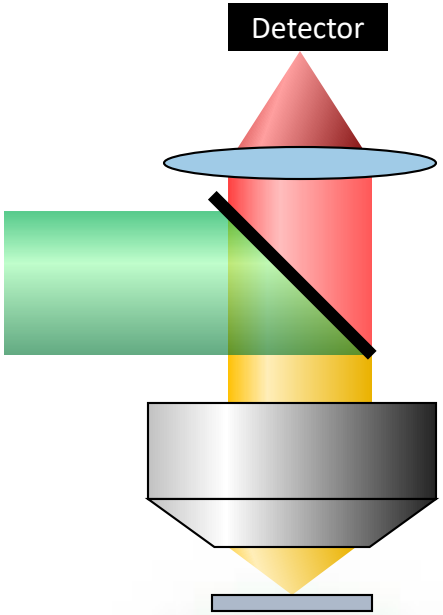
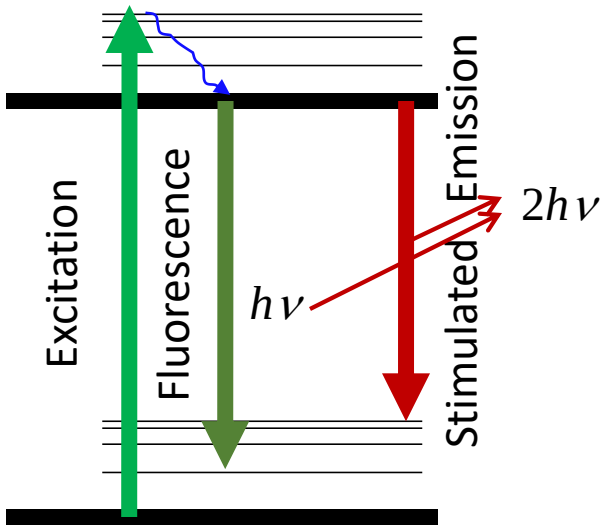
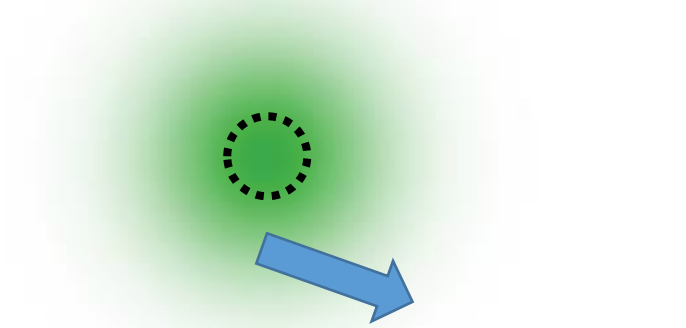
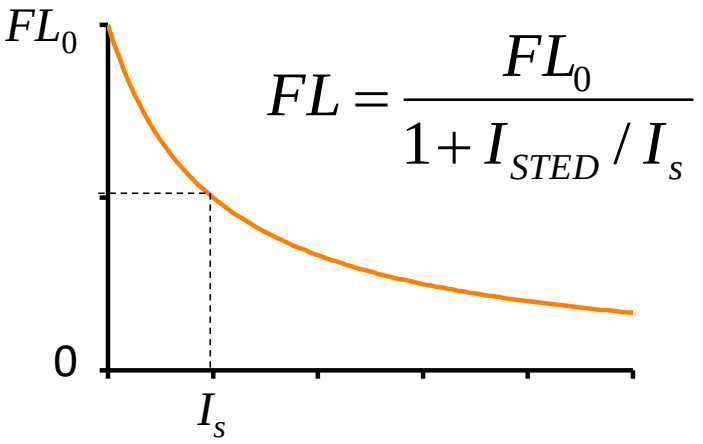
Stimulated Emission Depletion (STED)



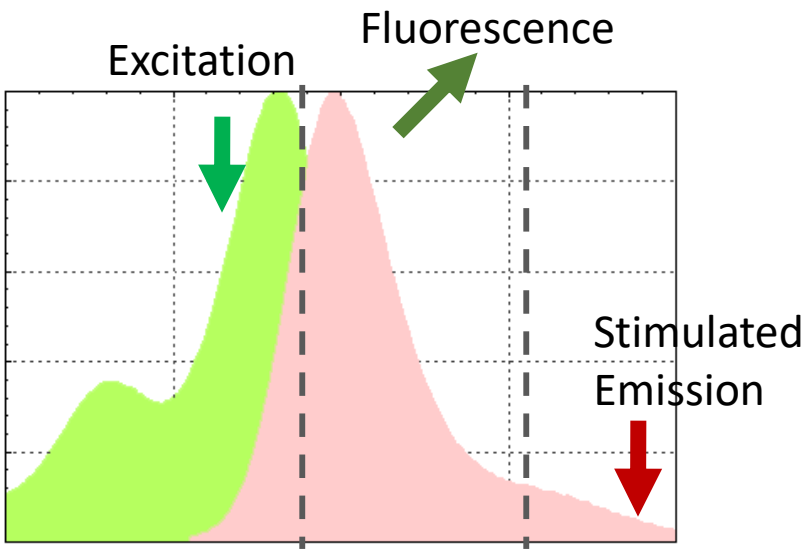


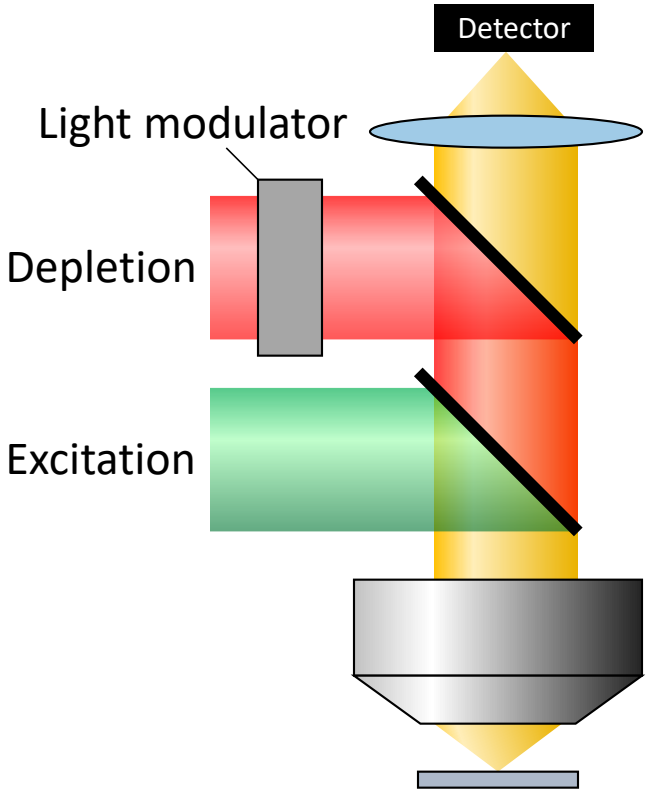




http://nic.ucsf.edu/dokuwiki/lib/exe/fetch.php?media=qb3_2013:super-resolution_optical_microscopy.pdf

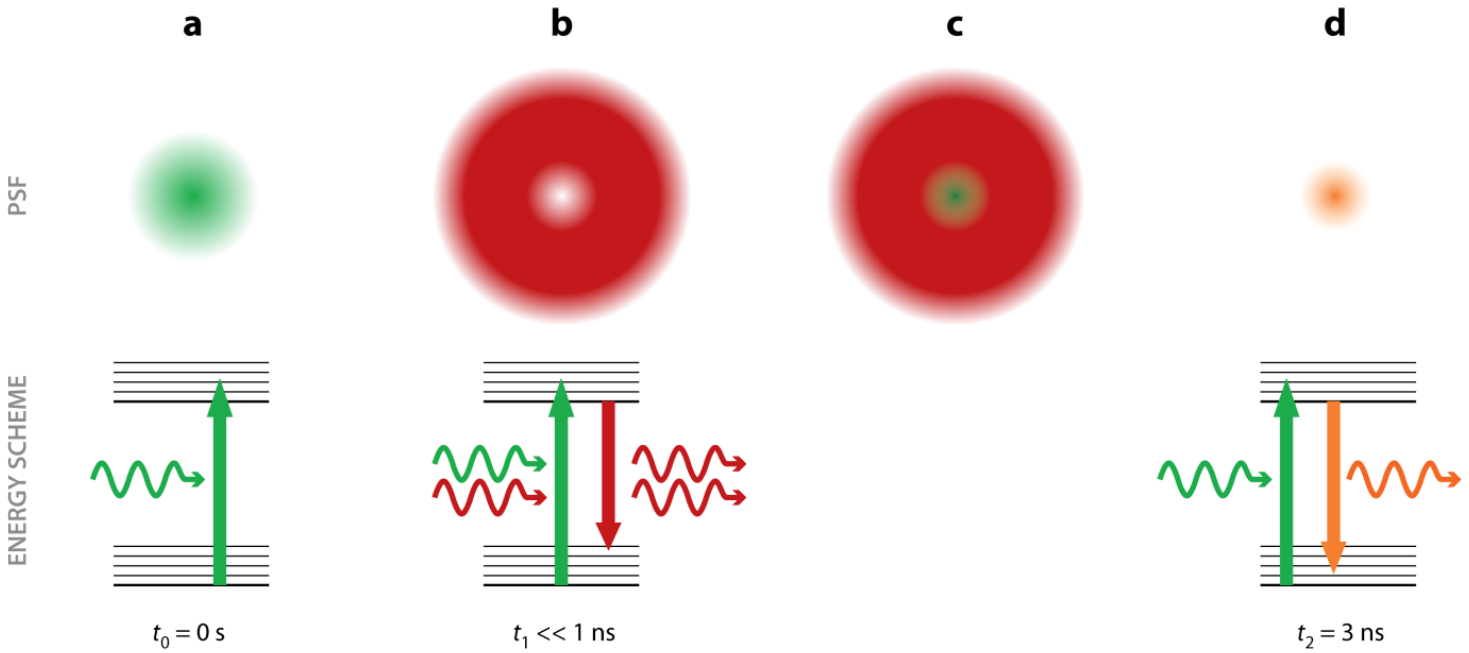
Stimulated Emission Depletion (STED)





Hell 1994, Hell 2000

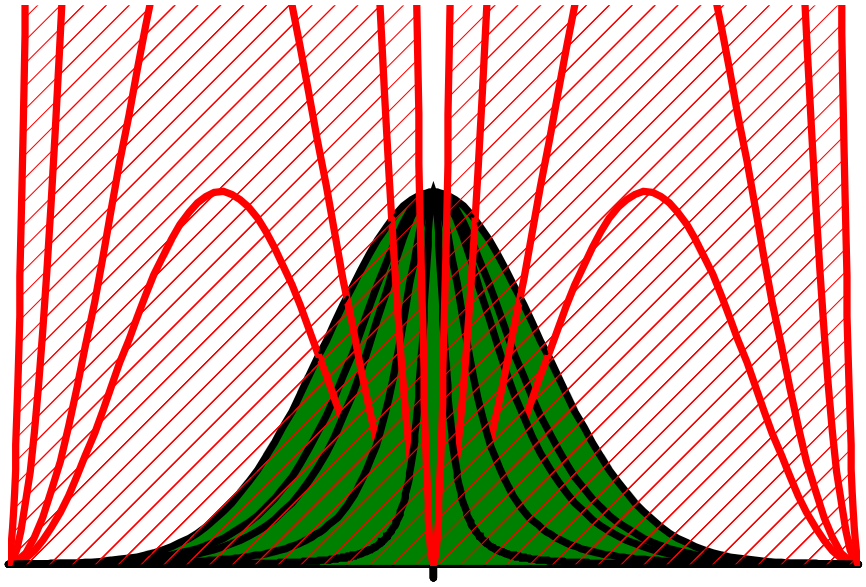
http://nic.ucsf.edu/dokuwiki/lib/exe/fetch.php?media=qb3_2013:super-resolution_optical_microscopy.pdf



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

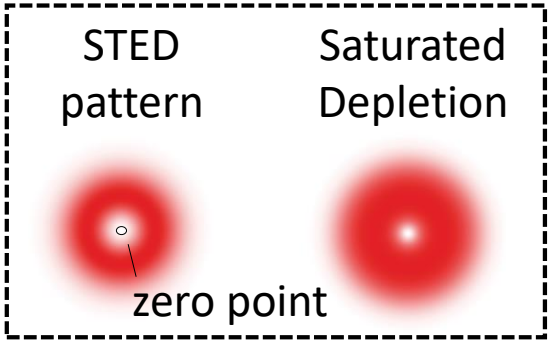
The excitation and de-excitation processes in stimulated emission depletion (STED). (*a*) (*Top*) Excitation point spread function (PSF). Its size is limited by diffraction of the excitation light through the objective. (*Bottom*) Corresponding transition in the Jablonski energy scheme. (*b*) (*Top*) Stimulated emission doughnut-shaped beam. (*Bottom*) The fluorophores within the region of the doughnut are forced to the ground state by the stimulated emission process driven into saturation. Note that the fluorophores in the very center of the doughnut beam do not see any STED light and thus remain in the excited state. (*c*) The two previous beams are superimposed and scanned along the region of interest. (*d*) (*Top*) Effective PSF of the combined effects. (*Bottom*) The fluorophores that have not been de-excited by the STED beam can emit fluorescent photons, which come from a region of much smaller radius. A careful selection of the emission filter can prevent the usually further redshifted stimulated emission light from reaching the detector.

Saturated depletion



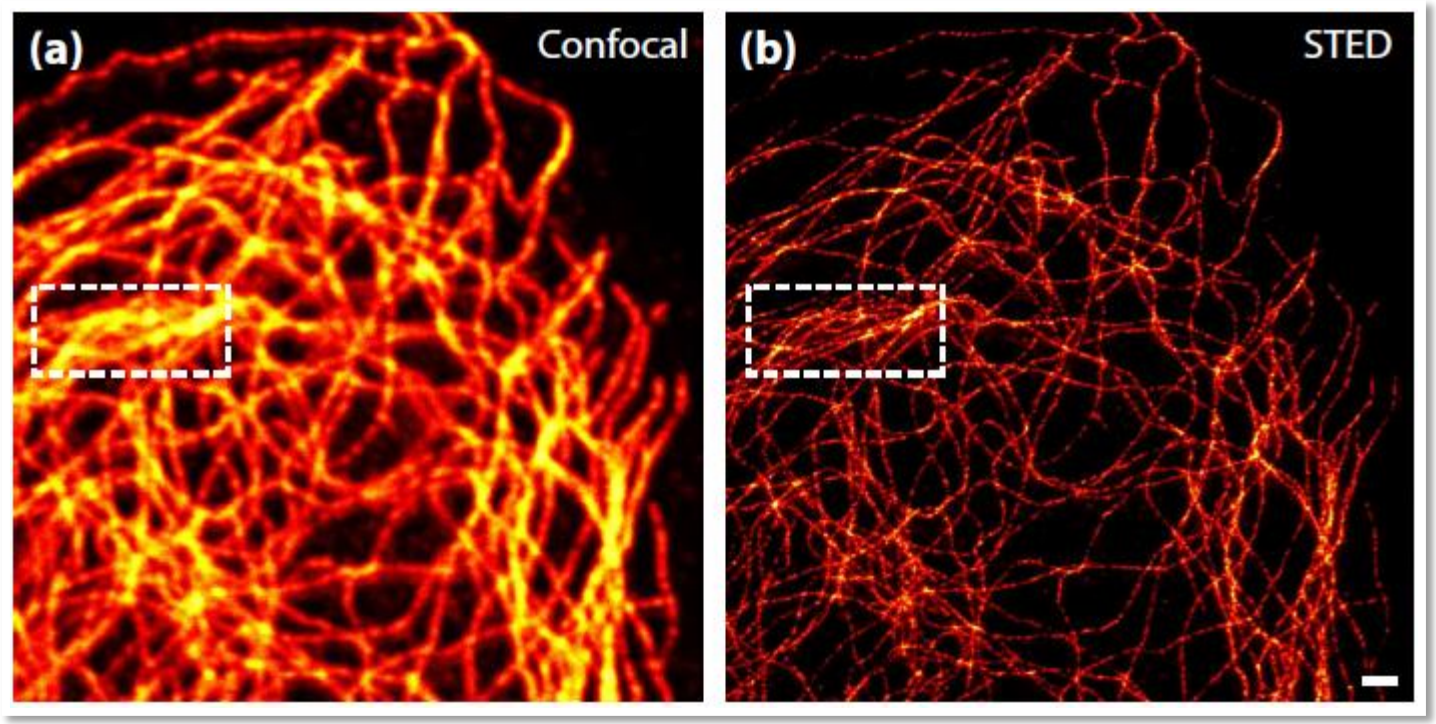
$$I_{\mathrm{STED}} = 100 I_S$$

$$d = \frac{1}{\sqrt{1 + I / I_s}} \cdot \frac{\lambda}{2NA}$$



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

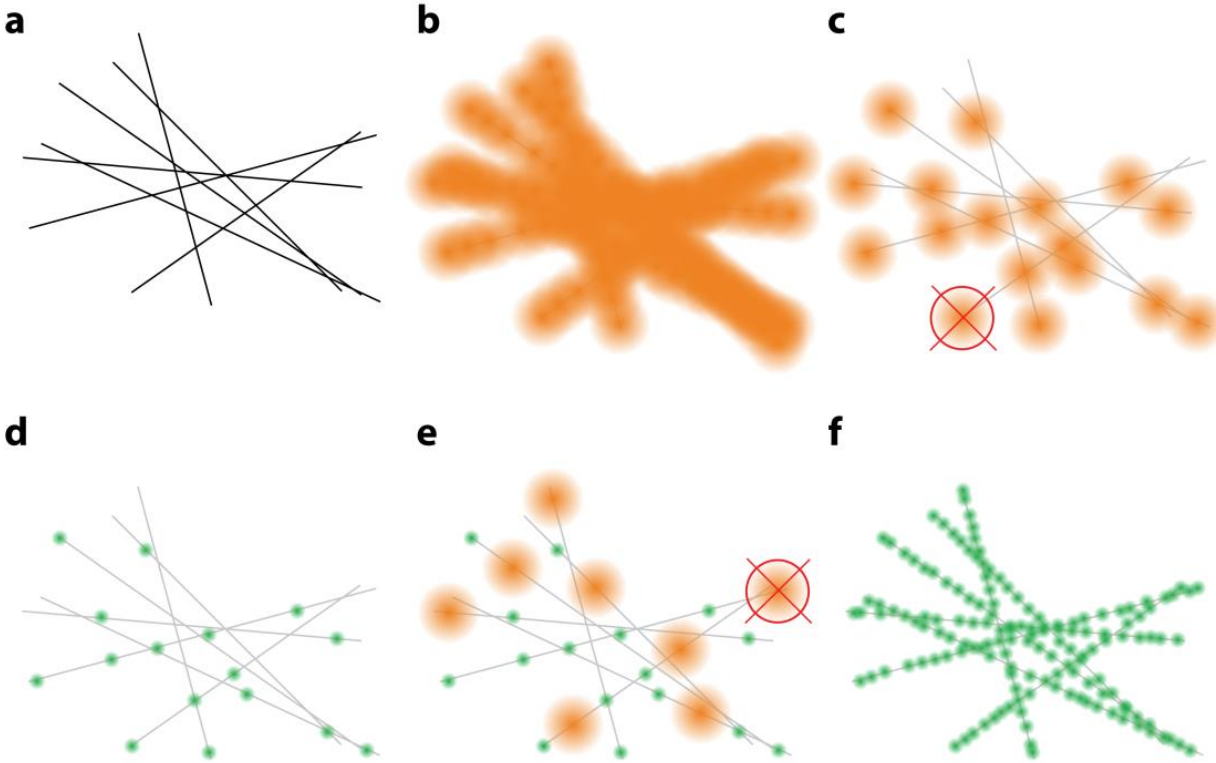
Images STED de microtubules



Wildanger et al., 2009

PALM et STORM

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

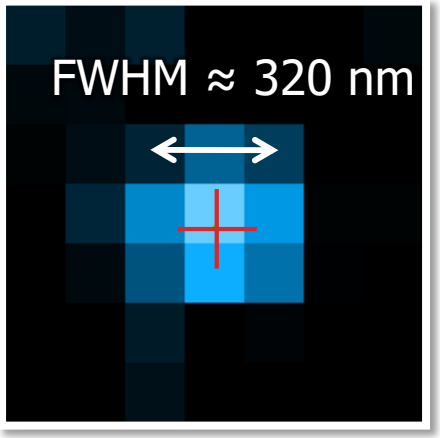


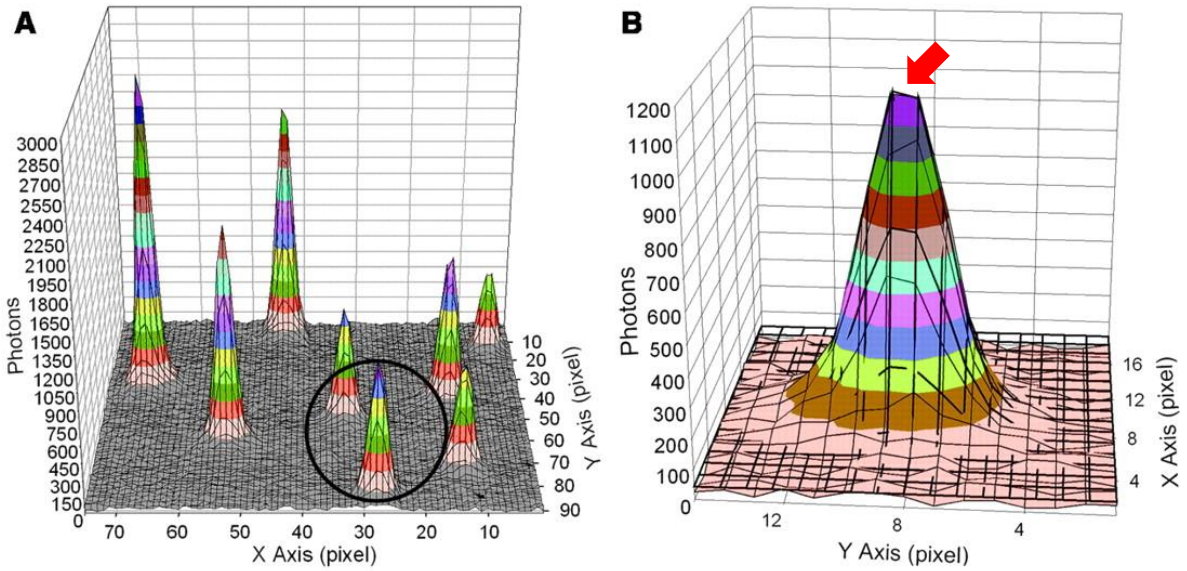
Jost A, Heintzmann R. 2013.
Annu. Rev. Mater. Res. 43:261–82

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

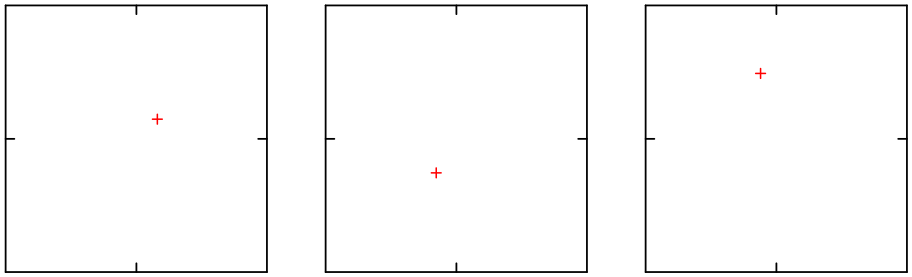




Ahmet Yildiz et al. Science 2003;300:2061-2065

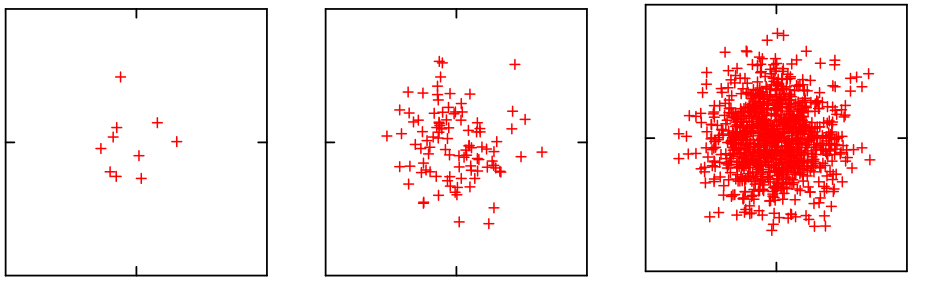
PSF with 0.5-s integration time of several individual Cy3-dyes attached to a coverslip.

Précision de la méthode

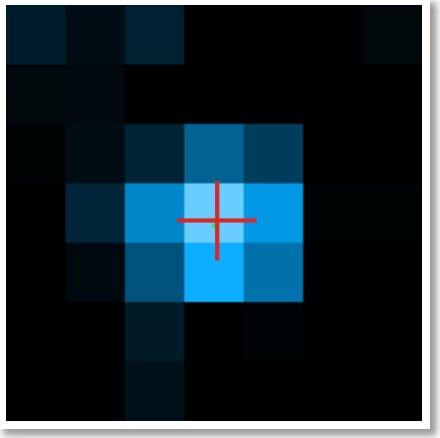


1 photon

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



10 photons 100 photons 1000 photons

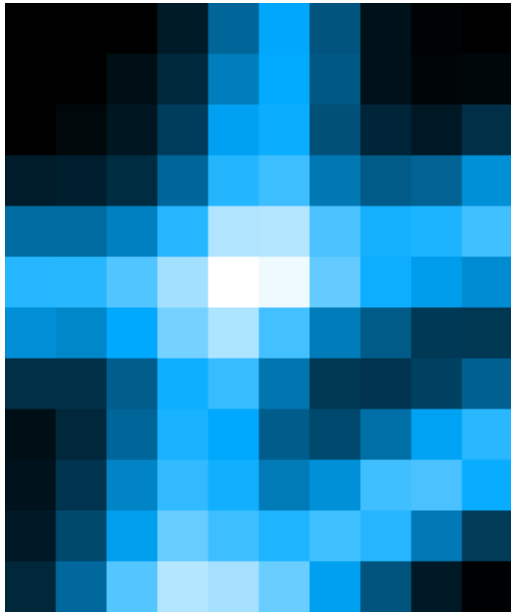


$$d = \frac{1}{\sqrt{N}} \cdot \frac{\lambda}{2NA}$$

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

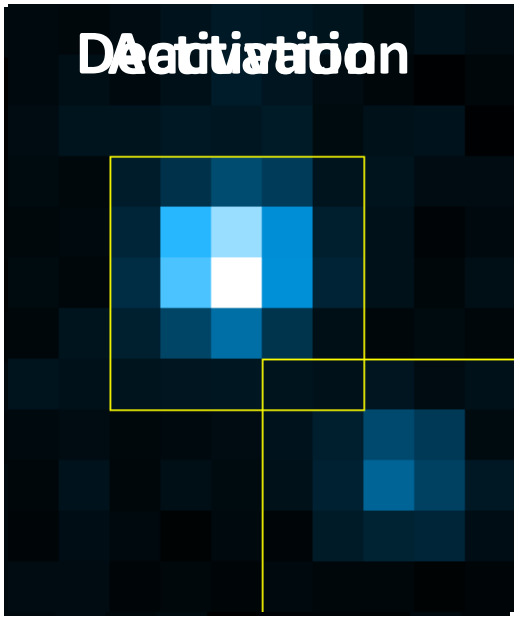
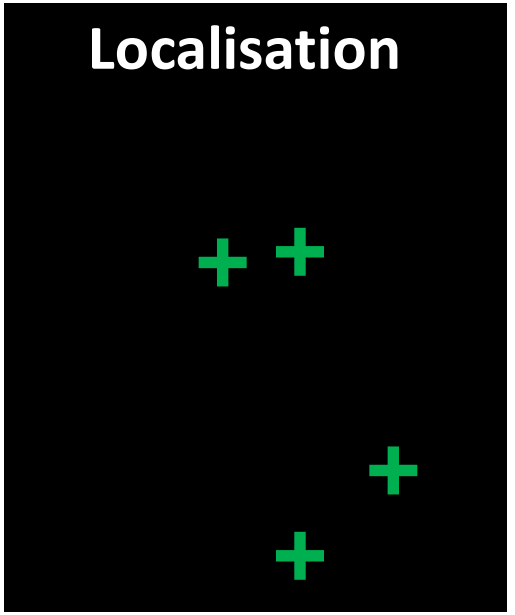


Image STORM



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