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 a 2-photon microscope?

A phone:
317-278-0436

Internet connection:
kwdunn@iupui.edu

12% Ethanol

Espresso Machine
“Gaggia”
Building Your Own 2-Photon Microscope

Turn Key System

Why?

1) Budget
   1) Initial expenses
   2) Maintenance

More expensive
$400K-500K

2) Flexibility

$150K Laser

3) Upgrades

Build your own

Buy Confocal microscope

Convert to a 2-photon

First step (first major decision)

Which Platform?

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

Motion artifacts

“All custom made holding device specifically designed for the organ of interest”

“Positioning and securing the organ to the coverslip”

Upright

All the organs

Inverted

All the organs but the brain
Live Cell Imaging
All the organs

Upright vs. Inverted

Upright

All the organs

Inverted

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

Upright converted to inverted

All the organs
Laser

488 nm
Laser combiner 561 nm
633 nm
UV laser 405 nm

1) Microscope

Scanning unit

Microscope

Ti-sapphire lasers

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
High power lasers (3-4 W)

Laser combiner: 488 nm, 561 nm, 633 nm
UV laser: 405 nm

Laser output power

2) Laser

Ti-sapphire lasers

1) Microscope

Scanning unit

Microscope

Graph: Output Power vs. Wavelength (nm)

Chamaleon Ultra II
Loss of power throughout the optics

1) Microscope
2) Laser

Beam expander

Dichroic mirrors

- UV laser 405 nm
- Laser combiner 488 nm, 561 nm, 633 nm

- Ti-sapphire lasers

- Scanning unit

- Microscope

Chamaleon Ultra II

Output Power (mW)

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Power (mW)</th>
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<tbody>
<tr>
<td>400</td>
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<tr>
<td>1100</td>
<td>3500</td>
</tr>
<tr>
<td>1200</td>
<td>4000</td>
</tr>
</tbody>
</table>

(A) 800 nm – 3520 mW (100%)
(B) 3280 mW (93%)
(C) 864 mW (25%) – S.H. cuts 20%
(D) 340 mW (10%) – 60x N.A 1.2

Loss of power throughout the optics
Control the power at the specimen

2) Laser

High power lasers (3-4 W)

1) Microscope

Laser combiner  488 nm, 561 nm, 633 nm
UV laser  405 nm

3) Control output power

Ti-sapphire lasers

Beam expander

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

2) ND continuous filter wheel

Circular ND filters (800 nm)

Beam attenuation (%) vs. O.D.
Control the power at the specimen

1) Microscope

2) Laser

3) Control output power

- Ti-sapphire lasers

4) Beam expander

Laser combiner 488 nm, 561 nm, 633 nm

UV laser 405 nm

High power lasers (3-4 W)

1) ND filters

2) ND continuous filter wheel

3) AOM (Acousto-optic modulator)

4) EOM (Electro-optic modulator)

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

1) Microscope
2) Laser
3) Control output power
4) Autocorrelator

Ti-sapphire lasers

Beam expander
Laser combiner 488 nm, 561 nm, 633 nm
UV laser 405 nm

Pulses: 100-150 fs

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

![Graph showing pulse width vs. wavelength](image)
Size of the laser beam

1) Control the size of the beam
2) Control the power at the specimen

Laser combiner: 488 nm, 561 nm, 633 nm
UV laser: 405 nm

Ti-sapphire lasers: 488 nm, 561 nm, 633 nm

1) Microscope
2) Laser
3) Control output power
4) Autocorrelator
5) Beam expander
Filling the back aperture of the lens

Essential for large lenses such as the 20X

Control the power at the specimen by overfilling
Challenge: alignment of the beam

1) Microscope
2) Laser
3) Control output power
4) Autocorrelator
5) Beam expander

Laser combiner 488 nm, 561 nm, 633 nm
UV laser 405 nm

Ti-sapphire lasers
Proper optics

1) Microscope

2) Laser

3) Control output power

4) Autocorrelator

5) Beam expander

6) Optics

Laser combiner 488 nm, 561 nm, 633 nm
UV laser 405 nm

Scanning unit

Microscope

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

2) Laser
   Ti-sapphire lasers

1) Microscope
   Scanning unit

3) Control output power

4) Autocorrelator

5) Beam expander

6) Optics

Laser combiner: 488 nm, 561 nm, 633 nm

UV laser: 405 nm

7) Detectors

A) Descanned detectors
B) Non-descanned detector

Positioning
Non-descanned detectors

3 Cooled PMT from Hamamatsu R6060-11
1 Gallium Arsenide PMT
Non-descanned detectors

Objective inverter with PMT
2) Laser
3) Control output power
4) Autocorrelator
5) Beam expander
6) Optics
7) Detectors

1) Microscope

Scanning unit

- Ti-sapphire lasers
- Laser combiner: 488 nm, 561 nm, 633 nm
- UV laser: 405 nm

- CCD camera
- Microscope

Confocal microscopy

Two-photon microscopy