Image acquisition by two-photon microscopy

Microscopy Methods in Biomedicine

13 October 2025, IMG, Prague, CR

Daniel Hadraba

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+420 776 441 811

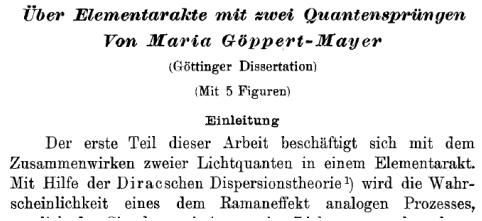
History

INSTITUTE OF PHYSIOLOGY CAS

1931

Maria Göeppert-Mayer

Über Elementarakte mit zwei Quantensprüngen," Ann. Phys. 9(3), 273–294



Über Elementarakte mit zwei Quantensprüngen
Von Maria Göppert-Mayer

(Göttinger Dissertation)

(Mit 5 Figuren)

Einleitung

Der erste Teil dieser Arbeit beschäftigt sich mit dem Zusammenwirken zweier Lichtquanten in einem Elementarakt. Mit Hilfe der Diracschen Dispersionstheorie[1]) wird die Wahrscheinlichkeit eines dem Ramaneffekt analogen Prozesses,

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Kaiser and Garrett

Two-Photon Excitation in CaF2:Eu2+," Phys. Rev. Lett. 7(6), 229–231

VOLUME 7, NUMBER 6 PHYSICAL REVIEW LETTERS SEPTEMBER 15, 1961

TWO-PHOTON EXCITATION IN $CaF_2:Eu^{2+}$

W. Kaiser and C. G. B. Garrett
Bell Telephone Laboratories, Murray Hill, New Jersey
(Received August 28, 1961)

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responding to the diameter of the incident light beam. When pure CaF_2 was illuminated by the optical maser in the same way, no light with

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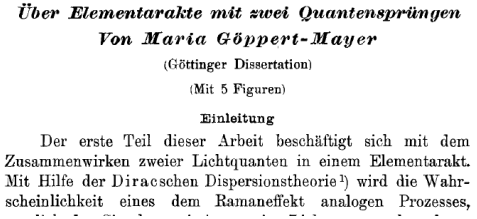

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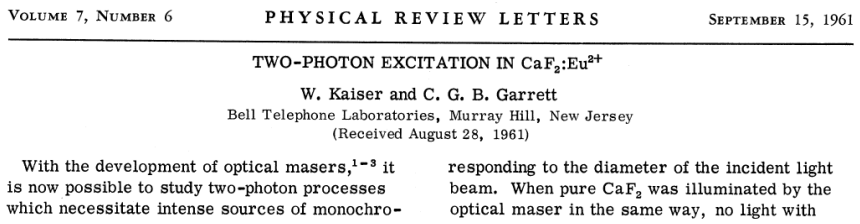

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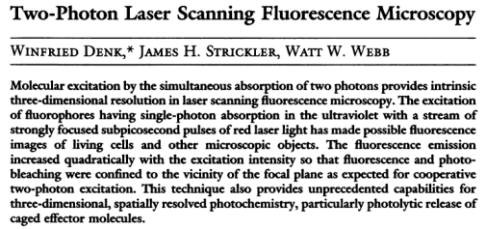
1990

Denk, Strickler and Webb

Two-photon laser scanning fluorescence microscopy,
Science. 1990 Apr 6; 248(4951):73-6.



Two-Photon Laser Scanning Fluorescence Microscopy

WINFRIED DENK,* JAMES H. STRICKLER, WATT W. WEBB

Molecular excitation by the simultaneous absorption of two photons provides intrinsic three-dimensional resolution in laser scanning fluorescence microscopy. The excitation of fluorophores having single-photon absorption in the ultraviolet with a stream of strongly focused subpicosecond pulses of red laser light has made possible fluorescence images of living cells and other microscopic objects. The fluorescence emission increased quadratically with the excitation intensity so that fluorescence and photobleaching were confined to the vicinity of the focal plane as expected for cooperative two-photon excitation. This technique also provides unprecedented capabilities for three-dimensional, spatially resolved photochemistry, particularly photolytic release of caged effector molecules.

Samples & Light Interaction

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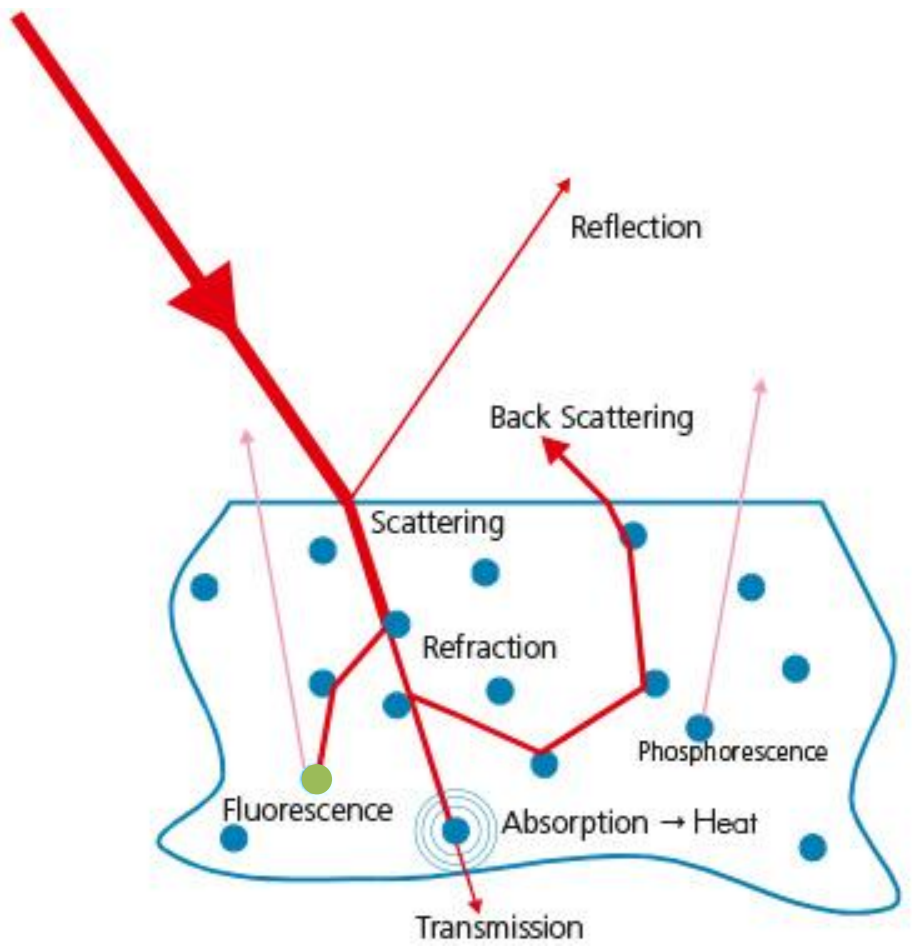

Samples & Light Interaction

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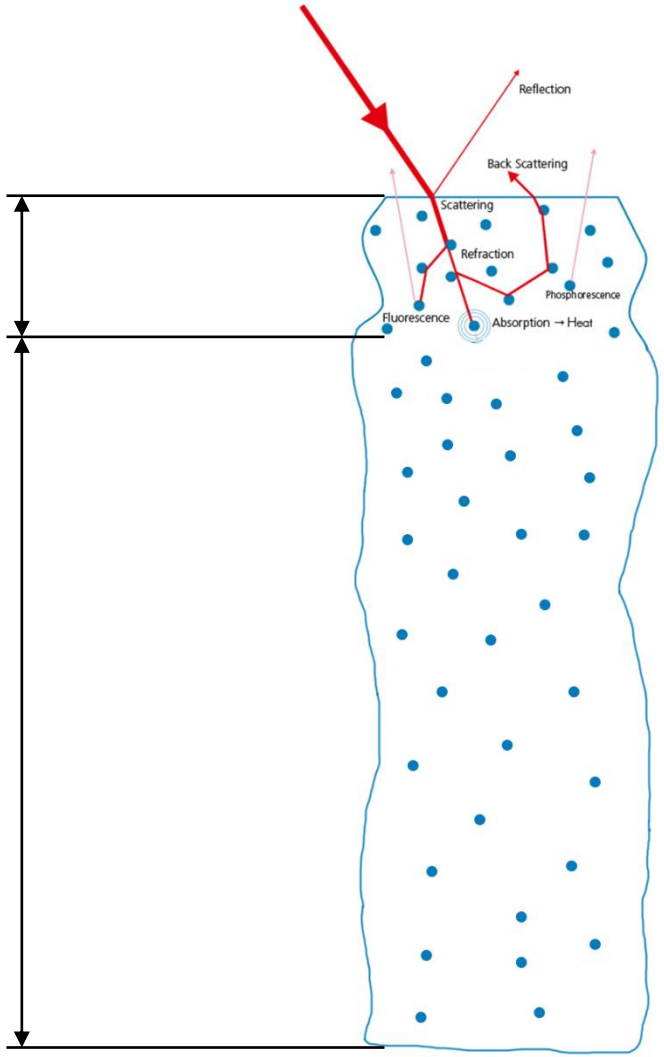
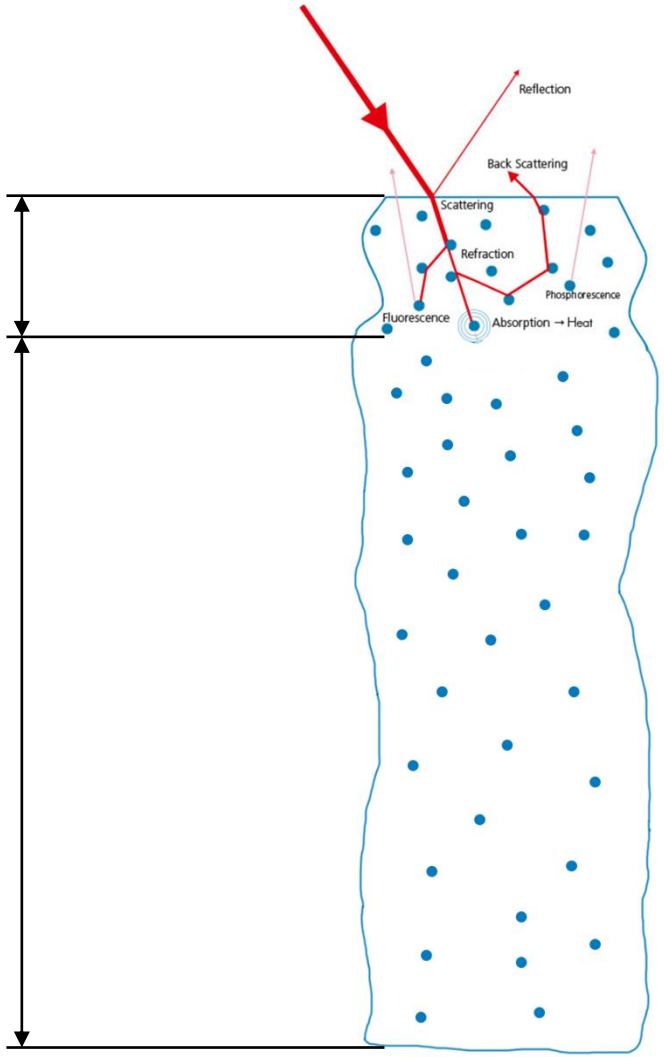
50 µm

450 µm



3D sample

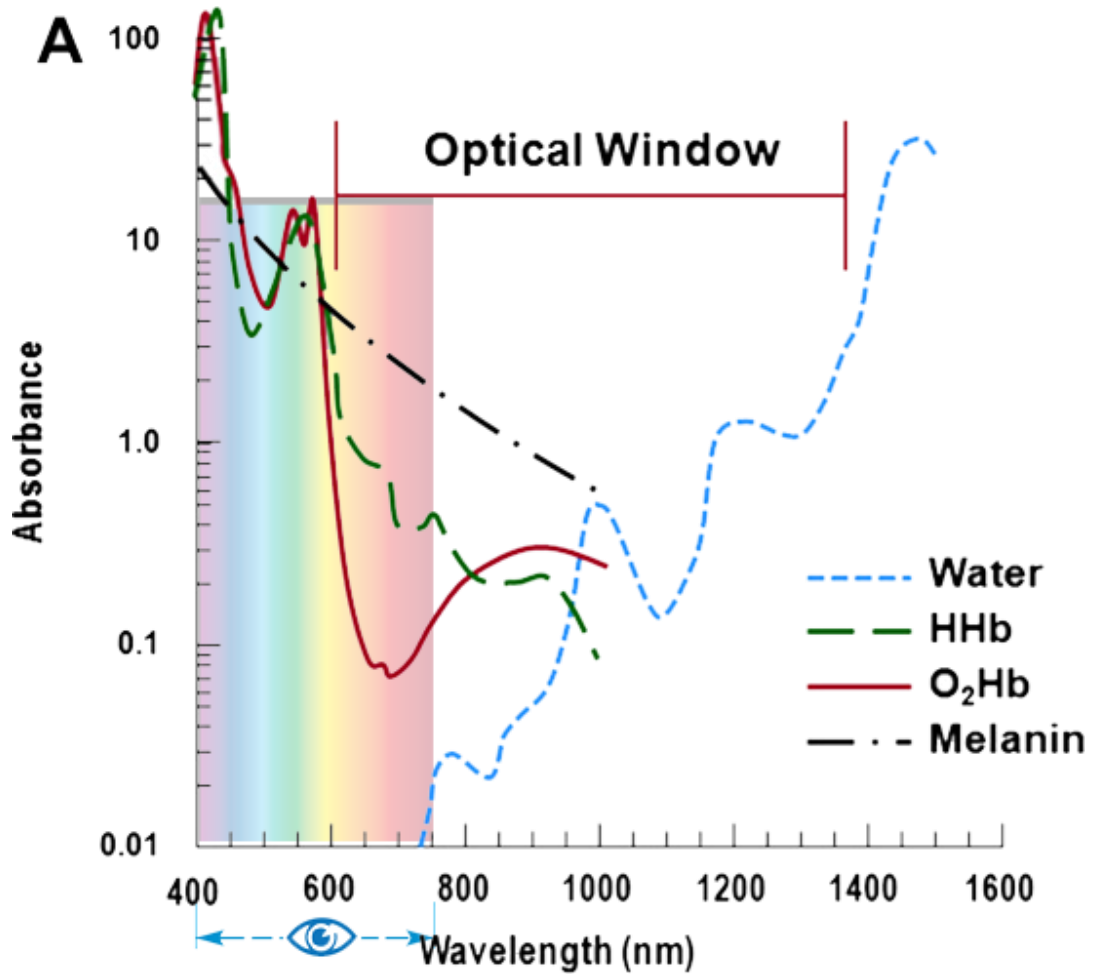
Samples & Light Interaction



- Deeper to the sample

- Less damage to the sample

Deeper into the sample



Pellicer and Bravo (2011)

Deeper into the sample

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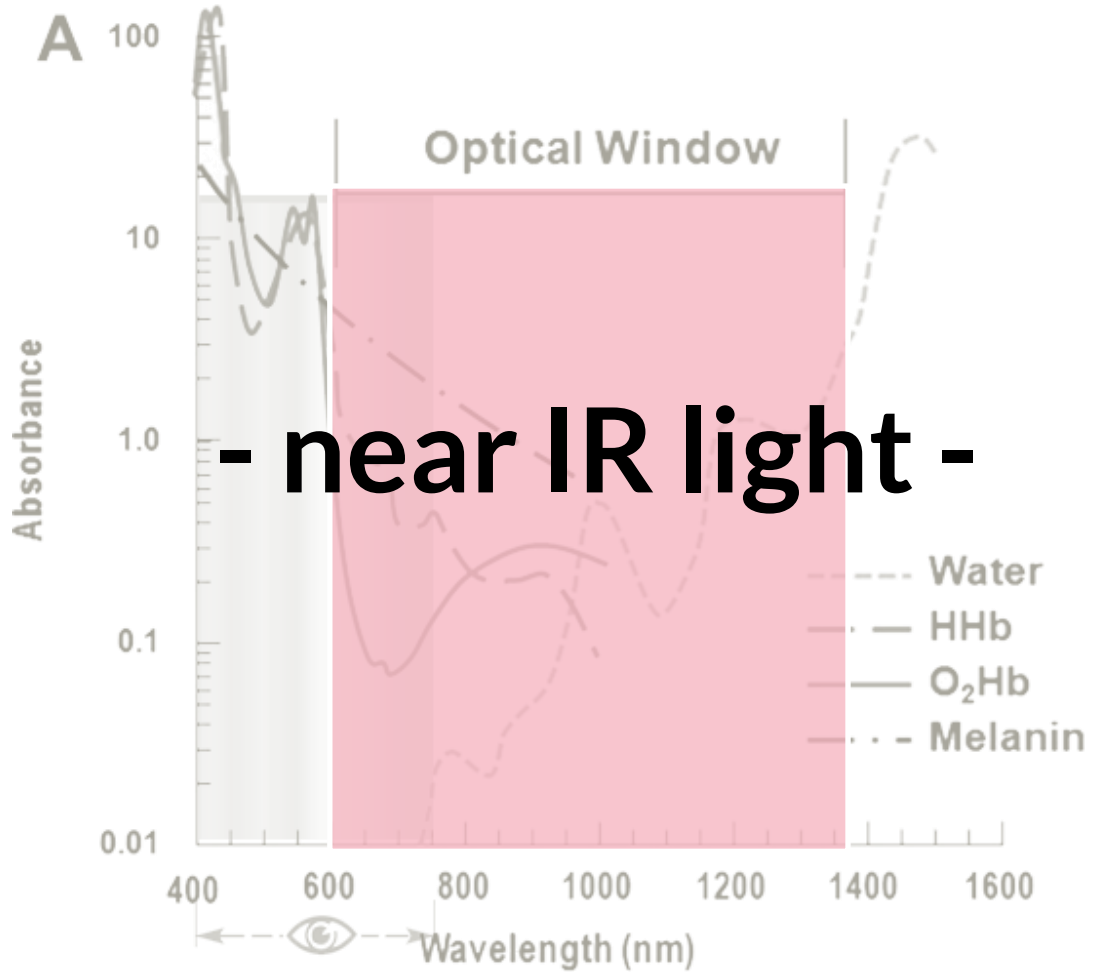

- near IR light -

Pellicer and Bravo (2011)

Deeper into the sample

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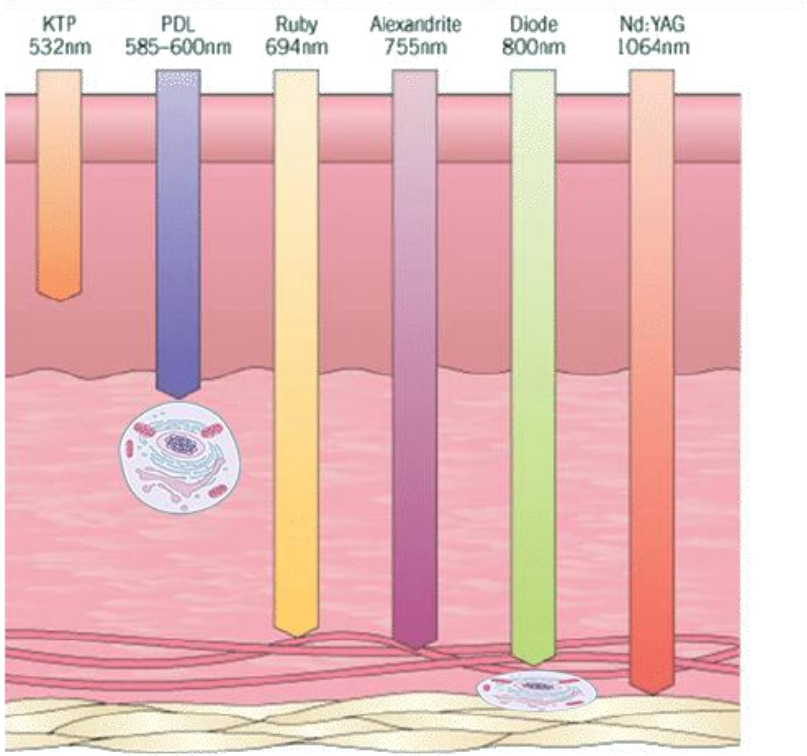
IR light



Less damage to the sample

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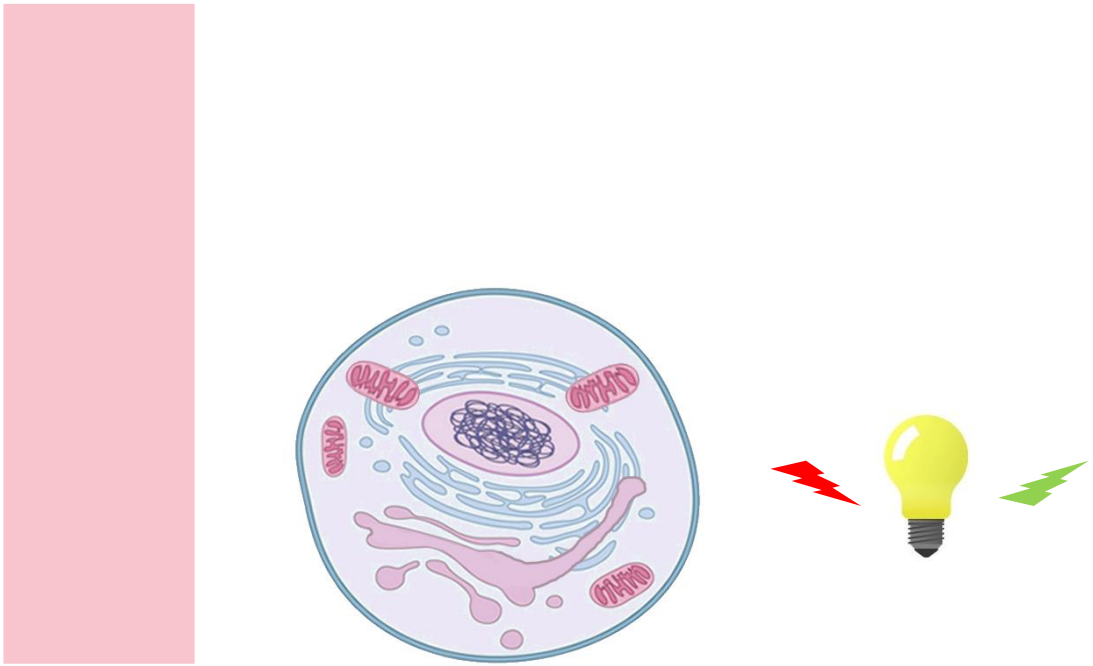
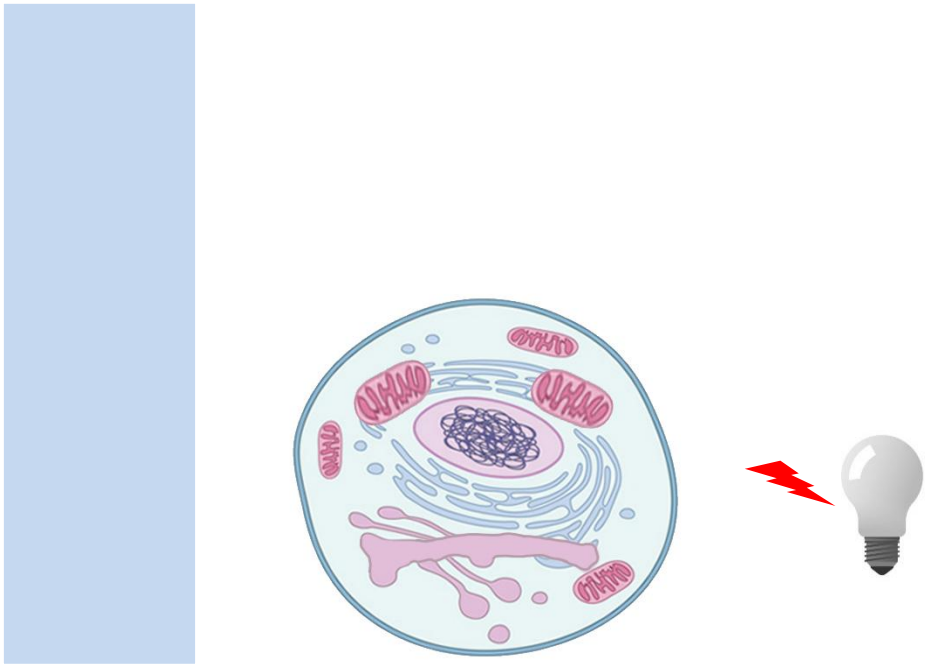
IR light



UV light



Less damage to the sample

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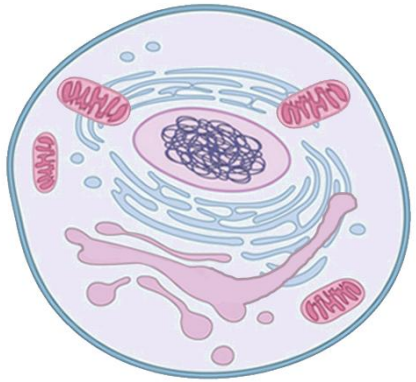
IR light



- No UV light
- Excites only ROI

1-photon experiment

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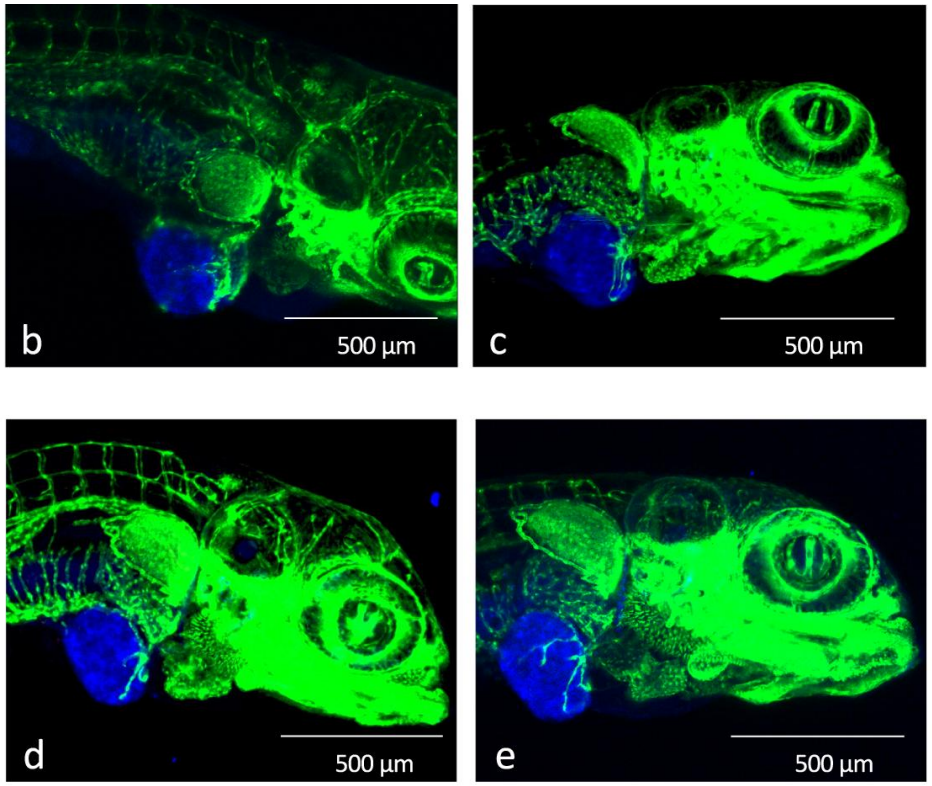
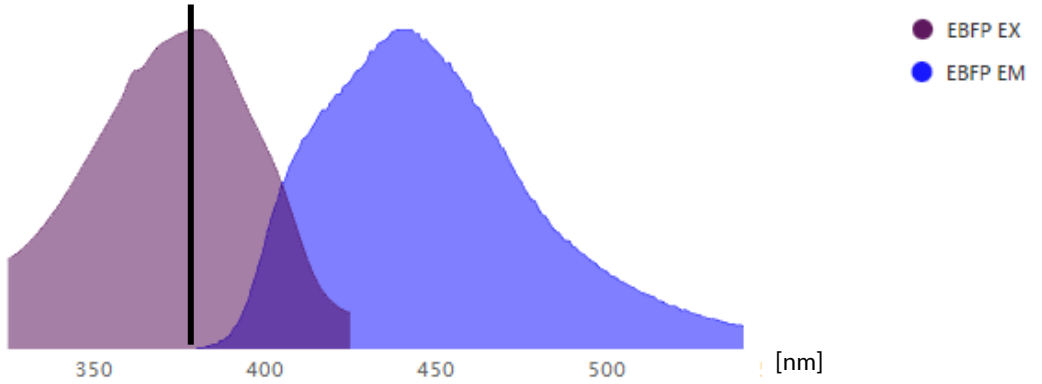
BFP-encoding mTagBFP2-Lifeact-7 plasmid



Mealiea D. et al. (2020)



- UV – blue excitation light

1-photon experiment

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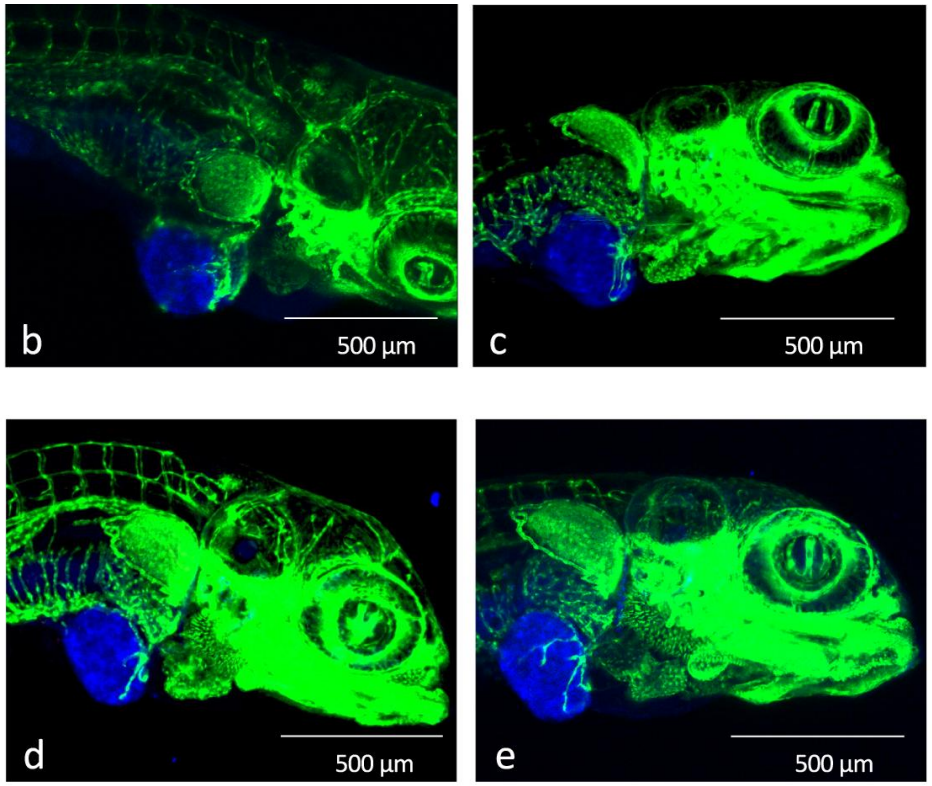

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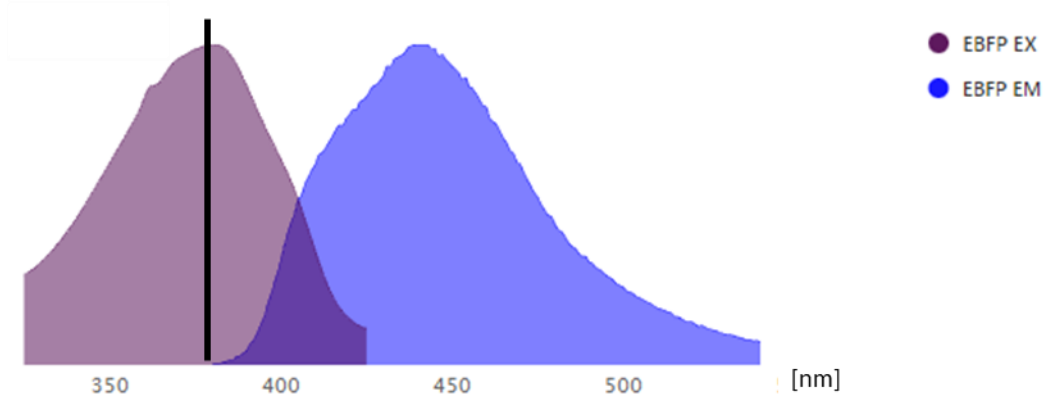

- UV – blue excitation light
- Not only ROI excited

1-photon experiment

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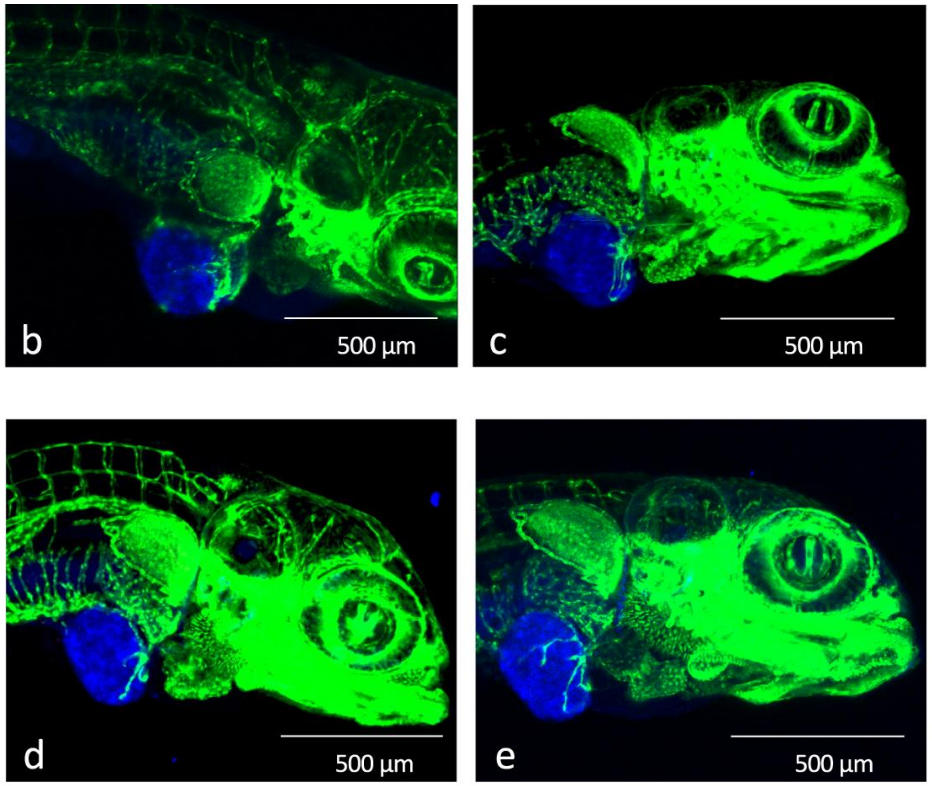

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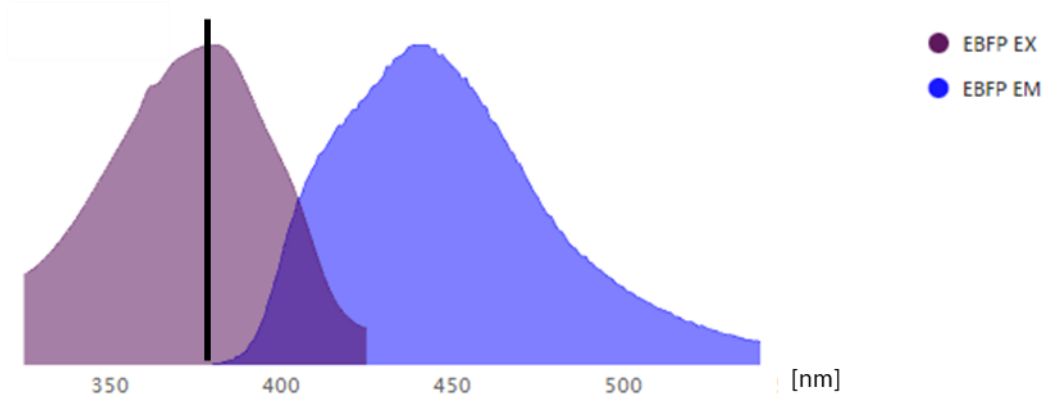
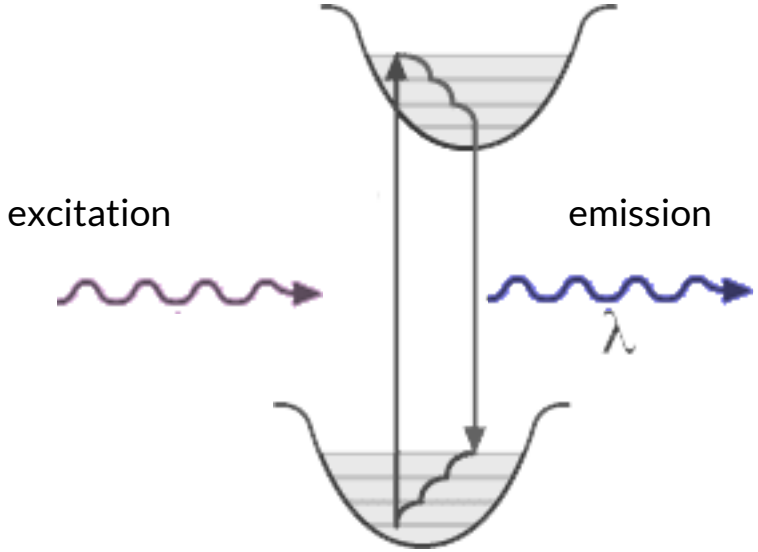
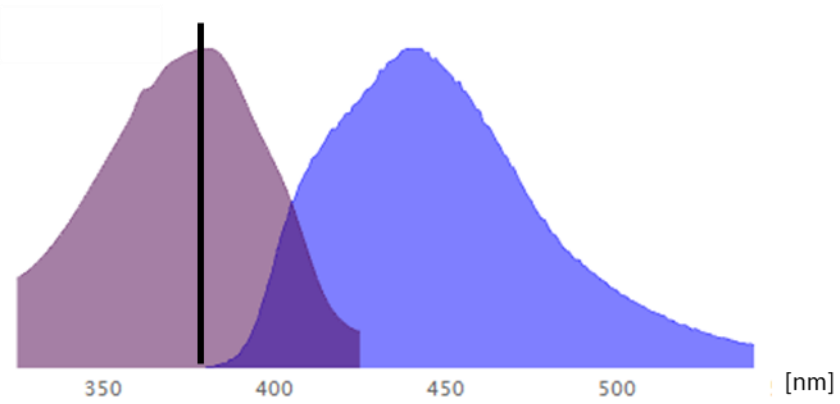


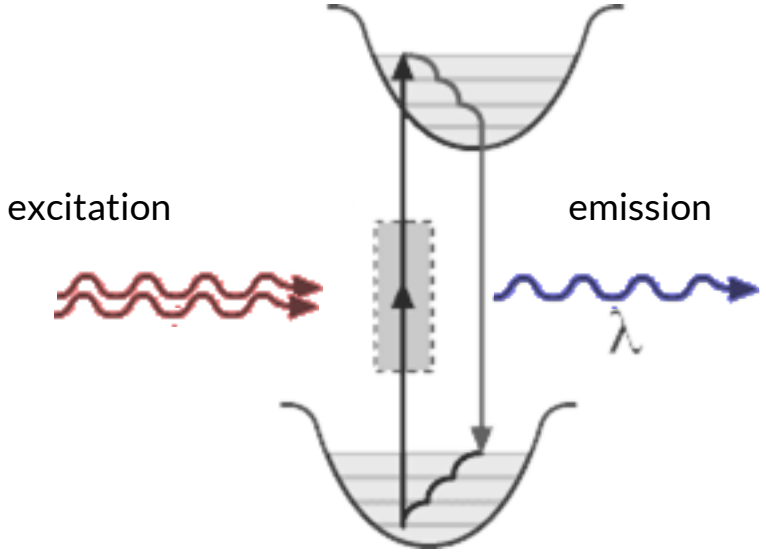
- UV – blue excitation light
- Not only ROI excited
- Penetration depth

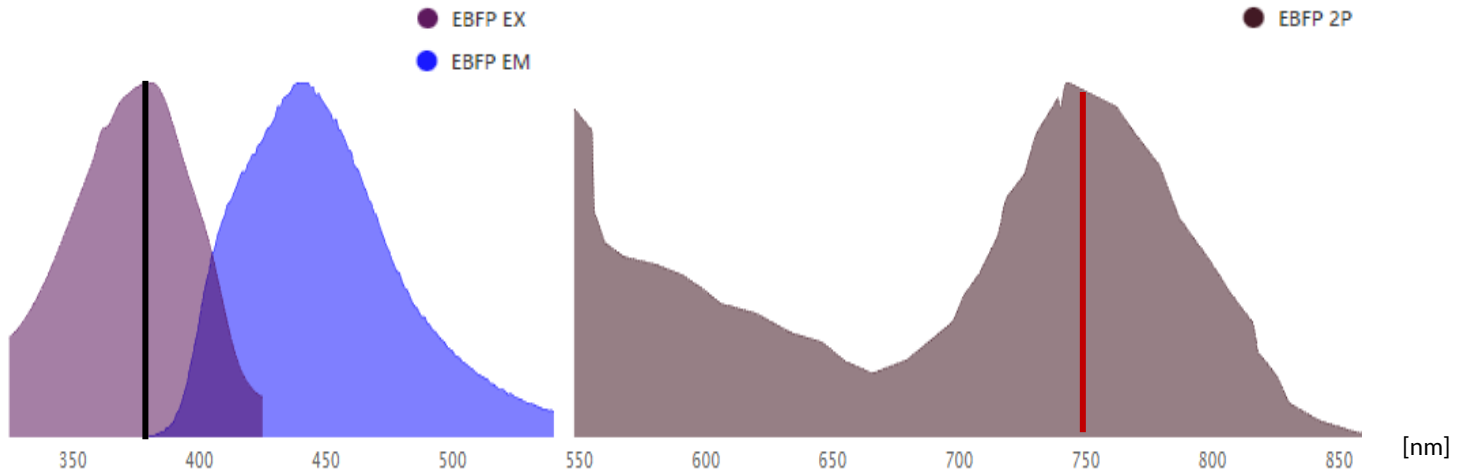
1-photon experiment





1-photon vs 2-photon experiment





1-photon vs 2-photon

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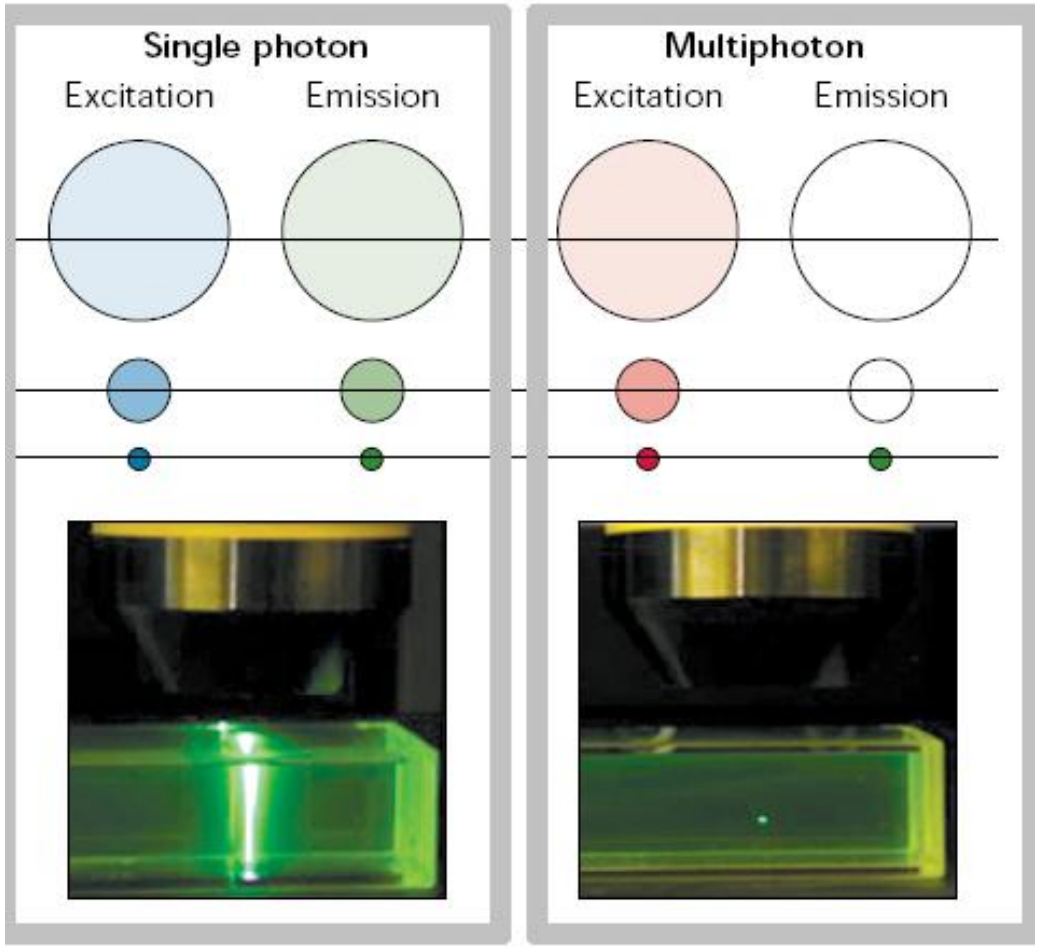
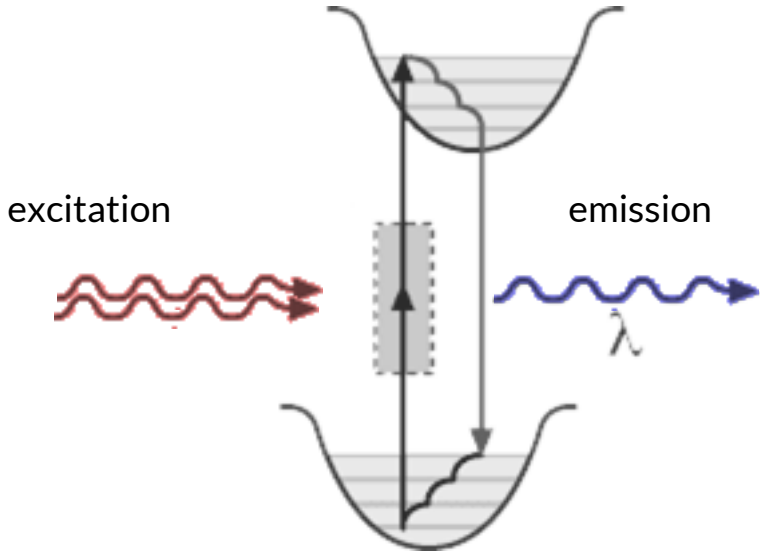
focal plane



1-photon vs 2-photon

waiting for …

2-photon



$$n_{2PE} = \frac{p_{ave}^2 \delta}{\Delta\tau \, \Delta\upsilon^2} \left(\frac{NA^2}{2\hbar c \lambda} \right)^2$$

Denk et al. – ref 9. (1990)

- High average (peak) power
- High NA
- Short pulse duration
- Low repetition rate

2-photon

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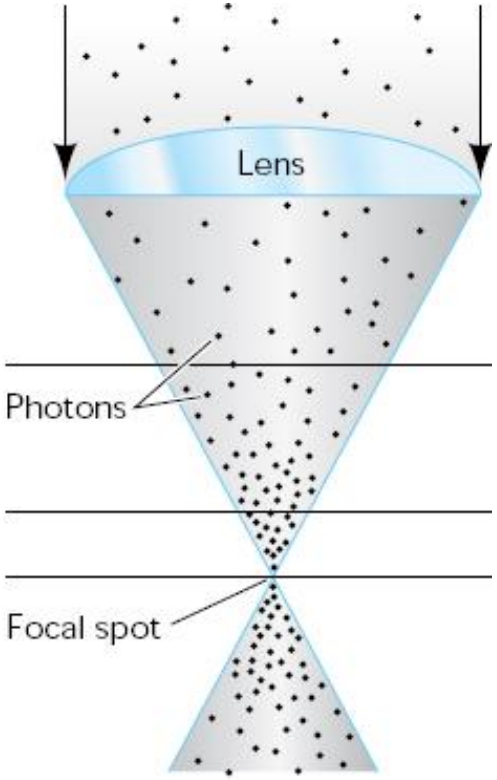
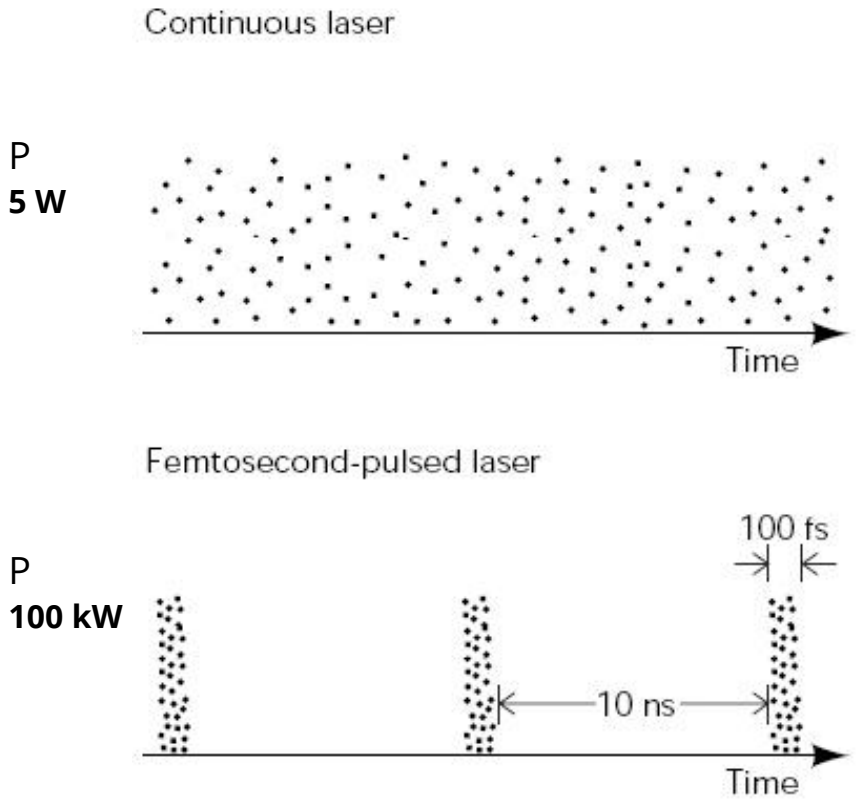
spatial compression



temporal compression



2-photon hardware – laser source

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$$n_{2PE} = \frac{p_{ave}^2 \delta}{\Delta\tau \, \Delta\upsilon^2} \left(\frac{NA^2}{2\hbar c \lambda} \right)^2$$

Output A	Specification
Wavelength Range (nm)	680-1300nm
Output power (mW)	
700nm	1300
800nm	1400
900nm	1300
1000nm	1100
1200nm	800
1300nm	600
Output B	
Wavelength (nm)	1040nm
Output power (mW)	>1500

2-photon hardware – objective

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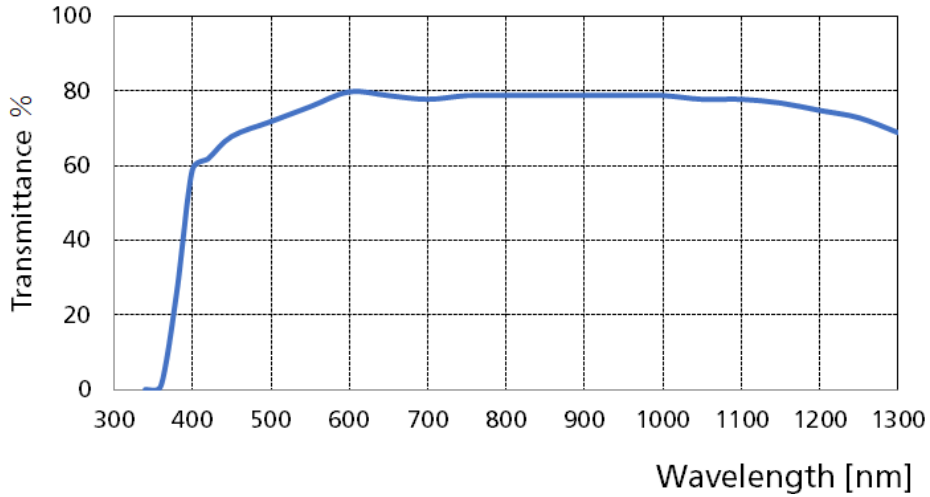
$$n_{2PE} = \frac{p_{ave}^2 \delta}{\Delta\tau \, \Delta\upsilon^2} \left(\frac{NA^2}{2\hbar c \lambda} \right)^2$$

- High NA
- High WD
- Large FOV
- High transmittance



2-photon hardware

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Bruker Ultima

Leica Stellaris 8

2-photon experiment

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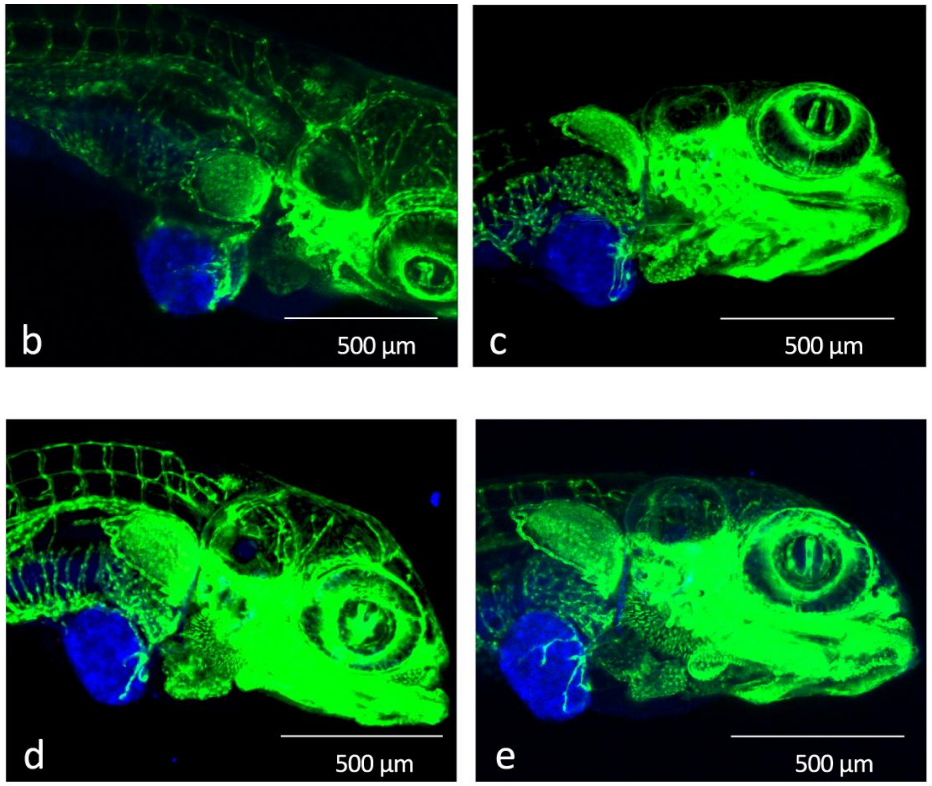

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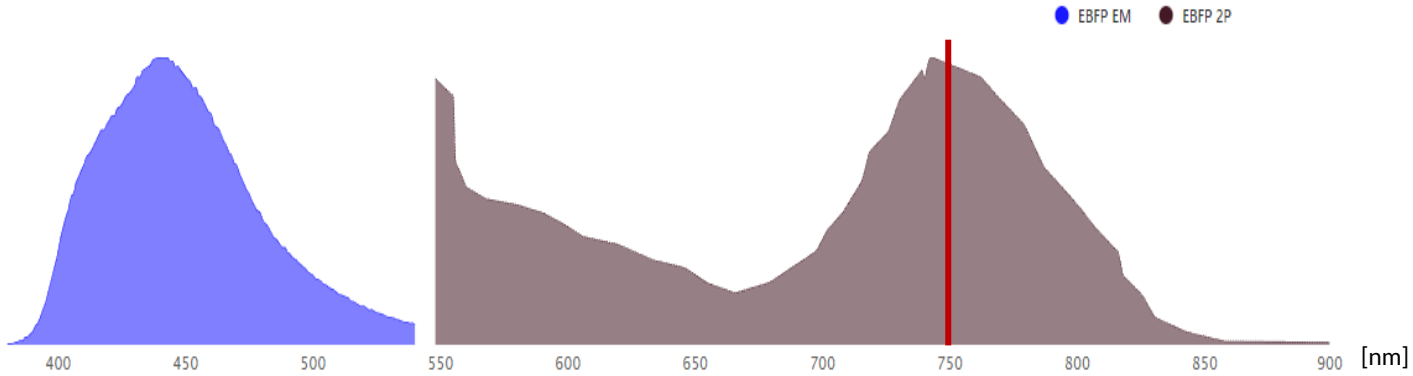

- IR light
- Only ROI excited
- Penetration depth

2-photon experiment

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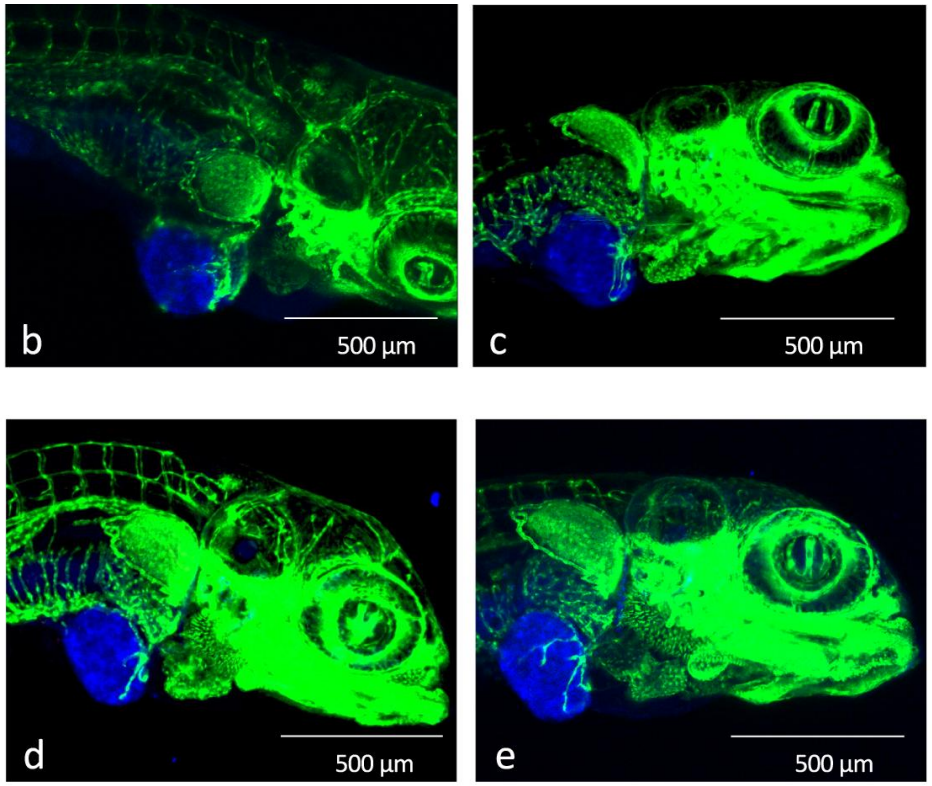

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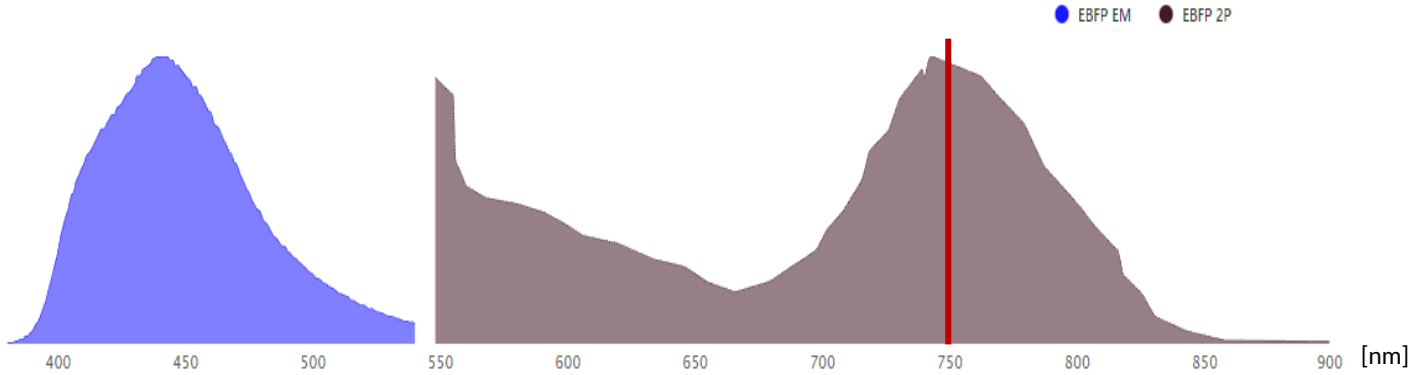

- Lower resolution
- Broader exaction spectra
- Less photons
- Photobleaching

2-photon – standard imaging

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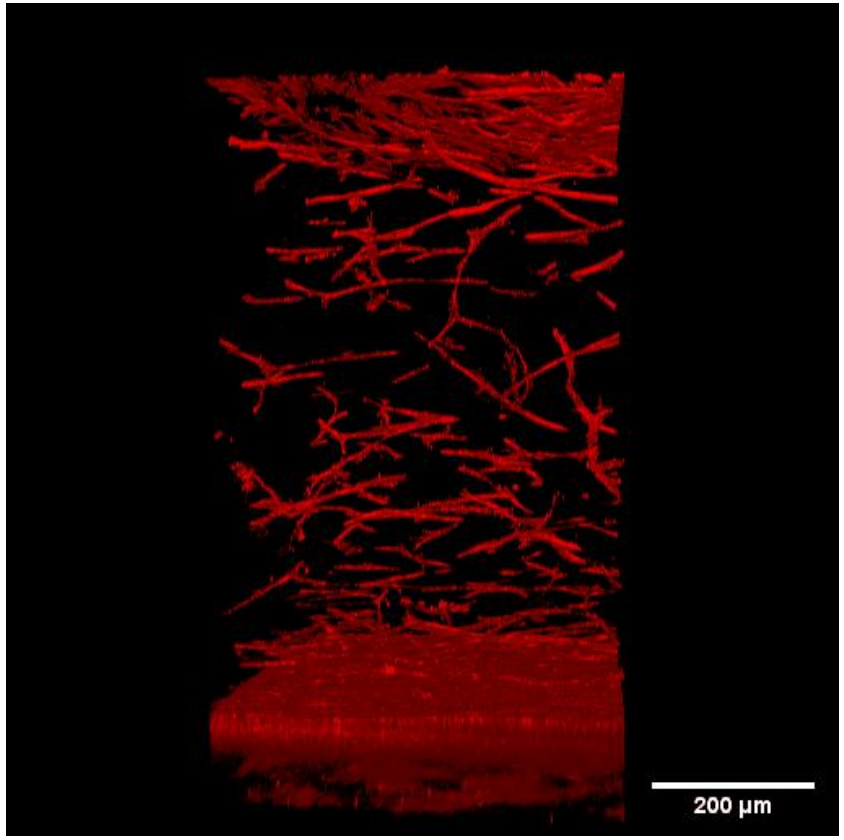
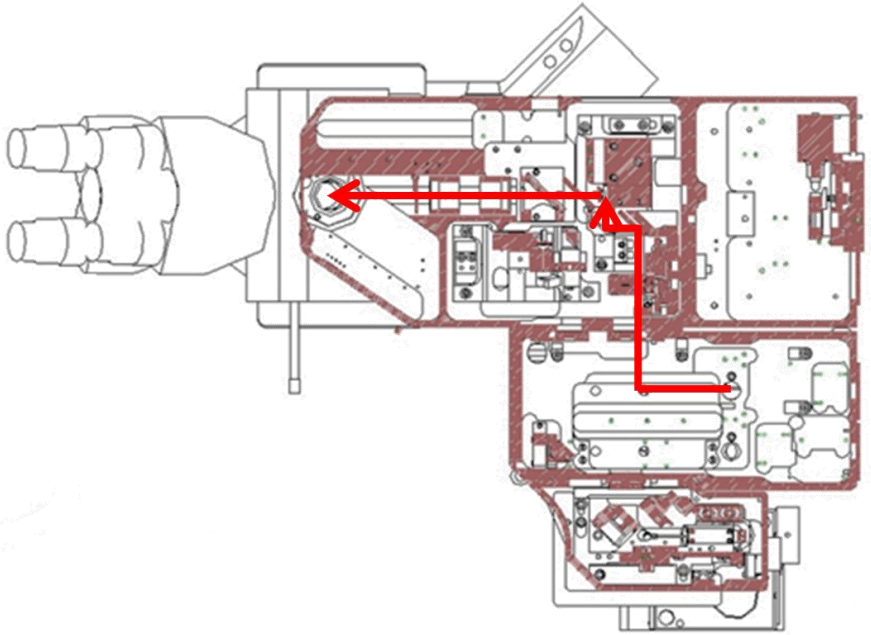
3D tissue engineered skin construct



Zikmundova M.



2-photon – standard imaging

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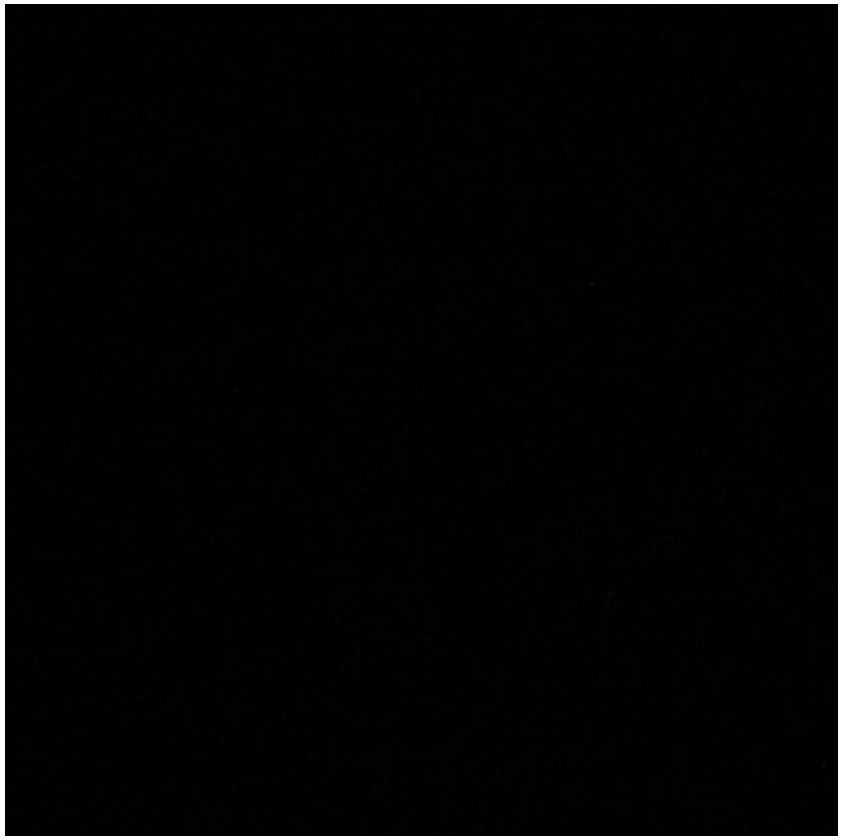
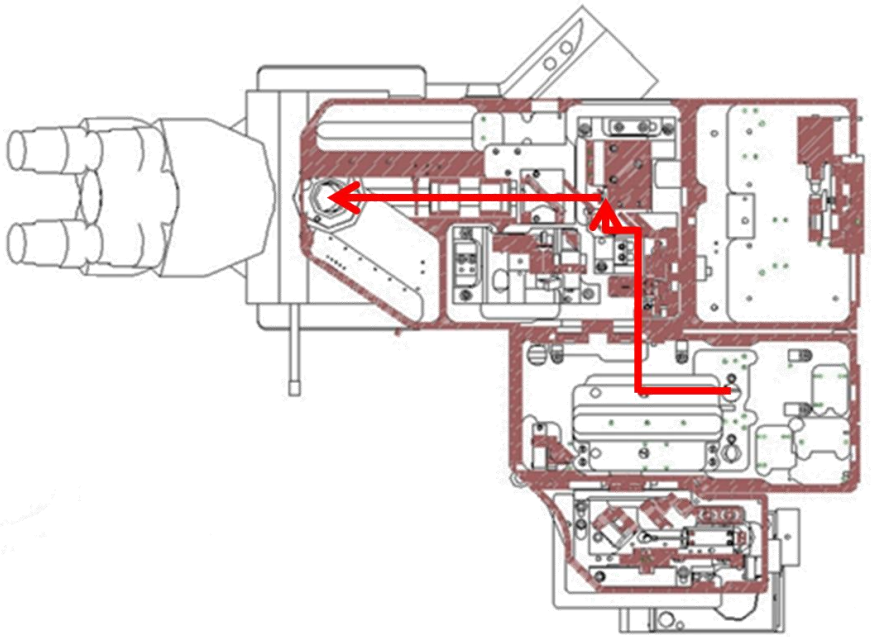
Fluorescein – blood flow through cranial window





Kala D.

2-photon – photoactivation

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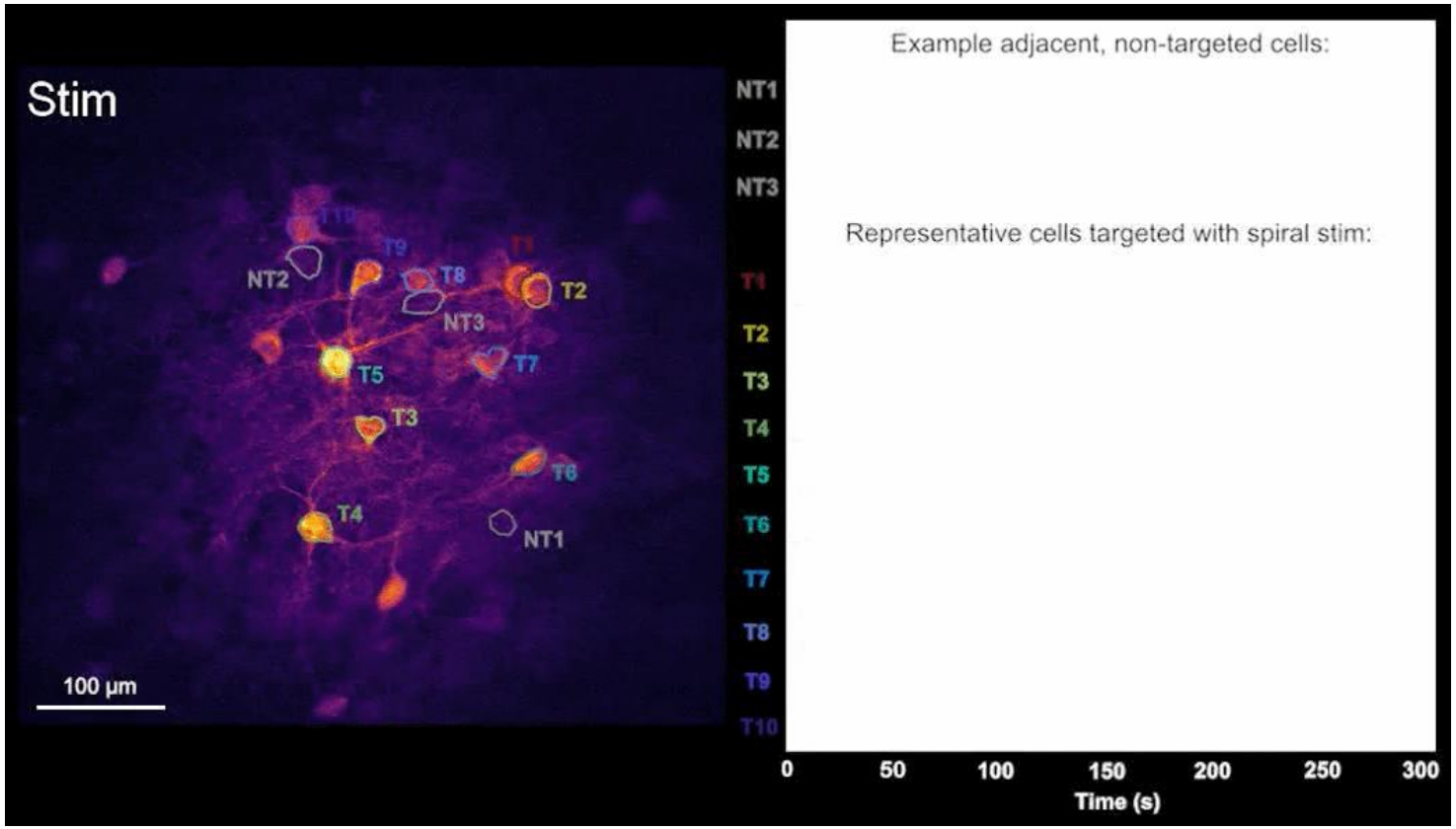
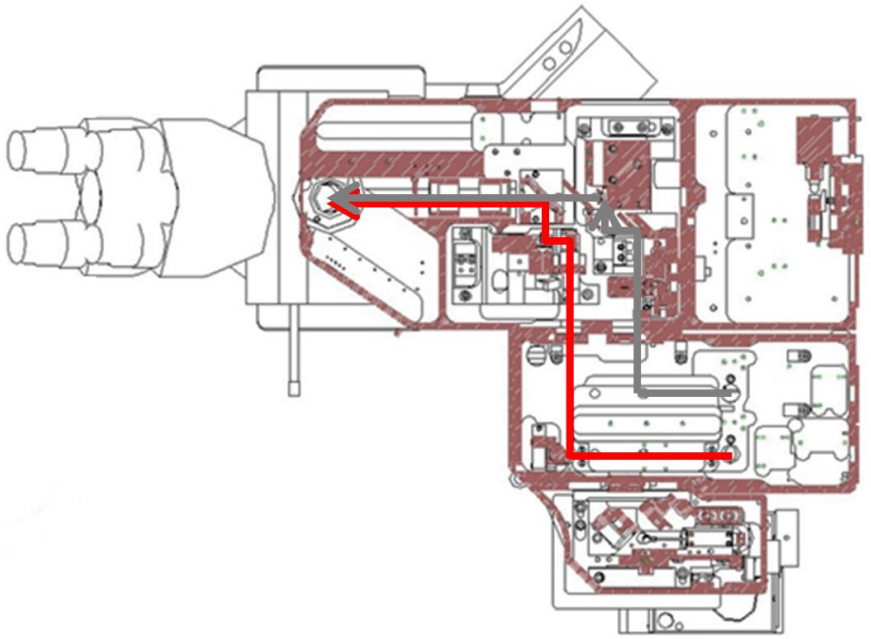
Activation of social-stimuli responsive neurons is linked to feeding behavior





Jennings et al. Nature 2019, Deisseroth Lab, Stanford

2-photon – resonant imaging

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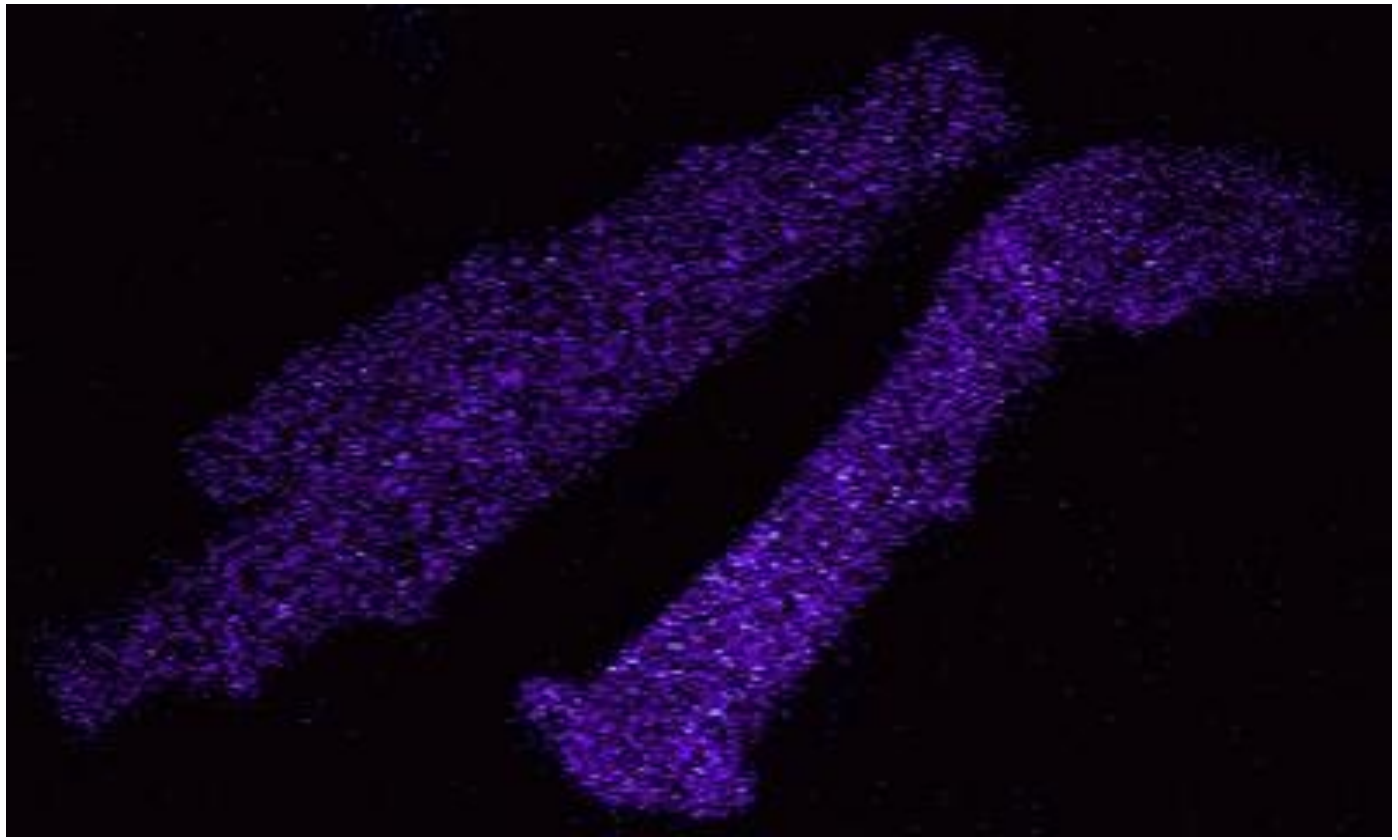
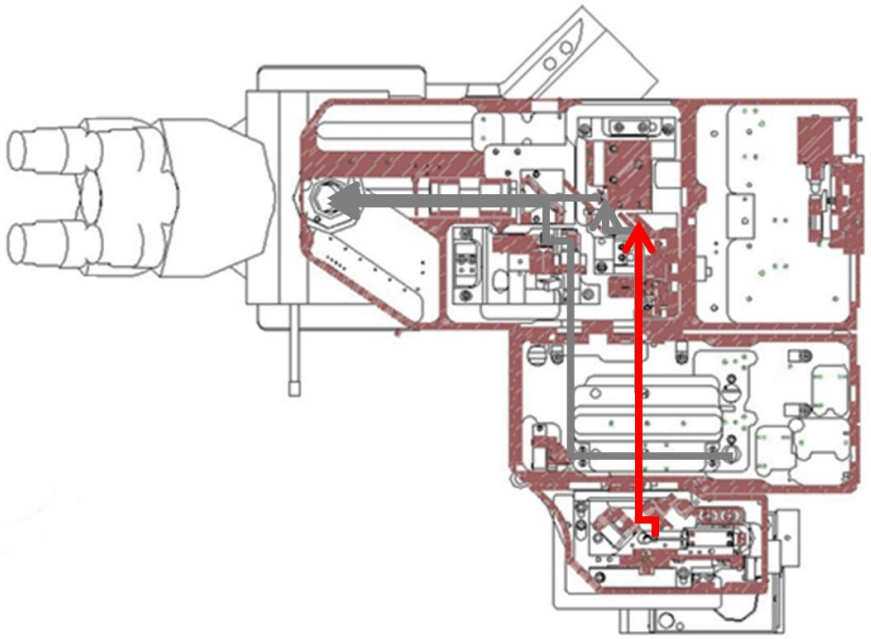
Calcium waves in cardiac muscles



G. Ellis-Davies, Mount Sinai School of Medicine New York



2-photon – volumetric imaging

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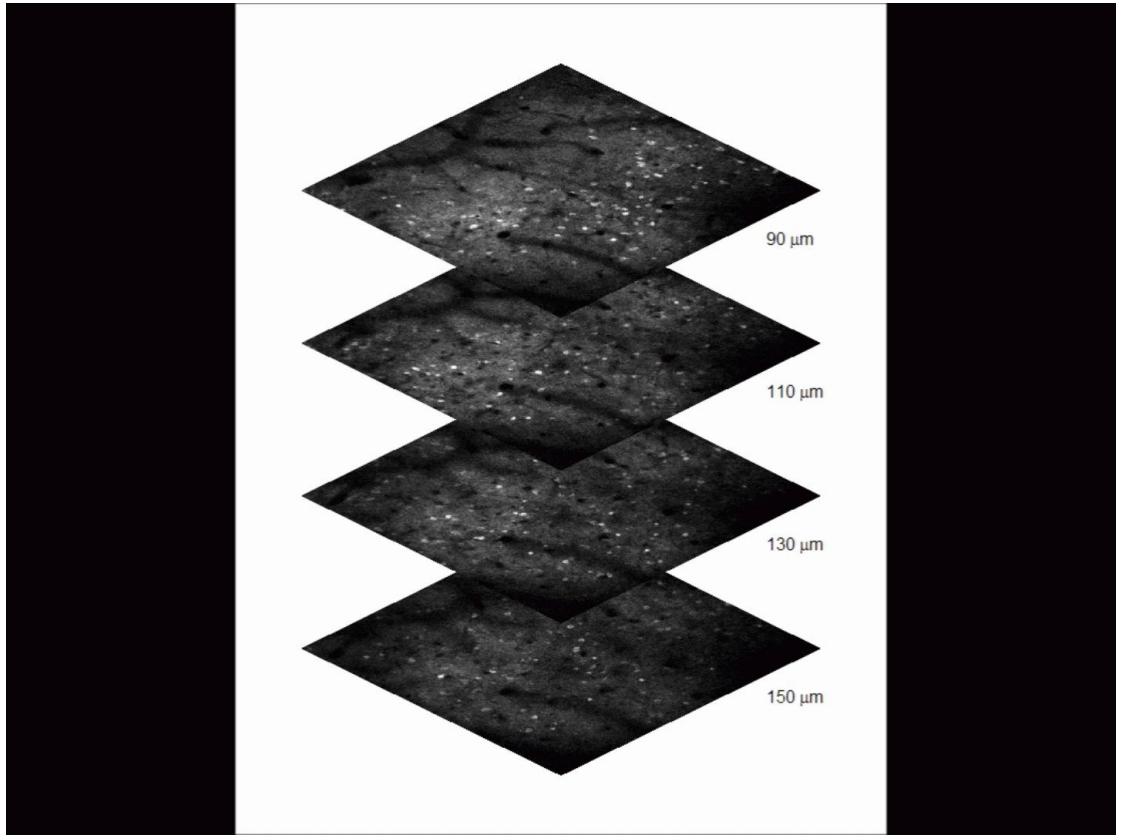
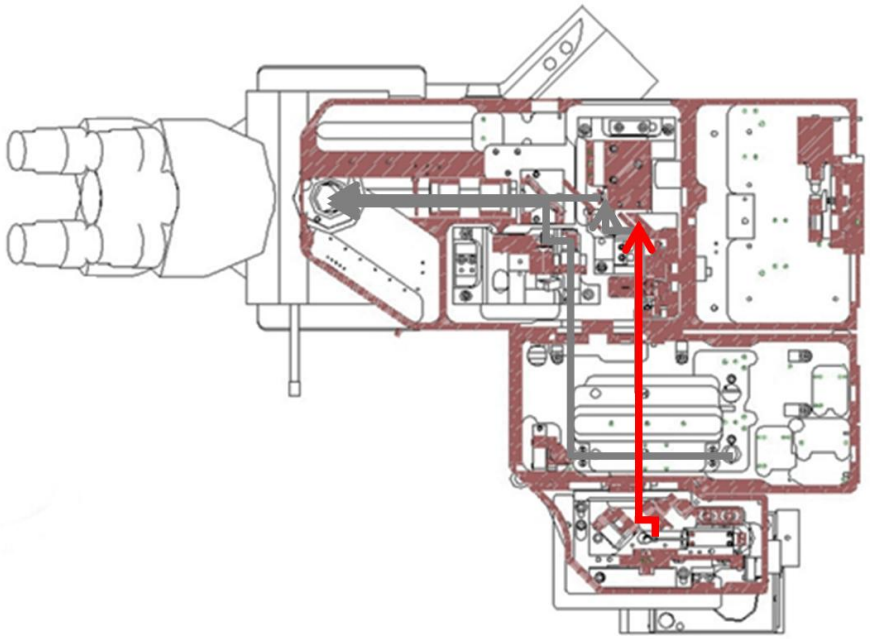
Volumetric imaging through cranial window

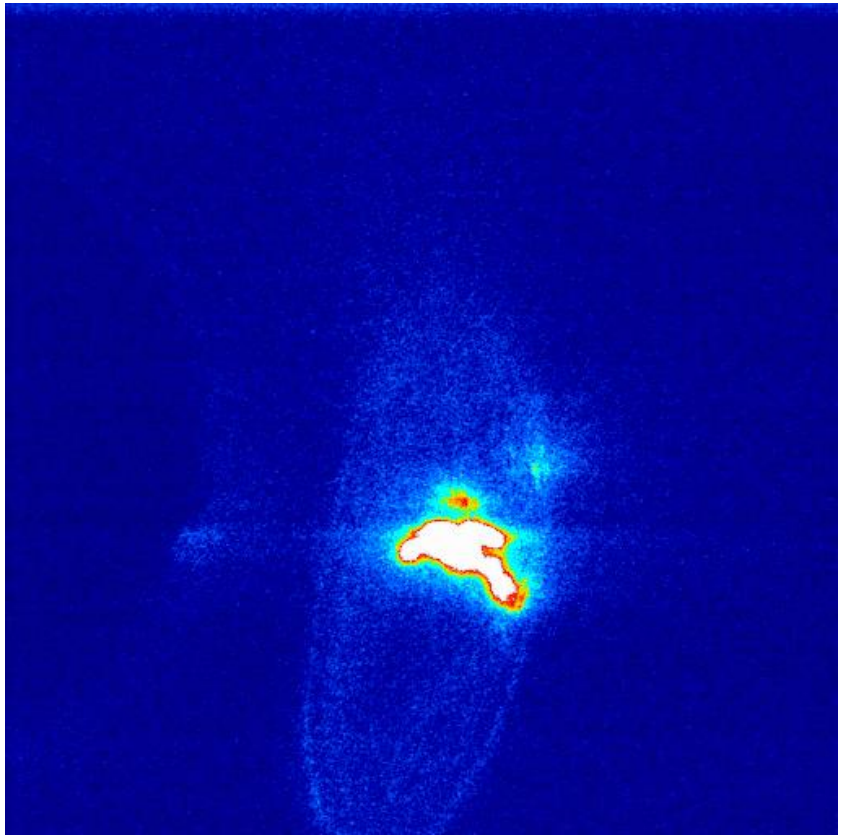




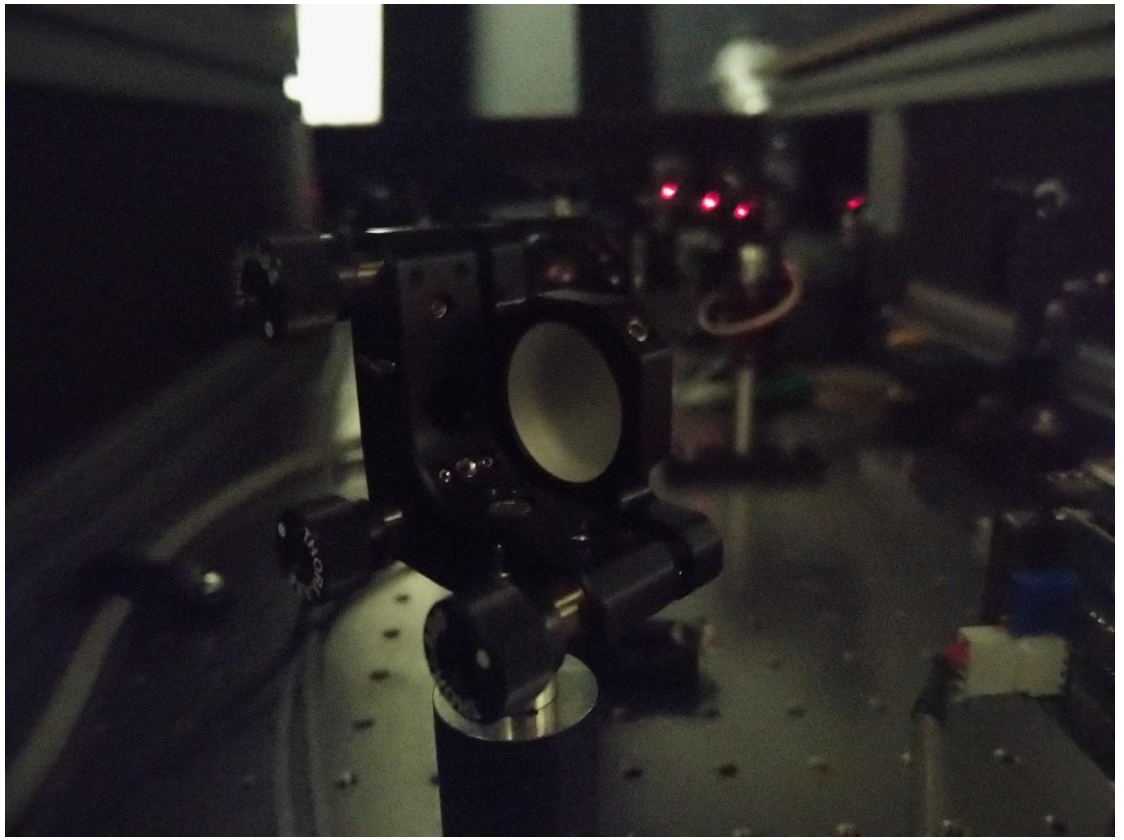
M. Goard et al., eLife 2016, Sur lab, MIT

2-photon – flexibility

Automated stage correction – API



E. Scheer, Cori Bargmann Laboratory, Rockefeller University



What next …

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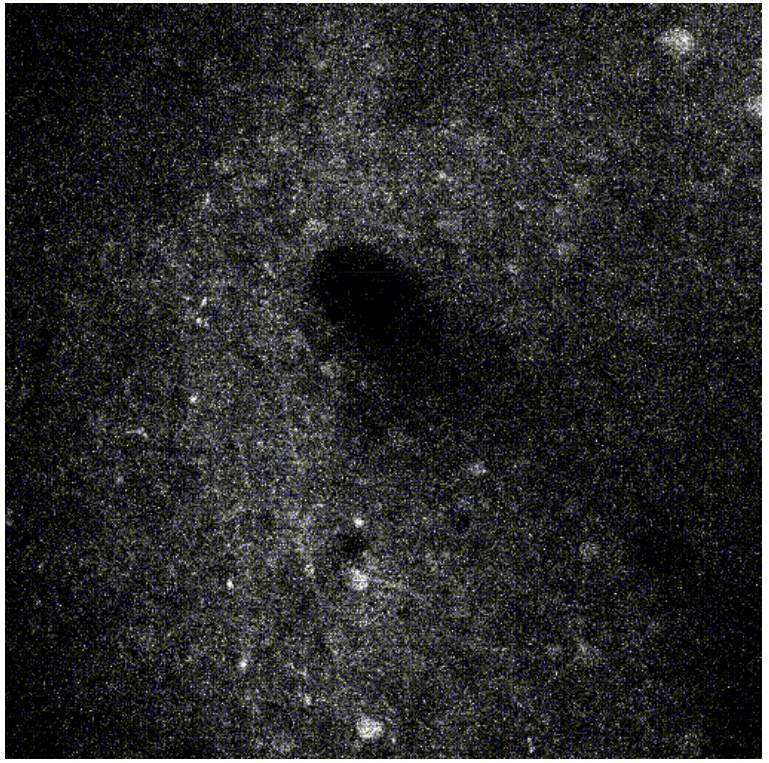
3-photon fluorescence imaging IEM

Label-free techniques

- Autofluorescence
- Raman (CARS, SRS)
- Second Harmonic Generation (SHG)
- Third Harmonic Generation (THG)

Spinning disk with Brillouin modality IPHYS



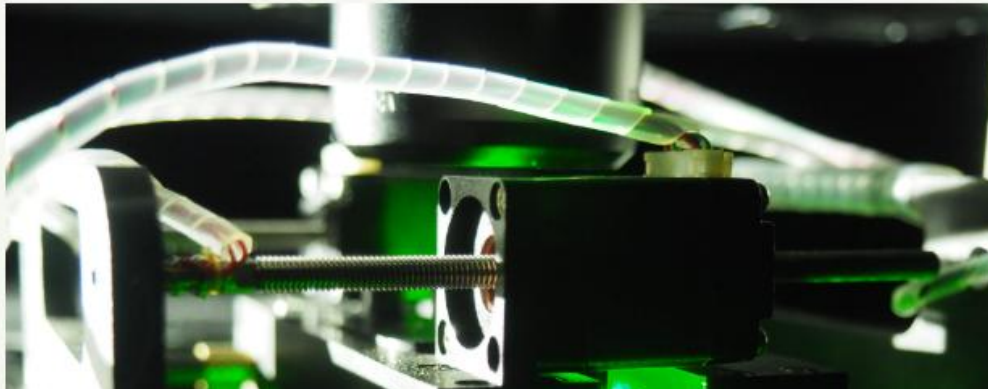
Prakash Kara lab, U.Minnesota

upcoming events 2025!

10. – . 11.12. 2025	**Mechanical characterization of biological samples using correlative methods** - registration open
September 2026	**Advanced Course on Preclinical Imaging** - registration in 2025

Mechanical characterization of biological samples using correlative methods

Time: 09.12.2025 – 10.12.2025
Location: Building Da I. Institute of Physiology, CAS, Vídeňská 108
142 00 Praha 4, Czech Republic



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