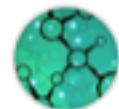


Introduction to Digital Image Processing, Analysis and Visualisation

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Bordeaux Imaging Center

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 @fab_cordelieres, @BIC_Bordeaux



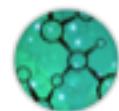
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What is an image ?

The word cloud illustrates the following concepts:

- Image**: The central concept.
- format**: A major category.
- compression**: A sub-category of format.
- JPEG**: A specific image format.
- file**: Another major category.
- TIFF**, **Tiff**: Image formats.
- PNG**: An image format.
- Lossless**, **GIF**, **Vector**, **HEIF**, **ndpi**, **nd2**, **ZVI**: Various image file types.
- algorithm**, **BMP**, **lossless**, **bit**, **page**, **support**, **store**, **provide**, **truecolor**, **information**, **number**, **original**: Technical terms related to image processing and storage.
- channel**, **quality**, **language**, **graphics**, **text**, **example**, **compressed**, **JPEG/JFIF**: Descriptive terms and file types.
- computer**, **camera**, **storage**, **CGM**, **Exif**, **Save**: Device and file system-related terms.
- application**, **24-bit BPG**, **one**, **depth**, **uncompressed**, **smaller**, **digital**, **also**, **algorithm**, **Lossless**, **widely**, **bit**, **Vector**, **color**: Specific file characteristics and usage.



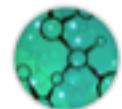
What is an image ?

The image IS NOT the object



Ceci n'est pas une pipe.

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



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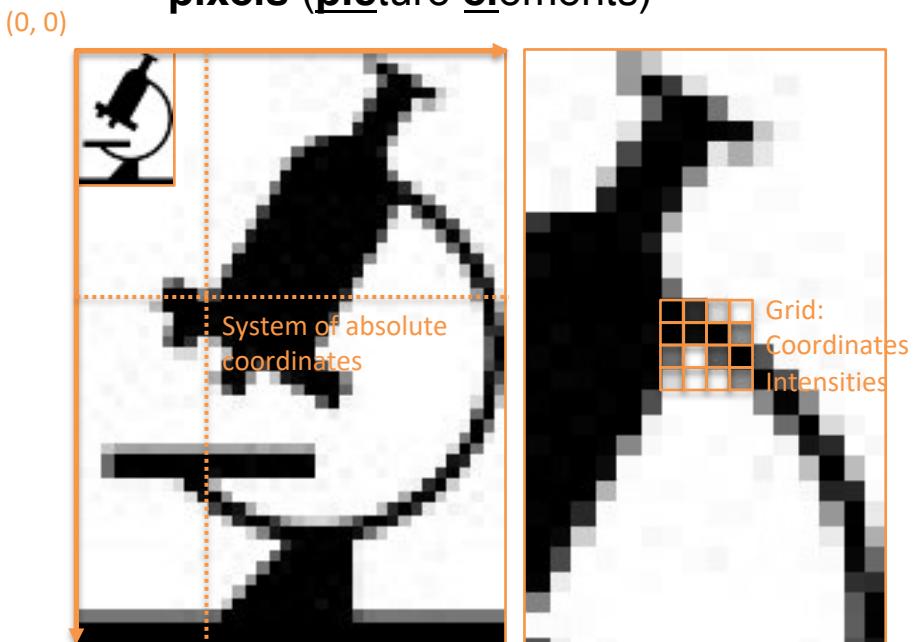


What is an image ?

The nature of data

Raster image

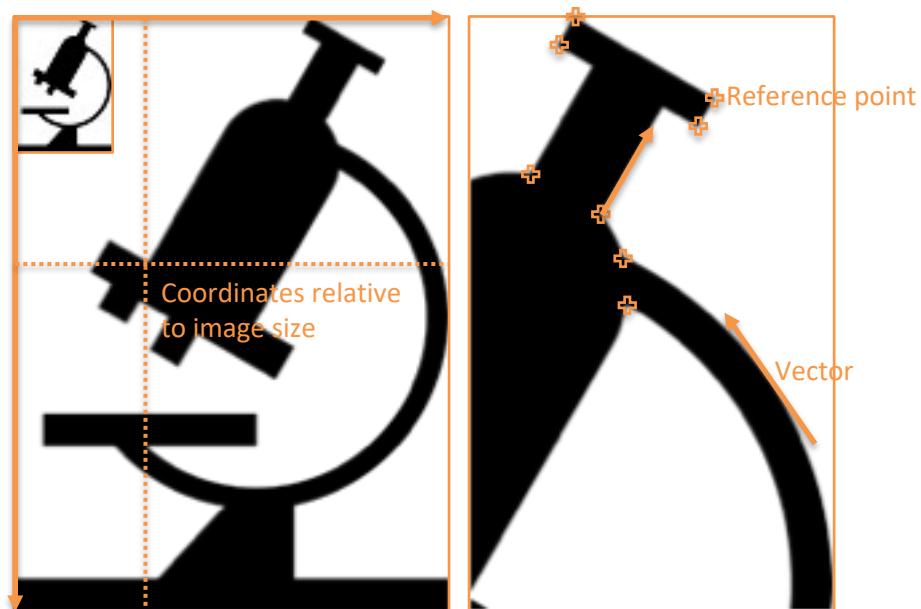
- Painted using individual elements: **pixels (picture elements)**



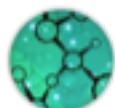
- Printing quality **depends** on a compromise between **dimension** and **resolution**

Vector image

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



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



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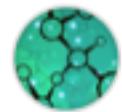
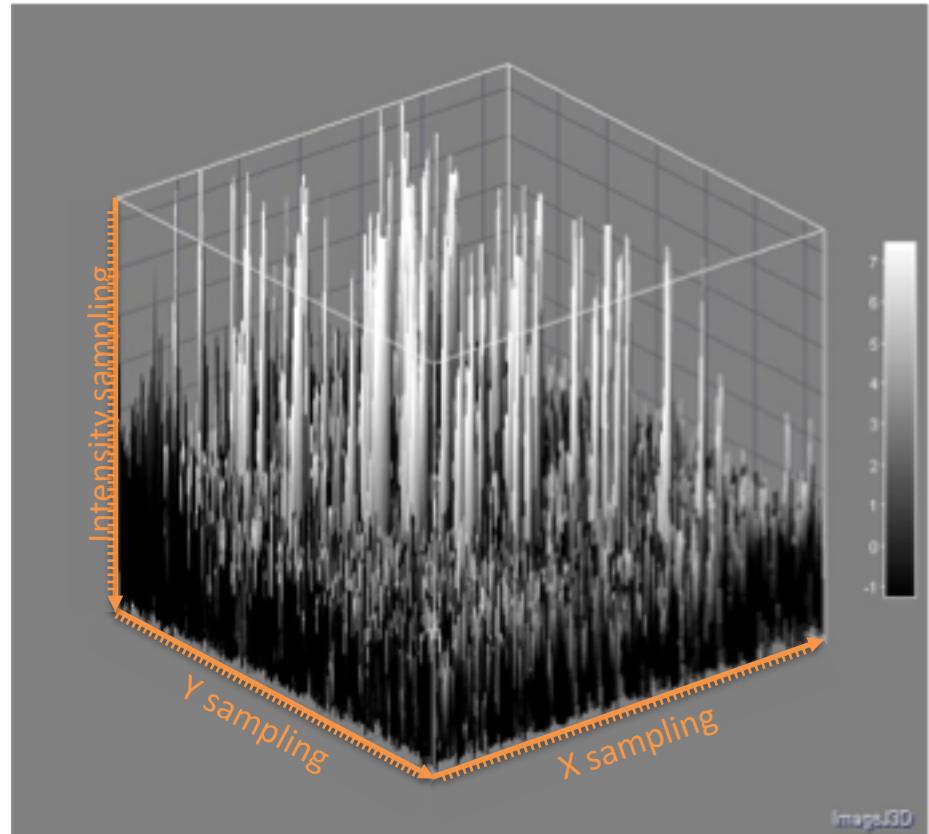
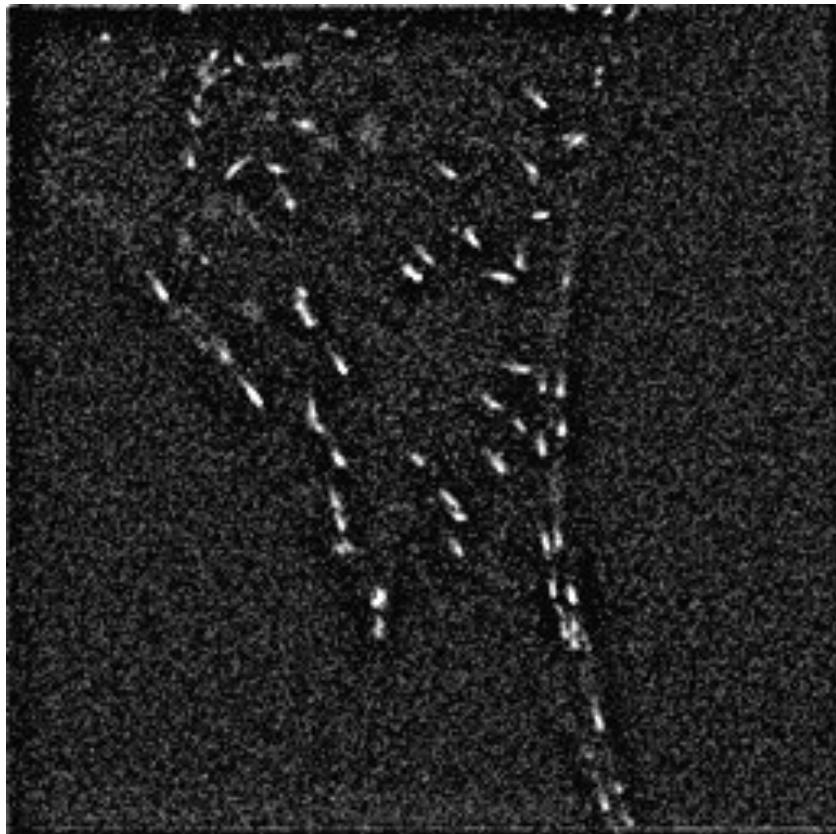
What is an image ?

An image is a matrix



What is an image ?

The image stores data originating from a dual sampling



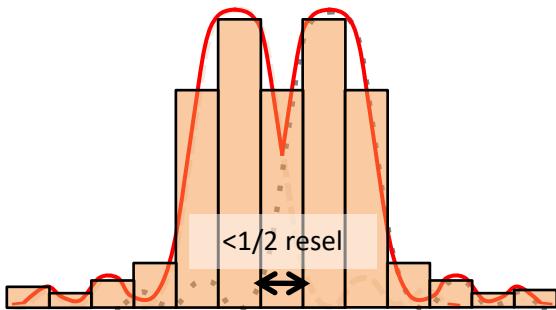
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What is an image ?

How to sample properly ?

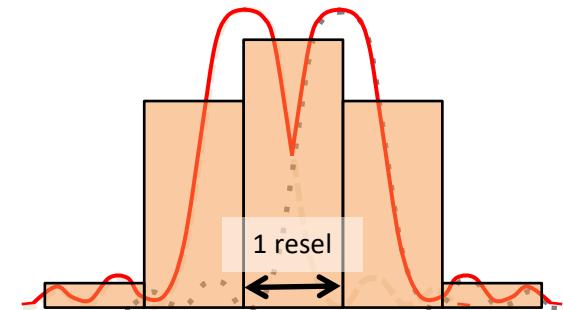
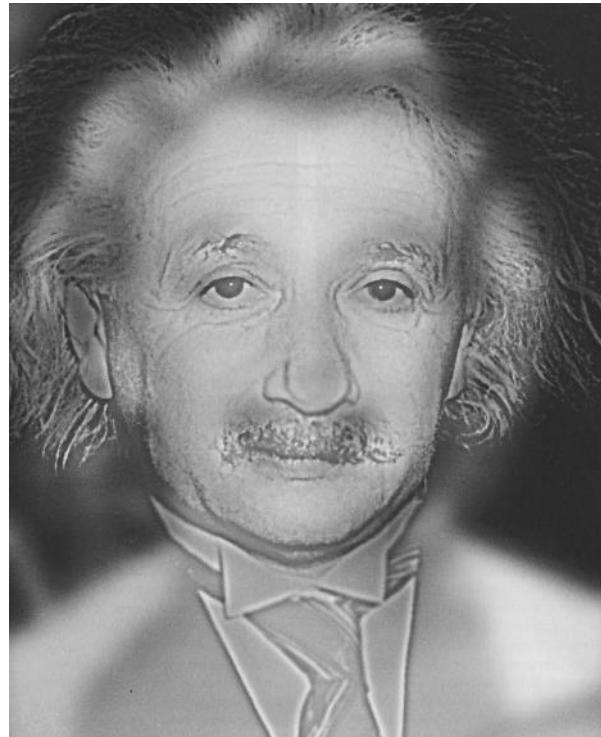
Let's take 2 points, their size below the optical resolution, spatially close one from the other...



Appropriate sampling

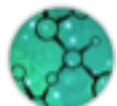
Shannon-Nyquist's theorem:

The image pixel size should be just below half the resolution (not more)



Inappropriate sampling

Inappropriate sampling might impairs our perception of the actual scene !

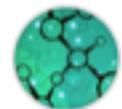
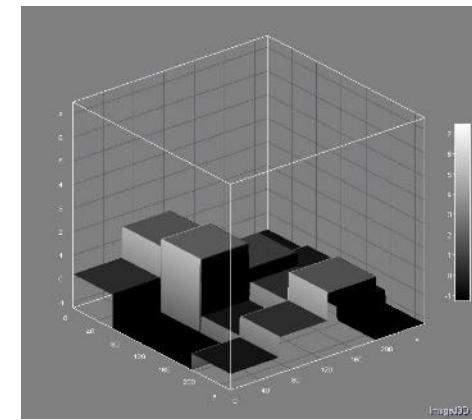
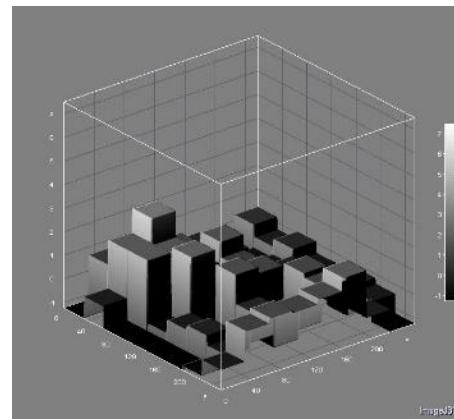
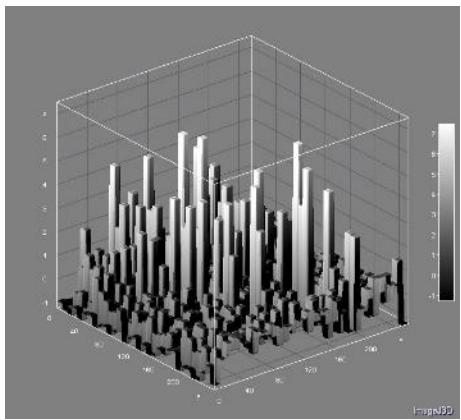
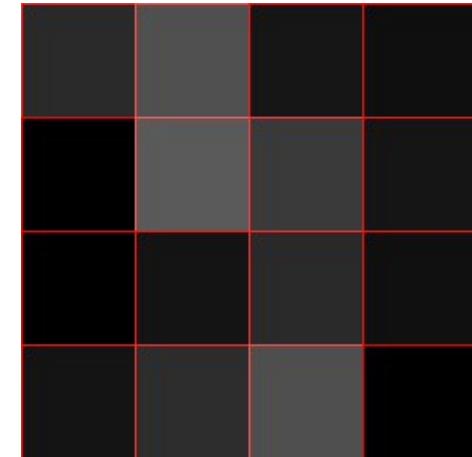
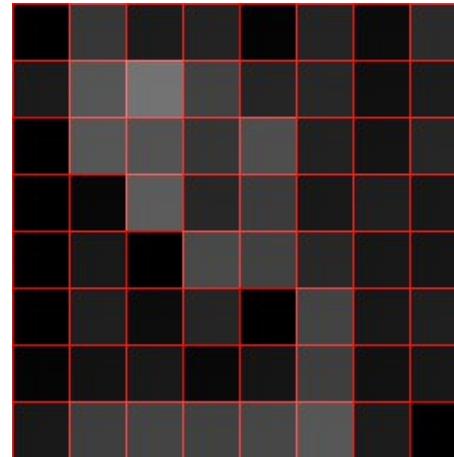
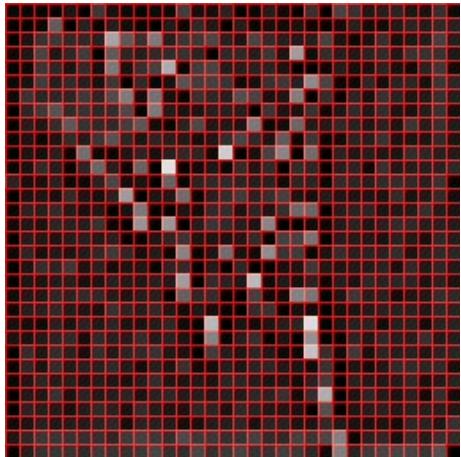


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What is an image ?

The image stores data originating from a dual sampling

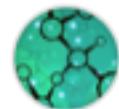
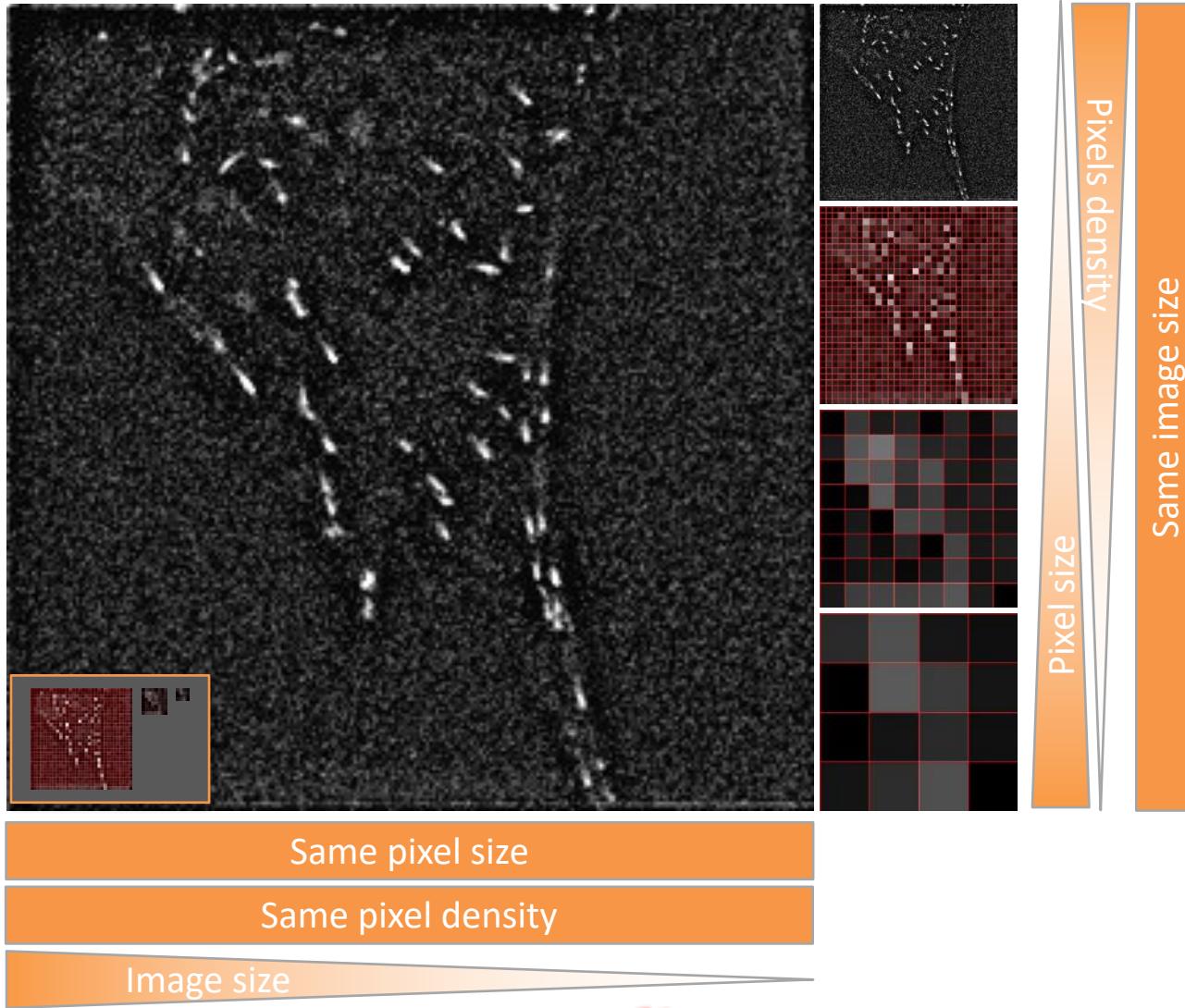


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What is an image ?

Dual sampling: spatial sampling, density and display

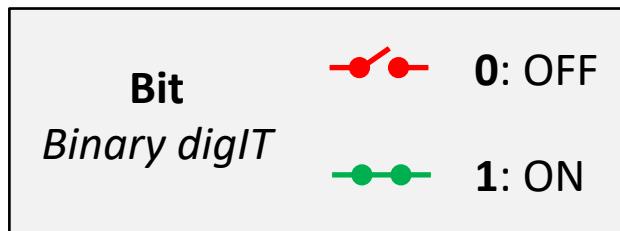


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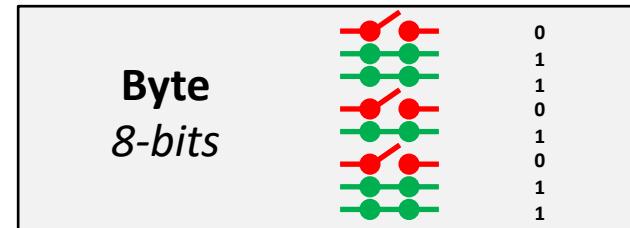


What is an image ?

Data handling in a computer: bits and bytes



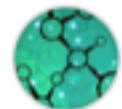
2 states per bit



2⁸ states per byte

| | | | | | | | | | |
|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | |
| Little endian | 2^0 | 2^1 | 2^2 | 2^3 | 2^4 | 2^5 | 2^6 | 2^7 | |
| | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | |
| | 0 | 2 | 4 | 0 | 16 | 0 | 64 | 128 | 214 |
| Big endian | 2^7 | 2^6 | 2^5 | 2^4 | 2^3 | 2^2 | 2^1 | 2^0 | |
| | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | |
| | 0 | 64 | 32 | 0 | 8 | 0 | 2 | 1 | 107 |

"There are only 10 types of people in the world: those who understand binary, and those who don't."

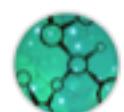
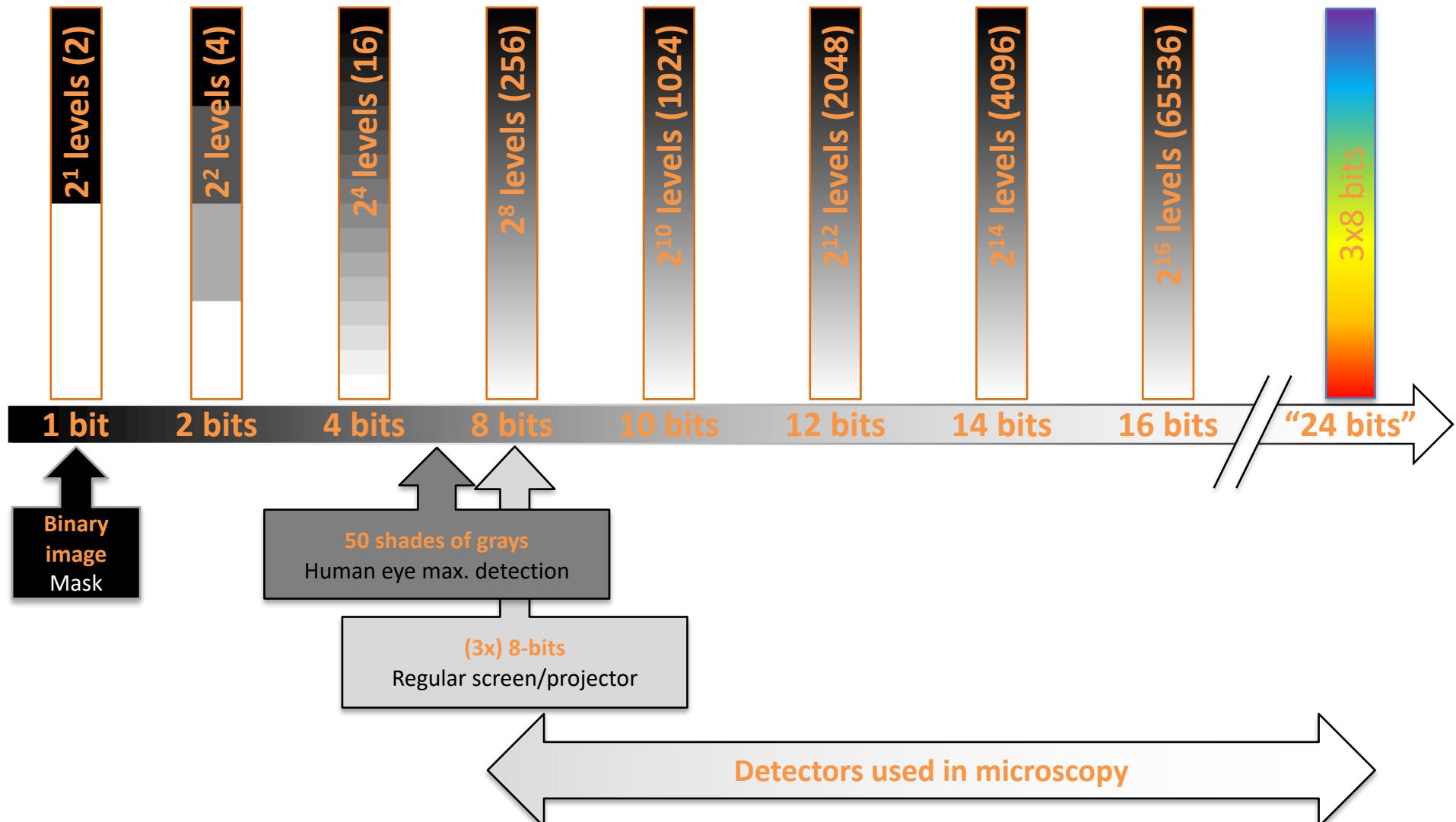


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What is an image ?

Dual sampling: intensity and bit depth

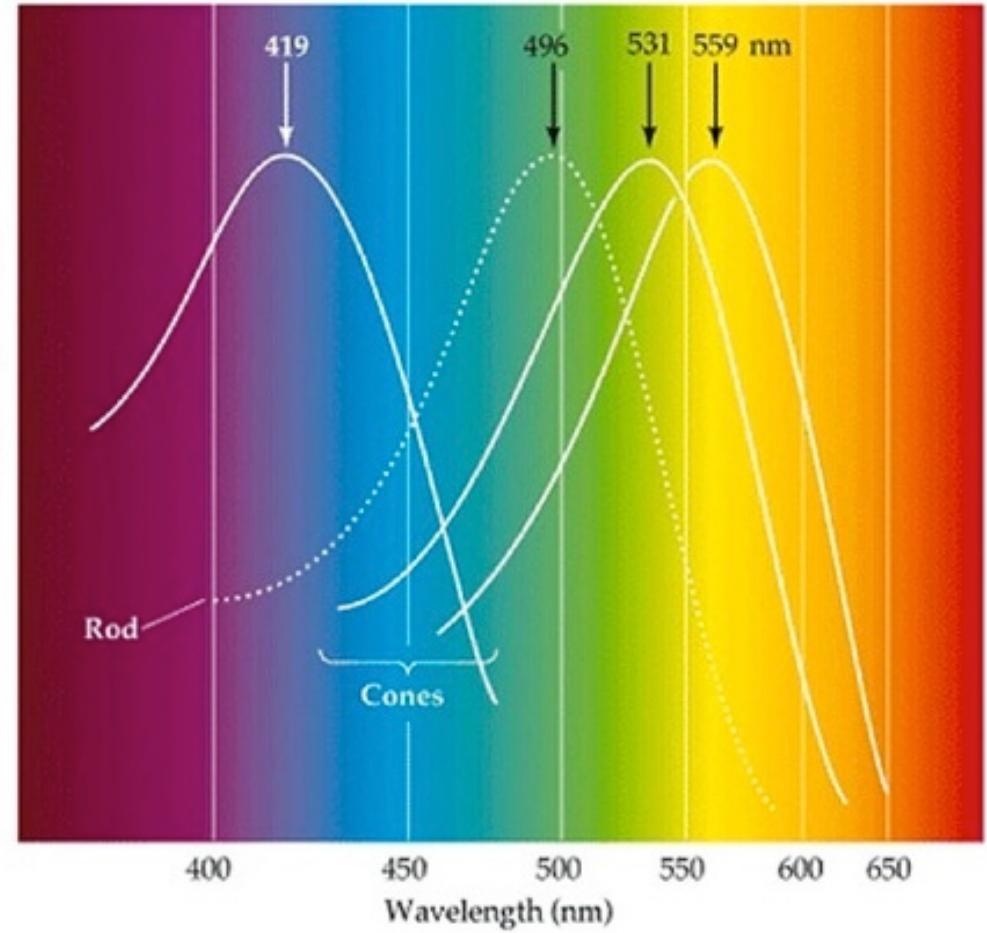


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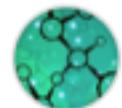


What is an image ?

Seeing in color



<http://webvision.med.utah.edu/>



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What is an image ?

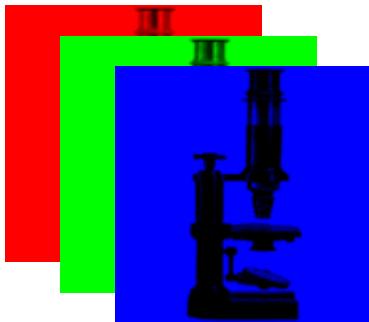
Color versus colorised image



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

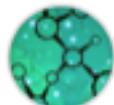
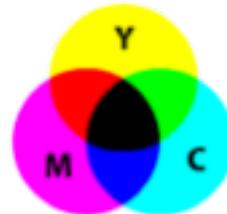
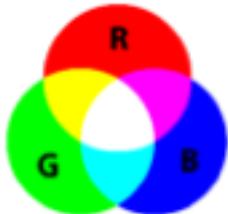
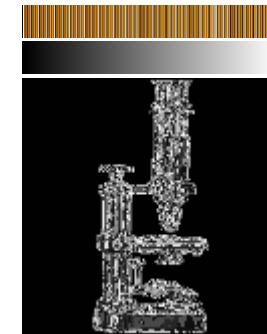


Subtractive mixing



Indexed colors

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

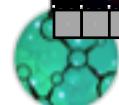
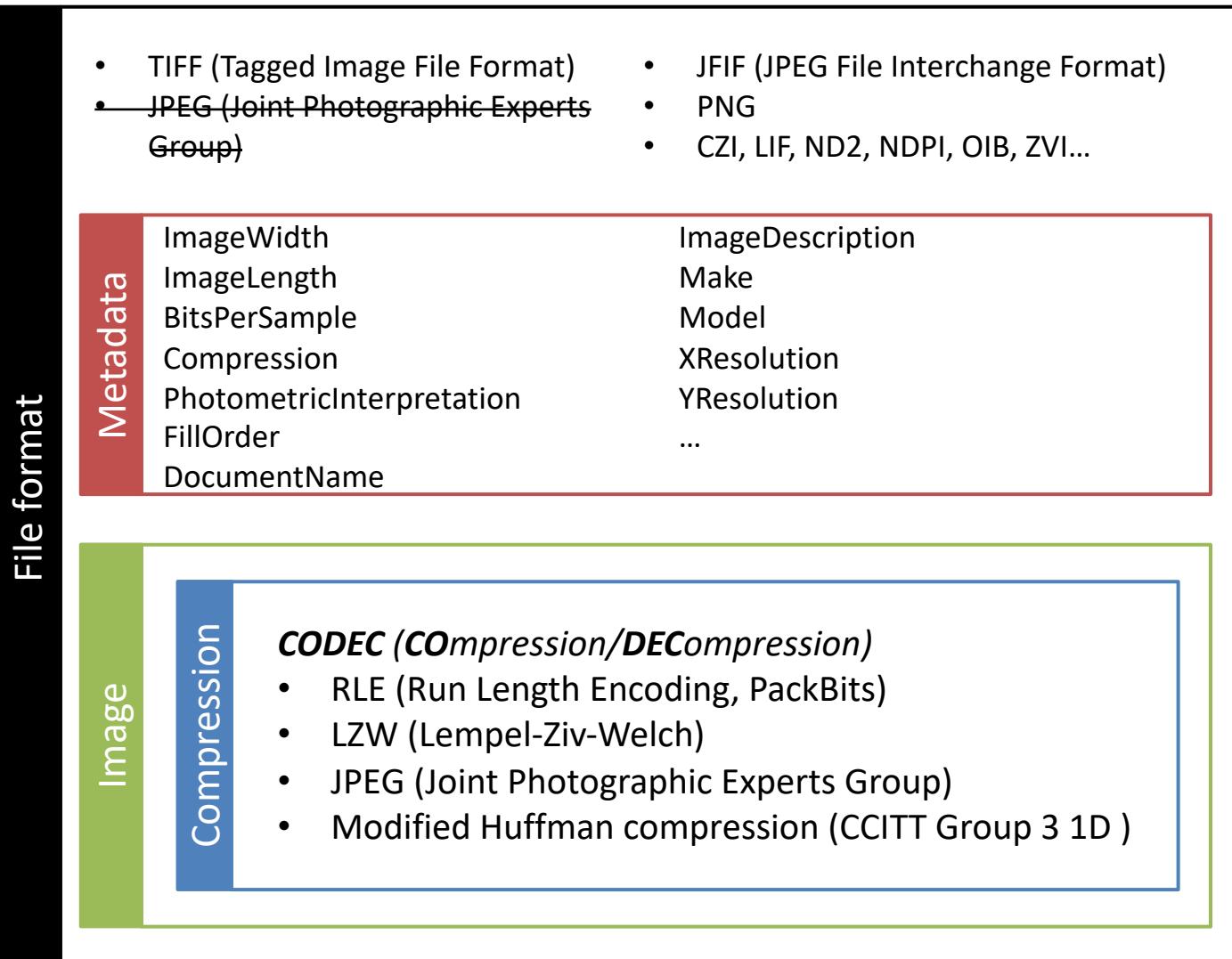
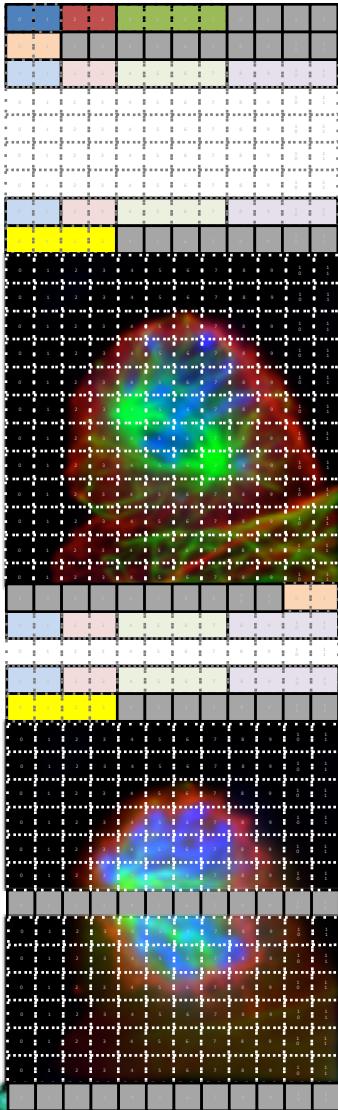


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What is an image ?

Storing an image: the container



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What is an image ?

Storing an image: saving space, compression strategies

Run-length encoding

Sentence:

AAABBBBBBAAACCCCCAAAAAB

Dictionary-based compression

Sentence:

ABCDEFABCEFEABC

- For each value, count the number of occurrence

- Identify individual words

ABC DDEF ABC EFE ABC

Compressed sentence:

3A6B3A5C5A1B

- Build a dictionary:

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

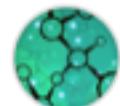
- Re-write the sentence using the dictionary:

Compressed sentence:

1231341

Used in LZW/ZIP compressions

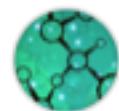
***Only non destructive compressions should be used for image processing and analysis
JPEG is a destructive compression: to be ONLY used for mail or presentation purposes***



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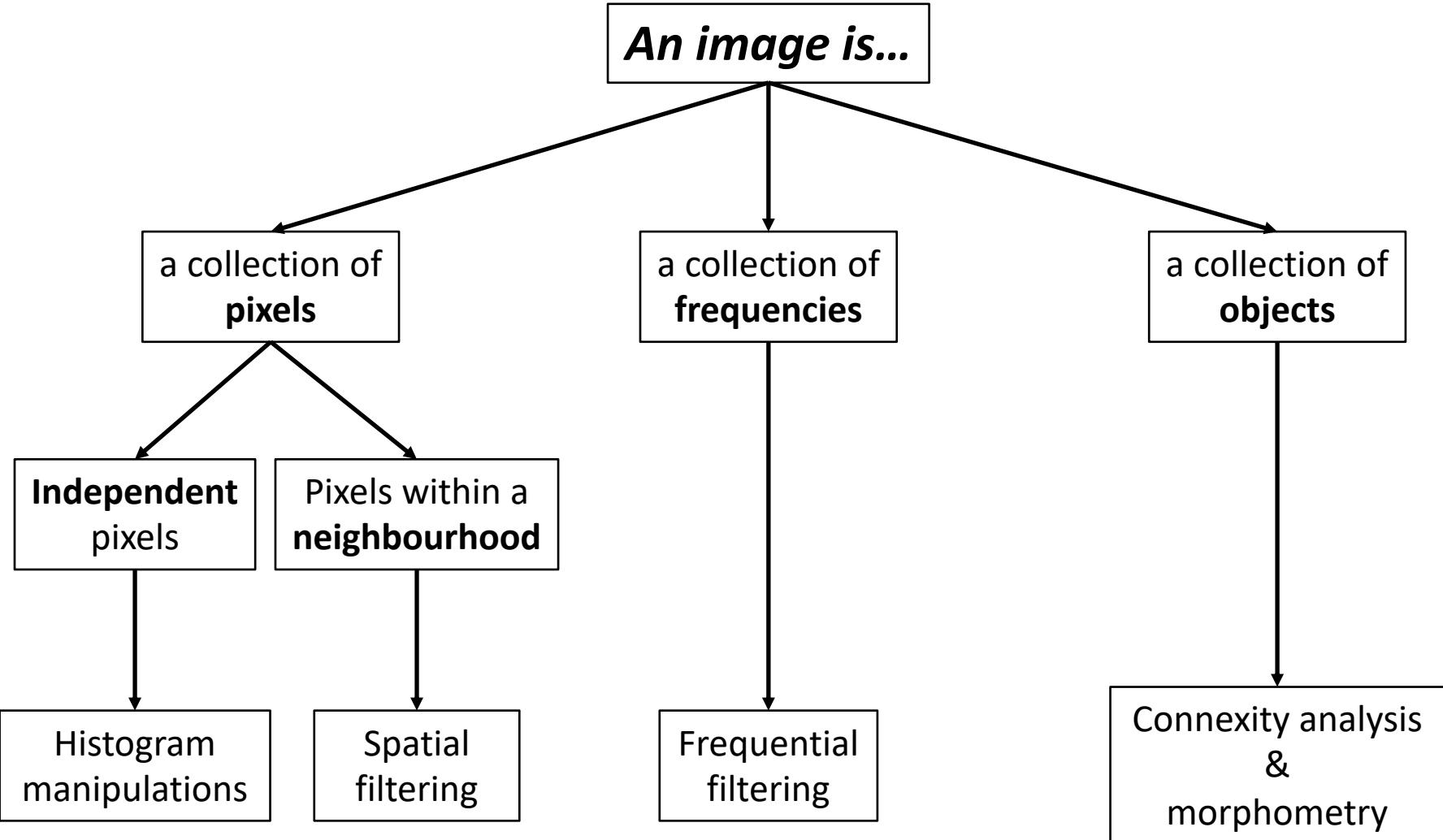


Image Processing



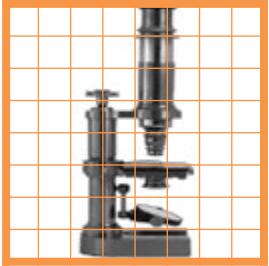
Several way to consider a single image

And associated processing techniques

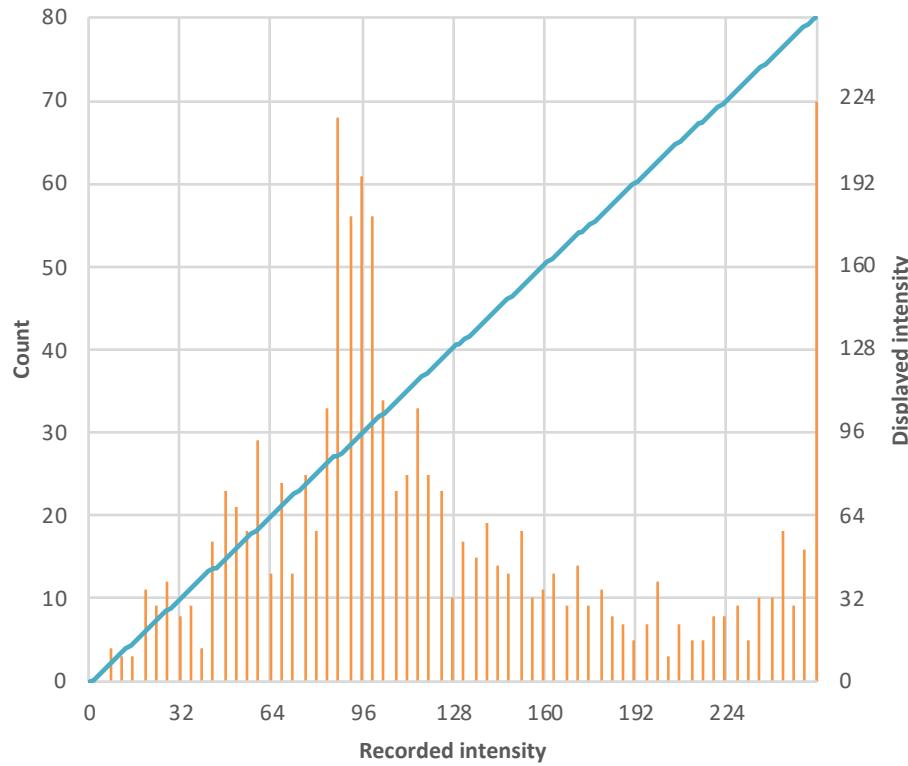


The image is a collection of intensities

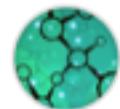
Working with the image's histogram



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...*

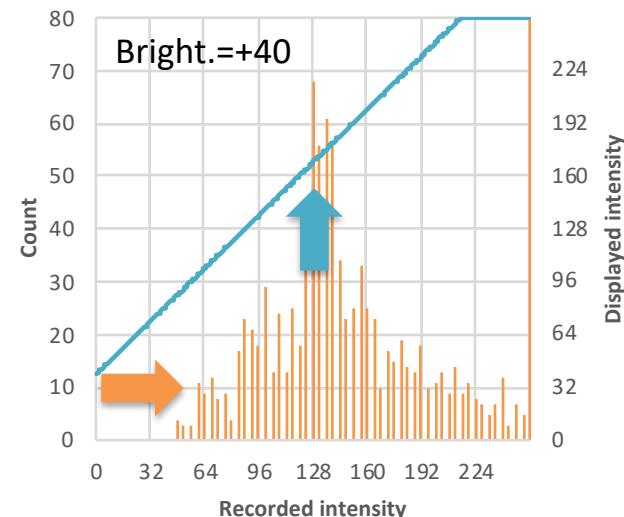
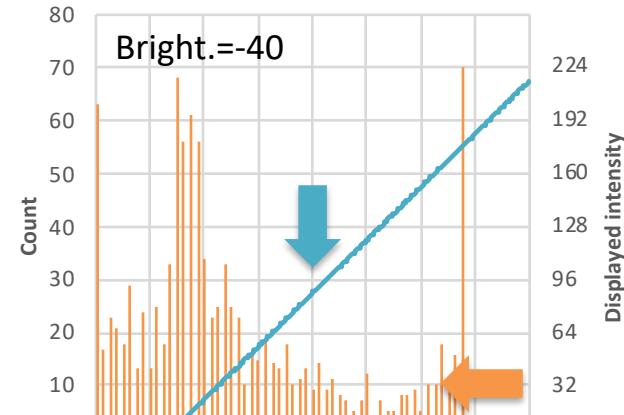
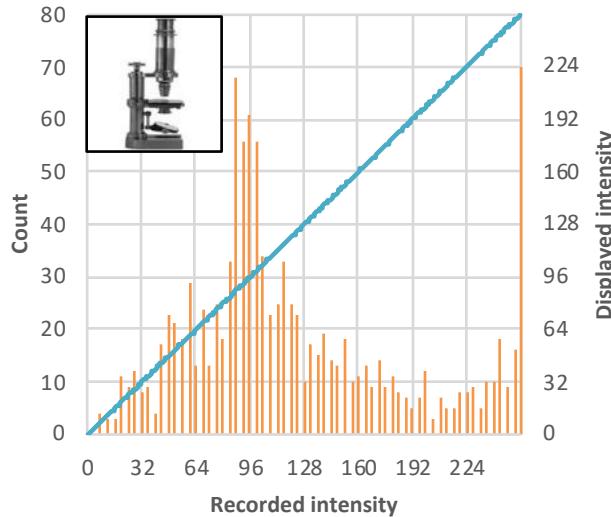


FRANCE-BIOIMAGING



The image is a collection of intensities

Linear histogram modification: brightness



Brightness:

The same value is added to all intensities

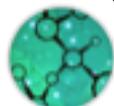


Thresholding:

Negative values are shifted to zero

Saturation:

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

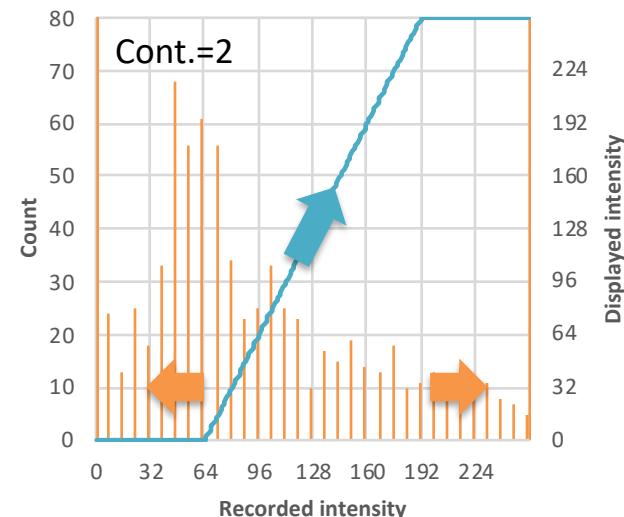
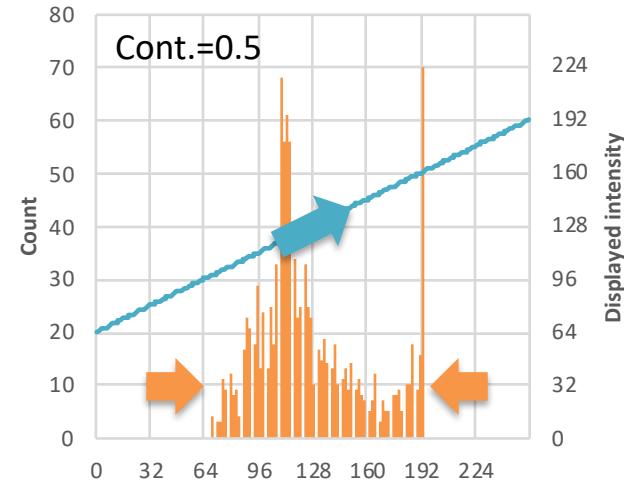
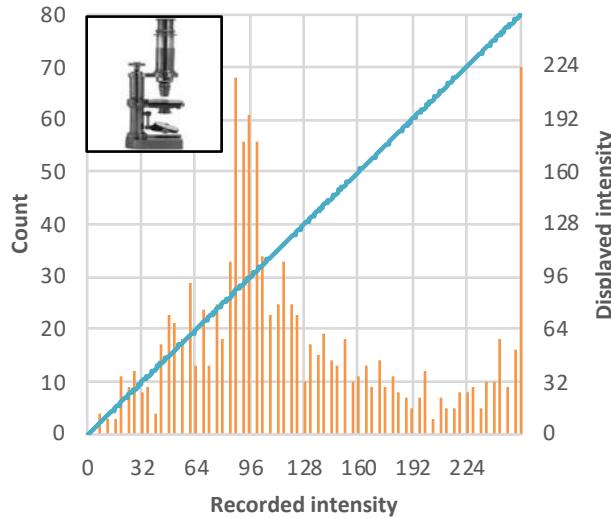


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The image is a collection of intensities

Linear histogram modification: contrast



Contrast:

Response line: the slope is changed

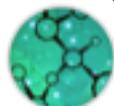
The mid-range value remains constant

Thresholding:

Negative values are shifted to zero

Saturation:

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

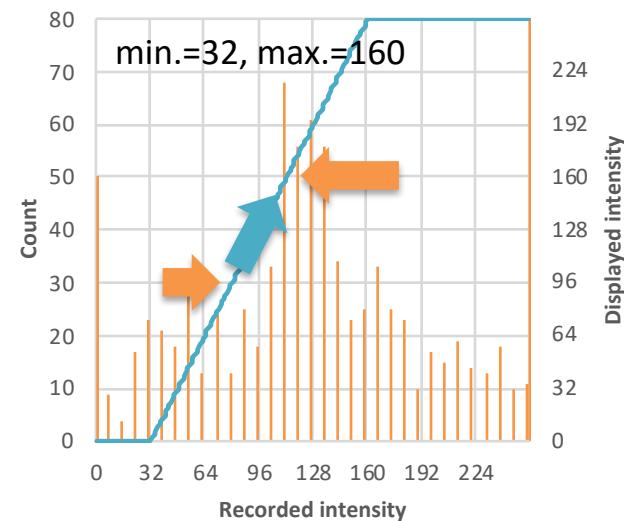
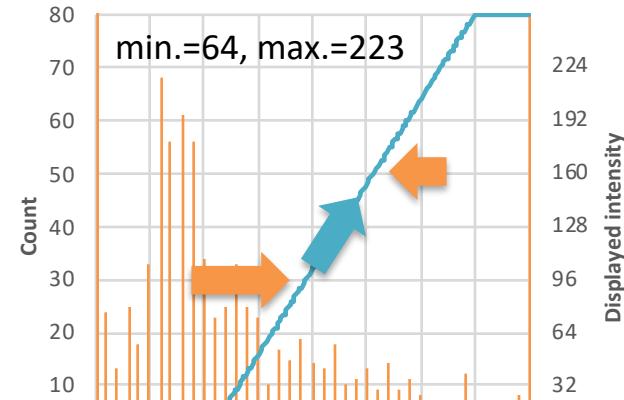
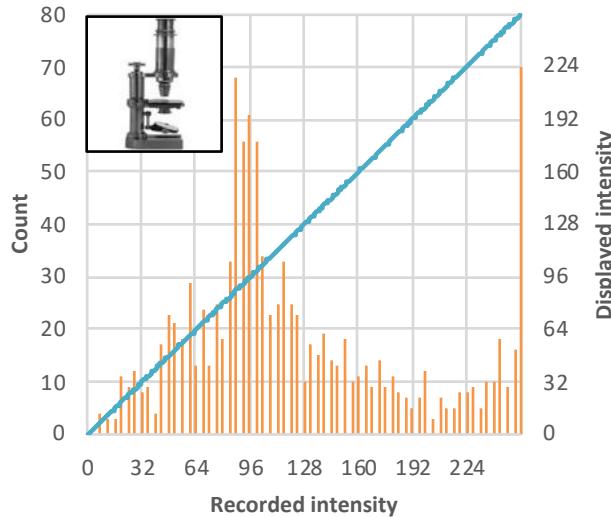


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The image is a collection of intensities

Linear histogram modification: min-max



Min-Max:

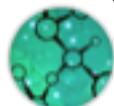
Intensities are linearly distributed between the two newly set limits

Thresholding:

Negative values are shifted to zero

Saturation:

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

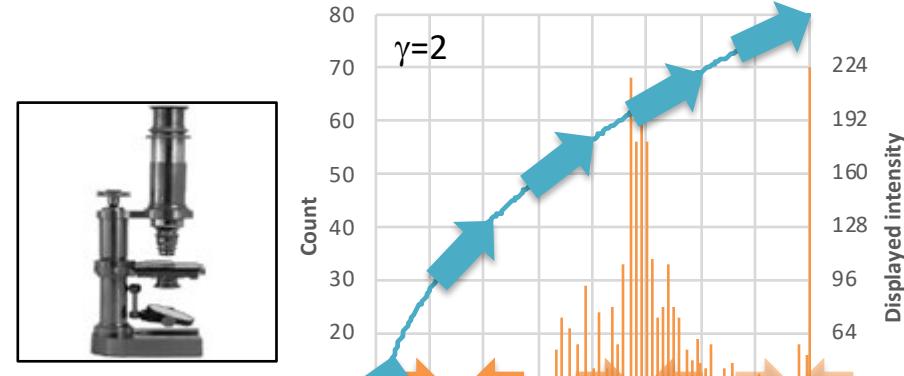
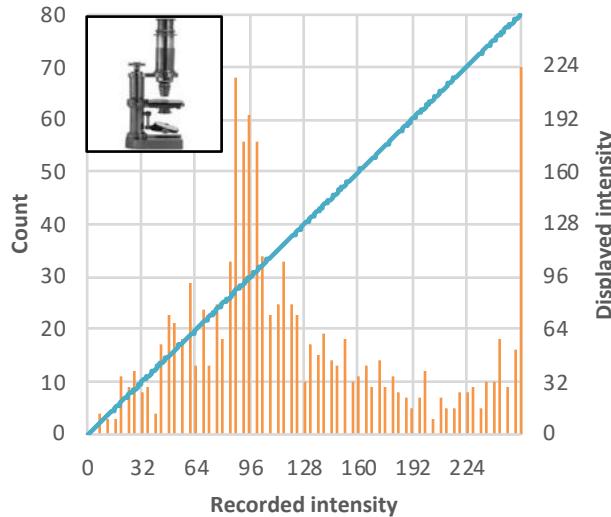


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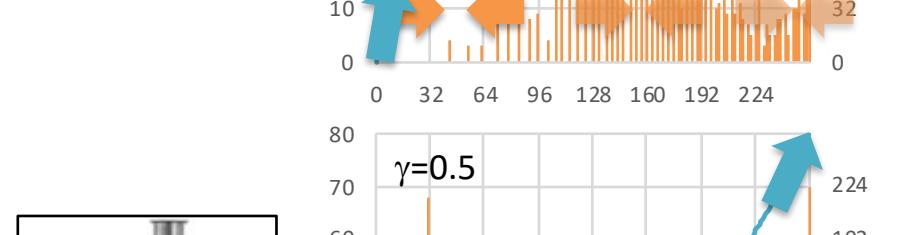


The image is a collection of intensities

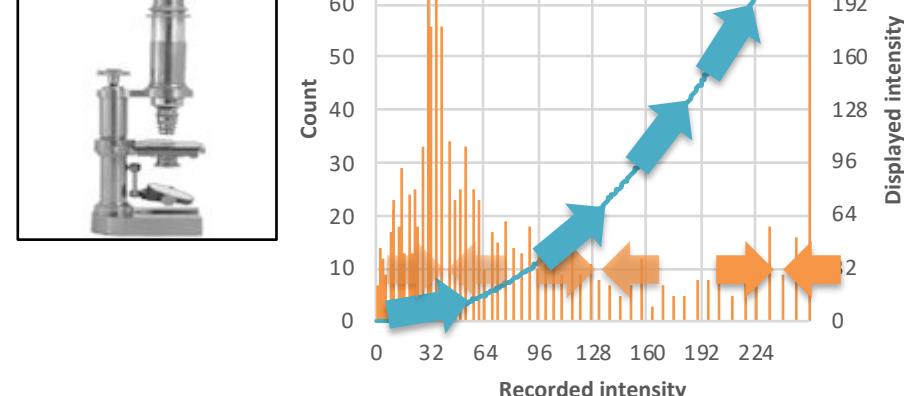
Non-linear histogram modification: gamma



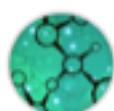
Gamma:
Contrast is variable



Thresholding:
Minimum stays at zero



Saturation:
Maximum remains the same

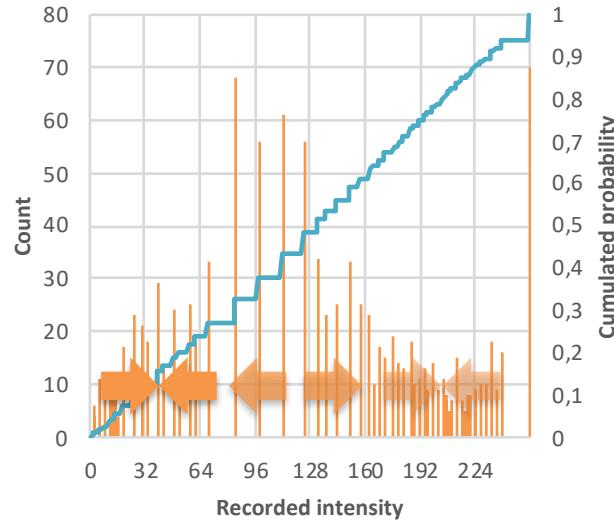
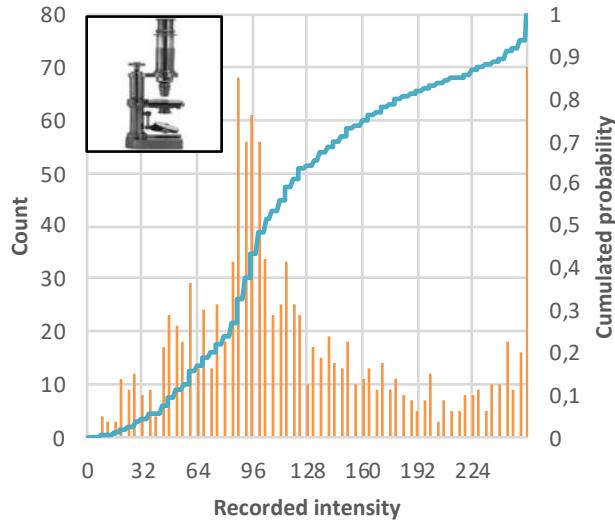


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The image is a collection of intensities

Non-linear histogram modification: histogram egalisation



Histogram egalisation:

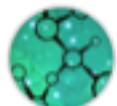
Contrast stretched for highly represented intensities

Thresholding:

Minimum stays at zero

Saturation:

Maximum remains the same



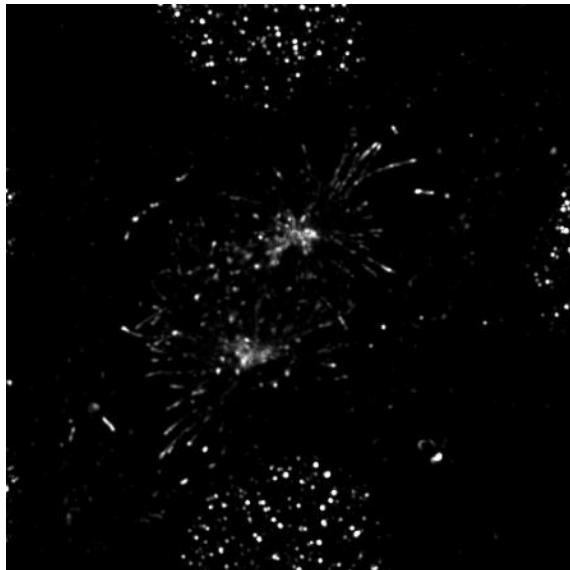
FRANCE-BIOIMAGING



The image is a collection of intensities

FAQ: Can I use non-linear histogram modifications ?

Raw image

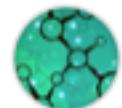


- No saturation
- Spindle poles visible
- MTs tips visible

Synopsis:

- You know the protein is slightly located on MTs and heavily on poles
- You want to show LOCATIONS
- You can't go back to the microscope
- You dropped your slide and broke it
- You have a group meeting in just 5 minutes

What will you do ???



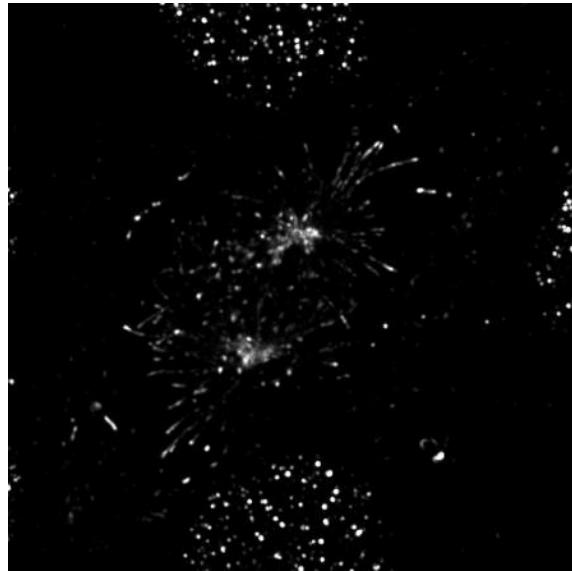
FRANCE-BIOIMAGING



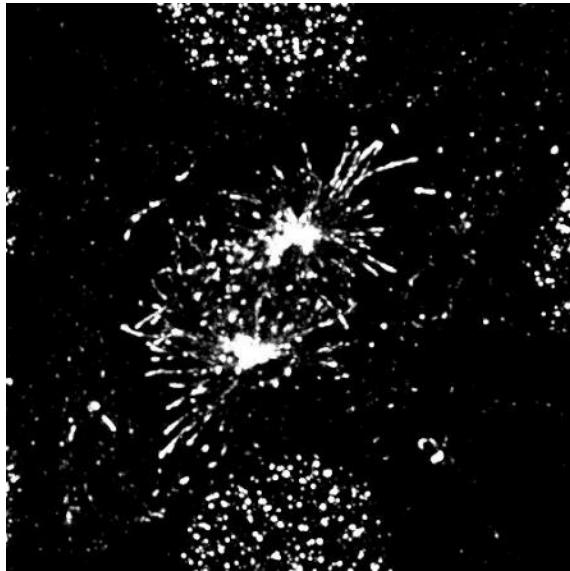
The image is a collection of intensities

FAQ: Can I use non-linear histogram modifications ?

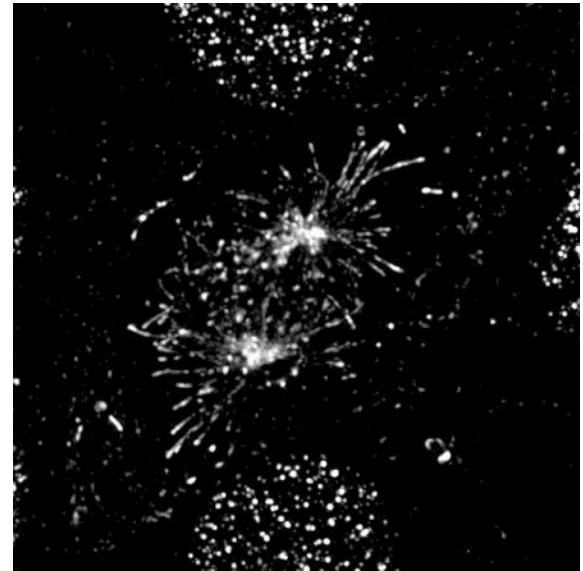
Raw image



Boosted contrast



Gamma



- No saturation
- Spindle poles visible
- MTs tips visible

- Highly saturated
- Spindle poles size increased
- MTs tips visible
- Staining visible all along astral MTs

- No saturation
- All localisations visible
- Relative intensities lost

The image is a collection of intensities

FAQ: Can I use non-linear histogram modifications ?

YES, but clearly state you've been using it !

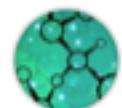
"Image manipulation policy

Digital images in manuscripts accepted for publication will be scrutinized by our production department for any indication of manipulation. If evidence of inappropriate image manipulation is detected, the Journal's Editors may ask for the original data to be supplied.

Do not add to, alter, enhance, obscure, move or remove a specific feature of an image – the focus should be on the data rather than its presentation (e.g. do not 'clean up' backgrounds or remove/obscure imperfections and non-specific bands)

*Adjustments should be applied to the whole image so no specific feature of the original data, including background, is obscured, eliminated or misrepresented as a consequence. **Any non-linear adjustments must be disclosed in the appropriate figure legends** and in the Materials and Methods section.” (From **Journal of Cell Science** website)*

Conclusion: always have a look at the « instructions to authors » !



FRANCE-BIOIMAGING



Spatial filtering

Linear filtering : taking care of the neighbours, based on values

Original image

| | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|
| 168 | 151 | 131 | 114 | 89 | 62 | 118 |
| 141 | 183 | 182 | 148 | 122 | 60 | 102 |
| 160 | 254 | 254 | 201 | 106 | 66 | 112 |
| 162 | 254 | 255 | 235 | 105 | 74 | 132 |
| 162 | 254 | 255 | 244 | 128 | 78 | 150 |
| 163 | 254 | 255 | 252 | 165 | 100 | 189 |
| 121 | 196 | 211 | 203 | 188 | 175 | 213 |

Filter

| | | |
|---|---|---|
| 1 | 1 | 1 |
| 1 | 1 | 1 |
| 1 | 1 | 1 |

$$168 \times 1 + 151 \times 1 + 131 \times 1 + \\ 141 \times 1 + 183 \times 1 + 182 \times 1 + \\ 160 \times 1 + 254 \times 1 + 254 \times 1 = 1624$$

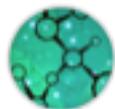
$$1624 / 9 = 180.44444$$

→ 180

| | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|
| 168 | 151 | 131 | 114 | 89 | 62 | 118 |
| 141 | 183 | 182 | 148 | 122 | 60 | 102 |
| 160 | 254 | 254 | 201 | 106 | 66 | 112 |
| 162 | 254 | 255 | 235 | 105 | 74 | 132 |
| 162 | 254 | 255 | 244 | 128 | 78 | 150 |
| 163 | 254 | 255 | 252 | 165 | 100 | 189 |
| 121 | 196 | 211 | 203 | 188 | 175 | 213 |

Destination image

| | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|
| 168 | 151 | 131 | 114 | 89 | 62 | 118 |
| 141 | 183 | 182 | 148 | 122 | 60 | 102 |
| 160 | 254 | 254 | 201 | 106 | 66 | 112 |
| 162 | 254 | 255 | 235 | 105 | 74 | 132 |
| 162 | 254 | 255 | 244 | 128 | 78 | 150 |
| 163 | 254 | 255 | 252 | 165 | 100 | 189 |
| 121 | 196 | 211 | 203 | 188 | 175 | 213 |



FRANCE-BIOIMAGING



Spatial filtering

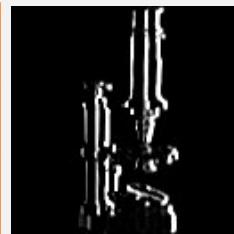
Most currently used filters

Original



Sobel
Horizontal

| | | |
|----|---|---|
| -1 | 0 | 1 |
| -2 | 0 | 2 |
| -1 | 0 | 1 |



Laplacian
4-connected

| | | |
|----|----|----|
| 0 | -1 | 0 |
| -1 | 4 | -1 |
| 0 | -1 | 0 |



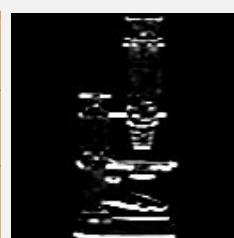
Gaussian
($\sigma=1$)

| | | | | |
|---|----|----|----|---|
| 1 | 4 | 7 | 4 | 1 |
| 4 | 16 | 26 | 16 | 4 |
| 7 | 26 | 41 | 26 | 7 |
| 4 | 16 | 26 | 16 | 4 |
| 1 | 4 | 7 | 4 | 1 |



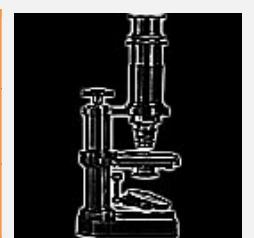
Sobel
Vertical

| | | |
|----|----|----|
| -1 | -2 | -1 |
| 0 | 0 | 0 |
| 1 | 2 | 1 |



Laplacian
8-connected

| | | |
|----|----|----|
| -1 | -1 | -1 |
| -1 | 8 | -1 |
| -1 | -1 | -1 |



Sharpen

| | | |
|----|----|----|
| -1 | -1 | -1 |
| -1 | 12 | -1 |
| -1 | -1 | -1 |

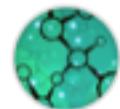


Sobel
 $\sqrt{\text{Horizontal}^2 + \text{Vertical}^2}$



Your turn:
be creative !

| | | |
|---|---|---|
| ? | ? | ? |
| ? | ? | ? |
| ? | ? | ? |



FRANCE-BIOIMAGING



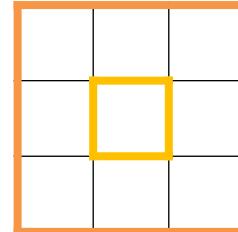
Spatial filtering

Rank filtering: taking care of the neighbours, based on order

Original image

| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|--|
| 168 | 151 | 131 | 114 | 89 | 62 | 118 | |
| 141 | 183 | 182 | 148 | 122 | 60 | 102 | |
| 160 | 254 | 254 | 201 | 106 | 66 | 112 | |
| 162 | 254 | 255 | 235 | 105 | 74 | 132 | |
| 162 | 254 | 255 | 244 | 128 | 78 | 150 | |
| 163 | 254 | 255 | 252 | 165 | 100 | 189 | |
| 121 | 196 | 211 | 203 | 188 | 175 | 213 | |

Filter



Destination image

| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|--|
| 168 | 151 | 131 | 114 | 89 | 62 | 118 | |
| 141 | 183 | 182 | 148 | 122 | 60 | 102 | |
| 160 | 254 | 254 | 201 | 106 | 66 | 112 | |
| 162 | 254 | 255 | 235 | 105 | 74 | 132 | |
| 162 | 254 | 255 | 244 | 128 | 78 | 150 | |
| 163 | 254 | 255 | 252 | 165 | 100 | 189 | |
| 121 | 196 | 211 | 203 | 188 | 175 | 213 | |

131 | 141 | 151 | 160 | 168 | 182 | 183 | 254 | 254

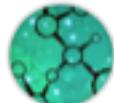
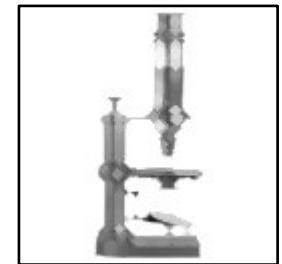
Minimum



Median



Maximum



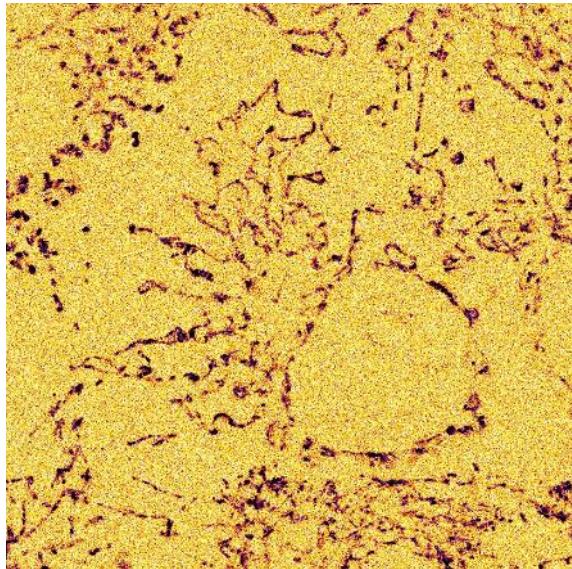
FRANCE-BIOIMAGING



Spatial filtering

The noisy case: linear vs rank filtering

Raw (Gaussian noise)

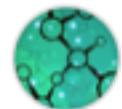


- Noise appears as rapid local intensity variations

Synopsis:

- You can't go back to the microscope
- You dropped your slide and broke it
- You have a group meeting in just 5 minutes

***What will you do ???
(Not a lucky day)***



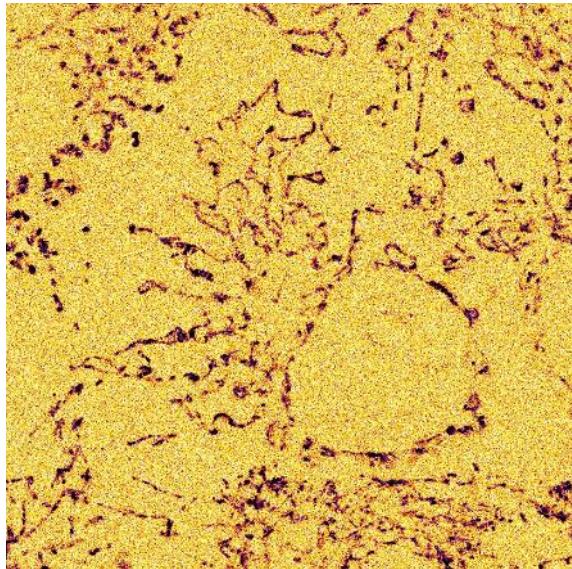
FRANCE-BIOIMAGING



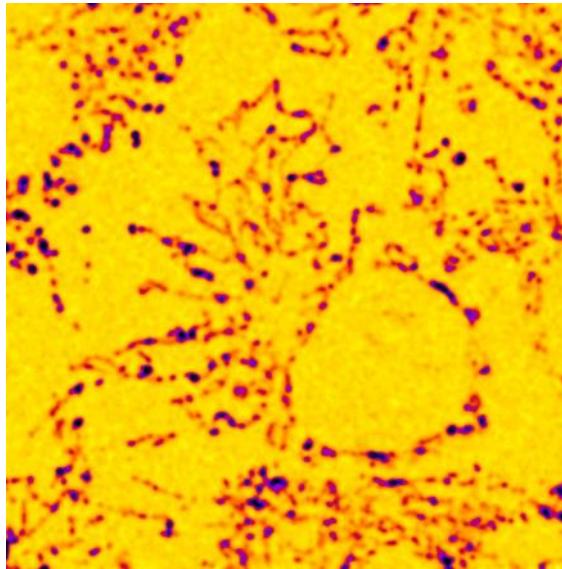
Spatial filtering

The noisy case: linear vs rank filtering

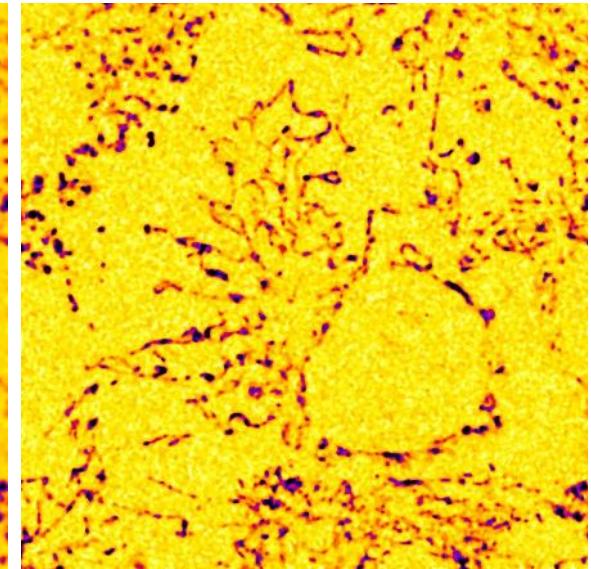
Raw (Gaussian noise)



Gaussian filter



Median filter



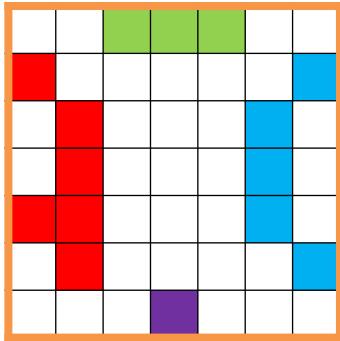
- Noise appears as rapid local intensity variations
- Image has been heavily smoothed
- Noise is less visible
- Structures' borders have been spread
- Image has been slightly smoothed
- Noise is less visible
- Structures' borders have been almost preserved

There is nothing like a good sample preparation and a proper image acquisition !

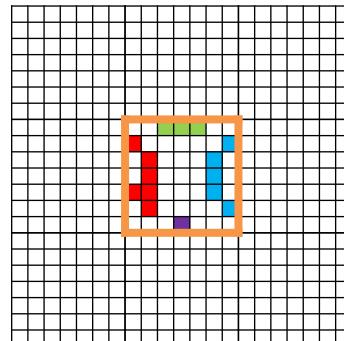
Spatial filtering

Taking care of the borders

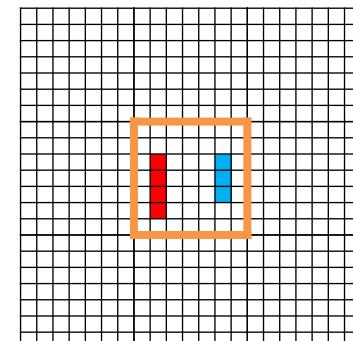
Original image



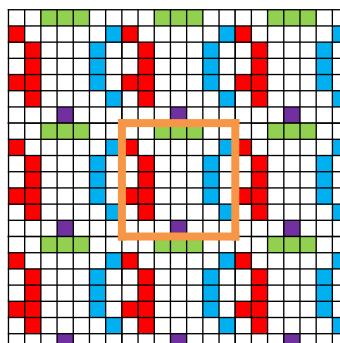
No borders



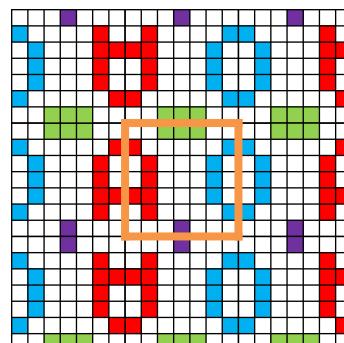
No processing



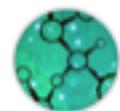
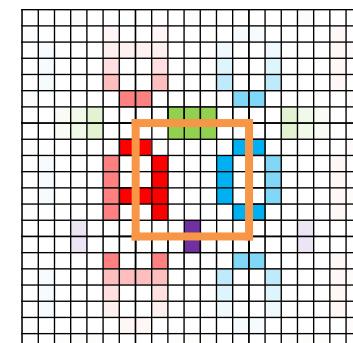
Repetition



Symmetry



Weighted symmetry



FRANCE-BIOIMAGING



Spatial filtering

Influence of parameters: size and shape

Original



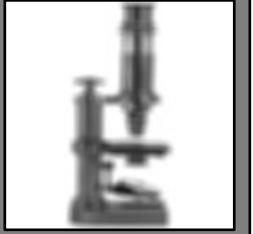
3x3

| | | |
|---|---|---|
| 1 | 1 | 1 |
| 1 | 1 | 1 |
| 1 | 1 | 1 |



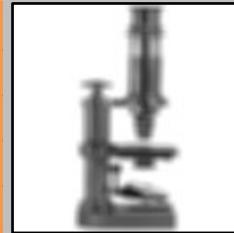
Square

| | | | | |
|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |



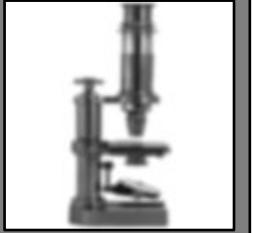
5x5

| | | | | |
|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |



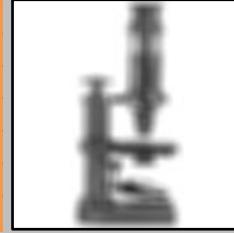
Diamond

| | | | | |
|---|---|---|---|---|
| 0 | 0 | 1 | 0 | 0 |
| 0 | 1 | 1 | 1 | 0 |
| 1 | 1 | 1 | 1 | 1 |
| 0 | 1 | 1 | 1 | 0 |
| 0 | 0 | 1 | 0 | 0 |



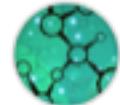
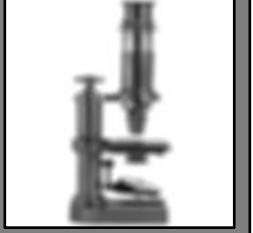
7x7

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 |



Circle

| | | | | |
|---|---|---|---|---|
| 0 | 1 | 1 | 1 | 0 |
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 0 | 1 | 1 | 1 | 0 |

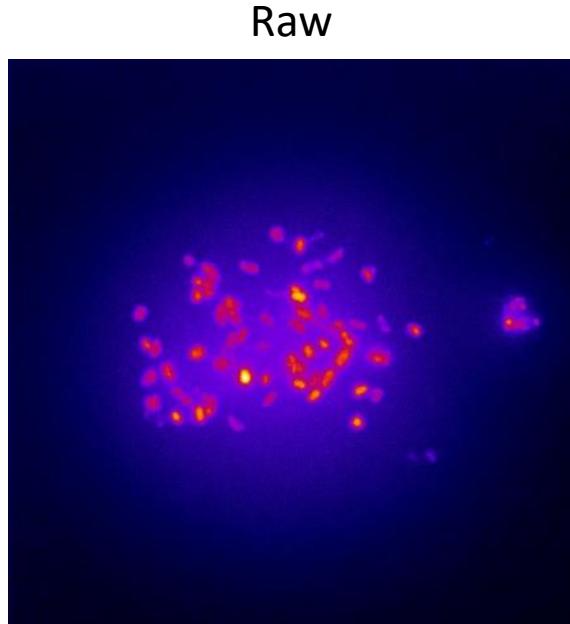


FRANCE-BIOIMAGING



Spatial filtering

Influence of parameters: size and shape



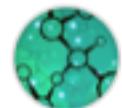
- Small details (kinetochores, width: 5-6 pixels)
- Large intensity variation: blur+uneven illumination

Synopsis:

- You can't go back to the microscope
- You didn't acquire an image of a blank field
- You dropped your slide and broke it
- You have a group meeting in just 5 minutes

***What will you do ???
(Still not a lucky day...)***

There is nothing like a good sample preparation and a proper image acquisition !

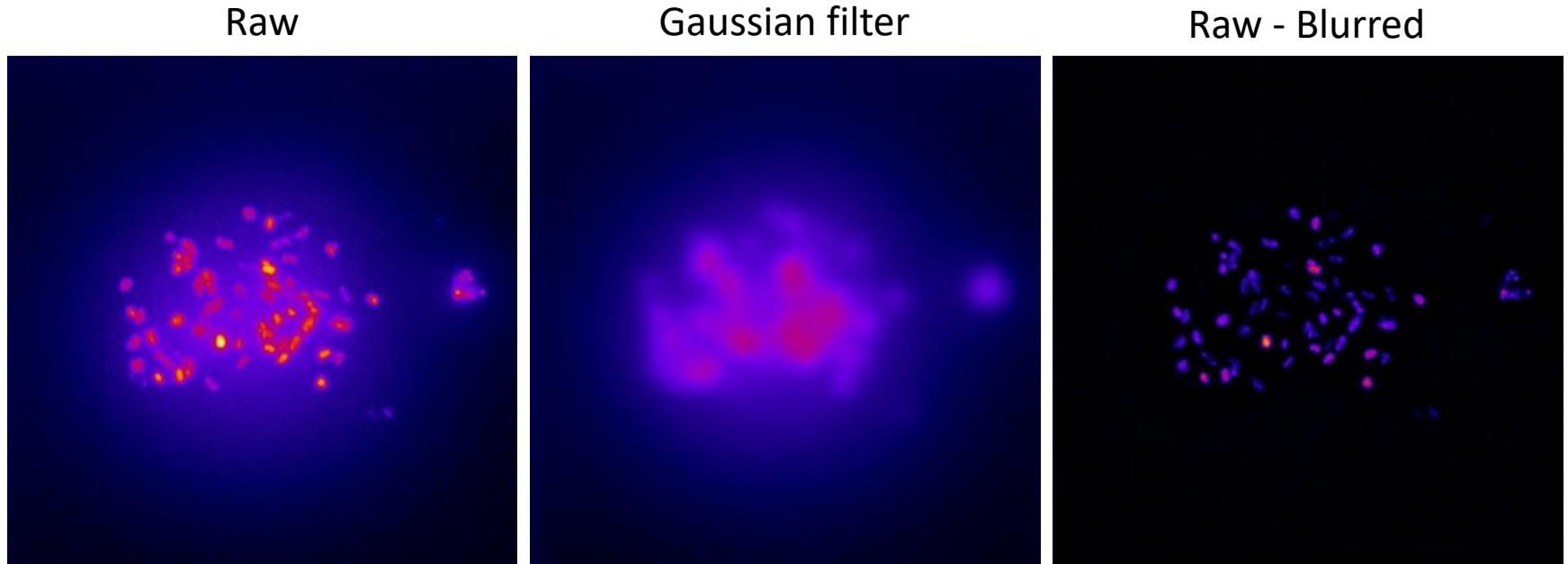


FRANCE-BIOIMAGING



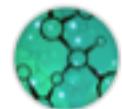
Spatial filtering

Influence of parameters: size and shape



- Small details (kinetochores, width: 5-6 pixels)
- Large intensity variation: blur+uneven illumination
- Gaussian blur, radius larger than the largest KT
- Smallest details are removed
- The large intensity variation remains
- Basic arithmetic between images is possible (image is a matrix)
- Smallest details remain
- The large intensity variation has been removed

There is nothing like a good sample preparation and a proper image acquisition !



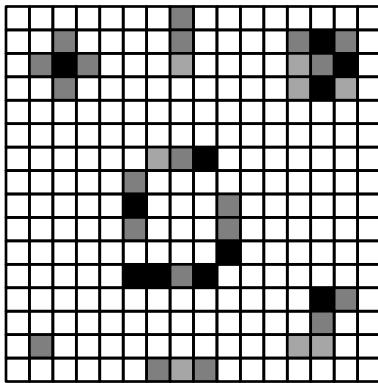
FRANCE-BIOIMAGING



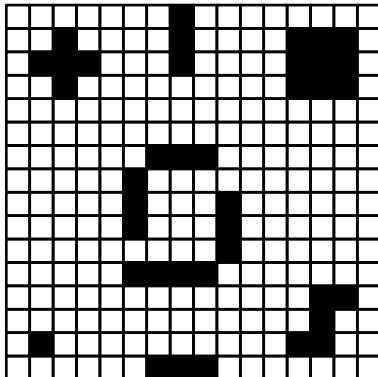
Morphomathematics

From intensity image to binary image (mask)

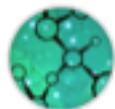
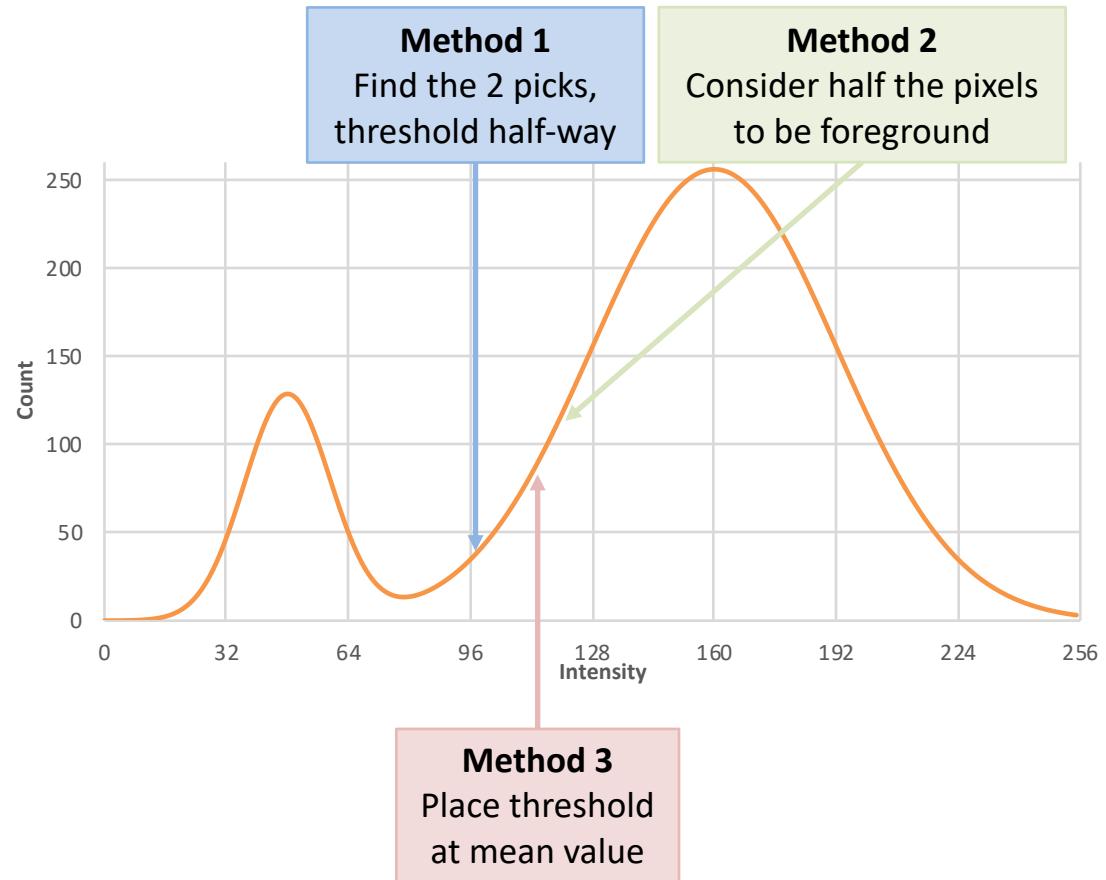
Intensity image



Binary image



How to partition image in object vs background pixels ?



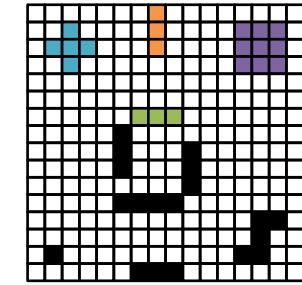
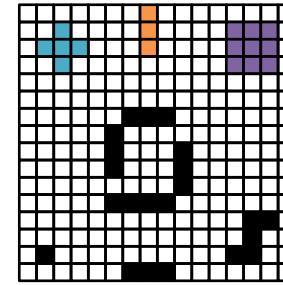
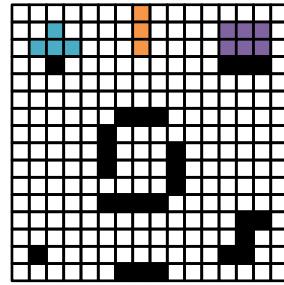
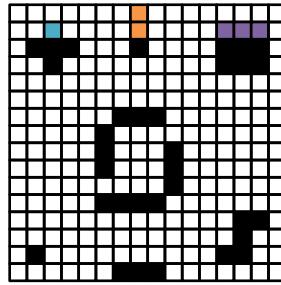
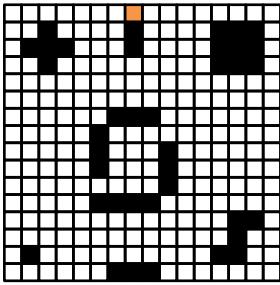
FRANCE-BIOIMAGING



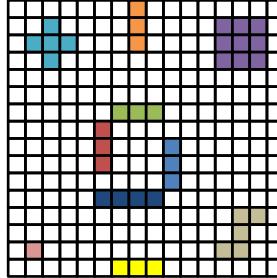
Image as a collection of objects

Connexity analysis

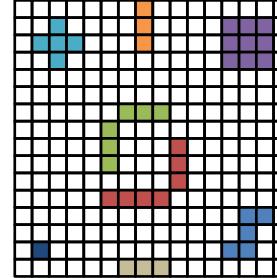
Pixel tagging, line per line → Objects' map



4-connected



8-connected



Features extraction:

- Count objects: maximum of all tags
- Total intensity: sum intensities for each tag
- Area: number of pixels per tag x pixel surface
- Perimeter: number of pixels lacking at least 1 neighbour x pixel size

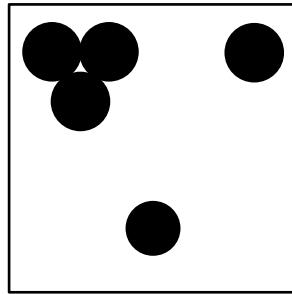
Extension to the 3D case:

6 or 26-connected

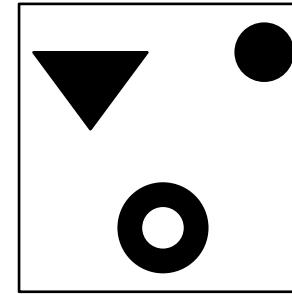
Working with binary images

Logical operators

Mask A

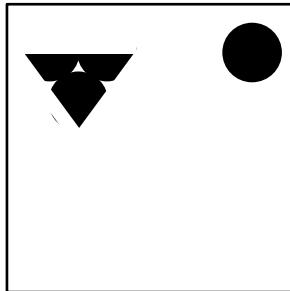


Mask B

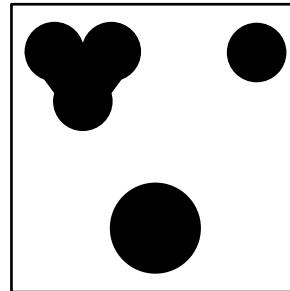


| A | B | AND | OR | XOR |
|---|---|-----|----|-----|
| 1 | 1 | 1 | 1 | 0 |
| 1 | 0 | 0 | 1 | 1 |
| 0 | 1 | 0 | 1 | 1 |
| 0 | 0 | 0 | 0 | 0 |

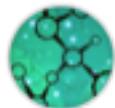
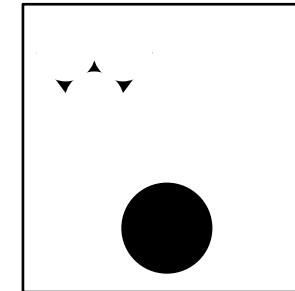
A AND B



A OR B



A XOR B



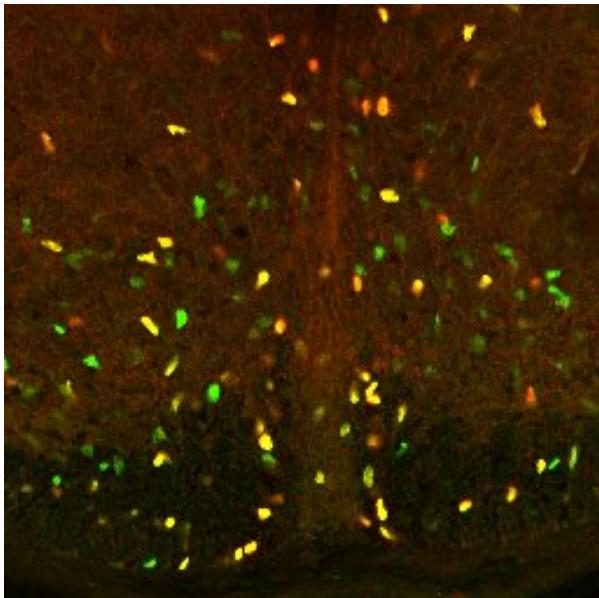
FRANCE-BIOIMAGING



Working with binary images

Logical operators and co-expression analysis

Original image



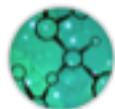
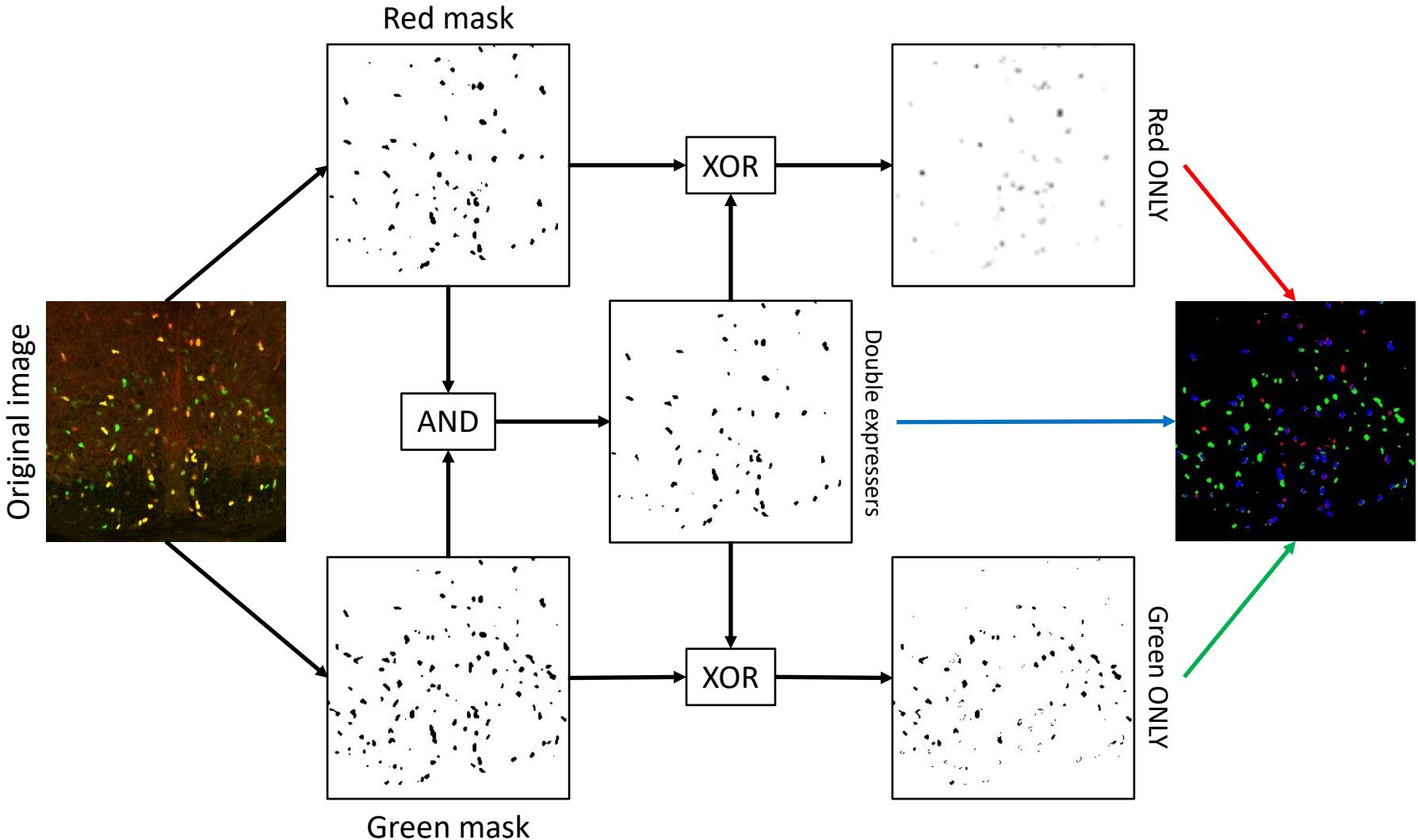
Synopsis:

- The image presents population of cells expressing either:
 - Marker A only
 - Marker B only
 - Both Marker A and marker B
- You want to isolate each type of cell
- You want to count each type of cell

How would you do ???

Working with binary images

Logical operators and co-expression analysis

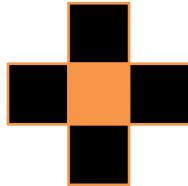


FRANCE-BIOIMAGING

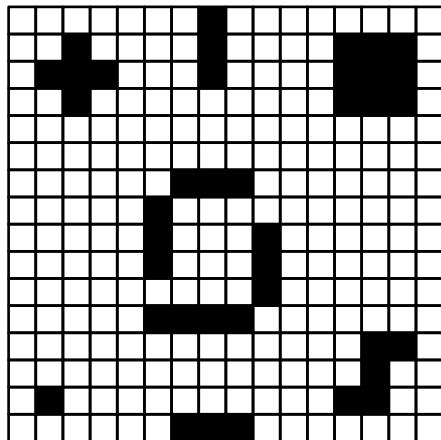


Mathematical morphology

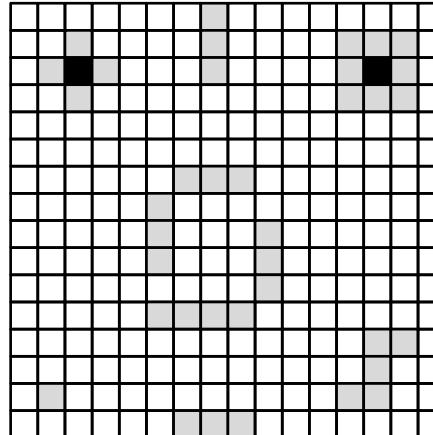
Morphological operators: 4-connected case



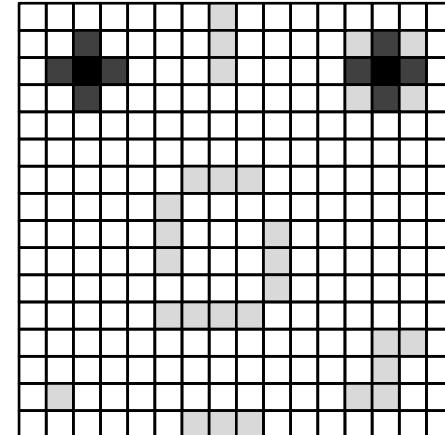
Structuring element



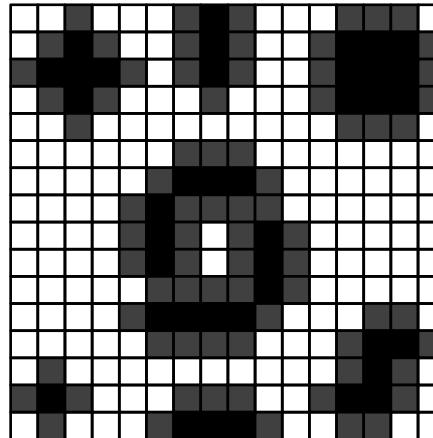
Binary image



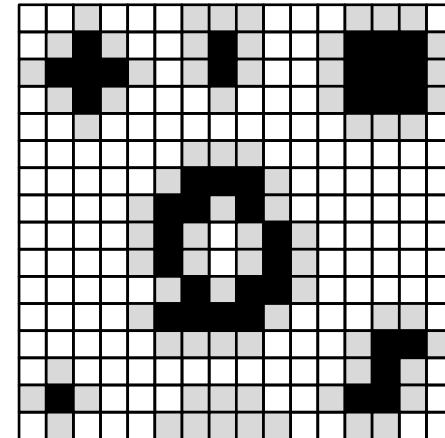
Erosion



Erode → Dilate = Open



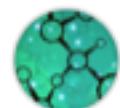
Dilation



Dilate → Erode = Close

Erode: propagate background

Dilate: propagate objects

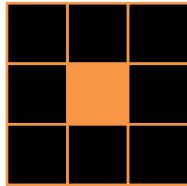


FRANCE-BIOIMAGING

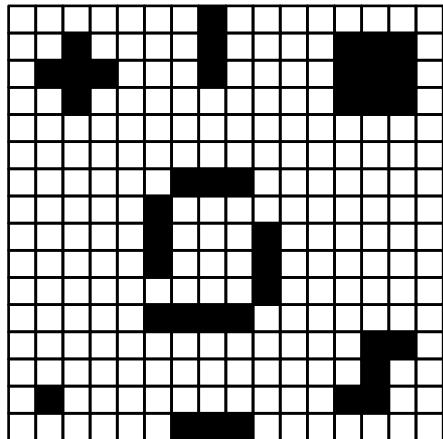


Mathematical morphology

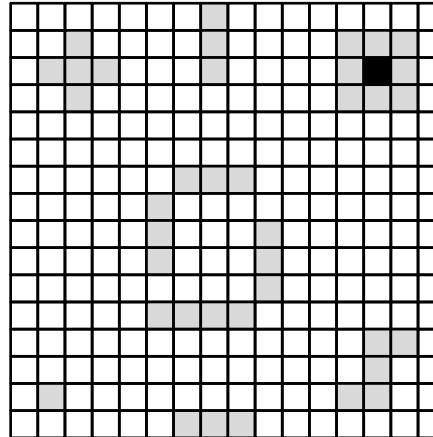
Morphological operators: 8-connected case



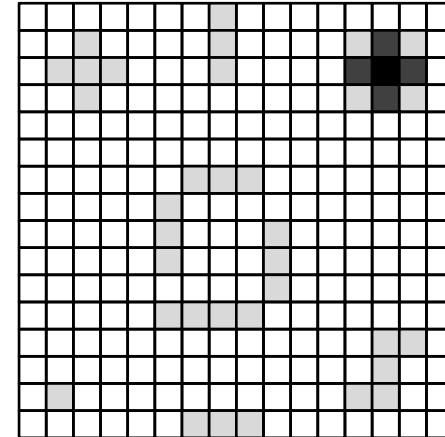
Structuring element



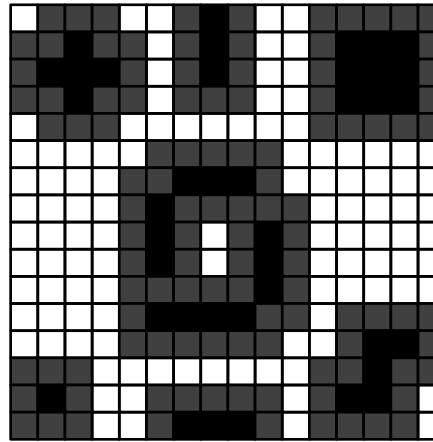
Binary image



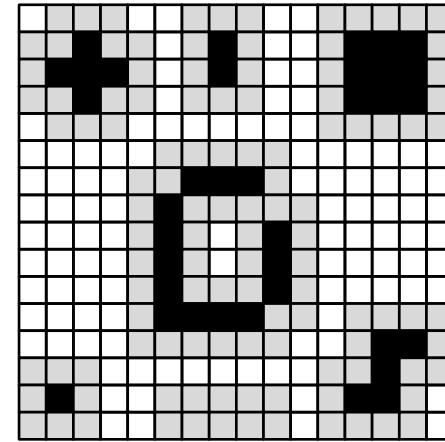
Erosion



Erode → Dilate = Open



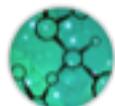
Dilation



Dilate → Erode = Close

Erode: propagate background

Dilate: propagate objects

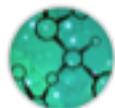
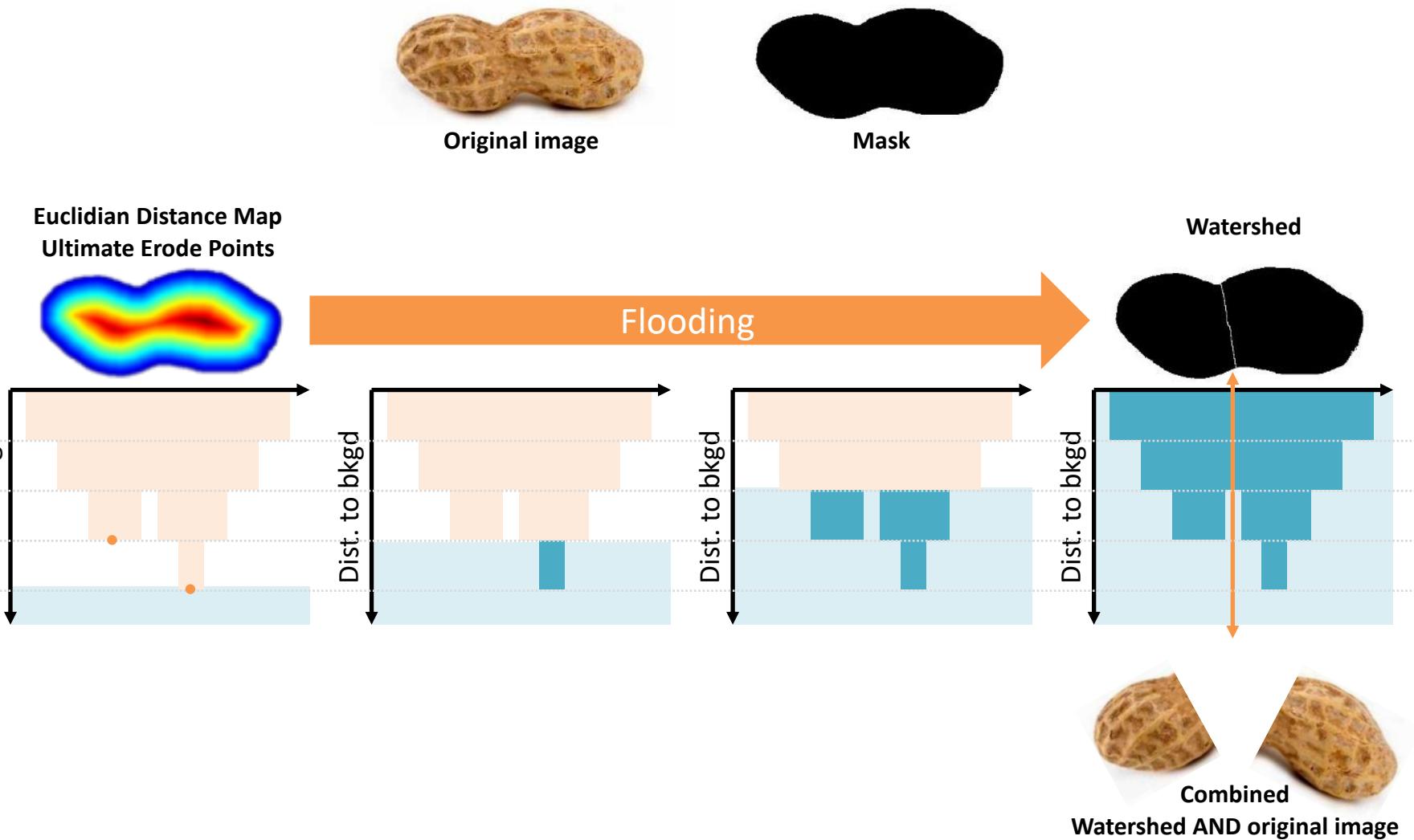


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Mathematical morphology

How to separate peanuts? Watershed transform



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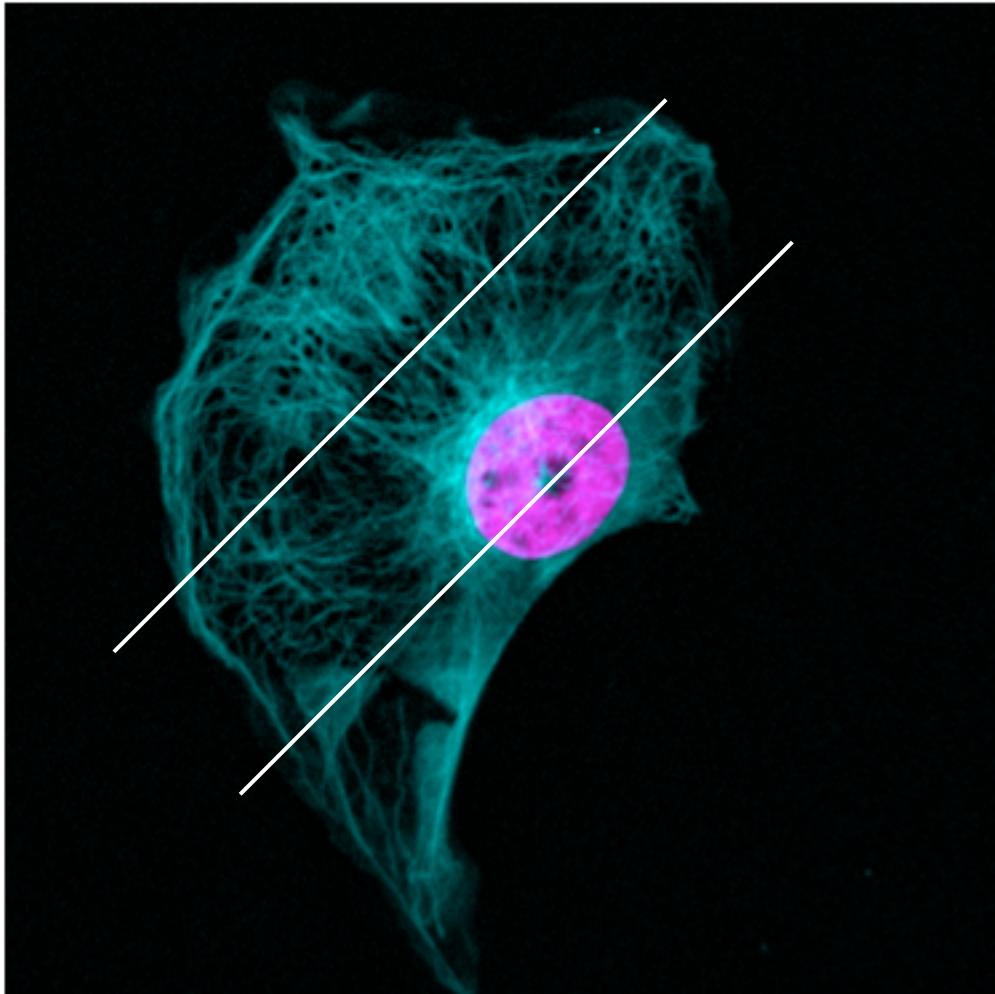


Combined
Watershed AND original image



Frequential filtering

The image is a collection of frequencies



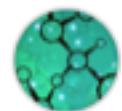
Microtubules

Small details = high frequencies



Nucleus

Rough details = low frequencies



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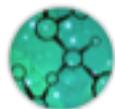
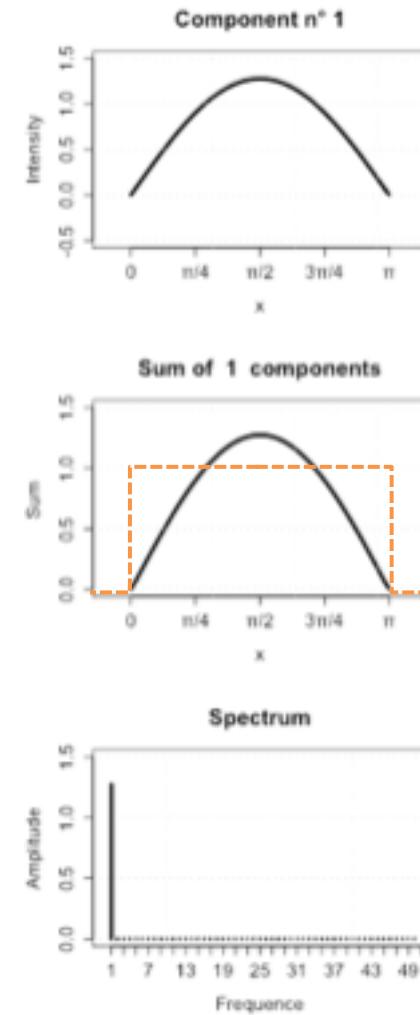
Frequential filtering

Fourier's transform in a nutshell



Any signal
might be
decomposed
into a sum of
sines/cosines

Jean Baptiste Joseph Fourier
Fourier's series were first introduced in 1822



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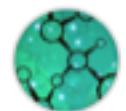
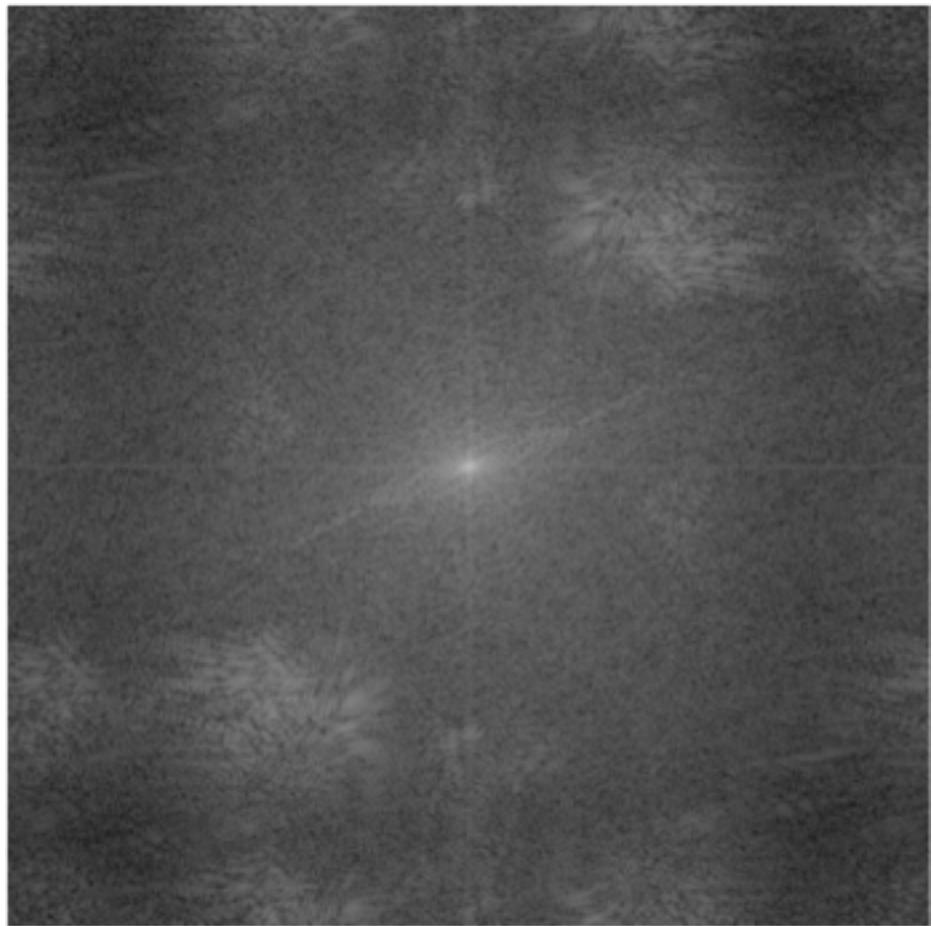


Frequential filtering

Fourier's transform of Fourier's portrait, in 2D



Jean Baptiste Joseph Fourier
Fourier's series were first introduced in 1822



FRANCE-BIOIMAGING

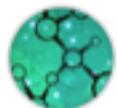
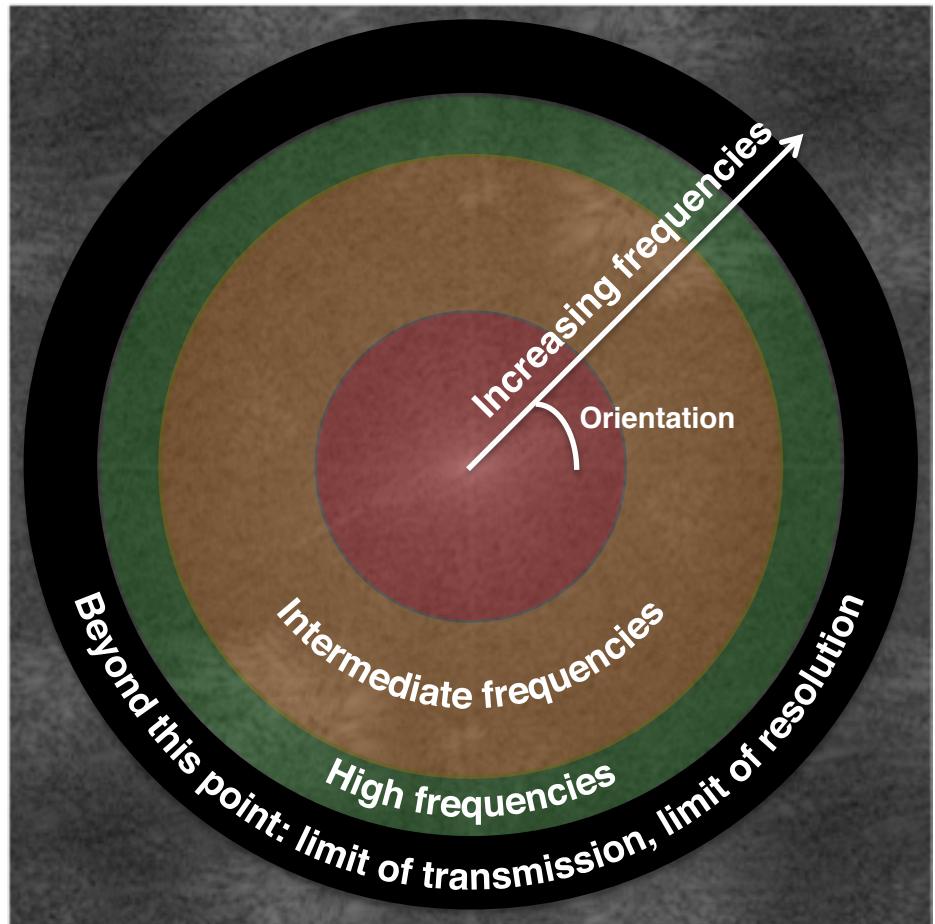


Frequential filtering

Fourier's transform of Fourier's portrait, in 2D



Jean Baptiste Joseph Fourier
Fourier's series were first introduced in 1822



FRANCE-BIOIMAGING

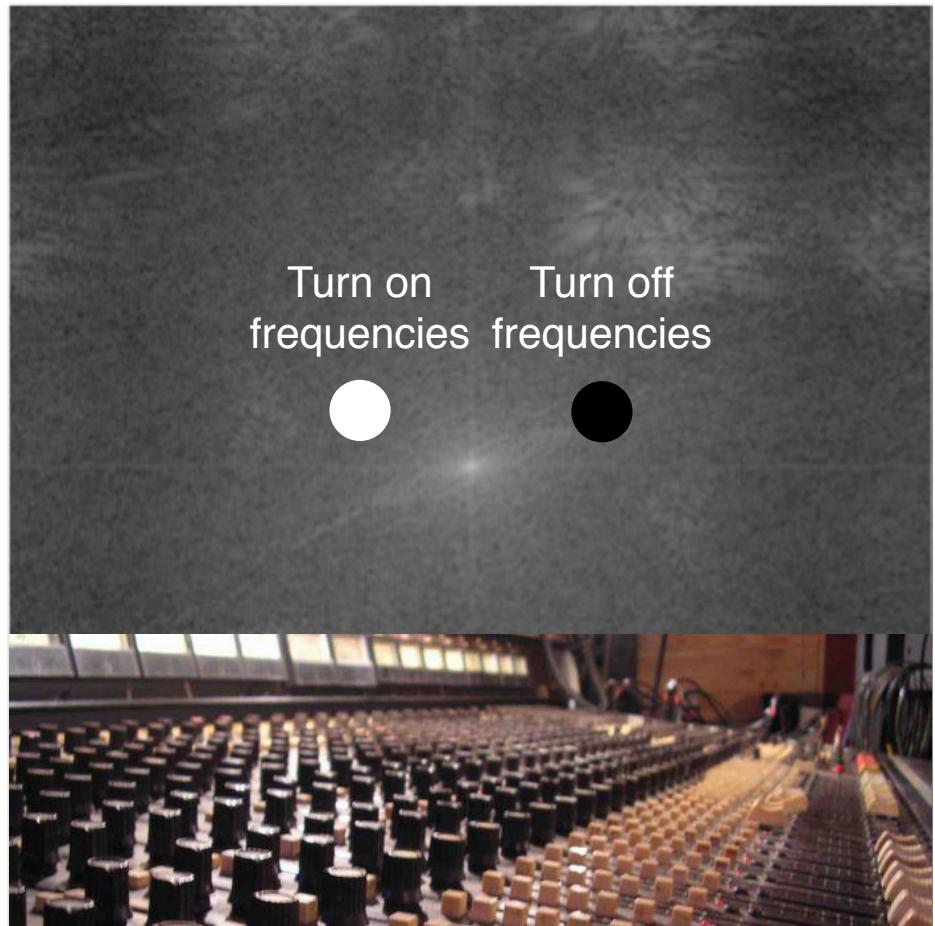


Frequential filtering

Fourier's transform of Fourier's portrait, in 2D



JBJ Fourier, imaging DJ
Scratching with phonographs since 1822



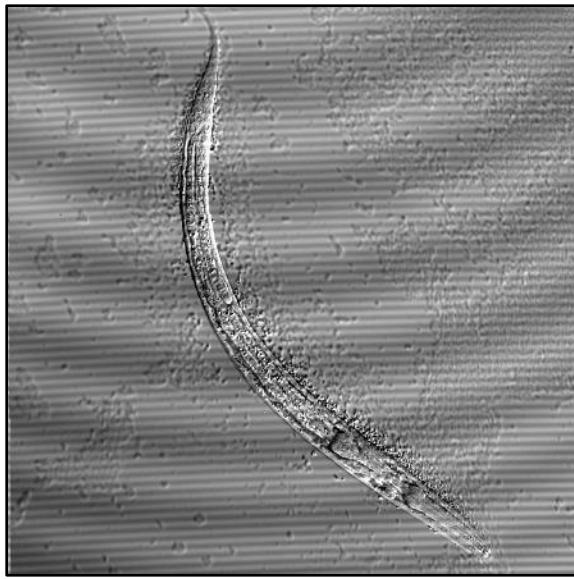
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Frequential filtering

Fourier's transform of Fourier's portrait, in 2D

Image: MN Soler

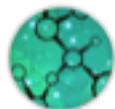


- HF: probably due to neon tube while acquiring the image on a confocal
- LF: ???

Synopsis:

- You can't go back to the microscope
- You dropped your slide and broke it
- You have a group meeting in just 5 minutes

***What will you do ???
(Really not a lucky day:
maybe go back home and have a nap ?)***



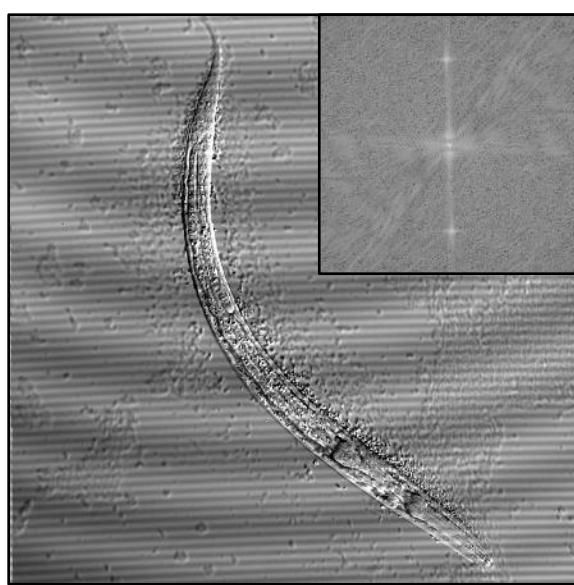
FRANCE-BIOIMAGING



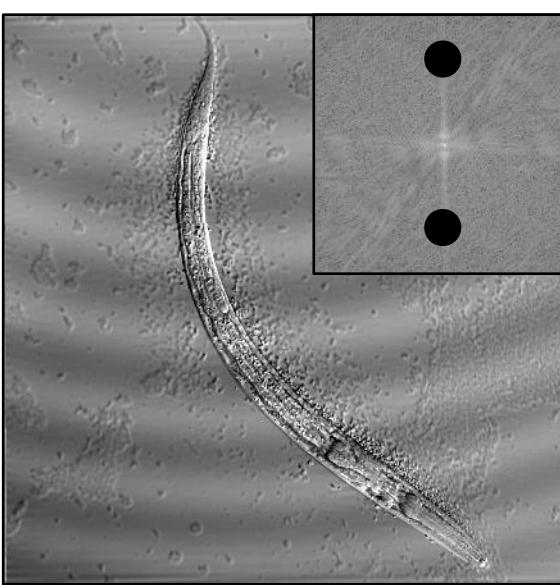
Frequential filtering

Fourier's transform of Fourier's portrait, in 2D

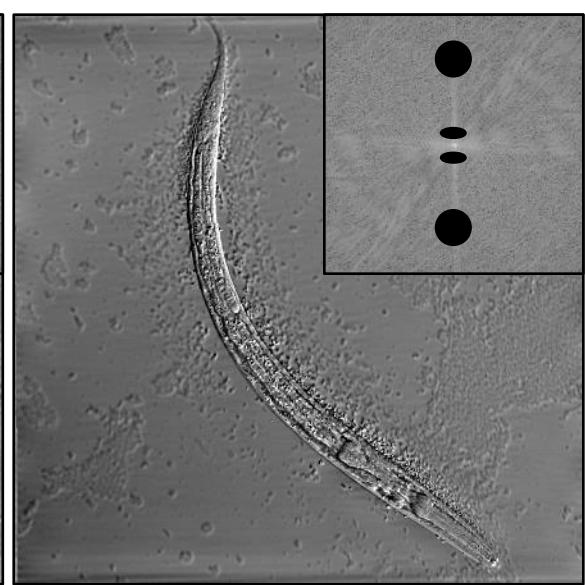
Image: MN Soler



Raw



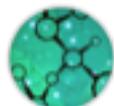
Remove HF



Remove HF + LF

- HF: probably due to neon tube while acquiring the image on a confocal
- LF: ???
- HF removed
- Simply set contribution of the bandwidth to 0
- Carefully set the orientation
- LF removed
- Simply set contribution of the bandwidth to 0
- Carefully set the orientation

There is nothing like a good sample preparation and a proper image acquisition !



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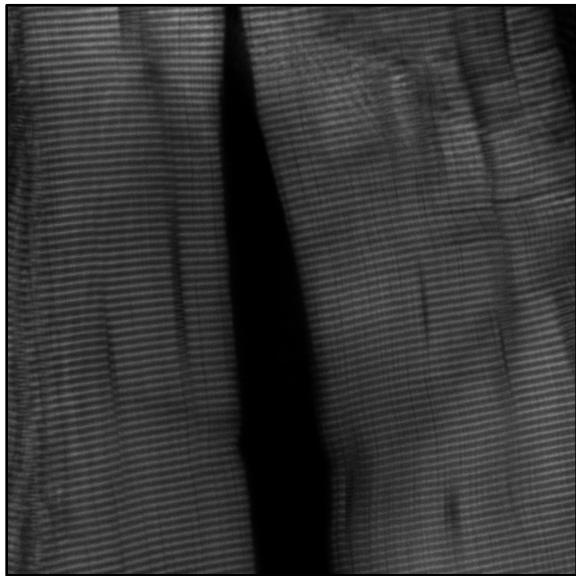


Frequential filtering

Fourier's transform: not just a filtering method

Image: S Marais

Original image

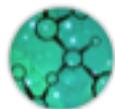


- Muscle fibres
- Periodical structures
- Oriented bundles

Synopsis:

- The image presents muscle fibres
- You want to measure the average spacing between stripes

How would you do ???



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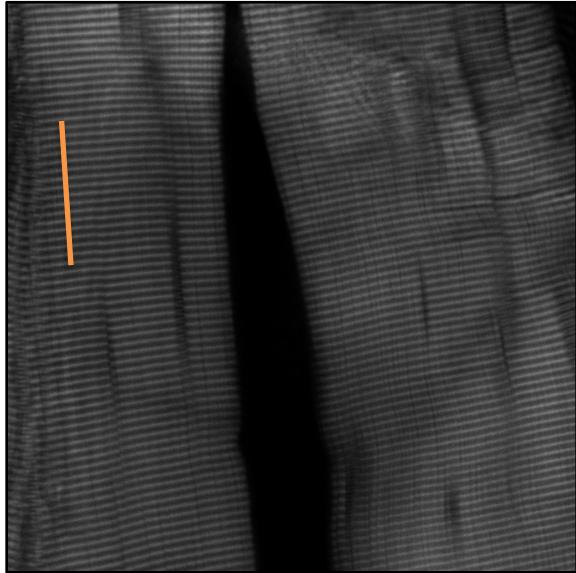


Frequential filtering

Fourier's transform: not just a filtering method

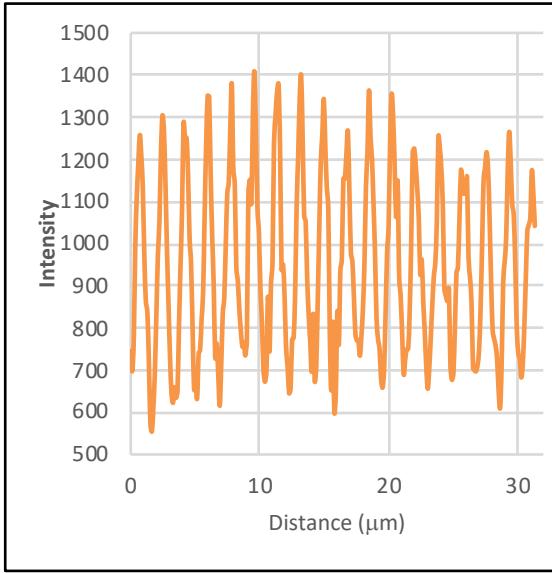
Image: S Marais

Original image



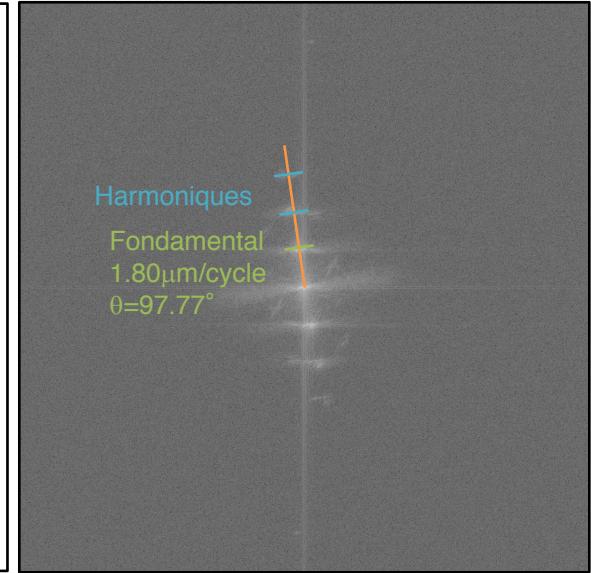
- Muscle fibers
- Periodical structures
- Oriented bundles

Intensity profile

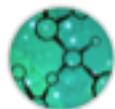


- Draw a line parallel to main orientation (get orientation)
- Retrieve intensity profile
- Define periodicity

FFT



- Perform FFT
- Point at local maxima close
- Get both orientation and size per cycle



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Image Visualisation

Image visualisation

Fact, challenge and method

Fact:

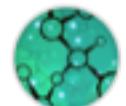
Most commonly used output media are 2D

Challenge:

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

Method:

Understand how we perceive volumes

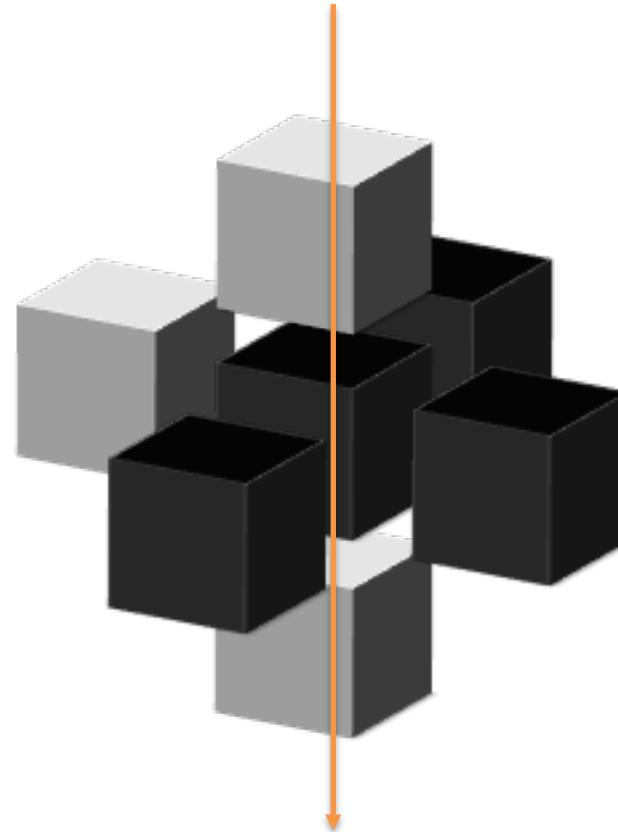
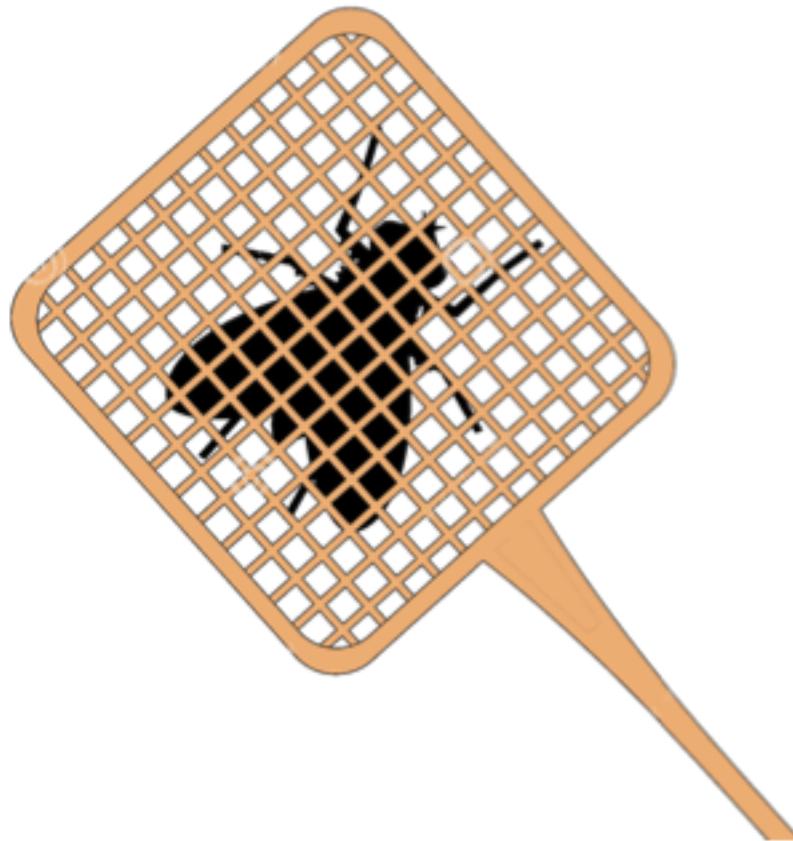


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Image visualisation

Rough method: squeezing everything onto a single plane

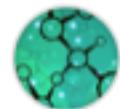


Min

Med

Max

Other
rule



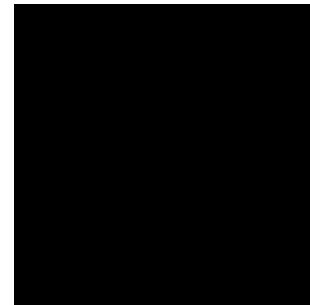
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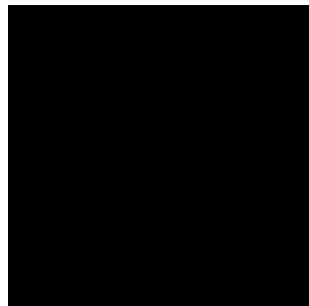
Image visualisation

Rough method: squeezing everything onto a single plane

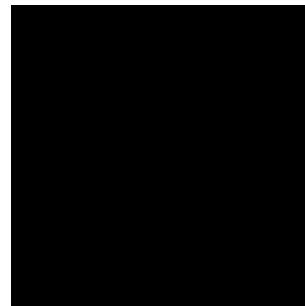
Original data



Minimum



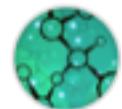
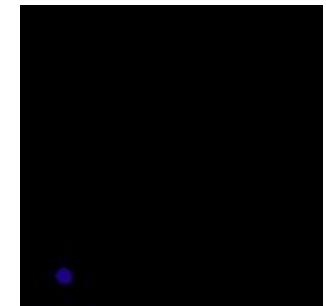
Median



Maximum



Mean



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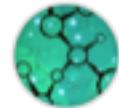


Image visualisation

Refined method: taking into account psychological parameters



Golconda, Magritte, 1953



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Image visualisation

Refined method: taking into account psychological parameters

Size of the image on retina (bigger= closer)



Texture fades away in the distance



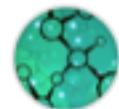
2 eyes: made to have 2 different points of view



When rotating, closest points move slower



Overlap: incomplete figures are in background



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Image visualisation

Refined method: mimicking binocular disparity

2 eyes: made to have 2 different points of view



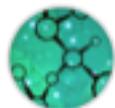
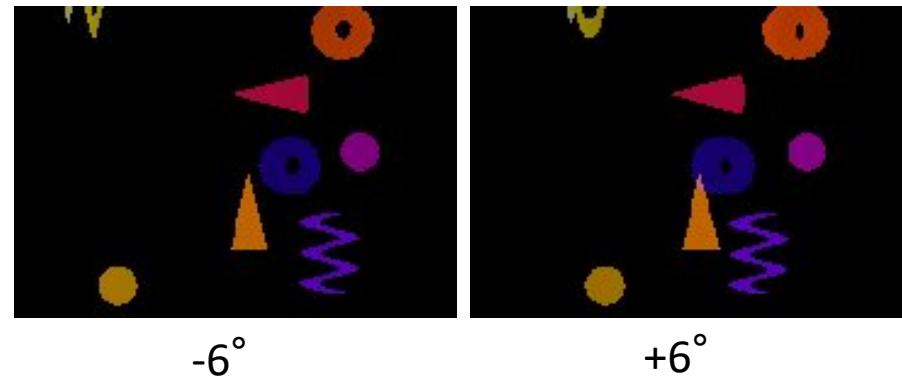
Anaglyph



Original data



Stereogram



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Image visualisation

Refined method: taking benefit of motion parallax

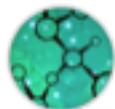
When rotating, closest points move slower



Original data



Multiple angle view



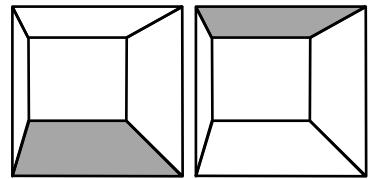
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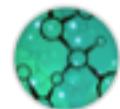
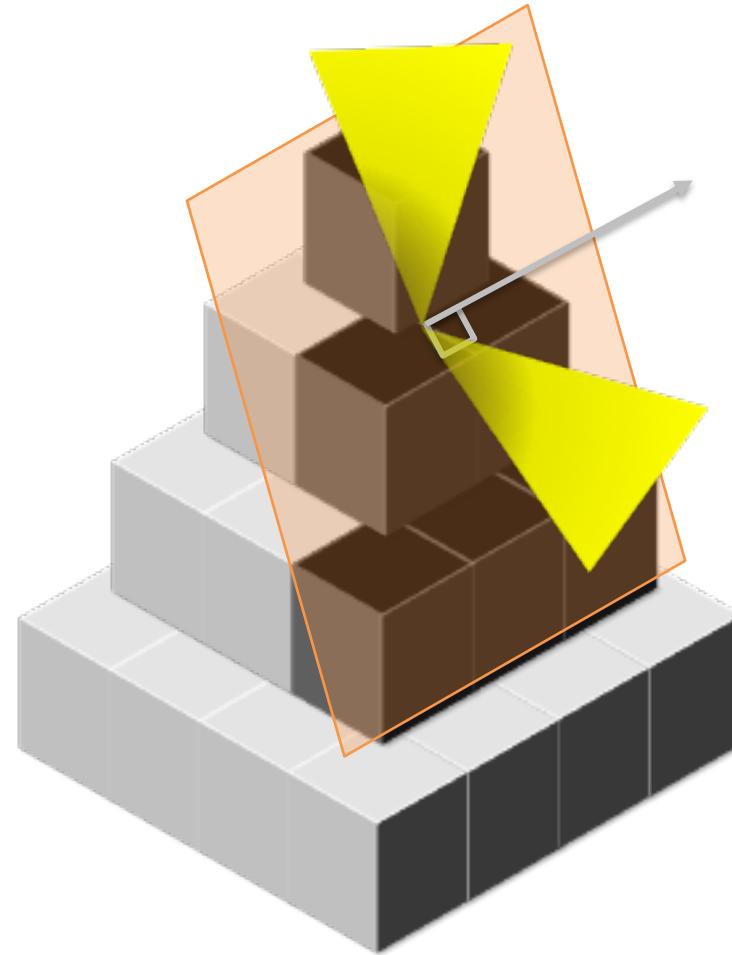
Image visualisation

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



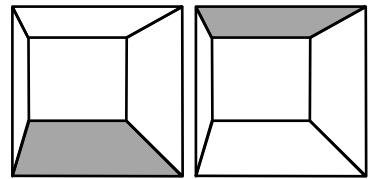
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Image visualisation

Refined method: taking care of rendering

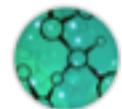
**Knowledge from
experience: light comes
from above**



Original data



Surface rendering



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Concluding remarks

Resources in image processing

- **Software:**

- *ImageJ and sons:*



- Canonical ImageJ
 - μ -Manager: Image processing and microscope control
 - FIJI: ImageJ and (too) many tools
 - ImageJ2: next generation, fully rewritten



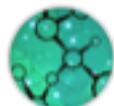
- *Icy: a good alternative*



- Original design
 - Includes ImageJ and μ -Manager
 - Increasing number of plugins, with excellent search engine
 - An exceptional 3D viewer
 - Visual macro editor

- **Some resources:**

- Catalogue of tools: BioImage Information Index, biii.info
 - Open textbook: <http://www.imaging-git.com/applications/bioimage-data-analysis-0>



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