

Procesamiento de Imágenes I



Curso Binacional 2023
Uruguay - Chile

Microscopía para el Estudio de Biofilms Bacterianos

Instituto de Investigaciones Biológicas Clemente Estable (IIBCE)
Instituto de Neurociencia Biomédica (BNI), ICBM, F-Med, U-Chile

2 - 6 de octubre - Teórico
9 - 13 de octubre - Práctico Uy.
30 de octubre - 3 de noviembre - Práctico Cl.

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

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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>



ISTA Institute of Science and Technology Austria

- ↑ TIME SPENT ON SAMPLE PREP
- ↓ TIME SPENT ON THE SCOPE
- ↓ TIME SPENT ON THE ANALYSIS
- ↓ TIME SPENT CRYING

2022 19

Incluye preparación de scope/adquisición

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/emj.2020105889>

- Reproducibility

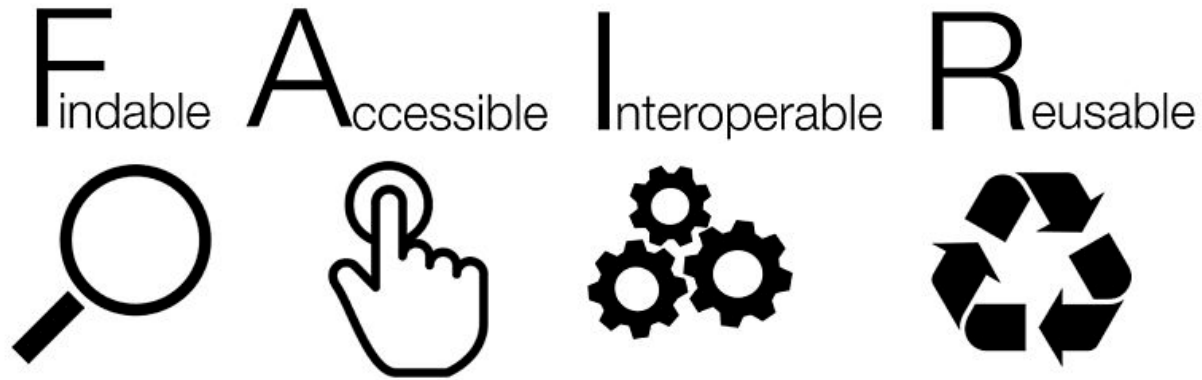
- Methods
- Results
- Inferential

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

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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](#) 

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Abstract

[Nature Methods](#) (2023) |

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 Ideal

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 Ideal

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 Ideal

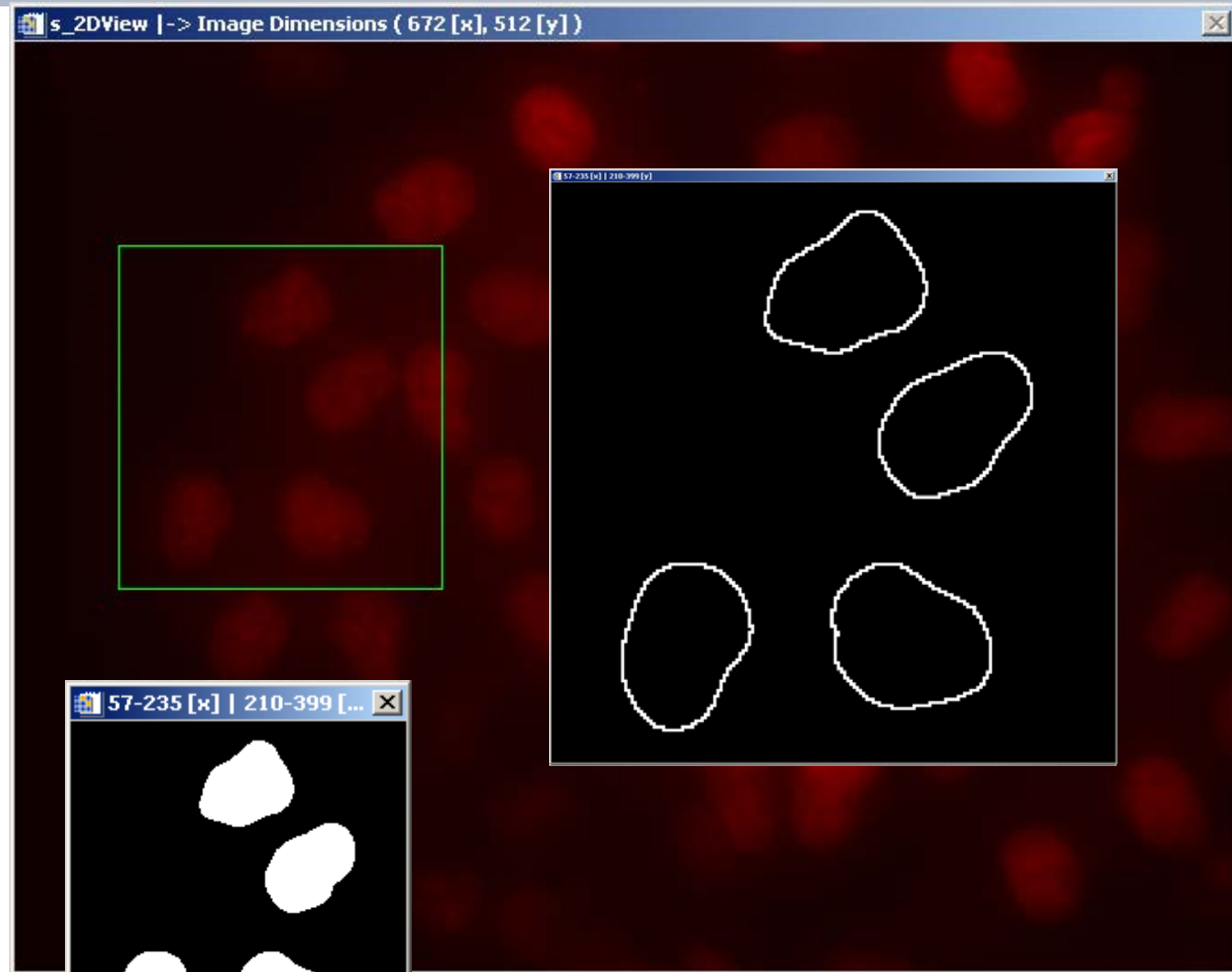
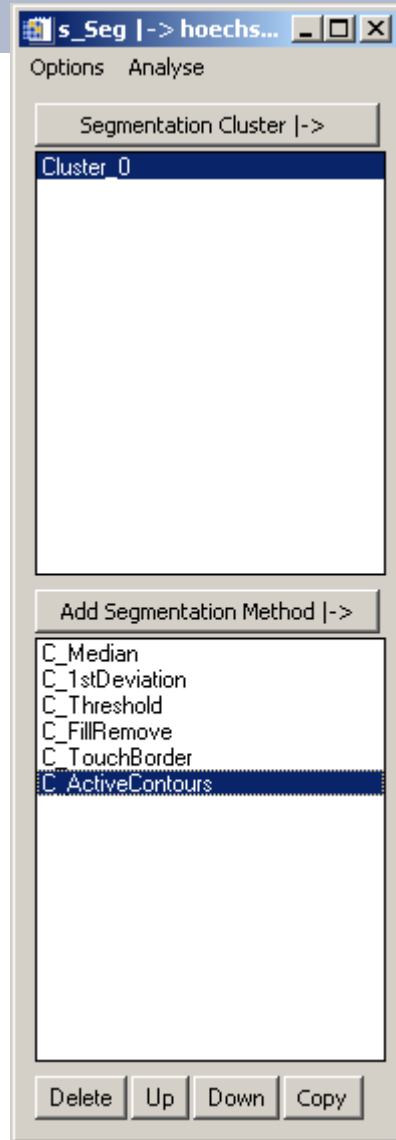
Machine learning workflows

-  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)

> Conceptos

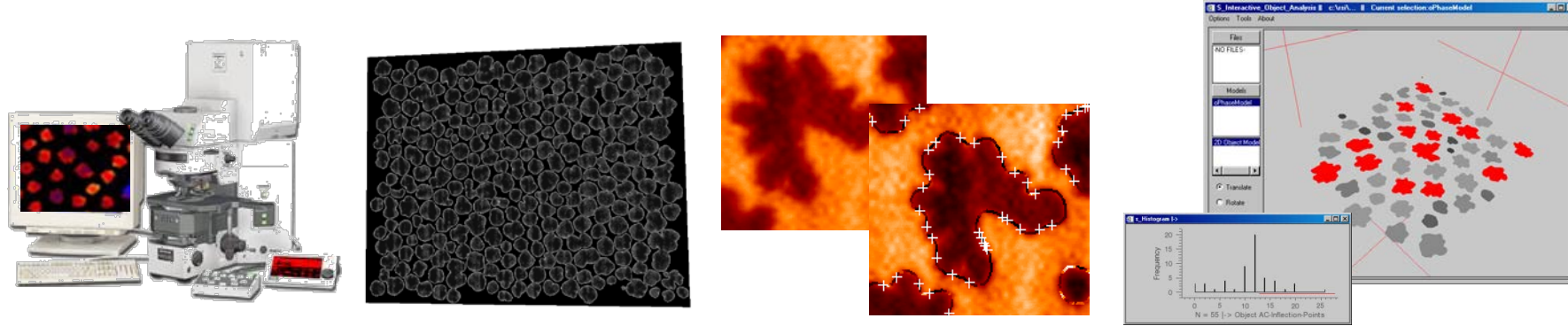
- Signal & noise
 - SNR
- Signal amplitude
- Signal frequency/frequencies
- Signal amplitude
 - Fluorescence intensity
- Segmentation
- Pixel / Voxel
- Bit
- (Sample) Bit depth
- Sampling frequency/interval
 - Pixel size or spacing
- (Intensity) Histogram
- Filter

> Conceptos



> 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

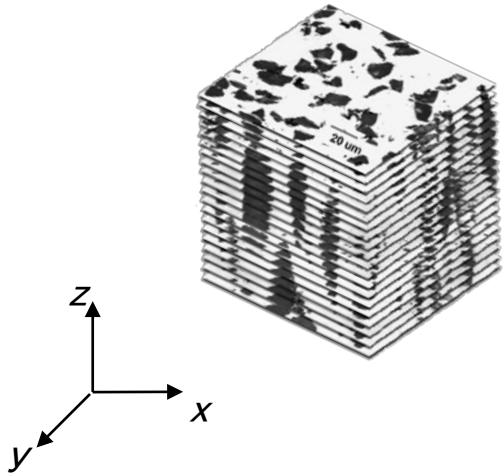
images +
descriptions



> Conceptos

- **Digital image processing** means...

- Digital... discrete, finite



- A discrete set is composed by elements which are “isolated” one from another

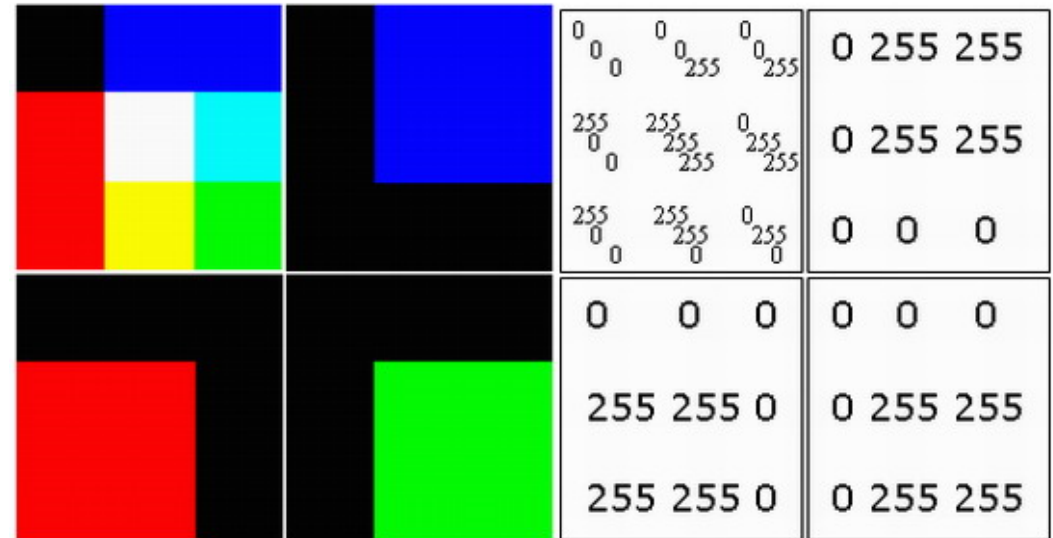
Examples:

- Natural numbers $\{1, 2, 3, \dots\}$ (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



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

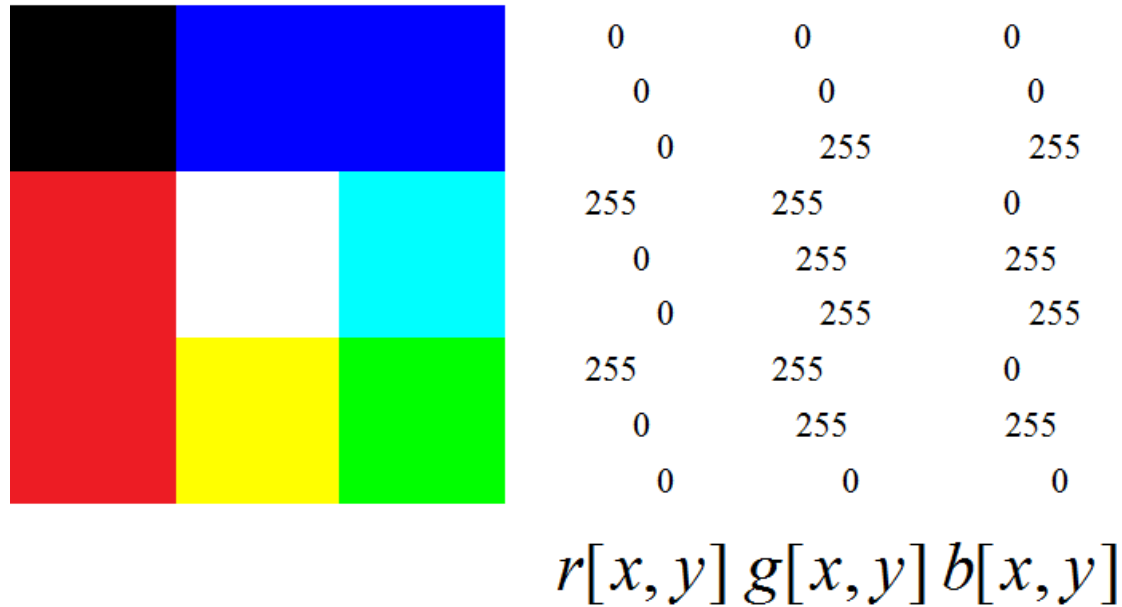
$$(x, y) \in [0, \dim_x - 1] \times [0, \dim_y - 1]$$

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

> Conceptos

- **Color space** (through examples)

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



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

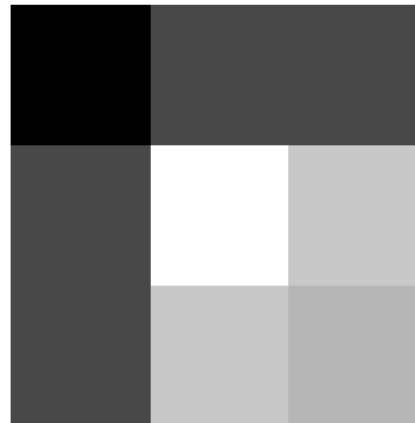
> Concepts

- **Greyscale image**

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

0	85	85
85	255	170
85	170	85

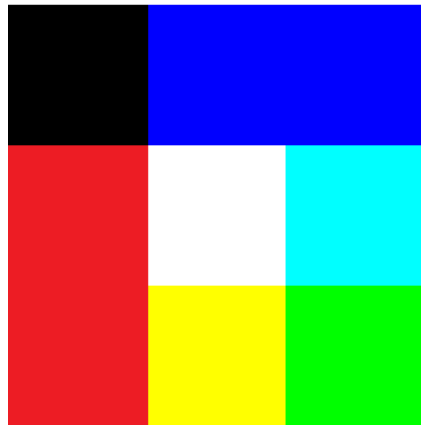
$I[x,y]$



How to go from an RGB to a greyscale image?

> 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

$I[x,y]$



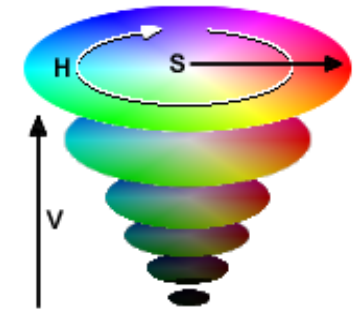
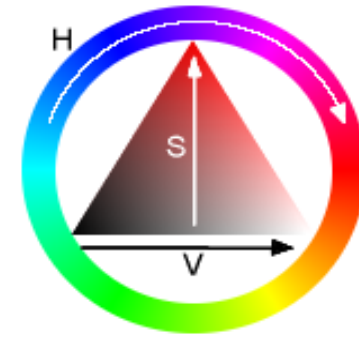
How good is the human eye resolving colors in R, G or B tones?

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.



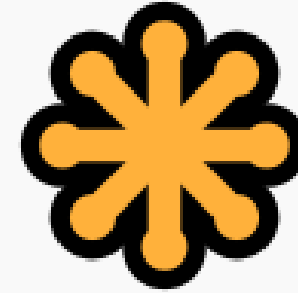
$$H = \begin{cases} \Theta & G \geq B \\ 2\pi - \Theta & G < B \end{cases}$$
$$S = 1 - 3 \frac{\min(R, G, B)}{R + G + B}$$
$$I = \frac{R + G + B}{3}$$

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

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

Scalable Vector Graphics



```
<?xml version="1.0" encoding="UTF-8" standalone="no" ?>
<svg version="1.0" xmlns="http://www.w3.org/2000/svg" >
  <defs>
    <linearGradient x1="99.7" x2="0" y1="0" y2="0" />
  </defs>
  <use xlink:href="#box_gr" />
  <use xlink:href="#circle" />
  <use xlink:href="#circle" />
  <line x1="100" y1="300" />
  <!--add more content here-->
  <circle cx1="90" cy1="300" />
</svg>
```



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

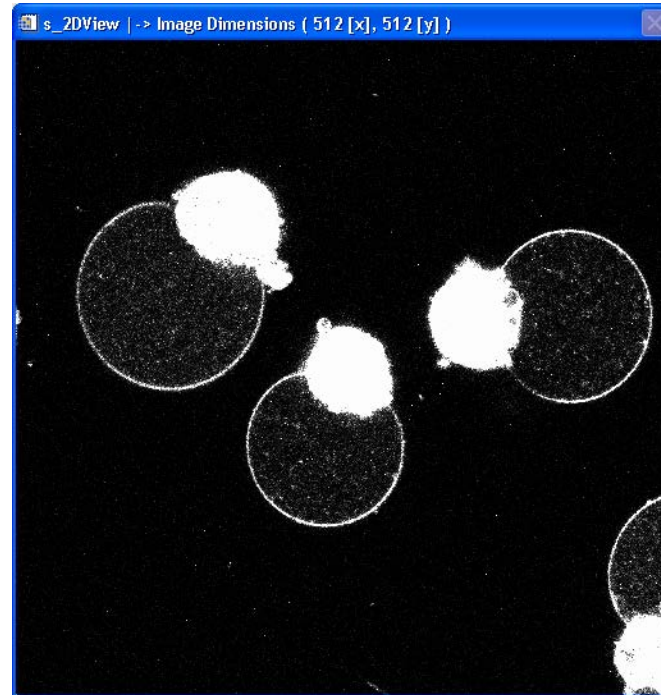
> Conceptos

- ...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 \equiv binary

> 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

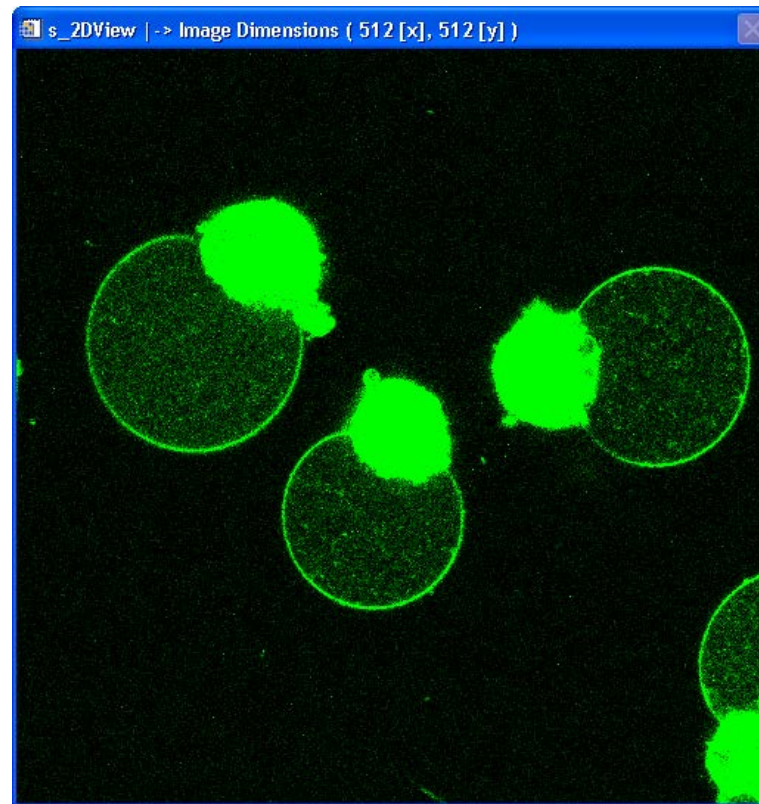
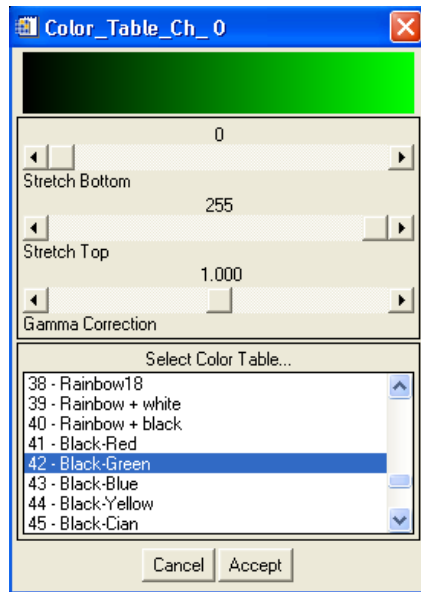
$$I(290,267) = 220$$

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...

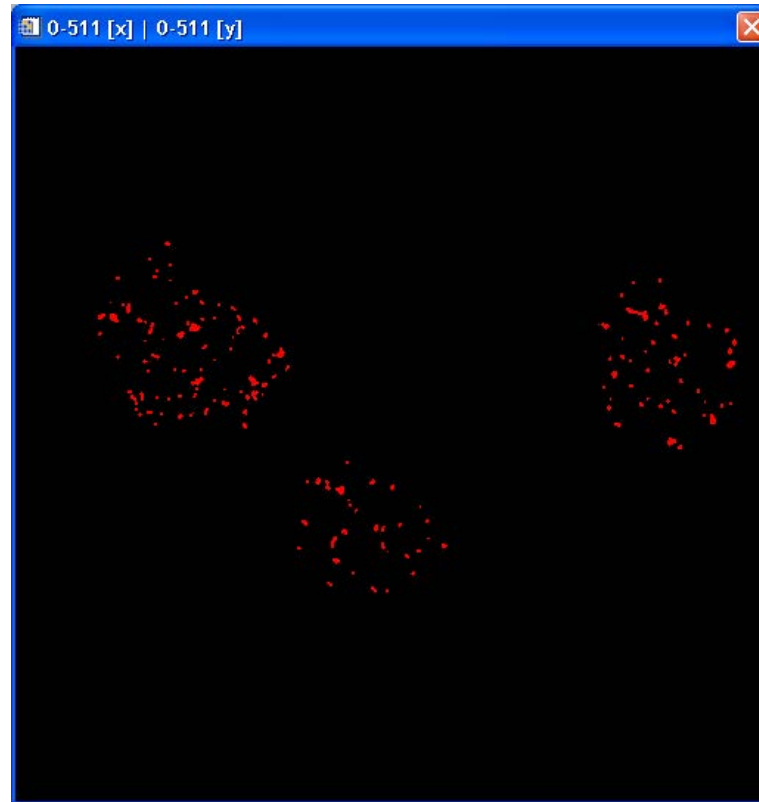
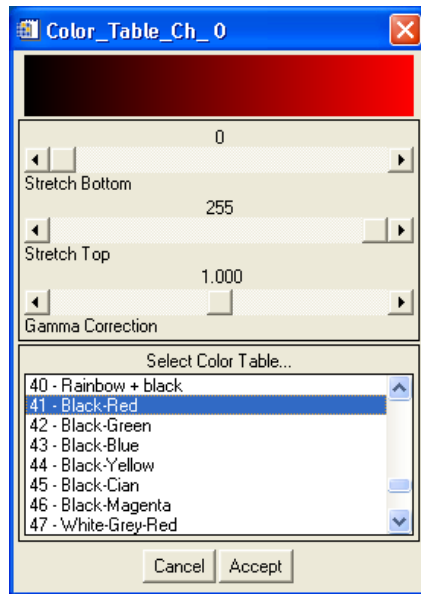
	r	g	b
0	0	0	0
0	1	0	0
0	2	0	0
:	:	:	:
:	:	:	:
:	:	:	:
:	:	:	:
0	200	0	0
:	:	:	:
:	:	:	:
0	255	0	0



> Conceptos

- Black-to-red LUT...

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

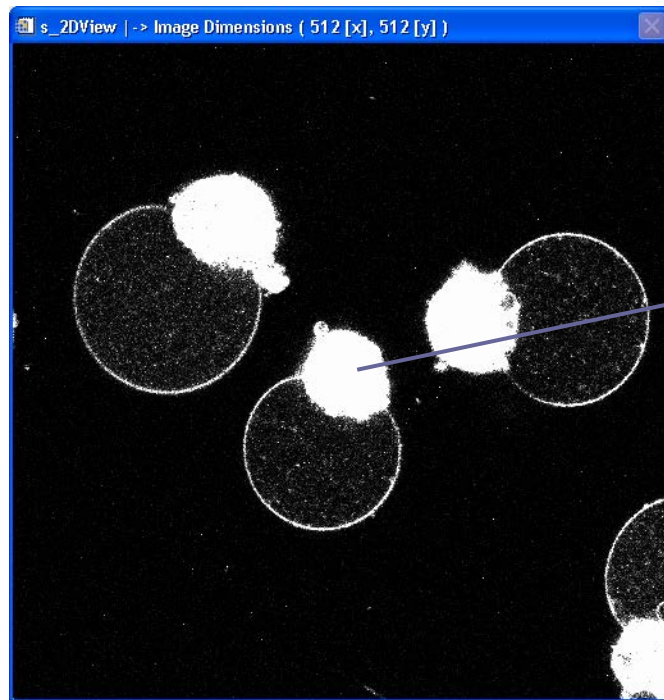


In ImageJ/FIJI: Go to the "Image" -> "Lookup Tables" menu

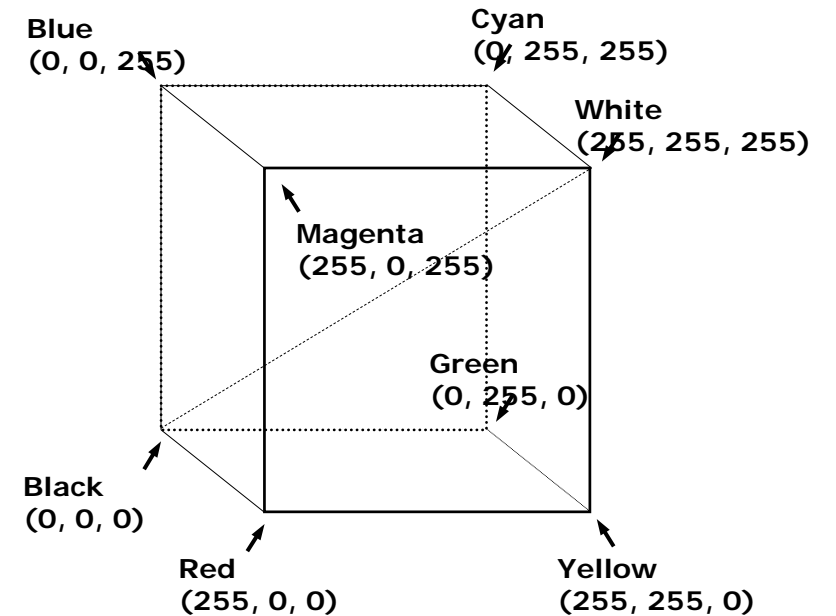
> Conceptos

- ...or any custom color table

r	g	b
0	0	0
⋮	⋮	⋮
⋮	⋮	⋮
⋮	⋮	⋮
⋮	⋮	⋮
⋮	⋮	⋮
⋮	⋮	⋮
⋮	⋮	⋮
220	220	220
⋮	⋮	⋮
⋮	⋮	⋮
255	255	255

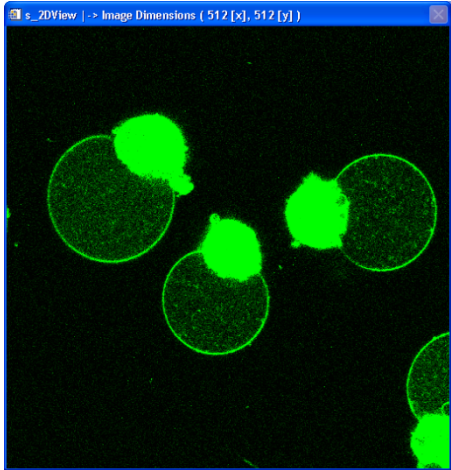


$I(290,267) = 220$



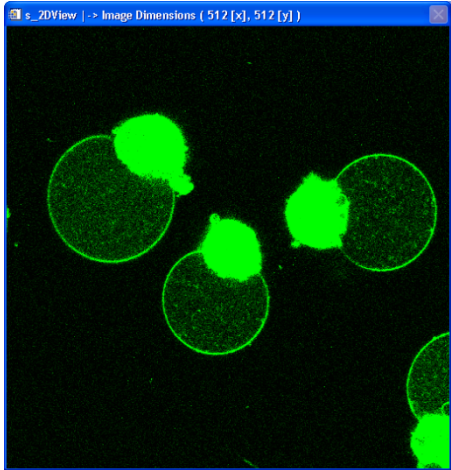
Red = $[r_0, r_1, \dots, r_{255}]$
Green = $[g_0, g_1, \dots, g_{255}]$
Blue = $[b_0, b_1, \dots, b_{255}]$

> Conceptos



Occlusions may occur in 3D visualization

> Conceptos



[R, G, B, α]

opacity values can
be associated to
pixels (or voxels in
3D) for visualization
purposes

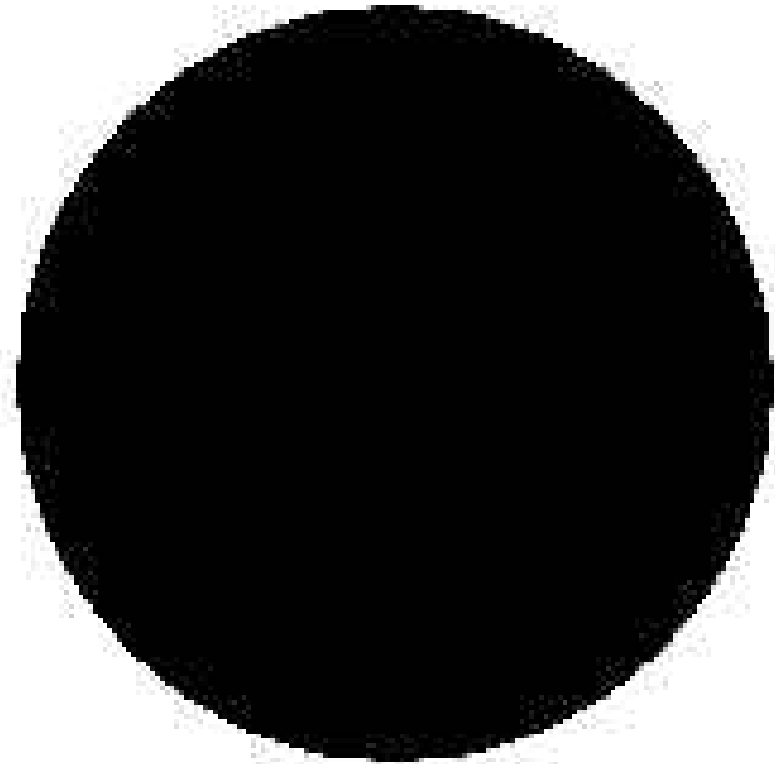
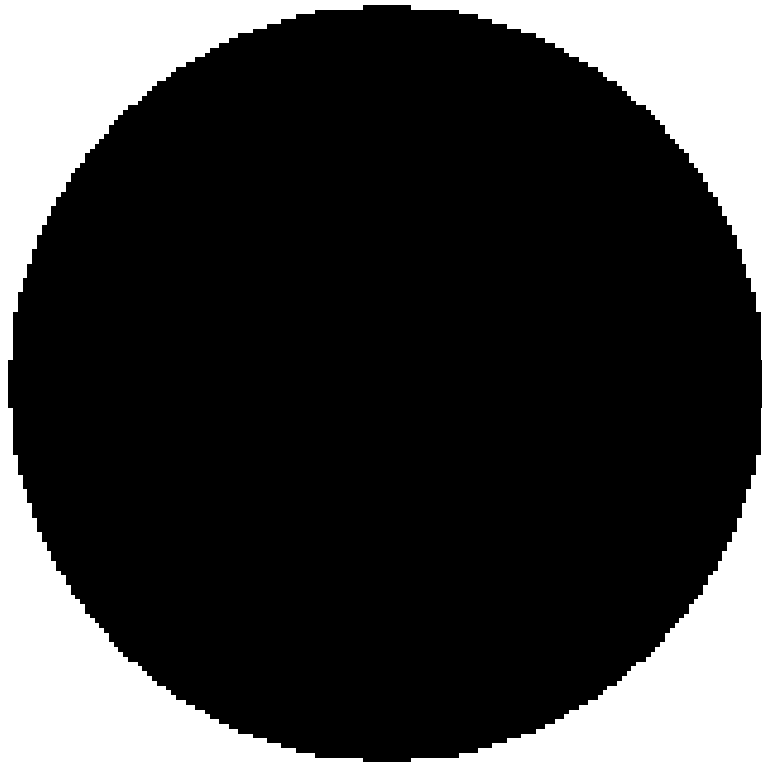


> Conceptos (resumen parcial)

- 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

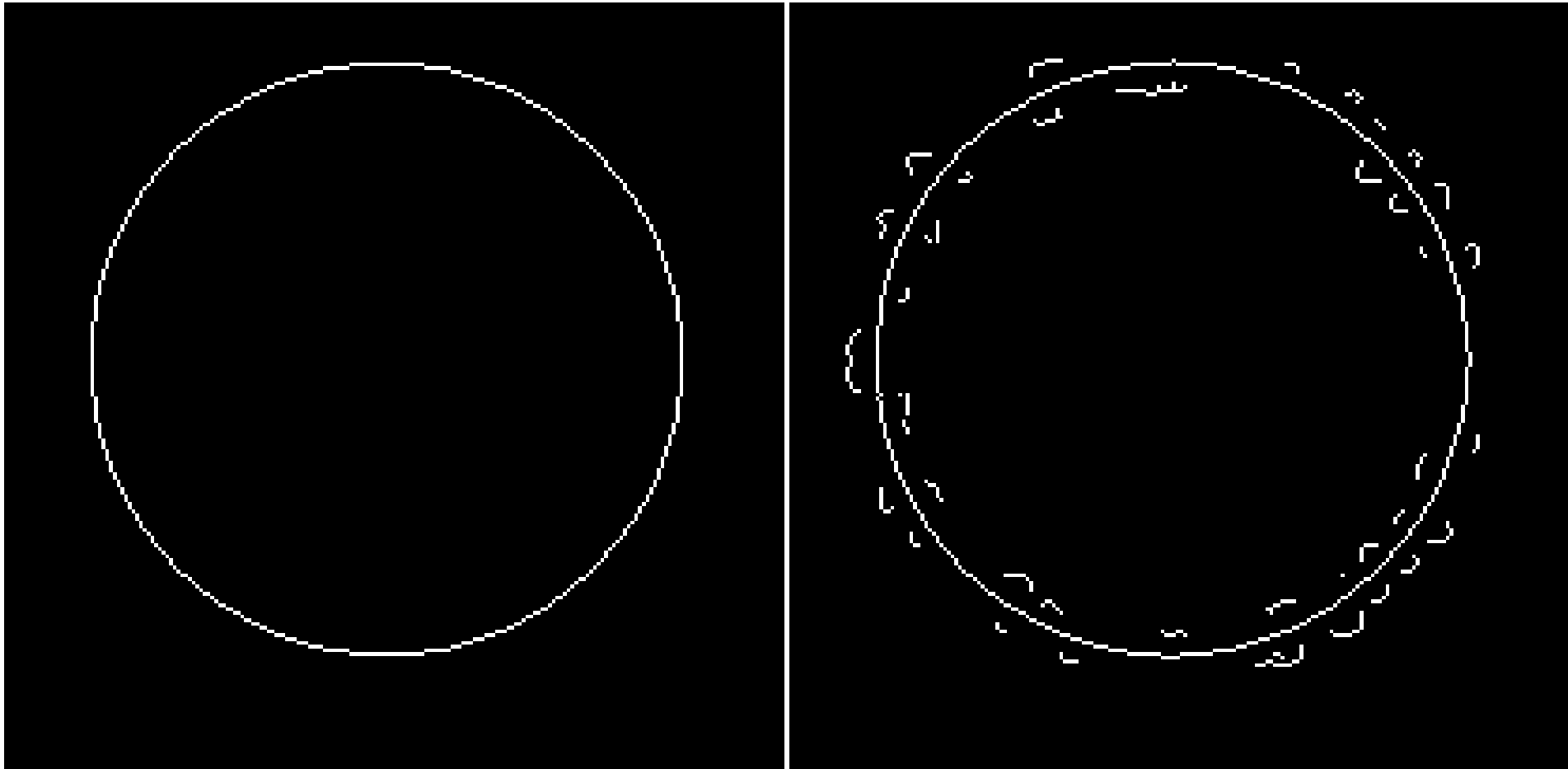
- **Lossless** (left) and **Lossy** (right) file/compression



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

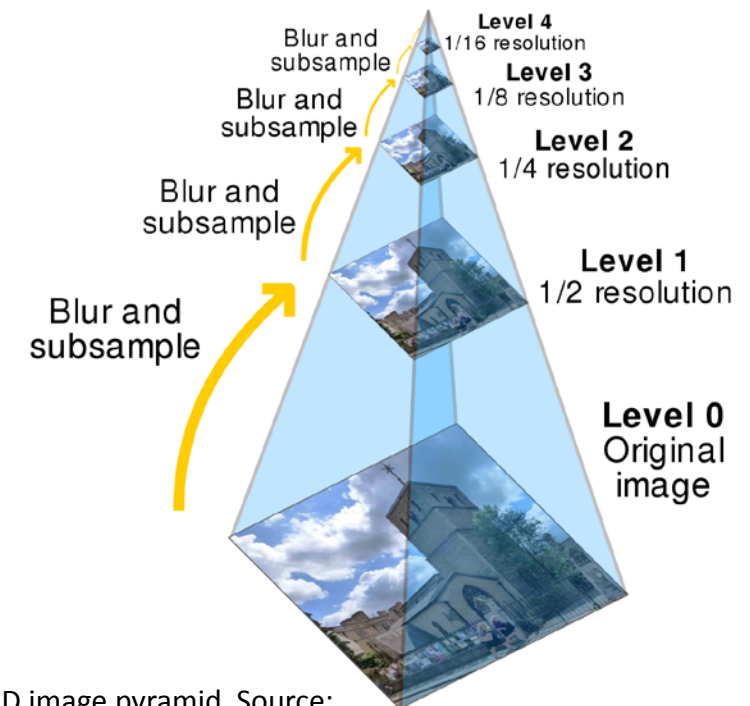
- N5
- Zarr

<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_\(image_processing\)](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