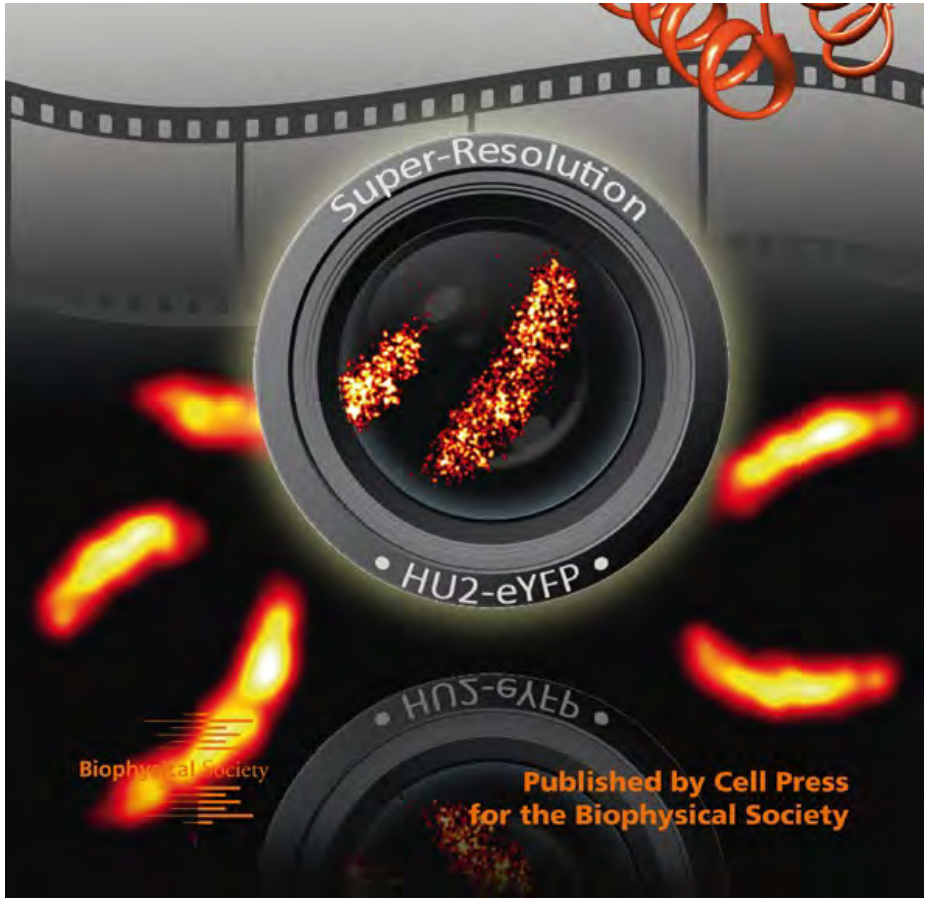
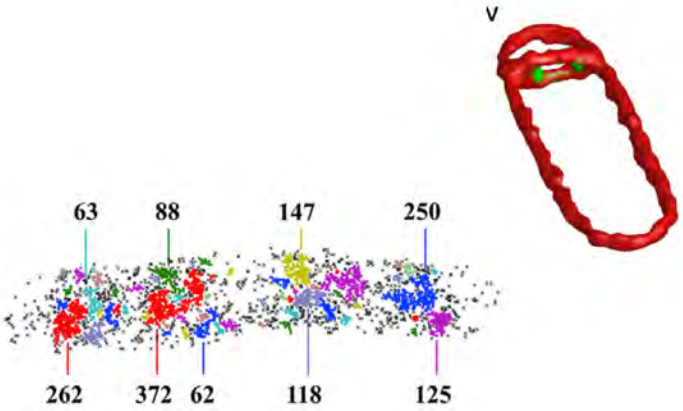
Super-resolution and single molecule imaging of bacteria

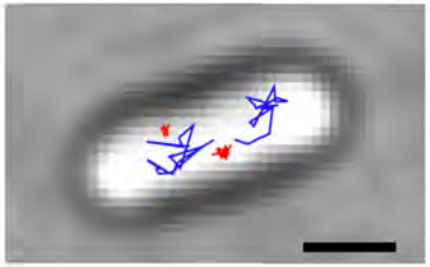


Steven Lee

Dr Seamus Holden
Centre for Bacterial Cell Biology
seamus.holden@ncl.ac.uk

Super-resolution and single molecule imaging for microbiology

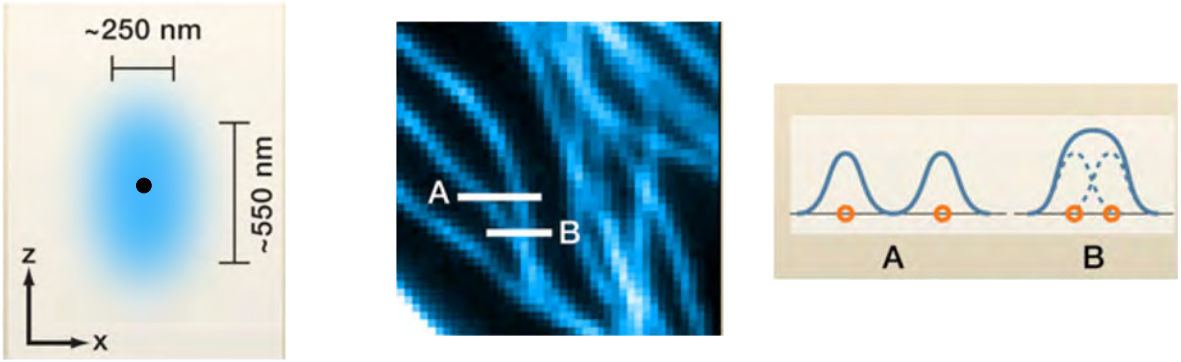




- These techniques dramatically increase resolution and allow us to probe the behaviour of single proteins in live cells
- Revolutionary throughout biology
- But particularly useful in bacteria due to their small size and their relative simplicity

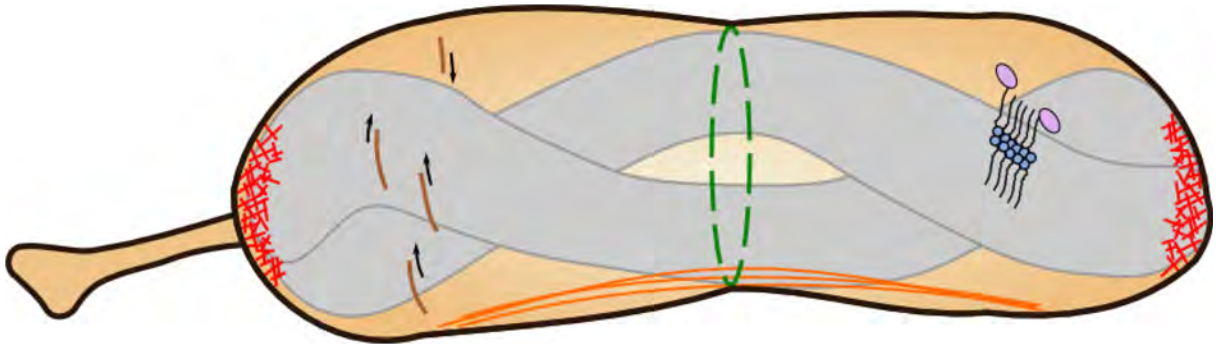
Diffraction poses serious problems in bacteria…

Diffraction limits the resolution of light microscopy:



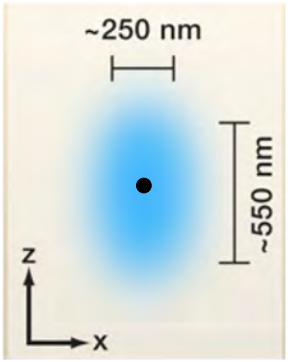
Huang et al, *Cell* (2010)

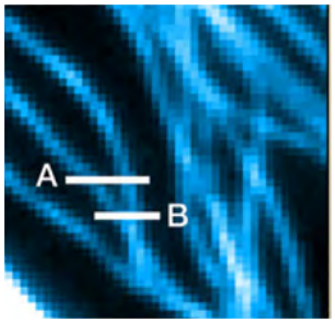
In practice this is a serious limitation!

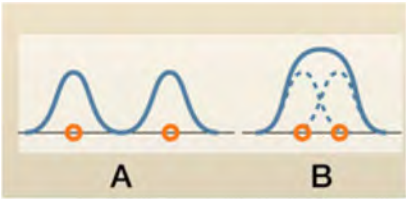


Super-resolution microscopy resolves this problem

Diffraction limits the resolution of light microscopy:







Huang et al, *Cell* (2010)

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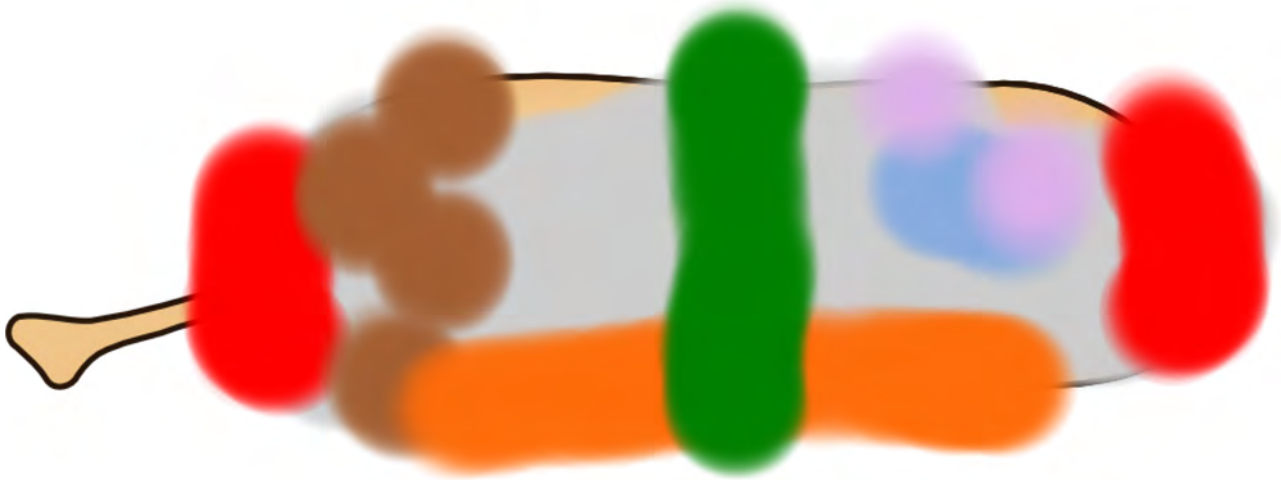


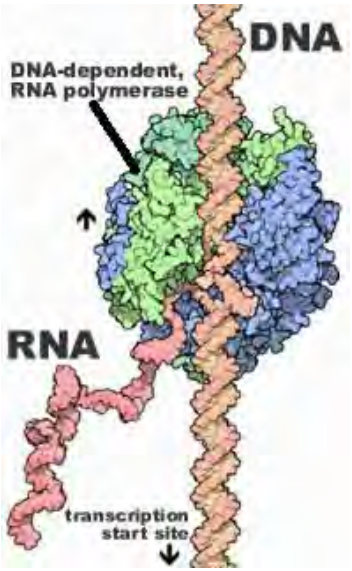
Super-resolution microscopy to the rescue…

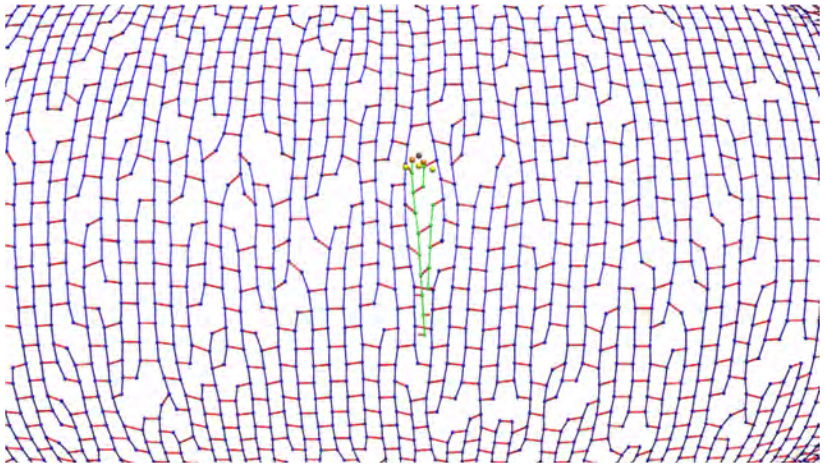
Single molecule microbiology

Biology works at the single molecule level!

Examples:

- Chromosome is a single molecule!
- Gene expression is performed by a single molecule nanomachine - RNA polymerase
- Cell wall remodelling is performed by single multi-enzyme complexes

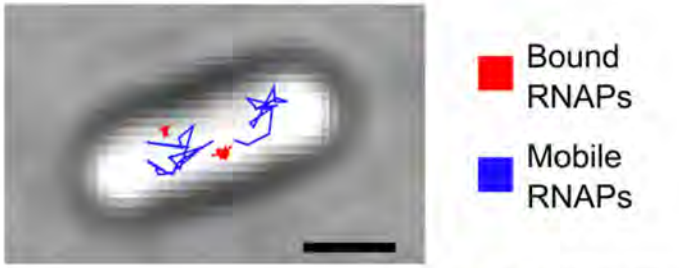




Nguyen et al PNAS 2015

Single molecule microbiology

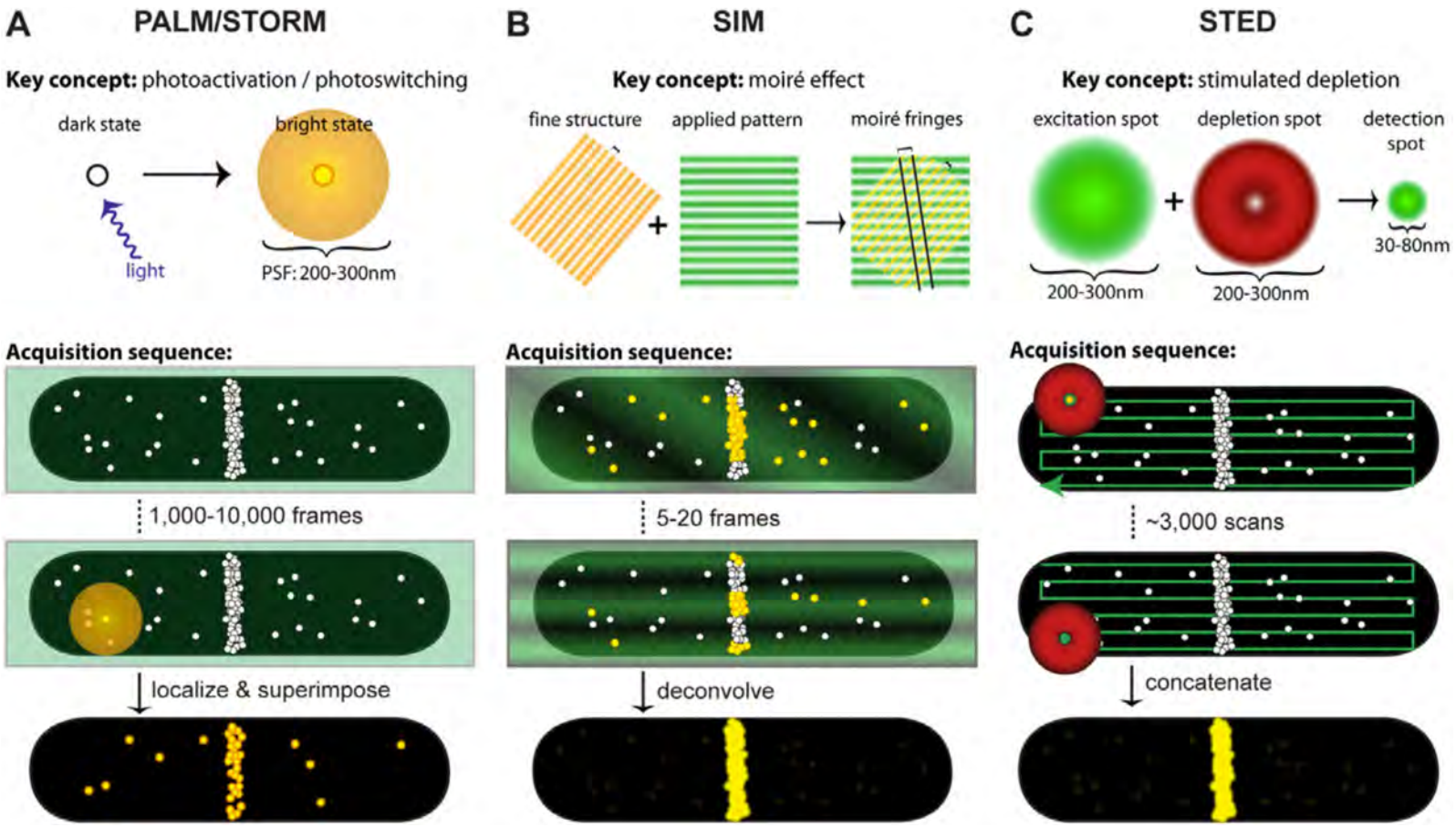
Different copies of a protein will be in multiple different states in the cell
Eg, RNAP bound/ unbound to DNA:



Stracy et al PNAS 2015

"Ensemble" methods average over these different states -
To get accurate information we need to measure one molecule at a time

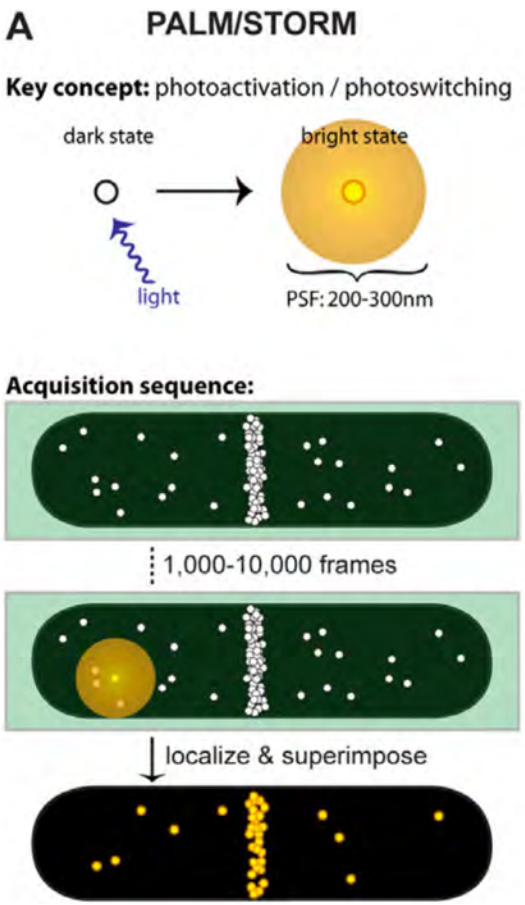
Super-resolution methods



Resolution: 20 nm 100 nm ~50 nm

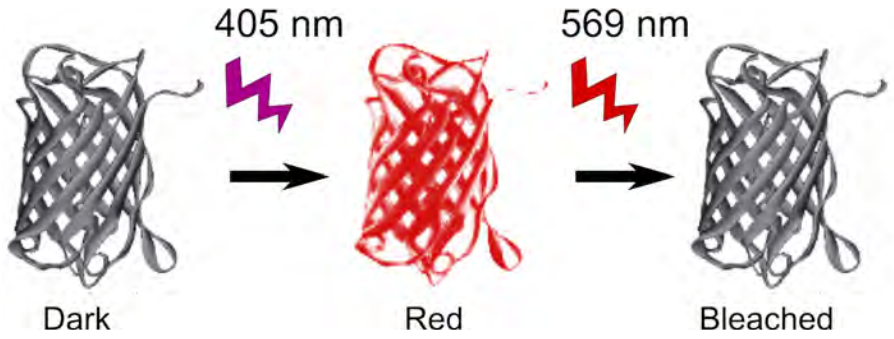
Coltharp & Xiao, Cell Microbiol 2012

Localization microscopy: principle

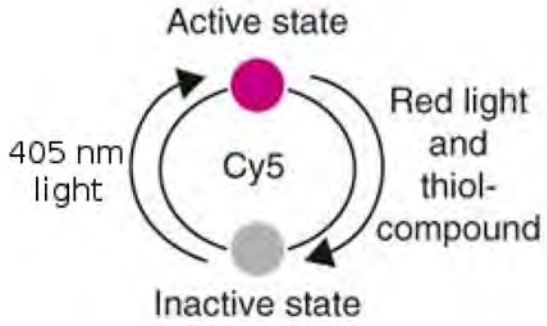


It’s all about making fluorophores blink!

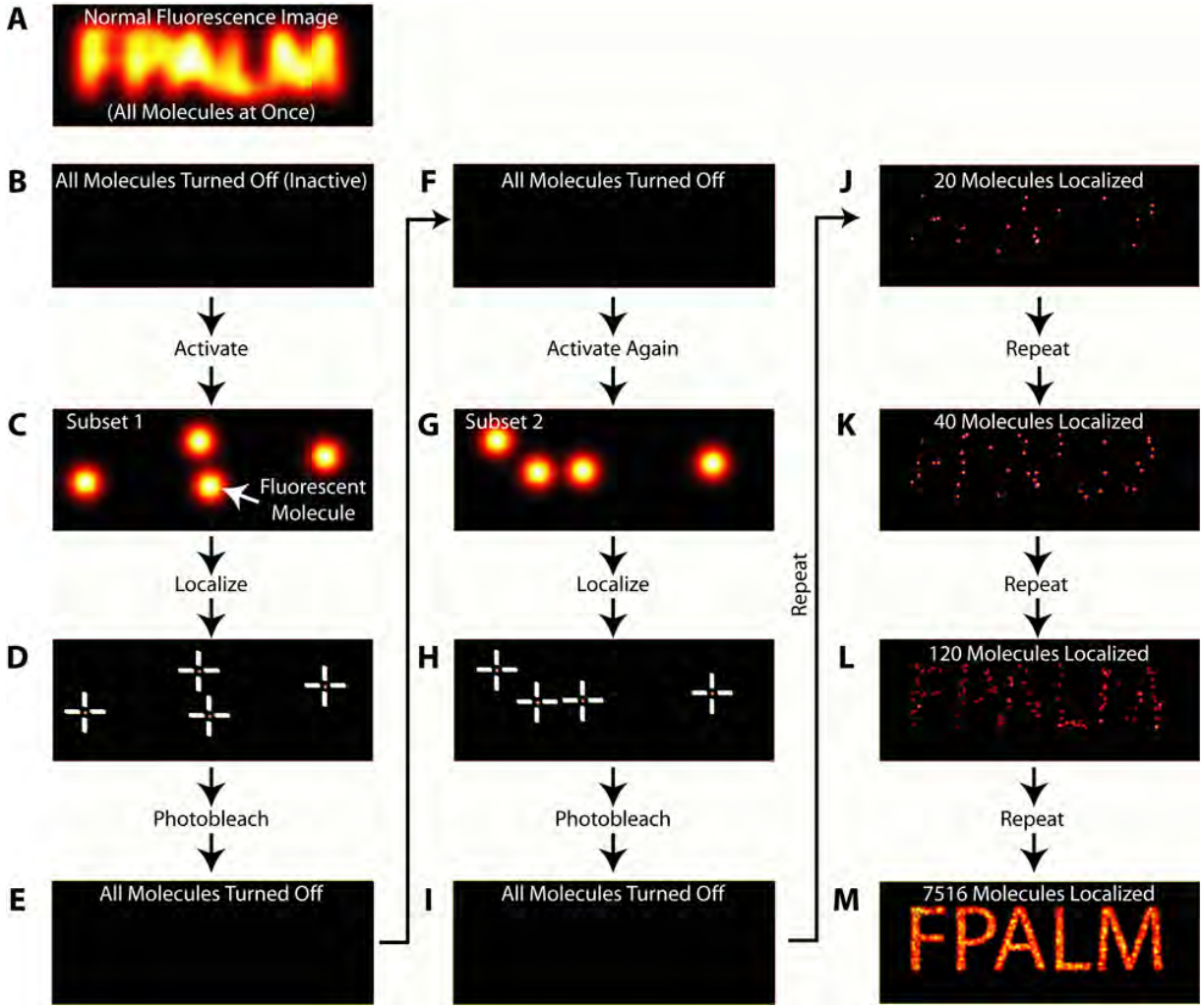
Photoactivatable fluorescent proteins:



Photoswitchable organic dyes



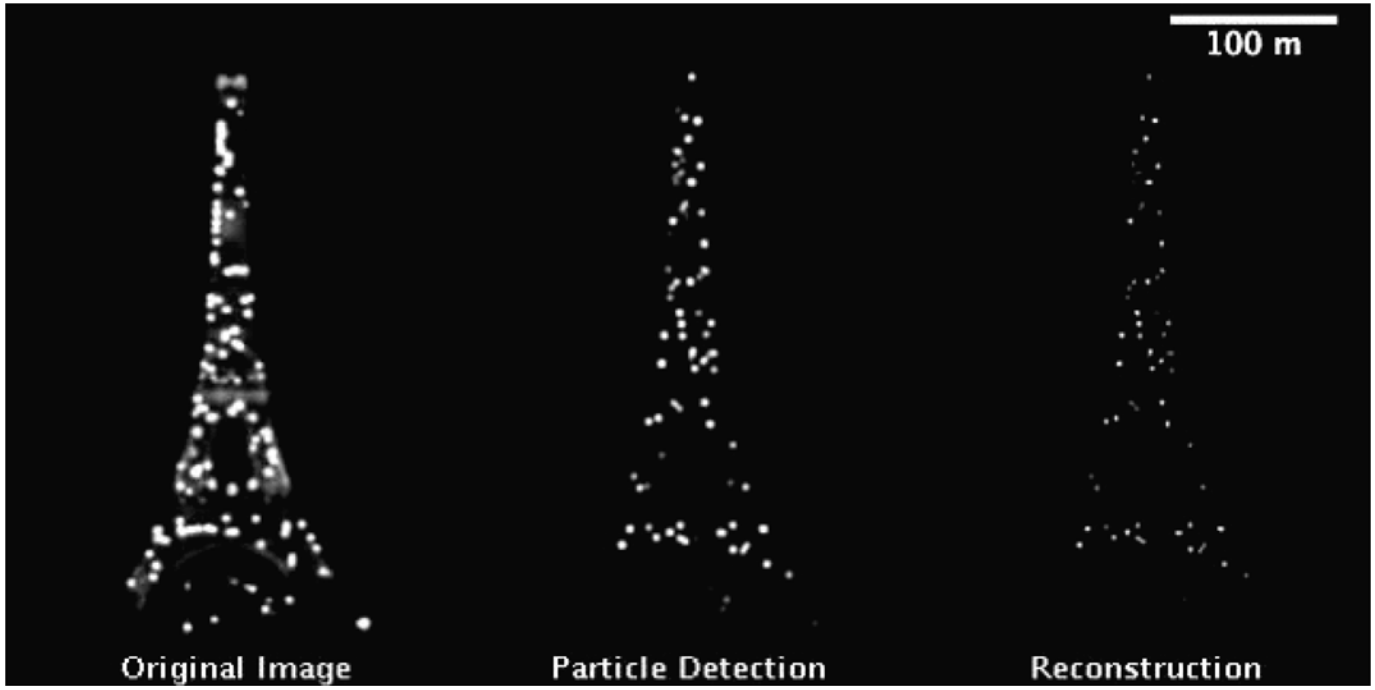
… and then finding their centres



Stochastic Optical Reconstruction Microscopy (STORM)/
Photoactivation Localization Microscopy (PALM)

Betzig et al., *Science (*2006)
Rust et al, *Nat. Methods* (2006)
Hess et al., *Biophys. J.* (2006)

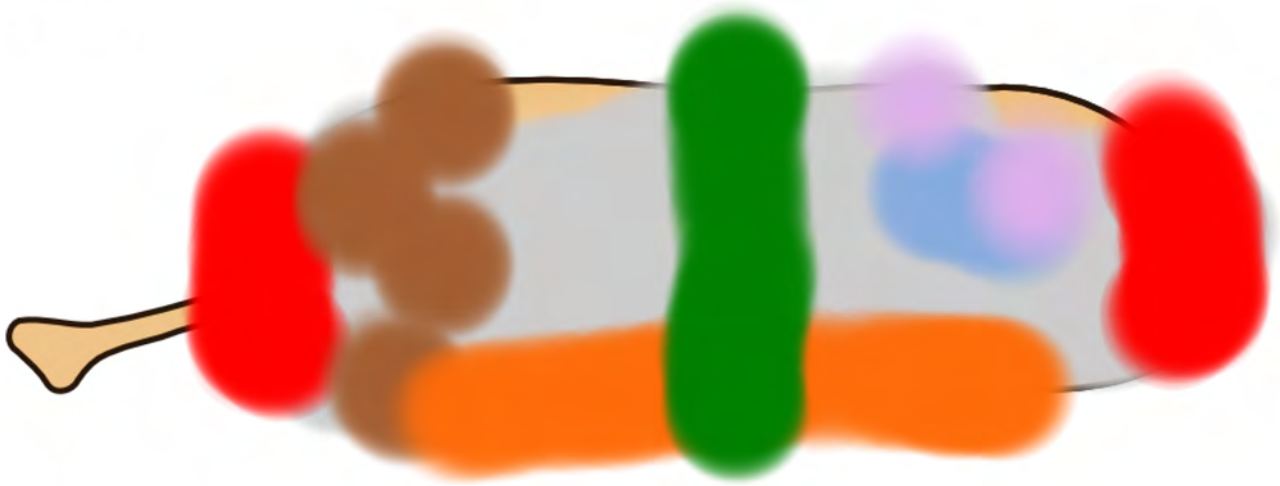
STORM over the Eiffel Tower



Henriques et al., *Nat. Methods* (2010)

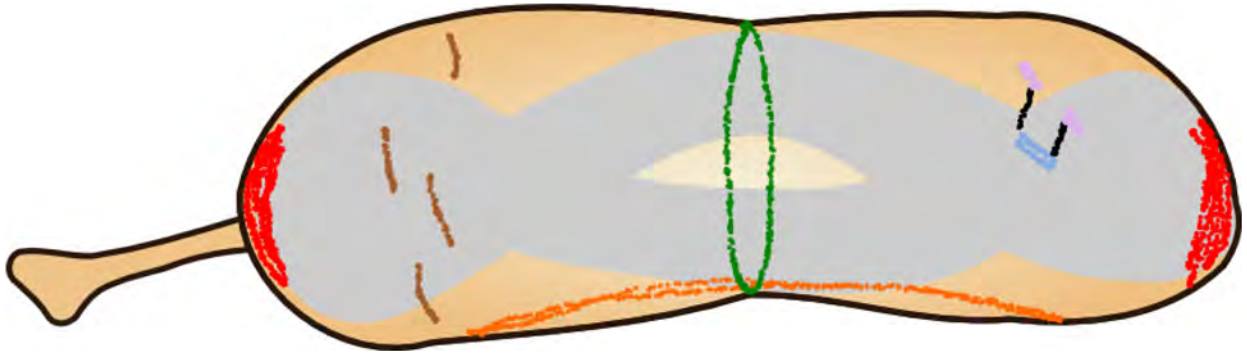
Resolution

Spatial resolution
XY: 25 nm
Z: 100 nm



Resolution

Spatial resolution
XY: 25 nm
Z: 100 nm



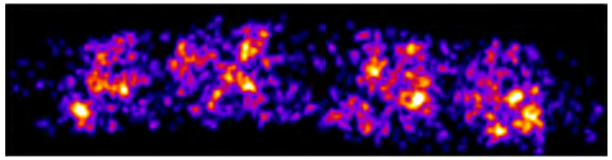
Time (typ.): 3 – 5 mins
Time (best): 2 s (FPs), 30 ms (dyes)

Advantages:
- Highest resolution of SR microscopies
- Single molecule information

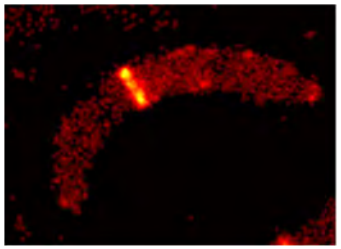
Disadvantages:
- Requires high laser powers

→ phototoxicity problems

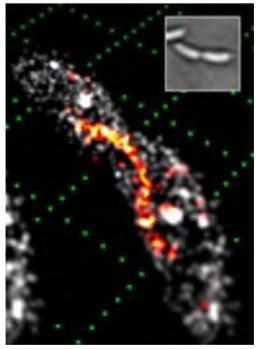
Localization microscopy: applications



RNA polymerase

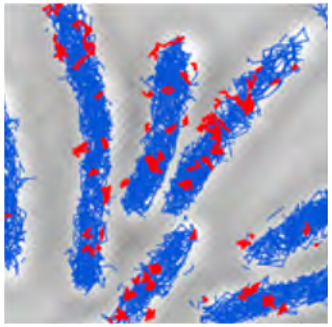


FtsZ



Crescentin

CheY

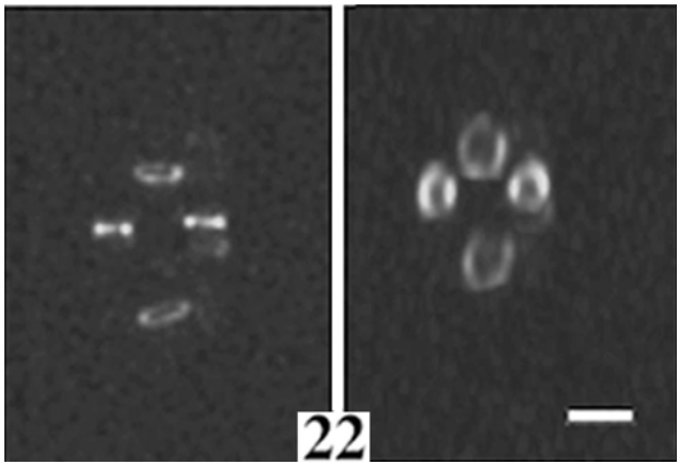


DNA polymerase

Endesfelder, Finan, Holden et al., *Biophys J.* (2013)
Holden et al, *PNAS* (2014)
Greenfield et al, *Plos Biol* (2009)
Lew et al, PNAS (2011)
Uphoff et al, *PNAS* (2013)

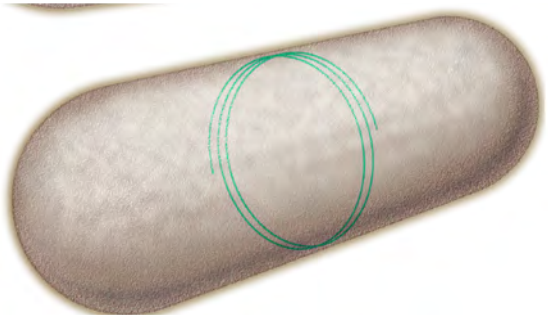
FtsZ ultrastructure

Diffraction limited imaging of the cell division cytoskeletal “Z-ring” look continuous:



Sun & Margolin J. Bac 1998

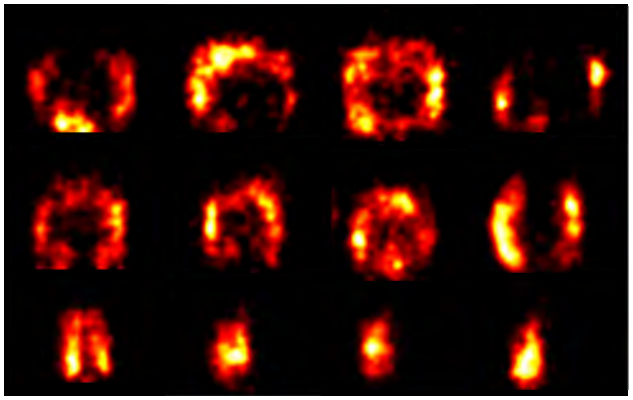
Consistent with the idea of a force generating constrictive ring:



Erickson et al. Mirco & Mol Bio Rev 2010

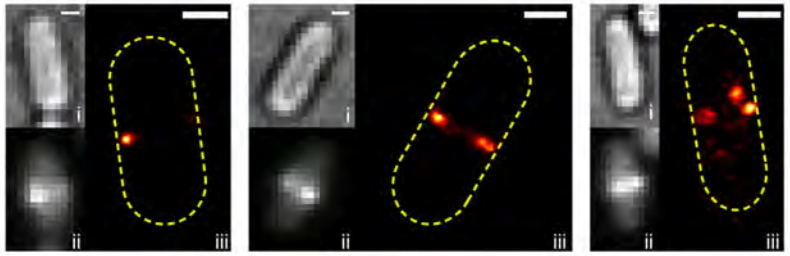
Super-resolution suggests a patchy Z-ring

C. crescentus 3D PALM



Holden et al PNAS 2014

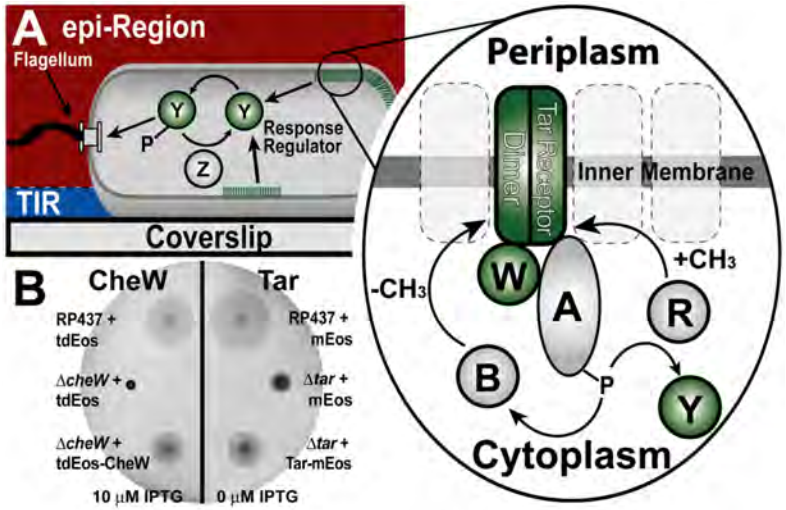
E. coli 2D PALM



Buss et al PLoS Genetics 2015

More on this next time…

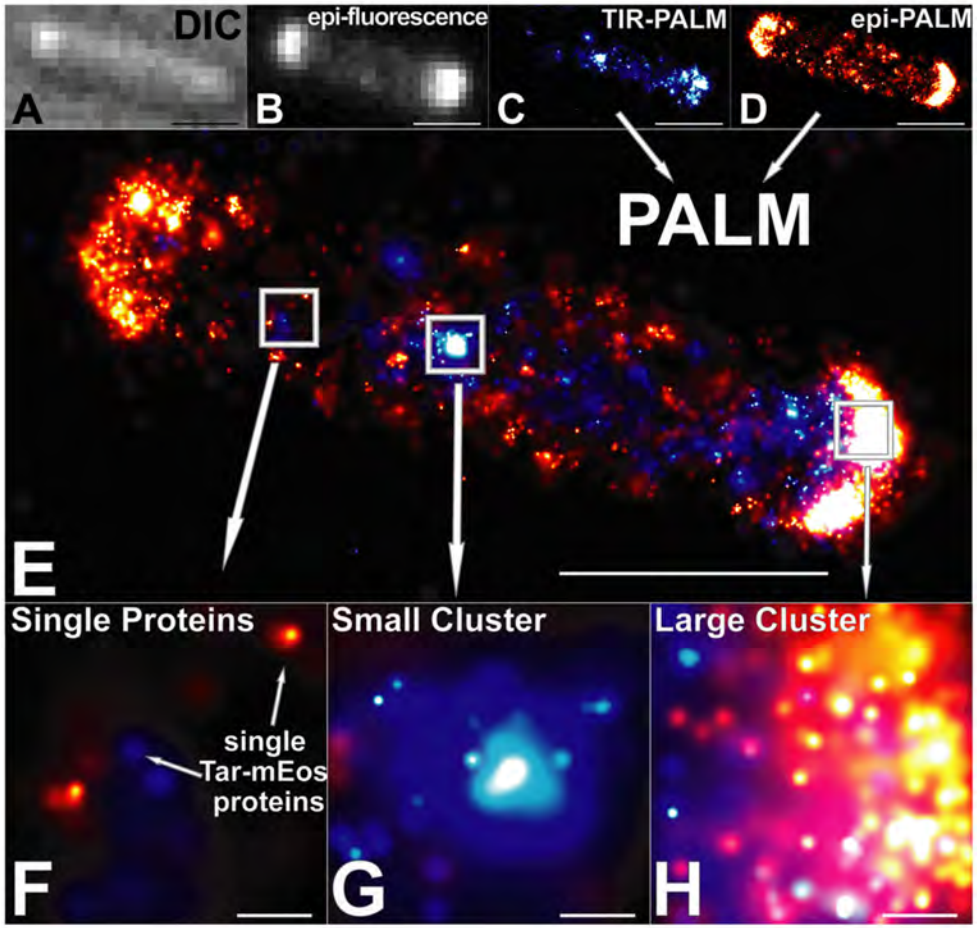
Chemotaxis sensors



Tar proteins senses chemicals outside of cell
Large clusters of Tar act cooperatively to amplify signals
How are clusters organised?

Greenfield et al PLoS Biol 2009

Chemotaxis sensors



Continuously varying distribution of cluster sizes

→ Suggests stochastic nucleation (ie no defined cluster size)

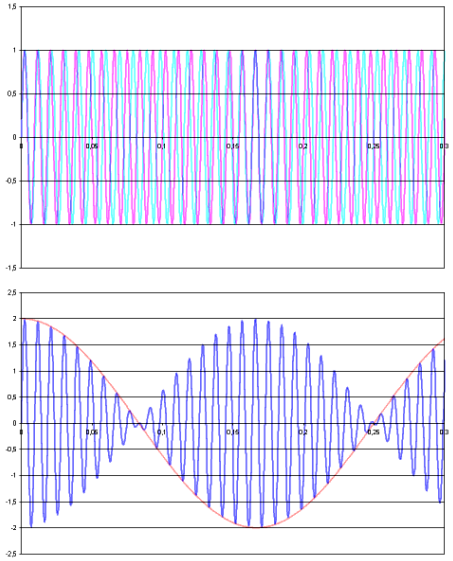
→ Potential explanation for spontaneous polar clusters

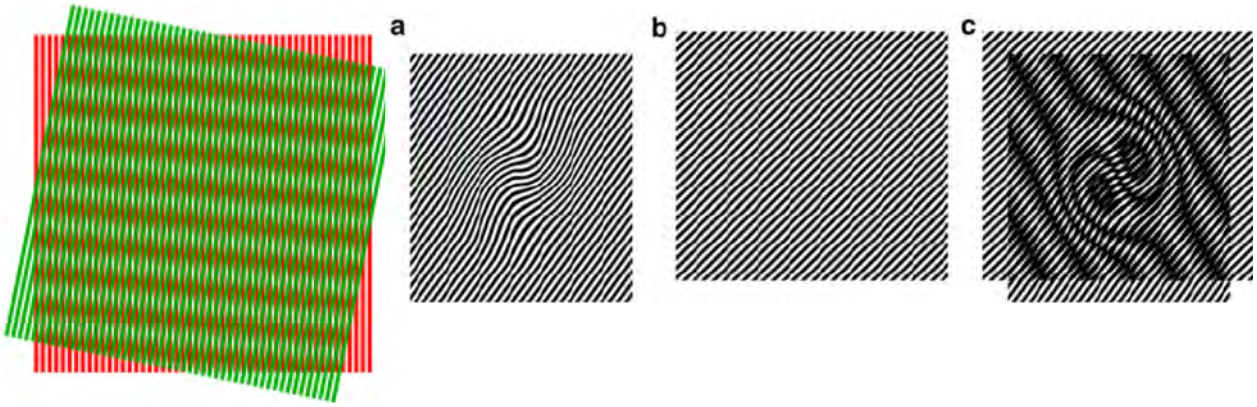
Greenfield et al PLoS Biol 2009

Structured illumination microscopy: principle

It’s kind of subtle…
Let’s discuss it with the ukulele…

Moire fringes project high frequency information (invisible) to lower frequency





Example in practice:
http://zeiss-campus.magnet.fsu.edu/tutorials/superresolution/hrsim/indexflash.html

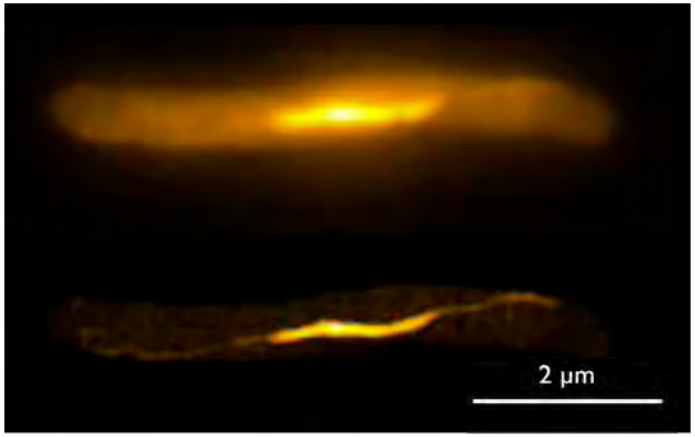
Related techniques: iSIM, Airyscanning

Moiré fringes Wikipedia
Rego, Shao, Methods Mol Biol 2015

Resolution

Spatial resolution
XY: 115 nm
Z: 350 nm

Time (typ.): 0.6- 1 s



E. coli RecA

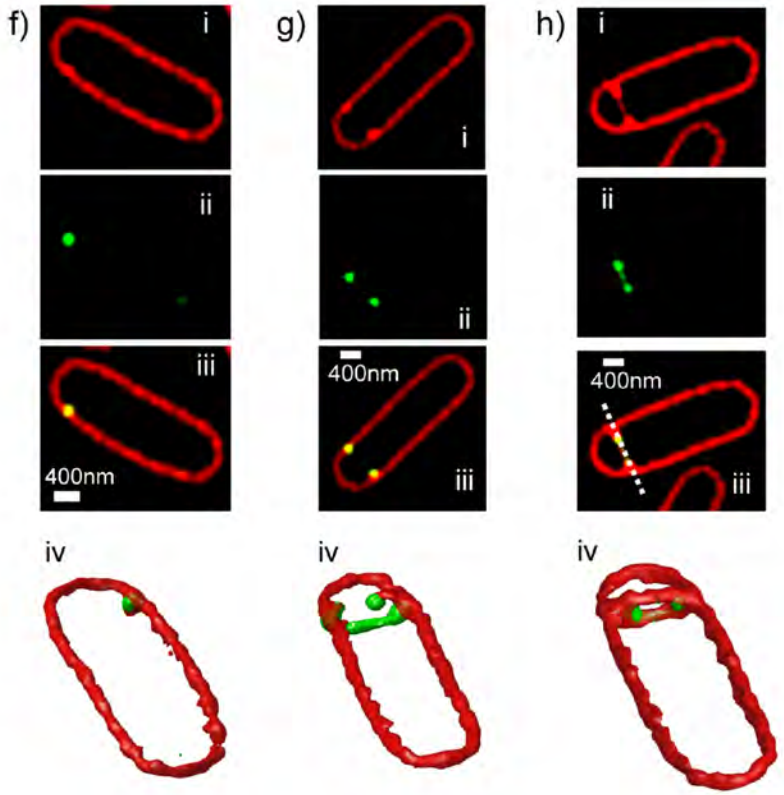
Lesterlin et al Nature 2014

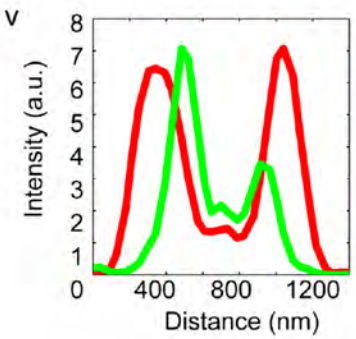
Advantages:
- FAST!
- Low laser power

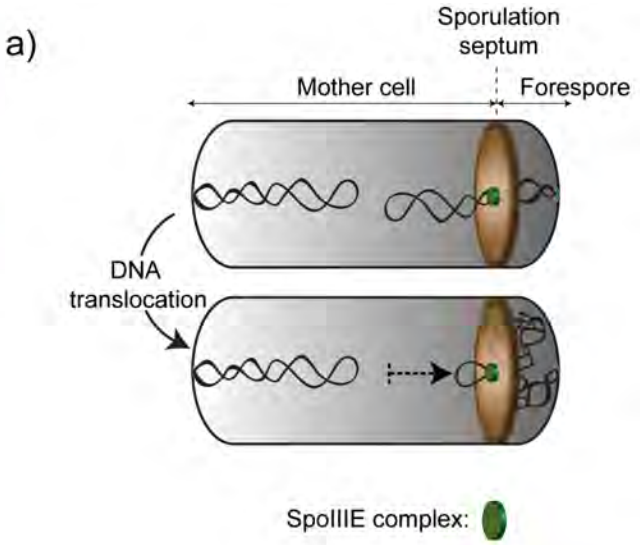
→ Low phototoxicity
→ Extended time lapse
-Really good at multicolour

Disadvantages:
- Lower resolution

SpoIIIE DNA pump recruitment to *B. subtilis* septation sites



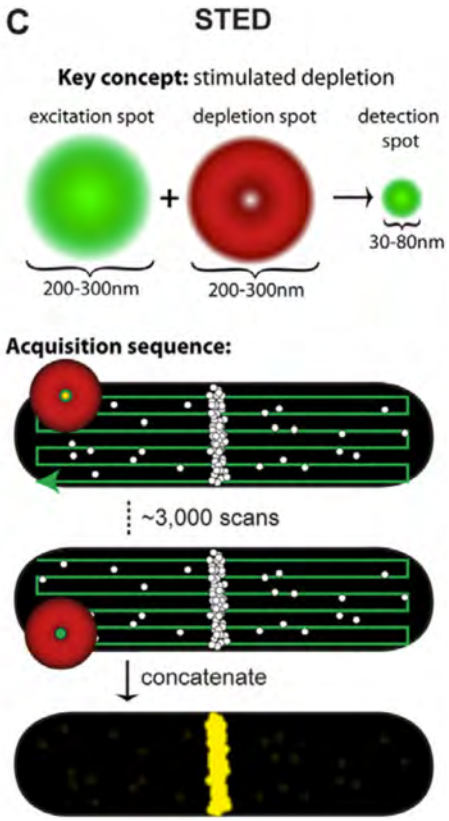




SpoIIIE is a translocase – pumps chromosome into forespore
Directly visualized localization to leading edge of closing septum

Fiche et al PLoS Biol 2013

Stimulated Emission Depletion microscopy



Resolution: 50 nm. Time resolution: 1-2s

My view:

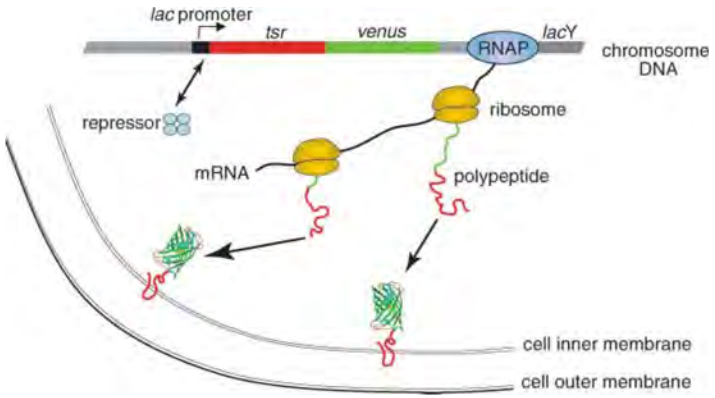
- Serious problems with photobleaching make this unsuited to live cell imaging…
- STORM is better at fixed cell

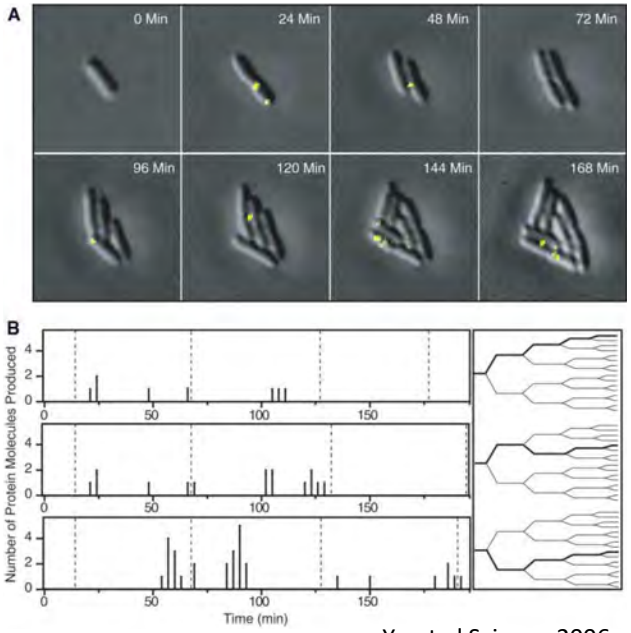
Single molecule imaging

- Closely related to localization microscopy

- Key techniques
  - Single molecule tracking
  - Molecule counting

Single molecule gene expression

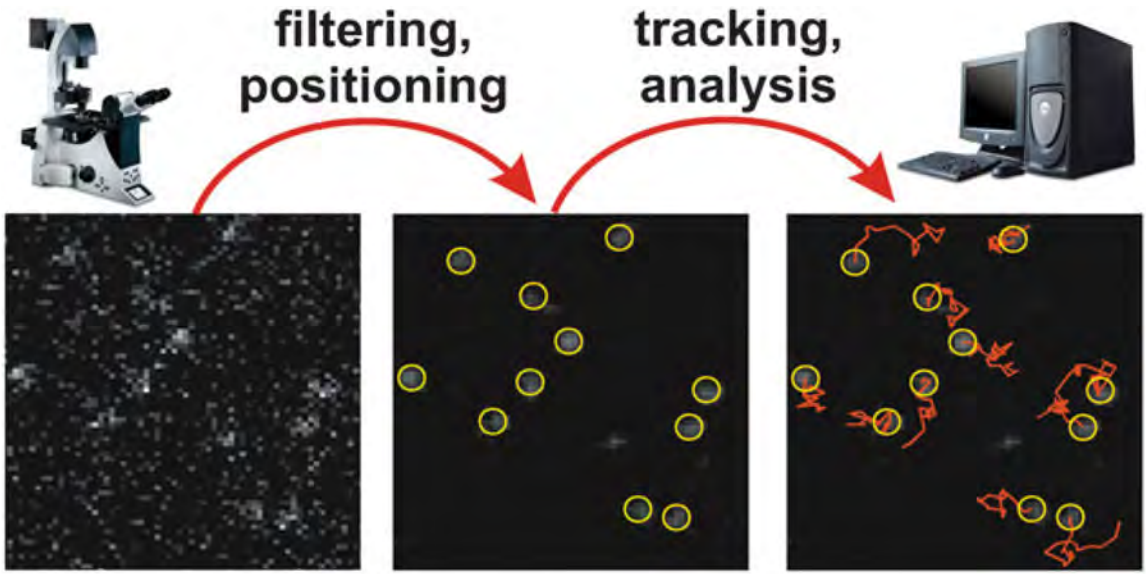




Yu et al Science 2006

One of the earliest really powerful applications of single molecule imaging
Proteins are expressed and observed in real time
Direct observation of "bursty" expression
– ie. multiple protein expressed rapidly after transcription of a single mRNA

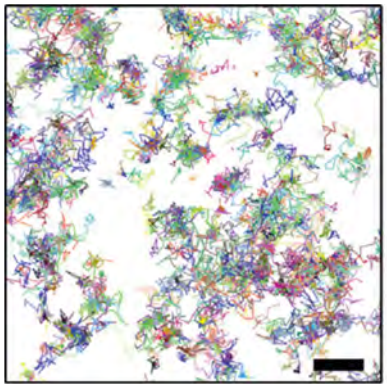
Single molecule tracking: principle



Woll et al Phy Chem Chem Phys 2013

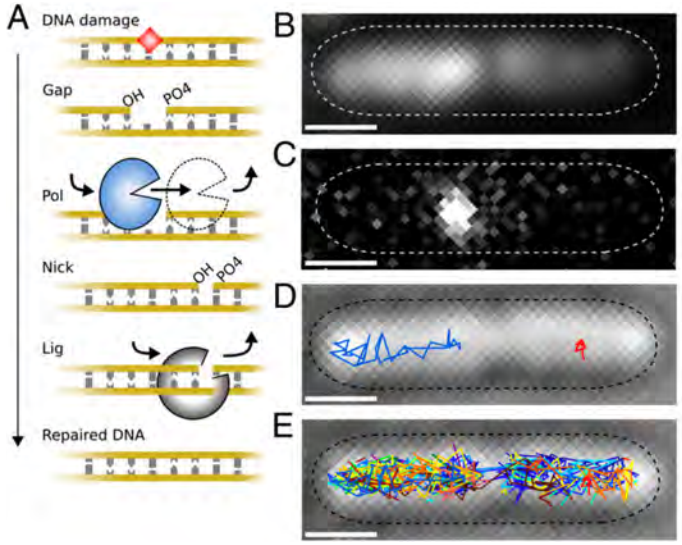
These days normally combined with photoactivation to obtain 1000s of tracks
→ Single particle tracking PALM (sptPALM) – extremely powerful in bacteria

Can study the binding/ diffusion of **all** the copies of a labelled protein in a cell



Manley et al Nat Methods 2008

Single molecule tracking of E. coli DNA polymerase I



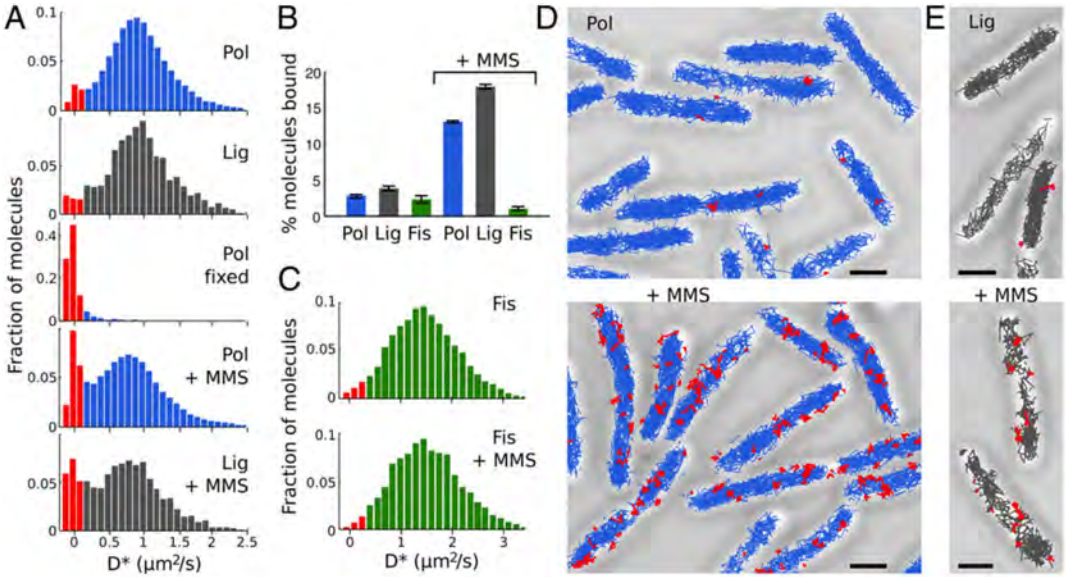
DNAP I is a repair polymerase

Track its motion –

- Fast diffusion – DNA unbound
- Slow diffusion – DNA bound

Uphoff et al PNAS 2013

Single molecule tracking of E. coli DNA polymerase I

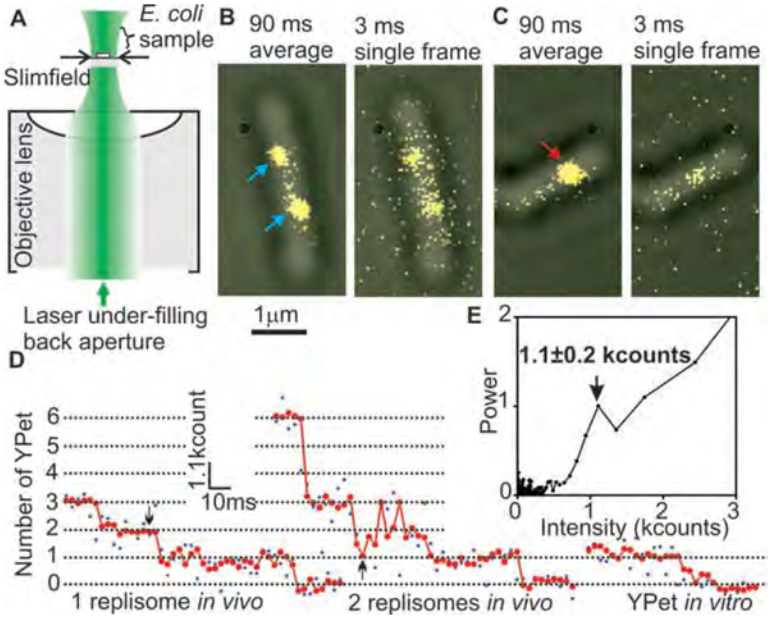


Direct observation of DNAPs actively repairing DNA gaps & nicks

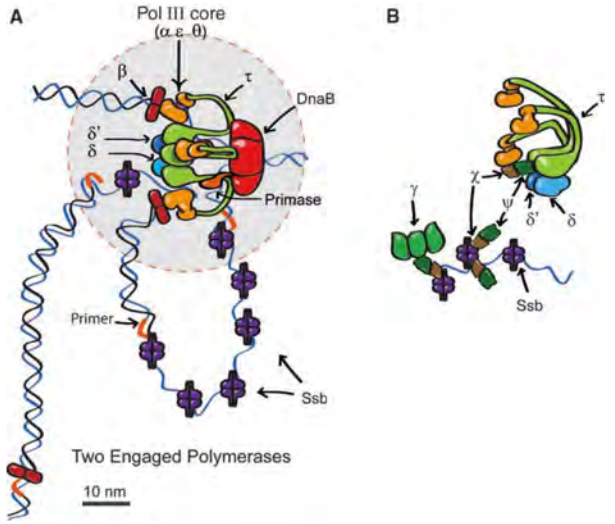
- Repair times
- Search times

Uphoff et al PNAS 2013

Single molecule counting by photobleaching



Watch foci bleach step-by-step
→ Tells you how many proteins are in the focus



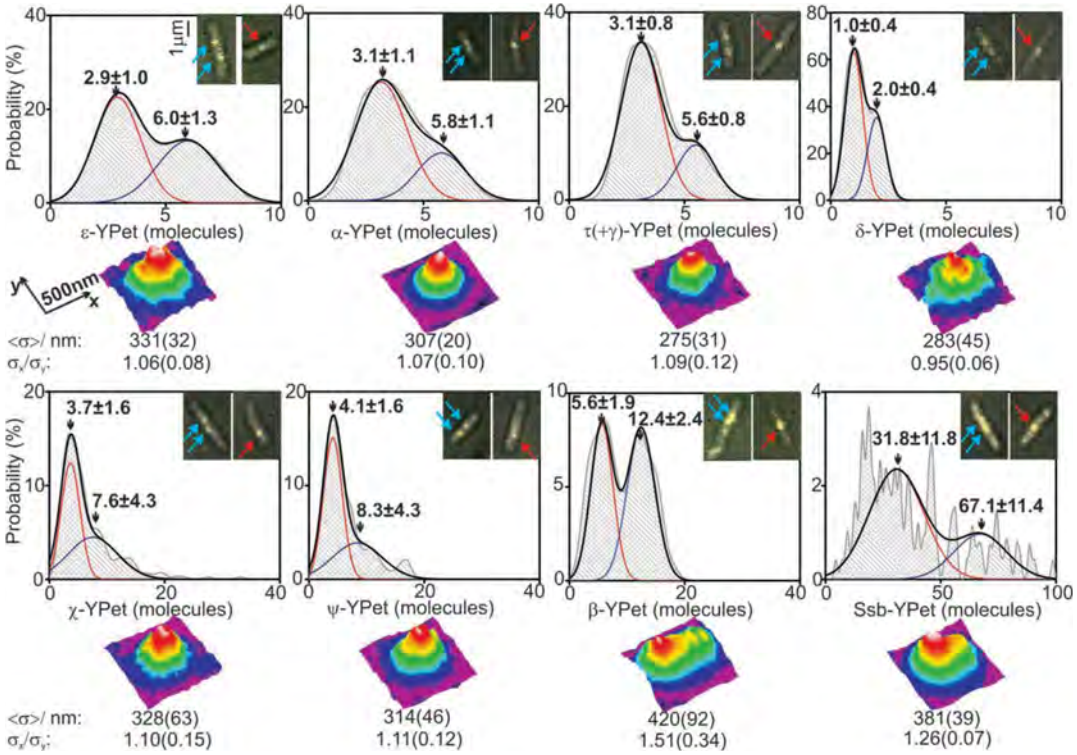
Reyes-Lamothe et al, Science 2010

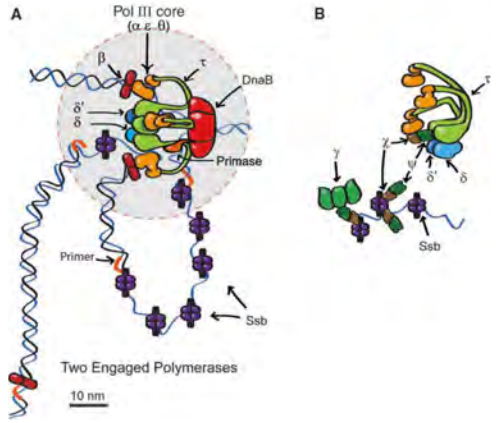
Single molecule counting by photobleaching

Very cool paper

By measuring numbers of all the key replisome proteins, determined in vivo stoichiometry of replisome

They found an extra polymerase!

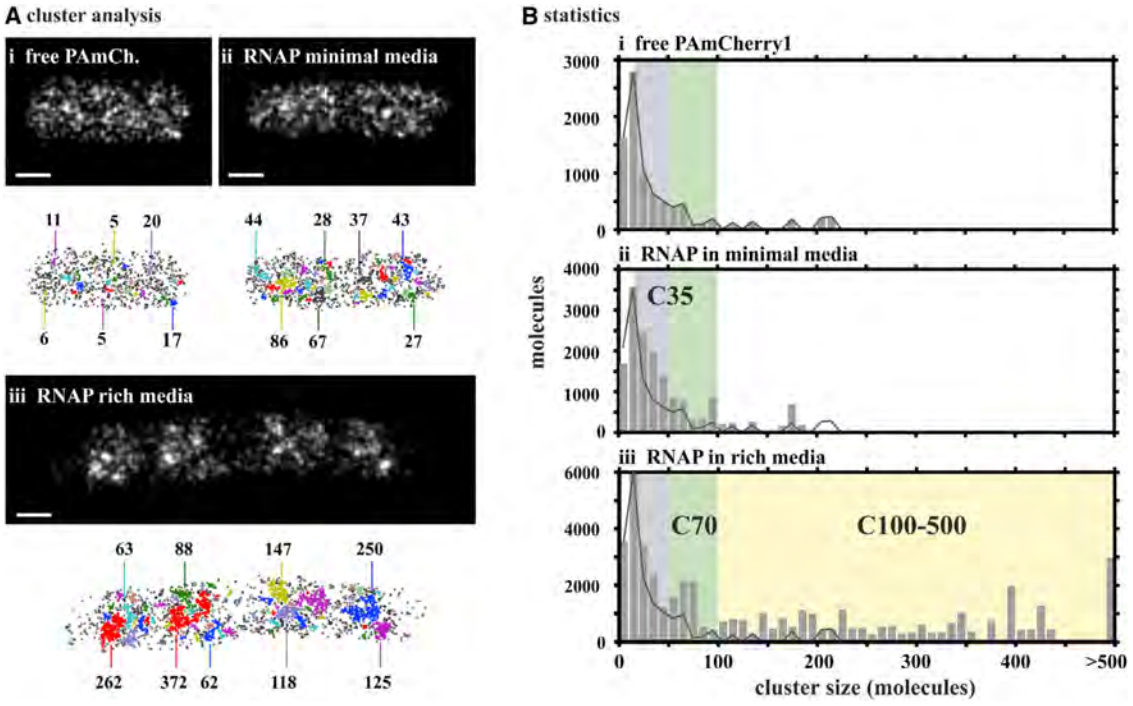




Reyes-Lamothe et al, Science 2010

Single molecule counting by localization microscopy

Since you localize the molecules one-by-one, why not count them?



Endesfelder et al, Biophys J 2012

Potentially very powerful for large complexes where photobleaching would not work
BUT - determining absolute numbers (rather than relative stoichiometry) is an ongoing challenge - mainly due to difficulty establishing 'dark' fraction of FPs
Need good "counting standards"

Summary

- Single molecule imaging and super-resolution are powerful tools for both bacterial cell biology and in vivo biophysics
- Rapidly moving, exciting area

- Biology, one molecule at a time!

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