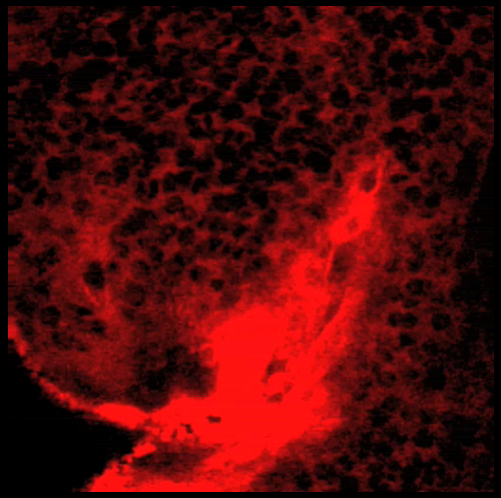
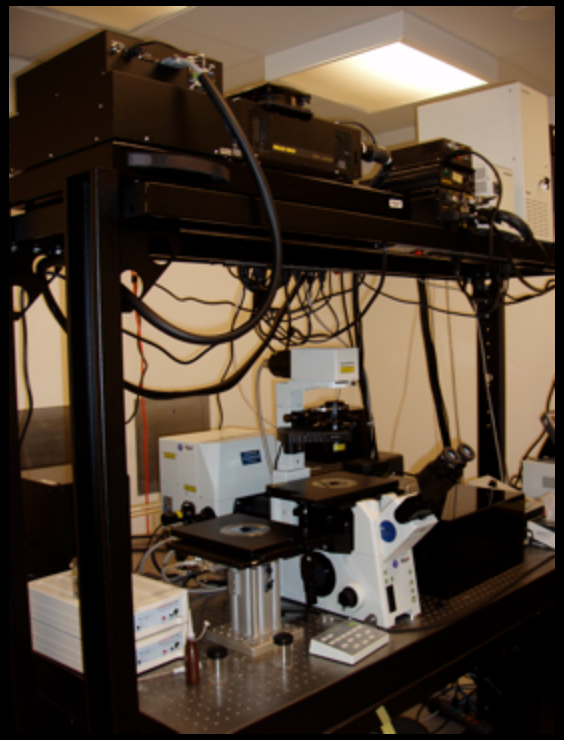
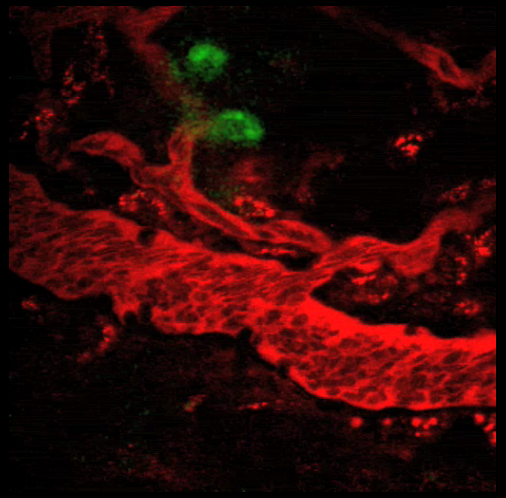
Building Your Own 2-Photon Microscope: Challenges, Advantages and Limitations







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Building Your Own 2-Photon Microscope: Challenges, Advantages and Limitations

How did we manage to ~~build~~ put together a 2-photon microscope?

12% Ethanol (Preferably Italian)

Caffeine (Strictly Italian Espresso)

Being a pain in the neck…….

Building Your Own 2-Photon Microscope

Turn Key System

More expensive

~~$400K-500K~~

$150K

$150K Laser

Why?

1) Budget
   1) Low-Start up package
   2) Maintenance
2) Flexibility
3) Upgrades

Build your own

Buy Confocal microscope → Convert to a 2-photon

First step (first major decision)

Which Platform?

Thorlabs
Labvision

Olympus

1) We copied the system built here
2) Flexibility
3) Support

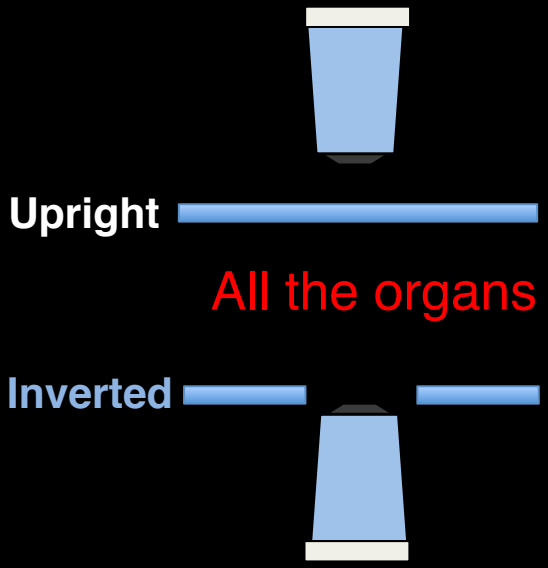
Upright vs. Inverted

Flexibility:

1) 2-photon
2) Confocal

1) Intravital imaging
2) Live Cell imaging

1) All the organs



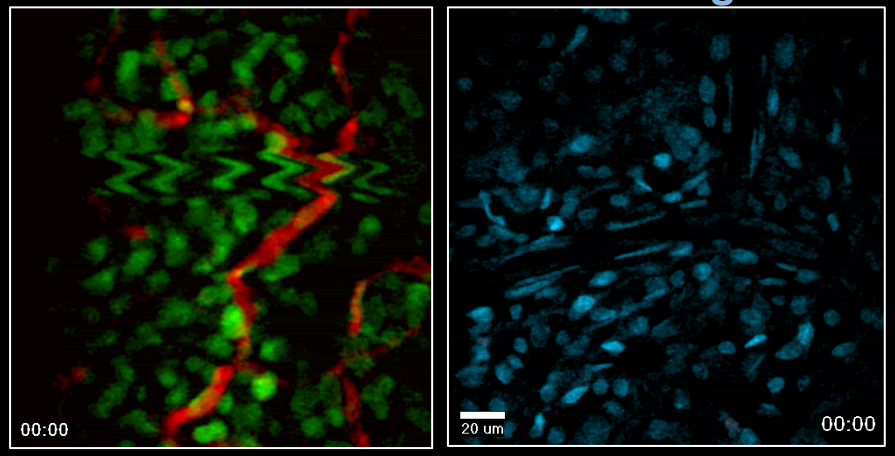
All the organs

All the organs but the brain
Live Cell Imaging

Motion artifacts

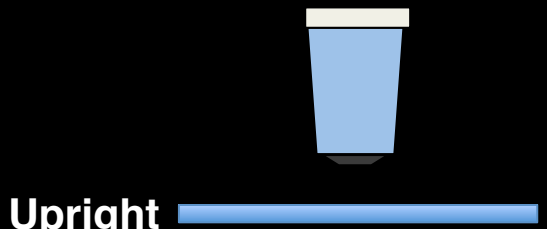
"Custom made holding device specifically designed for the organ of interest"

w/o holding device **with holding device**

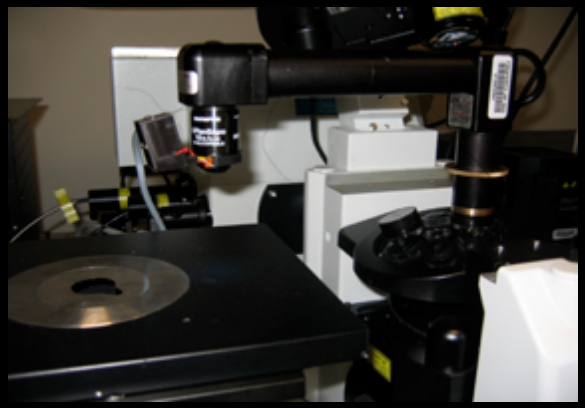


"Positioning and securing the organ to the coverslip"

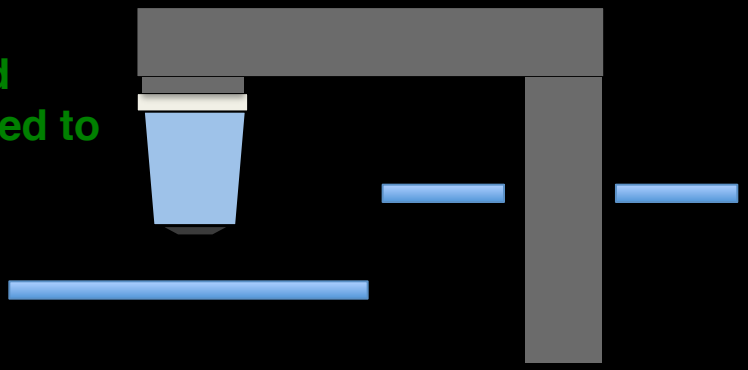
Upright vs. Inverted

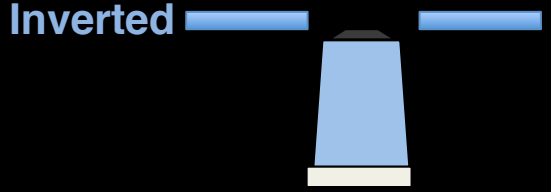


All the organs



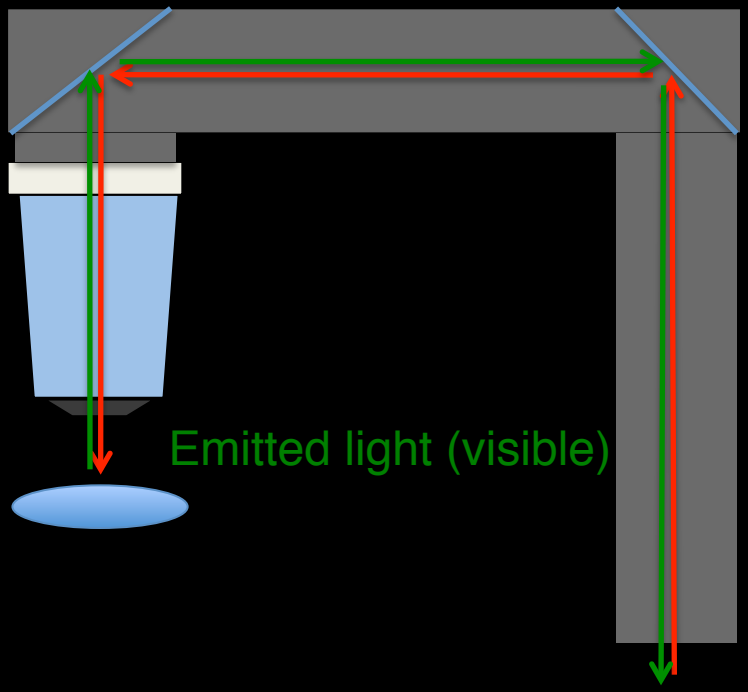
Inverted converted to upright





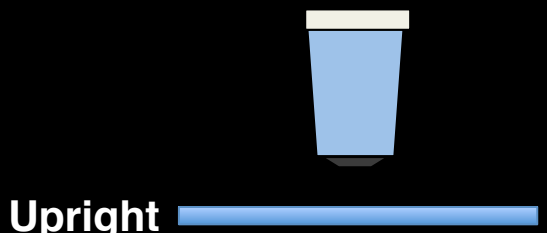
All the organs but the brain
Live Cell Imaging

Upright vs. Inverted



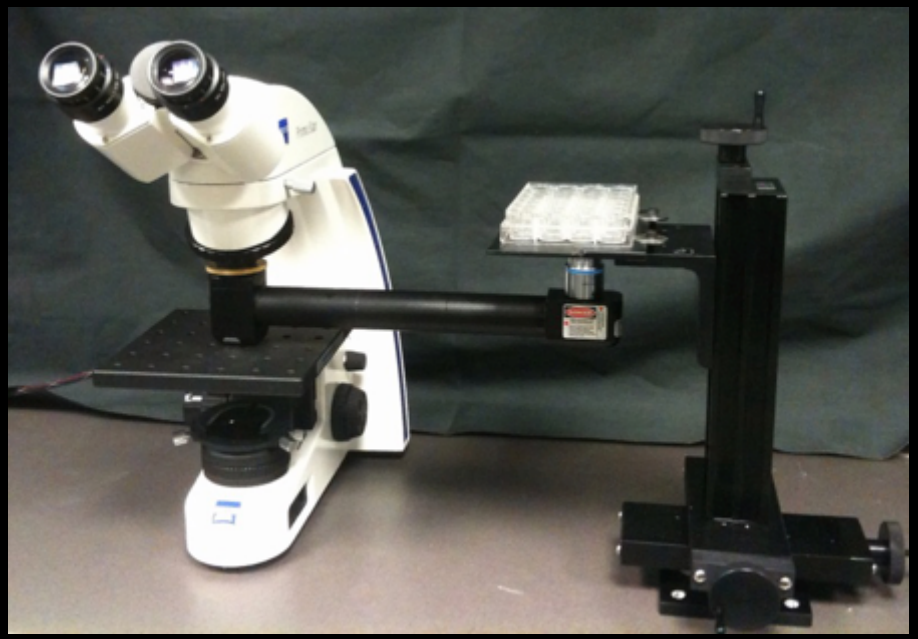
1) Optimized for visible light
2) Increase the light path
    1) Model available with PMT on top
3) Loss of power (5-10%)
4) No effects on laser pulse width
5) Requires extra stage
6) Head can be rotated
7) Adaptors for lenses

Upright vs. Inverted



All the organs

Upright converted to inverted

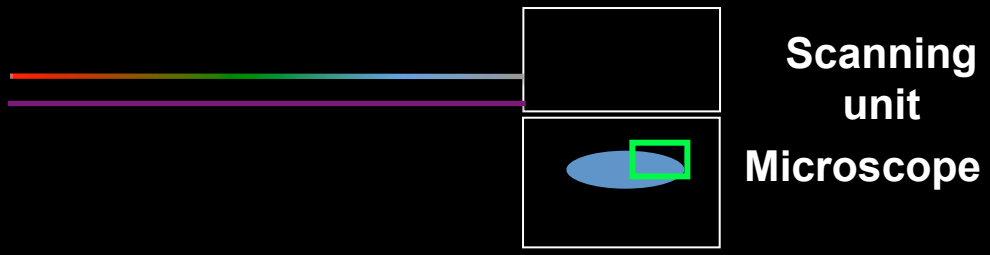


Laser

Laser combiner

488 nm
561 nm
633 nm
UV laser 405 nm

1) Microscope



Ti-sapphire lasers

"Laser"

Tunable vs. single wavelength

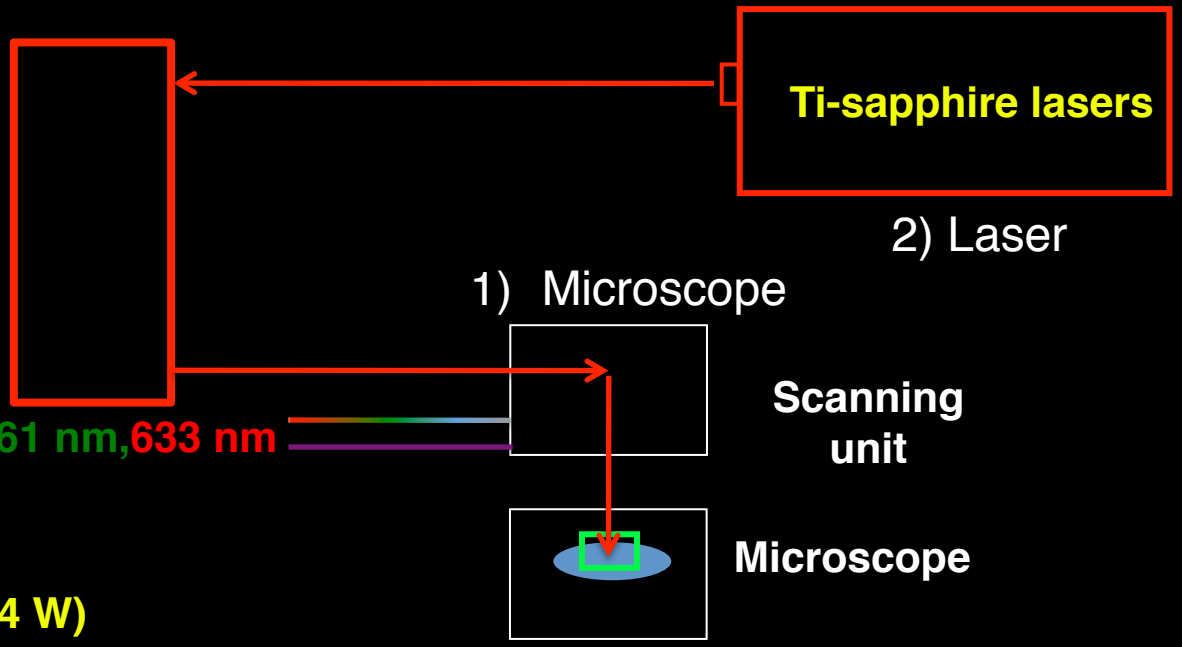
High power lasers (3-4 W)
Repetition rates: 80-100 Hz

Pulses: 100-150 fs

Beam diameter: 1.2 +/- 0.2 mm

Tunable: 680-1080 nm

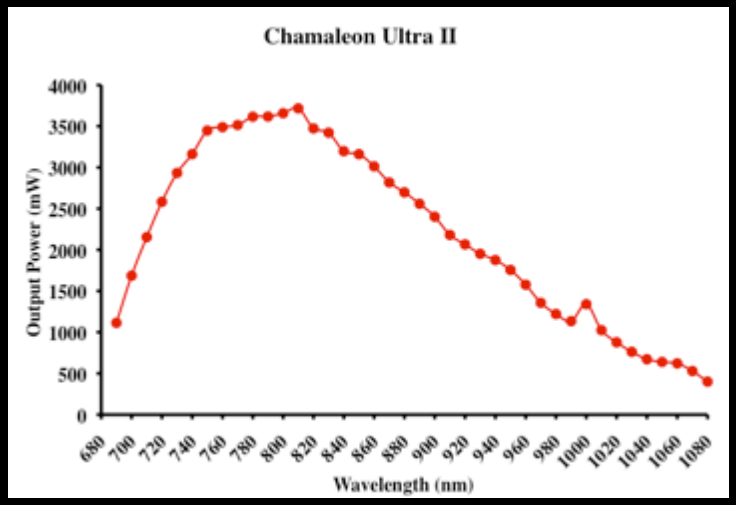
Laser output power

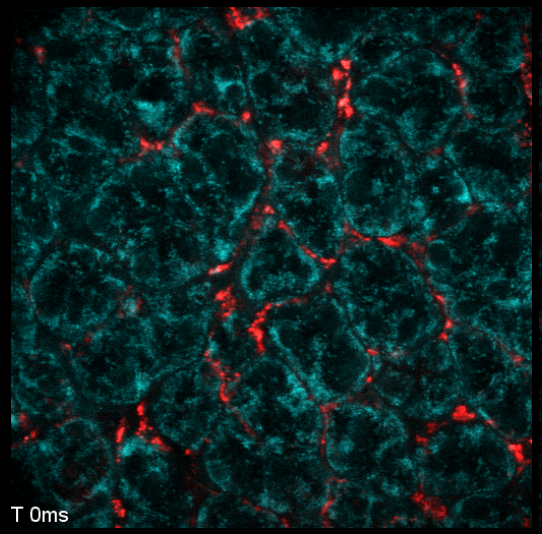


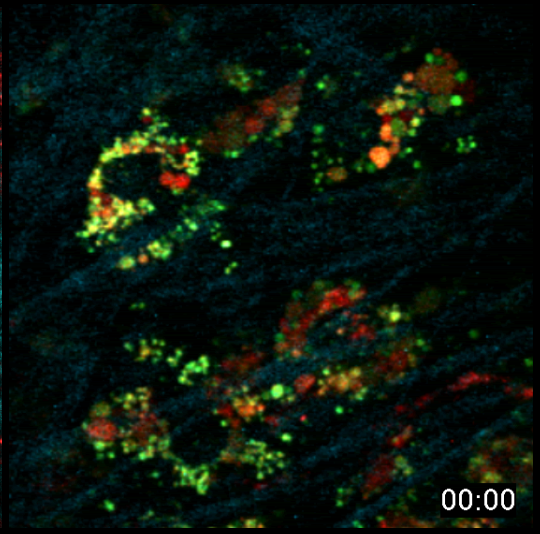
Laser combiner 488 nm,561 nm,633 nm

UV laser 405 nm

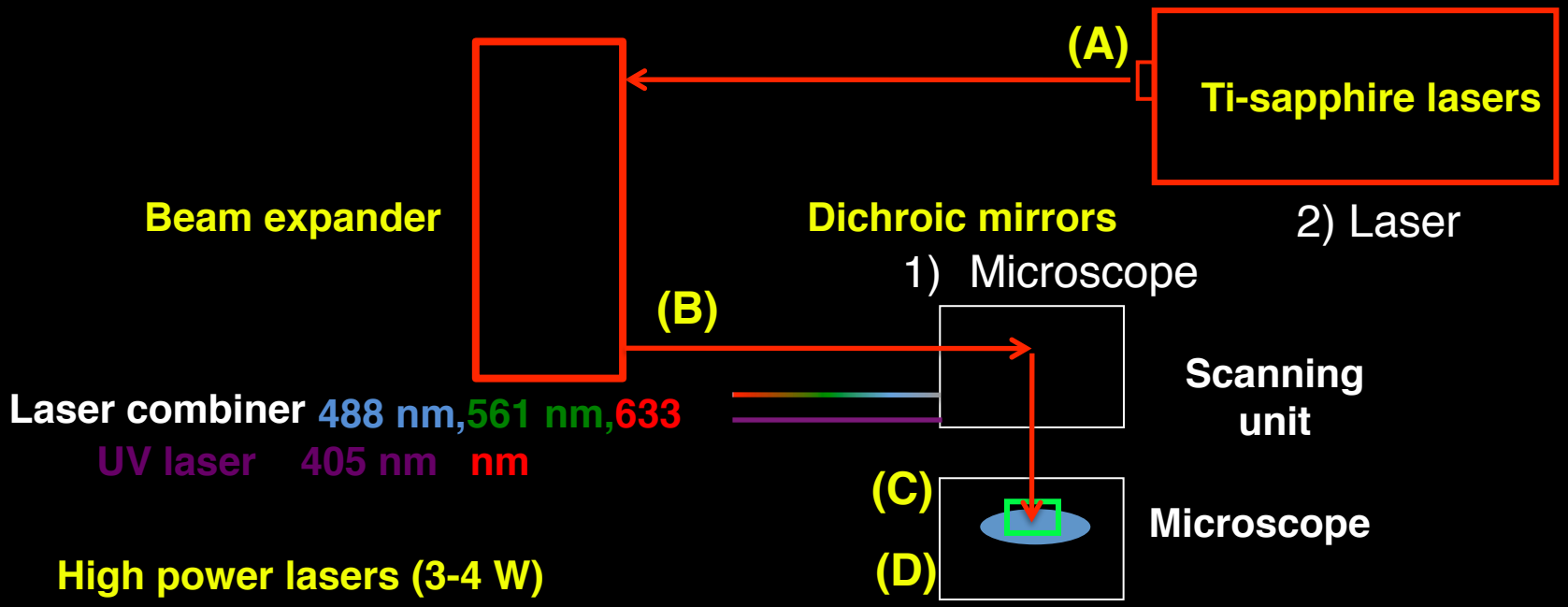
High power lasers (3-4 W)



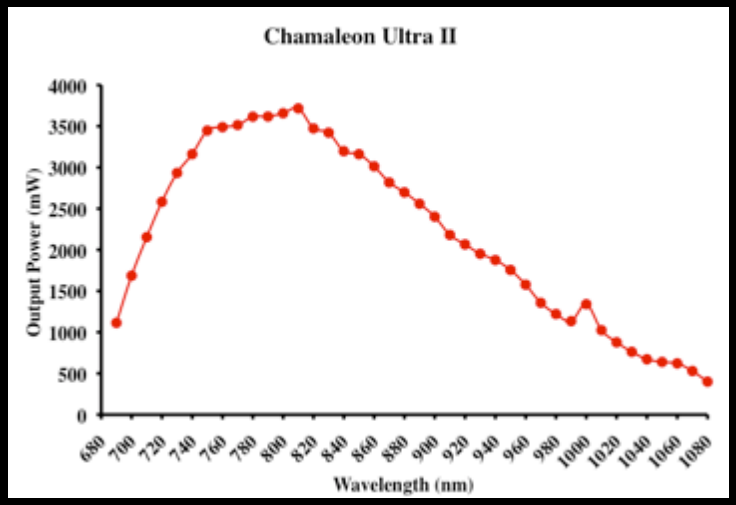




Loss of power throughout the optics

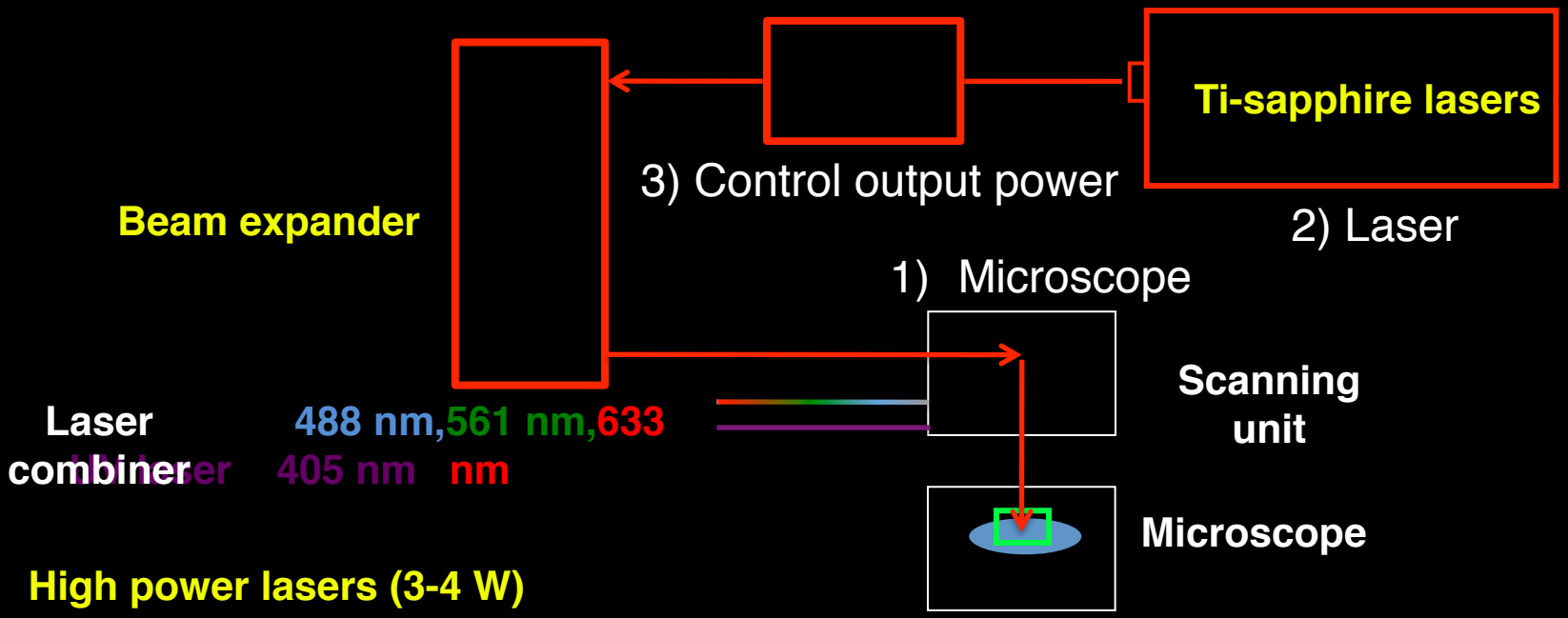


High power lasers (3-4 W)



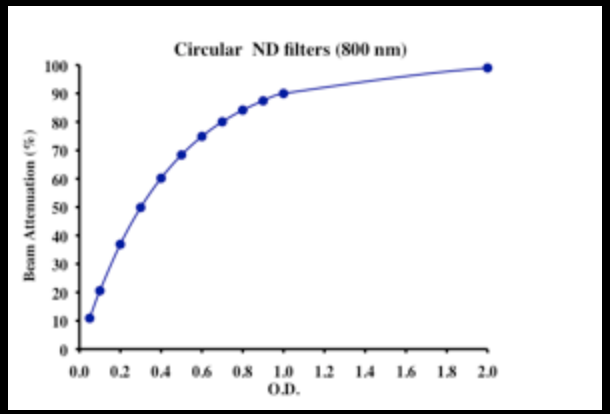
(A) 800 nm – 3520 mW (100%)
(B) 3280 mW (93%)
(C) 864 mW (25%)
(D) 340 mW (10%) – 60x N.A 1.2

Control the power at the specimen

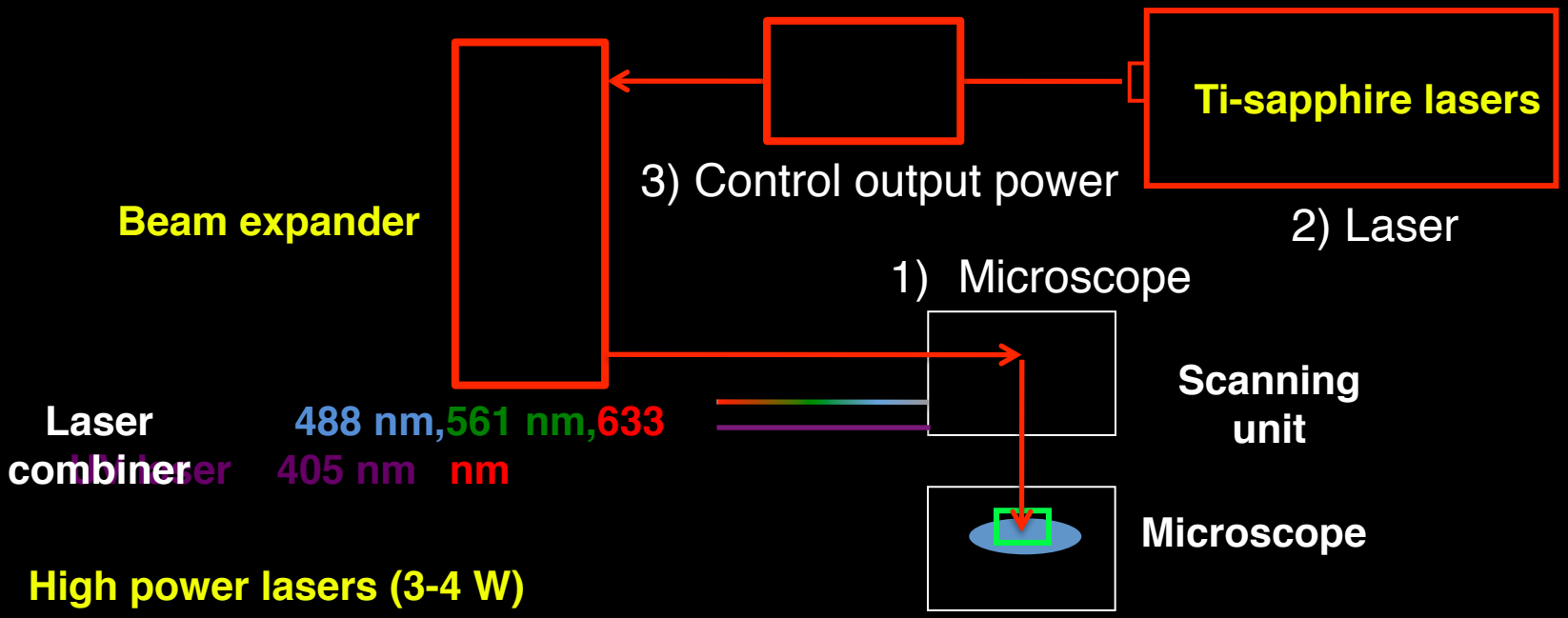


1) ND filters
   a) Single
   b) Carousel with multiple filters (8-10)
      a) Manually or software controlled

2) ND continuous filter wheel



Control the power at the specimen



1) ND filters

2) ND continuous filter wheel

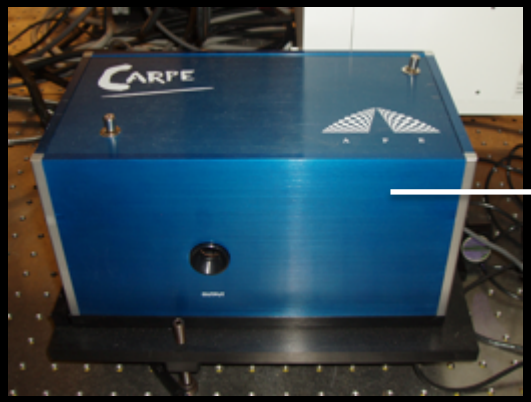
3) AOM (Acousto-optic modulator)

4) EOM (Electro-optic modulator)

a) Easy integration with the software
b) Size of the beam matching the aperture of the AOM
c) Significant pulse broadening (up to 600 fs)
   a) Need for a pre-chirping system
d) Deflection of the beam
   a) Not practical if different wavelengths are needed
   b) Need for an automatic realignment set up (expensive)

Broadening of the pulse width

(a)



4) Autocorrelator 3) Control output power

(a)

Ti-sapphire lasers

2) Laser

1) Microscope

Beam expander

Laser combiner 488 nm,561 nm,633 nm

UV laser 405 nm

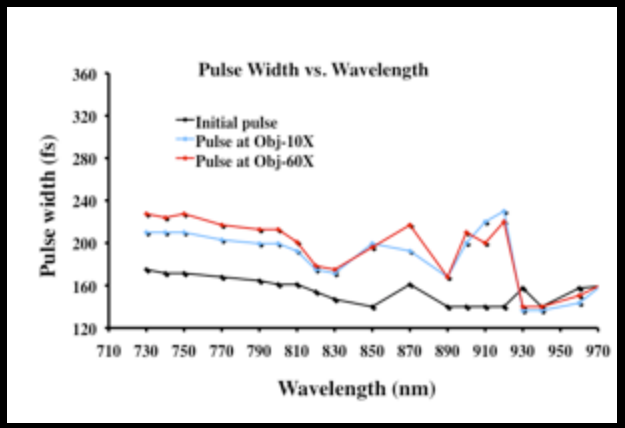
Pulses: 100-150 fs

Scanning unit

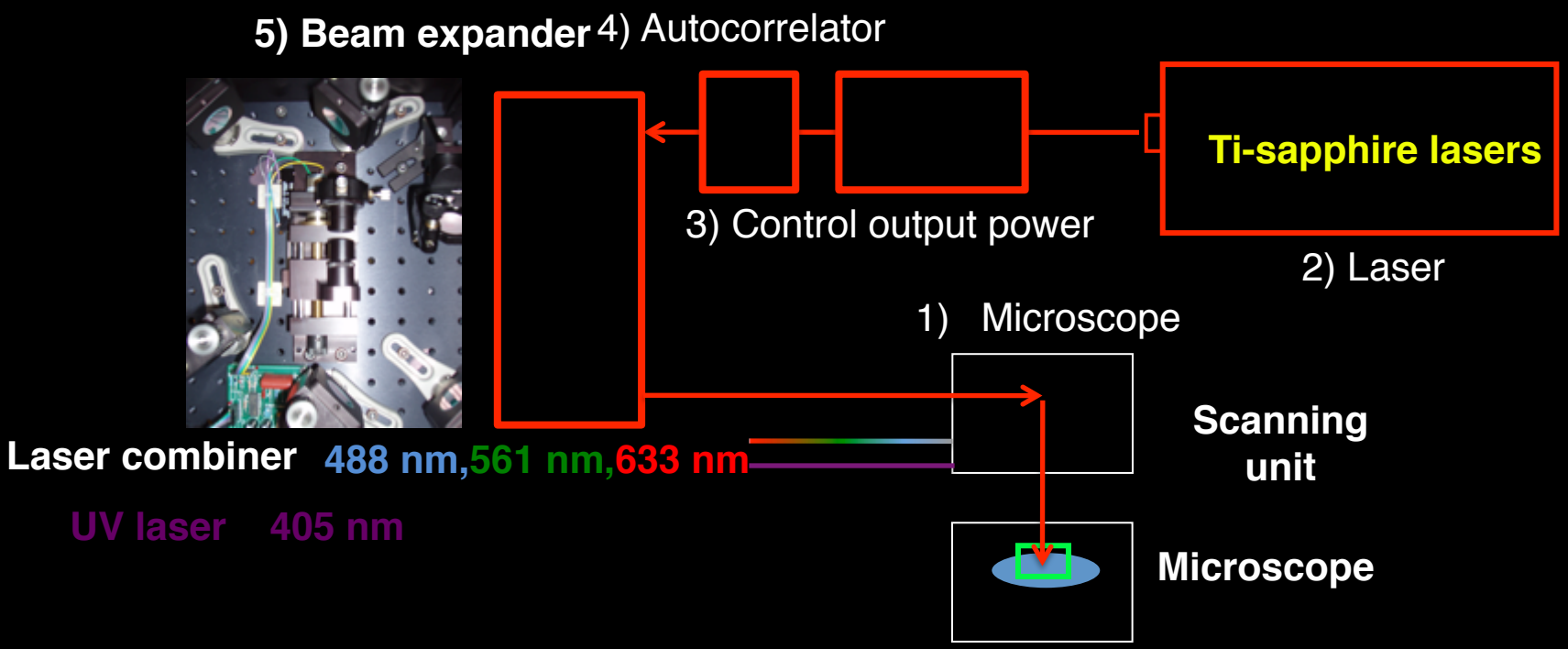
Microscope

(b)

(a) Measure the pulse at the “source”
(b) Measure the pulse and the power at the “specimen”



Size of the laser beam

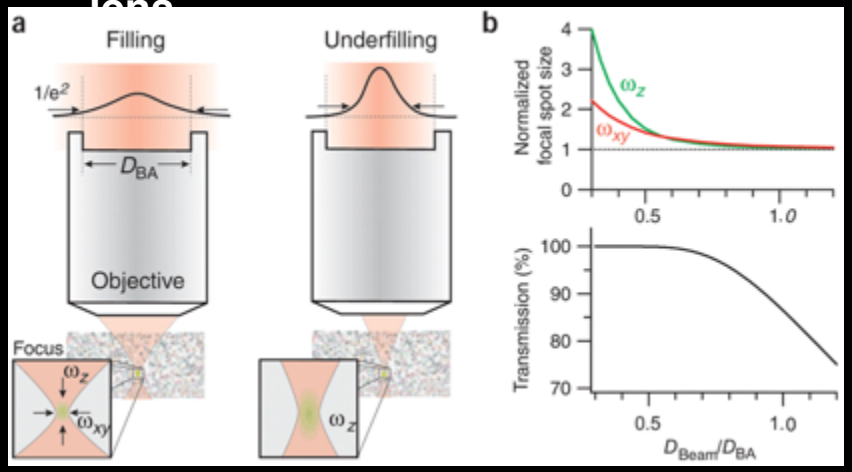


1) **Control the size of the beam**

2) **Control the power at the specimen**

Filling the backaperture of the lens

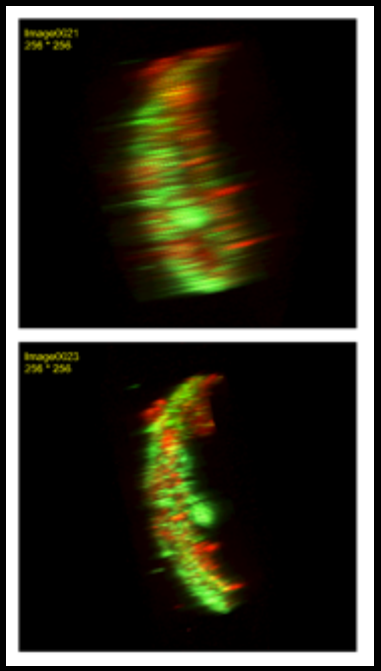
Filling the back aperture of the



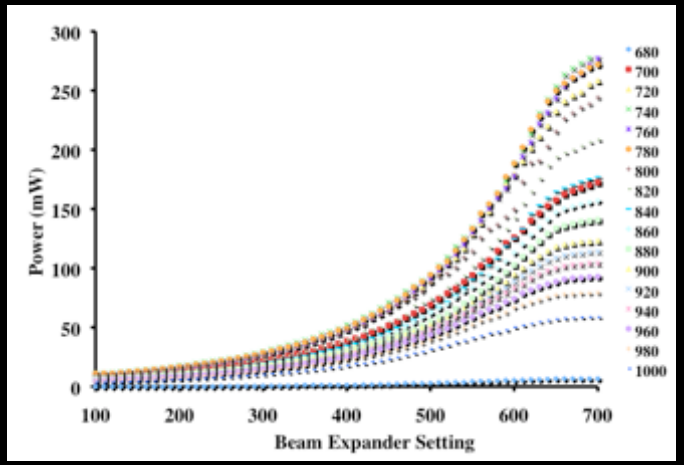
Essential for large lenses such as the 20X

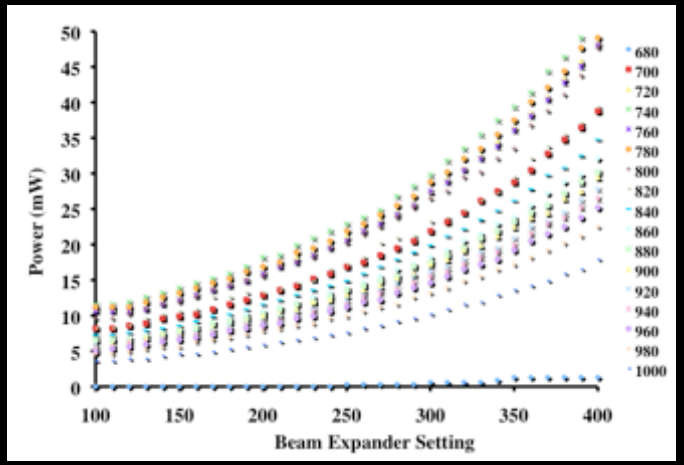
Underfilling

Filling

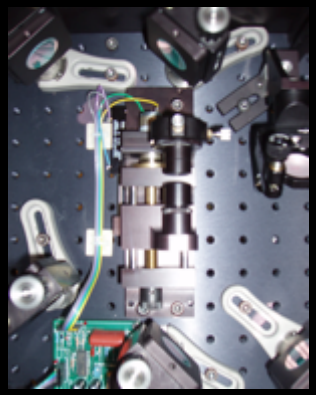


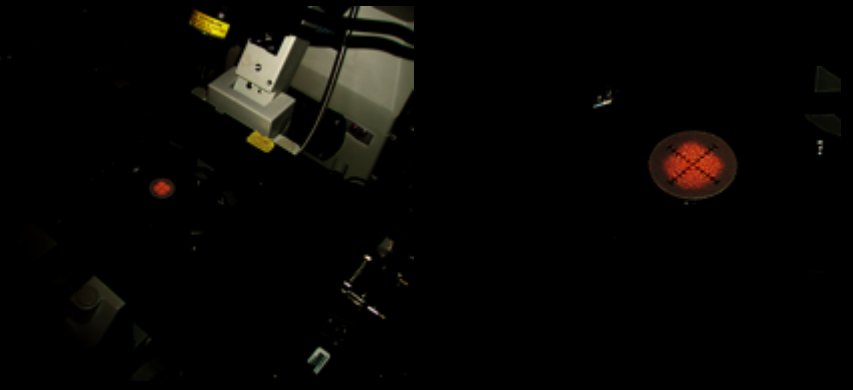
Control the power at the specimen by overfilling



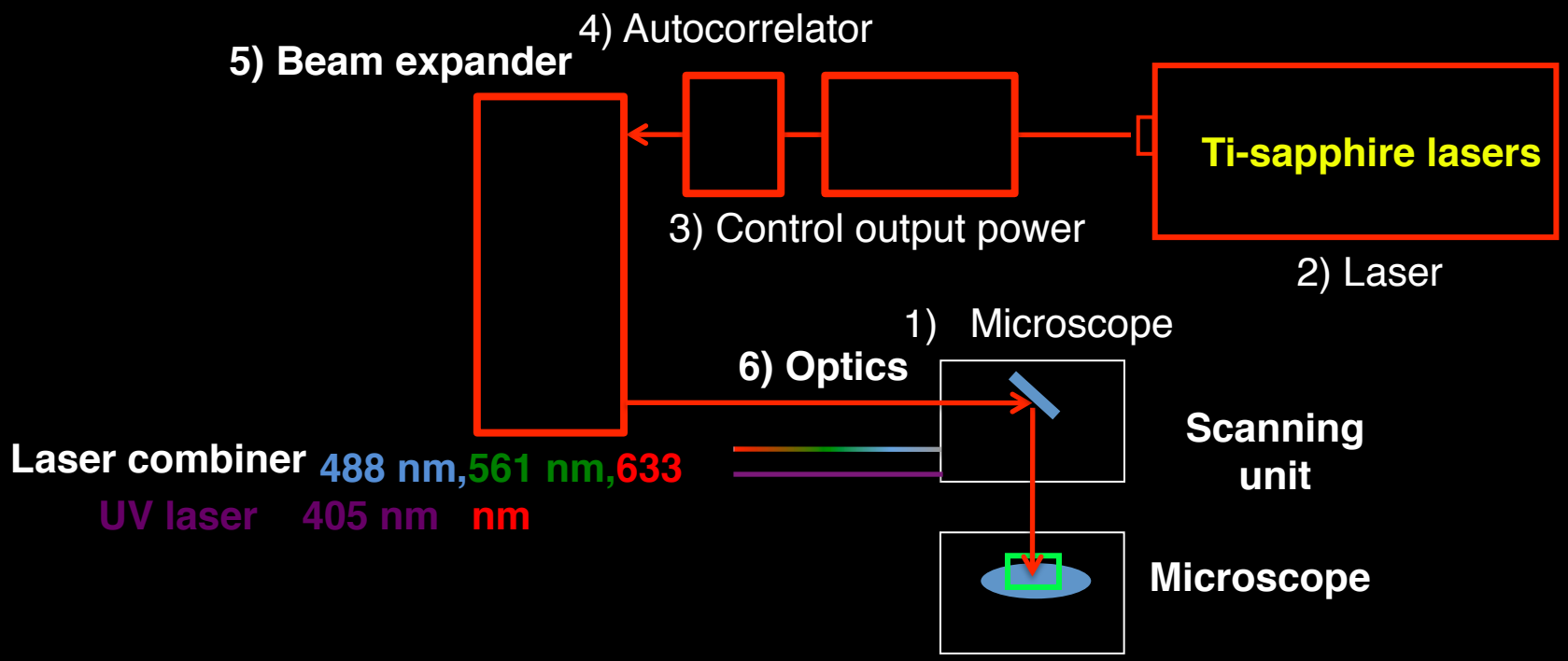


Challenge: alignment of the beam



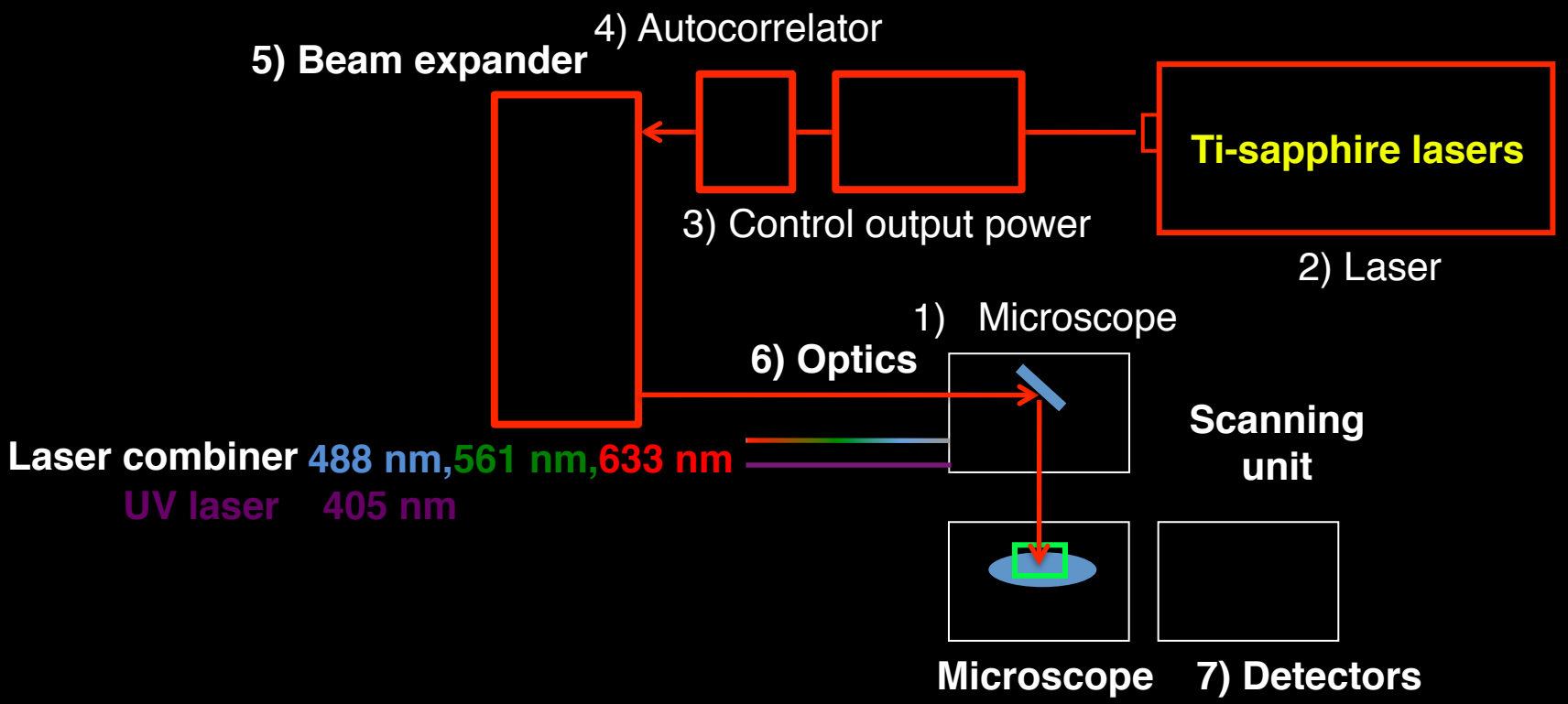


Proper optics



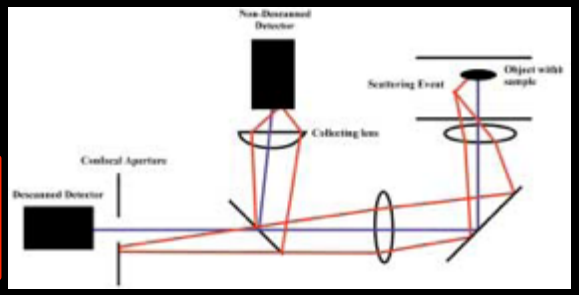
6) Excitation Dichroic mirror – reflect above 675-680 nm

Non-descanned detectors



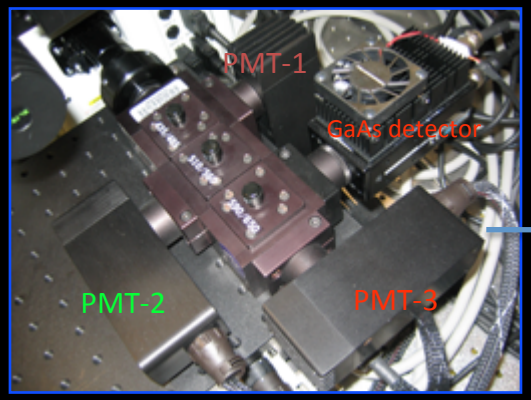
A) Descanned detectors

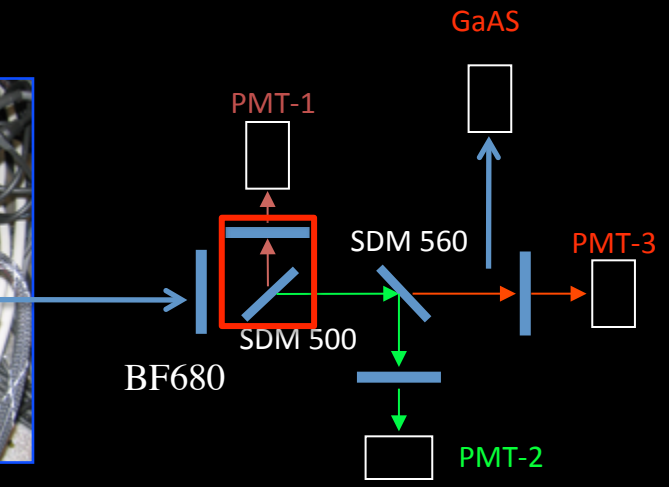
B) Non-descanned detector



Positioning

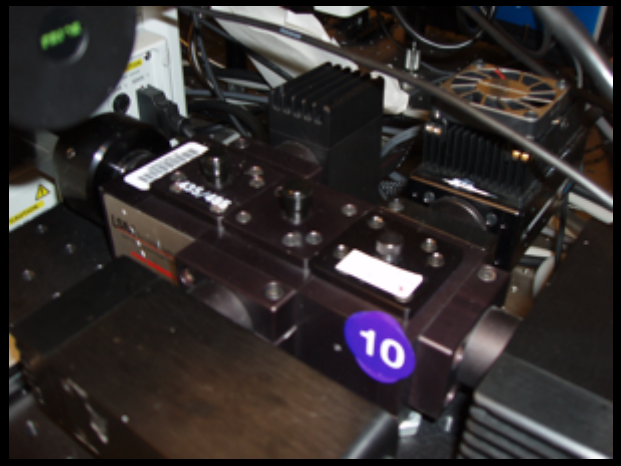
Non-descanned detectors

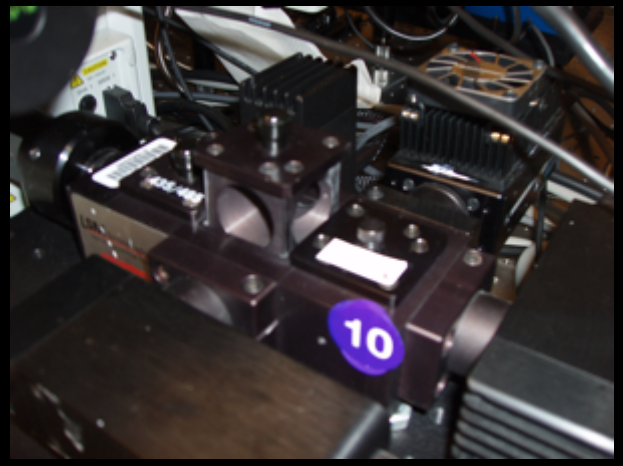


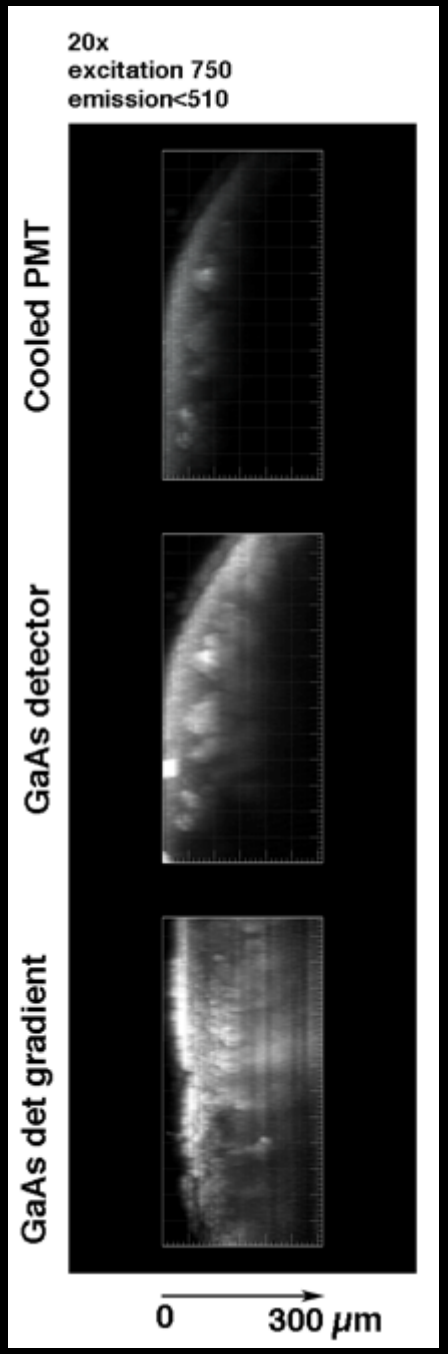


3 Cooled PMT from Hamamatzu R6060-11

1 Gallium Arsenide PMT

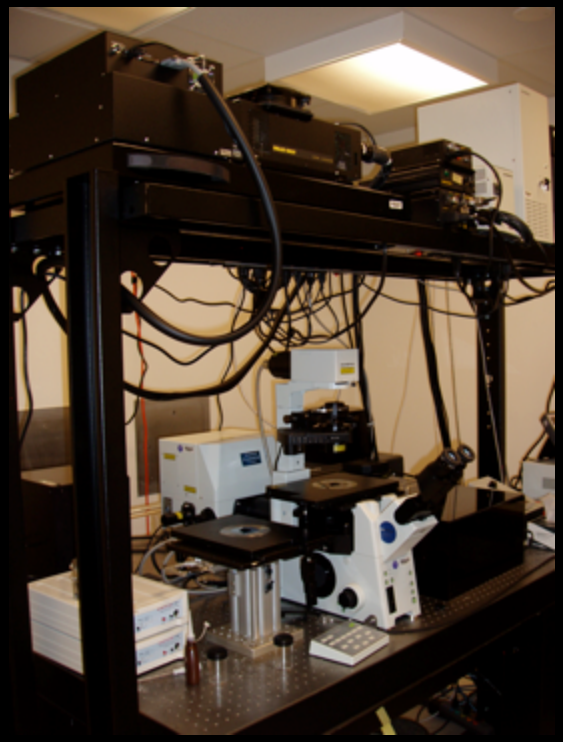


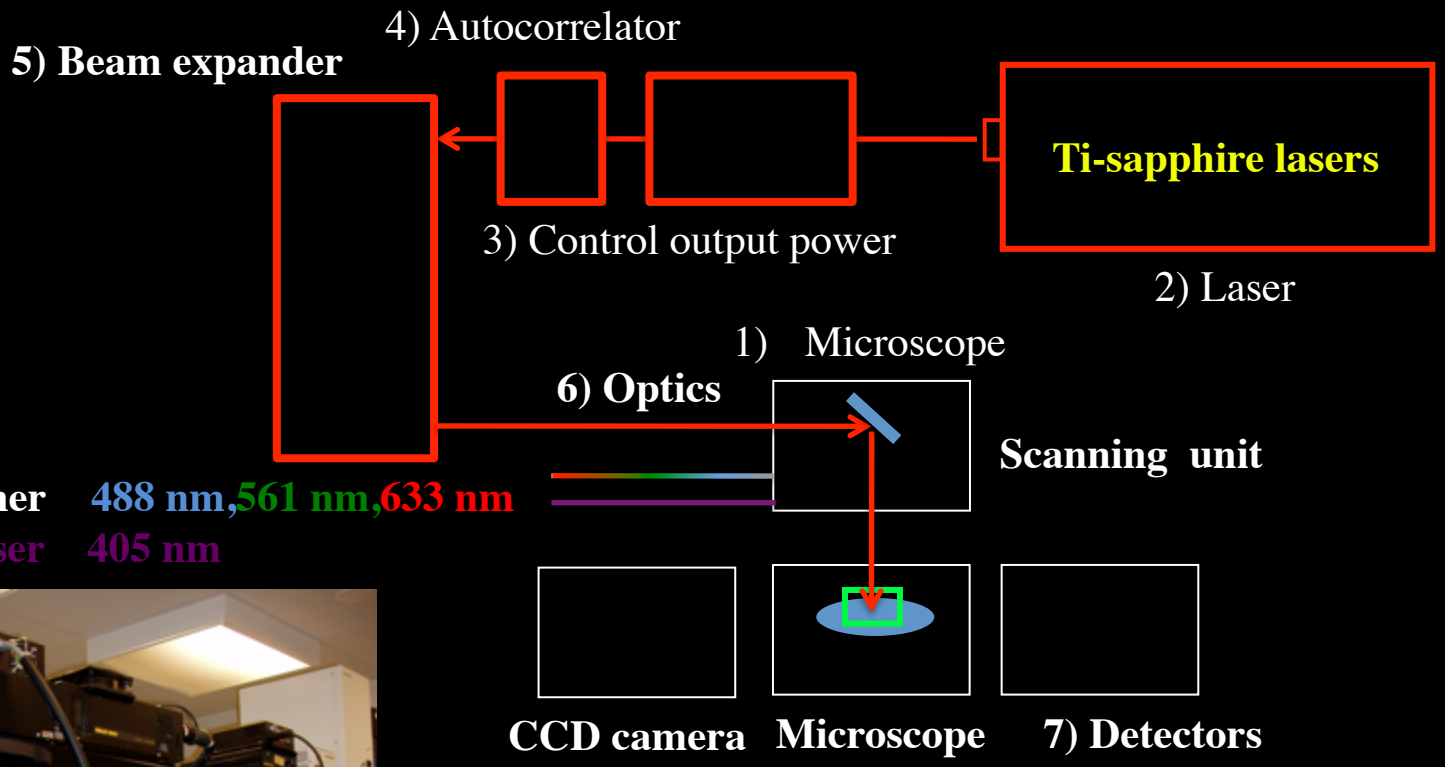




Non-descanned detectors

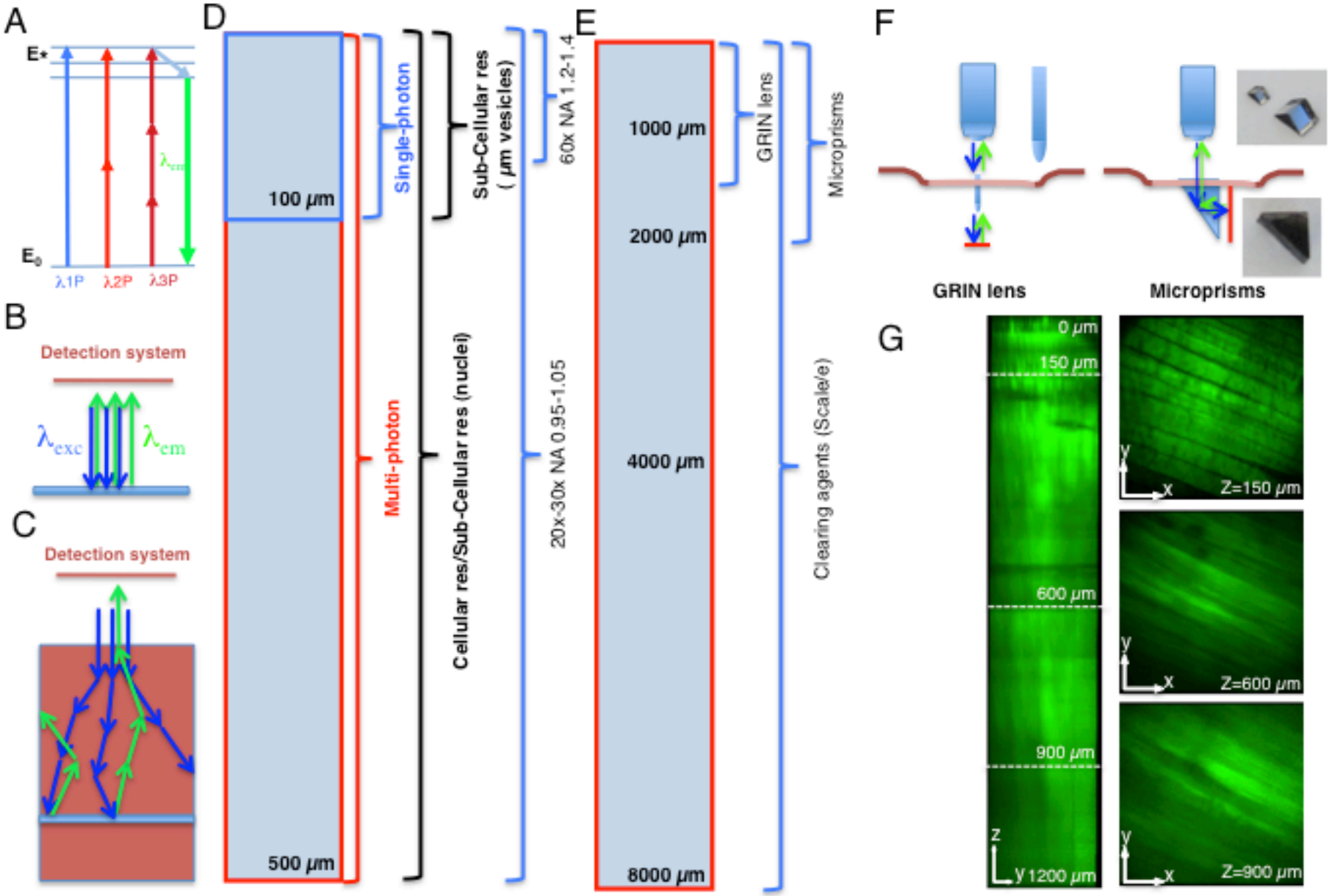
Objective inverter with PMT



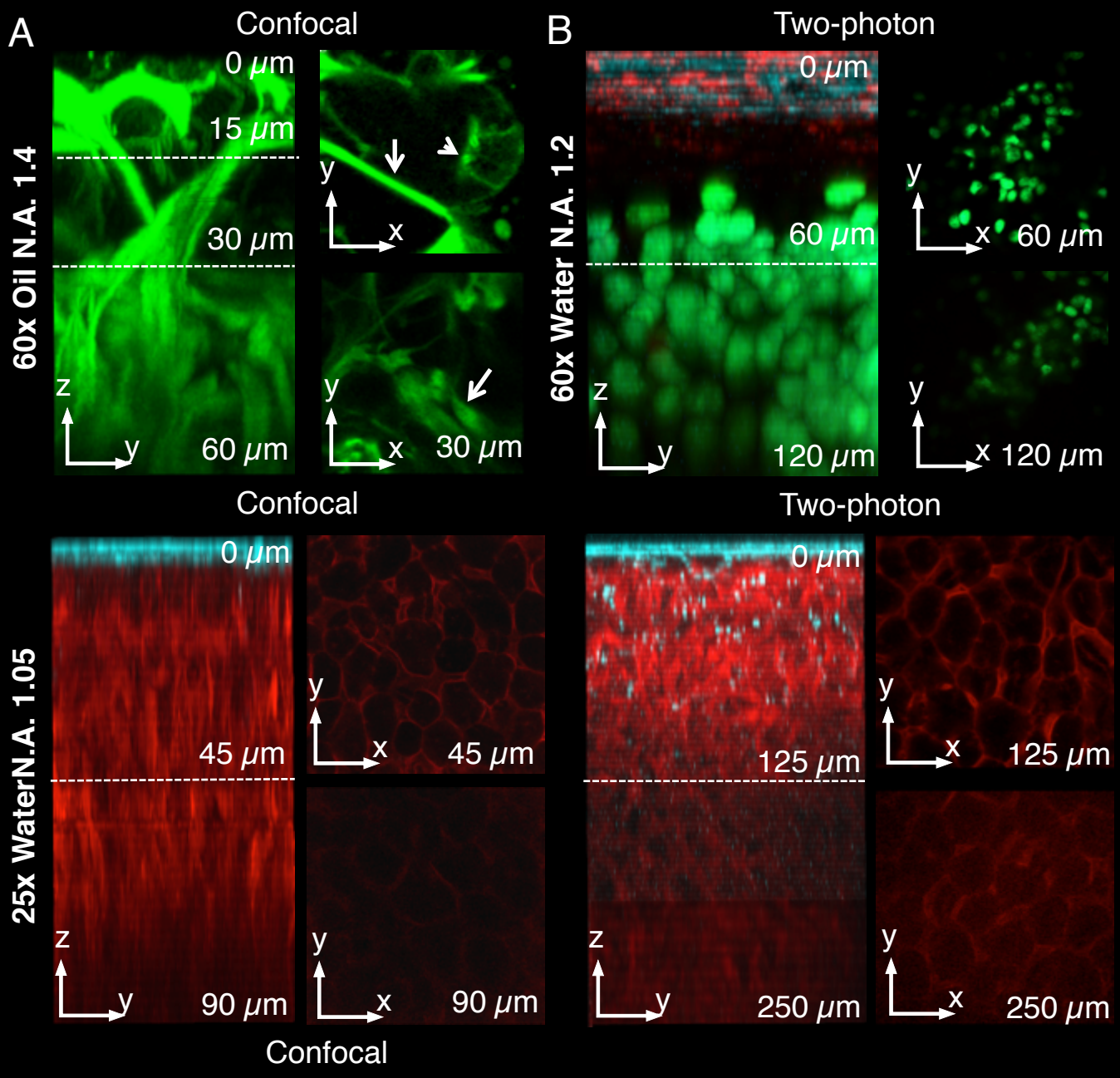


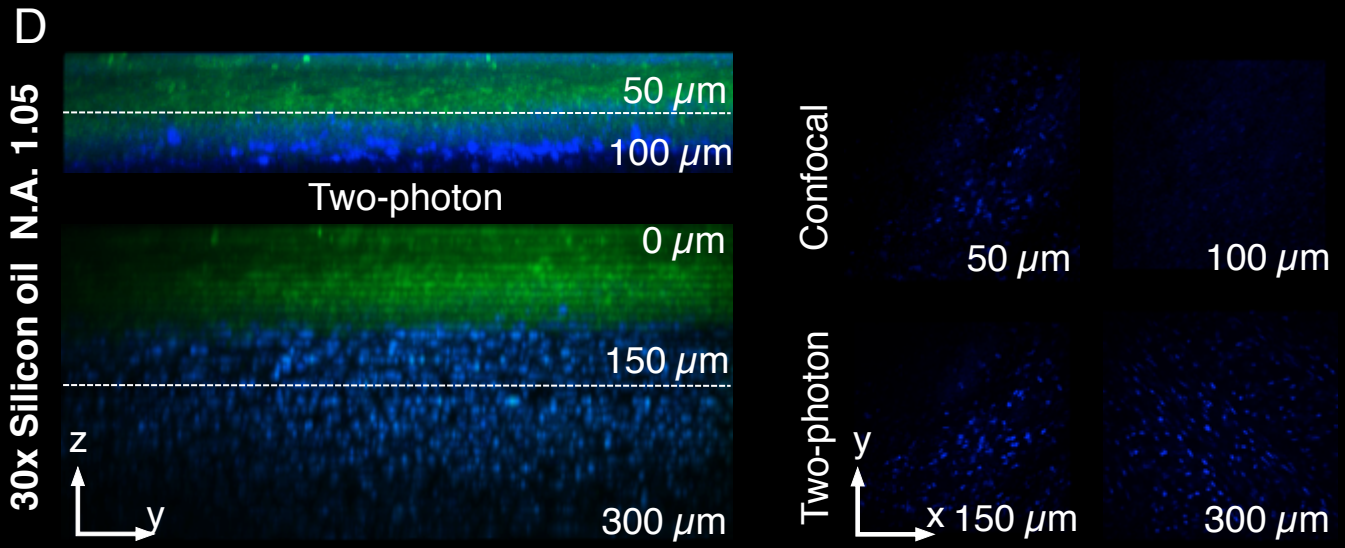
Confocal microscopy

Two-photon microscopy



Masedunskas et al. Figure 1


A
Confocal
B
Two-photon
60x Oil N.A. 1.4
60x Water N.A. 1.2
25x WaterN.A. 1.05
0 μm
15 μm
30 μm
60 μm
120 μm
45 μm
90 μm
125 μm
250 μm
x
y
z


D
30x Silicon oil N.A. 1.05
50 μm
100 μm
Two-photon
0 μm
150 μm
z
y
300 μm
Confocal
50 μm
100 μm
Two-photon
y
x 150 μm
300 μm