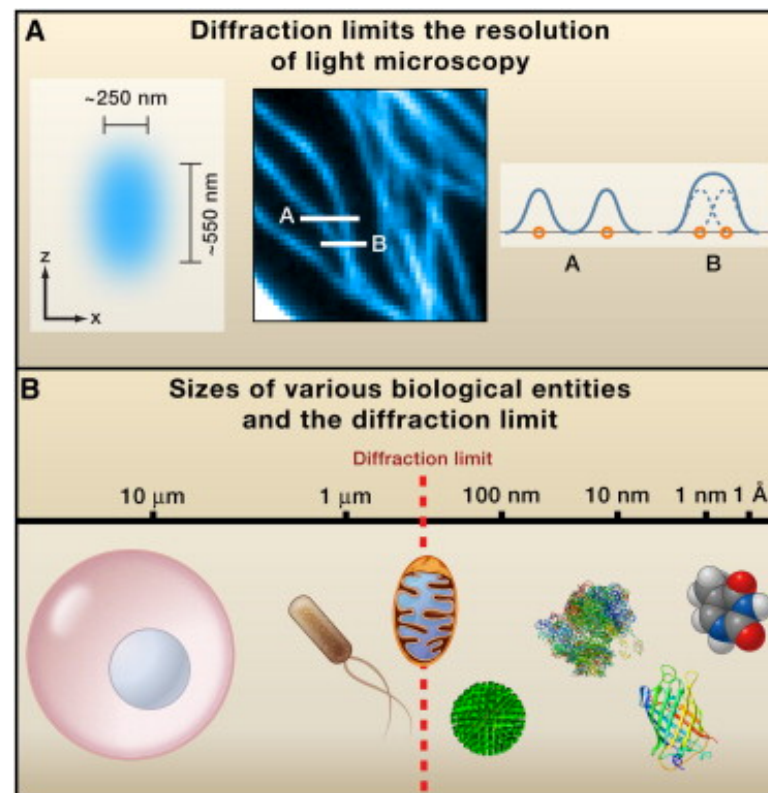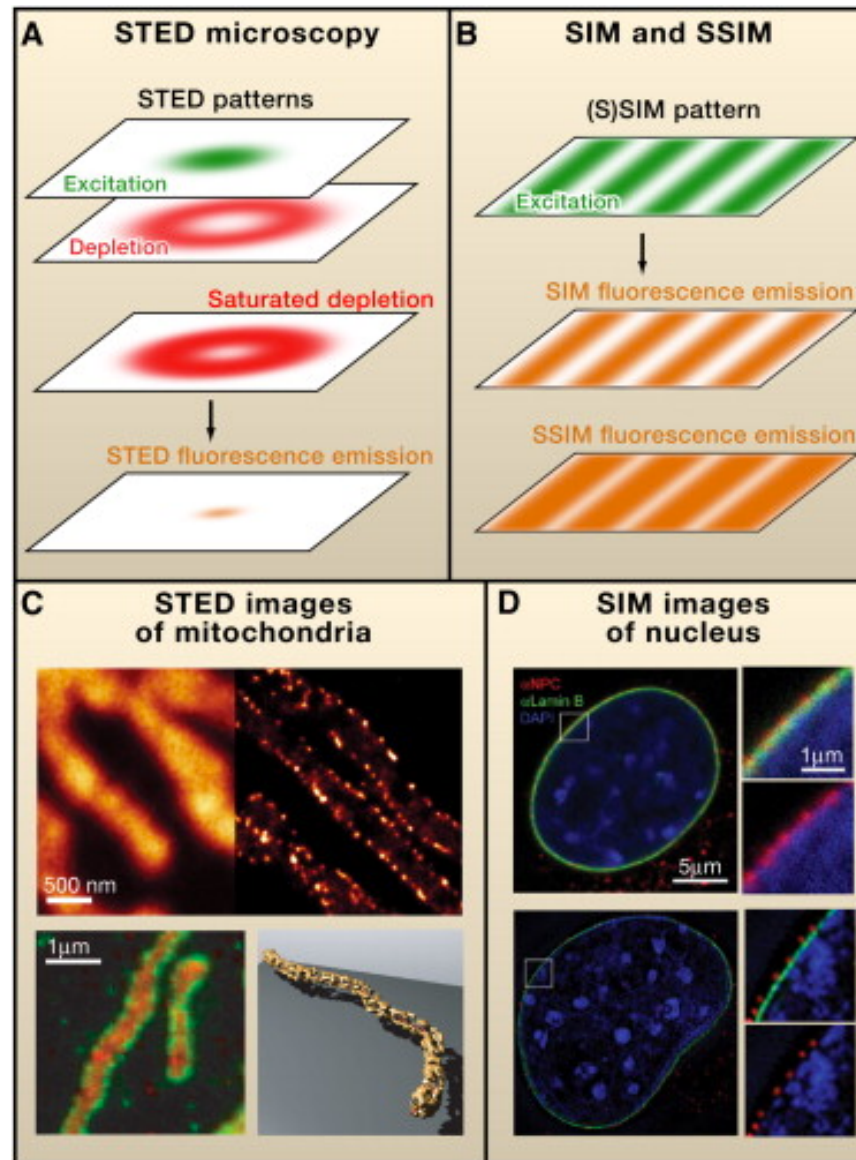# Why is detecting single molecules difficult?

$$d = \frac{\lambda}{2n \sin \theta} = \frac{\lambda}{2NA}$$
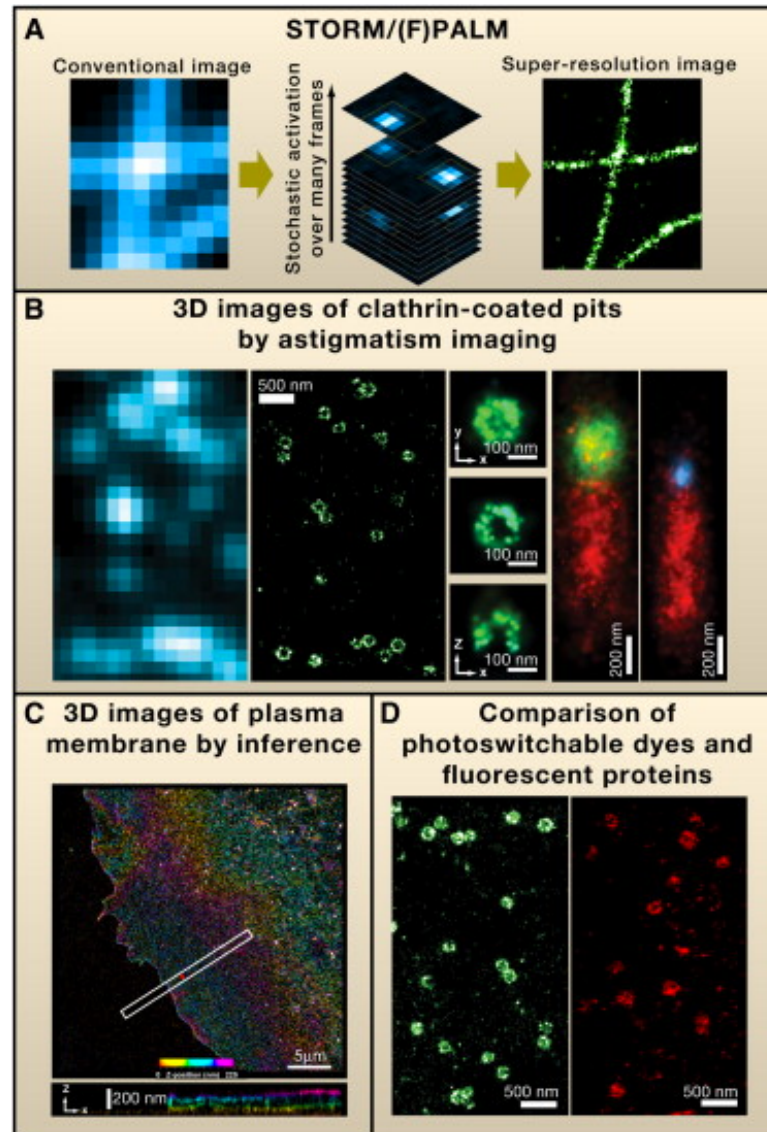

Huang et al Cell 2010: "Breaking the diffraction limit"

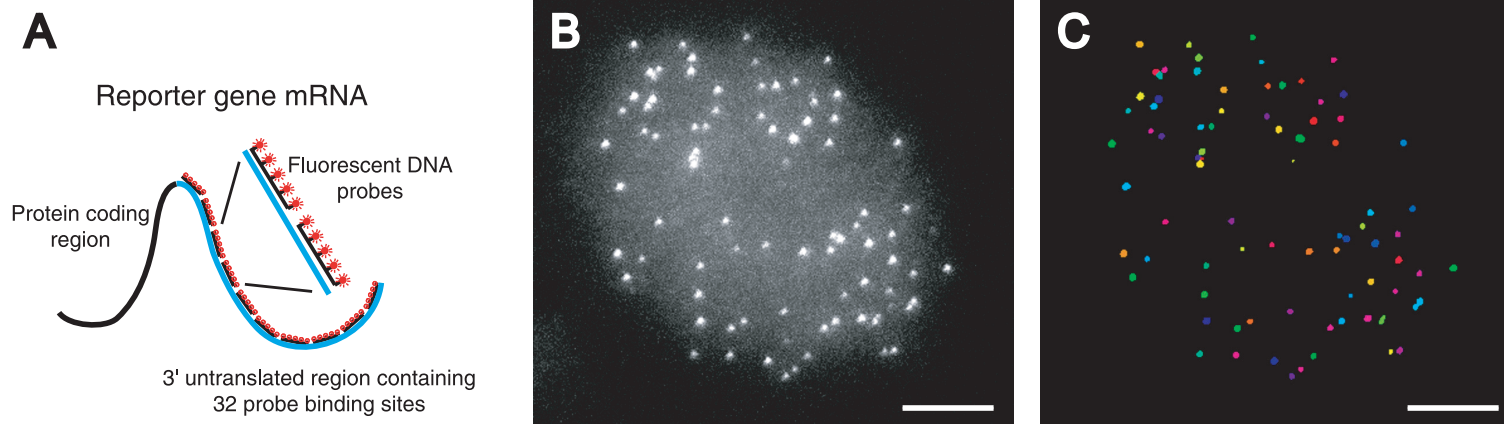| A | **STED microscopy** | B | **SIM and SSIM** |

A — STED microscopy

STED patterns

Excitation

Depletion

Saturated depletion

STED fluorescence emission

B — SIM and SSIM

(S)SIM pattern

Excitation

SIM fluorescence emission

SSIM fluorescence emission

C — STED images of mitochondria

500 nm

1µm

D — SIM images of nucleus

αNPC
αLamin B
DAPI

1µm

5µm

stimulated emission depletion (STED) microscopy

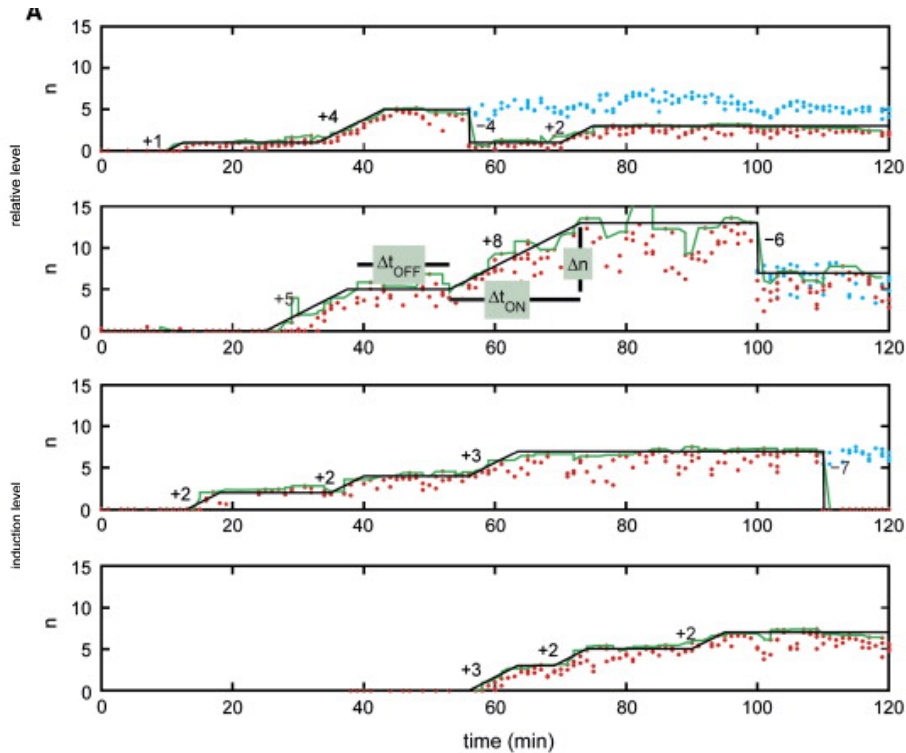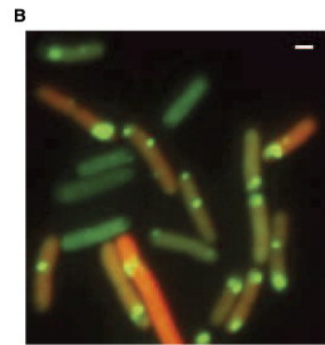Structured illumination microscopy (SIM) and saturated SIM (SSIM)

A STORM/(F)PALM

Conventional image → Stochastic activation over many frames → Super-resolution image

B 3D images of clathrin-coated pits by astigmatism imaging

500 nm

100 nm

100 nm

100 nm

200 nm

200 nm

C 3D images of plasma membrane by inference

5μm

200 nm

D Comparison of photoswitchable dyes and fluorescent proteins

500 nm

500 nm

Visualizing single molecule mRNA events

Visualizing mRNA molecules

FISH: fluorescence in situ hybridization

A Reporter gene mRNA

Fluorescent DNA probes

Protein coding region

3' untranslated region containing 32 probe binding sites

B

C

Must fix cells- but high throughput/ spatial information

Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S. Stochastic mRNA Synthesis in Mammalian Cells. PLoS Biol 2006; 4(10): e309

Visualizing mRNA molecules

MS2-GFP tags

Get kinetics- but have to modify genes

Visualizing single molecule protein events

Proteins produced in bursts
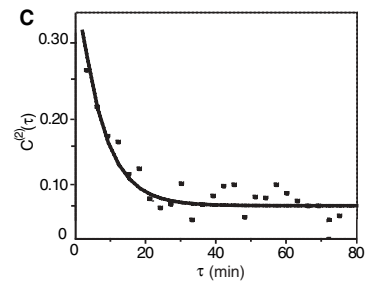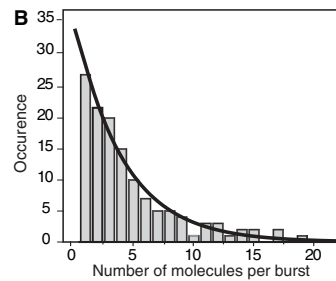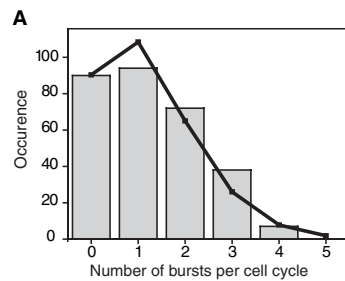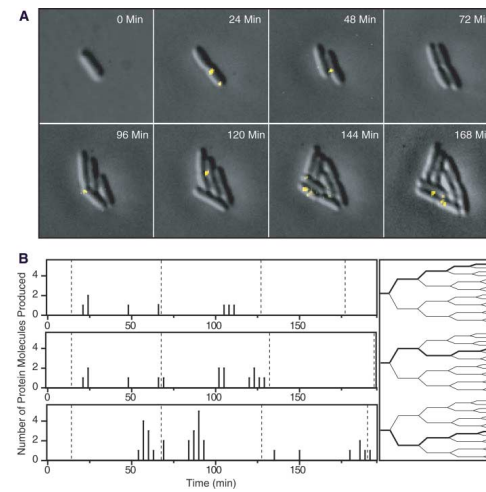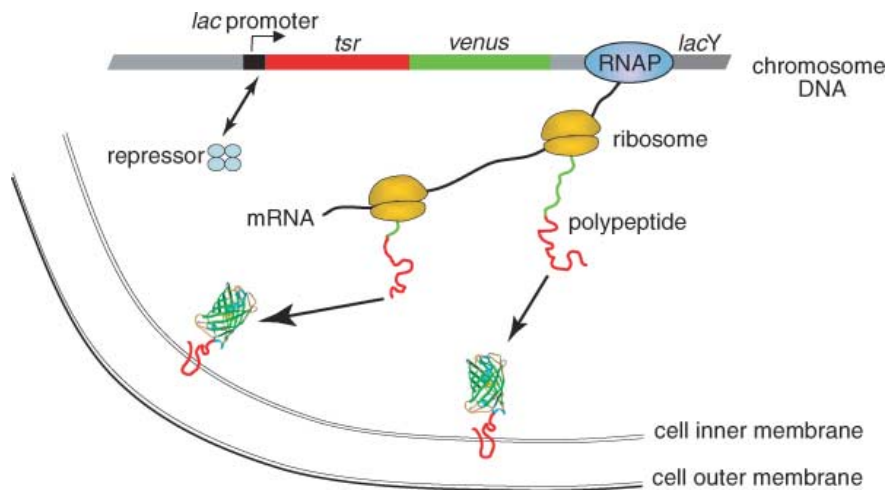
Yu et al Science 2006

a

PDMS

Cells

Closed chamber

Laser focus

Control channels

Flow channel

Objective

b

FDG $\xrightarrow{\beta\text{-gal}}$ Fluorescein + 2 Galactose

c

100 μm

d

Budding yeast

20 μm

e

Fluorescein (nM)

Time (min)

f

Probability

Slope (pM min^{-1})

Cai, Long, Nir Friedman, and X. Sunney Xie. Nature 440.7082 (2006): 358.

a

b

c

a

b *E. coli*, pLac

c *S. cerevisiae*, pGAL1
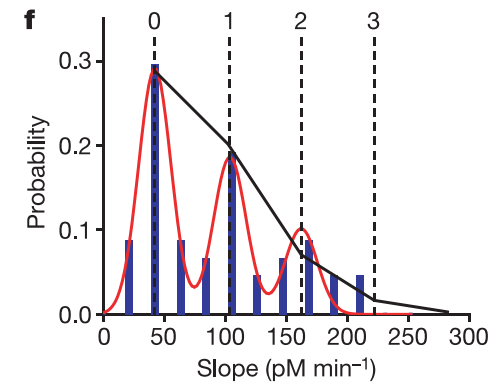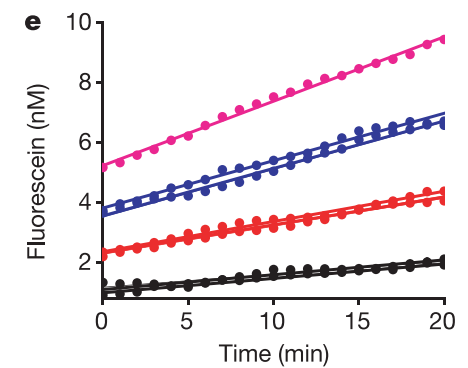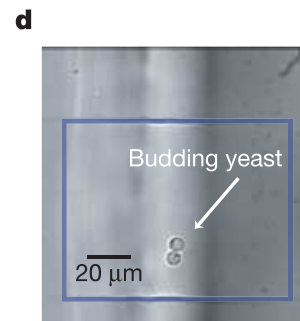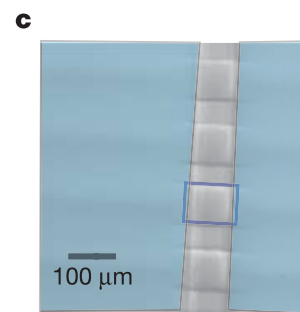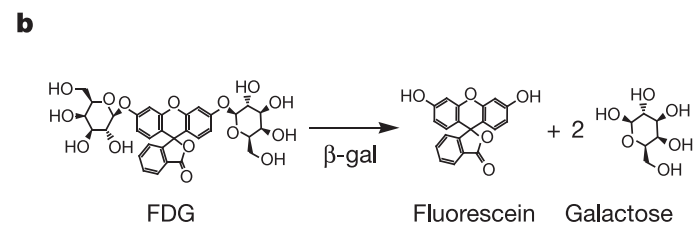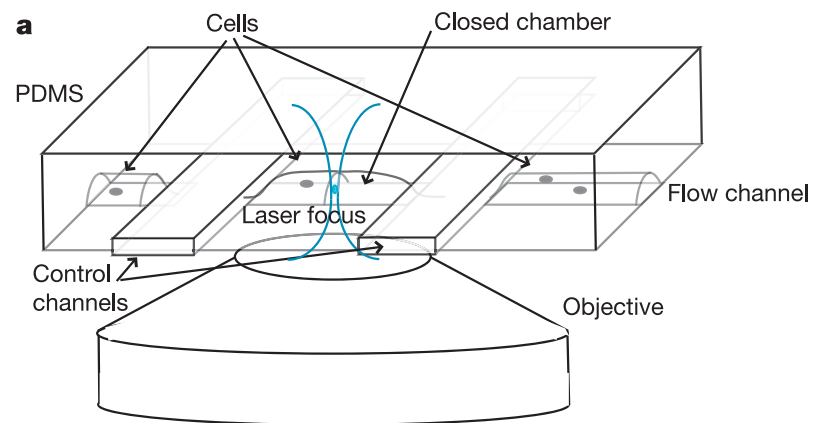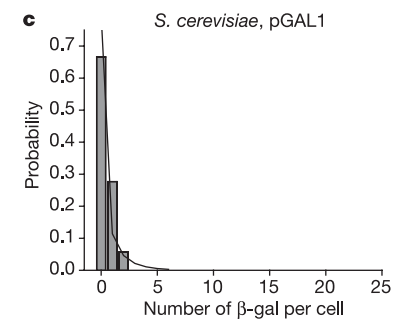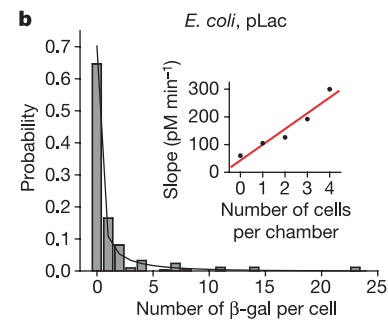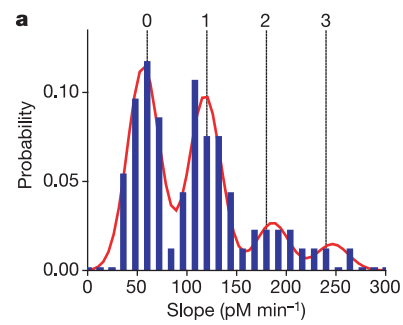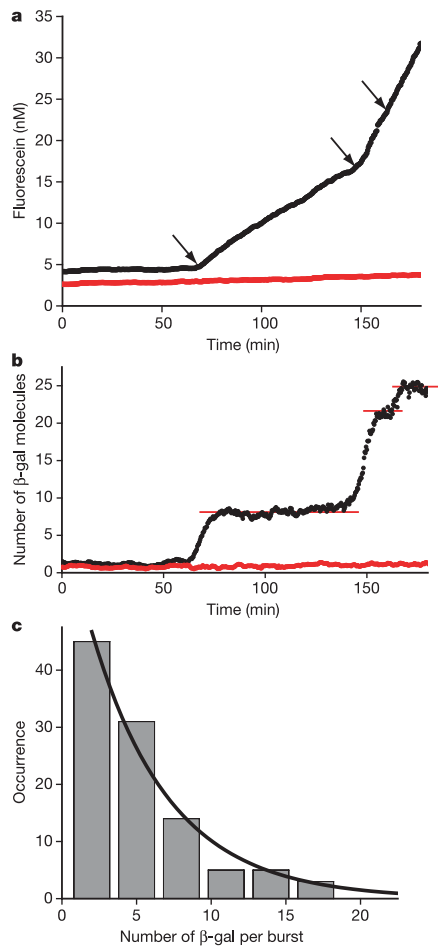
Protein distribution
well described
by Gamma distribution

$$p(x) = \frac{x^{a-1}e^{-x/b}}{b^a\Gamma(a)}$$

Cai, Long, Nir Friedman, and X. Sunney Xie. Nature 440.7082 (2006): 358.