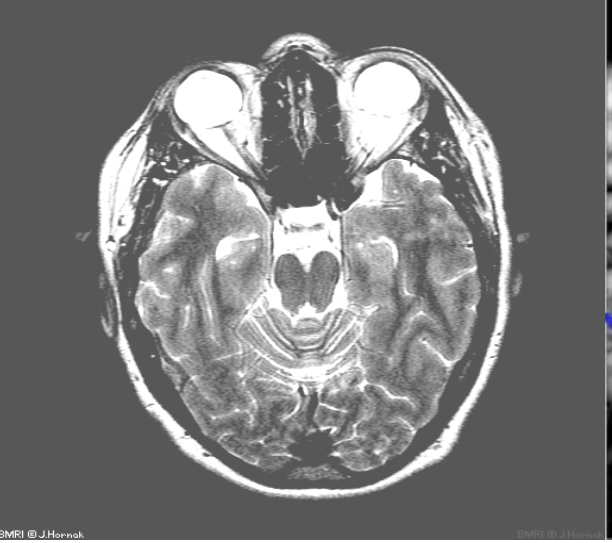
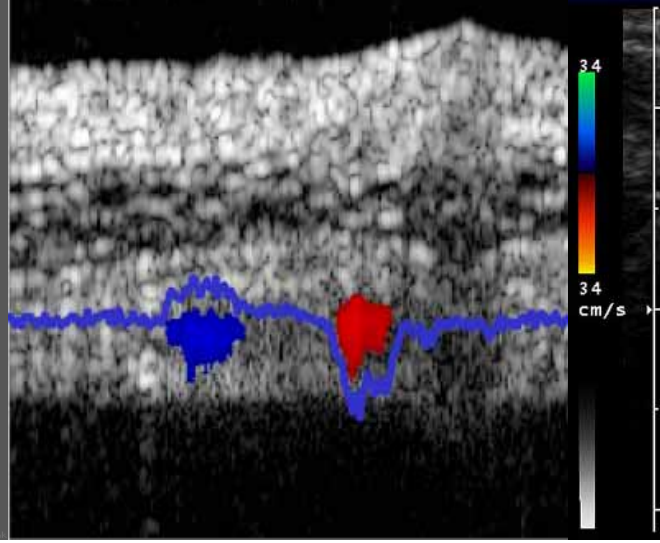
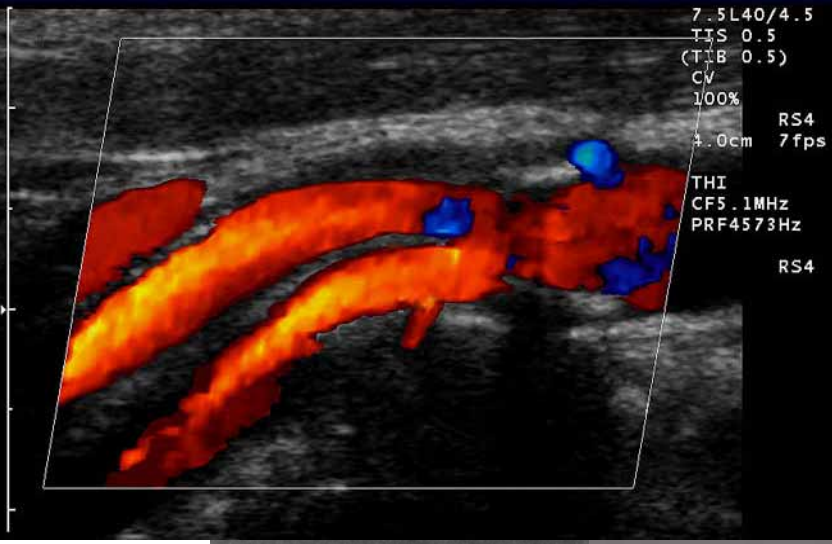




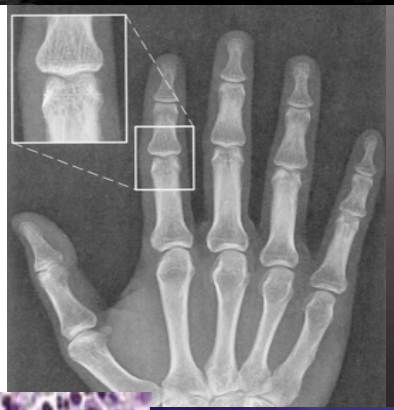


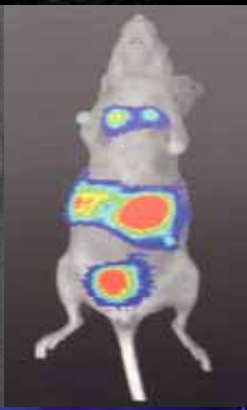
Biomedical Optical Imaging

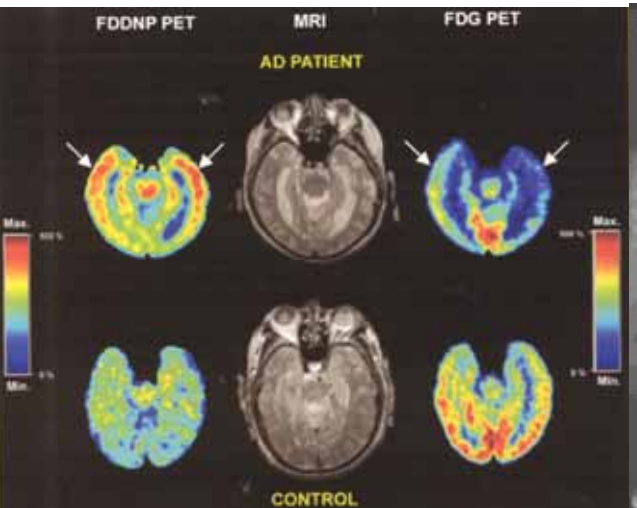
Martin Frenz

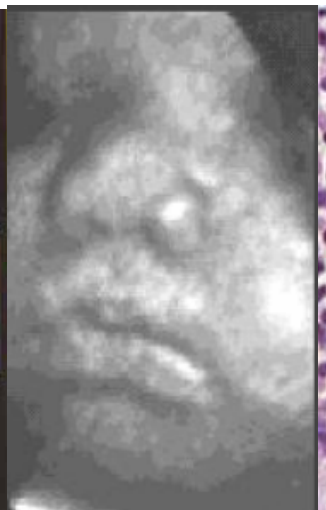
Biomedical Photonics Department

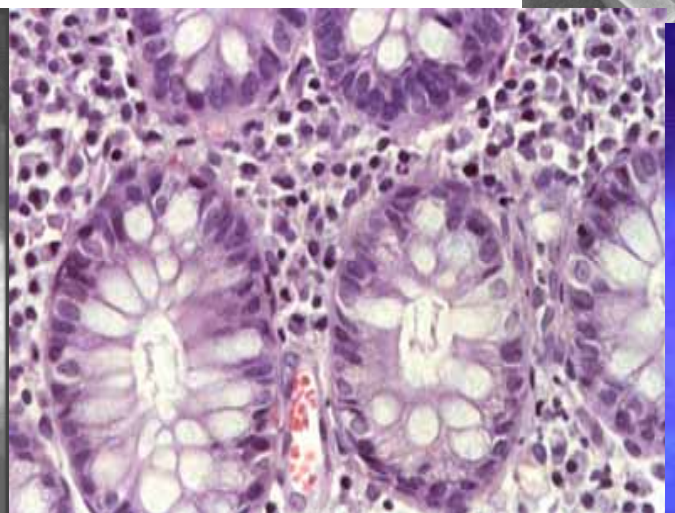
Institute of Applied Physics, University of Bern

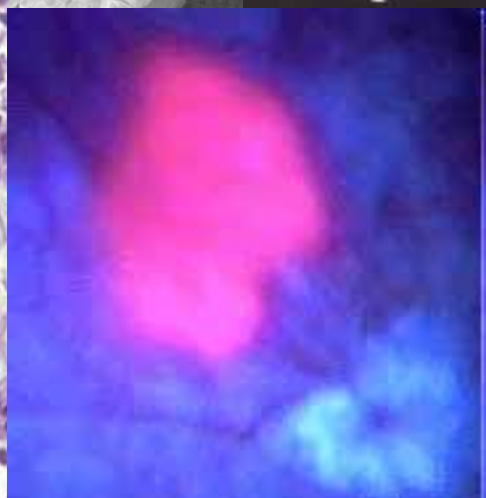






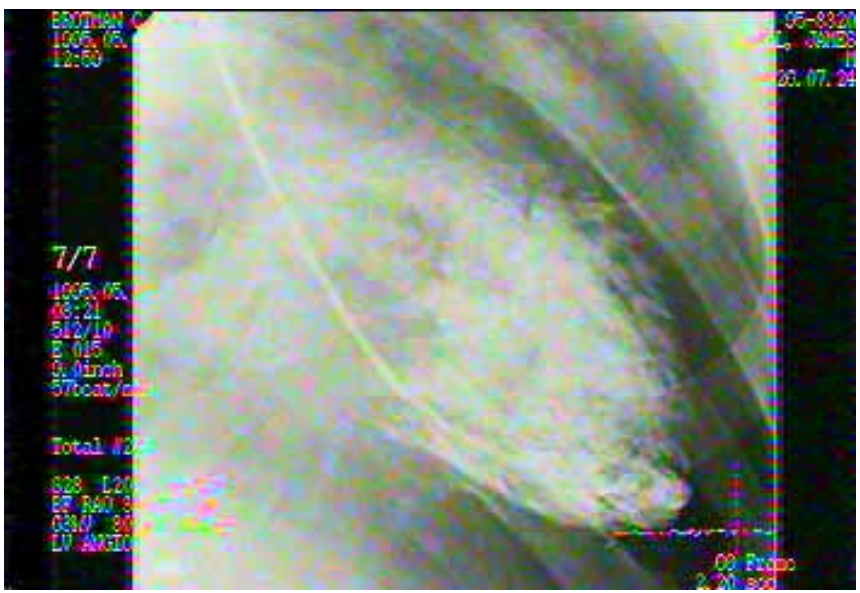






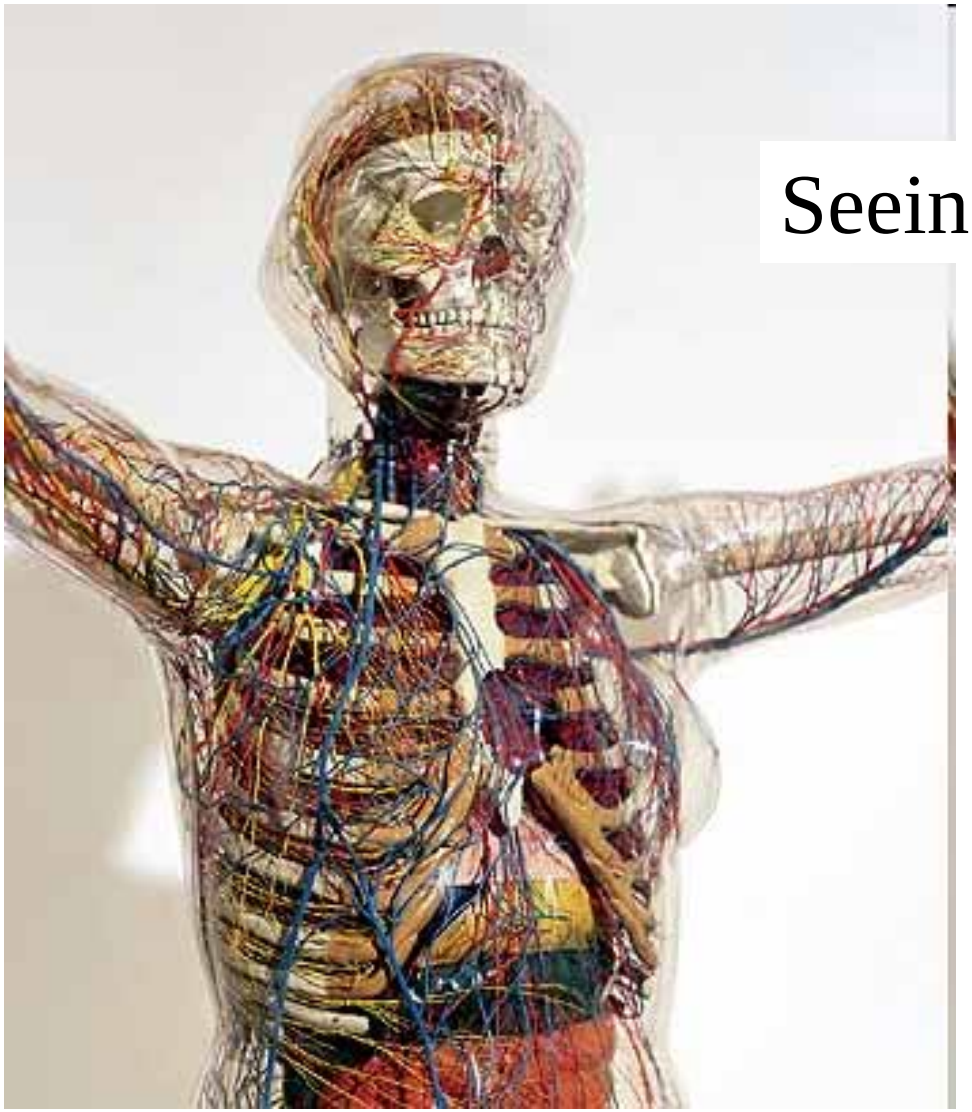
Imaging is one of the most powerful tools in biomedical research. The impact and amount of information contained in visual data is almost impossible to underestimate.

„A picture is worth ten thousand words“



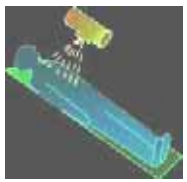
„A movie is almost priceless“

Seeing inside the body with light

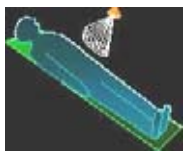


Problem: strong scattering of light in biological tissues over the whole spectral range!

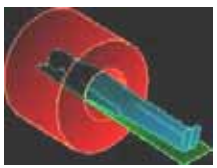
Imaging techniques



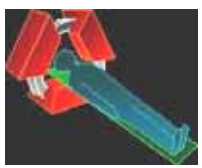
x-ray techniques:	planar (i.e. mammography)	(1895)
	computer tomography	(1970s)



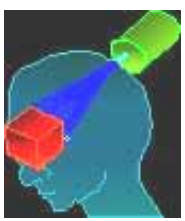
ultrasound:	A/B-mode	(1940)
	Doppler	



MR techniques:	magnetic resonant imaging	(1970s)
	magnetic resonant spectroscopy	



nuclear imaging:	positron emission tomography	(1975)
	single photon emission computer tomography	

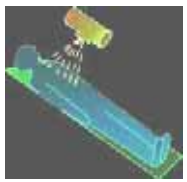


optical techniques:	confocal microscopy	(1986)
	optical coherence tomography	(1991)
	transillumination techniques	
	fluorescence techniques	

Optimum requirements

- spatial resolution range of λ
- temporal resolution 1 ms (real time)
- field of view μm up to cm
- no ionizing radiation — ~~X-ray~~, ~~nuclear imaging, PET~~
- no restrain or anesthesia
- show structures and function
- see anywhere in the body
- low cost and easy to use — ~~MRI~~

Imaging techniques



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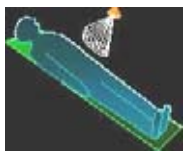
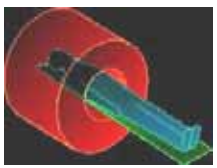
computer tomography (1970s)



ultrasound: A/B-mode (1940)
Doppler



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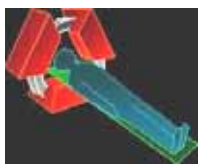
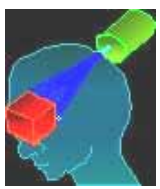
magnetic resonant spectroscopy



nuclear imaging: positron emission tomography (1975)
single photon emission computer tomography



optical techniques: confocal microscopy (1986)
optical coherence tomography (1991)
transillumination techniques
fluorescence techniques

Optical imaging techniques

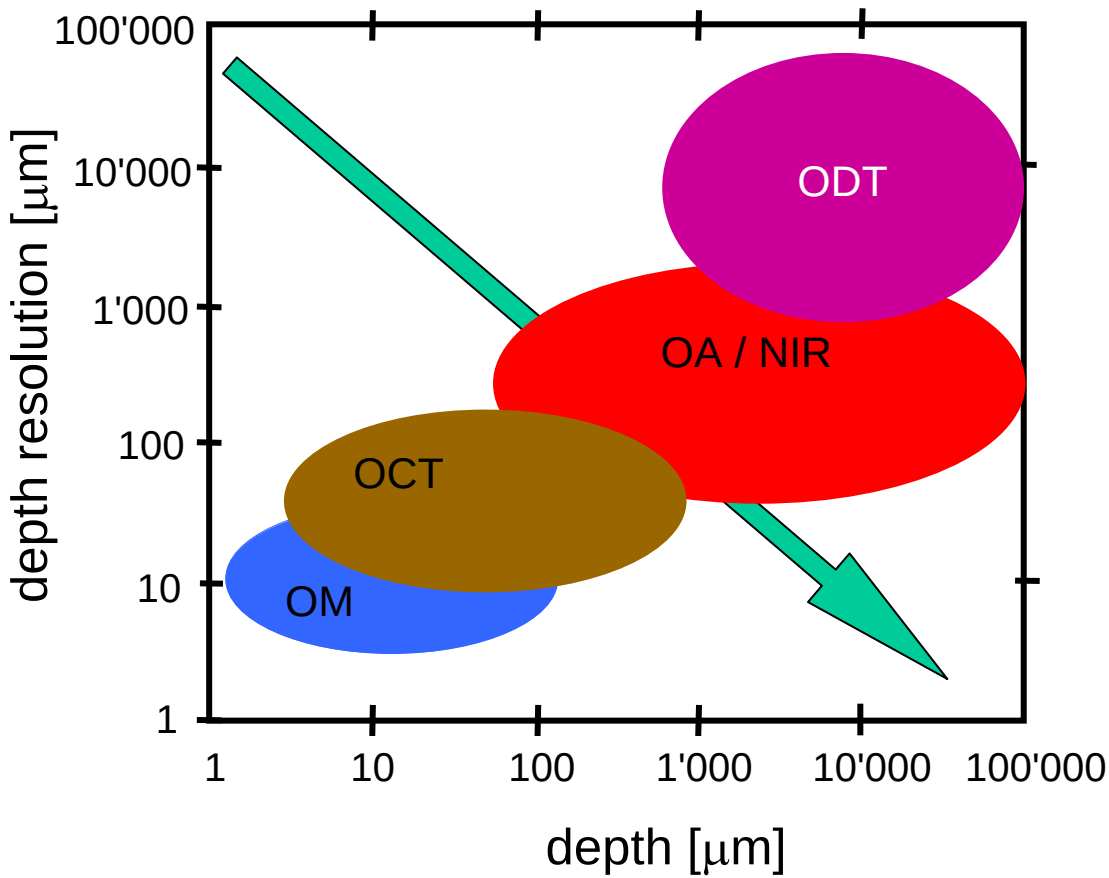
- high spatial resolution
- deep penetration

OM: confocal microscopy
multi-photon microscopy
(Markus Kohler)

OCT: Optical coherence tomography
(Rainer Leitgeb)

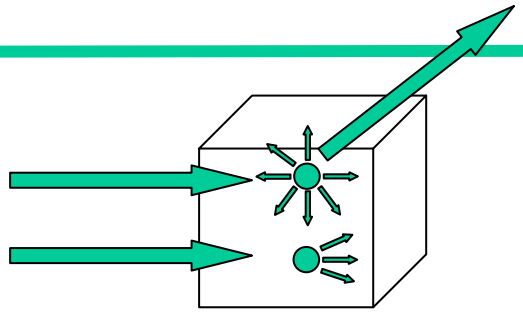
OA: Optoacoustic

ODT: Optical diffusion tomography
time of flight tomography
frequency modulated tomography



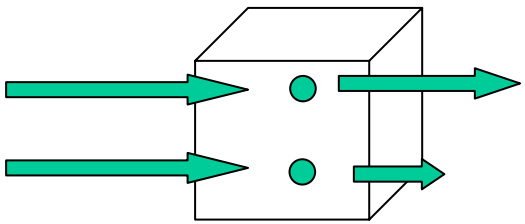
Optical detection

scatter



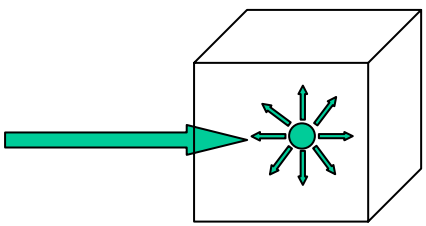
- intrinsic scatter of tissue
- exogenous scatters (gold and silver particles)

absorption



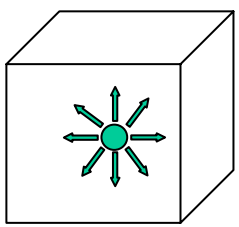
- intrinsic tissue absorption
- exogenous scatters (dyes, gold and silver particles)

fluorescence



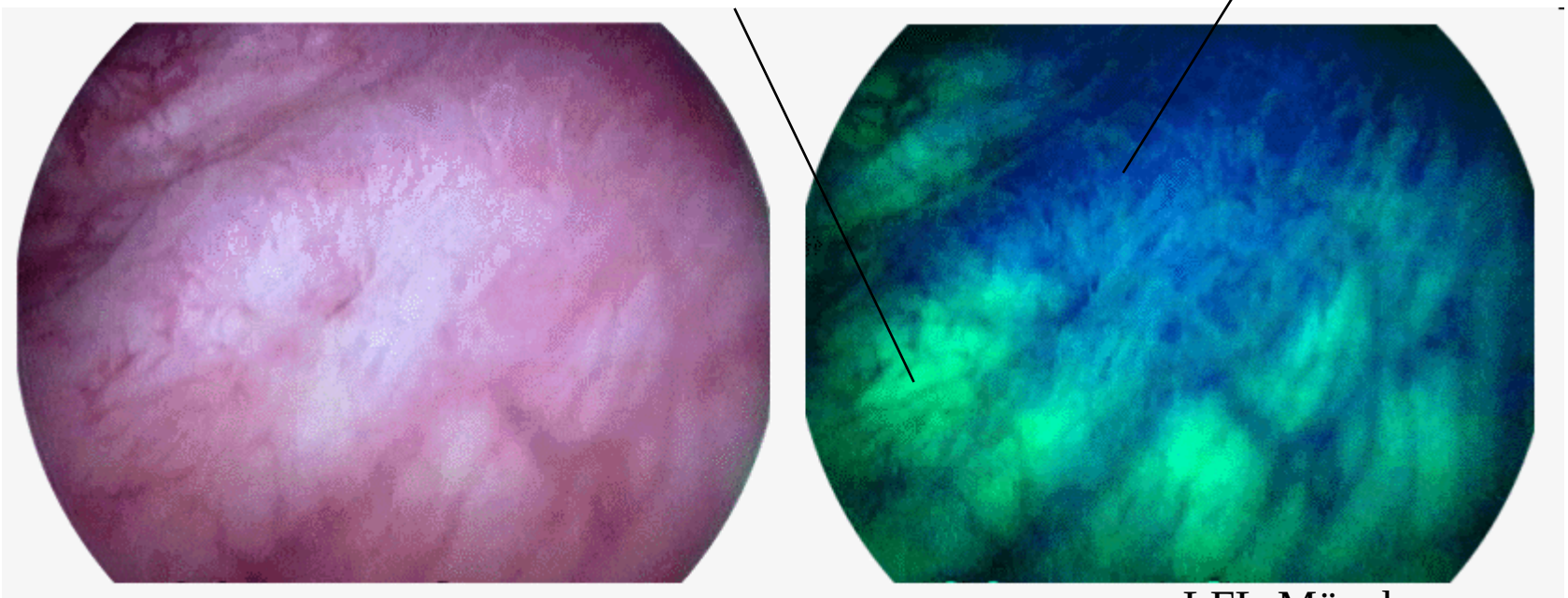
- tissue auto-fluorescence (NADPH)
- fluorescence dyes (porphyrin)
- fluorescence protein-reporter genes (GFP)

bioluminescence



- bioluminescence protein-reporter genes (luciferase)

Autofluorescence

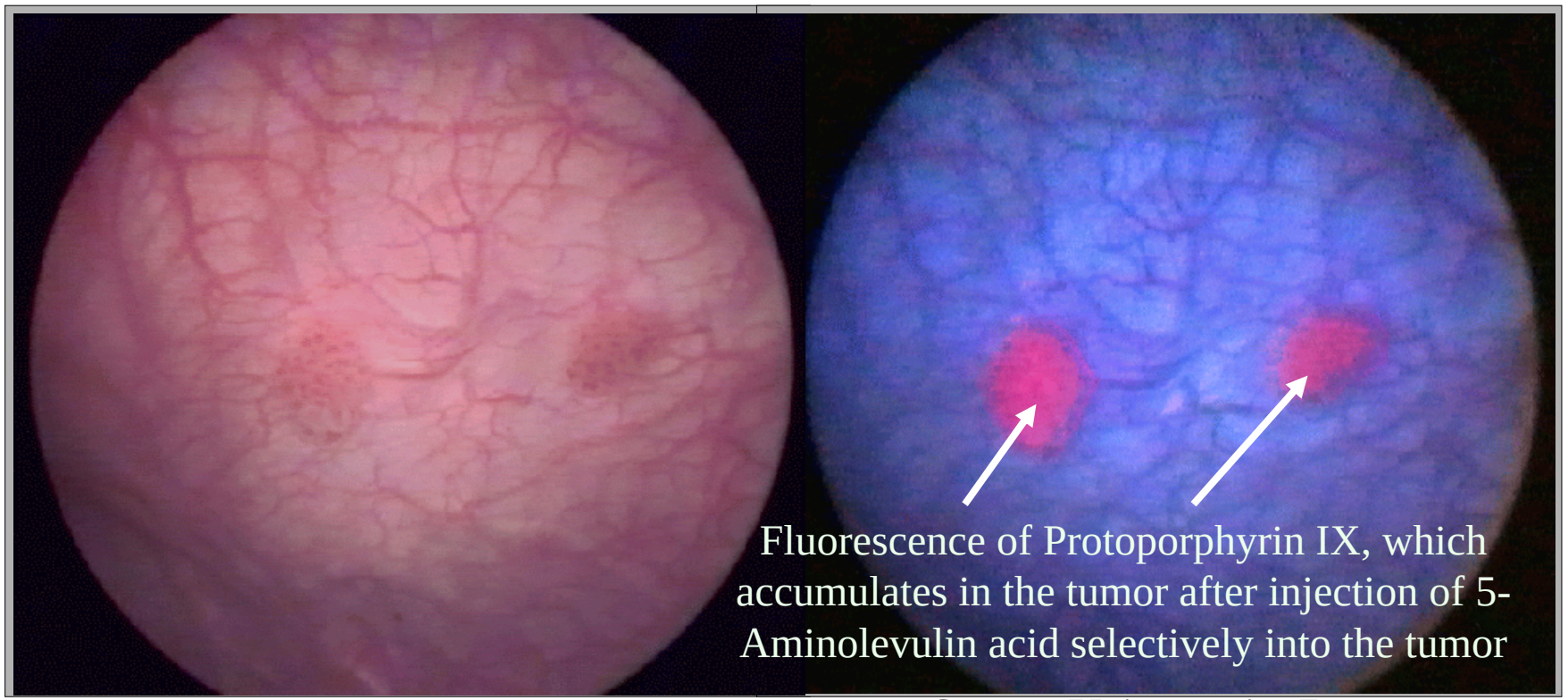


white light and autofluorescence of a flat pTaG1-tumor in the bladder after excitation with light at 400 nm. The thickened mucosa (start of a tumor growing) shows a good contrast to the healthy green fluorescent tissue.

Photodynamic diagnosis

UNIVERSITÄT BERN

application in urology: bladder carcinoma



Stepp, Kriegmair, Baumgartner

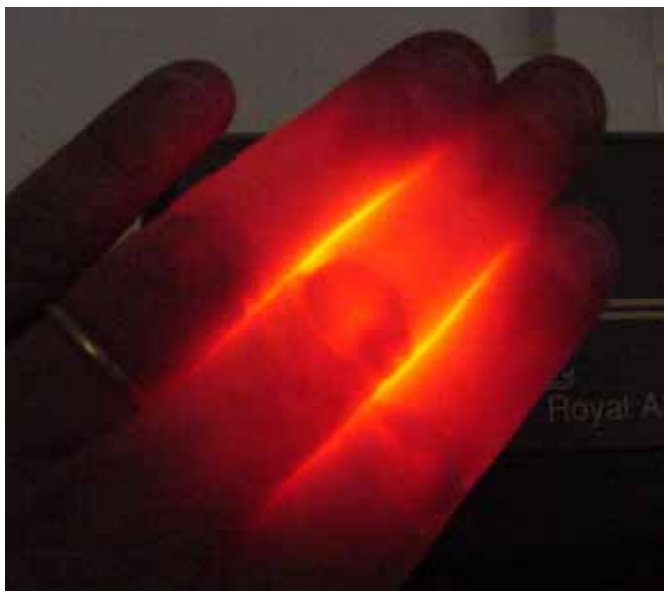
excitation: 380 - 430 nm
detection: > 445 nm

red fluorescence
blue background

Light propagation in tissue

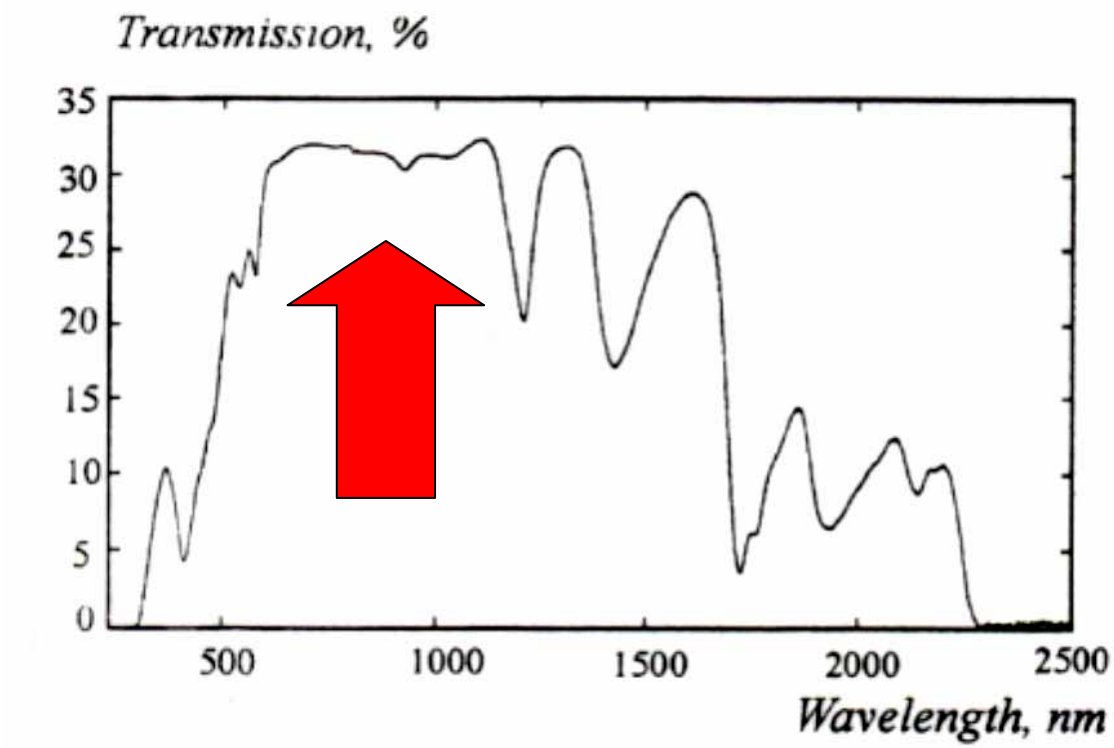
- optically inhomogeneous (1 g tissue contains about 10^9 cells)
- index of refraction higher than in air (Fresnel reflection)

diffusion of light in tissue



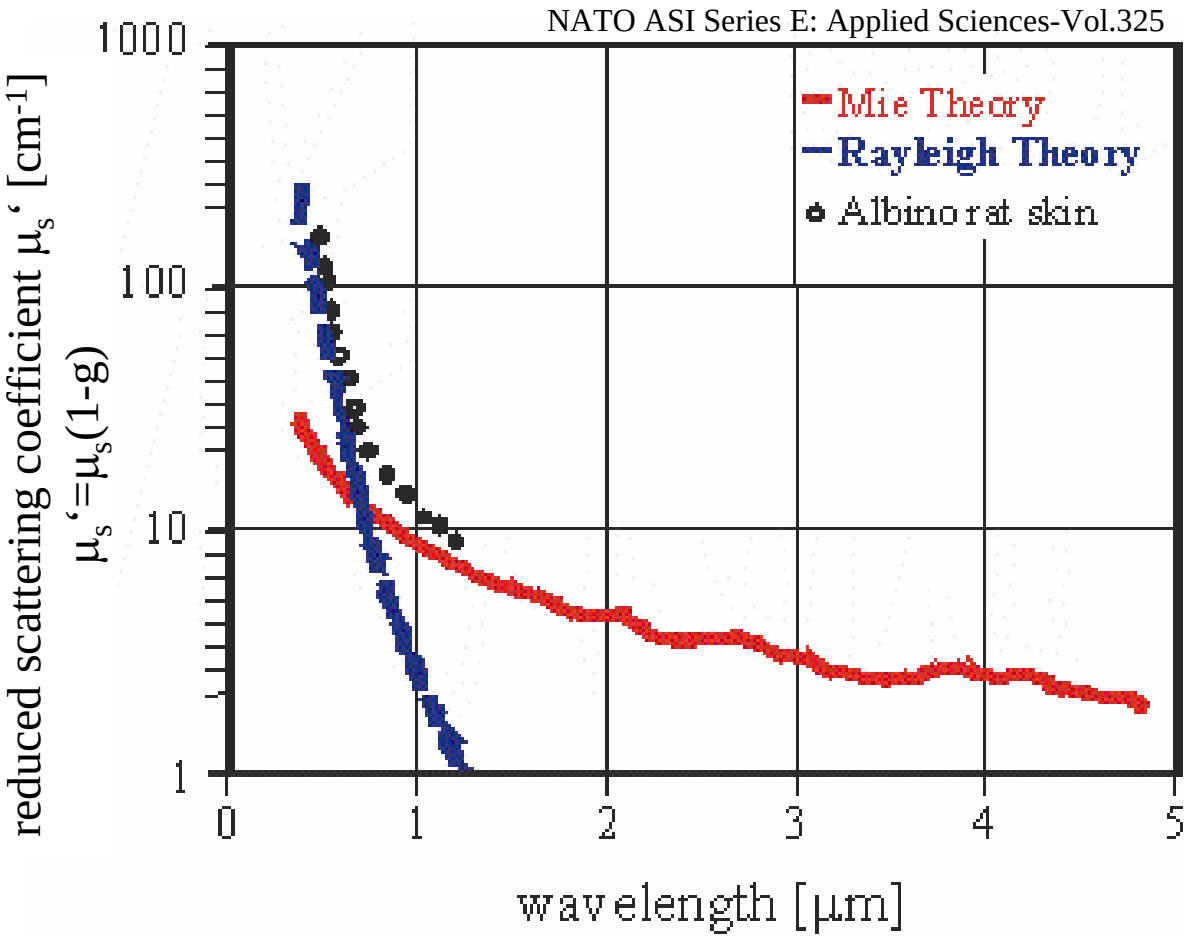
➡ **strong scattering**

3 mm thick slab of female breast tissue



F.A. Marks, Proc. SPIE 1641, p 227 (1992)

Rayleigh- and Mie-scattering



Mie scattering:

particle size ≥ λ

(macromolecular structures, membranes)

Rayleigh scattering

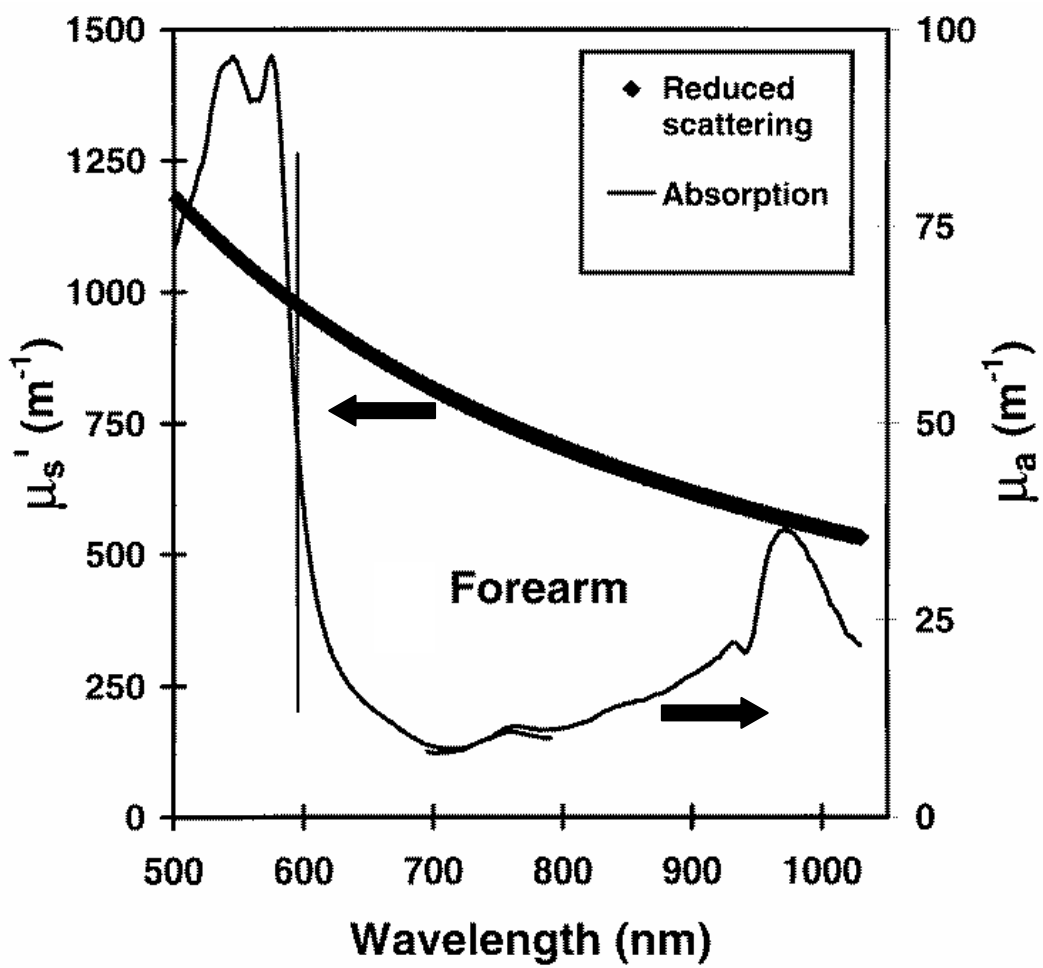
particle size << λ

organelles, collagen fibers, cells)

optical scattering spectra provide information about size distribution of scatters

Optical tissue properties

scattering and absorption



Doornbos et al. Phys.Med.Biol. 44, p 967 (1999)

Beer-Lambert law

$$I(d) = (1 - R_F) \cdot I_0 \cdot \exp(-\mu_t d)$$

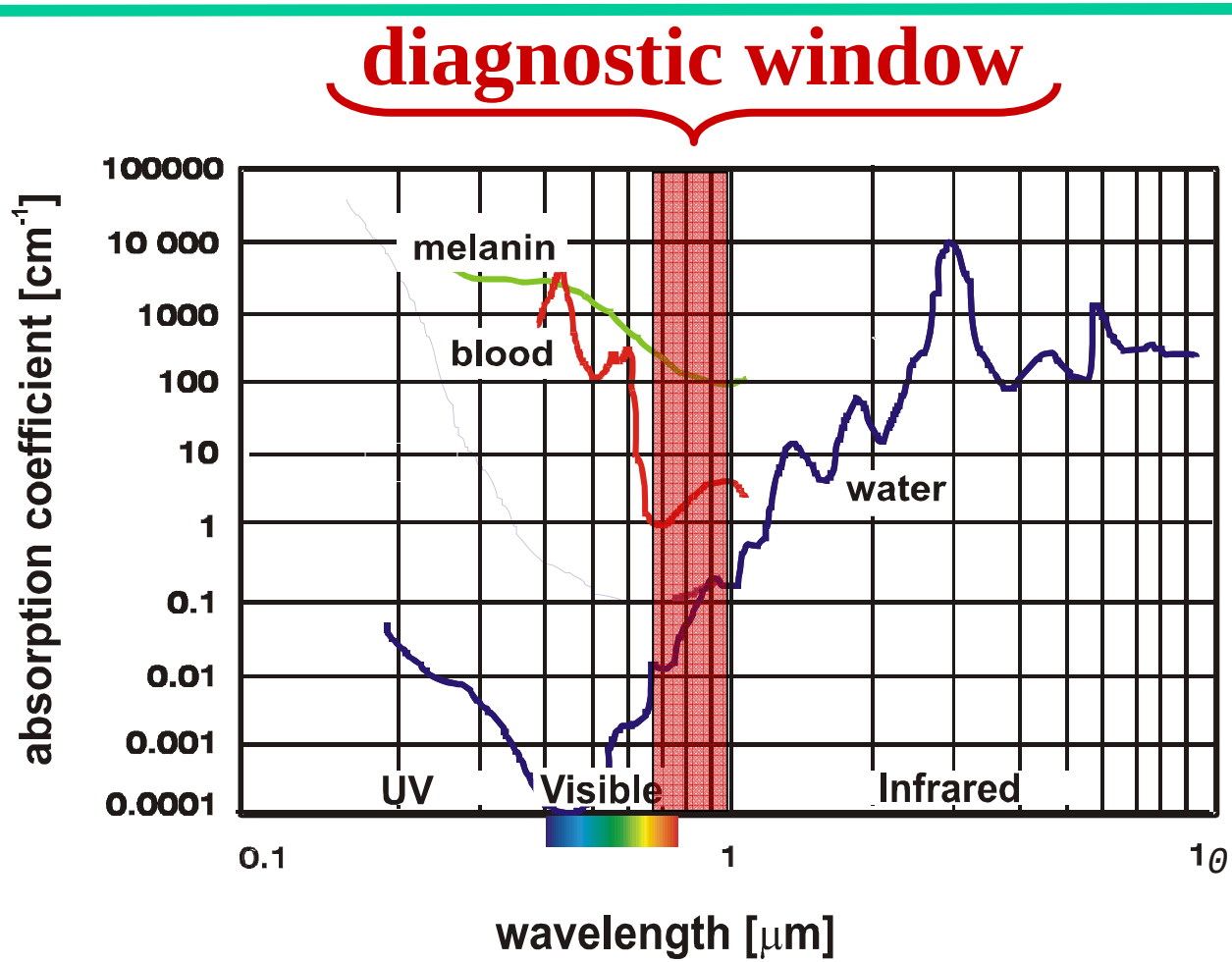
$$\mu_t = \mu_a + \mu_s$$

I_0 = collimated irradiance [W/cm^2]
R_F = Fresnel reflection

Mean free path length

$$l_{path} = \frac{1}{\mu_t}$$

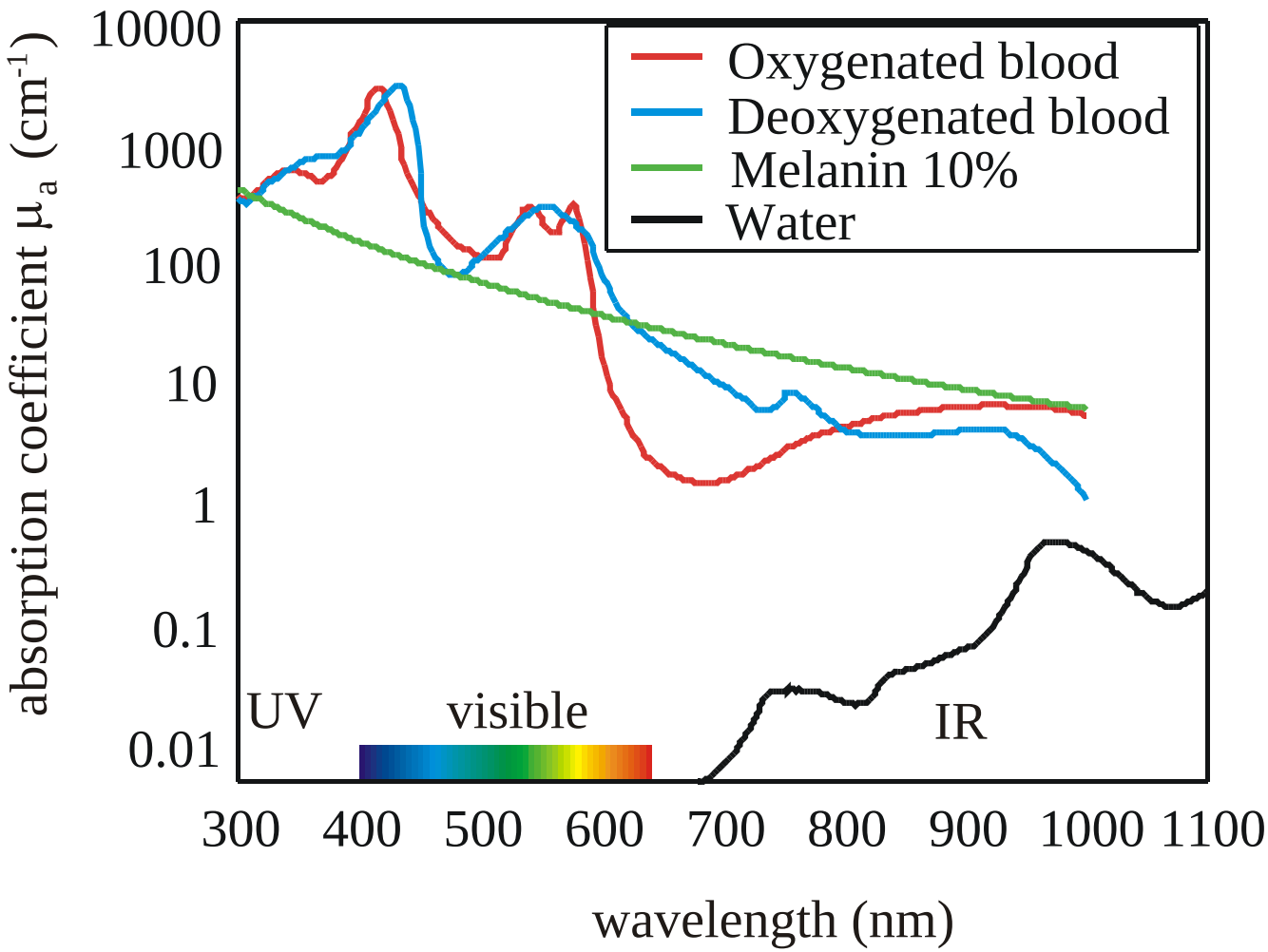
Tissue absorption



Hemoglobin and water have relatively low absorption in the near-IR

➡ near-IR "window" enables optical imaging and near-IR spectroscopy

Optical imaging



angiogenesis

(blood concentration)

+

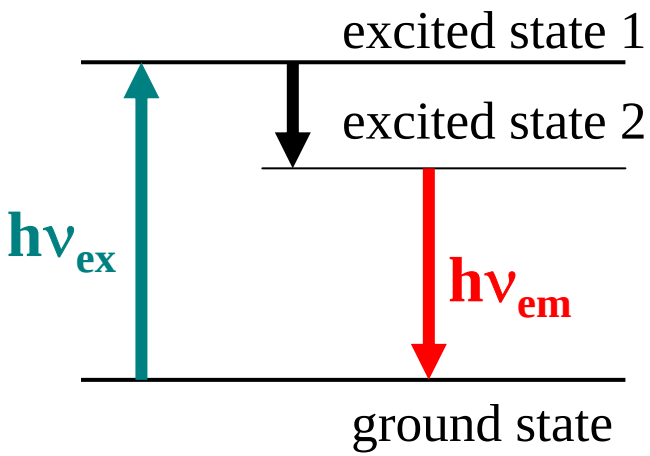
hypermetabolism

(oxygen saturation)

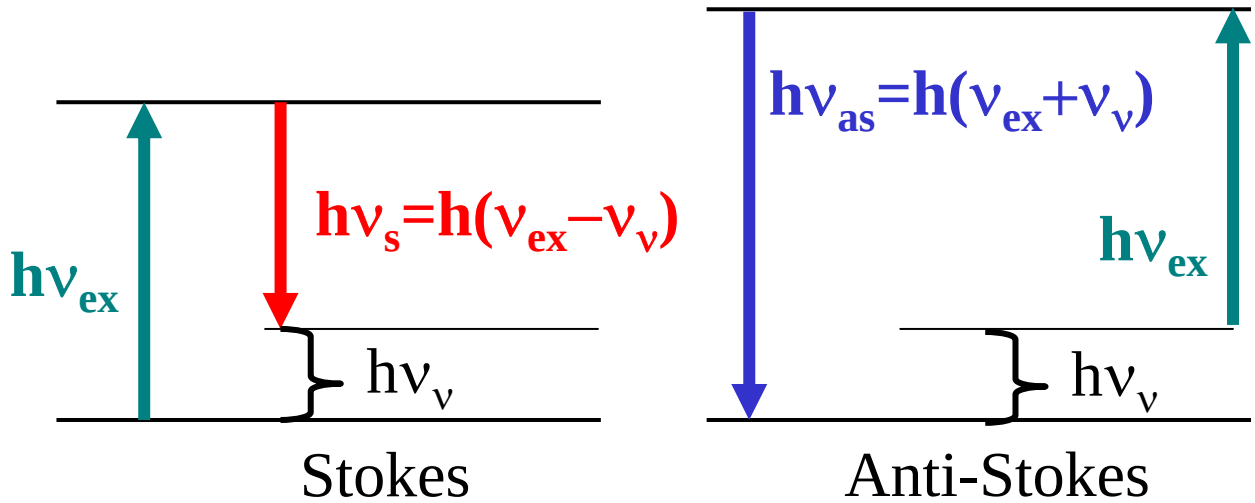
optical absorption provides contrast for functional imaging

Fluorescence and Raman scattering

Fluorescence



Raman

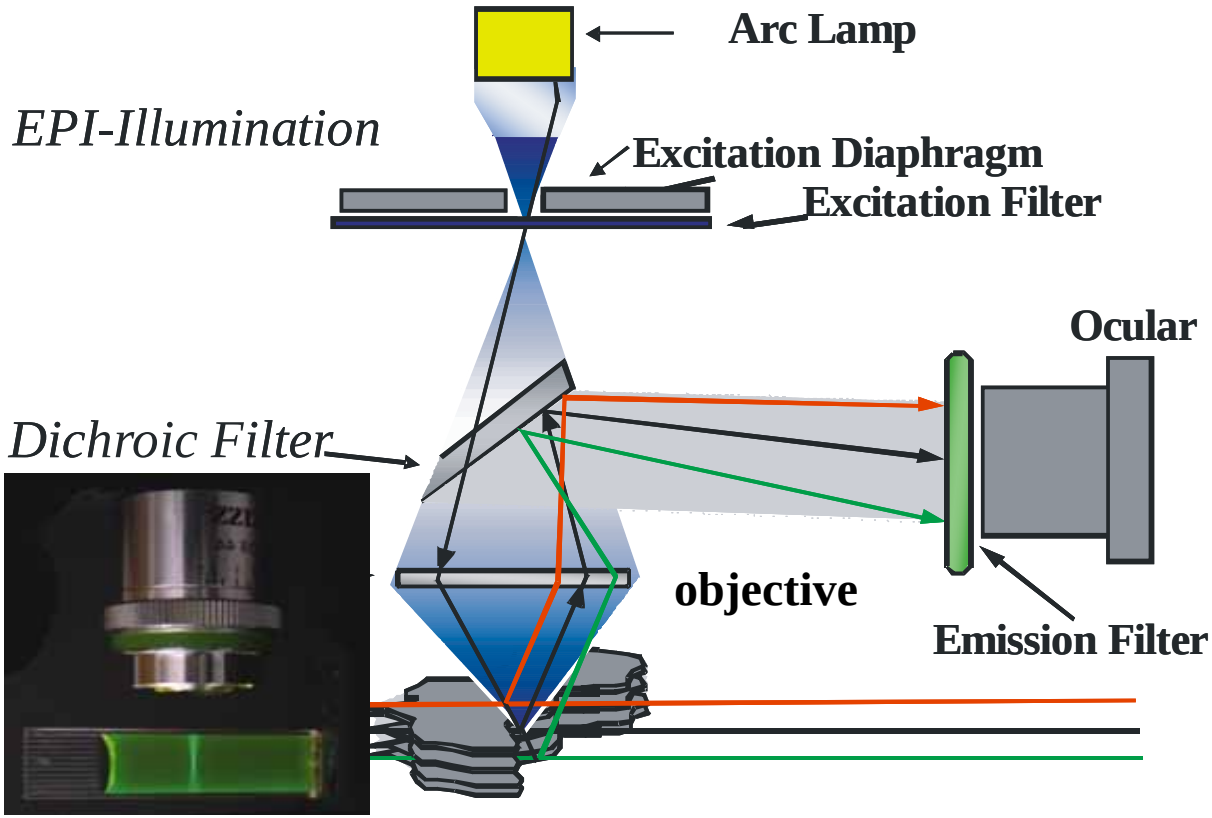


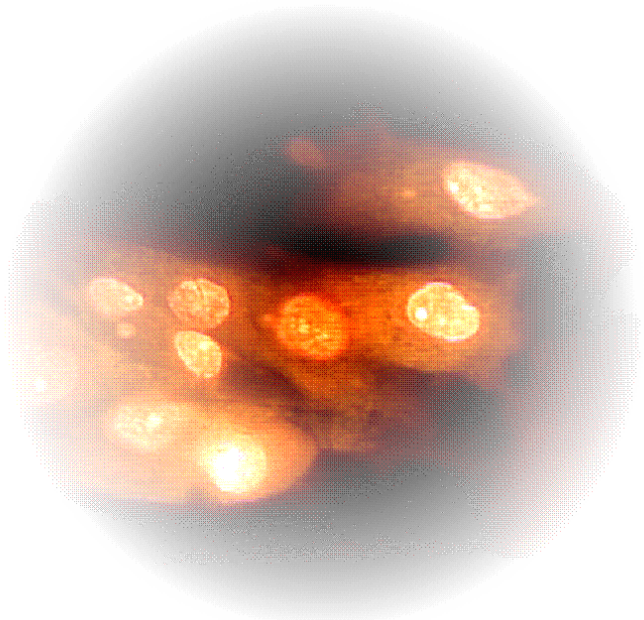
➡ radiative decay from an excited state

➡
- coupling to molecular vibrations
- each molecule has unique Raman spectrum
- typical Raman cross-section
  $\sigma \sim 10^{-29}$ $cm^2 molecule^{-1} sr^{-1}$ (very weak signal)

Fluorescence or Raman scattering provides biochemical information because they are related to molecular conformation

Fluorescence microscopy

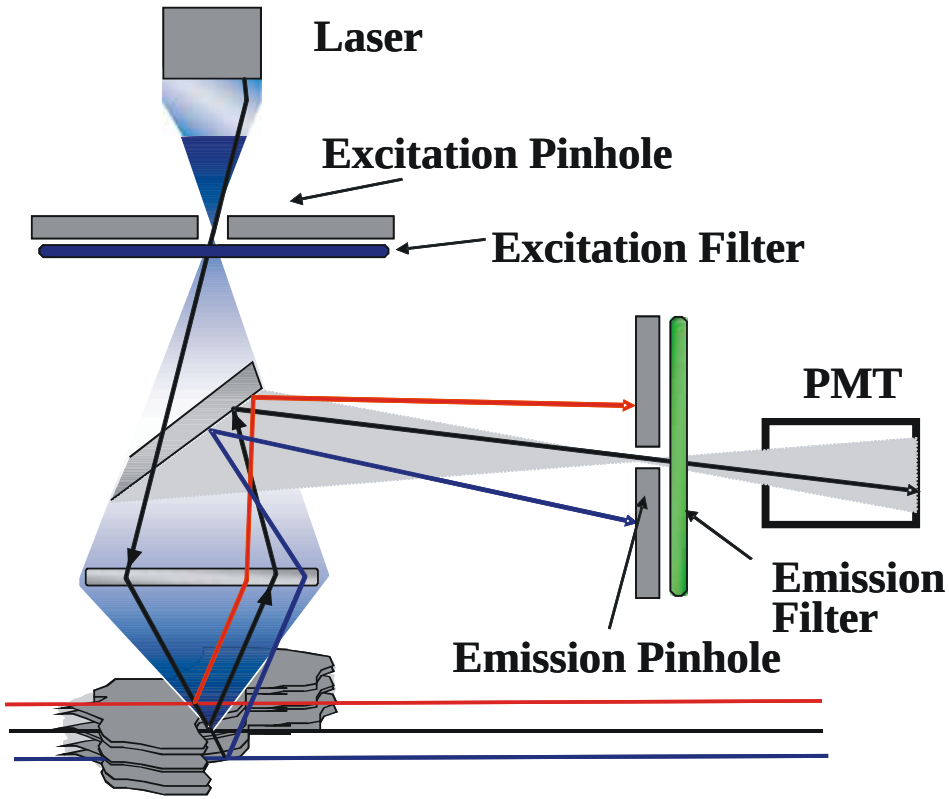


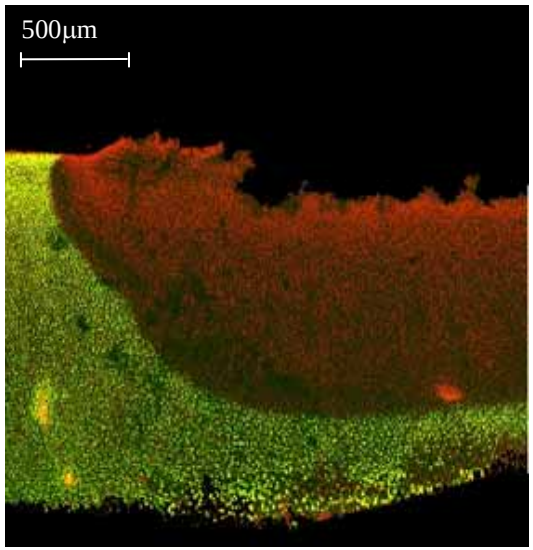


- provides higher contrast than conventional optical microscopy
- image is blurred due to fluorescence from out-of-focus regions
- bleaching of the chromophore

www.micro.magnet.fsu.edu/primer

Confocal microscopy





- The fluorescence emission that occurs **above** and **below** the focal plane is not confocal with the pinhole aperture.
- bleaching of the dye
- excitation in the UV region

3D imaging

www.micro.magnet.fsu.edu/primer

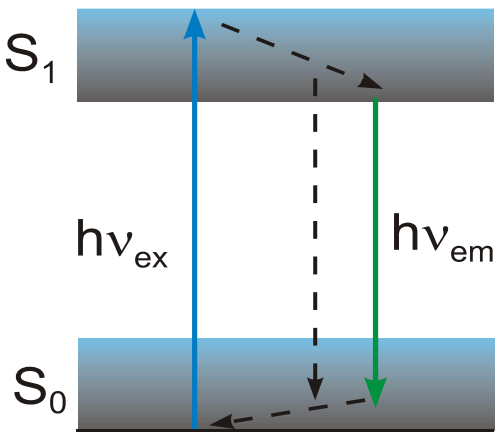
Two-photon vs. one-photon fluorescence

$$Fluorescence \propto I(r,t)$$

$$Fluorescence \propto I^2(r,t)$$

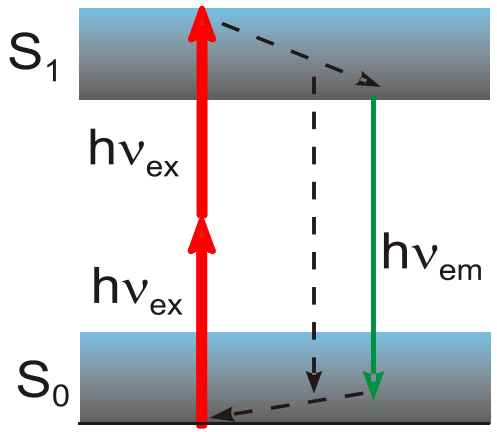
Single-photon excitation

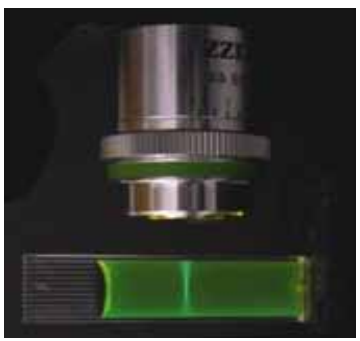
linear

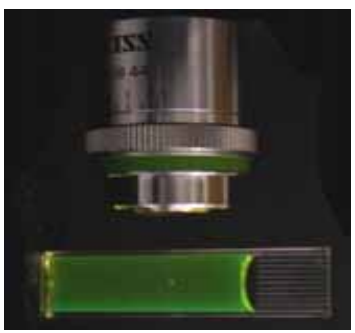


Two-photon excitation

nonlinear







- λ_{em} shorter than λ_{ex}
- less scattering
- low probability event

Quantitative two-photon microscopy

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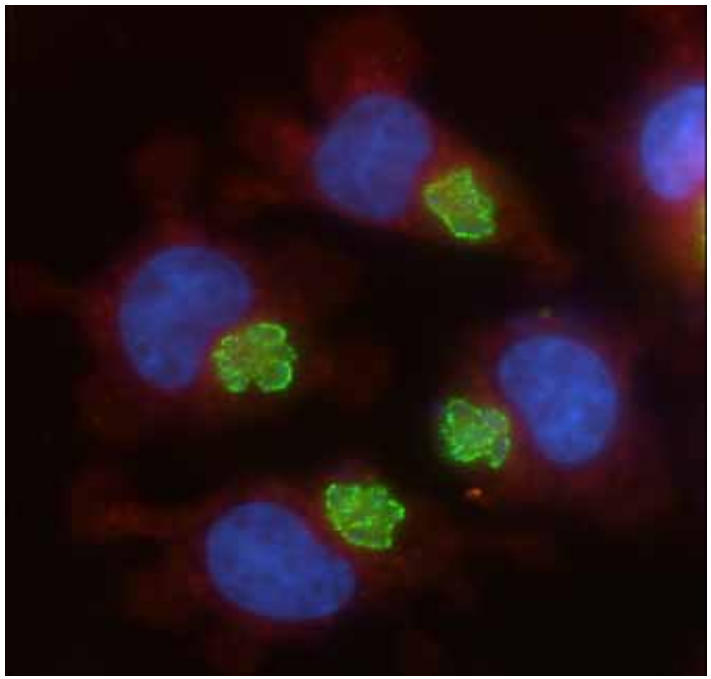
Theileria infected macrophages



- near-infrared radiation enhances the penetration depth
- reduces image deterioration due to scattering when passing through biological tissue
- less photo-bleaching and photo-damage
- regions above and below the excitation light cone are not excited (no background fluorescence)

→ **imaging limited to a small depth**

Targeted contrast agents

- Clinical diagnostic imaging often relies on different uptake behavior of contrast agents between tumors and the surrounding tissue
- Non-targeted dyes may accumulate in tumor due to increased vascular density or capillary permeability
- Some imaging agents specifically target certain receptors, which are overexpressed in malignant cells
- Examples of targeting ligands for delivery of diagnostic imaging agents include antibodies, hormones, or small peptides.

Markers

- Fluorescent dye (e.g. GFP, ICG)
- quantum dots
- Bioluminescence (Luciferase)

emission →

- fluorescence
- spectroscopy
- imaging
- ...

- gold nanoparticles
  (nano spheres, shells, rods)

emission ↔ absorption

- opt. spectroscopy
- confocal
- OCT
- photon migration
- optoacoustics
- .....

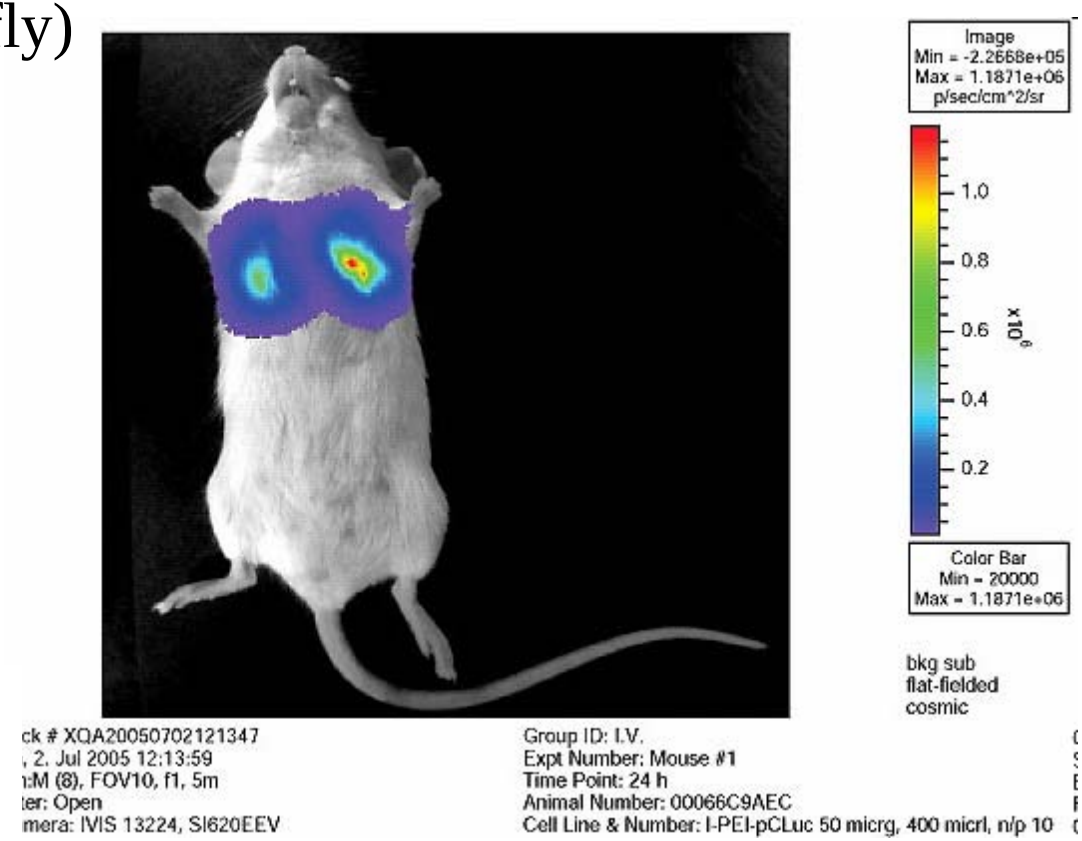
Bioluminescence (Luciferase)

- Bioluminescence: encymes catalyze a bio-chemical process inside the animal that emits light
- Luciferase emits light when it combines with luciferin, ATP and molecular oxygen (light from firefly)
- no external light required
- emission between 400-600 nm
- molecular imaging

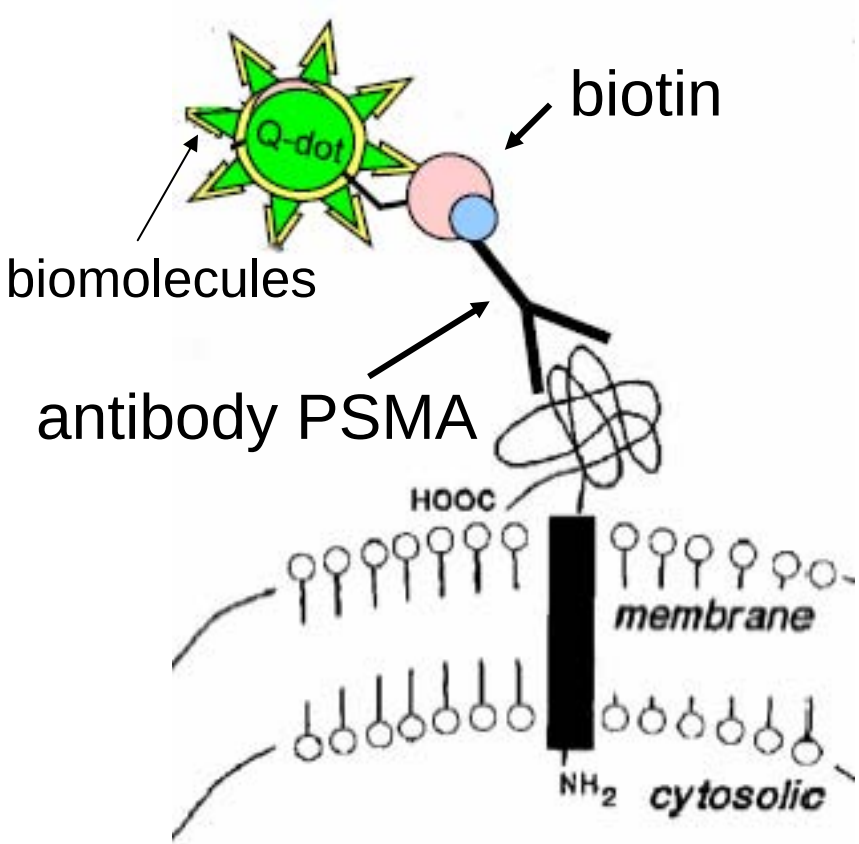
high sensitivity

but

poor spatial resolution due to light scattering

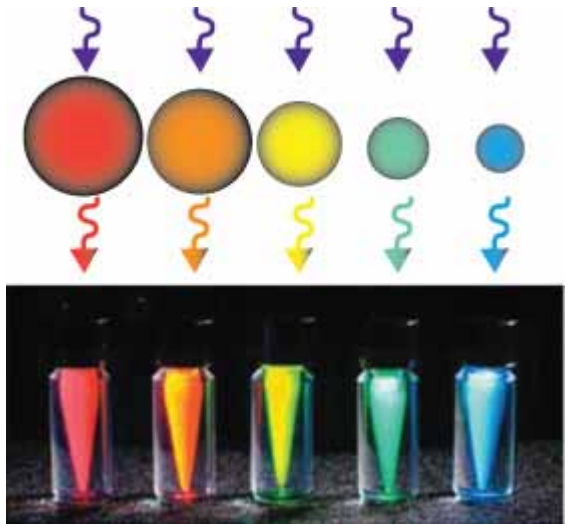


Quantum dots



Q-dots: ∅ = 15 - 20 nm

- tuneability
- no bleaching
- 10 – 20 nm size
- high quantum yield (> 90%)
- broadband excitation
- long fluorescence lifetime



http://probes.invitrogen.com

Quantum dots

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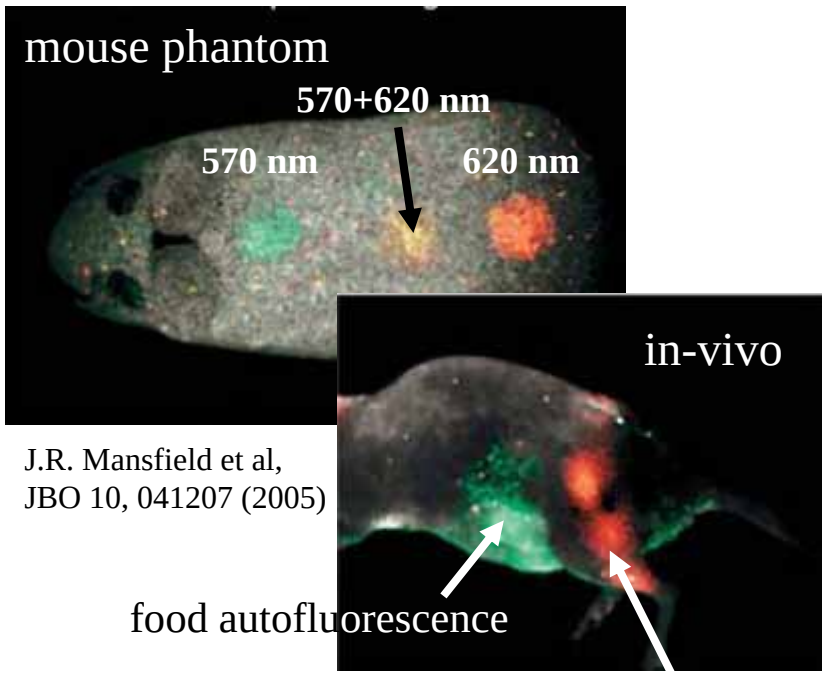
multispectral images using different Q-dots

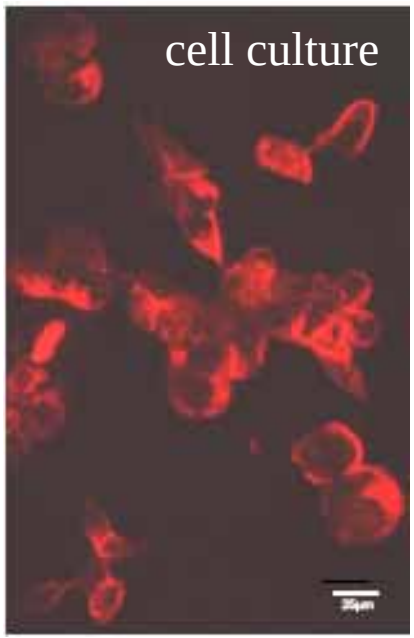


J.R. Mansfield et al,
JBO 10, 041207 (2005)

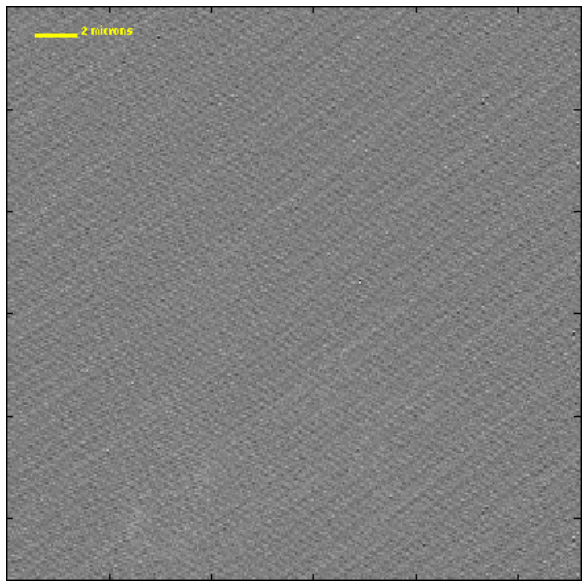
C4-2 prostate tumor cells

- injection of anti-PSMA antibody coupled to 640 nm Q-dots
- background = autofluorescence

LNCaP prostate tumor cells



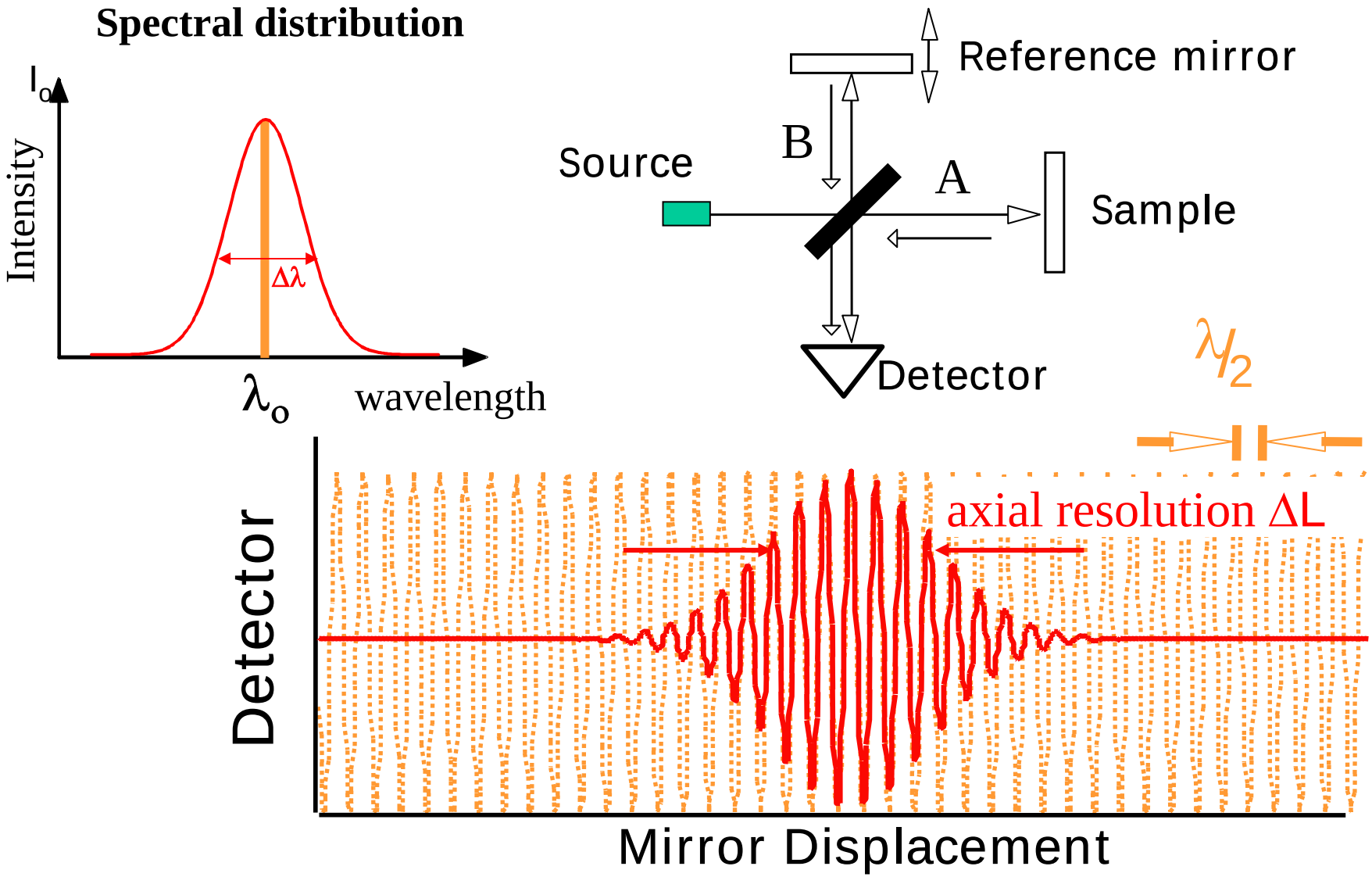
Q-dots
PSMA antibody
conjugate



gold nanorods

- 2 photon luminescence
- membrane binding

Optical Coherence Tomography (OCT)



Light sources

wavelength and bandwidth determine axial resolution (ΔL):

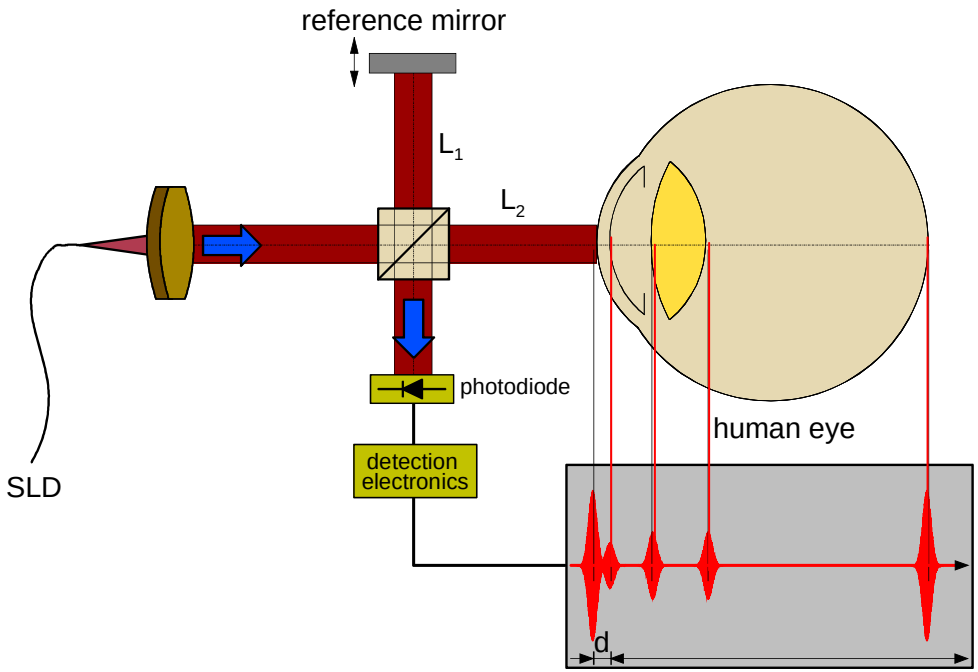
$$\Delta L = \frac{2\ln 2}{\pi}\frac{\lambda^2}{\Delta\lambda}$$

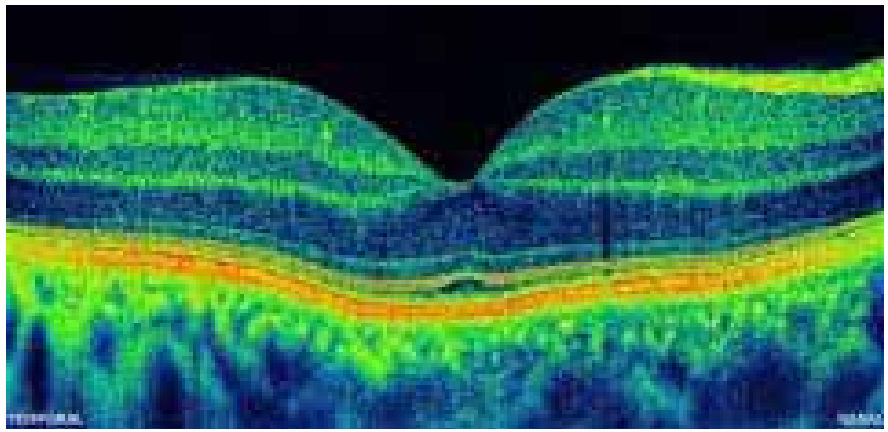
Typical resolution:

	SLD	*Ti:Sapphire laser*
λ_0 =	1300 nm	800 nm
$\Delta\lambda$ =	50 nm	125 nm
ΔL =	15 μm	2 μm

spatial resolution is given by the spot diameter of the laser

Optical Coherence Tomography





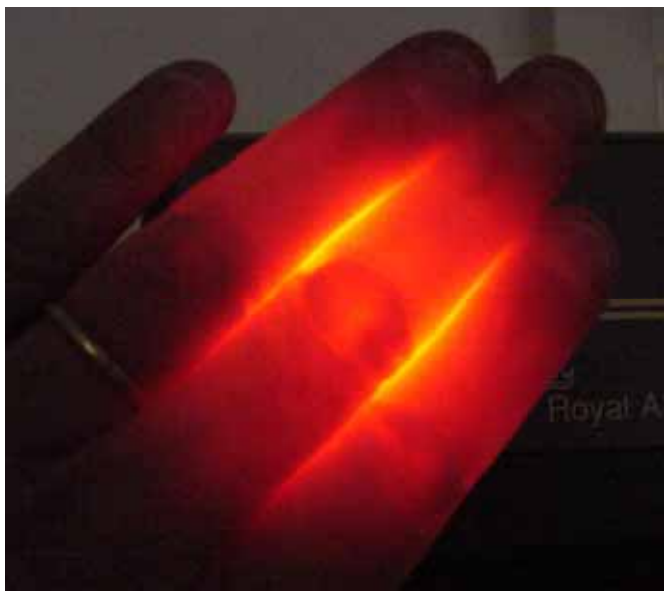
V.J. Srinivasan et al., SPIE Vol. 6079, p607907-1-5 (2006)

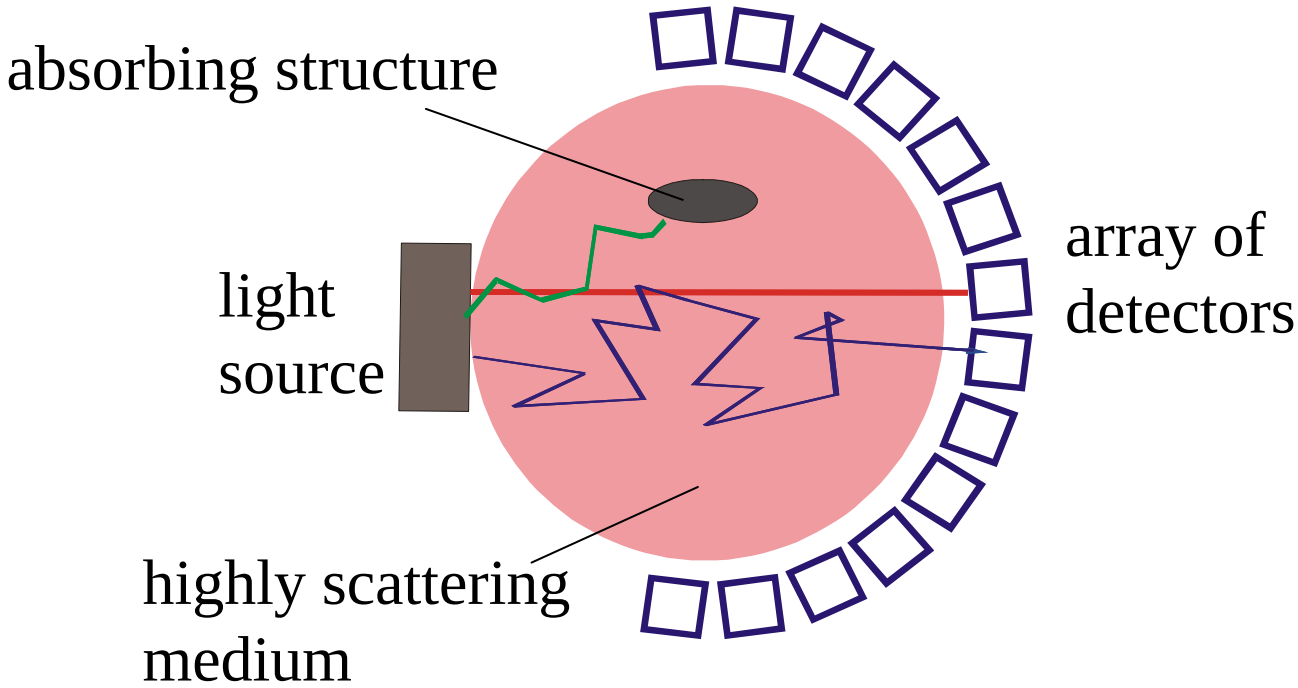
- 2D and 3D images based on interferometric measurement of optical back-reflection or back-scattering from internal tissue mircrostructures
- z-direction (longitudinal scan) by moving of reference mirror
- x-y direction (transverse scan) by moving the beam
- usually implemented with fiber optics

Optical transparency of the eye provides unique opportunity for high resolution imaging of the retina

Diffuse optical tomography

- measure light that passes through a highly scattering tissue
- frequency domain: measure amplitude and phase of modulated light

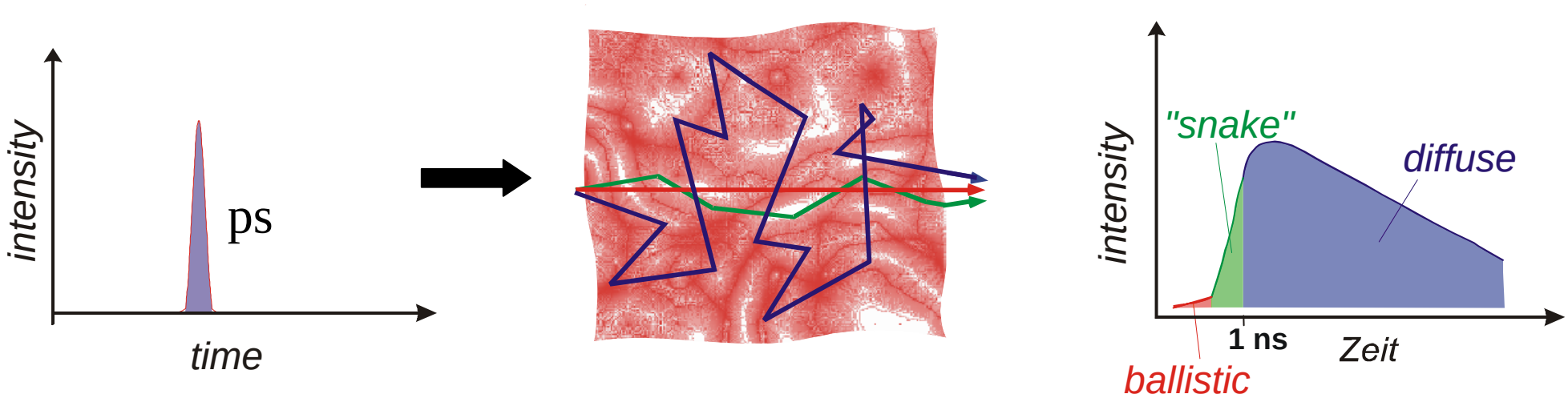




➡ **determine absorption and/or diffusion cross-section**

Optical tomography

- time domain: measure delay of light pulse at detector

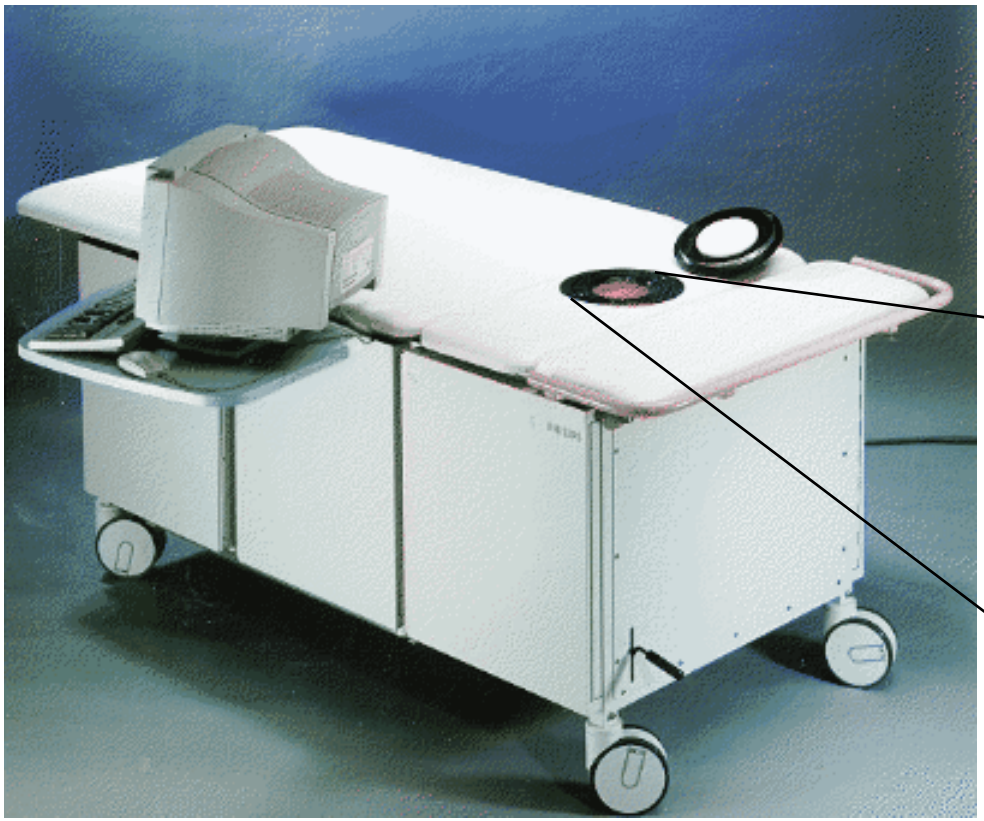


laser pulse | **highly scattering medium** | **detected laser light**

$$P(t,R) \propto \frac{1}{(4D\,t)^{3/2}} e^{-\frac{R^2}{4Dt} - \mu_a t}$$

D = diffusion coefficient [m^2/s]
μ_a = absorption coefficient [1/s]
R = distance between source and detector

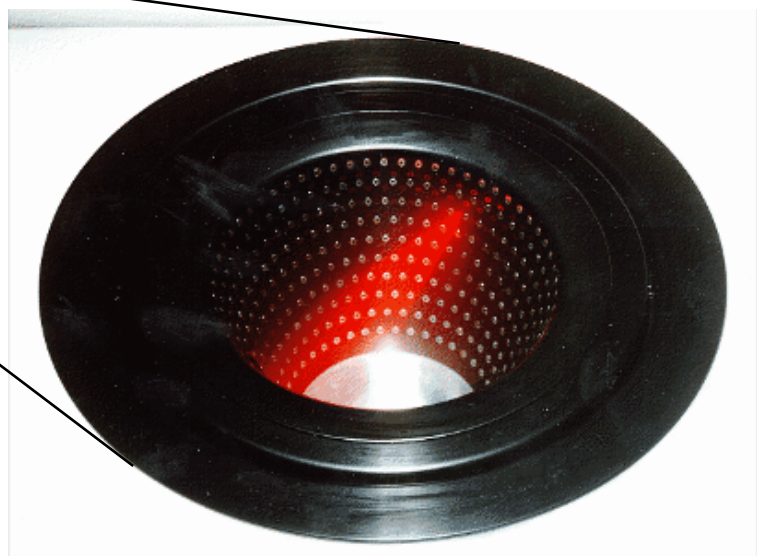
Optical mammography



Philips Medical Systems

laser: λ = 780 nm

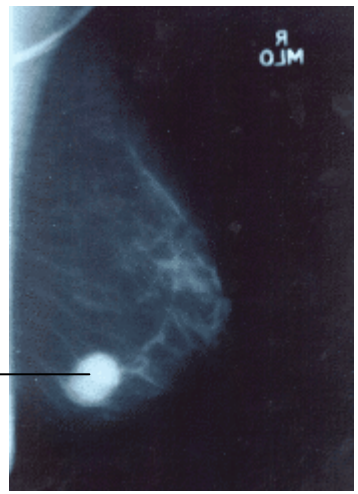
255 pairs of laser sources and detectors



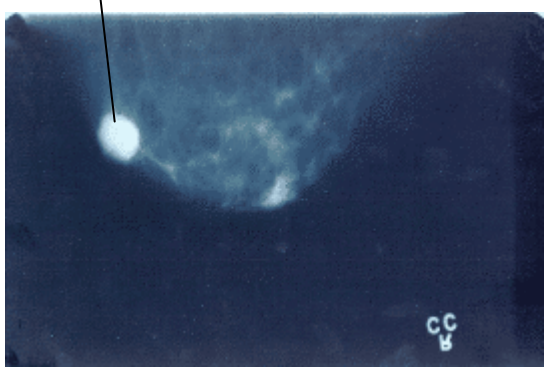
Optical mammography

x-ray

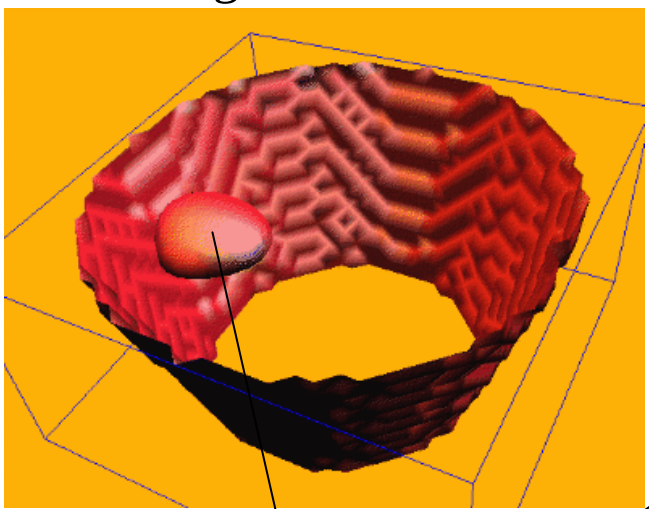
laser illumination



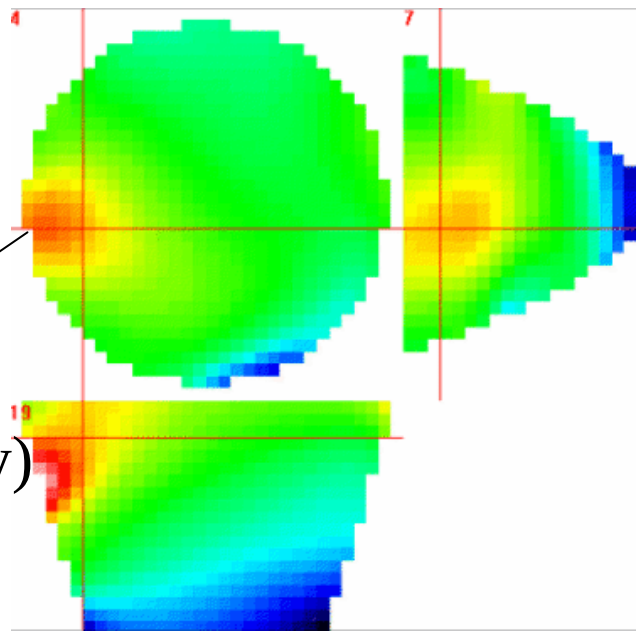
tumor
(1-2 cm)



3D-image-reconstruction



tumor
(increased vascular density)

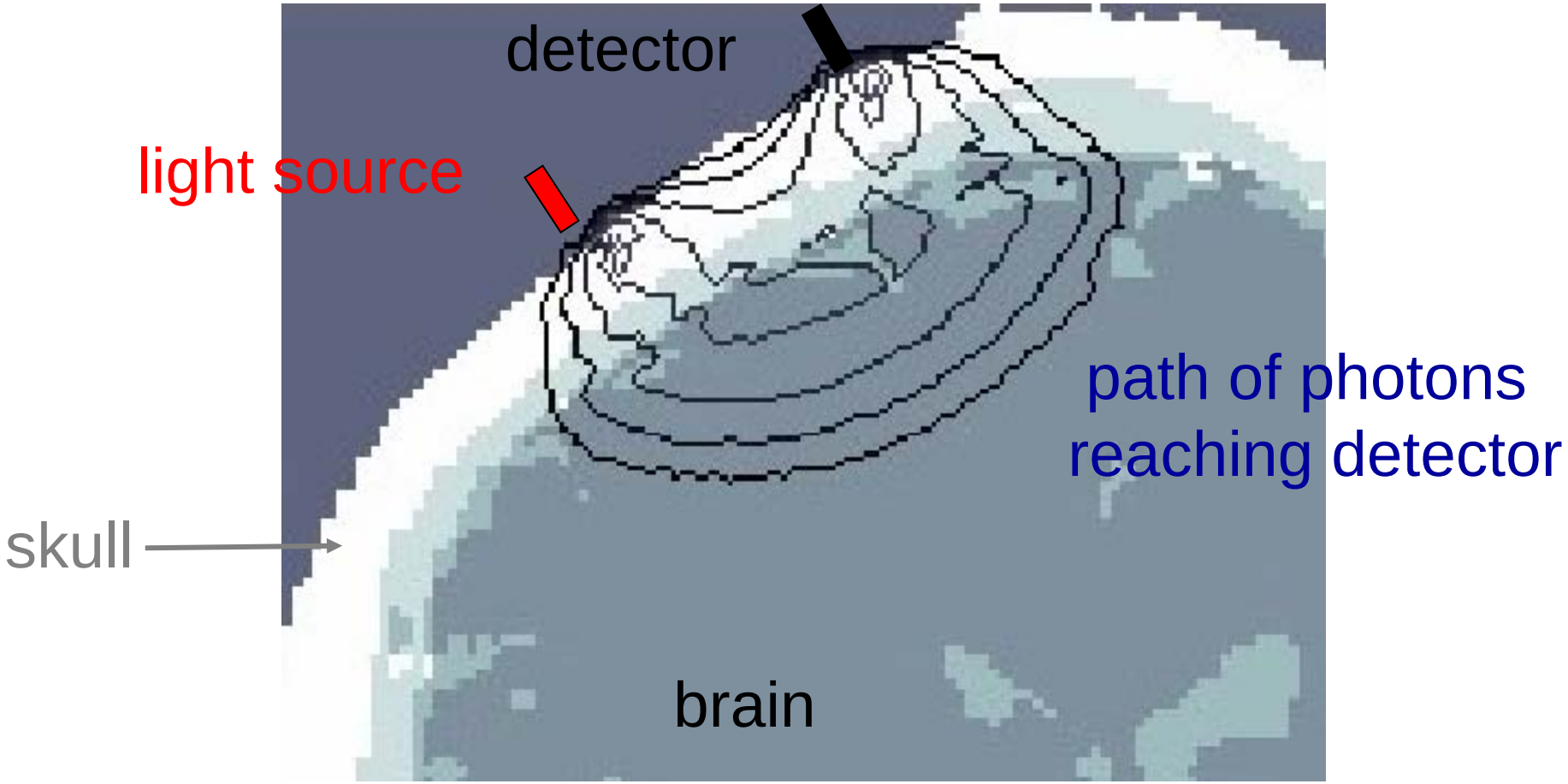


imaging of inhomogenities

structural changes

absorption changes

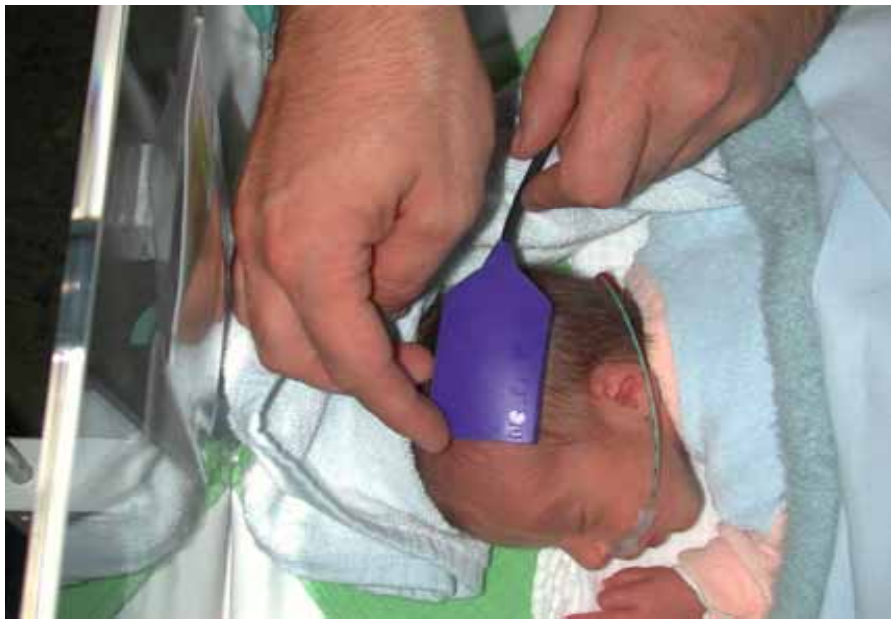
Path of light



- non-invasive and painless
- continuous measurement i.e. long-term monitoring
- real-time display

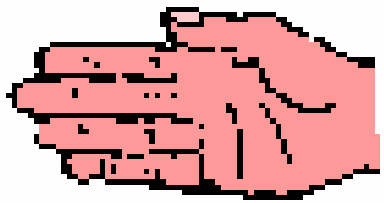
Functional brain imaging by near-infrared spectroscopy

Neonatology University Hospital Zurich (M. Wolf)

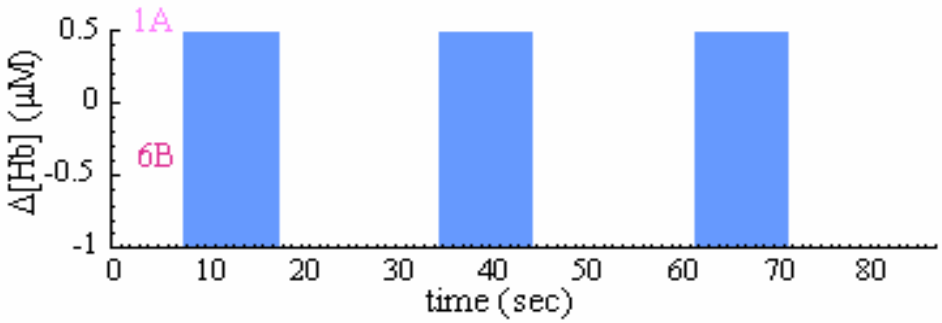


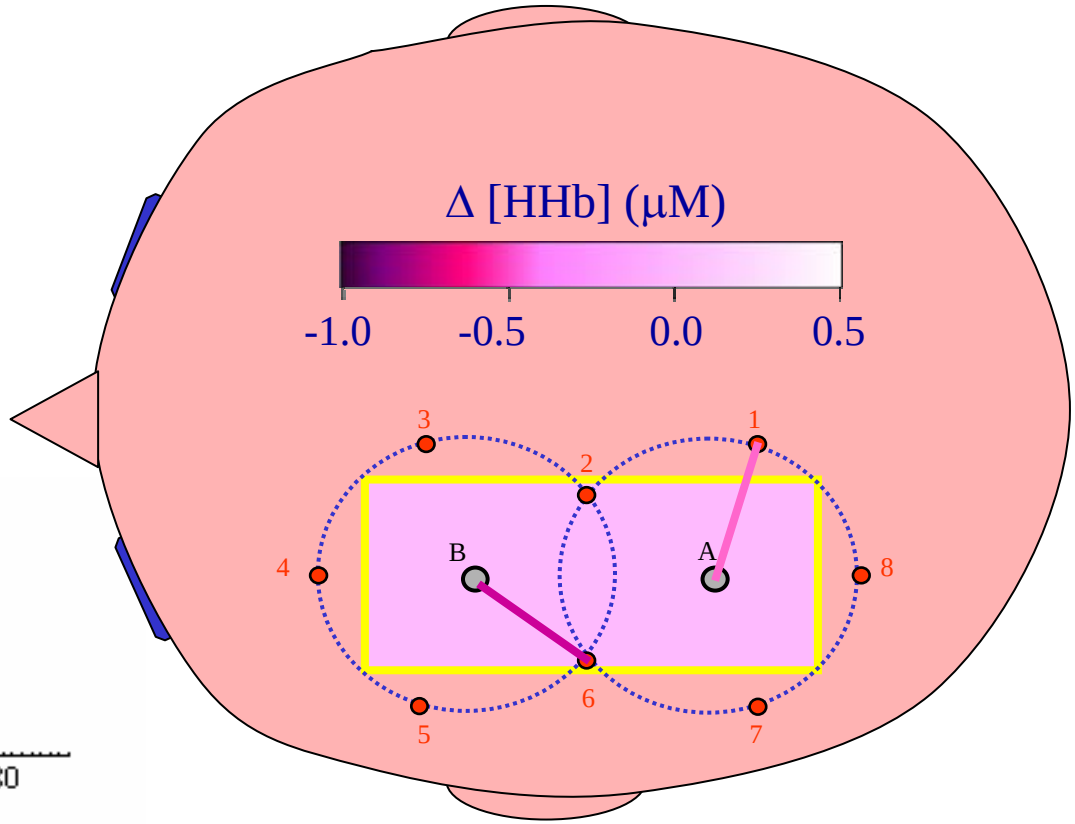
- Diagnosis of lesions and impending danger is vital for outcome
- Brain functional disorder
  - Attention deficit hyperactive disorder (ADHD), epilepsy, psychiatric disorders
- Research on brain function will
  - lead to improved understanding of brain and brain development
  - enhance prevention of diseases and complications
  - improve treatment

Video of an activation



moving average of 5 points
acquisition time 800 ms



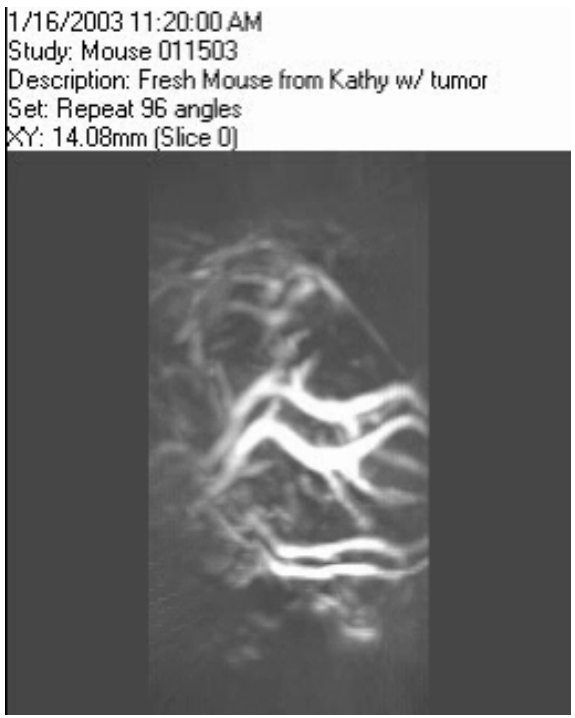


Franceschini MA et al. Opt. Express 2000; 6: 49-57

- local changes in oxy-and deoxyhemoglobin concentration indicate brain activity
- quantification possible due to absorption at different wavelengths
- high temporal resolution

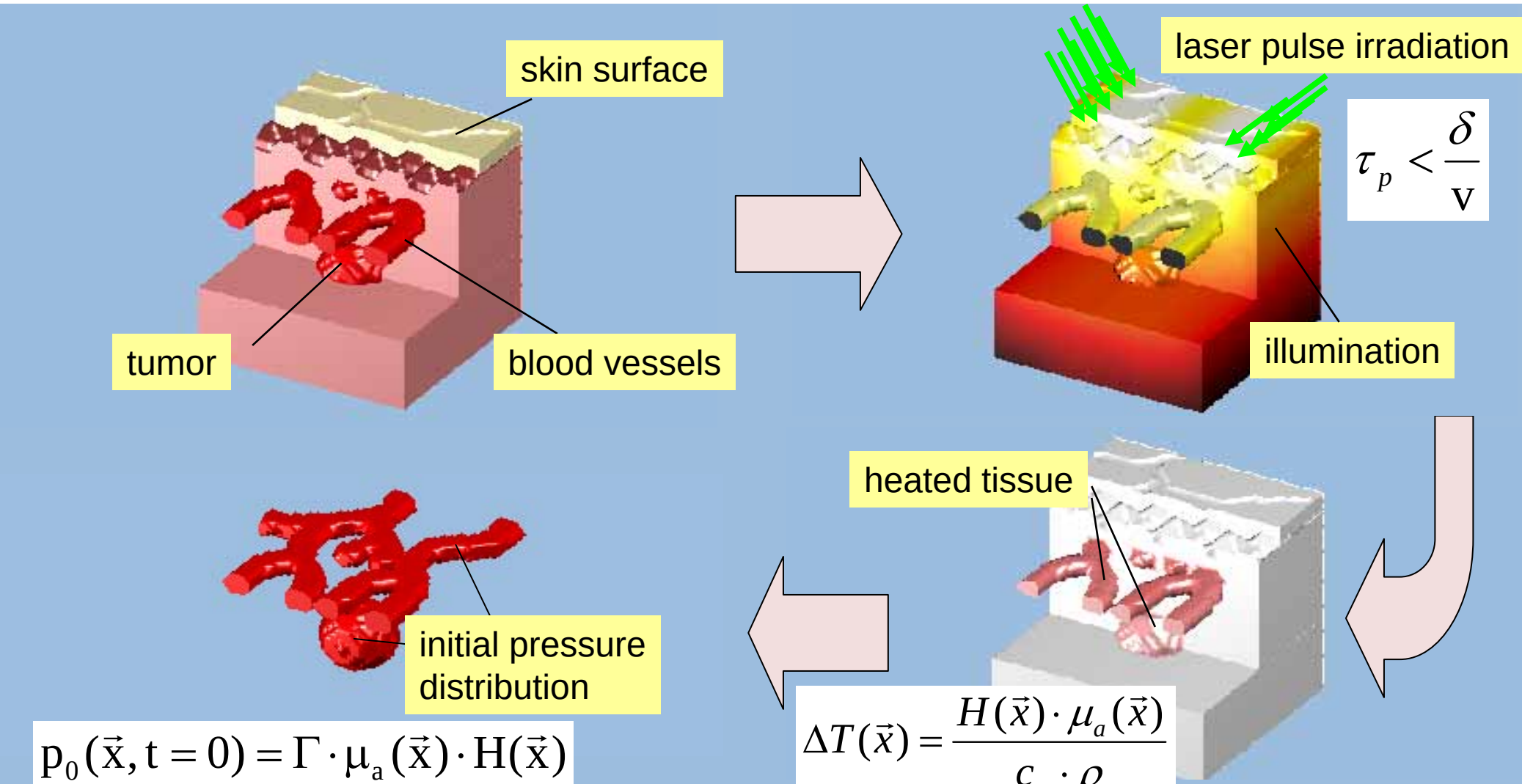
Optoacoustic imaging

Combines the excellent contrast of optical absorption with the good spatial resolution of ultrasound for deep imaging in the optical diffusive regime.



OptoSonics

What is optoacoustic imaging?



What is optoacoustic imaging?

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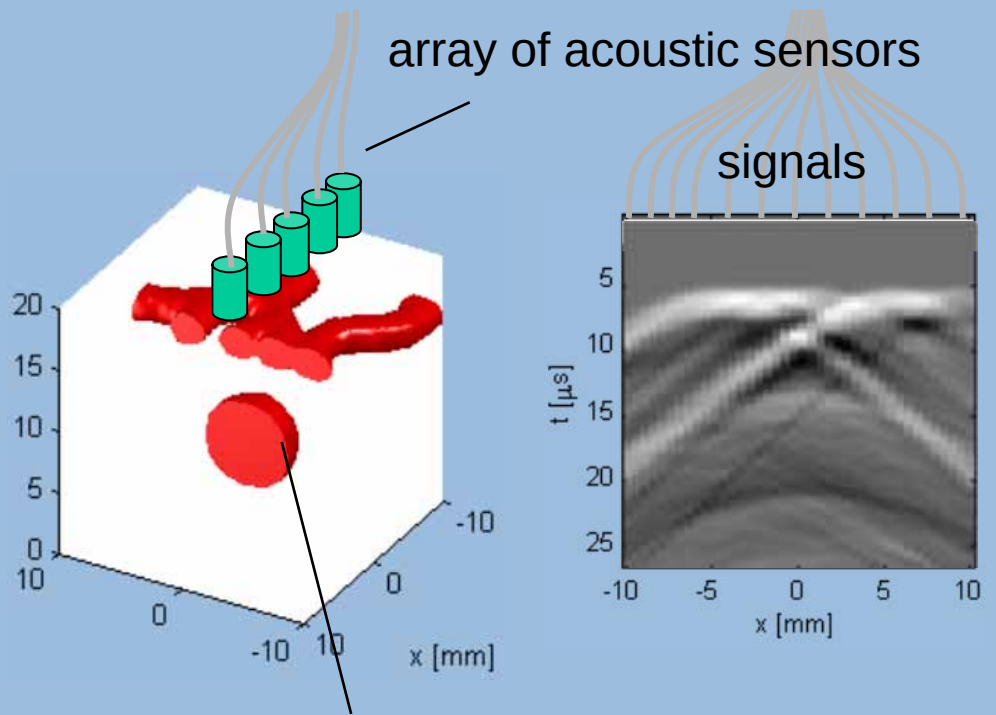
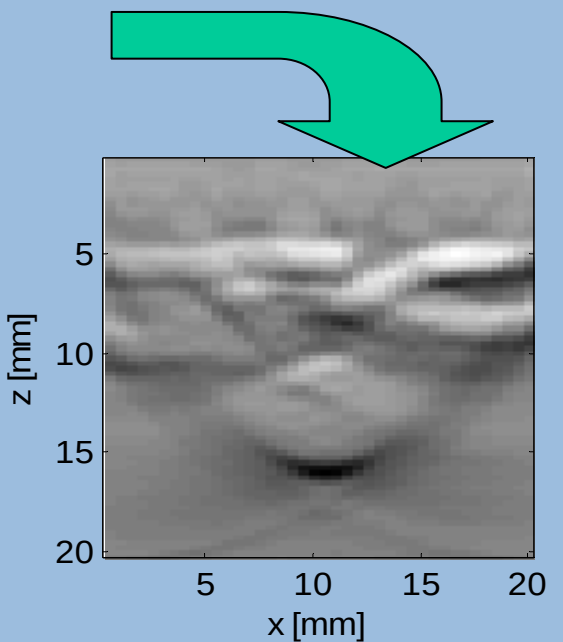
ultrasound propagation and detection



reconstruction



$$\frac{\partial^2 p(\vec{x},t)}{\partial t^2} - v^2 \Delta p(\vec{x},t) = \Gamma \frac{\partial}{\partial t} S(\vec{x},t)$$

- backward projection
- FFT - algorithm
- .....

Absorbers

endogenous and exogenous chromophors

- blood
- melanin
- .....

- dyes
- nanoparticles
- .....

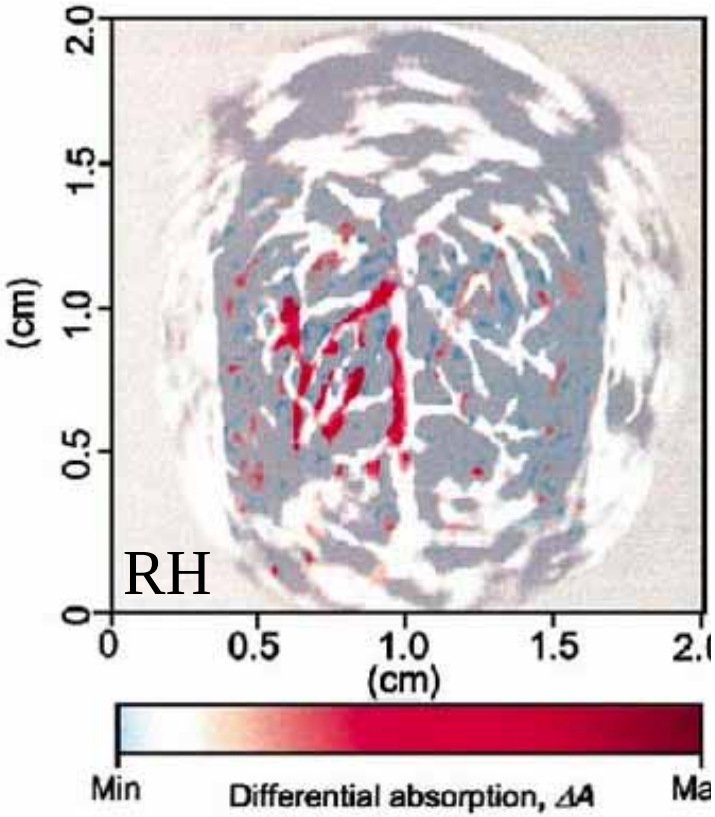
contrast enhancement

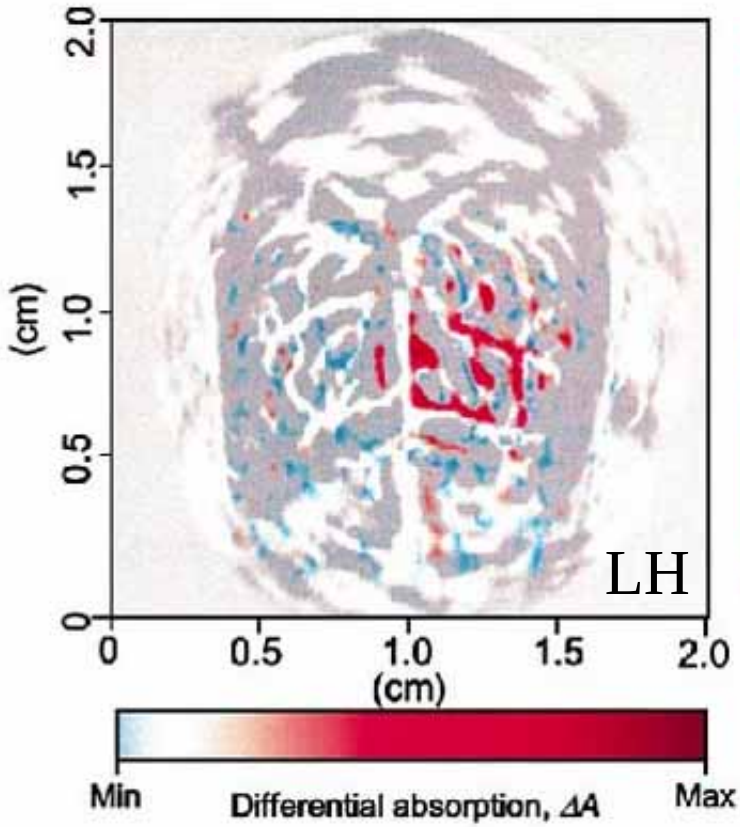
goal:
strong near-infrared absorption

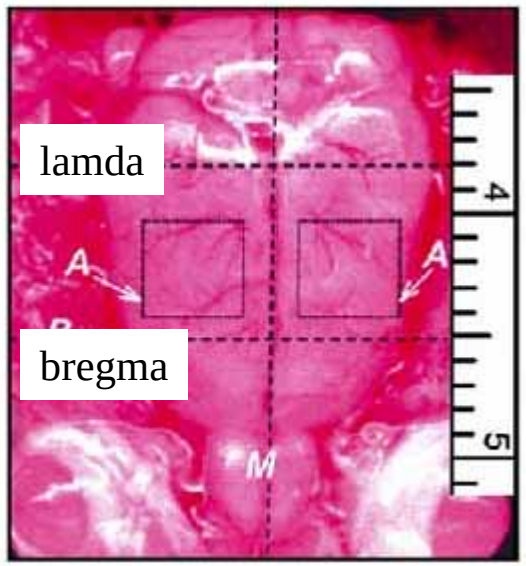
→ **functional imaging**

Functional optoacoustic imaging

whiskers-stimulation







open skull image

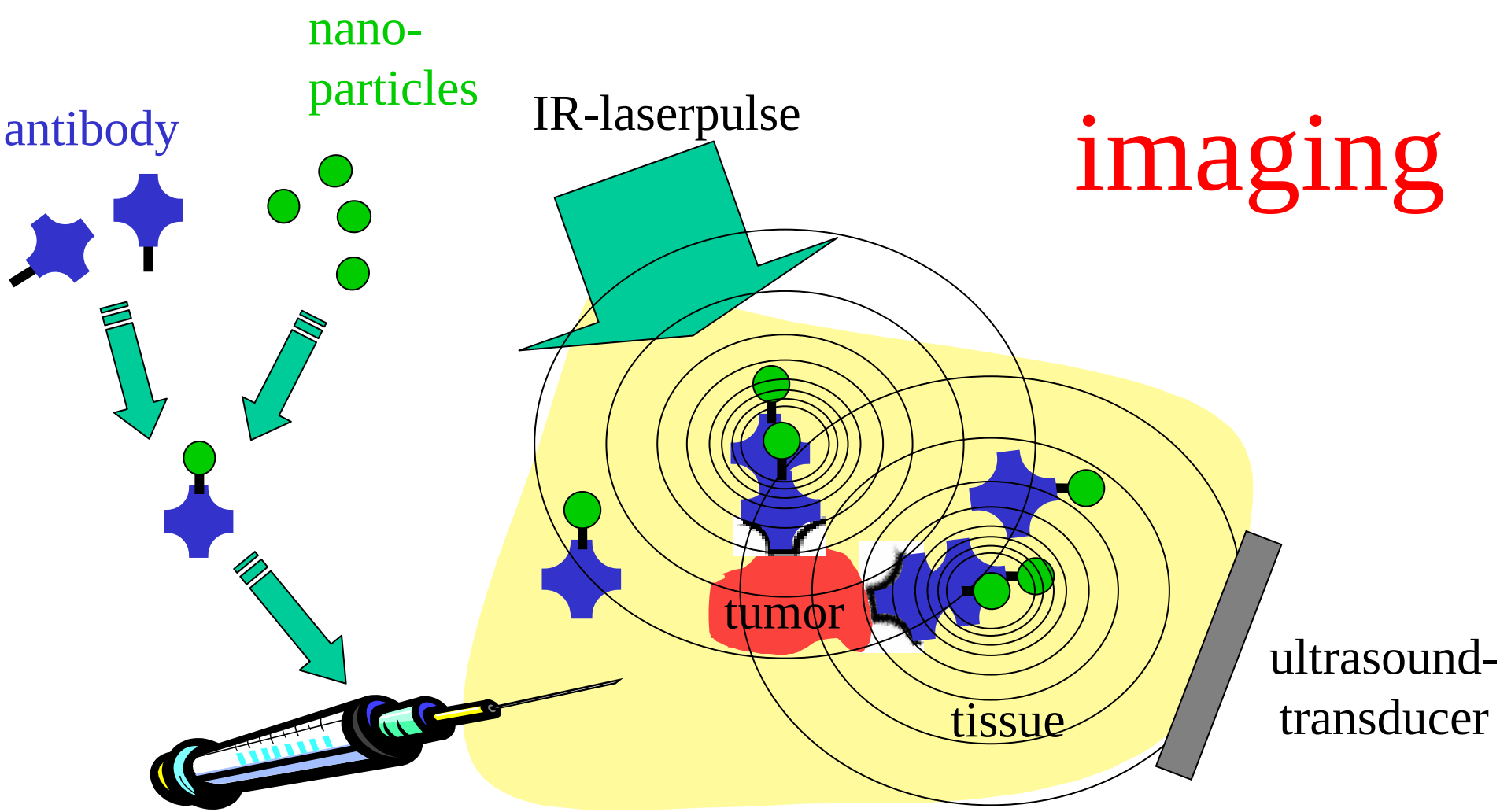
left-side

right-side

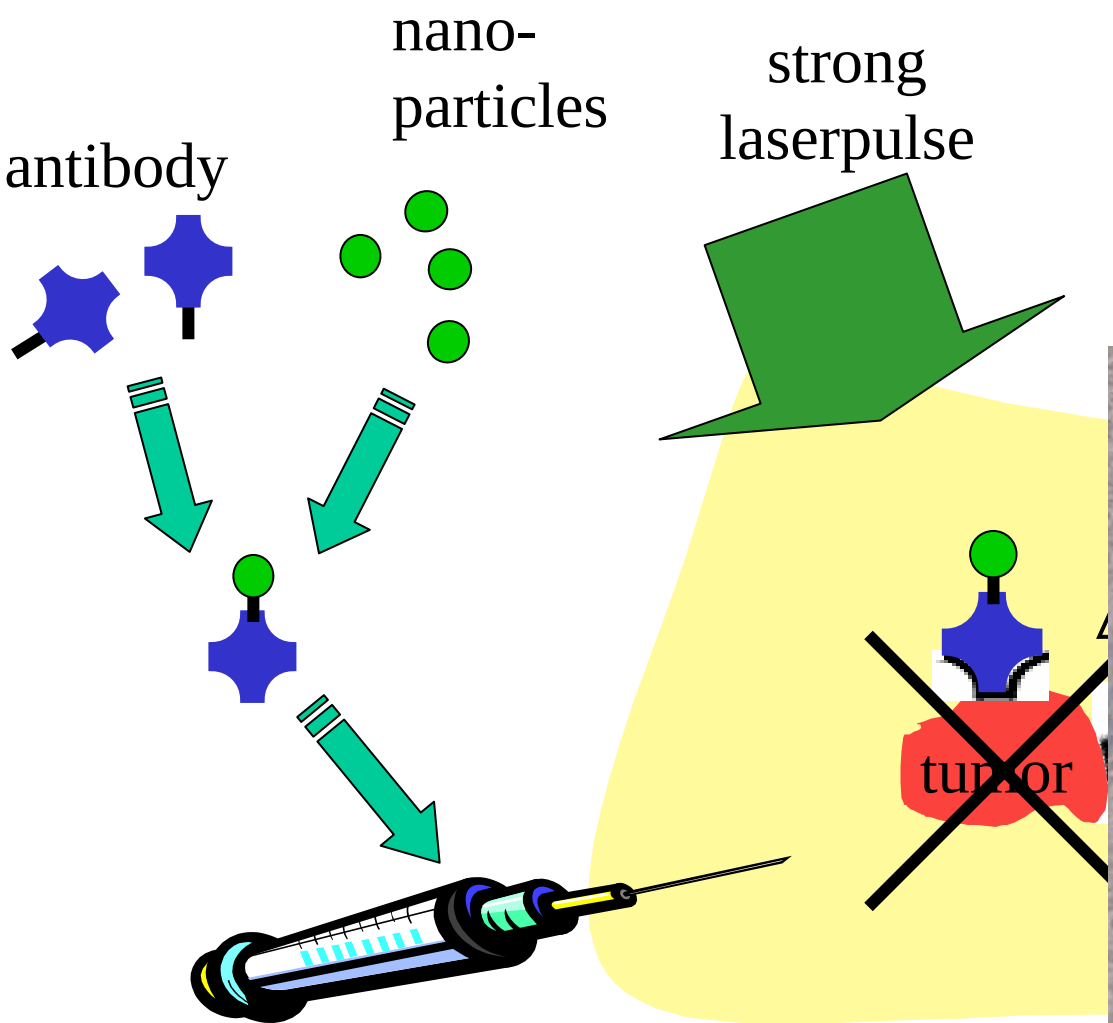
➡ increase in vascular blood volume or flow

Nature Biotechnology (2003) Vol 21, 803

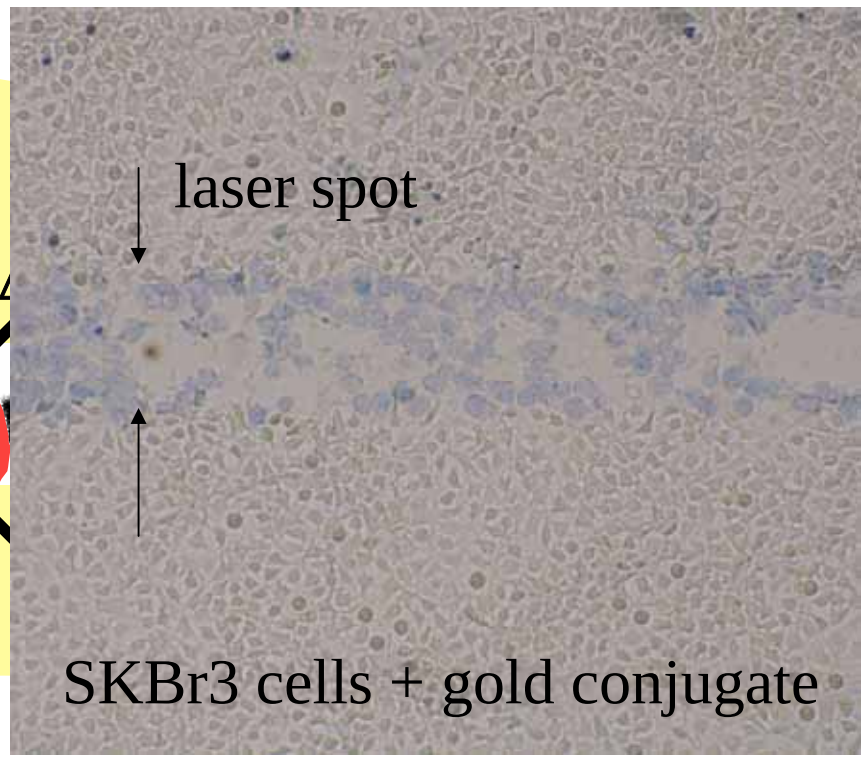
Exogenous absorbers



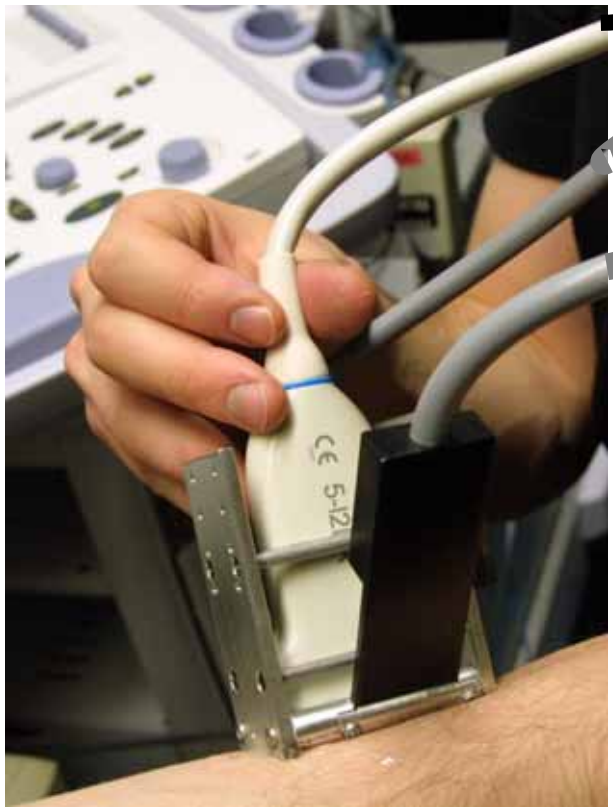
Selective tumor treatment

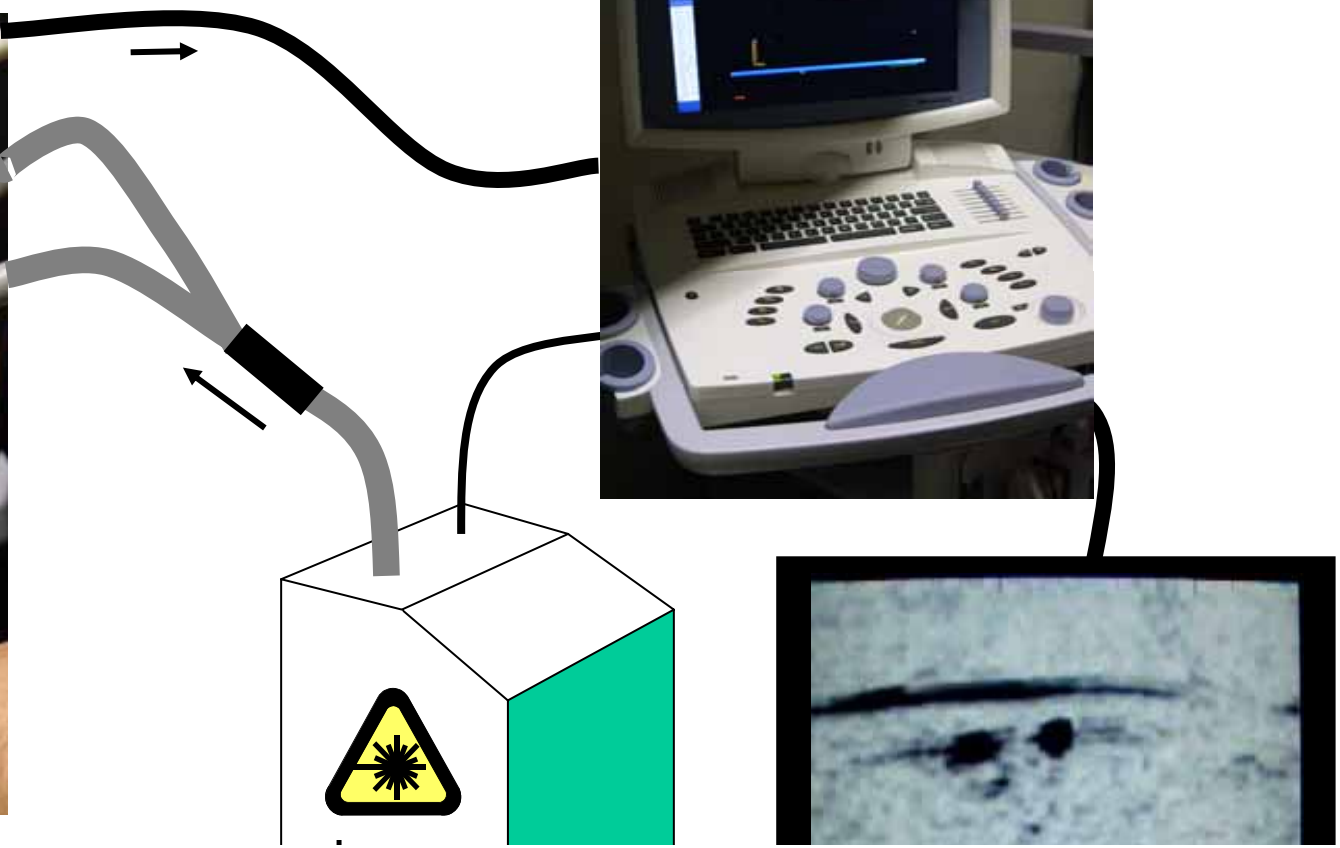


therapy



Optoacoustic Imaging

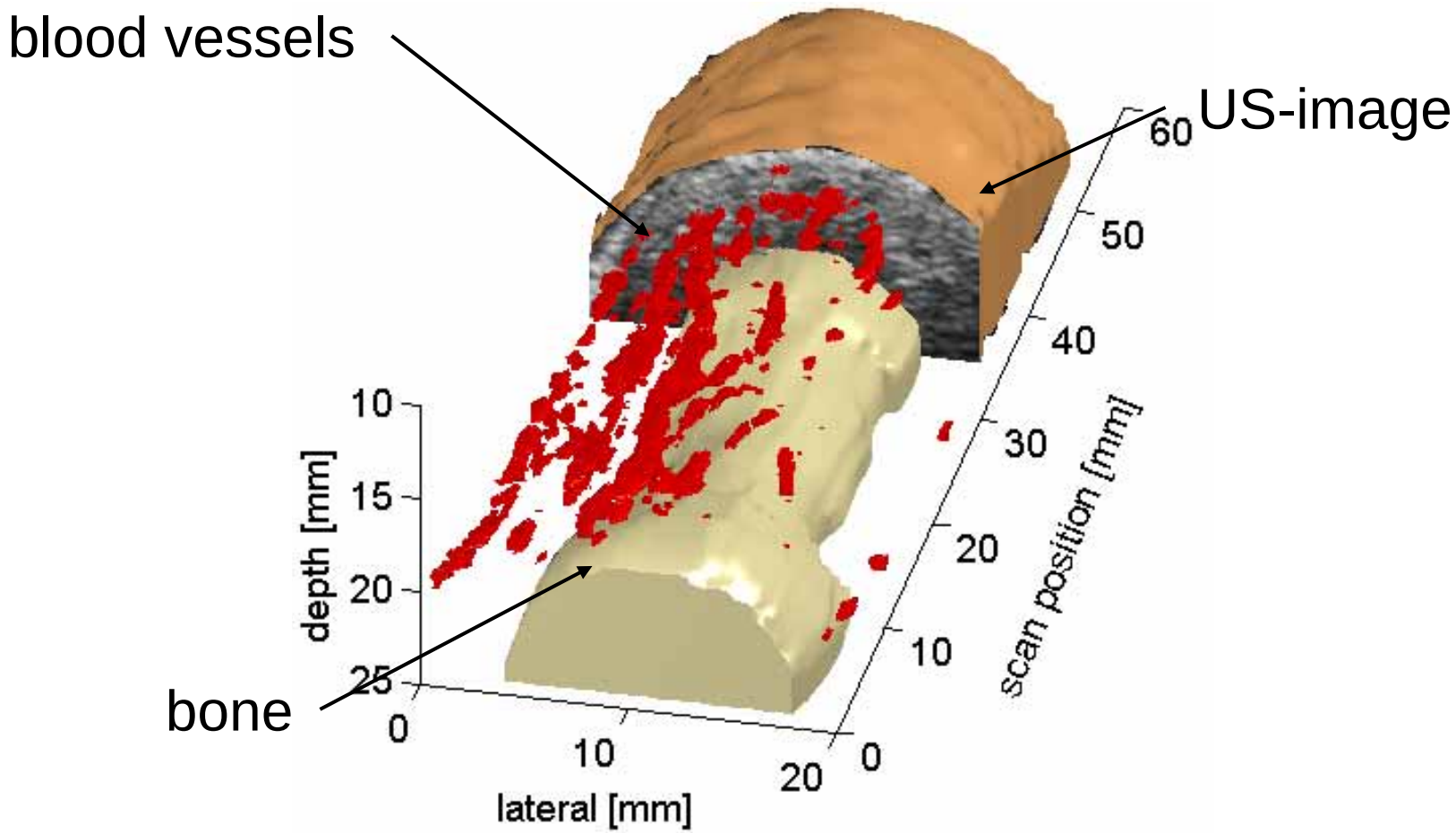




- 10 Hz, 5 ns Q-Switched Nd:YAG laser with 3ω and OPO (400-2000 nm)
- triggered laser pulse replaces transmitted ultrasound pulse
- optoacoustic images recorded with US-system (adapted receiver timing)

Comparison optoacoustic - US

vascularization of a finger tip



Conclusion

- Optical photons provide non-ionizing and safe radiation for medical applications.
- Optical scattering spectra provide information about the size distribution of scatters, such as cell nuclei.
- Optical absorption provides contrast for functional imaging
- Optical spectra-based on absorption, fluorescence, bioluminescence or Raman scattering provide biochemical information because they are related to molecular conformation.

➡ optical imaging covers a wide range of applications

➡ future is multimodal imaging