Kick-Off Interdisciplinary Workshop

Image processing techniques for object reconstruction

Your name, e-mail, institution
Ivan Amat-Roldan, ivan@ymaging.com, Ymaging

Outline

1. Microscopy techniques

2. Imaging processing techniques for object reconstruction depends on the optical setup

1. ChipScope

1. Microscopy techniques

Wide field microscopy

Confocal and Multiphoton Microscopy

TIRF microscopy

NSOM

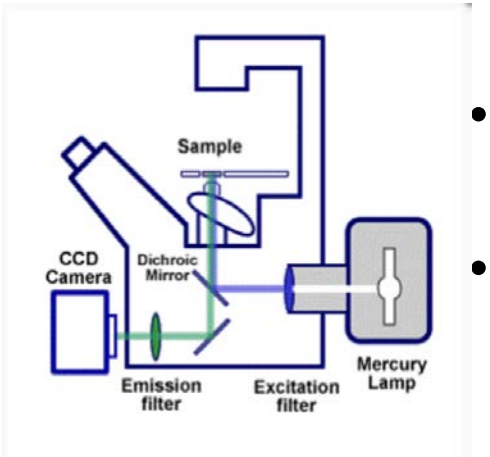
Structured Illumination

STED

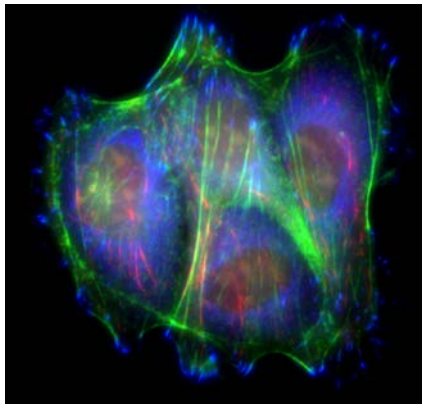
PALM and STORM

Wide field microscopy

- Contrast: scattering, fluorescence, phase contrast.



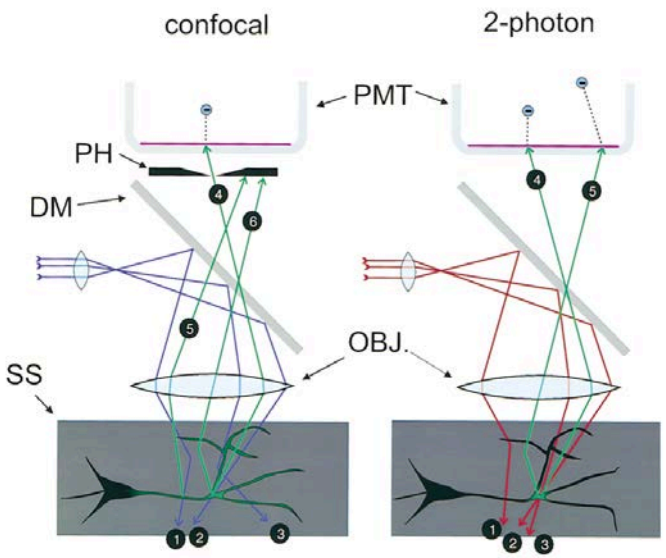
- Advantages: cost-effective, extremely simple to use.
- Limitations: diffraction limited, without depth information.

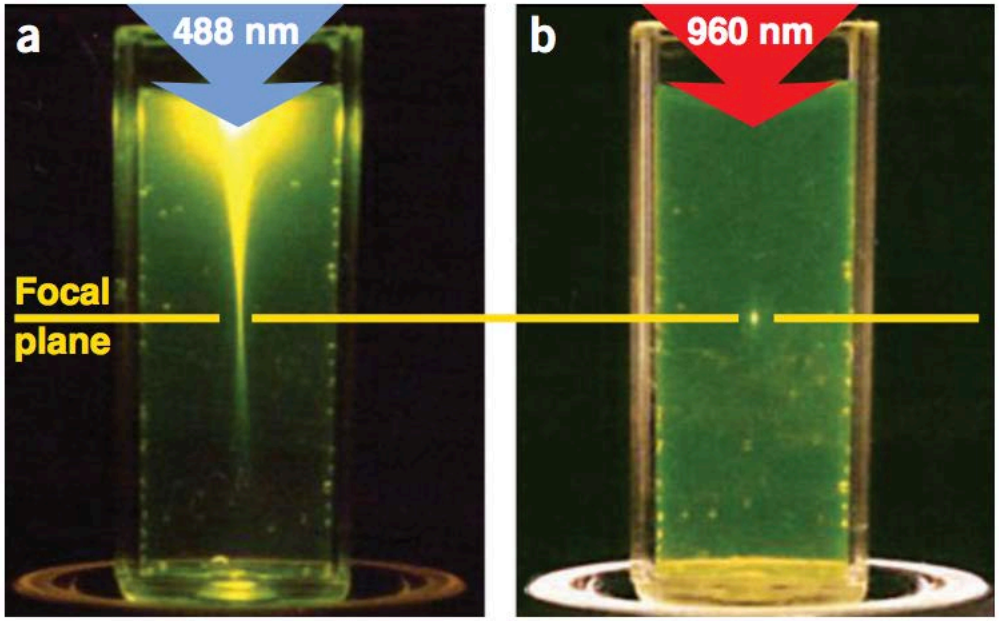


http://www.pages.drexel.edu/~bio/cores/cic/wide-field-microscopy.html

Confocal and Multiphoton Microscopy

- Contrast: scattering, fluorescence, and multiphoton processes (SHG, CARS,…).





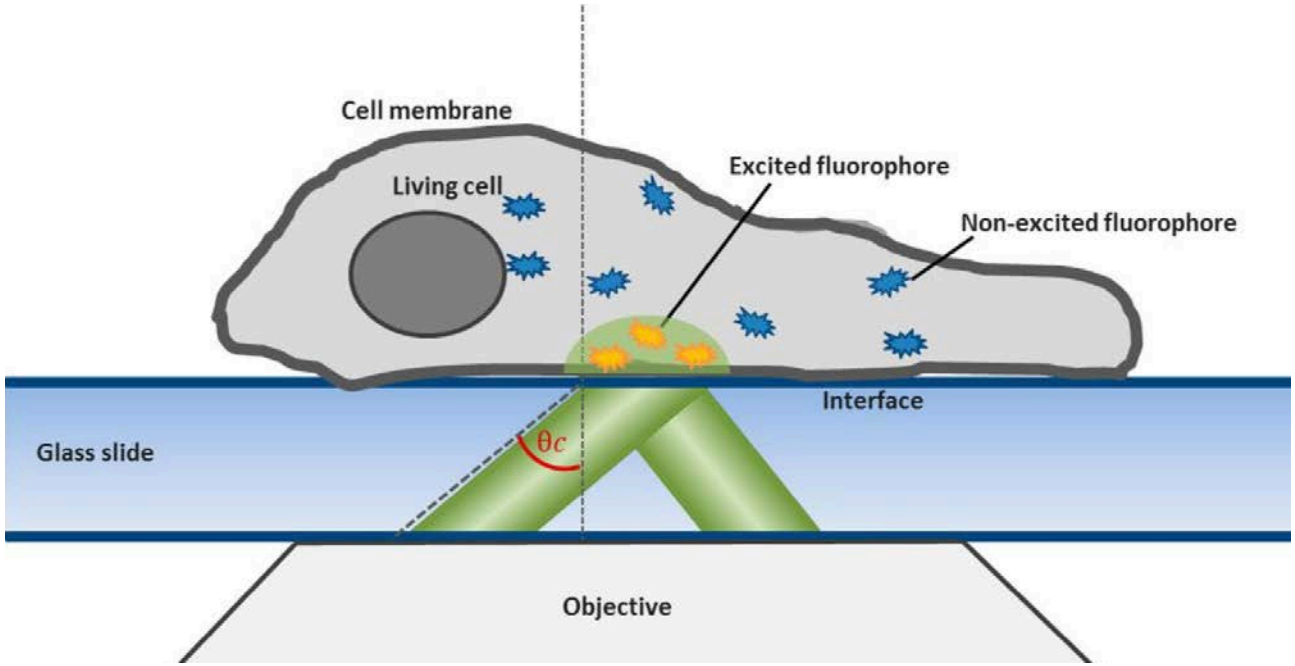
Advantages: 3D high resolution, compatible with many imaging modalities.

Limitations: diffraction limited, scanning laser (moving parts), 10-1000 fps, expensive and bulky optics.

Denk, W., & Svoboda, K. (1997). Photon upmanship: why multiphoton imaging is more than a gimmick. *Neuron*, *18*(3), 351-357.l
Zipfel, W. R., Williams, R. M., & Webb, W. W. (2003). Nonlinear magic: multiphoton microscopy in the biosciences. *NATURE BIOTECHNOLOGY*, *21*(11), 1369.

TIRF microscopy

- Contrast: fluorescence.



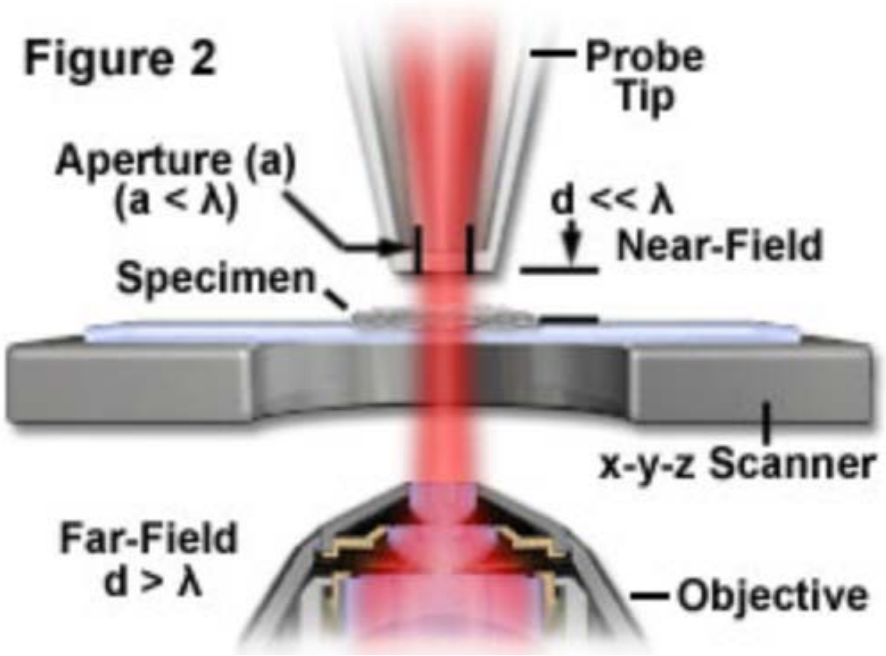
Advantages: strong light confinement in axial direction, easy-to-use.

Limitations: diffraction limited in transversal view, no depth resolution.

http://www.leica-microsystems.com/science-lab/universal-paint-dynamic-super-resolution-microscopy/

NSOM

- Contrast: polarization, fluorescence, phase contrast.



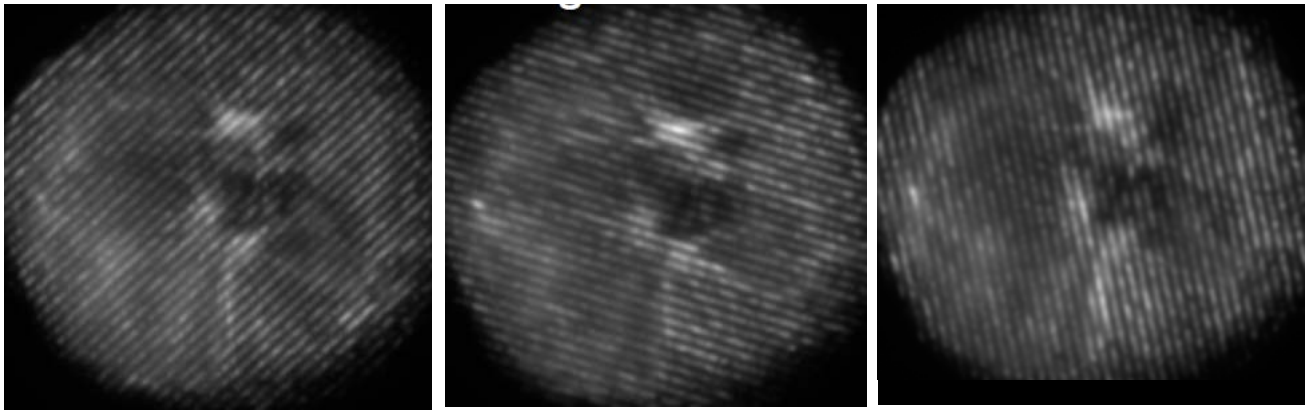
Advantages: beyond diffraction limit (superresolution).

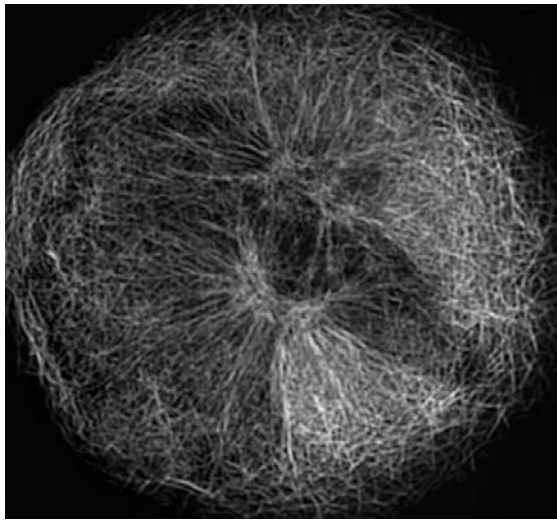
Limitations: needs to scan a tip which is very slow.

http://www.olympusmicro.com/primer/techniques/nearfield/nearfieldintro.html

Structured Illumination Microscopy

- Contrast: scattering, fluorescence.





Advantages: easy to adapt a wide-field microscope, enhancement resolution by multiple projections and processing, nonlinear version enables to go beyond diffraction limit, concept works in 3D.

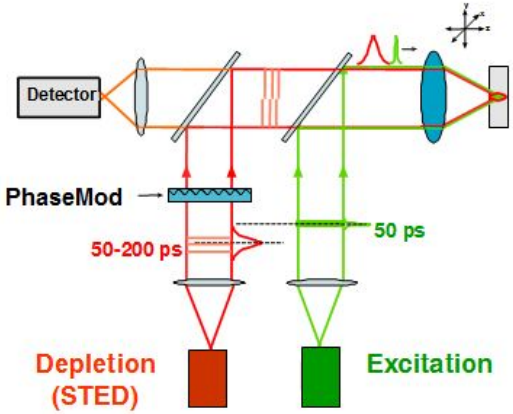
Limitations: needs multiple acqusitions to build an image.

Gustafsson, M. G. (2000). Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *Journal of microscopy*, *198*(2), 82-87.

STED

- Contrast: fluorescence in combination to material saturation.

$$D = \frac{\lambda}{2n \sin\alpha \sqrt{1 + \frac{I}{I_{sat}}}}$$



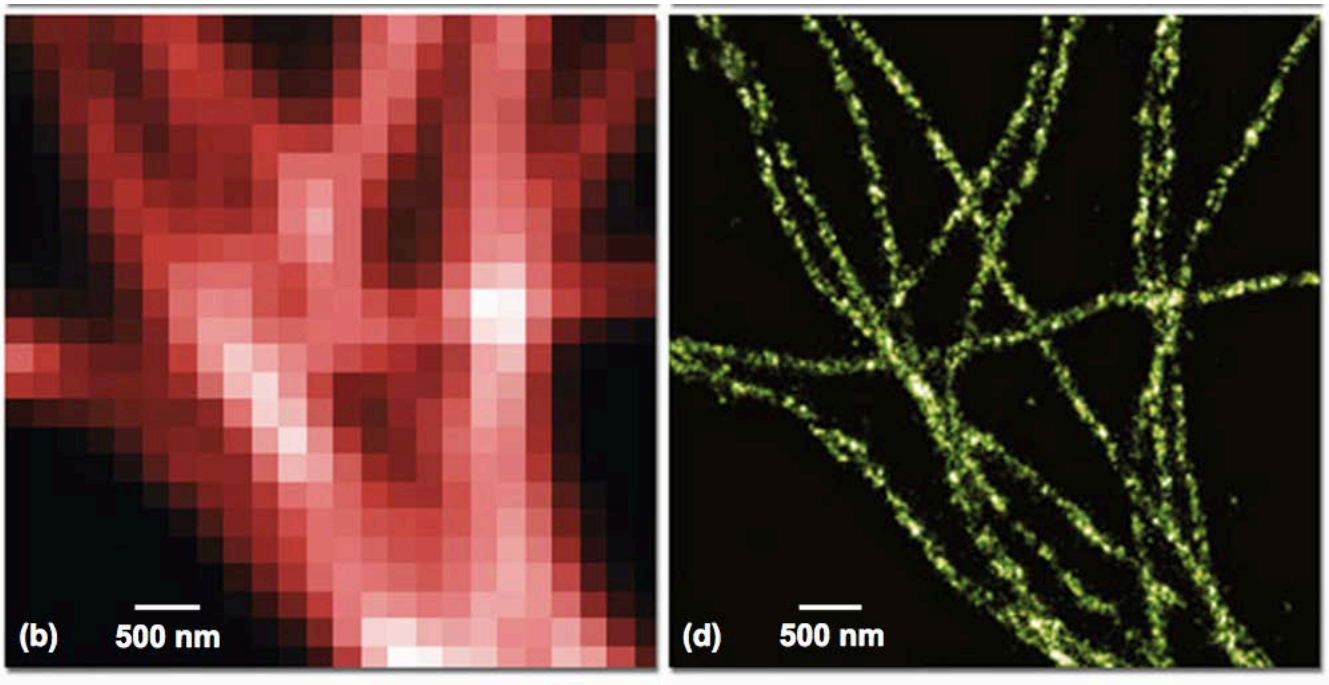
Advantages: superresolution.

Limitations: bulky optics (beam forming, temporal synchronization, scanning unit,…), limited number of dyes as spectral range of depletion needs no to overlap with excitation.

https://en.wikipedia.org/wiki/STED_microscopy

PALM and STORM

- Contrast: fluorescence of photoswitchable or photoactivable dye.



Advantages: superresolution without scanning, a standard microscope is equipped with a EMCCD camera and laser is set to low power.

Limitations: high demands on dyes related to photoswitching speed, expensive camera EMCCD

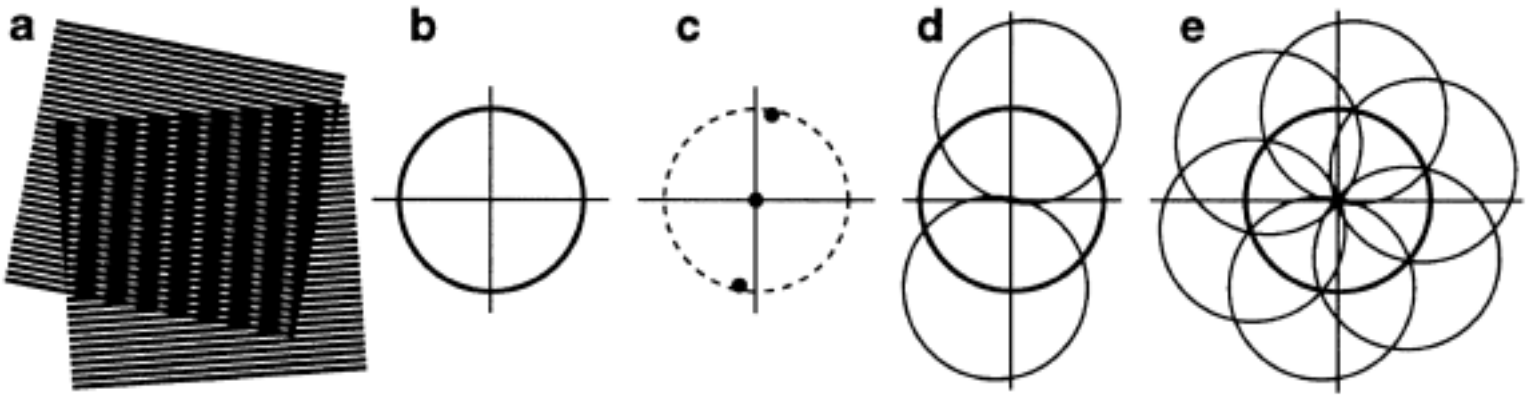
https://www.microscopyu.com/techniques/super-resolution/single-molecule-super-resolution-imaging

Depending on the optical set-up of the microscope image reconstruction requires different processing means:

- **Wide field microscopy** has noprocessing (image is directly provided by the CCD), similarly other recent imaging techniques such as SPIM have no processing (but stacking for 3D acquisitions).

- **Scanning techniques** (NSOM, confocal, multiphoton, STED) they require basic syncronisation for frame start (sometimes also line start) and minimal postprocessing includes temporal mapping of the imaging points into a 2D image. This principle is directly extended for a third dimension when the imaging plane is also scanned.

- **Structured Illumination,** a sequence of images at different depths and then added with the correct phase in the Fourier domain. This can be further extended to 3rd dimension (axial).

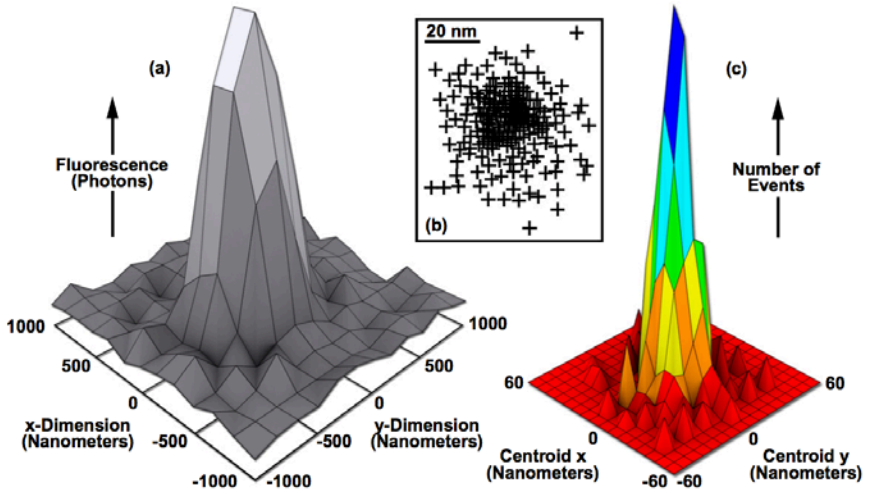


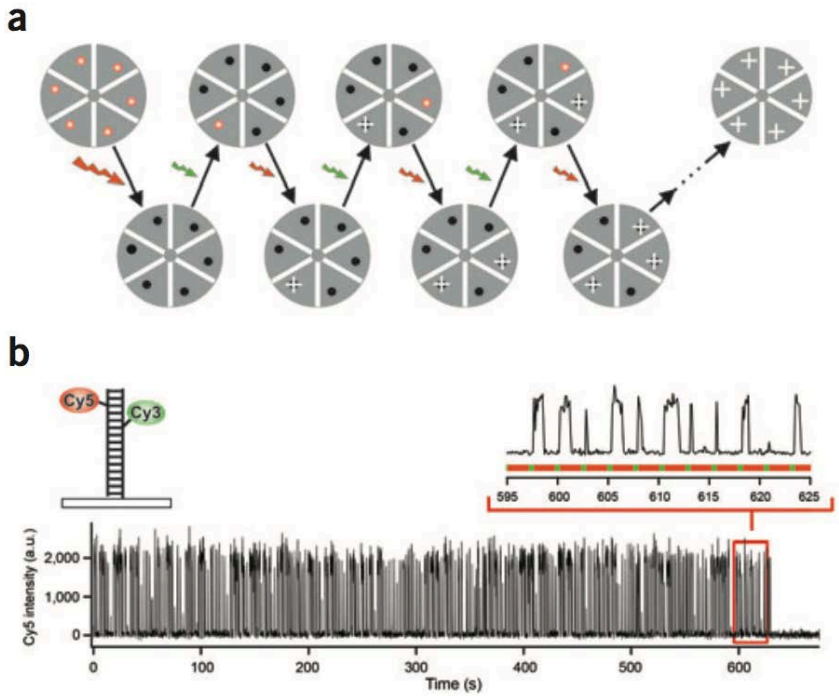
Limitations: motion, bleaching, drift, low signal, low modulation, spherical aberration.

For this additional quality control based on image processing can be computed to detect or even correct some of this artifacts.

Gustafsson, M. G. (2000). Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *Journal of microscopy*, *198*(2), 82-87.

- **PALM and STORM**, are methods based on single molecule detection so they try to estimate with highest accuracy the position of the emitters, which highly depends on the number of photons.





Limitations: background noise needs to be reduced, sparsely excited fluorescent molecules must be suffeciently appart (diffraction limit), constraints of fluorophores fast swithcing speed and bleaching sets the limit of resolution (number of photons).

Rust, M. J., Bates, M., & Zhuang, X. (2006). Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nature methods*, *3*(10), 793-796.

- **Chipscope** is a breakthrough in imaging technology as it can exploit currently unavailable aspects compared to other techniques:
  - it can provide unprecedented spatial resolution and temporal information of the excitation source
  - fast photodetector response
  - extremely cheap packaging

However, many questions are open about the functioning and how processing can help to improve ChipScope performance:

- What is the impact of light propagation in thick specimens in terms of transversal and axial localization. Can we model that? Can we build an algorithm to compensate that?
- Can we use similar principles to structured illumination?
- Do we still need photoswitchable emitters with this technology? Can we further use them to increase resolution?
- Can we make faster acquisitions compared to STORM?
- How much can we improve life cell imaging?
- How much data can we stream in our first prototype?

Thank you for your attention!