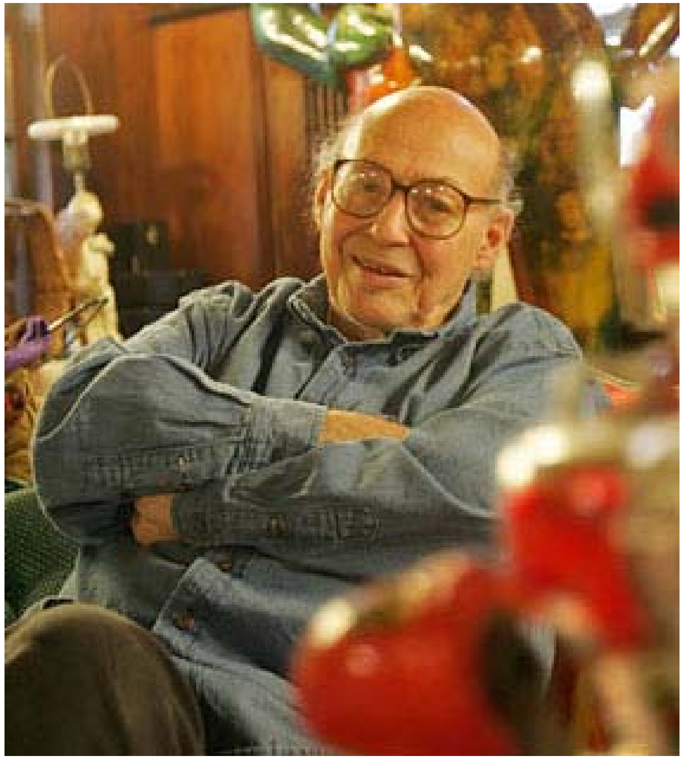
BioPhotonics

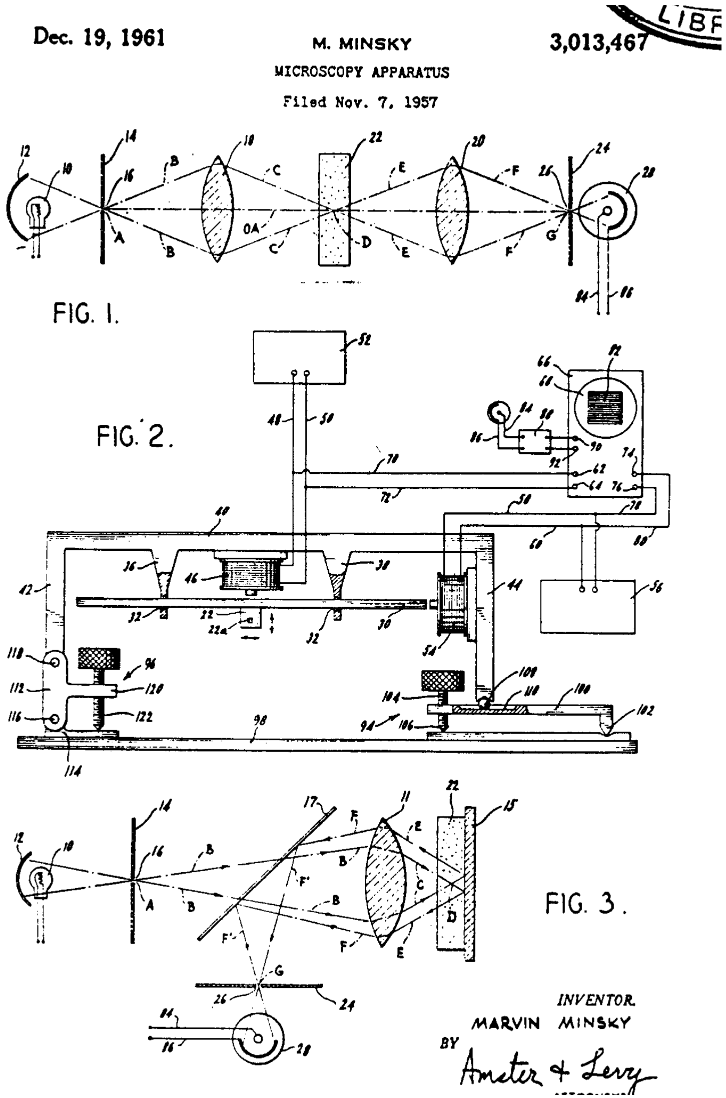
1. Confocal Microscopy
2. Multiphoton Microscopy
3. OCT
4. Optical Neural Interface

Introduction – Confocal Microscopy

- In 1957, the basic concept was developed by Marvin Minsky. (patented in 1961)



Marvin Minsky (MIT Media Lab)



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.

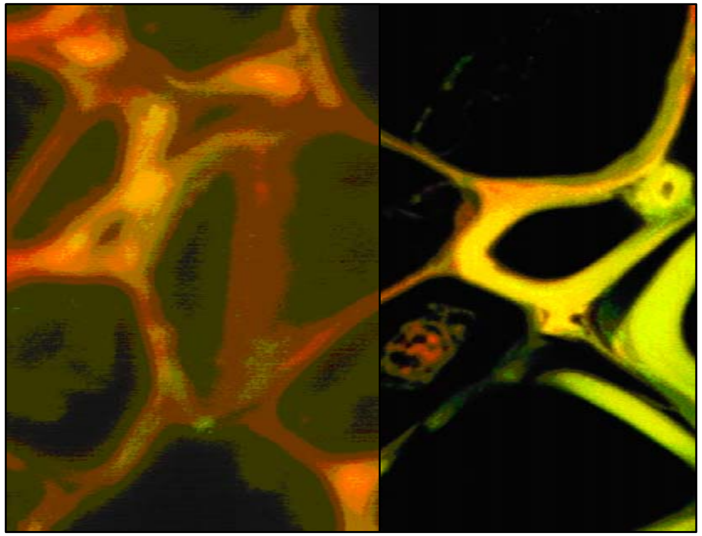


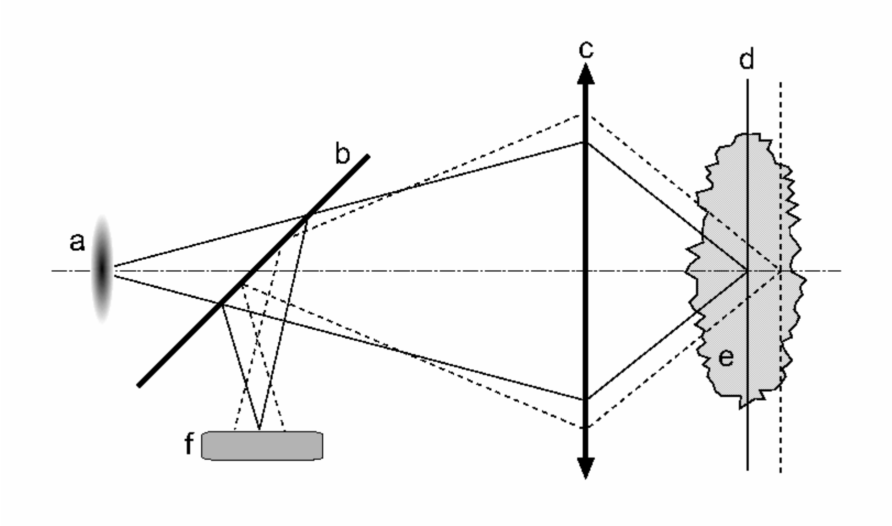
Photo from

H. Brismar,

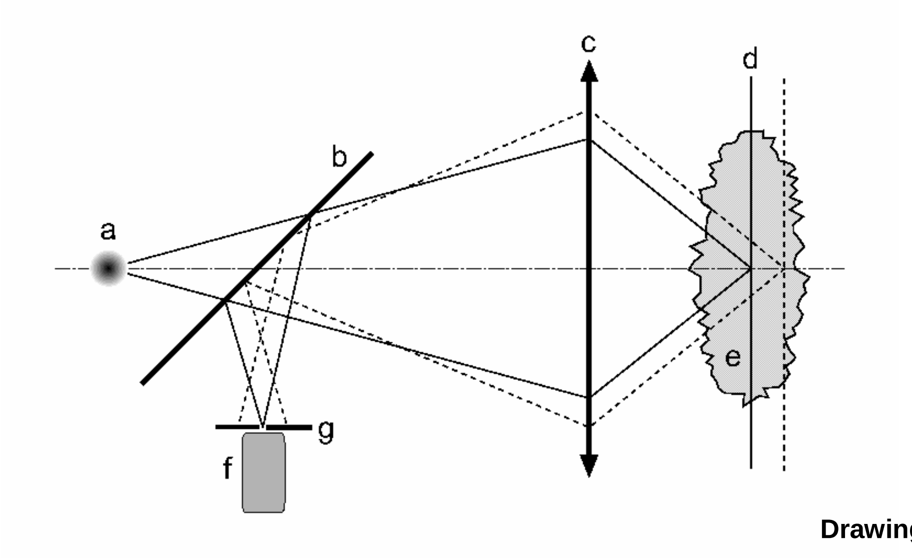
Cell physics, KTH

Widefield **Confocal**

Wide-field vs. Confocal Microscopy



Wide-field Microscopy



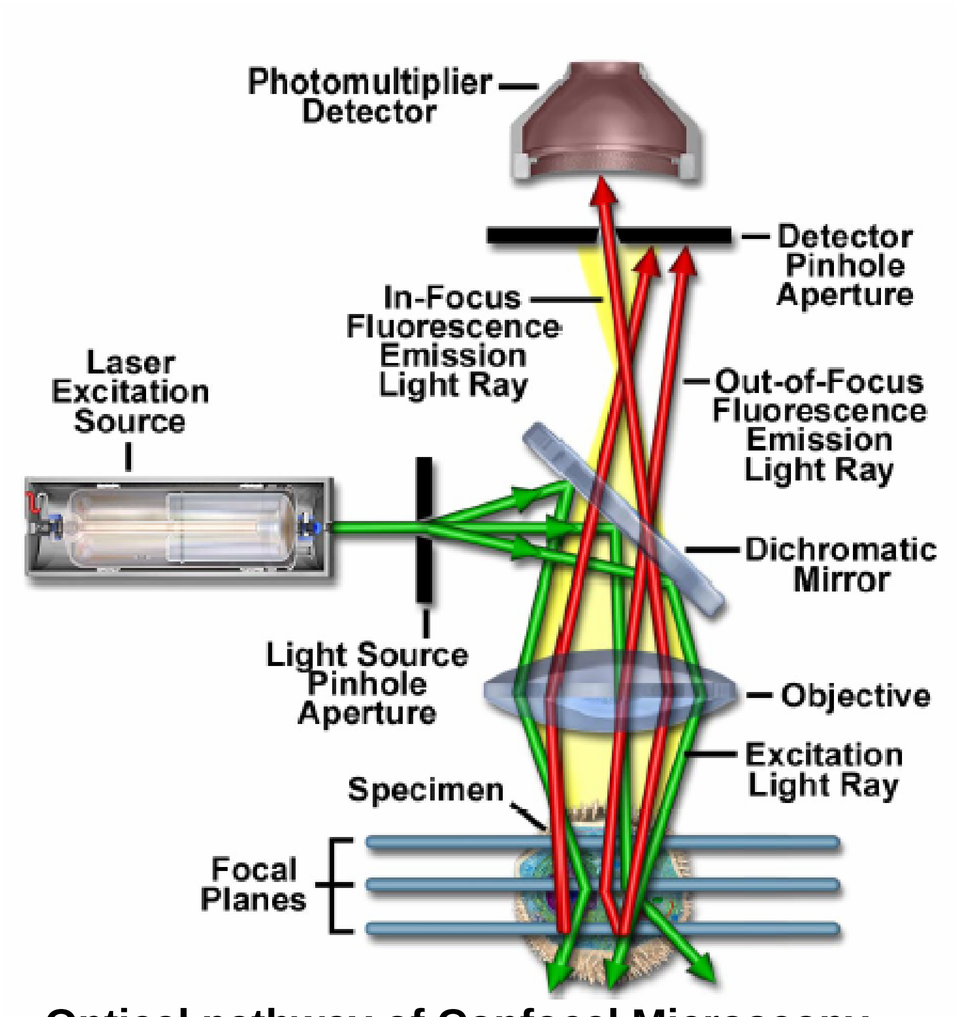
Confocal Microscopy

Drawing by

J.P. Robinson. @ PUCL

- Wide-field Microscopy (Conventional Microscopy)
  - The entire specimen is illuminated and observed.
- Confocal Microscopy
  - Only one object point is illuminated and observed at a time.
  - Scanning is required to build up an image of the entire field.

Confocal Laser Scanning Microscope

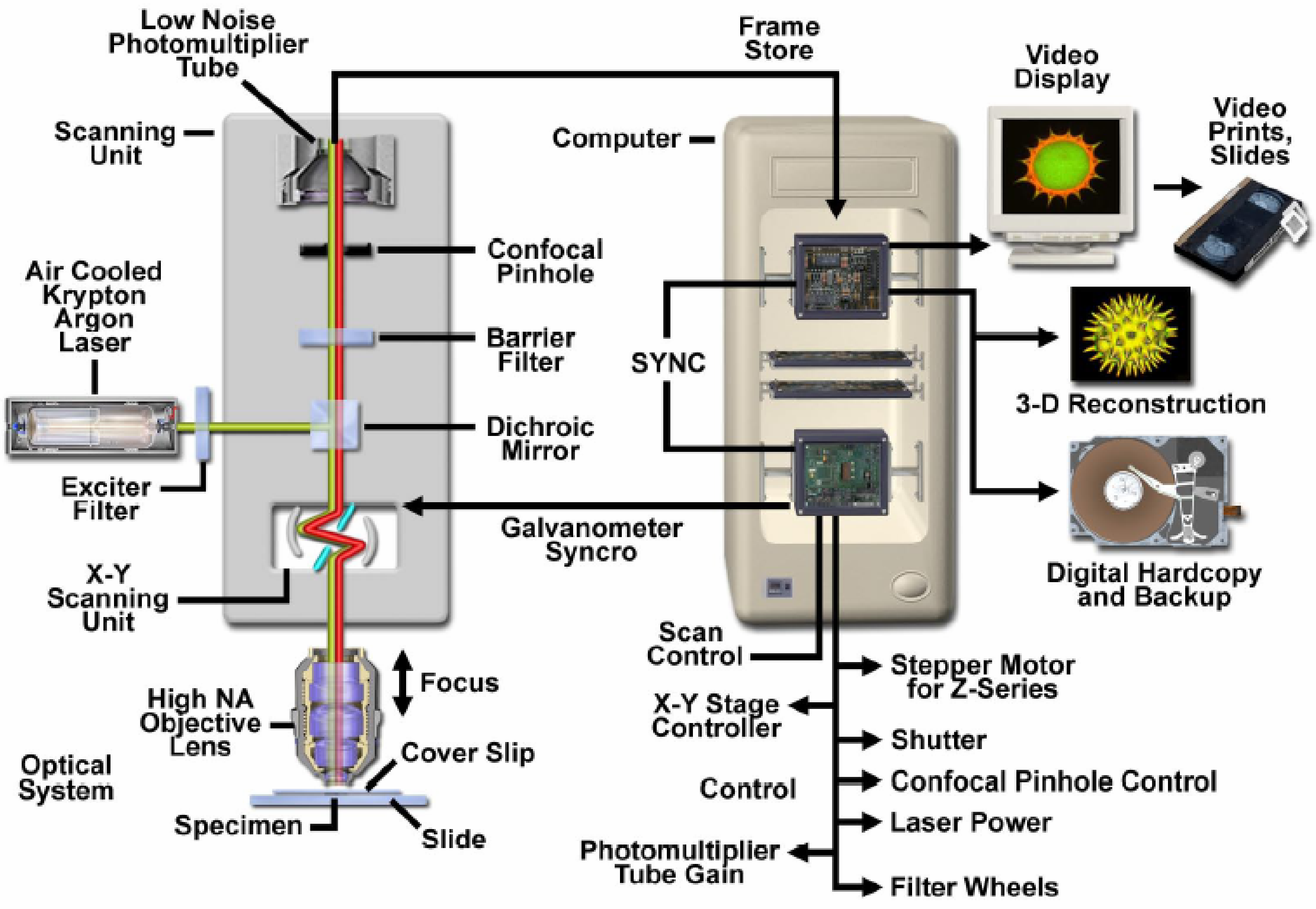


Optical pathway of Confocal Microscopy

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*

Confocal Laser Scanning Microscope System



Nathan et al., “Laser Scanning Confocal Microscopy”

Wide-field vs. Confocal Microscopy

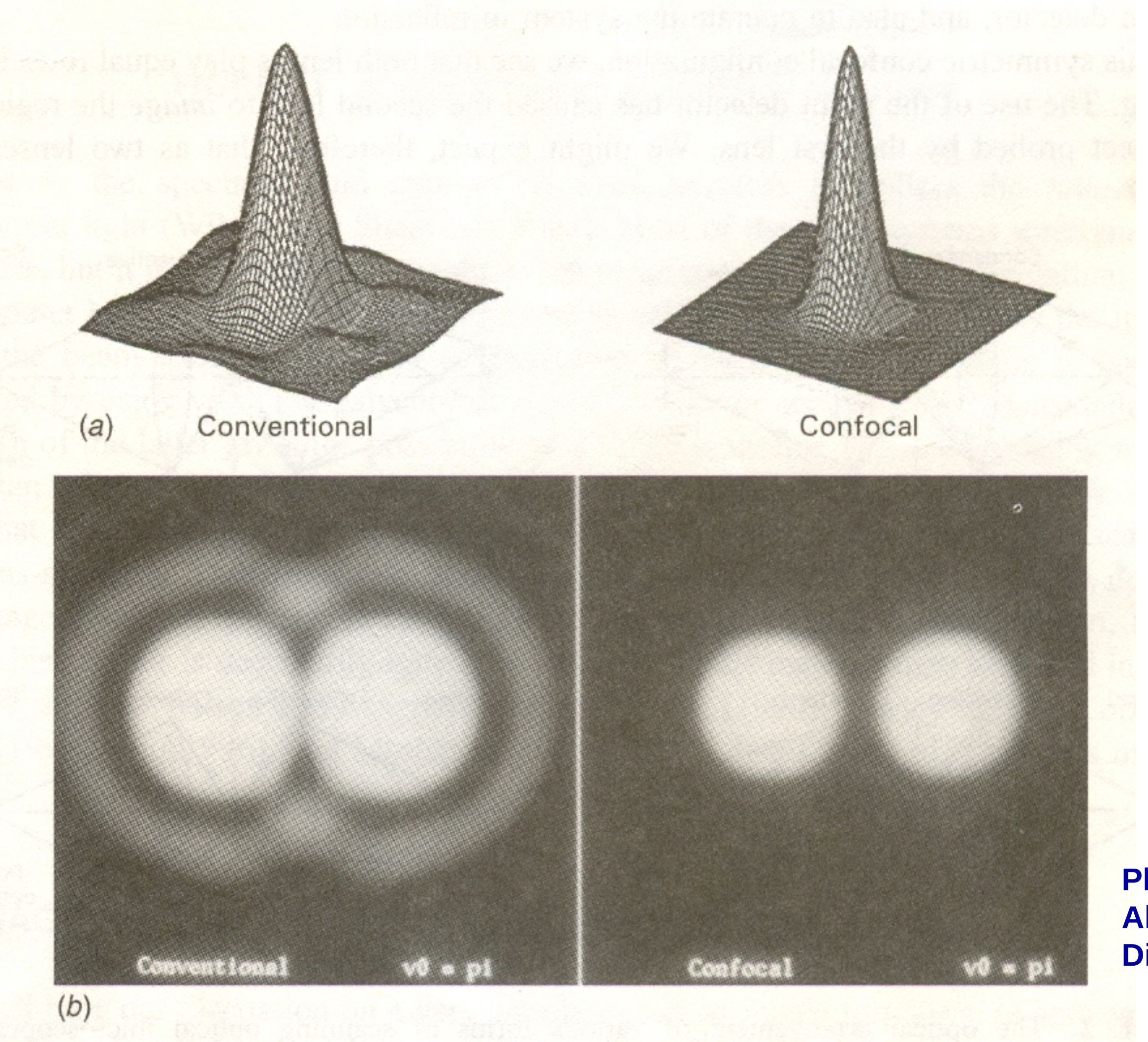
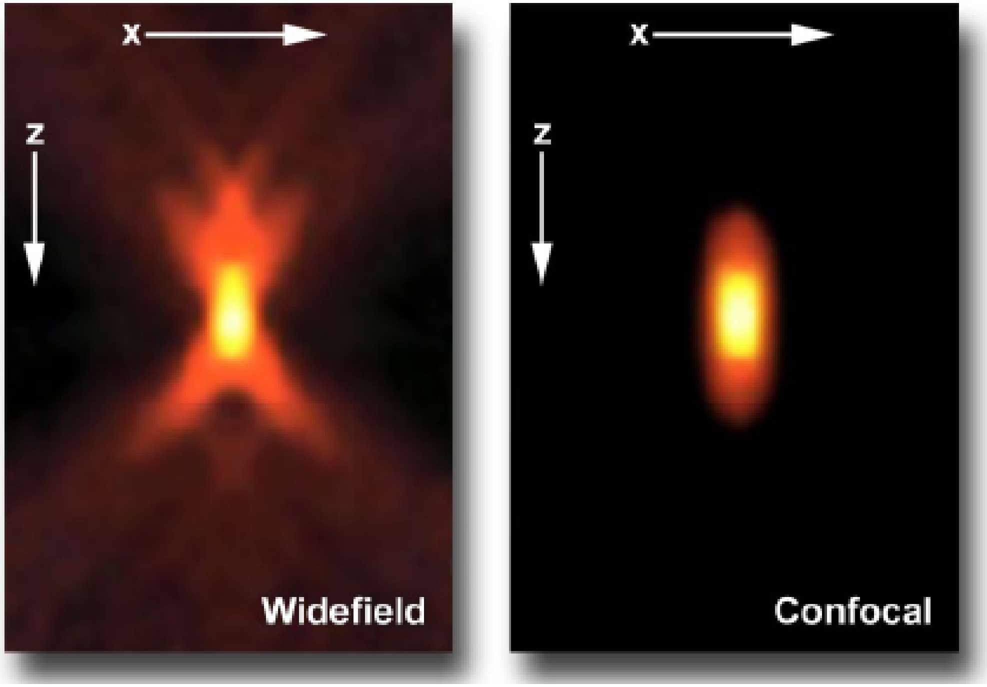


Photo from Alberto Diaspro

Comparison of (a) Point spread function, (b) two-point objects image
Improvement of lateral resolution (x-y) is apparent !

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

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

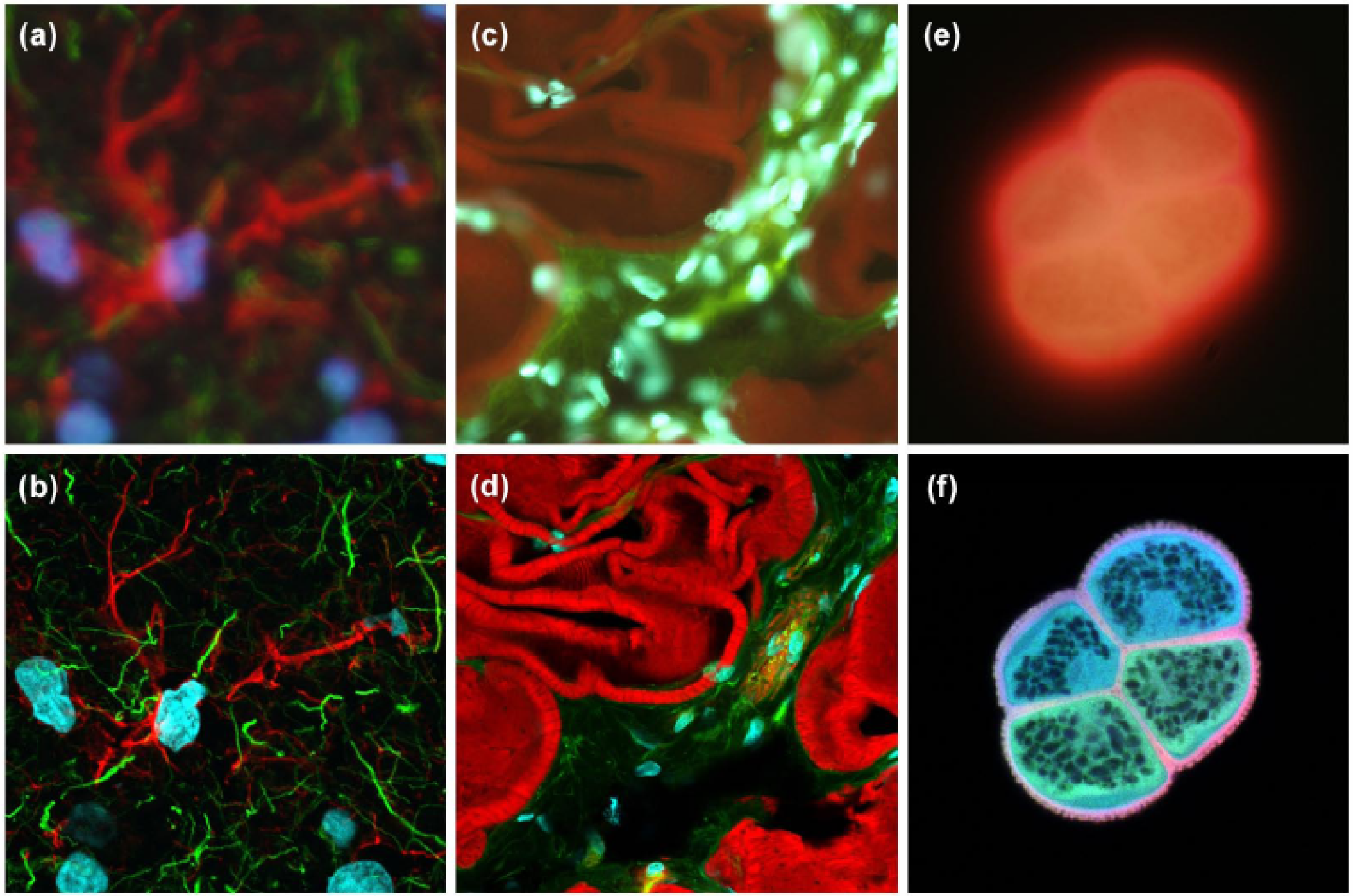
$$r_{z,\text{confocal}} \approx 1.4\ \lambda \bullet \eta / NA^2$$

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"

Confocal Microscopy Optical Sections

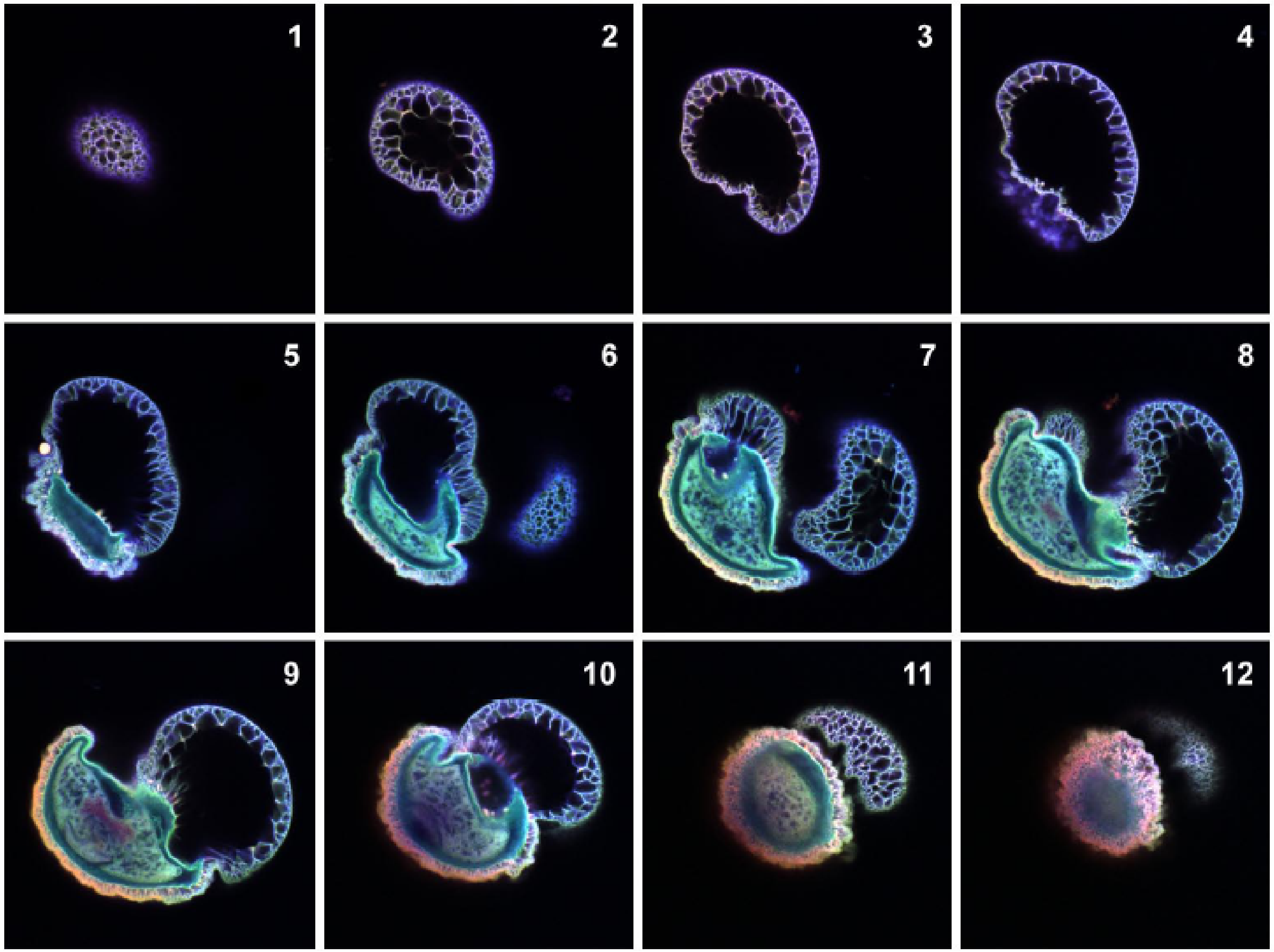


Photo from Nathan

Lodgepole pine pollen grain optical sections.
Each image in the sequence (1-12) represents the view obtained from steps of 3 micrometers.

Multi-dimensional View of Living Cells

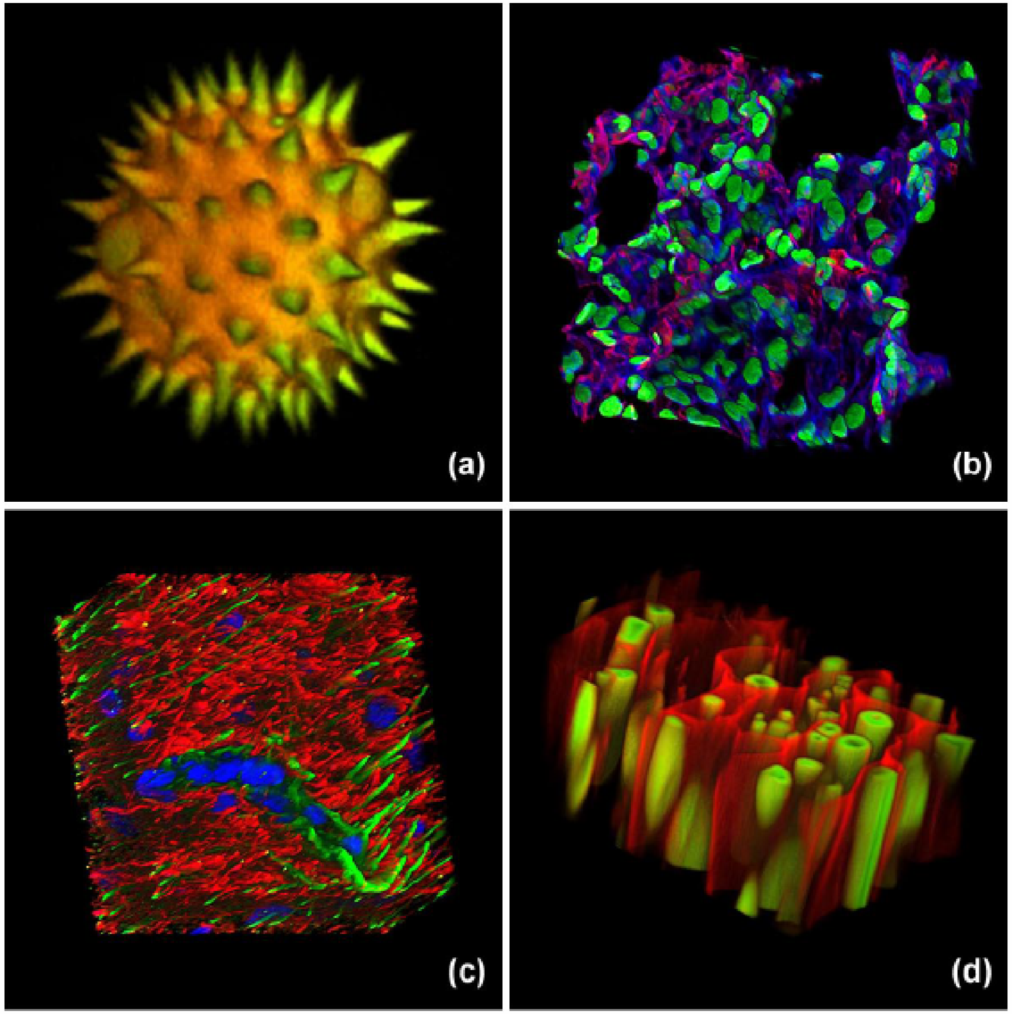


Photo from Nathan

3-D volume renders from confocal microscopy optical sections.
(a)Sunflower pollen grain, (b) Mouse lung tissue,
(c) Rat brain thick section, (d) Fern root.

Disadvantages of Confocal Microscopy

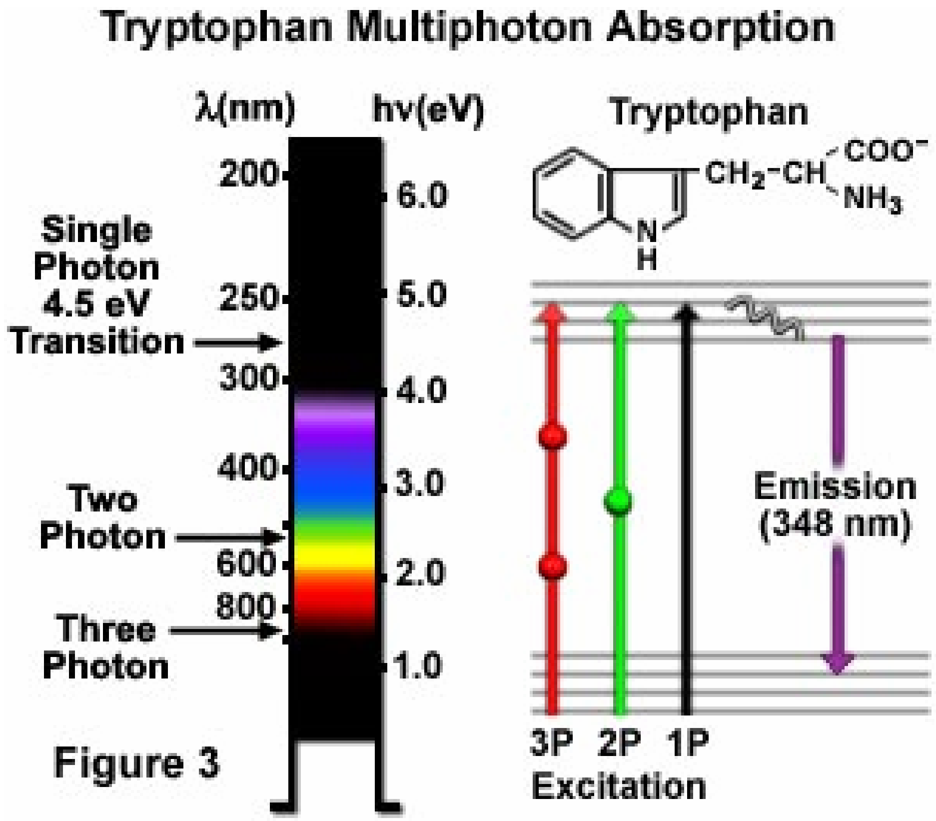
- Limited number of excitation wavelengths are available with common lasers, which occur over very narrow bands and are expensive to produce in the ultraviolet region.
- High-intensity laser irradiation to living cells and tissues could be harmful.
- The high cost of purchasing and operating multi-user 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 Microscopy Principles

- The number of photons absorbed per fluorophore per pulse :

$$n_a \approx \frac{p_0^2 \delta}{\tau_p f_p^2} \left(\frac{(NA)^2}{2\hbar c \lambda} \right)^2$$

τ_p : the pulse duration.

δ : the fluorophore's two-photon absorption at wavelength.

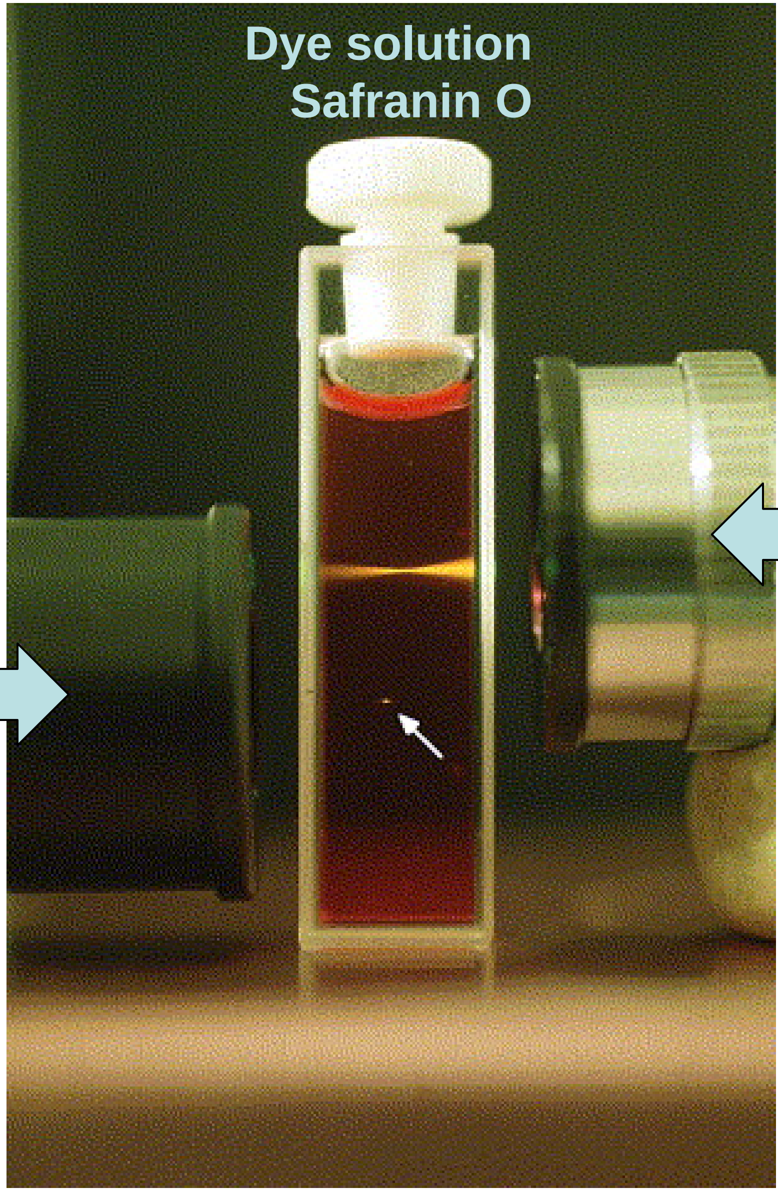
p_0 : the average laser intensity.

f_p : the laser's repetition rate.

NA: the numerical aperture of the focusing objective.

- Lasers typically used in two-photon microscope provide 100-fs pulses at about 100 MHz.

Two-photon vs. One-photon Excitation Volume

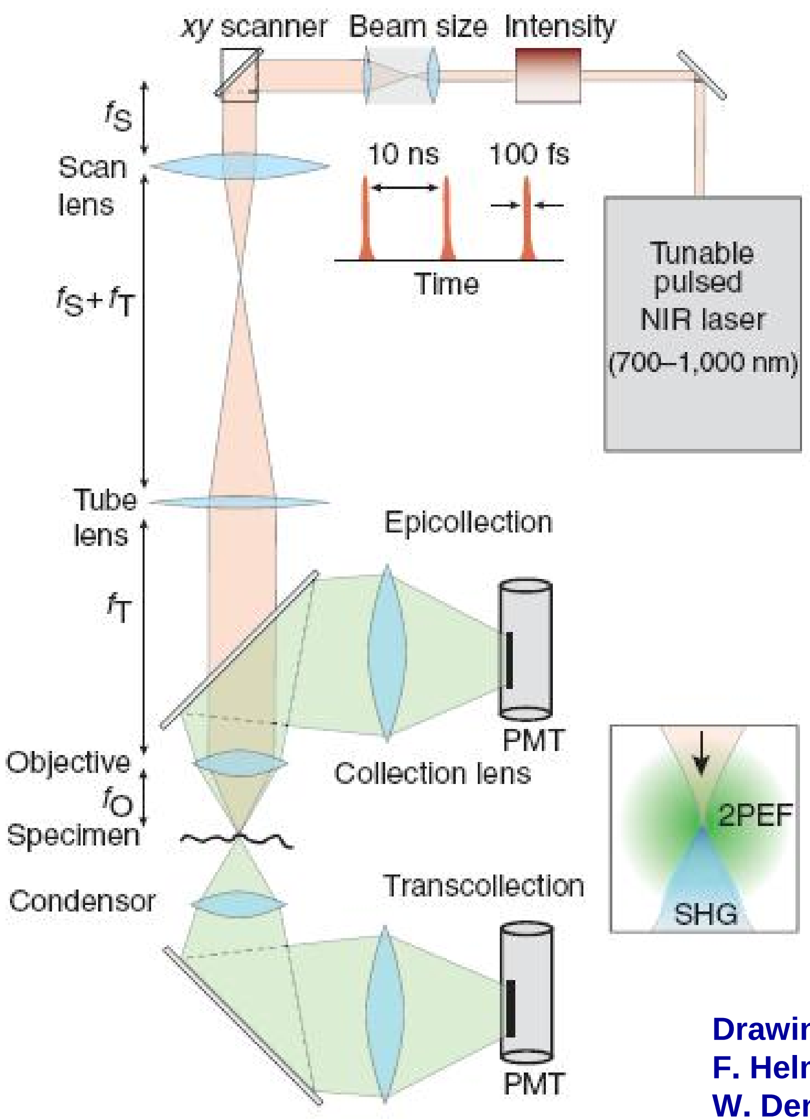


Two-photon Excitation (1046 nm, IR Laser)

One-photon Excitation (543 nm, Green laser)

Photo from Brad Amos

Two-photon Microscopy Configuration



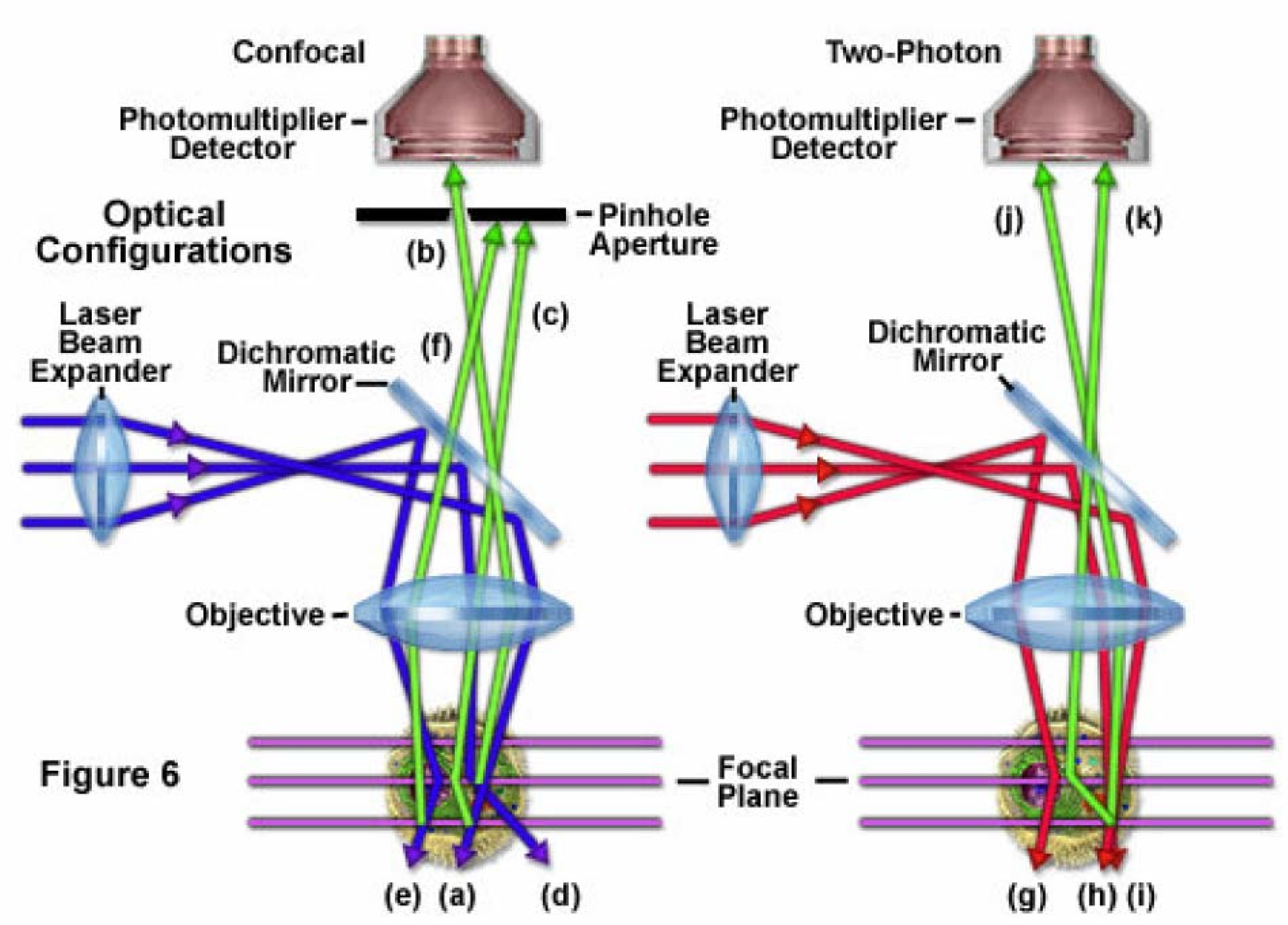
Drawing by F. Helmchn & W. Denk

- Generic two-photon laser-scanning microscope
  - NIR femto-second pulsed laser source

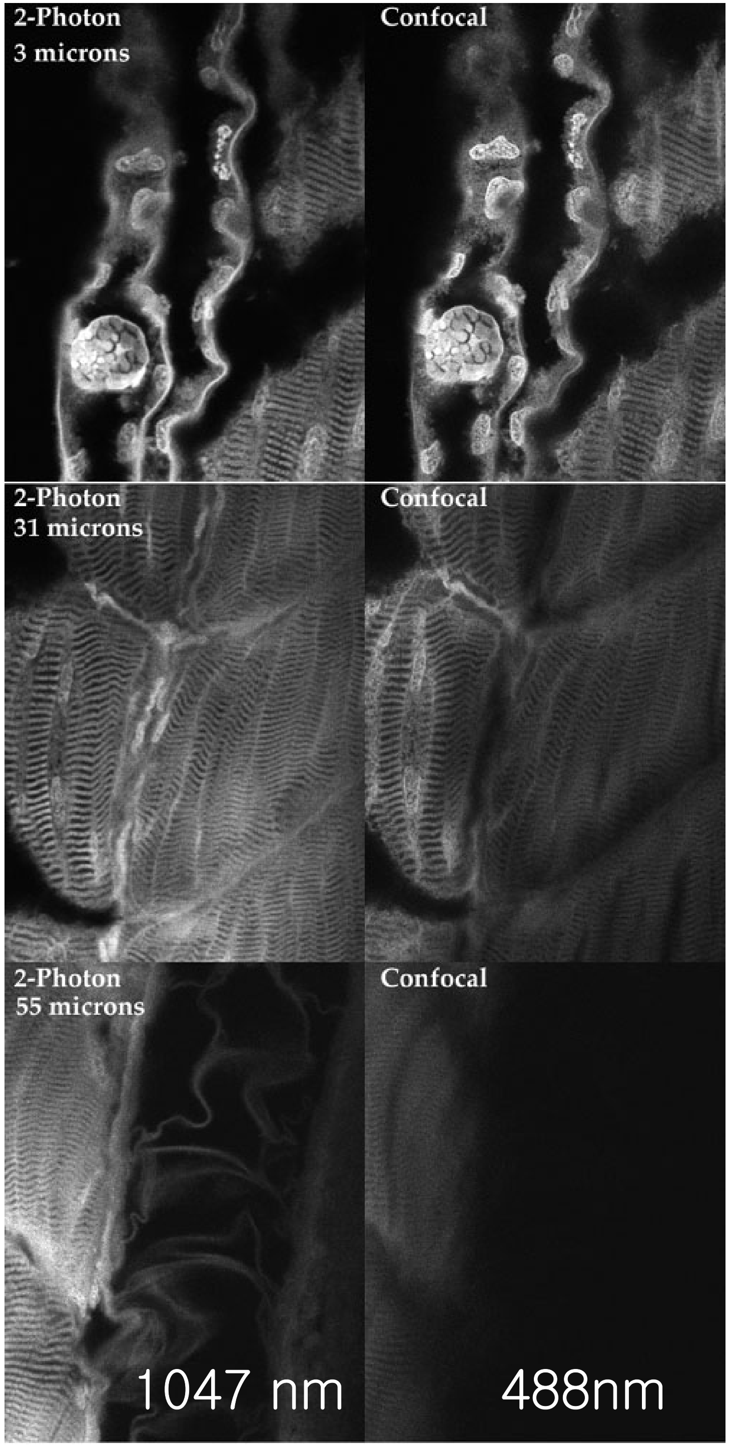
  (f_S – scan lens focal lengths,
  f_T – tube lens focal lengths,
  f_O – objective lens focal lengths)

- Two-photon excited fluorescence is isotropically emitted, can be collected in epi- and trans-collection mode by photomultiplier tubes (PMTs).

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.

Confocal vs. Two-photon

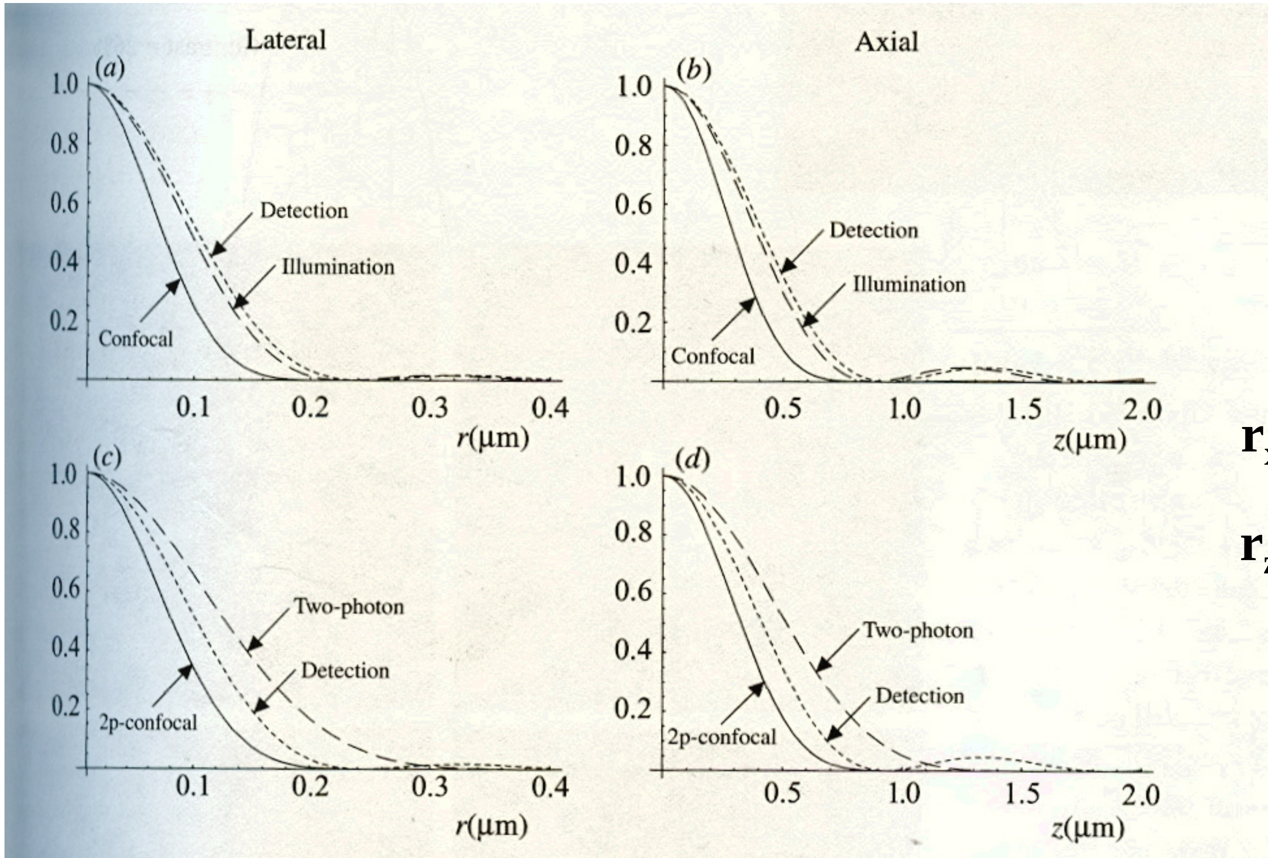


FIGURE 3 Orthogonal extents of the illumination, detection, and confocal PSFs calculated under exactly the same conditions as the contour plots: (*a*) lateral ($z = 0$) and (*b*) axial ($r = 0$) extents. Orthogonal extents of the two-photon, detection, and 2*p*-confocal PSFs in (*c*) lateral ($z = 0$) and (*d*) axial ($r = 0$) directions. Note the different scales for the lateral and axial plots. The plots show clearly that all three techniques are capable of distinguishing point objects along the optical axis. With such objects, every conventional microscope will perform better than a two-photon microscope.

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

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

Confocal microscopy

$$\mathbf{r_{xy,two\text{-}photon} \approx 0.7\,\lambda\,/\,NA}$$

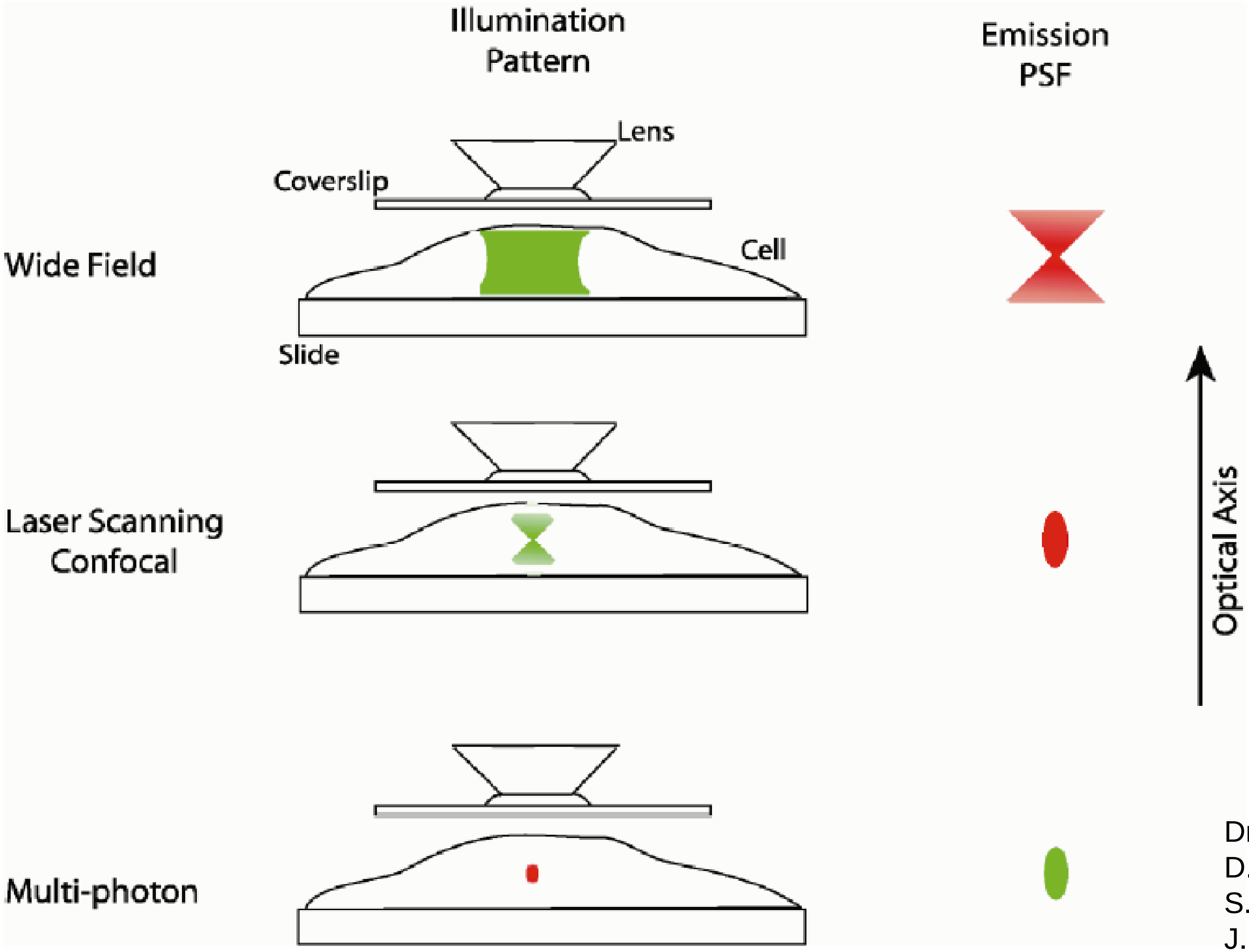
$$\mathbf{r_{z,two\text{-}photon} \approx 2.3\,\lambda \bullet \eta\,/\,NA^2}$$

Two-photon microscopy

Two-photon + Confocal shows higher resolution!!

Graph from Alberto Diaspro

Summary



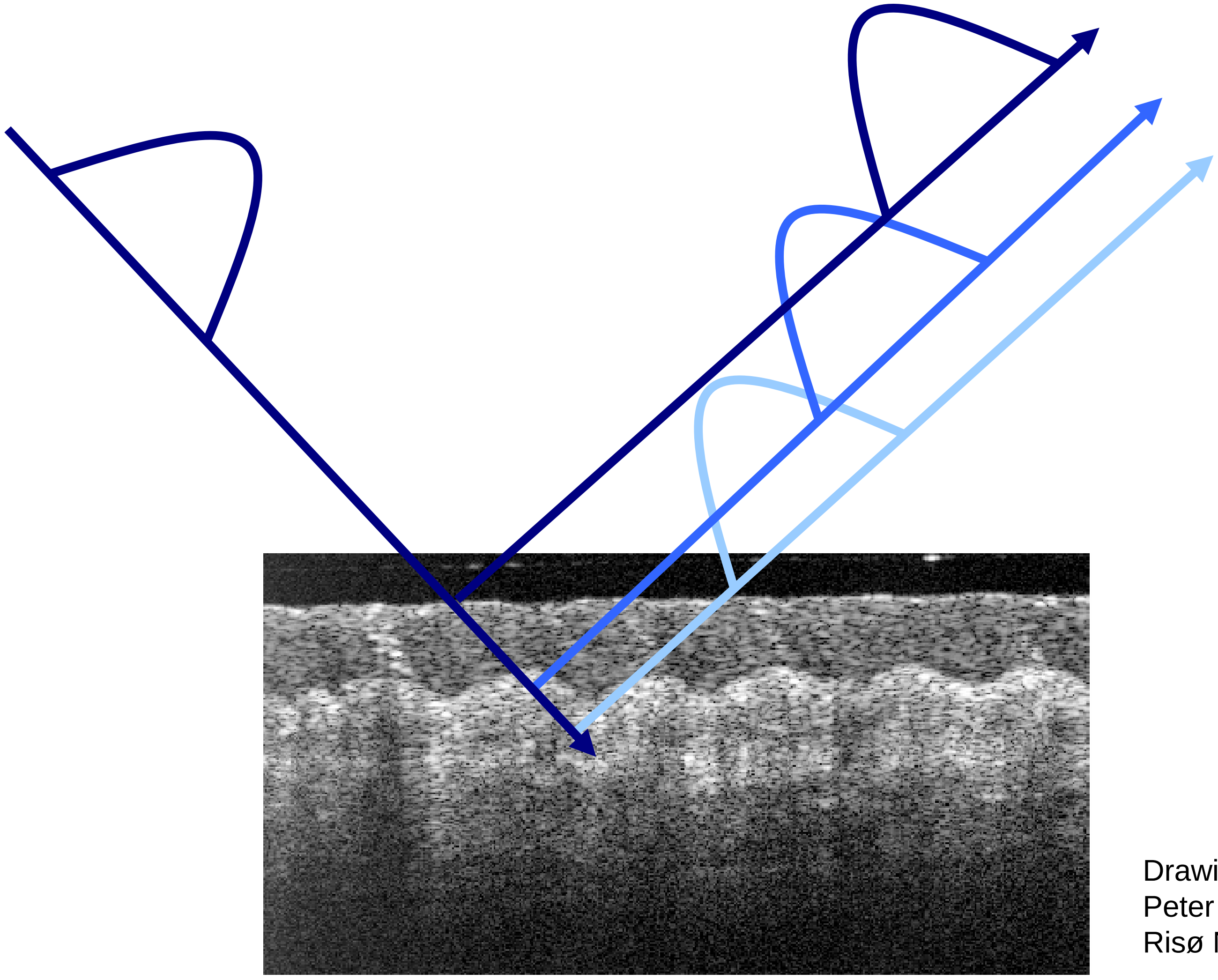
Drawing by P. D. Andrews, I. S. Harper and J. R. Swedlow

3. Optical Coherence Tomography (OCT)

Introduction – Optical Coherence Tomography

- OCT is an interferometric imaging technique that provides cross-sectional views of the subsurface microstructure of biological tissue.
- It measures reflected light from tissue discontinuities
  - e.g. the epidermis-dermis junction.
- Even in highly scattering media, it provide high spatial resolution cross-sectional view of tissues without excision.

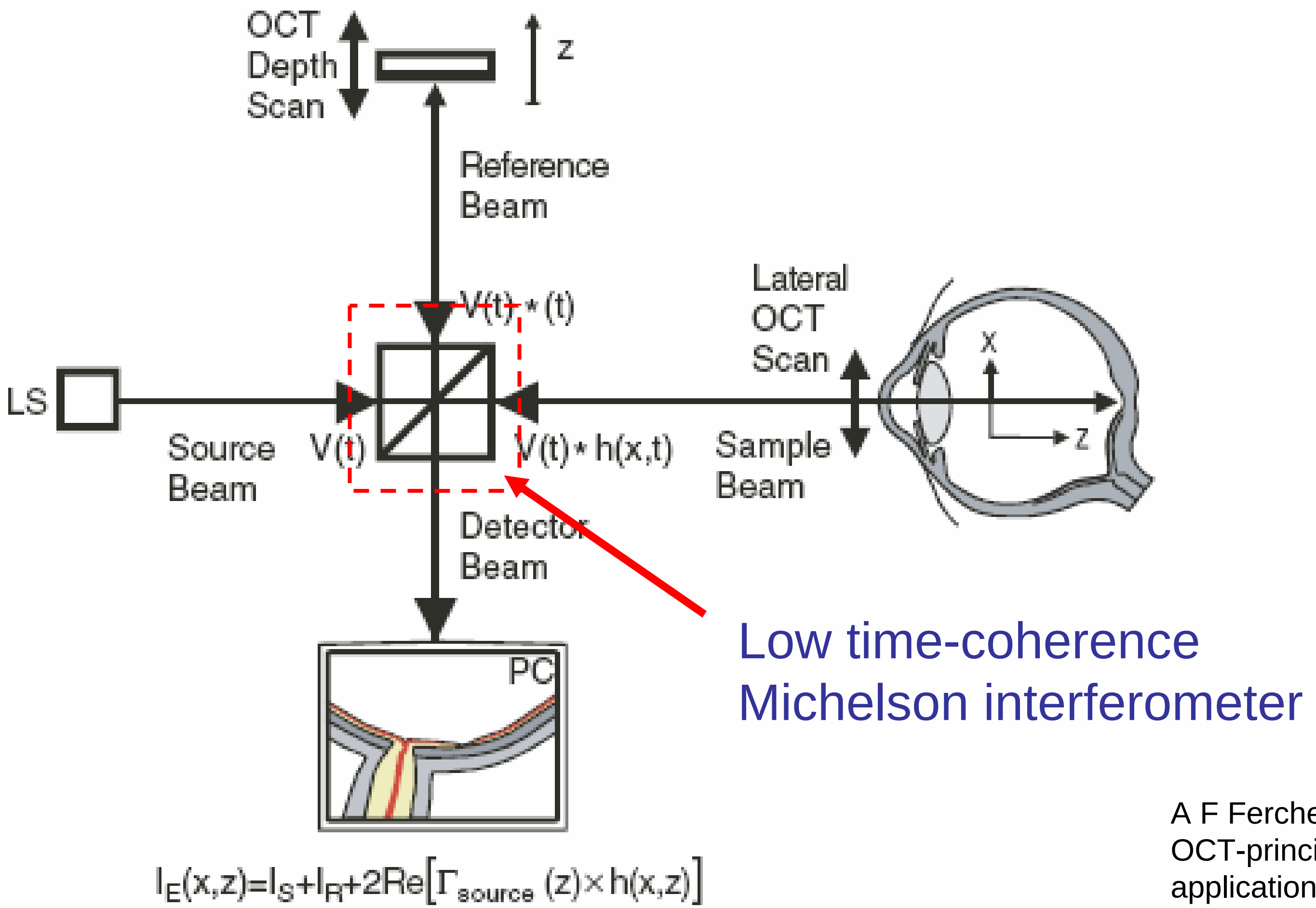
Optical Coherence Tomography



Drawing by
Peter E. Andersen
Risø National Laboratory

OCT measures reflected light from tissue interfaces !

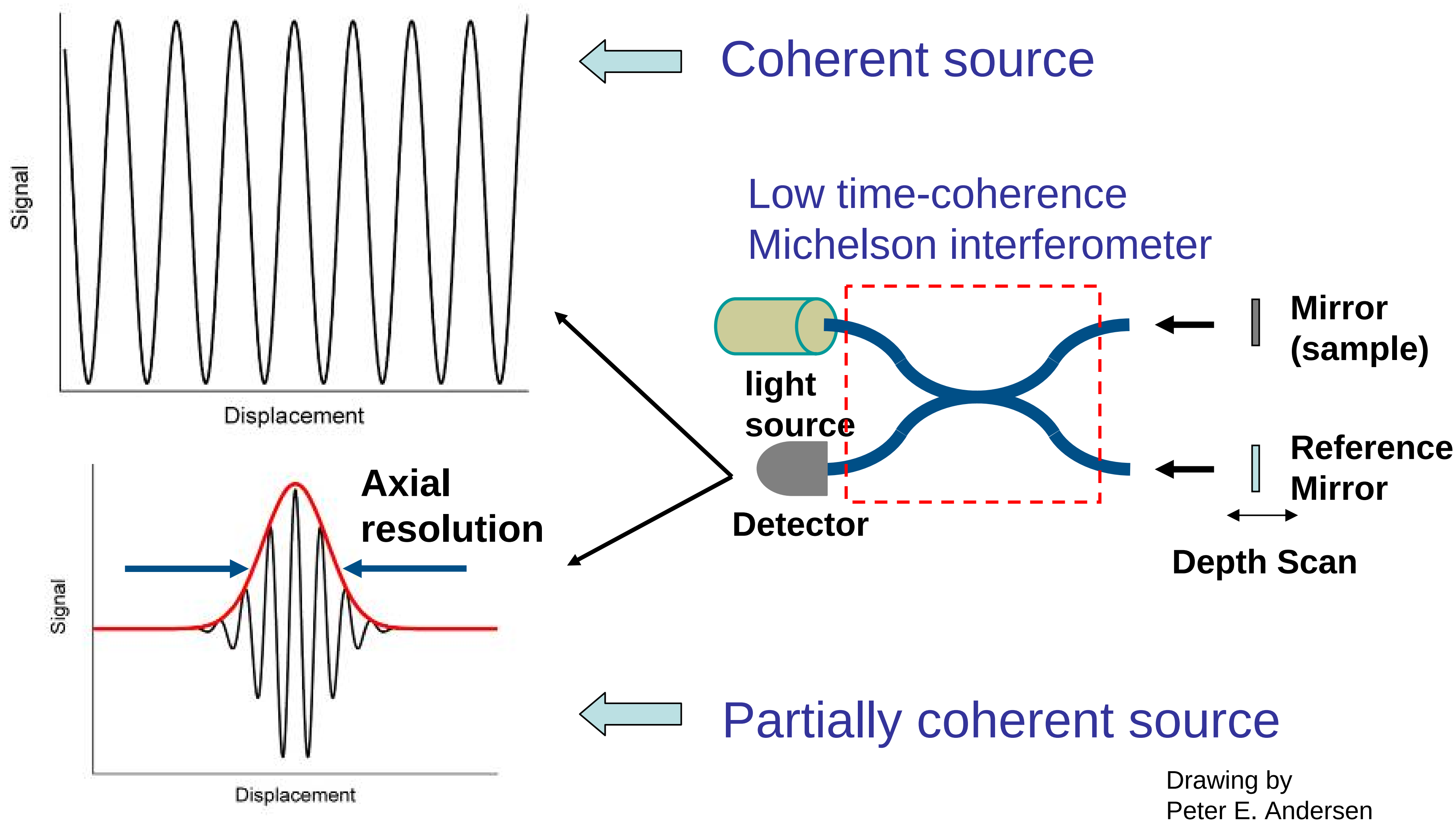
Standard OCT scheme



A F Fercher et al., OCT-principles and applications

Figure 1. Standard OCT scheme based on a low time-coherence Michelson interferometer. The intensity I_E at the interferometer exit depends on the sample response $h(x, z)$ convolved with the source coherence function $\Gamma_{Source}(z)$. LS = low time-coherence light source; PC = personal computer.

Low-Coherence Interferometry



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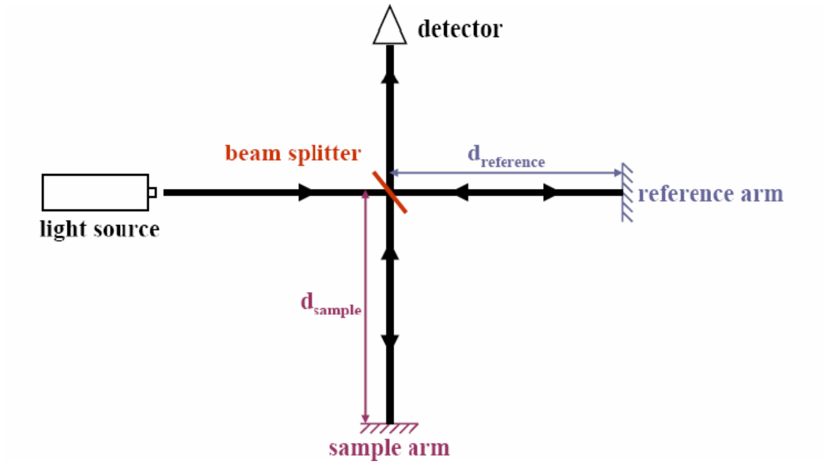
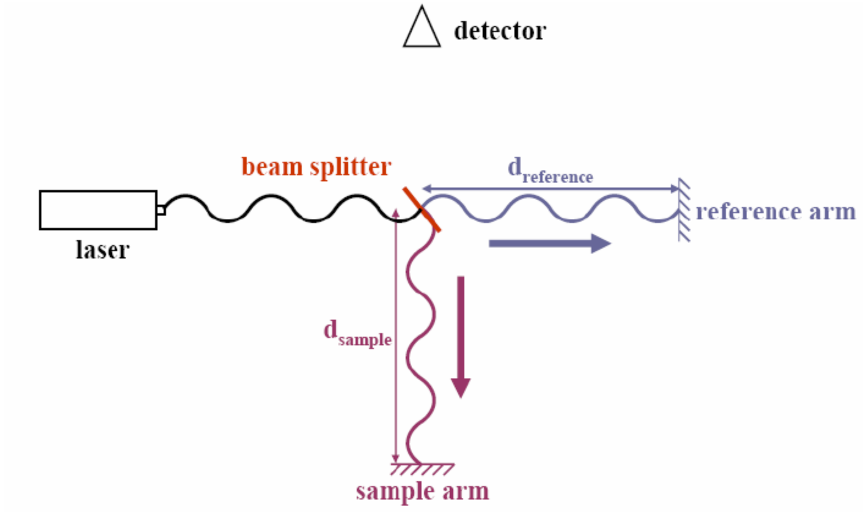
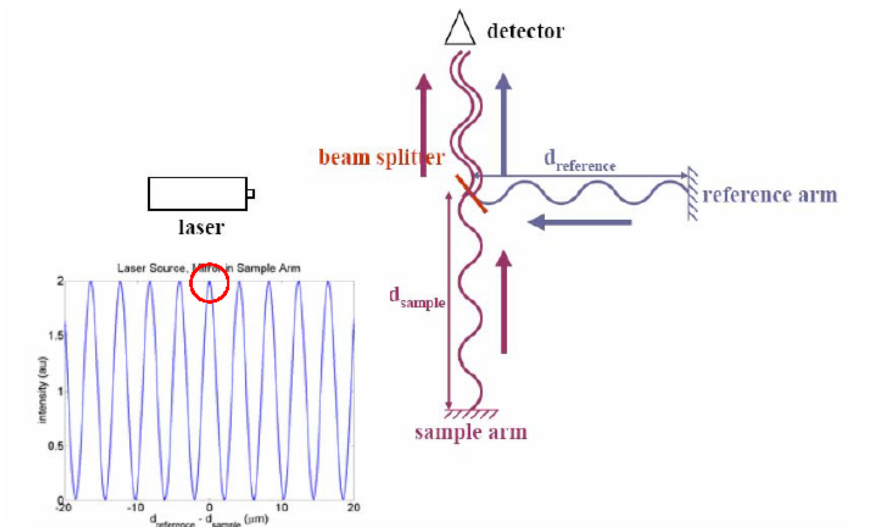
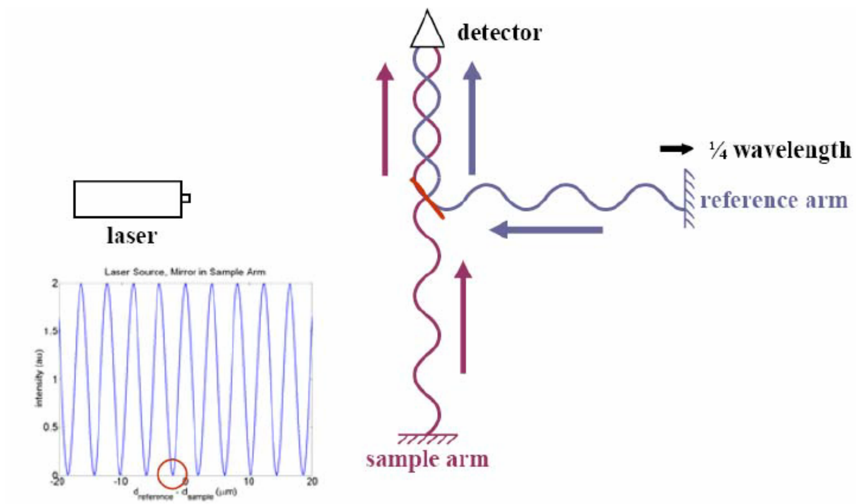
Michelson Interferometer

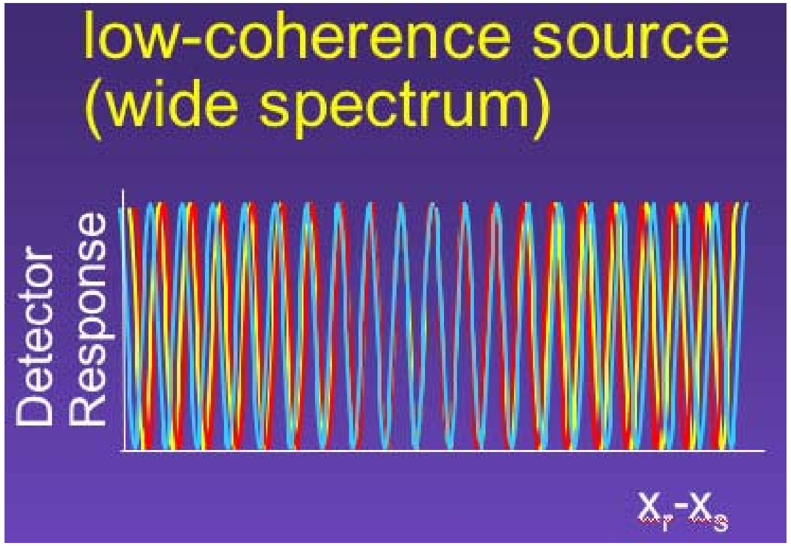


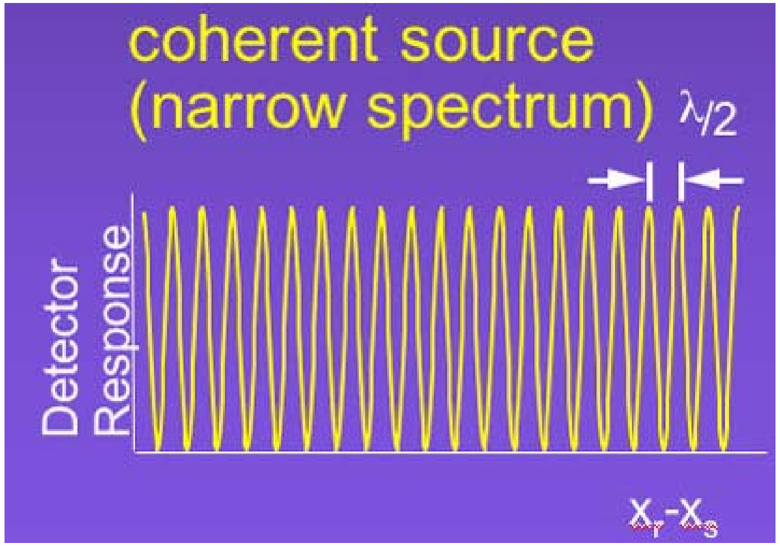


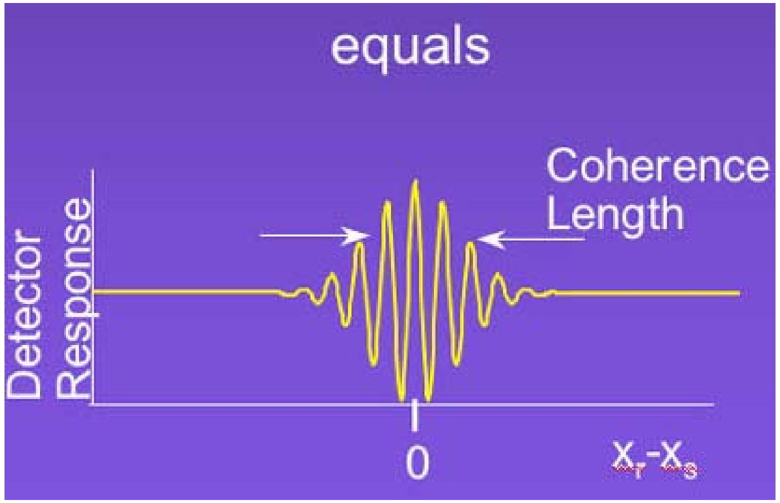


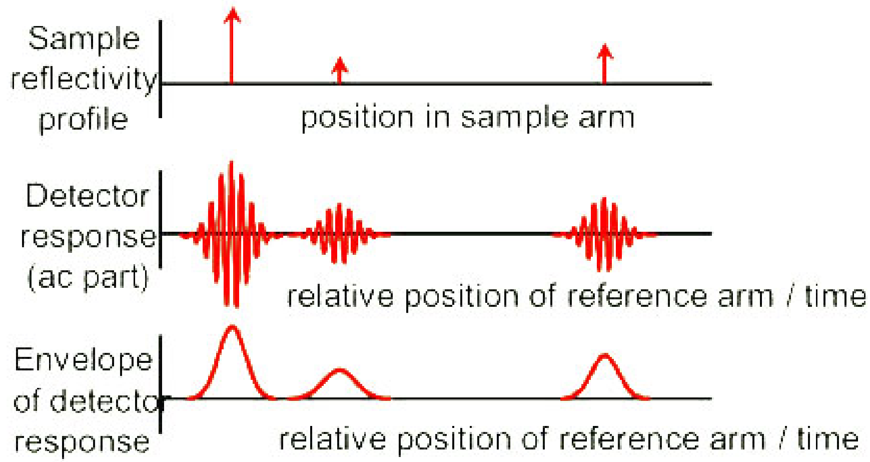


Low-Coherence Interferometry

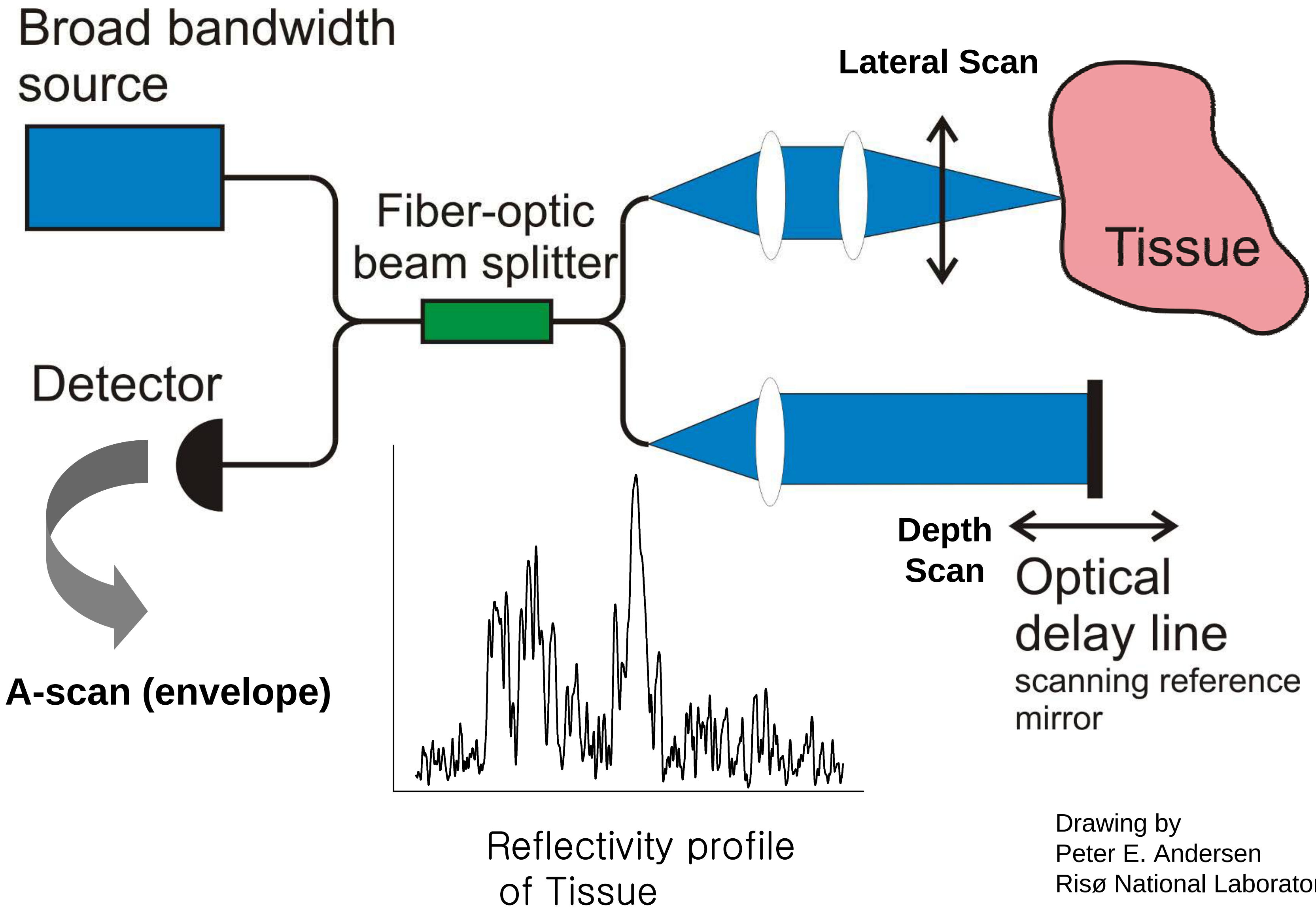








OCT – Principles



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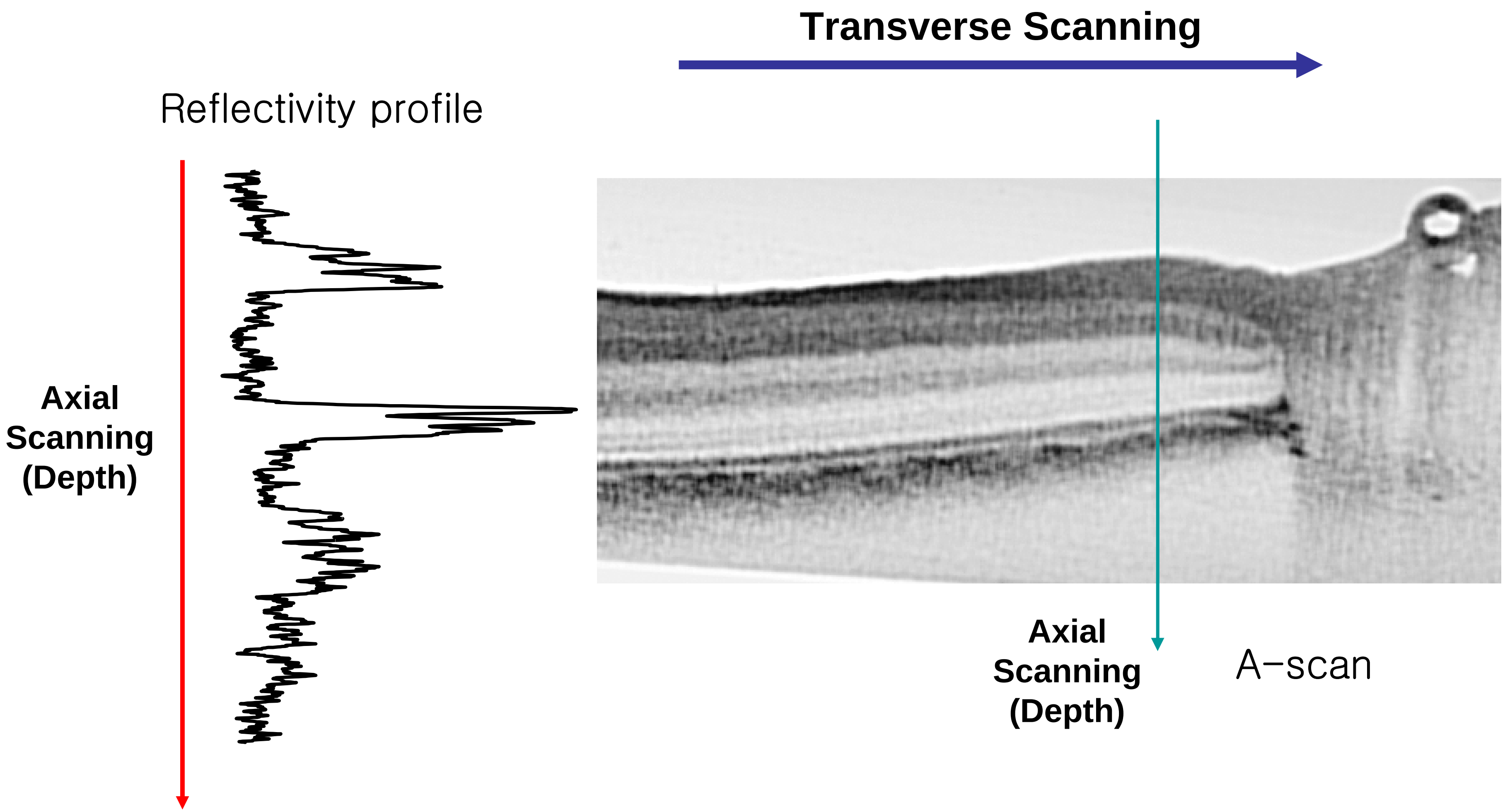
Peter E. Andersen
Risø National Laboratory

OCT – Image construction



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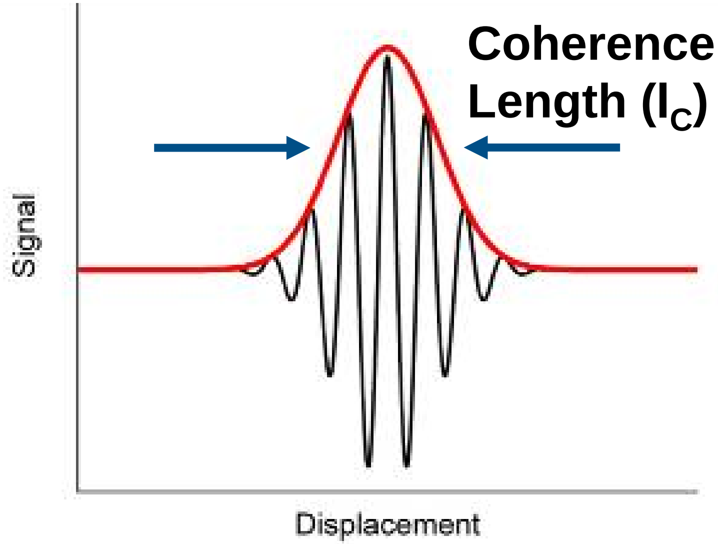
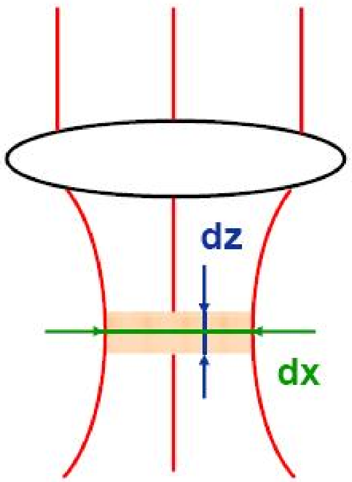
OCT – Spatial Resolution



- OCT depth (axial) resolution (dz) is defined by the coherence length.

$$l_c = \frac{2c\ln 2}{\pi}\frac{1}{\Delta\nu} = \frac{2\ln 2}{\pi}\frac{\lambda_0^2}{\Delta\lambda} \approx 0.44\frac{\lambda_0^2}{\Delta\lambda}$$

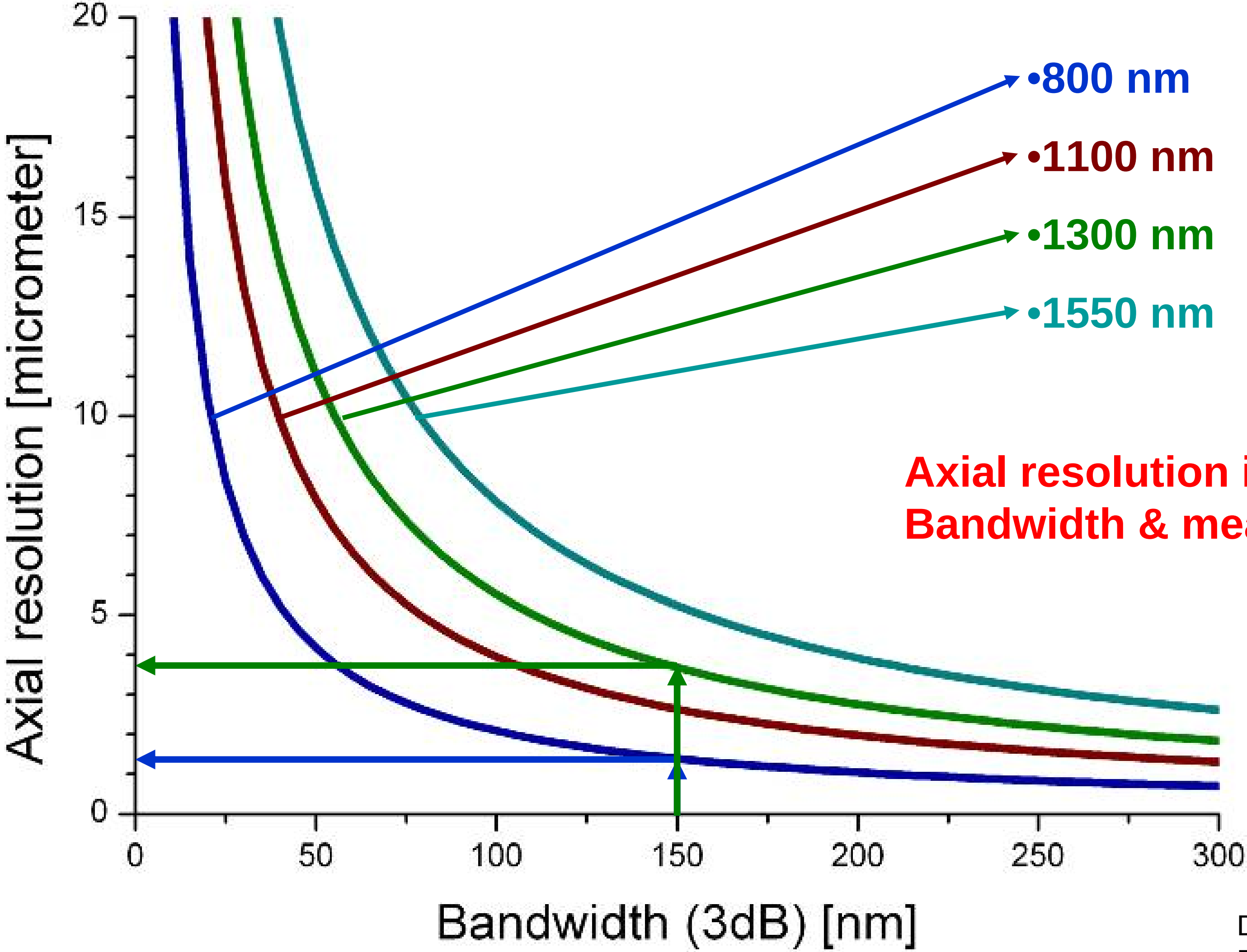
$\Delta\lambda$ is the 3dB−bandwidth
λ_0 is the mean wavelength



- OCT transverse (lateral) resolution (dx) depends on
  - Optics.
  - Lateral scan size step.

- Axial and lateral resolutions ar e decoupled !

OCT – Axial resolution



Axial resolution is related to Bandwidth & mean wavelength

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OCT – Light Sources

- The general requirements
  - Emission in the near infrared
    - Penetration of light into tissue is important.
    - 1200 ~ 1800 nm wavelengths shows the deepest penetration.
  - Short temporal coherence length
    - The Broader the emission bandwidth of the source, the better resolution and contrast that can be achieved.
  - High irradiance
    - For wide dynamic range and high detection sensitivity.

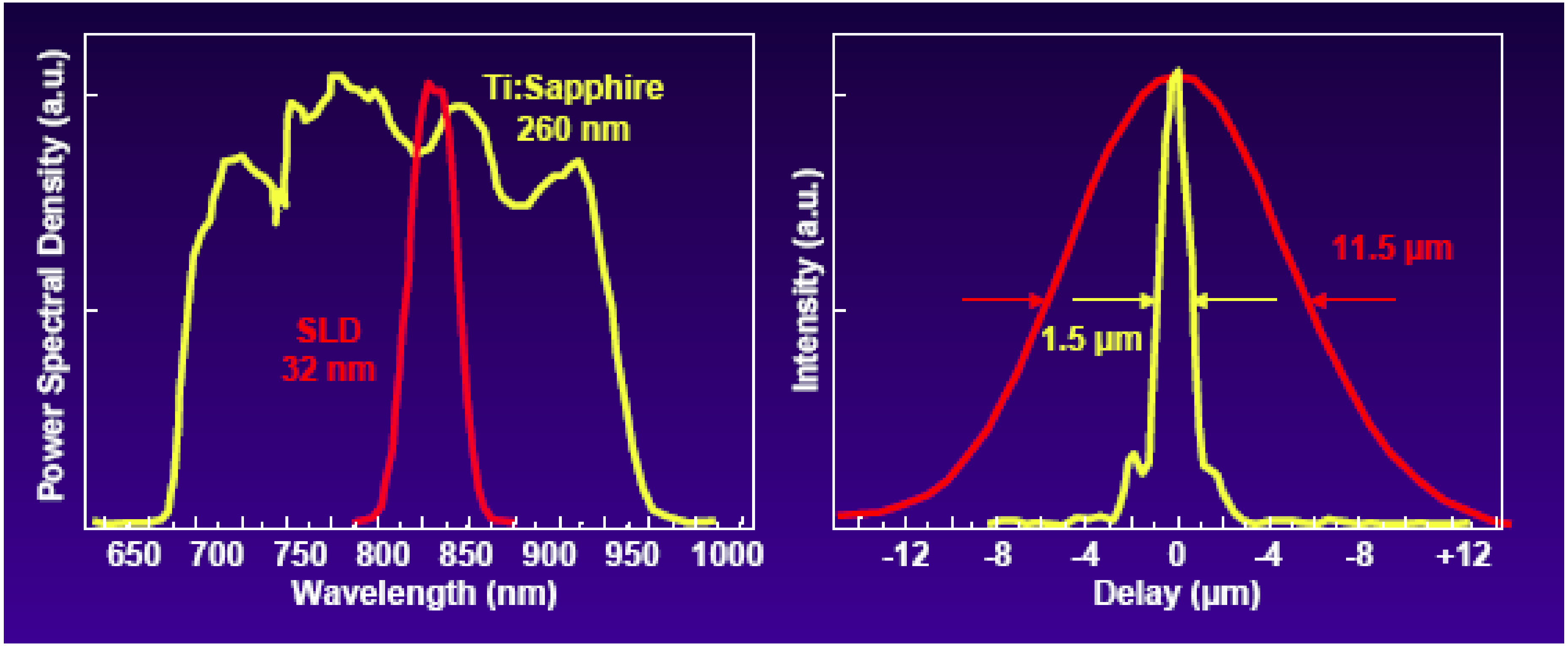
OCT – Light Sources

- SLD (Super Luminescent Diode)
  - Most popular light source in OCT
  - 800 nm, 1300 nm (similar to fiber optic communication bands)
  - High irradiance (1~10 mW) and low cost
  - Coherence lengths of SLD (15 ~ 30 um) are not short enough to achieve the resolution required for many medical applications.

- ELED (Edge-emitting LED)
  - Low cost & coherence length (17 um)
  - Low irradiance (20 ~ 300 uW)

- Pulsed laser (Mode-locked Ti:sapphire laser)
  - High resolution – 1.5 um coherence length
  - High irradiance – 400 mW
  - Used in **Ultra-High-Resolution OCT (UHR-OCT)**

OCT – Light Sources



Comparison of SLD & Pulsed Laser source

Pulsed laser source shows higher axial resolution !!

OCT – Light Sources

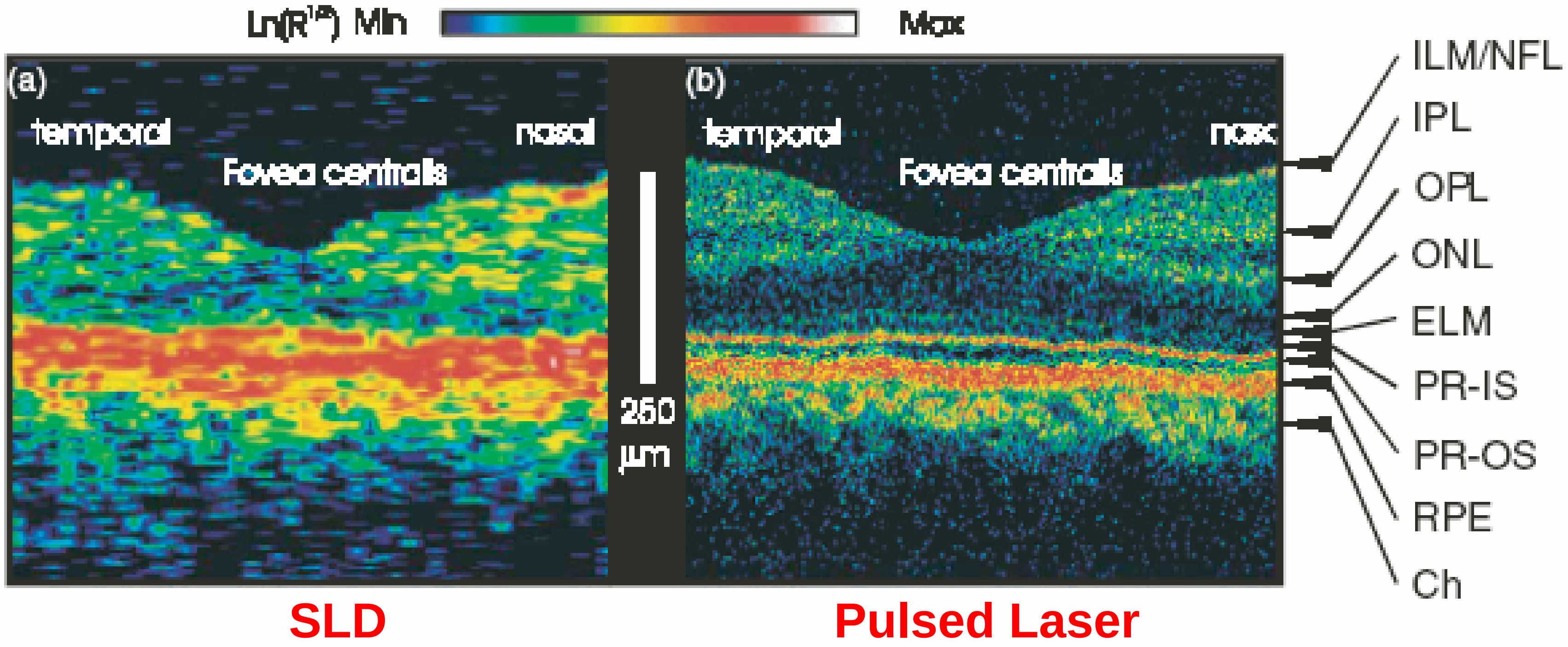


Figure 9. Topographical *in vivo* mapping of retinal layers at the *Fovea centralis* along ~3 mm of the papillomacular axis. The logarithm of the LCI signal is represented on a false-colour scale shown on top of the figure. (*a*) SLD: mean wavelength $\bar{\lambda} = 843$; $\Delta\lambda = 30$ nm; depth resolution 10 μm. (*b*) Ti : Al_2O_3 laser: mean wavelength $\bar{\lambda} = 800$; $\Delta\lambda = 260$ nm; 3 μm depth resolution. The layers are (from top): ILM/NFL = inner limiting membrane/nerve fibre layer; IPL = inner plexiform layer; OPL = outer plexiform layer; ONL = outer nuclear layer; ELM = external limiting membrane; PR–IS = photoreceptors inner segment; PR–OS = photoreceptors outer segment; RPE = retinal pigment epithelium; Ch = choriocapillaris and Choroid. Adapted from Drexler *et al* (2000). Courtesy of Fujimoto, MIT. Reprinted by permission from Kugler Publications, The Netherlands.

OCT – Applications

- Ophthalmology
  - diagnosing retinal diseases.
- Dermatology
  - skin diseases,
  - early detection of skin cancers.
- Cardio-vascular diseases
  - vulnerable plaque detection.
- Endoscopy (fiber-optic devices)
  - gastrology

- Functional imaging
  - Doppler OCT,
  - spectroscopic OCT,
  - PS-OCT.

- Guided surgery
  - brain surgery,
  - knee surgery.

OCT in ophthalmology

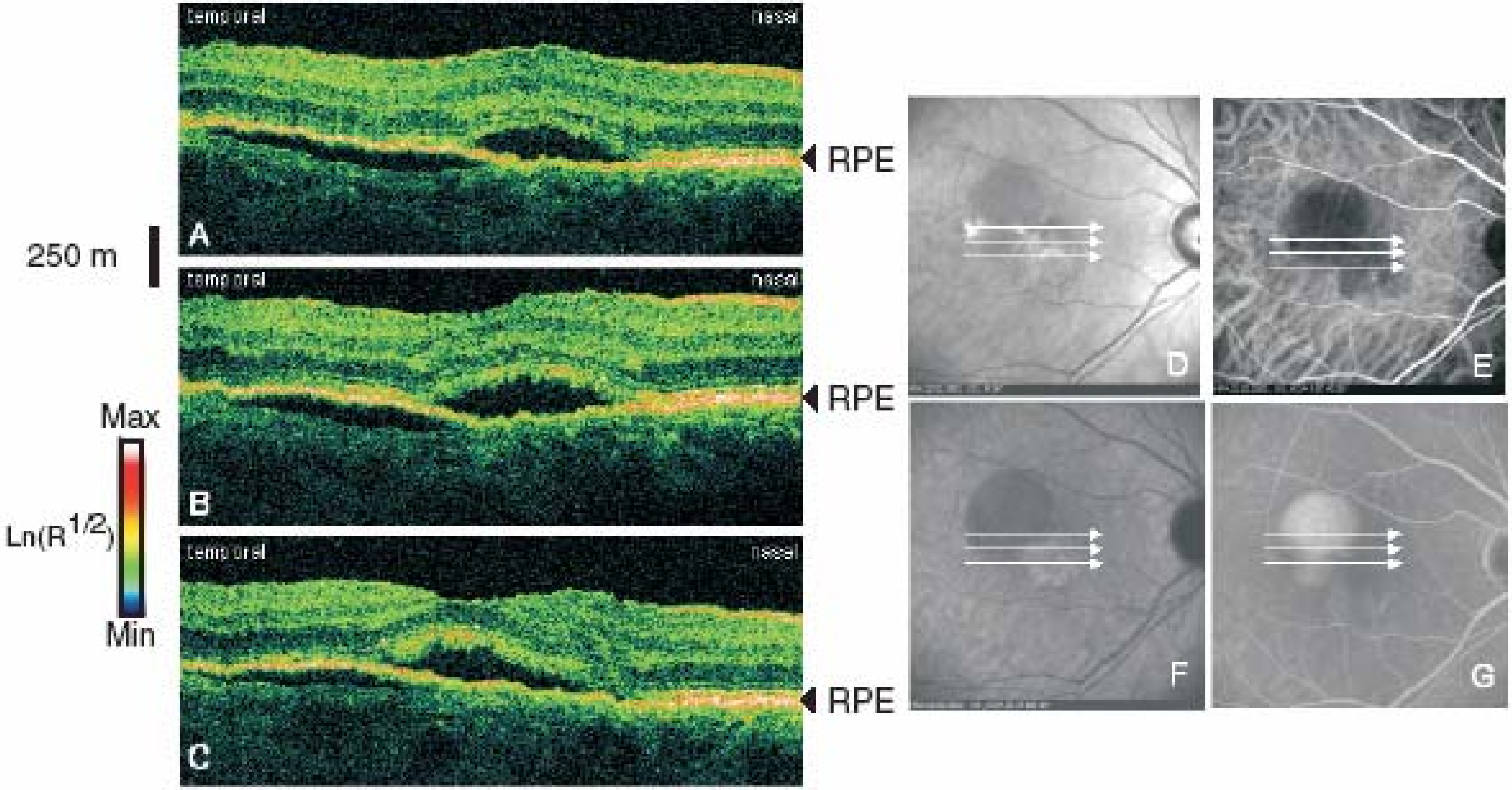
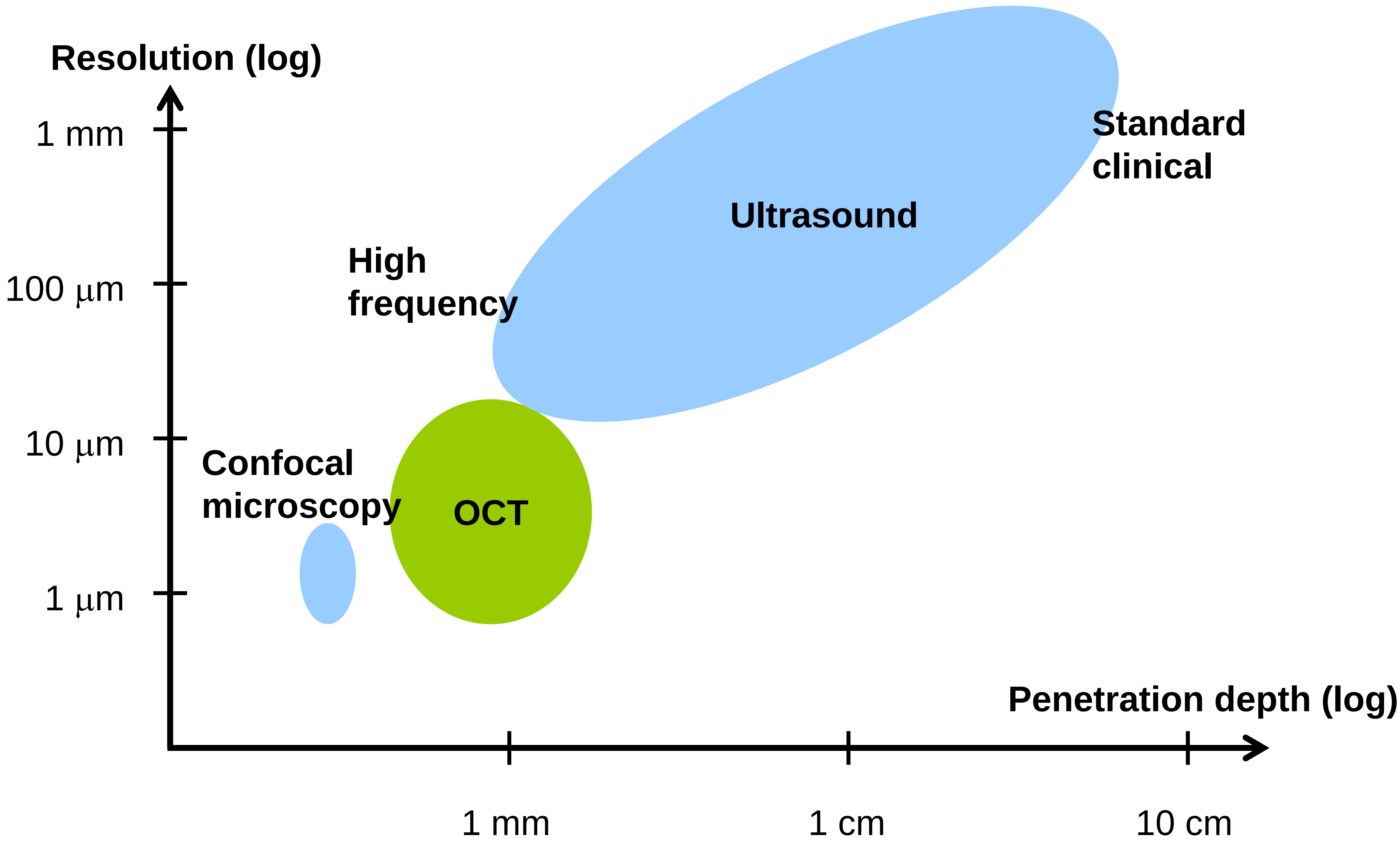


Figure 24. Patient with age-related macular degeneration with occult classic neovascularization and serous detachment of the RPE. (*a*)–(*c*) Ultra-high-resolution OCT images through the foveal region (the logarithm of the LCI signal is represented on a false-colour scale shown left of the figures). These pictures clearly delineate the subretinal (above RPE) and RPE detachments (below RPE). (*d*) Corresponding scan positions on an infrared. (*e*) and (*f*) Early and late fluorescein angiography photos. (*g*) ICG fundus photo.

OCT vs. Standard Imaging



Drawing by Peter E. Andersen Risø National Laboratory

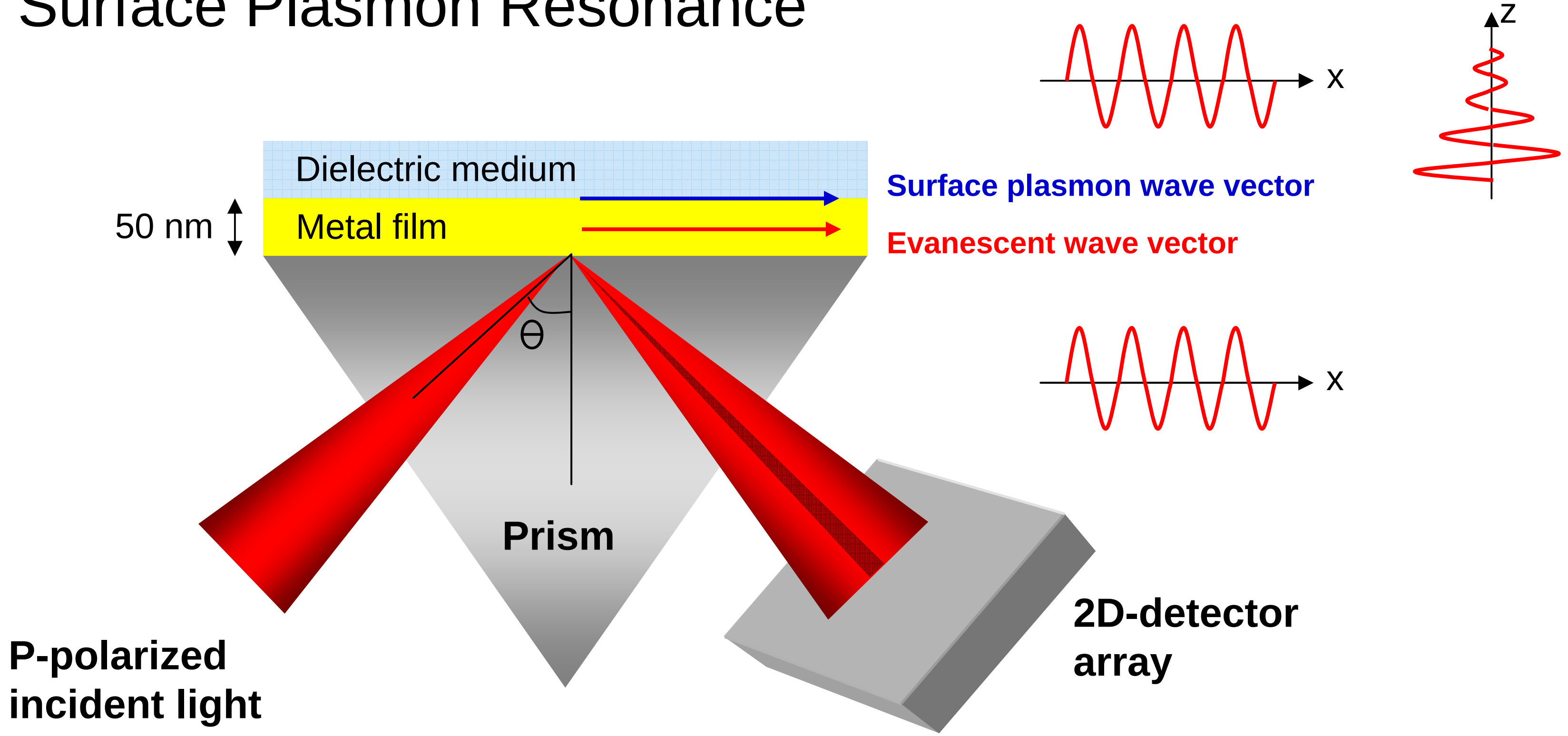
Characteristics of OCT

- Advantages
  - High depth and transversal resolution
  - Contact-free and non-invasive operation
- Disadvantages
  - Limited penetration depth in scattering media compared to alternative imaging modalities (MRI, CT, Ultrasound…)

4. Optical Neural Interfaces
4-1 SPR NI

Neural Signal Detection using SPR

- Surface Plasmon Resonance

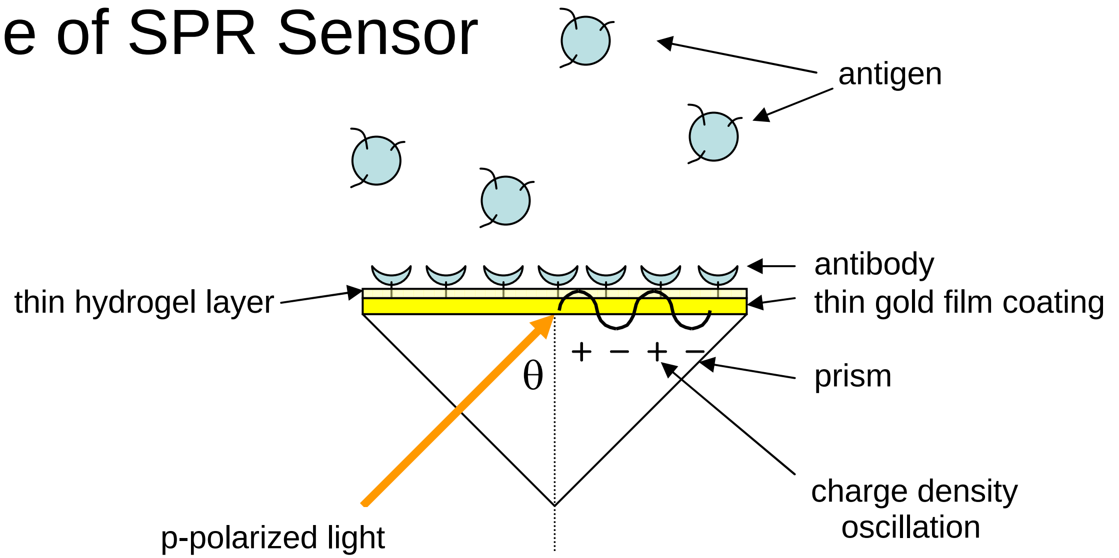


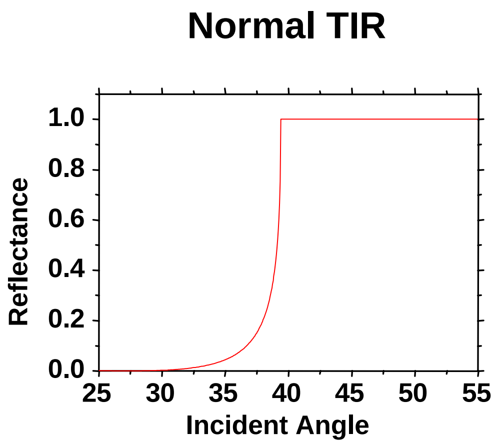
$$k_x = \frac{2\pi}{\lambda} n_p \sin\theta \,, \quad k_{sp} = \frac{2\pi}{\lambda}\sqrt{\frac{n_m^{\ 2} n_d^{\ 2}}{n_m^{\ 2} + n_d^{\ 2}}}, \quad k_x = k_{sp}$$

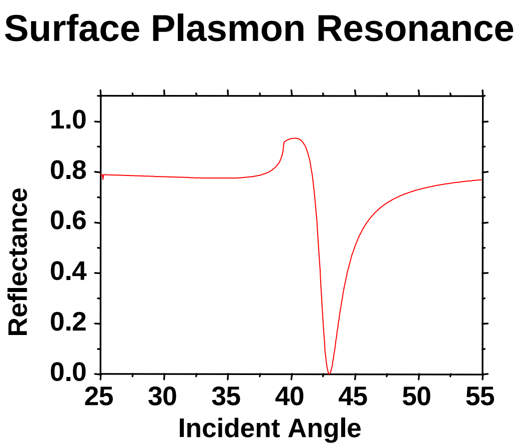
(n_p, n_m, and n_d: Refractive index of prism, metal, and dielectric medium)

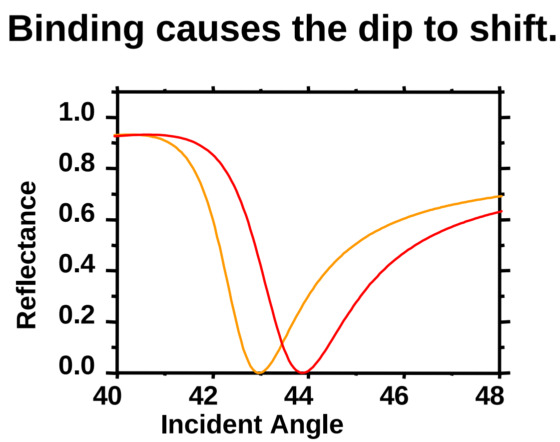
Neural Signal Detection using SPR

- Principle of SPR Sensor





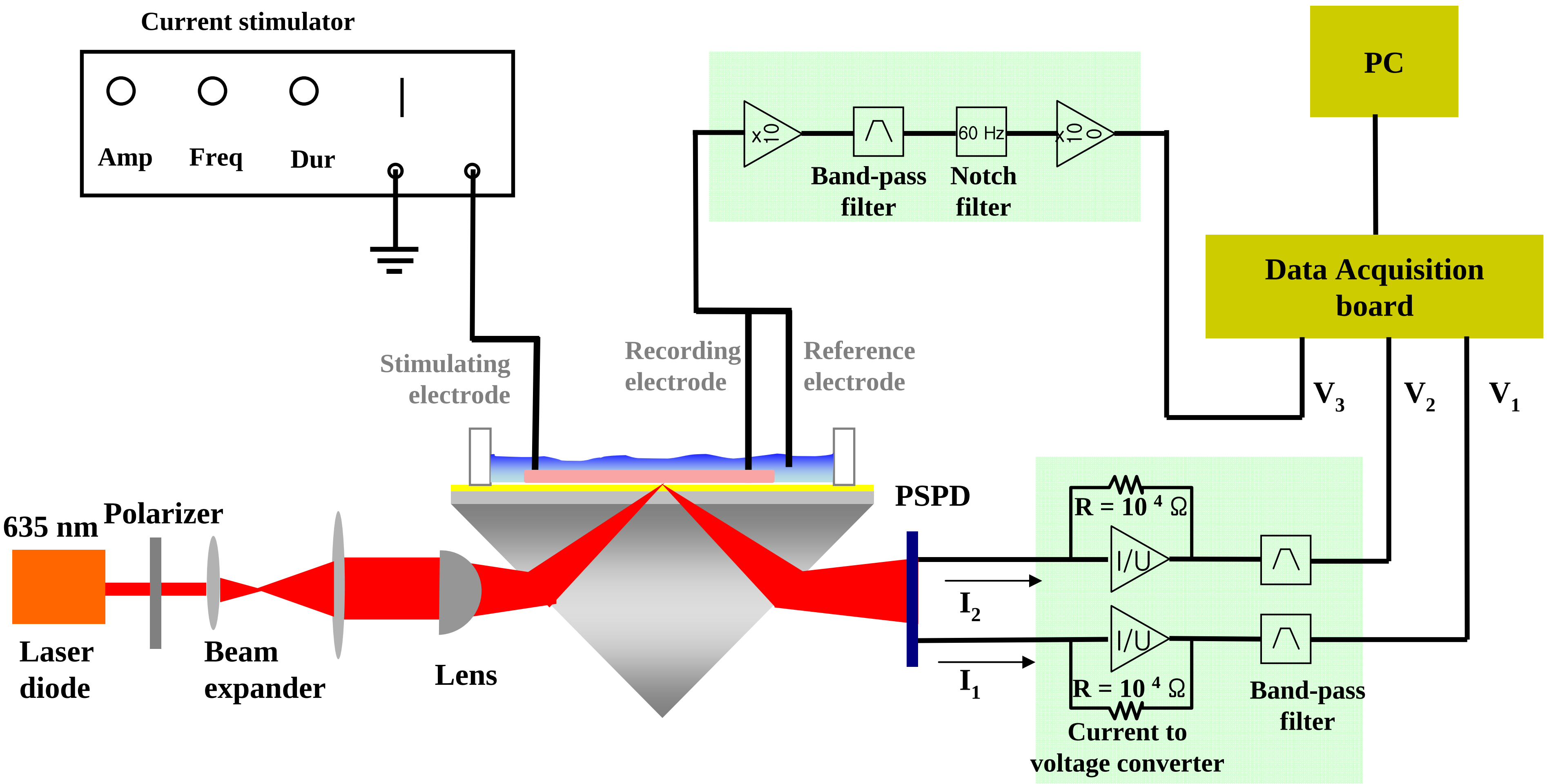




Rothenhäusler *et al.*, Nature 1988
Wilson, Science 2002

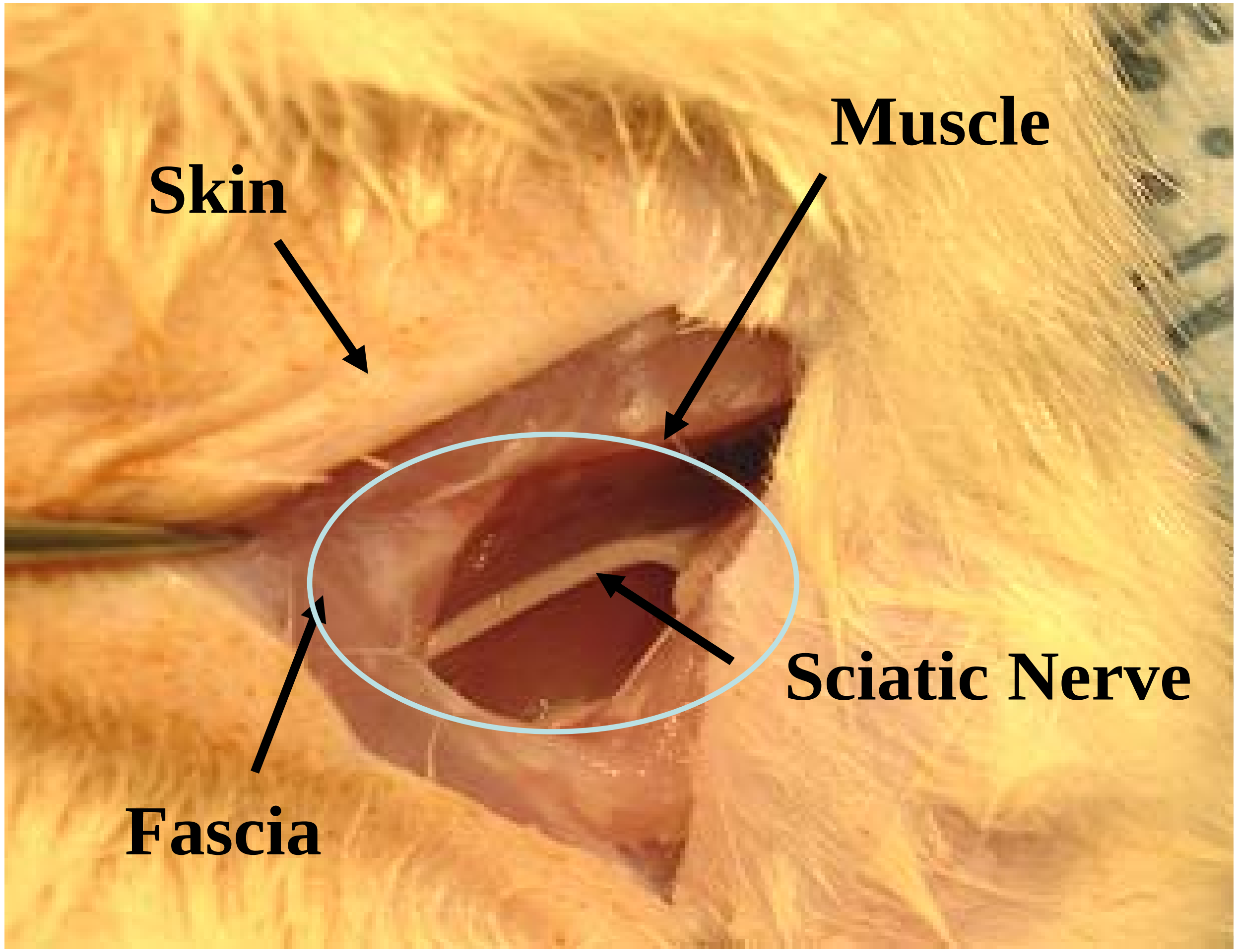
Neural Signal Detection using SPR

- Instrumentation



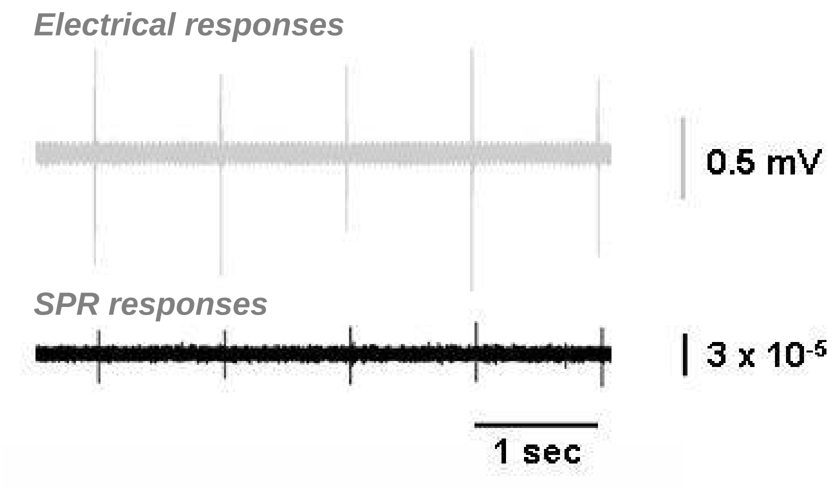
Neural Signal Detection using SPR

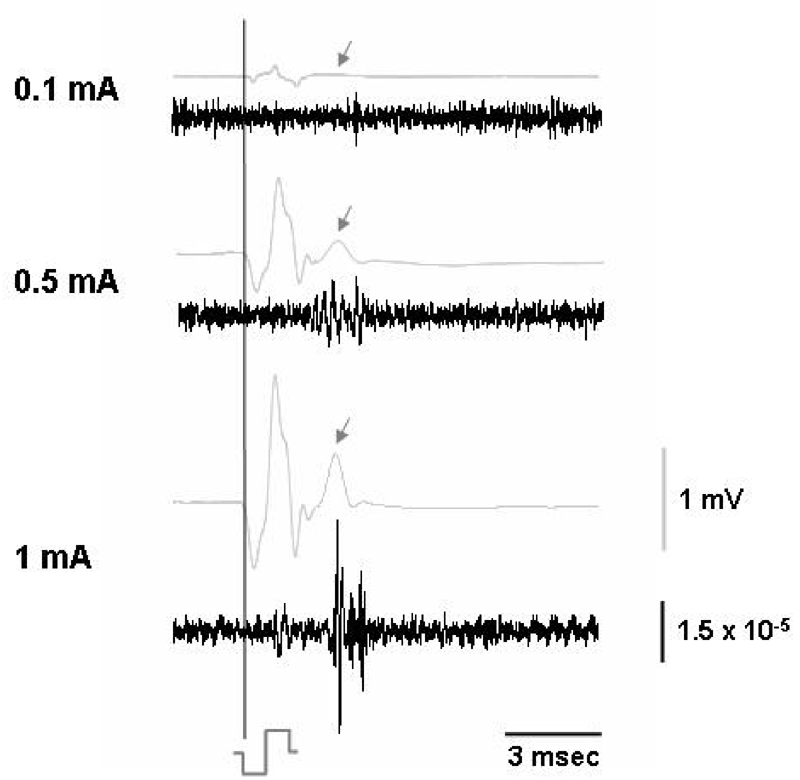
- Material: Rat Sciatic Nerve



Neural Signal Detection using SPR

- Electrical (gray) and SPR (black) Responses during Neural Activation





Kim *et al.* Opt. Letters 2008

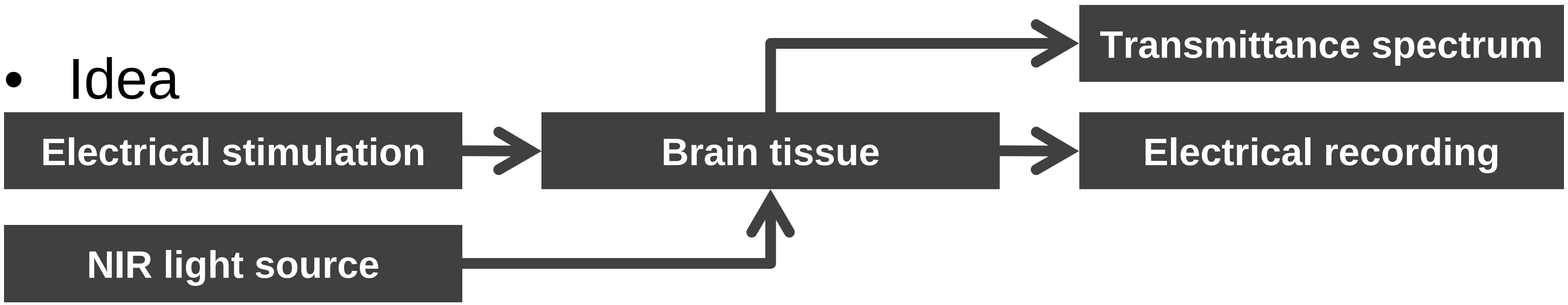
4-2 NIR BCI

Neural Signal Detection using NIR Spectrum

- Motivation

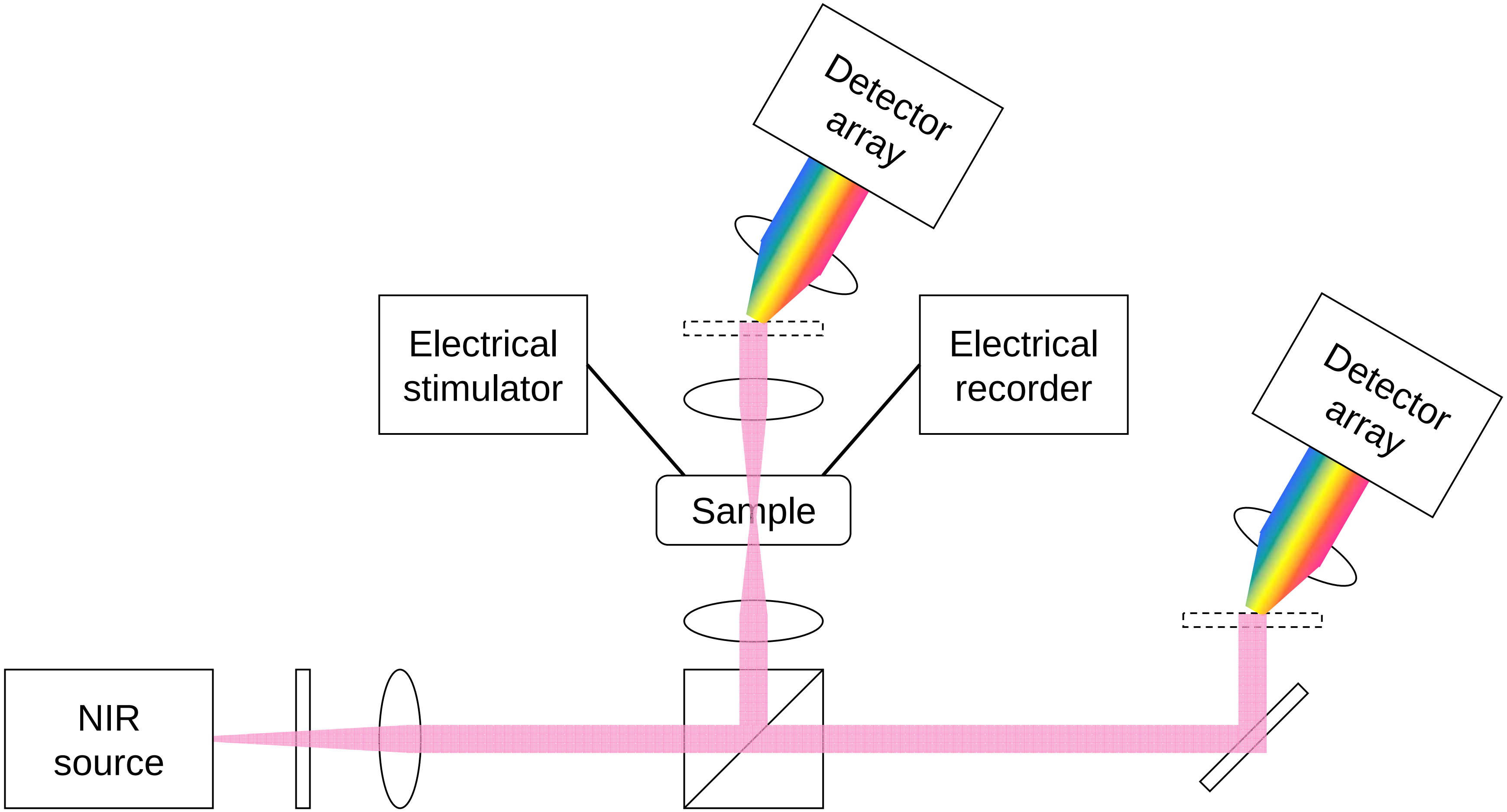
	Contact free	Label free	Whole field imaging	Brain tissue
Electrode		V		V
Voltage sensitive dye	V		V	V
Dark field microscope	V	V		
OCT	V	V		
New method needed	**V**	**V**	**V**	**V**

- Idea



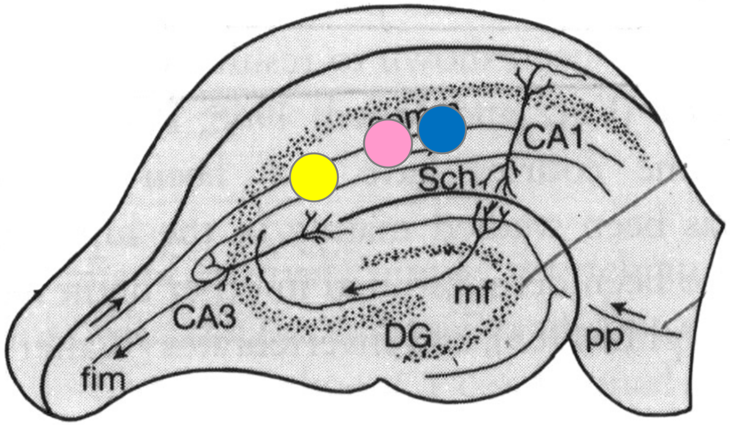
Neural Signal Detection using NIR Spectrum

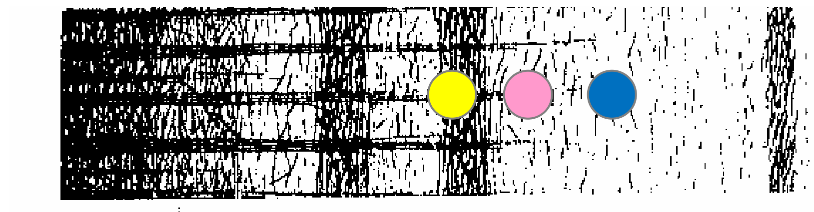
- Instrumentation: High-speed NIR Transmission Spectrometer



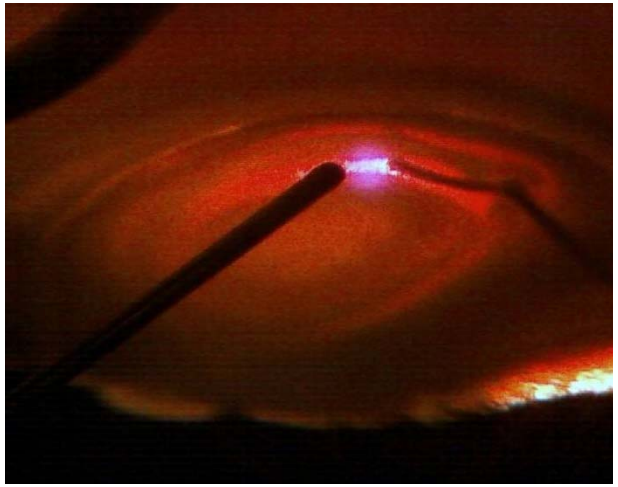
Neural Signal Detection using NIR Spectrum

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



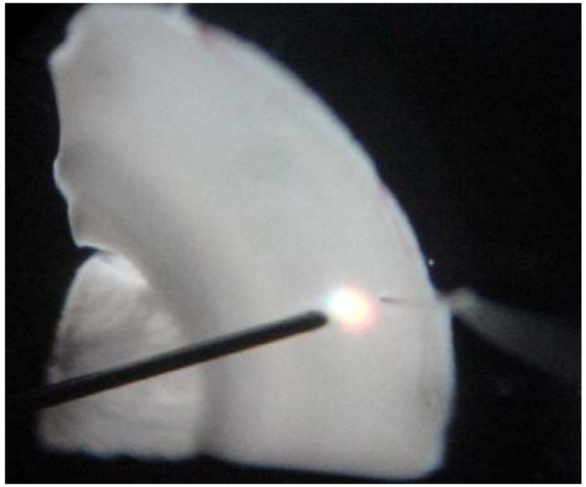


IV III II



Electrical stimulation
Optical recording
Electrical recording

1 mm



Neural Signal Detection using NIR Spectrum

- Preliminary Results