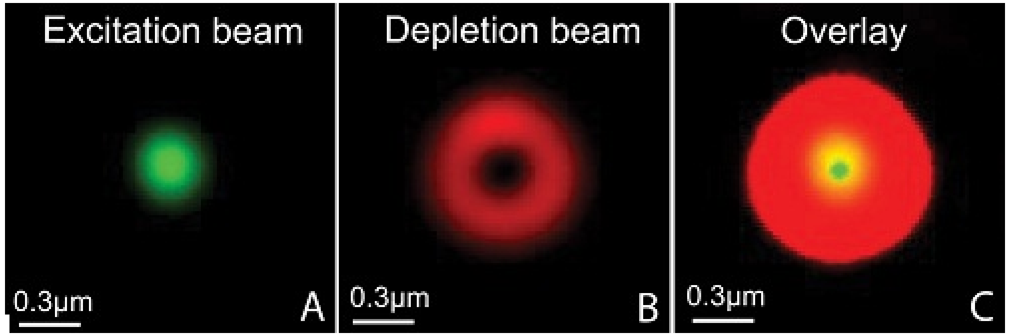
More STED, Fourier transforms

- Last class
  - PALM/BALM
  - STED
- This class
  - Finish STED
  - Intro to Fourier transforms



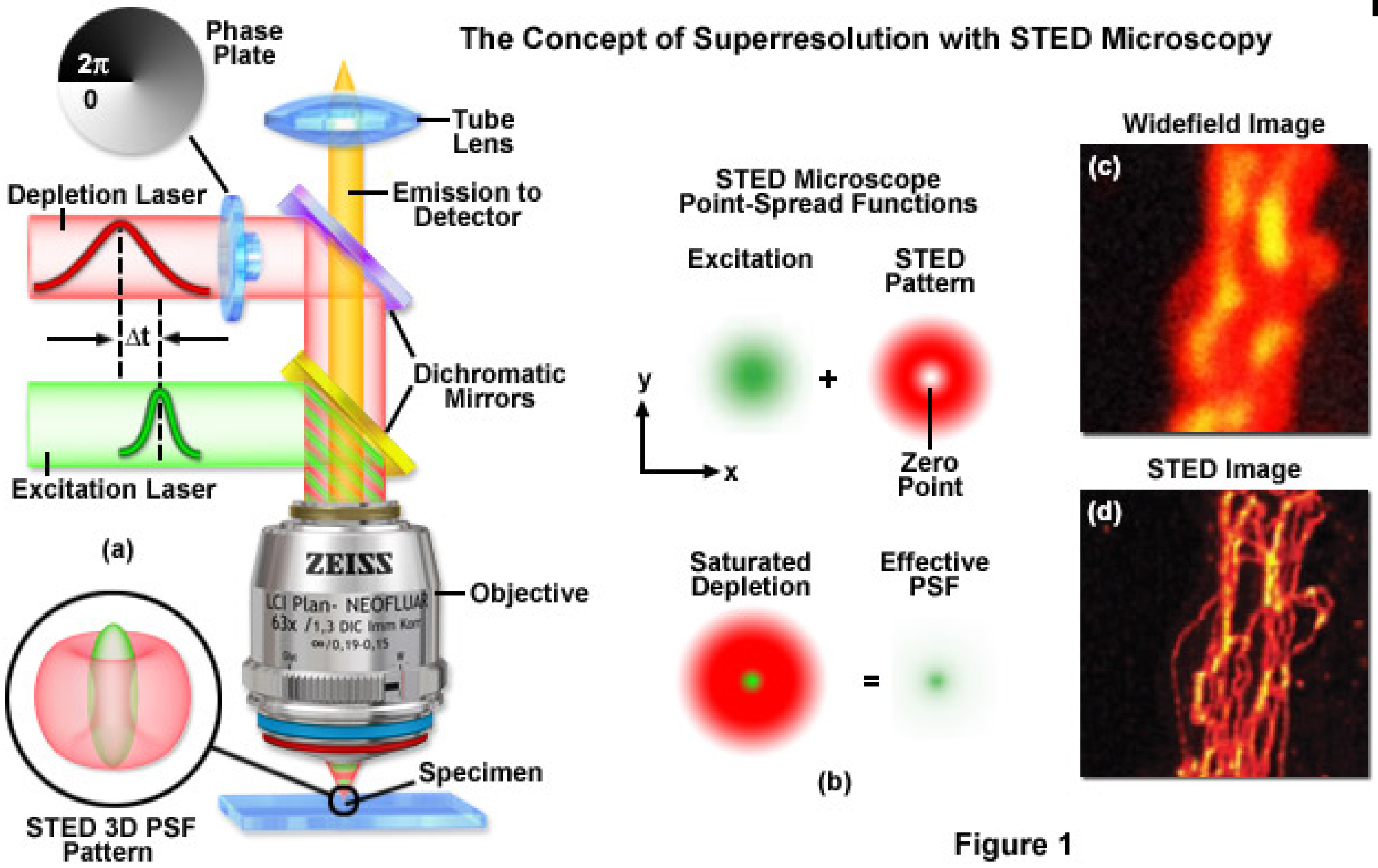


Figure 1

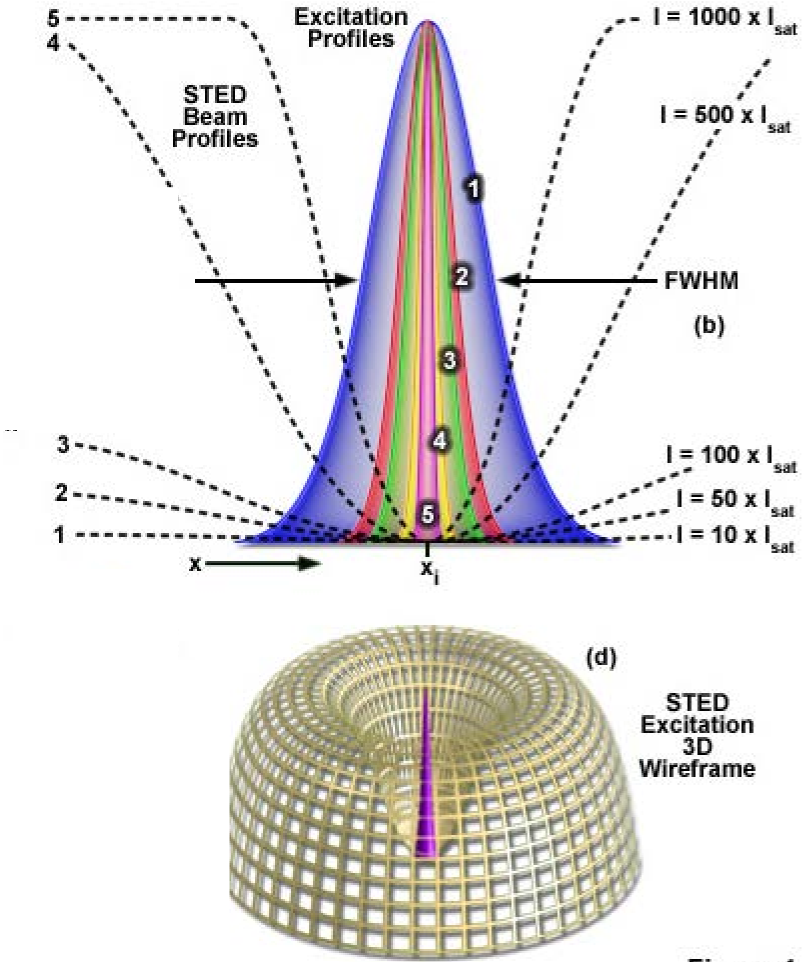
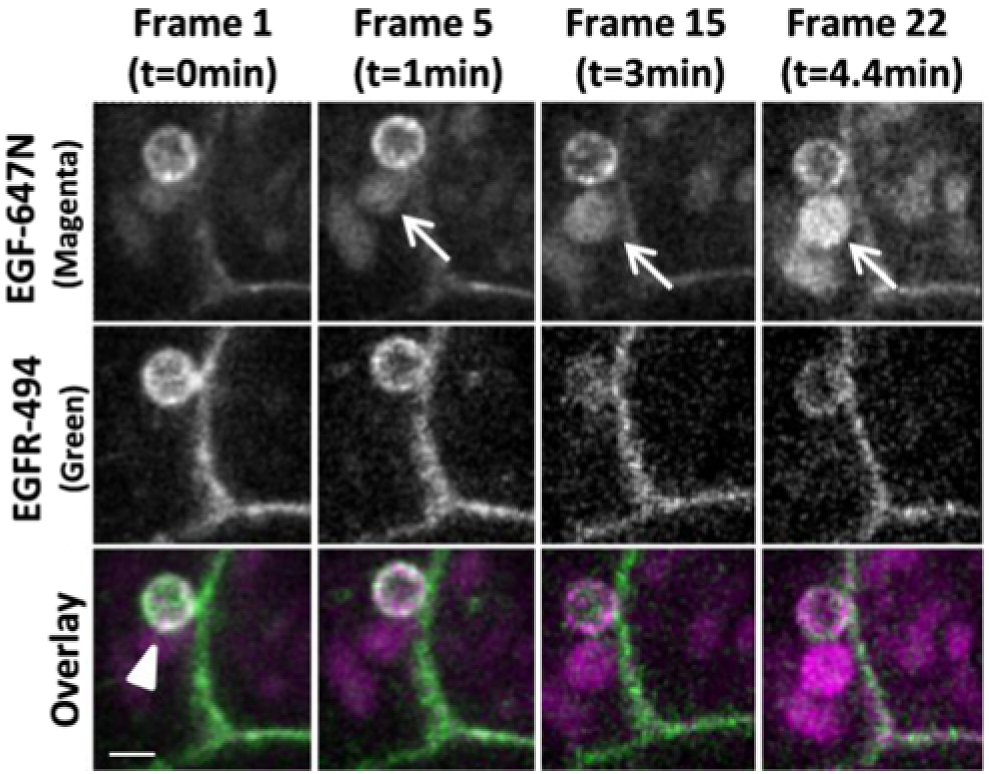


Figure 4

Multi color STED

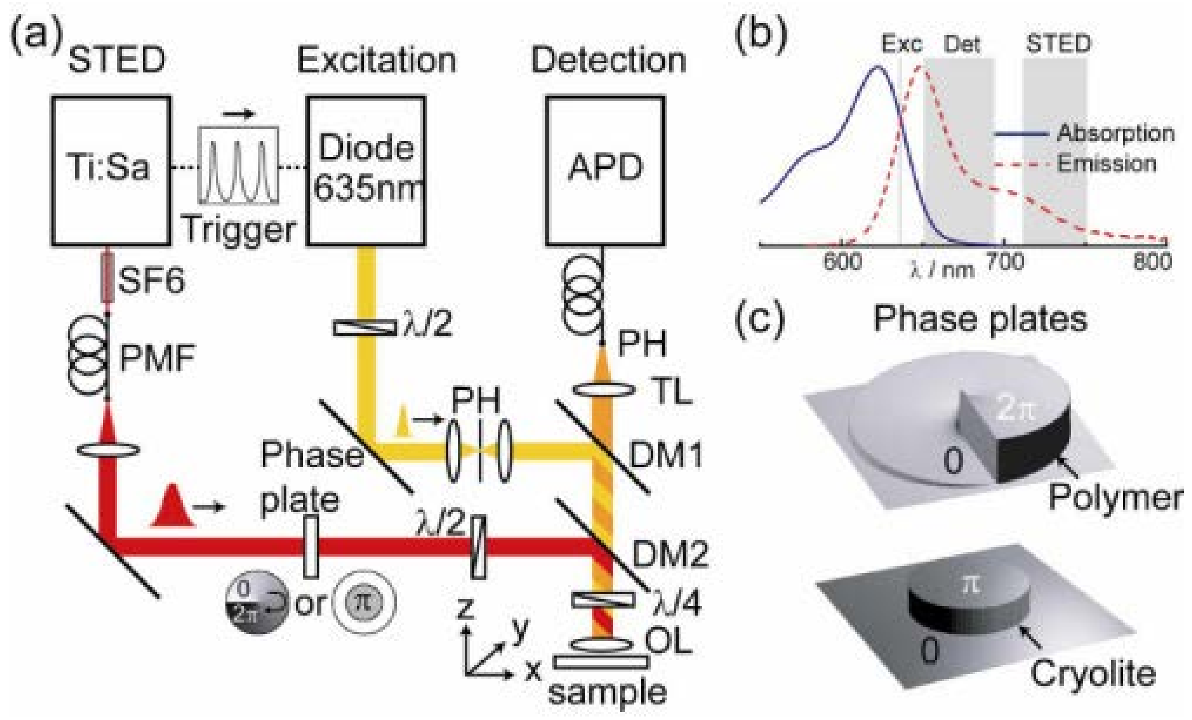
- Possible, but hard to deal with
- Due to intense STED depletion beam, it's hard to combine with additional red dye
- Has been achieved, including 3 color by measuring one lifetime



HEK cells with labeled growth factor and receptor

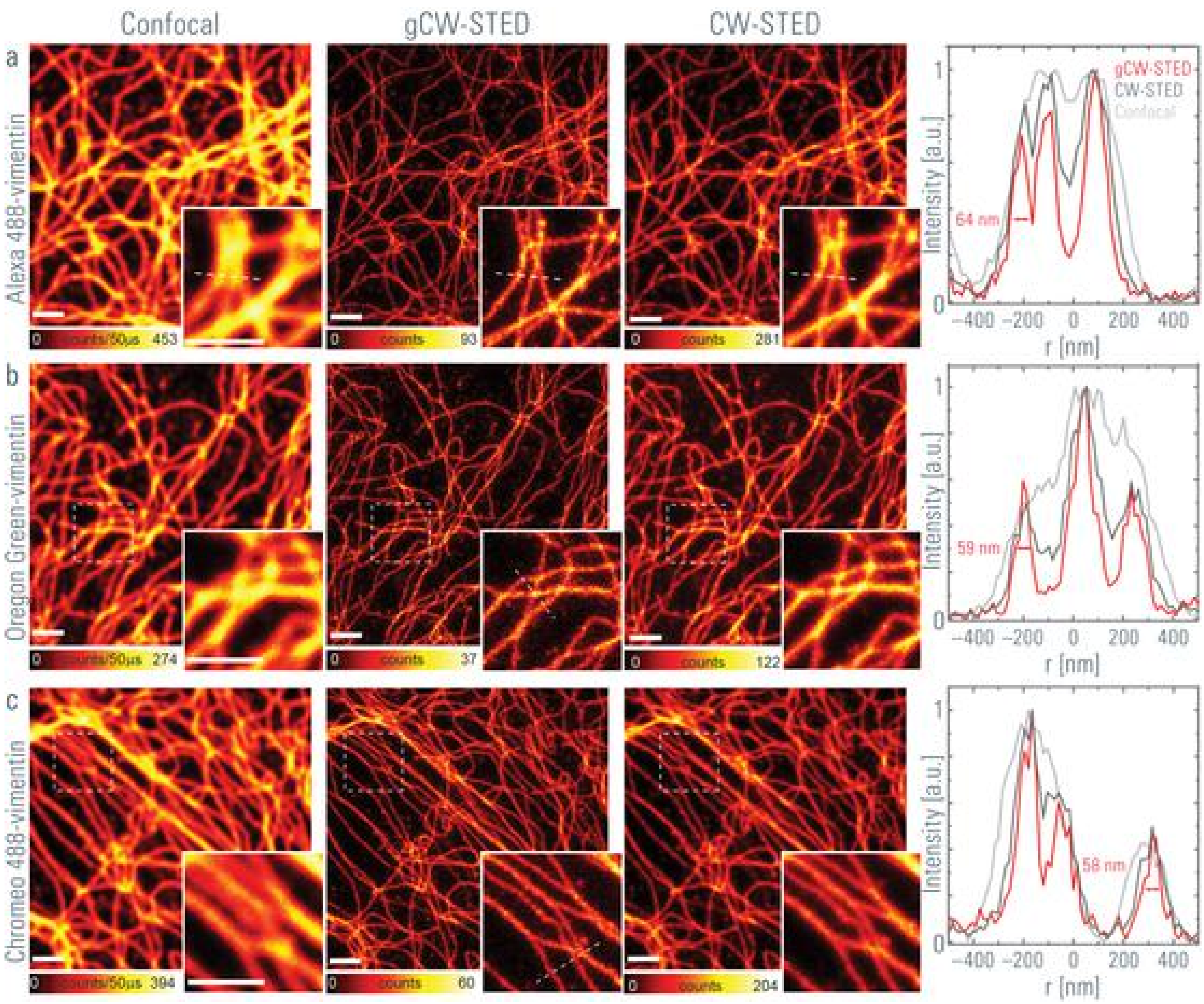
STED timing

- Fluorophores live in excited state for ~ns
- Have to apply the depletion beam within < 1 ns of excitation
- Use ultra-fast lasers



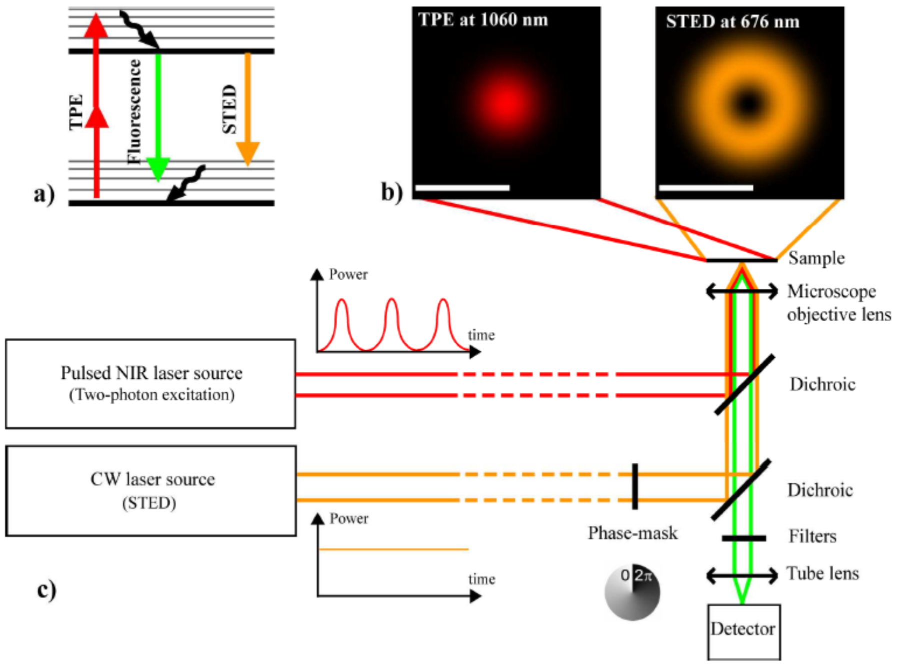
CW STED

- Possible to use CW lasers, but you lose the picosecond time control with the ultrafast laser
- Gated CW STED is the preferred method
- STED laser is on continuously, (High power, Low noise)
- Excitation laser is pulsed (gated)
- Pulsing prevents re-excitation of a quenched fluorophore

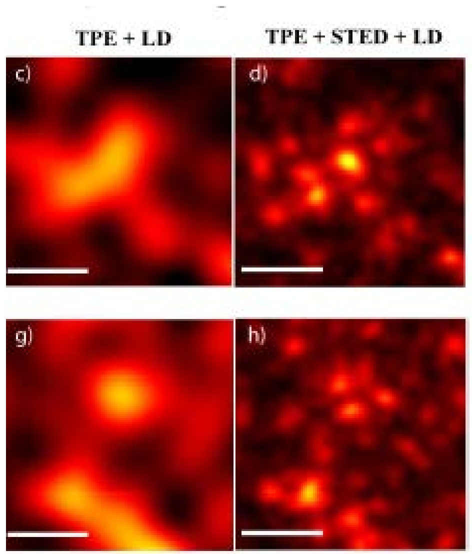


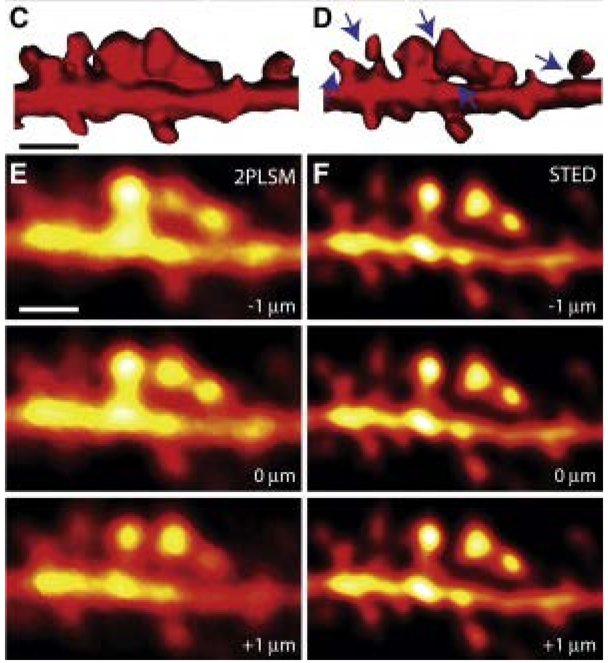
Two photon STED

- Use two photon source to generate initial fluorescence
- STED beam stays the same
- Allows 3D sectioning and deeper penetration into tissue
- Some use in brain slice
- Scattering is a challenge to keep the STED beam focused



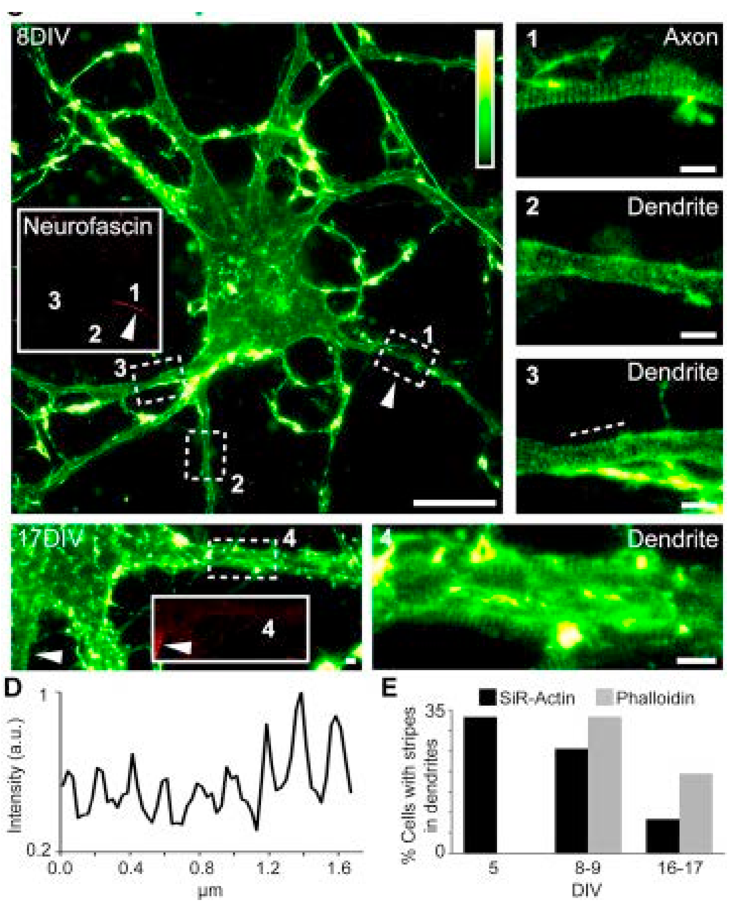
NFKb in PtK2

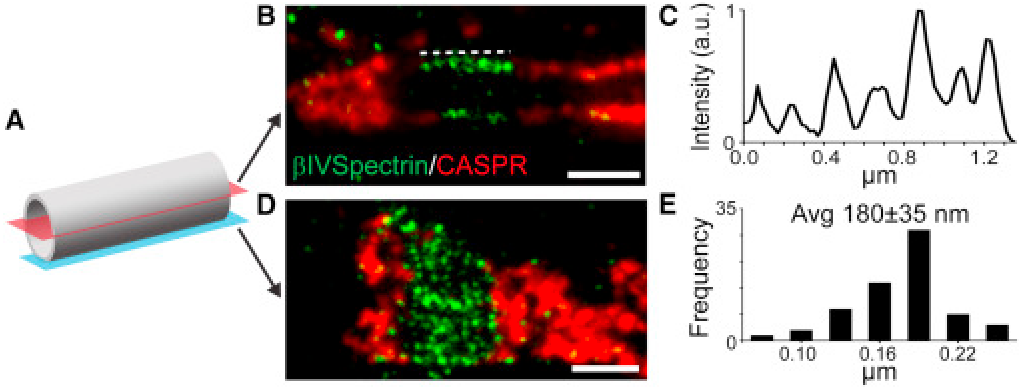


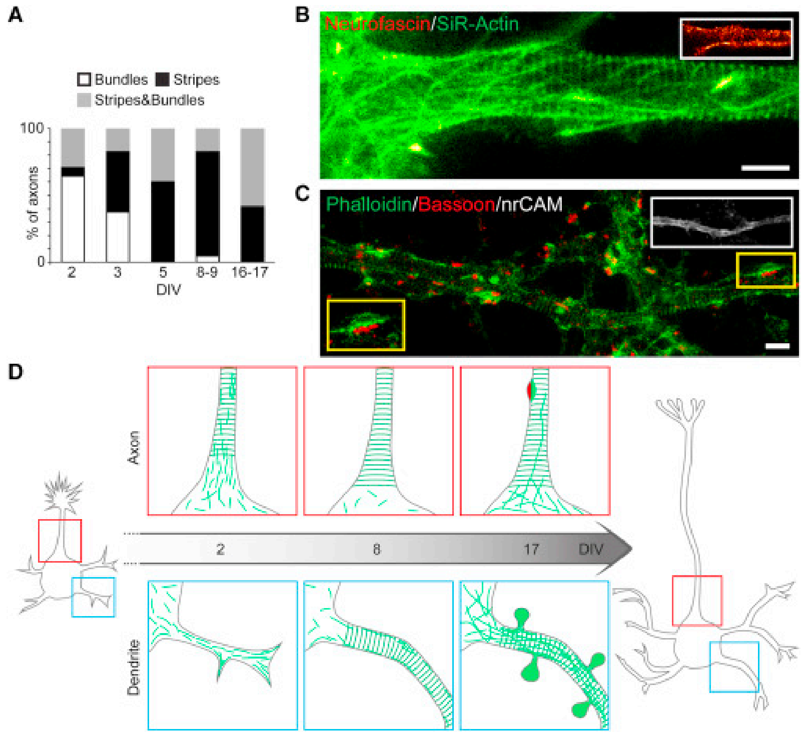


STED applications – neuronal cytoskeleton

- Live cell STED showed periodic organization in both axons and dendrites
- Also found in peripheral nervous system at Nodes of Ranvier
- Enabled by live-cell STED

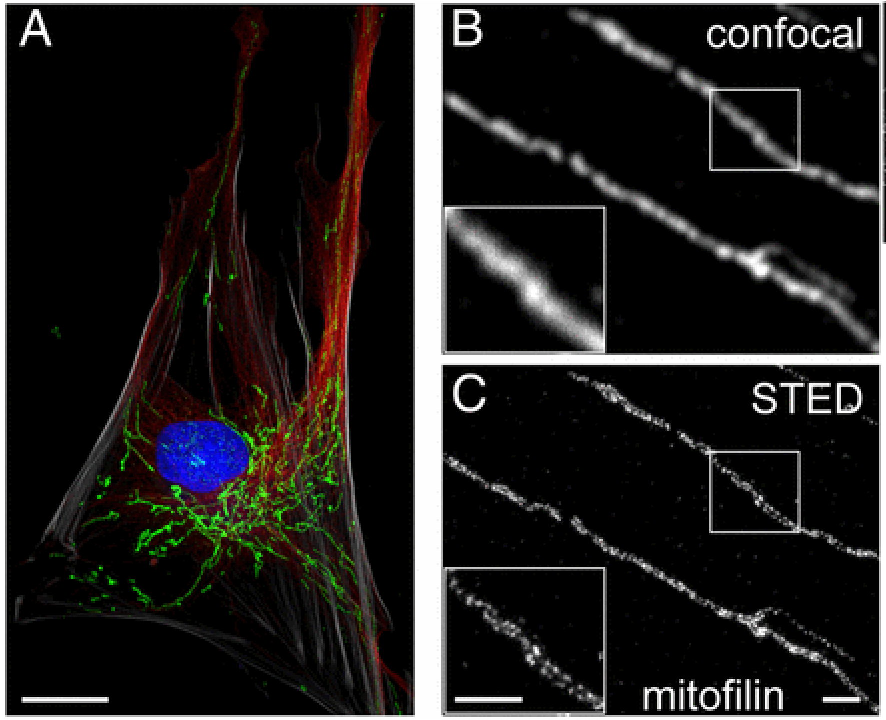


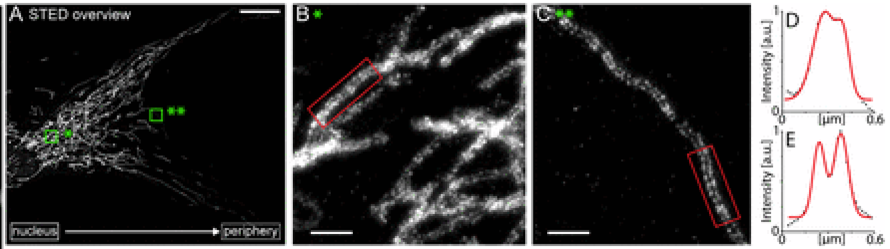


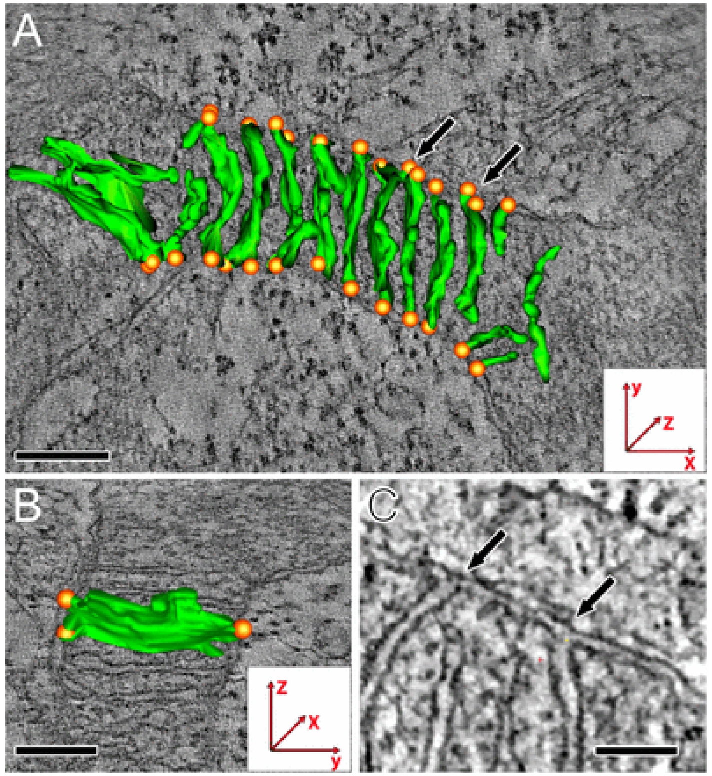


STED applications – organelle imaging

- Mitofilin is localized in clusters that are blurred in confocal
- Clusters were concentrated on mitochondria near nucleus
- Hypothesize mitofilin is part of mitochondrial skeleton

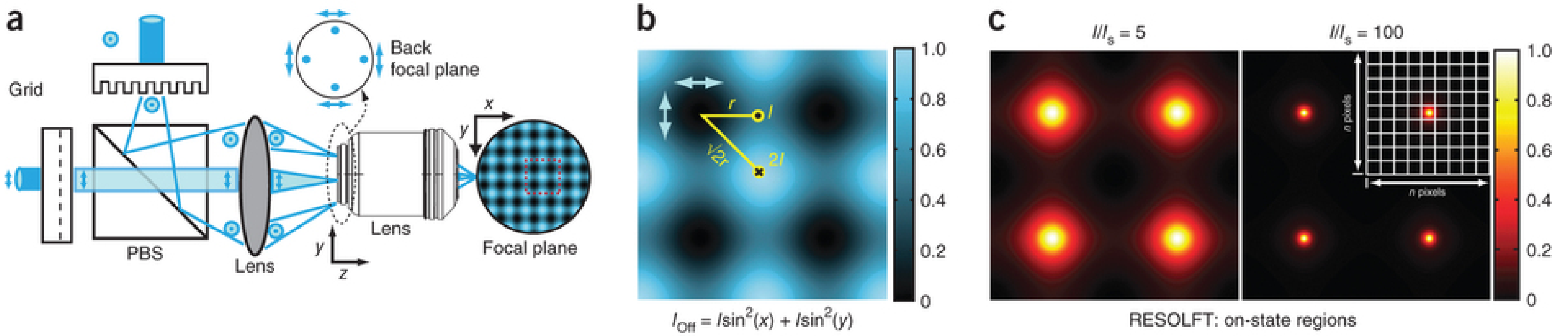


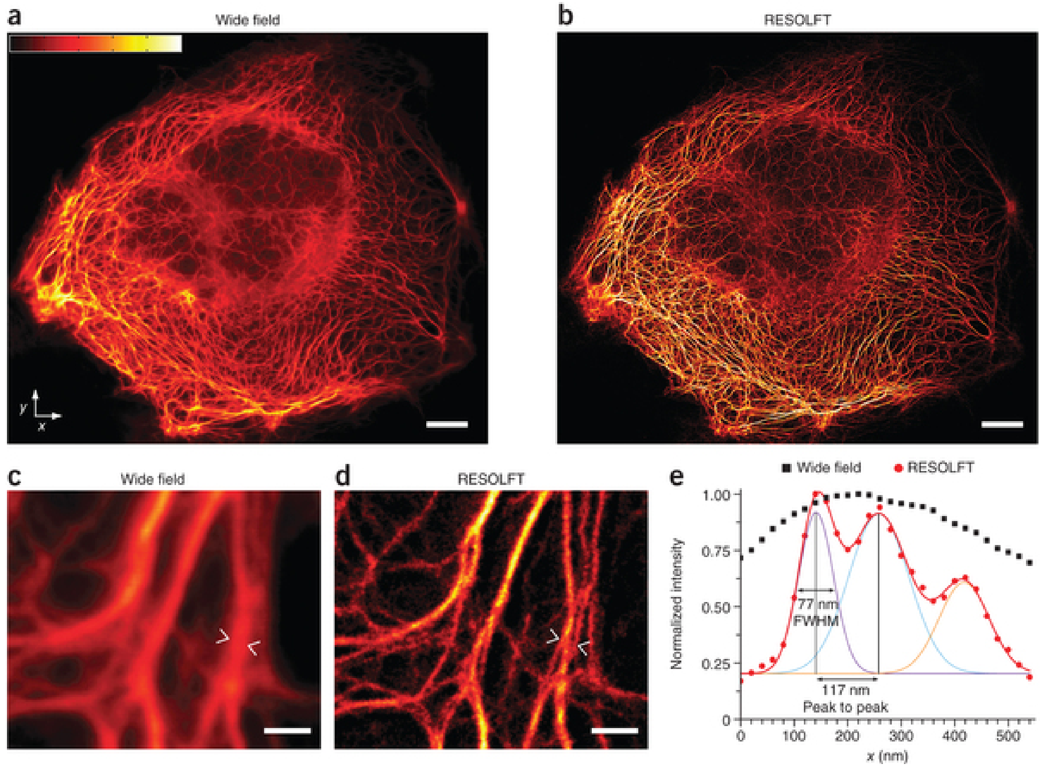




Parallelized STED

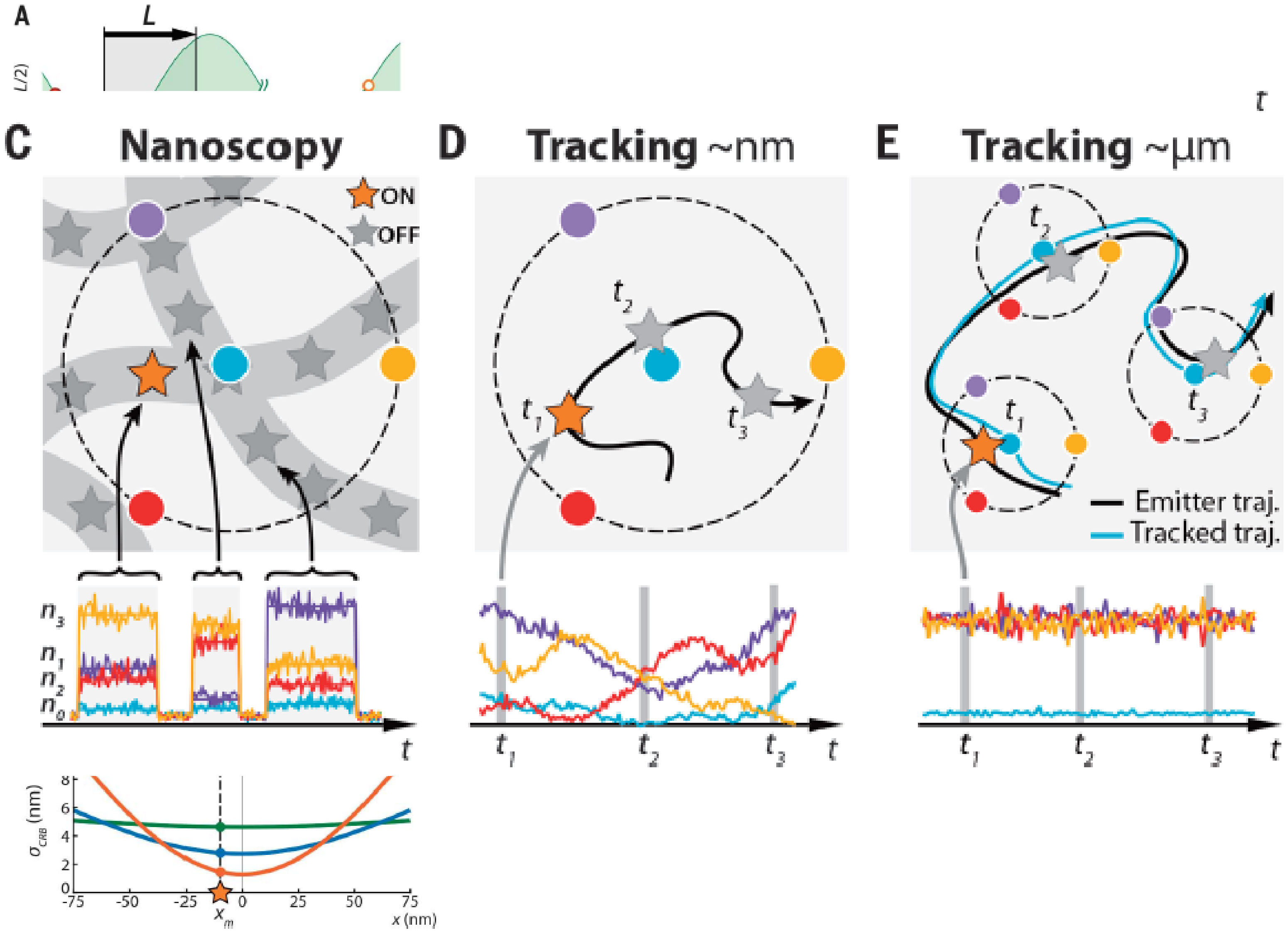
- Similar to spinning disk, can you create a grating of small spots with STED beam
- Small translations of the array will fill in entire image
- Very fast imaging with high spatial resolution
- Also allows lower intensities
- Limited only by transition rate of fluorophores and intensity of lasers

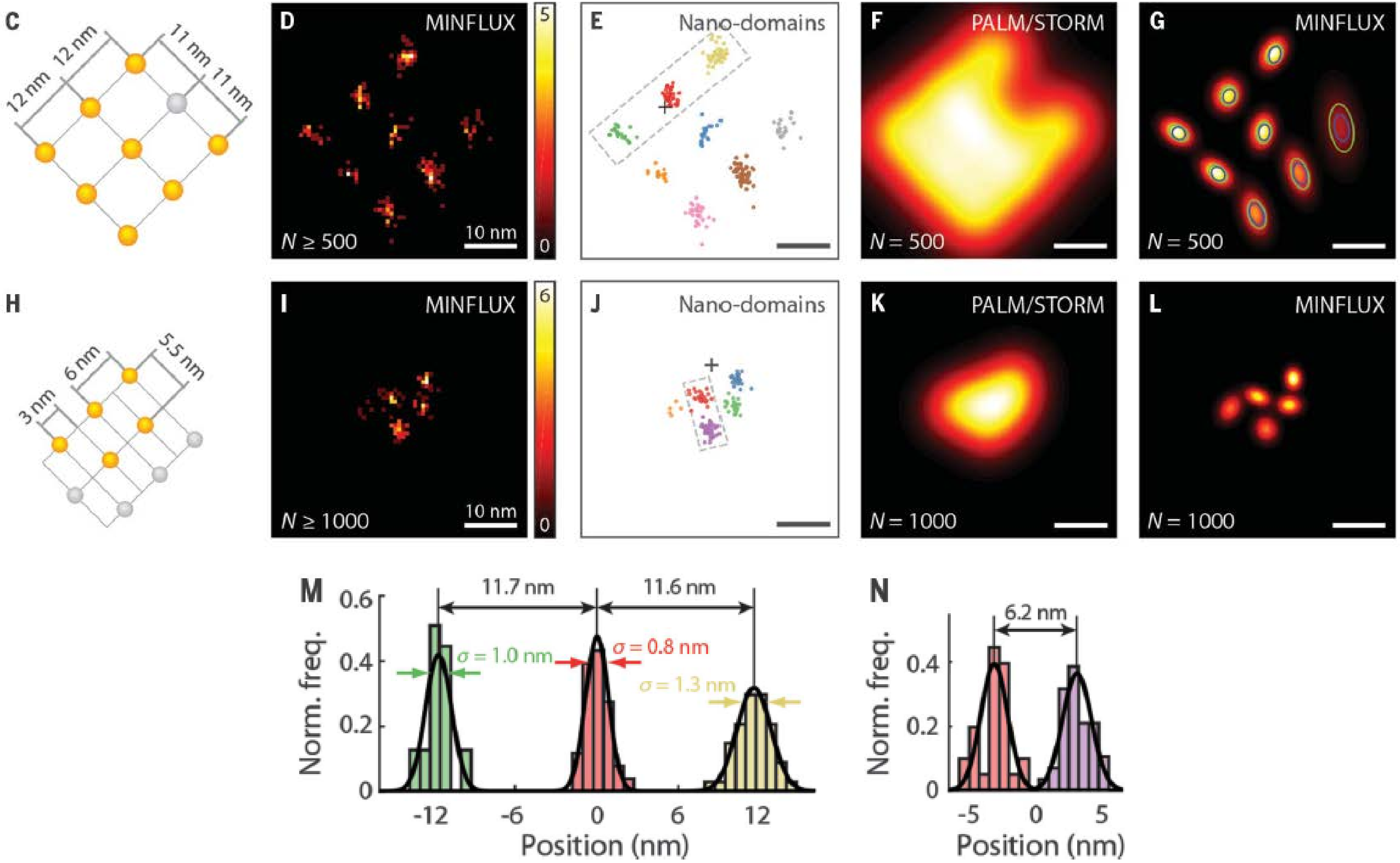


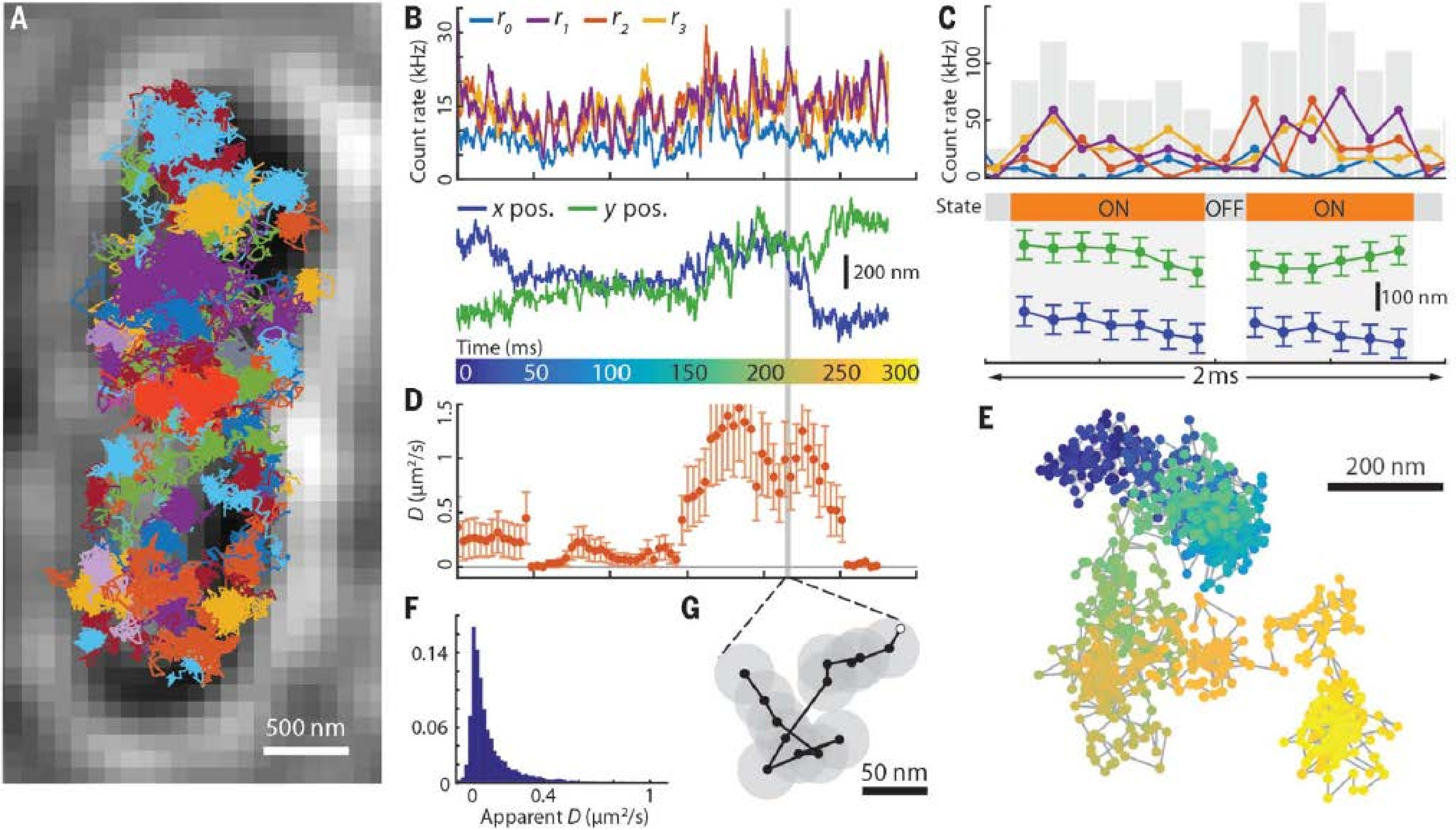


Minflux – the latest and greatest

- Use four excitation points of your donut, moving it around your sample
- Trade in photons for multiple measurements to decrease uncertainty
- Can be combined with PALM/STORM for imaging




C
12 nm
12 nm
12 nm
11 nm
11 nm
D
MINFLUX
N ≥ 500
10 nm
5
0
E
Nano-domains
F
PALM/STORM
N = 500
G
MINFLUX
N = 500
H
3 nm
6 nm
5.5 nm
I
MINFLUX
N ≥ 1000
10 nm
6
0
J
Nano-domains
K
PALM/STORM
N = 1000
L
MINFLUX
N = 1000
M
11.7 nm
11.6 nm
σ = 1.0 nm
σ = 0.8 nm
σ = 1.3 nm
Norm. freq.
Position (nm)
N
6.2 nm
Norm. freq.
Position (nm)



A
500 nm
B
r_0 r_1 r_2 r_3
Count rate (kHz)
30
15
0
x pos. y pos.
200 nm
Time (ms)
0 50 100 150 200 250 300
C
Count rate (kHz)
100
50
0
State
ON
OFF
ON
100 nm
2 ms
D
D (µm²/s)
1.5
1
0.5
0
E
200 nm
F
0.14
0.06
0
0 0.4 1
Apparent D (µm²/s)
G
50 nm

Fourier transforms…

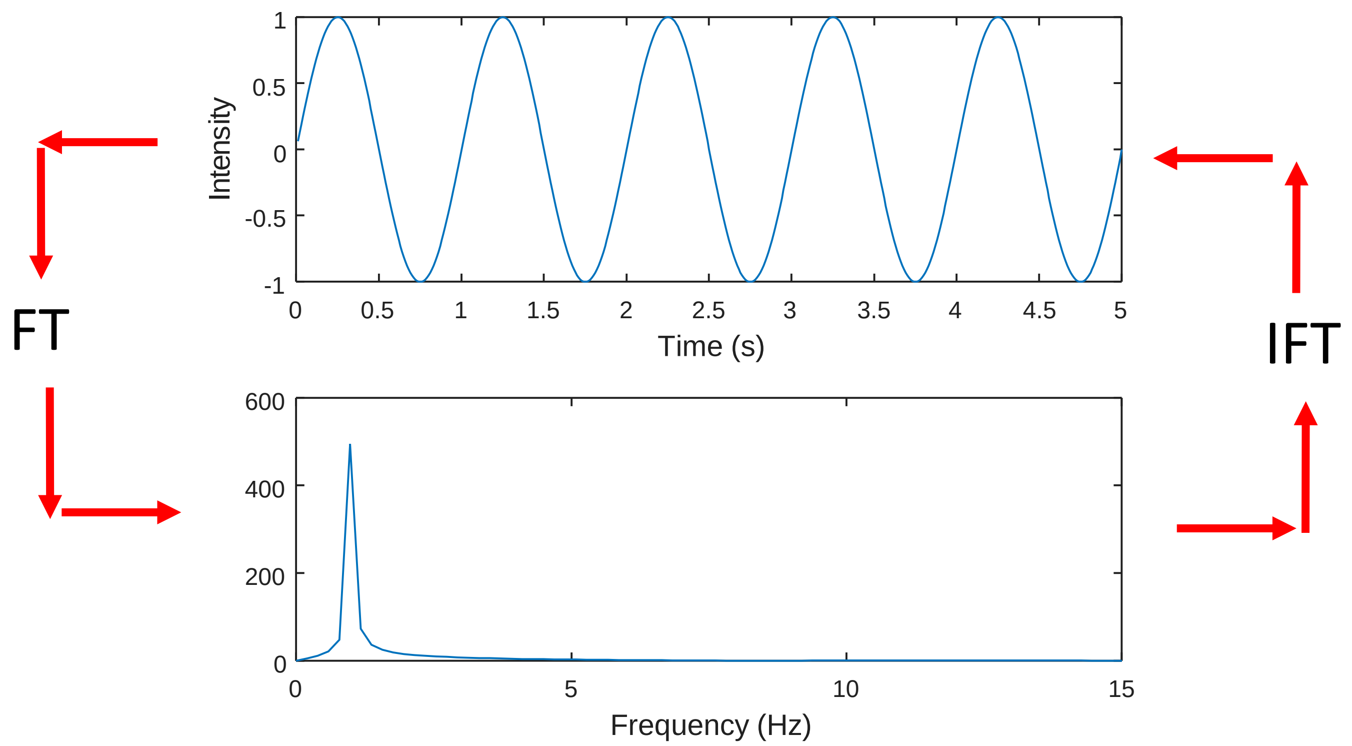
- Don't be afraid
- Maybe just a little…
- Essential to understanding SIM

Intro to FT

$$f(t) = \sum_n c_n \sin(2\pi n f)$$

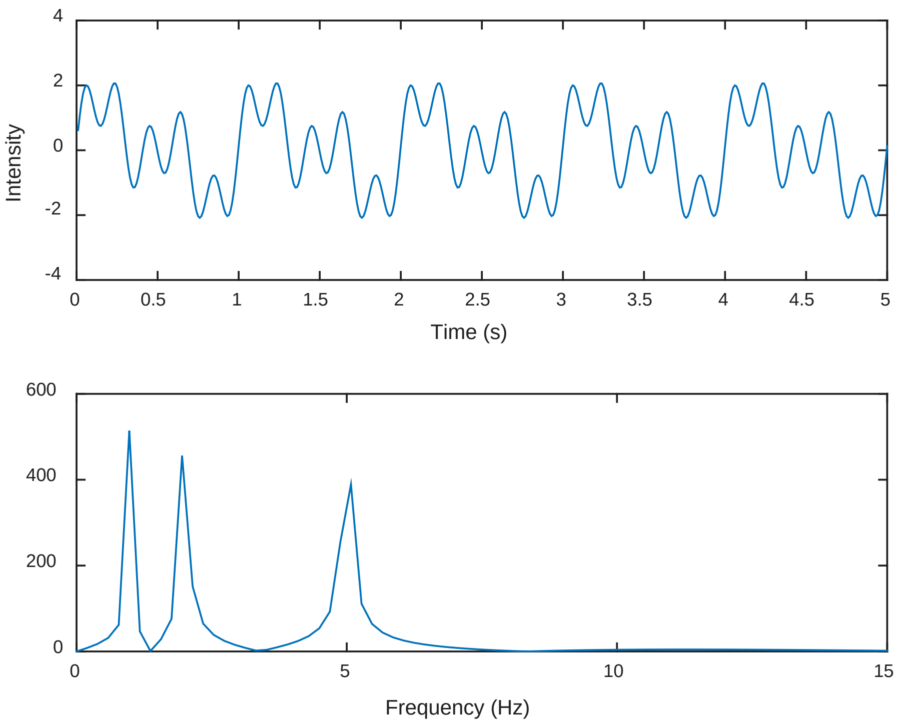
- Consider any time varying signal
- The Fourier argument says that ANY time varying function can be equivalently represented by a sum of sine waves with varied frequencies
- Each description is equivalently valid
- This means that we can think of things varying in time (time domain) or frequency (frequency domain)

If we can figure out c_n we can describe any function as a sum of sine waves

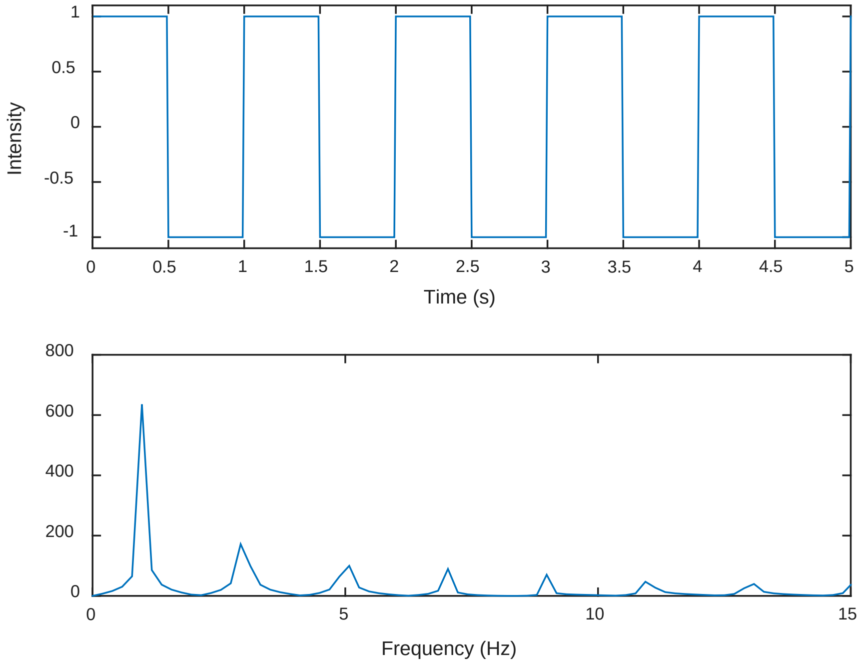


FFT decomposition

- More complicated functions will need more Fourier components
- The fast Fourier transform (FFT) will calculate all the C_n components for us



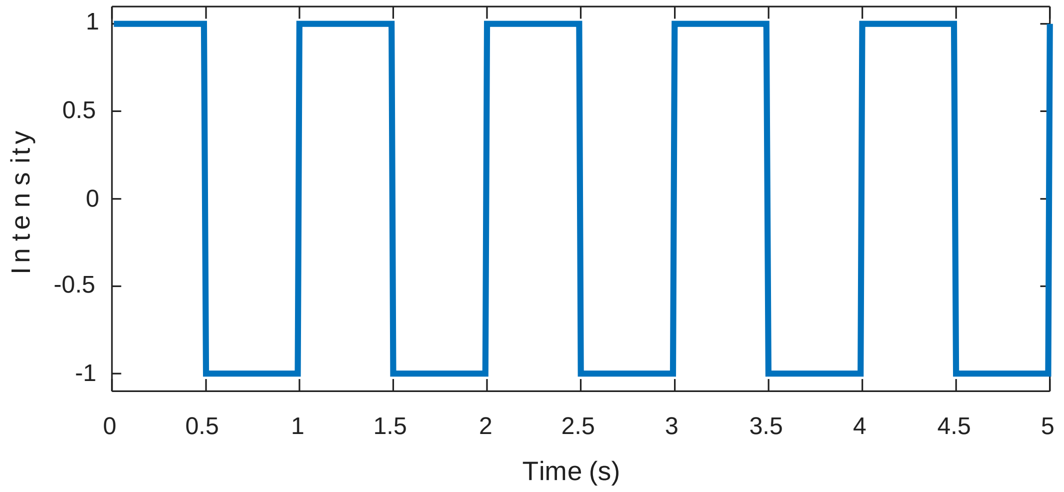
3 sine waves at different frequencies

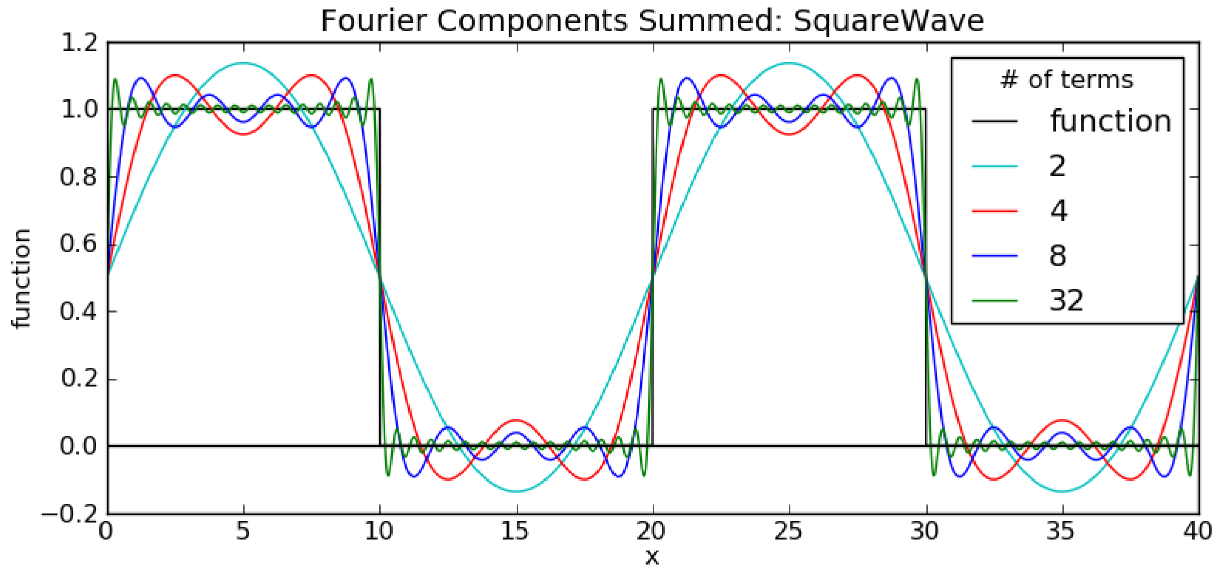


Square wave decomposes into large numbers of waves

Fourier reconstruction

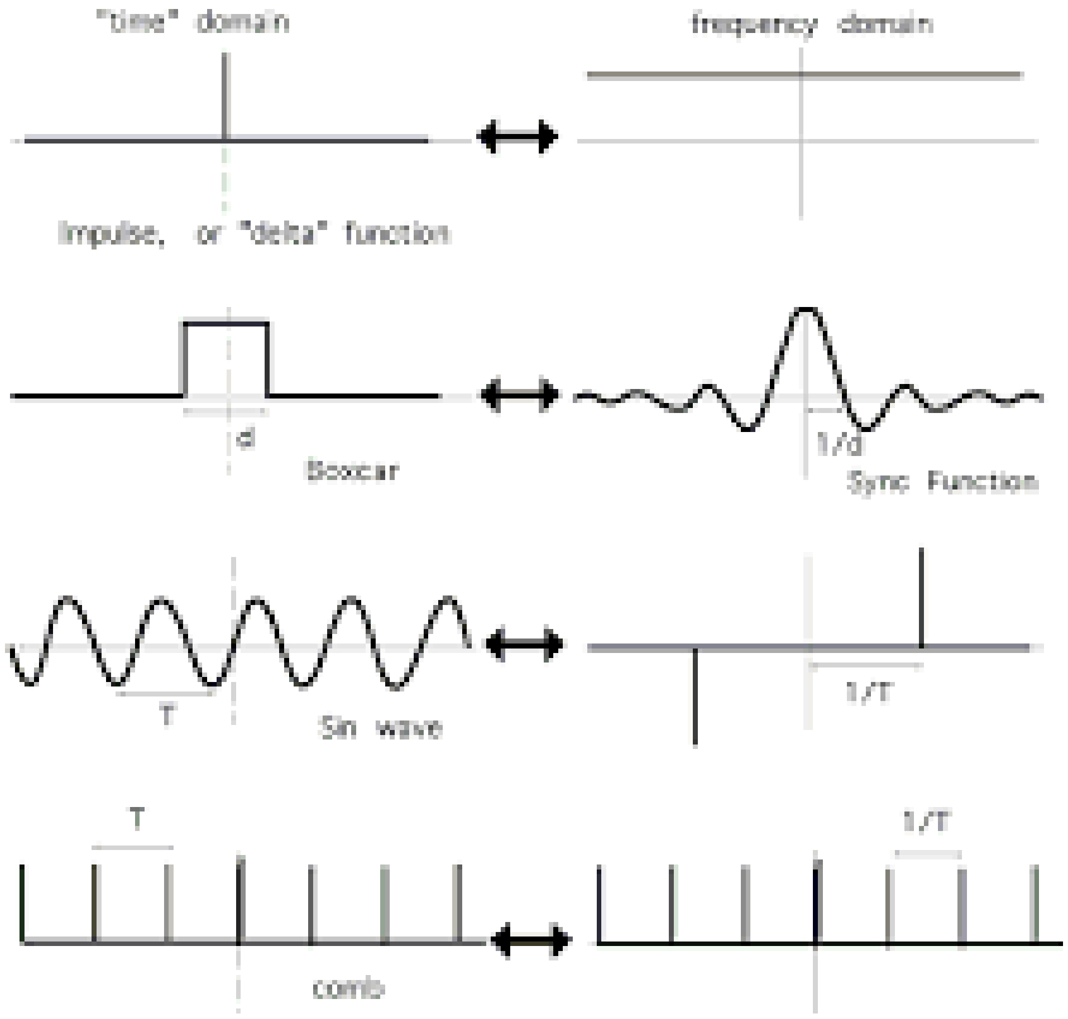
- We can also build back arbitrary functions by taking the Inverse fast fourier transform (IFFT)
- The more frequencies we allow, the closer we can represent the original signal
- Sudden changes (steps) are VERY high frequency, need lots of components to model
- Ringing pattern at edges gets reduced with increasing reconstruction frequencies





Common Fourier Transforms

- A delta function goes up and down infinitely fast, we need equal contribution from infinite frequencies
- A rectangle function gets turned into a sinc function (aperture -> airy function)
- A sine wave is a single line (one frequency)
- Bonus – Guassian becomes Gaussian



On to Matlab…