BioPhotonics

1. Confocal Microscopy
2. Multiphoton Microscopy
3. Optical Neural Interface

Introduction – Confocal Microscopy

- Confocal microscopy is an optical imaging technique used to increase micrograph contrast and to reconstruct 3-D images by using a spatial pinhole to eliminate out-of-focus light (flare) in specimens that are thicker than the focal plane.

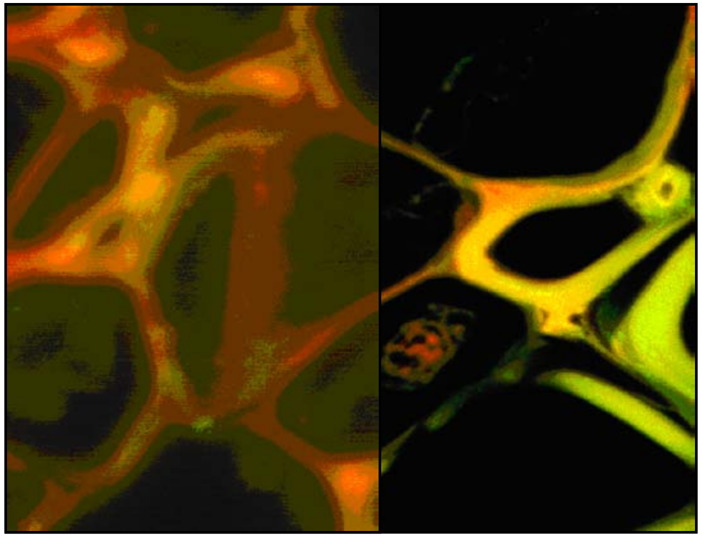
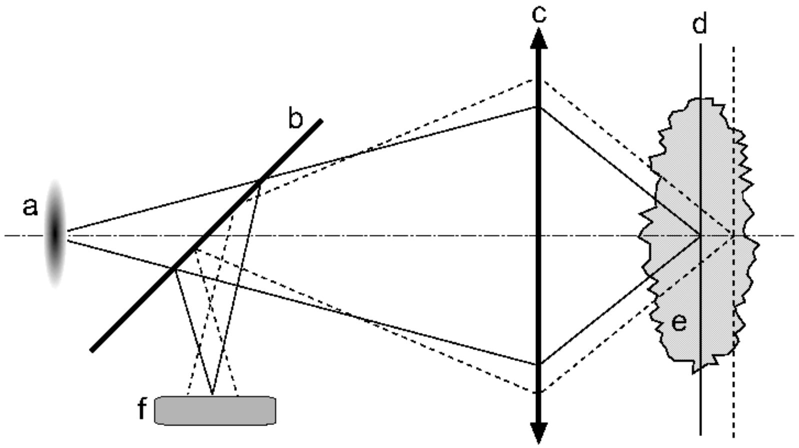


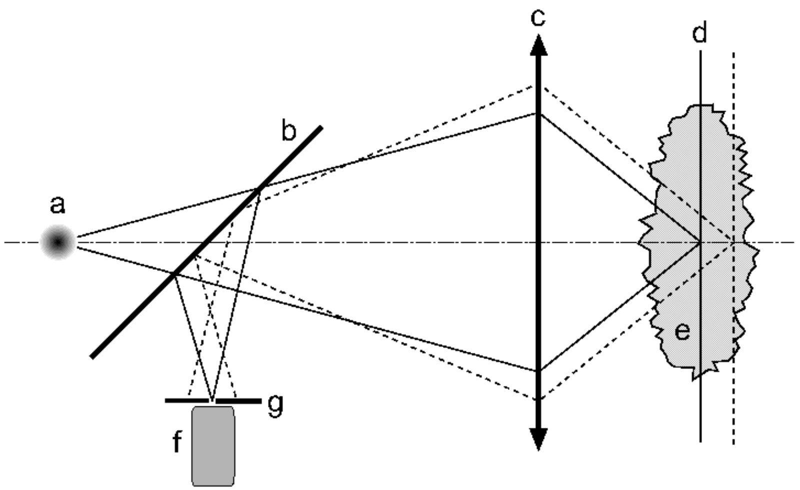
Photo from
H. Brismar,
Cell physics, KTH

Widefield **Confocal**

Wide-field vs. Confocal Microscopy



Wide−field Microscopy



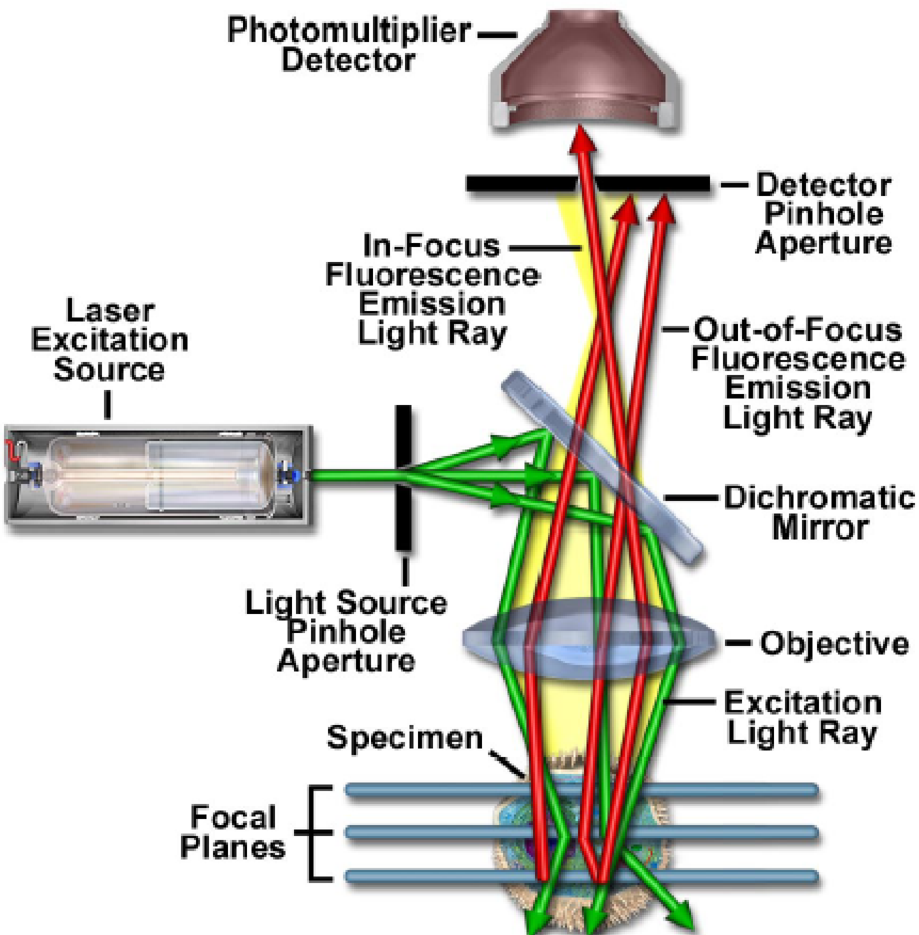
Confocal Microscopy

- Wide-field Microscopy (Conventional Microscopy)
  - Uses light source with broad spectrum
  - The entire specimen is illuminated and observed.
- Confocal Microscopy
  - Uses light source with single wavelength (laser)
  - Only one object point is illuminated and observed at a time.
  - Scanning is required to build up an image of the entire field.

Drawing by

J.P. Robinson. @ PUCL

Confocal Laser Scanning Microscope

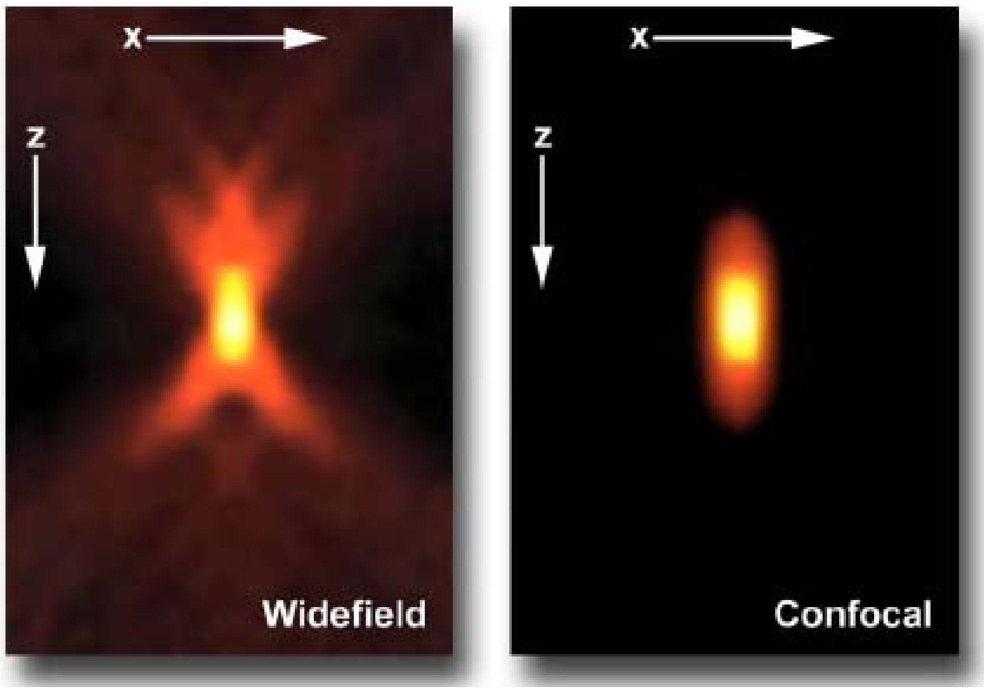


Optical pathway of Confocal Microscopy

Nathan et al., "Laser Scanning Confocal Microscopy"

- Coherent light emitted by the laser system passes through
  1) Light Source Pinhole Aperture
  2) Detector Pinhole Aperture
  – *Confocal*
- Out-of-Focus Fluorescence Emission Light is not detected by the Photomultiplier tube (PMT).
  – *High Resolution*
- Confocal microscopy can produce in-focus images of thick specimens.
  – *Optical Sectioning*

Wide-field vs. Confocal Microscopy



Comparison of axial (x-z) point spread functions.

Photo from Nathan

Lateral & Axial extent of point spread function is reduced by about 30% in confocal microscope. -> *Resolution improved!*

$$r_{xy,\text{wide-field}} \approx 0.6\,\lambda / NA$$

$$r_{z,\text{wide-field}} \approx 2\,\lambda \bullet \eta / NA^2$$

Wide-field microscopy

$$r_{xy,\text{confocal}} \approx 0.4\,\lambda / NA$$

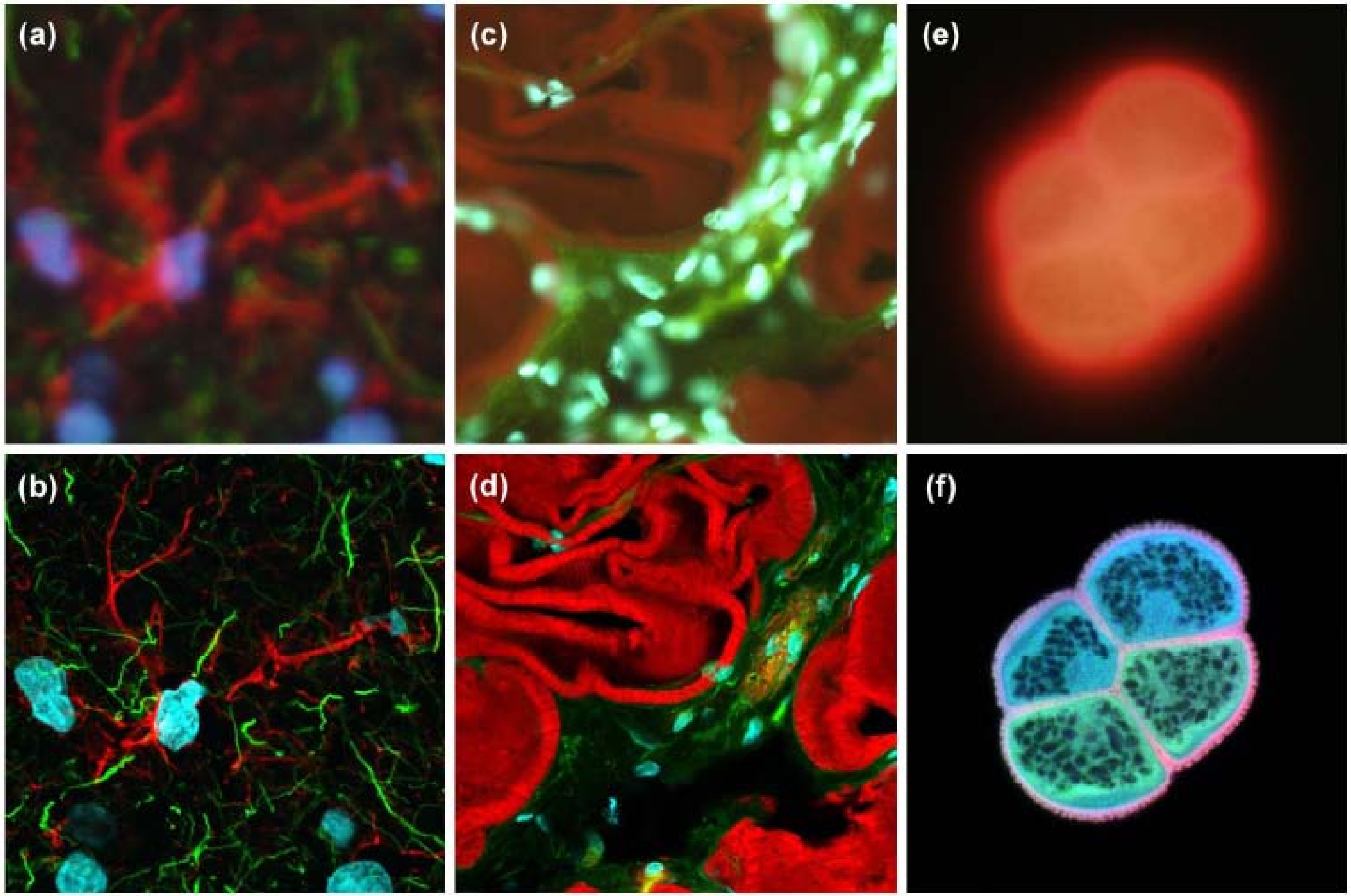
$$r_{z,\text{confocal}} \approx 1.4\,\lambda \bullet \eta / NA^2$$

Confocal microscopy

Wide-field vs. Confocal Microscopy

Wide-field Microscopy

Confocal Microscopy



(a), (b) – Mouse brain hippocampus thick section
(c), (d) – Rat smooth muscle thick section
(e), (f) – Sunflower pollen grain

Nathan et al., "Laser Scanning Confocal Microscopy"

Disadvantages of Confocal Microscopy

- Limited number of excitation wavelengths are available with common lasers, which occur over very narrow bands and are expensive to produce in the ultraviolet region.

- High-intensity laser irradiation to living cells and tissues could be harmful.

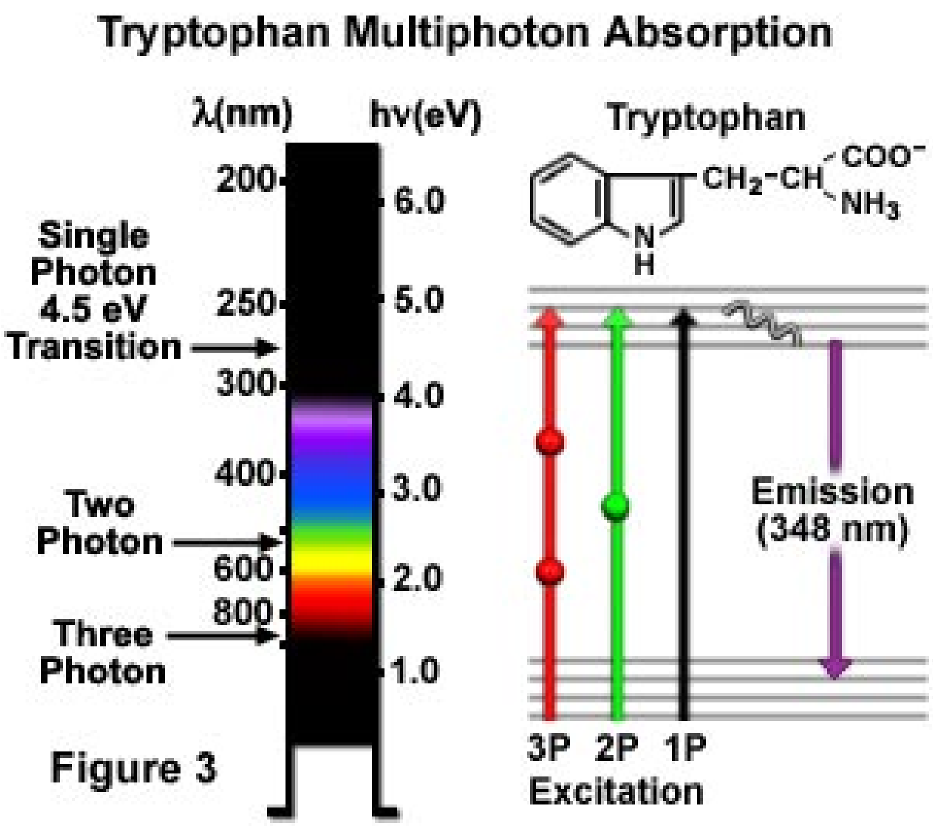
- The high cost of purchasing and operating multi-user confocal microscope systems can range up to an order of magnitude higher than comparable wide-field microscope.

2. Two-photon Microscopy

Introduction – Two-photon Microscopy

- Two-photon excitation employs a concept first described by Maria Göppert-Mayer in her 1931 doctoral dissertation.
- Two-photon Microscopy has been patented by Winfried Denk, James Strickler and Watt Webb at Cornell University.
- Two-photon excitation microscopy (multi-photon excitation microscopy) is a fluorescence imaging technique that allows imaging living tissue up to a depth of one millimeter.
- Two-photon microscopy may be a viable alternative to confocal microscopy due to its deeper tissue penetration and reduced photo-toxicity.

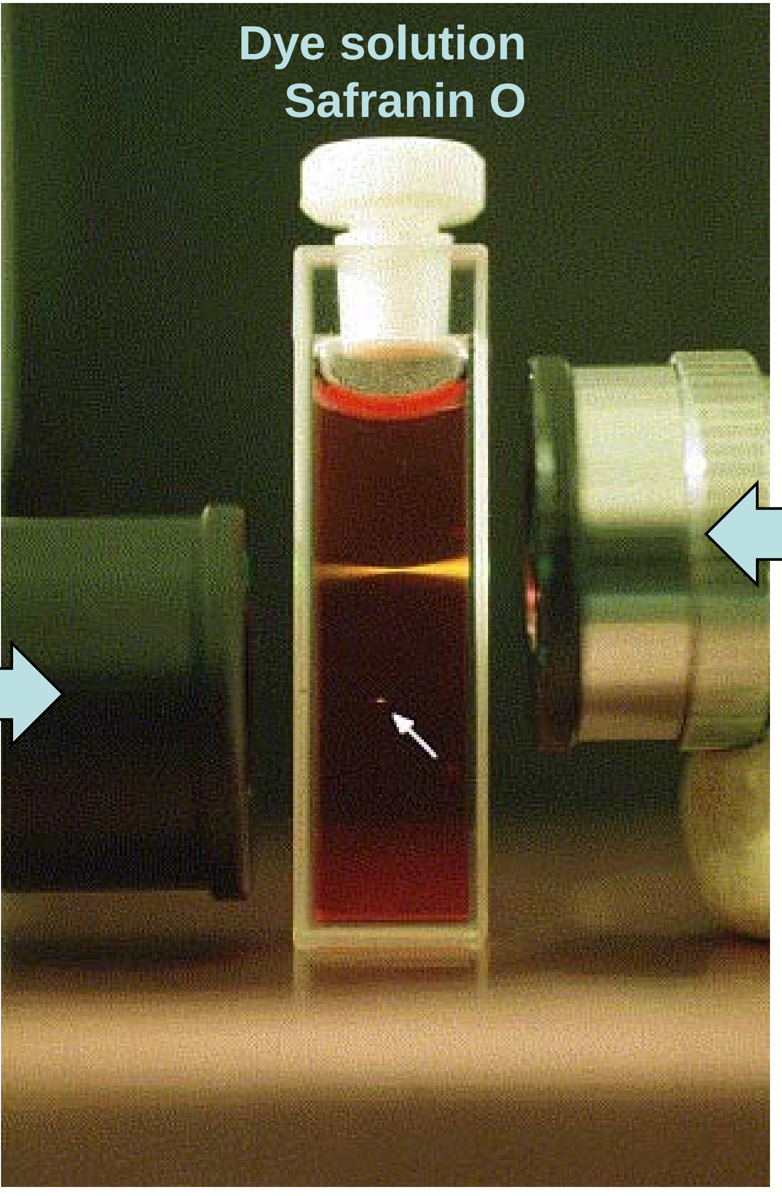
Two-photon Microscopy Principles



Jablonski diagram, illustrating multi-photon absorption.

- Two-photons (or multi-photons) of low energy can promote the molecule to an excited state, which then proceeds along the normal fluorescence-emission pathway.
- The probability of absorption of two-photons is extremely low.
- Therefore a high flux of excitation photons is required. (femtosecond laser)

Two-photon vs. One-photon Excitation Volume

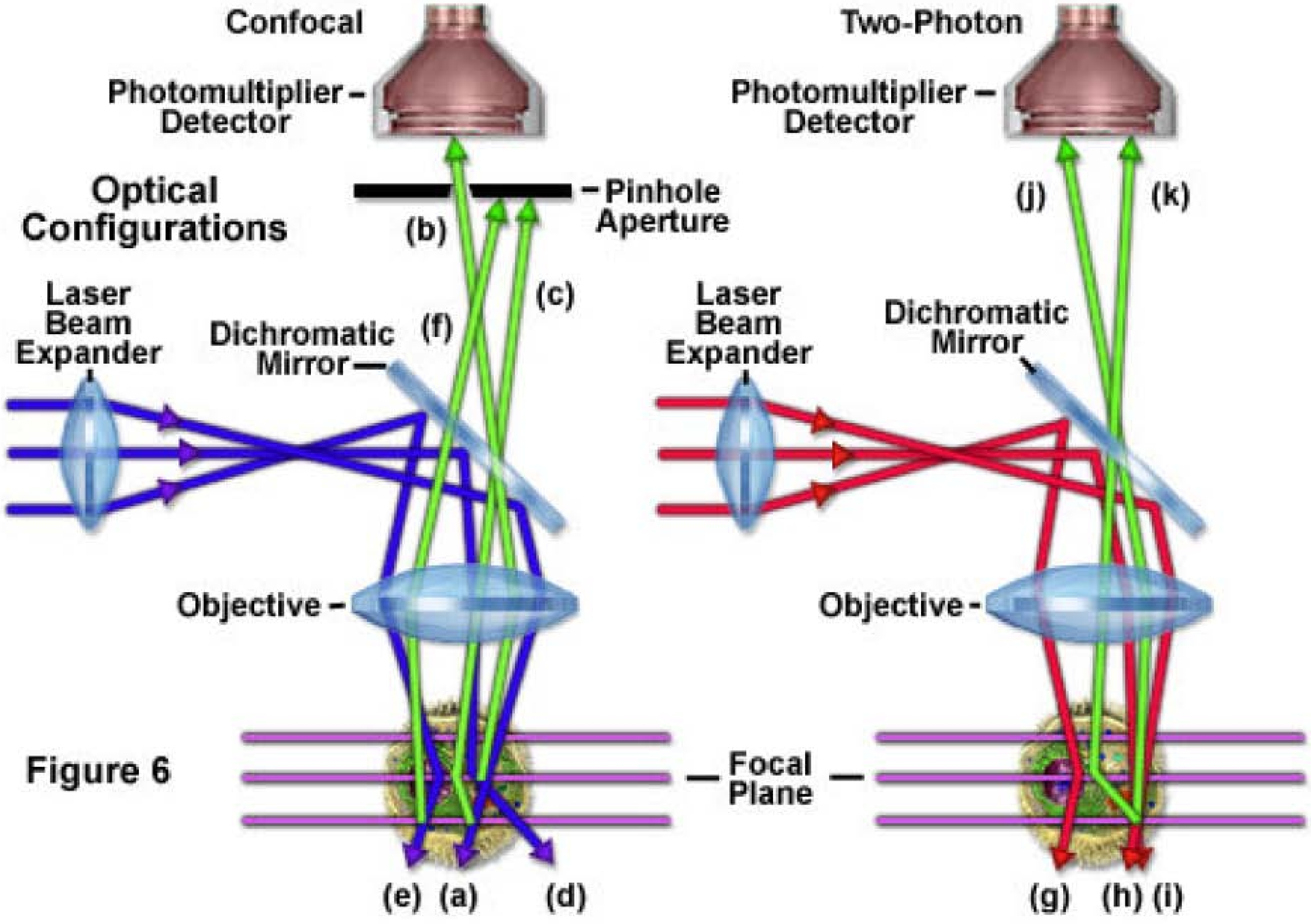


Two-photon Excitation (1046 nm, IR Laser)

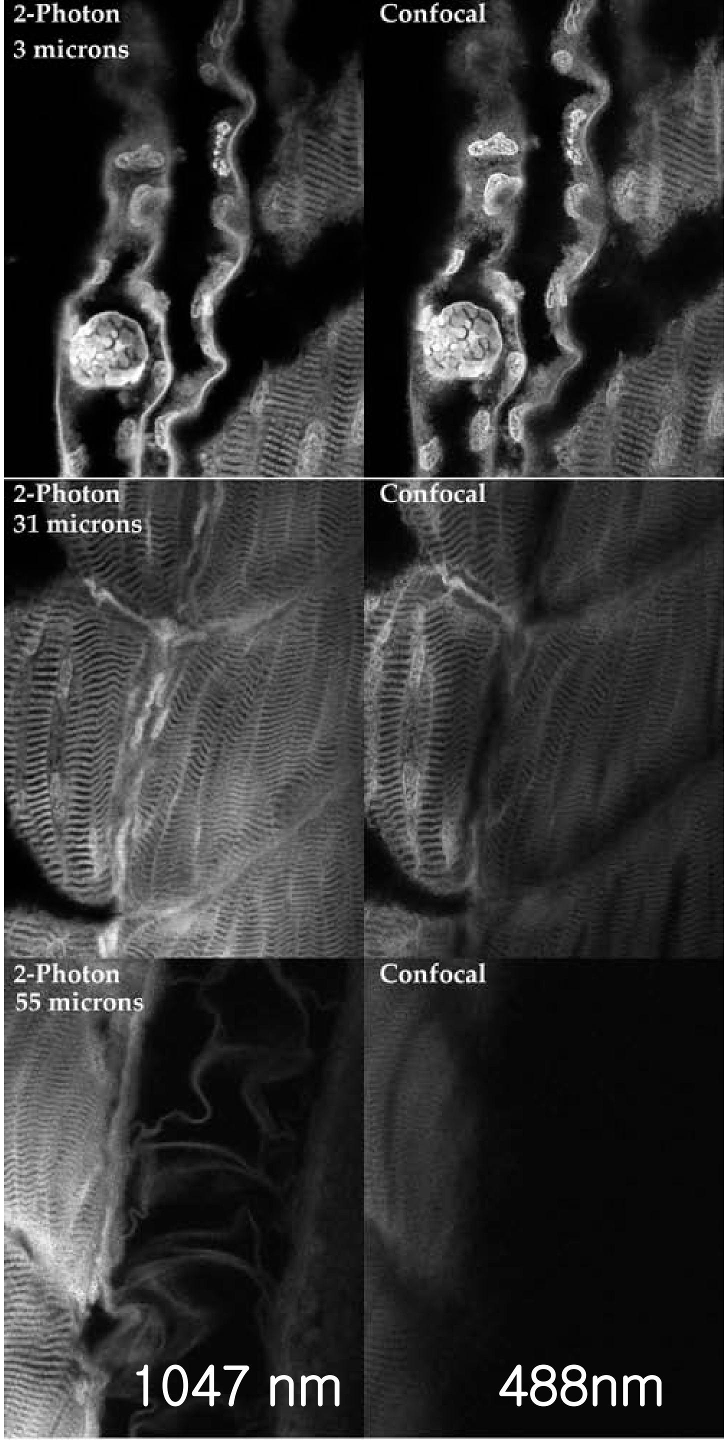
One-photon Excitation (543 nm, Green laser)

Photo from Brad Amos

Confocal vs. Two-photon



No pinhole aperture is required in two-photon microscopy !



Confocal vs. Two-photon Microscopy

- Sequence of images showing a comparison between confocal imaging (488nm excitation) and two-photon imaging (1047nm excitation).
- The sample is a zebra fish that is heavily stained with safranin (the sample was prepared by B. Amos).
- Two-photon imaging is able to give much better images deep into the specimen.

Photo from: Multi-Photon Excitation Fluorescence Microscope Coordinator, Madison, WI

Advantages of Two-photon Microscopy

- Fluorescence excitation is confined to a femto-liter volume – less photo-bleaching.

- Excitation wavelengths are not absorbed by fluorophore above plane of focus.

- Longer excitation wavelengths penetrate more deeply into biological tissue.

- Inherent optical sectioning.

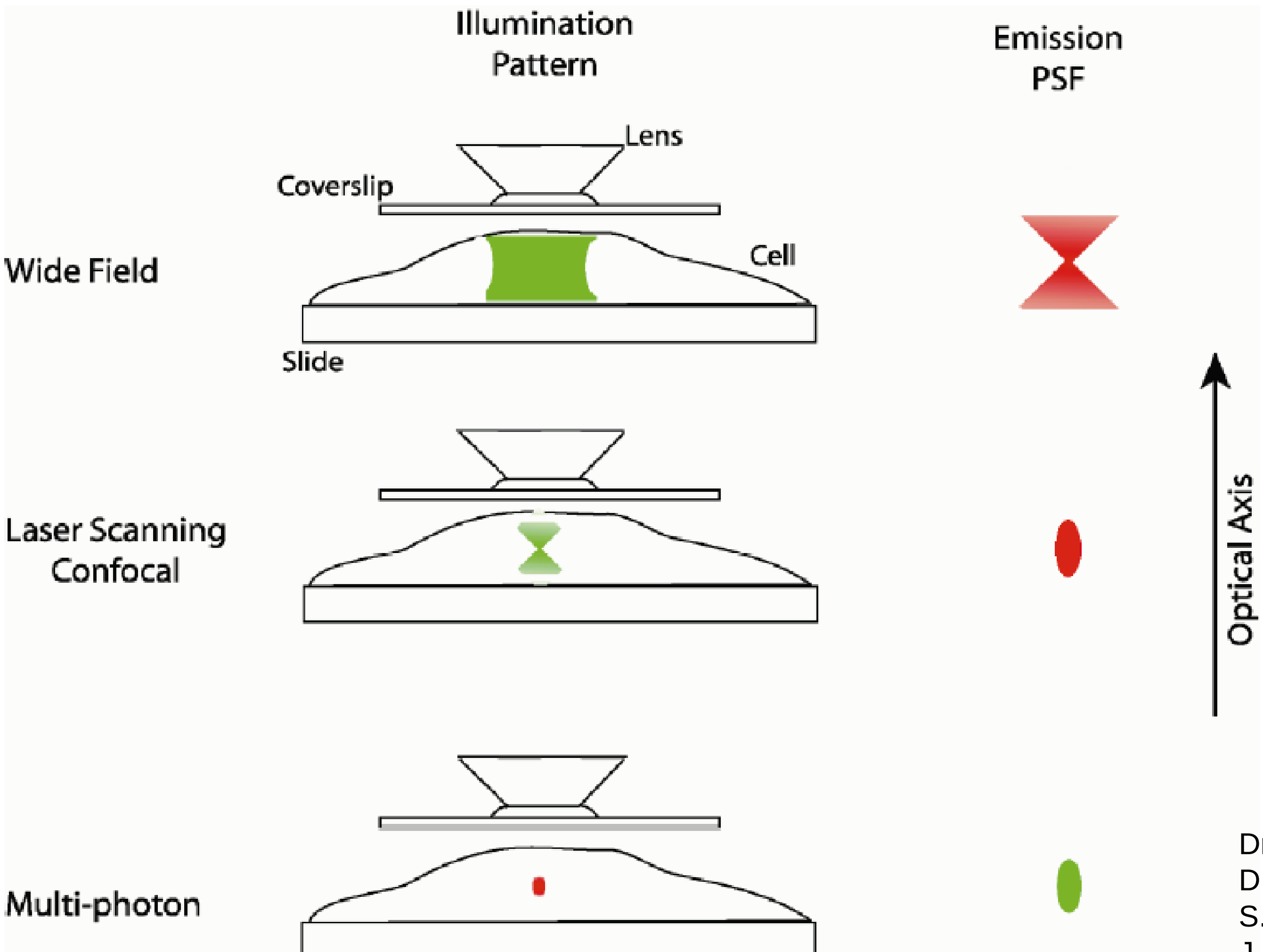
Limitations of Two-photon Microscopy

- Slightly lower resolution with a given fluorophore when compared to confocal imaging. This loss in resolution can be eliminated by the use of a confocal aperture at the expense of a loss in signal. (two-photon + confocal !!)

- Thermal damage can occur in a specimen if it contains chromophores that absorb the excitation wavelengths, such as the pigment melanin.

- Only works with fluorescence imaging.

Summary



Drawing by P. D. Andrews, I. S. Harper and J. R. Swedlow

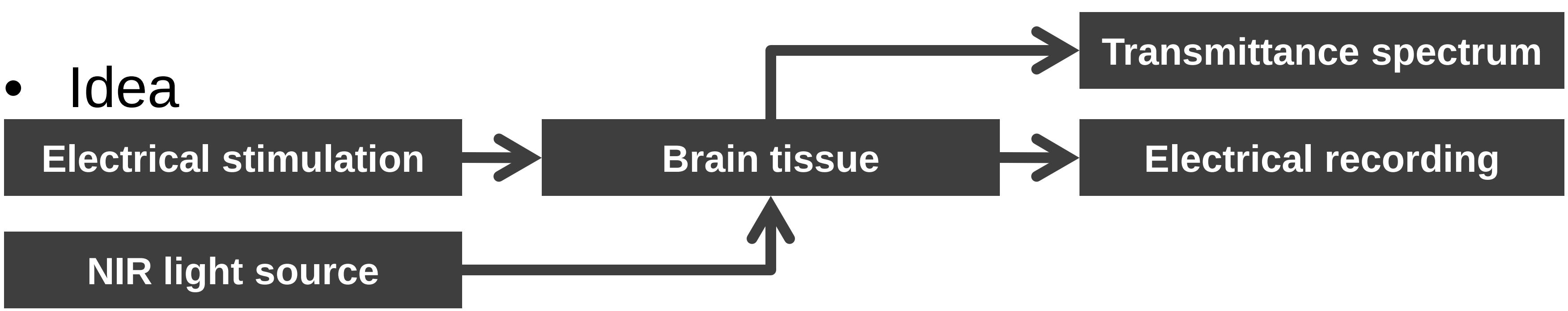
Optical Neural Interface

Neural Signal Detection using NIR Spectrum

- Motivation

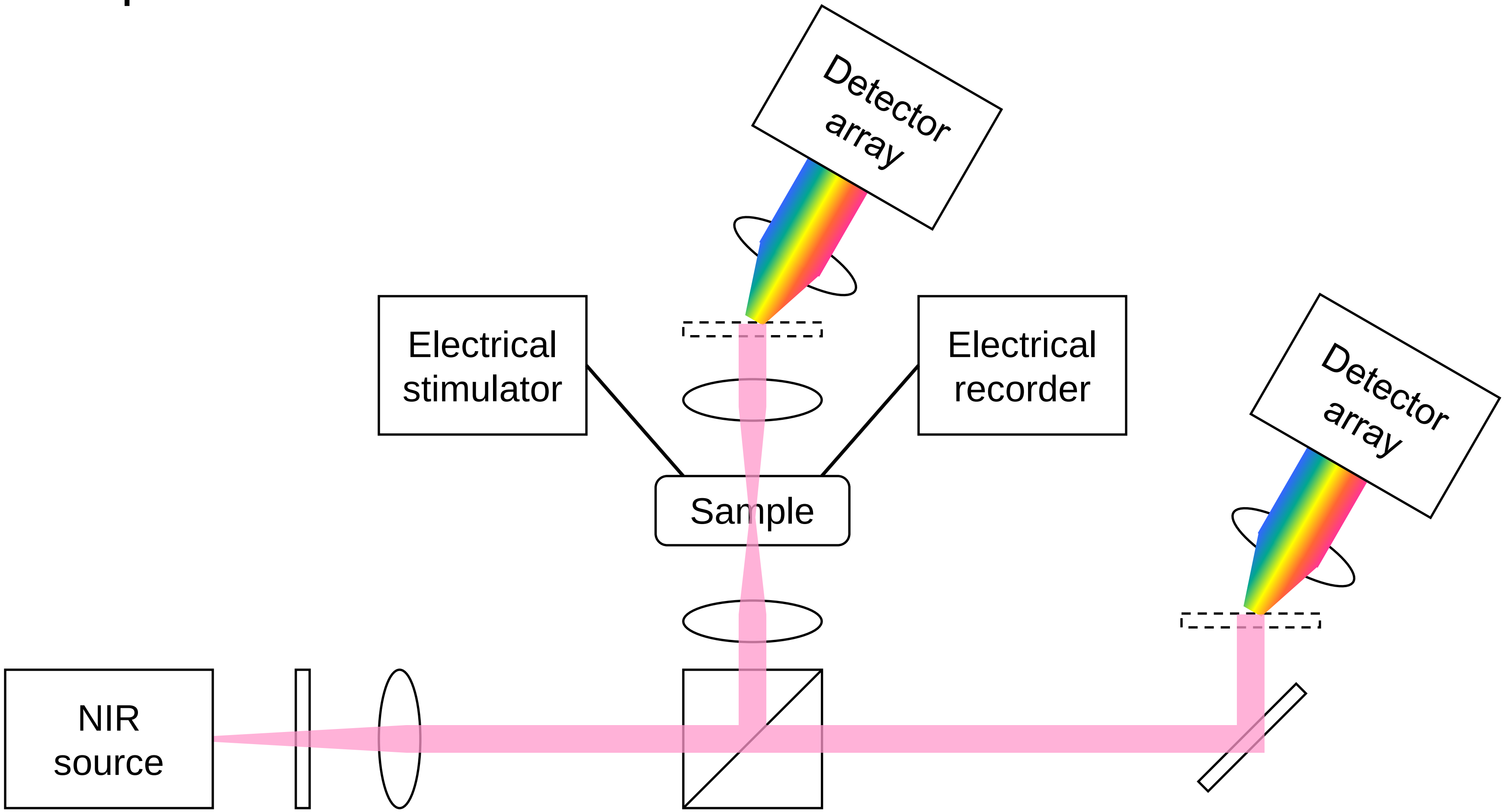
	Contact free	Label free	Whole field imaging	Brain tissue
Electrode		V		V
Voltage sensitive dye	V		V	V
Dark field microscope	V	V		
OCT	V	V		
New method needed	**V**	**V**	**V**	**V**

- Idea



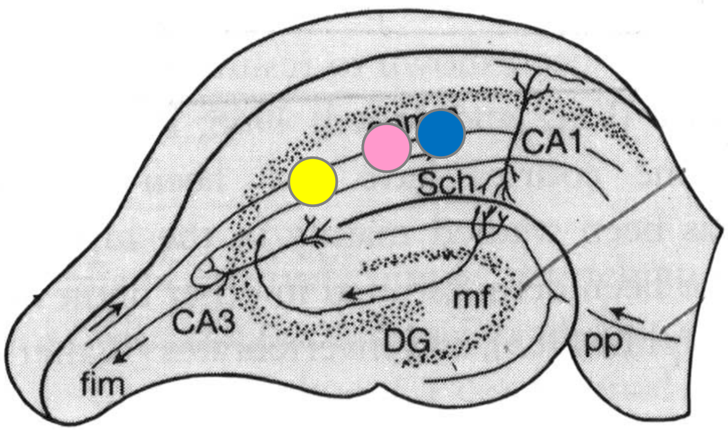
Neural Signal Detection using NIR Spectrum

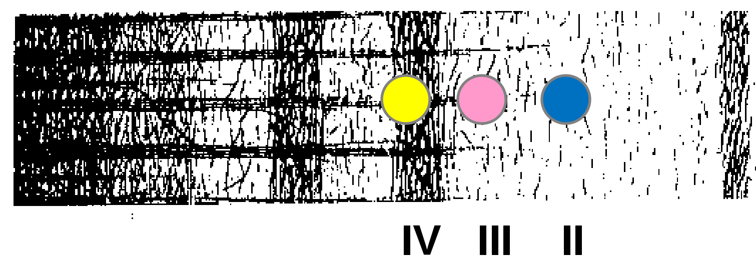
- Instrumentation: High-speed NIR Transmission Spectrometer

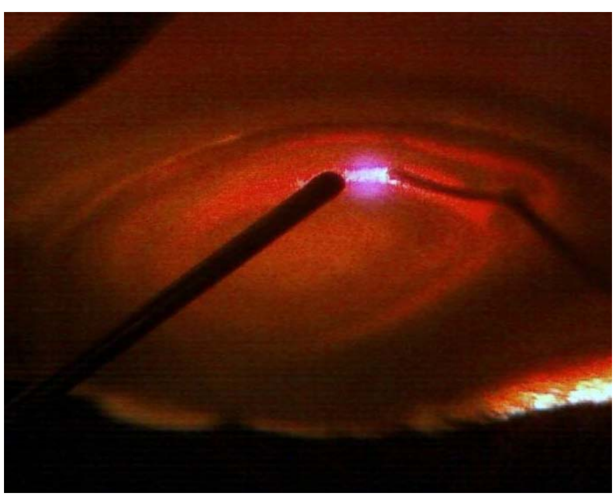


Neural Signal Detection using NIR Spectrum

- Material: Rat Brain Slices (Hippocampal Slice & Cortical Slice)

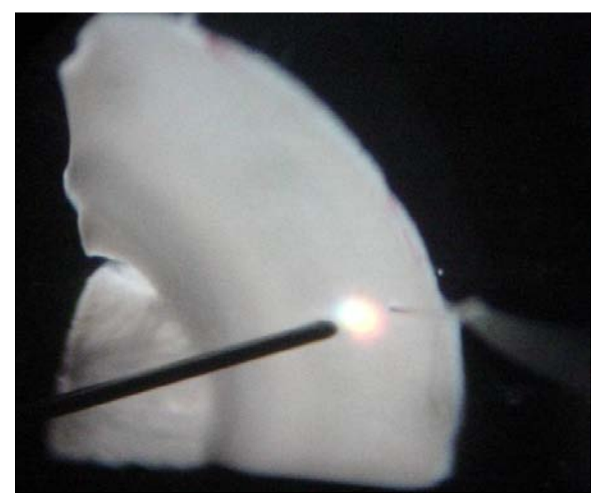






- **Electrical stimulation**
- **Optical recording**
- **Electrical recording**

1 mm



Neural Signal Detection using NIR Spectrum

- Preliminary Results