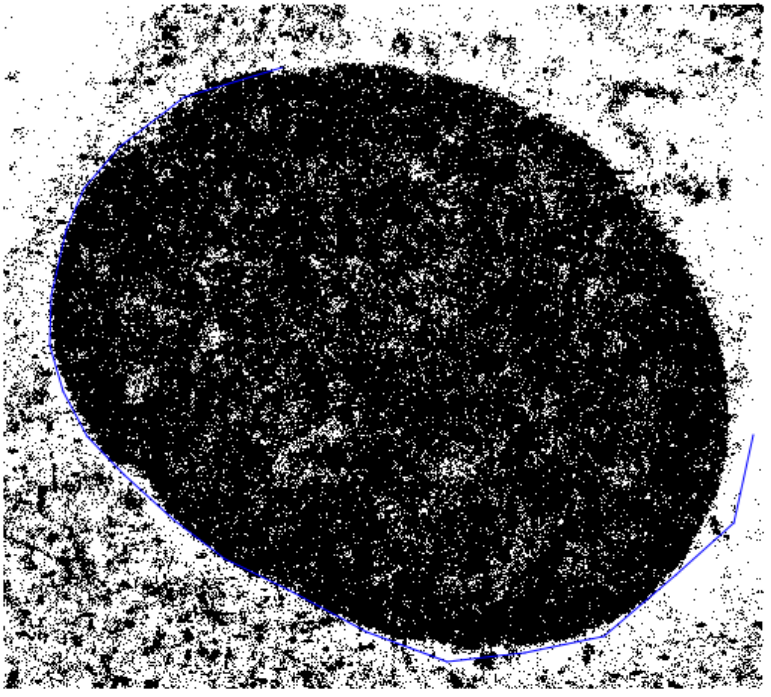
Corrélation spatiale de la chromatine et de la machinerie transcriptionnelle



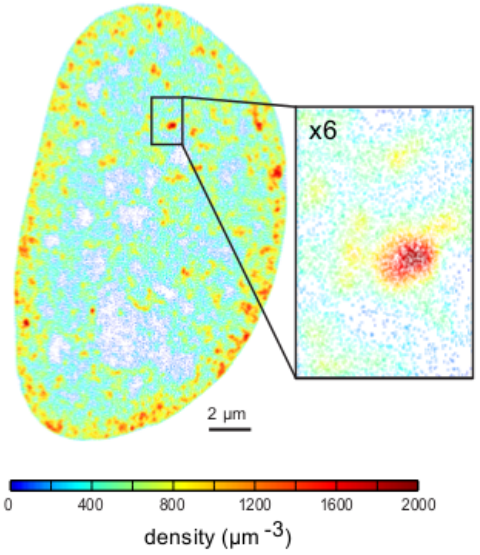
Maxime Woringer

sous la direction de :
Vincent Récamier,
Ignacio Izeddin
et Xavier Darzacq

Laboratoire d'Imagerie Fonctionnelle de la Transcription (FIT), IBENS, Paris

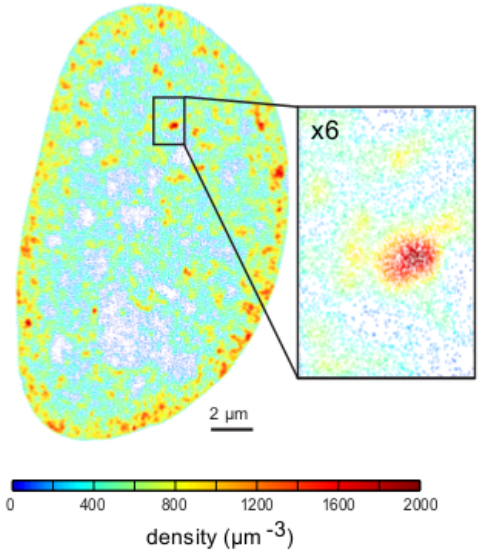
Introduction

- De l'ADN/ de la chromatine

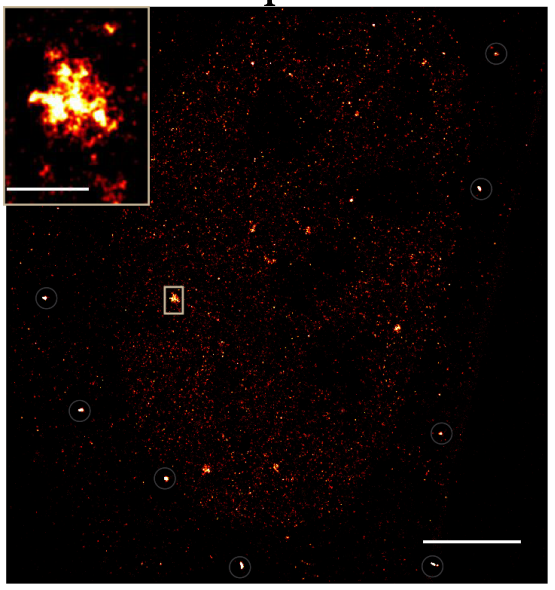


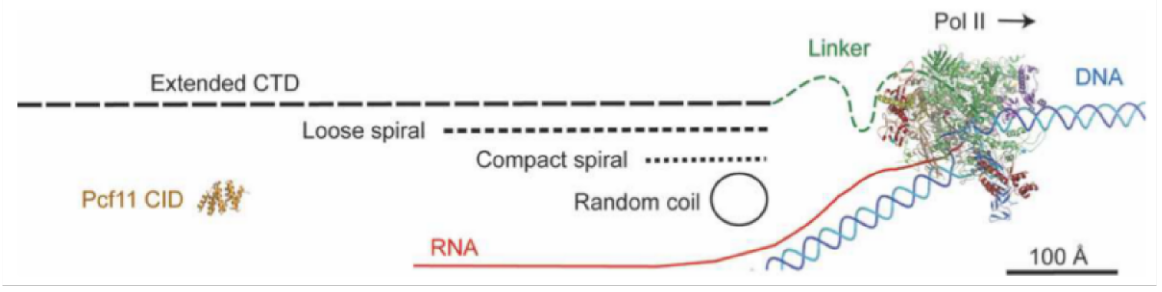
Introduction

- De l'ADN/ de la chromatine



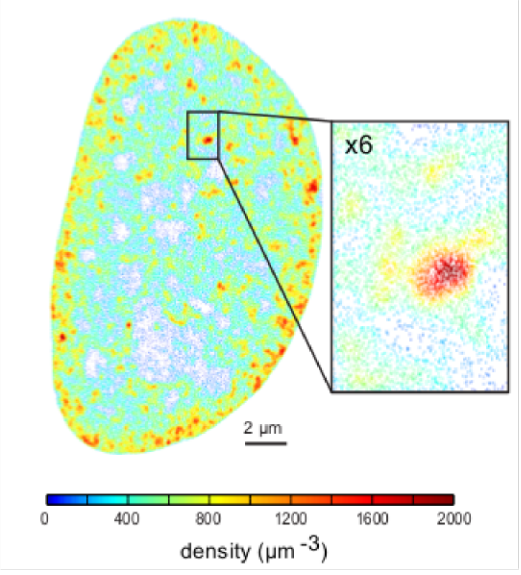
- De l'ARNpolII



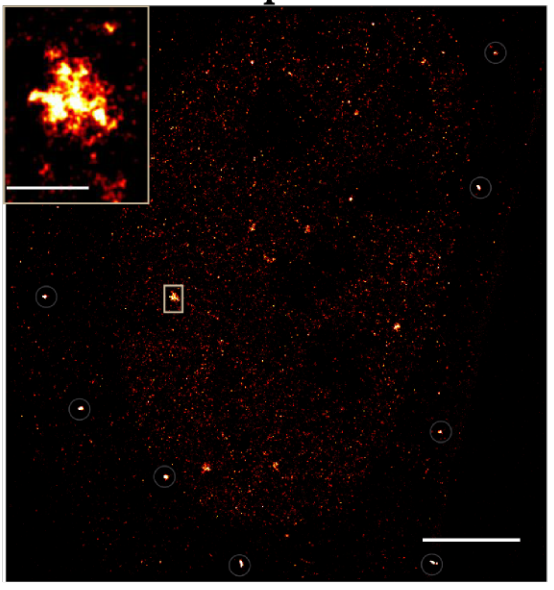


Introduction

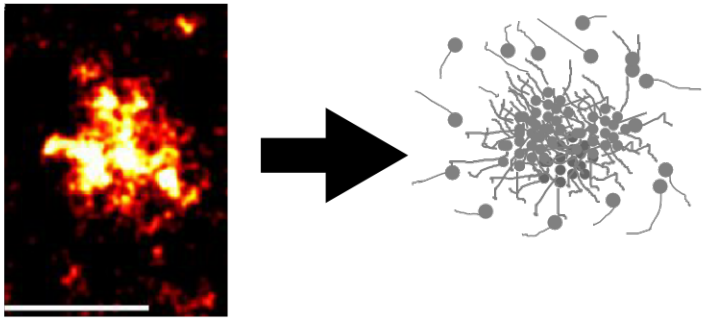
- De l'ADN/ de la chromatine



- De l'ARNpolII



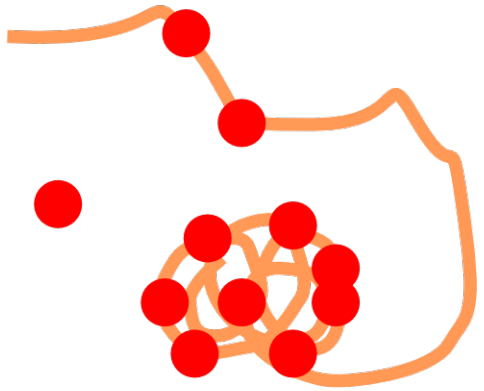
- Des réseaux



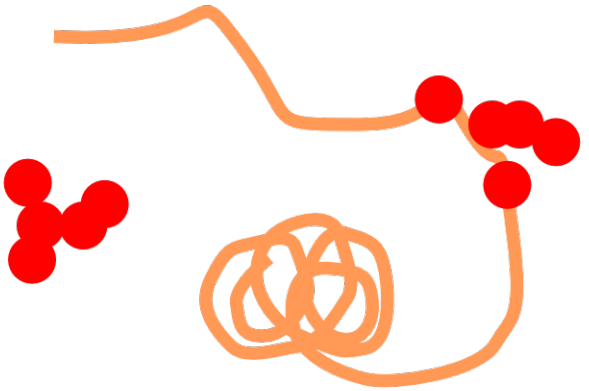
?

Corrélations spatiales

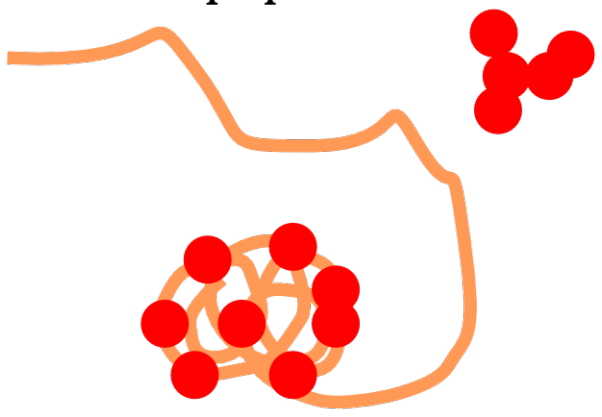
Colocalisation ?



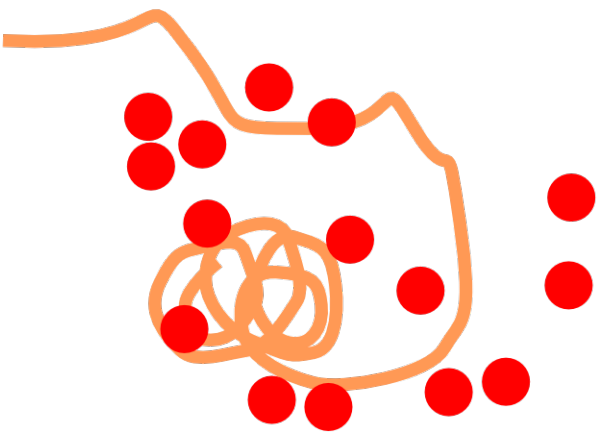
Exclusion ?



Plusieurs populations ?

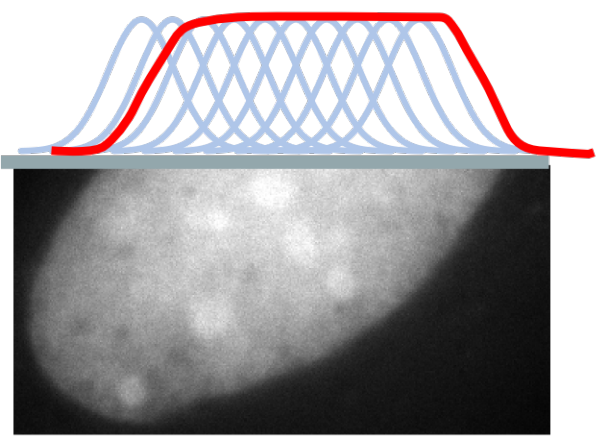


Pas de corrélation ?

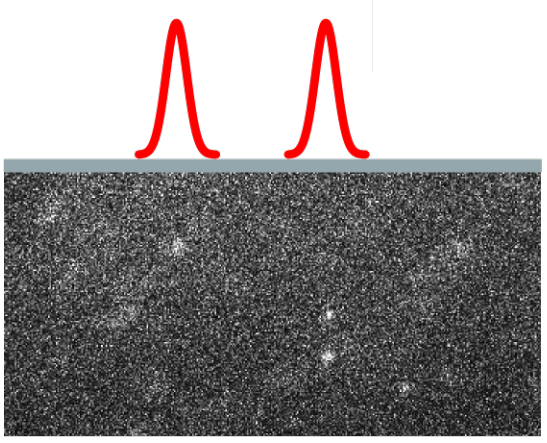


Superrésolution

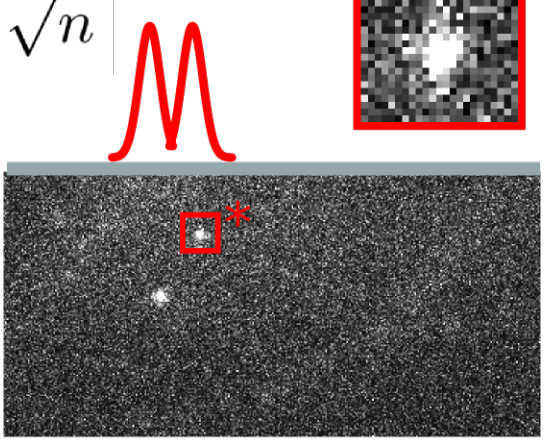
$$SE = \frac{\sigma}{\sqrt{n}}$$



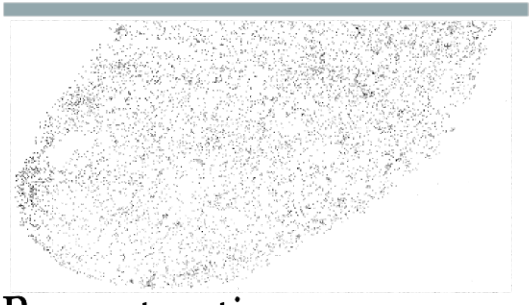
Imagerie traditionnelle



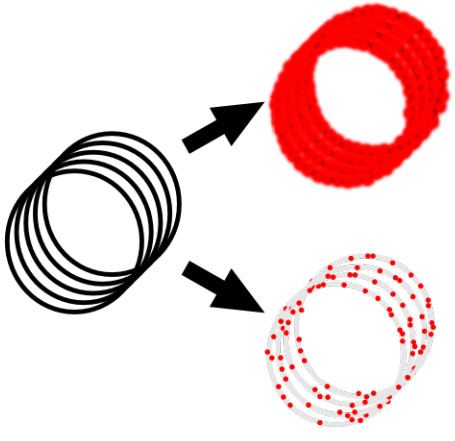
Images du film



Superrésolution



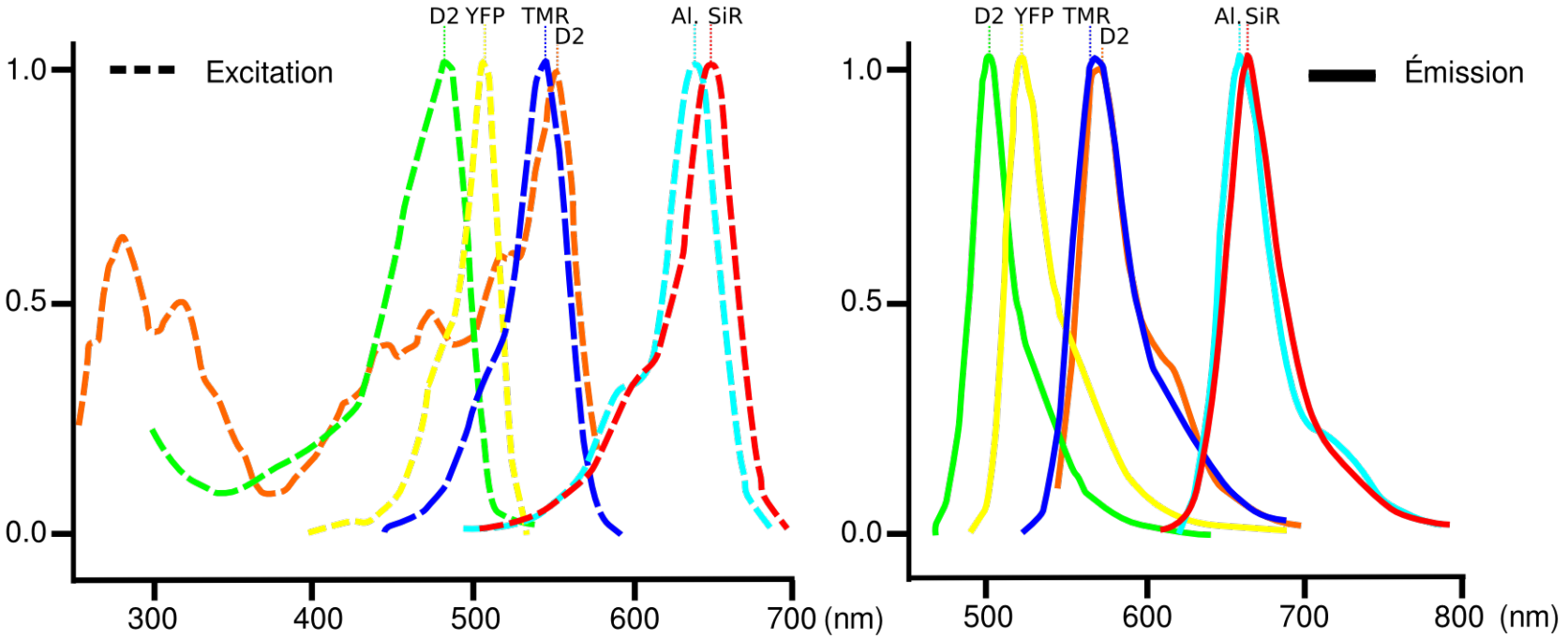
Reconstruction



PALM
- fluorophore protéique
- faible nombre de photons
- marquage de toutes les protéines d'intérêt

STORM
- fluorophore organique
- marquage chimique limité
- Grande photostabilité
- photoblanchiment majeur avant l'observation

Choix des fluorophores



- Dendra2 (non activée) et pa-GFP (*D2*)
- Silicon-Rhodamin (*SiR*)
- YFP
- Dendra2 (photoconvertie)
- TMR
- Alexa 647 (*Al.*)

Protocole

Culture des cellules

Transfection

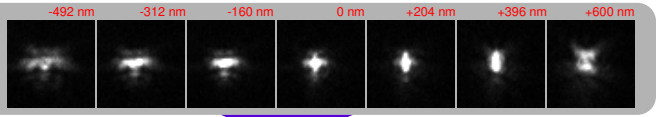
± Fixation

Marquage

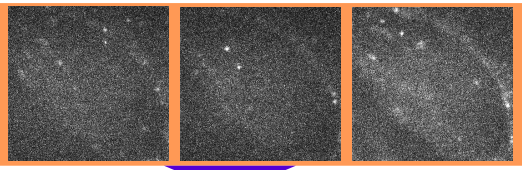
Calibration du front d'onde

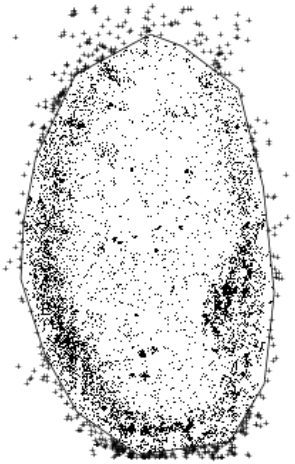
Calibration de l'image

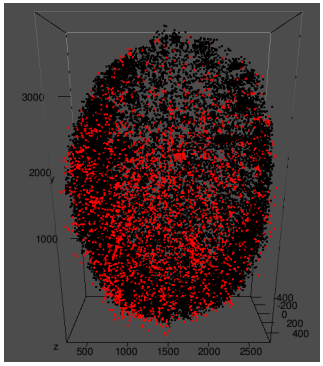
"PSF shaping"



Acquisitions

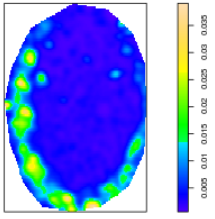






Détection des PSF

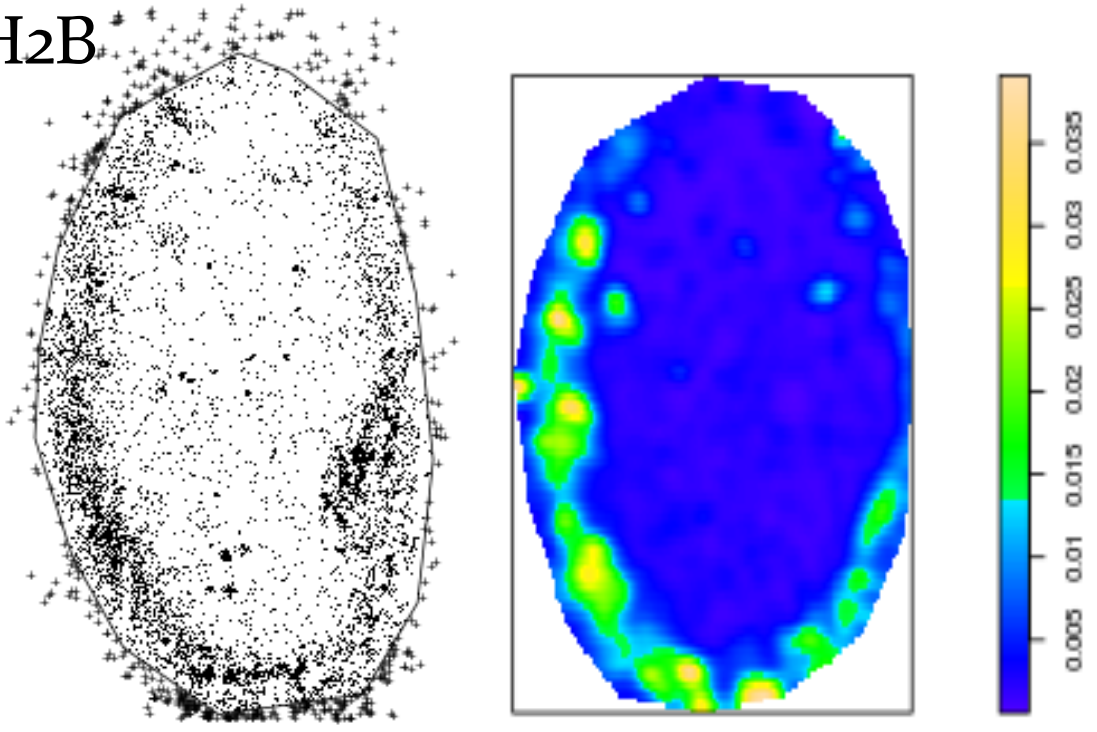
Calibration en z

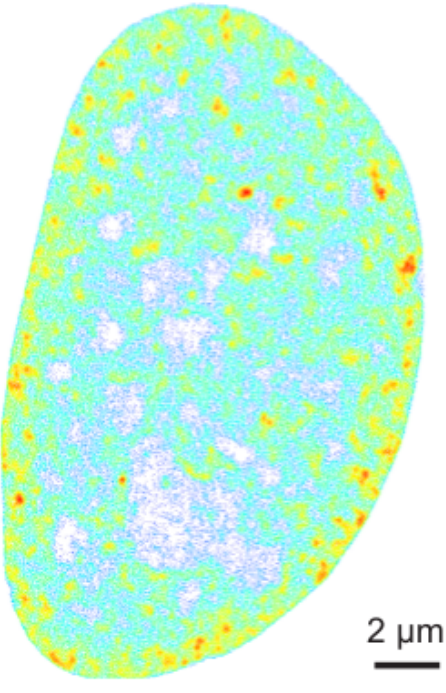


Analyses

Qualité des acquisitions

H2B



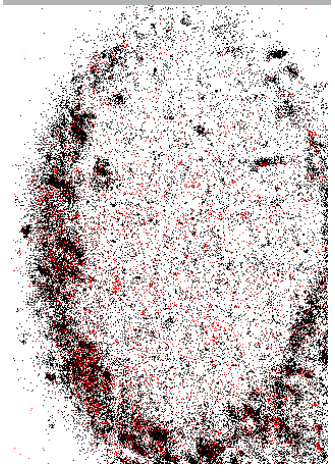


- ~30000 points
- ~10% hors de la cellule
- Qualité beaucoup plus faible pour PolII (5000 points dont beaucoup de détections multiples).

Récamier et al., soumis

Analyses

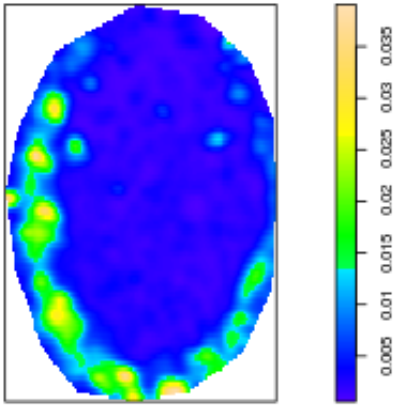
Détections



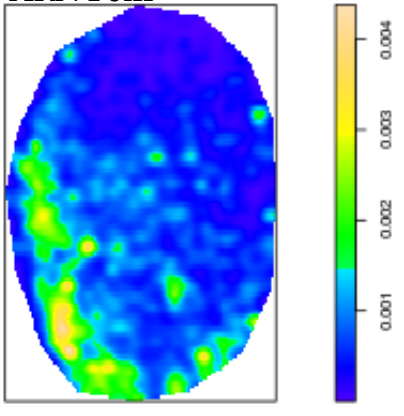
- H2B
- ARN PolII

Densité (à 500 nm)

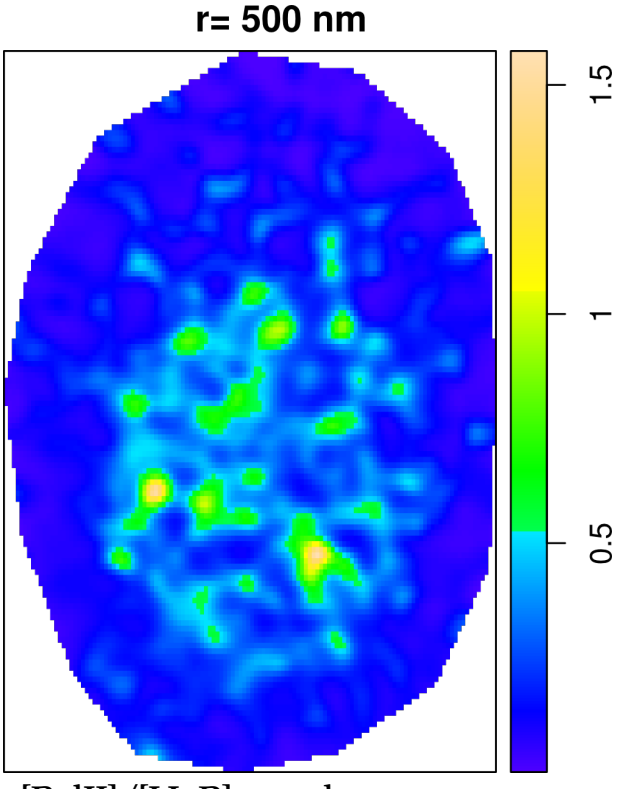
H2B



ARN PolII



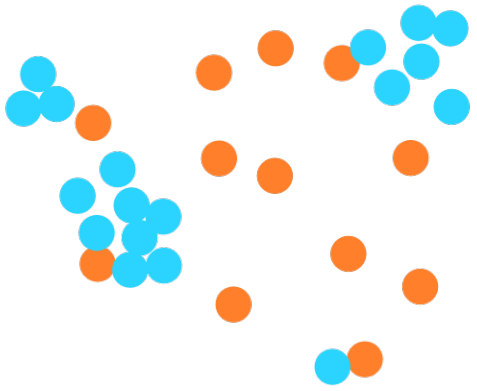
Abondance relative

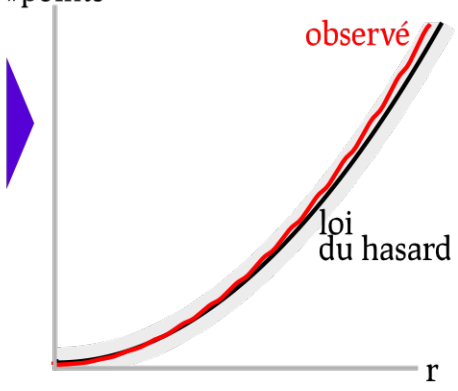


[PolII]/[H2B], pas de 500 nm

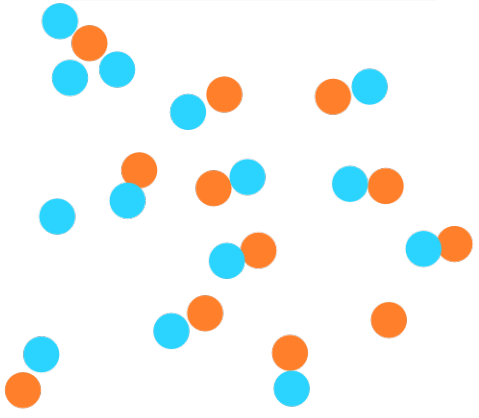
Analyses

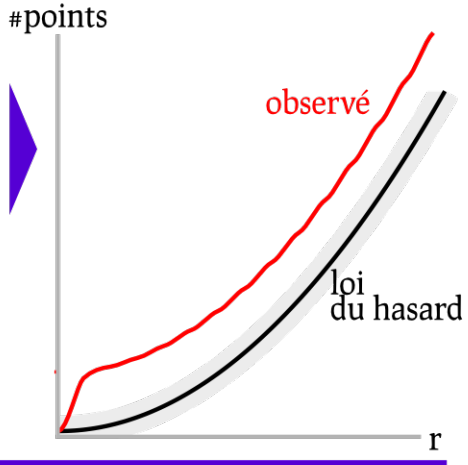
CSR (complete spatial randomness)



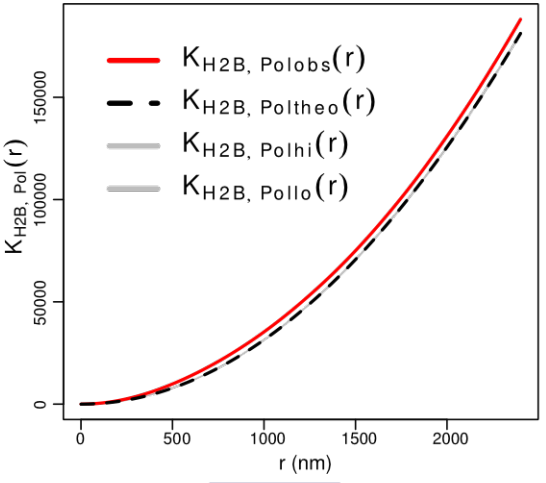


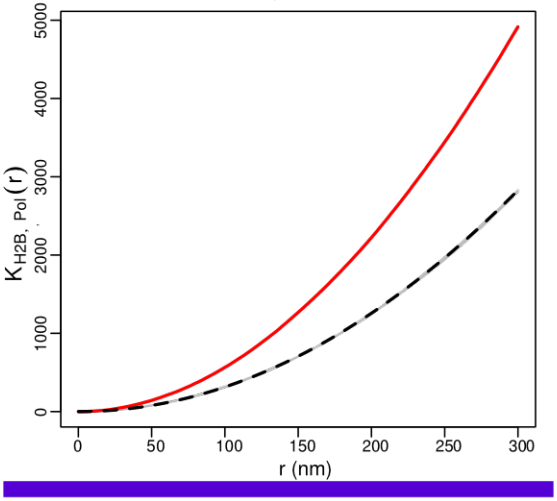
Colocalisation





Principe





Observations

Conclusion

Expérimental

Faisabilité du PALM/STORM en deux couleurs sur le modèle H2B/PolII,

Faible qualité des données acquises,

Nombreuses pistes prometteuses d'amélioration.

Théorique

On dispose des outils théoriques pour traiter les données,

Les indicateurs déterminés sont calculables en un temps raisonnable (~ 1 jour).

Catalyse hétérogène

Surface spécifique immense de la chromatine (fractale) : *adsorption facilitée*

Dimensionnalité réduite de la chromatine : *surface réactive*

Fortes connexions avec la polymérase (?) : *convergence des facteurs de transcription.*