

# Medical Fluorescence Imaging
# -
# Tutorial

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LUND UNIVERSITY

*BRIGHTER*
*Berlin, 26 June 2009*

# Aim with the presentation

## Motivate

- the use of in vivo fluorescence imaging for:
  - Early cancer diagnostics
  - Identification of tumour boundaries
  - Assessment of blood vessels
  - Visualisation of lymph vessels
  - Treatment response assessments
- the use of laser parameters necessary:
  - Average power required
  - Pulsed mode
  - Robustness, compactness and user friendlyness

# Clinical use of fluorescence imaging

## In-vivo kinetics of inhaled ALA-Induced PpIX fluorescence in bronchial tissue

White-light image (A1) 5-ALA-induced PPIX fluorescence image (A2) of a patient with squamous cell carcinoma.

## Fluorescence-guided resection of malignant gliomas

## Fluorescence angiography

Fluorescein angiographic features before and after PDT for choroidal neovascularization (CNV)

Hautmann *et al*. Respir. Res., **8** (2007)

Stummer *et al.* Lancet Oncol. (2006)

Hikichi *et al*., RETINA **21** (2001)

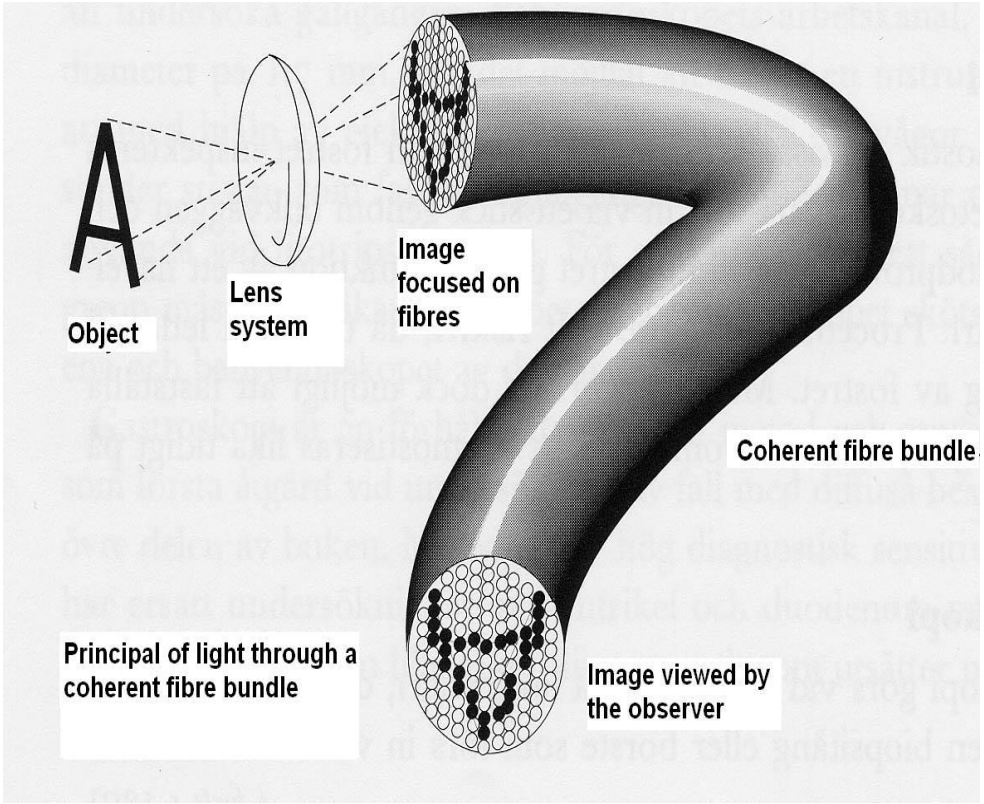
# Fluorescence detection of malignancies in the urinary bladder


The Storz D-Light system

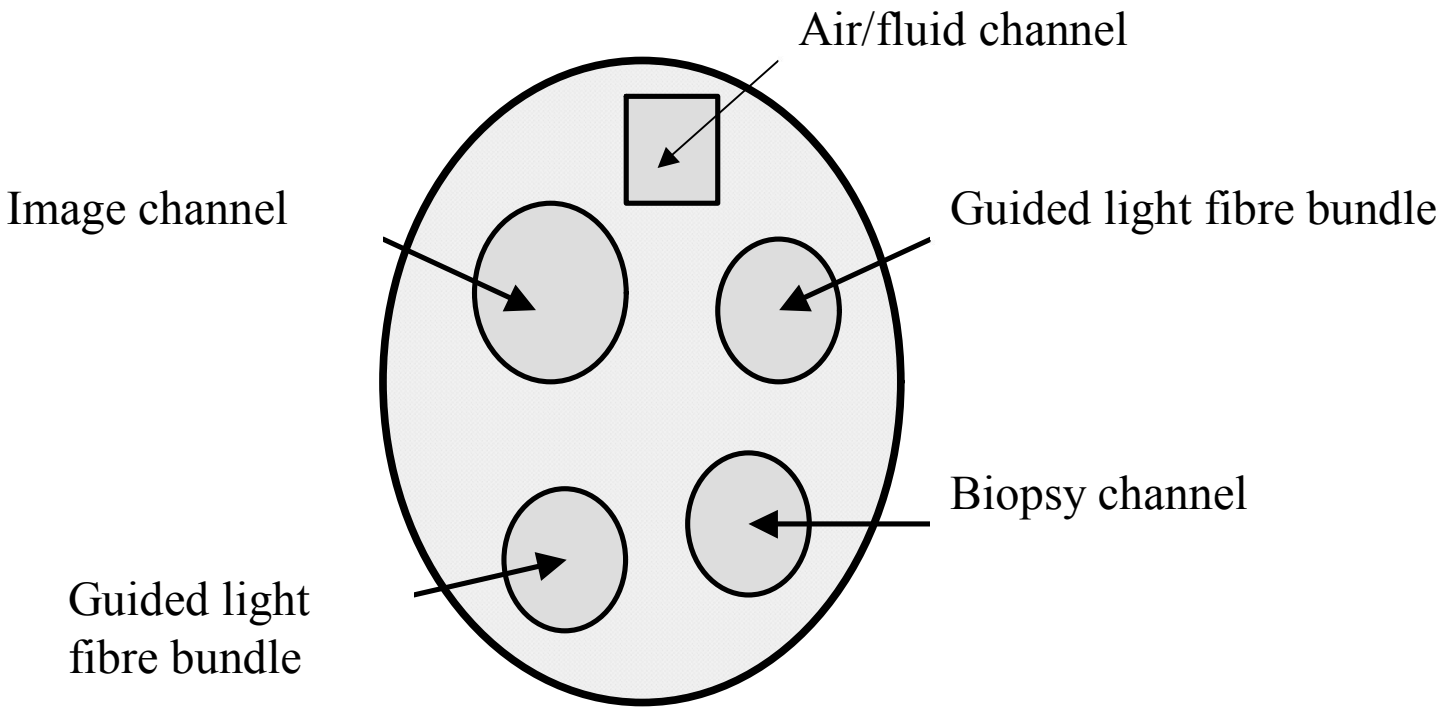
M. Kriegmair *et al.* Munich

# Fibre endoscope

## Coherent fibre bundle



# Cross-section of the distal end



# Endoscopical PDT – in combination with fluorescence detection

## Vocal fold carcinoma in situ

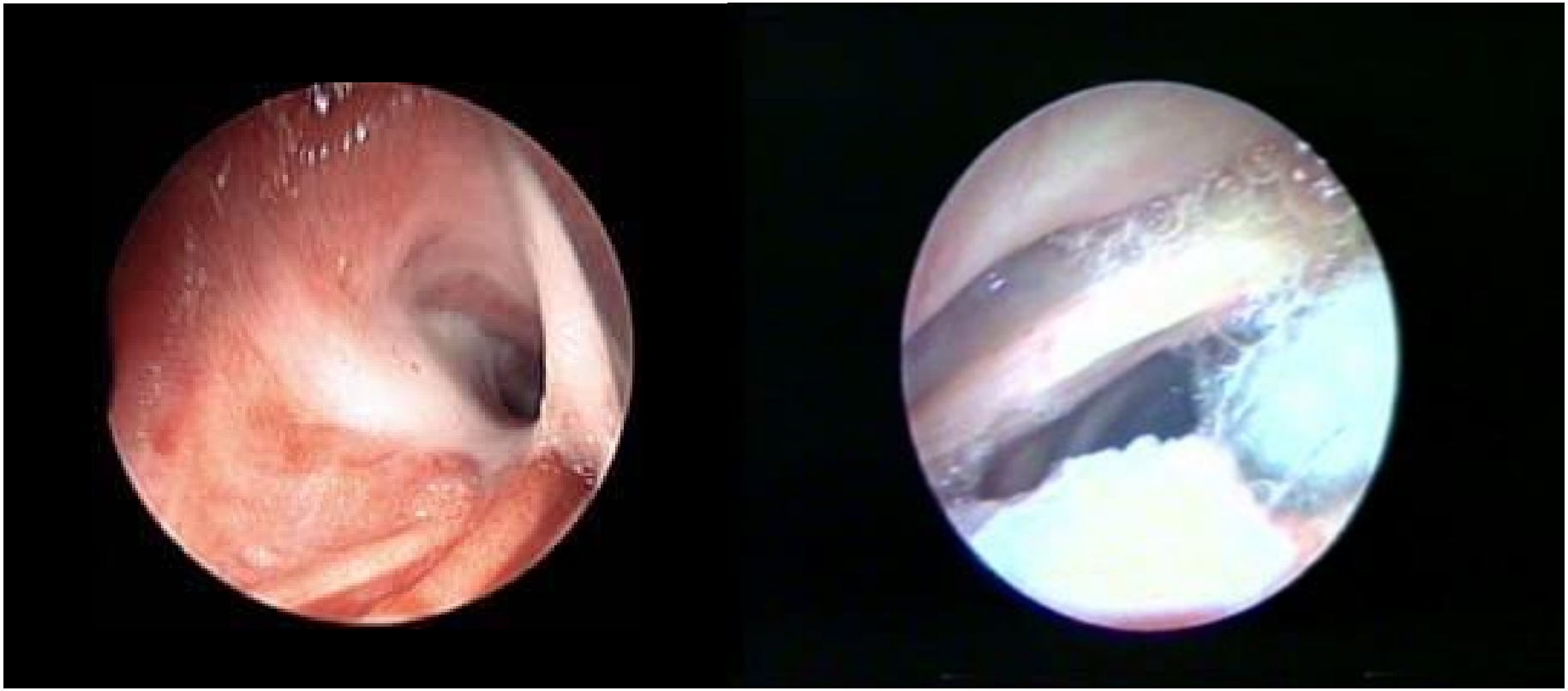
*Clinical, laser-based, fluorescence diagnostics and photodynamic therapy of a malignant tumour.*
*Insert: Endoscopic view of the vocal cords (left) and the same view with a colour-coded fluorescence image superimposed (right). The yellow area indicates the presence of a squamous cell carcinoma.*

ENT-Department with Dr. Roland Rydell
Lund University Hospital

# The diagnosis

White-light mode

Fluorescence video mode



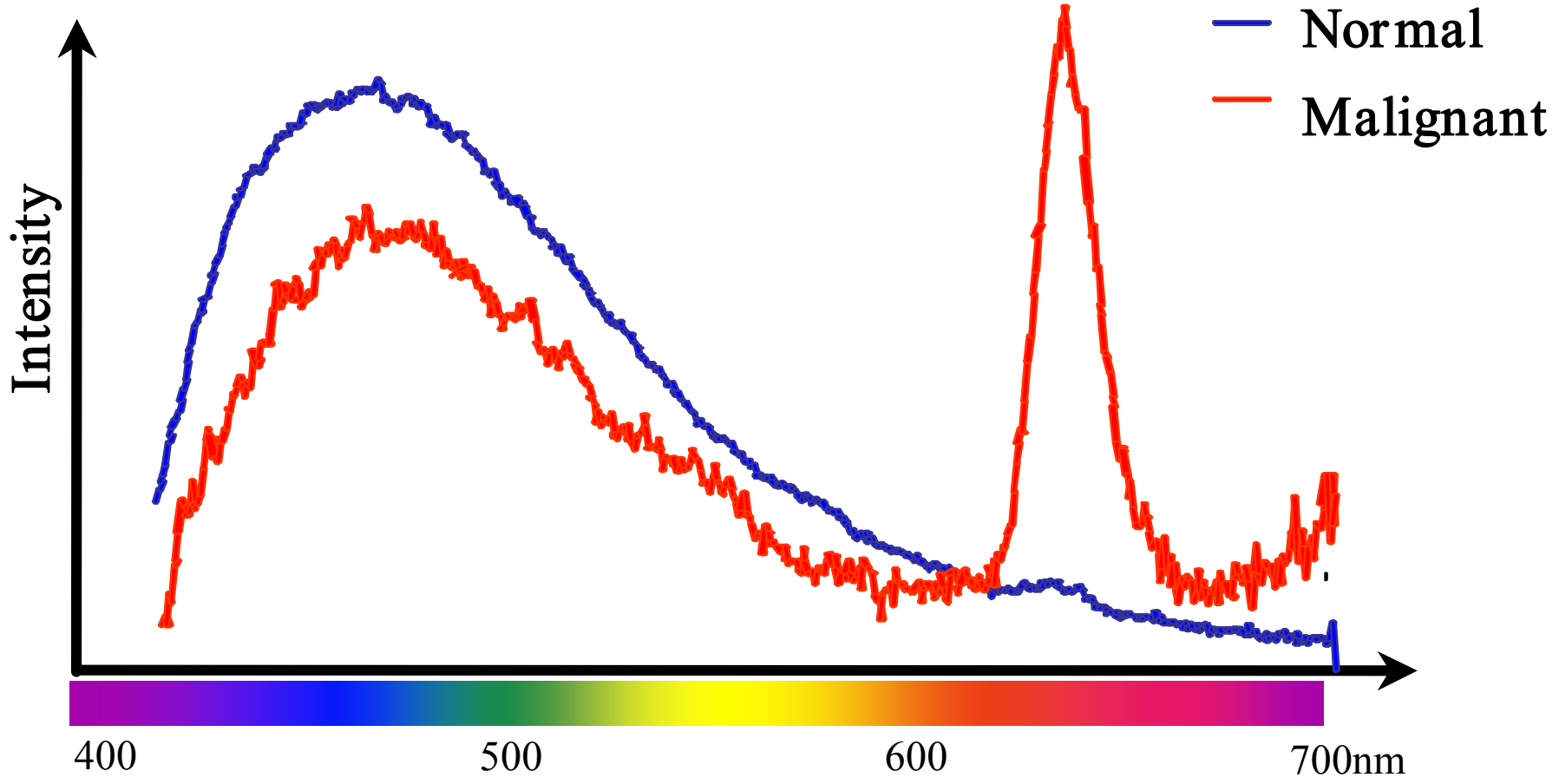
Imaging using the Storz D-light system for larynx diagnostics

**Important developments: Multispectral analysis**

**Simultaneous white light and fluorescence**

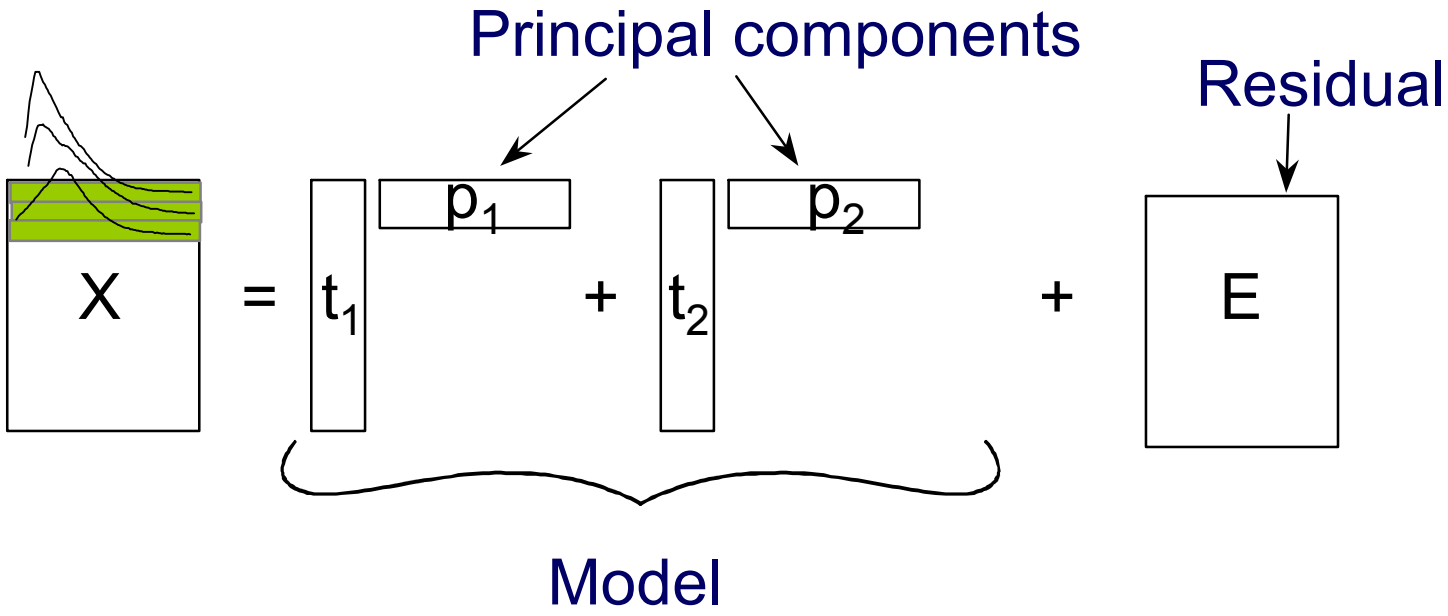
Rydell *et al.* Head & Neck (2008), Rydell *et al* (unpublished)

# Typical fluorescence spectra



# Multivariate analysis

## Decomposition:

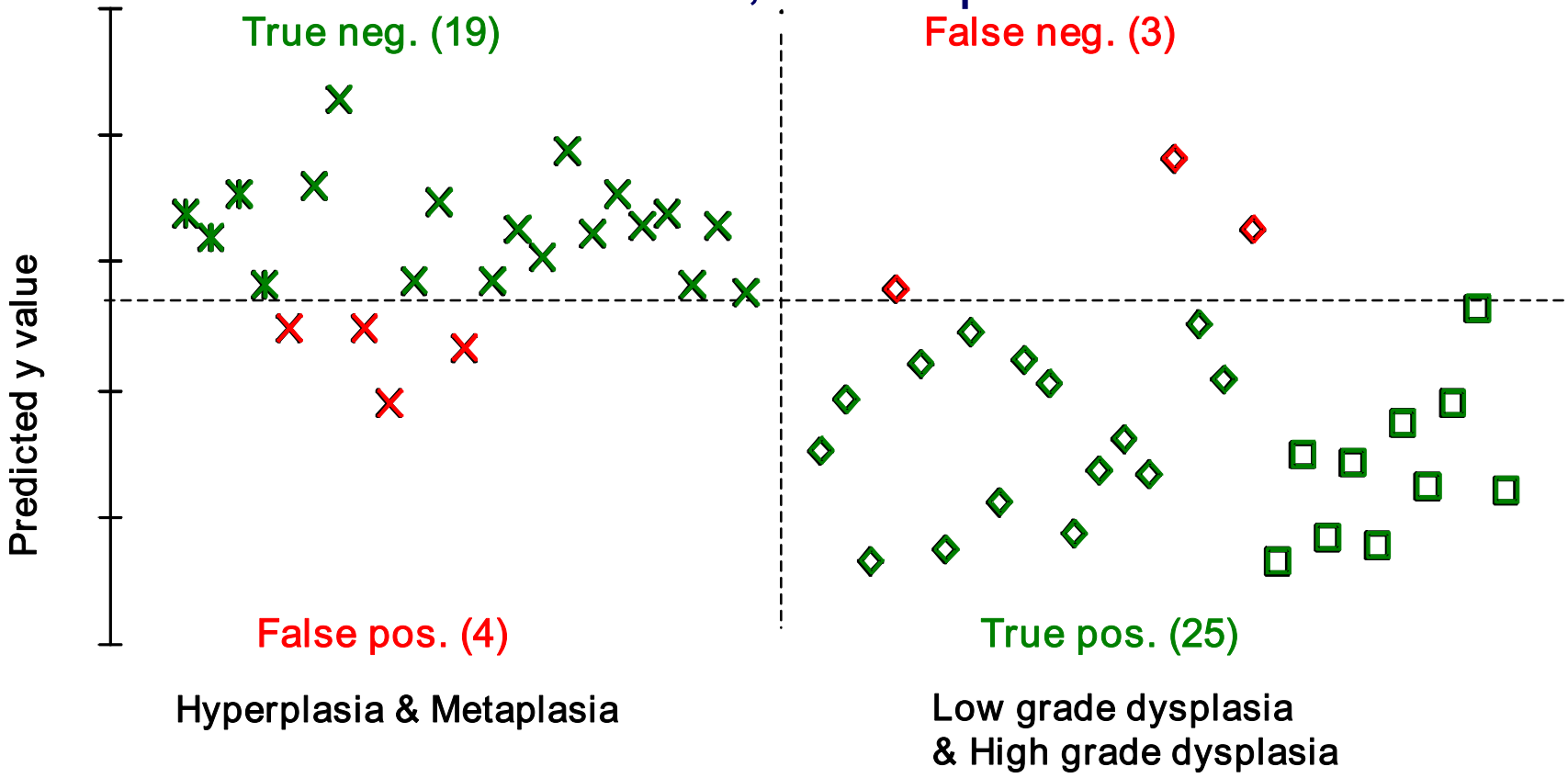



**Partial Least Squares (PLS): Principal components chosen for best correlation with y-variable (histopathology)**

# Diagnostic potential

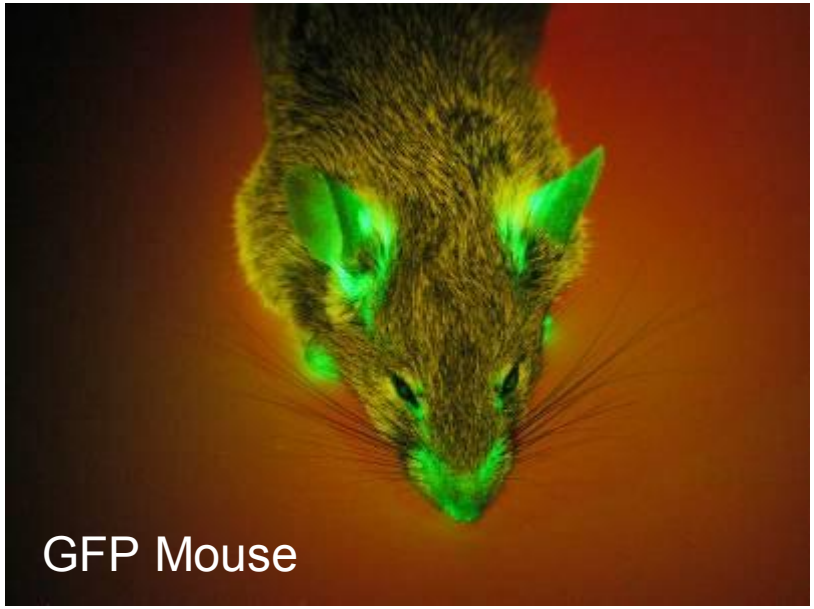
## To be useful for diagnostic purpose separation is often required on individual point basis

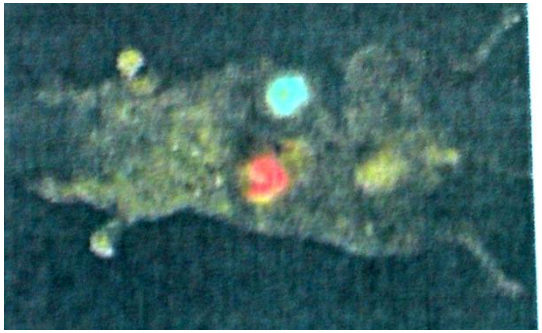
- 89% sens., 83% spec.



# Biological applications of *in vivo* fluorescence imaging

- Animal models widely used in biomedical research
- More than 90% of animals used are mice
- Non-invasive imaging studies very valuable tool
- Allow non-invasive **longitudinal** and **dynamic studies**



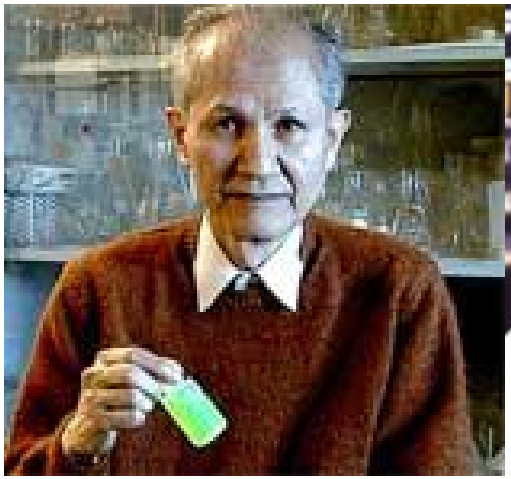
Hoffman and Yang Nature Protocols (2006)

Sharma *et al.* Am. J. Physiol. (2007)


KUNGL.
VETENSKAPSAKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

# 2008 Nobel Prize in Chemistry

## for the discovery and development of the green fluorescent protein, GFP



*Osamu Shimomura*

Japanese citizen, born 1928 in Japan. Ph.D. in organic chemis 1960, from Nagoya University, Professor emeritus at Marine B Laboratory (MBL), Woods Hole MA, USA a nd Boston Universi Medical School, MA, USA.

*Martin Chalfie*

US citizen, born 1947, grew u Chicago, IL, USA. Ph.D. 197 neurobiology
from Harvard University. William R. Kenan, Jr. Profess Biological Sciences at Columbia University, New York, NY, USA, since 1982.

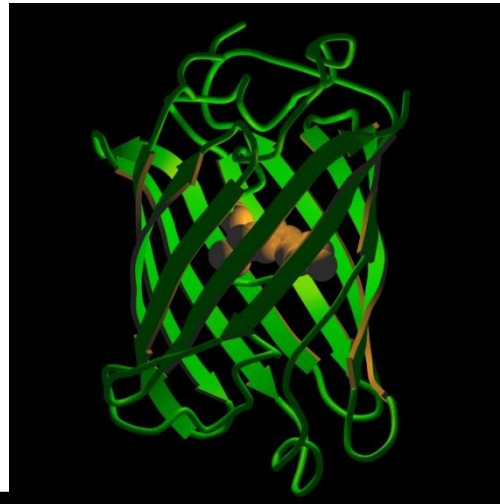
*Roger Y. Tsien*

US citizen, born 1952 in New York, NY, USA. Ph.D. in physiology 1977 from Cambridge University, UK. Professor at University of California, San Diego, La Jolla, CA, USA since, 1989.

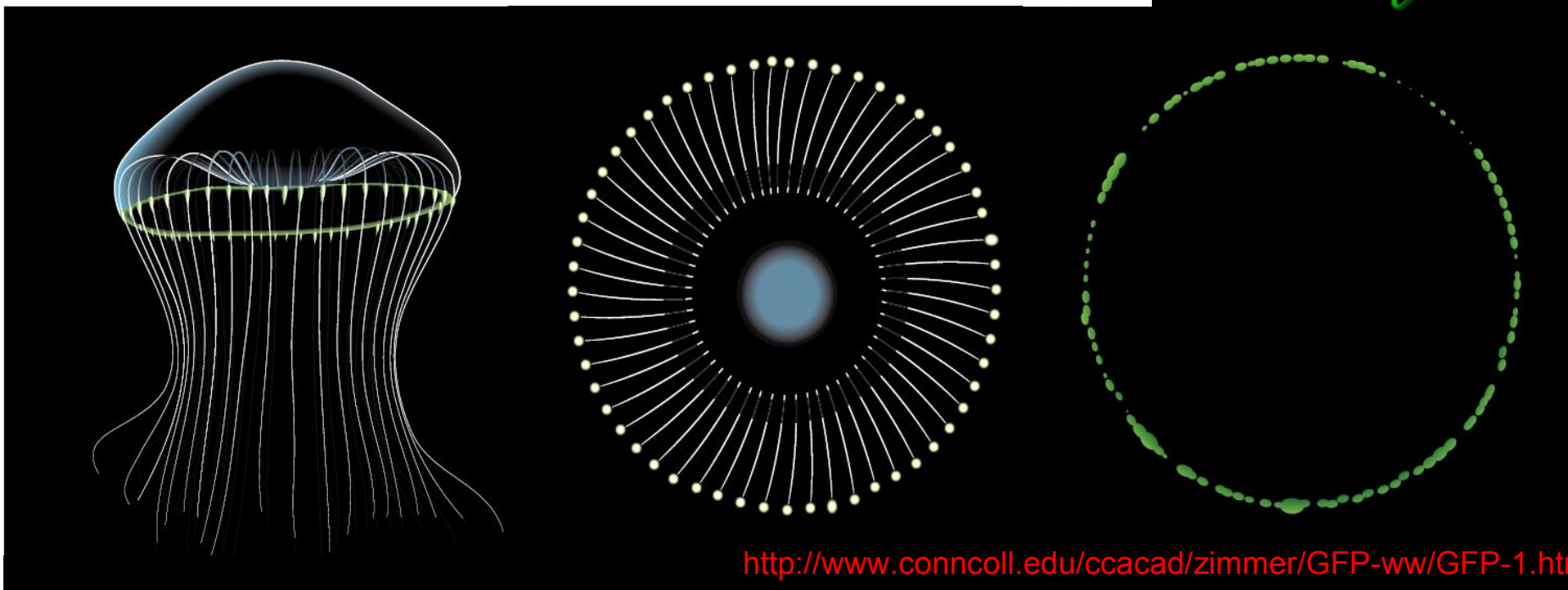
# Green Fluorescence Protein - GFP

What is GFP?

A small naturally occuring protein which is highly fluorescent. GFP consists of 238 amino acids, linked together in a long chain. This chain folds up into the shape of a beer can. Inside the beer can structure the amino acids 65, 66 and 67 form the chemical group that absorbs UV and blue light, and fluoresces green.



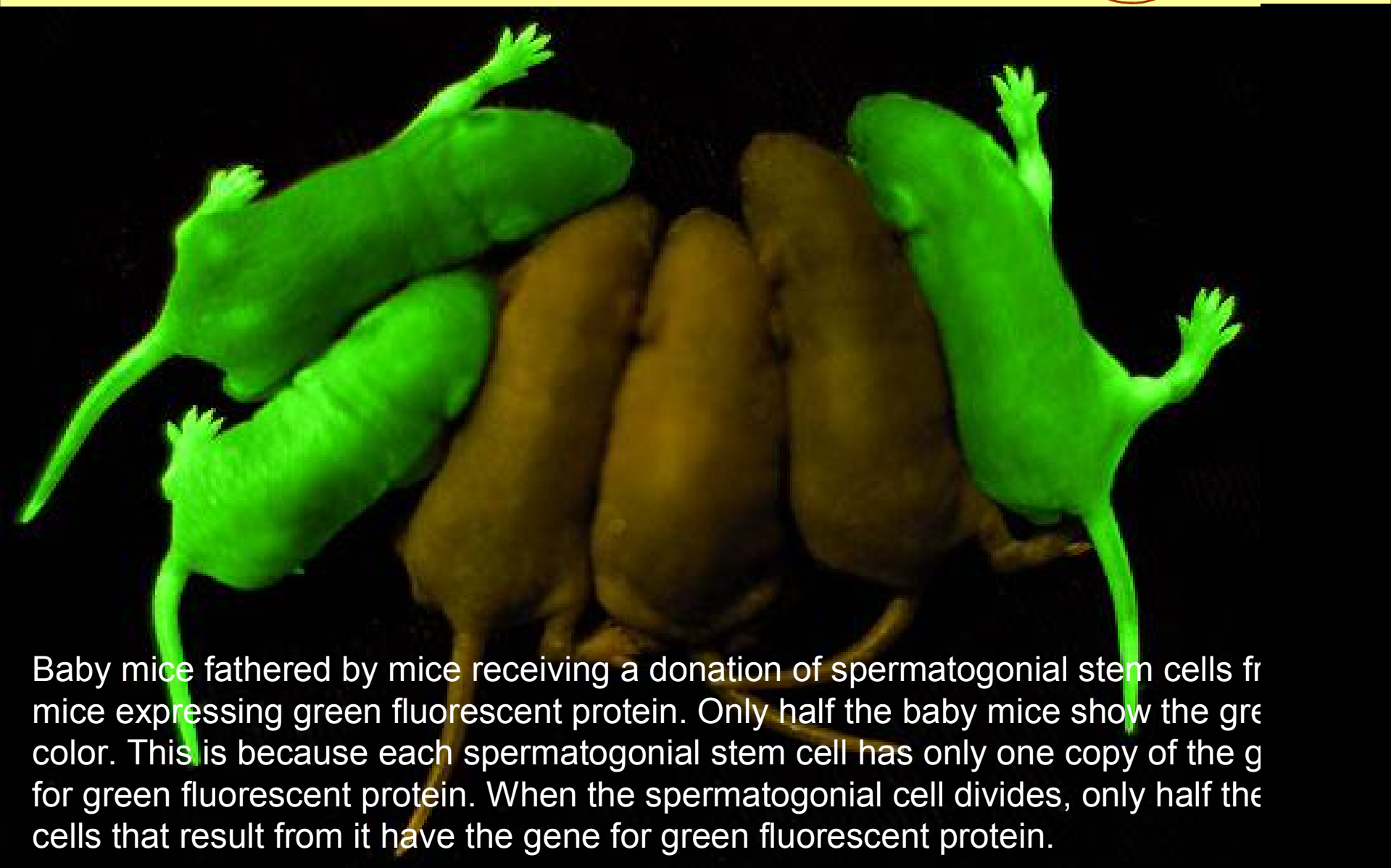
*Aequorea victoria – a jellyfish in the Northern Pacific Ocean*



http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP-1.ht

# Fluorescence protein labeling in biology

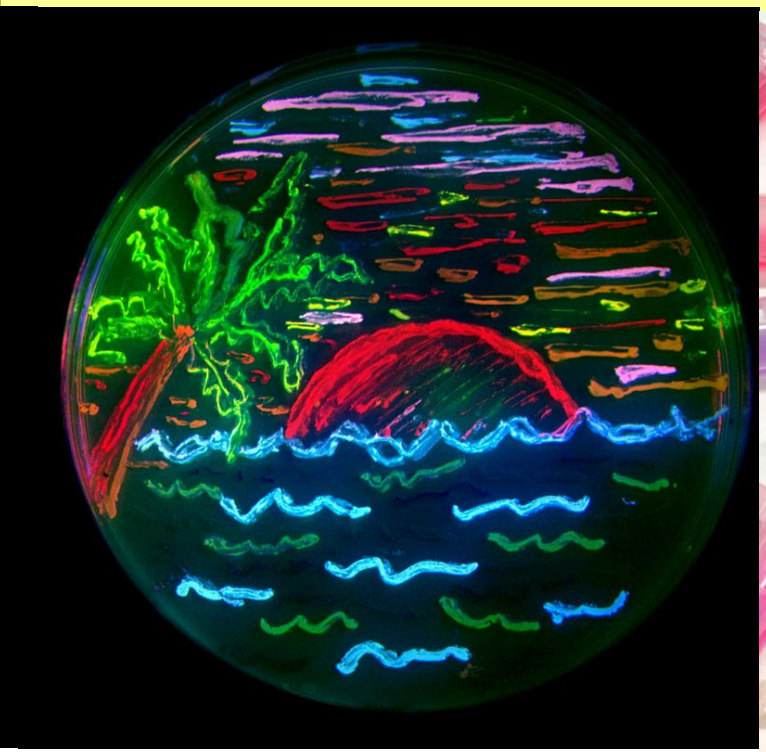
http://www.nichd.nih.gov/news/releases/green_brown_mice.cfm



Baby mice fathered by mice receiving a donation of spermatogonial stem cells fr
mice expressing green fluorescent protein. Only half the baby mice show the gre
color. This is because each spermatogonial stem cell has only one copy of the g
for green fluorescent protein. When the spermatogonial cell divides, only half the
cells that result from it have the gene for green fluorescent protein.

# Fluorescence Proteins in all colours – Roger Y. Tsien

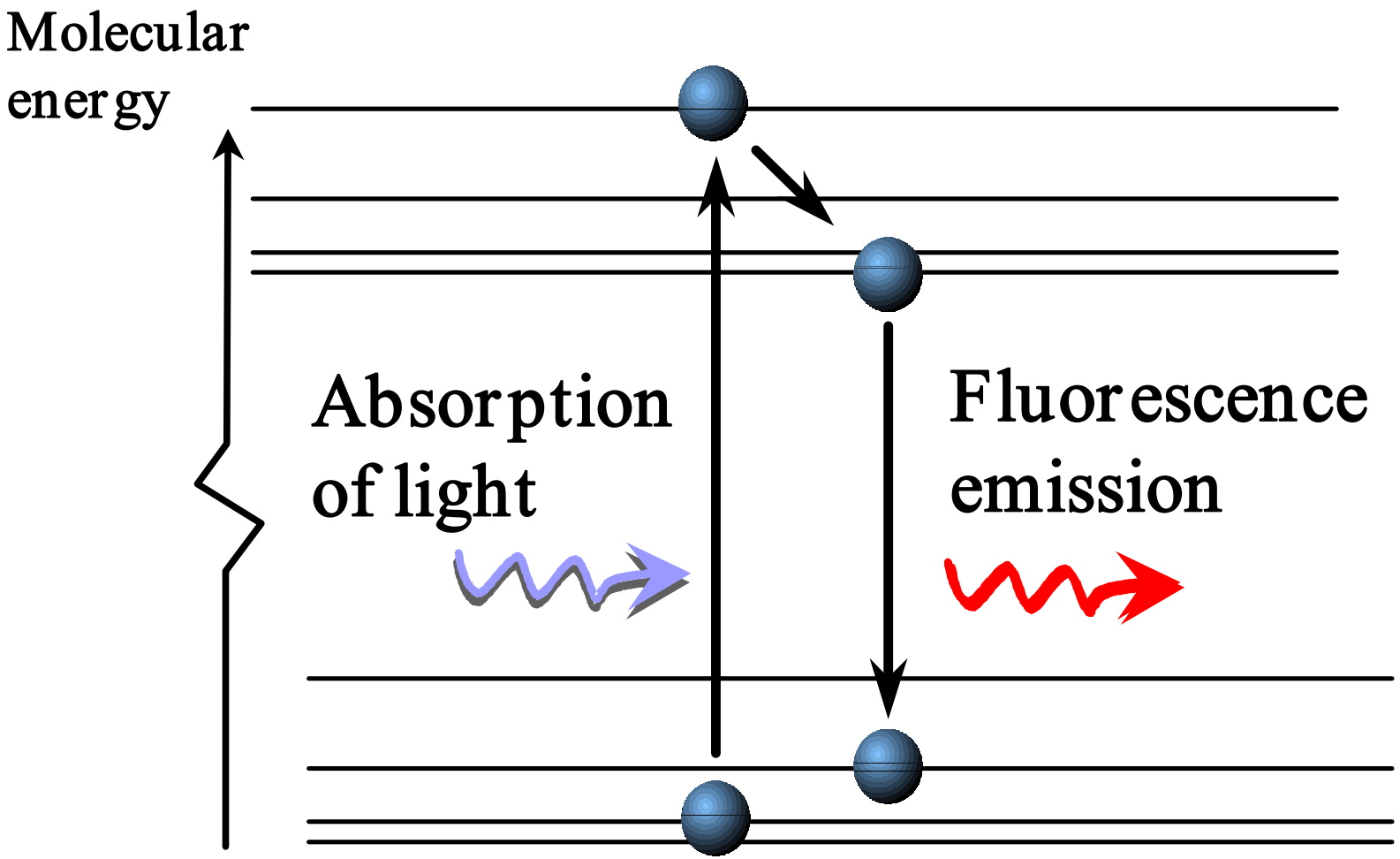
Using DNA technology, various amino acids in different parts of GFP were exchanged



Agar Plate of Fluorescent Bacteria Colonies


http://www.tsienlab.ucsd.edu

# What is fluorescence?



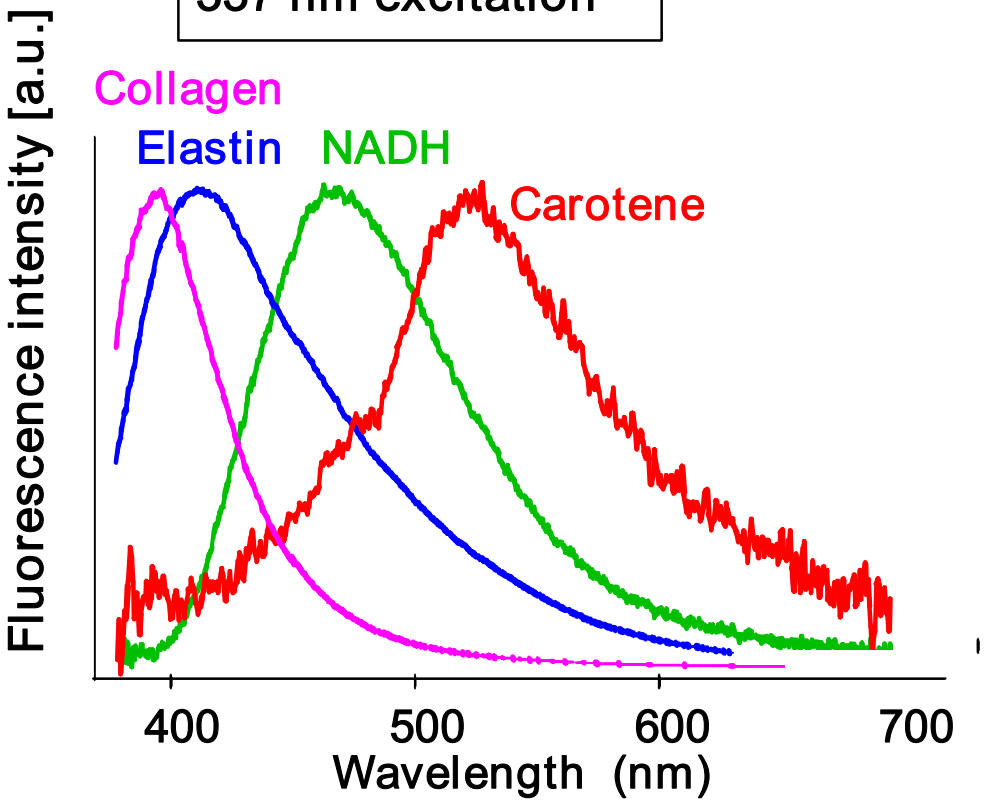
Fluorescence induced by exposure to ultraviolet light in vials containing various sized Cadmium selenide (CdSe) quantum dots. This is a file from the Wikimedia Commons, http://en.wikipedia.org/wiki/Fluorescence

Fluorescent minerals. This is a file from the Wikimedia Commons, http://en.wikipedia.org/wiki/Fluorescence

# Fluorescence

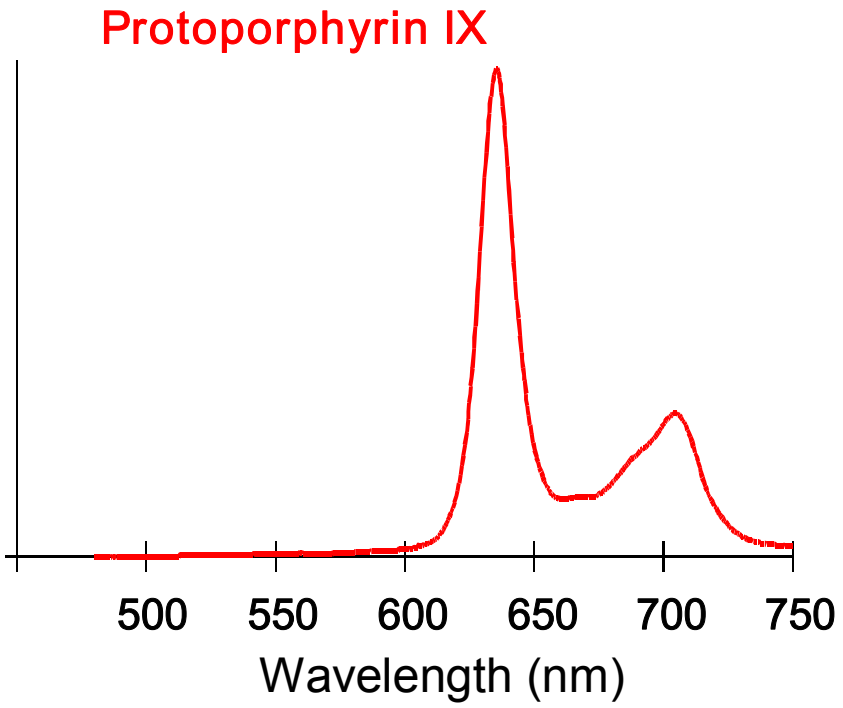
## Tissue autofluorescence

337 nm excitation




## Protoporphyrin IX

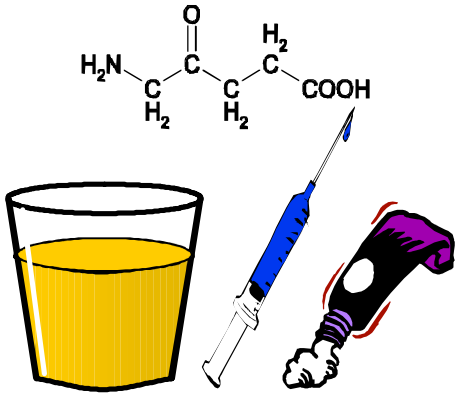
405 nm excitation



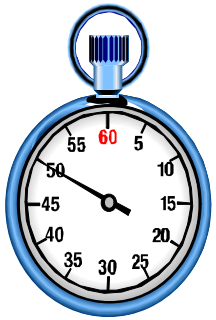

J. Johansson, Dissertation thesis, LTH (1993).
af Klinteberg *et al.* (1999)

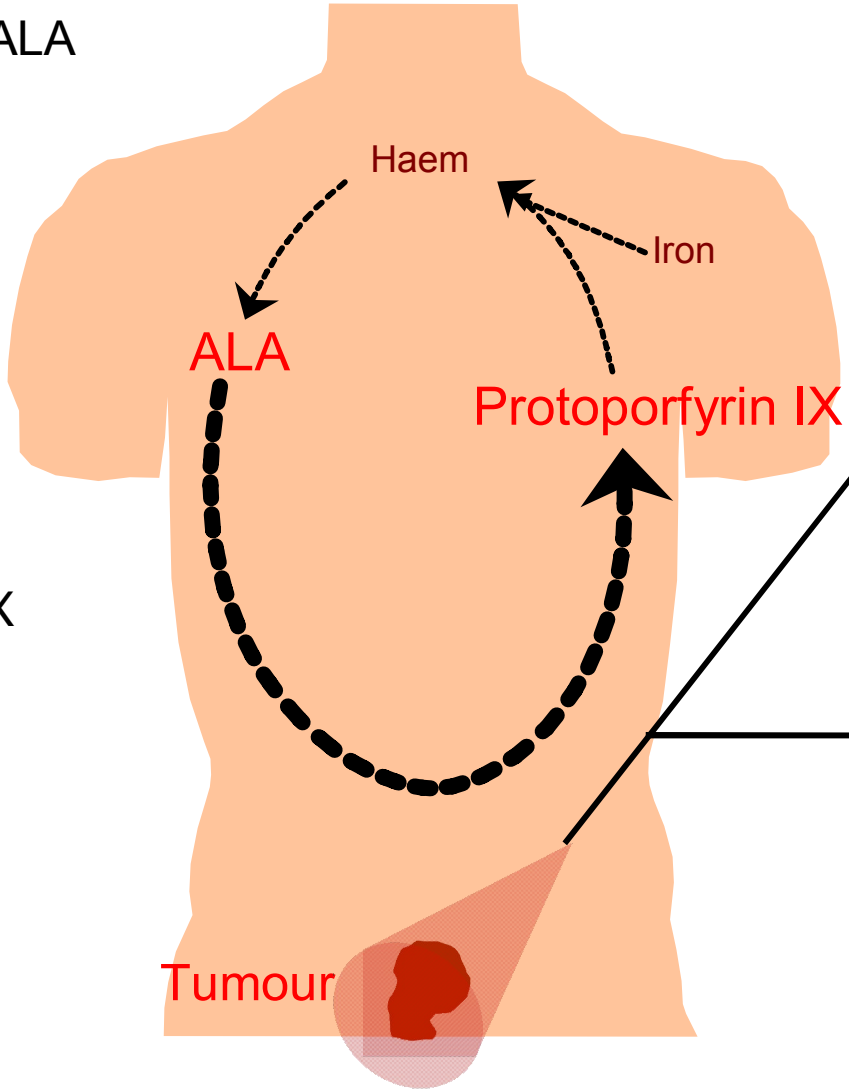
# Fluorescence diagnostics of skin tumour following ALA administration

## 1. Administration of ALA

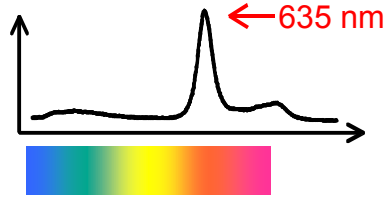


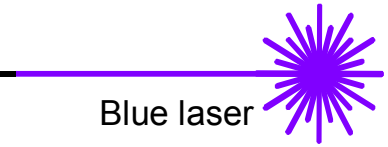

## 2. Production of PpIX



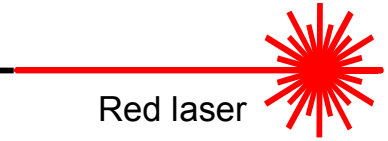




## 3. Diagnostics



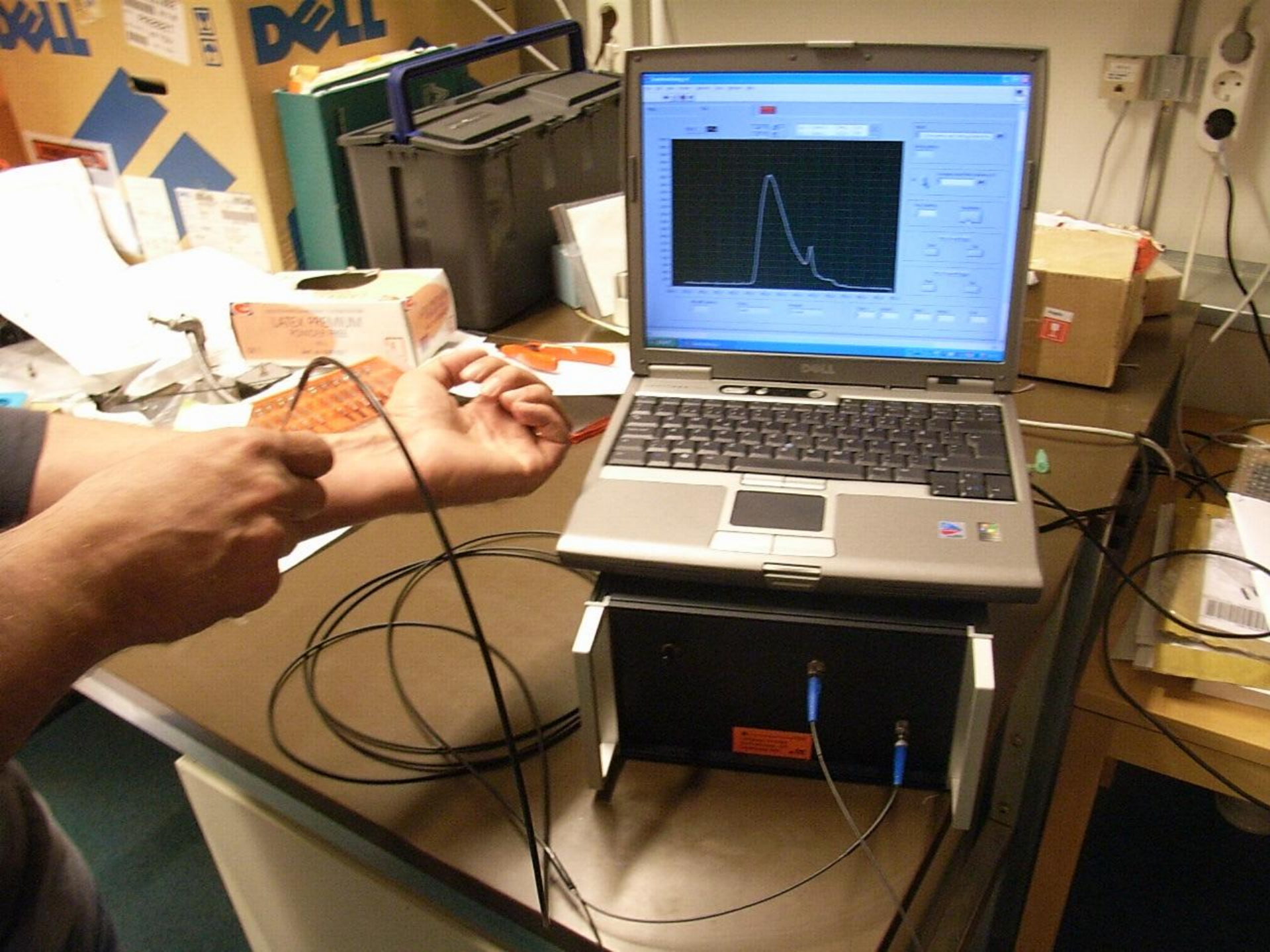




## 4. Treatment



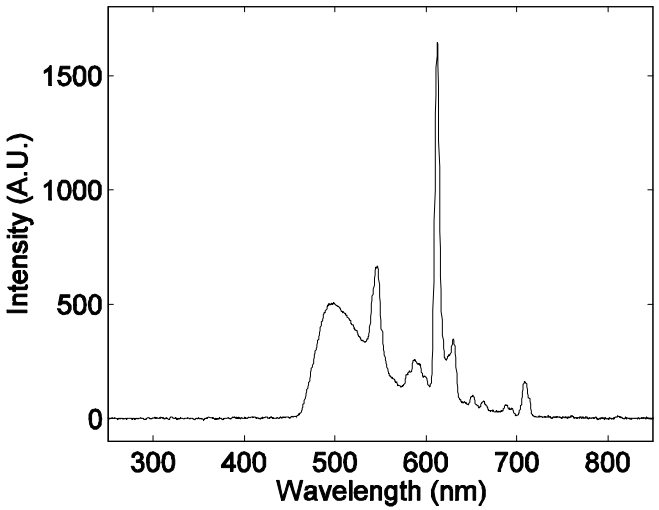
# Tumour localising agents

| Photosensitisers (PDT)<br>Fluorescent tumour markers (LIF) | RED<br>Absorption<br>Peak |
|---|---|
| Haematoporphyrin derivative (HpD), (Photofrin) | 630 nm |
| δ-aminolevulinic acid (ALA) | 635 nm |
| Mesotetrahydroxyphenychlorin (mTHPC), (Foscan) | 652 nm |
| Tin Etiopurpurin (Pyrlytin) | 660 nm |
| Benzoporphyrin, (Verteporfin) | 690 nm |
| Phthalocyanins | 720 nm |
| Lutetium texaphyrin (Lutrin) | 732 nm |
| Bacteriochlorophyll (Tookad) | 760 nm |

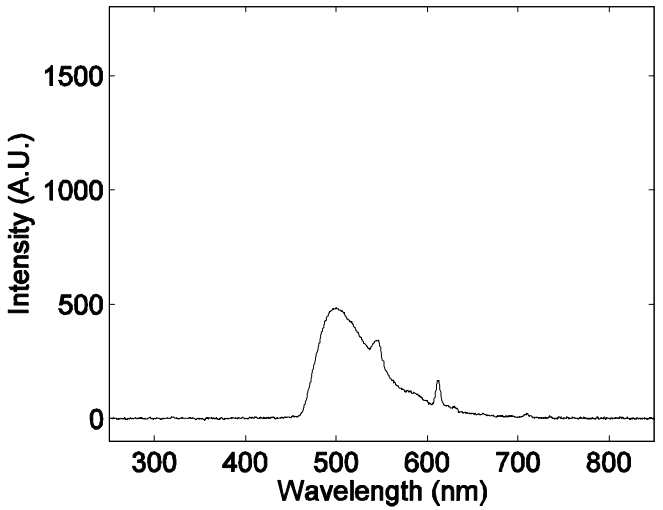

DELL

# Background light interference

Without funnel

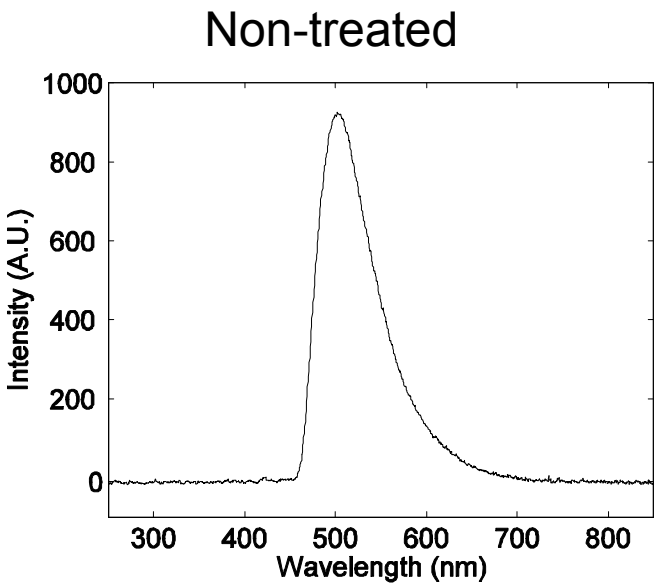


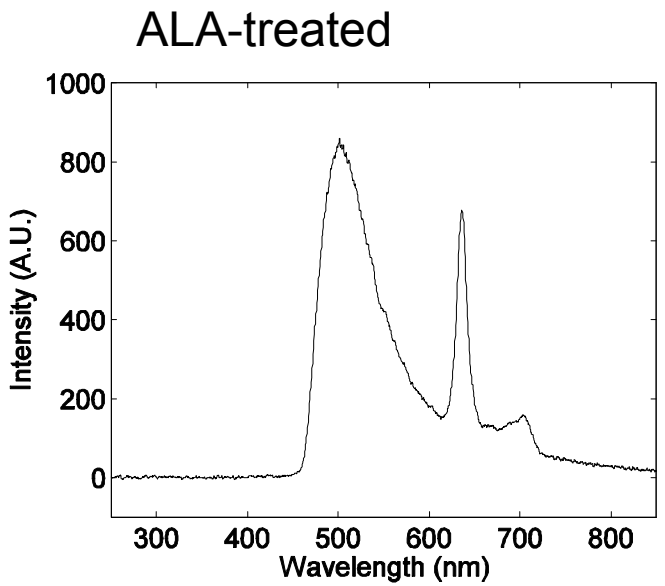

With funnel




Haj-Hossini et al. unpublished (2009)

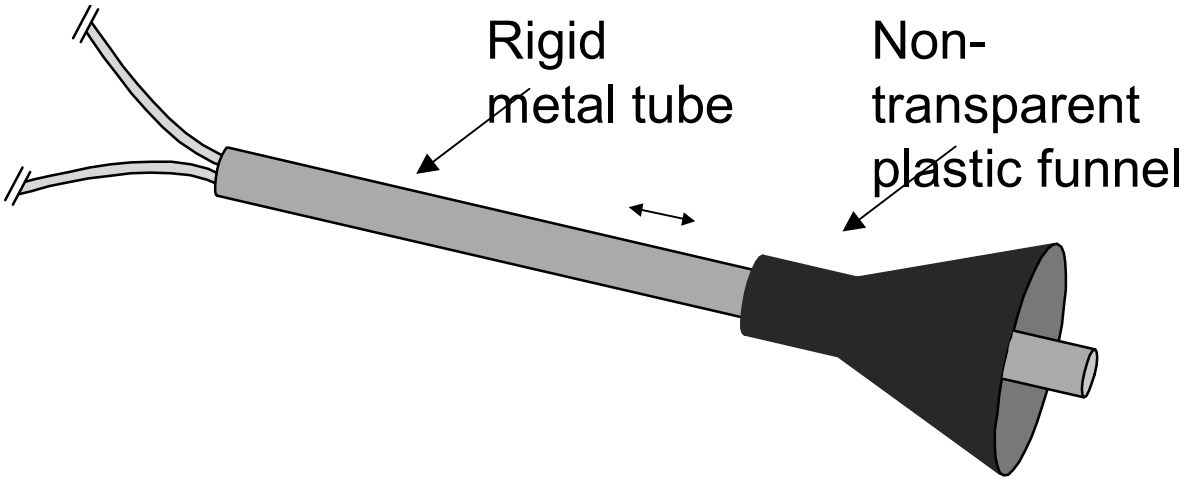
# Skin measurements




Haj-Hossini et al. unpublished (2009)

# Ambient light shielding

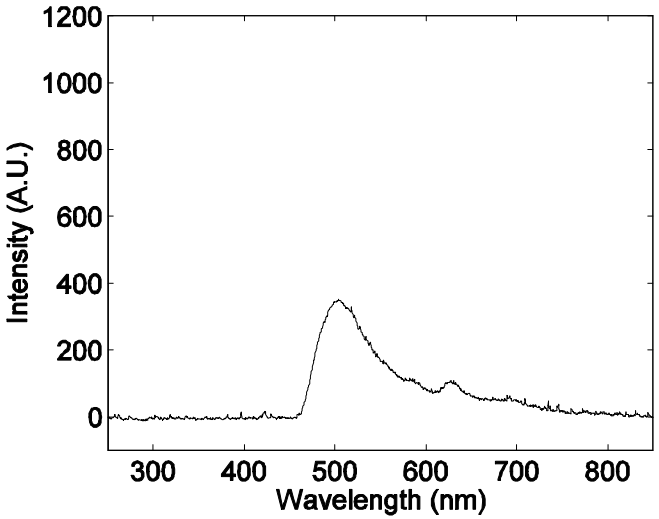



Haj-Hossini et al. unpublished (2009)

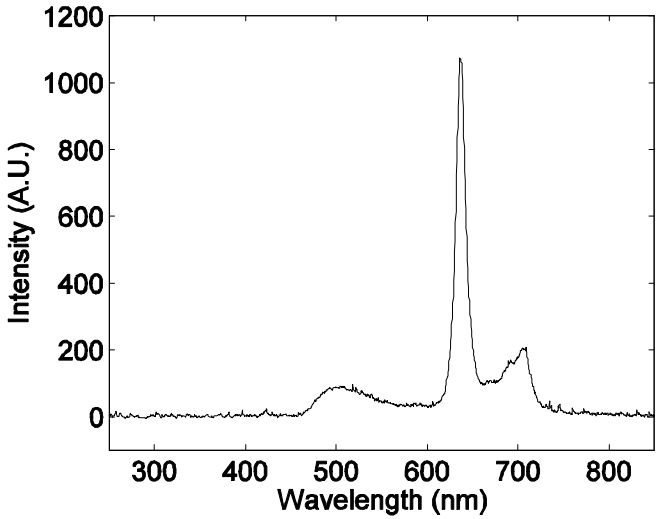

MEDICAL
SWEDEN

# Brain measurement

Healthy tissue



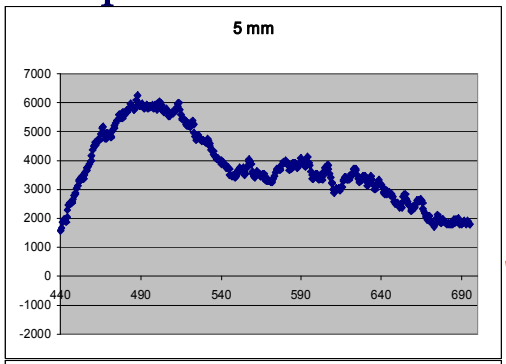

GBM

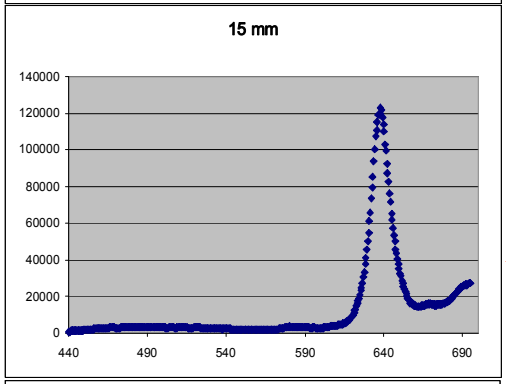


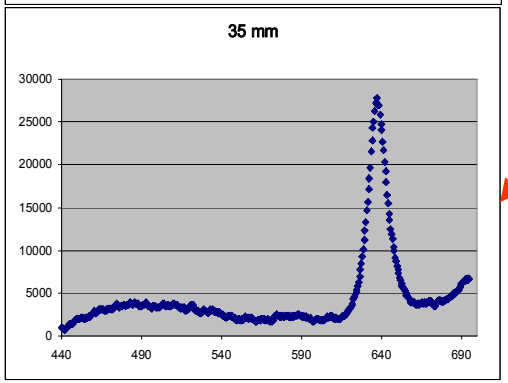

Richter *et al.* Unpublished (2009)

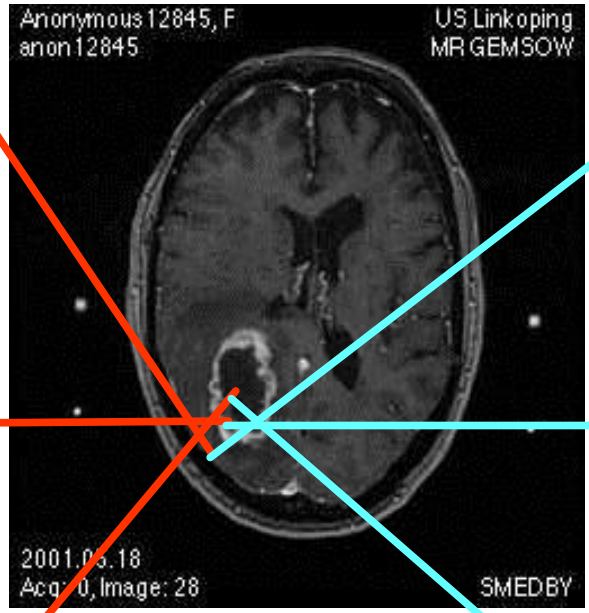
# Astrocytoma

## PpIX fluorescence

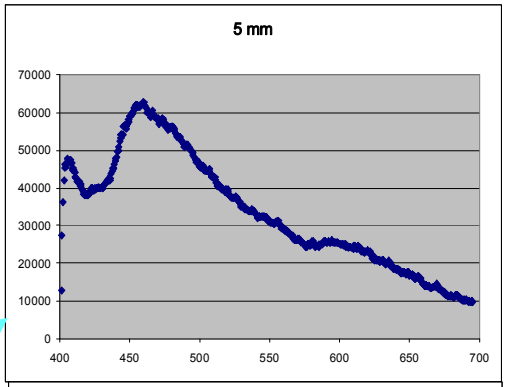


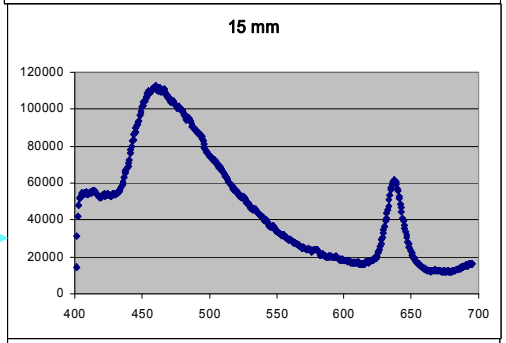



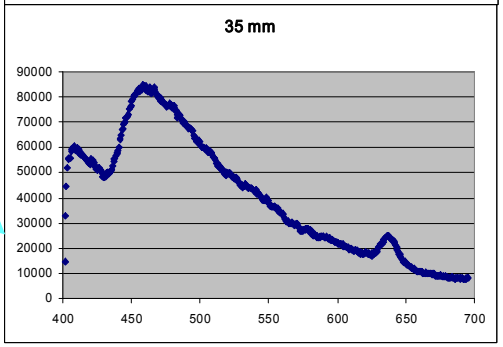




## Autofluorescence

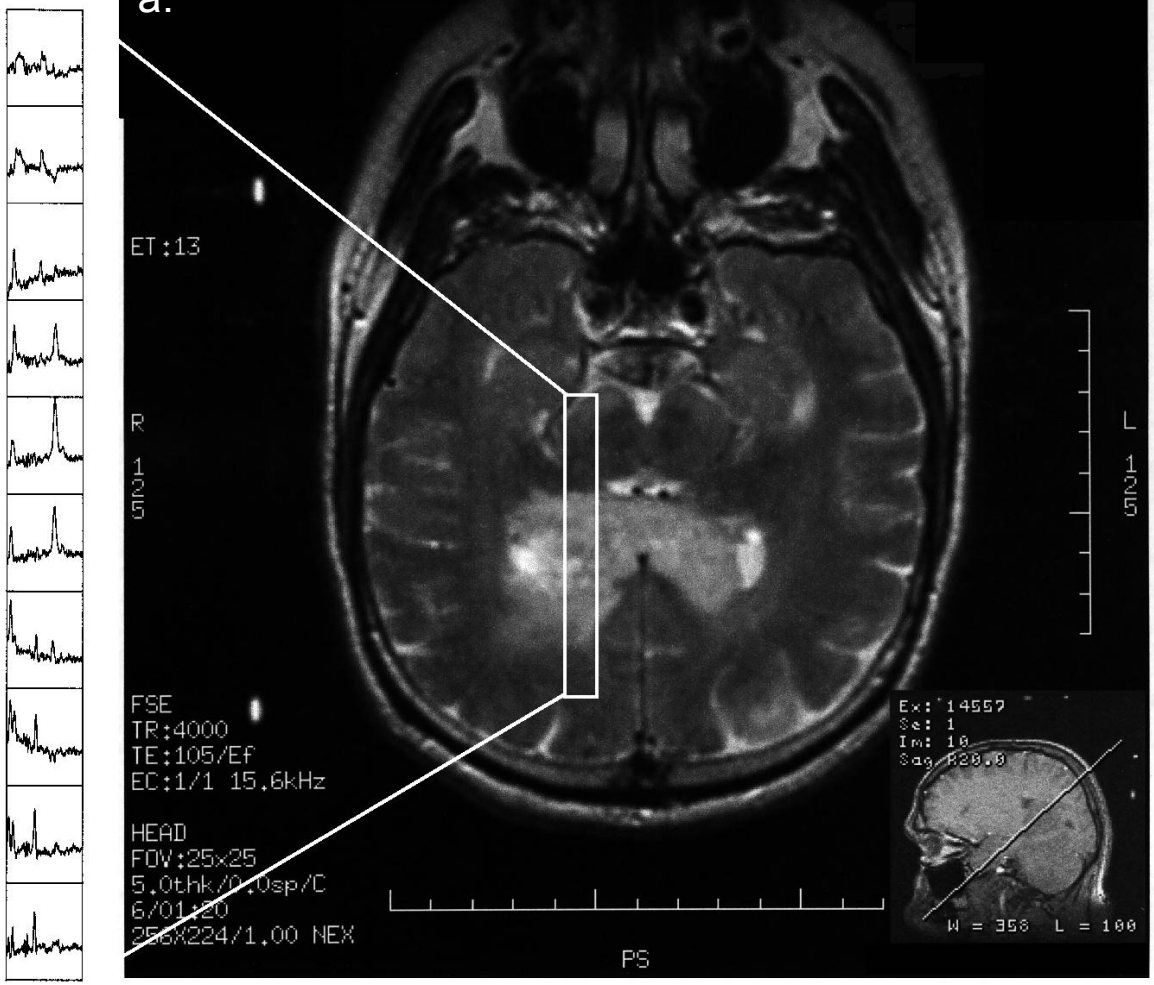


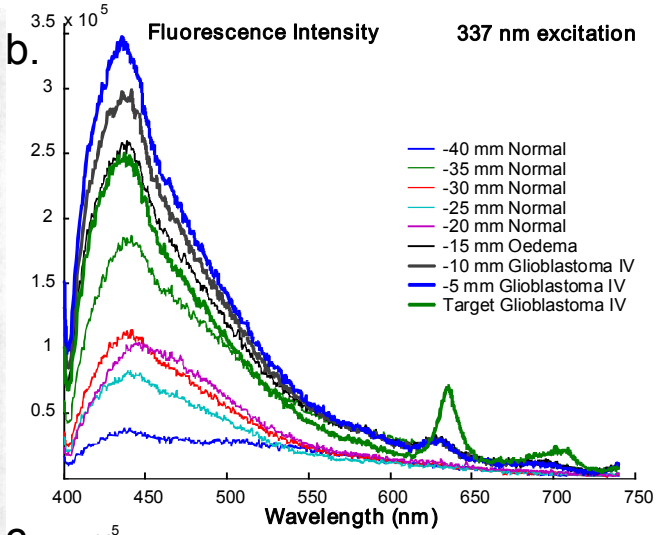



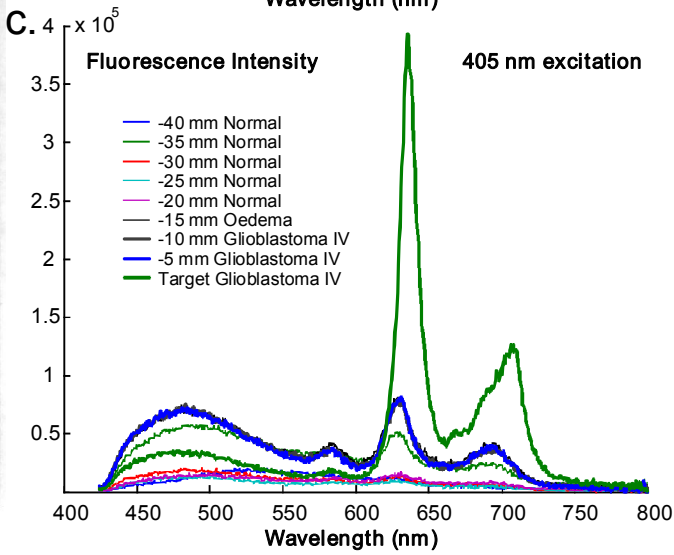




Pålsson et al. unpublished (200

# MR and fluorescence spectra



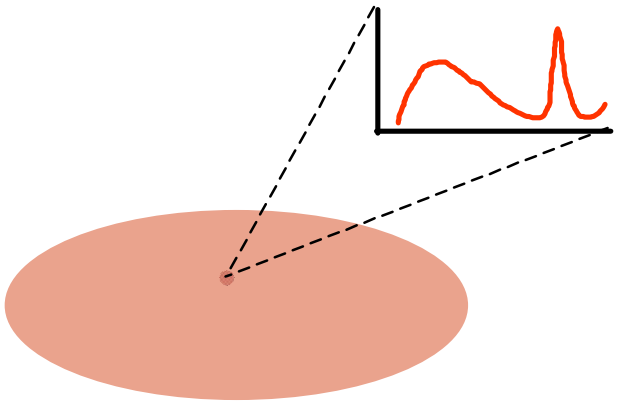



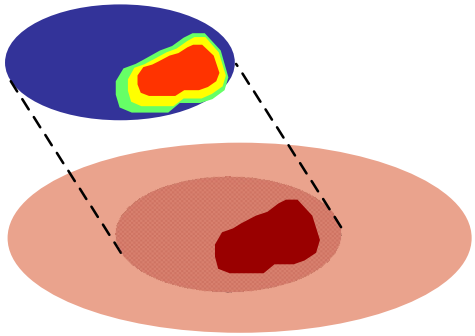




Pålsson et al. unpublished (200

# Fluorescence diagnostics

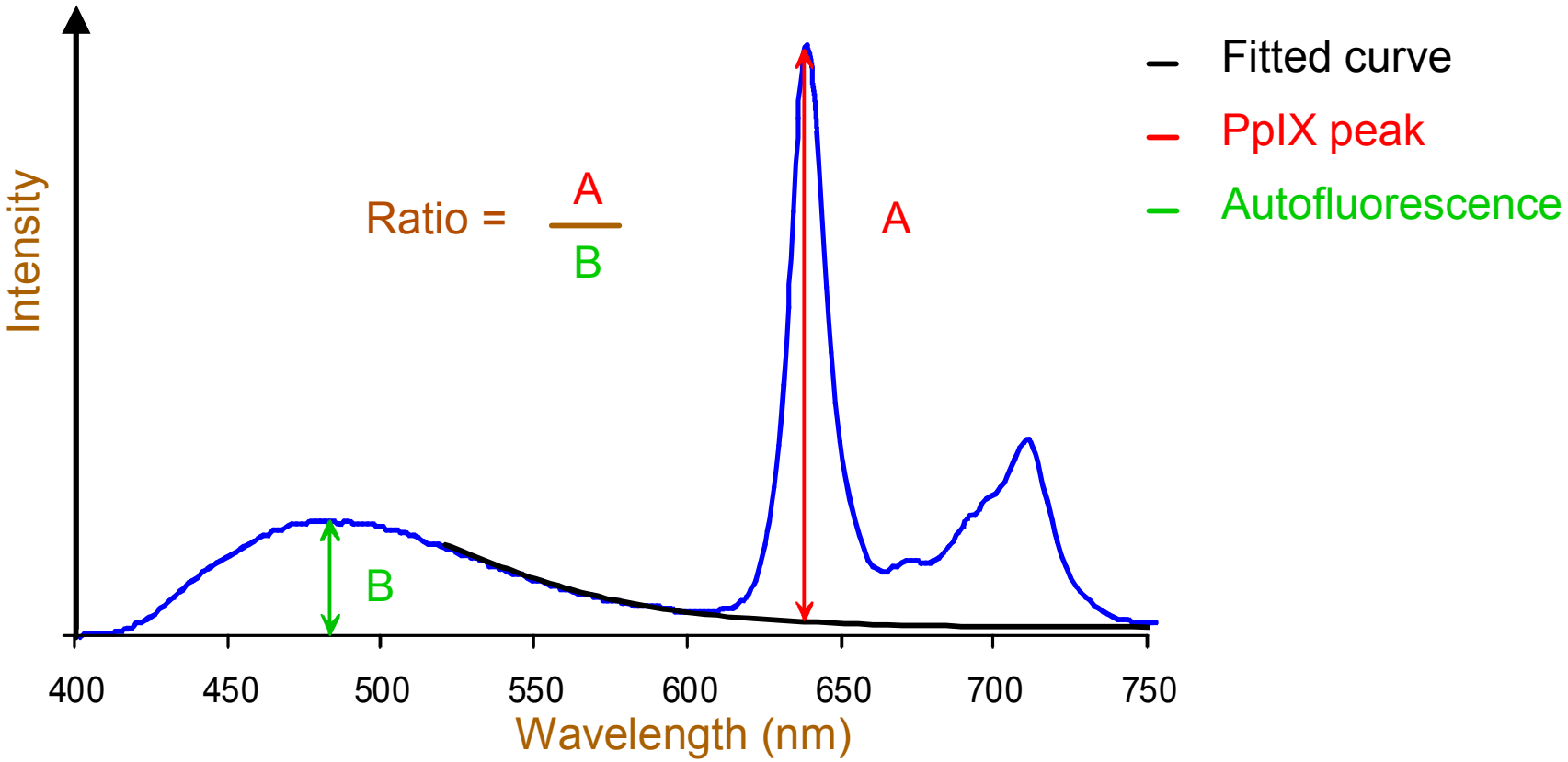
Point monitoring:
Whole spectrum in
one small tissue site



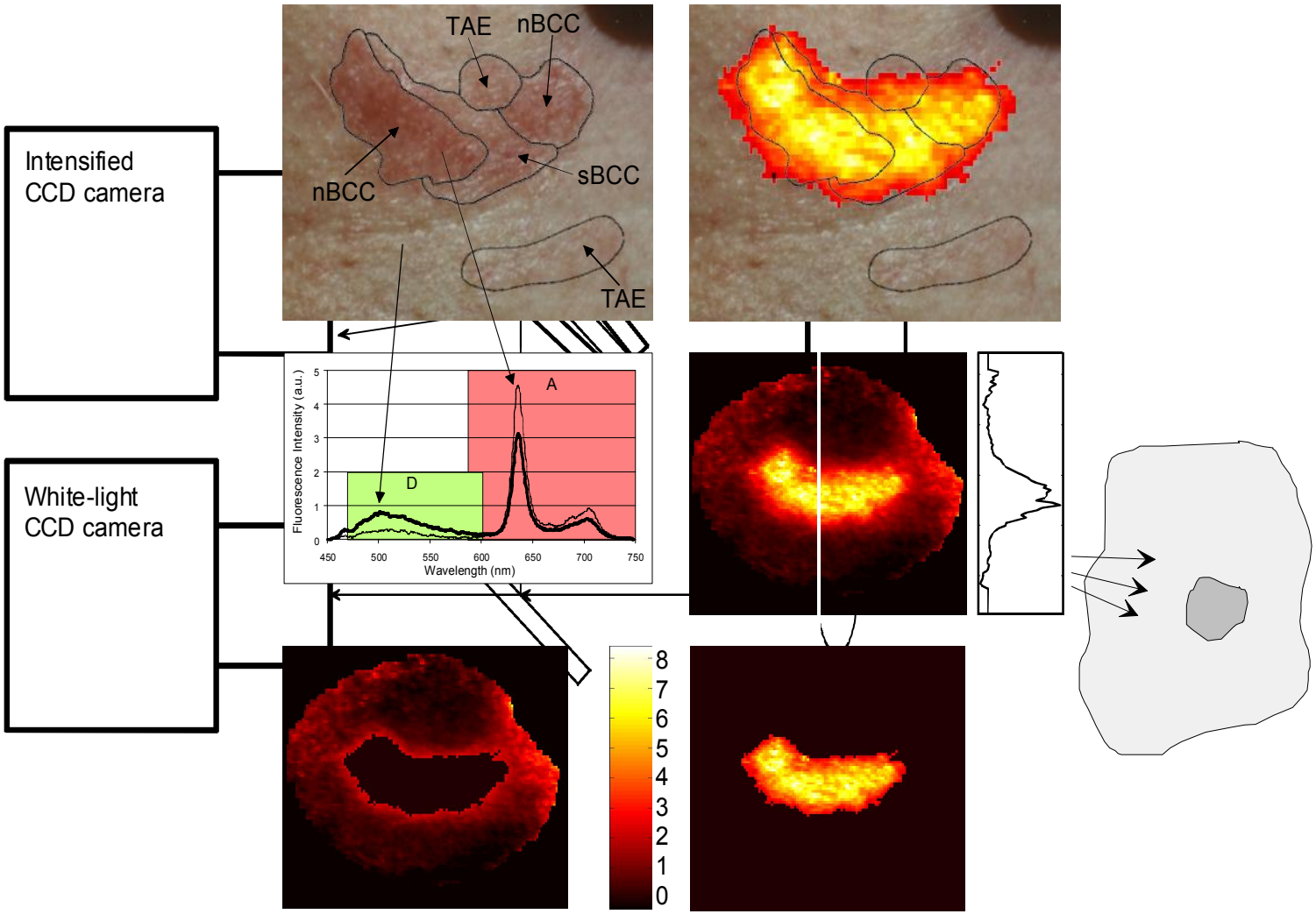


Imaging:
Less spectral information
but in larger area

# Fluorescence specrum



# Dermatological Multicolour Fluorescence imaging

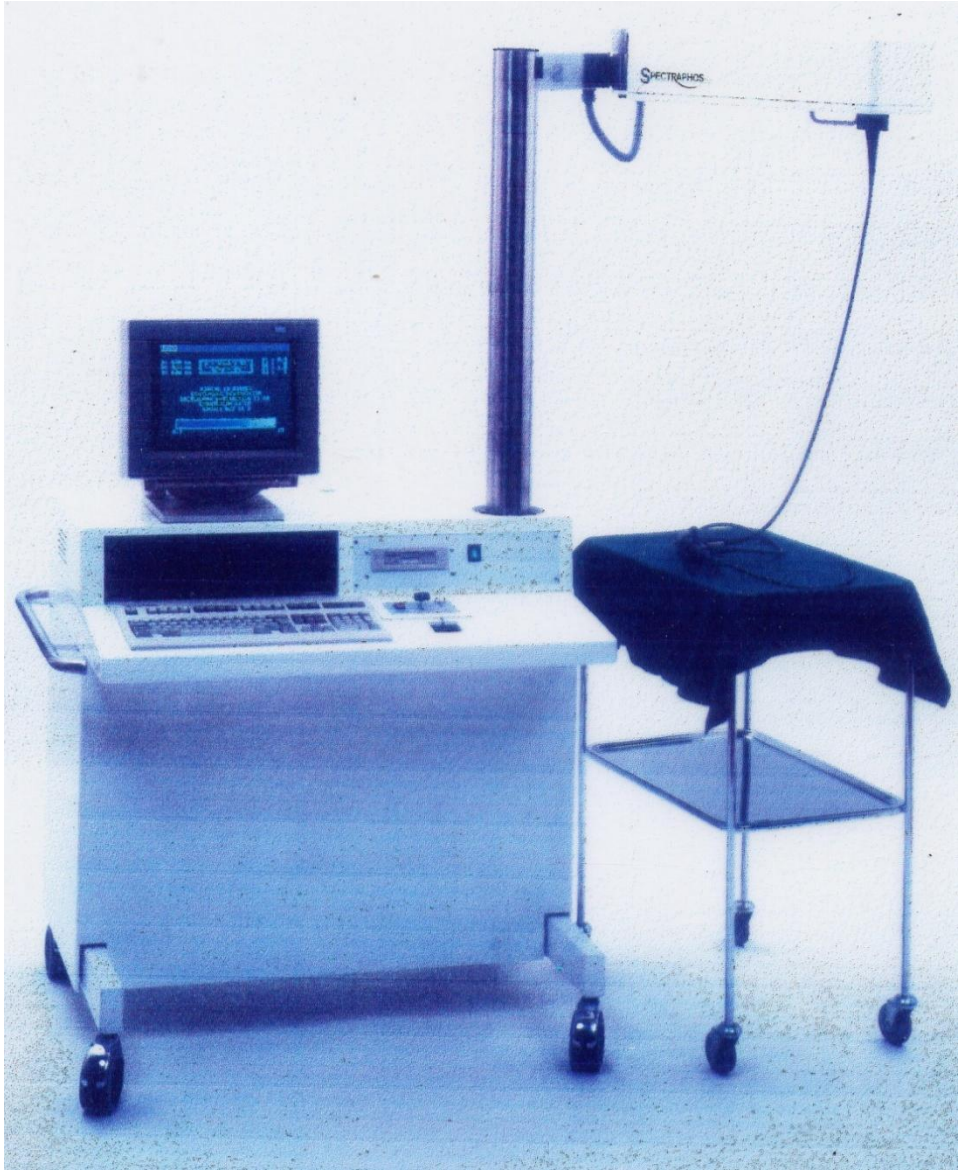


# Multicolour Fluorescence Imaging

White light image

Digitally processed image

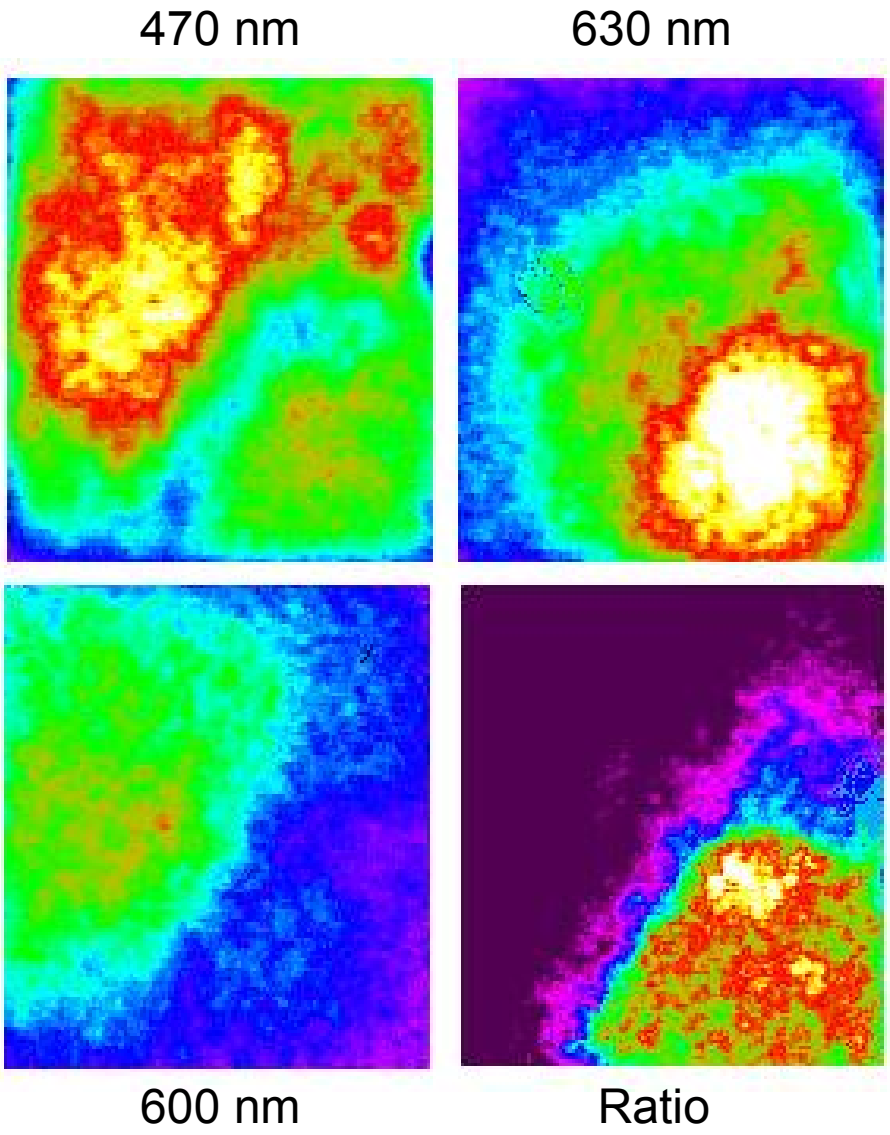
# Spectraphos multispectral fluorescence imaging system




Svanberg et al. Acta Radiol (199

# Multicolour fluorescence imaging

Rodent brain fluorescence following i.v. administration of ALA




Andersson-Engels *et al.* Bioimaging (1995)
Andersson-Engels *et al.* Appl Opt (1992)

# Hyper Spectral Diagnostic Imager

Combines:
- video image
- reflectance scan
- fluorescence scan

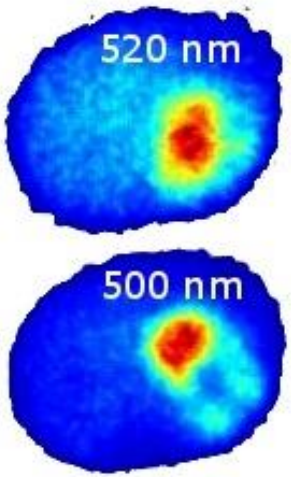
Aim:
- interactive diagnostics
- integrated colposcope

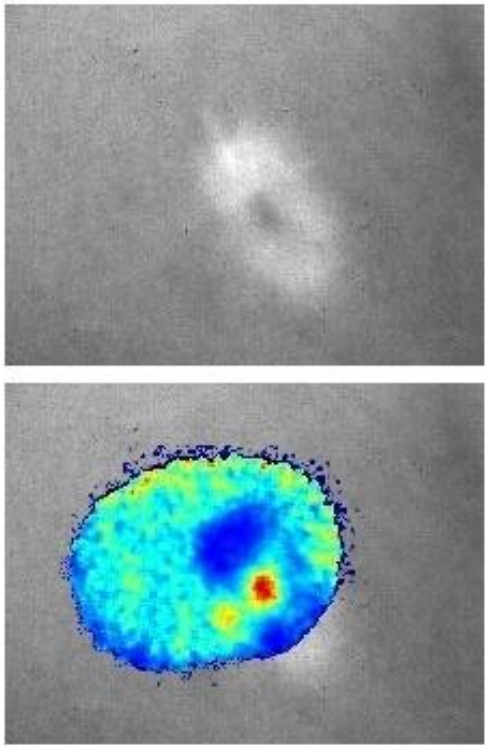
Science & Technology Inc, USA

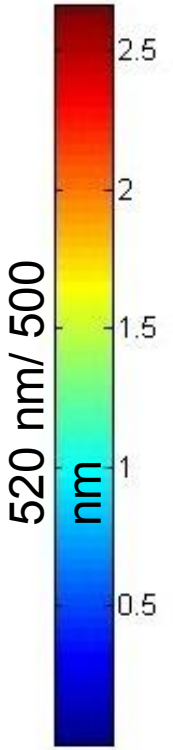
# Halo naevus

16 year old male

Located at left waist

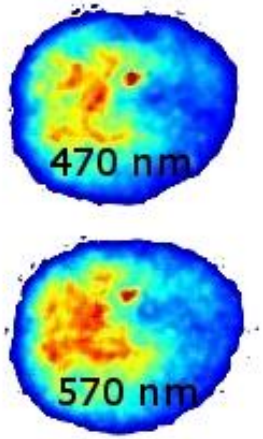


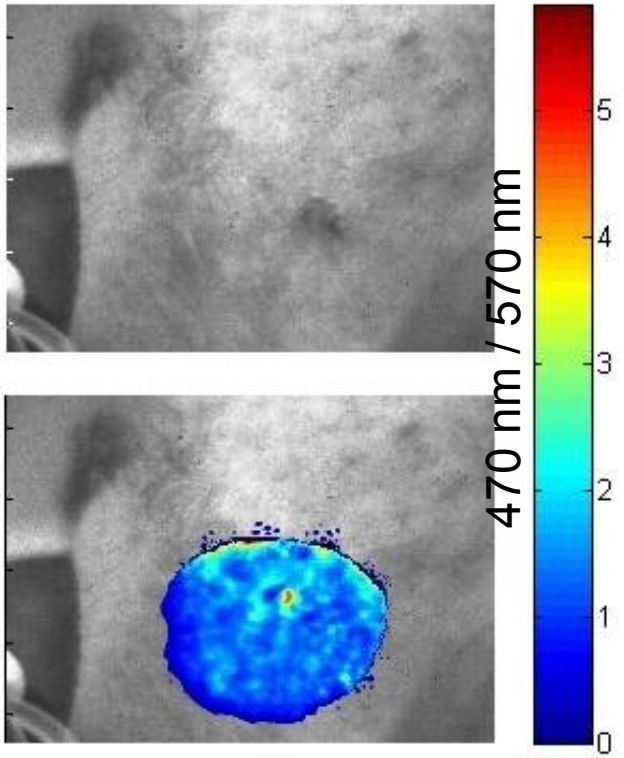





# Pigmentented BC or malignant melanoma

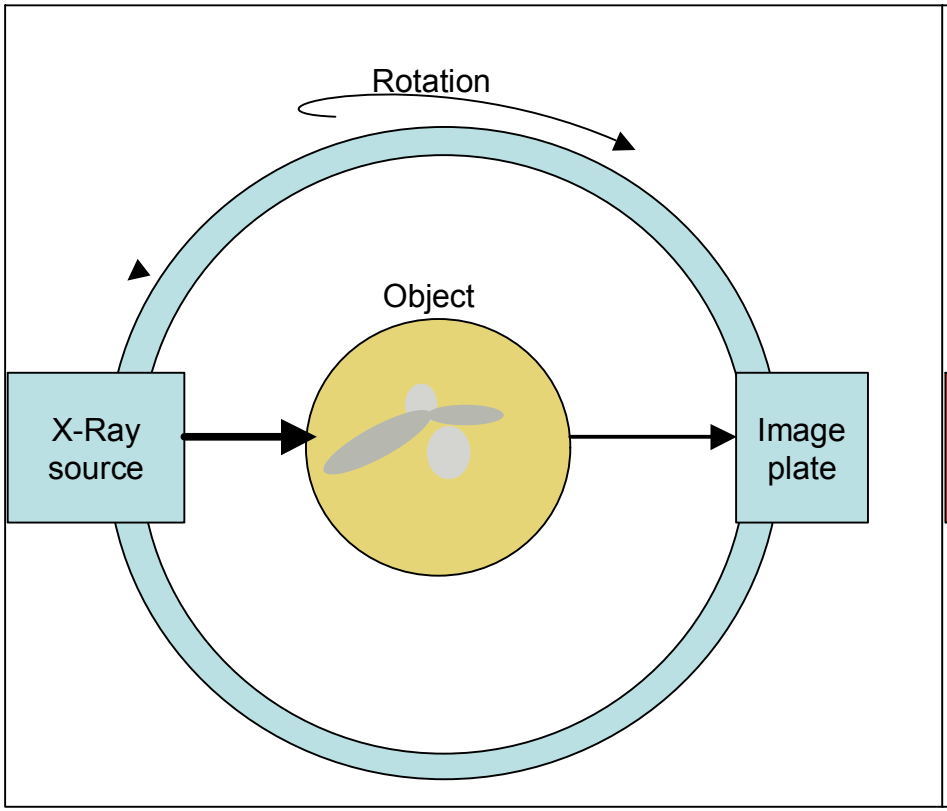
83 years old female
located next to the eye
went for surgery



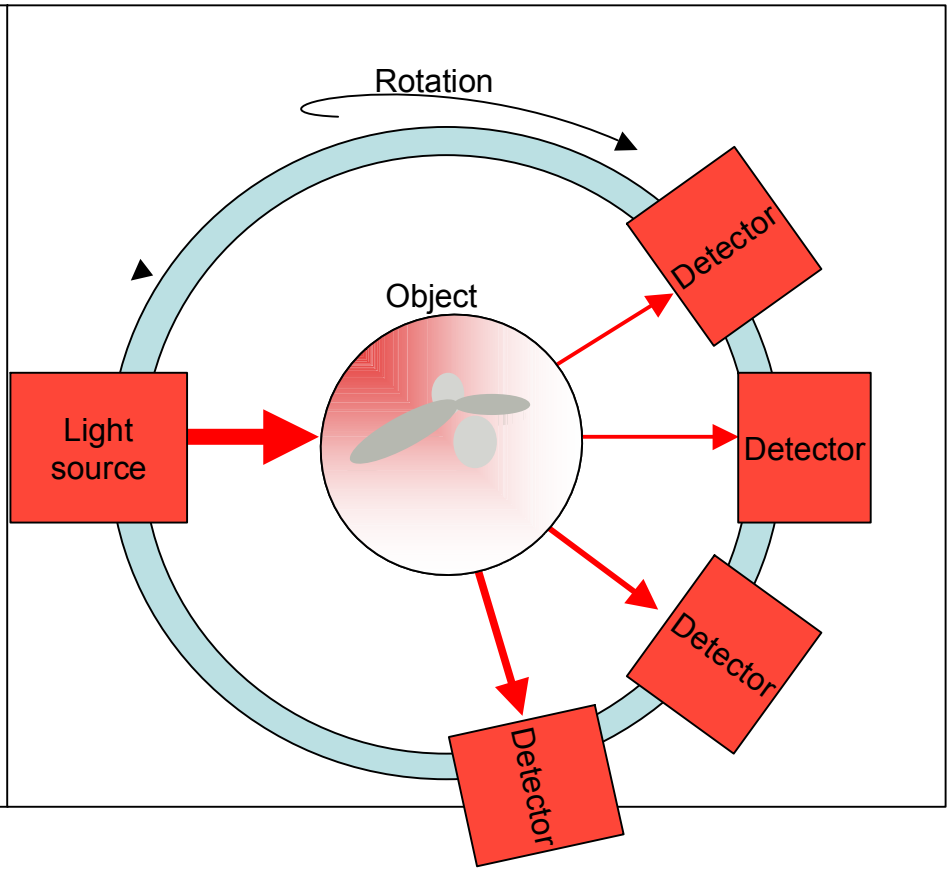



# Diffuse Optical Tomography

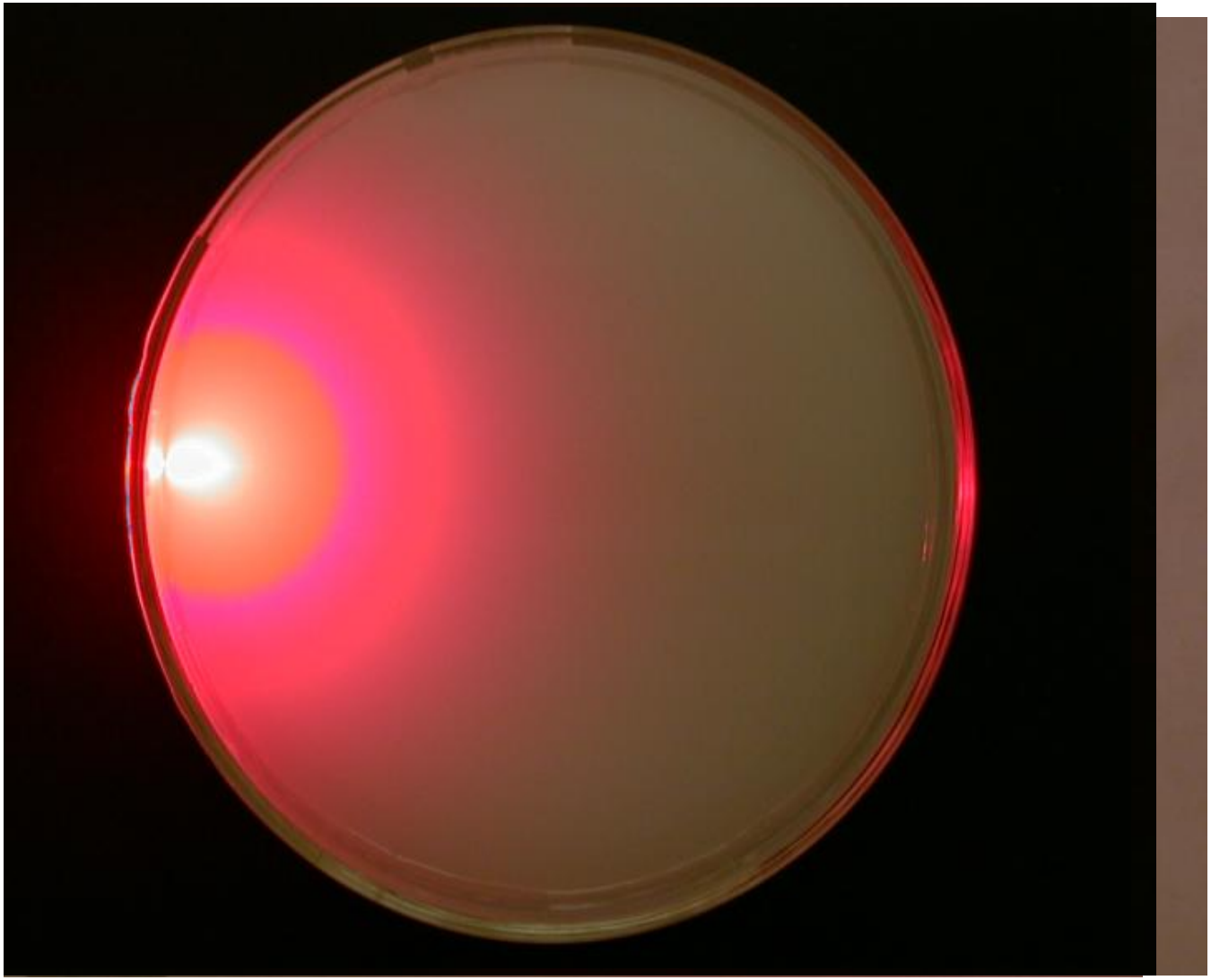
Linear image reconstruction

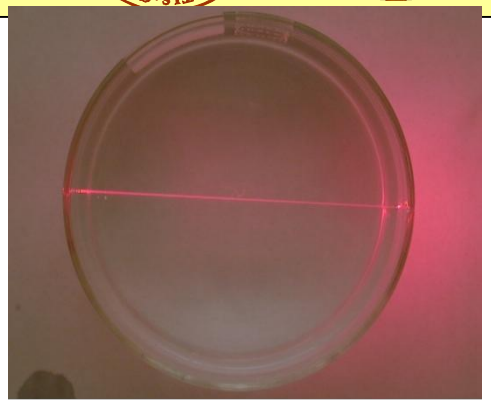


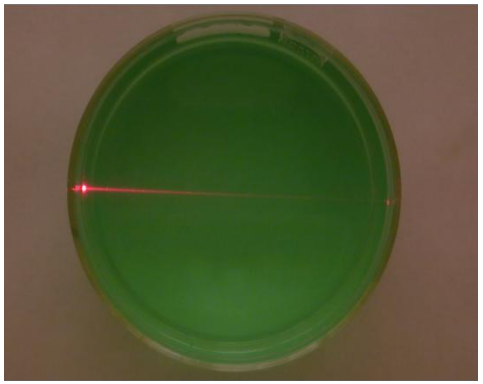

Non-linear image reconstruction

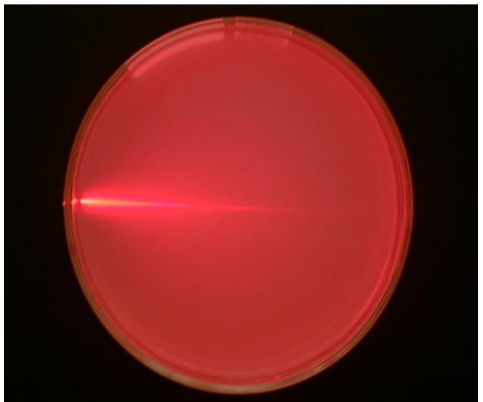


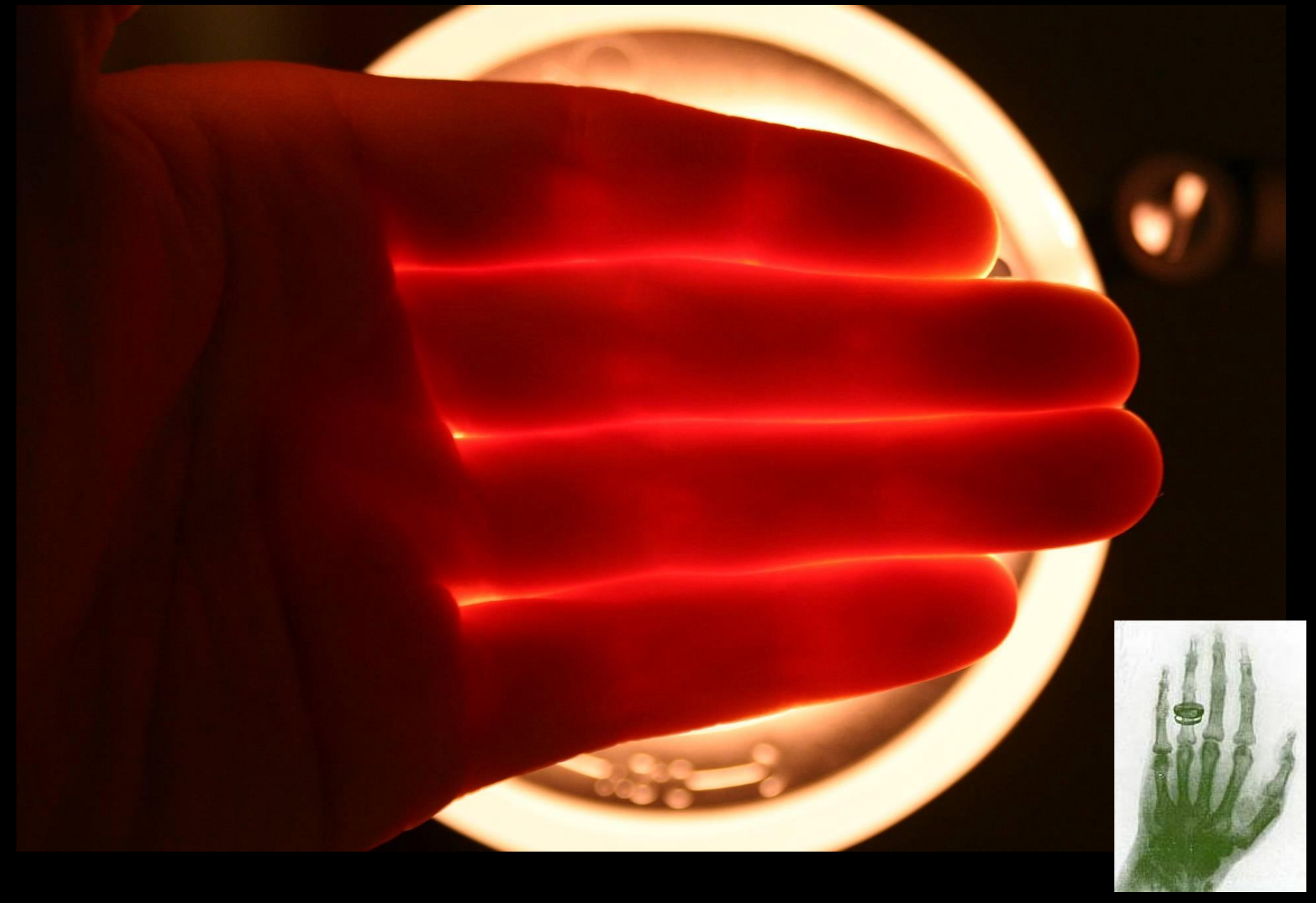
# Absorption and scattering



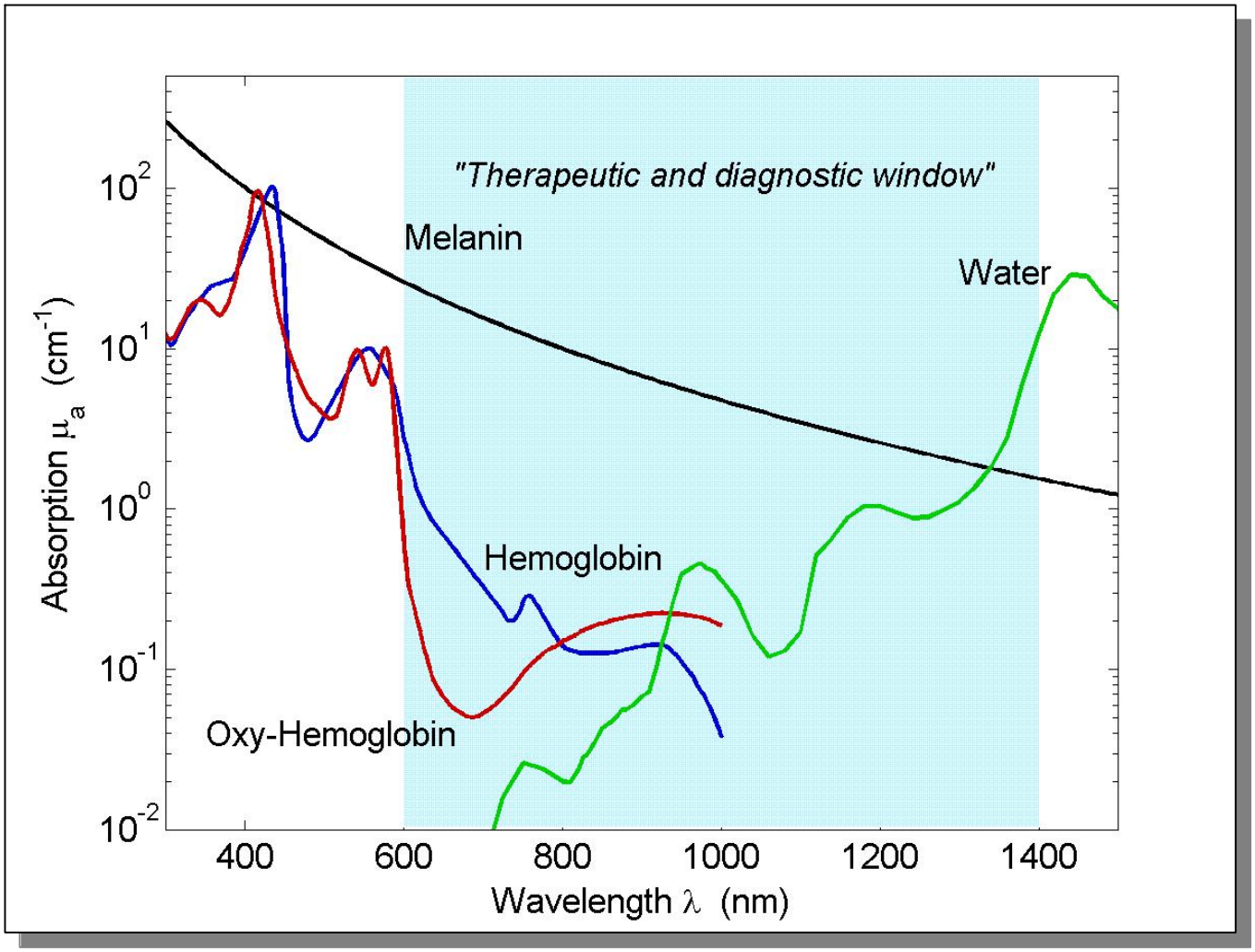




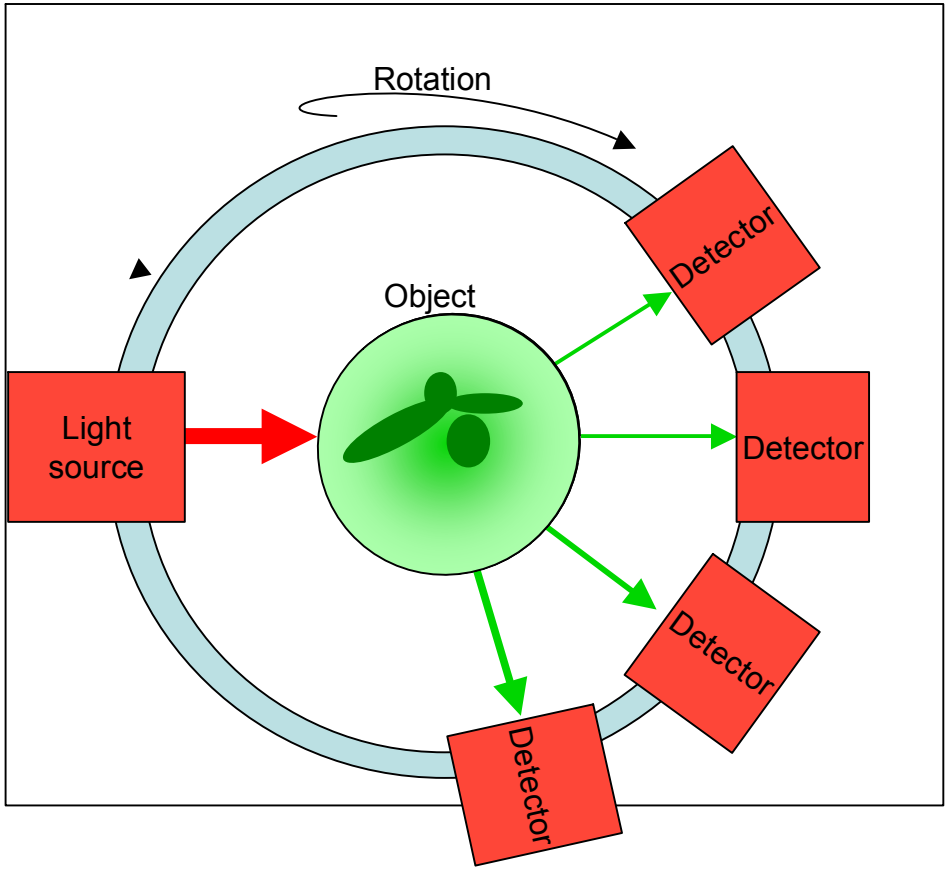




# Absorption spectra of important tissue chromophores



# Fluorescence-mediated tomography

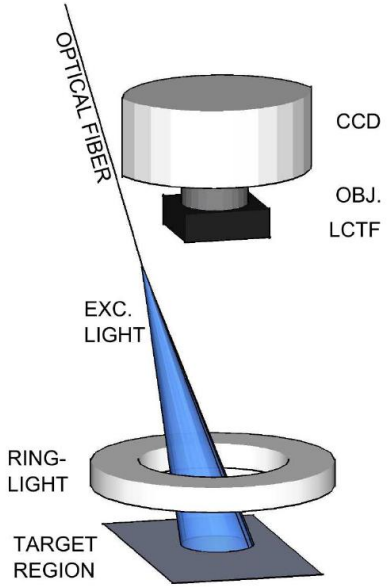


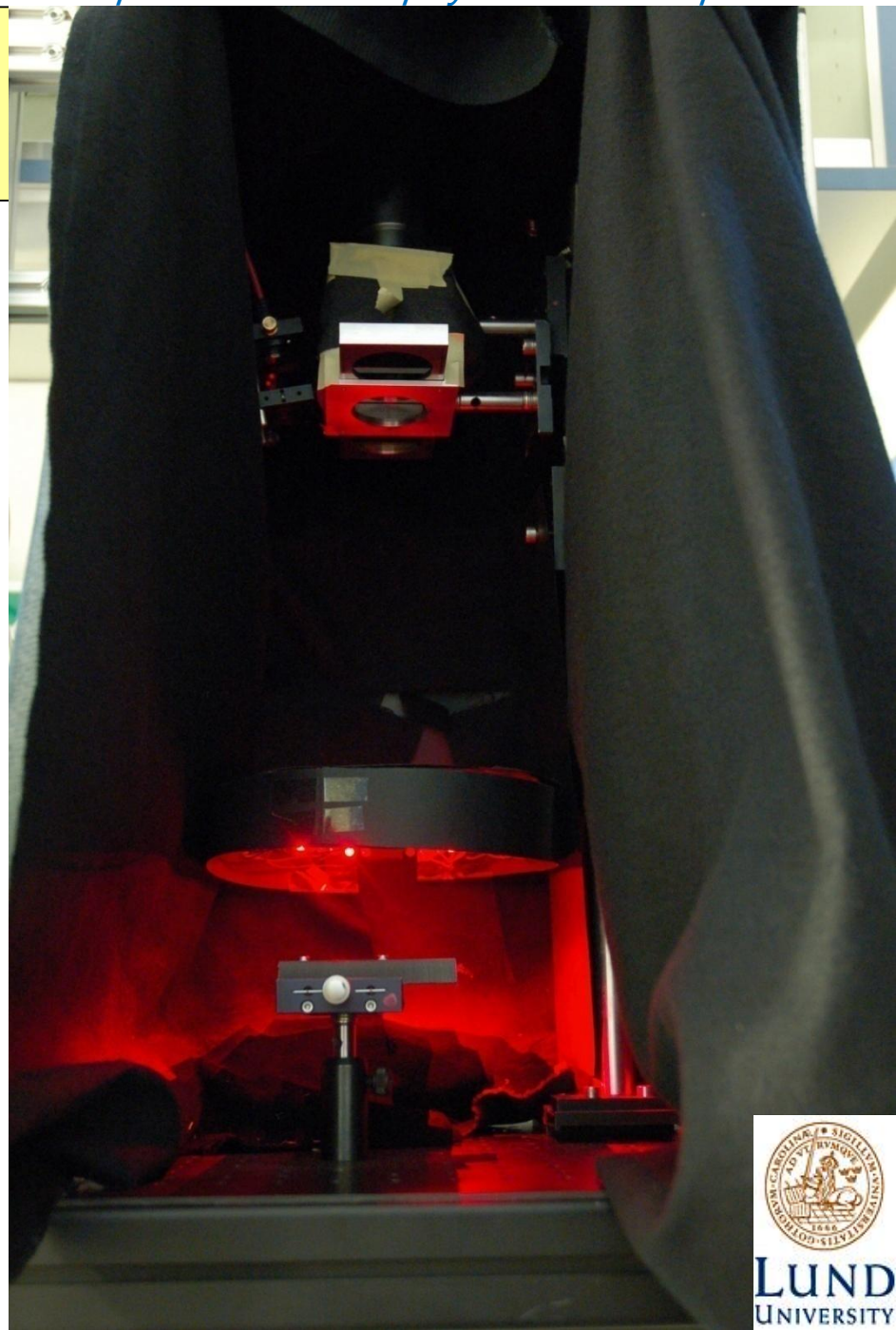
# Instrumentation from Lund Medical Laser Centre on its way to National Technical University in Athens

Collaboration with BioLitec, Bonn, Germany

# Joint WP6 campaign Instrumentation

During the last period we have spent much effort in evaluating the fluorescence imaging data from the measurement campaign in Jena.



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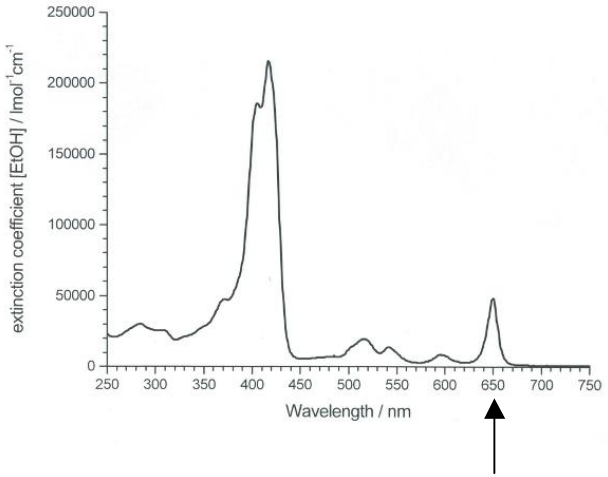
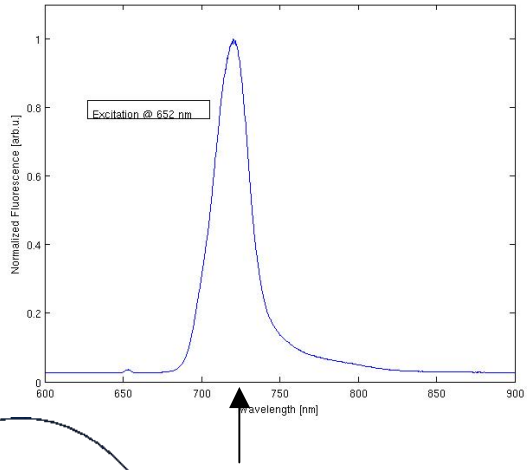
# Tomographic Reconstruction

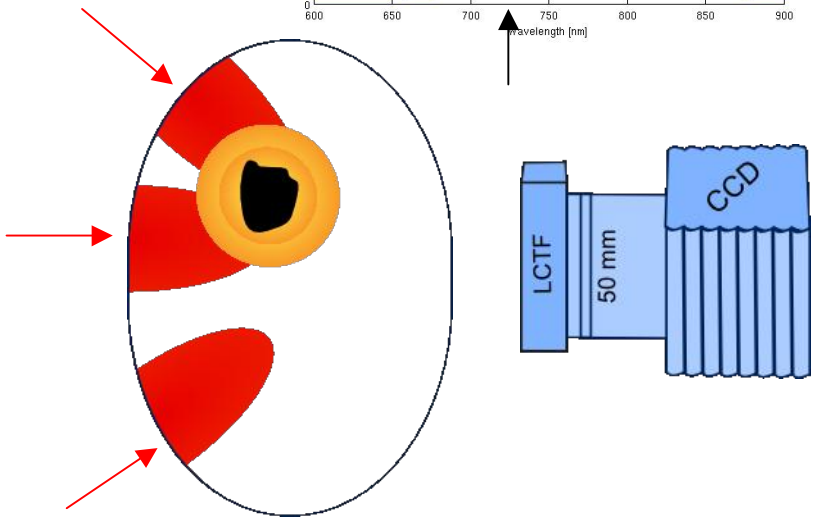
## Extinction coefficient




## Fluorescence Spectra



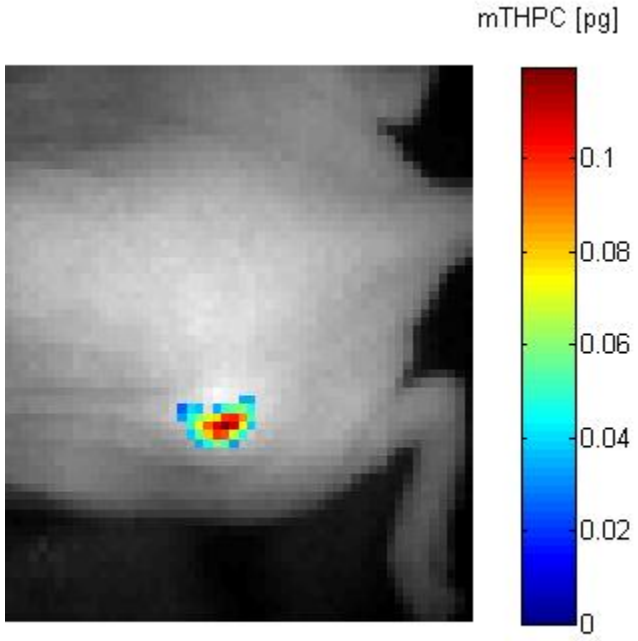

**Measure**
Excitation light, 652 nm
Fluorescence light, 720 nm



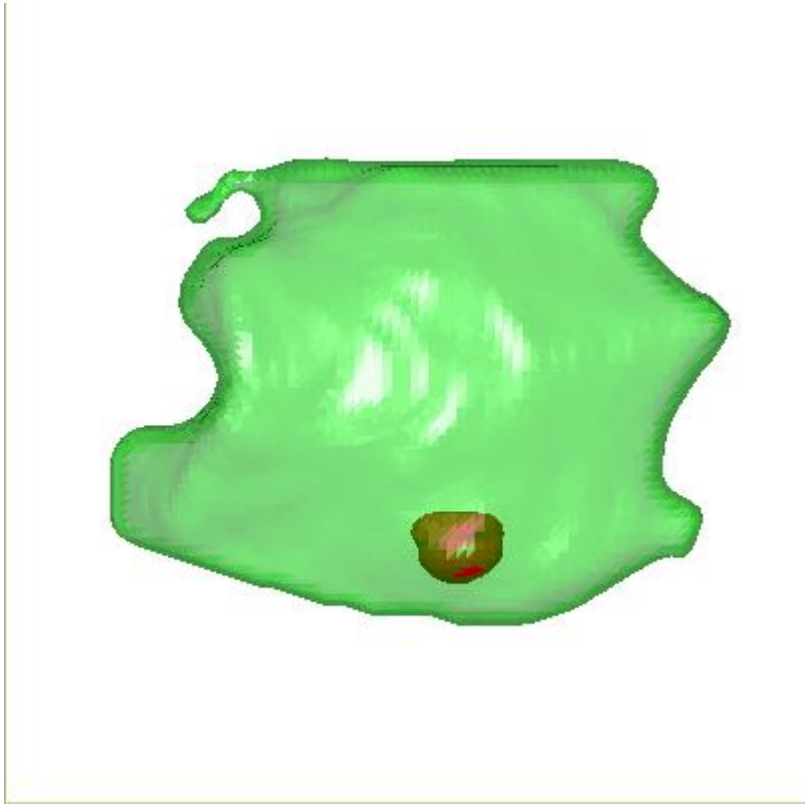

Svenmarker *et al.* unpublished (200

# *In vivo* non-invasive FosPeg Images

2D View



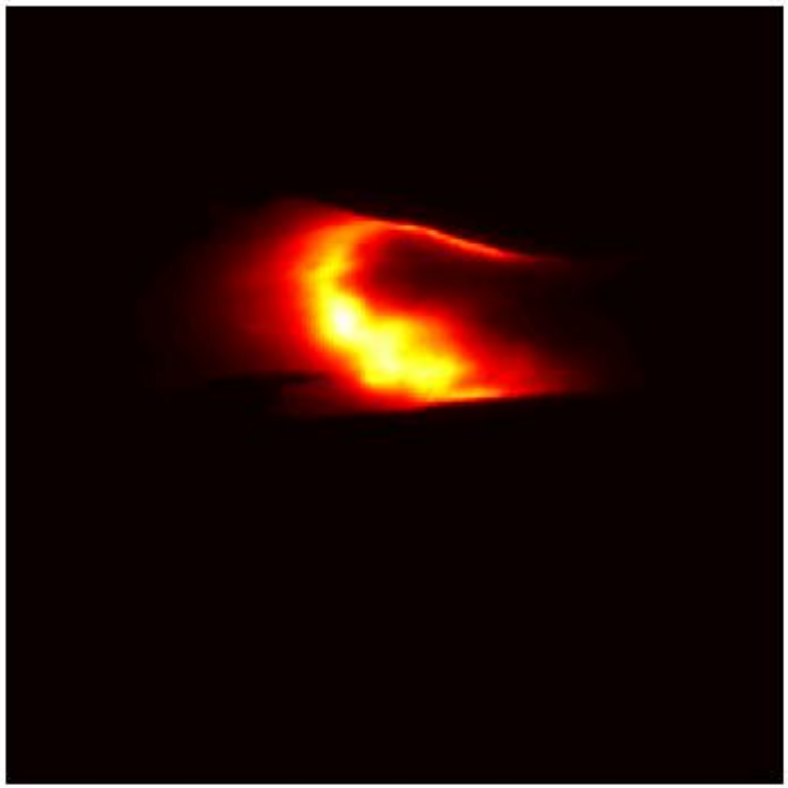

3D View



Svenmarker *et al.* unpublished (200

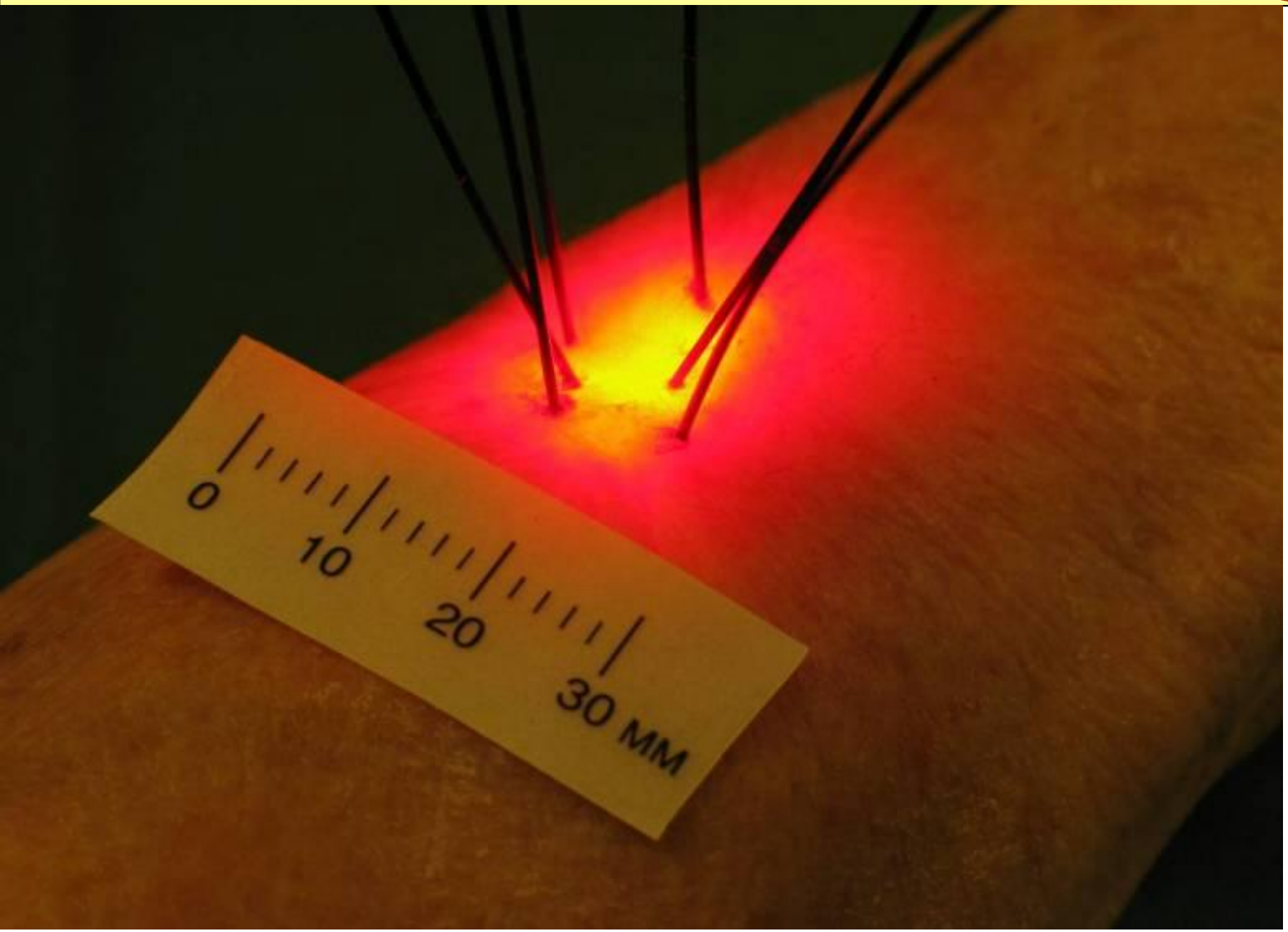
# Novel nanoparticles as fluorescence markers

Fluorescence imaging of a rat leg



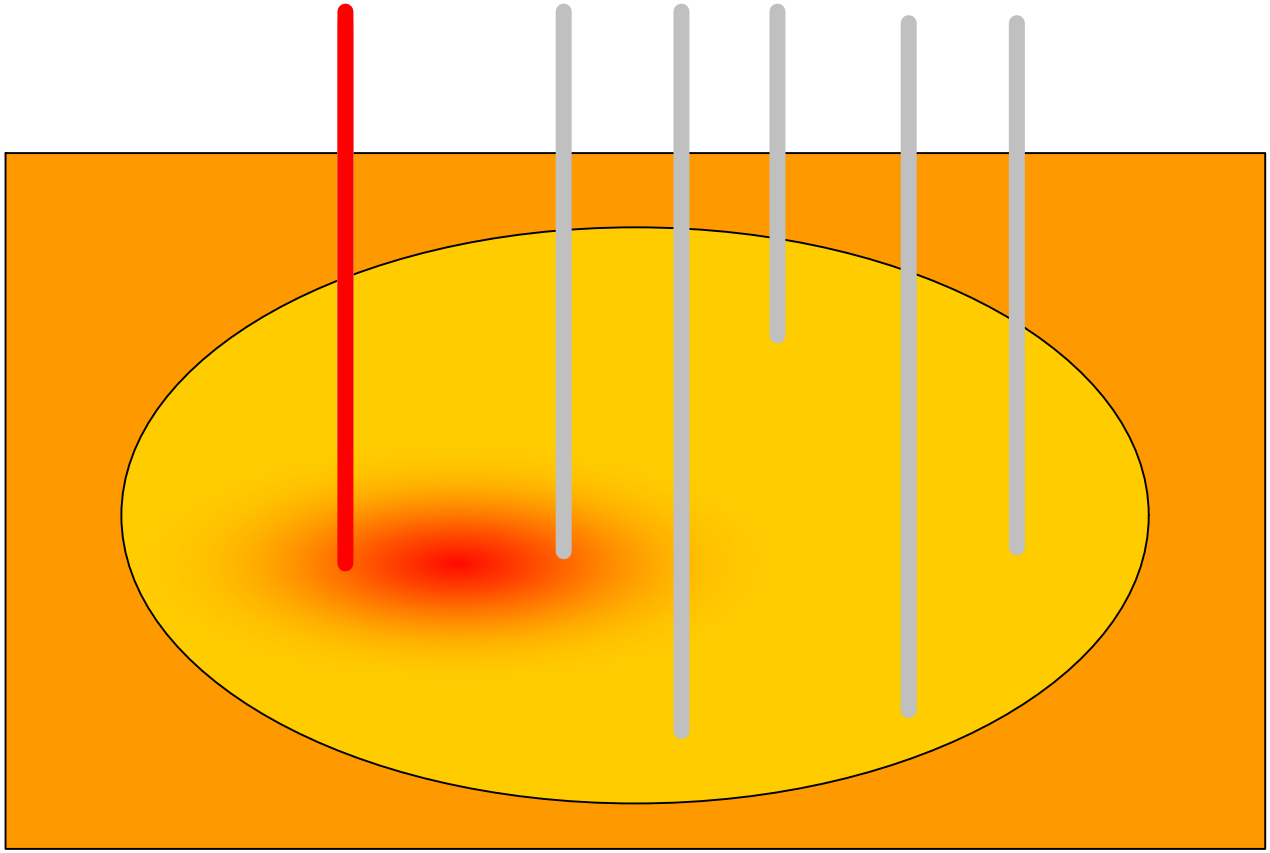
Xu et al. Appl Phys Lett (200
Xu et al. Appl Phys Lett (200

# IPDT during radiation




Soto Thompson *et al.* JEPTO (20
Johansson *et al.* JBO (2007)

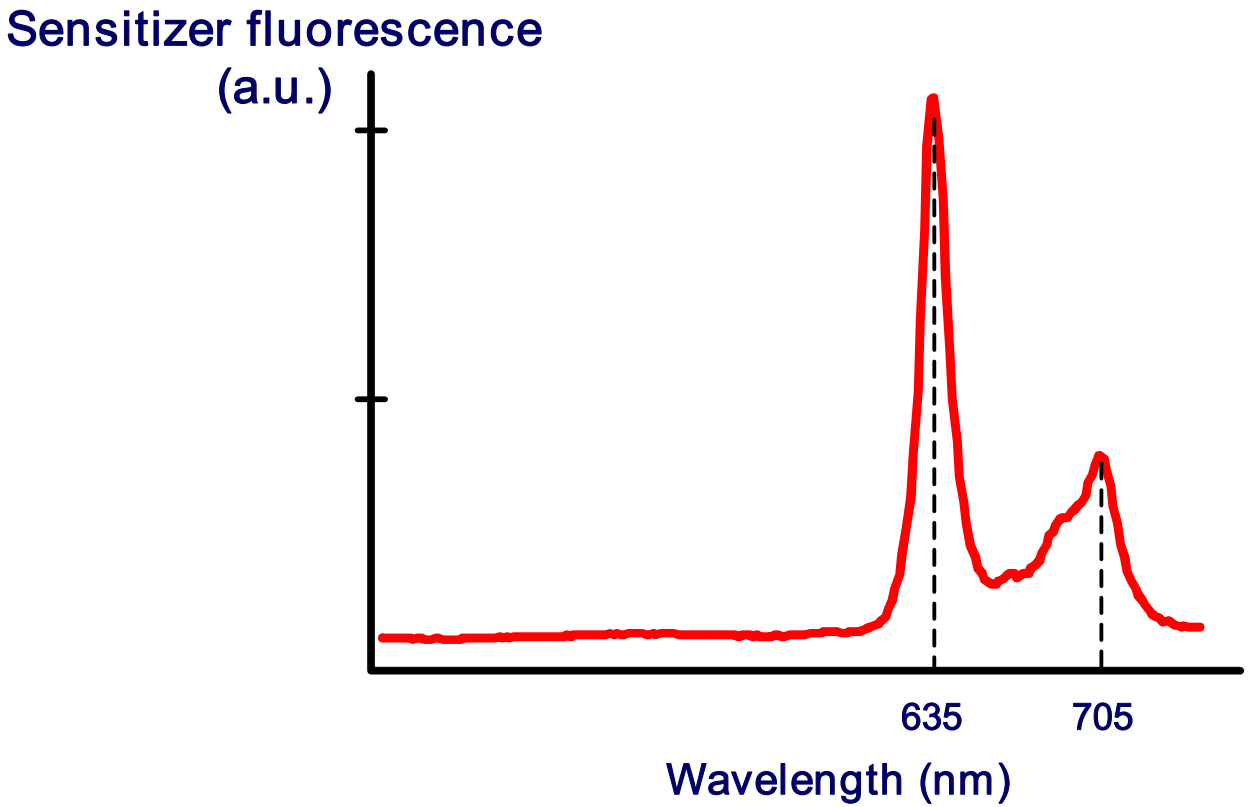
# Measurements of light fluence



Interactive dosimetry – eliminates treatment failure

Soto Thompson *et al.* JEPTO (200
Johansson *et al.* JBO (2007)

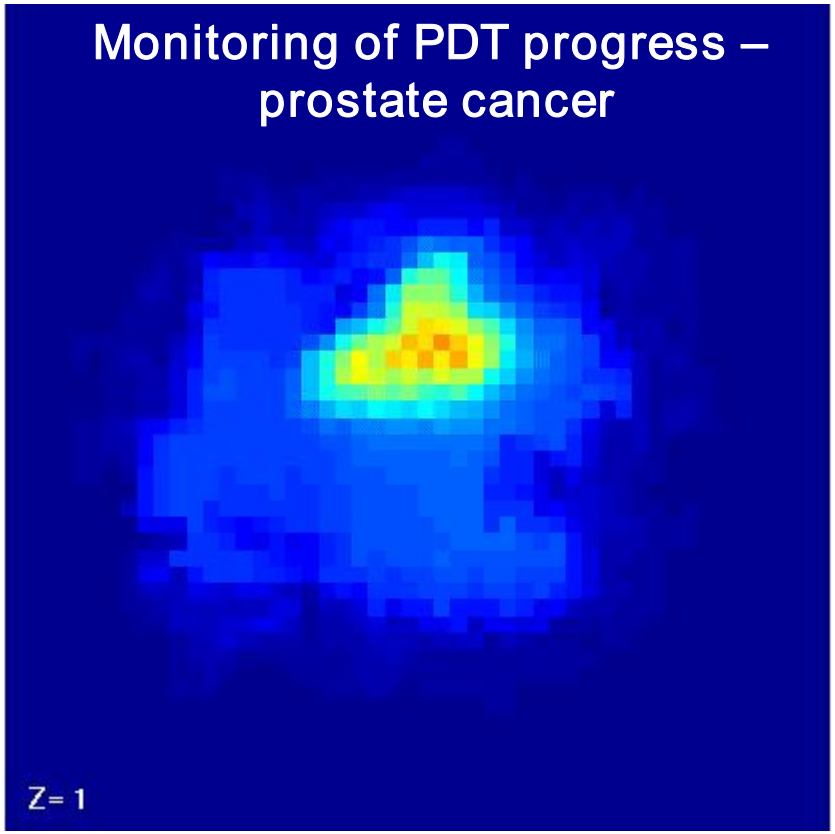
# Measurements of Sensitizer concentration




Assess sensitizer level using fluorescence

# Fluorescence tomography of mTHPC concentration during prostate cancer PDT

## Assessment of distribution of the photosensitiser mTHPC during photodynamic therapy of prostate cancer




Svenmarker et al. (m*anuscript*)
Axelsson et al. *Opt Lett.* (2009)

Axelsson et al. *Opt. Express* (2007)
Xu et al. *Appl. Phys. Lett.* (2008)

# The Group

http://www.atomic.physics.lu.se/biophotonics



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