Femtosecond laser microsurgery in live cells and multiphoton imaging

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Stanford Univeristy

MRS Meeting
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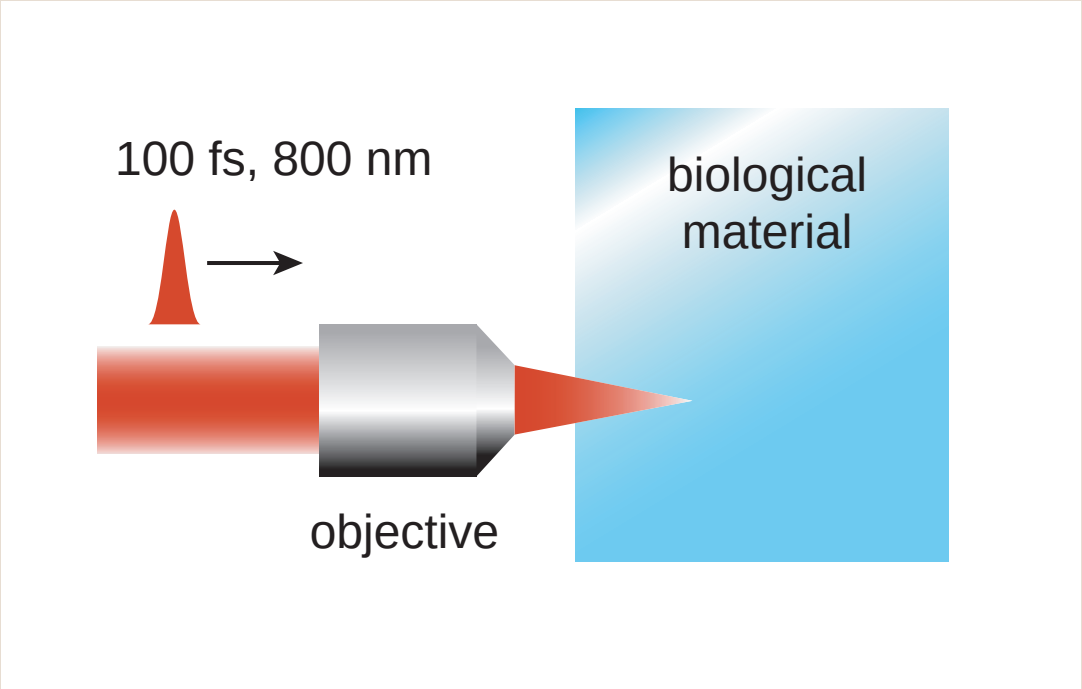
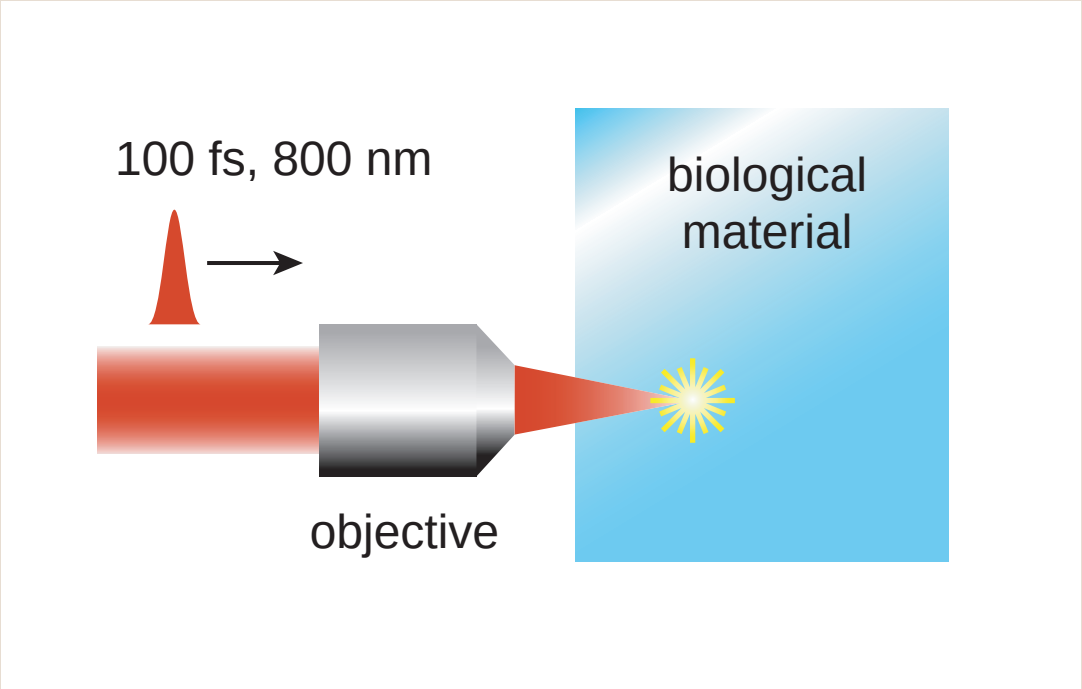
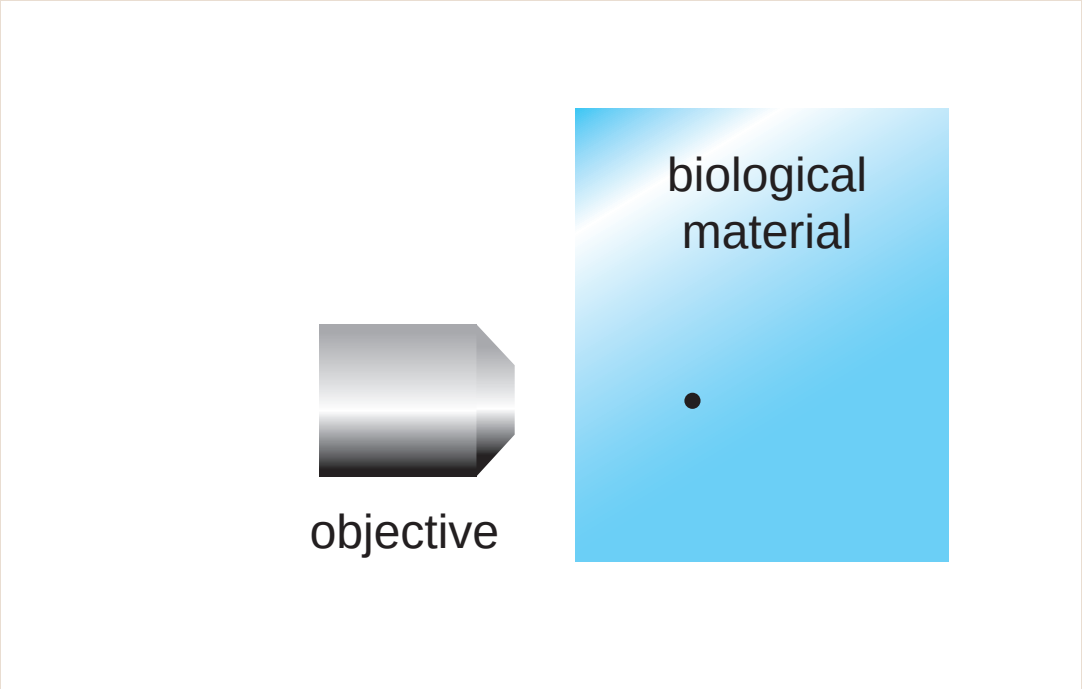
high intensity at focus



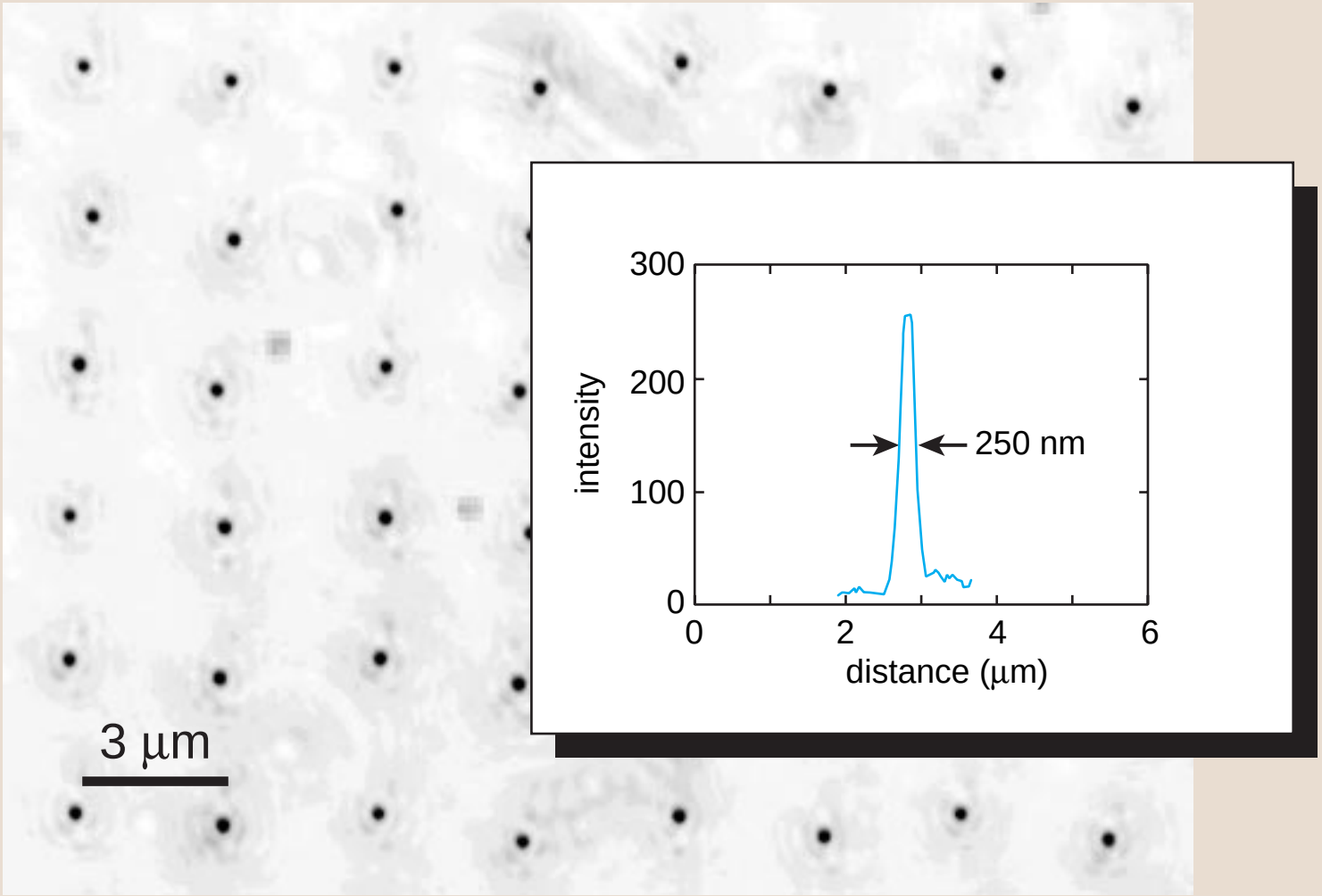
causes nonlinear ionization



producing microscopic bulk disruption



Introduction



40 nJ
100 fs
800 nm
0.65 NA
Corning 0211

top view

Outline

- Background
  - Absorption mechanism
  - Dynamics of photodisruption
- Microsurgery in live cells
- Multiphoton imaging
- Summary

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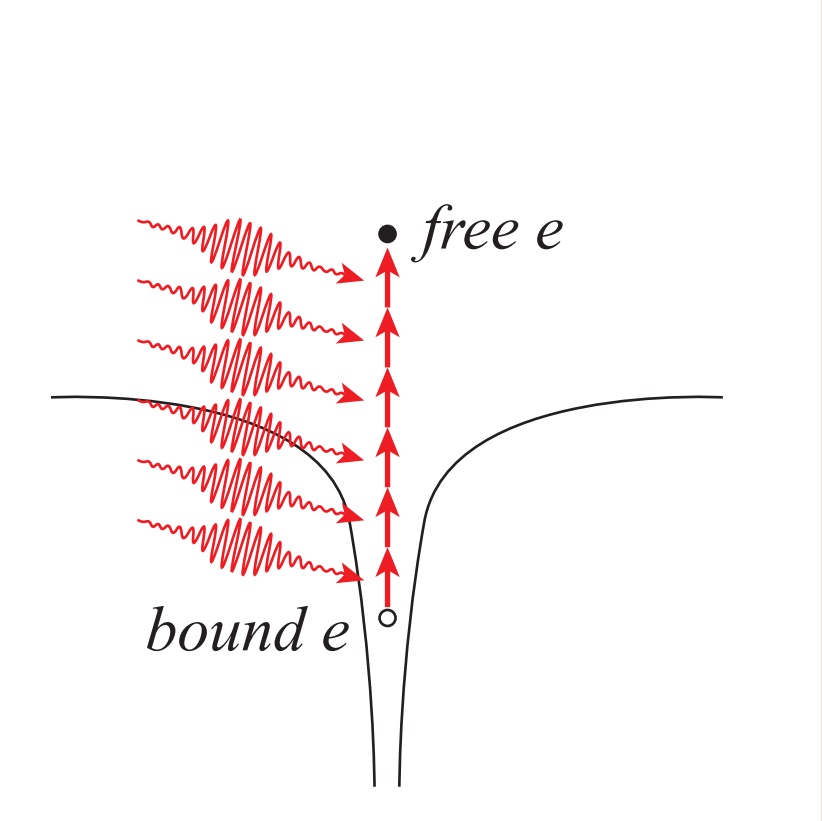
Mechanism

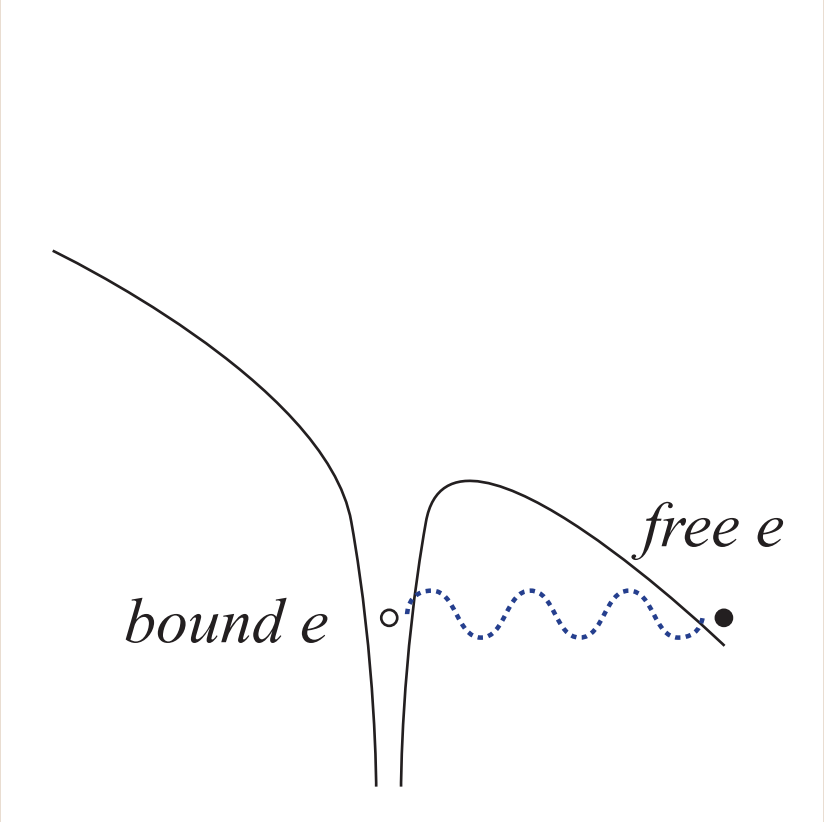
multiphoton ionization



ionization rate $\propto I^n$

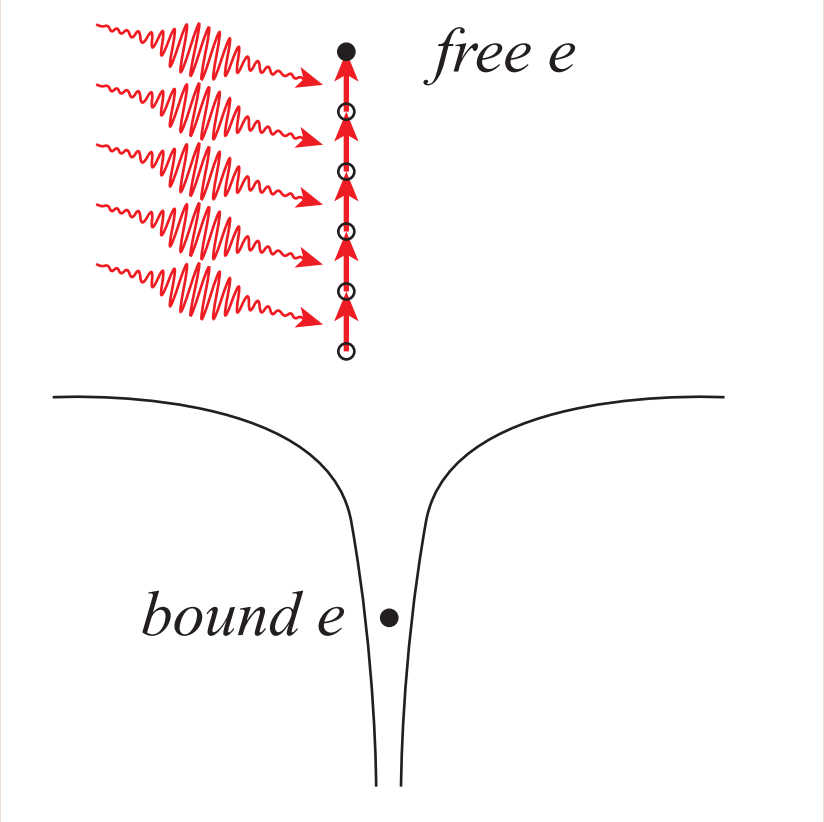
Mechanism

tunneling



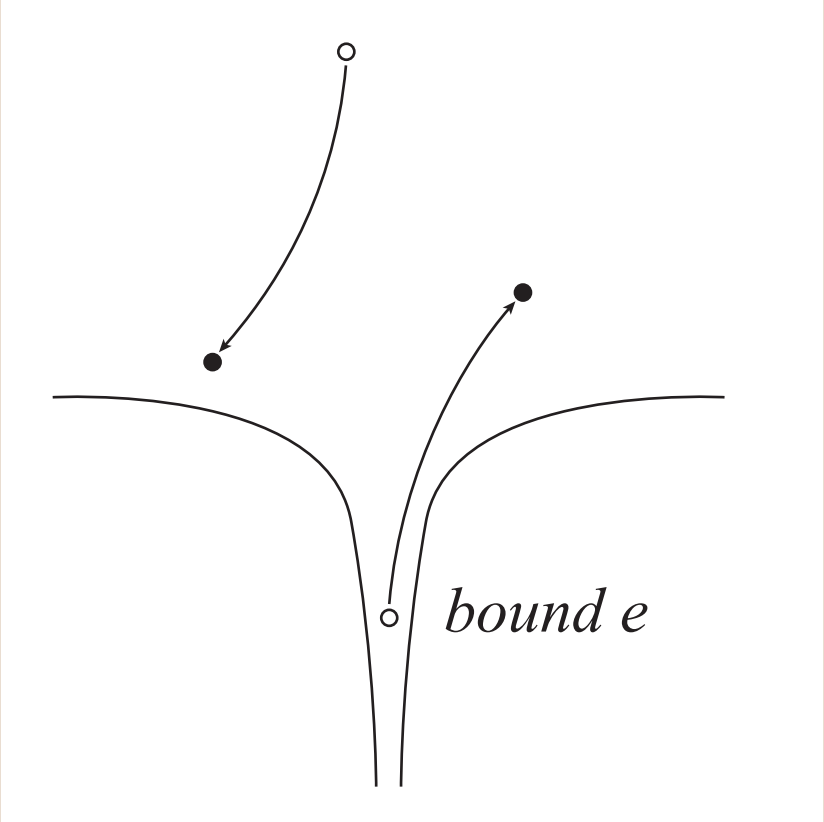
tunneling probability $\propto I^{-1/2}\exp(I^{-1/2})$

avalanche ionization



Mechanism

avalanche ionization



ionization rate $\propto$ *nI*

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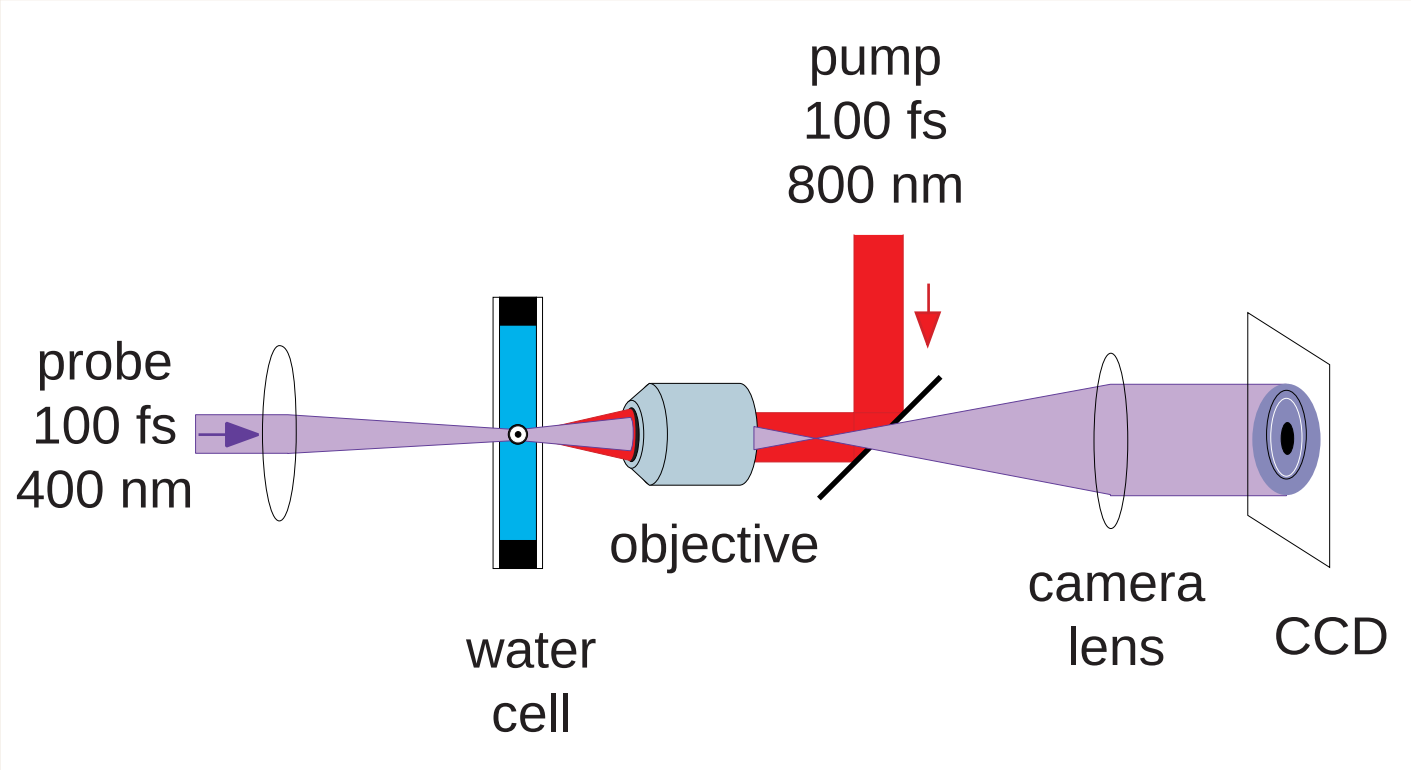
time-resolved imaging of microexplosion in water

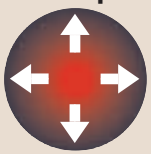


Dynamics

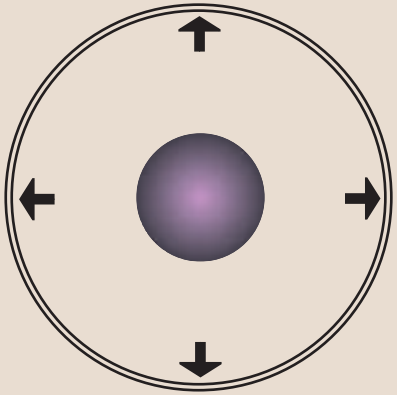
Nonlinear
Absorption

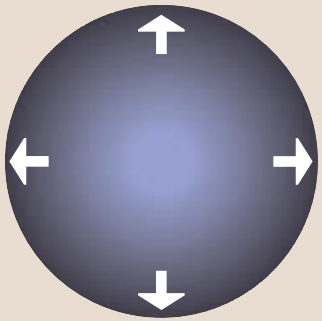
Plasma formation

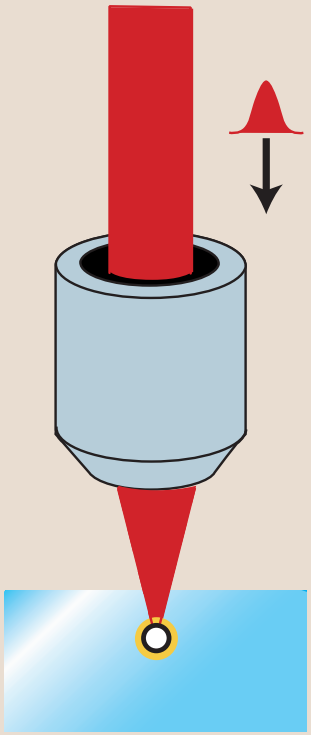
Plasma expansion



Mechanical effects

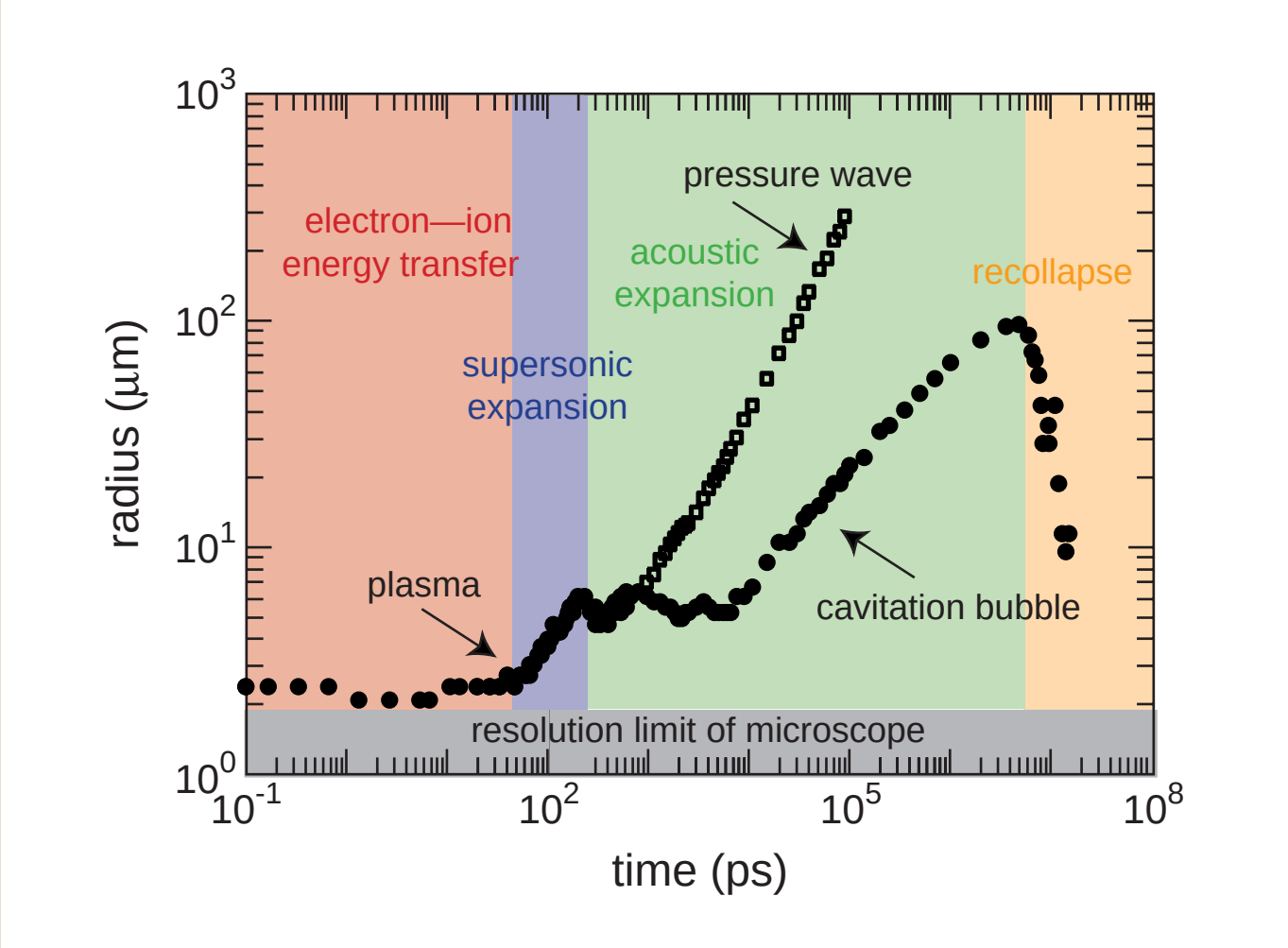




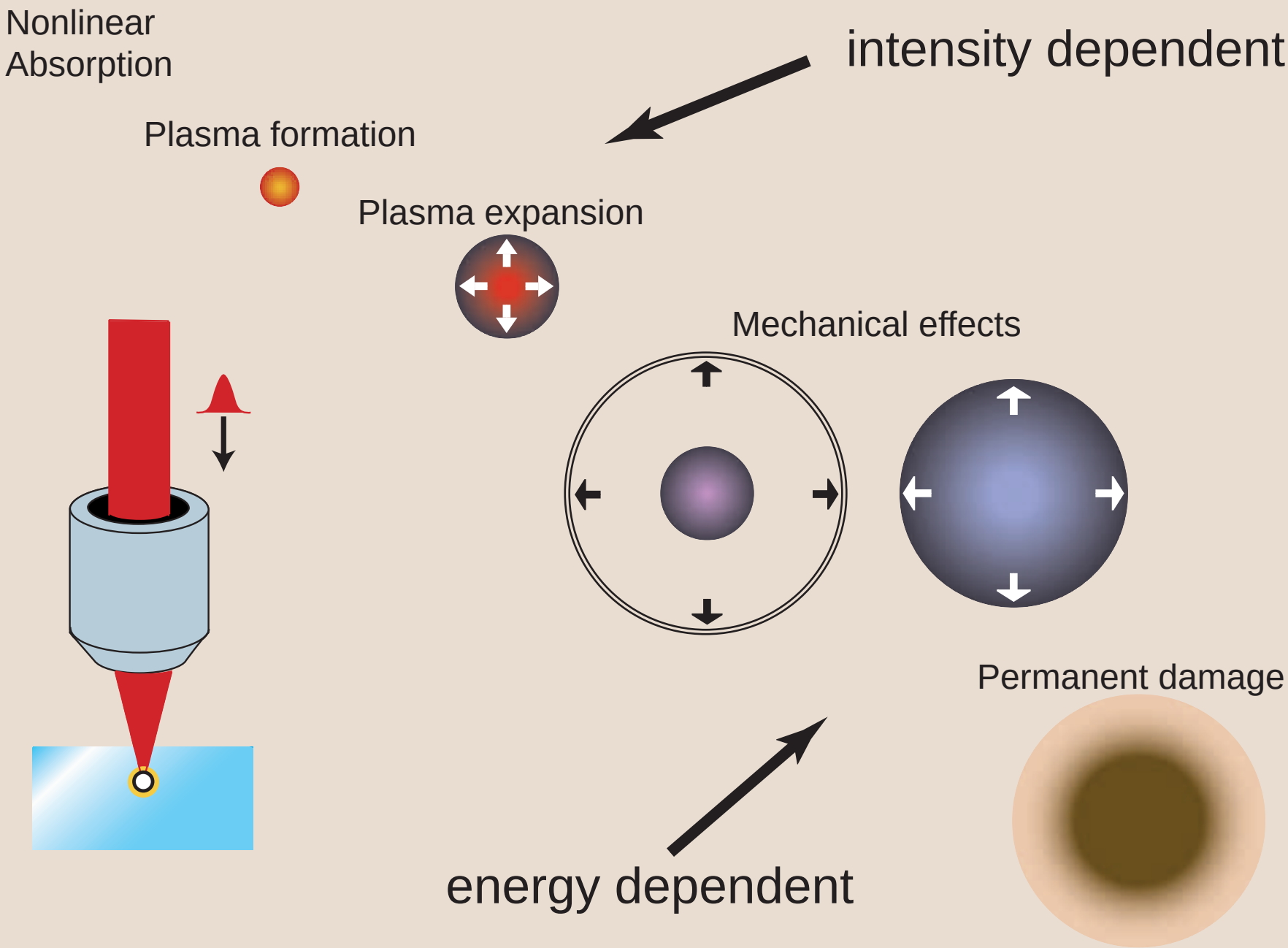


Permanent damage

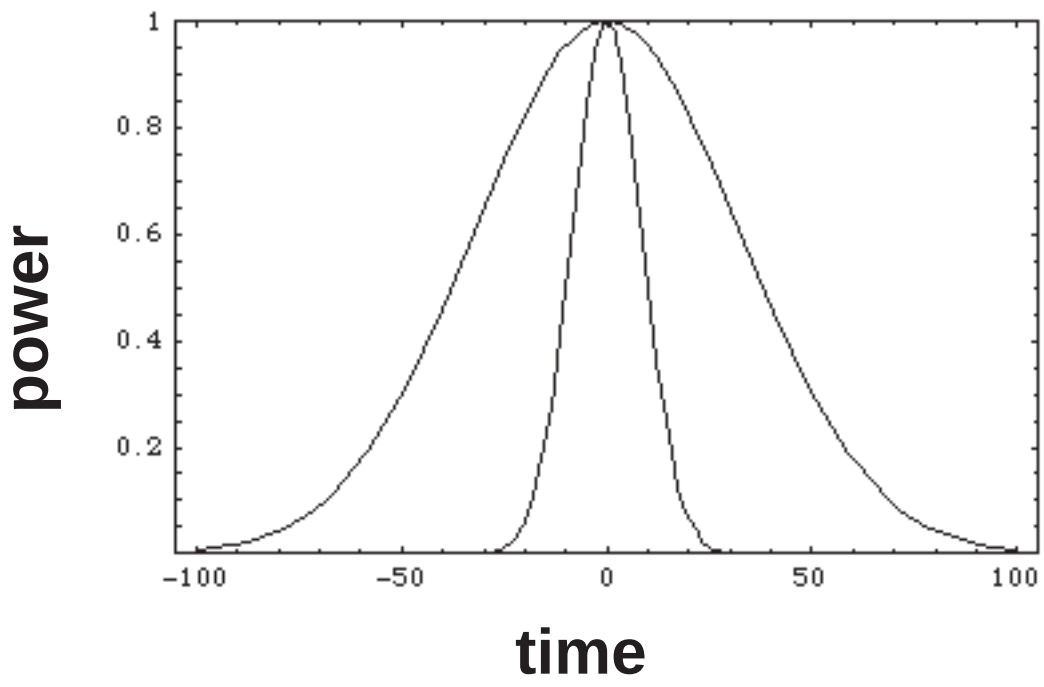
microexplosion radius vs. time



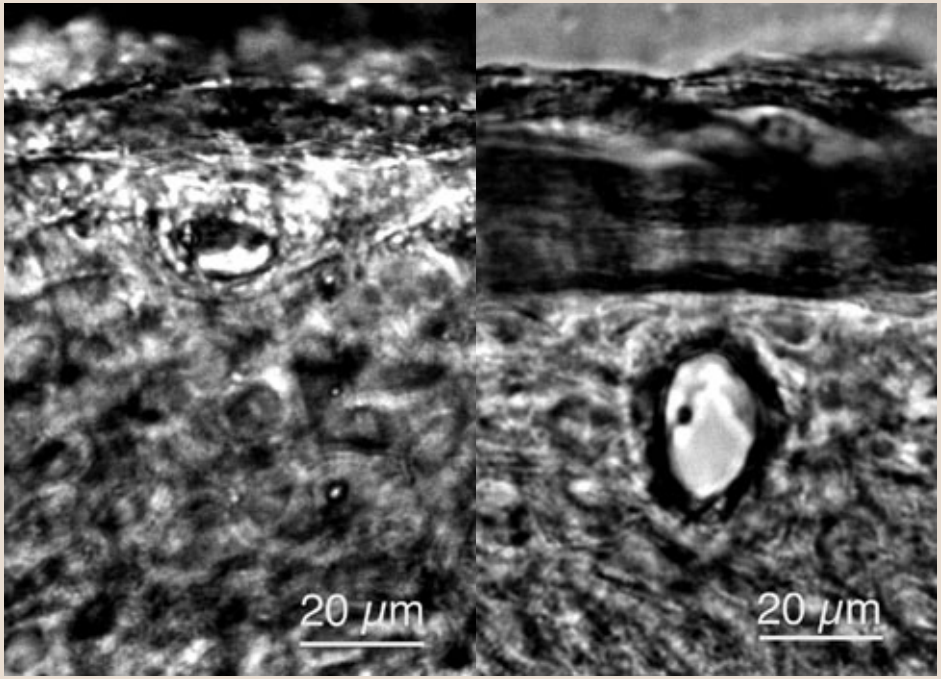
Dynamics


Nonlinear
Absorption
intensity dependent
Plasma formation
Plasma expansion
Mechanical effects
Permanent damage
energy dependent

short pulse produces the same power and intensity for less pulse energy



Dynamics

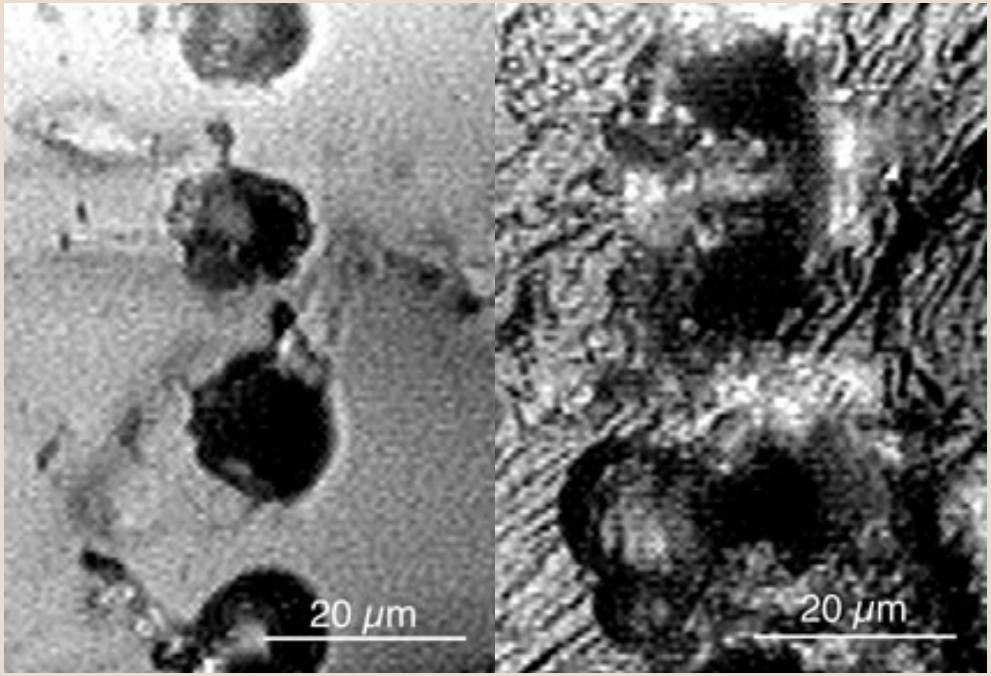


fs pulse ps pulse

subsurface damage at 20μJ

surface damage at 40μJ

fs pulse ps pulse



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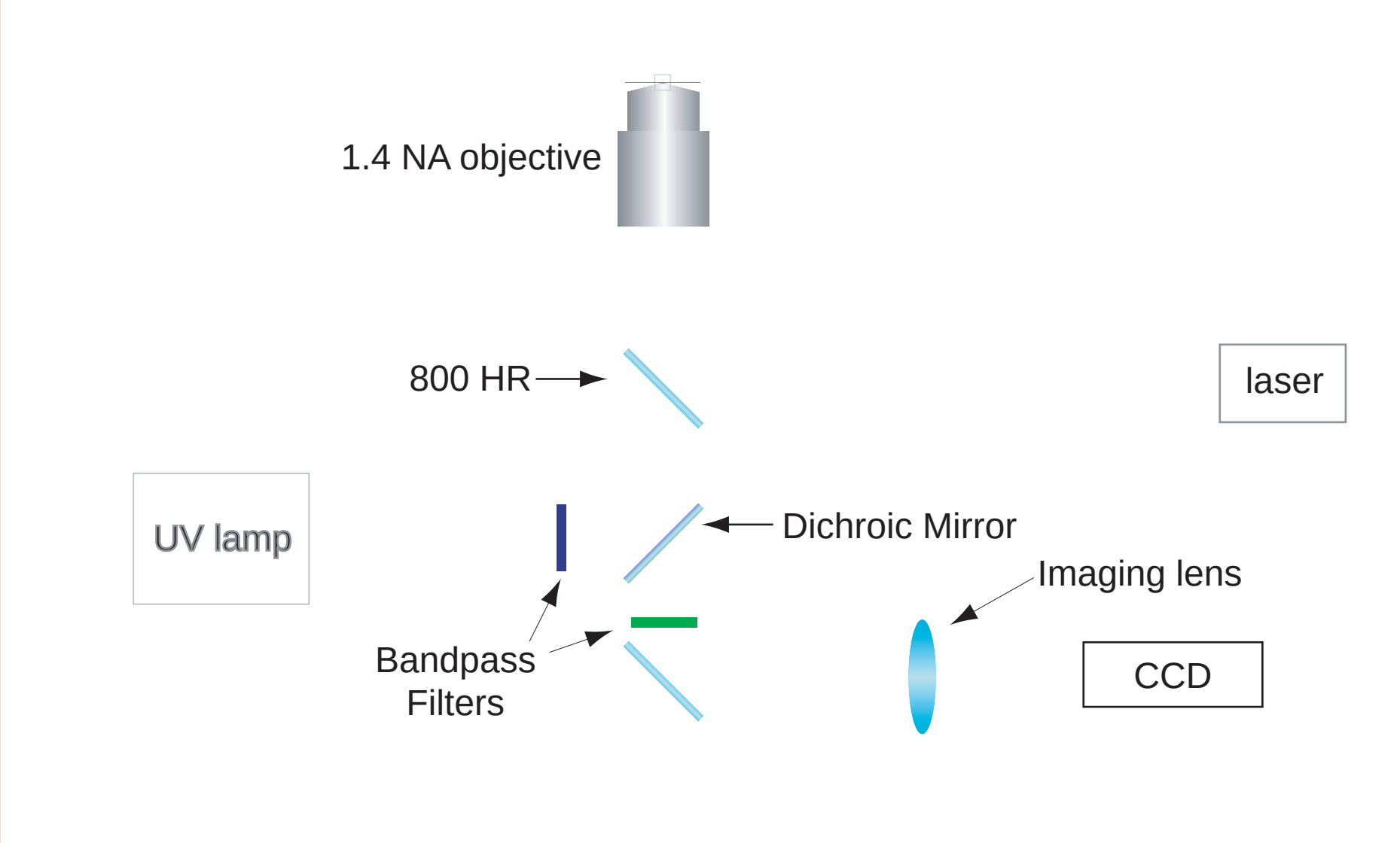
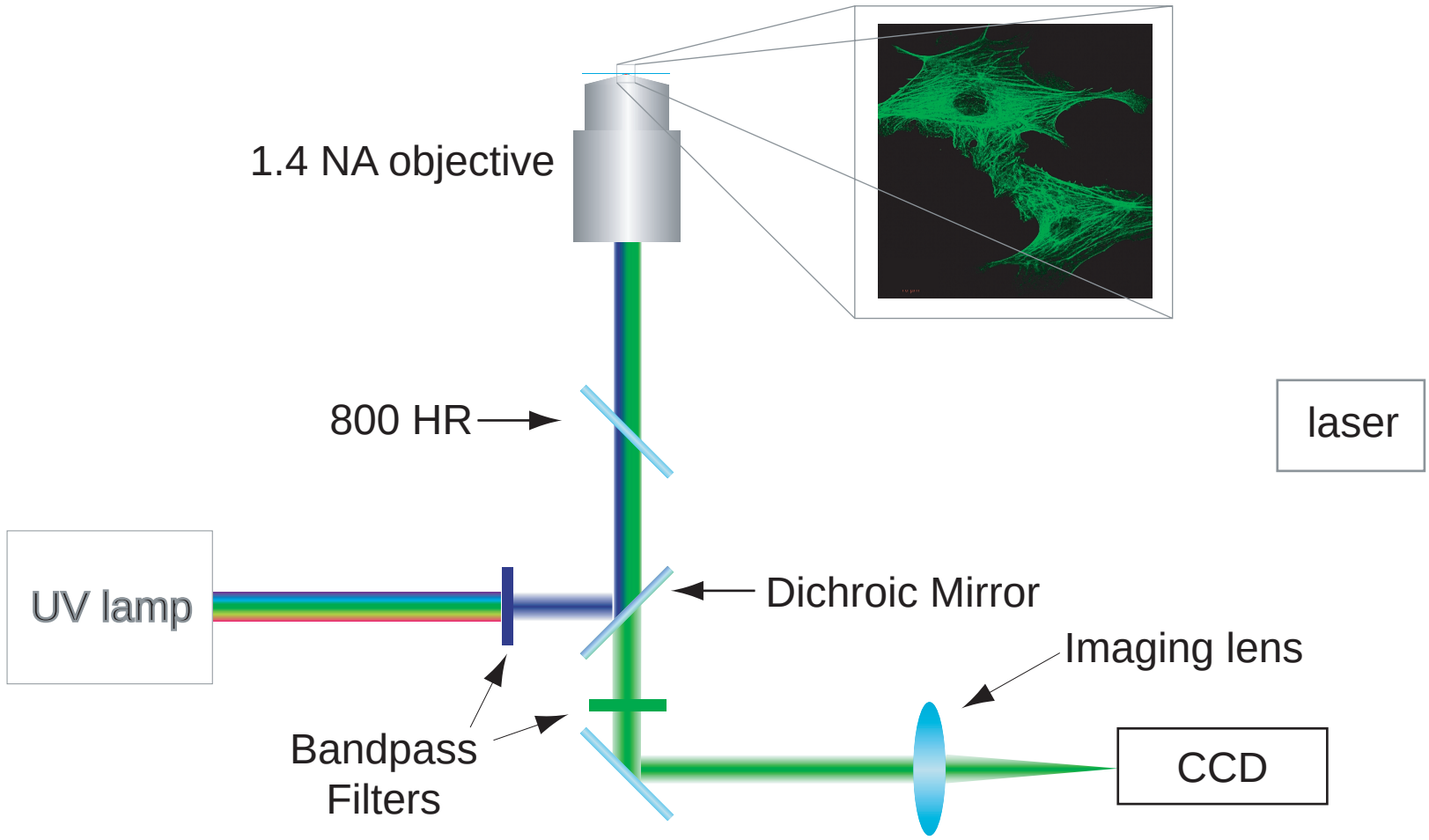
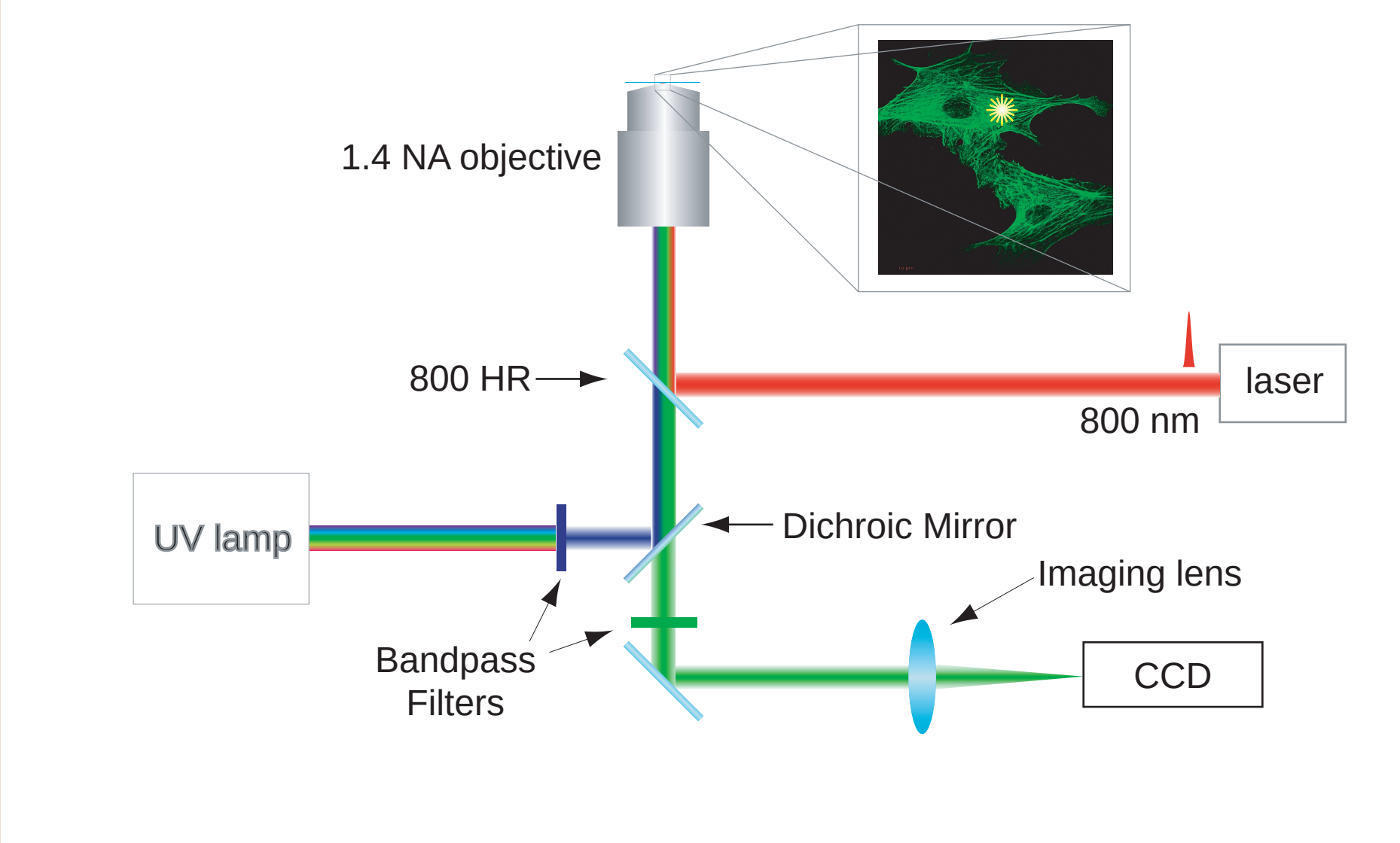
experimental setup



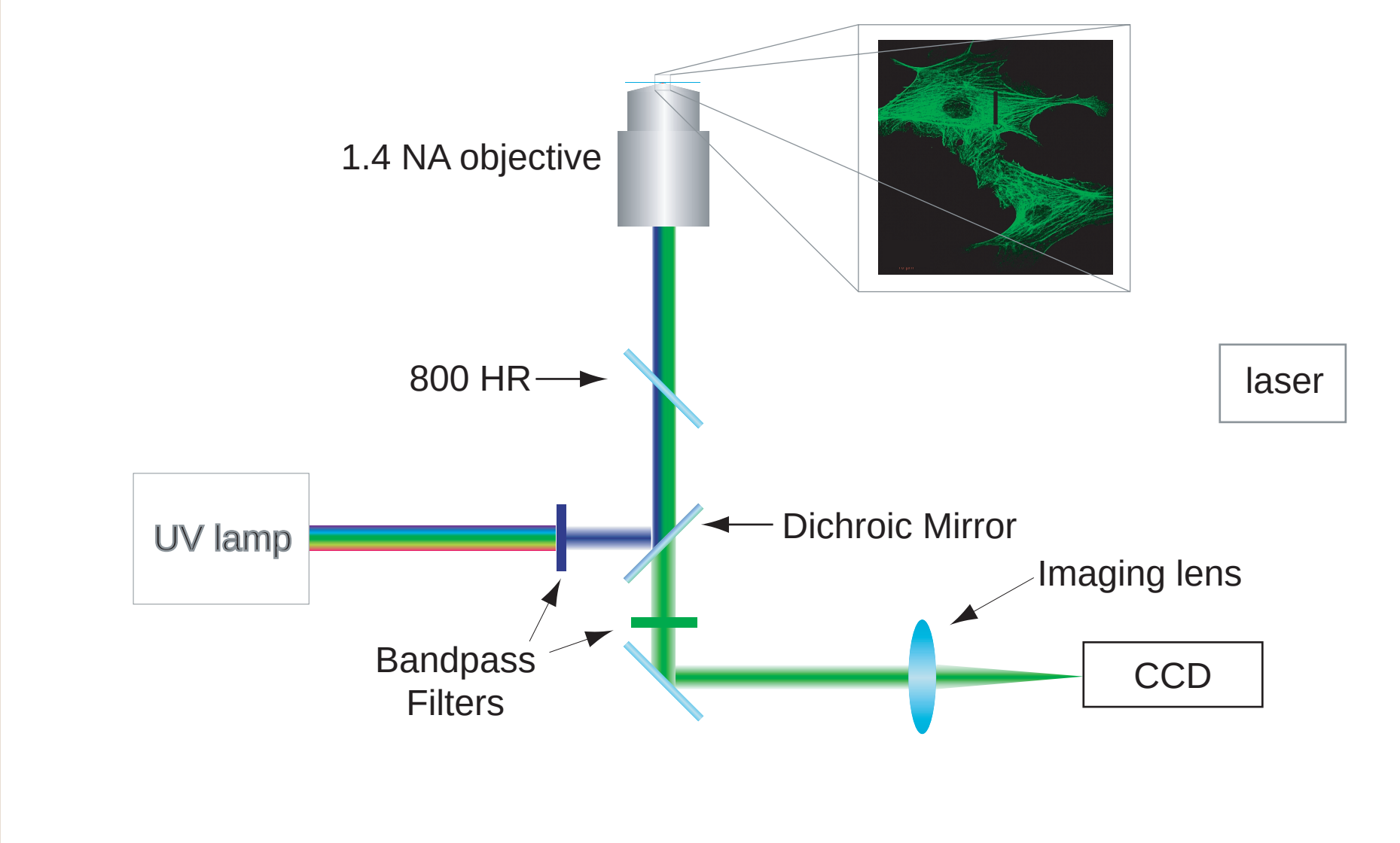
experimental setup



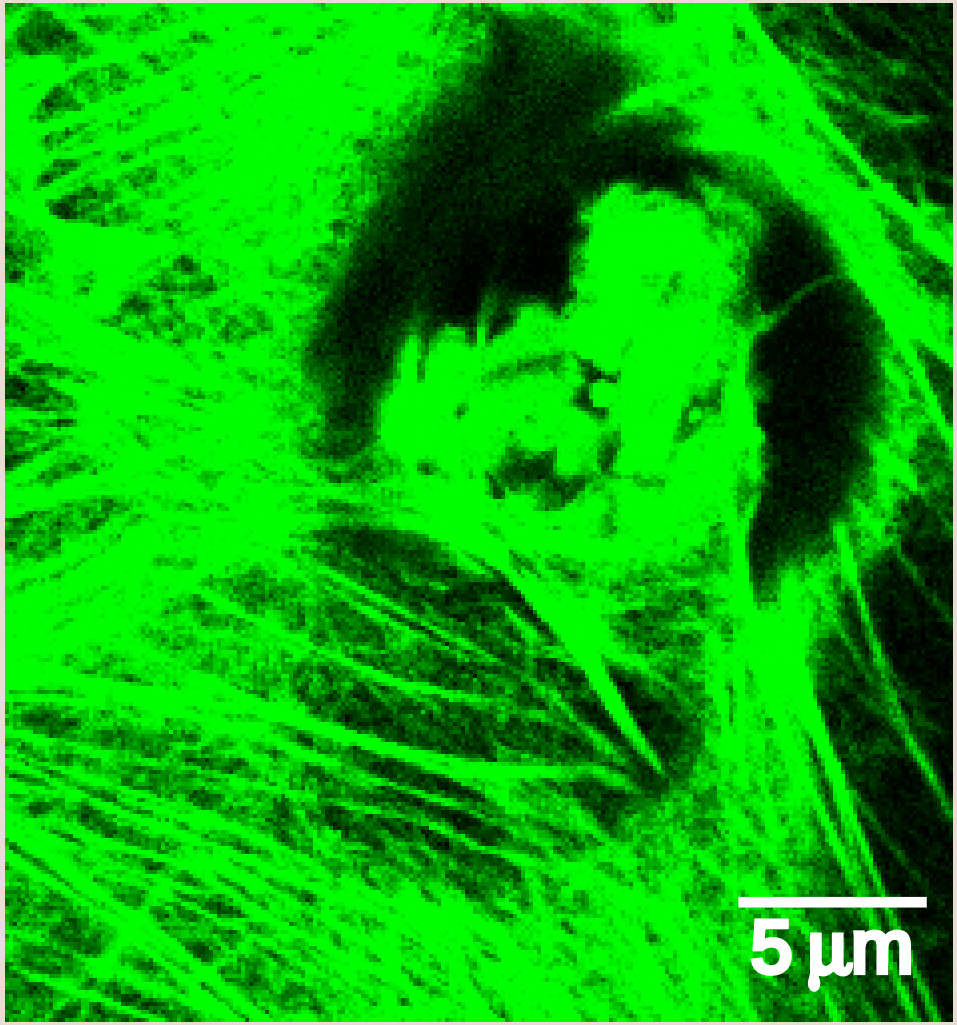
experimental setup



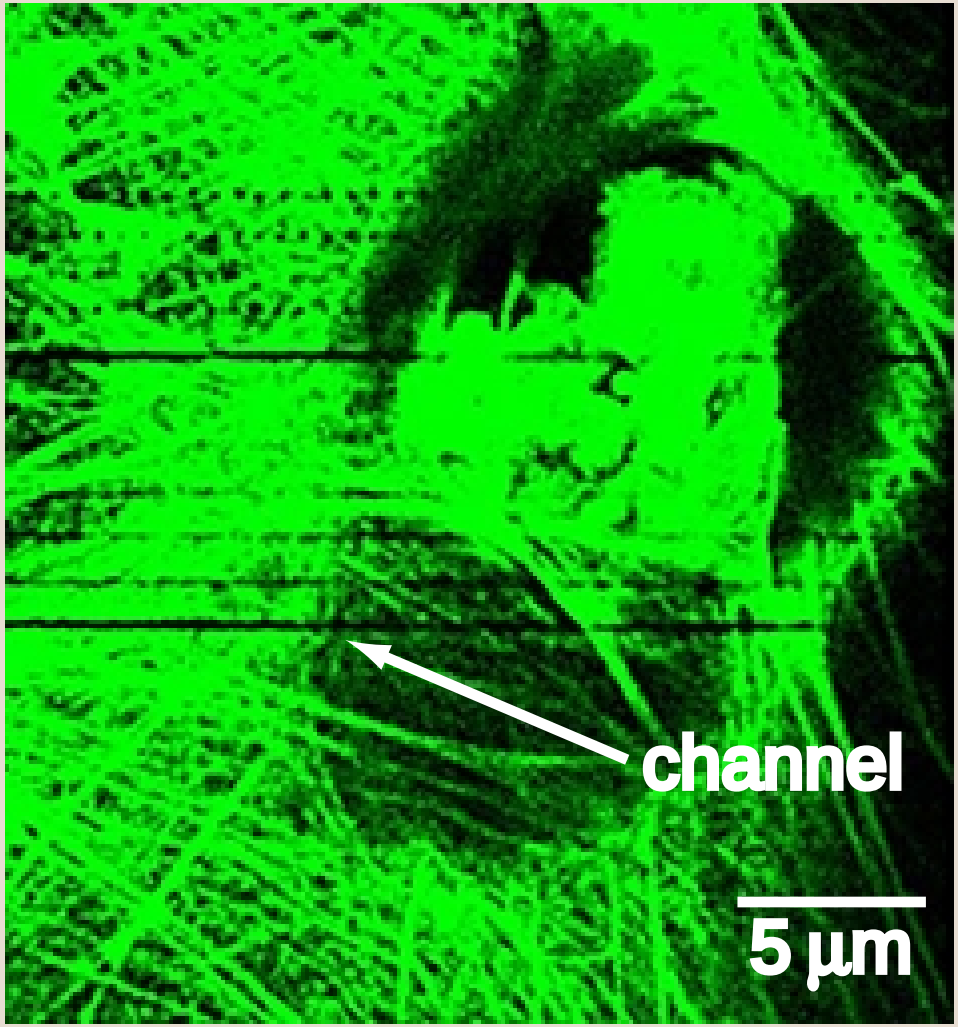
experimental setup



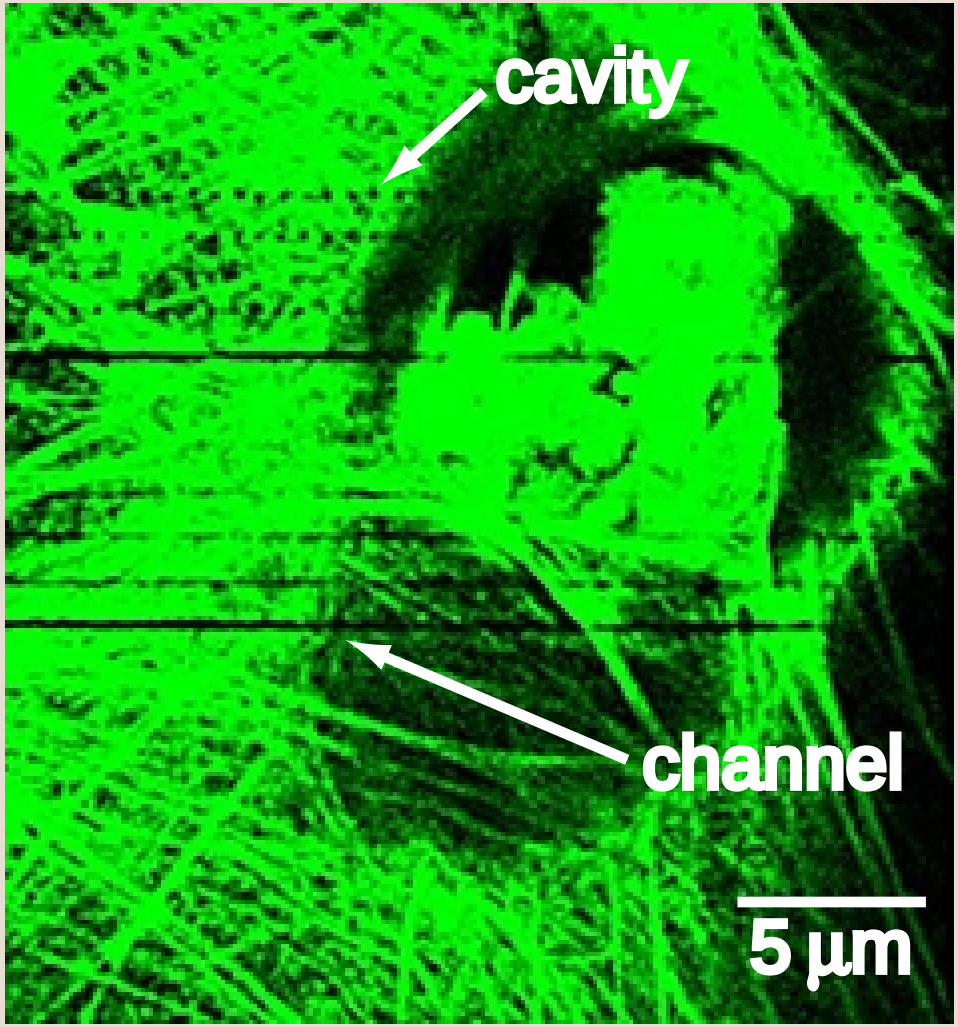
Single Cell



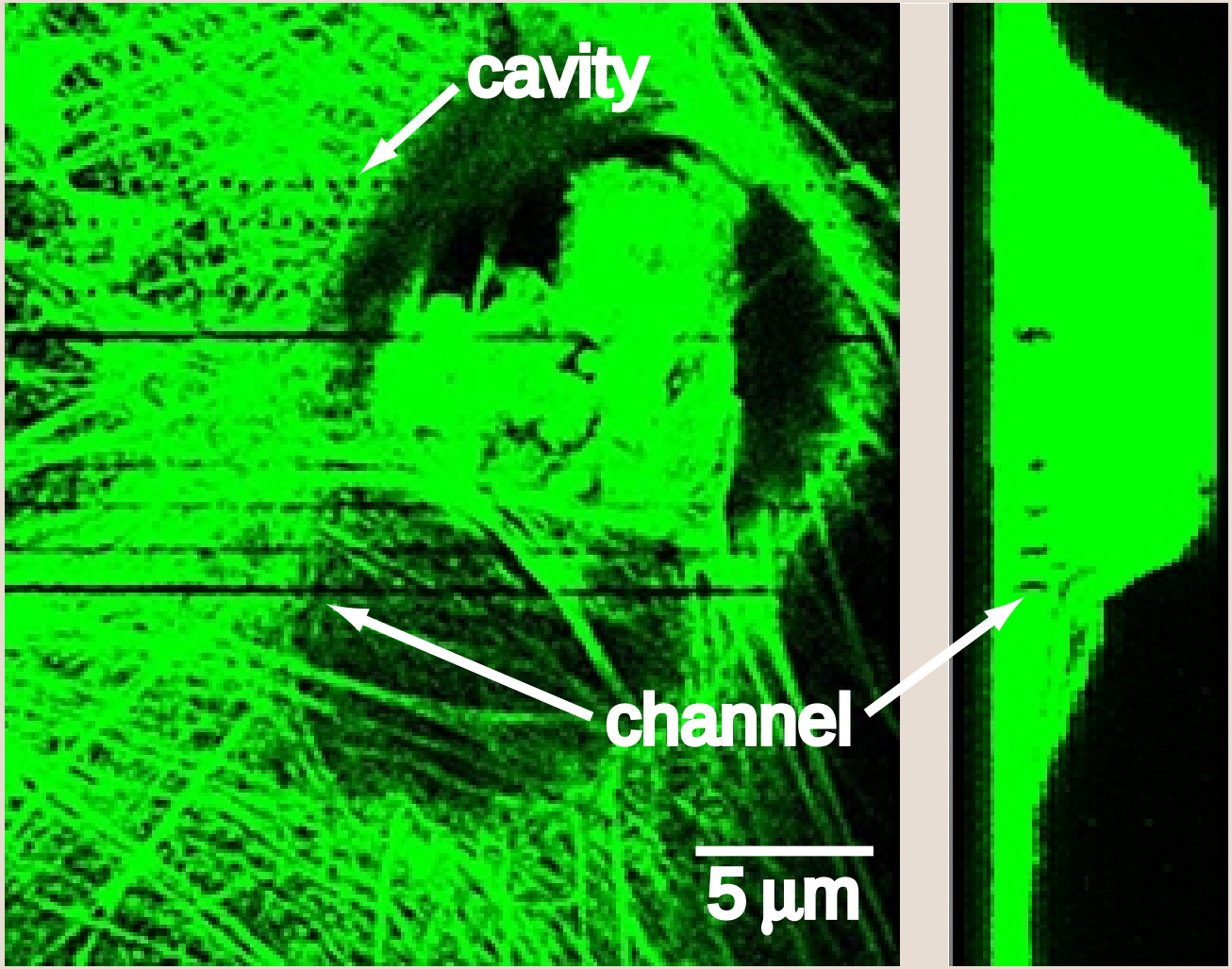
Single Cell



Single Cell



Single Cell



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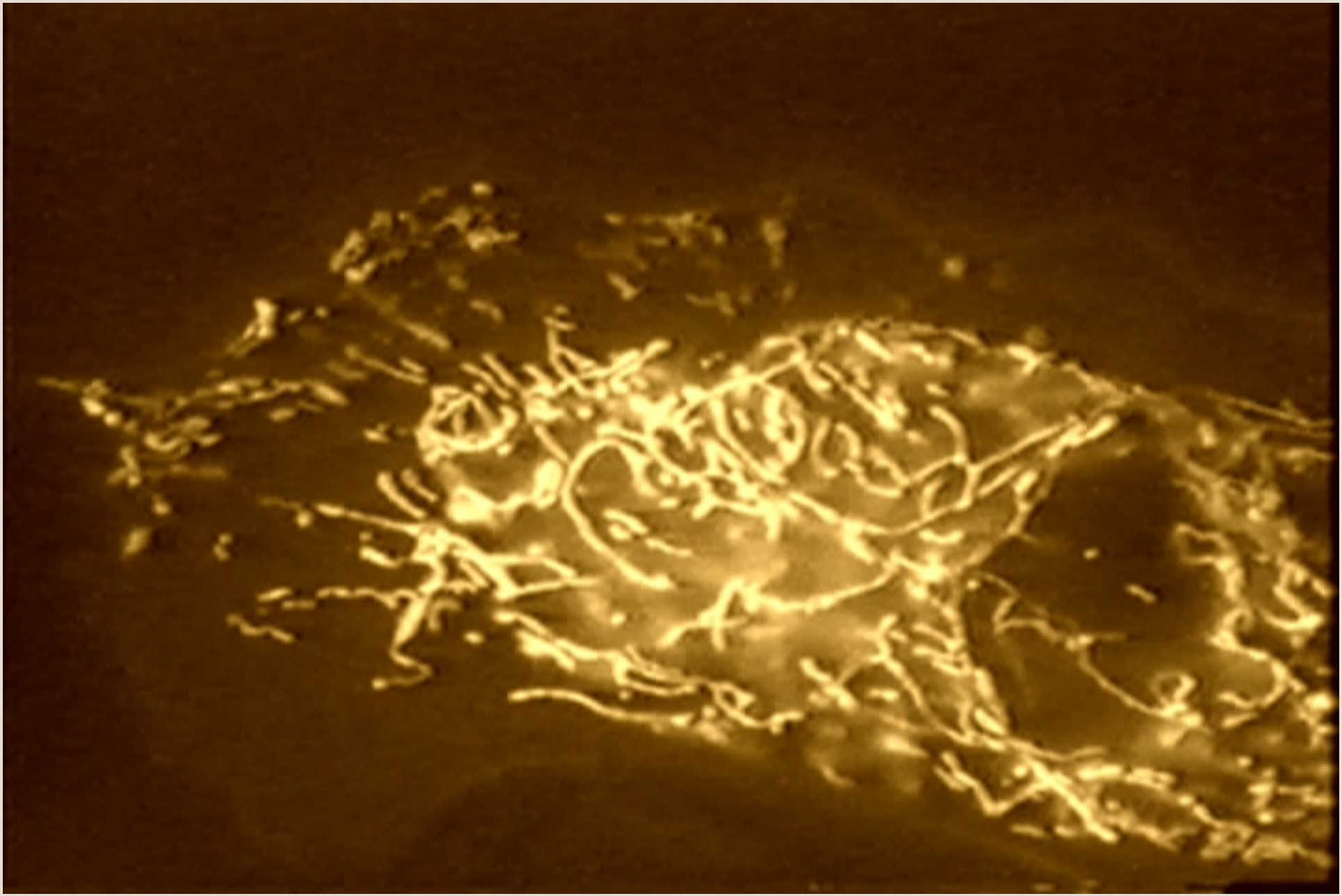
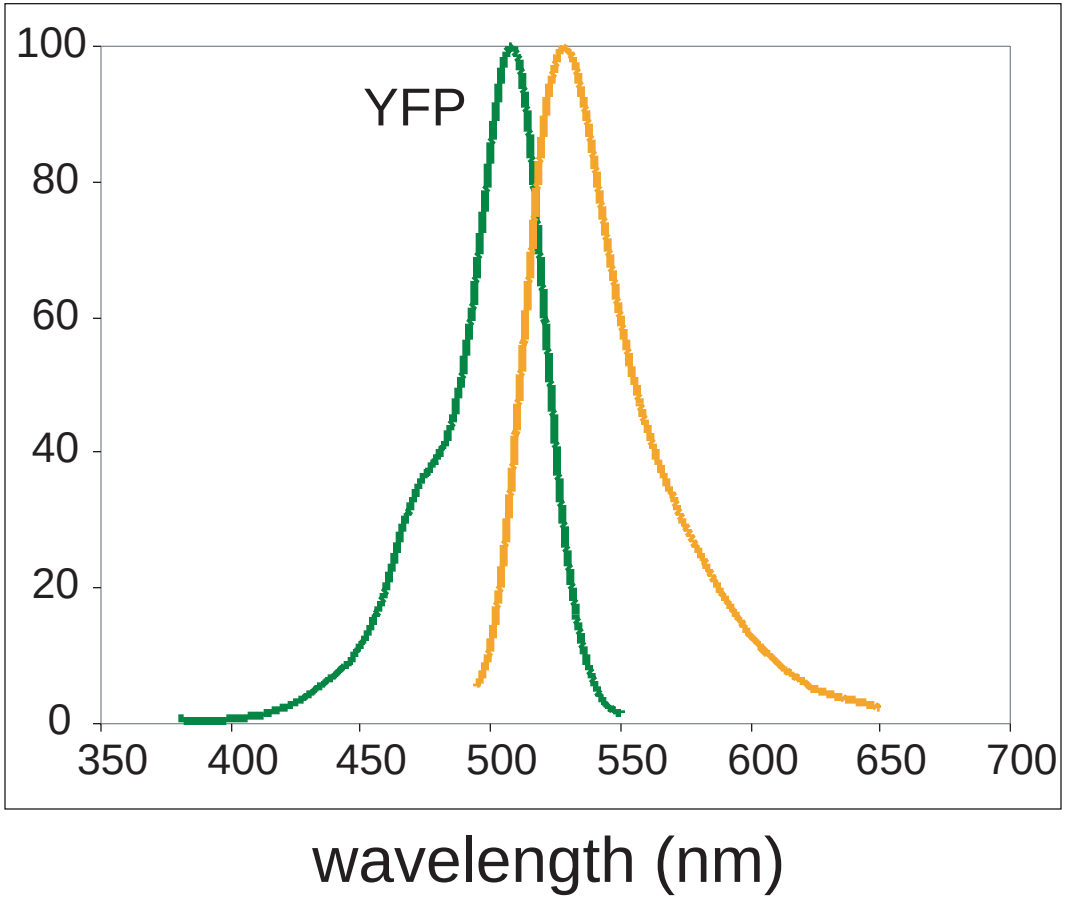
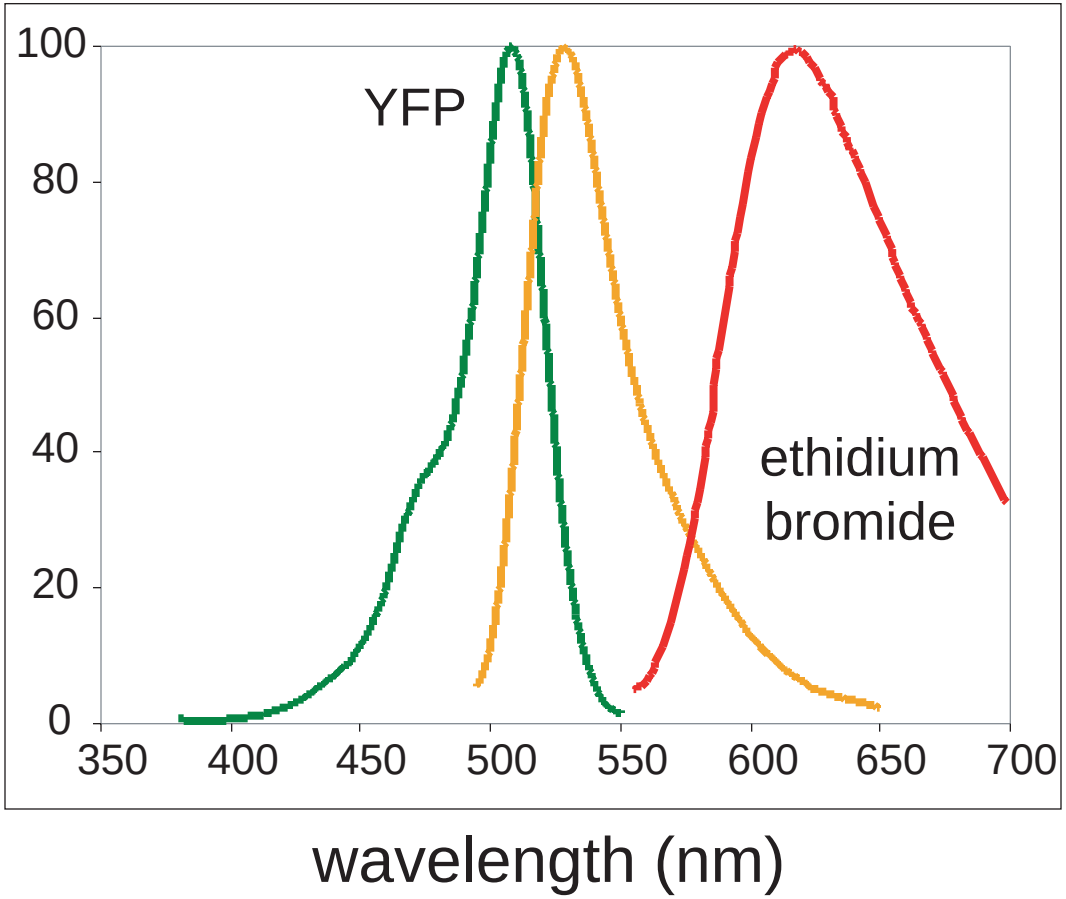


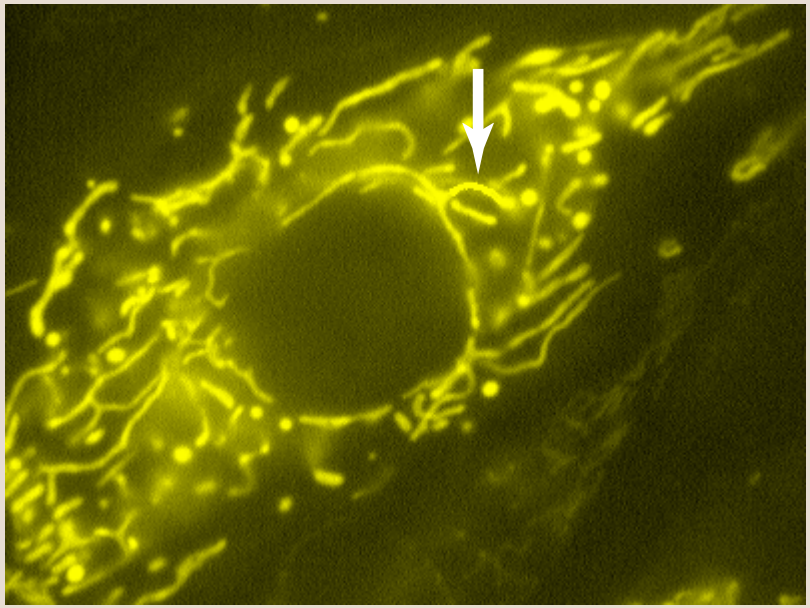
Single Cell


YFP
100
80
60
40
20
0
350
400
450
500
550
600
650
700
wavelength (nm)

Single Cell


100
80
60
40
20
0
350
400
450
500
550
600
650
700
YFP
ethidium
bromide
wavelength (nm)

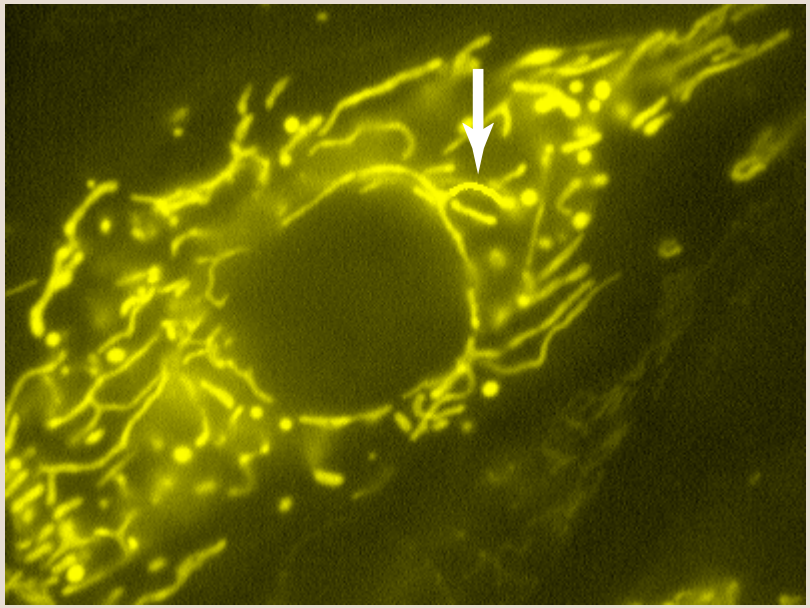
100 fs,
2 nJ/pulse



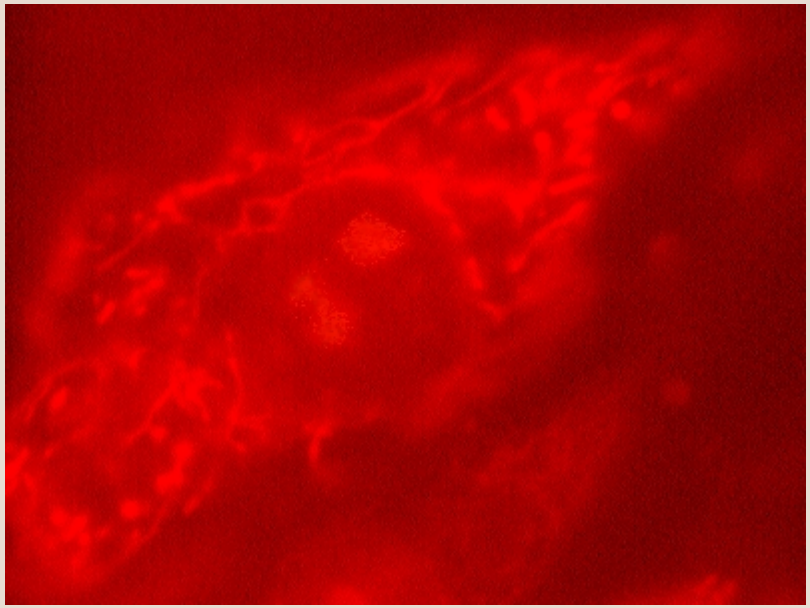
before

Single Cell

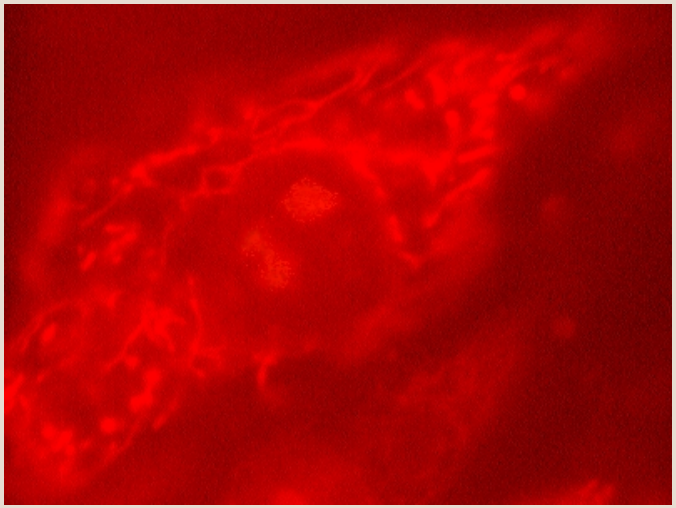
100 fs,
2 nJ/pulse



before



Single Cell



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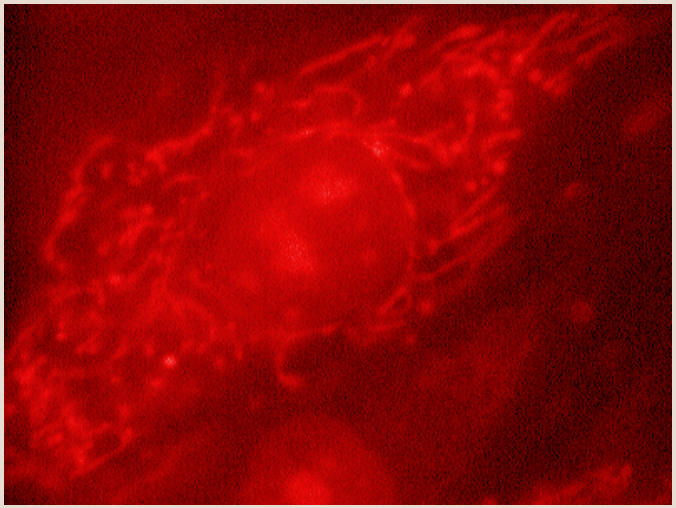
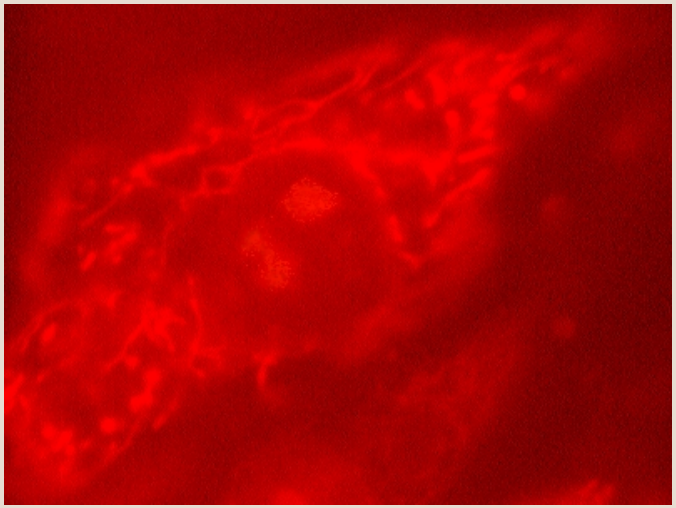


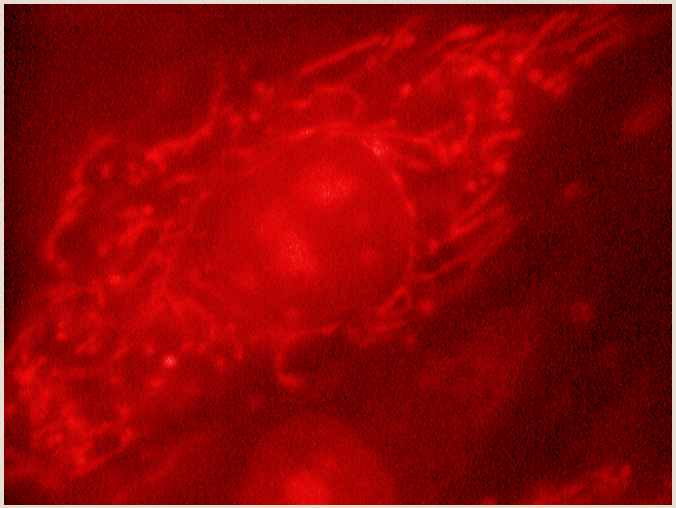
after

100 fs,
2 nJ/pulse

Single Cell

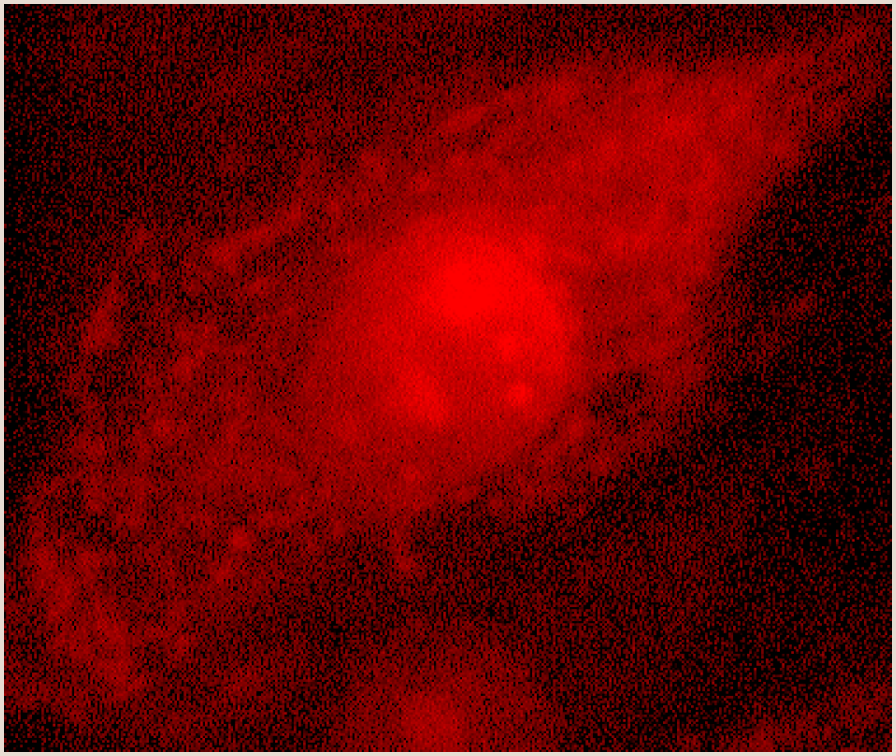


before

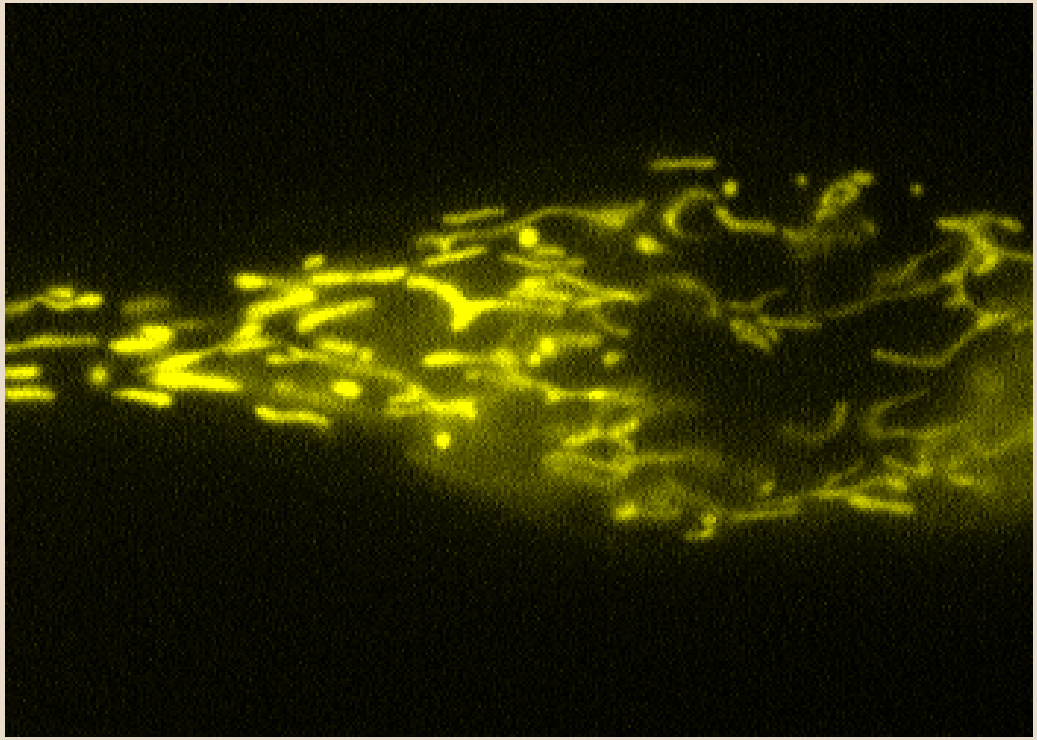


after

100 fs,
2 nJ/pulse



Mitochondrial organization:



continuous network

similar to the

endoplasmic reticulum?

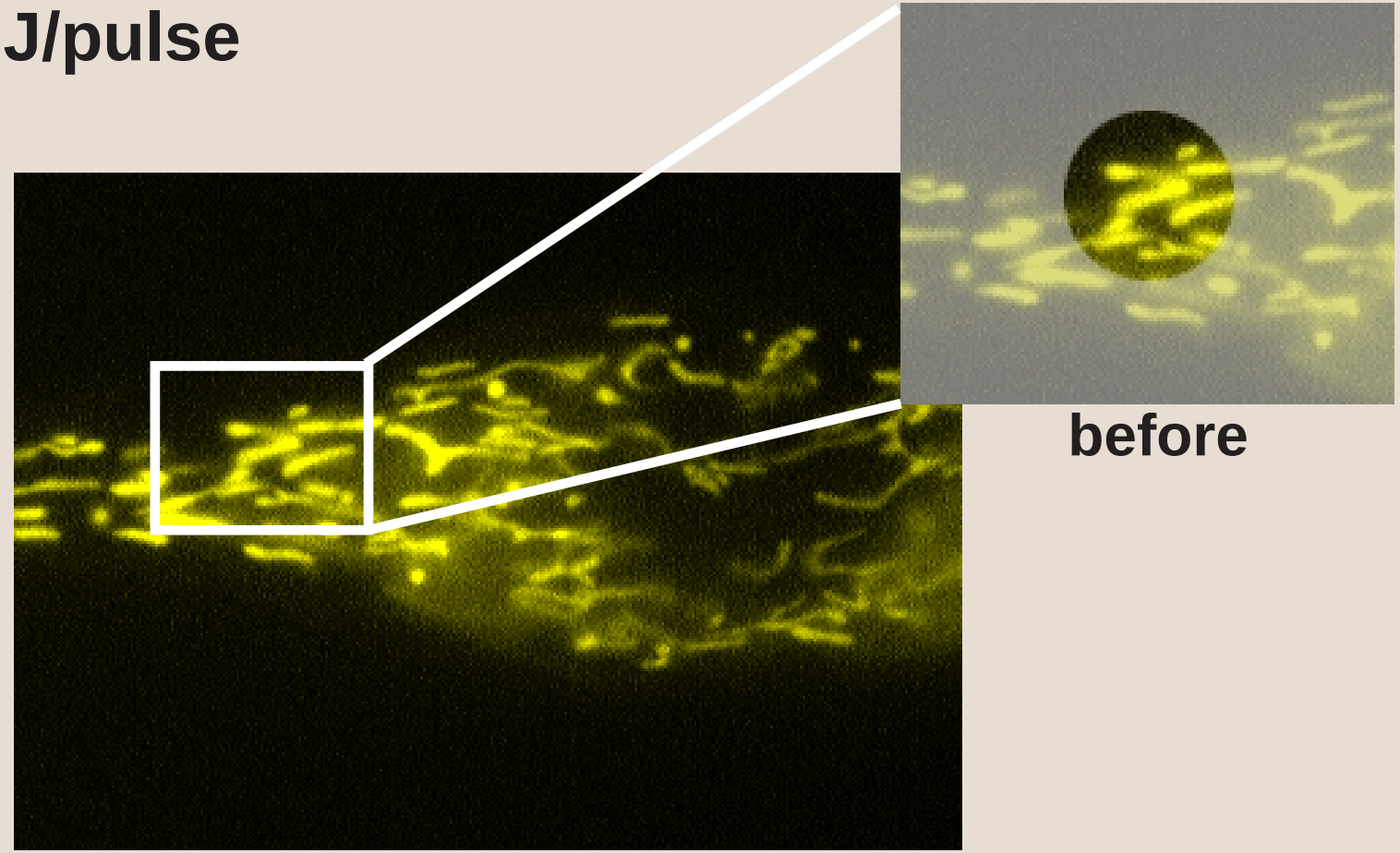
or

independent functional

units?

Single Cell

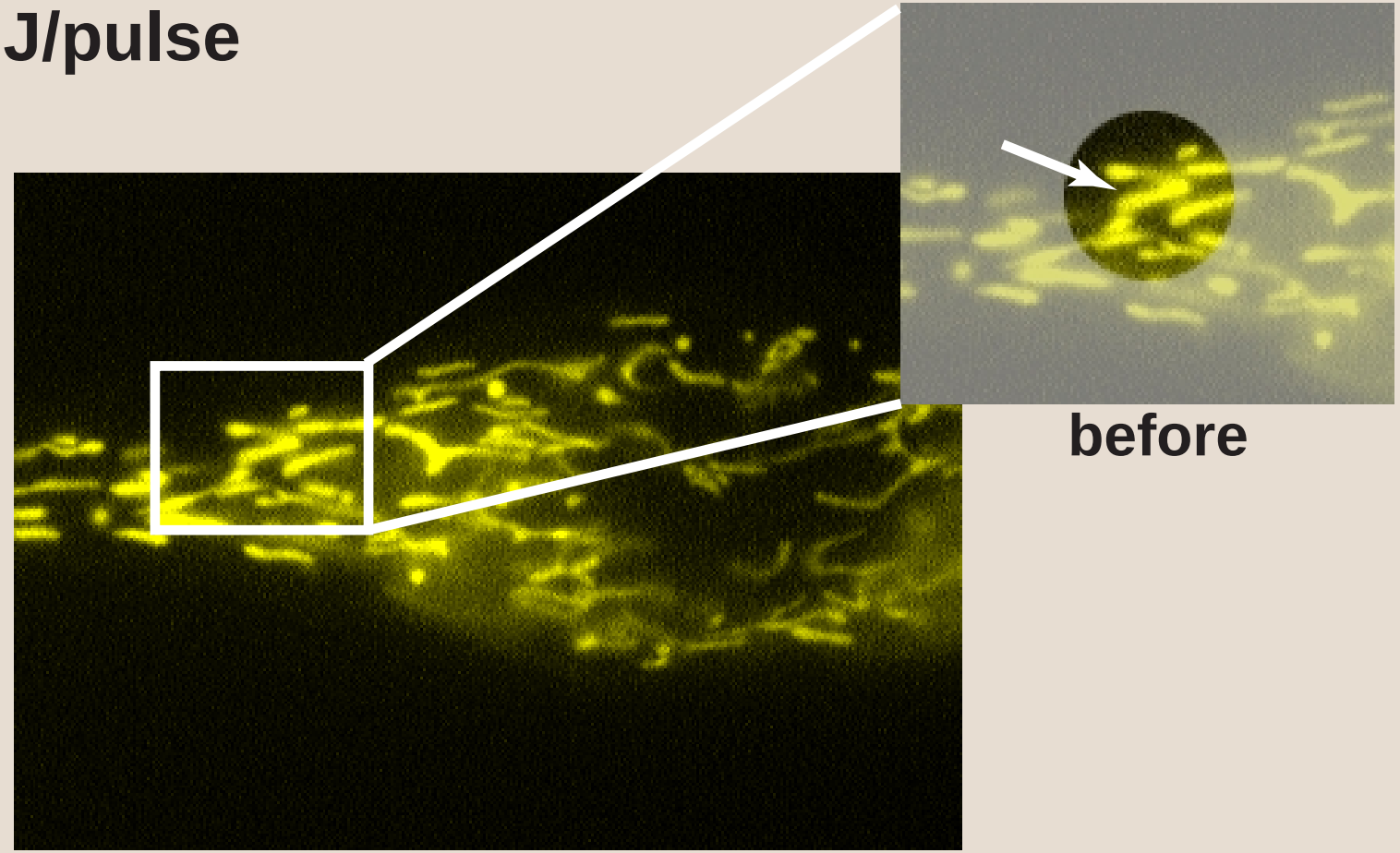
100 fs,
2 nJ/pulse



before

Single Cell

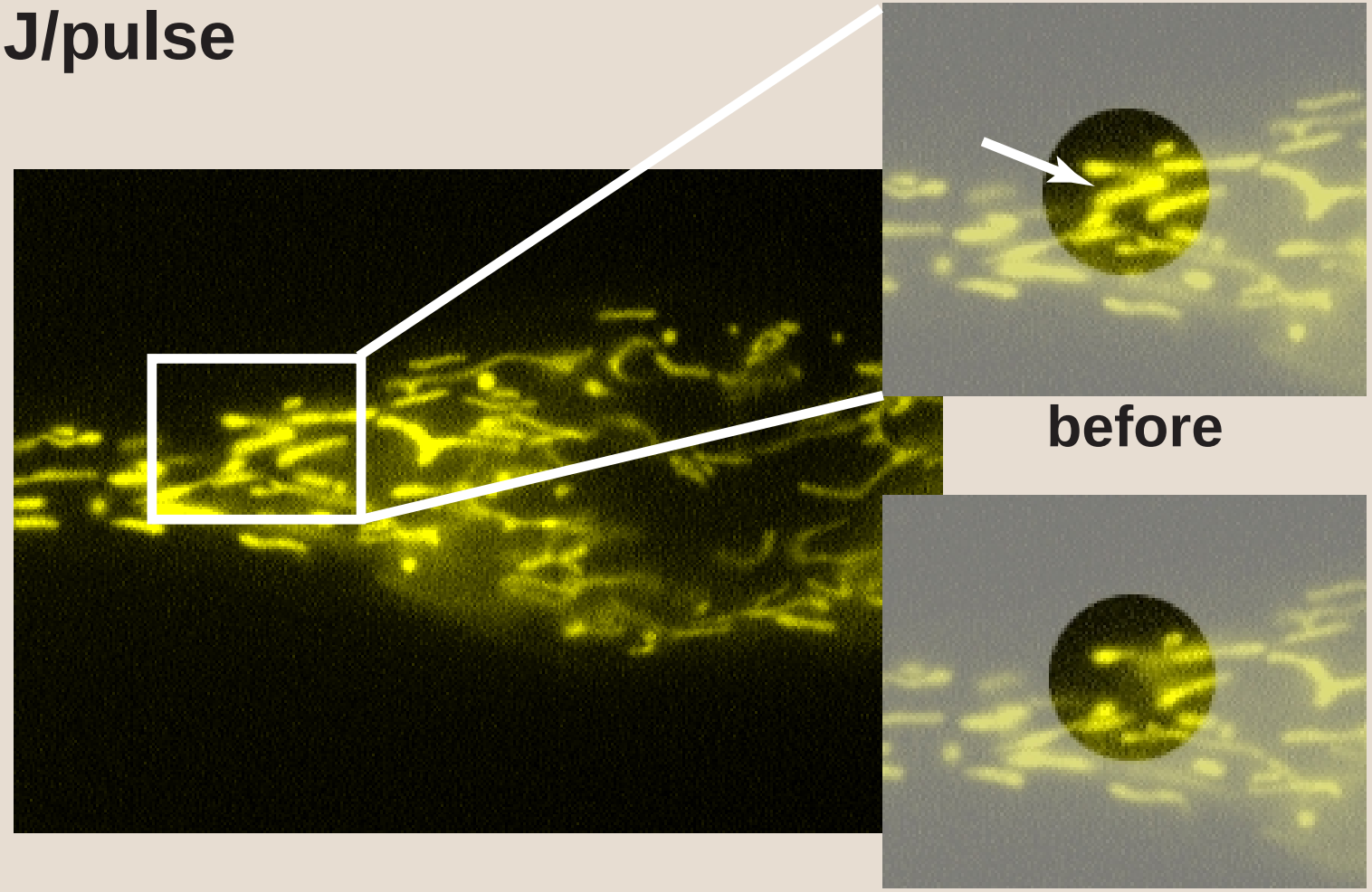
100 fs,
2 nJ/pulse



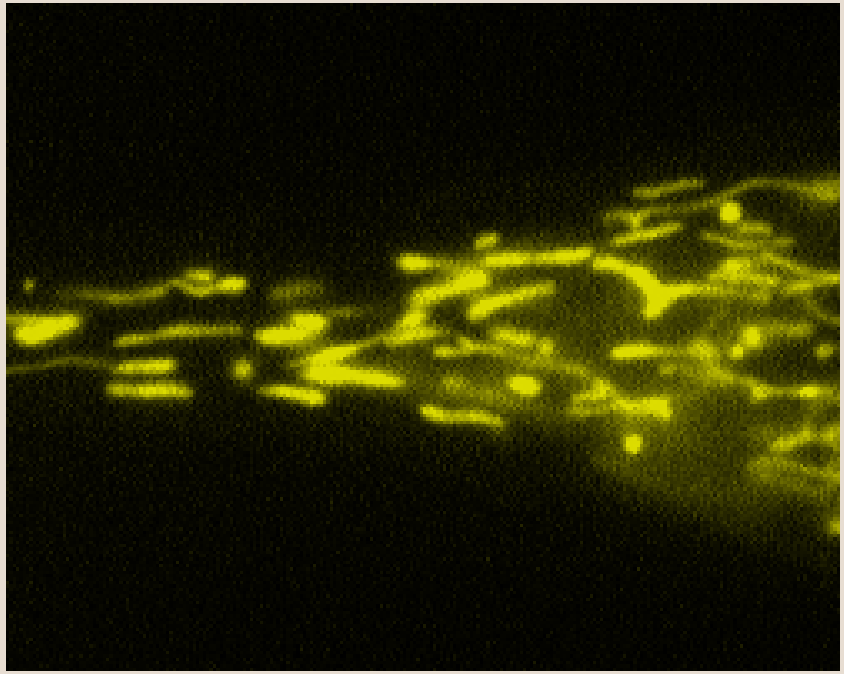
before

Single Cell

100 fs,
2 nJ/pulse



Single Cell



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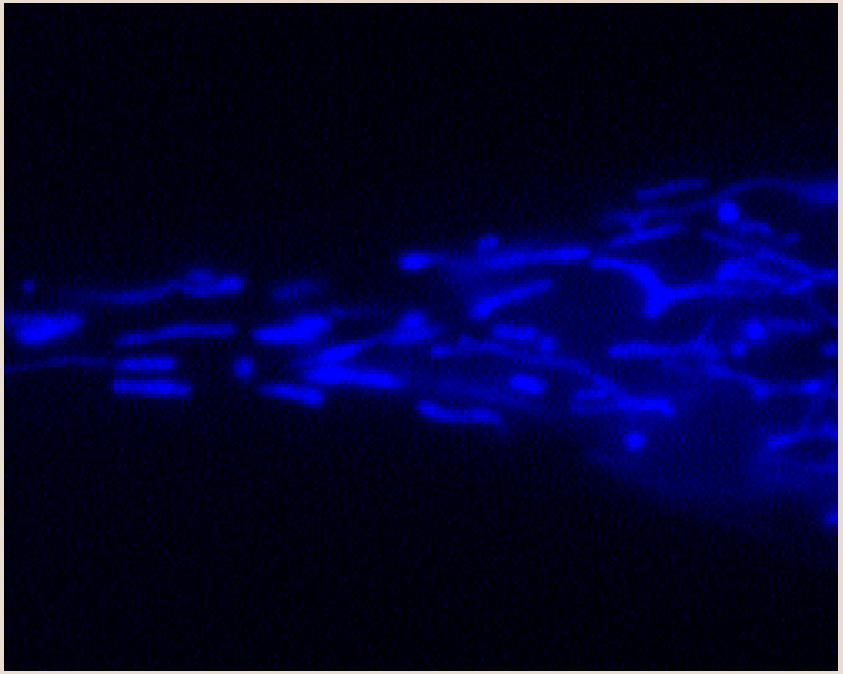
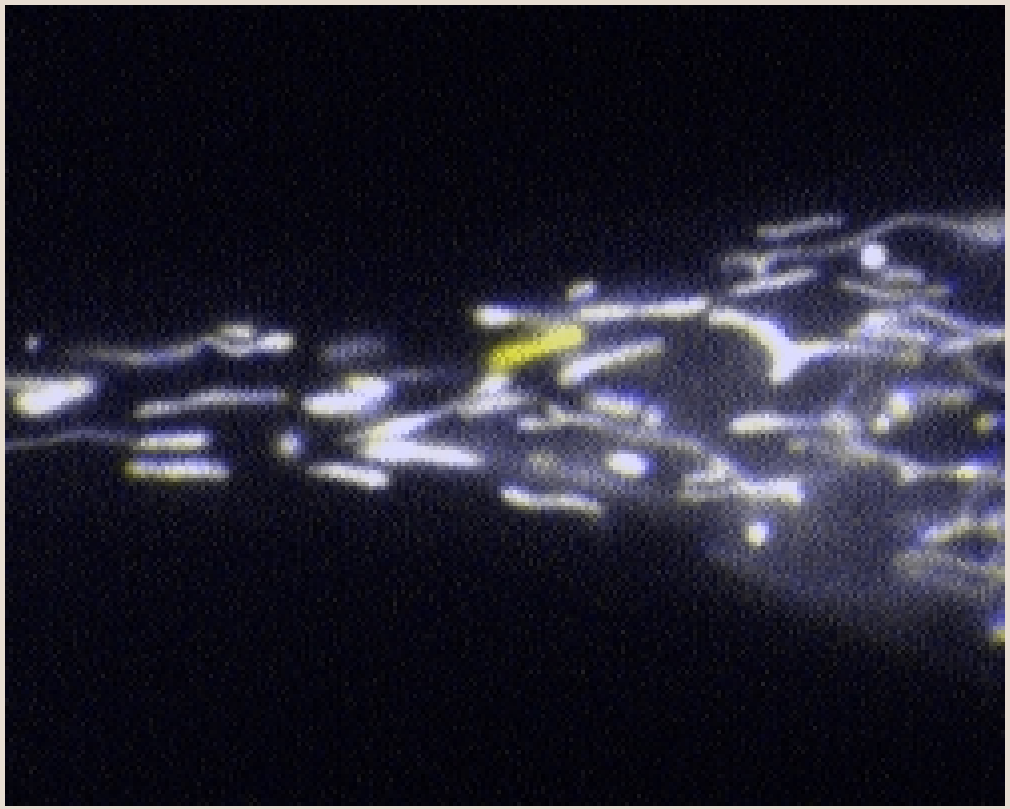
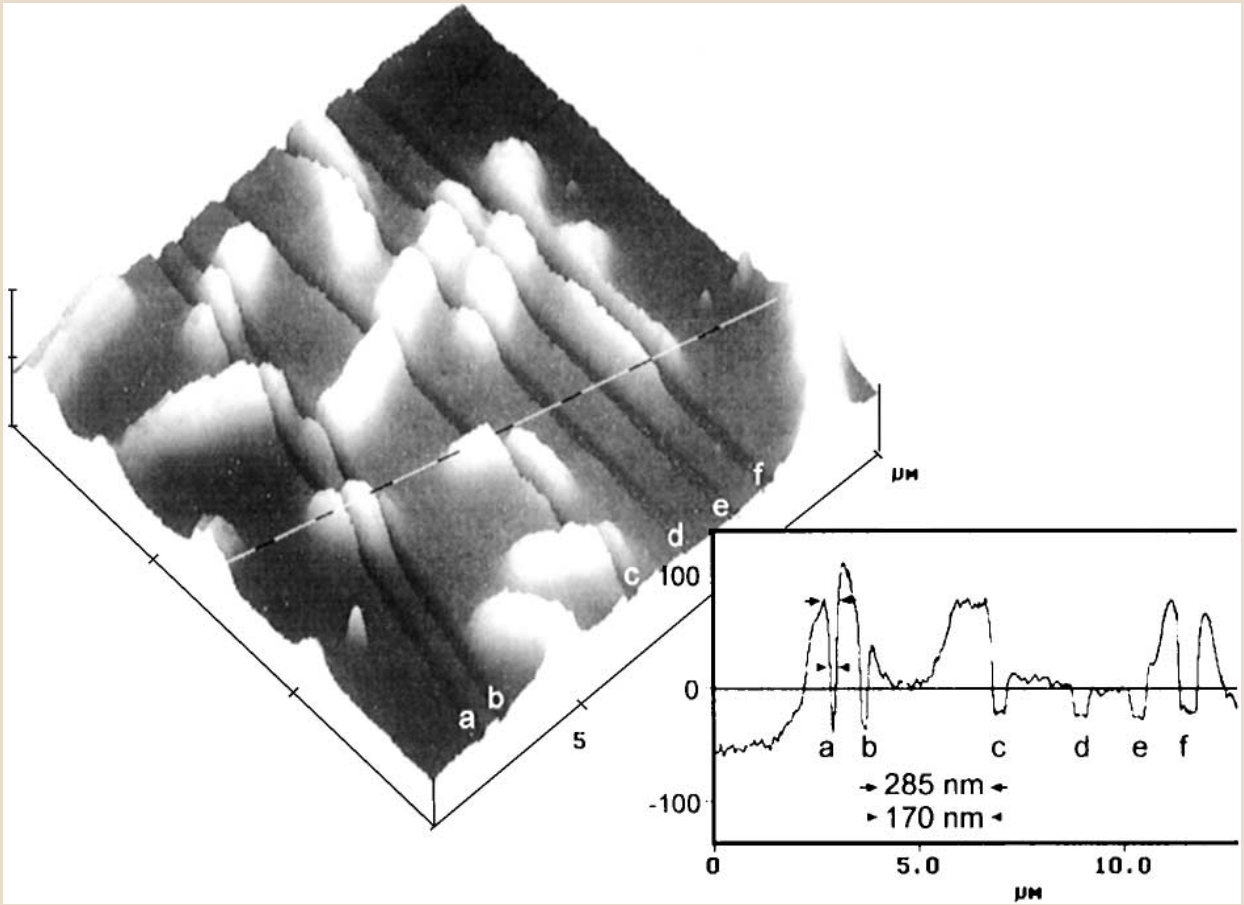


after

Single Cell

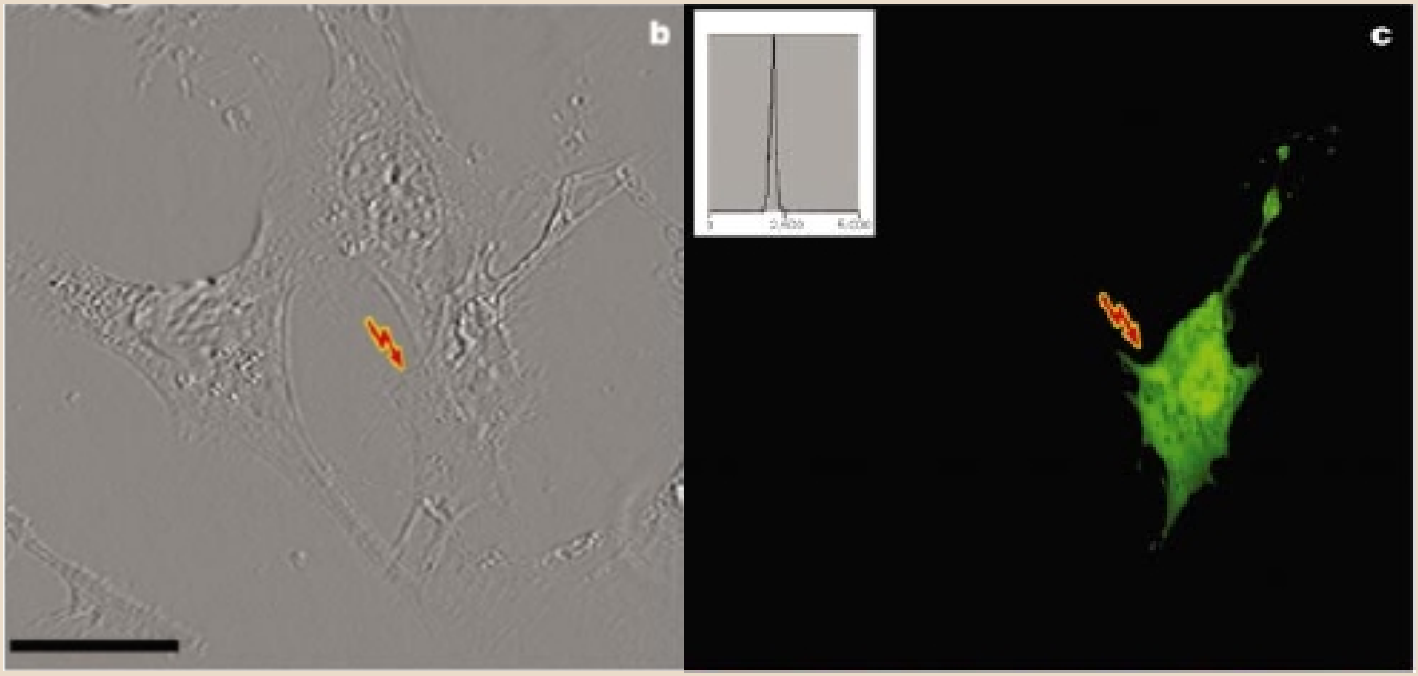


Single Cell



Ref. K. Konig, I. Riemann, and W. Fritzsche, *Opt. Lett.* **26** (2001)

Targeted transfection by femtosecond laser



800 nm, <100 fs, 80 MHz

Ref. Uday K. Tirlapur and Karsten Konig, *Nature* **418** (2001).

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Why multiphoton imaging?

Multiphoton imaging

Why multiphoton imaging?

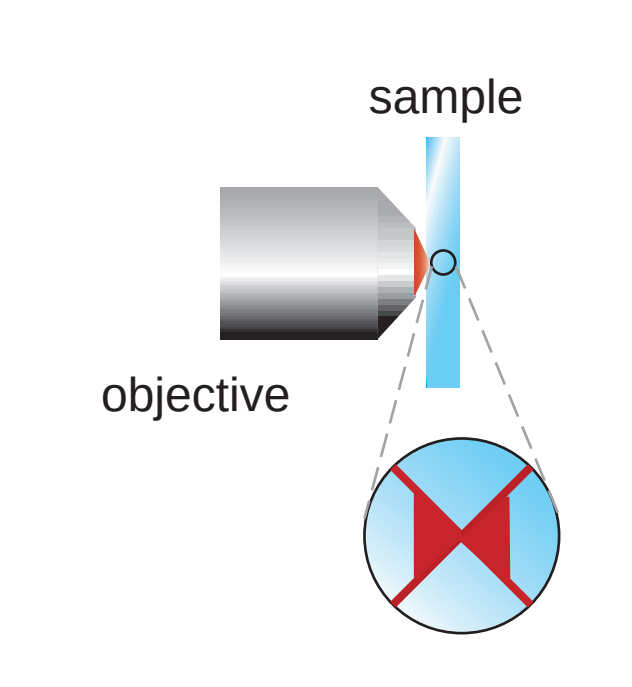
sectioning capability

Multiphoton imaging

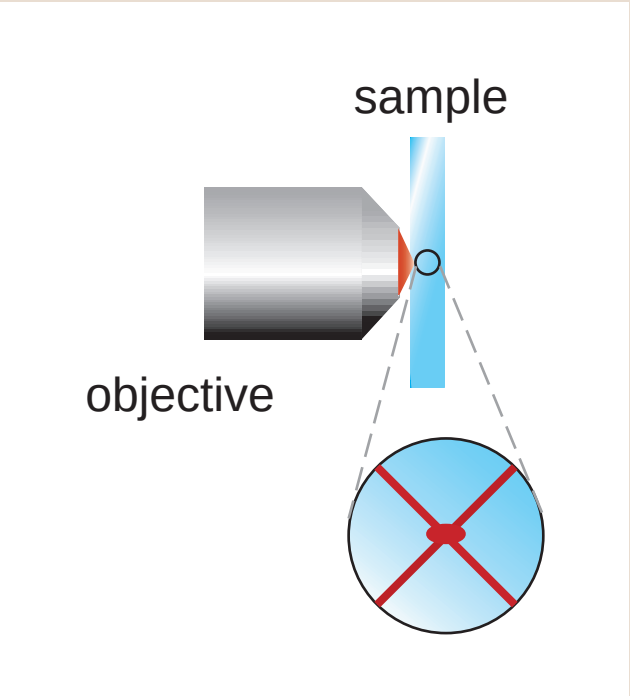
Why multiphoton imaging?

- sectioning capability
- less photobleaching of the fluorophore

Multiphoton Imaging



linear absorption of light at the focus



multiphoton absorption of fs pulses at the focus

Multiphoton imaging

Why multiphoton imaging?

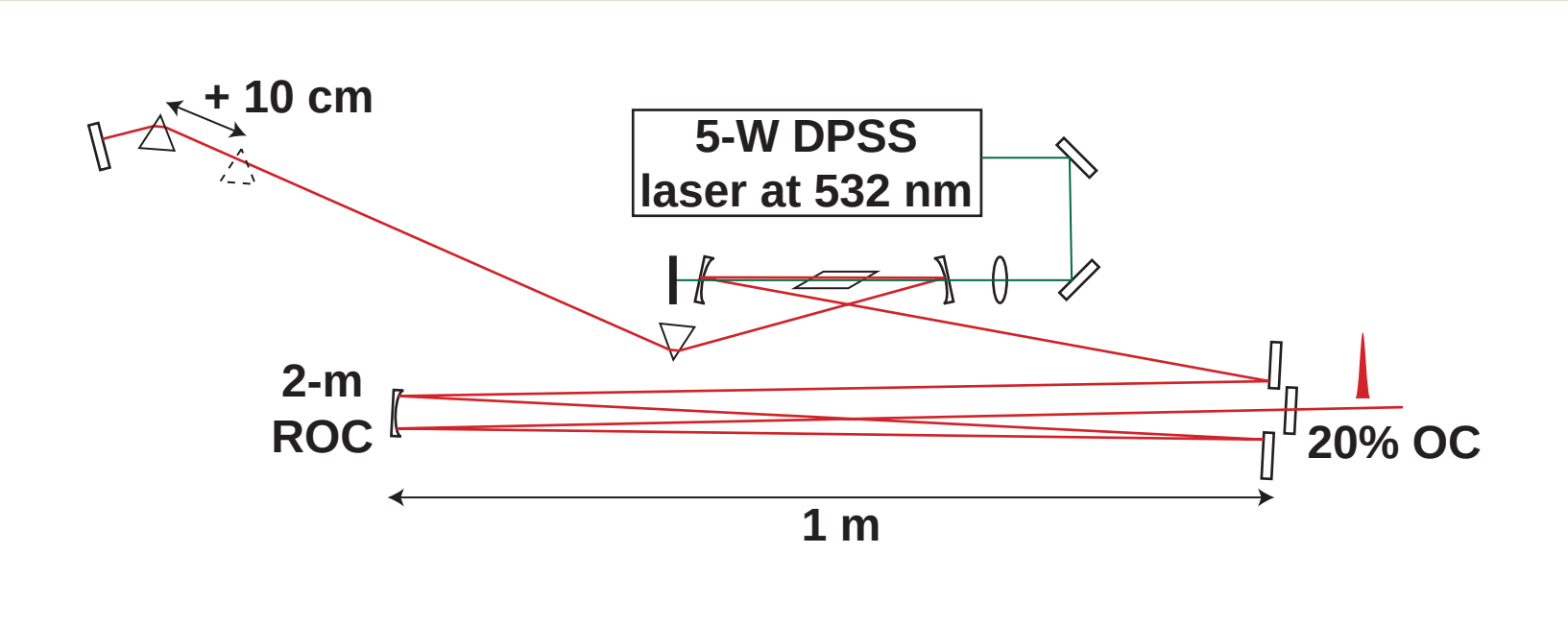
- sectioning capability
- less photobleaching of the fluorophore
- deeper penetration for thick samples

Multiphoton imaging

Why multiphoton imaging?

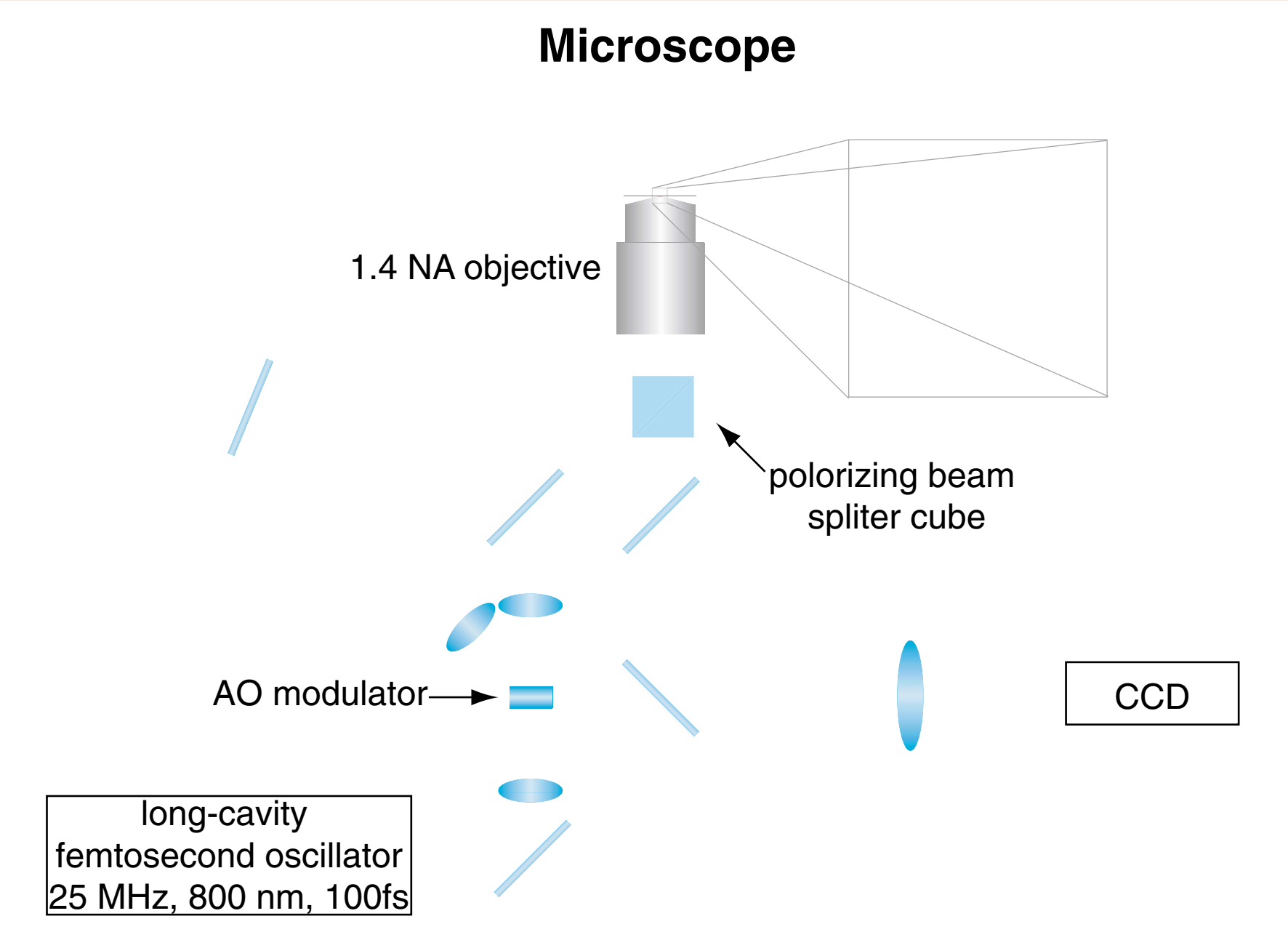
- sectioning capability
- less photobleaching of the fluorophore
- deeper penetration for thick samples
- easier alignment

... extend cavity of standard Ti:Sapph oscillator

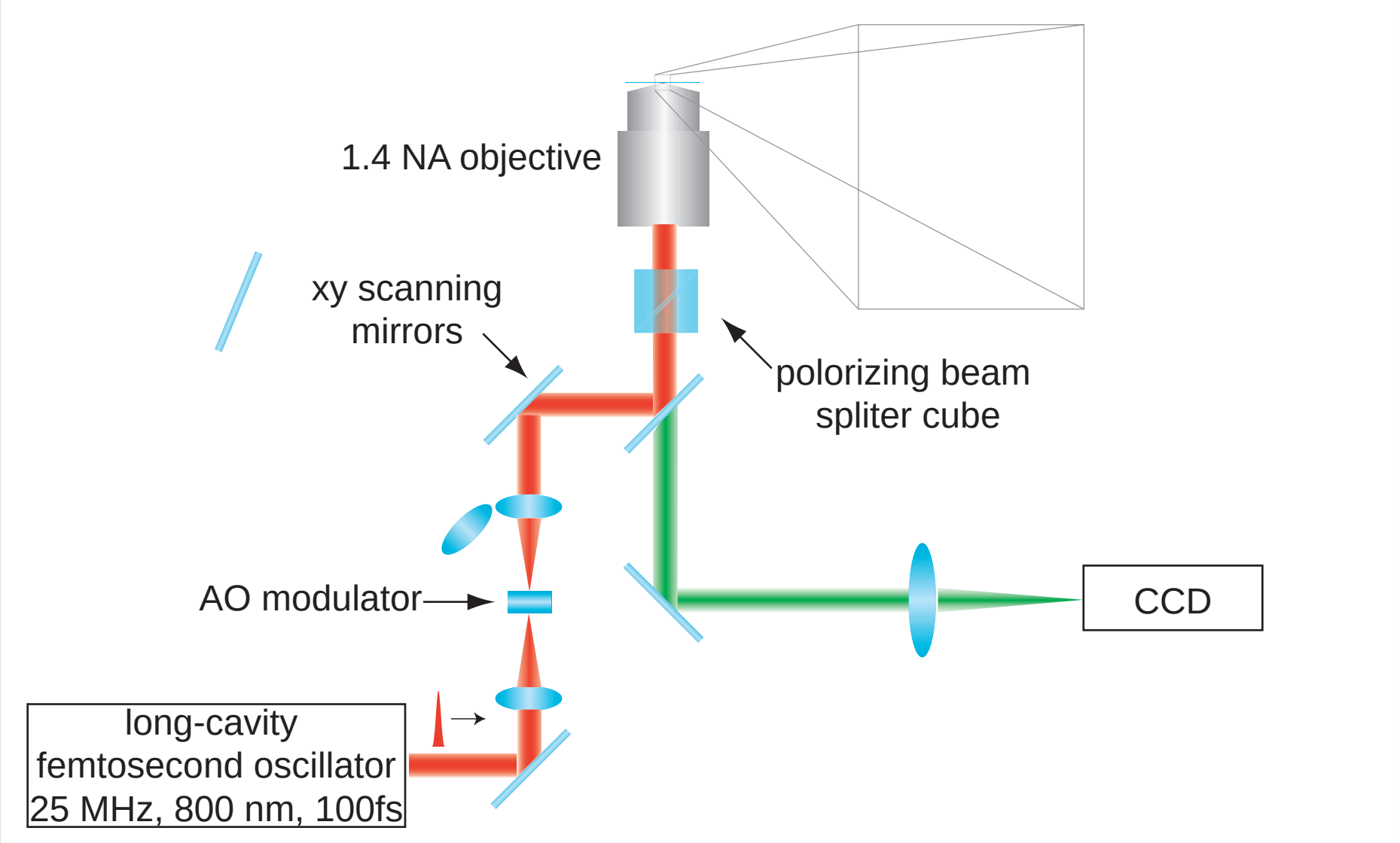


laser specs: 20 nJ, 25 MHz, 20 fs

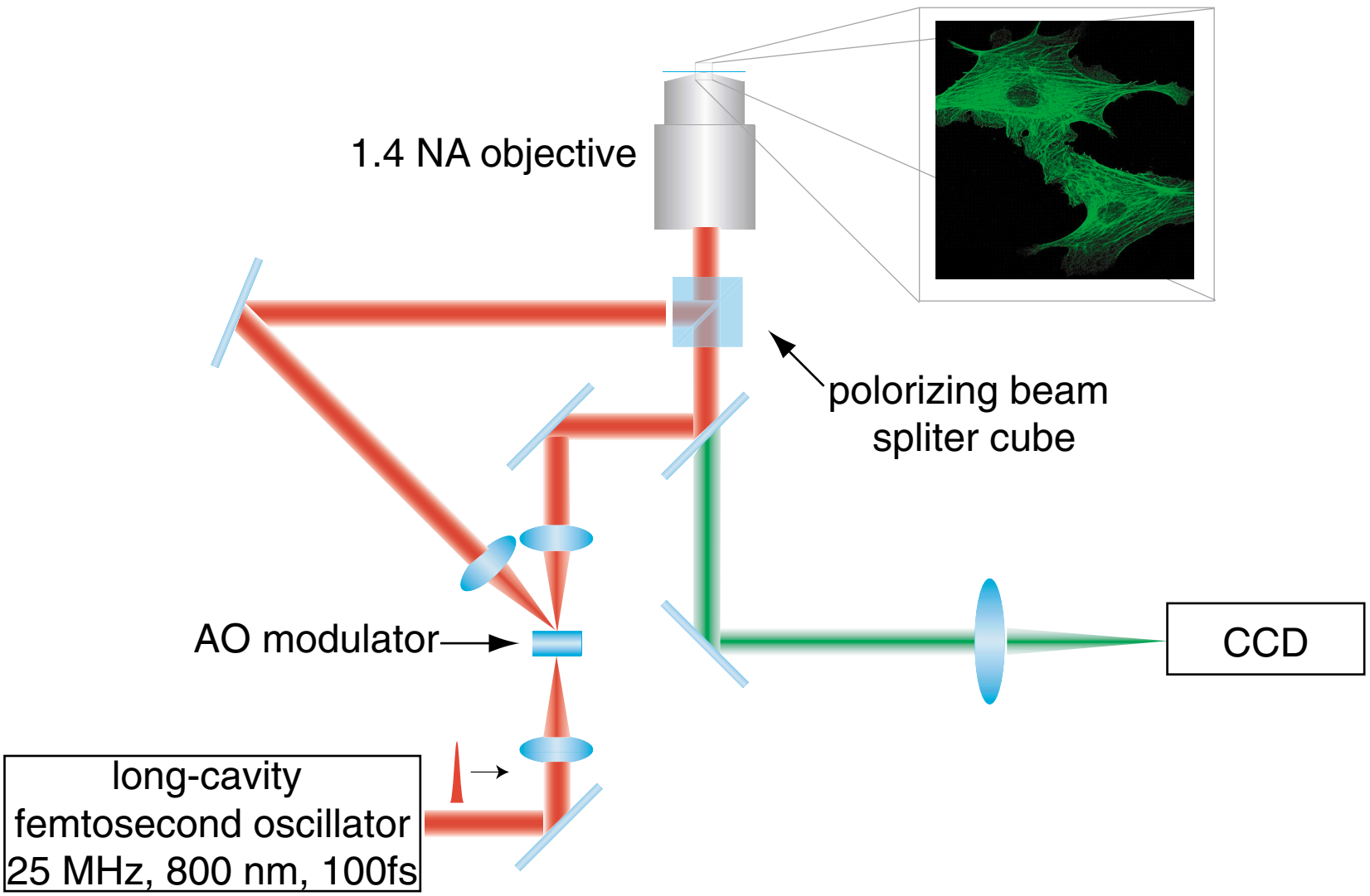
Ref: A.R. Libertun, et.al., CLEO 1999; S.H. Cho, et. al., CLEO 1999.


Microscope
1.4 NA objective
polorizing beam
spliter cube
AO modulator
CCD
long-cavity
femtosecond oscillator
25 MHz, 800 nm, 100fs

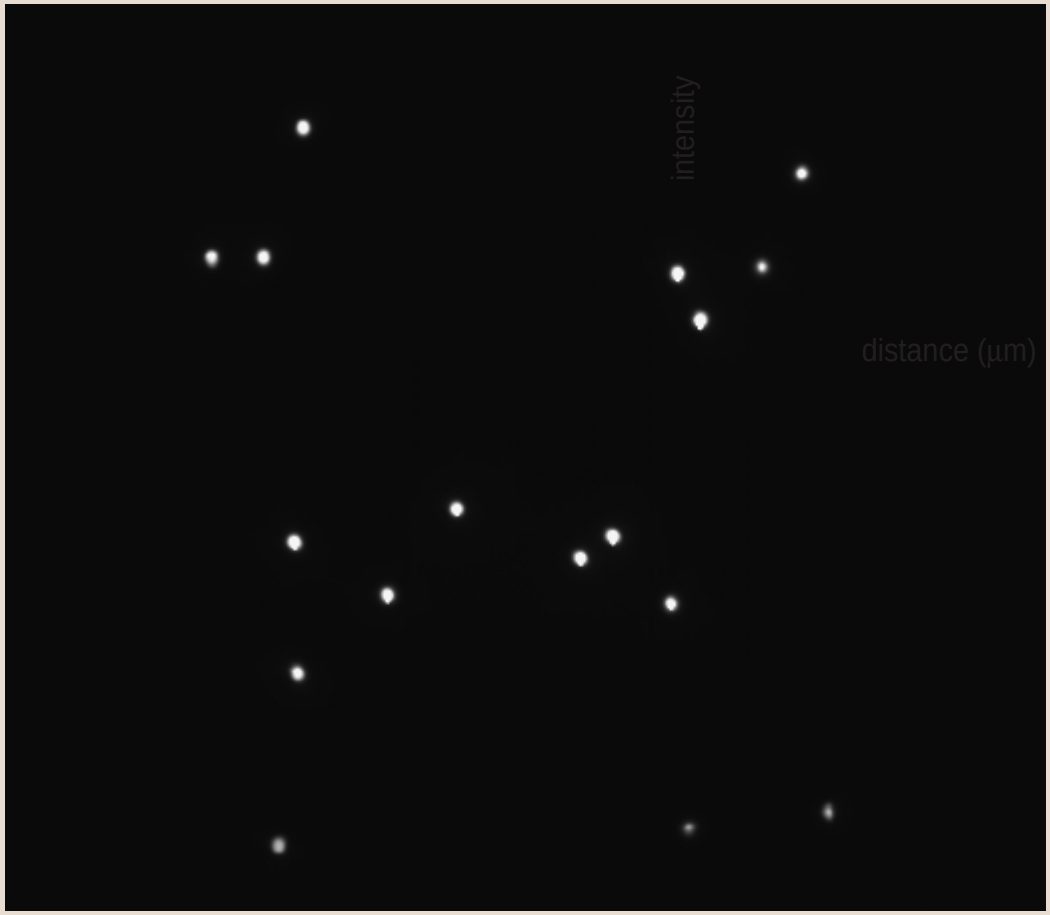
Microscope


1.4 NA objective
xy scanning mirrors
polorizing beam spliter cube
AO modulator
CCD
long-cavity femtosecond oscillator 25 MHz, 800 nm, 100fs

Microscope

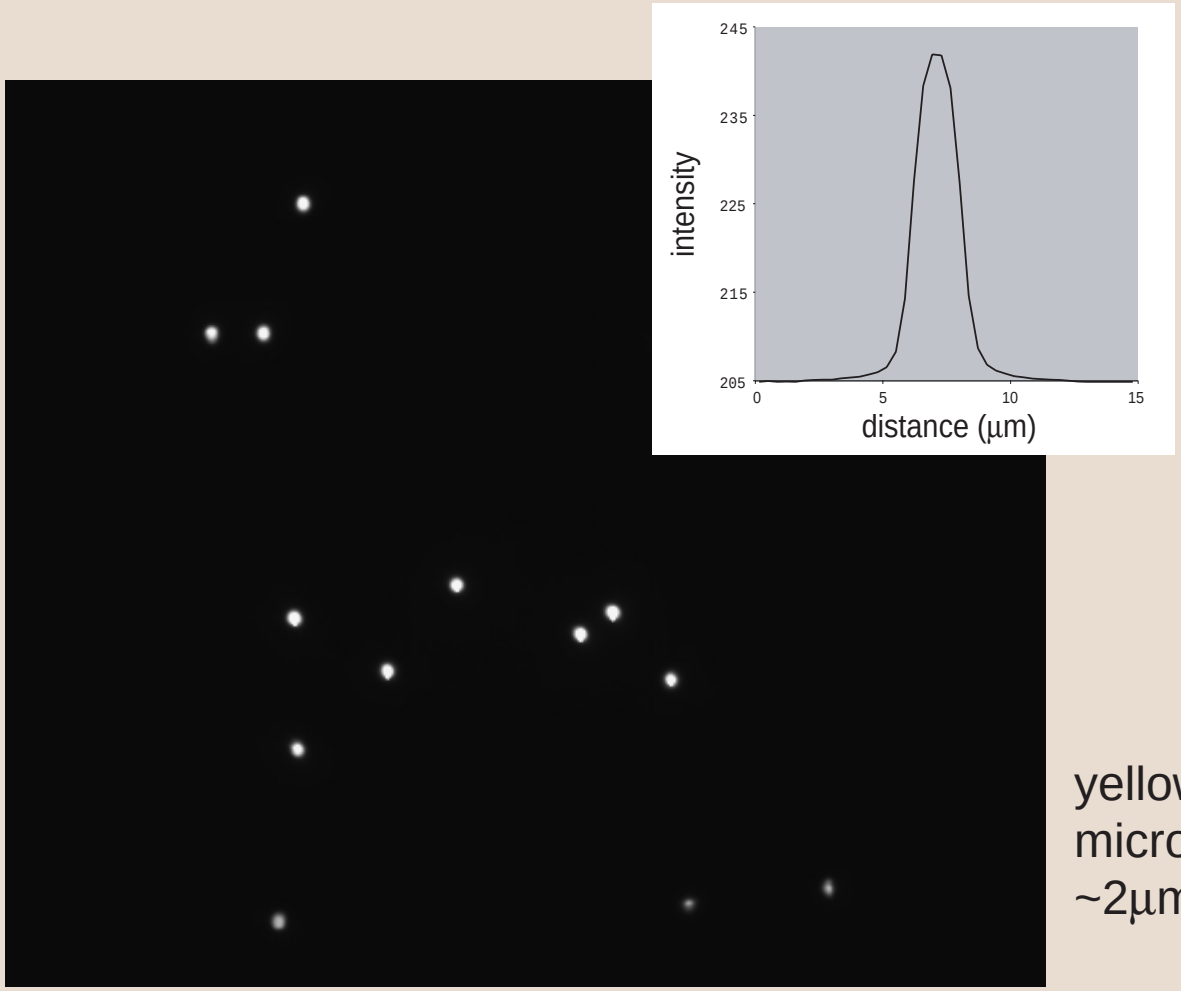

1.4 NA objective
polorizing beam
spliter cube
AO modulator
CCD
long-cavity
femtosecond oscillator
25 MHz, 800 nm, 100fs

Multiphoton Imaging



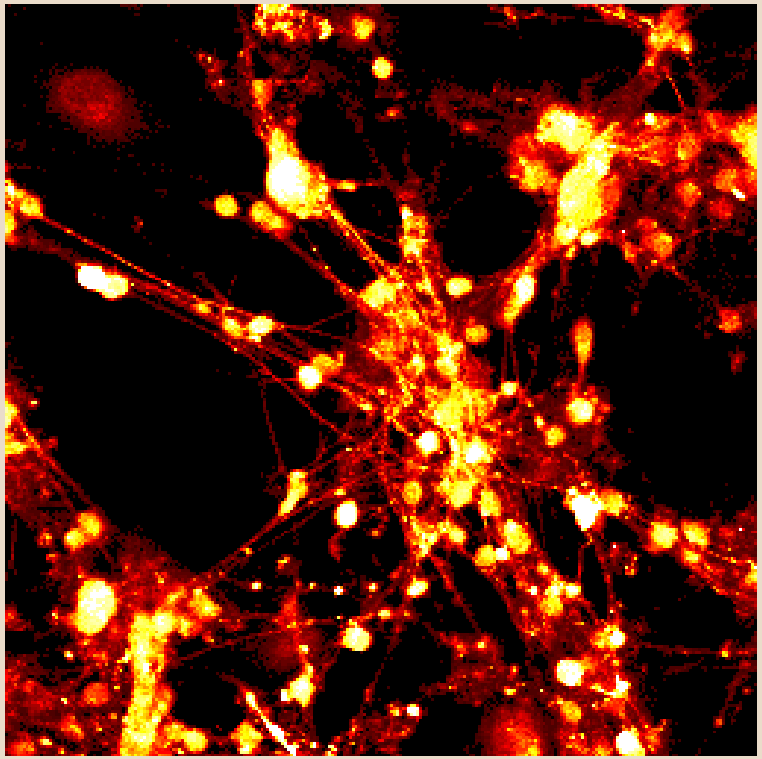
yellow-green microspheres with ~2μm radius

Multiphoton Imaging

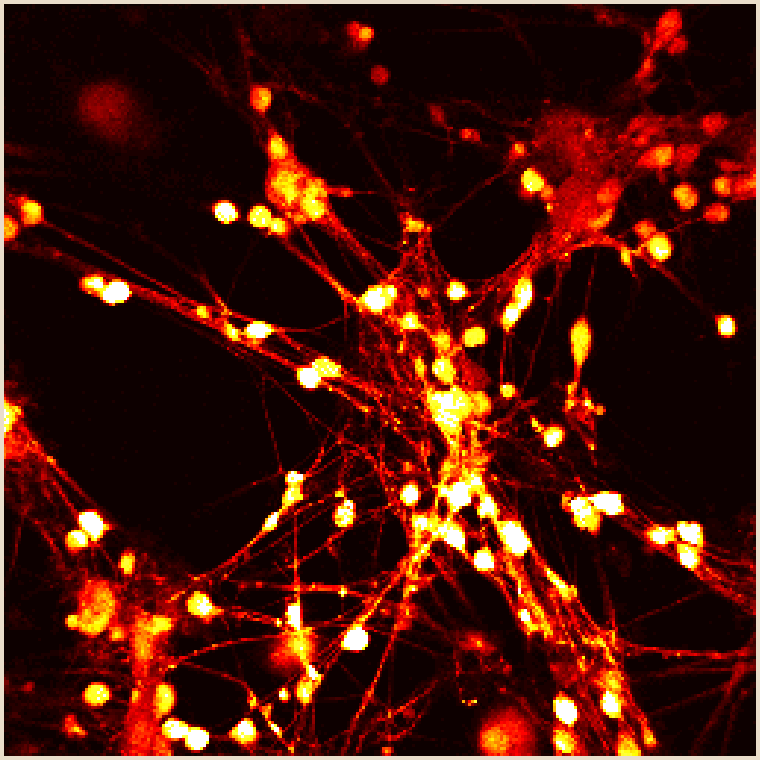


yellow-green microspheres with ~2μm radius

Multiphoton Imaging



Confocal microscopy image



Two-photon microscopy image

Ref: Neuroscience group, Department of Anatomy, University of Bristol

Summary

- photodisrupt subcellular organelles in live cells
- study cellular functions and processes
- combine microsurgery and multiphoton imaging

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Acknowledgments:

Mazur group

Melton group

Weitz group

For a copy of this talk and additional information, see:

http://mazur-www.harvard.edu