Fluorescence tracing

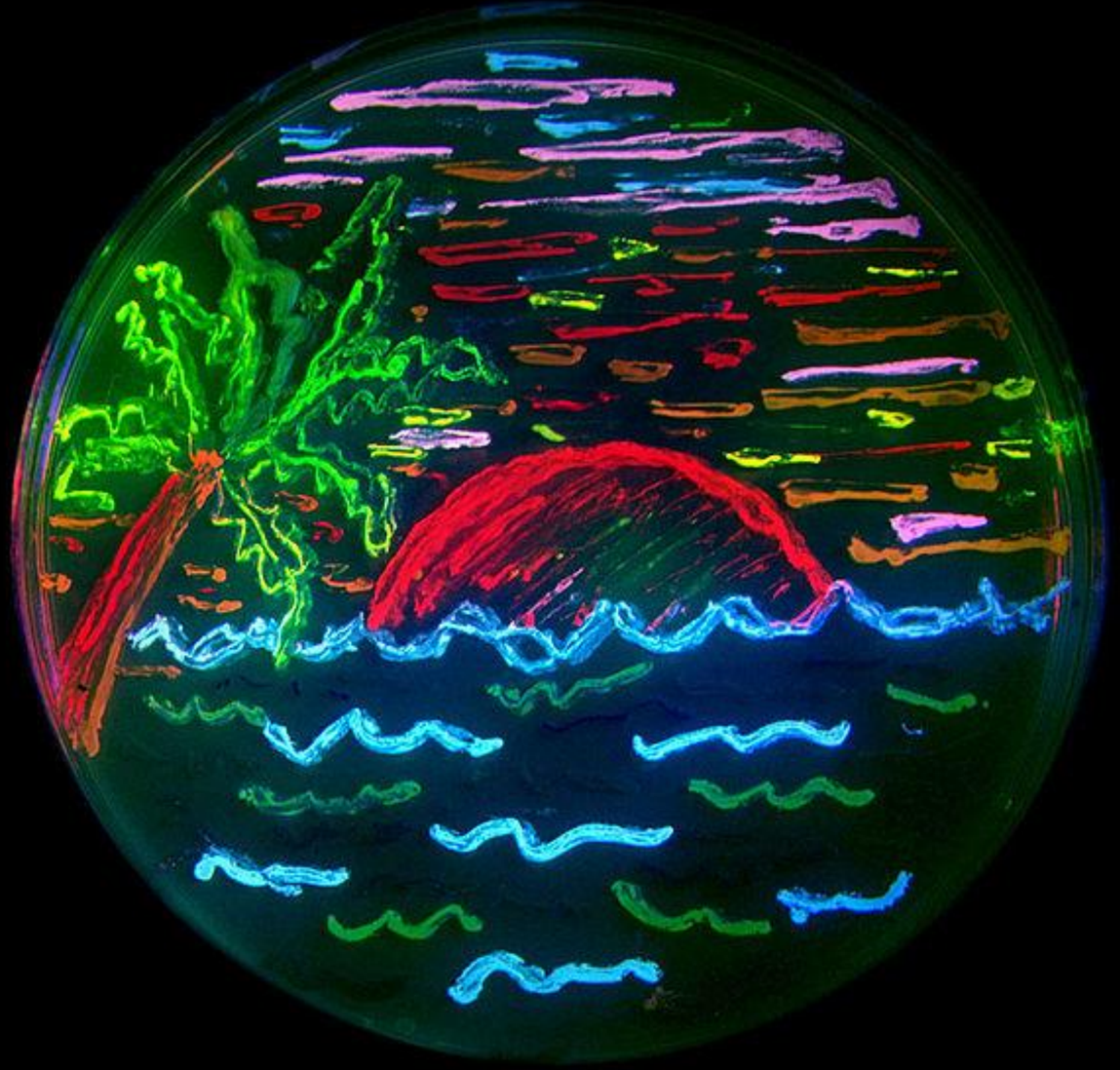
Shuangyin Wang

Fluorescence

- First described by British scientist Sir George G. Stokes in 1852
- Is the emission of light by a substance that has absorbed electromagnetic radiation of a different wavelength.
- In most cases, emitted light has a longer wavelength than the absorbed radiation.
- Initiated in biological investigations until 1930s

Applications in biochemistry

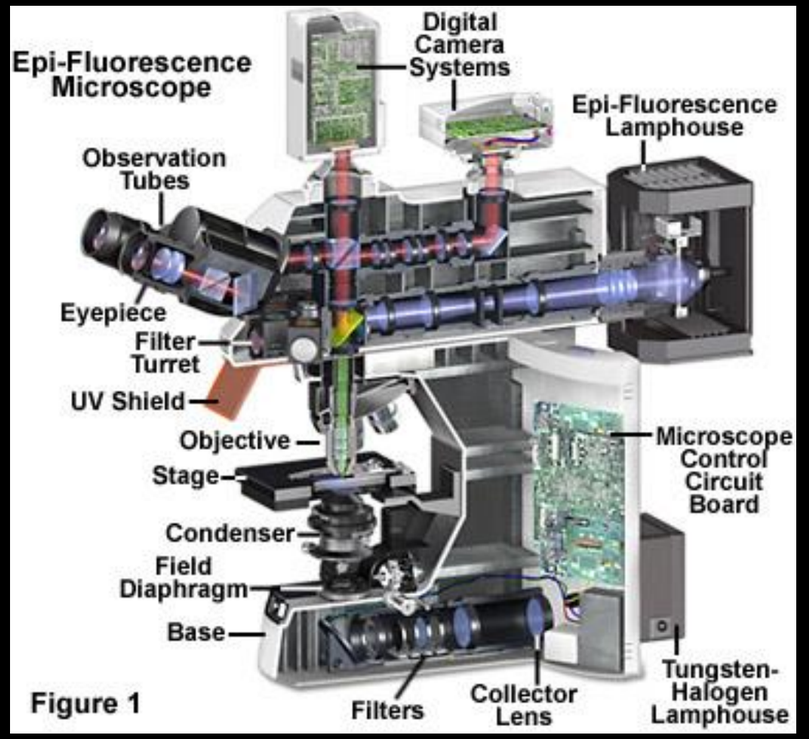
- Automated sequencing of DNA
- DNA microarrays
- DNA detection
- Fluorescent-activated cell sorting
- Fluorescent glucose biosensors



Living bacteria expressing 8 FP

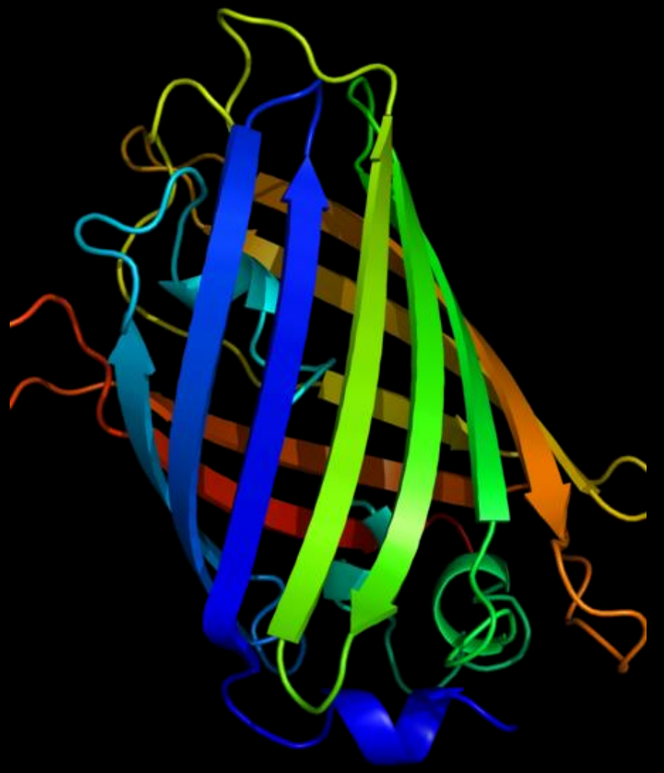
Fluorescence Microscope

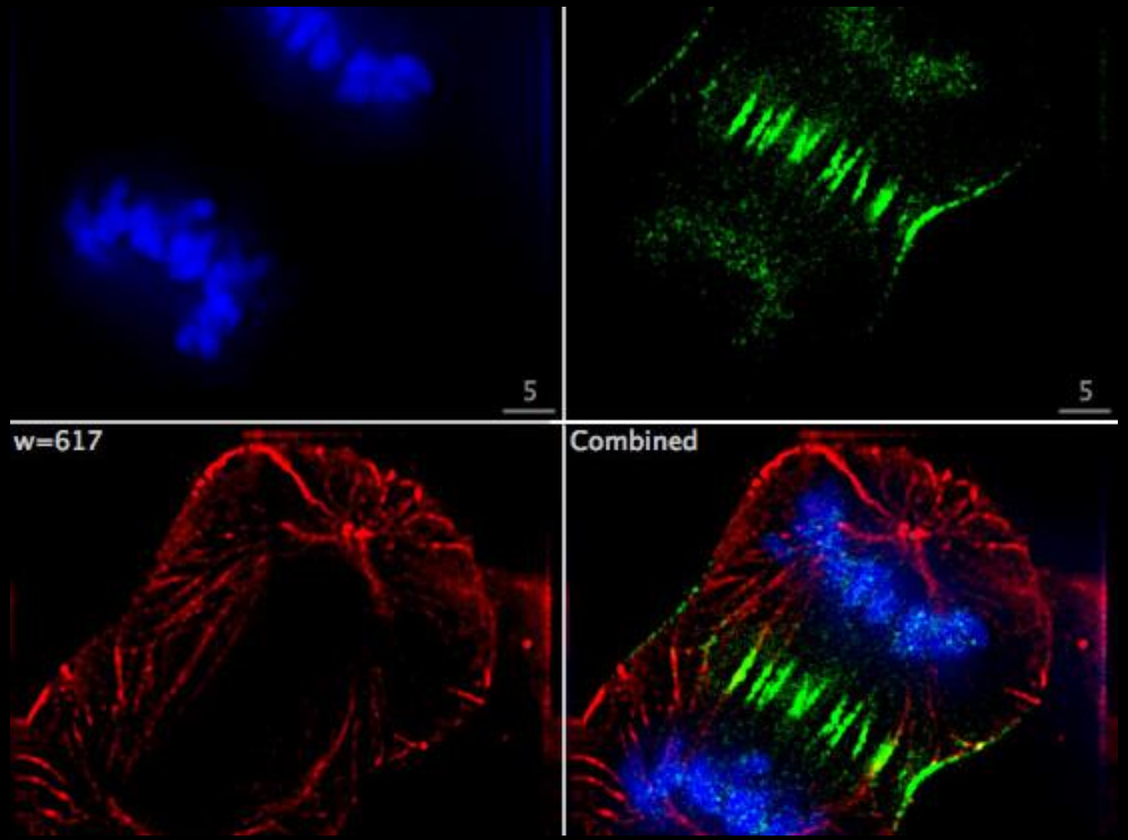
- is an optical microscope used to study properties of organic or inorganic substances using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption.



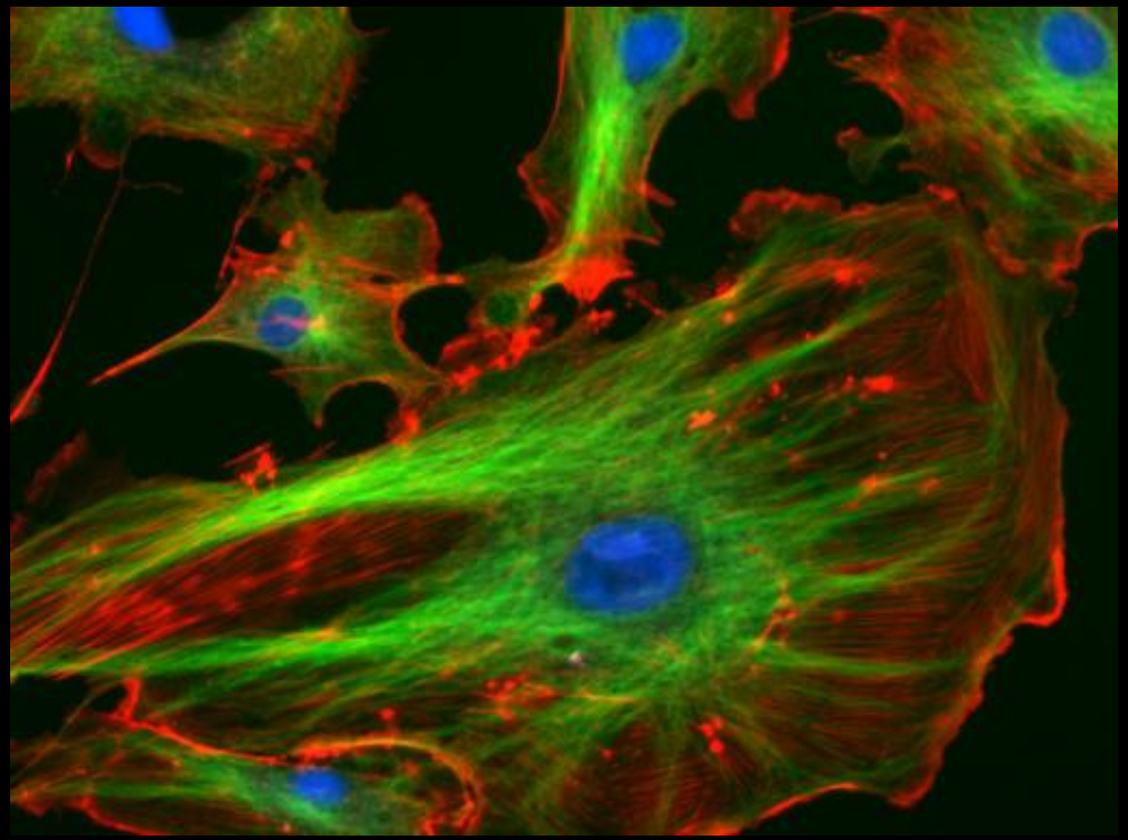
Green fluorescent protein

- 2008 Nobel Prize in chemistry
- first isolated from jellyfish *A. victoria*
- 238 amino acid residues
- exhibits bright green fluorescence when exposed to blue light

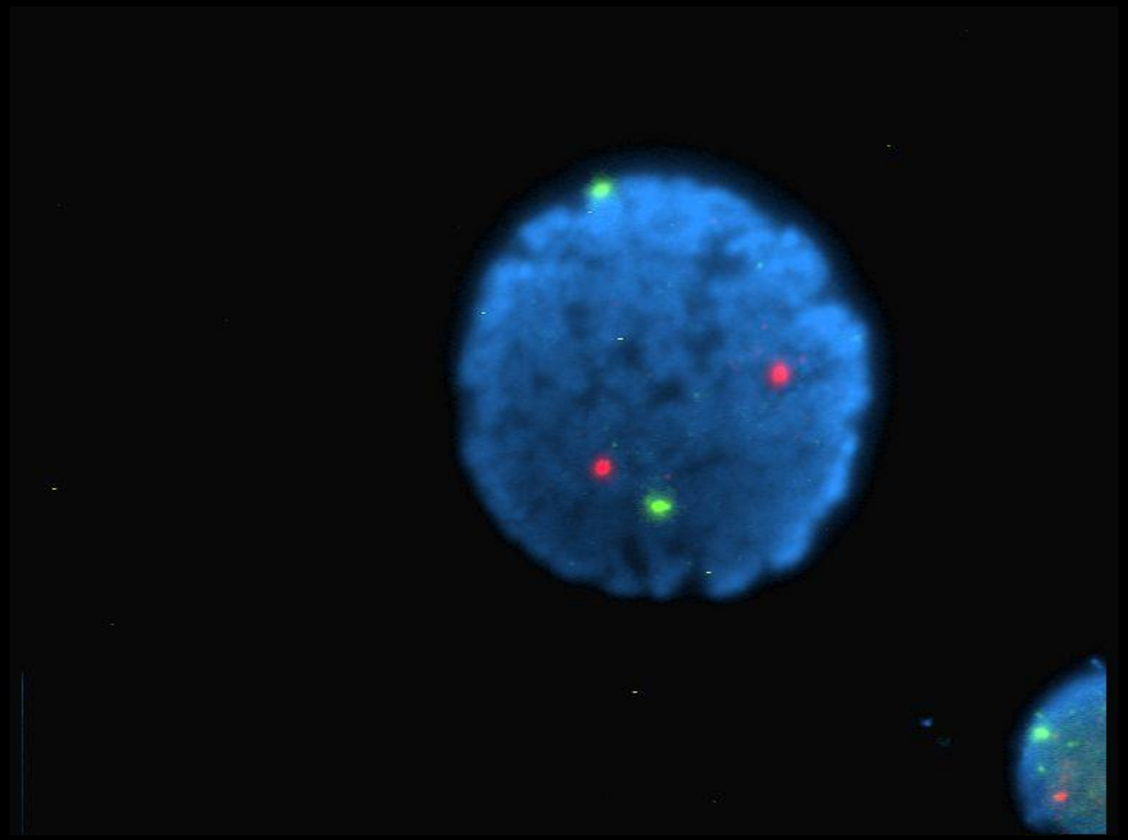




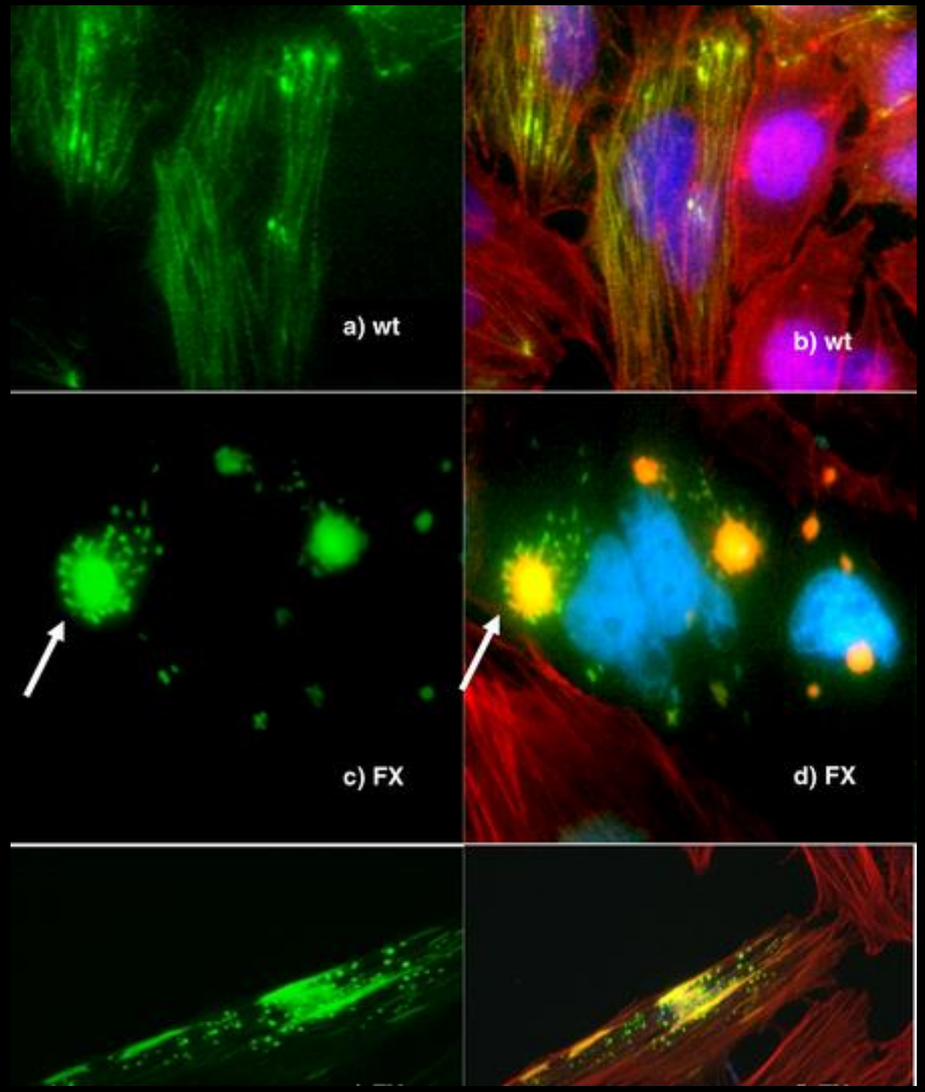
Human cancer cell dividing



Endothelial cells : nuclei (blue)
microtubles (green), actin filaments (red)



Human lymphocyte nucleus
FISH 13(green) & 21(red)



Expression of Human Wild-Type and P239S Mutant Palladin Constructs in HeLa Cells

Nature Methods : review of the year

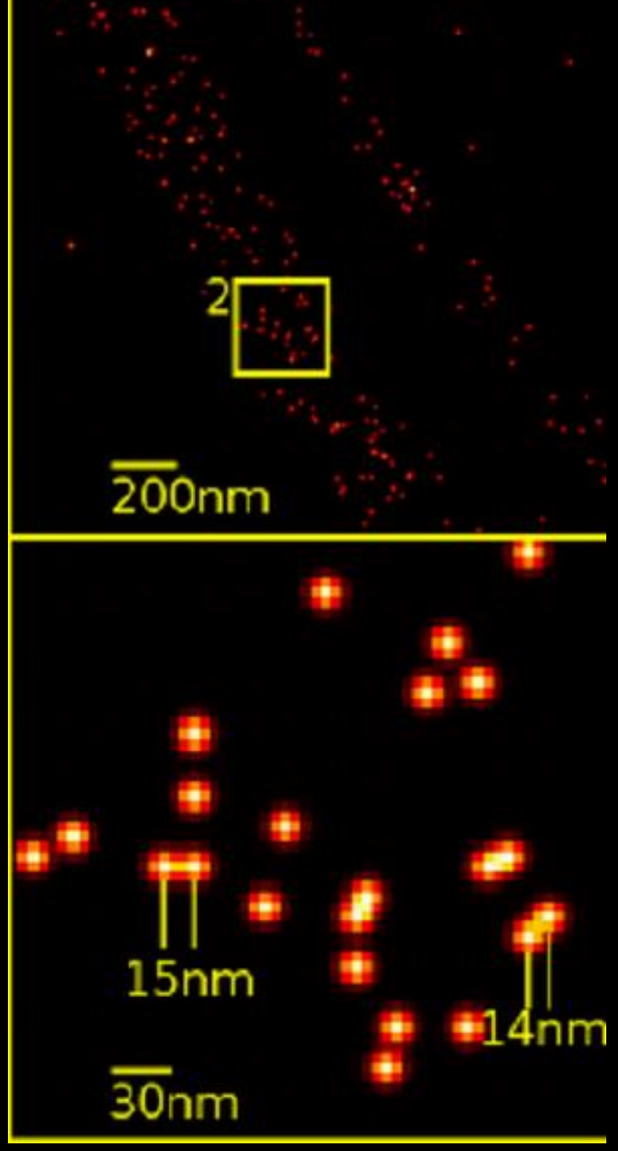
- 2007 : Next-generation sequence
- 2008 : Super-resolution fluorescence microscopy

- Confocal Laser Scanning Microscope (CLSM, LSCM)
- Epi-Fluorescence Microscopes
- Total internal reflection fluorescence microscopy (TIRFM)

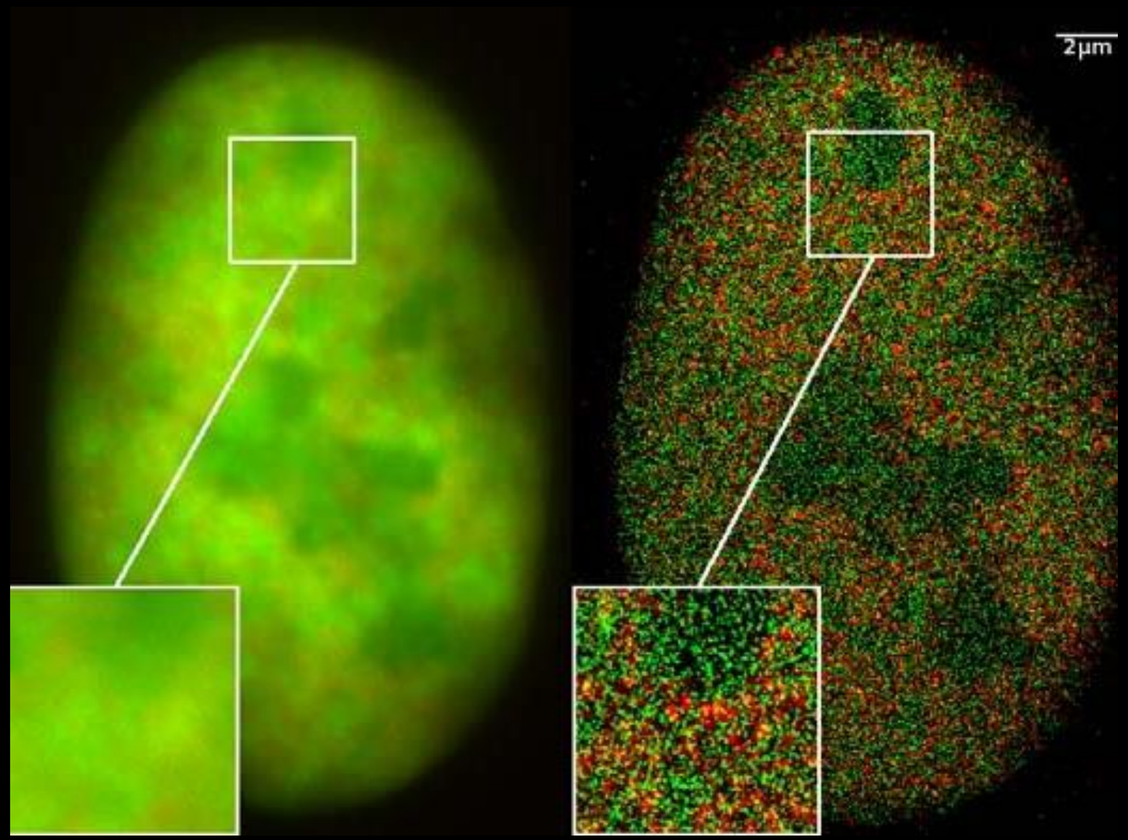
A new wave of cellular imaging

- stimulated emission depletion (STED)
- (fluorescence) photoactivation localization microscopy (fPALM)stochastic optical reconstruction microscopy (STORM)
- structured illumination microscopy (SIM)

- Annu Rev Cell Dev Biol. 2010 Nov 10;26:285-314.



A new localization microscopy technique
for standard fluorescent dyes



Red : histone molecules
Green : chromatin remodeling proteins

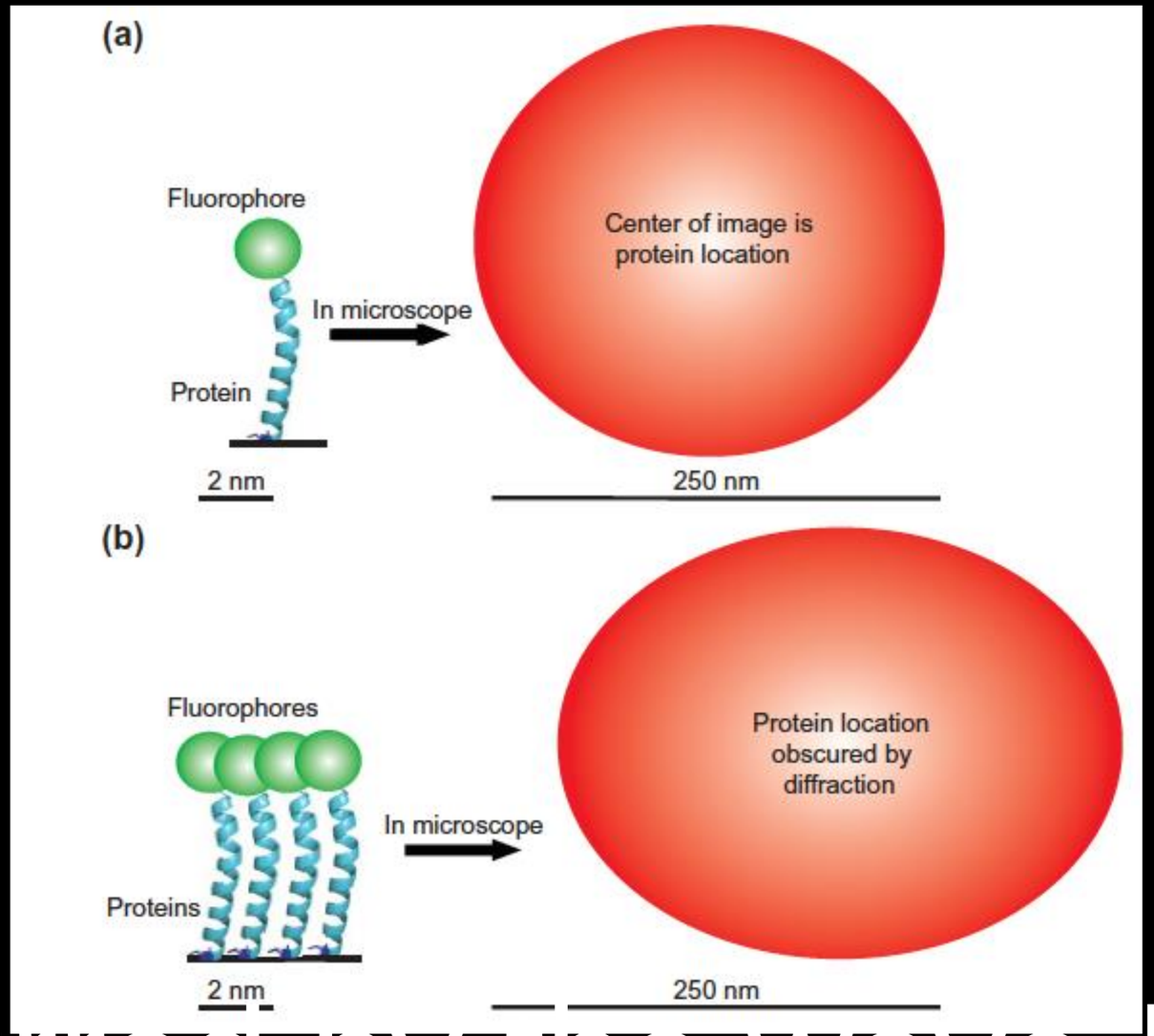
McEvoy *et al. BMC Biology* 2010, **8**:106
http://www.biomedcentral.com/1741-7007/8/106

BMC Biology
incorporating Journal of Biology

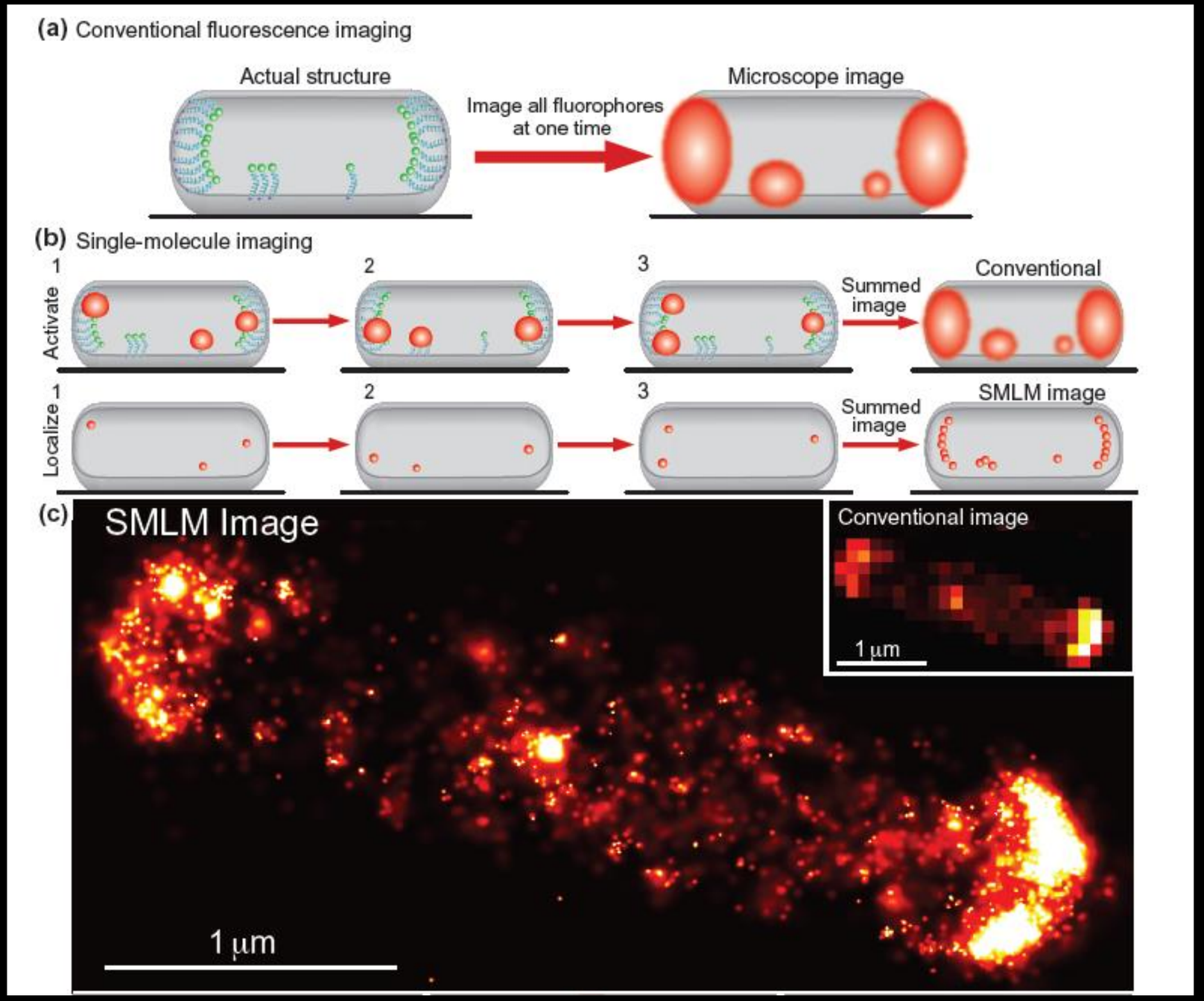
QUESTION & ANSWER Open Access

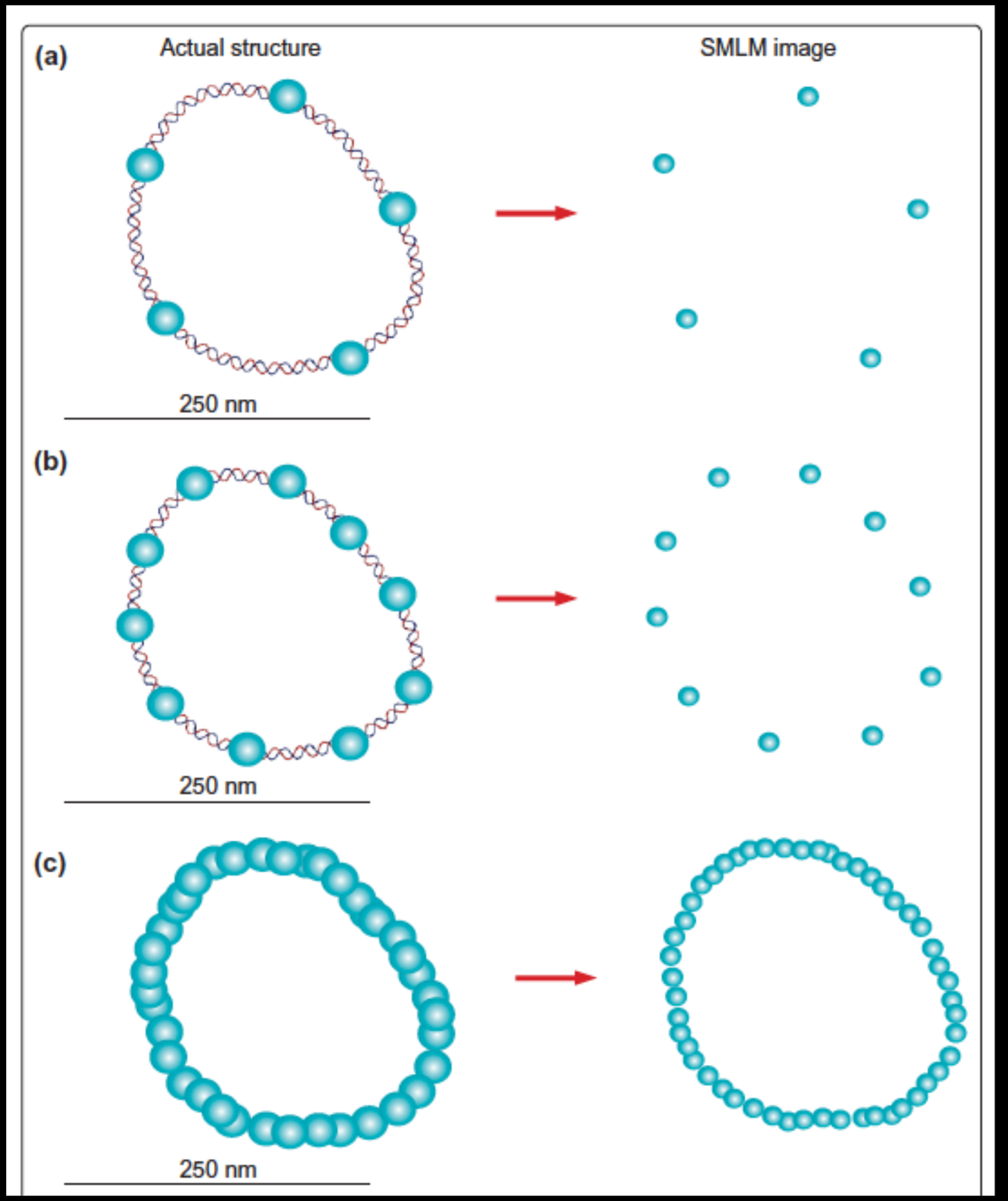
Q&A: Single-molecule localization microscopy for biological imaging

Ann L McEvoy[1], Derek Greenfield[1,2,5], Mark Bates[3] and Jan Liphardt[1,2,4*]



Blurred by the wave-like proerties


(a) Conventional fluorescence imaging
Actual structure
Image all fluorophores at one time
Microscope image
(b) Single-molecule imaging
1
2
3
Activate
Summed image
Conventional
Localize
SMLM image
(c)
SMLM Image
Conventional image
1 µm
1 µm


(a)
Actual structure
SMLM image
250 nm
(b)
250 nm
(c)
250 nm

Near-isotropic 3D optical nanoscopy with photon-limited chromophores

Jianyong Tang[1], Jasper Akerboom, Alipasha Vaziri, Loren L. Looger, and Charles V. Shank[1]

Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147

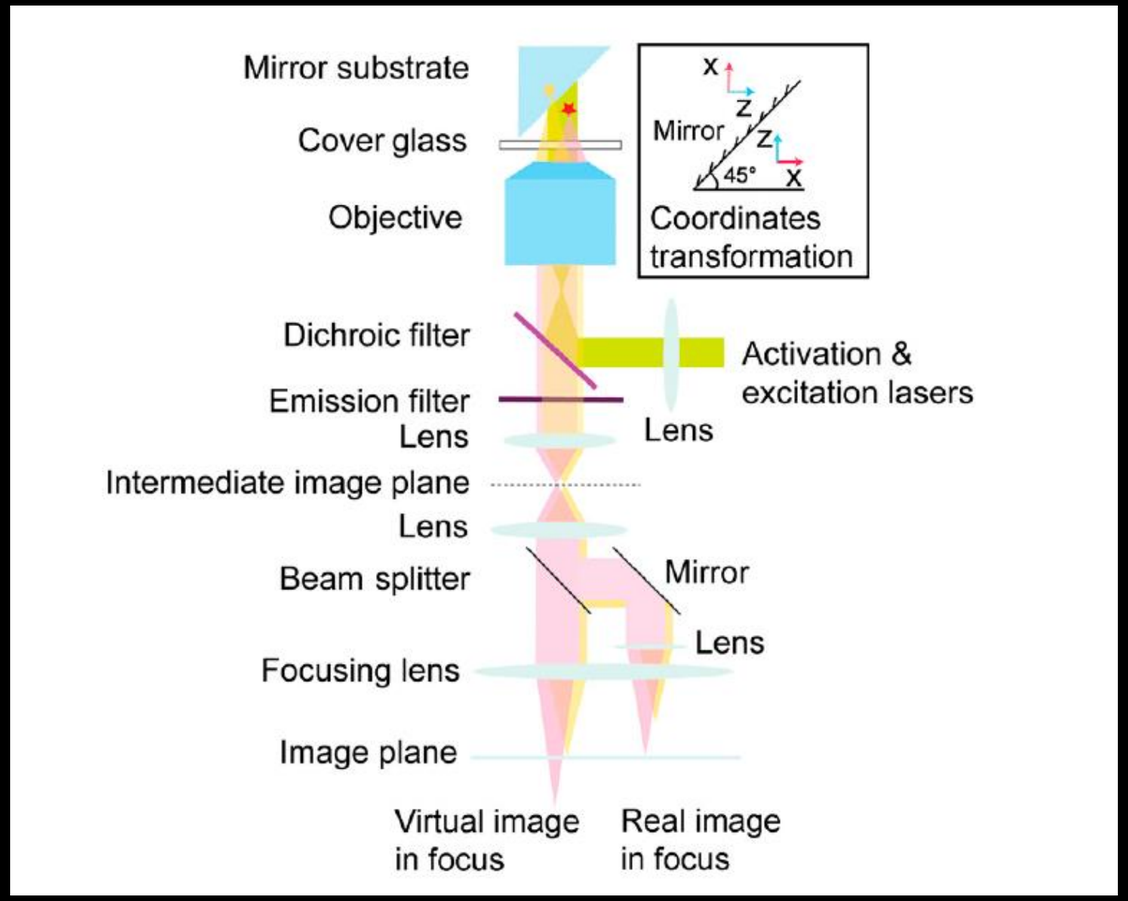
Contributed by Charles V. Shank, April 9, 2010 (sent for review December 28, 2009)

Imaging approaches based on single molecule localization break the diffraction barrier of conventional fluorescence microscopy, allowing for bioimaging with nanometer resolution. It remains a challenge, however, to precisely localize photon-limited single molecules in 3D. We have developed a new localization-based imaging technique achieving almost isotropic subdiffraction resolution in 3D. A tilted mirror is used to generate a side view in addition to

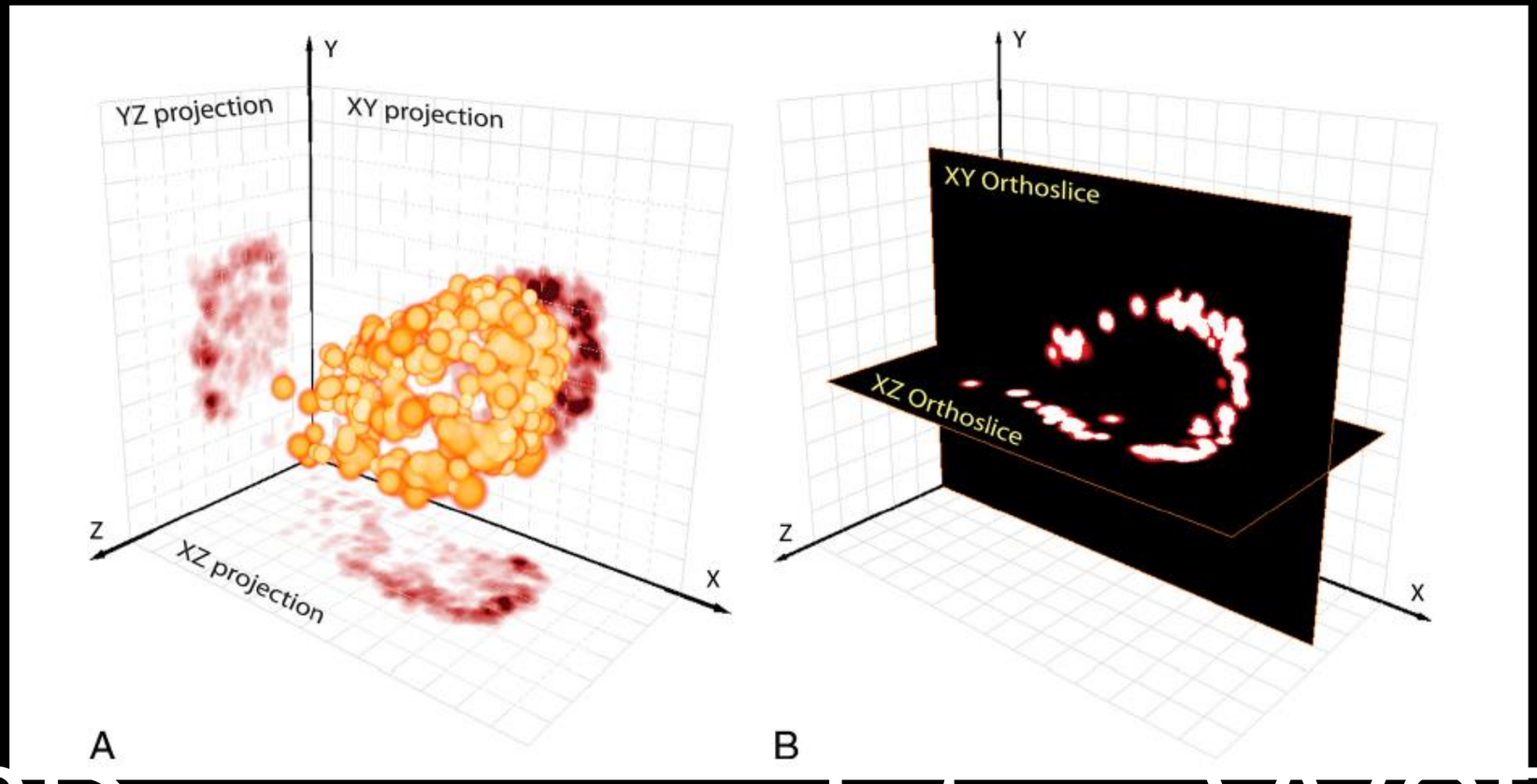
samples, allowing for higher imaging resolution according to the Nyquist criterion (21, 22).

However, each PA-FP molecule emits a limited number of photons (typically 200–1,000) (19, 23) before it bleaches (its "photon budget"), lower than typical synthetic dyes (>6,000) (24).

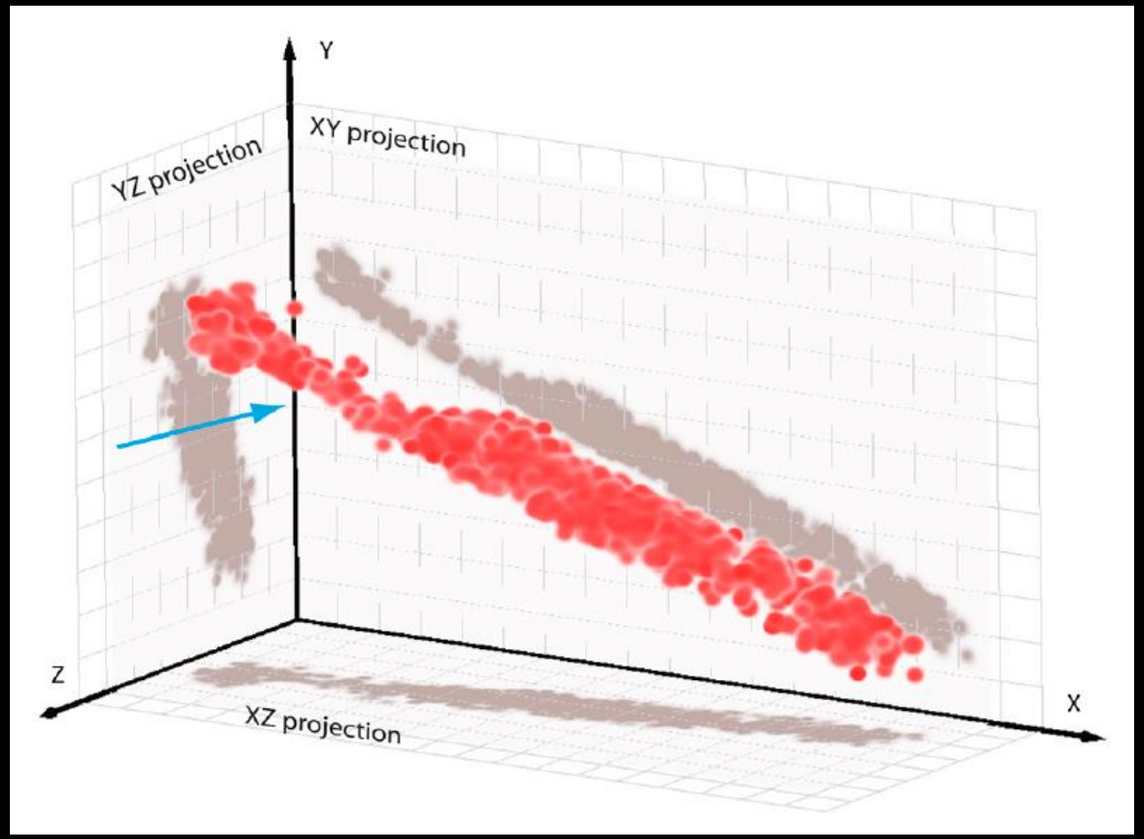
Recently, progress has been reported on extending single molecule-based SR techniques to three dimensions by generating



VVSRM principle and realization



A 3D-superresolution VVSRM image



A rat neuronal dendrite

Thank you ~ !