored images: additionally provide grayscale version for comparison	Ideal
Gamma adjustments: additionally provide linear-adjusted image for comparison	

Image annotation	
Add scale information (scale bar, image length in figure/figure legend)	Minimal
Explain all annotations (in figure/figure legend)	
Annotations should be legible (line width, size/point size, color)	
Annotations should not obscure key data	
Annotate imaging details important for interpreting the figure (depending on the main message and imaging technique, this may be, e.g., image pixel size, imaging intervals (time-lapse in movies), exposure time or anatomical section)	Recommended

Image availability	
Images are shared (lossless compression/microscope images)	
Image files are freely downloadable (public database)	Recommended
Image files are in dedicated image database (added-value database or image archive)	Ideal

Checklists for publication of image-analysis workflows

Established workflows	
Cite workflow and platform	Minimal
Key settings	
Example data	
Manual ROI	
Exact version	
All settings	Recommended
Public example	
Document usage (e.g., screen recording or tutorial)	Ideal
Cloud hosted or container	

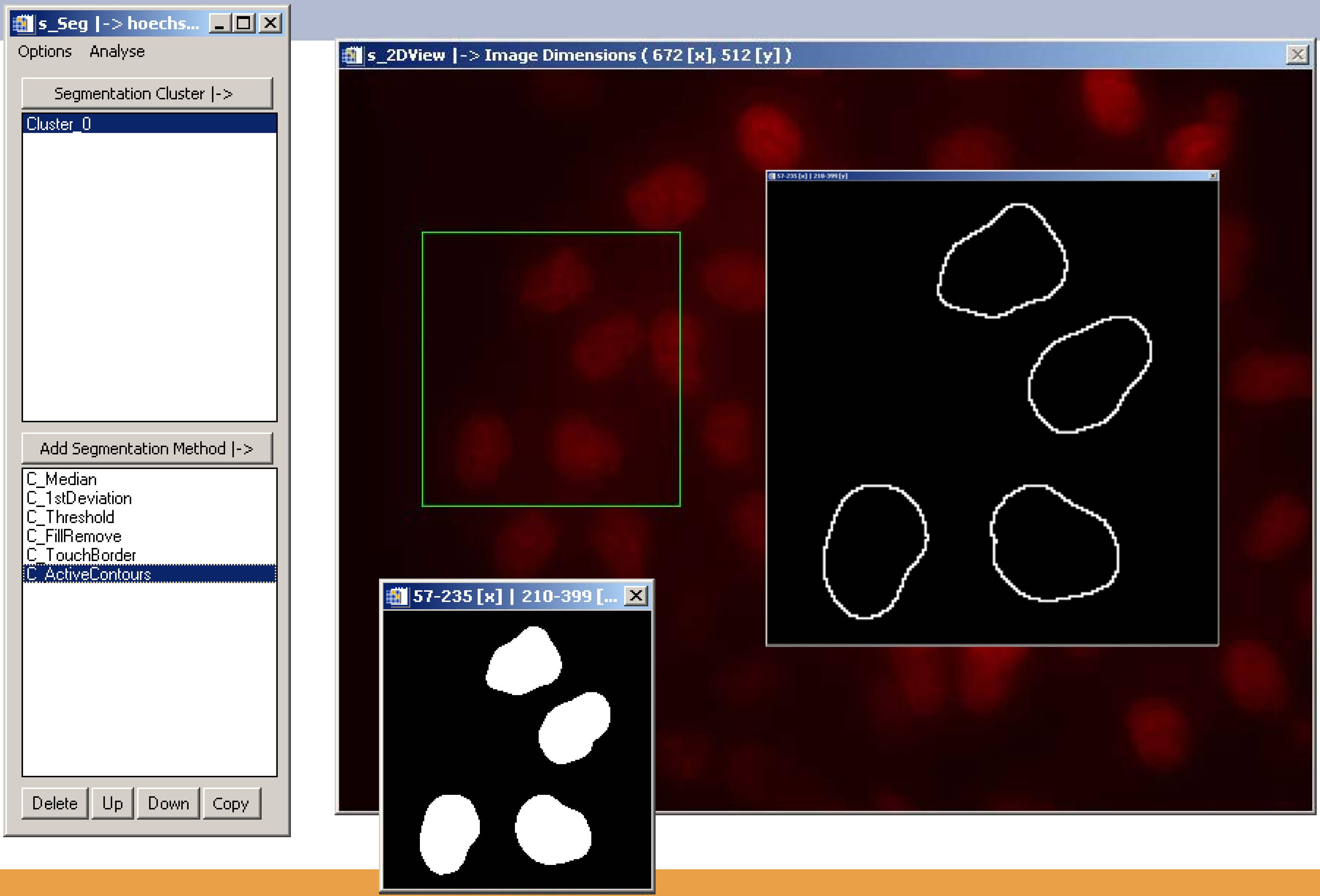
New workflows	
Cite components and platform	Minimal
Describe sequence	
Key settings	
Example data and code	
Manual ROI	
Exact versions	
All settings	Recommended
Public example data and code	
Rationale	
Limitations	
Screen recording or tutorial	Ideal
Easy install and usage, container	

Machine learning workflows	
Cite original method	Minimal (all models)
Access to model	
Example or validation data	
Training and testing data and metadata	Recommended (pretrained and new models)
Code available	
Limitations	
Cloud hosted or container	
Standardized format	Ideal (new models)

> Concepts | Wishlist

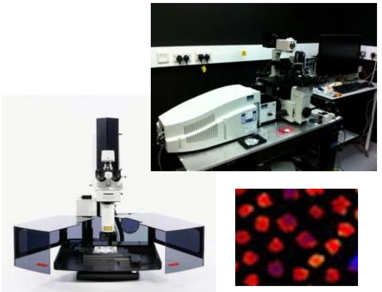
- Signal & noise
  - SNR
- Signal amplitude
- Signal frequency/frequencies
- Signal amplitude
  - Fluorescence intensity
- Segmentation
- Pixel / Voxel
- Bit
- (Sample) Bit depth
- Sampling frequency/interval
  - Pixel size or spacing
- (Intensity) Histogram
- Filter

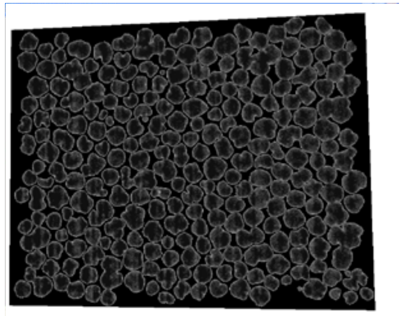
> Concepts

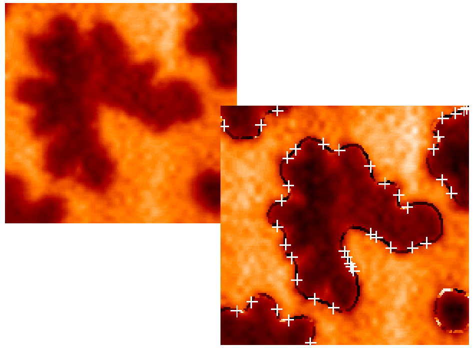

s_Seg |-> hoechs...
Options
Analyse
Segmentation Cluster |->
Cluster_0
Add Segmentation Method |->
C_Median
C_1stDeviation
C_Threshold
C_FillRemove
C_TouchBorder
C_ActiveContours
Delete
Up
Down
Copy
s_2DView |-> Image Dimensions (672 [x], 512 [y])
57-235 [x] | 210-399 [...

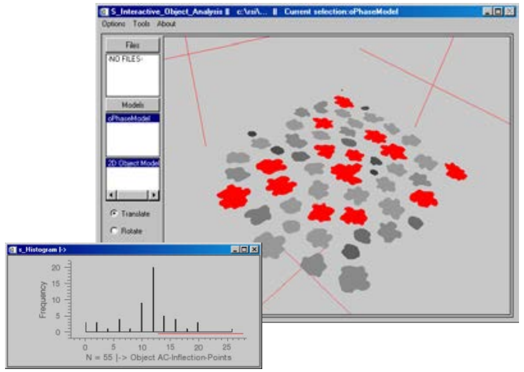
> Concepts

Image Processing









I. Acquisition
- Microscopes
- Cameras
- ...

II. (pre)Treatment or Filtering
- Restoring (e.g. deconvolution)
- Contrast enhancement
- Artifact removal
- ...

III. Analysis
- ROIs / models
- Measurements

IV. Understanding
- Higher level task
- Hypotheses support

images   images   images + descriptions

> Processing | Filters

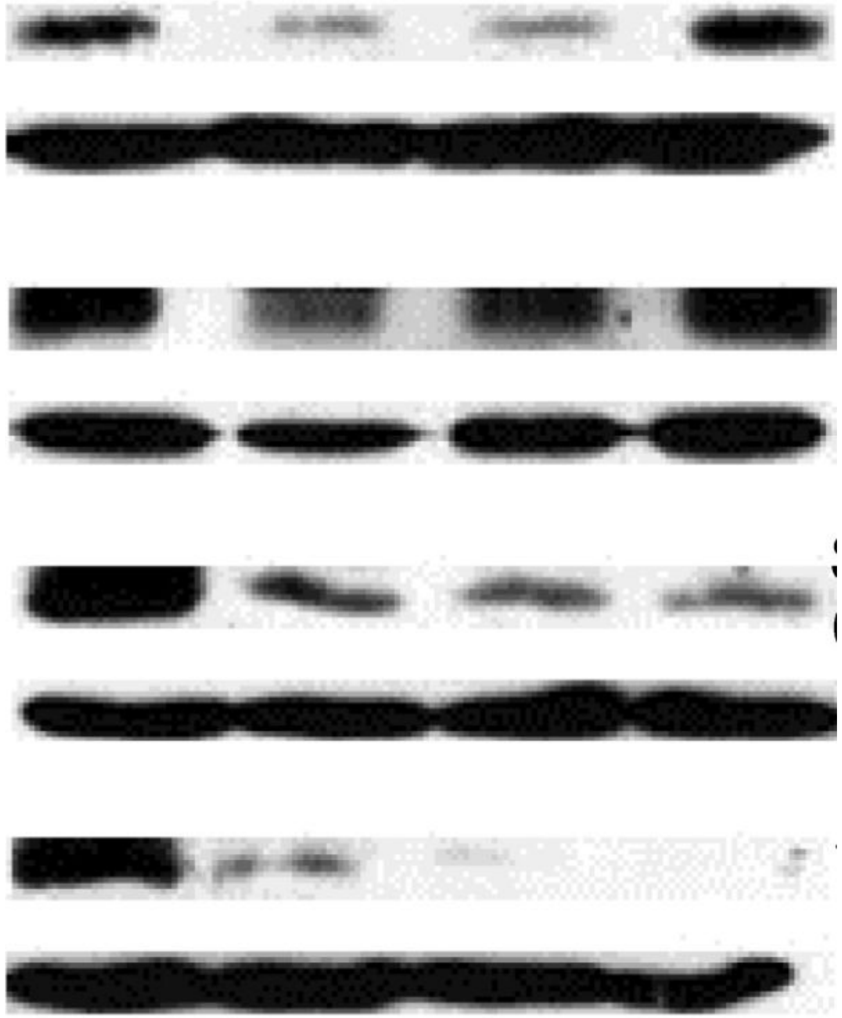


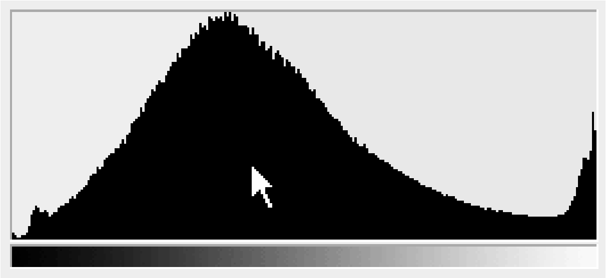
Image source: https://www.elsevier.com/editors-update/story/publishing-ethics/the-art-of-detecting-data-and-image-manipulation

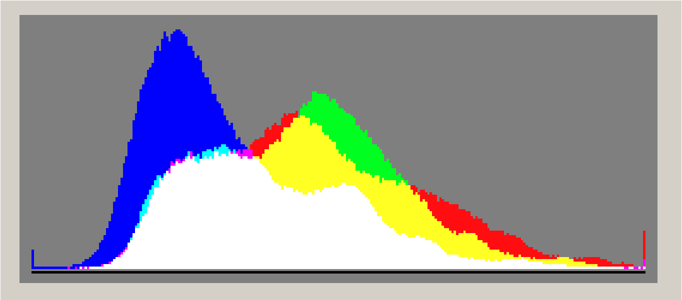
> Processing | Filters | Brightness & Contrast

Brightness? Contrast?

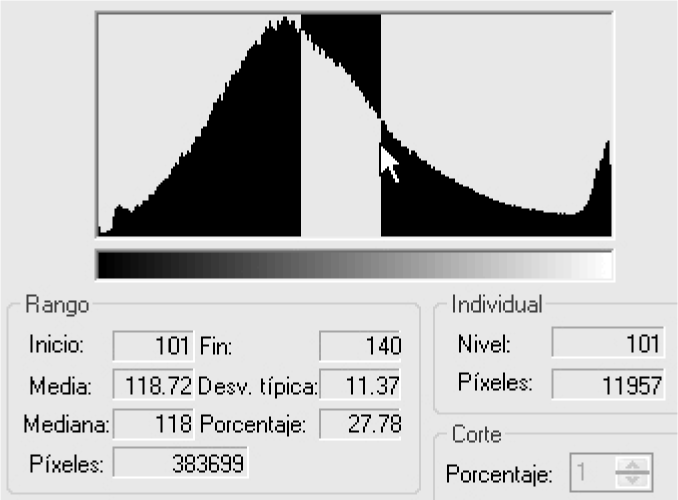
https://riunet.upv.es/bitstream/handle/10251/12711/El%20histograma%20una%20imagen%20digital.pdf?sequence=1

> Processing | Intensity Histograms





Podemos obtener información de un área determinada del histograma.

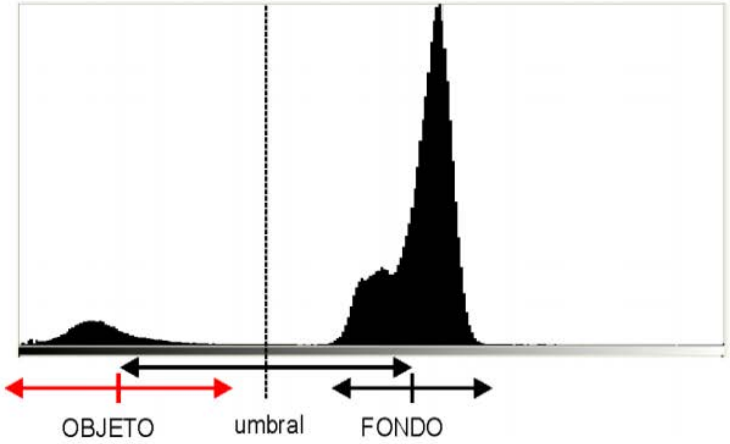


> Processing | Intensity Histograms

> Processing | Intensity Histograms

Si una fotografía está **sobreexpuesta** el histograma estará inclinado hacia la **derecha** (más píxeles iluminados)
Si una fotografía está **subexpuesta** el histograma estará inclinado hacia la **izquierda** (más píxeles oscuros)
Si una fotografía esta **correctamente expuesta** el histograma estará en el **centro o extendido**.

> Processing | Intensity Histograms



Biomodal distribution

Figura 12. Ejemplo de umbralización de una imagen con ayuda de su histograma.

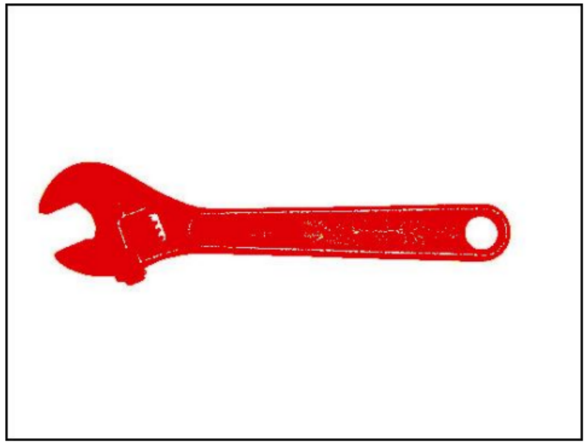
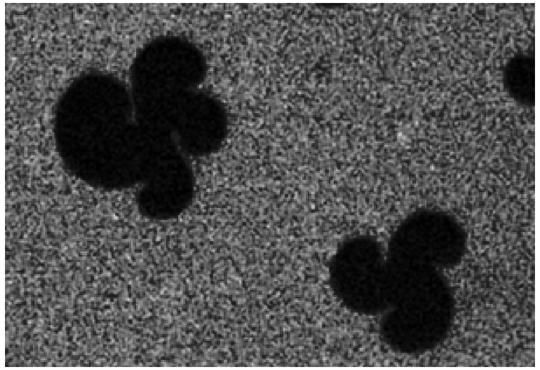
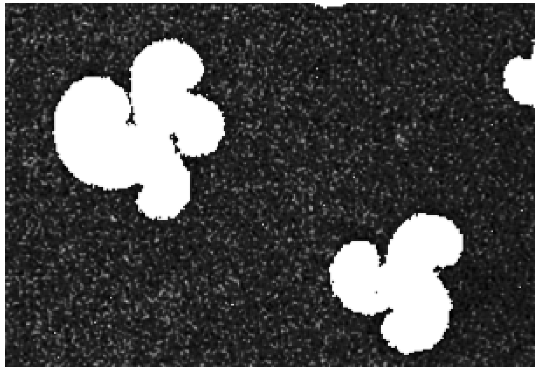


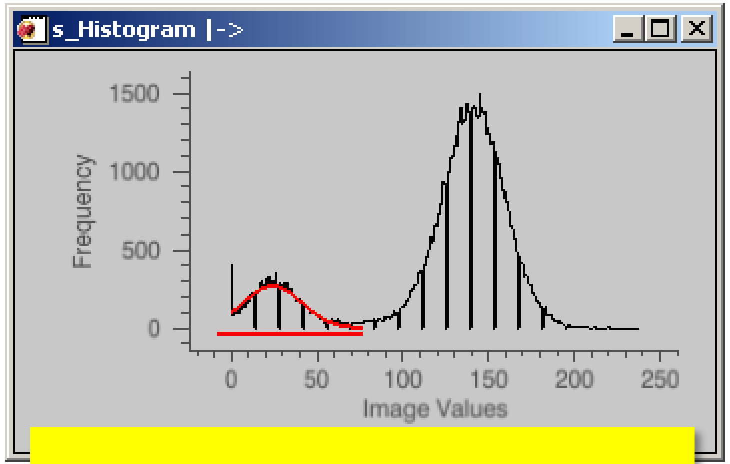
Figura 13. Resultado de la umbralización automática de la imagen anterior.

> Processing | Intensity Histograms

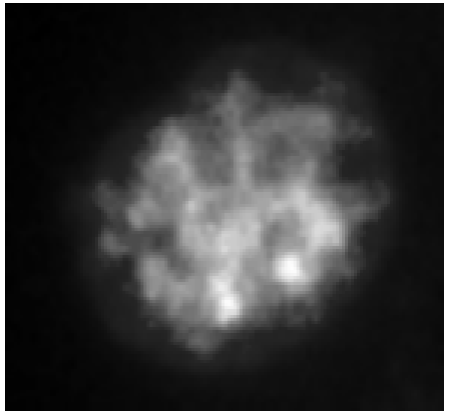
- Threshold filter segmentation: ROIs (white) / background (black)

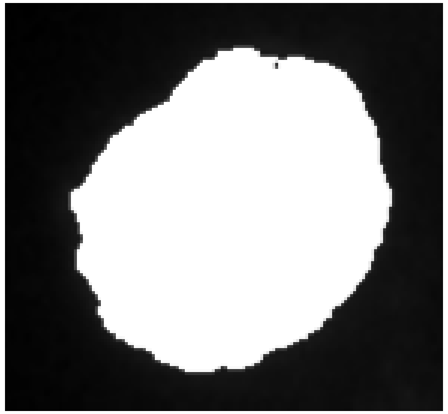




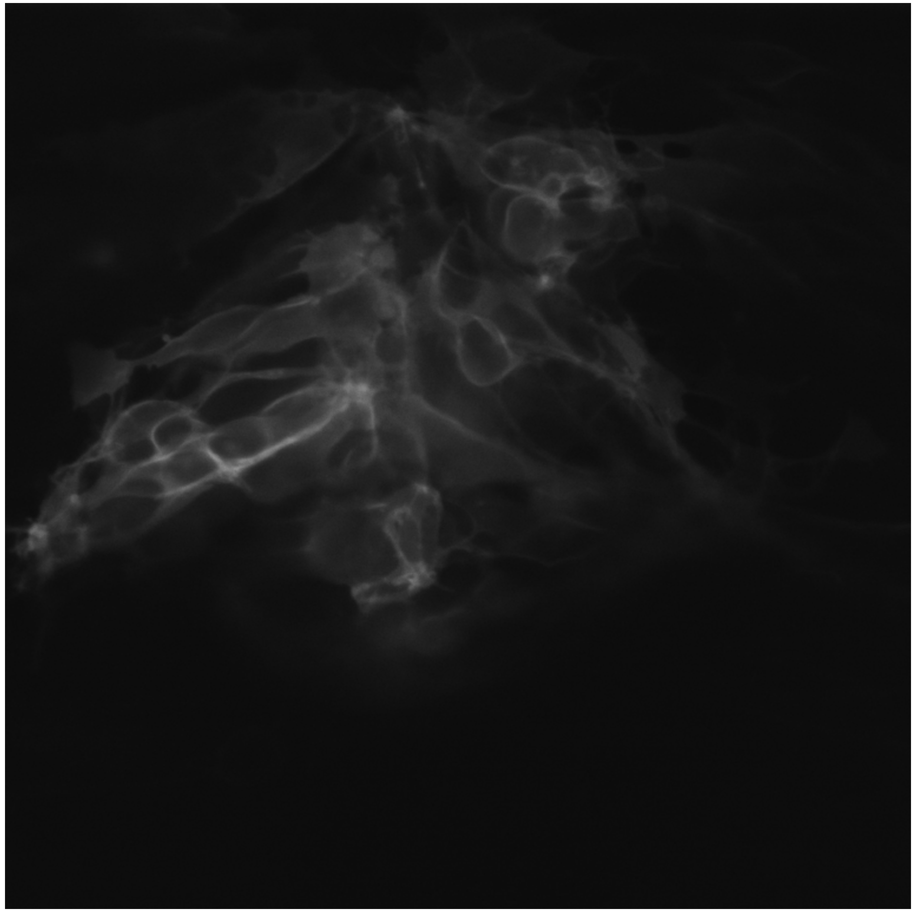


Intensity histograms





> Processing | Filters



Fluorescent zebrafish embryo parapineal complex

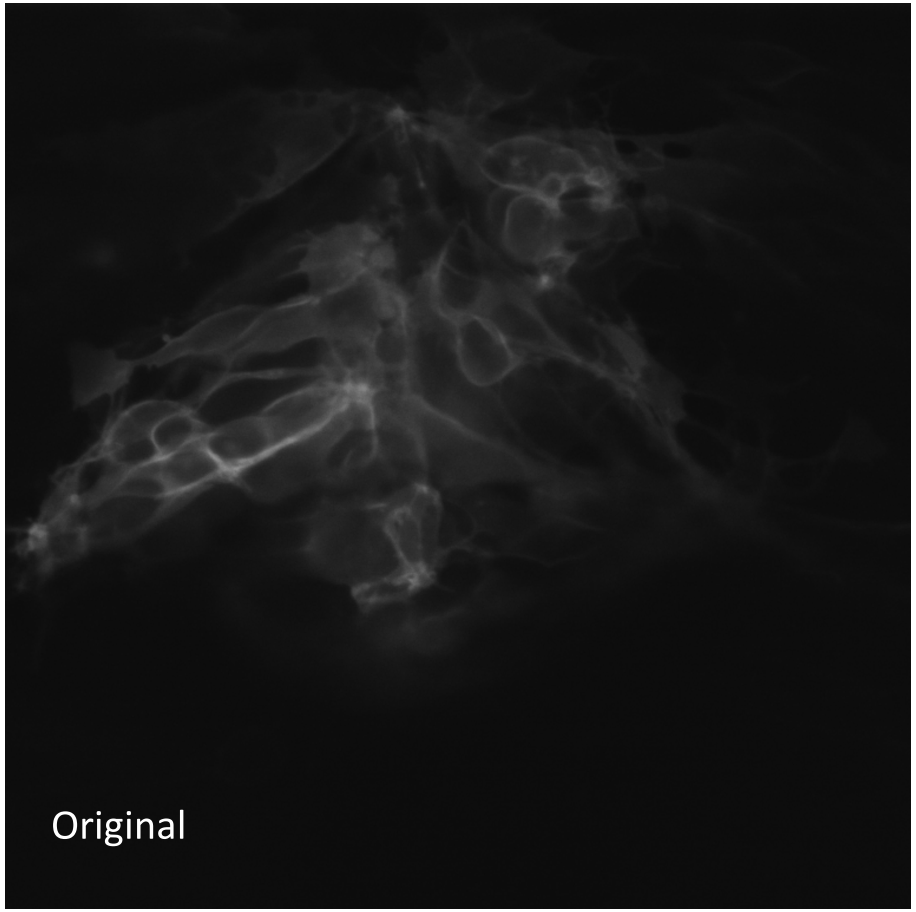
Transgenic *flh*::gap43-EGFP zebrafish embryo , 38 hpf (hours post-fertilization). Spinning disk microscopy

C.G. Lemus, M.L. Concha (LEO/SCIAN)

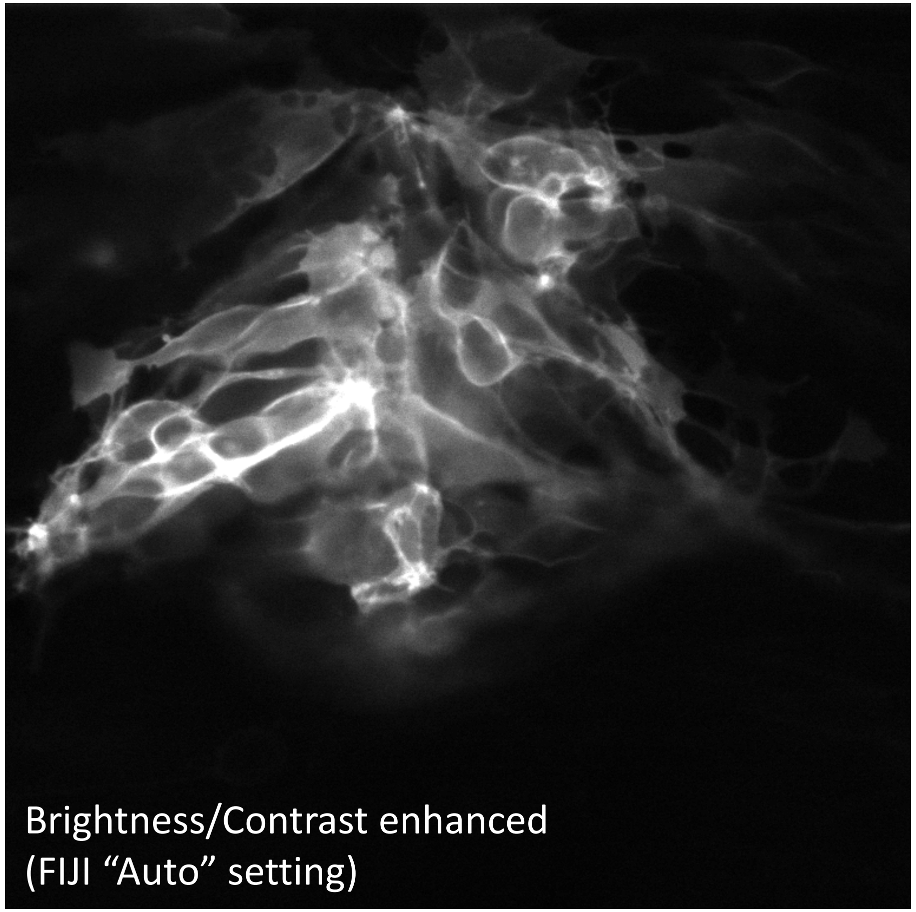
8 bits per pixel, 768x768x69 voxels

166x166x500 nm^3 voxel size

> Processing | Filters



Original



Brightness/Contrast enhanced
(FIJI “Auto” setting)

> Processing | Analysis

- **Image Analysis**

The extraction of meaningful descriptions of features of interest from images

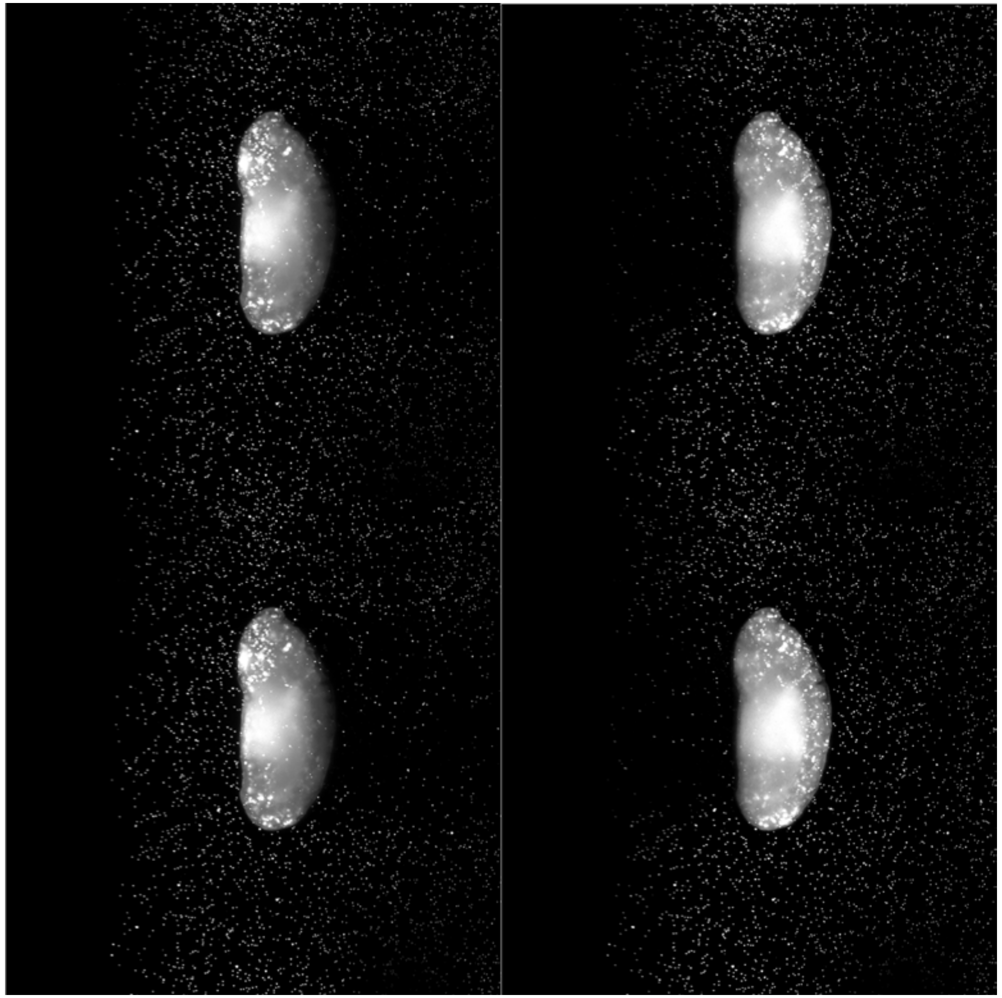
Adapted from
Young I, Gerbrands J, van Vliet L (1995)
Fundamentals of Image Processing. Delft: PH

> Processing | Analysis

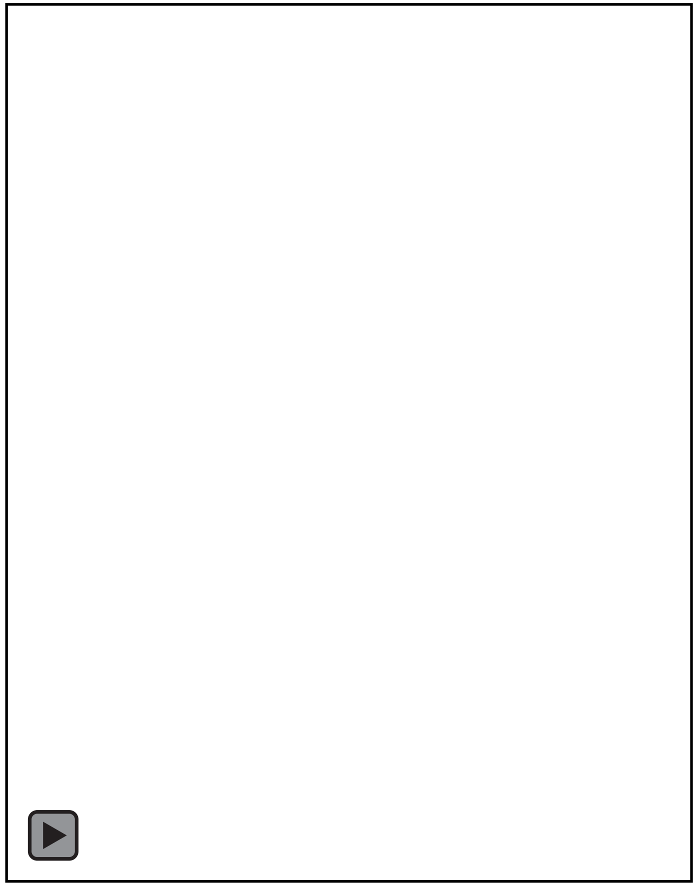
- Examples of analysis tasks
  - **Objects/regions identification (segmentation)**
    - Cells and/or their organelles
  - **Registration: image, region and/or feature “matching”**
    - Drift correction of the sample (from acquisition)
    - Relative speeds/displacements inside a given cell or reference 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

> Processing | Analysis

- 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)

> Processing | Analysis Tasks

- Identification (features, objects, scenes)

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

> Processing | Analysis Tasks

- **Segmentation**

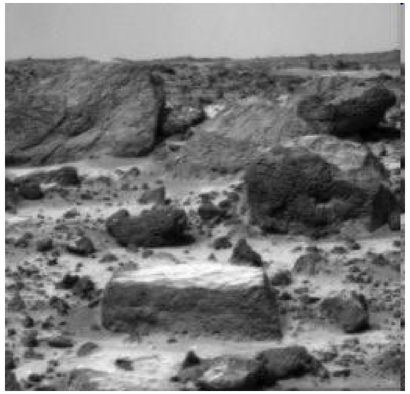
  - The partitioning of a given image into regions of interest (ROIs) according to given criteria (e.g. color).

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

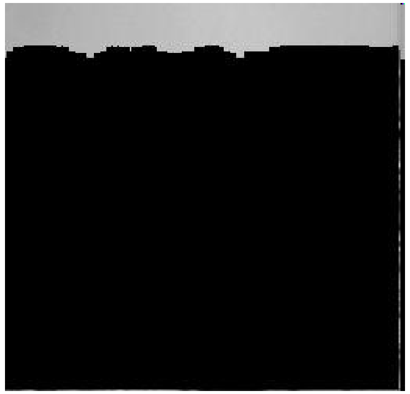
Shapiro LG and Stockman GC (2001):
“Computer Vision”, pp 279-325
New Jersey, Prentice-Hall

> Processing | Analysis Tasks

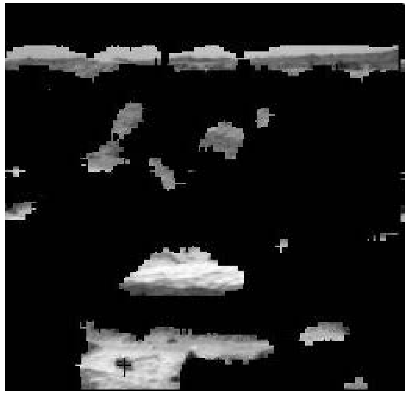
- **Segmentation** (“pre Machine Learning era”)



Sol 3, Mars
Pathfinder Mission

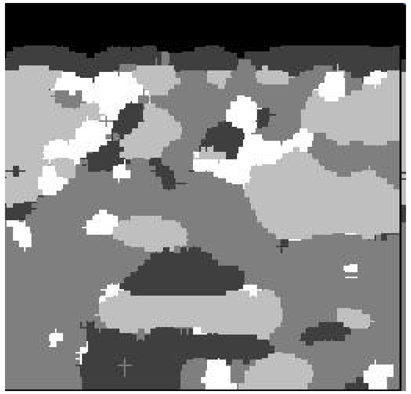


Sky / Flat



Dust / Horizon

...etc...

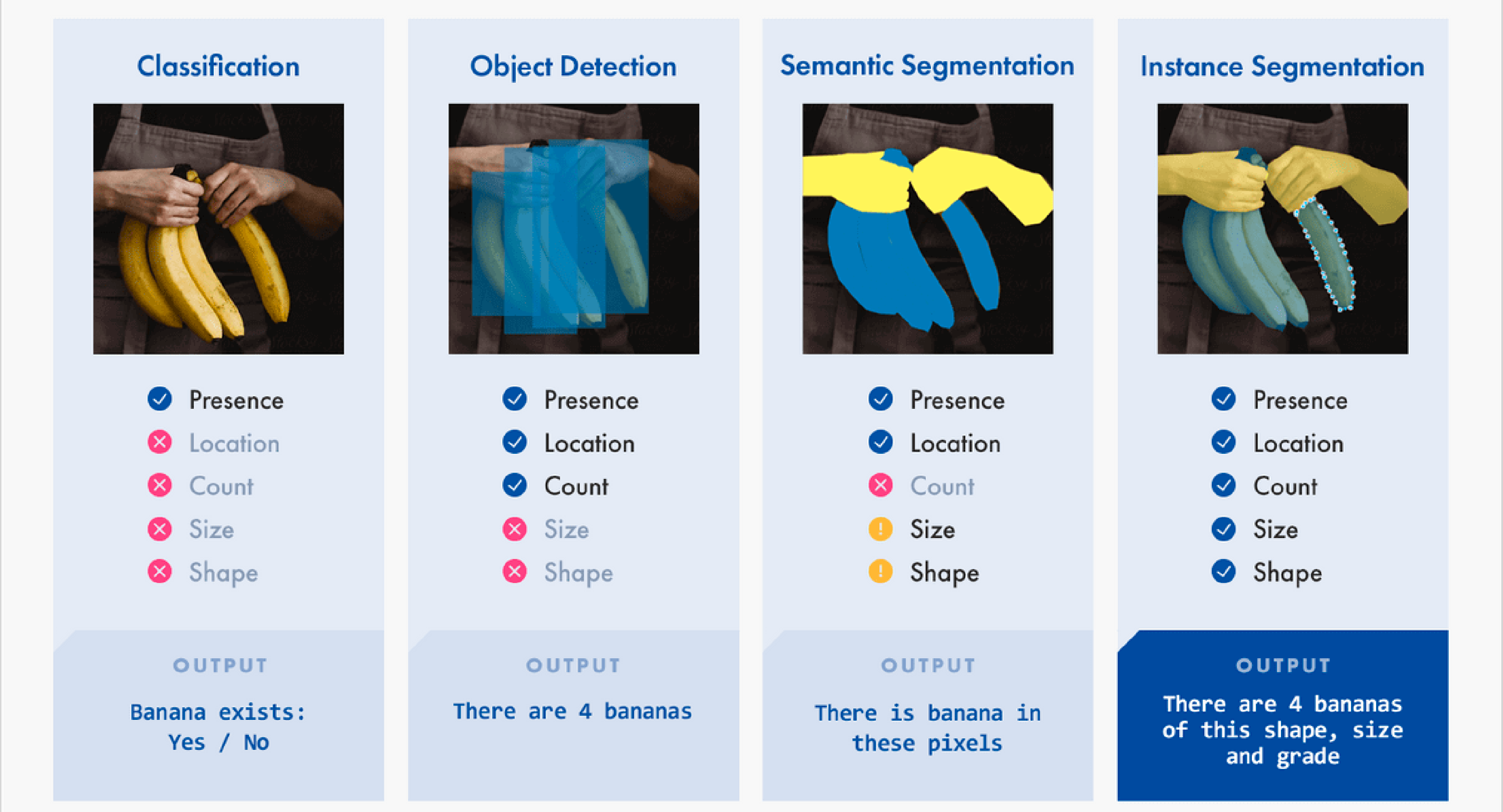


Final segmentation

Source: NASA

> Processing | Analysis Tasks

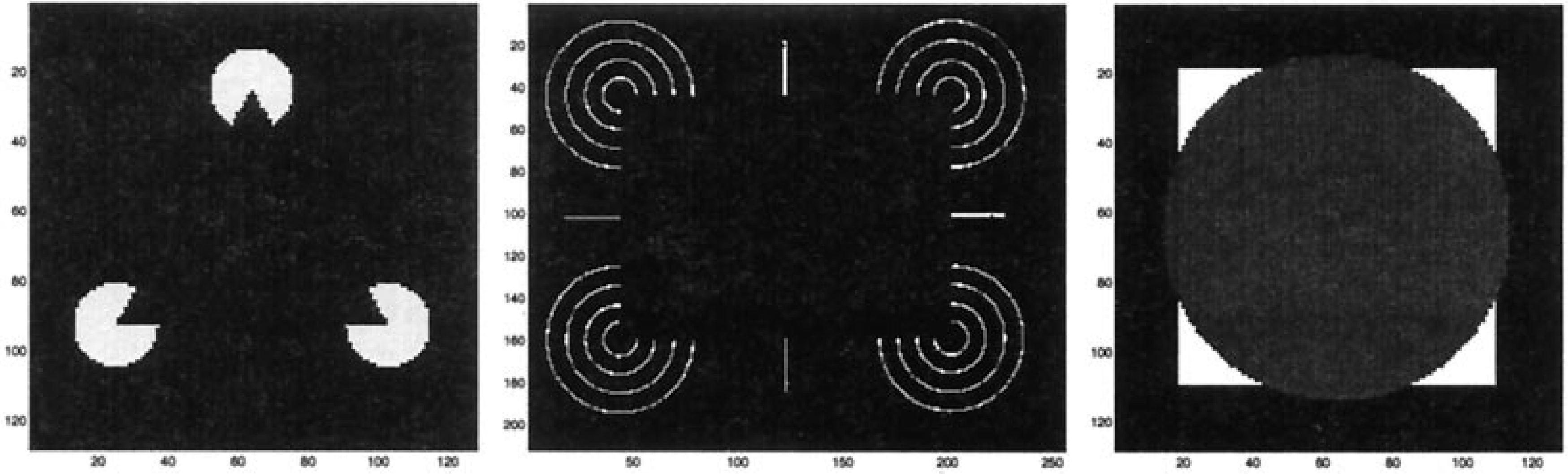
- **Segmentation** ("2020's Machine Learning era")



C. Vásquez
(UdeC/UCh)

> Processing | Analysis Tasks

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

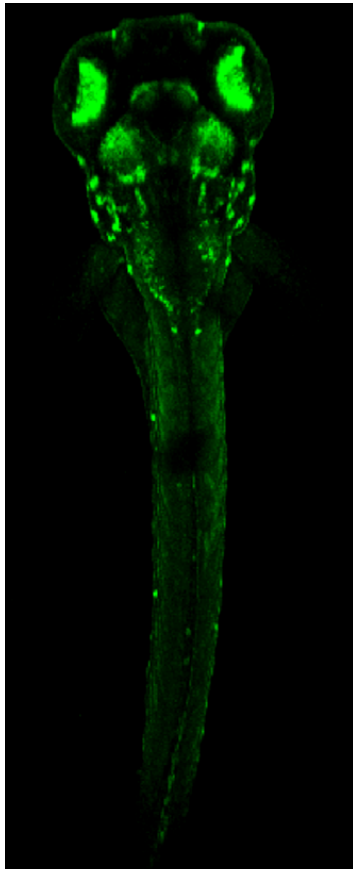


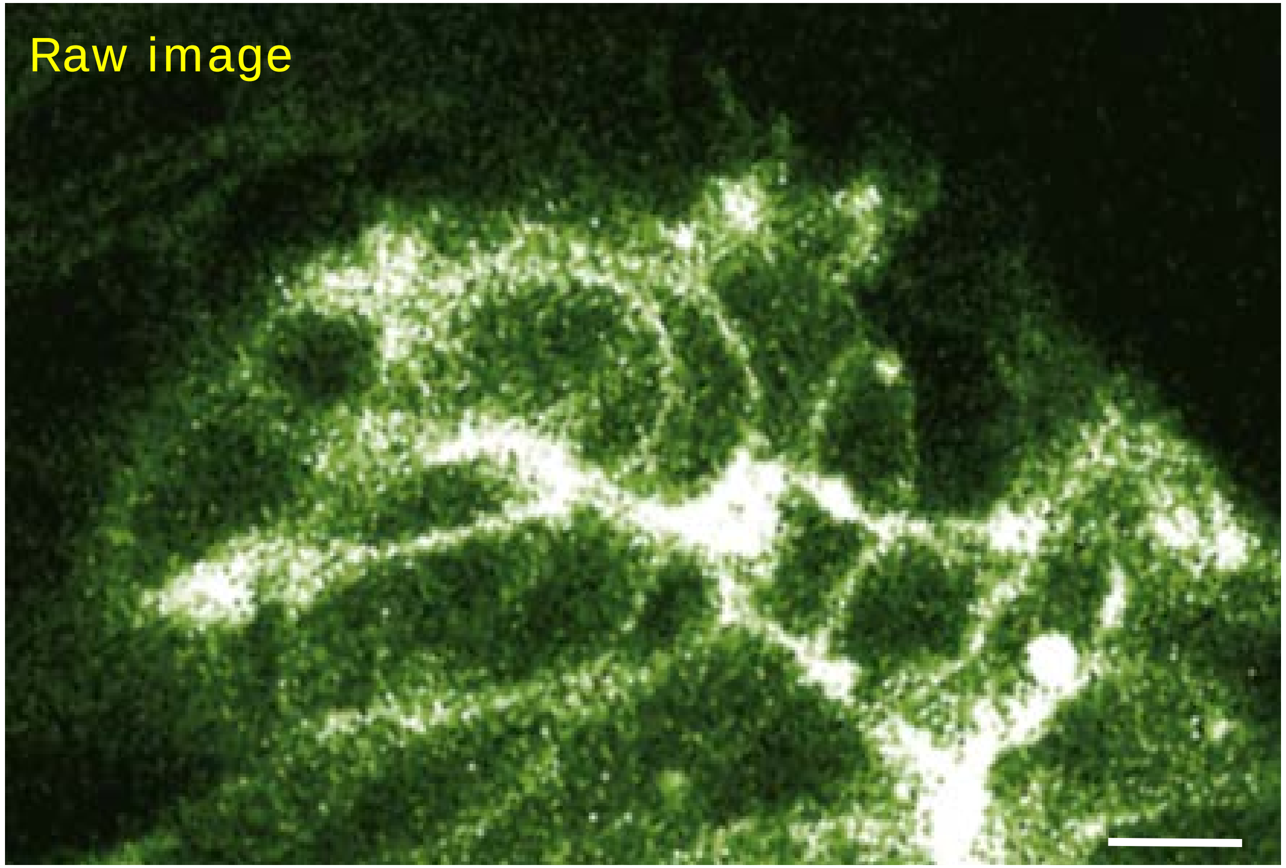
  - …not always (almost never) enough information for a 100% accurate segmentation.

D. Marr (1982) Vision

Sarti & Sethian (2000) Subjective surfaces. PNAS

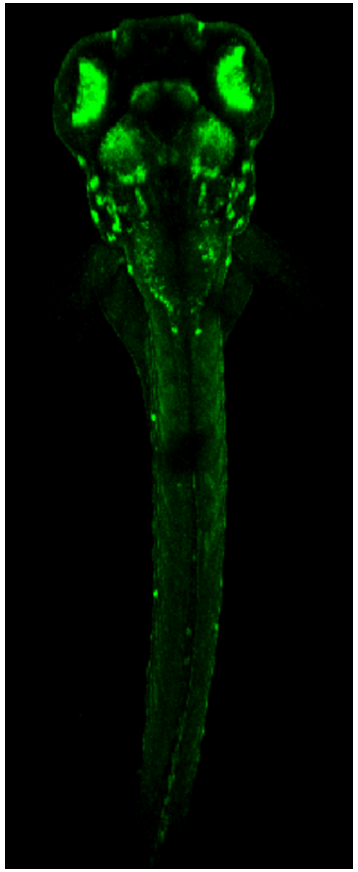
> Processing | Analysis Tasks | Segmentation

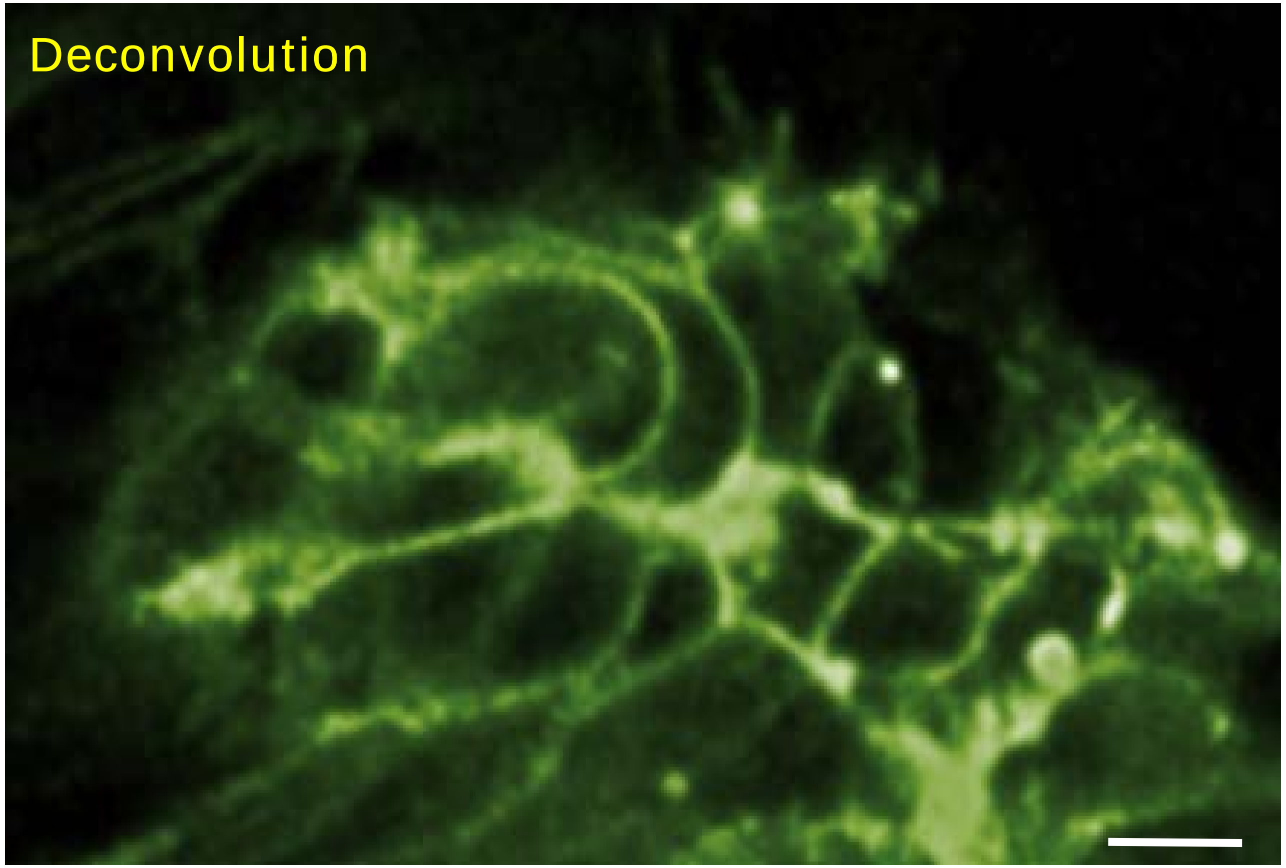




CG Lemus, S Härtel, ML Concha (LEO, SCIAN-Lab, BNI)

> Processing | Analysis Tasks | Segmentation

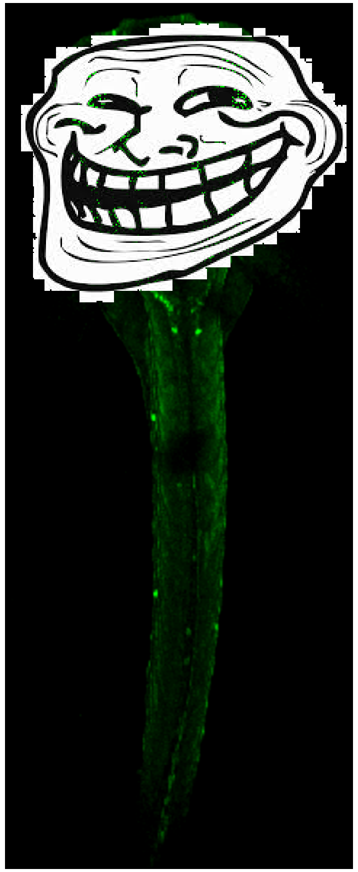


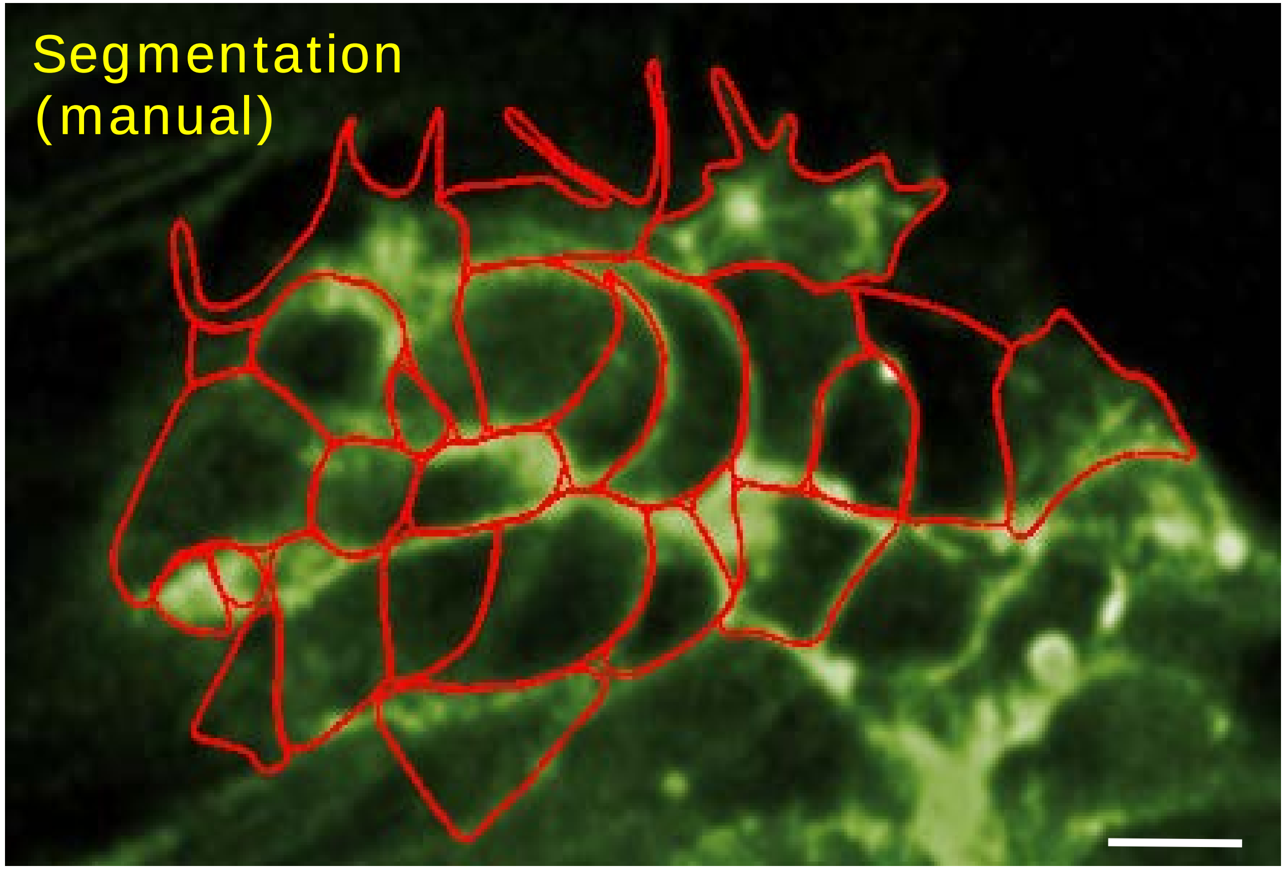


CG Lemus, S Härtel, ML Concha (LEO, SCIAN-Lab, BNI)

> Processing | Analysis Tasks | Segmentation

Problem?





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

> Processing | Analysis Tasks | Segmentation

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.

> 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