Resolution in Light Microscopy

Resolution is the distance that must separate two points in order for the points to be distinguishable*.

What is your resolution? E E E E E E E E

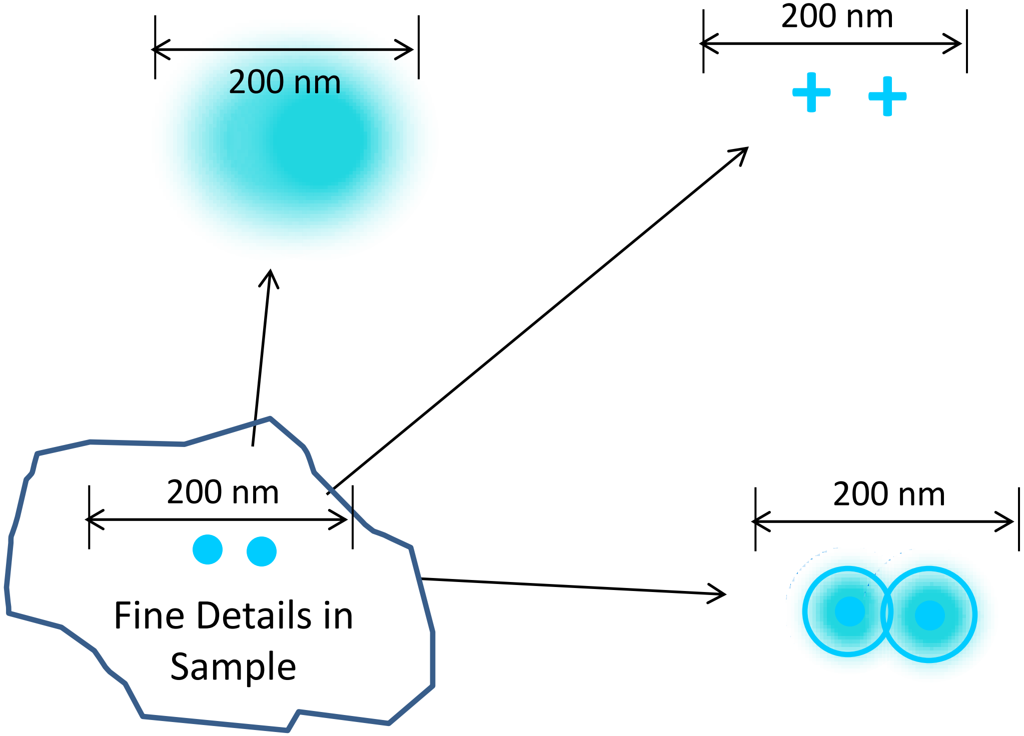
Due to a physical process called diffraction, even a perfectly designed microscope has an optical resolution no better than about 200 nm.

$$\text{Diffraction-limited resolution} = \frac{0.61\,\lambda}{1.4} = \sim 200\text{ nm}$$

Super Resolution Light Microscopy

Super resolution techniques offer resolution better than the traditional 200 nm diffraction-limit.

Wide-field or Confocal
(details lost)



<u>S</u>tochastic <u>O</u>ptical <u>R</u>econstruction <u>M</u>icroscopy (STORM)

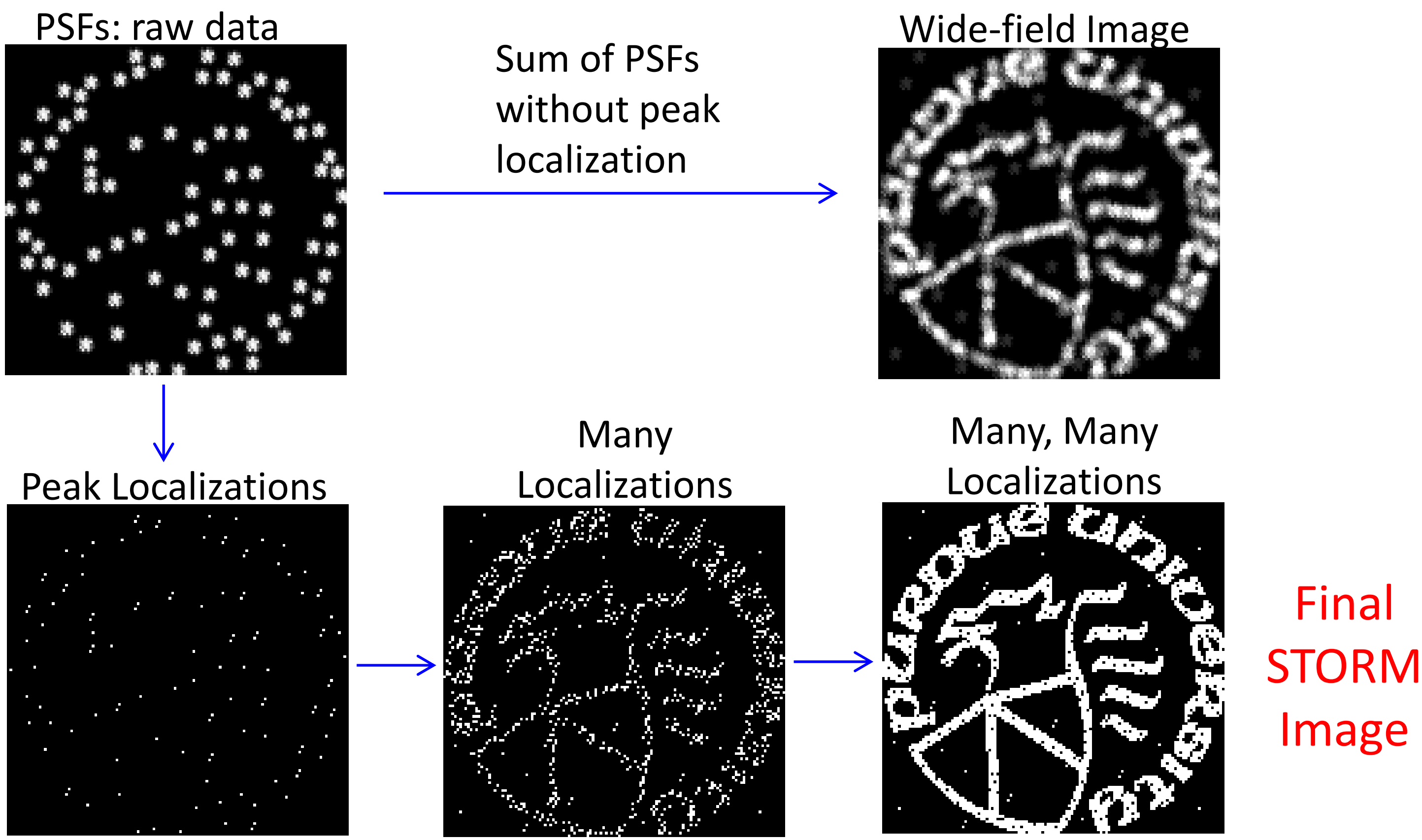
Localizes the fluorescent peaks of individual fluorophores. 20-50 nm resolution.

<u>S</u>tructured <u>I</u>llumination <u>M</u>icroscopy (SIM)

Uses the Moire effect to shift the sample's high frequencies to lower frequencies that can be resolved and then uses lots of math to recover the original. 100-200 nm resolution.

How STORM breaks the diffraction-limit

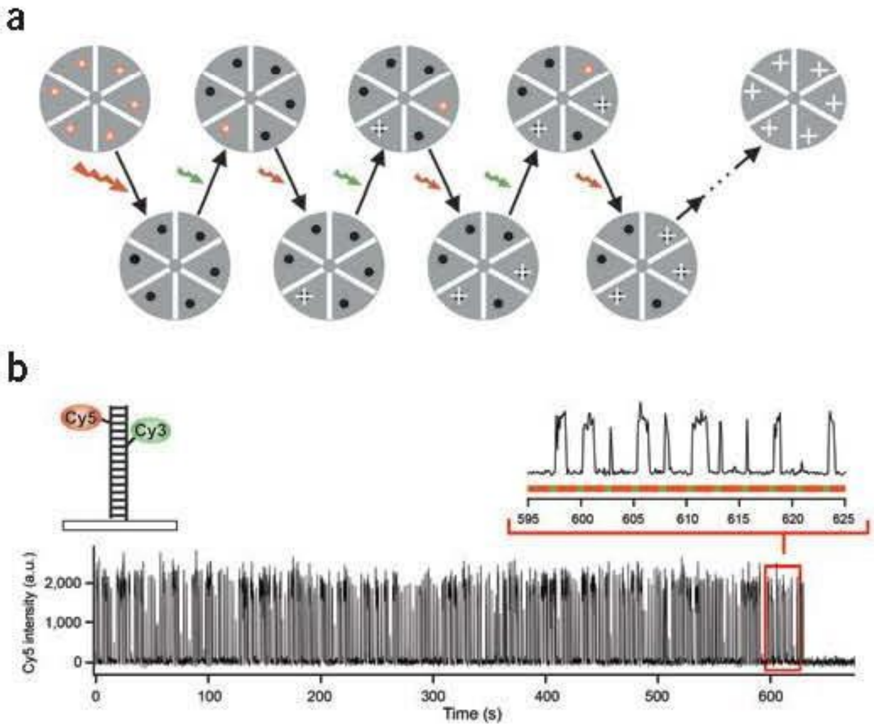
Using special dyes, it is possible to image only a few fluorophore molecules per frame. Thus, the peak of each molecule's image can be located. After thousands of localizations (& frames), the final 'image' is a graph of the location of the peak fluorescence from each molecule.



(aka Photoactivated Localization Microscopy, or PALM)

STORM dyes must be sparsely activatable

Photoswitchable dye pairs are best

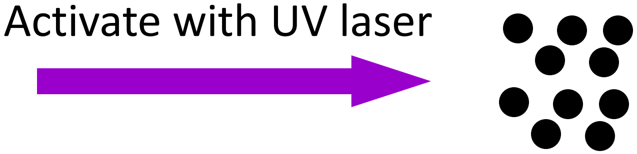


Rust, Nat Methods, 2006

Photoactivatable dyes can work

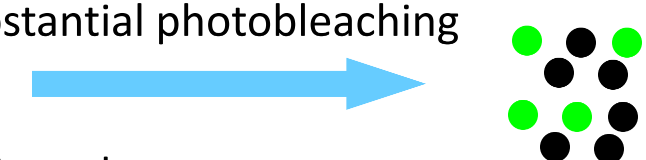
(aka PALM)

1. Activate with UV laser



2. Bleach with excitation laser

3. Collect image after substantial photobleaching

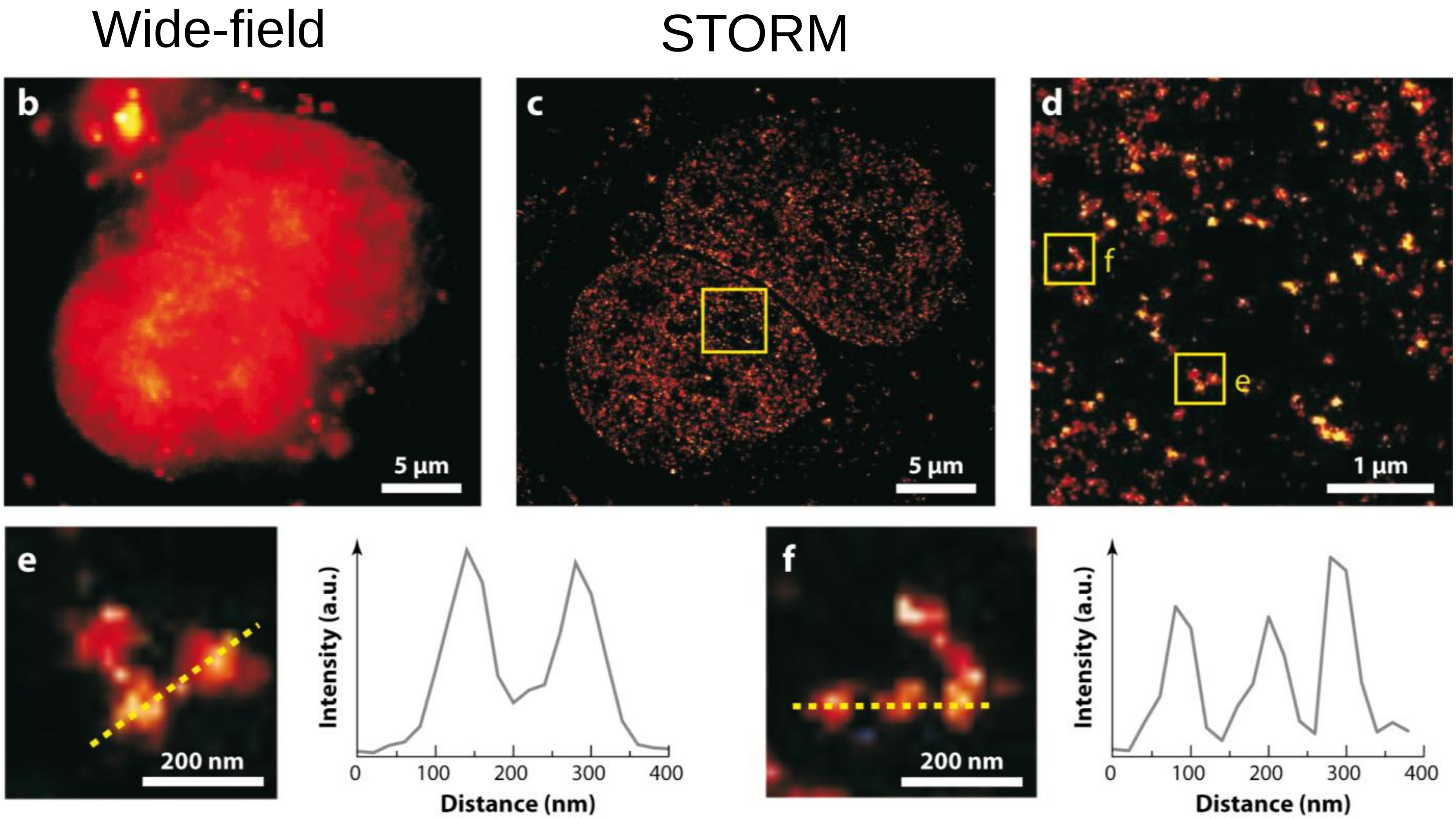


4. Repeat …

e.g. Betzig, Science, 2006

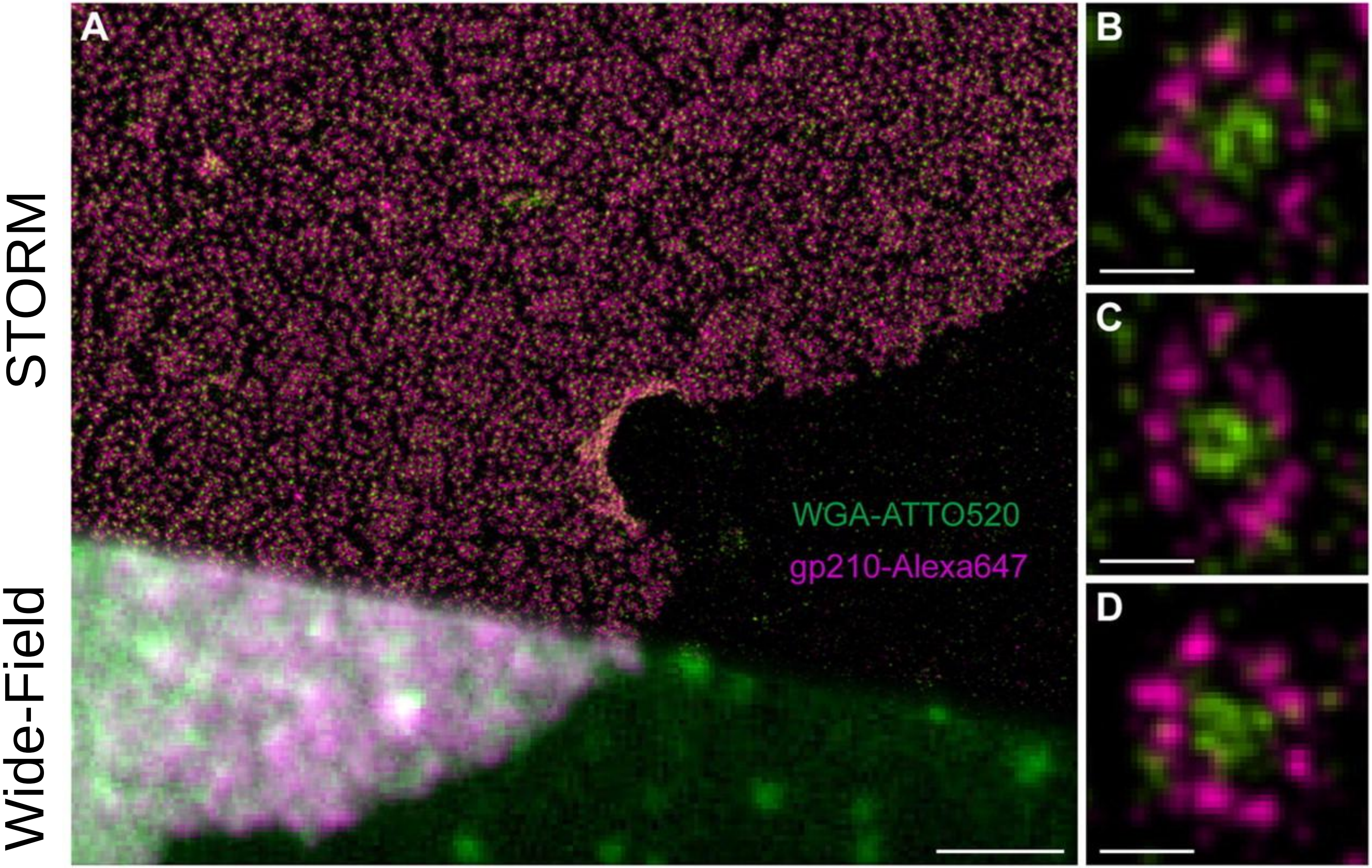
New dyes and strategies are emerging: e.g. dSTORM and the TMP tag / trimethoprim strategy for in vivo labeling (Wombacher, Nature, 2010)

Applications: Histone architecture in vivo



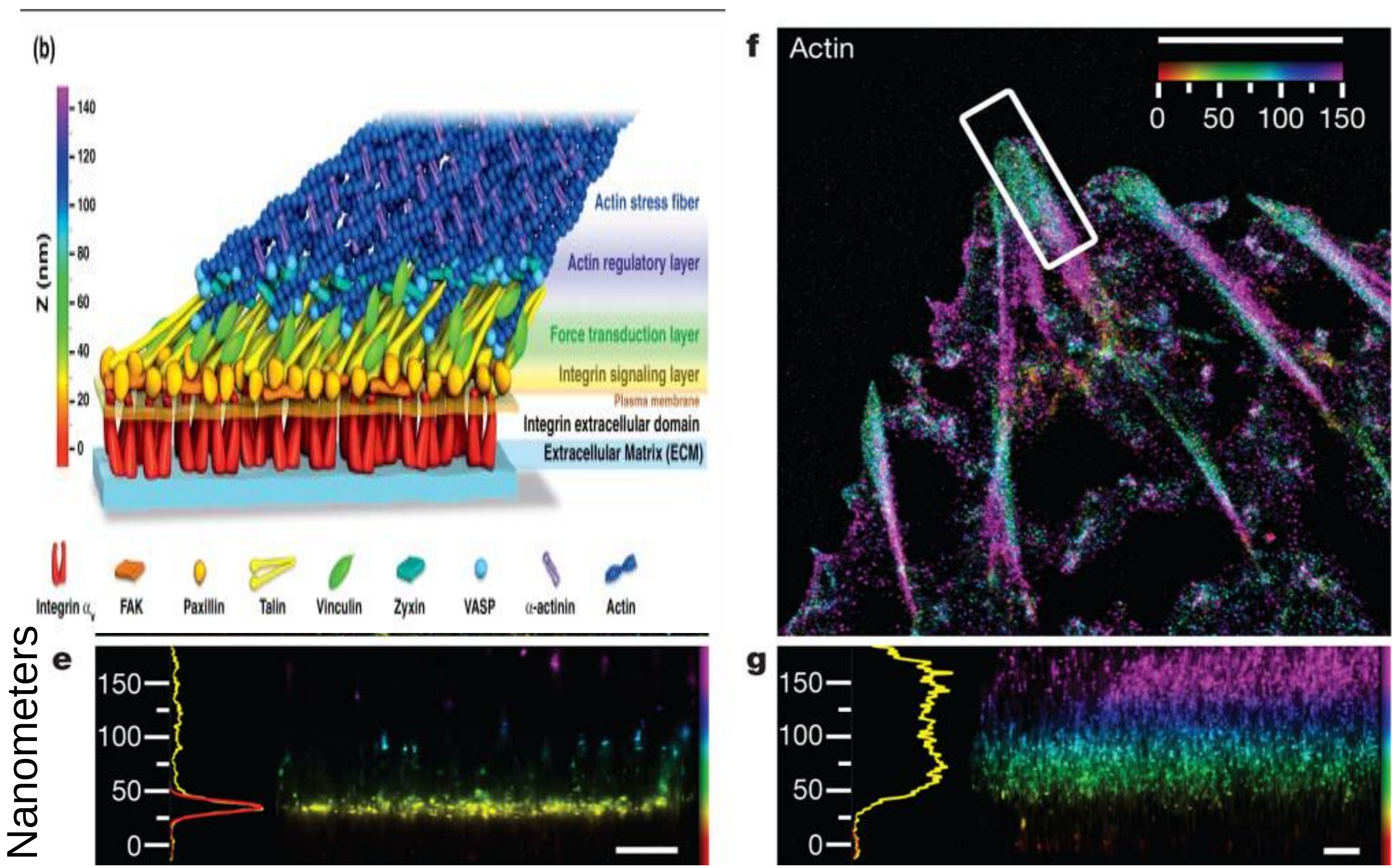
van de Linde S, et al. 2012.
Annu. Rev. Phys. Chem. 63:519–40

Applications: Nuclear Pore Structure



Löschberger A et al. J Cell Sci 2012;125:570-575

Applications: 3D Structure of Focal Adhesions (STORM works in 3D too!)



P Kanchanawong *et al. Nature* **468**, 580-584 (2010) doi:10.1038/nature09621