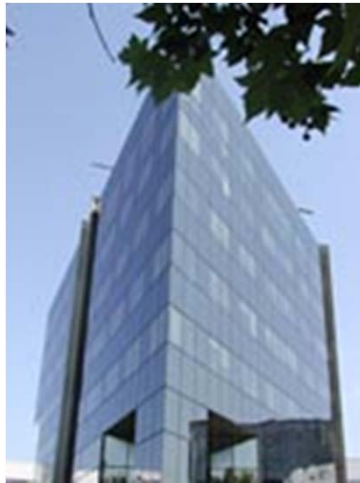


INTEGRATION de l'IMAGESTREAMX (AMNIS) DANS LE POLE LYONNAIS

Structure d'accueil
Tour CERVI



Plateau technique AniRA-Cytométrie

US8/UMS 3444/SFR Gerland Biosciences



RAQ : Patricia Barbot
patricia.barbot@inserm.fr

ERGONOMIE DU PLATEAU TECHNIQUE PRINCIPAL DE CYTOMETRIE ET INTEGRATION DE L'IMAGESTREAM X

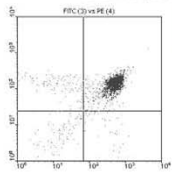
Imagestream X



Station de traitement des données

APPORT DE LA CYTOMETRIE D'IMAGE (IMAGESTREAM X)

Cytométrie

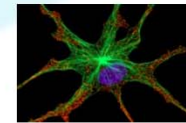


Instrument
interdisciplinaire

Imagerie en flux



Microscopie



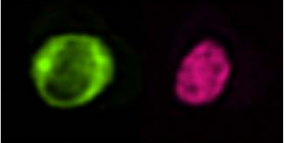
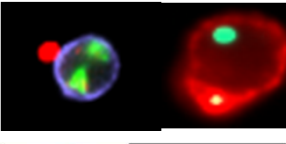
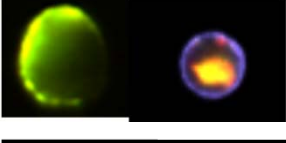
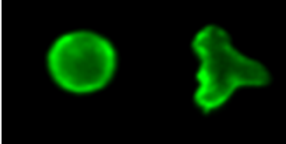
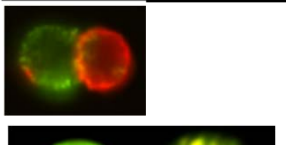
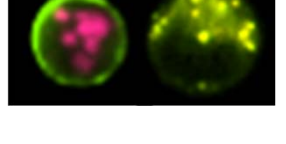
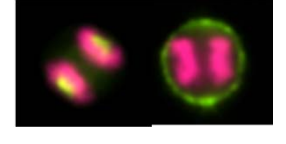
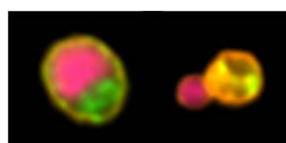
Acquisition rapide

Quantification numérique d'image

Analyse de populations rares

Analyse statistique sur un grand nombre de cellules

APPLICATIONS : Microbiologie, Immunologie, Parasitologie....

	ETUDES	EXEMPLES
	Signalisation cellulaire	Translocation NFkB , Localisation
	Internalisation et phagocytose	Internalisation, phagocytose de bactéries par des monocytes
	Colocalisation intracellulaire	Colocalisation de ligands avec les lysosomes
	Etude de forme	Differentiation cellulaire
	Interaction entre cellules	Formation de synapse
	Mort cellulaire et autophagie	Apoptose, fragmentation nucléaire, Activation caspase3
	Cycle cellulaire et mitose	Classification morphologique des phases de mitose
	Cellules souches	Différenciation erythrocytaire

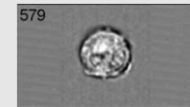
CONFIGURATION SELECTIONNEE DE L'IMASTREAM X - PHENOCAN

- ❖ **4 lasers:** - Violet (405nm)
- Bleu (488nm)
- Vert (561 nm)
- Rouge (642nm)



- ❖ **12 paramètres simultanés:**

2 x « brightfields »

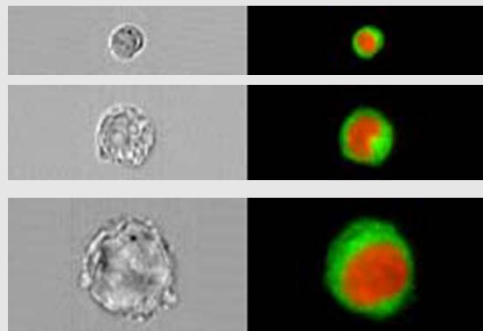


1 «darkfield » Ssc



9 fluorescences

❖ **3 tailles d'objectifs**



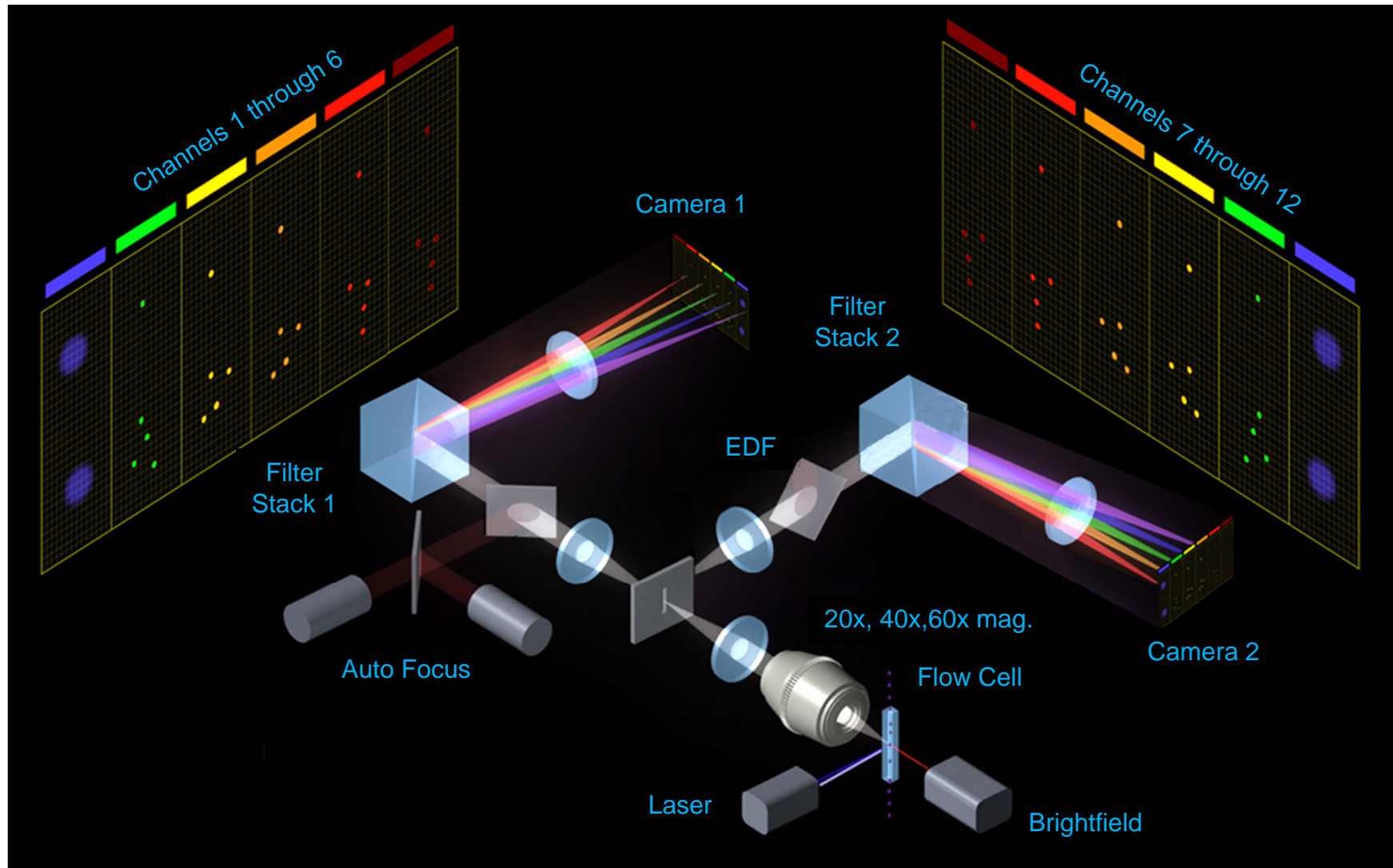
x20 (1 μm /pixel)

x40 (0.5 μm /pixel)

x60 (0.33 μm /pixel)

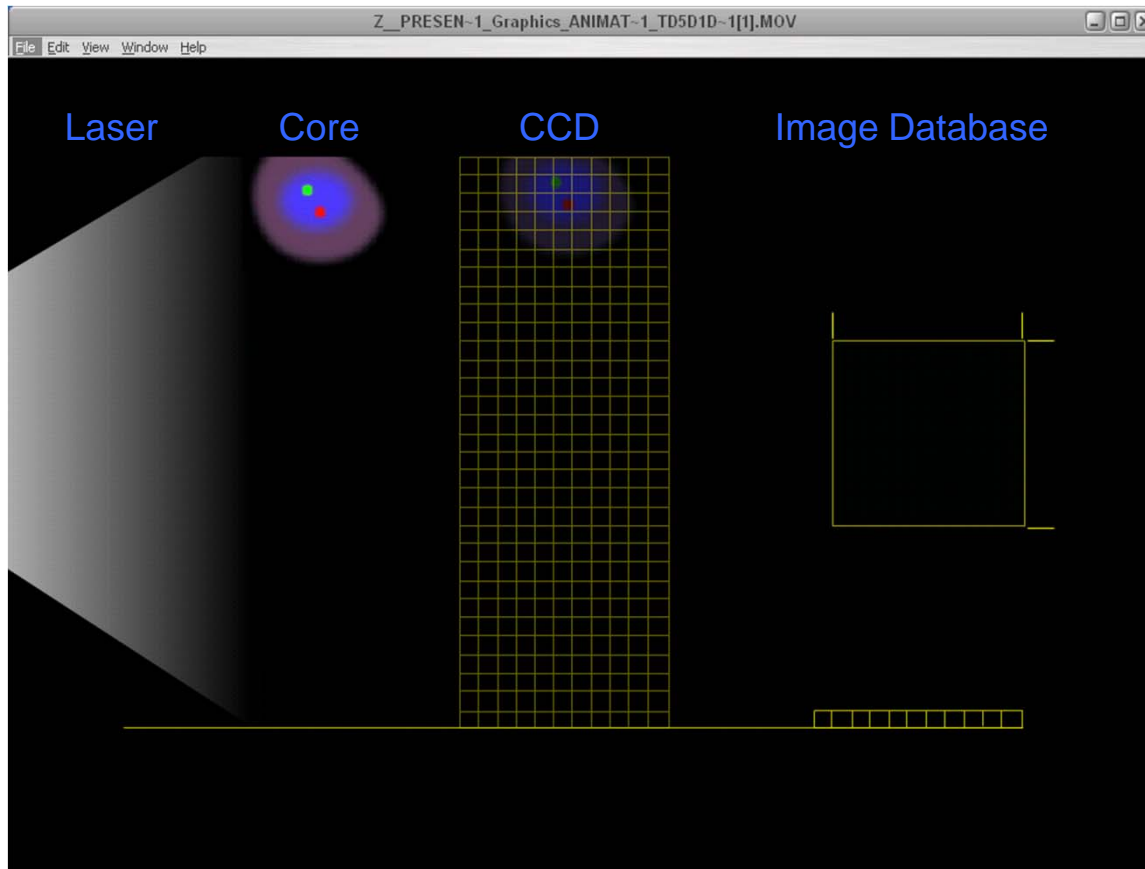
❖ **Module d'extention de champs « Extended Depth of Field » (EDF)**

BANC OPTIQUE: SCHEMA GENERAL



BANC OPTIQUE: INTEGRATION DU SIGNAL

Principe d'intégration du signal de fluorescence ou "Time Delay Integration"



CAMERA - TDI CCD

- Fluorescence recueillie durant toute la durée de passage devant le détecteur
- La lumière est détectée sur la première ligne de pixels, puis transférée au pixel situé au dessous en synchronisation avec la vitesse de passage de la cellule
- L'intensité lumineuse est intégrée sur toute la hauteur du détecteur

Augmentation de la sensibilité en conservant une image nette

Augmentation du débit= perte de sensibilité

PREPARATION DES ECHANTILLONS ET ACQUISITION DES DONNEES

➤ Préparation des échantillons

- ✓ Similaire à la cytométrie
- ✓ Simple marquage (compensation)
- ✓ Passage en **ependorf 1.5 ml**, 20µl/échantillons
- ✓ Concentration cellulaire: **10-100 M/ml**

➤ Acquisition des données

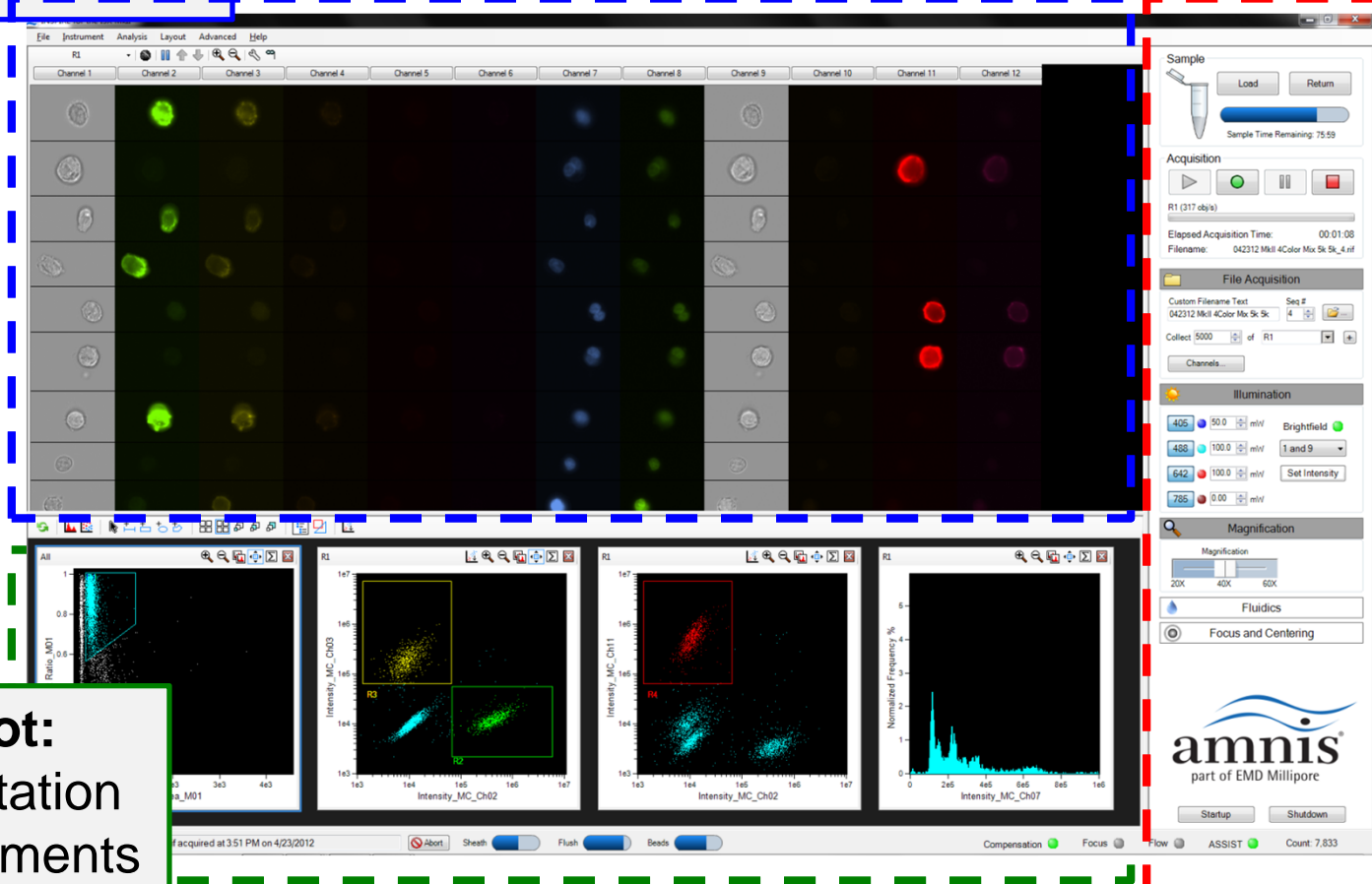
- ✓ Logiciel **Inspire**
- ✓ **1 000 evts/sec** maximum (moyenne de 5 min par tubes)
- ✓ Compensation automatique
- ✓ Simple d'utilisation

ACQUISITION DES DONNEES

Logiciel d'acquisition : **INSPIRE**

Galerie d'images:
Affichage en temps
réel

**Contrôle de
l'instrument:**

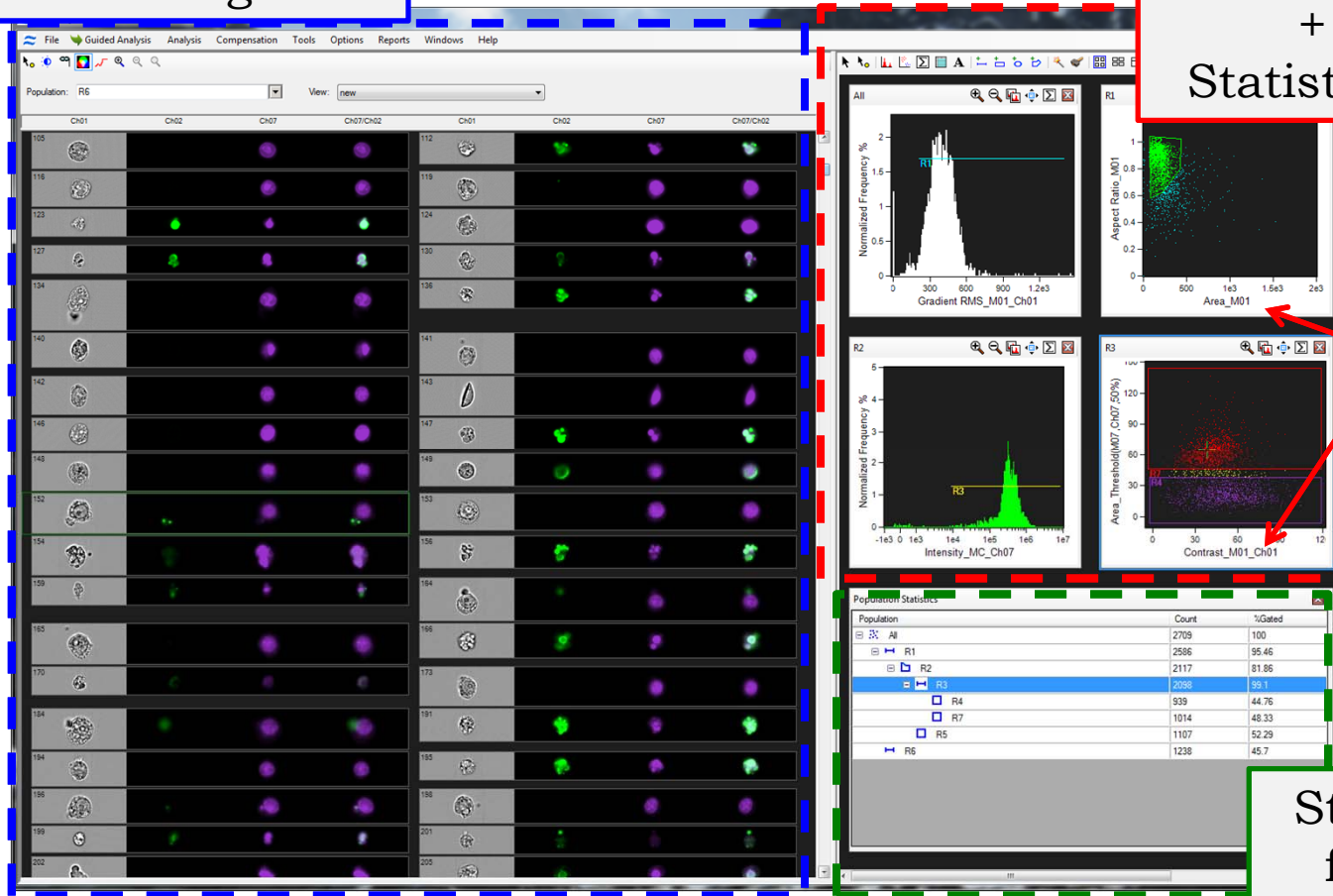


Dot plot:
Incrémentation
des événements
en temps réel

ANALYSE DES DONNEES 1/3

Logiciel d'analyse: IDEAS

Galerie d'images



Graphique
+
Statistique

Paramètres
de
quantification
des images

Stratégie de
fenêtrage

ANALYSE DES DONNEES 2/3

LISTE NON EXHAUSTIVE DES PARAMETRES DE QUANTIFICATION DES IMAGES

TAILLES (en microns)

Aire, Diamètre, Longueur ...

FORMES

Circularité, « Shape Ratio » ...

LOCALISATION (coordonnées X,Y)

Centroides XY ...

TEXTURE:
mesure des variation d'intensité locales

Comptage de spots, Gradient RMS ...

INTENSITE de signal

Intensité, Moy. Pixel, Intensité des spots....












COMPARAISON

Similarité, « Brigh Detail Similiraity » ...

ANALYSE DES DONNEES 3/3

WIZARD : UN OUTIL D'ANALYSE ASSISTEE












Select the wizard to use for analysis:

	Open File	Creates a template to facilitate analysis.
	Display Properties	Automatically sets image display properties.
	Begin Analysis	Identifies single, focused, fluorescent positive cells.
	Feature Finder	Assists the user in picking relevant features for separating populations. The file must contain members of each population.
	Apoptosis	Creates an analysis template for identifying apoptotic events based on brightfield and nuclear morphology.
	Cell Cycle - Mitosis	Creates an analysis template that distinguishes mitotic and apoptotic events.
	Co-localization	Creates an analysis template for measuring the co-localization of two probes on, in, or between cells in your sample.
	Internalization	Creates an analysis template for measuring the internalization of a probe.
	Nuclear Localization	Creates an analysis template for measuring the nuclear localization of a probe.
	Shape Change	Creates an analysis template for measuring circular morphology.
	Spot	Creates an analysis template for measuring texture based on spot counting.

ANALYSE DES DONNEES 3/3

WIZARD : UN OUTIL D'ANALYSE ASSISTEE

Select the wizard to use for analysis:

	Open File	Creates a template to facilitate analysis.
	Display Properties	Automatically sets image display properties.
	Begin Analysis	Identifies single, focused, fluorescent positive cells.
	Feature Finder	Assists the user in picking relevant features for separating populations. The file must contain members of each population.
	Apoptosis	Creates an analysis template for identifying apoptotic events based on brightfield and nuclear morphology.
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	Shape Change	Creates an analysis template for measuring circular morphology.
	Spot	Creates an analysis template for measuring texture based on spot counting.

« non supervisé » : détecteur de paramètres