Les étapes du traitement de l'analyse d'image

La capture → image brute

Prétraitement → niveaux de gris

Segmentation → image binaire

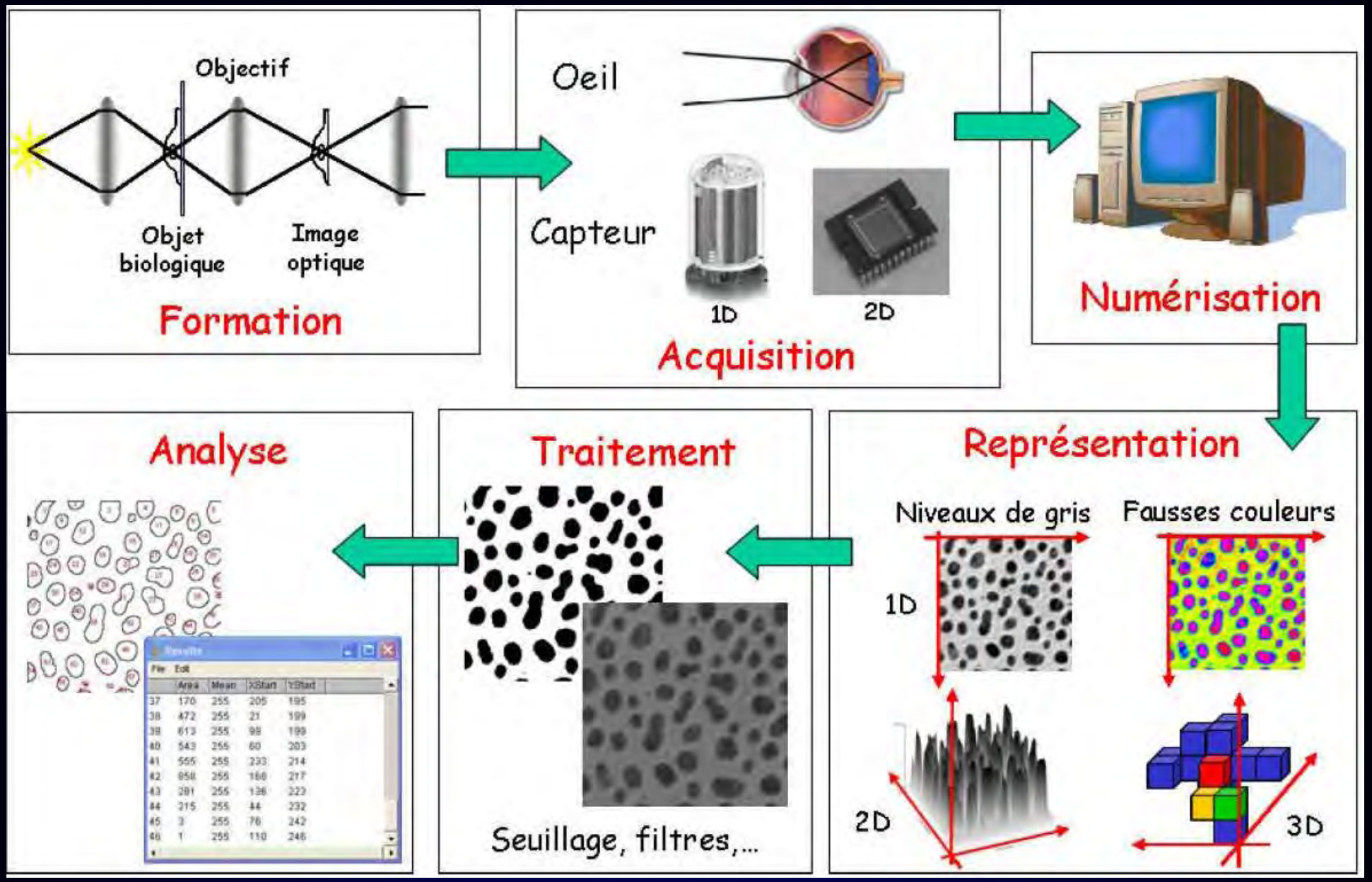
Post-traitement → régions d'intérêts

Quantification → données

Amélioration

Publication

Le traitement et l'analyse d'images



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

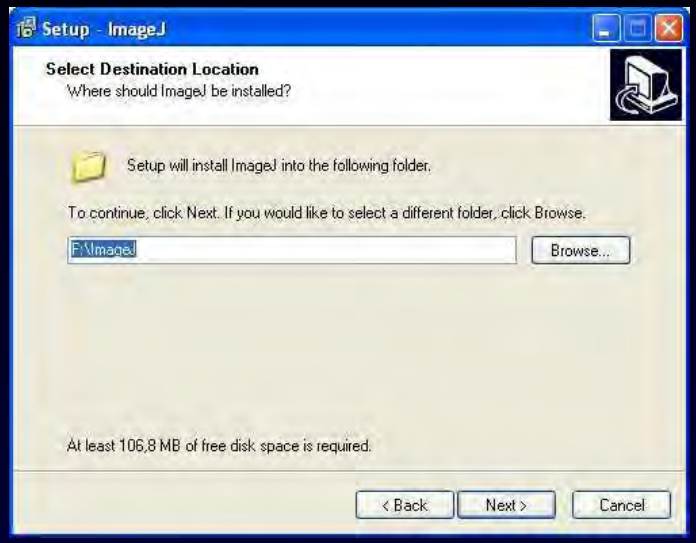
Traduction en Java du logiciel NIH Image
du National Institutes of Health (USA)
développé par Wayne Rasband

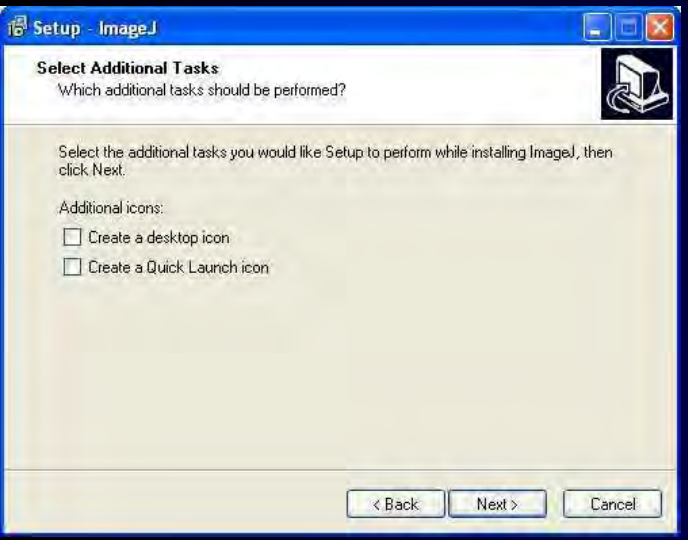
Il fonctionne sur de multiples plates-formes (Windows, Mac, Linux, Unix, …).

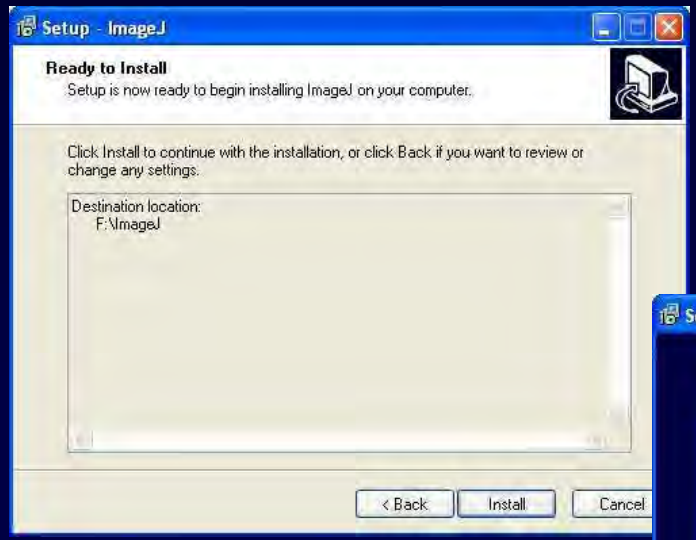
http://rsbweb.nih.gov/ij/

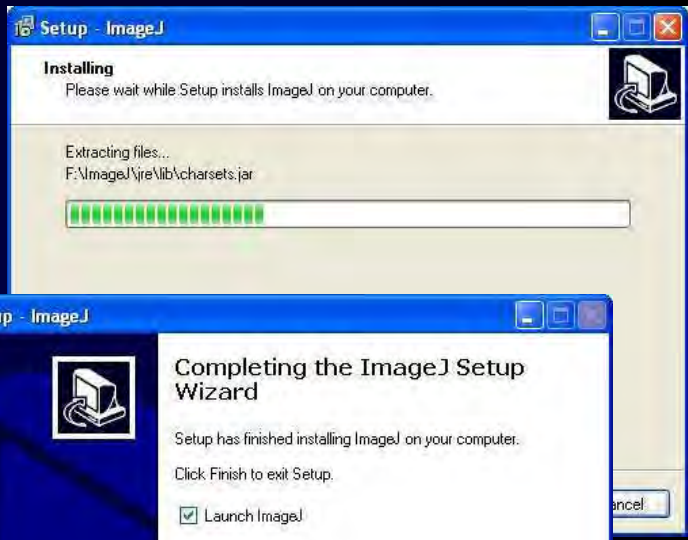
Installation

Installation



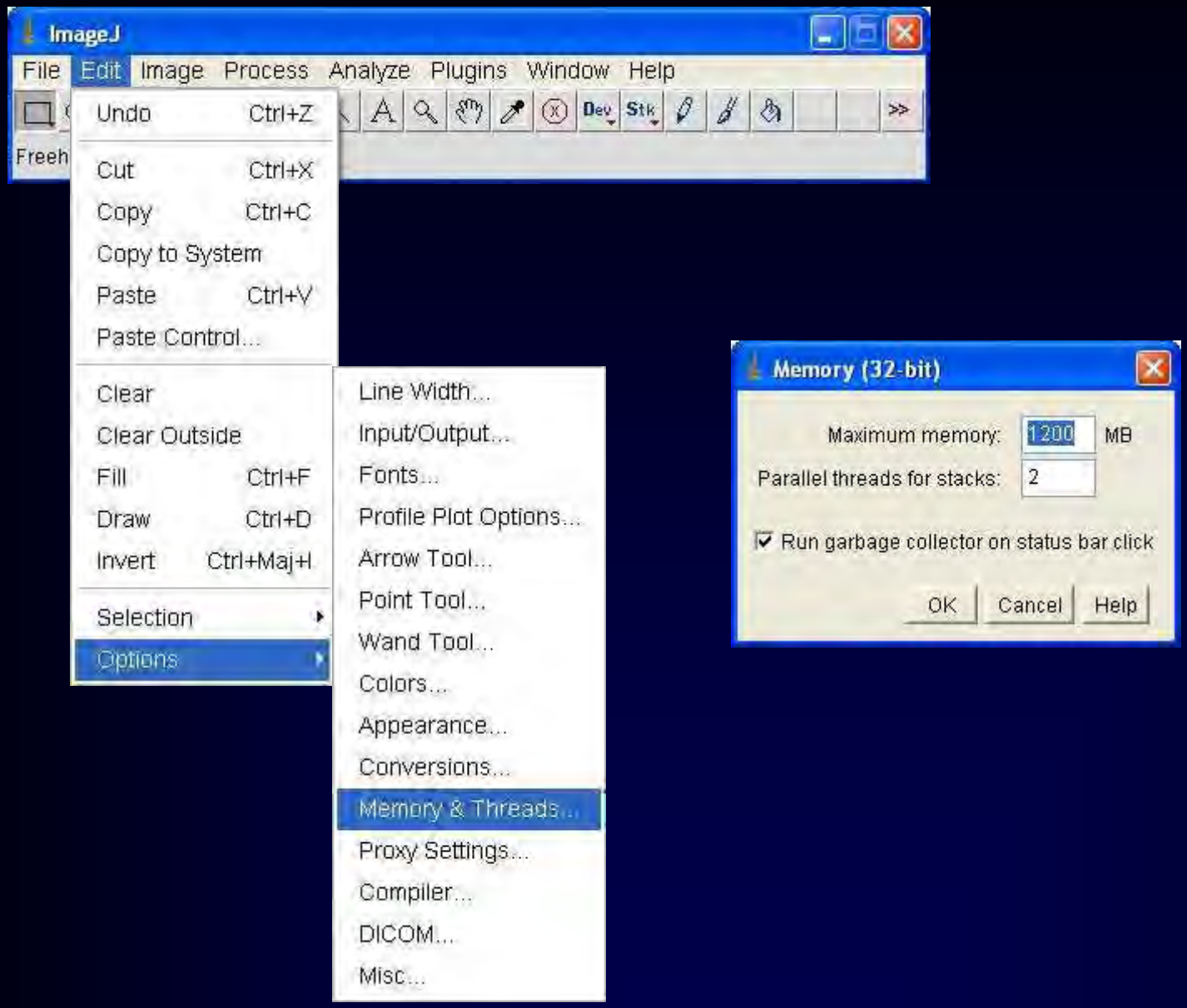






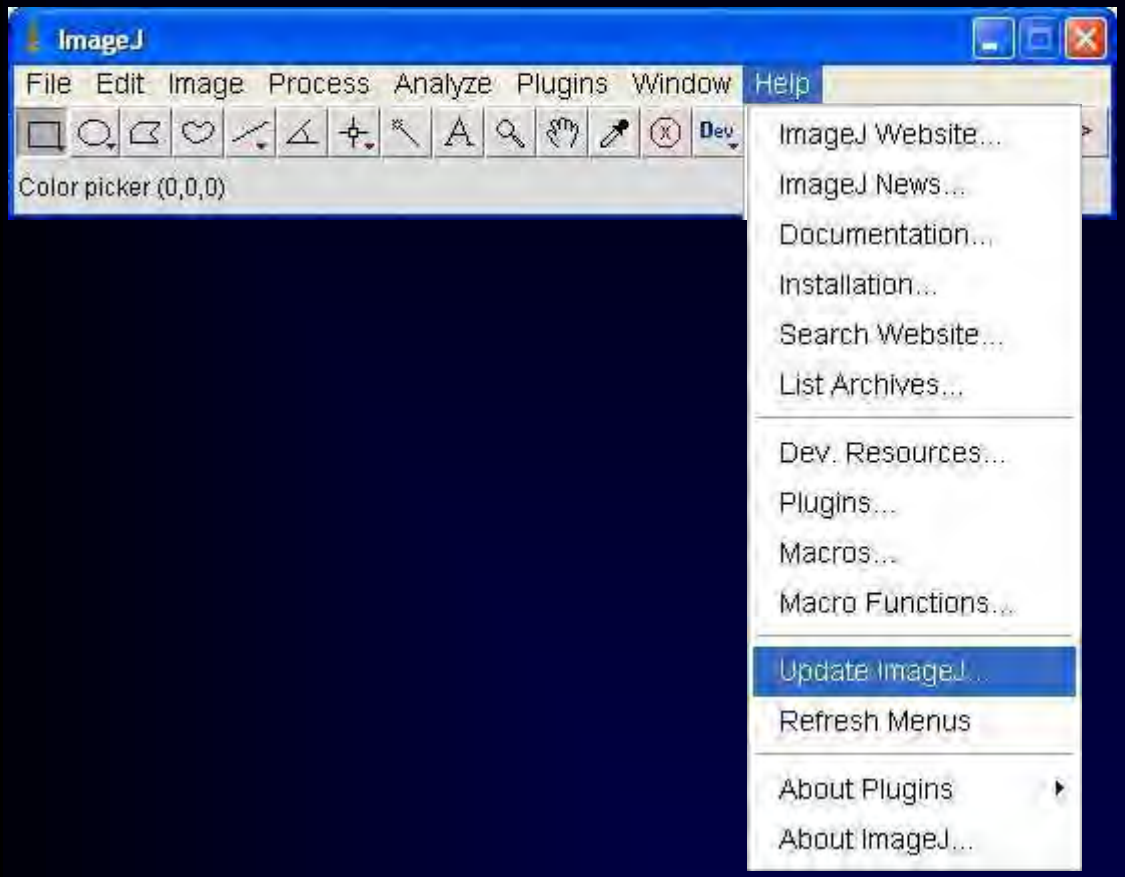
Configuration

Configuration



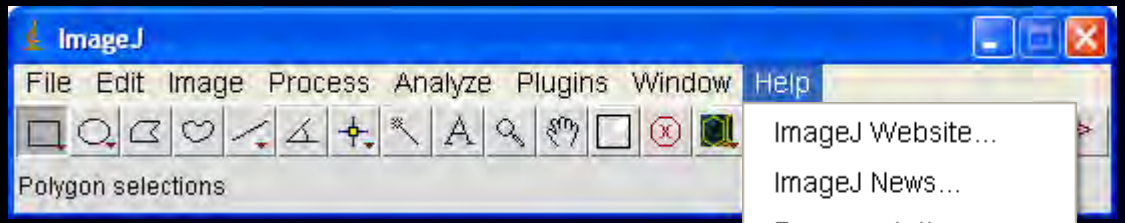
Edit → Option → Memory & Treads...

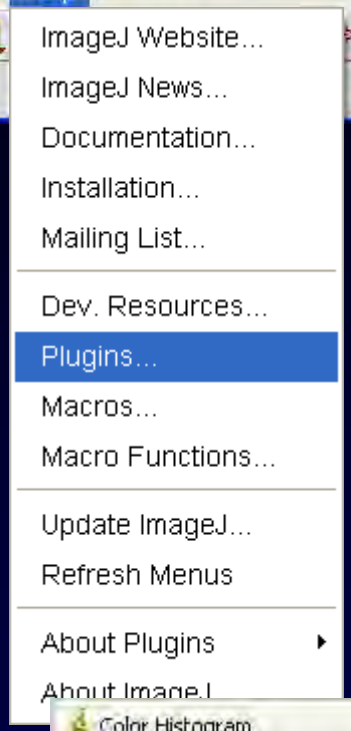
Mise à jour

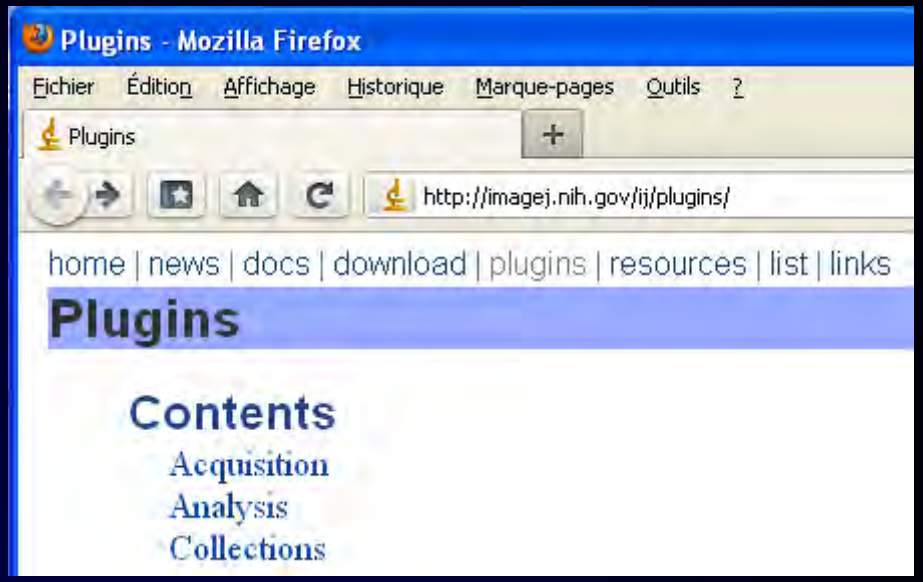


Help→ Update ImageJ...

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

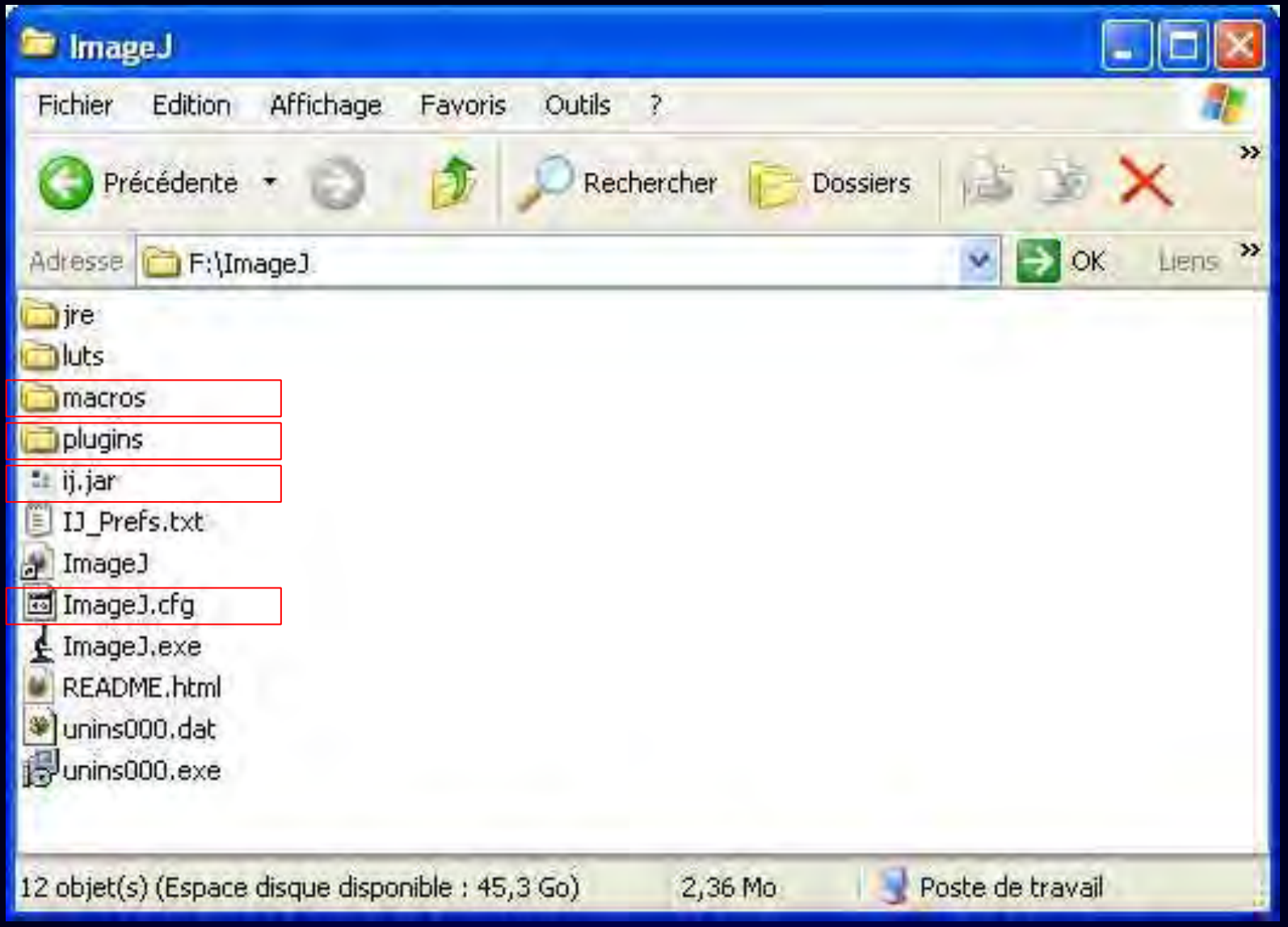
- Mettre à jour les menus Help--> Refresh Menus

- 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

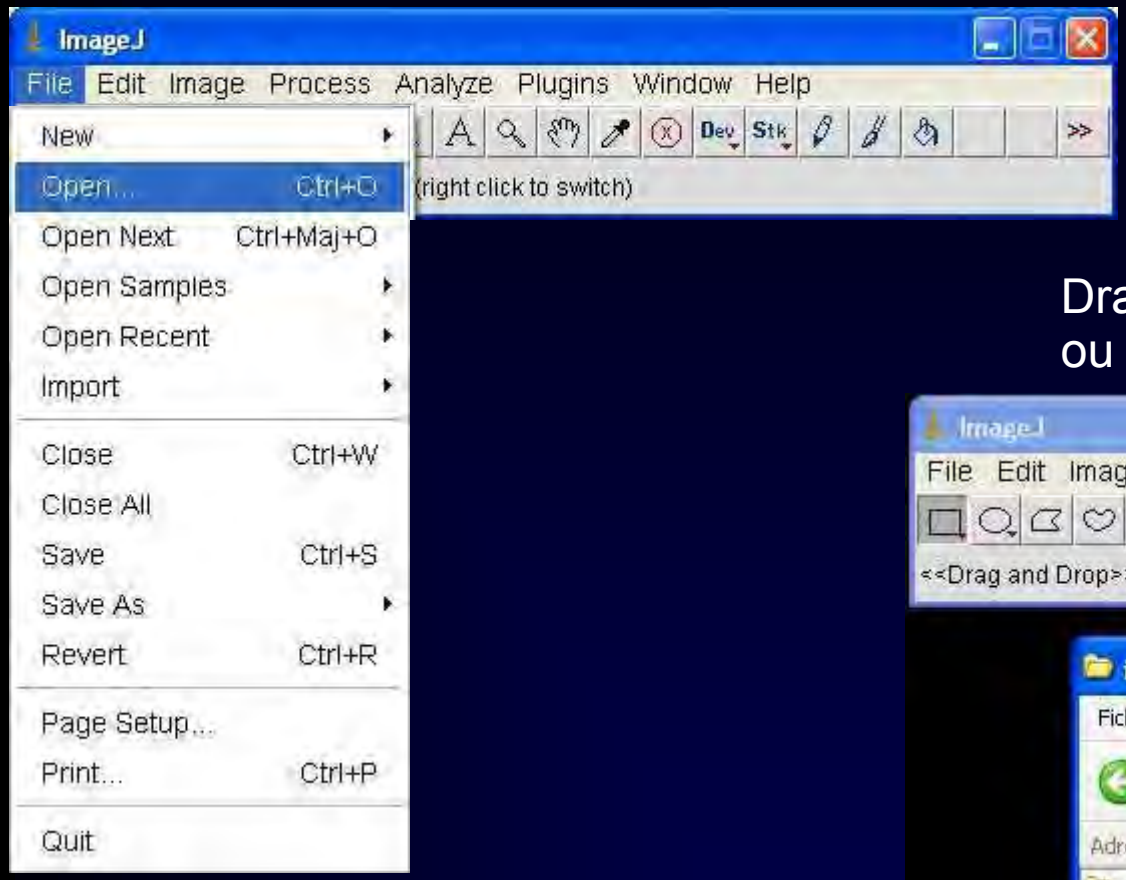
Barre de menu

Barre d'outils

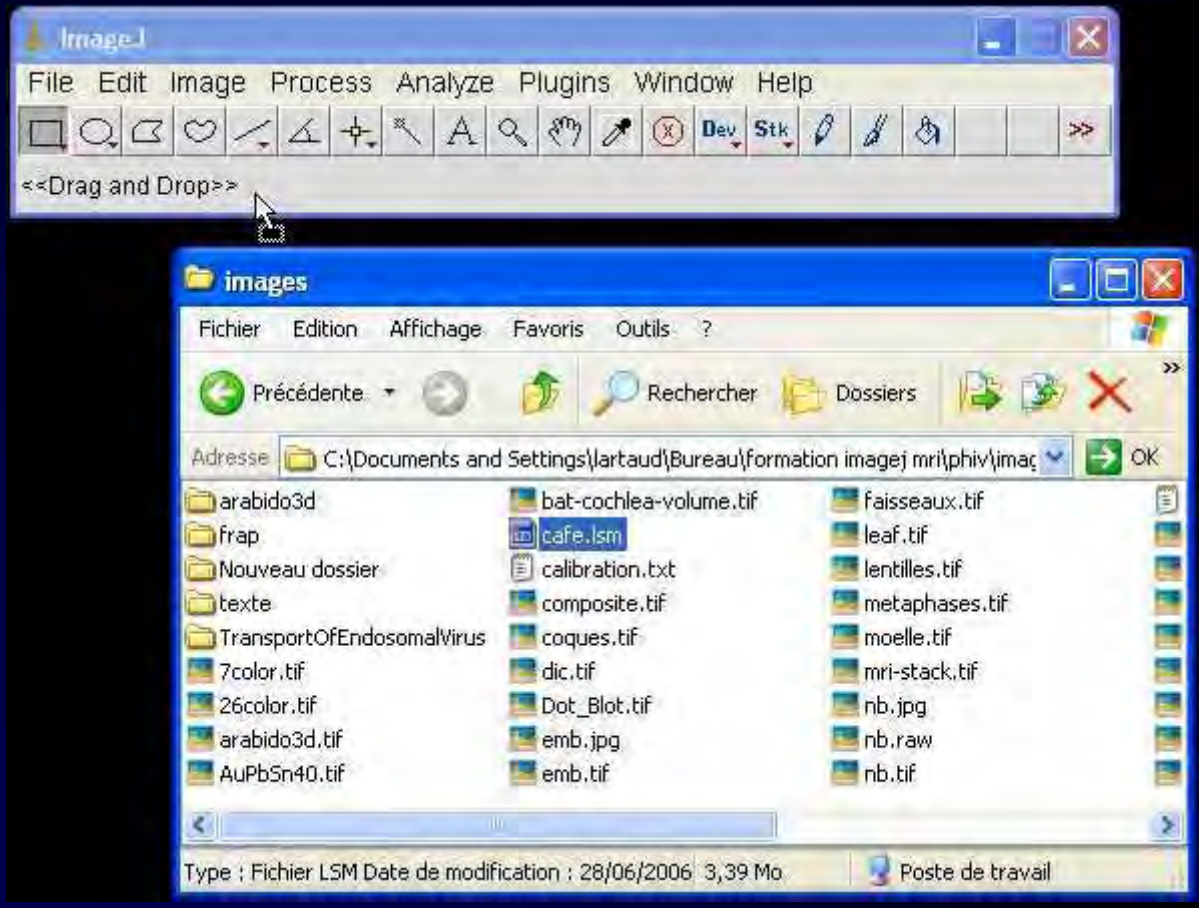
Barre d'état

Ouvrir une image

File → Open...



Drag and Drop sur la fenêtre
ou l'icône



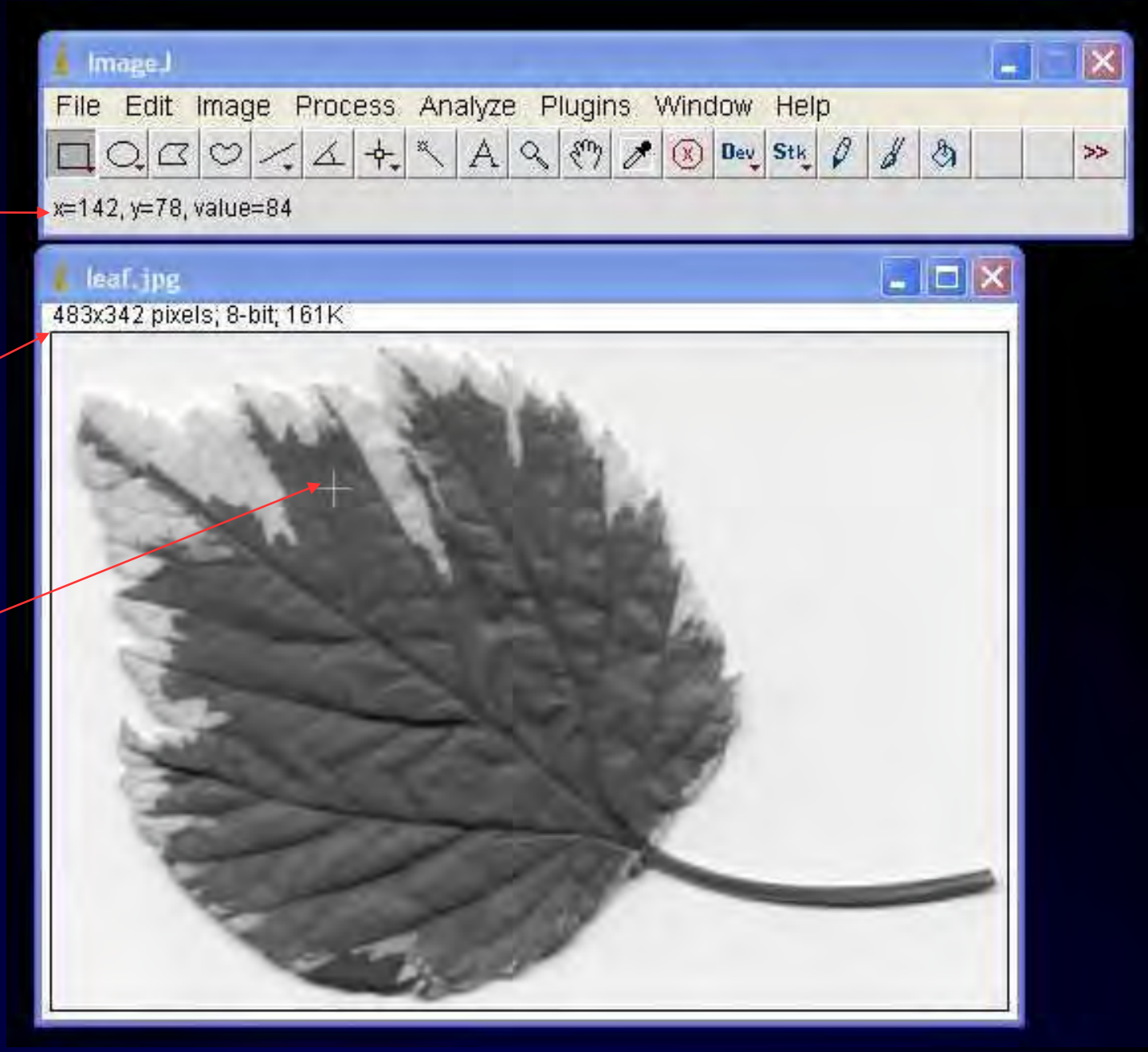
File → Open ...

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

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

Touche « - »

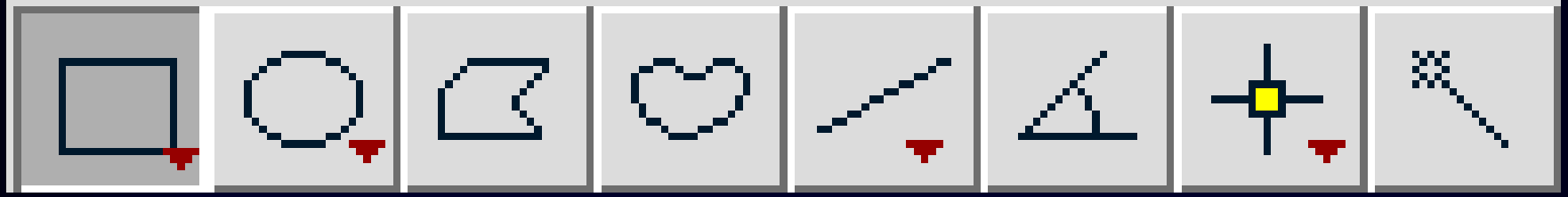
Touche « + »

Zone affichée

Rapport affichage/image

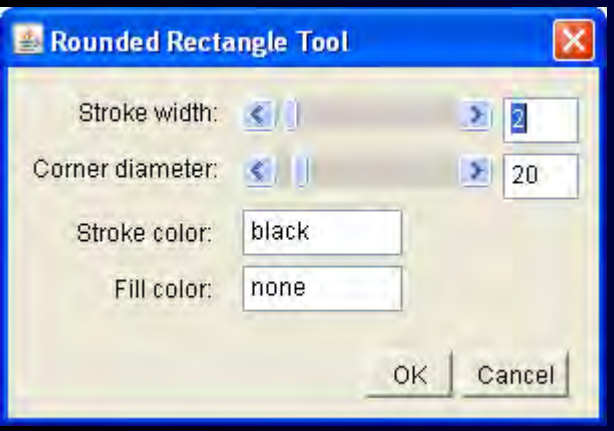
Outils de sélection (ROI)

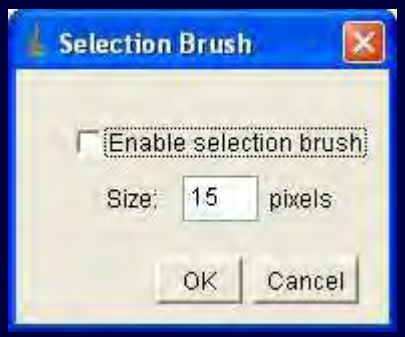
Rectangle | Ovale | Polygone | À main levée | Ligne | Angle | Point | Baguette



Choix d'outils différents : Clic droit sur triangle rouge

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

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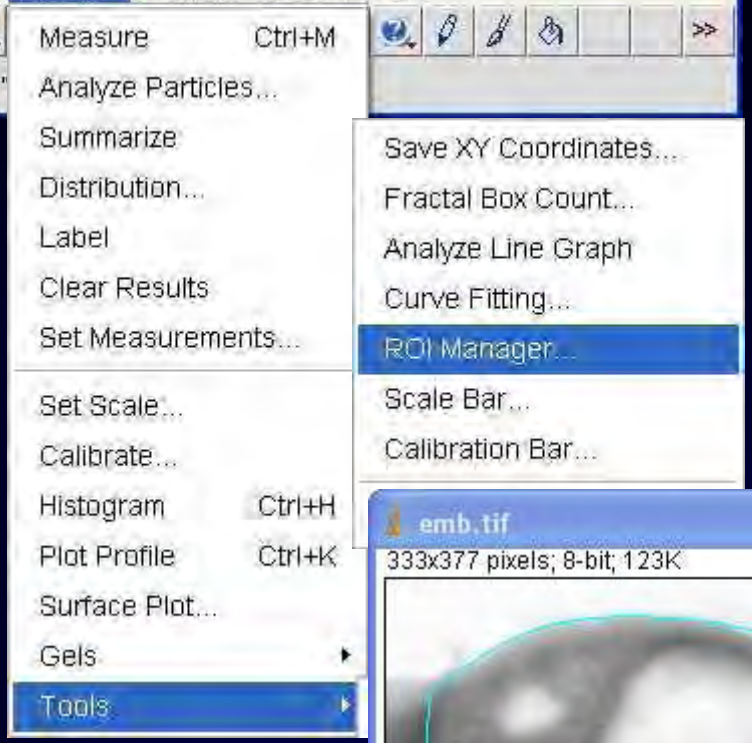
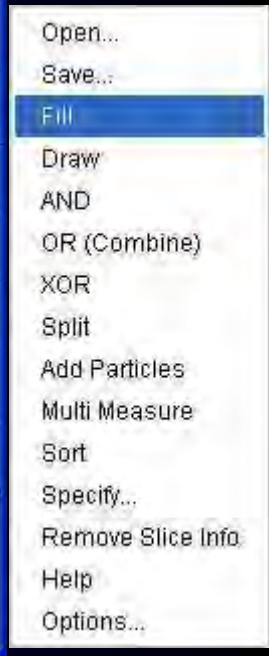
ROI Manager





Analyse → Tools → ROI Manager...

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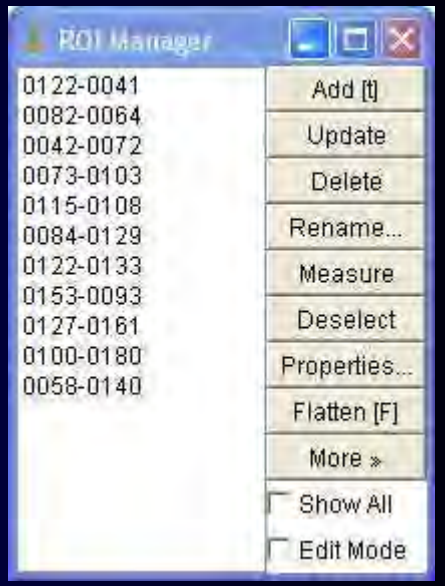
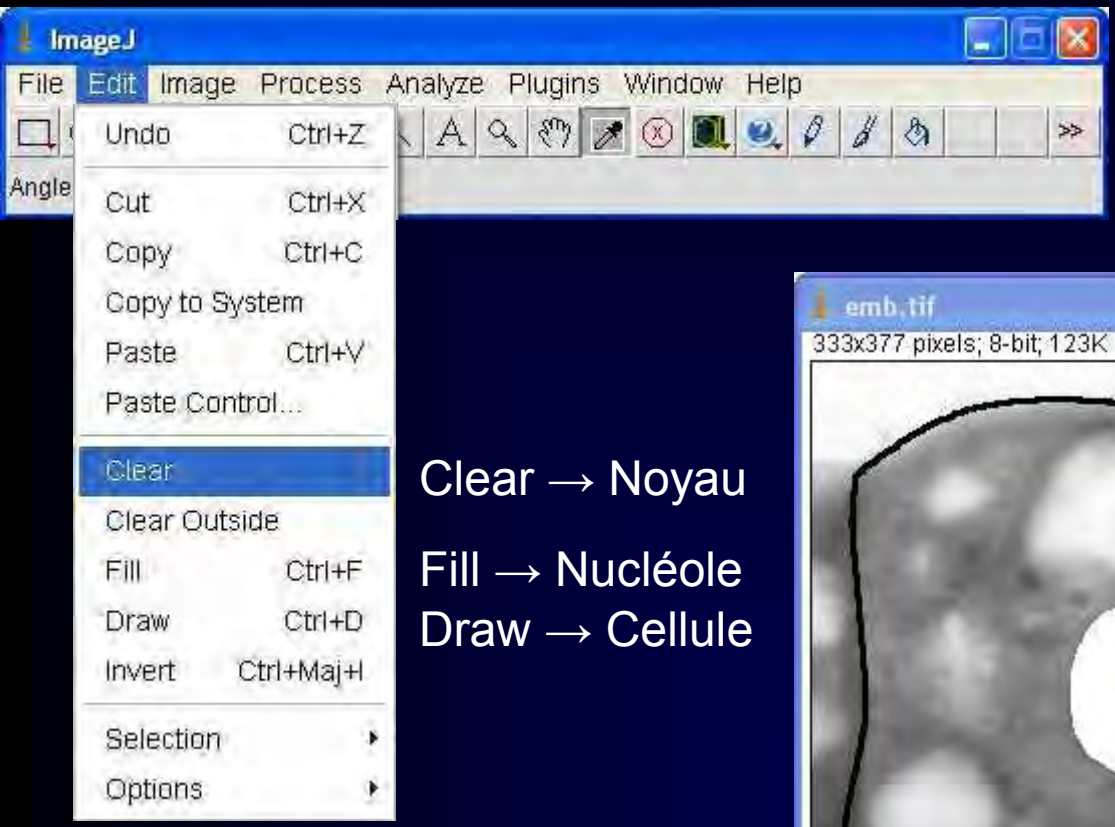
Outil Point pour le comptage



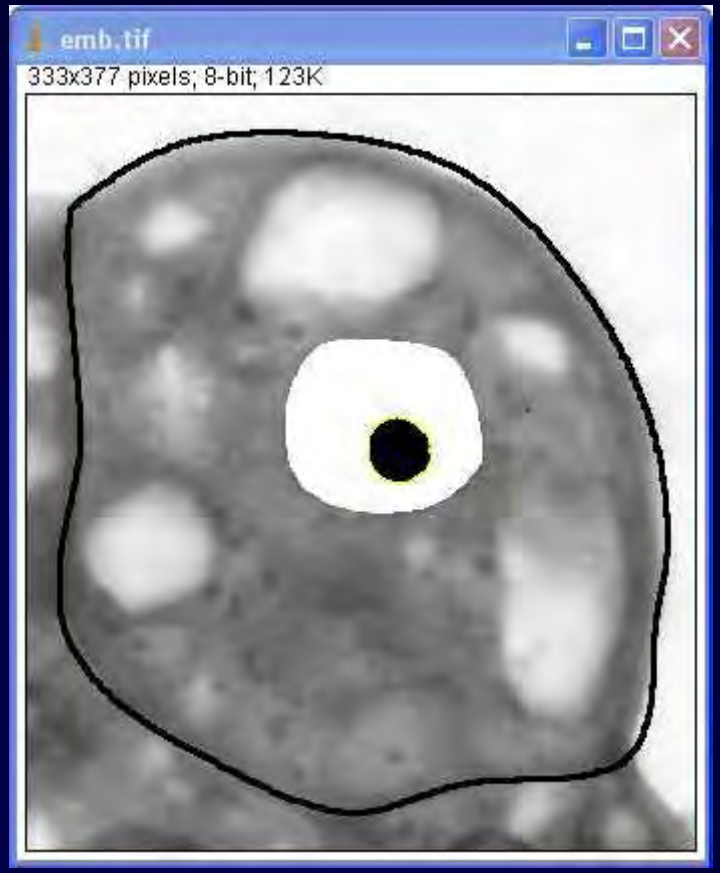
Dessin



Clear → Noyau

Fill → Nucléole

Draw → Cellule



Edit → Clear...

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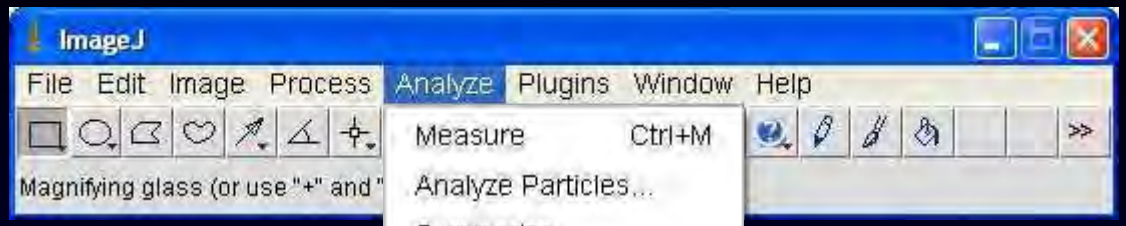
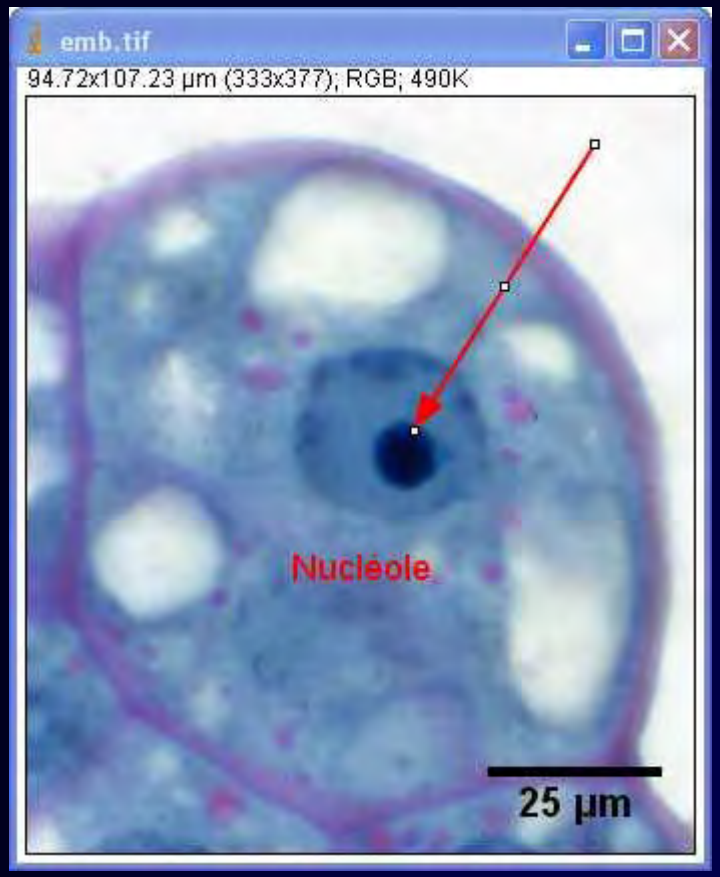
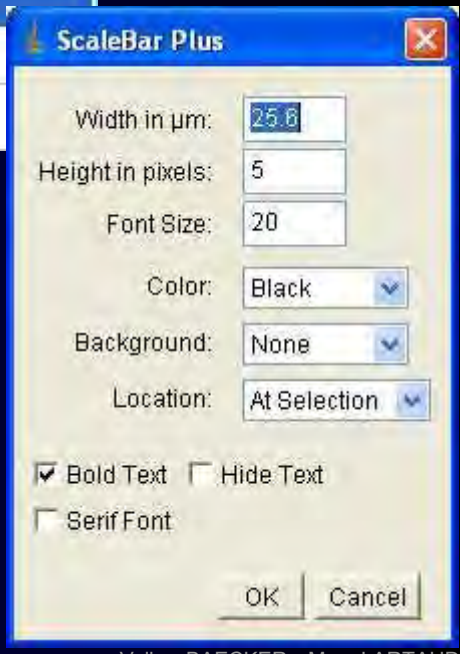
Annotations

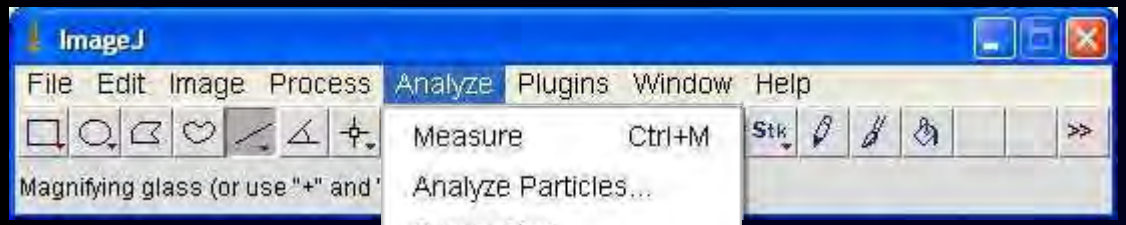


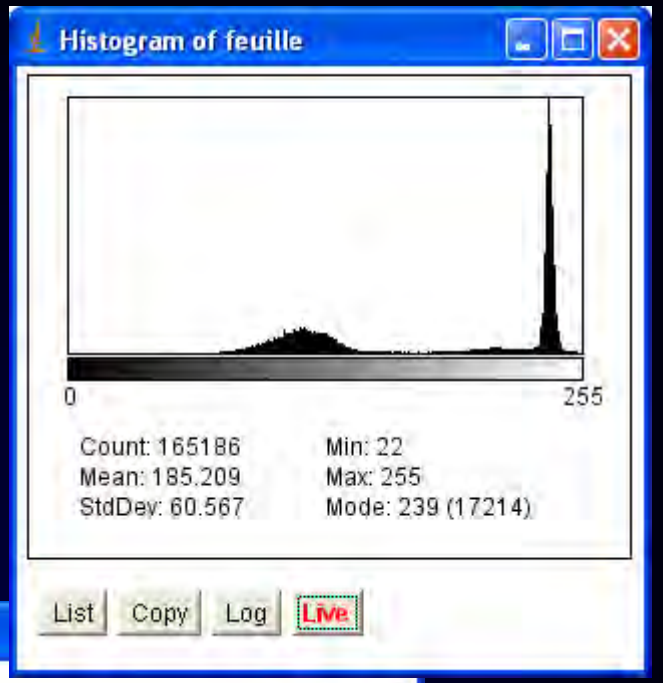




Analyse→Tools→Scale Bar...

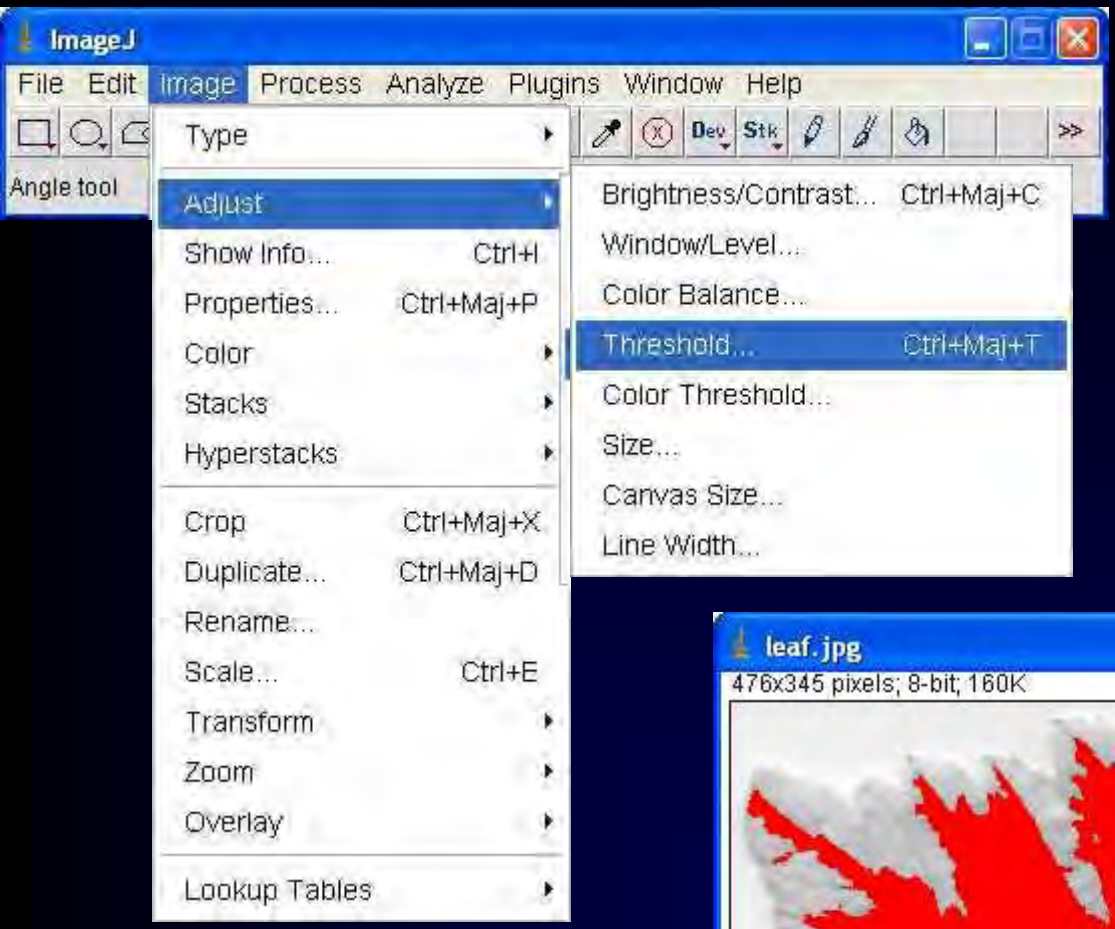
Histogramme

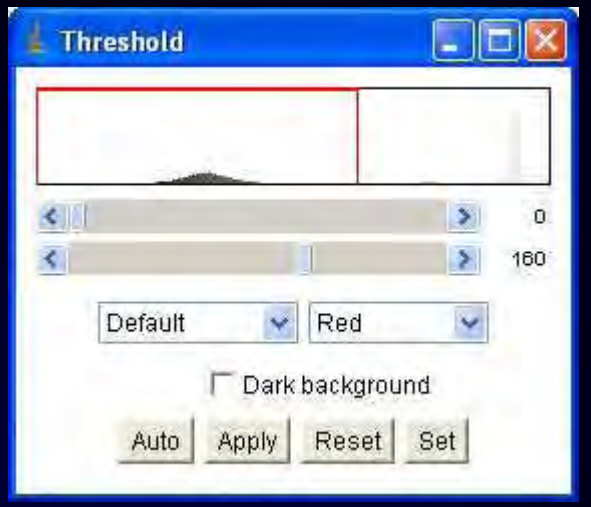


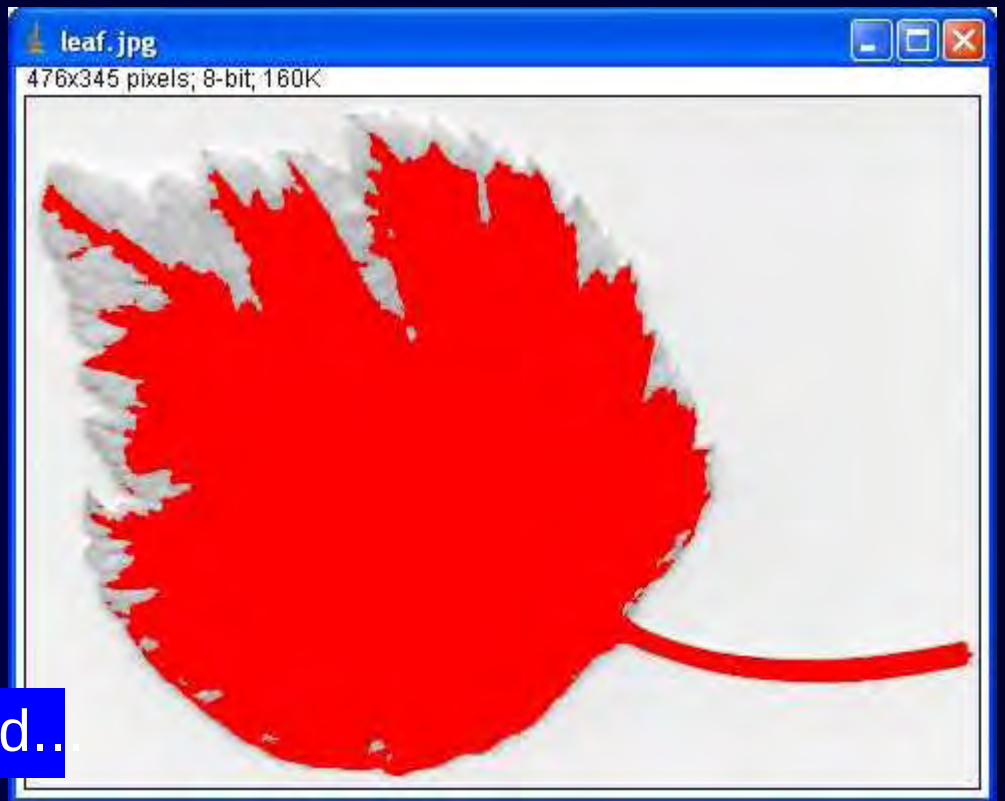


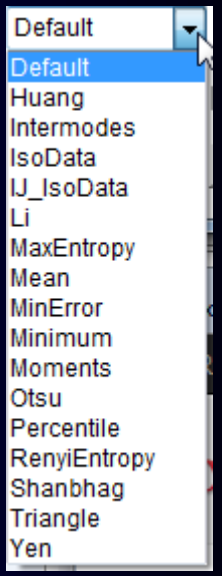
Analyse→Histogram

Seuillage



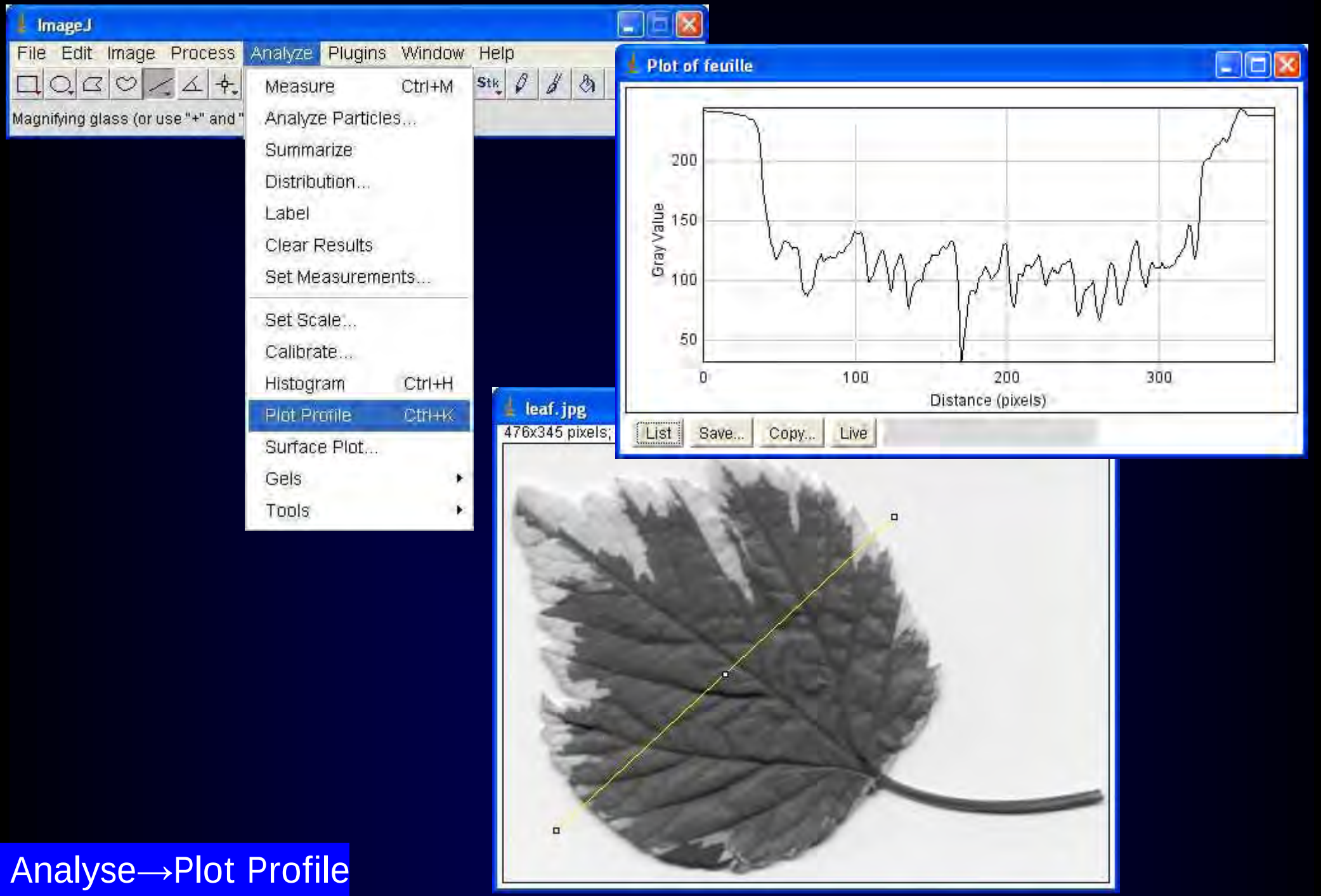






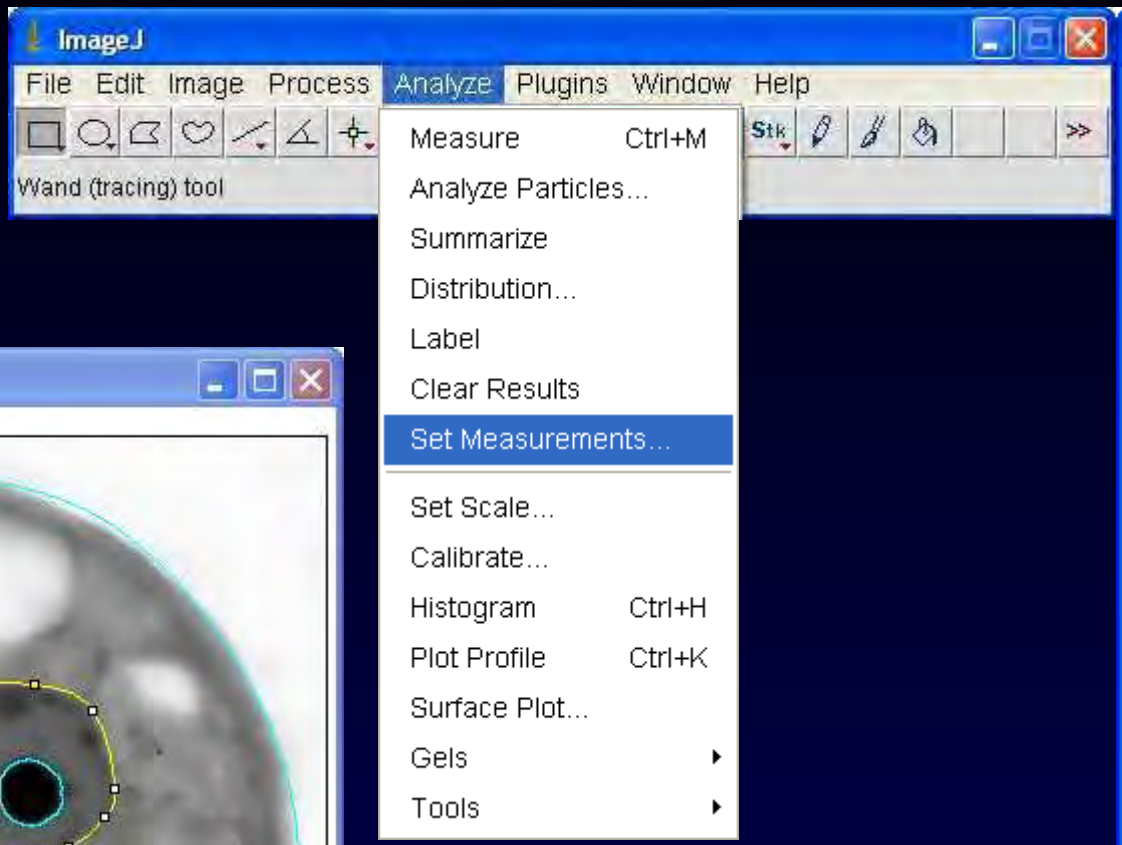
Image→Adjust→Threshold..

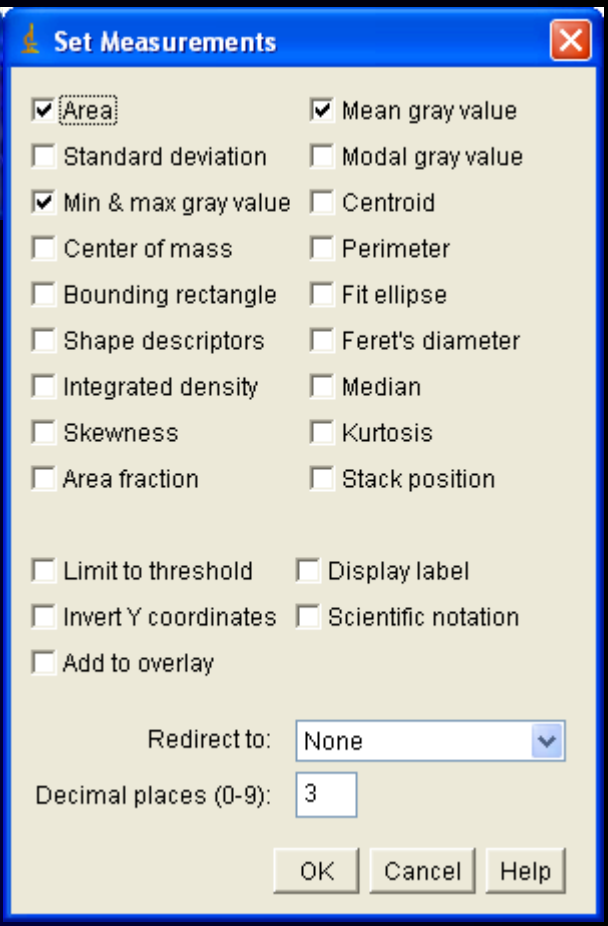
Plot Profile



Analyse→Plot Profile

Mesures de sélection





Analyse→Measure

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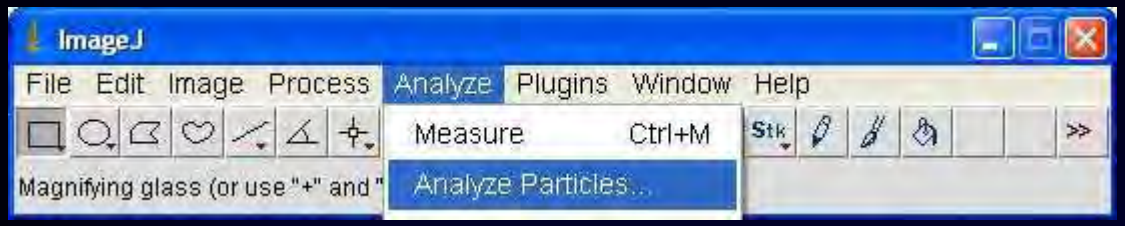
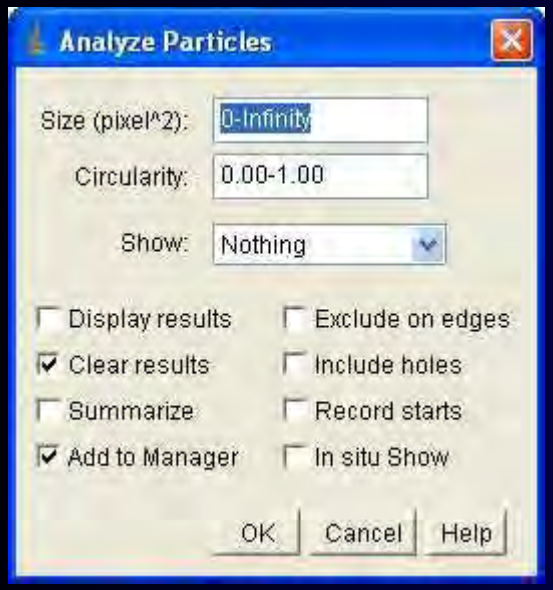
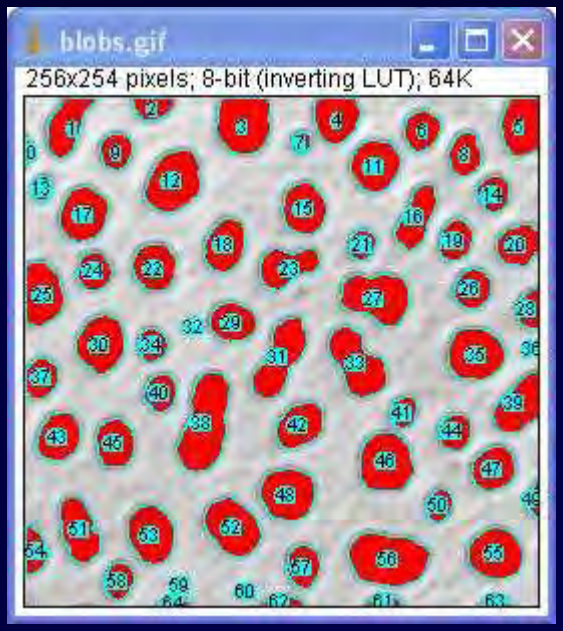
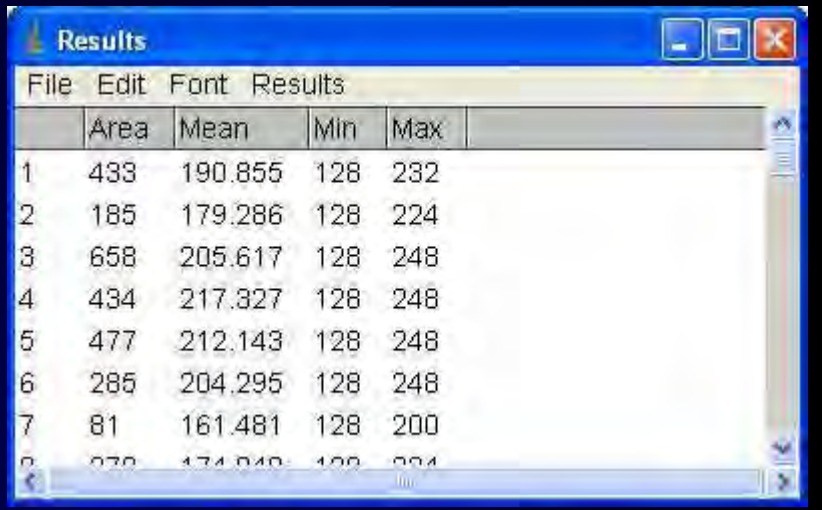
Mesures d'objets









Analyse→Analyse Particles...

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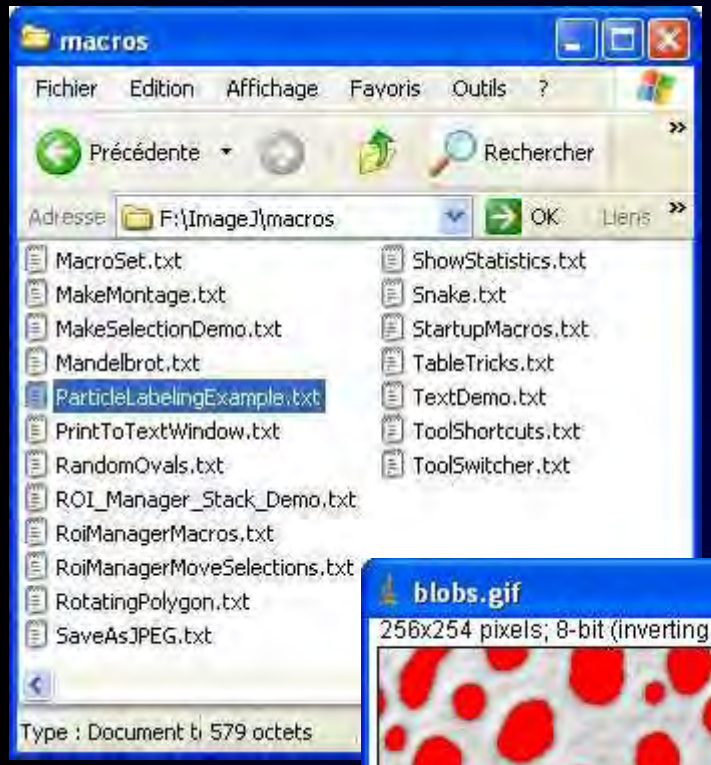
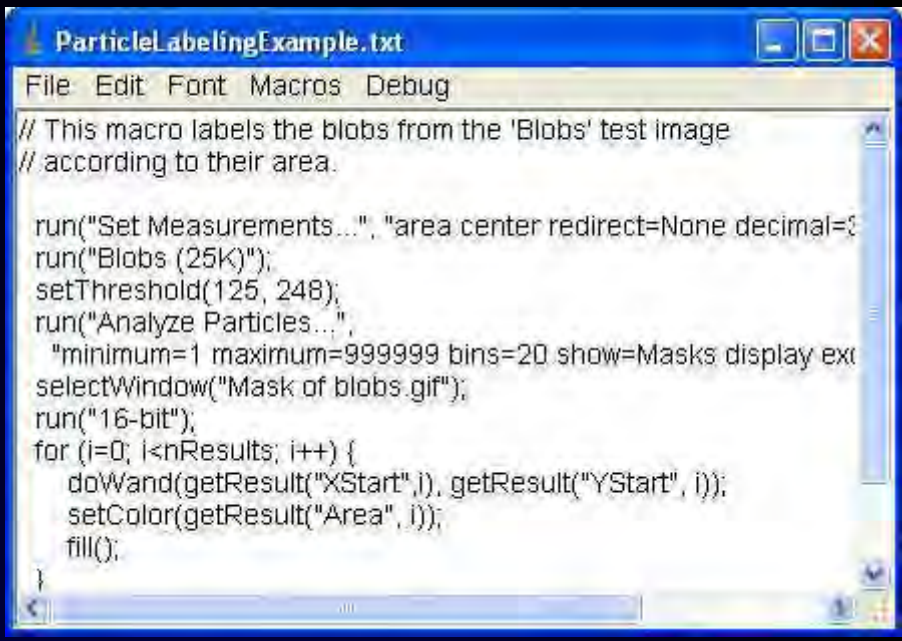
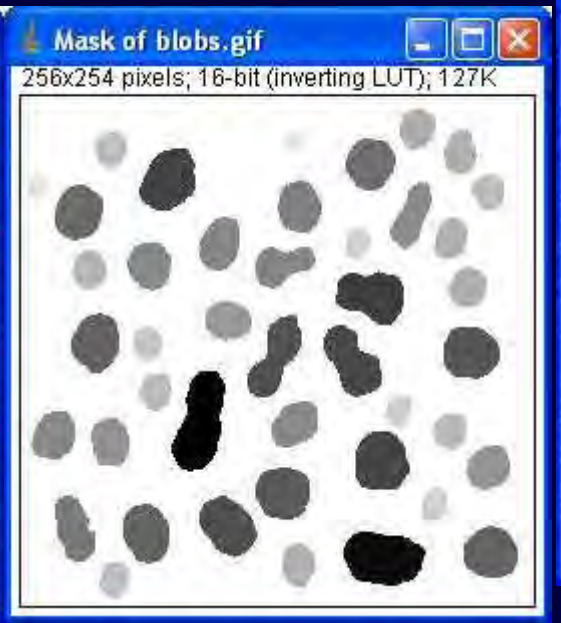
Macro

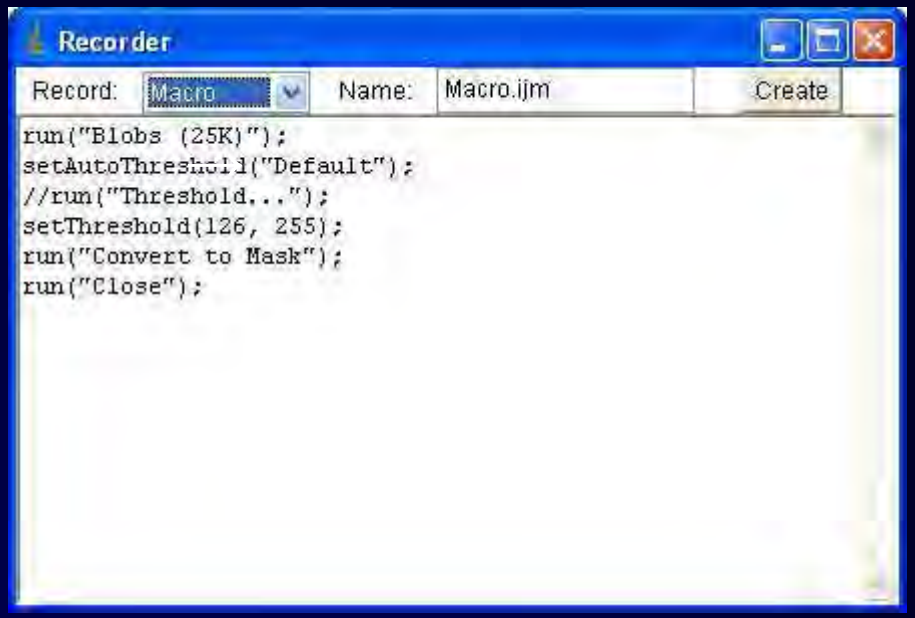






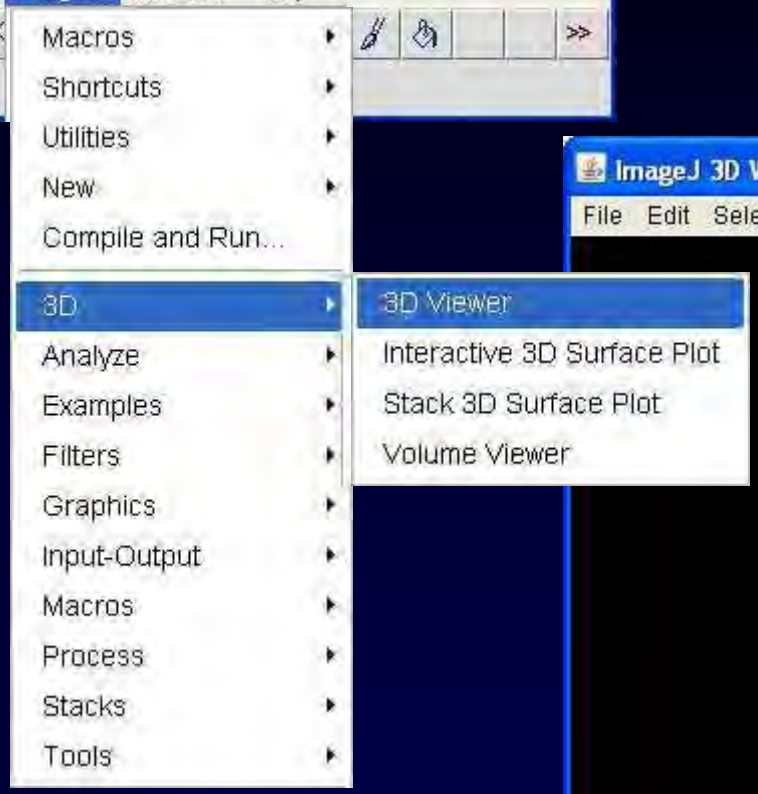
Plugins→Macros→Run

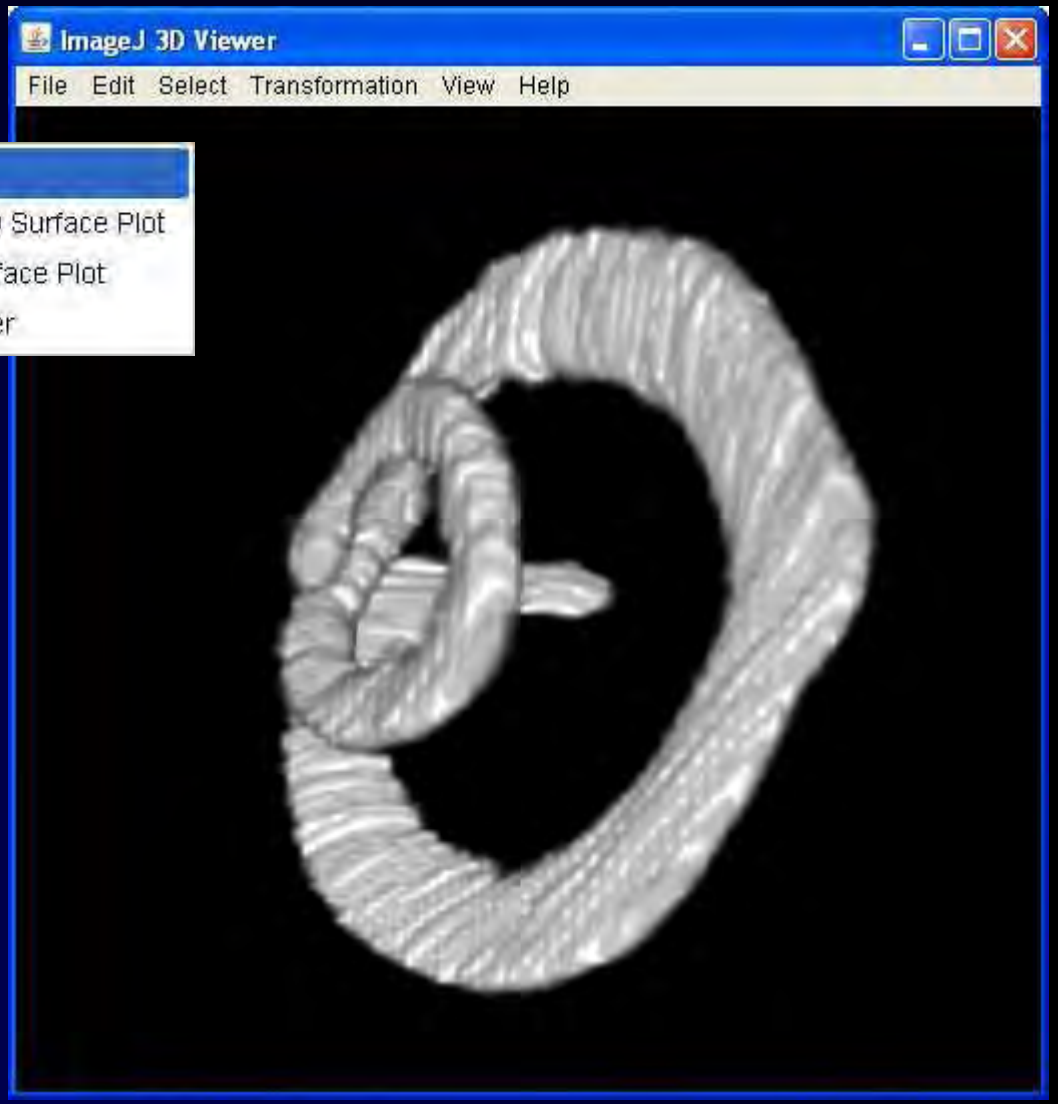
Enregistrement de macro



Plugins→Macros→Record...

Plugins

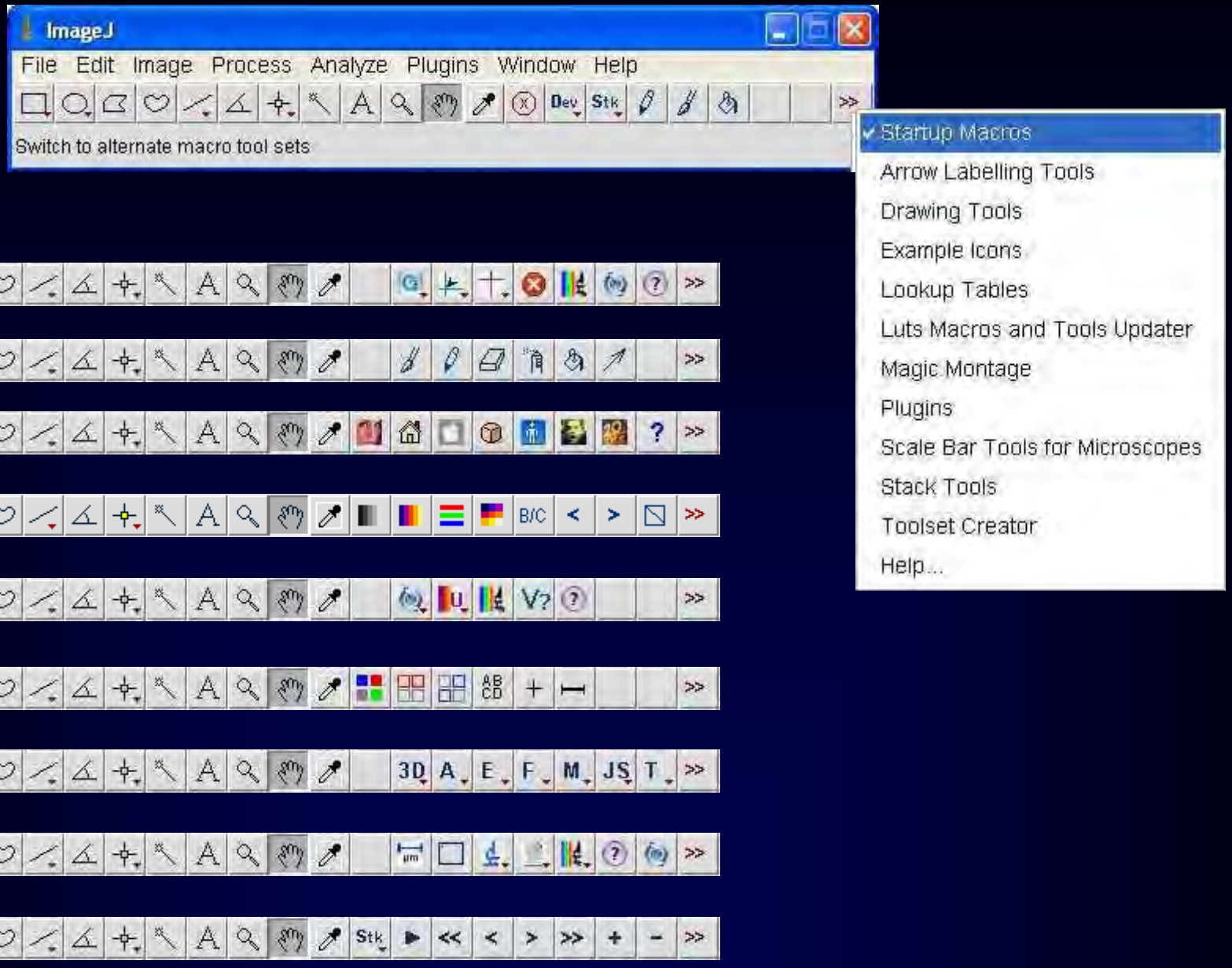




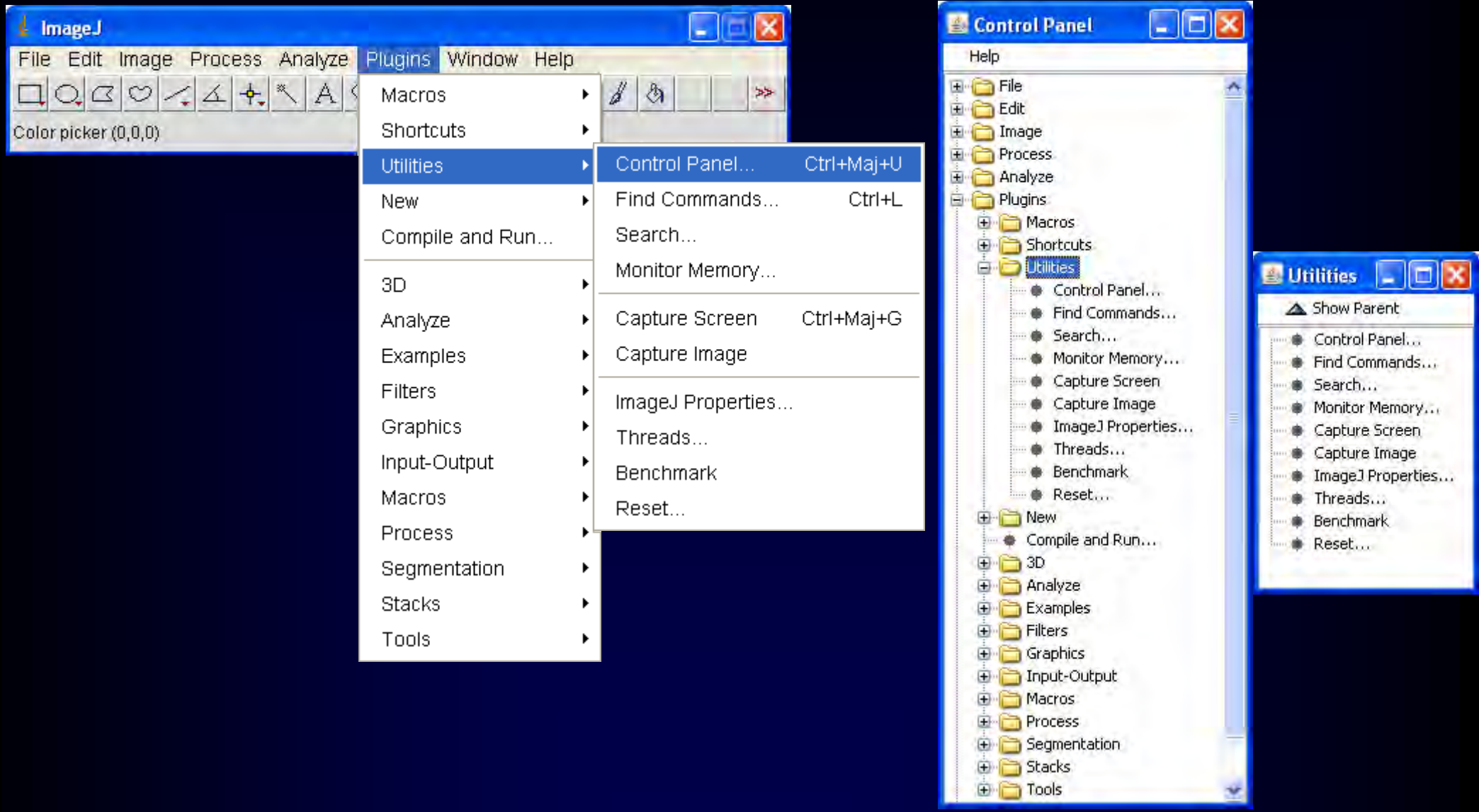
Stack

Plugins→3D→3D Viewer

Barres d'outils



Panneaux de commandes



Plugins→Utilities→Control Panel

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Topic 02 – Basic tools 1

Topic 03 – Basic tools 2

L’image numérique

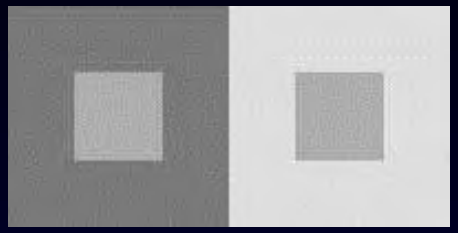
Les Prétraitements

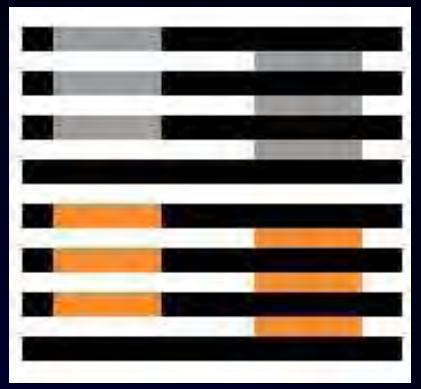
La Segmentation

Les Post-traitements

La Quantification

Image et perception





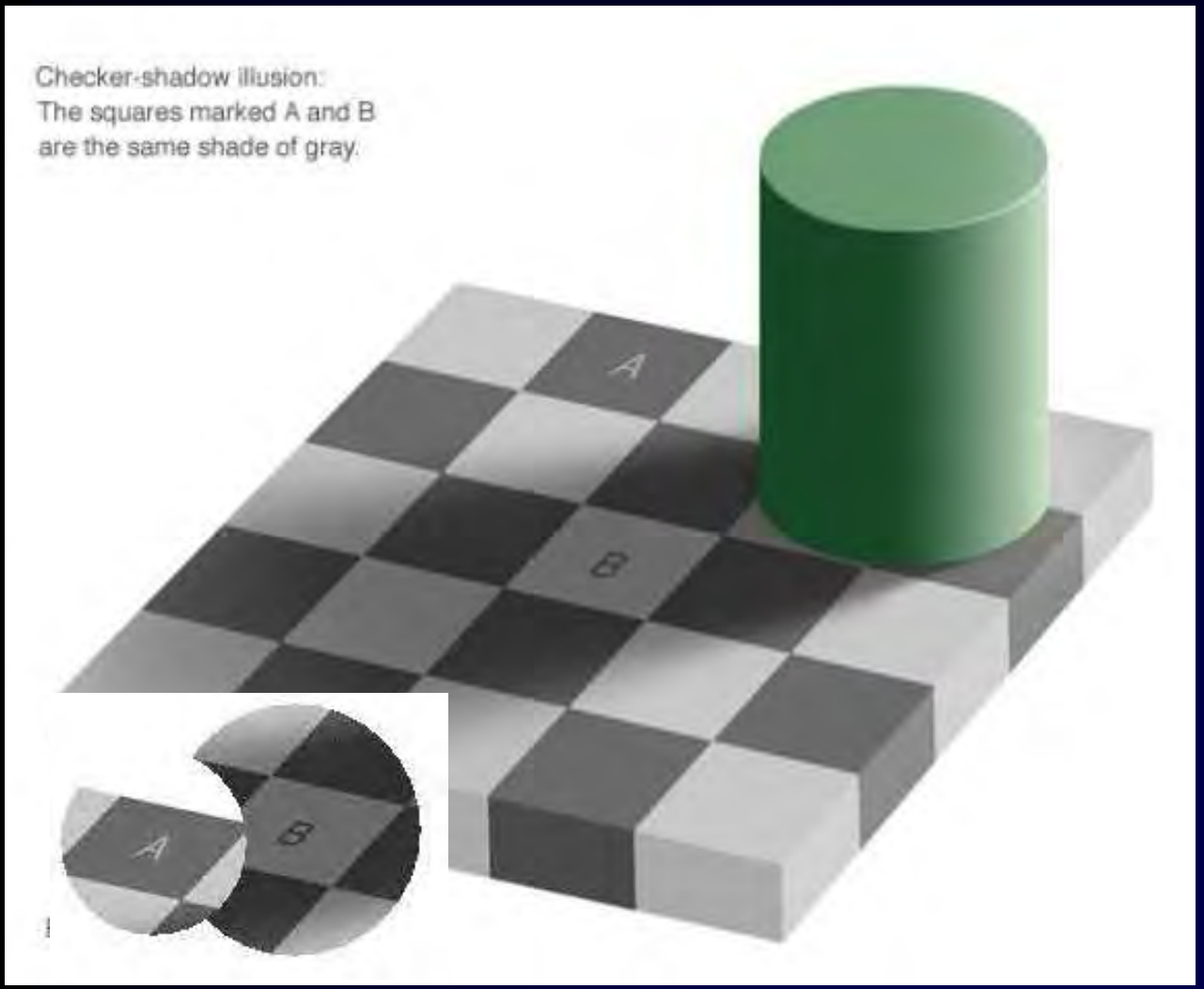
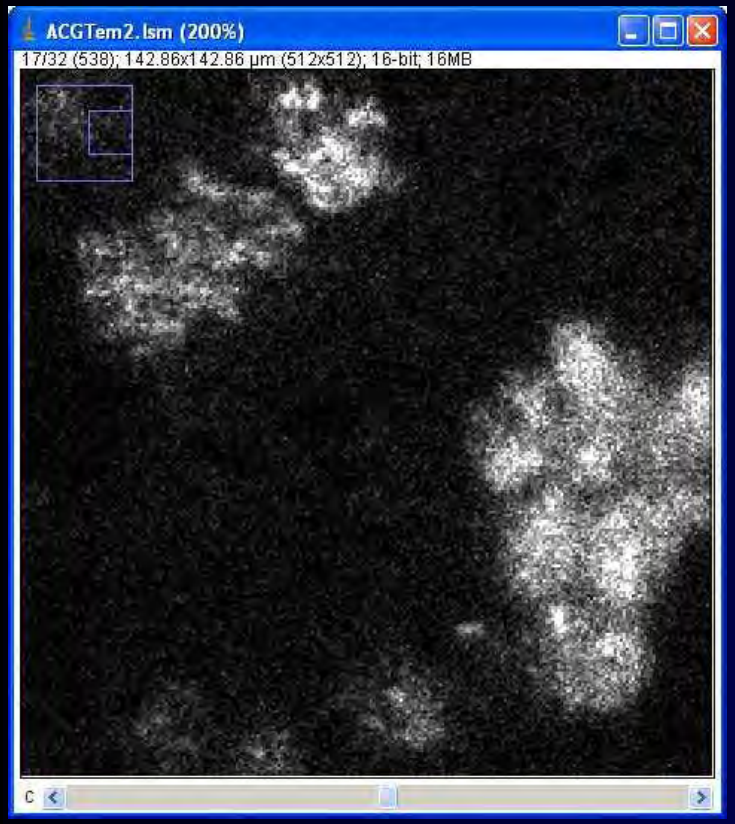
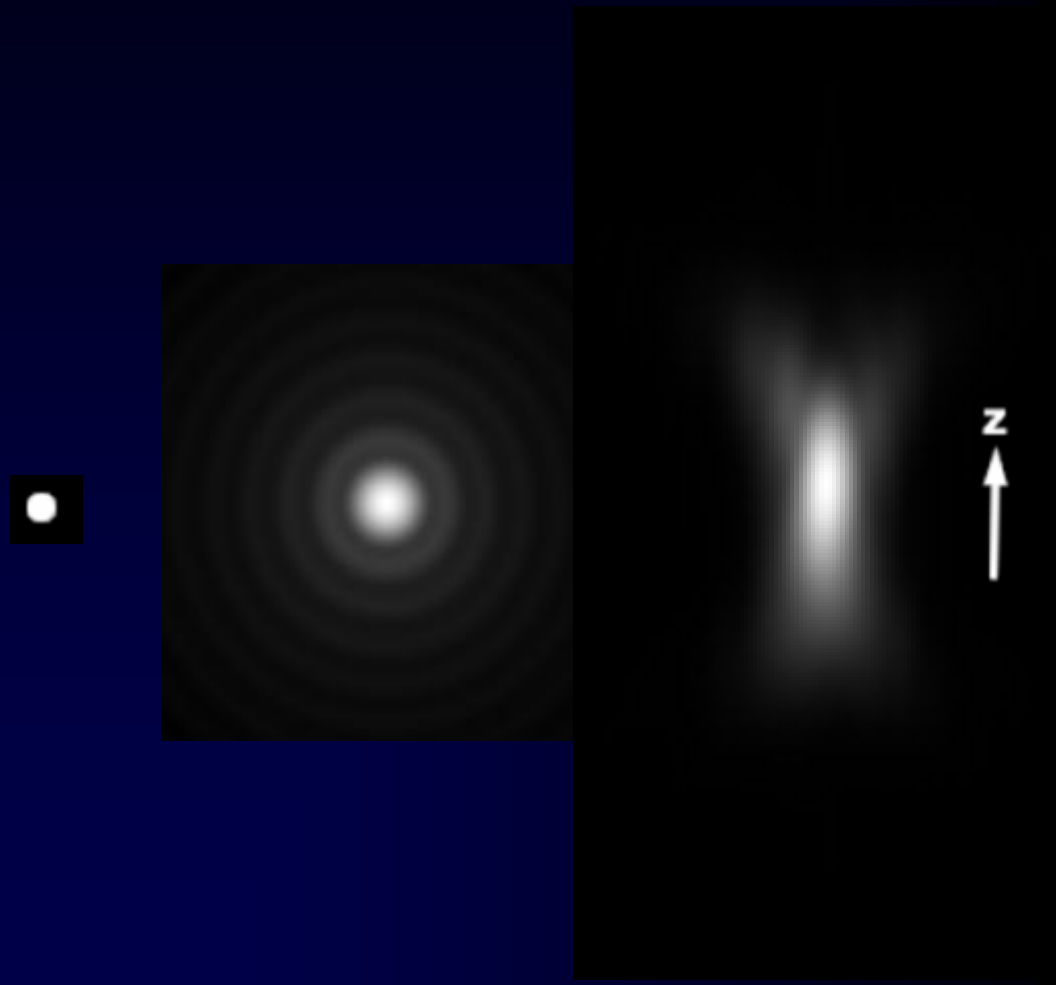


Image et déformations

Bruit



PSF

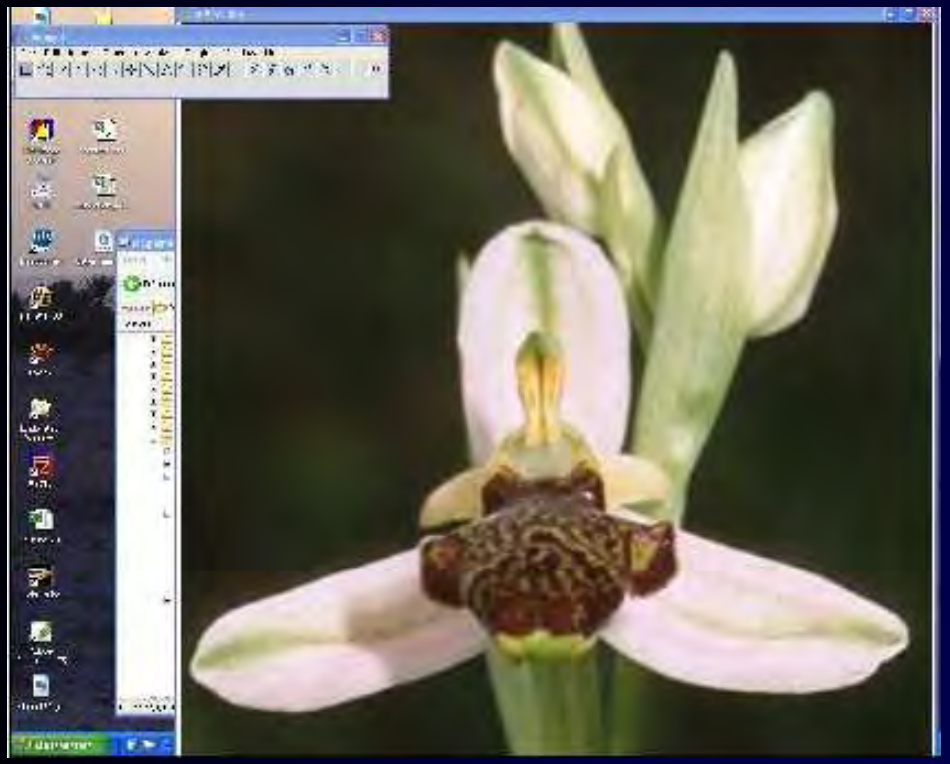


Résolutions : Nombre de pixels par pouce à l'affichage

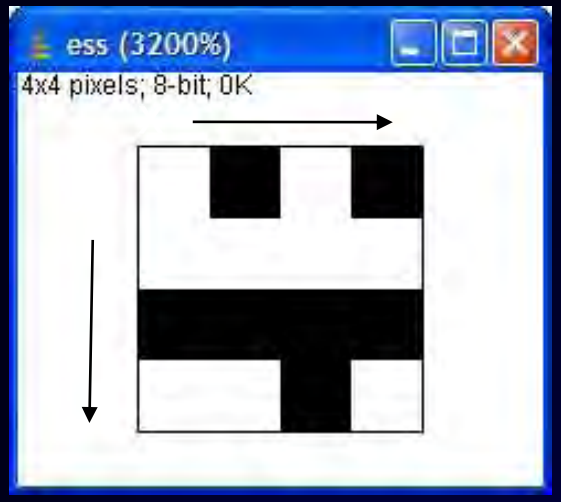
1024*1024
1 Mega pixels

Impression 300 dpi

Ecran 72 dpi

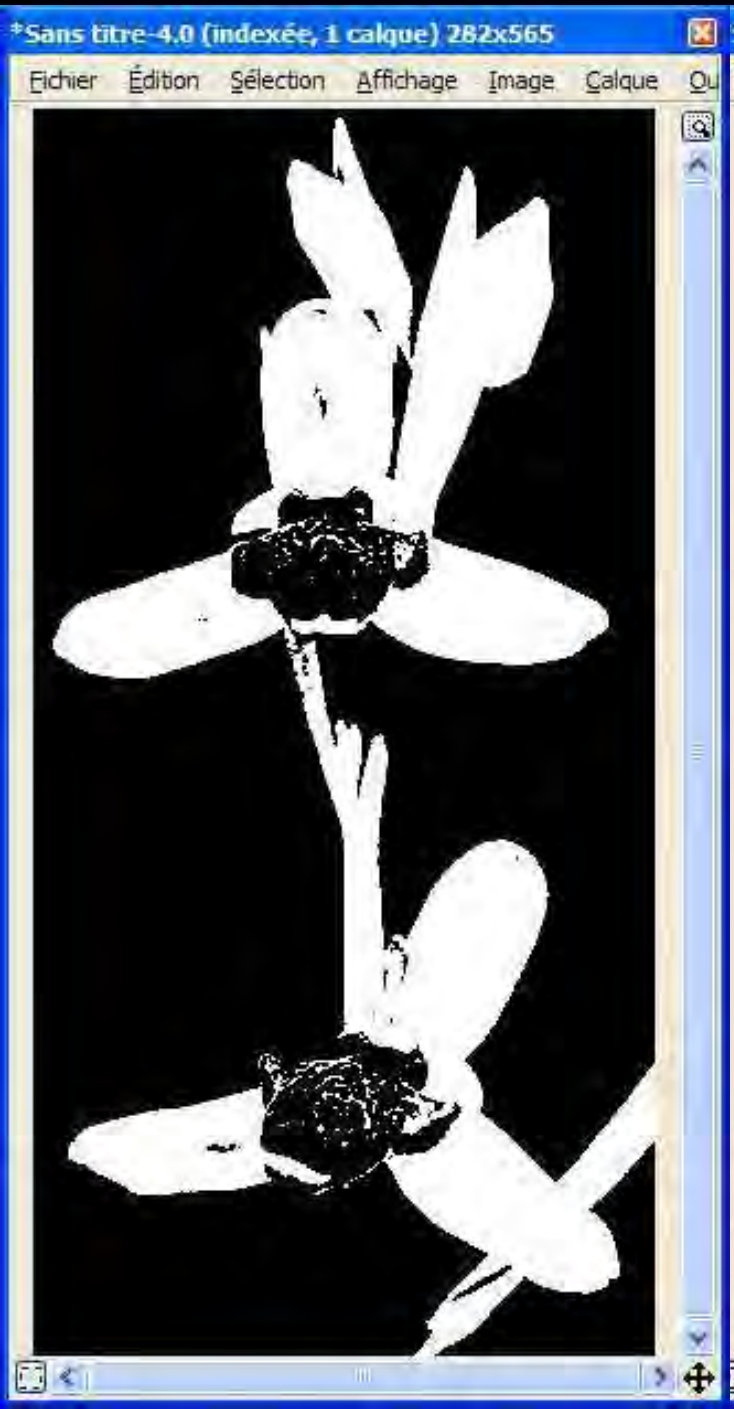


Codage binaire



Codage

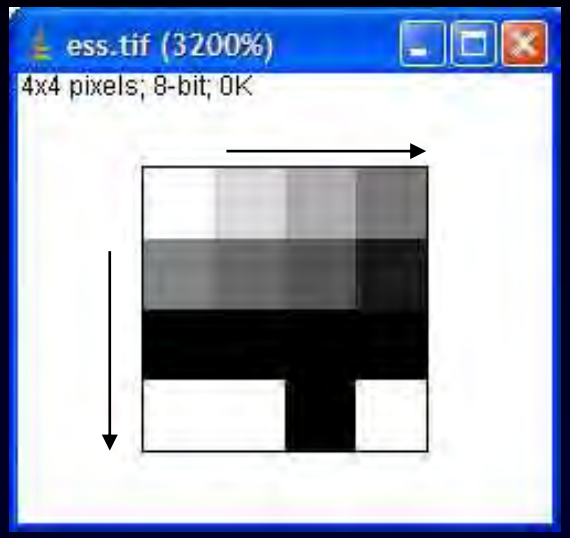
1bit→2 valeurs

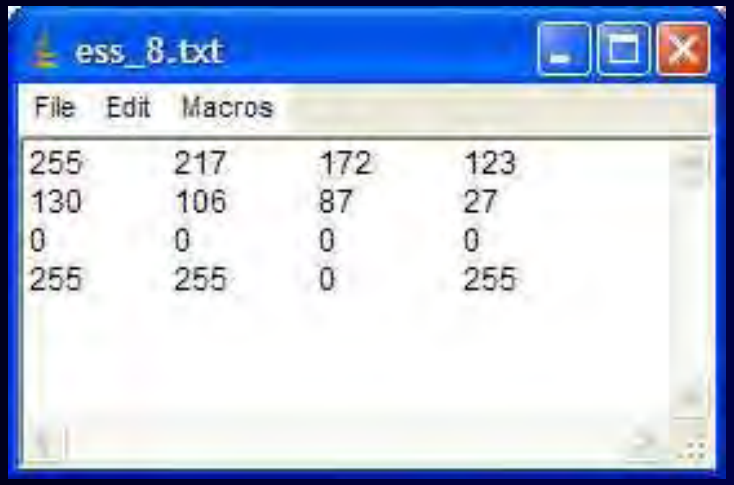


3bits→8 valeurs

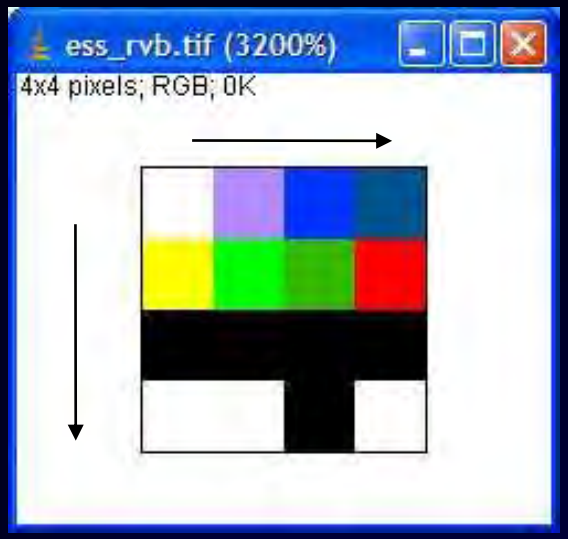
5bits→32 valeurs

Codage 8 bits 0→255 Valeurs de Niveaux de gris





Codage couleur 24 bits RVB

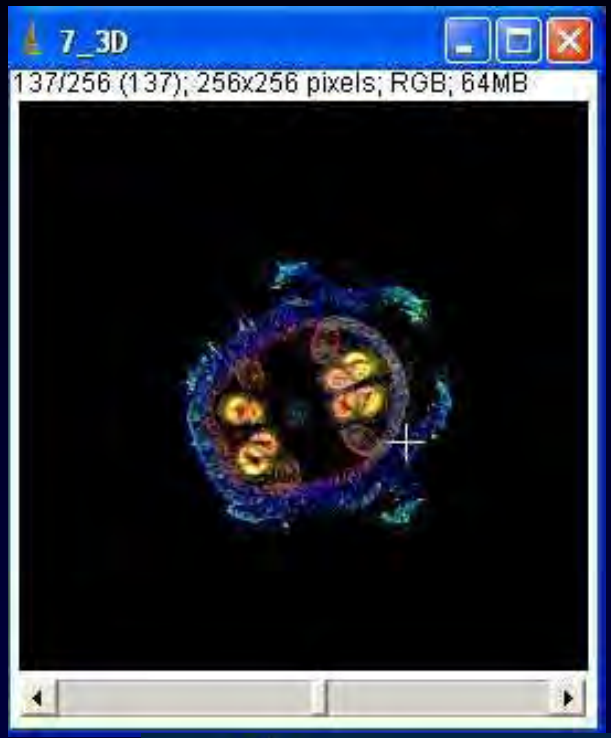


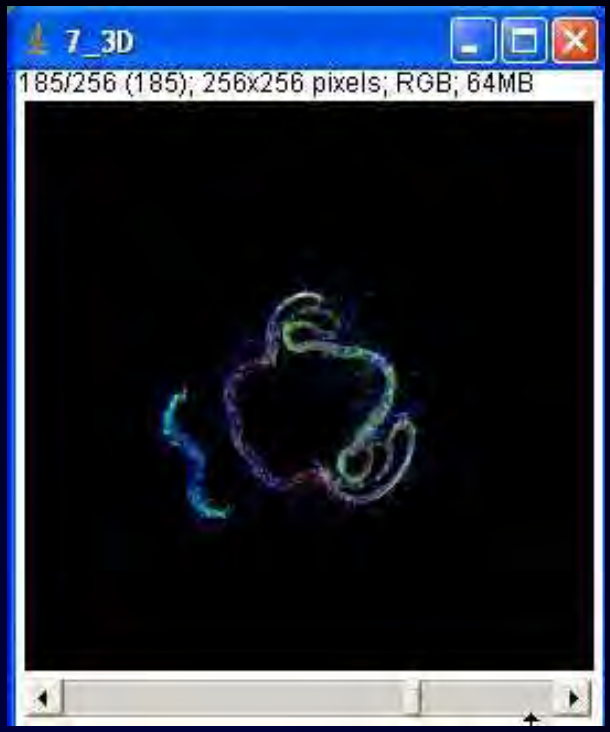
ess_rvb.txt

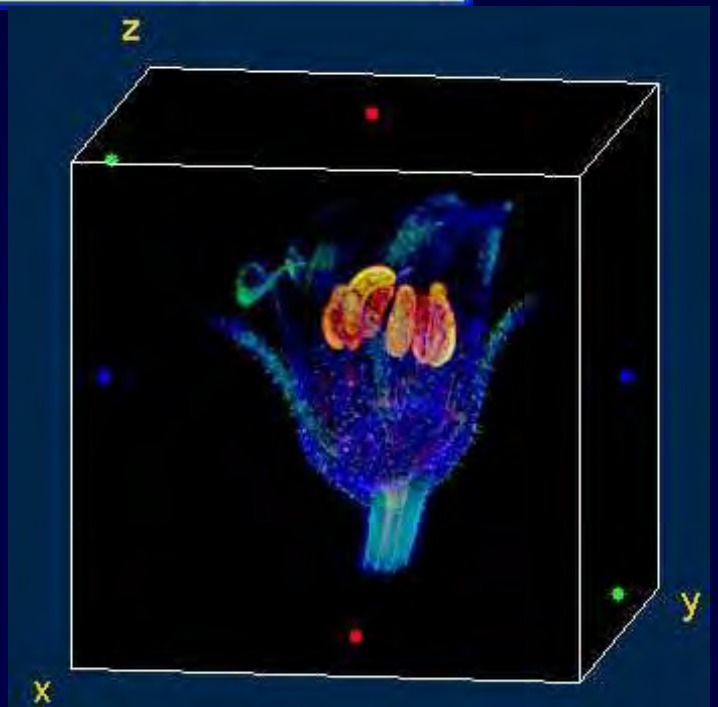
File Edit Macros

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







Format d'image

8bit [0,255]
16bit [0,65 535],
32bit [-3.4*10^38 , +3.4*10^38]
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

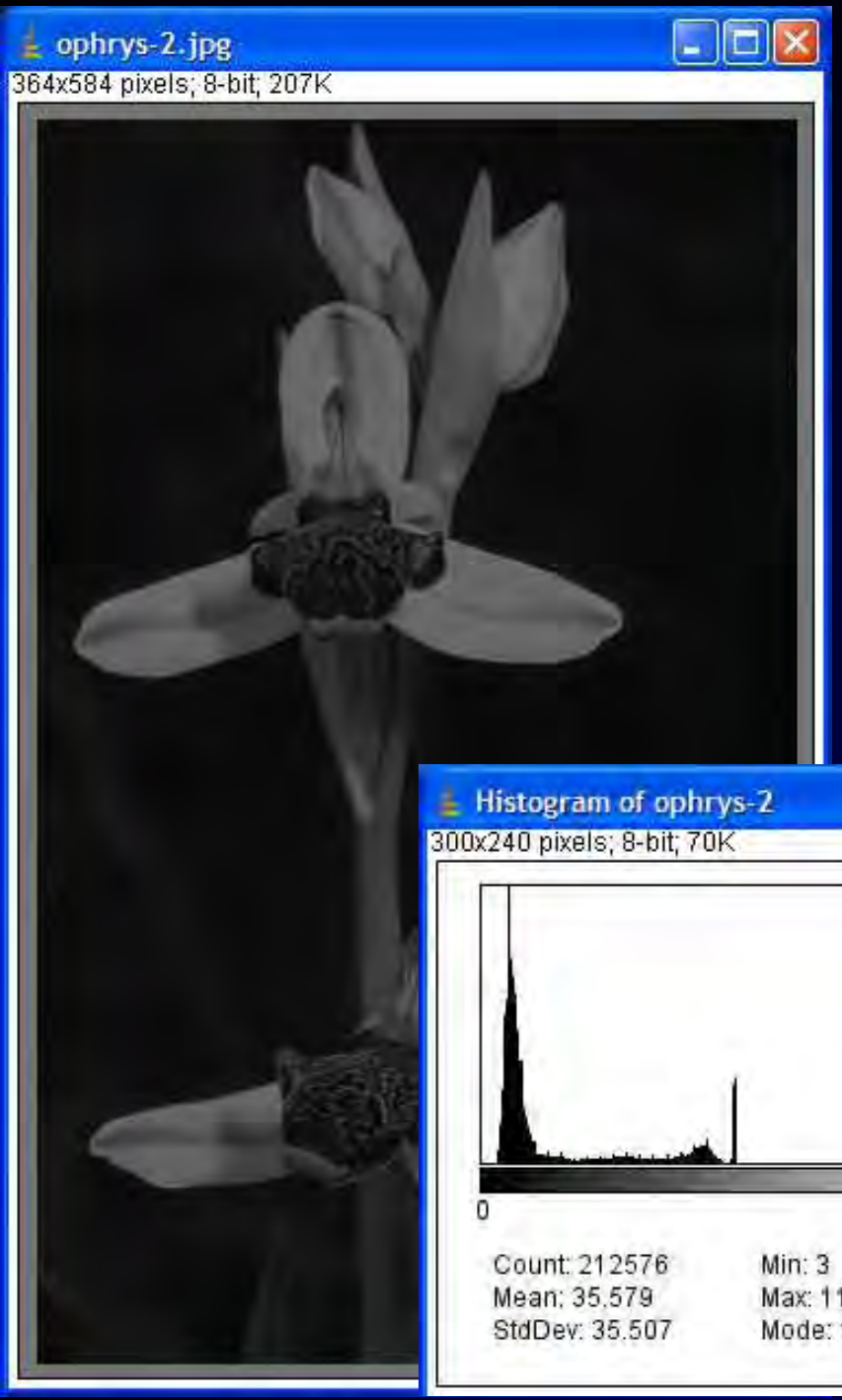
La Segmentation

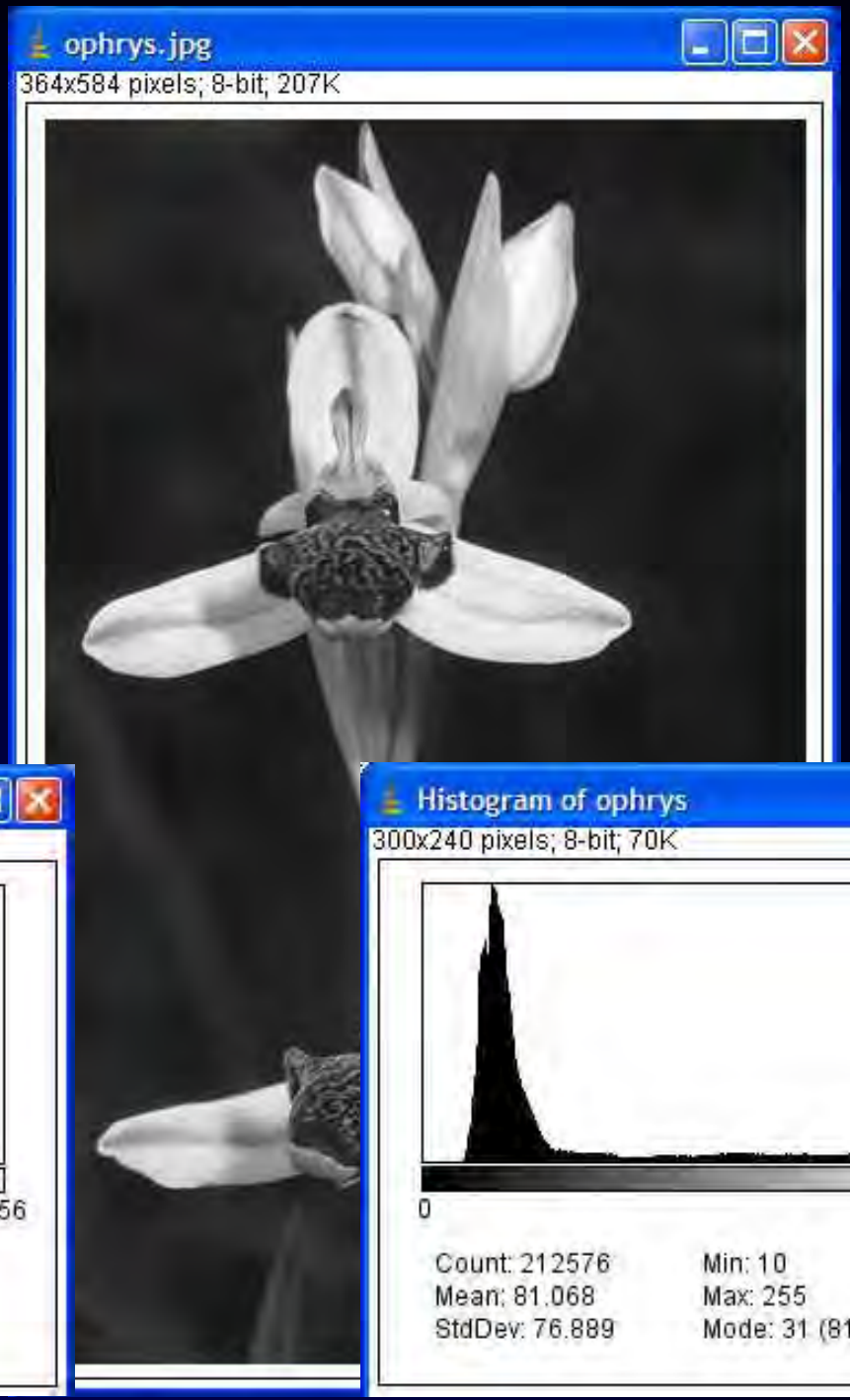
Les Post-traitements

La Quantification

Histogramme

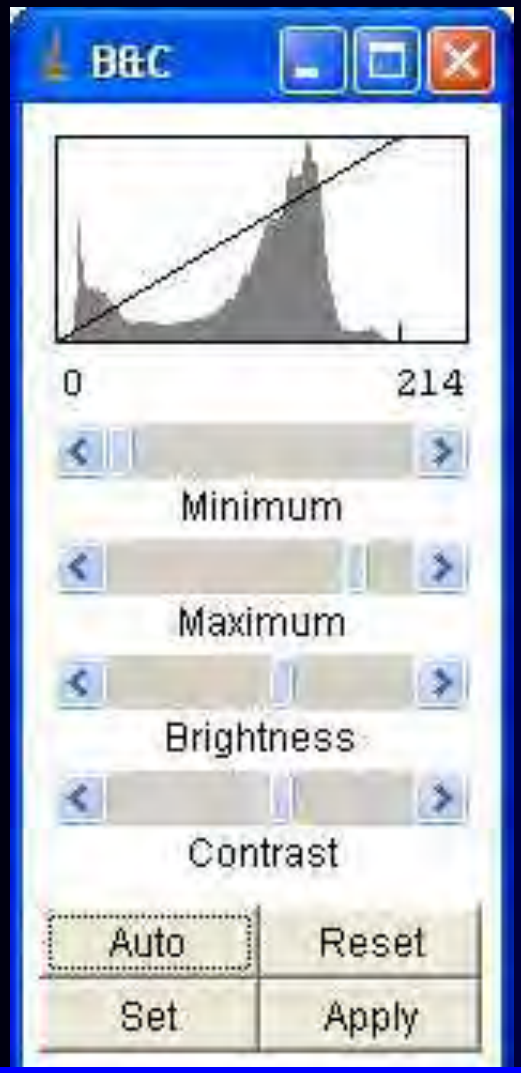
Dynamique = [valeur_mini , valeur_maxi]





Corrections linéaires

Luminosité Contraste



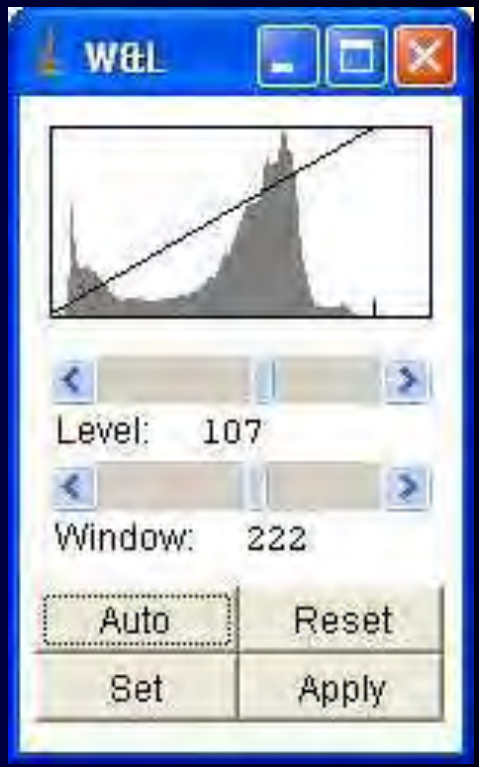
Mini

Maxi

Luminosité

Contraste

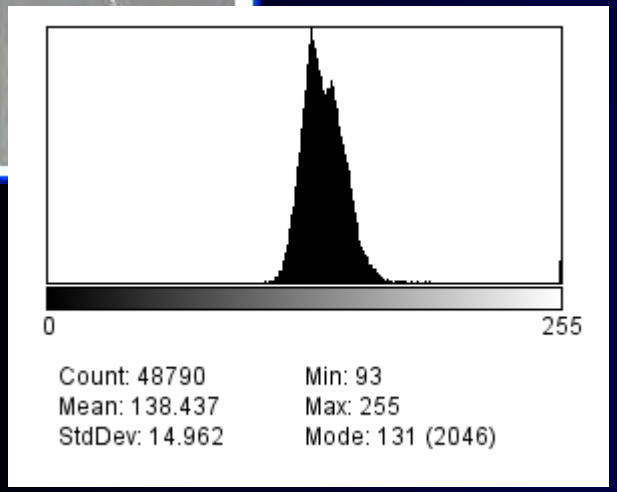
Niveaux

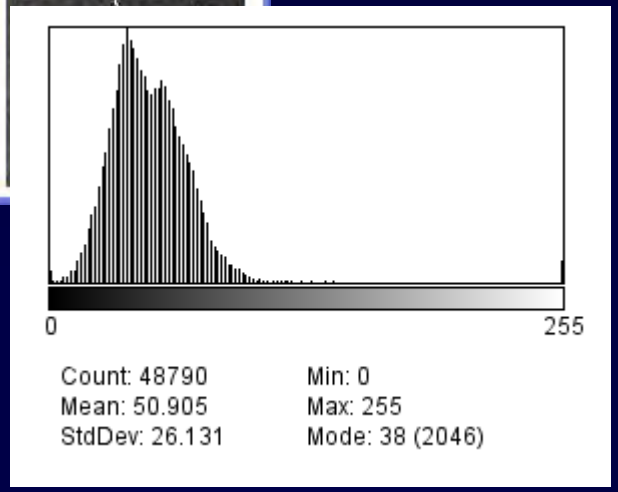


Image→Adjust→Brightness/Contrast...

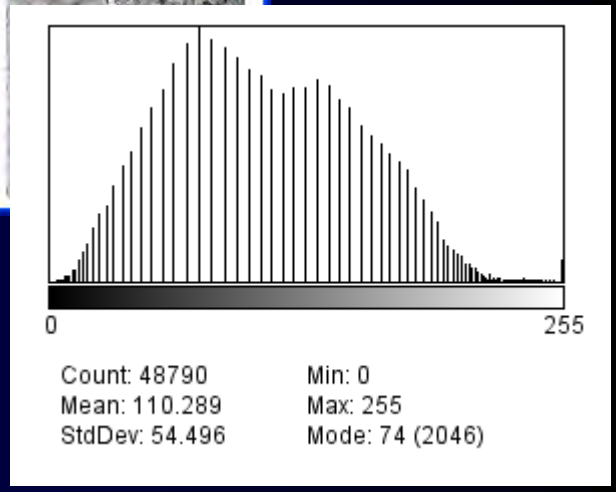
Correction non linéaire : Egalisation de l'histogramme

Densité de probabilité normalisée pour aplatir l'histogramme





Normalisation



Egalisation

Process→Enhance Contrast

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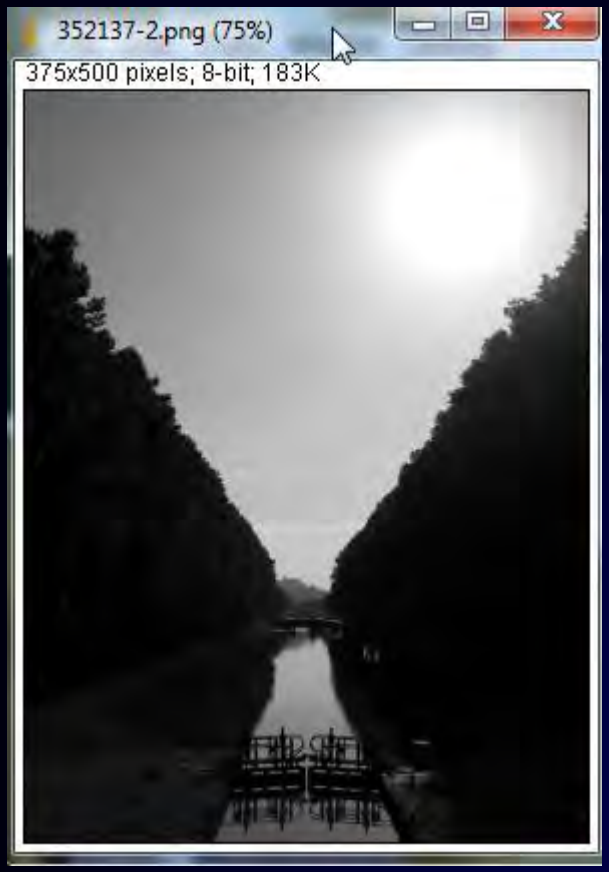
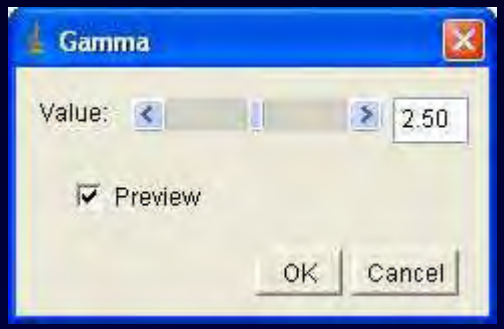
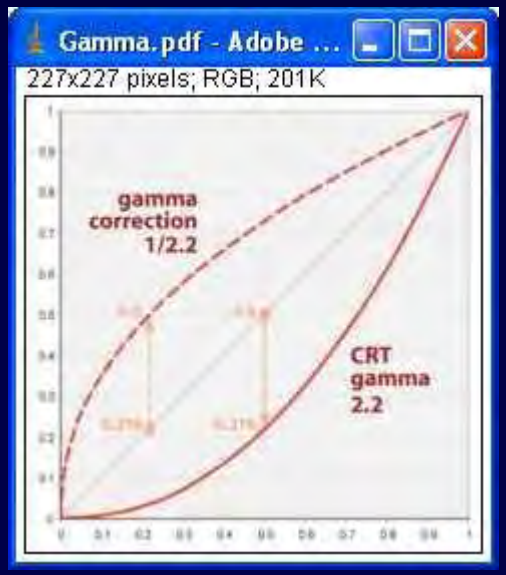
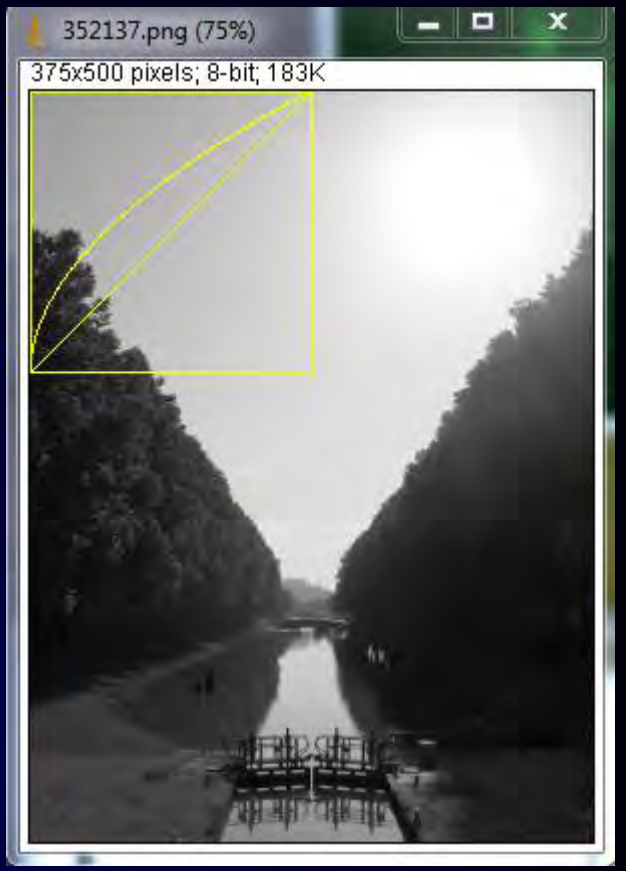
Correction non linéaire du Gamma

y = range * (x / range) ^ gamma
range = gamme de valeurs des pixels de l'image



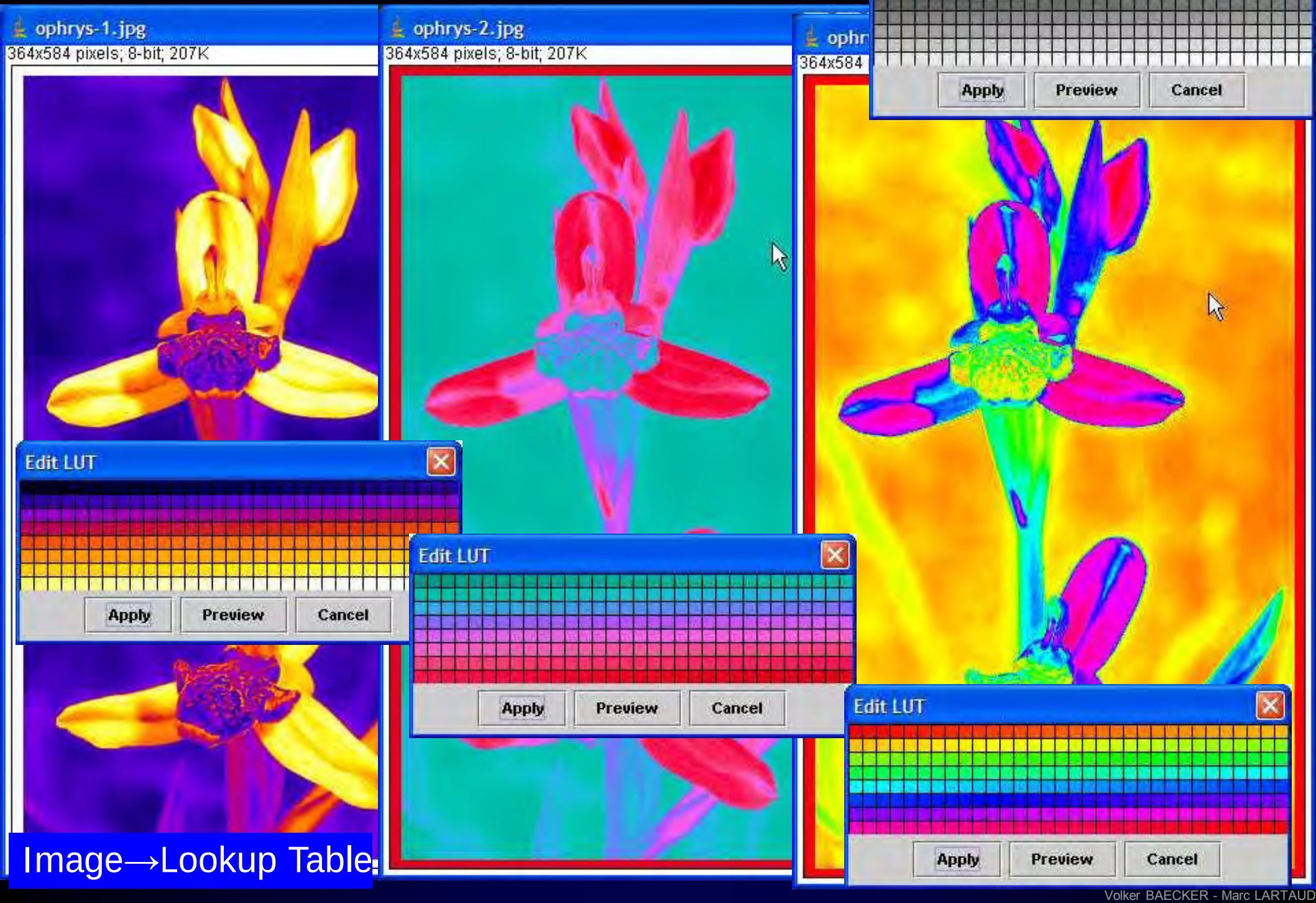




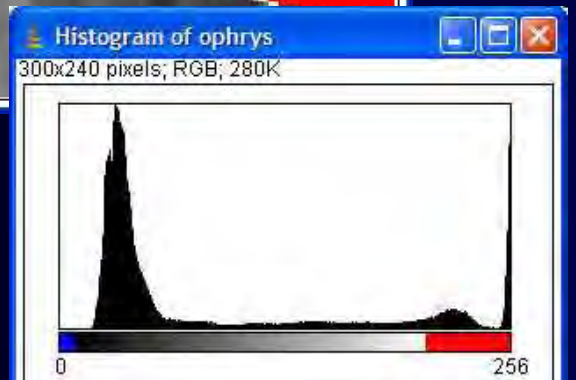


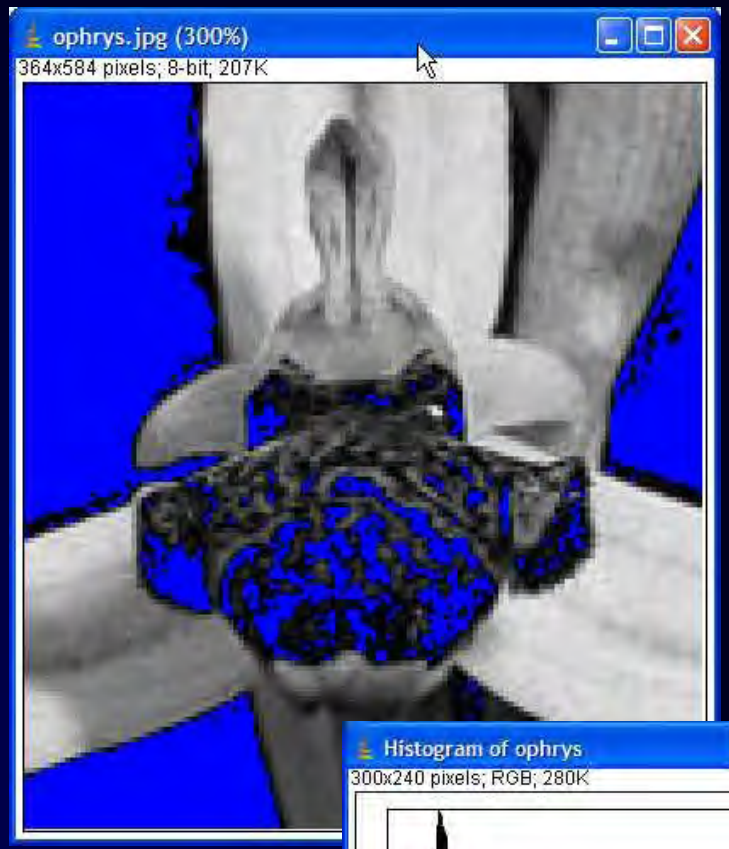
Process→Math→Gamma...

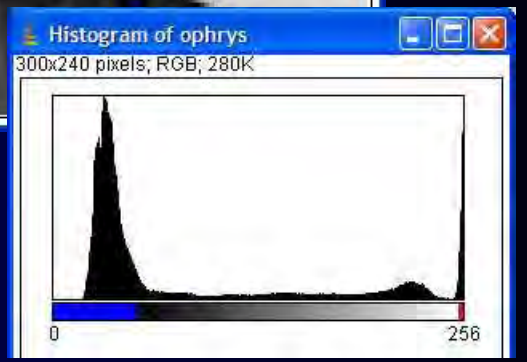
LUT : Lookup Unit Table



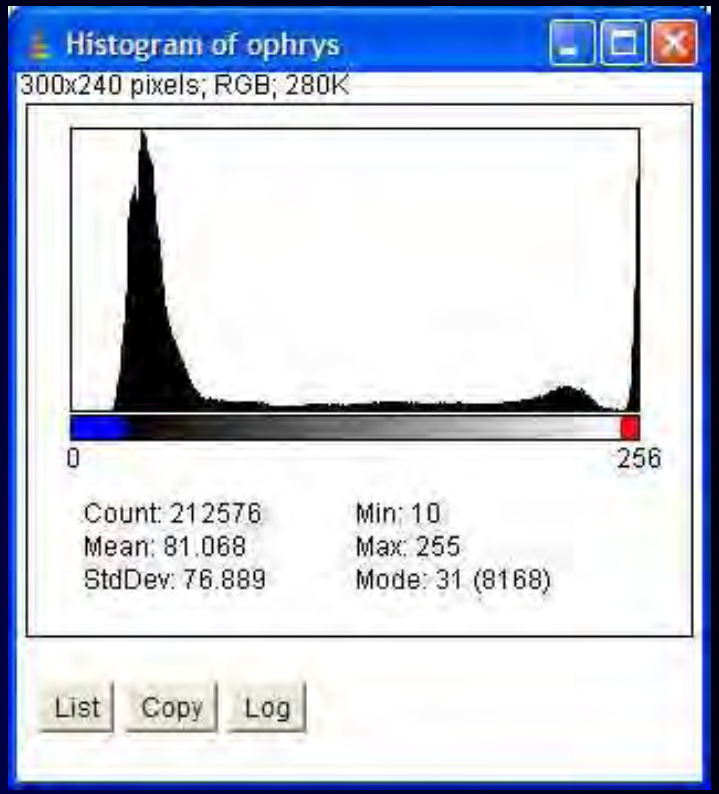
Réglages avec la LUT Hi Lo



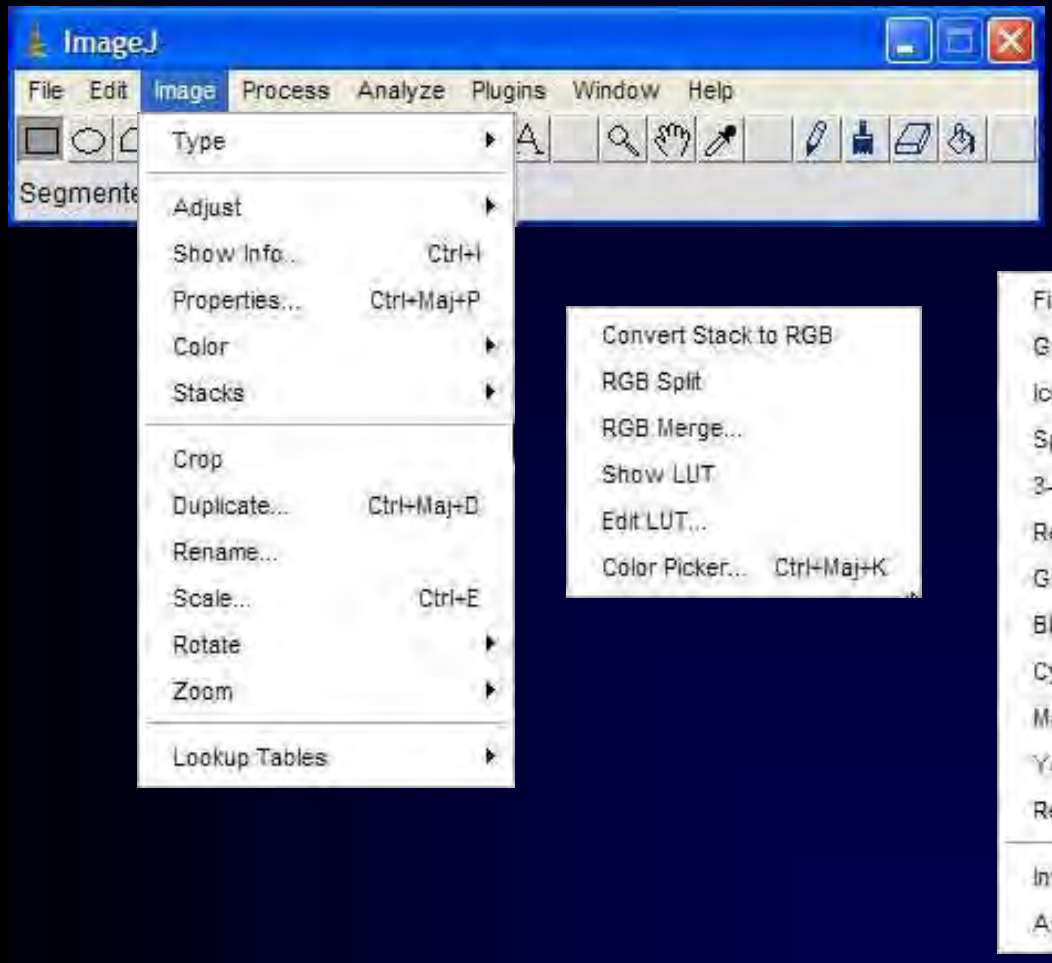


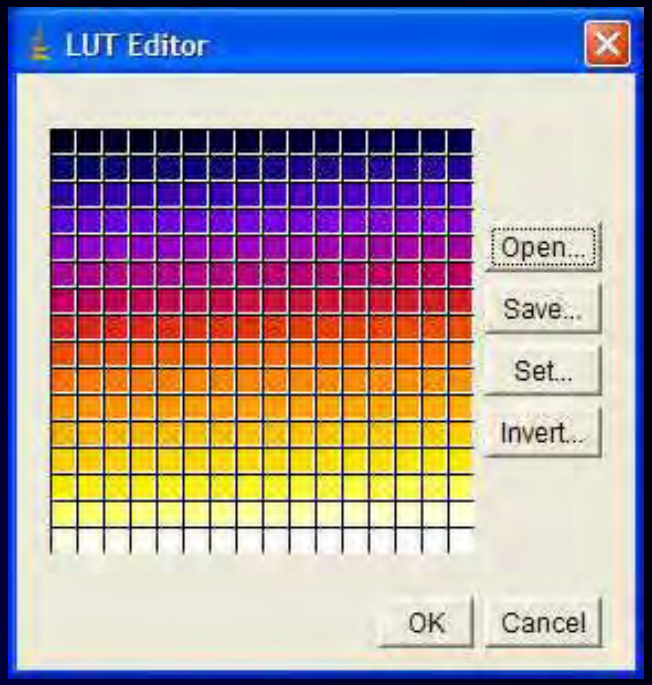


Réglages avec la LUT Hi Lo



Outils LUT dans ImageJ





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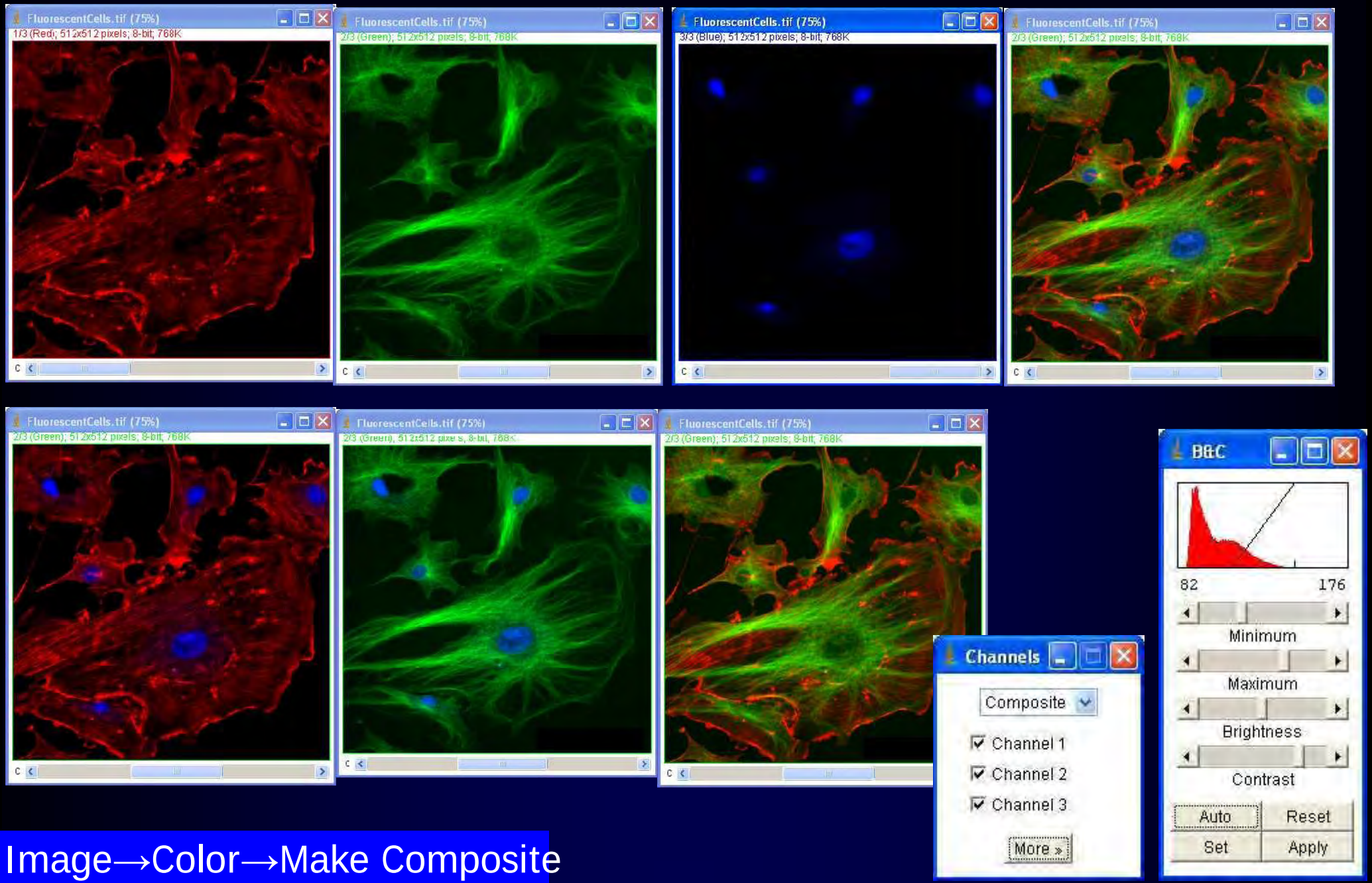
Réglages d'images composites



Image→Color→Make Composite

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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

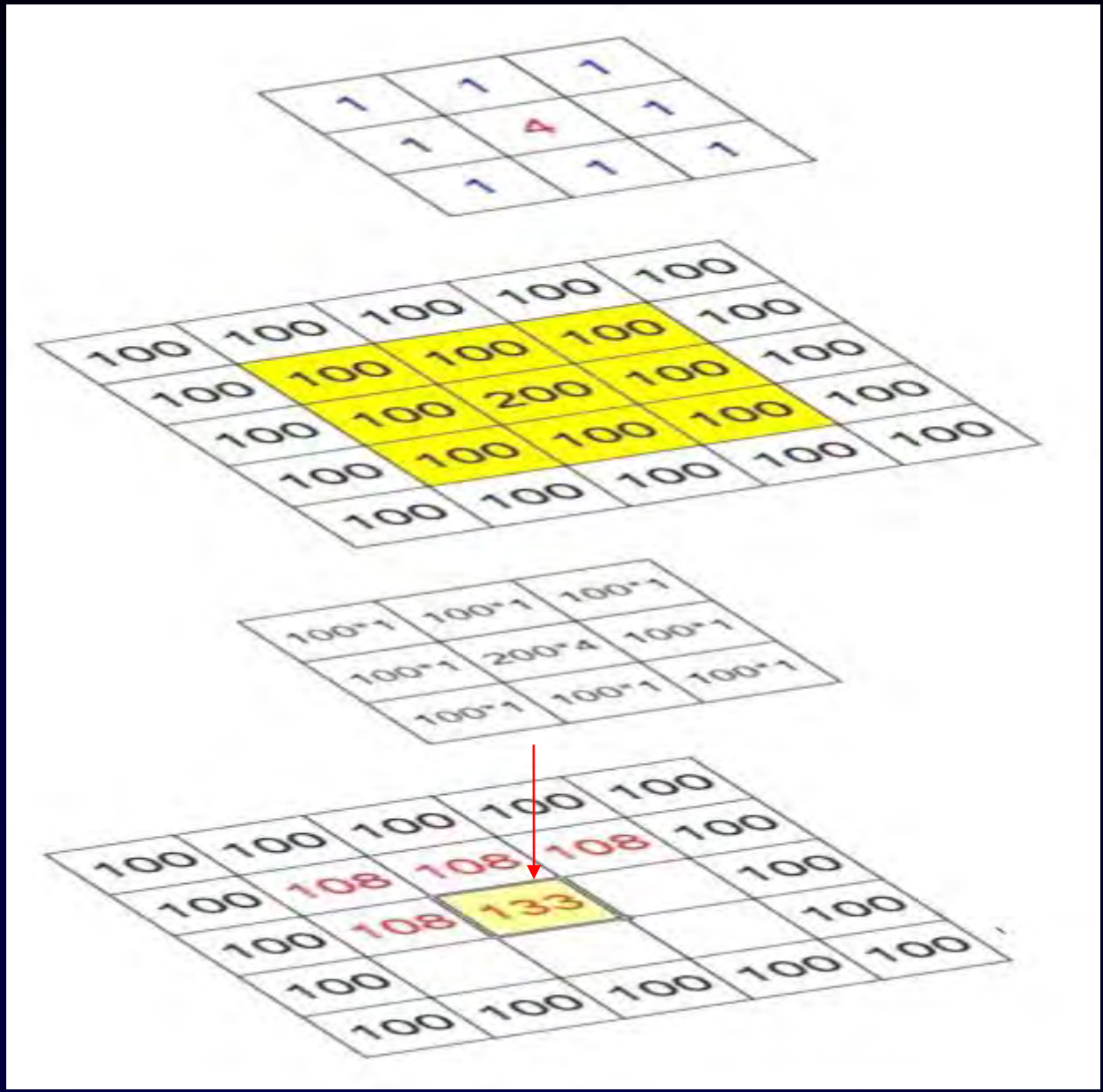
Filtres de convolution

Noyau

Image source

Produits du noyau et de la source

Image résultante



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

1600/12=133

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				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 | 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	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 | 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	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 | 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	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 | 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	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 | 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	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 | 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	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 | 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	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 | 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	108	108	100
100	108	133	108	100
100	108	108	108	100
100	100	100	100	100

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

Somme / 12 = 108

Filtres de convolution

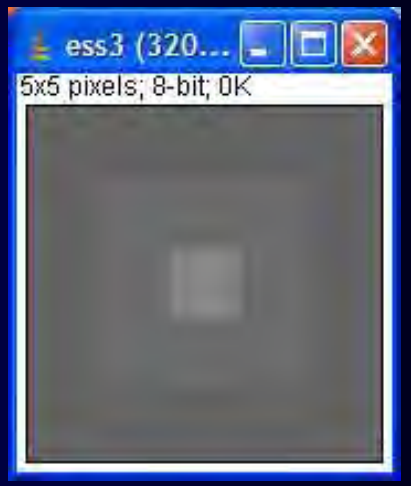
100	100	100	100	100
100	100	100	100	100
100	100	200	100	100
100	100	100	100	100
100	100	100	100	100

*

1	1	1
1	4	1
1	1	1

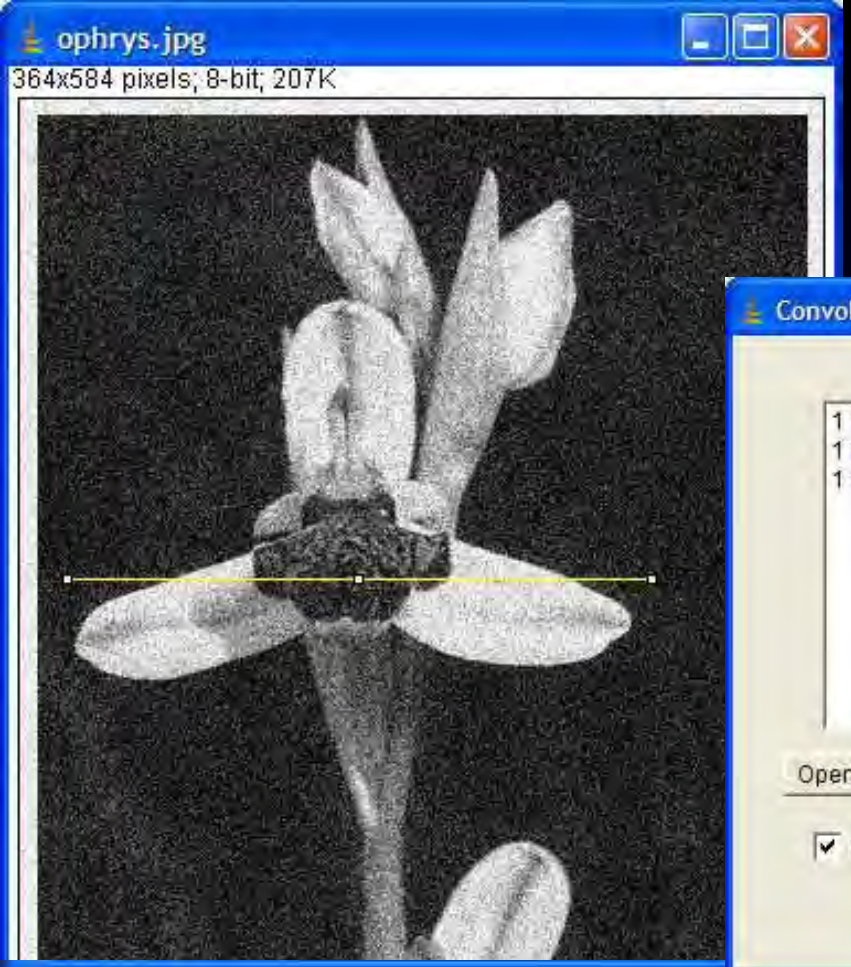
=

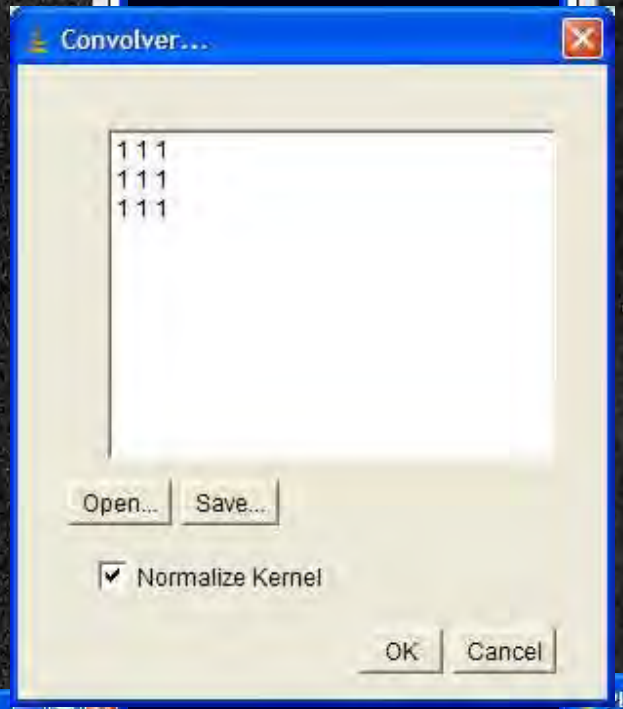
100	100	100	100	100
100	108	108	108	100
100	108	133	108	100
100	108	108	108	100
100	100	100	100	100

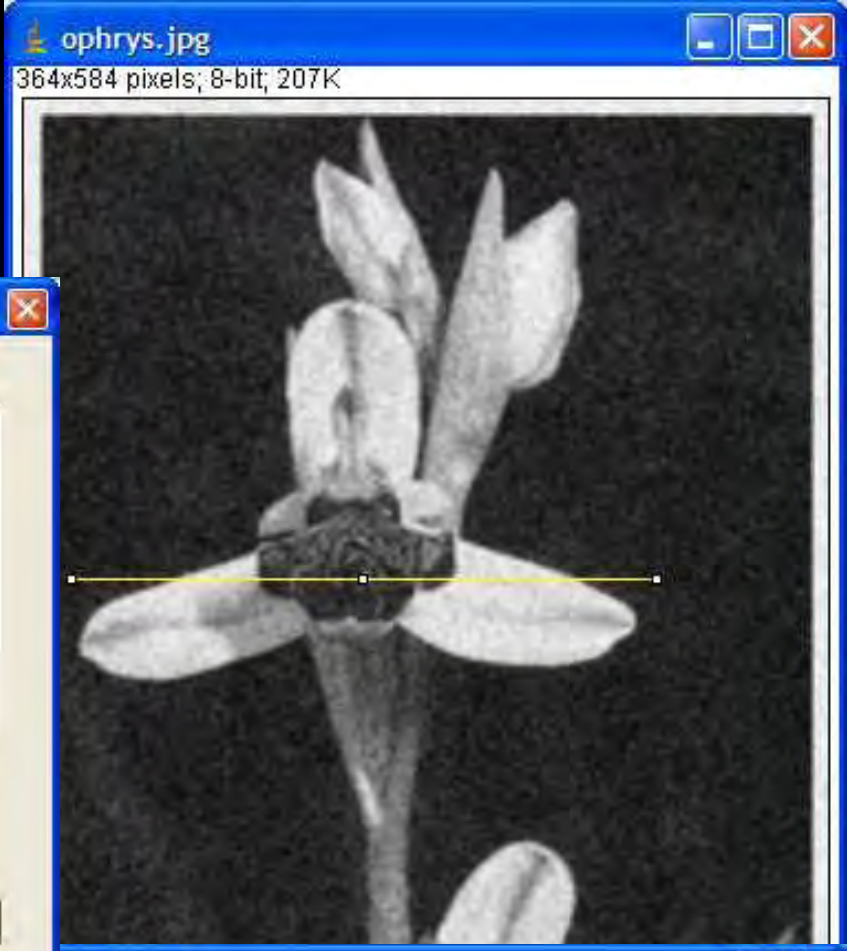


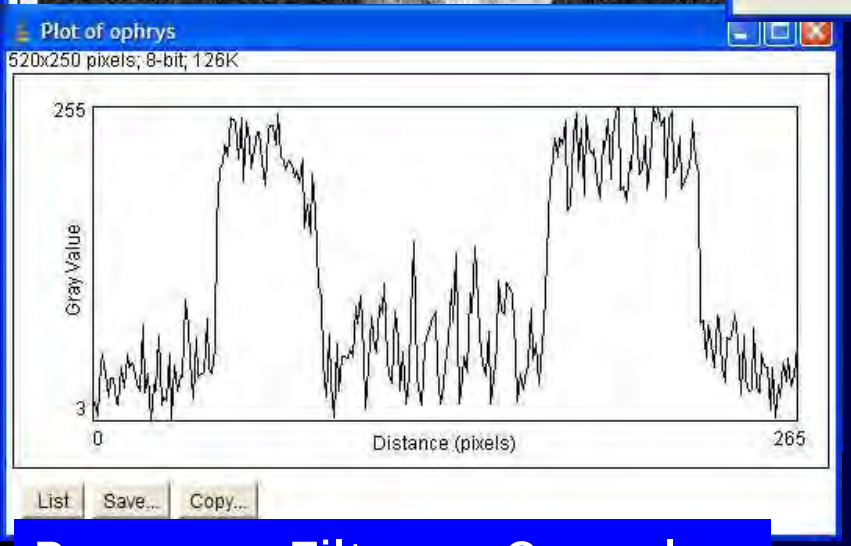
☞ Le résultat d'un tel filtrage est un lissage de l'image, il s'agit d'un **filtre passe-bas**.

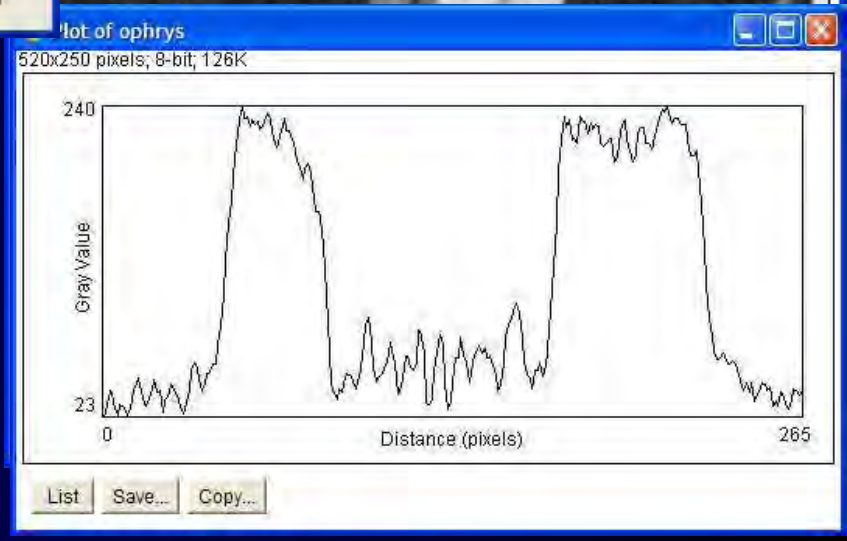
Filtre passe-bas moyenneur





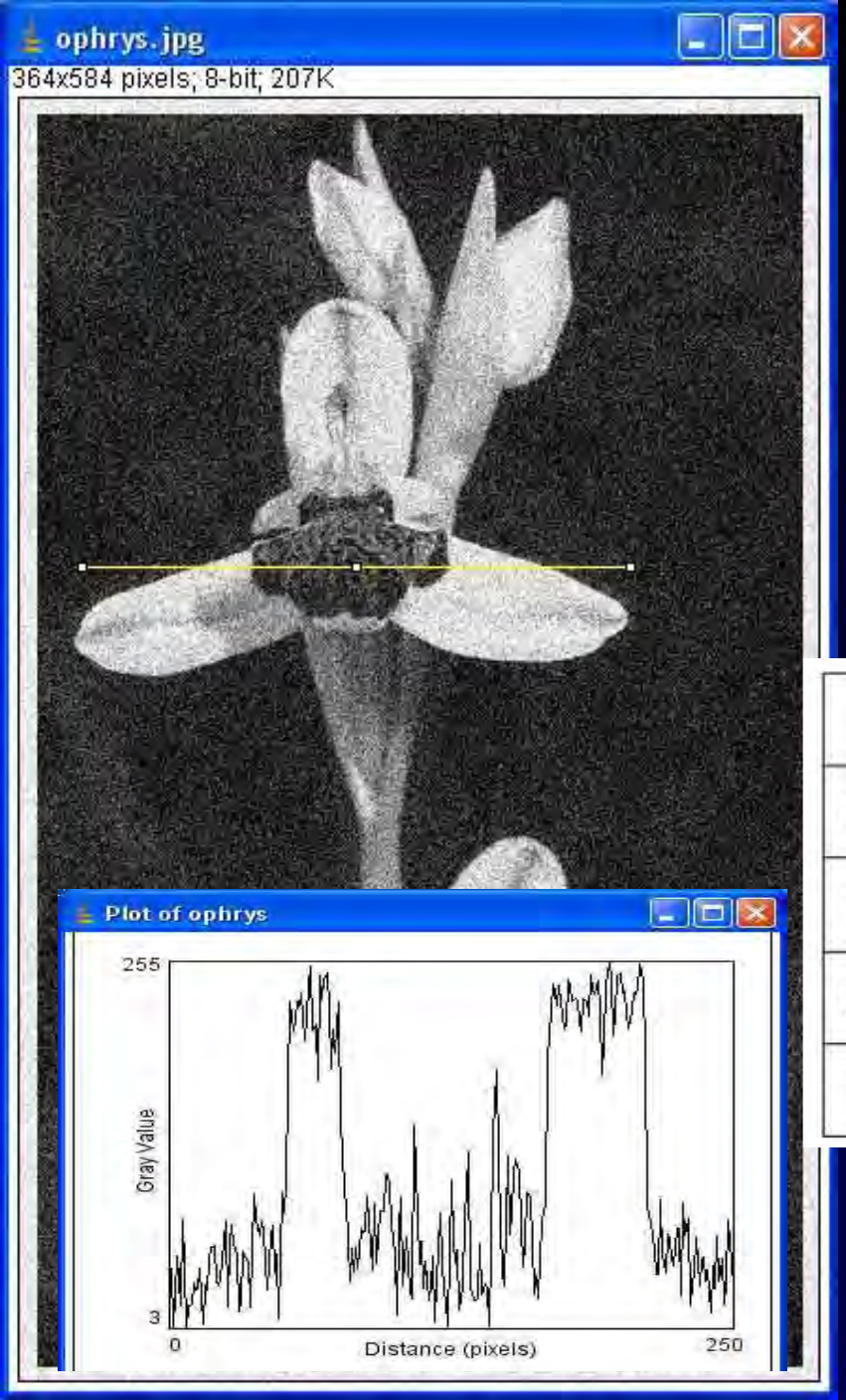




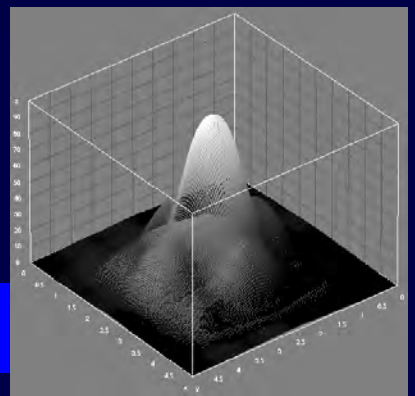


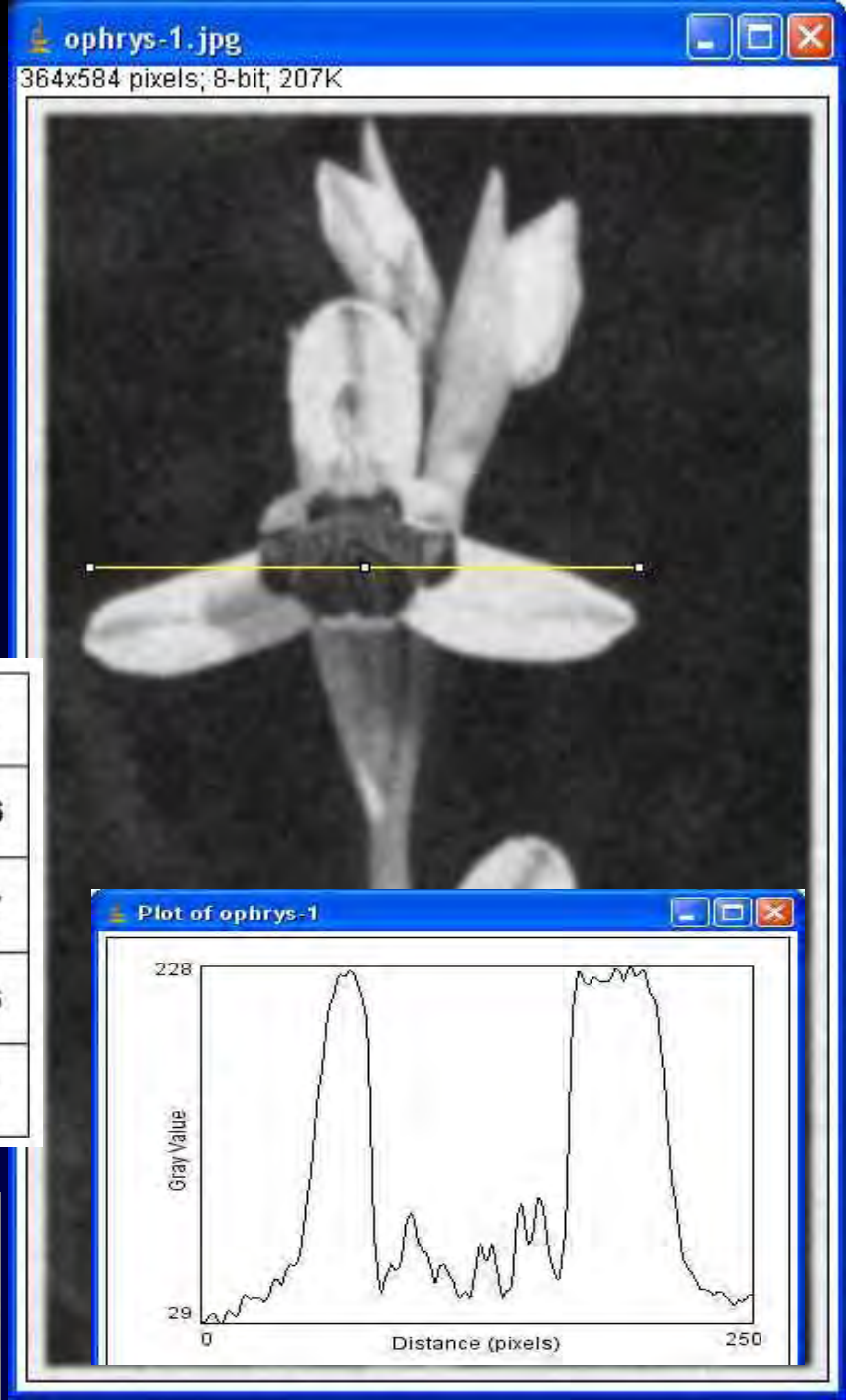
Process→Filters→Convolve...

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.

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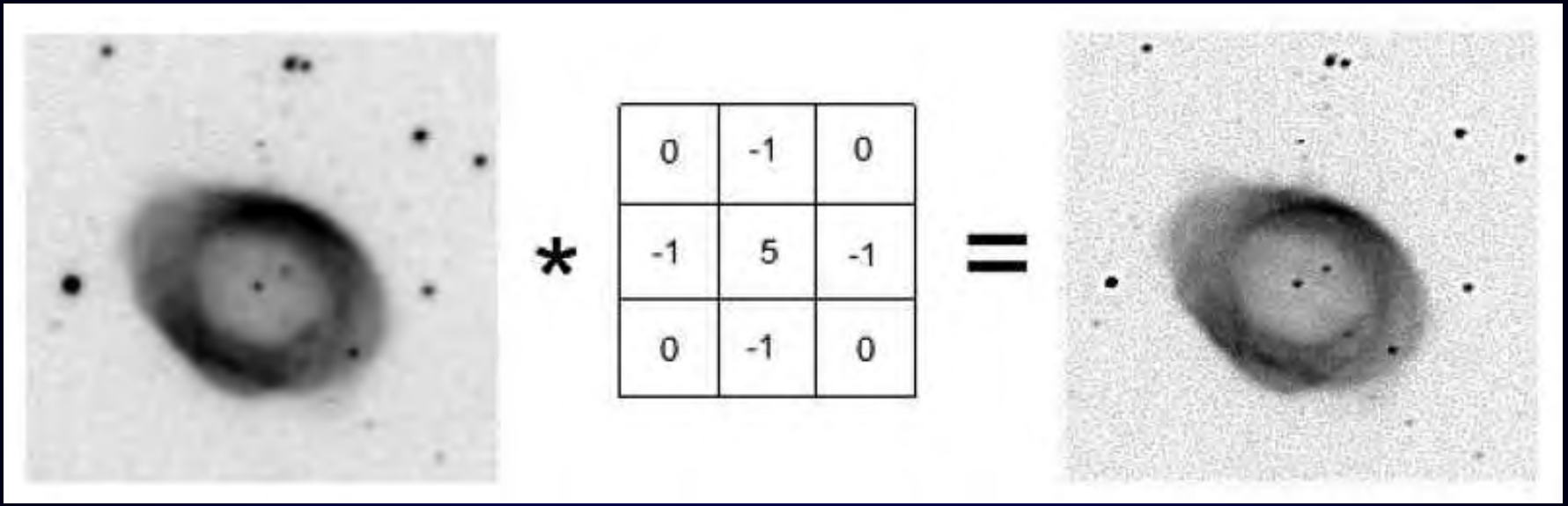
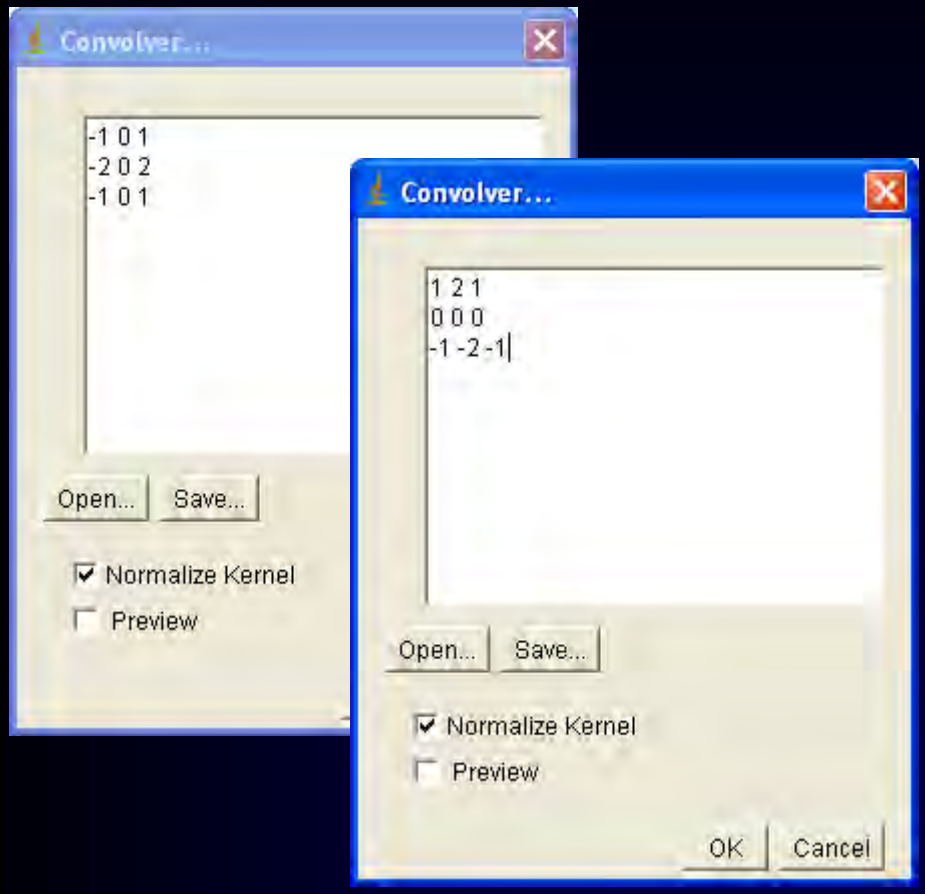
Filtres Passe-haut



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

Filtre Détection de bords



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 :

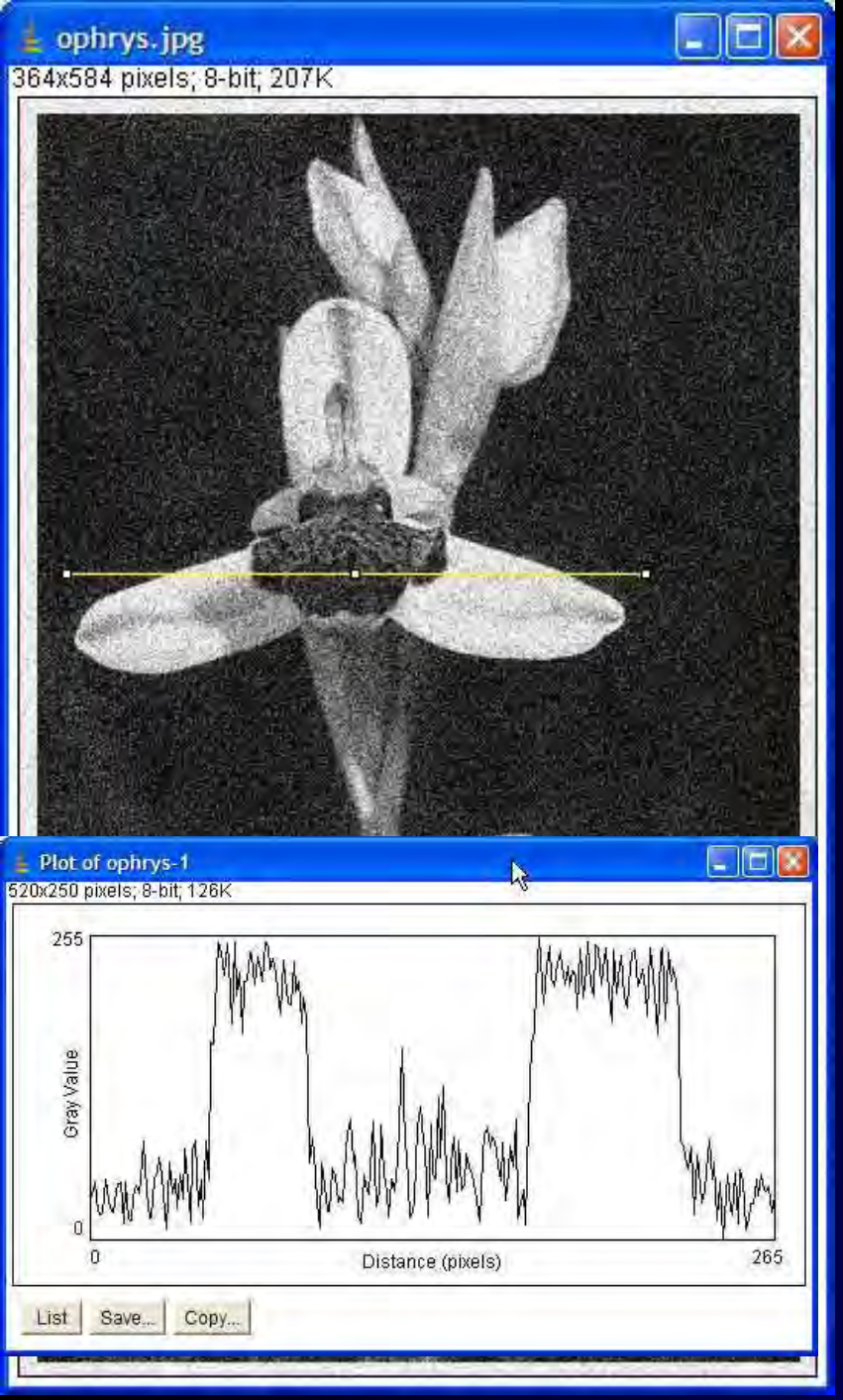
15	18	14
29	27	13
12	19	21

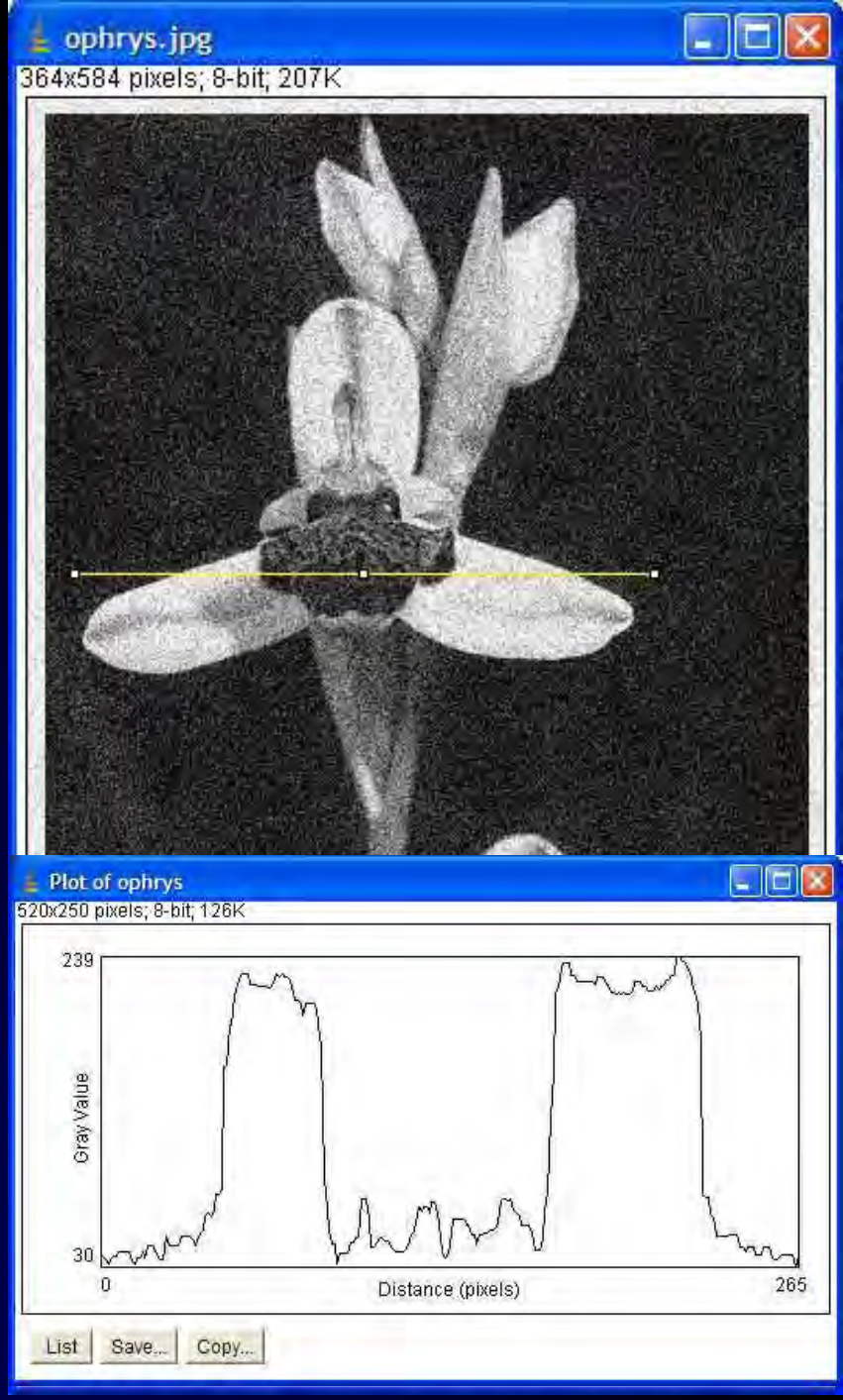
15	18	14
29	18	13
12	19	21

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

Filtre min → 12
Filtre max → 27

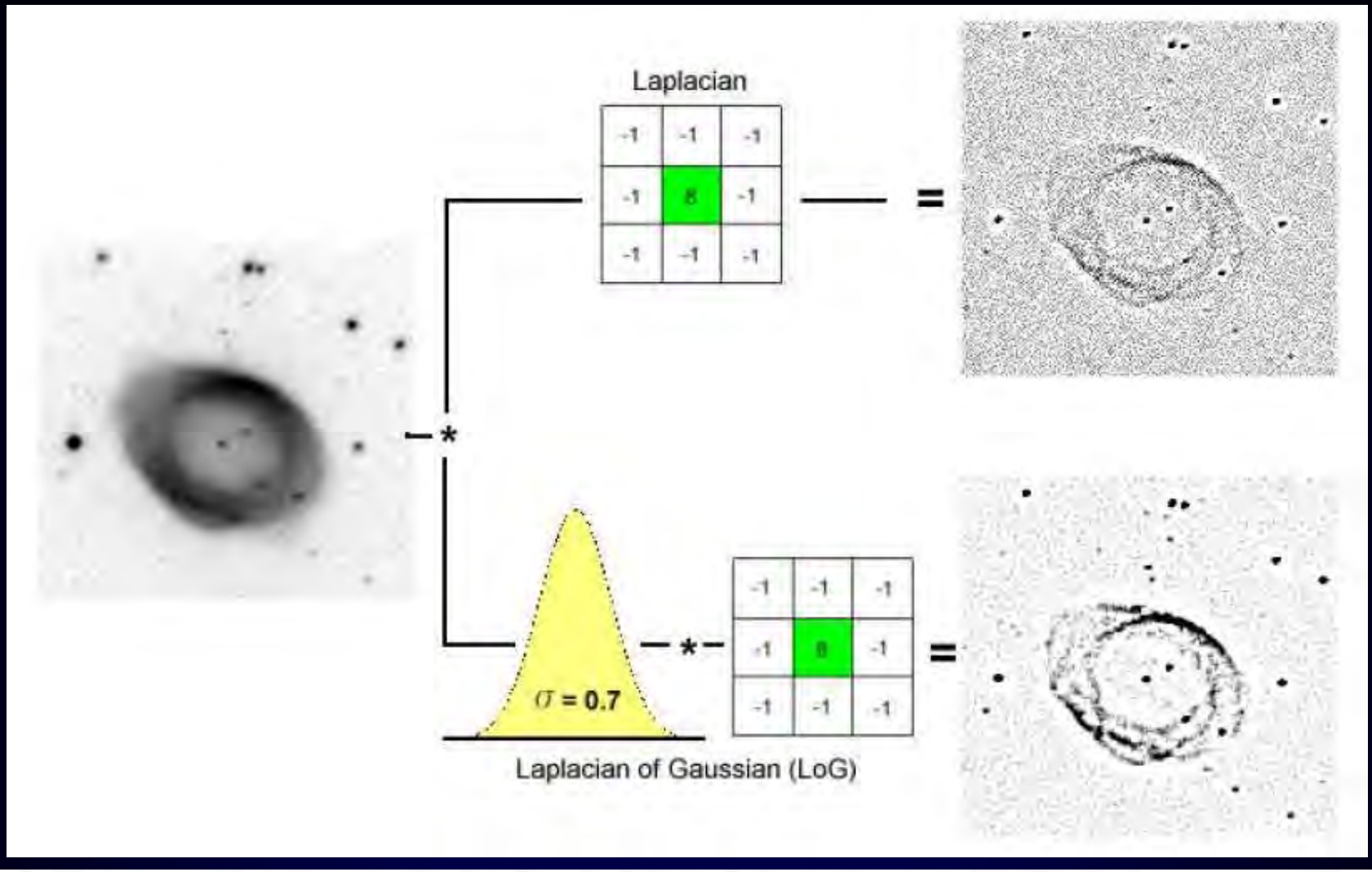
Filtre médian





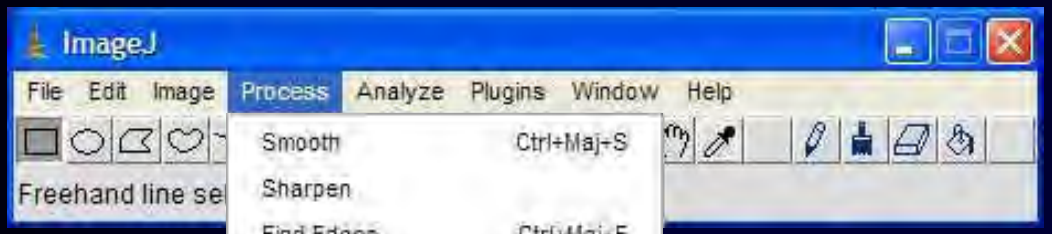
Process→Filters→Median...

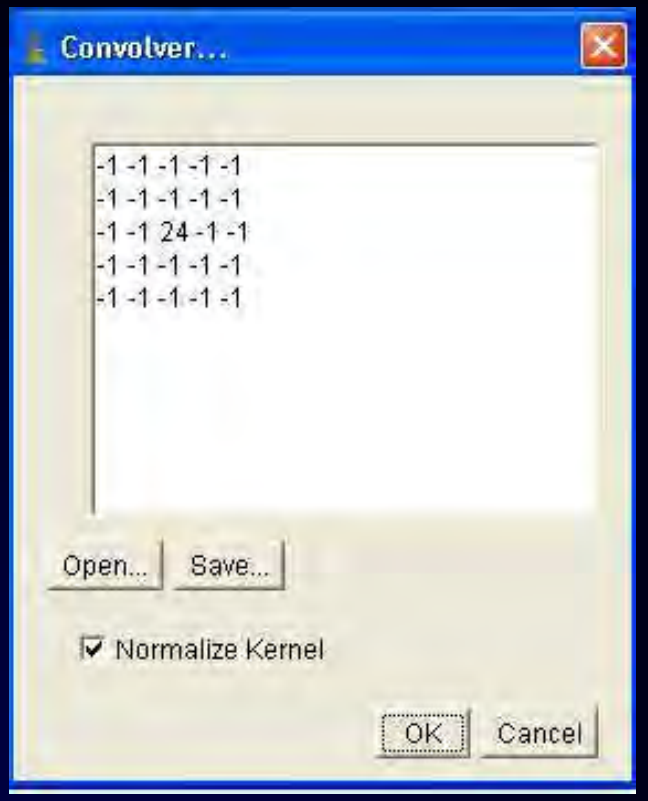
Combinaison Gaussien Laplacien

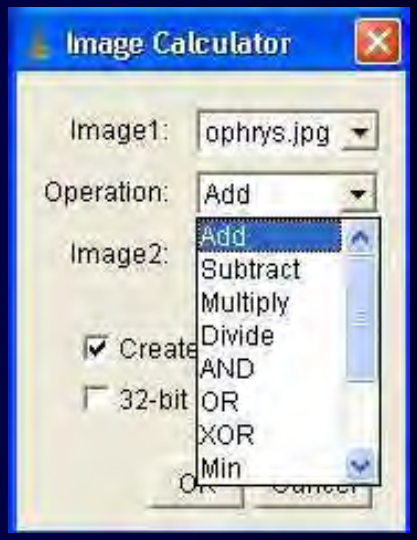


Les propriétés de réduction de bruit des filtres Gaussiens peuvent être utilisées en combinaisons avec d'autres filtres qui au contraire génèrent du bruit, comme les filtres Laplaciens. On peut par exemple choisir d'appliquer d'abord un filtre Gaussien pour réduire le bruit, avant d'appliquer un filtre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

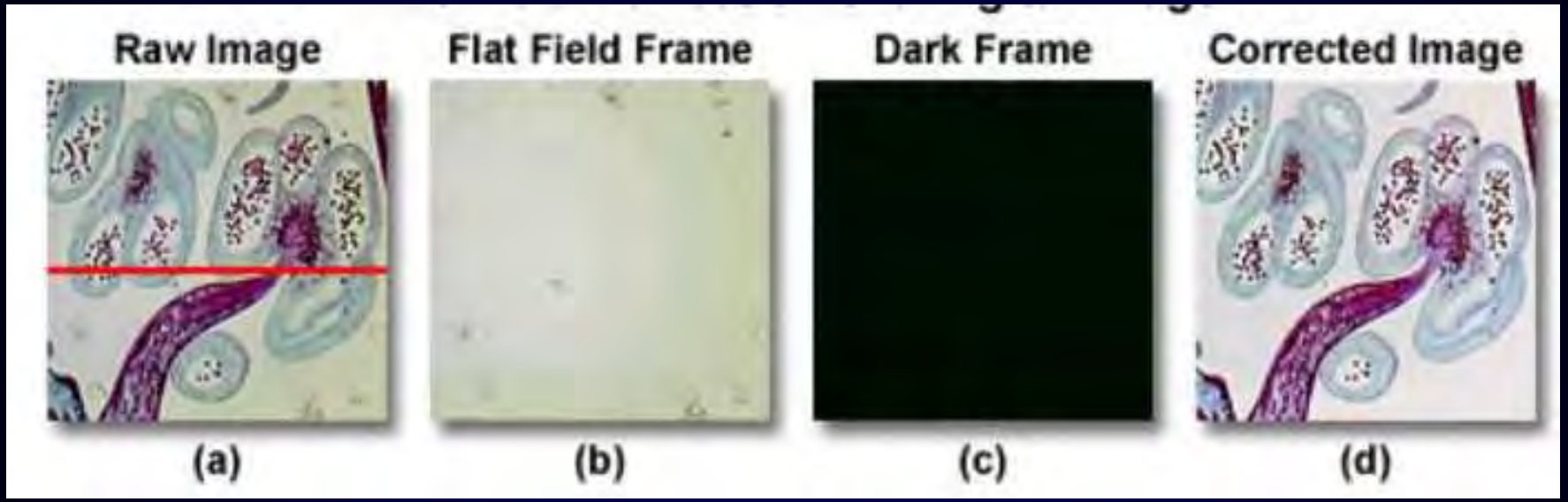
- Bruits de la caméra: bruit aléatoire, «pixels chauds» ,bruits périodiques
- Illumination non-homogène

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 = ((échantillon - CN) / (CC - CN)) * 255



Correction du fond

Correction après la capture

Bruit aléatoire : filtre gaussien ou médian mais perte de détail

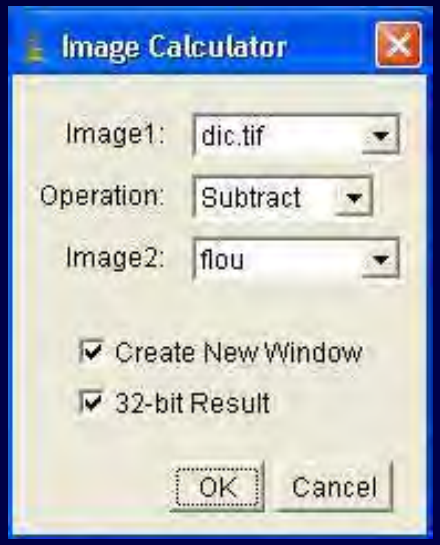
Pixels chauds : filtrer les pixels saturés isolés

Bruit périodique : filtre de Fourier

Illumination non-homogène :

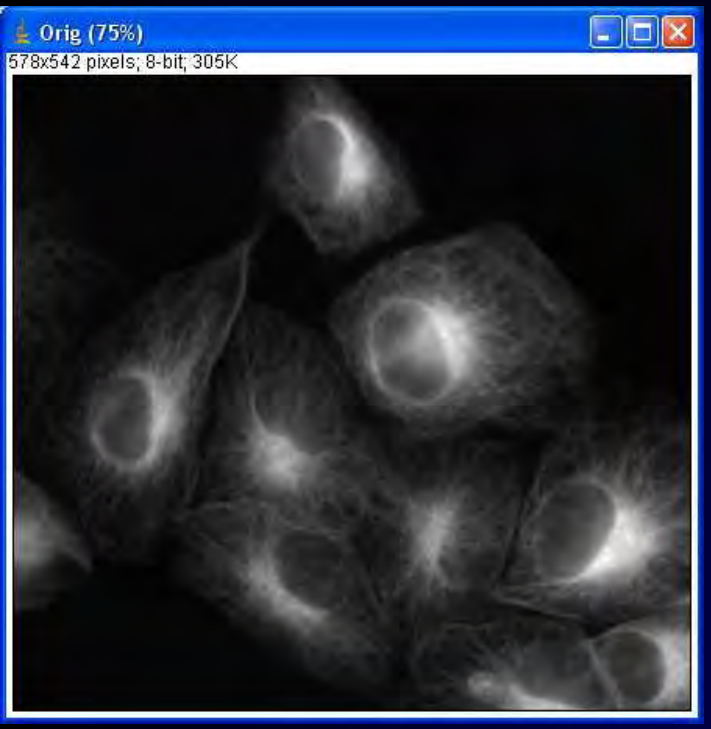
- Soustraire la même image très floue (filtre gaussien très large)
- Algorithme « rolling ball » (ImageJ Process→Substract Background)
- Toute une serie de plugins implémentés dans ImageJ (Fitting a polynomial surface)

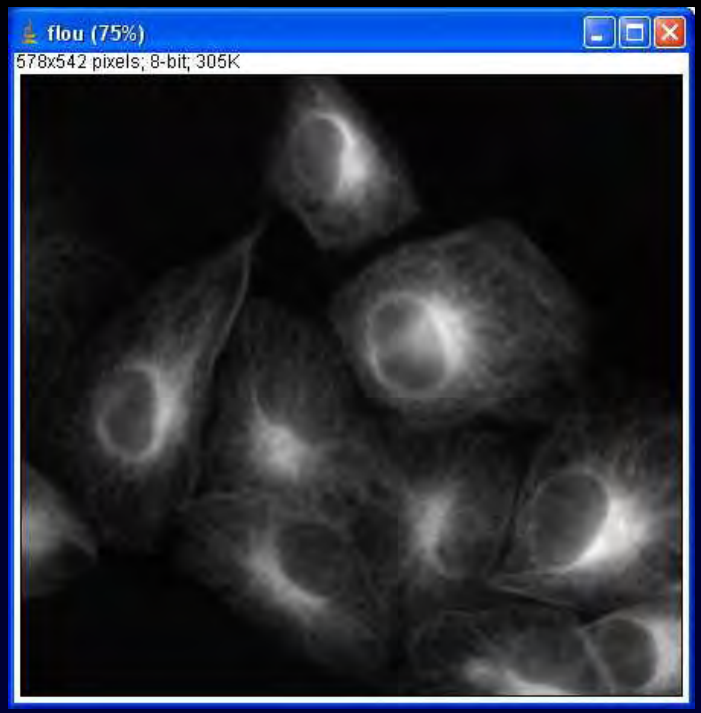
Masquer le fond



Masque de Flou

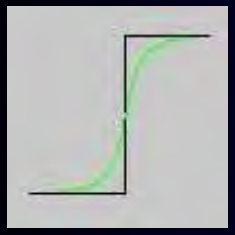
1 1 1
1 1 1
1 1 1

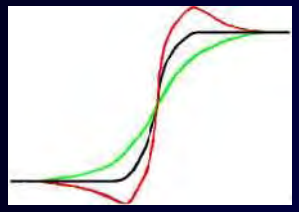




Flou - Orig

Orig –(Flou – Orig)





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Topic 07 – Background correction
Unsharp masking

L’image numérique

Les Prétraitements

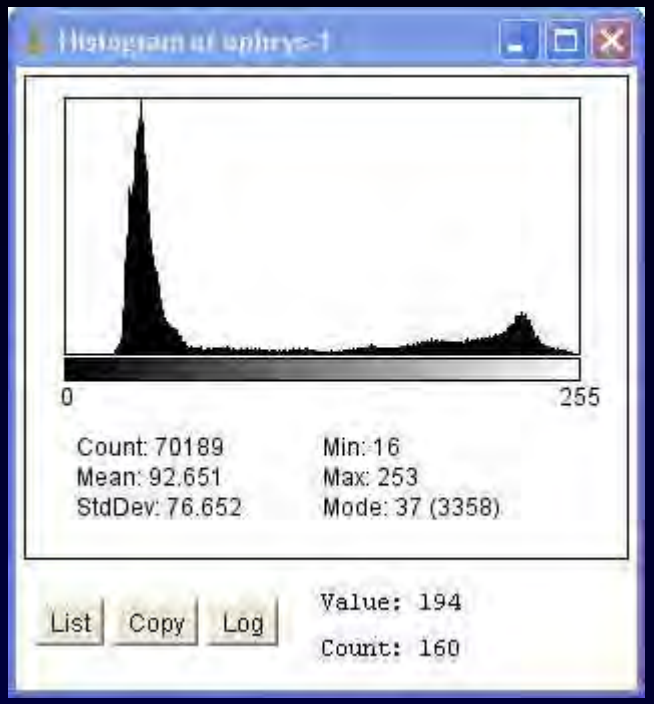
La Segmentation

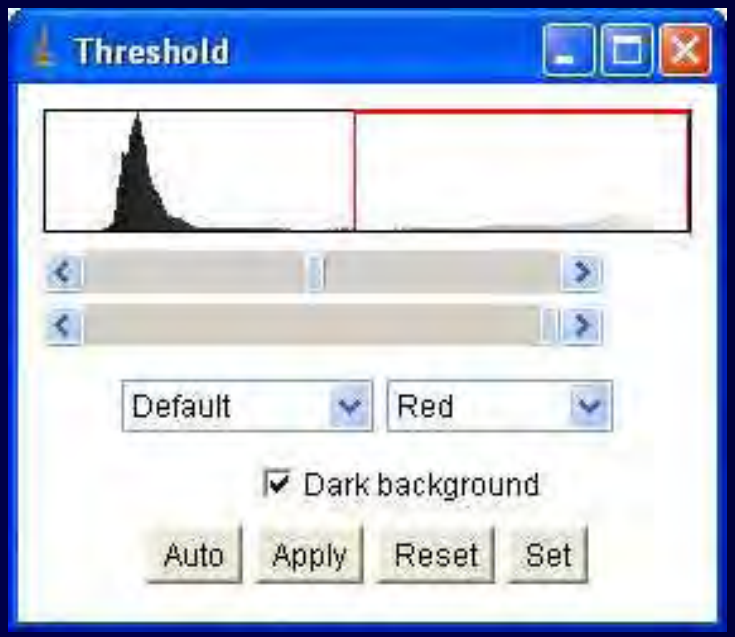
Les Post-traitements

La Quantification

Permet de séparer les régions d'intérêt du fond.

Le Seuillage automatique





Image→Adjust→Threshold…

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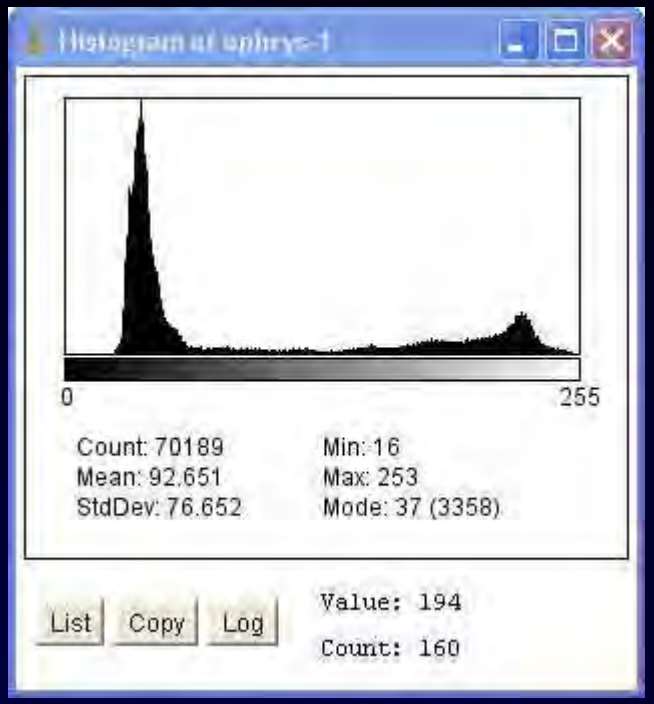
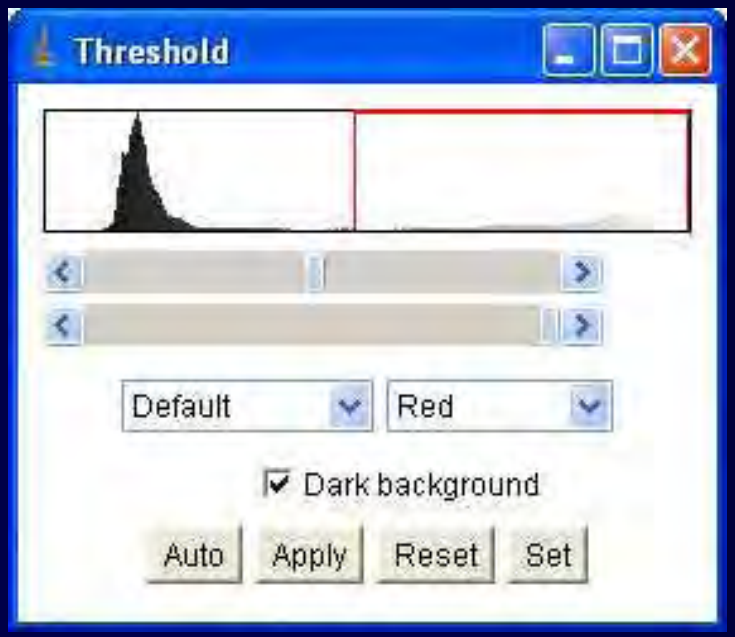
Le Seuillage automatique





Image→Adjust→Threshold…

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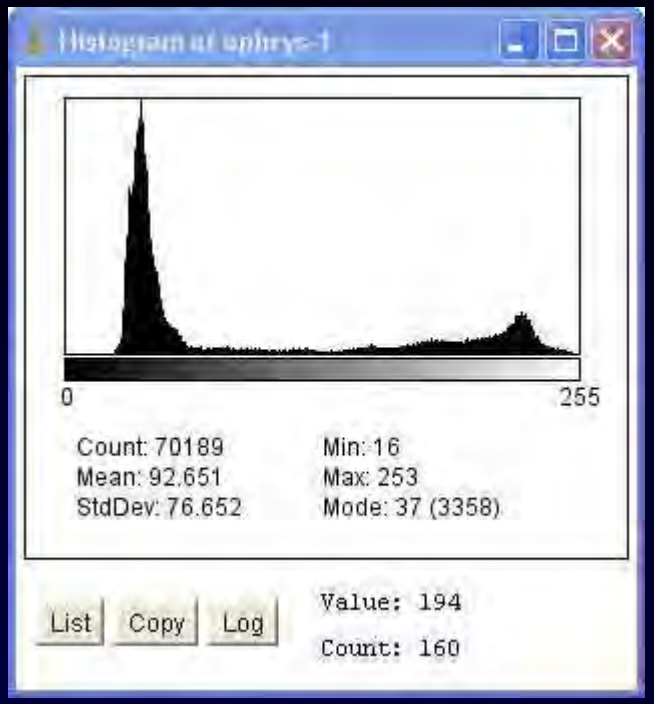
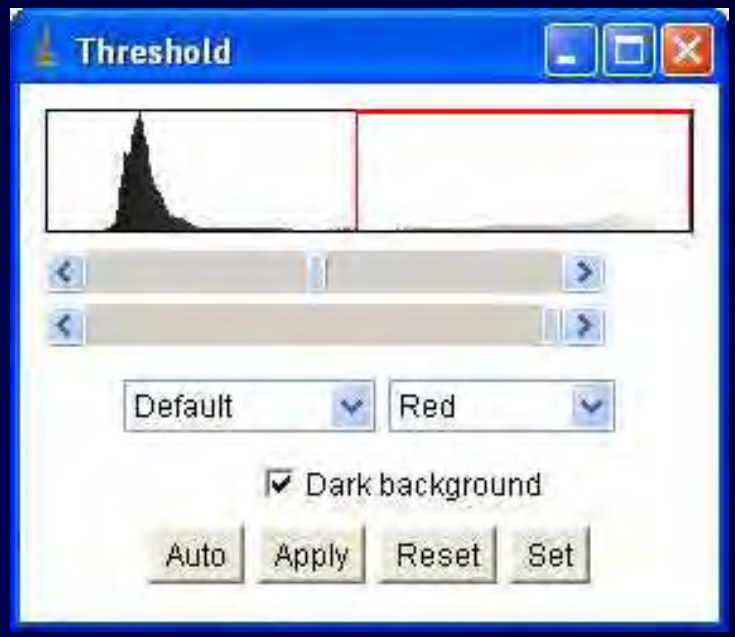
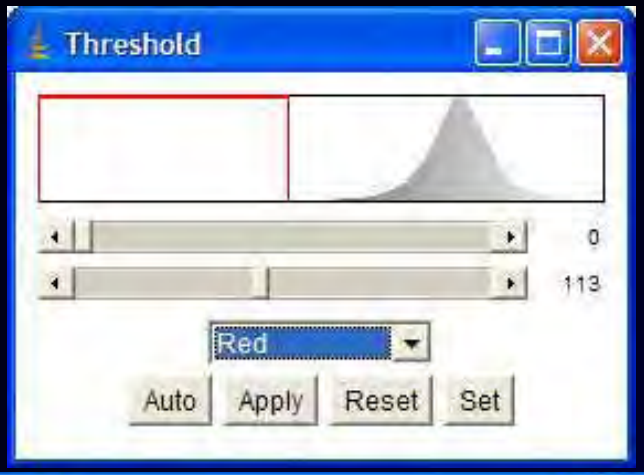
Le Seuillage automatique

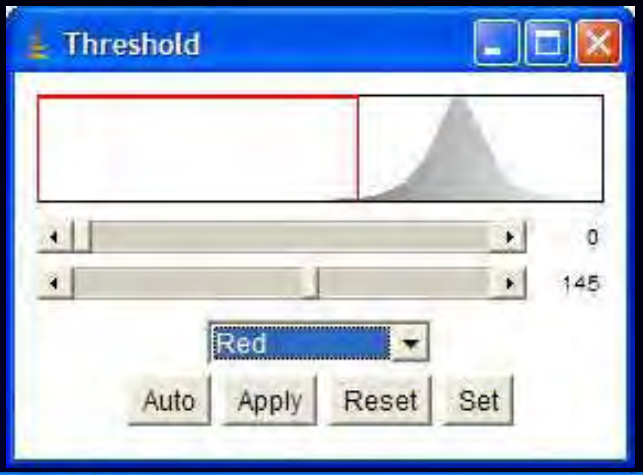


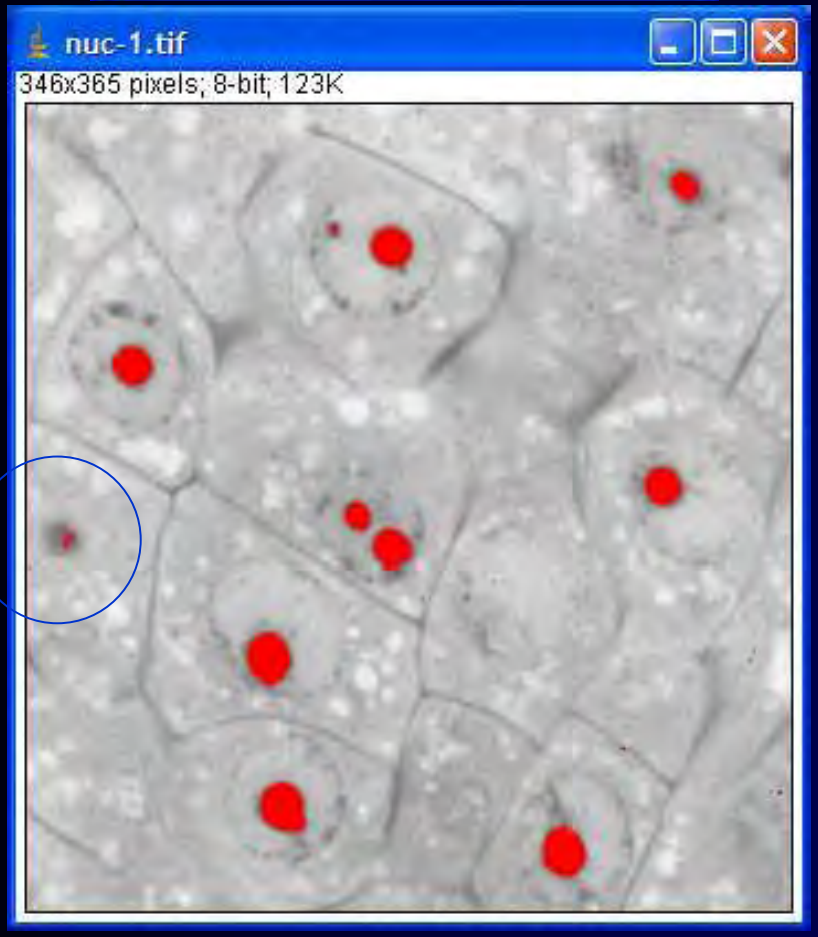


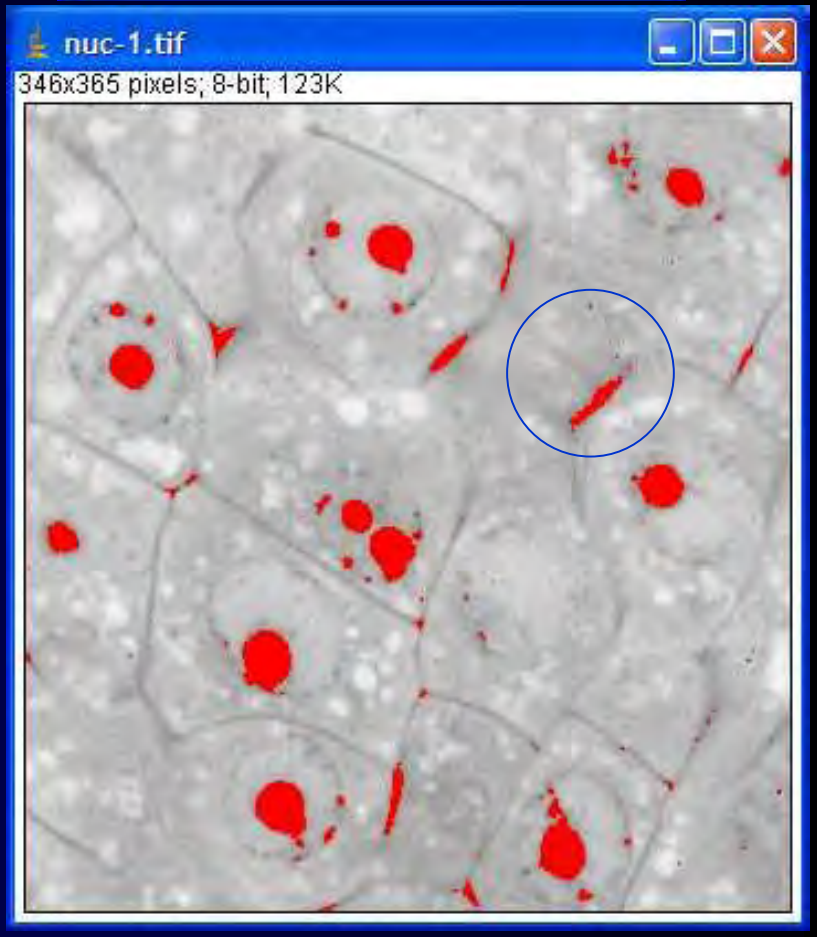
Image→Adjust→Threshold…

Le Seuillage manuel

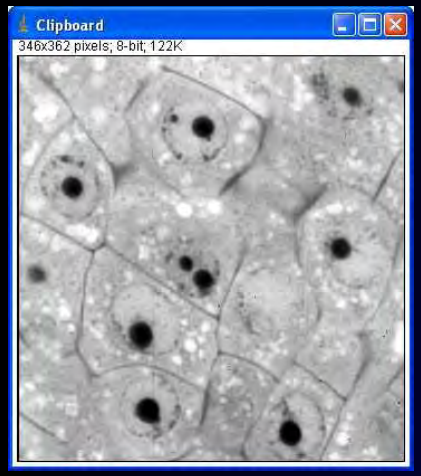


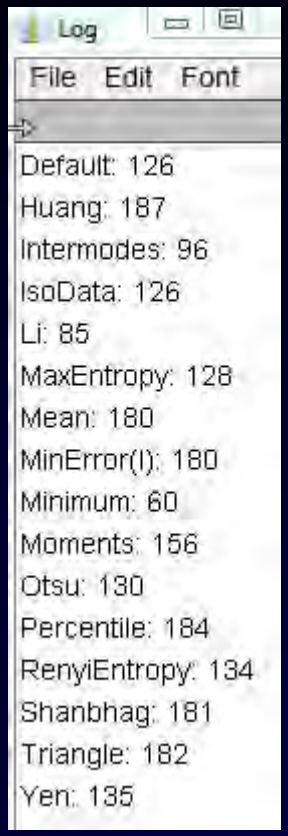


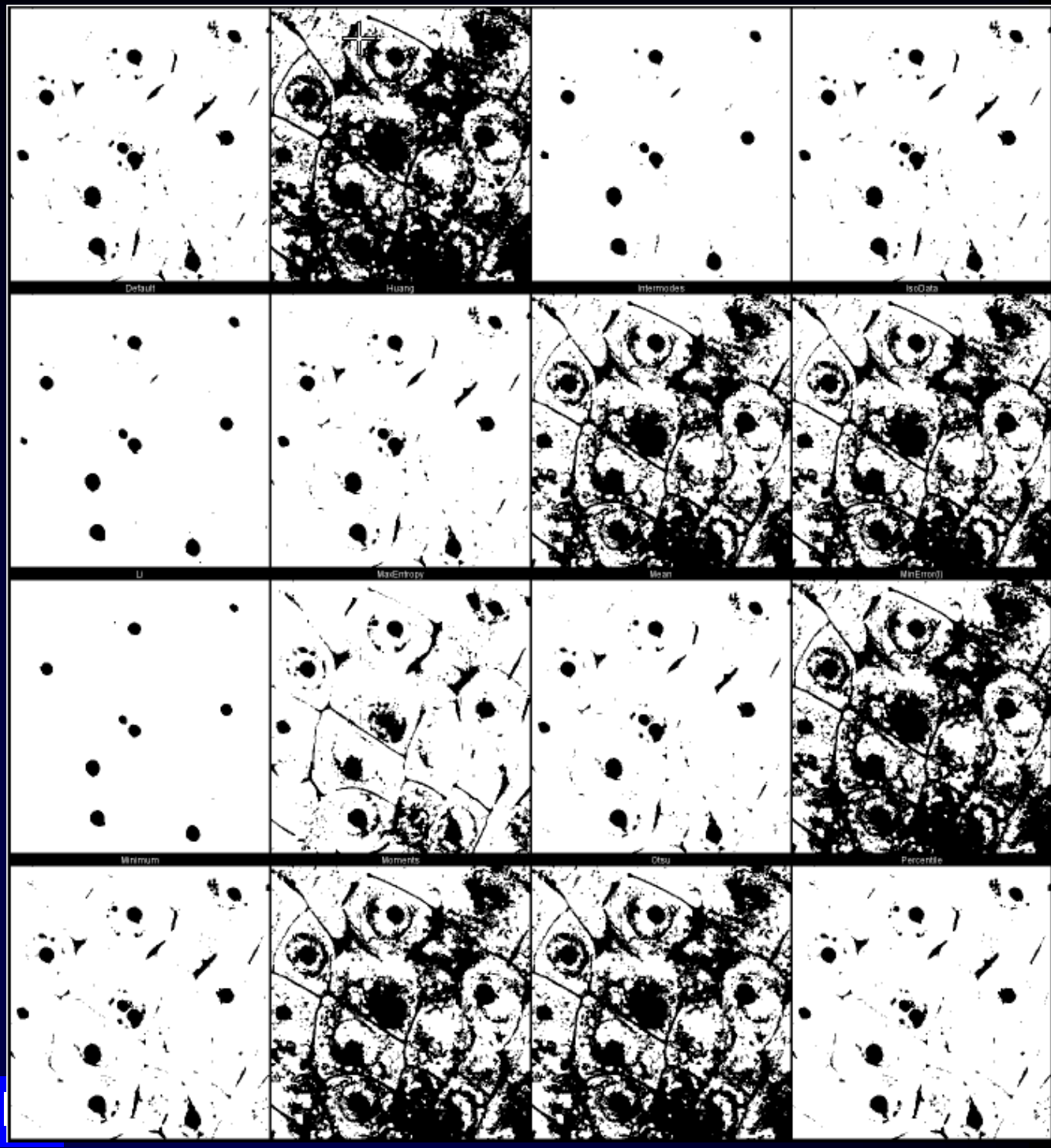




Le Seuillage automatique

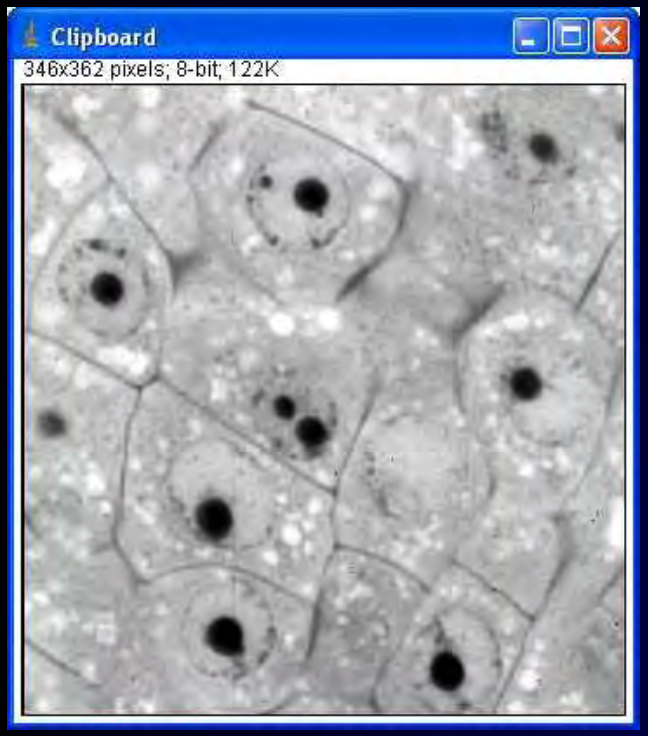


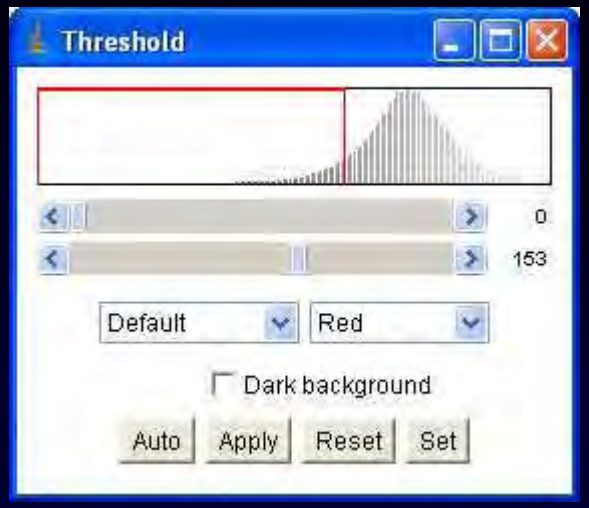


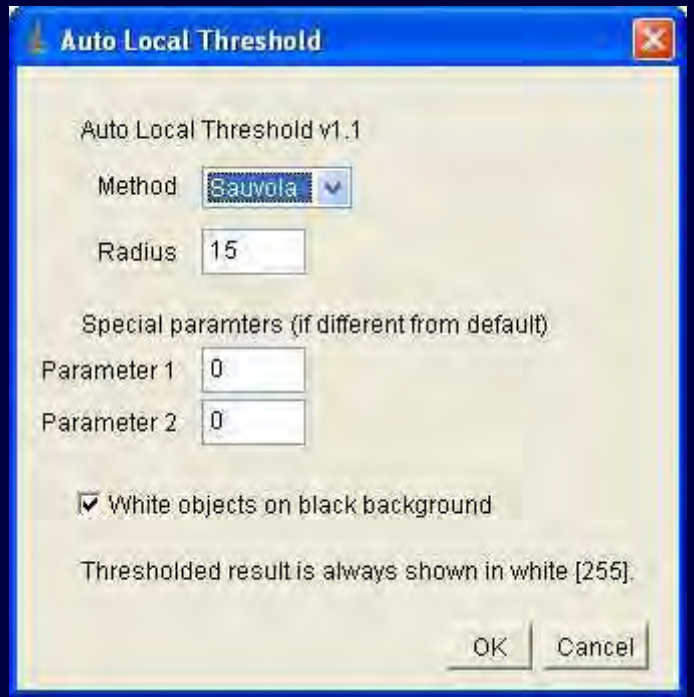


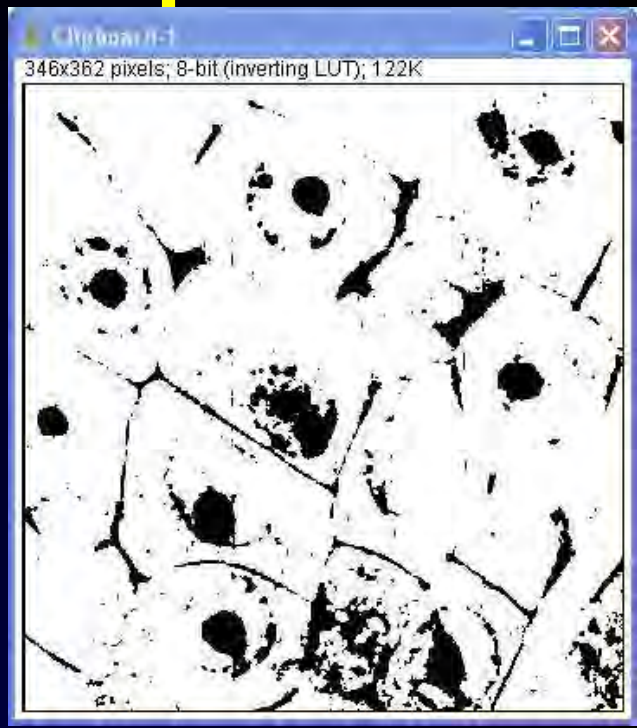
Image→Adjust→AutoThreshol

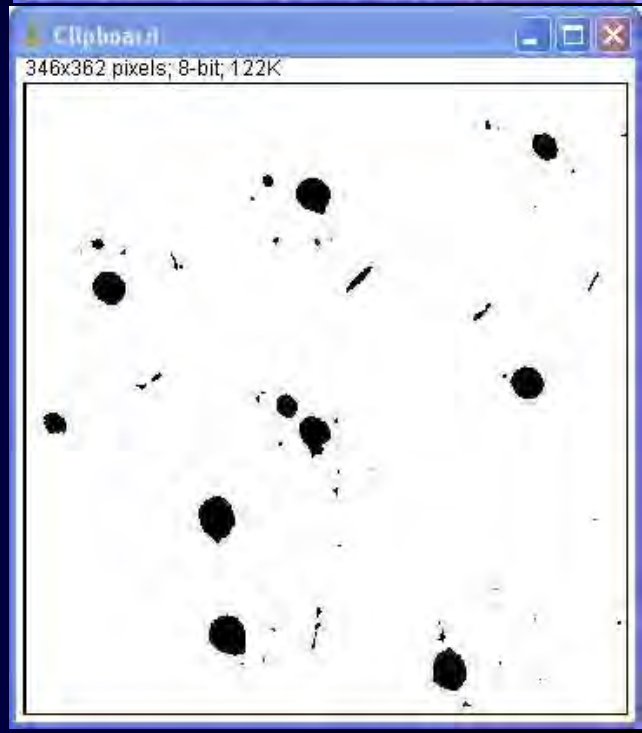
Seuillage local automatique



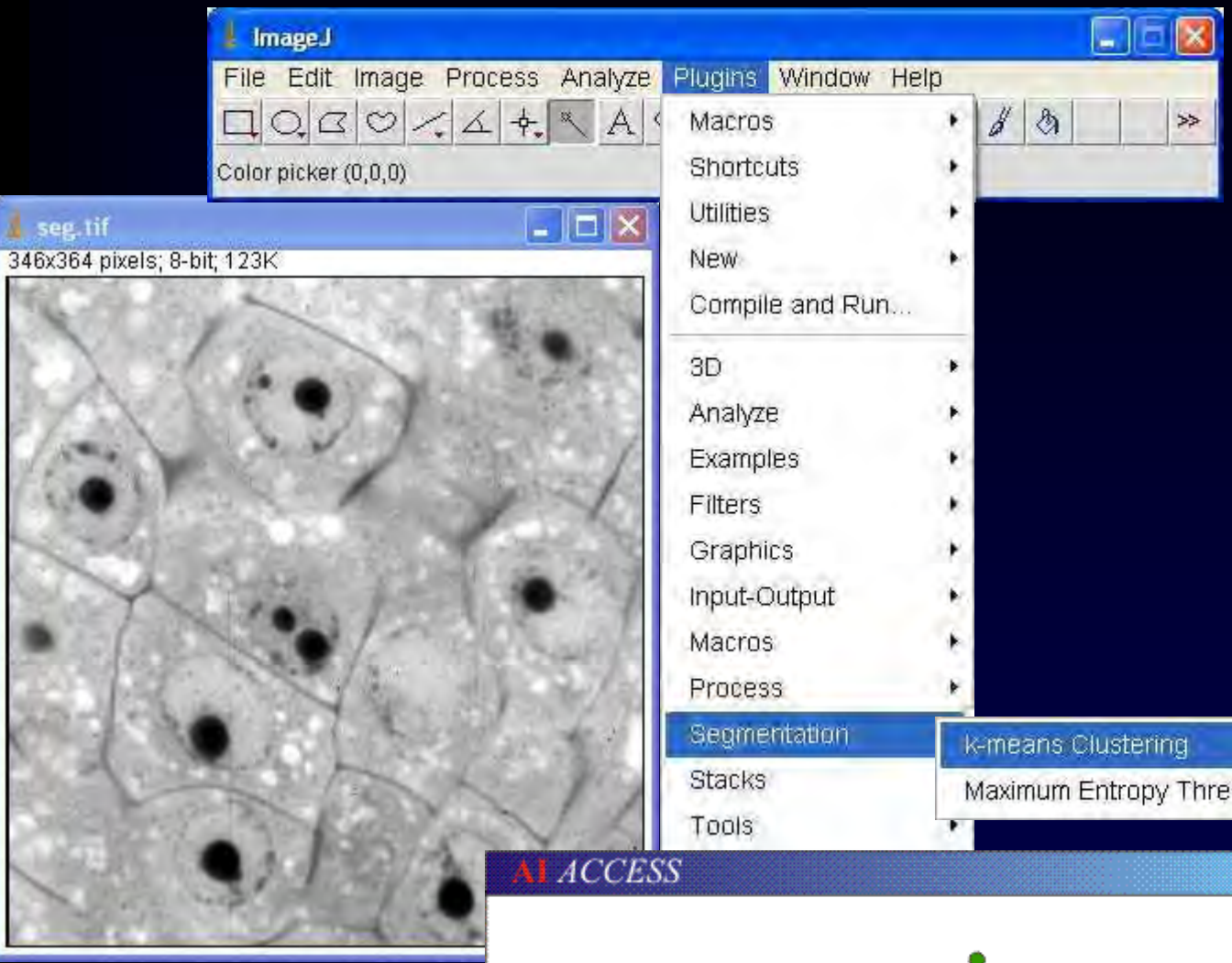


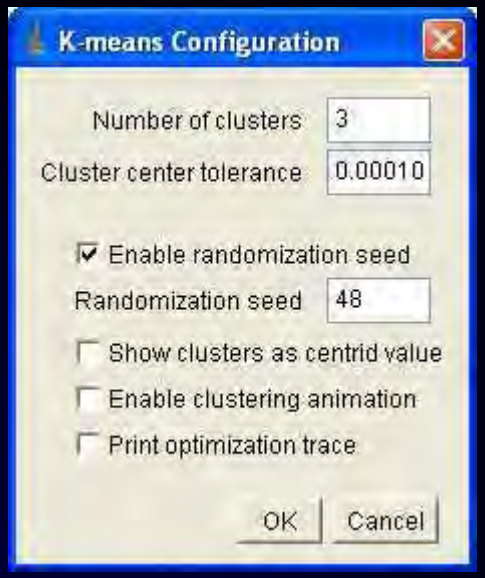


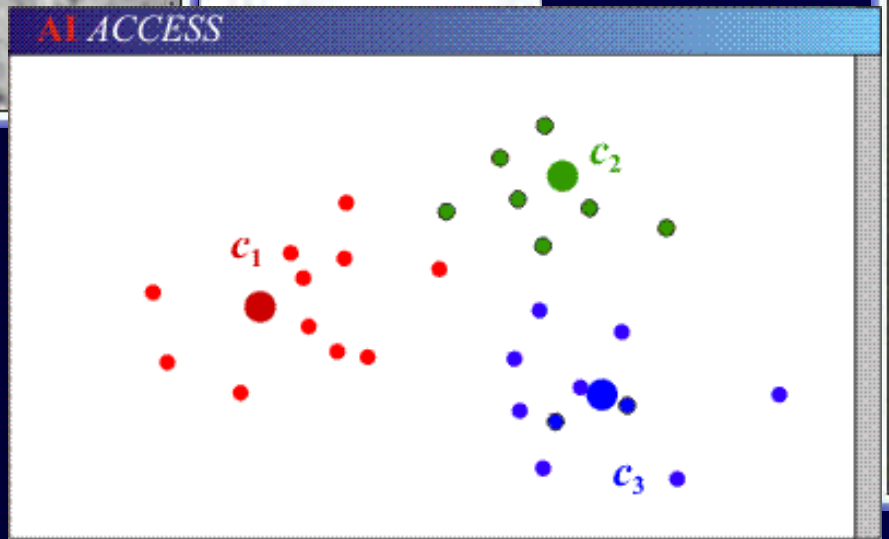


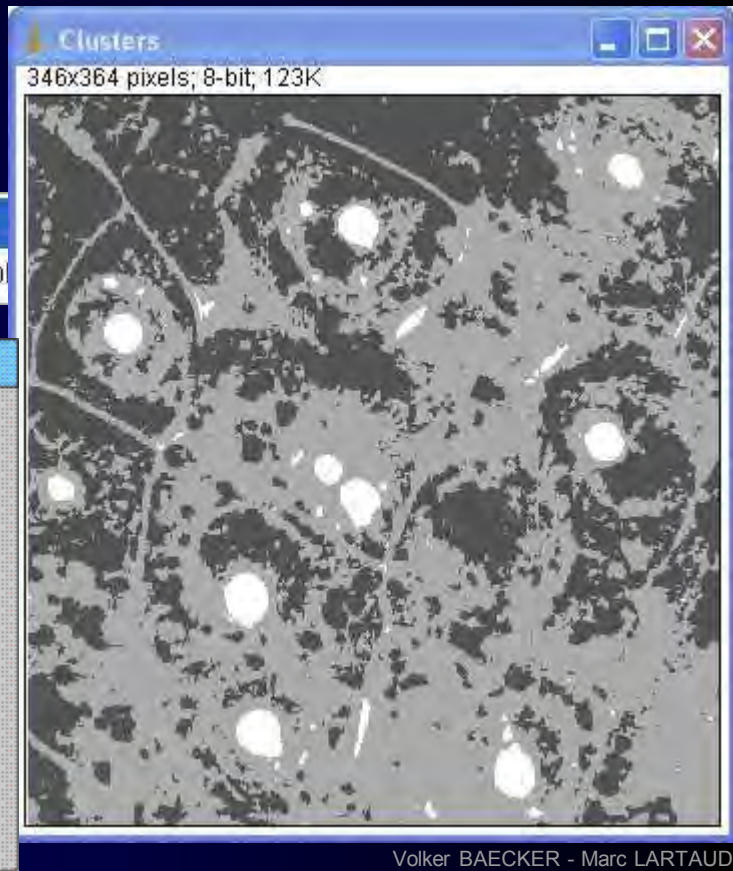


Plugin k-mean clustering









Filtrage avant le seuillage

FFT filtres dans le domaine de Fourier

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Couleur et segmentation séparation RGB

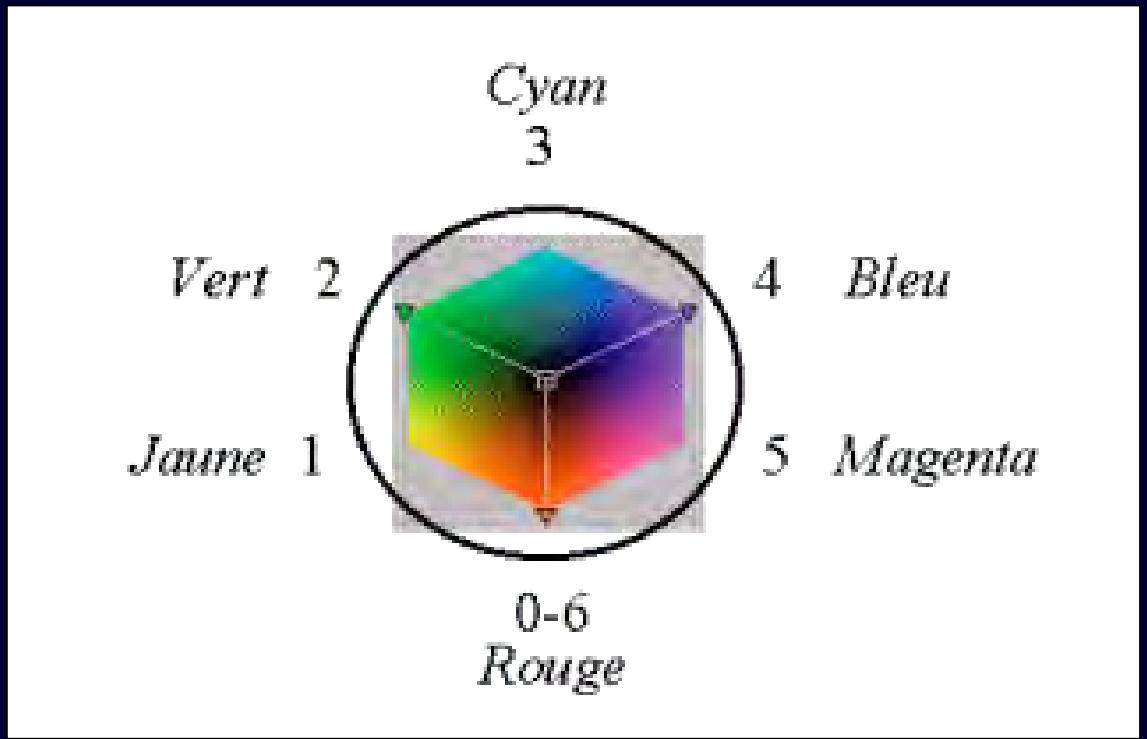
Image→Color→Split Channels...

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..

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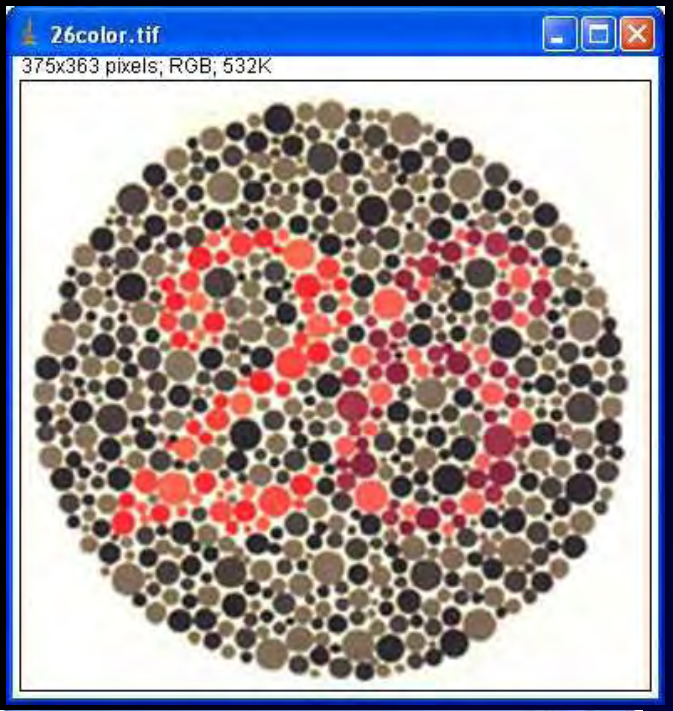
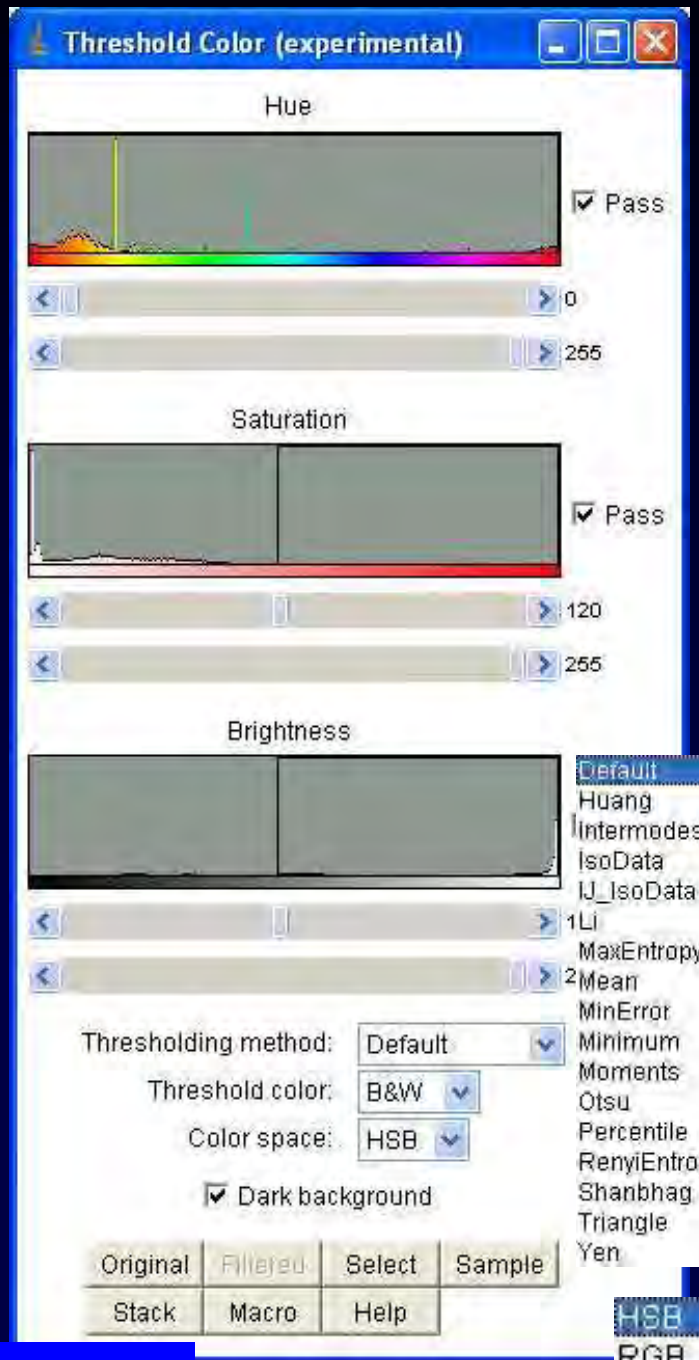
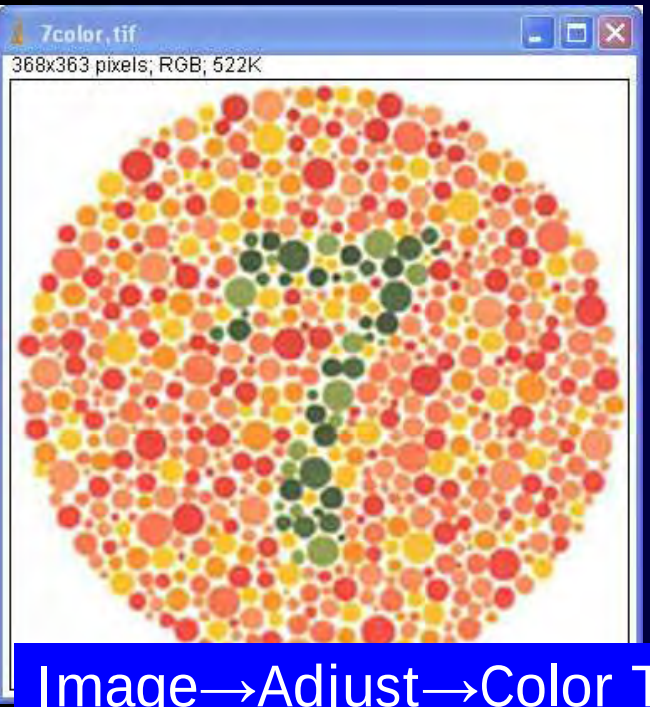
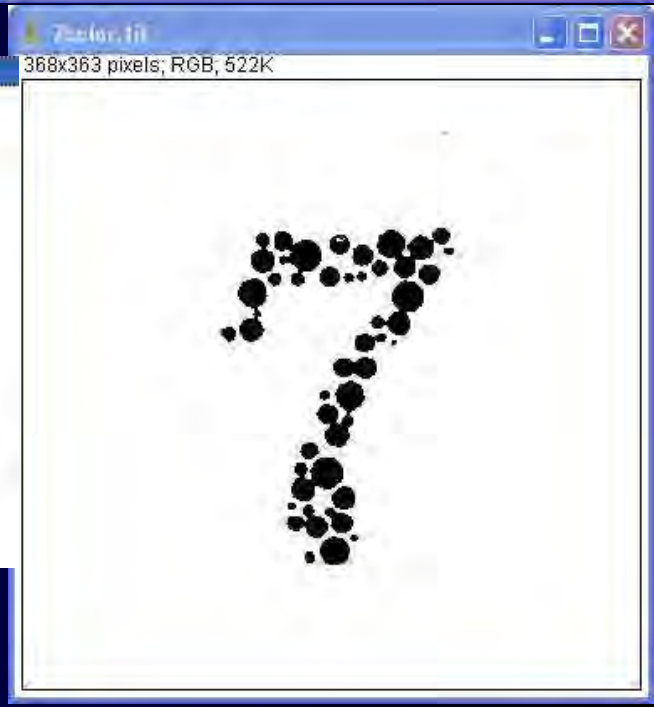
Seuillage couleur



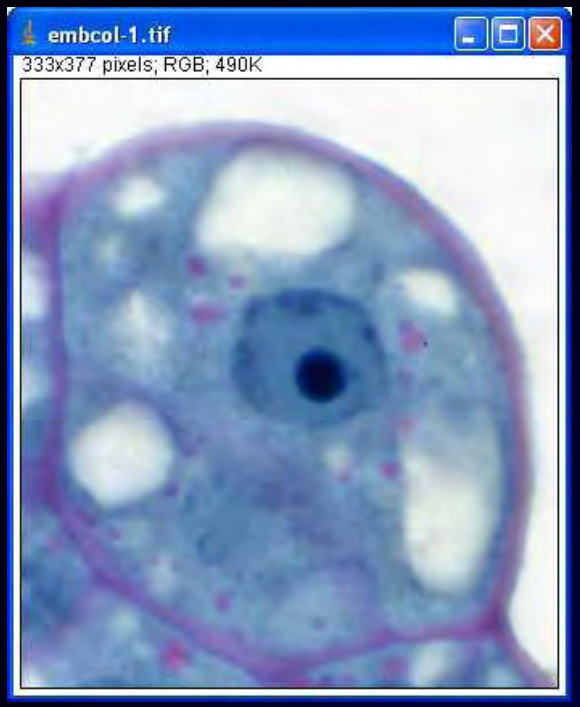


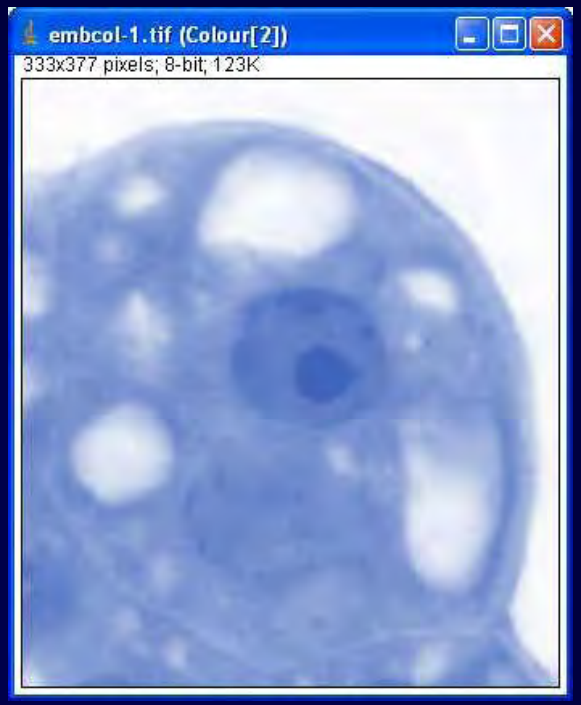




Image→Adjust→Color Threshold..

Plugin Colour Deconvolution





Topic 08 – Segmentation

L’image numérique

Les Prétraitements

La Segmentation

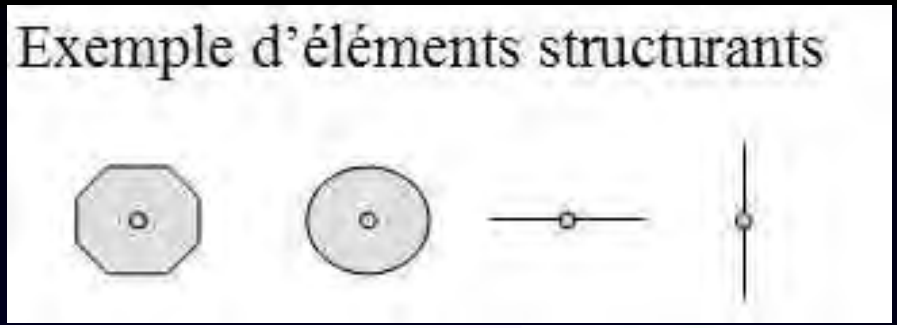
Les Post-traitements

Transformations de morphologie mathématique

La Quantification

Principe

Elément structurant

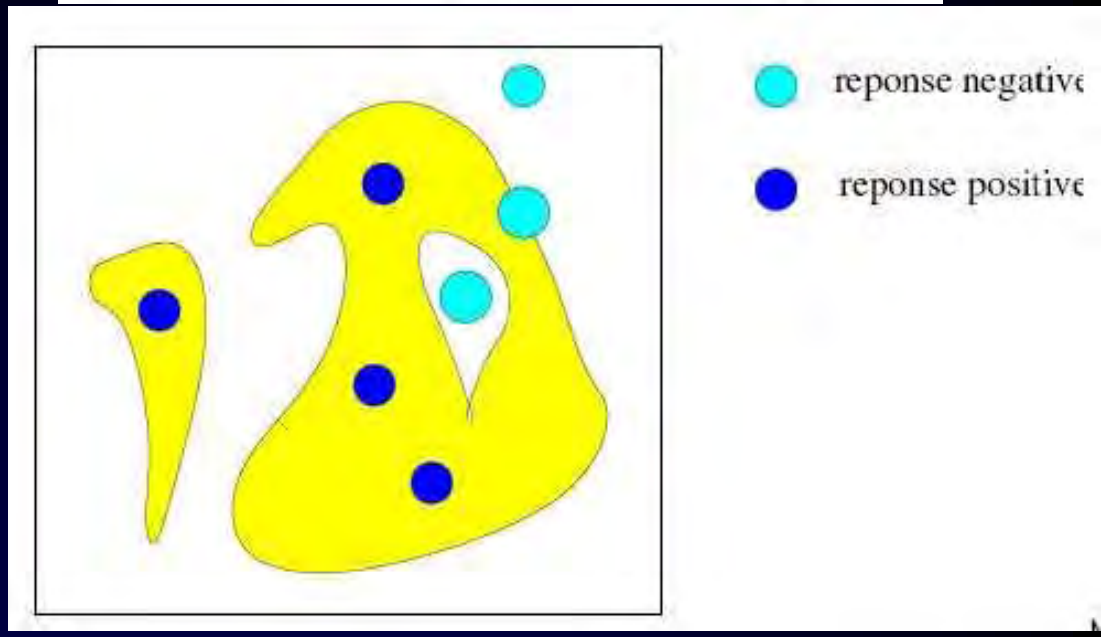


On déplace l'élément structurant sur toute l'image

Le pixel sera noir si:

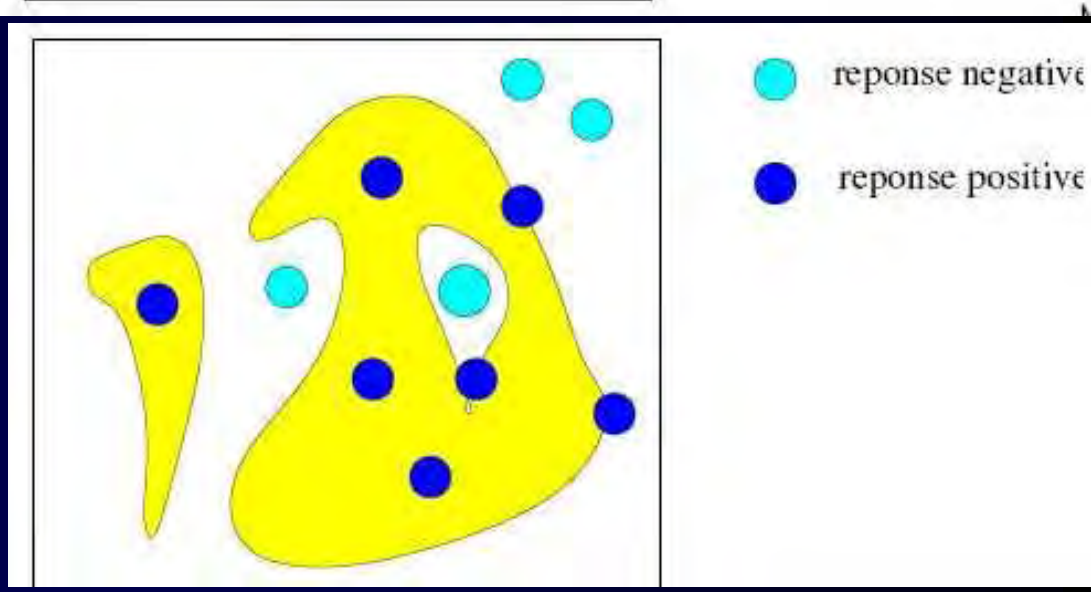
l'élément structurant est inclus dans un objet de l'image

→EROSION



l'élément structurant touche un objet de l'image

→ DILATATION



Morpho_math


panda.tif
286x258 pixels; 8-bit; 72K


erosion
286x258 pixels; 8-bit; 72K


dilatation
286x258 pixels; 8-bit; 72K


ouverture
286x258 pixels; 8-bit; 72K


fermeture
286x258 pixels; 8-bit; 72K

Morpho_math

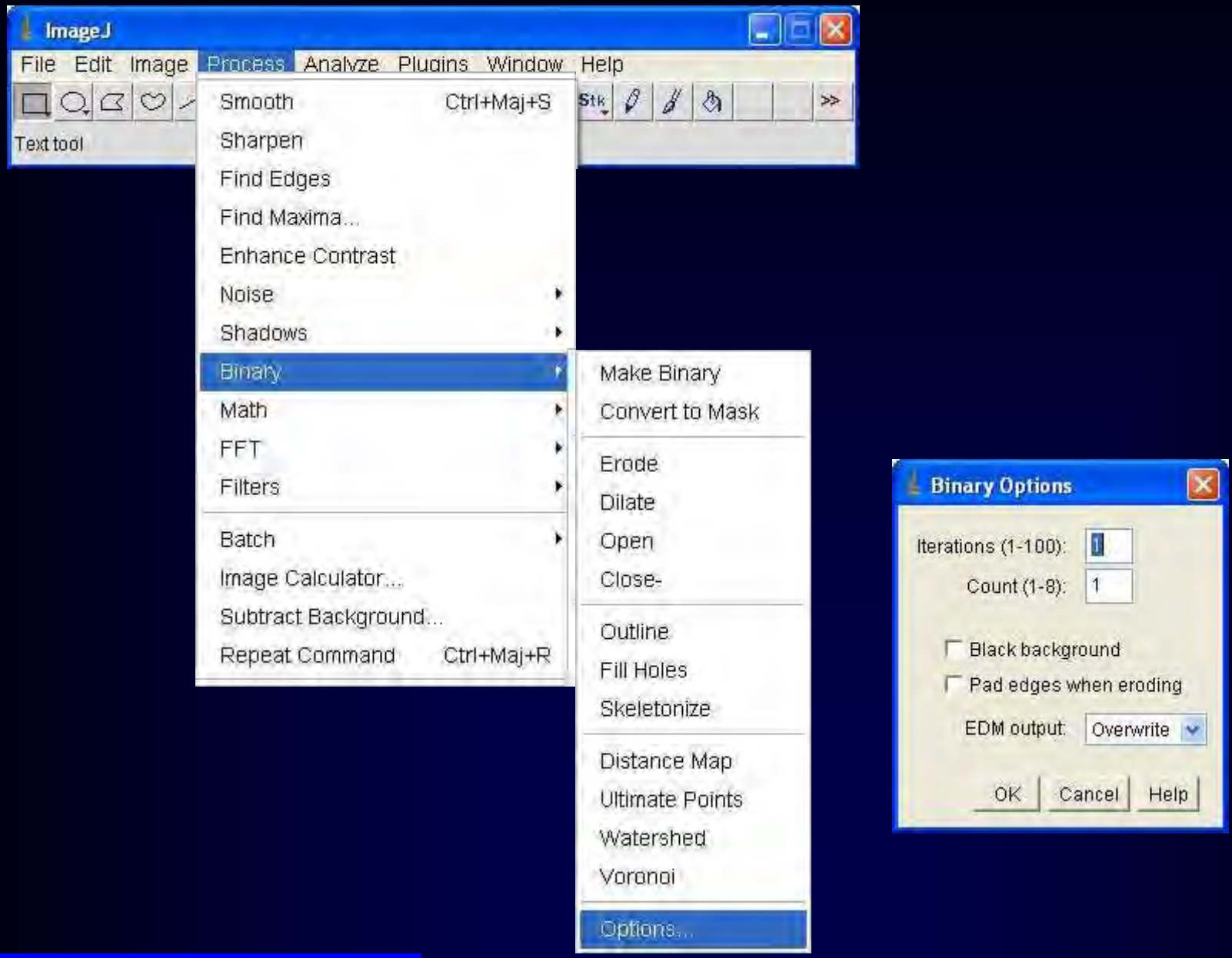

panda.tif
286x258 pixels; 8-bit; 72K


bouche trous
286x258 pixels; 8-bit; 72K


squelette
286x258 pixels; 8-bit; 72K


carte des distances
286x258 pixels; 8-bit; 72K

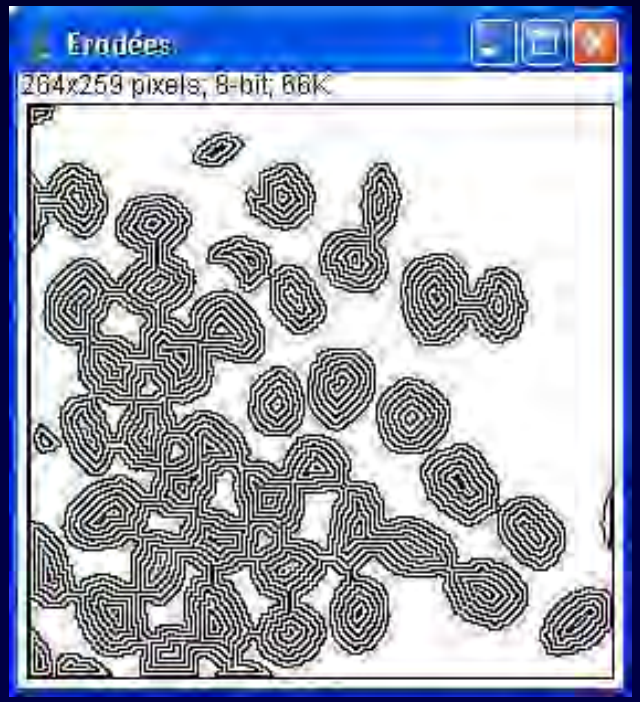
Menu Binary



Process→Binary→Options

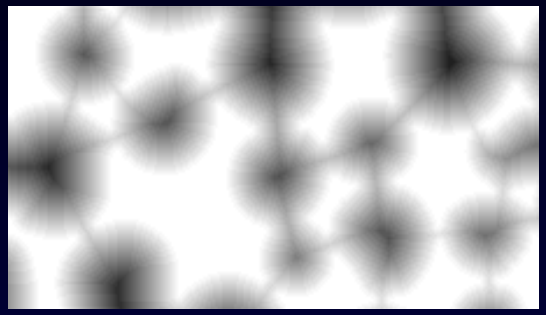
Erodé Ultime

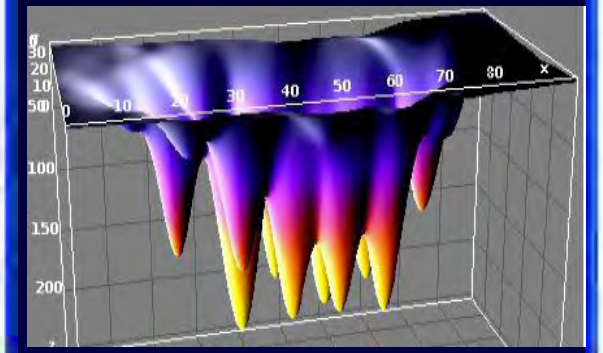
L’**érodé ultime** : la dernière fraction de l ’objet restant avant sa disparition lors d’érosions répétées.

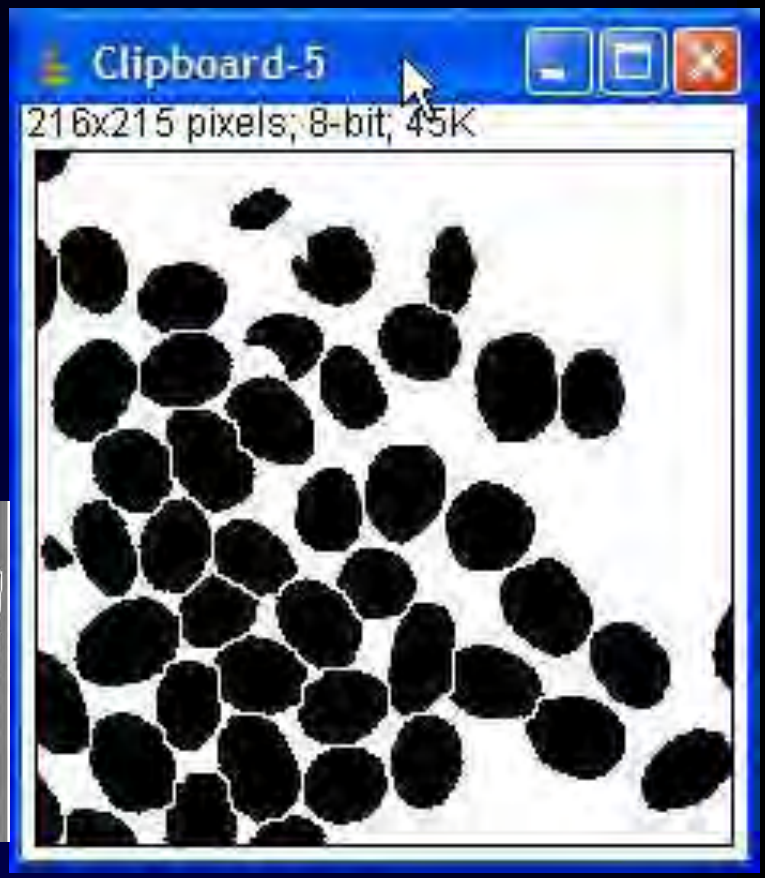


Process→Binary→Ultimate Points

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

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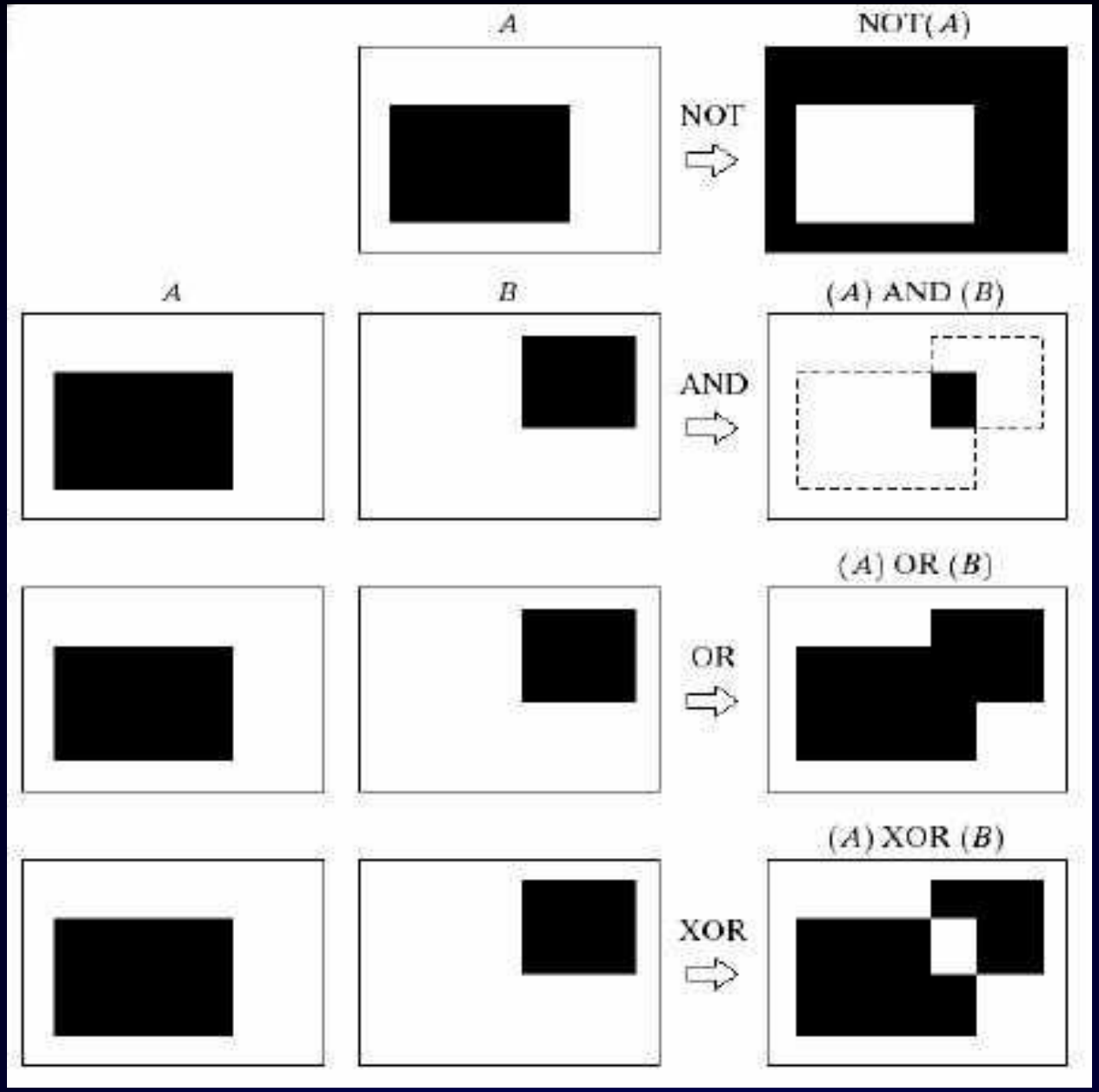
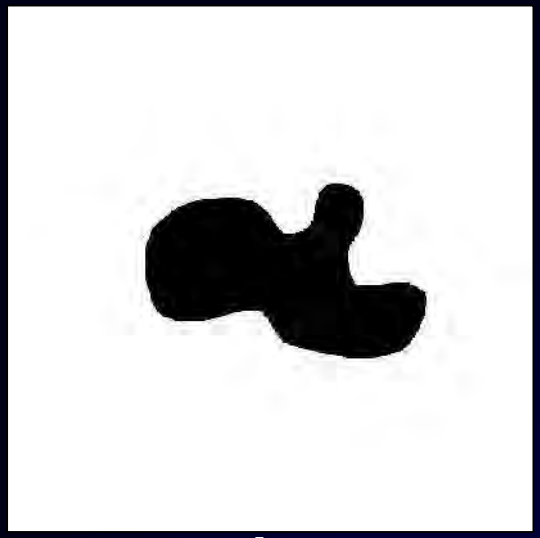
Opérations logiques

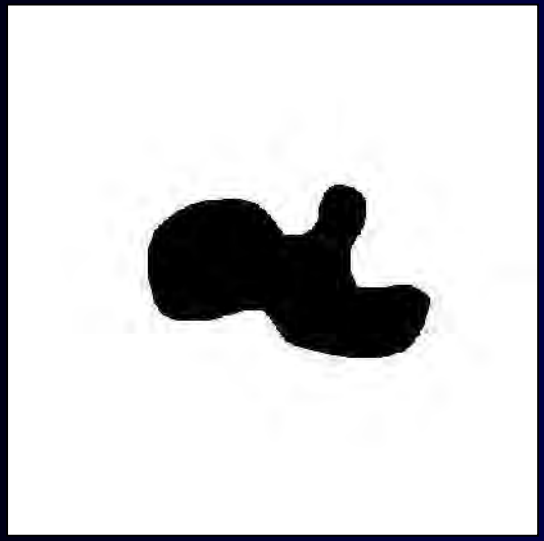


Process→Image Calculator...

Construction d'une fonction contour

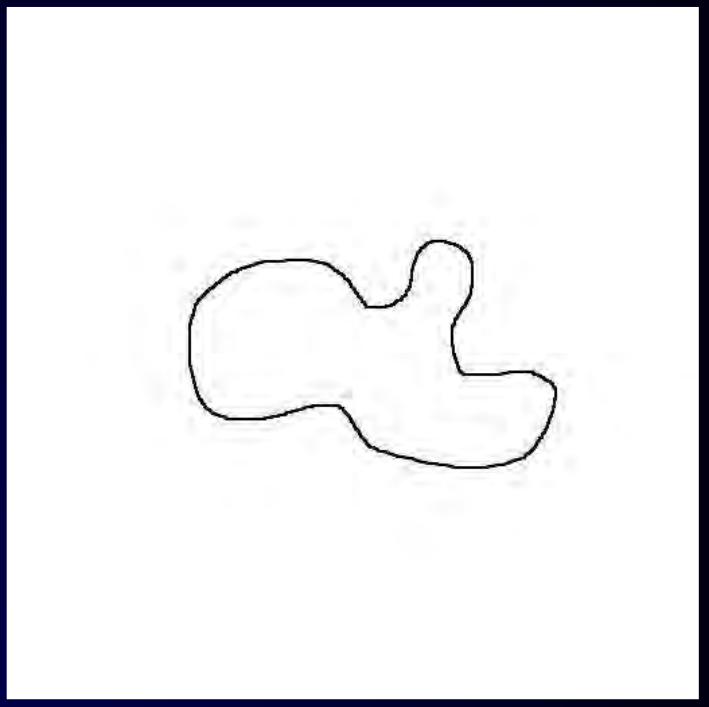


Erosion 1

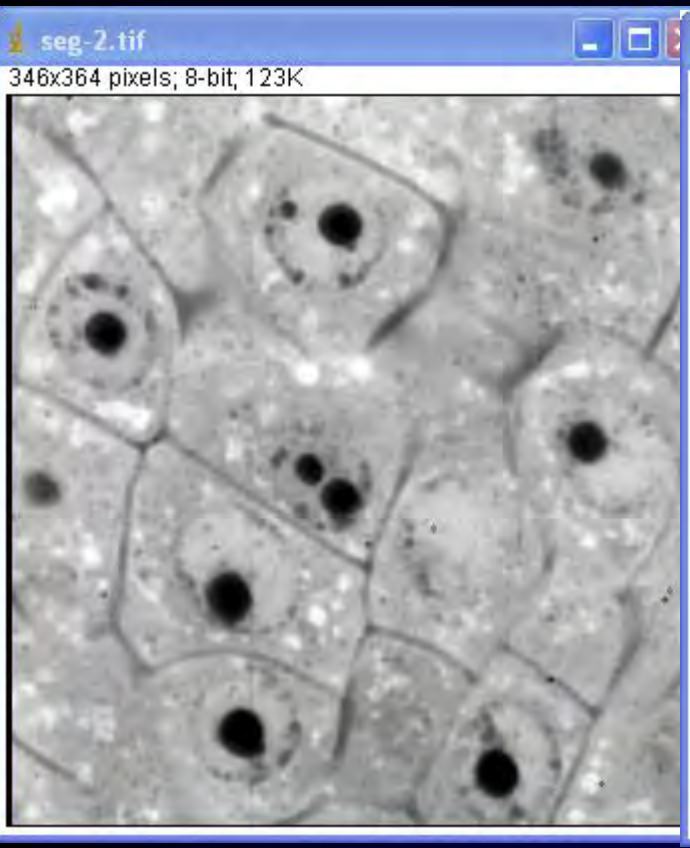


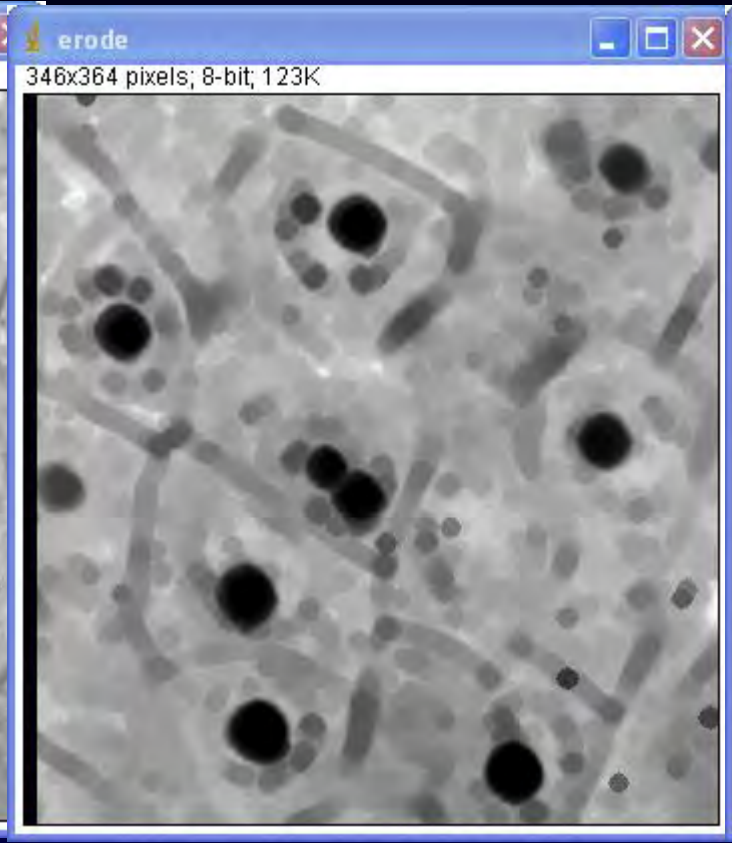
XOR

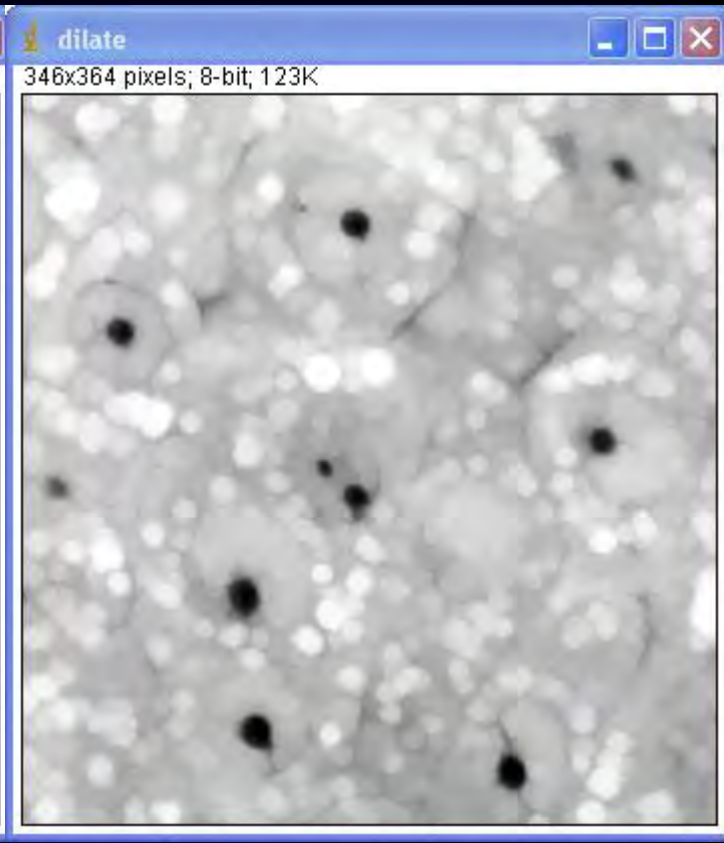
Contour



Morpho_math en niveaux de gris







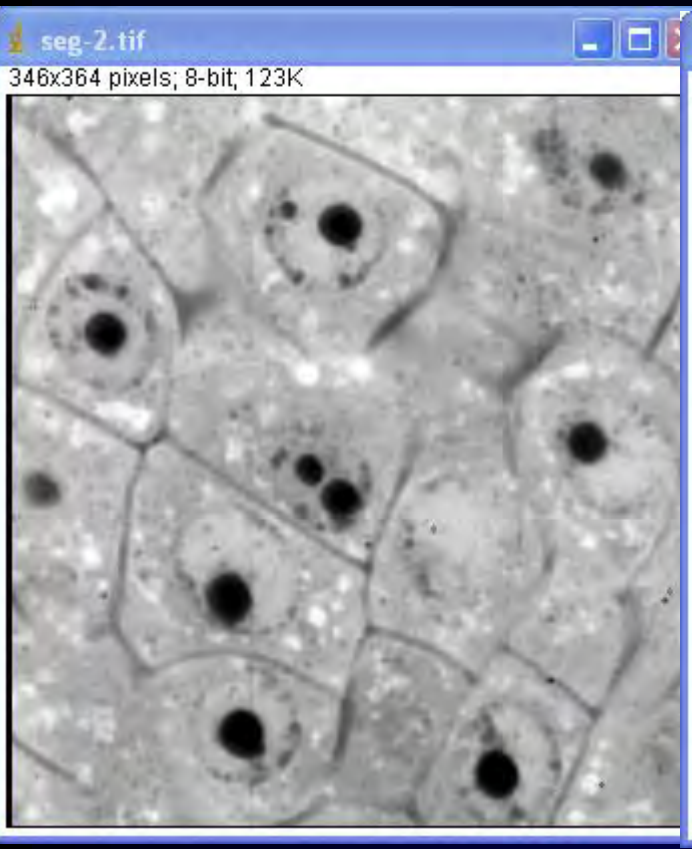
Plugin
Grayscale Morphology

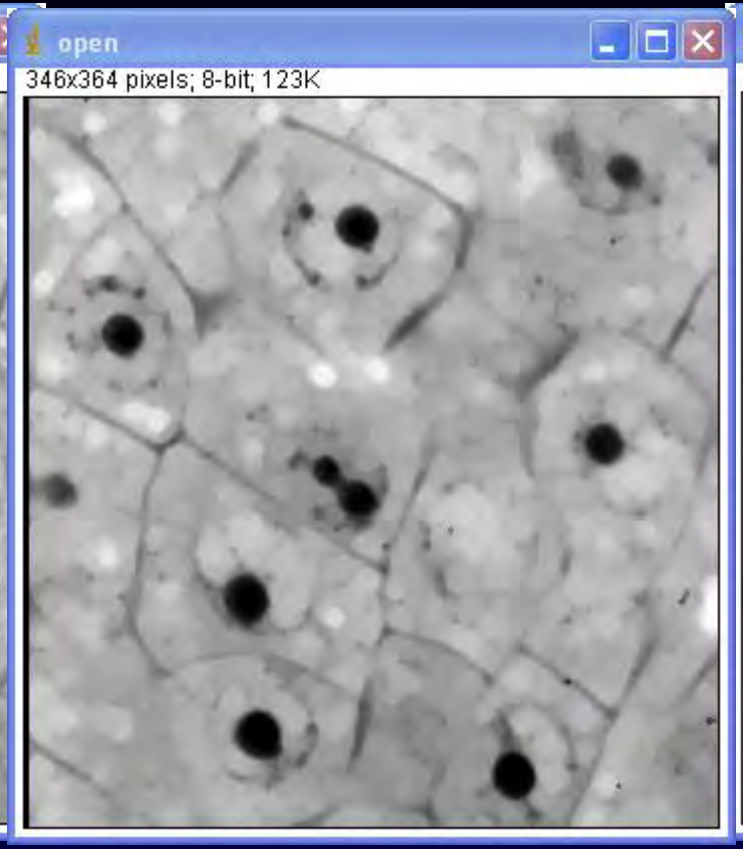
Erosion
agrandit les zones sombres

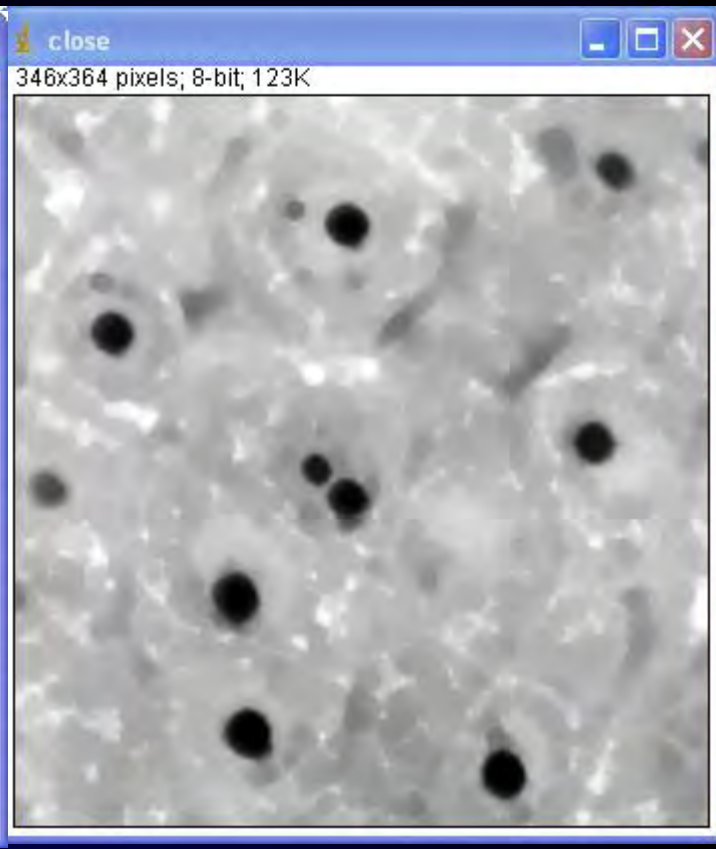
Dilatation
agrandit les zones claires

Plugins→Morphology→Gray Morphology

Morpho_math en niveaux de gris





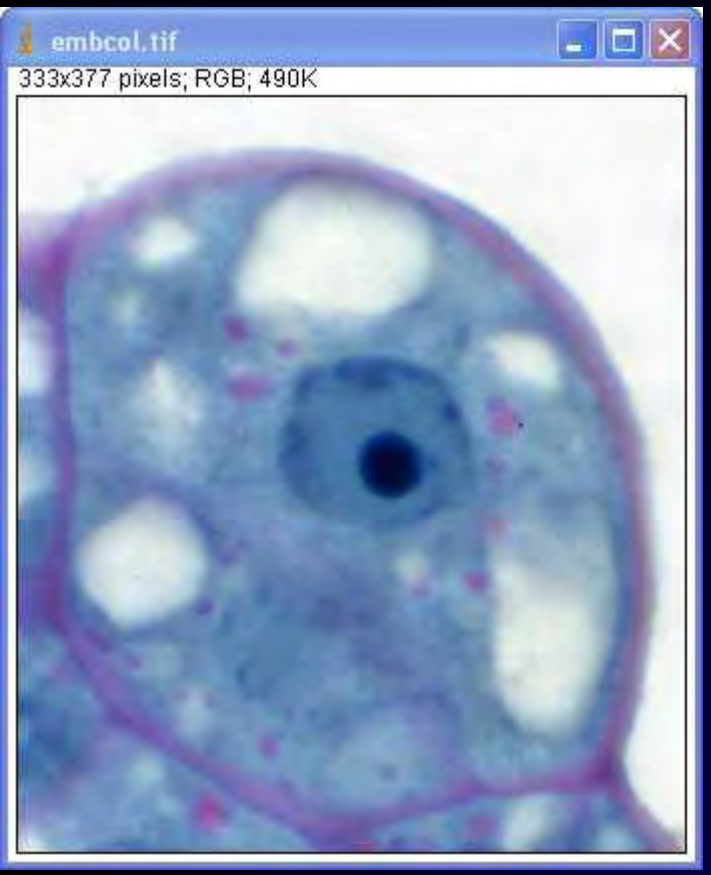


Ouverture
Erosion puis
Dilatation
supprime les petites
zones claires

Fermeture
Dilatation puis
Erosion
supprime les petites
zones sombres

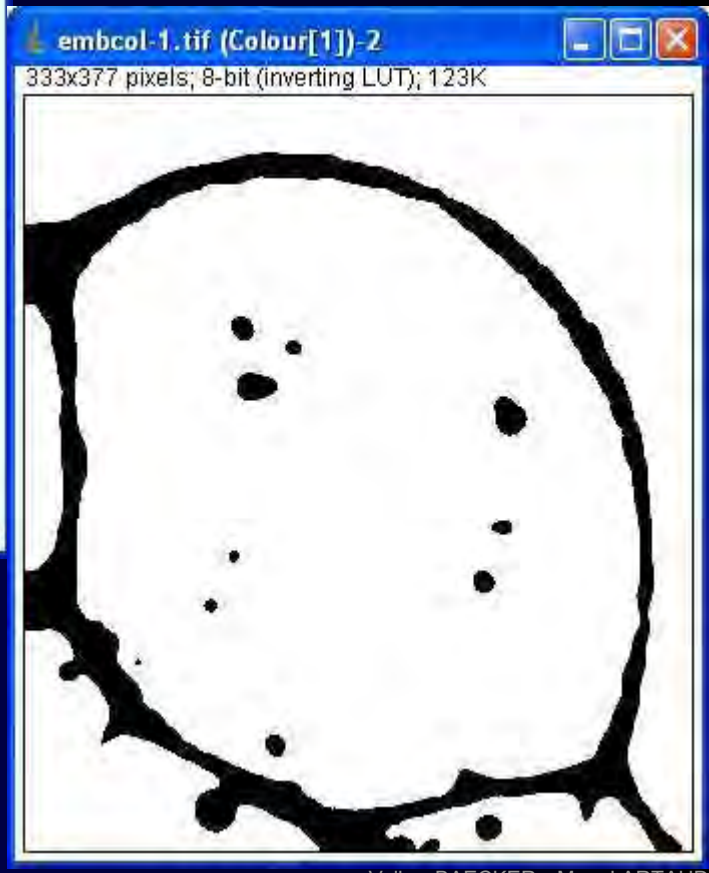
Plugins→Morphology→Gray Morphology

Exemple



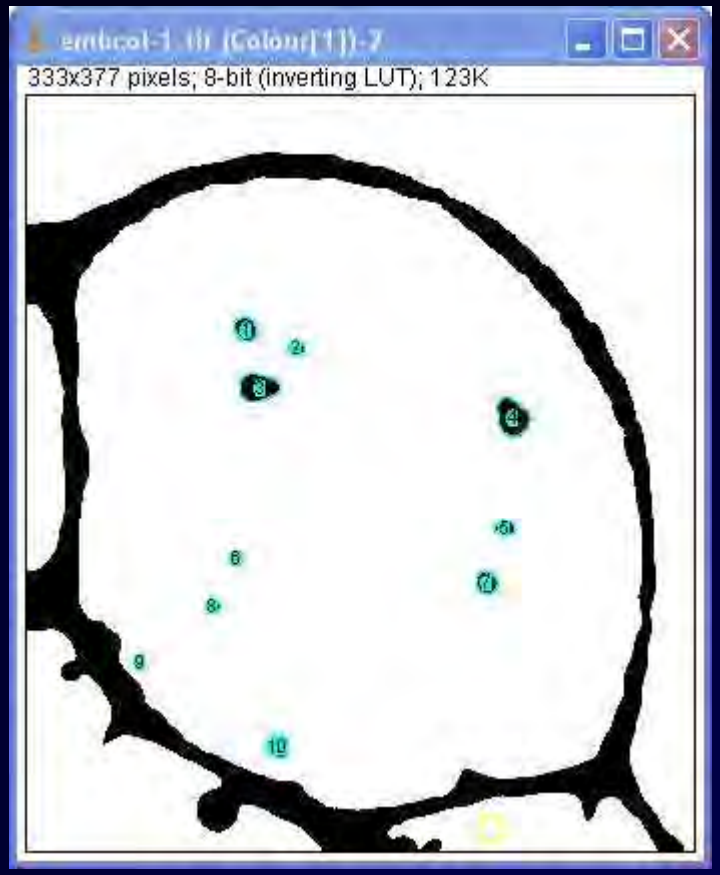
Colour Deconvolution

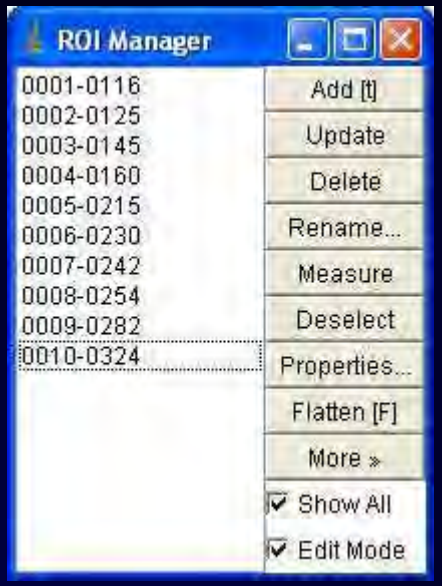
Seuillage



Exemple

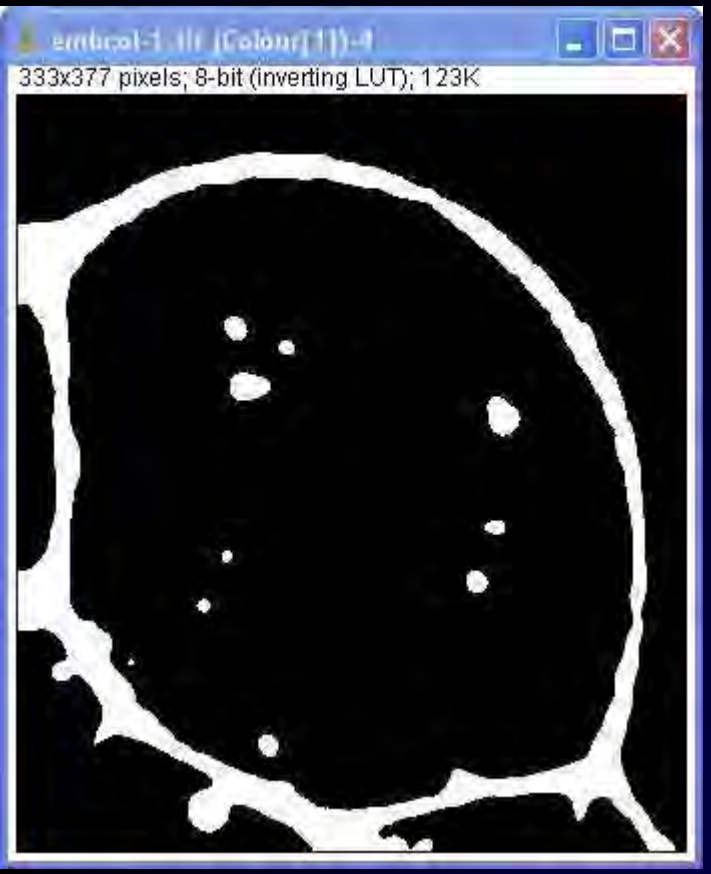
Analyse particules → ROI Manager



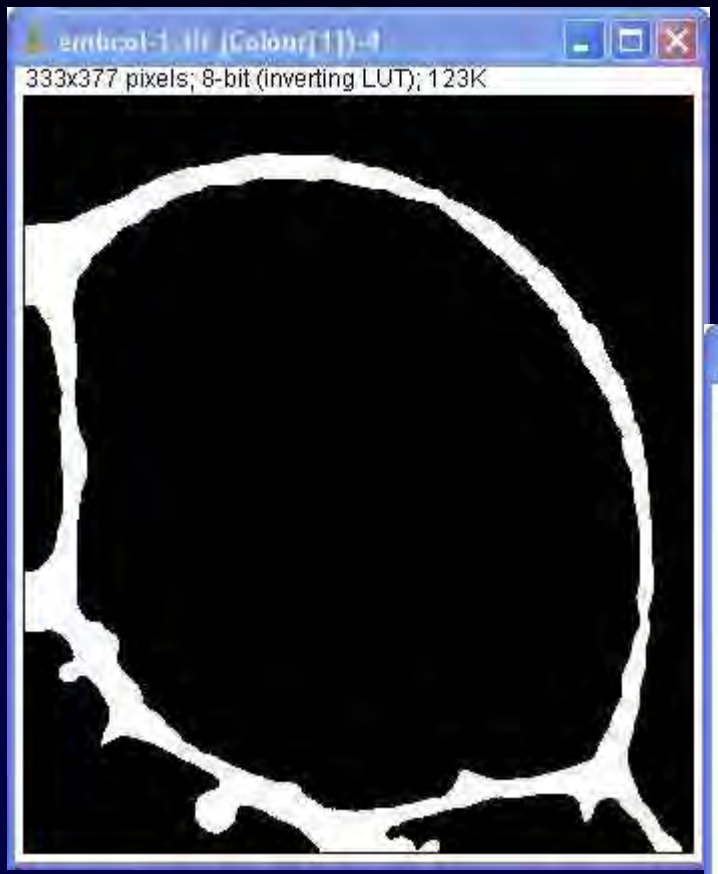


Exemple

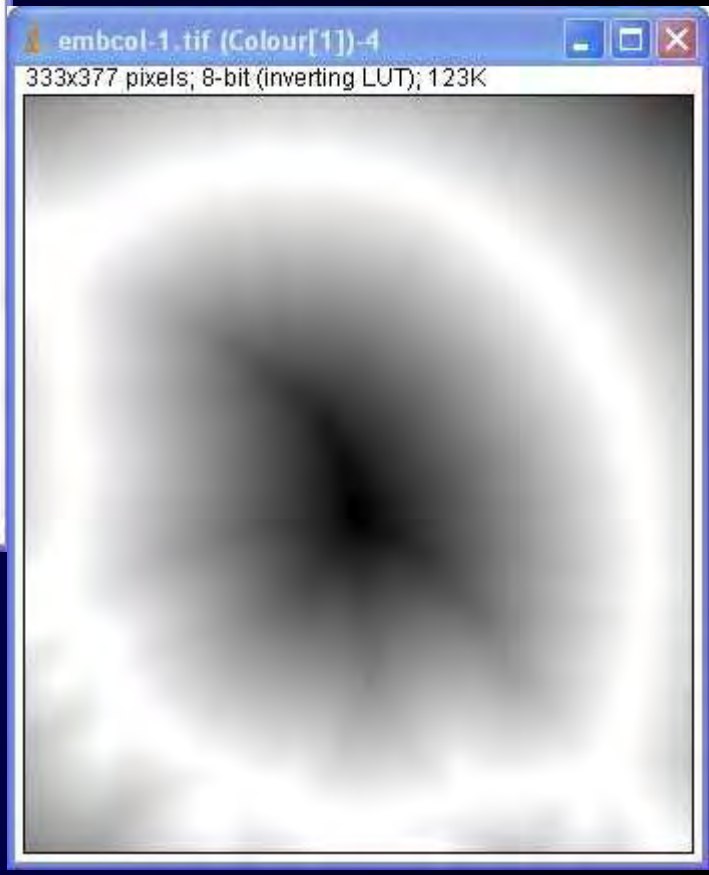
Inversion



Boucher les trous

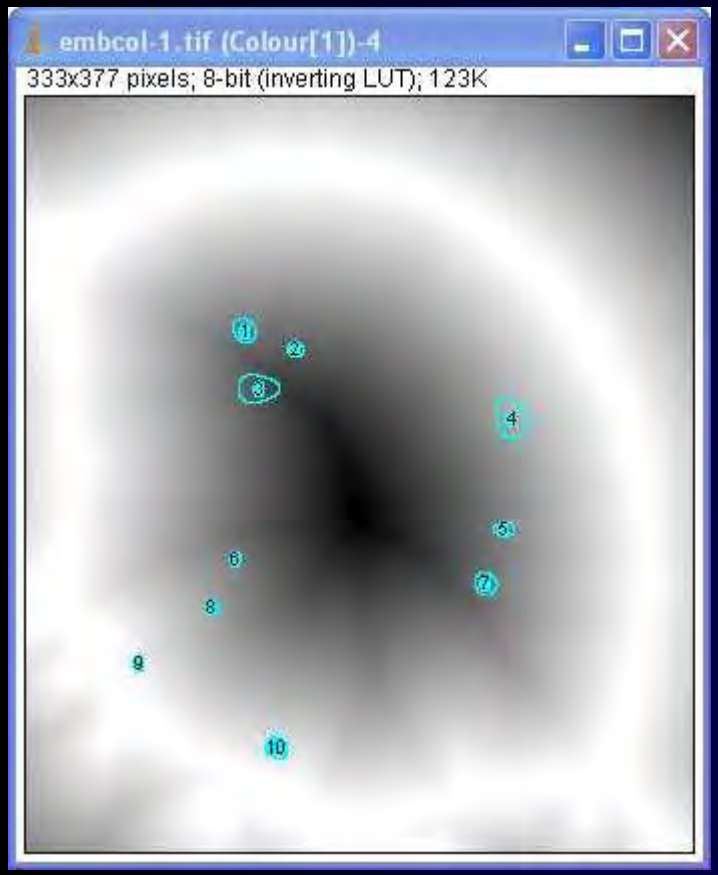


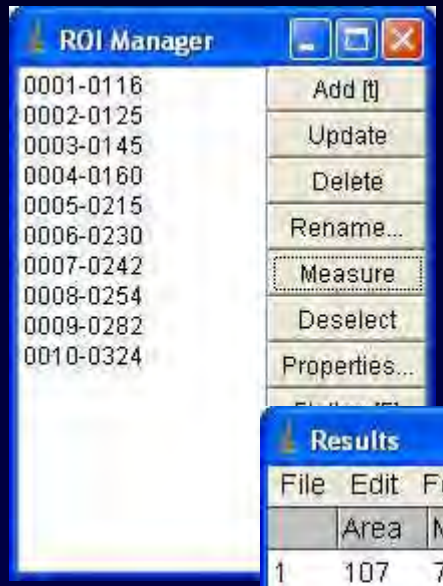
Carte des distances

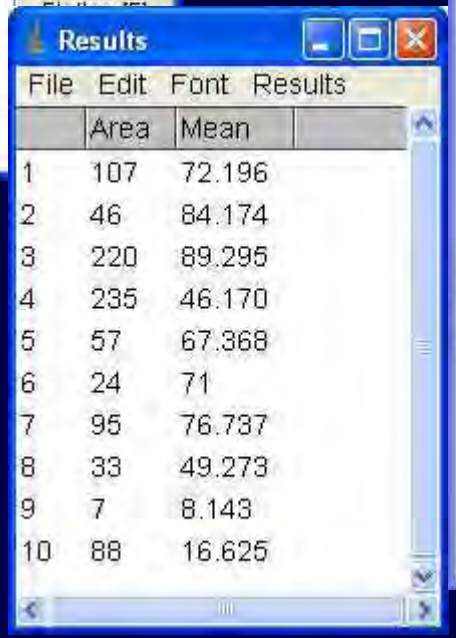


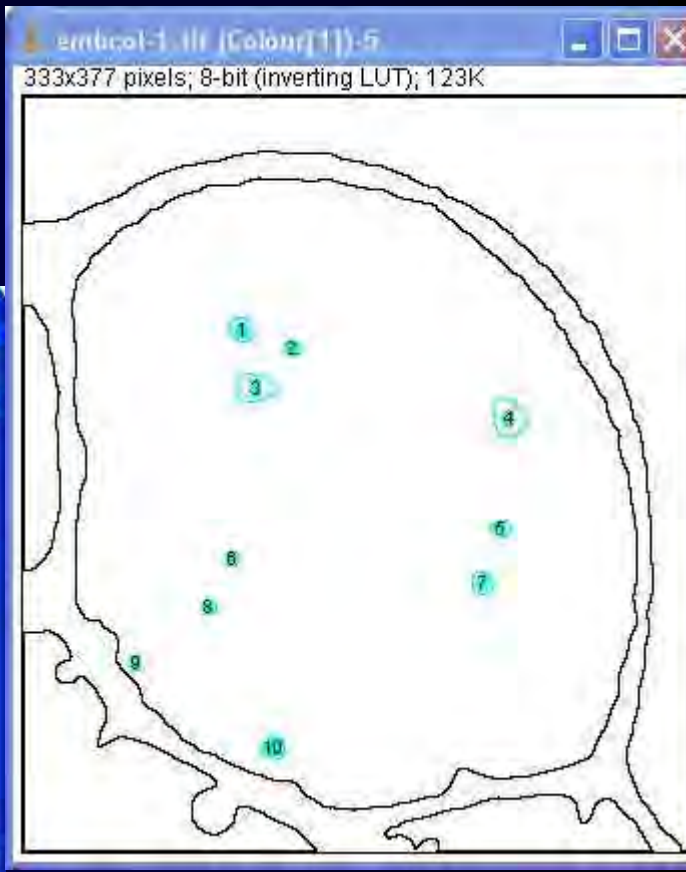
Exemple

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









Topic 09 – Matematical Morphology

L’image numérique

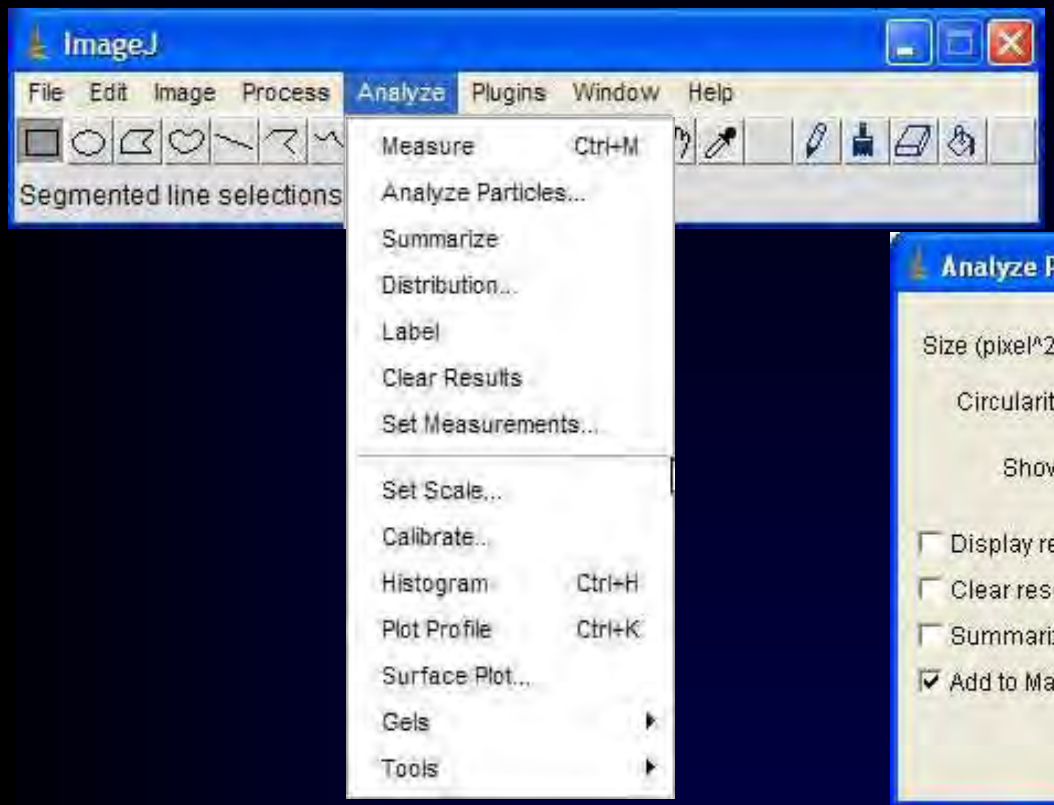
Les Prétraitements

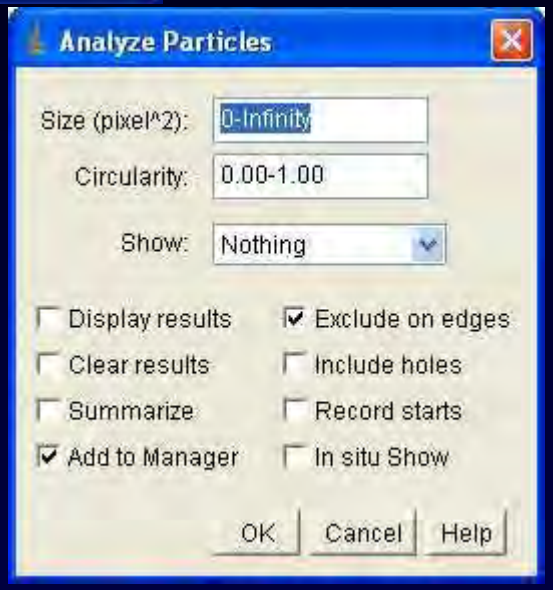
La Segmentation

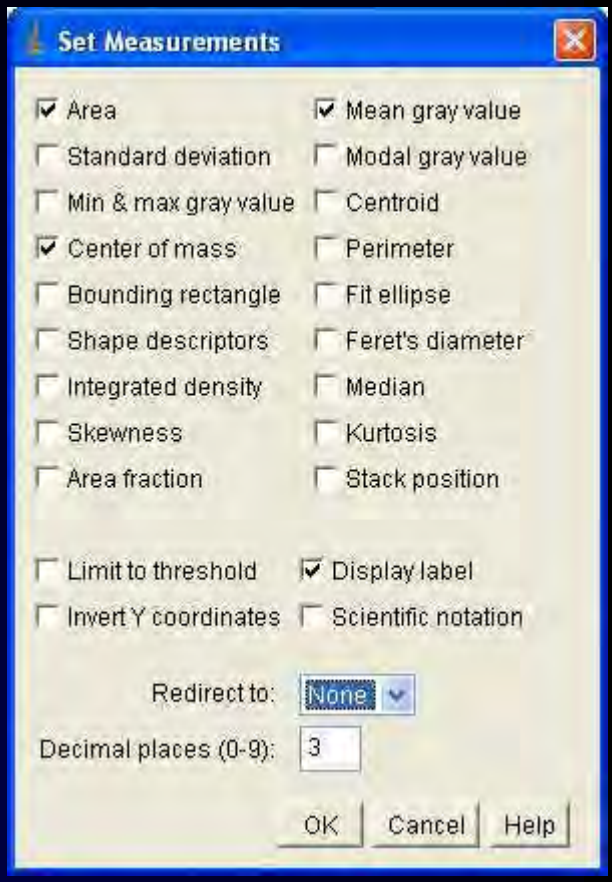
Les Post-traitements

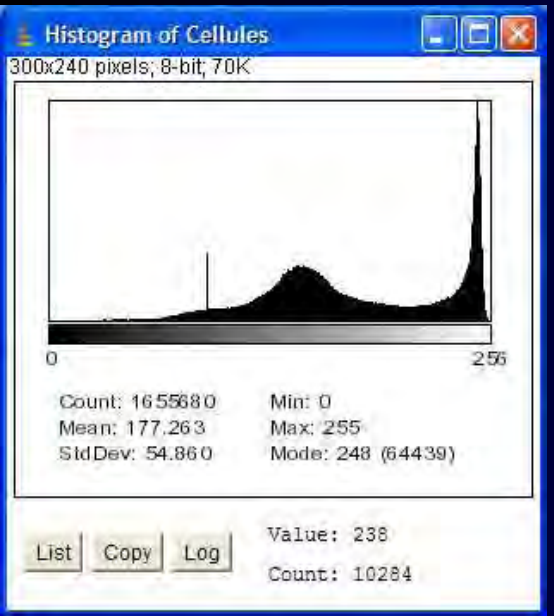
La Quantification

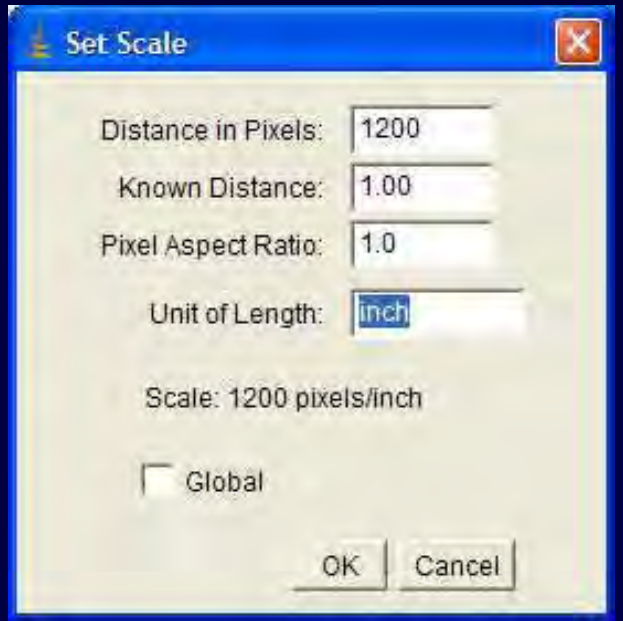
Mesures

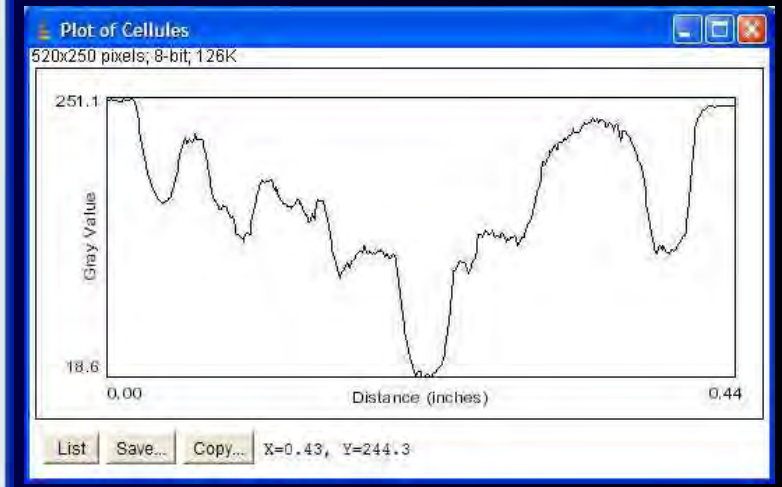

ImageJ
File Edit Image Process Analyze Plugins Window Help
Segmented line selections
Measure Ctrl+M
Analyze Particles...
Summarize
Distribution...
Label
Clear Results
Set Measurements...
Set Scale...
Calibrate...
Histogram Ctrl+H
Plot Profile Ctrl+K
Surface Plot...
Gels
Tools


Analyze Particles
Size (pixel^2): 0-Infinity
Circularity: 0.00-1.00
Show: Nothing
Display results
Clear results
Summarize
Add to Manager
Exclude on edges
Include holes
Record starts
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: None
Decimal places (0-9): 3
OK Cancel Help


Histogram of Cellules
300x240 pixels; 8-bit; 70K
0
256
Count: 1655680
Mean: 177.263
StdDev: 54.860
Min: 0
Max: 255
Mode: 248 (64439)
List Copy Log
Value: 238
Count: 10284


Set Scale
Distance in Pixels: 1200
Known Distance: 1.00
Pixel Aspect Ratio: 1.0
Unit of Length: inch
Scale: 1200 pixels/inch
Global
OK Cancel


Plot of Cellules
520x250 pixels; 8-bit; 126K
251.1
18.6
Gray Value
0.00
Distance (inches)
0.44
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 : 4pi(surface /périmètre²) = 1 pour un cercle
- Etc ...

Topic 10 – Quantification