Introduction to
Digital Image Processing and Analysis

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network of european bioimage analysts

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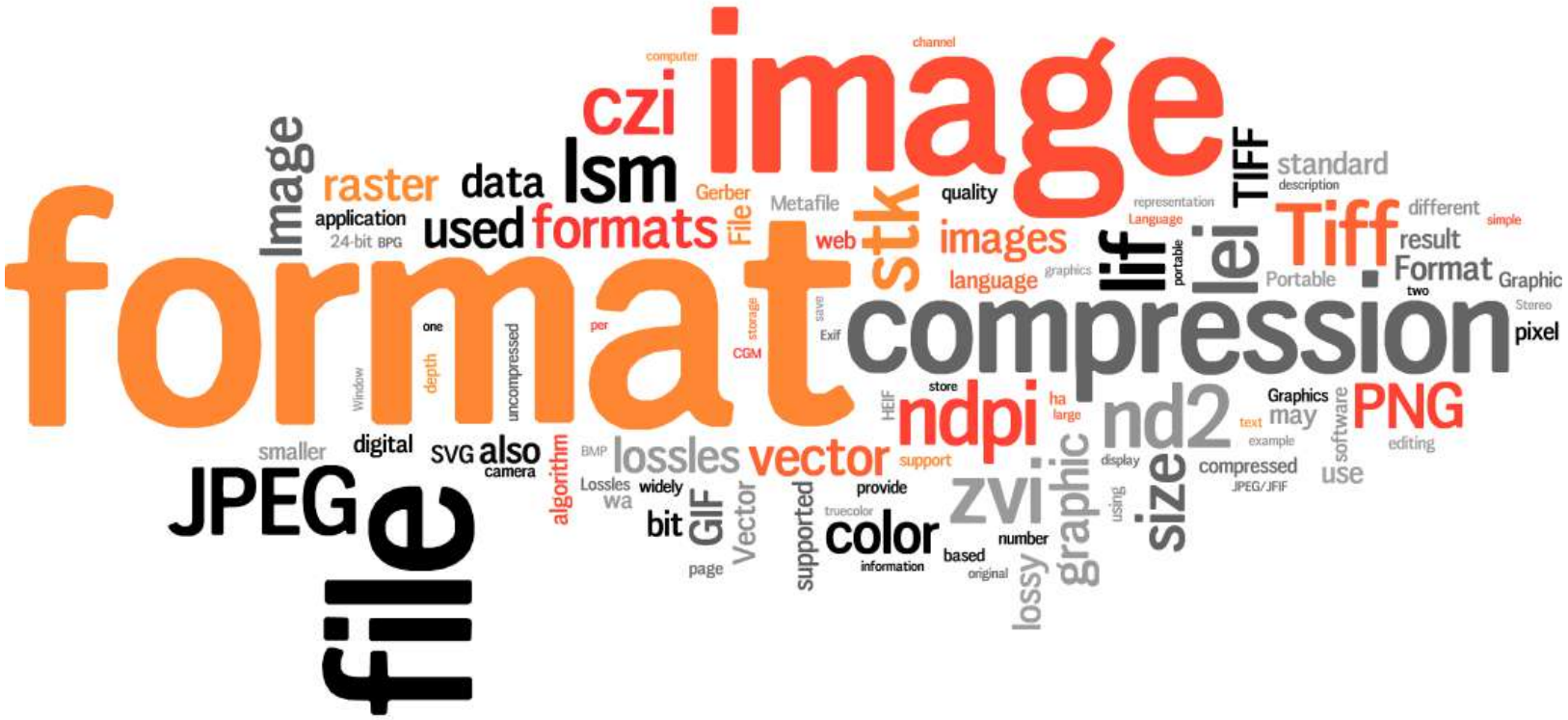
Before we start…

What will we learn during this session ?

Tutorial 1	What is an image ?
Tutorial 2	How to modify the image's display ?
Tutorial 3	How to overlay several views from the same scene ?
Tutorial 4	How to handle 3D images ?
Tutorial 5	How to quickly build a figure ?

What is an image ?



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What is an image ?

The image IS NOT the object

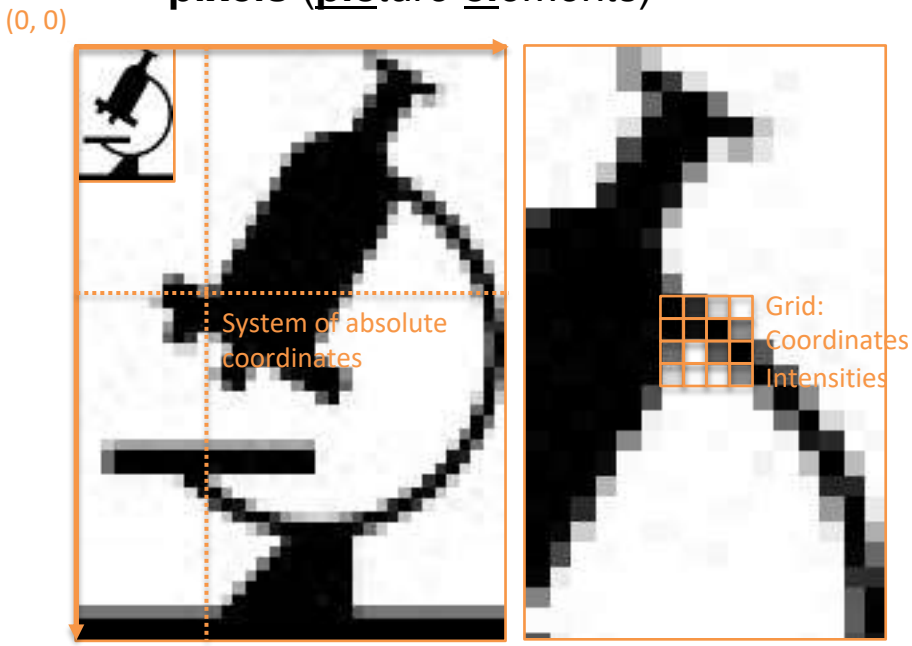
René Magritte, La trahison des images, 1928-29, huile sur toile, Los Angeles county Museum of Art, Los Angeles.

What is an image ?

The nature of data

Raster image

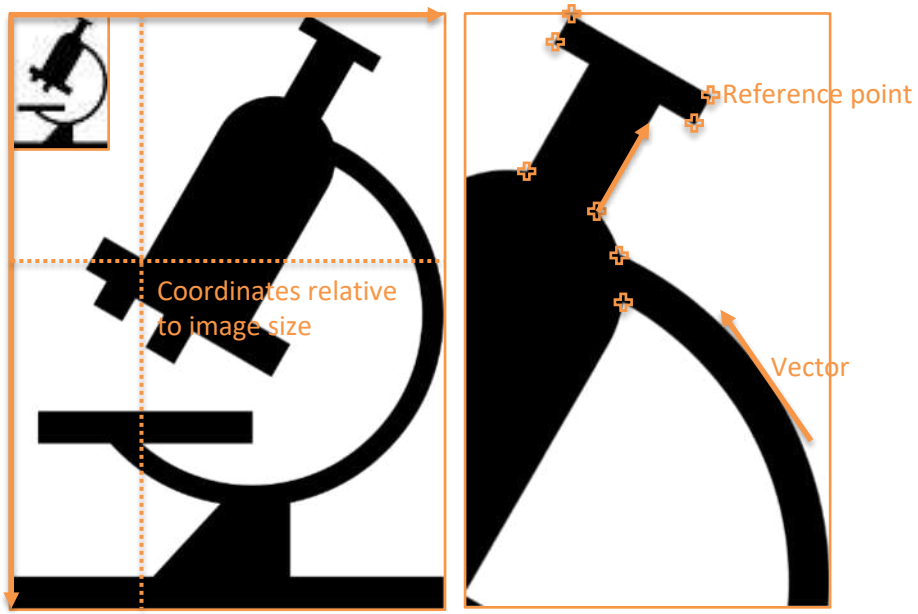
- Painted using individual elements: **pixels** (**pic**ture **el**ements)



- Printing quality **depends** on a compromise between **dimension** and **resolution**

Vector image

- Painted using **vectors** and **mathematical descriptors**



- Printing quality is **independent** of **dimension** and **resolution**

What is an image ?

An image is a matrix

255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	253	197	141	126	144	166	243	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	201	102	61	110	154	252	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	254	195	110	69	111	158	253	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	248	149	89	79	102	130	239	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	245	161	126	75	118	139	236	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	254	192	168	79	153	176	252	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	254	196	179	84	159	183	252	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	252	174	136	69	128	154	248	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	252	162	113	59	105	135	245	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	251	163	120	56	96	138	247	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	248	230	215	223	243	254	250	158	122	54	92	140	248	255	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	255	229	171	112	135	197	250	250	151	117	55	93	148	251	255	255	255	255	255	255	255	255
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255	255	255	255	255	255	255	255	255	234	137	84	99	162	254	255	235	105	74	132	246	255	255	255	255	255	255	255	255	255
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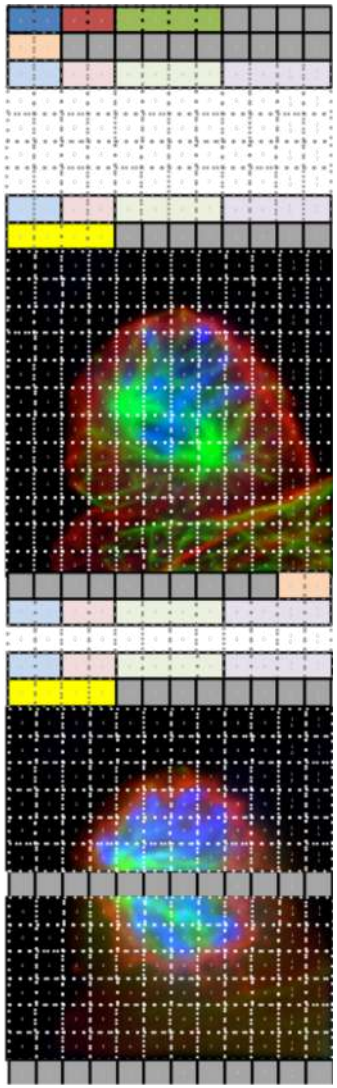
255	255	255	255	255	255	255	255	254	202	112	88	98	120	213	200	165	140	110	118	105	160	245	255	255	255	255	255	255	255
255	255	255	255	255	255	255	255	246	137	98	72	85	72	112	125	106	94	85	93	94	105	194	254	255	255	255	255	255	255
255	255	255	255	255	255	255	255	234	109	93	89	88	77	92	97	93	92	91	94	96	99	148	247	255	255	255	255	255	255
255	255	255	255	255	255	255	255	230	105	94	106	96	87	85	85	85	86	87	90	92	93	128	243	255	255	255	255	255	255
255	255	255	255	255	255	255	255	247	197	167	158	149	144	145	143	142	142	144	146	147	148	174	248	255	255	255	255	255	255

What is an image ?

Storing an image: the container



File format

- TIFF (Tagged Image File Format)
- ~~JPEG (Joint Photographic Experts Group)~~
- JFIF (JPEG File Interchange Format)
- PNG
- CZI, LIF, ND2, NDPI, OIB, ZVI…

Metadata

ImageWidth	ImageDescription
ImageLength	Make
BitsPerSample	Model
Compression	XResolution
PhotometricInterpretation	YResolution
FillOrder	…
DocumentName	

Image

Compression

***CODEC** (**CO**mpression/**DEC**ompression)*

- RLE (Run Length Encoding, PackBits)
- LZW (Lempel-Ziv-Welch)
- JPEG (Joint Photographic Experts Group)
- Modified Huffman compression (CCITT Group 3 1D)

What is an image ?

Storing an image: saving space, compression strategies

Run-length encoding

Sentence:

AAABBBBBBAAACCCCCAAAAAB

- For each value, count the number of occurrence

Compressed sentence:

3A6B3A5C5A1B

Dictionary-based compression

Sentence:

ABCDDEFABCEFEABC

- Identify individual words

  ABCDDEFABCEFEABC

- Build a dictionary:

  1=ABC; 2=DD; 3=EF; 4=E

- Re-write the sentence using the dictionary:

Compressed sentence:

1231341

Used in LZW/ZIP compressions

Only** non destructive compressions **should be used for image processing and analysis

JPEG is a destructive compression: to be ONLY used for mail or presentation purposes

Tutorial 1

What is an image ?

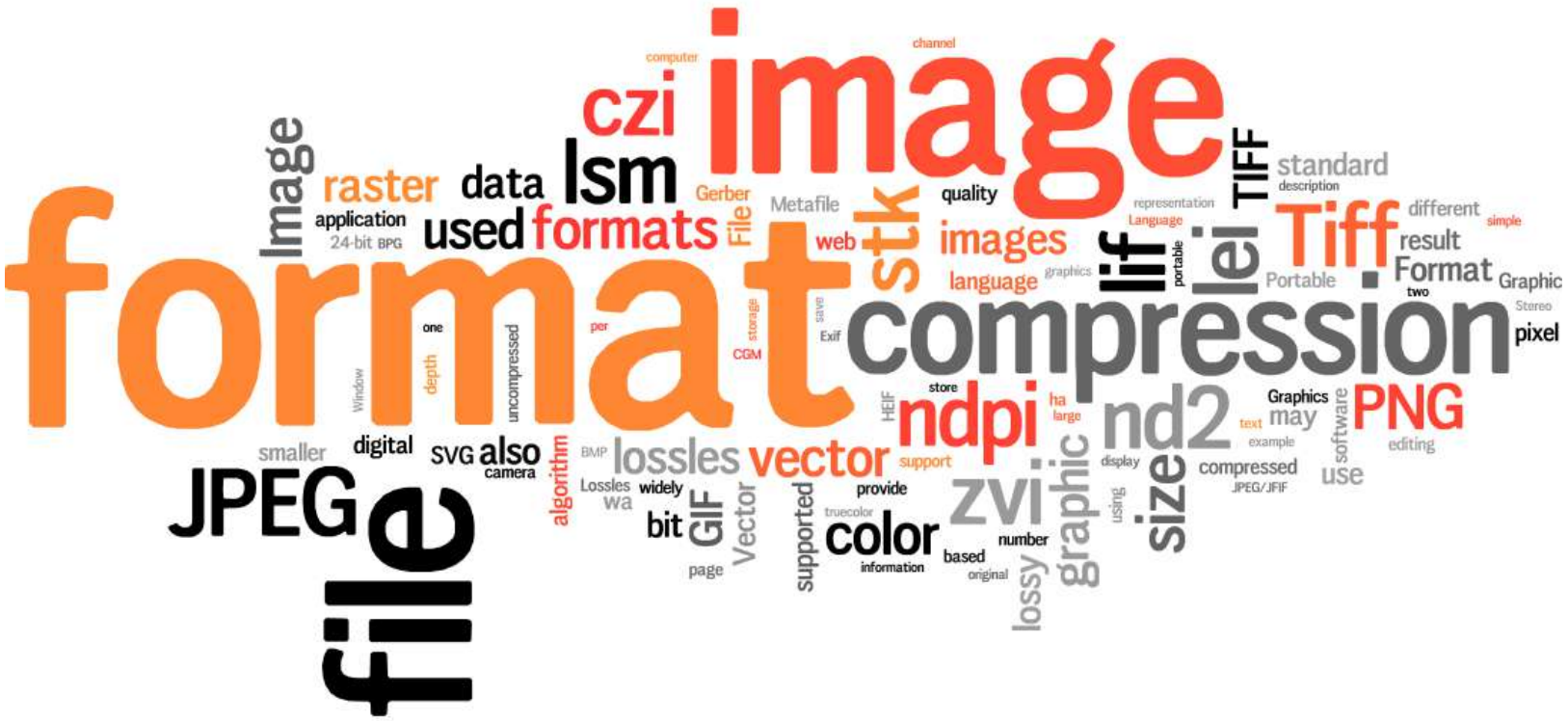
- Open an image

File>Open

- Open an image

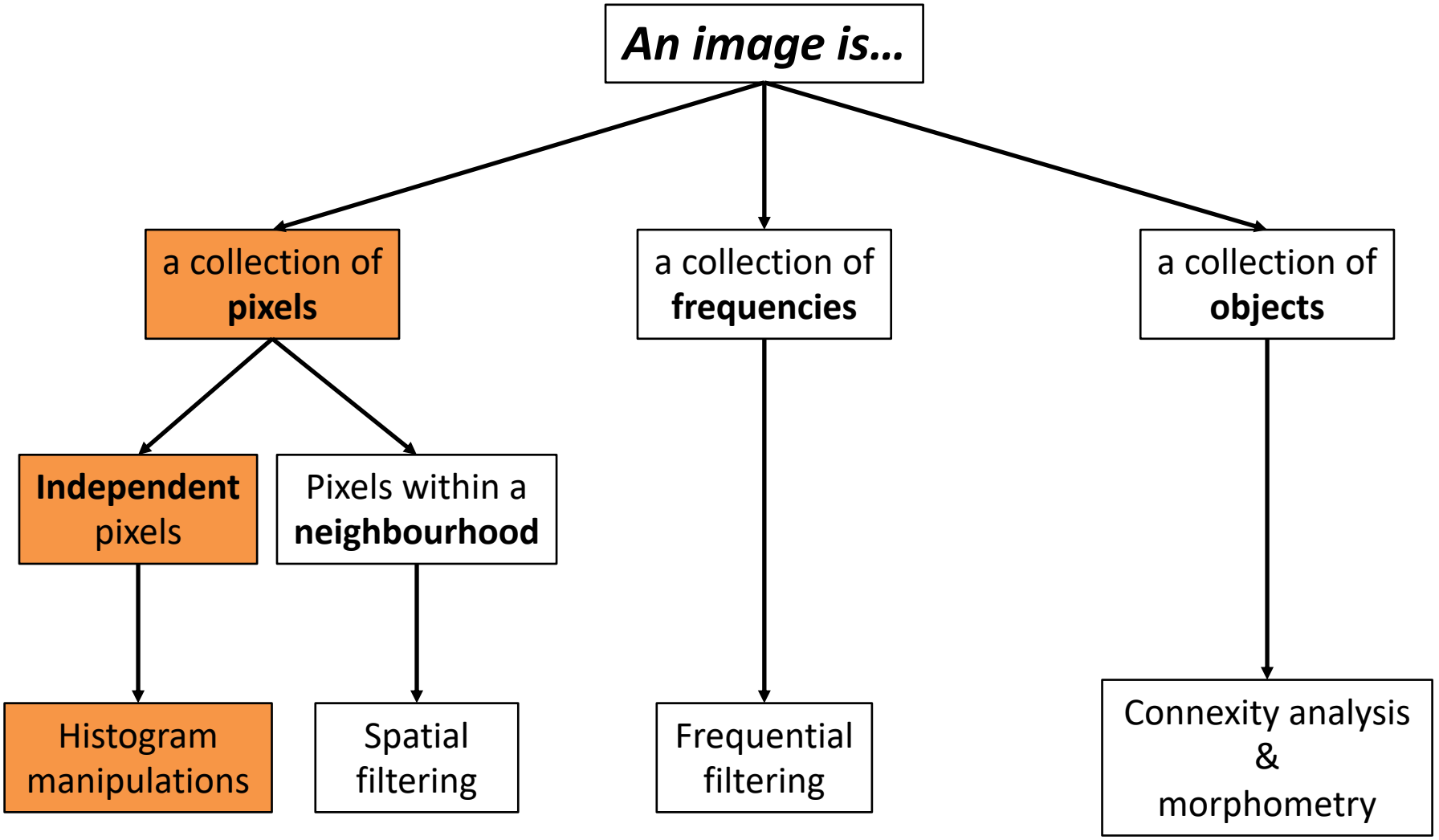
Drag & drop on ImageJ toolbar

Image Processing



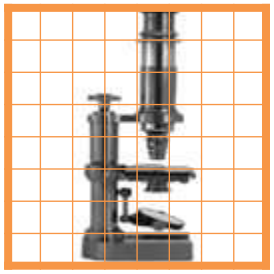
Several way to consider a single image

And associated processing techniques

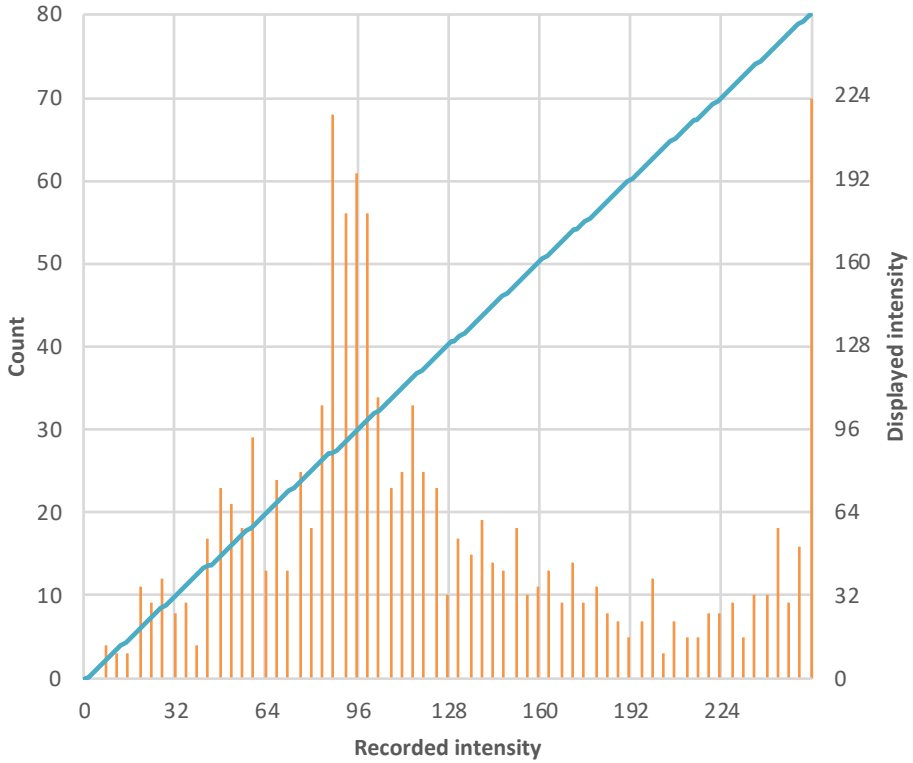


The image is a collection of intensities

Working with the image's histogram



1. Group pixels per increasing intensity
2. Count pixels per group
3. Plot count as a function of intensity



*This is a **REALLY BAD** histogram ! But a good support to illustrate histogram modifications…*

Tutorial 2

How to modify the image's display ?

- Get the histogram from the image

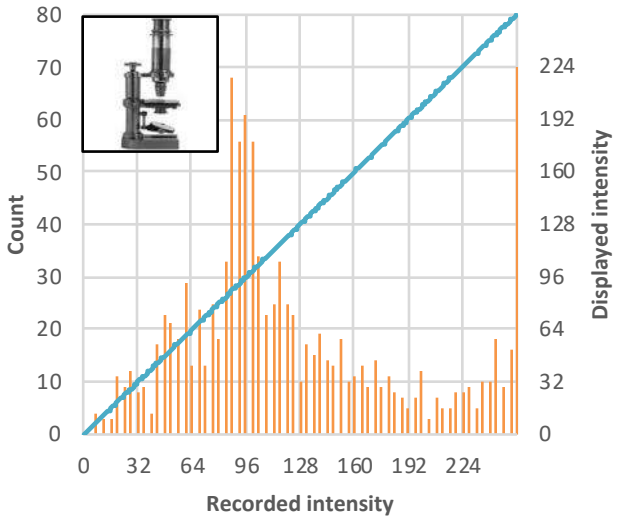
  Analyze>Histogram (try the « Live » mode)

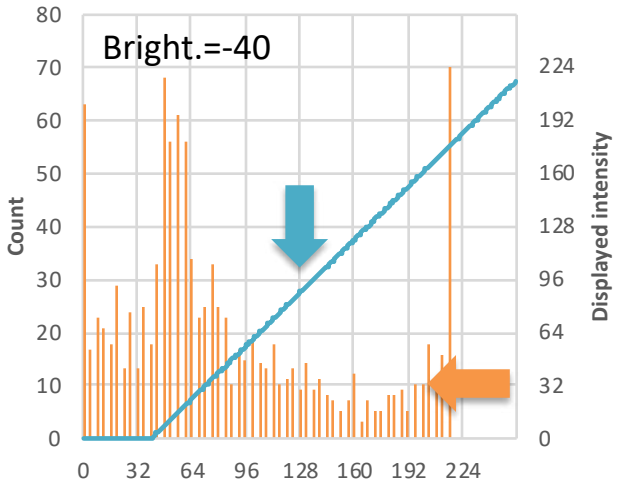
- Modify the image's display

  Image>Adjust>Brightness & Contrast

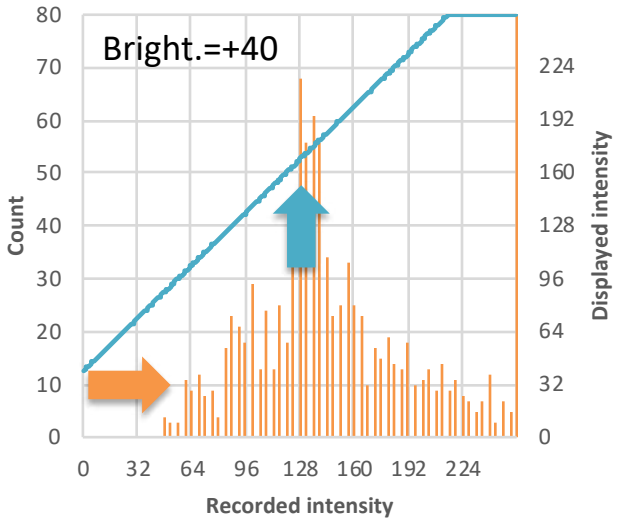
The image is a collection of intensities

Linear histogram modification: brightness





Brightness:
The same value is added to all intensities

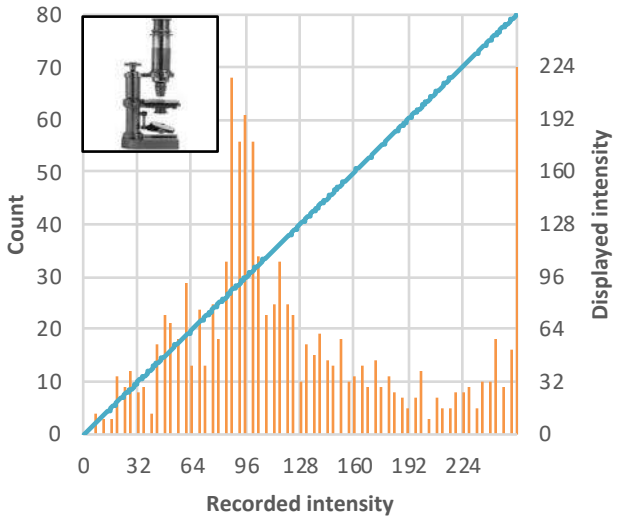


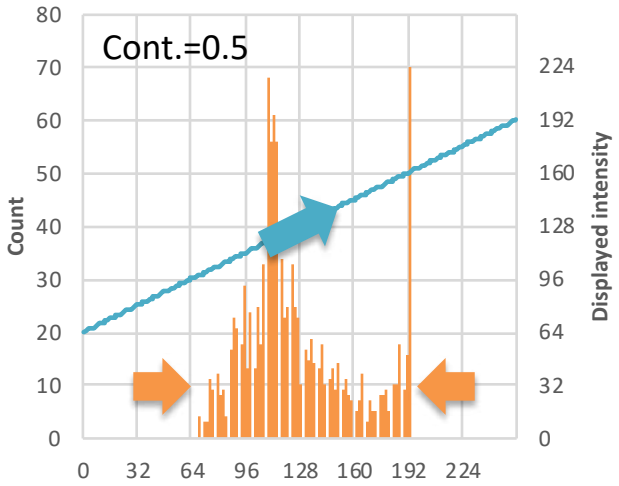
Thresholding:
Negative values are shifted to zero

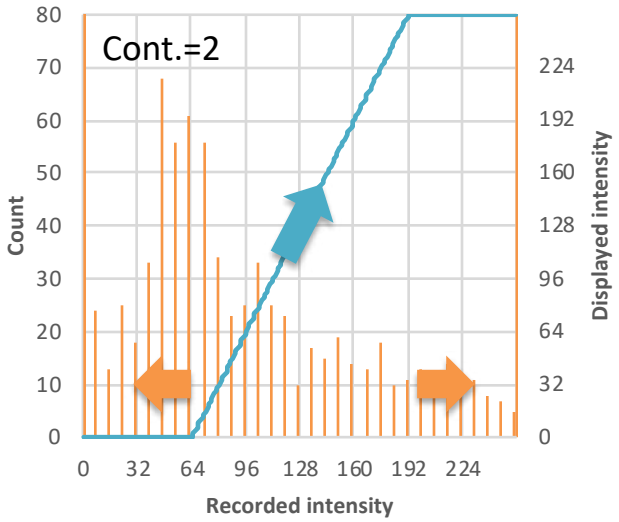
Saturation:
Values over the maximum of the range are clipped to the maximum of the range

The image is a collection of intensities

Linear histogram modification: contrast







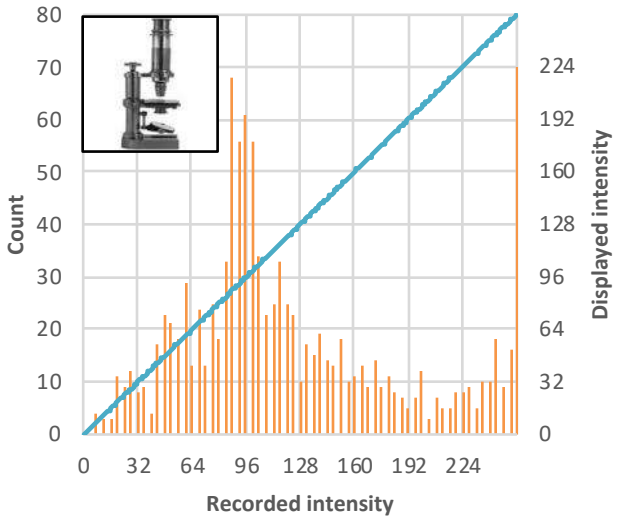
Contrast:
Response line: the slope is changed
The mid-range value remains constant

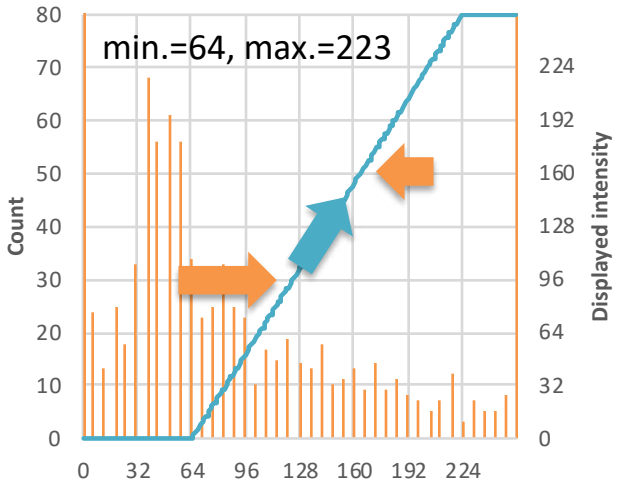
Thresholding:
Negative values are shifted to zero

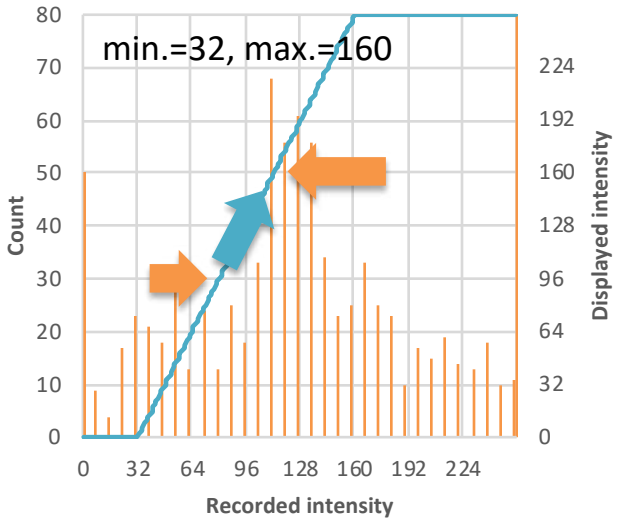
Saturation:
Values over the maximum of the range are clipped to the maximum of the range

The image is a collection of intensities

Linear histogram modification: min-max







Min-Max:
Intensities are linearly distributed between the two newly set limits

Thresholding:
Negative values are shifted to zero

Saturation:
Values over the maximum of the range are clipped to the maximum of the range

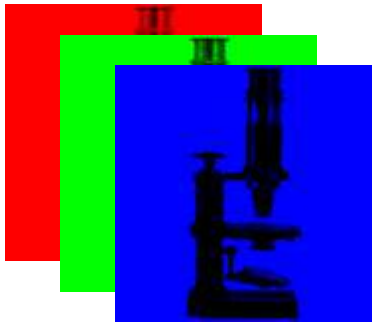
What is an image ?

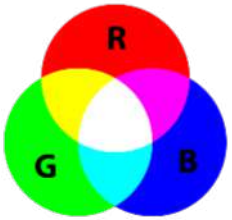
Color versus colorised image

Color image

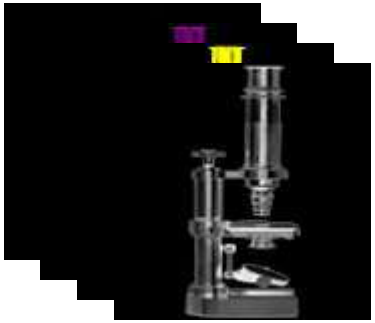
- A reference is selected
- One channel is generated per reference's component
- Each color is expressed as a weighted sum of each component

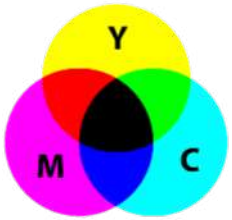
Additive mixing





Subtractive mixing





Indexed colors

- Build a dictionnary: 1 color=1 reference
- Replace each pixel's color by its reference

Tutorial 3

How to overlay several view from the same scene ?

- Overlay images

Image>Color>Merge channels

- Interact with the display

Image>Color>Channels tool

Image Visualisation

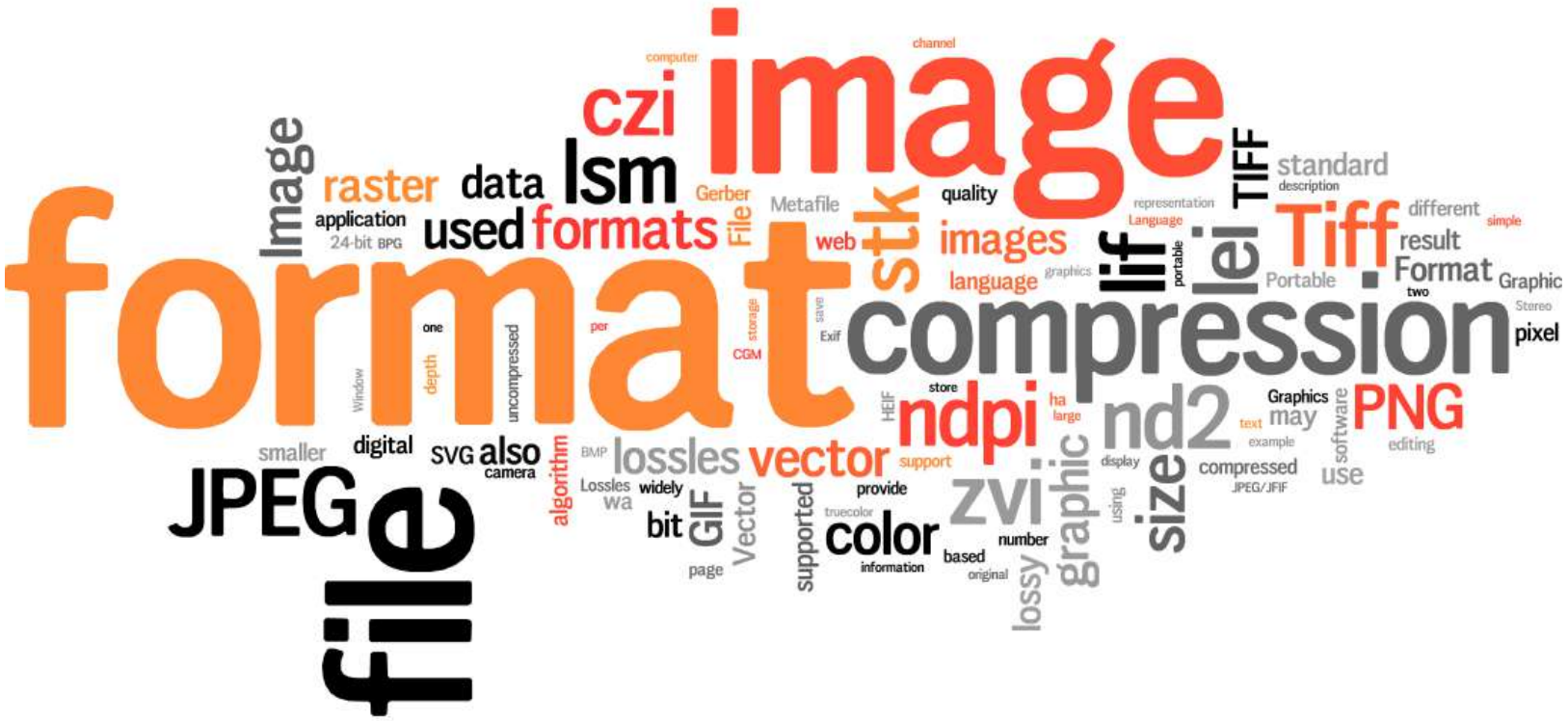


Image visualisation

Fact, challenge and method

Fact:

Most commonly used output media are 2D

Challenge:

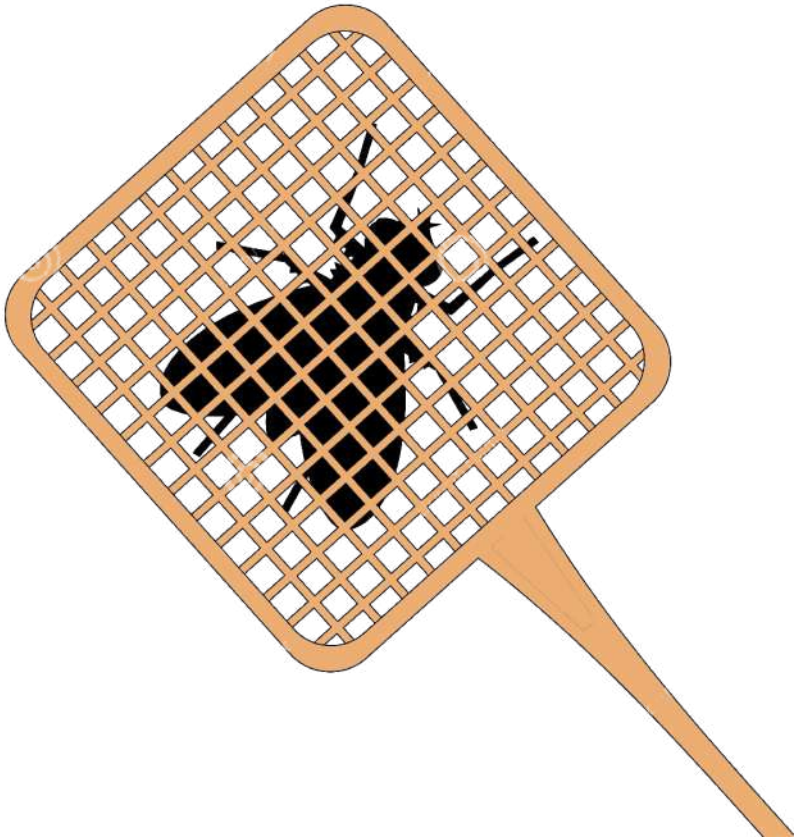
How to display a 3D dataset on a 2D medium ?

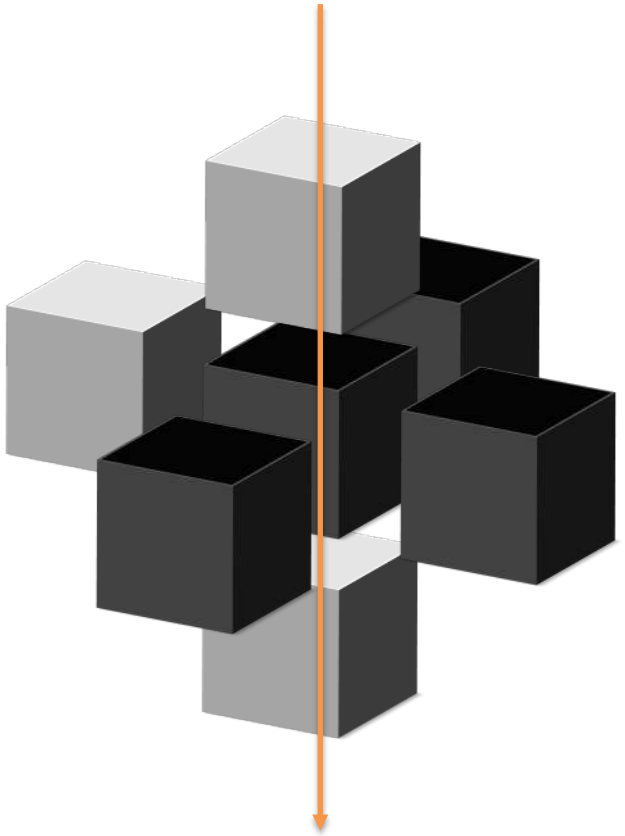
Method:

Understand how we perceive volumes

Image visualisation

Rough method: squeezing everything onto a single plane



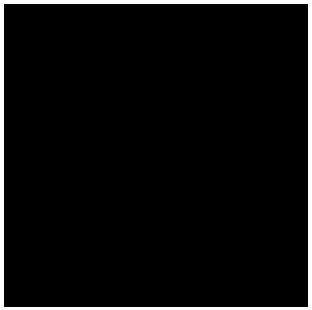


Min | Med | Max | Other rule

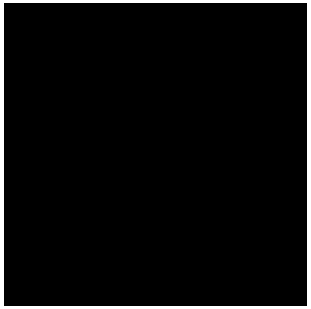
Image visualisation

Rough method: squeezing everything onto a single plane

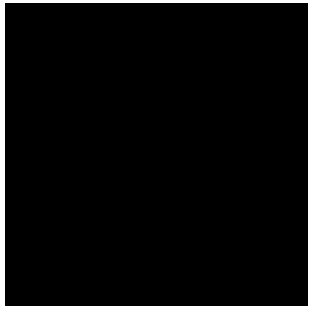
Original data



Minimum



Median



Maximum

Mean

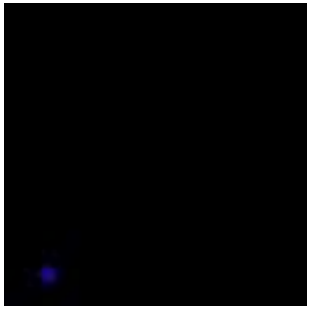


Image visualisation

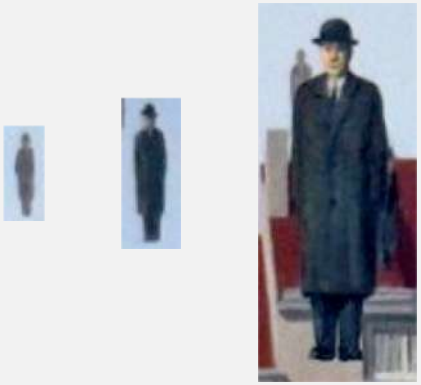
Refined method: taking into account psychological parameters

Golconda, Magritte, 1953

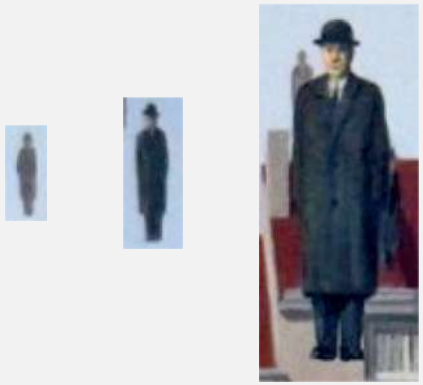
Image visualisation

Refined method: taking into account psychological parameters

Size of the image on retina (bigger= closer)



Texture fades away in the distance



2 eyes: made to have 2 different points of view

When rotating, closest points move slower

Overlap: incomplete figures are in background

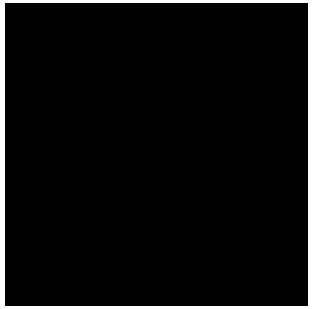
Image visualisation

Refined method: mimicking binocular disparity

2 eyes: made to have 2 different points of view

Anaglyph

Original data



Stereogram

-6°

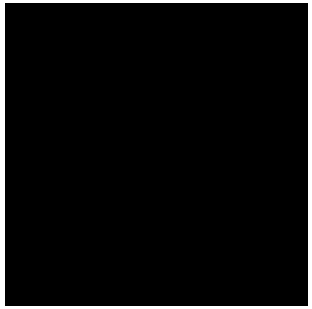
+6°

Image visualisation

Refined method: taking benefit of motion parallax

When rotating, closest points move slower

Original data

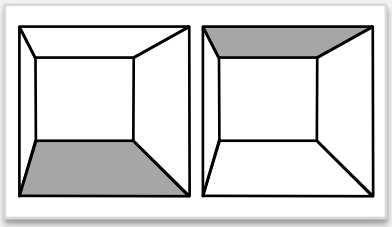


Multiple angle view

Image visualisation

Refined method: taking care of rendering

Knowledge from experience: light comes from above



1. Create a surface (keep only voxels connected to background)
2. Light it up

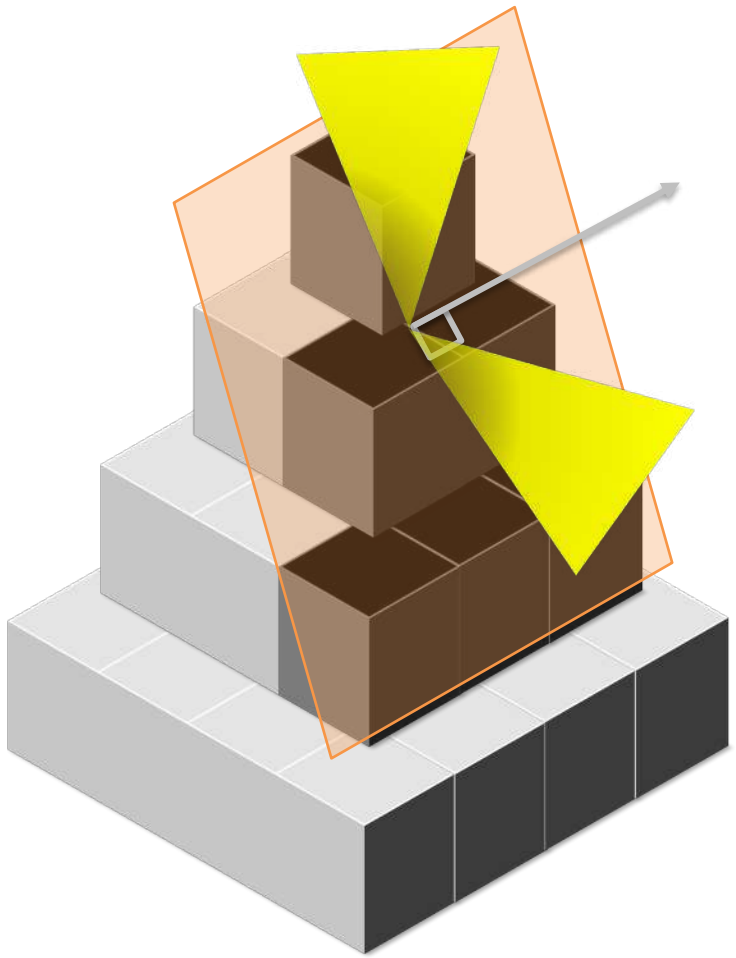
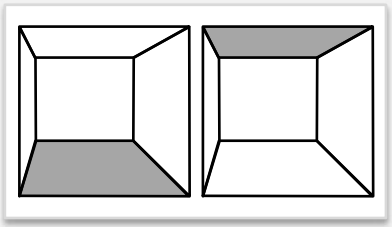


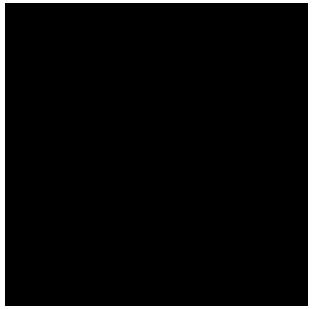
Image visualisation

Refined method: taking care of rendering

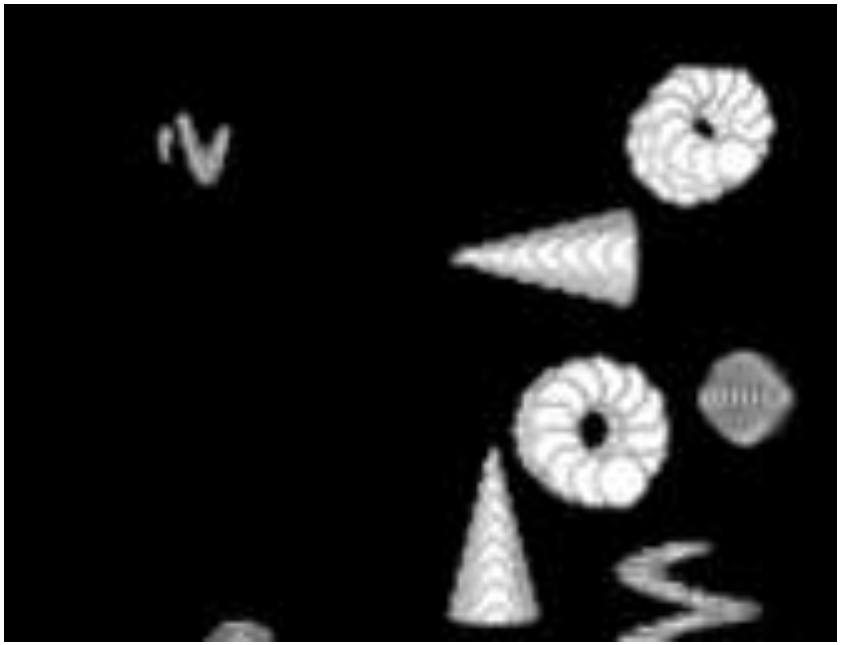
Knowledge from experience: light comes from above



Original data



Surface rendering



Tutorial 4

How to handle 3D images ?

- Generating 2D projections

  Image>Stacks>Z Project

- Generating variable angle views

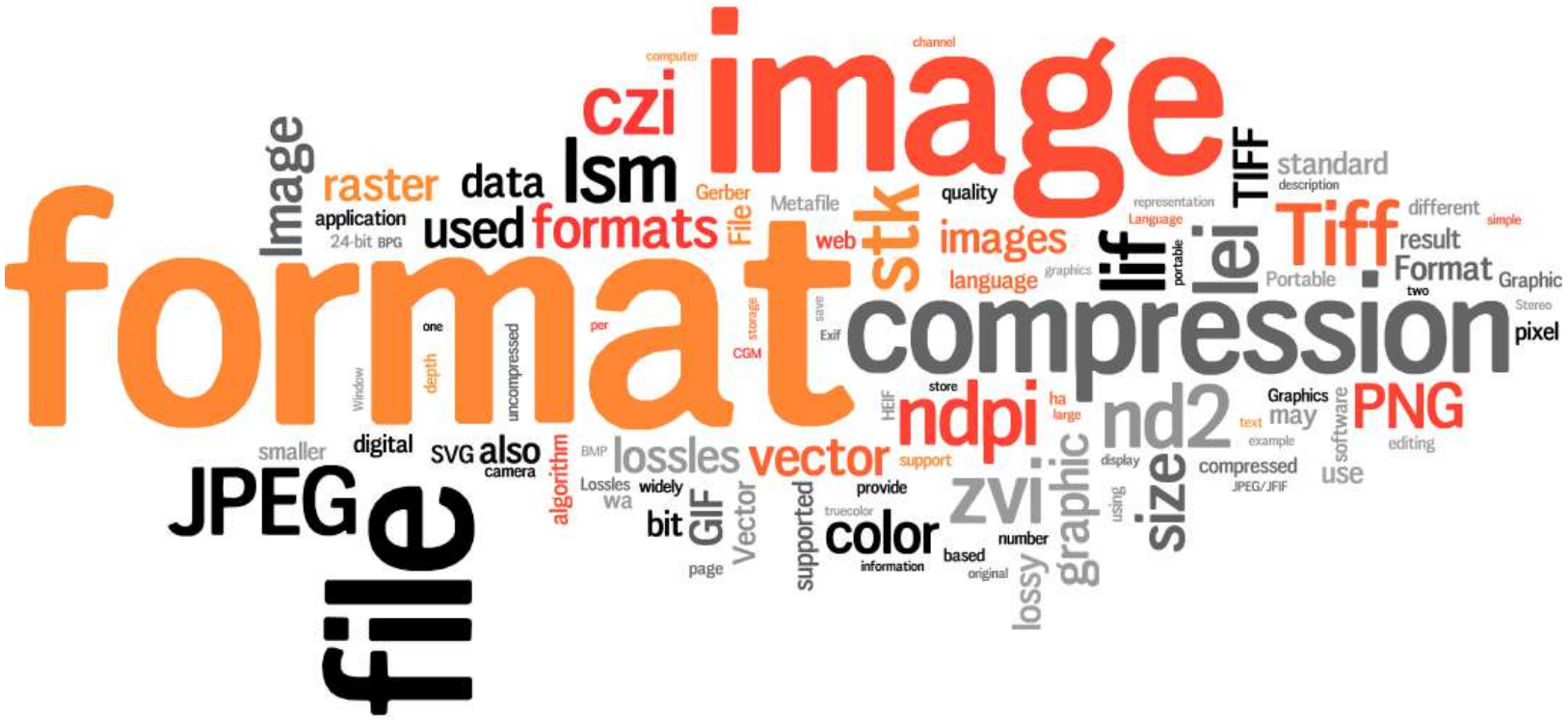
  Image>Stacks>3D Project

- Interact with the 3D display

  Plugins>3D Viewer

Tutorial 5

Building figures with FigureJ



Before getting started with FigureJ

Important:

This plugin is Open Source, freely available etc…

Please acknowledge the good job made by the authors !

Read and cite:

MUTTERER, J. and ZINCK, E. (2013), Quick-and-clean article figures with FigureJ. Journal of Microscopy, 252: 89–91. doi:10.1111/jmi.12069

Getting started with FigureJ

Installation

- The plugin is available from ImageJ's wiki: http://imagejdocu.tudor.lu/doku.php?id=plugin:utilities:figurej:start

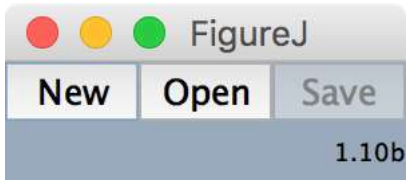
- For ImageJ:
  - Follow the instructions on the wiki: download the plugin + additional libraries (imagescience, BioFormat, LSM Reader).
  - Drag/drop all file to ImageJ toolbar and press "Ok" when asked for anything.

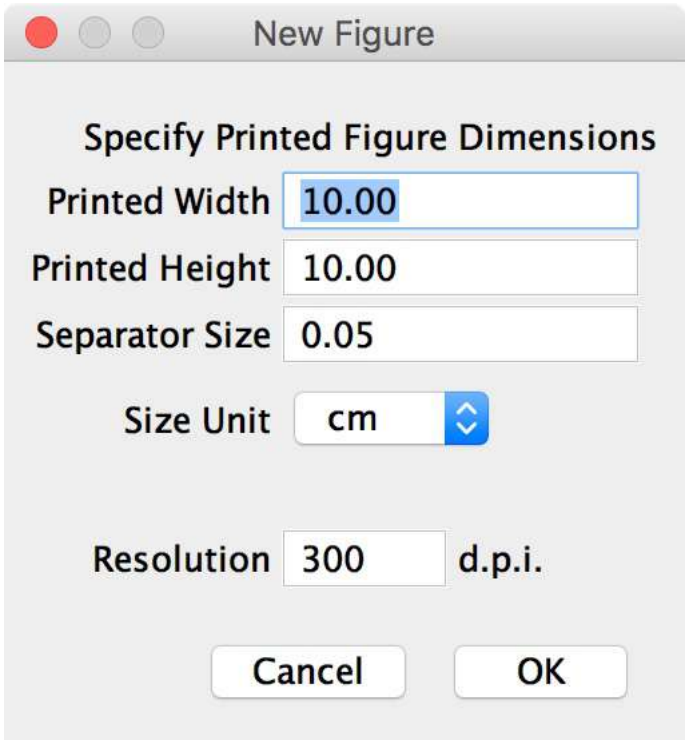
- For FiJi:
  - Go to Help/Update.
  - Add the IBMP-CNRS, ImageScience and BioFormats update sites.

Getting started with FigureJ

Creating a new figure

- Plugins/FigureJ/FigureJ
- Create a new canvas
- Setup the figure



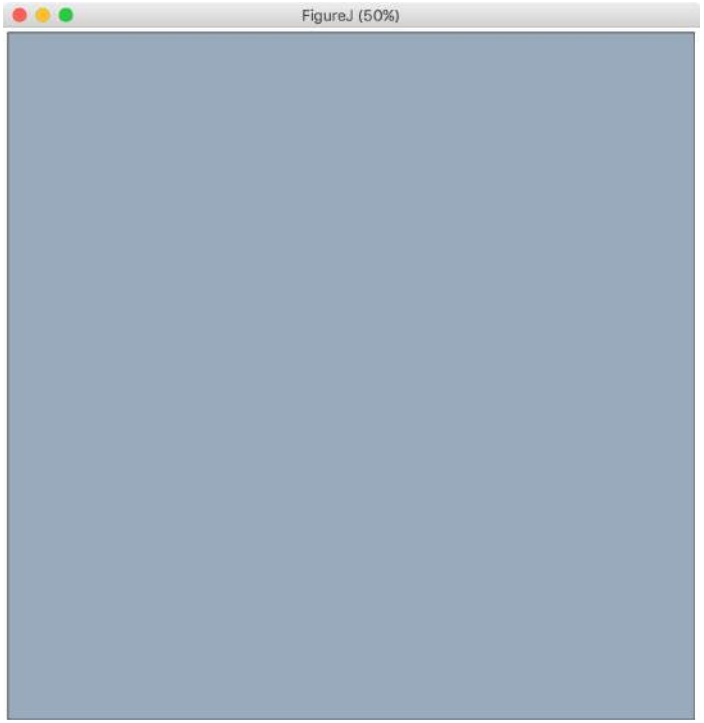


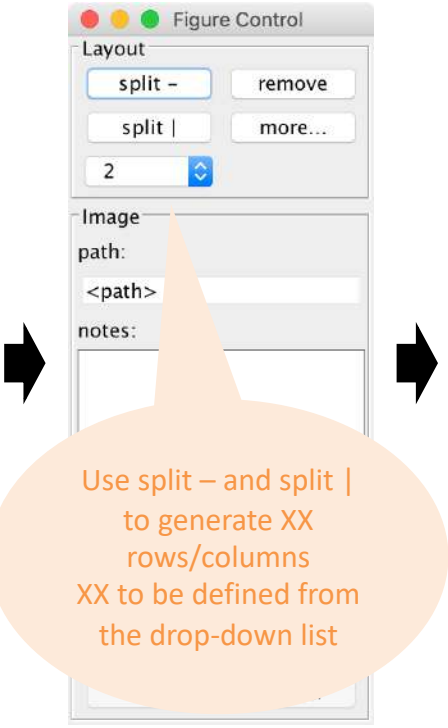
To be filled according to the journal's "instructions to authors"

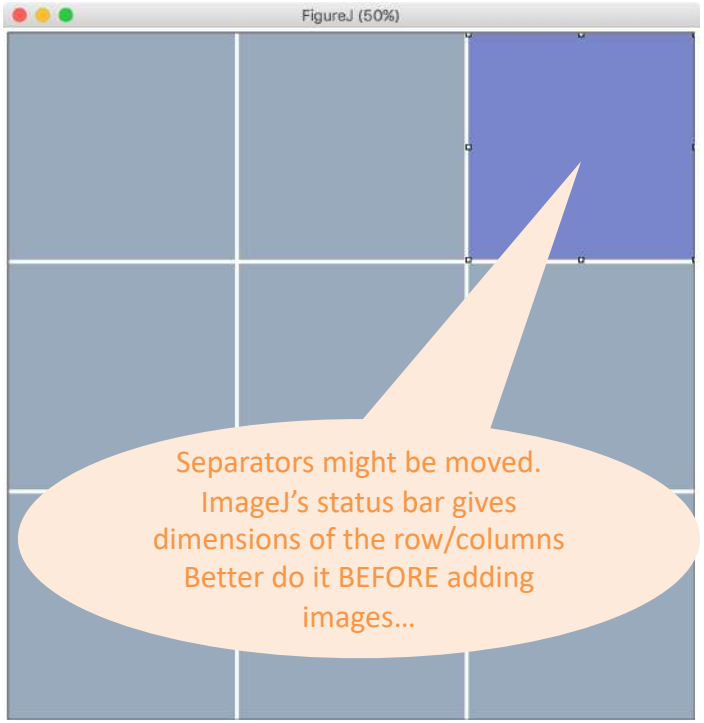
Getting started with FigureJ

Setting up the canvas

- Divide the empty canvas into containers for thumbnails
- Setup containers' dimensions







Getting started with FigureJ

Setting up the canvas

Most commonly encountered problem

If you can't select a thumbnail within the canvas, make sure FigureJ tool is active

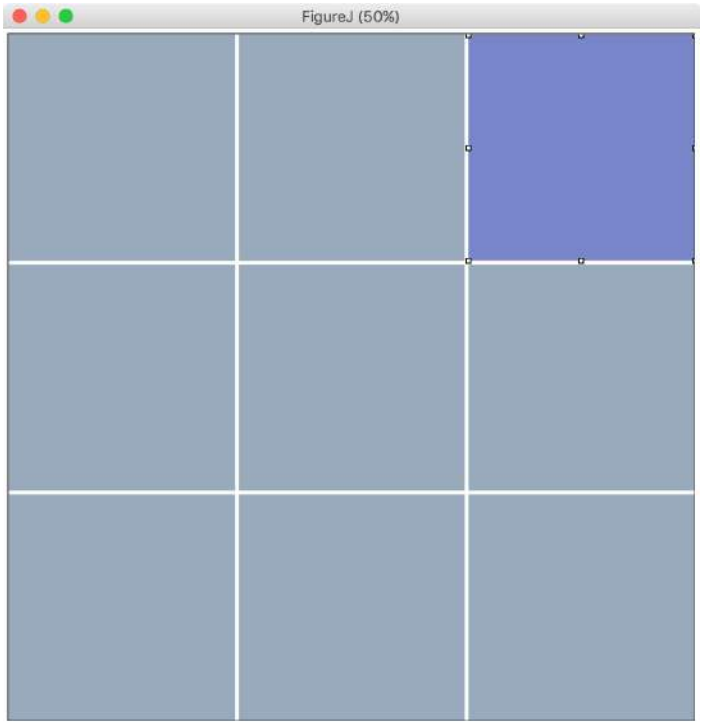
You might have one of those active

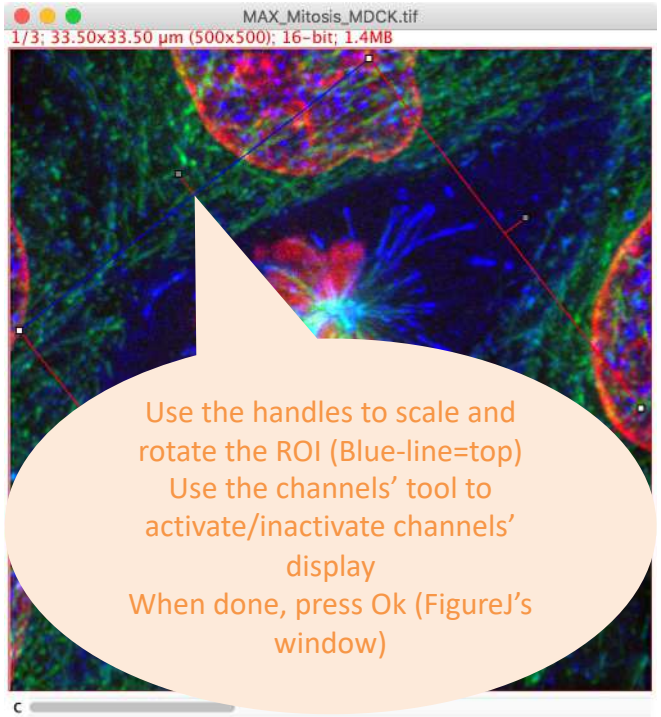
Click here !

Getting started with FigureJ

Filling up the canvas

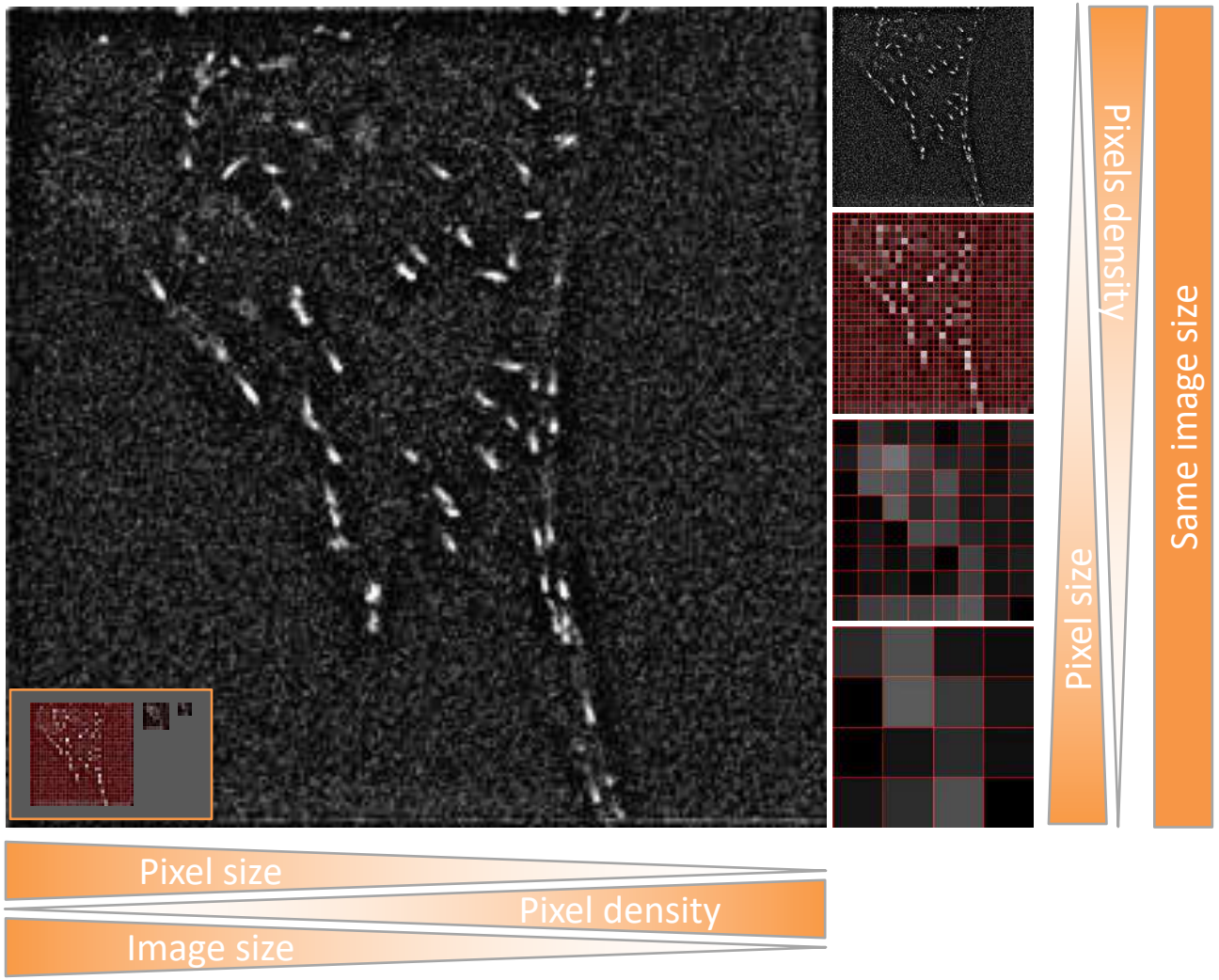
- Click on a thumbnail
- Press “Open” in FigureJ’s window
- Select the image to include and define the ROI to display





Getting started with FigureJ

Filling up the canvas: *Spatial sampling, density and display*

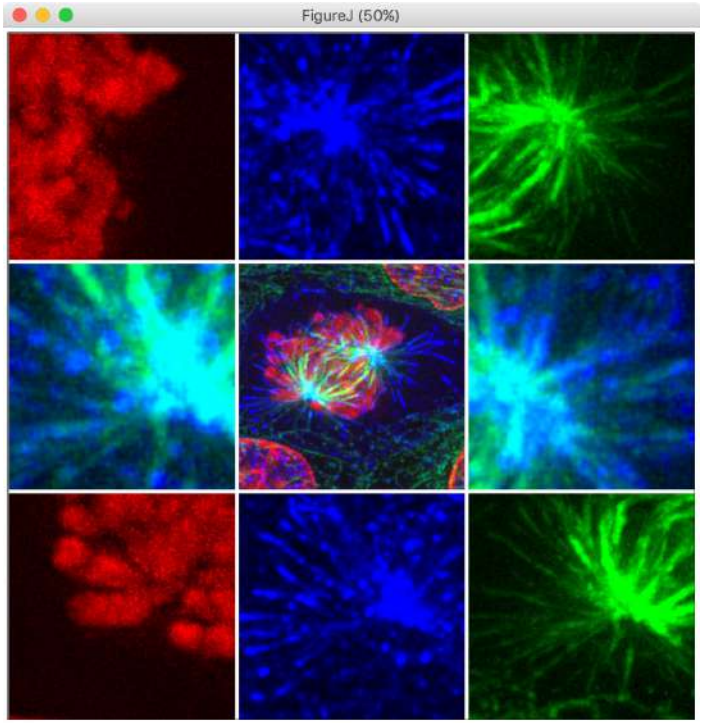


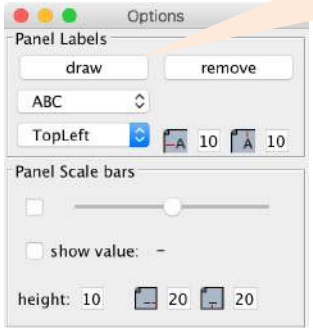
Getting started with FigureJ

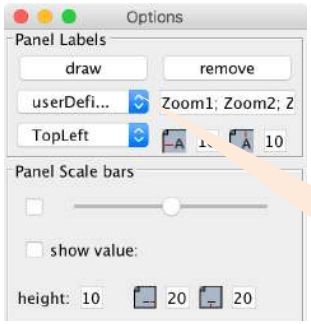
Annotating the figure: labels

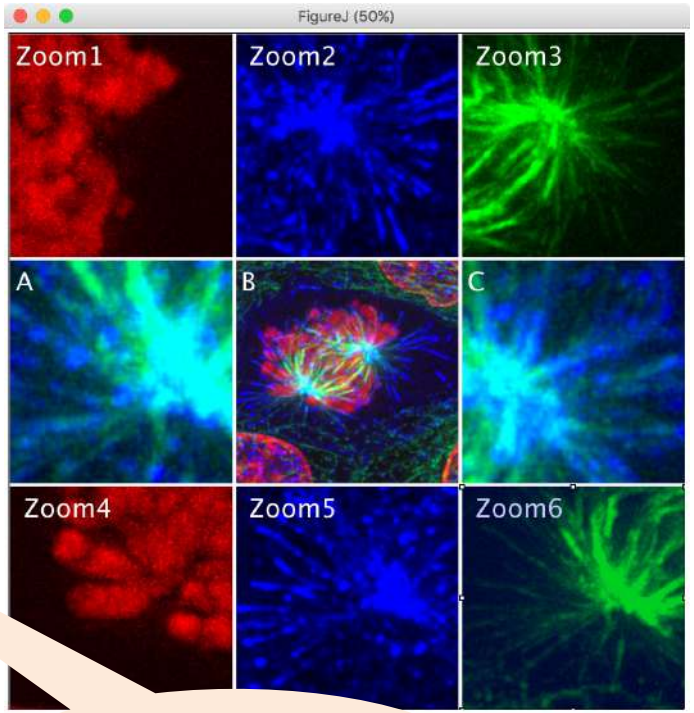
- Click on a thumbnail
- Press “More” in FigureJ’s window

Click draw: annotate
Shift+click draw: step back
Alt+click draw: reset counter
Use Edit/Options/Fonts to increase/decrease size etc..









User defined annotations: separate items with « ; »

FRANCE-BIOIMAGING

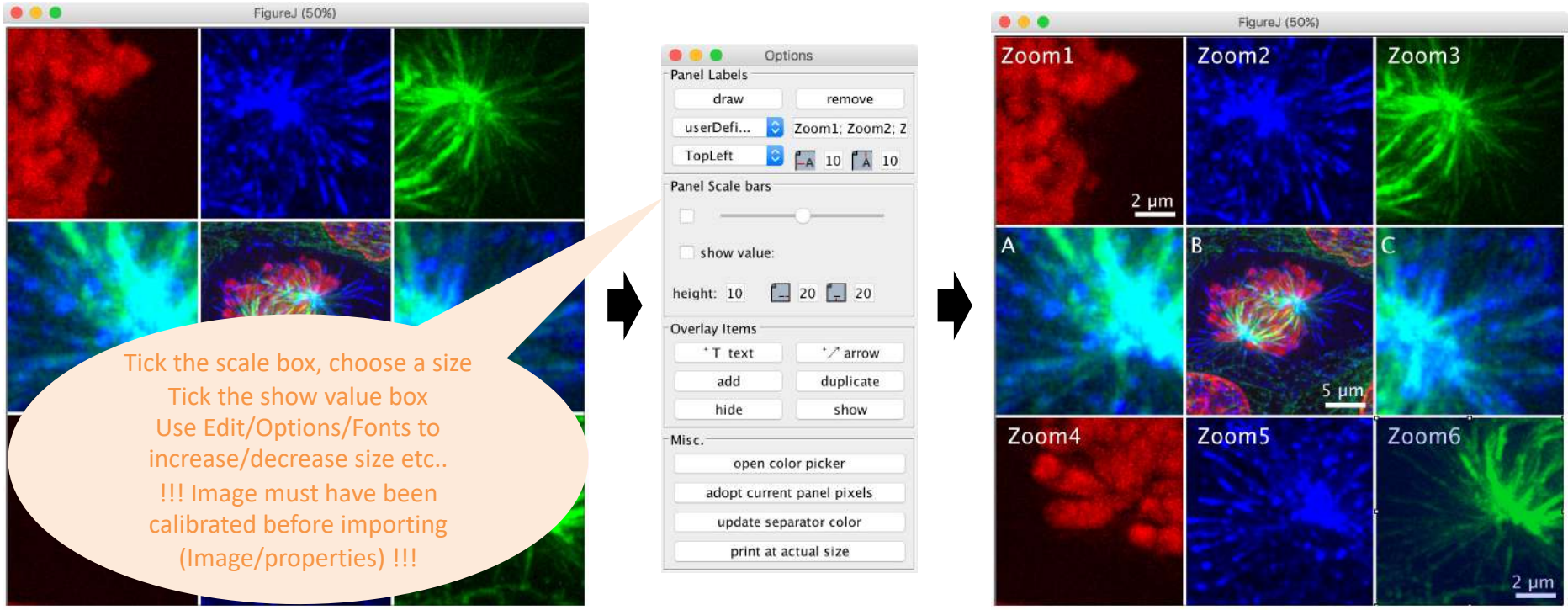
neubias
network of european bioimage analysts

BIC
Bordeaux Imaging Center

Getting started with FigureJ

Annotating the figure: scale bars

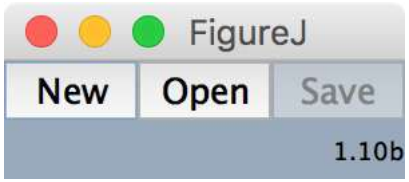
- Click on a thumbnail
- Press “More” in FigureJ’s window



Getting started with FigureJ

Exporting the figure

- Go back to FigureJ's small window
- Click on save button (not the ImageJ's one !!!)
- Select or create an output folder



File to recall using FigureJ's open to edit the figure

Full resolution image, to be sent to the editor

All notes taken for each single thumbnail (Note pas on FigureJ's window)

Low resolution, compressed image, for PPT and mail ONLY (jpg=EVIL)

Original image(s) used to build the figure