Procesamiento de Imágenes I

Jorge Jara, Dante Castagnini, Nicole C. Huerta

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

Laboratory for Scientific Image Analysis (SCIAN-Lab)
Centro de Informática Médica y Telemedicina (CIMT)
Biomedical Neuroscience Institute (BNI)
Programa de Biología Integrativa
Instituto de Ciencias Biomédicas (ICBM)

FACULTAD DE MEDICINA
UNIVERSIDAD DE CHILE

Temas/Clases

I. Principios, conceptos fundamentales

II. Procesamiento

III. Herramientas de software

Temas/Clases

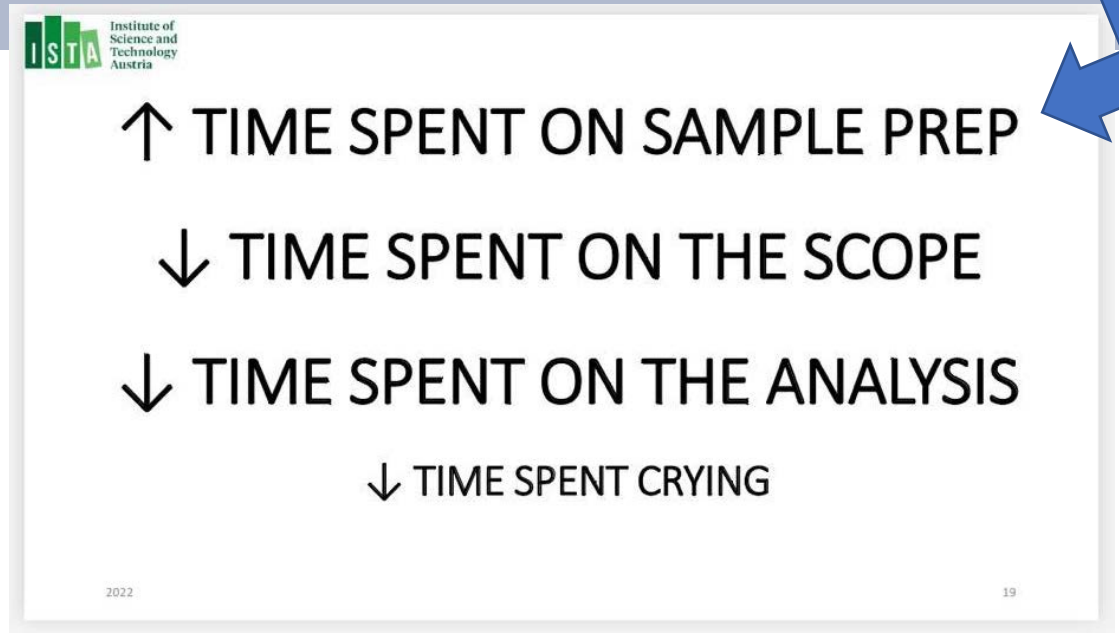
I. Principios, conceptos fundamentales

II. Procesamiento

III. Herramientas de software

> Principios

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



Incluye preparación de scope/adqusición

Source: BioImage Analysts all over the world in the 20's

> Principios

- “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)

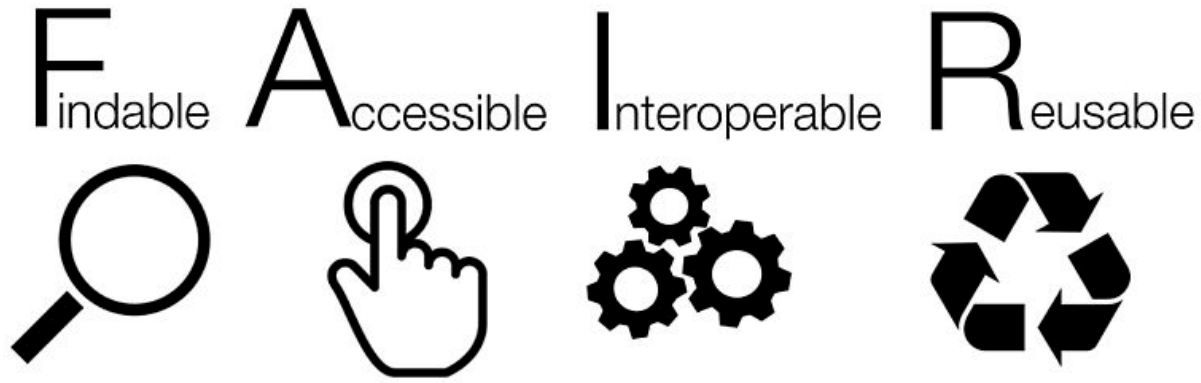
https://doi.org/10.15252/embj.2020105889

- 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

> Principios

- 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.

> Principios

nature methods

Explore content · About the journal · Publish with us

nature › nature methods › perspectives › article

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

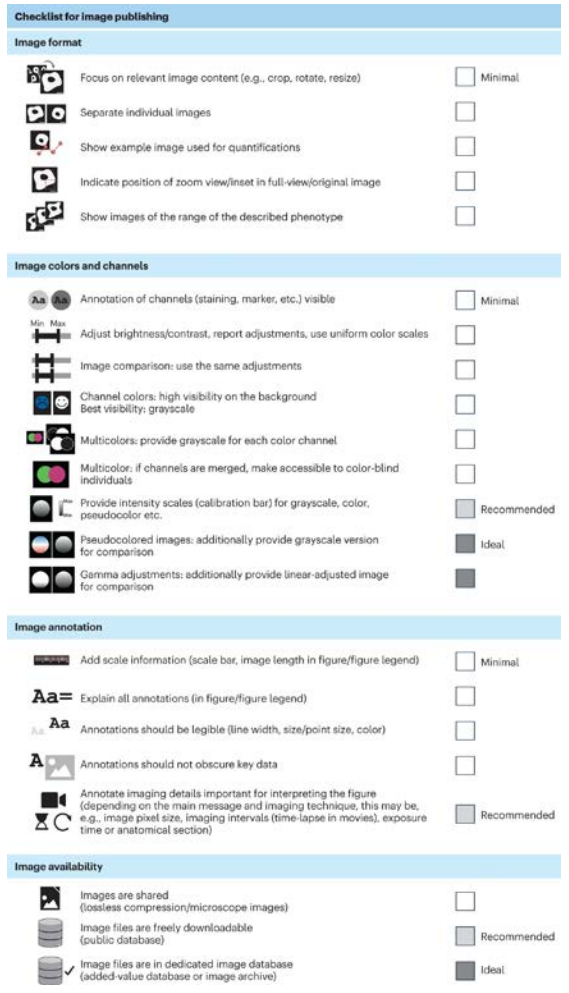
Show fewer authors

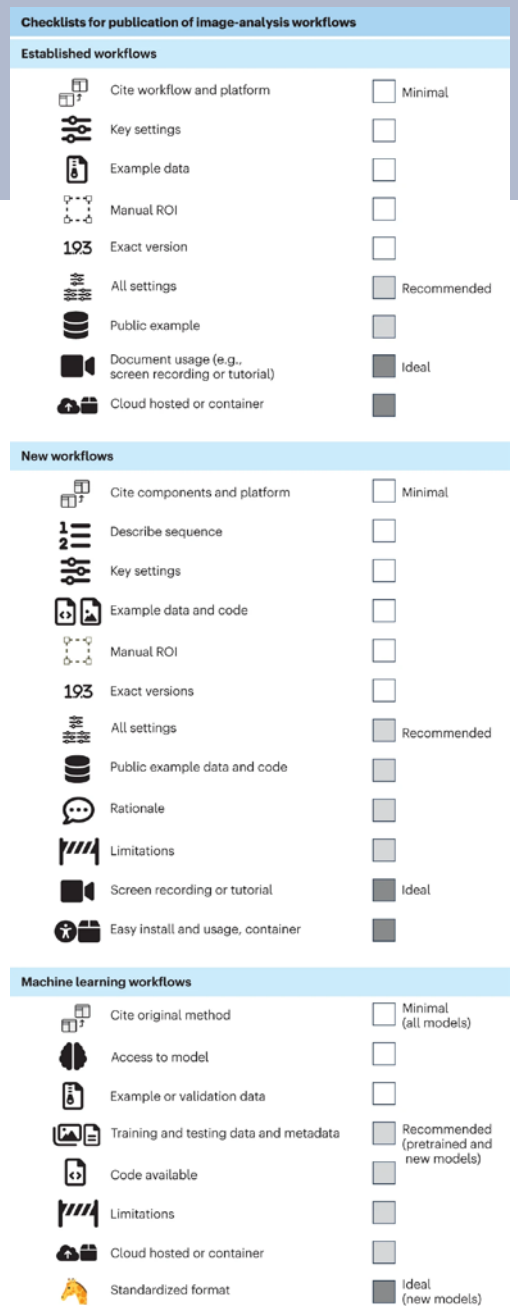
Nature Methods (2023) |

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

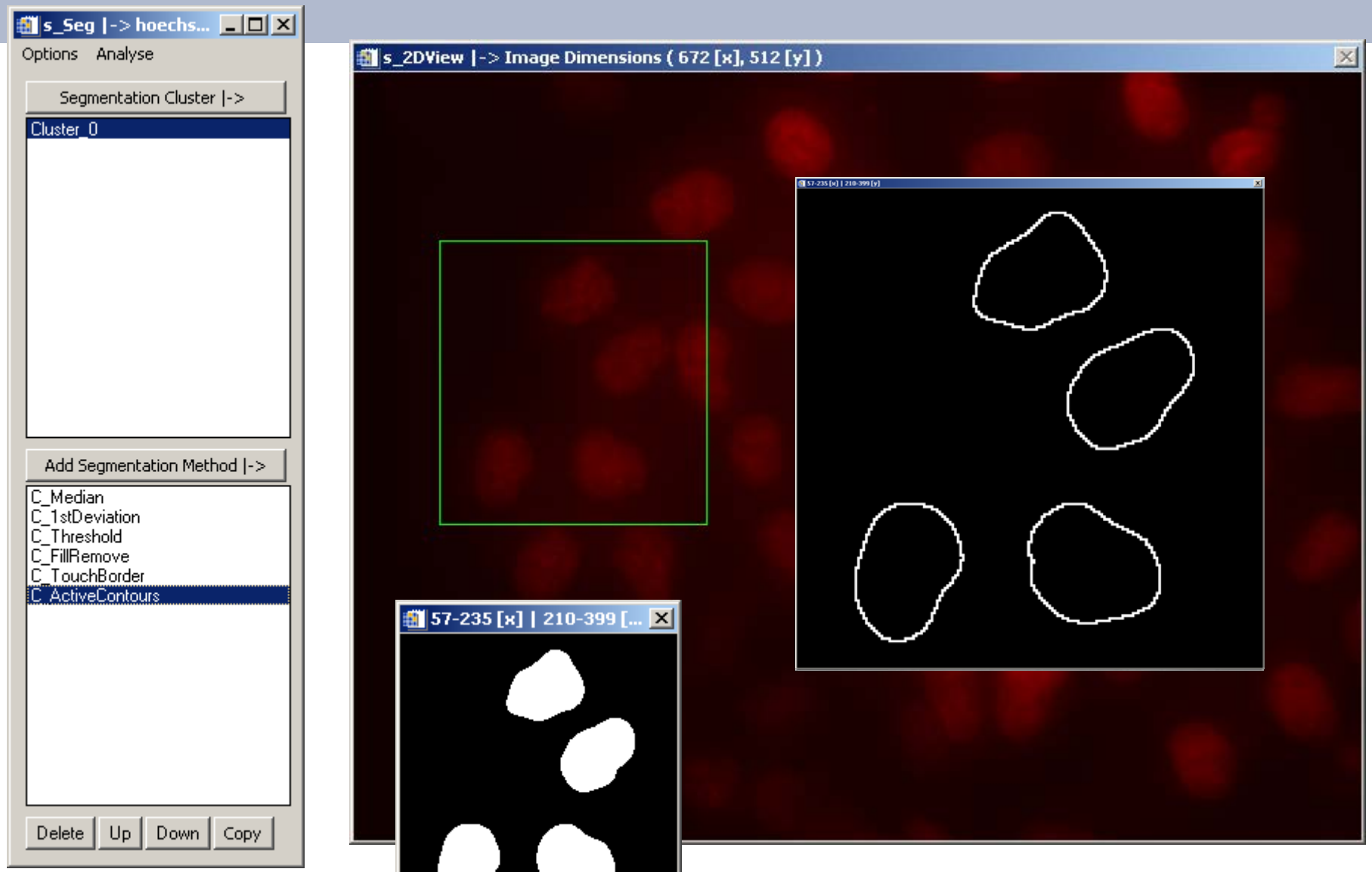




> Conceptos

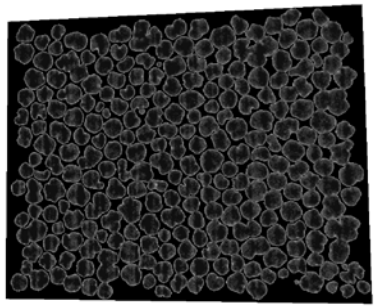
- Signal & noise
  - SNR
- Signal amplitude
- Signal frequency/frequencies
- Signal amplitude
  - Fluorescence intensity
- Segmentation
- Pixel / Voxel
- Bit
- (Sample) Bit depth
- Sampling frequency/interval
  - Pixel size or spacing
- (Intensity) Histogram
- Filter

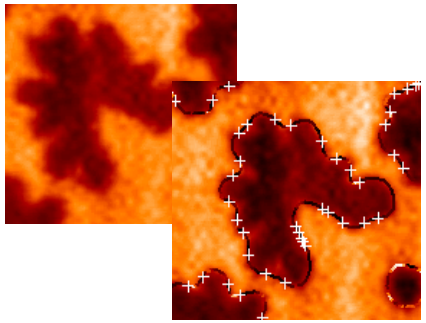
> Conceptos

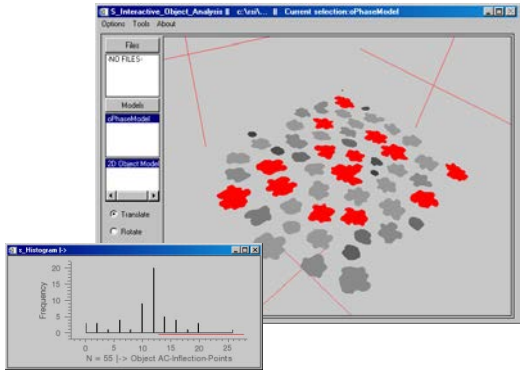

s_Seg |-> hoechs...
Options
Analyse
Segmentation Cluster |->
Cluster_0
Add Segmentation Method |->
C_Median
C_1stDeviation
C_Threshold
C_FillRemove
C_TouchBorder
C_ActiveContours
Delete
Up
Down
Copy
s_2DView |-> Image Dimensions (672 [x], 512 [y])
57-235 [x] | 210-399 [...

> Conceptos

Image Processing







Acquisition
- Microscopes
- Cameras
- …

(pre)Treatment or Filtering
- Restoring
- Contrast enhancement
- Artifact removal
- …

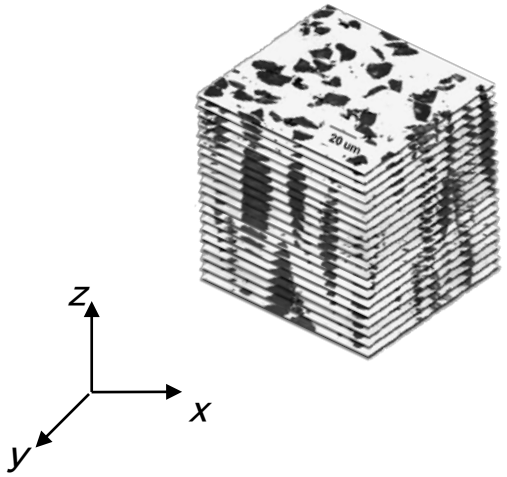
Analysis
- ROIs / models
- Measurements

Understanding
- Higher level task
- Hypotheses support

images **images** **images + descriptions**

> Conceptos

- **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)

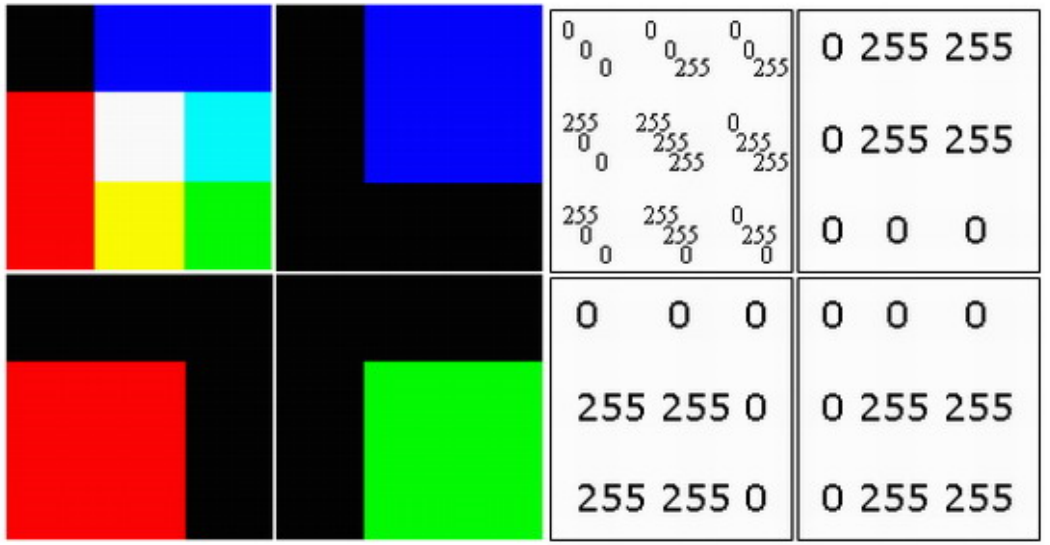
    - If not discrete? Continuum
      Example: real numbers in the [0,1] interval (infinite set)

> Conceptos

- 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



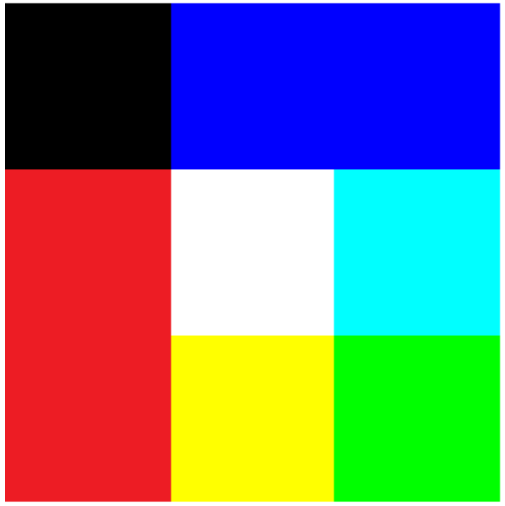
$$I = f(x, y)$$

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

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

> Conceptos

- **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

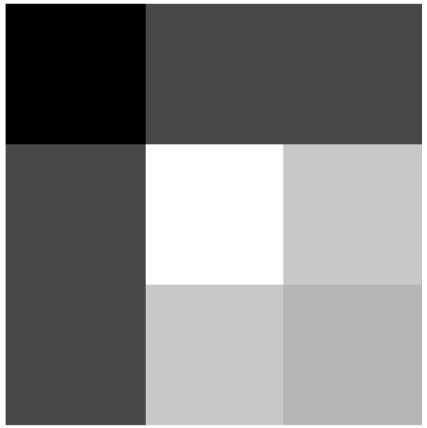
$$r[x,y]\ g[x,y]\ b[x,y]$$

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

> Conceptos

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

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

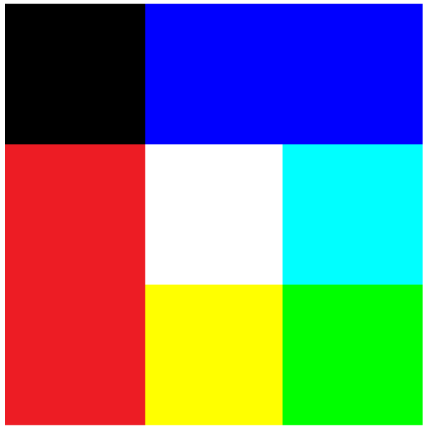


$I[x, y]$

How to go from an RGB to a greyscale image?

> Conceptos

- From RGB to greyscale

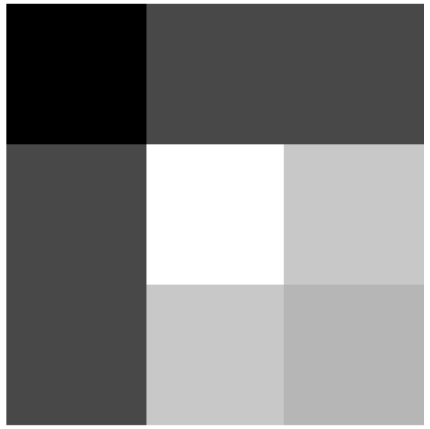


0	0	0
0	0	0
0	255	255
255	255	0
0	255	255
0	255	255
255	255	0
0	255	255
0	0	0

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

0	85	85
85	255	170
85	170	85

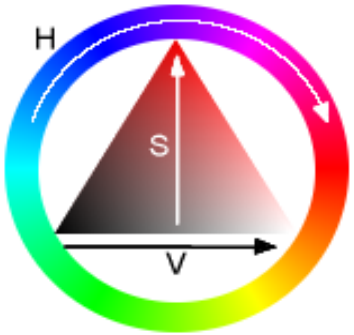
$I[x,y]$

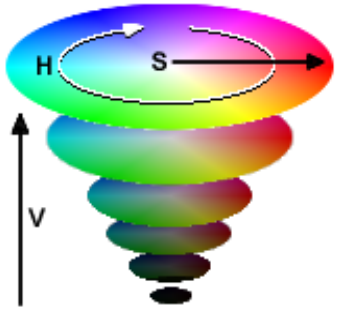


How good is the human eye resolving colors in R, G or B tones?

Conceptos

- **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.

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

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

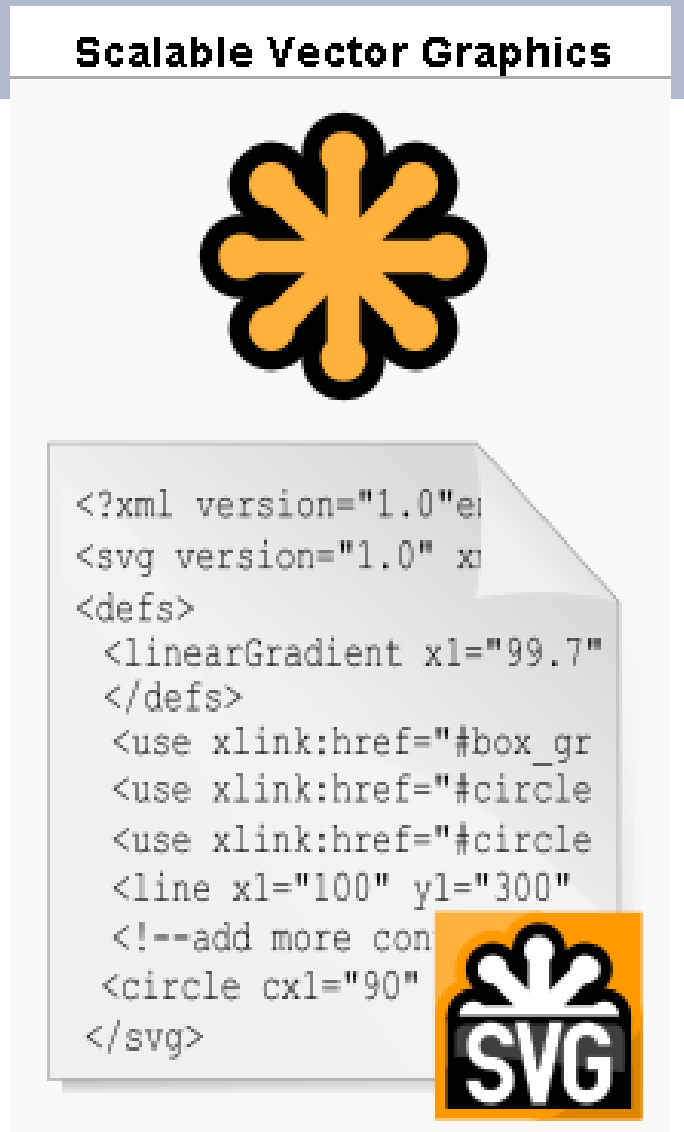
$$I = \frac{R+G+B}{3}$$

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

Conceptos

- 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

> Conceptos

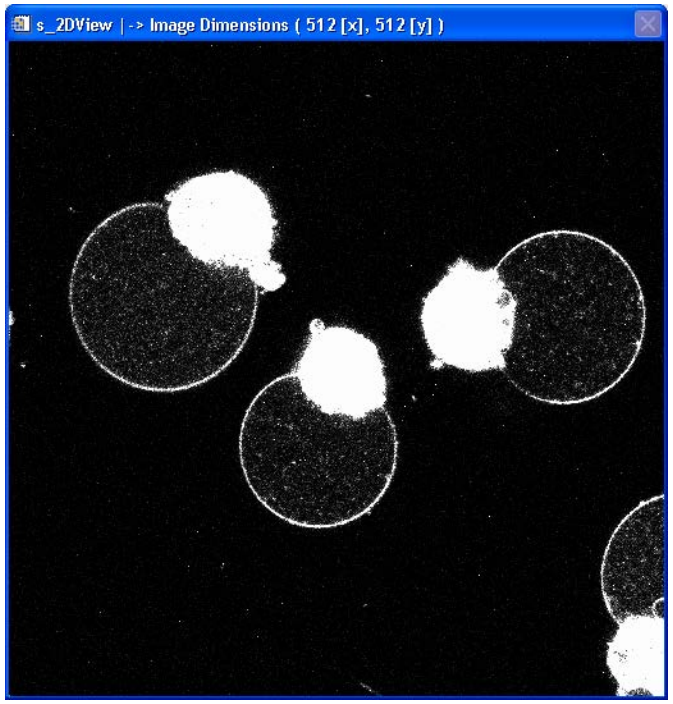
- …so, a digital image can be treated as a mathematical function (in the mathematical sense)…
  - on a discrete domain
  - with numeric values associated to each elements, representing a property (such as color, brightness, depth, etc.)
- Nowadays, digital ≡ binary

Aubert & Kornprobst (2006)
Mathematical Problems In Image Processing

> Conceptos

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

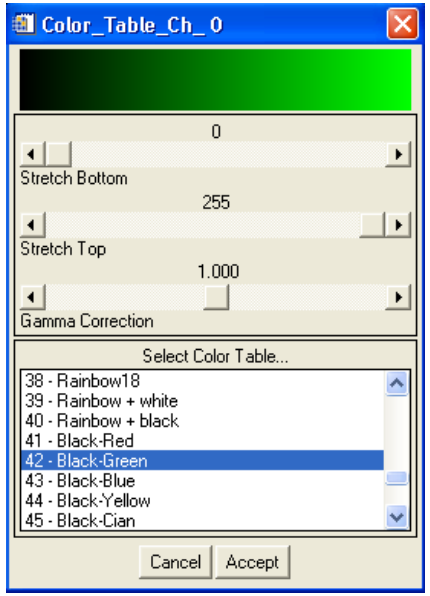
$I(290,267) = 220$

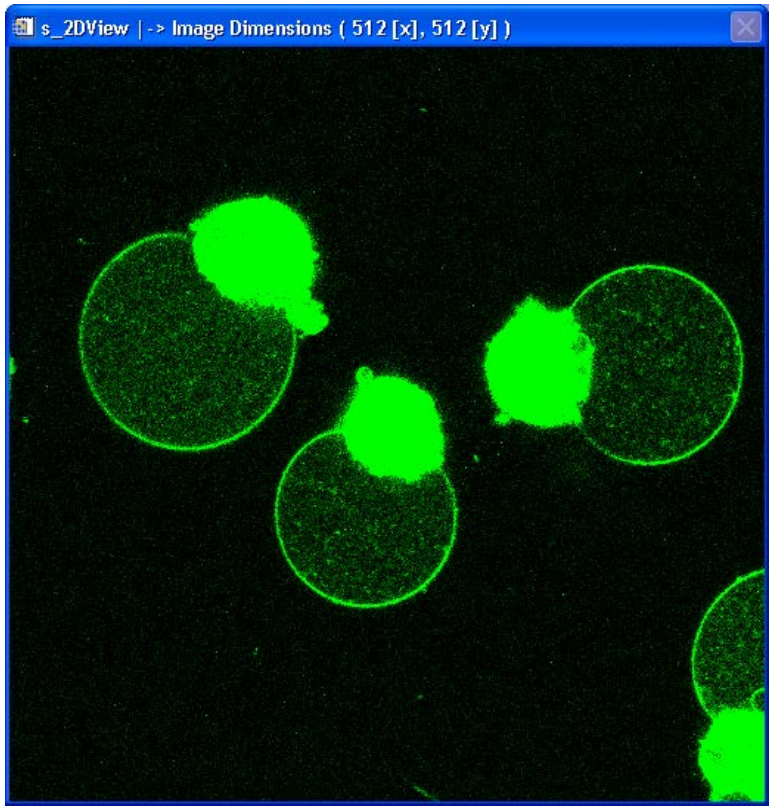
A n-bit greyscale image encodes up to 2^n intensity values

> Conceptos

- 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

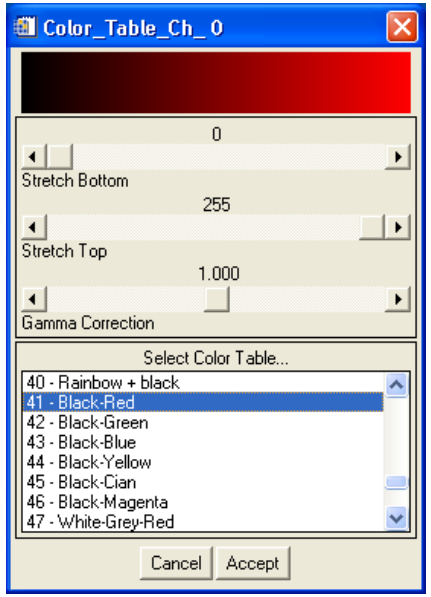




> Conceptos

- Black-to-red LUT…

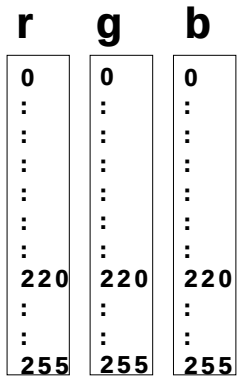
r	g	b
0	0	0
1	0	0
2	0	0
:	:	:
:	:	:
:	:	:
:	:	:
220	0	0
:	:	:
:	:	:
255	0	0

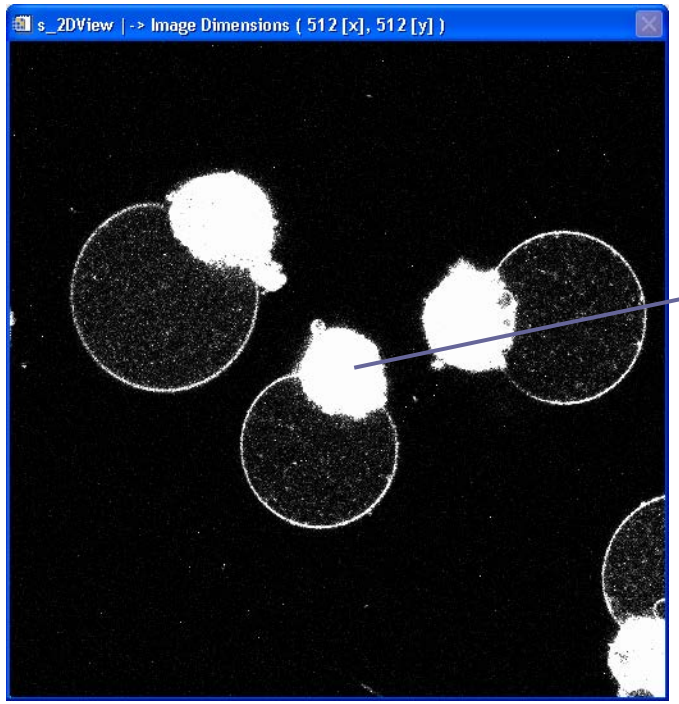


In ImageJ/FIJI: Go to the "Image"->"Lookup Tables" menu

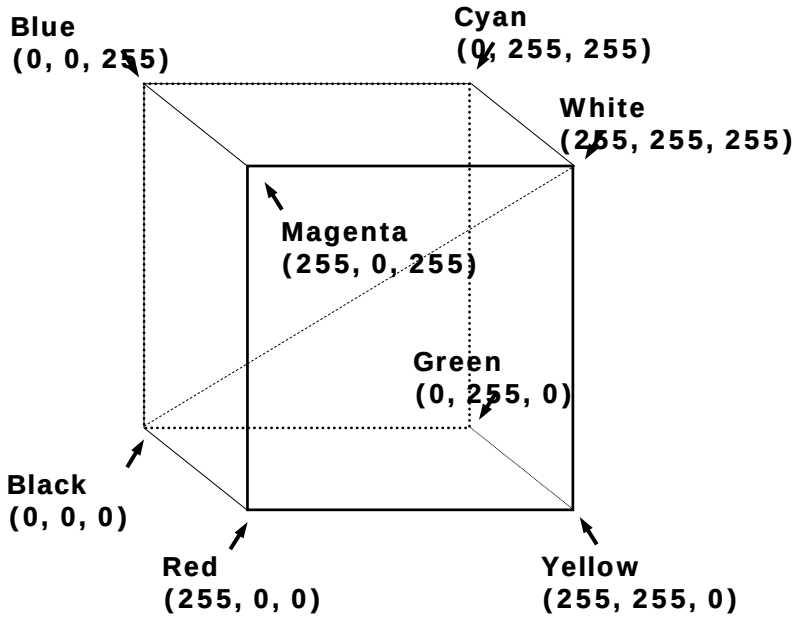
> Conceptos

- …or any custom color table



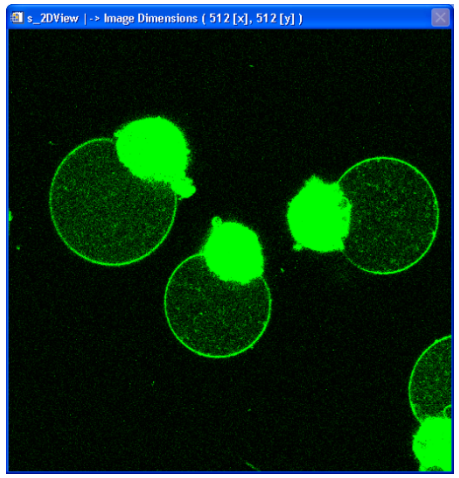


I(290,267) = 220



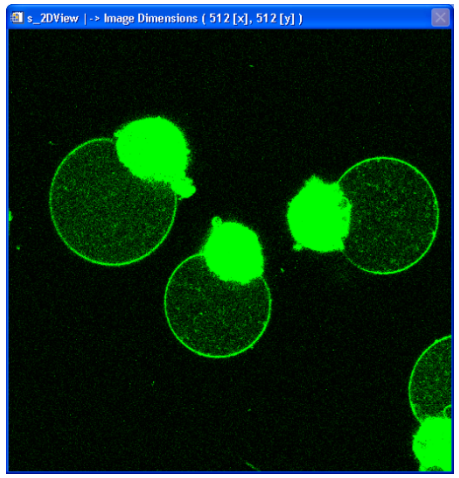
Red $= [r_0, r_1, \ldots, r_{255}]$
Green $= [g_0, g_1, \ldots, g_{255}]$
Blue $= [b_0, b_1, \ldots, b_{255}]$

> Conceptos



Occlusions may occur in 3D visualization

> Conceptos



[R, G, B, α]

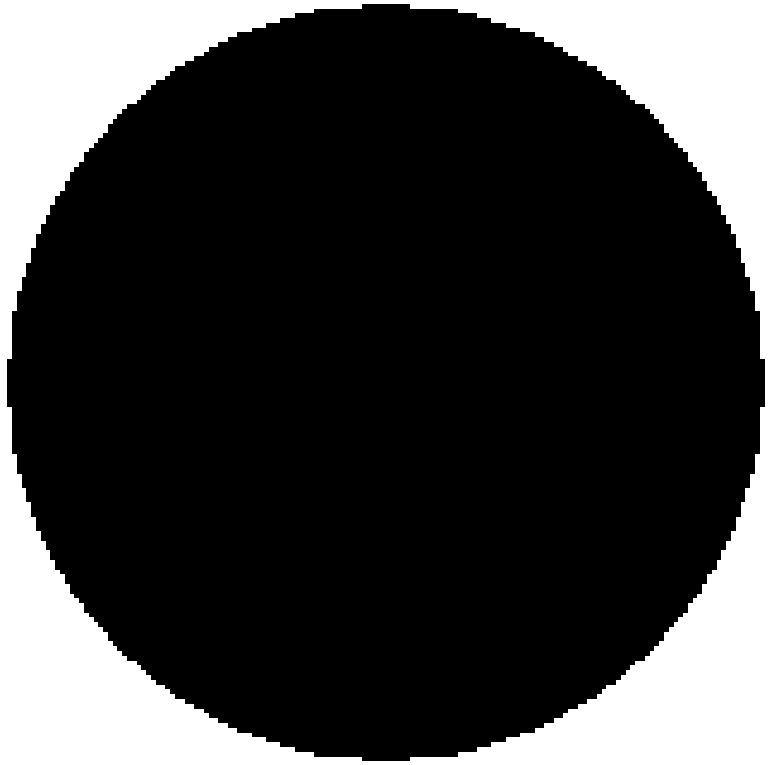
pacity values can
e associated to
ixels (or voxels in
3D) for visualization
purposes

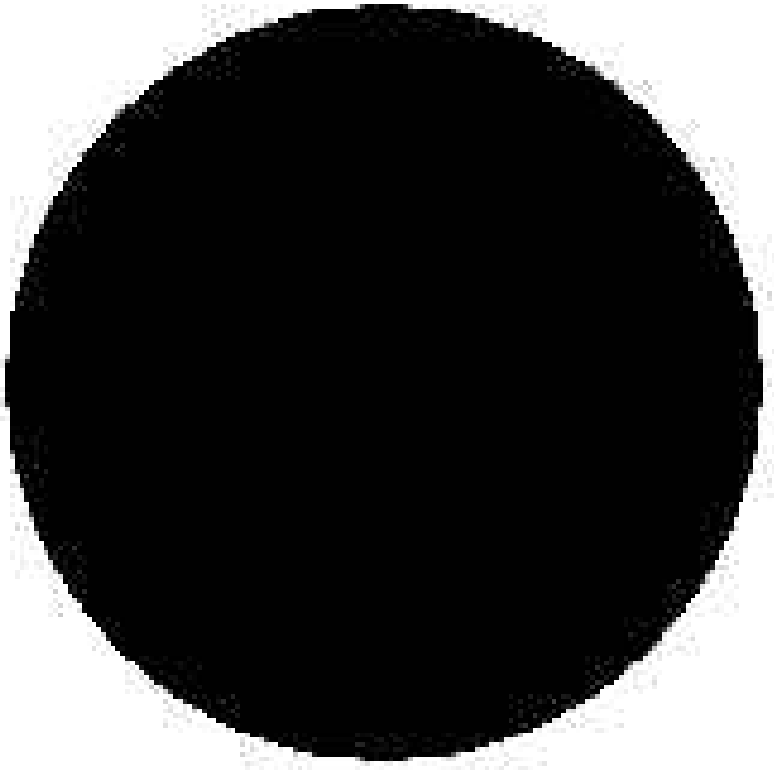
> Conceptos (resumen parcial)

- 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…

> Conceptos

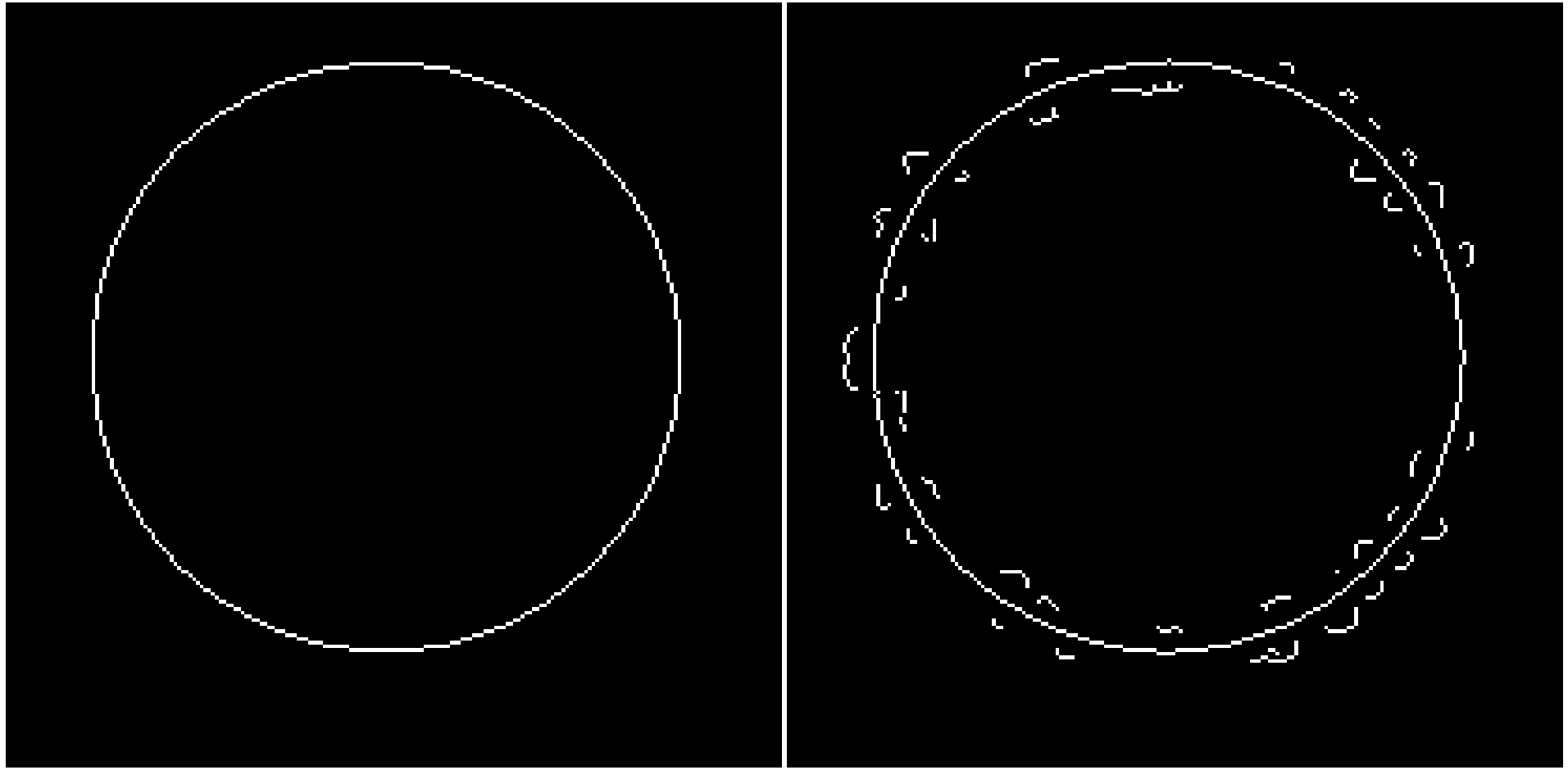
- **Lossless** (left) and **Lossy** (right) file/compression





Lossy

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



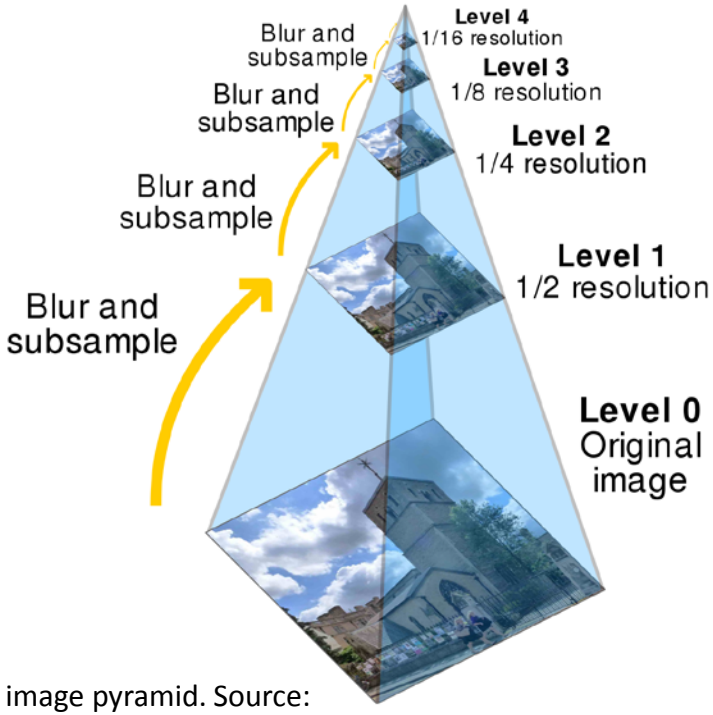
> Conceptos

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)

> Conceptos

- **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