

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

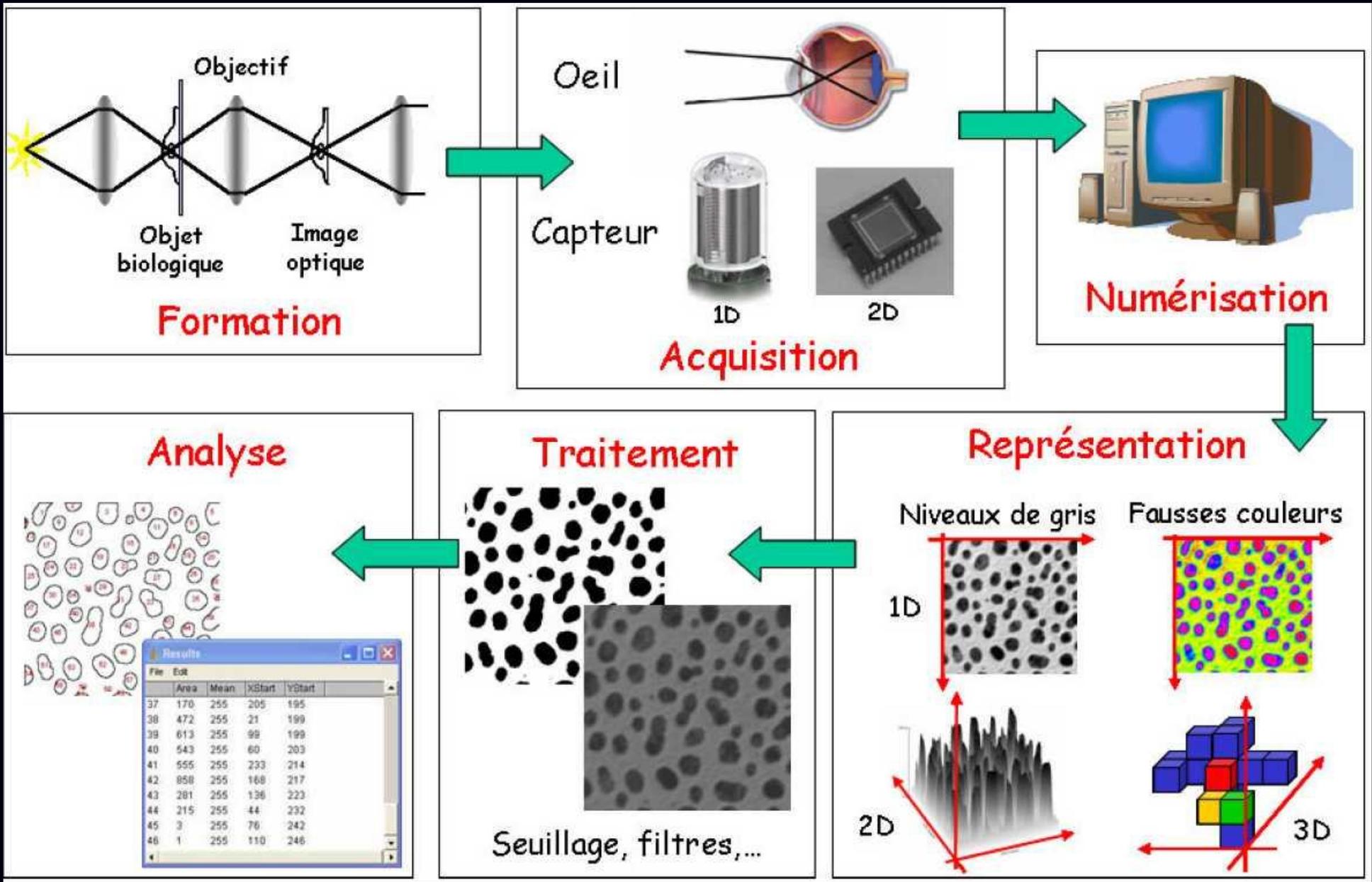
Quantification → données

Amélioration

Publication



Le traitement et l'analyse d'images





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

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 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, [ImageJ64](#), which uses Java 1.6 in 64-bit mode on Intel Macs running OS X 10.4 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](#))

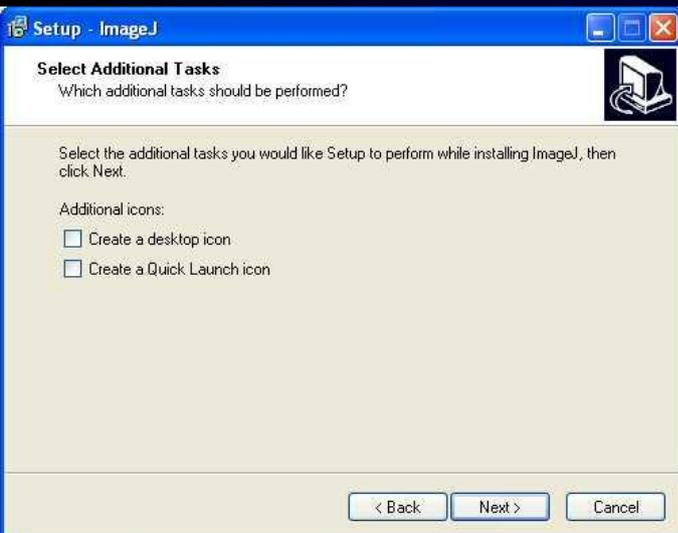
Ouverture de ij146-jdk6-setup.exe

Vous avez choisi d'ouvrir :

- ij146-jdk6-setup.exe**
qui est un fichier de type : Binary File (27.2 Mo)
à partir de : http://rsbweb.nih.gov

Voulez-vous enregistrer ce fichier ?

Installation



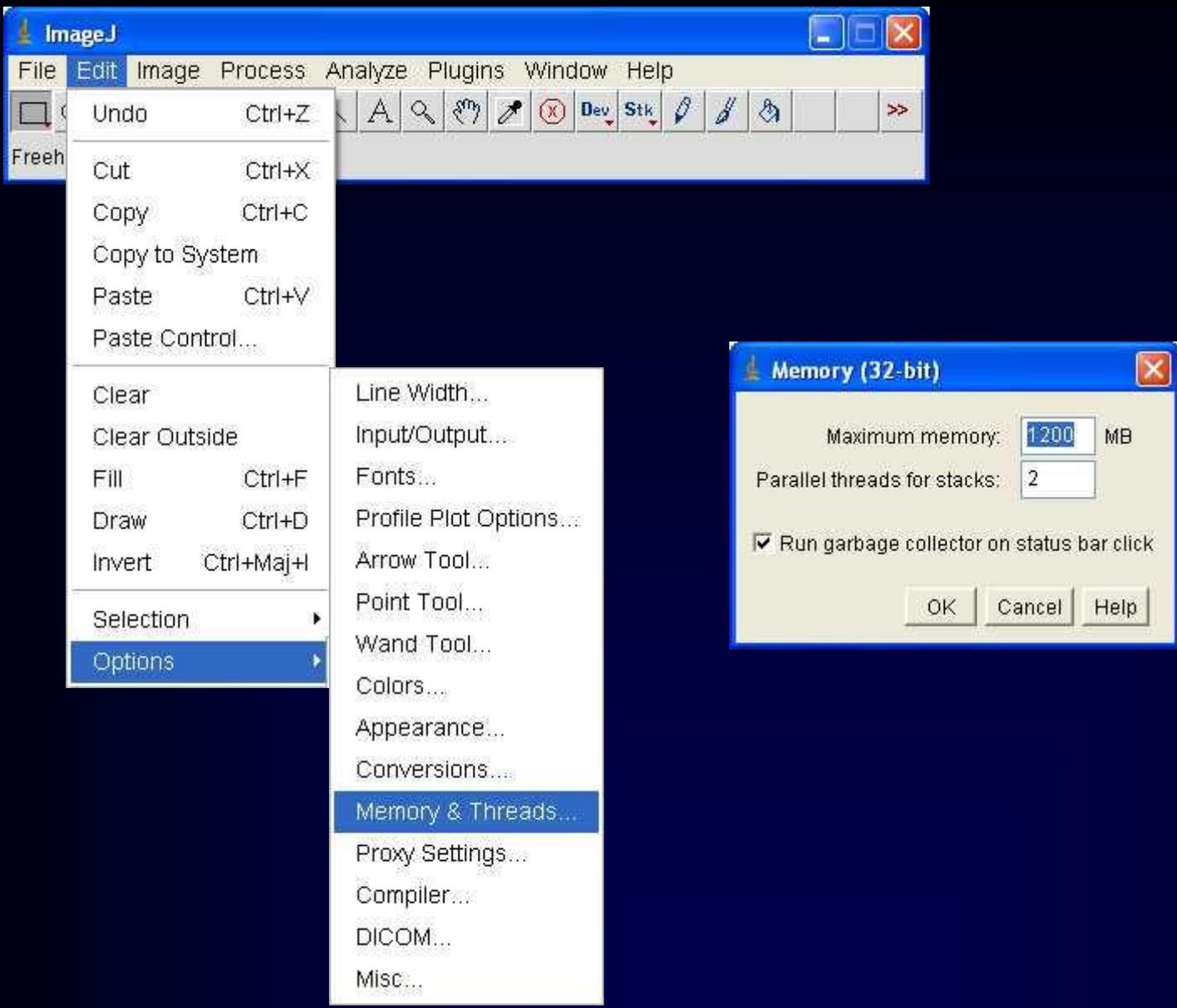


Configuration



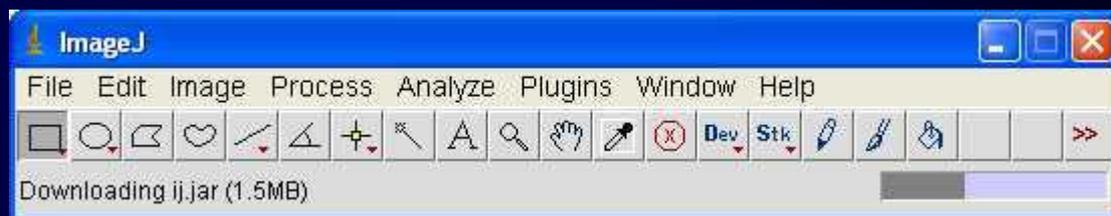
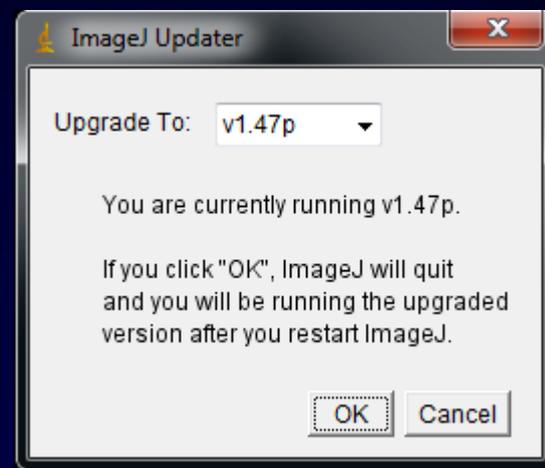
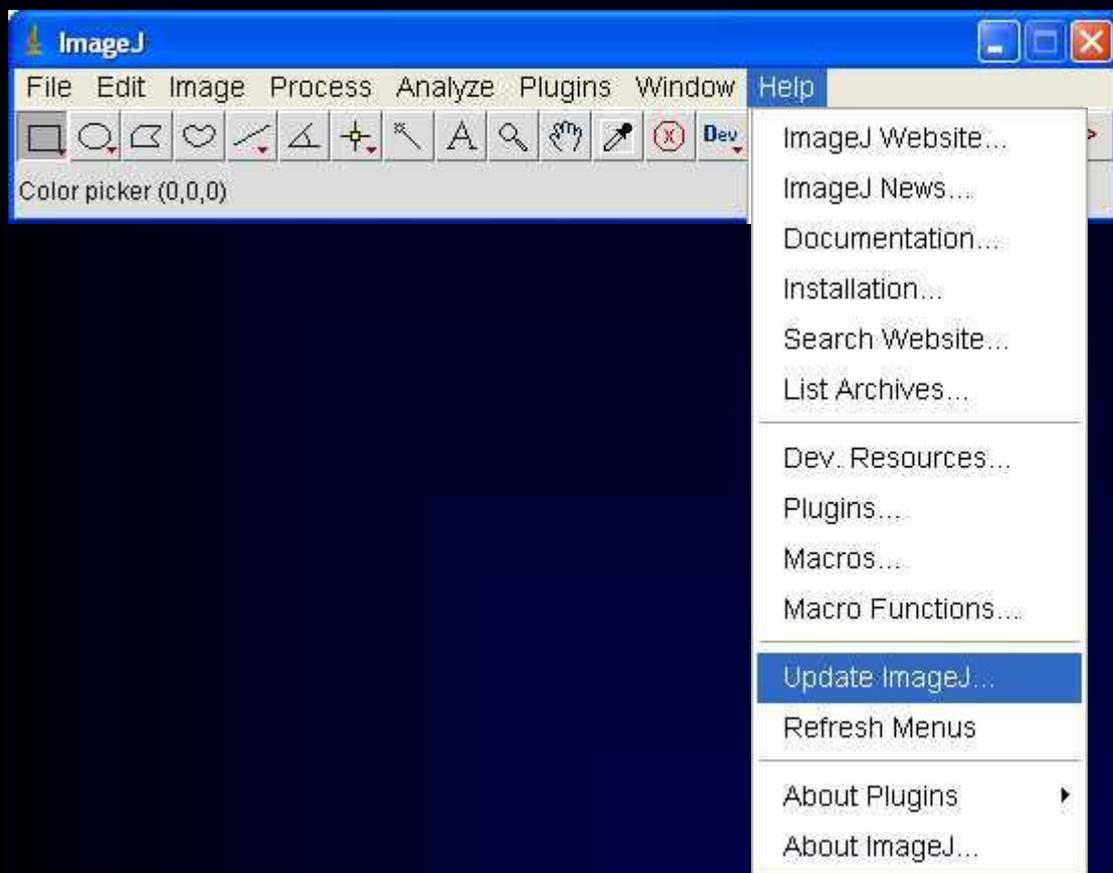


Configuration



Edit → Option → Memory & Treads...

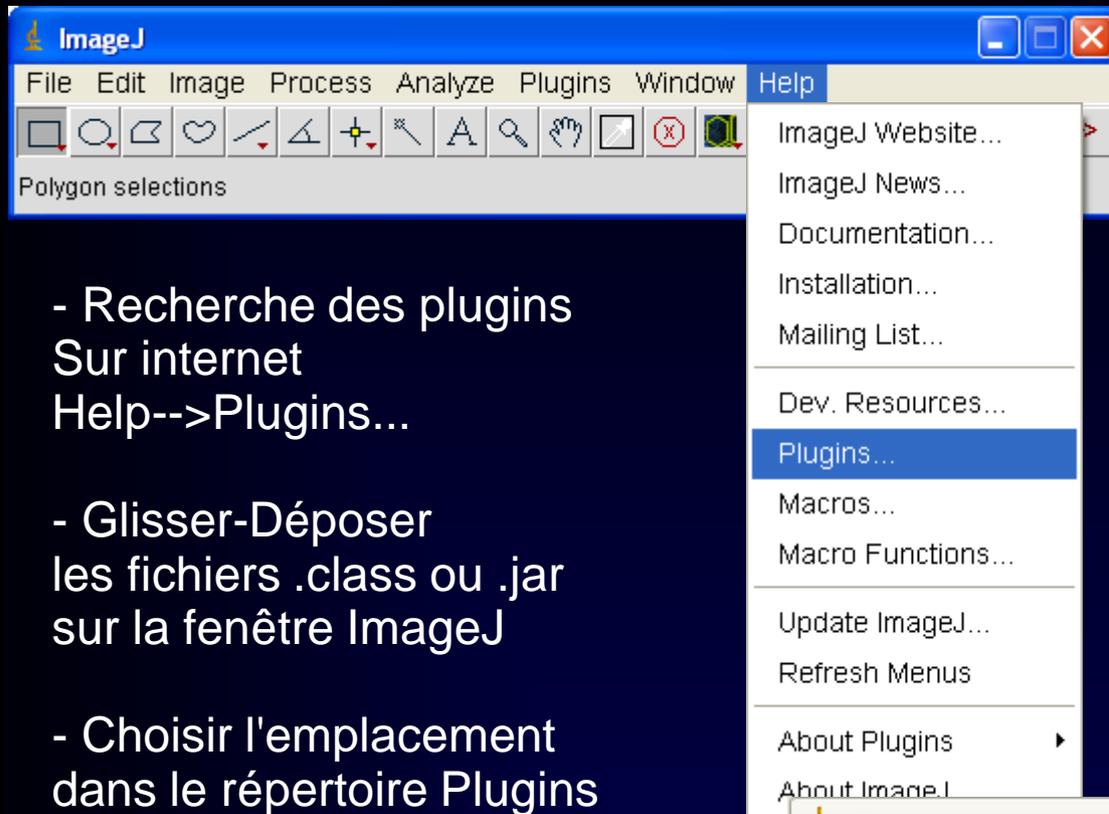
Mise à jour



Help → Update ImageJ...



Installation de Plugins



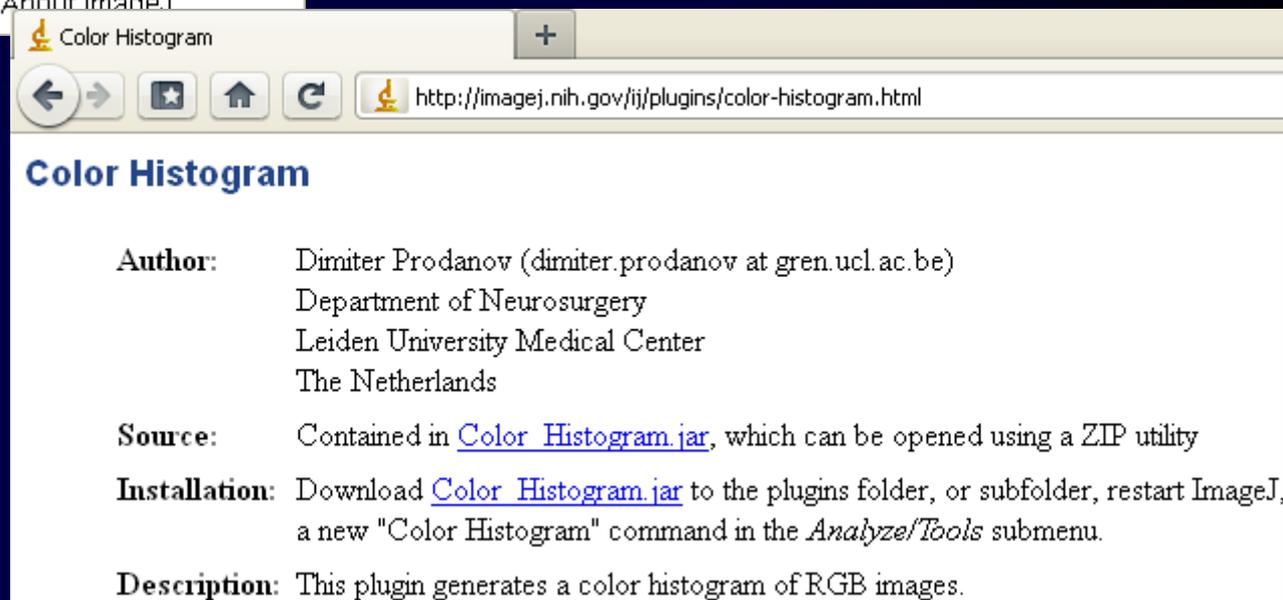
- Recherche des plugins
Sur internet
Help-->Plugins...

- 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

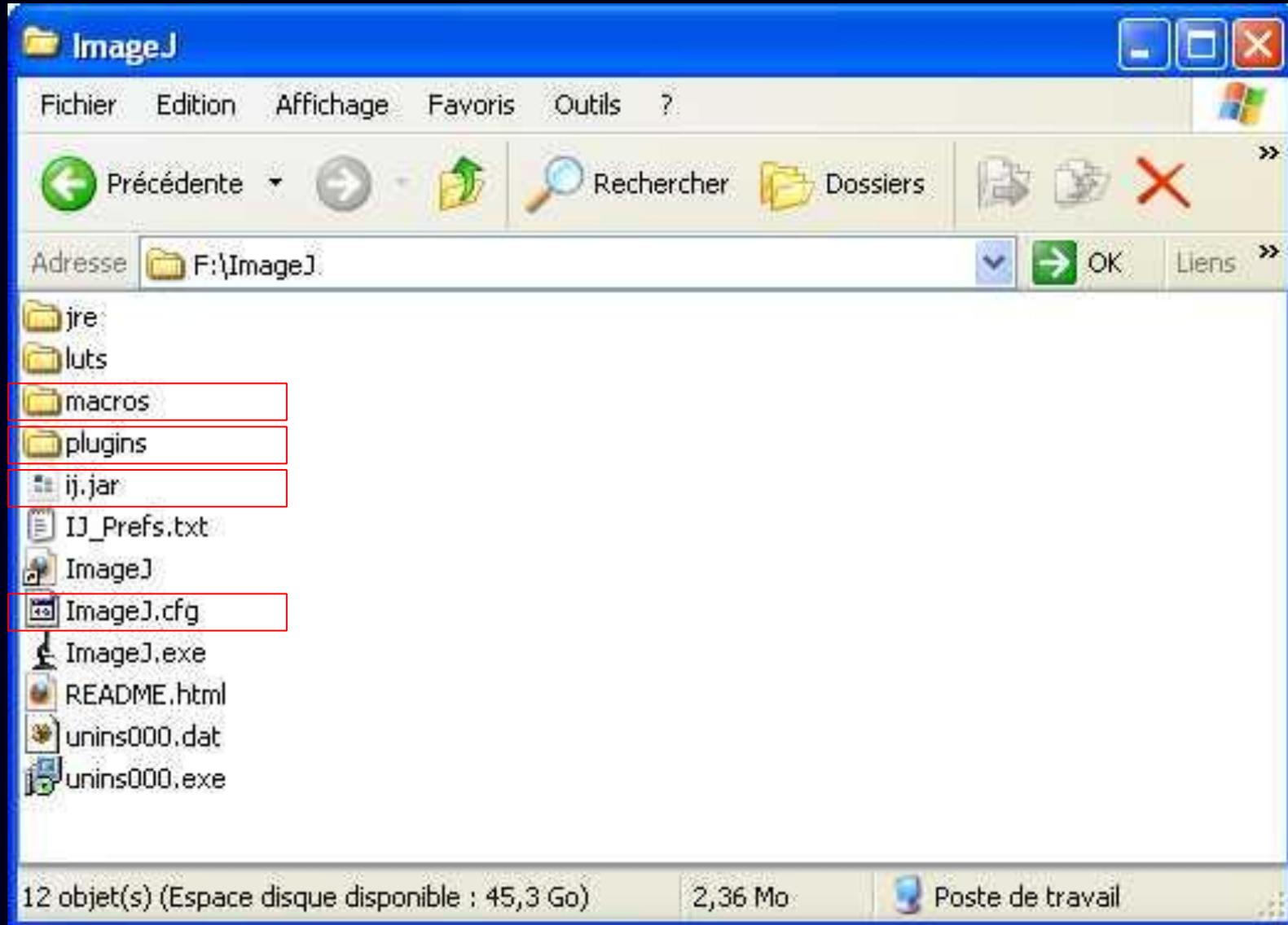
- Apparition de la
nouvelle commande
dans le menu Plugins



Help→ Plugins...

Help→ Refresh Menus

Dossiers ImageJ





Topic 01 - Installation, configuration, help and updates



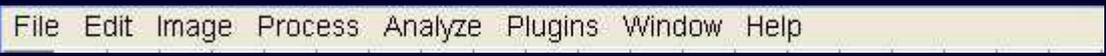
Les bases pour l'utilisation d'ImageJ



Interface



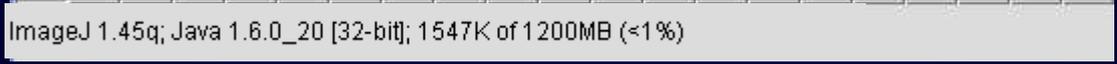
Barre de menu



Barre d'outils



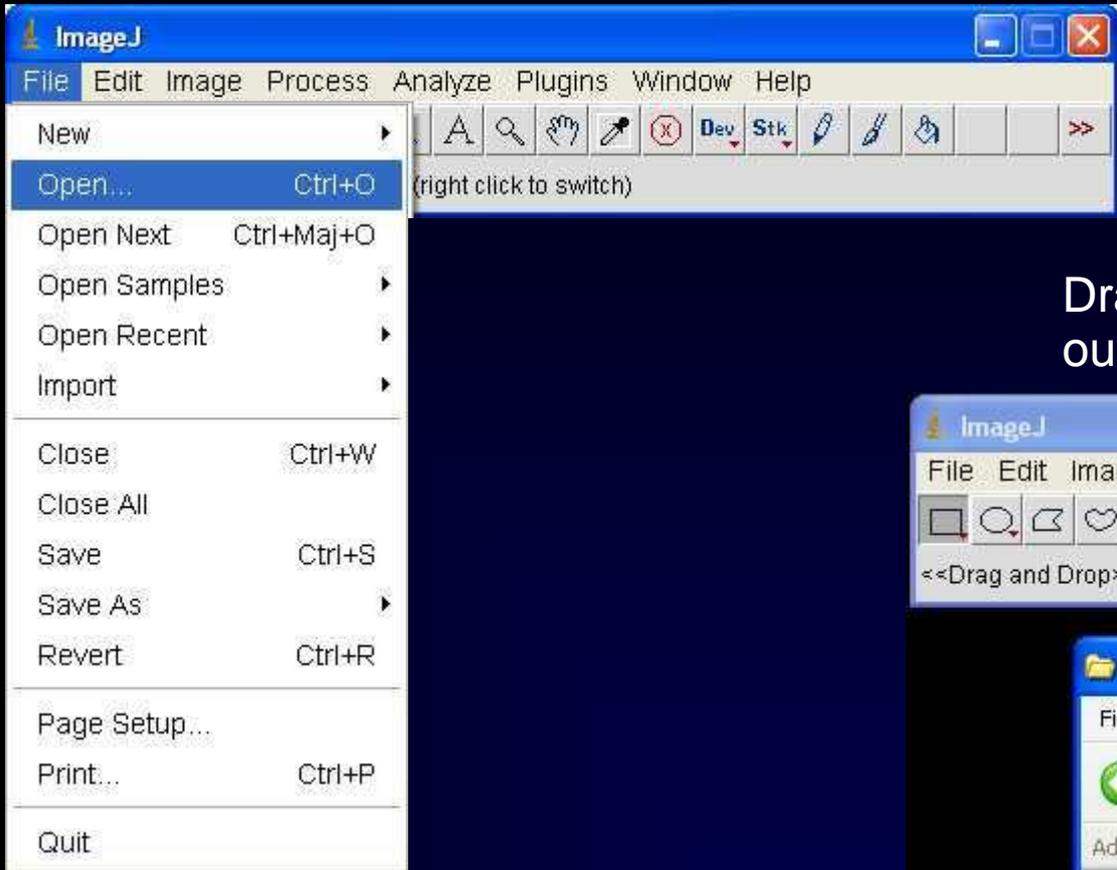
Barre d'état



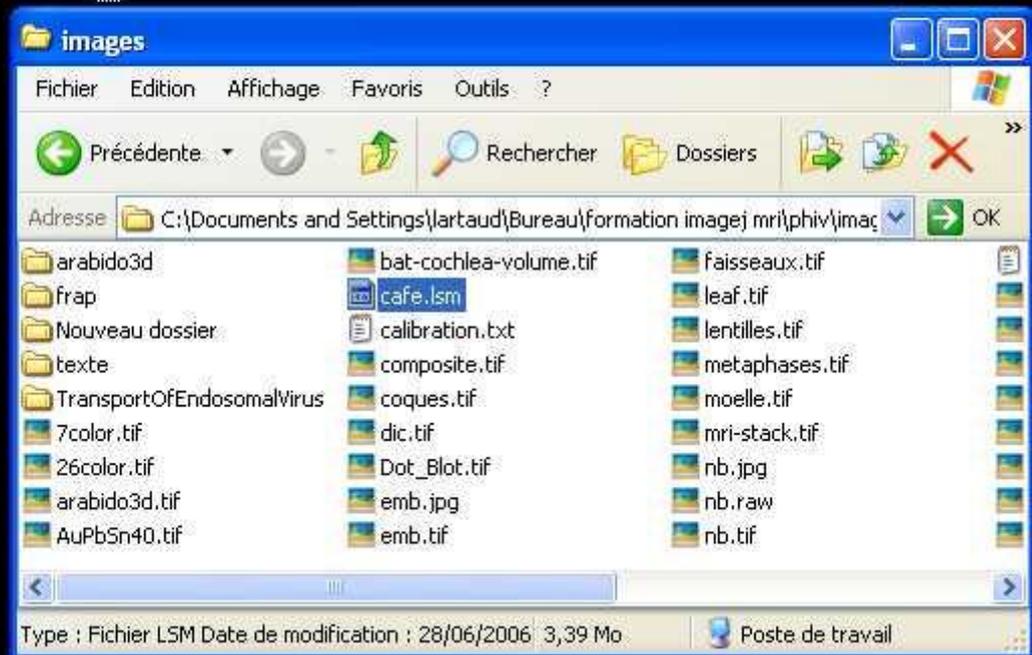


Ouvrir une image

File → Open...



Drag and Drop sur la fenêtre ou l'icône

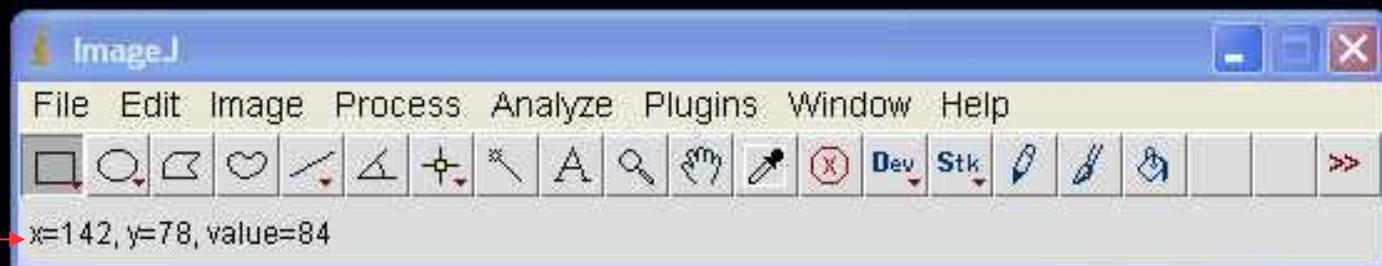


File → Open ...

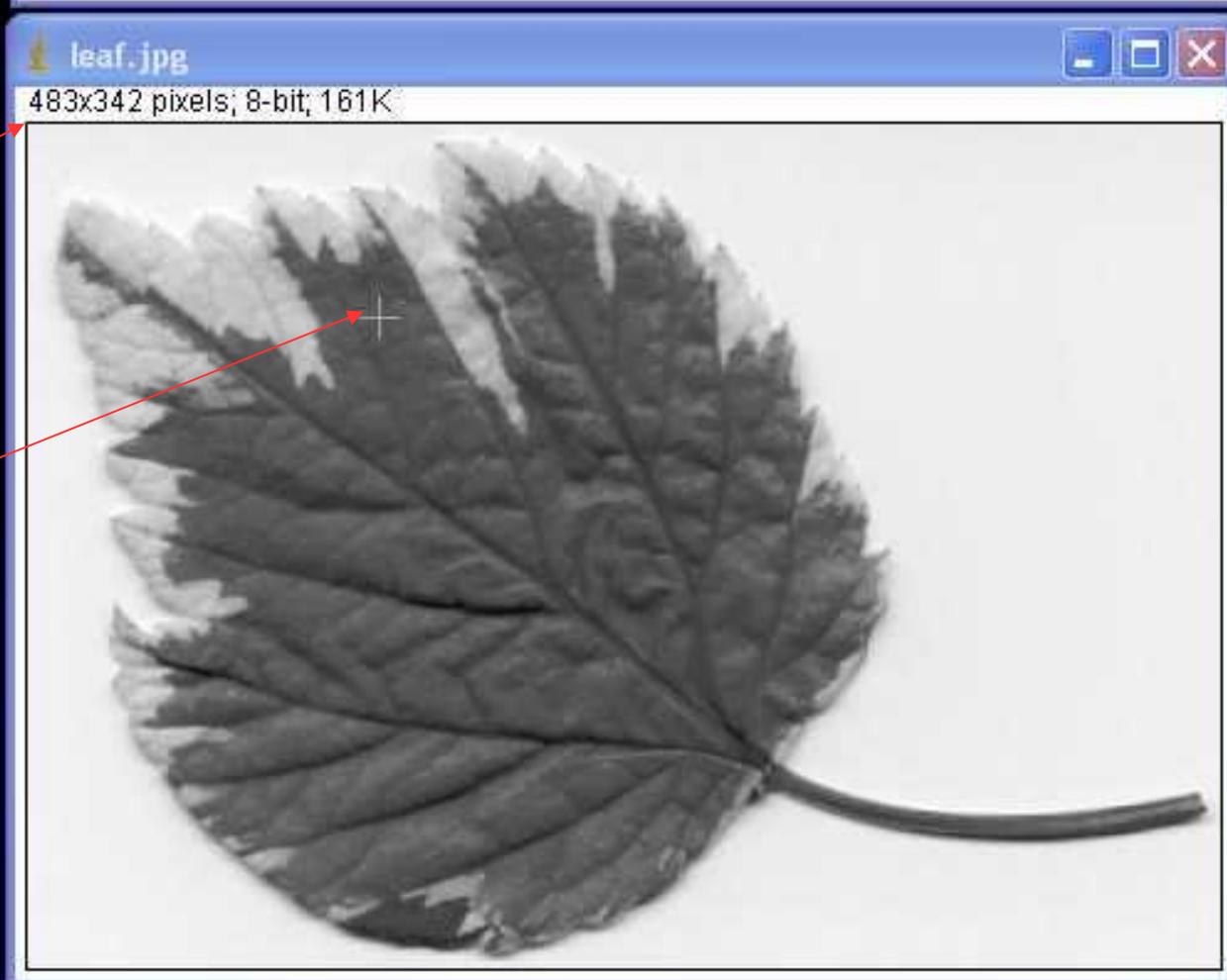


Informations pixel

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

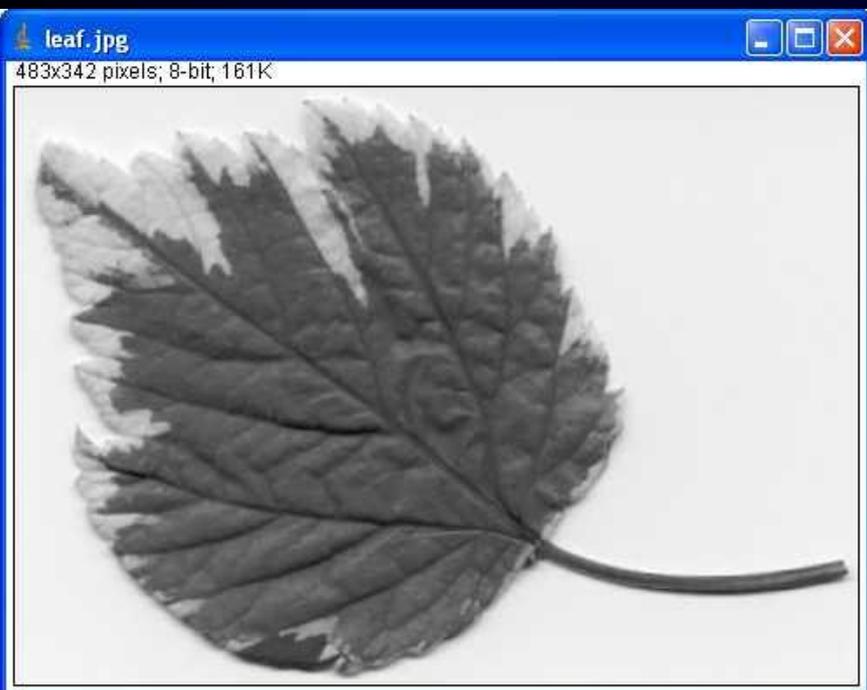
Touche « - »



Touche « + »

Zone affichée

Rapport affichage/image



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



Outils de sélection (ROI)

Rectangl
e

Ovale

Polygone

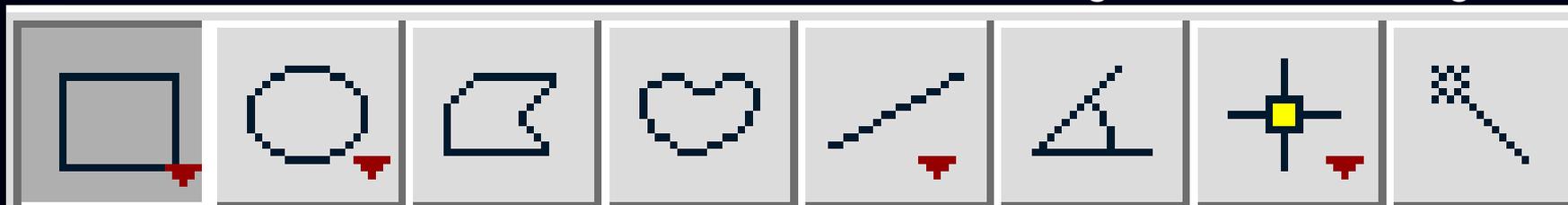
À main levée

Ligne

Angle

Point

Baguette



Choix d'outils différents : Clic droit sur triangle rouge

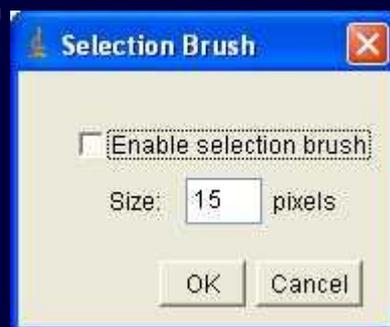
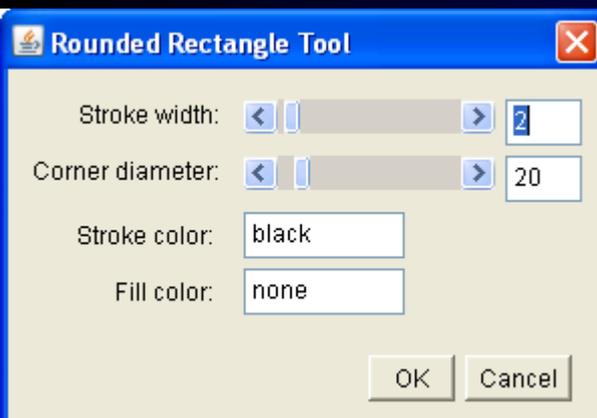
- ✓ Rectangle Tool
- Rounded Rectangle Tool

- ✓ 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

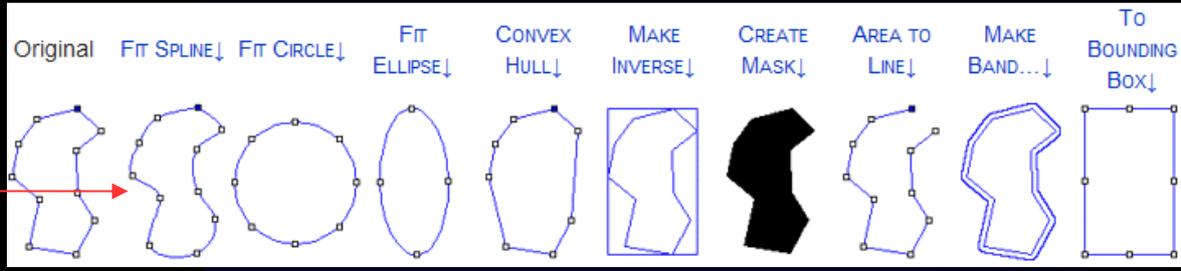
Retrouver la sélection : Edit → Selection → Restore Selection



Menus de sélection

- Select All Ctrl+A
- Select None Ctrl+Maj+A
- Restore Selection Ctrl+Maj+E
- Fit Spline
- Fit Circle
- Fit Ellipse
- Convex Hull
- Make Inverse
- Create Selection
- Create Mask

- Properties... Ctrl+Y
- Rotate...
- Enlarge...
- Make Band...
- Specify...
- Straighten...
- To Bounding Box
- Line to Area
- Area to Line
- Image to Selection...
- Add to Manager Ctrl+T



- ImageJ
- File
- Edit**
- Image
- Process

- 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

Specify

Width:

Height:

X Coordinate:

Y Coordinate:

Oval

Centered

OK Cancel

Edit → 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

emb.tif

333x377 pixels; 8-bit; 123K

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

Multi Plot

Sort

Specify...

Remove Slice Info

Labels...

List

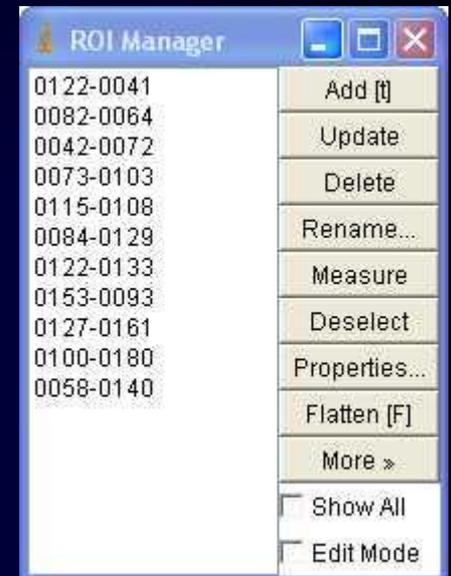
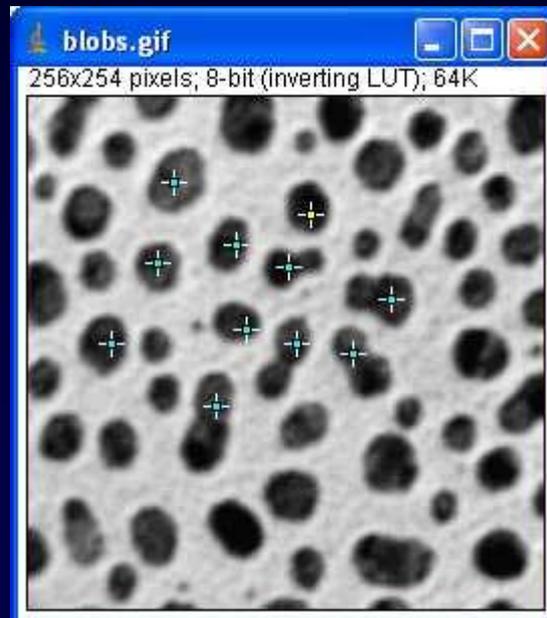
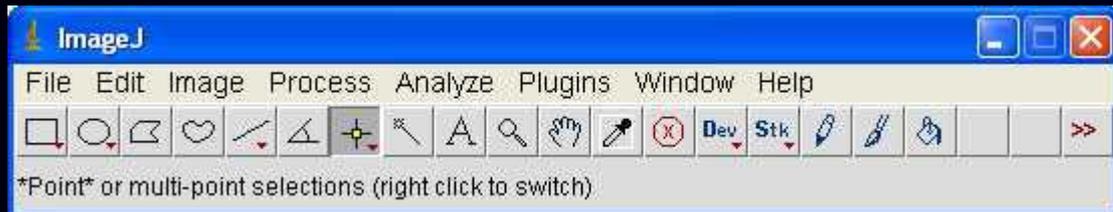
Help

Options...

Analyse → Tools → ROI Manager...



Outil Point pour le comptage





Dessin

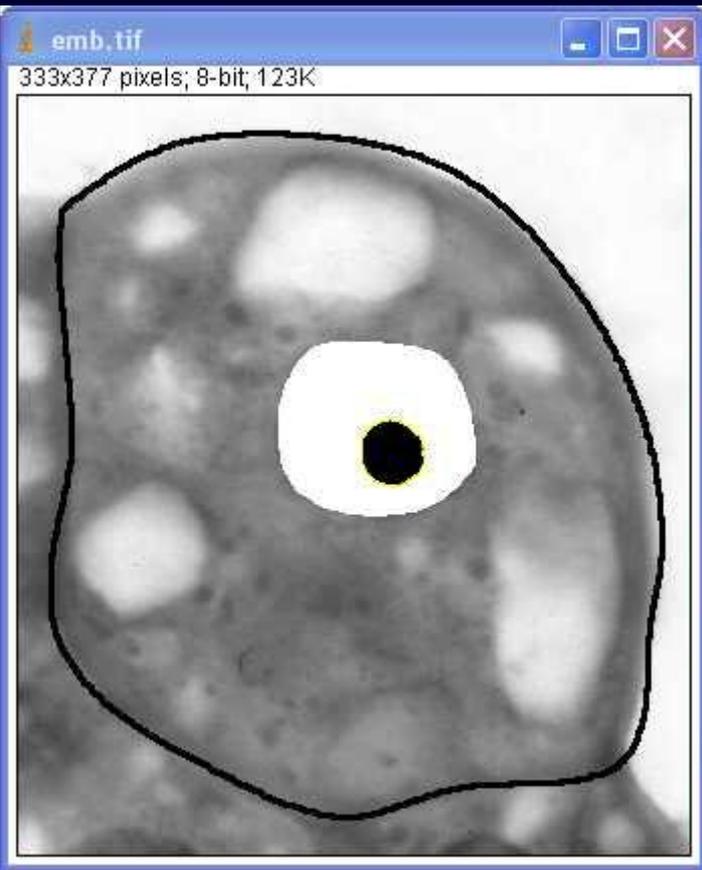


ImageJ

File Edit Image Process Analyze Plugins Window Help

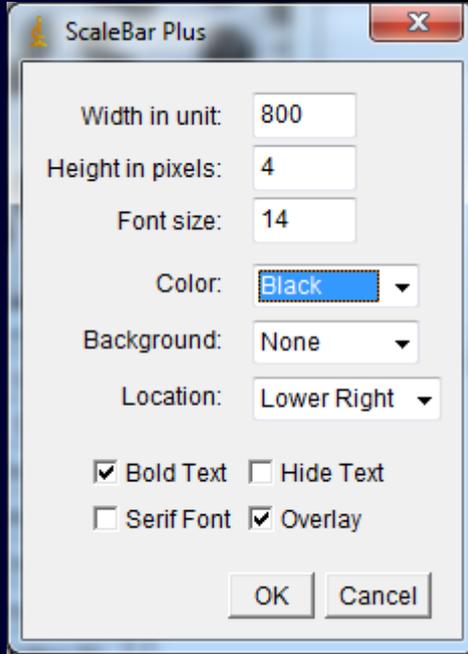
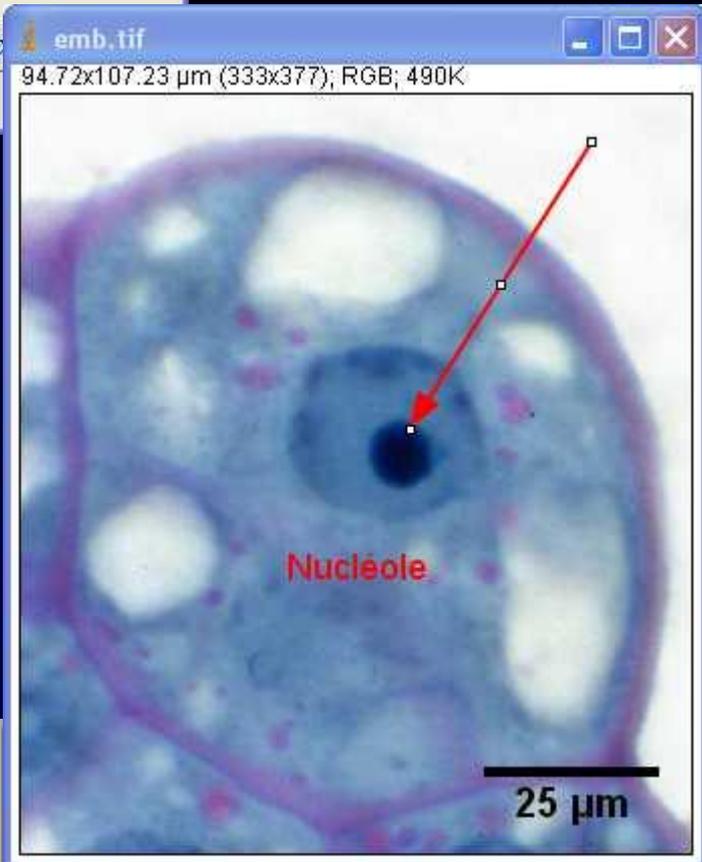
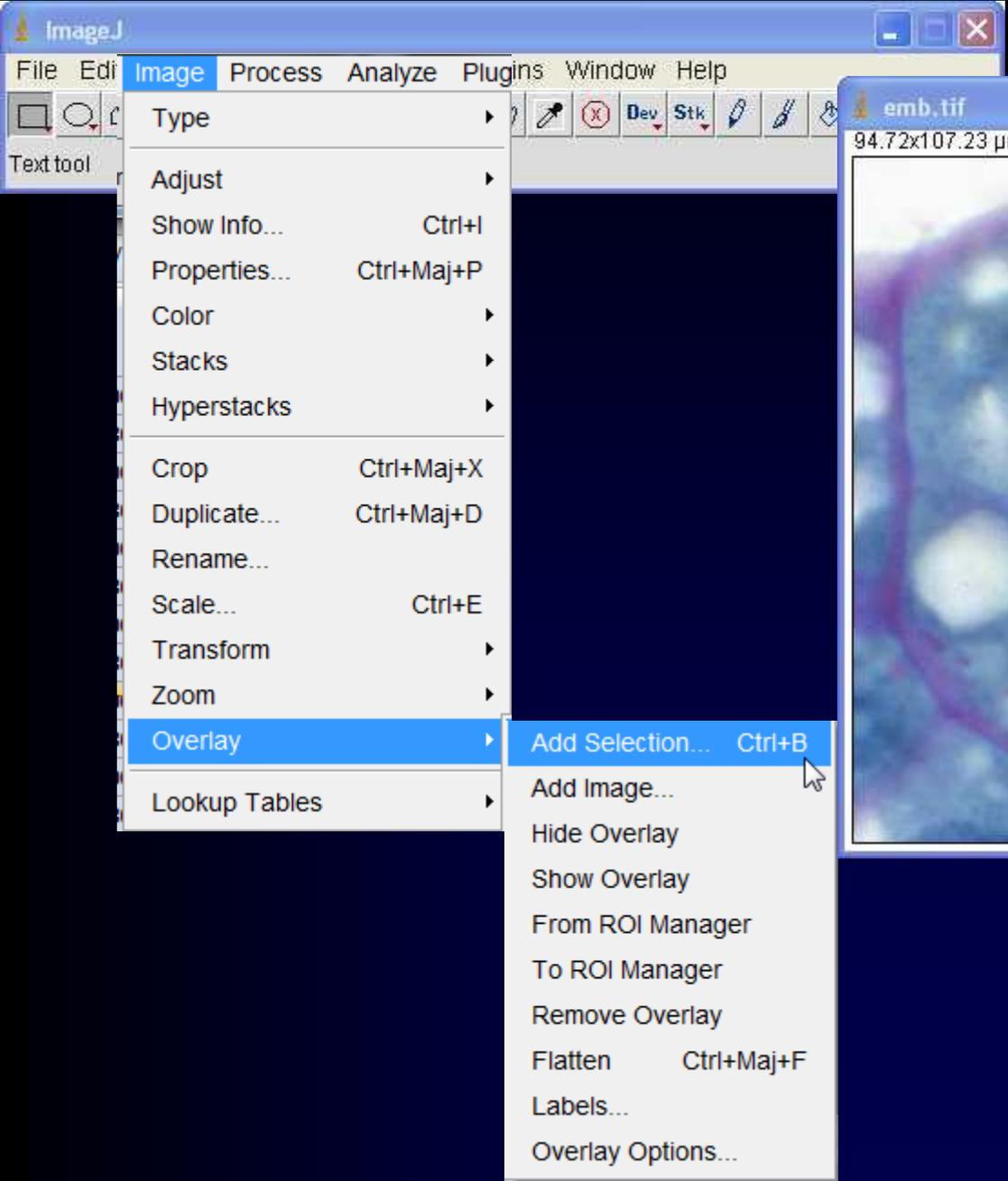
- Undo Ctrl+Z
- Cut Ctrl+X
- Copy Ctrl+C
- Copy to System
- Paste Ctrl+V
- Paste Control...
- Clear**
- Clear Outside
- Fill Ctrl+F
- Draw Ctrl+D
- Invert Ctrl+Maj+H
- Selection
- Options

Clear → Noyau
 Fill → Nucléole
 Draw → Cellule



Edit → Clear...

Annotations



Image→Overlay→Add Selection...

Analyse→Tools→Scale Bar...



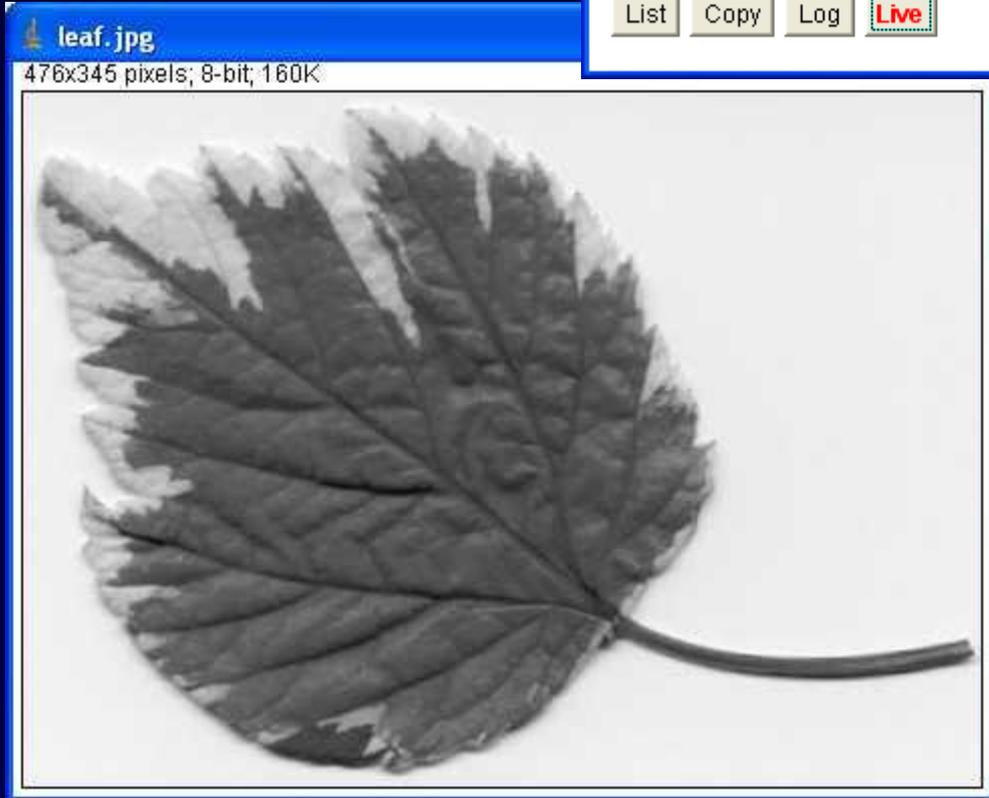
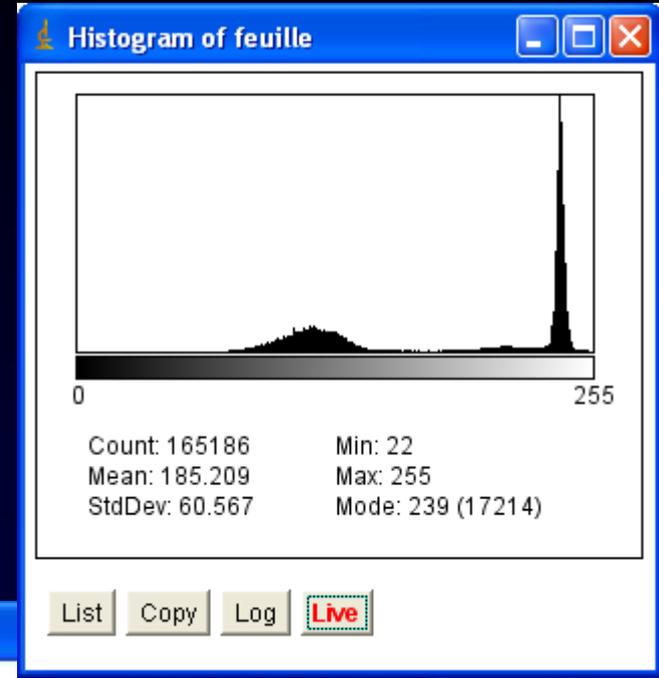
Histogramme

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

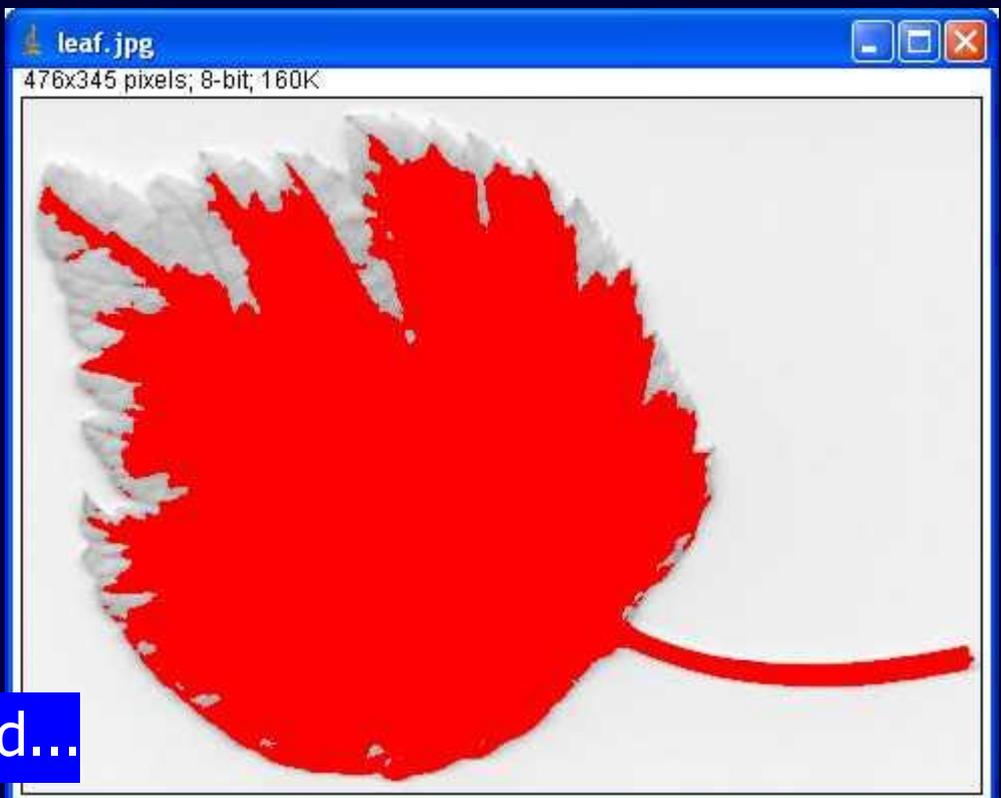
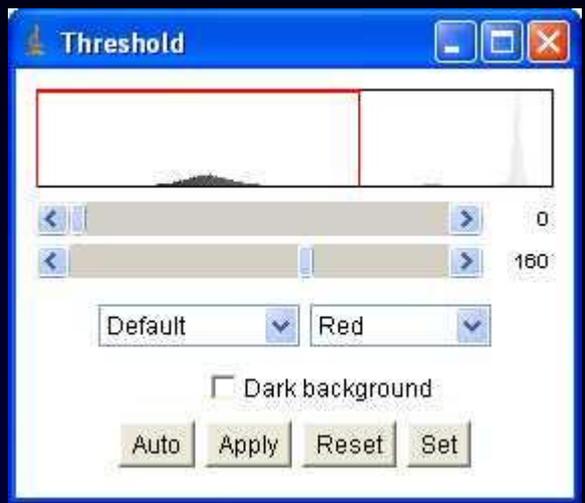
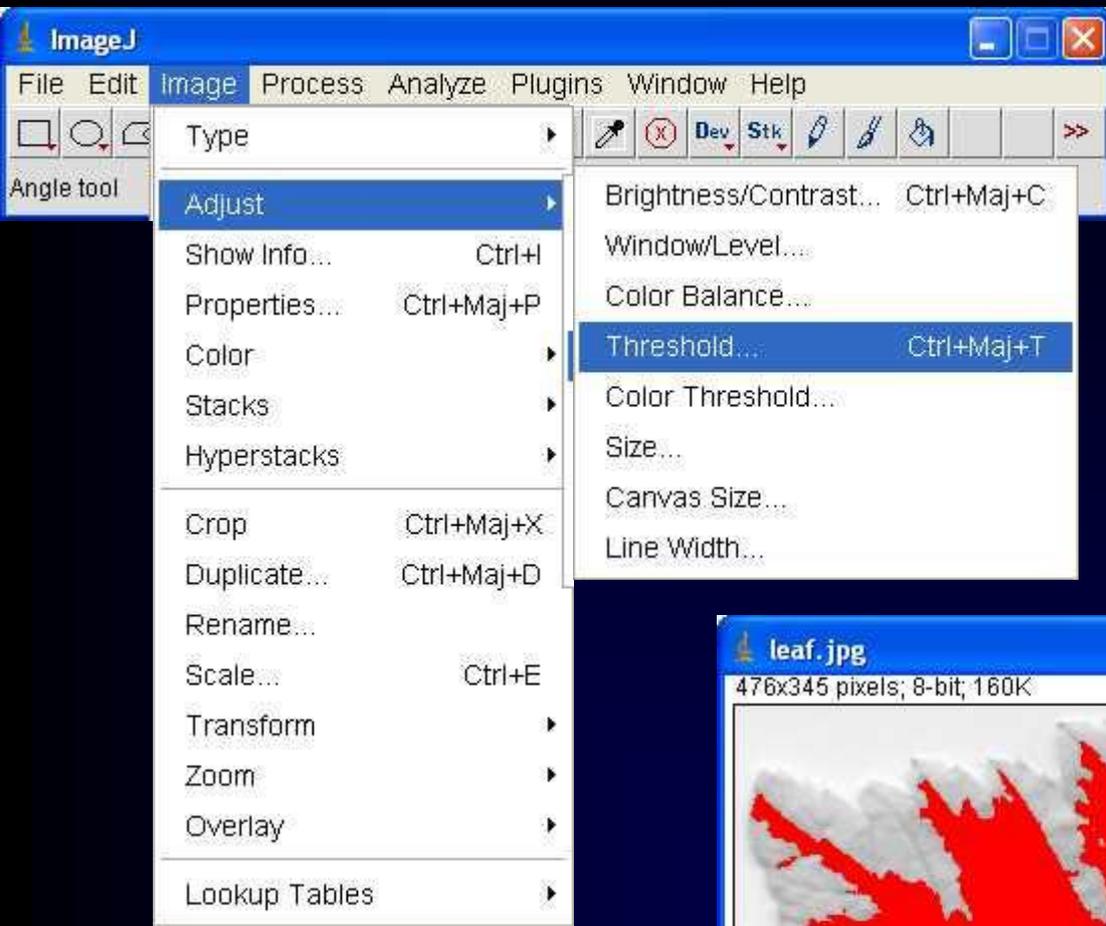
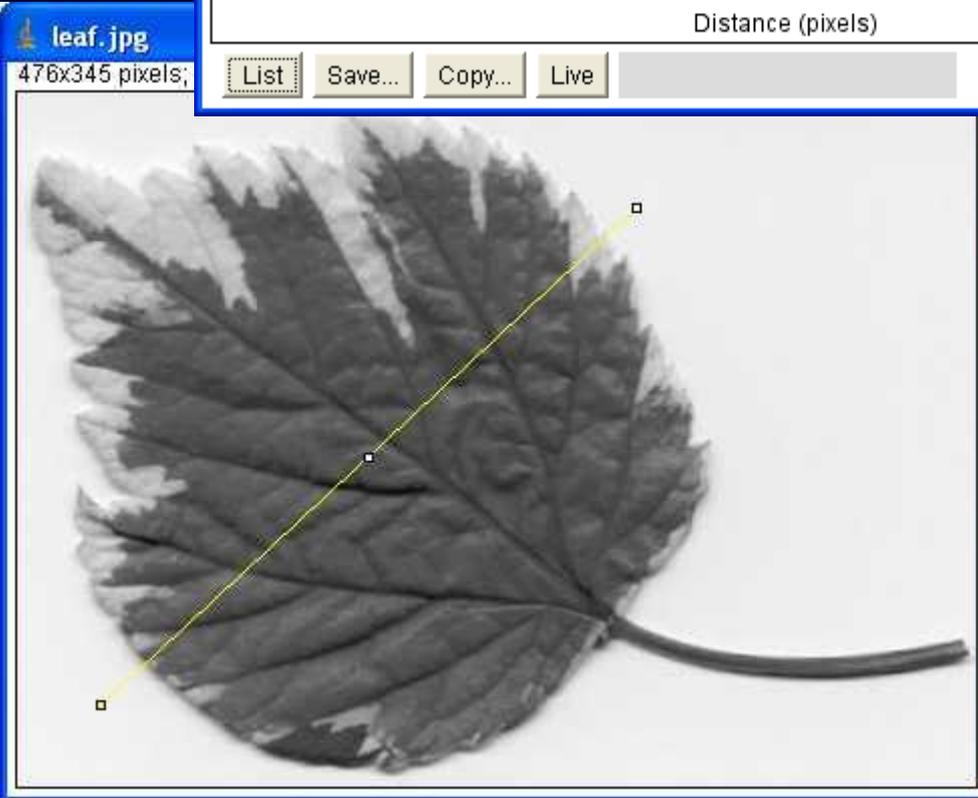
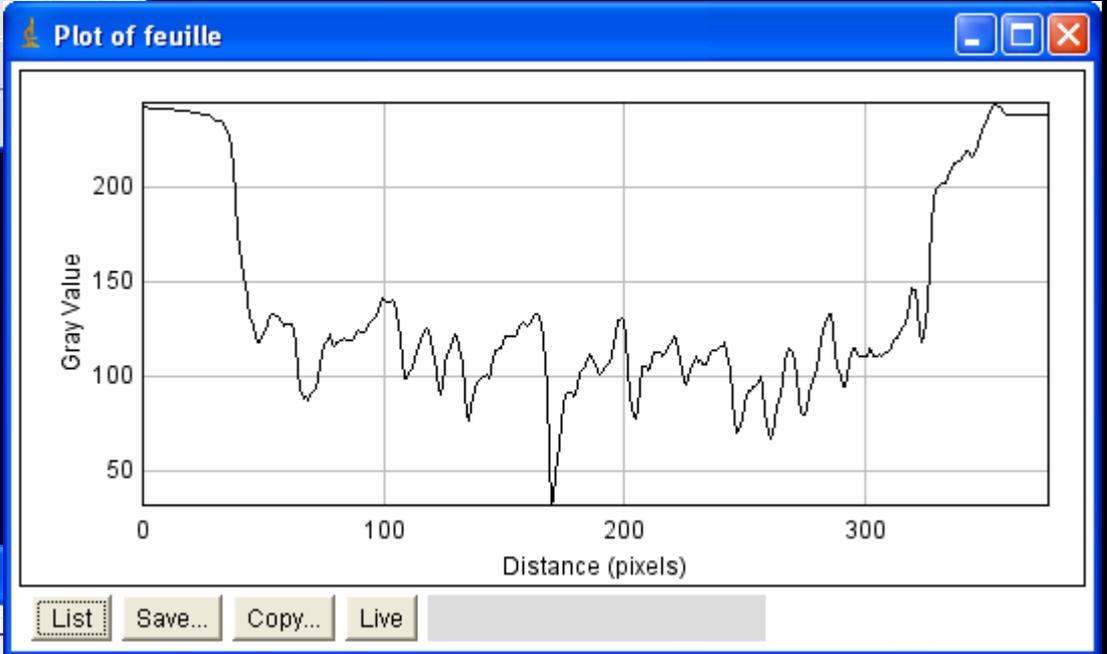
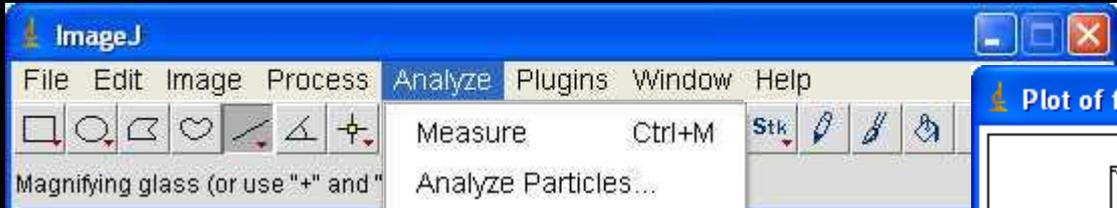


Image → Adjust → Threshold...



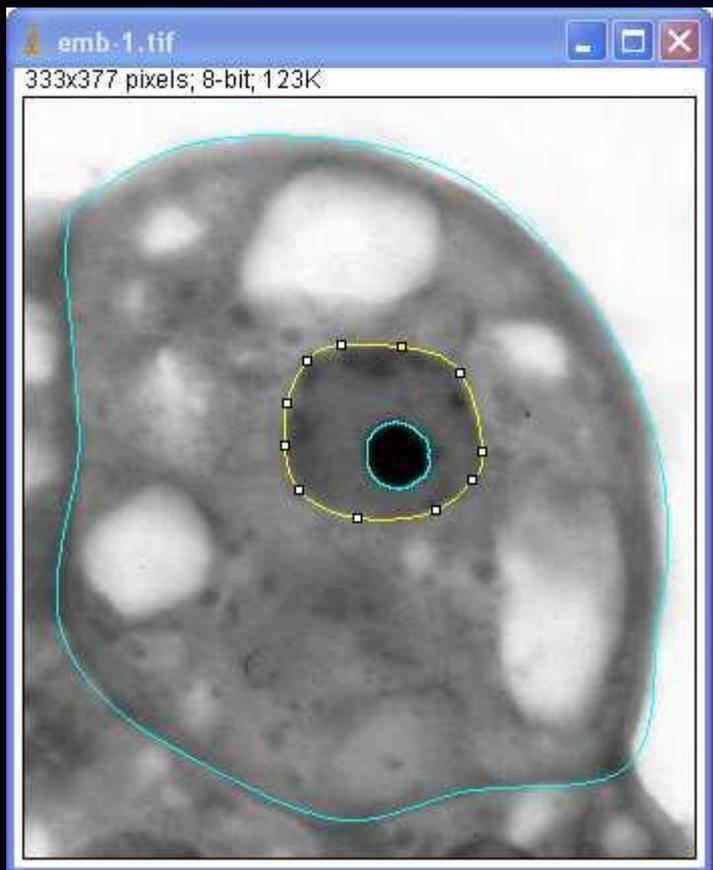
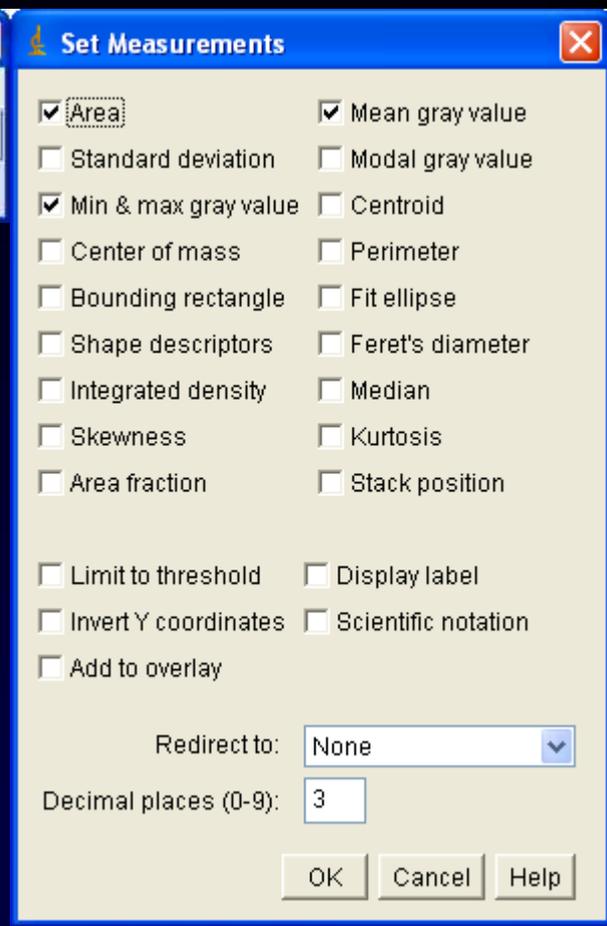
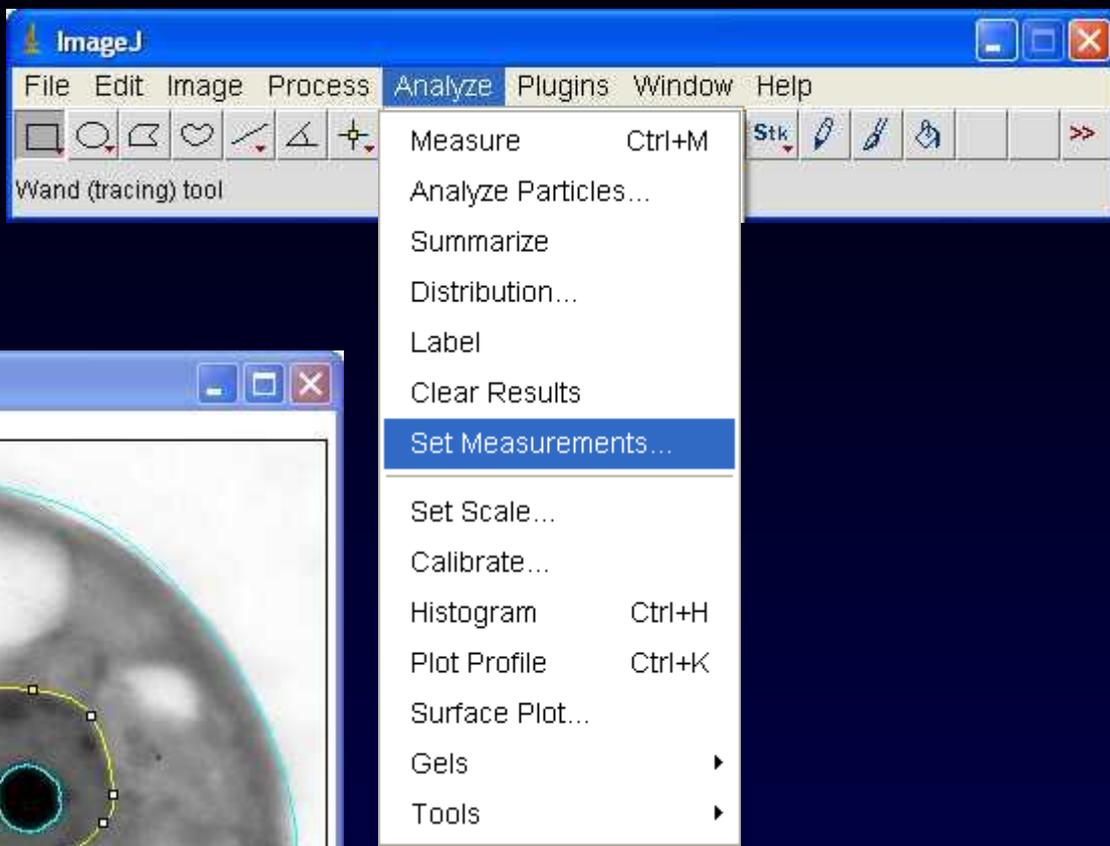
Plot Profile



Analyse → Plot Profile



Mesures de sélection

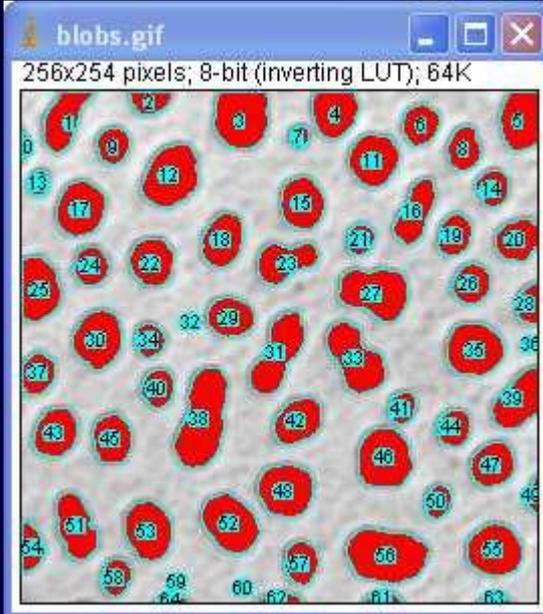
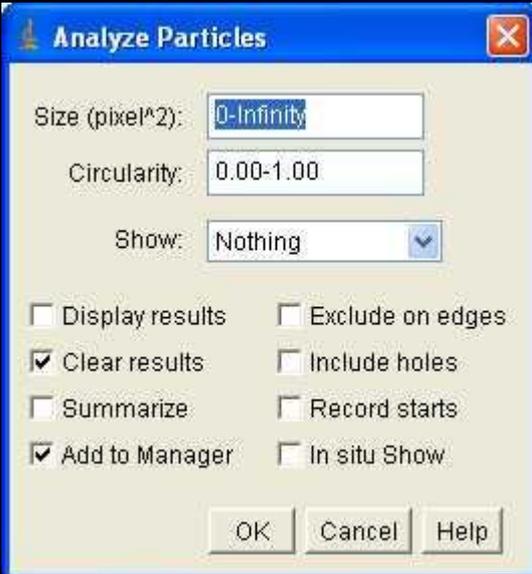
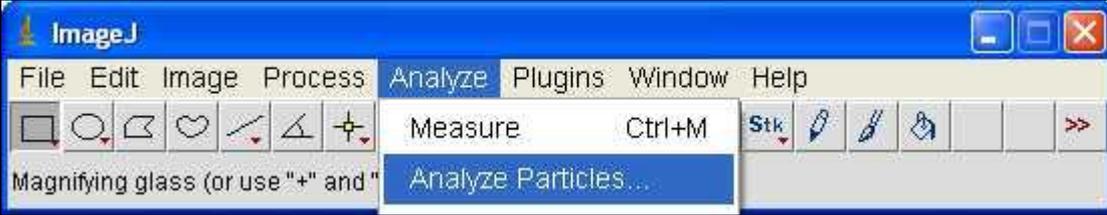


File	Edit	Font	Results	
	Area	Mean	Min	Max
1	7154	81.665	0	141

Analyse → Measure



Mesures d'objets



File	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...



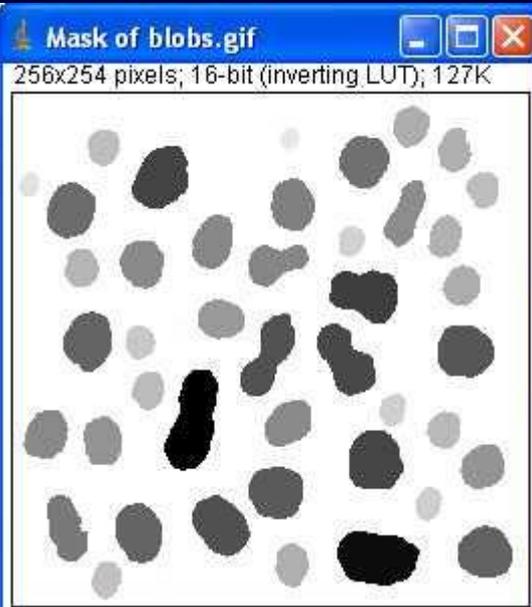
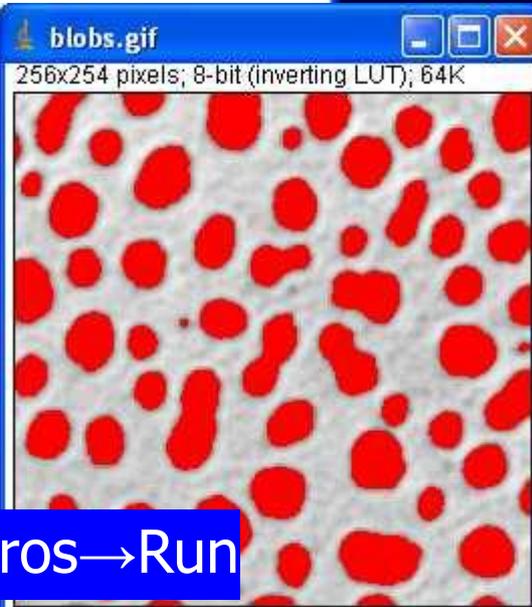
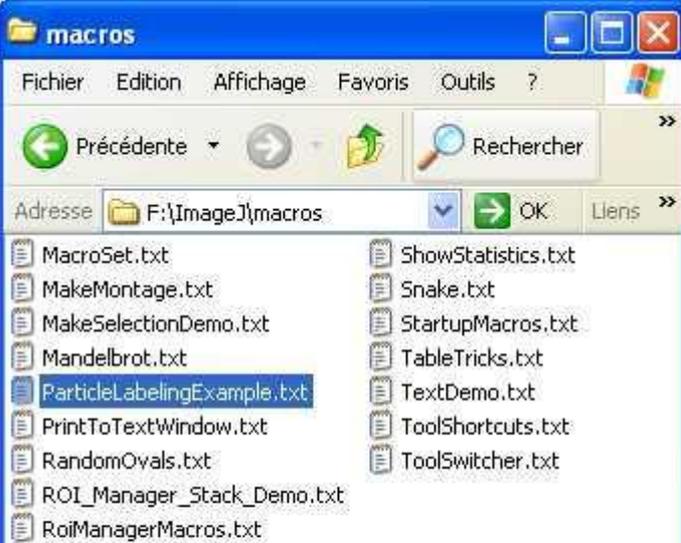
Macro



```

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

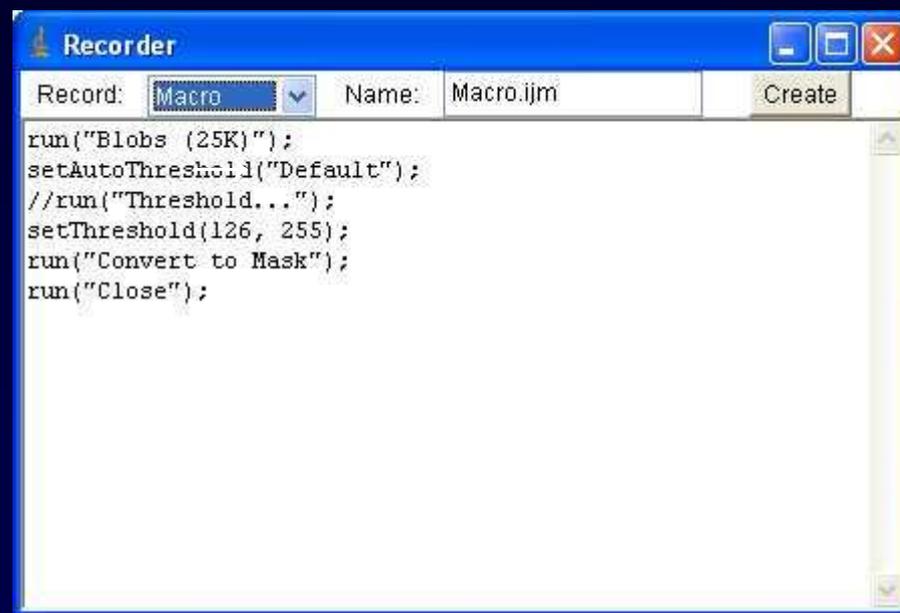
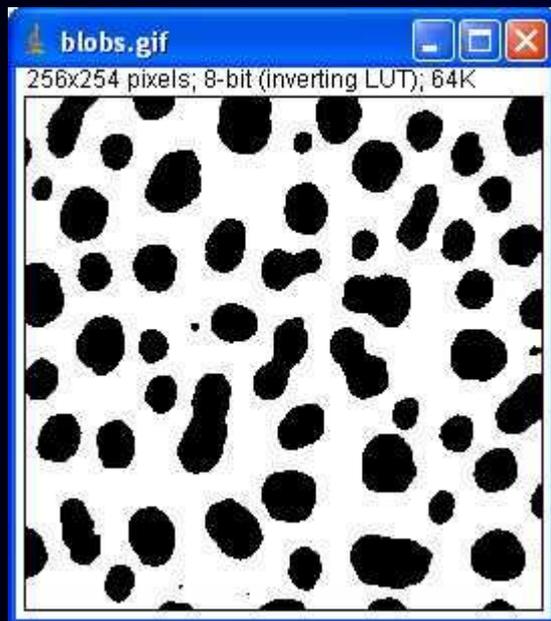
run("Set Measurements...", "area center redirect=None decimal=3");
run("Blobs (25K)");
setThreshold(125, 248);
run("Analyze Particles...",
    "minimum=1 maximum=999999 bins=20 show=Masks display exclude");
selectWindow("Mask of blobs.gif");
run("16-bit");
for (i=0; i<nResults; i++) {
    doWand(getResult("XStart",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

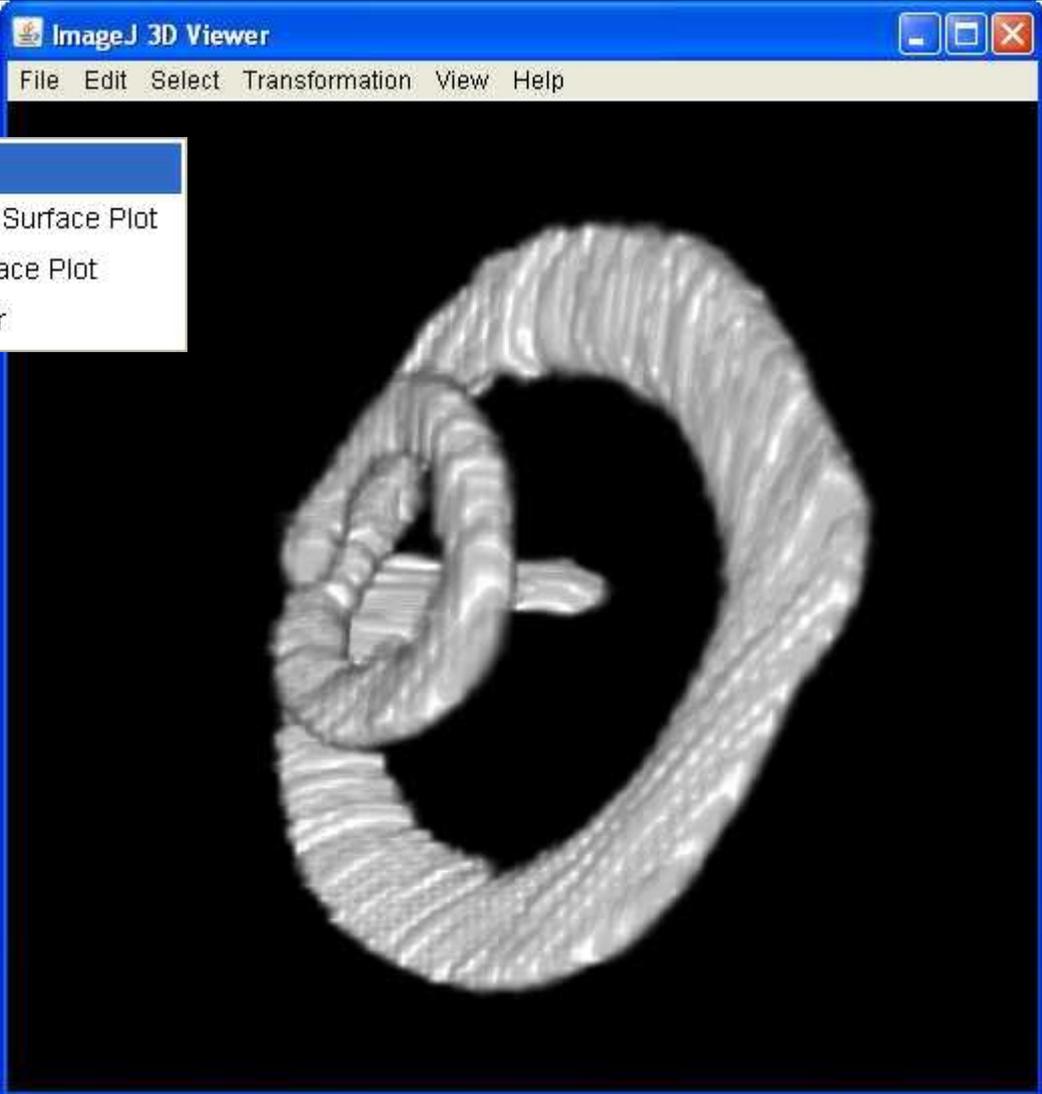
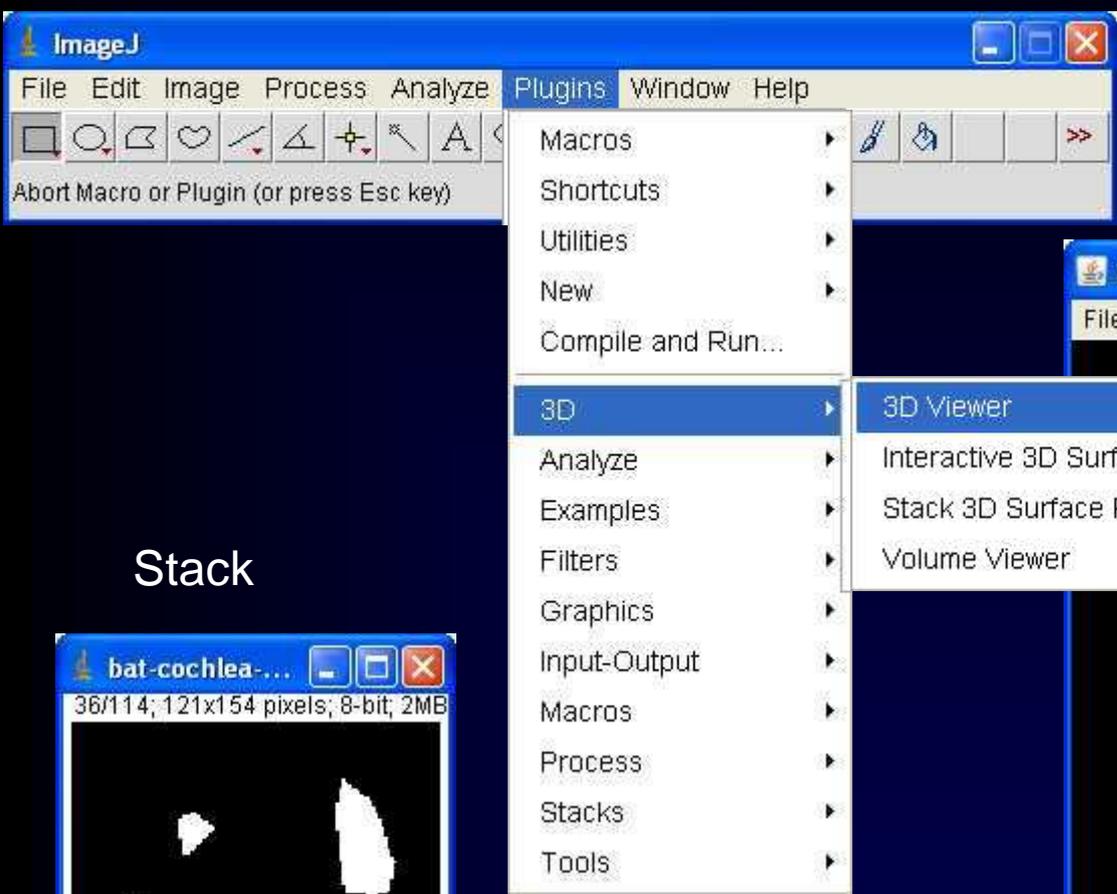
Enregistrement de macro



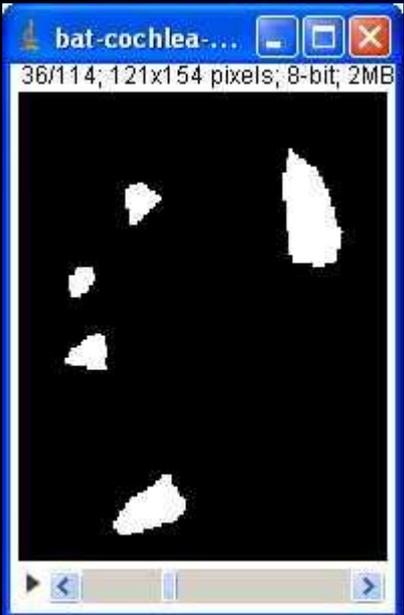
Plugins → Macros → Record...



Plugins



Stack



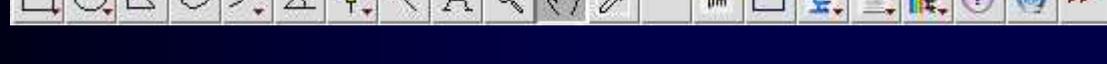
Plugins → 3D → 3D Viewer



Barres d'outils

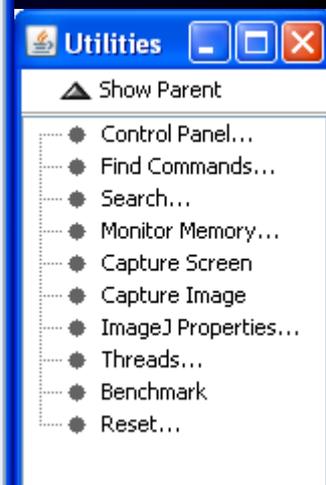
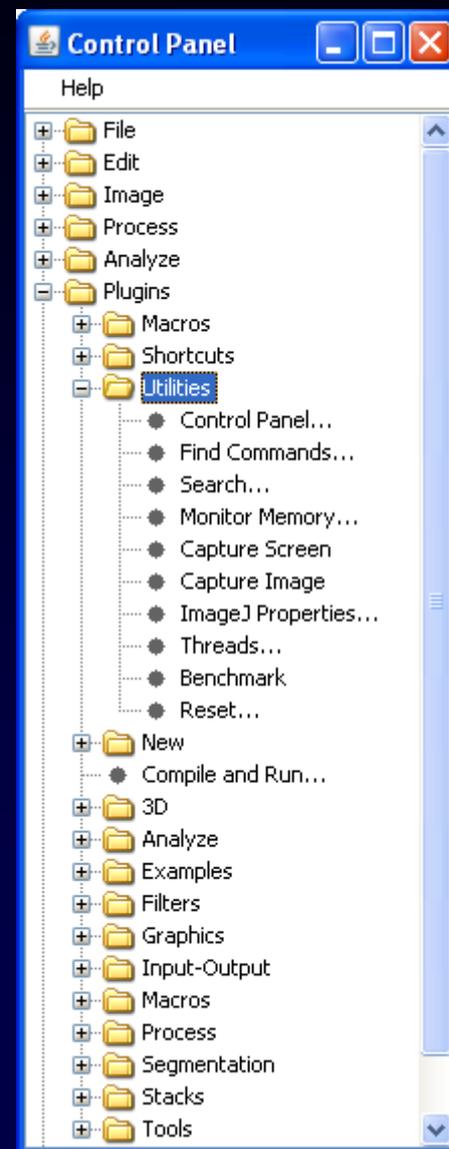
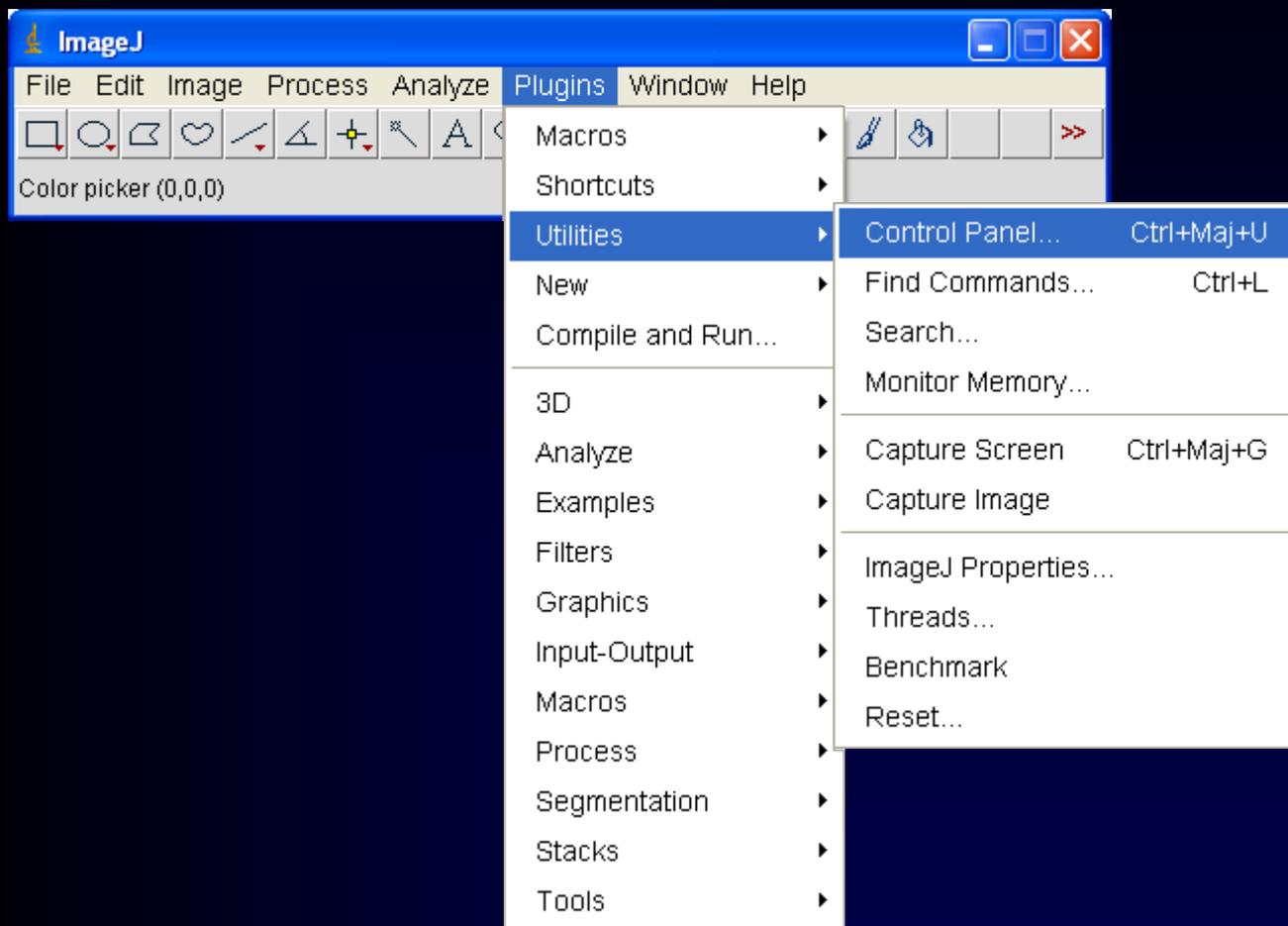


- ✓ Startup Macros
- Arrow Labelling Tools
- Drawing Tools
- Example Icons
- Lookup Tables
- Luts Macros and Tools Updater
- Magic Montage
- Plugins
- Scale Bar Tools for Microscopes
- Stack Tools
- Toolset Creator
- Help...





Panneaux de commandes



Plugins → Utilities → Control Panel



Topic 02 – Basic tools 1

Topic 03 – Basic tools 2



L'image numérique

Les Prétraitements

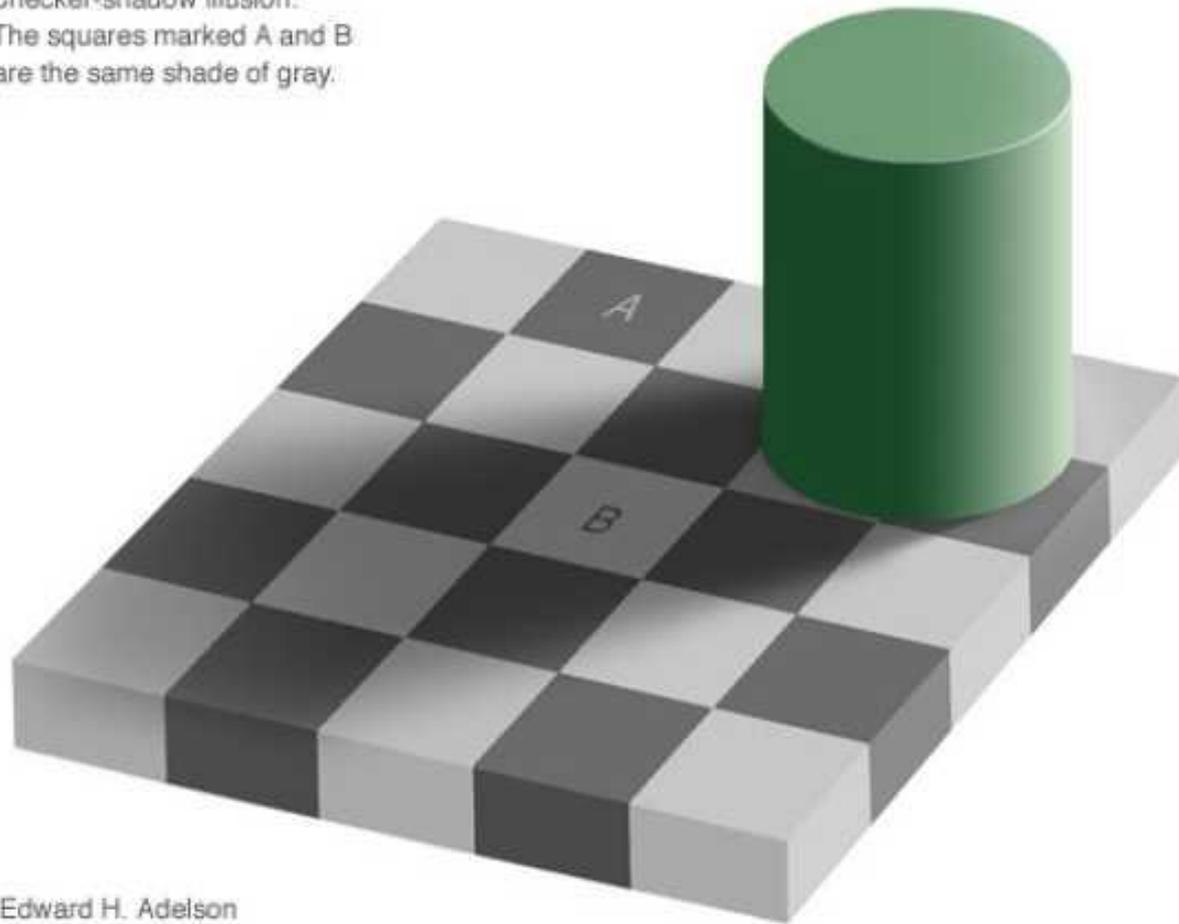
La Segmentation

Les Post-traitements

La Quantification

Image et perception

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



Edward H. Adelson

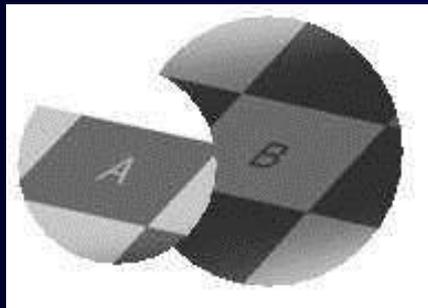
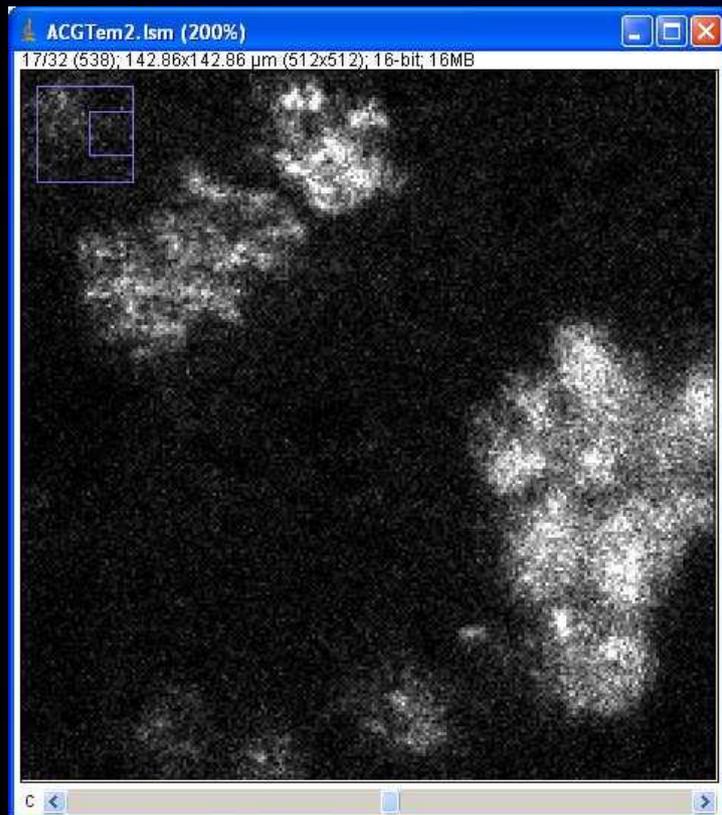


Image et déformations

Bruit



PSF

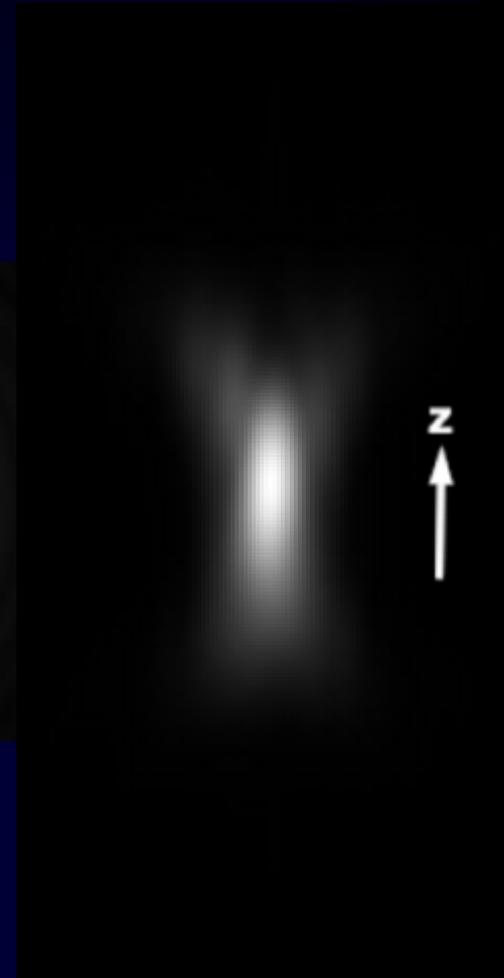
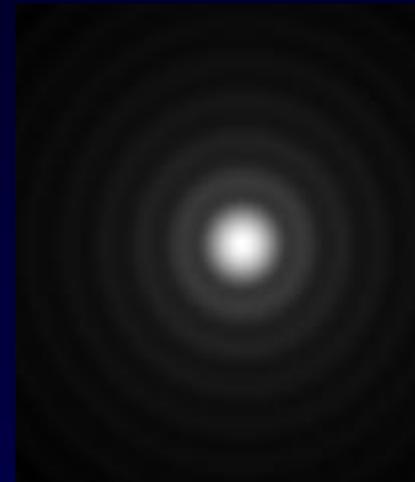
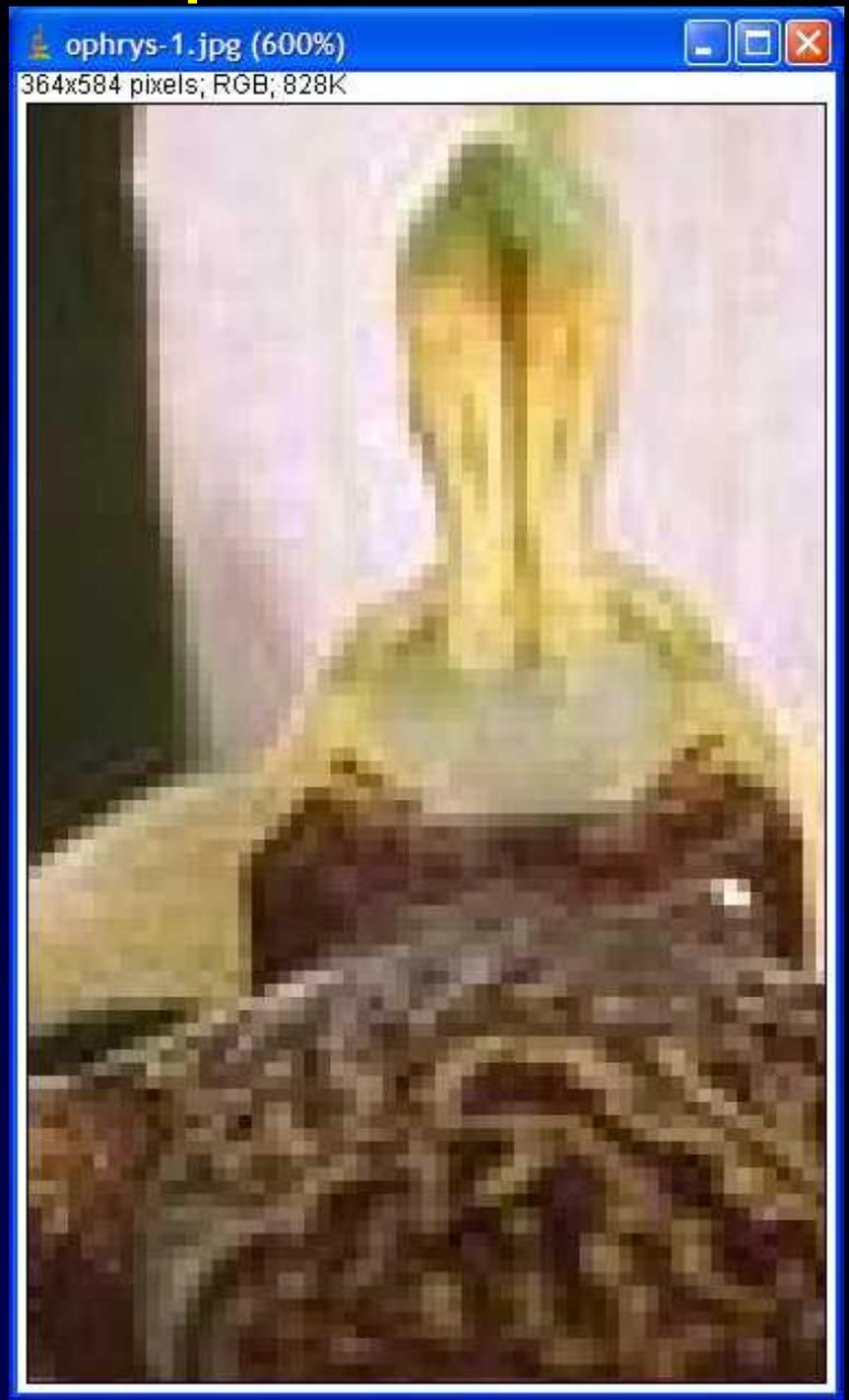




Image numérique = tableau de pixels



Détail = Affichage * 6



Résolution

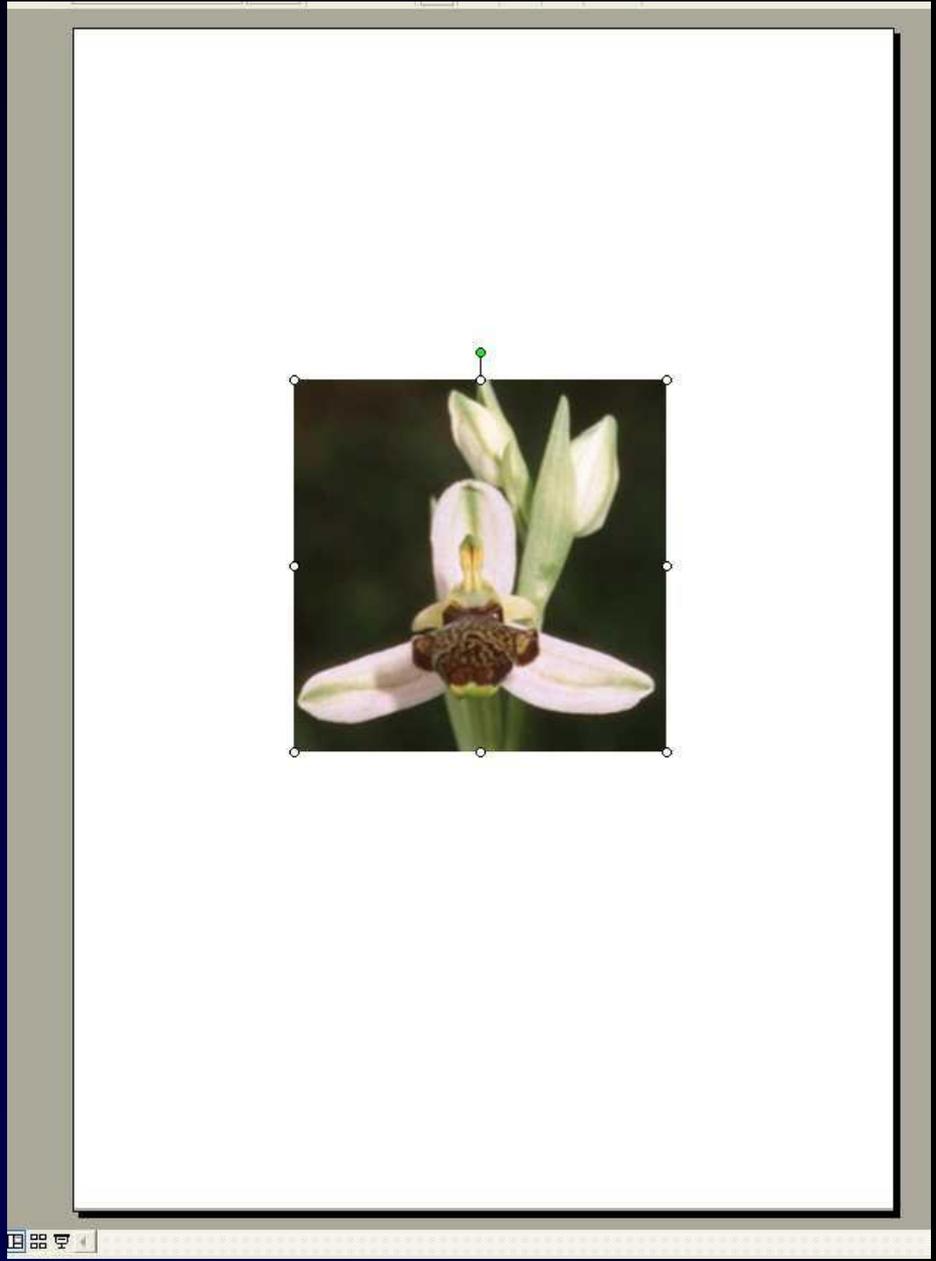
1024*1024
1 Mega pixels



Ecran 72 dpi → 35cm

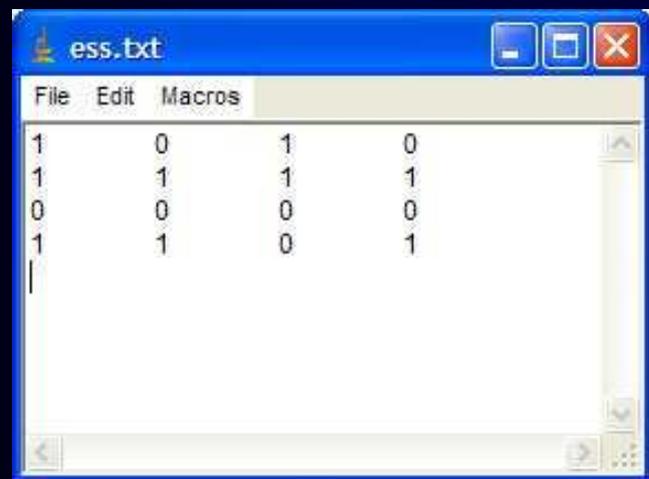
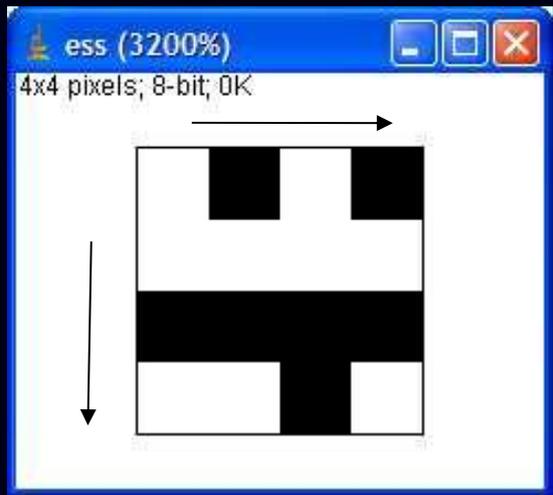


Impression 300 dpi → 8,5cm





Codage binaire



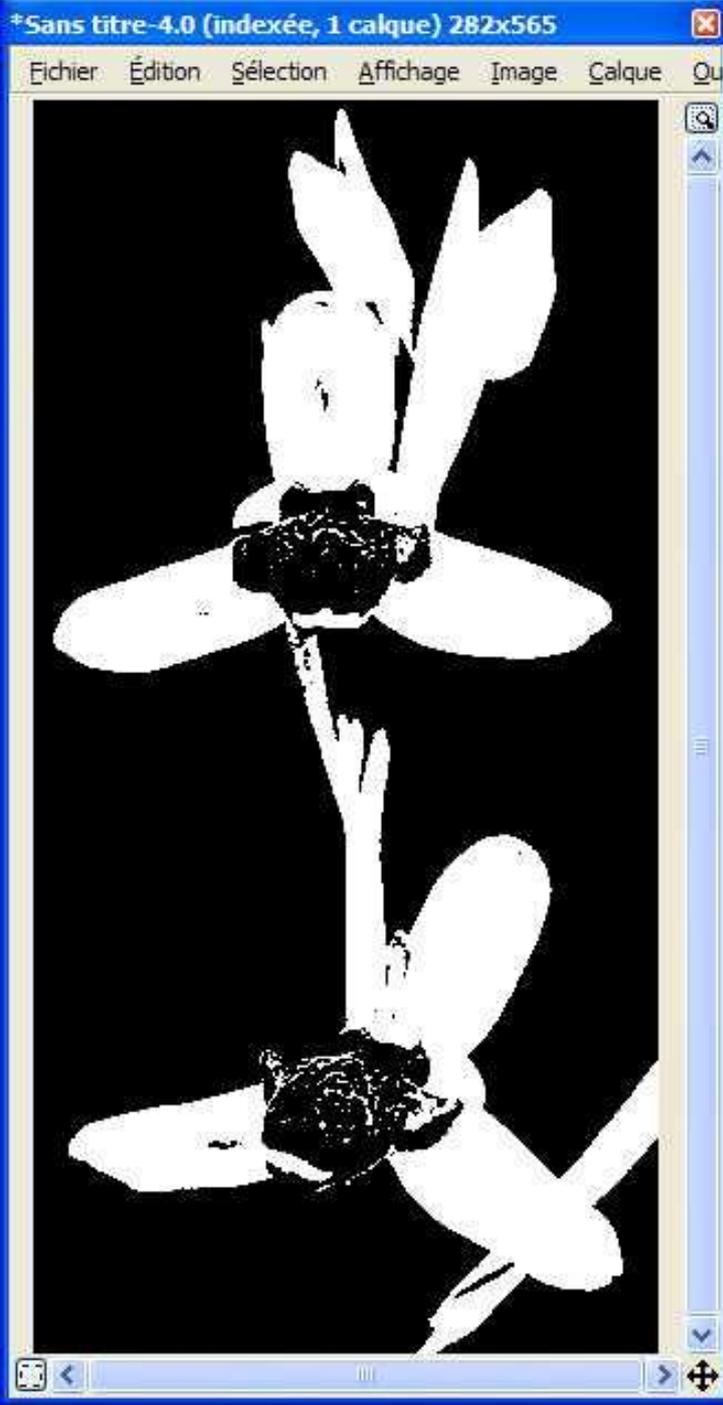


Codage

1bit \rightarrow 2 valeurs

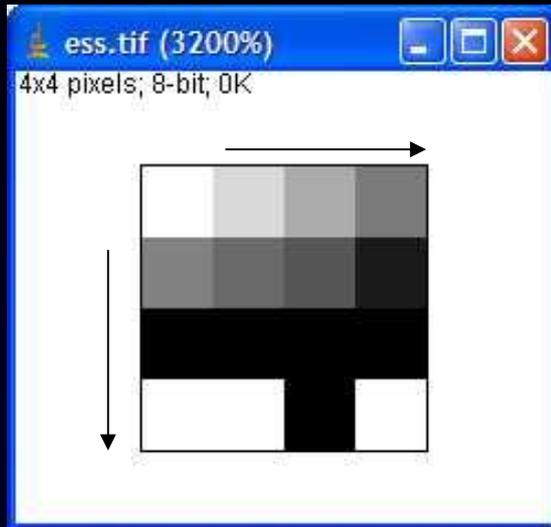
3bits \rightarrow 8 valeurs

5bits \rightarrow 32 valeurs

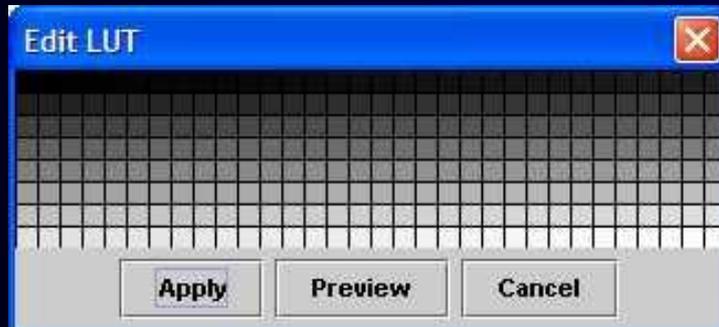




Codage 8 bits 0→255 Valeurs de Niveaux de gris

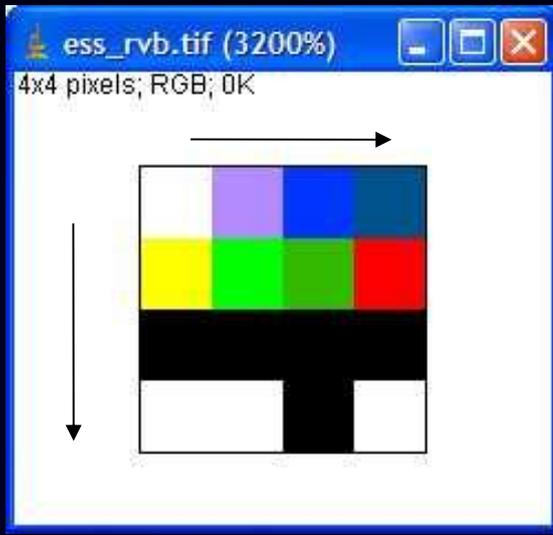


File	Edit	Macros	
255	217	172	123
130	106	87	27
0	0	0	0
255	255	0	255





Codage couleur 24 bits RVB



ess_rvb.txt

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

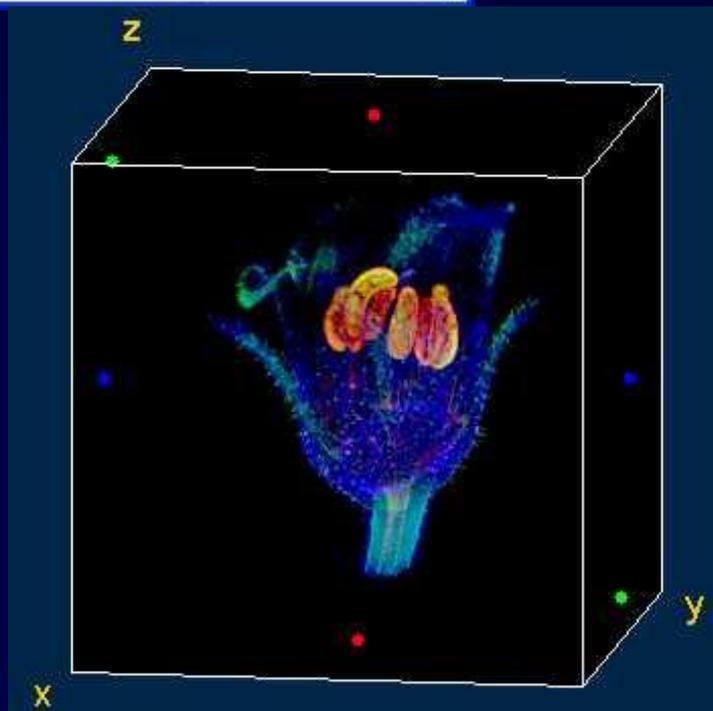
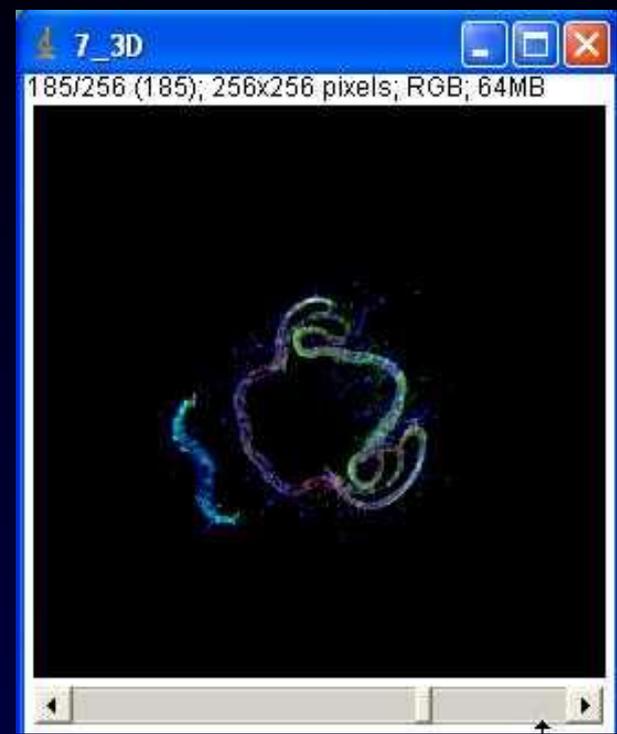
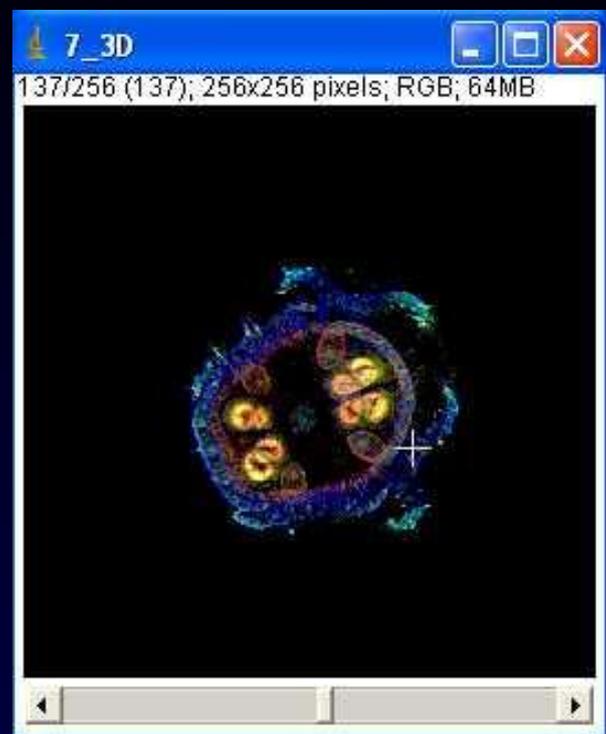
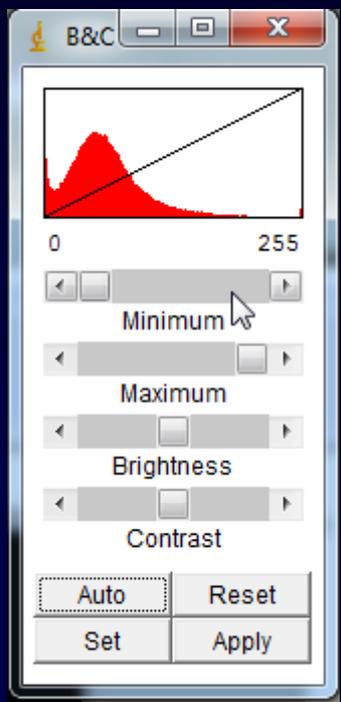
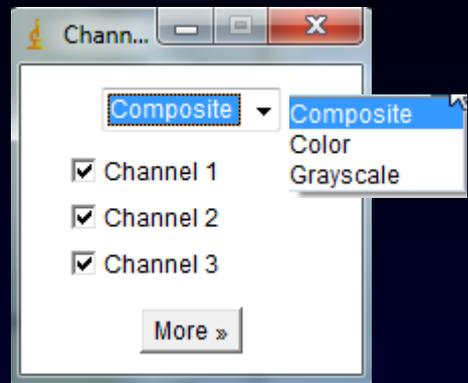
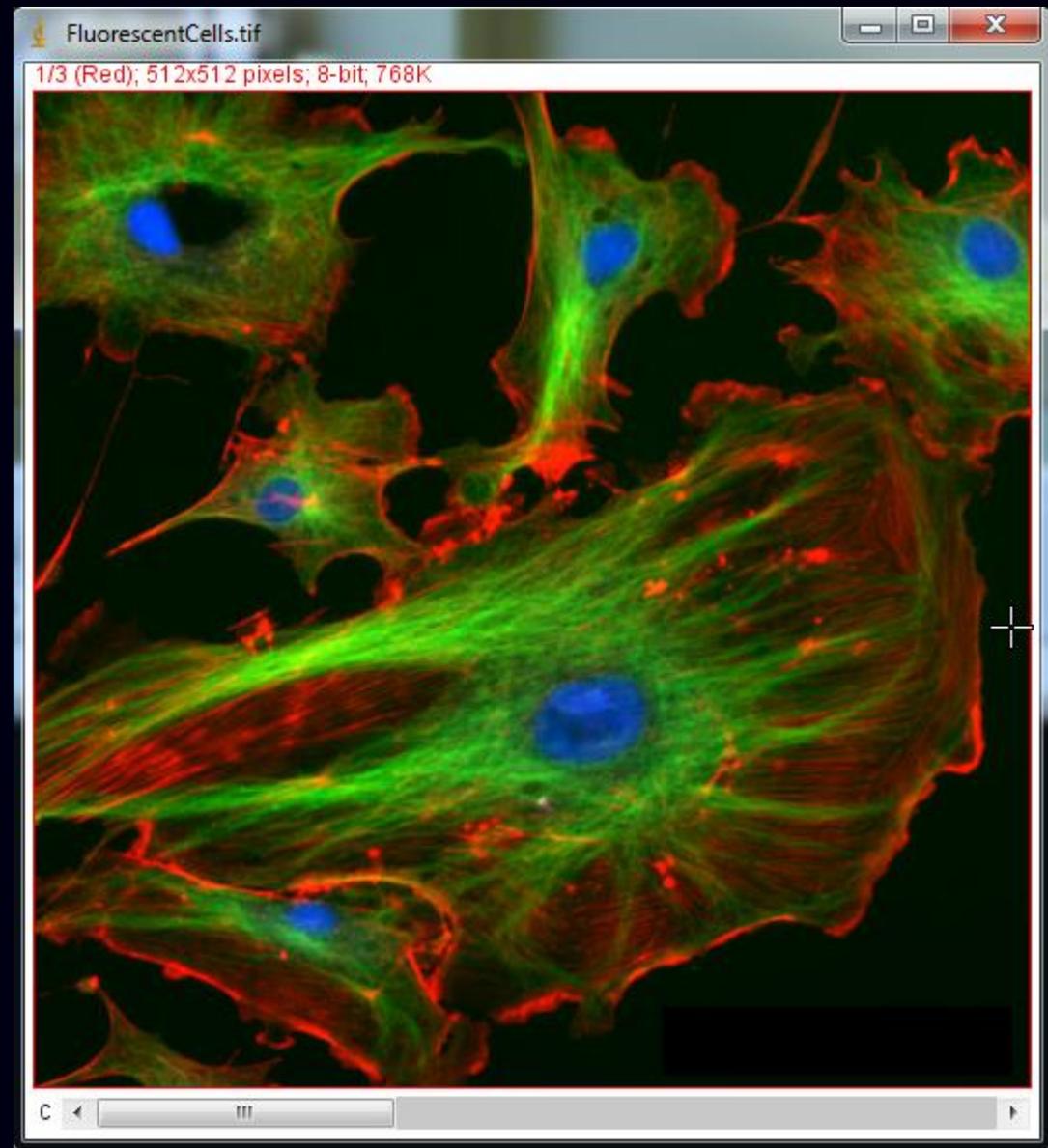




Image Composite





Format d'image

8bit [0,255]

16bit [0,65 535],

32bit [-3.4*10³⁸ , +3.4*10³⁸]

8bit color (LUT)

RGB 24bit [(0.0.0),(255.255.)]

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

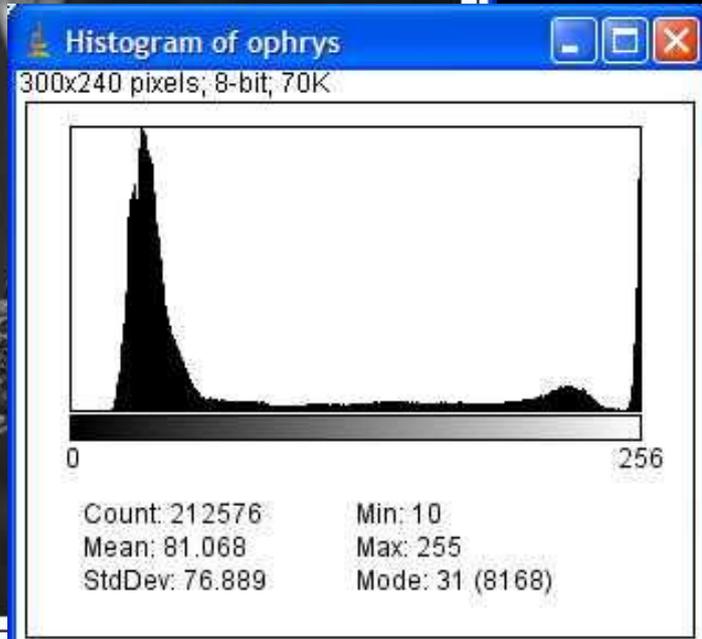
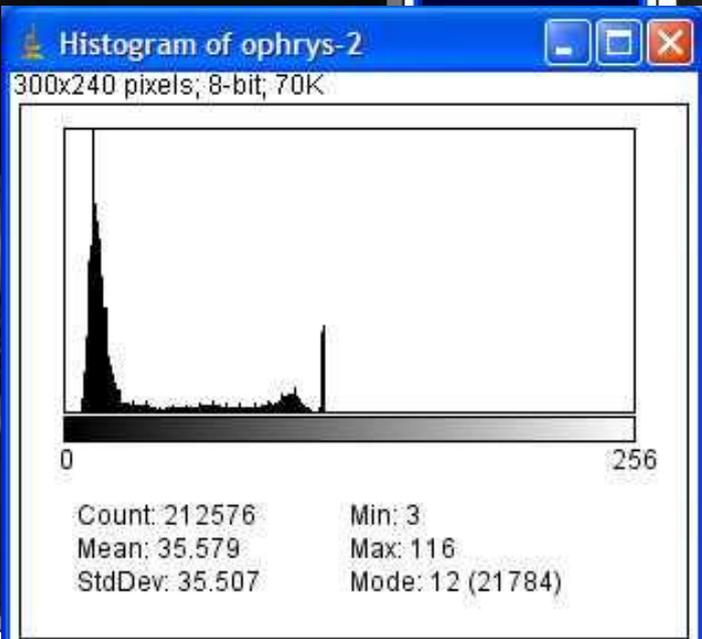
Les Post-traitements

La Quantification



Histogramme

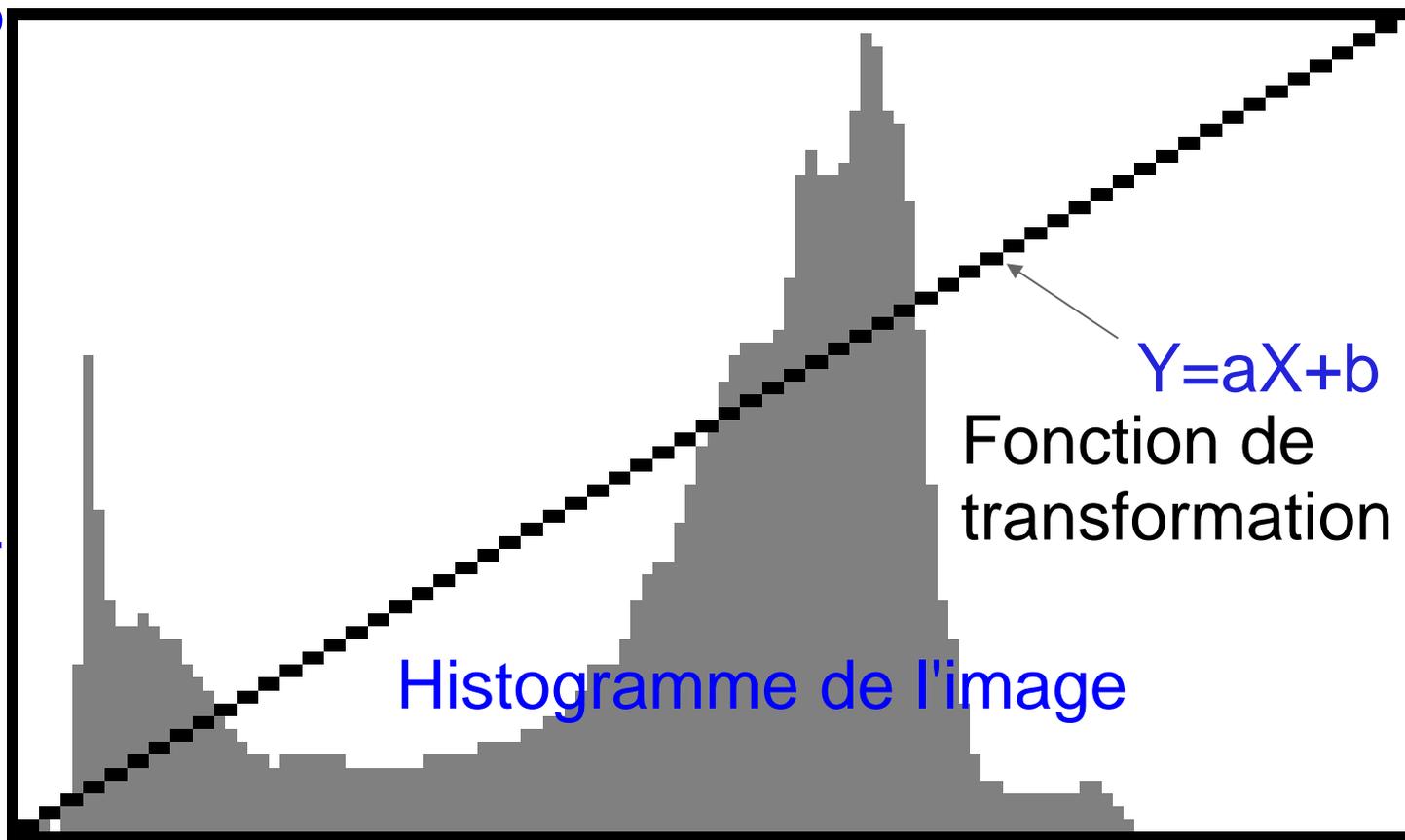
Dynamique = [valeur_mini , valeur_maxi]



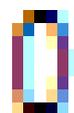


Corrections linéaires

Y=valeurs pixels de l'affichage



Mini



X=valeurs pixels de l'image

Maxi

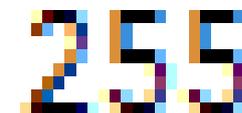


Image → Adjust → Brightness/Contrast...

a=Contraste
b=Luminosité

Corrections linéaires

mini maxi : les limites de la courbe de transformation

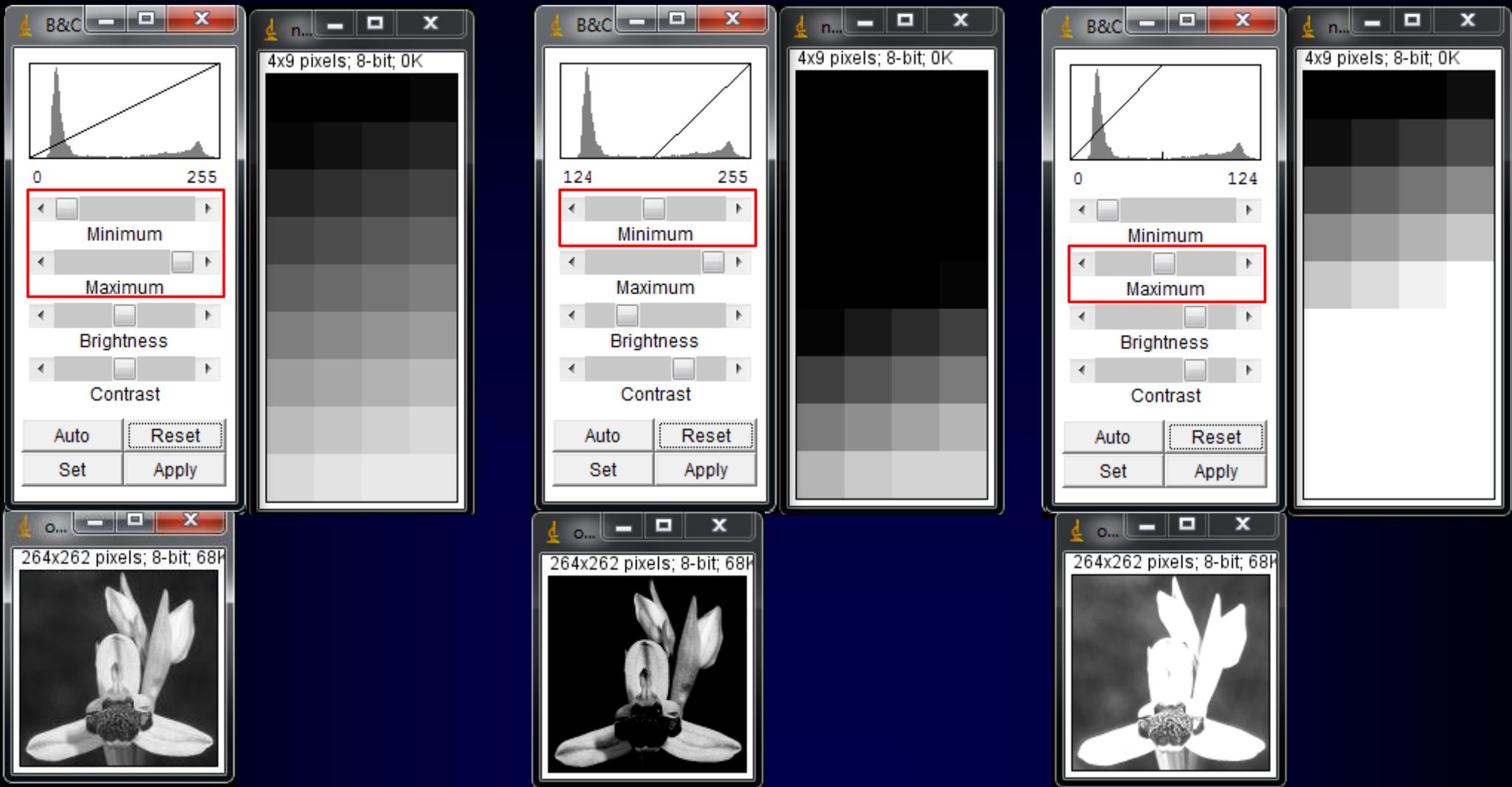


Image → Adjust → Brightness/Contrast...

Corrections linéaires

Luminosité « Brightness » déplace la courbe

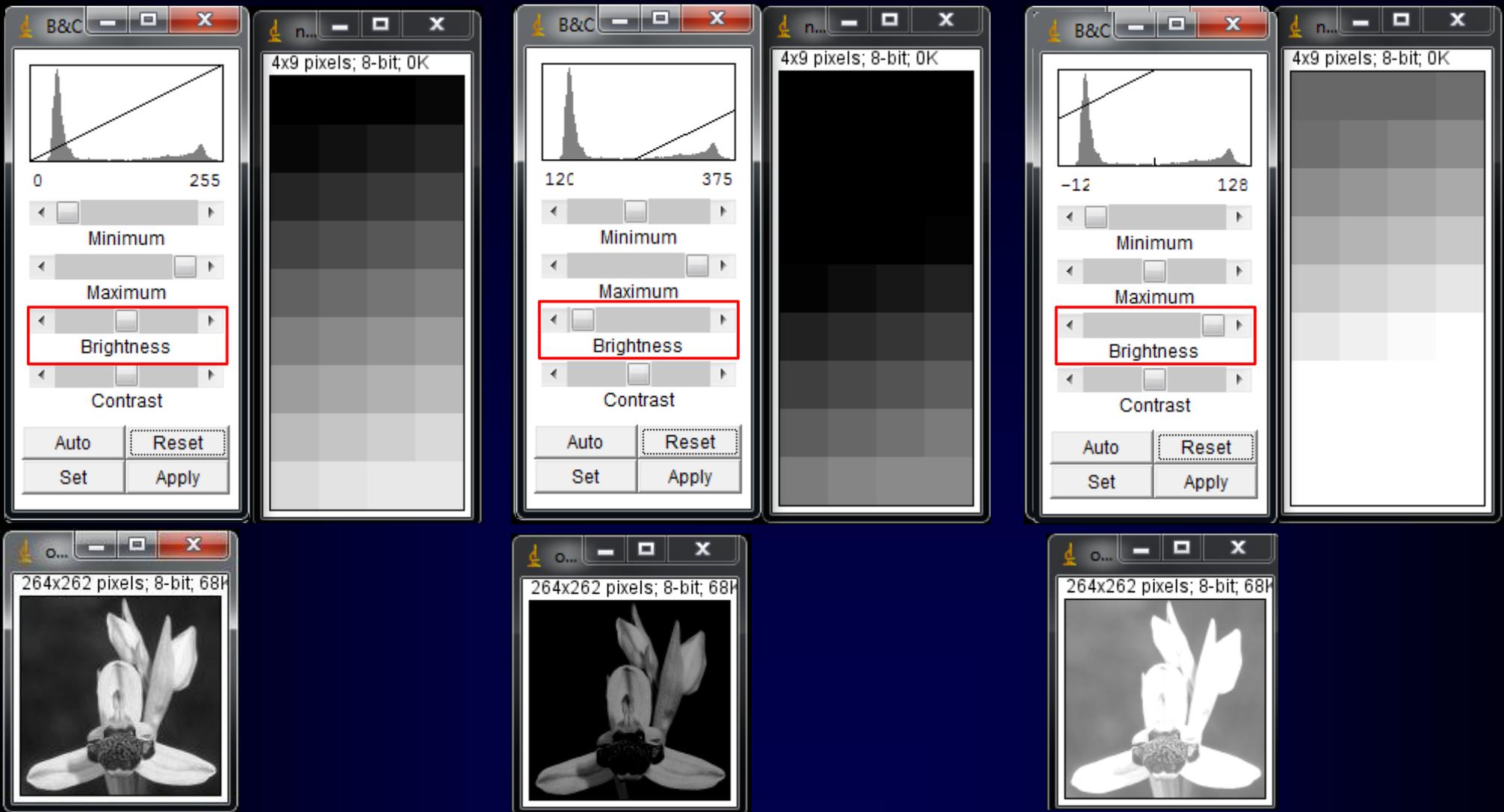


Image → Adjust → Brightness/Contrast...

Corrections linéaires

Contraste change la pente de la courbe

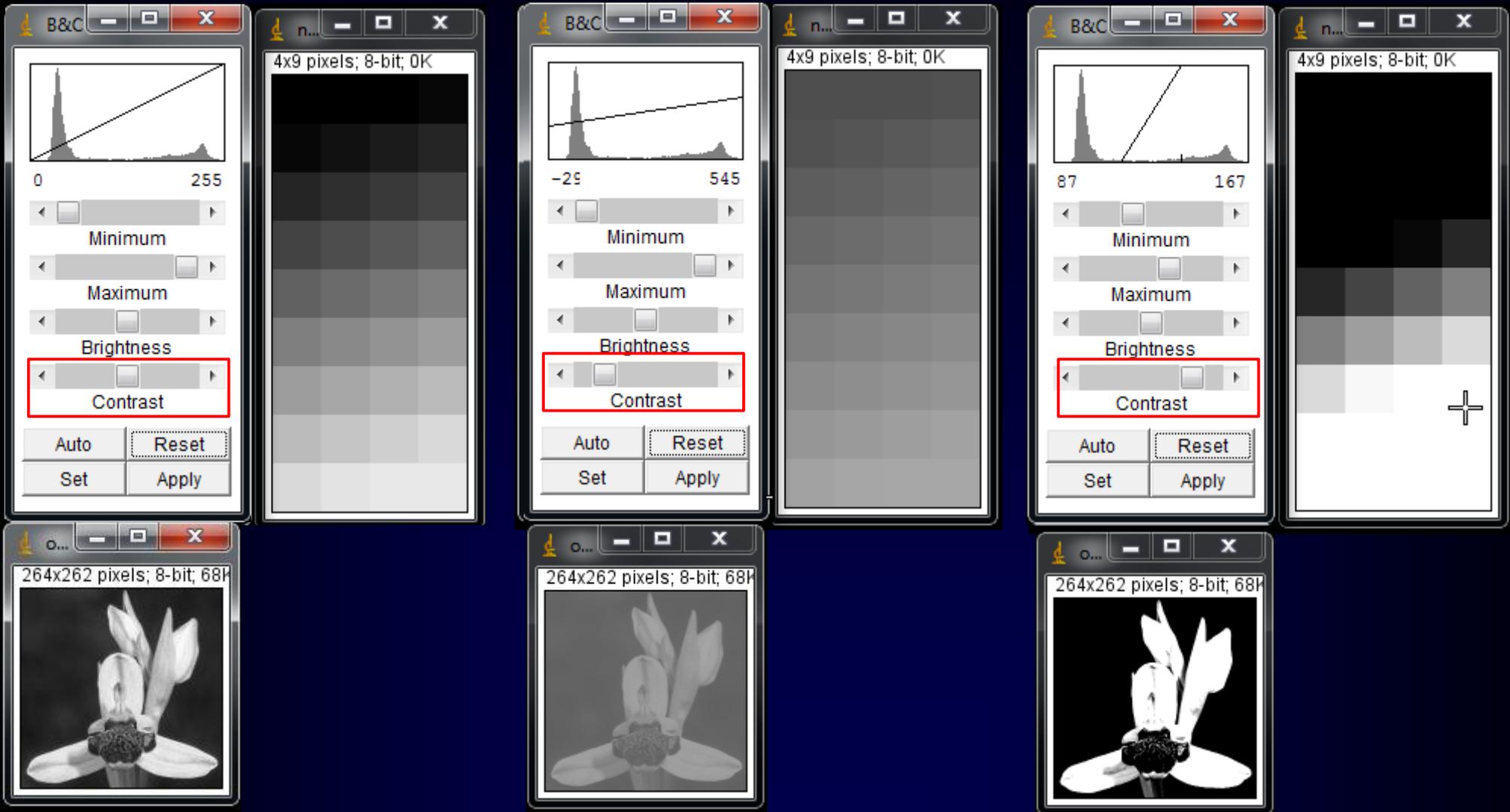


Image → Adjust → Brightness/Contrast...

Corrections linéaires

Niveaux « Level » valeur centre de la fenêtre $\langle \rangle$ b

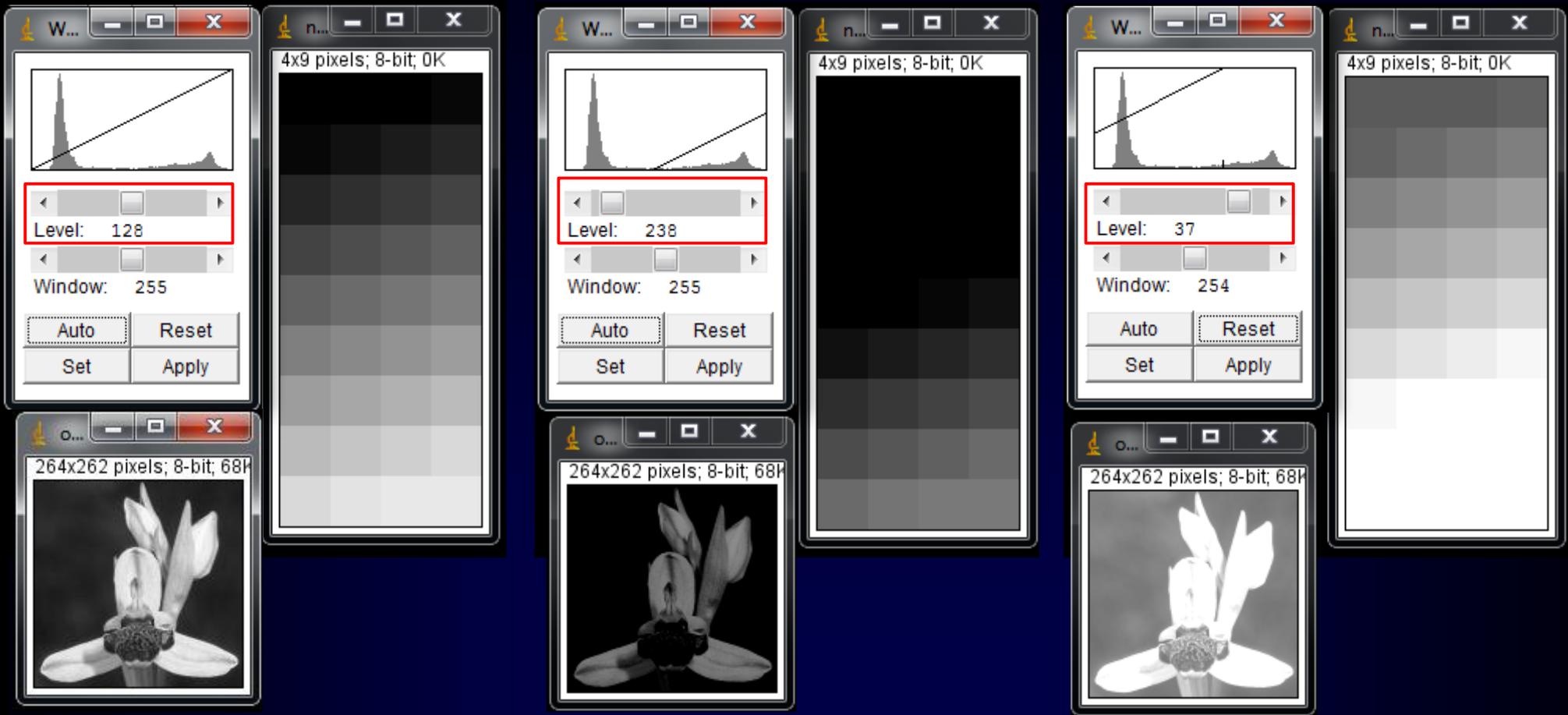


Image → Adjust → Window/Level...

Corrections linéaires

Window : largeur de la fenêtre \leftrightarrow a = pente

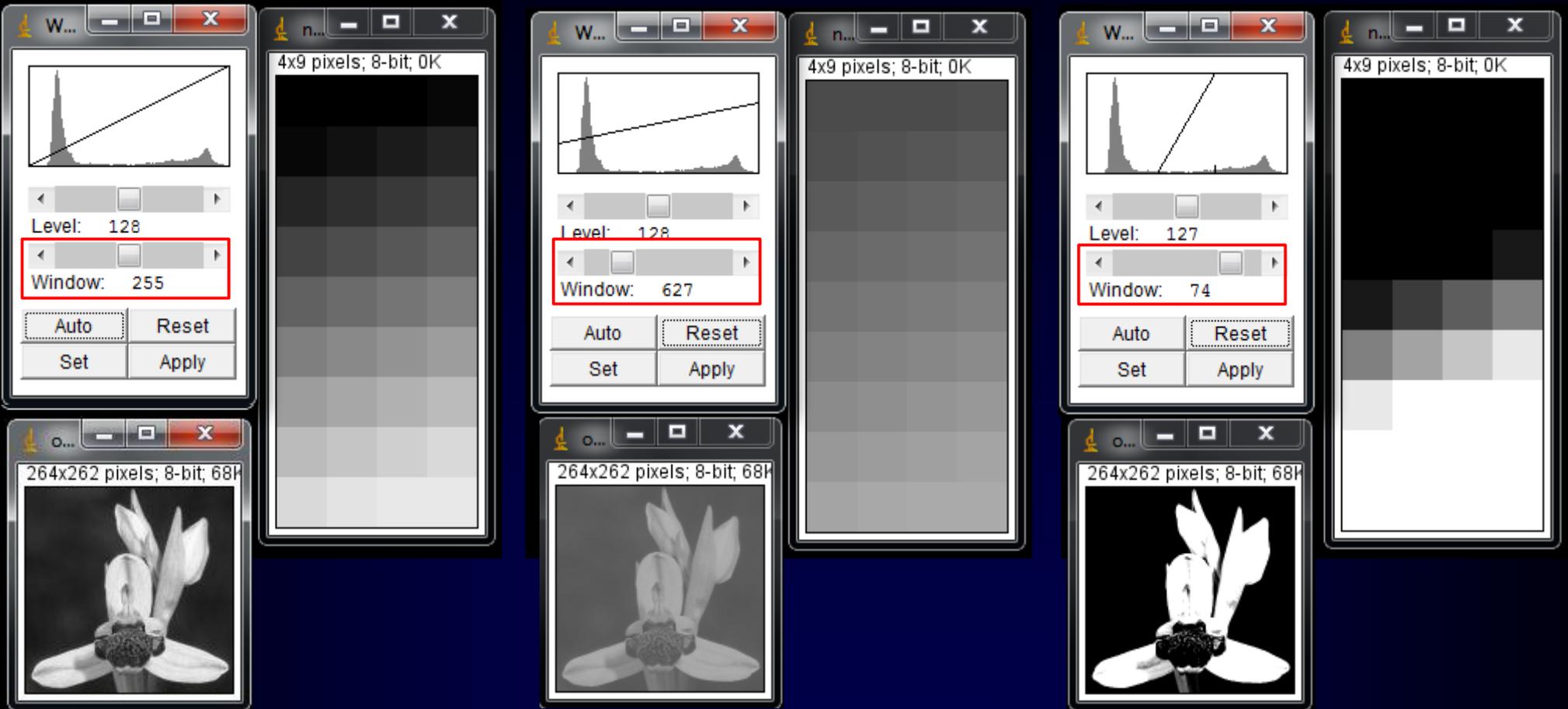
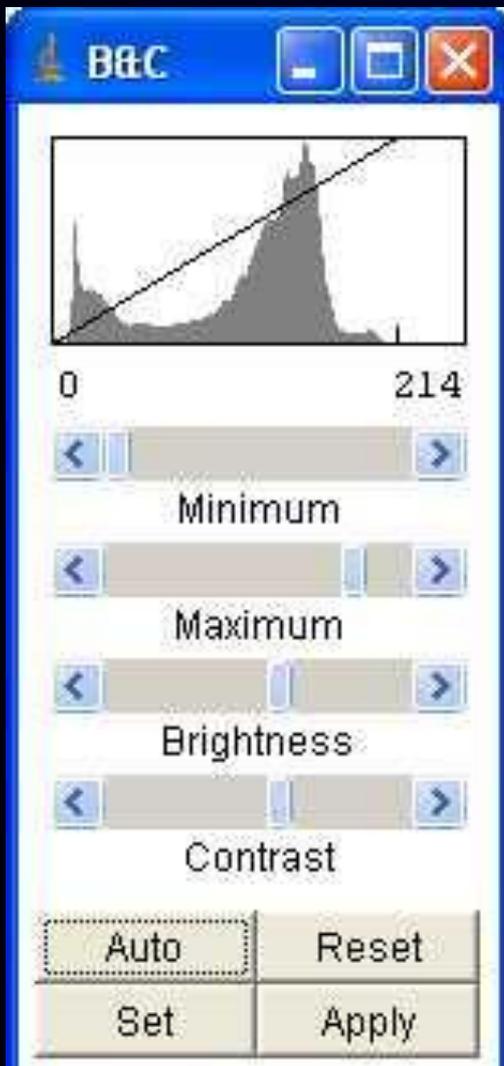


Image → Adjust → Brightness/Contrast...



Corrections linéaires

Luminosité Contraste



← Mini

← Maxi

← Luminosité →

← Contraste →

Niveaux

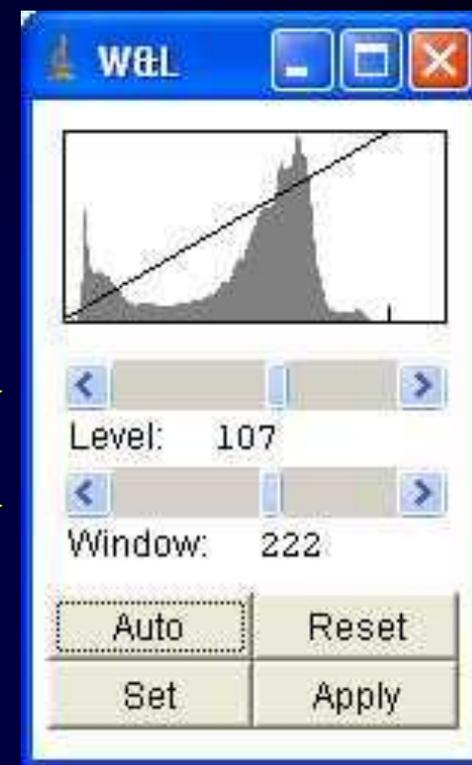
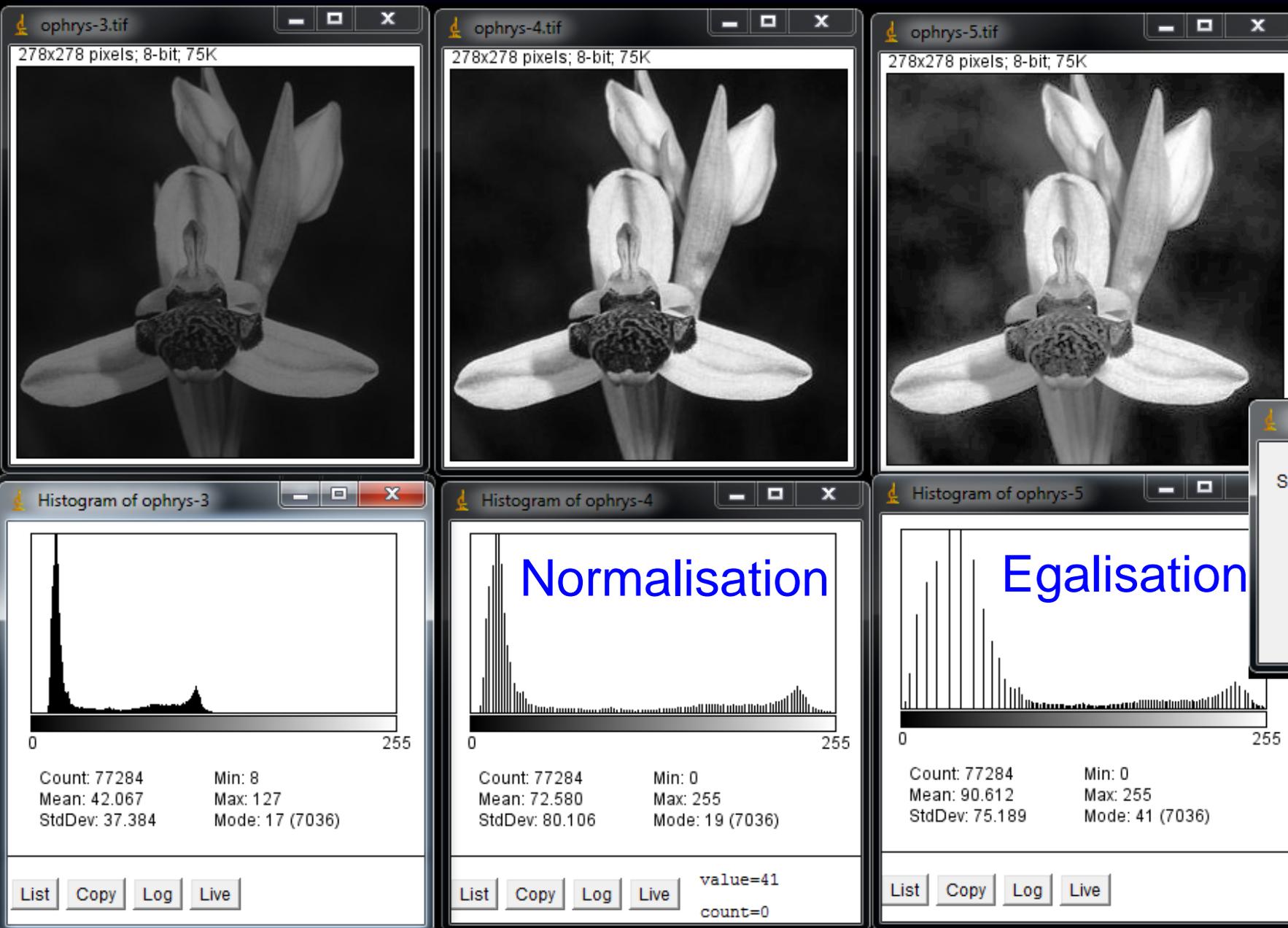


Image → Adjust → Brightness/Contrast...

Correction non linéaire : Egalisation de l'histogramme

Densité de probabilité normalisée pour aplatir l'histogramme



Enhance Contrast

Saturated pixels: 0.0 %

Normalize

Equalize histogram

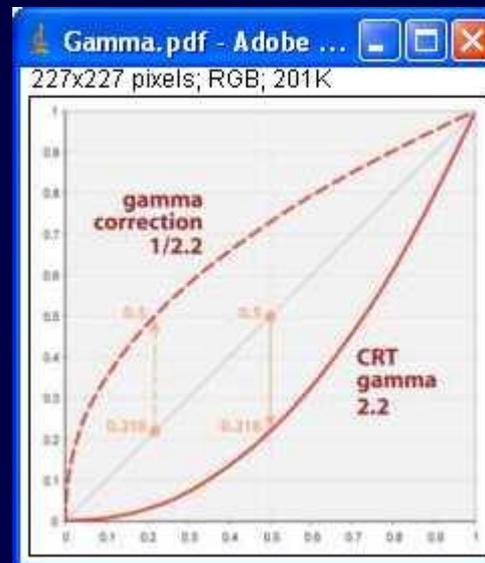
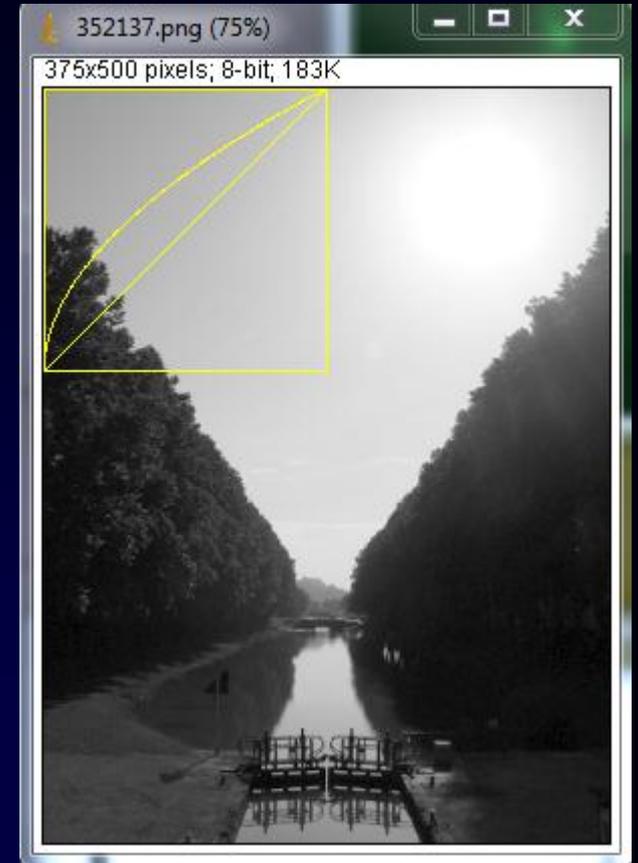
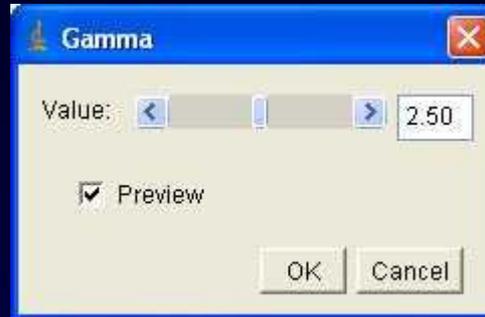
OK Cancel Help

Process → Enhance Contrast

Correction non linéaire du Gamma

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

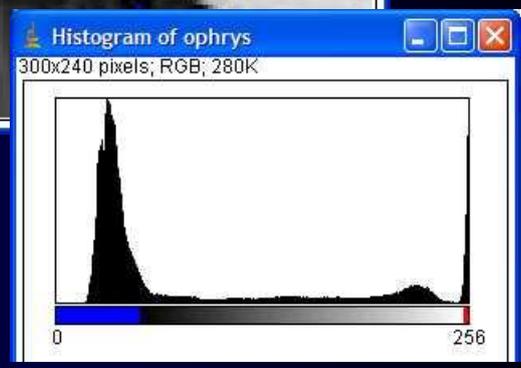
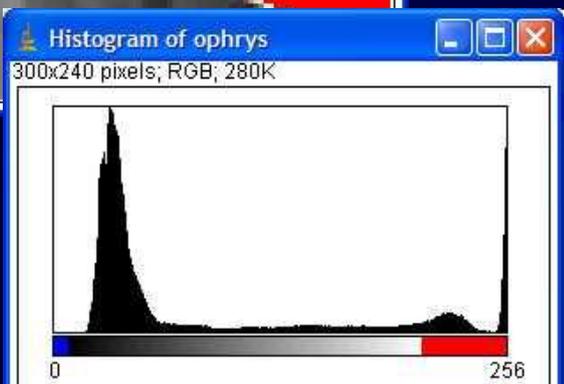
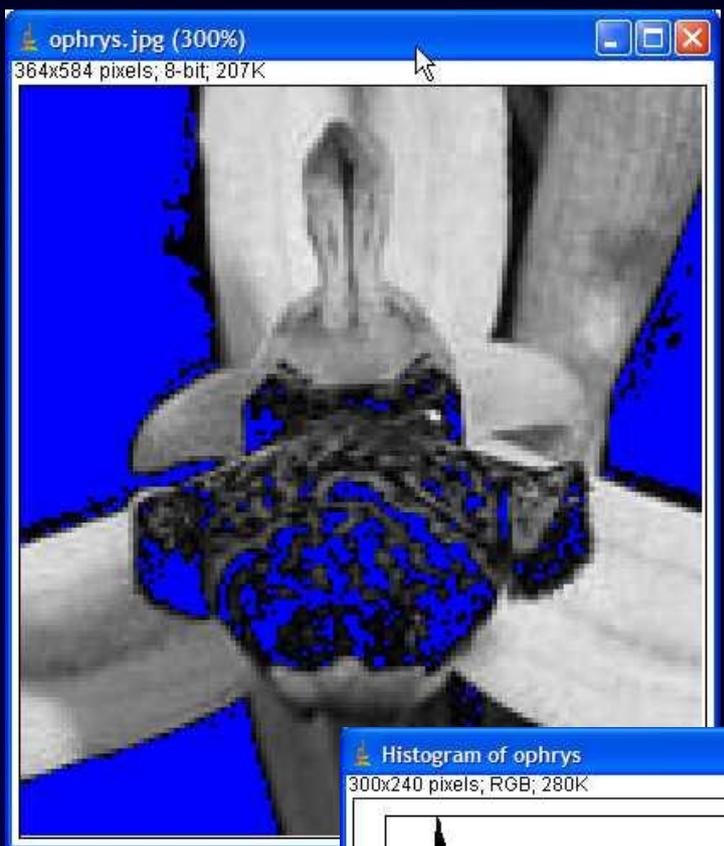
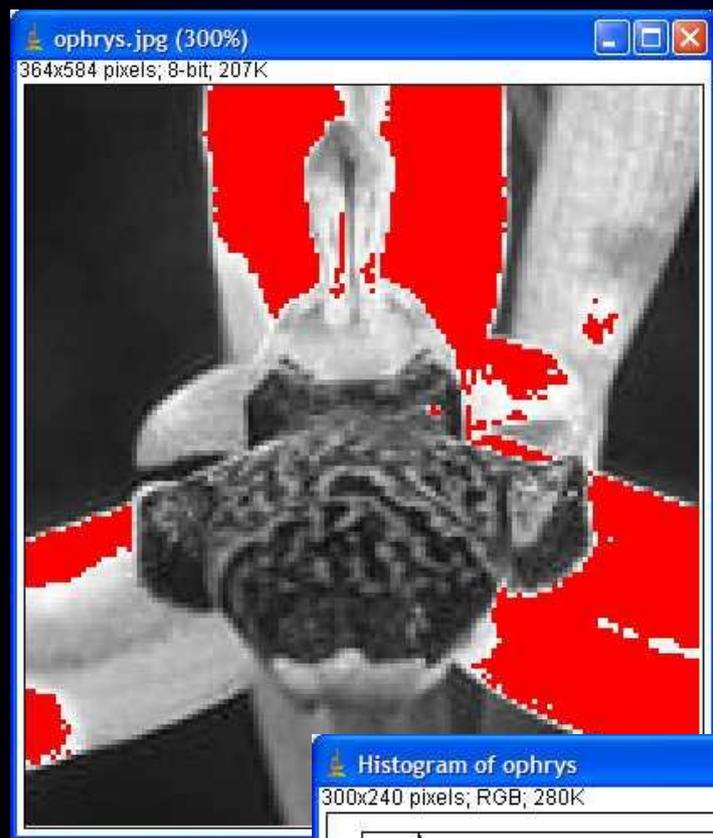
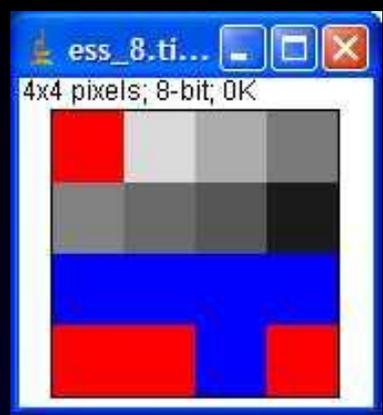
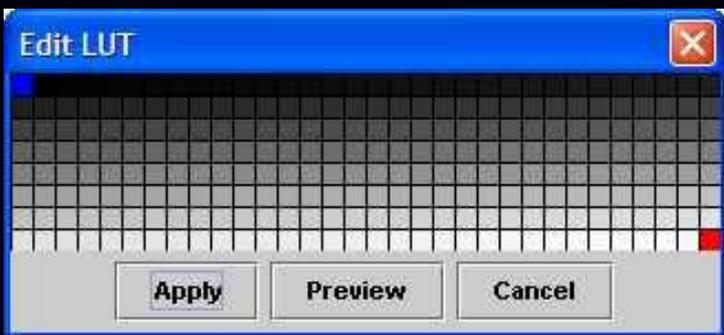
range = gamme de valeurs des pixels de l'image



Process → Math → Gamma...

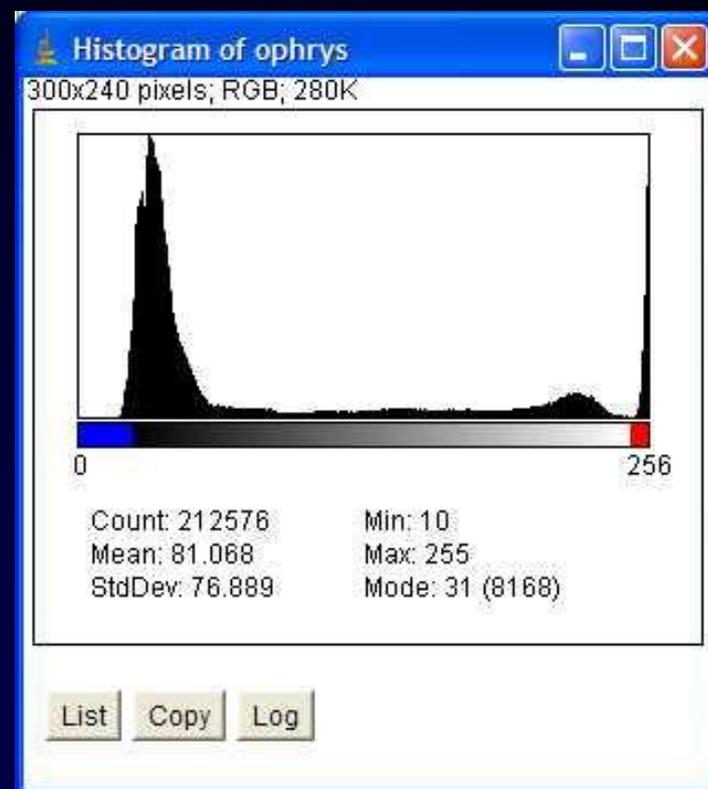


Réglages avec la LUT Hi Lo

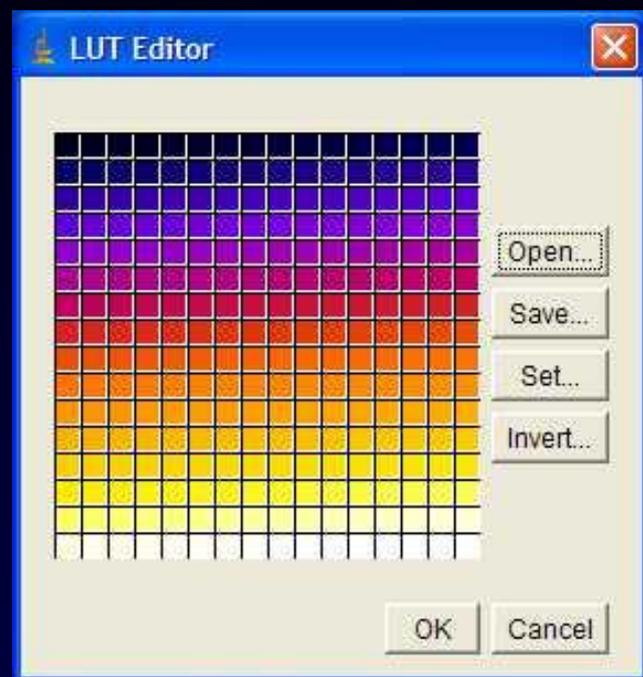
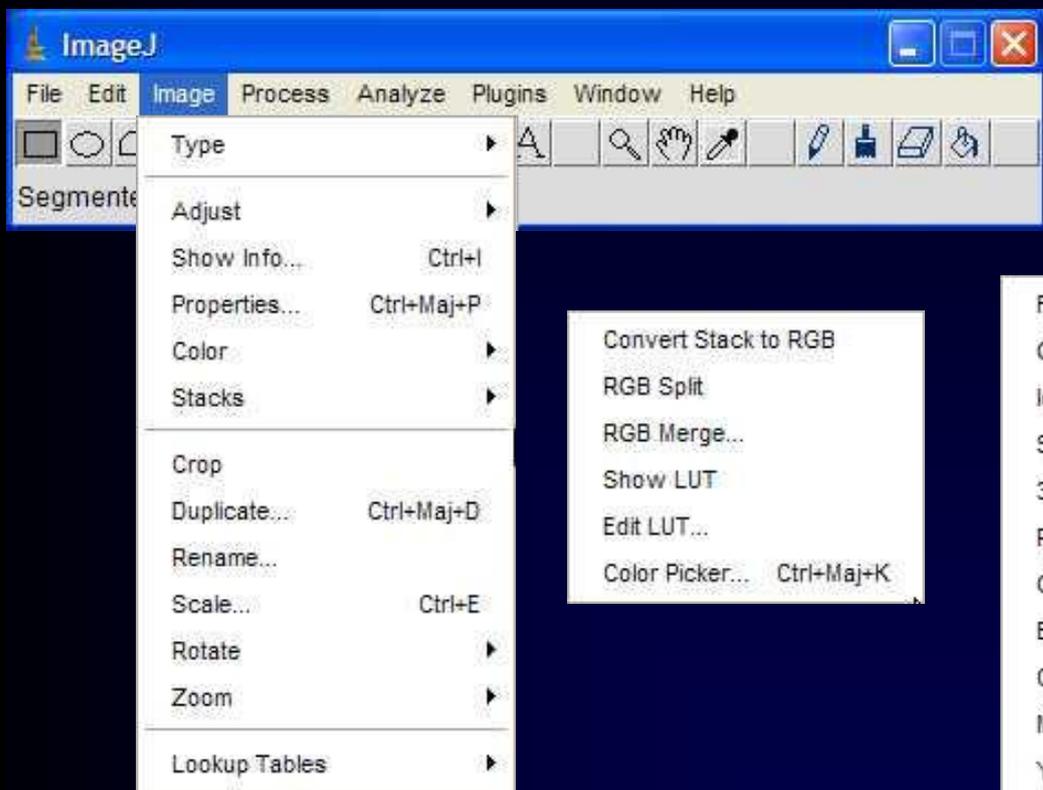




Réglages avec la LUT Hi Lo



Outils LUT dans ImageJ



Réglages d'images composites

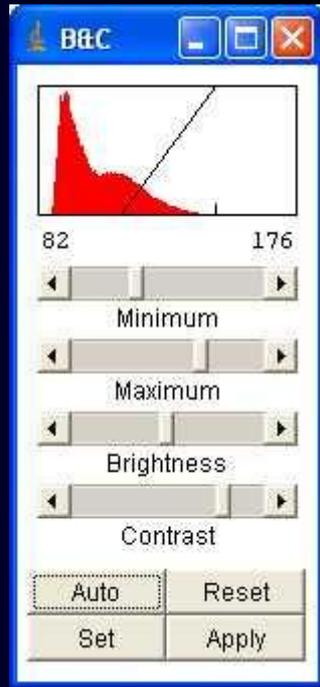
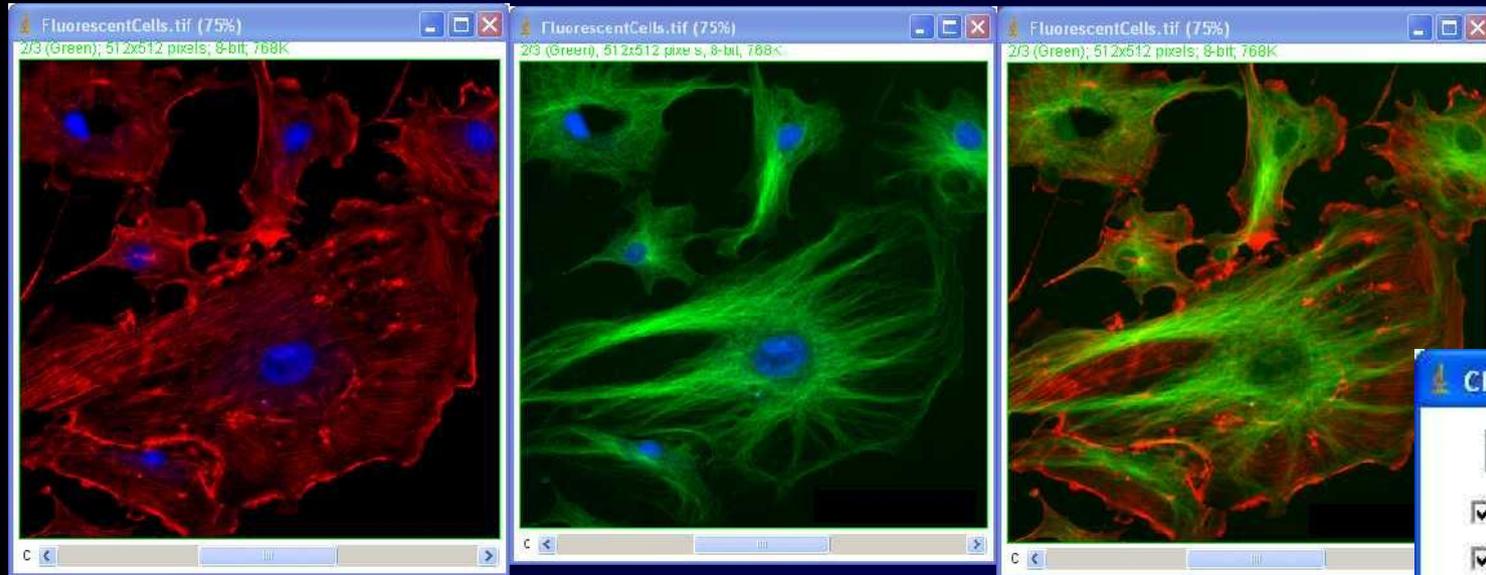
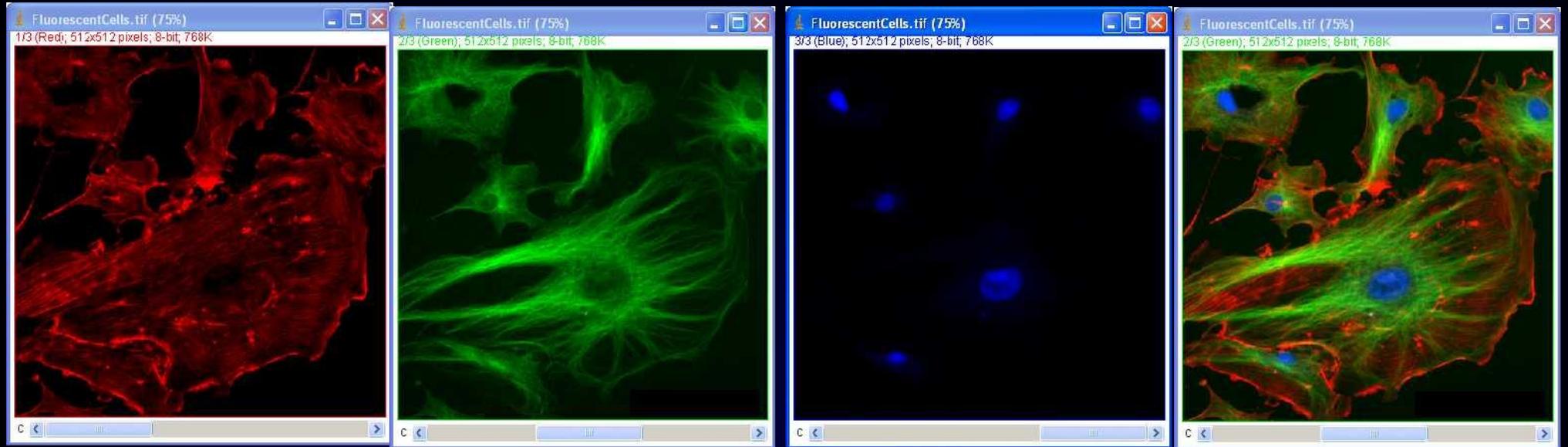


Image → Color → Make Composite



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

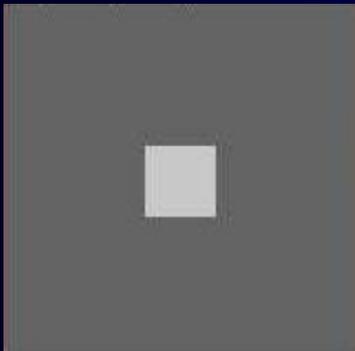
100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

Noyau

$$\begin{array}{|c|c|c|} \hline 1 & 1 & 1 \\ \hline 1 & 4 & 1 \\ \hline 1 & 1 & 1 \\ \hline \end{array} \quad =$$

Image résultante

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





Filtres de convolution

Image source

Noyau

Image résultante

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			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

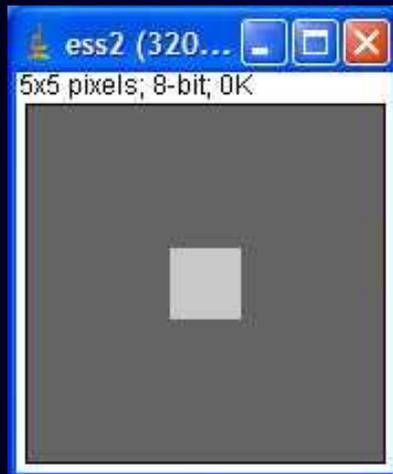
100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

*

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**.

Filtres de convolution

Noyau

1	1	1
1	4	1
1	1	1

$$\Sigma=12$$

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

Produits du noyau
et de la source

100×1	100×1	100×1
100×1	200×4	100×1
100×1	100×1	100×1

$$\Sigma=1600$$

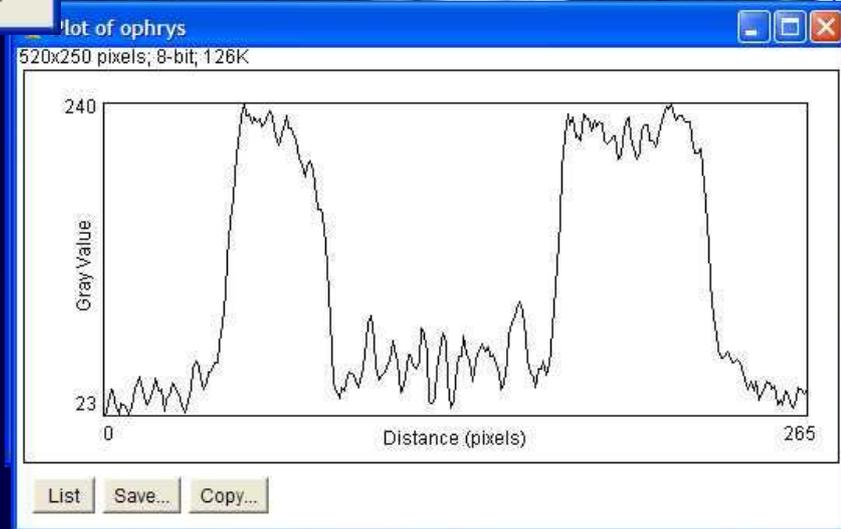
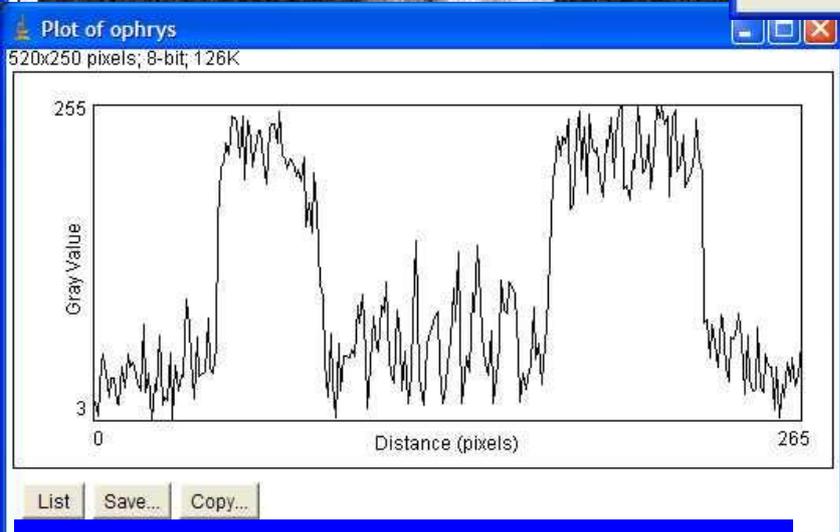
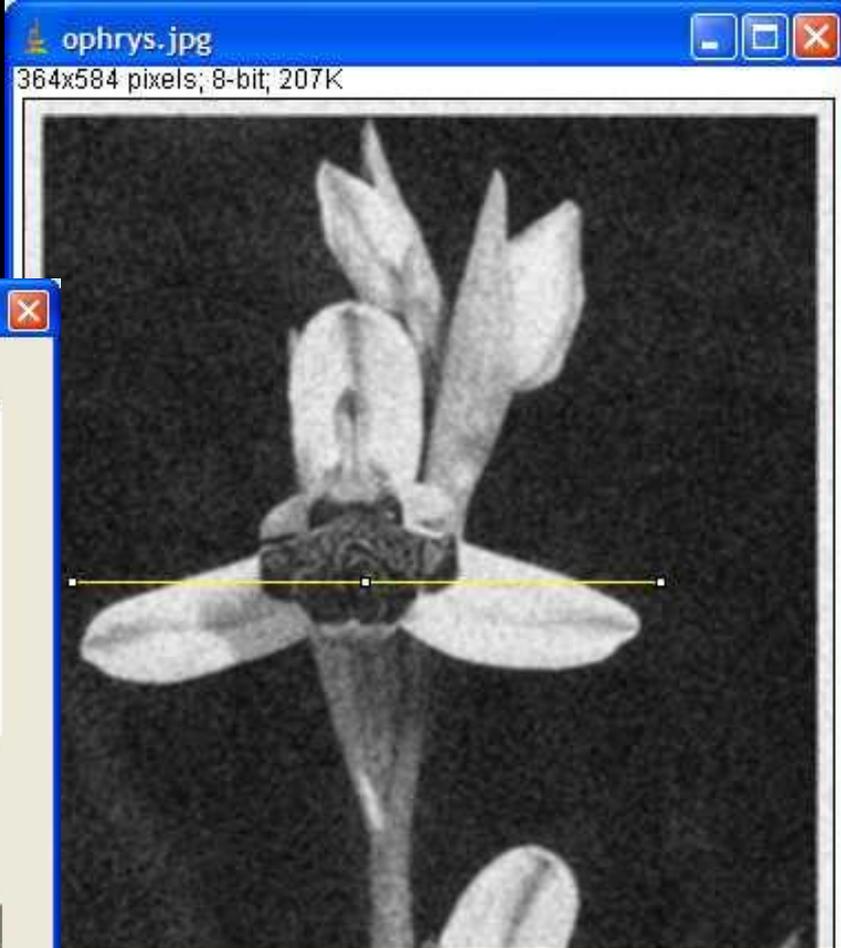
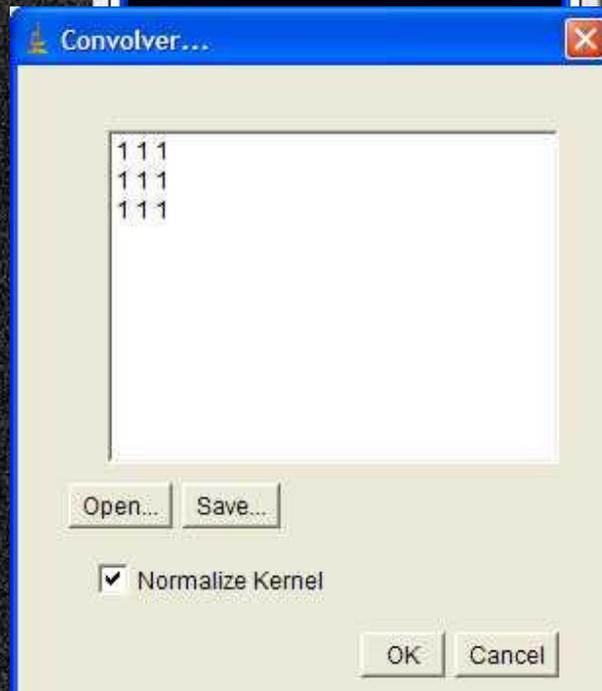
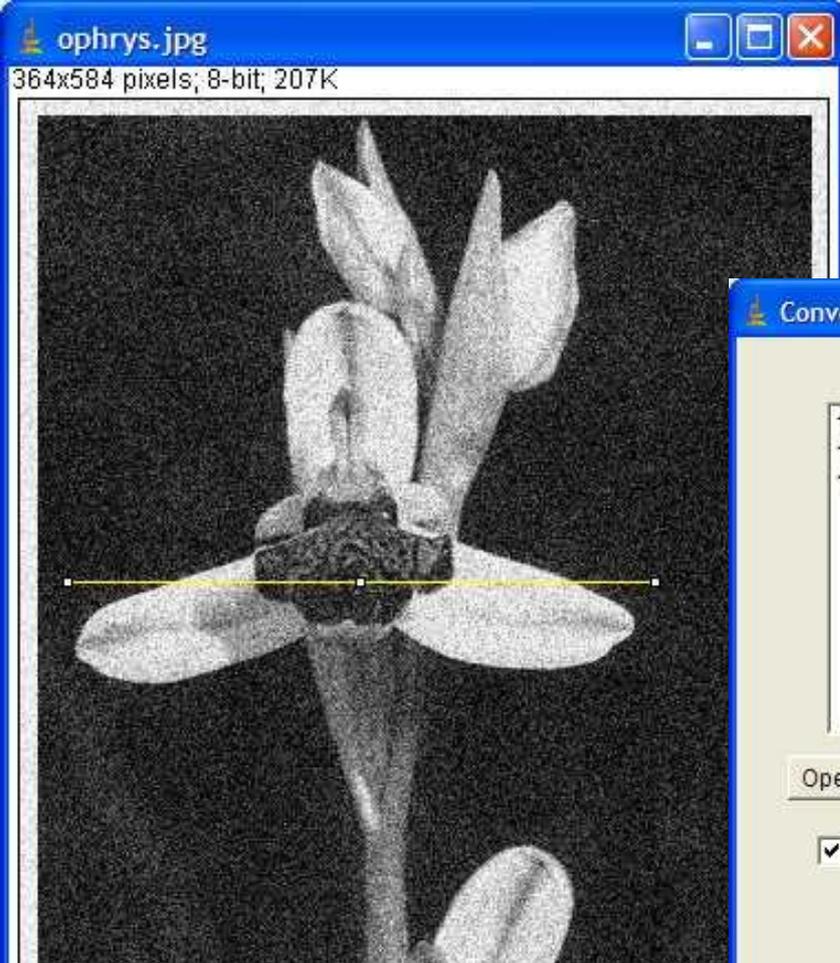
Image résultante

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

Le pixel cible prend la valeur de la somme des produits normalisée

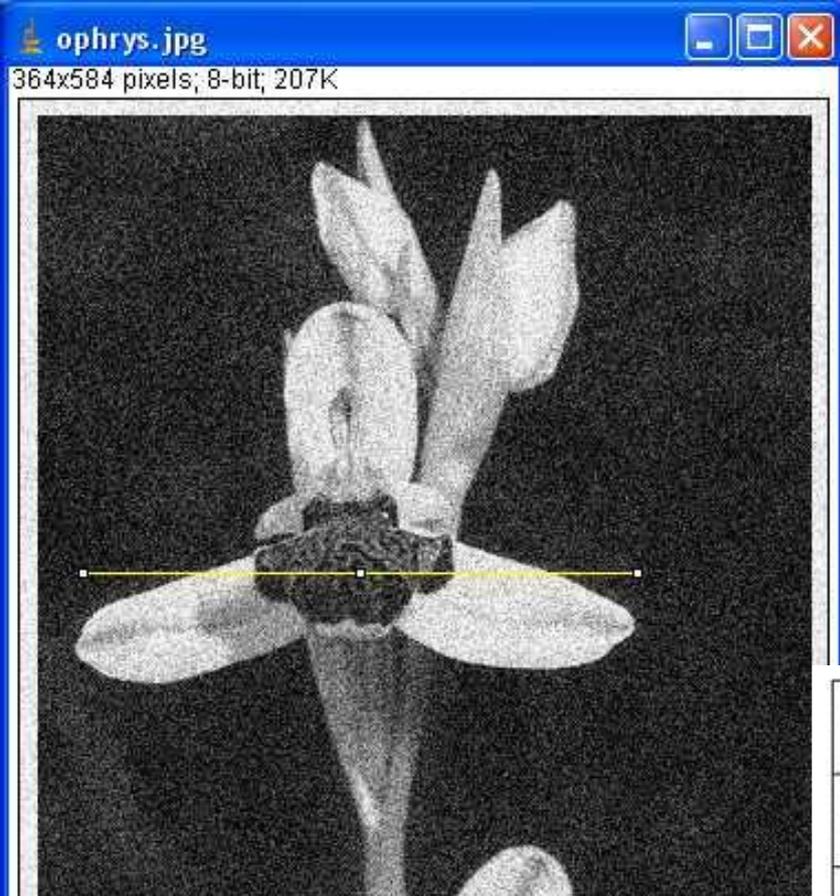
$$1600/12=133$$

Filtre passe-bas moyen

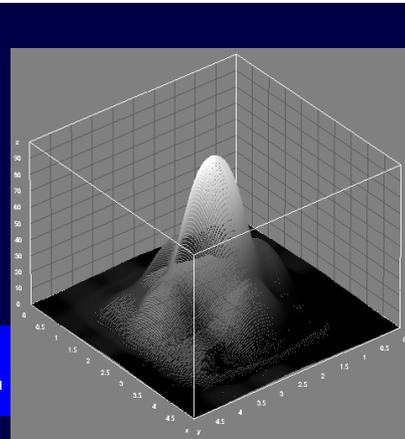
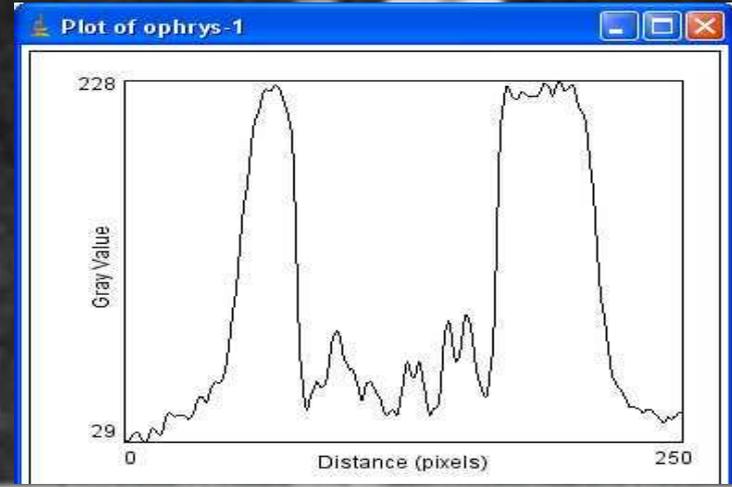
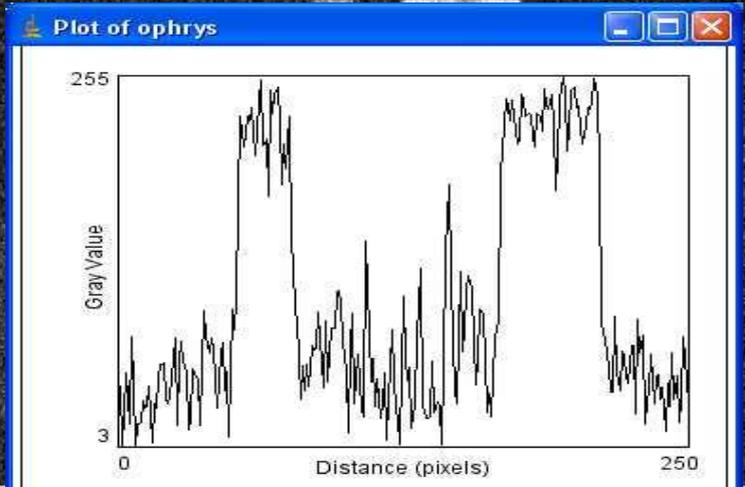
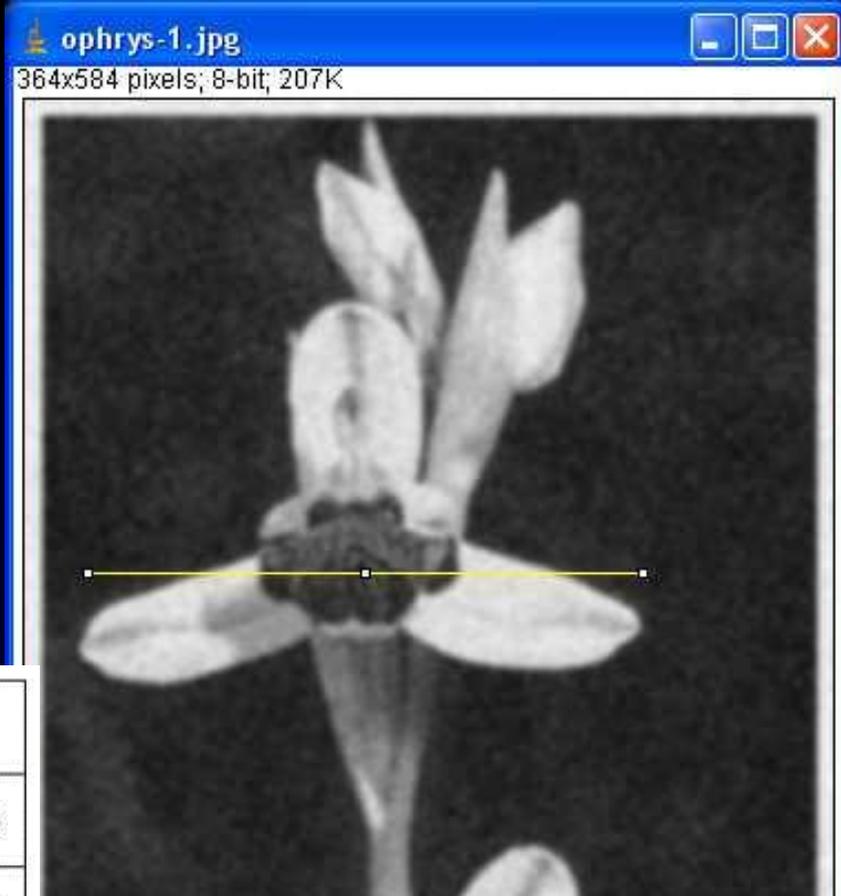


Process → Filters → Convolve...

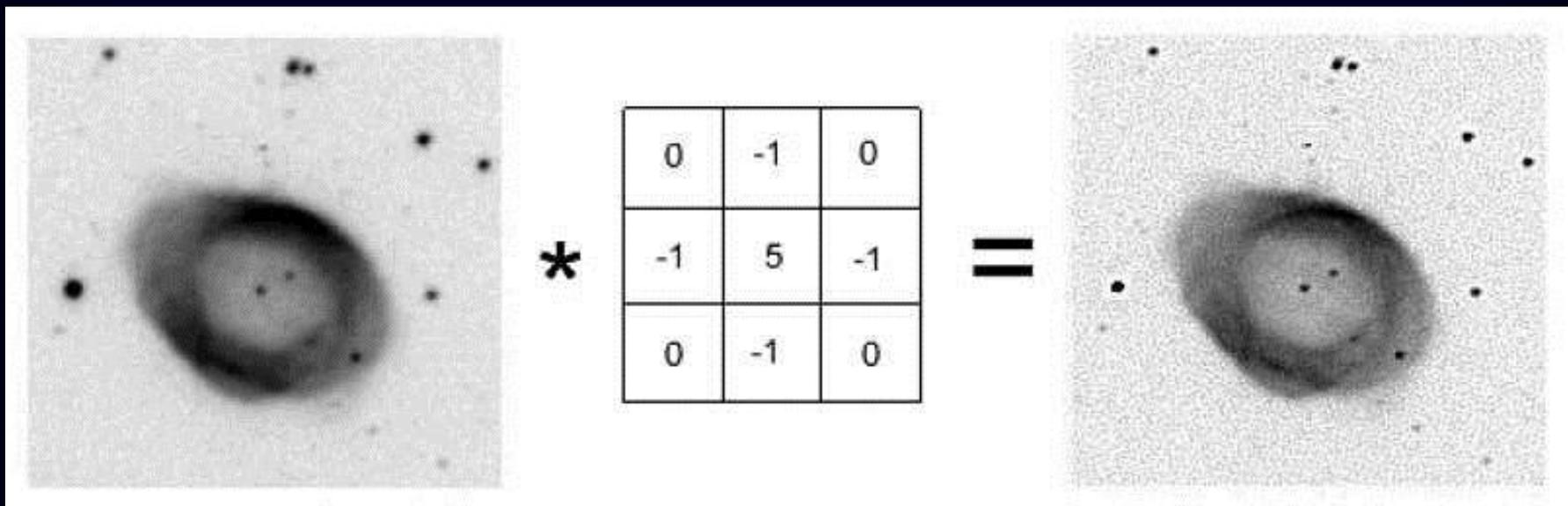
Filtre Gaussien



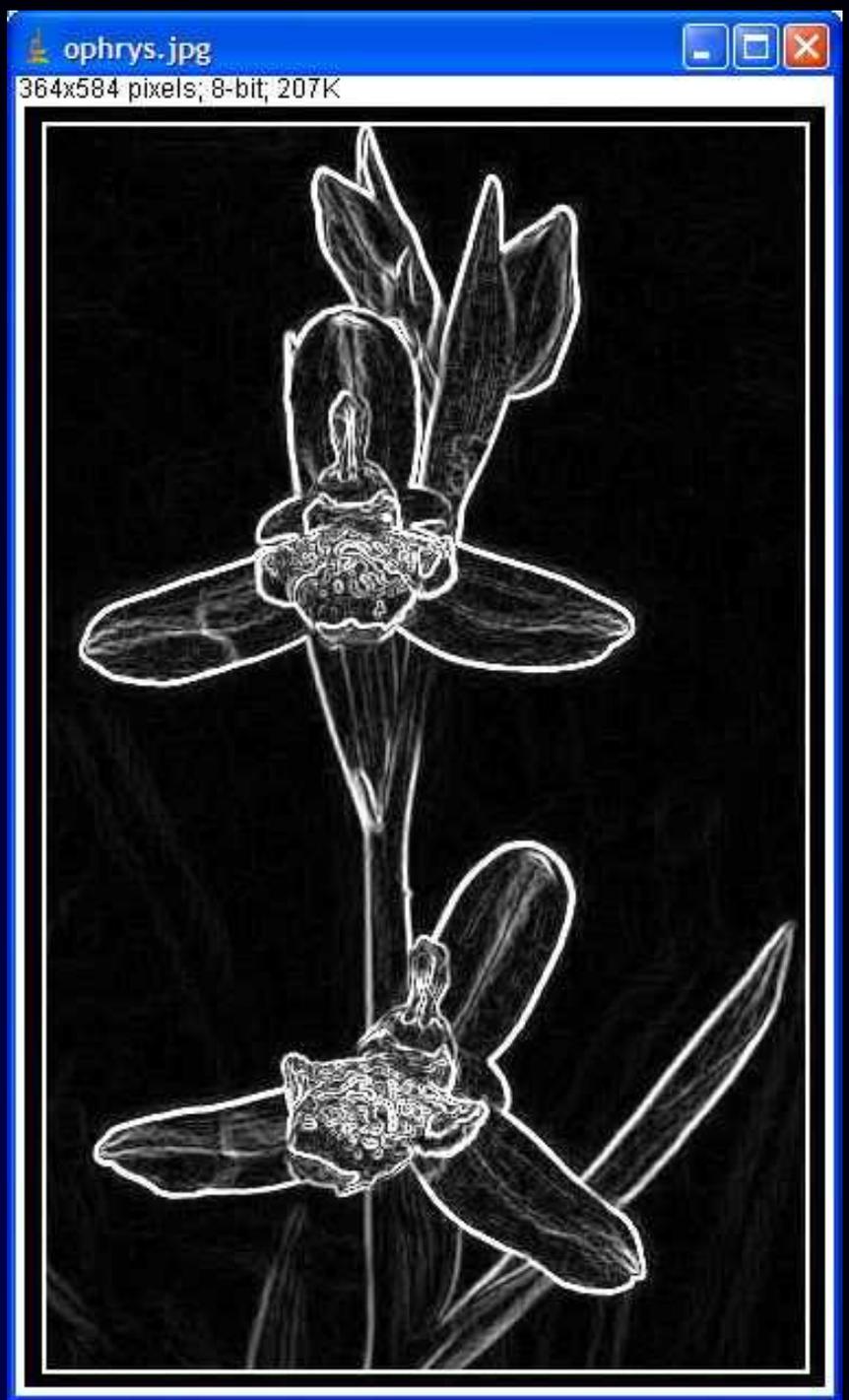
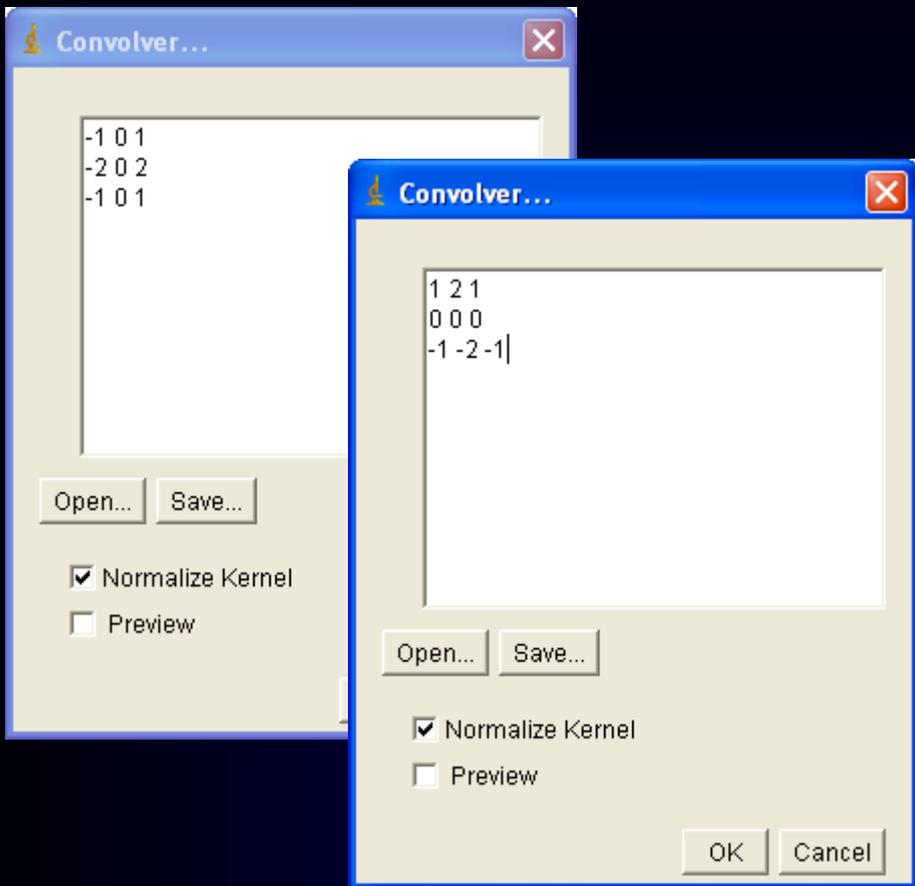
0	0.6	1.7	0.6	0
0.6	13	36	13	0.6
1.7	36	100	36	1.7
0.6	13	36	13	0.6
0	0.6	1.7	0.6	0



Process → Filters → Gaussian Blur.



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



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

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



Filtre non linéaire : le filtre médian

Un filtre médian affecte au pixel central la valeur médiane de la série :

15	18	14
29	27	13
12	19	21

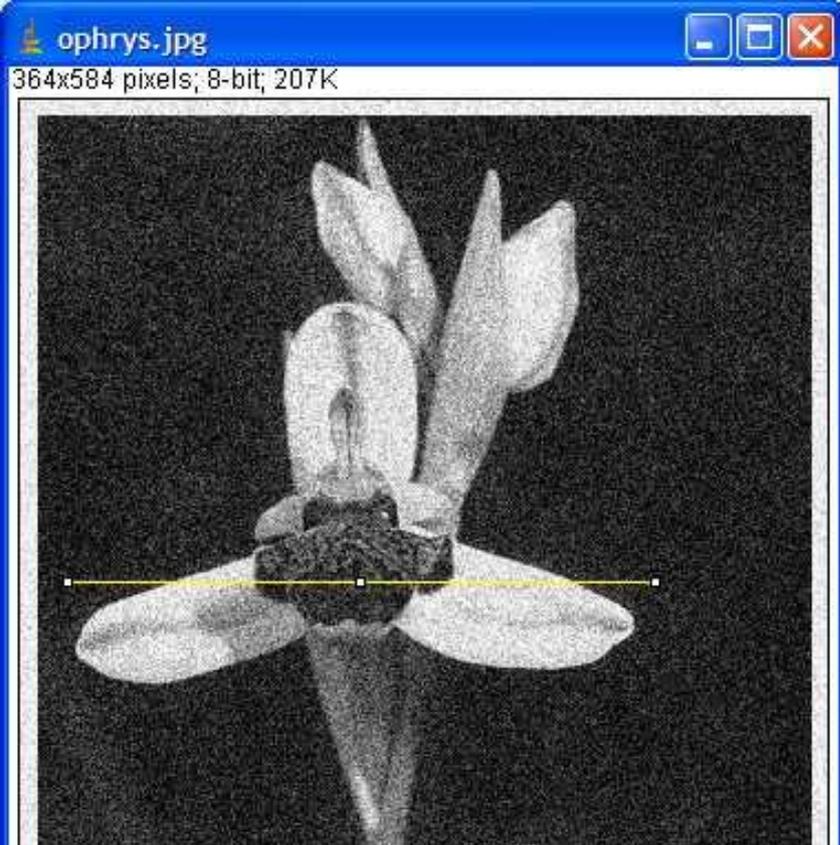


15	18	14
29	18	13
12	19	21

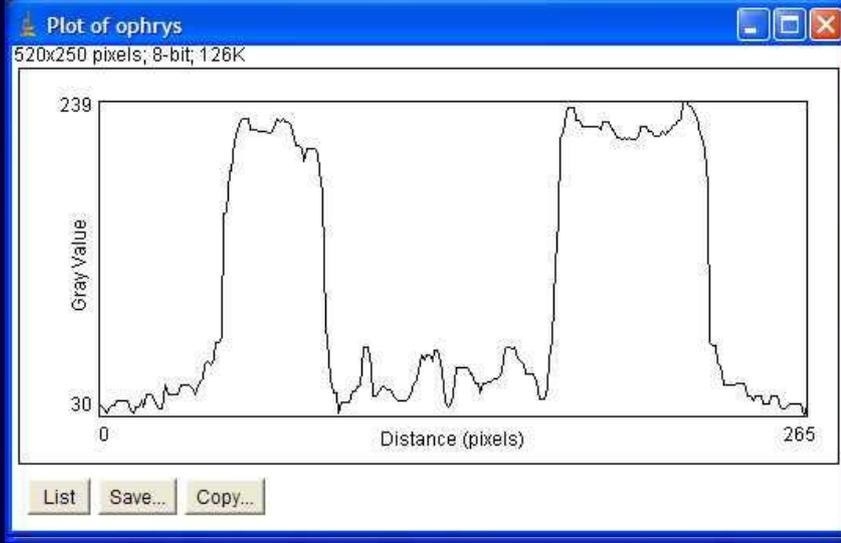
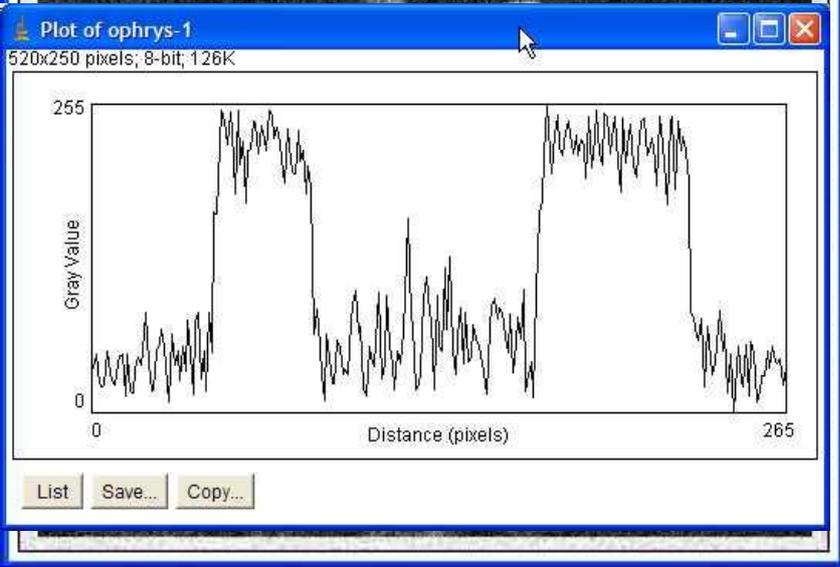
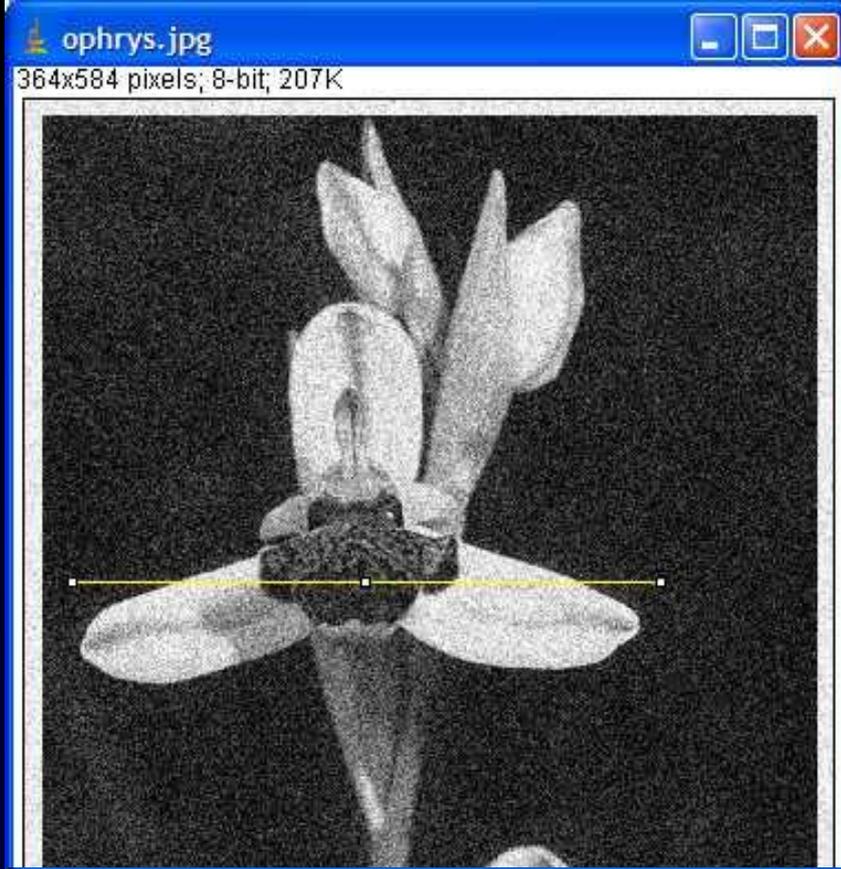
La valeur médiane de : 12,13,14,15,18,19,21,27,29 est **18**.

Filtre min → **12**

Filtre max → **27**

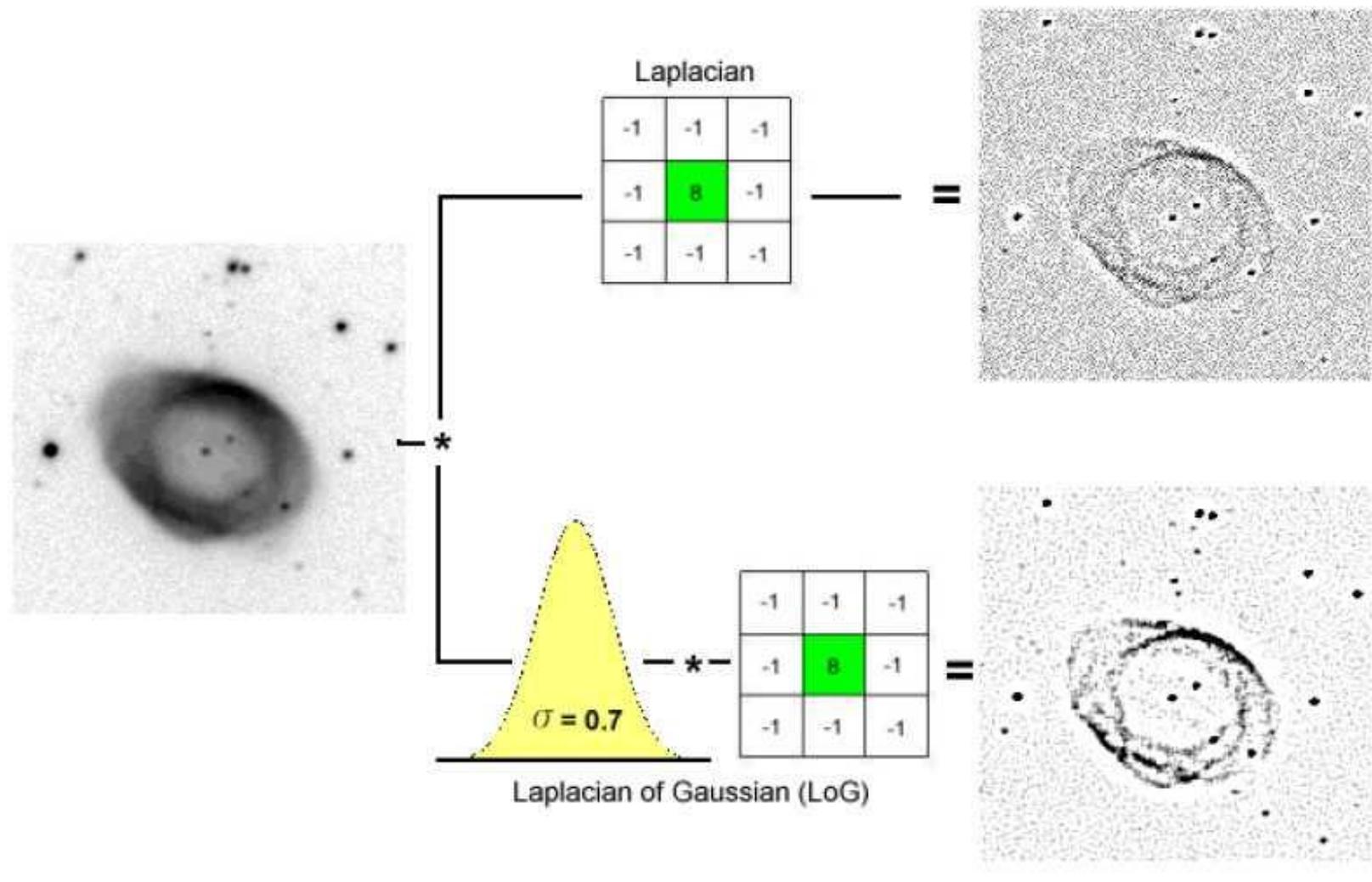


Filtre médian



Process → Filters → Median...

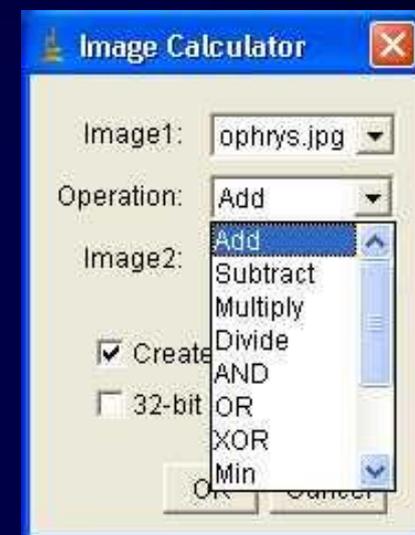
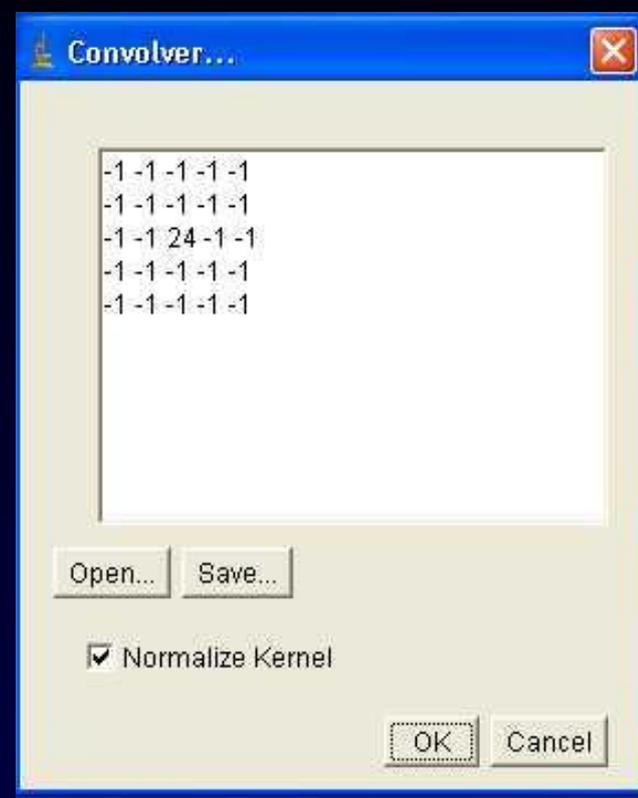
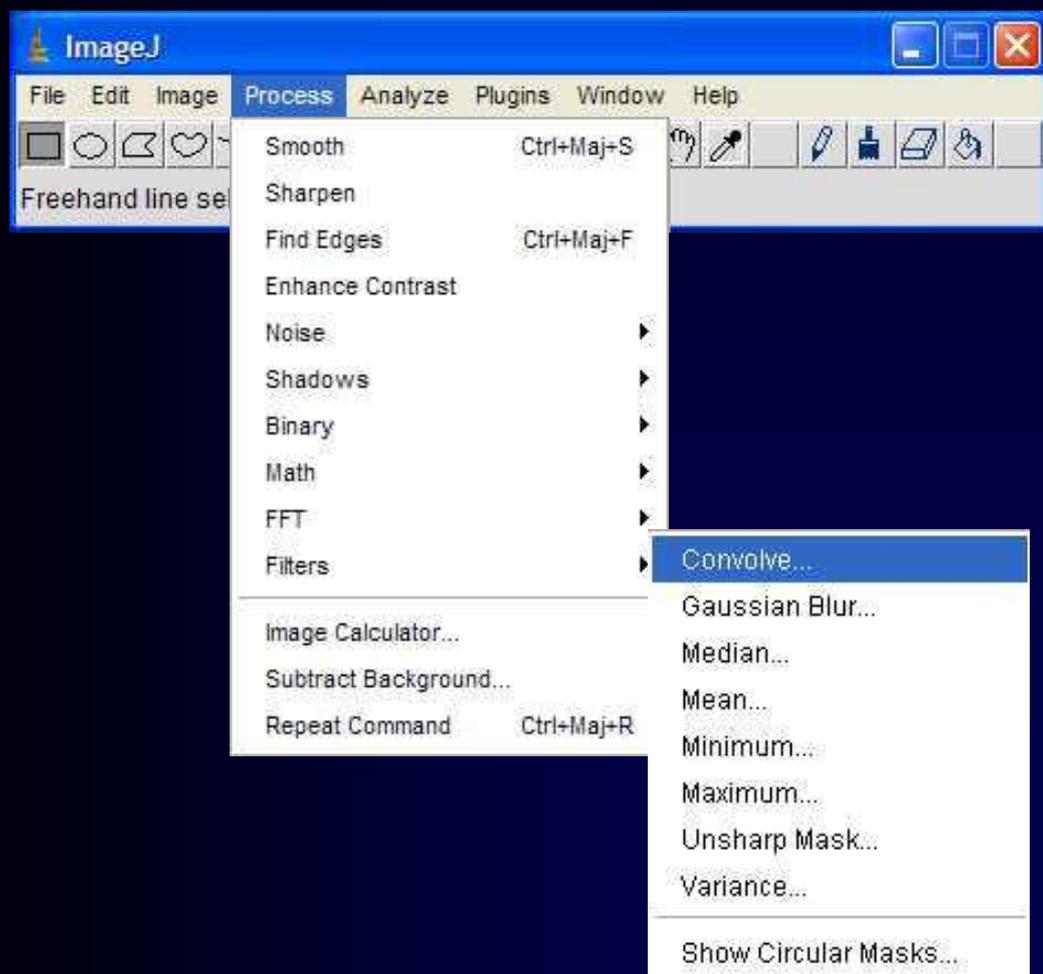
Combinaison Gaussien Laplacien



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

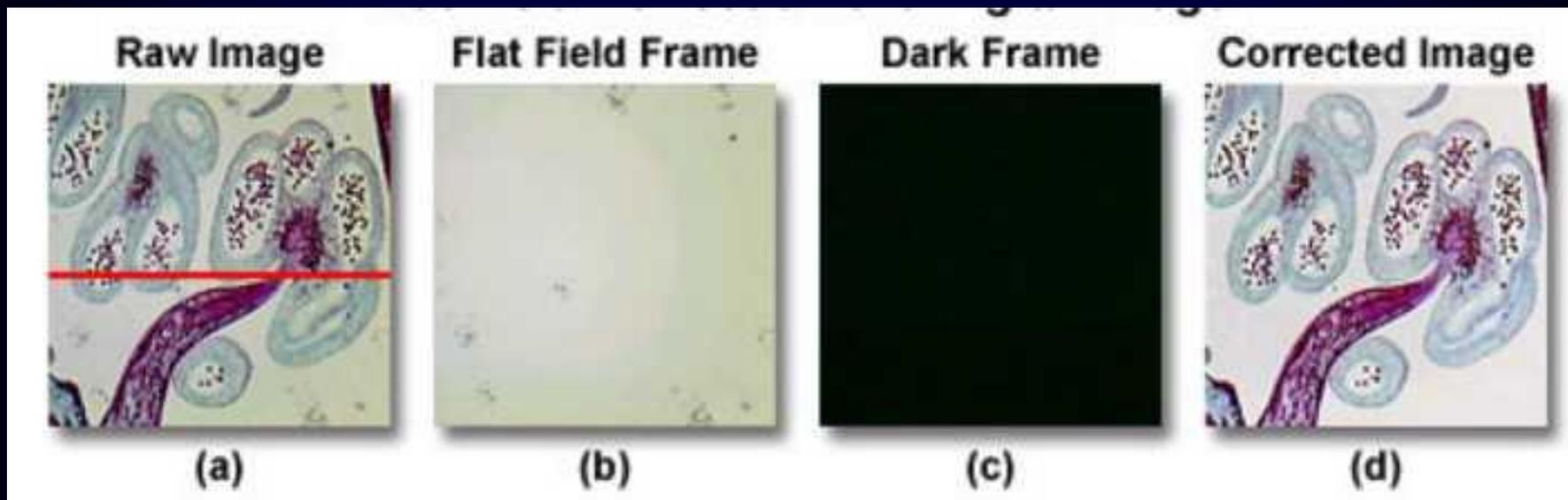
- Bruits de la caméra: bruit aléatoire, «pixels chauds», bruits périodiques
- Illumination non-homogène

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

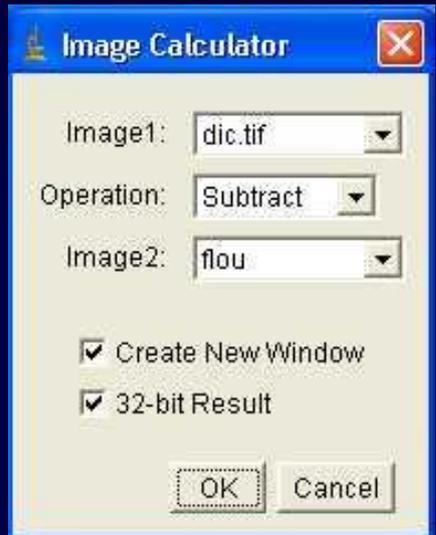
Bruit périodique : filtre de Fourier

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)



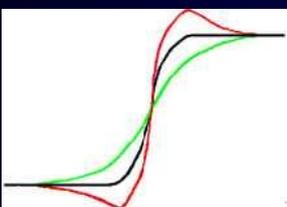
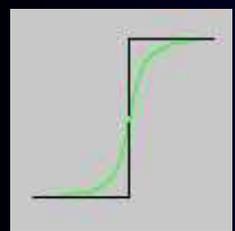
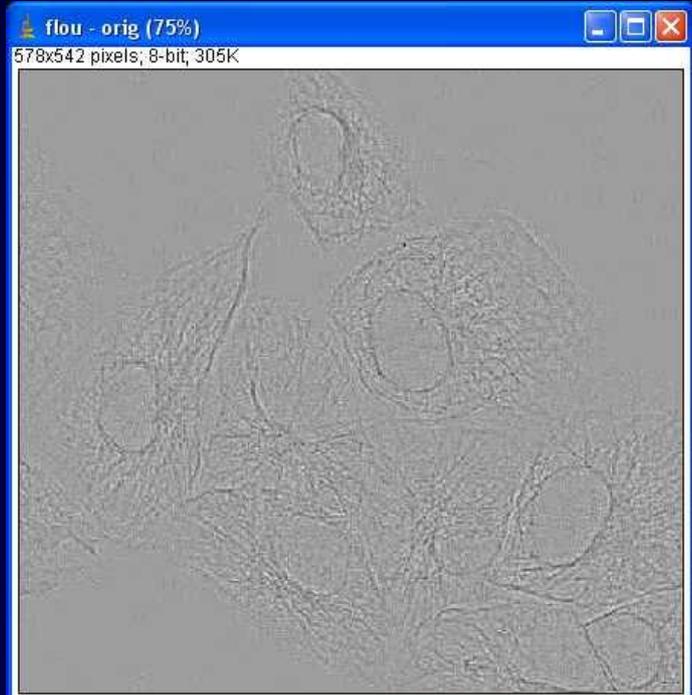
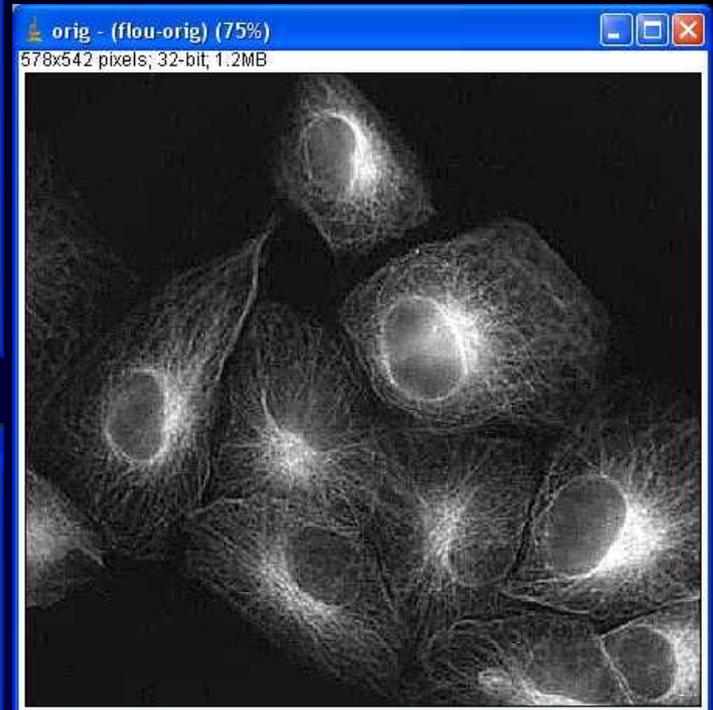
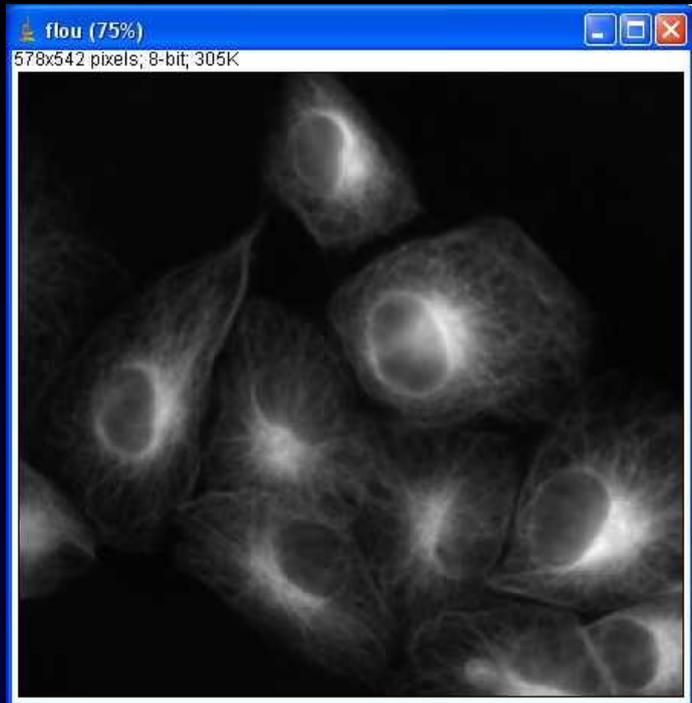
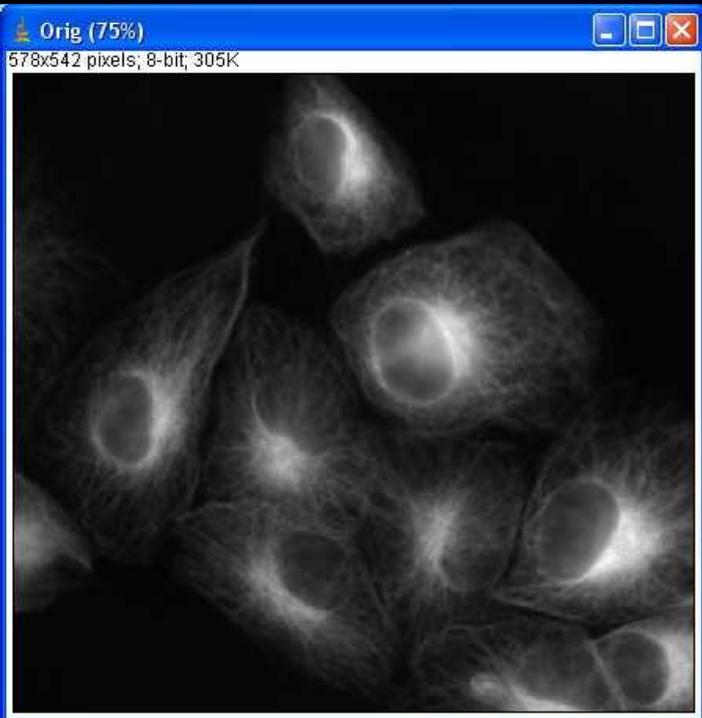
Masquer le fond





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

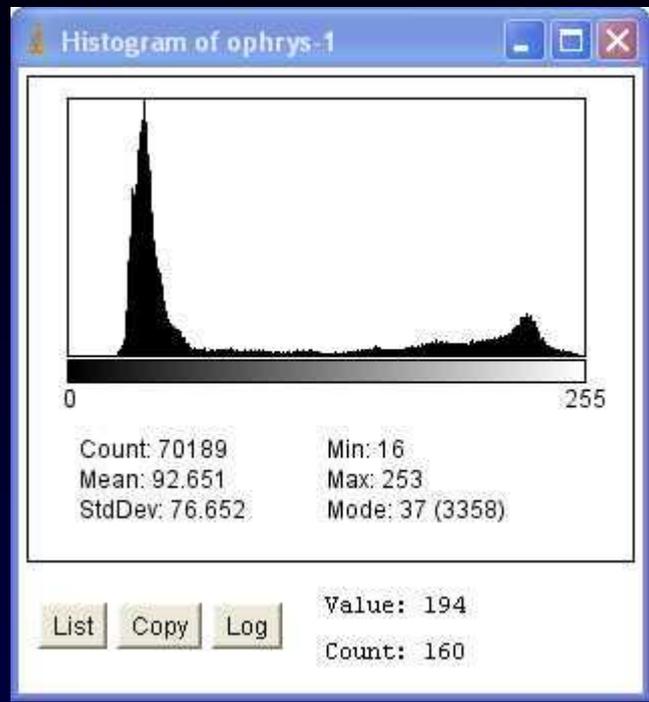
Permet de séparer les régions d'intérêt du fond.

Les Post-traitements

La Quantification



Le Seuillage automatique



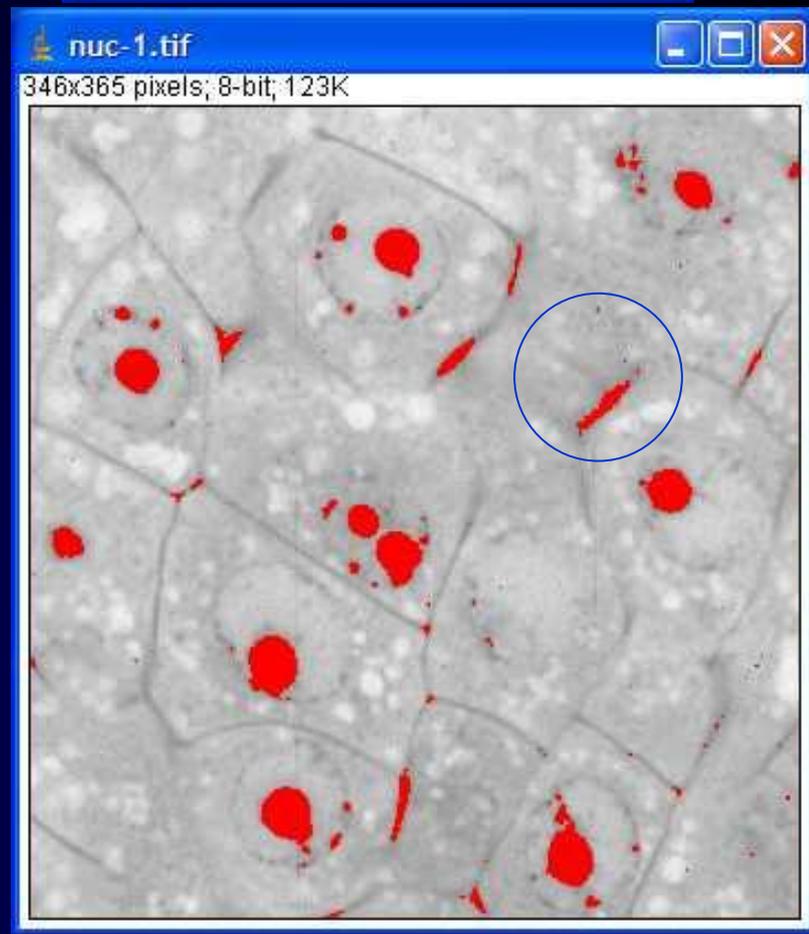
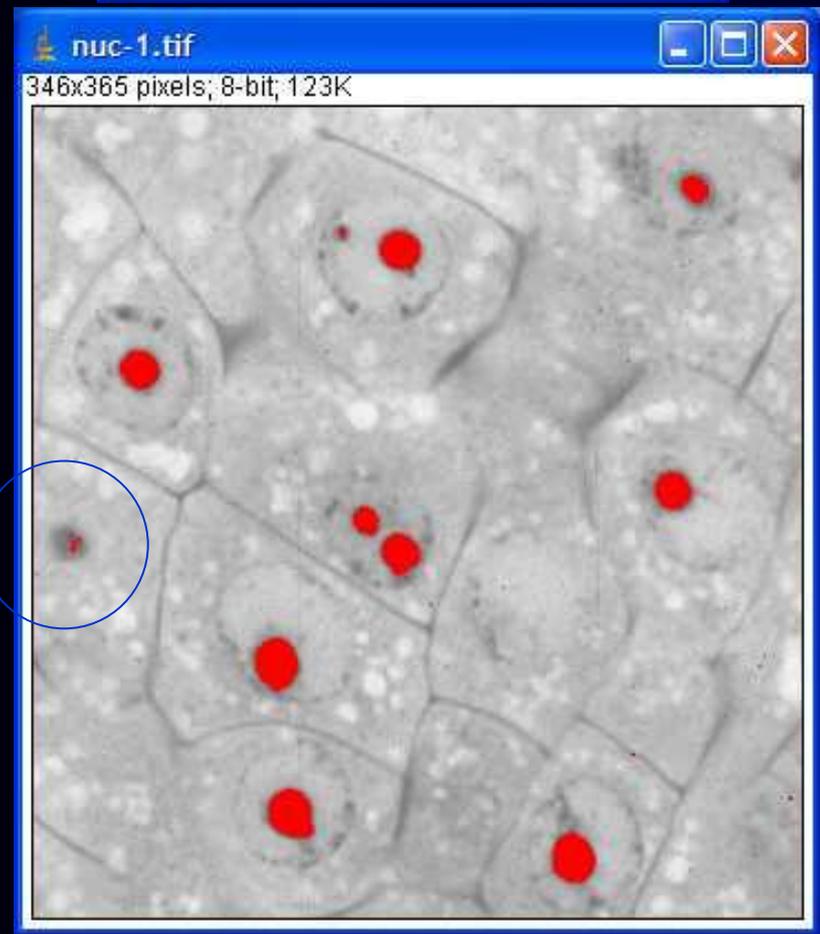
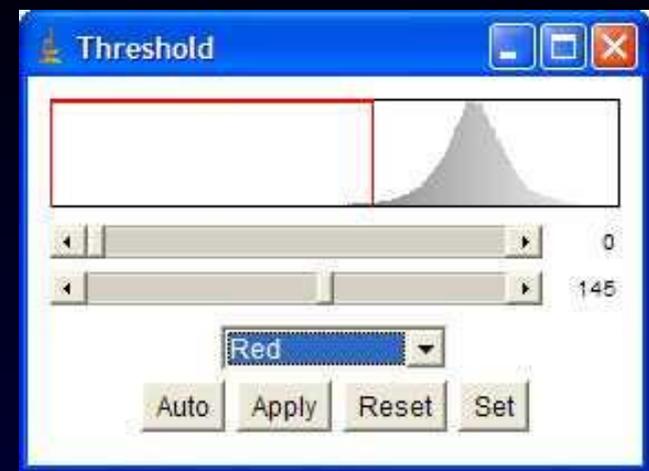
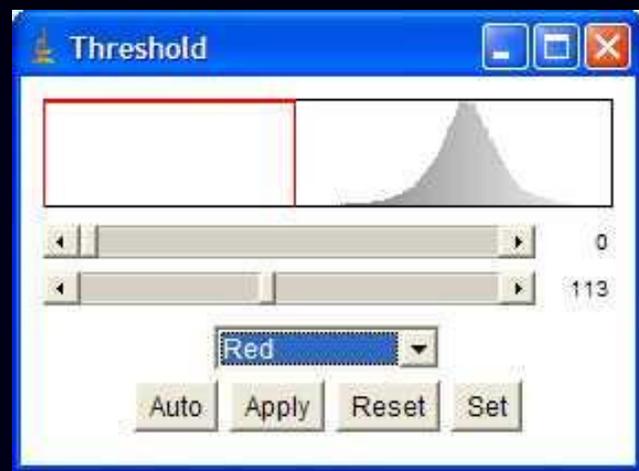
Threshold
 Default Red
 Dark background
 Auto Apply Reset Set

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

Image → Adjust → Threshold...



Le Seuillage manuel





Le Seuillage automatique

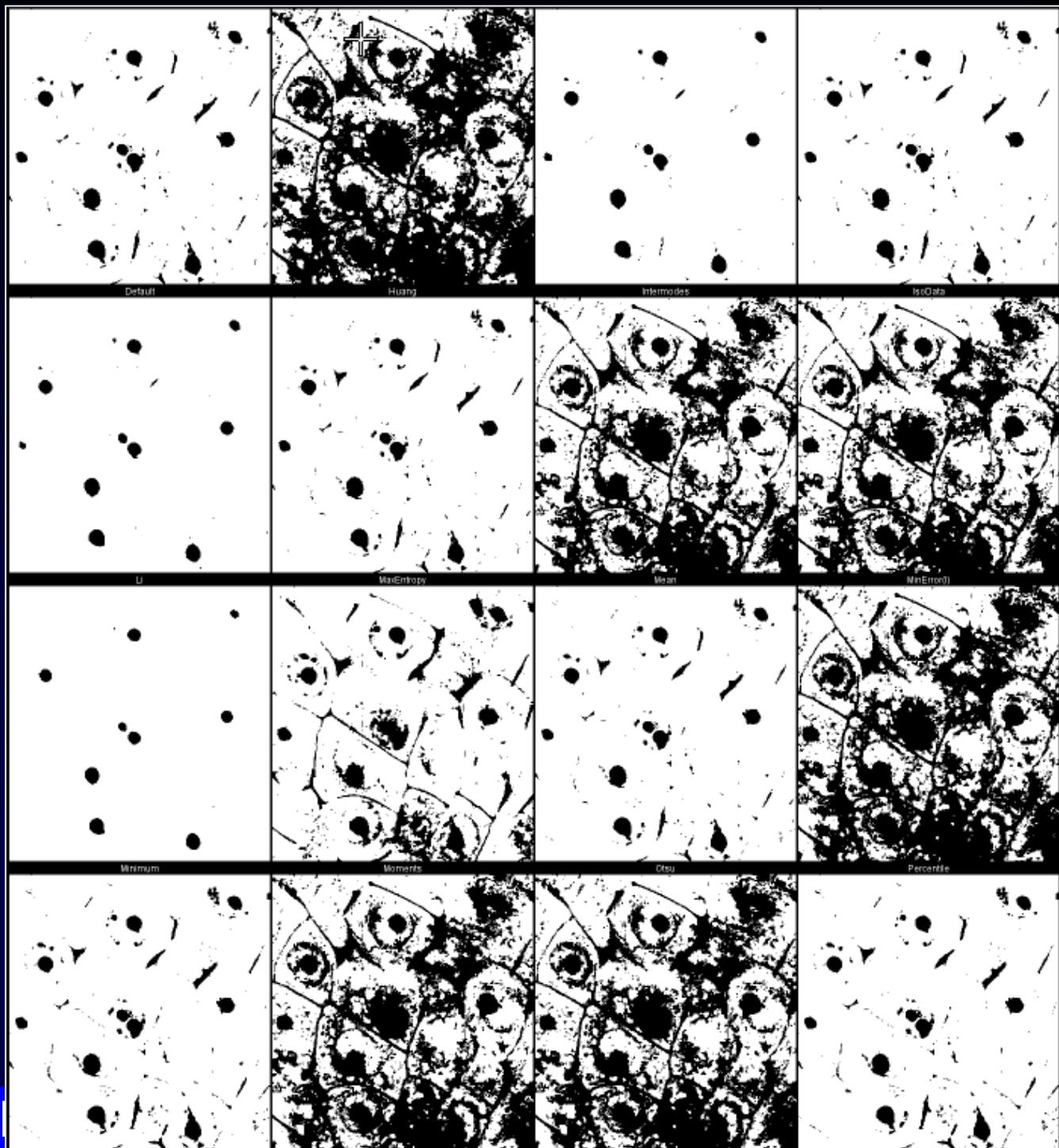
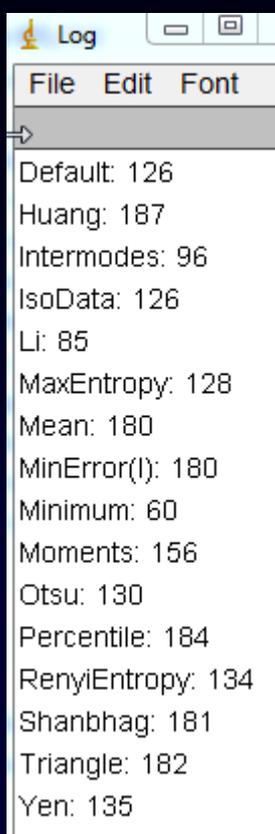
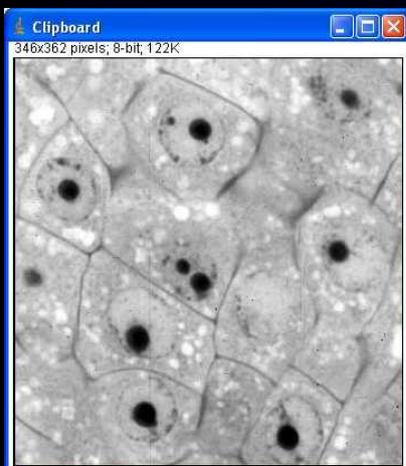
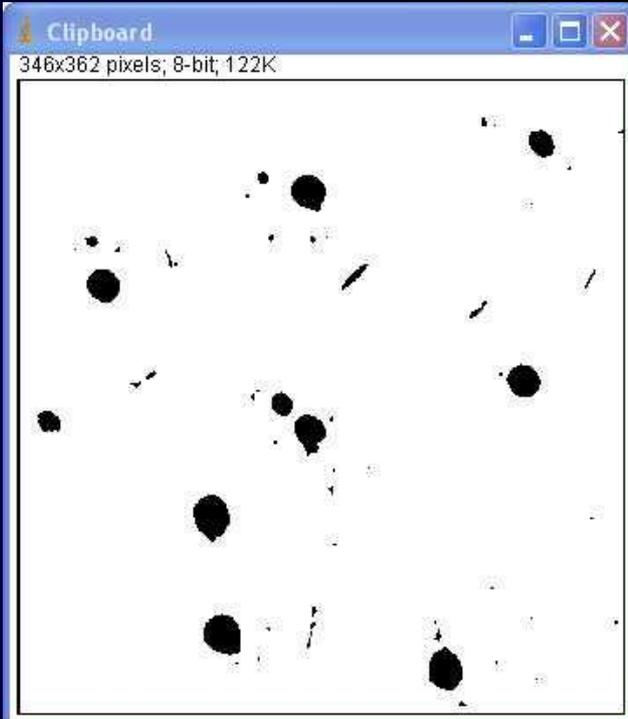
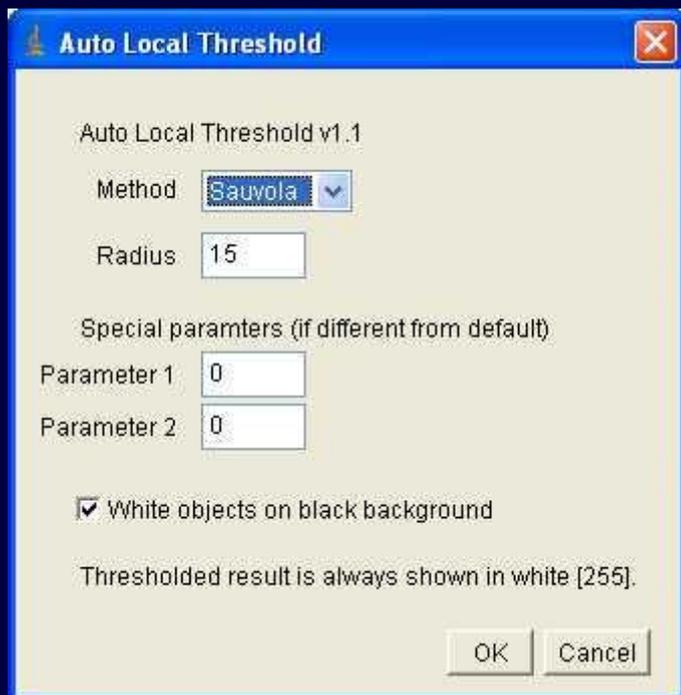
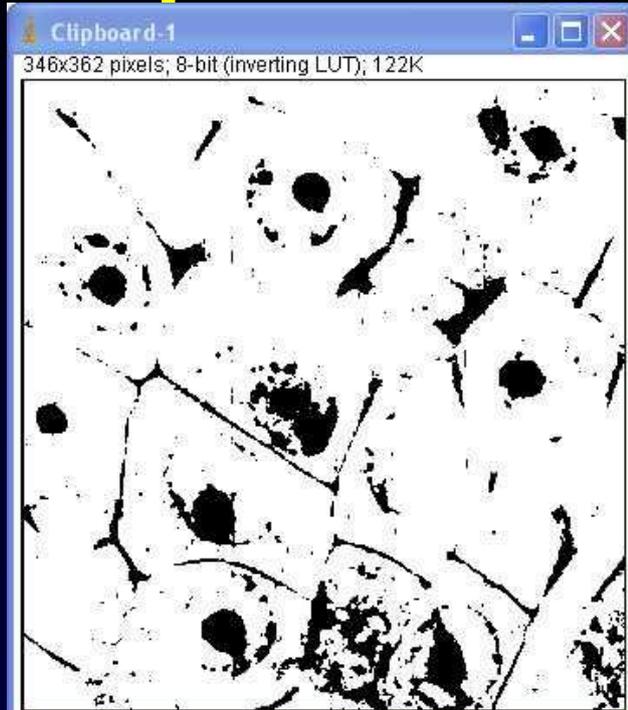
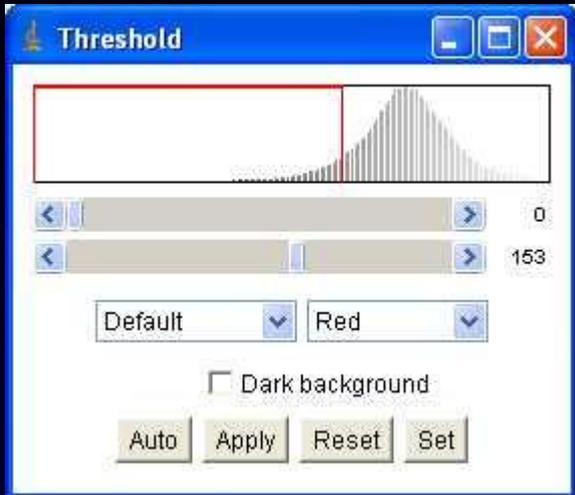
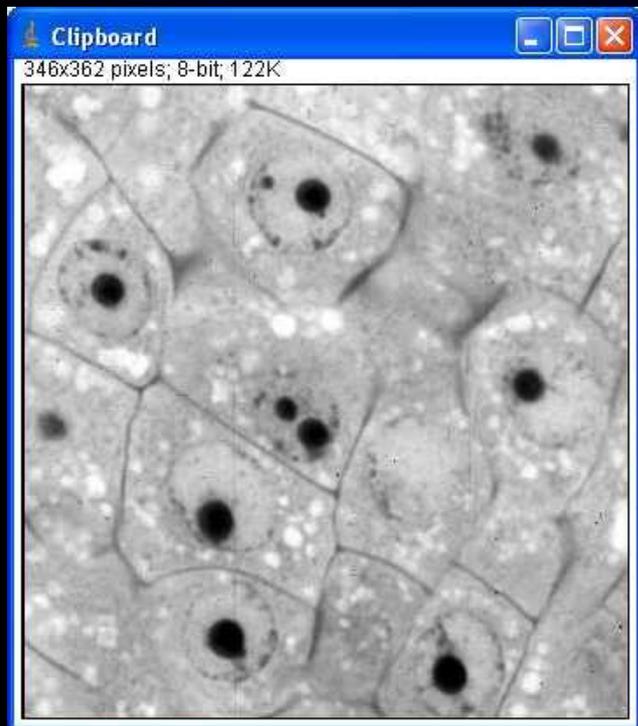


Image → Adjust → AutoThreshold

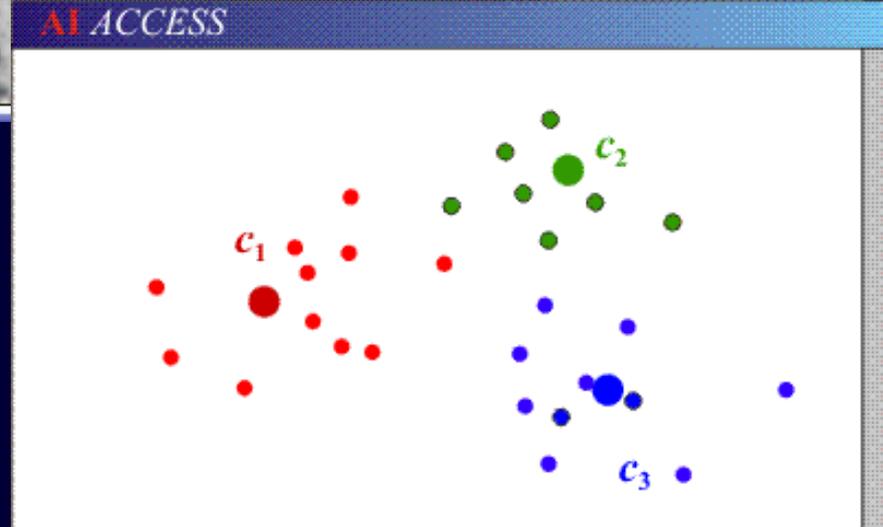
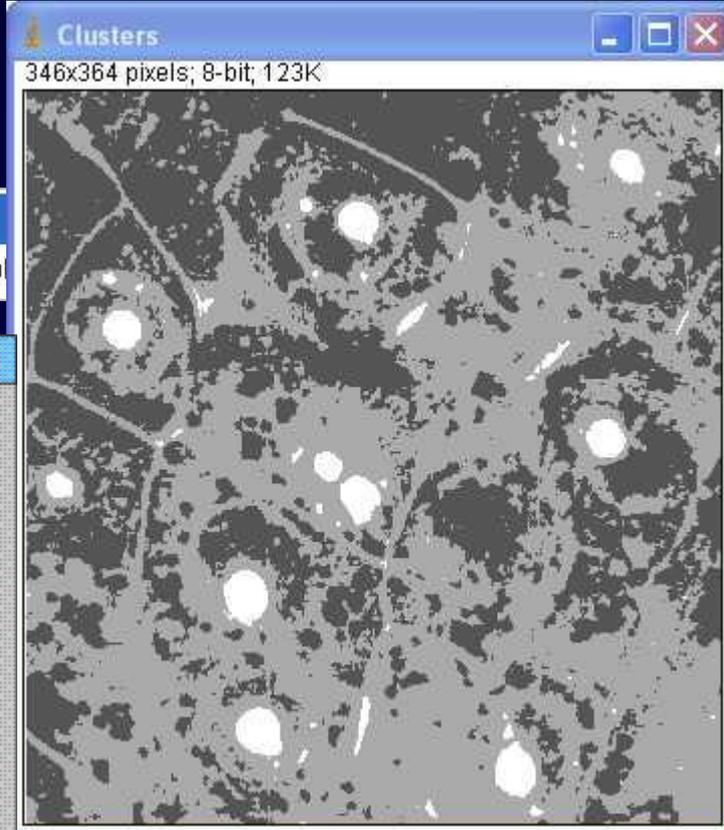
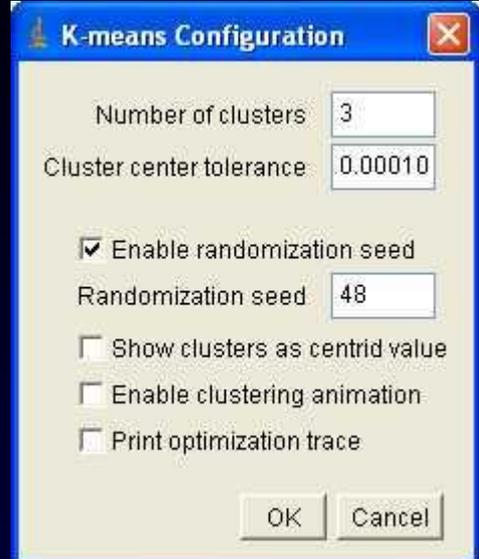
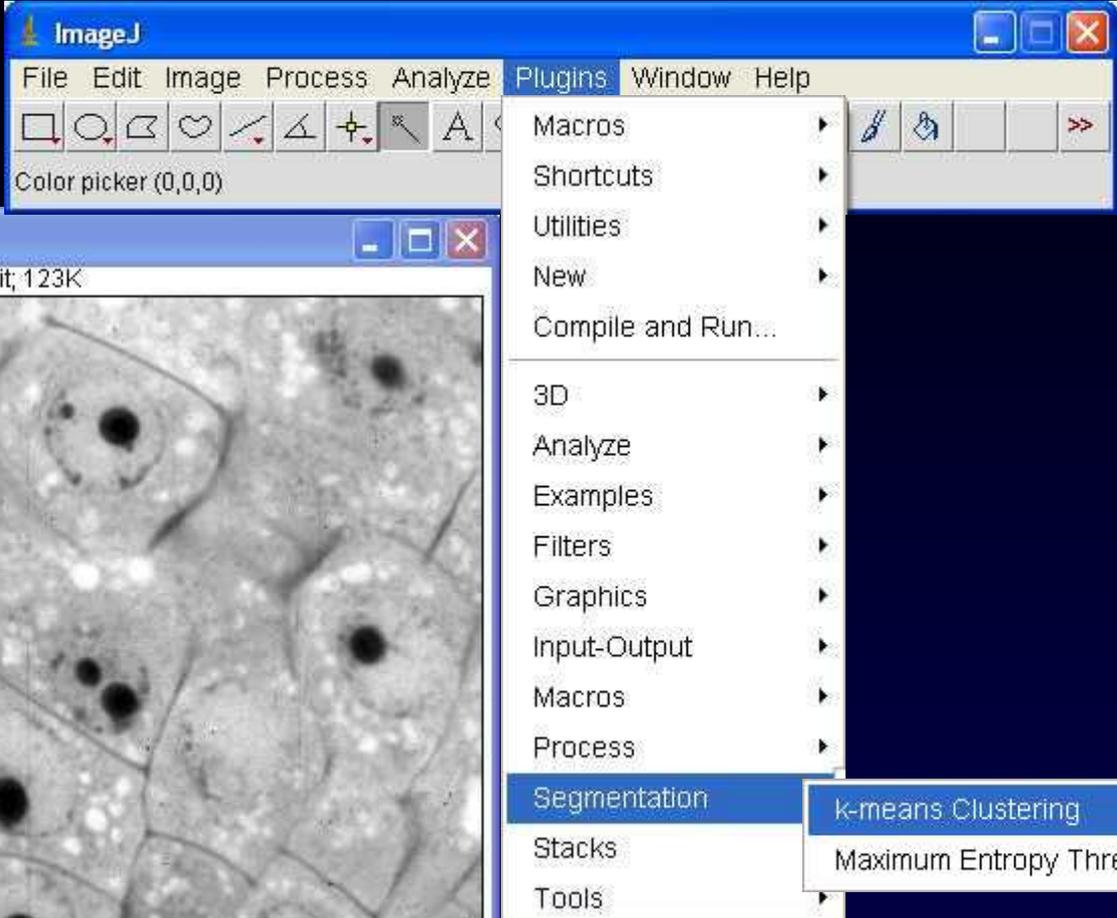


Seuillage local automatique



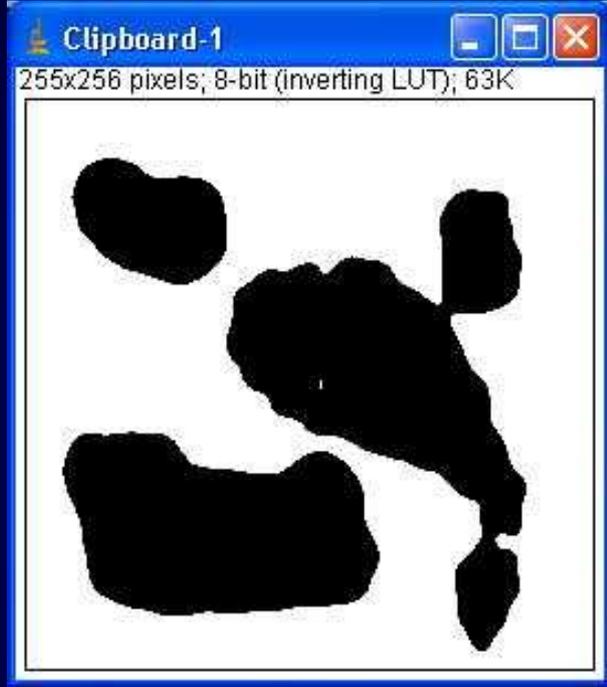
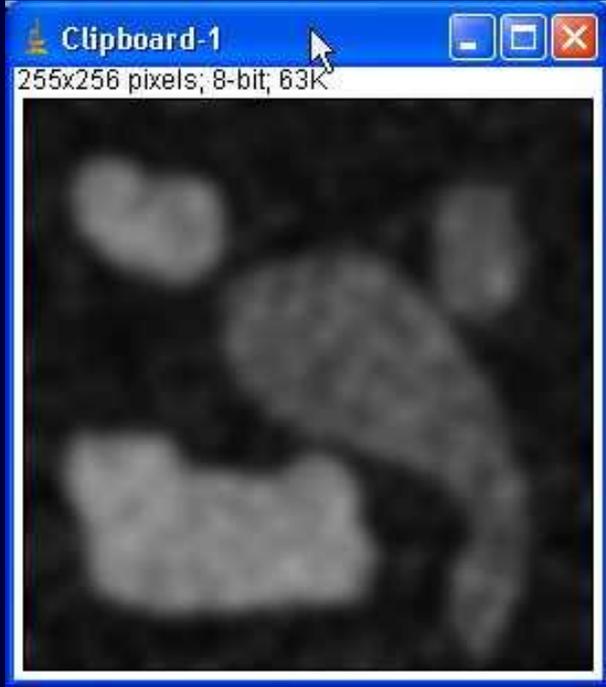
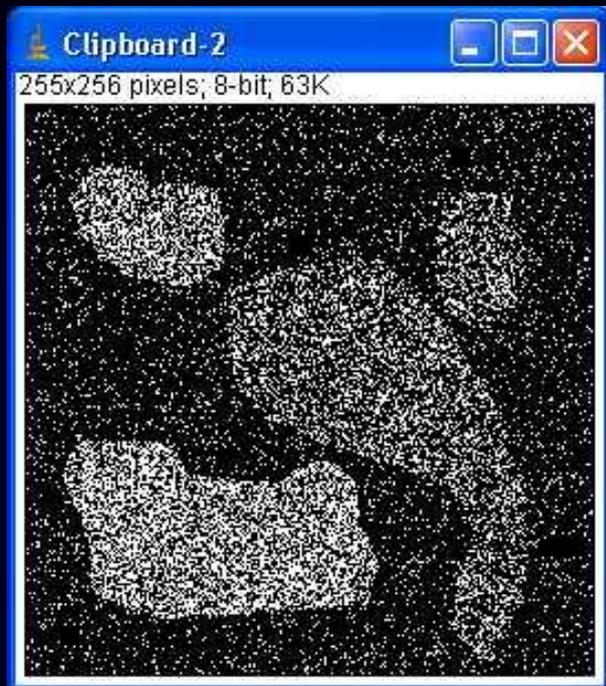


Plugin k-mean clustering



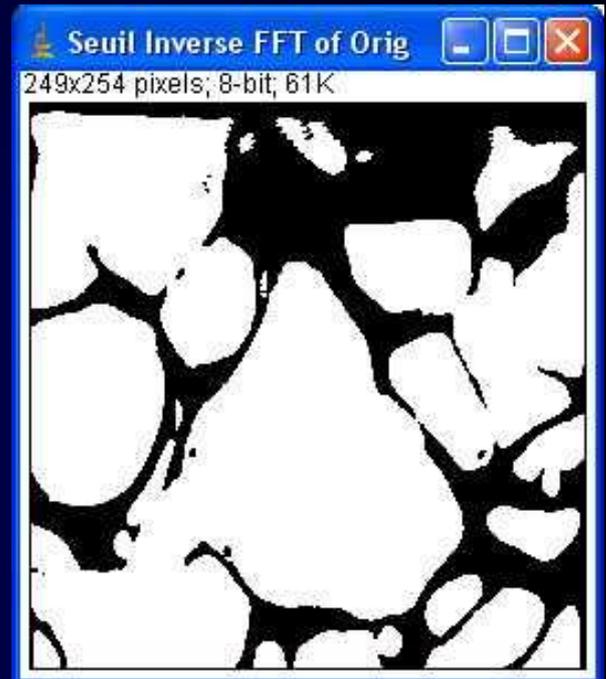
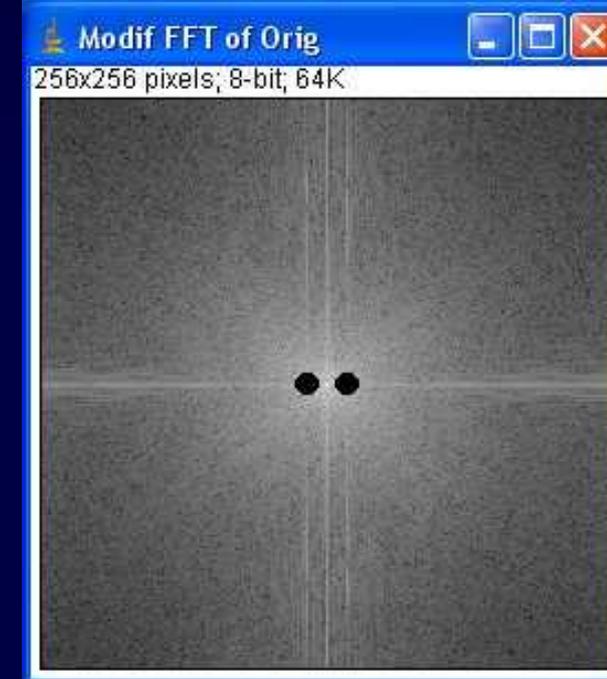
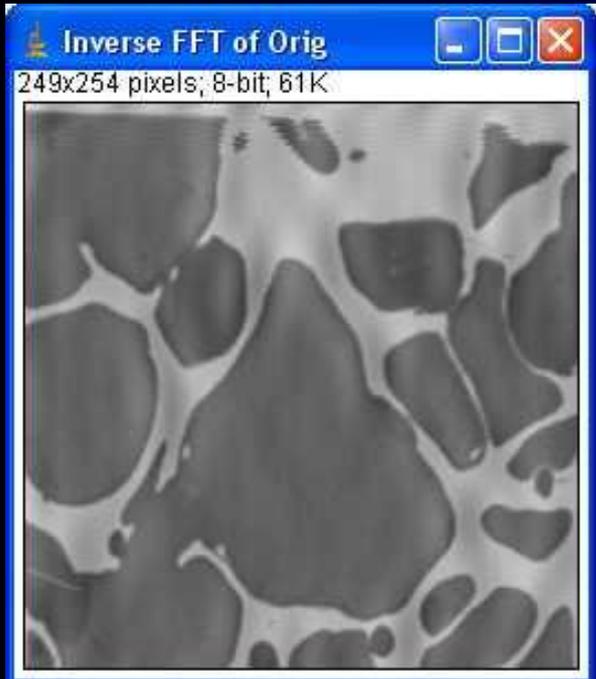
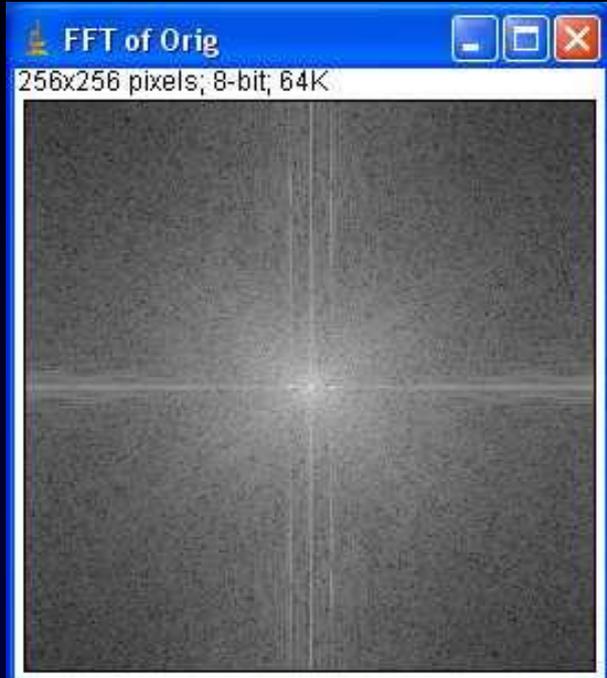
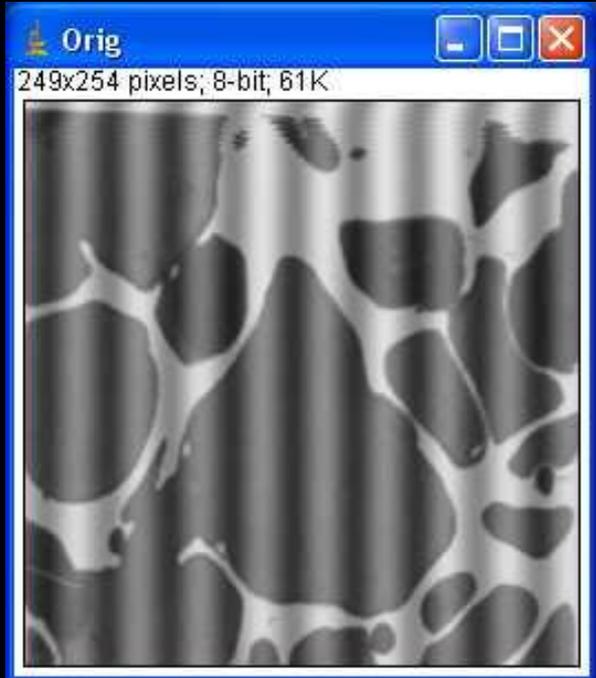


Filtrage avant le seuillage





FFT filtres dans le domaine de Fourier



Couleur et segmentation séparation RGB

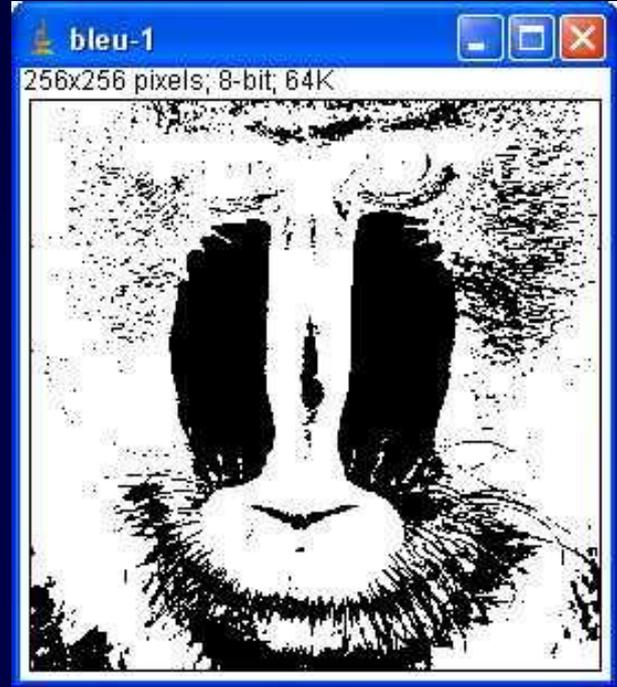
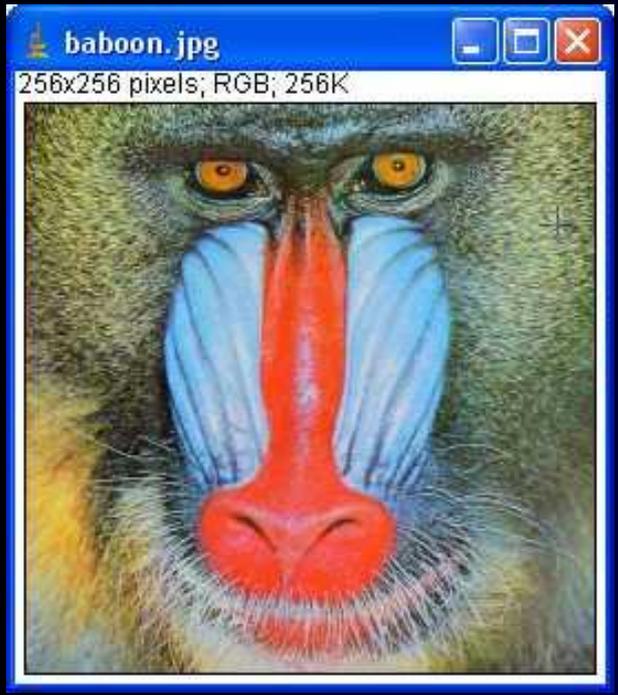


Image → Color → Split Channels...



Décomposition HSI

la Teinte ou Hue se référant à la couleur

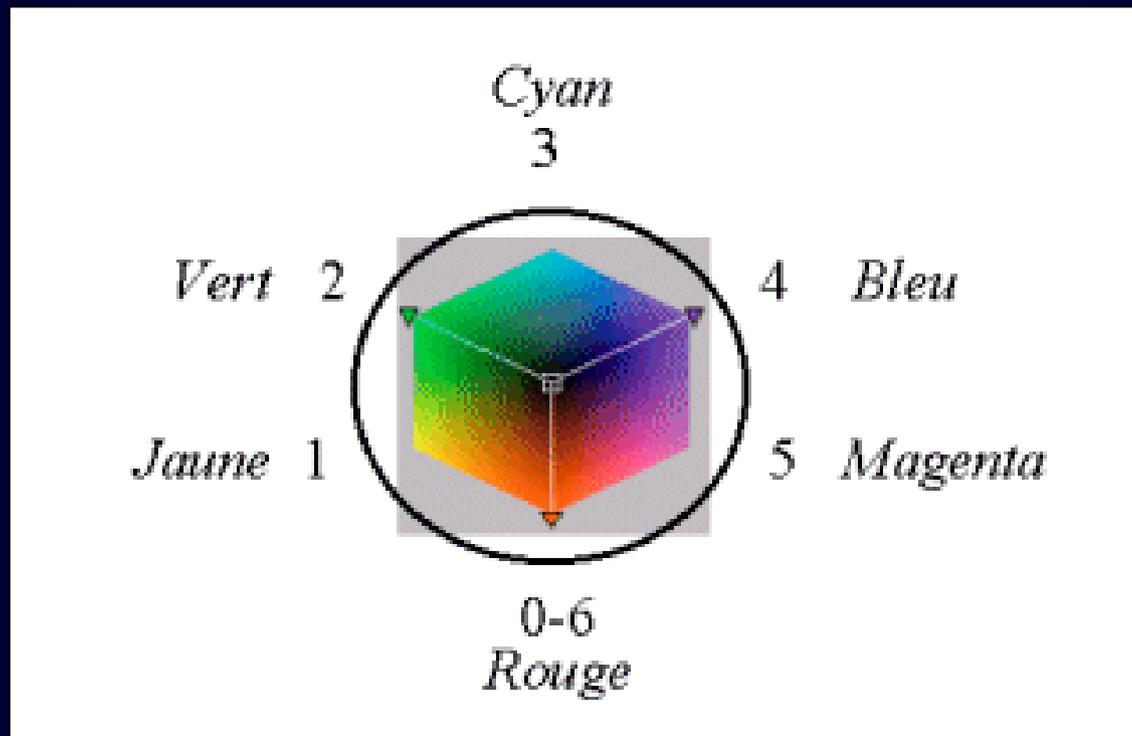
codée de 0 à 6 correspond à une distribution cyclique des couleurs

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

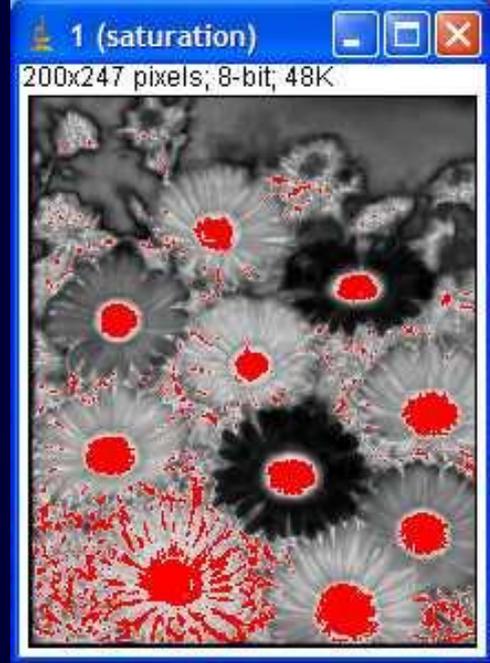
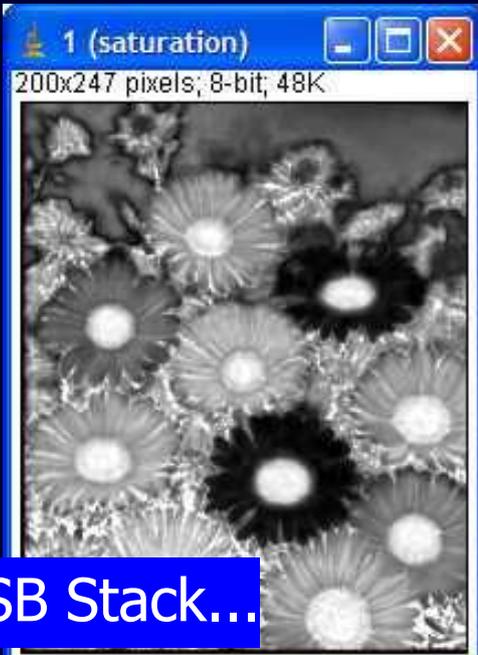
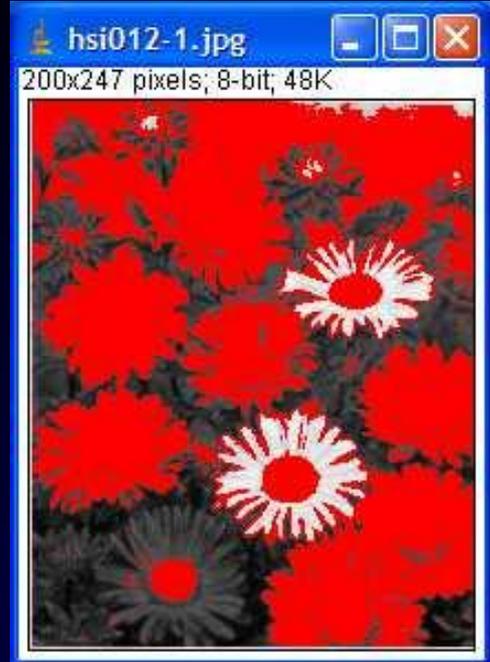
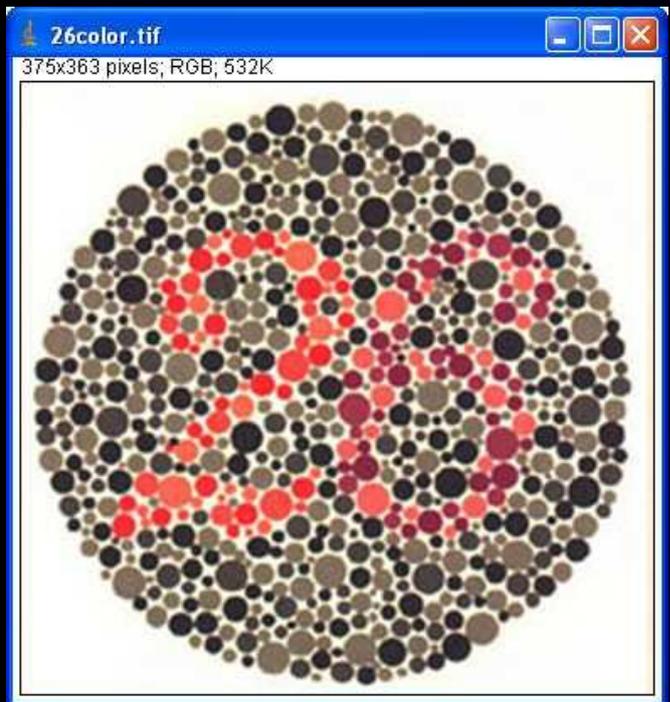


Image → Type → HSB Stack...

Seuillage couleur



Threshold Color (experimental)

Hue

Pass

Saturation

Pass

Brightness

Pass

Thresholding method: Default

Threshold color: B&W

Color space: HSB

Dark background

Original	Filtered	Select	Sample
Stack	Macro	Help	

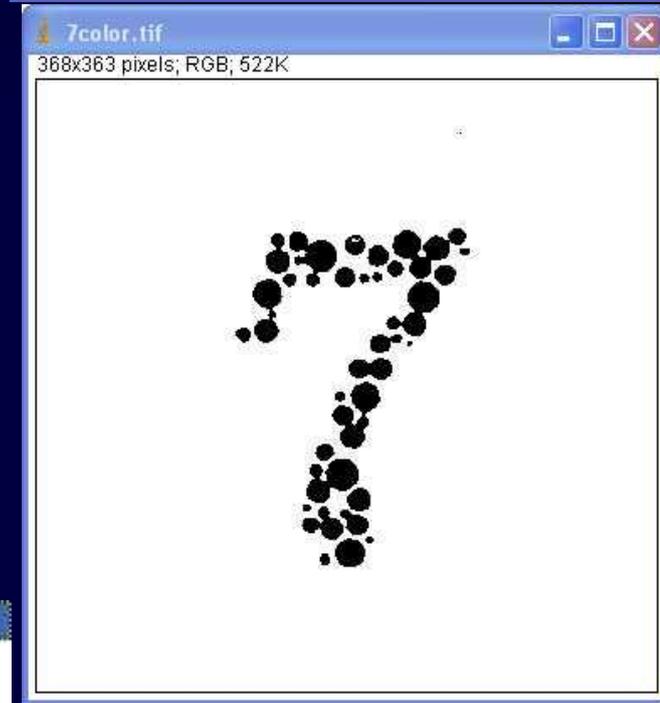
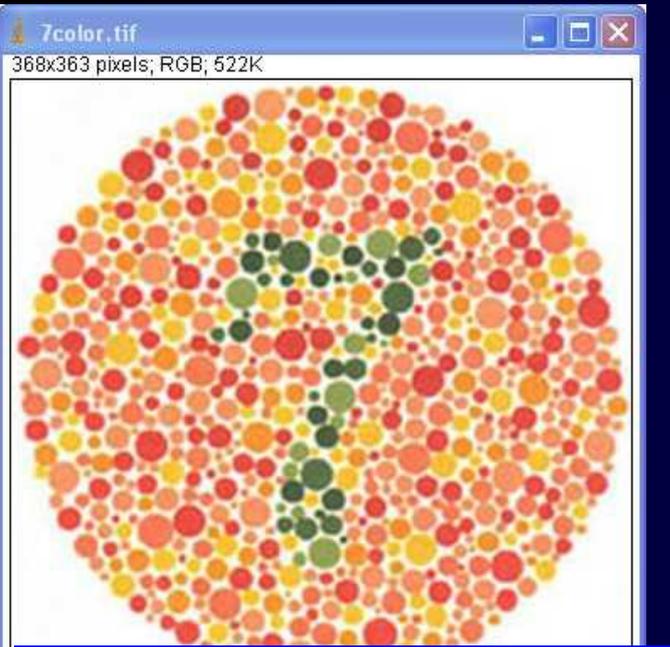
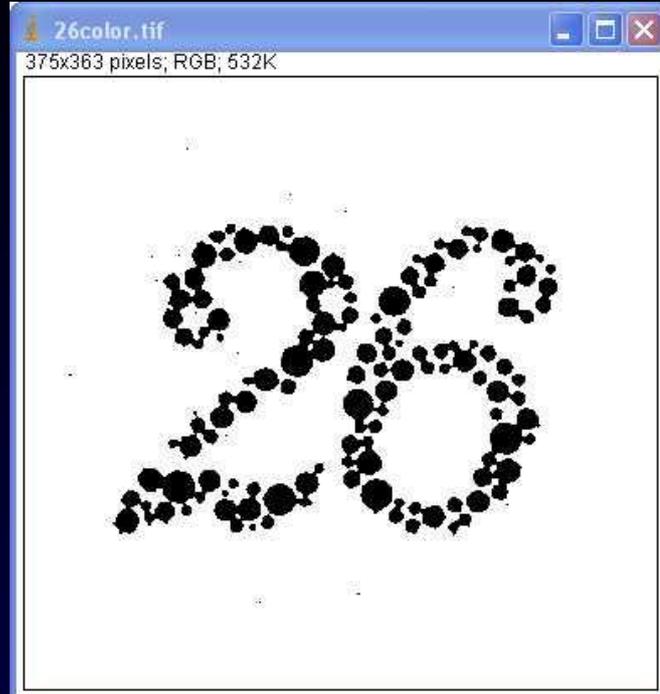
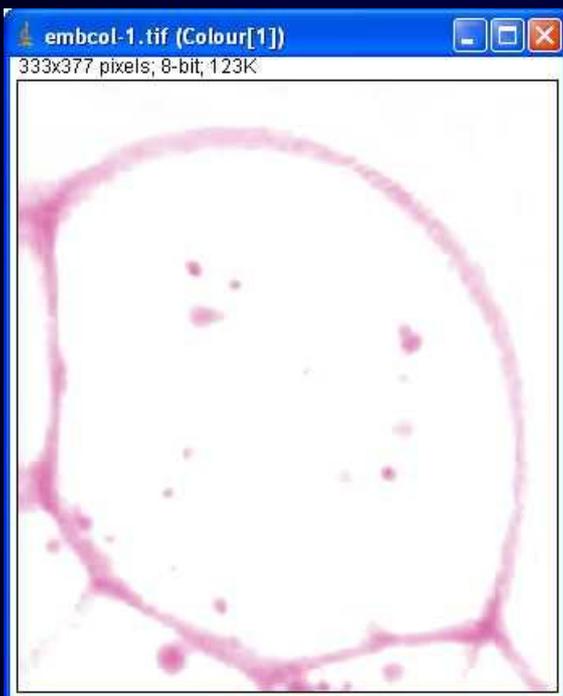
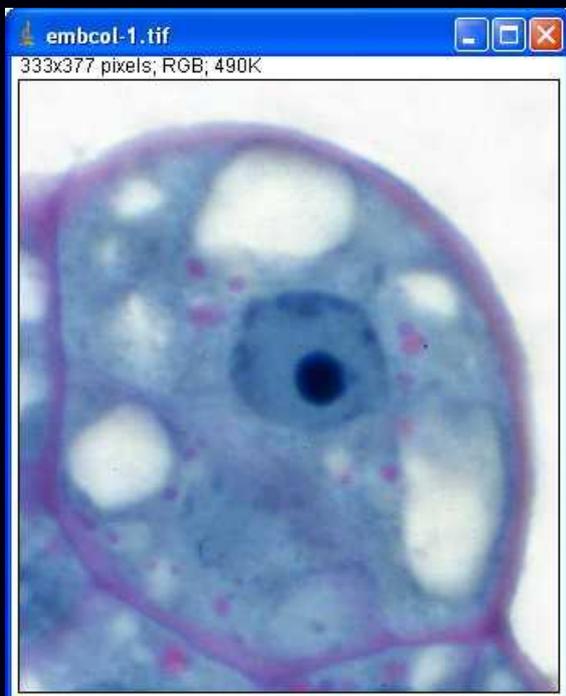


Image → Adjust → Color Threshold..

- HSB
- RGB
- Lab
- YUV



Plugin Colour Deconvolution





Topic 08 – Segmentation



L'image numérique

Les Prétraitements

La Segmentation

Les Post-traitements

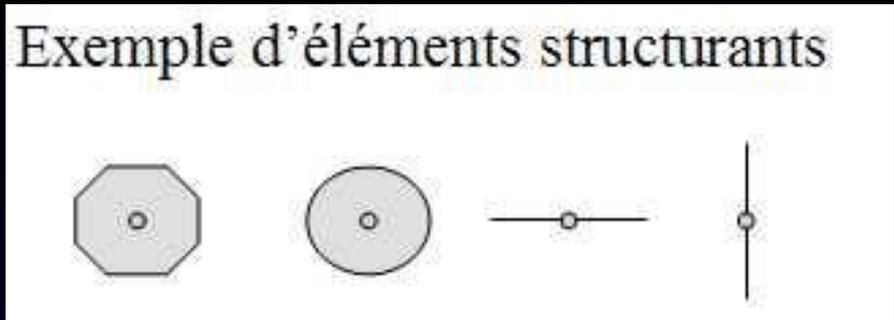
Transformations de
morphologie
mathématique

La Quantification



Principe

Elément structurant



On déplace l'élément structurant sur toute l'image

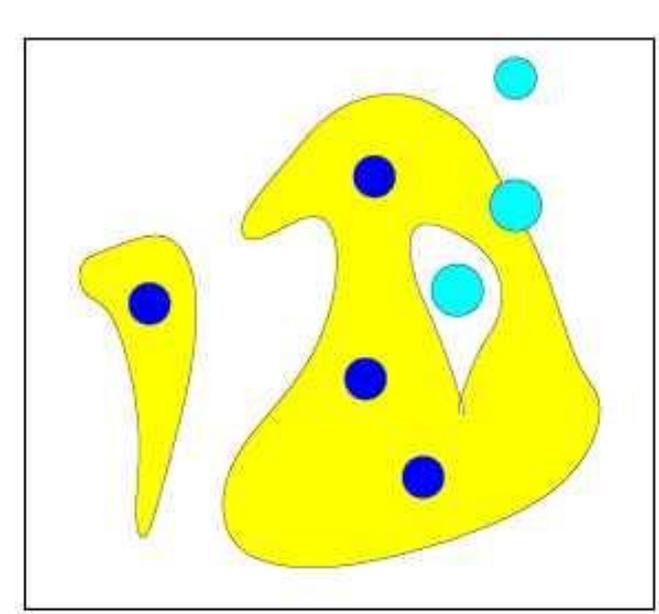
Le pixel sera noir si:

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

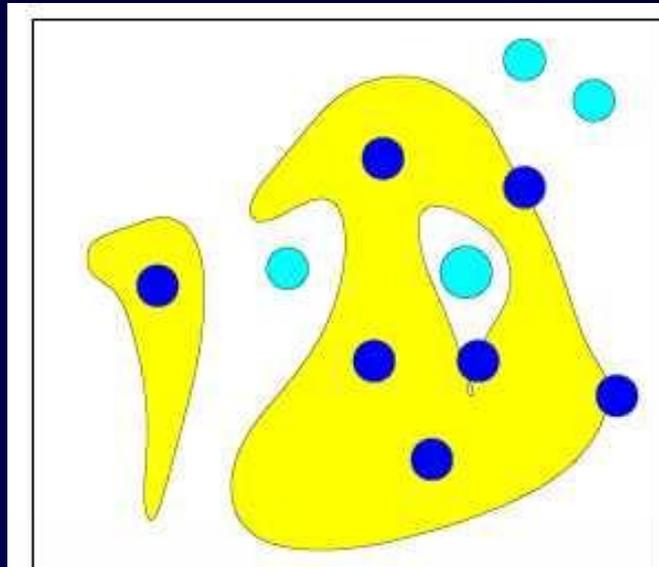
→ EROSION

l'élément structurant touche un objet de l'image

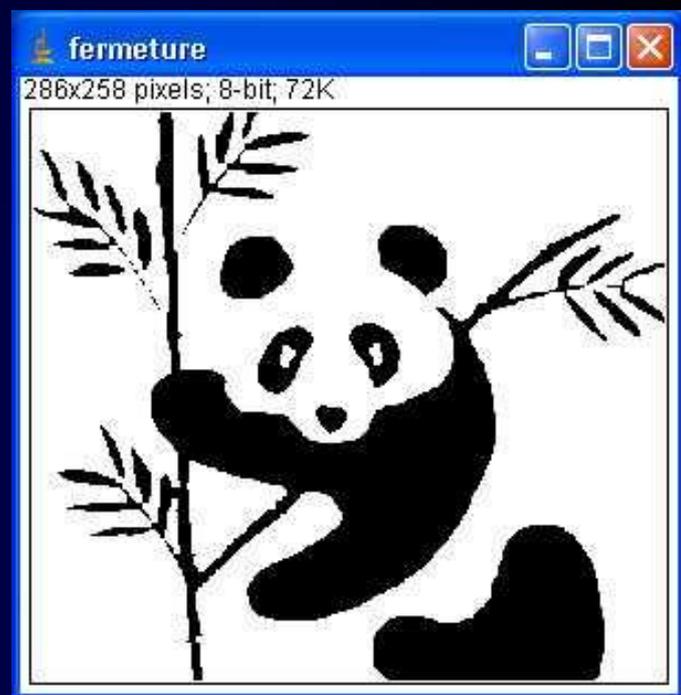
→ DILATATION



- reponse negative
- reponse positive

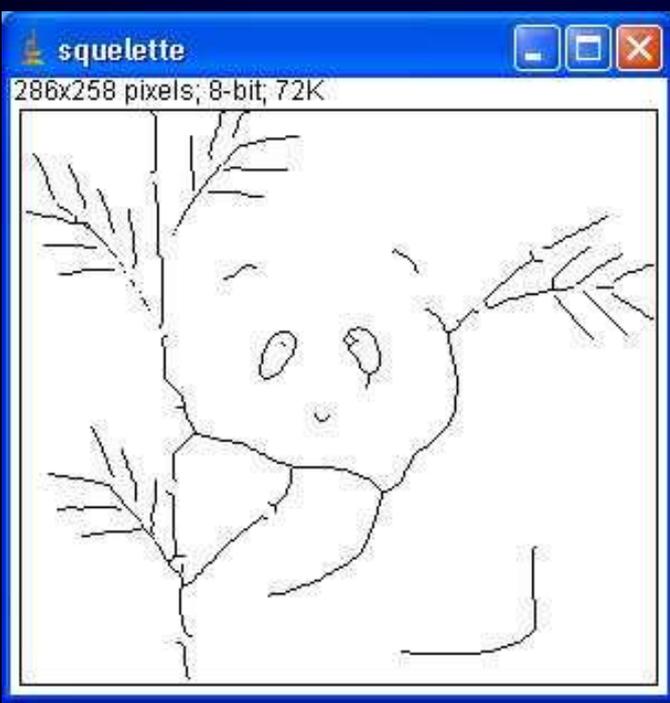
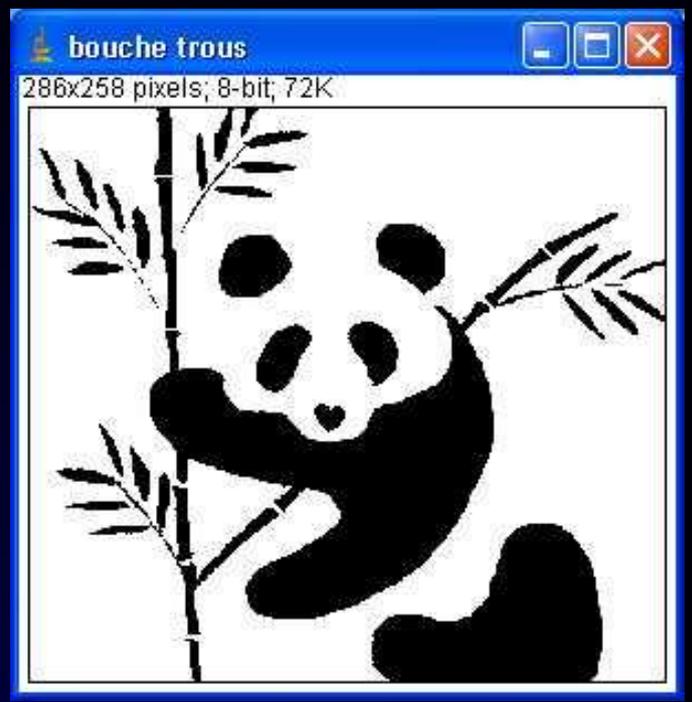


- reponse negative
- reponse positive



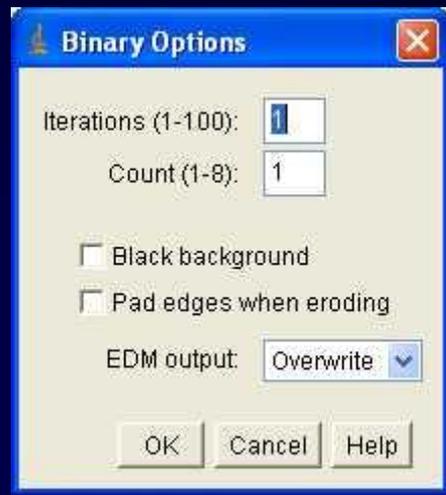
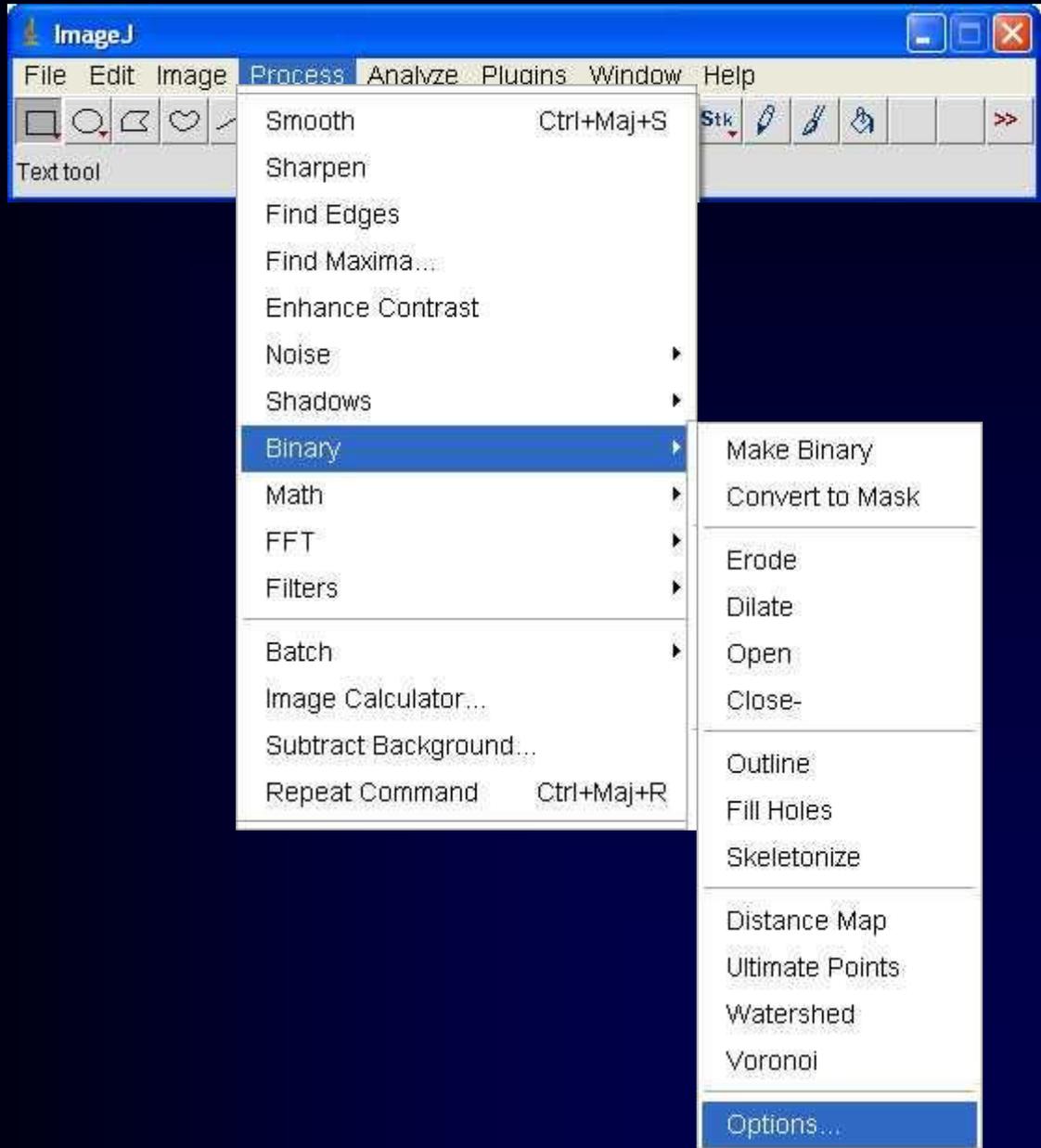


Morpho_math





Menu Binary

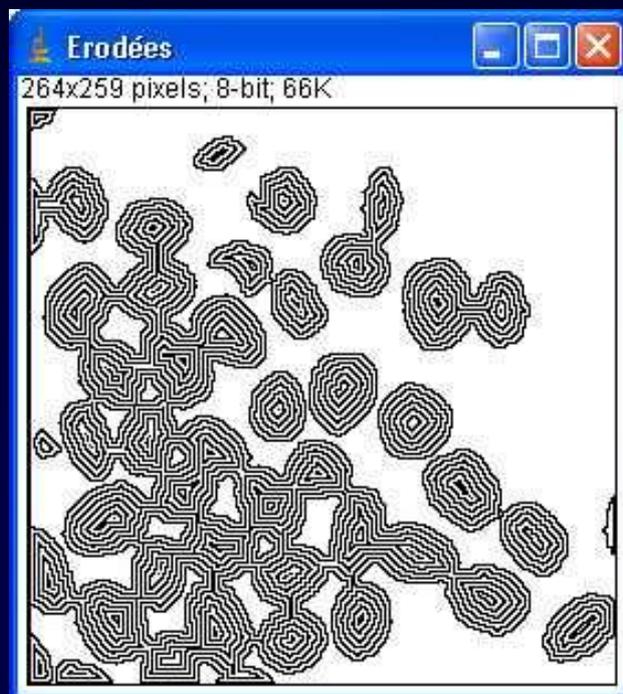
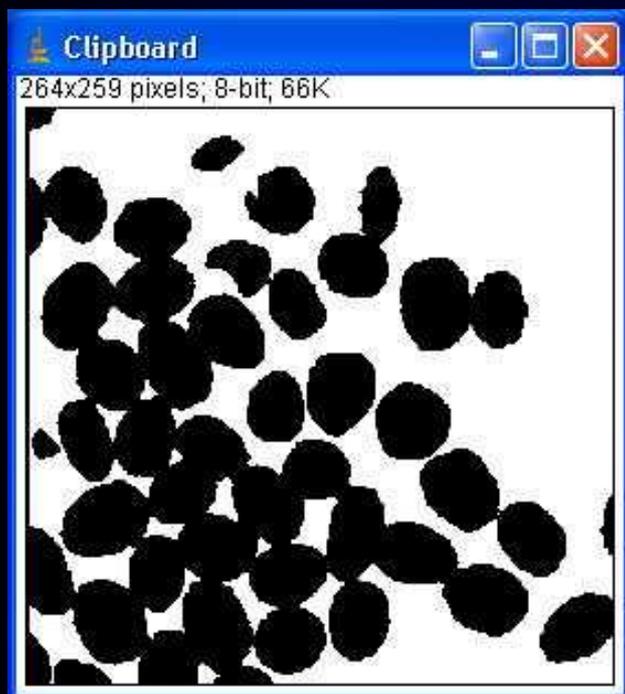


Process → Binary → Options



Erodé Ultime

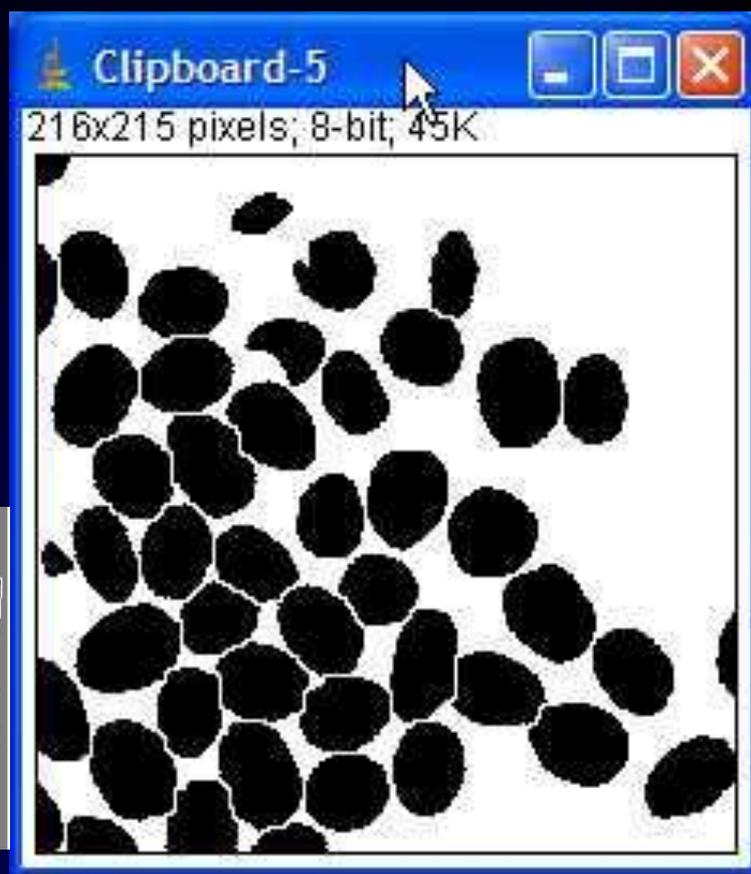
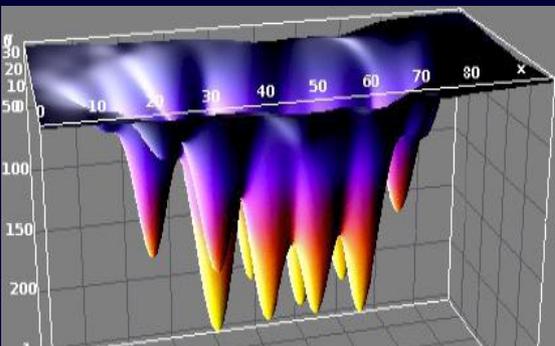
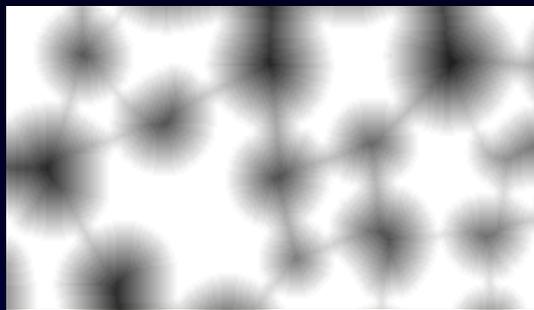
L'érodé ultime : la dernière fraction de l'objet restant avant sa disparition lors d'érosions répétées.



Process → Binary → Ultimate Points



La ligne de partage des eaux : watershed

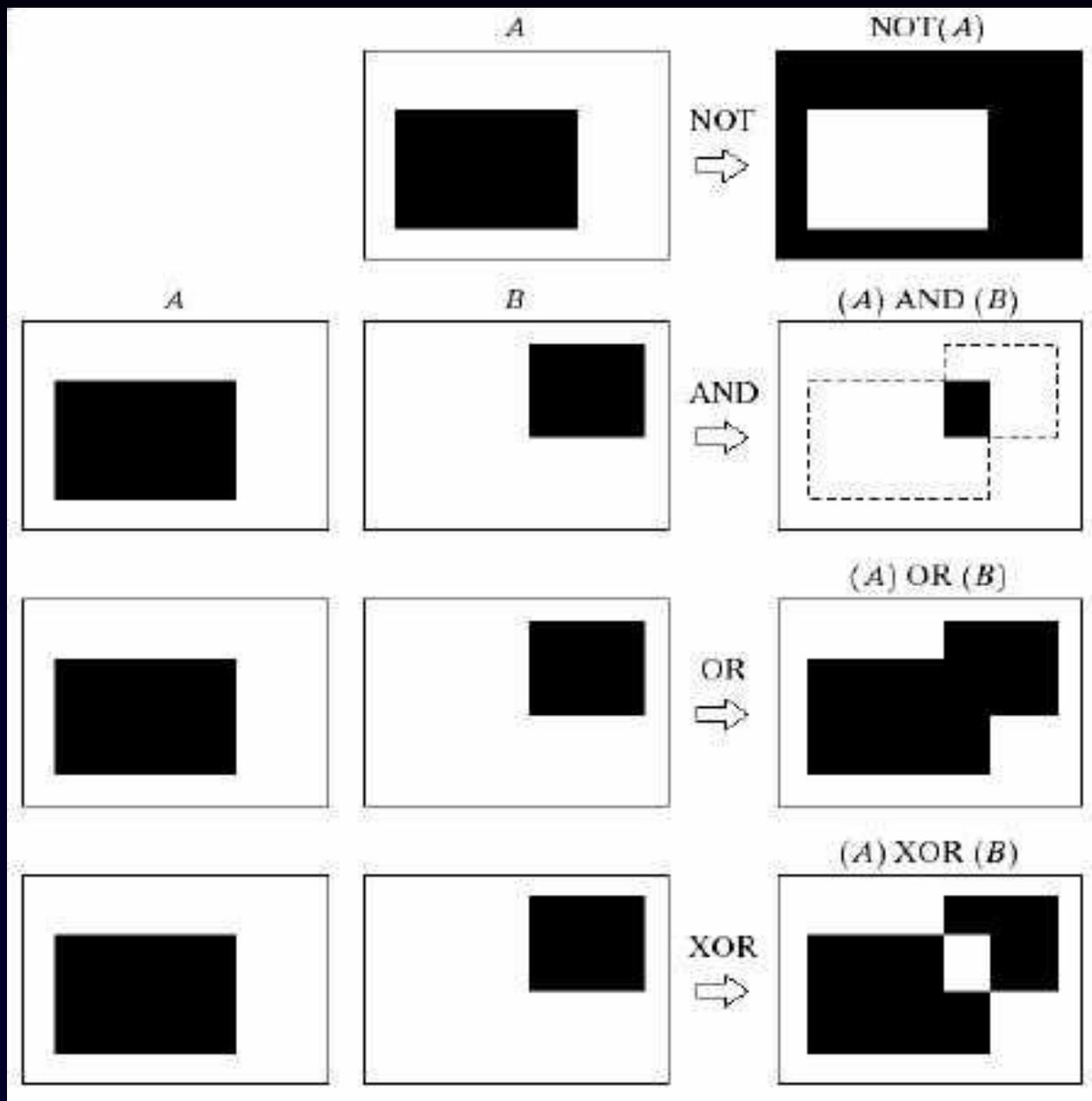


Cette transformation morphologique est la principale méthode de segmentation d'images proposées par la morphologie mathématique.

Process → Binary → Watershed



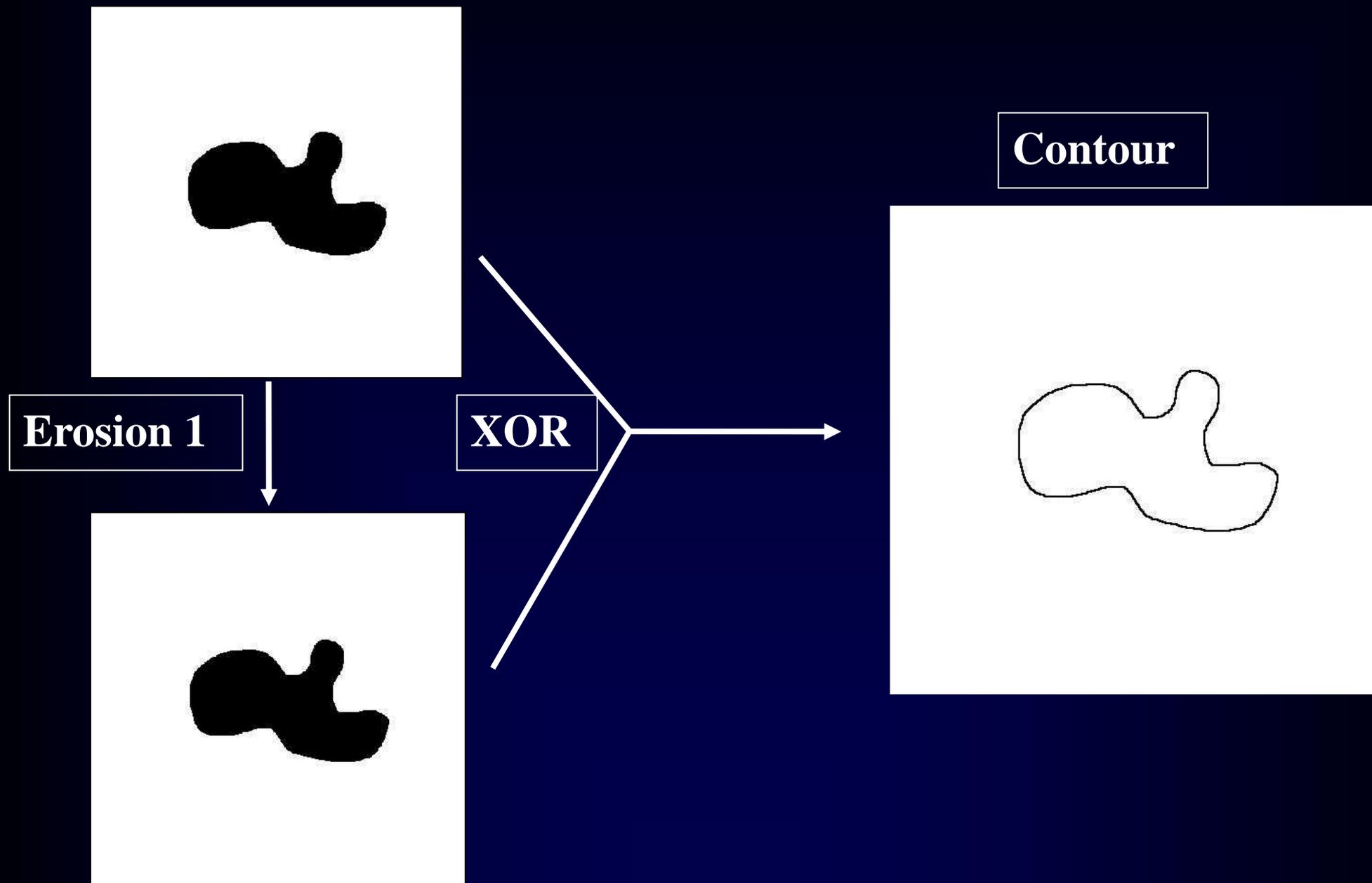
Opérations logiques

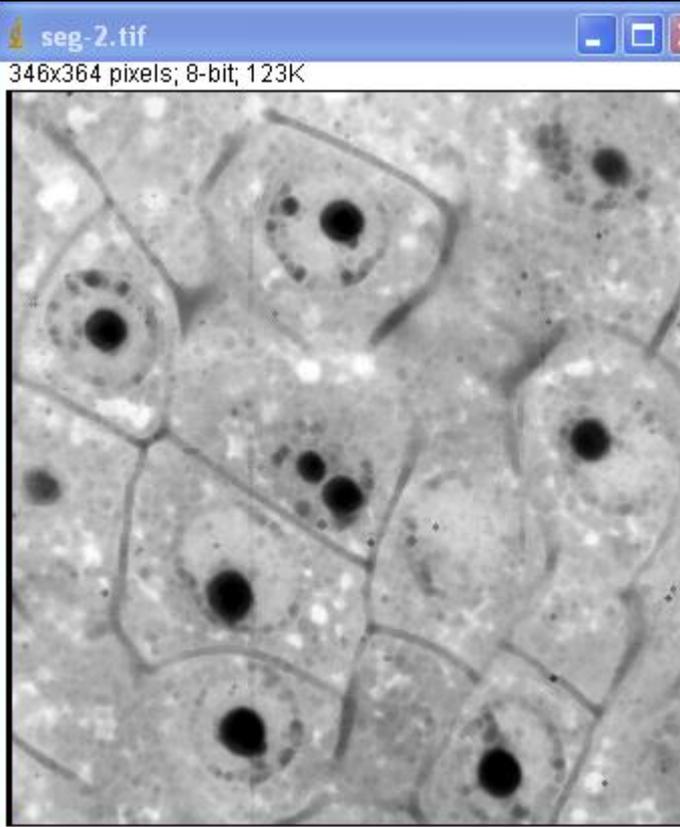


Process→Image Calculator...

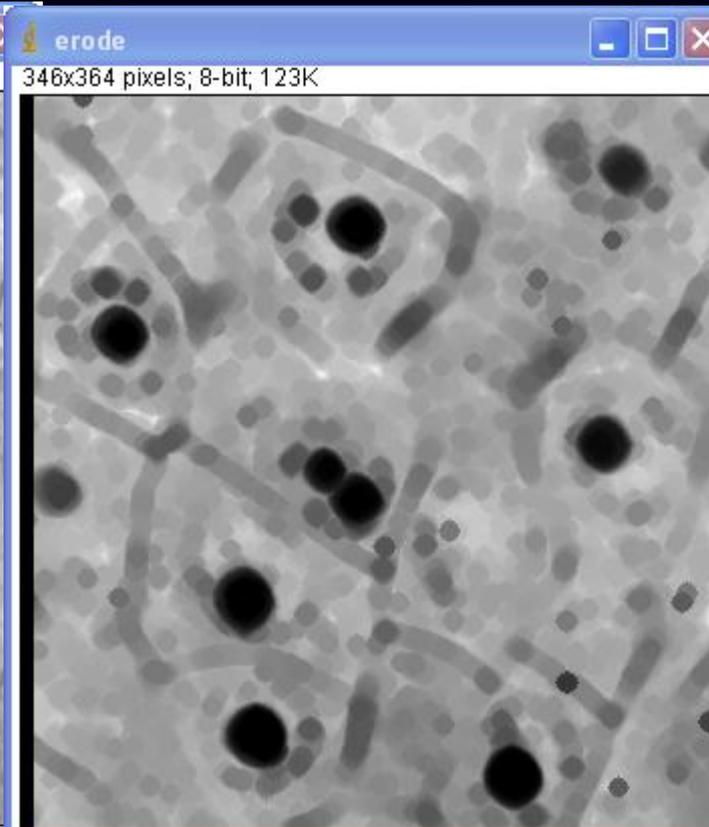


Construction d'une fonction contour

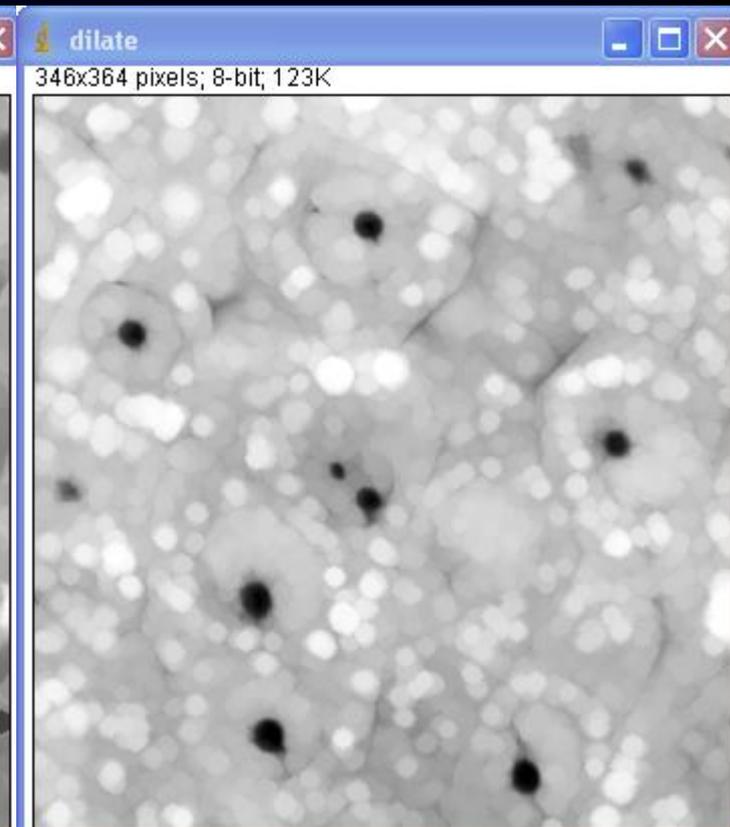




**Plugin
Grayscale Morphology**



**Erosion
agrandit les zones
sombres**

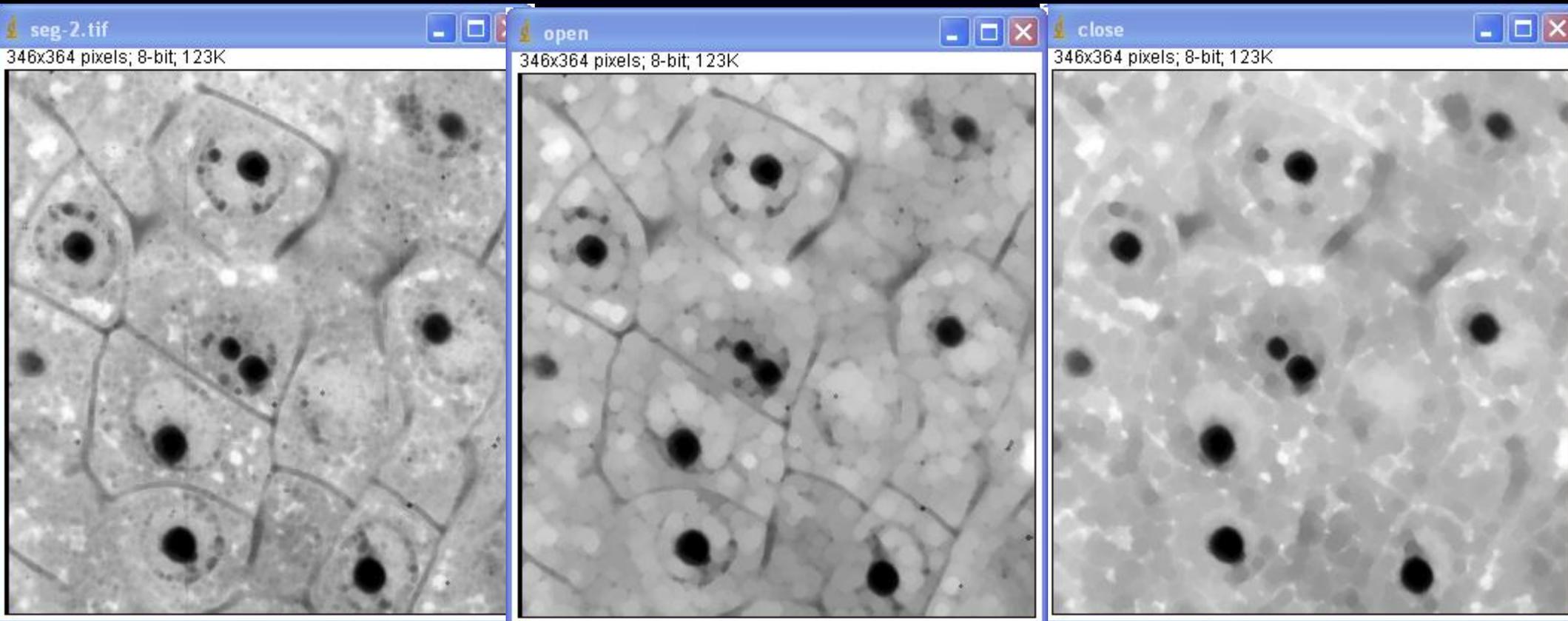


**Dilatation
agrandit les zones
claires**

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

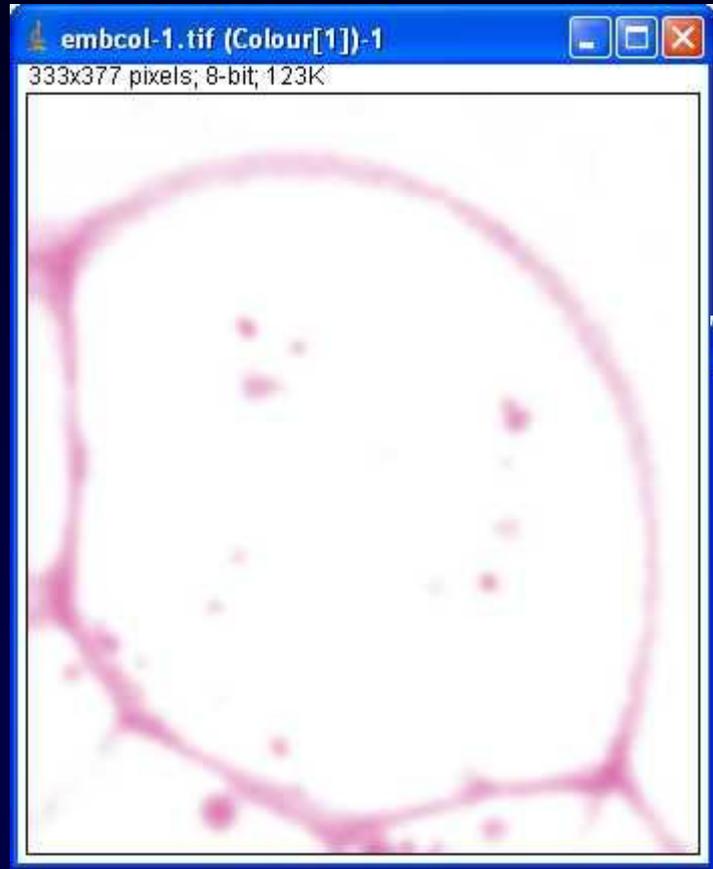
Plugins → Morphology → Gray Morphology



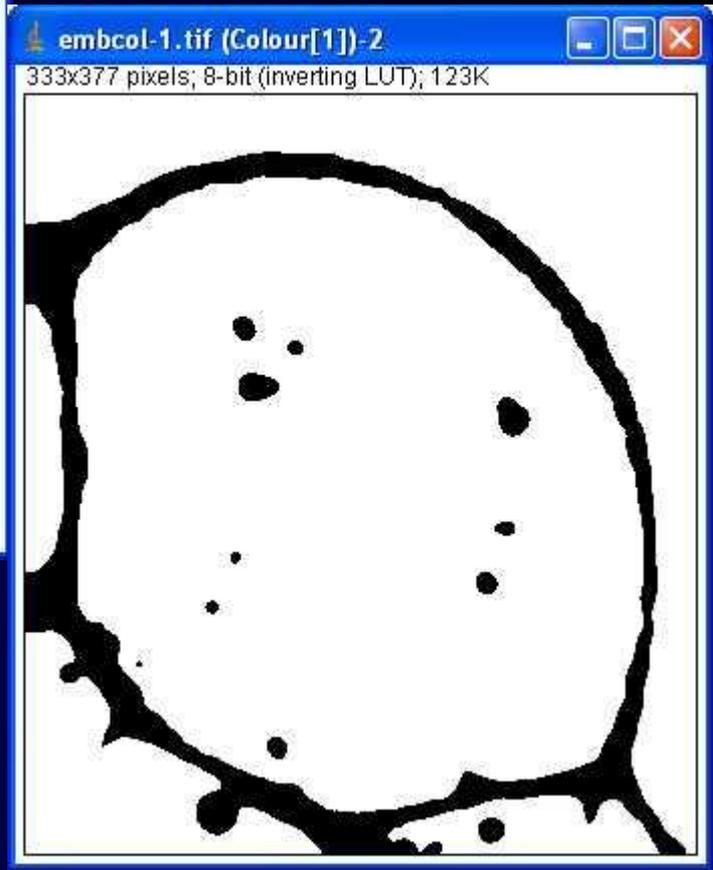
Exemple



Colour Deconvolution

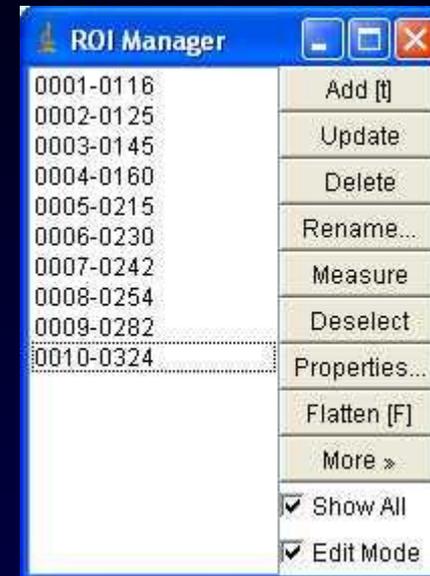
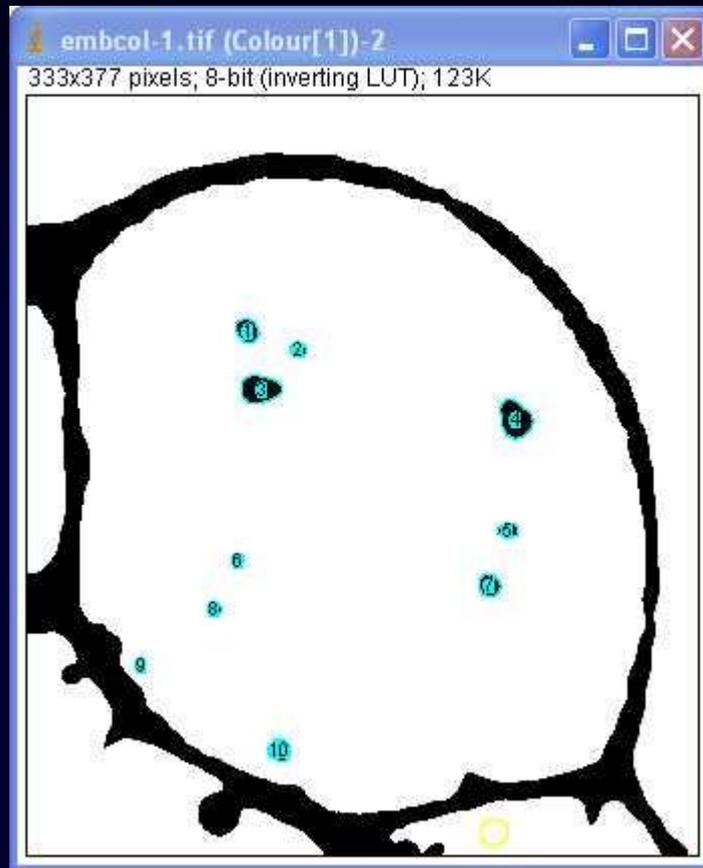


Seuillage



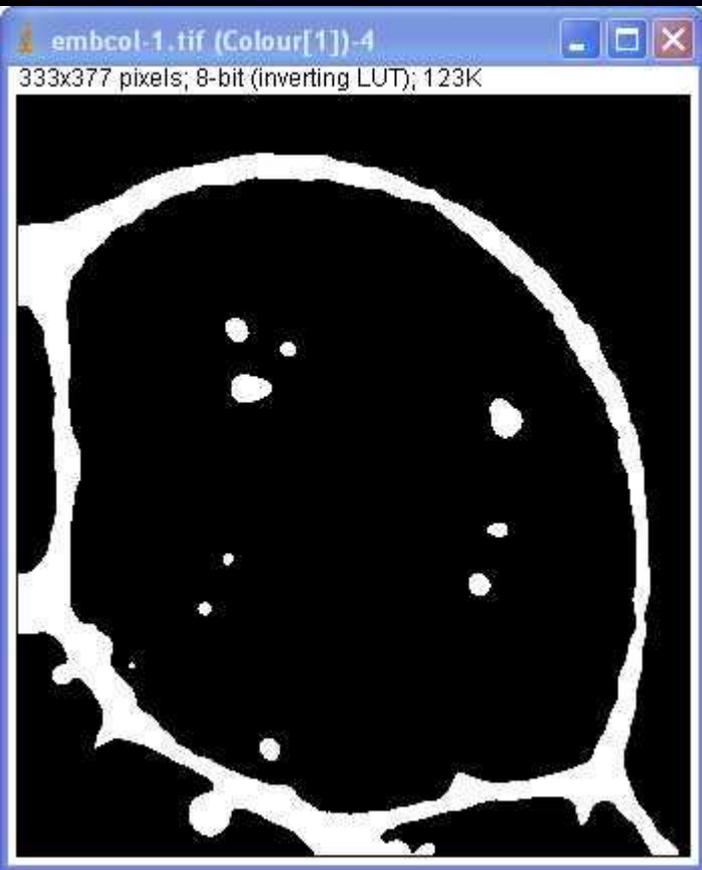
Exemple

Analyse particules → ROI Manager

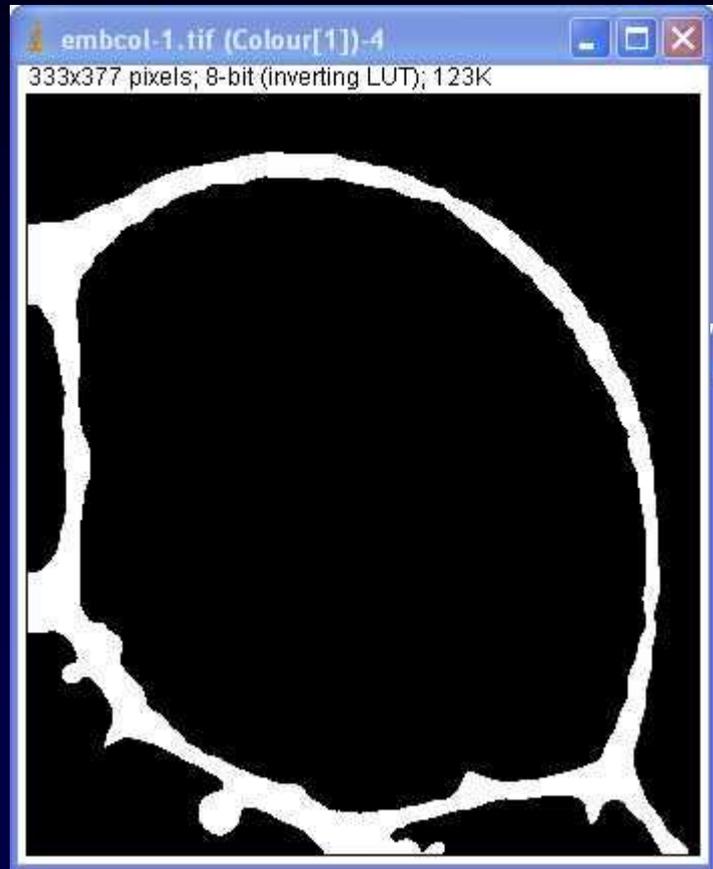


Exemple

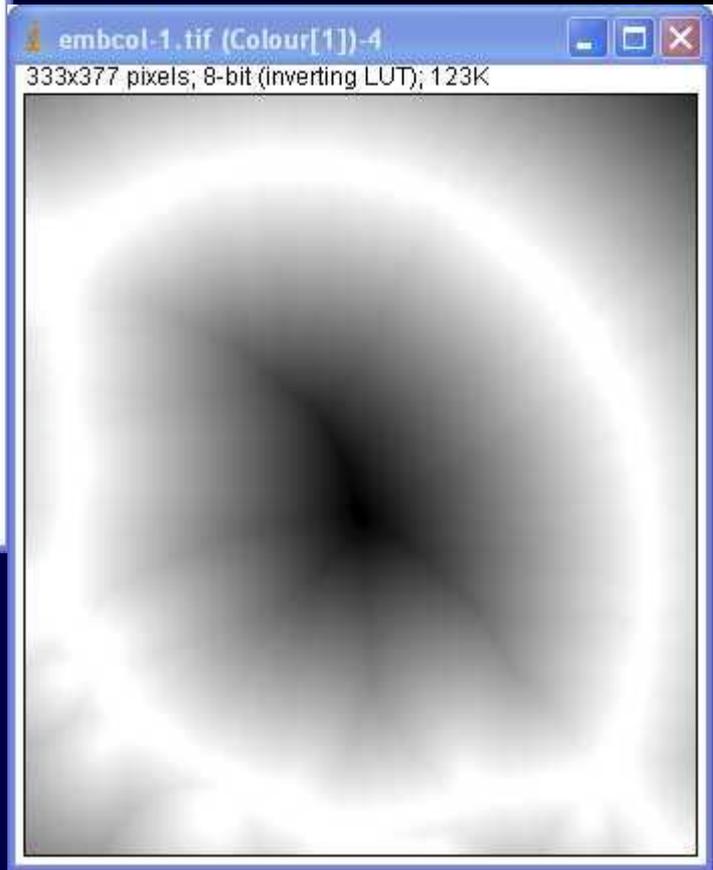
Inversion



Boucher les trous



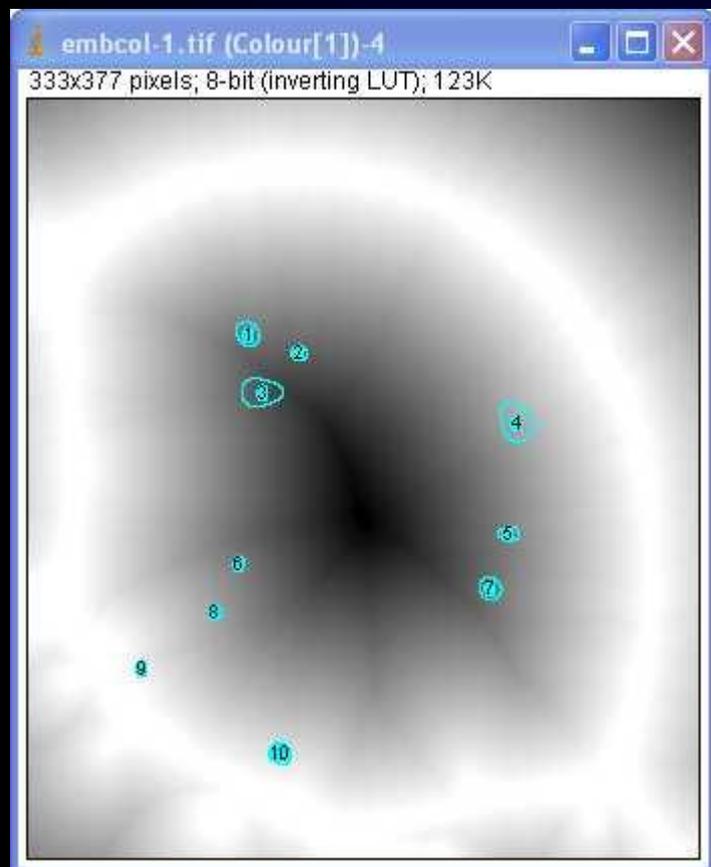
Carte des distances





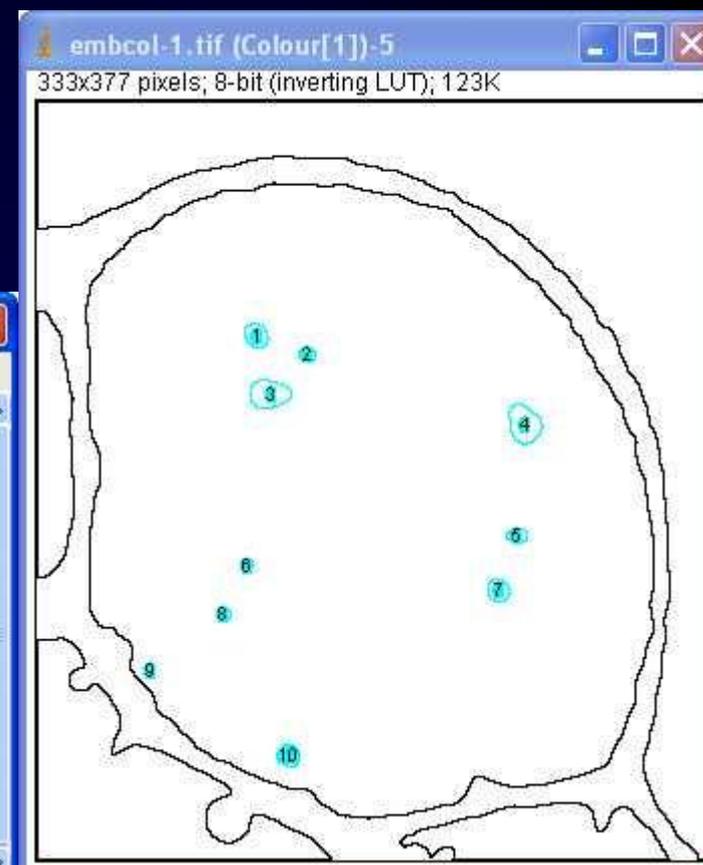
Exemple

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

ROI ID	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 (pixel²):

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

520x250 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

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

Ethique de l'édition d'images

Règles de base sur la retouche des images



-Les images numériques sont des données liées au système d'acquisition. Elles ne correspondent pas exactement à la réalité



- Sur une même figure les images doivent avoir subi le même traitement



-Les mesures d'intensité doivent être faites sur les images originales calibrées (attention aux variations de fluorescence!)



-Conserver l'image originale avant retouche



-Ajustements raisonnables sur l'image entière (luminosité, contraste.....)



-Recadrer une image

Ethique de l'édition d'images

Règles de base sur la retouche des images

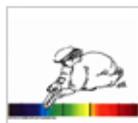
-  -Retouches spécifiques à une zone de l'image
-  -L'utilisation de filtres pour améliorer la qualité n'est pas recommandée
-  -Dupliquer des parties d'images est contraire à l'éthique, éviter de nettoyer l'image, ne pas ajouter d'objet !
-  -Ne pas compresser les images dans des formats qui changent les valeurs de pixel comme jpeg
-  -Faire attention à l'échantillonnage à l'acquisition, choisir un pas trois fois plus petit que la résolution du microscope
-  -Faire attention en changeant la taille (en pixel) de l'image, les pixels ajoutés ont des valeurs interpolées (artefacts) !



Guidelines

Guidelines for Best Practices in Image Processing

Please click on each guideline for further details. Also, see the guidelines demonstrated in [Photoshop Videos](#).



Treating Images as Data: Digital scientific images should be treated as data



Filters Degrade Data: Use of software filters to improve image quality is usually not recommended for biological images.



Saving the Original: Manipulations of digital images should always be done on a copy of the raw image data. The original must be retained.



Cloning Degrades Data: Cloning objects into an image or from other parts of the image is very questionable.



Making Simple Adjustments: Simple adjustments to the entire image are usually acceptable.



Making Intensity Measurements: Intensity measurements of digital images should be performed on raw data and the data should be calibrated to a known standard.



Cropping is usually OK: Cropping an image is usually acceptable.



Lossy Compression Degrades Data: Avoid the use of lossy compression.



Comparing Images: Digital images that will be compared to one another should be acquired under identical conditions.



Issues With Magnification: Magnification and resolution issues are important.



Manipulating the Entire Image: Manipulations that are specific to one area of an image and are not performed on other areas are



Issues With Pixels: Be careful when changing the size (in pixels) of a digital image.



Guides aux auteurs

-Format des fichiers :

TIFF pour les photos (compression LZW)

EPS pour les dessins vectoriel

PDF pour tout

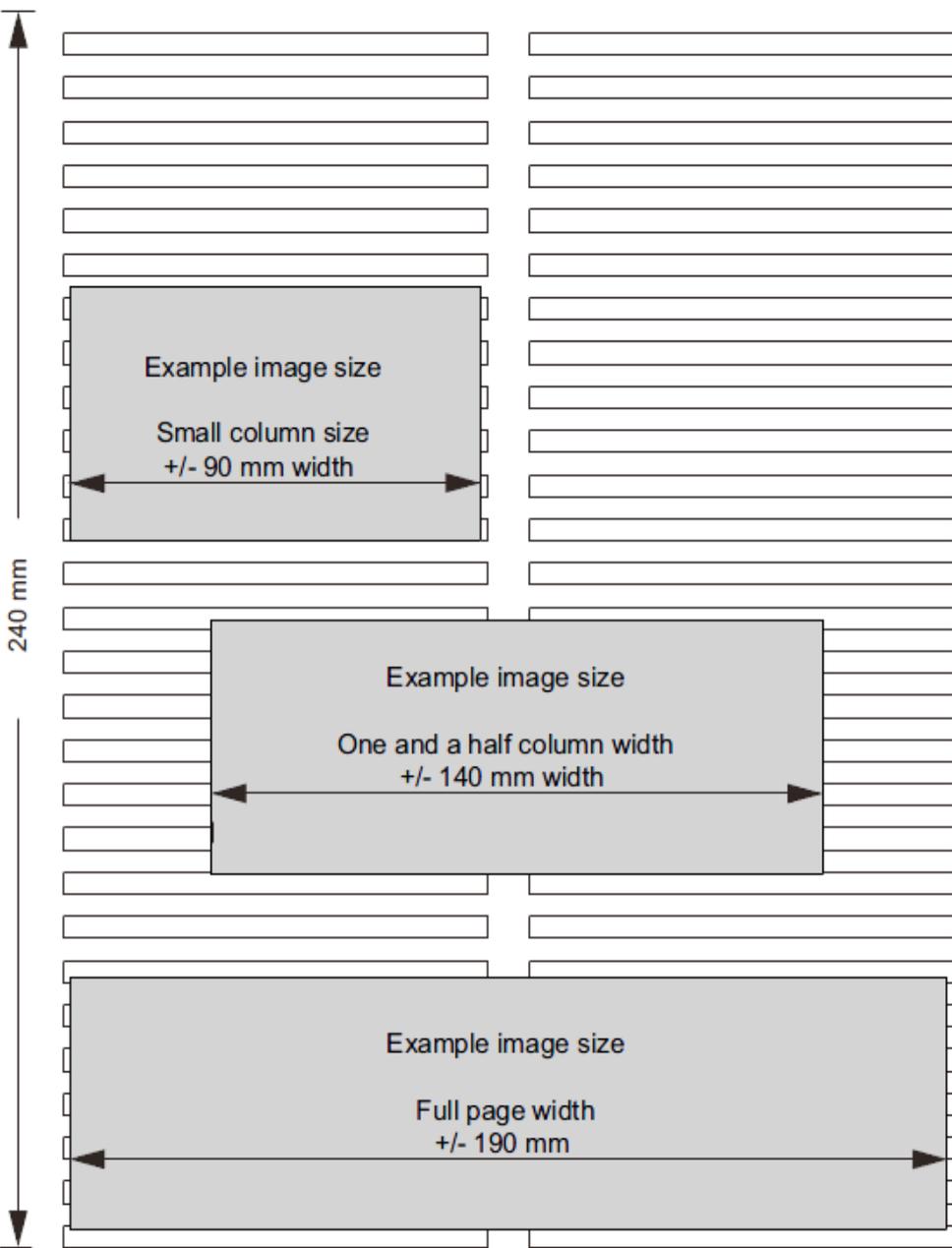
-Polices :

Arial, Helvetica, Courier, Symbol, Times

-Résolutions :

Photos 300 dpi

Graphiques 600-1000 dpi



Mise en page

Résolutions :
Largeur mini en pixel

	image width	A	B	C
Minimal size	30 mm	354	591	1181
Single column	90 mm	1063	1772	3543
1.5 column	140 mm	1654	2756	5512
Full width	190 mm	2244	3740	7480

- A: 300 dpi > Halftone images Gray/RGB
- B: 500 dpi > Combination art Gray/RGB
- C: 1000 dpi > Line art



FigureJ

Installation du plugin

Installation details for ImageJ

⚠ Update to the latest version of ImageJ.

The following files need to go to the ImageJ/plugins/ folder:

- figurej_101b3.jar

→ get it from  this link.

- imagescience.jar

→ get it from Eric Meijering website  <http://www.imagescience.org/meijering/software/download/imagescience.jar>

- loci_tools.jar

→ get it from the LOCI website at  <http://loci.wisc.edu/bio-formats/downloads>

- LSM_Reader.jar

→ get it from  http://imagejdocu.tudor.lu/lib/exe/fetch.php?media=plugin:inputoutput:lsm_reader:lsmplugins2012_07_23.zip



FigureJ

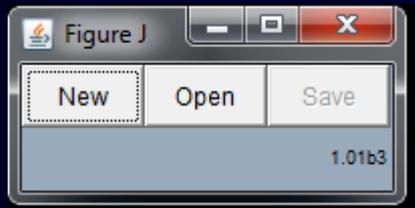
Lancer le plugin



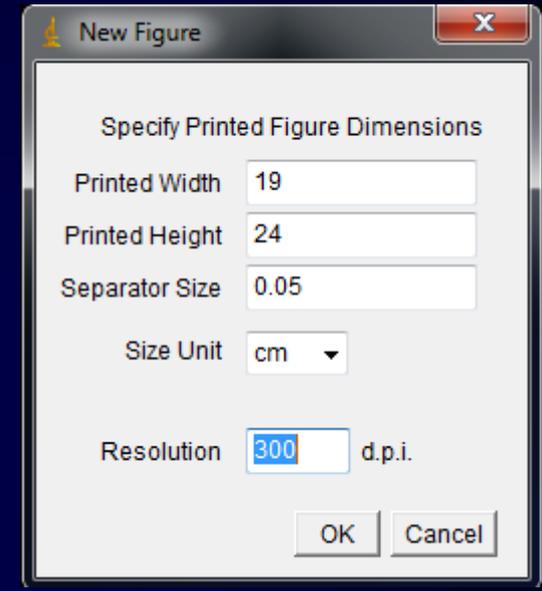
FigureJ

Insets Menu

panel sticking Tool



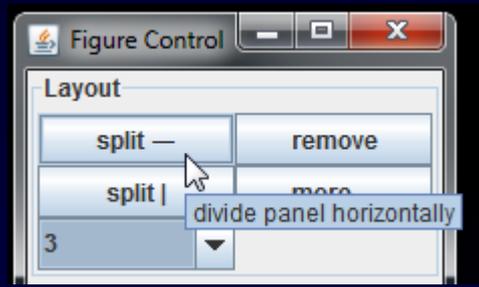
Determiner la taille de la figure
ainsi que sa résolution



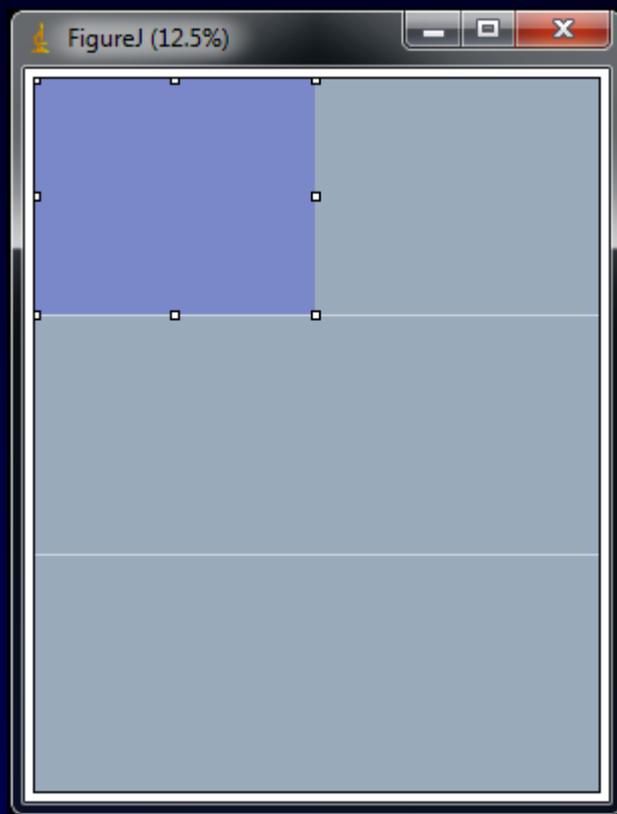
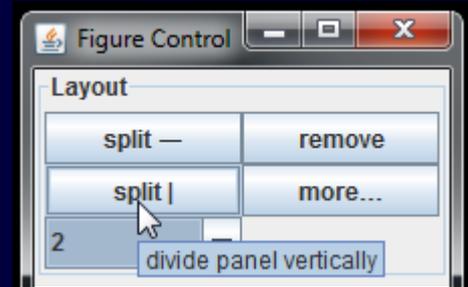
FigureJ

Maquette avec zones réservées aux photos
Le bouton agit sur la zone sélectionnée (en mauve)

Découpage horizontal

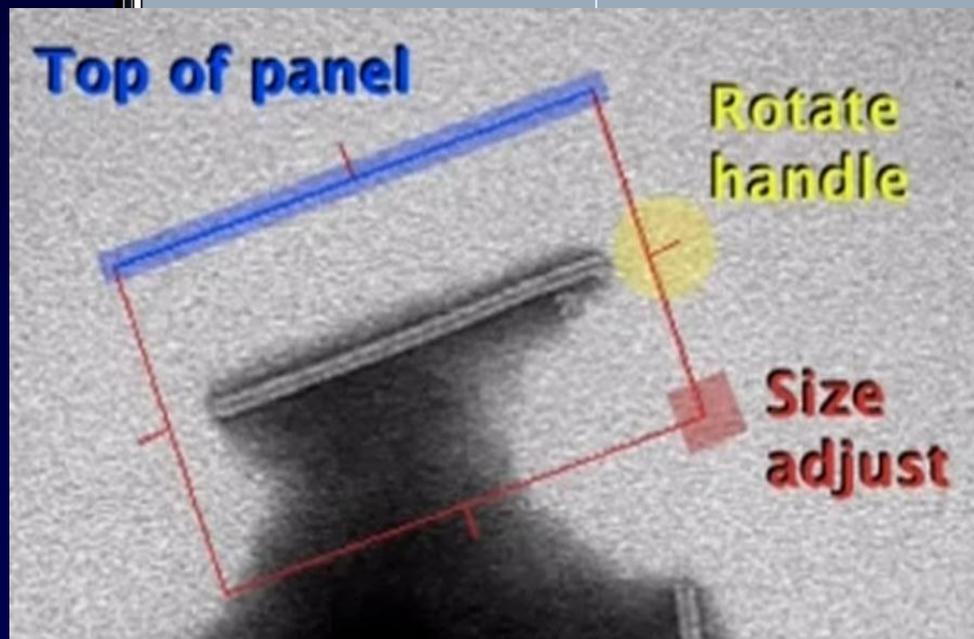
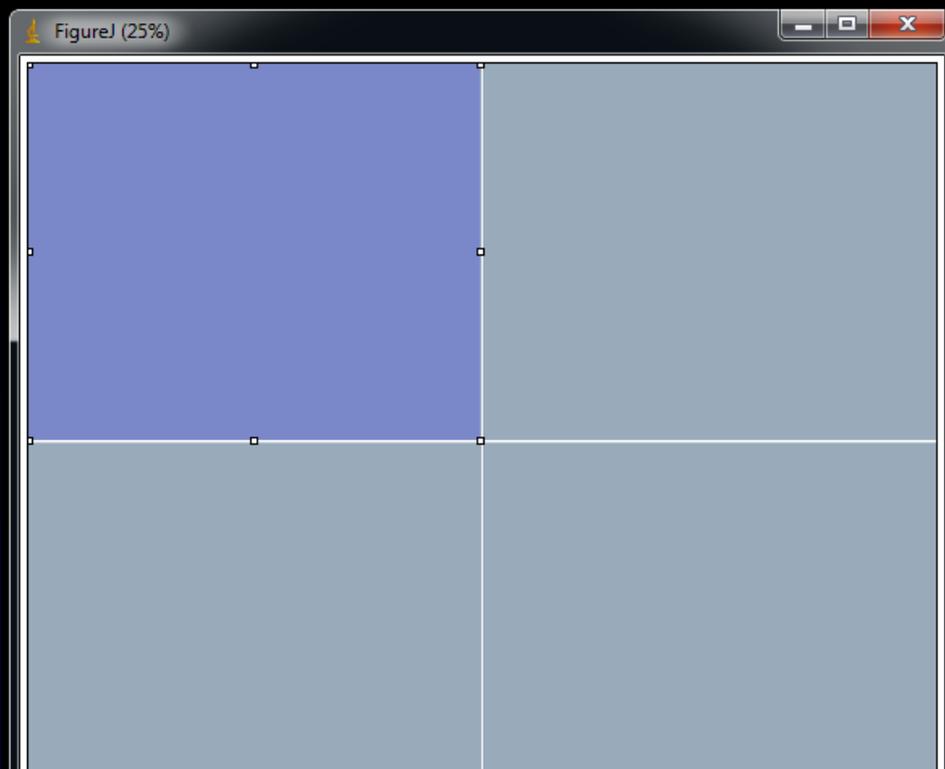
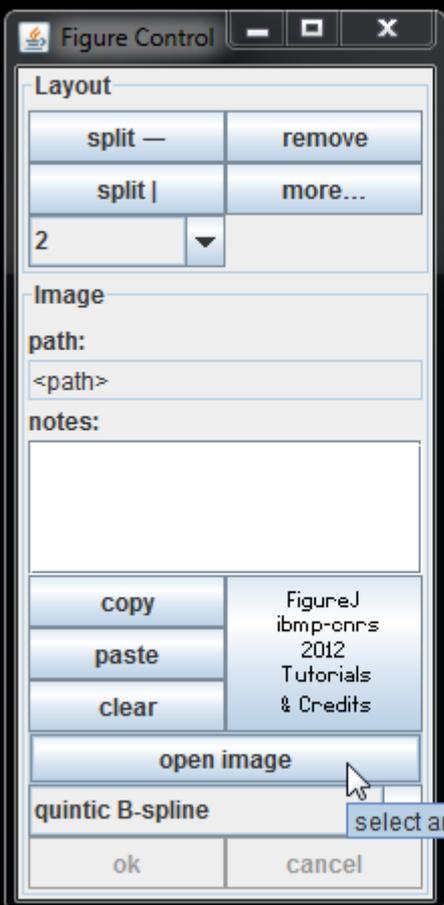


Découpage vertical



FigureJ

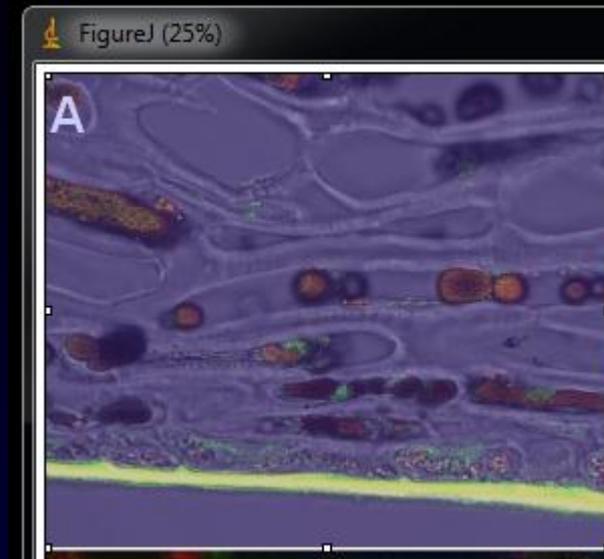
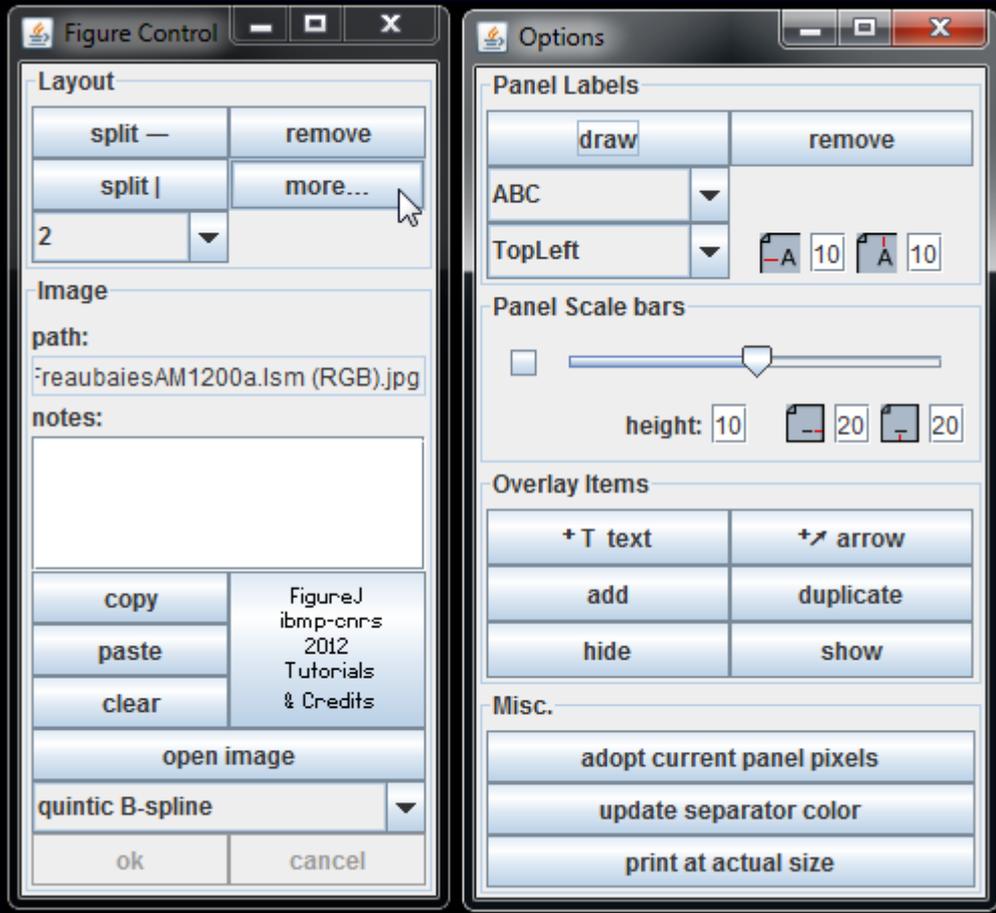
Importation des photos



- Choix de la zone à importer
- Agrandissement par les poignées en coin
- Rotation par les poignées des cotés

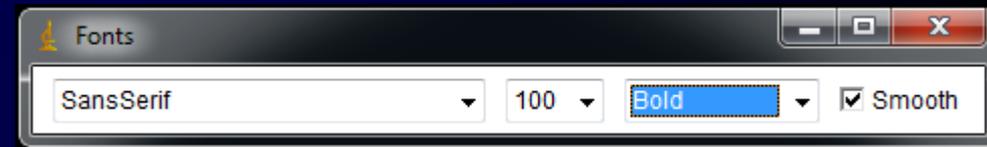
FigureJ

Lettrage ou numérotation



Choix de la couleur

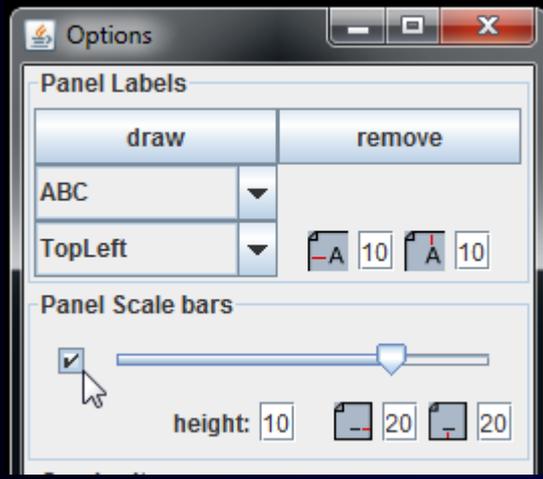
Choix de la police



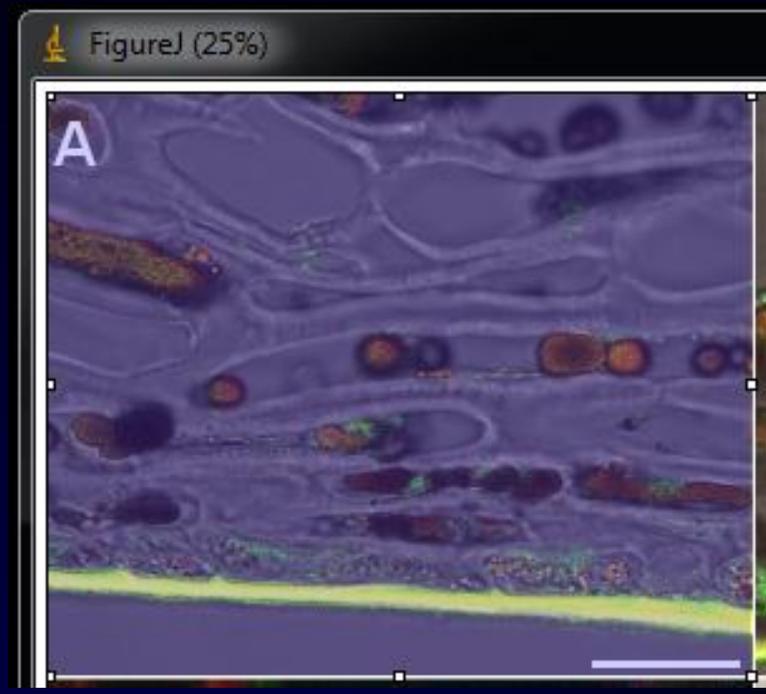
Pour remettre a zéro « alt+draw »

FigureJ

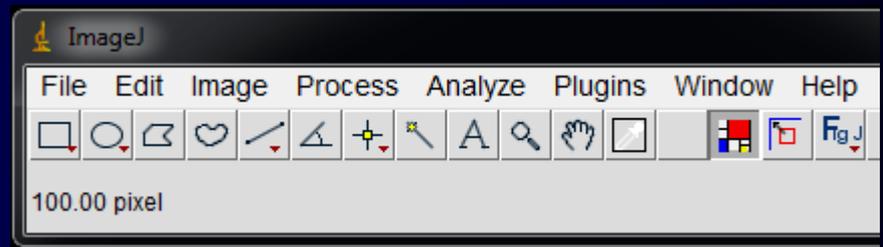
Barre d'échelle pour photos calibrées



Choix de la taille



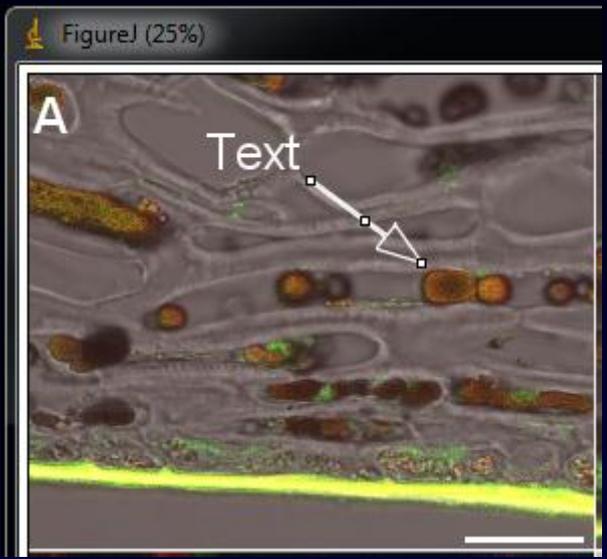
Affichage de la taille dans la barre d'état



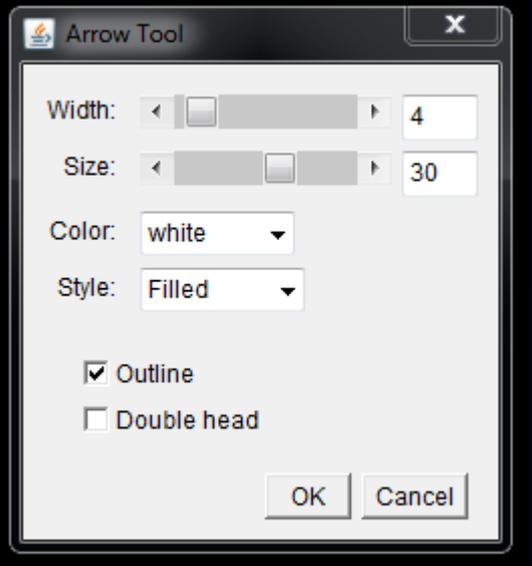
FigureJ

Ajout de texte
Et de flèches

Overlay Items	
+ T text	+ ↗ arrow
add	duplicate
hide	show



Paramètres pour les flèches



Paramètres pour le texte

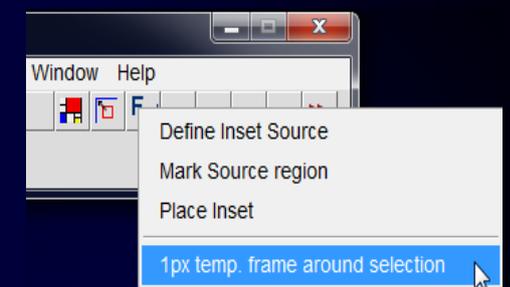
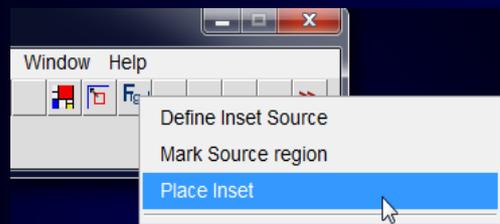
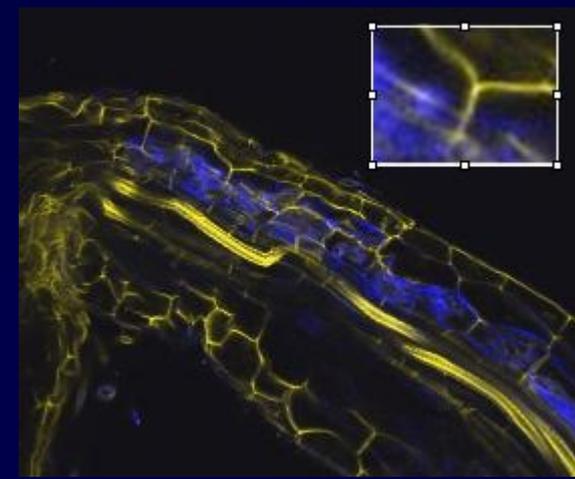
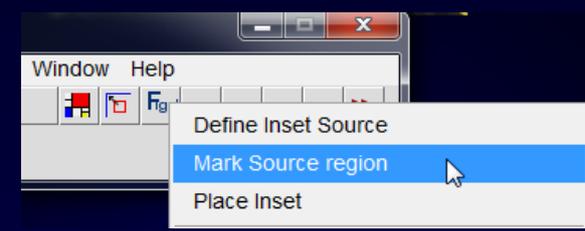
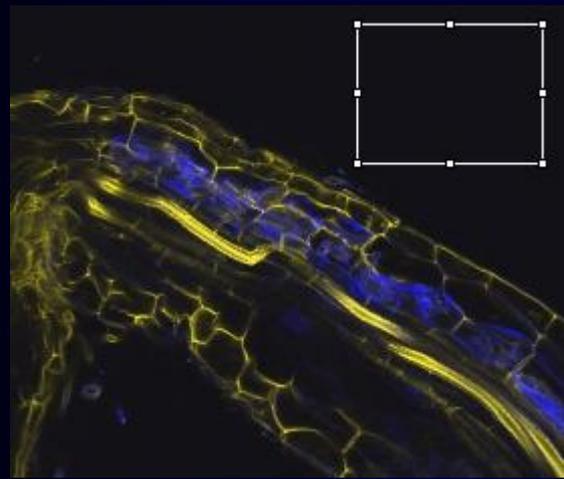
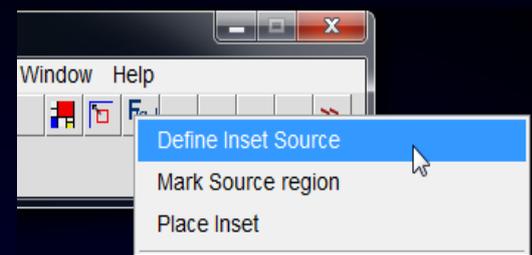
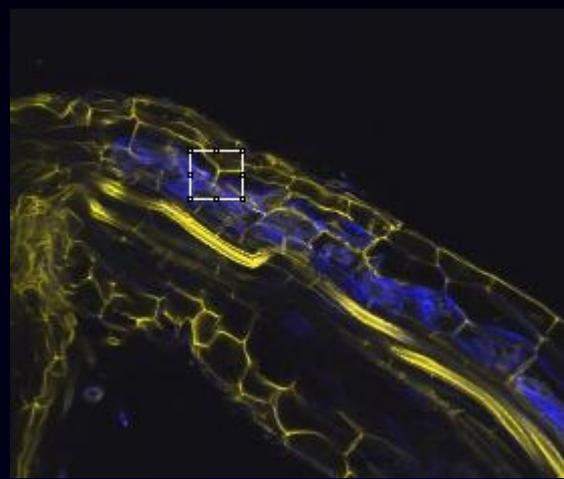


Astuce : mettre tout dans le ROI Manager
puis overlay from ROI Manager



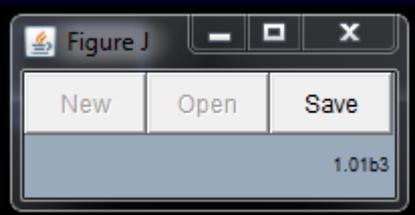
FigureJ

Inset



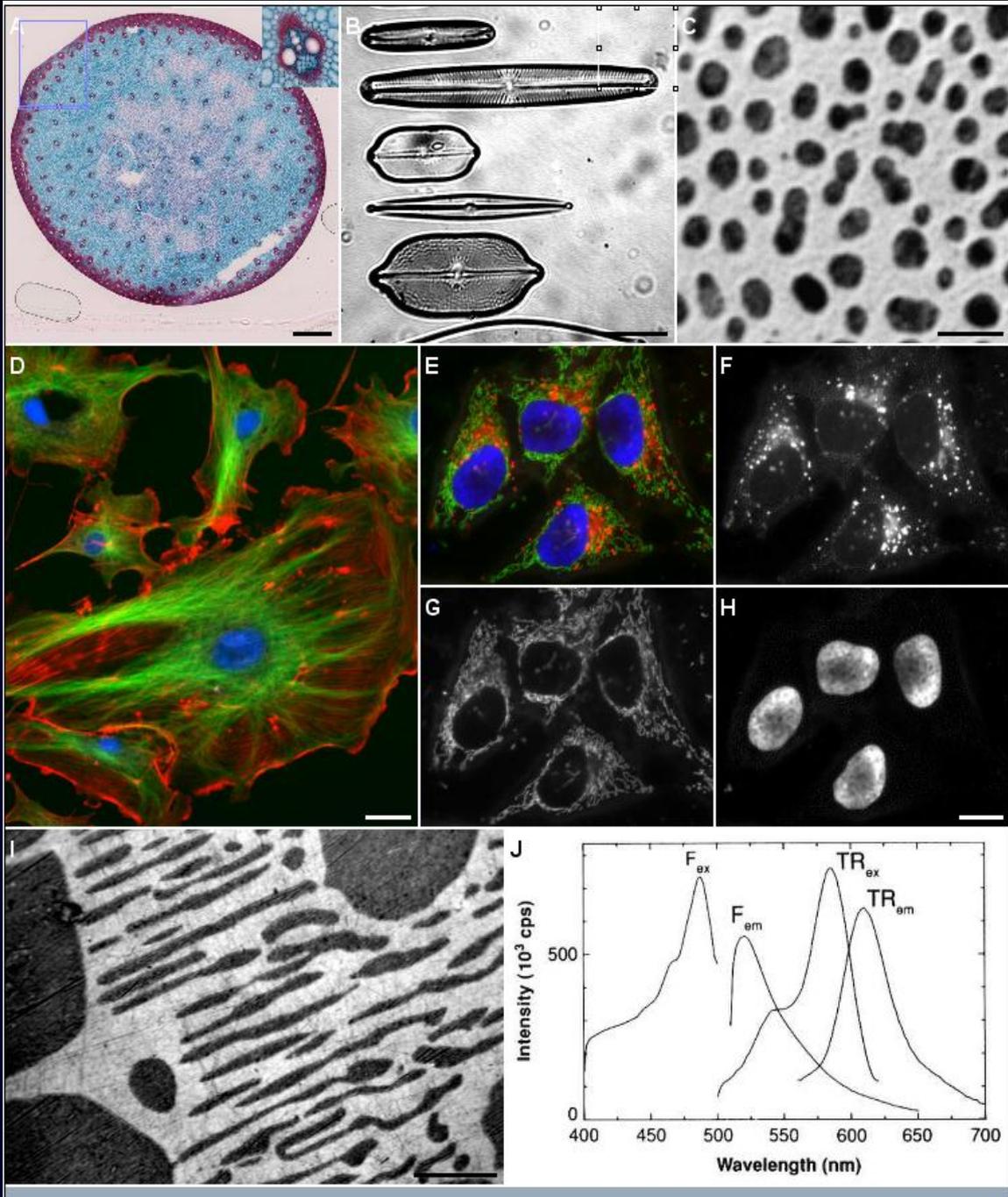
FigureJ

Dossier de sauvegarde



- save_figurej.figurej
- agnesFreaubaiasAM1200a.lsm (RGB).jpg
- agnesSyrahbaiesAM1200488543D.lsm (RGB).jpg
- brillouetChamaerops690nmDCVROI.lsm (RGB).jpg
- florence and huichen S14A2laser488nme.lsm (RGB).jpg
- jlv 120Louvert6.lsm (RGB).jpg
- JpegCompressed_save_figurej.jpg
- sergio lame3_arb12.lsm (RGB).jpg
- FullResolution_save_figurej.tif
- RoiSet.zip
- imageNotes.txt

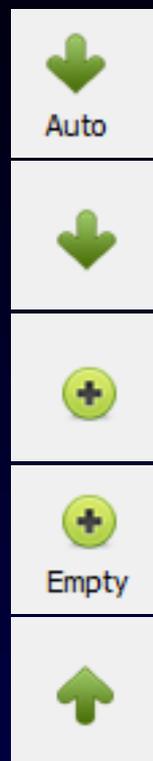
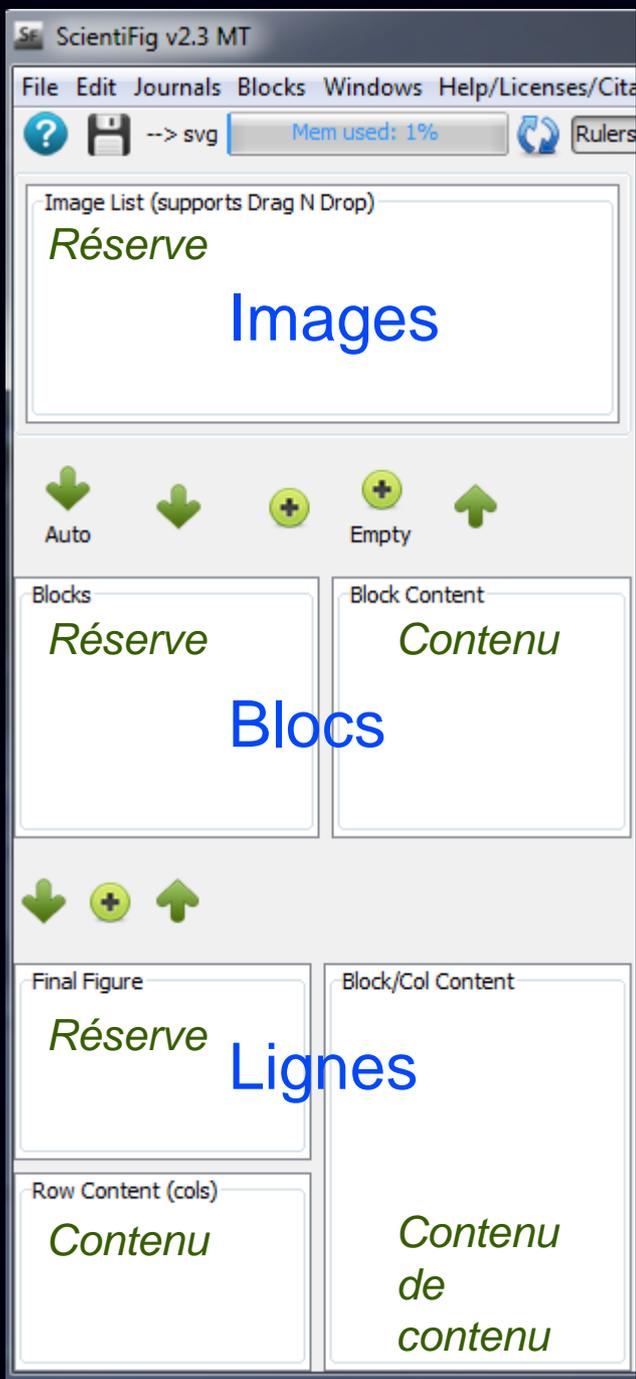
```
Panel: A
Image Datasource: agnesFreaubaiasAM1200a.lsm (RGB).jpg
External Datasource: none
Original folder: D:\Mes donnees\formation\figures\save_figurej\
Display range: [0.0-255.0]
Current [Slice,Frame,Channel]: [1,1,1]
Active Channels: []
User notes:
Scale bar: 100.0 pixel
Preprocessing:
```



Installation du plugin


New!

Description:	ScientiFig is a free tool to help you create, format or reformat scientific figures © 2012-2013 Benoit Aigouy
Author:	Benoit Aigouy (benoit.aigouy@udamail.fr)
Installation as a stand-alone:	Unzip the file and double click on "launcher.jar" To run ScientiFig directly from the command line type: <code>java -Xmx1024m -jar /path/to/ScientiFig.jar</code>
Installation as an ImageJ/Fiji plugin:	Please have a look at this video demo Unzip the ScientiFig ImageJ or FIJI version to a destination directory then copy this entire destination directory to the ImageJ/Fiji "plugins" directory.
Installation as a Fiji plugin: (FIJI update site parameters)	Please have a look at this video demo Name: ScientiFig URL: http://srv-gred.u-clermont1.fr/labmirouse/software/FIJI_update_site/ NB: I strongly recommend that you delete previous ScientiFig installations from your FIJI 'plugins' directory
Requirements:	ScientiFig works under Linux, Windows and Mac OS X. It requires java 1.6 or > to be installed on your system.
Downloads:	ScientiFig (Standalone version around 5 Mb) ScientiFig (ImageJ version around 3.6 Mb) ScientiFig (FIJI version around 600 kb) ScientiFig (Standalone version with the old interface, not supported anymore please don't use 5 Mb)



Crée un bloc pour chaque image de la liste

Crée un bloc pour l'image sélectionnée

Ajoute une image au bloc actif

Ajoute un espace blanc au bloc actif

Détruit le bloc et remet les images en réserve

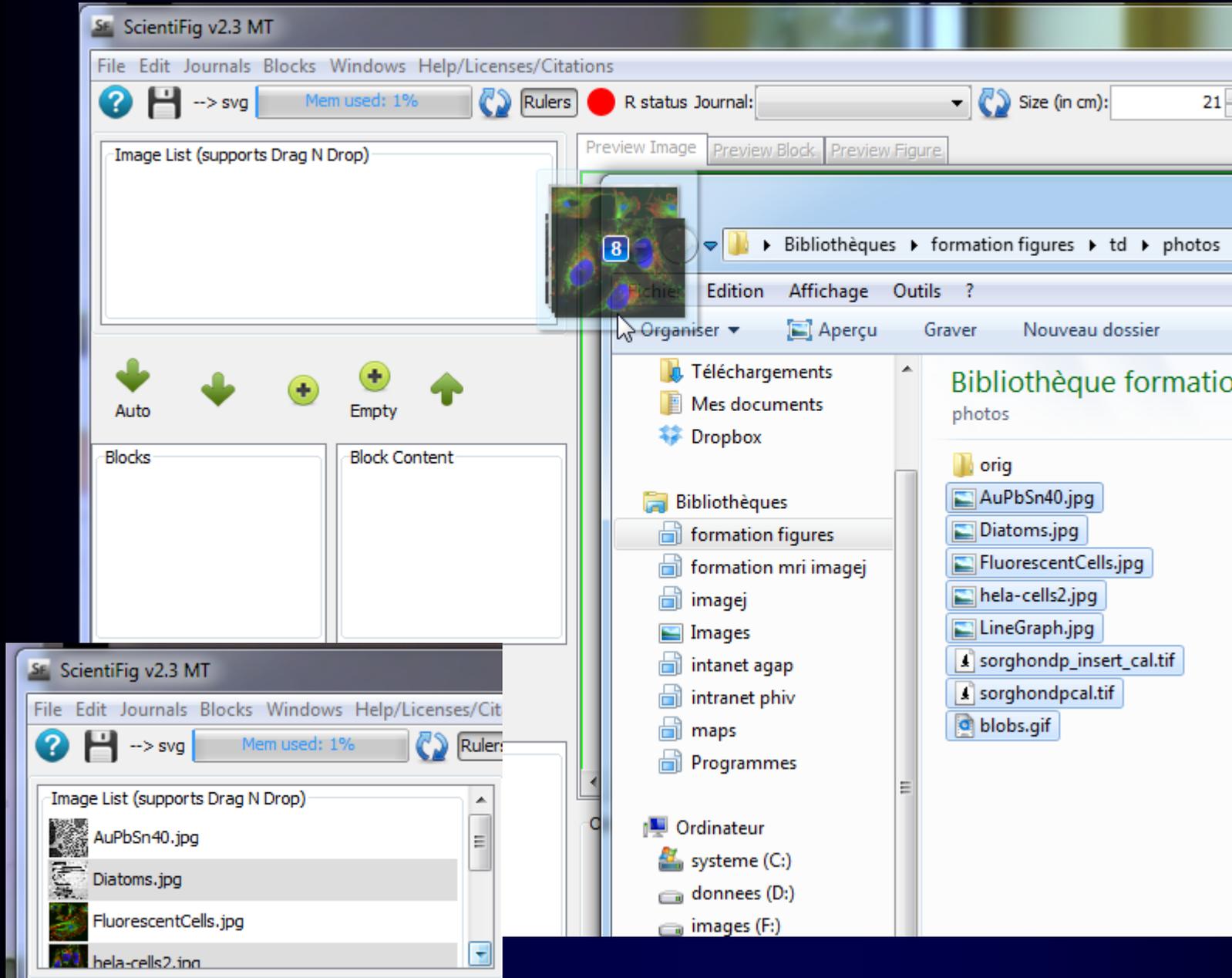


Crée une ligne avec le bloc sélectionné

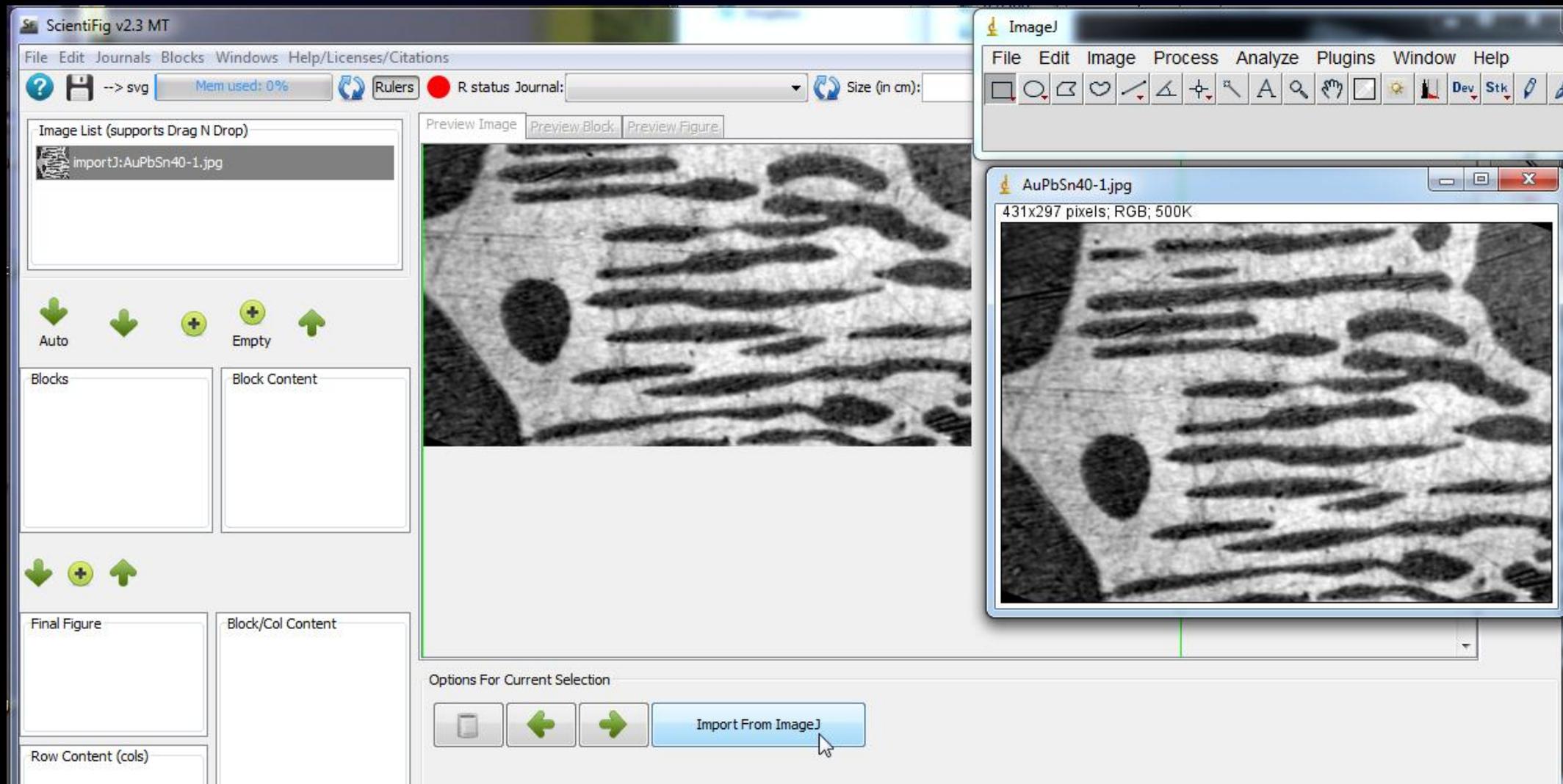
Ajoute un bloc à la ligne active

Enlève la ligne et remet les blocs en réserve

Import images par drag and drop



Import de l'image active d'ImageJ (après recadrage et rotation)



The screenshot displays the ScientiFig v2.3 MT software interface. The main window shows a preview of a grayscale electron microscopy image of a biological specimen. The interface includes a menu bar (File, Edit, Journals, Blocks, Windows, Help/Licenses/Citations), a toolbar with various icons, and a sidebar with an 'Image List' containing 'importJ: AuPbSn40-1.jpg'. Below the sidebar are sections for 'Blocks', 'Block Content', 'Final Figure', and 'Block/Col Content'. At the bottom, the 'Options For Current Selection' panel is visible, featuring a button labeled 'Import From ImageJ' which is being clicked by a mouse cursor. An 'ImageJ' window is also open, showing the same image with a bounding box around it, and its title bar reads 'AuPbSn40-1.jpg' with dimensions '431x297 pixels; RGB; 500K'.

ScientiFig v2.3 MT

File Edit Journals Blocks Windows Help/Licenses/Citations

Mem used: 2% R status Journal: Size (in cm): 21 (or px): 595 (or cols)

Image List (supports Drag N Drop)

Auto Empty

Blocks

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8

Block Content

hela-cells2:D

Final Figure

Block/Col Content

Row Content (cols)

Options For Current Selection

Space (px): 3 1st Letter: A Update Letters

Crop Left: 0 Right: 0 Up: 0 Down: 0 Angle: 0 Rotate Left Rotate Right

Annotate Remove Annotations Replace Image width: 672 Image height: 512 A

Create a Block

Channels To Display

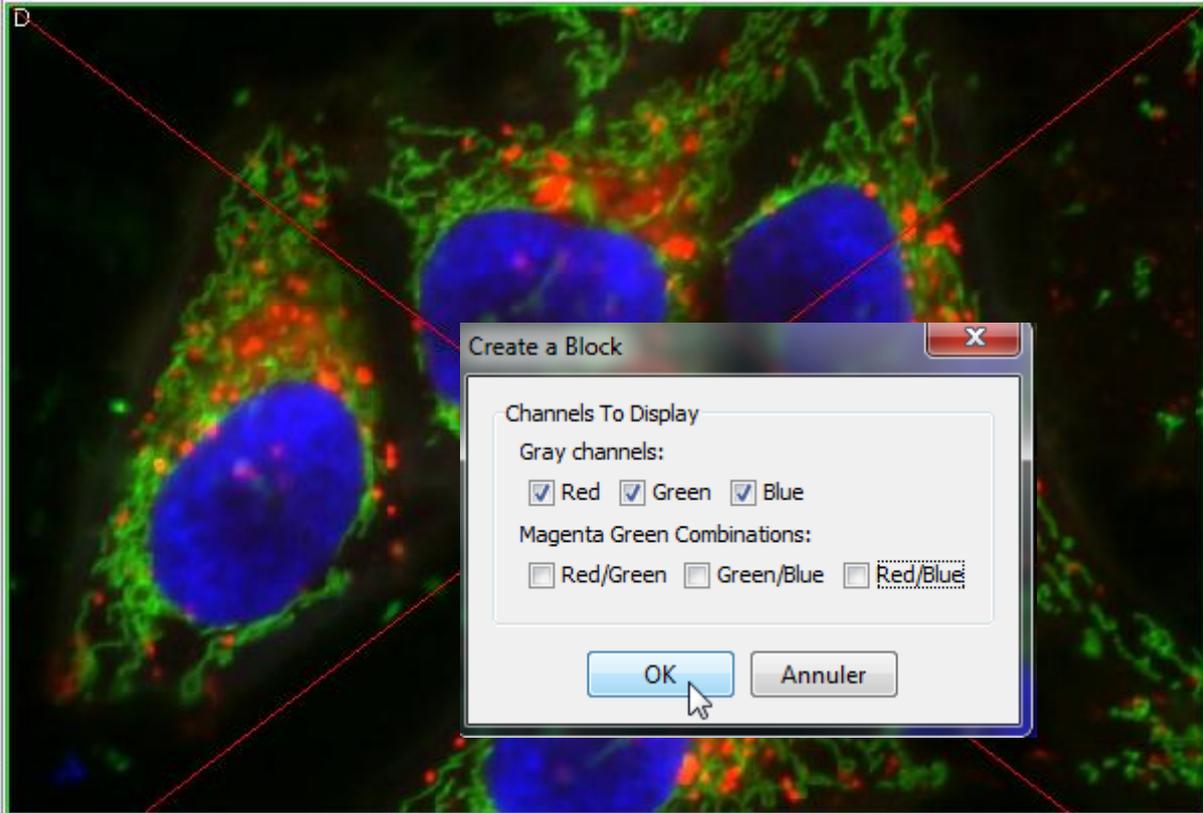
Gray channels:

Red Green Blue

Magenta Green Combinations:

Red/Green Green/Blue Red/Blue

OK Annuler



Options For Current Selection

Reformat Table Space (px): 3 1st Letter: A Update Letters

Edit Block Format (change nb of rows and columns)

Create a Block

Parameters

Nb Rows: 2

Nb Cols: 2

Fill Order: Meander Comb

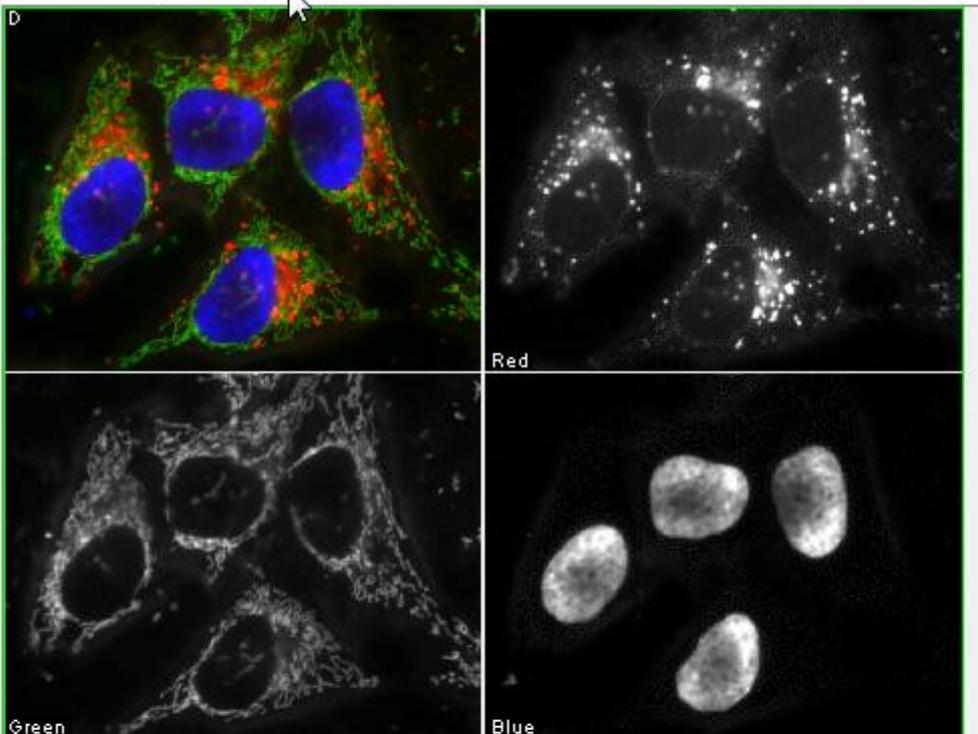
Rows X Columns (total nb of images in the block): 4

Number of images to fit in the block: 4

Infos: Everything is fine (Rows X Cols >= Number of images to fit)

OK Annuler

Preview Image Preview Block Preview Figure



Options For Current Selection

Reformat Table Space (px): 3 1st Letter: A Update Letters

ScientiFig

ScientiFig v2.3 MT

File Edit Journals Figure Windows Help/Licenses/Citations

Mem used: 2% R status Journal: Size (in cm): 21

Image List (supports Drag N Drop)

- sorghondp_insert_cal.tif

Auto Empty

Blocks Block Content

Final Figure

- 7+2+8
- 3+4
- 1+5

Block/Col Content

- sorghondpca:G

Row Content (cols)

- 1
- 2
- 3

Preview Image Preview Block Preview Figure

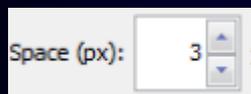
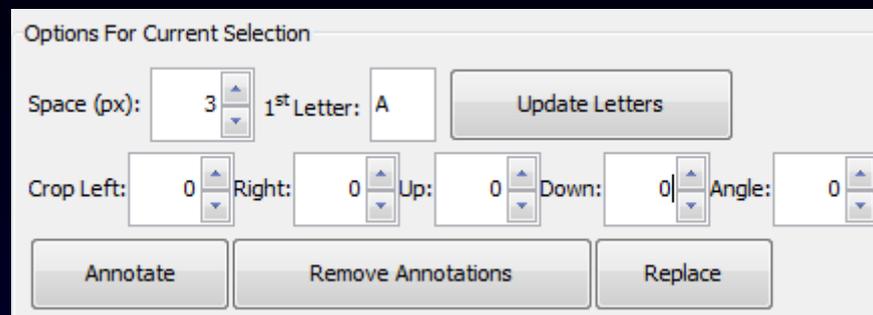
Options For Current Selection

Space (px): 3 1st Letter: A Update Letters

Crop Left: 0 Right: 0 Up: 0 Down: 0 Angle: 0

Annotate Remove Annotations Replace

Menu image active



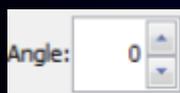
Largeur des espaces entres les images



Choix de la première lettre et remise à jour du lettrage



Recadrage de l'image active



Rotation de l'image active



Annotations : création et annulation



Remplace l'image active

Déplacement des des lignes ou blocs actifs

Final Figure

7+2+8
3+4
1+5

Row Content (cols)

1
2

Block/Col Content

AuPbSn40:I

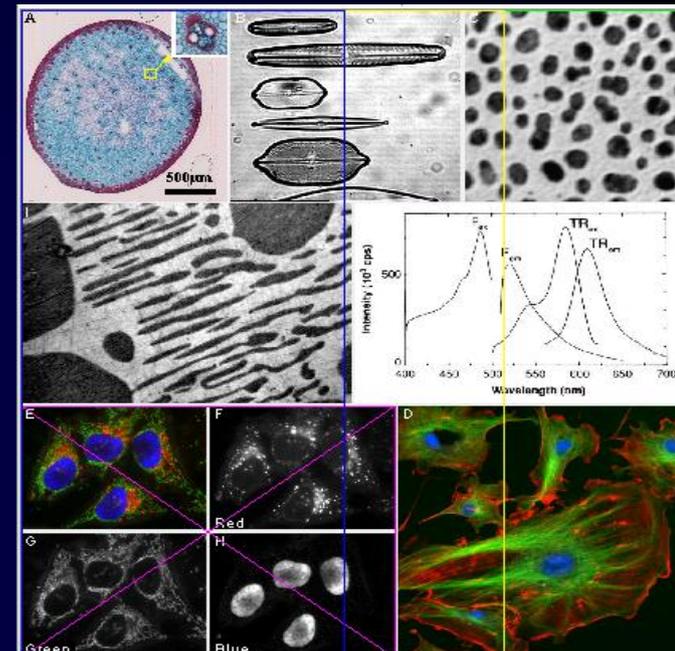
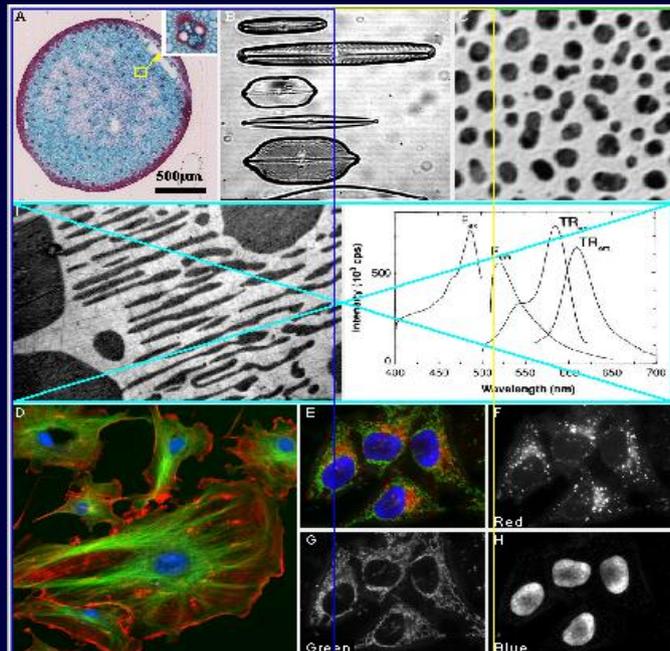
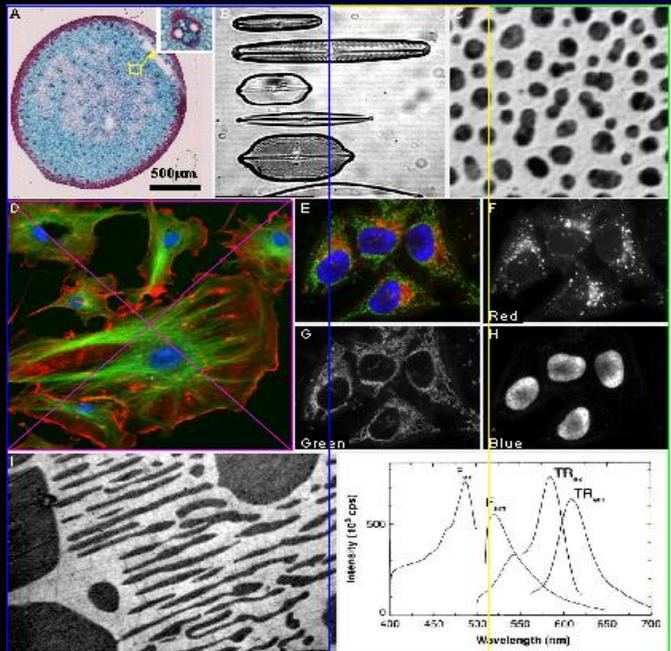
Options For Current Selection

←

→

Space (px):

Move block to the left



Lettrage

Inset

Barre d'échelle

Texte

Edit Image

Image/Text

Letter Upper Right Corner
 Upper Left Corner

Select the position of the text to edit

 Lower Right Corner
 Lower Left Corner Scale Bar

Stroke Size:

NB: changing stroke by pressing the 'Force' button is not

Inset/PIp

Fraction of the image width (dedicated for the inset):

Inset spacer:

Inset Scale Bar Size (px):

Inset Border Color:

Inset position:

upper left corner upper right corner
 lower left corner lower right corner

ScaleBar Parameters

Bar Size in Px Of The Non Scaled Image:

Bar Size in whatever unit (µm, mm,...):

Px Size In the Same unit (Conversion Factor):

Bar thickness/height (in px):

Bar Color:

Text: Above Scale Bar

 text color **B** *I* sub_{script} super^{script}
aßy no Bg Black Bg White Bg

Image Comments (just for you, they will not be displayed)

Inset/PIP

Fraction of the image width
(dedicated for the inset):

Inset spacer:

Inset Scale Bar Size (px):

Inset Border Color:

Inset position:

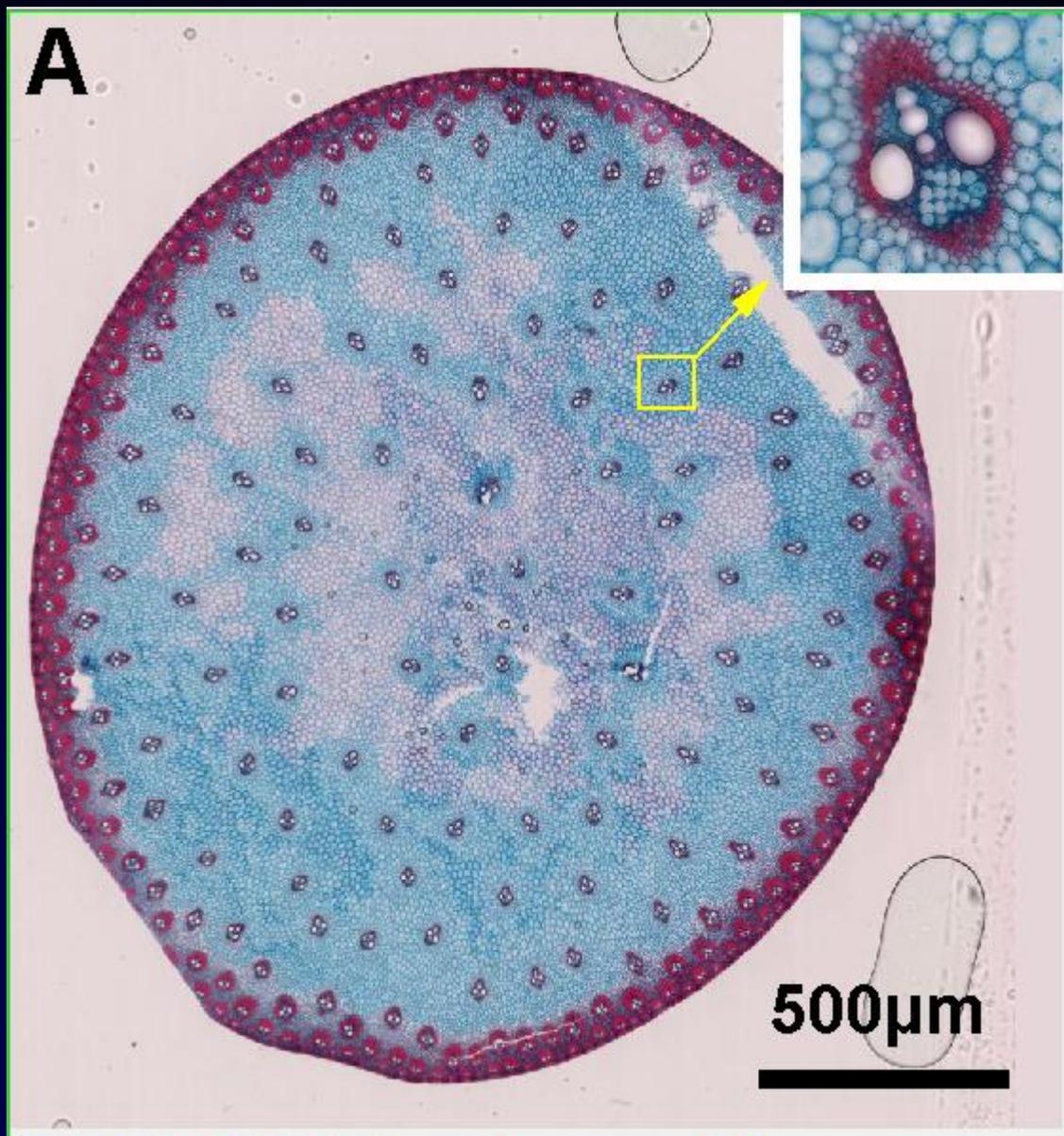
upper left corner upper right corner

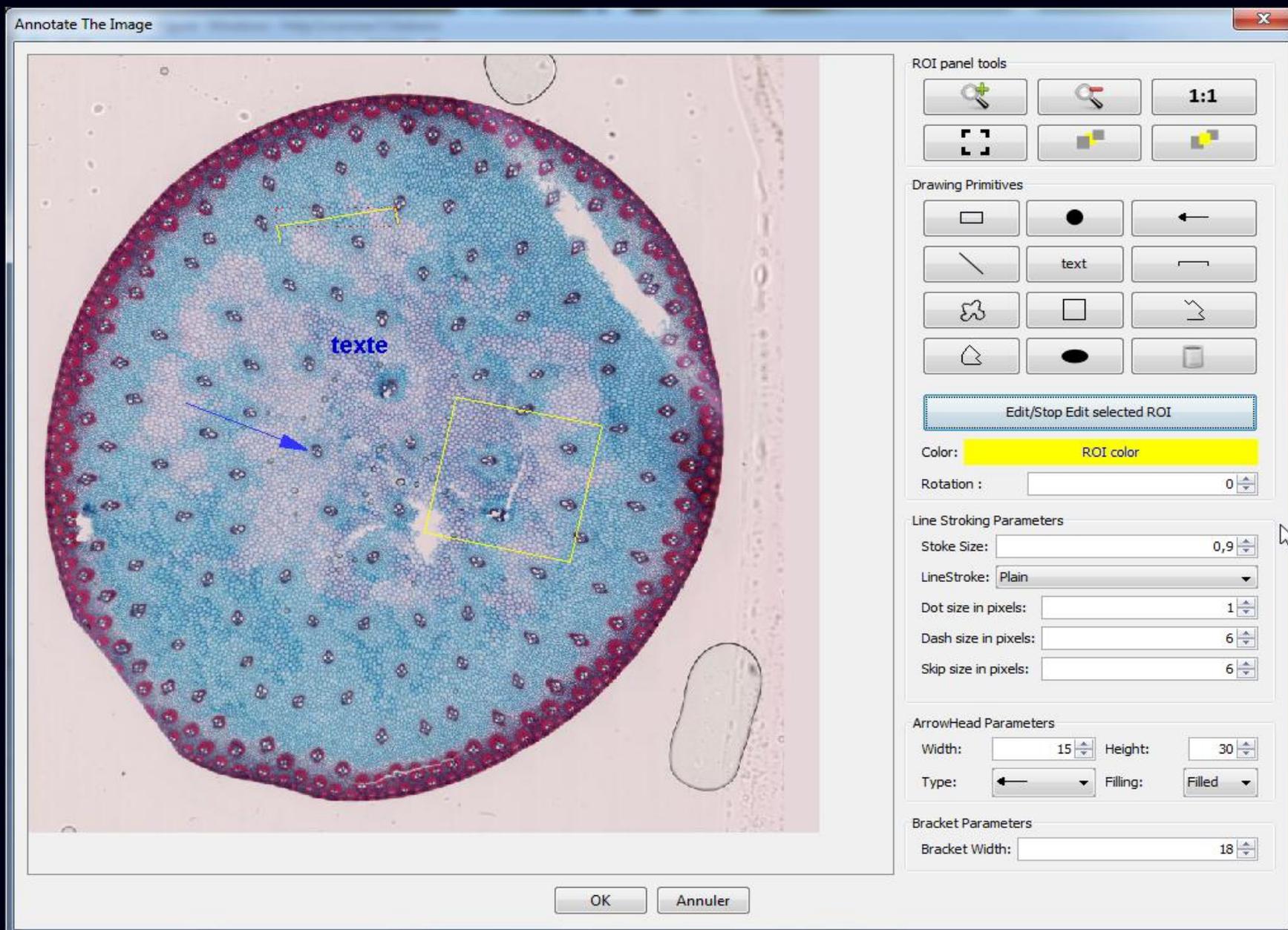
lower left corner lower right corner

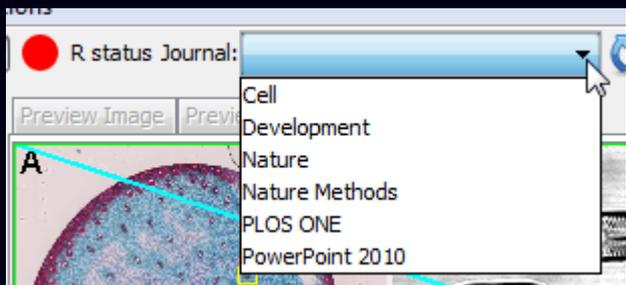
Add inset

Image List (supports Drag N Drop)

 sorghondp_insert_cal.tif







Warning

Text Parameters

- Override bolding
- Override italic
- Override bolding (letters only Recommended)

Graphs (FiguR)

- Change Line Width
- Change Point Size

NB: Changing line stroke or point size is irreversible

ROIs associated to images

- Change ROI Stroke size

NB: Changing ROI stroke size is irreversible

SVG images

Size (in cm): 18,3 (or px): 519 (or cols): 2 Cols

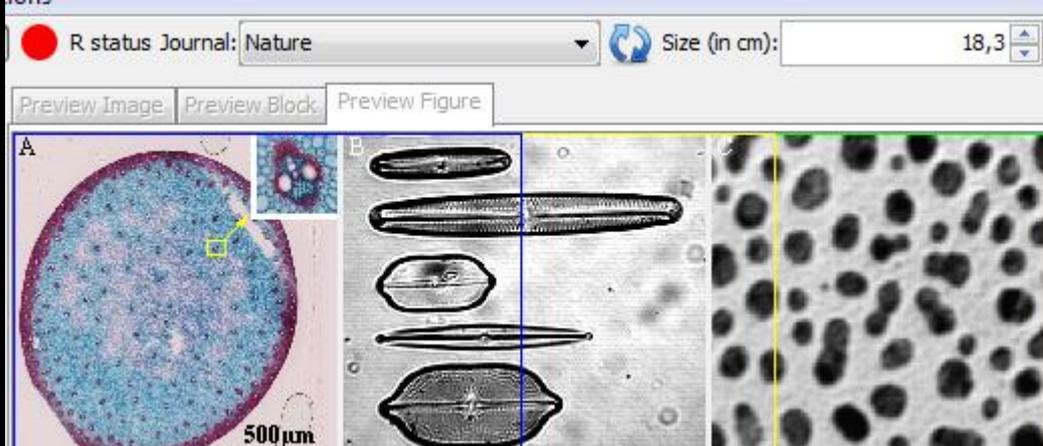
le and will permanently affect ink twice before changing svg stroke.

Illustrator handle stroke size : you might be using to open to export as raster please use

all the images in the current table

DO YOU REALLY WANT TO CONTINUE ?

OK Annuler



FiguR v0.7 beta

File Journals Data Themes Legend Facets Intercepts Axes About/Citations

Journal Style : R status Mode : Safe (use most likely factors) Unsafe (any column can be a factor)

Parameters

Input File:

Main Title:

Legend Title:

x Axis Title:

y Axis Title:

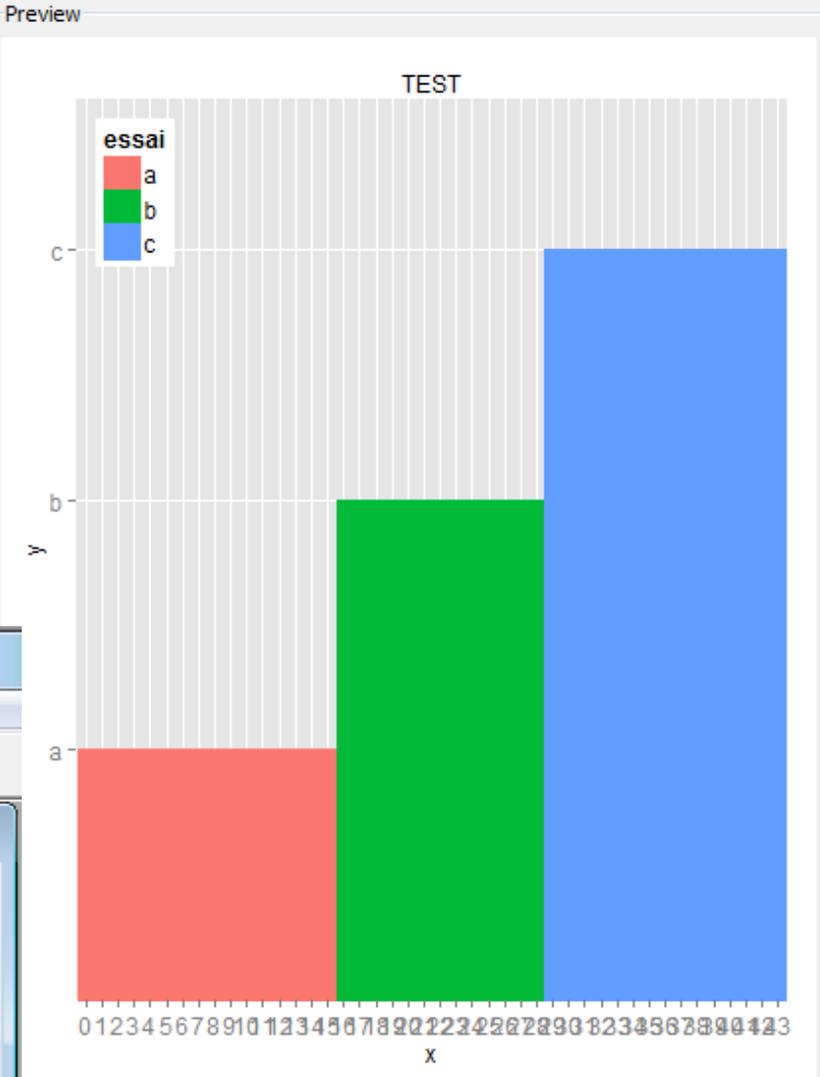
Select A Plot style then click on +

Plots List

```
geom_bar(aes(x=as.factor(x), y=azert, fill=as.factor(azert), color=as.factor(azert)), stat=
```

Colors: Colors

Preview



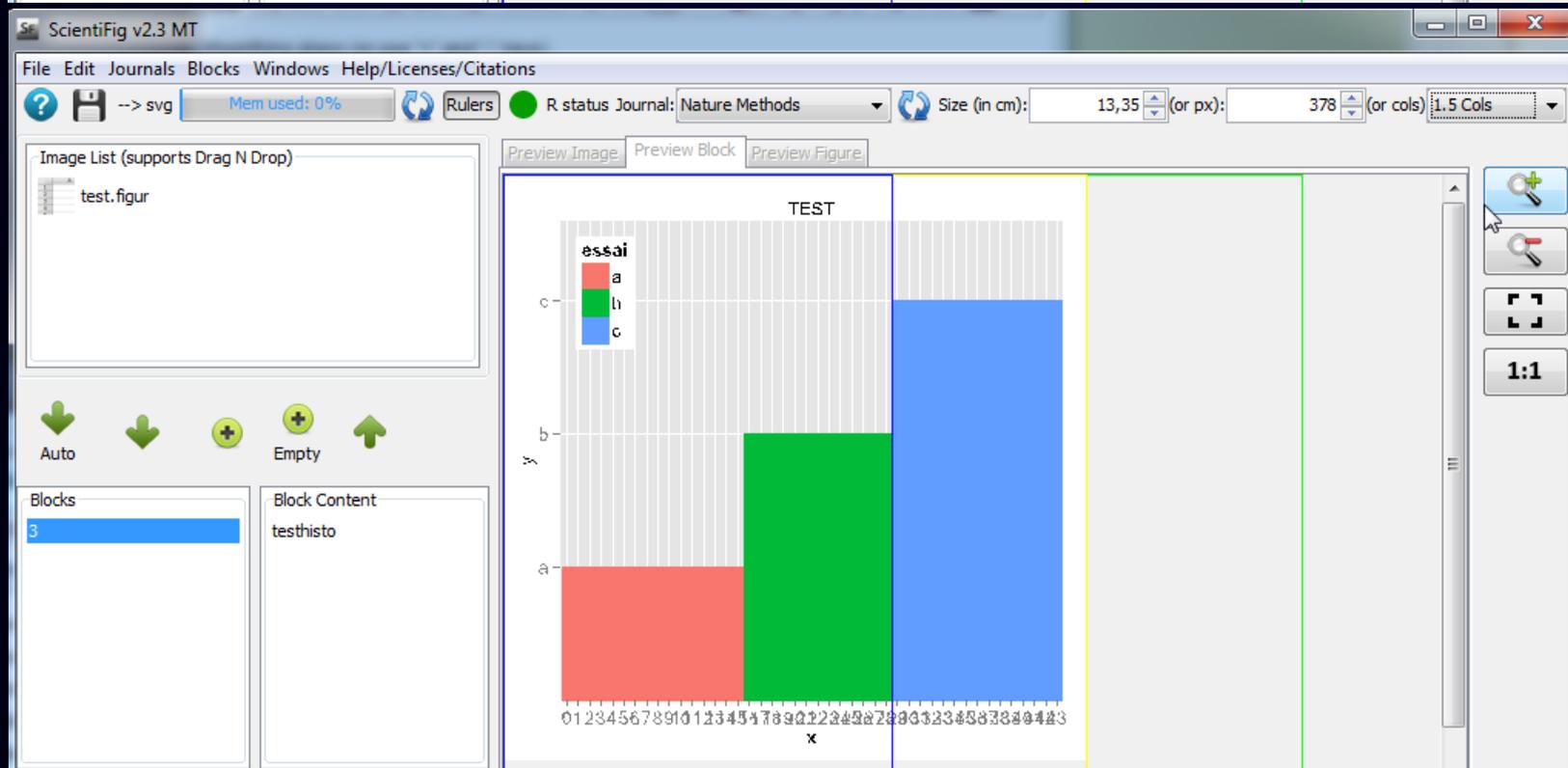
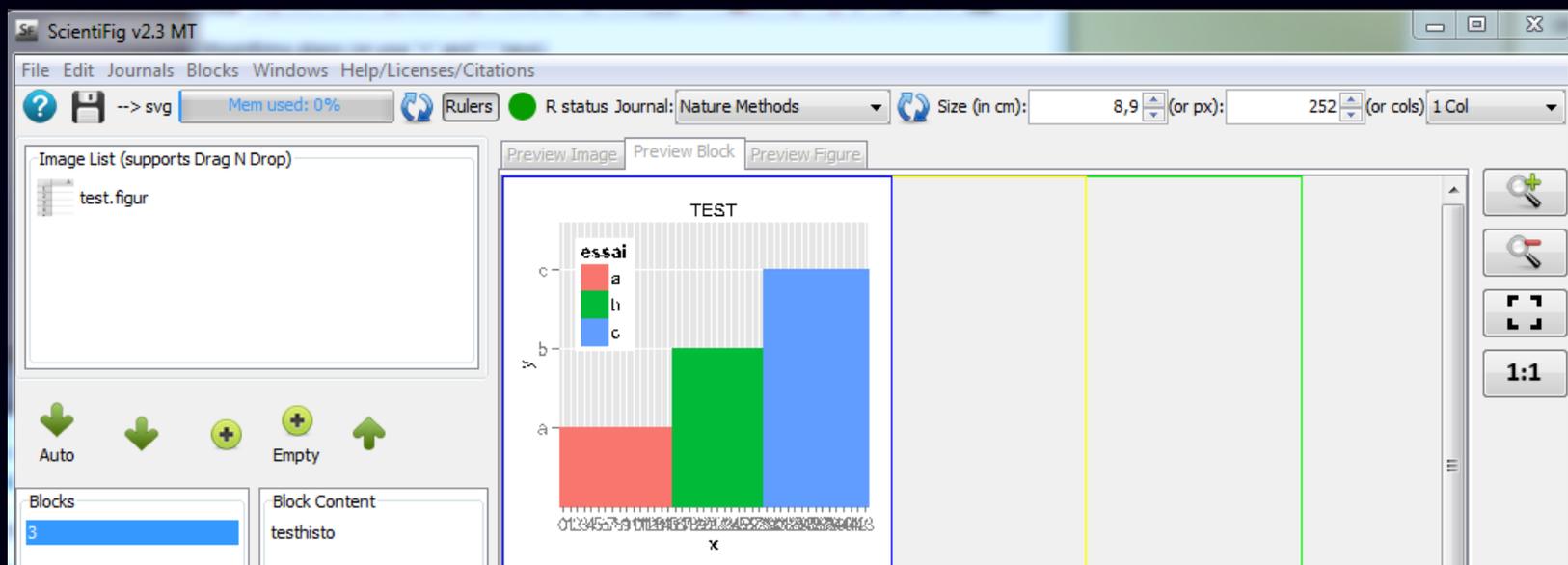
Category (x-axis)	Value (y-axis)	Color
a	~10	Red
b	~20	Green
c	~30	Blue

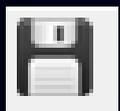
RGui (64-bit)

Fichier Edition Voir Misc Packages Fenêtres Aide

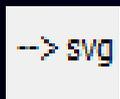
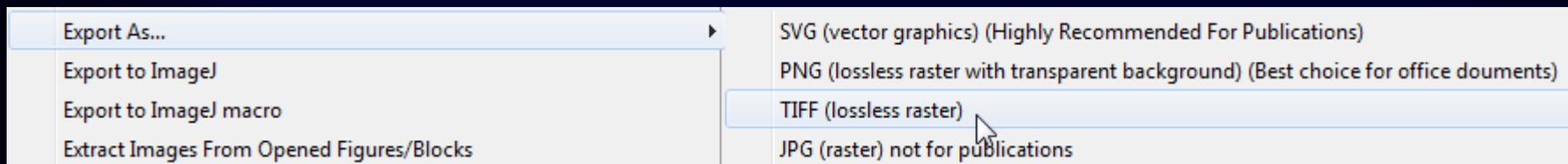
R Console

```
> library(Rserve)
> Rserve(args='--vanilla')
Starting Rserve...
"C:\PROGRA~1\R\R-215~1.3\library\Rserve\libs\x64\Rserve.exe" --vanilla
> |
```





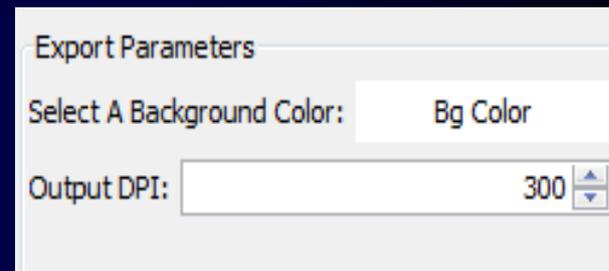
Au format ScientiFig .yf5m



Au format vectoriel SVG



Au format TIFF avec le choix de la résolution





FigureJ / ScientiFig

Maquette	Rapide	Modifiable
Import photos	Recadrage et rotation	
Lettrage		Automatique
Barre d'échelle		Option texte
Annotations		Objets
Inset		Facile
Lien graphique	(R)	R dynamique via FiguR
Style		Journal
Export	Tiff	Tiff SVG

Logiciels libres lisant le format vectoriel SVG : Scribus, Inkscape