

# Biomedical Applications of Fluorescence Spectroscopy

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# The Agenda

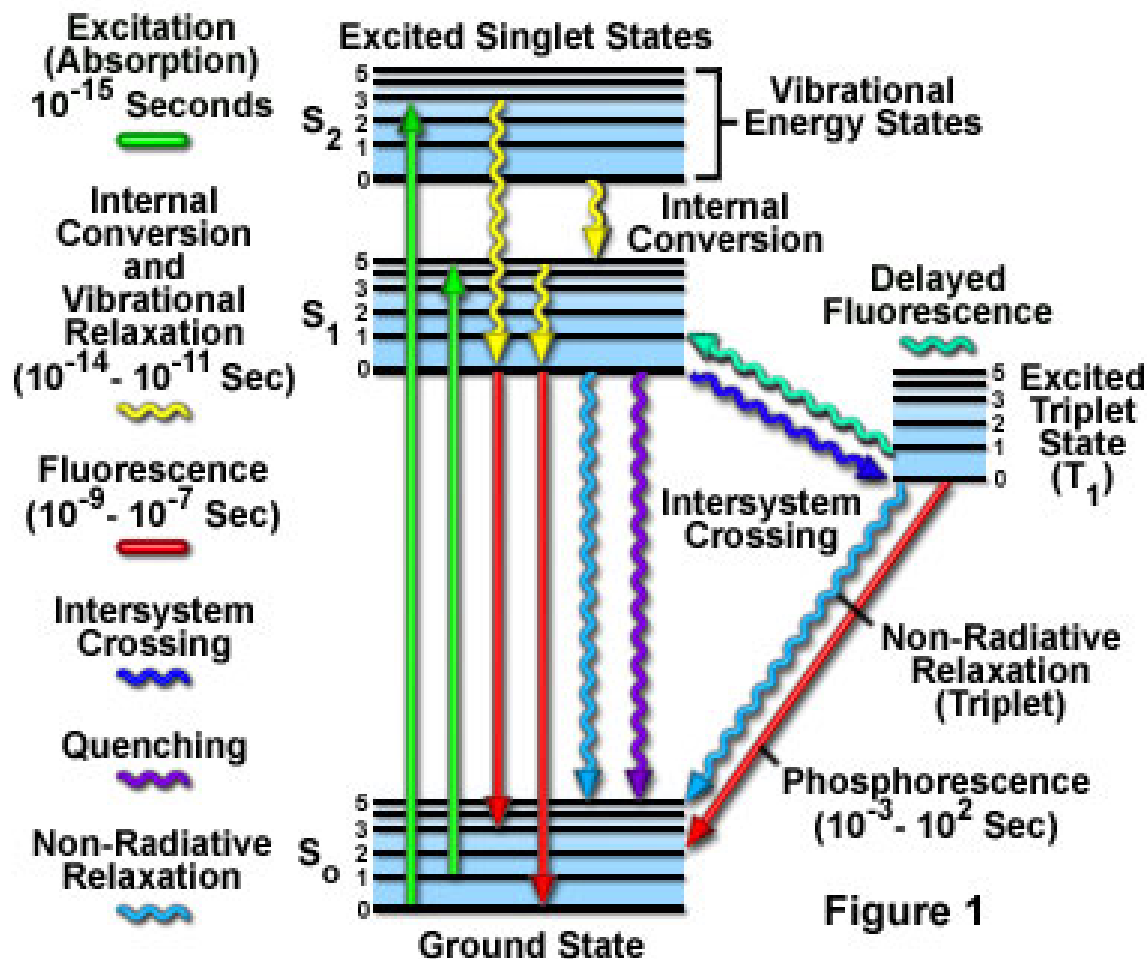
- Fluorescence Imaging
  - Physics and Terminology of Fluorescence
  - Life Induced Fluorescence Imaging
    - Fluorescence Endoscopy
  - Lifetime Imaging
    - Portable FLIM system & Glioma Imaging
- Photodynamic Therapy (PDT)
  - Theory of PDT
  - Examples of treatment

# What and Why of Fluorescence

- Fluorescence Spectroscopy: Study of interactions of radiation with matter; In specific, fluorescence radiation that is emitted from a sample
- Commonly used as a marker or for cell staining; Differentiating structures
- In biomedical applications we can use these properties of fluorescence to increase the specificity and sensitivity in imaging diagnosis
  - i.e. Early Cancer Detection
- Systems can be made small and portable

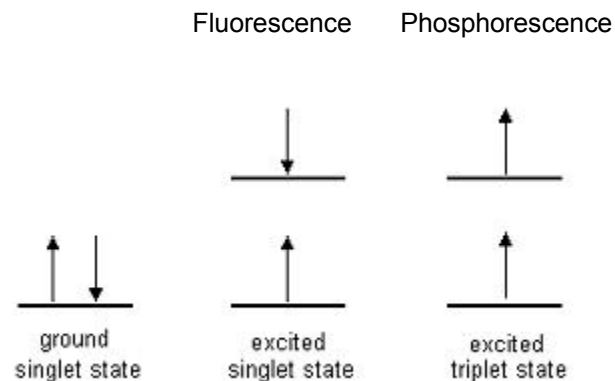
# Physics of Fluorescence

Jablonski Energy Diagram



Photoluminescence typically occurs in aromatic compounds.

Fluorescence almost always occurs from the  $S(1)$  state.  $S(n)$  to  $S(1)$  is very rapid.



# Terminology

**Fluorophores** are the components in molecules that cause them to fluoresce.

Endogenous : Naturally found in an environment

Exogenous: Inserted as a dye

**Quantum Efficiency (Intensity):** Number of photons absorbed compared to number emitted.

Calculated by time constants of the depopulation of a state.

$\Gamma$  = emissive rate of fluorophore

$k_{nr}$  = rate of non-radiative decay

$$Q = \frac{\Gamma}{\Gamma + k_{nr}}$$

Always less than 1 (Stokes shift)

**Fluorescence Lifetime:** The time it takes for an electron to go from  $S(n)$  to  $S(n-1)$

i.e. For an impulse excitation how long the molecule fluoresces

$$I(t) = I_0 \exp(-t/\tau) \quad I_0 \text{ initial intensity. } \tau \text{ is fluorescence lifetime.}$$

This is a fingerprint for a molecule

**Light Induced Fluorescence Imaging** is the observation of an objects emission spectra in response to an excitation at a specific wavelength(s)

# Photobleaching

## Differential Photobleaching in Multiply-Stained Cell Cultures

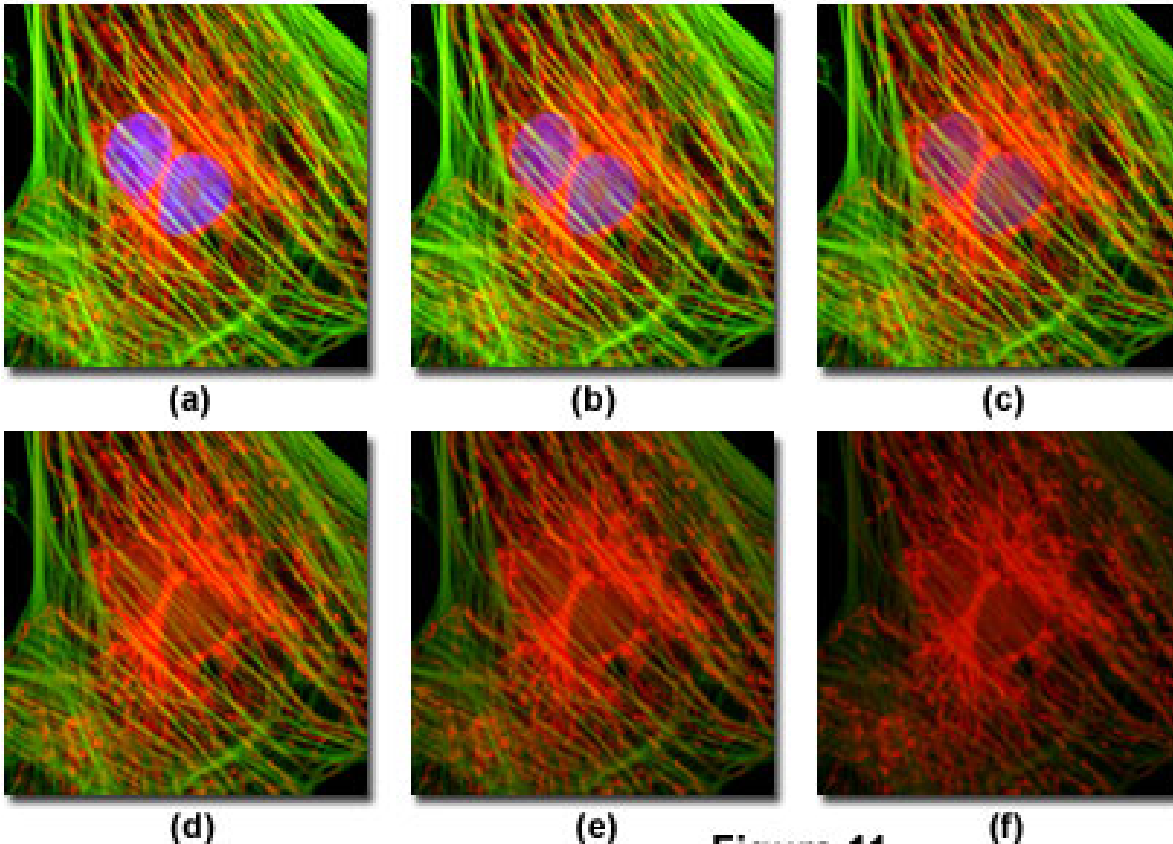


Figure 11

The permanent loss of fluorescence of a fluorophore

Causes:

1. High intensity
2. Excessive excitation

Deerskin fibroblasts cells

**Green – Actin skeletons**  
(Alexa Fluor 488)

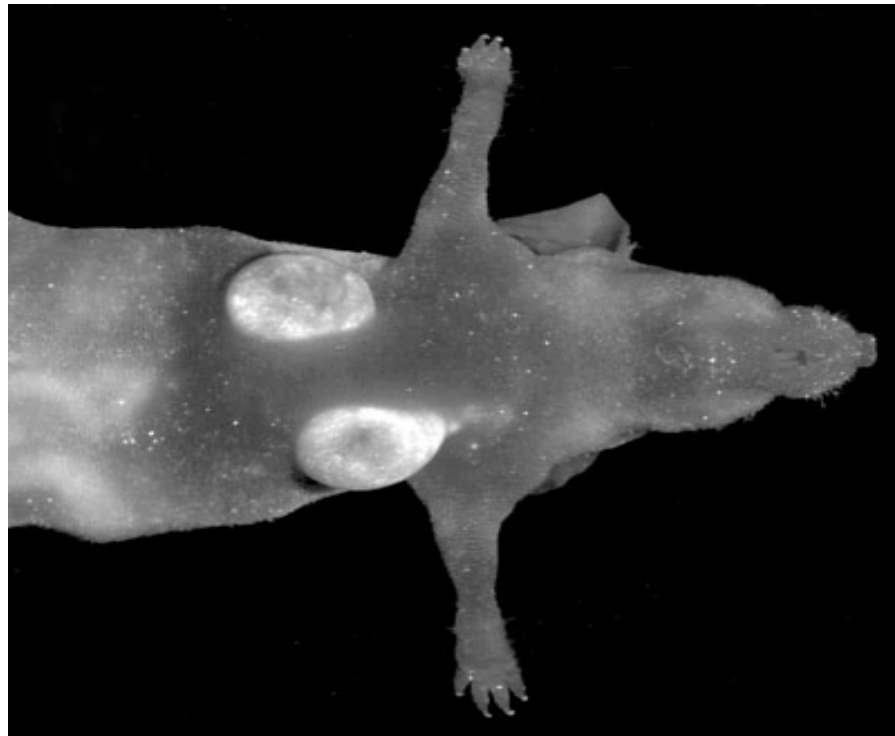
**Red – mitochondria**  
(MitoTracker Red)

**Blue – Nuclei**  
(DAPI)

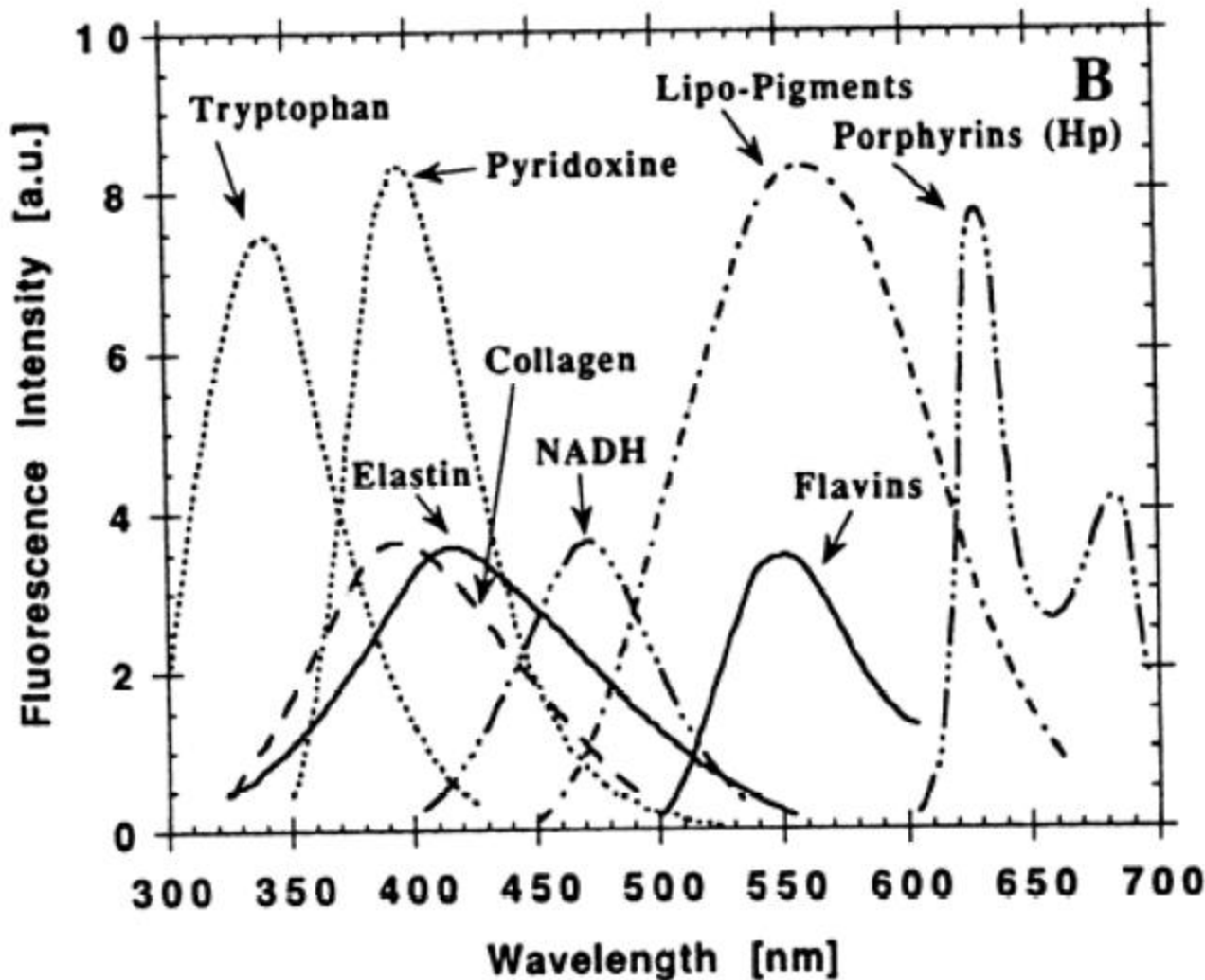
<http://www.olympusconfocal.com/theory/fluorophoresintro.html>

# Light Induced Fluorescence Imaging (LIFE)

Excitation  Emission



# Endogenous Fluorophores



NADH is an enzyme cofactor that plays a major role in metabolism. It is commonly found bounded to proteins throughout body

Collagen and Elastin also commonly found throughout the body

Fluorescent mechanisms of these tissues not entirely known



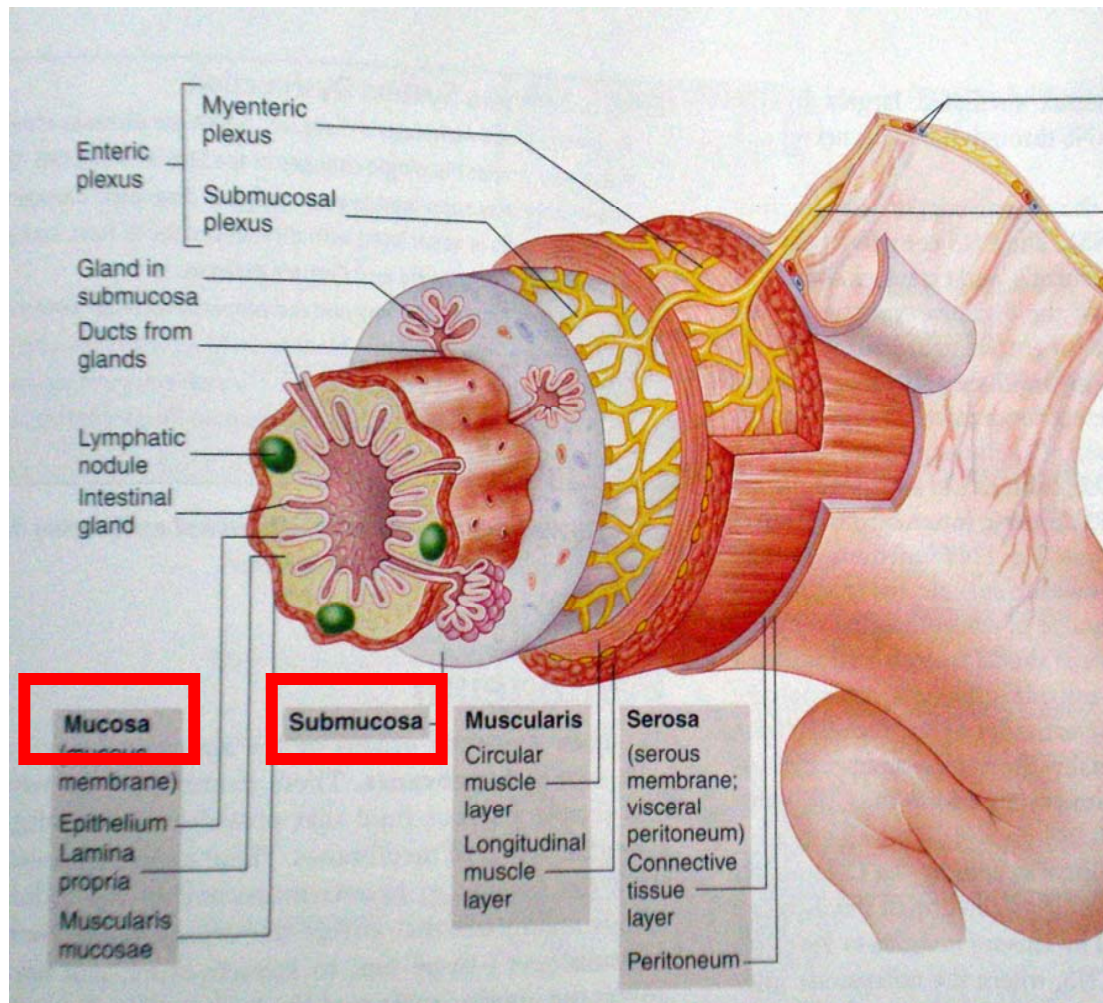
# Fluorescence Endoscopy

## Early Cancer Detection

- 95% of all colorectal cancers believed to arise from Adenomas. Cancerous lesions difficult to detect at early stages of development. If found early more treatment options available.
- Ulcerative colitis is a rare disease in which surface adenomas also appear but are usually hard to differentiate in early stages.
  - Can lead to colorectal cancer
  - Commonly confused with Crohn's disease

In Both cases early and proper diagnosis is aided by knowing where to take a tissue biopsy. Fluorescence imaging can increase the chances of earlier detection

# Fluorescence Endoscopy Anatomy



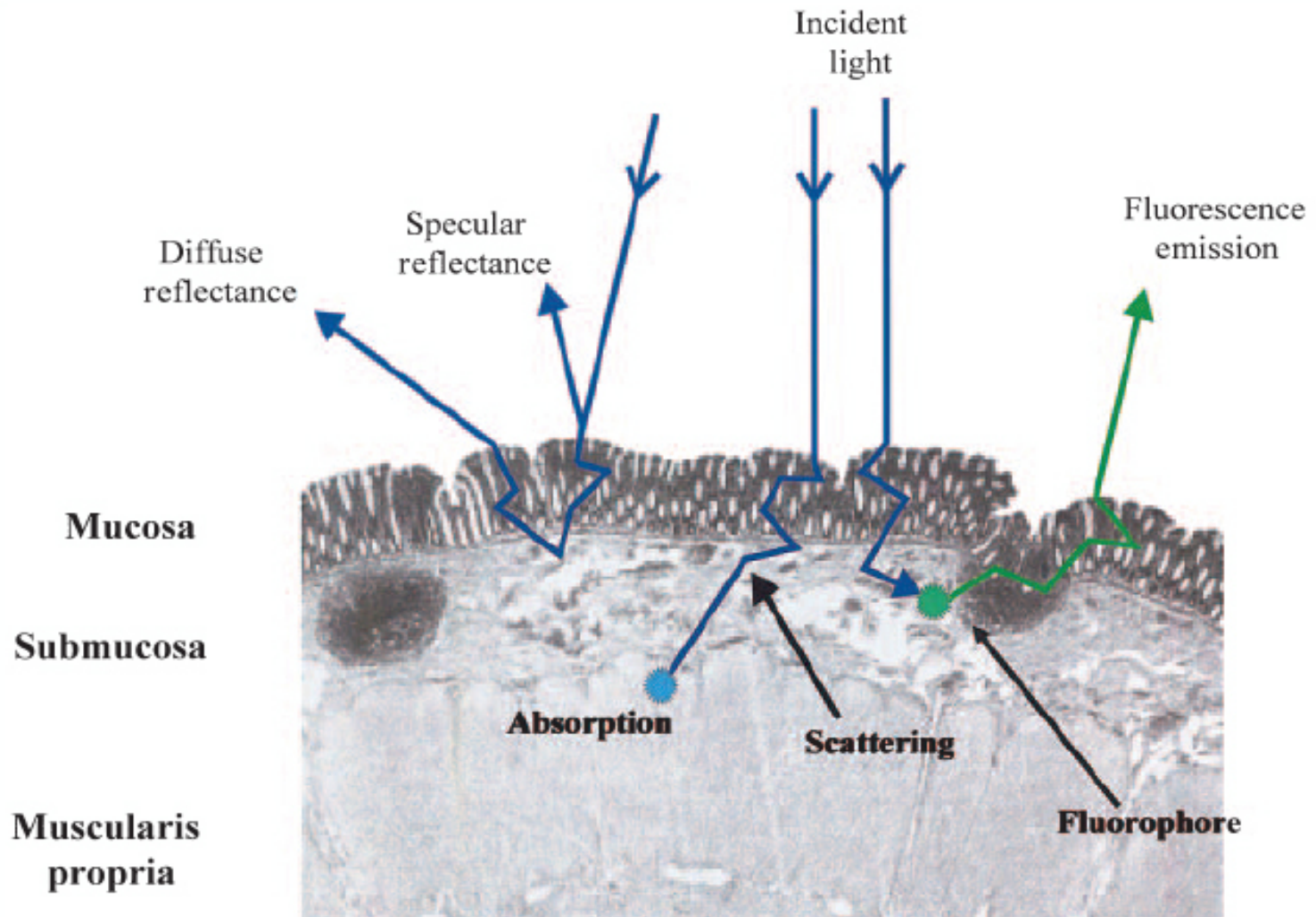
## Submucosa

- Main source of fluorescence is the large abundance of collagen.
- Believed that it has undergone glycosylation to increase intensity
- Other stronger fluorophores have been shown to be in almost negligible concentrations relative to collagen. (Flavins, NADH, pyridoxal 5'phosphate)
- Hemoglobin accumulation a top connective tissues reduces fluorescence

## Mucosa

- Mucous membrane comprised of squamous and columnar epithelium cells
- Membrane acts as a screen decreasing fluorescence excitation and emission

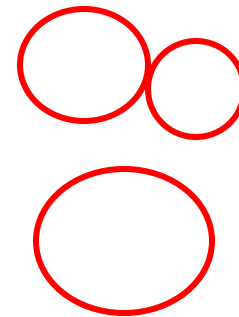
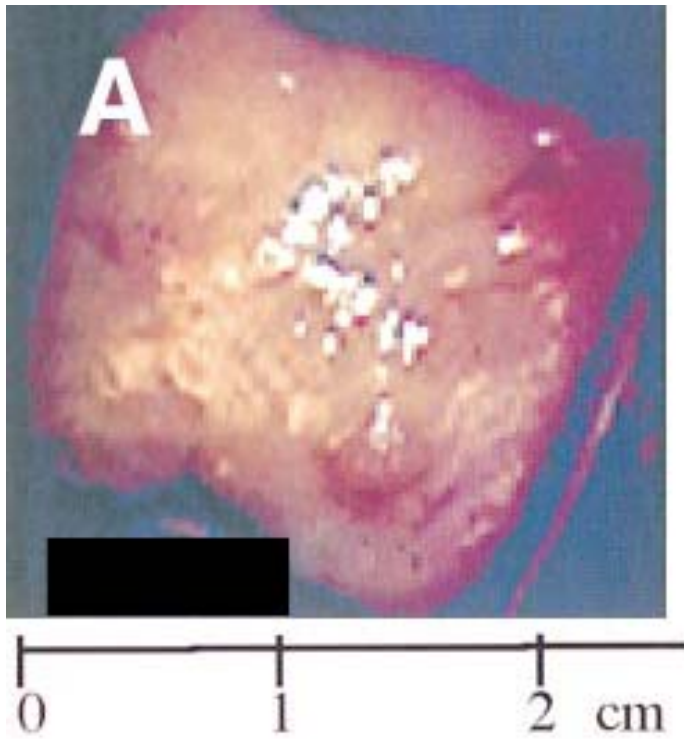
# Fluorescence Endoscopy Principles



# Fluorescence Endoscopy

Adenomas have a reduced fluorescent intensity (Aprox. factor of 3).

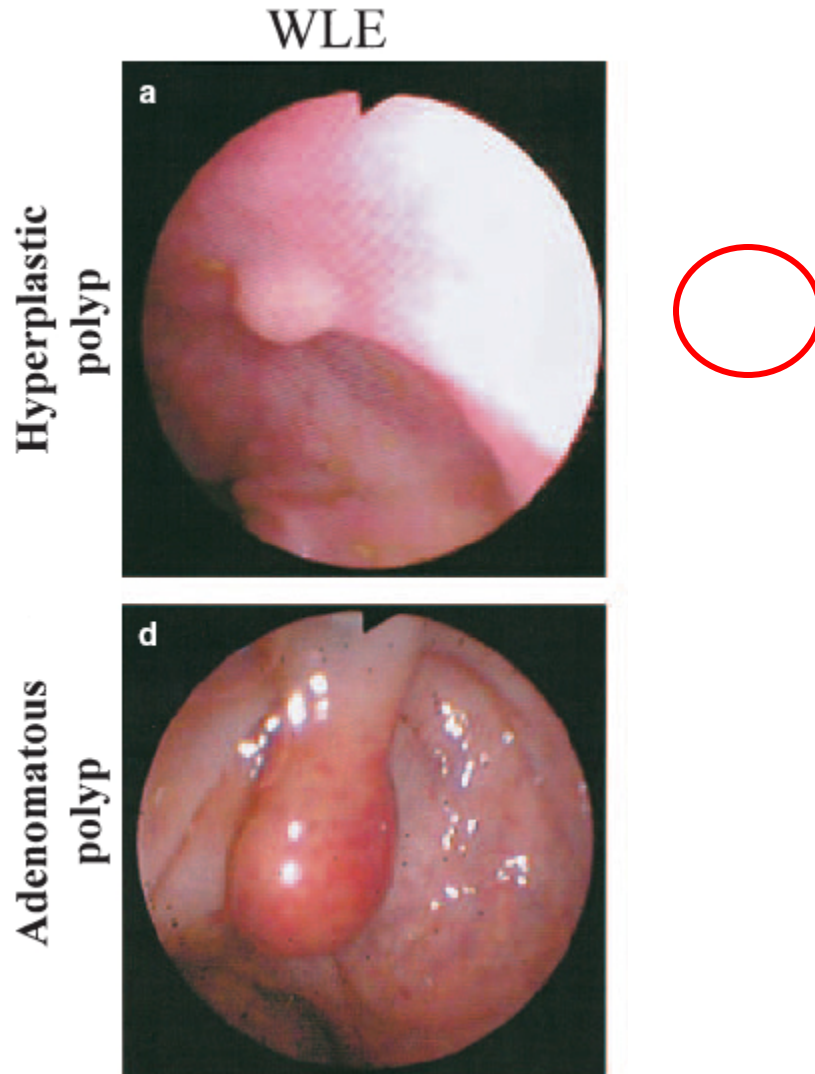
- 1) Decrease in quantum yield
- 2) Tissue architecture is different
- 3) Increase of blood volume (micro-vascular density)



# Fluorescence Endoscopy

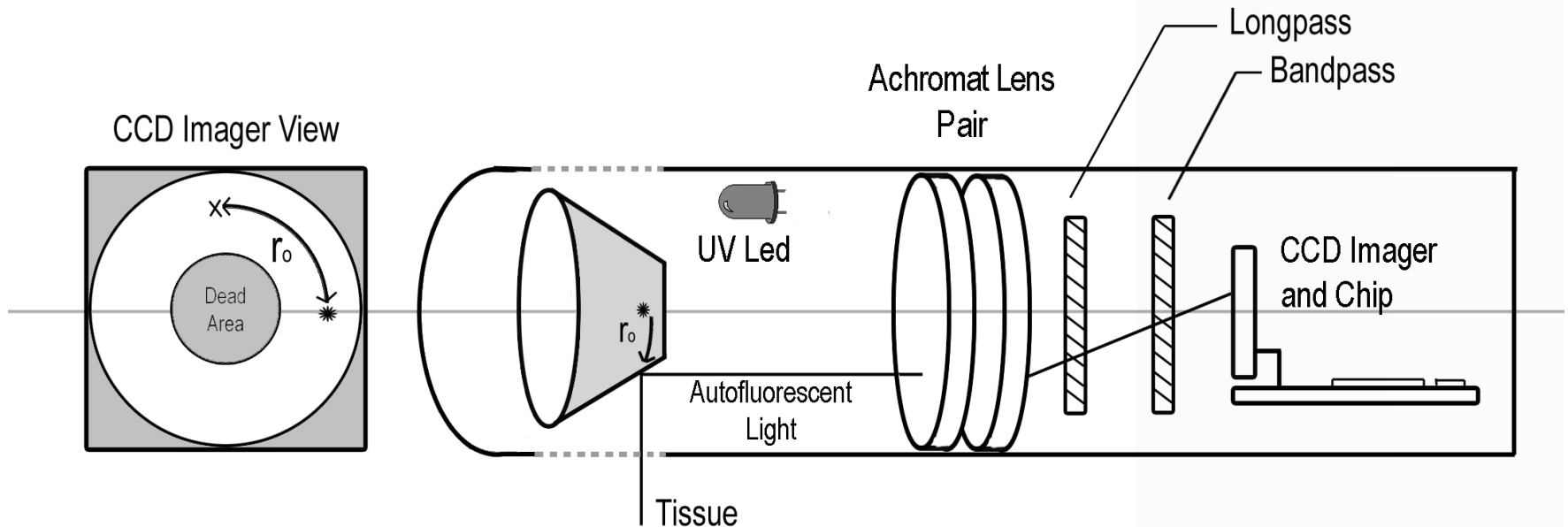
False identifications of polypoid adenomas can lead to unnecessary labour intensive surgeries to remove them.

WLE – White light endoscopy  
LIFE – light induced fluorescence endoscopy



# Fluorescence Endoscopy Capsule

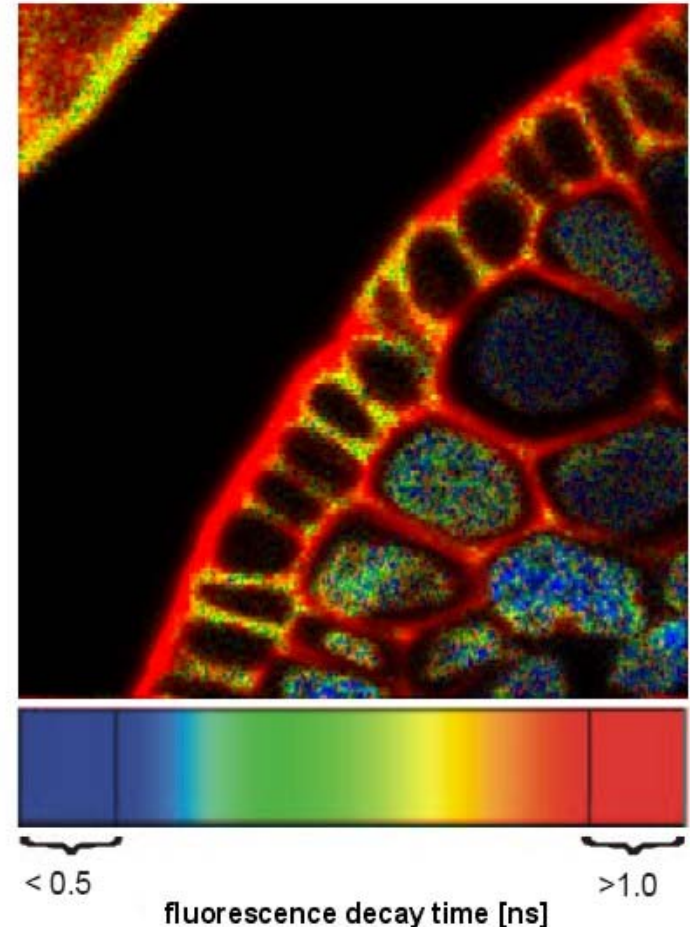
Excitation at  $\sim 365\text{nm}$  using 4mW UV Led's



# Fluorescence Lifetime Imaging (FLIM)

- Spectral imaging not always precise. Need another more specific tool for differentiation of cellular and Tissue structures
- Every Fluorophore has a distinct fluorescence lifetime (almost a fingerprint) that can be used to enhance specificity of images

Apple seed excited at 635nm at 10Mhz  
4 minutes to create image

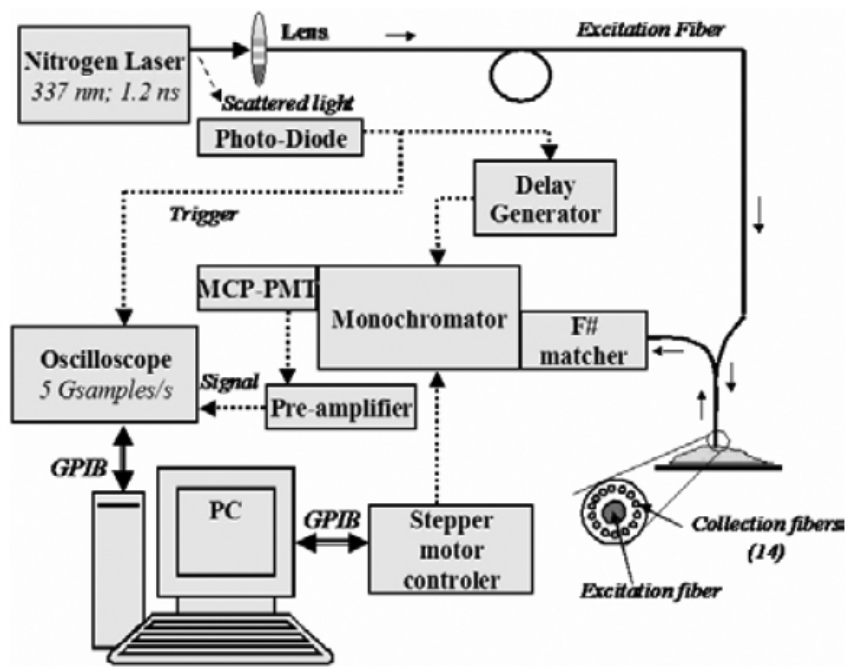


# FLIM

## A Portable Clinical System

The system also uses a digital pulse generator (DG-535) as the master clock for syncing of the components.

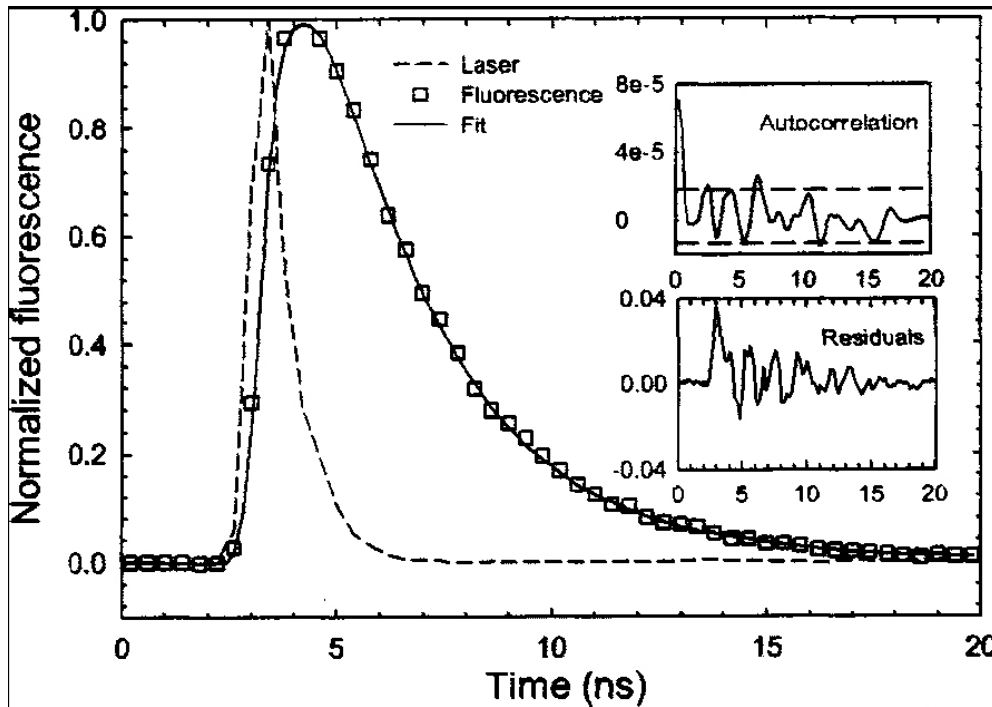
- First pulse train triggers laser pulse (which triggers Oscilloscope capture)
- Second triggers ICCD and PMT (Detectors)





# FLIM

## A Portable Clinical System

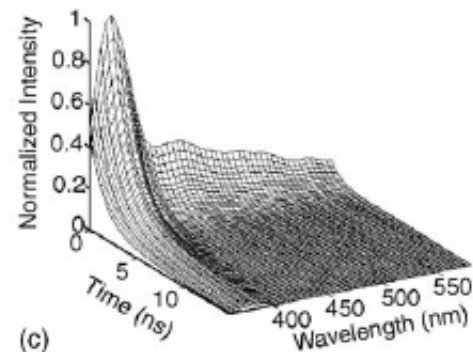
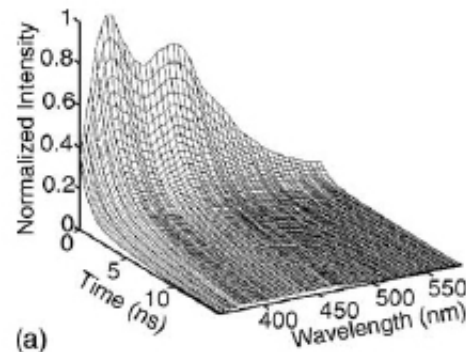


Rhodamin B Fluorescent Dye

Time-domain laser-induced fluorescence spectroscopy apparatus for clinical diagnostics – Q. Fang; Review of Scientific Instruments Vol 75 Issue 1: 151-162

a) Normal-atherosclerotic c) Thick lesion macrophage

In vivo detection of macrophages in a rabbit atherosclerotic model by time-resolved laser-induced fluorescence spectroscopy – Q. Fang; Atherosclerosis 181 (2005) 295–303



# FLIM Application

## Glioma detection in Brain Tissue

CNS tumors originating from glial cells. 60% of all brain tumors (US)

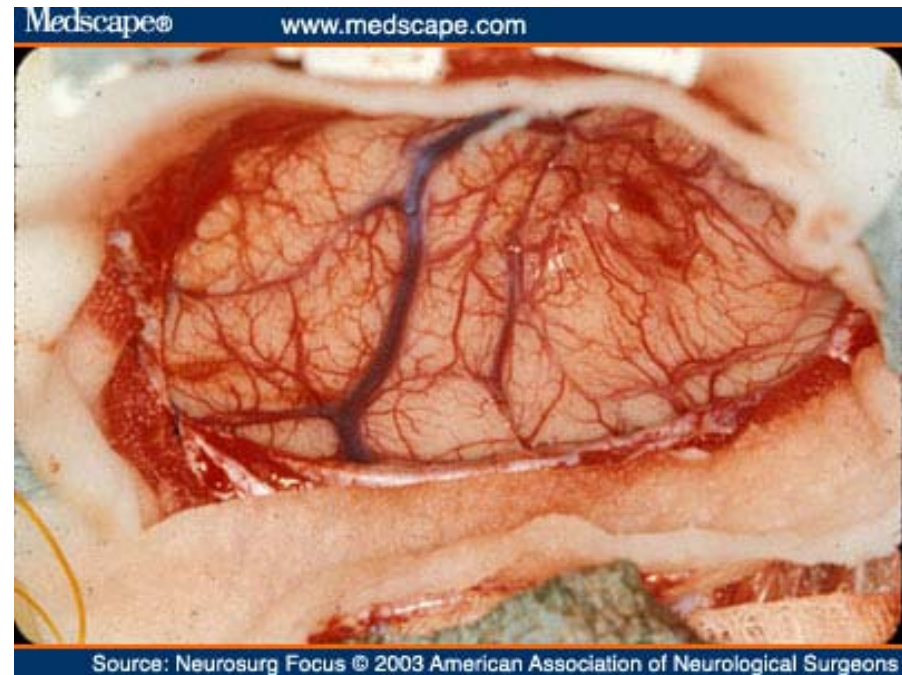
- Pilocytic astrocytoma (benign)
- Low-grade astrocytoma (benign)
- Anaplastic or Malignant astrocytoma
- Glioblastoma multiforme (very malignant)

- In Adults 70% of cases located superior to tentorium cerebelli. In Children 70% located in the brainstem.

- Glioblastoma are found in white and grey matter. Cause a breakdown in the blood brain barrier.

- FLIM aids as a clinical analysis tool to determine which tissues to freeze and analyze (biopsy) in a short amount of time.

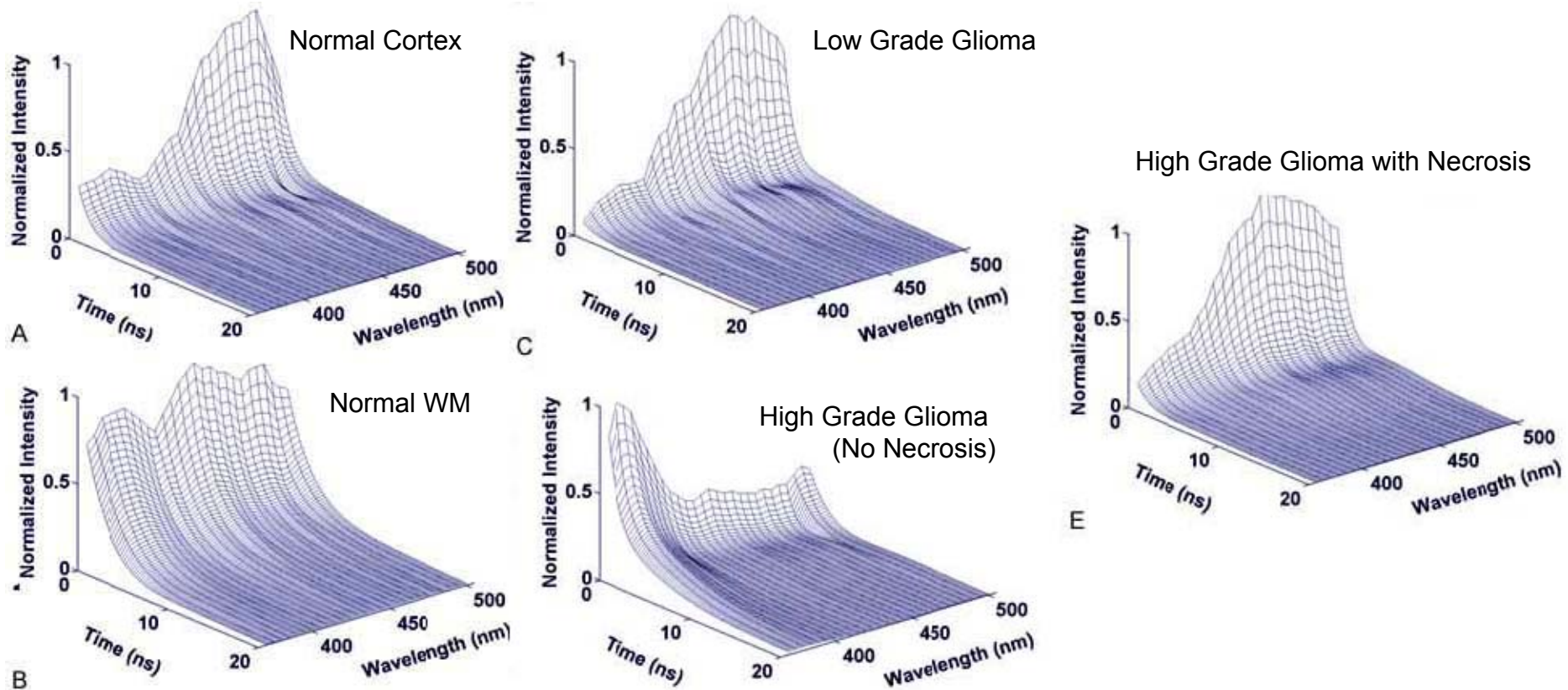
High Grade Glioma on Cortical surface



Source: Neurosurg Focus © 2003 American Association of Neurological Surgeons

# FLIM Application

## Time-Resolved Fluorescence Emission



# Future Directions in Imaging

- Multi-Photon Excitation in NIR

- 3D and 4D Imaging
- Greater tissue penetration
- Better SNR and image quality
- Requires femtosecond lasers

- Quantum Dot Imaging

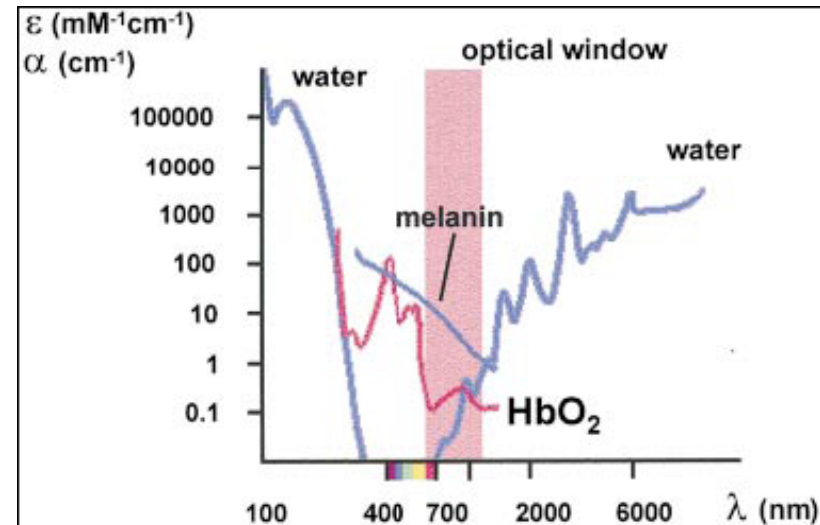
- Nano-sized exogenous fluorophores

- Multimodality Imaging

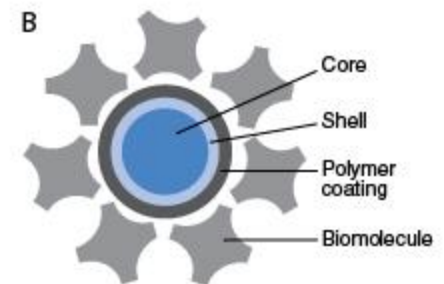
- Combination of different types of imaging technologies

- Multi-Spectral Imaging

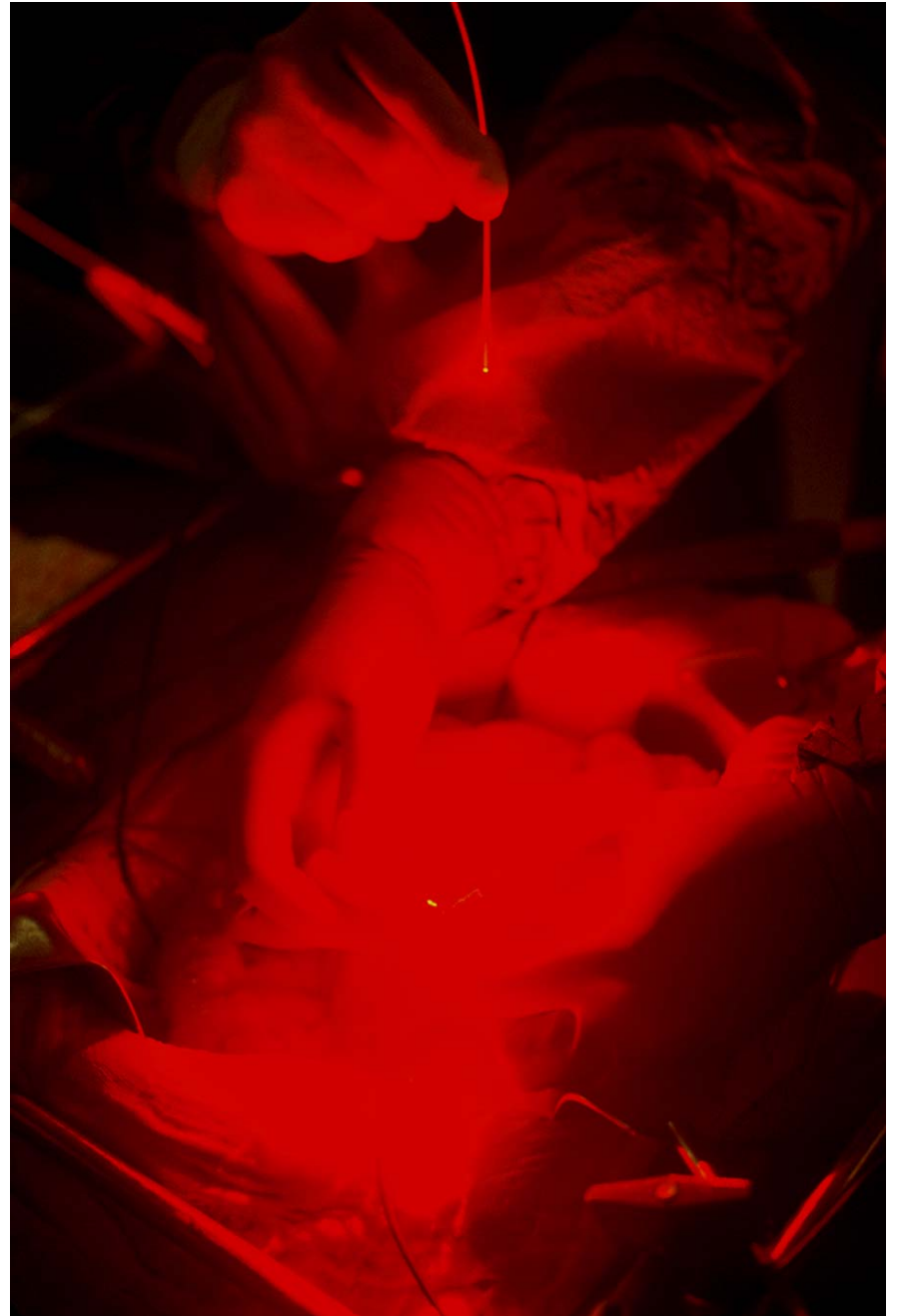
- Multiple excitation wavelengths



Multi-photon microscopy in life sciences – K. König; Journal of Microscopy, Vol. 200, Pt 2, November 2000, pp. 83±104.



# Photodynamic therapy



# Cancer treatments...

**Surgery:** can remove tumors, but may not be effective against cancer

tumor vs cancer?

**Chemotherapy:** affects all fast dividing cells - hair, intestinal lining, white blood cells. numerous side effects.

**Radiation therapy:** exposing nearby healthy cells to radiation is unavoidable.

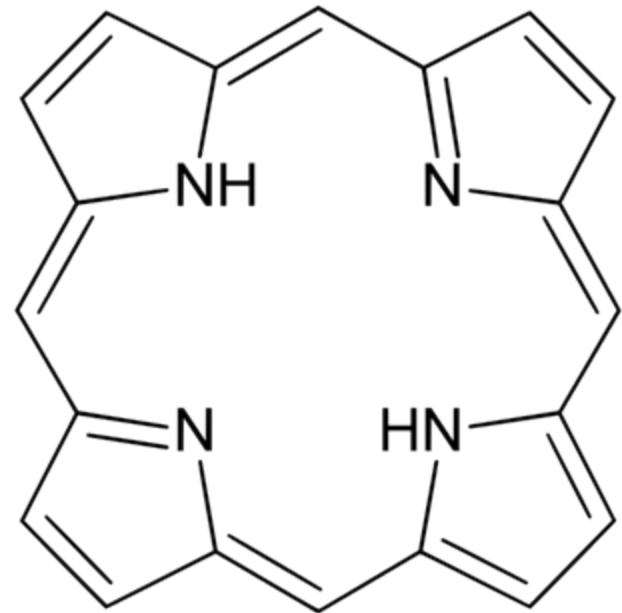
**Ideal cancer treatment:** kill all cancerous cells and leave healthy ones alone



# Theory

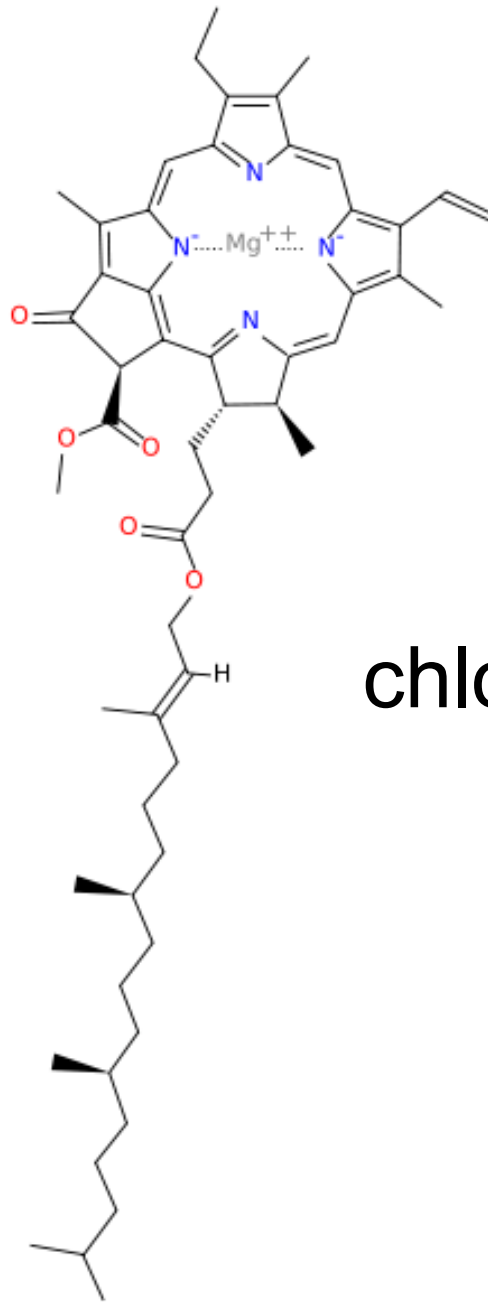
1. Photosensitizer is injected into patient
2. Photosensitizer leaves normal tissue but remains in cancerous tissue
3. Photosensitizer is exposed to light and releases singlet oxygen which kills nearby cells

Photosensitizer must be present in the cell and exposed to have any effect!



## Porphine

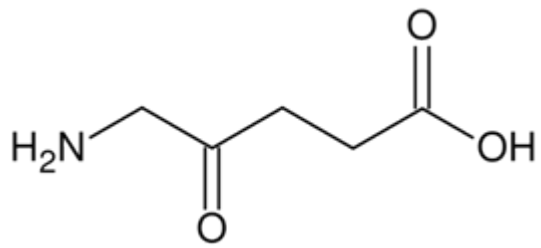
highly conjugated system



chlorophyll

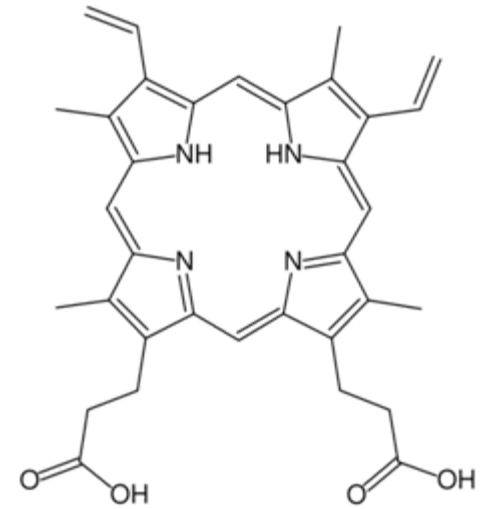




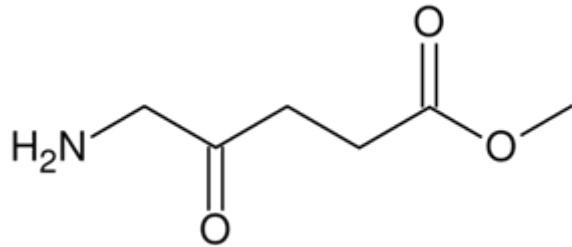


Aminolevulinic acid

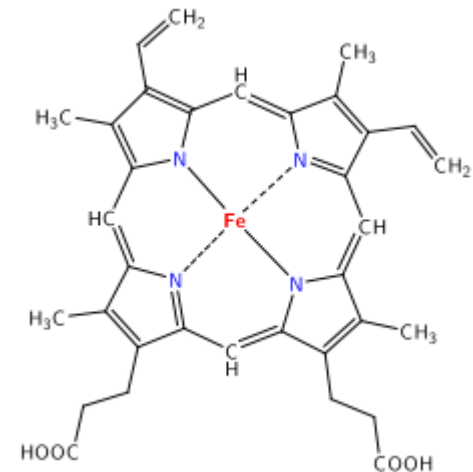
very complicated chemistry



protoporphyrin IX



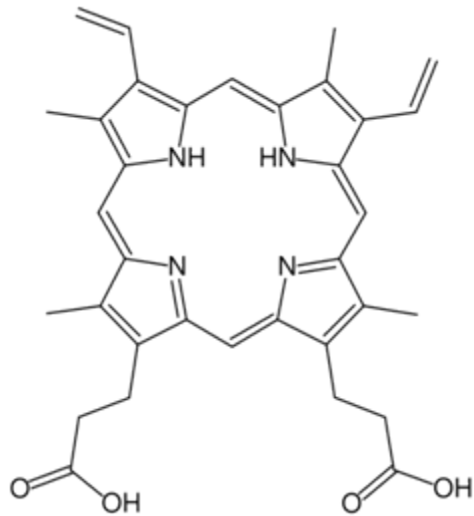
Methyl aminolevulinate



Heme – found in hemoglobin

a metabolic precursor to the photosensitizer can also be used. Aminolevulinic acid or Methyl aminolevulinate are precursors to protoporphyrin IX.

Why would the body do this?



light



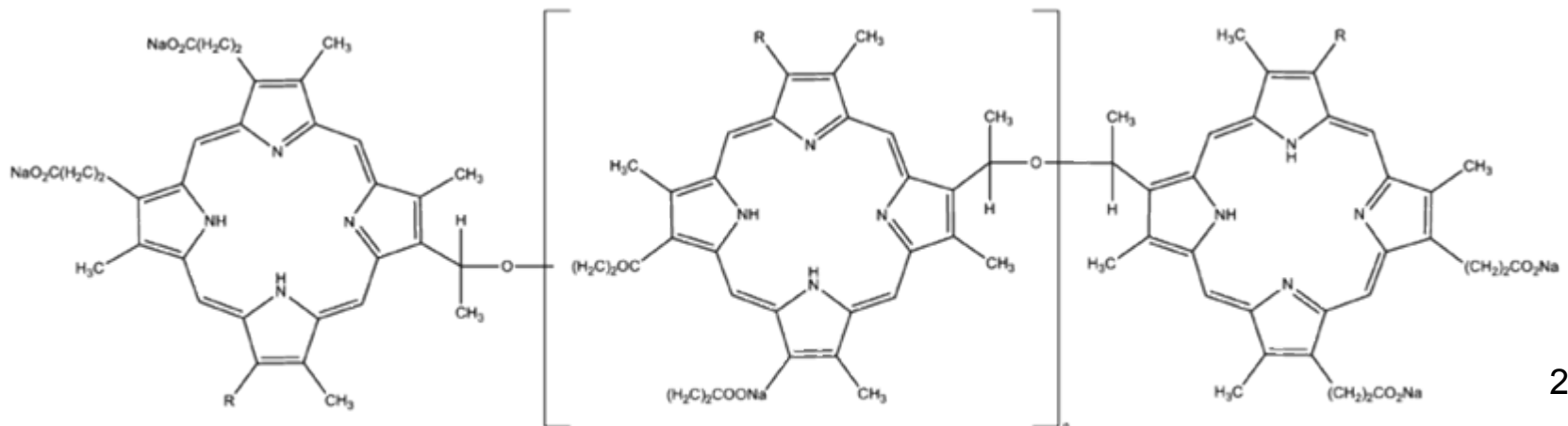
singlet oxygen

singlet oxygen can participate in radical reactions and damage cells.

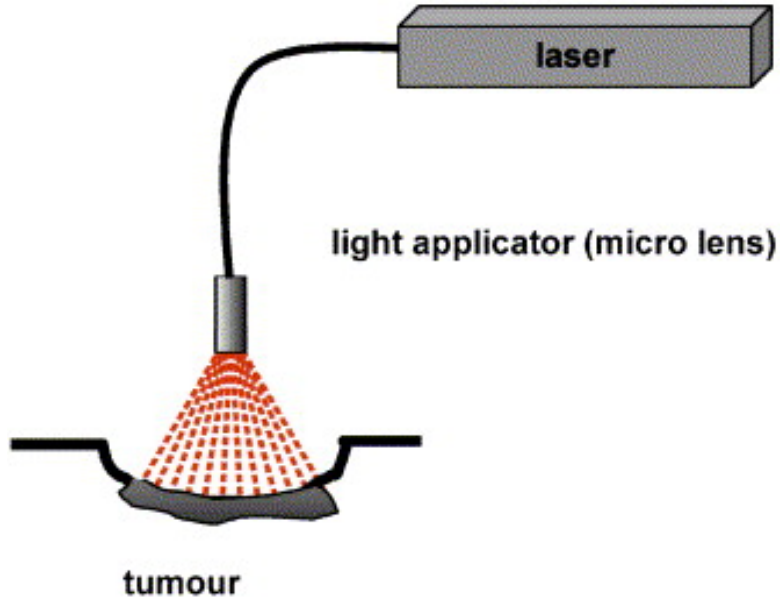
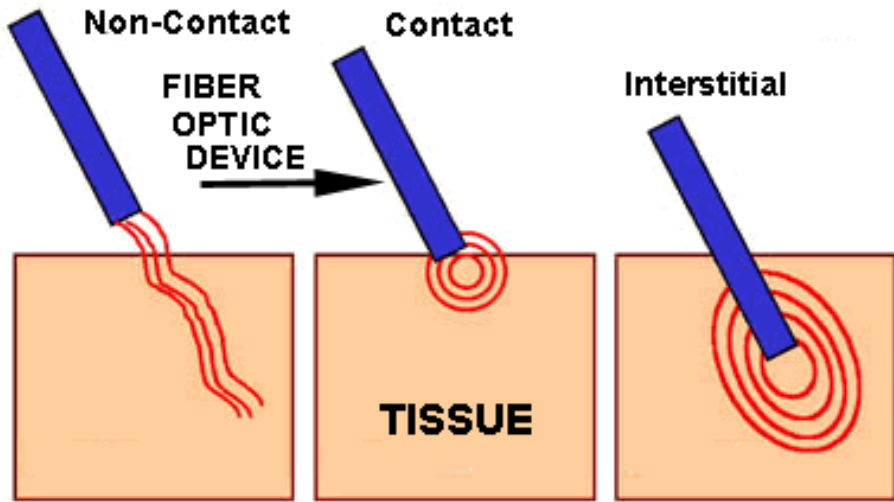
- Higher energy state of oxygen.
- Highly reactive.
- Formed through an energy transfer process when the photosensitizer is exposed to the correct wavelength of light.

# Example treatment

- Photofrin (porfimer sodium) given
- Wait 40-50 hours for drug to be eliminated from non-cancerous tissue (how?)
- Application of laser or led light at 630 nm for 5-40 minutes.
- Inflammation, swelling, pain, sensitivity to bright light for 30 days



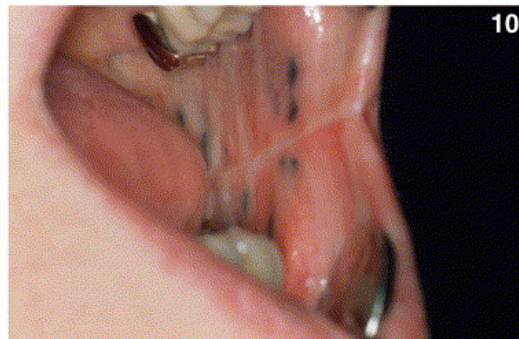
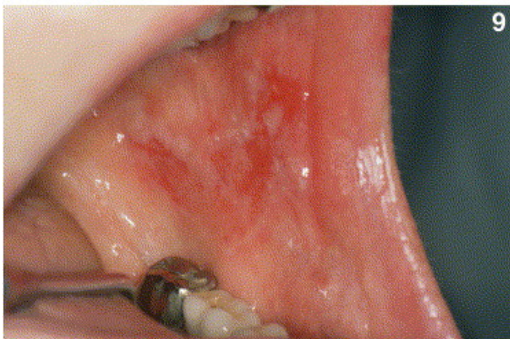
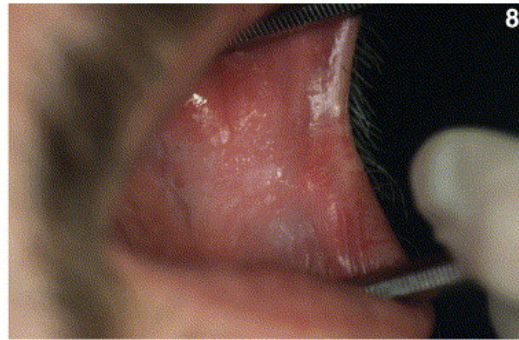
# TYPES OF LASER TREATMENTS



Light delivered via fiber optic, laser or led



PDT can be used to clean up remaining cancerous cells after surgically removing a tumor



**5.** Patient with actinic keratosis prior to PDT using topical application of 20% ALA.

**6.** Patient after ALA-mediated PDT.

**7.** Patient with oral leukoplakia prior to topical application of ALA.

**8.** Patient after ALA-mediated PDT.

**9.** Patient with histologically proven field cancerisation (early invasive cancer) at multiple location of the cheek. Prior to Foscan®-mediated PDT.

**10.** Patient 3 month after PDT with normal mouth opening (note: artificial black tattoo marks).

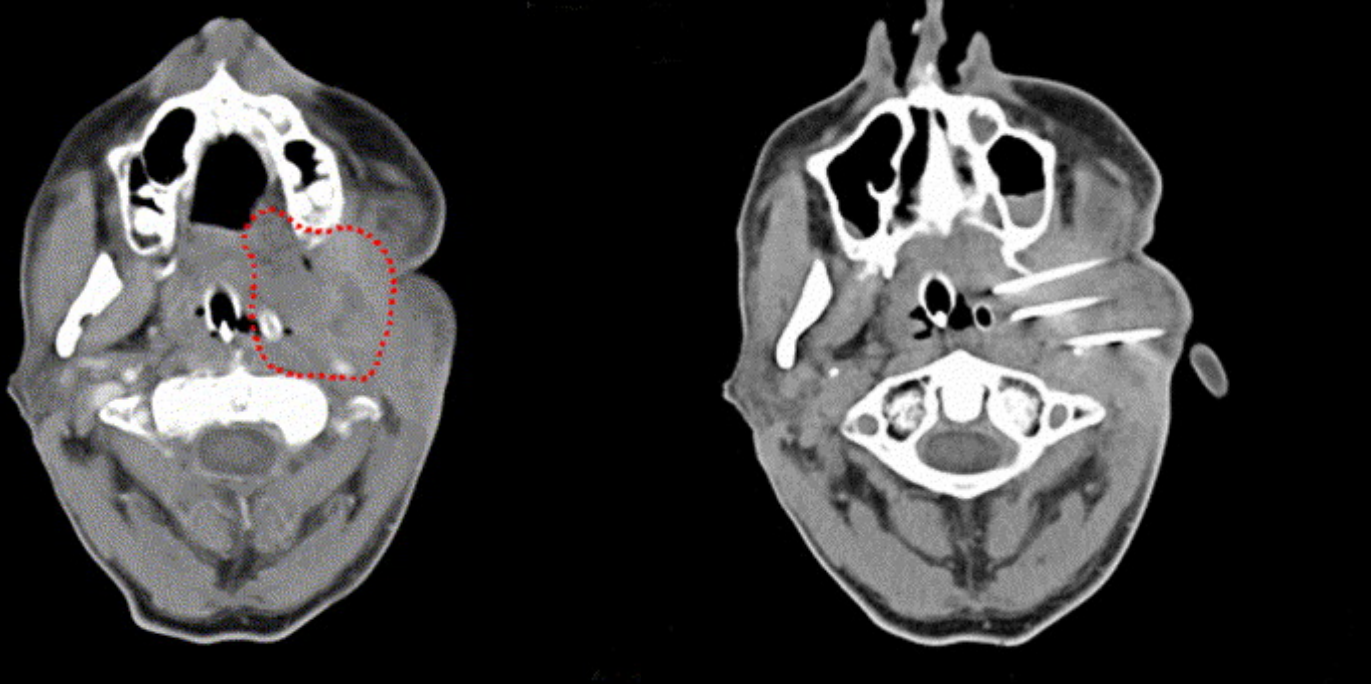


Fig. 11. CT scan of a patient with recurrent SCC after surgery and radiotherapy, prior to Foscan®-mediated PDT (note: tumour mass marked).

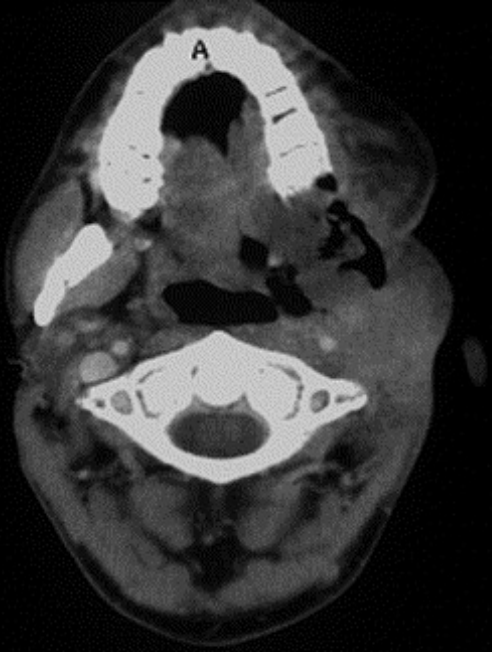


Fig. 12. CT scan with needles/laser fibres stabbed into the tumour during PDT.

Fig. 13. CT scan 2 months after PDT with significant tumour mass reduction.

# Applications / Success rate

Currently used to treat:

- skin cancer
- esophagus cancer

Clinical trials:

- brain
- prostate
- stomach
- liver
- peritoneal

Success rate

- Limited clinical data so far
- 50-80% for BCC
- as effective as traditional techniques such as chemotherapy, surgery, and radiation therapy
- unlike chemotherapy and radiation therapy, PDT can be repeated as many times as needed

drawbacks

- light attenuation by tissue - only penetrates 1 cm
- used to treat cancers close to surface of organ
- dependent on the presence of oxygen

# Questions

- i.e. Where am I and What just happened these past 45 minutes?



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