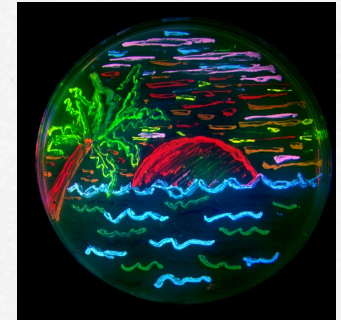


Détection & tracking de particules fluorescentes

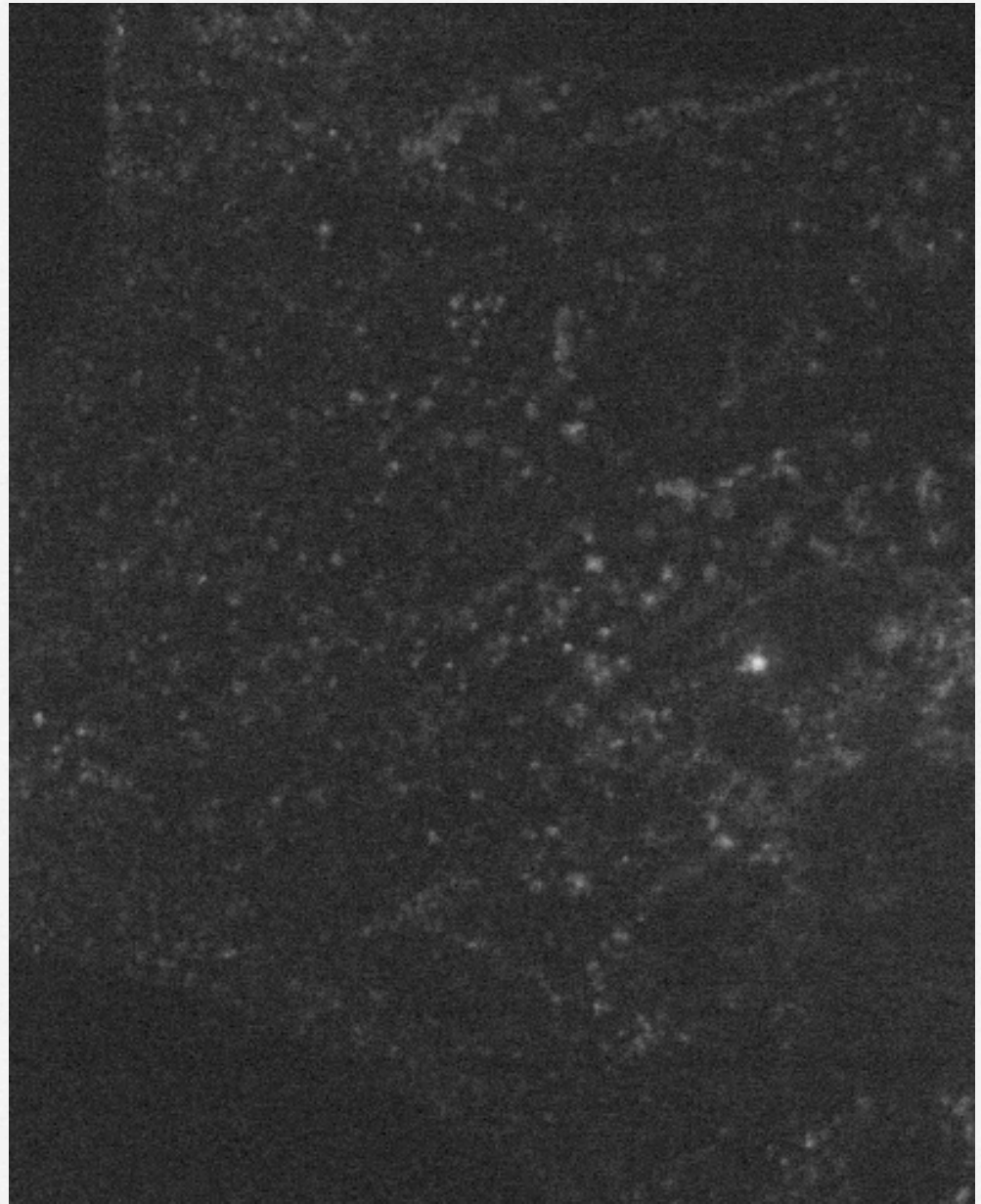
Institut Pasteur - CNRS URA 2582
Unité d'Analyse d'Image Quantitative
<http://www.bioimageanalysis.org>

Motivations et objectifs

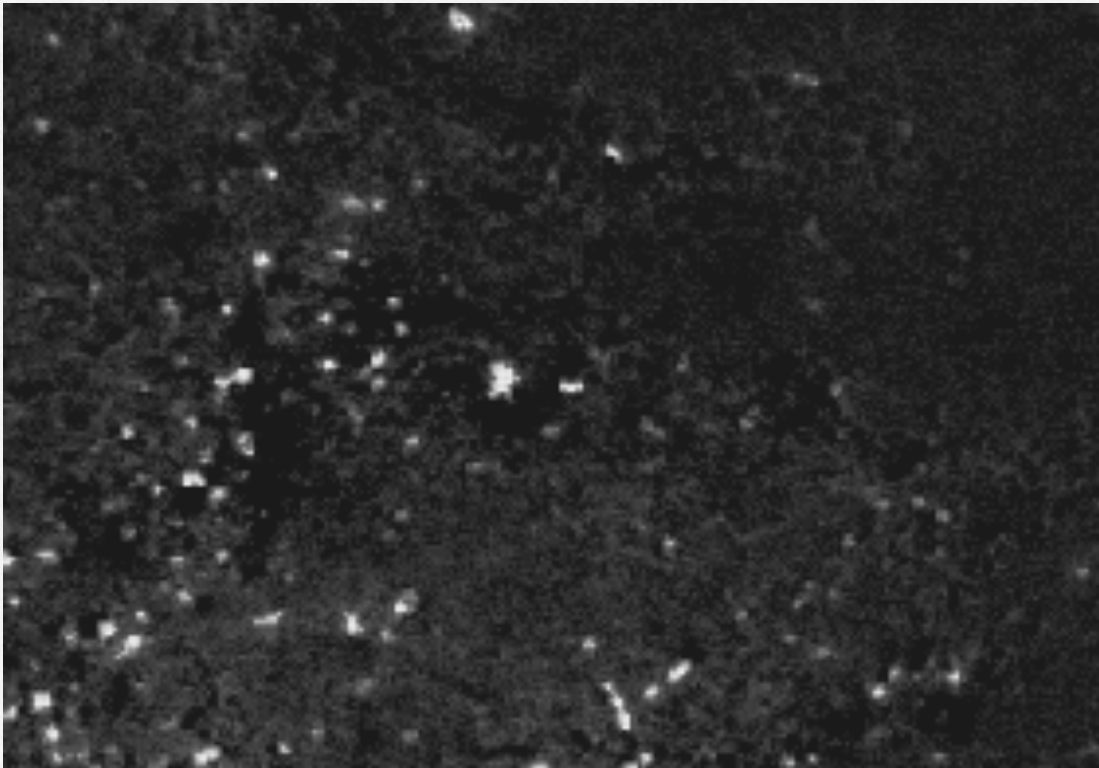


- Les techniques modernes de microscopies permettent d'observer efficacement divers compartiments cellulaires et entités intracellulaires (vésicules, endosomes, virus, etc.)
- Etude de la dynamique d'objets biologiques en microscopie 2D/3D+t
- But : fournir des méthodes permettant de
 - gérer la grande variabilité des données biologiques
 - augmenter la sensibilité et la spécificité de l'analyse
 - fournir des résultats robustes, quantitatives et reproductibles

Golgi units in
Hela cells



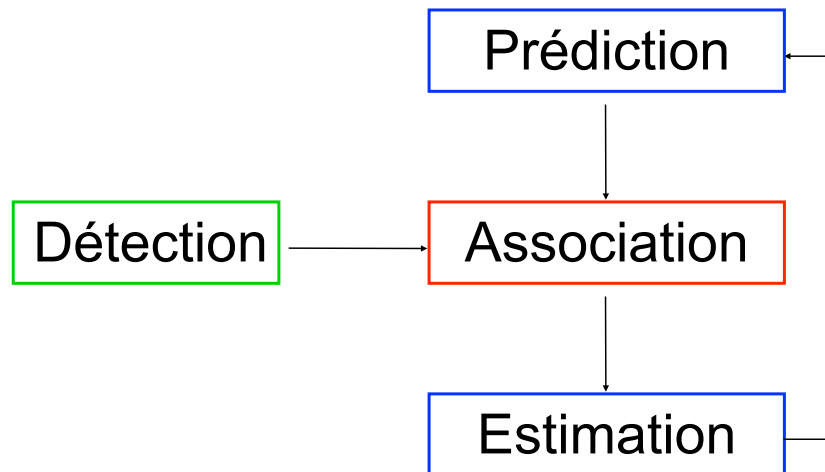
Problématique



Difficultés:

- aspect difficilement modélisable
- très bruité, faible SNR
- nombre élevé d'objets
- peuvent apparaître/disparaître
- peuvent s'agglomérer

Tracking bayésien



► Détection

- Pour obtenir des mesures des objets

► Filtrage bayésien

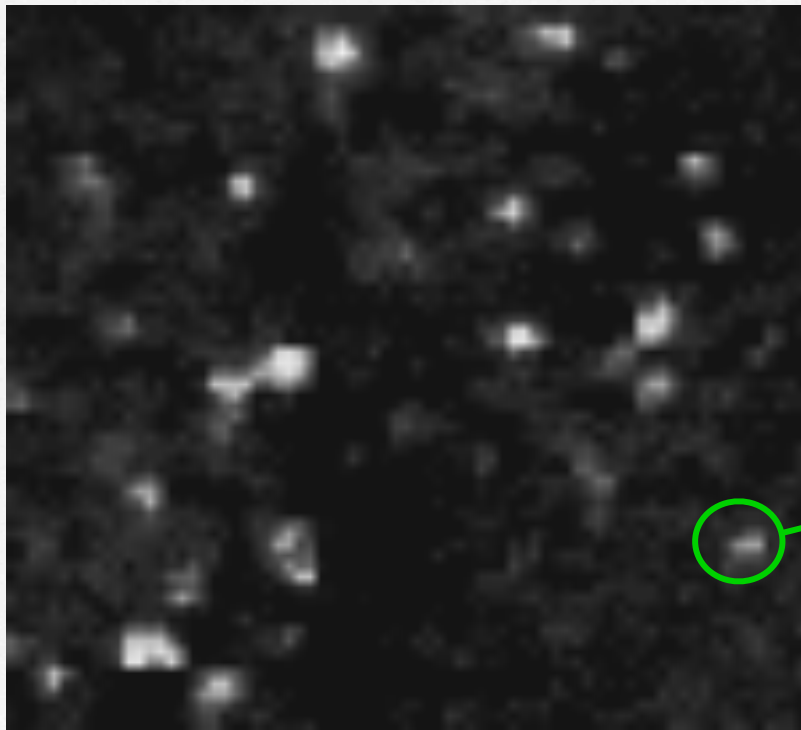
- Pour créer des prédictions puis des estimations des objets

► Association

- Pour déterminer quelle mesure provient de quel objet, si elle provient d'un objet

Détection

- ▶ objectif : produire des mesures des objets



$$\mathbf{z}_t = \begin{bmatrix} x_t \\ y_t \\ z_t \\ v_t \\ i_t \end{bmatrix}$$

Détection de taches
fluorescentes

[Olivo-Marín:2002]

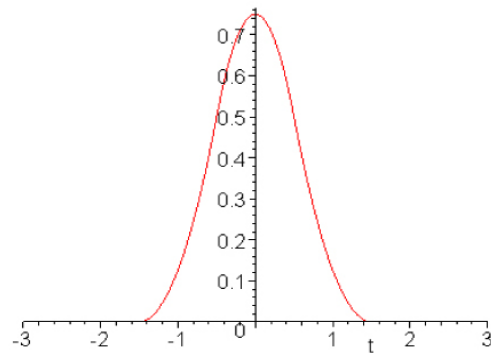
[Cuartero:2001]

[Zhang:2003]

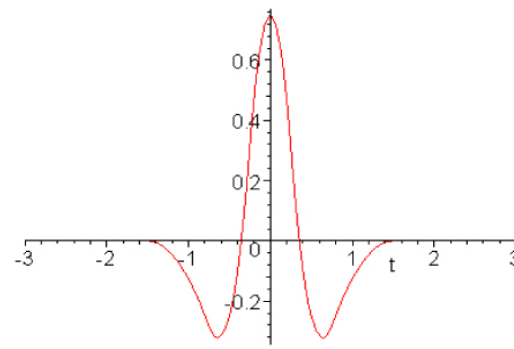
Spot Detection by Wavelet Transform

Features of the wavelet transform

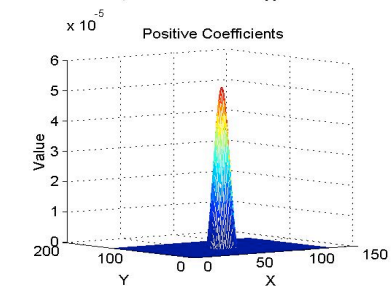
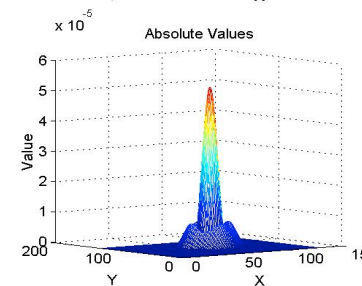
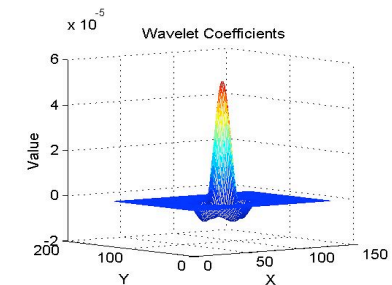
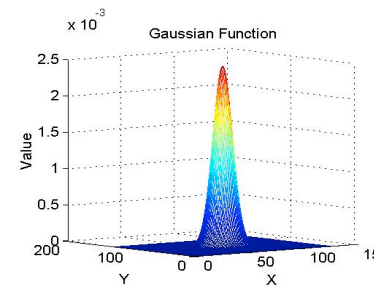
- Based on the convolution of the signal with a family of functions derived from a “mother” function by translation and dilation



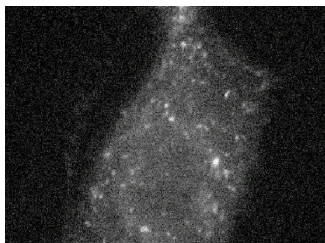
B2 - scale function



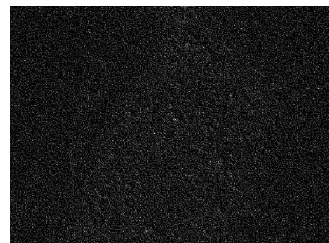
B2 - wavelet function



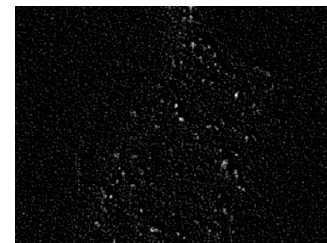
- Analysis tool that cuts up data into different frequency components and studies each with a resolution adapted to its scale



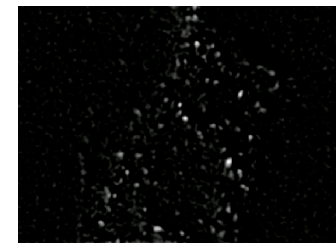
Original



1st scale



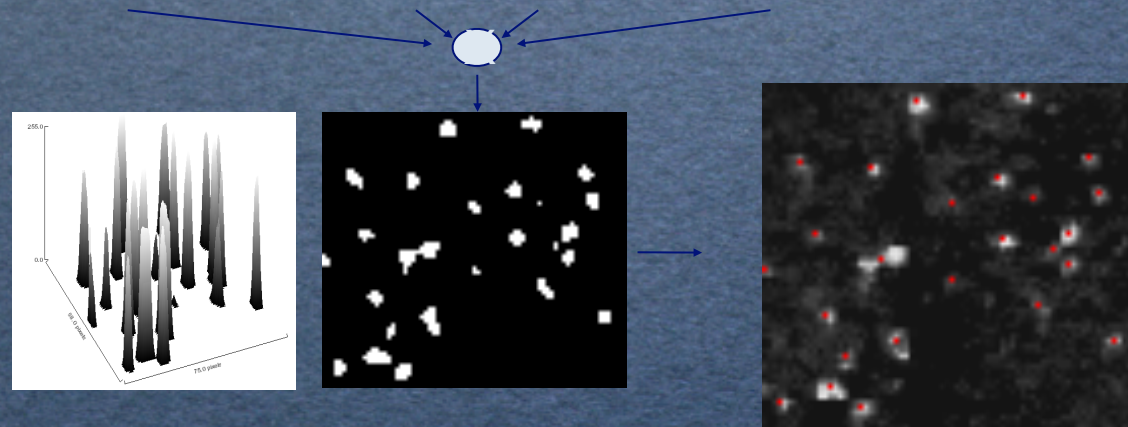
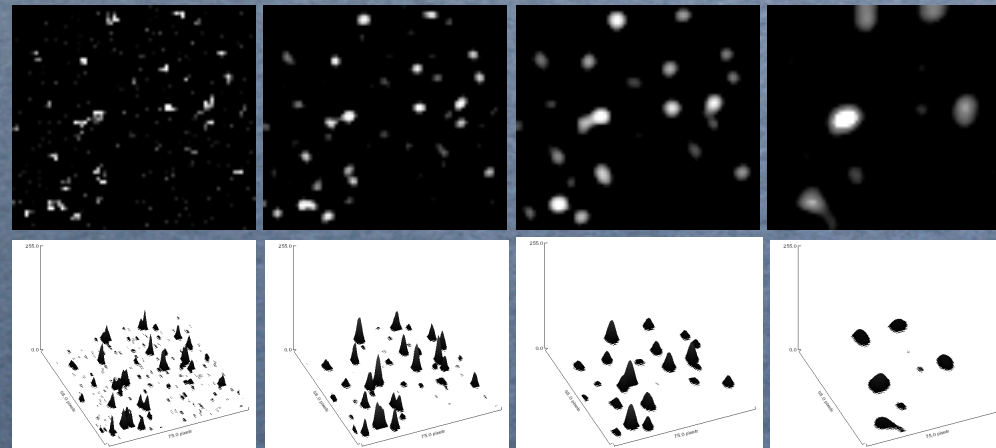
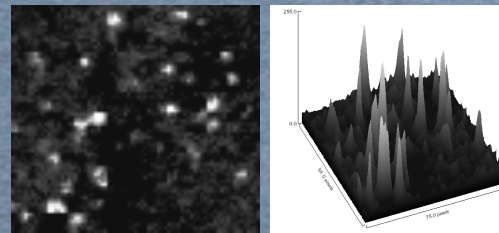
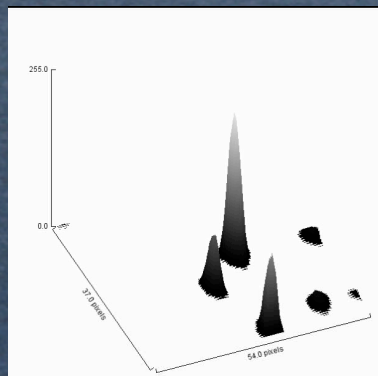
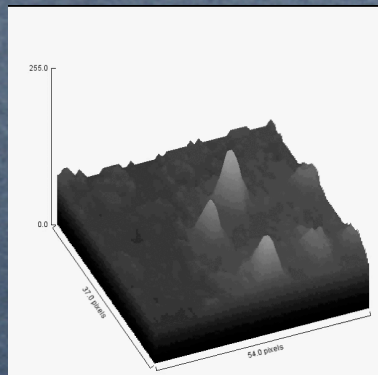
2nd scale



3rd scale

Spot Detection

Noise
reduction



Improved
detection

Olivo-Marin, J.-C., *Pattern Recognition* (2002)

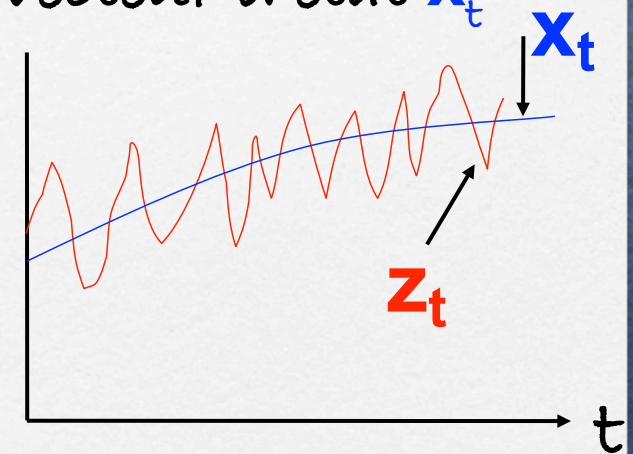
Filtre de Kalman

- ▶ Algorithme récursif de traitement de données
- ▶ Génère un estimé optimal des quantités désirées à partir d'un ensemble de mesures
- ▶ Optimal ?
 - ▶ Pour un système linéaire avec du bruit blanc gaussien, le filtre de Kalman est le 'meilleur' estimé
 - ▶ pour un système non linéaire, l'optimalité est 'qualifié'
- ▶ Récursif ?
 - ▶ Ne nécessite que de connaître que les précédentes mesures

Filtrage bayésien

► Obtenir la densité de probabilité d'un vecteur d'état \mathbf{x}_t récursivement depuis :

- les vecteurs de mesure $\mathbf{z}_1, \dots, \mathbf{z}_t$.
- La vraisemblance $p(\mathbf{z}_t | \mathbf{x}_t)$.
- L'a priori temporel $p(\mathbf{x}_t | \mathbf{x}_{t-1})$



► Donc :

- obtenir $p(\mathbf{x}_t | \mathbf{z}_1, \dots, \mathbf{z}_t)$ depuis $p(\mathbf{x}_{t-1} | \mathbf{z}_1, \dots, \mathbf{z}_{t-1})$

Filtrage bayésien

- ▶ Prédiction : Si \mathbf{x}_t est un processus de Markov

$$p(\mathbf{x}_t / \mathbf{z}_1, \dots, \mathbf{z}_{t-1}) = \int p(\mathbf{x}_t / \mathbf{x}_{t-1}) p(\mathbf{x}_{t-1} / \mathbf{z}_1, \dots, \mathbf{z}_{t-1}) d\mathbf{x}_{t-1}$$

- ▶ Mise à jour : Bayes' rule

$$p(\mathbf{x}_t / \mathbf{z}_1, \dots, \mathbf{z}_t) = \frac{1}{C} p(\mathbf{z}_t / \mathbf{x}_t) p(\mathbf{x}_t / \mathbf{z}_1, \dots, \mathbf{z}_{t-1})$$

$$C = \int p(\mathbf{z}_t / \mathbf{x}_t) p(\mathbf{x}_t / \mathbf{z}_1, \dots, \mathbf{z}_t) d\mathbf{x}_t$$

- ▶ Ne peut, en général, pas être obtenu

- ▶ Kalman filter (analytique optimale)
- ▶ Extended Kalman filter (analytique sous-optimale)
- ▶ Grid Based filter (optimale)
- ▶ Particle filter (stochastique sous-optimale)
- ▶ Interacting Multiple Models (analytique sous-optimale)

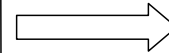
Filtrage bayésien

- ▶ Kalman filter (analytique optimale)
- ▶ Extended Kalman filter (analytique sous-optimale)
- ▶ Grid Based filter (optimale).
- ▶ Particle filter (stochastique sous-optimale).
- ▶ Interacting Multiple Models (analytique sous-optimale)

Filtrage bayésien

Kalman filter (analytique optimale)

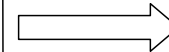
$p(z_t | x_t), p(x_t | x_{t-1})$ linéaire gaussienne
 $p(x_{t-1} | z_1, \dots, z_{t-1})$ gaussienne



$p(x_t | z_1, \dots, z_t)$ gaussienne
avec paramètres connus

Extended Kalman filter (analytique sous-optimale)

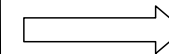
$p(z_t | x_t), p(x_t | x_{t-1})$ non gaussienne linéaire.
 $p(x_{t-1} | z_1, \dots, z_{t-1})$ gaussienne



$p(x_t | z_1, \dots, z_t)$ gaussienne avec
paramètres approximatés

Grid Based filter (optimale)

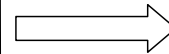
$p(z_t | x_t), p(x_t | x_{t-1})$ non gaussienne, non linéaire.



$p(x_t | z_1, \dots, z_t)$

Particle filter (stochastique sous-optimale)

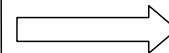
$p(z_t | x_t), p(x_t | x_{t-1})$ non gaussienne, non linéaire.
 $q(x_t | x_{t-1}, z_t)$ func.d'imp.



$p(x_t | z_1, \dots, z_t)$ approximatée
par échantillonnage

Interacting Multiple Models (analytique sous-optimale)

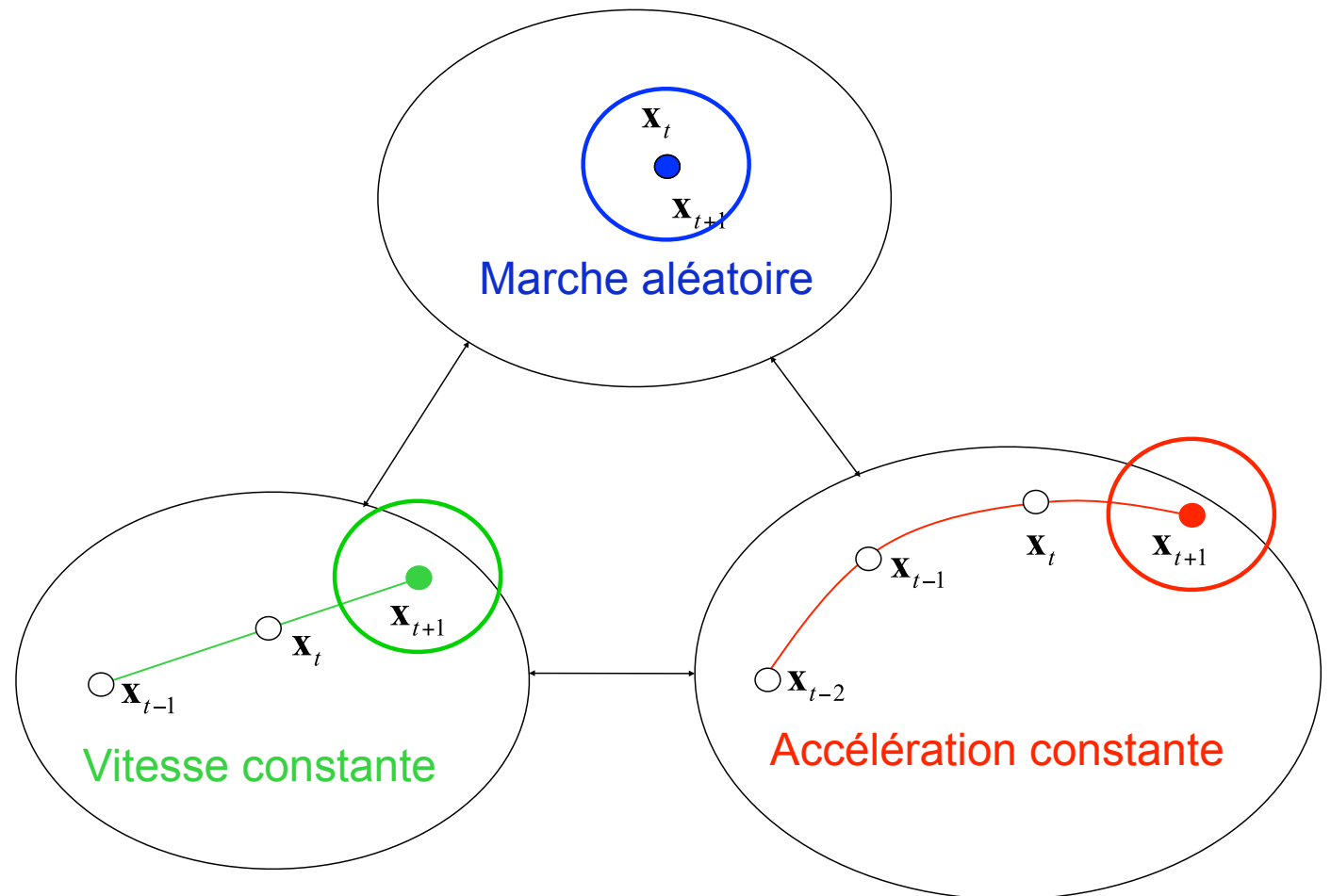
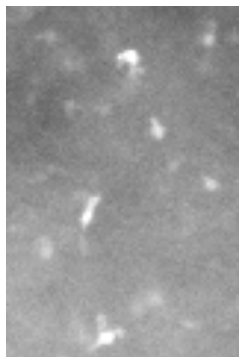
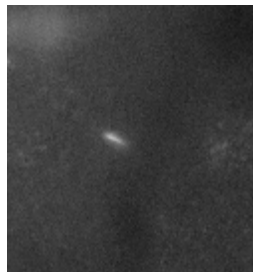
Banc de filtres de Kalman



$p(x_t | z_1, \dots, z_t)$ approximatée par une
somme de gaussiennes

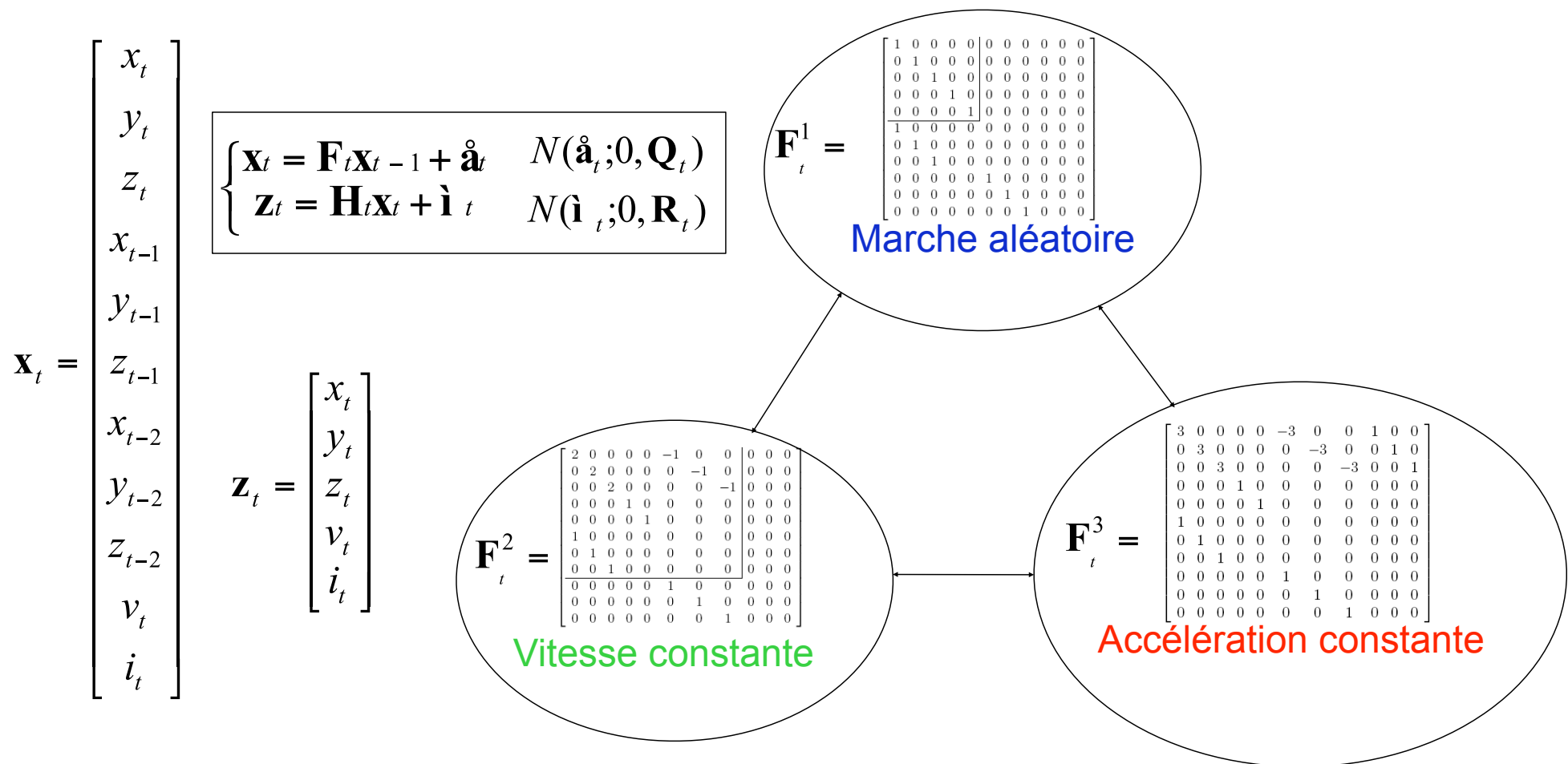
Filtrage bayésien

► Proposition 1 : Modèle d'état



Filtrage bayésien

► Modèle d'état proposé



Filtrage bayésien

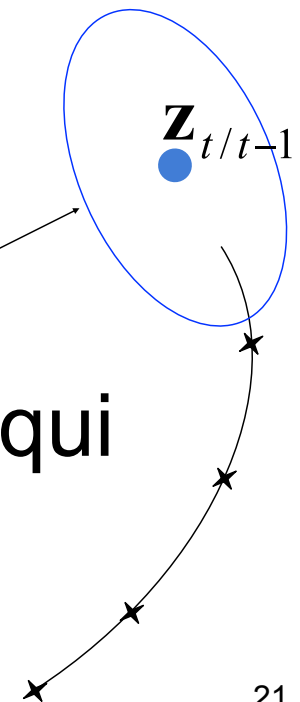
- ▶ Une fenêtre de recherche peut être obtenue avec les KF, EKF et IMM
- ▶ Exemple avec un Filtre de Kalman :

$$\mathbf{z}_{t/t-1} = \mathbf{H}_t \mathbf{F}_t \mathbf{x}_{t-1/t-1}$$

$$\mathbf{S}_t = \mathbf{H}_t \mathbf{Q}_t \mathbf{F}_t \mathbf{P}_{t-1/t-1} \mathbf{F}_t^T \mathbf{H}_t^T + \mathbf{R}_t$$

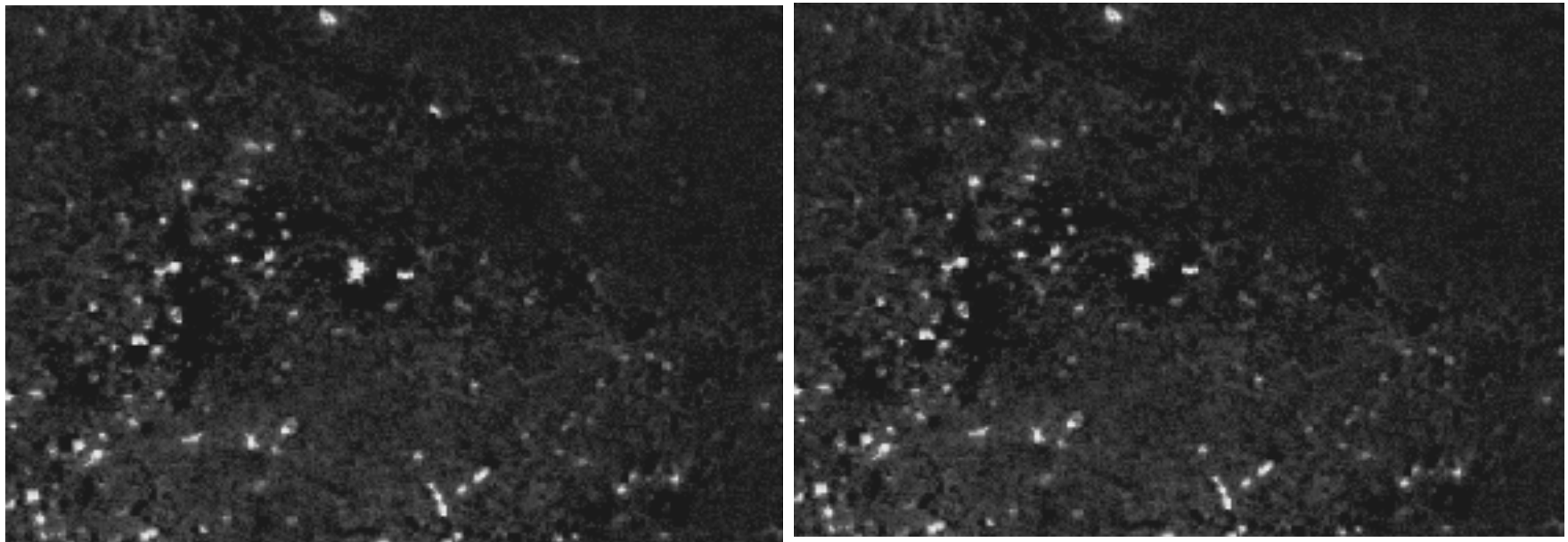
Pr > 95% avec $N(\mathbf{z}_t; \mathbf{z}_{t/t-1}, \mathbf{S}_t)$

- ▶ Proposition 2 : une mise à jour de \mathbf{Q}_t qui adapte la fenêtre de recherche

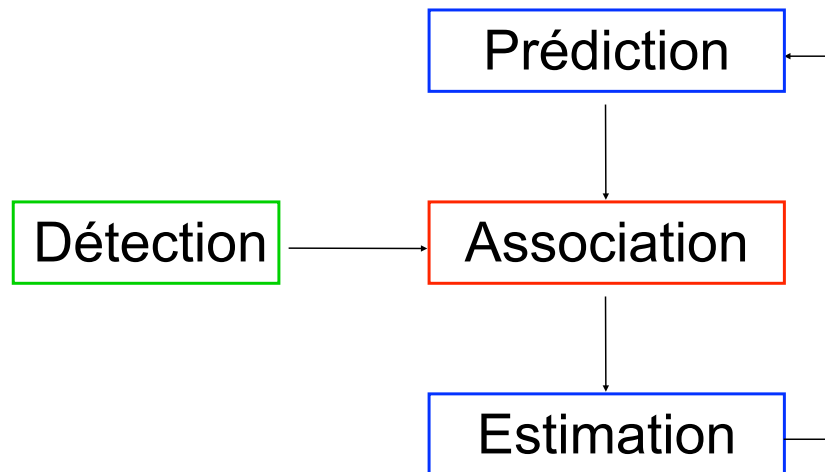


Adaptation de fenêtre de recherche

- ▶ endosomes



Association



► Détection

- Pour obtenir des mesures des objets

► Filtrage bayésien

- Pour créer des prédictions puis des estimations des objets

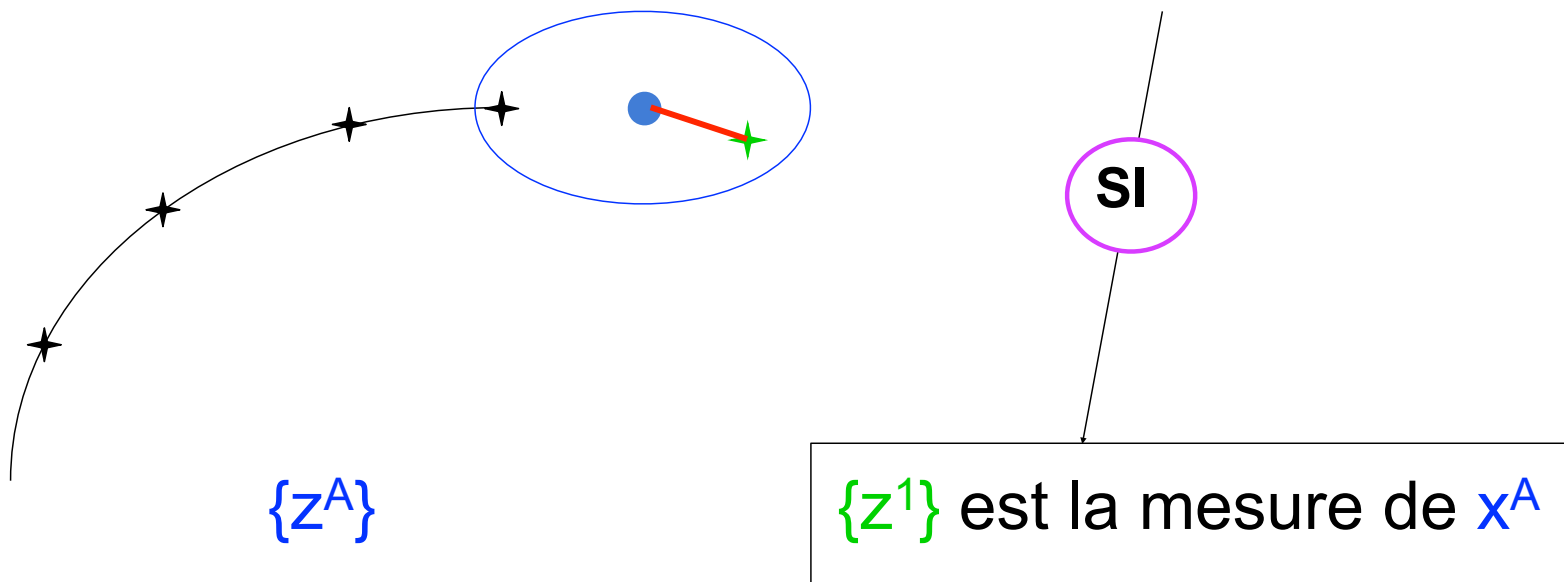
► Association

- Pour déterminer quelle mesure provient de quel objet, si elle provient d'un objet

Association

- ▶ Principe pour 1 objet (4 étapes)
 - prédiction, détection, association et estimation

▶ Pas de problème d'association !

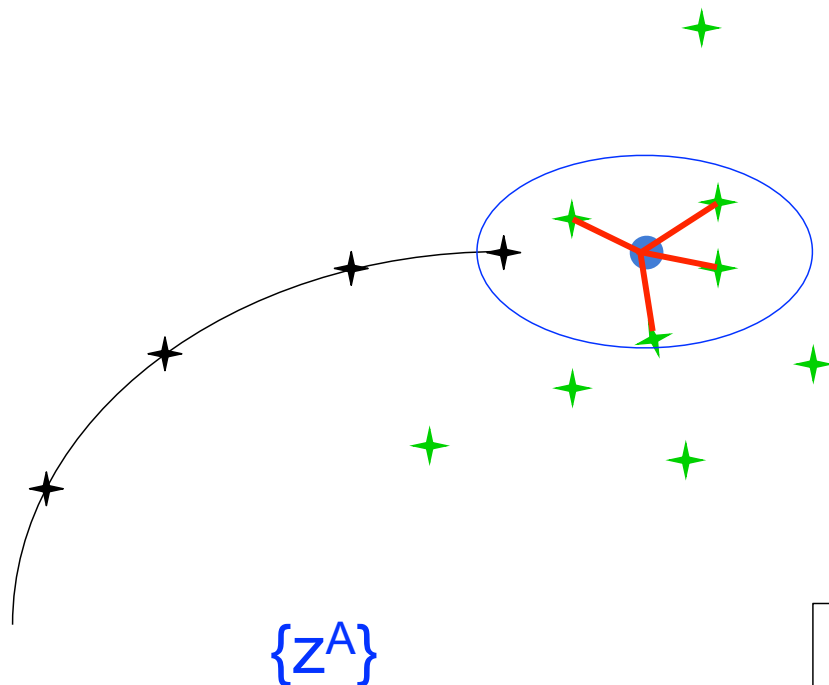


Association

- ▶ Principe pour 1 objet (4 étapes)
 - prédiction, détection, association et estimation

▶ Association avec :

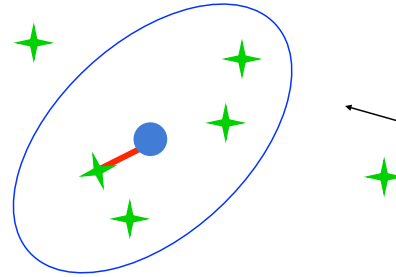
- ▶ ML
- ▶ PDA
- ▶ MHT



SI

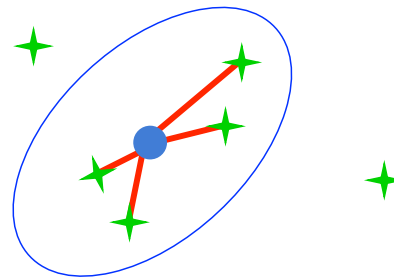
Association

▶ ML



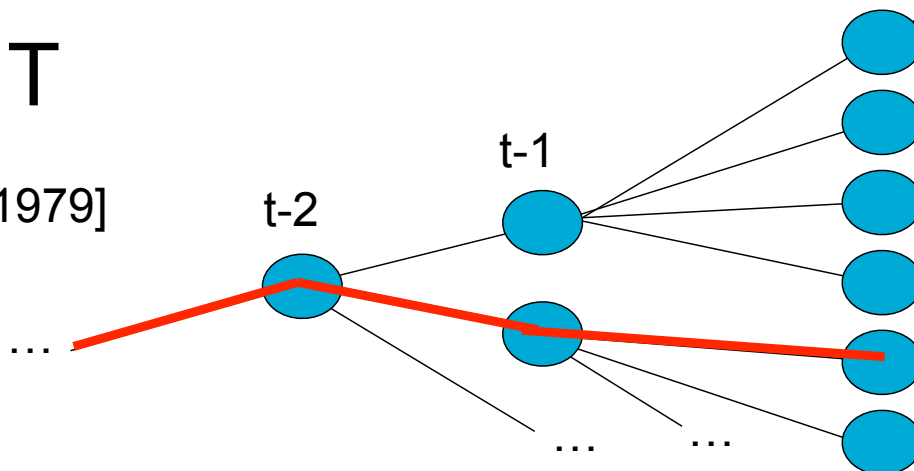
▶ PDA

[Fortmann:1980]



▶ MHT

[reid:1979]

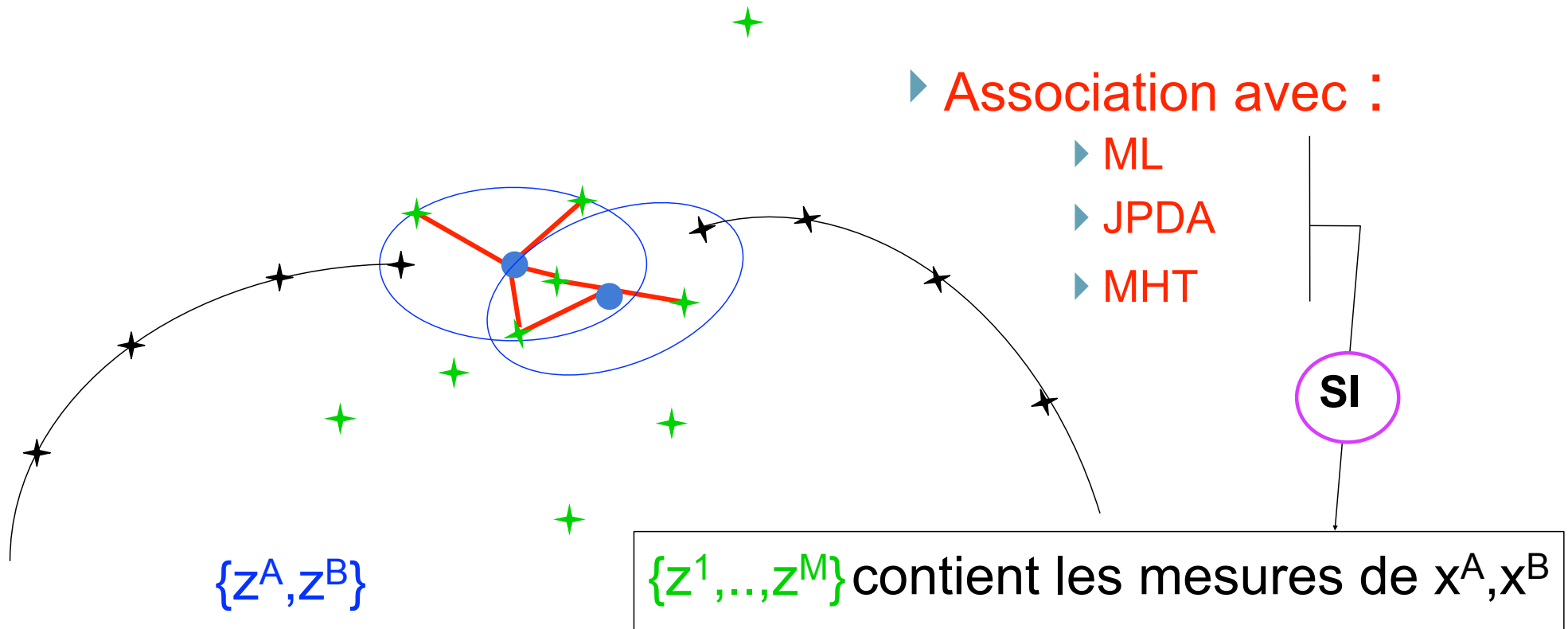


Association

- ▶ Principe pour plusieurs objets (4 étapes)
 - prédiction, détection, association et estimation

- ▶ Association avec :

- ▶ ML
- ▶ JPDA
- ▶ MHT



Association

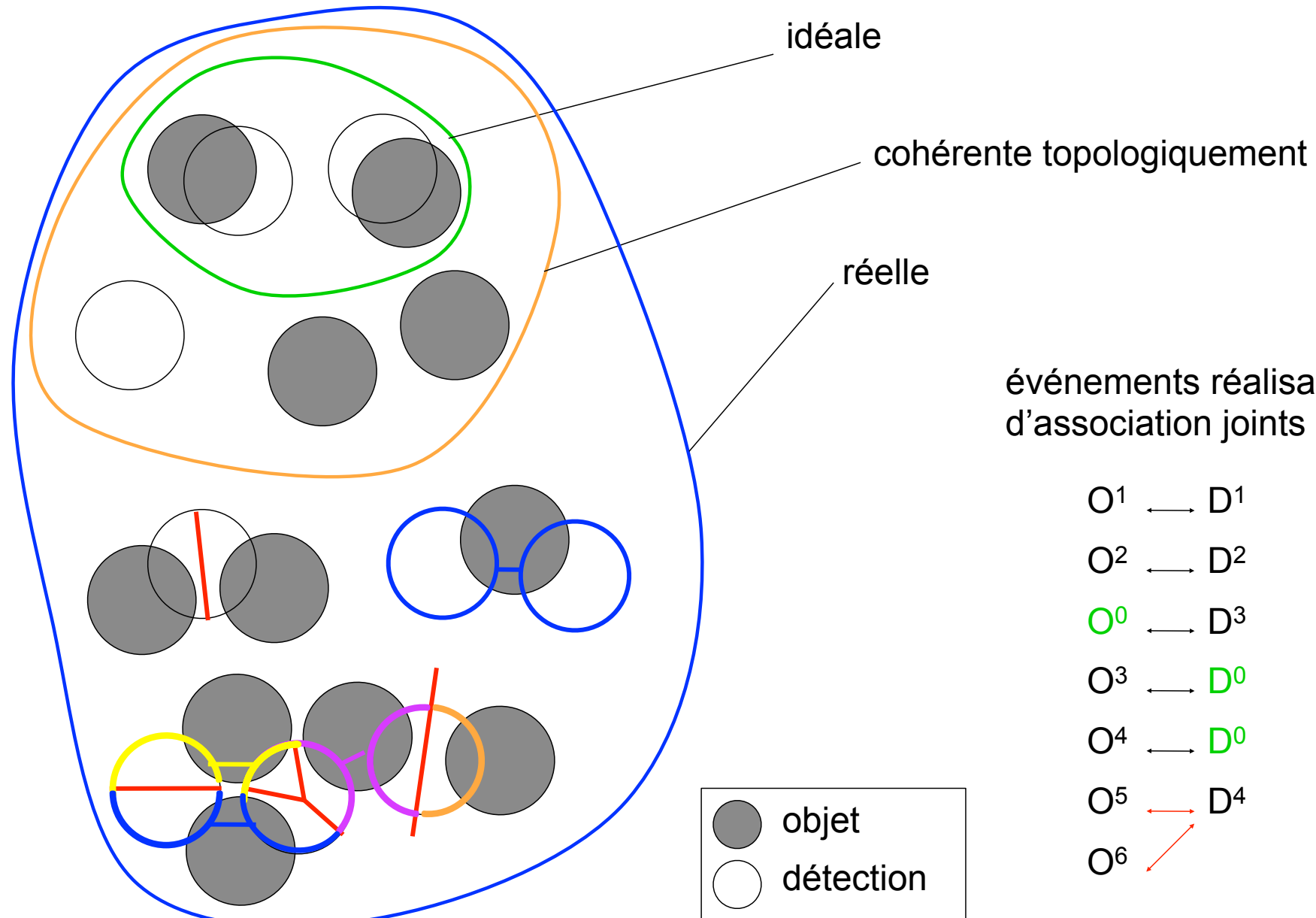
▶ Tracking multi-objets en pratique

Toutes ces méthodes font
l'hypothèse suivante :

- ▶ ML
- ▶ JPDA
- ▶ MHT
- ✓ une mesure est produite par un objet au plus
- ✓ un objet produit au plus une mesure

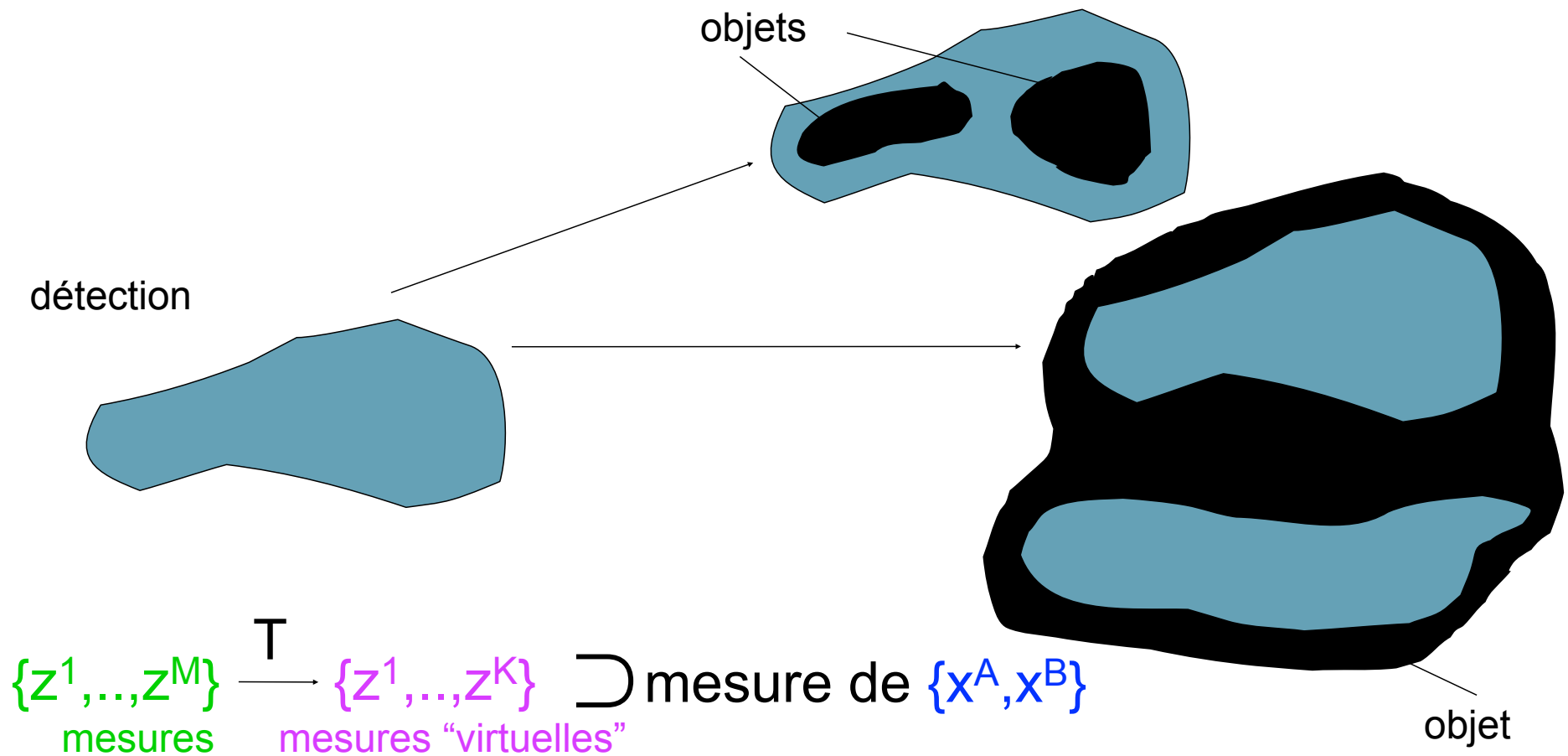
Dans la plupart des applications, ces hypothèses ne sont pas validées, conduisant à l'échec de ces méthodes

Association

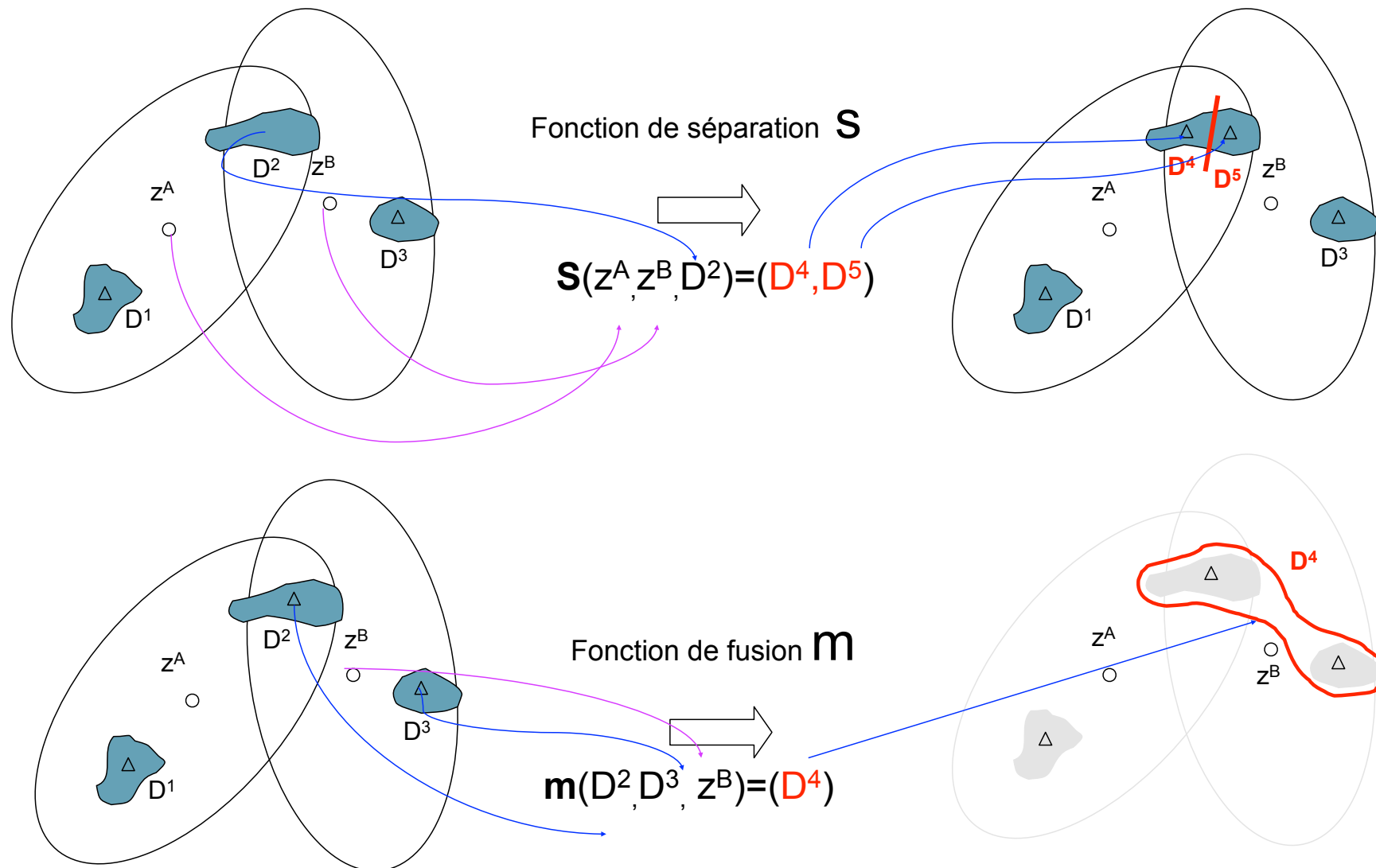


Association

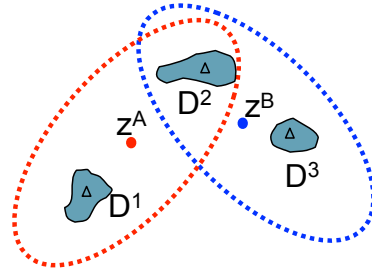
► Détection fusionnée et détection partielle



Association séparation-fusion



Association séparation-fusion



Séparation des détections conflictuelles

$$D^{j_{i_1}}, \dots, D^{j_{i_q}} = s_q(D^j, \mathbf{z}_{t/t-1}^{i_1}, \dots, \mathbf{z}_{t/t-1}^{i_q})$$

Détections non conflictuelles et séparées

$$E'_i = N_i \cup \bigcup_j D^{j_i}$$

Toutes les fusions possibles pour i

$$Z'_i = \left\{ m_q(e^j, \mathbf{z}_{t/t-1}^i), e^j \in \mathcal{P}(E'_i) \right\}$$

Toutes les fusions possibles (ensemble des mesures virtuelles)

$$Z' = \bigcup_i Z'_i$$

$Z' \times \dots \times Z' \supset \Theta$ contient des "événements réalisables d'association joints" approximés pour les x^1, \dots, x^N

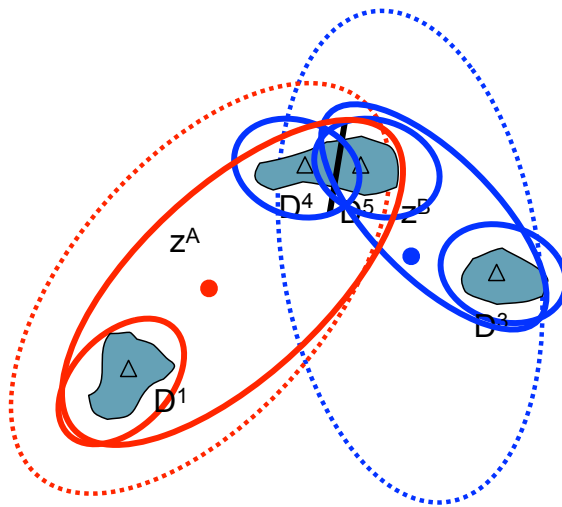
$$\theta^* = \arg \max_{\theta \in \Theta} P(\theta / \mathbf{Z}_{1:t})$$

[dépend du choix de $s(\cdot)$ et $m(\cdot)$]

$$\theta^* = \arg \max_{\theta \in \Theta} \frac{1}{c} \frac{\phi!}{V^\phi} \prod_j (\Lambda_{i,j})^{\tau_j} \prod_i \left[P_D^i \right]^{\delta_i} \left[(1 - P_D^i) \right]^{-\delta_i}$$

Association séparation-fusion

Évènement réalisable d'association joint $\theta \in \Theta \subset Z' \times Z'$



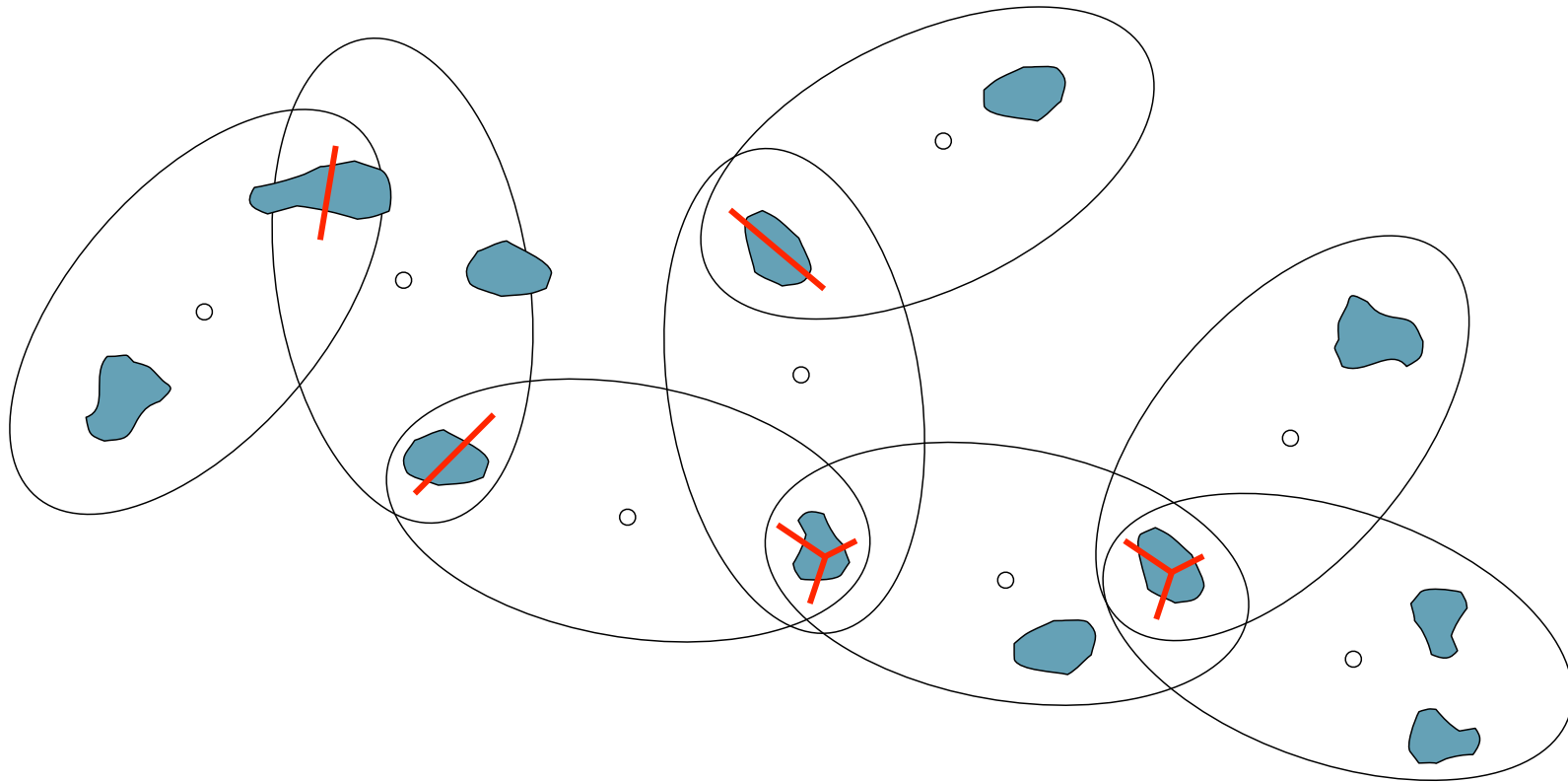
$$|Z' \times Z'| = 64$$

$$|\Theta| = 36$$

	z^A	z^B
θ_1	D^1	D^3
θ_2	D^1	D^4
θ_3	D^1	D^5
θ_4	D^1	$m(D^3, D^5)$
θ_5	D^1	$m(D^3, D^4)$
..
θ_{36}	$m(D^1, D^4, D^5)$	\emptyset

Association séparation-fusion

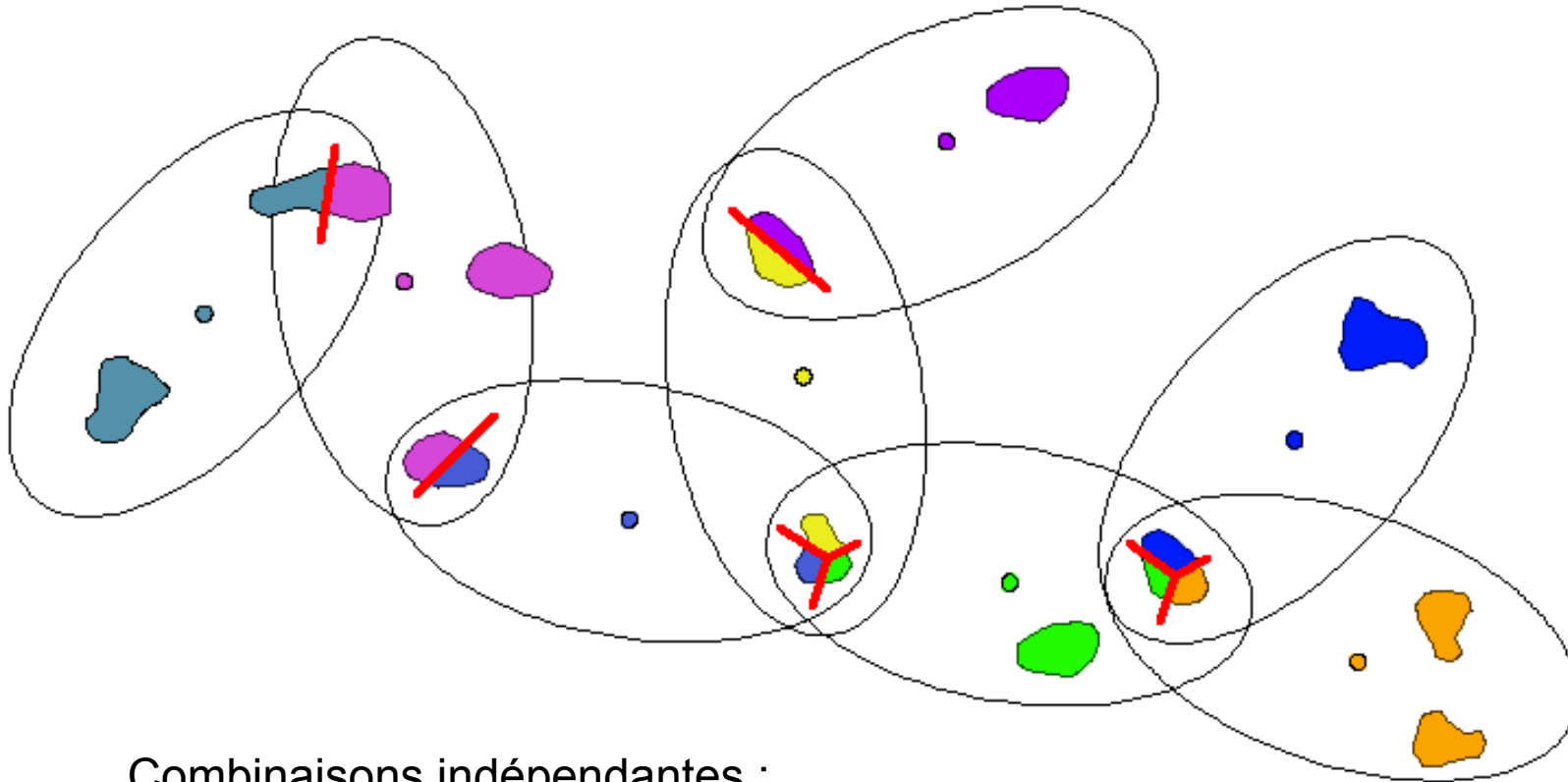
- ▶ Complexité : implémentation « naïve »



$$2^3 \times 2^5 \times 2^5 \times 2^5 \times 2^3 \times 2^7 \times 2^4 \times 2^5 = 2^{37} = 137.438.953.472$$

Association séparation-fusion

- ▶ Complexité : implémentation « globale »

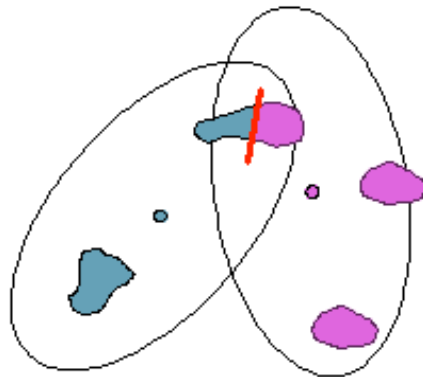


Combinaisons indépendantes :

$$2^2 + 2^3 + 2^2 + 2^2 + 2^2 + 2^3 + 2^2 + 2^3 = 44$$

Association séparation-fusion

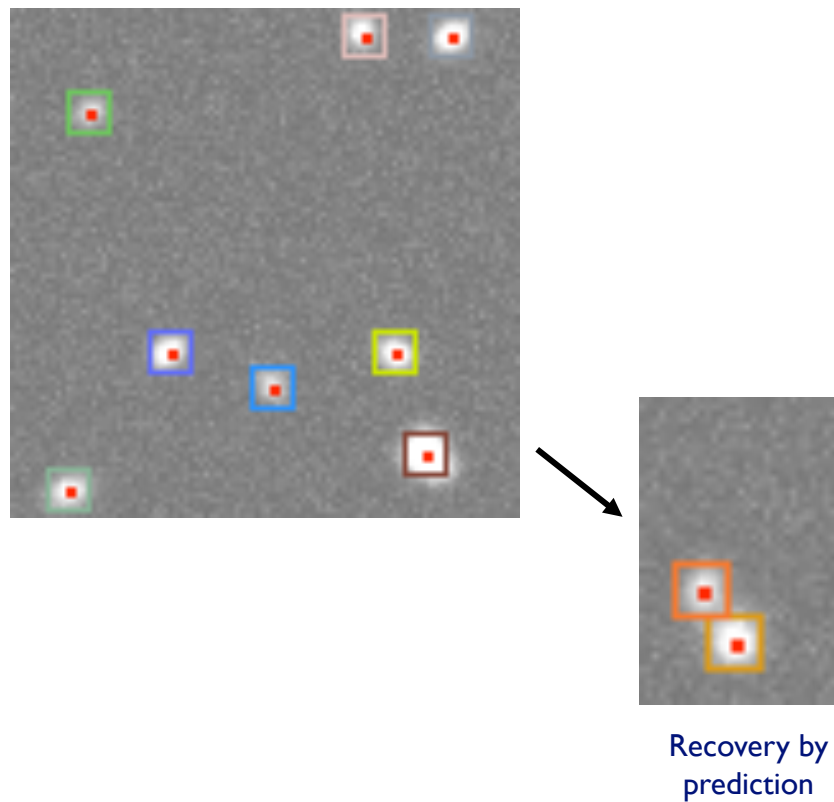
- ▶ Fonction de séparation $s(\cdot)$: k-moyennes contraintes



- ▶ Fonction de fusion $m(\cdot)$: union

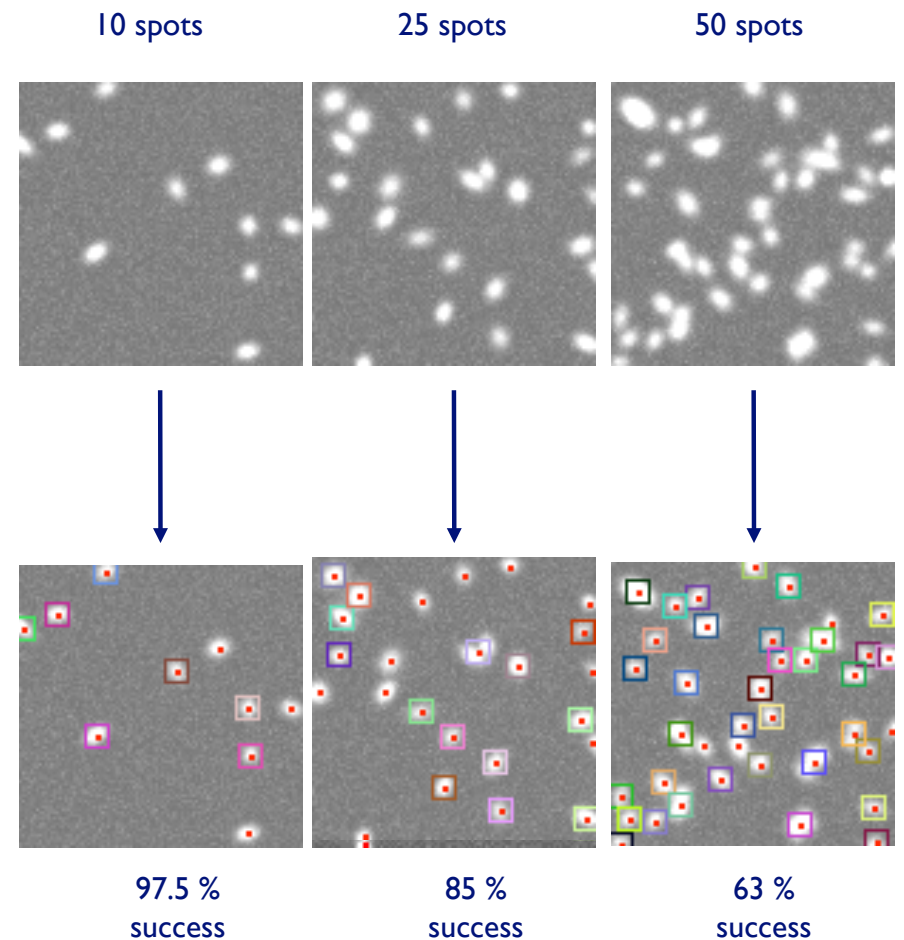
Spot Detection and Tracking

Robustness to missing detections



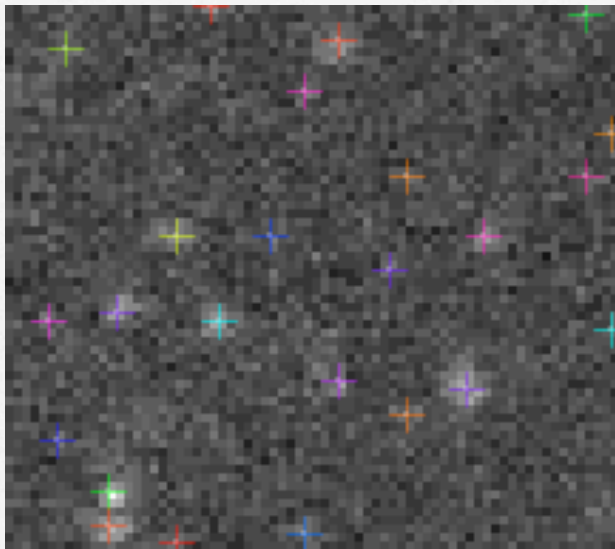
Robustness to density

(100 x 100 x 50 sequences)

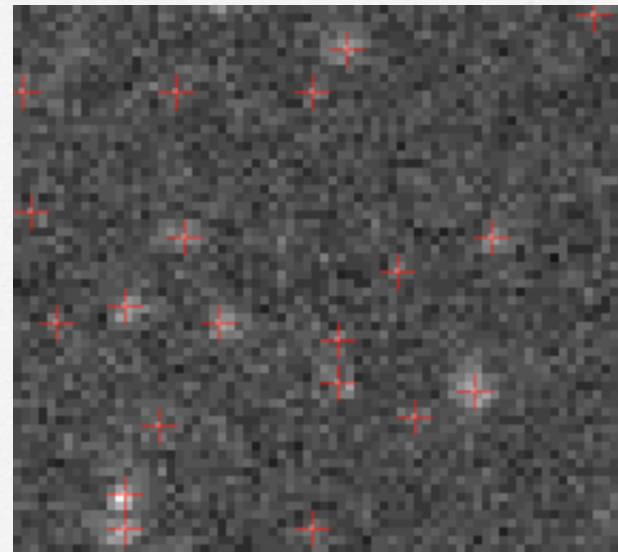


Limitations

■ Golgi units tracking in Hela cells

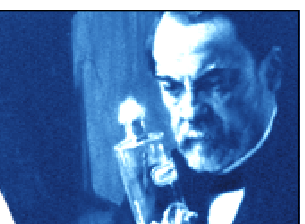


Instantaneous Maximum
Likelihood (IML) tracking

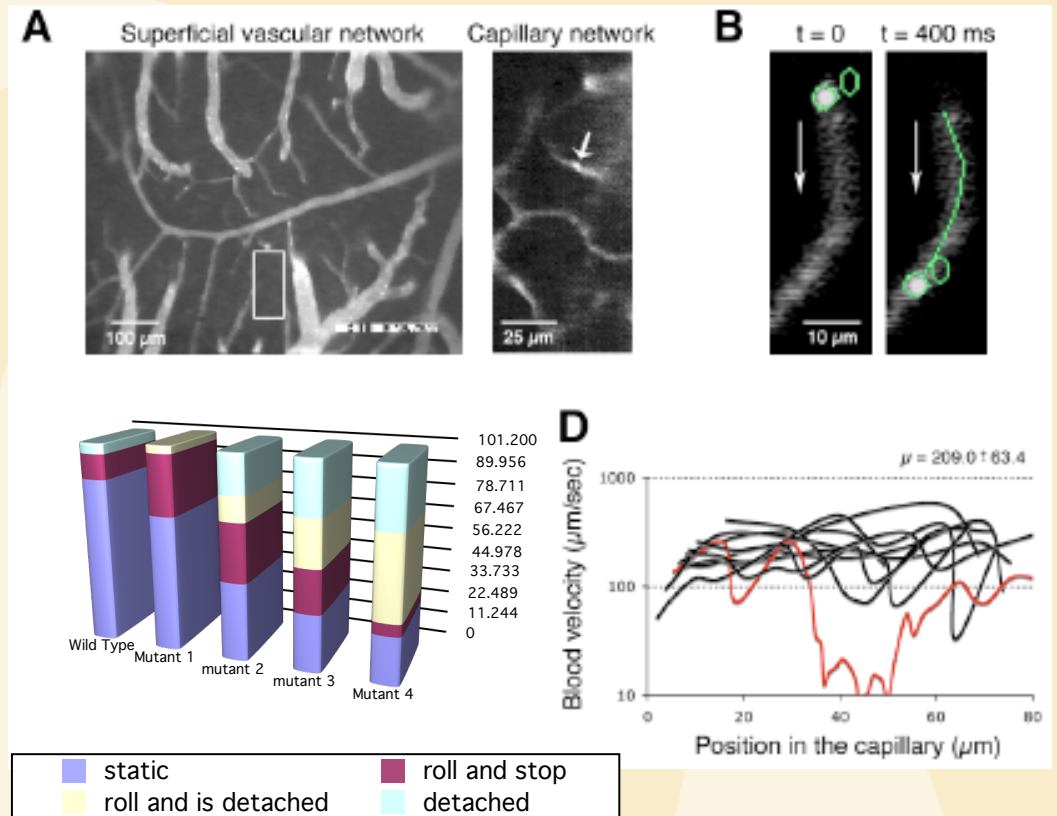
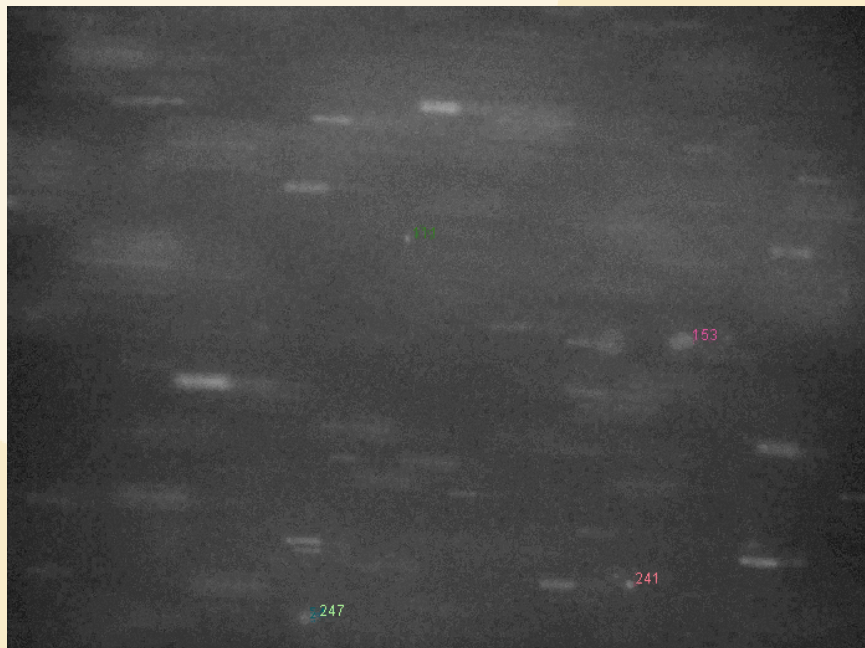
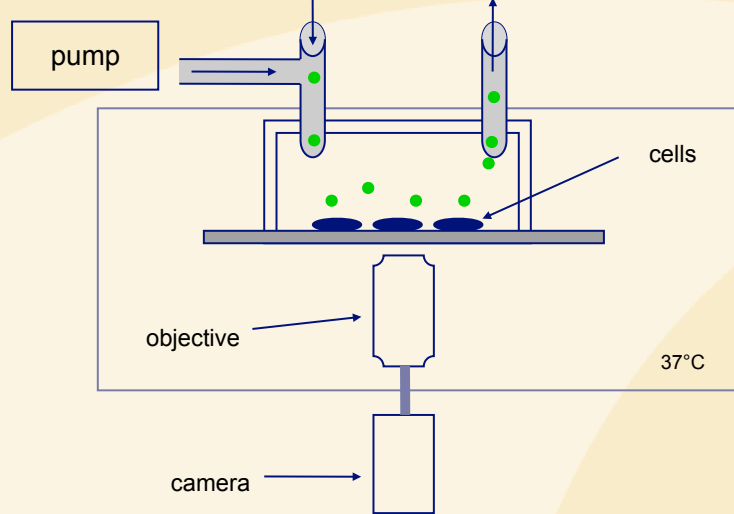


MHT
prenant en compte le futur

Applications



Introduction of fluorescent bacteria

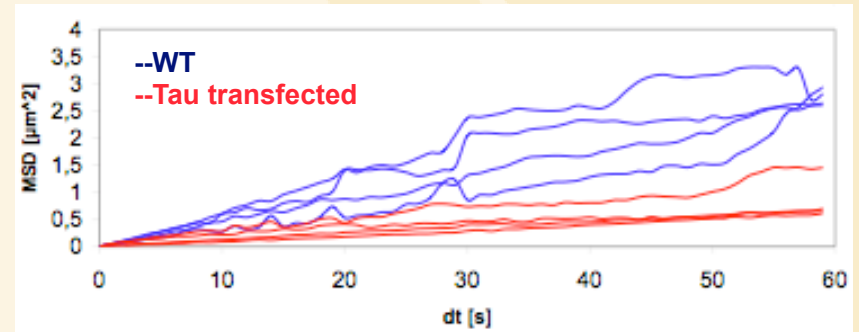
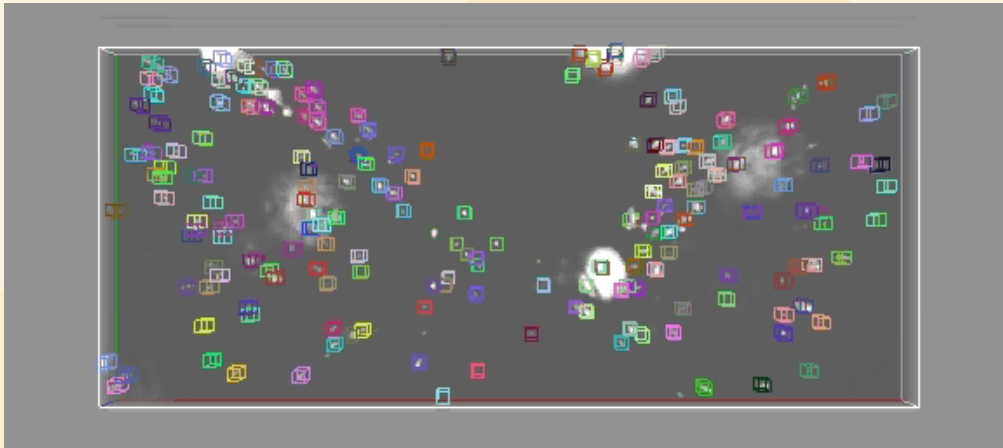
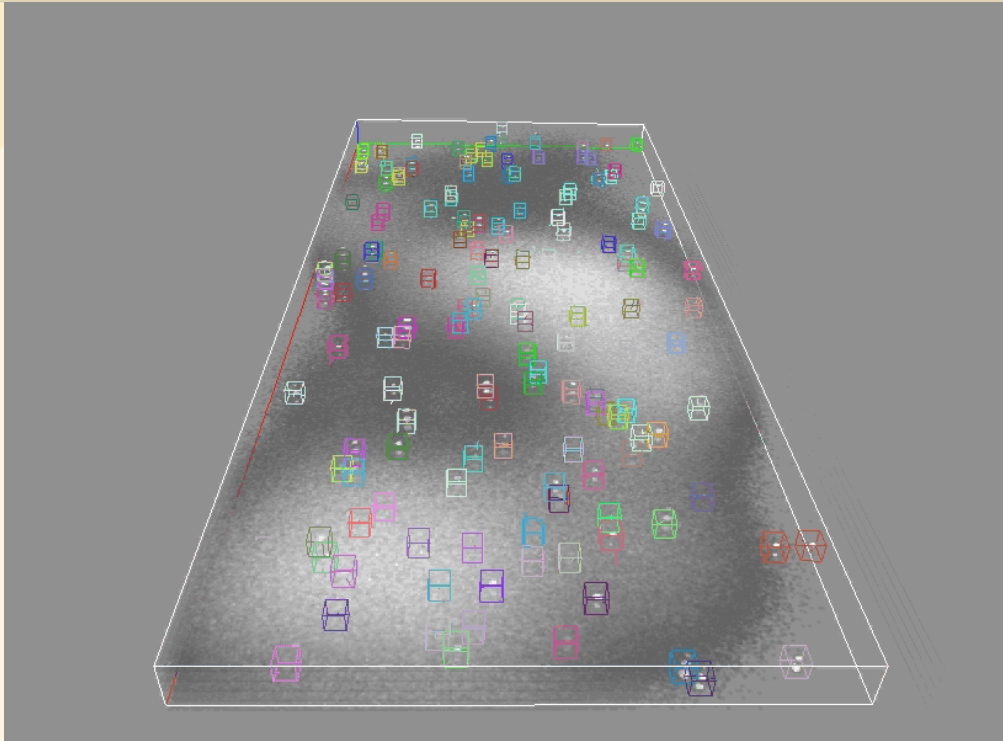
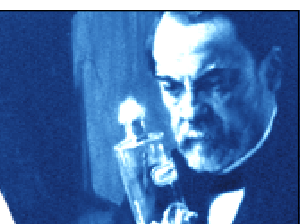


***Neisseria meningitidis* adhesion to capillary walls is a key factor for invasion**

E. Mairey, A. Genovesio, J.-C. Olivo-Marin, X. Nassif, G. Dumenil, *J. Ex. Med.* (2006)



Application in 3D + t

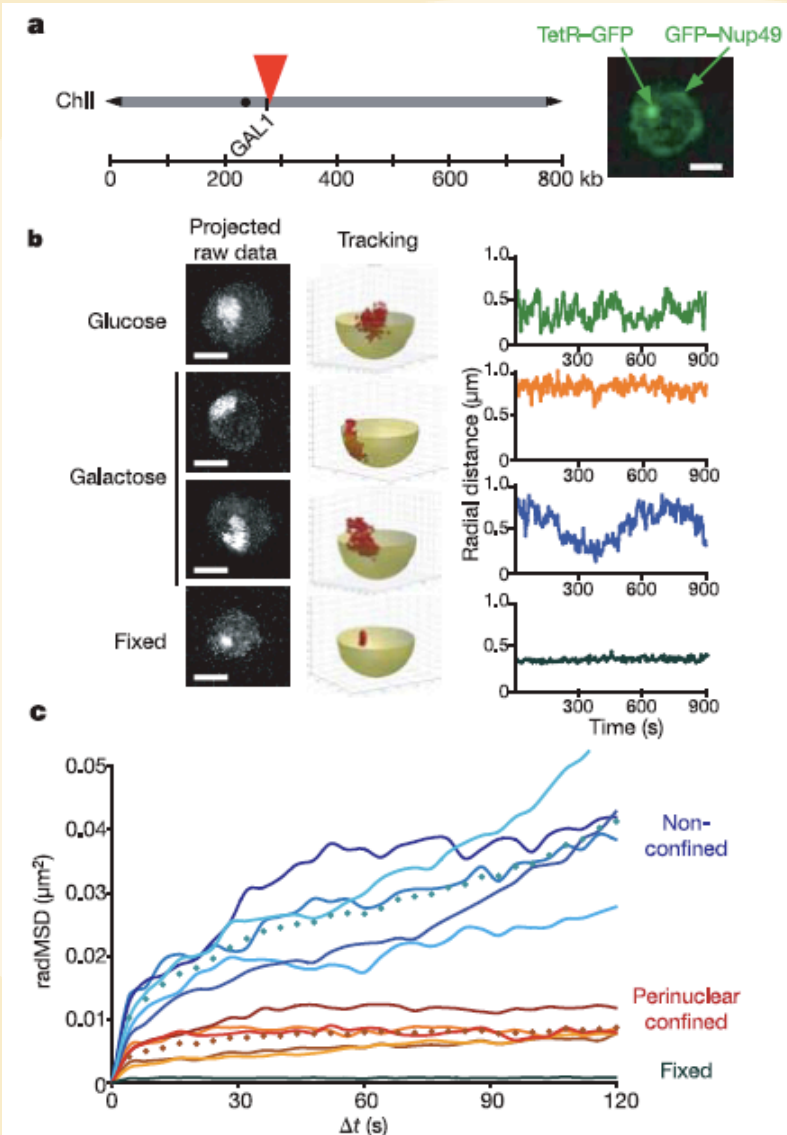
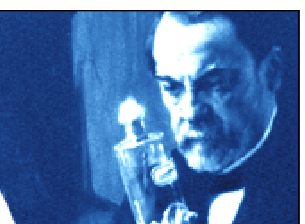


Tau reduces endosomes motility

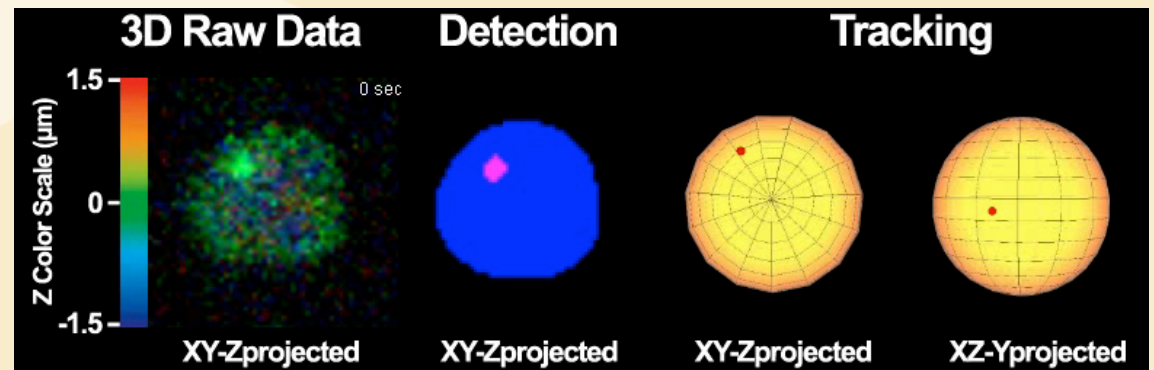
A. Genovesio, V. Emiliani, W. Parack, M. Copepy, J.-C. Olivo-Marin, *IEEE Trans. Image Processing* (2006)



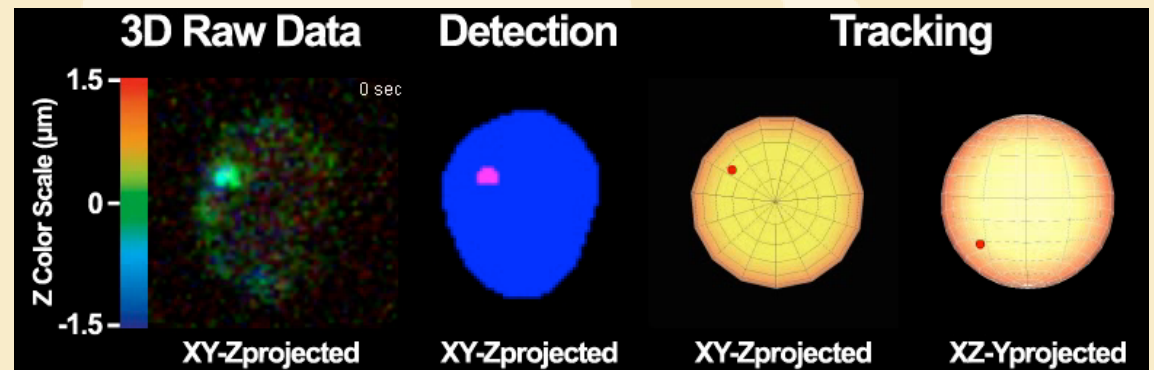
Application in 3D + t



Inactivated gene



Activated gene

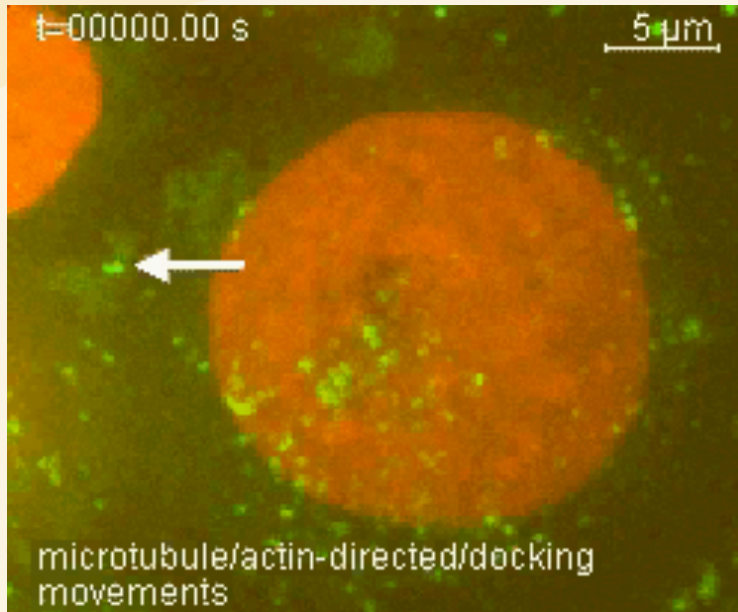
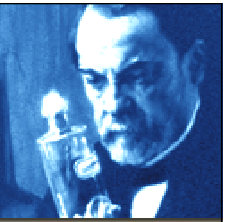


The GAL locus is subject to sub-diffusive movement and the activation of GAL genes leads to a confinement in dynamic motility.

G. Cabal, A. Genovesio, S. Rodriguez-Navarro, C. Zimmer, O. Gadal, A. Lesne, H. Buc, J.-C. Olivo-Marin, E. Hurt, U. Nehrbass, *Nature* (2006)

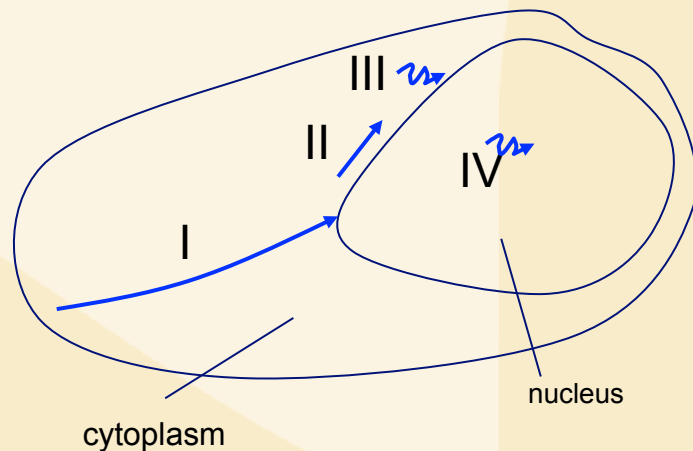


Application in 3D + t



HIV exhibits four types of movements

	Type	Velocity	3D tracks	Movement characteristics
Mouvement I	Microtubule-directed	peaks at 0.1 to 1 $\mu\text{m/s}$		Directed
Mouvement II	Actin-directed	under 0.03 $\mu\text{m/s}$		Directed
Mouvement III	Docking at the nuclear membrane	under 0.03 $\mu\text{m/s}^{\text{a}}$		Confined
Mouvement IV	Intranuclear movement	under 0.005 $\mu\text{m/s}^{\text{a}}$		Diffusive

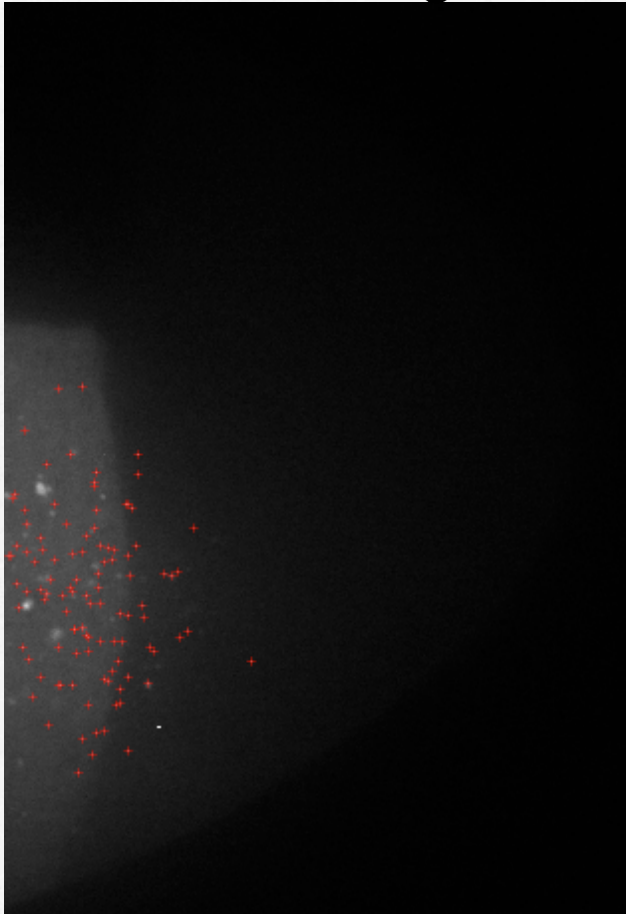


N. Arhel, A. Genovesio, J.-C. Olivo-Marin, S. Shorte, P. Charneau, *Nature Methods* (2006)

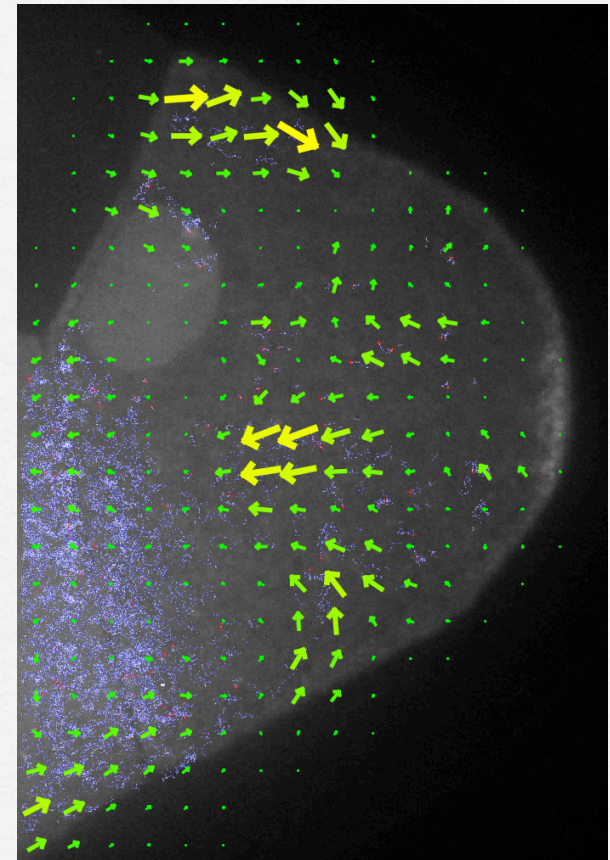


Applications

- Rabb6 tracking in *Drosophila* oocytes



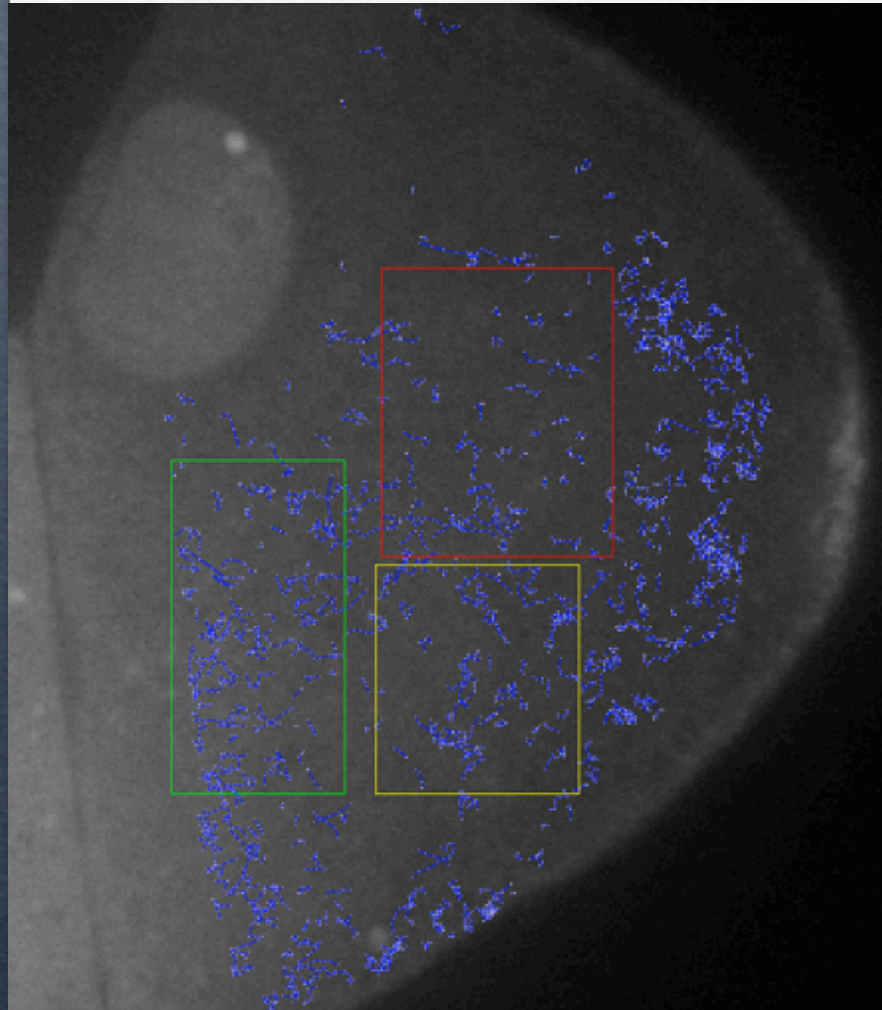
MHT tracking



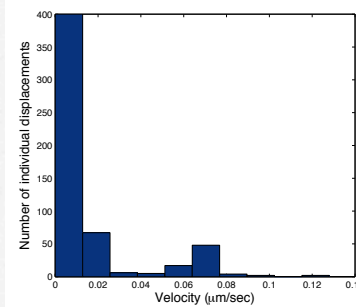
Flow visualization

Applications

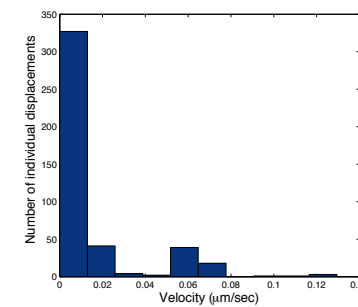
□ Jump length classification



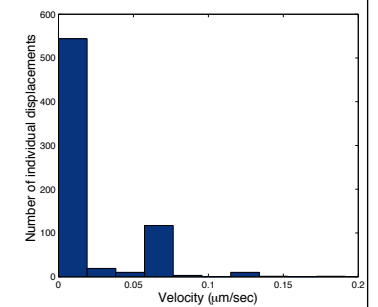
Histograms of jump length



Red area.



Yellow area.

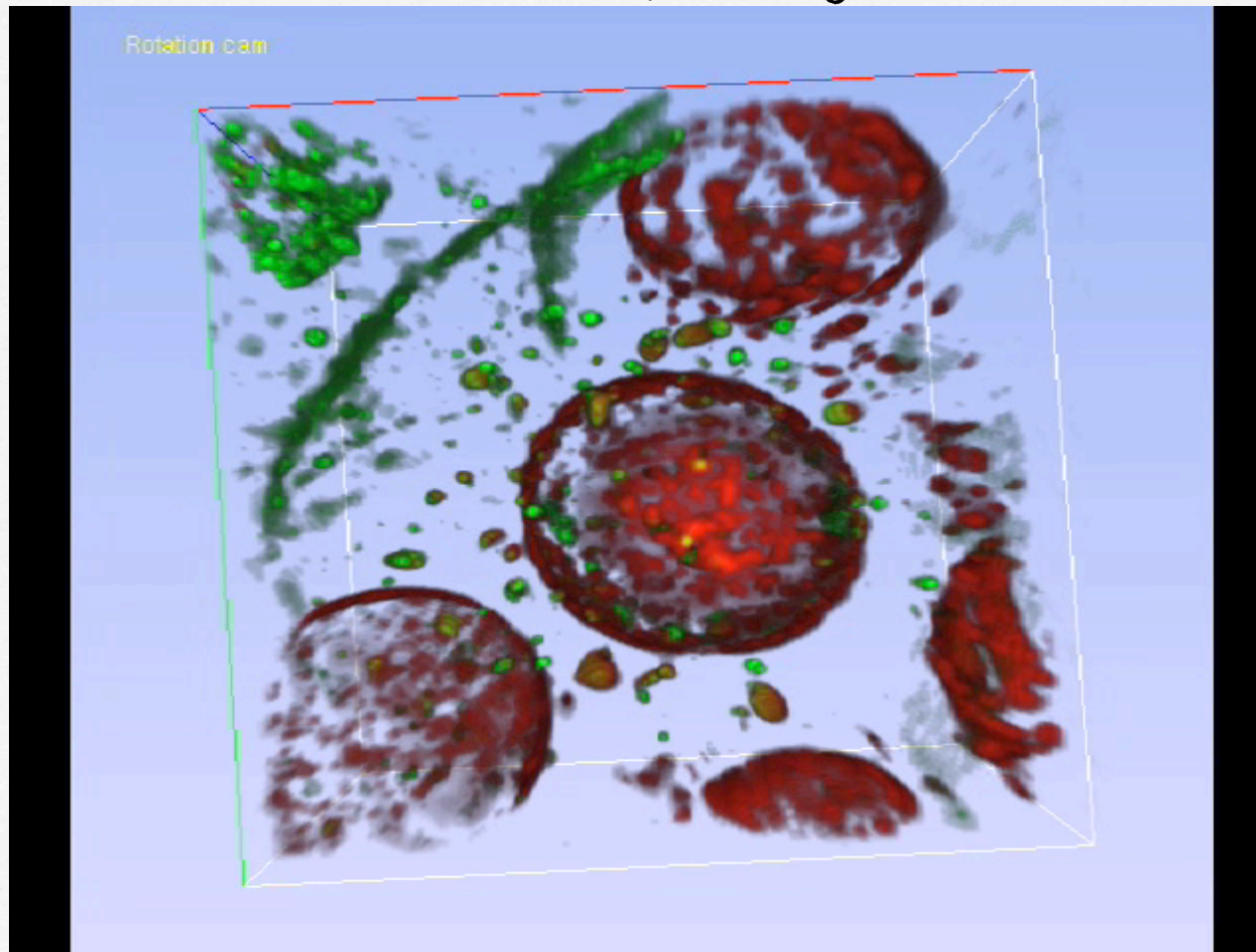


Green area.

Mainly two velocities ($0.01 \mu\text{m/s}$ and $0.06 \mu\text{m/s}$) whatever the location in the oocyte.

Applications

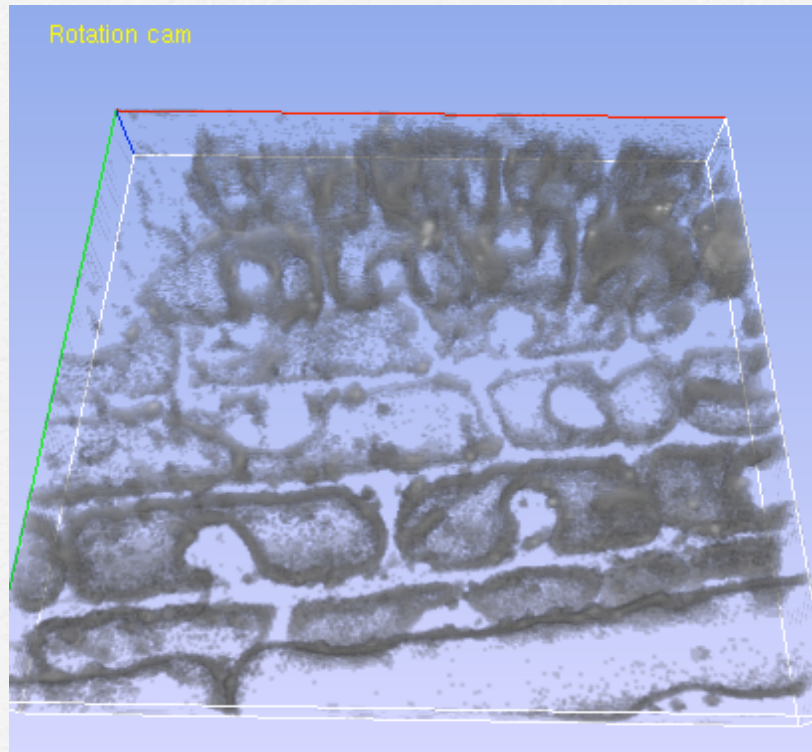
- 3D track co-localization in the *Drosophila* oocyte



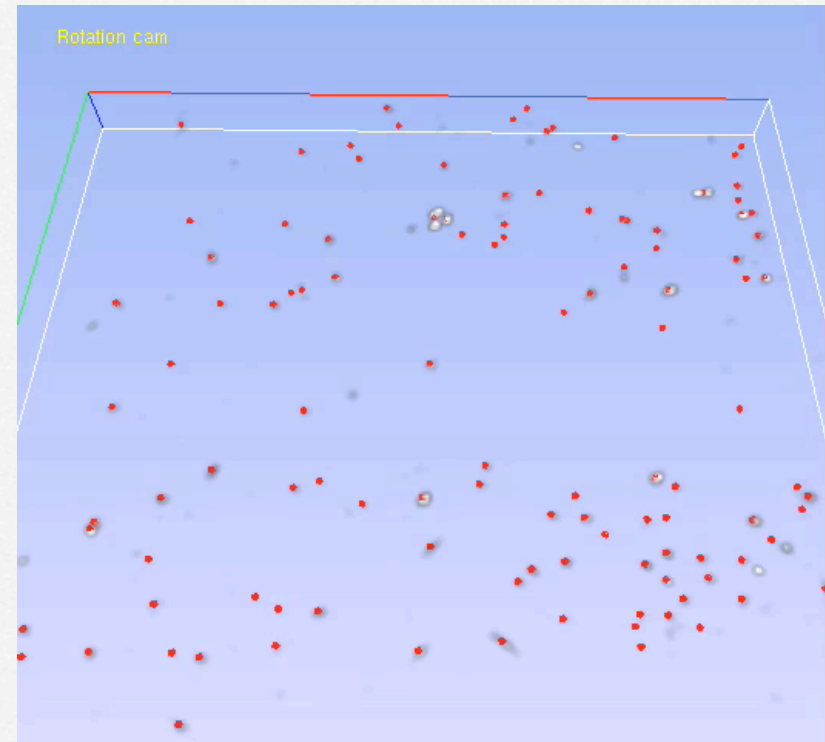
Mhlanga *et al.*, "In vivo colocalisation of oskar mRNA and trans-acting proteins revealed by quantitative imaging of *Drosophila* oocyte", *PLoS one* 2009. 40

Applications

□ Tracking KOR1 compartments in plant cells



Original 3D movie.



Particles only movie after source separation and tracking.

*Chenouard *et al.*, Morphological source separation for particle tracking in complex biological environments, ICPR, 2008.

*Chenouard *et al.*, Particle tracking in fluorescent microscopy images improved by morphological source separation, ICIP 2009

Remerciements

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 - *...*