Super-resolution microscopy

Introduction:

- Super-resolution microscopy is a form of light microscopy.
- Due to the diffraction of light, the resolution of conventional light microscopy is limited
- A good approximation of the resolution attainable is the full width at half maximum (FWHM) of the point spread function.
- a precise wide field microscope with high numerical aperture and visible light usually reaches a resolution of ~250 nm.
- Super-resolution techniques allow the capture of images with a higher resolution than the diffraction limit.

Types of super-resolution microscopy:

1) "true" super-resolution techniques
2) "functional" super-resolution techniques

Most of the biological imaging fall into Functional category

There are two major groups of methods for functional super-resolution microscopy:

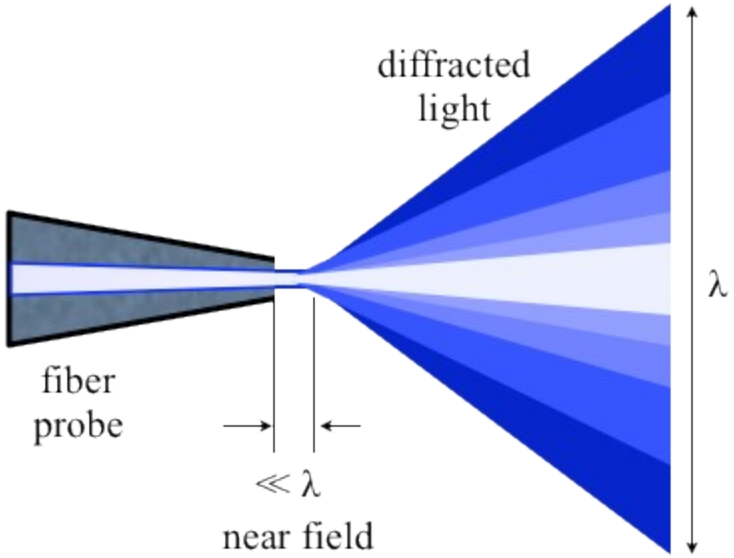
1) Deterministic super-resolution: The most commonly used emitters in biological microscopy, fluorophores, show a nonlinear response to excitation, and this nonlinear response can be exploited to enhance resolution. These methods include stimulated emission depletion microscopy (STED), ground state depletion microscopy (GSD), reversible saturable optical fluorescence transitions (RESOLFFT).

2) Stochastic super-resolution: The chemical complexity of many molecular light sources gives them a complex temporal behavior, which can be used to make several close-by fluorophores emit light at separate times and thereby become resolvable in time. These methods include all single-molecule localization methods (SMLM).

This year Nobel prize was for the development of super-resolved fluorescence microscopy which brings optical microscopy into the nanodimensions

Near-field scanning optical microscopy (NSOM/SNOM)

- A microscopic technique for nanostructure investigation that breaks the far field resolution limit.
- This is done by placing the detector very close (distance much smaller than wavelength λ) to the specimen surface.
- This allows for the surface inspection with high spatial, spectral and temporal resolving power.
- With this technique, the resolution of the image is limited by the size of the detector aperture and not by the wavelength of the illuminating light. In particular, lateral resolution of 20 nm and vertical resolution of 2–5 nm have been demonstrated.



ANSOM: ANSOM is aperture less NSOM: it uses a tip very close to a fluorophore to enhance the local electric field the fluorophore sees. Basically, the ANSOM tip is like a lightning rod, which creates a hot spot of light.

Near-field optical random mapping (NORM) microscopy

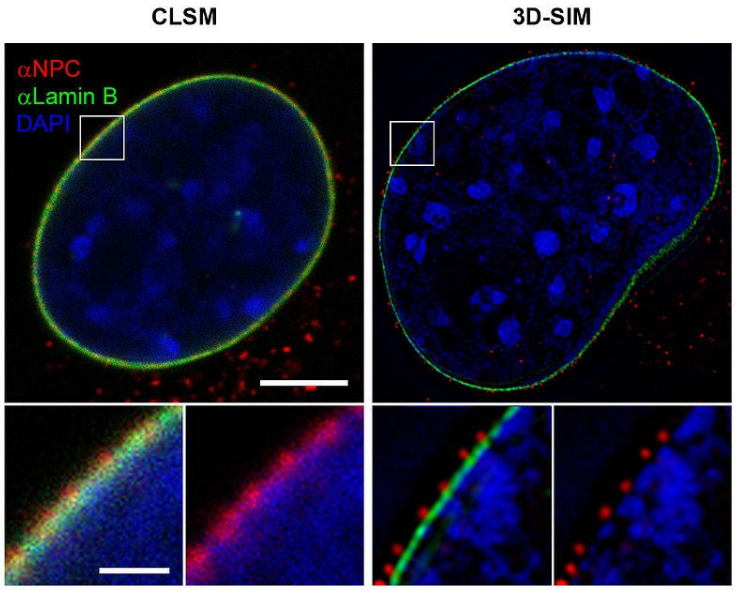
- NORM (Near-field Optical Random Mapping) microscopy is a method of optical near-field acquisition by a far-field microscope through the observation of nanoparticles Brownian motion in an immersion liquid.

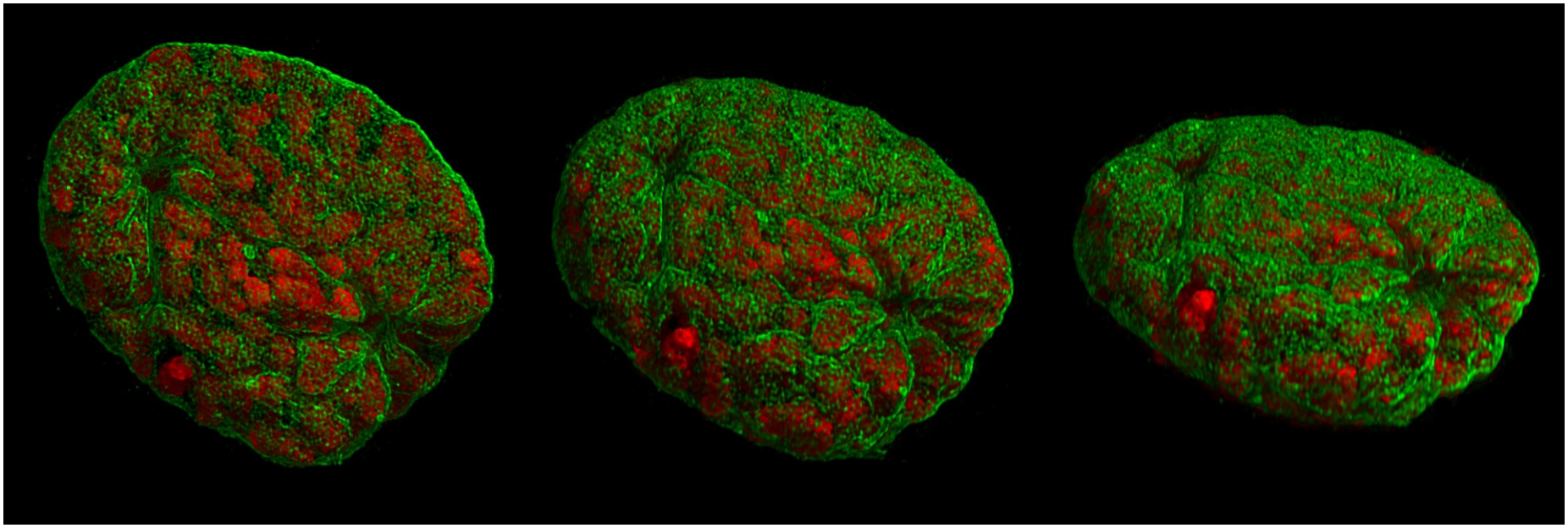
4Pi

- A 4Pi microscope is a laser scanning fluorescence microscope with an improved axial resolution.
- The improvement in resolution is achieved by using two opposing objective lenses both of which focused to the same geometrical location.
- By this, molecules residing in the common focal area of both objectives can be illuminated coherently from both sides and also the reflected or emitted light can be collected coherently, i.e. coherent superposition of emitted light on the detector is possible

Structured illumination microscopy (SIM)

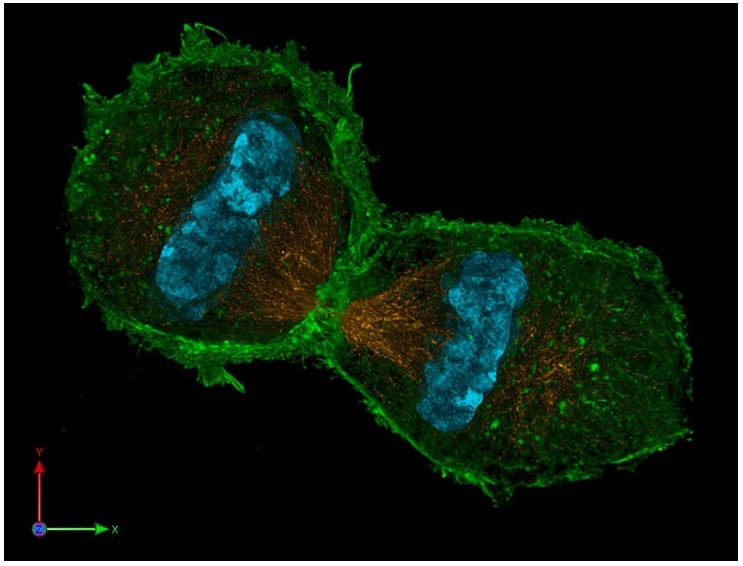
- The main concept of SI is to illuminate a sample with patterned light and increase the resolution by measuring the fringes in the Moire pattern (from the interference of the illumination pattern and the sample)
- SI enhances spatial resolution by collecting information from frequency space outside the observable region
- This process is done in reciprocal space: The Fourier transform (FT) of an SI image contains superimposed additional information from different areas of reciprocal space
- It is possible to computationally separate and reconstruct the FT image, which has much more resolution information
- The reverse FT returns the reconstructed image to a super-resolution image.





Cell nucleus in prophase from various angles

Two mouse cell nuclei in prophase



Mouse cell in telophase