Neue Fluoreszenz- und OCT-Methoden für Augendiagnostik

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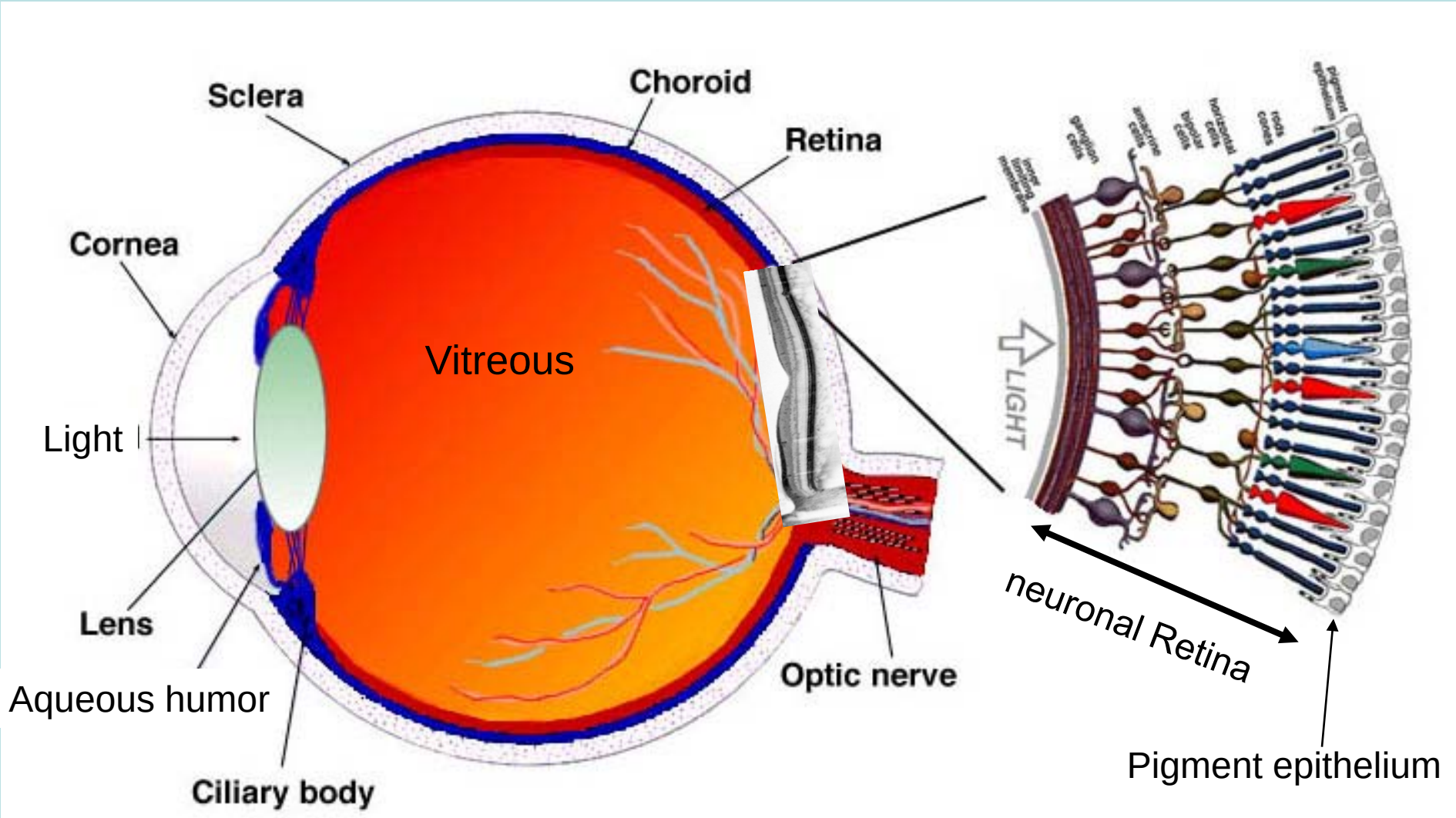
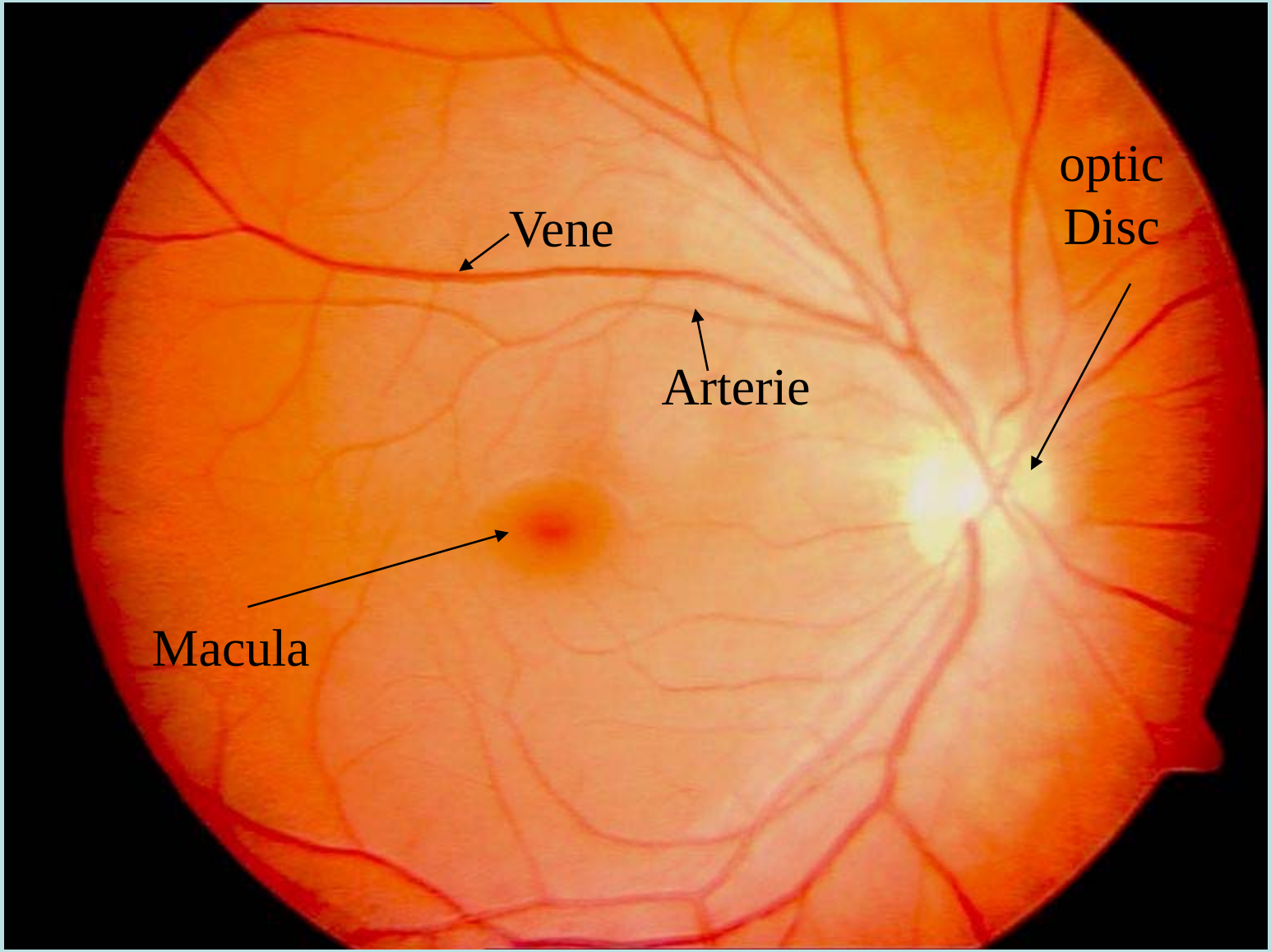
Anatomie des Auges



Normal Fundus (Funduskamera)

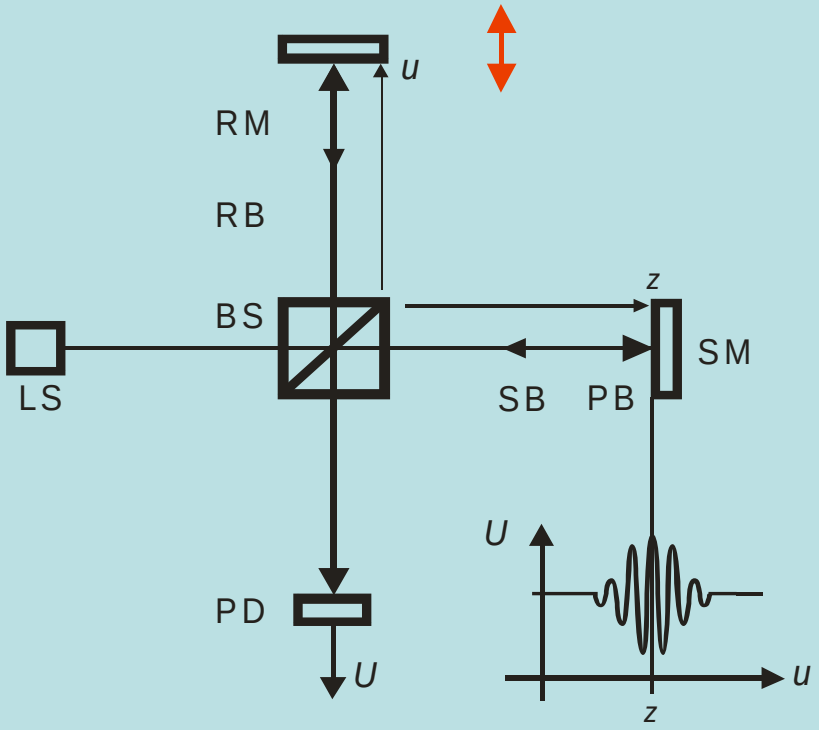


Methoden zur Untersuchung des Augenhintergrundes

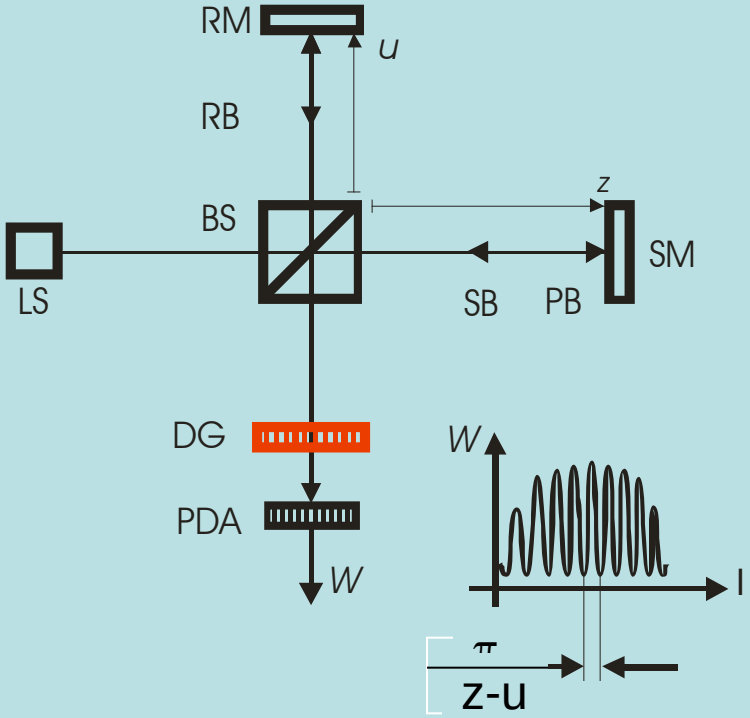
- Bilder des Augenhintergrundes
  - simultane Informationen aus allen Schichten
  - *erforderlich:*
  1. tomographische Information (Geometrie der Schichtstruktur)
  2. funktionelle Information (Stoffwechsel)

Prinzipien der optischen Kohärenz Tomographie (OCT)

Zeitdomäne



Frequenzdomäne



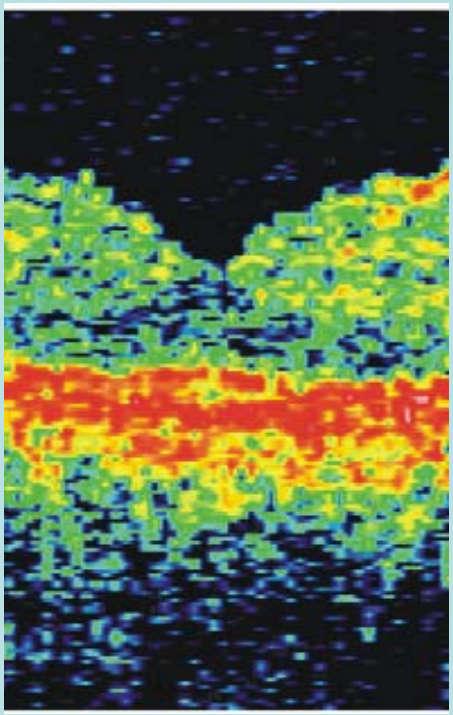
Transversale Auflösung

$$d = \frac{2\sqrt{\ln 2}}{\pi} \cdot \frac{\bar{\lambda}}{NA}$$

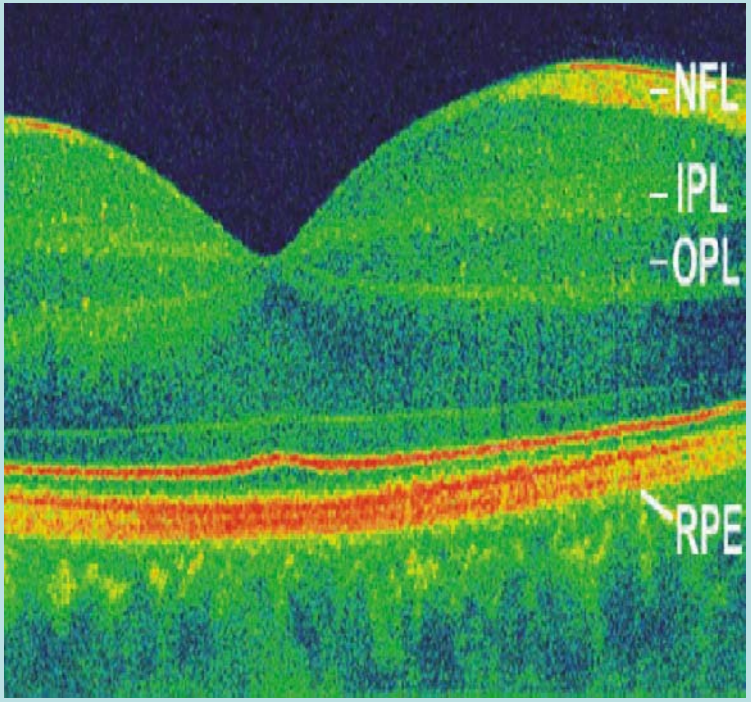
Tiefenauflösung

$$l_c = \frac{2\ln 2}{\pi} \cdot \frac{\bar{\lambda}^2}{\Delta\lambda}$$

OCT in time and in frequency domain

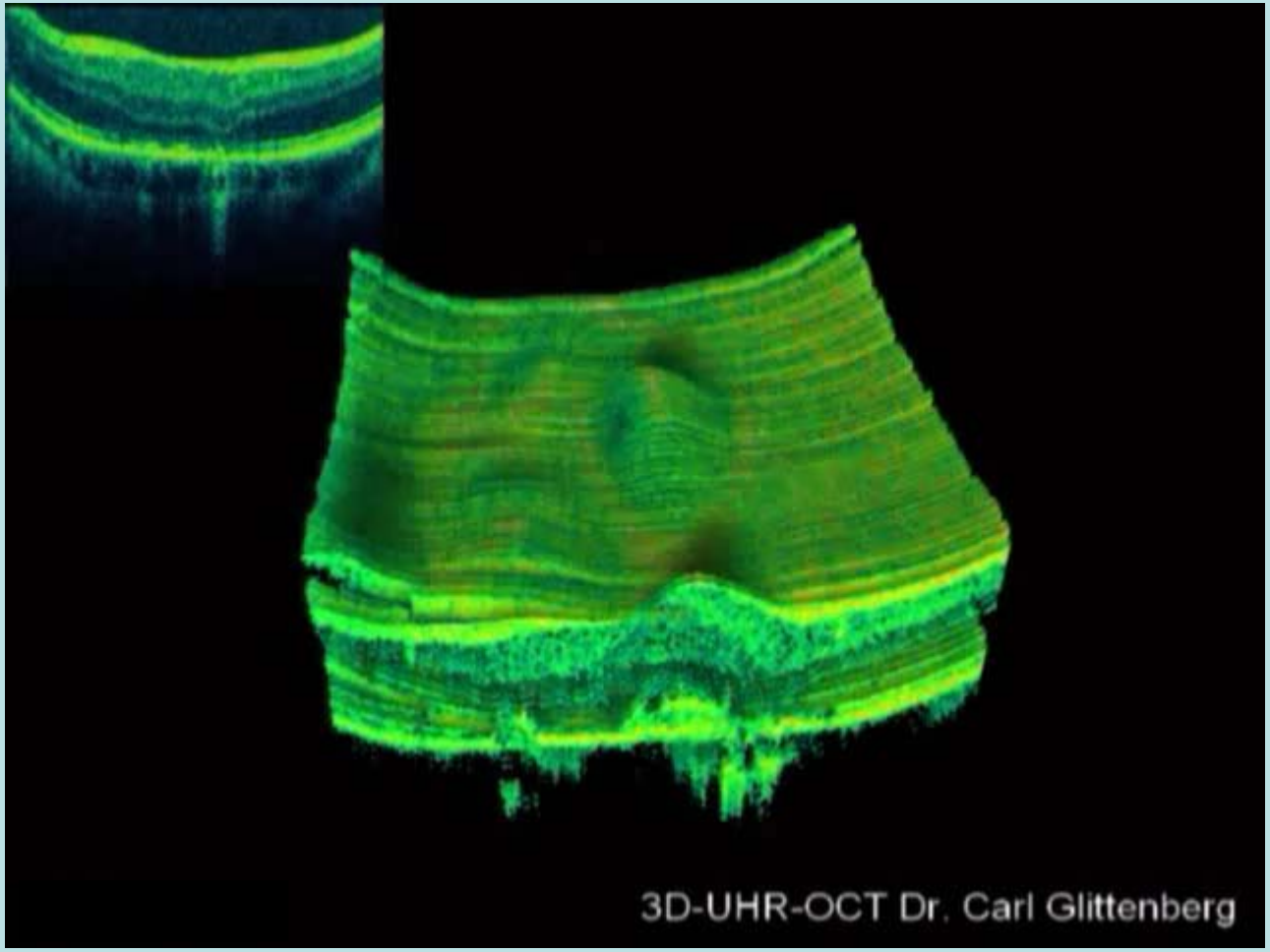


Hee et al., 1993



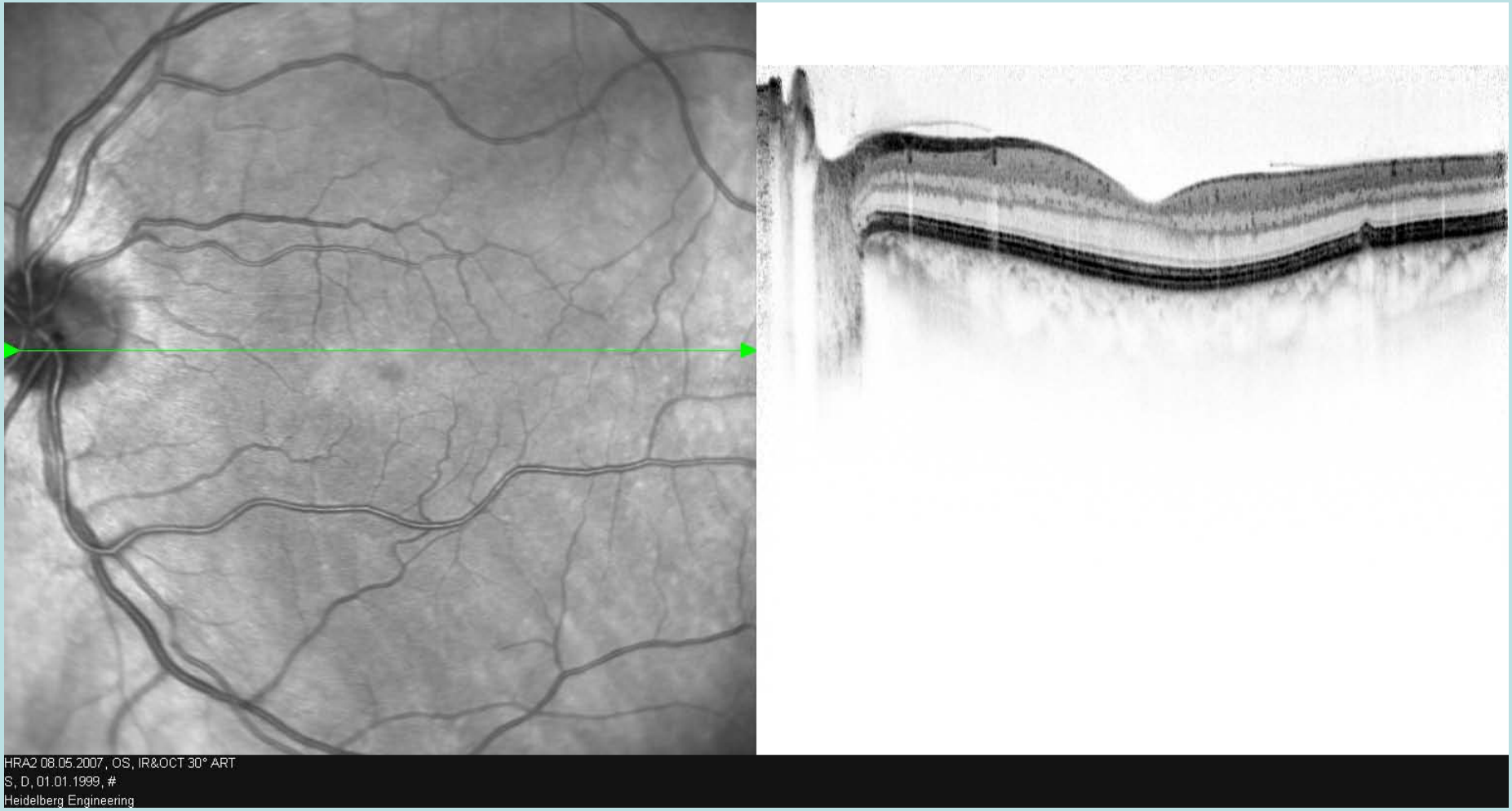
Wojtkowski et al., 2005

3 D Kubus des Augenhintergrundes

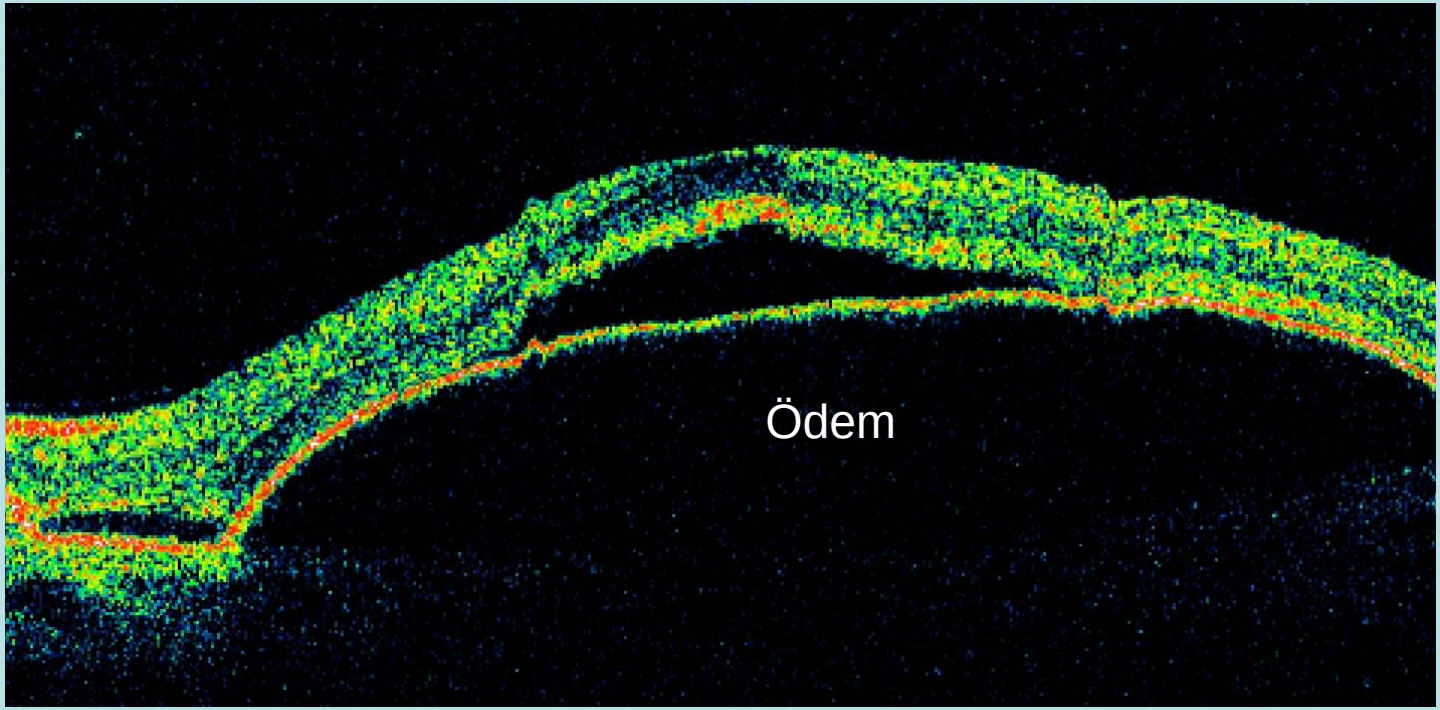


[Courtesy of W. Drexler; CDL-MUW, 2005]

Frequency domain OCT based on Laser Scanner Ophthalmoscope



Ödem mit Netzhautabhebung

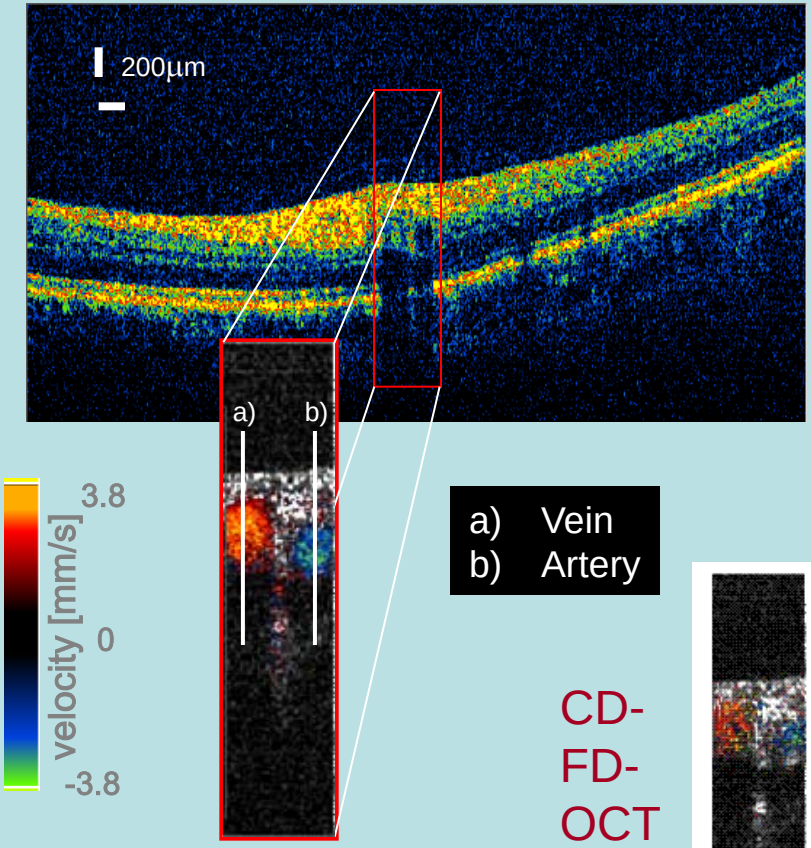


Doppler OCT Techniques

yield structural and flow data

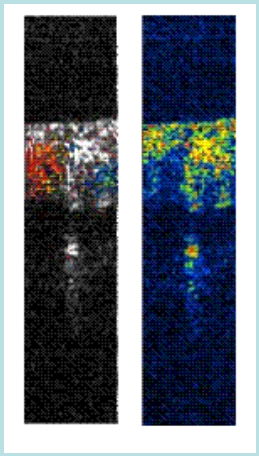
Funktional OCT

retinal blood flow in vivo



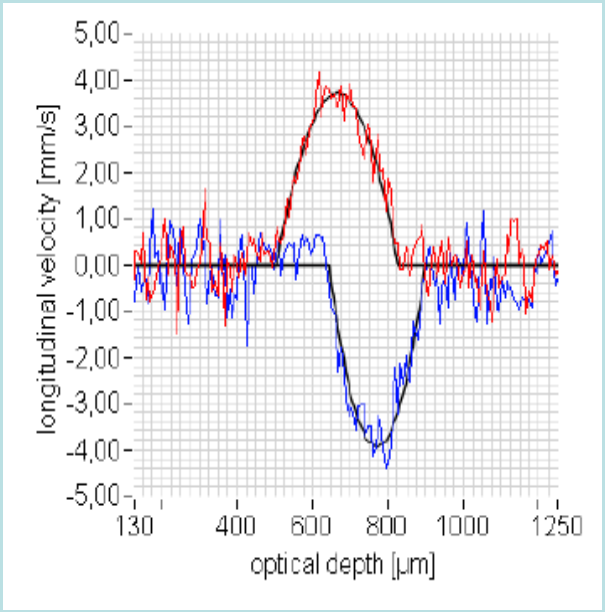
a) Vein
b) Artery

CD-
FD-
OCT



2-4 images/sec for 160 x 512 points
Resolution 17 µm;
Sensitivity ~90 dB
Volunteer heart beat 85 per minute

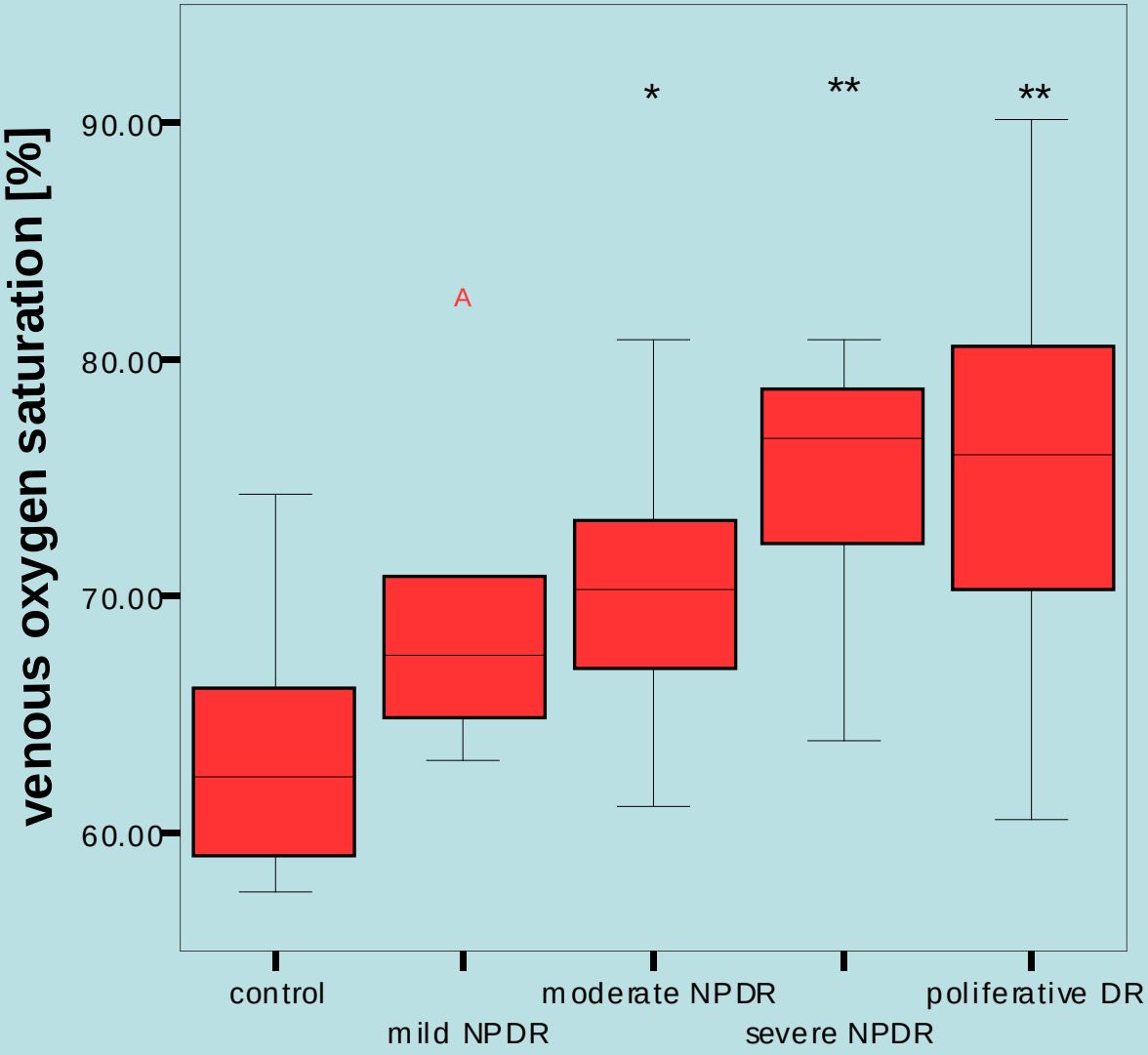
Velocity sensitivity ~200µm/s in tissue



Courtesy of A. Fercher

Medical University Vienna

Venous OS in diabetic Retinopathy

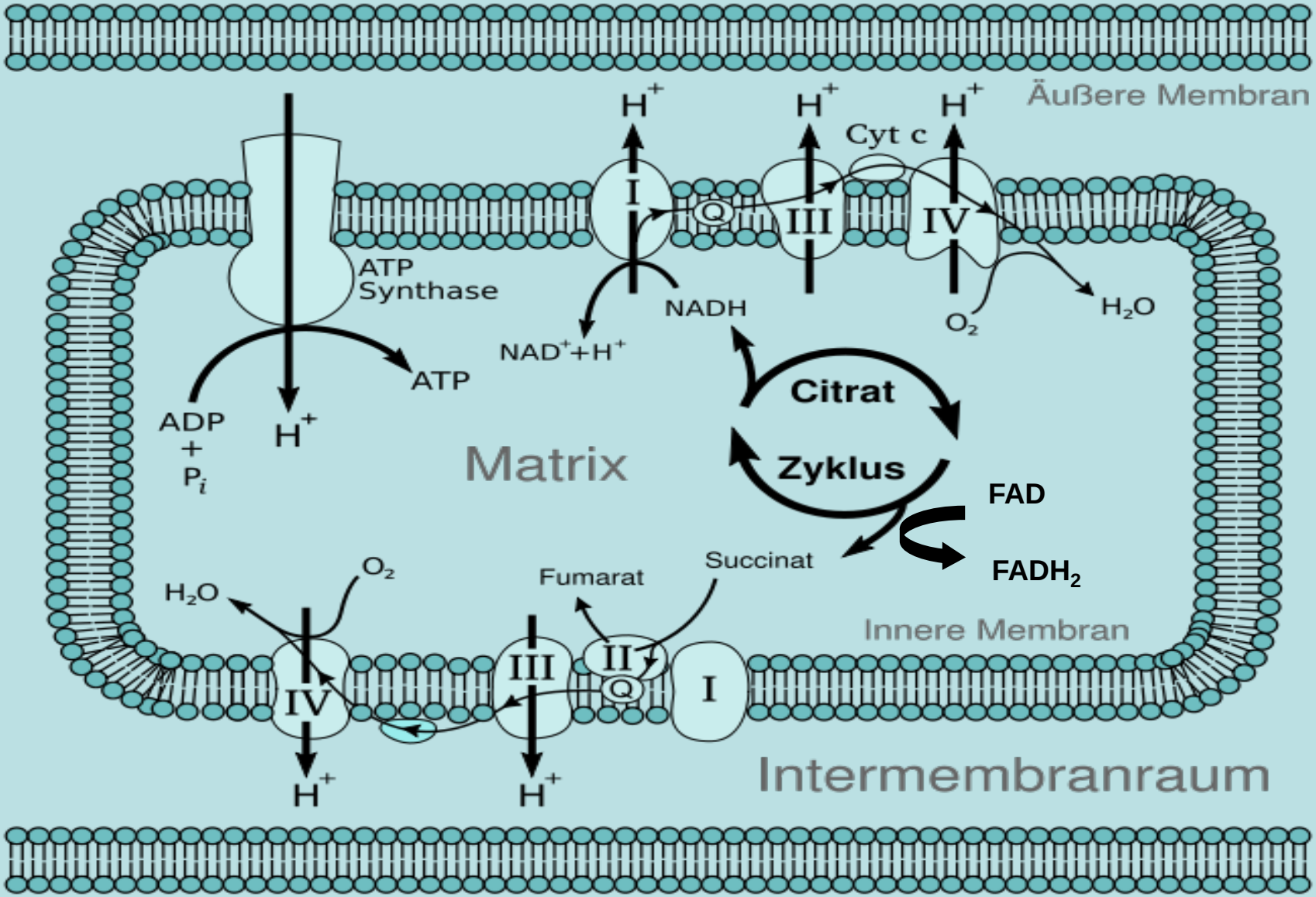


Hammer M et al Graefe´s Arch. Clin. Exp. Ophthalmol. DOI 10.1007 s00417-009-1078-6 (2009)

Charakterierung des zellulären Metabolismus durch endogene Fluorophore

(Britton Chance 1968)

Energieproduction in Mitochondrien



modifiziert nach Wikipedia

Auto-fluorescence of Coenzymes **NADH** und **FAD**

Redox pairs

NAD^+ – **NADH**

FAD^+ – $FADH_2$

sufficient O_2	rel. O_2- lack
no Fluorescence	intensive Fluorescence of **NADH**
intensive Fluorescence of **FAD^+**	no Fluorescence

further endogenous Fluorophores

Ageing pigment Lipofuscin, Melanin,
all-trans-retinal dimer λex=430 nm, λem=510 nm**,**

➡ age-related macular degeneration

Advanced glycation endproducts (AGE)

➡ Diabetes mellitus

Connective tissue Collagen, Elastin

➡ Glaucoma, Arterio-Sclerosis

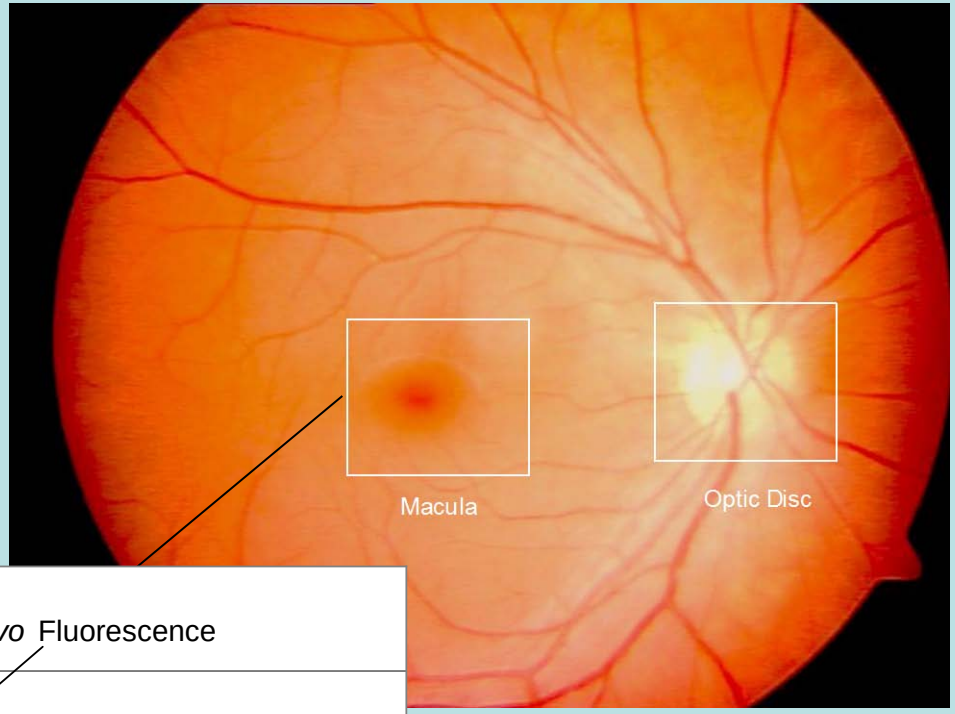
Pyridoxal phosphat, Protoporphyrin IX

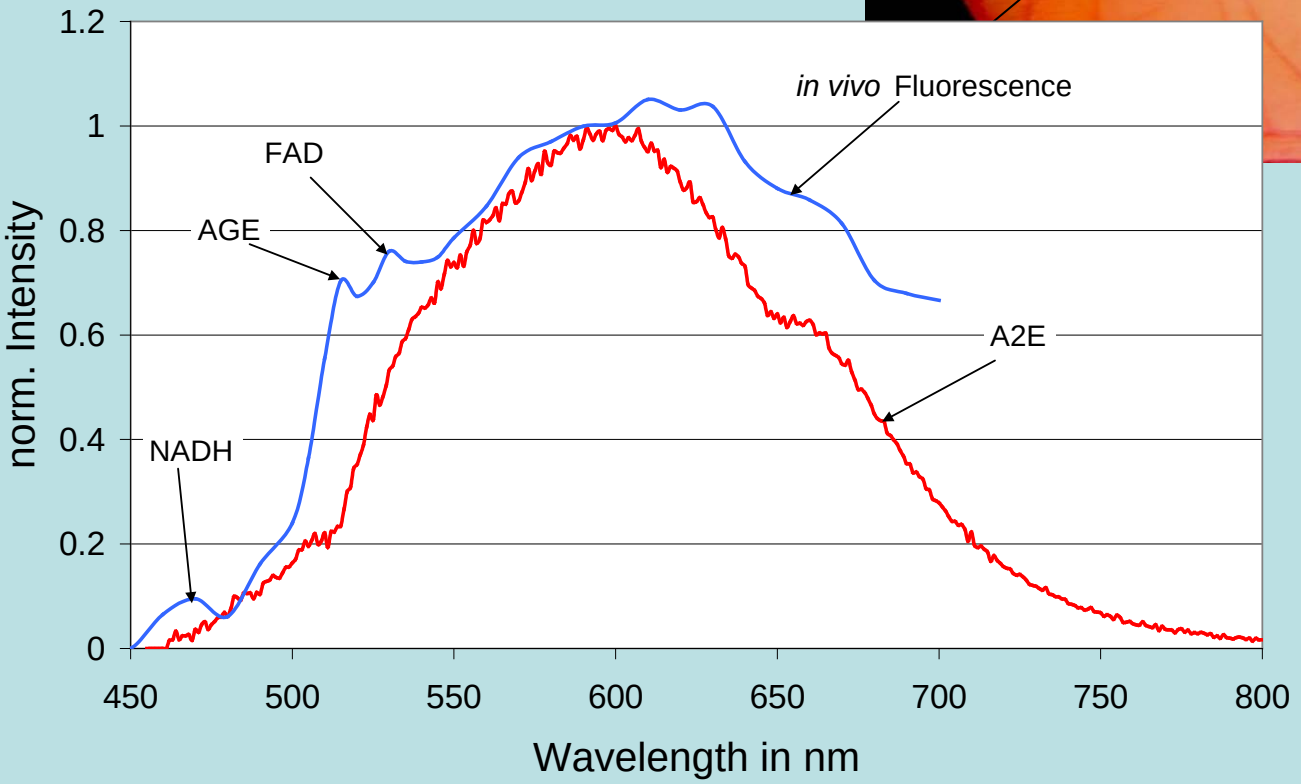
➡ Haemsynthesis

Amino acids Tryptophan, Kynurenin, Phenylalanin

➡ Cataracta

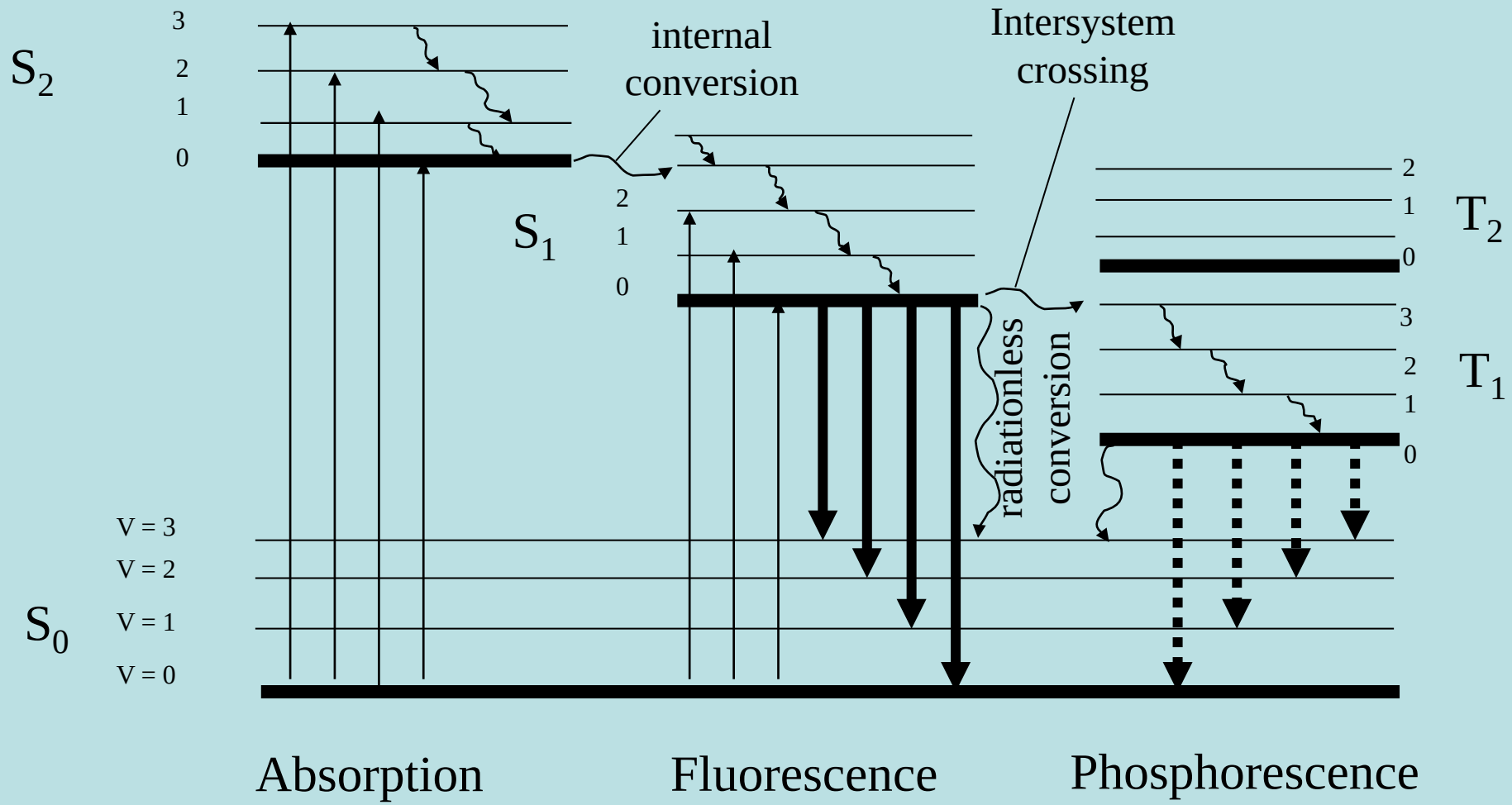
In vivo macular fluorescence spectrum





corrected for:
- lens fluorescence,
- ocular transmission,
- xanthophyll absorption

Jablonski Energie Diagramm

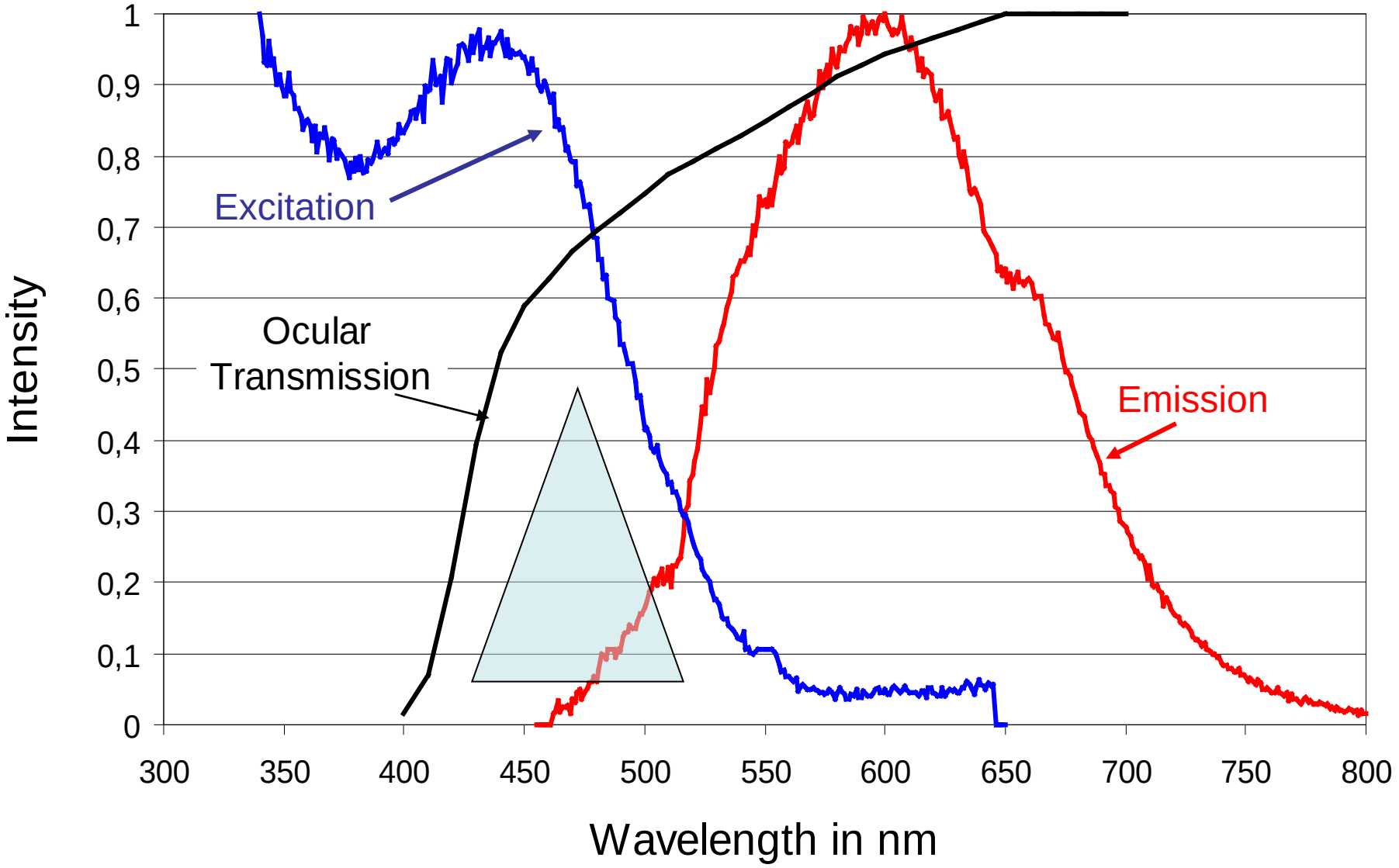


Preliminary studies

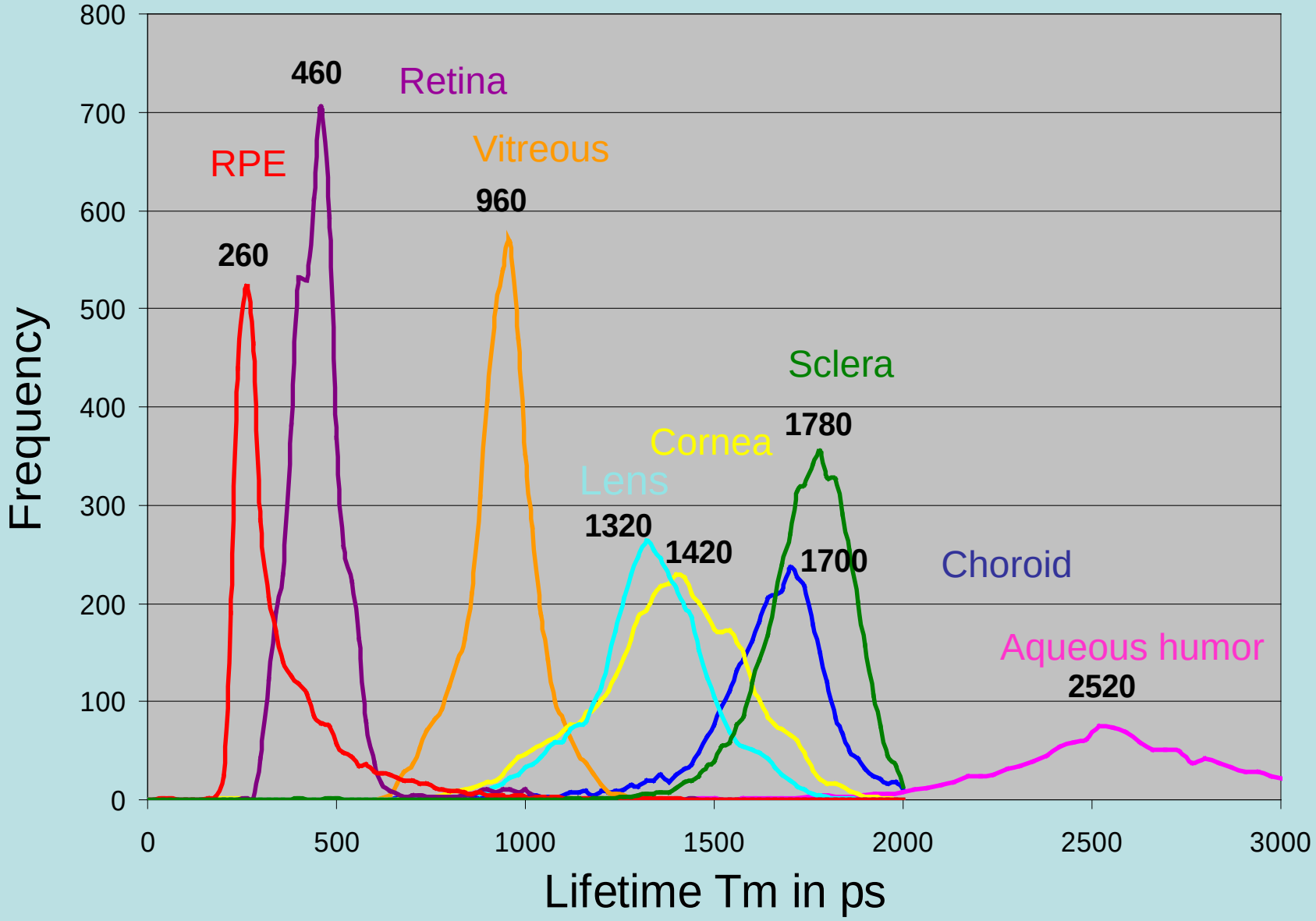
Excitation – and emission spectra as well as fluorescence lifetimes

- of endogenous fluorophores, expected at the eye ground
- of isolated anatomical structures of porcine eyes

A2E (Lipofuscin): **Excitation- and Emission spectra, compaired with Ocular transmission**

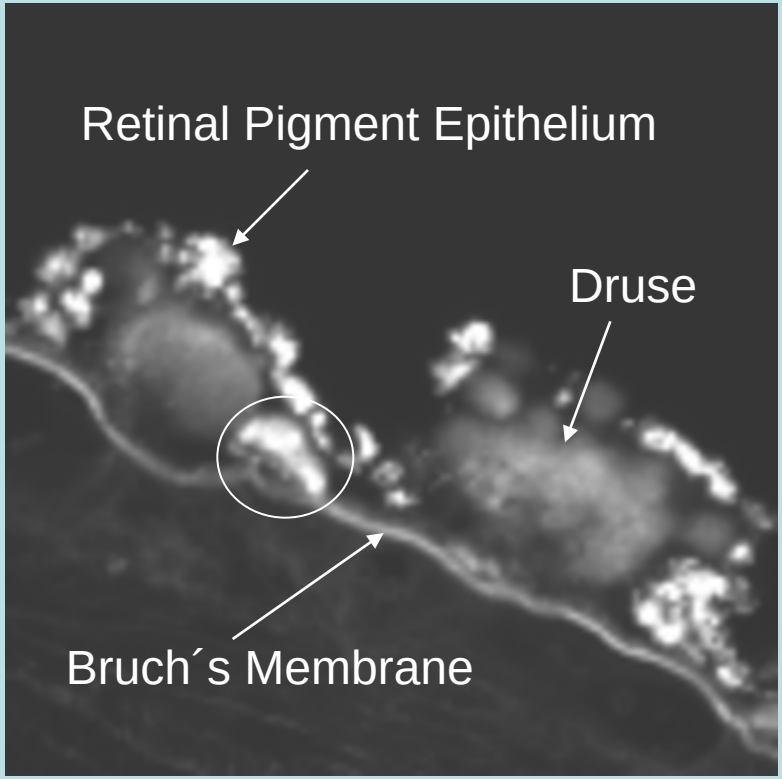


Lifetime T_m of ocular structures excitation 446 nm, emission 500 – 700 nm

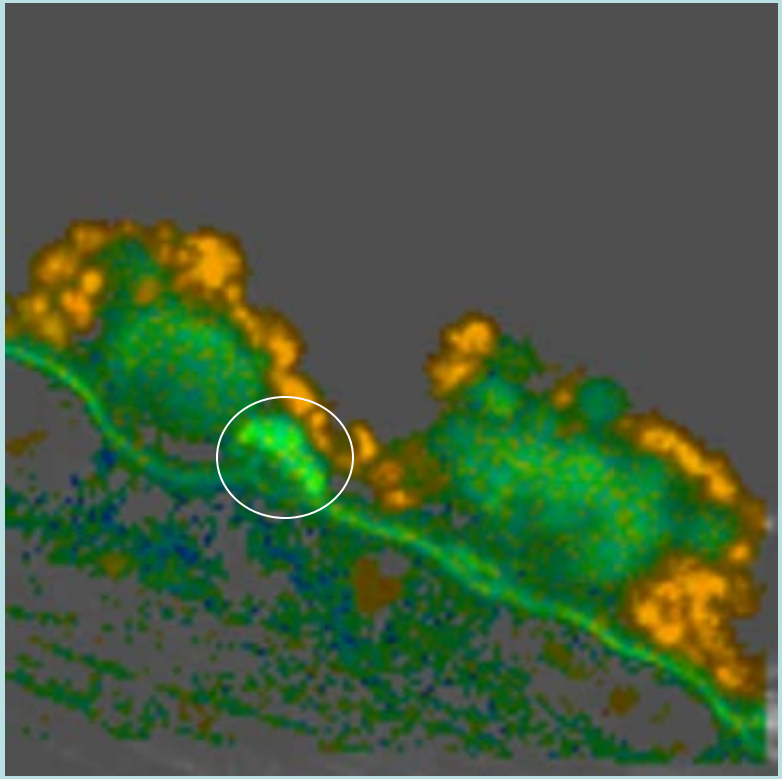


Fundus Sample of a human Donor (AMD)

Intensity



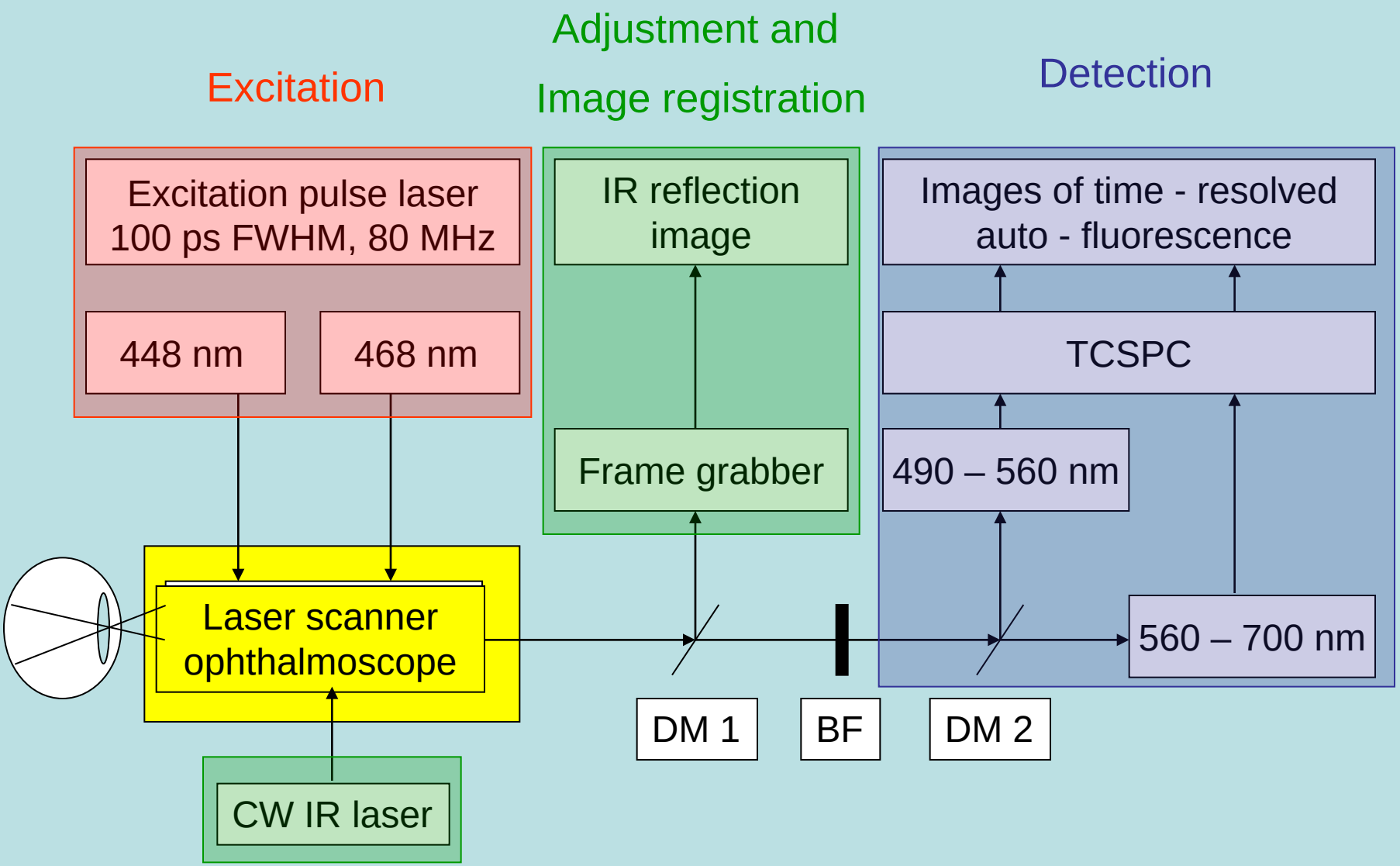
Lifetime Tm



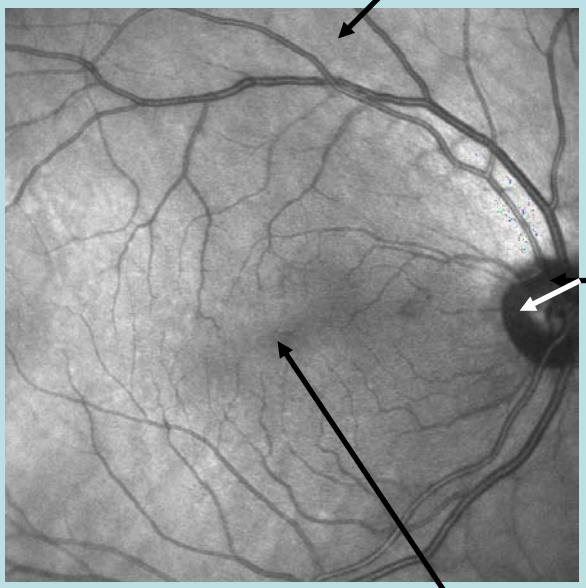
0

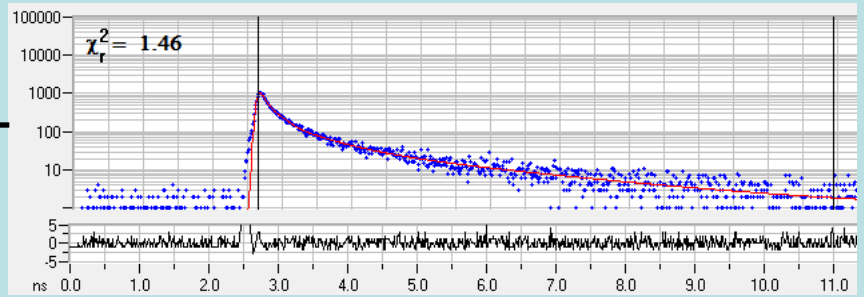
3500 ps

Fluorescence Lifetime Laser Scanner Ophthalmoscope selectable Excitation and Emission in 2 spectral bands

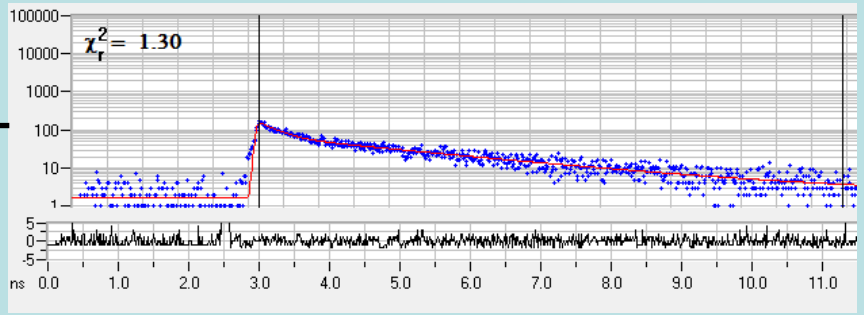


Decay at macula, surroundings, and optic disc

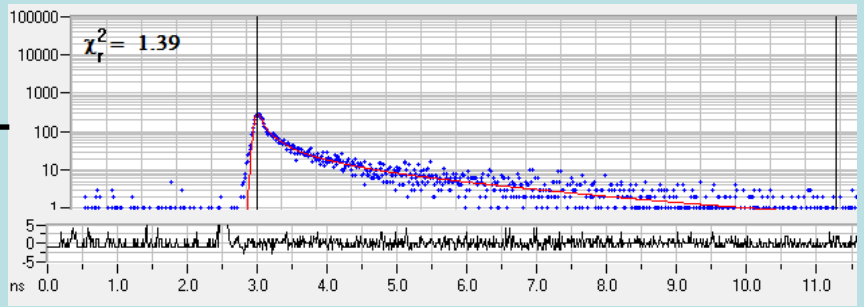




τ1=97 ps, τ2=518 ps, τ3=2123 ps



τ1=202 ps, τ2=2096ps, τ3=2984 ps



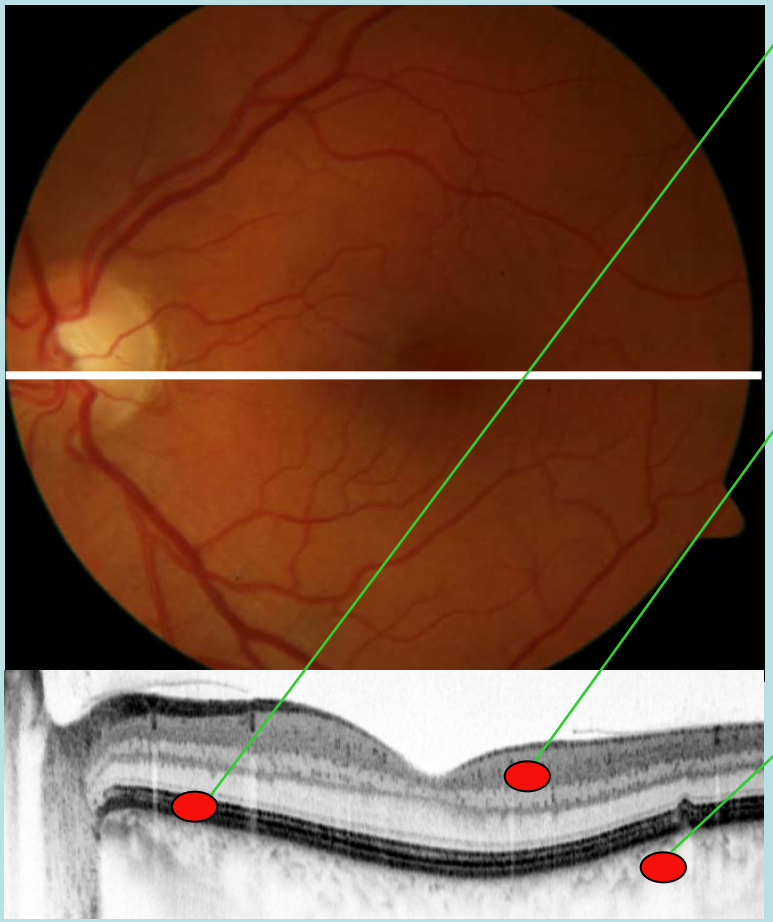
τ1=71 ps , τ2=480 ps, τ3=2082ps

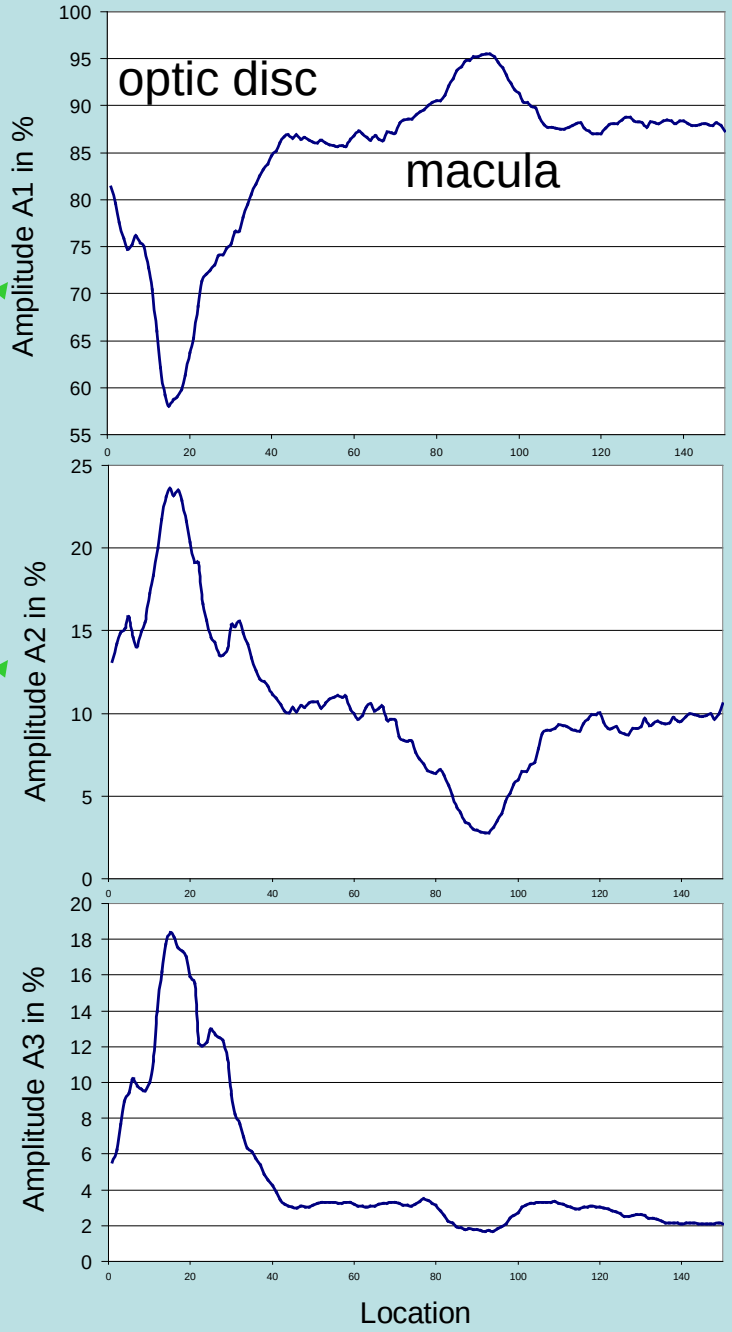
Model function

$$\frac{I_1(t)}{I_0} = \sum_i a_i \cdot e^{-\frac{t}{\tau_i}} + b$$

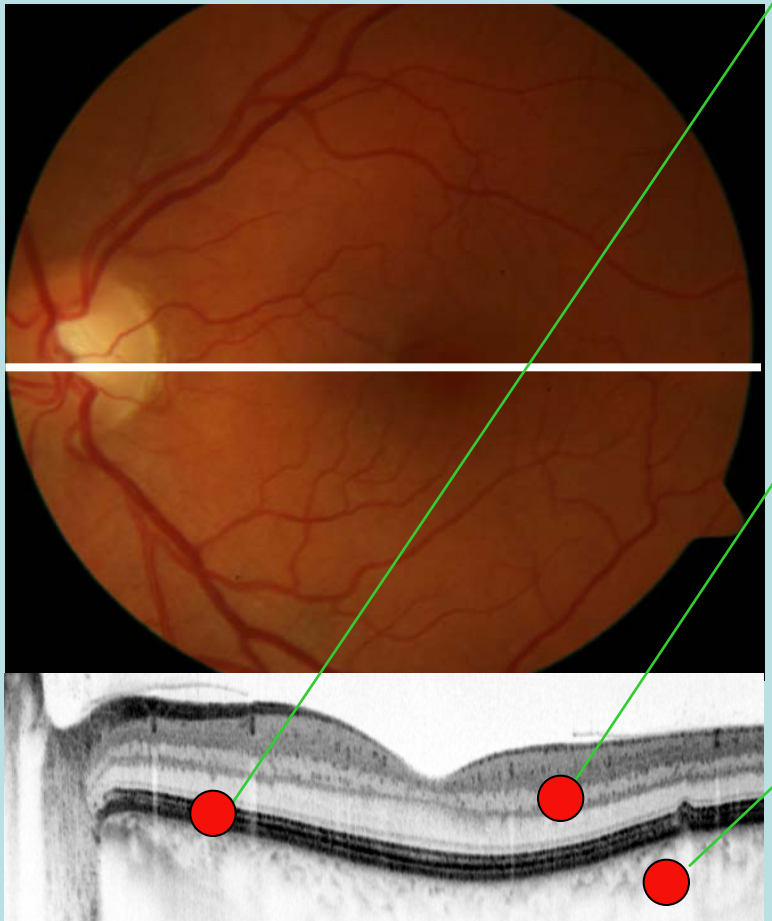
$I_1(t)$ - Fluorescence intensity at time t
I_0 - Fluorescence intensity at t = 0
α_i - Amplitude
τ_i - Lifetime
b - Background

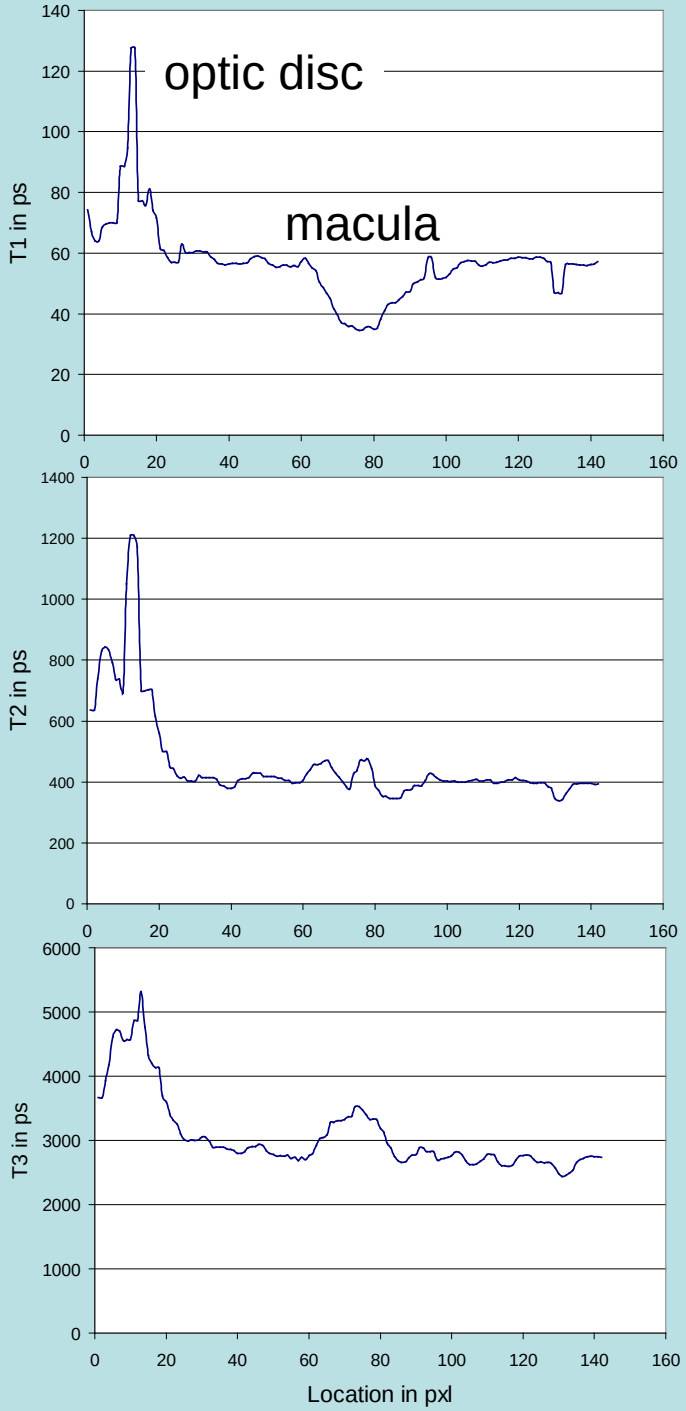
Local distribution of relative amplitudes A1, A2, A3 in %





Local distribution of lifetimes T1,T2,T3 in ps

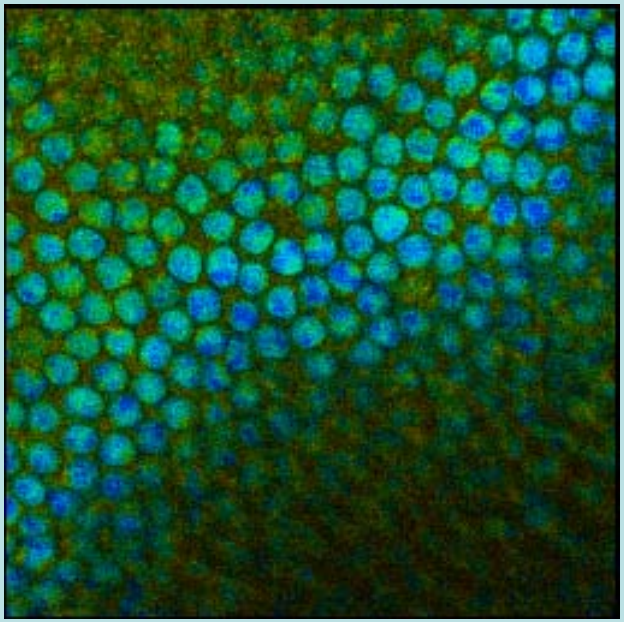


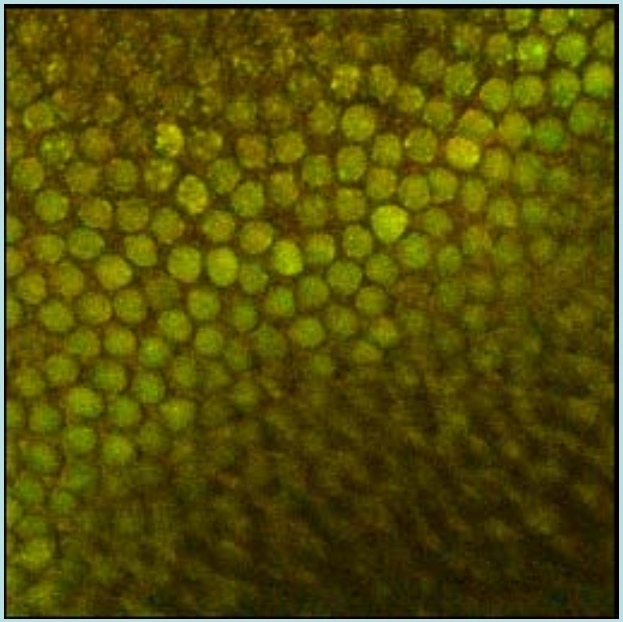


2-Photon FLIM of a fundus sample

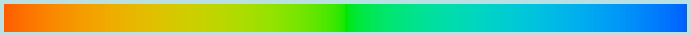
500 - 550 nm

560 - 700 nm





Receptors



50

500 ps

Relation of Lifetimes in Receptors (490-560 nm)

T1 median= 110± 53 ps
protein-bound FAD

T2 median= 1417±388 ps
protein-bound NADH

$$\frac{Q_1}{Q_2} = \frac{A_1 \cdot T_2}{A_2 \cdot T_2} = \frac{43\%}{57\%}$$

➡ Respiration

➡ Glycolysis

Evaluation of fluorescence lifetime measurements

Local alterations:

-Images of lifetimes, amplitudes, and relative contribution Q

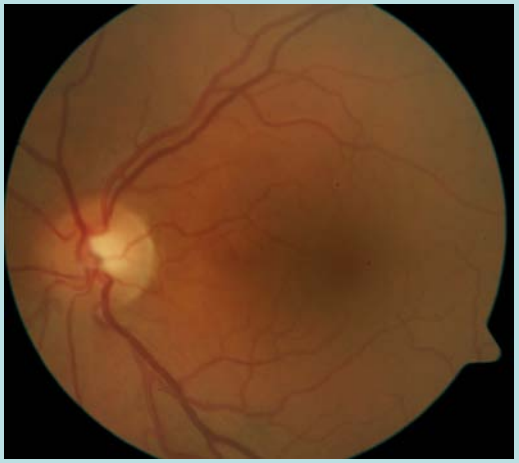
Global alterations:

-Histograms of lifetimes and amplitudes for statistical comparison between healthy subjects and diseases

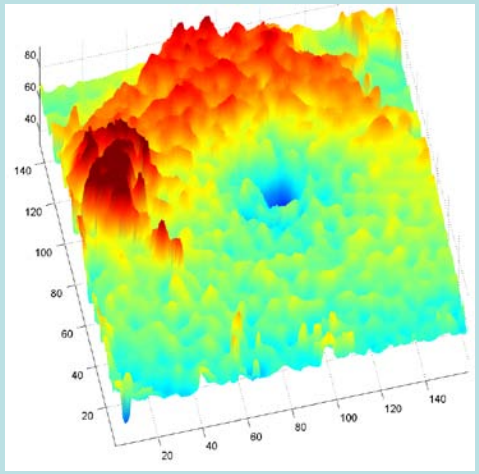
-Pre-classification by cluster diagrams

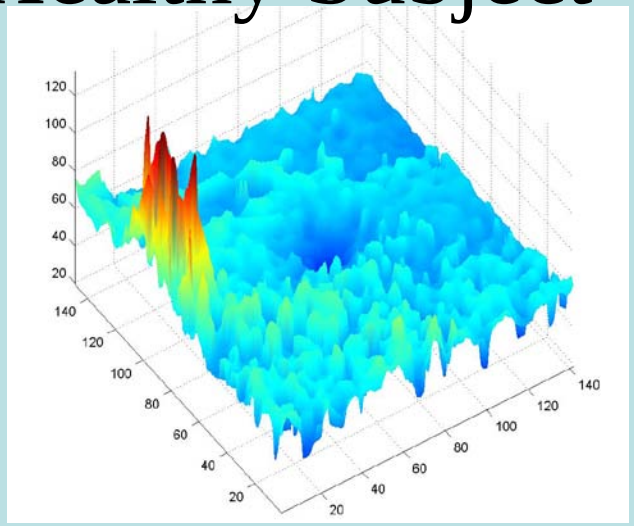
Diabetic Patient

Healthy Subject

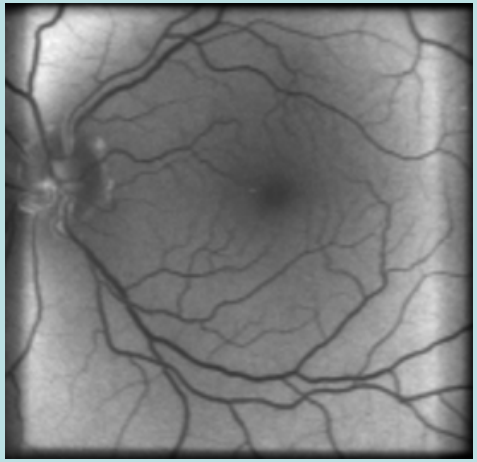


Fundus photography

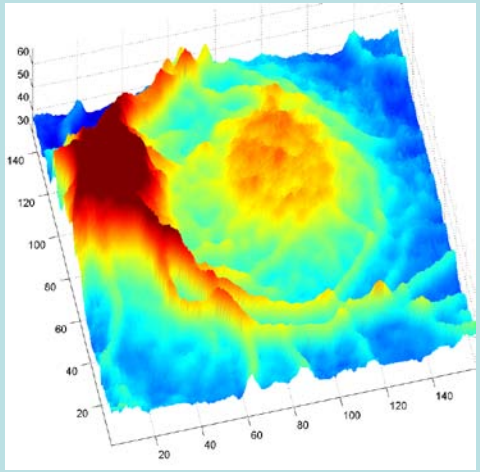


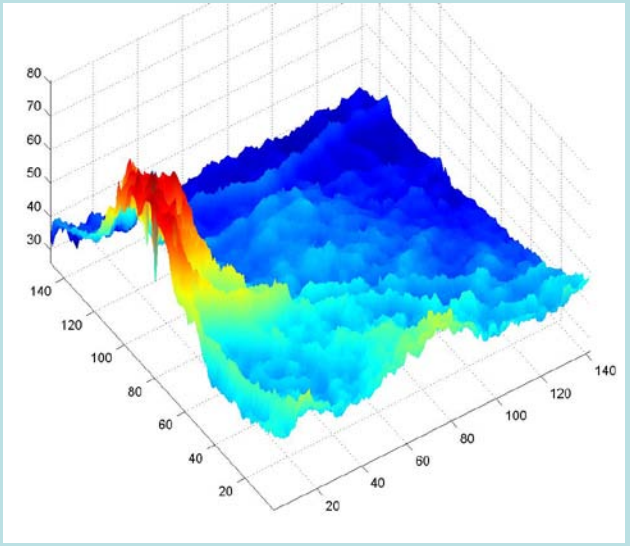


Lifetime T1 in ps (490-560 nm)



Fluorescence Intensity (490-560 nm)

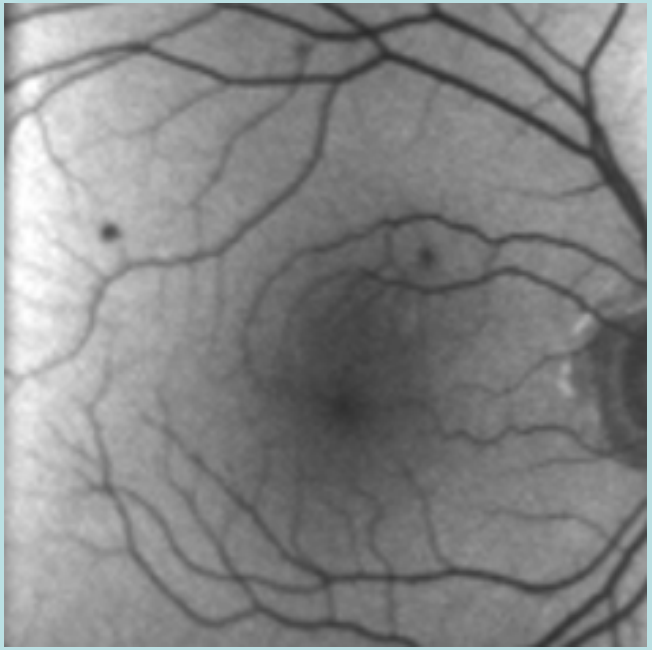




Contribution Q3 in % (490-560 nm)

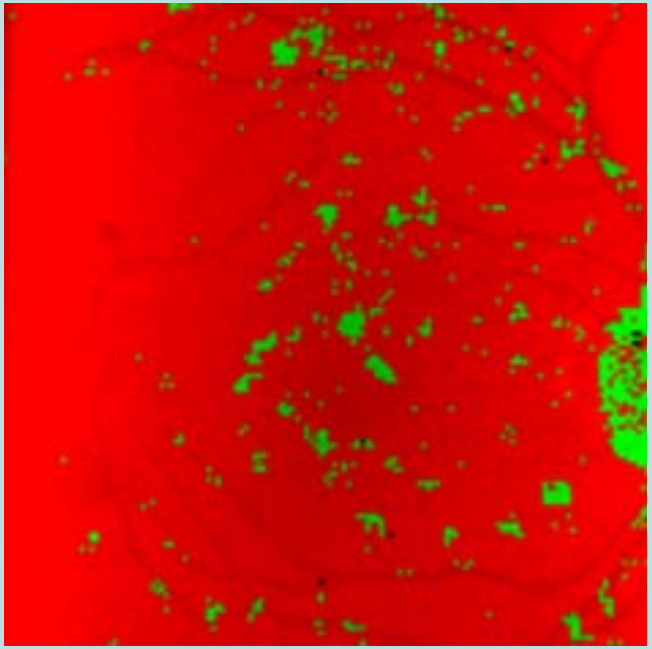
Undersupplied Fields in early diabetic Retinopathy

Fluorescence intensity



K2 560 - 700 nm

Fluorescence lifetime T2



green: T2>1000 ps

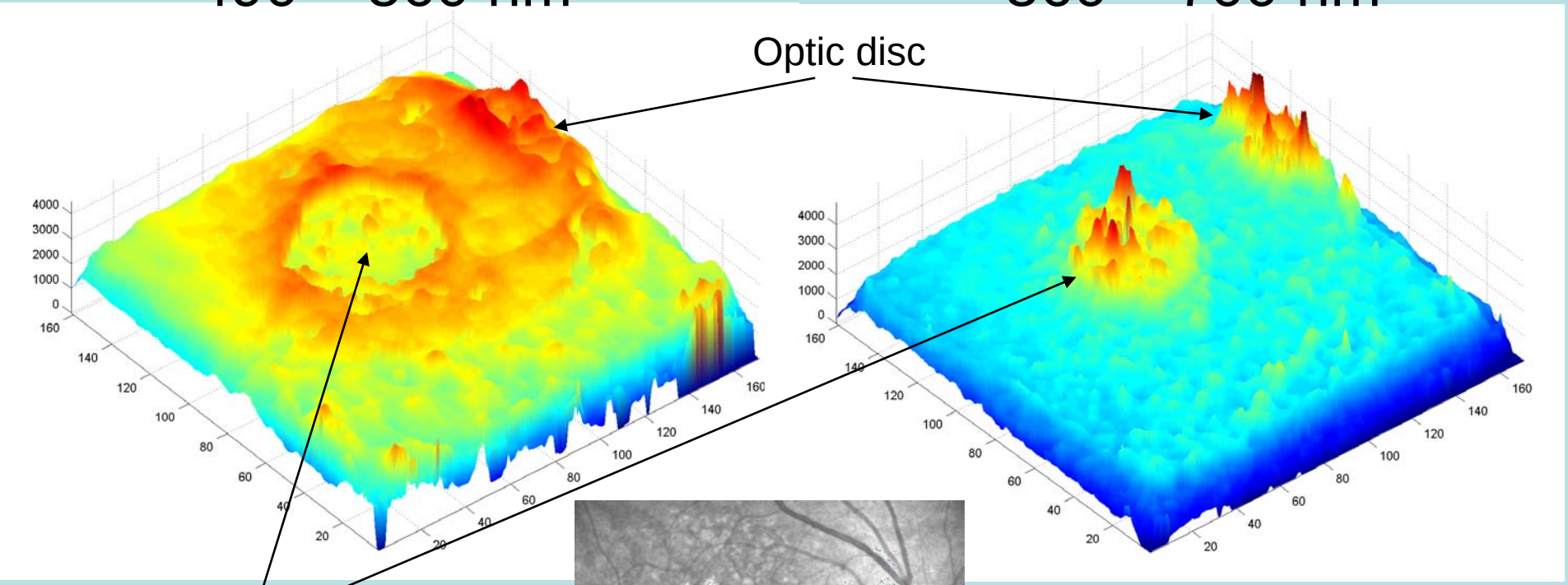
red: T2<1000 ps

K1 490 - 560 nm

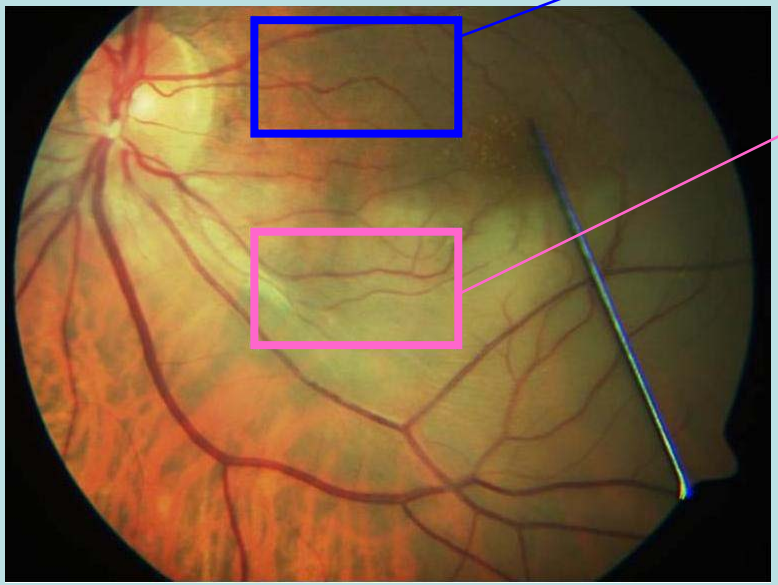
Non-exudative AMD – T3

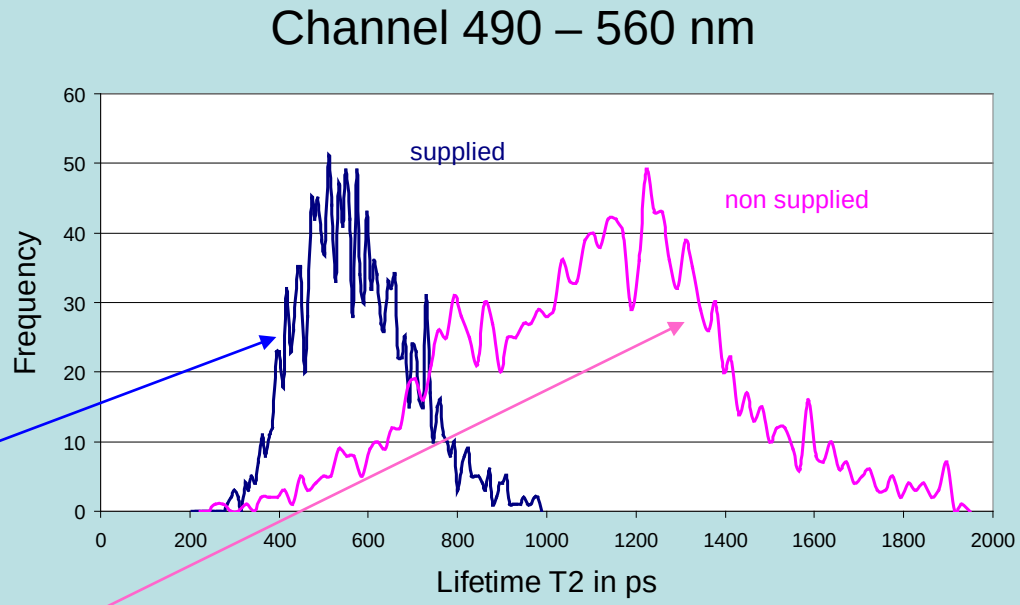
490 – 560 nm

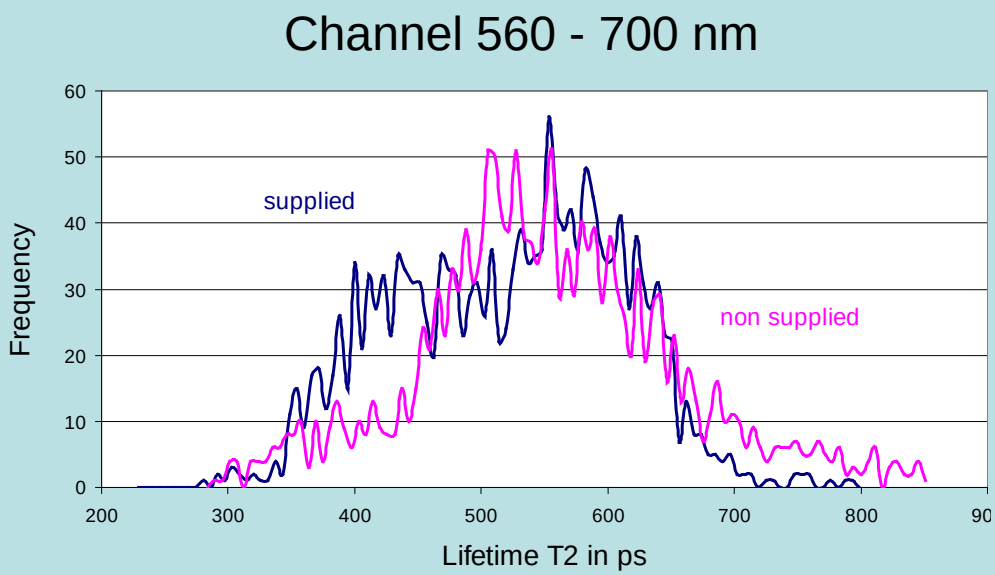
560 – 700 nm



Lifetime in branch arterial occlusion

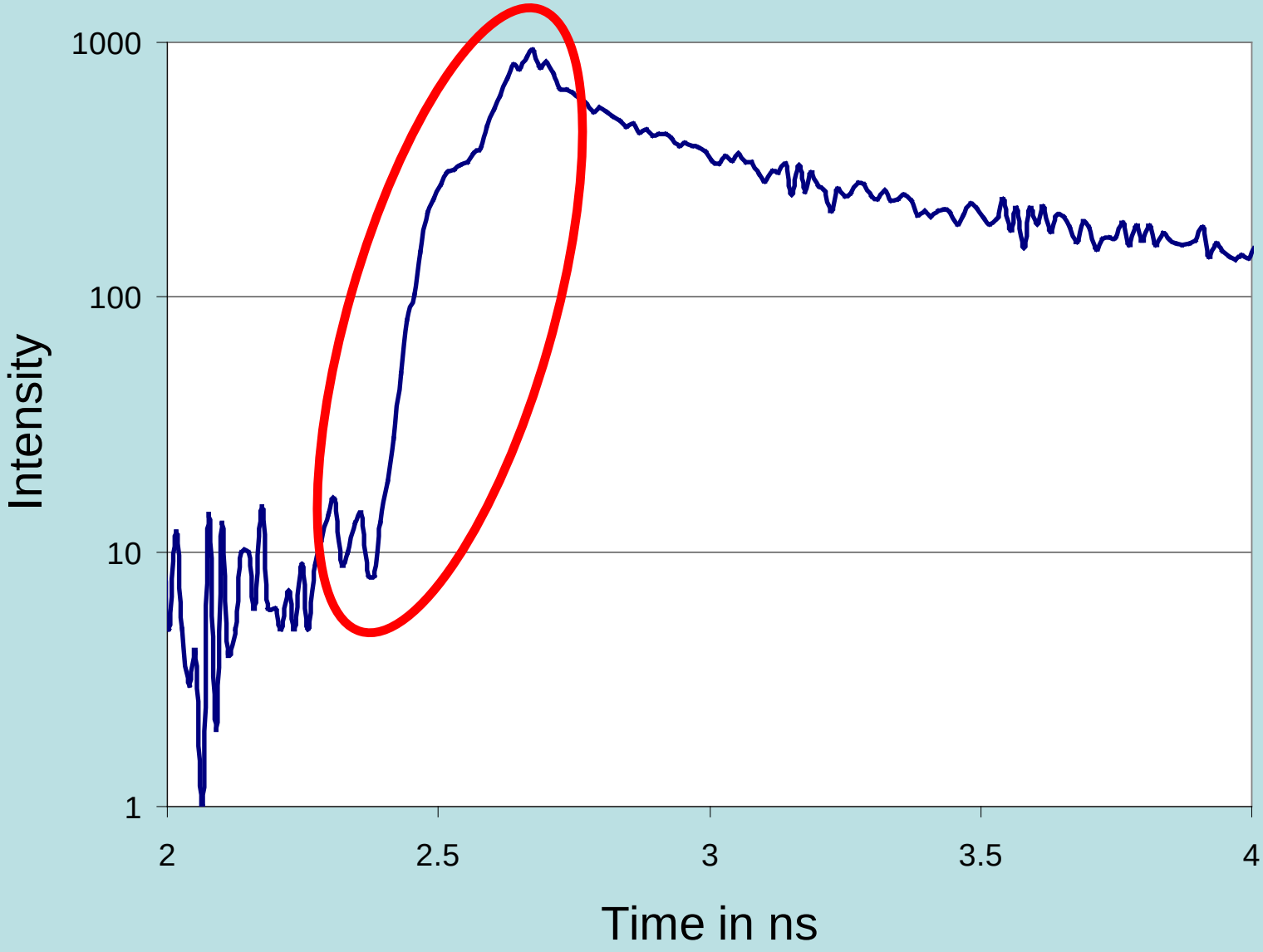




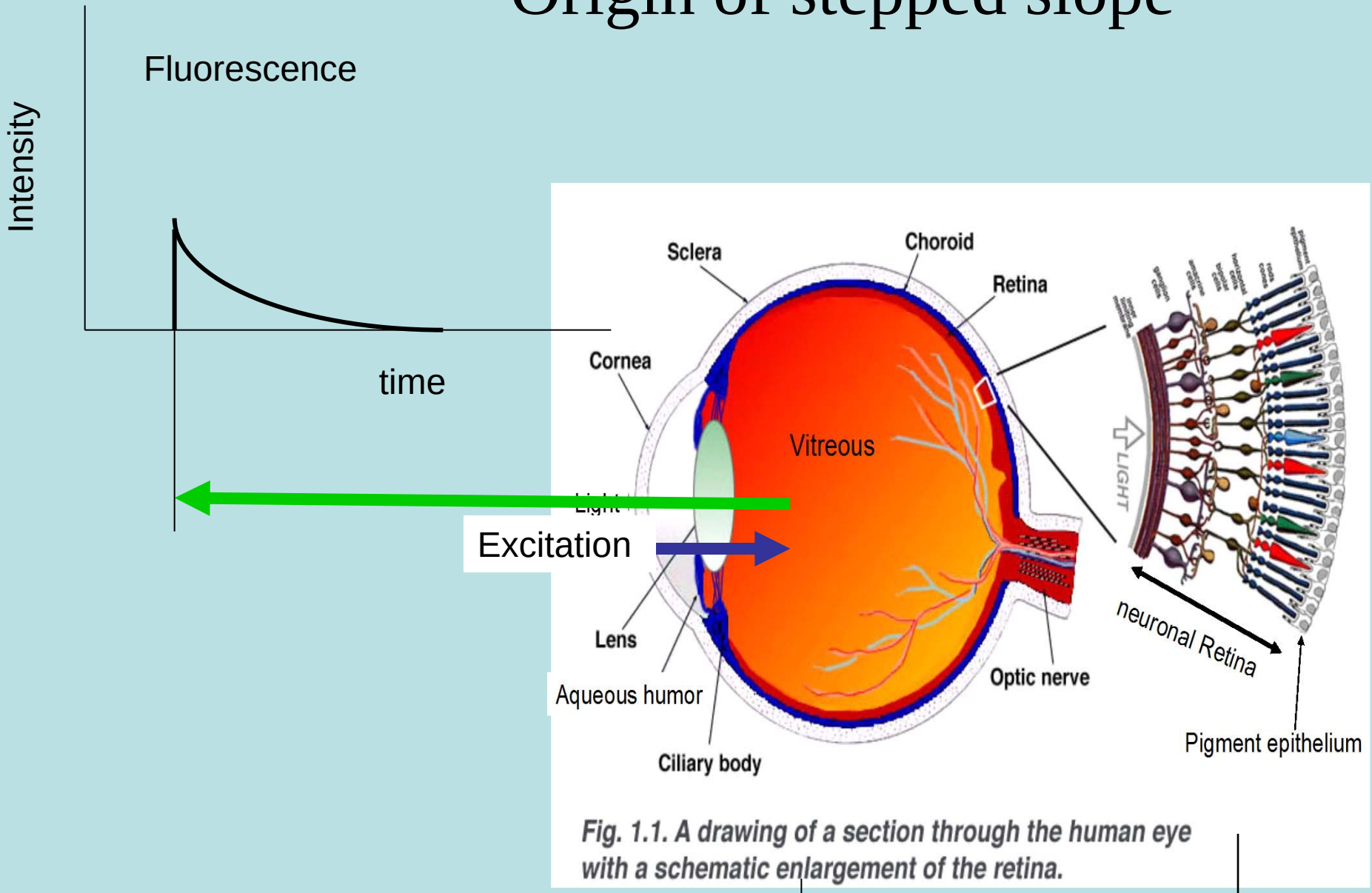


Approach for functional Tomography

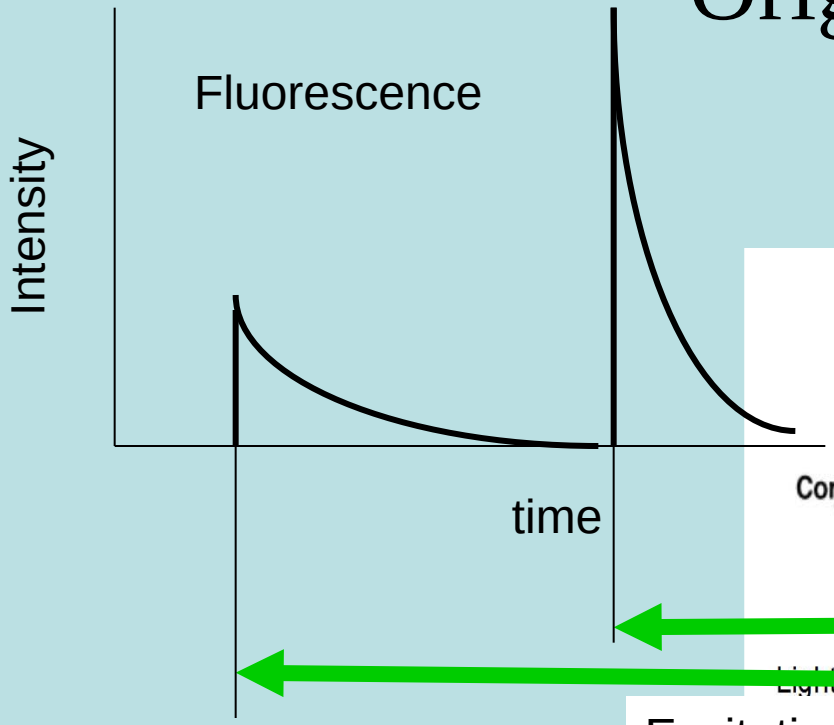
Zoom of slope of fluorescence



Origin of stepped slope



Origin of stepped gradient



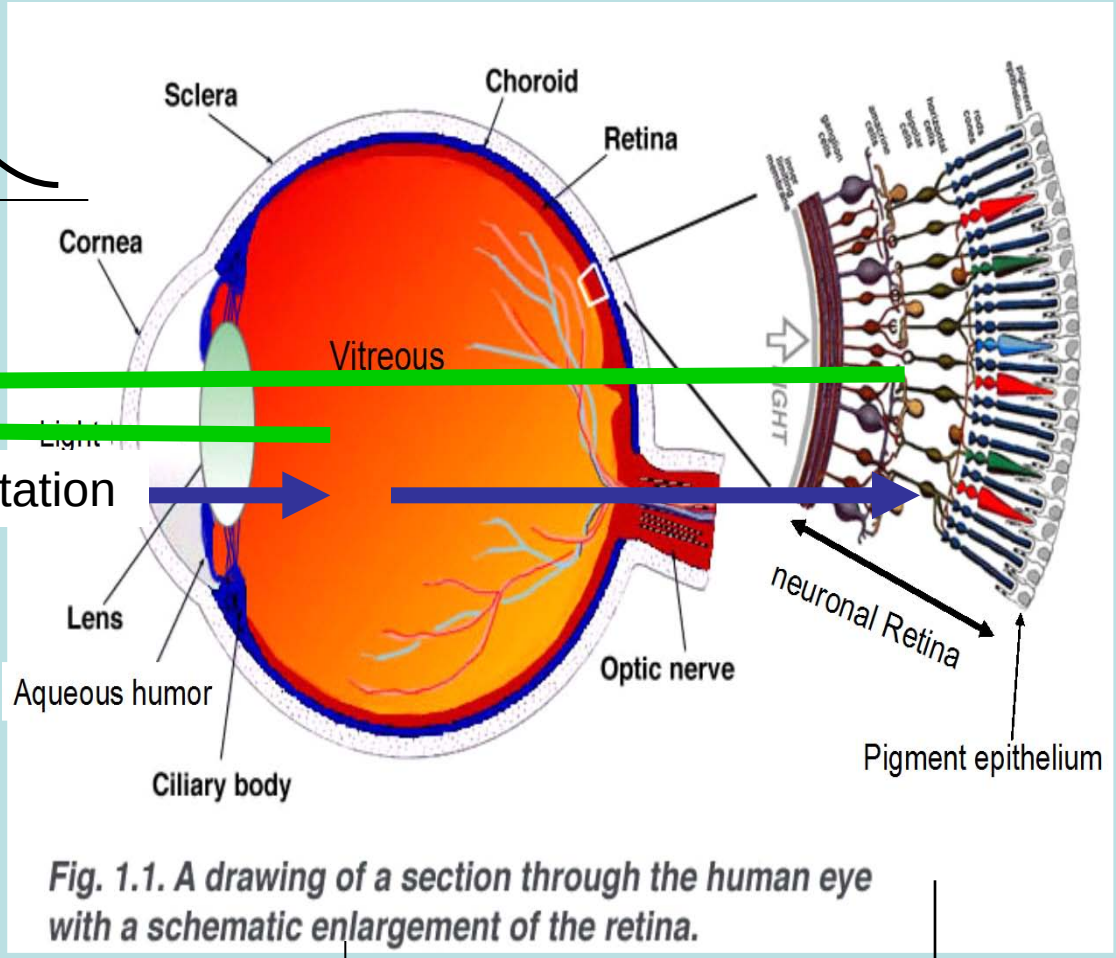
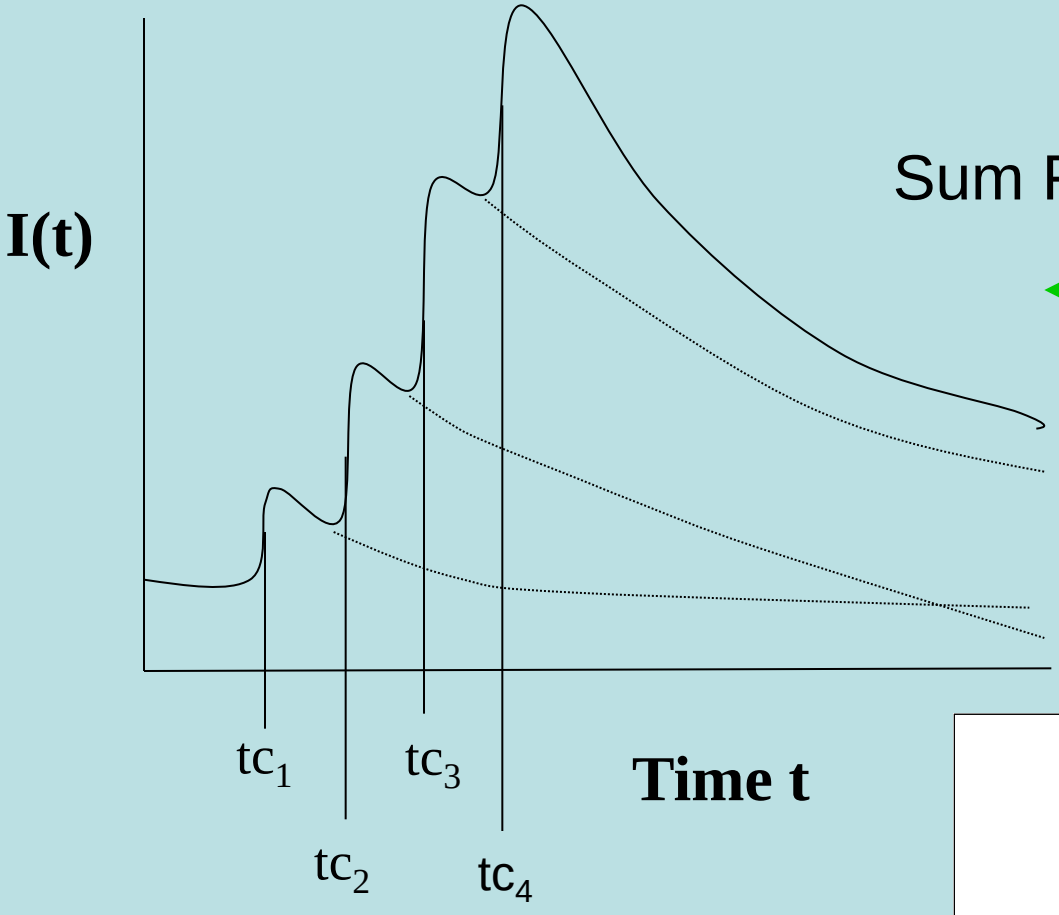
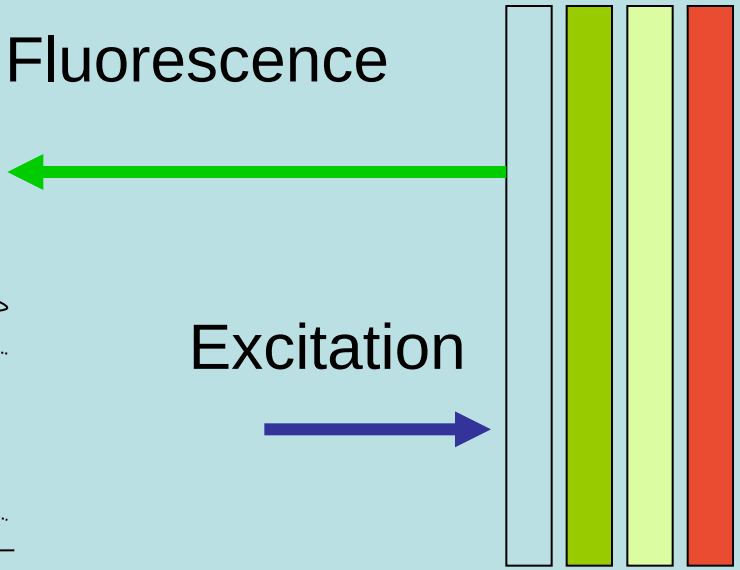


Fig. 1.1. A drawing of a section through the human eye with a schematic enlargement of the retina.

d

Fluorescence of a layered structure





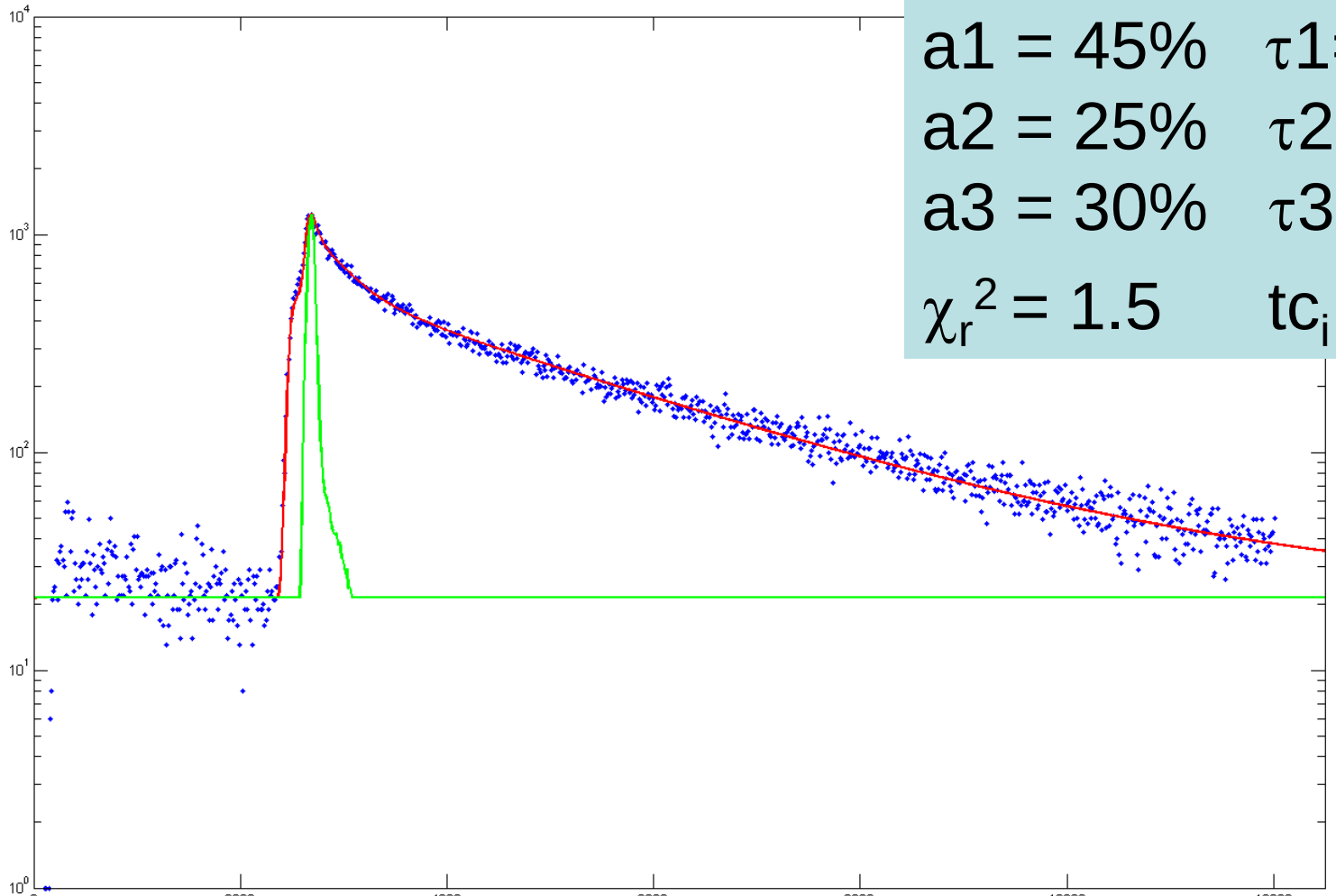
$$\frac{I(t)}{I_0} = \sum_{i,j=1}^{i=p,j=r} \alpha_i \cdot e^{-\frac{t-tc_i}{\tau_{i,j}}} + b$$

Connection between Functionality and Tomography

- **Functionality**:
  Decay behaviour in each layer:
  mono- or bi– exponential decay

- **Tomography**
  Distance between layers: $d = \frac{(tc_i - tc_j) \cdot c}{2 \cdot n}$

Optimal Fit of Sum Fluorescence



Results:

a1 = 45% τ1= 60 ps

a2 = 25% τ2= 320 ps

a3 = 30% τ3= 2660 ps

χ_r^2 = 1.5 tc_i =173.3 ps

Example

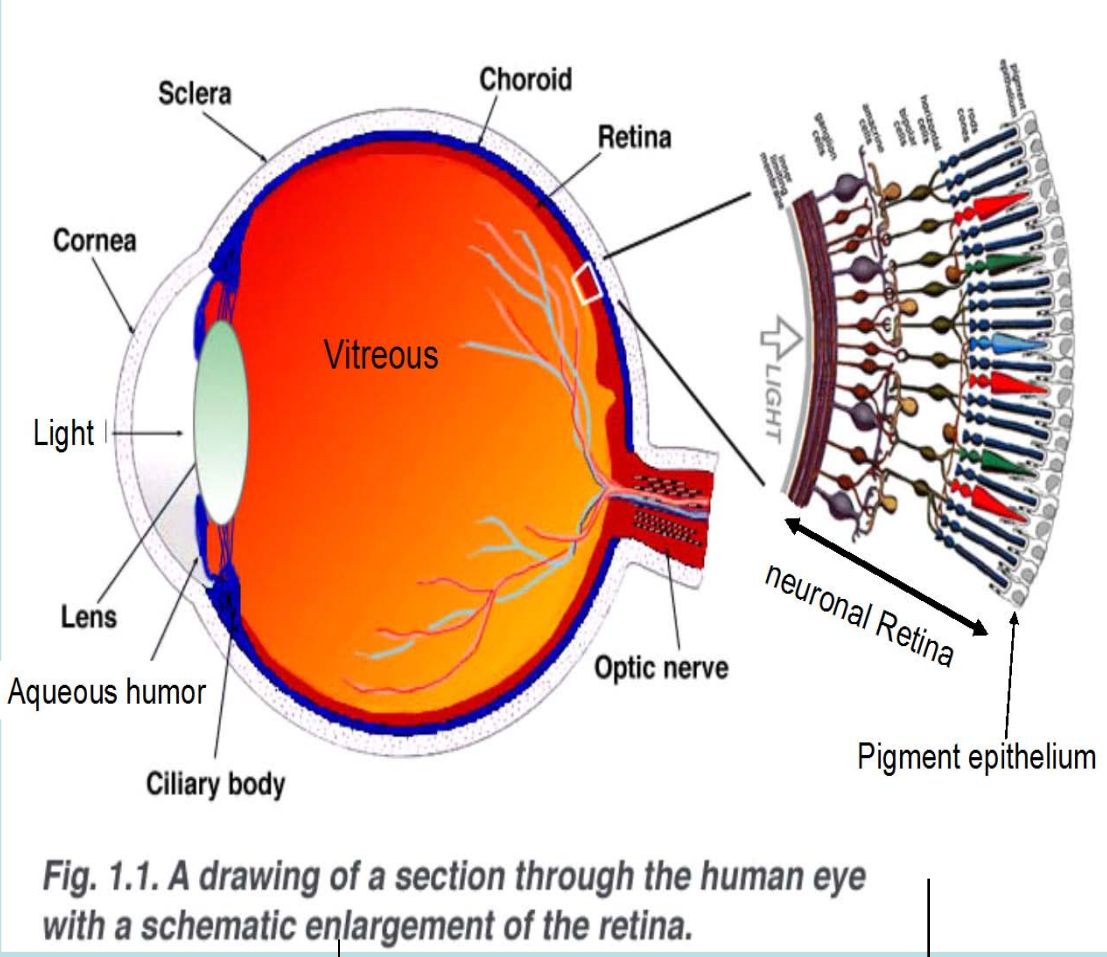
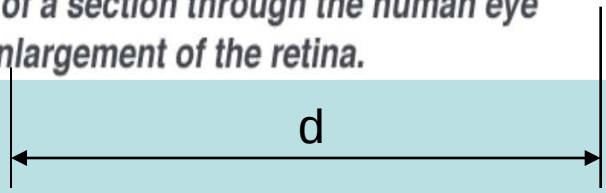


Fig. 1.1. A drawing of a section through the human eye with a schematic enlargement of the retina.



$tc_i = 173.29$ ps

$n_{vitreous} = 1.3668$

$c = 0.3$mm/ps

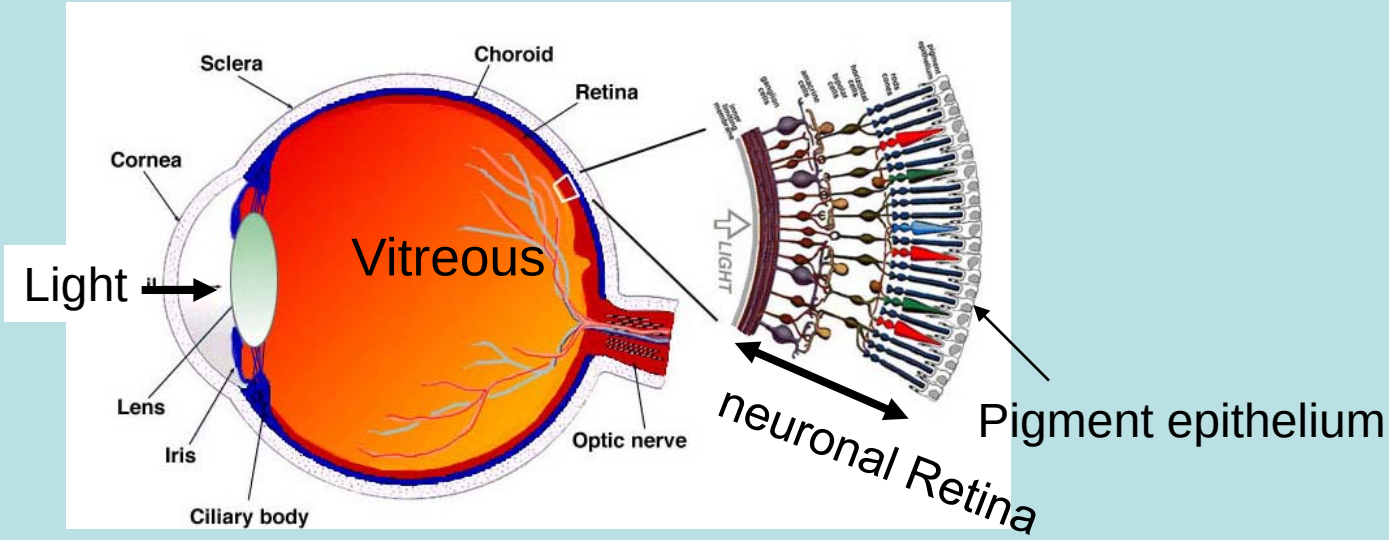
$\underline{d = 19.01\text{mm}}$

Distance
centre lens – retina
d = 18.8 mm

Requirement
for spatial resolution of fundus layers

spatial resolution ≈ time resolution

$\Delta d < 10\ \mu m$ ≈ 30 fs



Zusammenfassung

OCT in Frequenzdomäne:

- *in vivo* Tomographie (mikroskopischer Auflösung)
- funktionelle Tomographie
  (Mikrozirkulation, Polarisations-OCT,
  kontrastverstärkende Marker)

Zeitaufgelöste Autofluoreszenz

- Stoffwechsel auf zellulärem Niveau
- Potential für funktionelle Tomographie
  (Fluorophore und Schichtabstand)