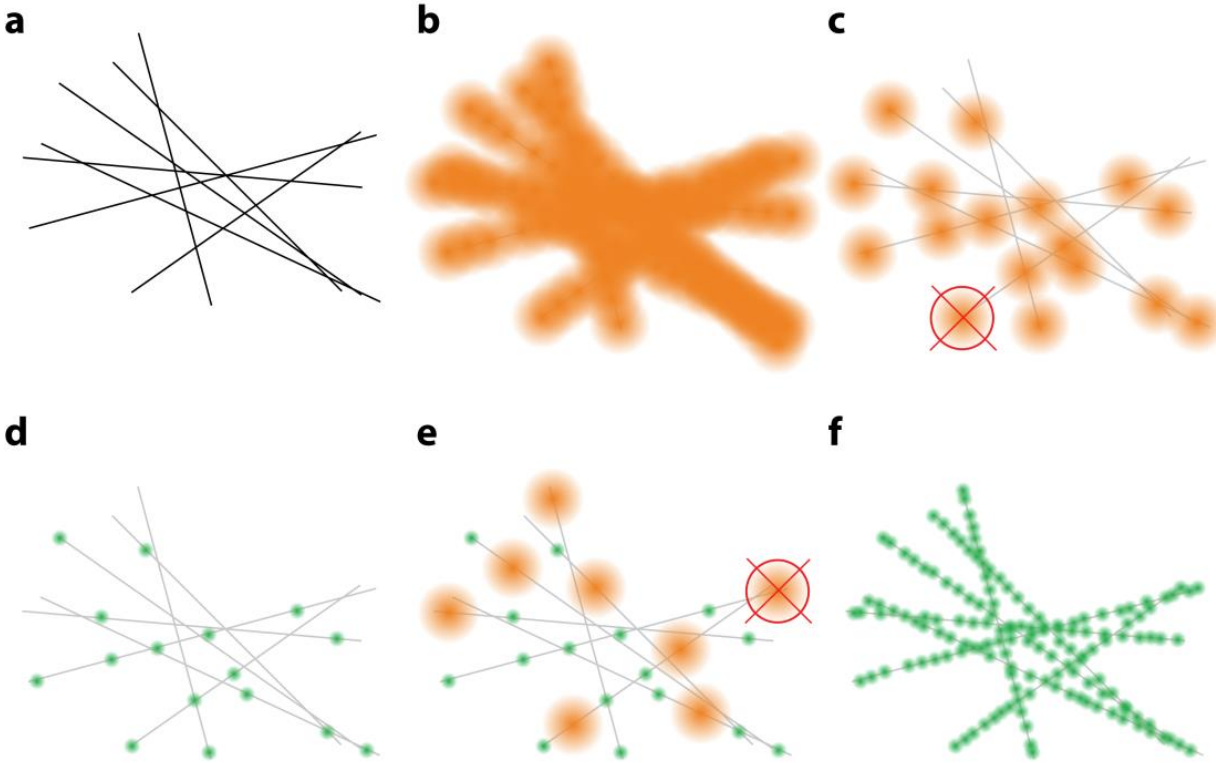
PALM et STORM

autres approches de super-résolution basées sur la modulation du fluorophore

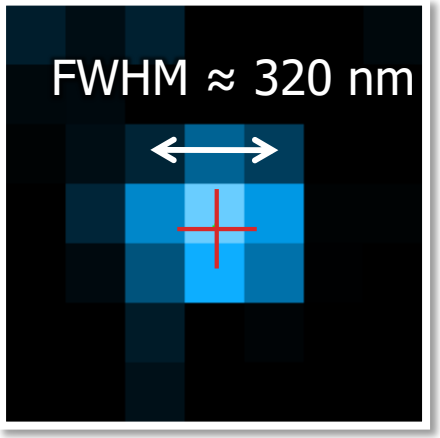


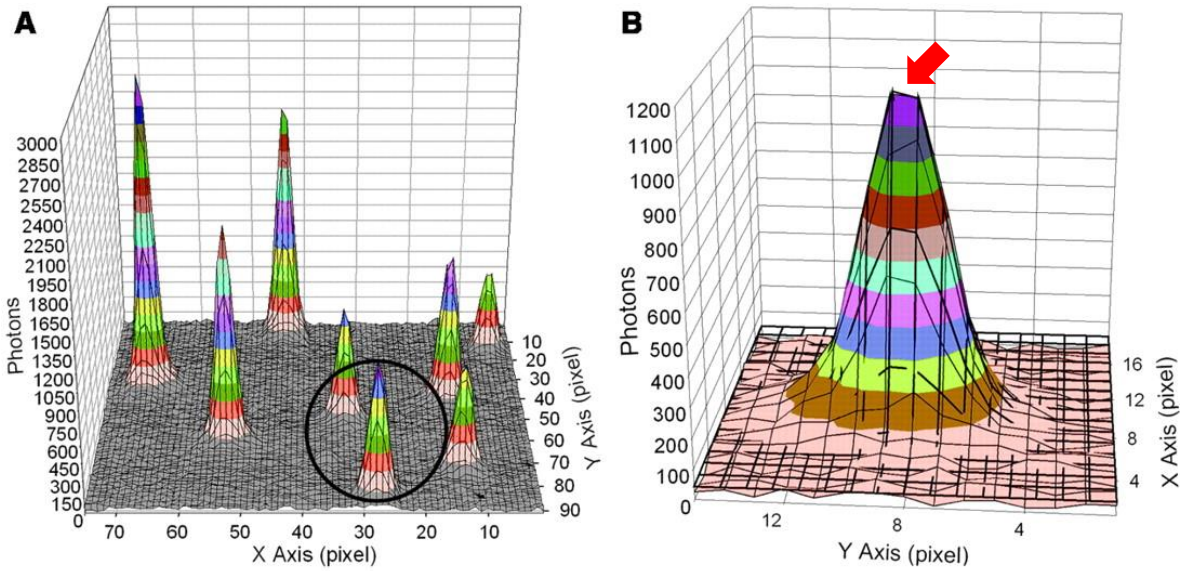
Jost A, Heintzmann R. 2013.
Annu. Rev. Mater. Res. 43:261–82

Principles of PALM and STORM. (*a*) Sample consisting of many point sources. (*b*) Simultaneous emission of all the fluorescent markers. (*c*) When photoactivatable (PALM) or photoswitchable (STORM) dyes are used, only very few photons will emit light in a given frame. The images of the individual points will therefore be sparse. A Gaussian fit (*red circle*) is applied to each of these single-molecule images. The center (*red cross*) corresponds to the most probable position of the initial point source. (*d*) Location map after processing of the single frame shown in panel *c*. The small green dots represent the estimation of the position with nanometer precision. (*e*) Another camera frame in which another set of fluorophores are shown. The processing procedure is repeated frame by frame. (*f*) After acquisition and processing of many frames, structural information about the sample is pointillistically reconstructed at a much better resolution.

Localisation d'une molécule

Image d'une molécule fluorescente

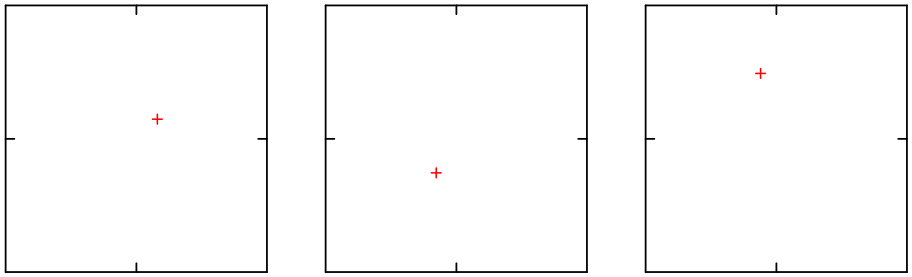




Ahmet Yildiz et al. Science 2003;300:2061-2065

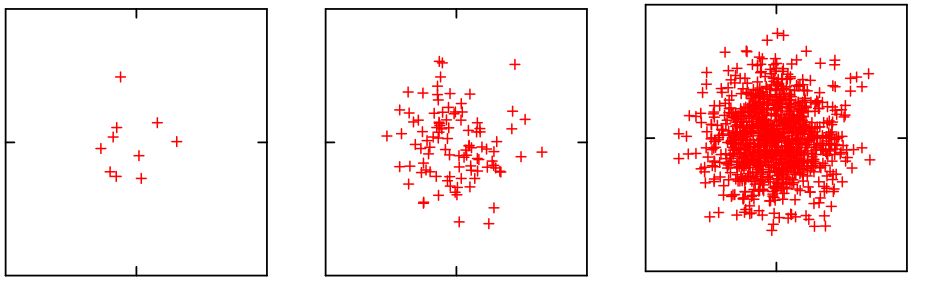
PSF with 0.5-s integration time of several individual Cy3-dyes attached to a coverslip.

Précision de la méthode

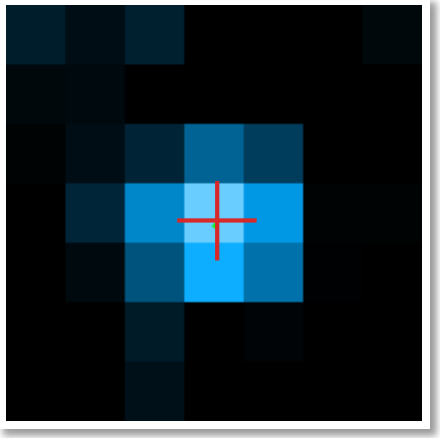


1 photon

$$d \approx \frac{\lambda}{2\,NA}$$



10 photons 100 photons 1000 photons

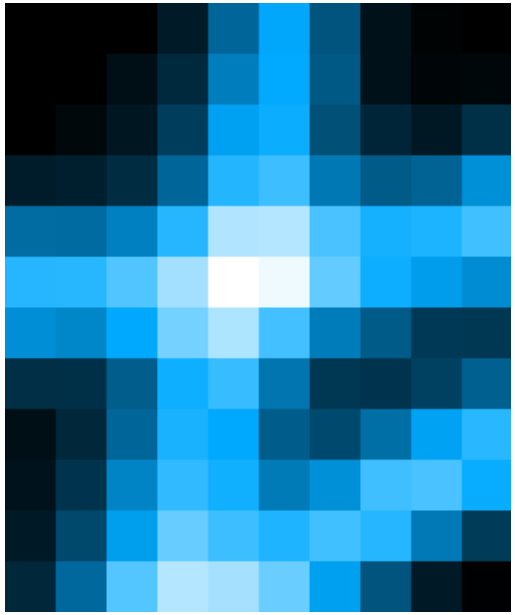


$$d = \frac{1}{\sqrt{N}} \cdot \frac{\lambda}{2NA}$$

http://nic.ucsf.edu/dokuwiki/lib/exe/fetch.php?media=qb3_2013:super-resolution_optical_microscopy.pdf

Imagerie de super-résolution par localisation

Fluorescence conventionelle



Images brutes

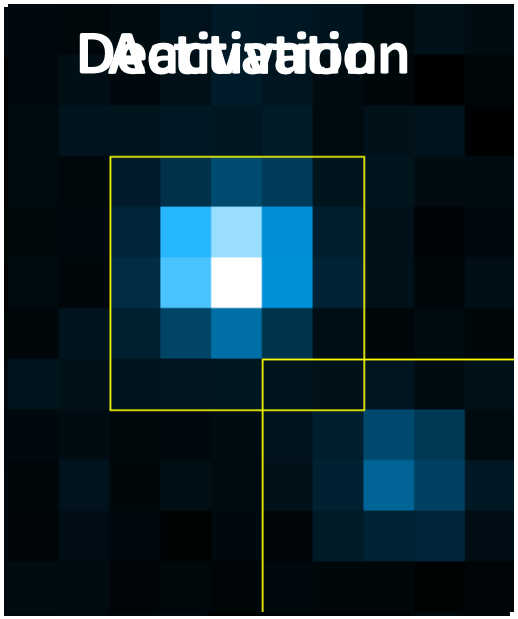
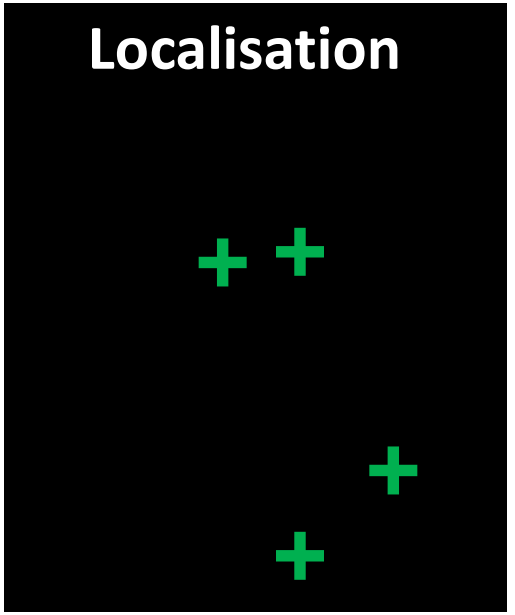


Image STORM



Stochastic Optical Reconstruction Microscopy = STORM

Also named as PALM (Betzig et al., Science, 2006) and FPALM (Hess et al., Biophys. J. 2006)

Huang et al., Annu Rev Biochem, 2009

http://nic.ucsf.edu/dokuwiki/lib/exe/fetch.php?media=qb3_2013:super-resolution_optical_microscopy.pdf