Reconstruction d'embryons à partir d'images 4D

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Module Image - LIRMM - ICAR
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Plan présentation

1 Introduction Biologie du développement

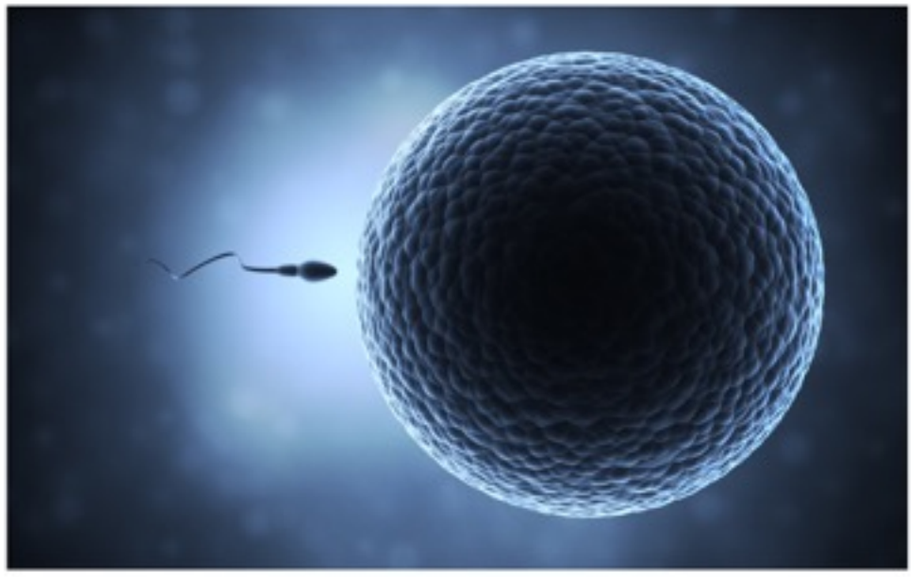
2 Protocoles d’acquisition et images 4D

3 Reconstruction phénoménologique de la morphogenèse à partir d’images 4D
(Filtres, Détection, Segmentation, Suivi cellulaire)

4 Visualisation de données 4D

5 Validation de données

Embryogenèse



?

Un peu d'histoire …

IV siècle a.v. J.C.

Théorie de la Préformation

Dans l'œuf, l'adulte préexiste
en version miniature

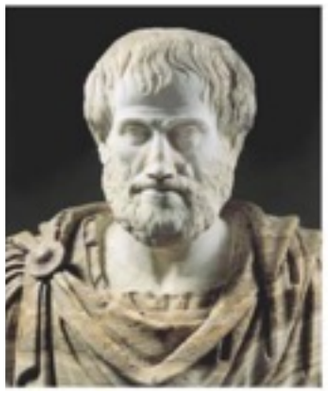
Préformationismes

Ovistes

Amimalculistes

Théorie Epigénétique

De nouvelles structures
s'ajoutent progressivement
au cours du développement



Aristote

Hypocrate pensait que nous héritions de petits morceaux de toutes les parties de nos parents.

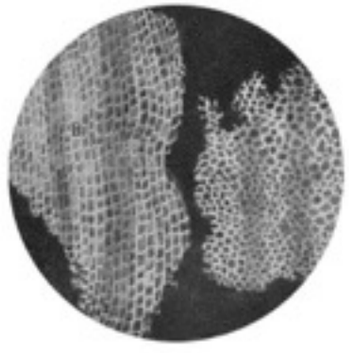
Aristote pensait que nous grandissions à partir de formes indistinctes qui se développent à l'intérieur de maman à cause de papa.

19ème siècle

Théorie cellulaire

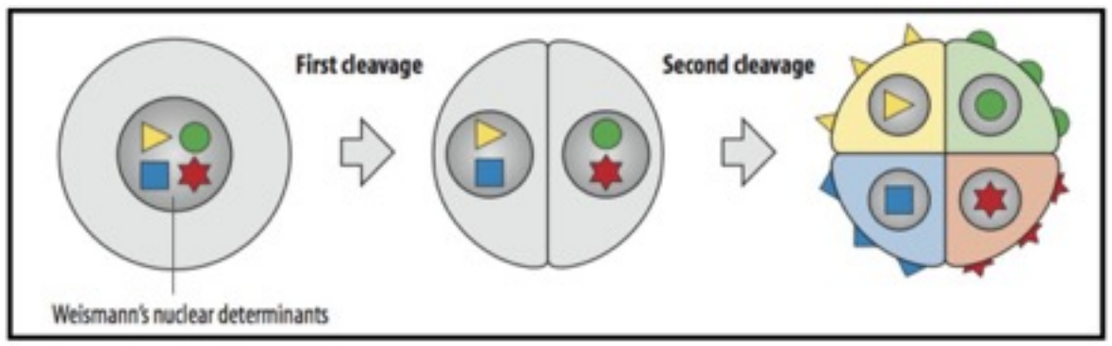
- Tout organisme vivant est composé d'une ou plusieurs cellules
- La cellule est élémentaire de la vie
- Tout cellule provient d'une autre cellule

Le microscope de R. Hooke



Cellules de liège

Théorie du développement mosaïque

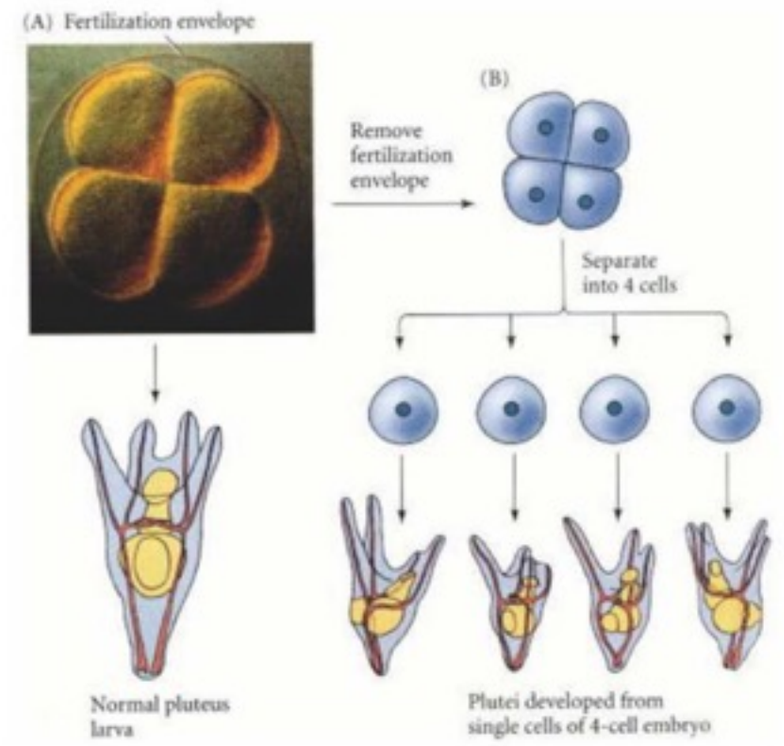


August Weismann

Le noyau comporte des déterminants qui sont partagés de manière asymétrique entre les filles

Embryologie experimentale

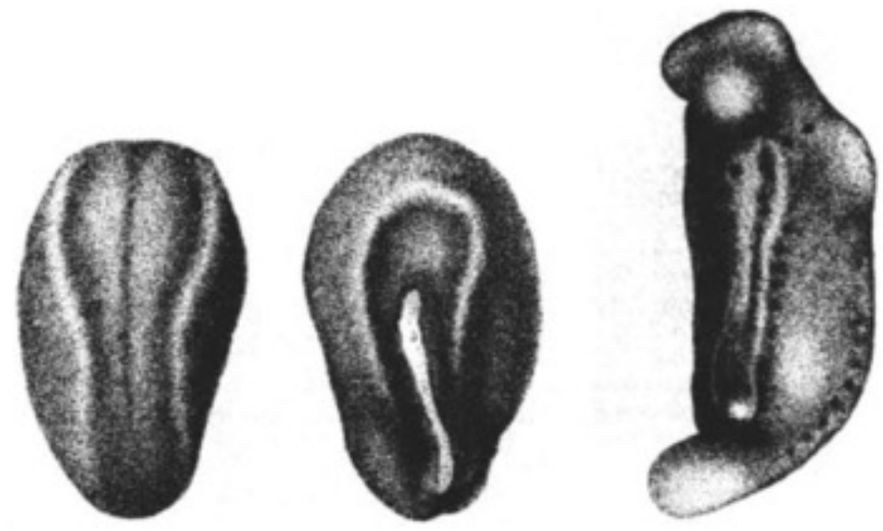
19ème siècle



Hans Drisch

Chaque cellule d'un embryon d'oursin précoce a la capacité de donner un oursin ….

Embryogenèse

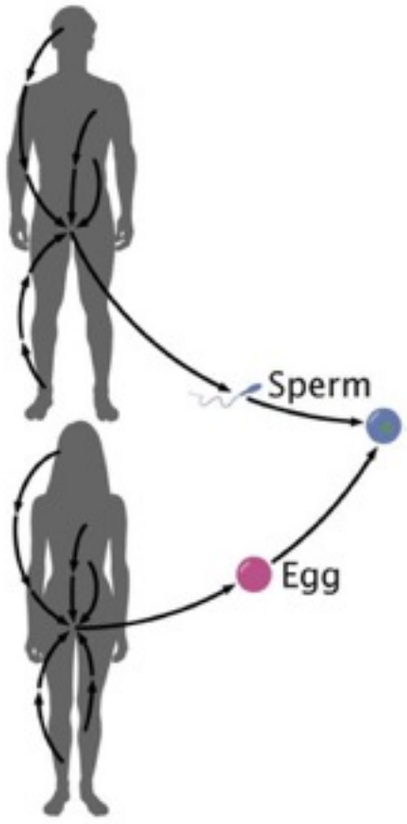


Hans Spemann

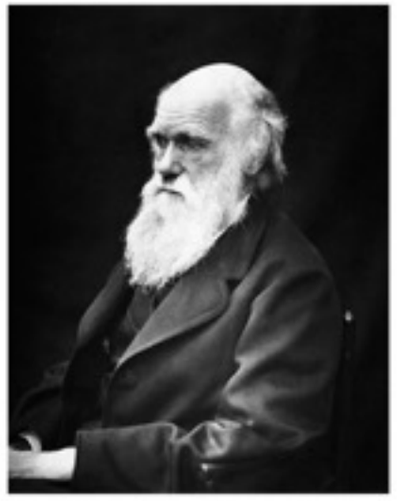
Différentiation cellulaire par signaux chimiques

Pangenèse

19ème siècle

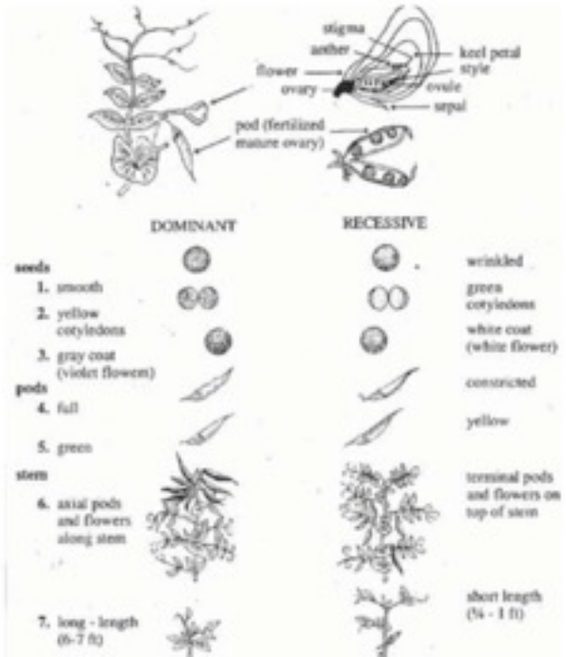


L'ensemble de l'organisme participe à l'hérédité



Charles Darwin

Lois de Mendel

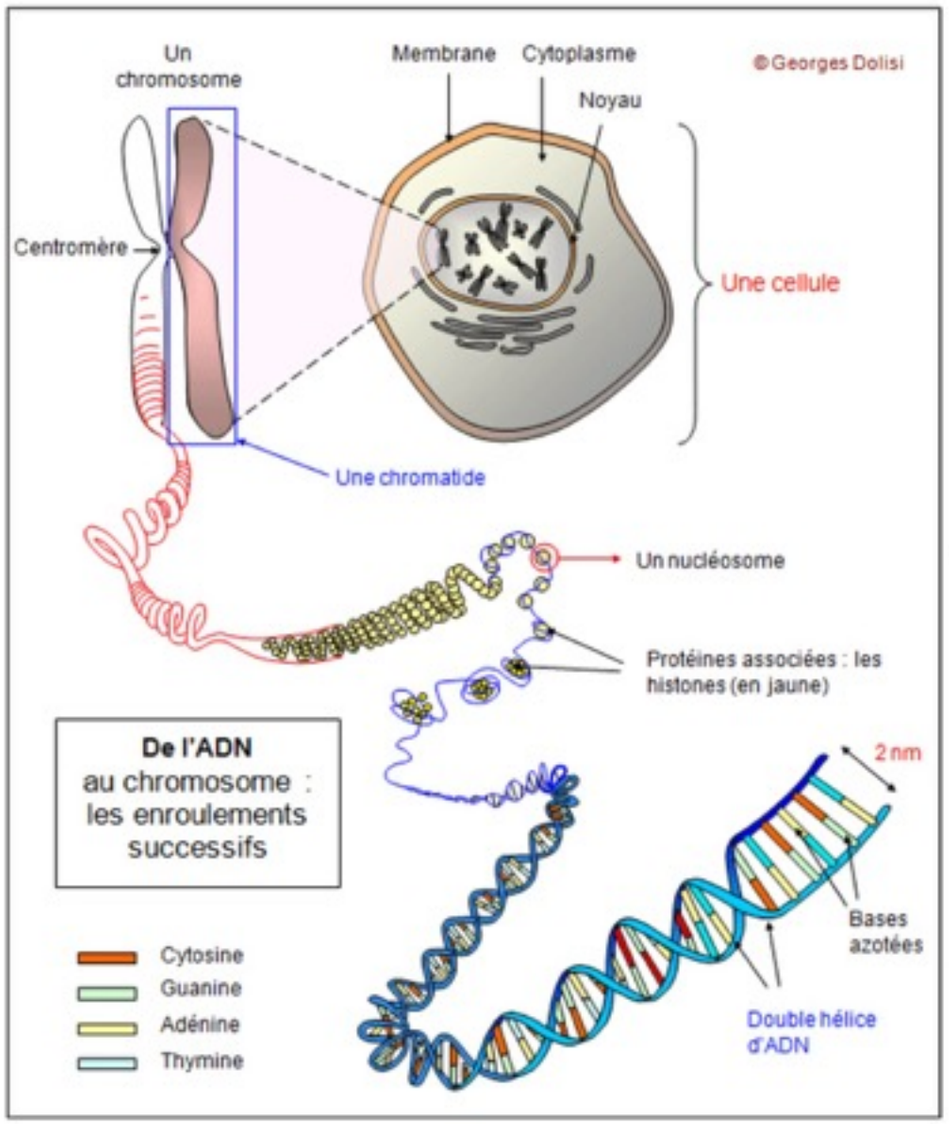


Facteurs héréditaires

Gregor Mendel

20ème siècle

Comment l'information génétique contrôle le développement ?



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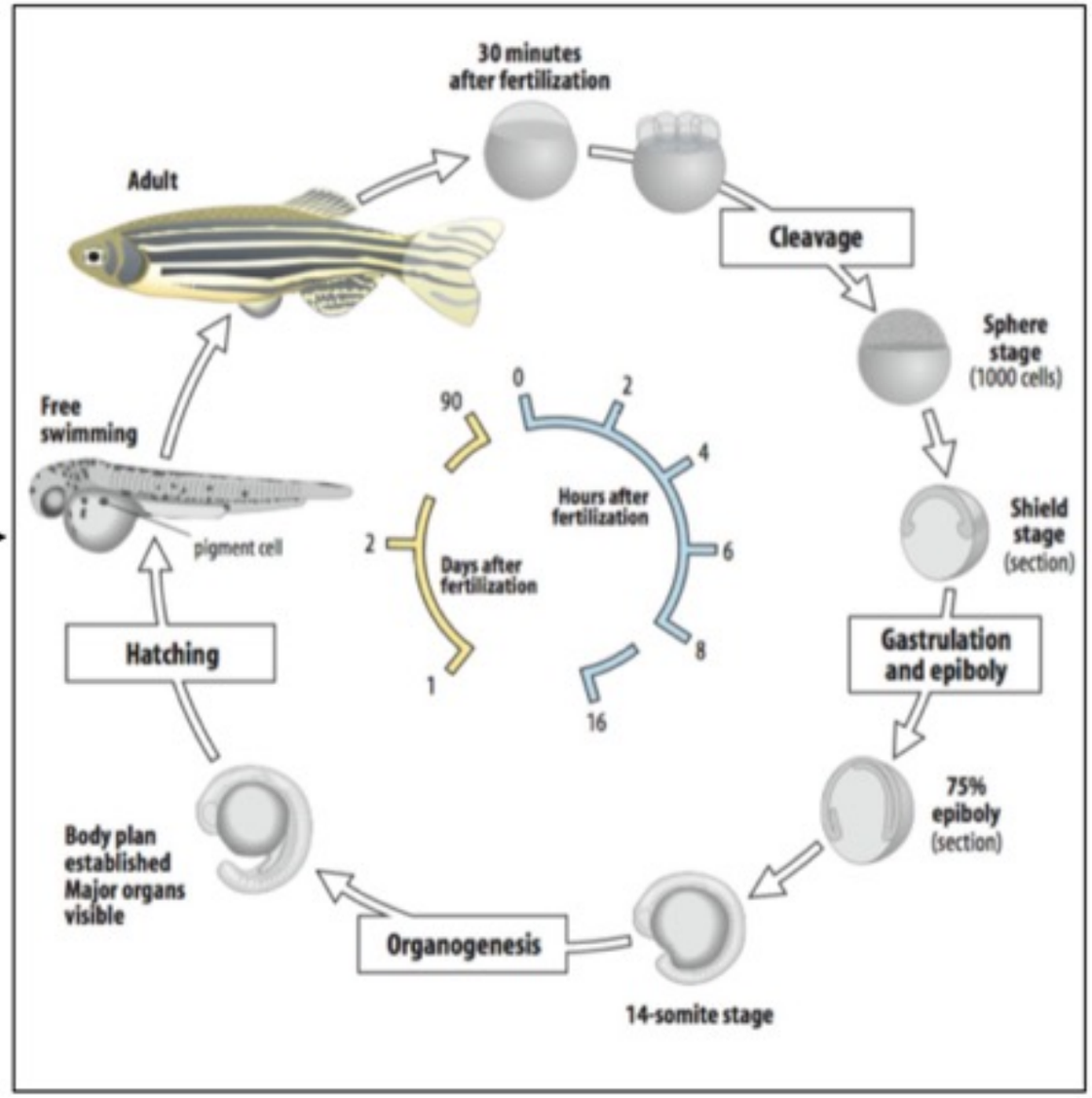
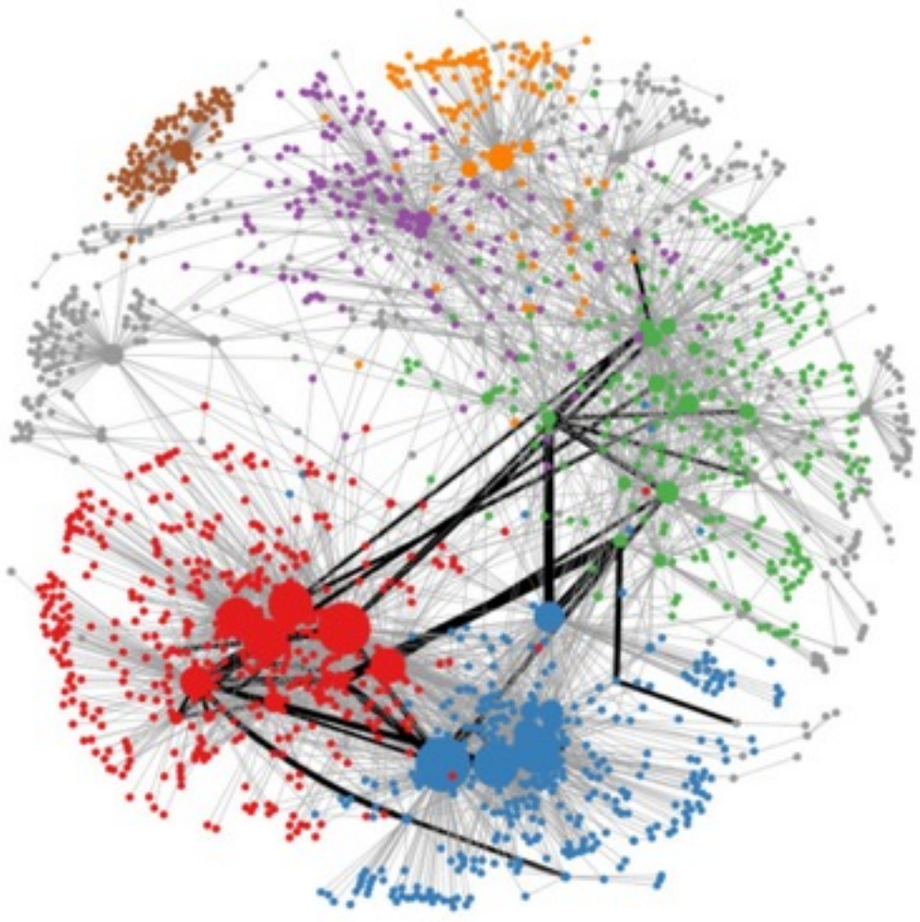


20ème siècle

Comment l'information génétique contrôle le développement ?

Réseau de régulation génétique



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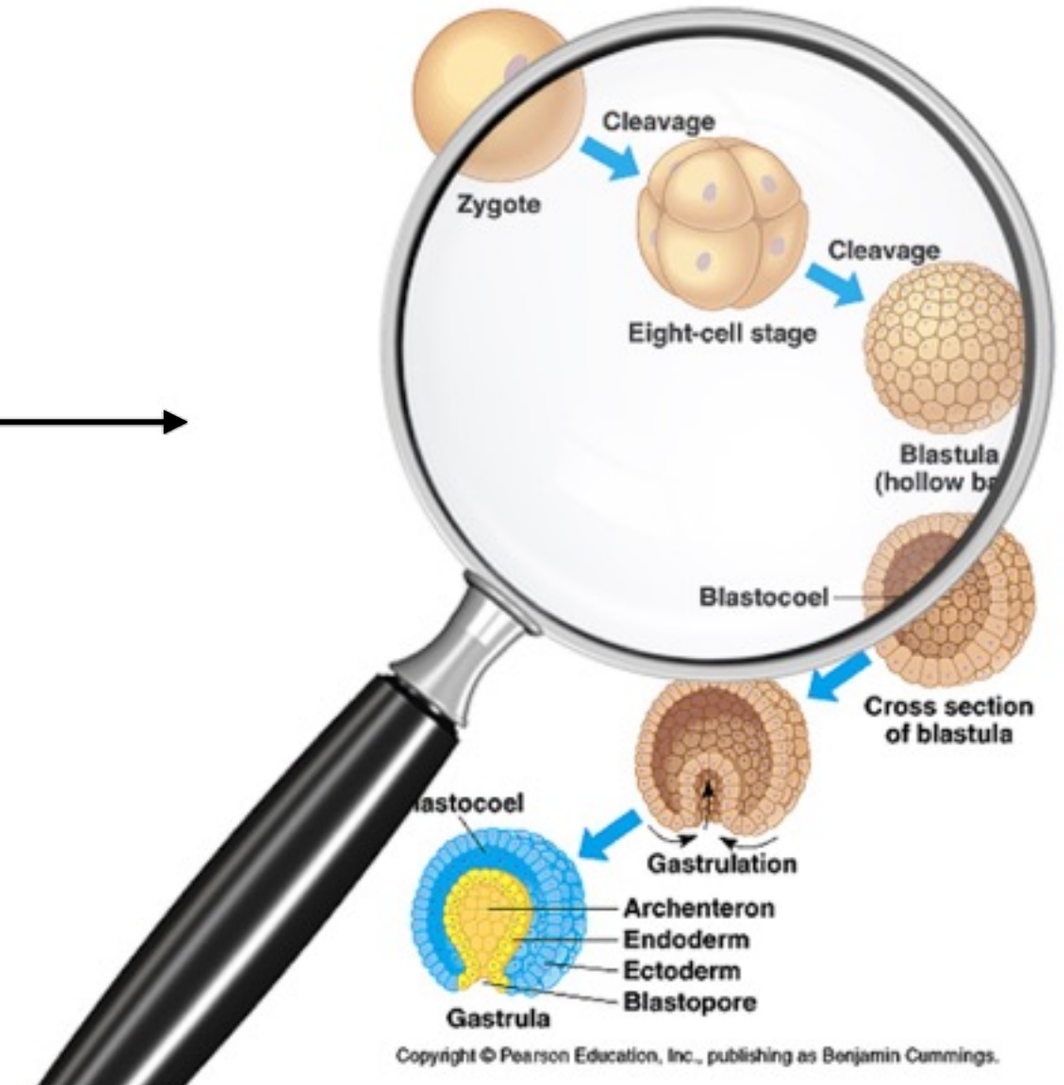
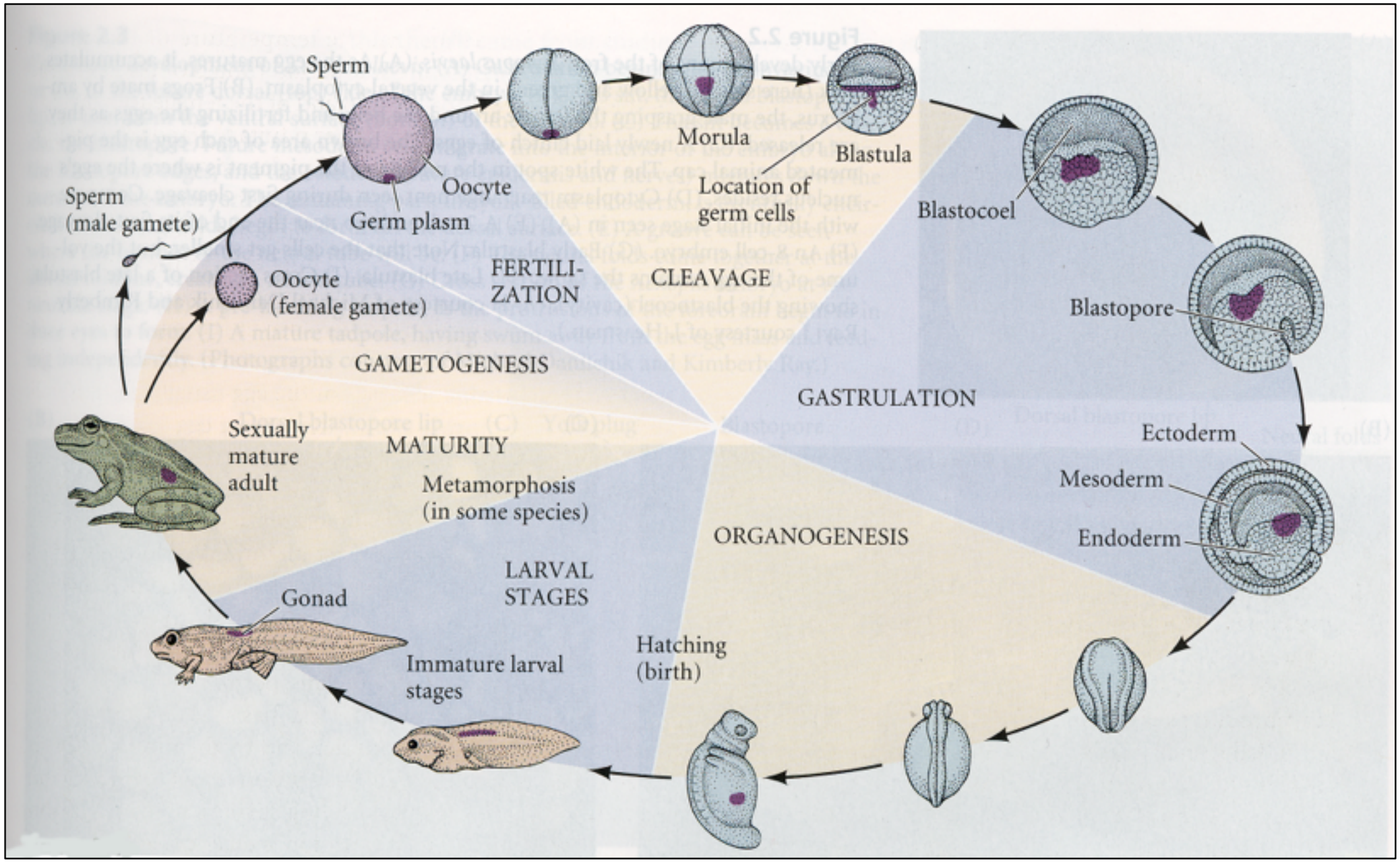
Stade de développement



Que veut on observer ?

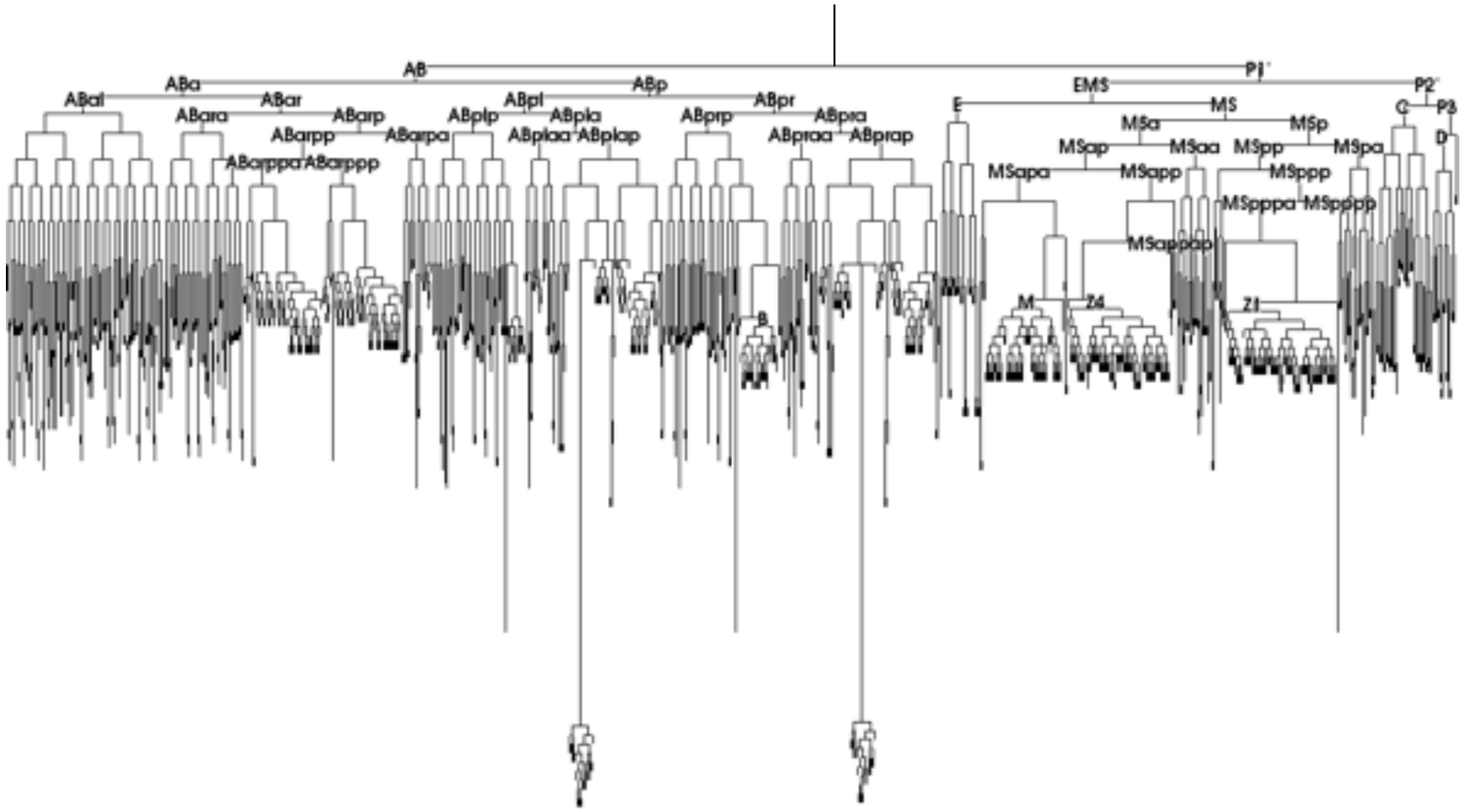


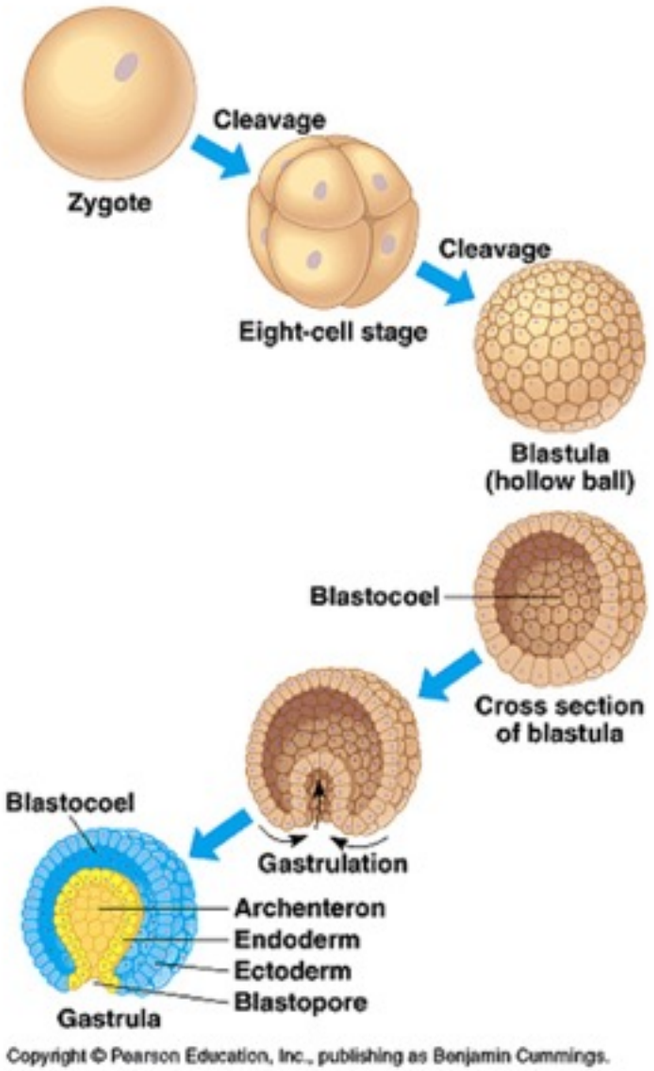
L’embryon à l’échelle cellulaire !

Suivi cellulaire

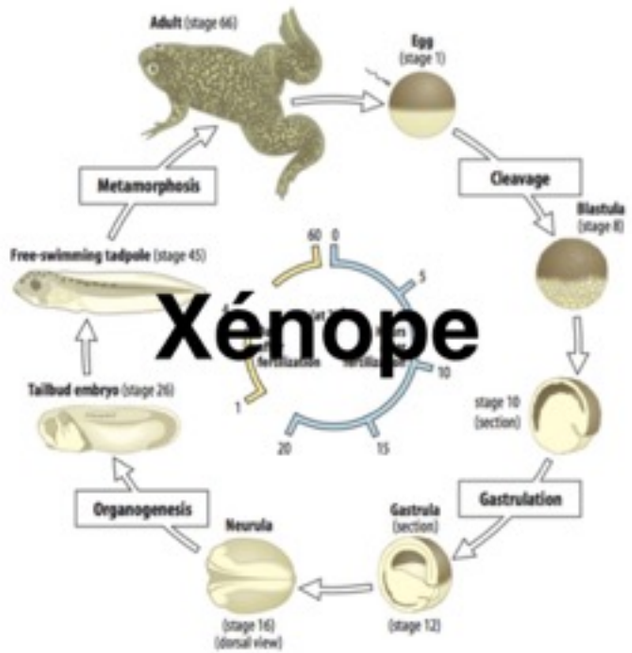
Arbre de lignage cellulaire

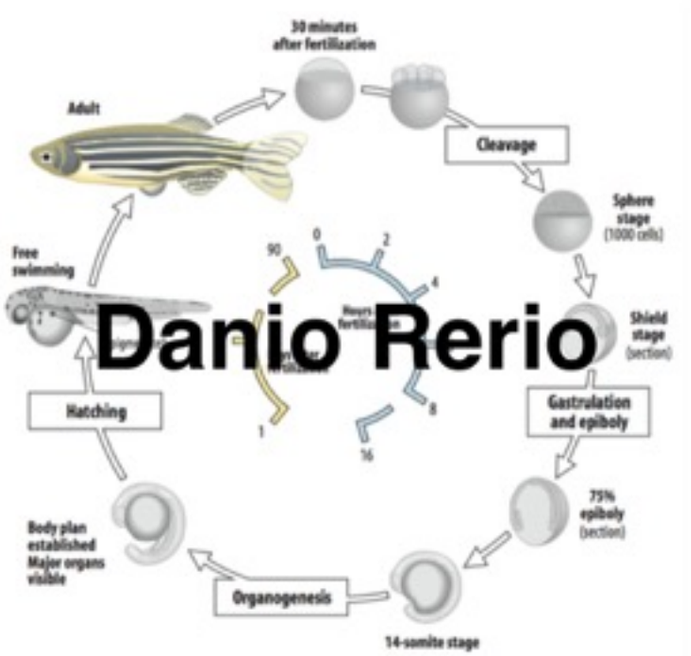
[Caenorhabditis elegans , Sulston 1983]



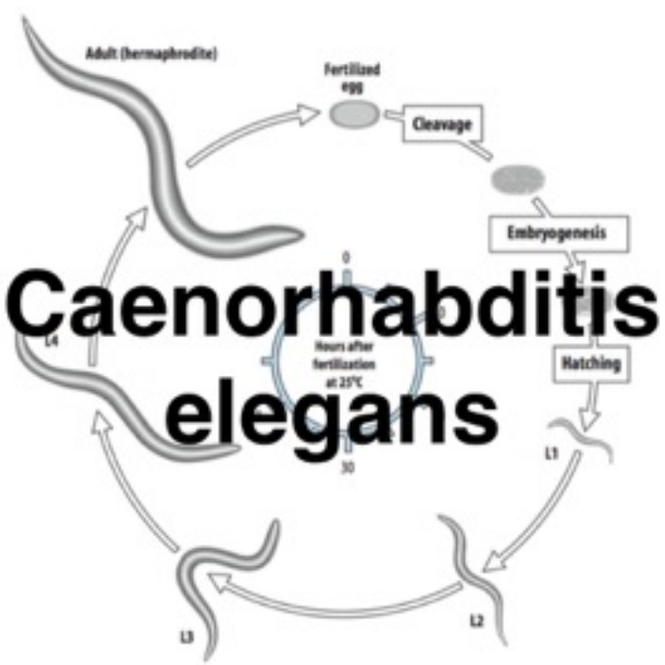


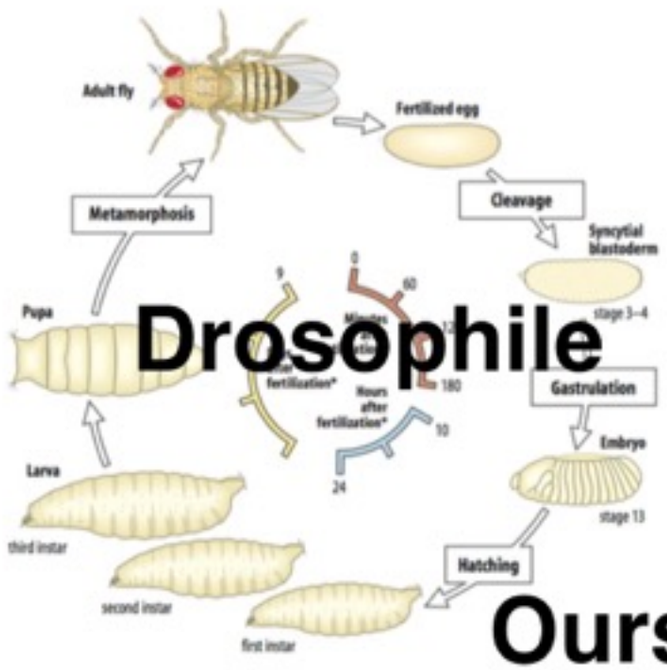
Modèle de la biologie du développement





- taille
- position phylogénétique
- transparence
- accessibilité
- cycle de vie
- ….



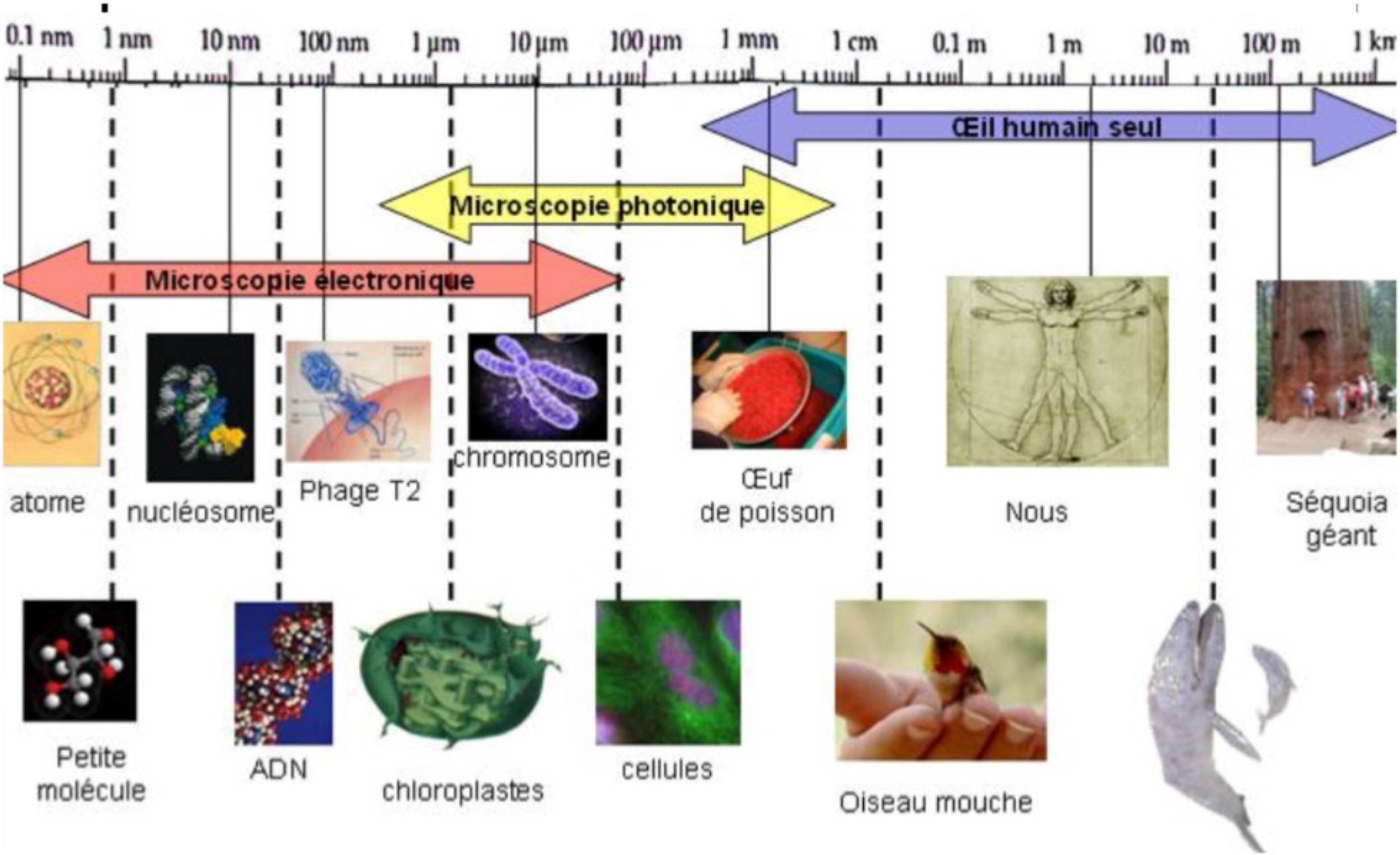


Oursin, Ascidies, Souris ! …

Comment peut-on l'observer ?

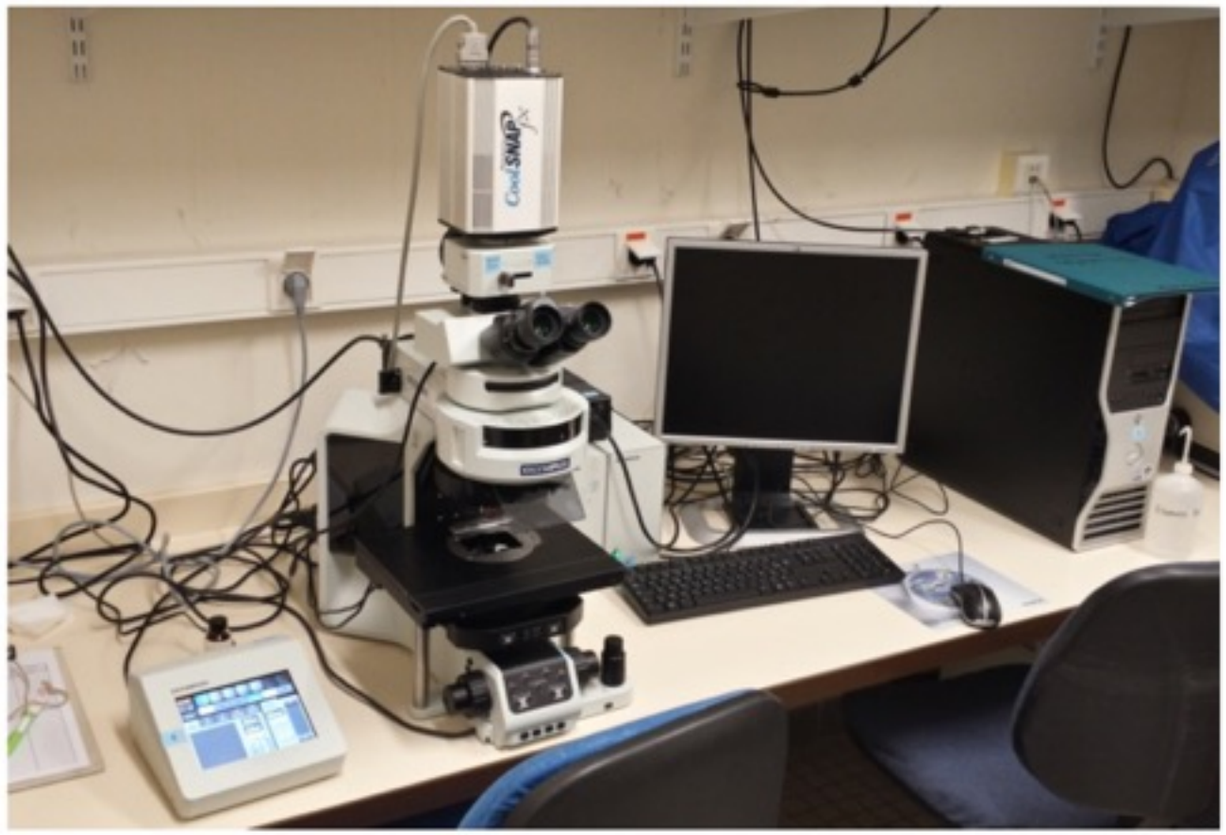
au microscope !

Quel microscope ?



Microscope à contraste interférentiel
(Normarski 1950)

exploite les interférences de deux faisceaux d'une onde lumineuse traversant un échantillon

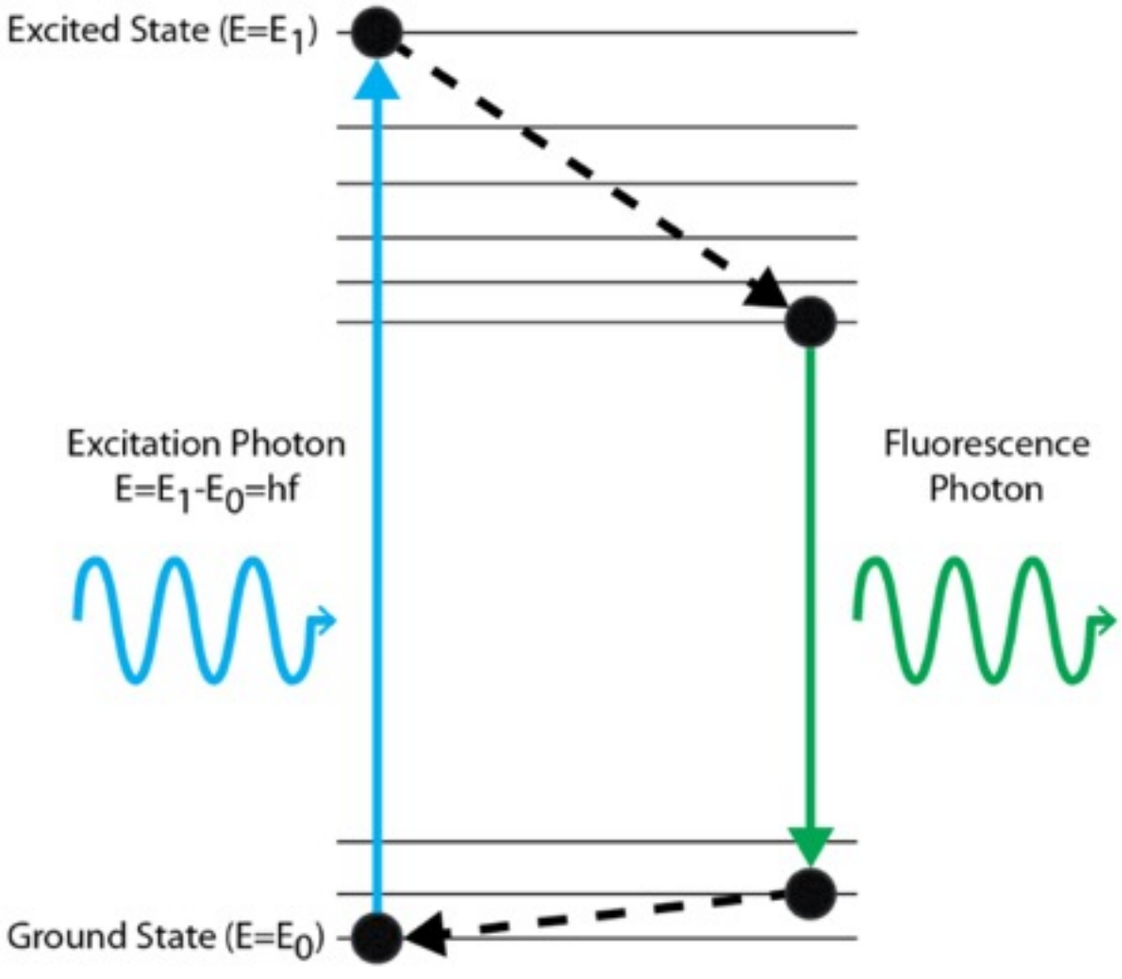


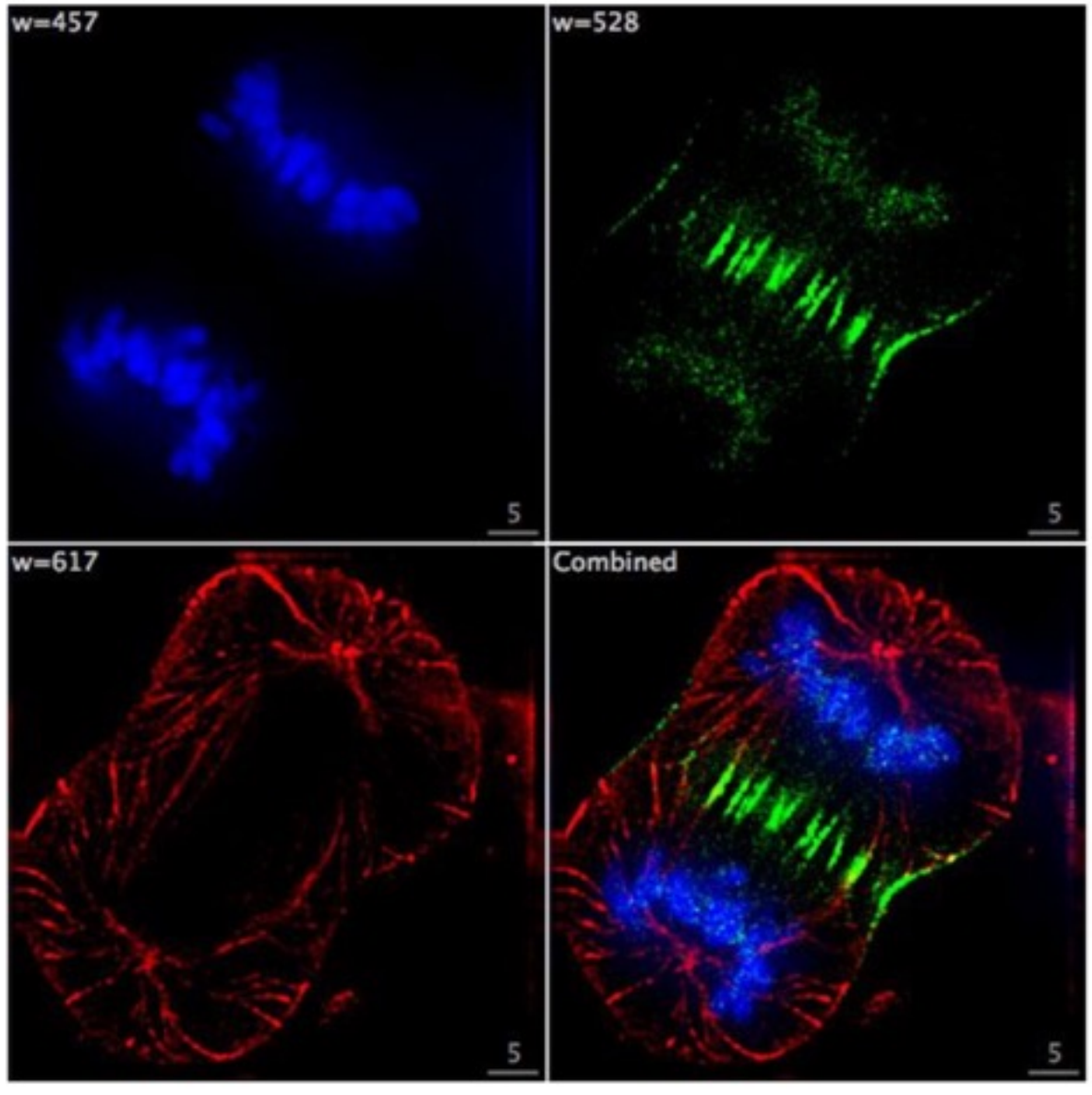
Kimmel 1995

⟶ Images 2D + temps

Fluorescence
(Stokes 1852)

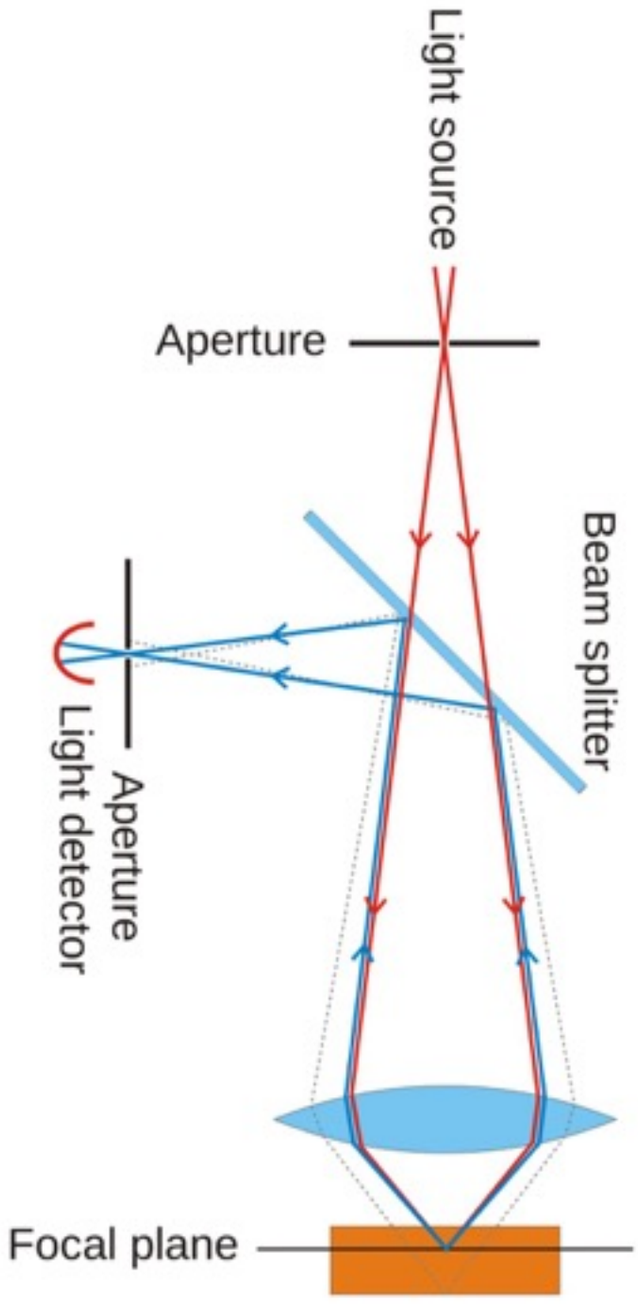
La fluorescence est la propriété que certains corps ou molécules ont à émettre une lumière après avoir été excités avec une lumière d'énergie supérieure. Ainsi, un objet excité par une longueur d'onde émettra une fluorescence à une longueur d'onde supérieure.

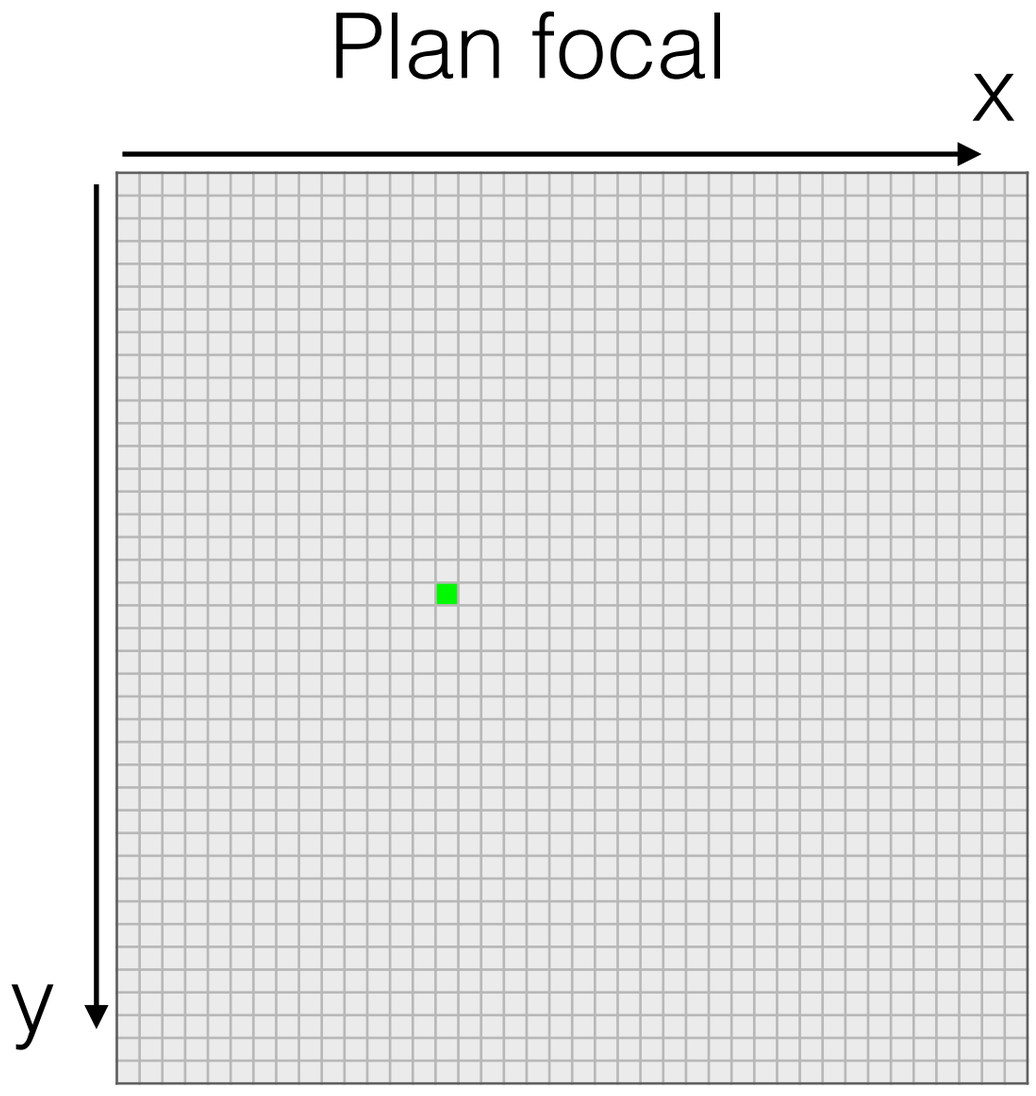




Microscope confocal

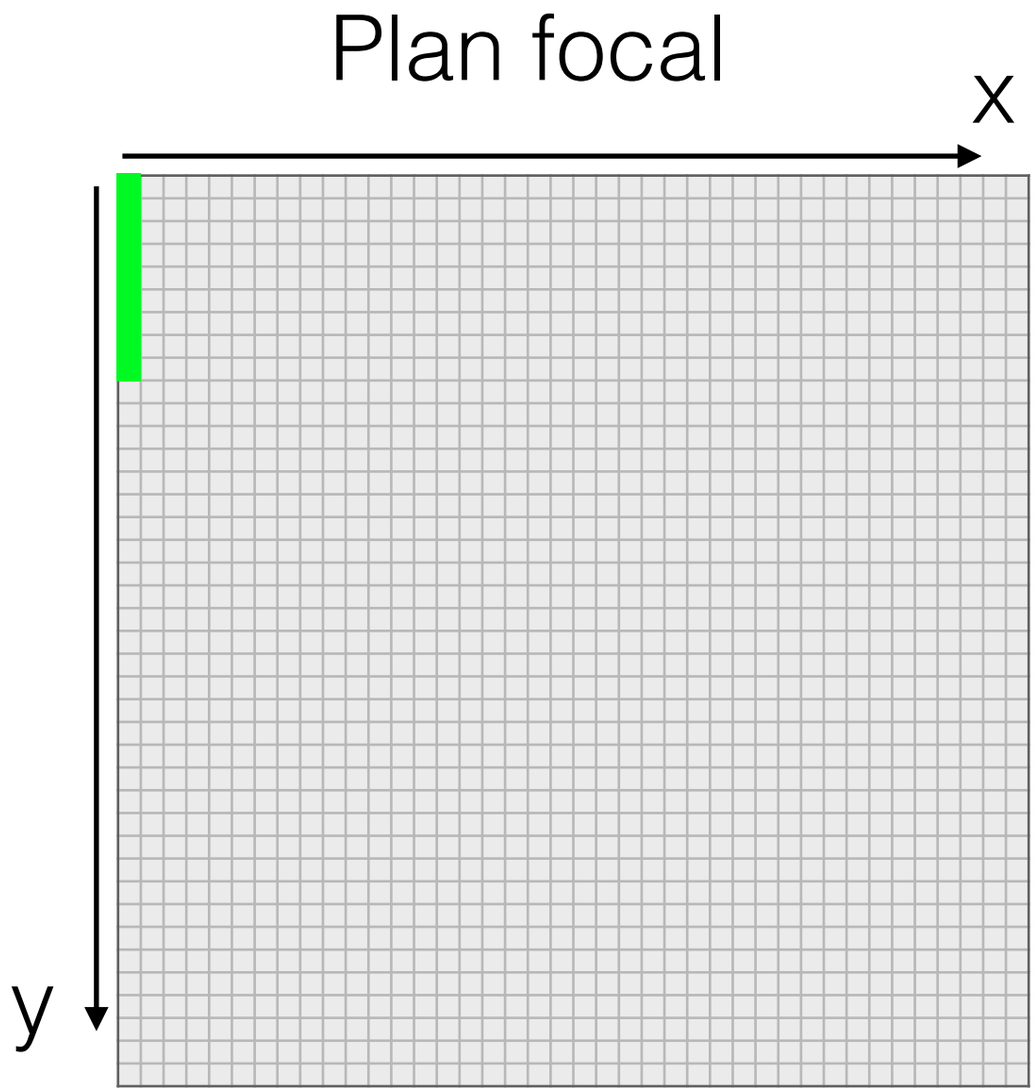
(Minsky 1953 , commercialisé en ~1980)

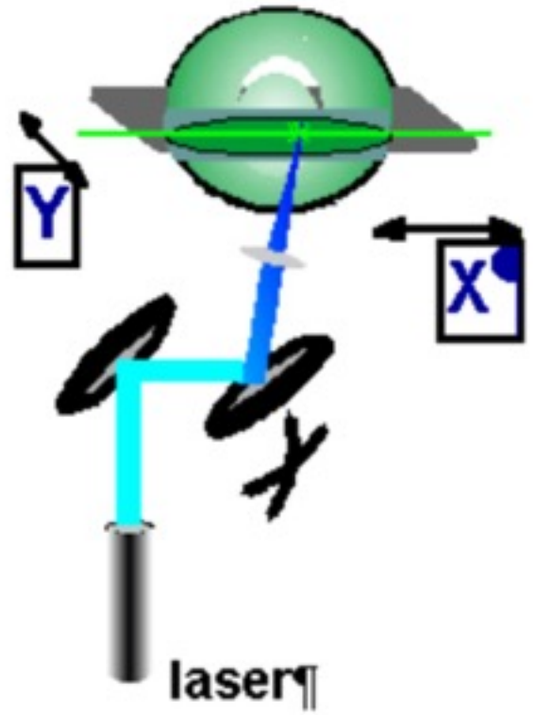




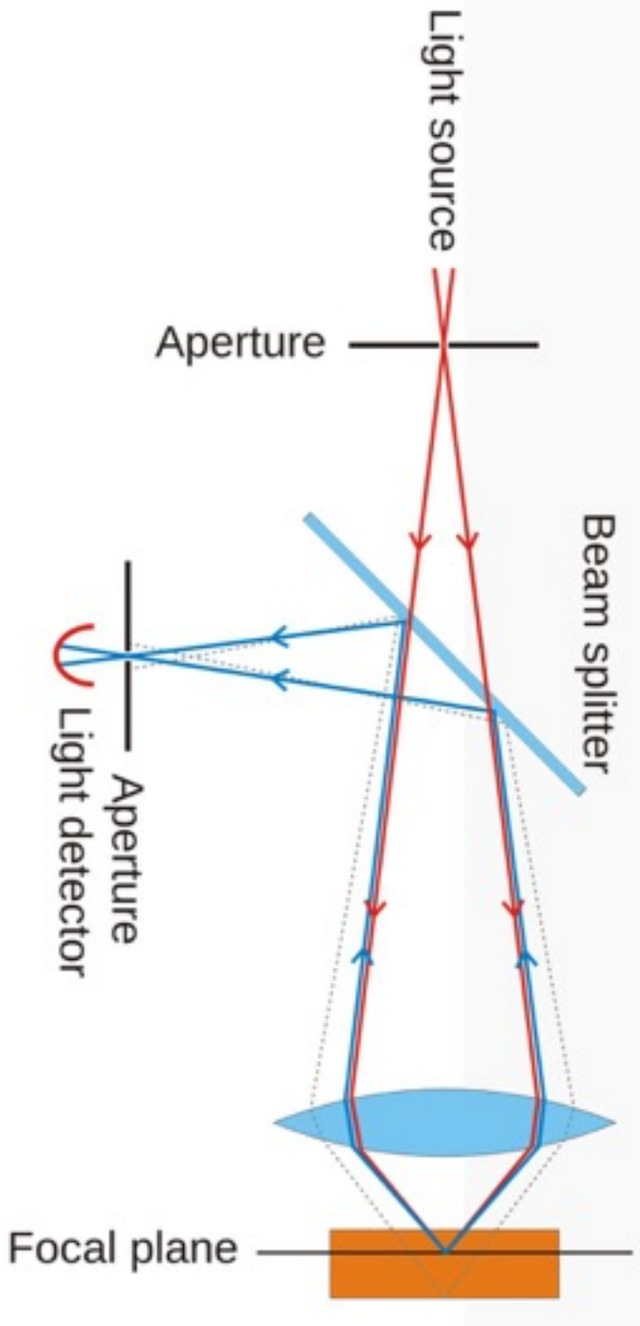
Microscope confocal

balayage laser





Microscope confocal



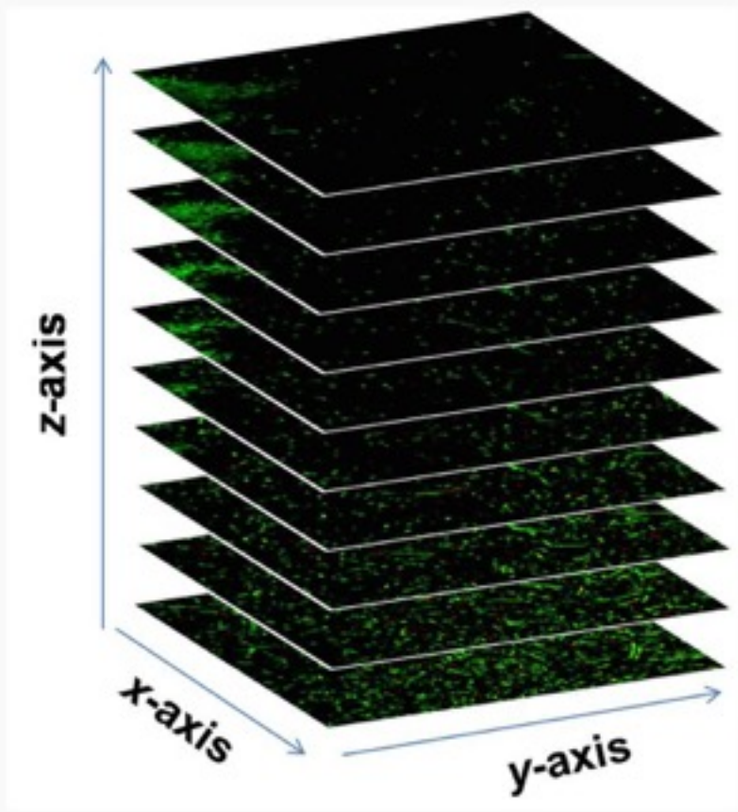
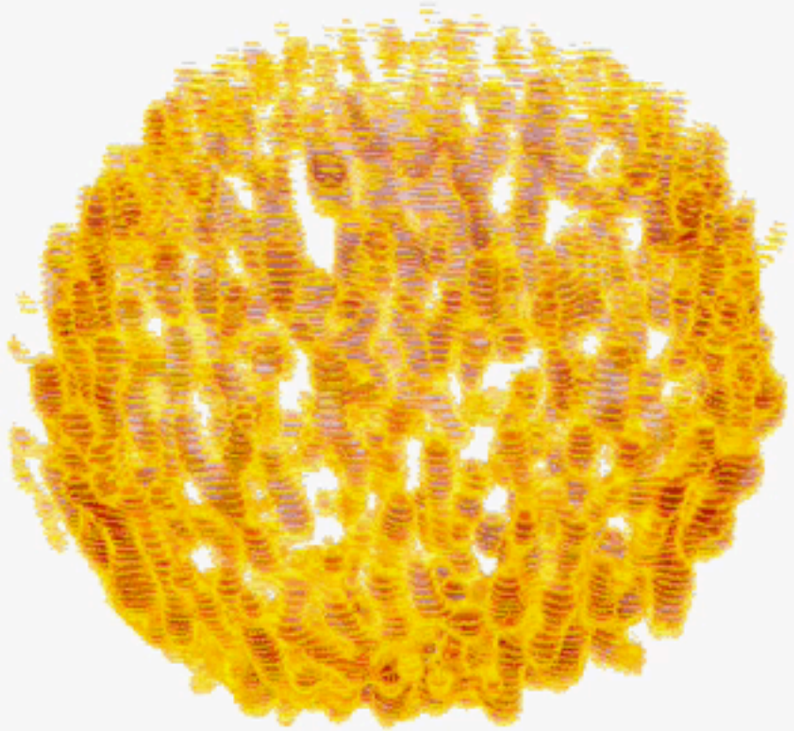
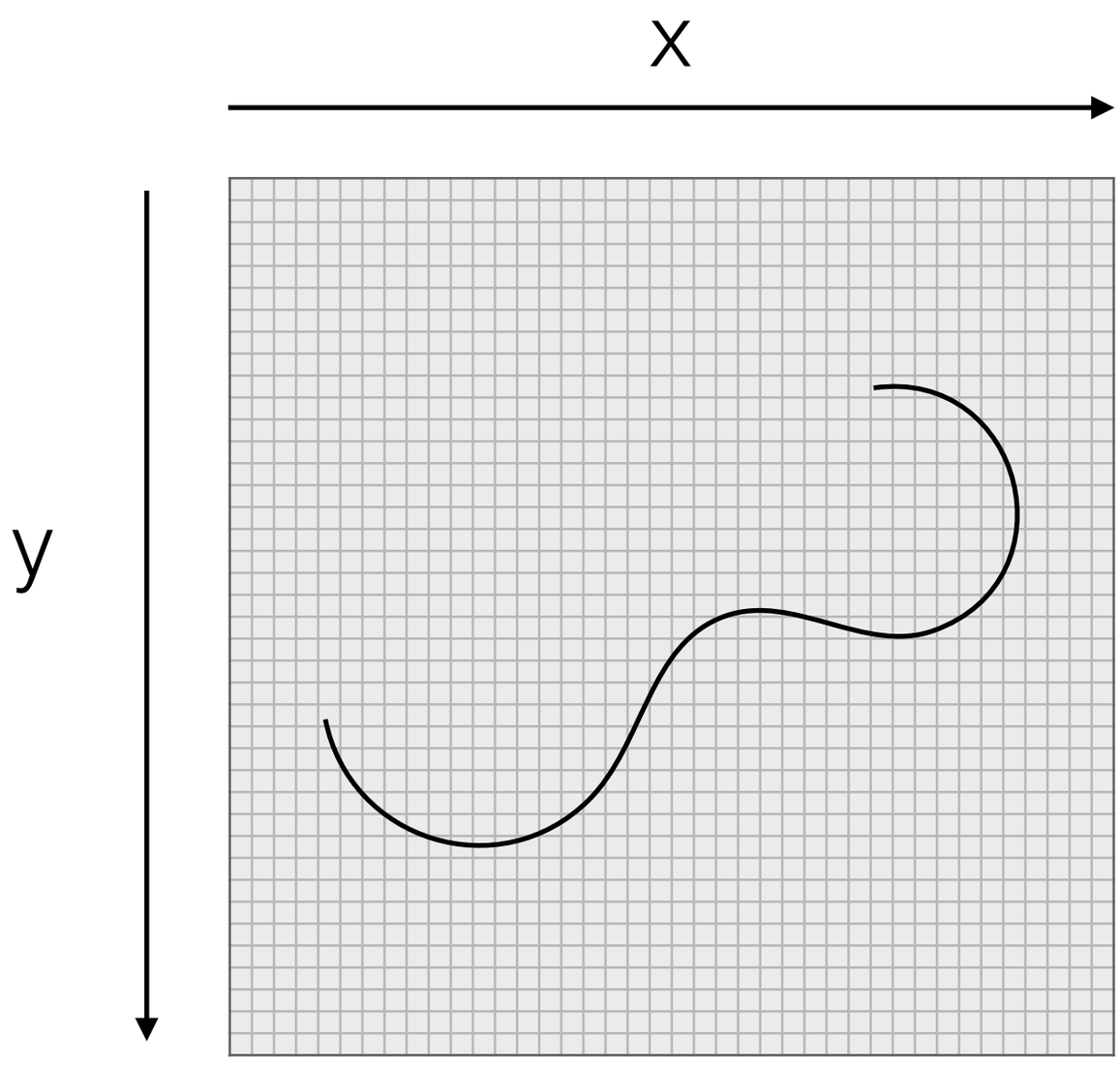


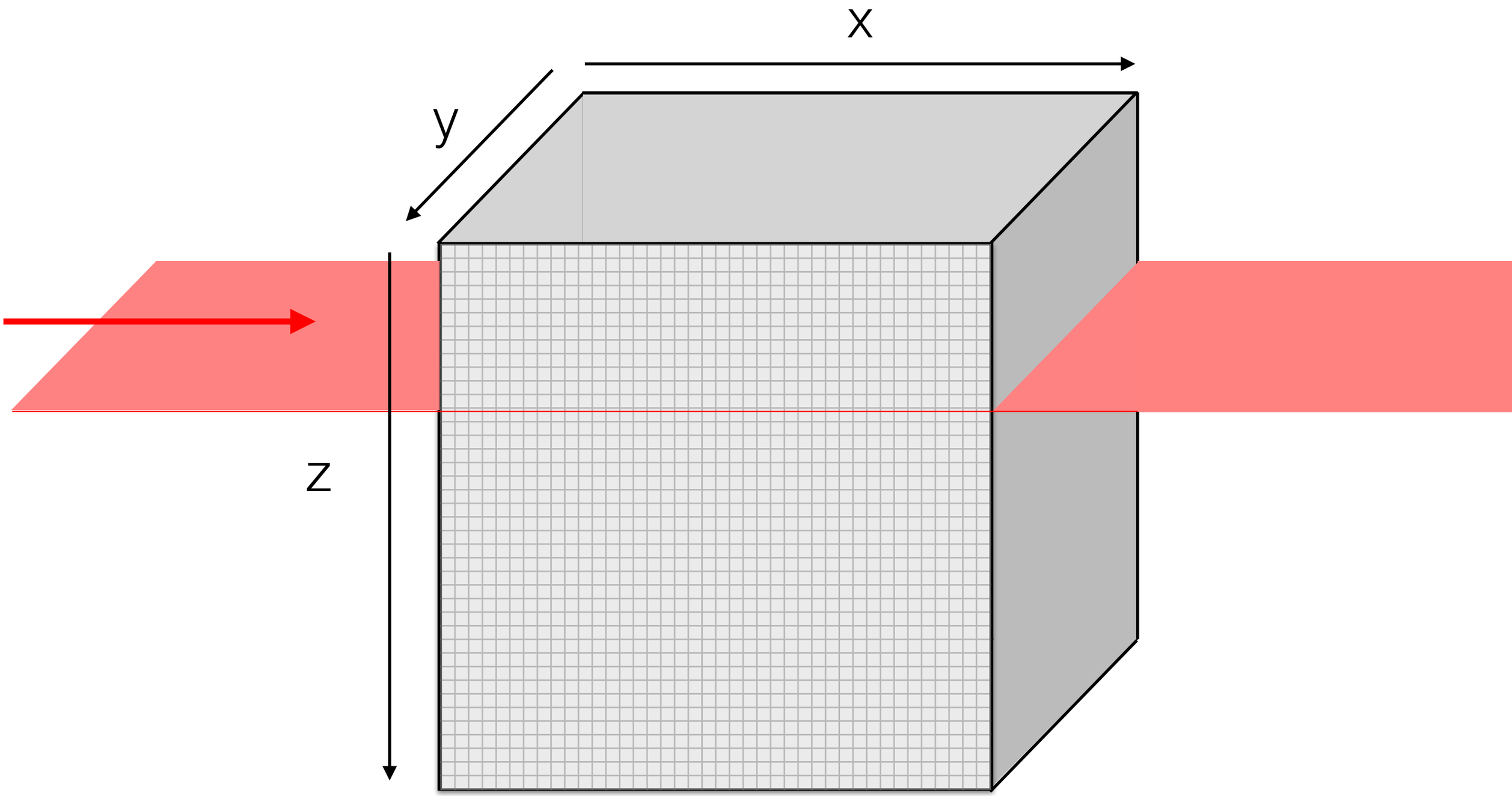
Image 3D

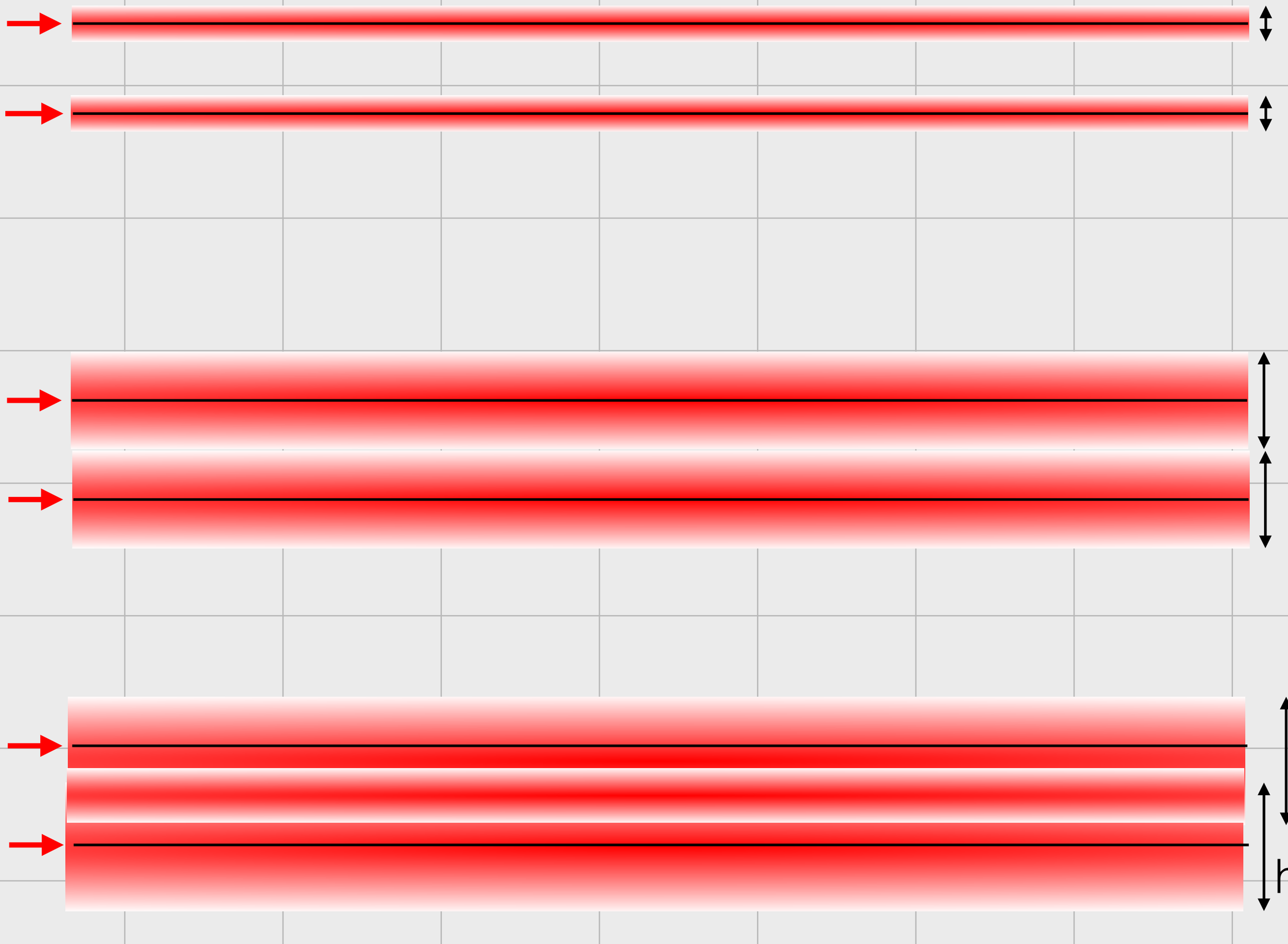


Découpage Image 2D



Découpage Image 3D

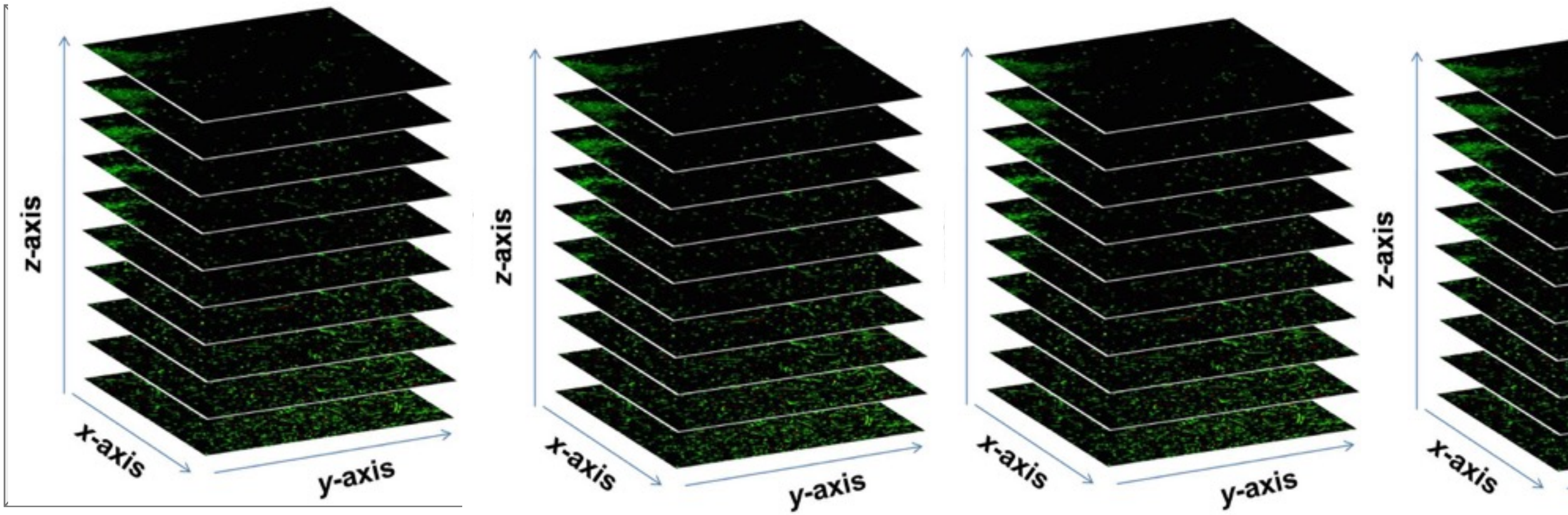



h

Microscope confocal

Image 4D

2min



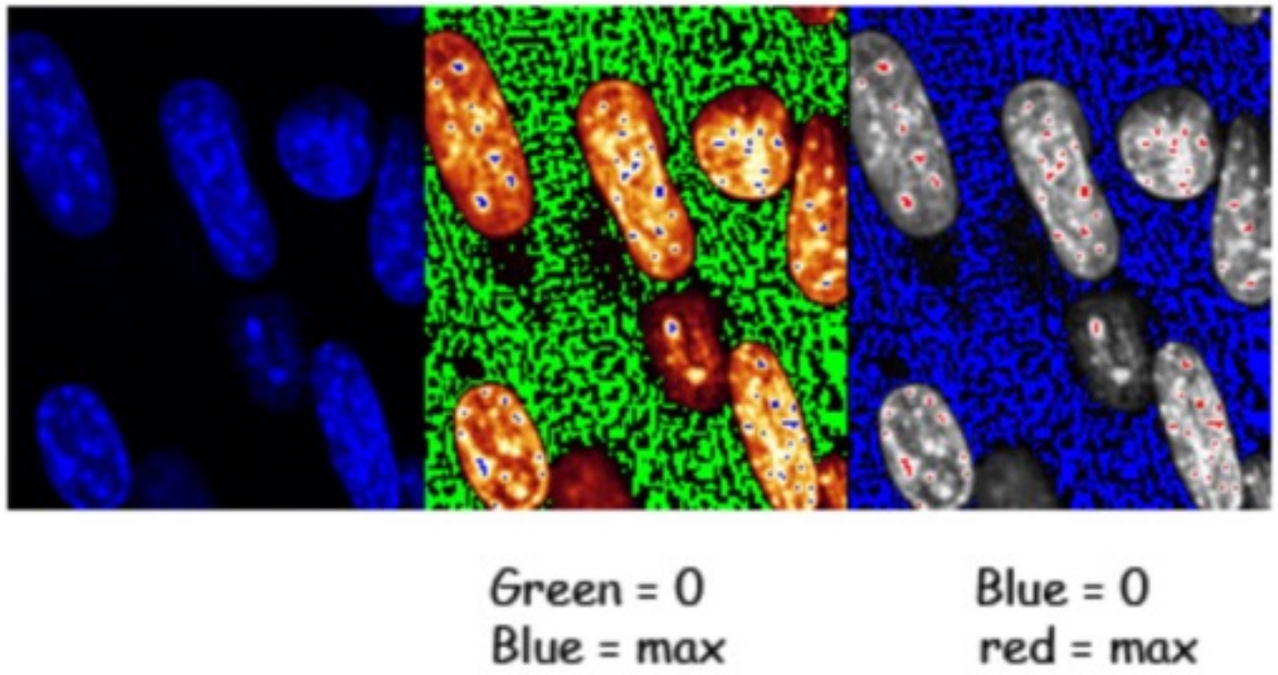
temps

Microscope confocal

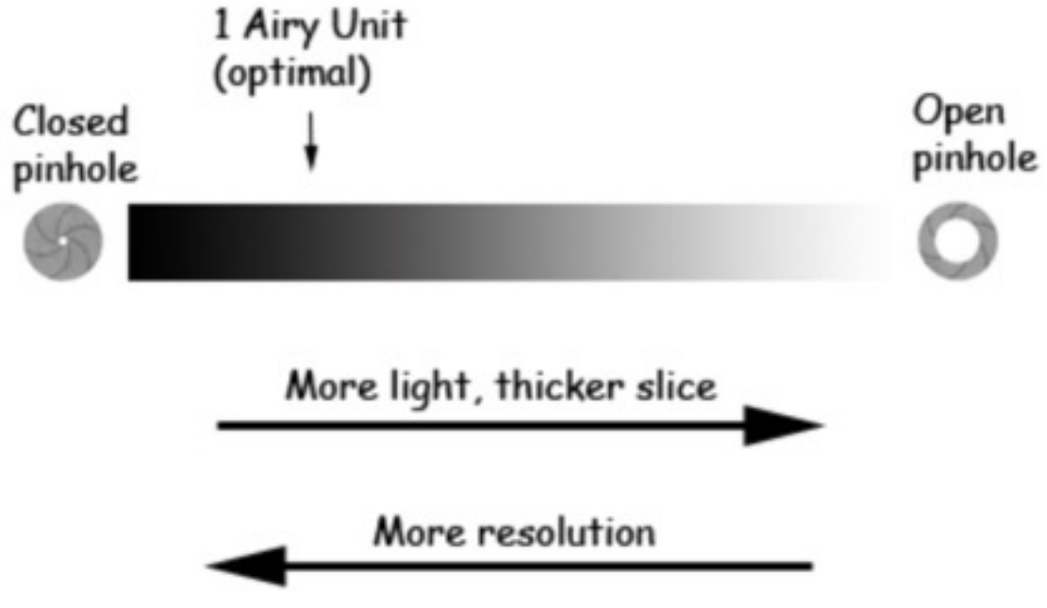
Microscope confocal

Puissance Laser

Sensibilité des détecteurs

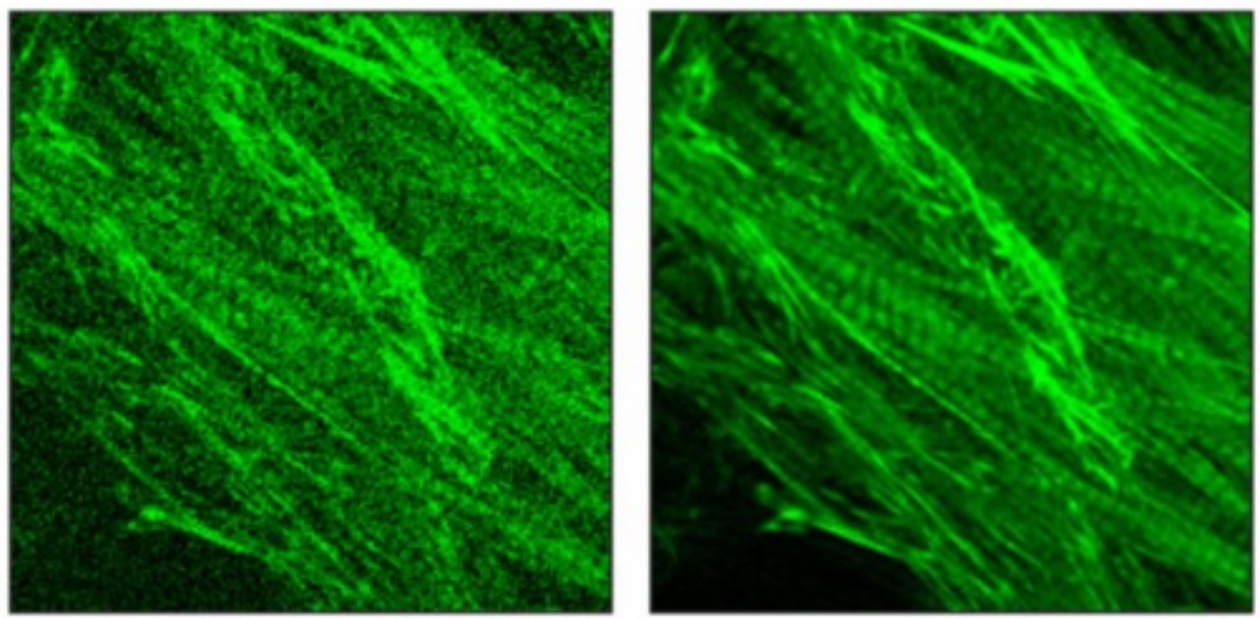


Ouverture

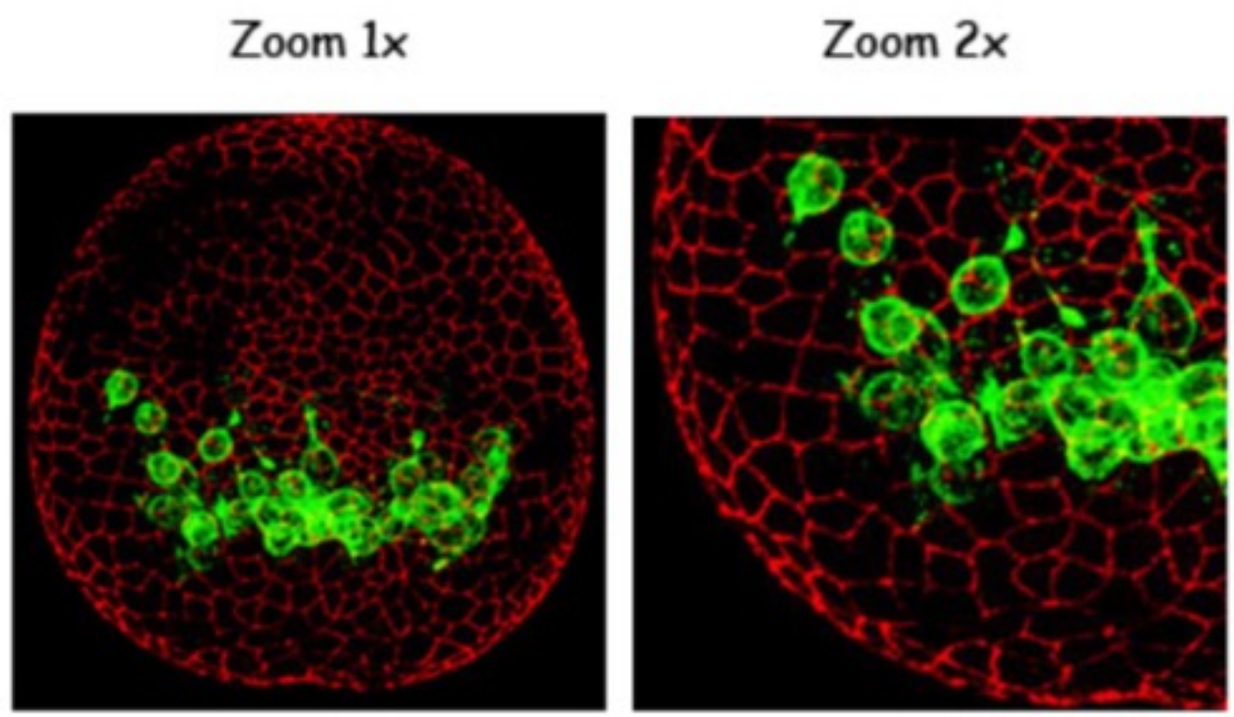


Microscope confocal

Nombre de passes



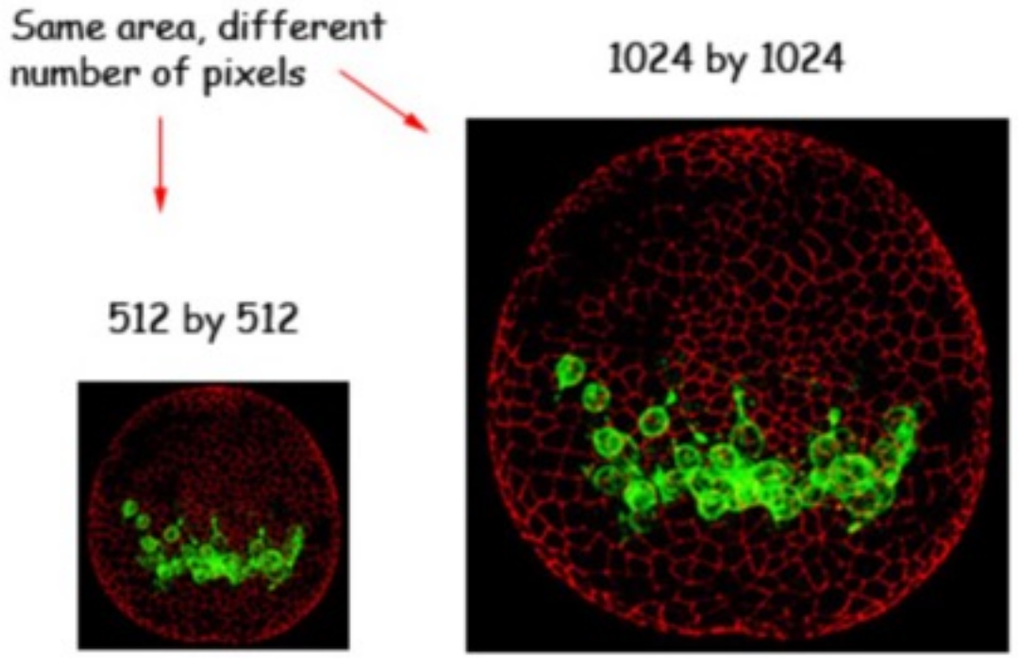
Zoom

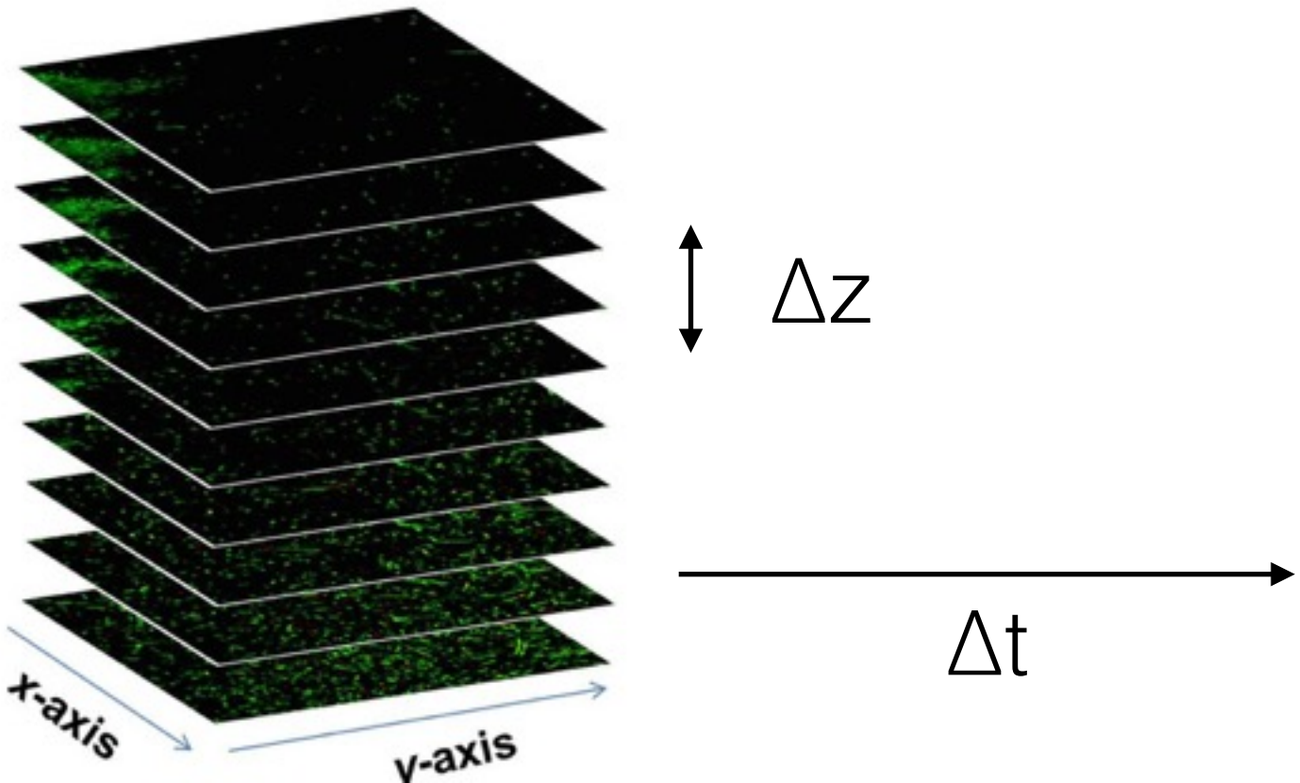


Microscope confocal

Nombre de pixel

Δxy





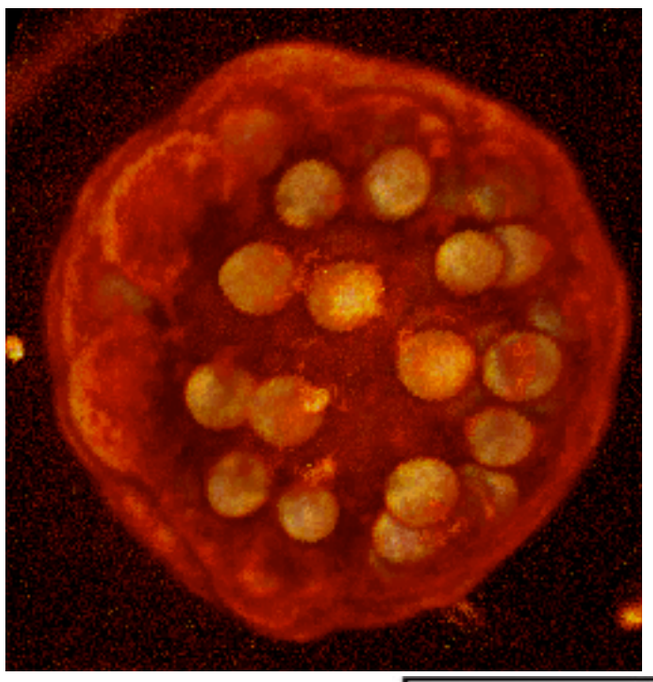
Microscope confocal

Objectifs

Résolution spatial
Résolution temporelle
Ratio Signal sur bruit

Photoblanchiment

Phototoxicité



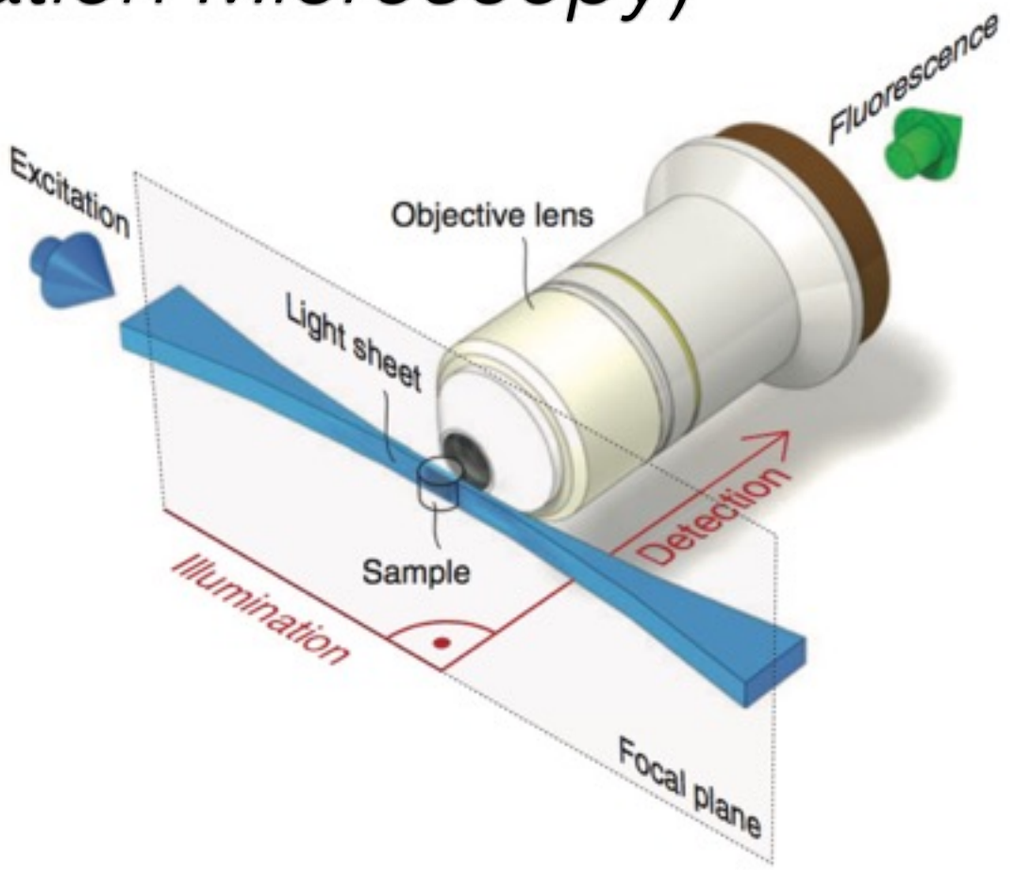
Les embryons bougent

$$\#\,\text{photons} \sim \text{density} \cdot \text{object size} \cdot \text{resolution} \cdot \text{signal} \cdot \text{time period} \cdot \text{repetitions}$$

[Stelzer 2013]

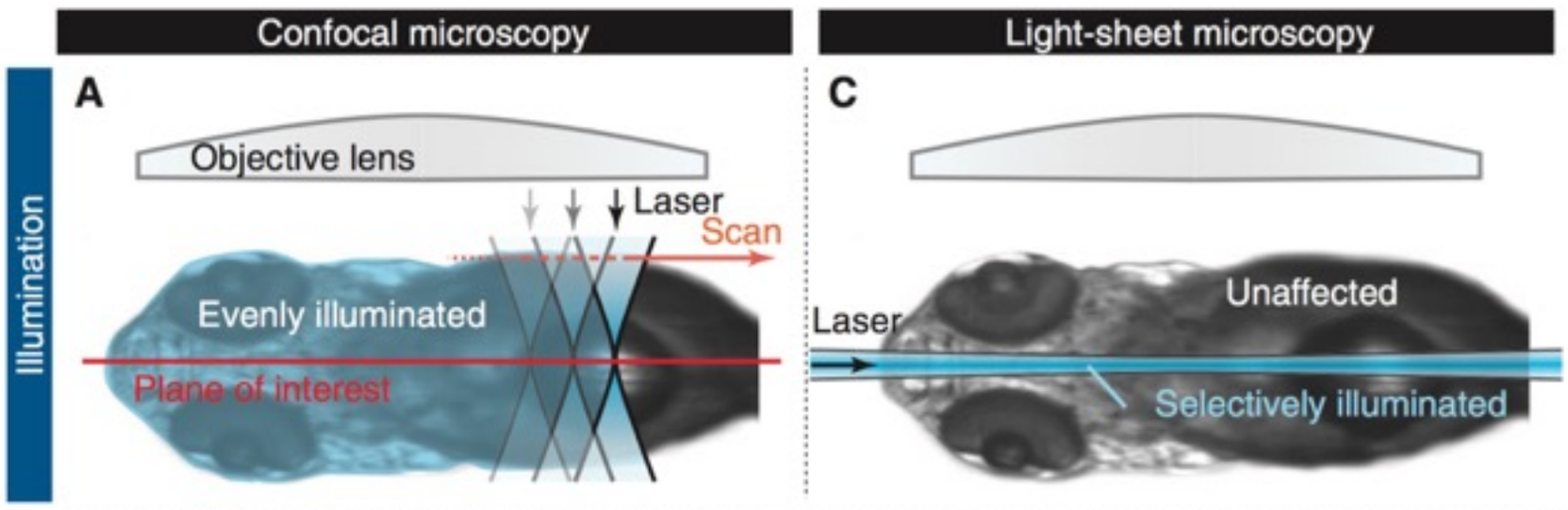
Microscope SPIM

(*Selective Plane Illumination Microscopy*)



Huisken 2004

Beaucoup plus rapide

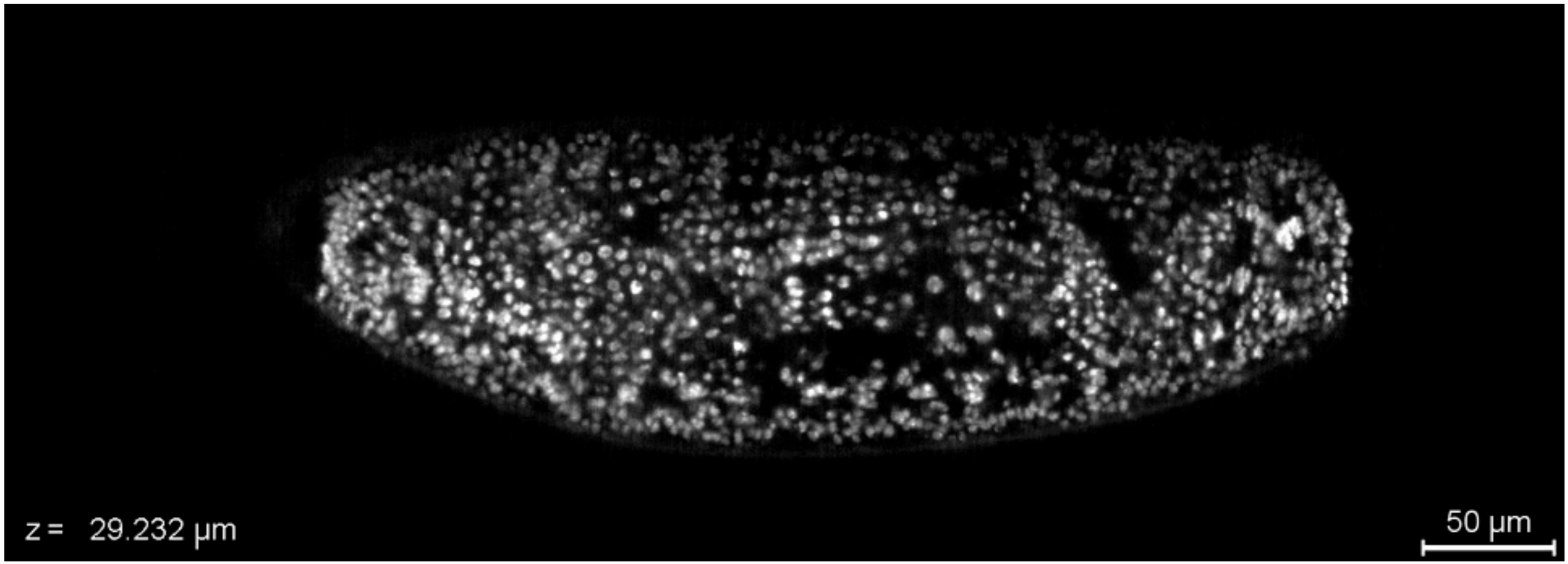


Huisken 2009

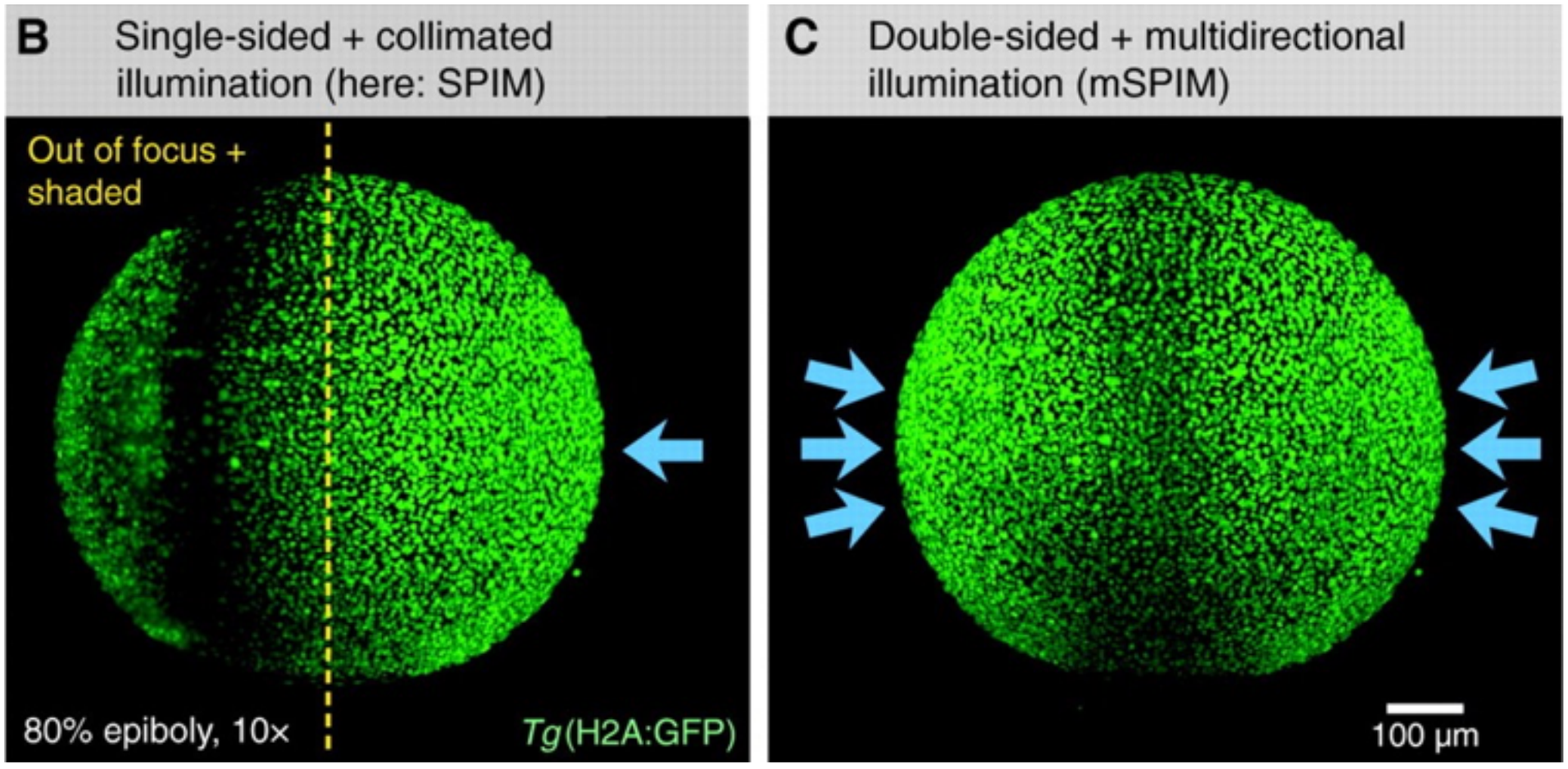
Moins d’illumination

Microscope SPIM

(*Selective Plane Illumination Microscopy*)

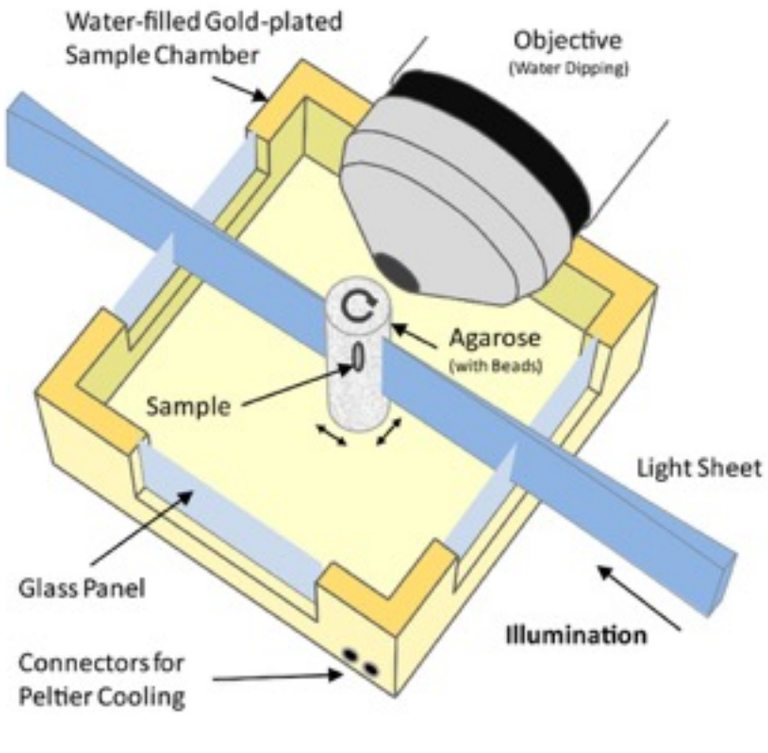


SPIM : Multiples Illuminations

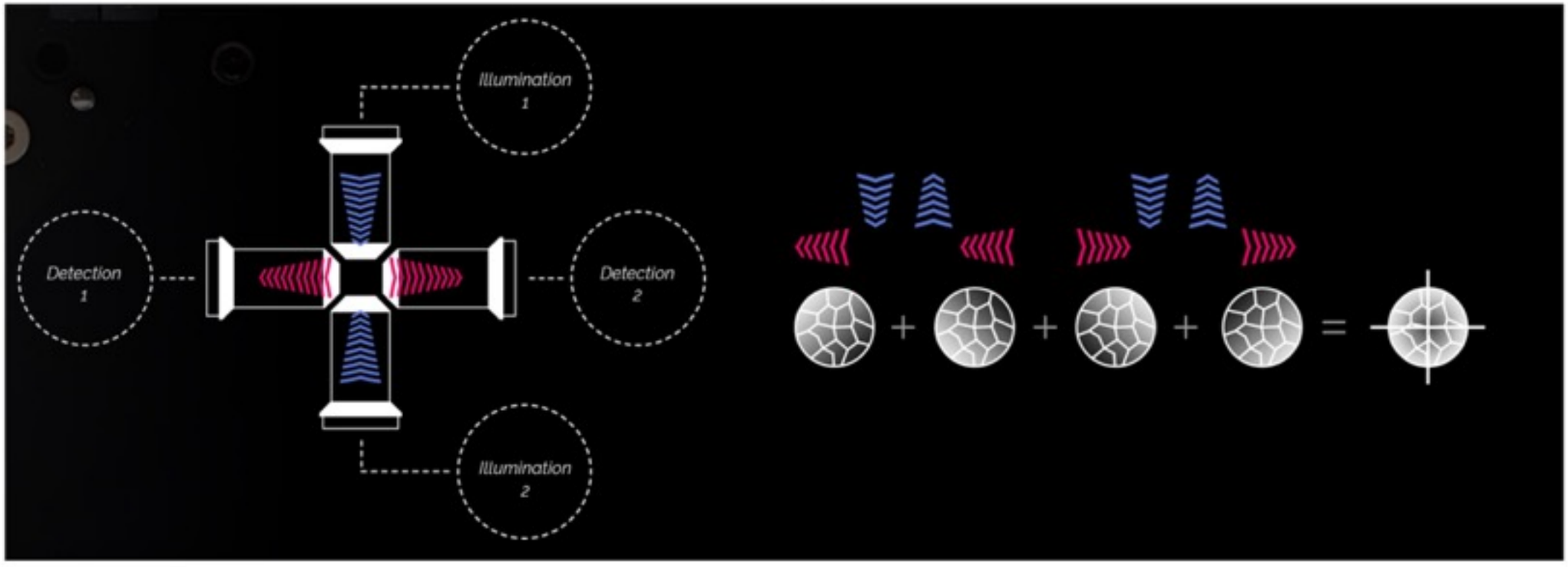


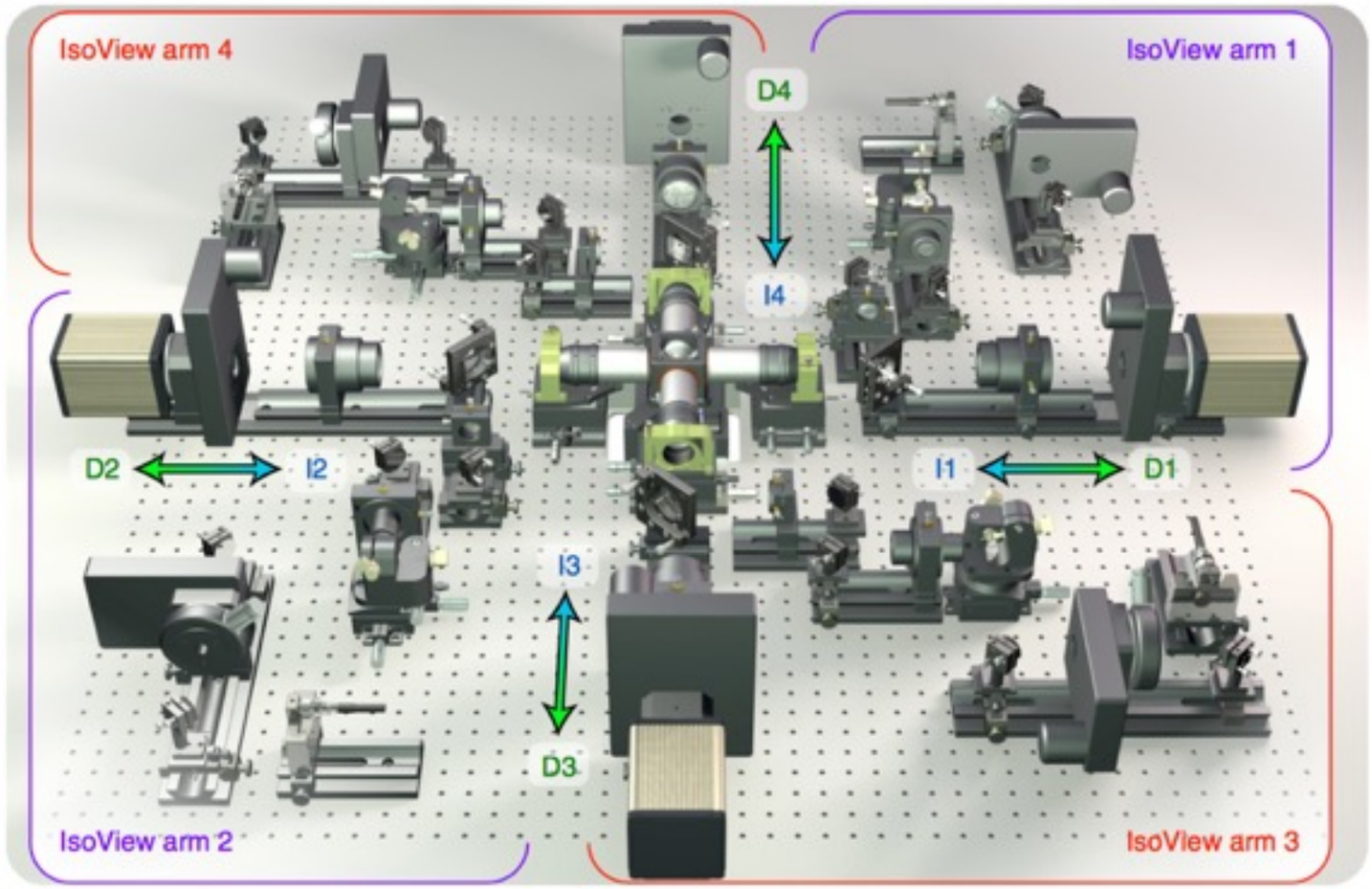
Huisken 2009

SPIM : Multiples Angles



SPIM : Multiples Vues

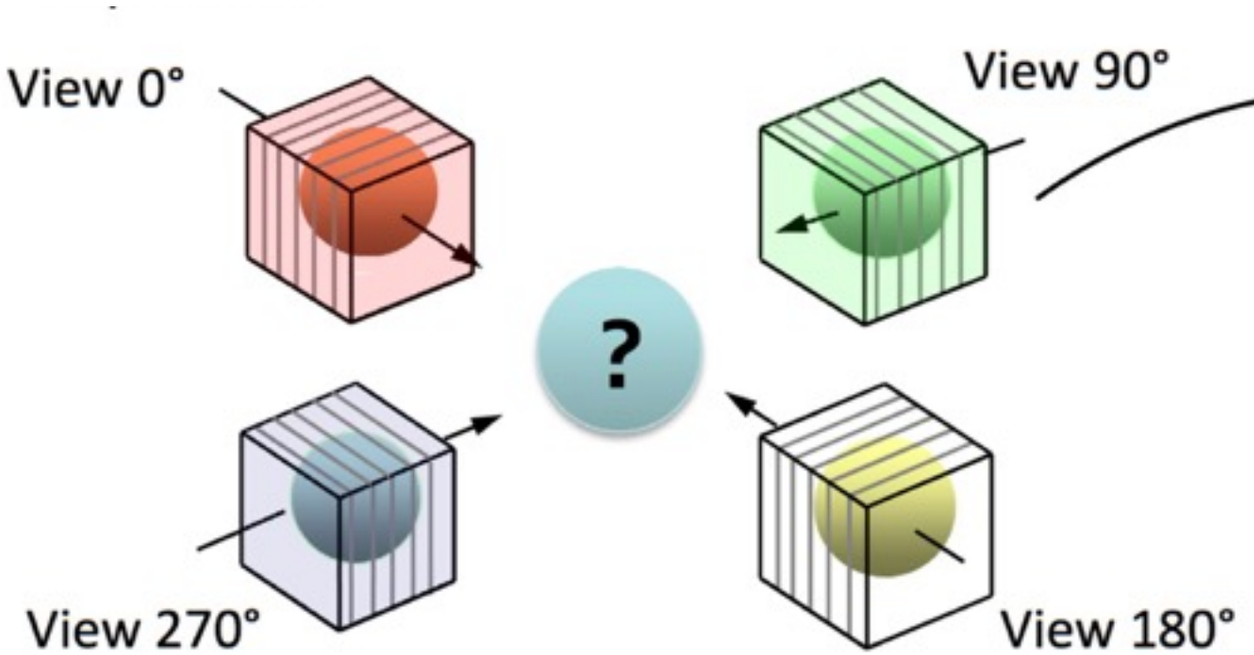


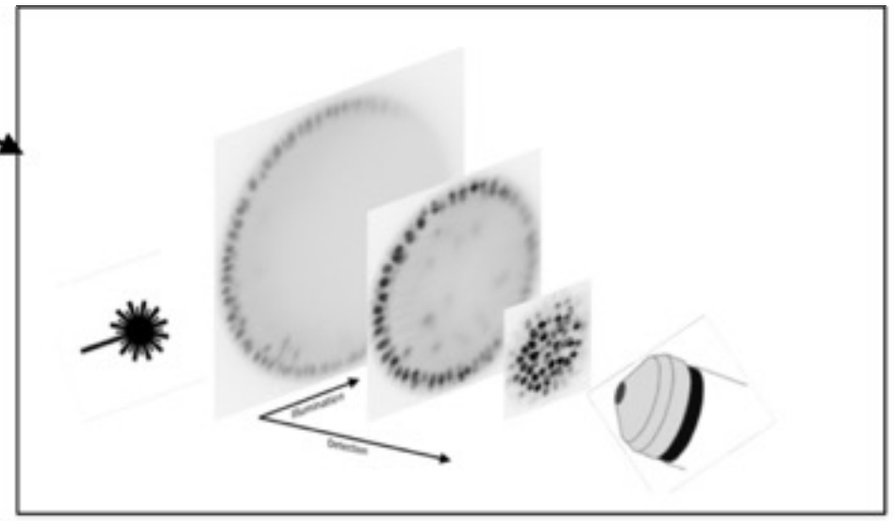


Chhetri 10/2015

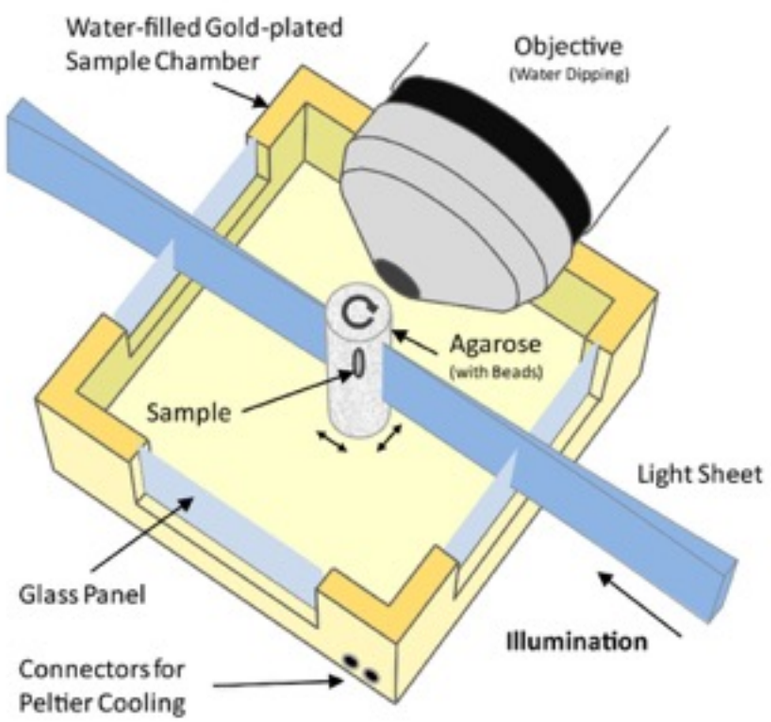
SPIM : Recalage et Fusion

Spatial





Dégradation du signal

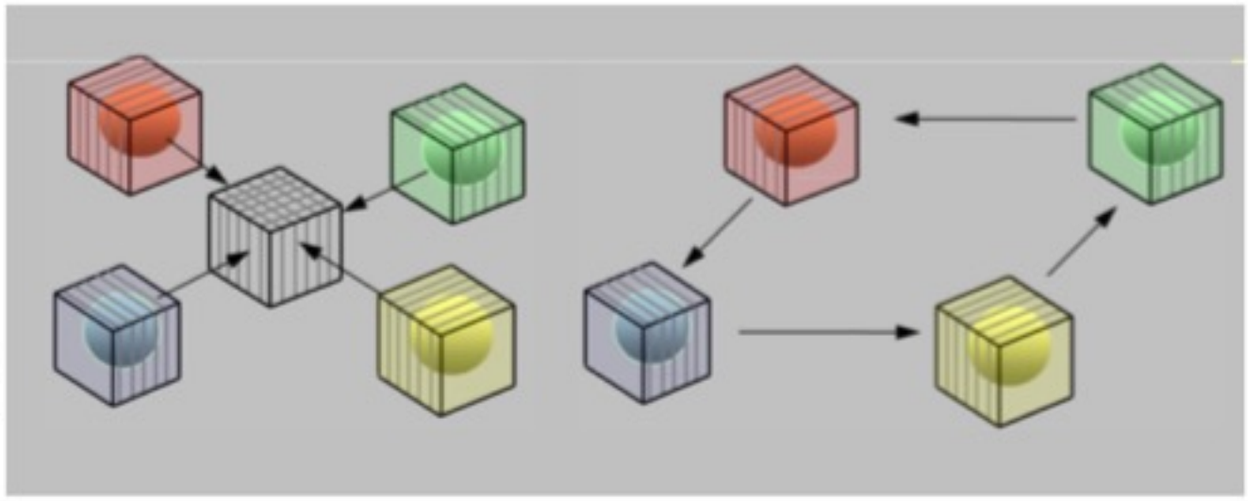


SPIM : Recalage et Fusion

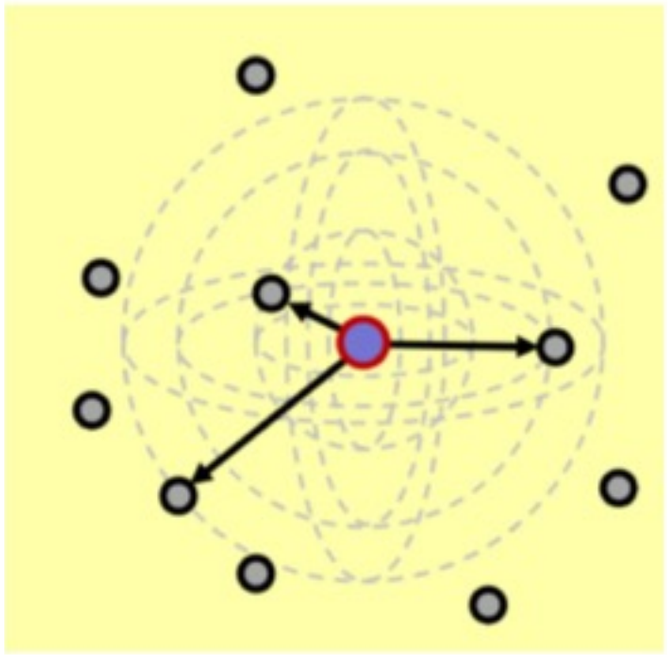
Temporel

Recalage sur l'intensité

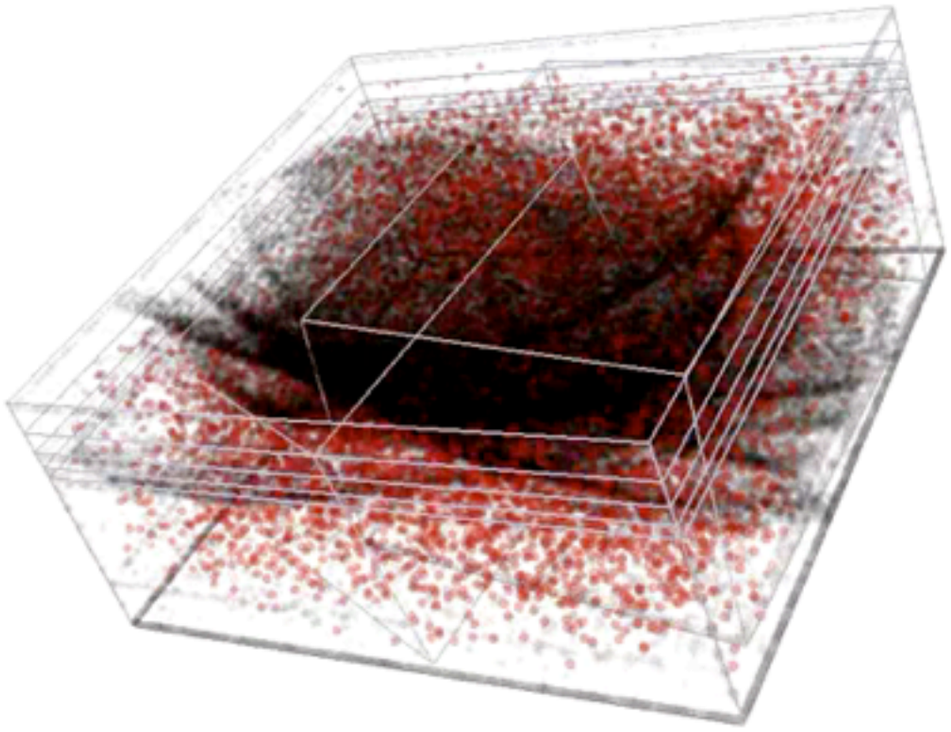
Très lent

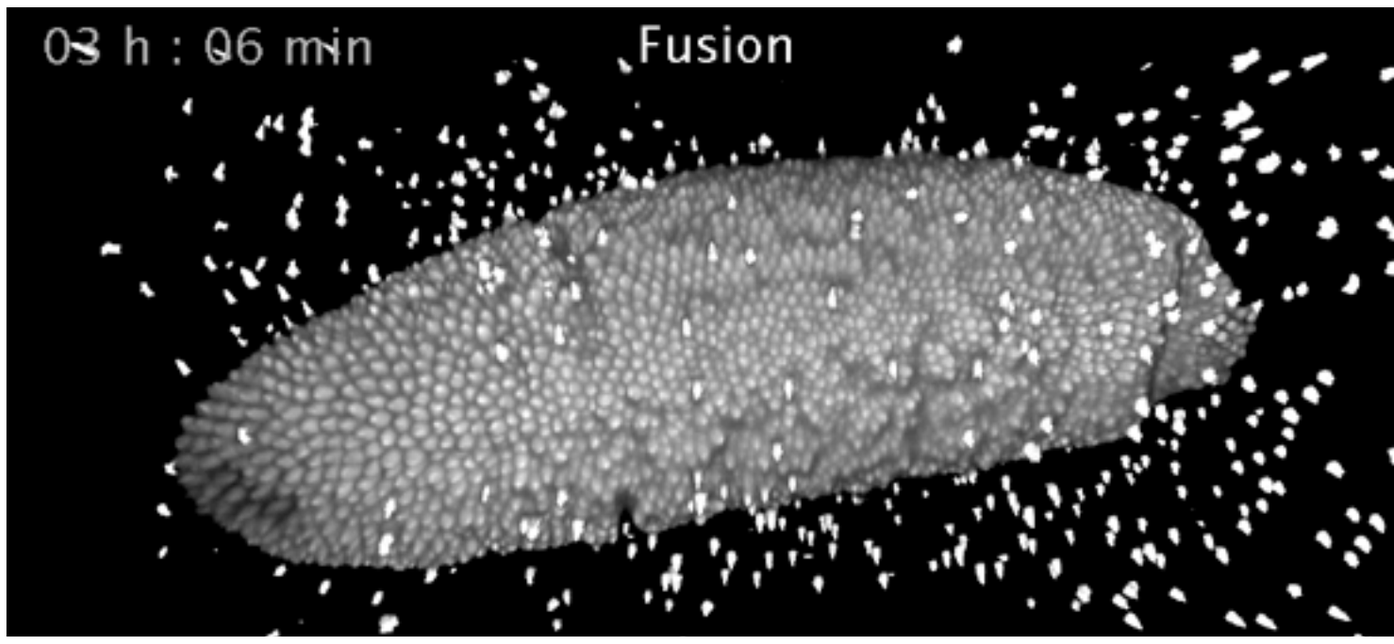


Recalage à l'aide de repères



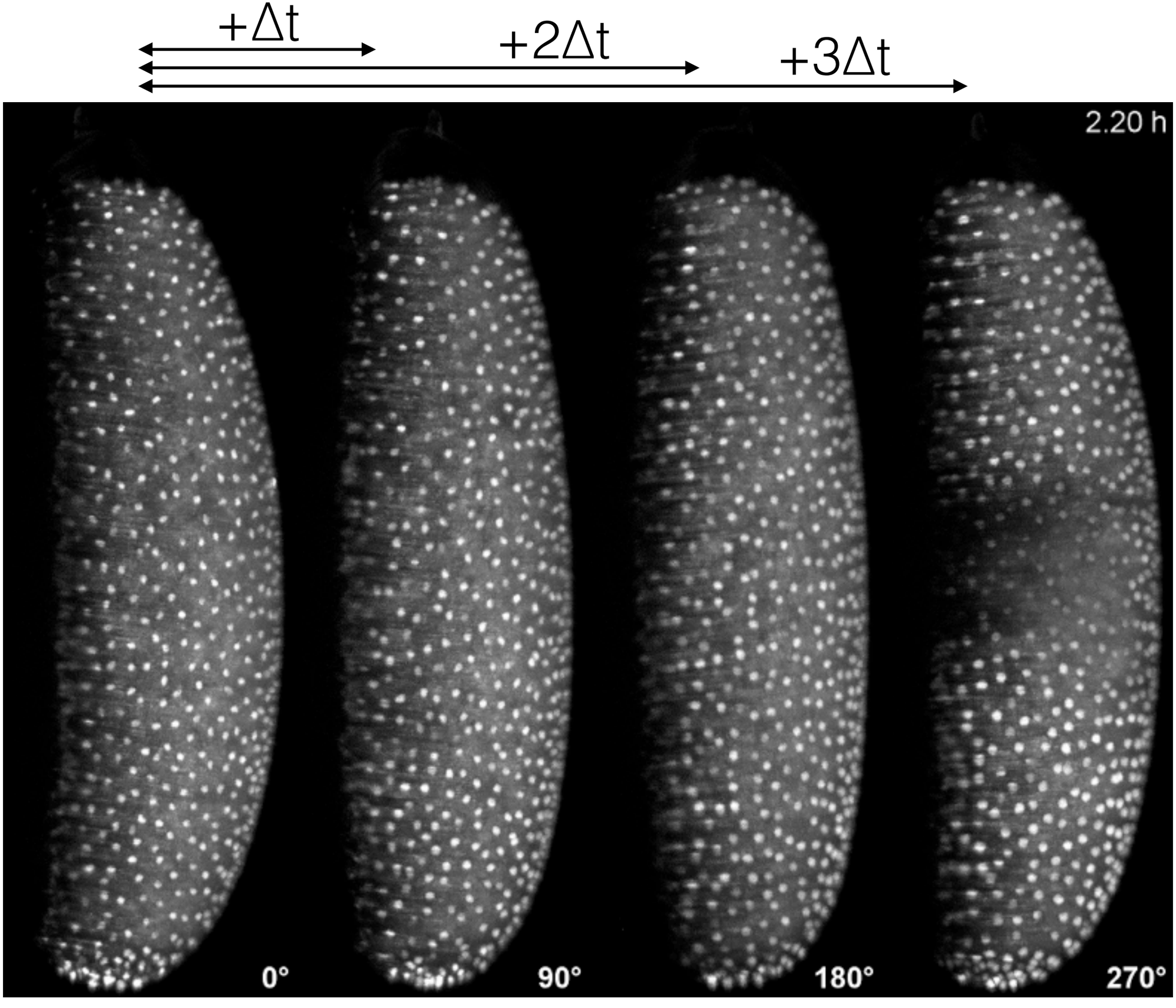
Très rapide, et très bon résultats mais nécessite d'injecter des micro-billes



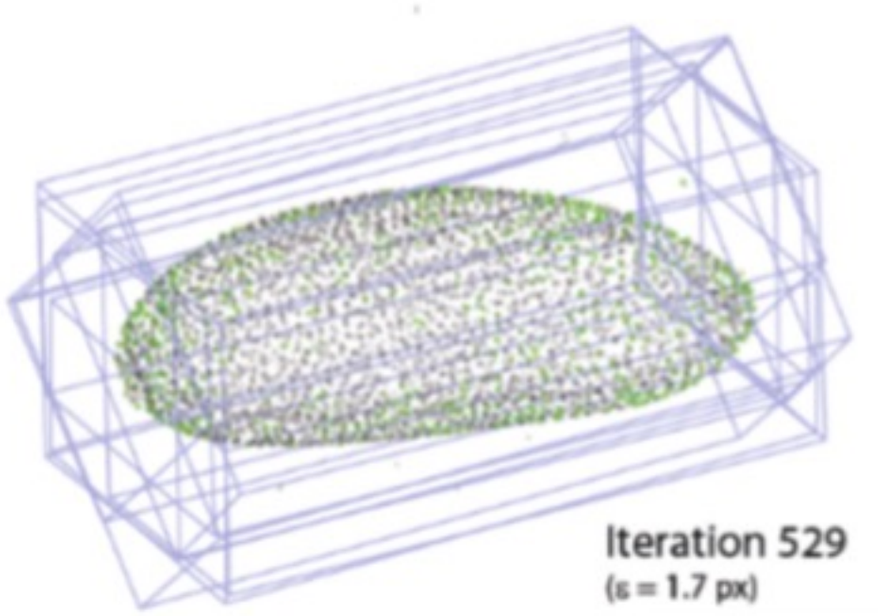


Preibish 2010

Problèmes du recalage à partir d'images



Recalage à l'aide des segmentations



Rapide, mais dépend de la qualité des images et des algorithmes de segmentations

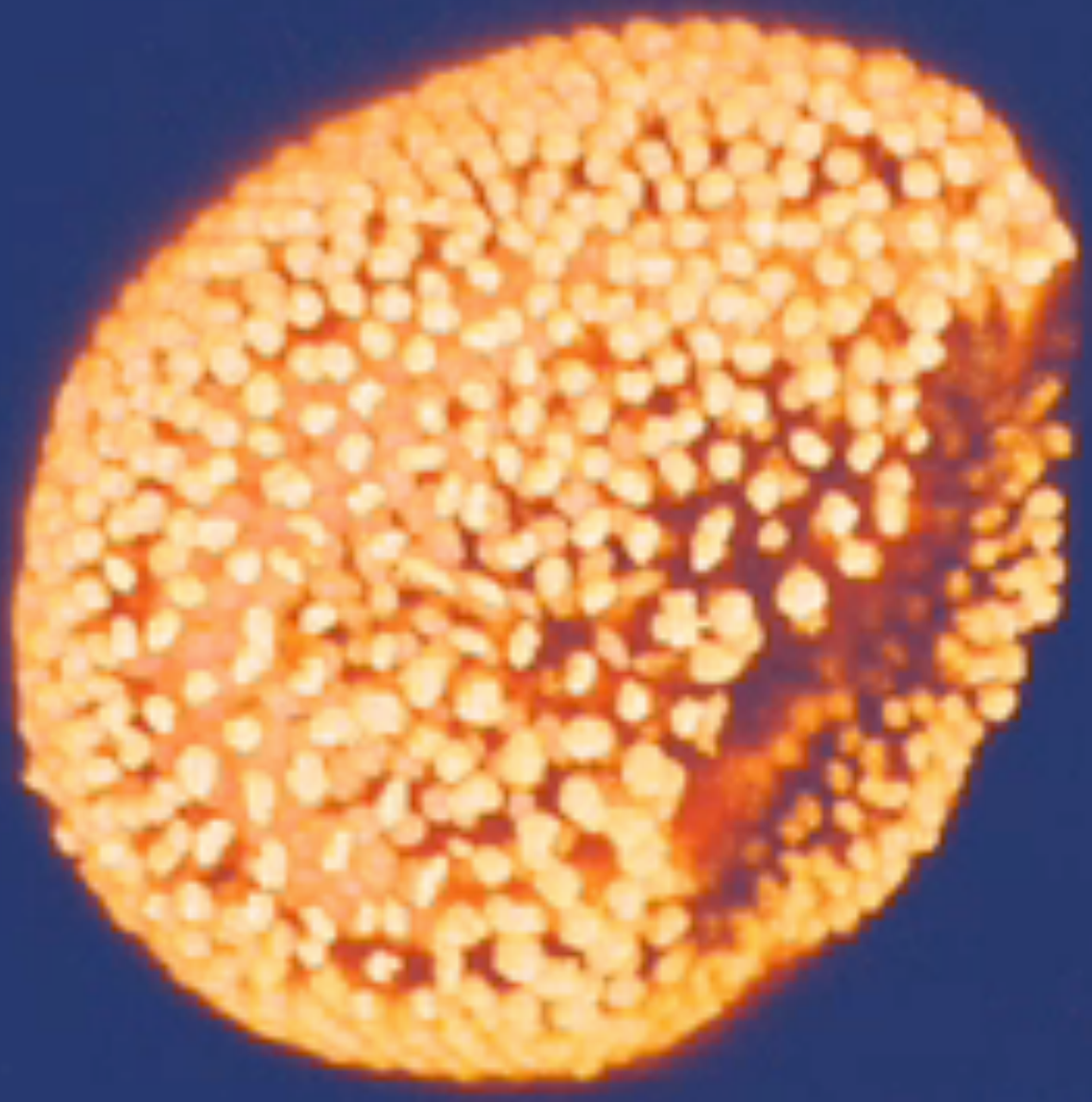
Keller 2008

Qu’observe t’on ?

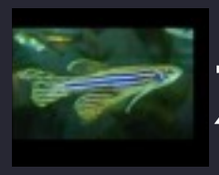
Sea Urchin

Phallusia Mamillata

Amphioxus



Zebrafish



Zebrafish

T = 63

Zebrafish

Zebrafish

Zebrafish

Zebrafish

Taille Image 4D

Acquisition standard:

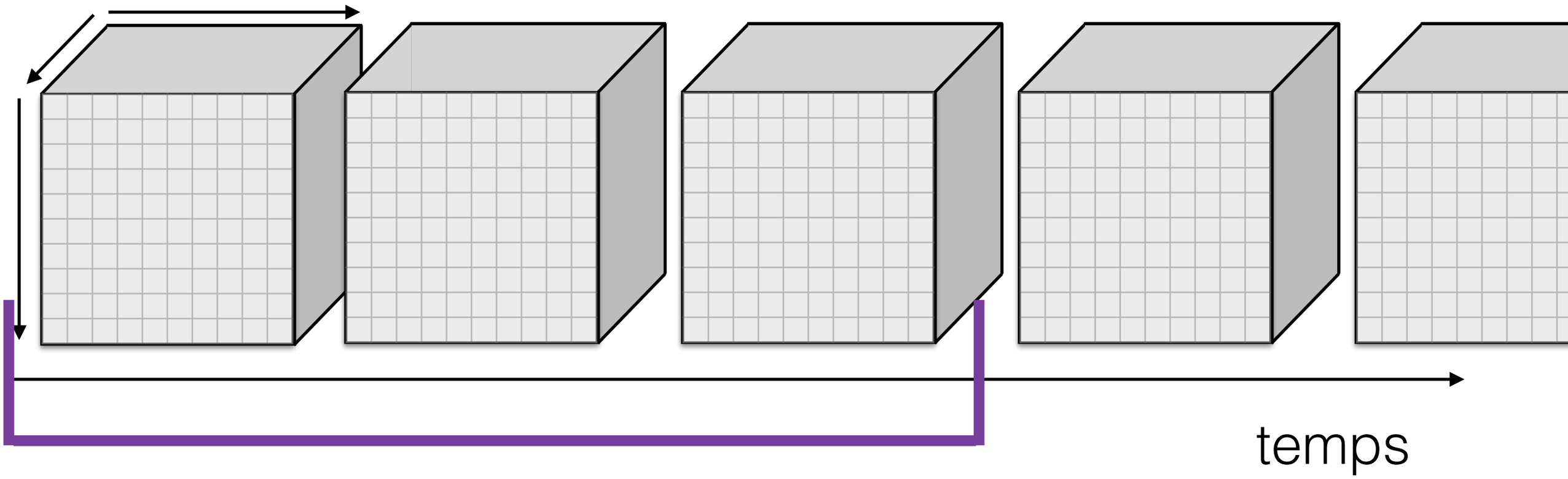
X * Y * Z * Temps * Canaux * Angle : 1000 * 1000 * 200 * 1000 * 2 * 4

⟶ + de mille milliards de voxels / embryons

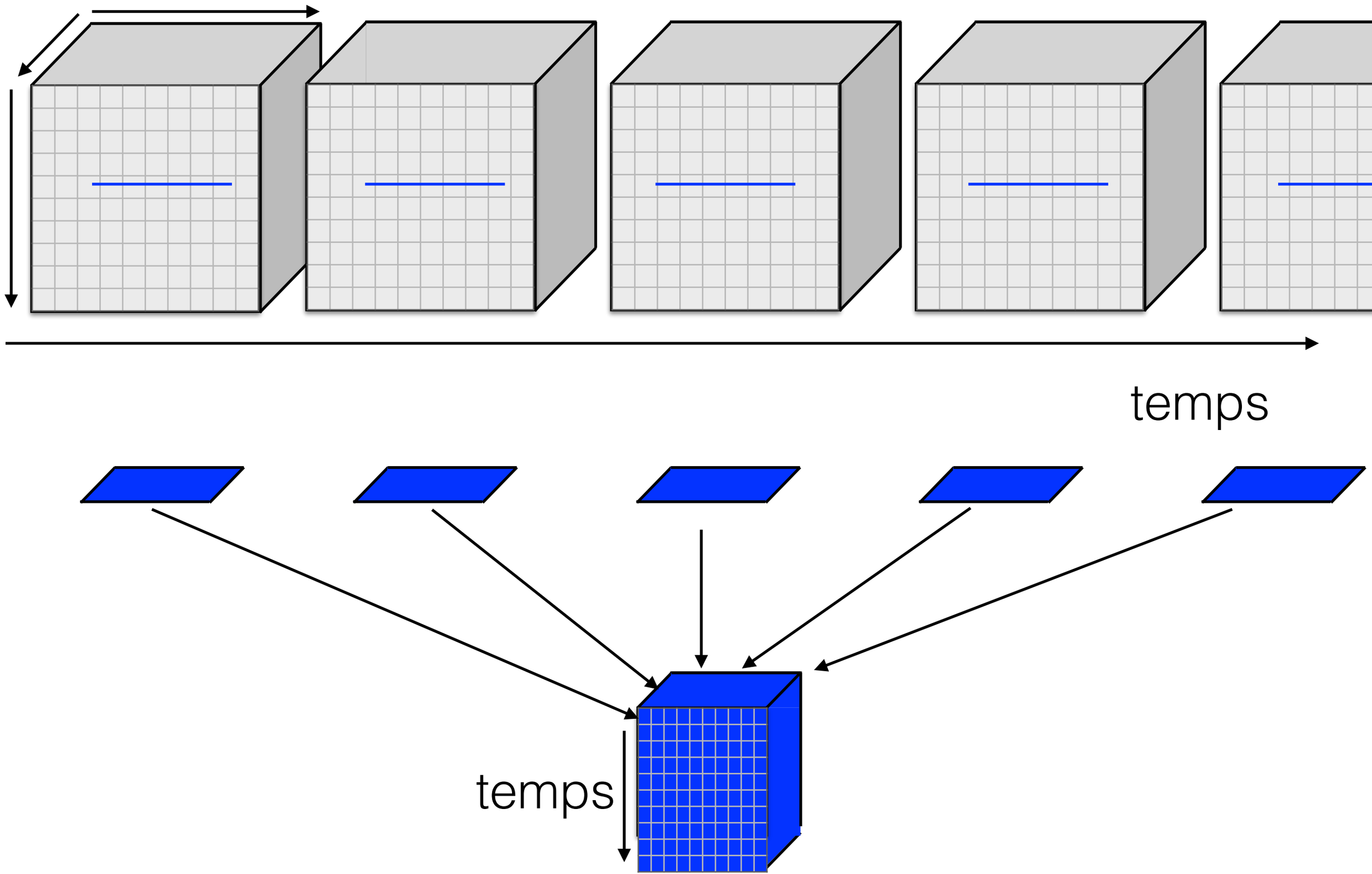
⟶ plusieurs terabytes / embryons

⟶ très bon taux de compression ~90%

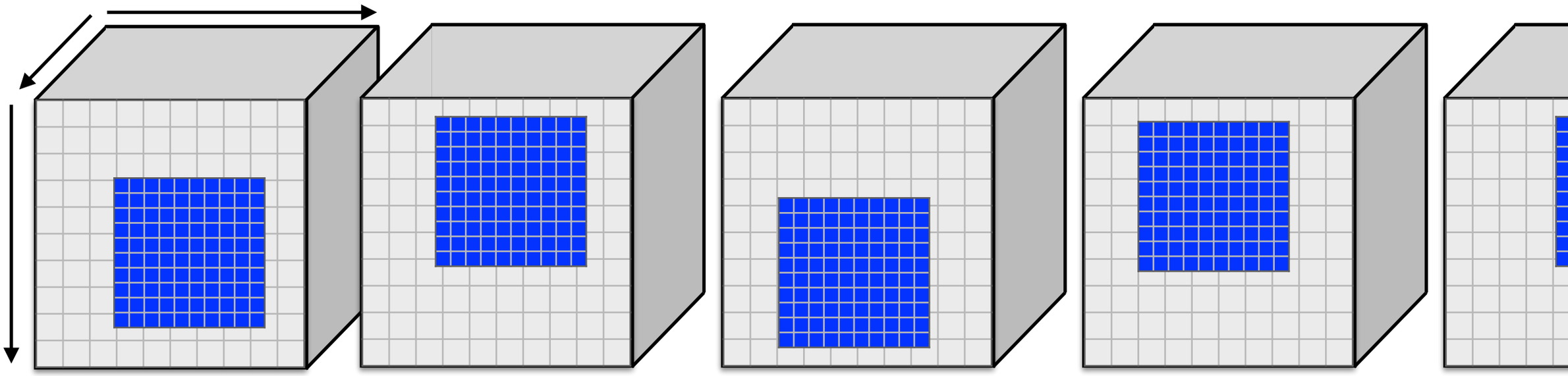
Comment parcourir les images 4D ?

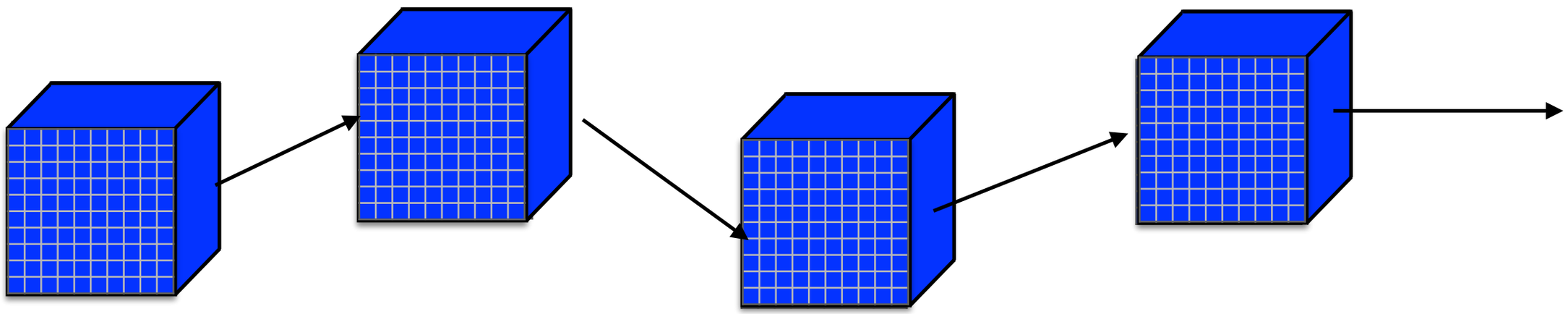


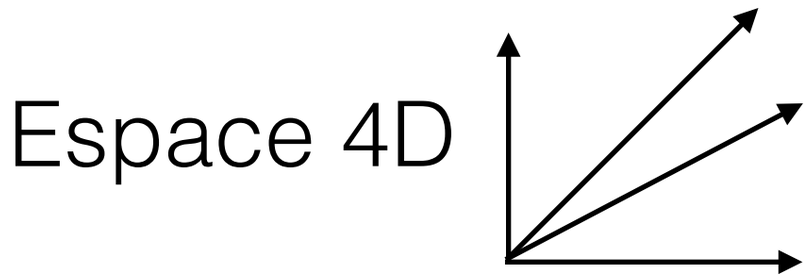
Comment parcourir les images 4D ?



Comment parcourir les images 4D ?

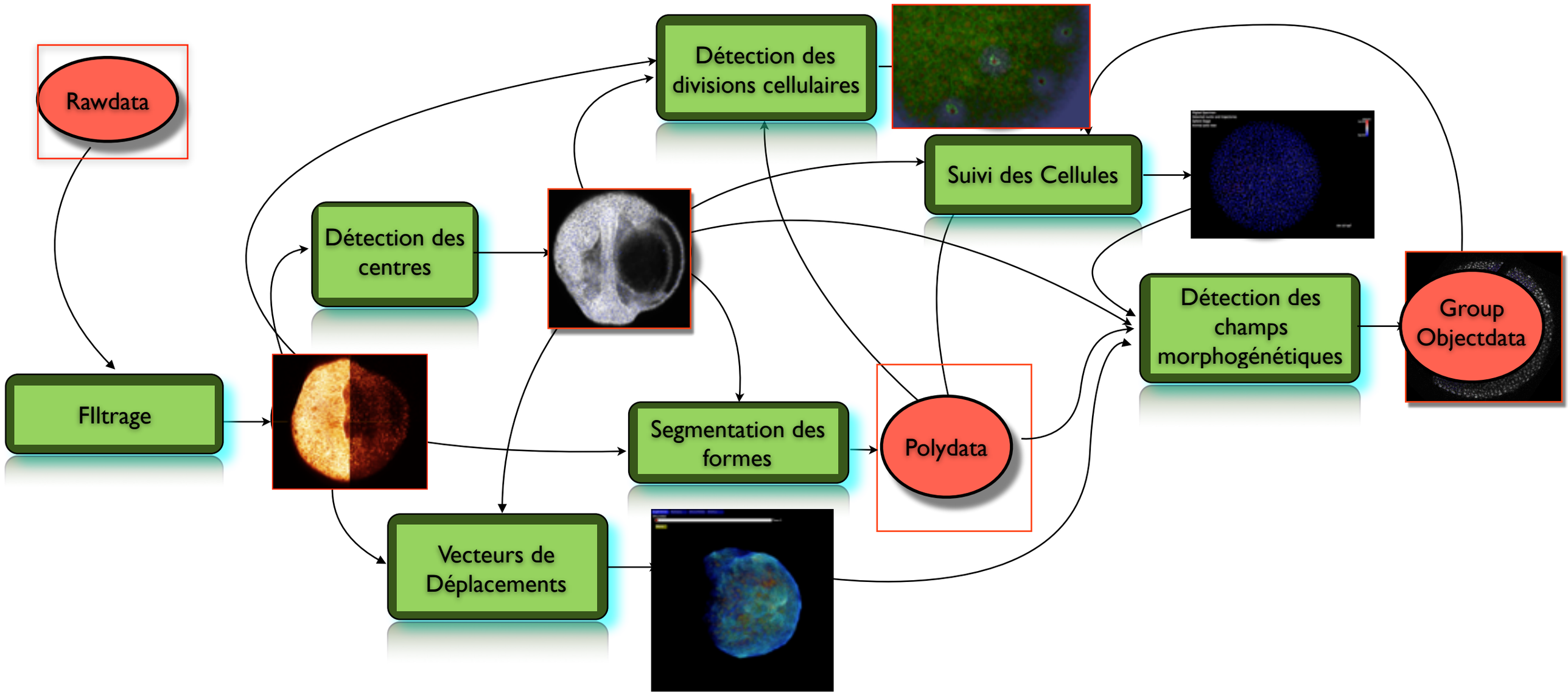




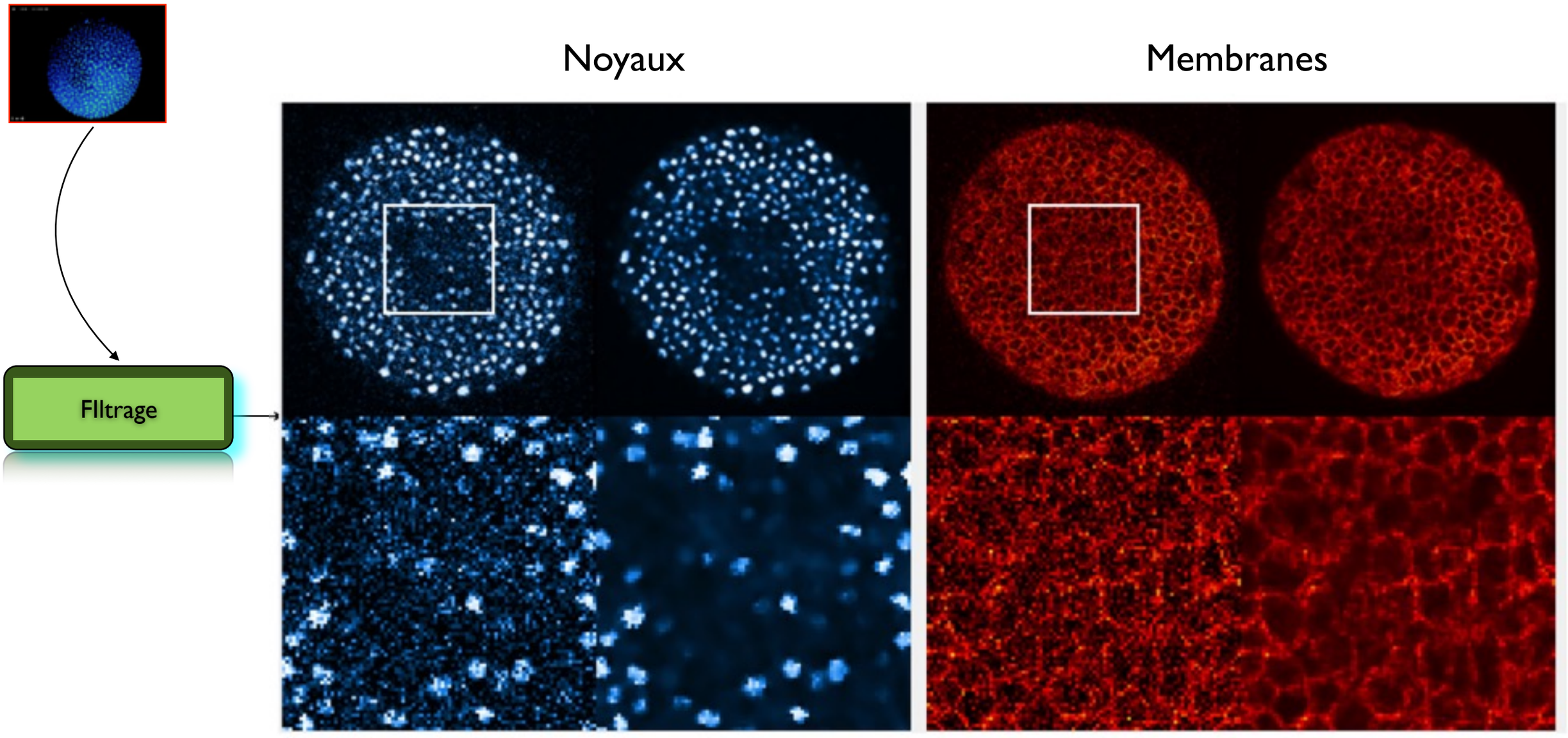


Reconstruction d’images 4D

Reconstruction Image 4D

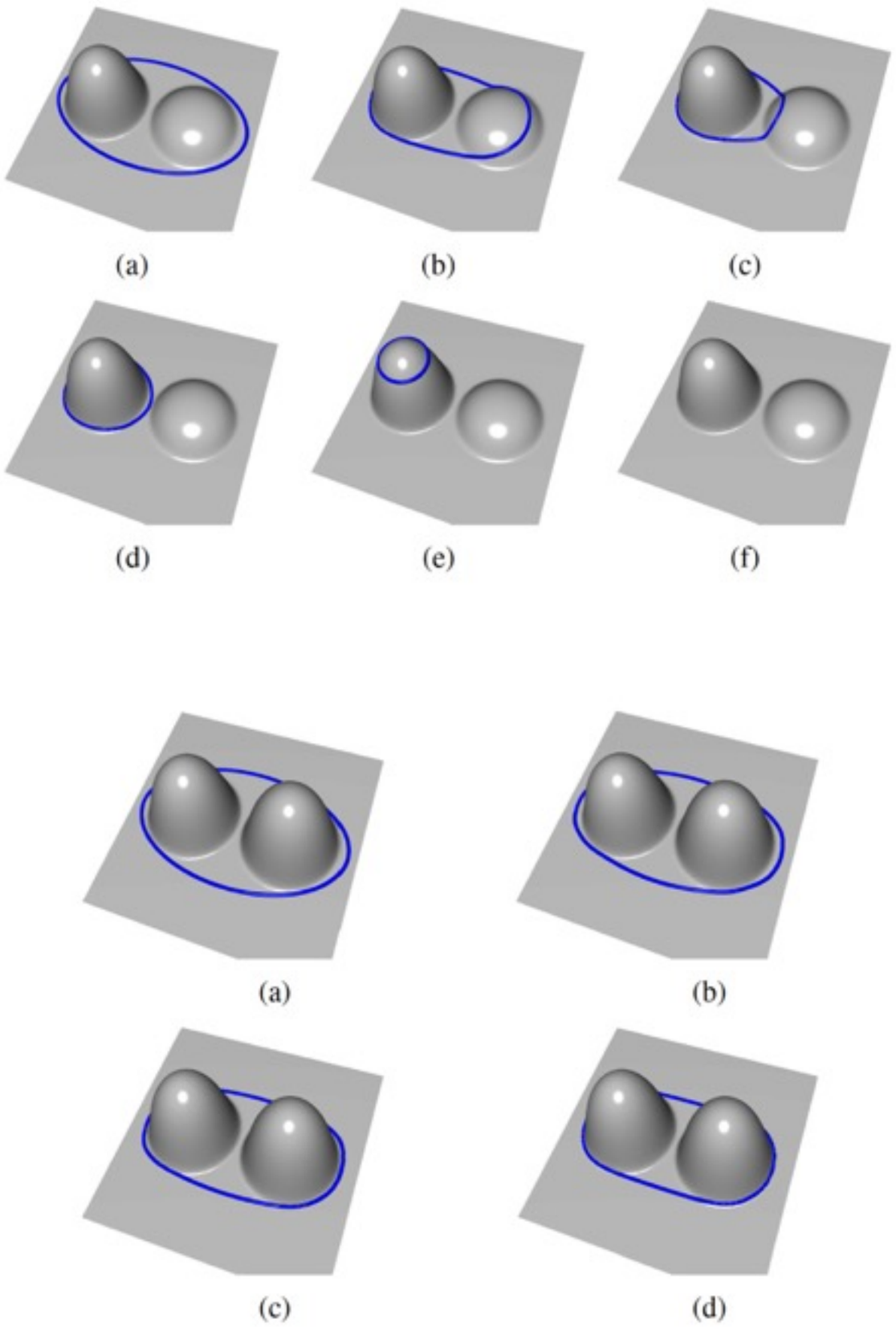


Filtres non linéaire

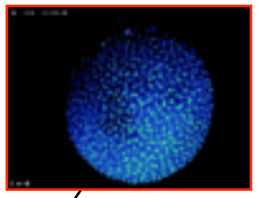


Intégrité des contours : on souhaiterait éliminer le bruit sans rendre flous les frontières des objets.

Geodesic Mean curvature Flow

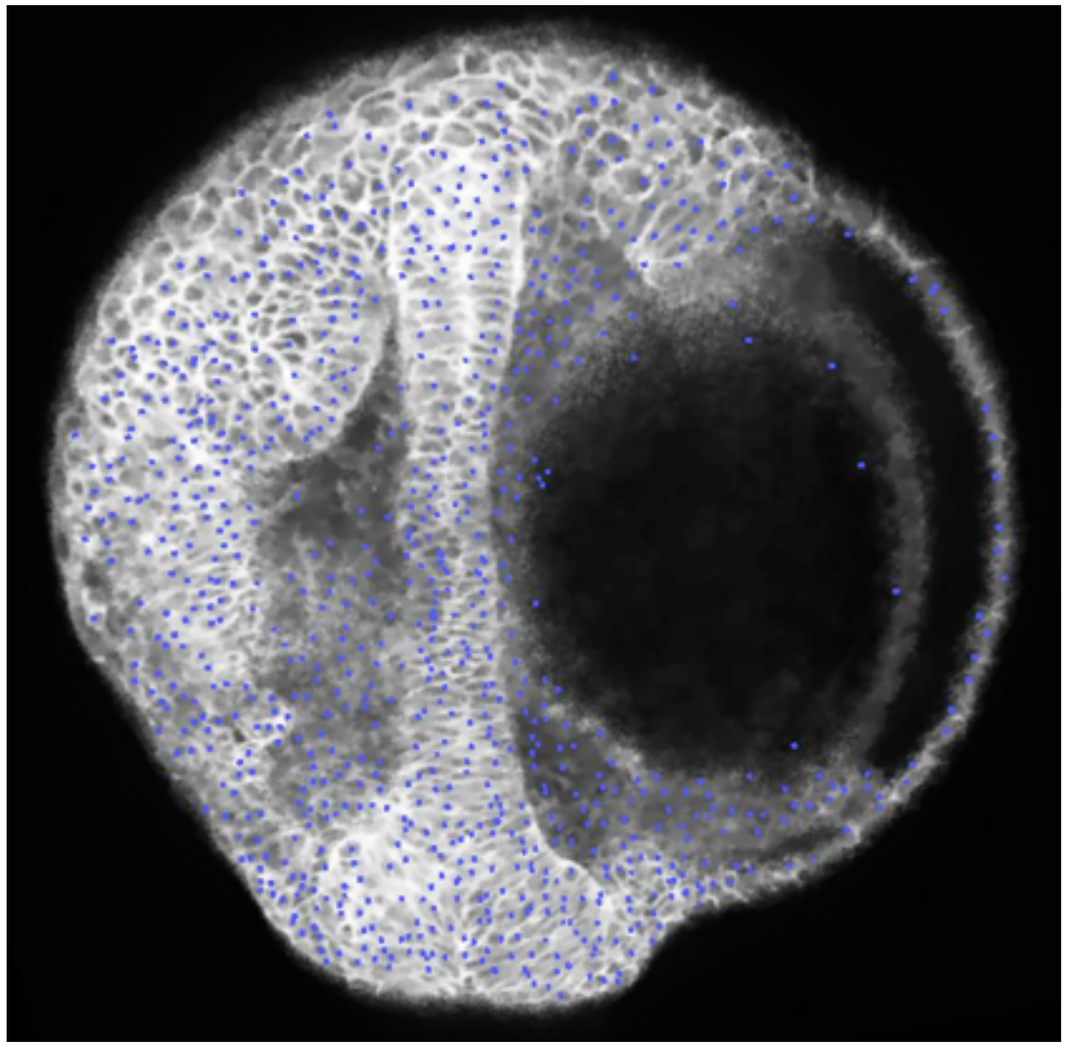


Identification des cellules

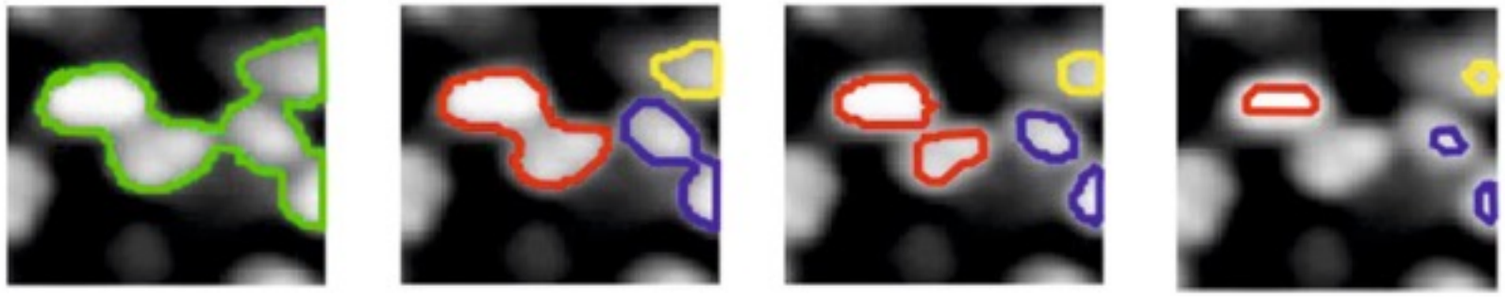


Détection des centres

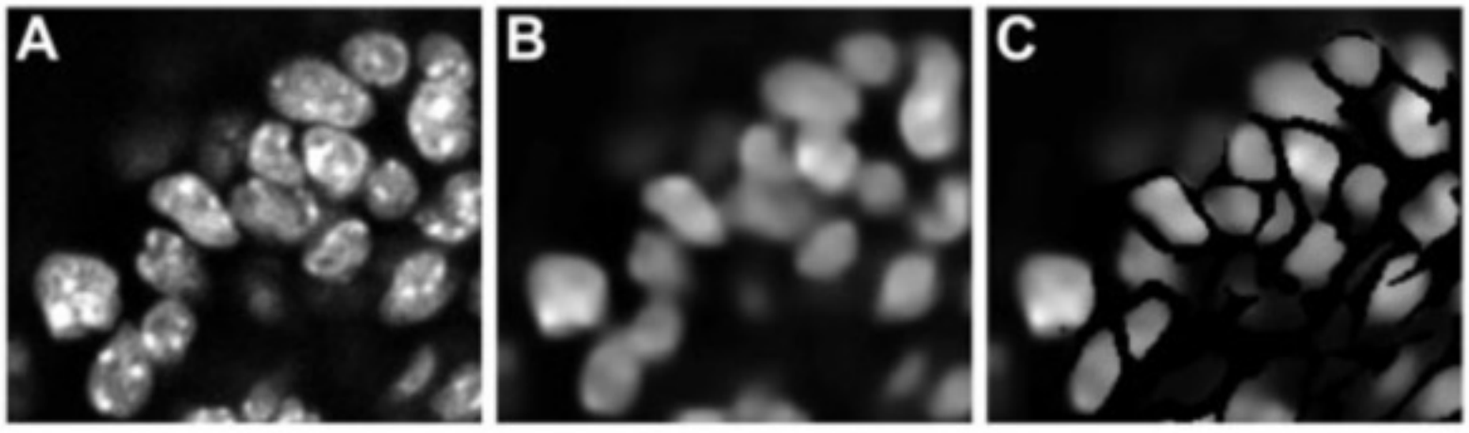
Filtrage



Morphologie Mathématique

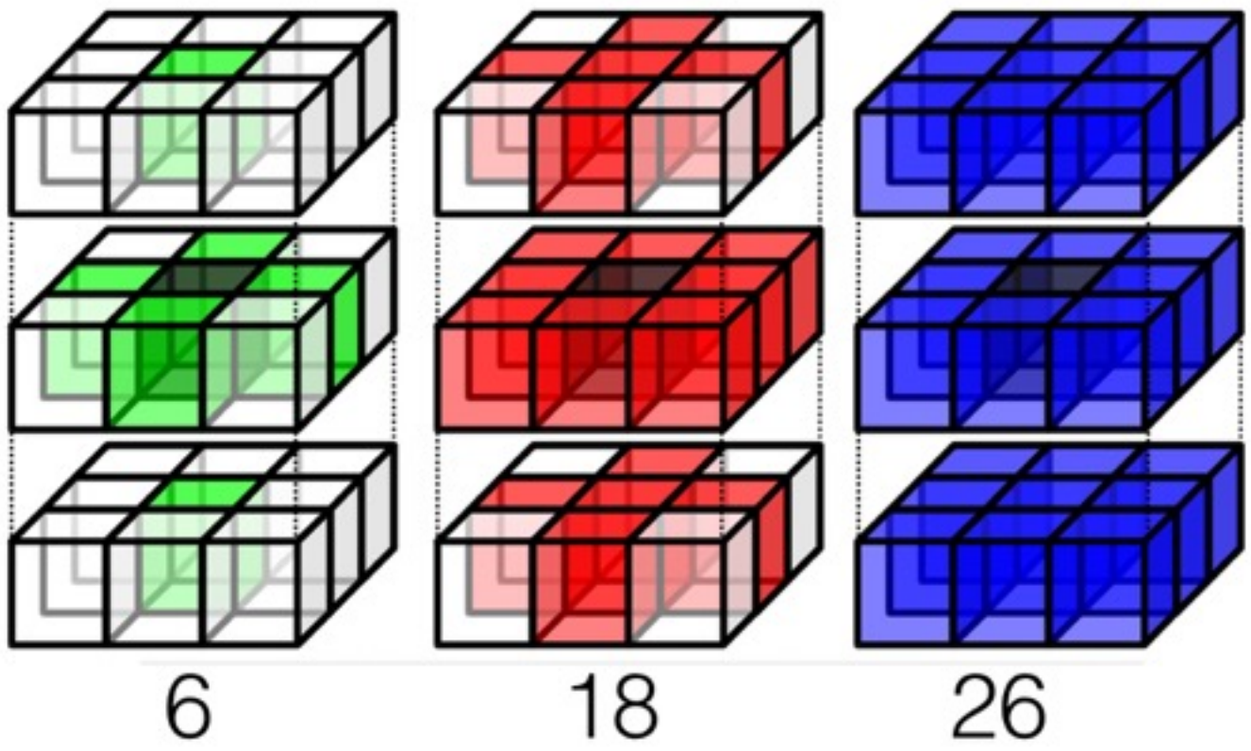


Soustraction de l'image des membranes

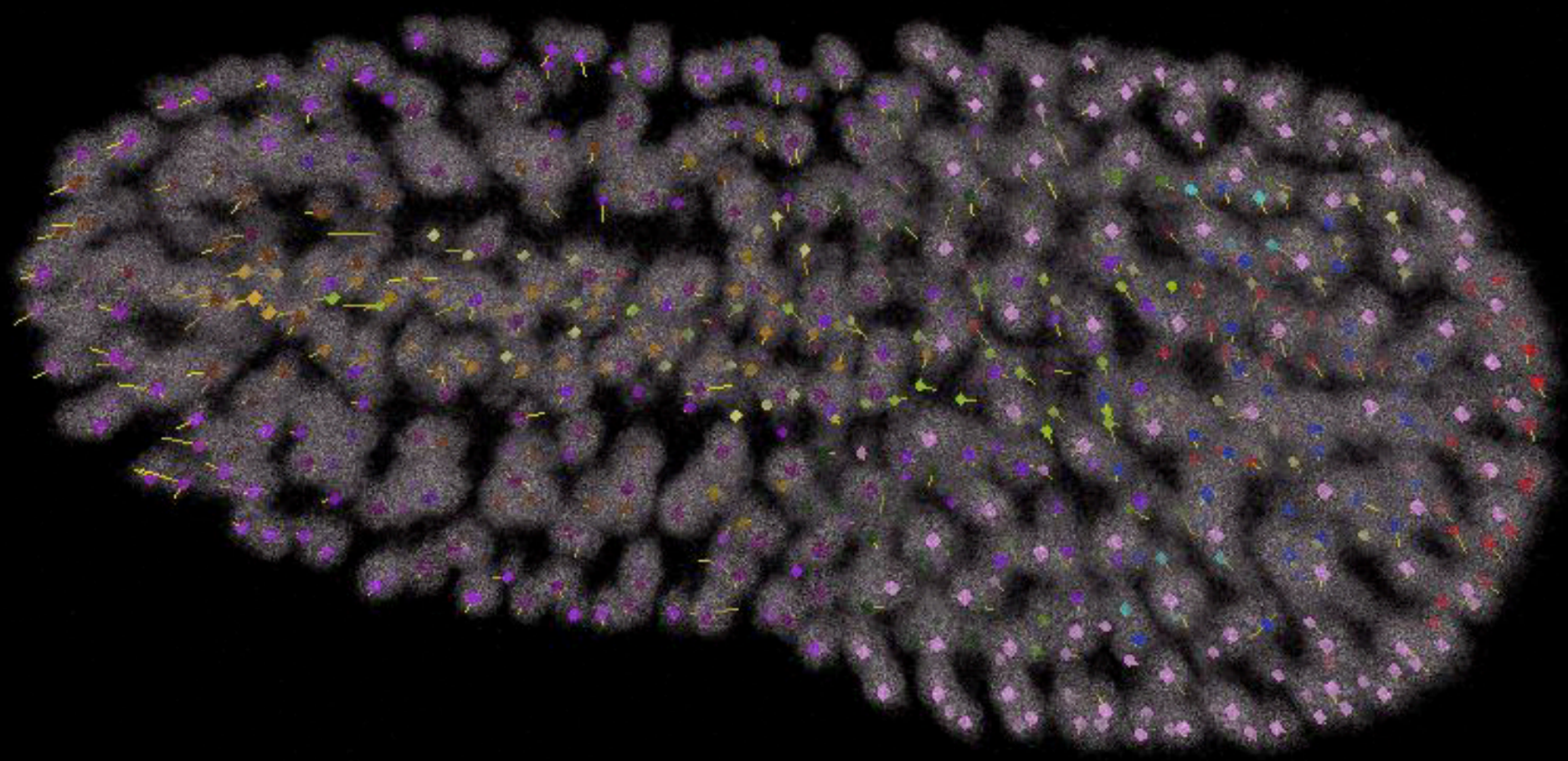


Pop 2013

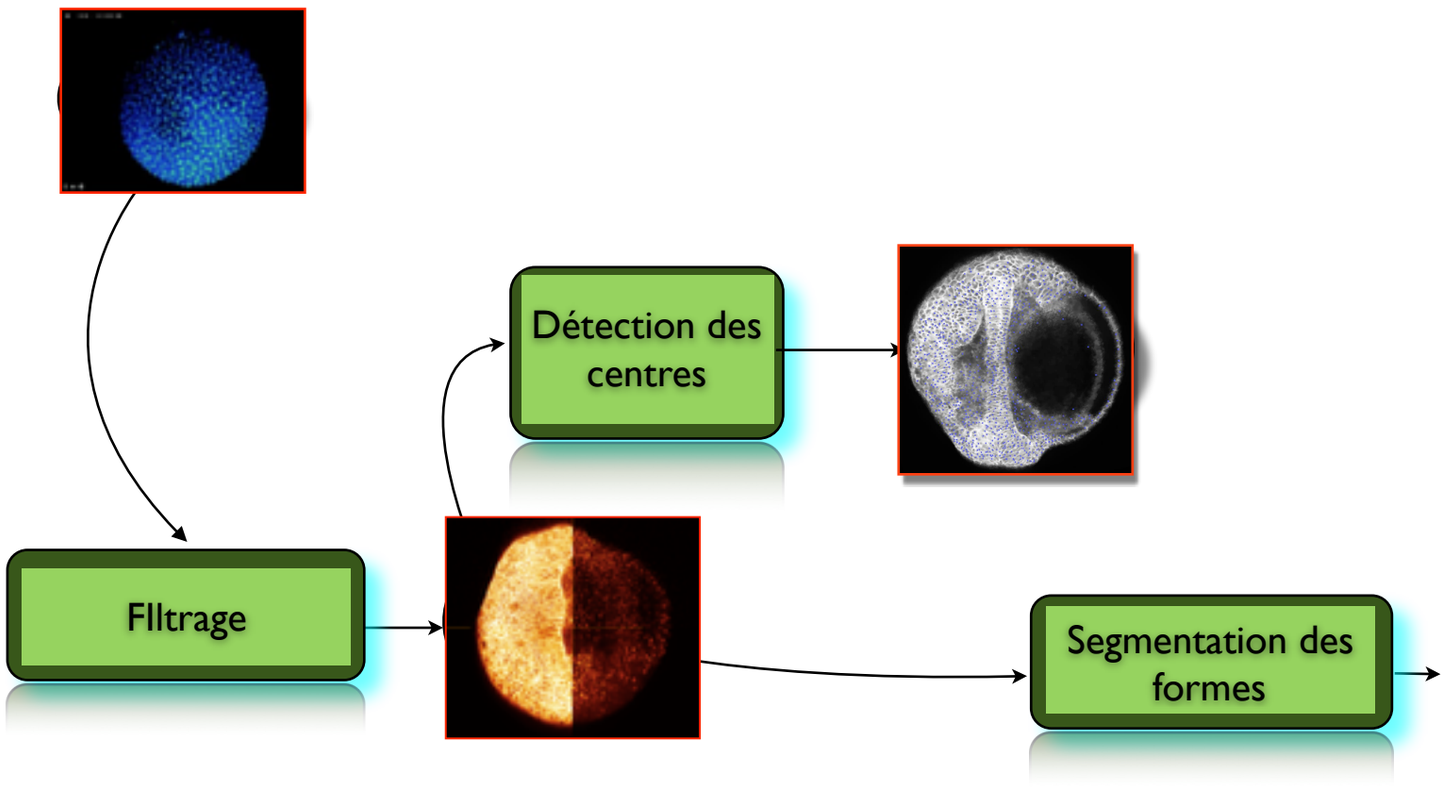
Labels par composantes connexes



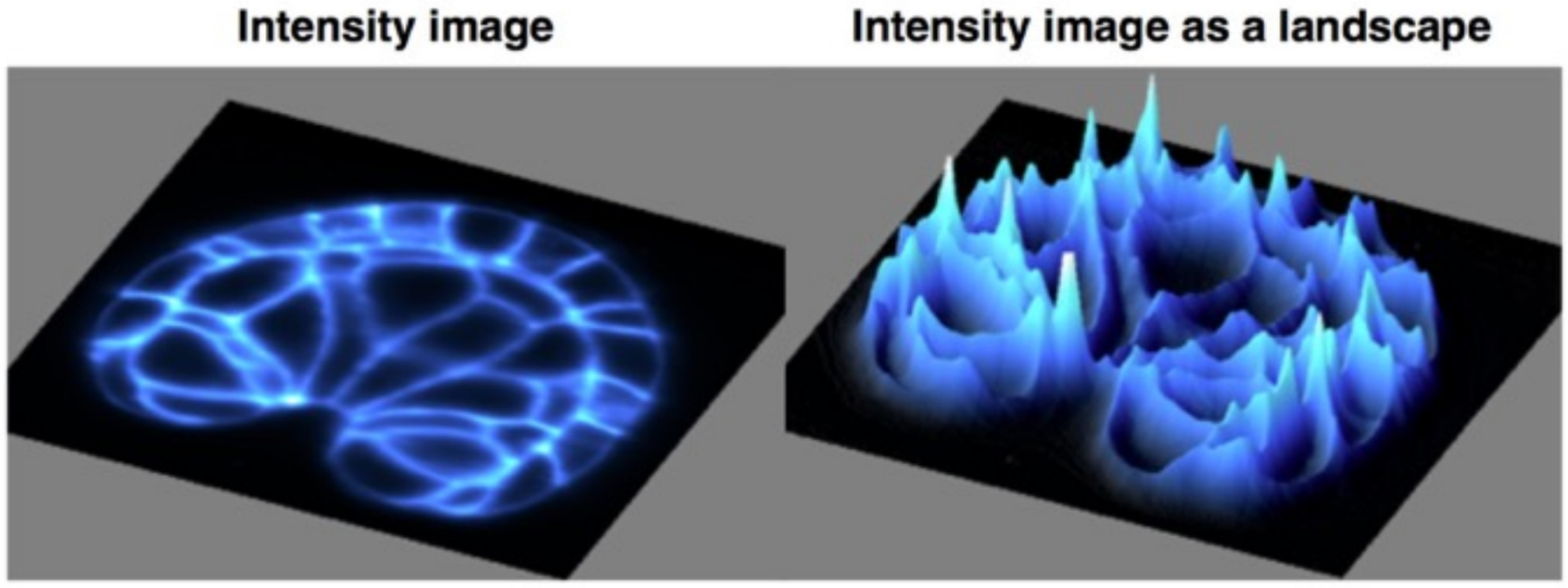
time 62

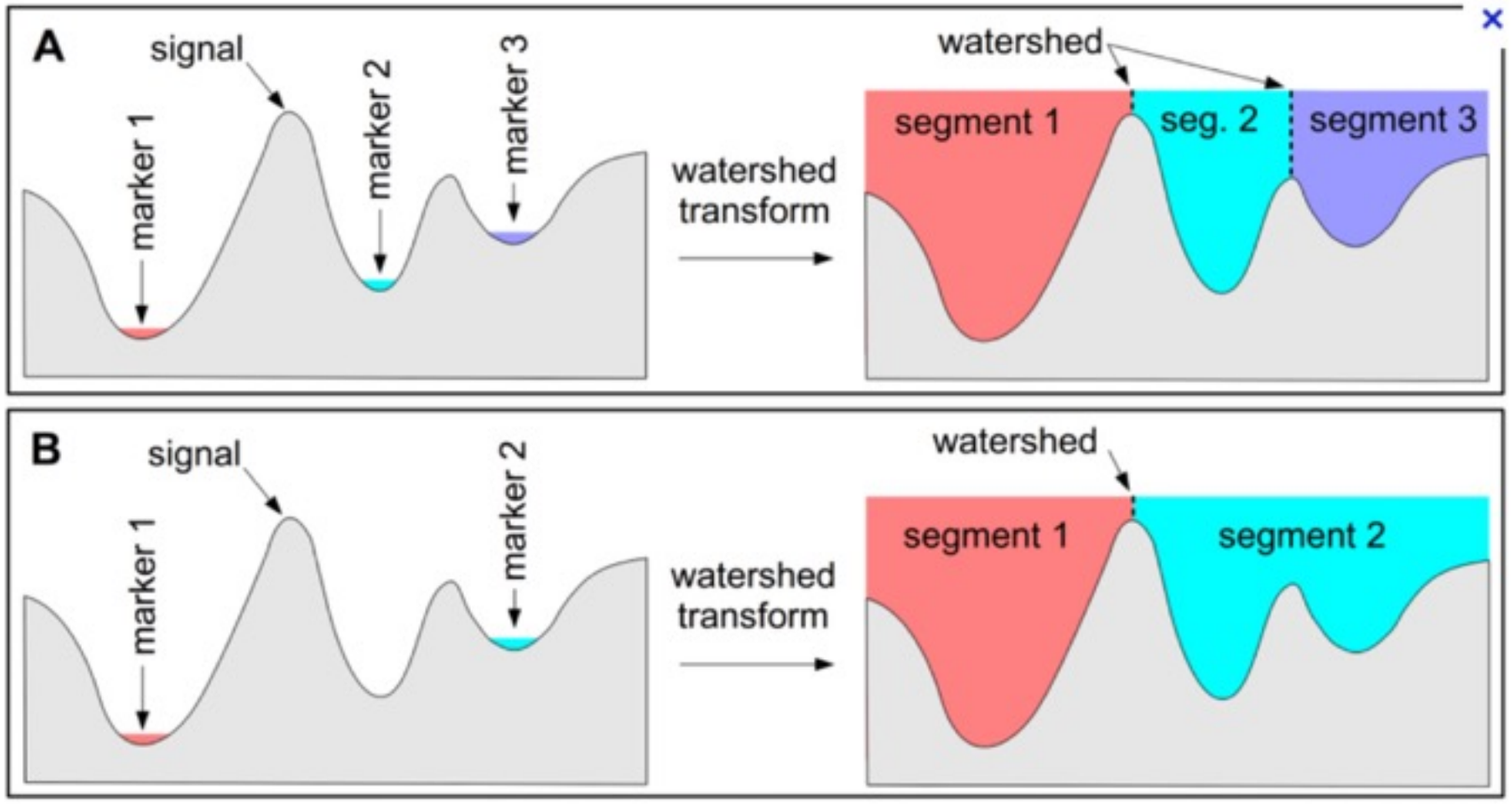


Segmentation 3D

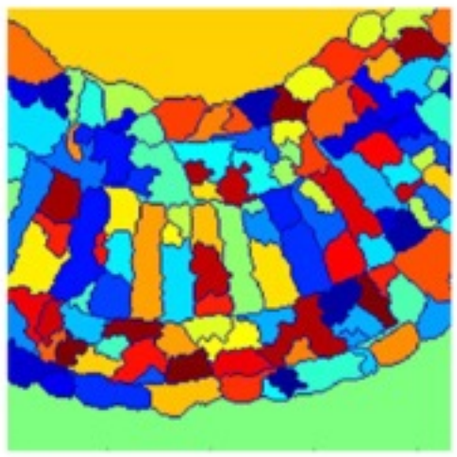
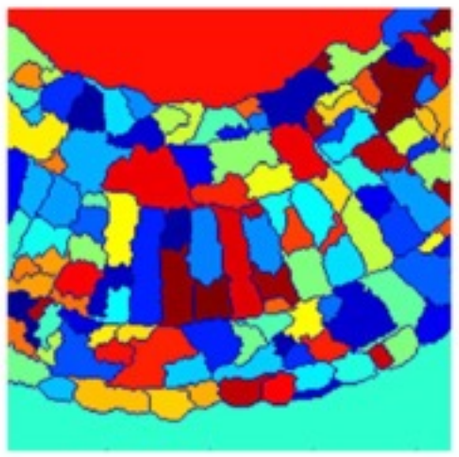
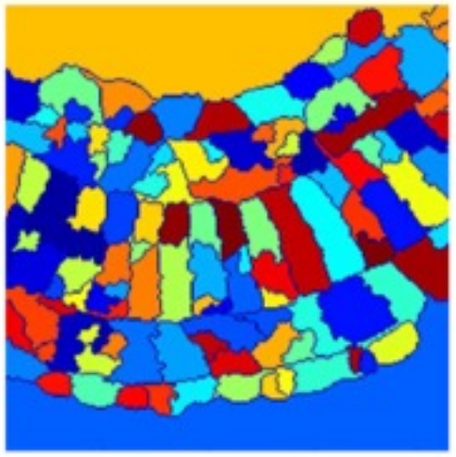


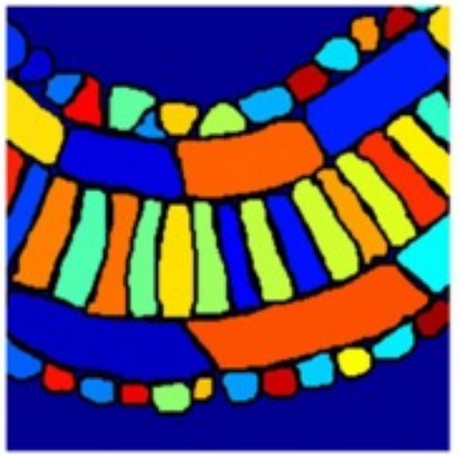
Watershed


Intensity image
Intensity image as a landscape


A
signal
marker 1
marker 2
marker 3
watershed transform
watershed
segment 1
seg. 2
segment 3
B
signal
marker 1
marker 2
watershed transform
watershed
segment 1
segment 2

Watershed

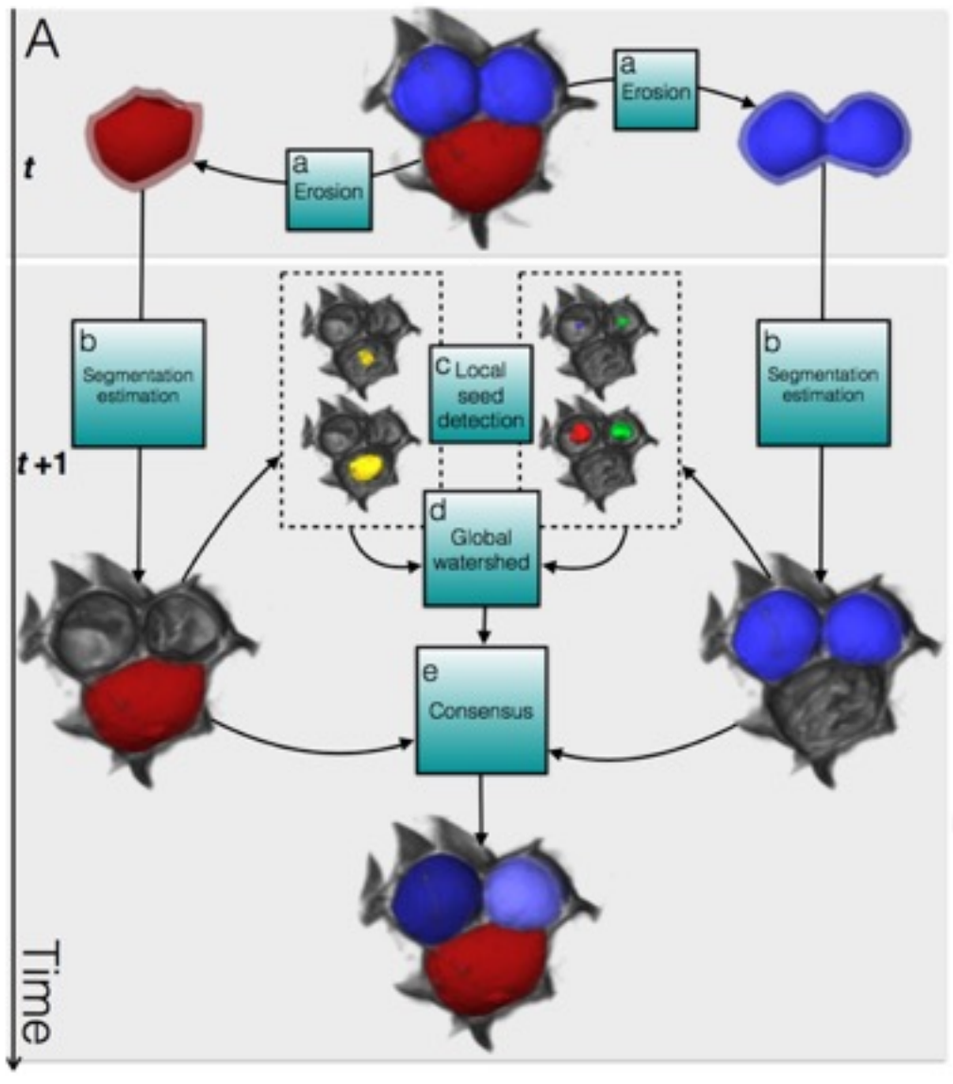






Random seed

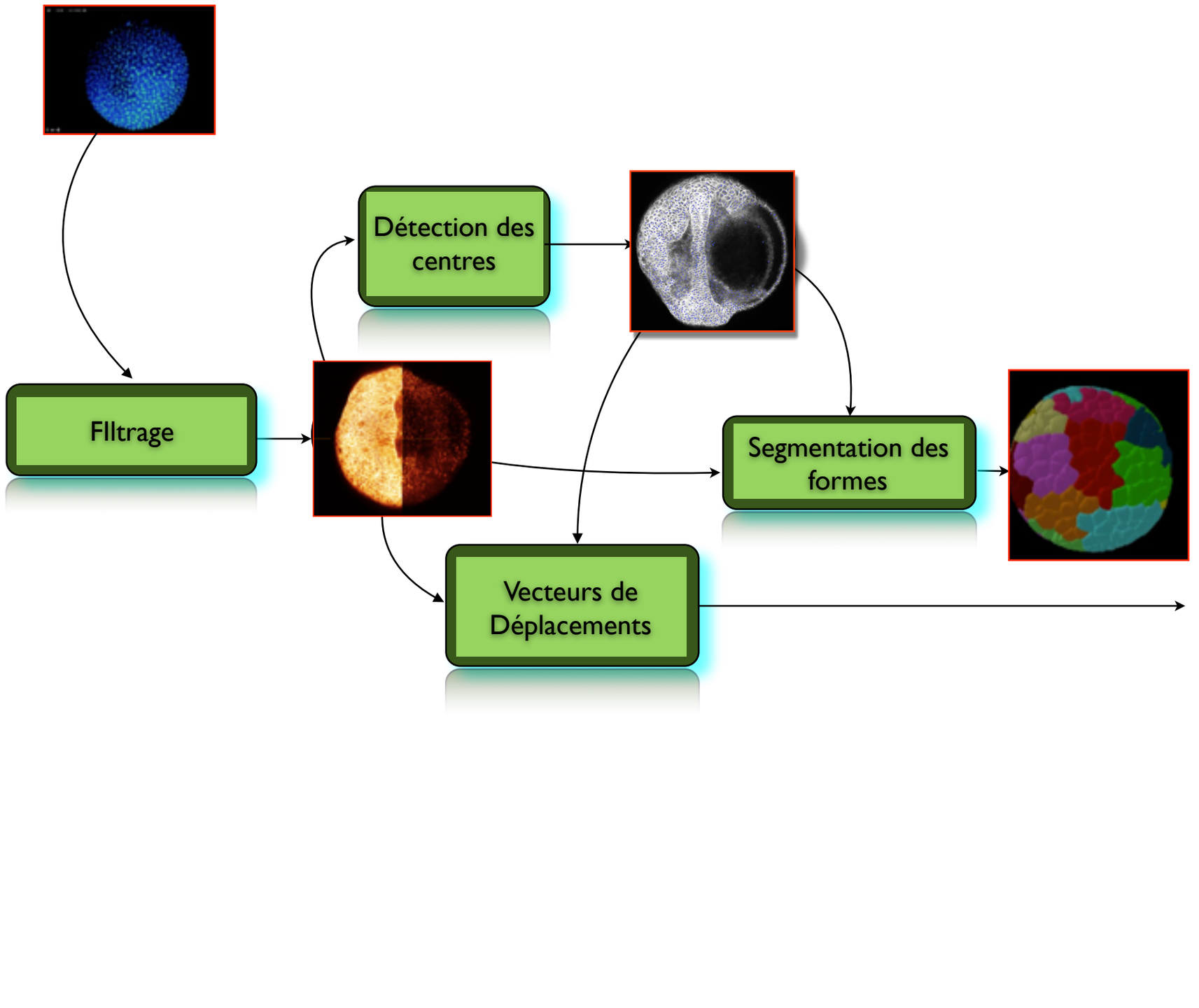
Delibaltov 2013

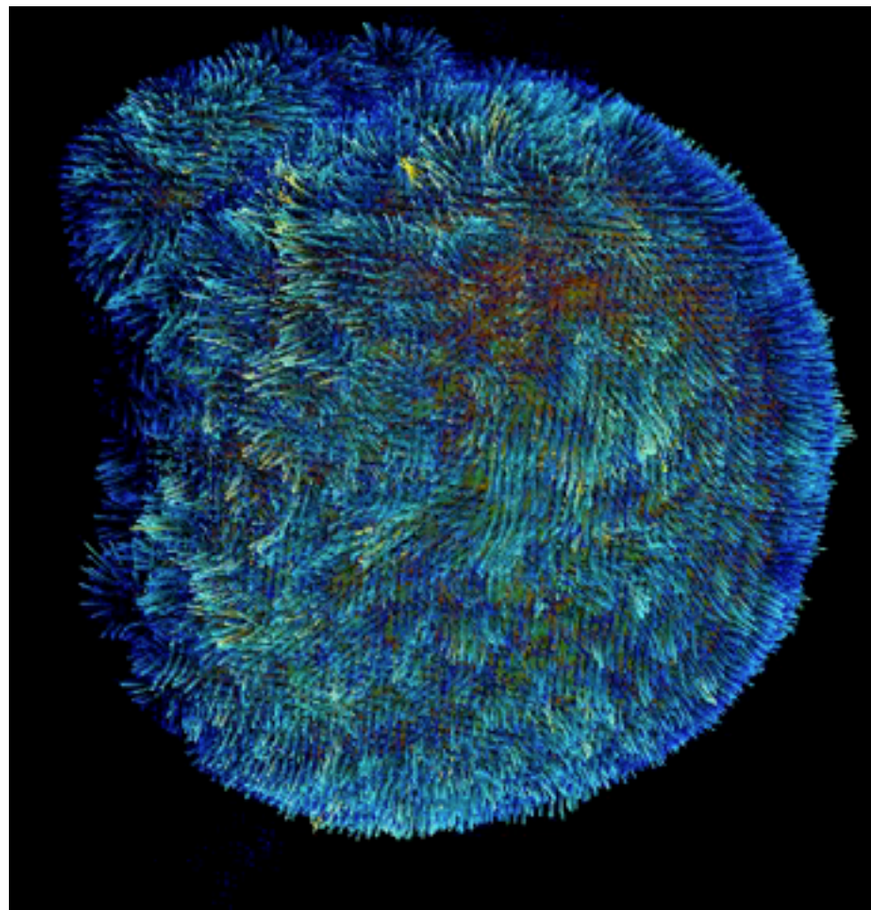
Watershed 4D



Guignard 2015

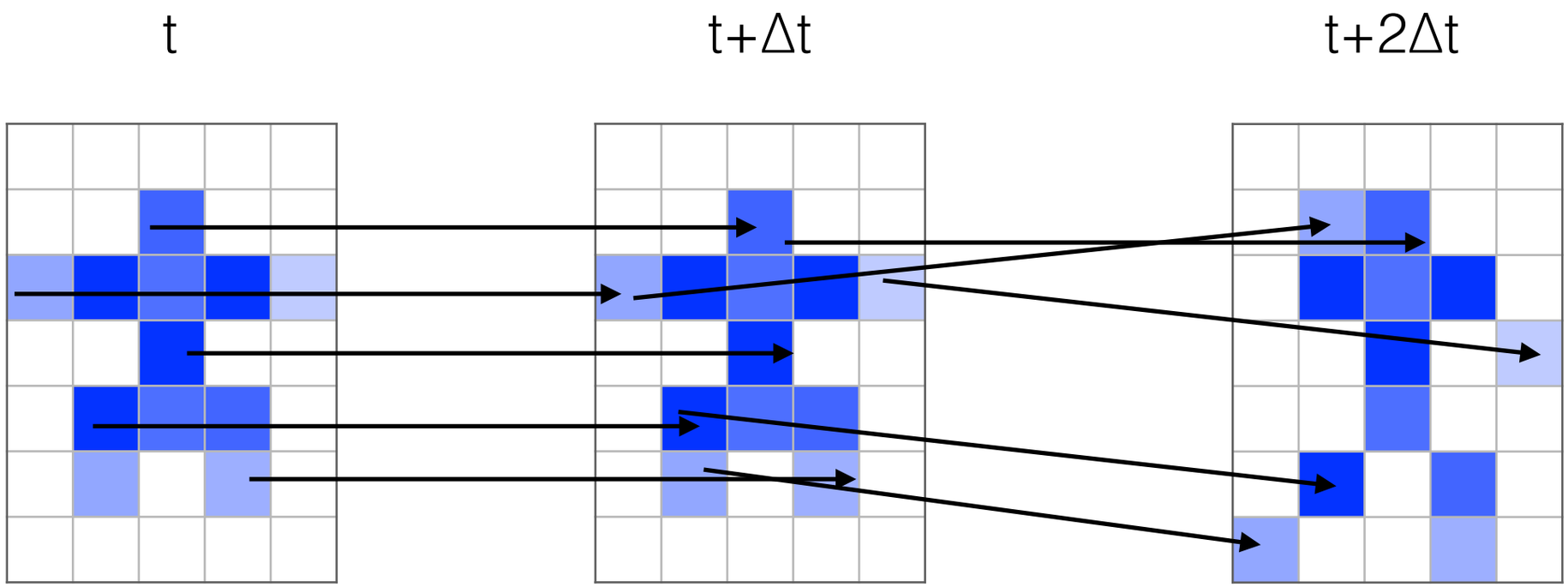
Reconstruction Image 4D

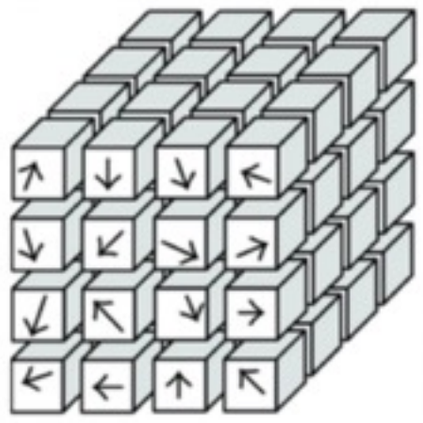




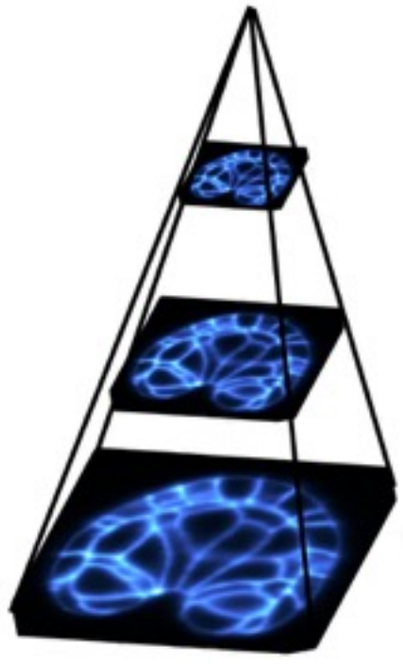
Vecteurs de déplacements (Flot optique)

Recalage non rigide



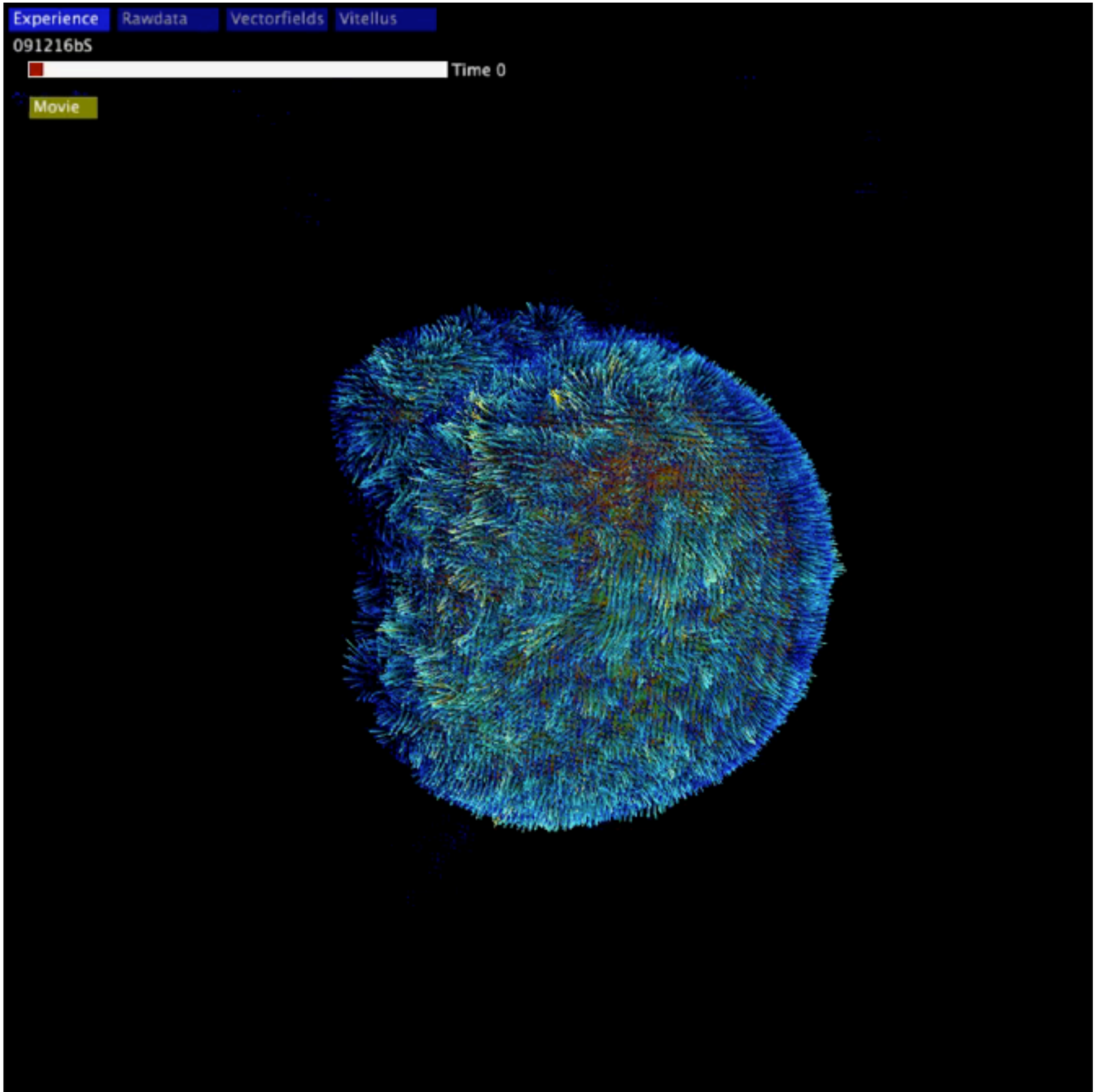


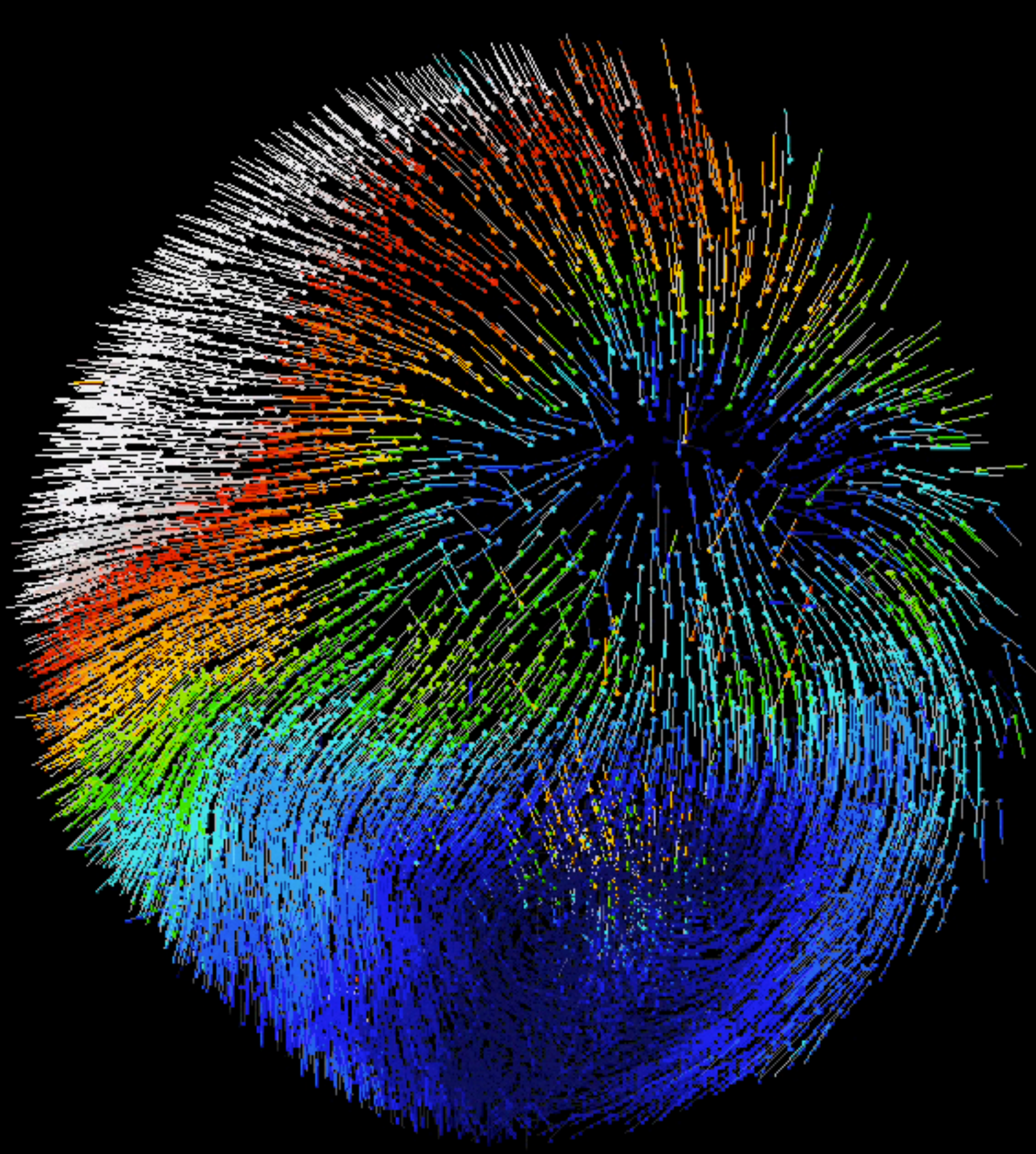
Hypothèse : invariance de l'intensité de chaque voxel entre 2 images



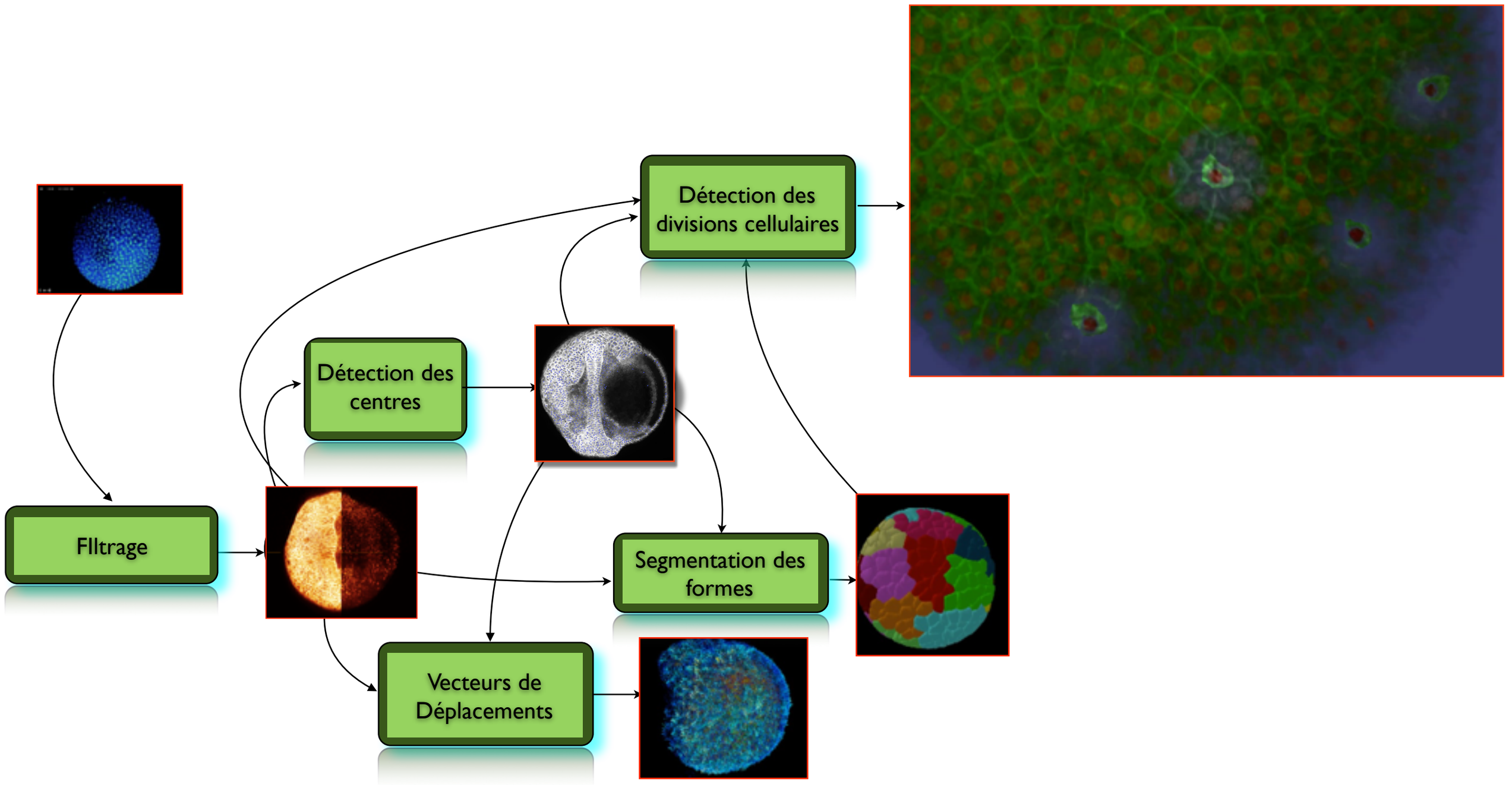
Approche multi-résolution

Moyenne spatial et temporel

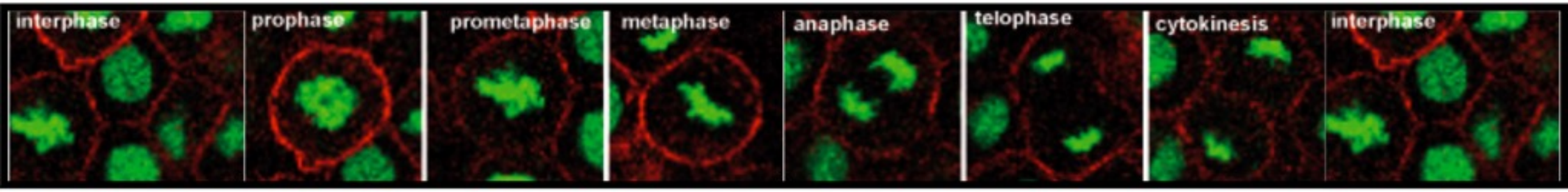


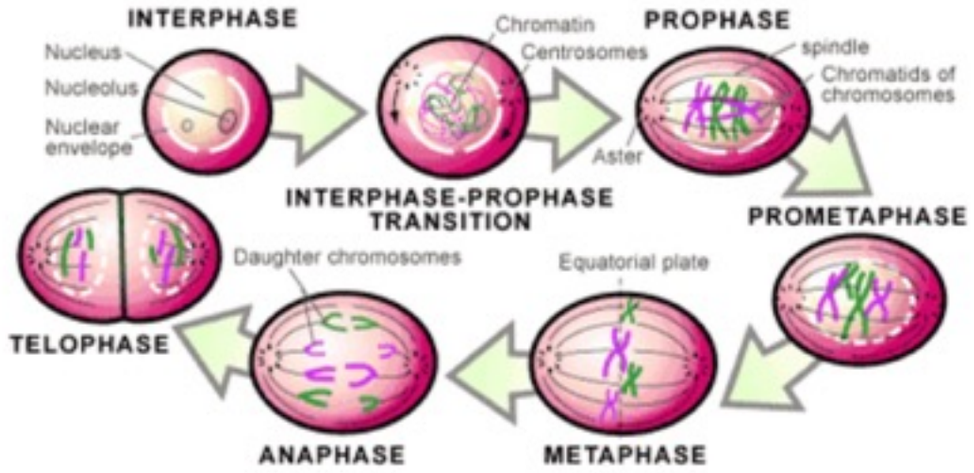


Reconstruction Image 4D



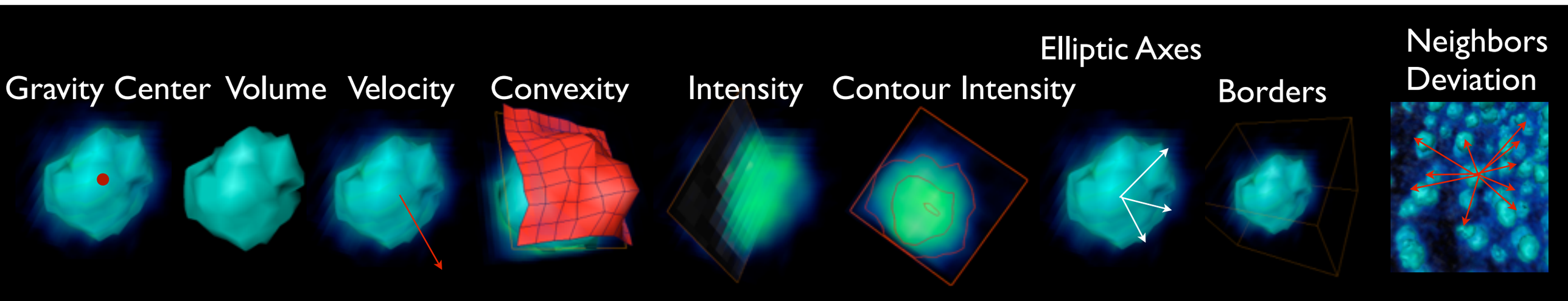
Détection des divisions cellulaires



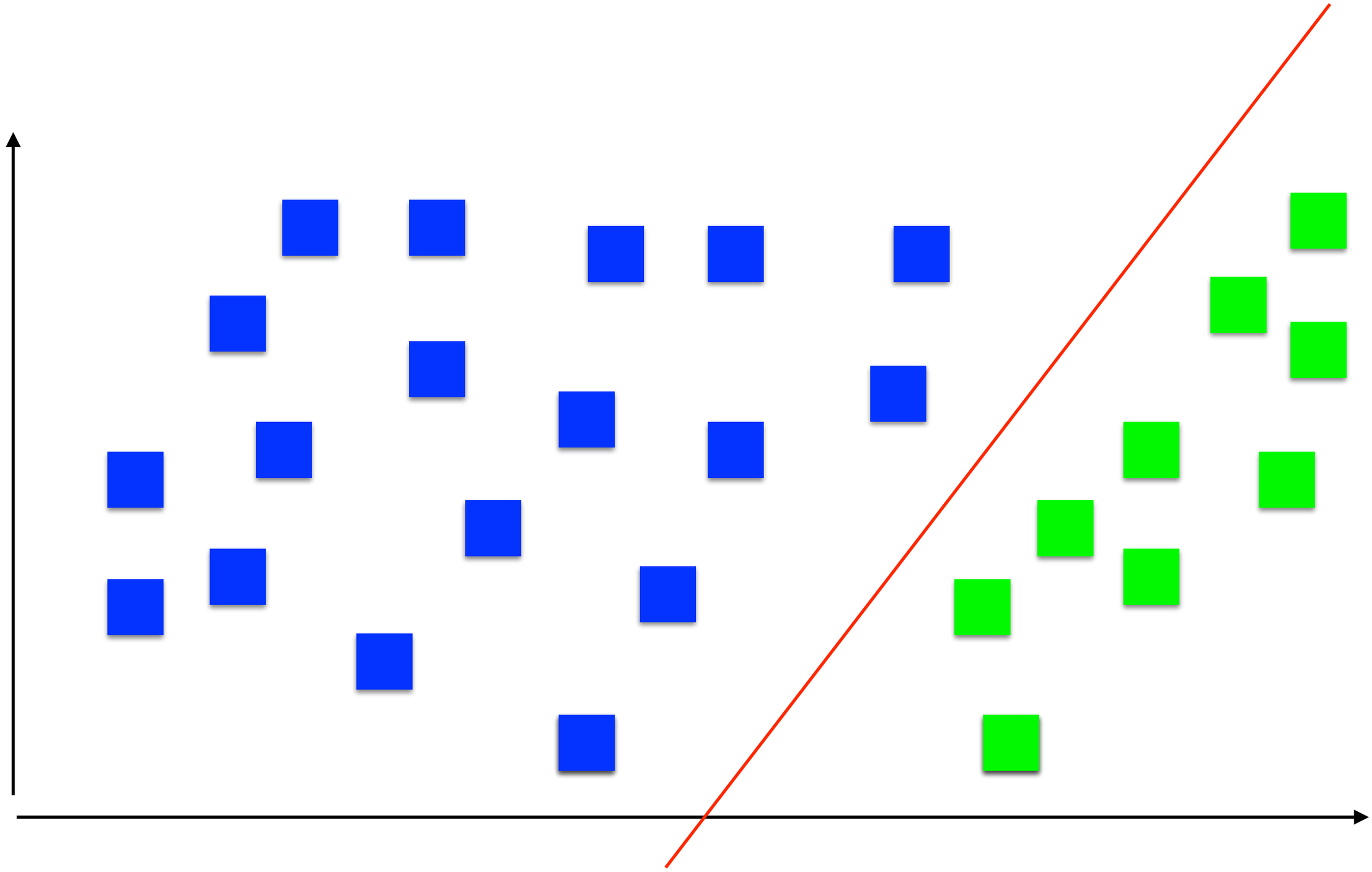


– **Prophase** : Membrane : shape + convex
– **Prometaphase** : Nuleus : volume down
– **Metaphase :** Nucleus : higher intensity
– **Anaphase** : 2 closest nuclei , 1 Membrane.
– **Telophase** : Membrane : shape change
– **Cytokinesis** : 2 Membranes

+ Interphase ⟶ 7 classes

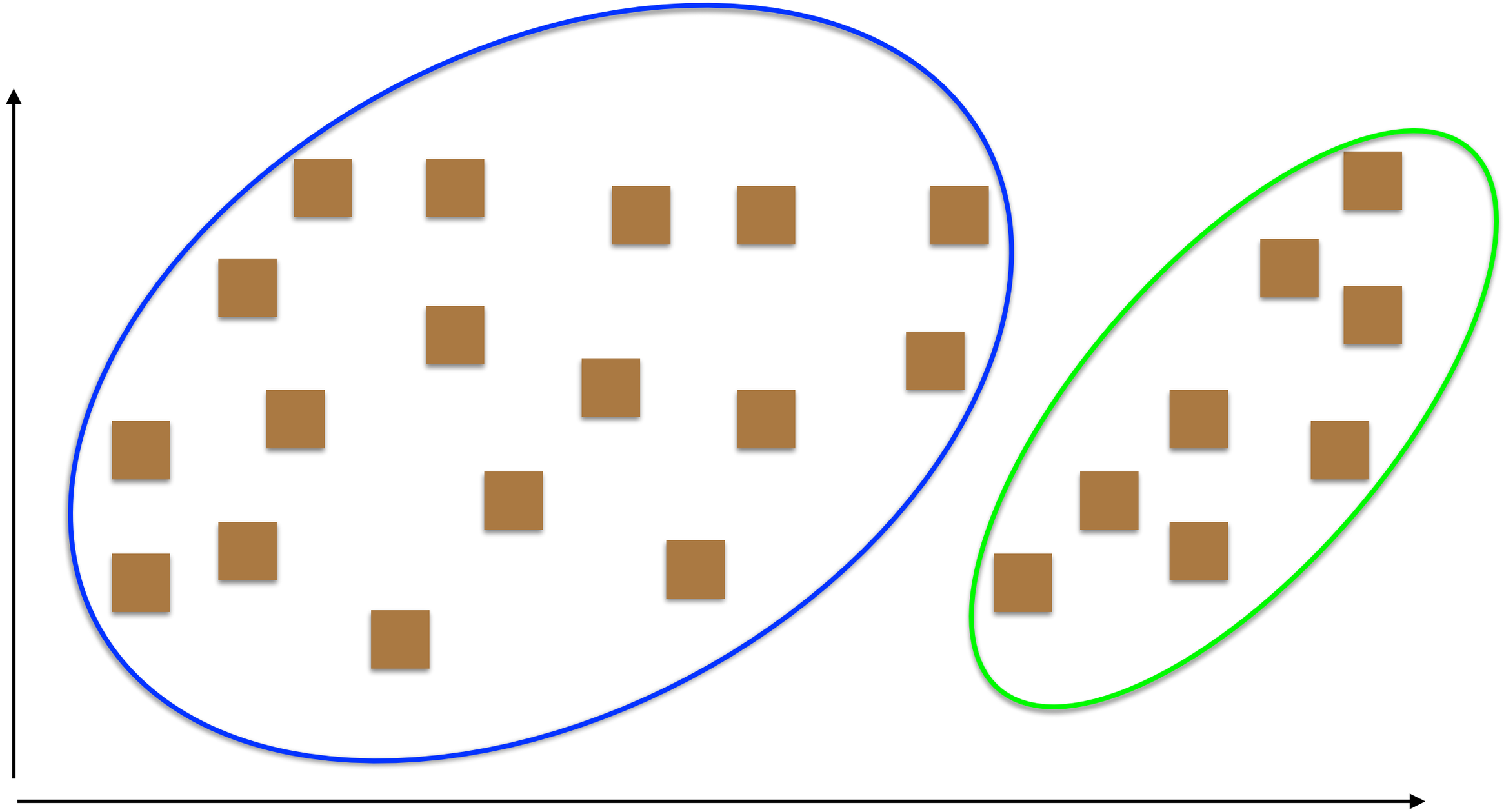


Très courte introduction à la classification



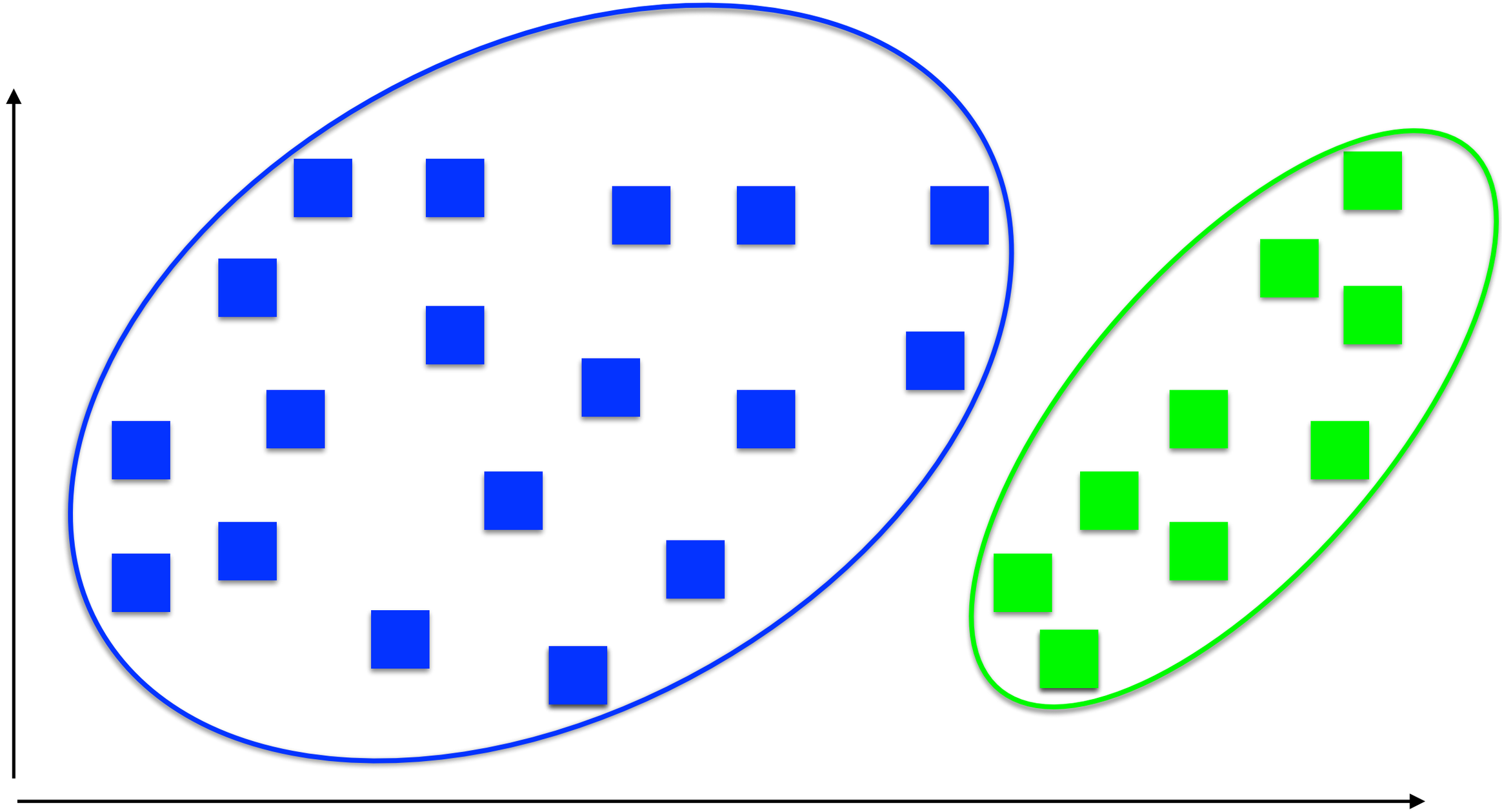
Classification supervisée

Très courte introduction à la classification



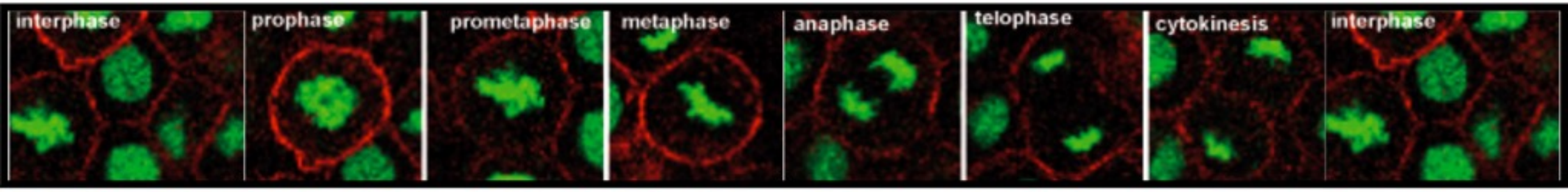
Classification non-supervisée

Très courte introduction à la classification



Classification non-supervisée

Détection des divisions cellulaires

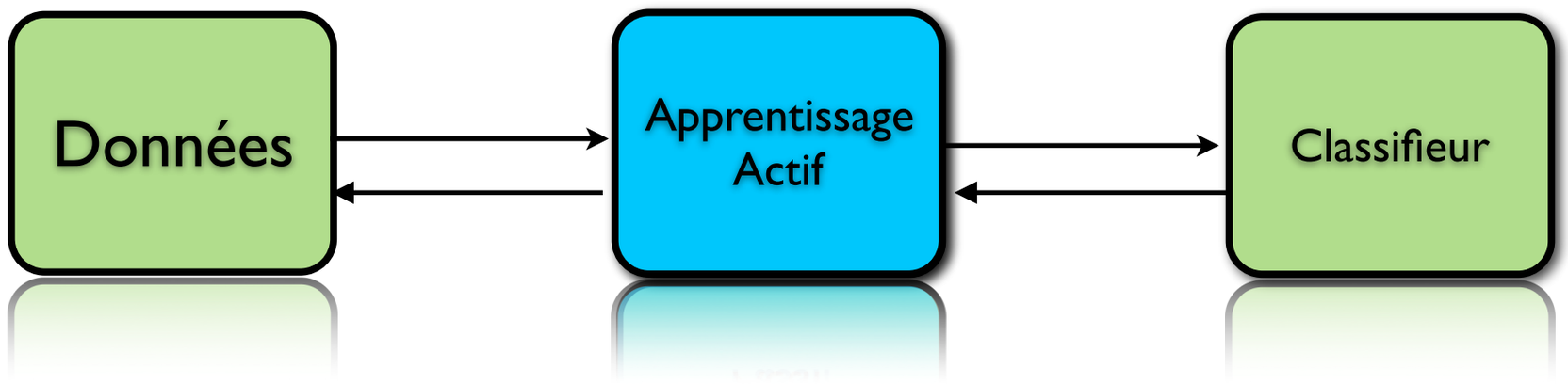


Apprentissage supervisé

annotation experte très longue

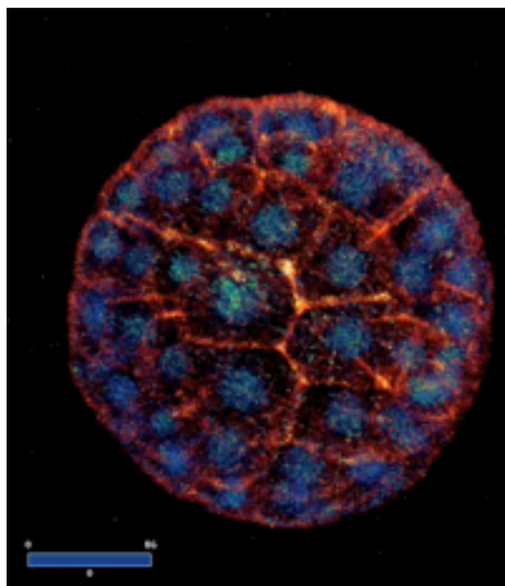
Apprentissage non supervisé

~0.5% cellules en division

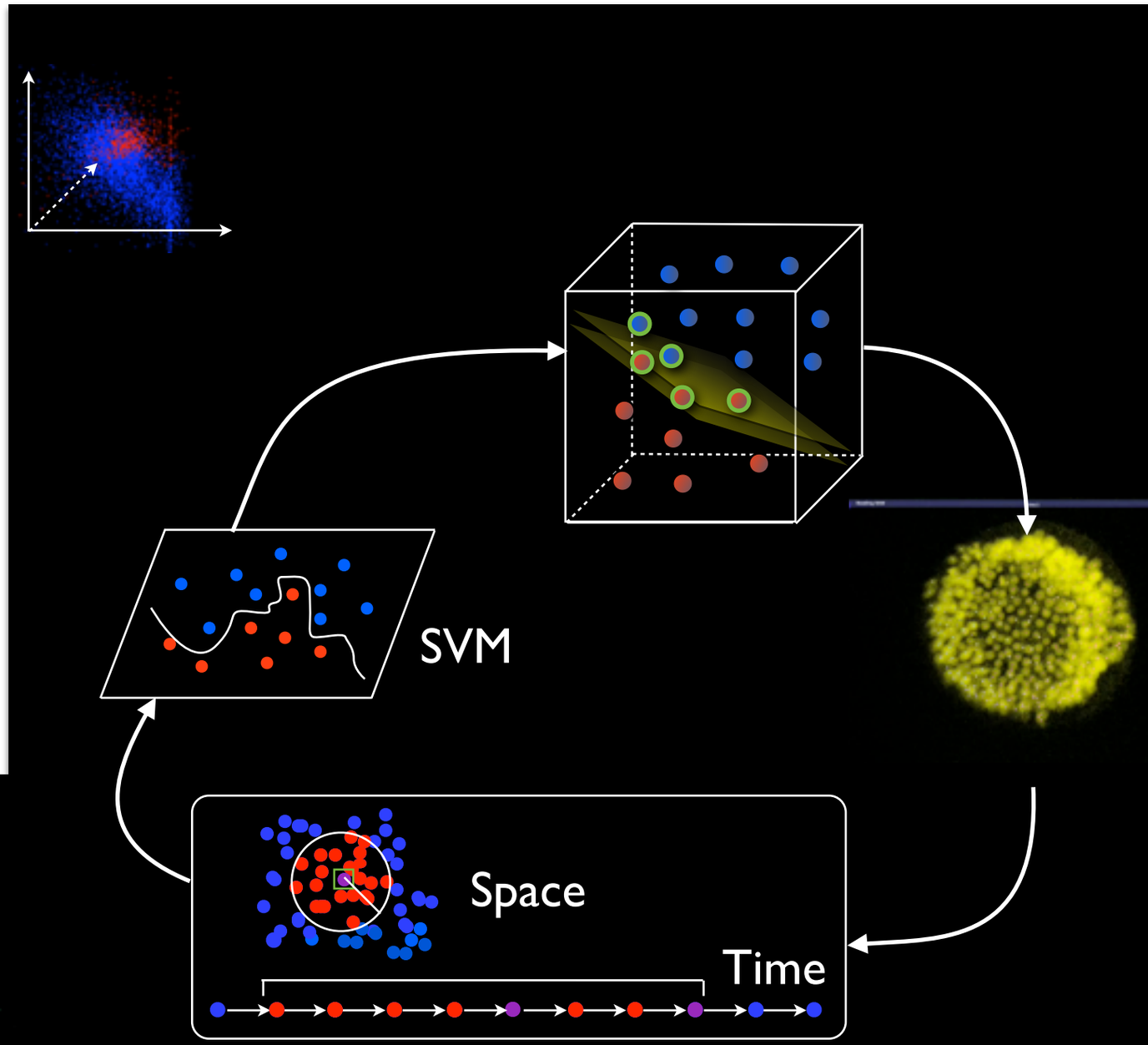


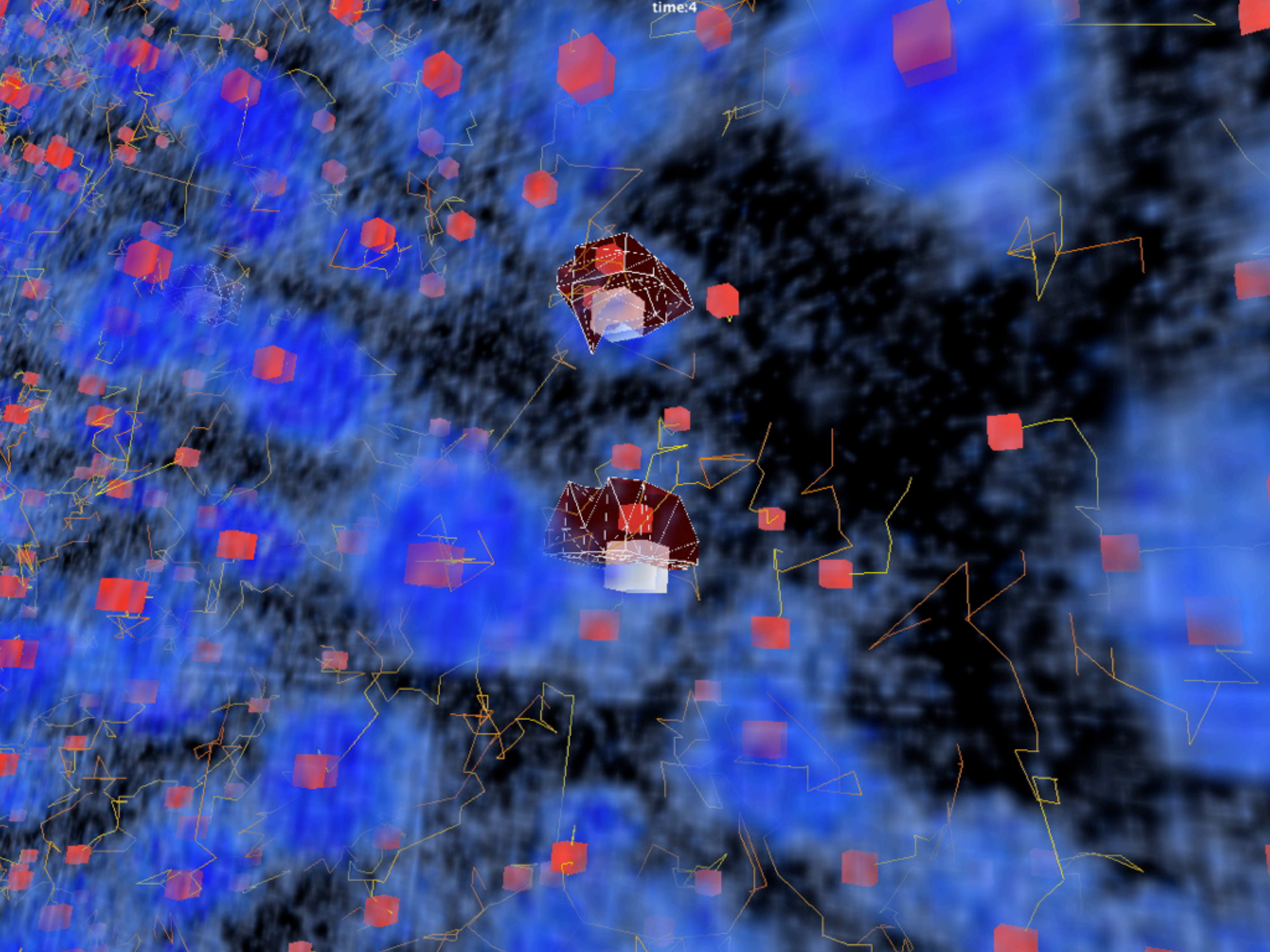
Détection des divisions cellulaires

- Pré - classification définie par la connaissance
- Estimation de densité
- 1er embryon
  - a) Annotation manuelle à la frontière des clusters
  - b) Extension des labels dans l'espace et le temps
  - c) Redéfinition des frontières
- 2ème Embryon

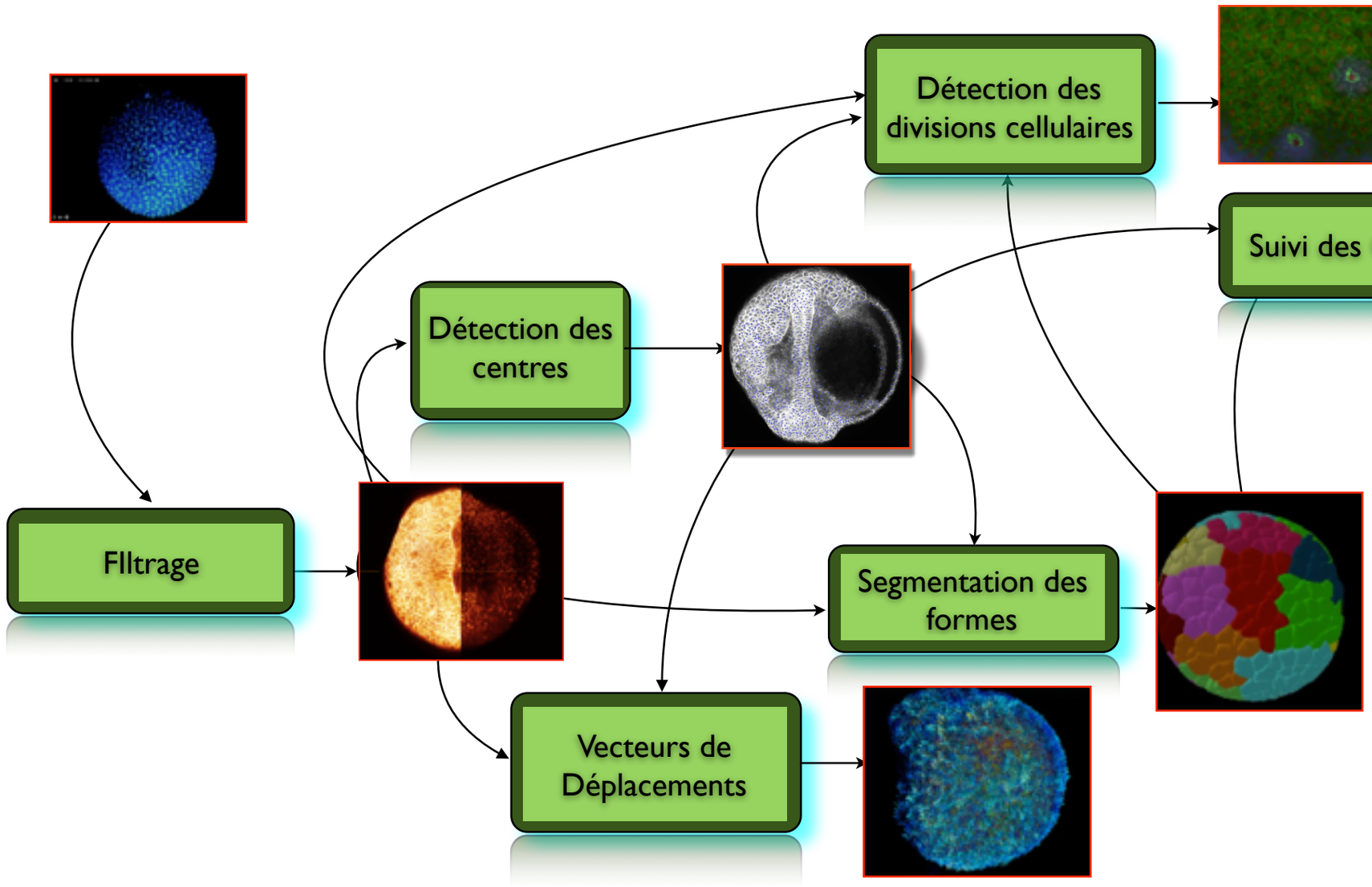


Apprentissage actif

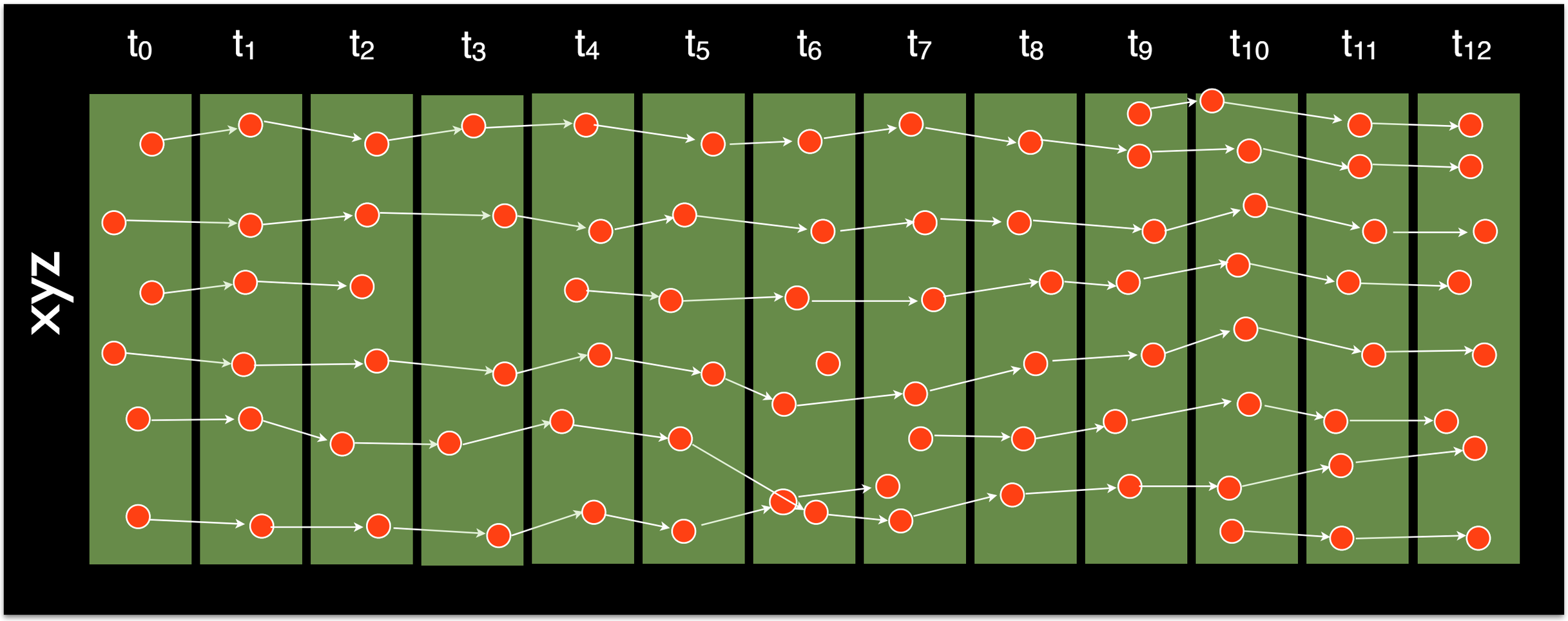



time:4

Reconstruction Image 4D

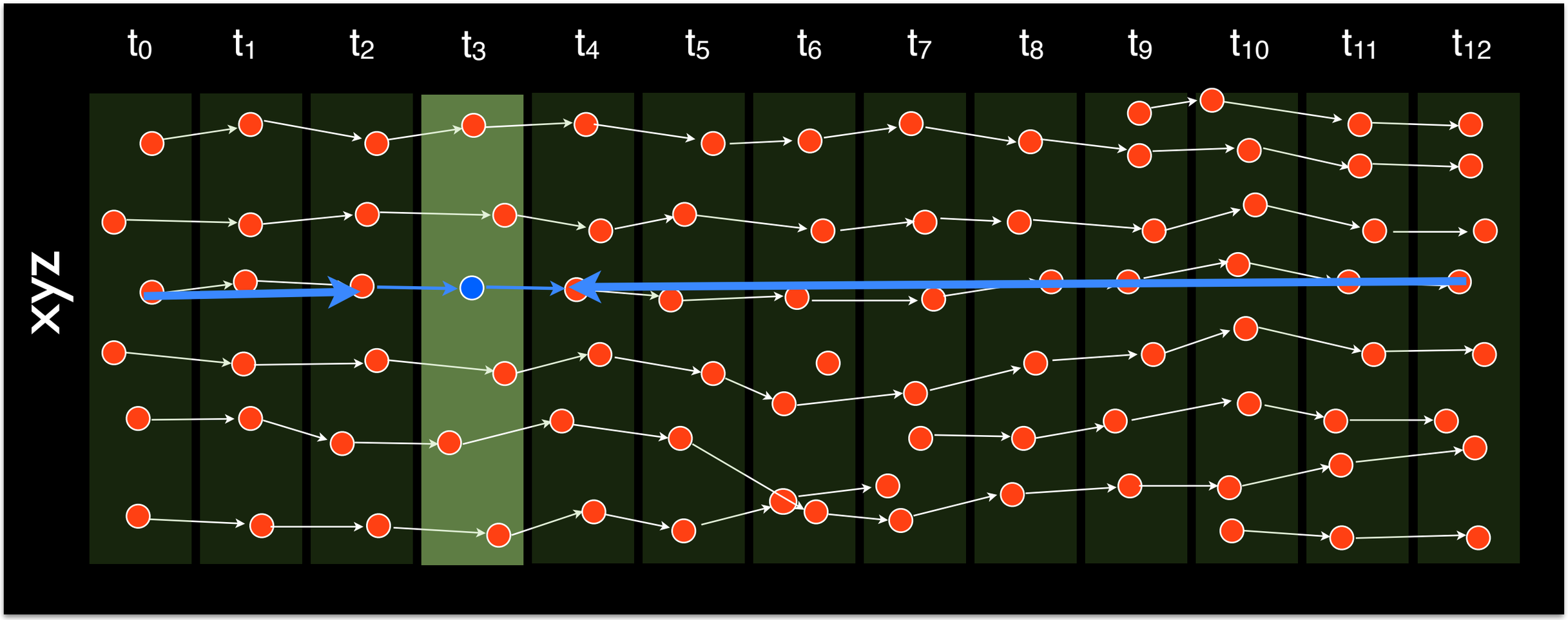


Suivi cellulaire par plus proche voisins



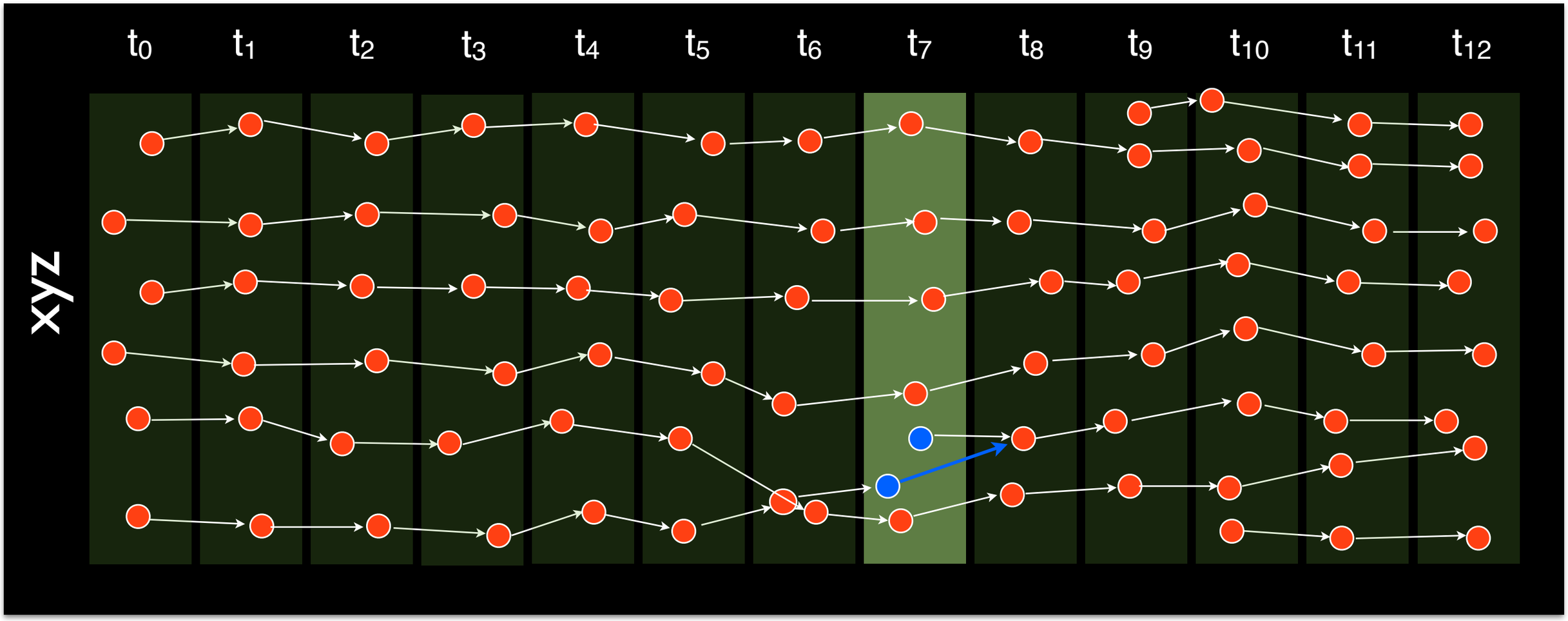
~ 90% de bons liens

Erreurs ….



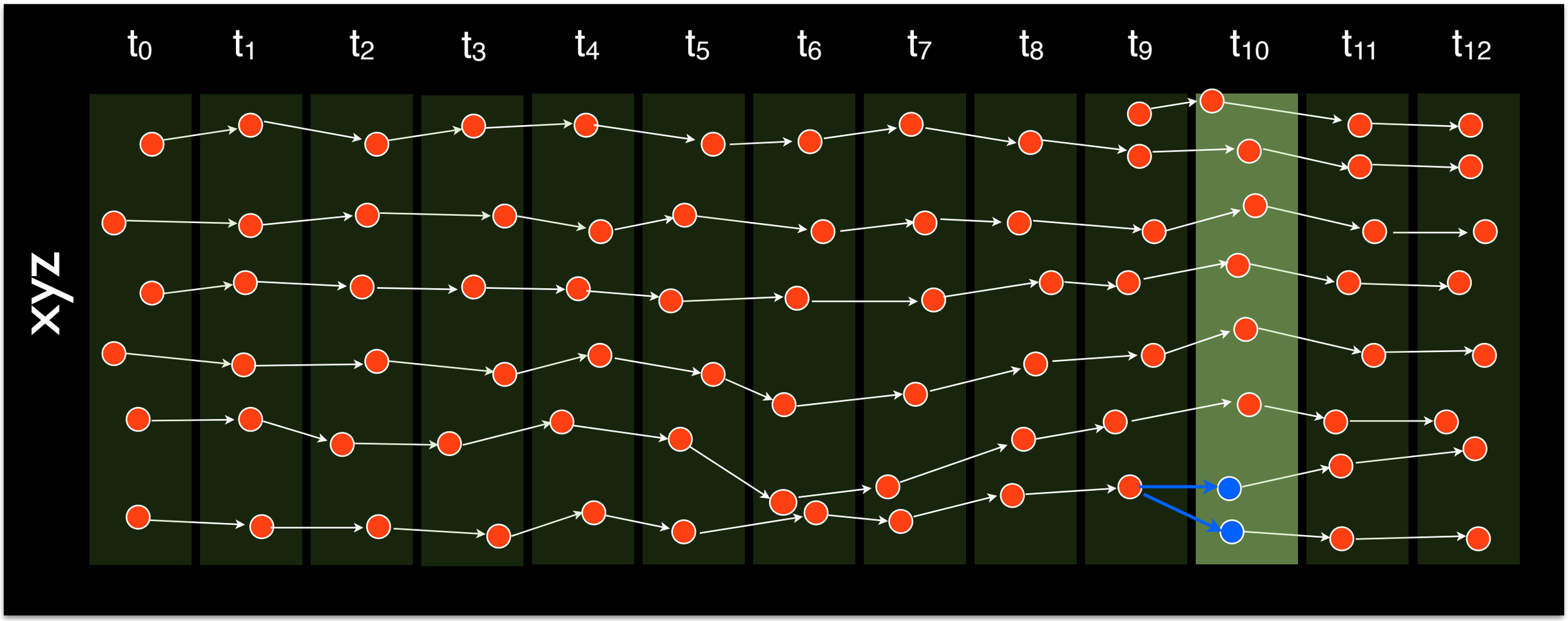
Faux Négatif

Erreurs ….



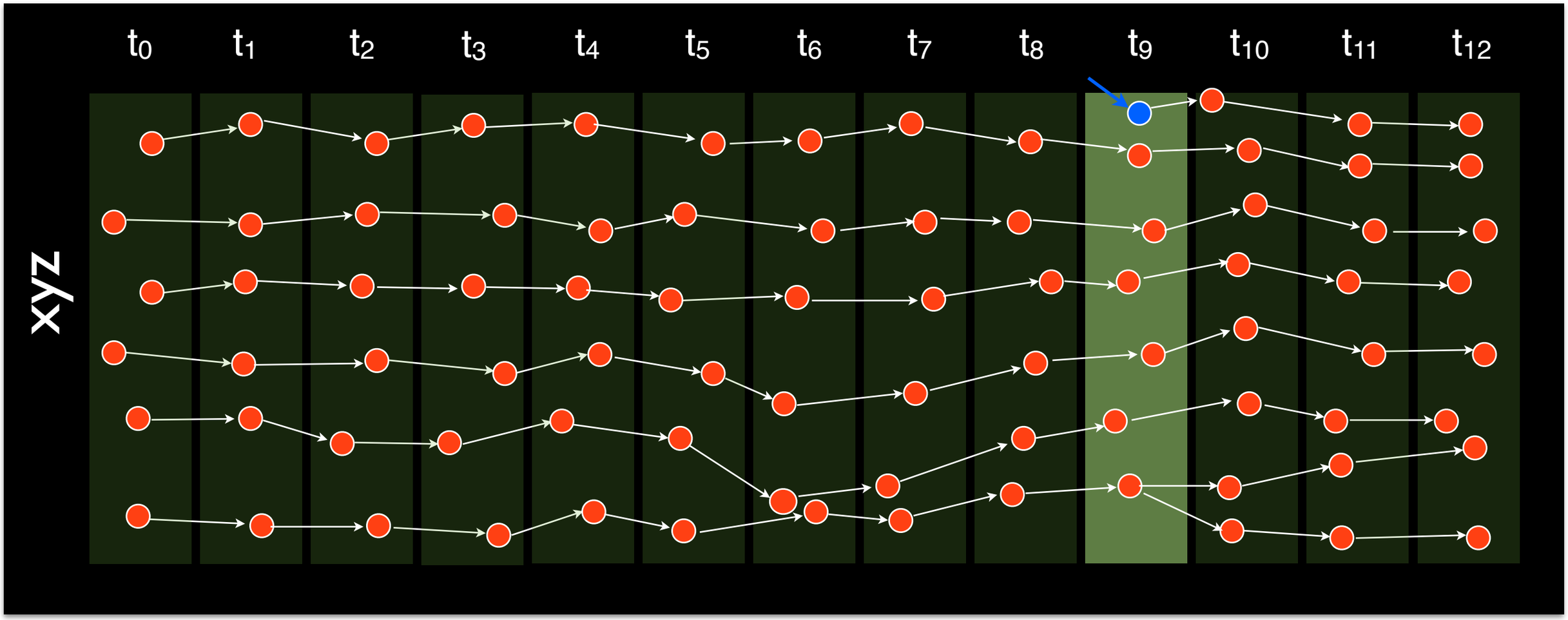
Faux positifs

Erreurs ….



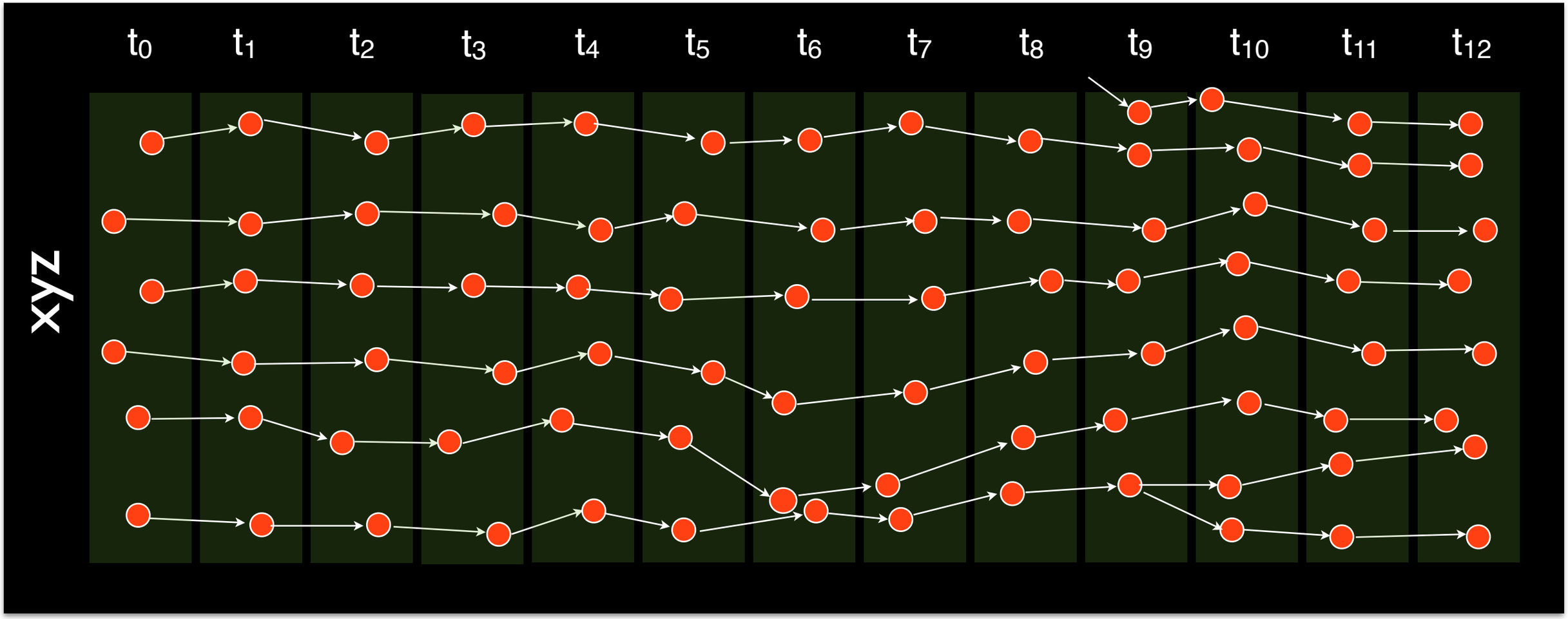
Cellule en division

Erreurs ….



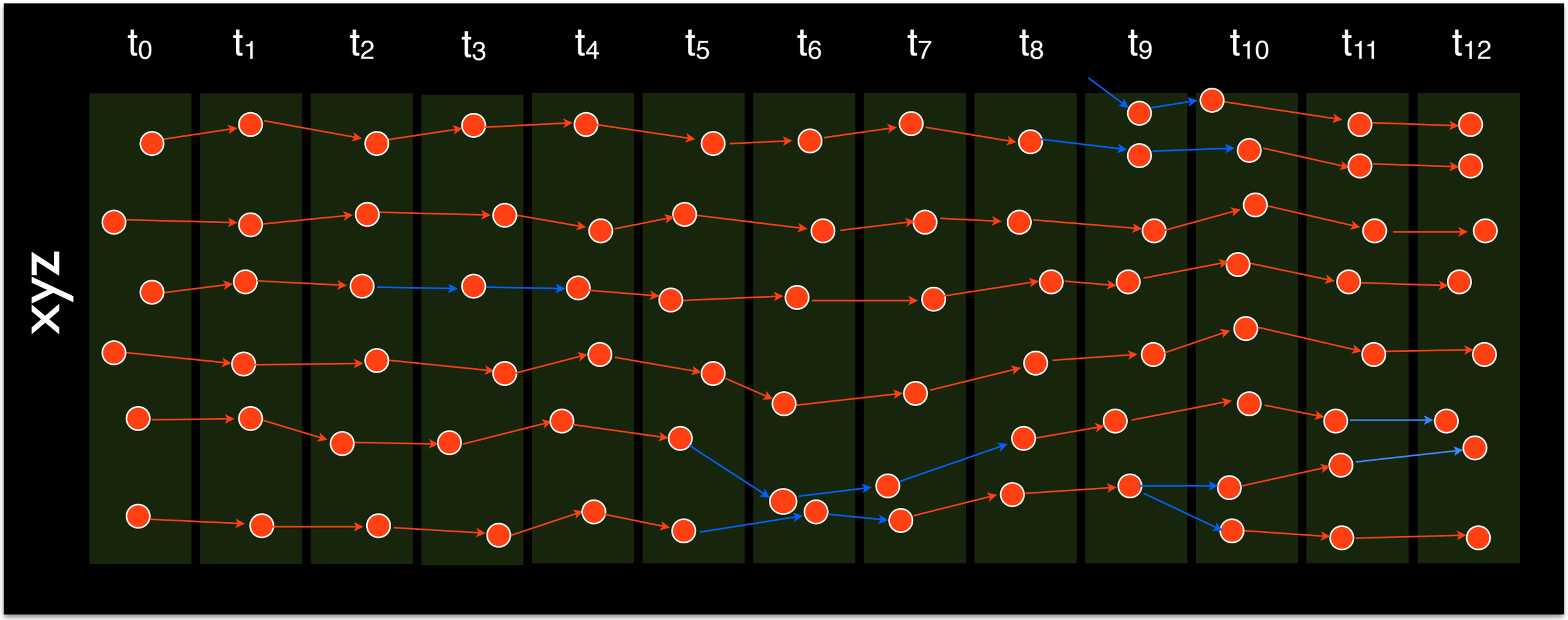
Limite de l’image

Erreurs ….



100% bon liens ?

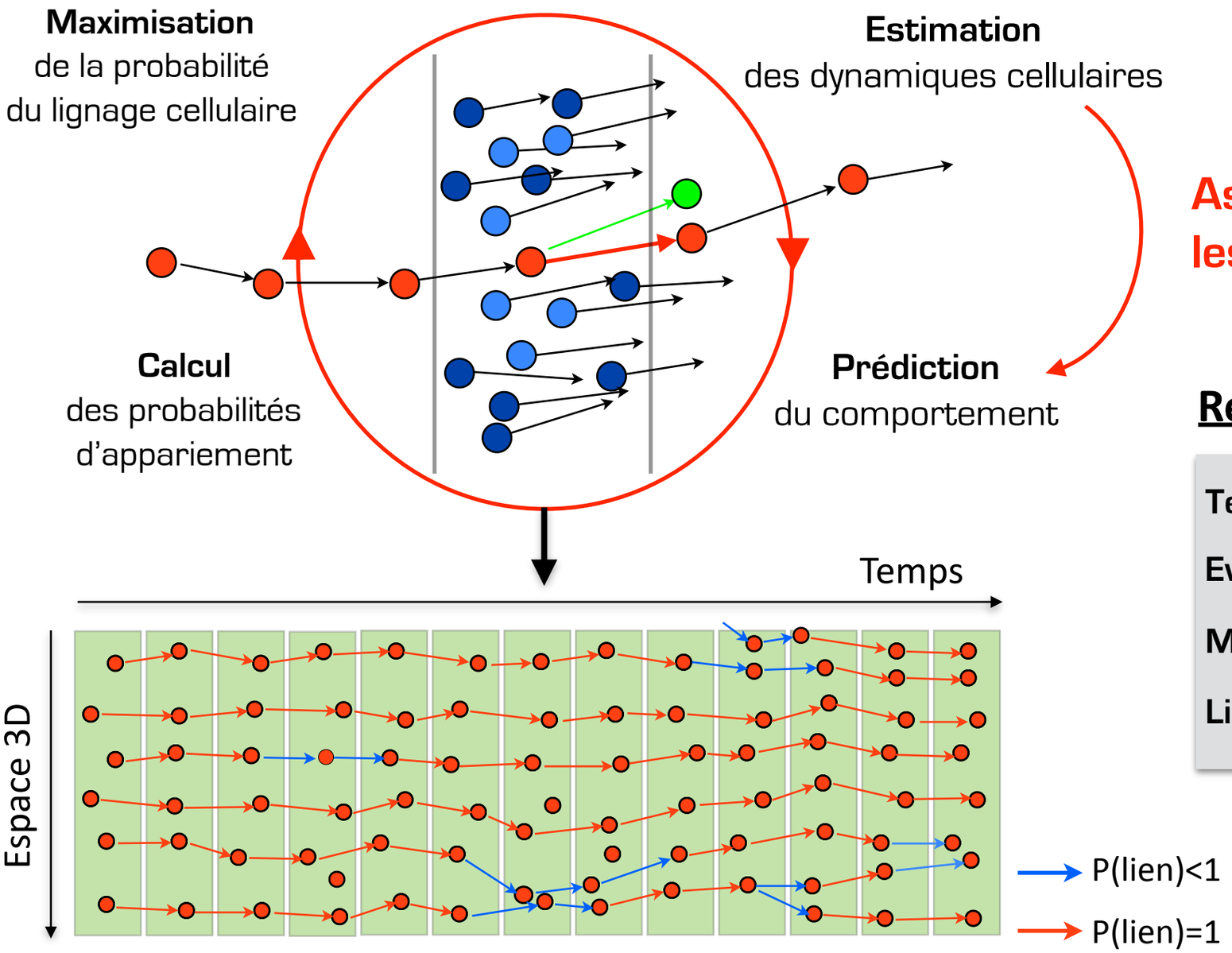
Reconstruction probabiliste



P(link)=1 P(link)<1

Suivi cellulaire probabiliste par maximisation de la vraisemblance

Propagation des certitudes par itération (Algorithme EM)



Assimilation par les données

Résultats :

Test : plusieurs organismes

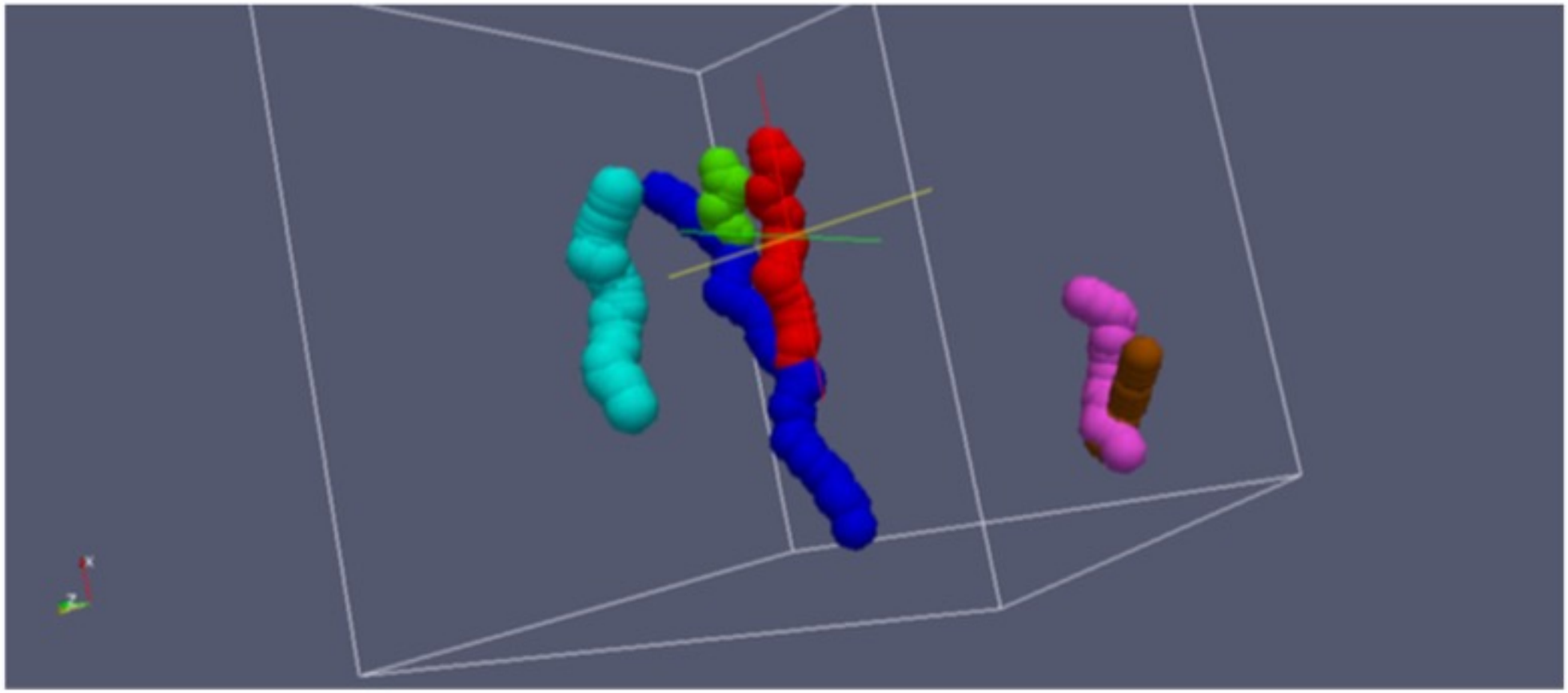
Evaluation : % d'erreurs ≦ autres méthodes

Méthode sans paramètres

Lignage cellulaire facilement corrigible

Embryon numérique avec une probabilité associée à chaque lien

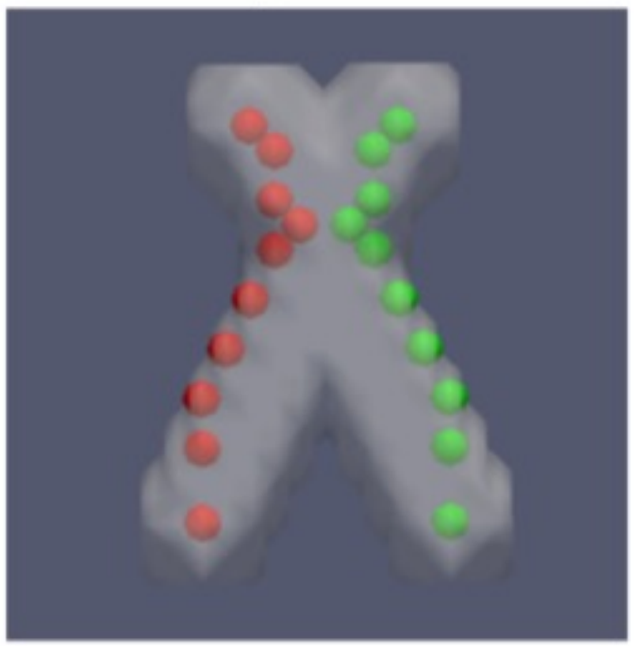
Segmentation de trajectoires 4D : 3D projection

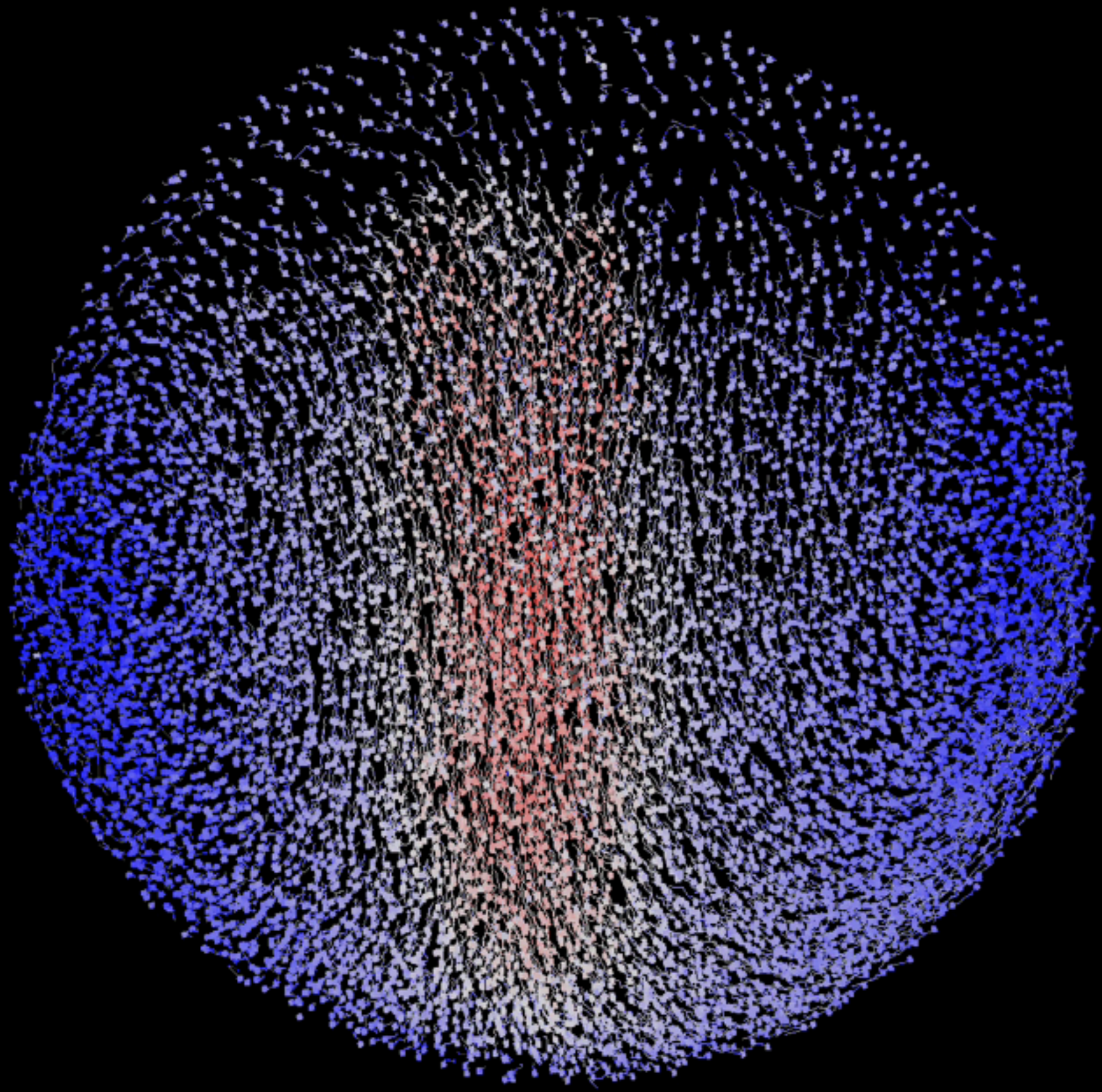


Mikula 2014

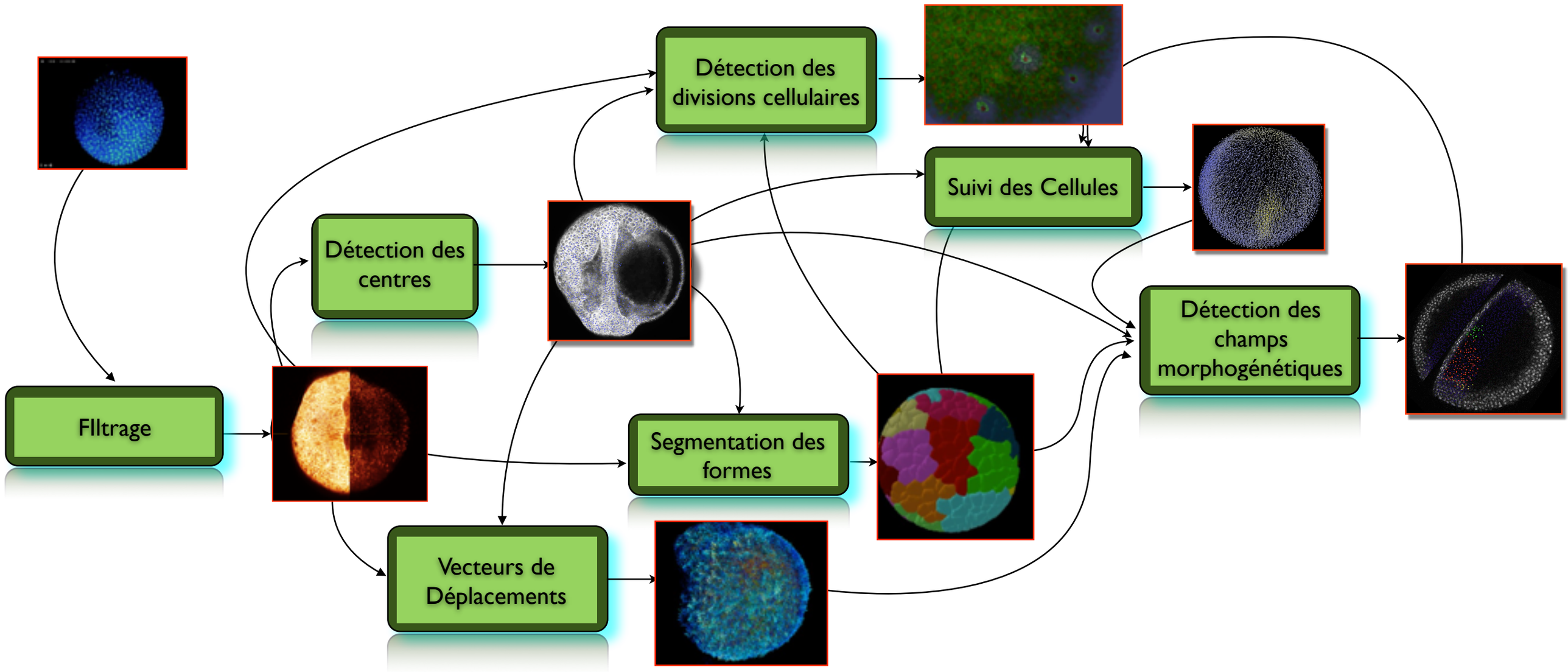
Divisions

2 trajectoires

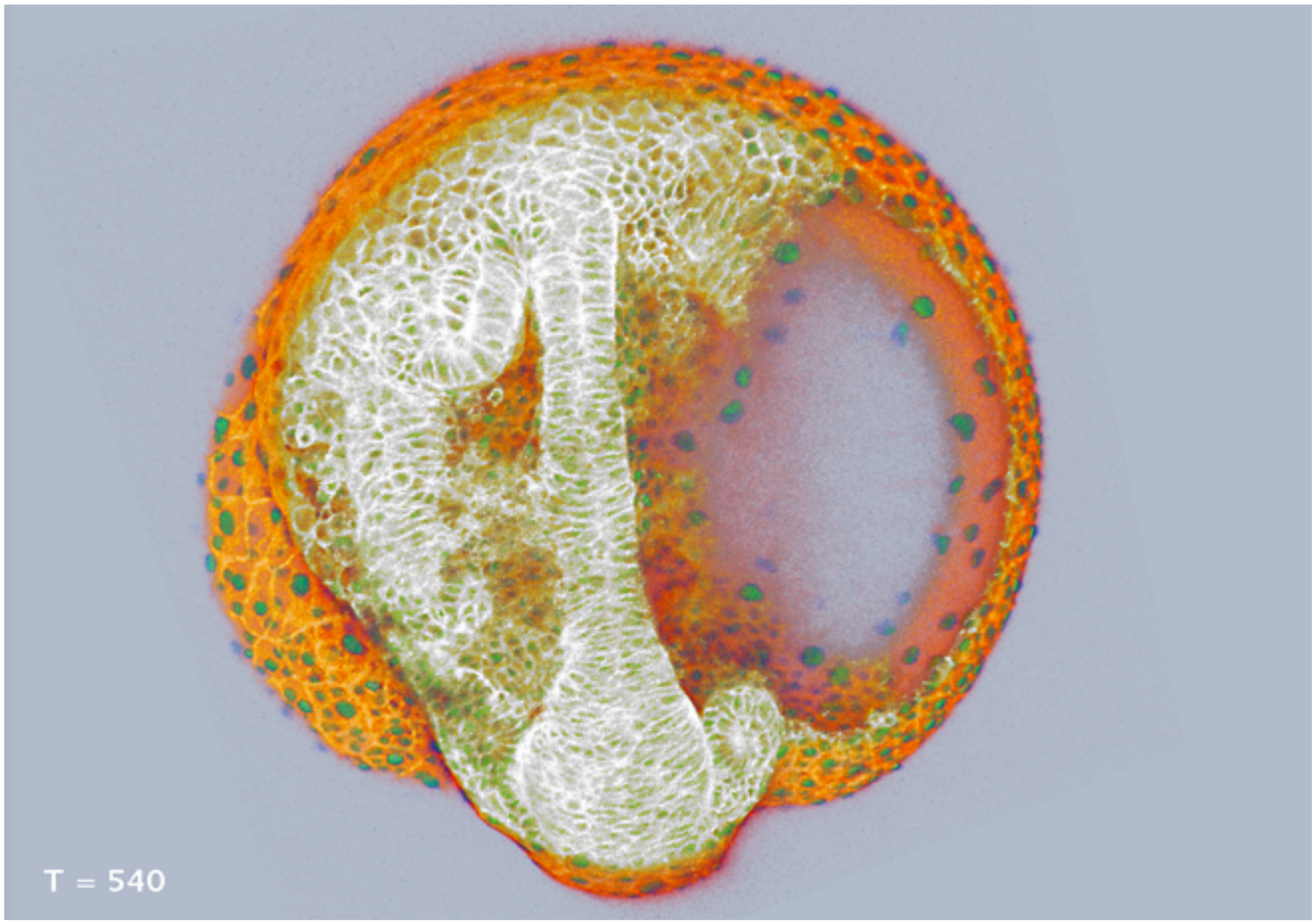




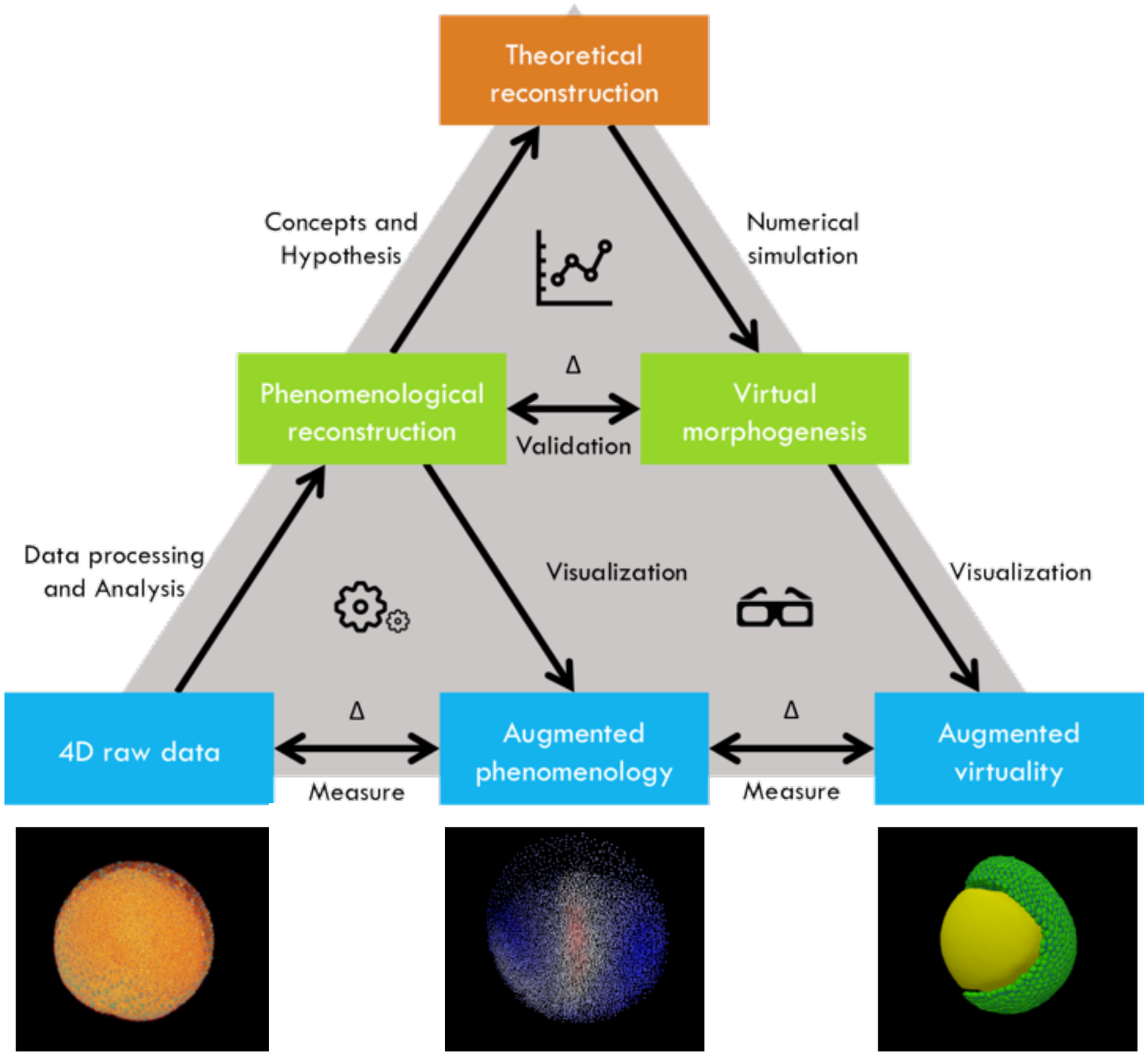
Reconstruction Image 4D



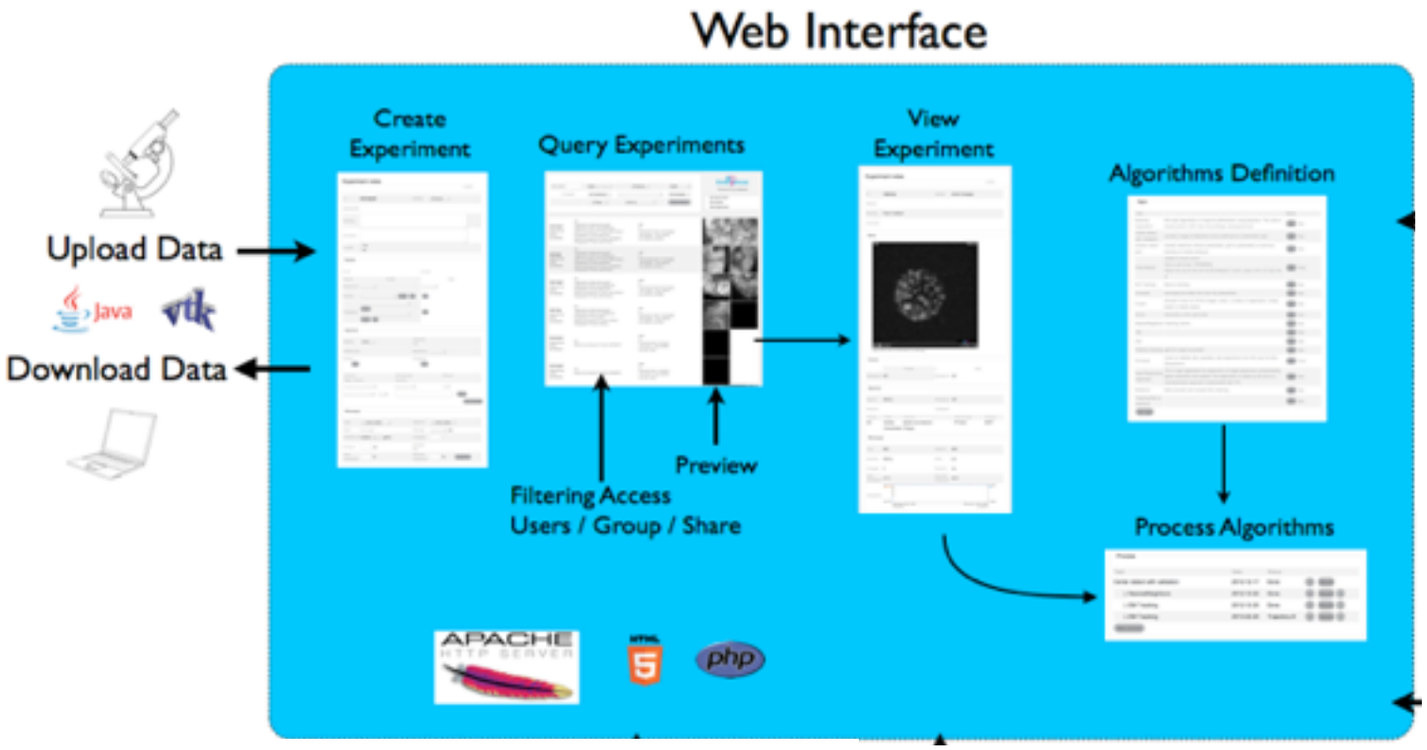
Champs morphogénetiques



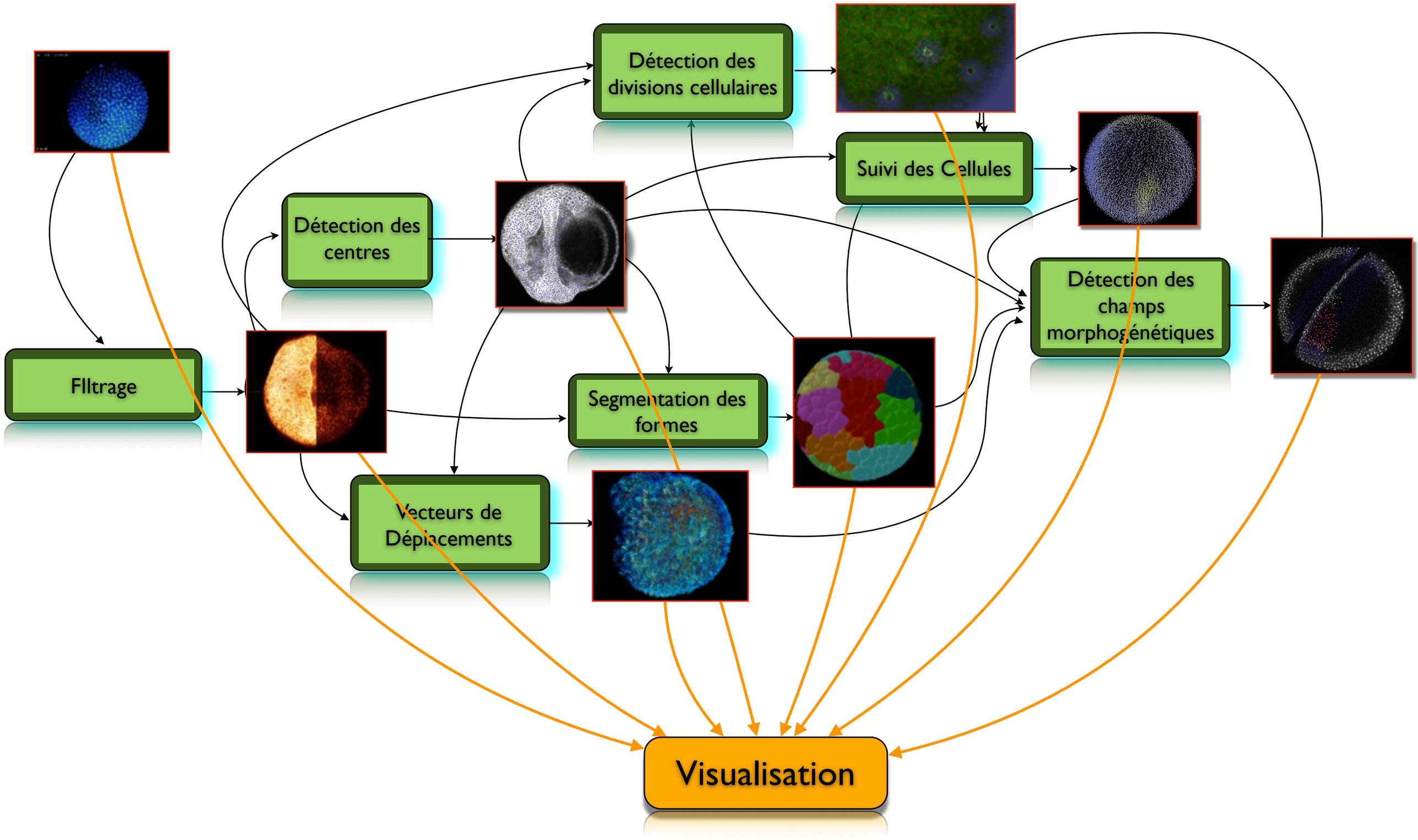
Approche épistémologique



Traitement des données 4D



Visualisation d'images 4D

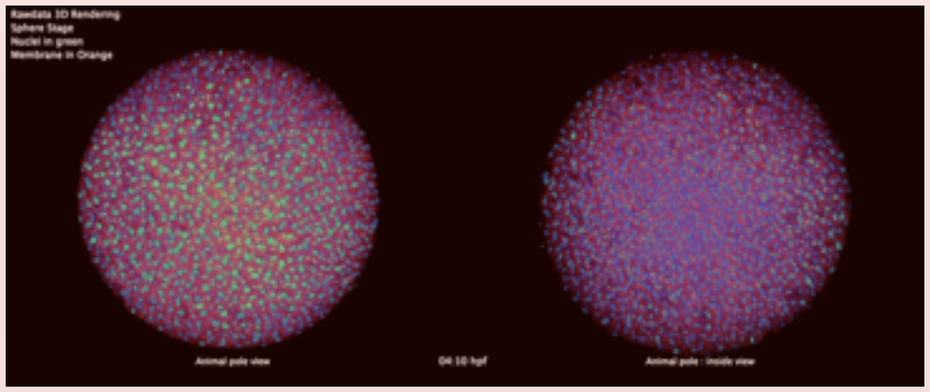


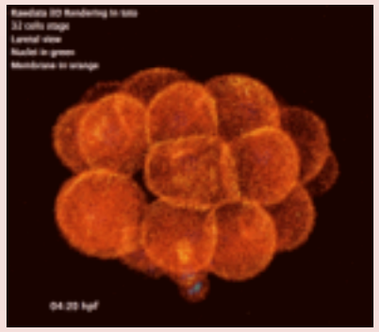
MovIT : Interface de Visualisation Interactive

[Faure & al Nature Communication 2016]

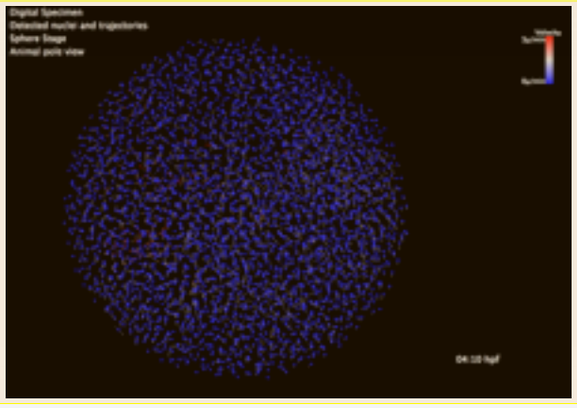
Zebrafish

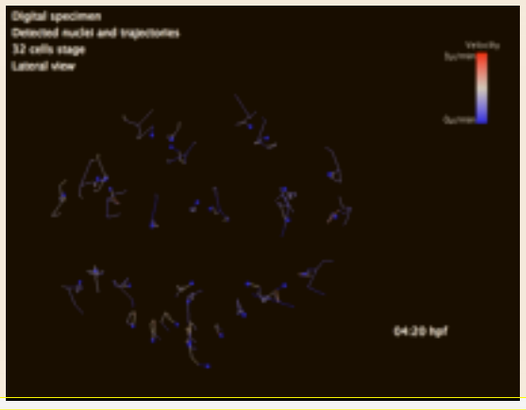
Sea Urchin



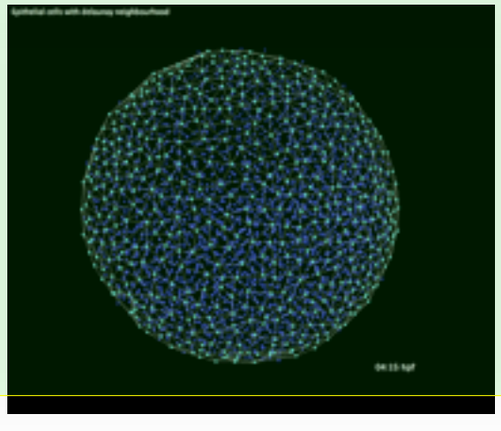


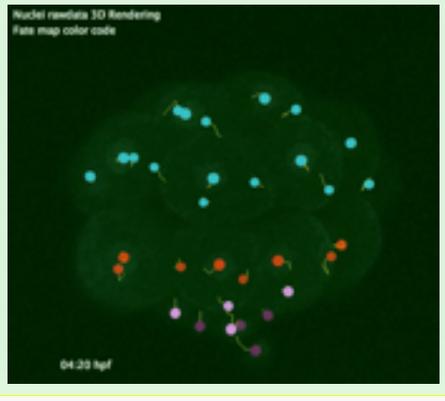
Observation



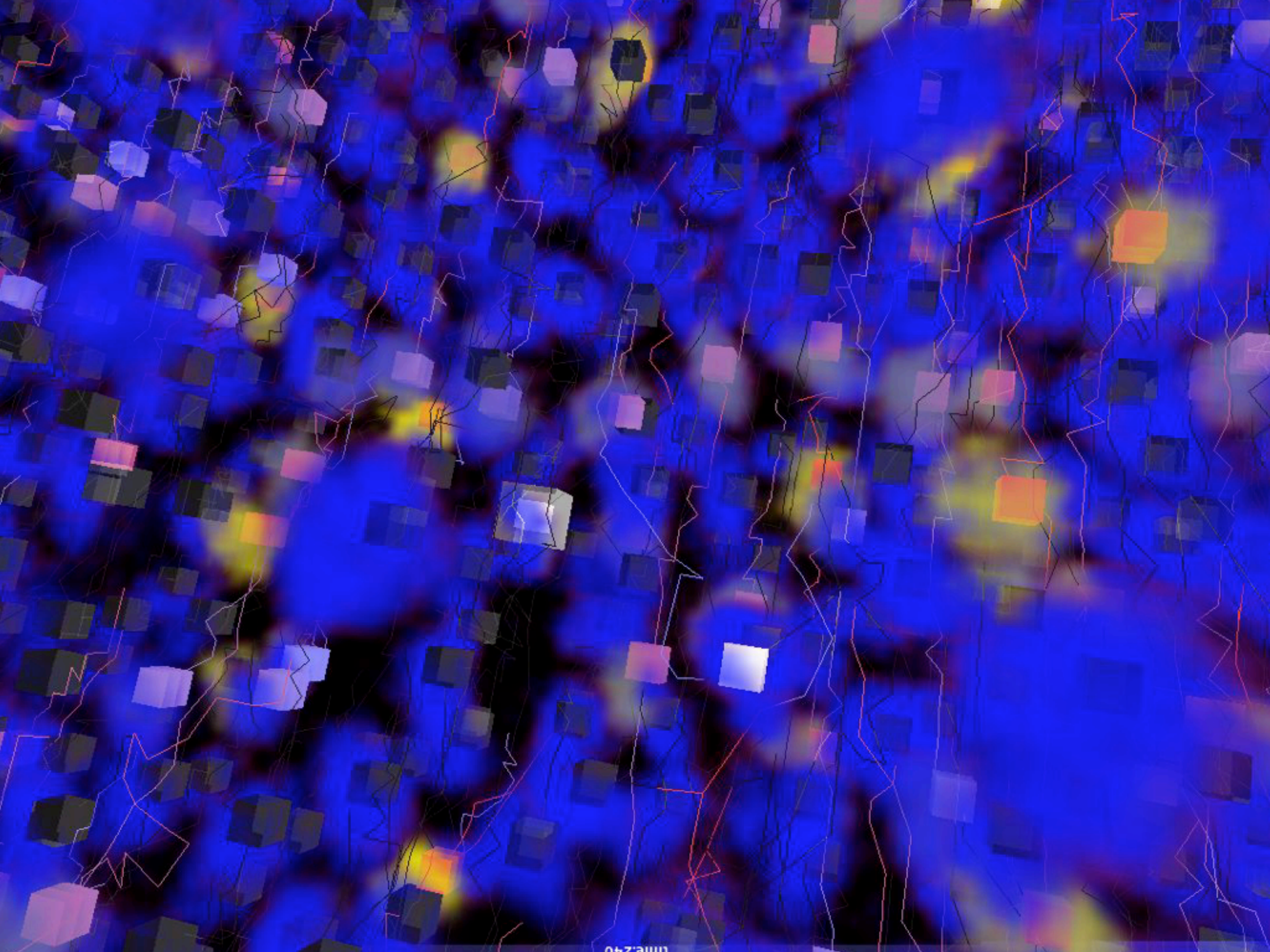


Digitizing





Abstraction



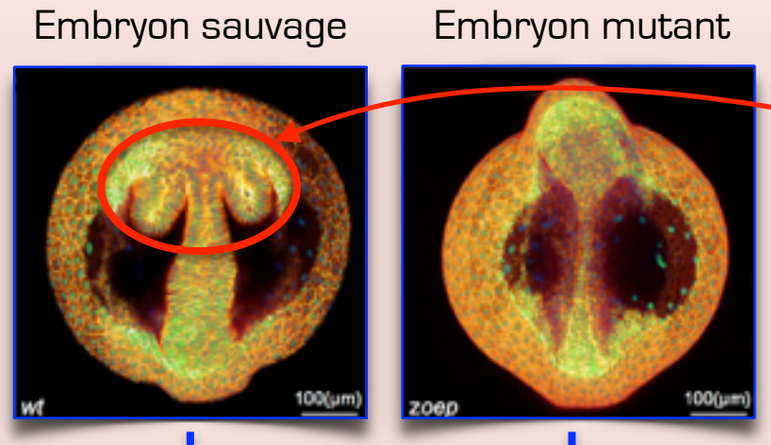
Reconstruction des dynamiques multi-échelles de la morphogenèse animale

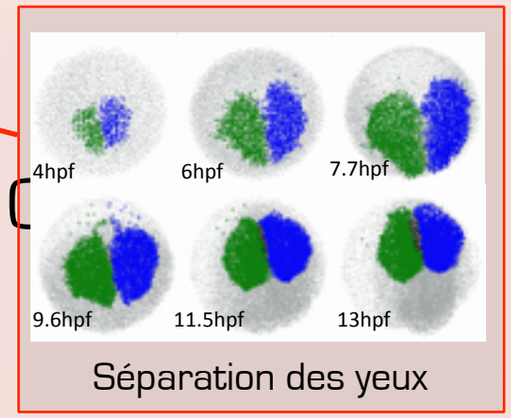
[Faure & al Nature Communication 2016]

BioEmergences

Résultats :

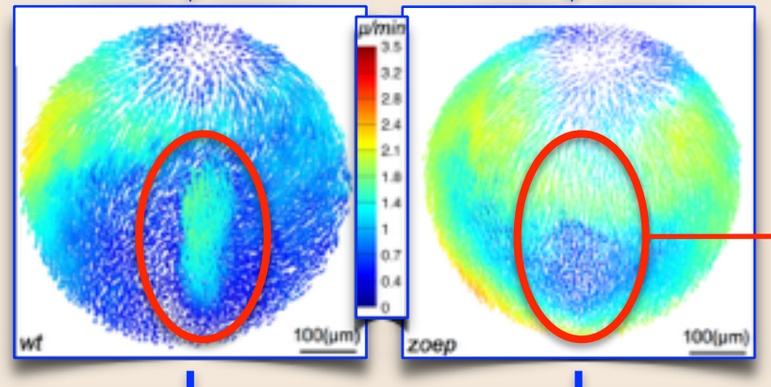
Enjeu : Compréhension des dynamiques cellulaires





Objectif : Comparaison d'embryons numériques

Méthode : Reconstruction automatisée par un web-service



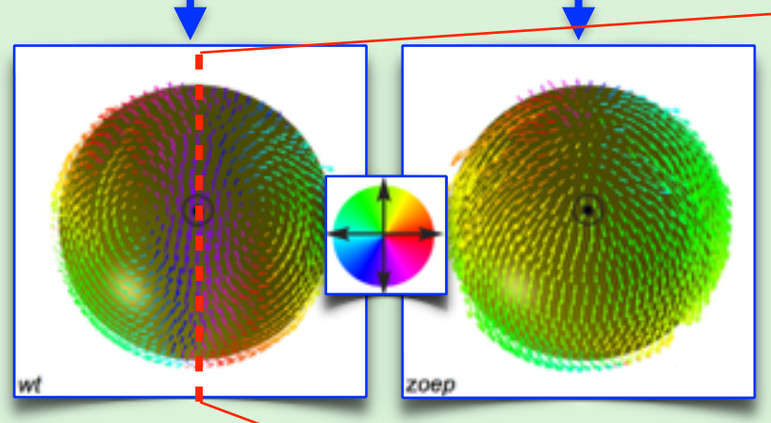
Digitalisation

zoep dépourvu d'une partie de l'hypoblaste

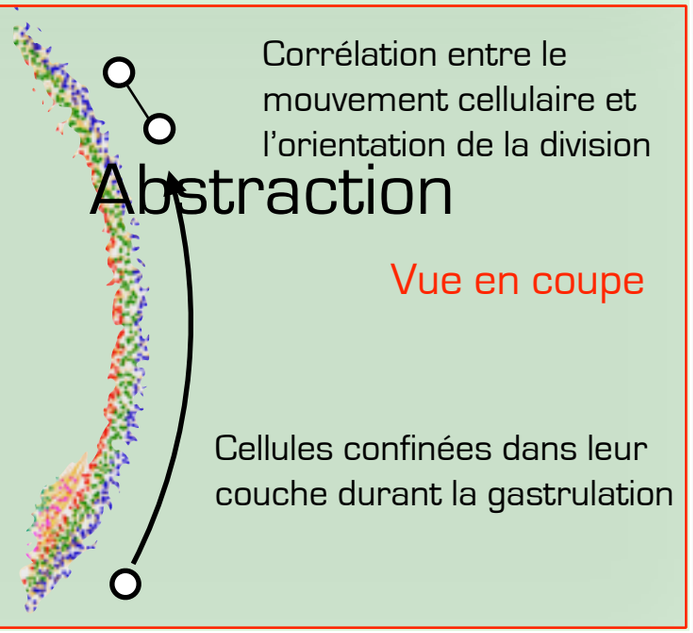
Objectif : Construction de prototypes (développement moyen)

Méthodes :

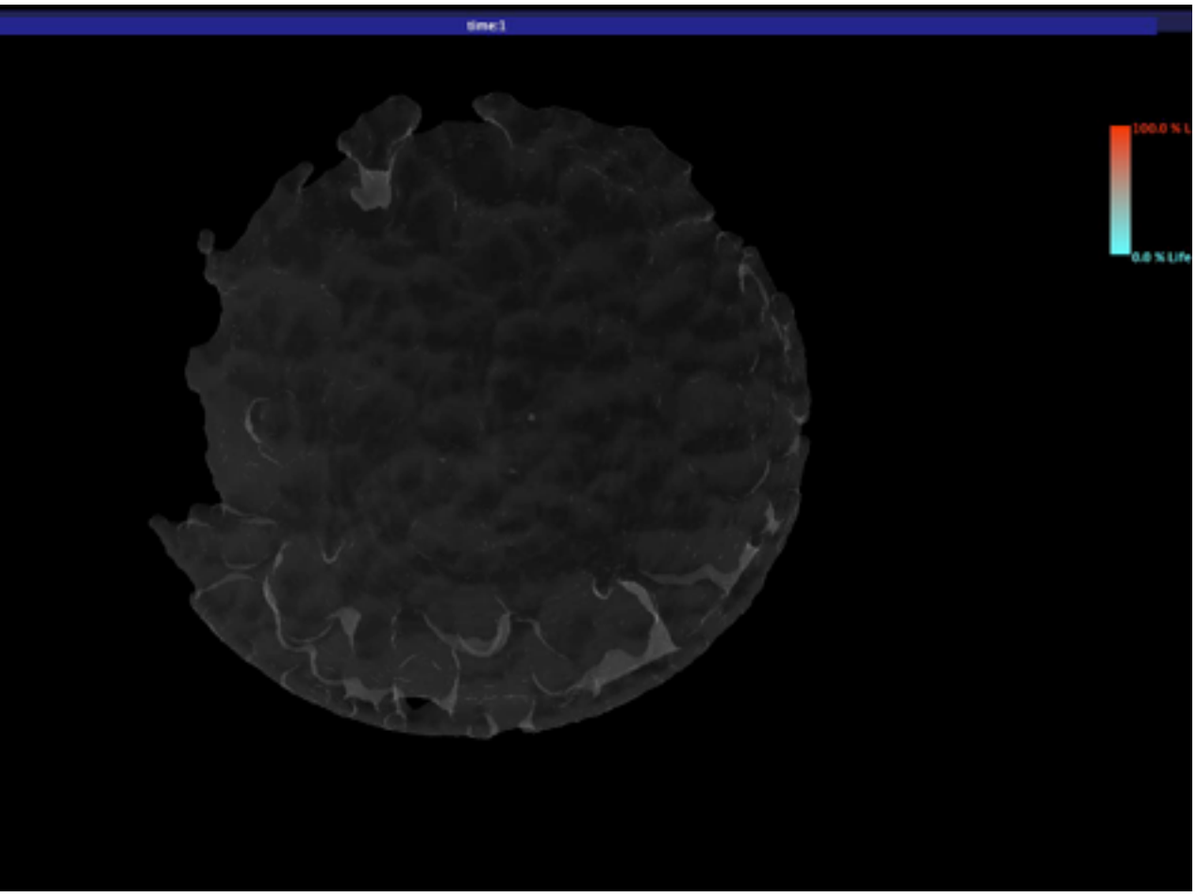
- Recalage 3D (Images et objets)
- Recalage temporel (Dynamiques)
- Moyennage local



Prototypes



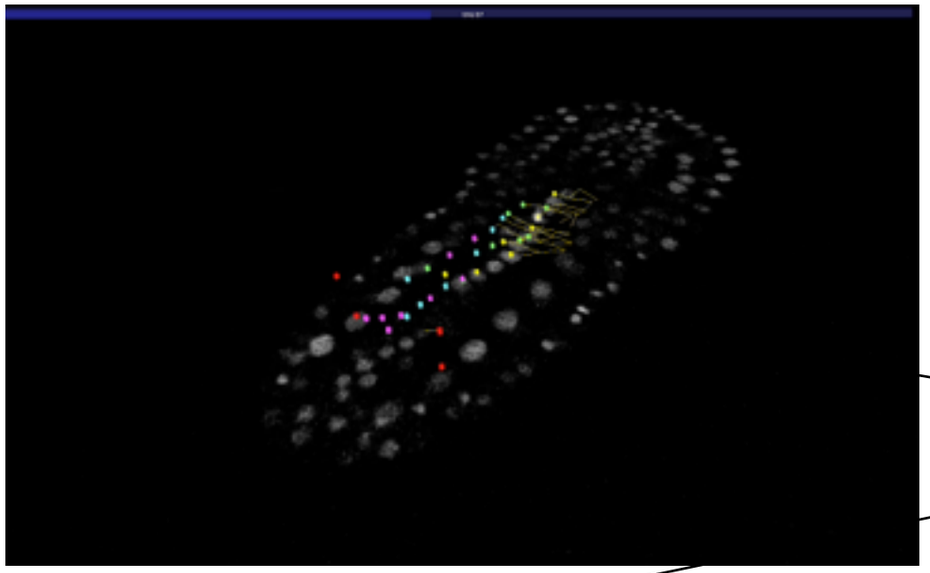
Division Prototype for zebrafish early embryo cell lineage

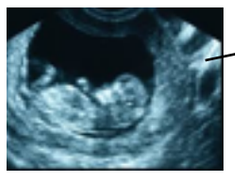


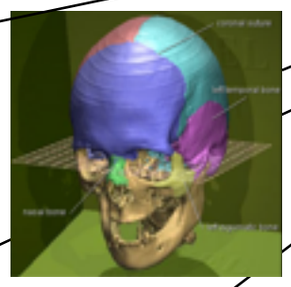
[Olivier & al Science 2010]

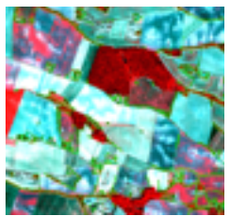
Ground truth

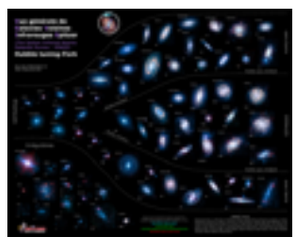
→ analyses des données
→ amélioration des algorithmes
→ évaluation des méthodes





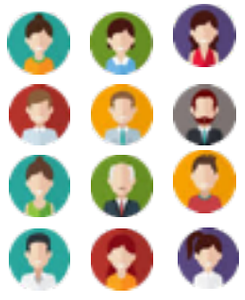






Plateforme de validation

- → détection
- → classification
- → segmentation
- → tracking



Jeux Sérieux : Validation d'images scientifiques

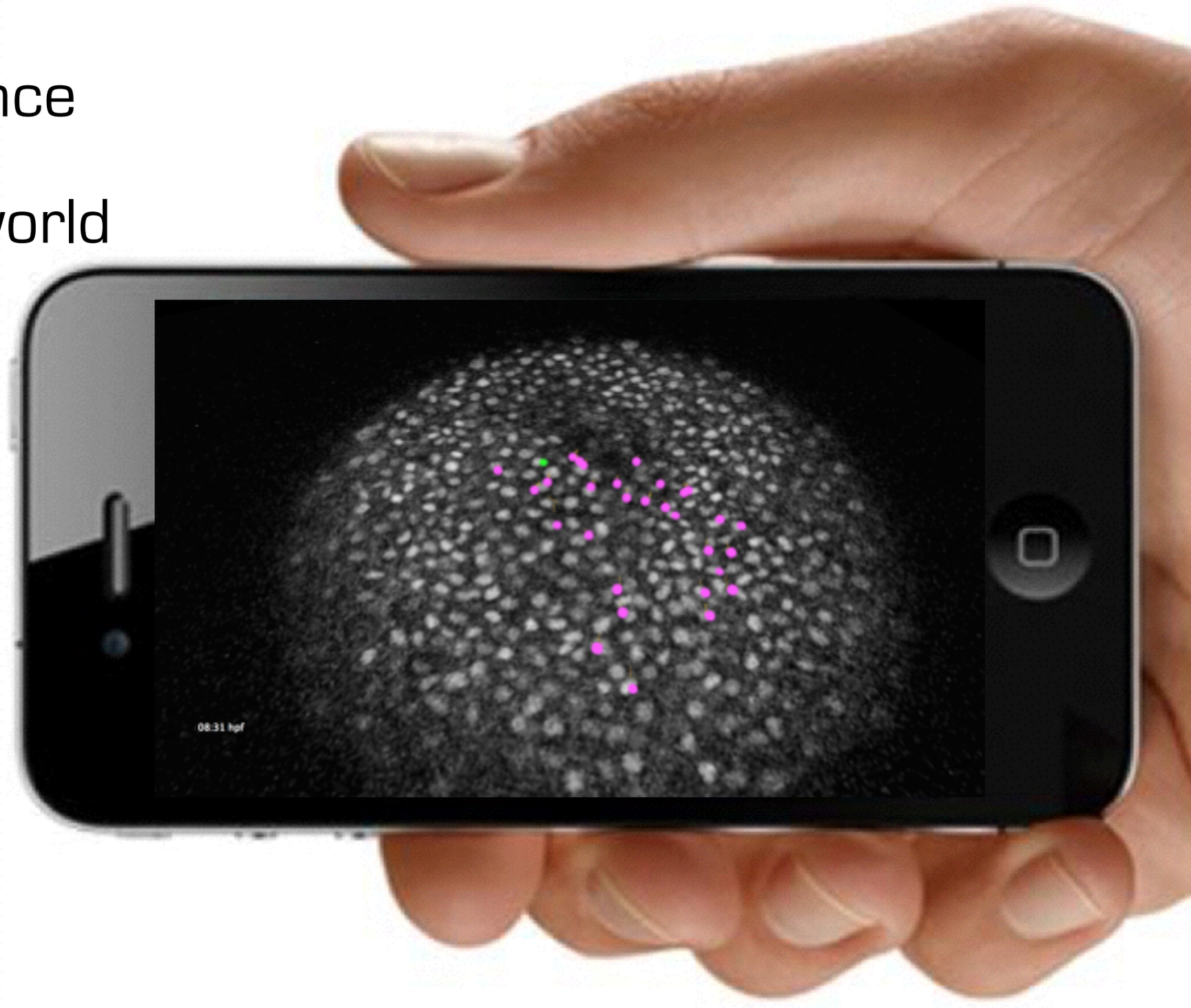
Citizen Science

Varieties of world

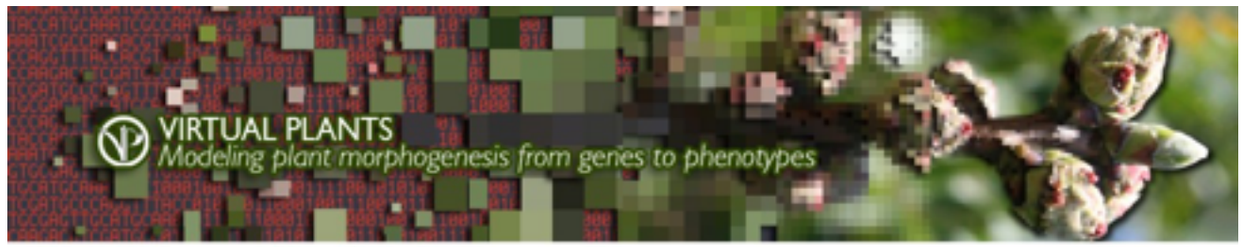
Education

Levels

Diffusion



Merci !



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Patrick Lemaire