



# Les étapes du traitement de l'analyse d'image

La capture → image brute

Prétraitement → niveaux de gris

Segmentation → image binaire

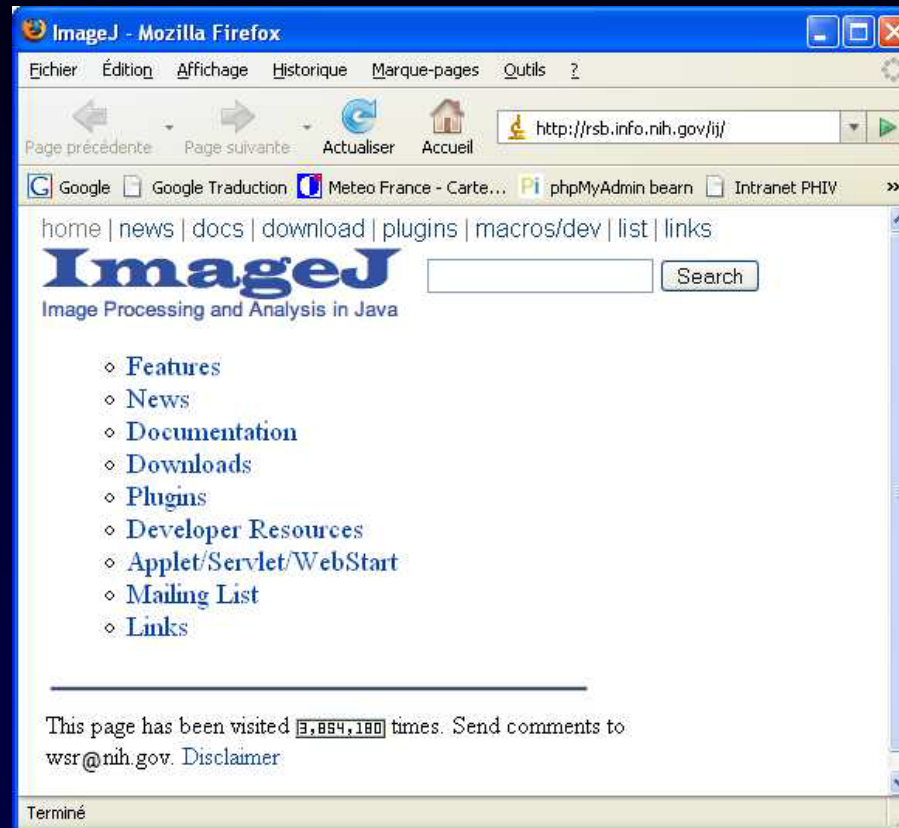
Post-traitement → régions d'intérêts

Quantification → données

Amélioration

Publication

# ImageJ est un logiciel libre de traitement et d'analyse d'images



Traduction en Java du logiciel NIH Image  
du National Institutes of Health (USA)  
développé par Wayne Rasband

Il fonctionne sur de multiples plates-formes (Windows, Mac, Linux, Unix, ...).

<http://rsbweb.nih.gov/ij/>



# Installation

The screenshot shows a Mozilla Firefox browser window displaying the download page for ImageJ. The browser's address bar shows the URL `rsbweb.nih.gov/ij/download.html`. The page content includes navigation links, a 'Download' header, and instructions for installing ImageJ on different operating systems. A Windows file dialog box is overlaid on the right side of the browser window, showing the selection of the file `ij144-jdk6-setup.exe` for opening. The dialog box asks if the user wants to register the file, with 'Enregistrer le fichier' and 'Annuler' buttons.

Download - Mozilla Firefox

Fichier Édition Affichage Historique Marque-pages Outils ?

Download +

rsbweb.nih.gov/ij/download.html

imagej

Les plus visités Intranet du Cirad AGAP intra Intranet DAP Intranet PHIV DAP phpMyAdmin PMA phiv | phpMyAdmin

home | news | docs | download | plugins | resources | list | links

## Download

### Platform Independent

To install ImageJ 1.44 on a computer with Java pre-installed, or to upgrade to the latest full distribution (including macros, plugins and LUTs), download [ij144.zip](#) (3MB) and extract the ImageJ directory. Use the *Help>Update ImageJ* command to upgrade to the latest pre-release version.

### Mac OS X

Download [ImageJ 1.44](#) (5.4MB) as a double-clickable Mac OS X application. Includes ImageJ64, which uses Java 1.6 in 64-bit mode on Intel Macs running OS X 10.5 or later. ([Instructions](#))

### Linux

Download ImageJ 1.44 [bundled with 32-bit Java](#) (46MB) or with [64-bit Java](#) (40MB). Both versions include Java 1.6.0\_20 from Sun and the ImageJ source code. ([Instructions](#))

### Windows

Download ImageJ 1.44 [bundled with 32-bit Java 1.6.0\\_20](#) (28MB), with [64-bit Java 1.6.0\\_20](#) (24MB; requires 64-bit Windows) or [without Java](#) (3MB). ([Instructions](#))

Ouverture de ij144-jdk6-setup.exe

Vous avez choisi d'ouvrir

**ij144-jdk6-setup.exe**  
qui est un fichier de type : Binary File  
à partir de : <http://rsbweb.nih.gov>

Voulez-vous enregistrer ce fichier ?

Enregistrer le fichier Annuler



# Installation

**Setup - ImageJ**

### Welcome to the ImageJ Setup Wizard

This will install ImageJ 1.44p on your computer.

It is recommended that you close all other applications before continuing.

Click Next to continue, or Cancel to exit Setup.

Next > Cancel

**Setup - ImageJ**

### Select Destination Location

Where should ImageJ be installed?

Setup will install ImageJ into the following folder.

To continue, click Next. If you would like to select a different folder, click Browse.

F:\ImageJ Browse...

At least 106,8 MB of free disk space is required.

< Back Next > Cancel

**Setup - ImageJ**

### Select Start Menu Folder

Where should Setup place the program's shortcuts?

Setup will create the program's shortcuts in the following Start Menu folder.

To continue, click Next. If you would like to select a different folder, click Browse.

ImageJ Browse...

Don't create a Start Menu folder

< Back Next > Cancel

**Setup - ImageJ**

### Select Additional Tasks

Which additional tasks should be performed?

Select the additional tasks you would like Setup to perform while installing ImageJ, then click Next.

Additional icons:

- Create a desktop icon
- Create a Quick Launch icon

< Back Next > Cancel

**Setup - ImageJ**

### Ready to Install

Setup is now ready to begin installing ImageJ on your computer.

Click Install to continue with the installation, or click Back if you want to review or change any settings.

Destination location:  
F:\ImageJ

< Back Install Cancel

**Setup - ImageJ**

### Installing

Please wait while Setup installs ImageJ on your computer.

Extracting files...  
F:\ImageJ\jre\lib\charsets.jar

Progress bar (100%)

Cancel

**Setup - ImageJ**

### Completing the ImageJ Setup Wizard

Setup has finished installing ImageJ on your computer.

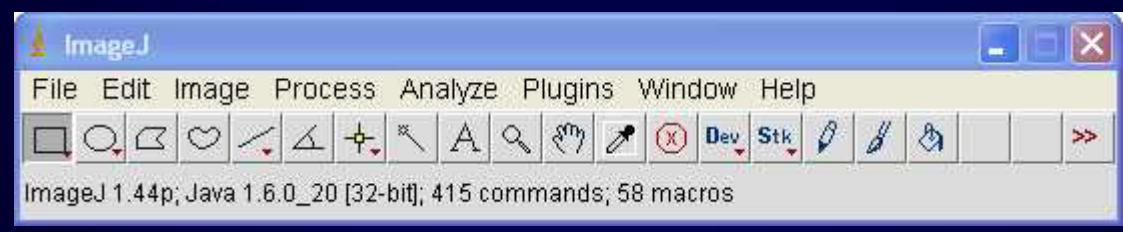
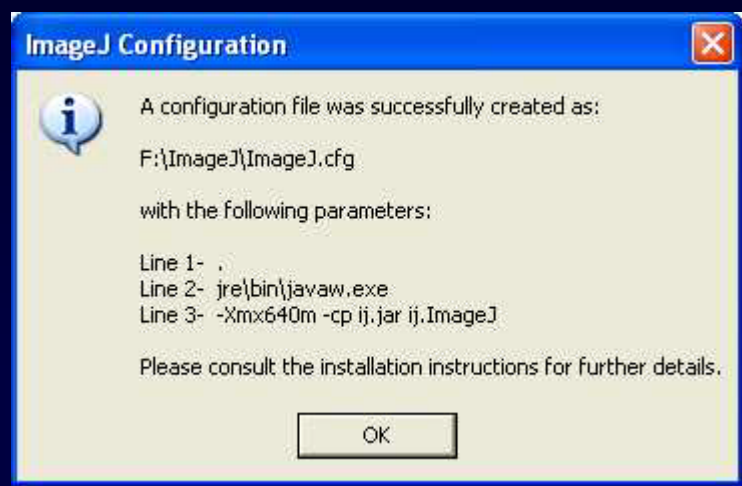
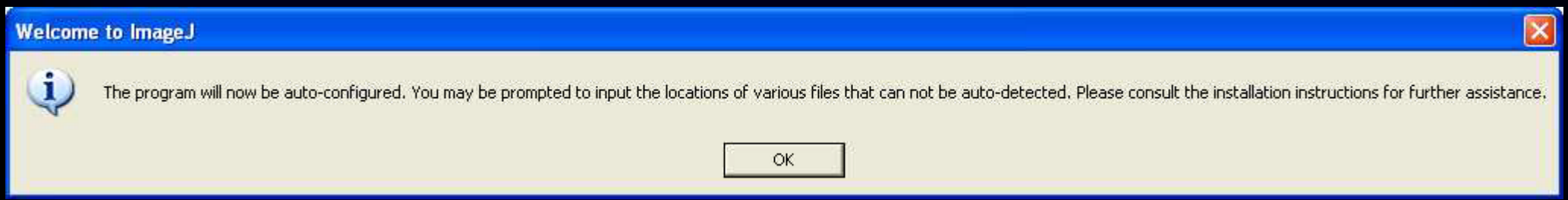
Click Finish to exit Setup.

Launch ImageJ

Finish

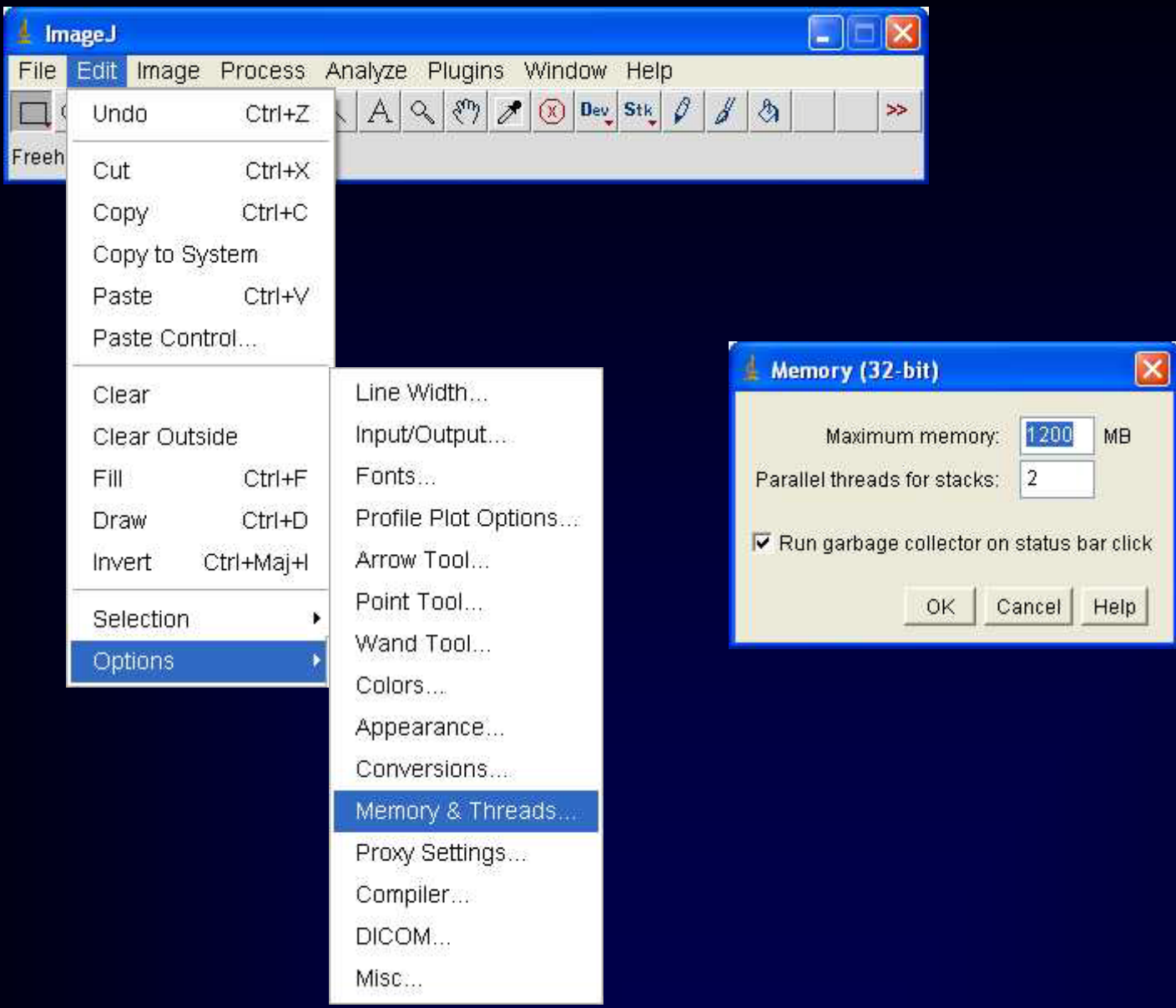


# Configuration



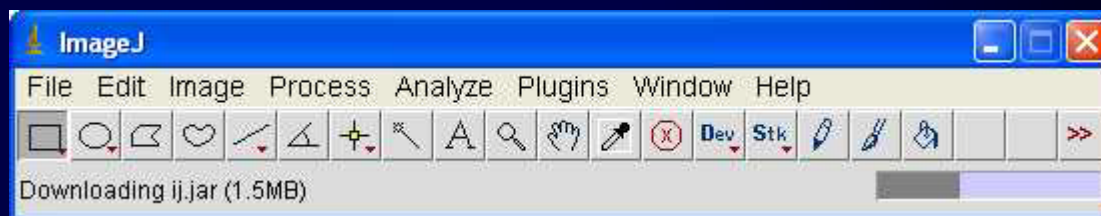
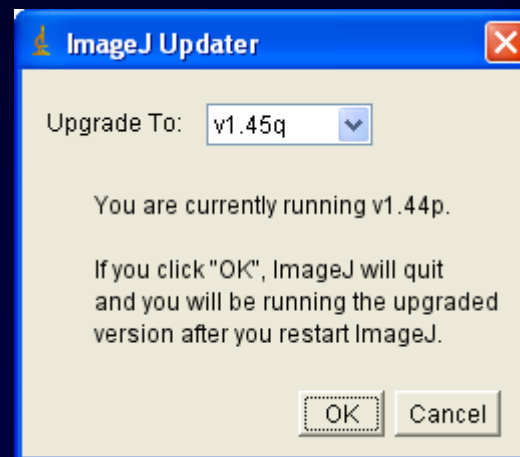
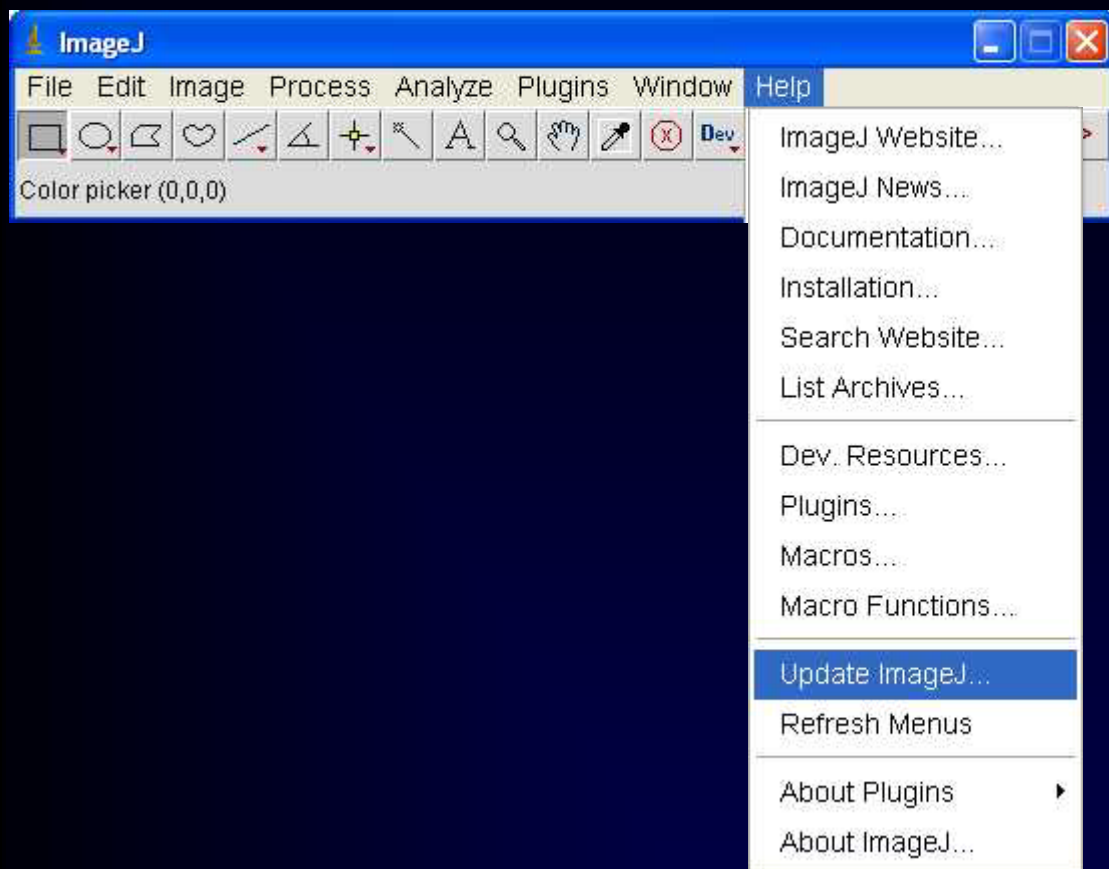


# Configuration



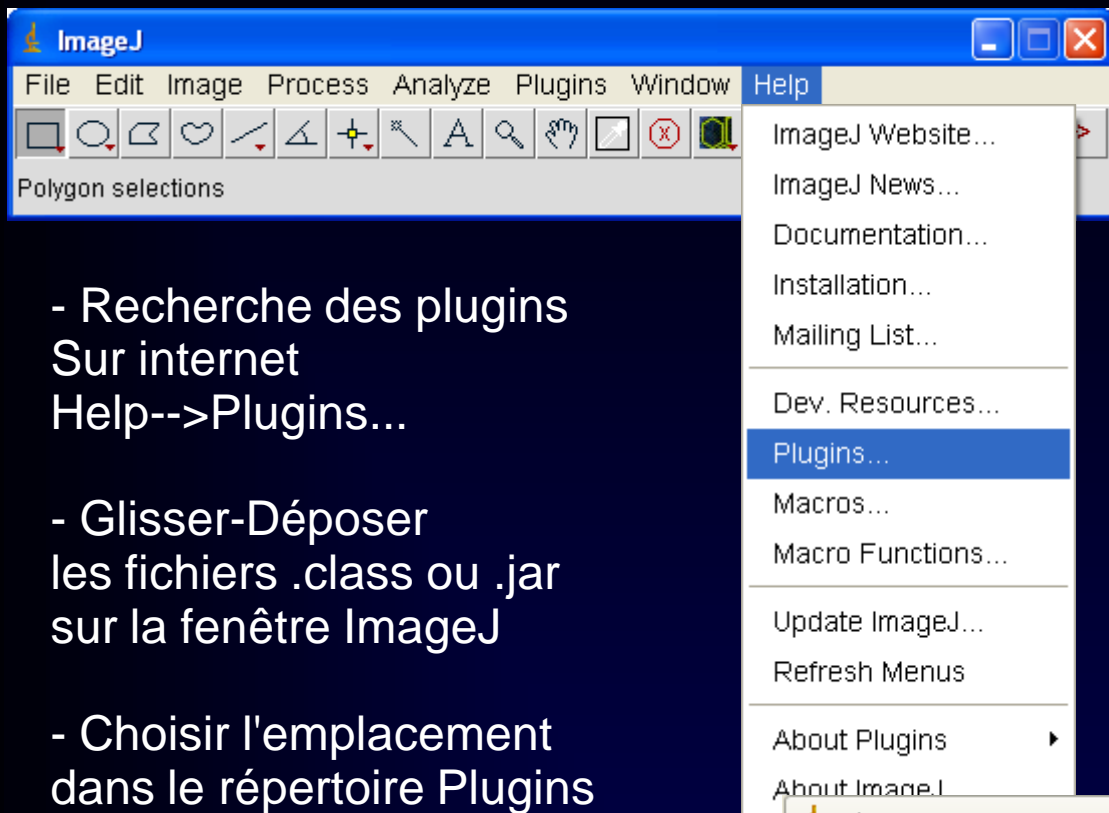
Edit → Option → Memory & Treads...

# Mise à jour



Help → Update ImageJ...

# Installation de Plugins



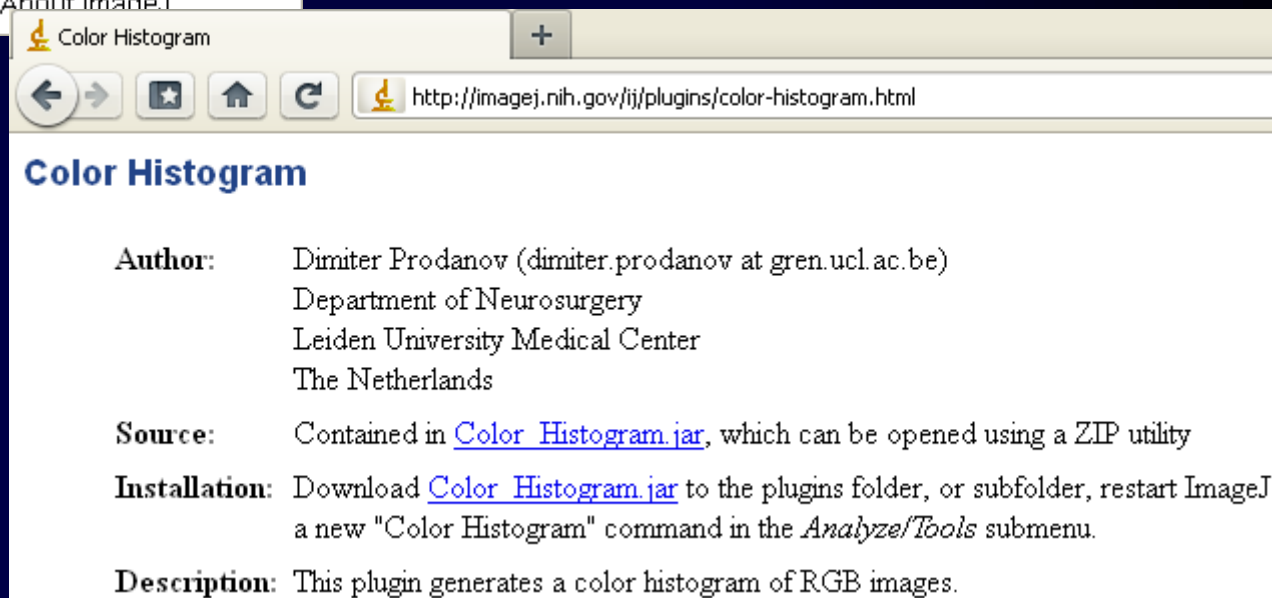
- Recherche des plugins  
Sur internet  
Help-->Plugins...

- Glisser-Déposer  
les fichiers .class ou .jar  
sur la fenêtre ImageJ

- Choisir l'emplacement  
dans le répertoire Plugins

- Mettre à jour les menus  
Help--> Refresh Menus

- Apparition de la  
nouvelle commande  
dans le menu Plugins



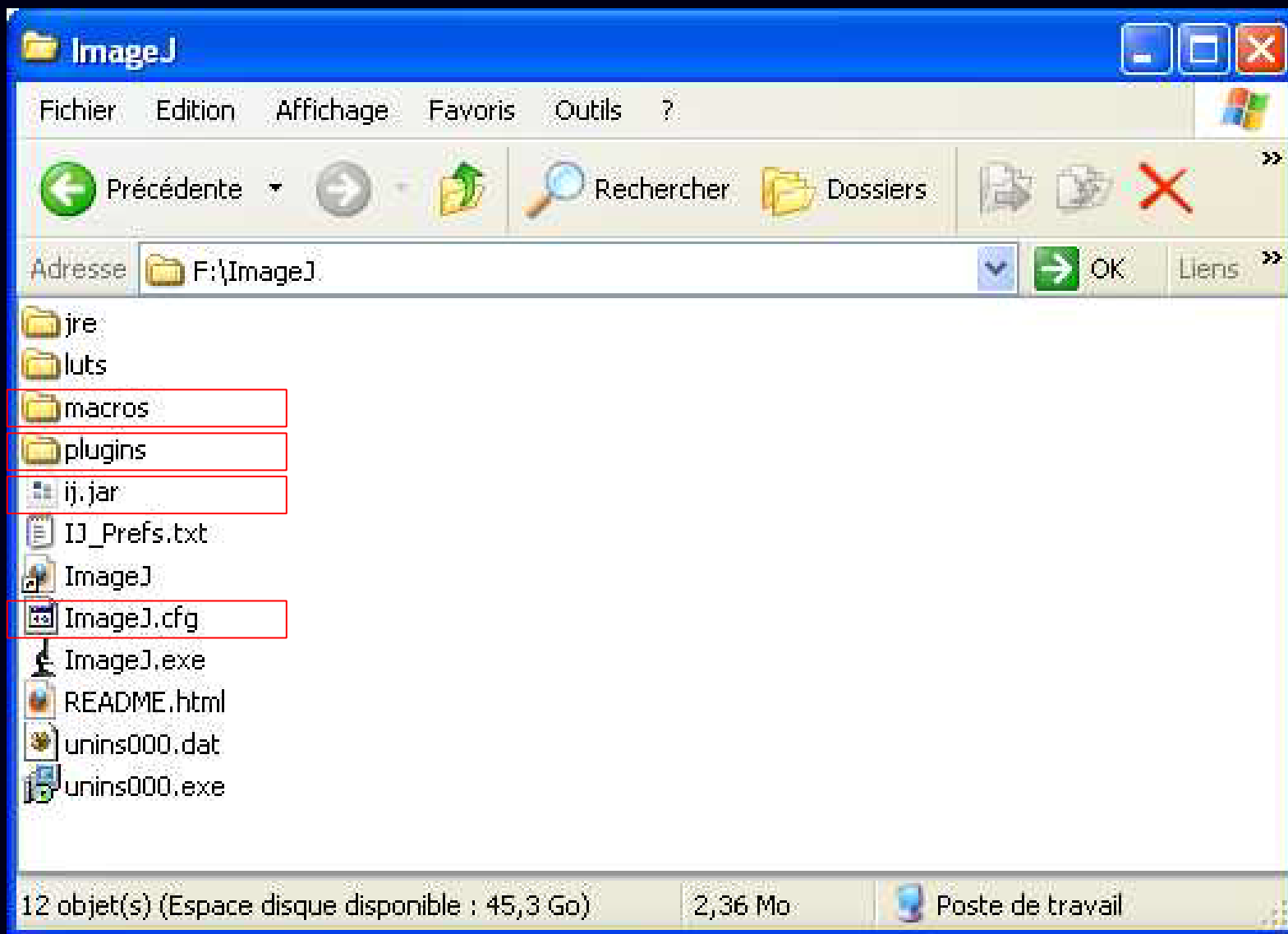
Help→ Plugins...

Help→ Refresh Menus





# Dossiers ImageJ





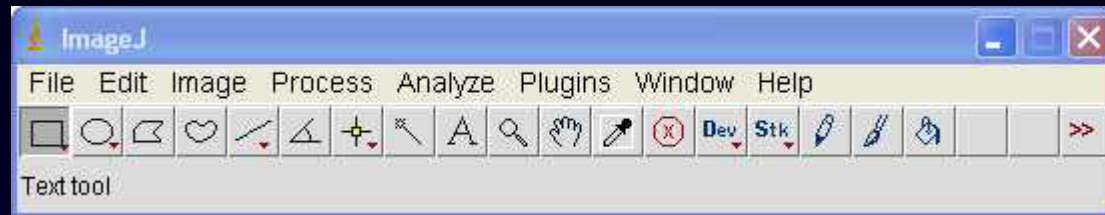
# Topic 01 - Installation, configuration, help and updates



# Les bases pour l'utilisation d'ImageJ



# Interface



## Barre de menu

File Edit Image Process Analyze Plugins Window Help

## Barre d'outils



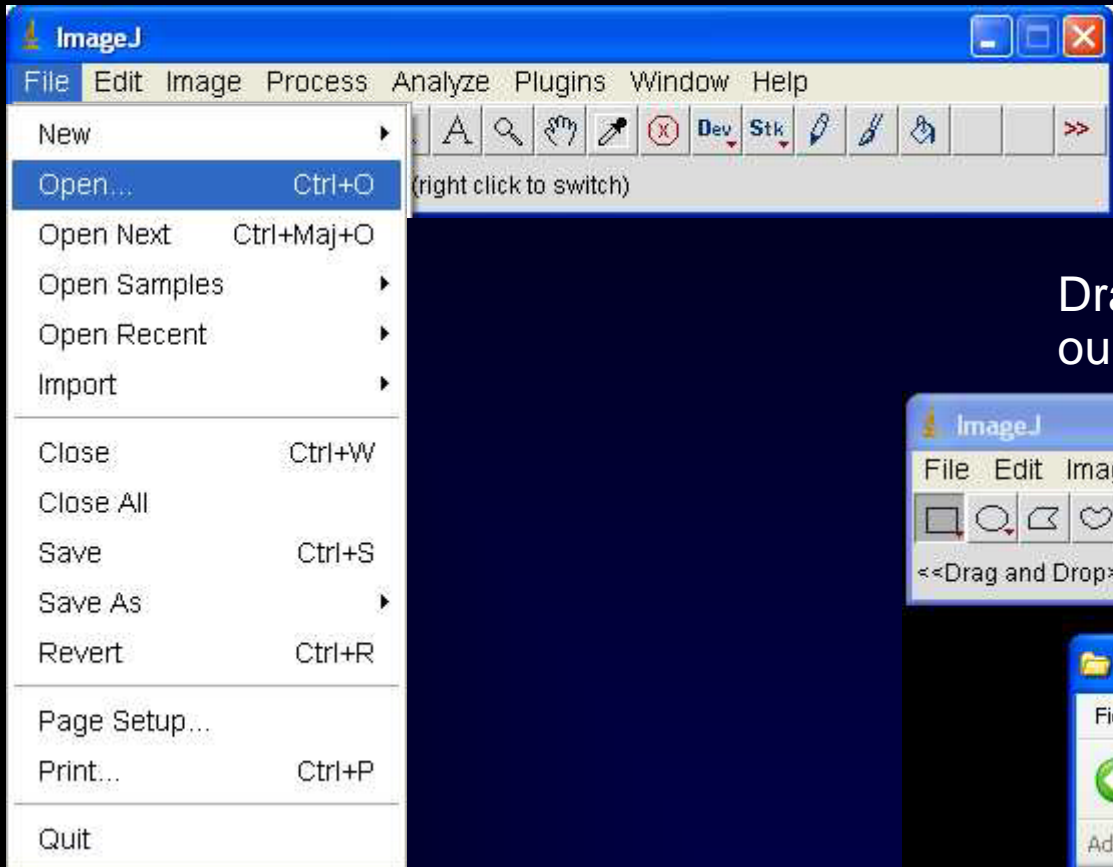
## Barre d'état

ImageJ 1.45q; Java 1.6.0\_20 [32-bit]; 1547K of 1200MB (<1%)

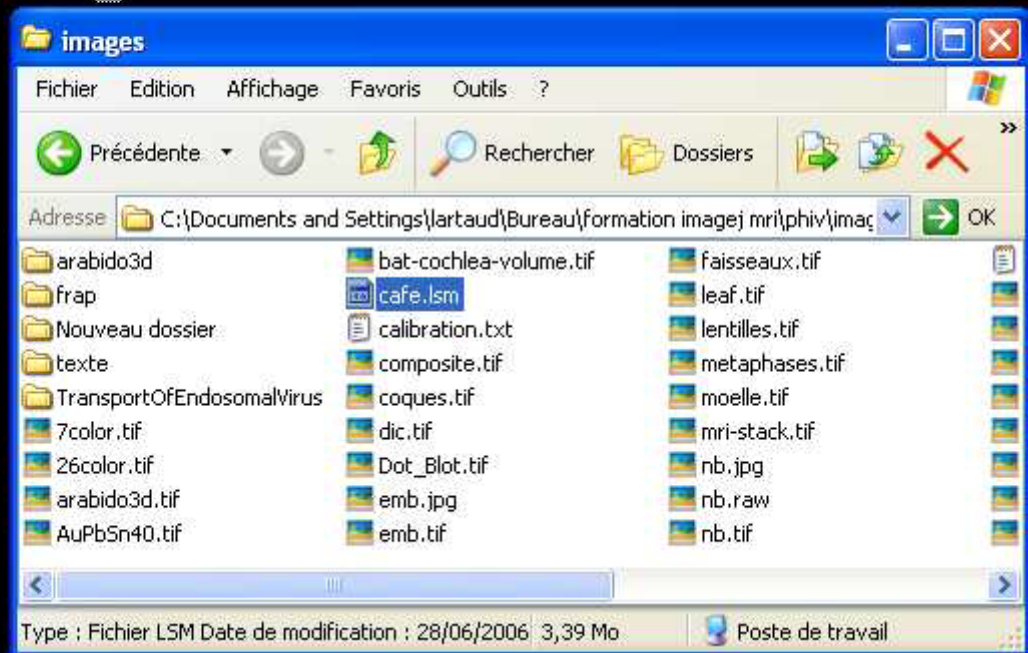
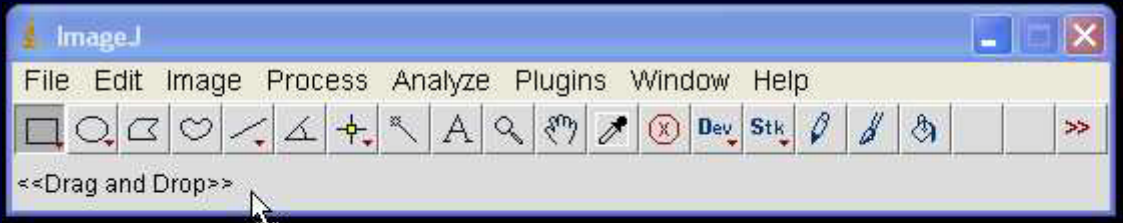


# Ouvrir une image

File → Open...



Drag and Drop sur la fenêtre ou l'icône

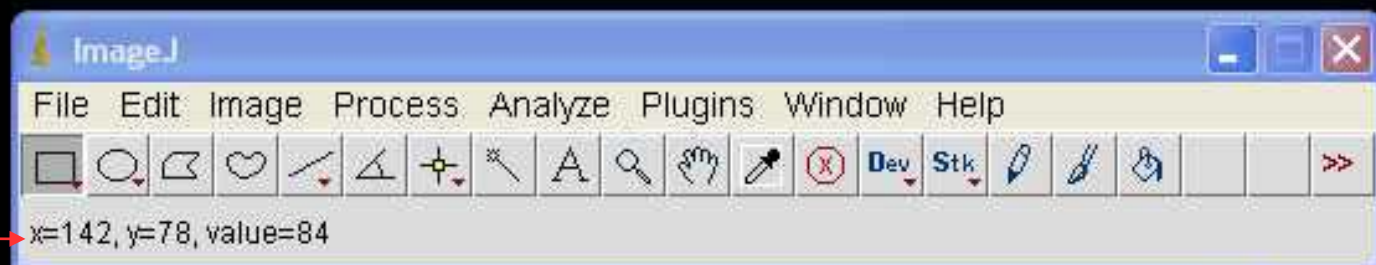


File → Open ...

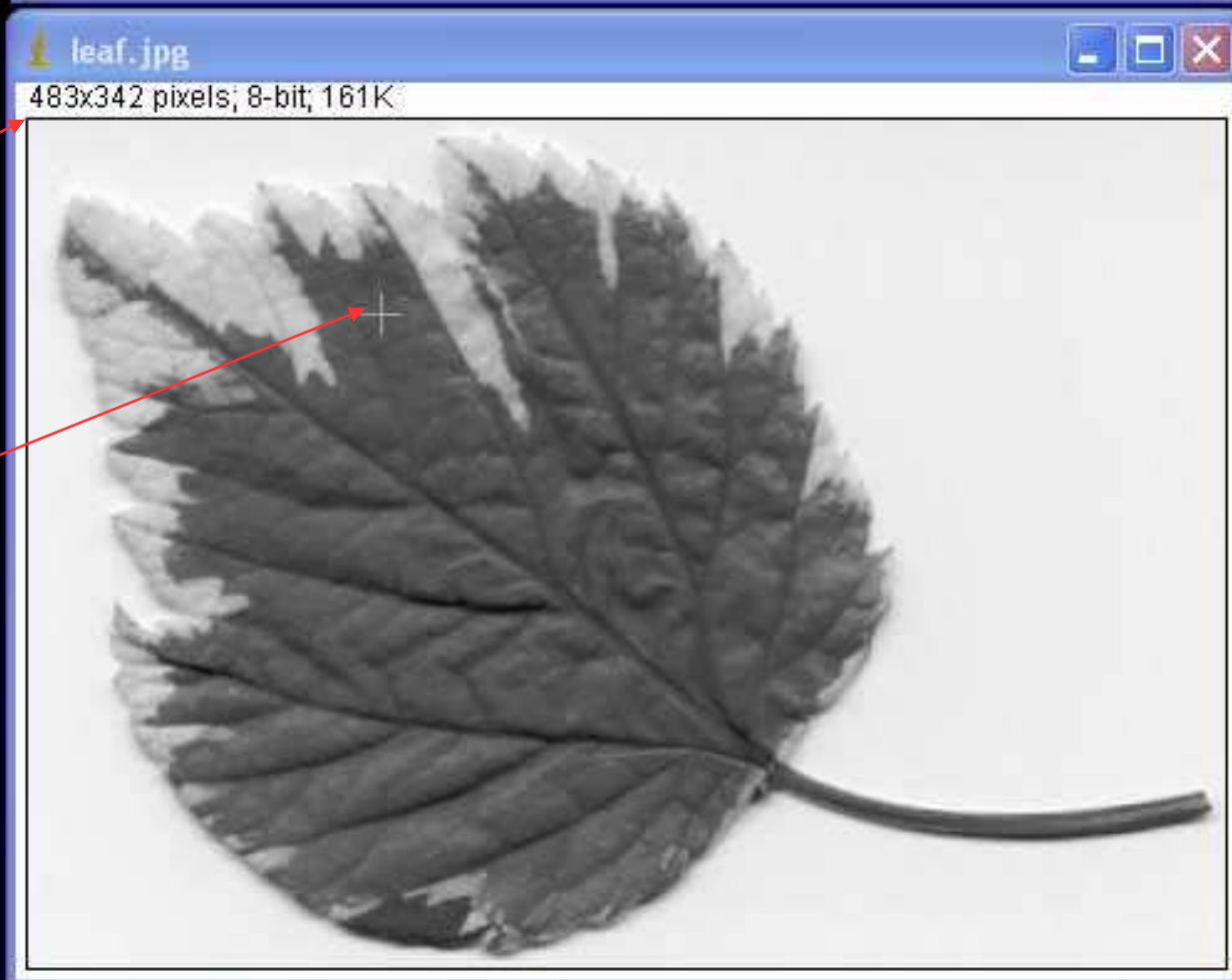


# Informations pixel

Informations sur le pixel  
sous le curseur de la souris  
position en x,y  
et valeur en niveau de gris



Informations image



Curseur de souris



# Zoom



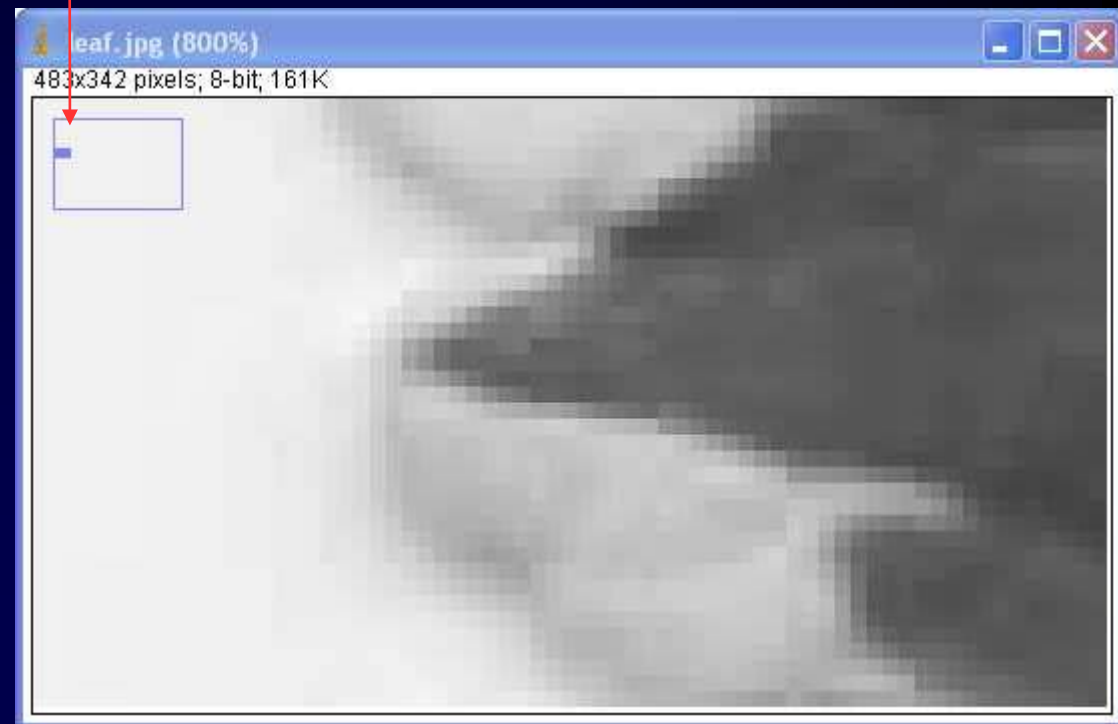
Déplacement de l'image dans la fenêtre  
Avec la souris et la barre d'espace appuyée

Touche « - »



Zone affichée

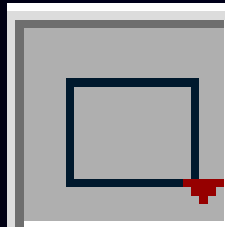
Touche « + »



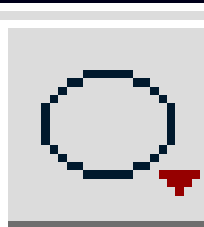


# Outils de sélection (ROI)

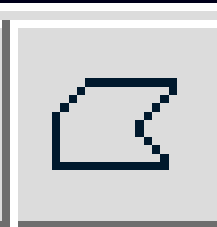
Rectangl  
e



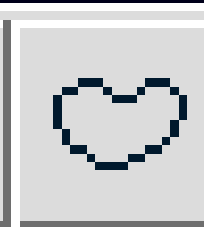
Ovale



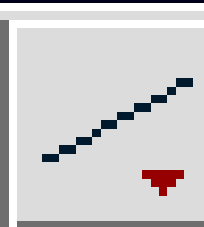
Polygone



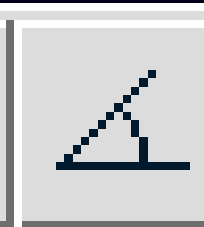
À main levée



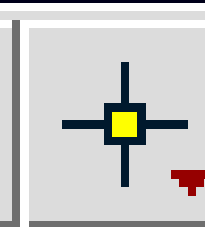
Ligne



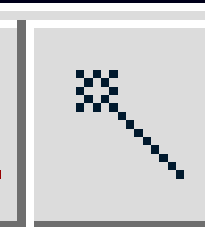
Angle



Point



Baguette



Choix d'outils différents : Clic droit sur triangle rouge

✓ Rectangle Tool

Rounded Rectangle Tool

✓ Oval selections

Elliptical selections

Selection Brush Tool

✓ Straight Line

Segmented Line

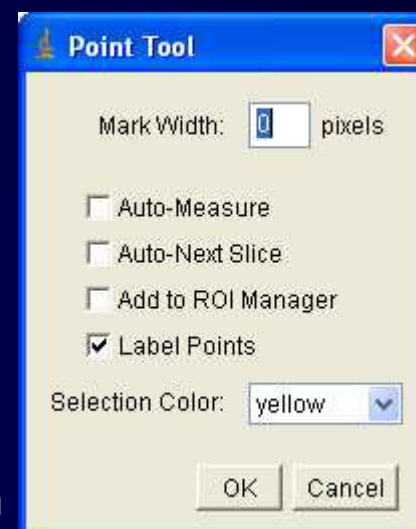
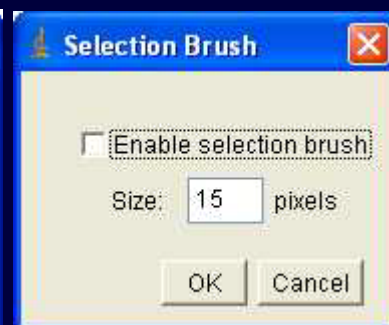
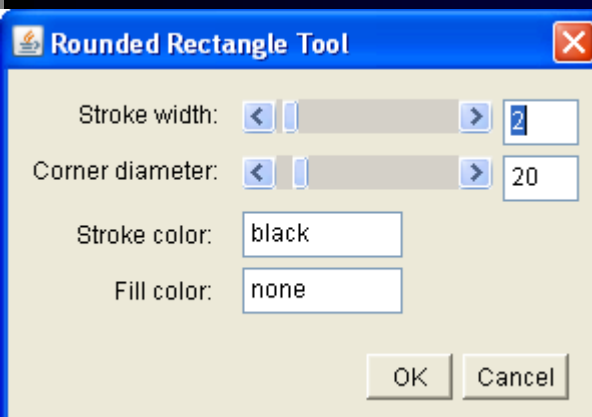
Freehand Line

Arrow tool

✓ Point Tool

Multi-point Tool

Options : Double clic sur l'icône de l'outil



Ajouter à la sélection : Maj-clic

Supprimer de la sélection : Alt-clic

Retrouver la sélection : Edit → Selection → Restore Selection





# ROI Manager

ImageJ

File Edit Image Process Analyze Plugins Window Help

Magnifying glass (or use "+" and "-")

Measure Ctrl+M

Analyze Particles...

Summarize

Distribution...

Label

Clear Results

Set Measurements...

Set Scale...

Calibrate...

Histogram Ctrl+H

Plot Profile Ctrl+K

Surface Plot...

Gels

Tools

Analyze

Save XY Coordinates...

Fractal Box Count...

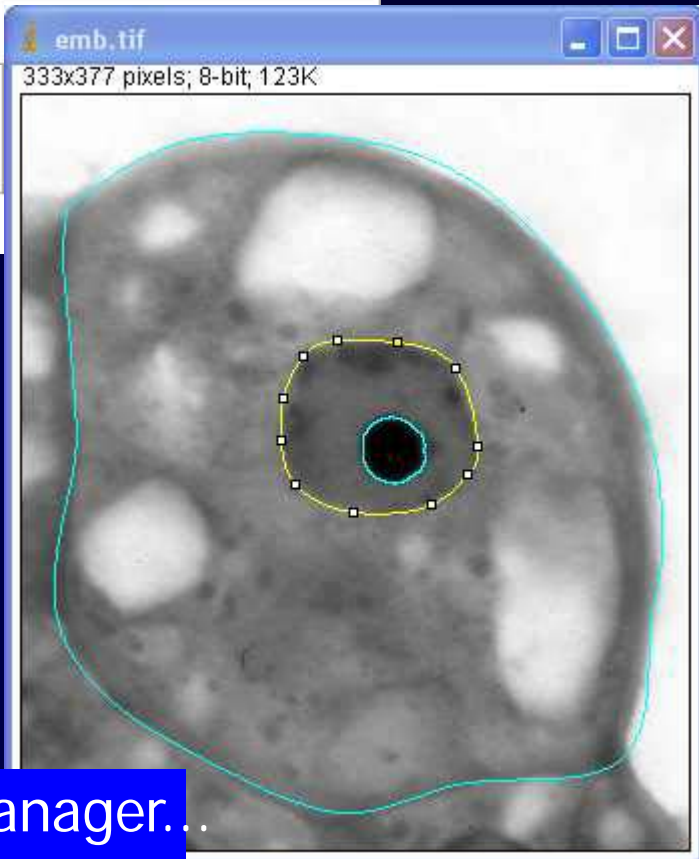
Analyze Line Graph

Curve Fitting...

**ROI Manager...**

Scale Bar...

Calibration Bar...



ROI Manager

Cellule	Add [t]
Noyau	Update
Nucléole	Delete
	Rename...
	Measure
	Deselect
	Properties...
	Flatten [F]
	More »
<input checked="" type="checkbox"/> Show All	
<input type="checkbox"/> Edit Mode	

Open...

Save...

**Fill**

Draw

AND

OR (Combine)

XOR

Split

Add Particles

Multi Measure

Sort

Specify...

Remove Slice Info

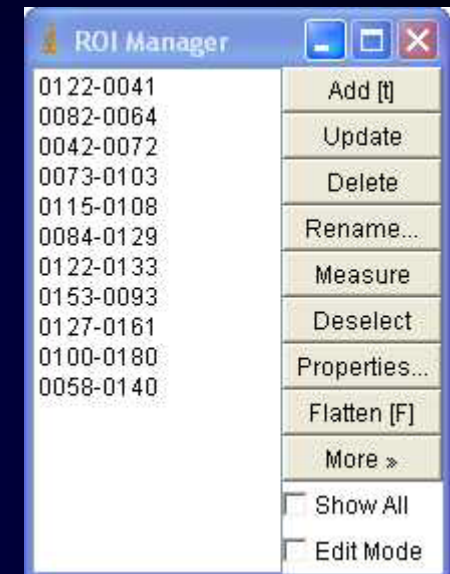
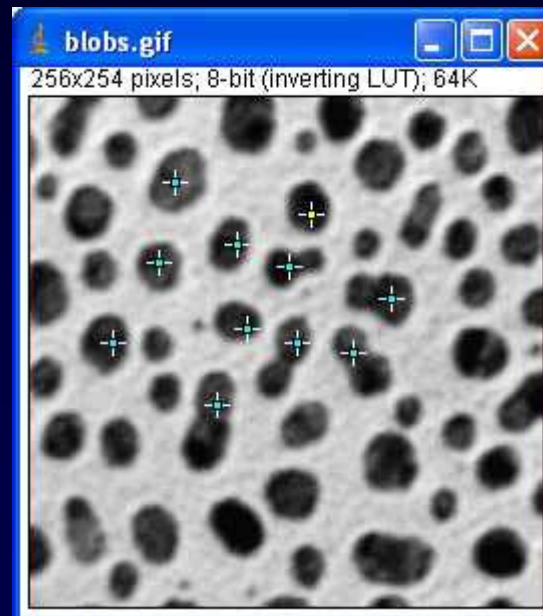
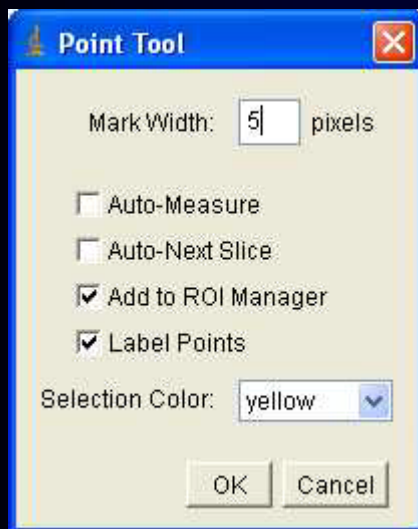
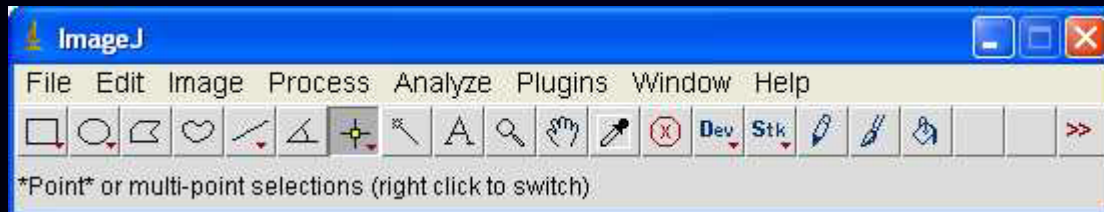
Help

Options...

Analyse → Tools → ROI Manager...



# Outil Point pour le comptage





# Dessin



ImageJ

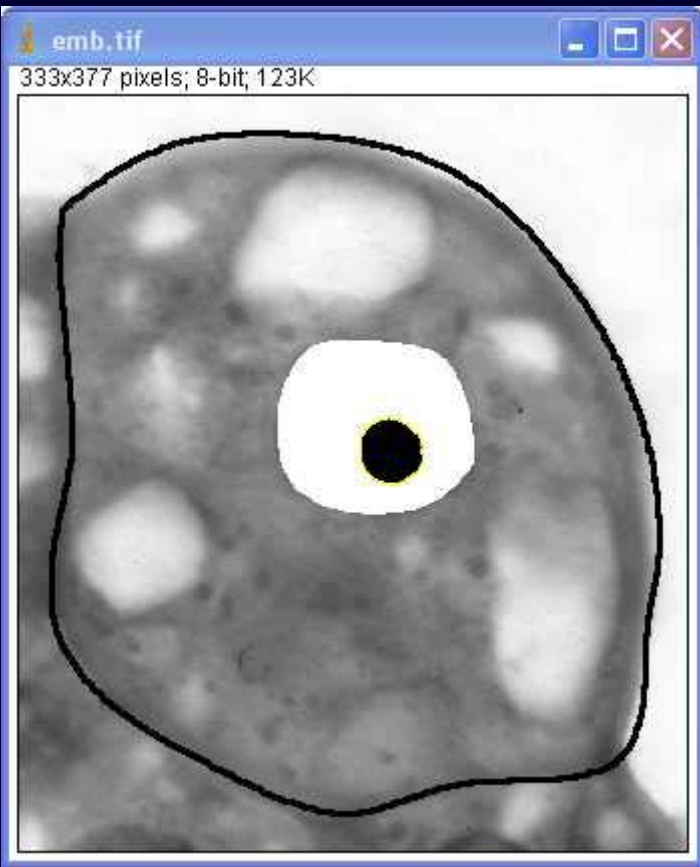
File Edit Image Process Analyze Plugins Window Help

- Undo Ctrl+Z
- Cut Ctrl+X
- Copy Ctrl+C
- Copy to System
- Paste Ctrl+V
- Paste Control...
- Clear**
- Clear Outside
- Fill Ctrl+F
- Draw Ctrl+D
- Invert Ctrl+Maj+I
- Selection ▶
- Options ▶

Clear → Noyau

Fill → Nucléole

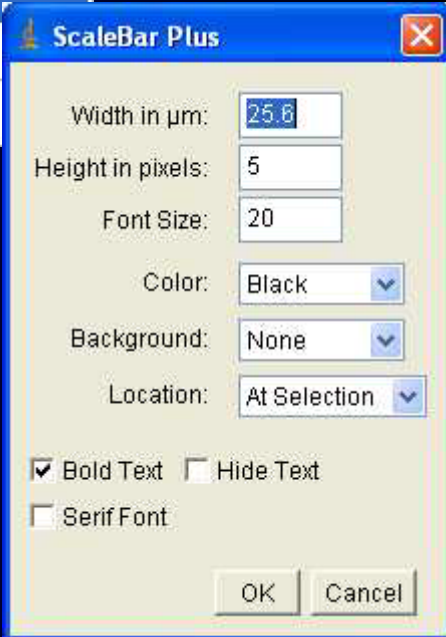
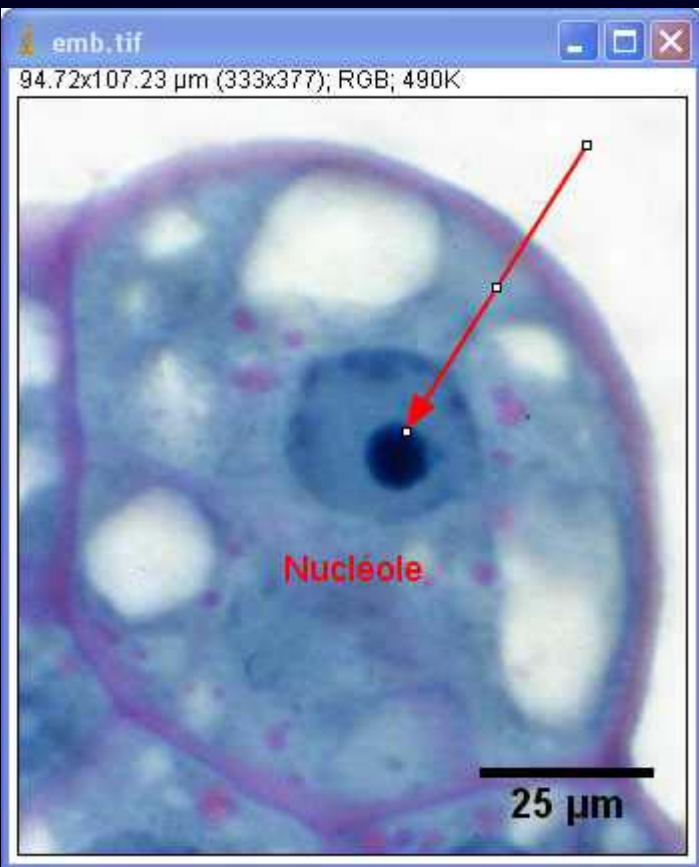
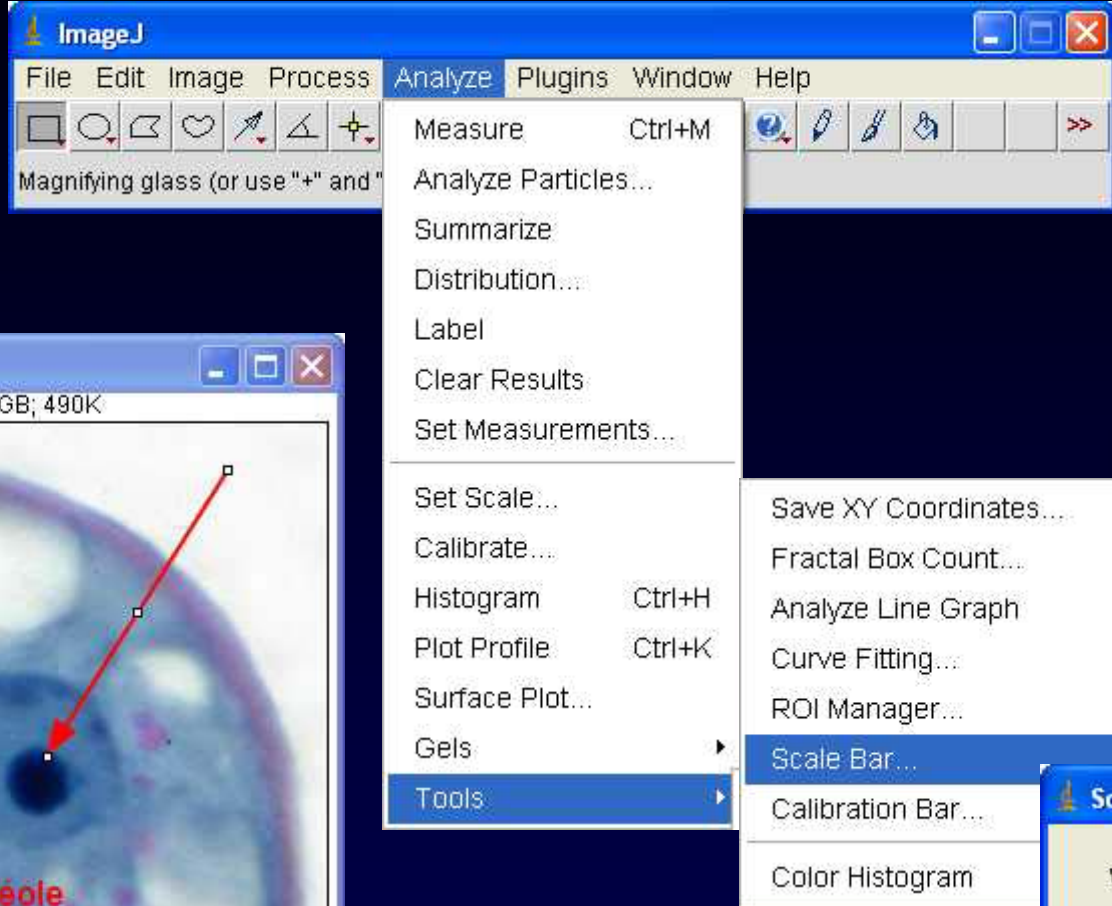
Draw → Cellule



Edit → Clear...



# Annotations



Analyse → Tools → Scale Bar...



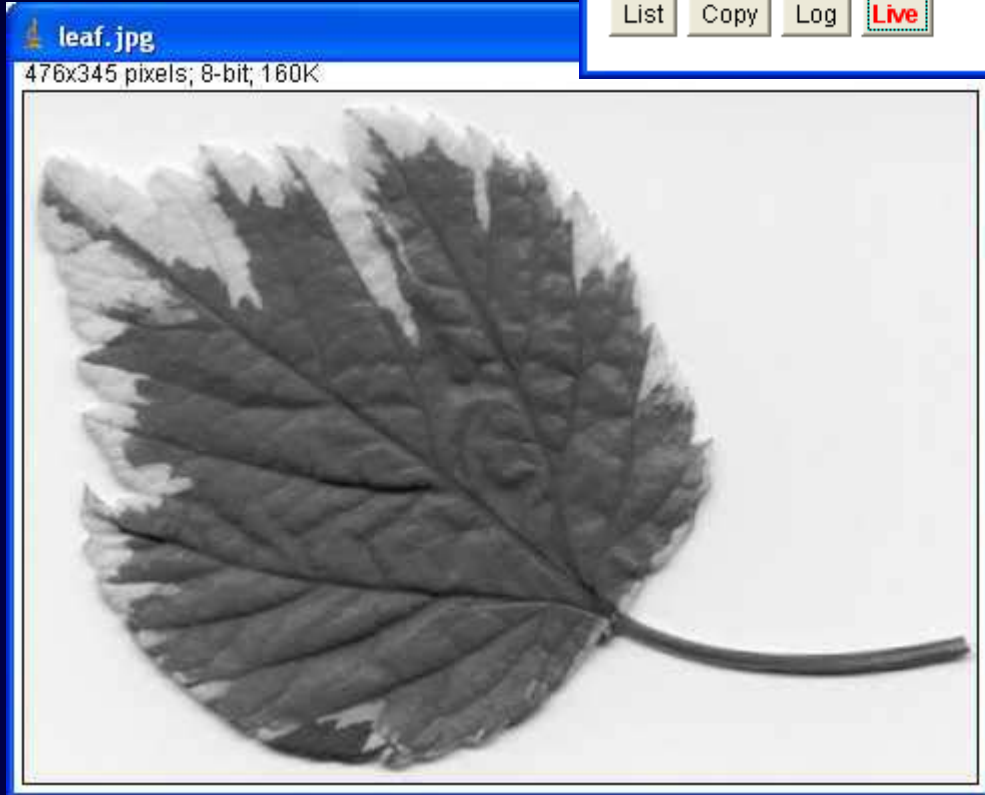
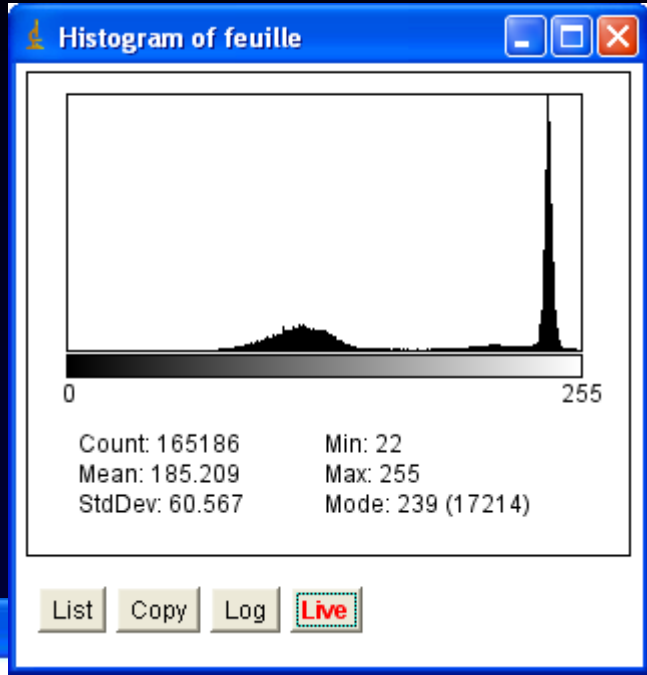
# Histogramme

ImageJ

File Edit Image Process Analyze Plugins Window Help

Magnifying glass (or use "+" and "-")

- Measure Ctrl+M
- Analyze Particles...
- Summarize
- Distribution...
- Label
- Clear Results
- Set Measurements...
- Set Scale...
- Calibrate...
- Histogram Ctrl+H**
- Plot Profile Ctrl+K
- Surface Plot...
- Gels ▶
- Tools ▶



Analyse → Histogram



# Seuillage

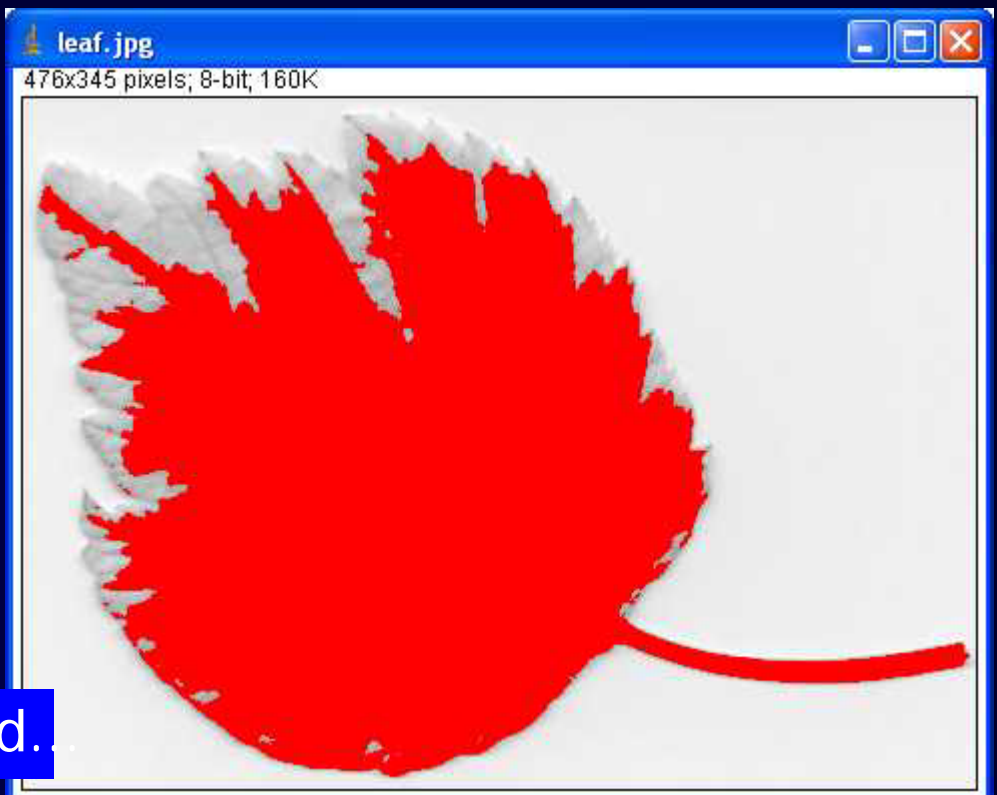
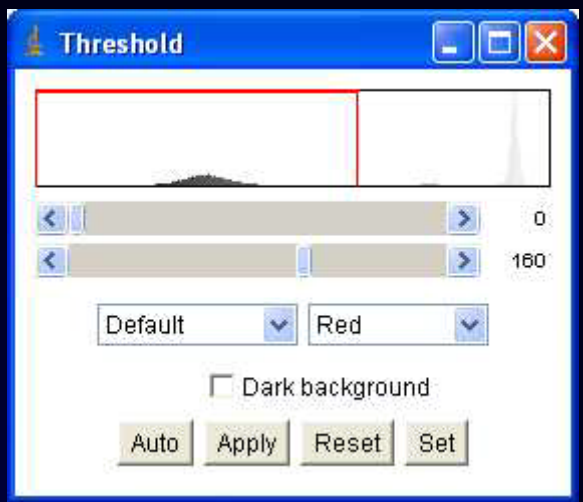
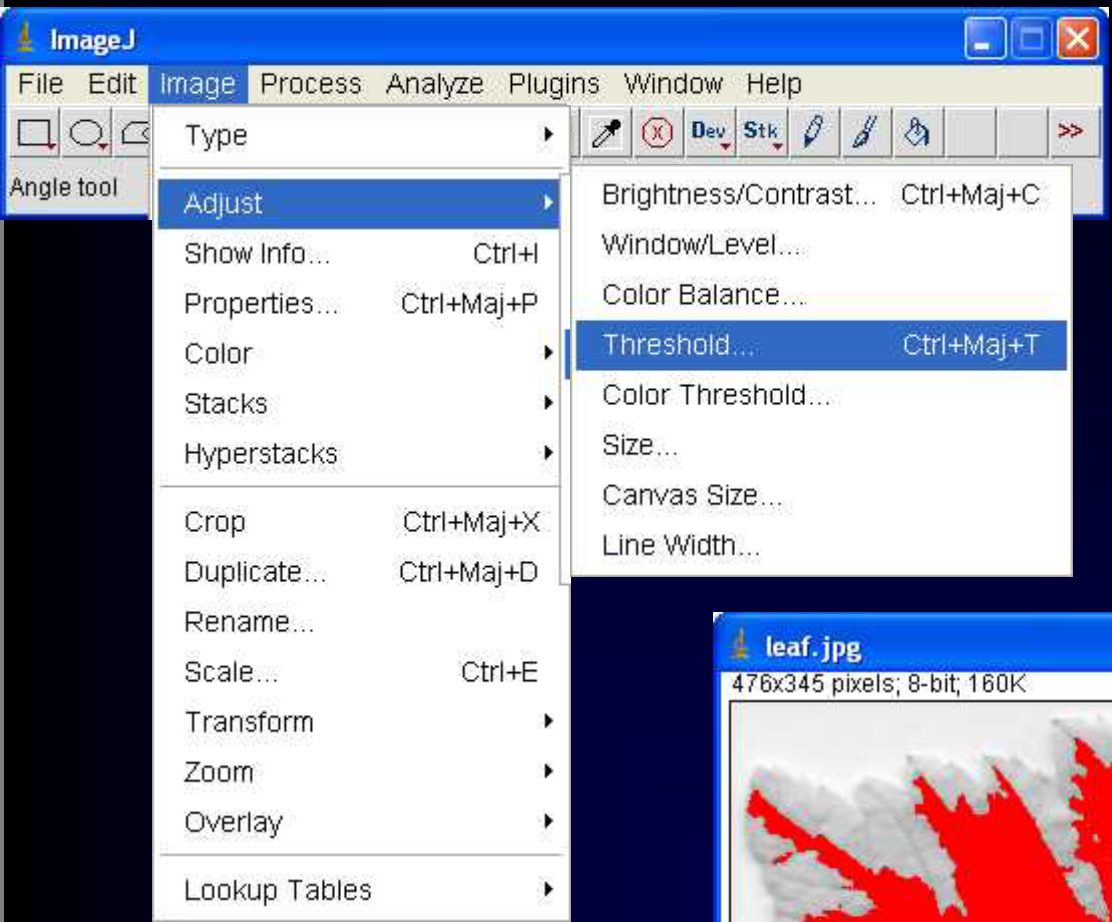
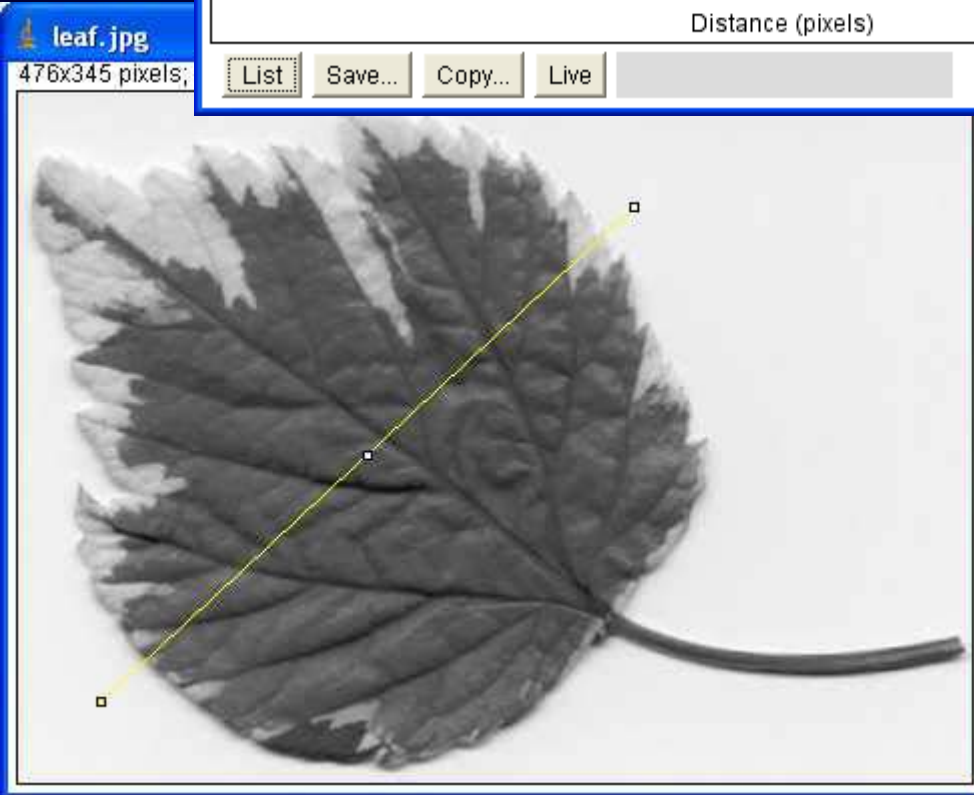
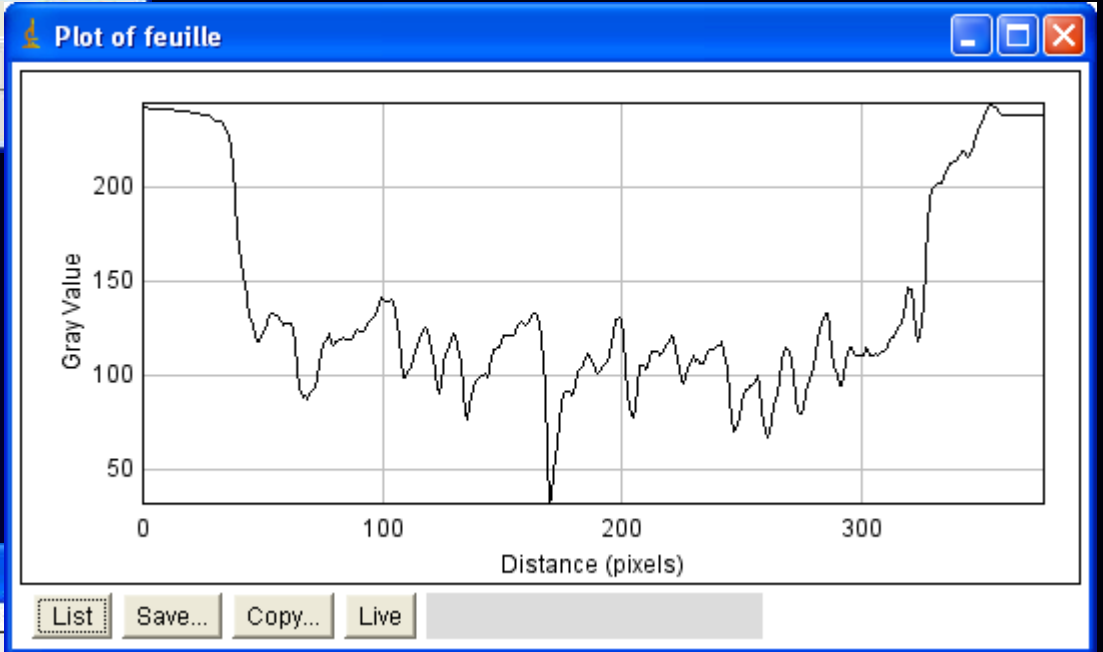


Image → Adjust → Threshold...



# Plot Profile



Analyse → Plot Profile



# Mesures de sélection

ImageJ

File Edit Image Process Analyze Plugins Window Help

Wand (tracing) tool

- Measure Ctrl+M
- Analyze Particles...
- Summarize
- Distribution...
- Label
- Clear Results
- Set Measurements...**
- Set Scale...
- Calibrate...
- Histogram Ctrl+H
- Plot Profile Ctrl+K
- Surface Plot...
- Gels ▶
- Tools ▶

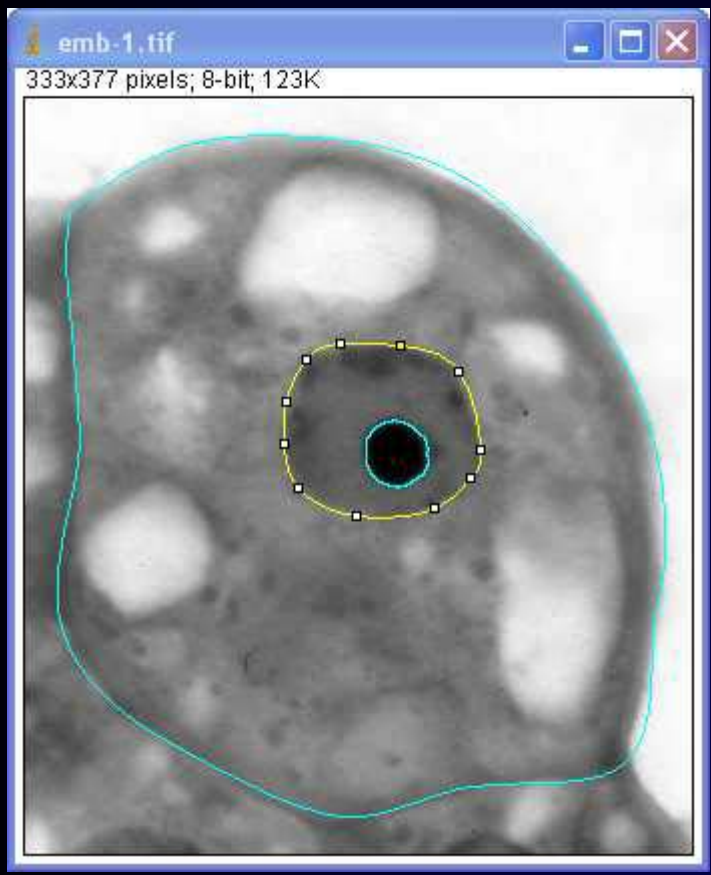
Set Measurements

- Area
- Standard deviation
- Min & max gray value
- Center of mass
- Bounding rectangle
- Shape descriptors
- Integrated density
- Skewness
- Area fraction
- Mean gray value
- Modal gray value
- Centroid
- Perimeter
- Fit ellipse
- Feret's diameter
- Median
- Kurtosis
- Stack position
- Limit to threshold
- Invert Y coordinates
- Add to overlay
- Display label
- Scientific notation

Redirect to:

Decimal places (0-9):

OK Cancel Help



Results

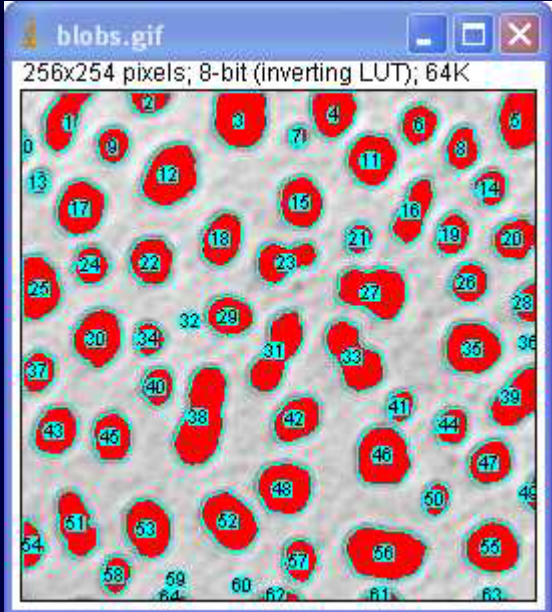
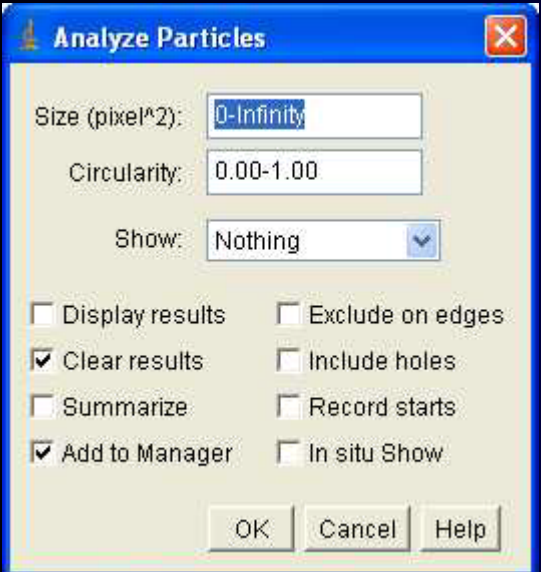
	Area	Mean	Min	Max
1	7154	81.665	0	141

Analyse → Measure





# Mesures d'objets

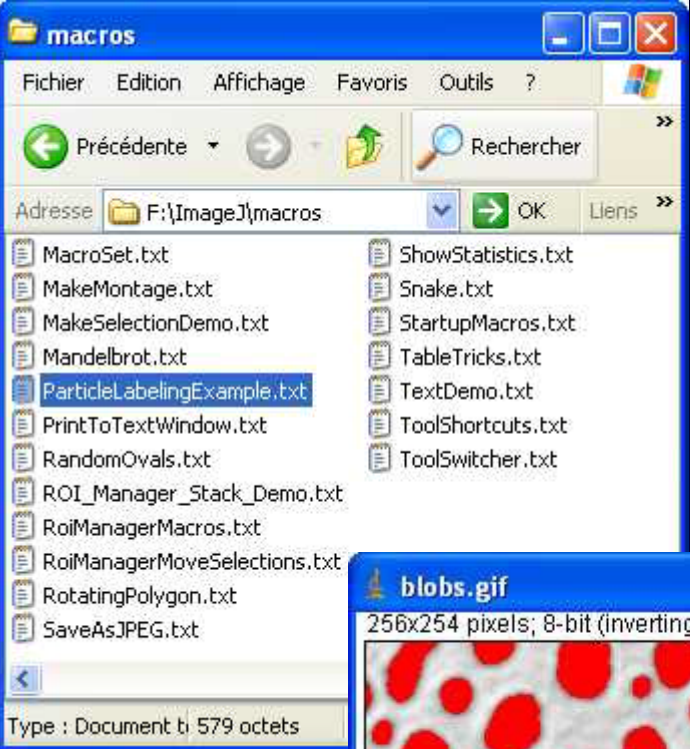


	Area	Mean	Min	Max
1	433	190.855	128	232
2	185	179.286	128	224
3	658	205.617	128	248
4	434	217.327	128	248
5	477	212.143	128	248
6	285	204.295	128	248
7	81	161.481	128	200
8	272	174.848	128	224

Analyse → Analyze Particles...



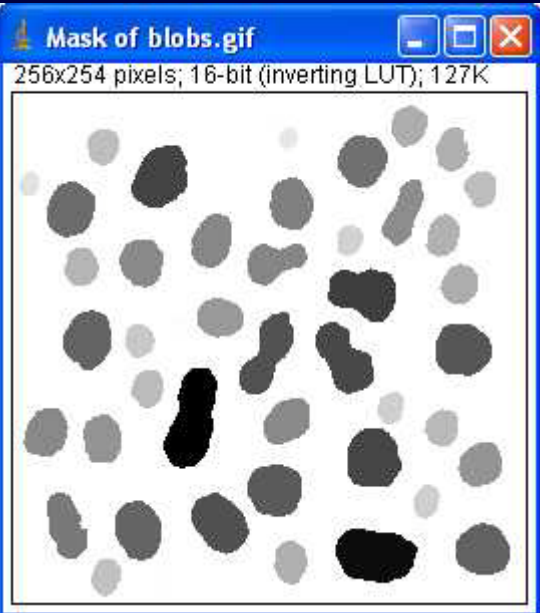
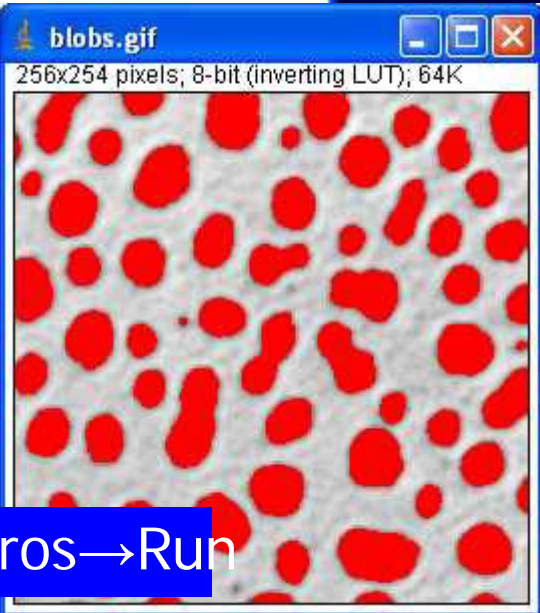
# Macro



```

ParticleLabelingExample.txt
File Edit Font Macros Debug
// This macro labels the blobs from the 'Blobs' test image
// according to their area.

run("Set Measurements...", "area center redirect=None decimal=3");
run("Blobs (25K)");
setThreshold(125, 248);
run("Analyze Particles...",
    "minimum=1 maximum=999999 bins=20 show=Masks display exclude");
selectWindow("Mask of blobs.gif");
run("16-bit");
for (i=0; i<nResults; i++) {
    doWand(getResult("XStart", i), getResult("YStart", i));
    setColor(getResult("Area", i));
    fill();
}
    
```

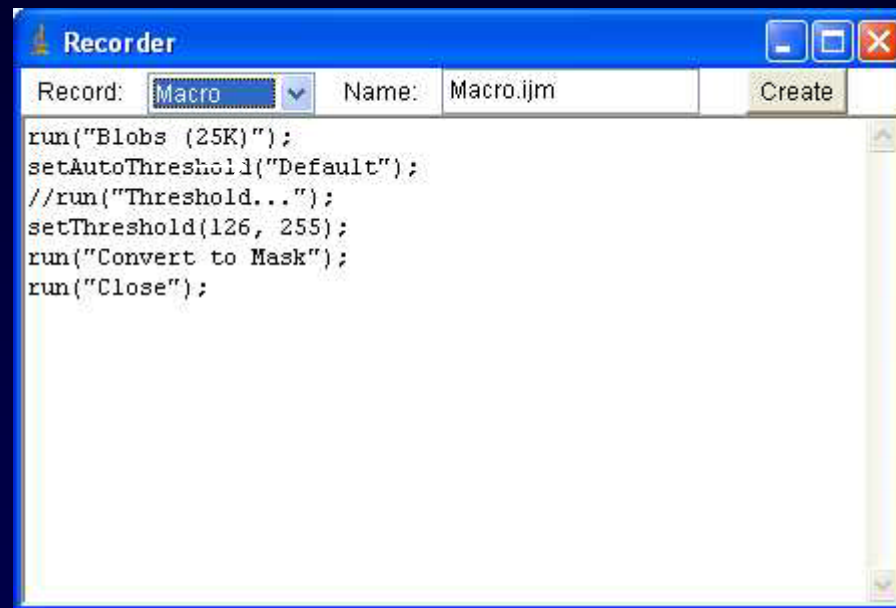


	Area	XM	YM	XStart	YStart
37	170	206.414	203.488	205	195
38	472	26.319	215.160	21	199
39	613	103.332	214.269	99	199
40	543	62.664	217.835	60	203
41	555	234.522	227.816	233	214
42	858	180.452	230.218	168	217
43	281	138.466	233.655	136	223
44	215	46.542	240.989	44	232
45	3	76.827	242.827	76	242
46	1	110.500	246.500	110	246

Plugins → Macros → Run



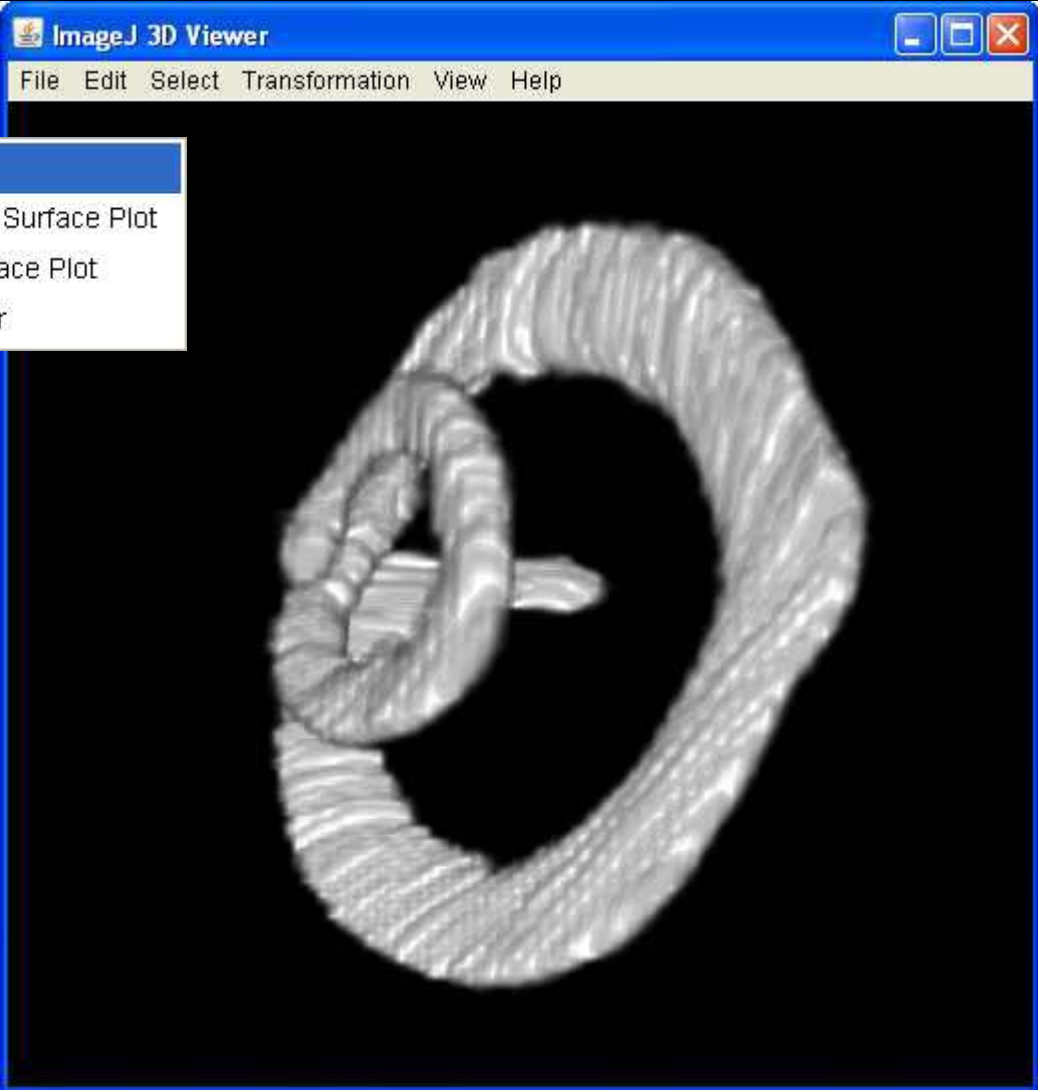
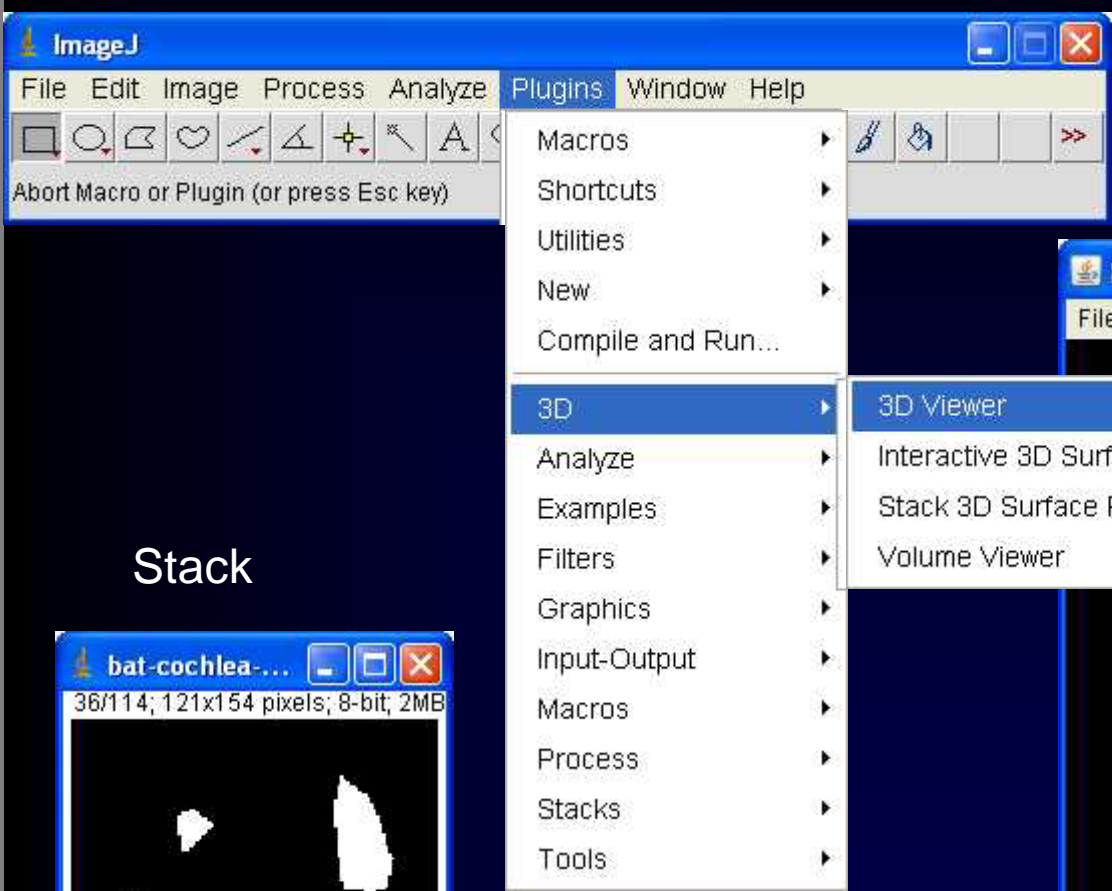
# Enregistrement de macro



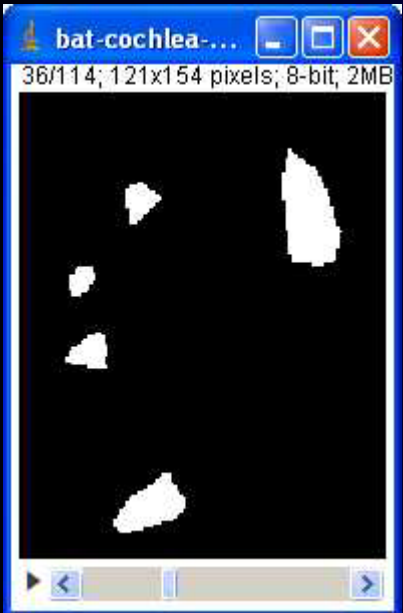
Plugins → Macros → Record...



# Plugins



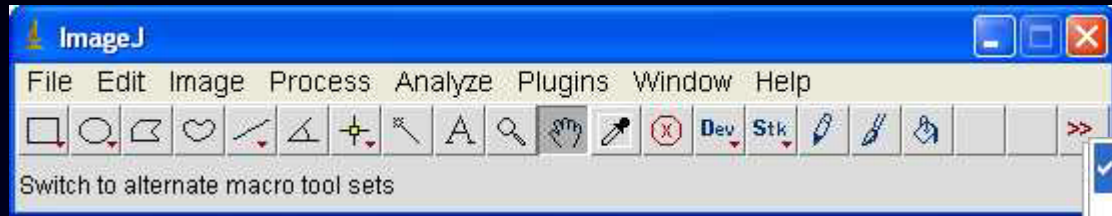
Stack



Plugins → 3D → 3D Viewer



# Barres d'outils

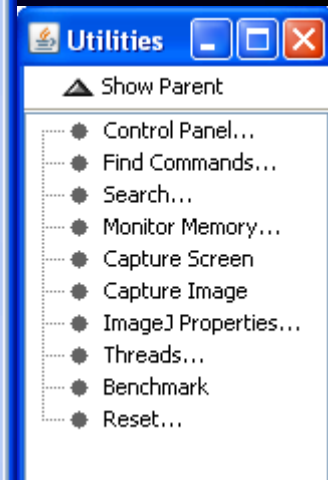
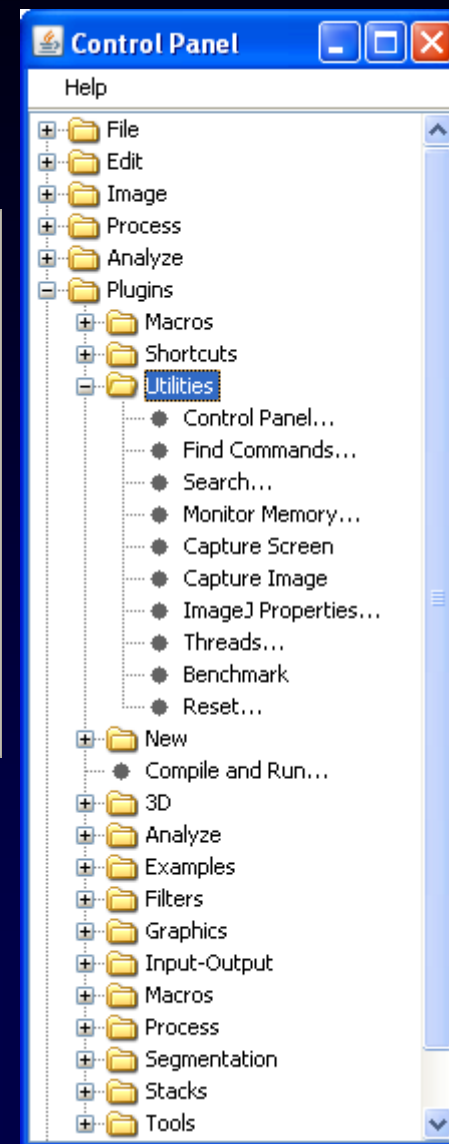
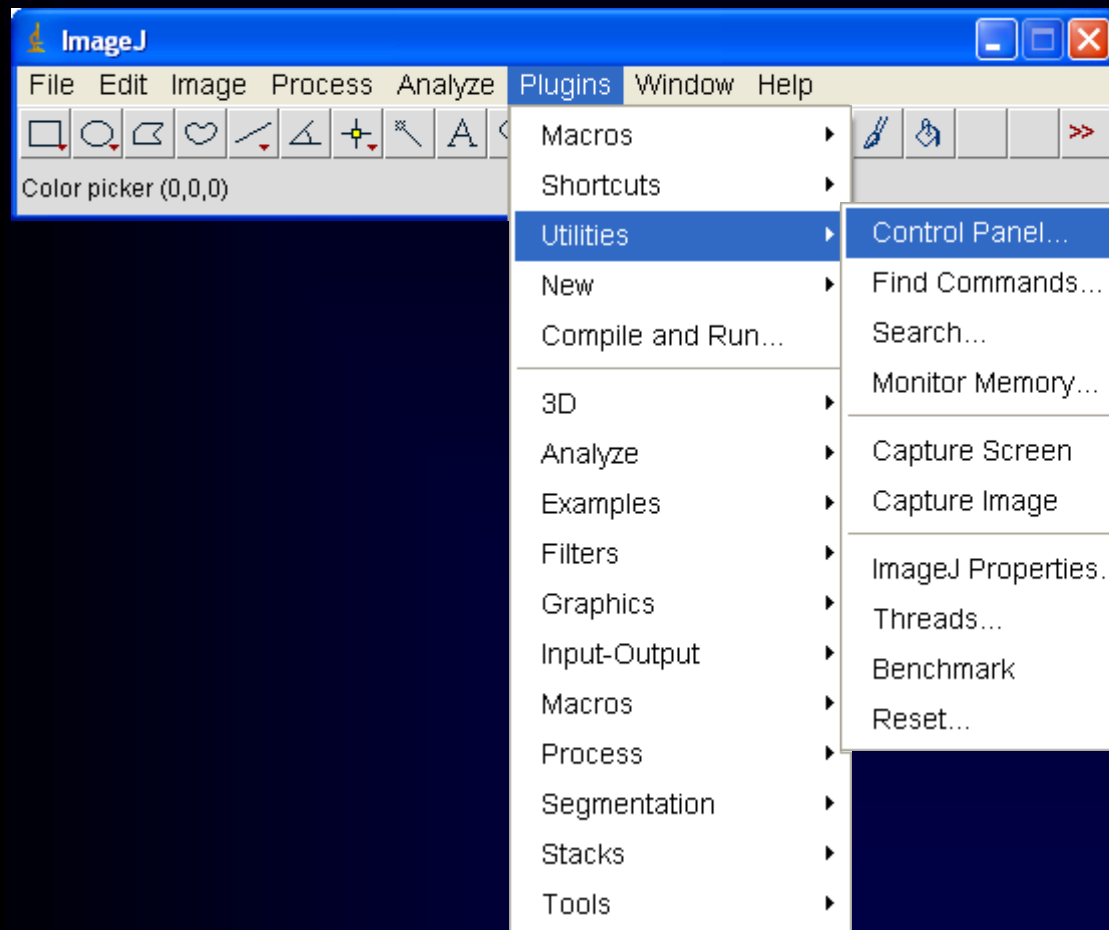


- ✓ Startup Macros
- Arrow Labelling Tools
- Drawing Tools
- Example Icons
- Lookup Tables
- Luts Macros and Tools Updater
- Magic Montage
- Plugins
- Scale Bar Tools for Microscopes
- Stack Tools
- Toolset Creator
- Help...





# Panneaux de commandes



Plugins → Utilities → Control Panel



**Topic 02 – Basic tools 1**

**Topic 03 – Basic tools 2**



# L'image numérique

Les Prétraitements

La Segmentation

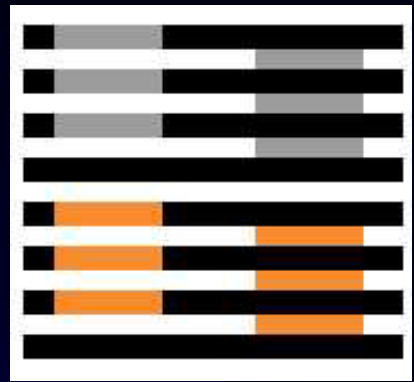
Les Post-traitements

La Quantification

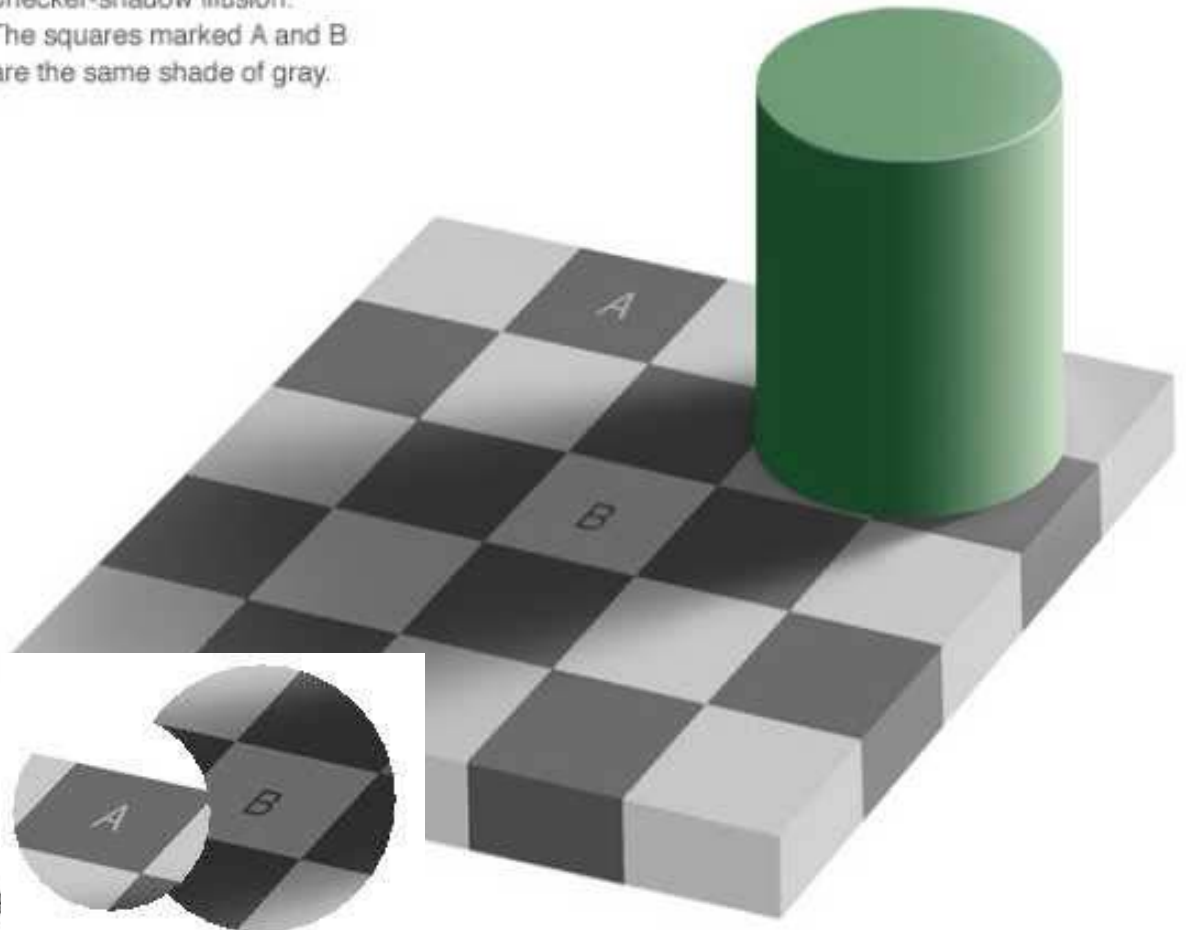




# Image et perception



Checker-shadow illusion:  
The squares marked A and B  
are the same shade of gray.





# Image numérique = tableau de pixels



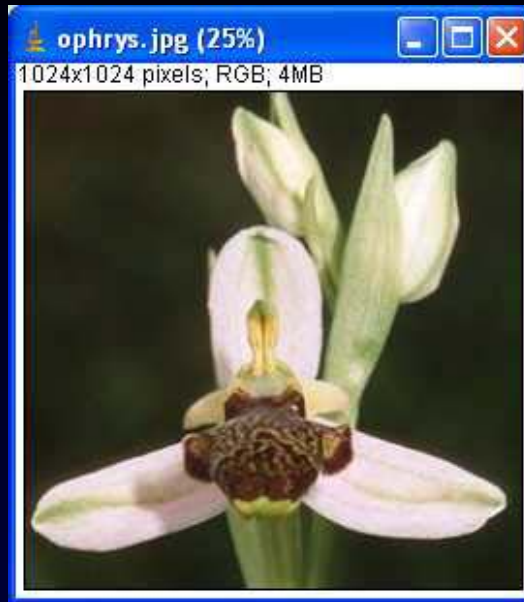
Détail = Affichage \* 6



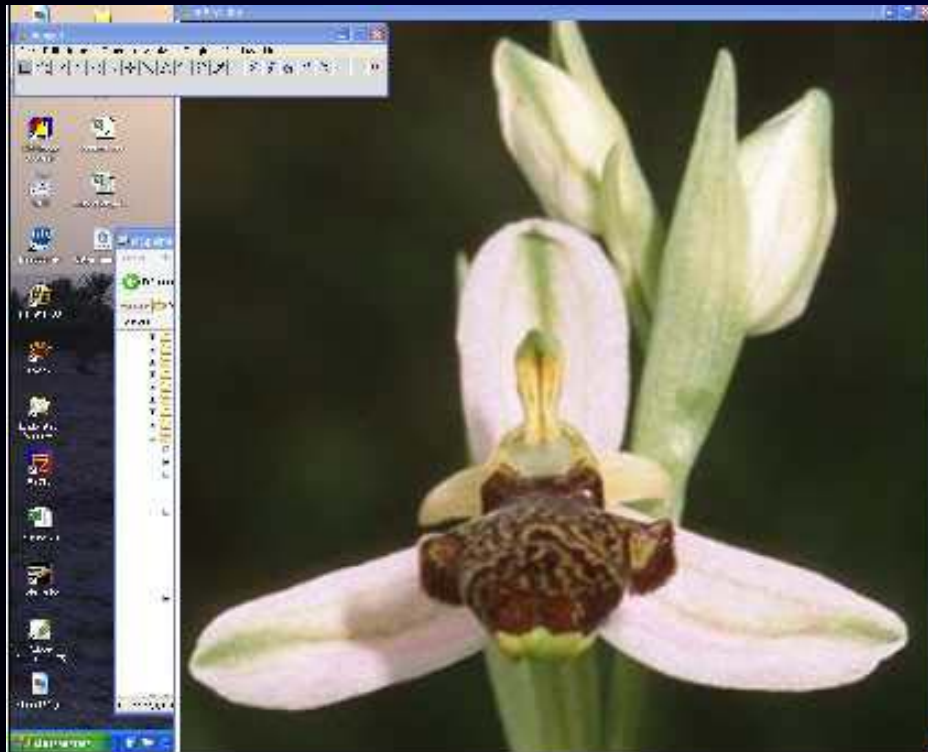
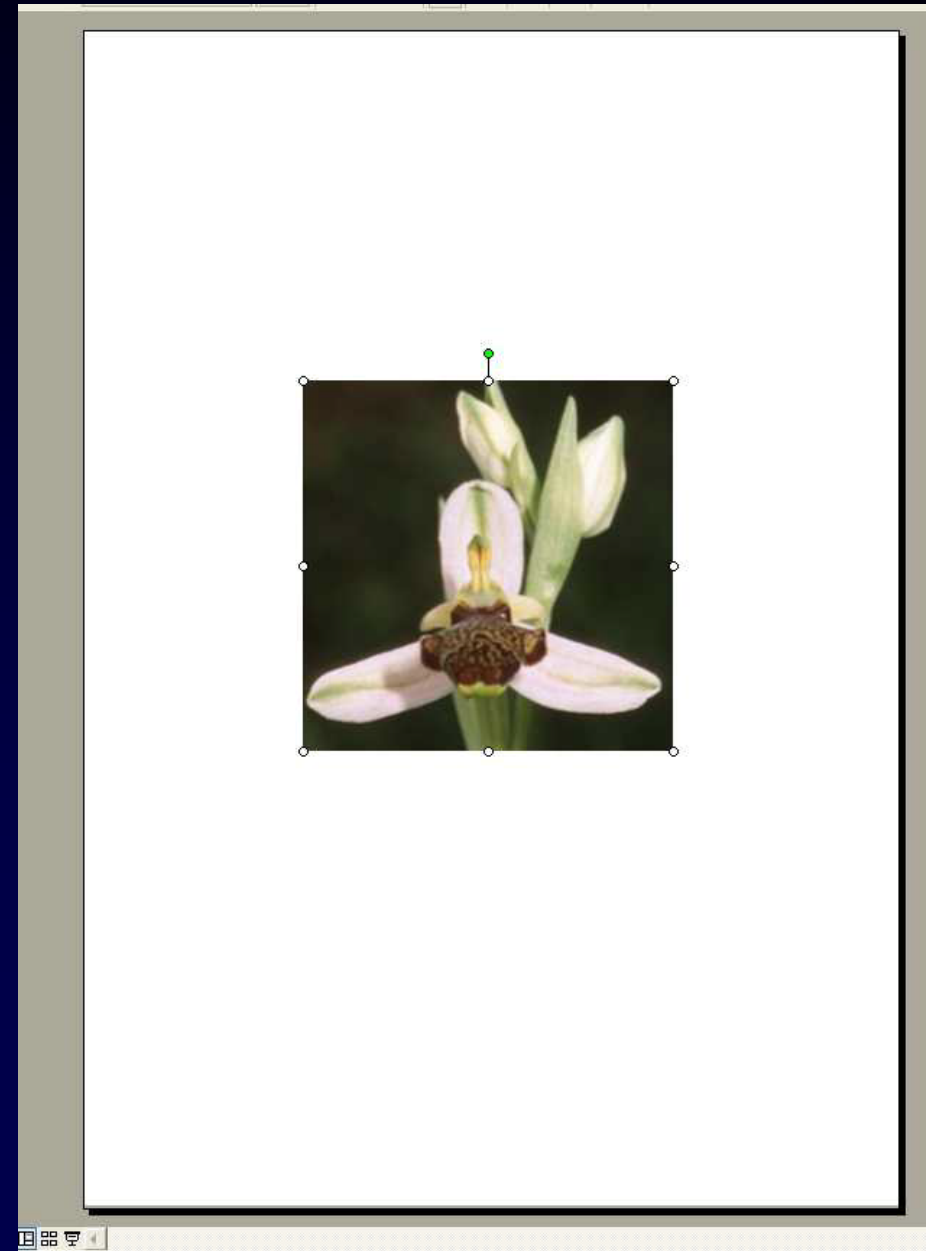
# Résolutions : Nombre de pixels par pouce à l'affichage

1024\*1024  
1 Mega pixels

Ecran 72 dpi

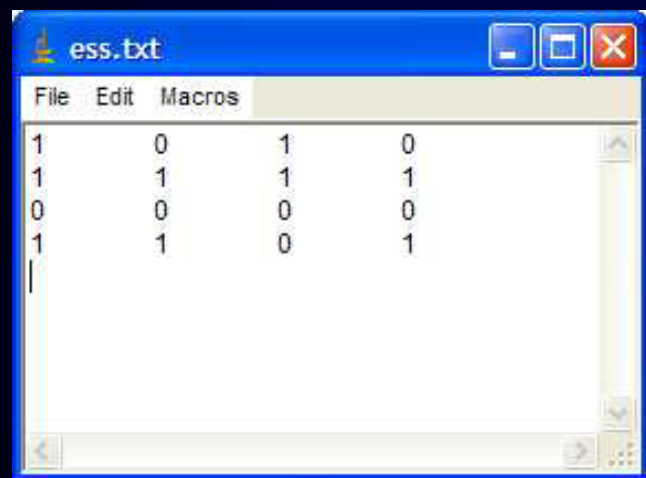
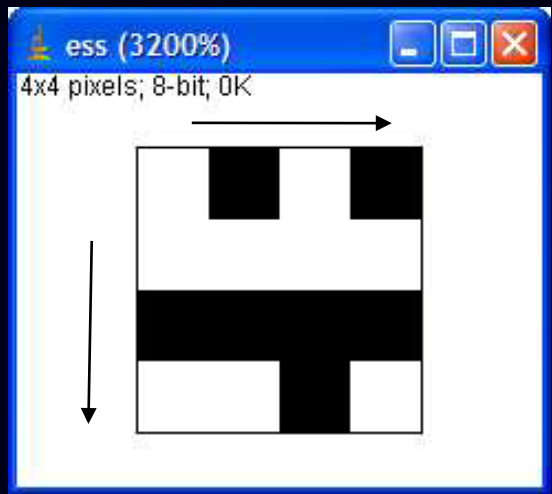


Impression 300 dpi





# Codage binaire



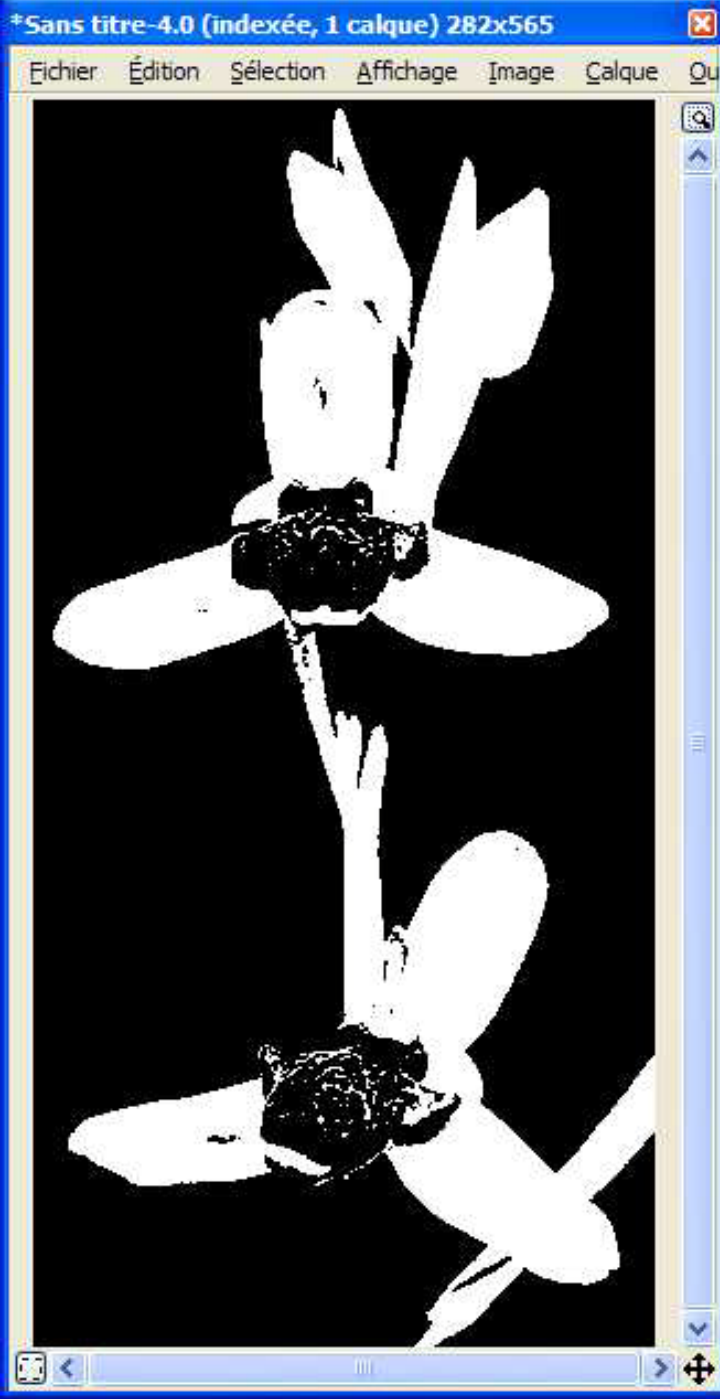


# Codage

1bit  $\rightarrow$  2 valeurs

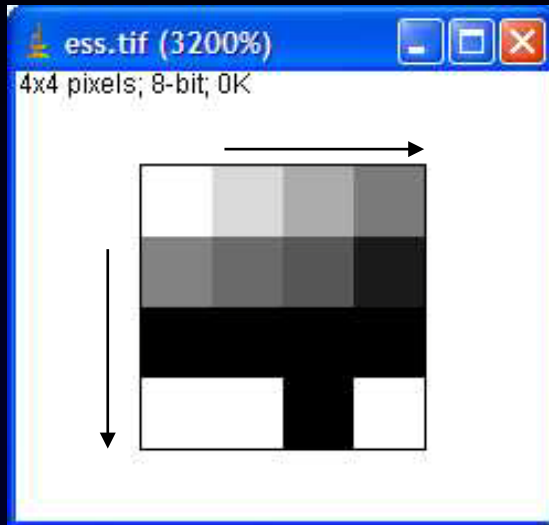
3bits  $\rightarrow$  8 valeurs

5bits  $\rightarrow$  32 valeurs



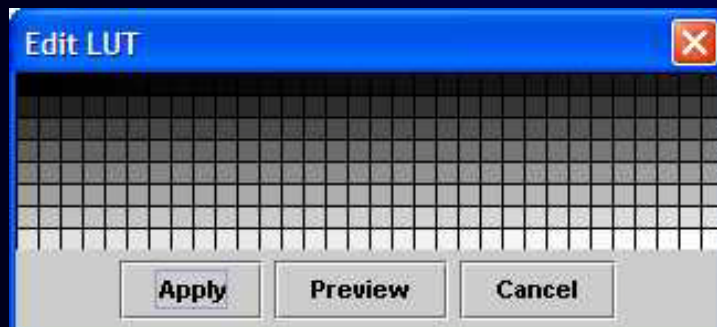


# Codage 8 bits 0→255 Valeurs de Niveaux de gris



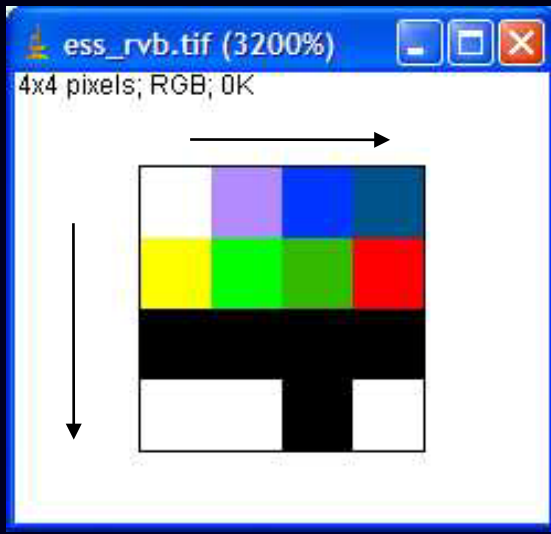
ess\_8.txt

File	Edit	Macros	
255	217	172	123
130	106	87	27
0	0	0	0
255	255	0	255





# Codage couleur 24 bits RVB

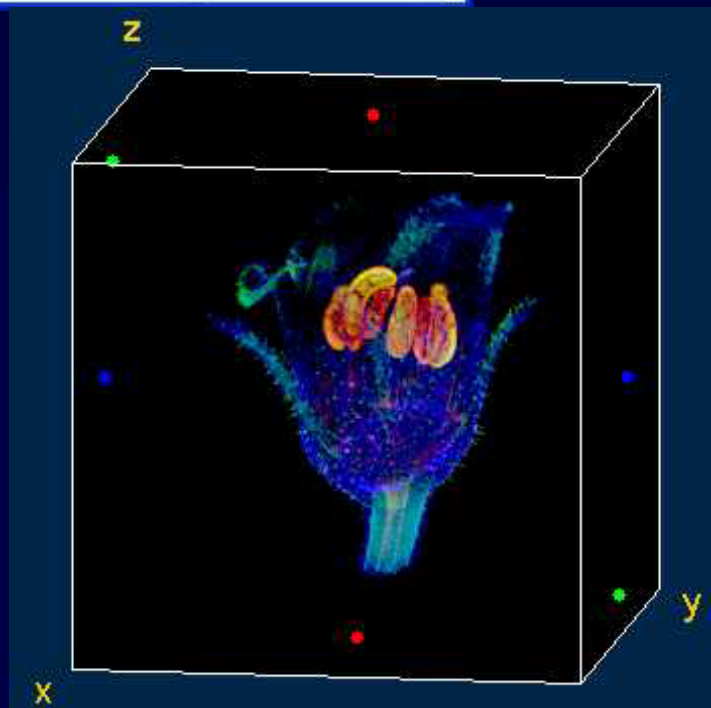
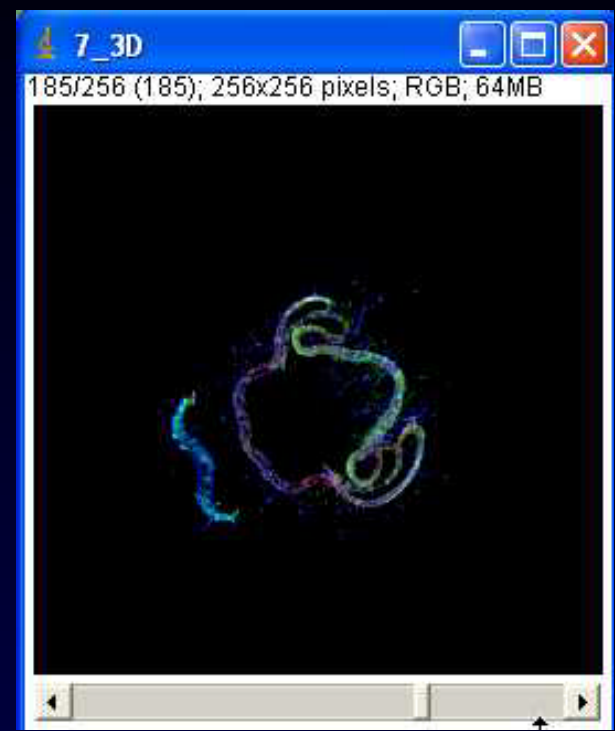
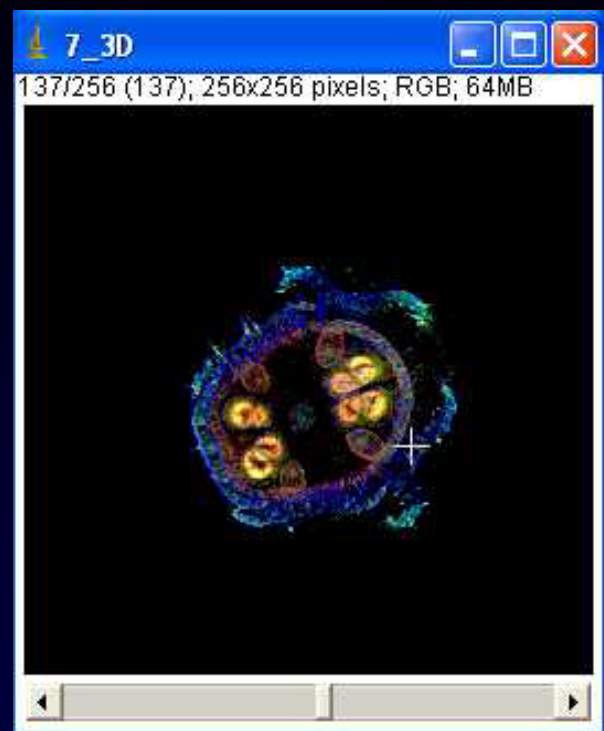


File	Edit	Macros		
255,255,255	180,139,125	0,54,255	0,82,139	
255,255,0	0,255,0	51,185,0	255,0,0	
0,0,0	0,0,0	0,0,0	0,0,0	
255,255,255	255,255,255	0,0,0	255,255,255	





# Piles d'images : Stack







# Format d'image

8bit, 16bit, 32bit, 8bit color

RGB

Stack

HyperStack

# Format de fichier

RAW : image brute

TIFF : sans perte + metadonnées

JPEG : compression avec perte d'information

LSM : format propriétaire de chez Zeiss

OME-TIFF : format Open Microscopy Environment



# Topic 04 – What is a digital image?



L'image numérique

## Les Prétraitements

Amélioration de la visualisation

Filtres et opérations

La Segmentation

Les Post-traitements

La Quantification

ophrys-1.jpg

364x584 pixels; 8-bit; 207K



ophrys-2.jpg

364x584 pixels; 8-bit; 207K



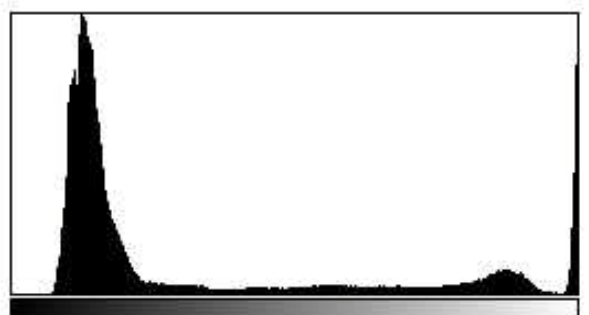
ophrys-2.jpg

364x584 pixels; 8-bit; 207K



Histogram of ophrys-1

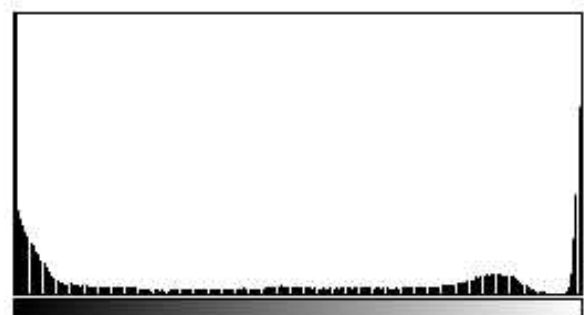
300x240 pixels; 8-bit; 70K



0 256

Histogram of ophrys-2

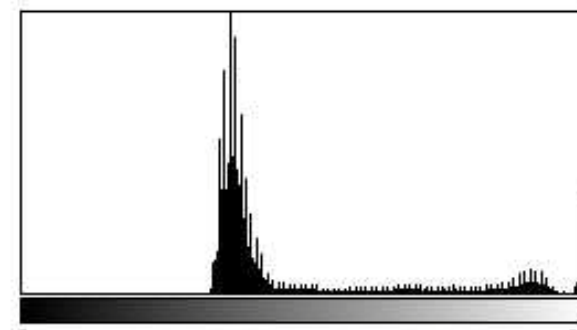
300x240 pixels; 8-bit; 70K



0 256

Histogram of ophrys-2

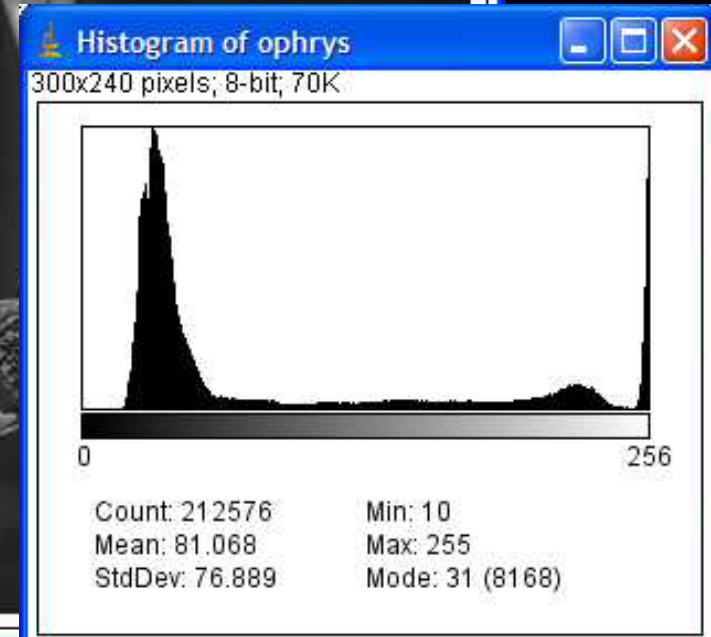
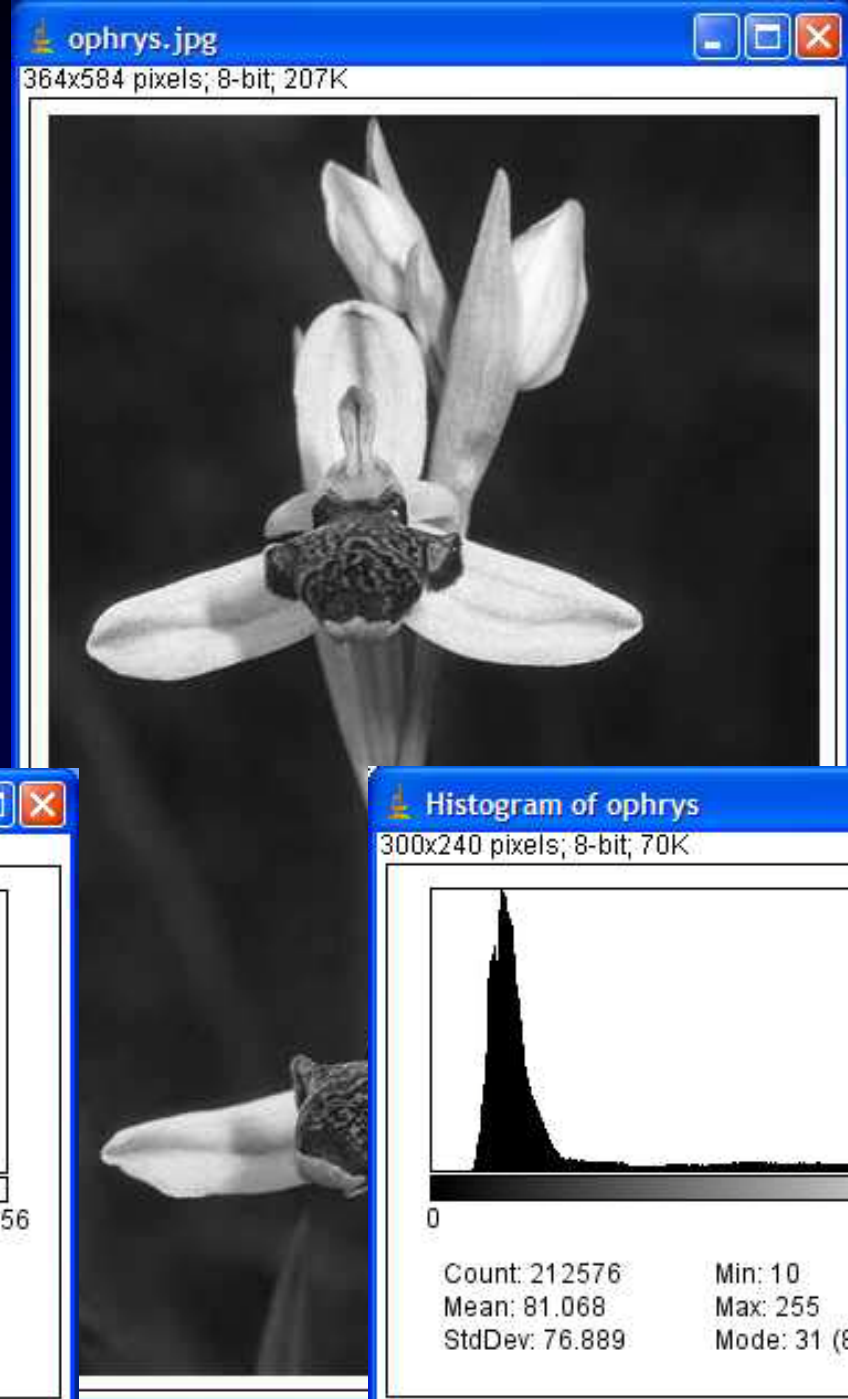
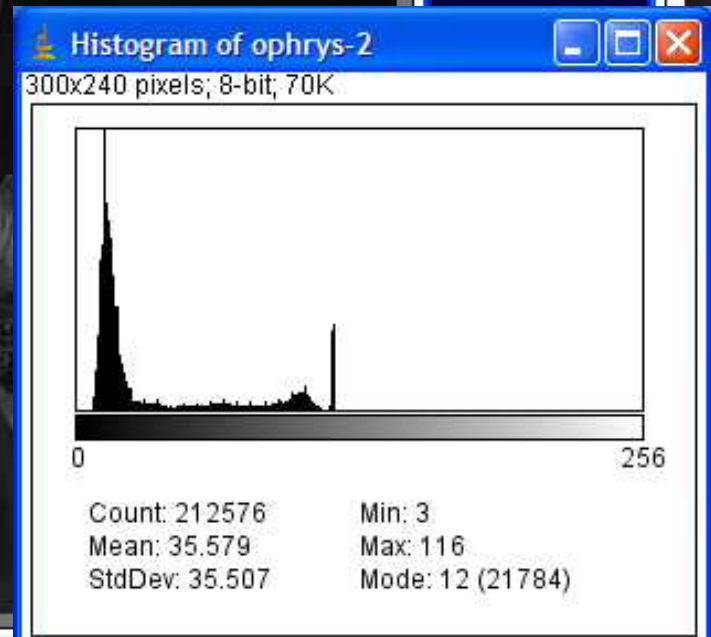
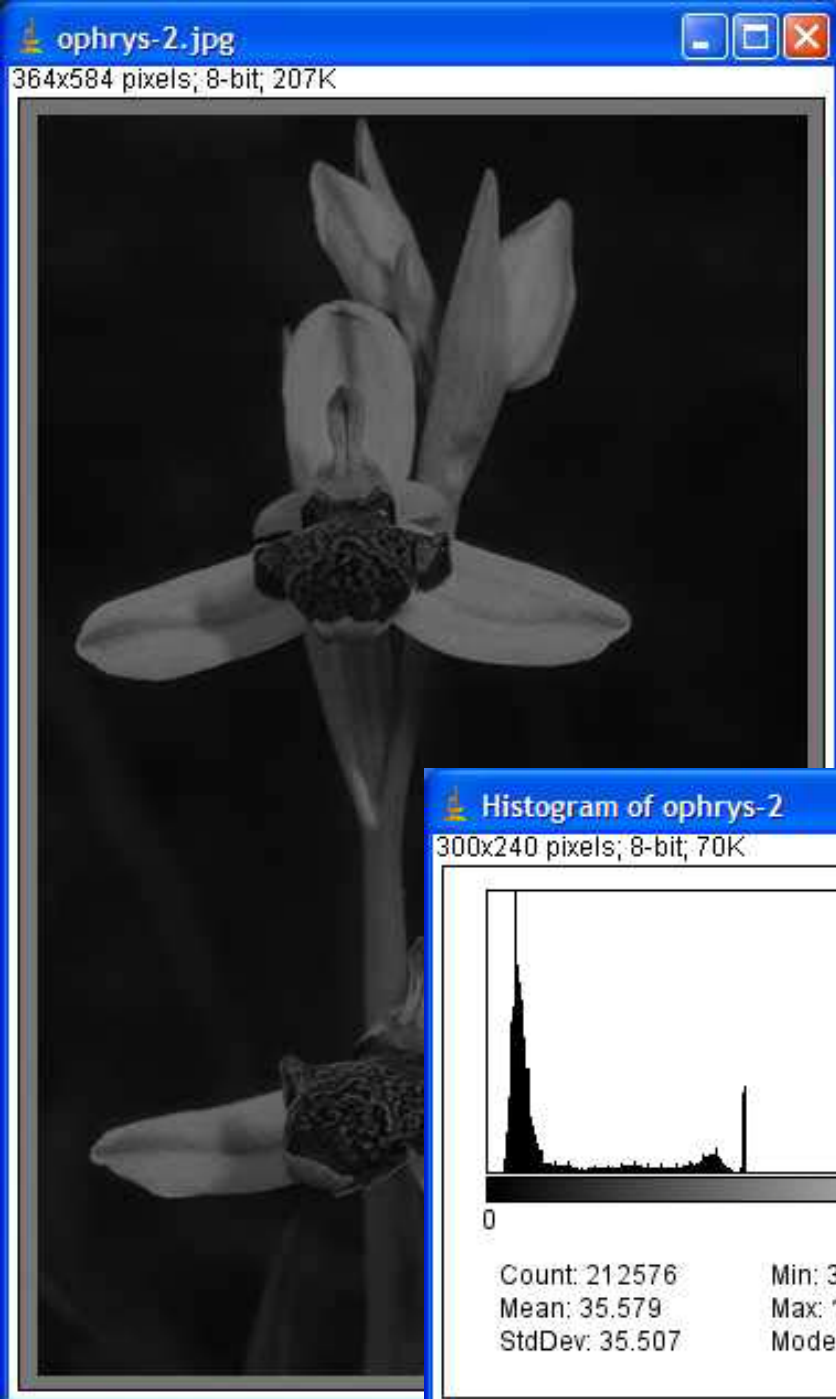
300x240 pixels; 8-bit; 70K



0 256

# Correction de la dynamique de l'image

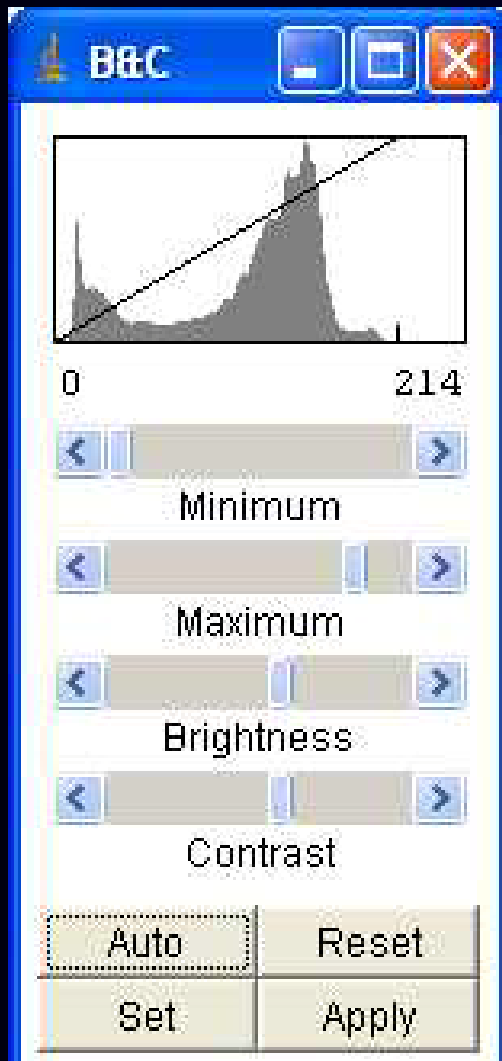
Dynamique = [ valeur\_mini , valeur\_maxi ]





# Corrections linéaires

## Luminosité Contraste



← Mini

← Maxi

← Luminosité →

← Contraste →

## Niveaux

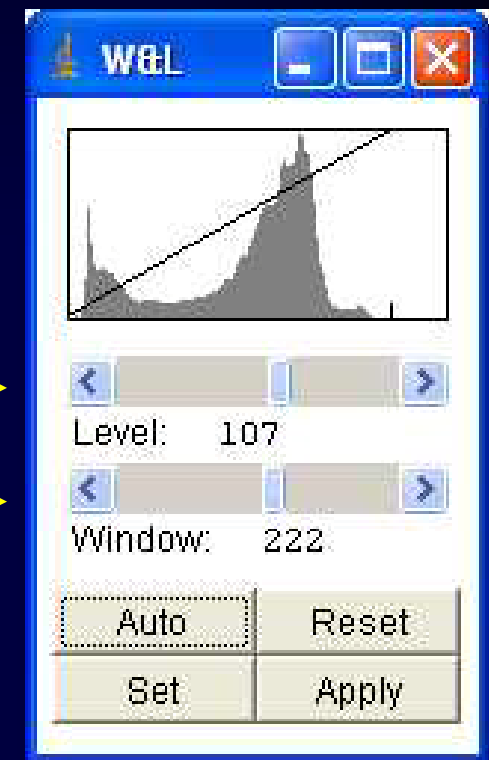


Image → Adjust → Brightness/Contrast...



# Correction non linéaire : Egalisation de l'histogramme

Densité de probabilité normalisée pour aplatir l'histogramme



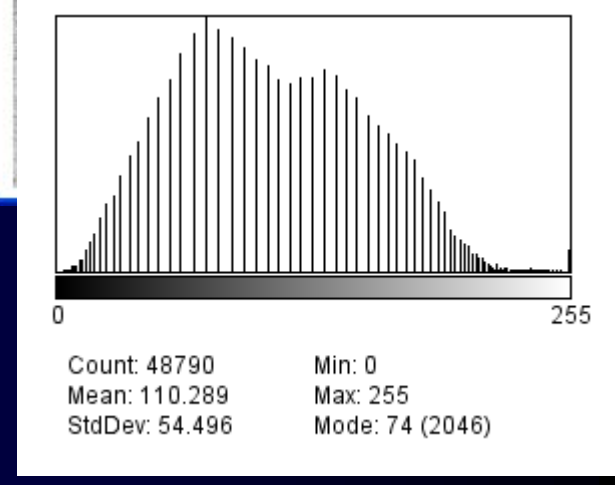
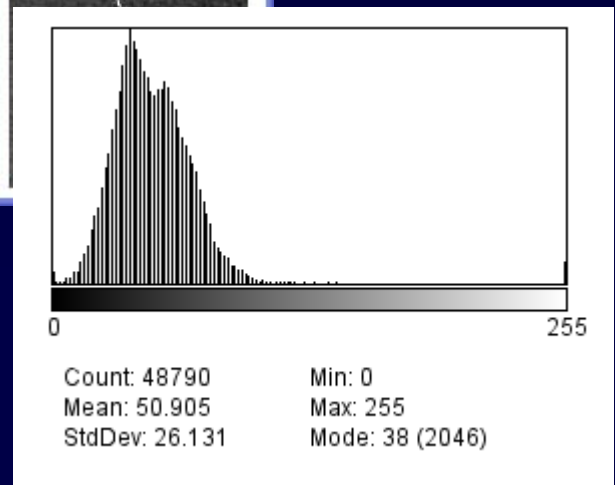
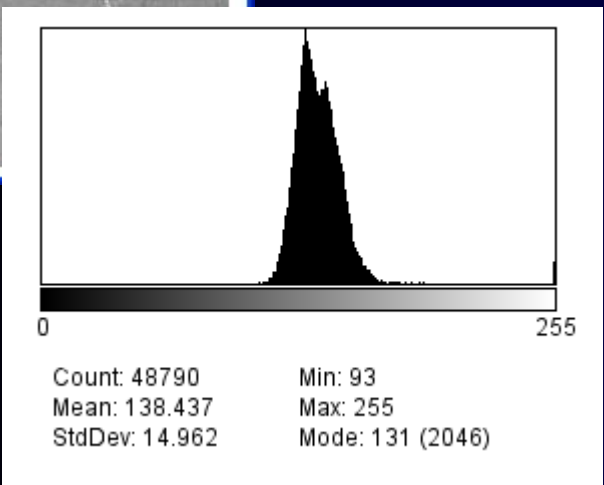
**Enhance Contrast**

Saturated Pixels: 0.4 %

Normalize

Equalize Histogram

OK Cancel



Normalisation

Egalisation

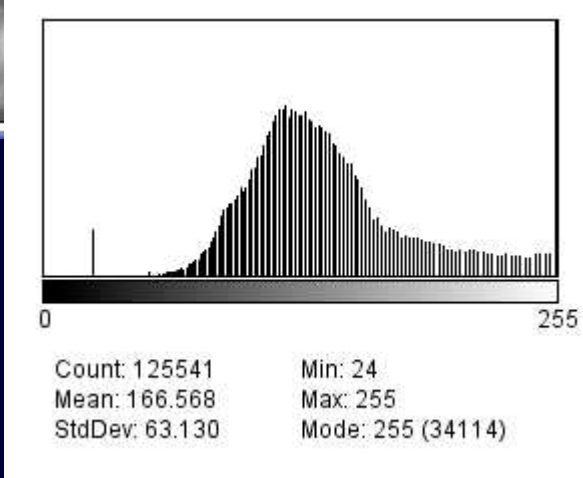
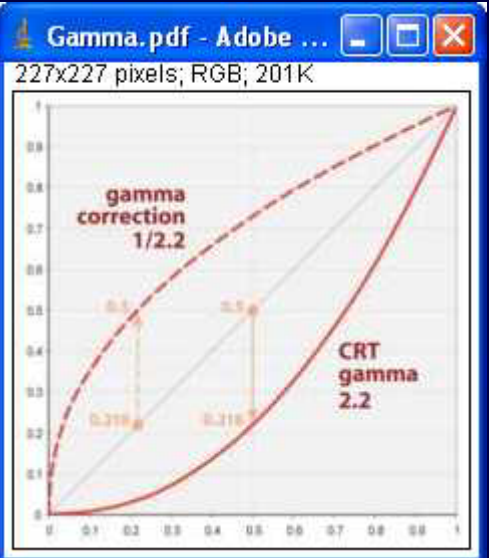
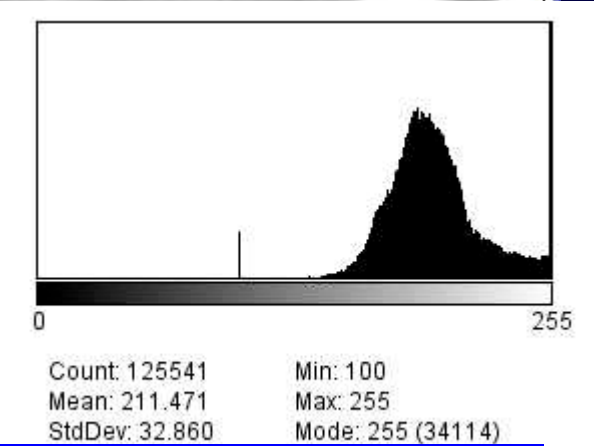
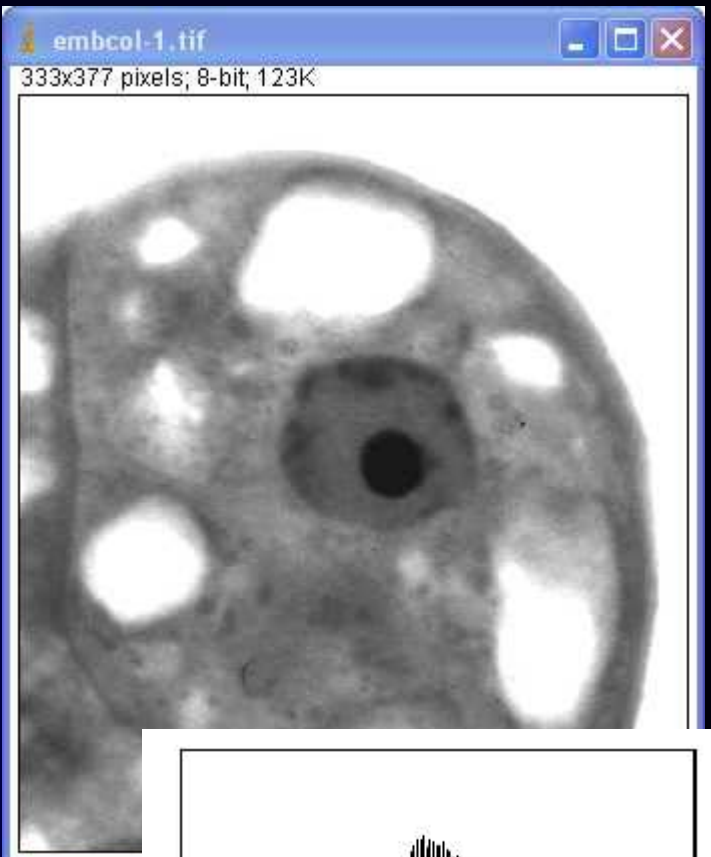
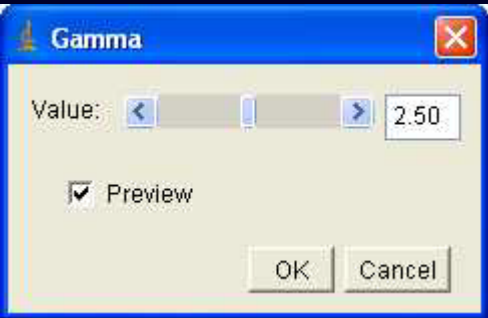
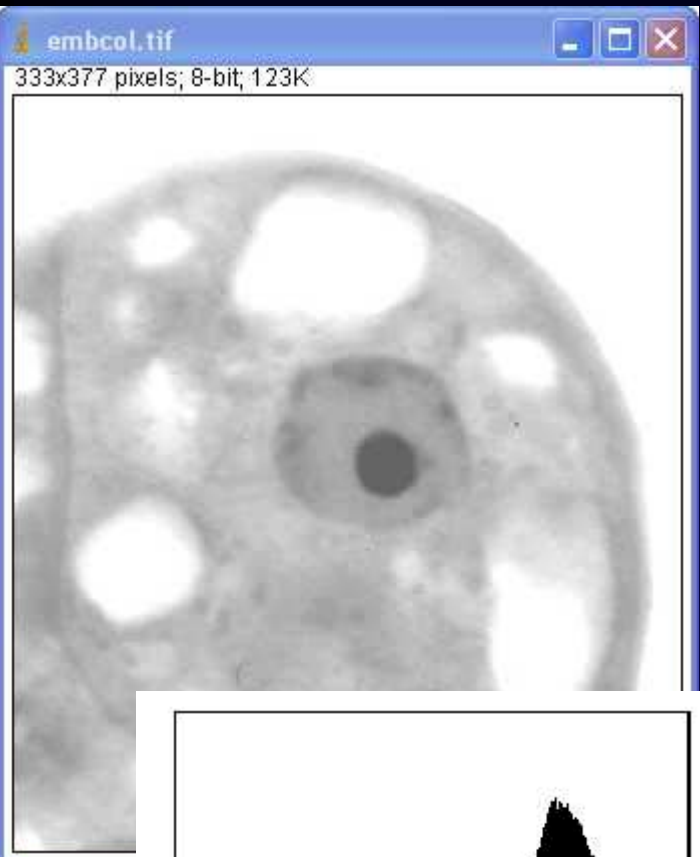
Process → Enhance Contrast



# Correction non linéaire du Gamma

$$y = \text{range} * (x / \text{range}) ^ \text{gamma}$$

range = gamme de valeurs des pixels de l'image



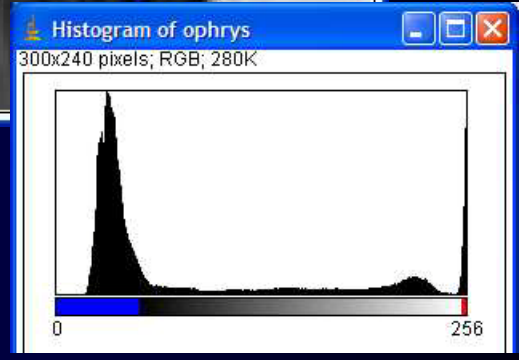
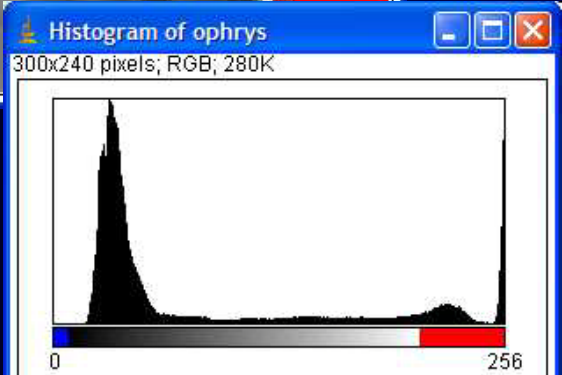
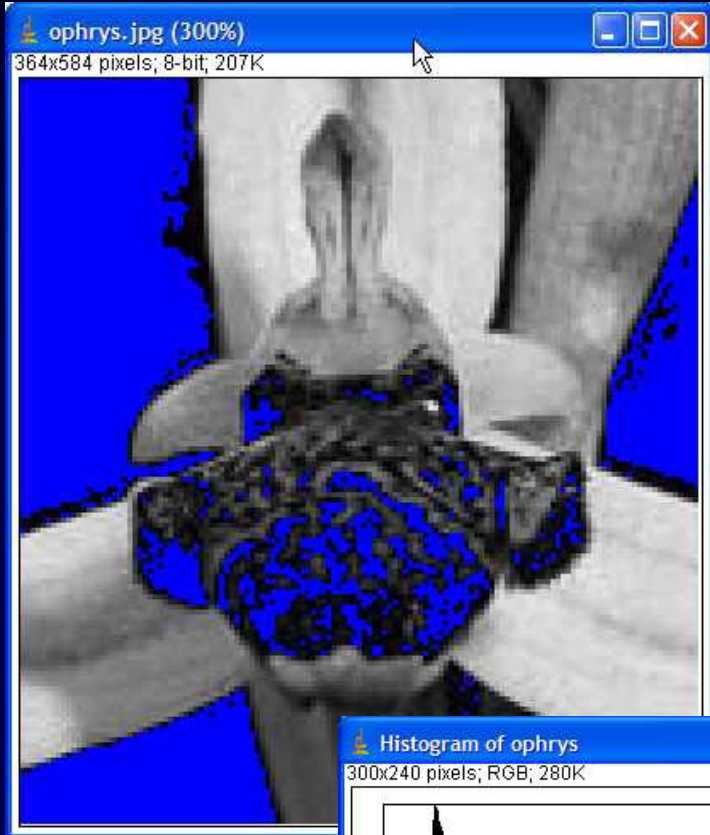
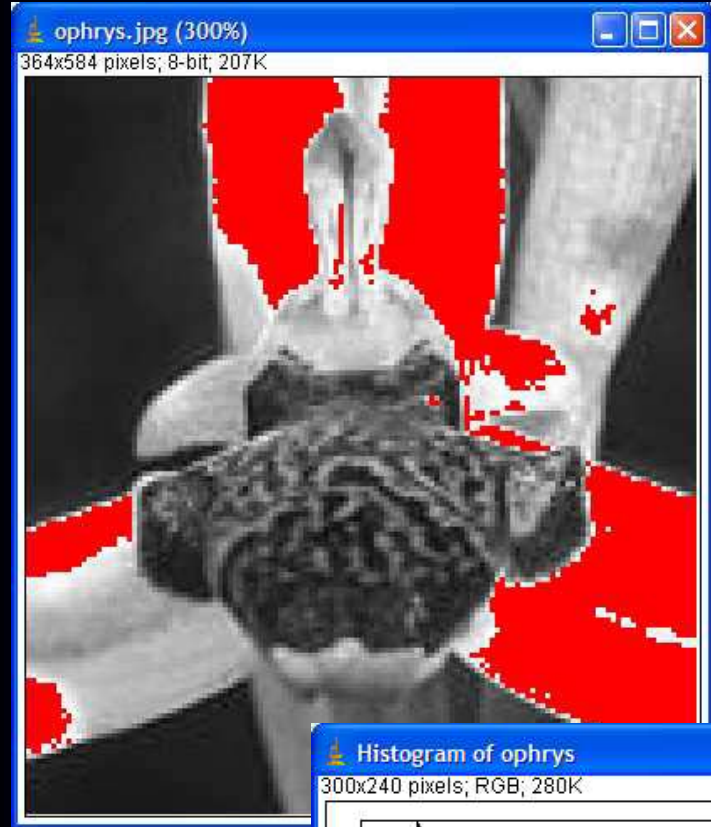
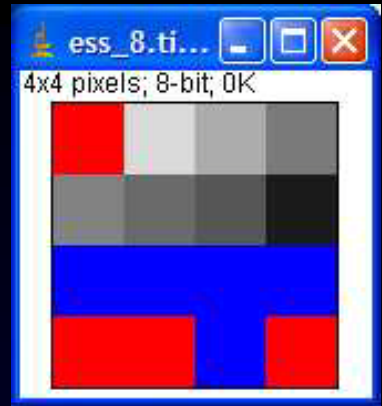
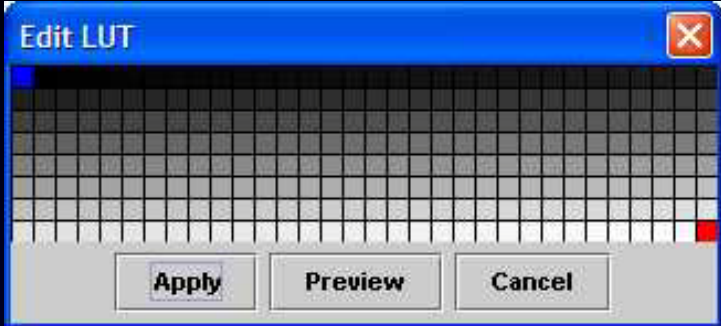
Process → Math → Gamma...







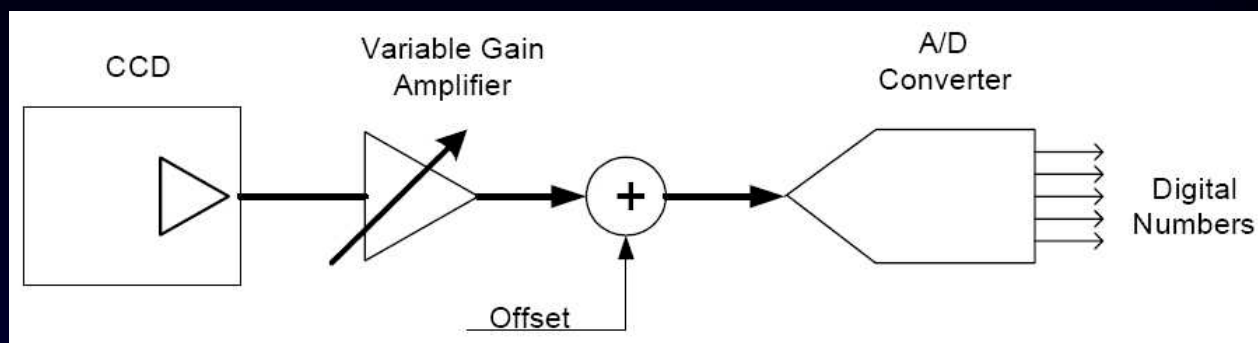
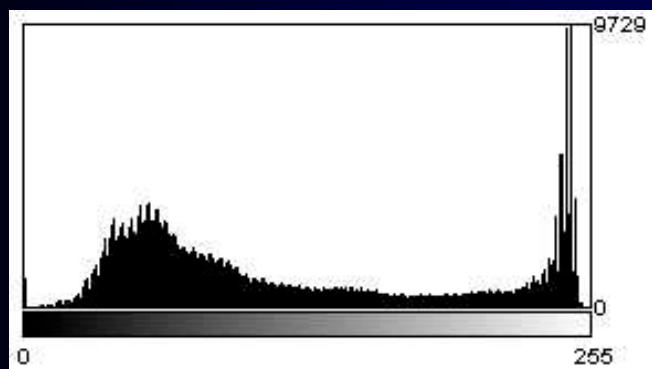
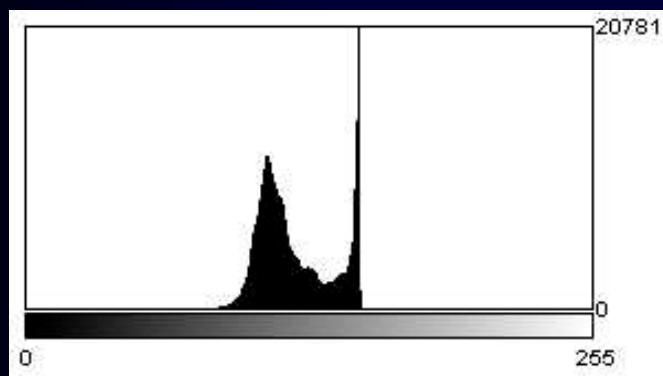
# Réglages avec la LUT Hi Lo



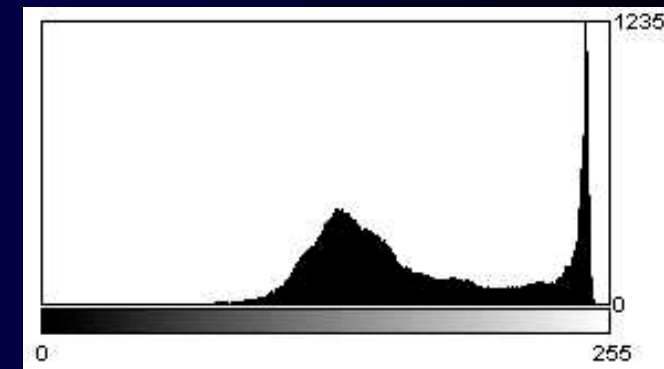
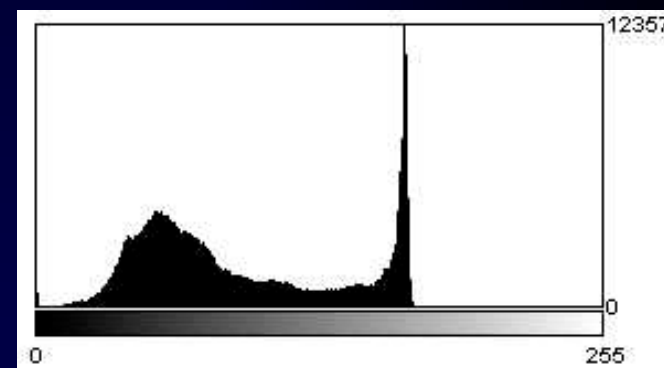


# Gain Offset

Le gain (contraste)  
joue sur la dynamique  
de l'histogramme

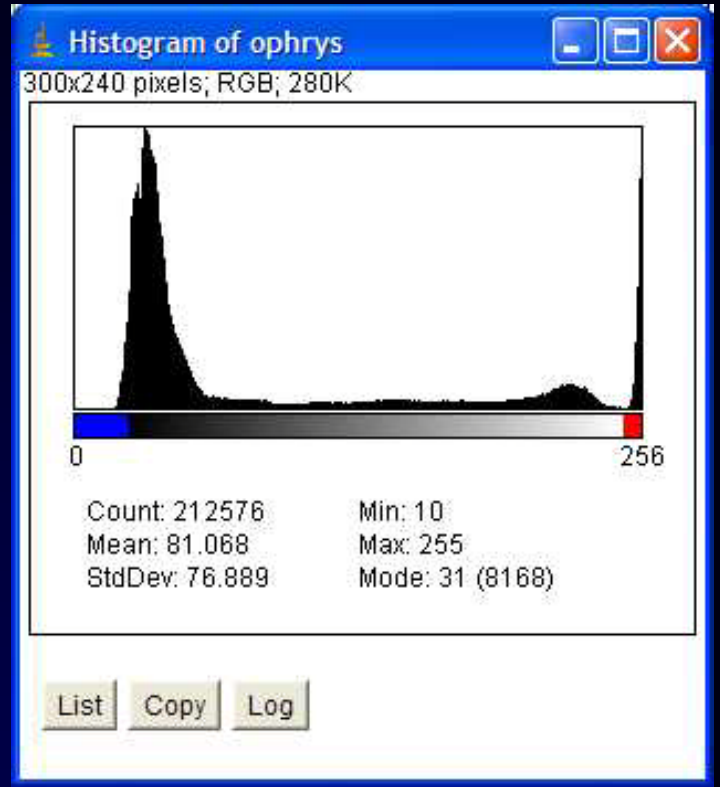
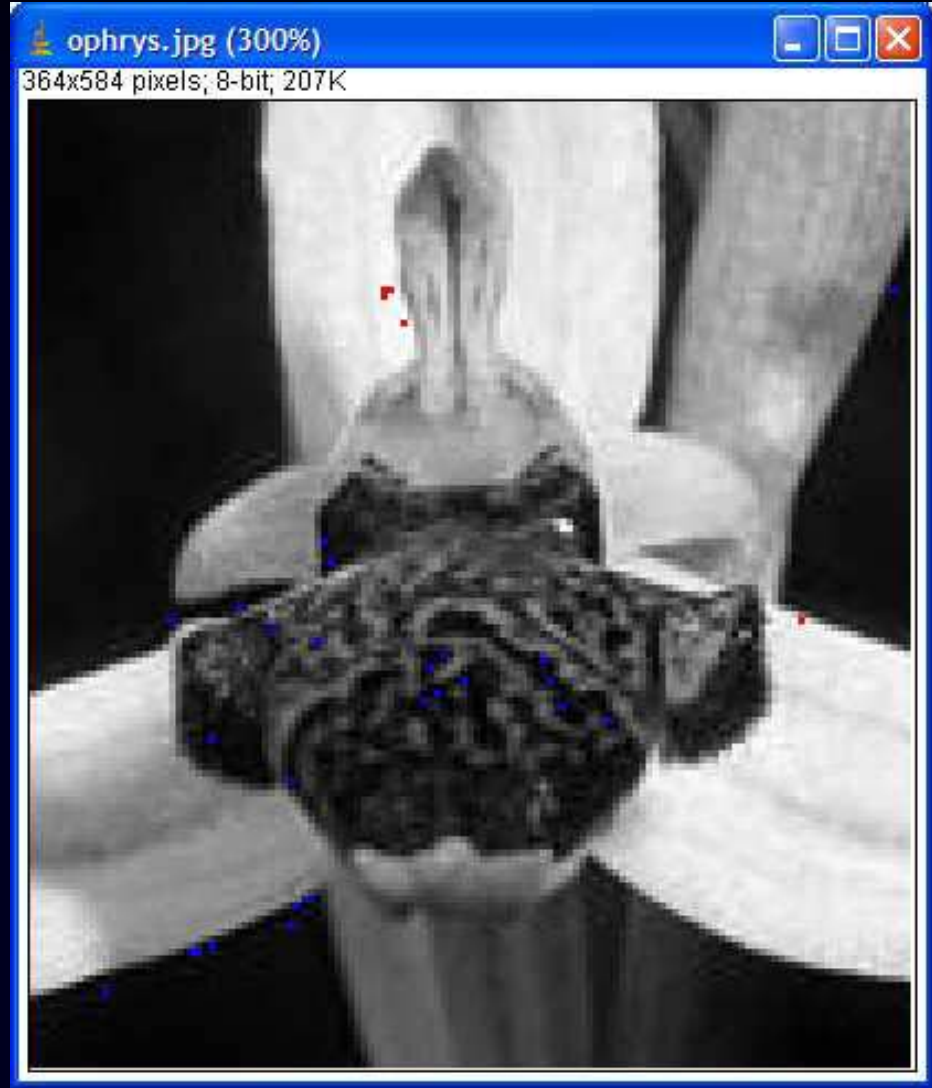


L'offset (luminosité)  
déplace l'histogramme



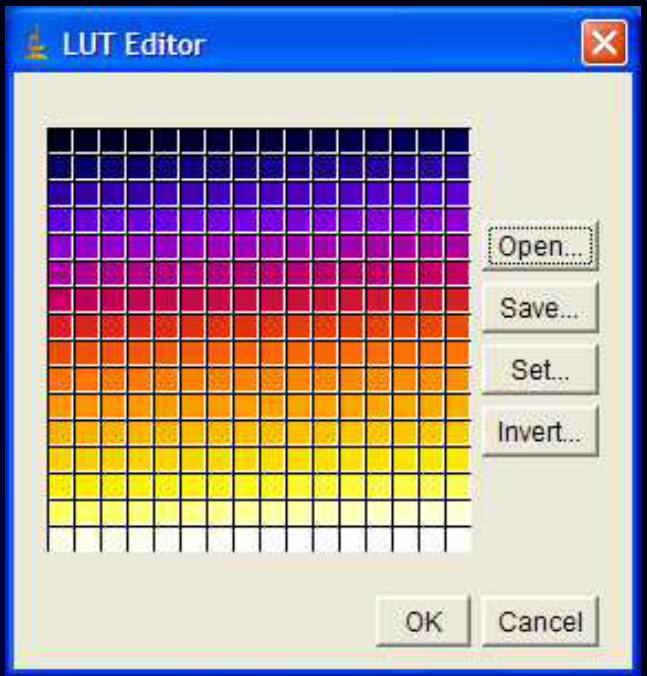
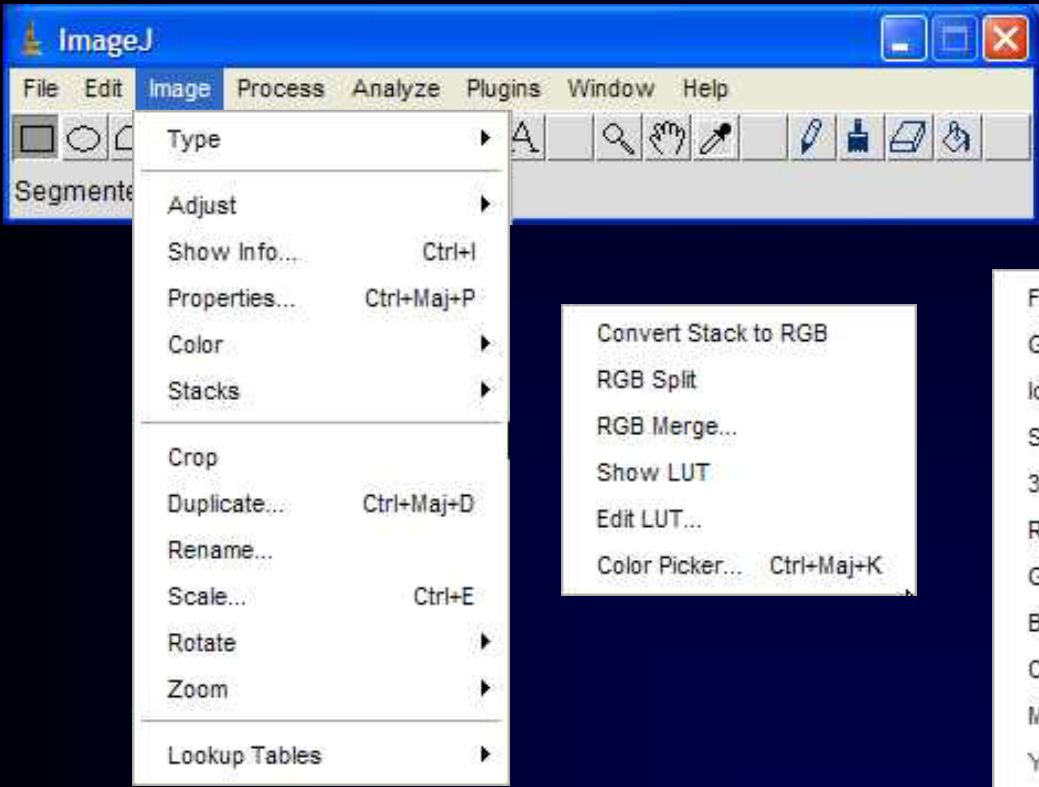


# Réglages avec la LUT Hi Lo





# Outils LUT dans ImageJ





# Réglages d'images composites

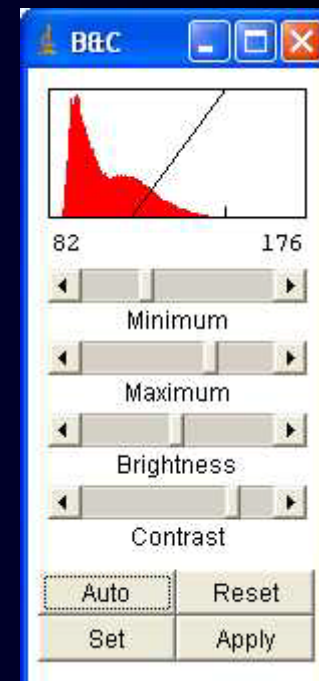
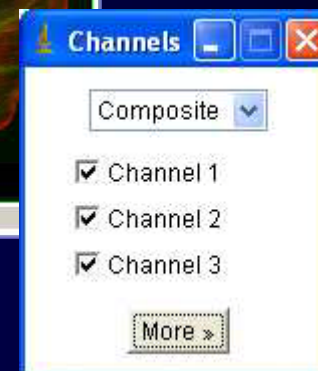
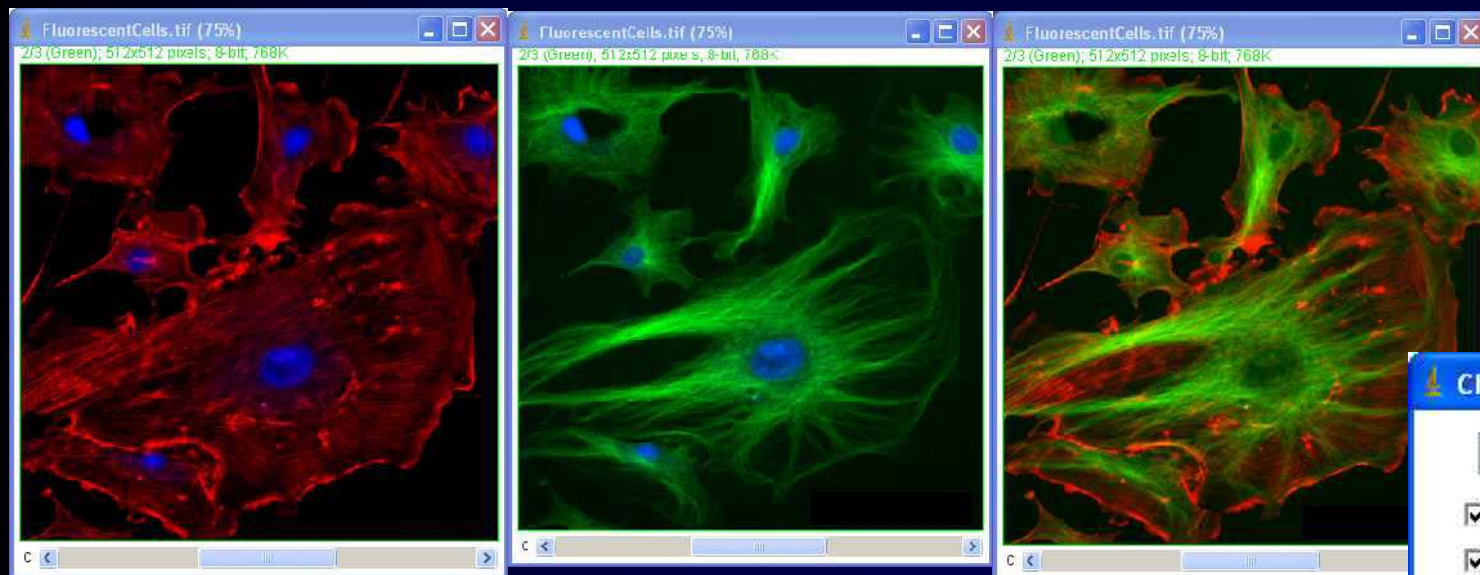
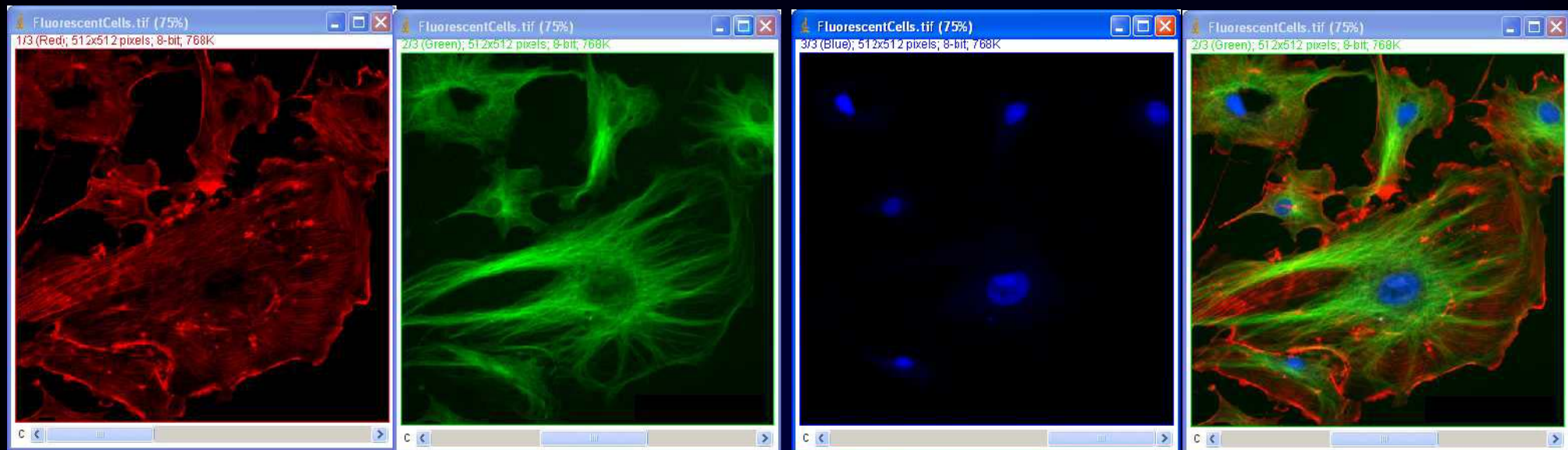


Image → Color → Make Composite



# Topic 05 – Brightness and Contrast Adjustment



L'image numérique

## Les Prétraitements

Amélioration de la visualisation  
Filtres et opérations

La Segmentation

Les Post-traitements

La Quantification





# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

\*

=

Image résultante

100	100	100	100	100
100				100
100				100
100				100
100	100	100	100	100



# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108			100
100				100
100				100
100	100	100	100	100

100*1	100*1	100*1
100*1	100*4	100*1
100*1	100*1	200*1

Somme / 12 = 108



# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108	108		100
100				100
100				100
100	100	100	100	100

100*1	100*1	100*1
100*1	100*4	100*1
100*1	200*1	100*1

Somme / 12 = 108



# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108	108	108	100
100				100
100				100
100	100	100	100	100

100*1	100*1	100*1
100*1	100*4	100*1
200*1	100*1	100*1

Somme / 12 = 108



# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108	108	108	100
100	108			100
100				100
100	100	100	100	100

100*1	100*1	100*1
100*1	100*4	200*1
100*1	100*1	100*1

Somme / 12 = 108



# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108	108	108	100
100	108	133		100
100				100
100	100	100	100	100

100*1	100*1	100*1
100*1	200*4	100*1
100*1	100*1	100*1

Somme / 12 = 133



# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108	108	108	100
100	108	133	108	100
100				100
100	100	100	100	100

100*1	100*1	100*1
200*1	100*4	100*1
100*1	100*1	100*1

Somme / 12 = 108



# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108	108	108	100
100	108	133	108	100
100	108			100
100	100	100	100	100

100*1	100*1	200*1
100*1	100*4	100*1
100*1	100*1	100*1

Somme / 12 = 108





# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108	108	108	100
100	108	133	108	100
100	108	108		100
100	100	100	100	100

100*1	200*1	100*1
100*1	100*4	100*1
100*1	100*1	100*1

Somme / 12 = 108



# Filtres de convolution

Image source

100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

1	1	1
1	4	1
1	1	1

Image résultante

100	100	100	100	100
100	108	108	108	100
100	108	133	108	100
100	108	108	108	100
100	100	100	100	100

200*1	100*1	100*1
100*1	100*4	100*1
100*1	100*1	100*1

Somme / 12 = 108



# Filtres de convolution

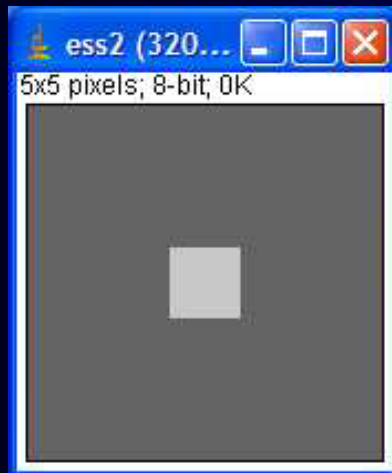
100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

\*

1	1	1
1	4	1
1	1	1

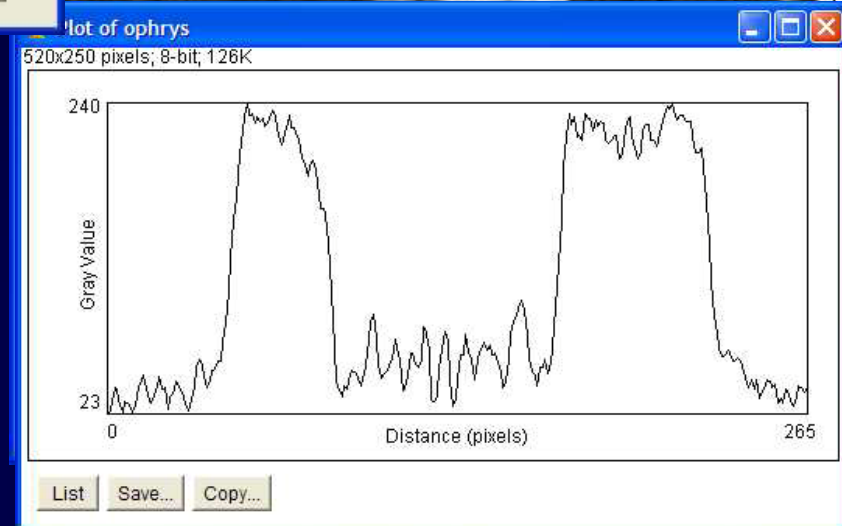
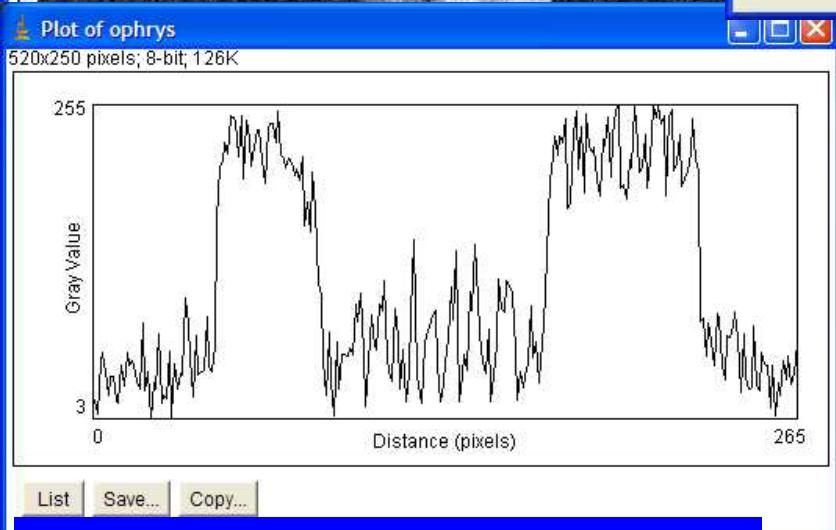
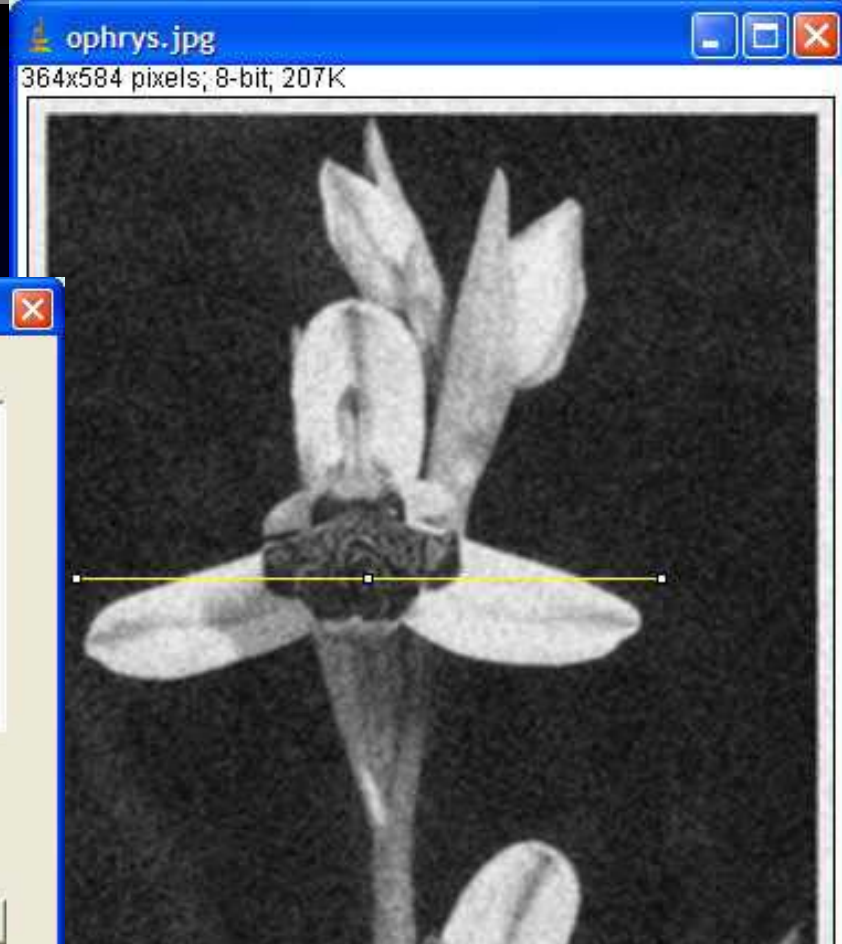
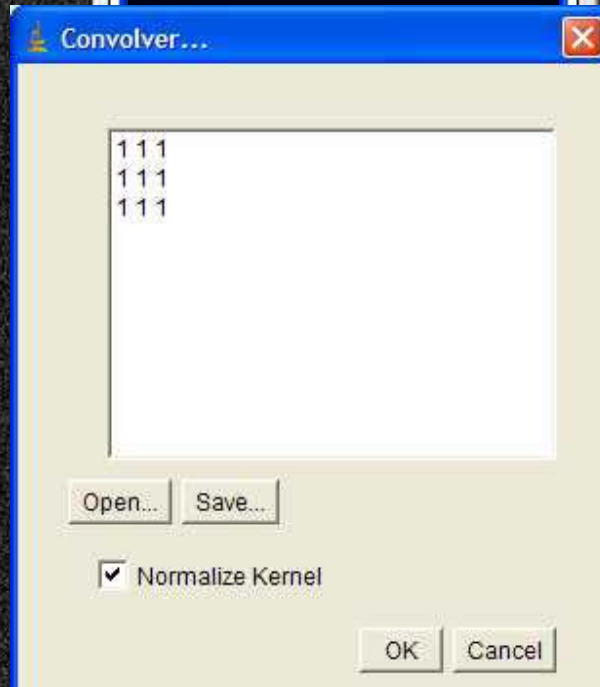
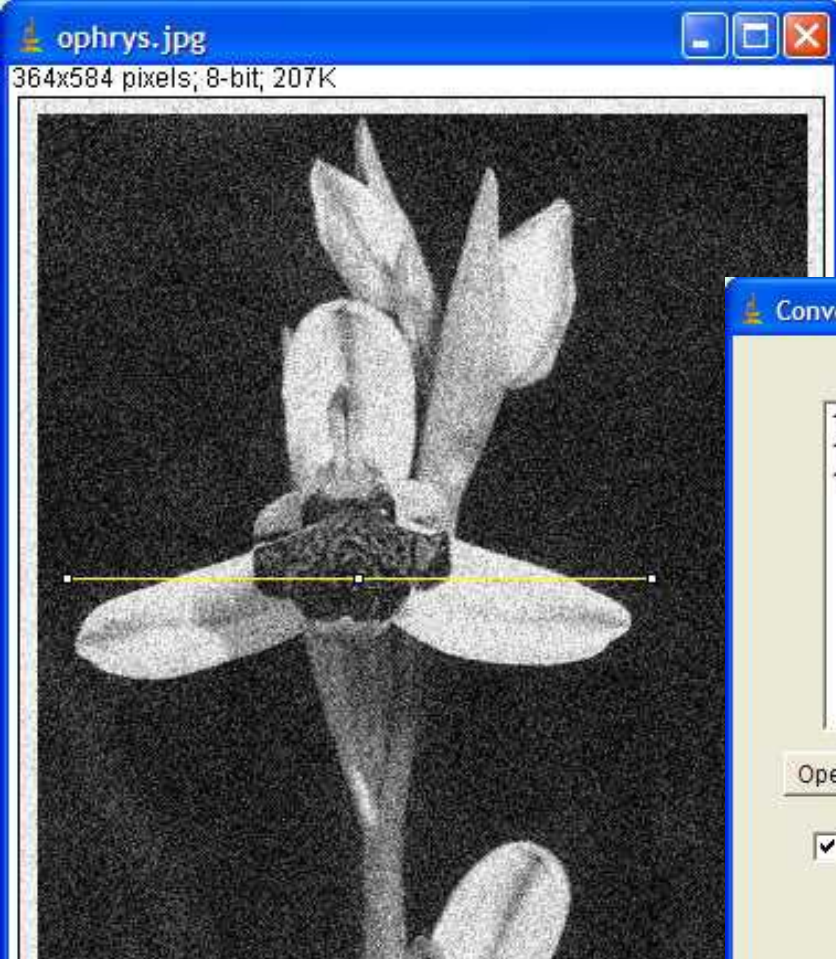
=

100	100	100	100	100
100	108	108	108	100
100	108	133	108	100
100	108	108	108	100
100	100	100	100	100



👉 Le résultat d'un tel filtrage est un lissage de l'image, il s'agit d'un **filtre passe-bas**.

# Filtre passe-bas moyen




Process → Filters → Convolve...



# Filtre médian

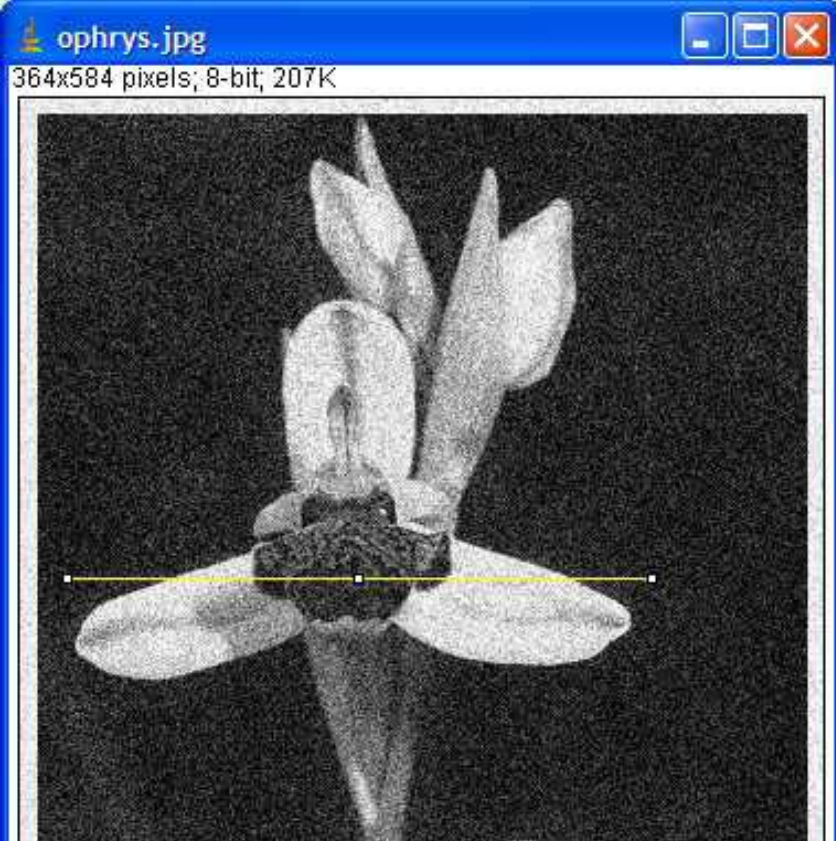
Un filtre médian non linéaire affecte au pixel central la valeur médiane de la série; ce qui nous donne, dans le cas de notre exemple, la série de pixels suivante :

15	18	14
29	27	13
12	19	21

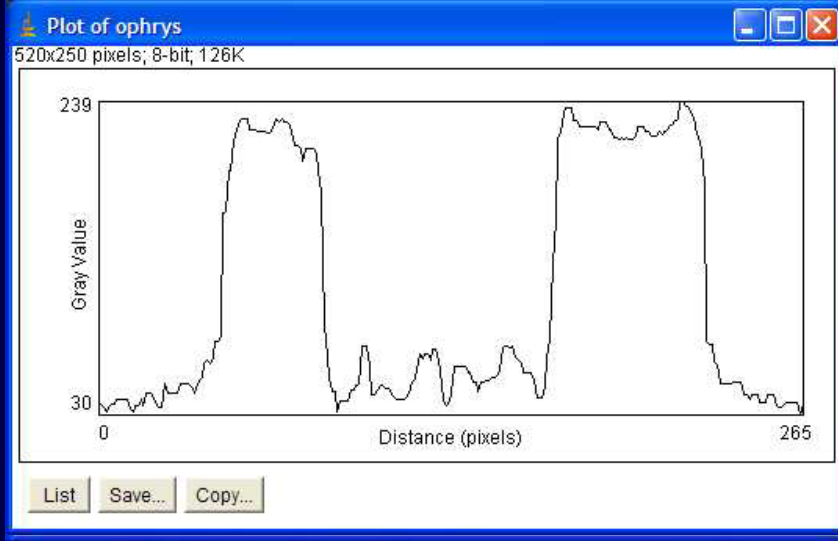
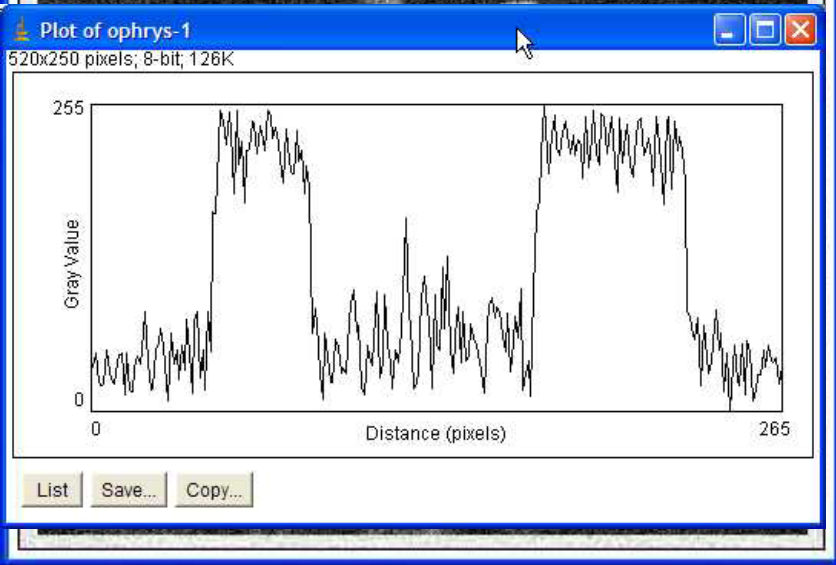
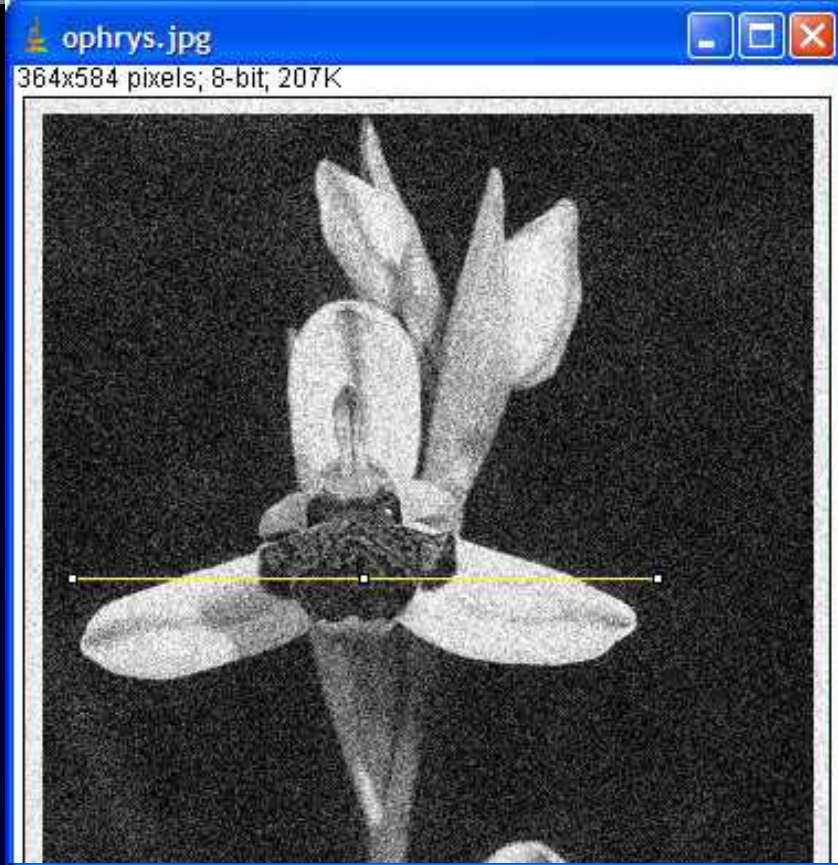


15	18	14
29	18	13
12	19	21

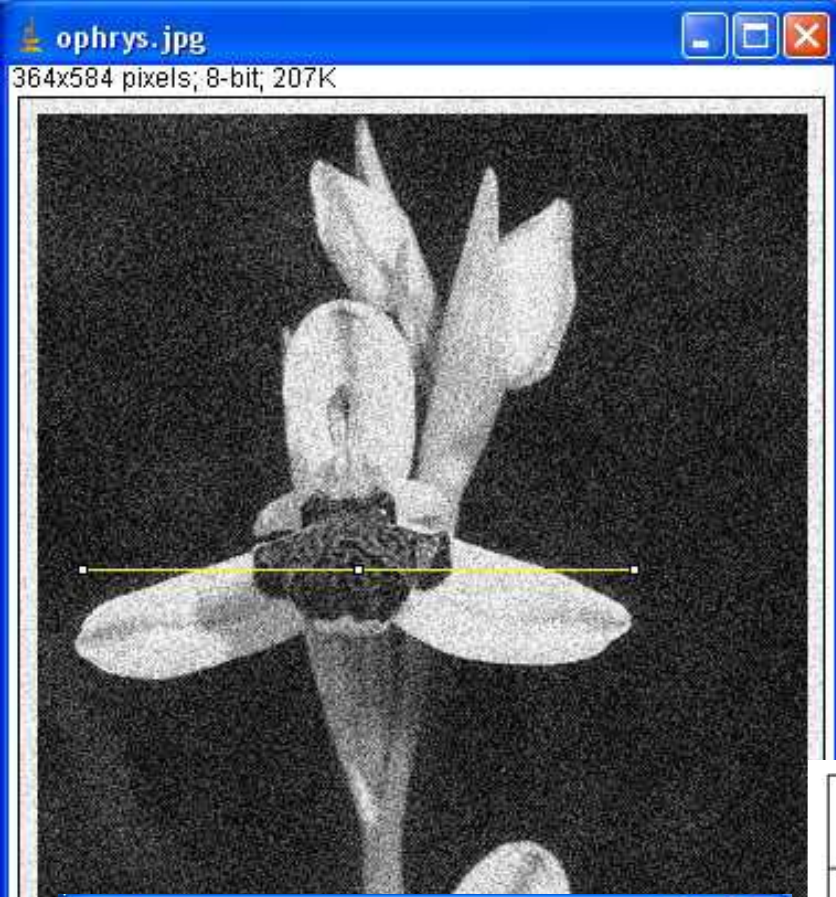
En effet, la médiane de : 12,13,14,15,18,19,21,27,29 est 18.



# Filtre médian



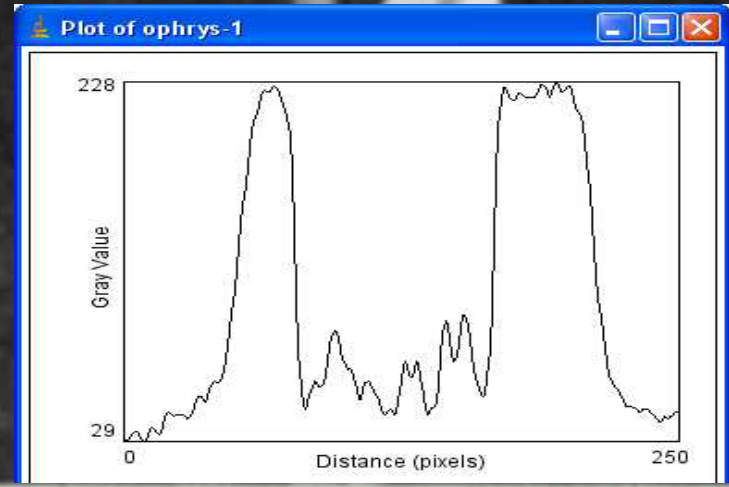
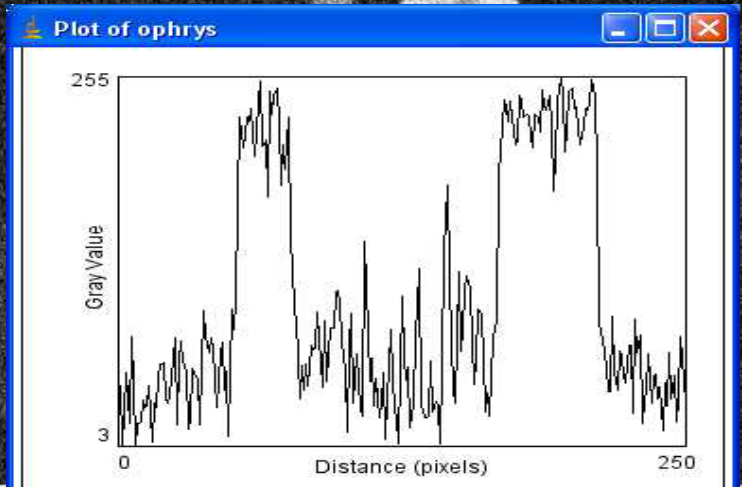
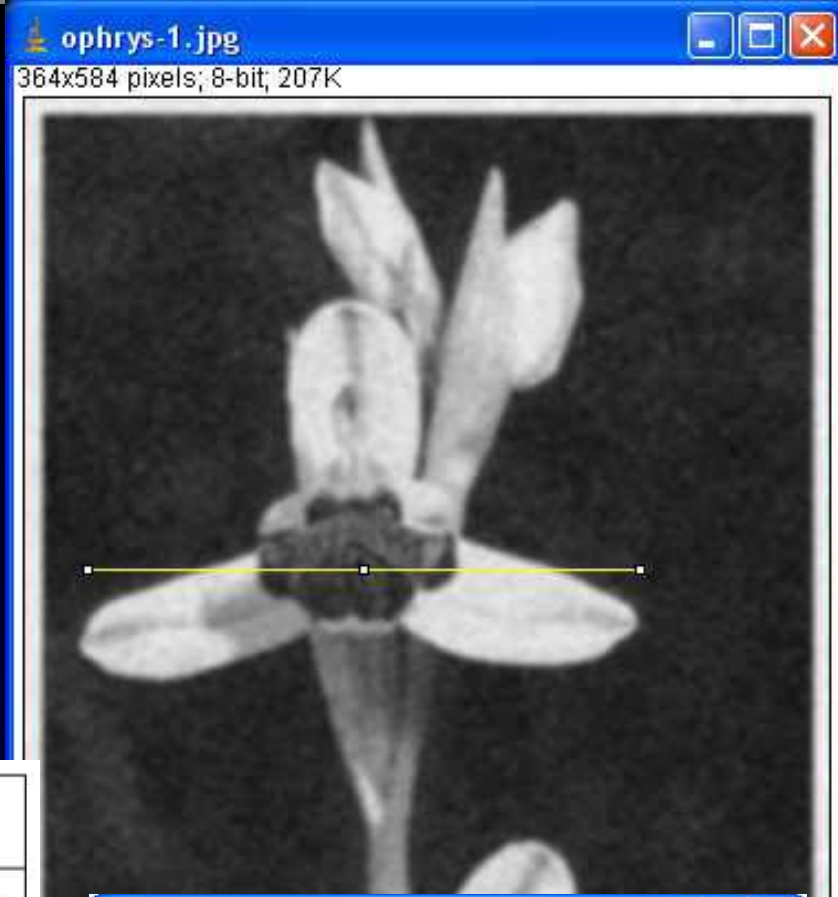
Process → Filters → Median...



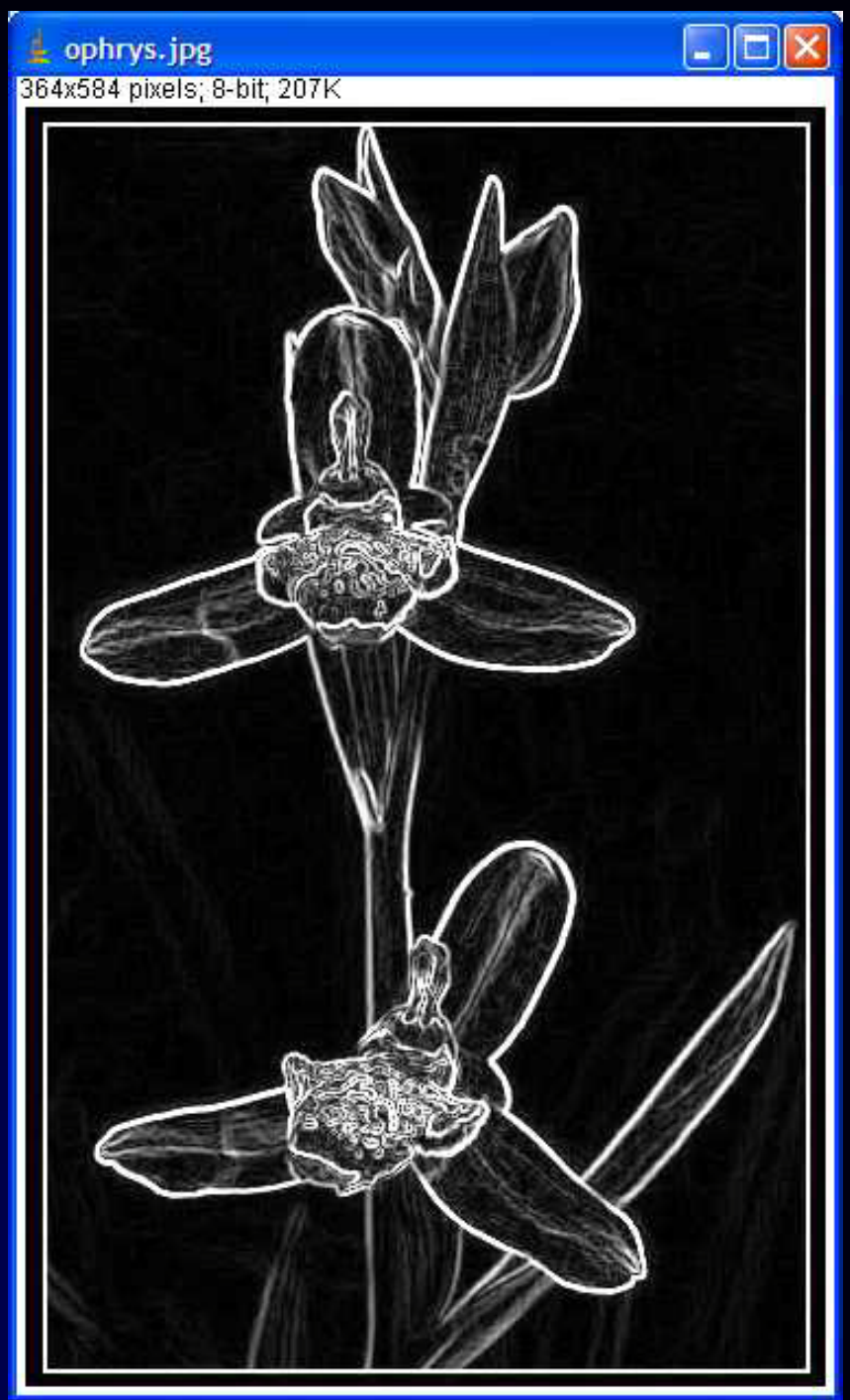
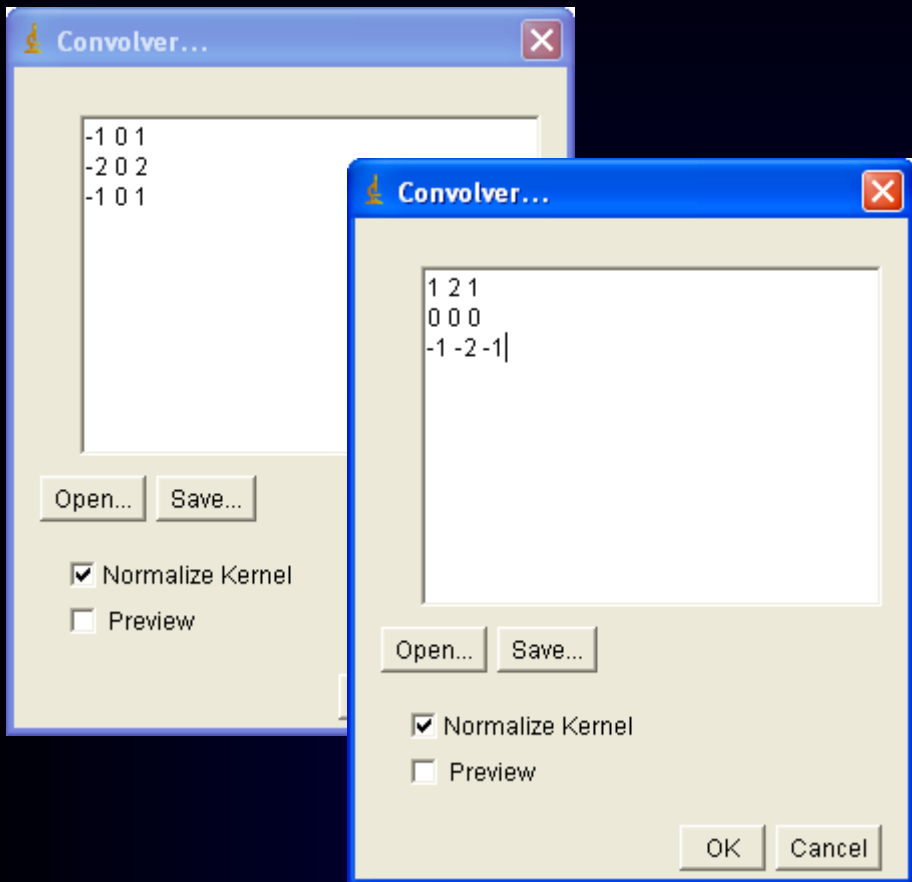
# Filtere Gaussien



0	0.6	1.7	0.6	0
0.6	13	36	13	0.6
1.7	36	100	36	1.7
0.6	13	36	13	0.6
0	0.6	1.7	0.6	0



Process → Filters → Gaussian Blur...

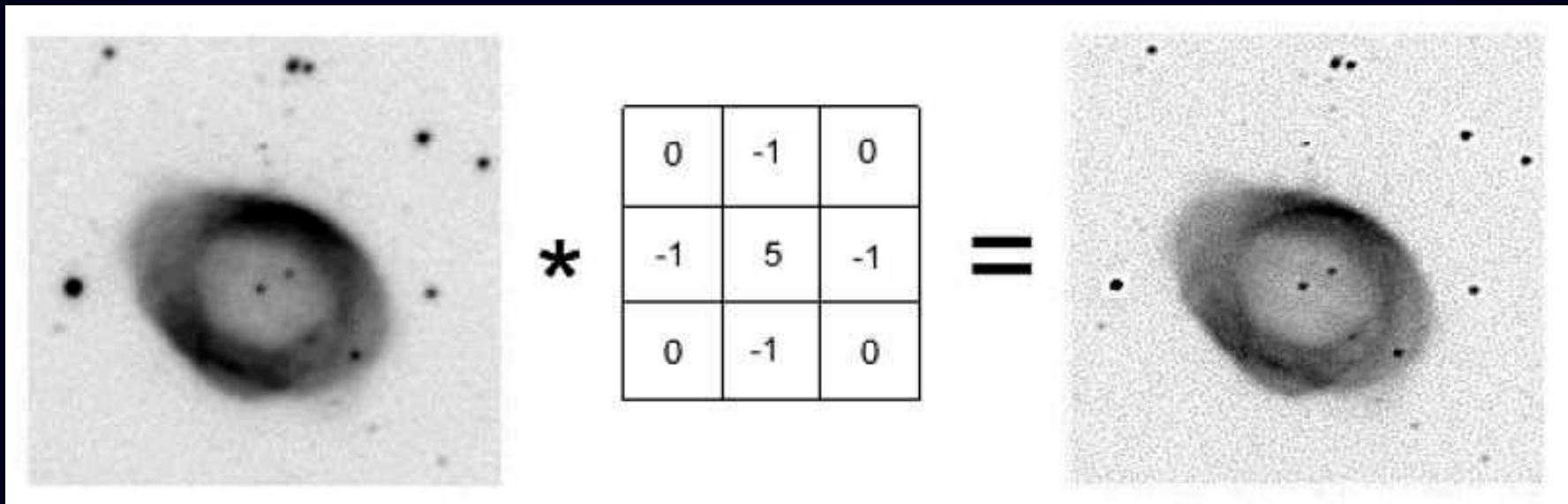


Les images sont convoluées par les filtres de Sobel  
Le résultat est la racine carrée de la somme des carrés des images

Process→Finds Edges

$$\sqrt{D_x^2 + D_y^2}$$

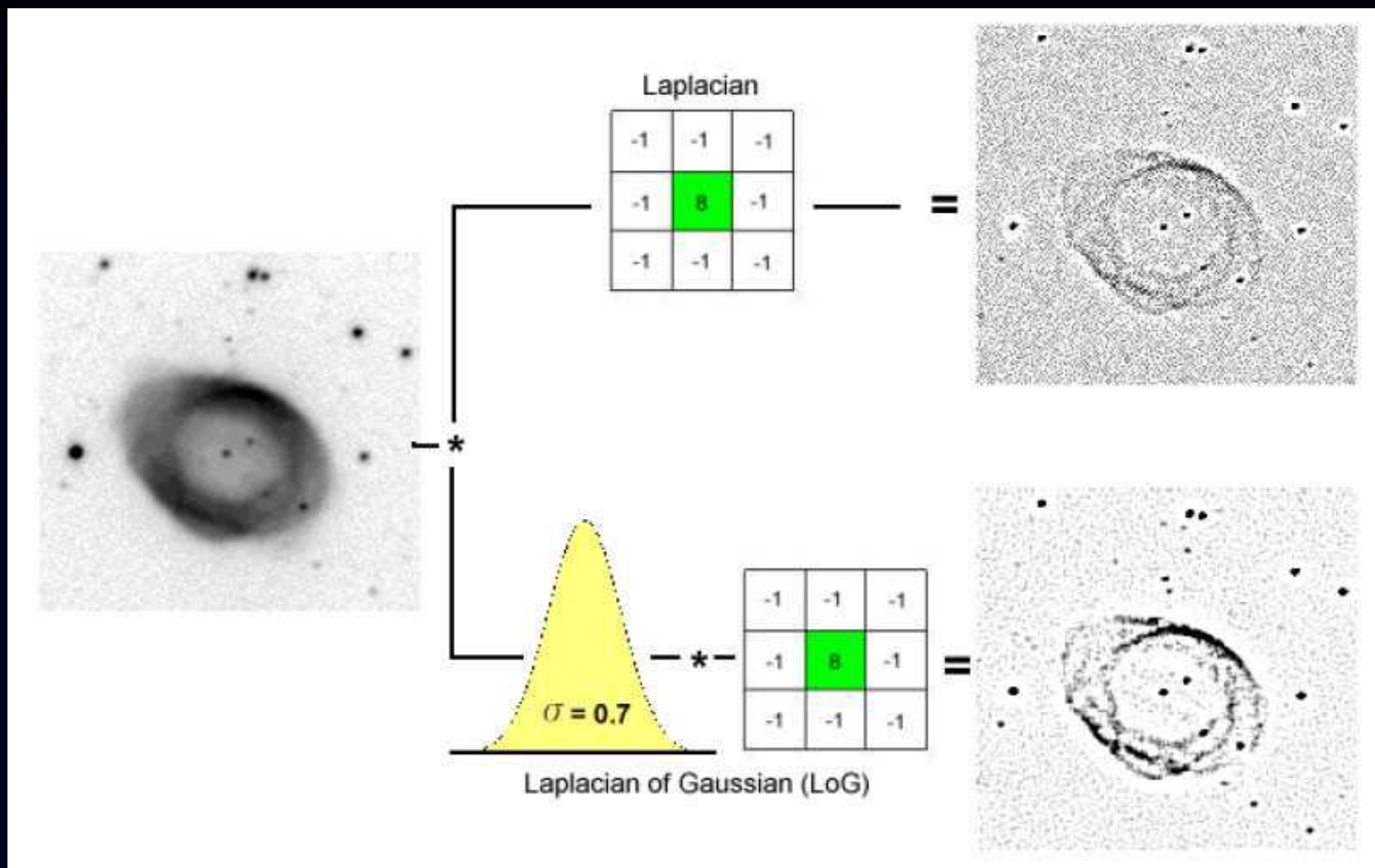




Le résultat est une accentuation des détails et du contraste mais aussi une augmentation du bruit



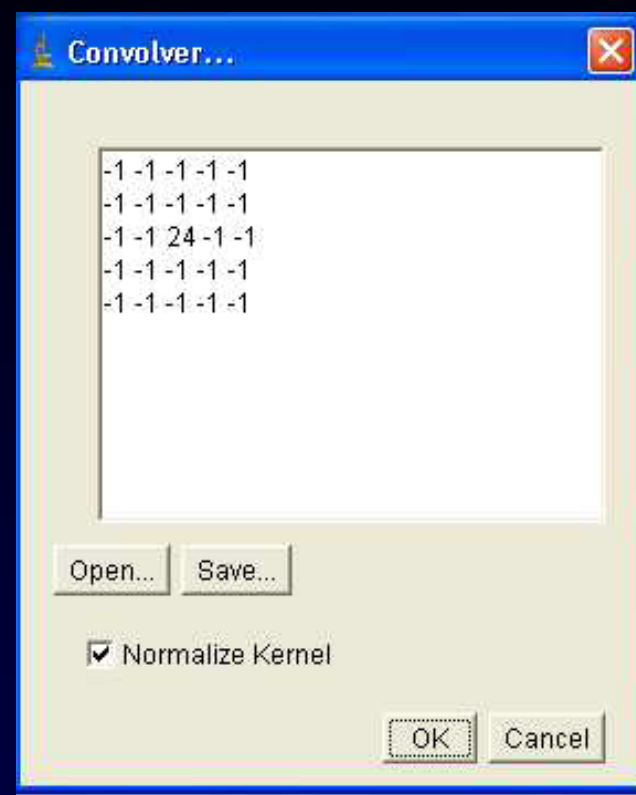
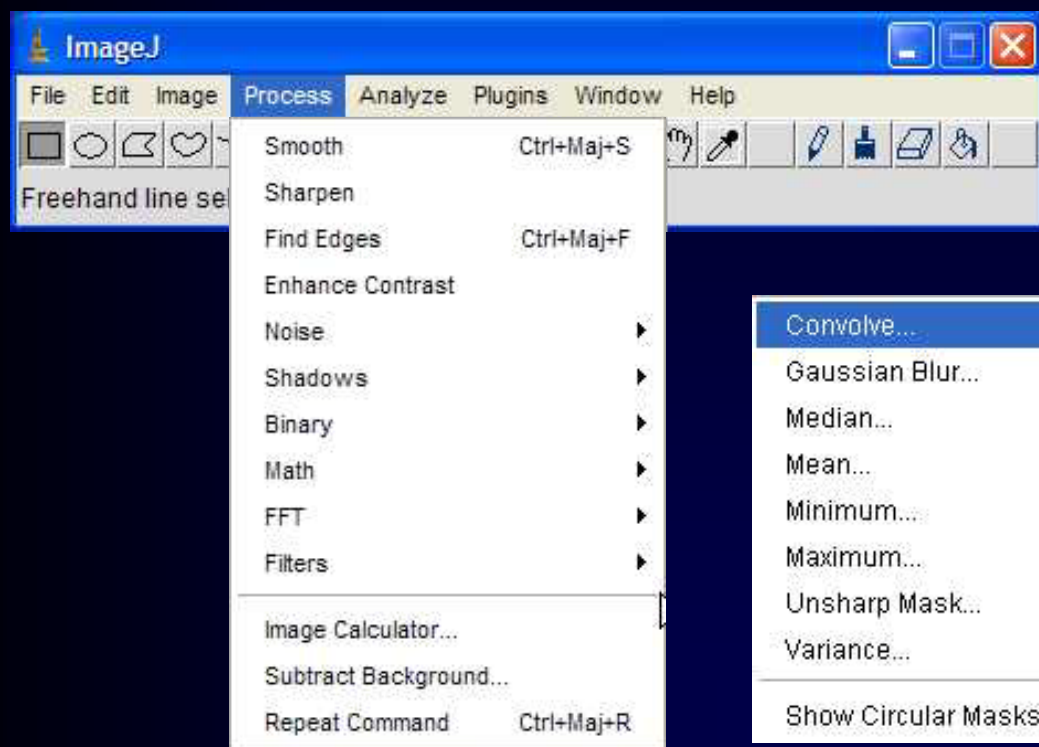
# Combinaison Gaussien Laplacien



Les propriétés de réduction de bruit des filtres Gaussiens peuvent être utilisées en combinaisons avec d'autres filtres qui au contraire génèrent du bruit, comme les filtres Laplaciens. On peut par exemple choisir d'appliquer d'abord un filtre Gaussien pour réduire le bruit, avant d'appliquer un filtre Laplacien pour détecter les points autour desquels les variations de luminosité sont importantes.



# Filtres et Opérations dans ImageJ





# Topic 06 – Noise and filter



# Correction du fond

## Sources de dégradation

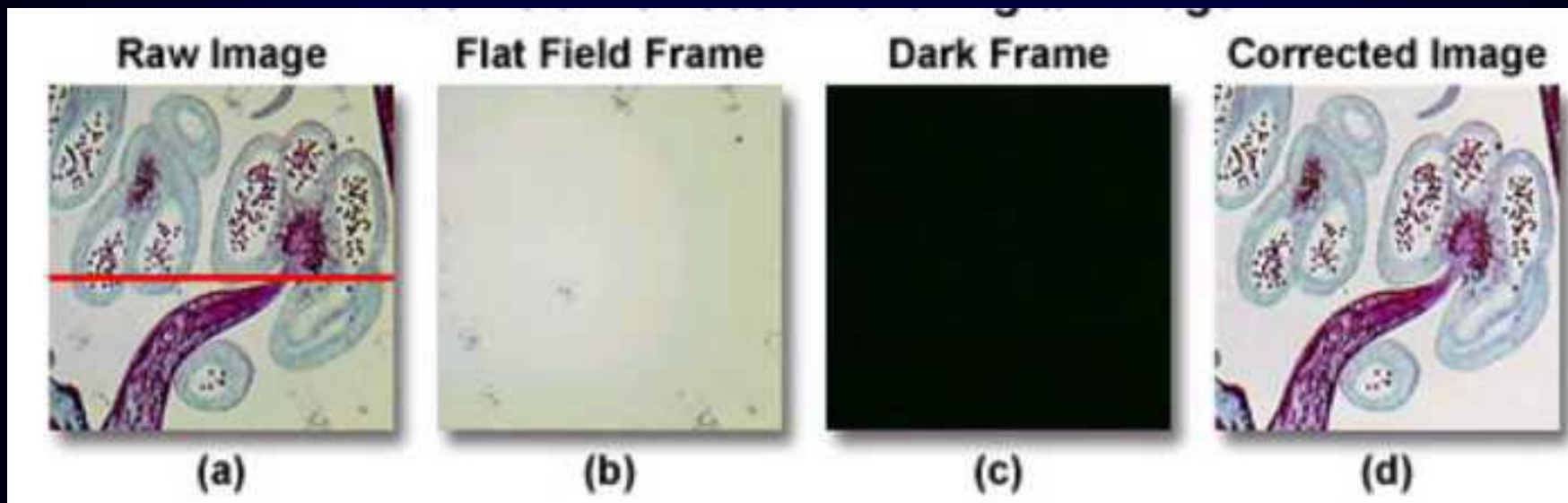
- Bruits de la caméra: bruit aléatoire, « pixels chauds », bruits périodiques
- Illumination non-homogène

## Correction à la capture

Fixer les réglages microscope – acquisition

Captures moyennées d'un champs noir CN (en coupant le trajet optique)  
d'un champs clair CC (lame sans échantillon) et de l'échantillon

Image corrigée =  $(\text{échantillon} - \text{CN}) / (\text{CC} - \text{CN}) * 255$





# Correction du fond

## Correction après la capture

Bruit aléatoire : filtre gaussien ou médian mais perte de détail

Pixels chauds : filtrer les pixels saturés isolés

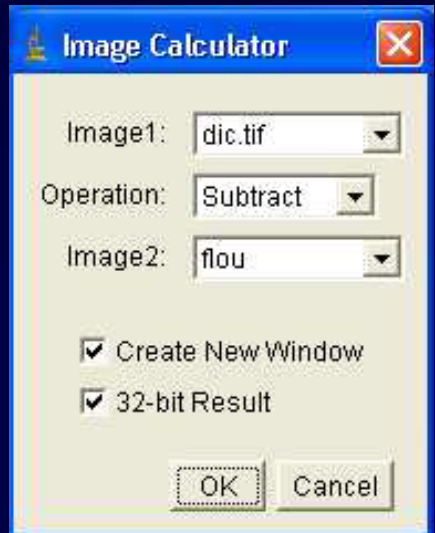
Bruit périodique : filtre de Fourier

Illumination non-homogène :

- Soustraire la même image très floue (filtre gaussien très large)
- Algorithme « rolling ball » (ImageJ Process → Subtract Background)
- Toute une série de plugins implémentés dans ImageJ (Fitting a polynomial surface)



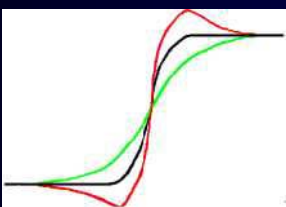
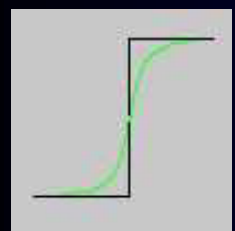
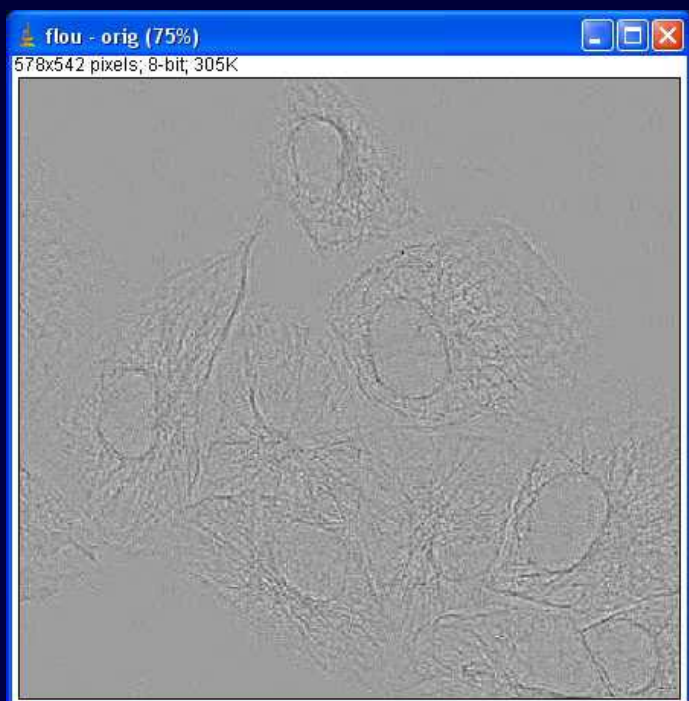
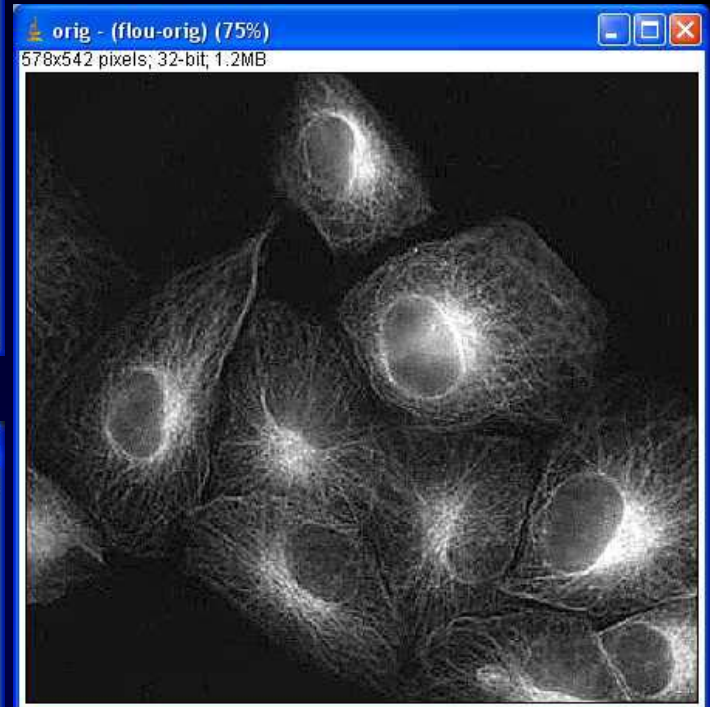
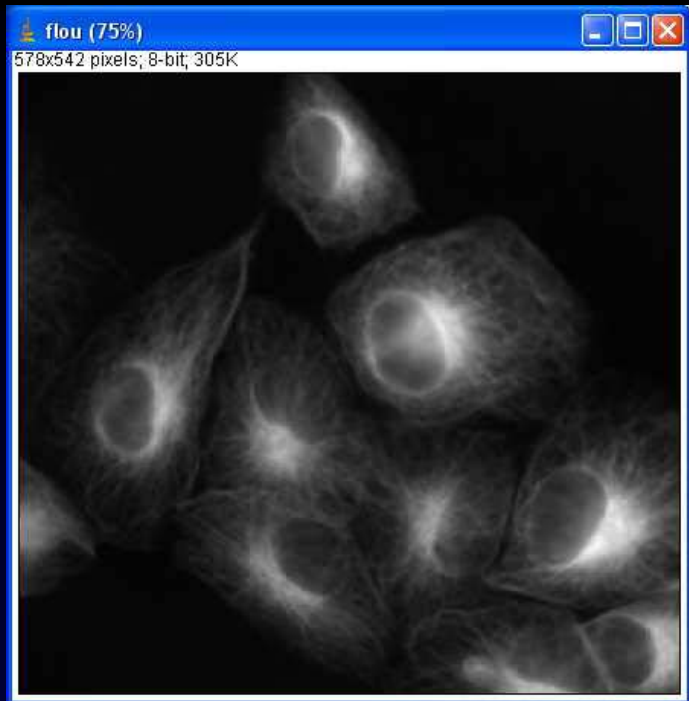
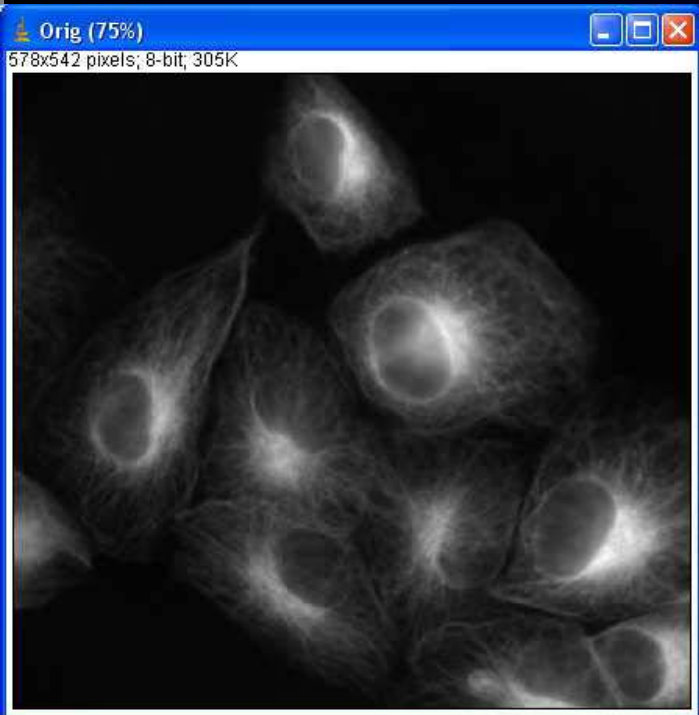
# Masquer le fond





# Masque de Flou

1 1 1  
 1 1 1  
 1 1 1



Orig - (Flou - Orig)

Flou - Orig





# Topic 07 – Background correction Unsharp masking



L'image numérique

Les Prétraitements

**La Segmentation**

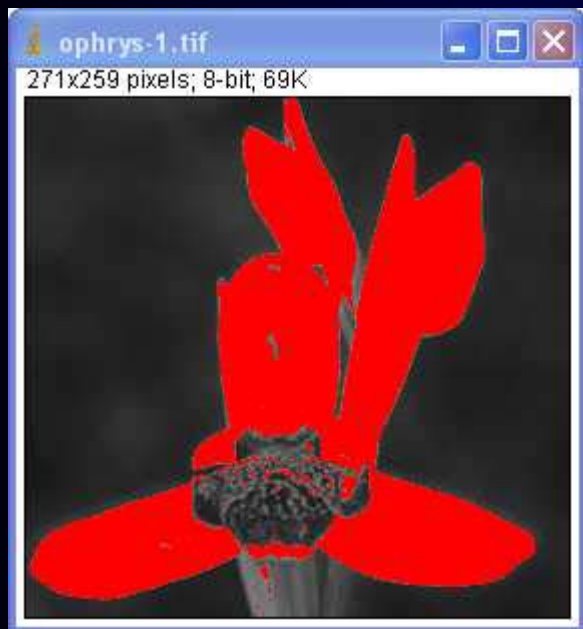
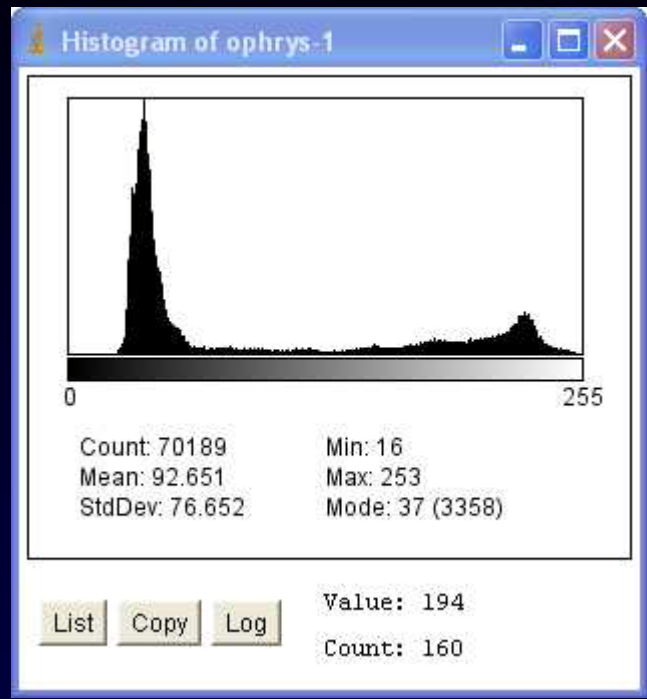
Permet de séparer les  
régions d'intérêt du fond.

Les Post-traitements

La Quantification



# Le Seuillage automatique



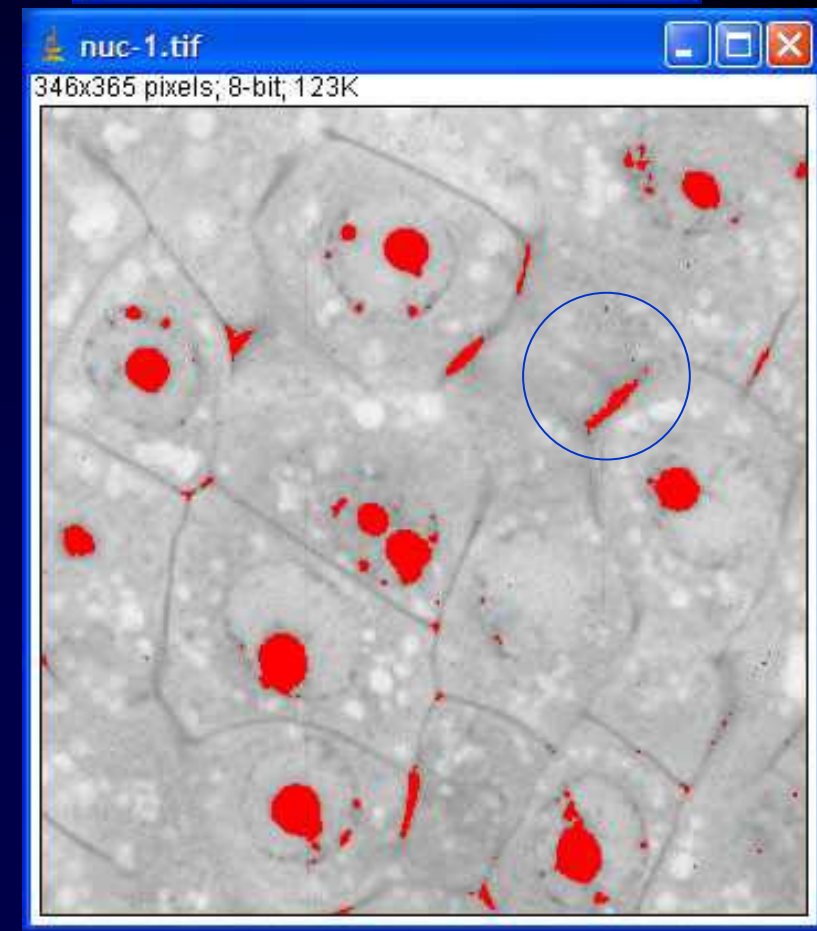
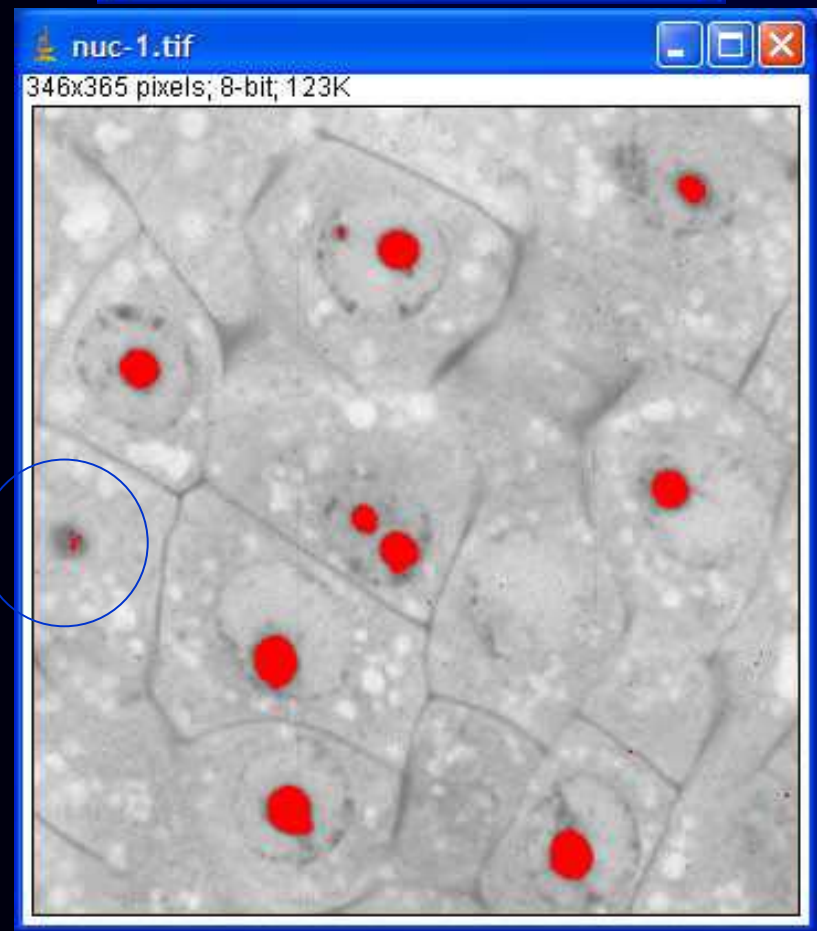
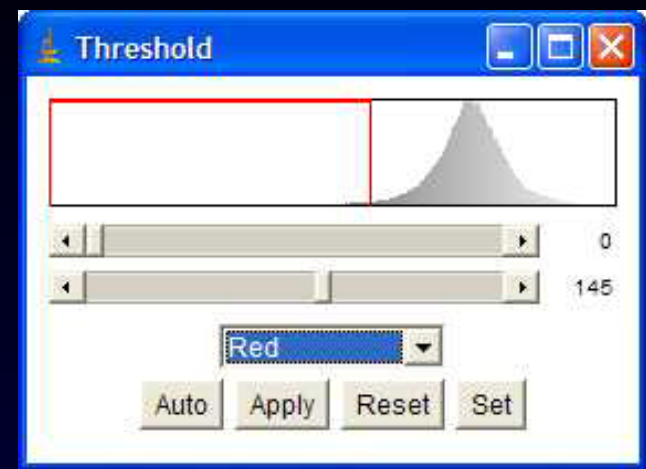
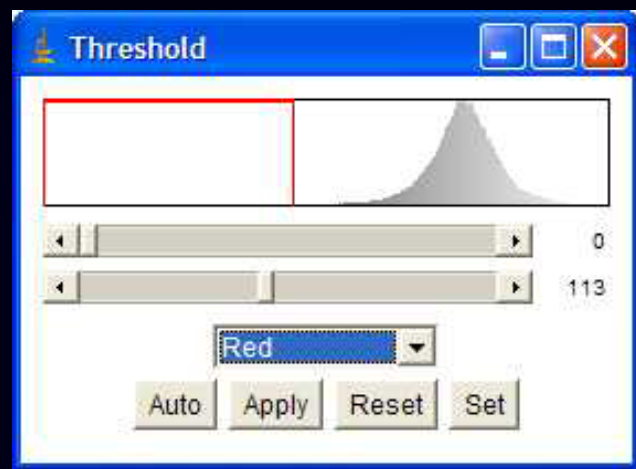
Threshold  
 Default    Red  
 Dark background  
 Auto    Apply    Reset    Set

- Default
- Huang
- Intermodes
- IsoData
- IJ\_IsoData
- Li
- MaxEntropy
- Mean
- MinError
- Minimum
- Moments
- Otsu
- Percentile
- RenyiEntropy
- Shanbhag
- Triangle
- Yen

Image → Adjust → Threshold...

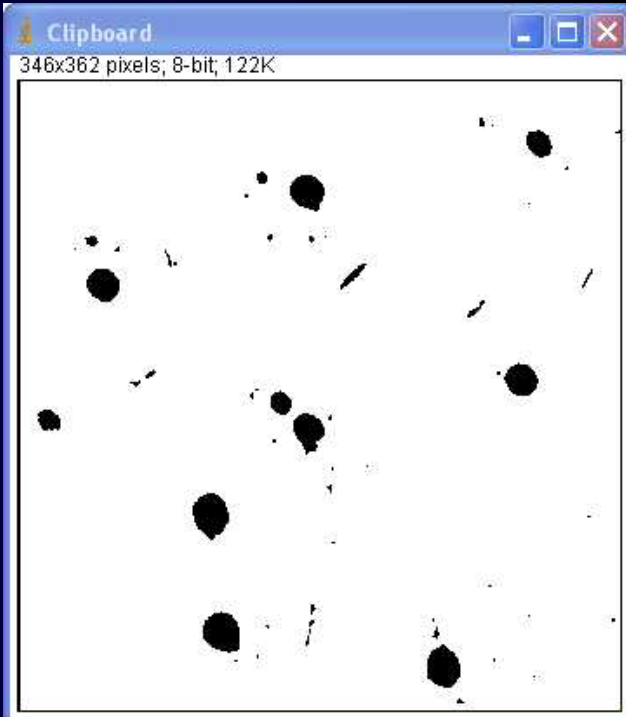
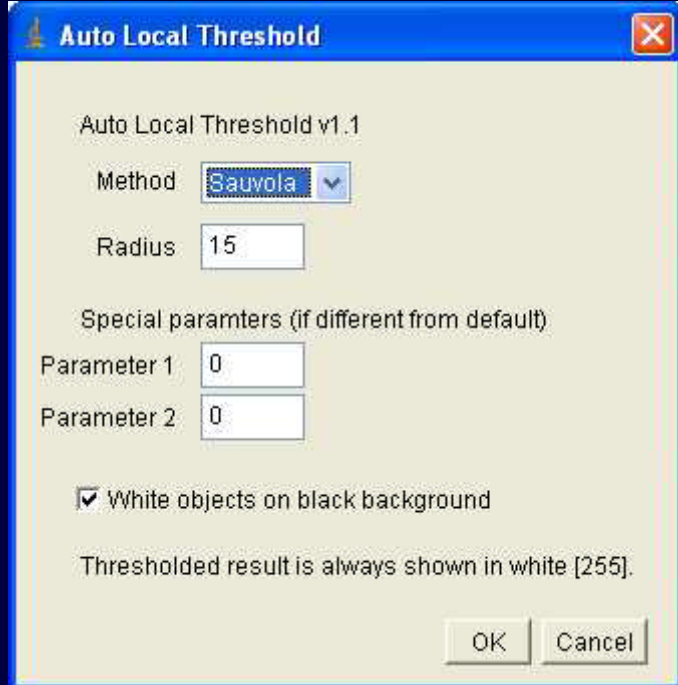
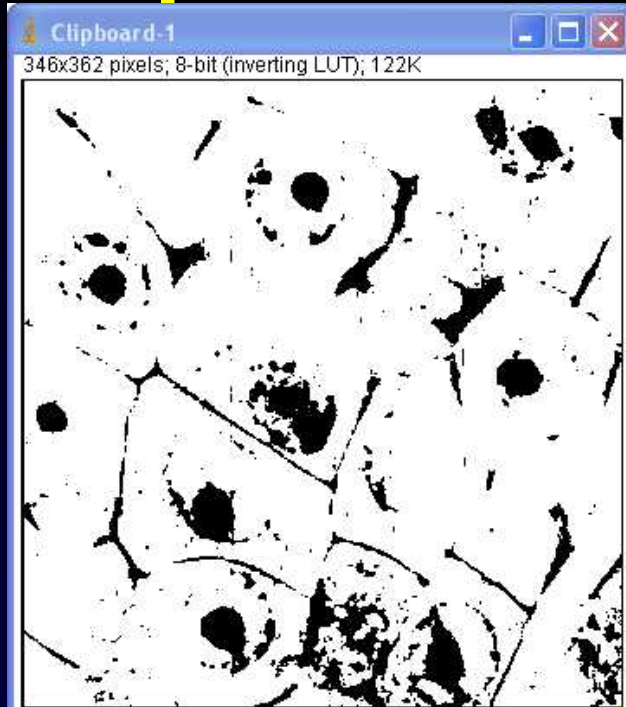
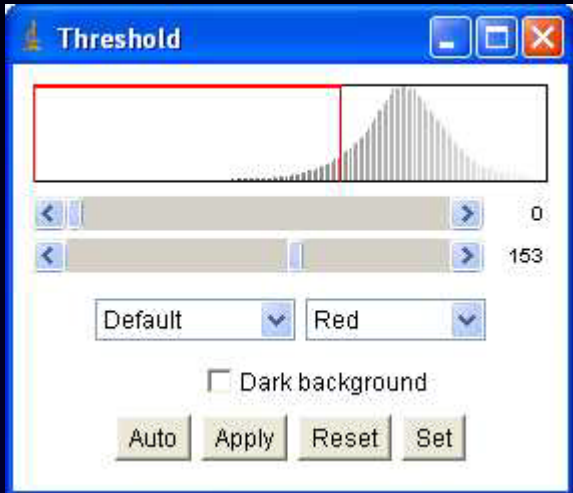


# Le Seuillage manuel



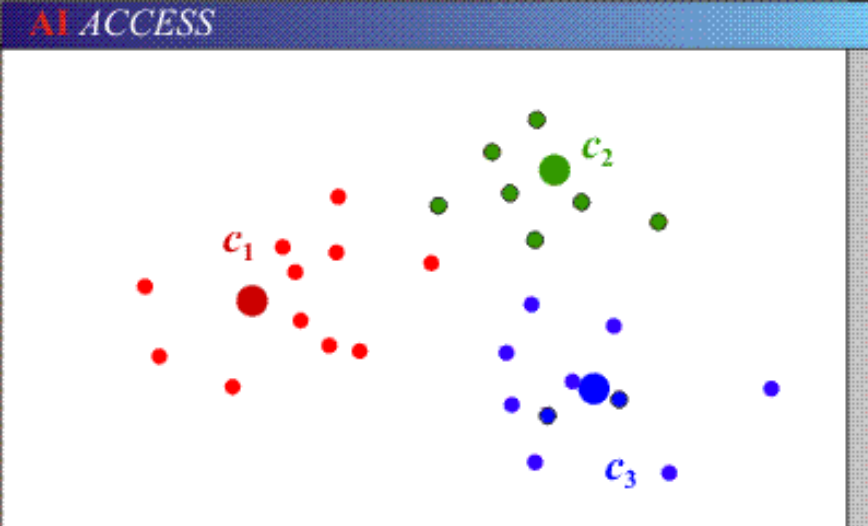
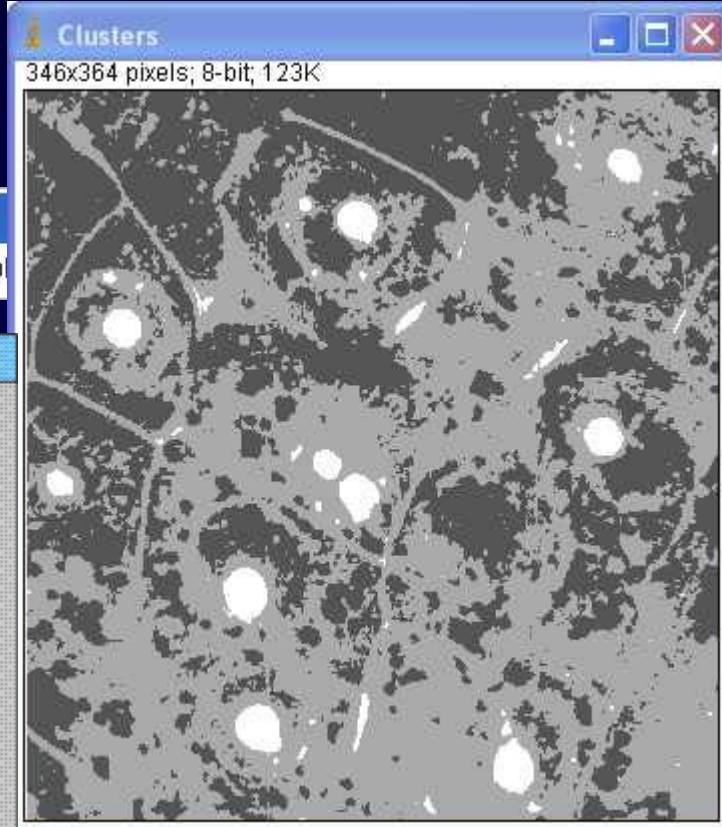
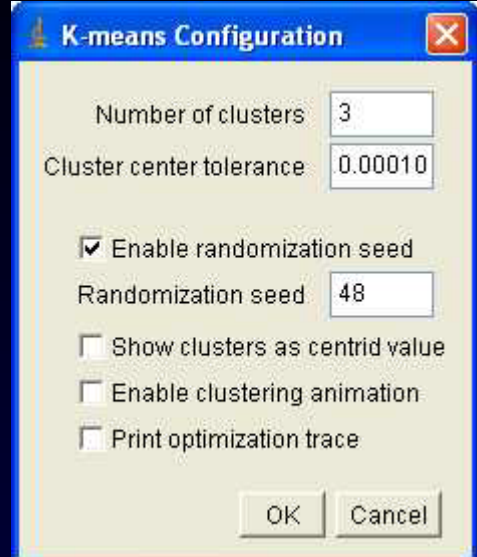
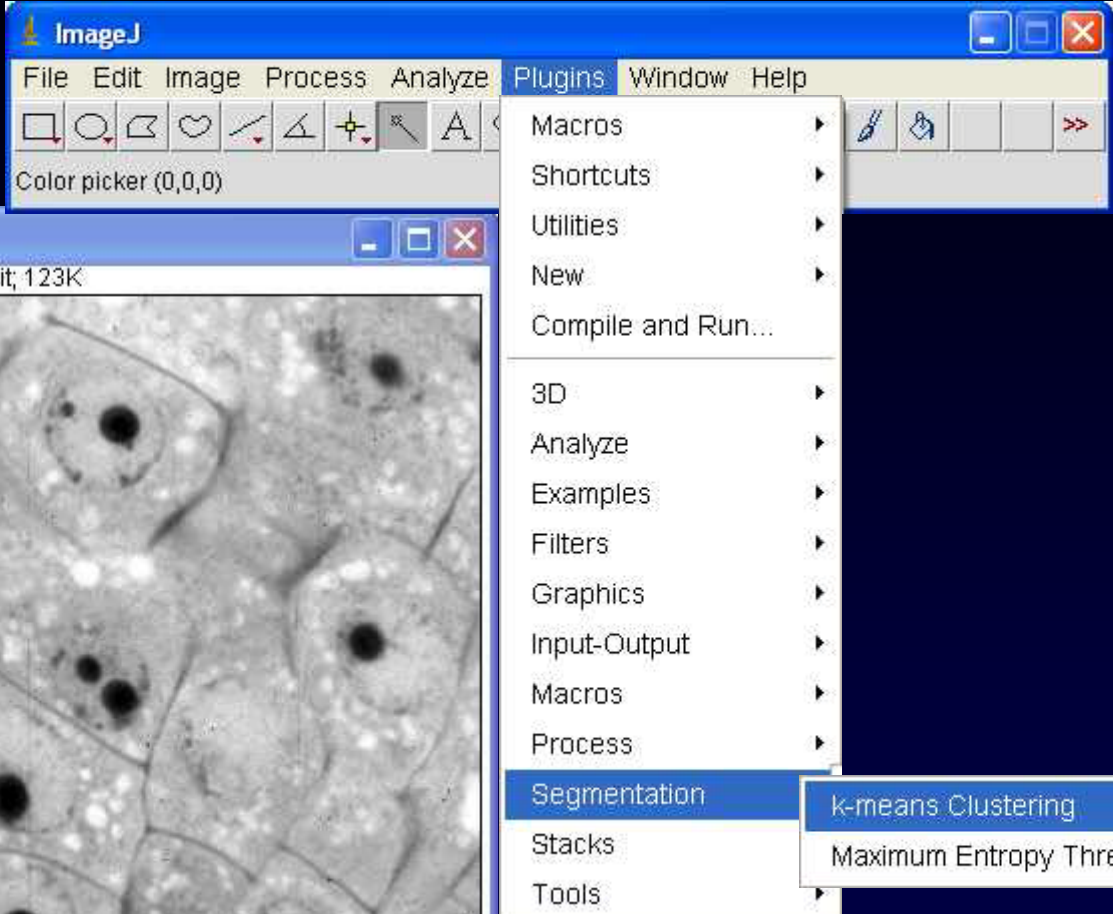


# Seuillage local automatique



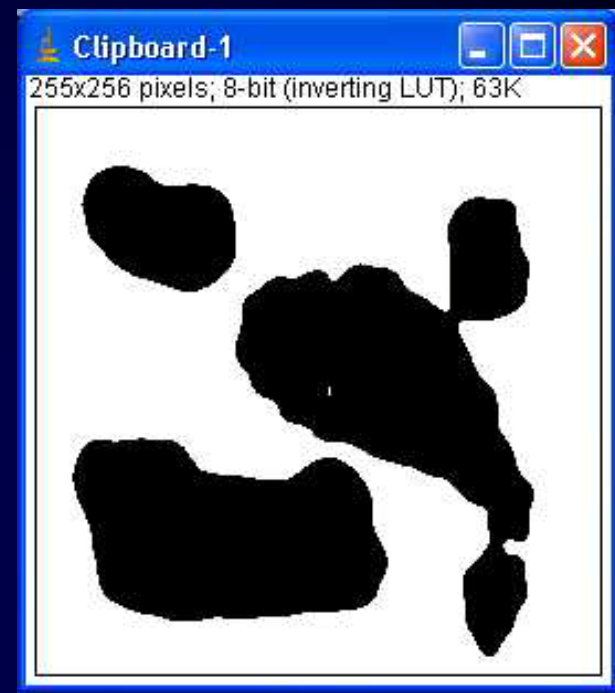
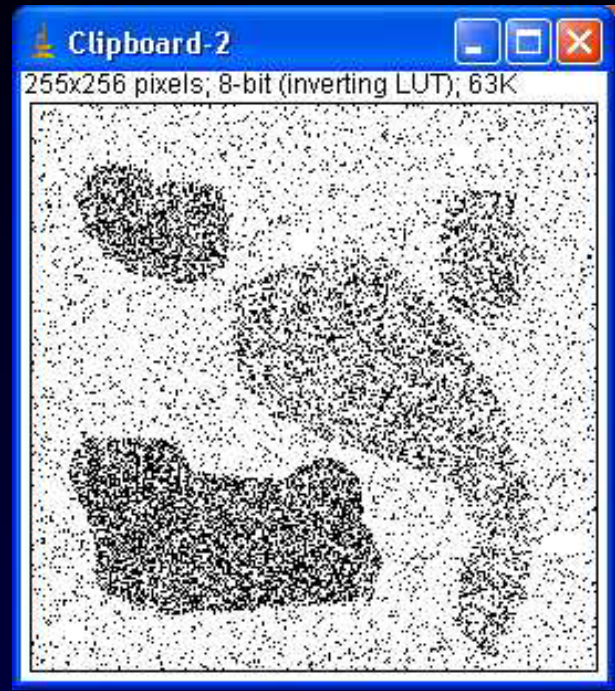


# Plugin k-mean clustering



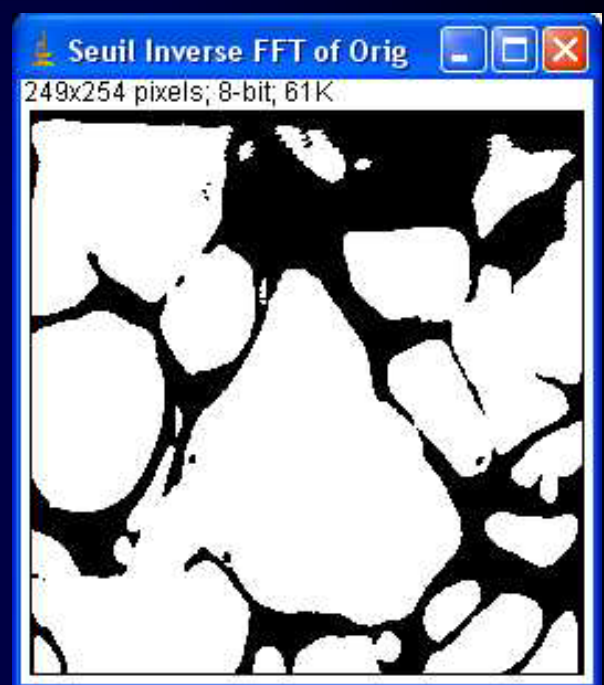
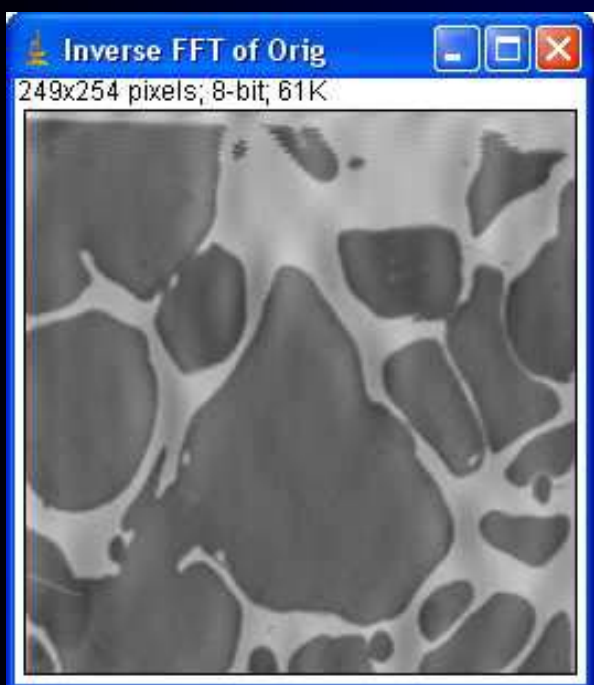
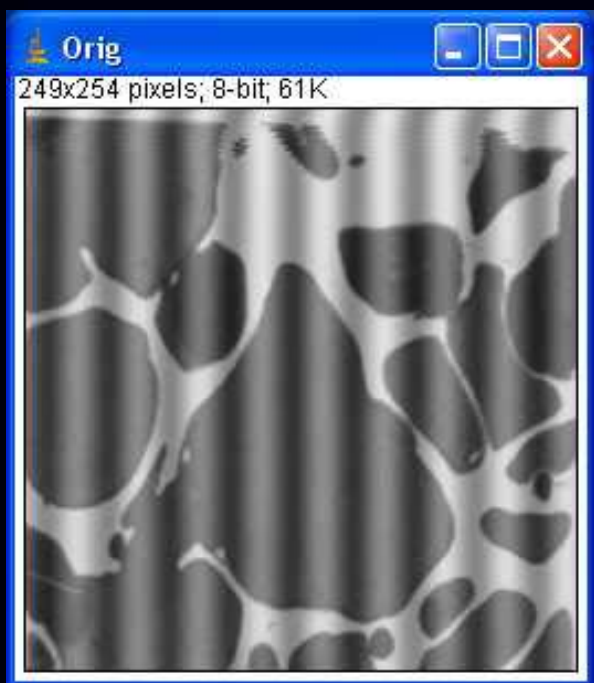


# Filtrage avant le seuillage





# FFT filtres dans le domaine de Fourier





# Couleur et segmentation séparation RGB

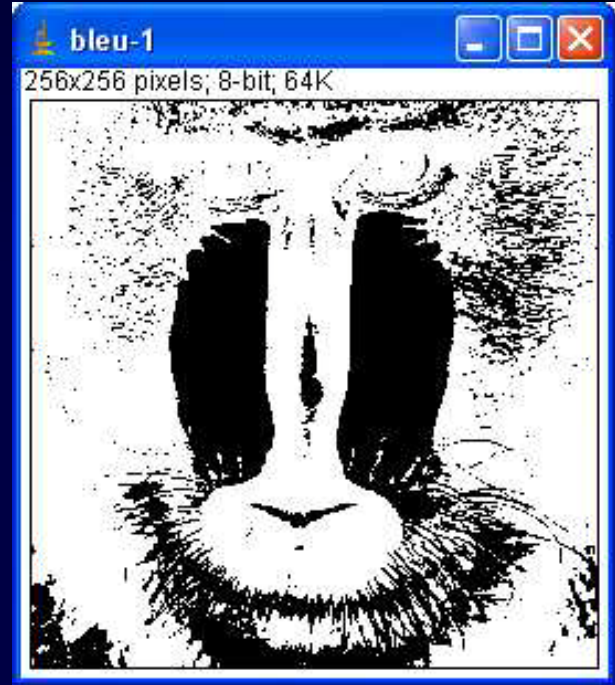
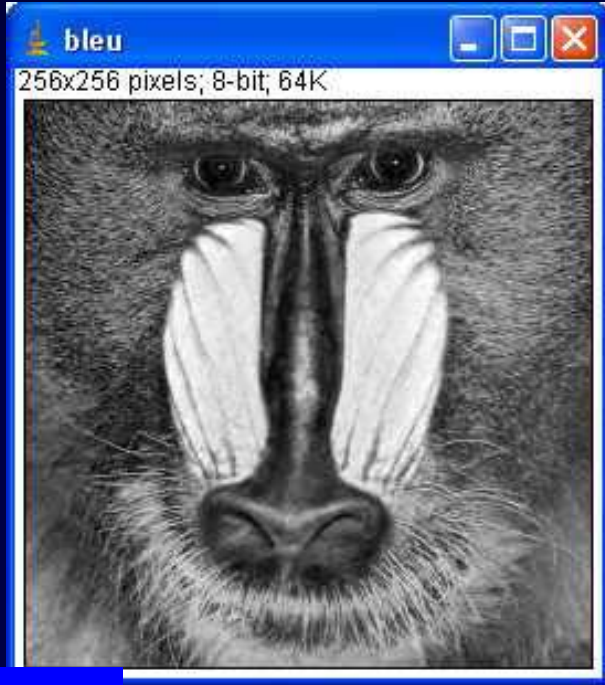


Image → Color → Split Channels...



# Décomposition HSI

la Teinte ou Hue se référant à la couleur

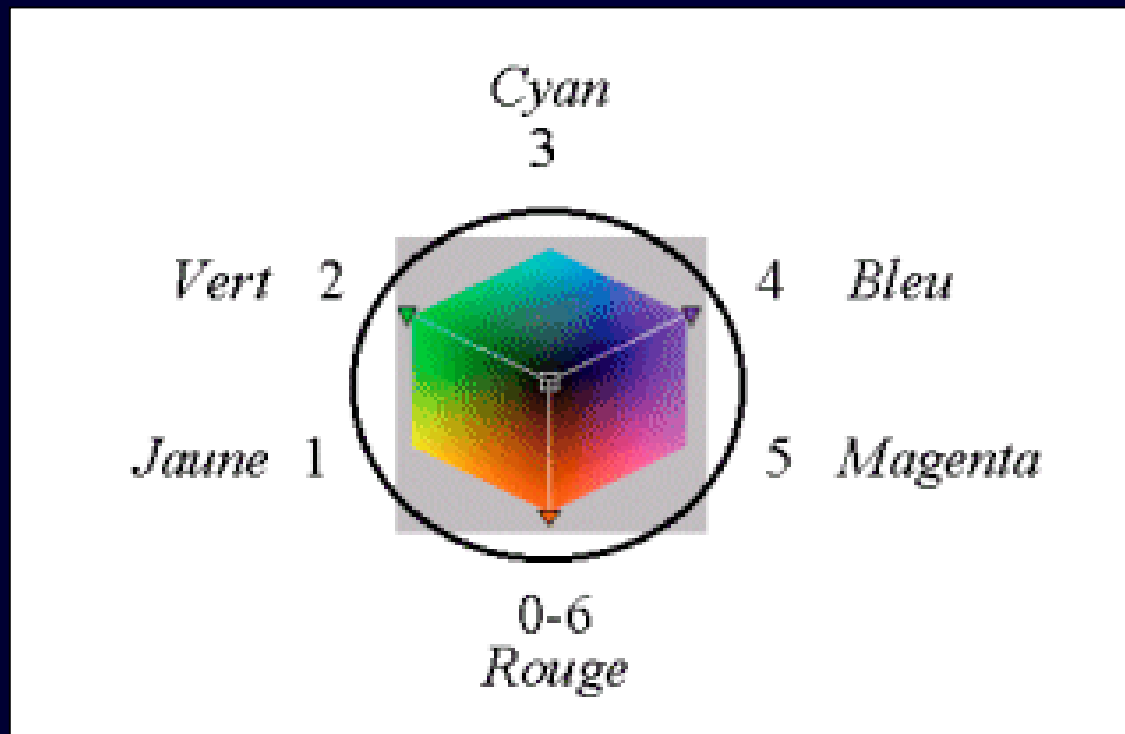
codée de 0 à 6 correspond à une distribution cyclique des couleurs

le noir, le blanc et les nuances de gris sont codés à 0, comme le rouge

la Saturation : mesure de l'absence de blanc dans une couleur

le " rouge pompier " étant une couleur saturée et le rose une couleur non saturée

l'Intensité ou Luminance : mesure de l'intensité d'une couleur, distinction entre clair et foncé





# Couleur et segmentation séparation HSI

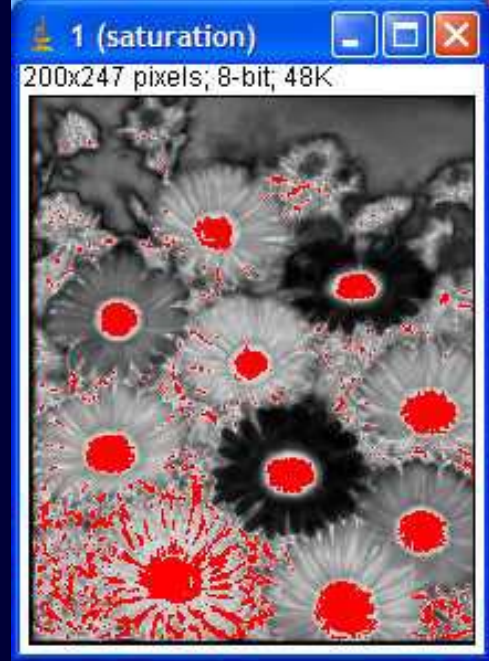
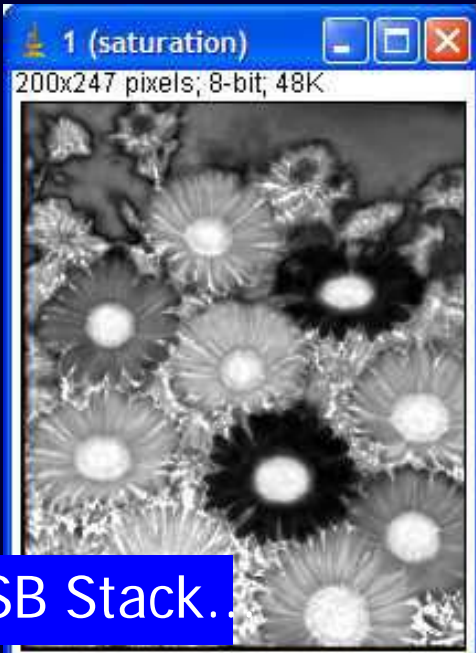
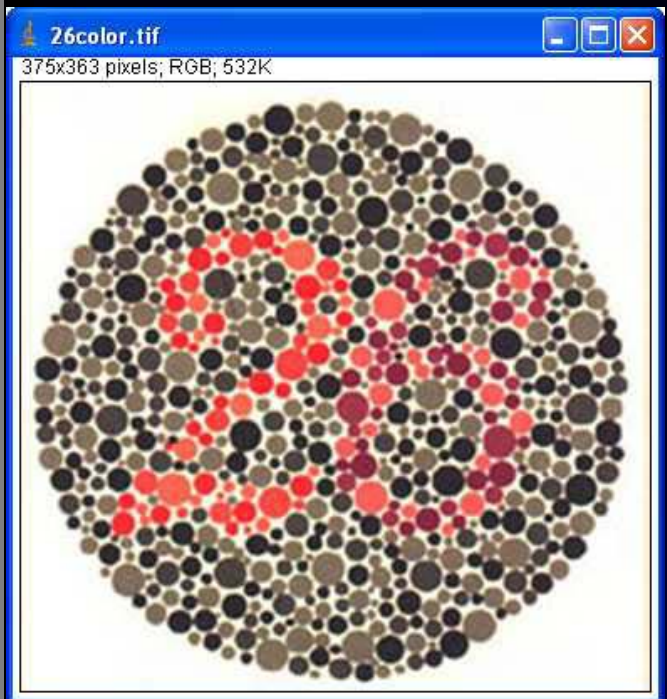


Image → Type → HSB Stack.



# Seuillage couleur



**Threshold Color (experimental)**

Hue

Saturation

Brightness

Thresholding method: Default

Threshold color: B&W

Color space: HSB

Dark background

Original Filtered Select Sample

Stack Macro Help

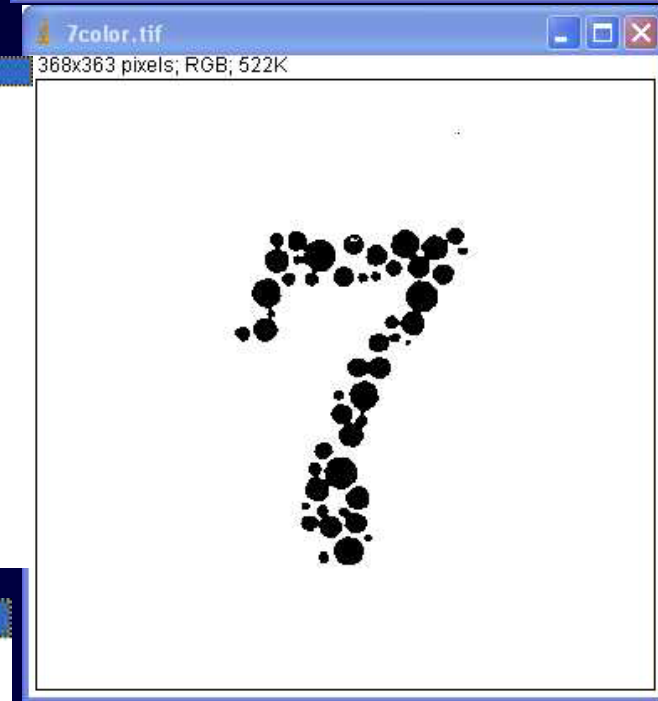
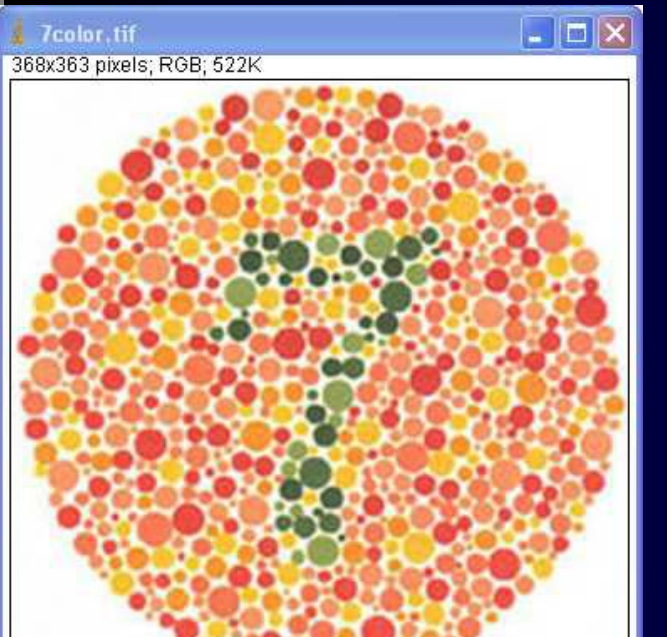
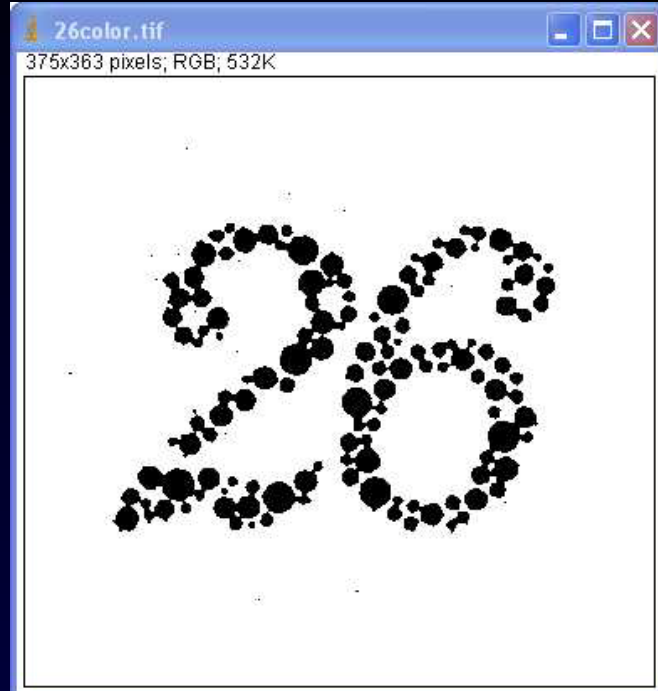
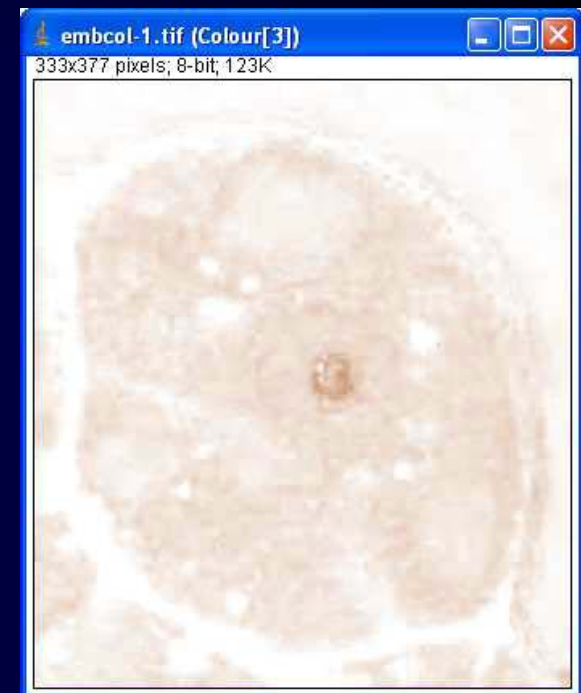
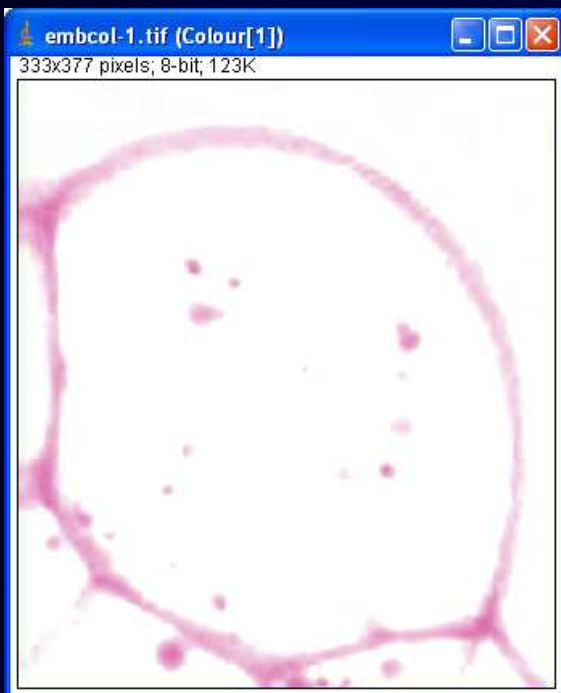
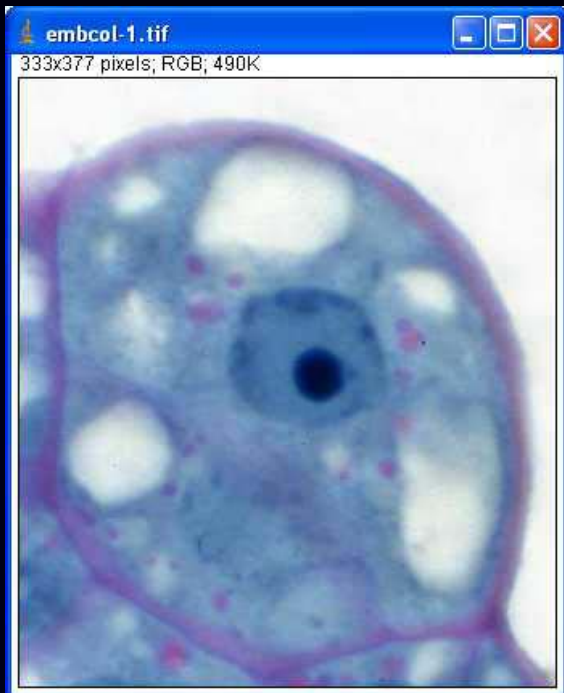


Image → Adjust → Color Threshold..

- Default
- Huang
- Intermodes
- IsoData
- IJ\_IsoData
- Li
- MaxEntropy
- Mean
- MinError
- Minimum
- Moments
- Otsu
- Percentile
- RenyiEntropy
- Shanbhag
- Triangle
- Yen
- HSB**
- RGB
- Lab
- YUV



# Plugin Colour Deconvolution





# Topic 08 – Segmentation



L'image numérique

Les Prétraitements

La Segmentation

**Les Post-traitements**

Transformations de  
morphologie  
mathématique

La Quantification



# Principe

Elément structurant

On déplace l'élément structurant sur toute l'image

Le pixel sera noir si:

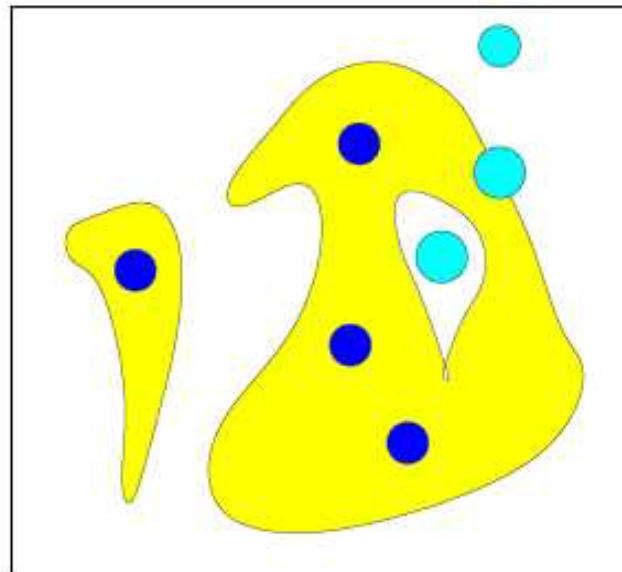
l'élément structurant est inclus dans un objet de l'image

→ EROSION

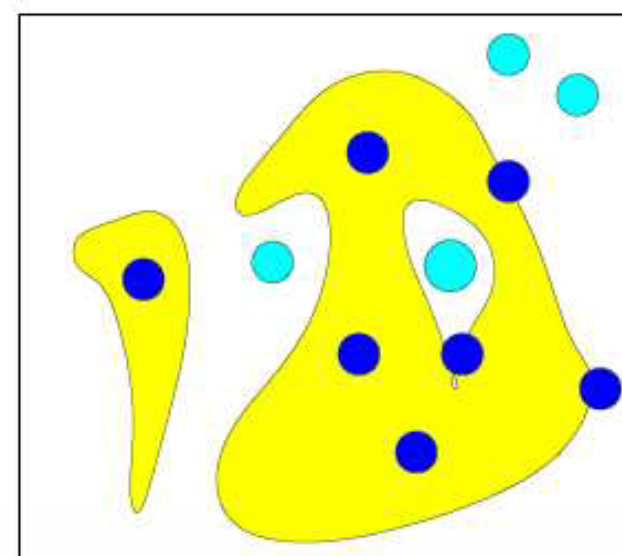
l'élément structurant touche un objet de l'image

→ DILATATION

Exemple d'éléments structurants

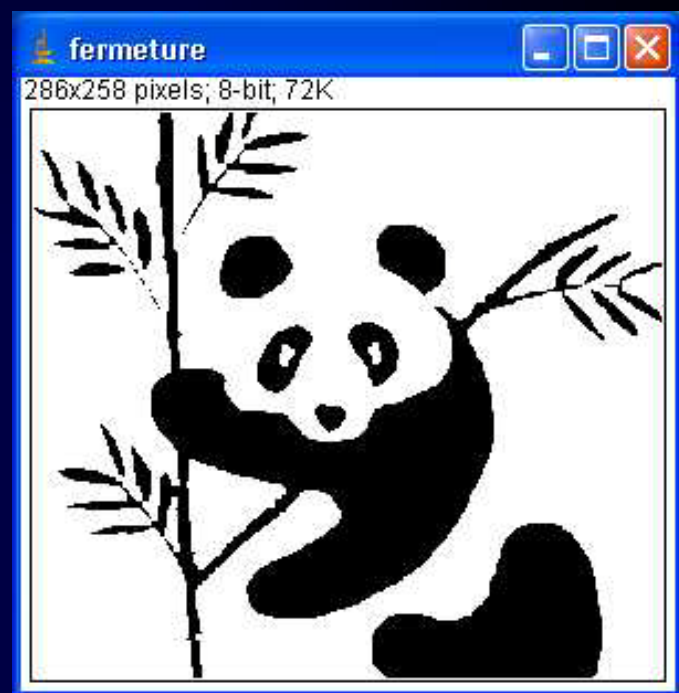


● reponse negative  
● reponse positive



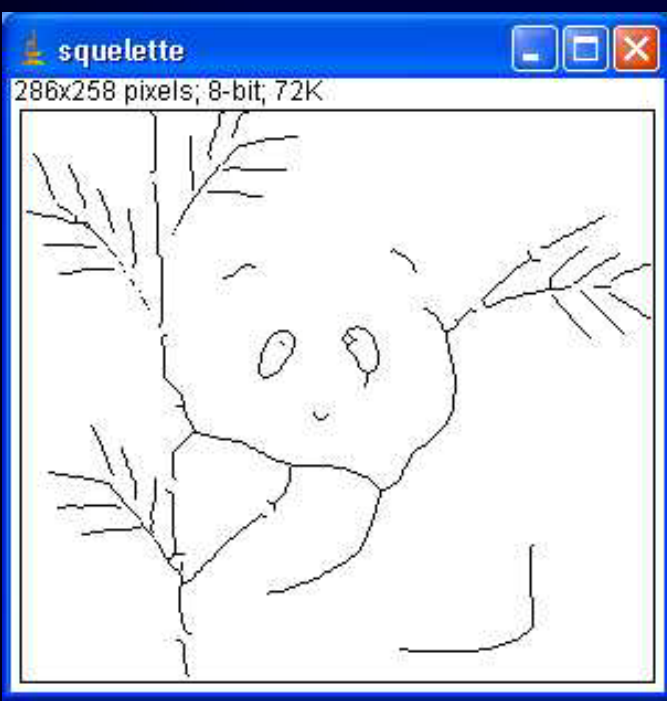
● reponse negative  
● reponse positive





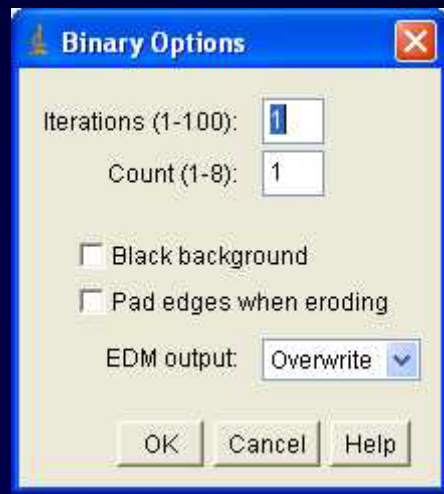
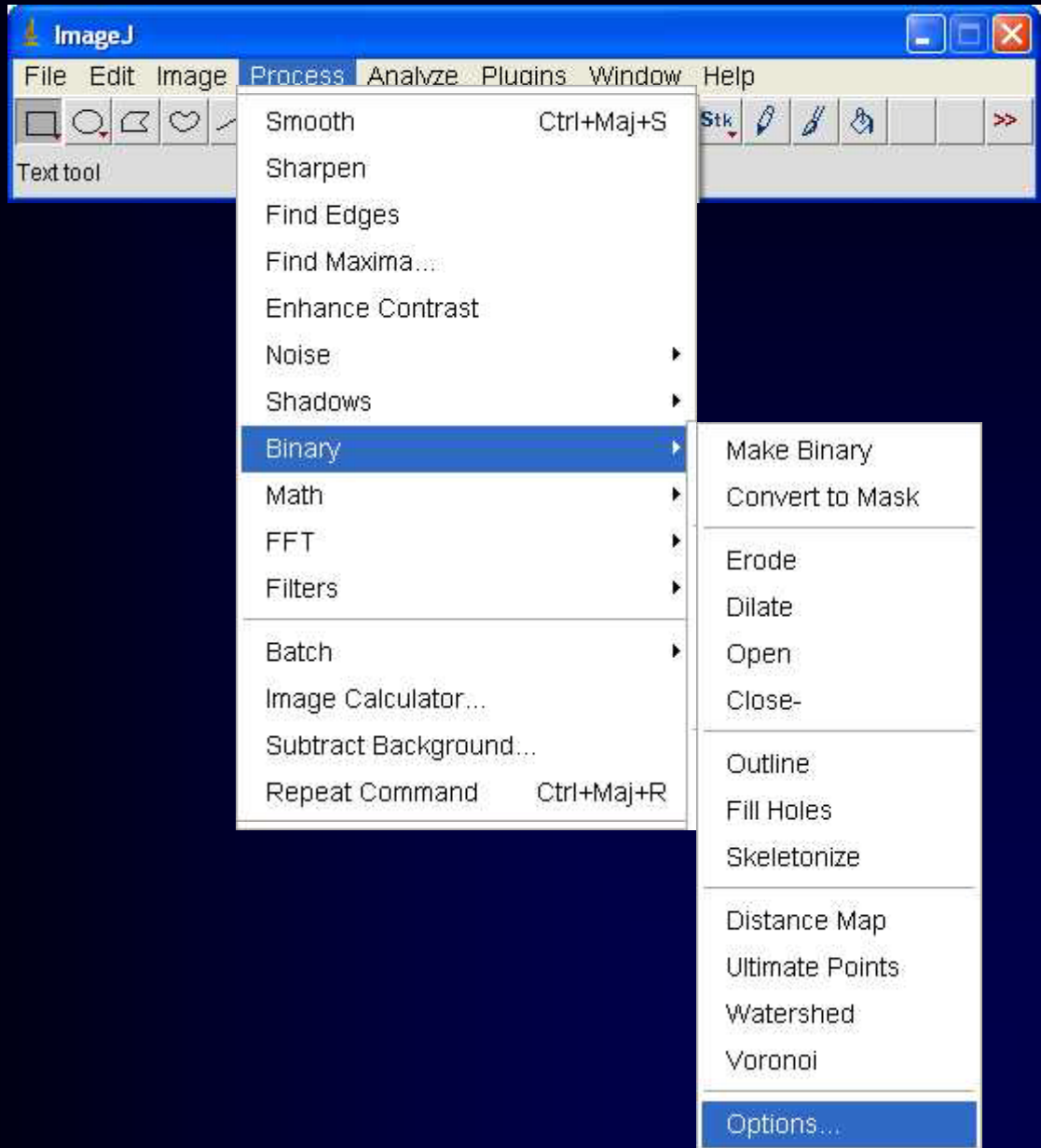


# Morpho\_math





# Menu Binary

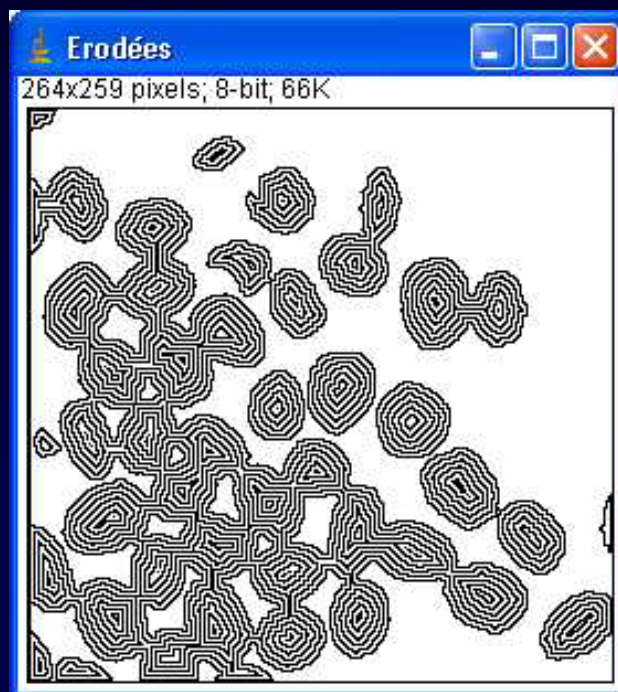
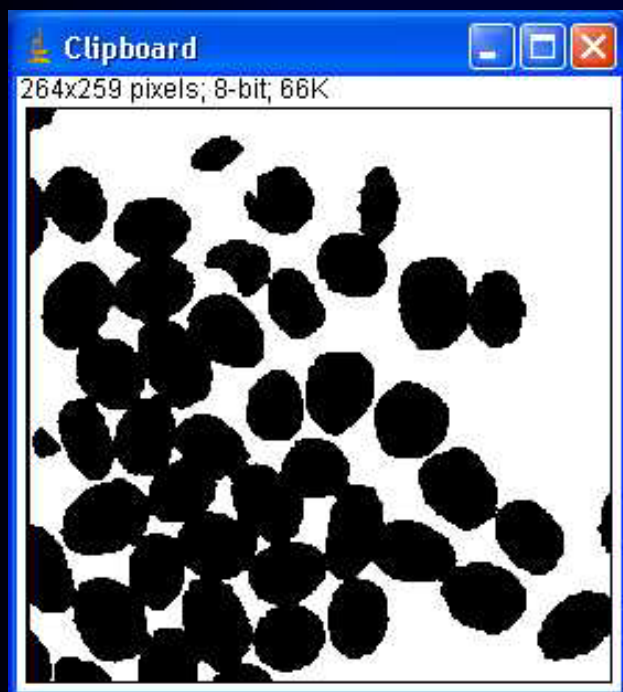


Process → Binary → Options



# Erodé Ultime

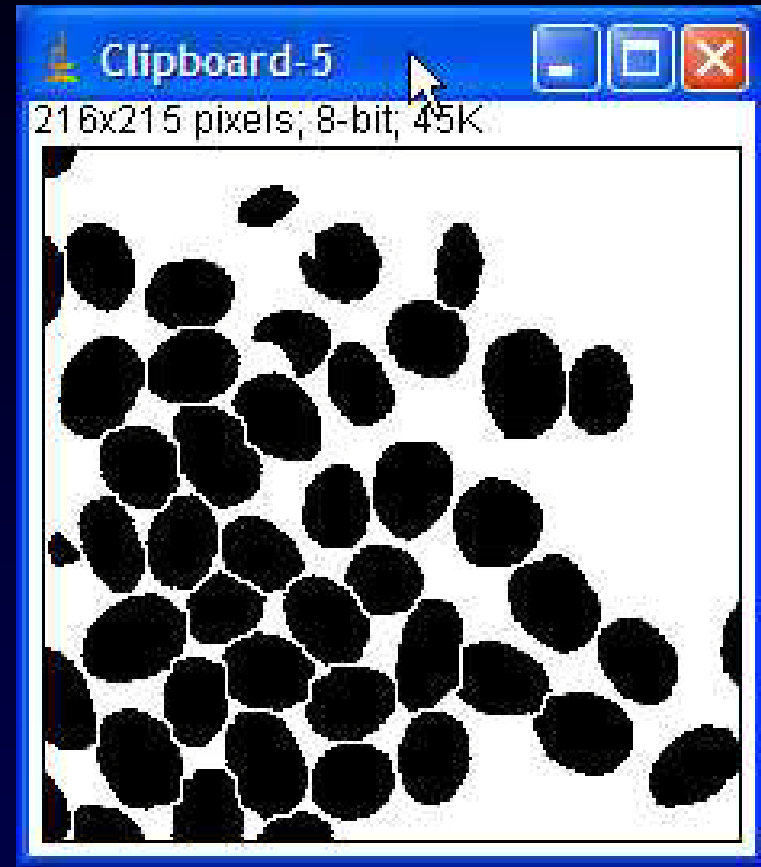
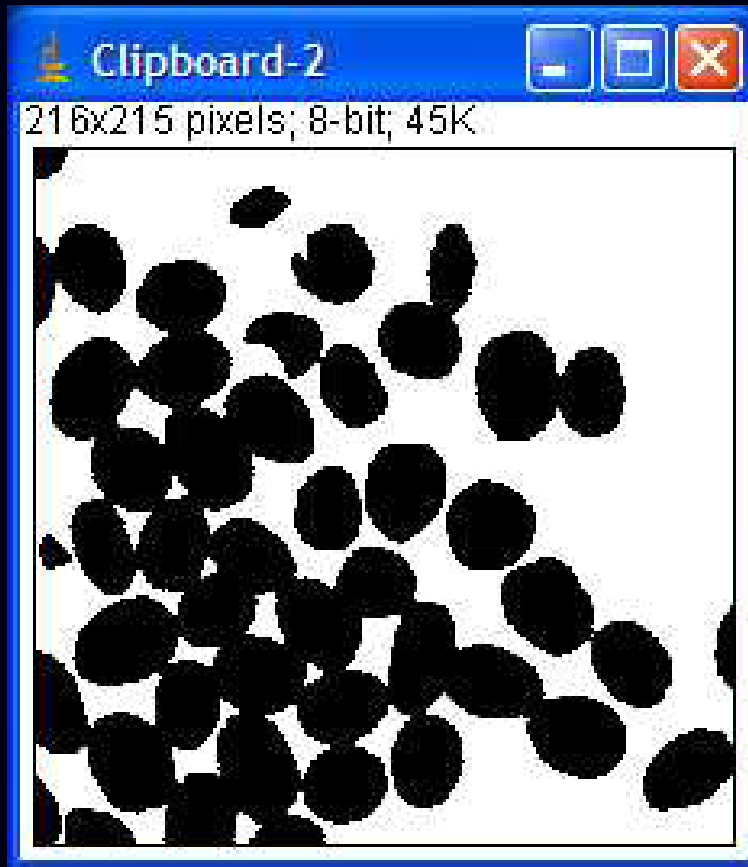
L'érodé ultime : la dernière fraction de l'objet restant avant sa disparition lors d'érosions répétées.



Process → Binary → Ultimate Points



# La ligne de partage des eaux : watershed

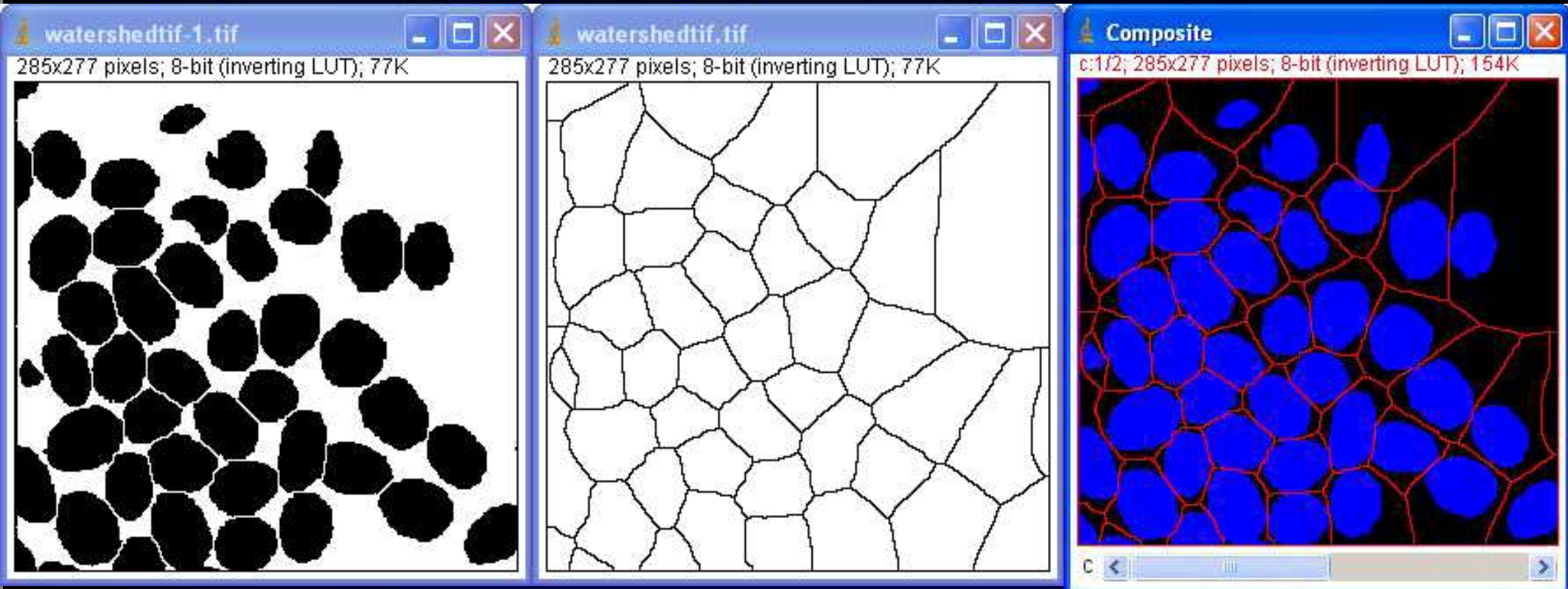


Cette transformation morphologique est la principale méthode de segmentation d'images proposées par la morphologie mathématique.

Process → Binary → Watershed



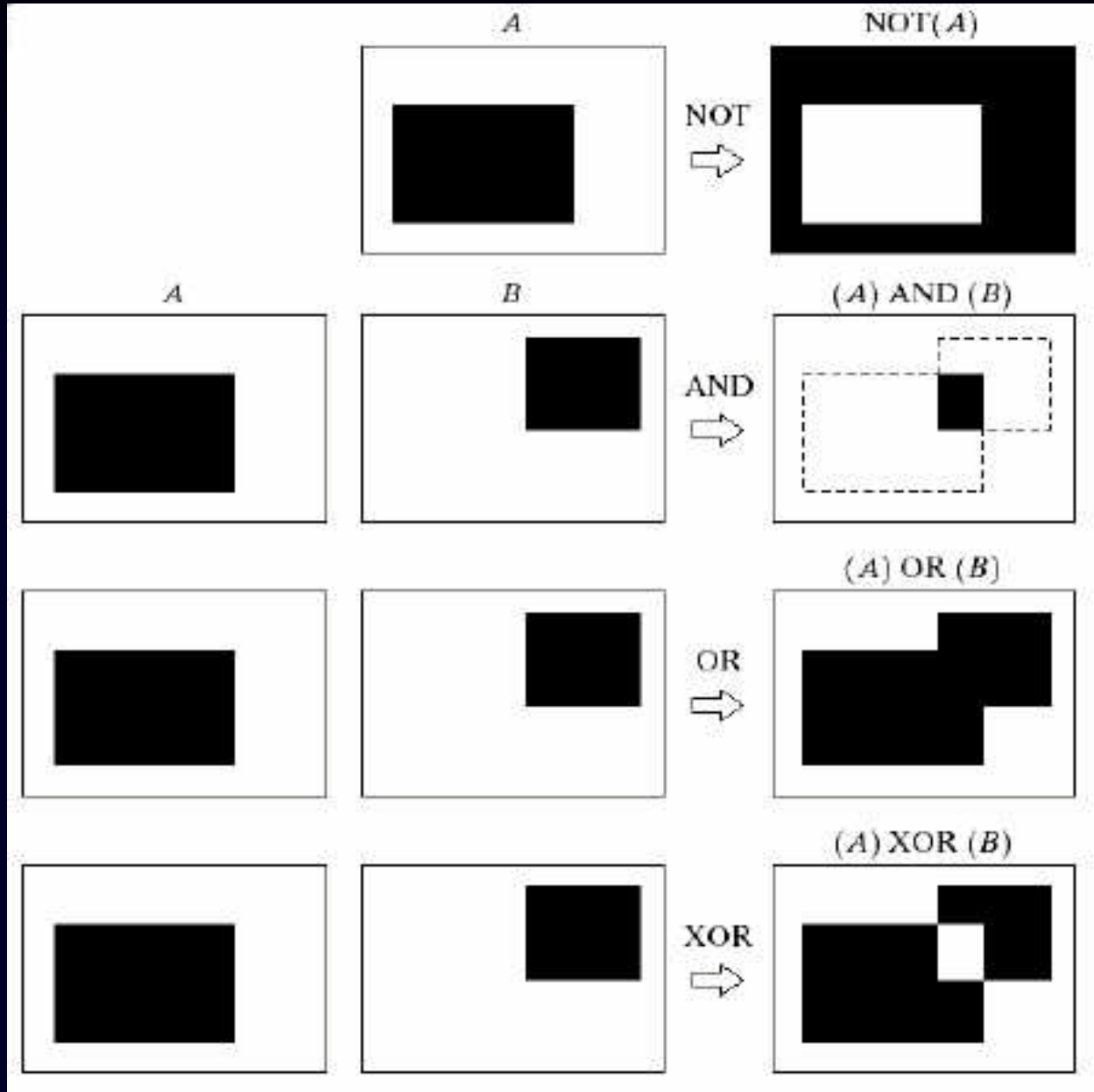
# Diagramme de Voronoï



Process → Binary → Voronoï



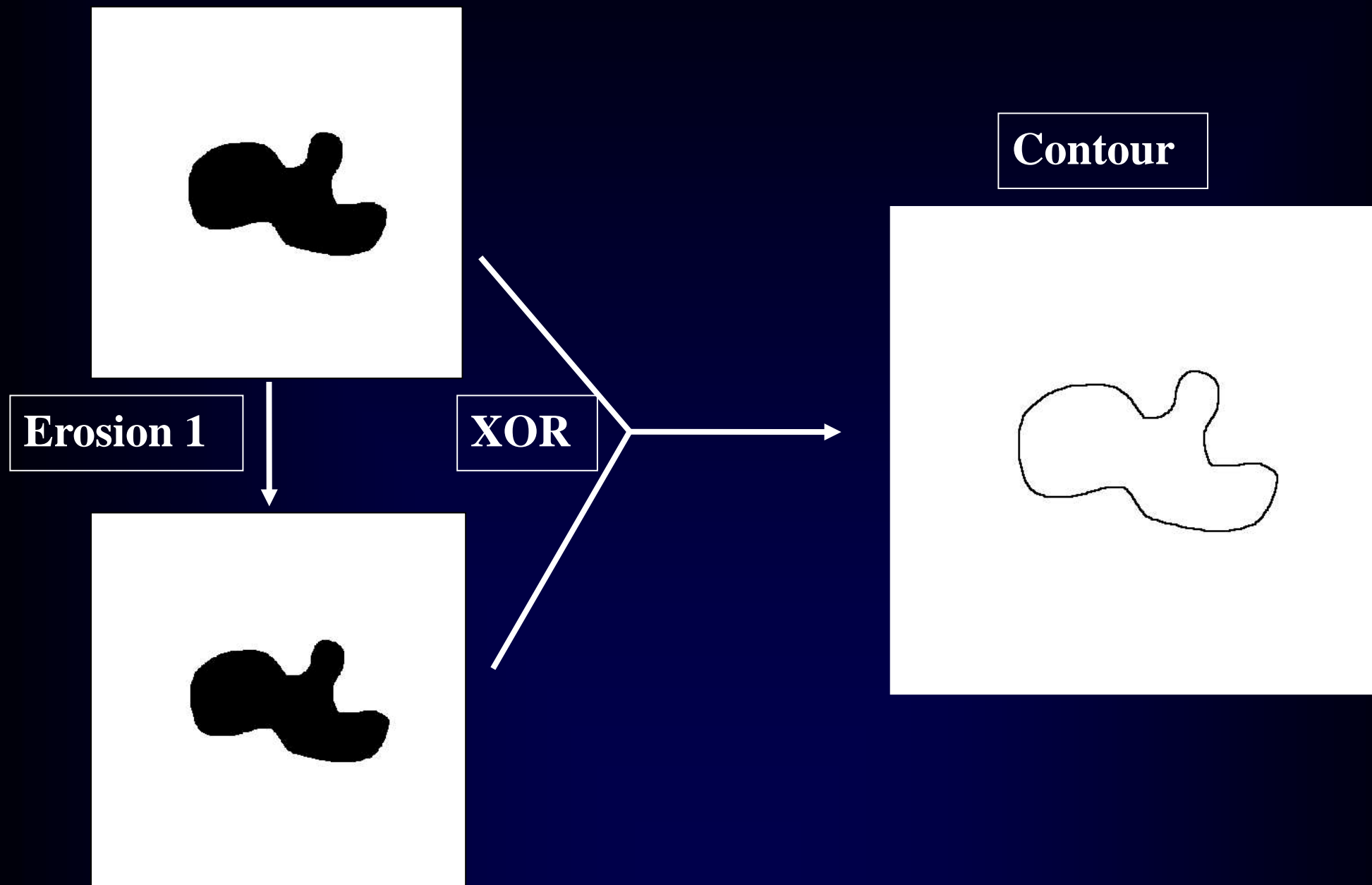
# Opérations logiques



Process→Image Calculator...



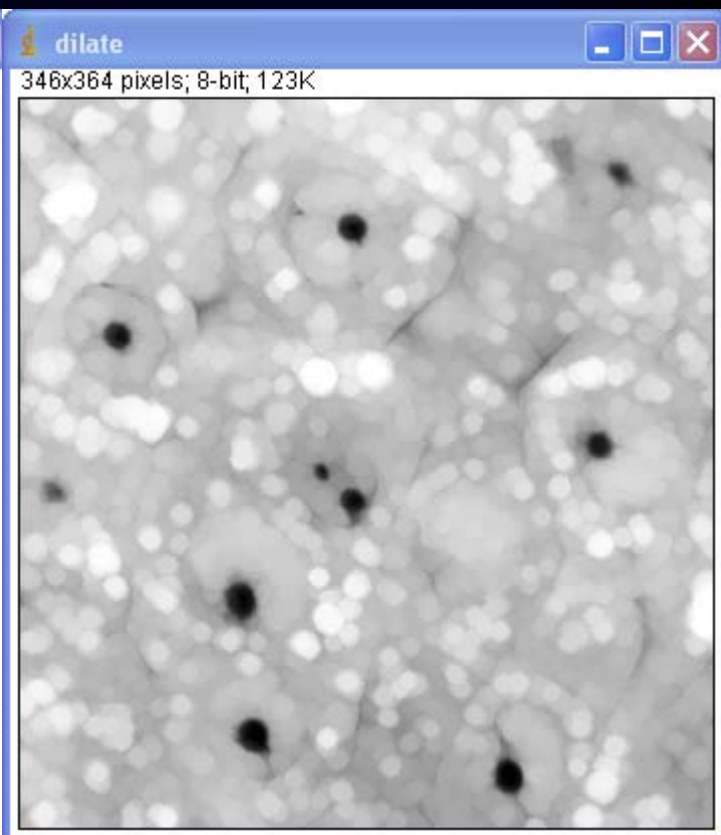
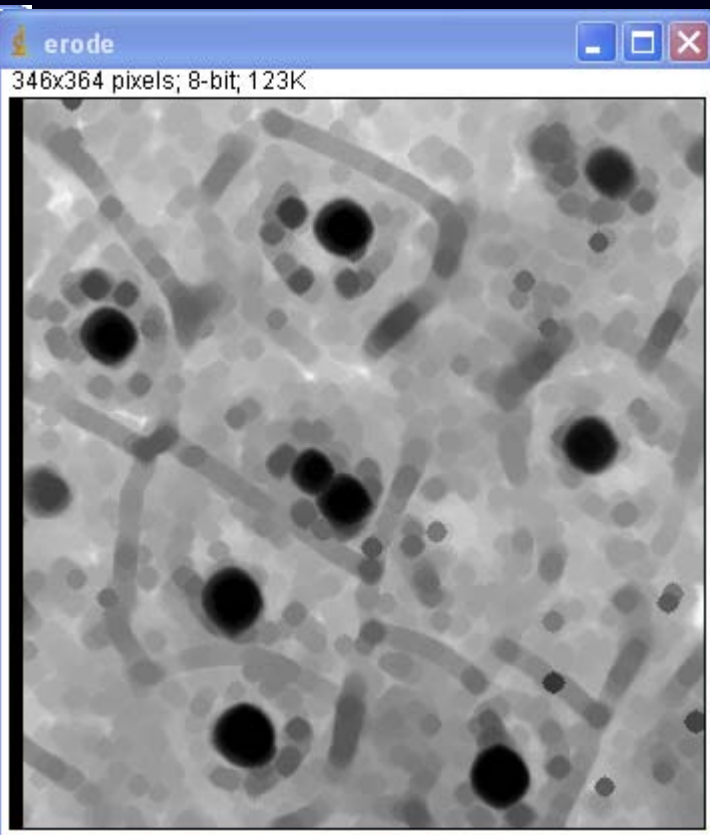
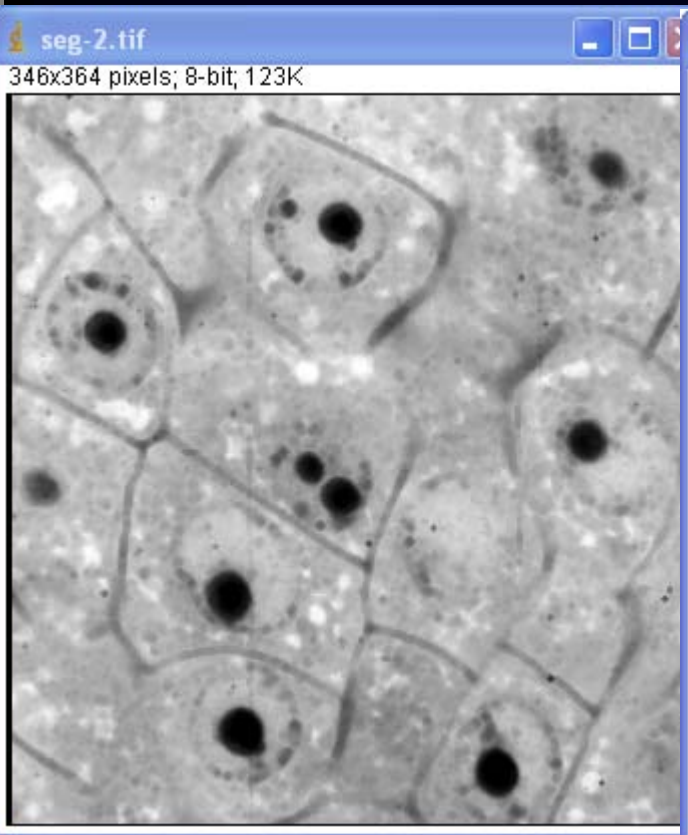
# Construction d'une fonction contour







# Morpho\_math en niveaux de gris



**Plugin  
Grayscale Morphology**

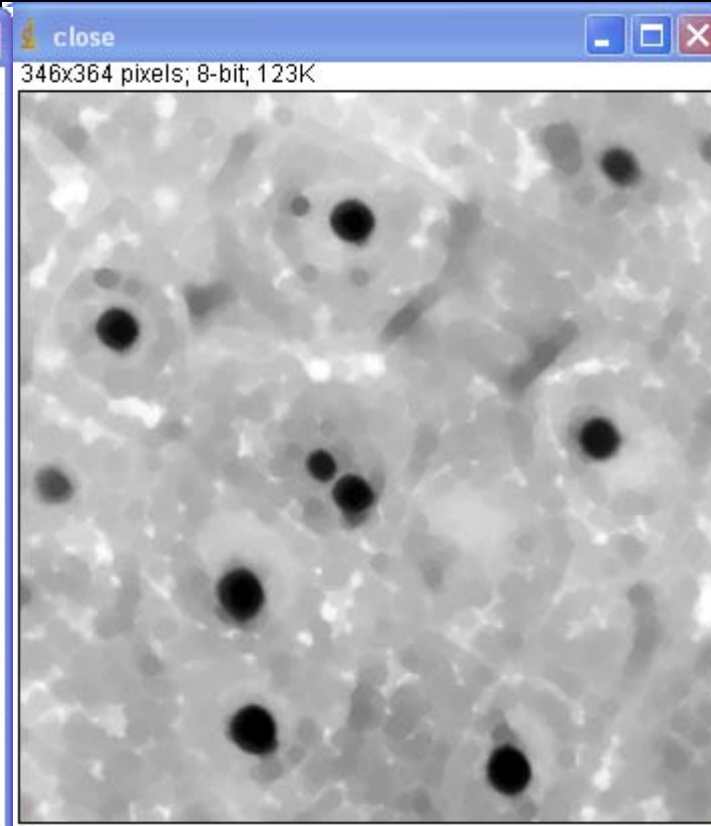
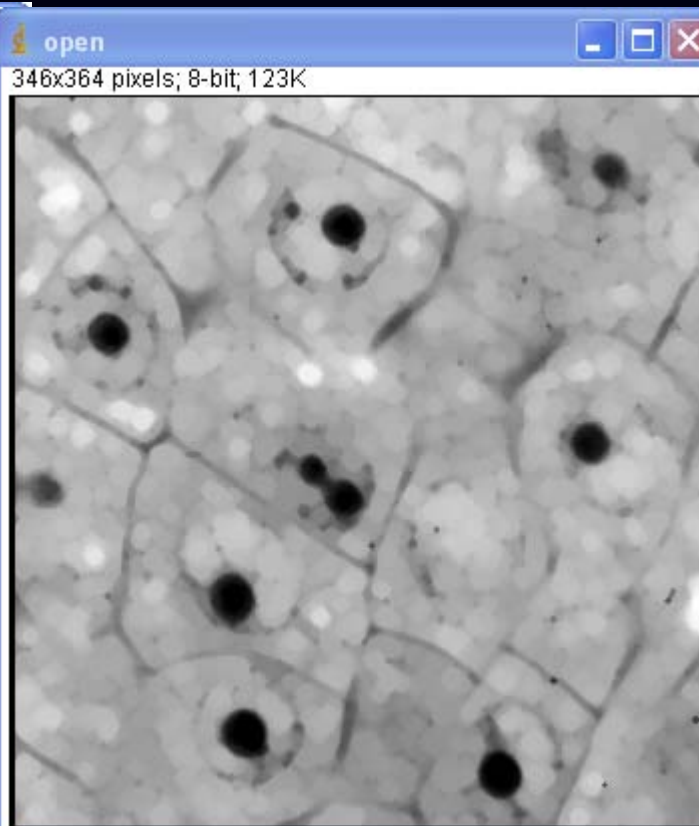
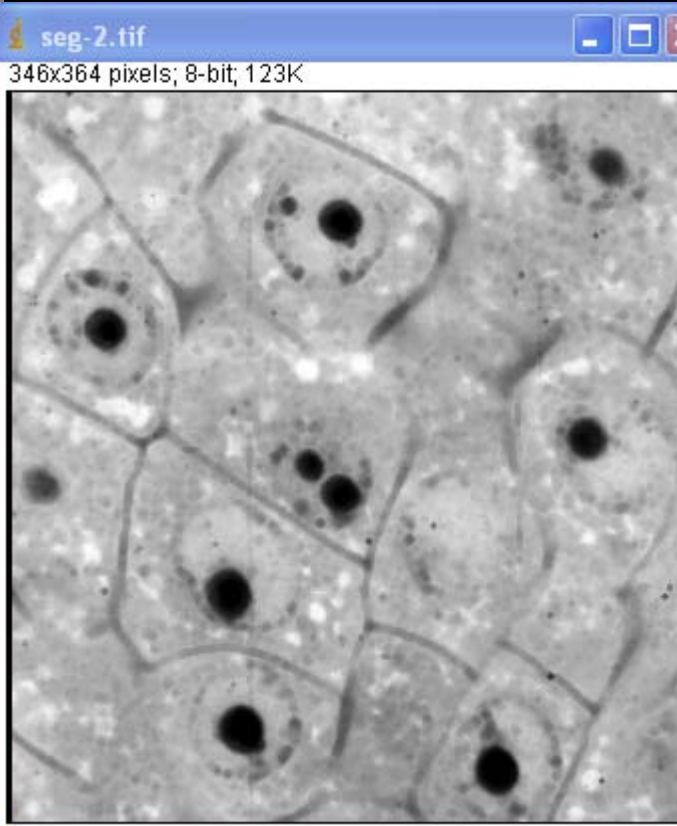
**Erosion  
agrandit les zones  
sombres**

**Dilatation  
agrandit les zones  
claires**

Plugins → Morphology → Gray Morphology



# Morpho\_math en niveaux de gris



**Ouverture**  
**Erosion puis**  
**Dilatation**  
**supprime les petites**  
**zones claires**

**Fermeture**  
**Dilatation puis**  
**Erosion**  
**supprime les petites**  
**zones sombres**

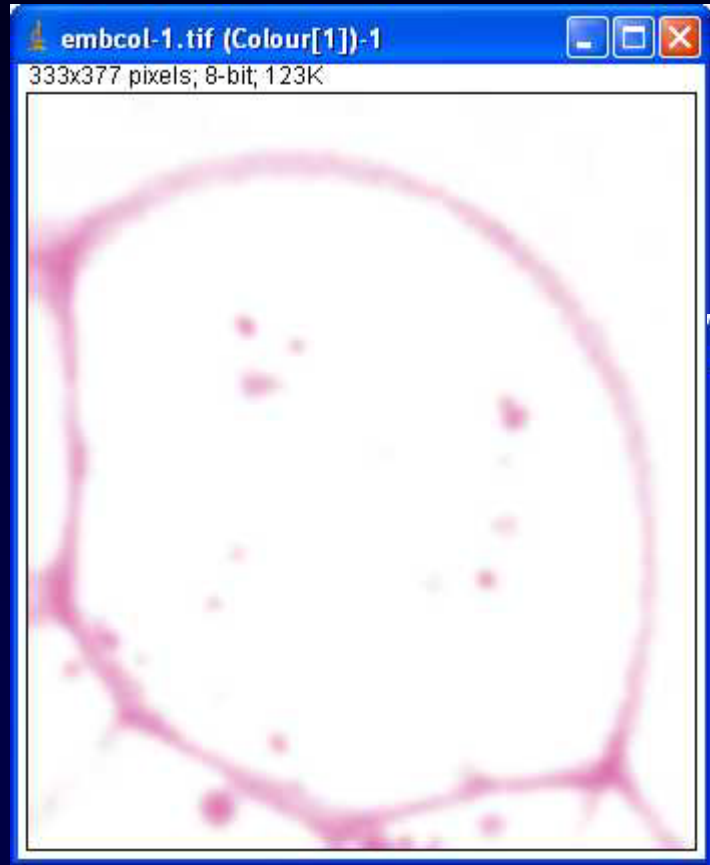
Plugins → Morphology → Gray Morphology



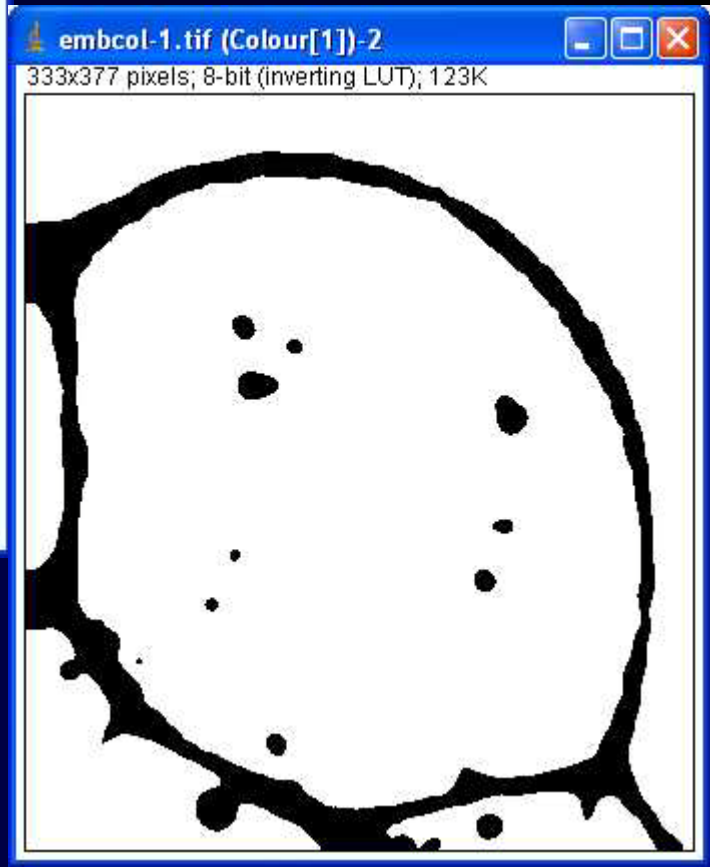
# Exemple



## Colour Deconvolution



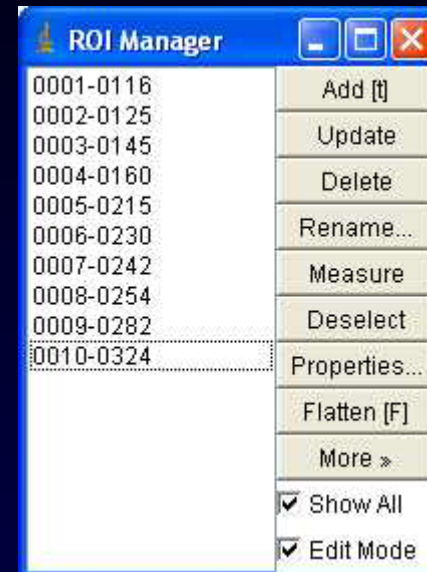
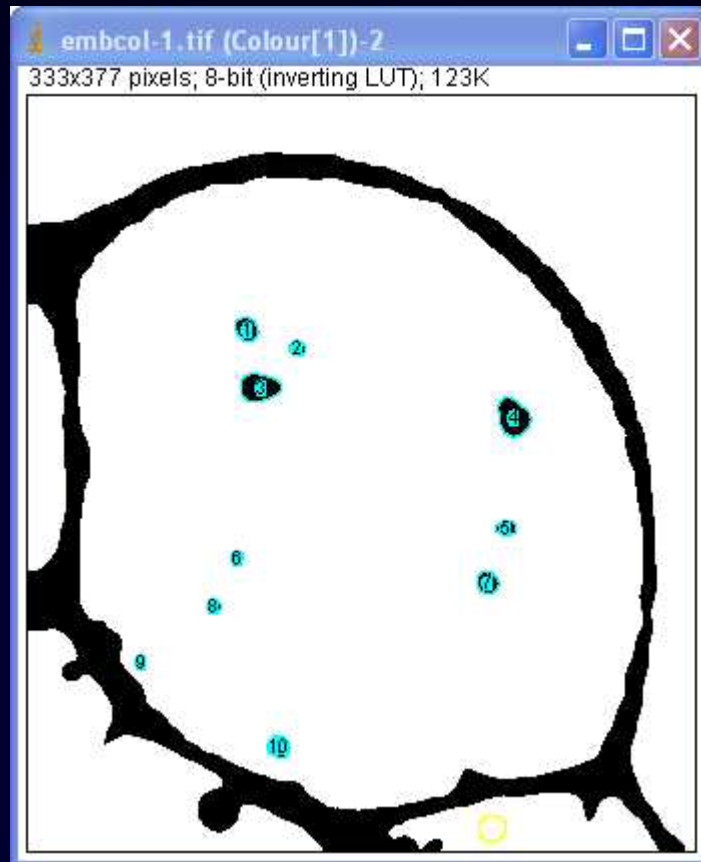
## Seuillage





# Exemple

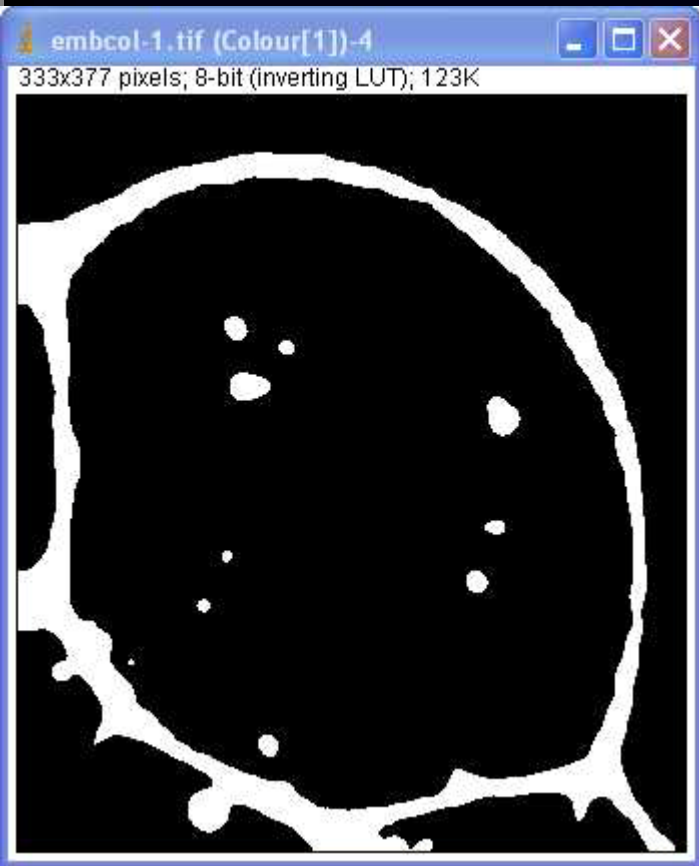
Analyse particules → ROI Manager



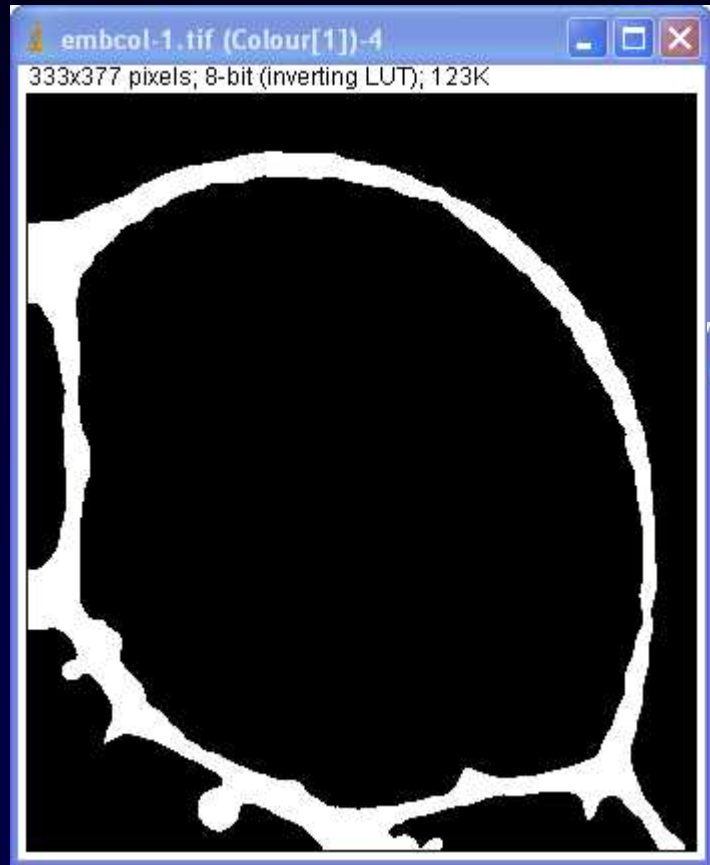


# Exemple

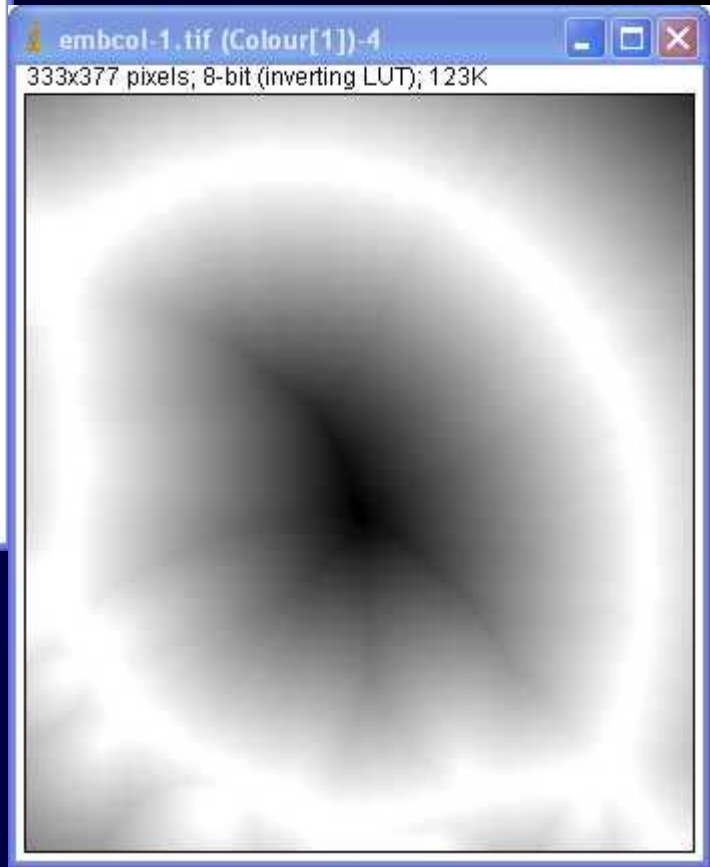
## Inversion



## Boucher les trous



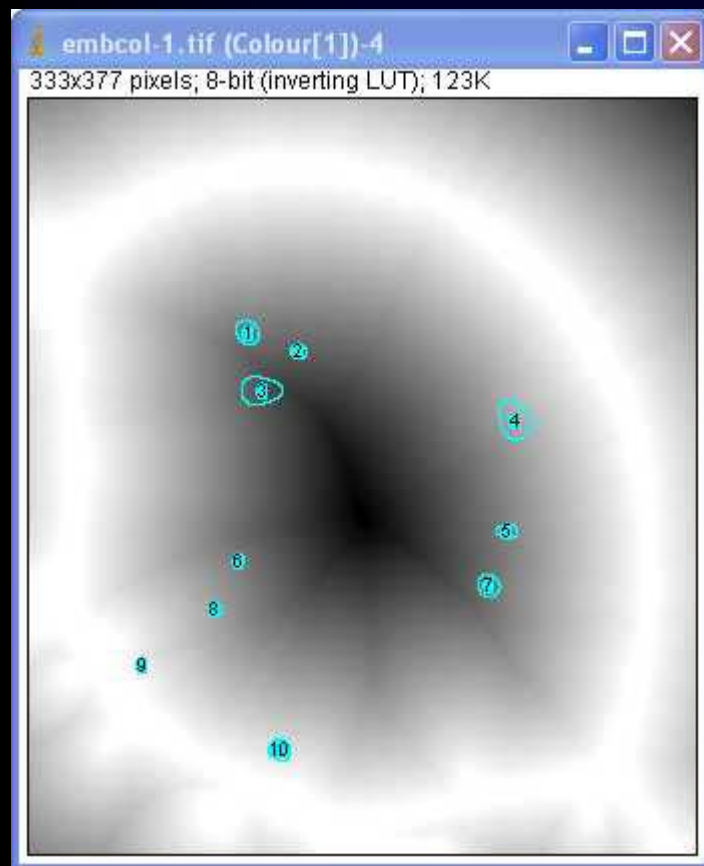
## Carte des distances





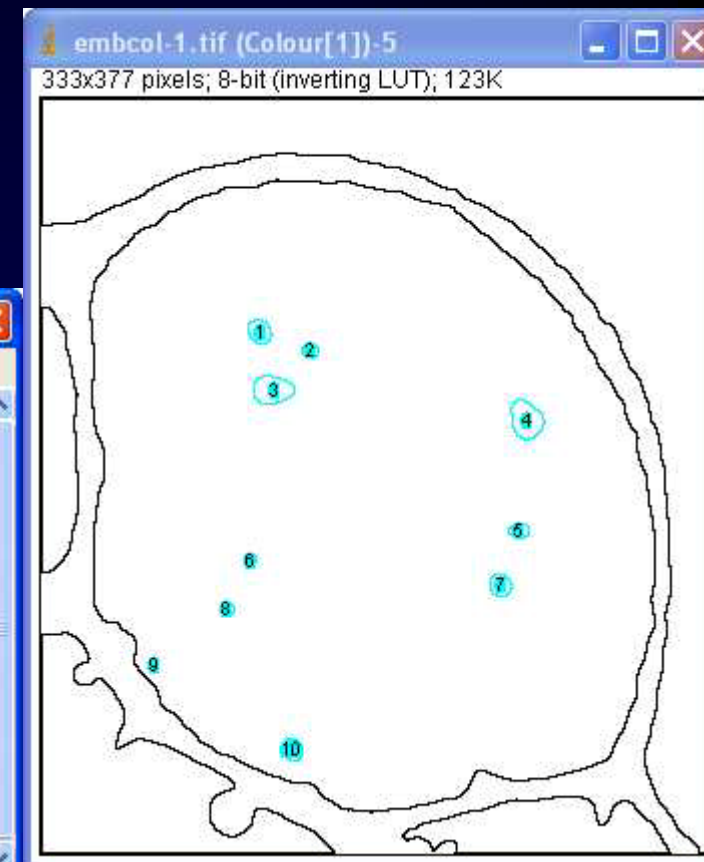
# Exemple

Mesures des moyennes de niveaux de gris  
sur la carte des distances →  
Estimation de la position des grains d'amidon  
dans la cellule



ROI ID	Bounding Box
0001	0116
0002	0125
0003	0145
0004	0160
0005	0215
0006	0230
0007	0242
0008	0254
0009	0282
0010	0324

File	Edit	Font	Results
	Area	Mean	
1	107	72.196	
2	46	84.174	
3	220	89.295	
4	235	46.170	
5	57	67.368	
6	24	71	
7	95	76.737	
8	33	49.273	
9	7	8.143	
10	88	16.625	





# Topic 09 – Mathematical Morphology



L'image numérique

Les Prétraitements

La Segmentation

Les Post-traitements

**La Quantification**





# Mesures

ImageJ

File Edit Image Process **Analyze** Plugins Window Help

Segmented line selections

- Measure Ctrl+M
- Analyze Particles...
- Summarize
- Distribution...
- Label
- Clear Results
- Set Measurements...
- Set Scale...
- Calibrate...
- Histogram Ctrl+H
- Plot Profile Ctrl+K
- Surface Plot...
- Gels
- Tools

Analyze Particles

Size (pixel<sup>2</sup>):

Circularity:

Show:

Display results  Exclude on edges

Clear results  Include holes

Summarize  Record starts

Add to Manager  In situ Show

OK Cancel Help

Set Measurements

Area  Mean gray value

Standard deviation  Modal gray value

Min & max gray value  Centroid

Center of mass  Perimeter

Bounding rectangle  Fit ellipse

Shape descriptors  Feret's diameter

Integrated density  Median

Skewness  Kurtosis

Area fraction  Stack position

Limit to threshold  Display label

Invert Y coordinates  Scientific notation

Redirect to:

Decimal places (0-9):

OK Cancel Help

Histogram of Cellules

300x240 pixels; 8-bit; 70K

Count: 1655680    Min: 0  
 Mean: 177.263    Max: 255  
 StdDev: 54.860    Mode: 248 (64439)

List Copy Log Value: 238  
 Count: 10284

Set Scale

Distance in Pixels:

Known Distance:

Pixel Aspect Ratio:

Unit of Length:

Scale: 1200 pixels/inch

Global

OK Cancel

Plot of Cellules

620x250 pixels; 8-bit; 126K

Gray Value

Distance (inches)

List Save... Copy... X=0.43, Y=244.3



# Mesures

- Longueur
- Surface
- Périmètre
- Moyenne des valeurs de niveaux de gris
- Niveau de gris le plus fréquent (sommet de l'histogramme)
- Min et Max des niveaux de gris
- Centroid : moyenne des x,y
- Center off mass : moyenne des x,y pondérée par les intensités de niveaux de gris
- Circularité :  $4\pi(\text{surface} / \text{périmètre}^2) = 1$  pour un cercle
- Etc ...



# Topic 10 – Quantification