# Introduction to imaging

# From image to acquisition

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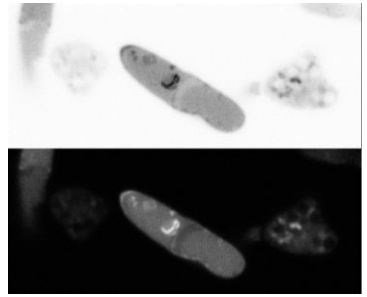
# An image is a partial representation of reality

Eye



Object

Sample preparation

Digital image

Image analysis and Communication of informations

Visualization system

microscope / stereomicroscope

Imaging system

*scanner

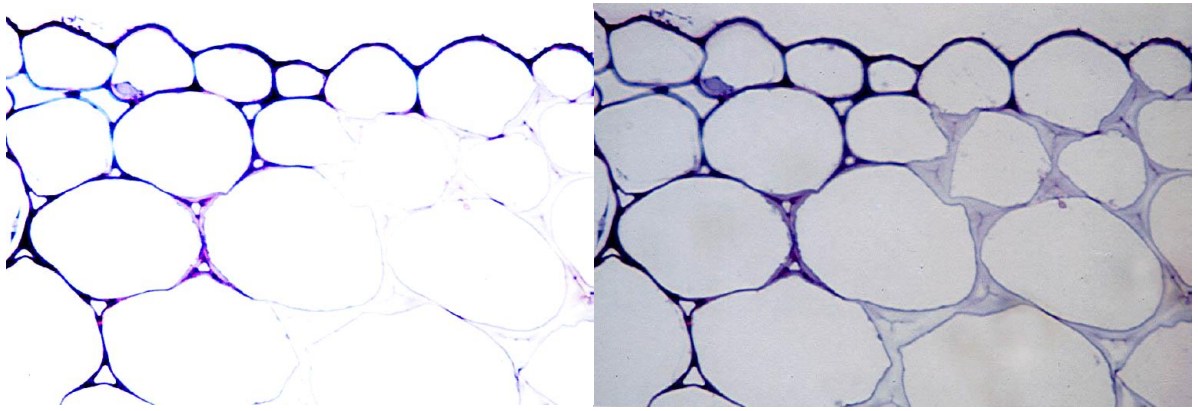
*camera

*Camera linked to the equipment:
microscope / stereomicroscope

**Is the information present in the acquired image, to answer my question?**

# Case 1 : Acquisition parameters

Detection of the signal/ information (Exposure time) : présence/absence



Detection of color  (white balance) : drowned information

# Case 2 : Information associated with the image

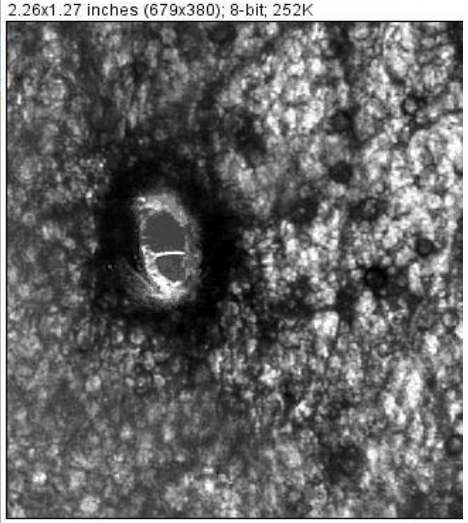
**One of the images doesn't contain information I am looking for**

necrotric_lesions_on_oilseed_rape_cotyledons-2.tif
679x380 pixels; RGB; 1008K

necrotric_lesions_on_oilseed_rape_cotyledons-3.tif
2.26x1.27 inches (679x380); 8-bit; 252K

necrotric_lesions_on_oilseed_rape_cotyledons-1.tif
2.26x1.27 inches (679x380); RGB; 1008K

necrotric_lesions_on_oilseed_rape_cotyledons-4.tif
2.26x1.27 inches (679x380); 8-bit; 252K



# Case 2 : Information associated with the image

necrotric_lesions_on_oilseed_rape_cotyledons-2.tif
679x380 pixels; RGB; 1008K

Missing information

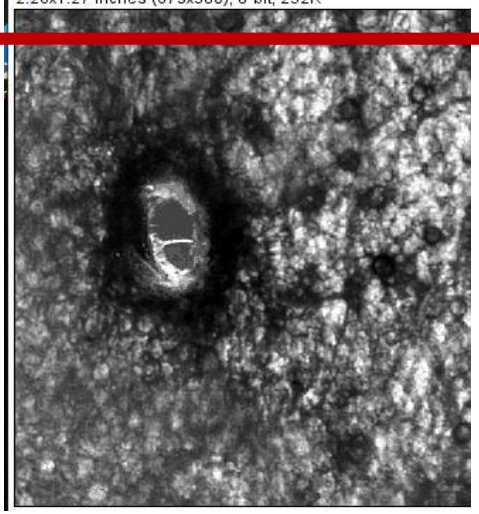
necrotric_lesions_on_oilseed_rape_cotyledons-3.tif
2.26x1.27 inches (679x380); 8-bit; 252K

Can get information

necrotric_lesions_on_oilseed_rape_cotyledons-1.tif
2.26x1.27 inches (679x380); RGB; 1008K

Can get information

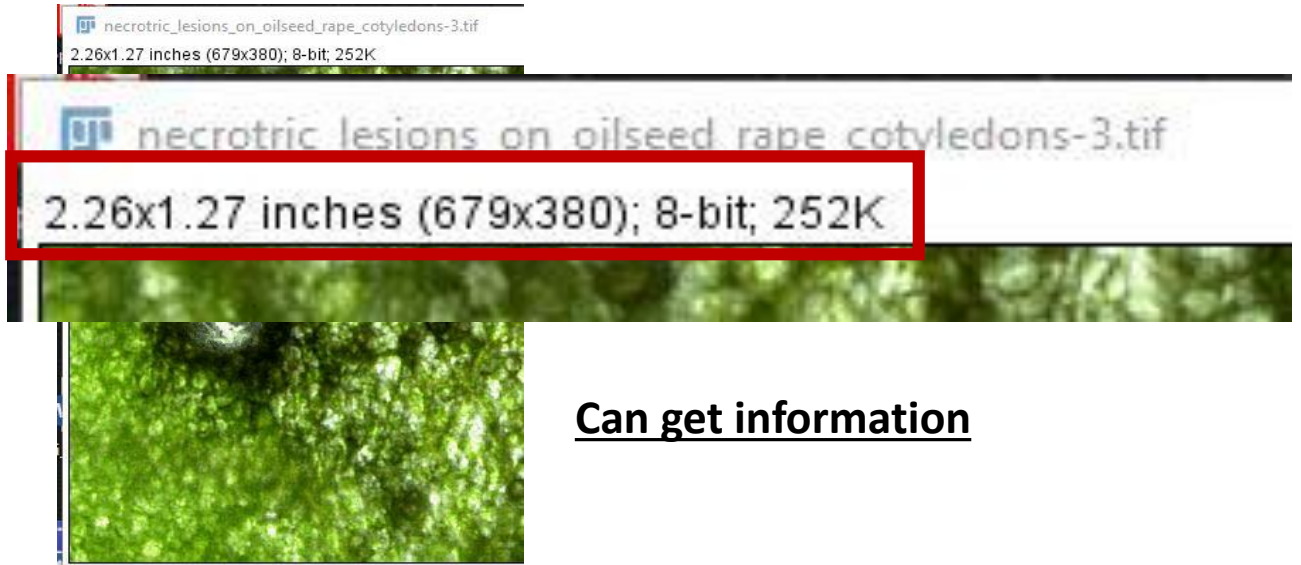
necrotric_lesions_on_oilseed_rape_cotyledons-4.tif
2.26x1.27 inches (679x380); 8-bit; 252K



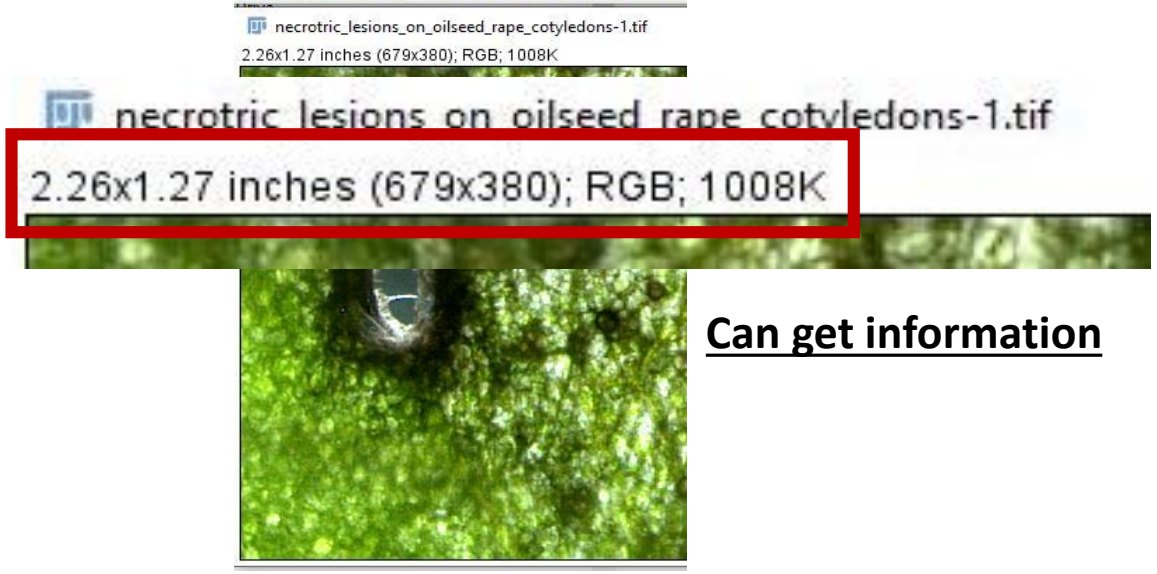
Can get information

# Case 2 : Information associated with the image

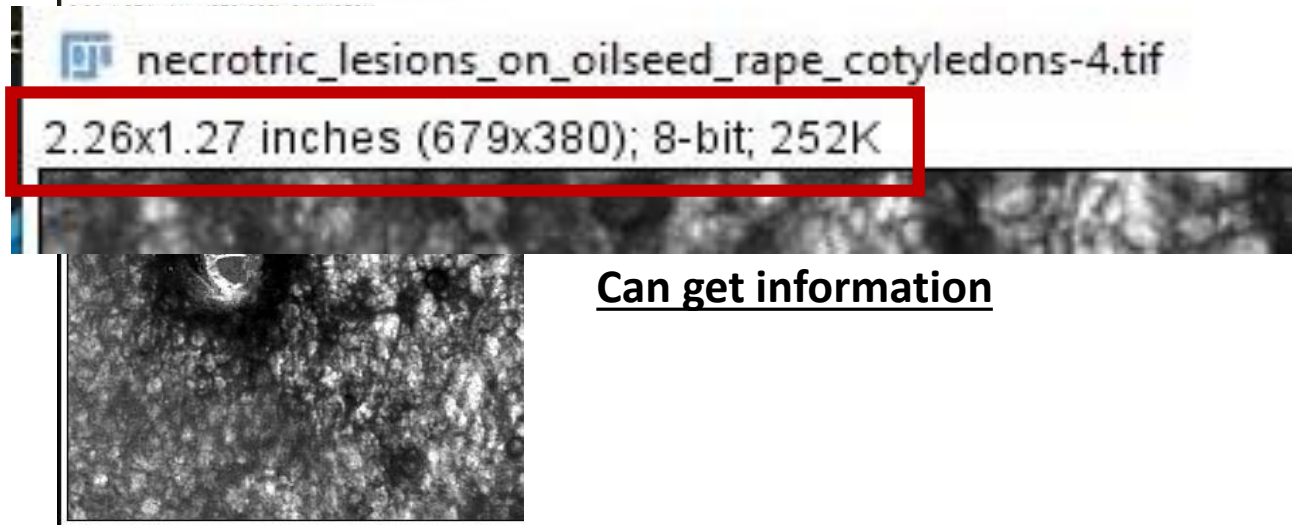

**Missing information**




**Can get information**




**Can get information**

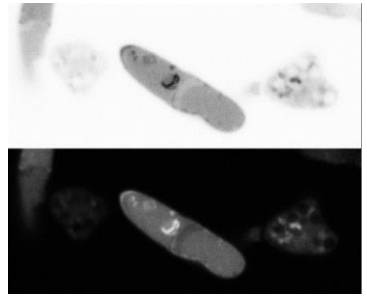



**Can get information**

# What is an image?

# An image is a partial representation of reality

Eye



Object

Sample preparation

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microscope / stereomicroscope

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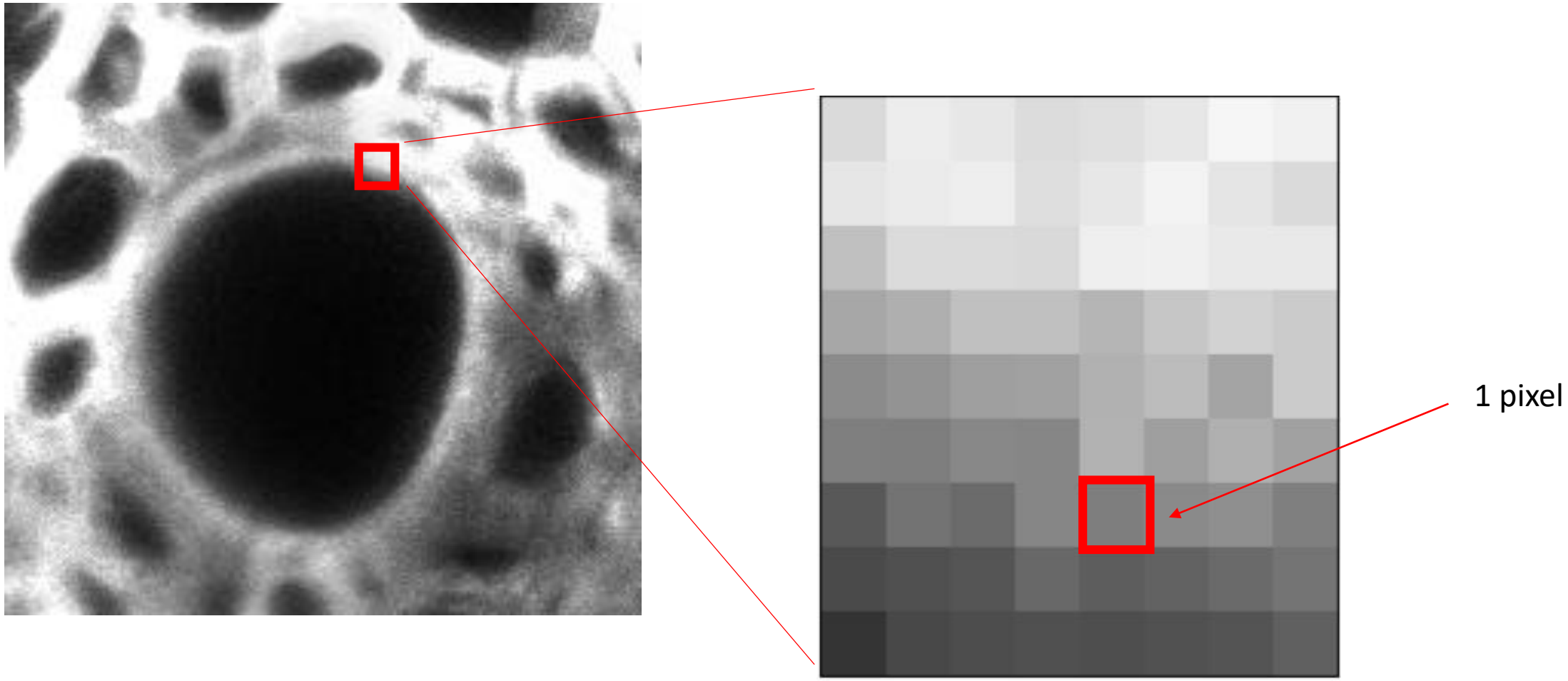
*scanner

*camera

*Camera linked to the equipment:
microscope / stereomicroscope

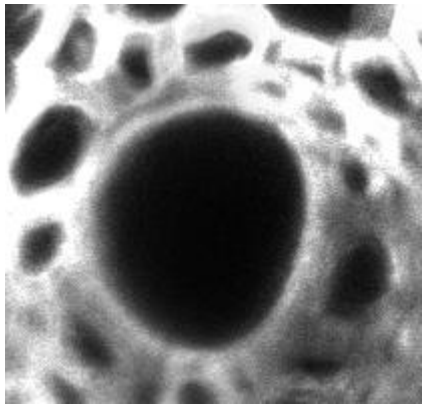
# What’s a digital image?

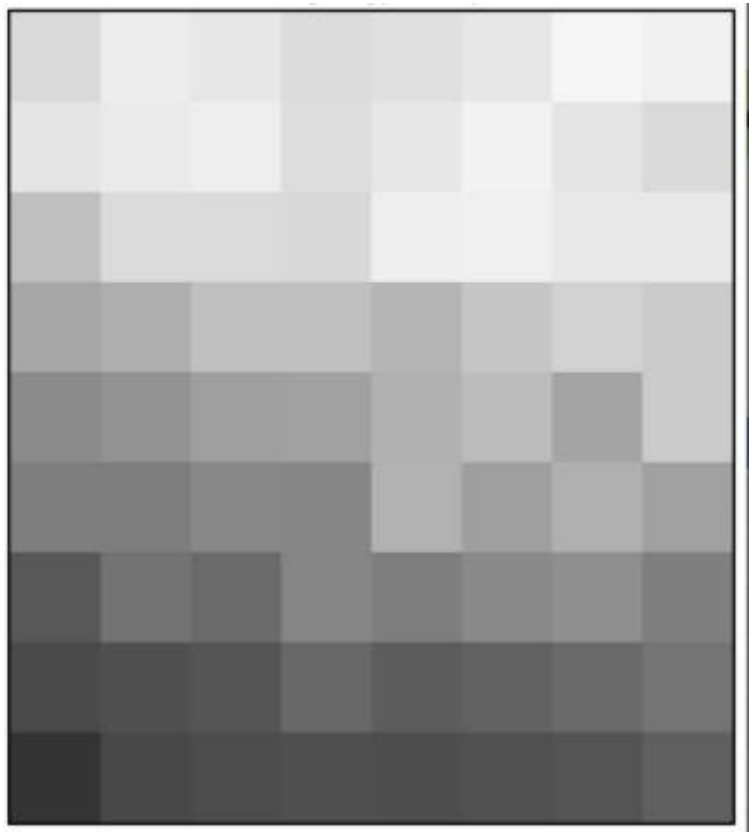
**1-Notion of pixel (picture element) :** fundamental element of an image



# What’s a digital image?

**2- A « digital » image is a tool which allows to better vizualise a huge table with lot of numbers**



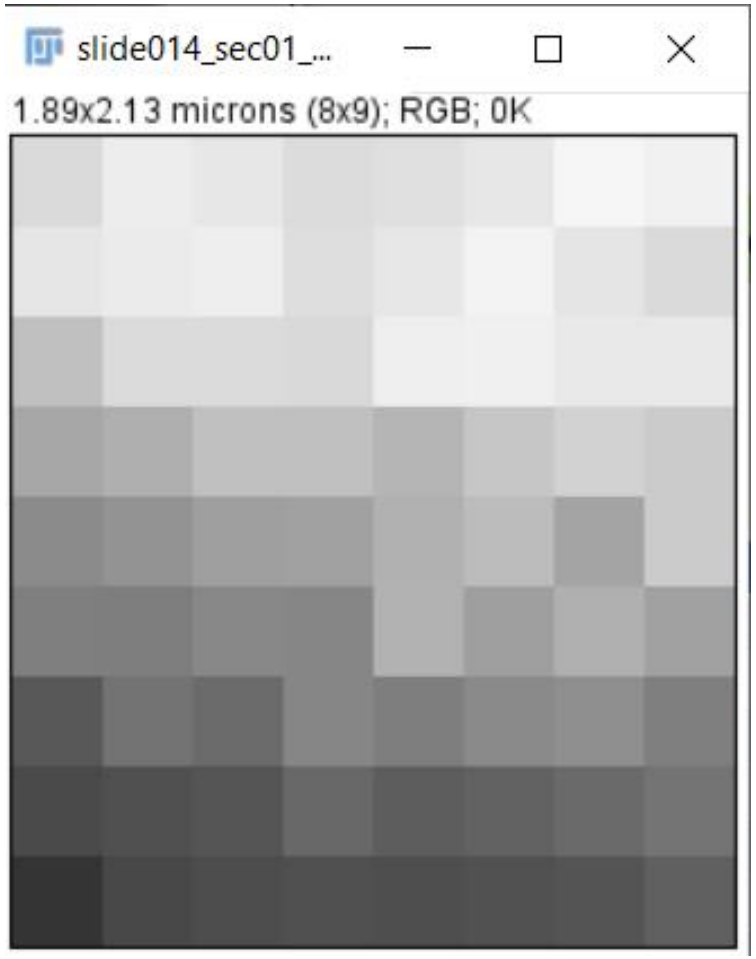


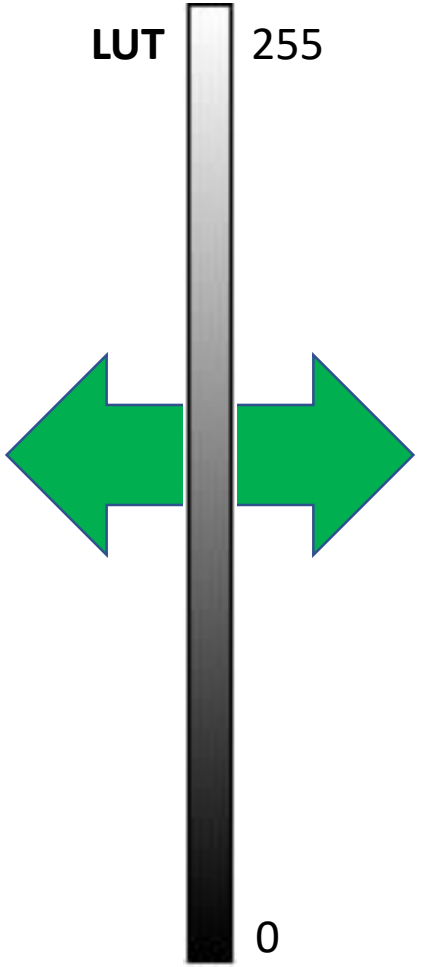
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **234** | **218** | **237** | **231** | **220** | **224** | **231** | **246** | **241** |
| 216 | 230 | 235 | 238 | 222 | 231 | 243 | 229 | 218 |
| 189 | 192 | 219 | 219 | 217 | 239 | 240 | 233 | 233 |
| 148 | 167 | 175 | 192 | 192 | 181 | 198 | 210 | 203 |
| 137 | 139 | 147 | 159 | 161 | 177 | 188 | 164 | 203 |
| 119 | 127 | 126 | 136 | 134 | 178 | 159 | 176 | 161 |
| 92 | 88 | 115 | 107 | 134 | 126 | 138 | 143 | 127 |
| 55 | 74 | 80 | 85 | 104 | 93 | 98 | 106 | 116 |
| 64 | 52 | 72 | 77 | 80 | 78 | 81 | 84 | 96 |

# What’s a digital image?

**3- LUT (Lookup Table) : Correlation table**

**Allows to correlate numerical value and the vizual aspect of the image.**




Grey level

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **234** | **218** | **237** | **231** | **220** | **224** | **231** | **246** | **241** |
| 216 | 230 | 235 | 238 | 222 | 231 | 243 | 229 | 218 |
| 189 | 192 | 219 | 219 | 217 | 239 | 240 | 233 | 233 |
| 148 | 167 | 175 | 192 | 192 | 181 | 198 | 210 | 203 |
| 137 | 139 | 147 | 159 | 161 | 177 | 188 | 164 | 203 |
| 119 | 127 | 126 | 136 | 134 | 178 | 159 | 176 | 161 |
| 92 | 88 | 115 | 107 | 134 | 126 | 138 | 143 | 127 |
| 55 | 74 | 80 | 85 | 104 | 93 | 98 | 106 | 116 |
| 64 | 52 | 72 | 77 | 80 | 78 | 81 | 84 | 96 |

# What's a digital image?

**4- Notion of bit (Binary digit) :** image dynamic

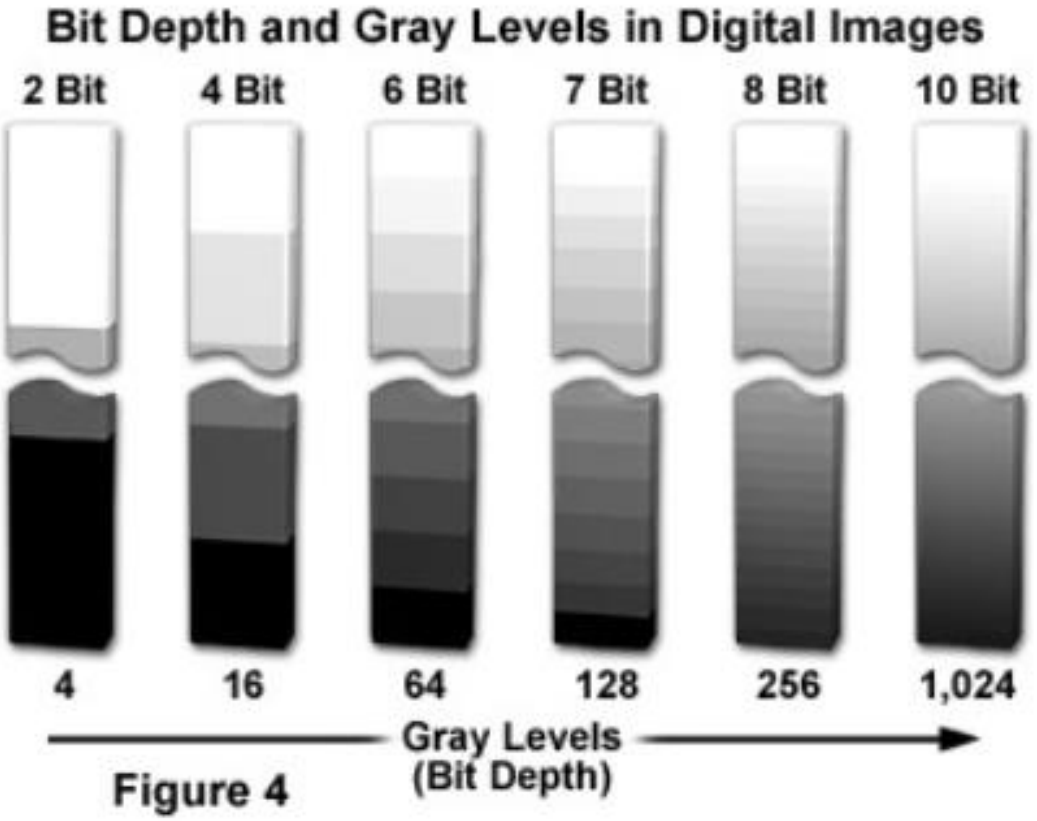


Figure 4

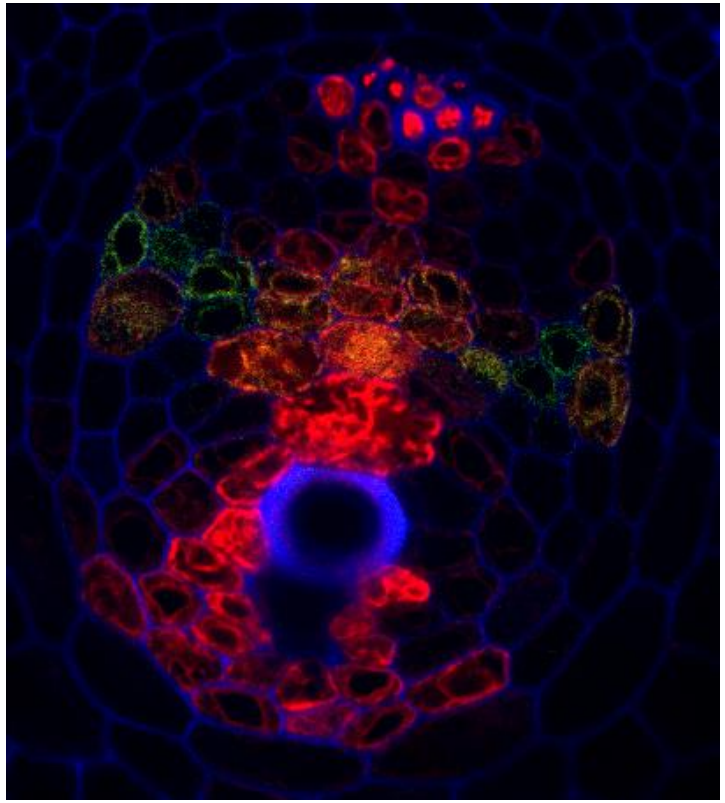
**… 16bit**

**A n bit image can contain up to $2^n$ bits of different grey levels.**

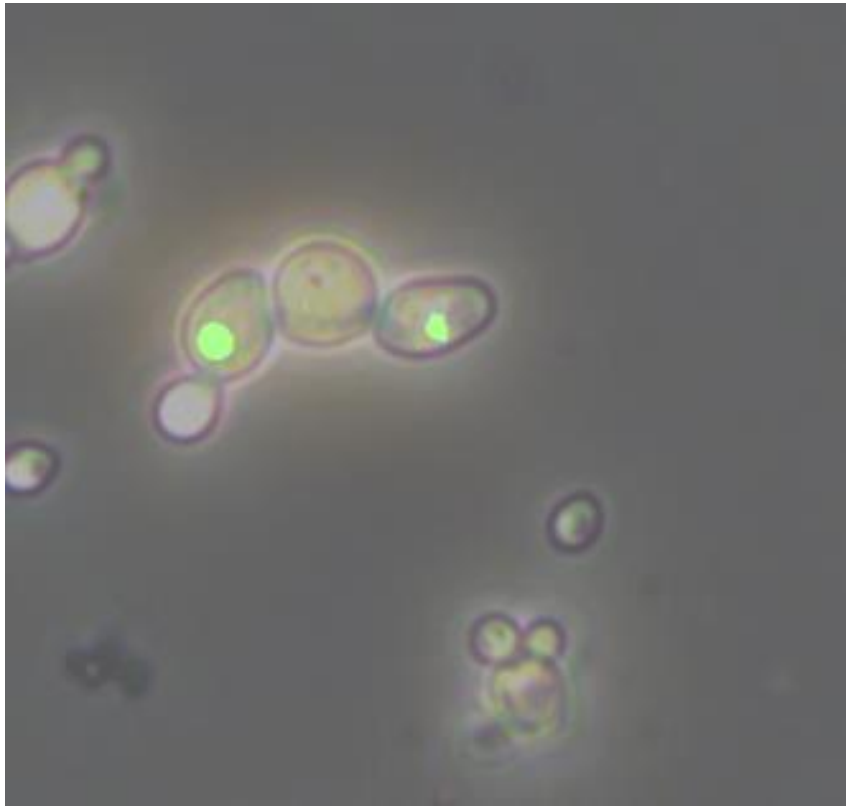
http://zeiss-campus.magnet.fsu.edu/articles/basics/digitalimaging.html

# What is a colour image?

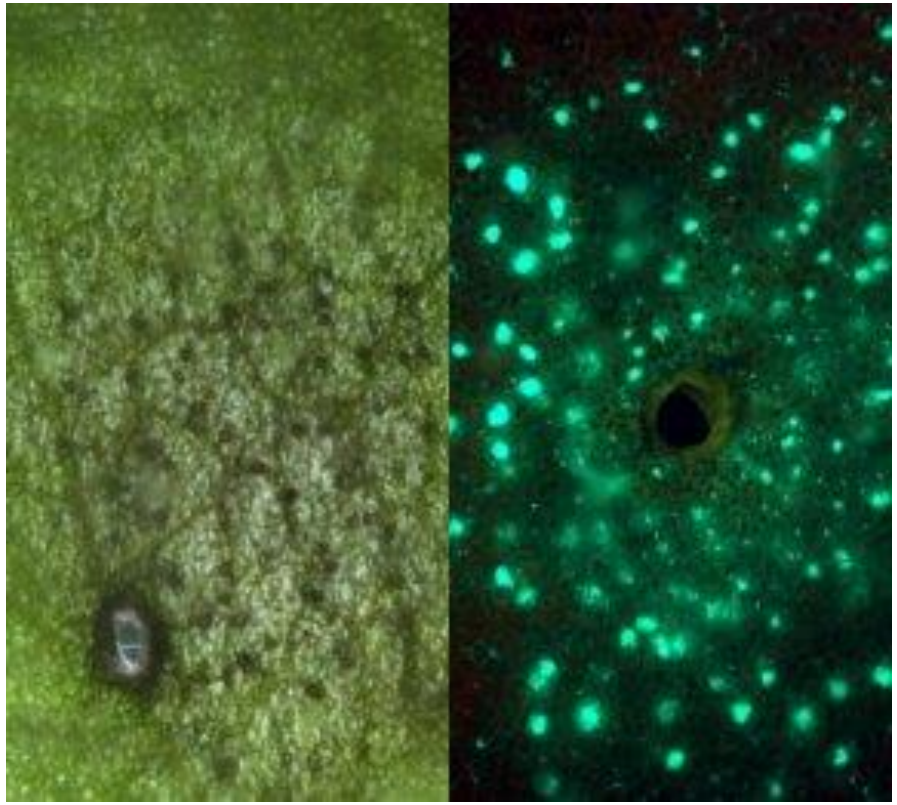
**One of the 3 images is not a colour image**



Confocal image, fluorescence



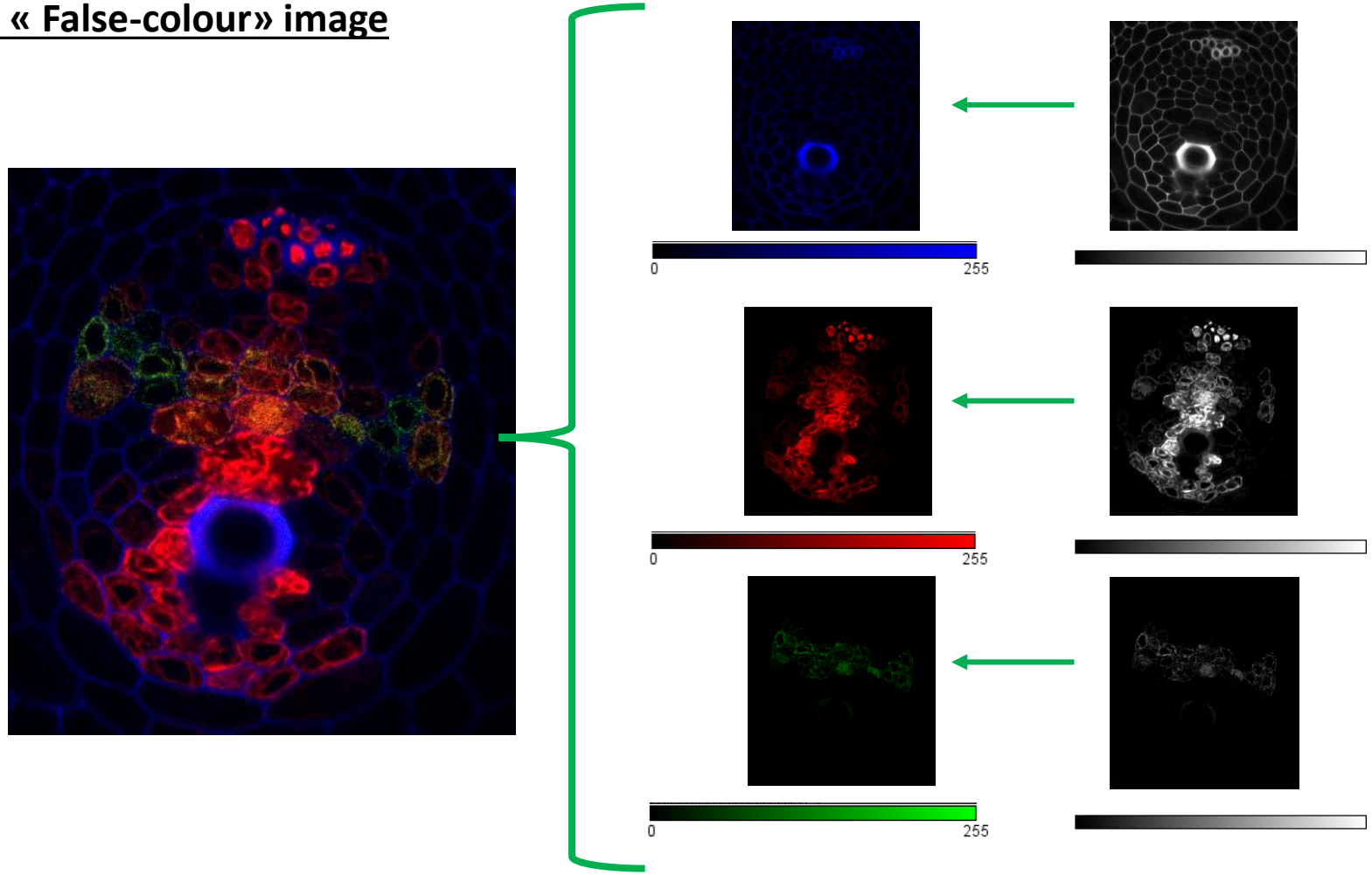
Epifluorescence image
(at BIOGER)



Stereomicroscope Image
(at BIOGER)

# What is a colour image?

**1- « False-colour» image**

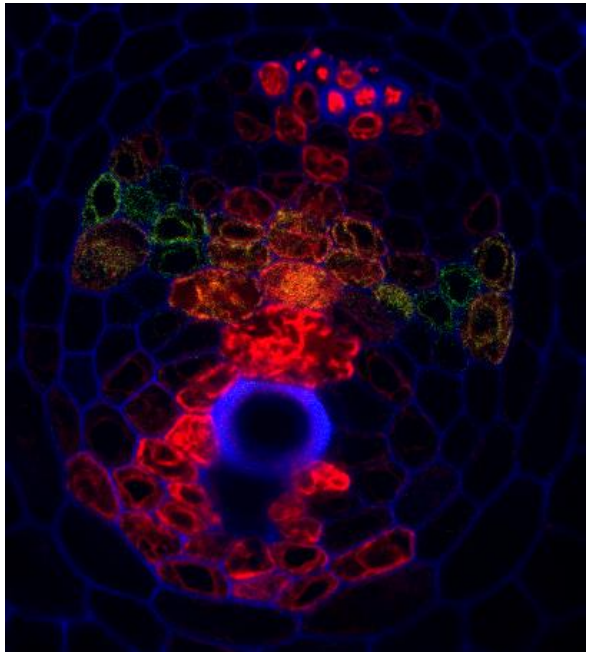


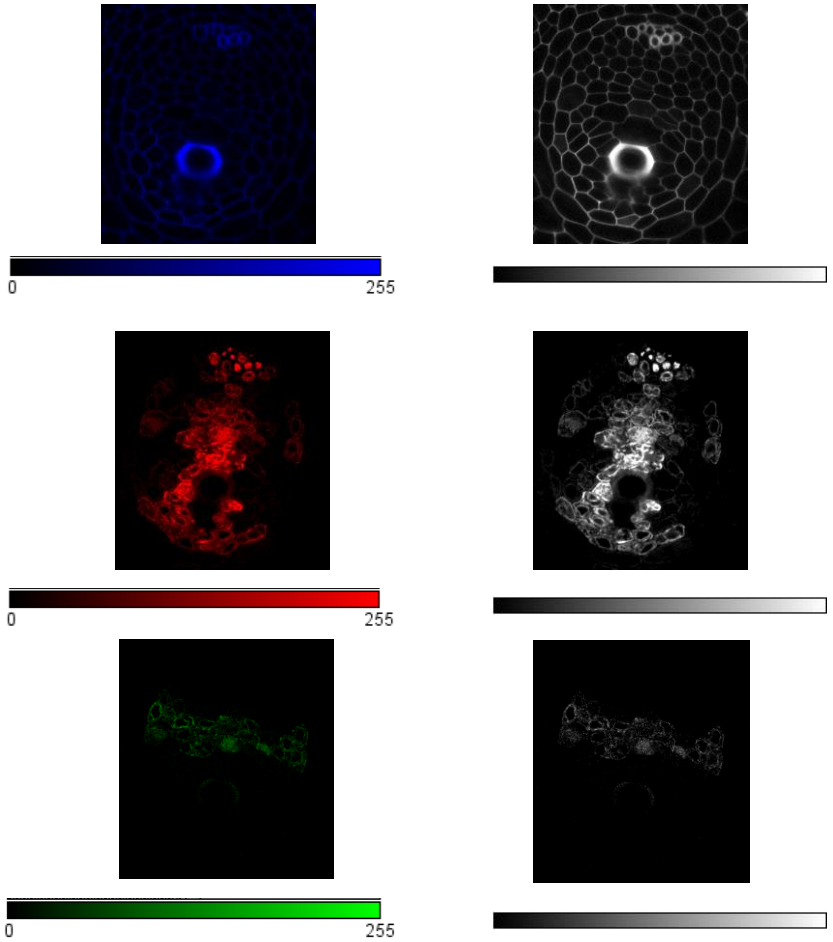

**Here, detection of 3 fluorecence chanels, but it is possible to increase up to 4,5 or 10!**

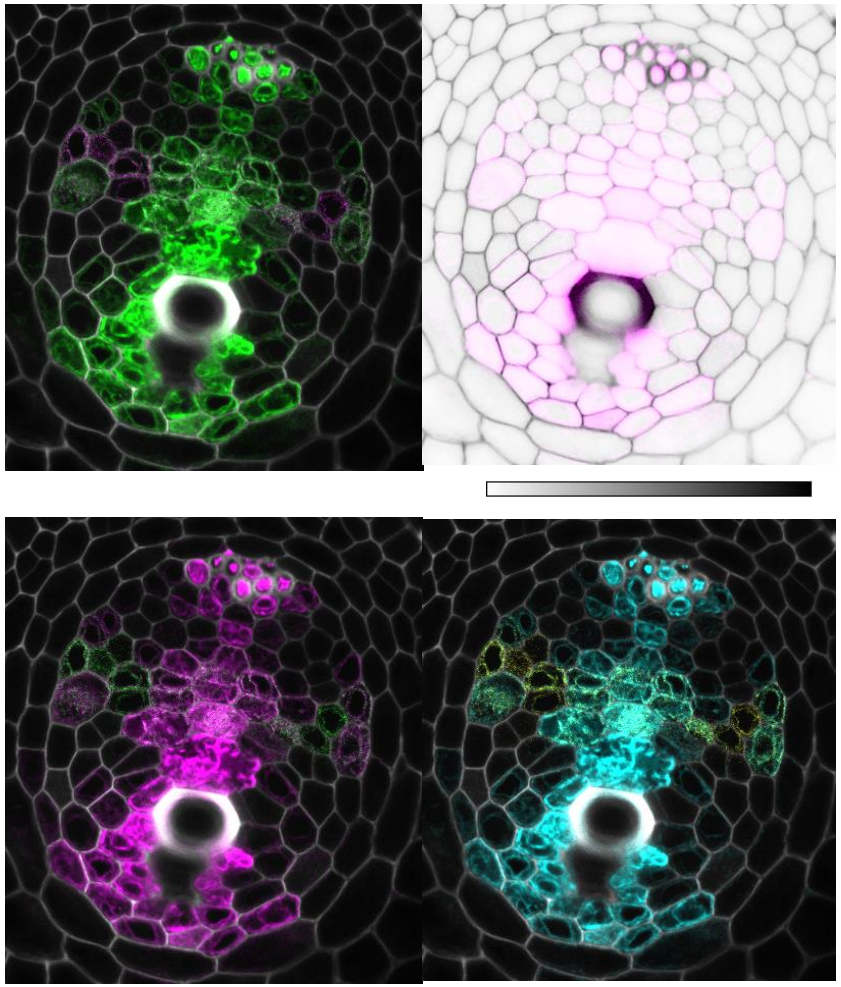
**In confocal microscopy (or with Black/White camera), detectors ONLY detect grey levels.**
**These grey levels are transformed to « False color ».**
**The false colour range is between black and the chosen monochrome colour.**

# What is a colour image?

**1- « False-colour» image**



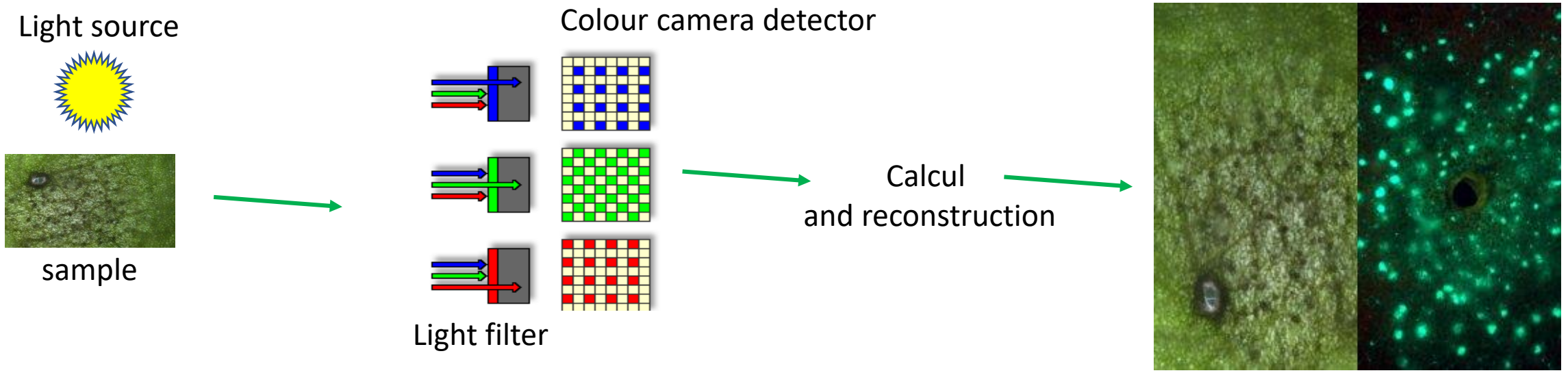


**Playing with LUTs, some information is better revealed!**

# What is a colour image?

**2- Colour image : Obtained with colour camera**




**RGB : information is coming from the detector on which the Red/Green/Blue parts of the light spectrum regarding specific standards (to imitate human eye physiology).**
**These informations are then encoded to color table from:**

**Black and blue**
**Black and green**
**Black and red**

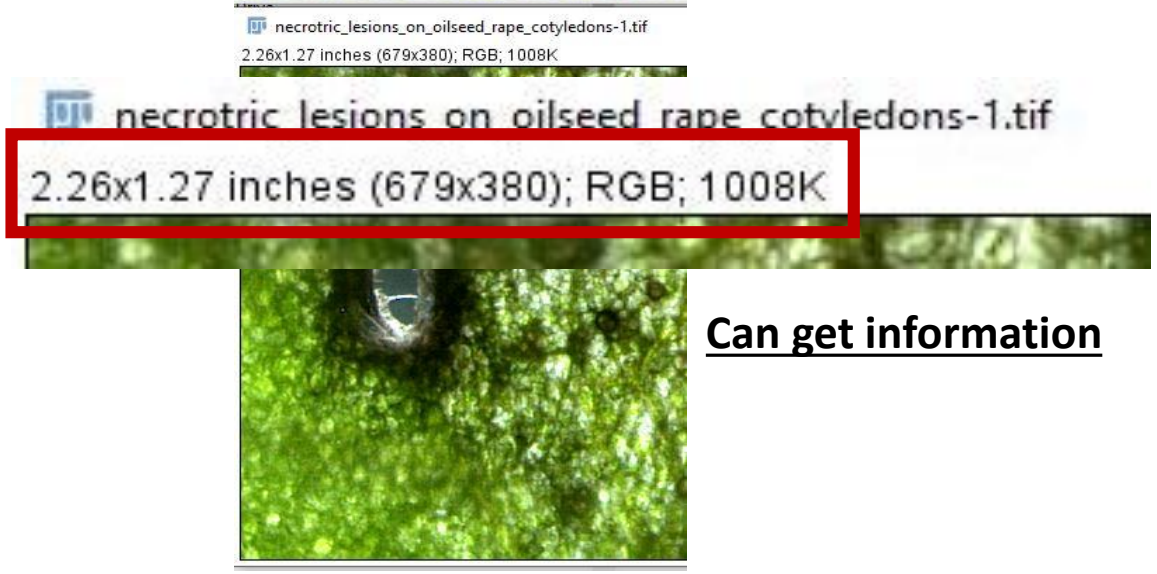
**to « reconstitute » a image with 3 channels.**

It is an RGB image

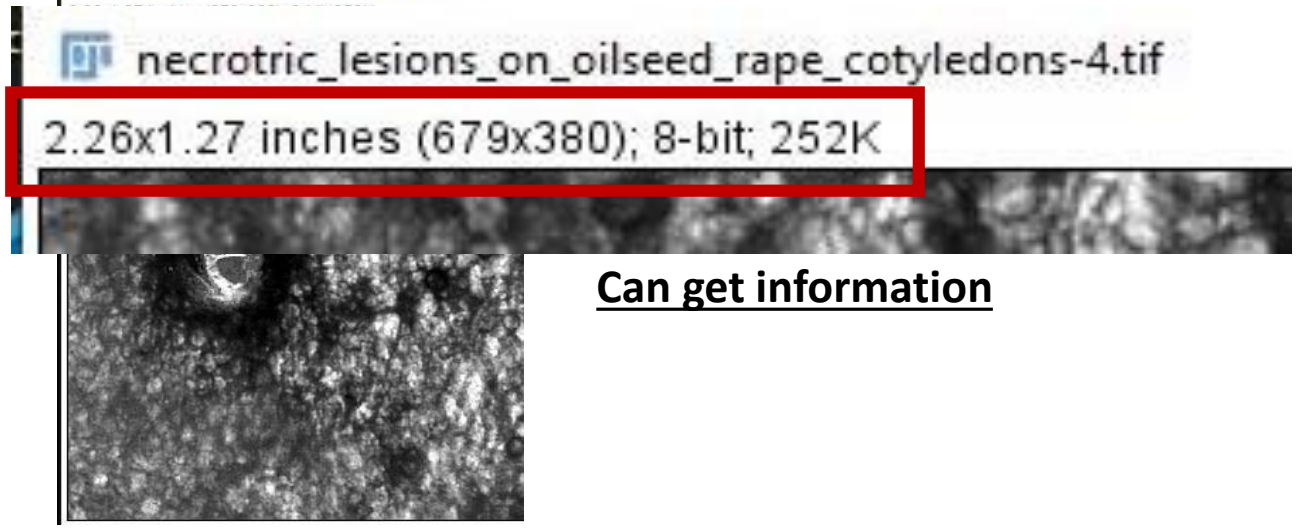
# Case 2 : Information associated to the image


**Missing information**


**Can get information**




**Can get information**




**Can get information**

# Image format
# (Major reason for mistakes during image analysis!!)

**Prioritize manufactuer format or OME tiff format when analysing image data or want to publish images.**

## Otherwise

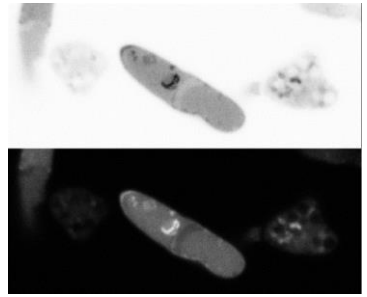
**2 type of information are lost**
Metadata : contain acquisition conditions (objectif, filter, exposure time, etc…)
Image data (pixels lost)

**Attention:  2 type of tiff format**
OME Tiff (Metadata kept) : Open Microscopy Environment
Tiff : Metadata lost

**Illustration : png/jpeg**
Compressed format OK, you don’t need details and it allows also to avoid a heavy file like over 200Mo.

# An image is a partial representation of reality

Eye



Object

Sample preparation

Digital image

Image analysis and Communication of informations

Visualization system

microscope / stereomicroscope

Imaging system

*scanner

*camera

*Camera linked to the equipment:
microscope / stereomicroscope

# What a microscope allows?

A microcope is a tool which allows to:

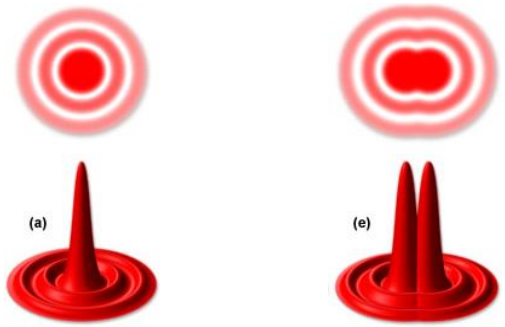
*obtain enlarged image of a small object

=**magnification**

*separate details of the object on the image

=**resolution** (pixel size, dpi-inch/ppp-pouce)

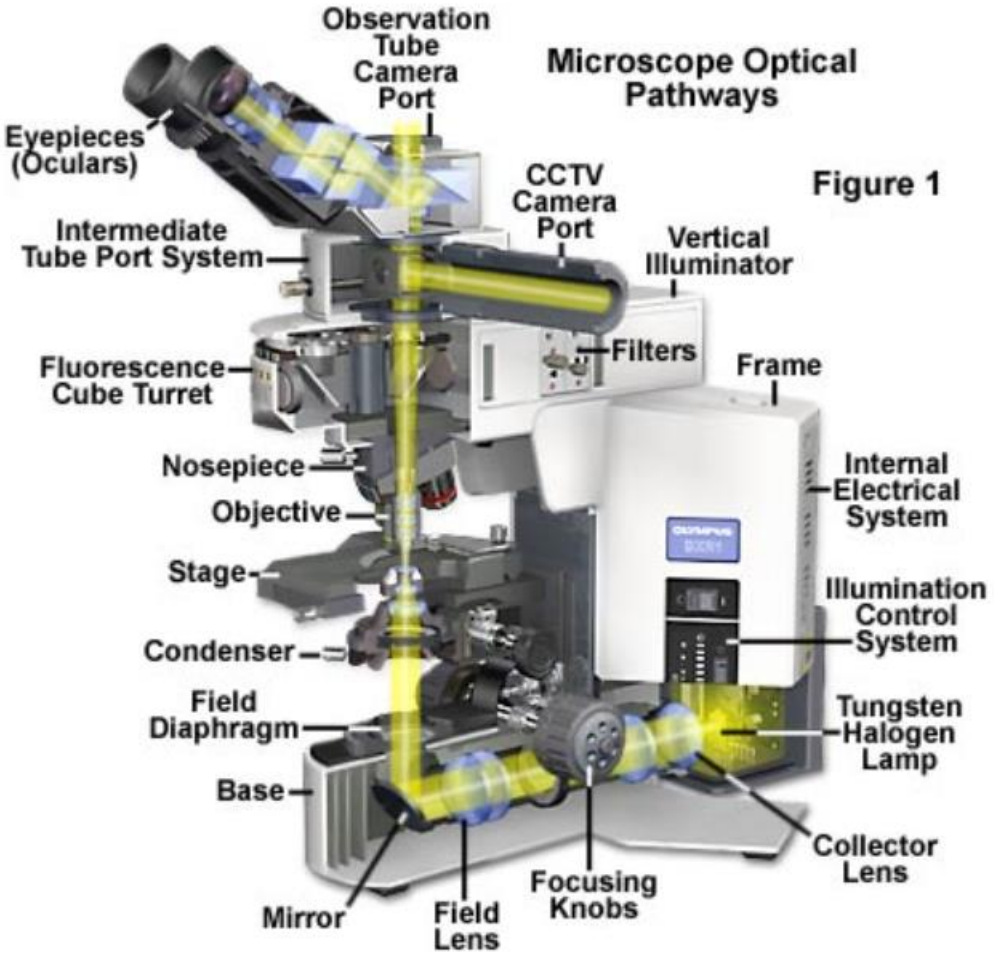
The resolution corresponds to the smallest distance needed to seperate two points

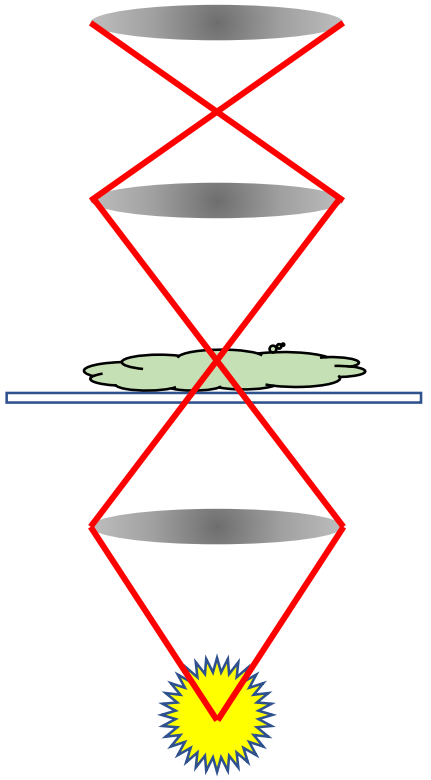



Resolved points Non resolved points

http://zeiss-campus.magnet.fsu.edu/articles/basics/imageformation.html

# The structure of the microscope



**Oculars**
Enlarge image produced by the objective

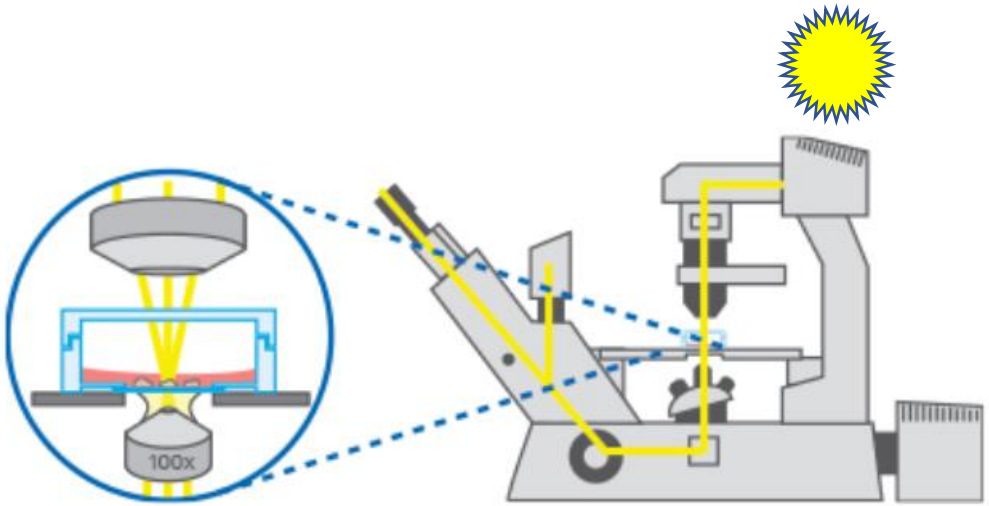
**Objective**
Produce an enlarged image of the sample

**Sample**

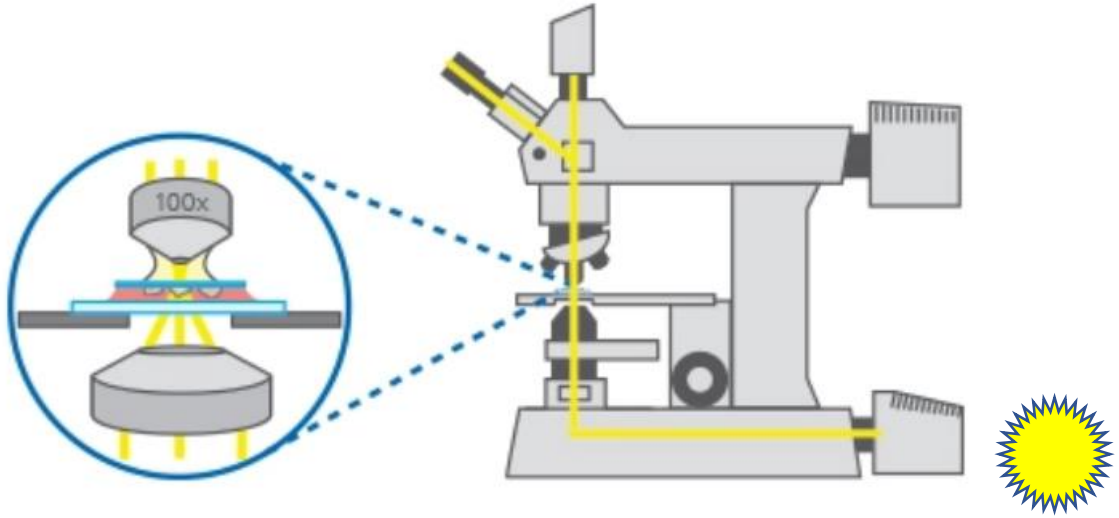
**Condenser**
Focus light on sample

**Light source**

# 2 assembly types for the microscope




**Inverted microscope**



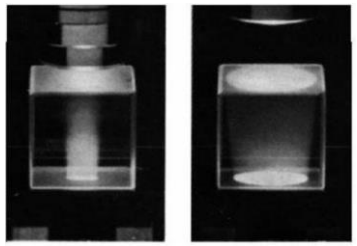

**Upright microscope**

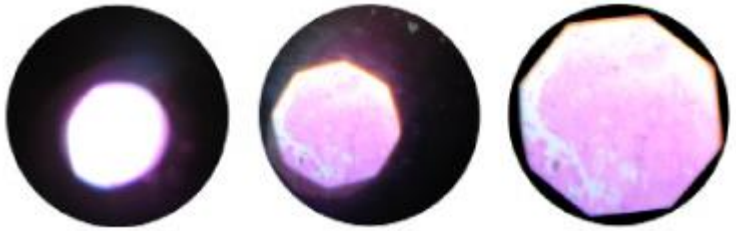
https://ibidi.com/content/212-inverted-and-upright-microscopy

# 3 components which allow to see your sample better

**1-Field diaphragm**:

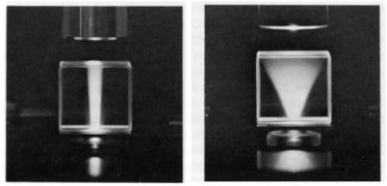
limit the **extention** of light beam

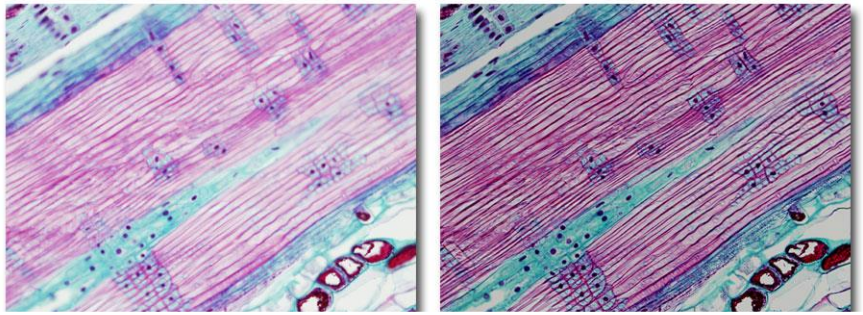




**2-Aperture diaphragm**:

limit the **opening** of light beam. It influence on the contrast and resolution of the image.





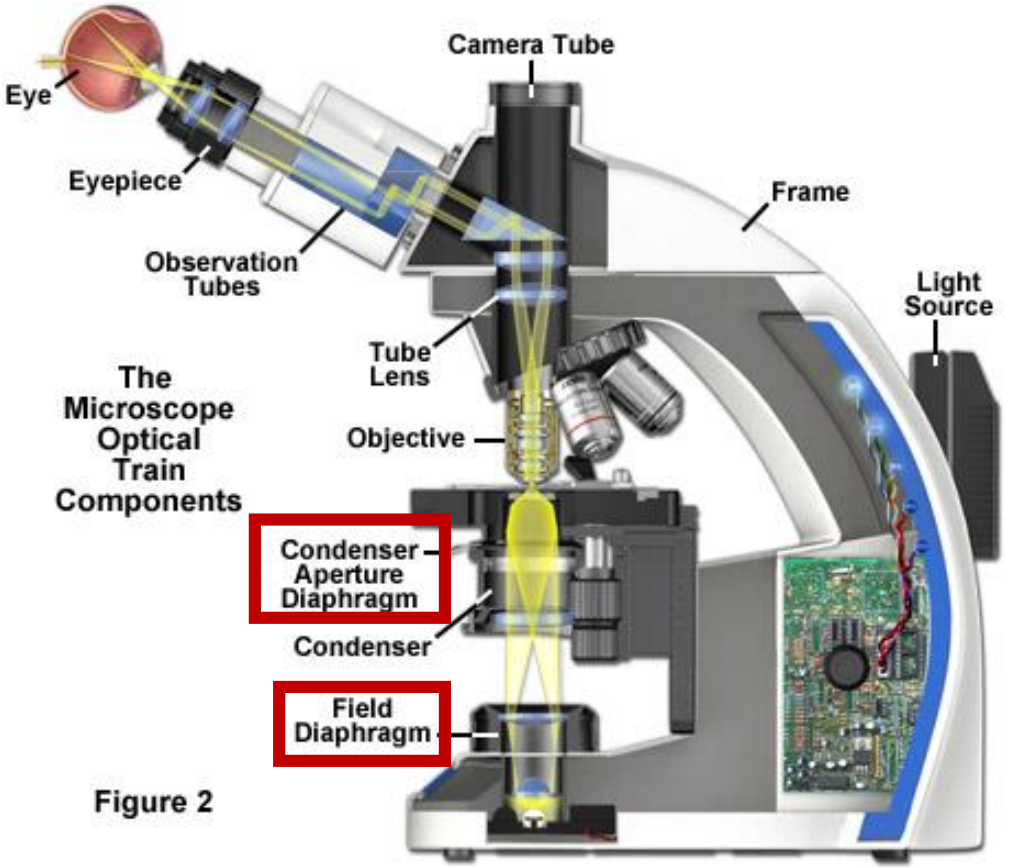



Figure 2

https://www.spotimaging.com/resources/white-papers/koehler-illumination

# Les 3 composants qui permettent de bien voir son échantillon

**3-Le condenser**

allows to **focus** light sourcce on sample

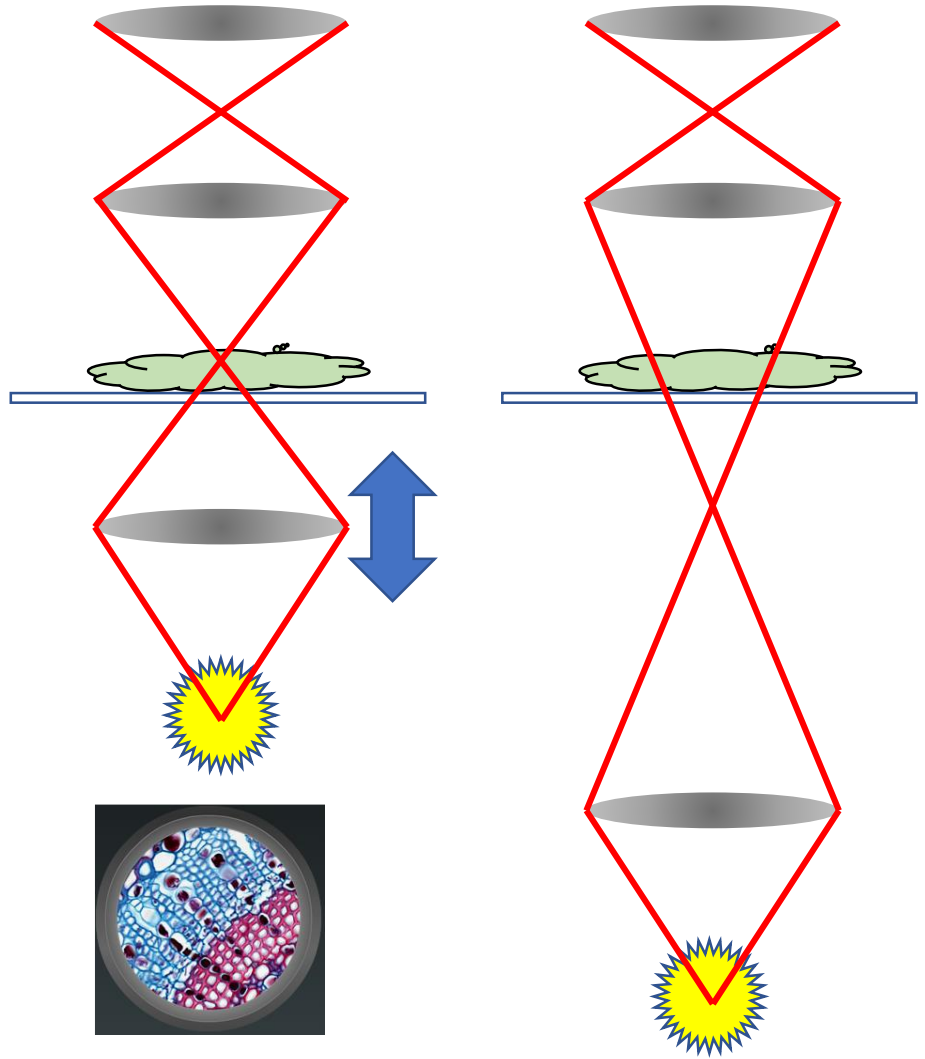
**Oculars**
Enlarge image produced by the objective

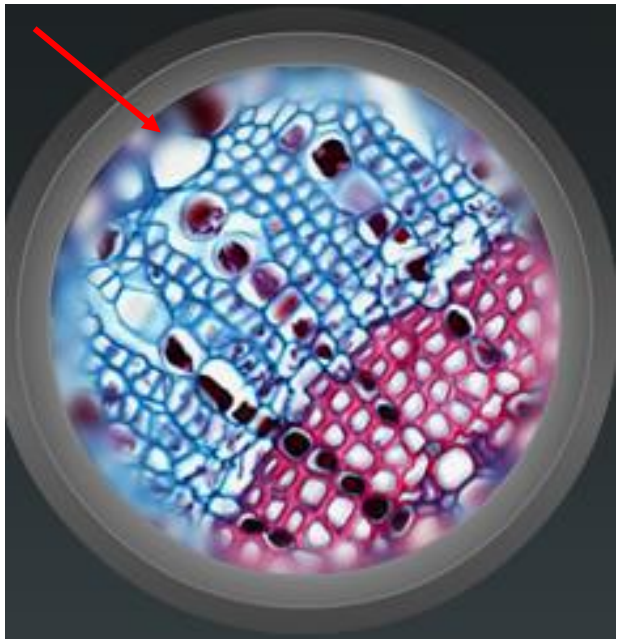
**Objective**
Produce an enlarged image of the sample

**Sample**

**Condenser**
Focus light on sample

**Light source**





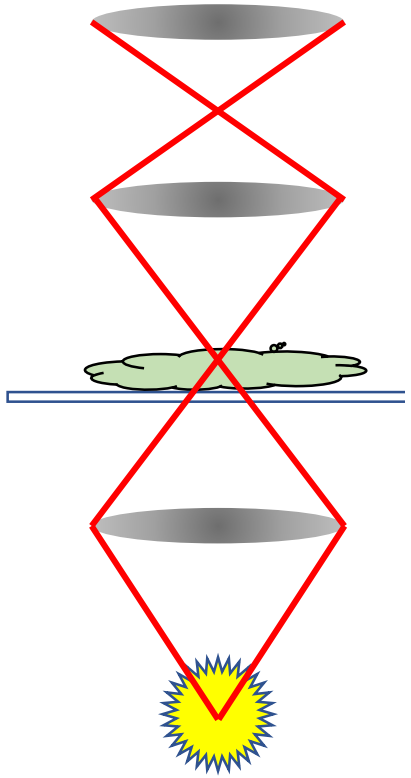
# To see well: homogenous illumination on sample

**Köhler illumination**

allows to **optimise** sample illumination

= Each point of light source is used to illuminate sample.

Thois technic allows to obtain homogenous illumination of samples.



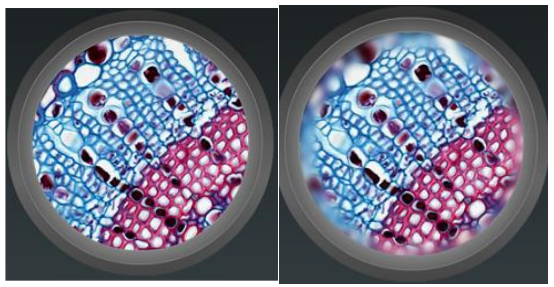
**Ocular**

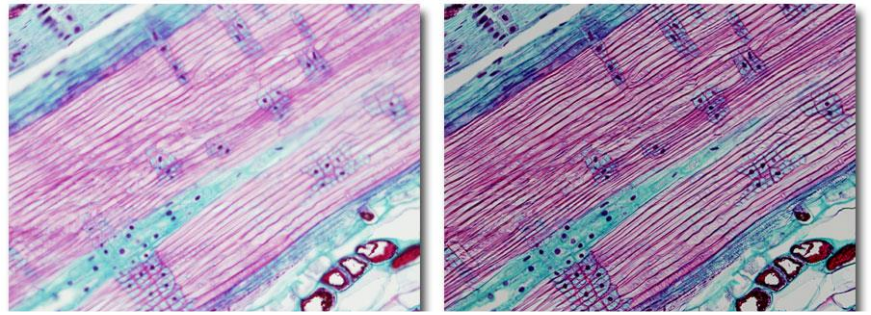
**Objective**

**Sample**

**Condenser**

**Light source**

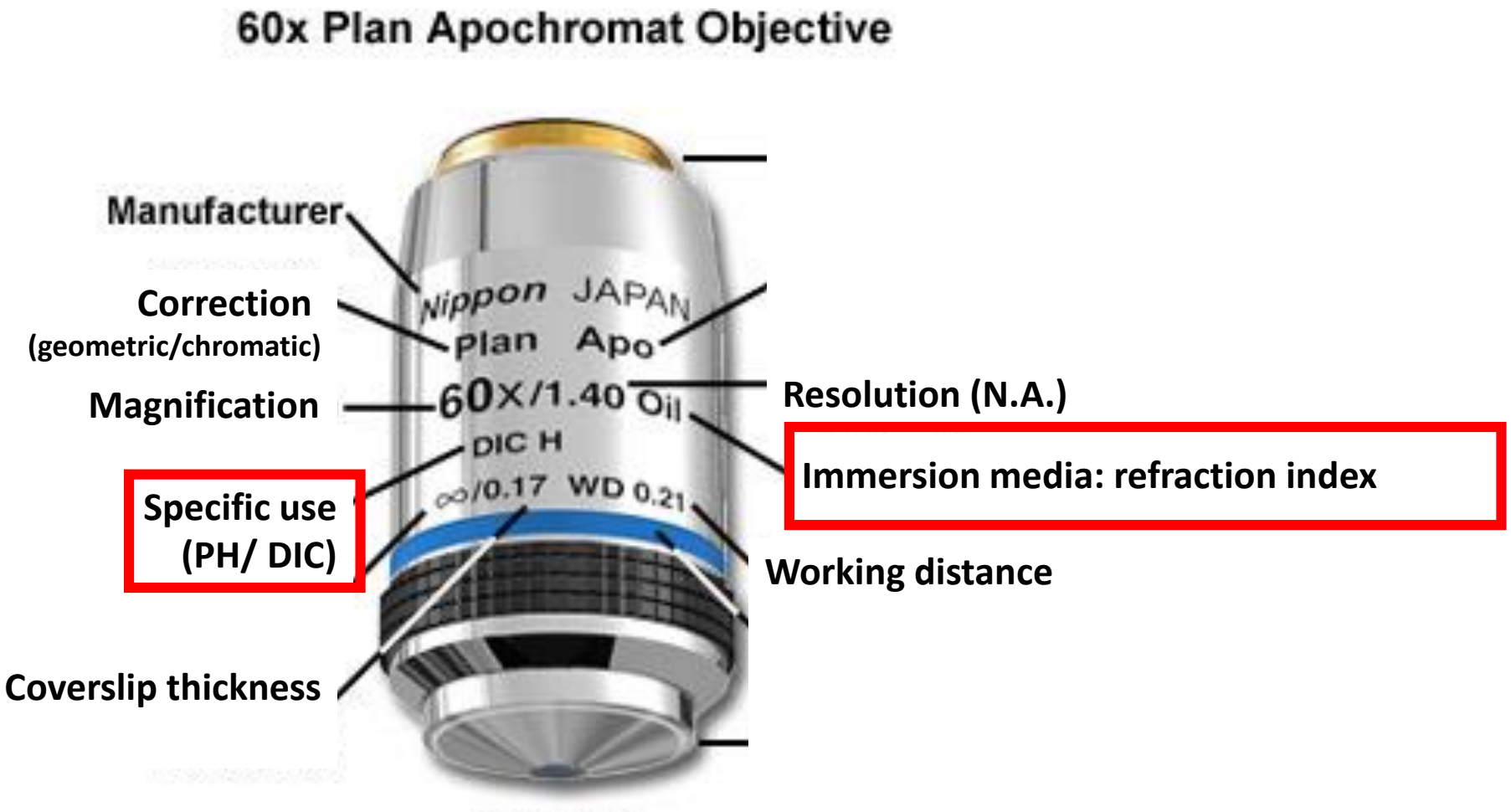




www.microscopyu.com/tutorials/kohler

https://www.olympus-lifescience.com/fr/microscope-resource/primer/java/kohler/contrast/

# To well see : objective




Lens on objective are handfinished

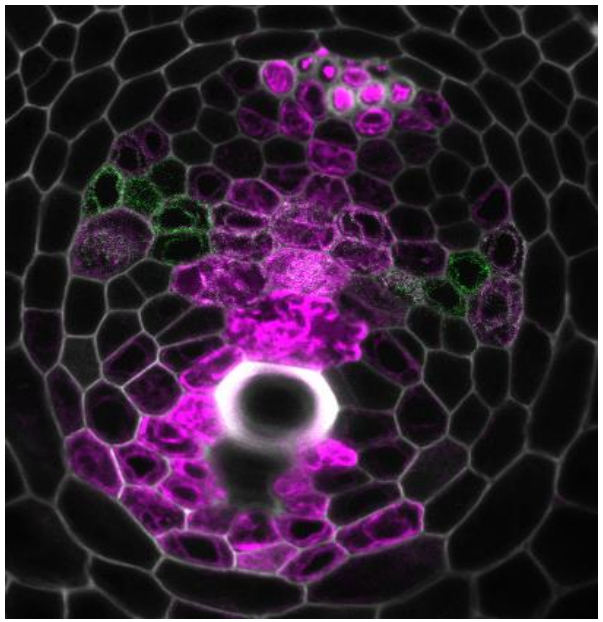
# What allows you to see well: contrast

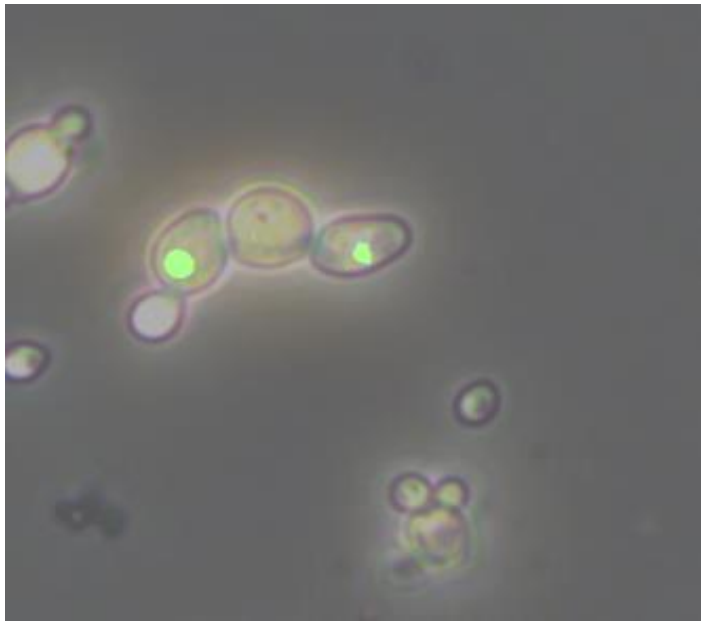
Bright field | Phase contrast | DIC

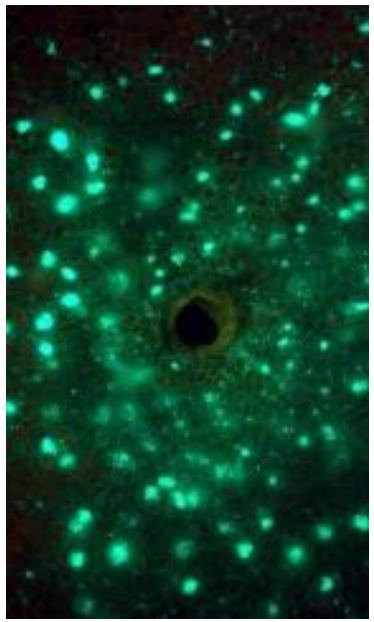
Specific Use
(PH/ DIC)

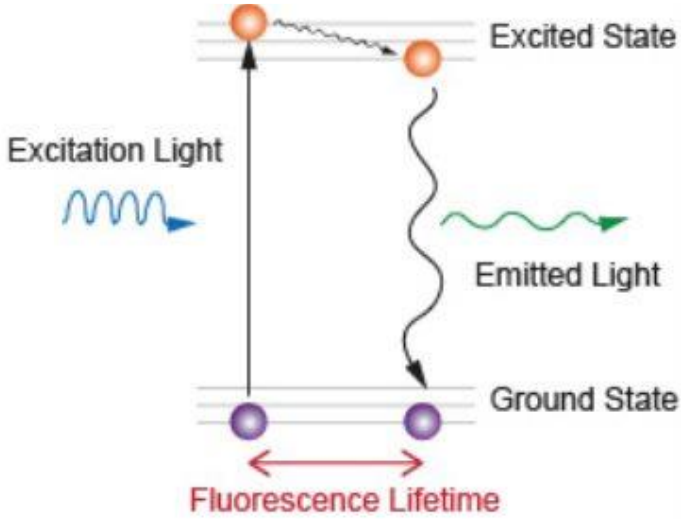
# More contrast!

Fluorescence allows to obtain a strong **specific contrast** of sample










The fluorescence is a chemical process which generate light emission after absoption of photon having a higher energy.

# To go further

**Resolution**

## Electron microscopy

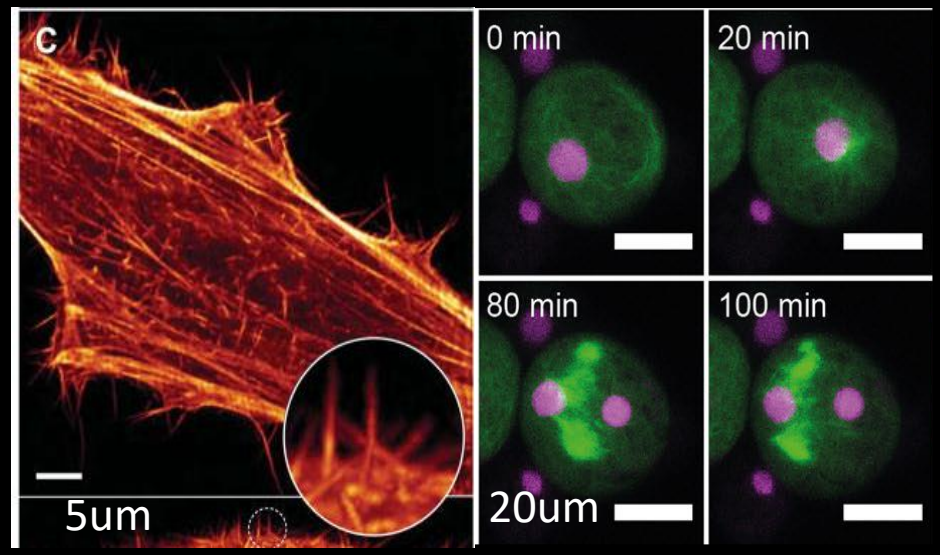
**Transmission Electron Microscopy**
Ultrastructure


Correlative microscopy

## Optical microscopy

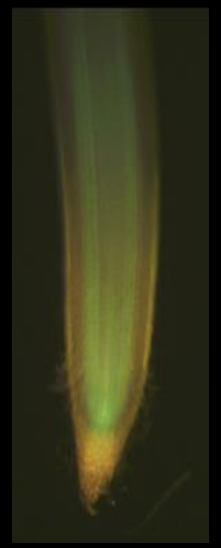
**Super Resolution**
Between confocal and TEM
(actin, tubulin)

**Confocal**
Small structure
in 3D




Hot topic, more and more commercial system

**Epifluorescence**
**(Thickness of sample)**

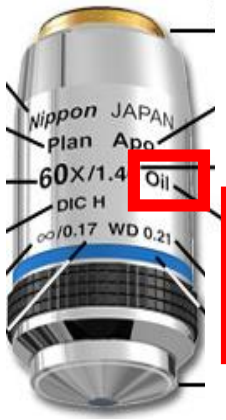


More and more « home made system » : 3D printing, micromanager, metamorph

# Just a few more points…

## Samples preparation

*Mounting media




Immersion media: refraction index

## Visualization system

Chemin optique propre
Pas de poussière!!

## Imaging system (camera)

Exposure time
File format when saving data

## Analyse et manipulation des images

Attention !! Improper handling of images can destroy information because it is based on "false" results.
(conserve as much as possible raw data!!)

Plastic surgery of image can change the conclusion of image analysis!!

https://www.youtube.com/watch?v=c_Oi2HKom_Y
In Defense of Image Data & Analysis Integrity

# Next: depending on what you want or need

**Fluorescence microscopy**
**And sample preparation**

*Fluorecent proteins and probes
*Epifluorecence VS confocal
*super resolution
*F technics
*Multiphoton microscopy

**Image processing, analysis and communication**

*raw data and different image format
*ImageJ/Fiji
*scale bar
*measure surface area ; count; semi-automation
*trick for the layout and communicaton

**Introduction to electron microscopy**


Thank you for your attention!