Multiphoton Imaging of the Retina

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- Second Harmonic Generation Imaging (SHGi)
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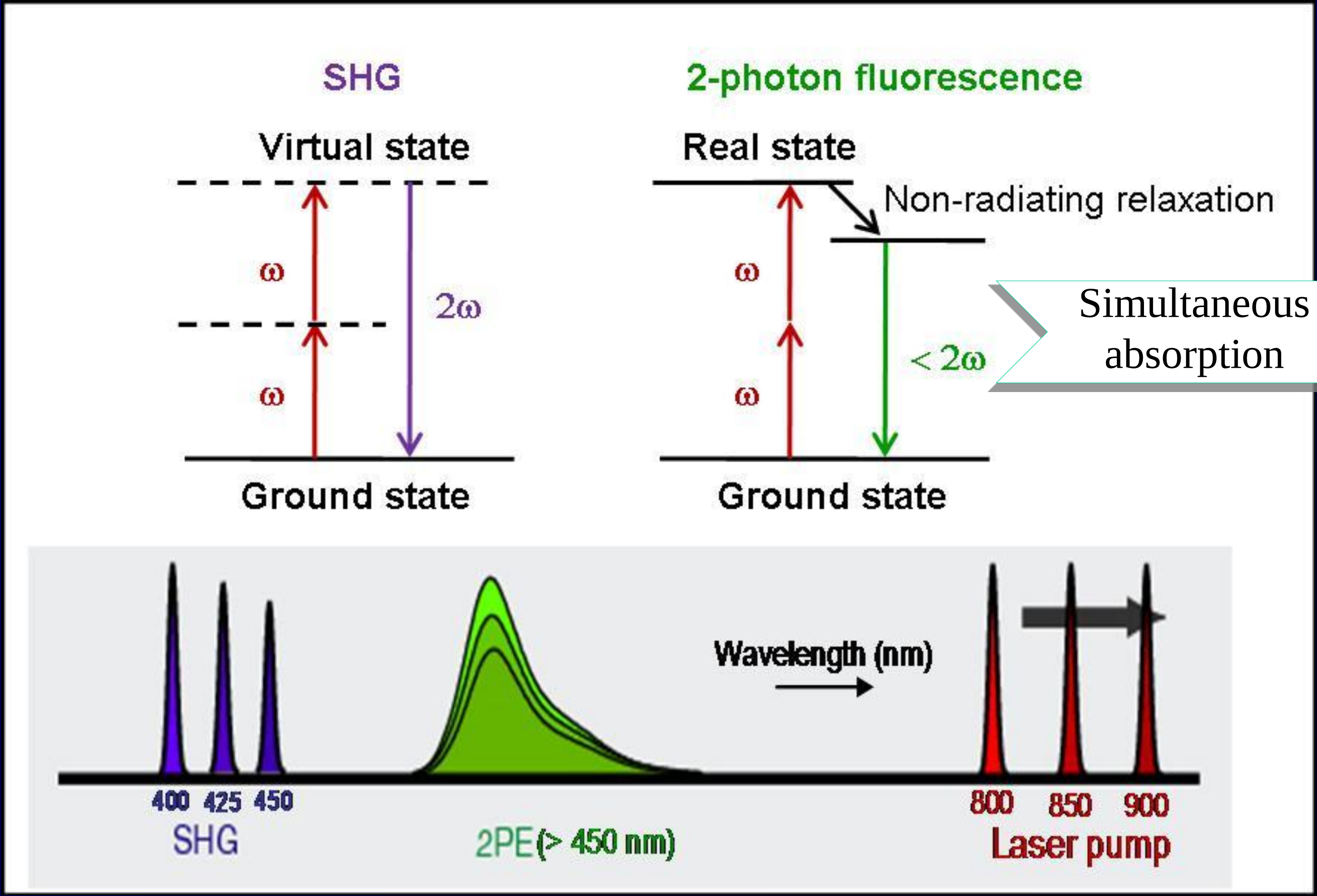
- Adaptive Optics Two-Photon Ophthalmoscope

Principle of two photon imaging



Simultaneous absorption

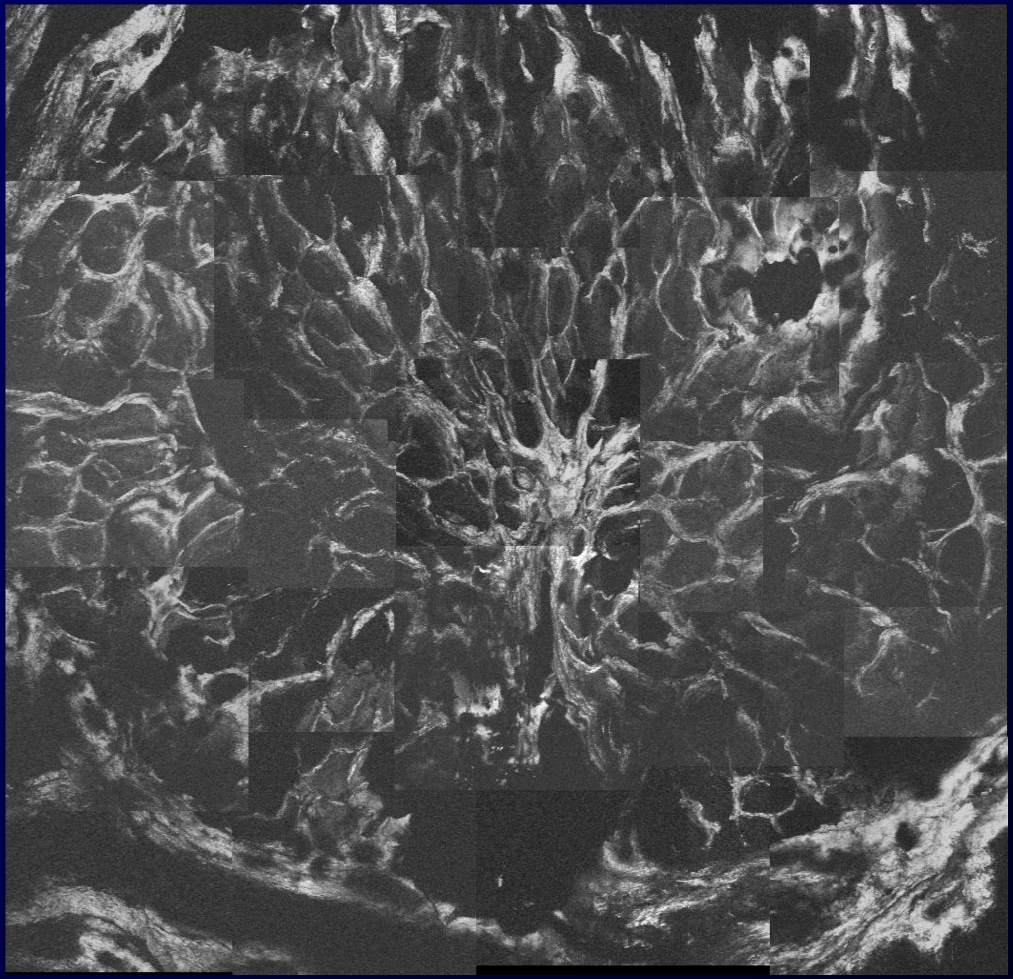
What is SHG?

SHG = Second Harmonic Generation
Second order nonlinear optical process
Two photons are effectively "combined" to form a new photon with twice the energy and therefore twice the frequency
Prerequisites: laser light, non-centrosymmetric media

Why SHG?

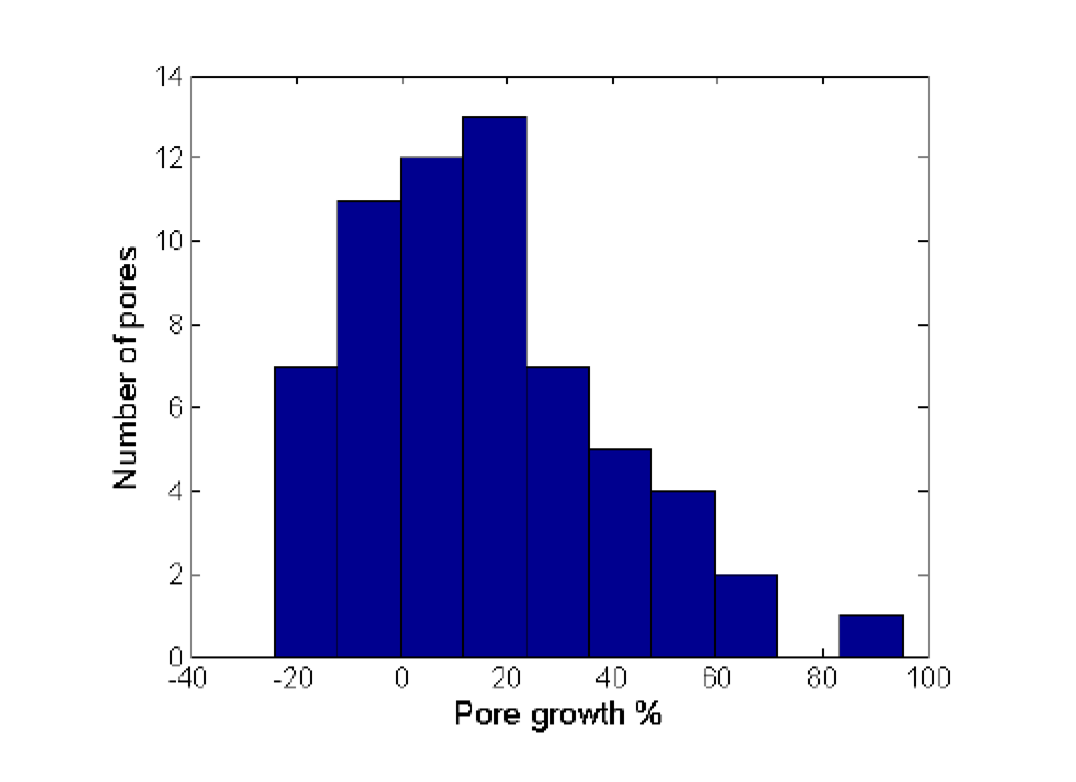
Non invasive
Less photo damage
Collagen gives a very strong SHG signal

SHG Imaging of the Lamina Cribrosa



Agopov et al, Lasers in Medical Sciences, Vol.24, Number 5,pp.787-792(2009)

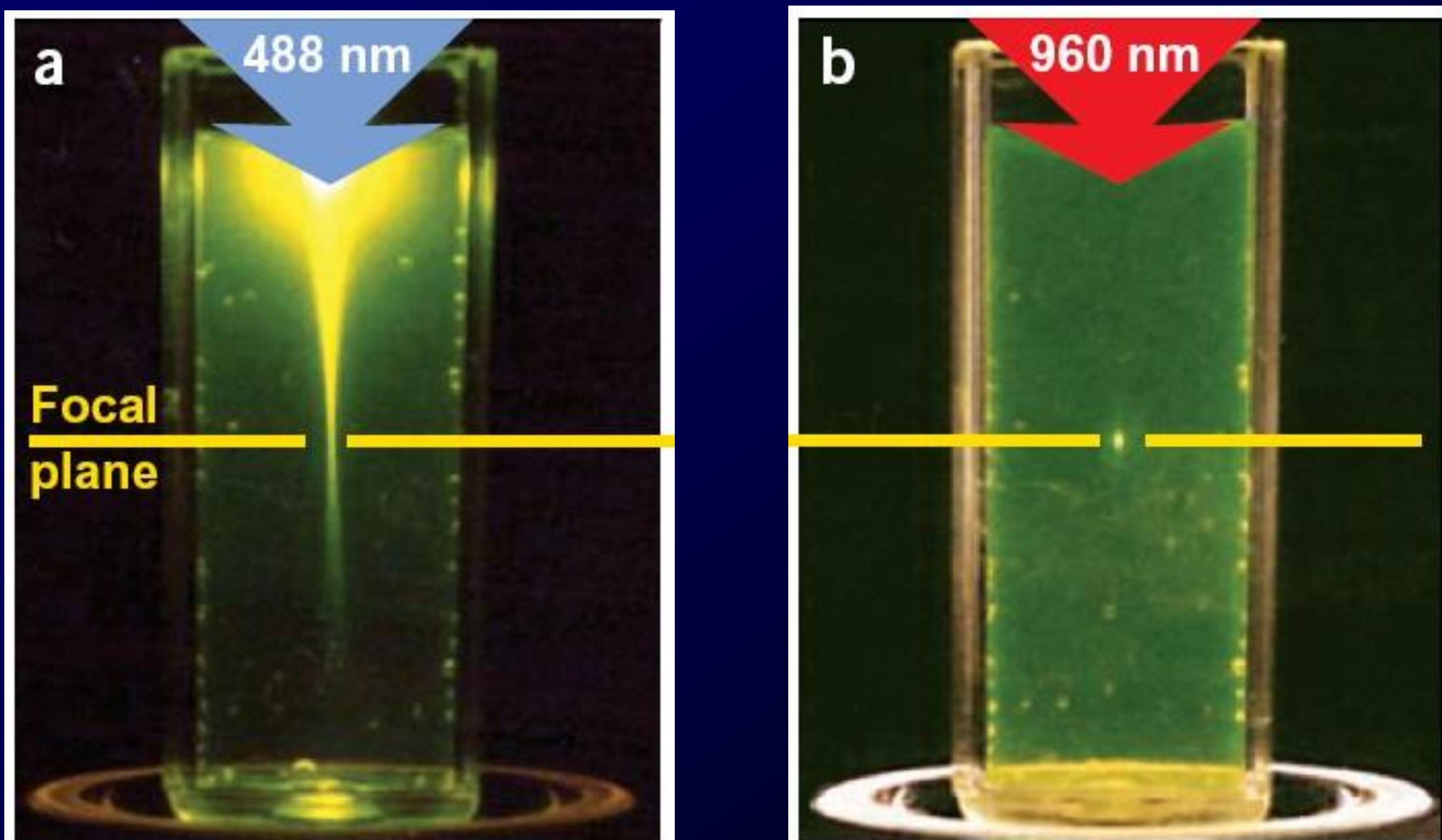
Pore size analysis of LC SHG images



The histogram of the pore growth.

Agopov et al, Lasers in Medical Sciences, Vol.24, Number 5,pp.787-792(2009)

1 photon v.s. 2 photon excitation



Advantages of Two-Photon exitation

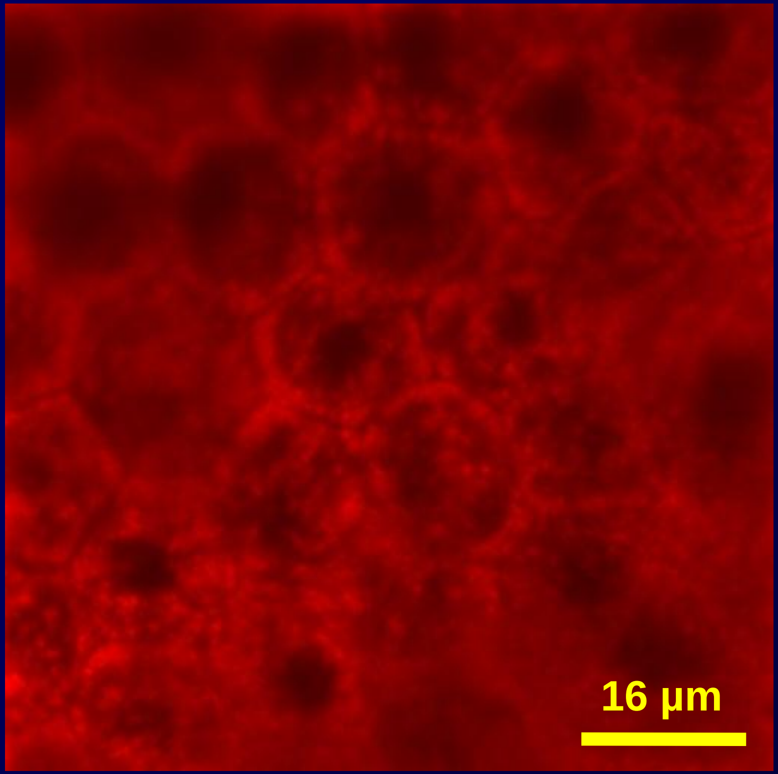
§ **Localized excitation:**

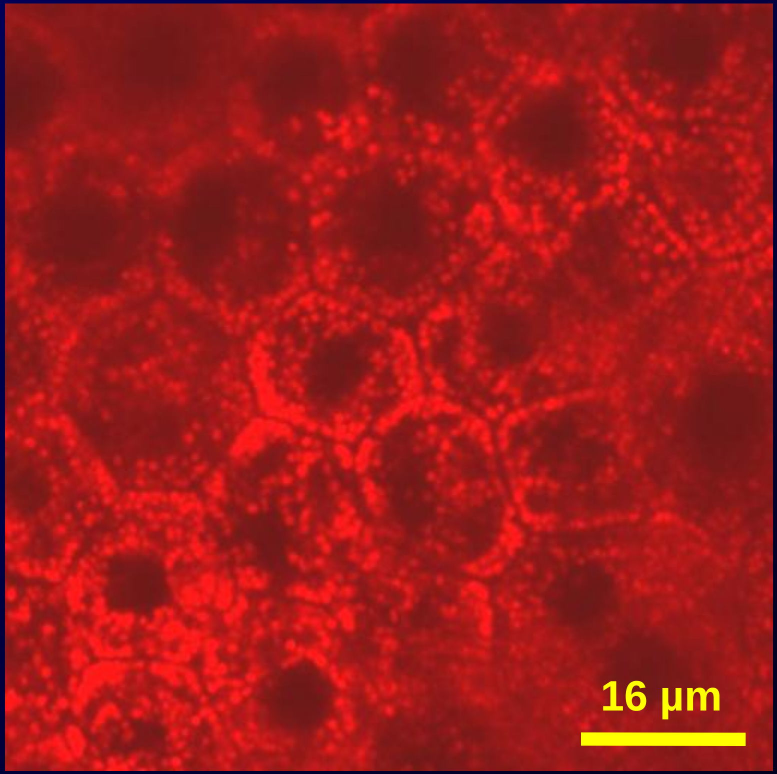
- Intrinsic three-dimensional resolution: eliminate out-of-focus and scattered fluorescence
- No confocal pinhole: also scattered fluorescence photons provide useful signal
- No out-of-focus photobleaching and photodamage related to fluorescence excitation

§ **Longer wavelength:**

- NIR excitation light generally less phototoxic than blue light
- Larger penetration depth due to less absoption, less scattering
- Expanded wavelength accessibility (UV excitation)

Autofluorescence from Retinal Pigment Epithelial (RPE) Cell



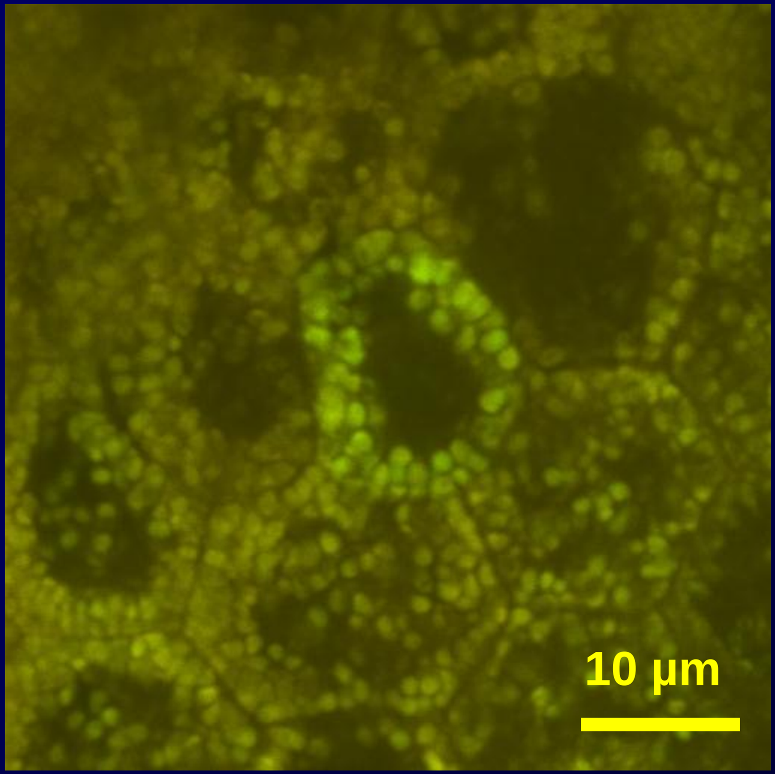


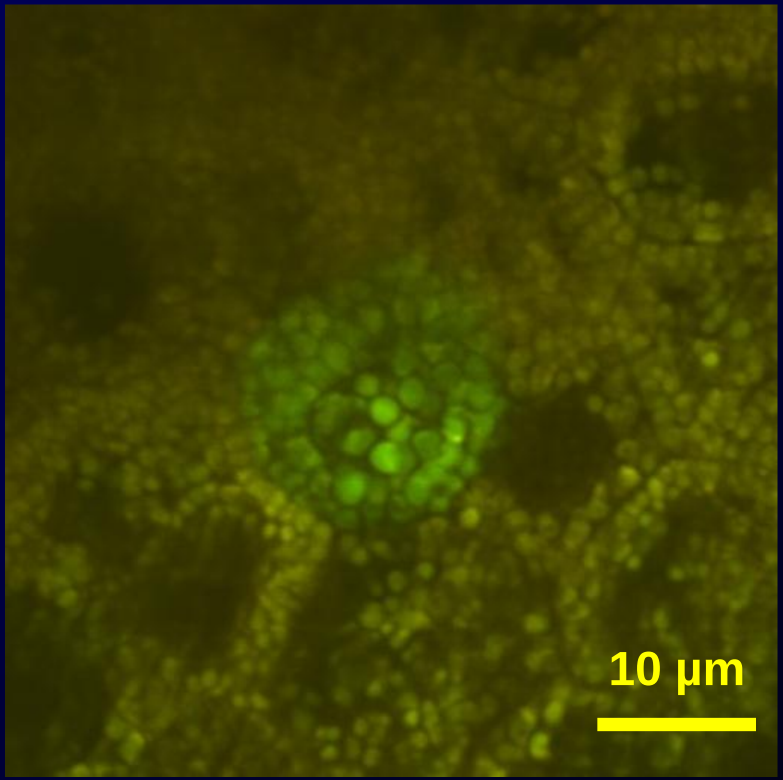
Confocal imaging
Argon laser 488 nm

Two photon imaging
Ti:Sapphire laser 800 nm

IOVS 47,4553(2005)

Abnormal spectrum of AF

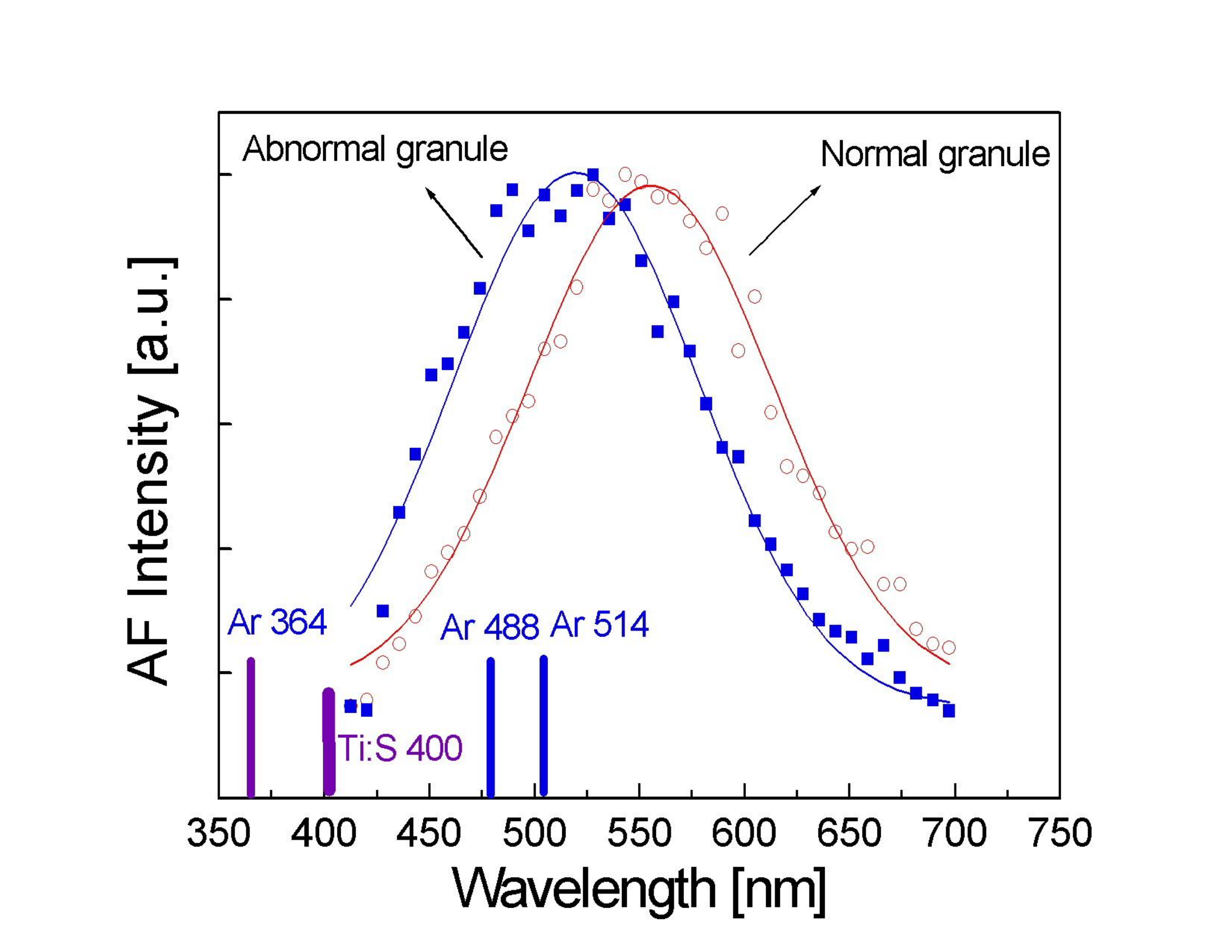




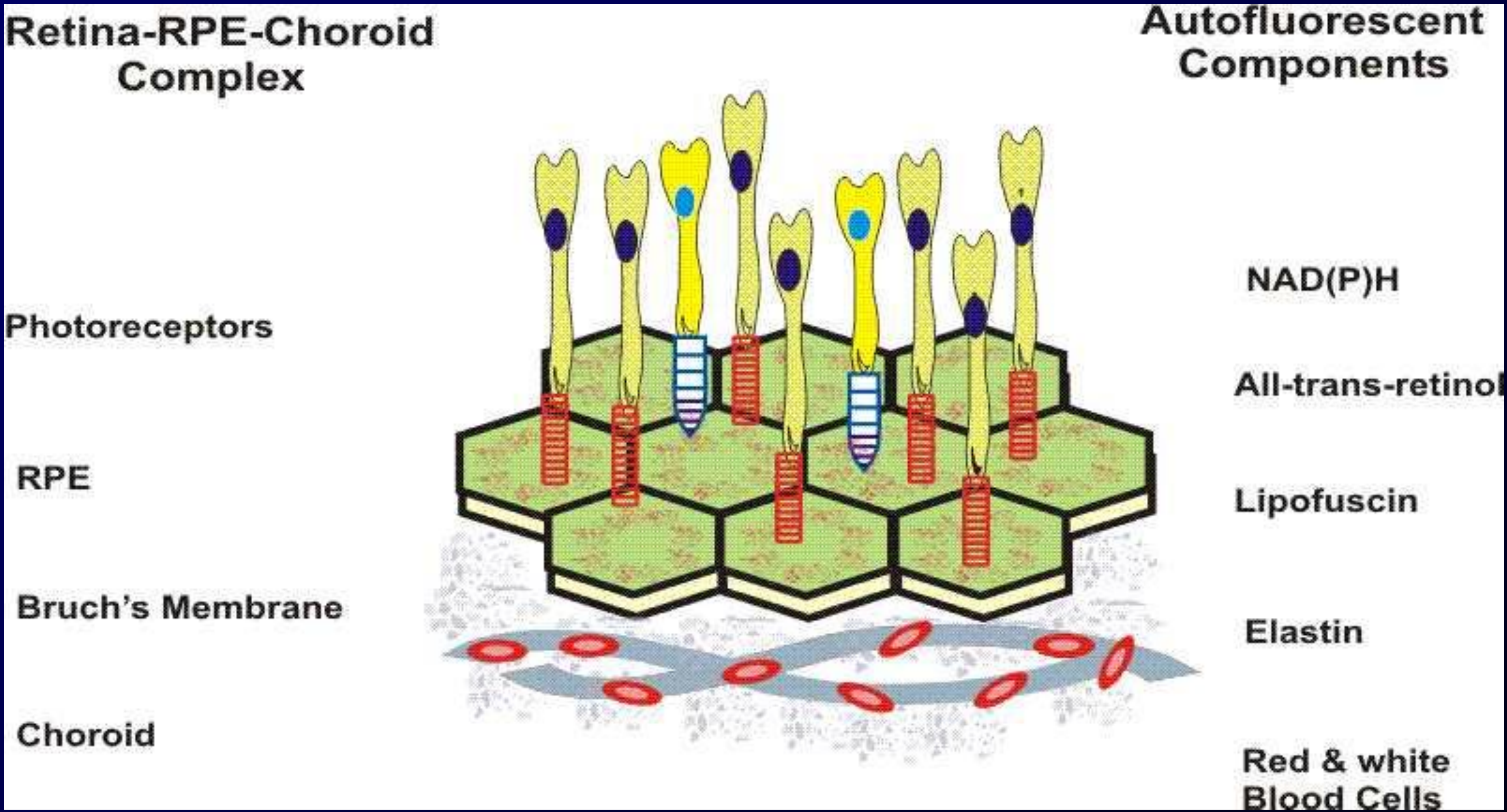
AF color coded: blue (500 – 550 nm) red (575-640 nm)

80 yrs patient, 16 hrs post mortem. RPE cells in the macula of retina.

Spectrum from abnormal granules

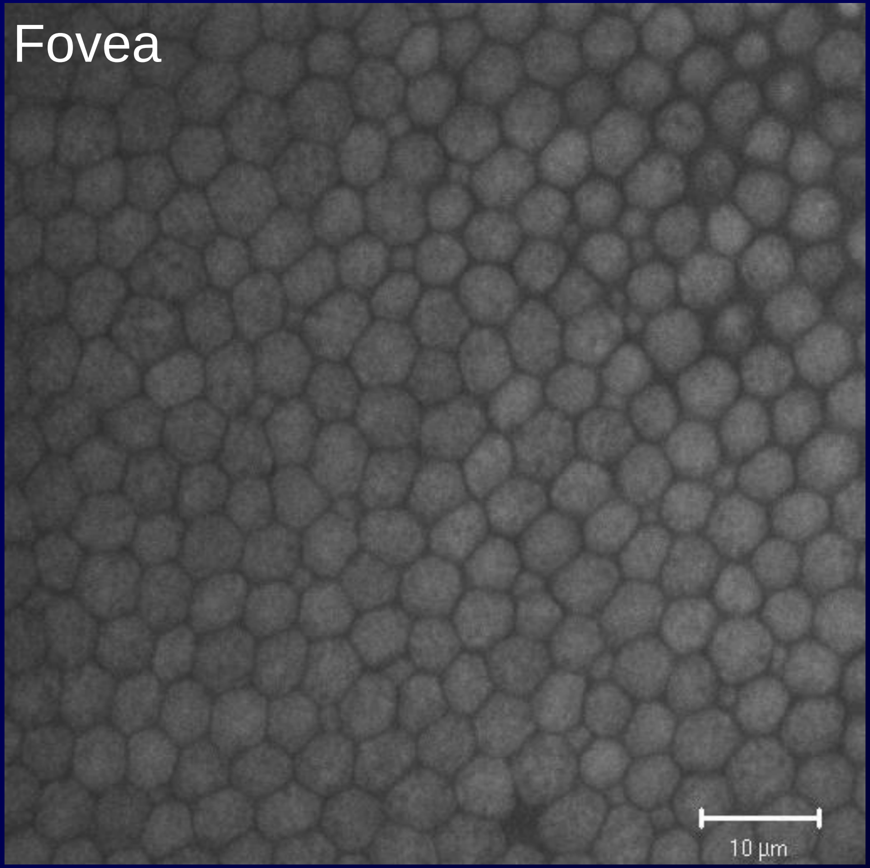


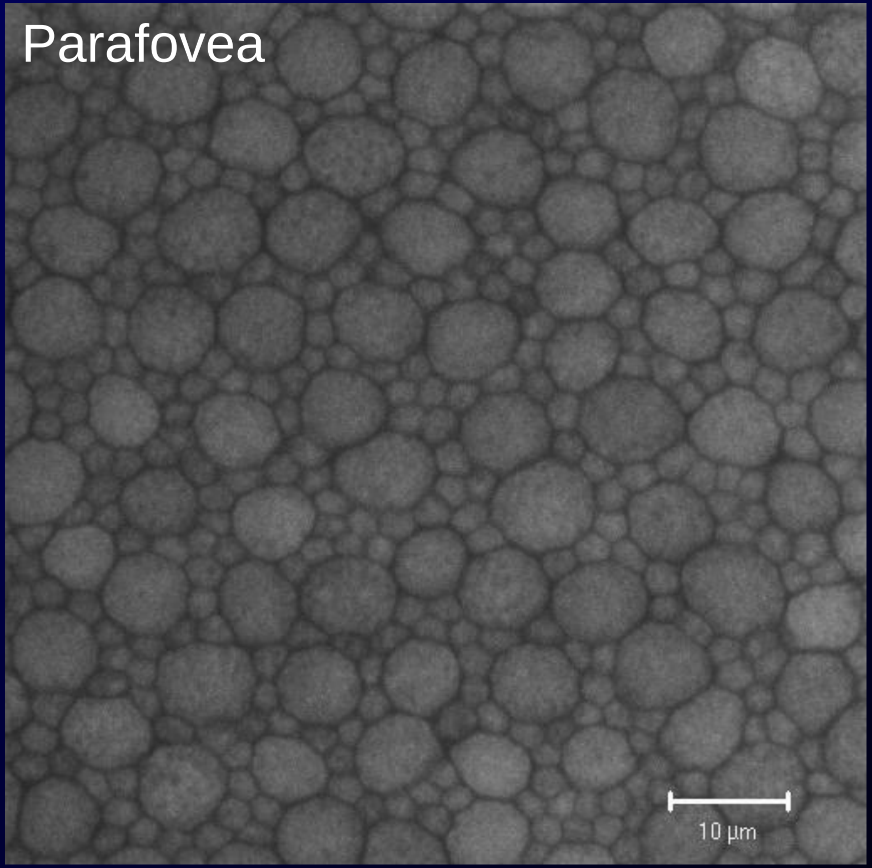
Autofluorescent Fluorophores in the retina-choroid complex



JBO 2007

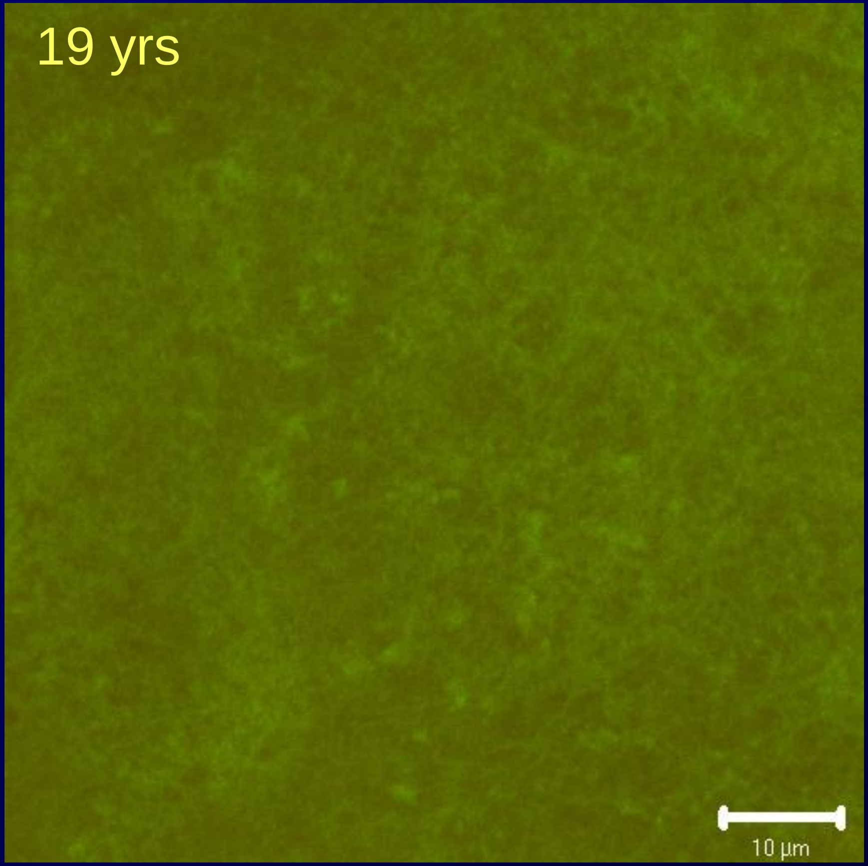
Foveal Cone/Rod Mosaic

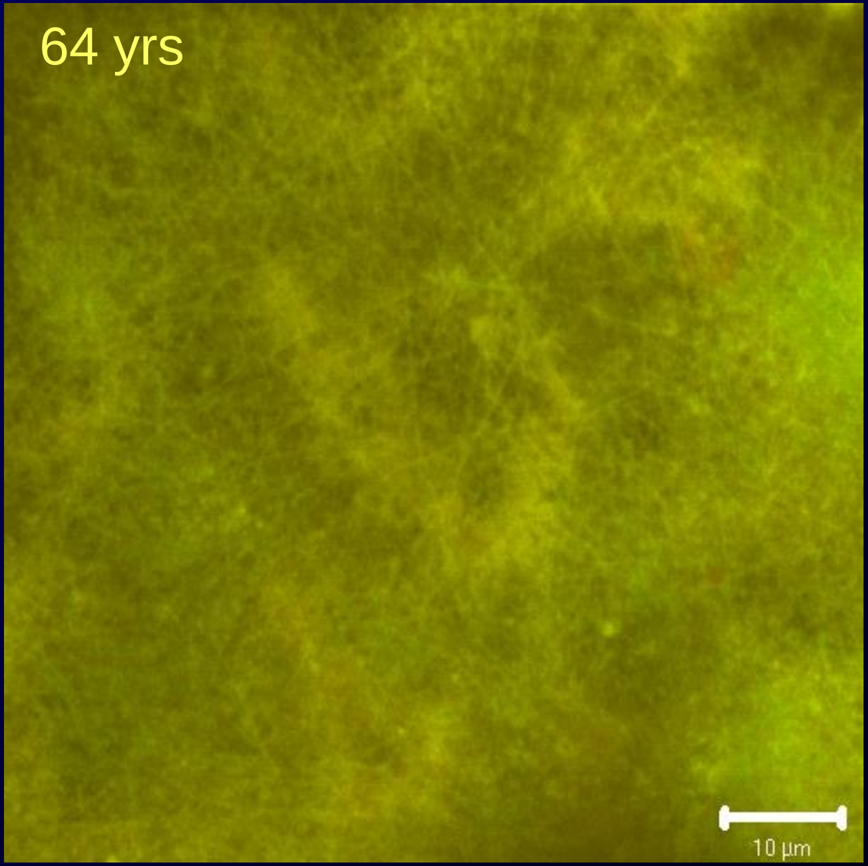




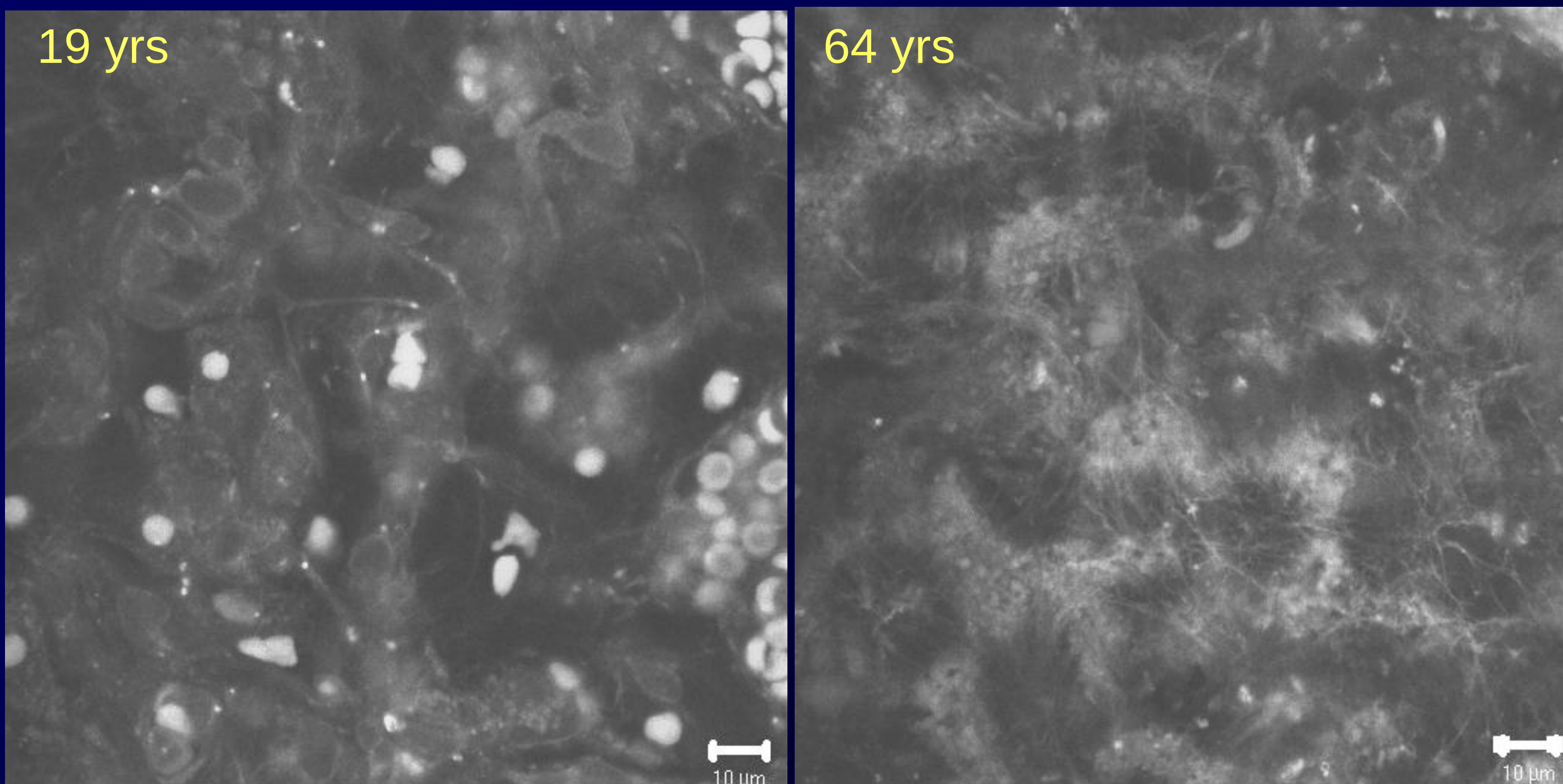
19 yr-old donor eye

Bruch‘s Membrane





Sub-choriocapillaries Layer



Future Developments

- In Vivo Two-Photon Imaging
- Bacterial Differentiation
- Laser Safety
- Customized Adaptive Optics

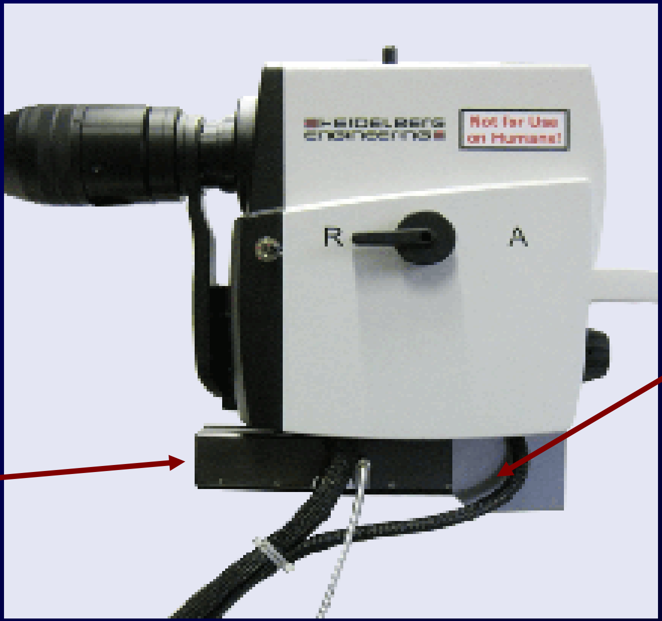
New In Vivo 2-Photon Ophthalmoscope

with 500mW Fiber-Laser and

AOM-controlled Intensity-Window

Two Photon Microscope with 500mW Laser

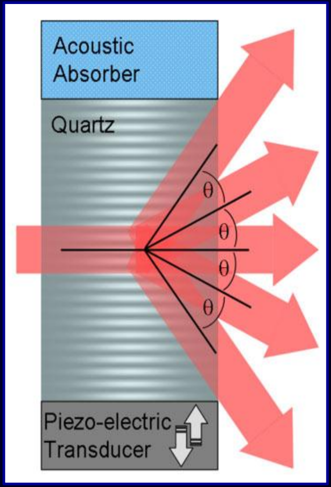
The Instrument



AOM

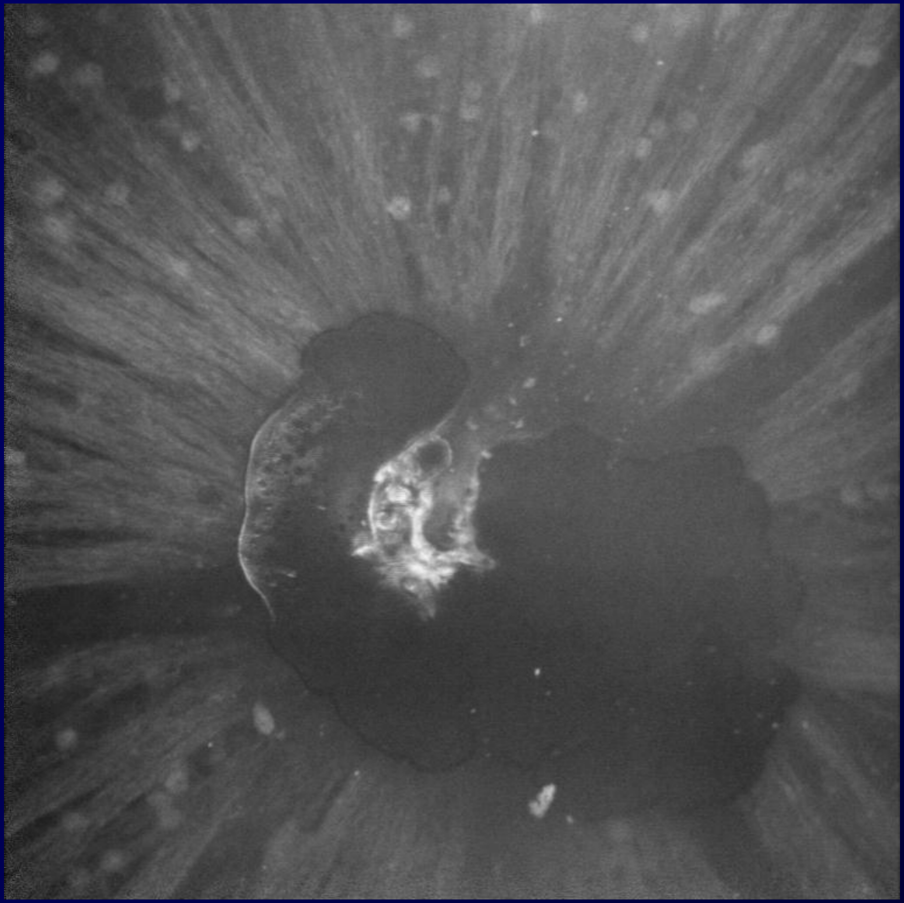
(Acousto Optic Modulator)

500mW Laser

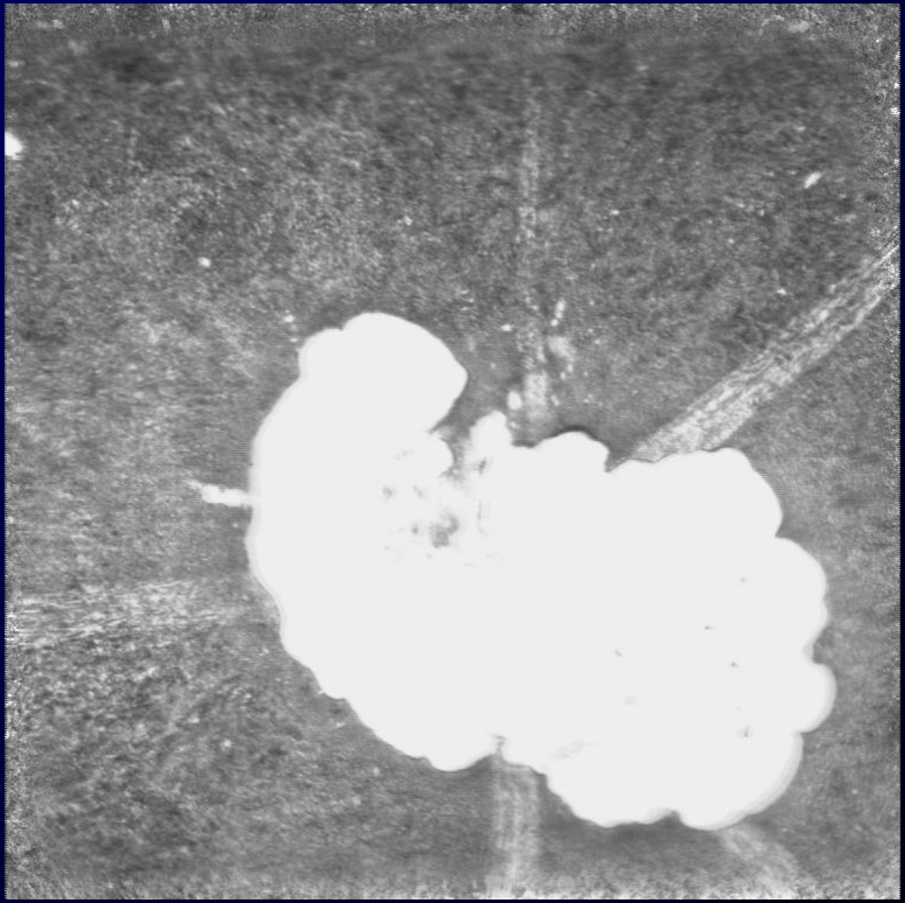


The AOM allows the instrument to modulate the intensity of 10 million spots per second inside a designated image area

Mice Optic Nerve Head

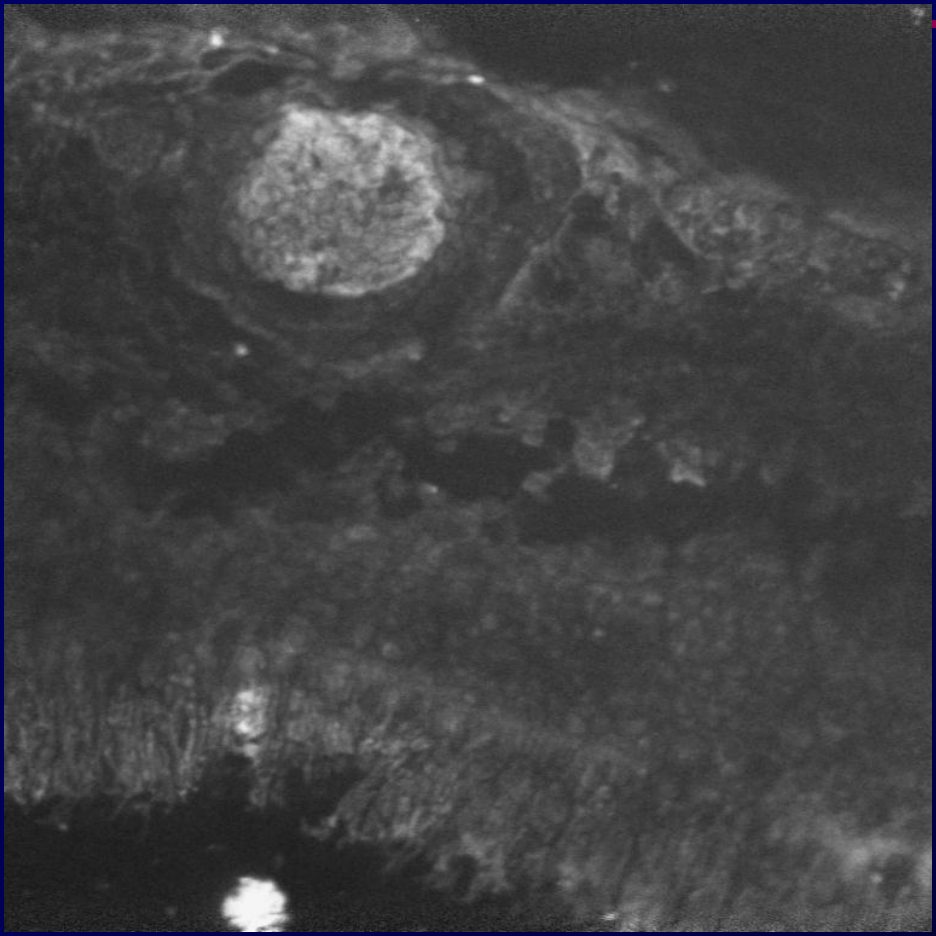


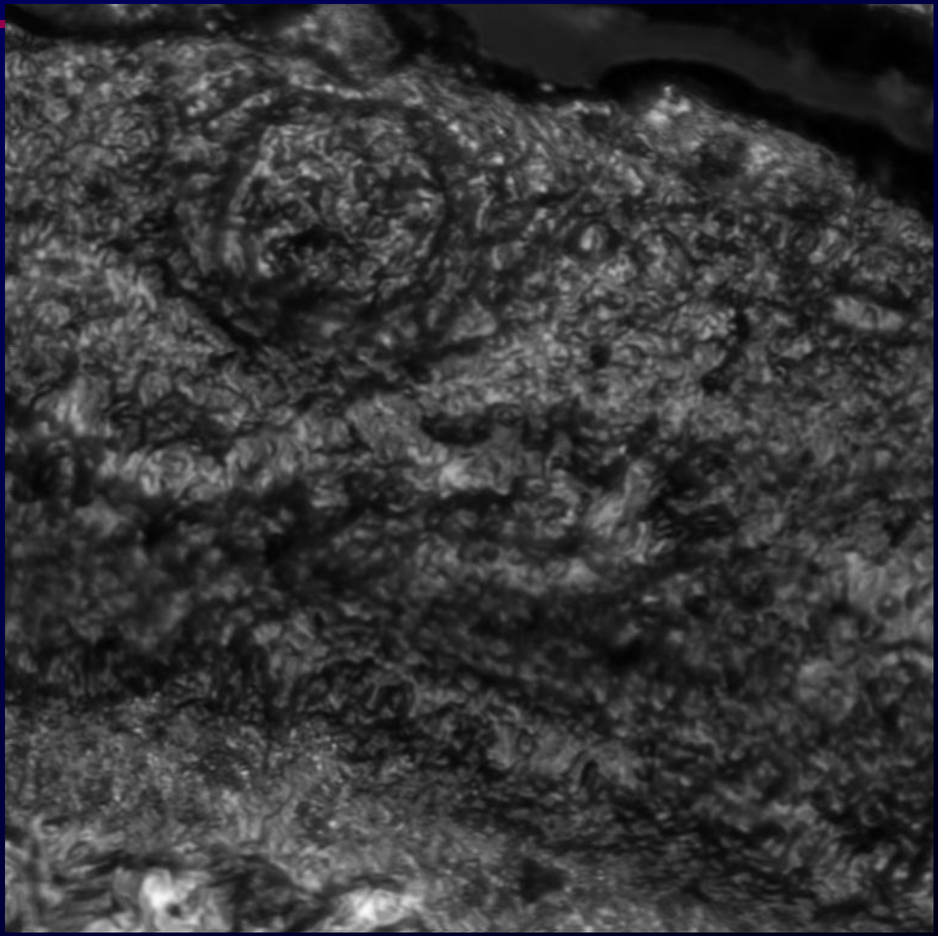
Two-Photon Image of Mice ONH.
190um * 190um



Confocal Image of Mice ONH.
190um * 190um

Abnormal Cell on Retina

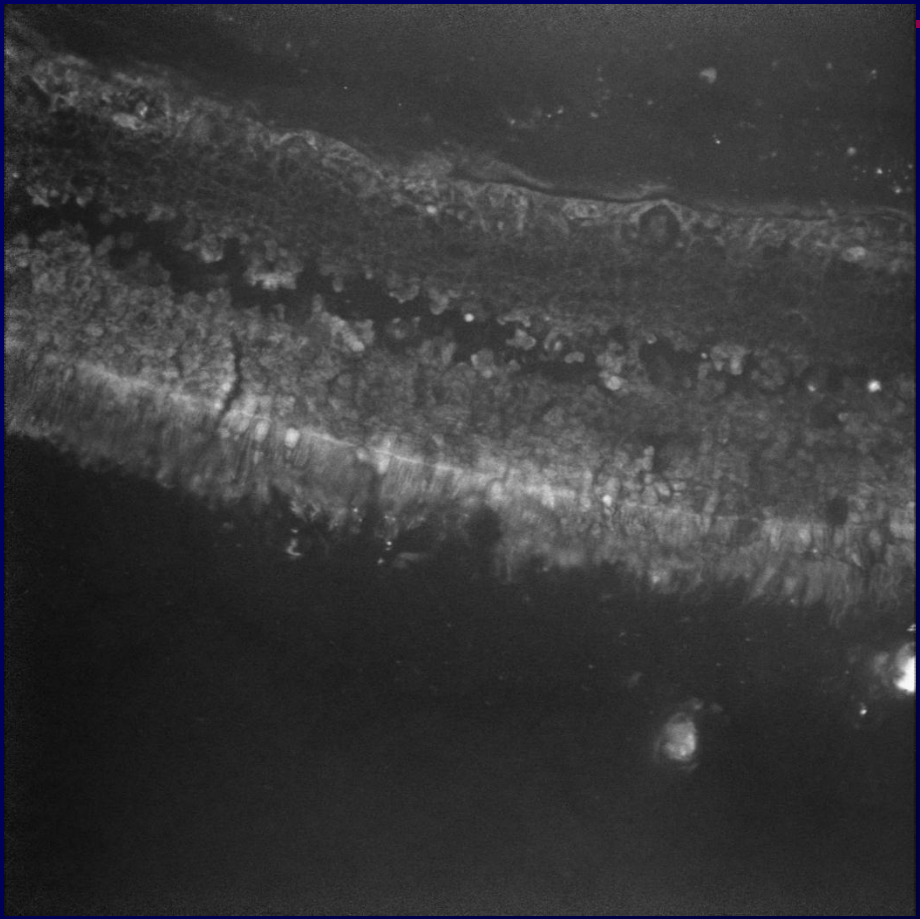




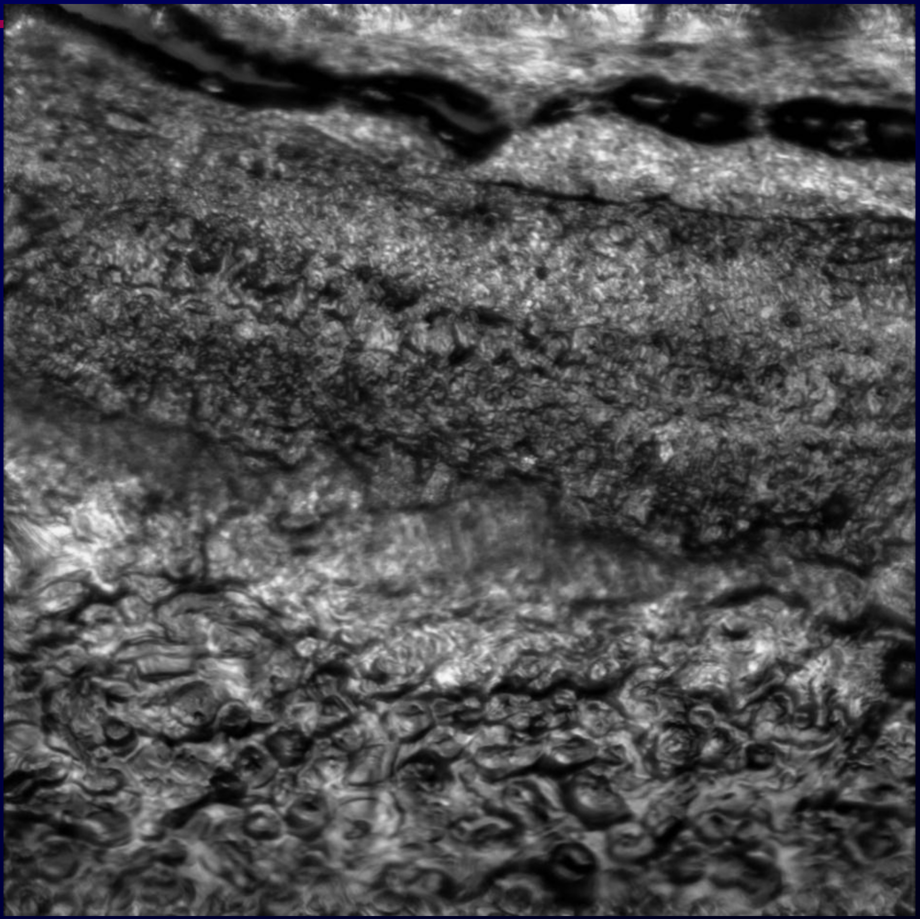
Two-Photon Image of abnormal cells on the retina 190um*190um

Confocal Image of abnormal cells on the retina 190um*190um

Cross Section of Retina

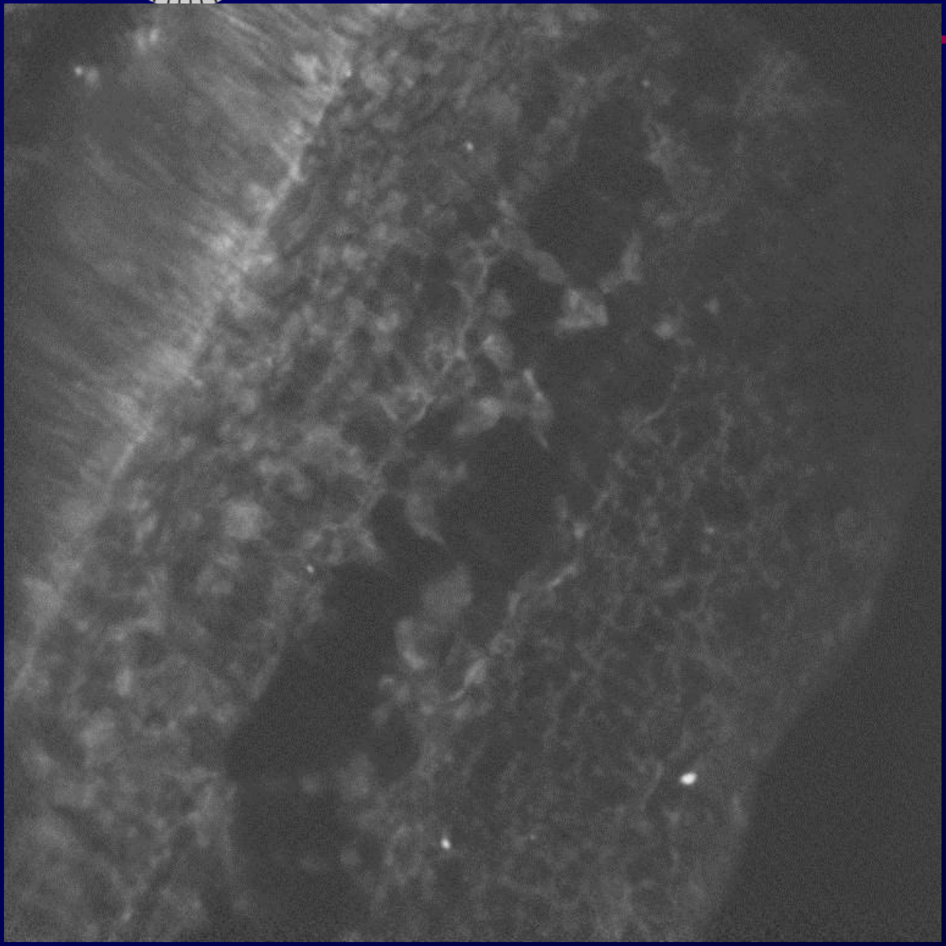


Two-Photon Image of Cross section of Retina 380um*380um

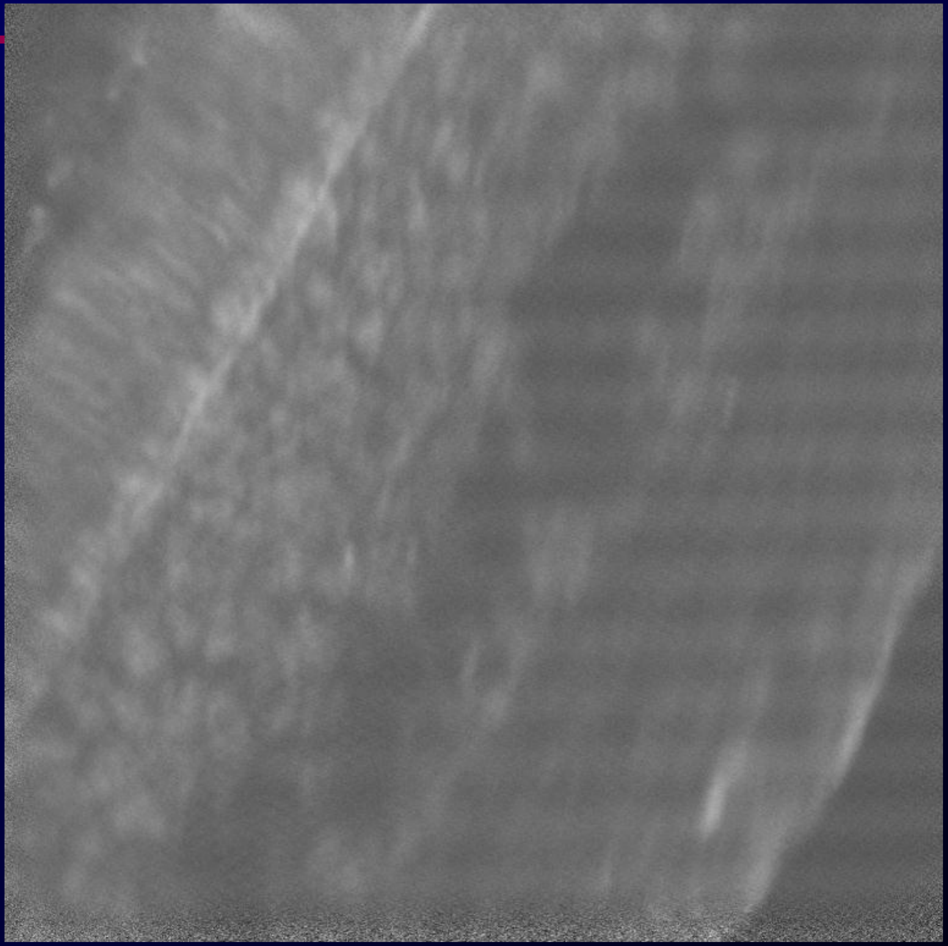


Confocal Image of Cross section of Retina 380um*380um

Cross Section of Retina by using different laser powers

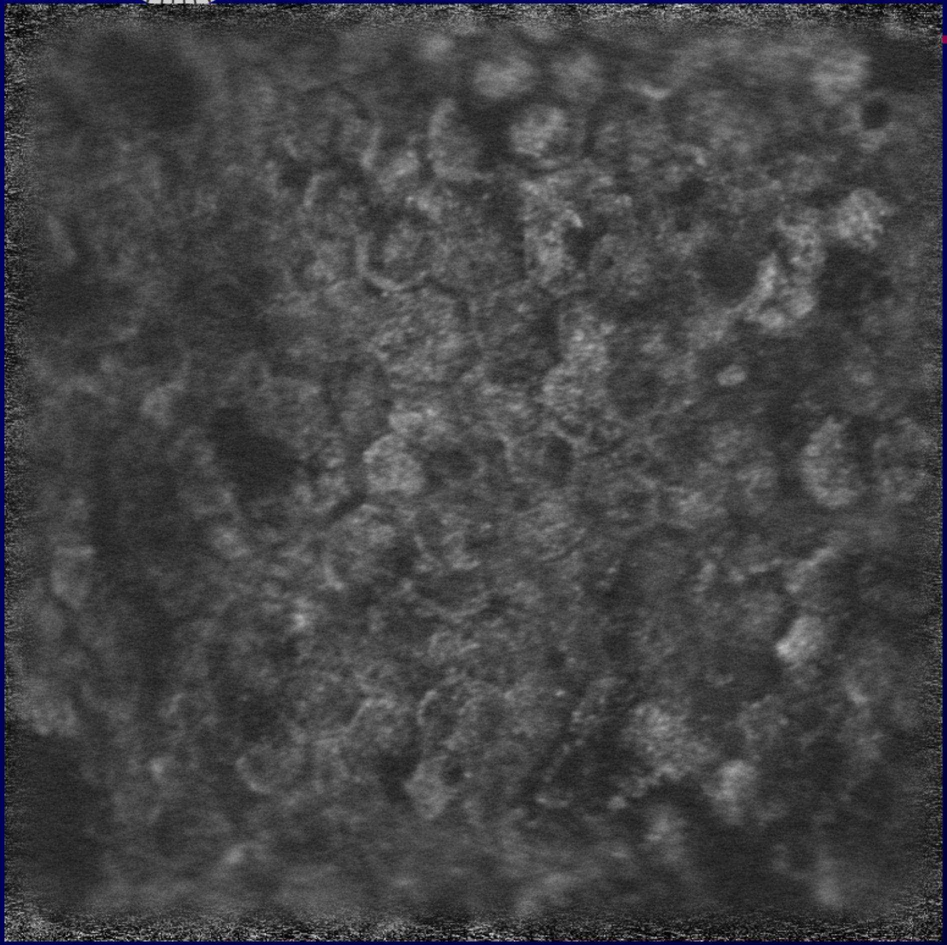


Two photon image of cross section of retina by using 100mW laser source (48mW before objective lens)

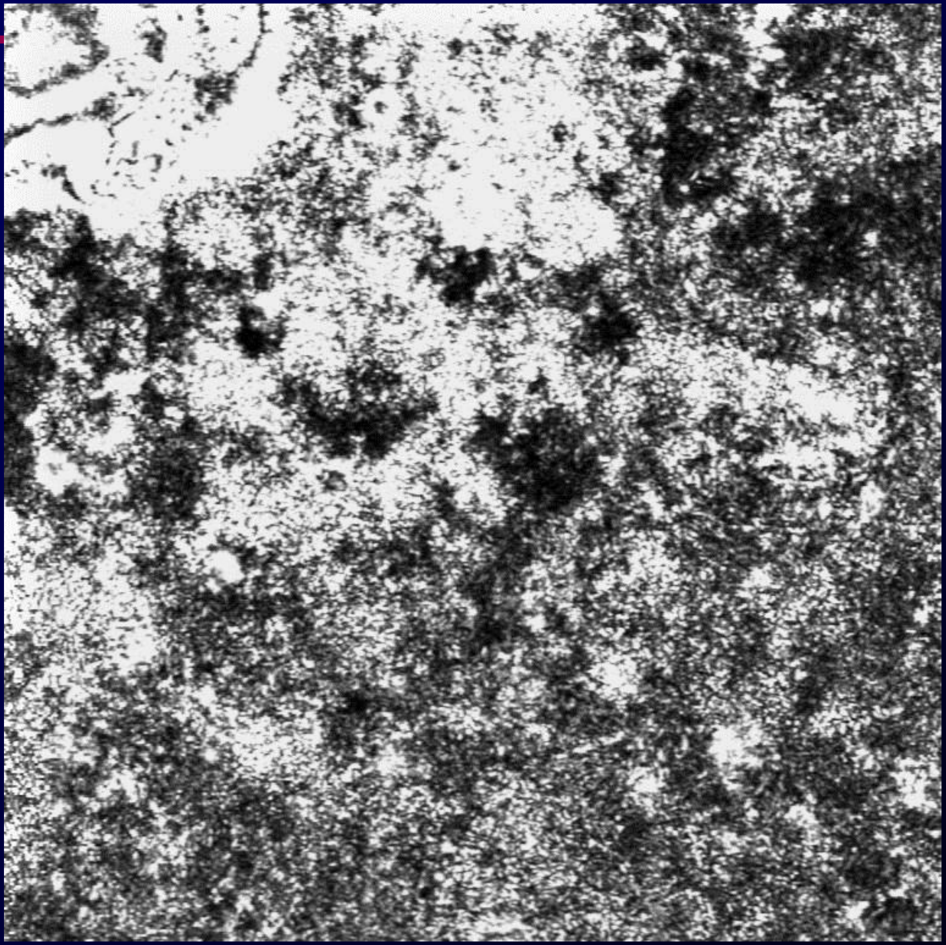


Two photon image of cross section of retina by using 40mW laser source(23mW before objectivce lens)

RPE Cells

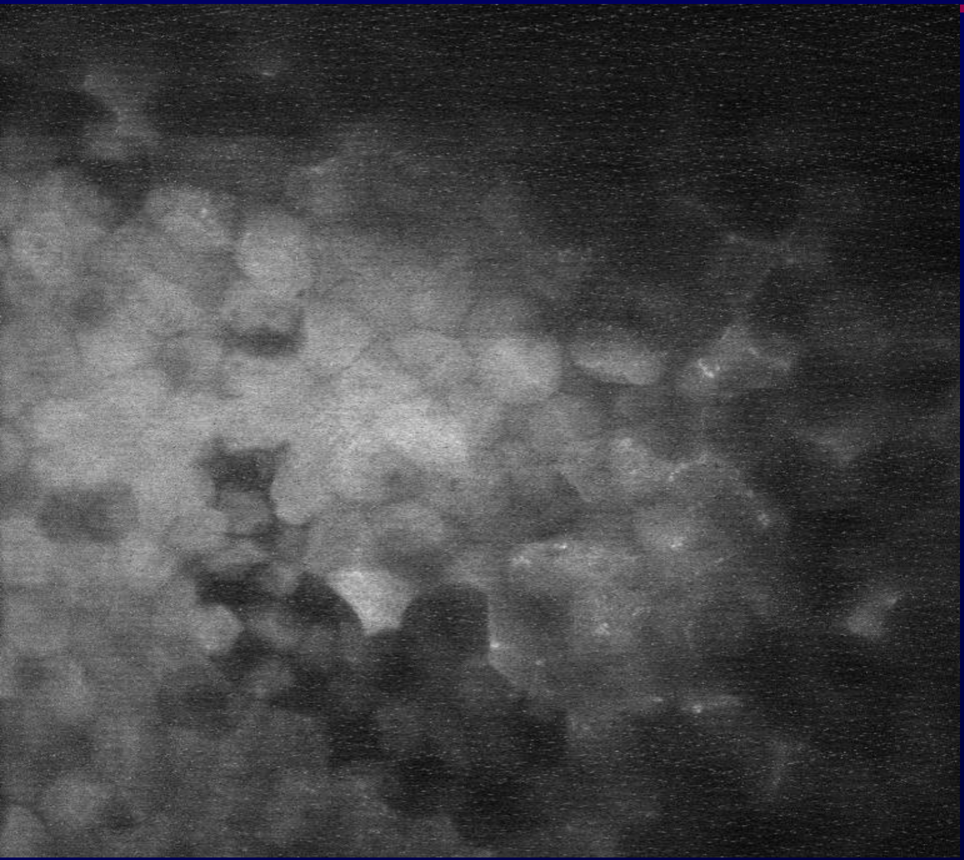


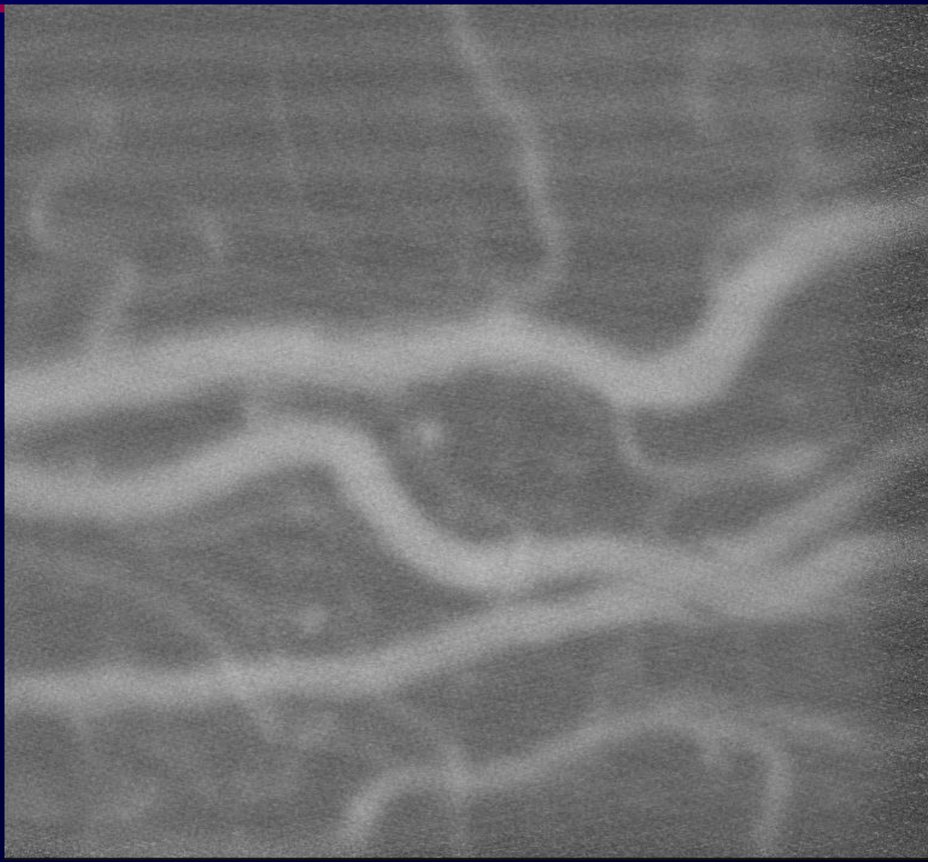
Two photon image of RPE cells



Confocal image of RPE cells

Two Photon images of living eyes



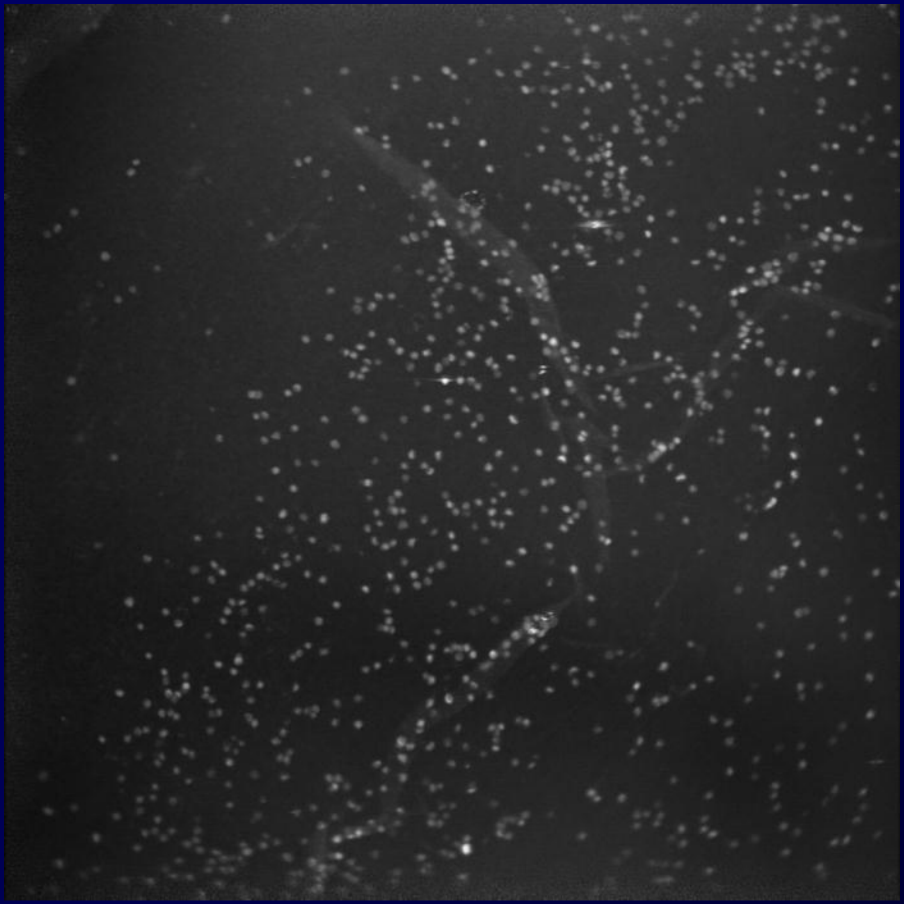


Two photon image of living rabbit cornea

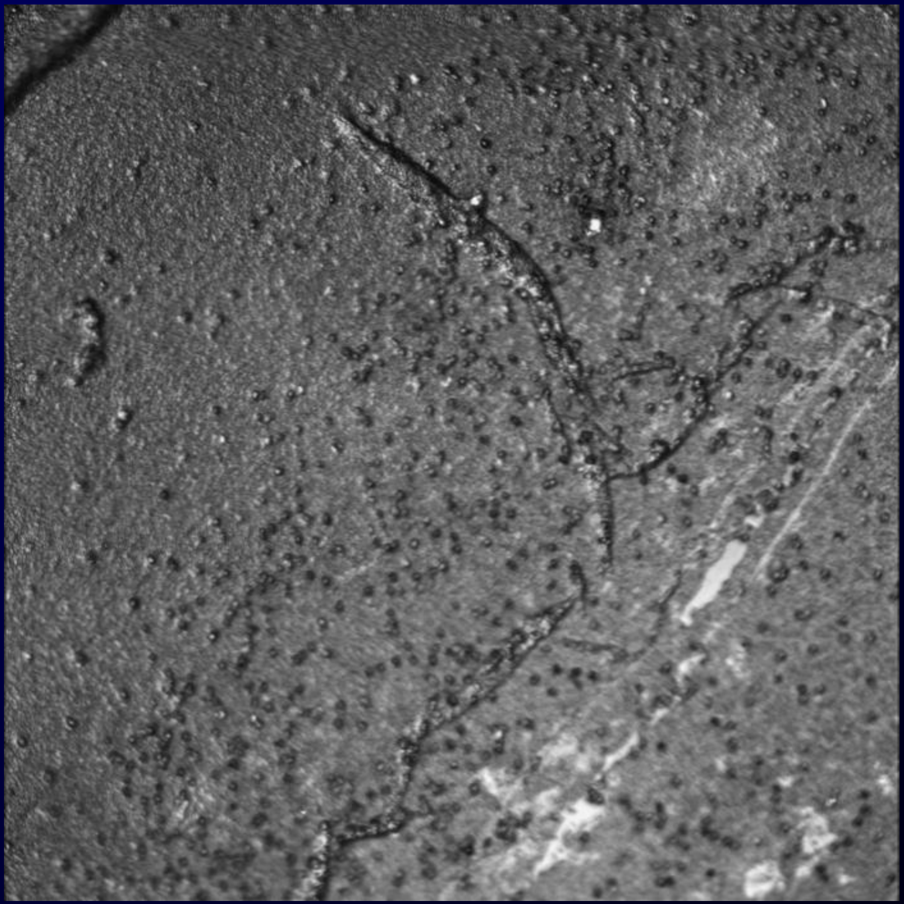
Two photon angiography of living rabbit retina

Two Photon Imaging of Bacteria

Streptococcus



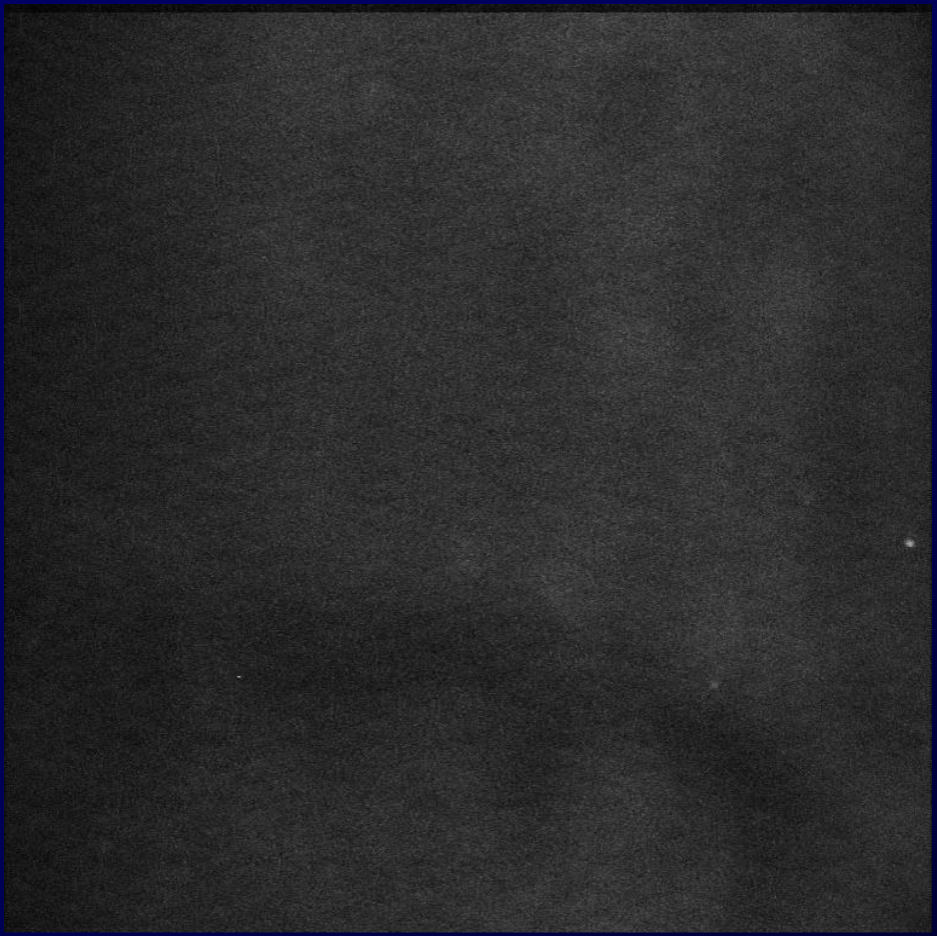
Two-Photon Image of *S. pneumoniae*.
380um * 380um



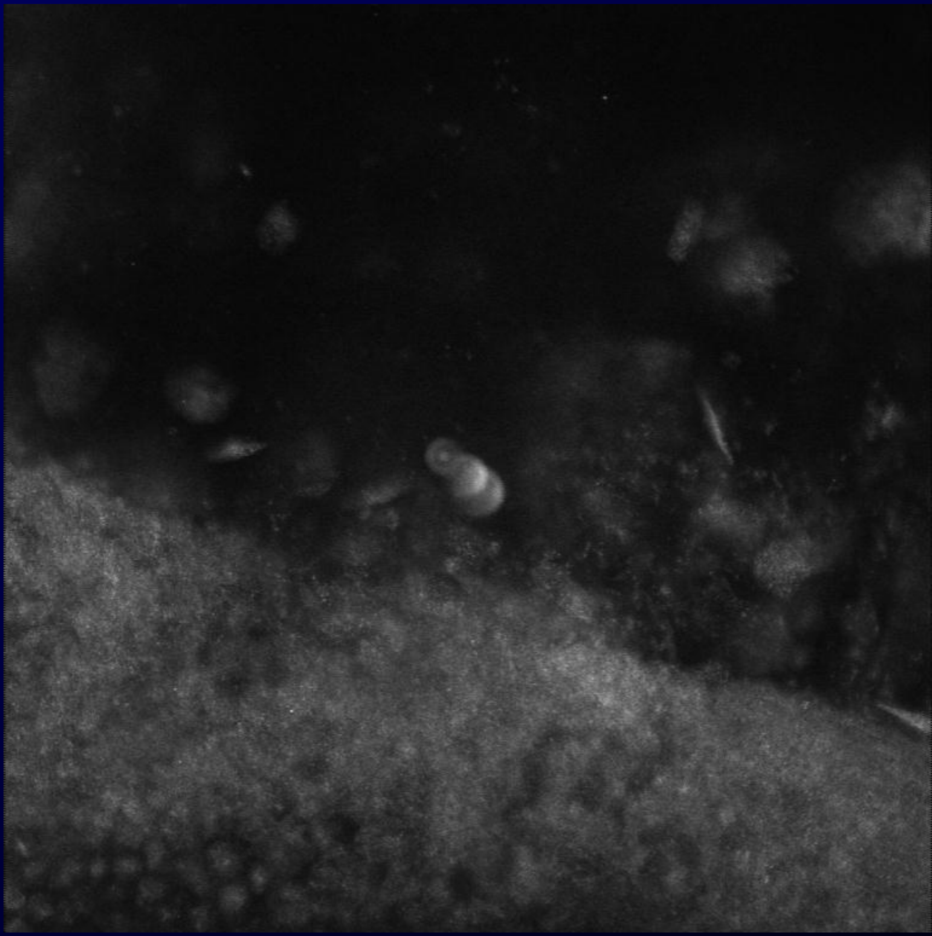
Confocal Image of *S. pneumoniae*.
380um * 380um

Karin E. Thomas, Yinhong Qu, Alexander K. Schuster, Tracy L. Purcell, Josef F. Bille, David J. Schanzlin. Two-Photon Ophthalmoscope Aids in Diagnosis of Infectious Keratitis. *Invest Ophthalmol Vis Sci* 2011; 52:ARVO E-Abstract 5851

Streptococcus Infected Cornea Button

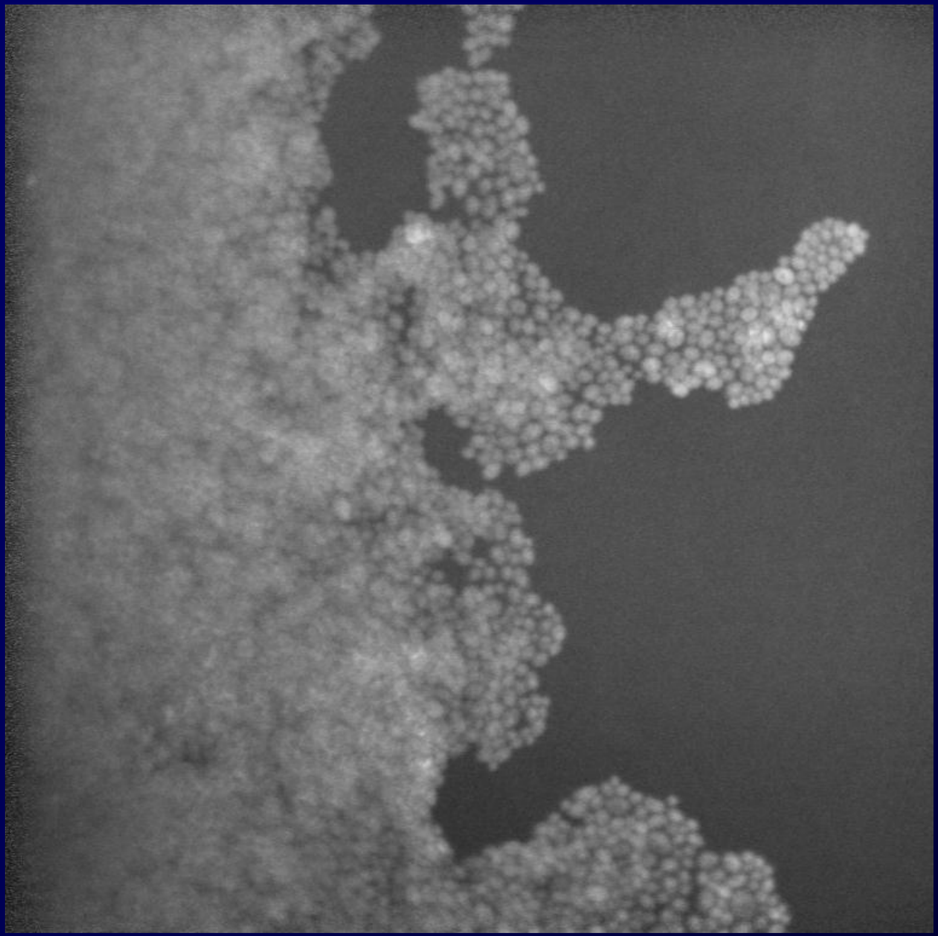


Two-Photon Scan through of *S. pneumoniae*. Infected Cornea Button

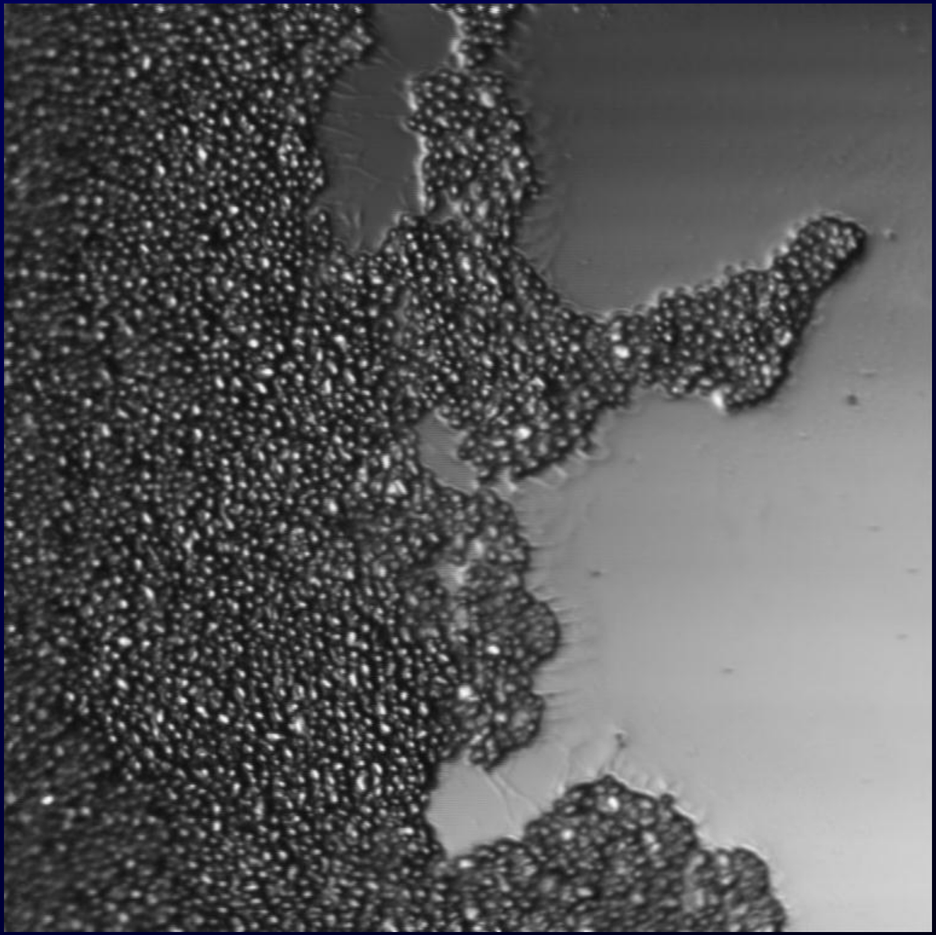


Confocal Scan through of *S. pneumoniae*. Infected Cornea Button

Candida Albicans

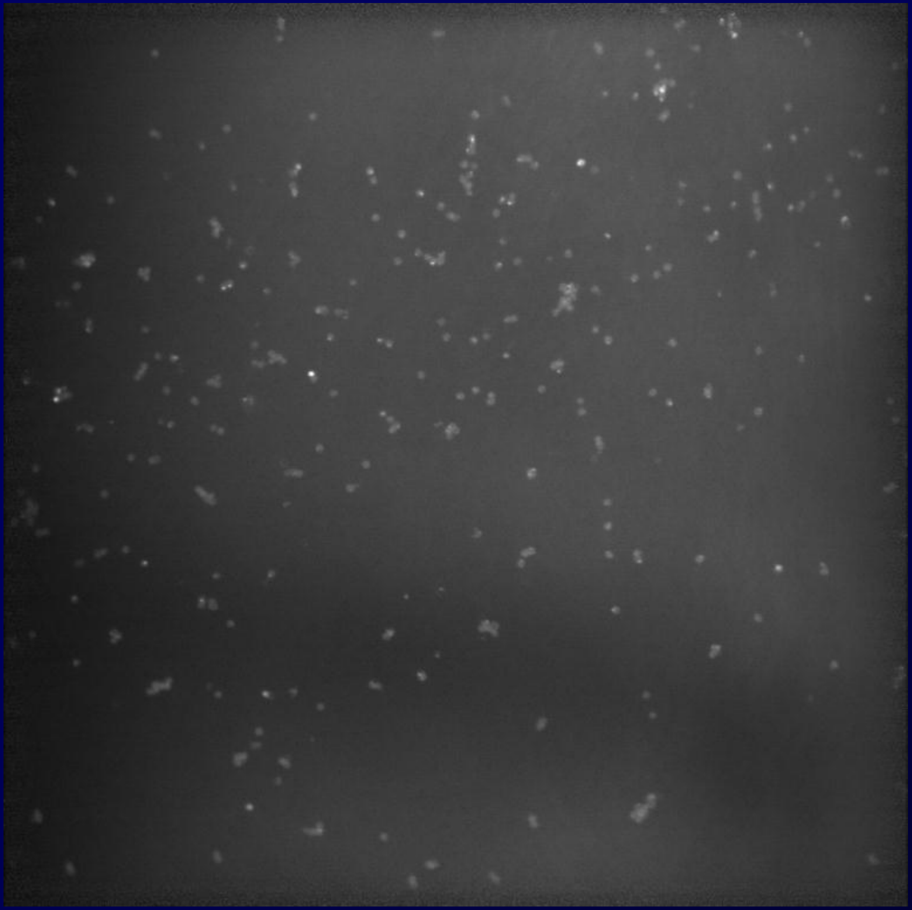


Two-Photon Image of *Candida albicans*
190um * 190um

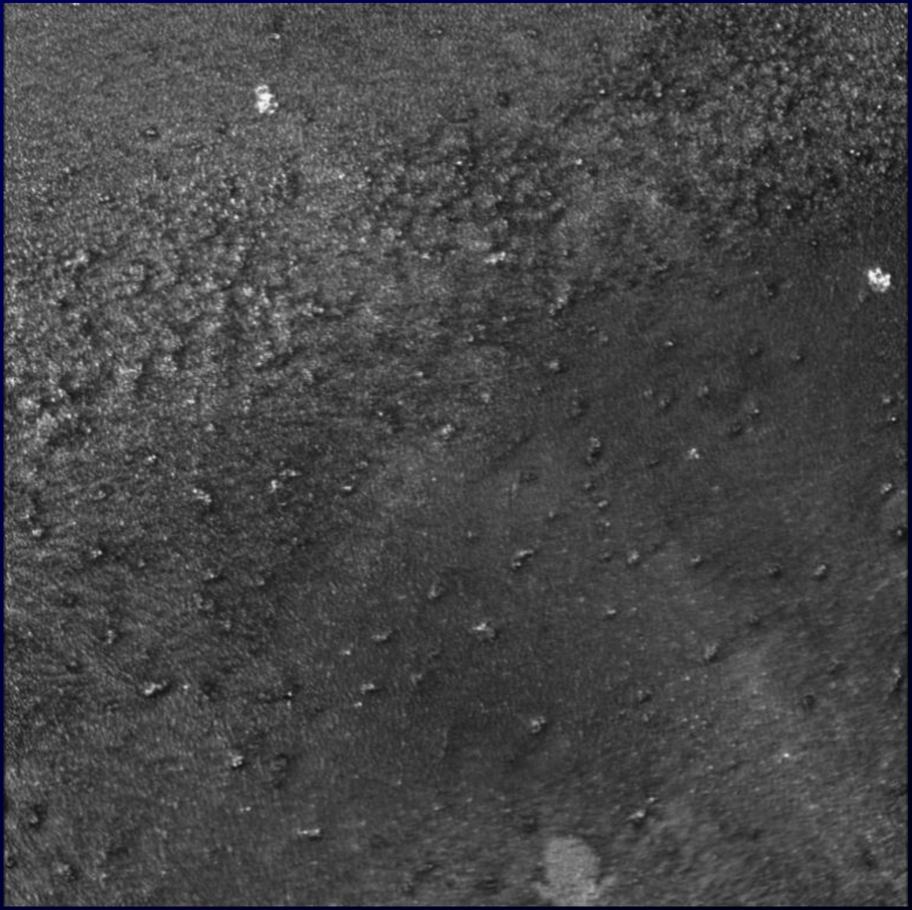


Confocal Image of *Candida albicans*
190um * 190um

Aspergillus

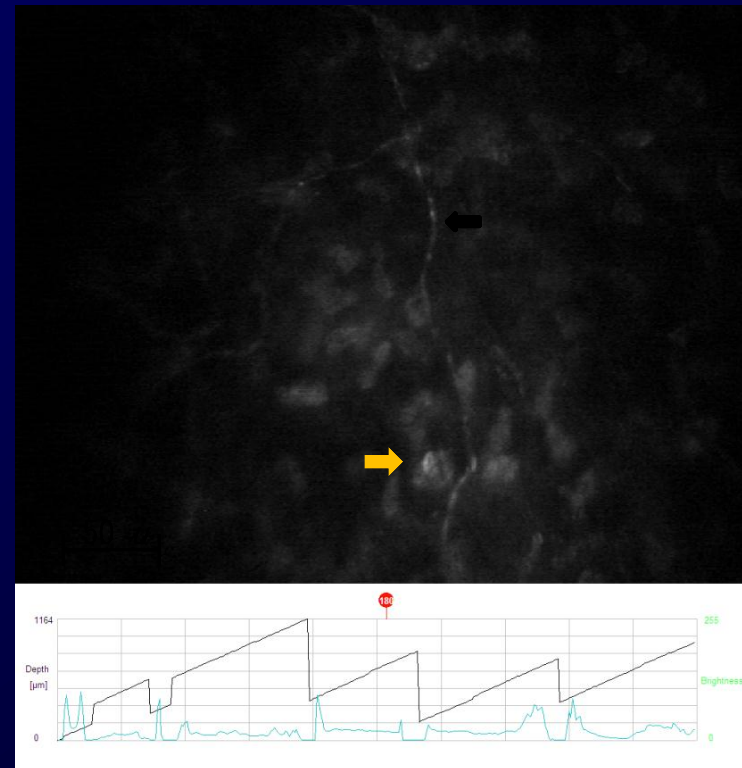


Two-Photon Image of *Aspergillus*
380um * 380um



Confocal Image of *Aspergillus*.
380um * 380um

Acanthamoeba Castellanii



Two-Photon Ophthalmoscope (50mW)

Note 3 cysts (black arrow) and one trophozoite (orange). Note increased image resolution compared to the Confoscan.

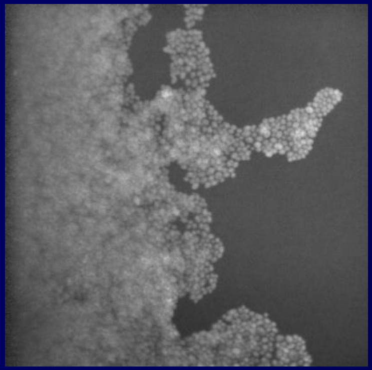
Amoeba provided by Dr. Visvesvara, Division of Parasitic Diseases,
Centers for Disease Control and Prevention

Nidek Confoscan Image

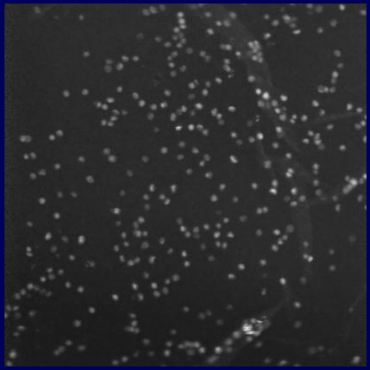
Cornea infected with *Acanthamoeba*. Note radial keratoneuritis (white arrow) and trophozoite (orange).

Image provided with permission from San Diego Eye Bank.

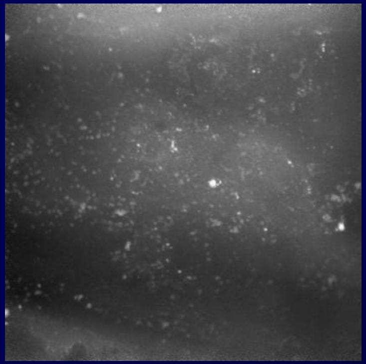
Identification of Microorganisms by using different laser powers



Candida albicans , 190um*190um
40mW,780nm,250fs



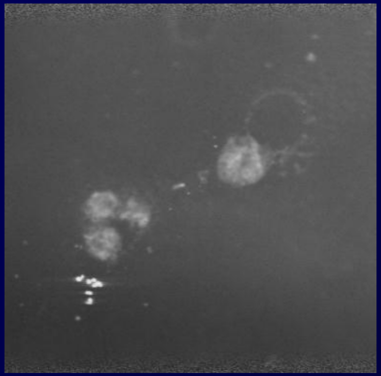
Streptococcus,
190um*190um
50mW,780nm,250fs



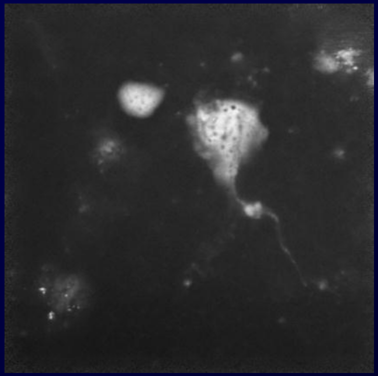
Pseudomonas Aeruginosa, 190um*190um
100mW, 780nm,250fs

30mW 50mW 100mW 150mW

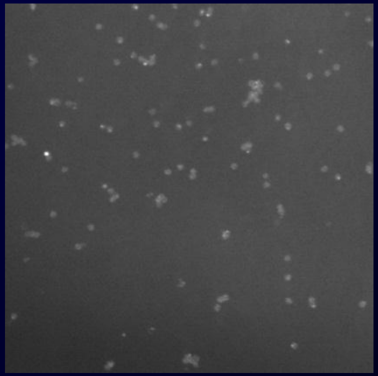
0mW 40mW 80mW 120mW



Acanthamoeba , 190um*190um
40mW,780nm,250fs



Microsporidium ,190um*190um
80mW,780nm,250fs

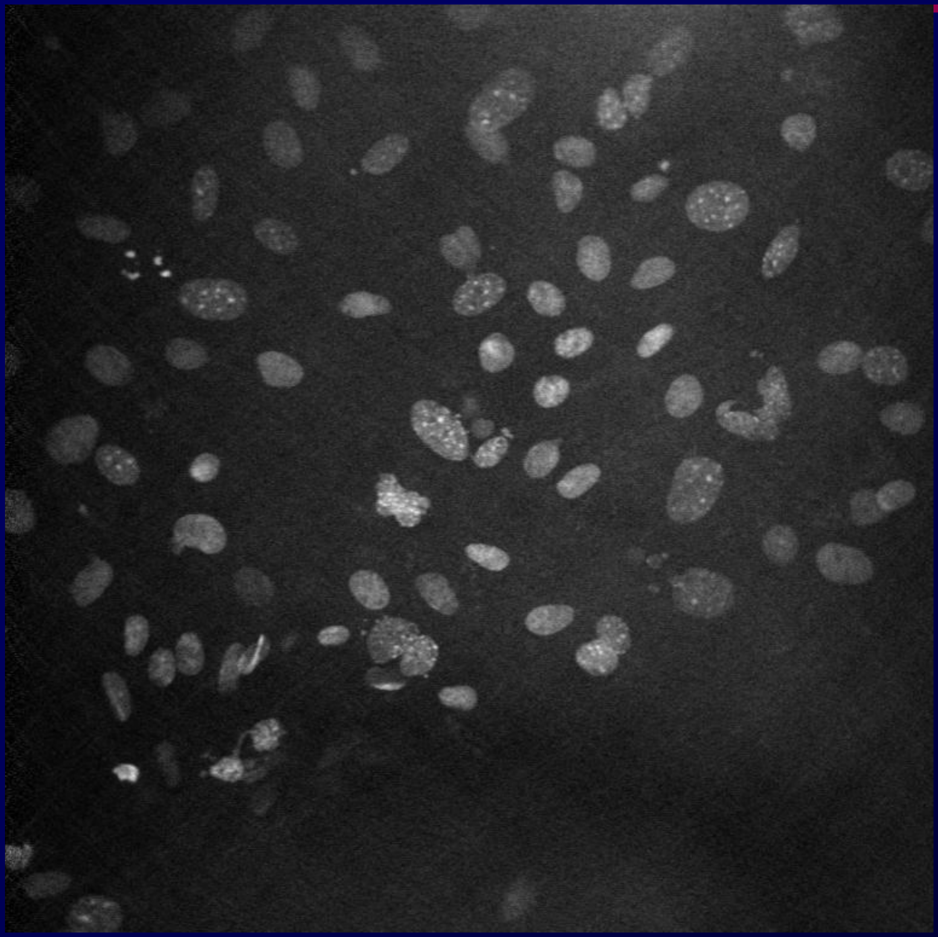


Aspergillus fungus,190um*190um
120mW, 780nm,250fs

Yinhong Qu, Karin E. Thomas, Alexander K. Schuster, Yi-Kai Wu, Tracy L. Purcell, II, Josef F. Bille, David J. Schanzlin. Identification of Microorganisms Utilizing the Two-Photon Ophthalmoscope. *Invest Ophthalmol Vis Sci* 2011; 52:ARVO E-Abstract 5823

Two Photon Imaging of Limbal Stem-Cells

Mice Limbal Stem Cells



Two-Photon Image of *Mice Limbal Stem Cells*
380um * 380um

Confocal Image of *Mice Limbal Stem Cells*
380um * 380um

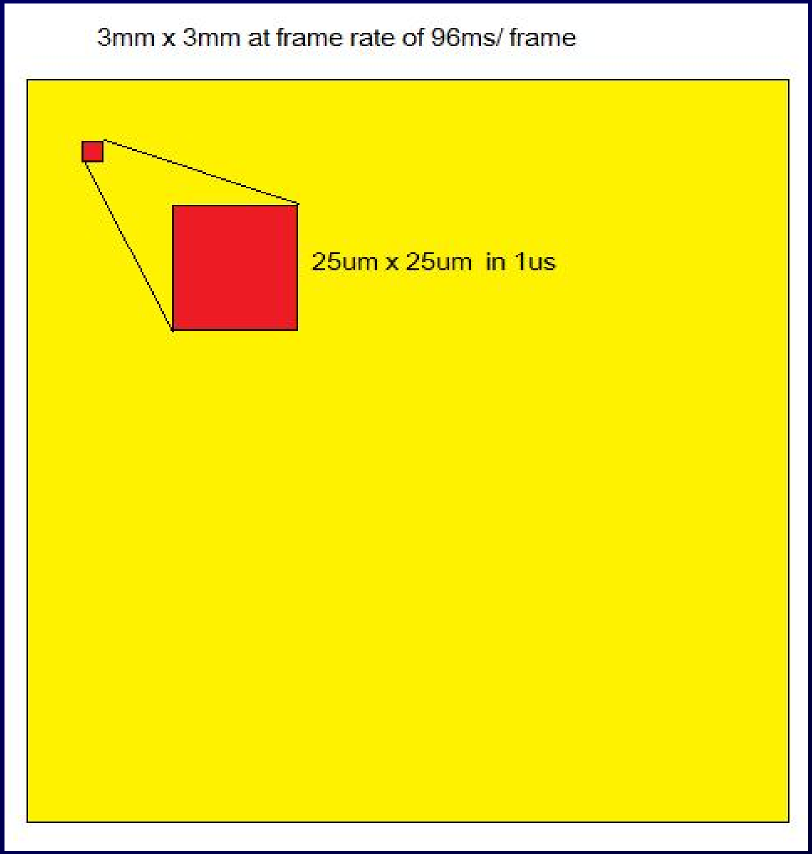
Laser Safety

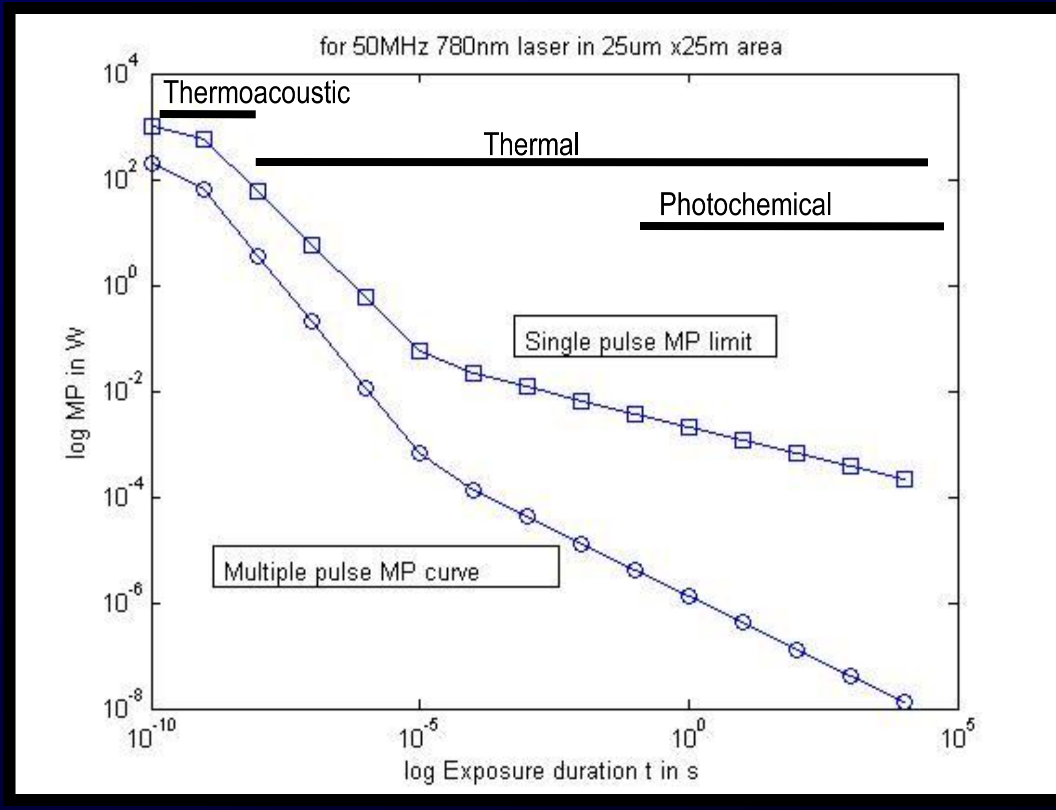
Maximum Permissible (MP) Corneal Irradiance

- Recommended MP corneal irradiance = $25*t^{-0.75}$ (W/cm^2) for t<10s
- Recommended MP corneal irradiance = 4.0 (W/ cm^2) for t>10s.
- The irradiated area is described by 1536µm * 1536µm = 0.0236cm^2 (HR-mode), therefore recommended MP is **94.4mW** for long exposure (t>10s) and for shorter exposure the power limit for 6 frames (1.2s) for HR-mode is **515mW**.

AOM

AOM-controlled Intensity Window / ANSI-Standard (Retinal Imaging)





MPΦ[150fs] =δ* 5.78*10^{-9} *CT *Cj* CE* t^{-1} = 625.5mW for one pulse

MPPav.25um[6.67us] = 1.93*10^{-7} *CT *CE* t^{-1} = 52.2mW (avearage power in $(25um)^2$ area)

Customized Adaptive Optics:

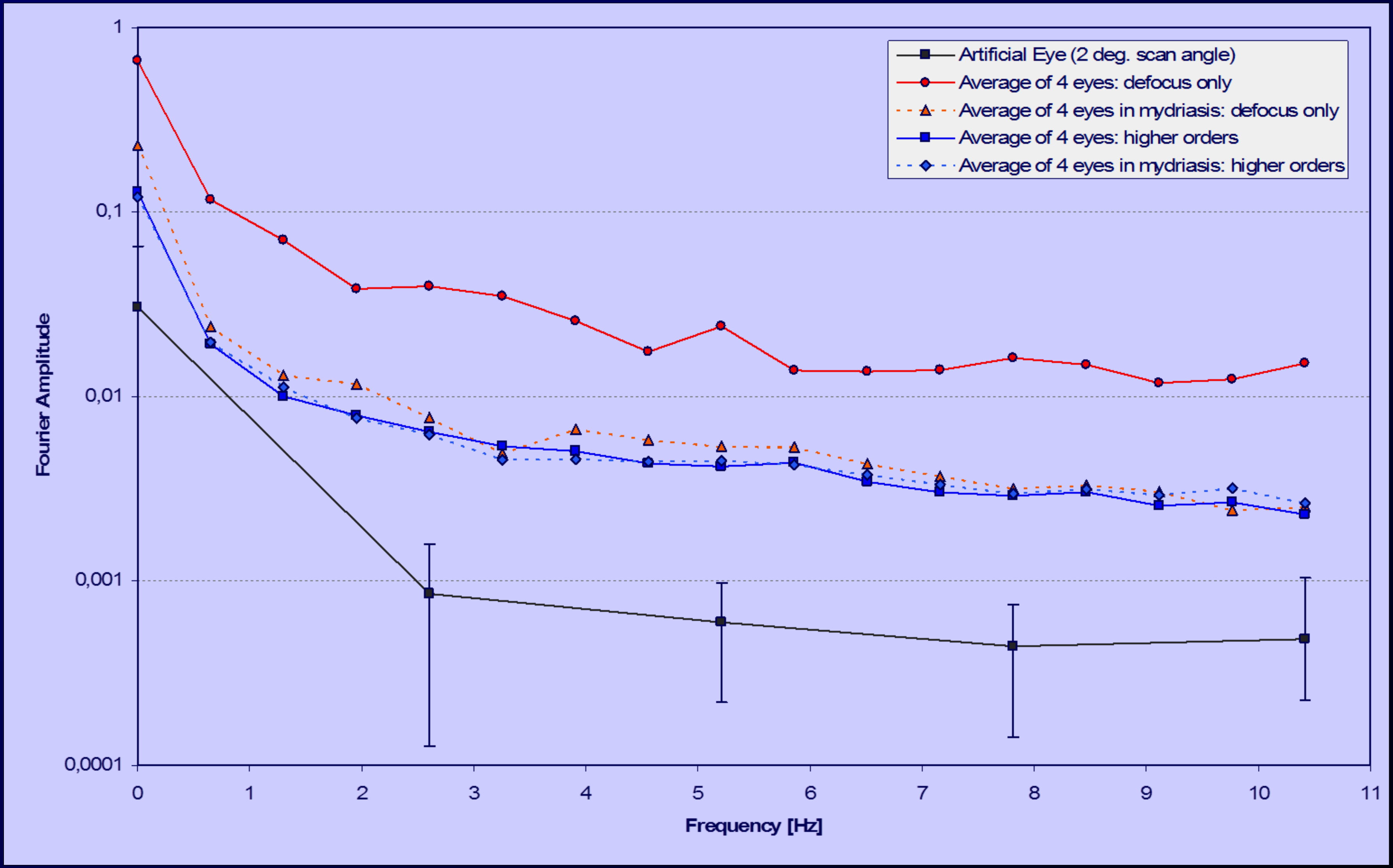
Adaptive Optics Two-Photon Ophthalmoscope

Compact Adaptive Optics System for Multiphoton Fundus Imaging

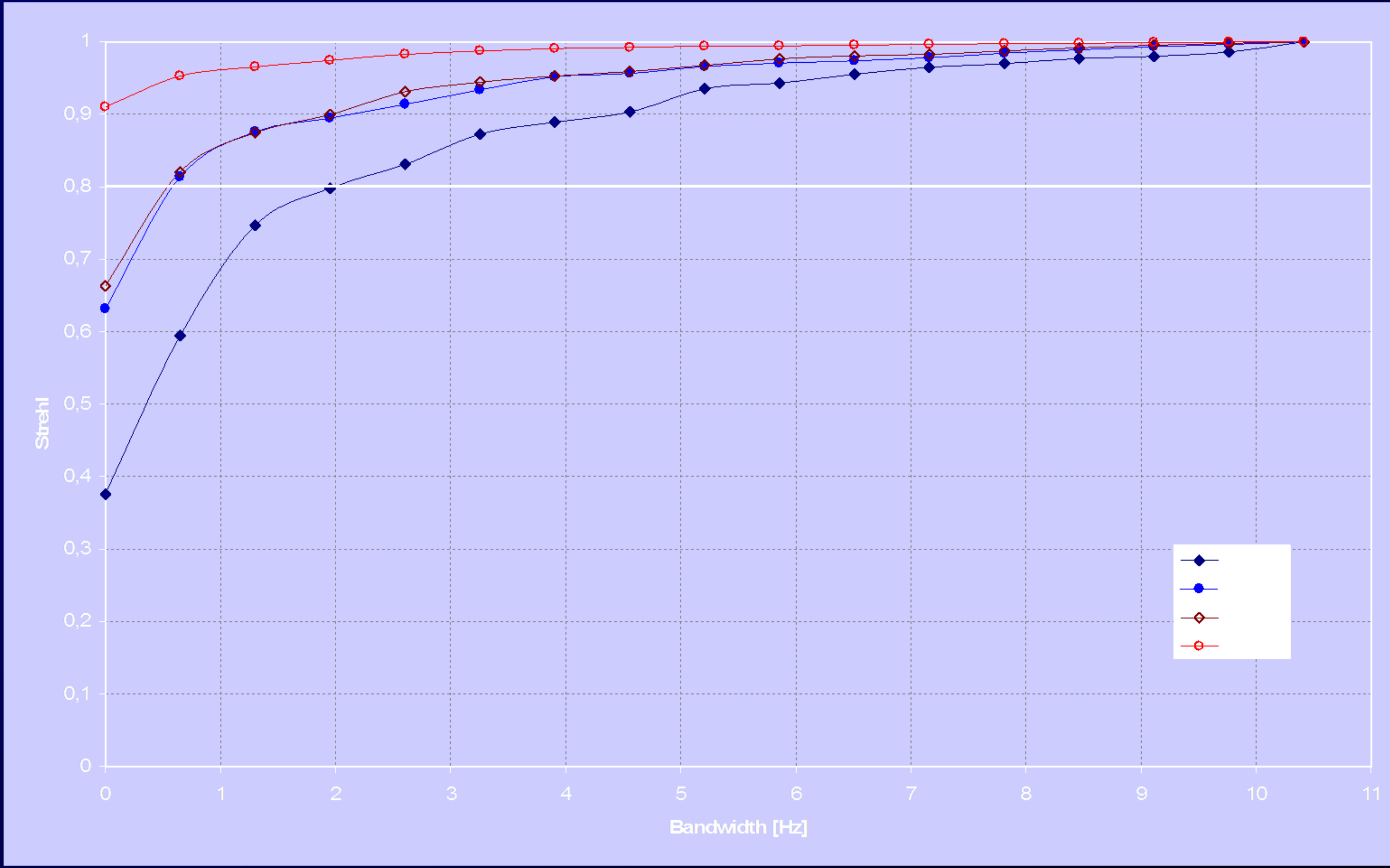
Josef Bille, Mikael Agopov, Cristina Alvarez-Diez, Meng Han, Nina Korablinova,
Olivier La Schiazza, Hongwei Zhang, Frank Mueller

Journal of Modern Optics
Vol. 55, No. 04, 2008, pp.749-758

Aberrations in dependence of frequency



Strehl in dependence of bandwidth

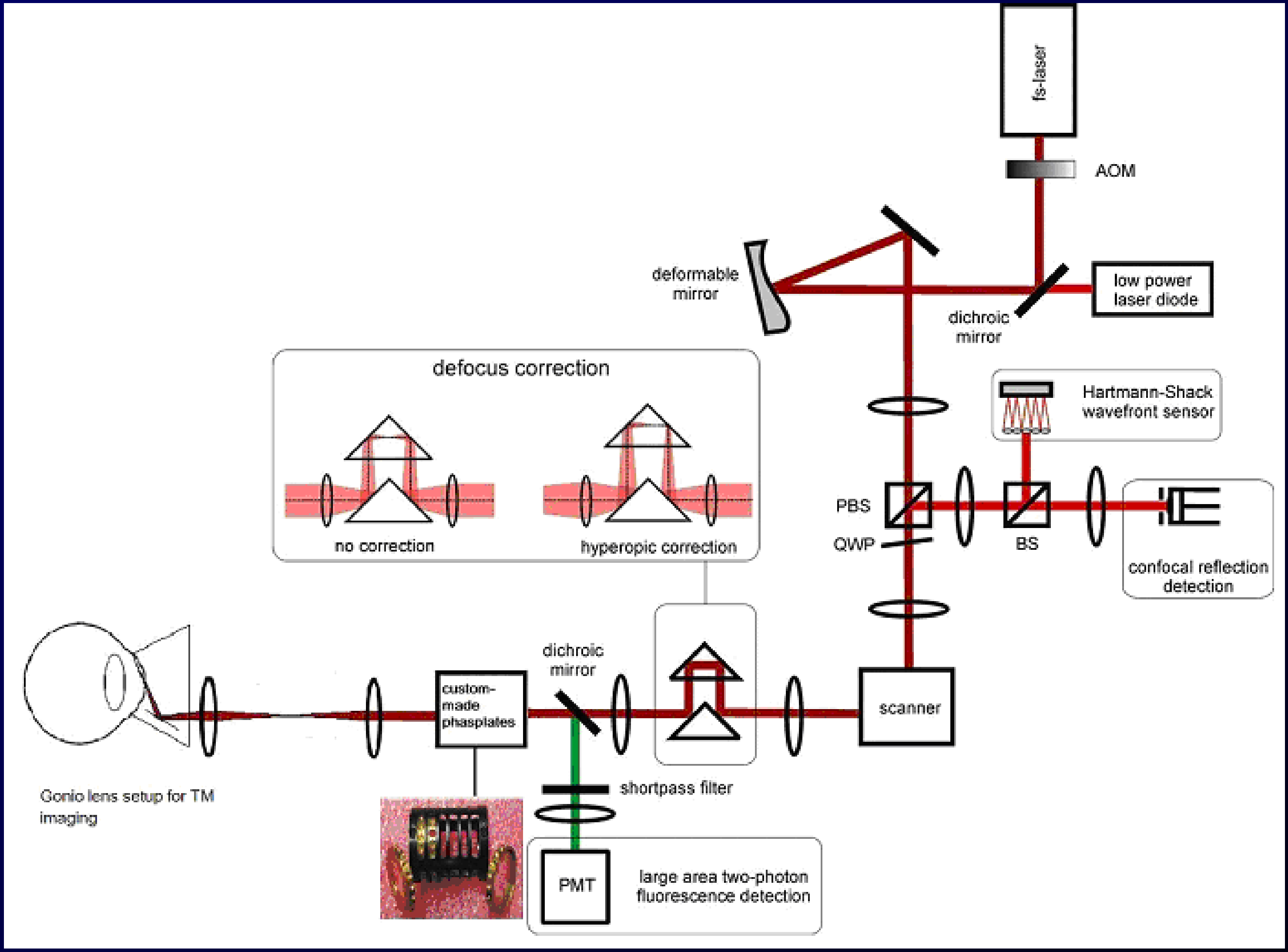


Strehl after static compensation

Proband	Eye	Strehl uncorrected (5.5 mm pupil)	Strehl after ideal spherocylindrical correction	Strehl after static correction of all aberrations
FM	OS	< 0.05	0.21	0.38
	OD	< 0.05	0.10	0.63
JC	OS	< 0.05	0.27	0.66
	OD	< 0.05	0.32	0.91

Bille J, Büchler-Costa J, Müller F, Aberration-free Refractive Surgery
Springer, 2001

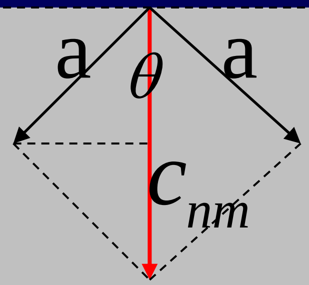
Optics of customized aberration-free Imaging



Precompensation of 3rd Order Aberrations using Phaseplates

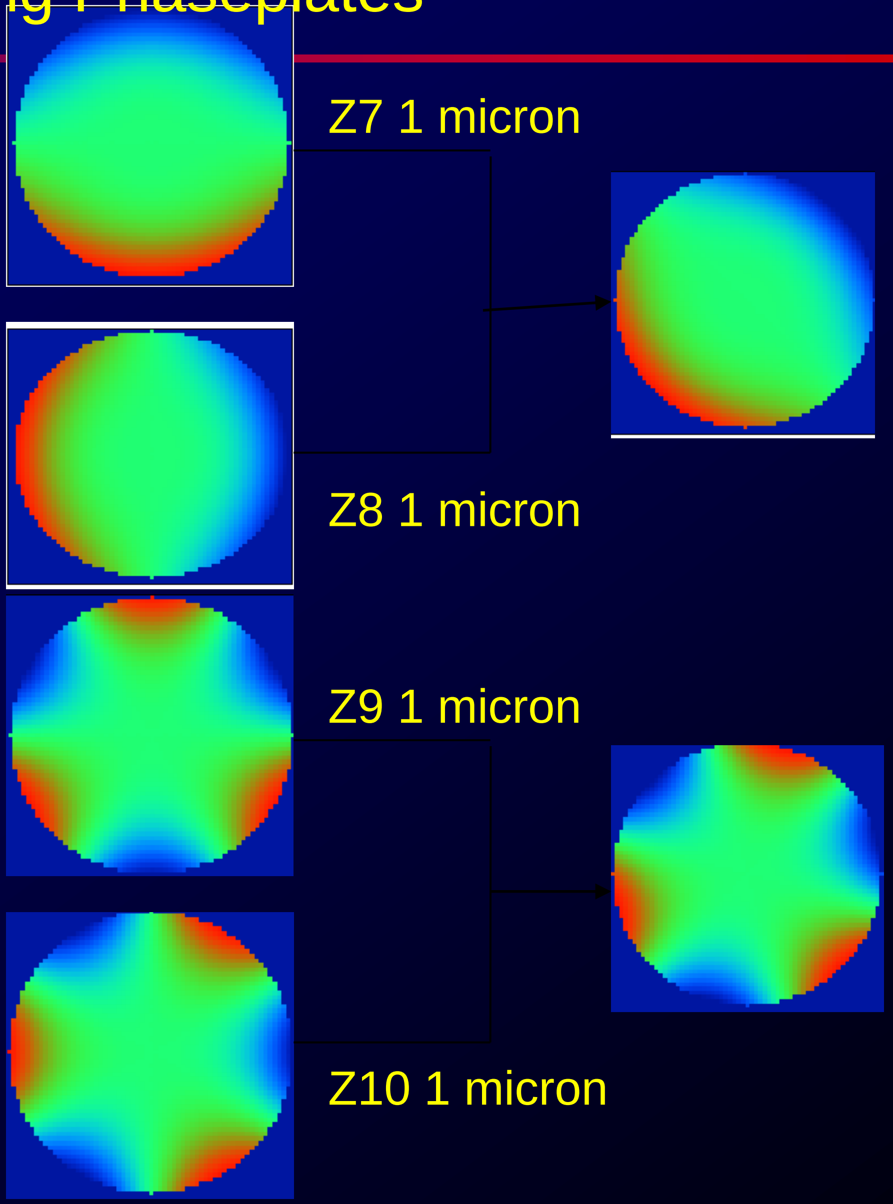
$$c_{nm} = \sqrt{(c_n^{-m})^2 + (c_n^m)^2}$$

$$\alpha_{nm} = \frac{a\tan(\frac{c_n^{-m}}{c_n^m})}{|m|}$$

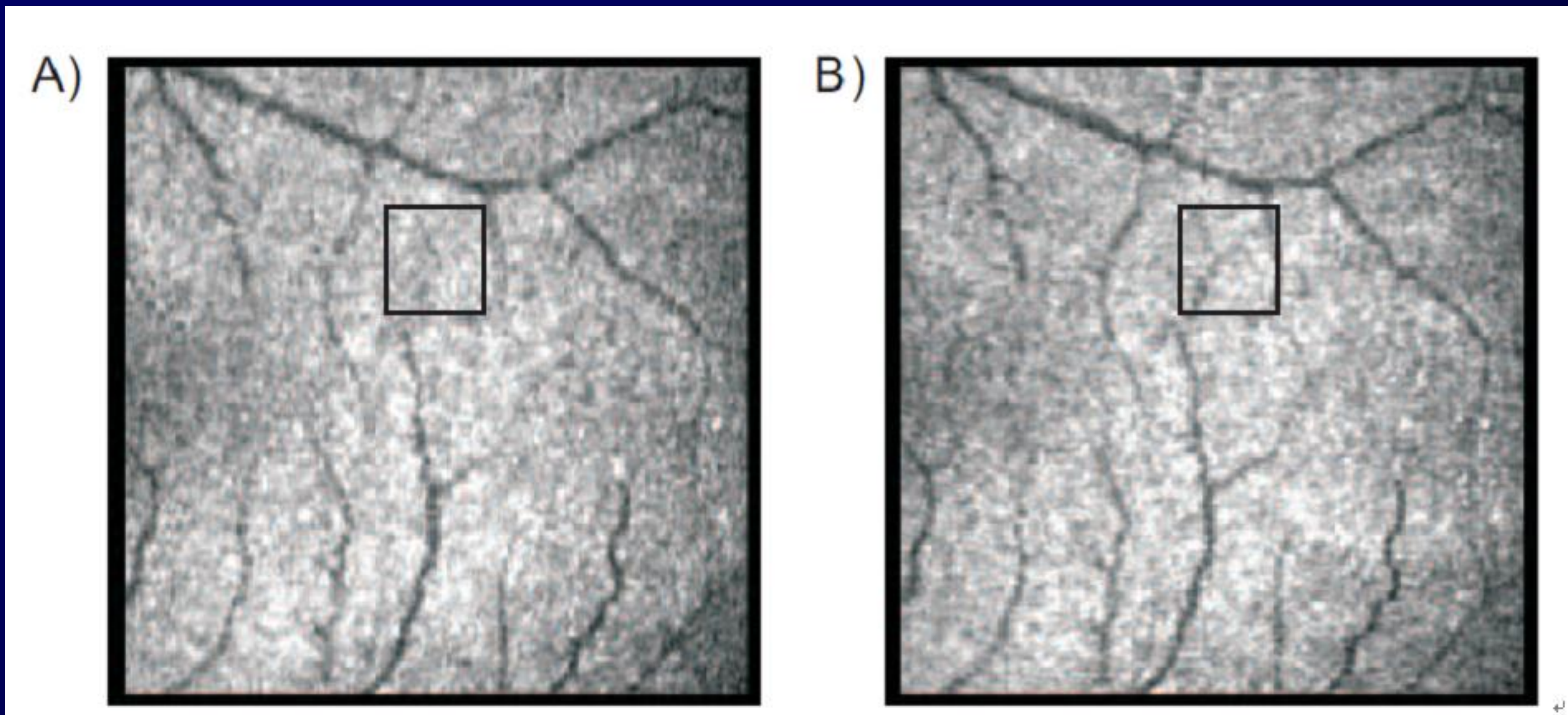


$$\theta = 2\arccos(\frac{c_{nm}}{2\,\mathrm{a}})$$

$$\beta_{all} = \alpha_{nm} + \frac{180}{m}$$



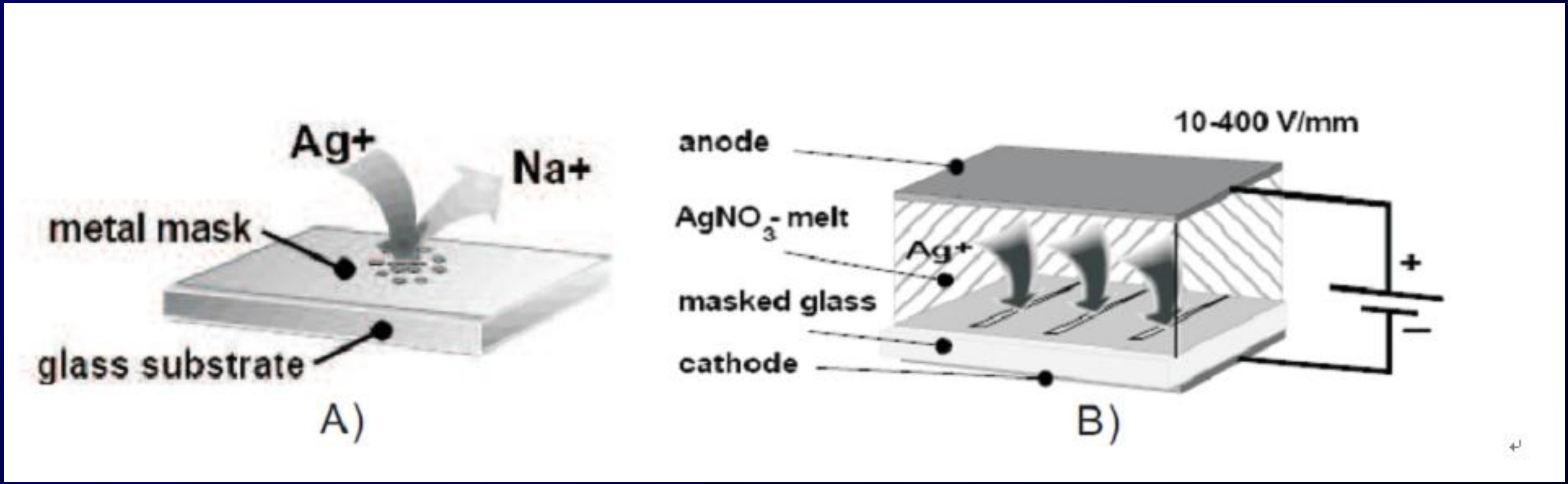
Coma Correction



A) Before coma correction

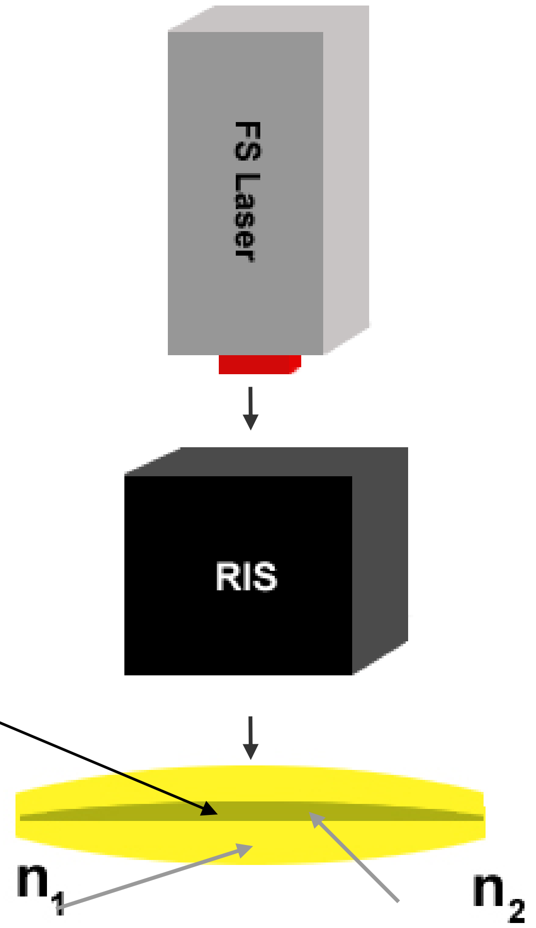
B) After coma correction

Mask Structured Ion Exchange Technique



A) Thermal diffusion: exchange of *Na+* by *Ag+* ions.
B) Field assisted process: *Ag*$^{+}$ ion current.

Mask-free Writing of Customized Phaseplates



FS = Femtosecond

780nm
100fs
50MHz
500mW

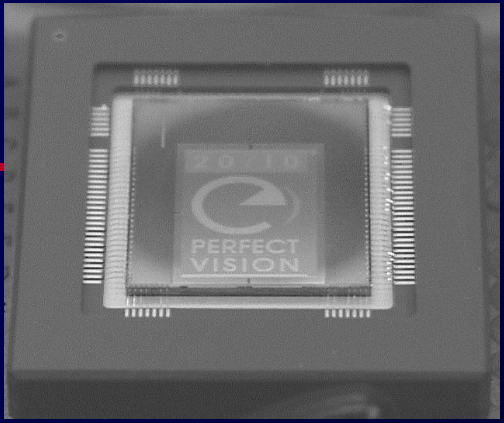
RIS = Refractive Index Shaping

(Alters chemical structure of "Lens")

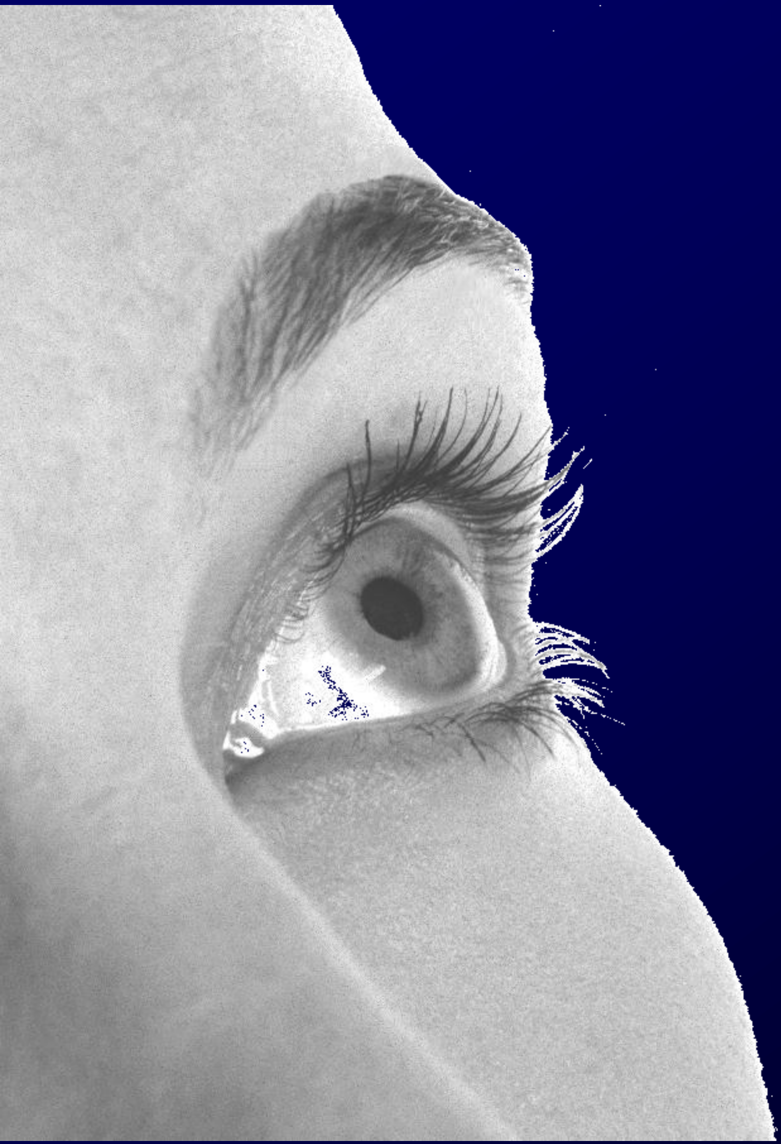
n_1 = Refractive index of IOL
n_2 = Refractive index of RIS "Lens"

R

Conclusion



- Second harmonic imaging provides new noninvasive structural information of the components of the human eye
- Two photon excited autofluorescence allows for structural and functional information of the retina
- Adaptive-optical beam shaping and AOM-controlled intensity-window contrast enhancement can provide cellular detail in in-vivo retina imaging



Thank you for your attention !