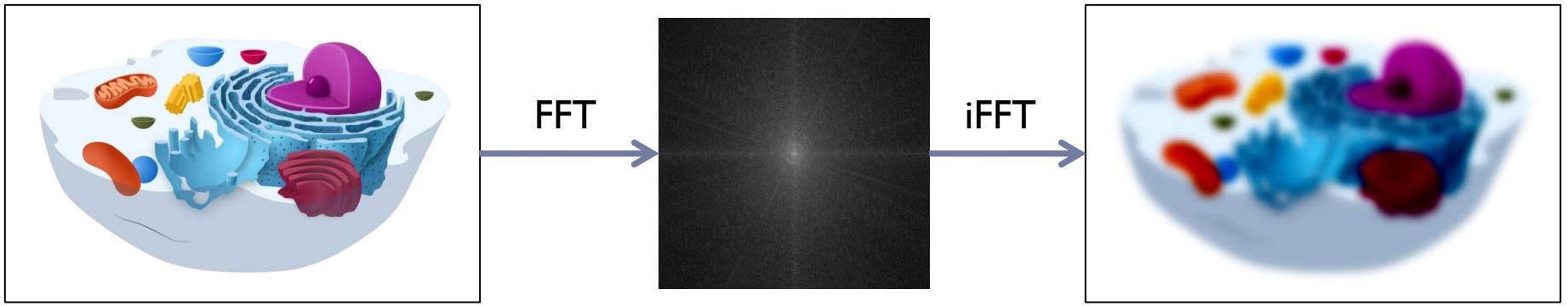
# R tools for spatial point pattern analysis applied to fluorescence localization nanoscopy

Julien Godet – MCU-PH

LBP UMR CNRS 7213 – Université de Strasbourg

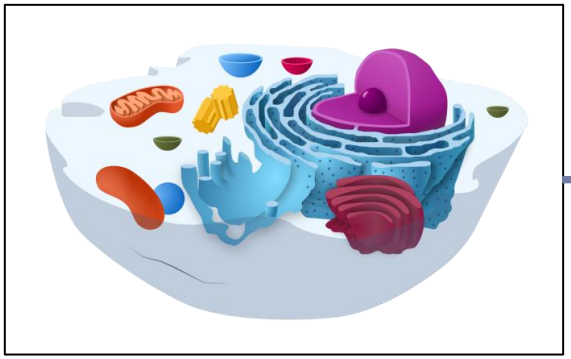
# Microscope: a resolution-limited instrument




object

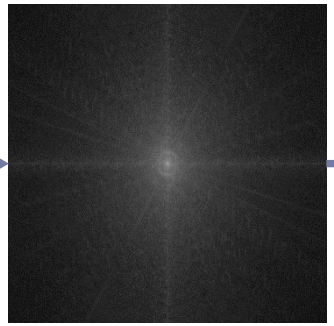
Blurry image

# Microscope: a resolution-limited instrument

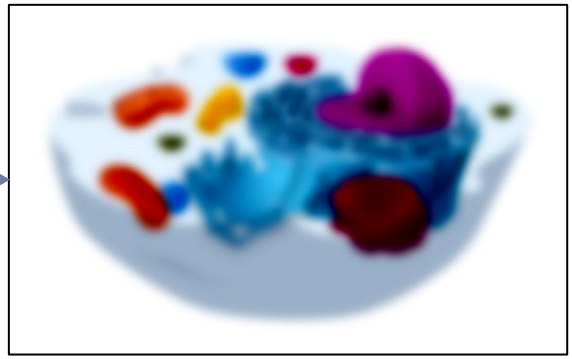


object

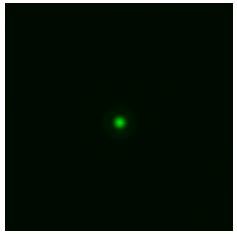
FFT



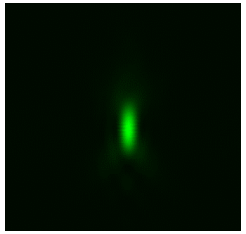
iFFT



Blurry image



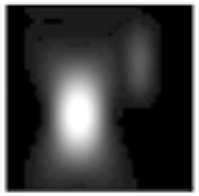
Point object



Point spread function (PSF)

Object

PSF



Image

$$d = \frac{\lambda}{2\,n\,\sin\alpha} \simeq \frac{\lambda}{2\,\mathrm{NA}}$$

# Beyond the diffraction limit

## Super-resolution techniques…

Deterministic functional techniques

- Stimulated Emission Depletion Microscopy STED
- Ground State Depletion GSD
- Saturated Structured Illumination Microscopy SSIM

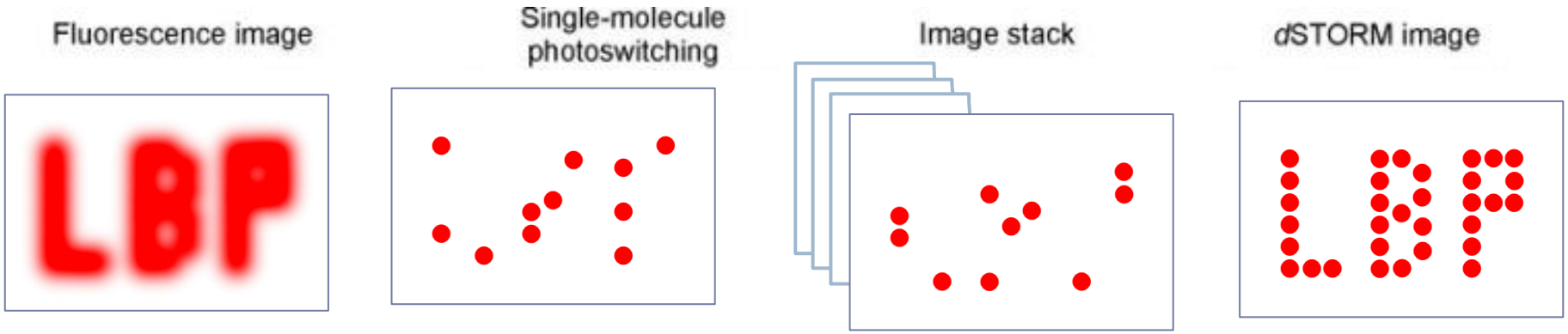
Stochastic functional techniques

- Photo-Activated Localization Microscopy PALM
- Stochastic Optical Reconstruction Microscopy STORM

… some among many

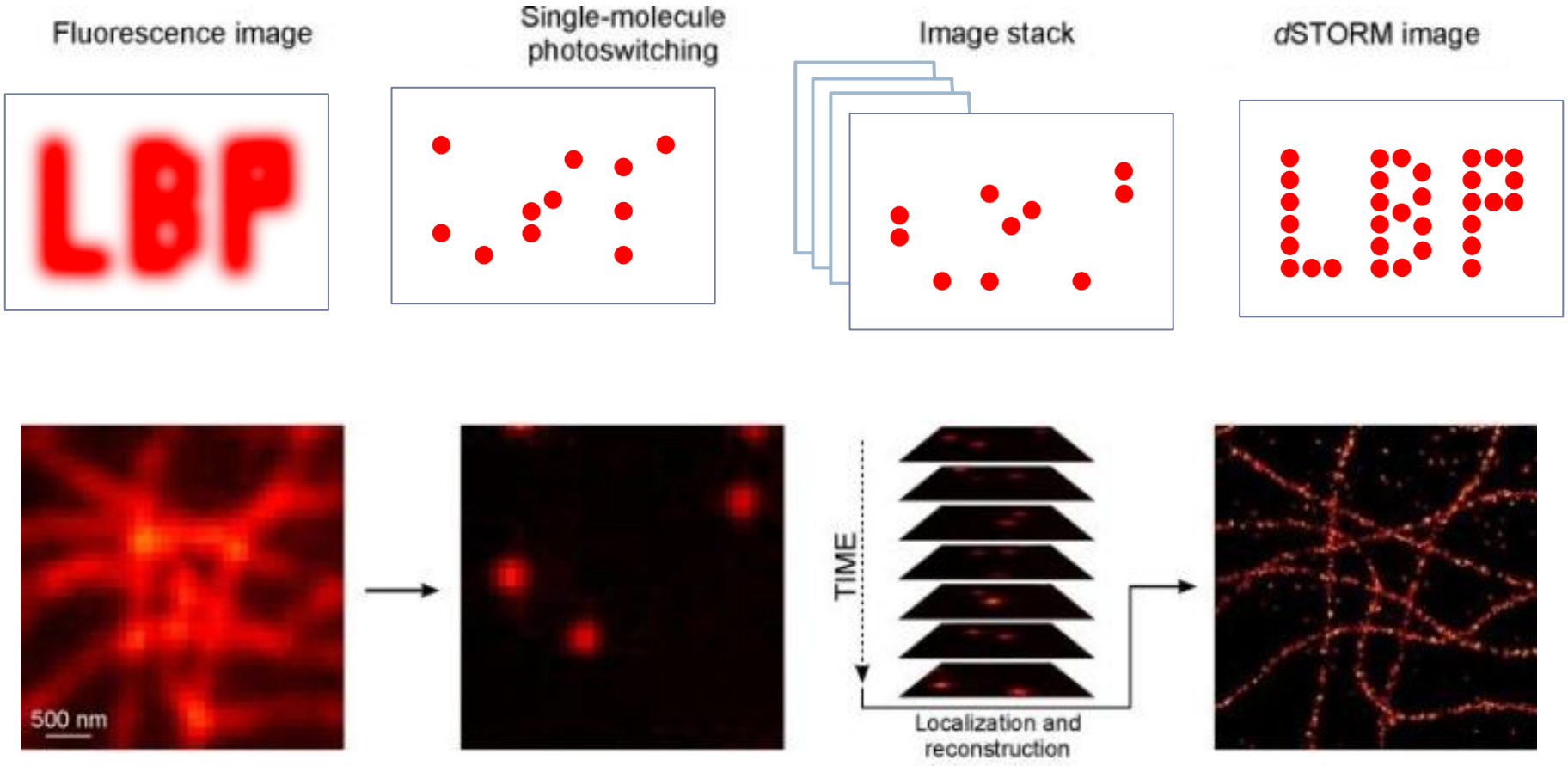
# Localization Microscopy: STORM principle

## STORM: Stochastic optical reconstruction microscopy

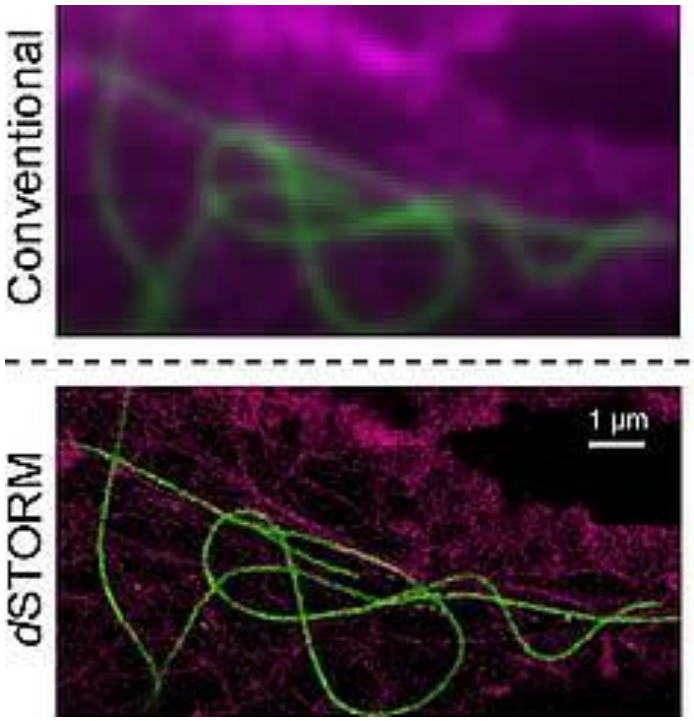


# Localization Microscopy: STORM principle

## STORM: Stochastic optical reconstruction microscopy

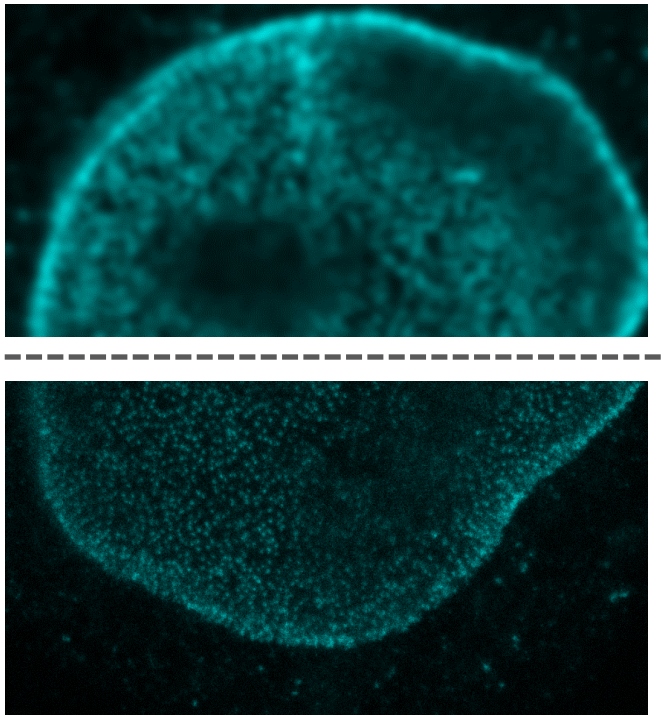


# STORM – examples of reconstructed images



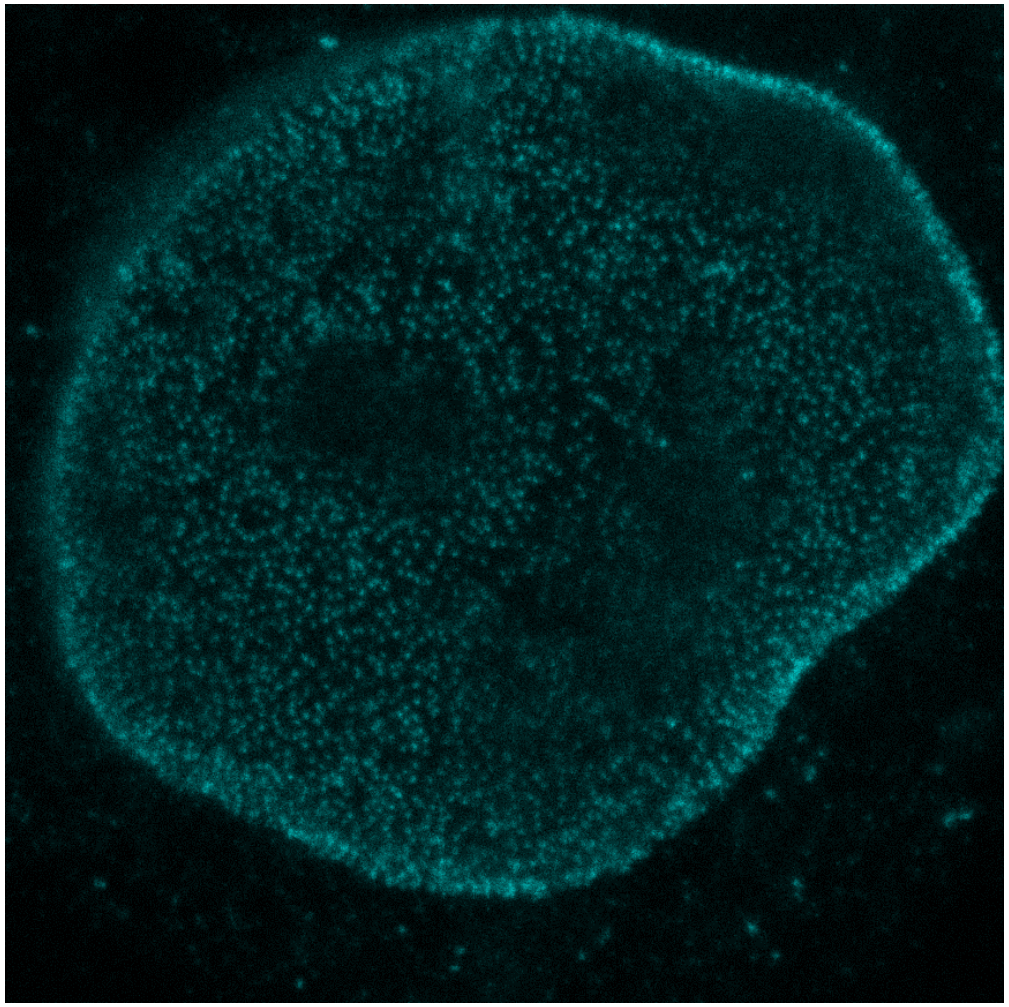

van de Linde et al. 2013

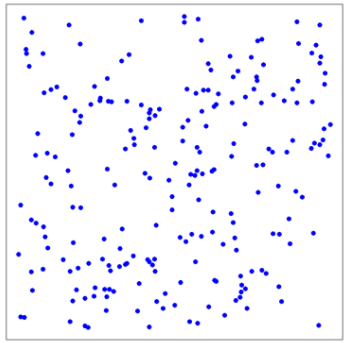
Actin (purple) and Tubulin (Green)-Cellular network



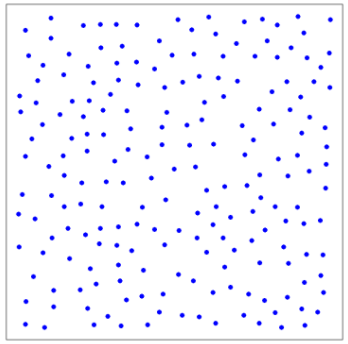
Nuclear Pores (Nup)

# How are the Nup spatially distributed?

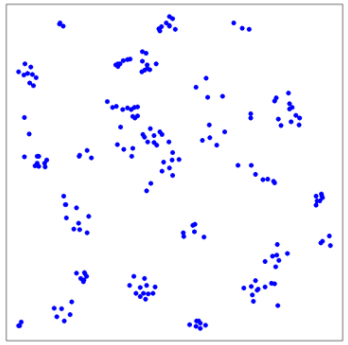




Randomly?
Independently distributed
Poisson process



Regularly?



In clusters?

# Spatial Point Pattern: Ripley's K function

```
> envelope(Y, fun=Kest,…) # envelope{spatstat}
```

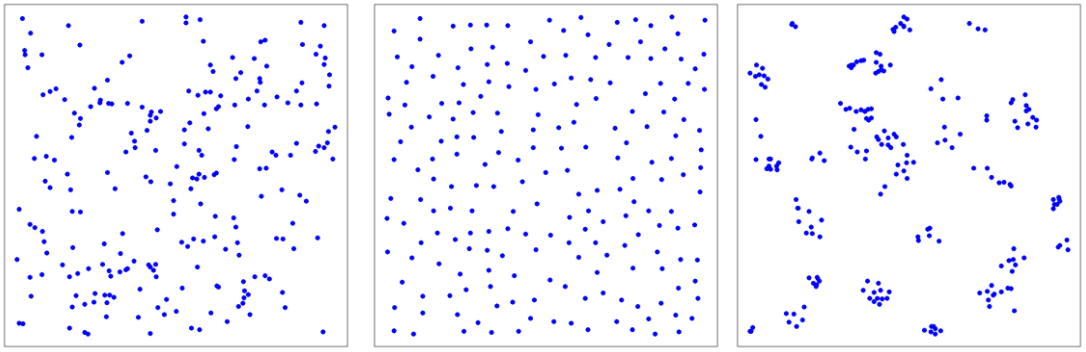
Complete Spatial Randomness (CSR)

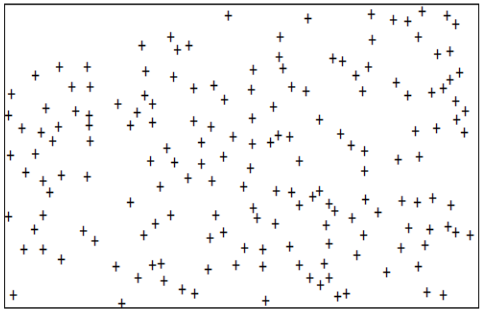
Regular

Clustered

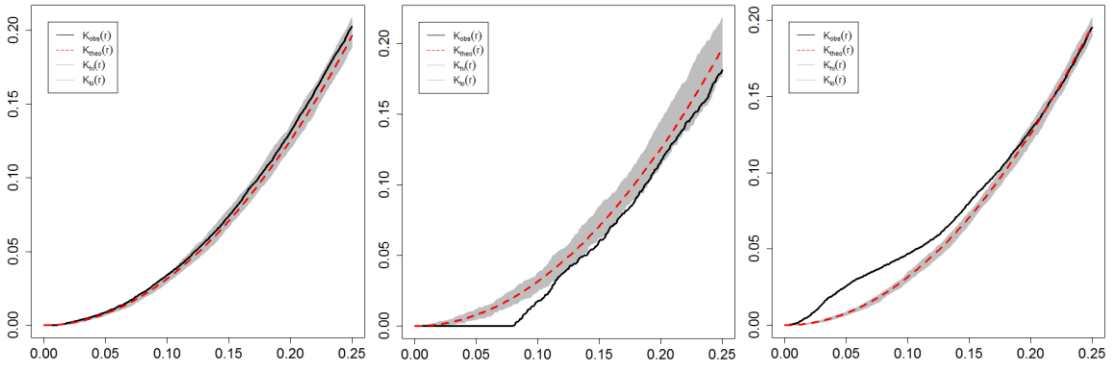
Nup localization

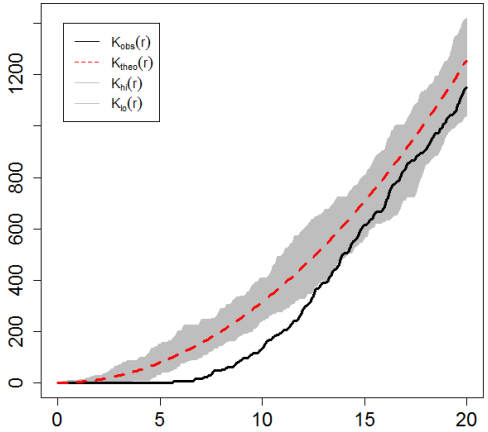
Pattern



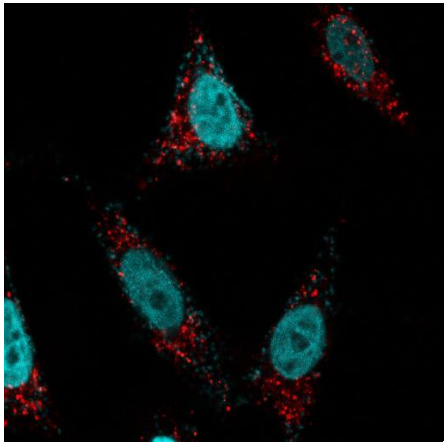


K function



# Smoothing estimate of the intensity of a point process as a function of a covariate



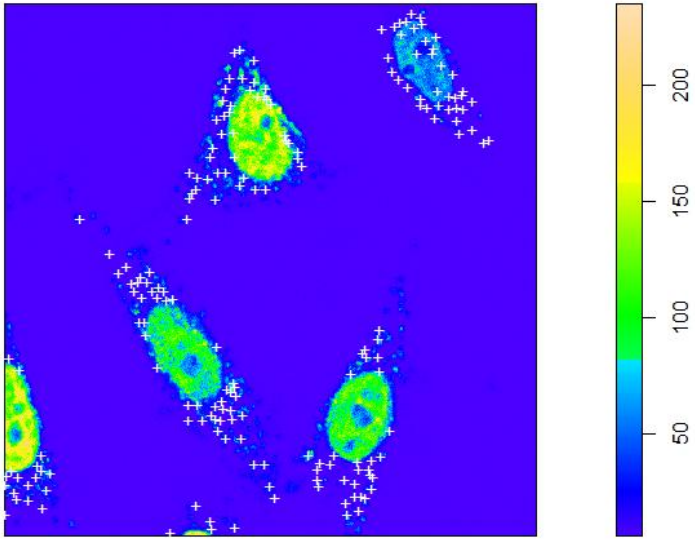
Relative distribution estimate $\lambda(u) = \rho(Z(u))$

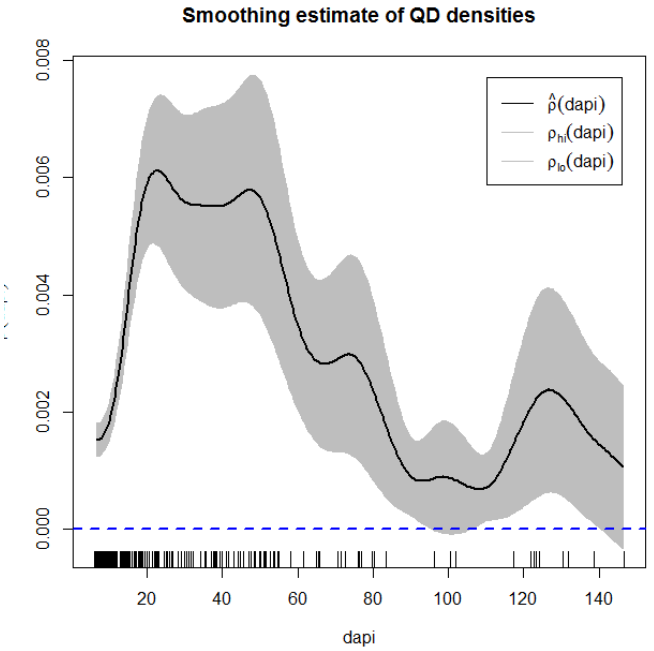
```
> rhohat(object, covariate, ...) #rhohat{spatstat}
```

Rho is the function describing how the intensity of points depends on the value of the covariate

Extract
- Points coordinates
- Covariate map

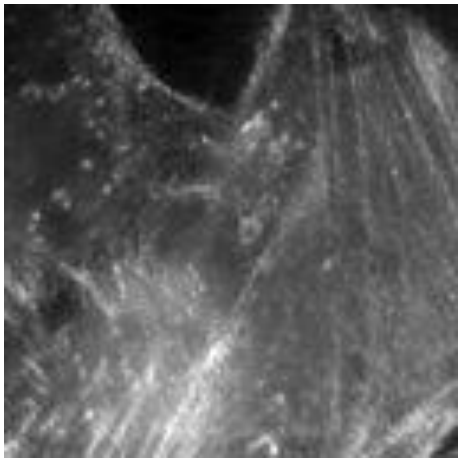
Import in R



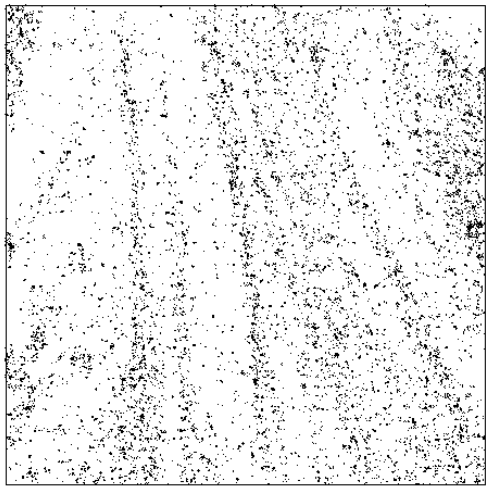



# Point patterns filtering: Choi-Hall sharpening

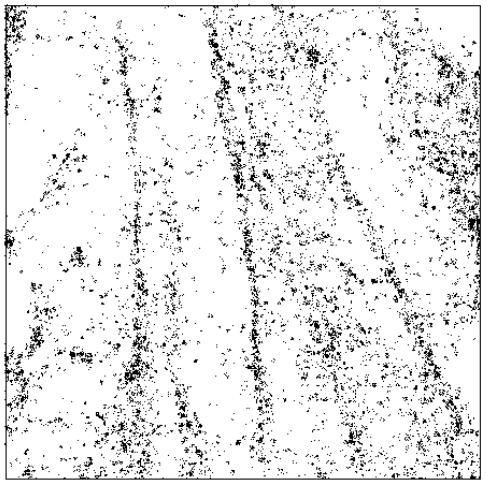
```
> sharpen(X, ...) #sharpen {spatstat}
```



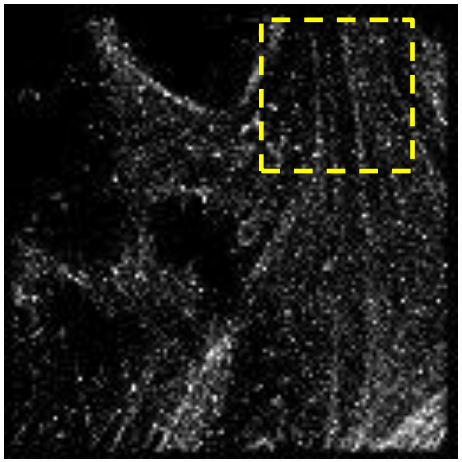
Classical image



Raw STORM image



'Sharpened' STORM image



STORM image: 1.2 $10^6$ points

Point patterns are believed to exhibit strong concentrations of points along the 'real' fiber

If the original data points are X[1],..., X[n], the new point X^[i] is a vector average of the nearby points X[j].

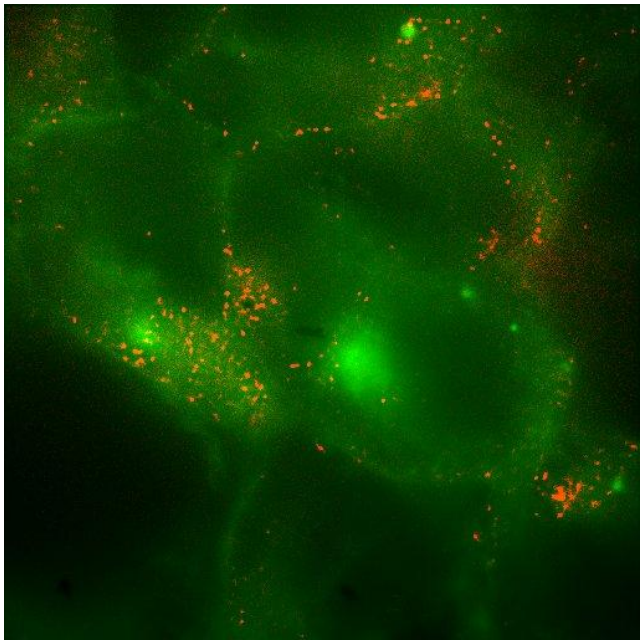
Data sharpening causes points to concentrate more tightly along the fibers.

# Point filtering: Nearest-neighbour cleaning

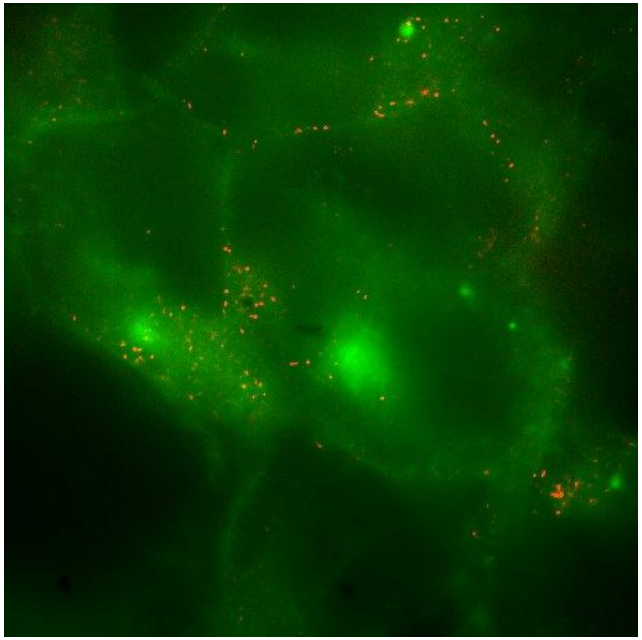
```
> nnclean(X, k, ...) #nnclean{spatstat}
```

Recognising features in a spatial point pattern in the presence of random clutter.

Group points into two classes 'feature' and 'noise' on the basis of their nearest-neighbour distances



Composite image
Raw STORM image (red) – Cy5NPY ligand
Classical TIRF image (green) – eGFPY1



Composite image
'Filtered' STORM image (red) – Cy5NPY ligand
Classical TIRF image (green) – eGFPY1

# Conclusion & Perspectives

## Why to use R for localization microscopy?

- Easy to use
- Extensive collection of tools for spatial point pattern analysis
- Functions available for 3D PPP
- Useful for - reconstructed-image analysis
  - image reconstruction (filtering)

Limitations

- Poor image processing capabilities

Perspectives

- Coupling R and Image J (Bio7 or equivalent projects)
- Real time analysis (optimize acquisition)