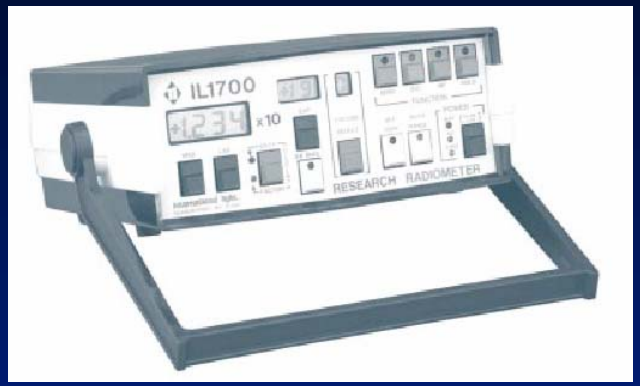
Instrumentational Setup



Some instruments we may encounter

Basics on PDT, PDD

Electromagnetic waves

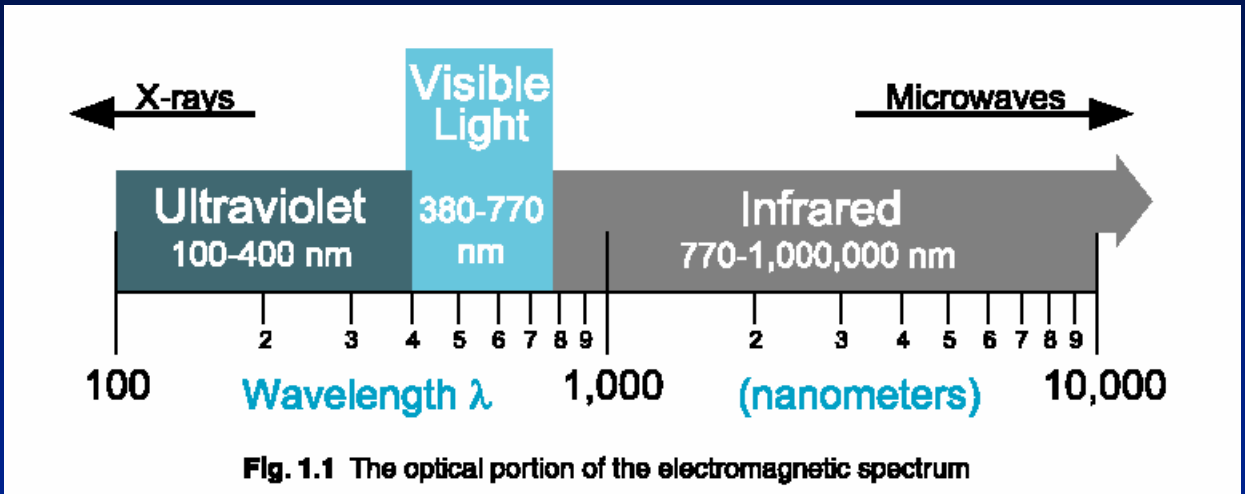
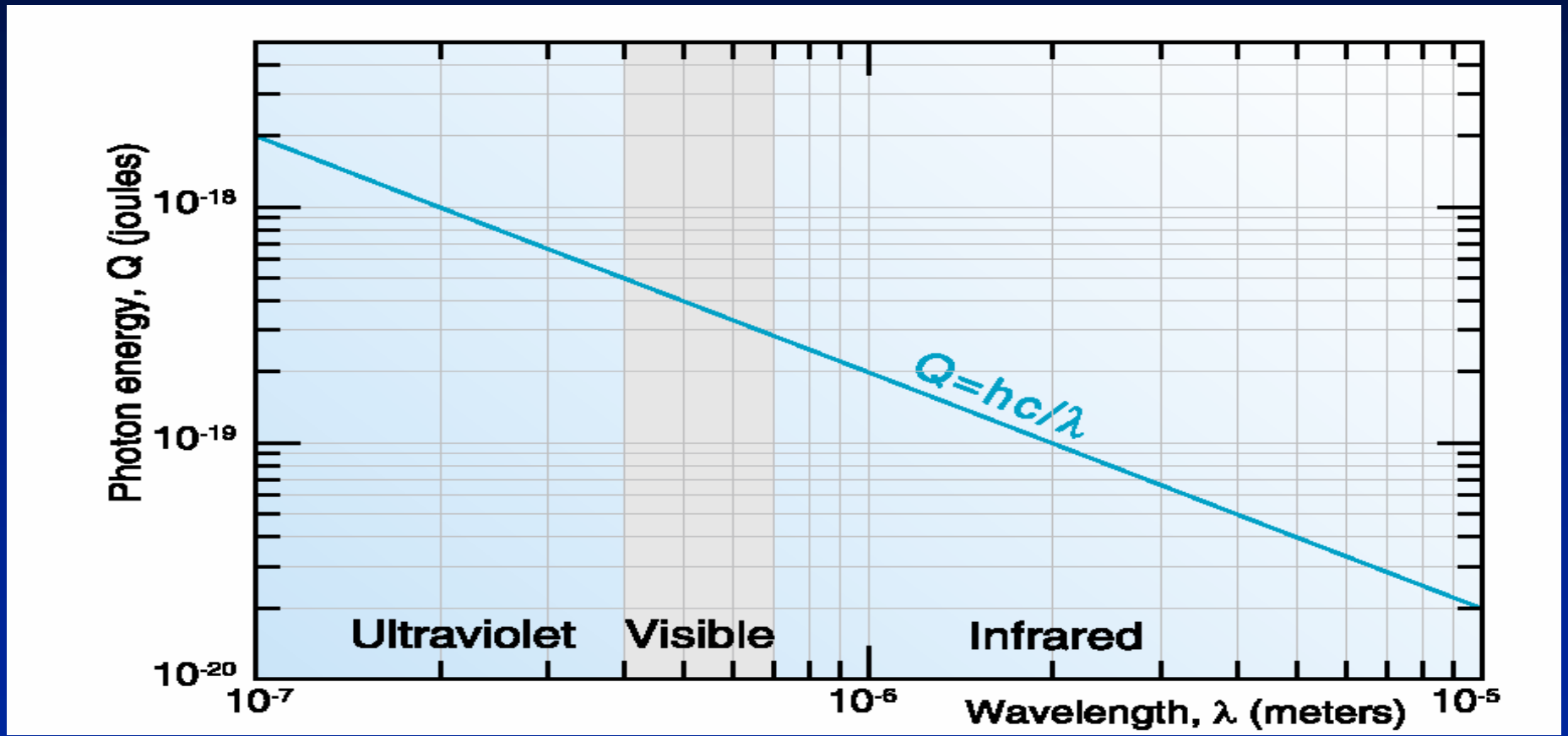
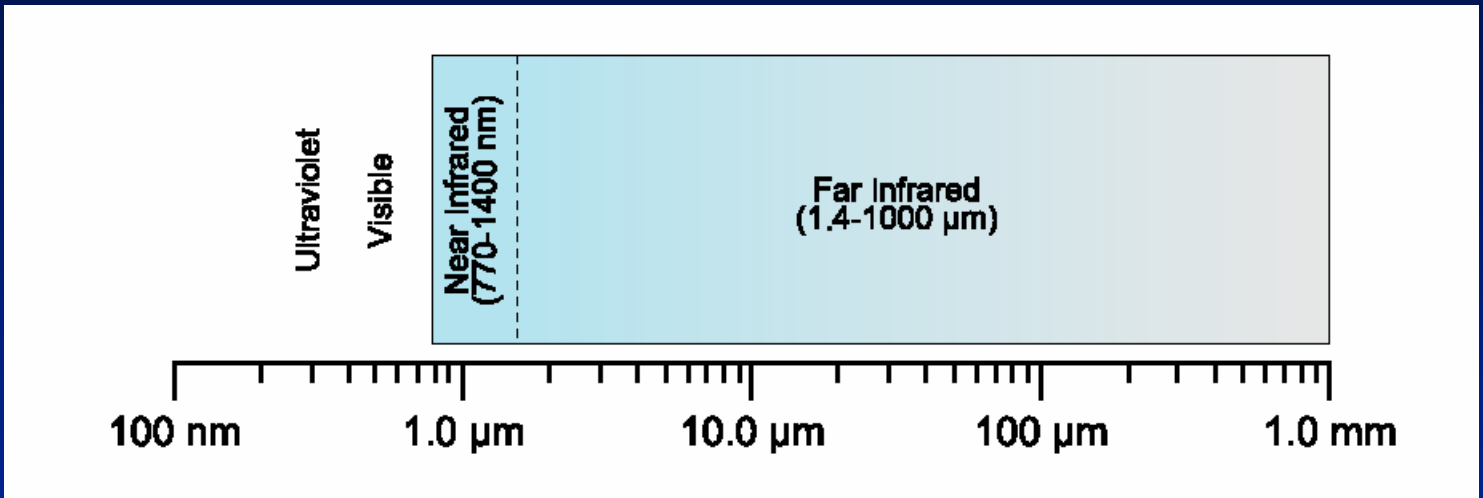


Fig. 1.1 The optical portion of the electromagnetic spectrum

Optical Power



Infrared Light



Detector Types

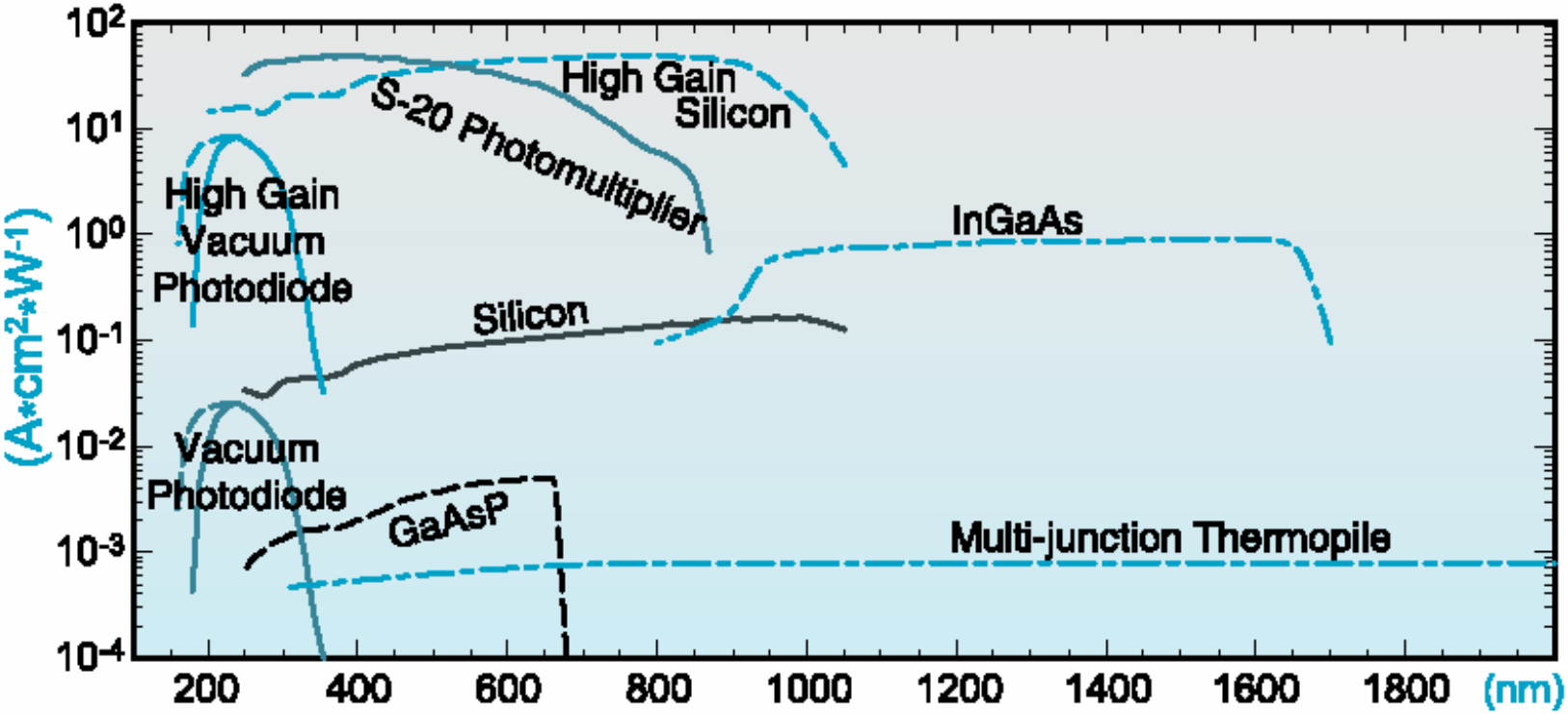
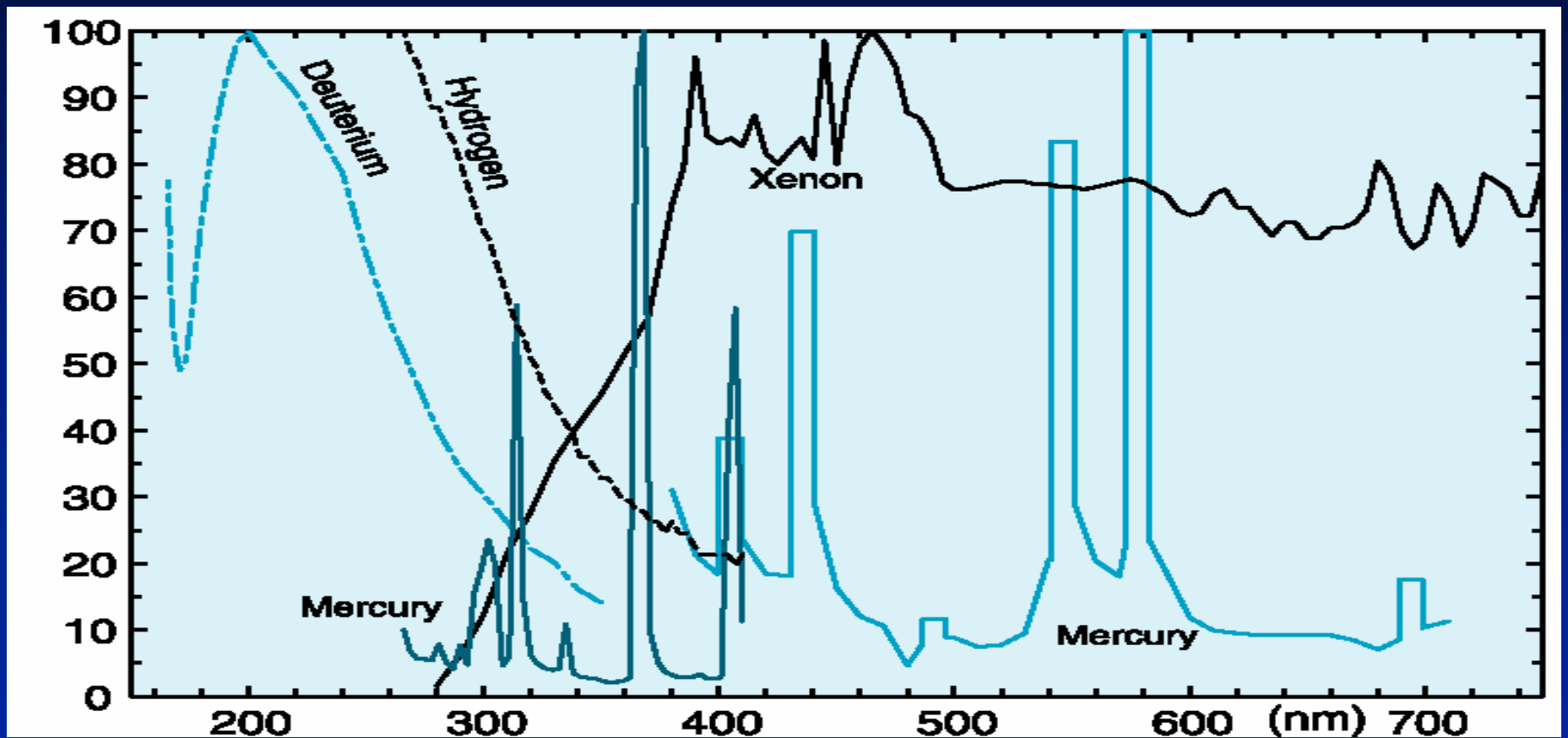
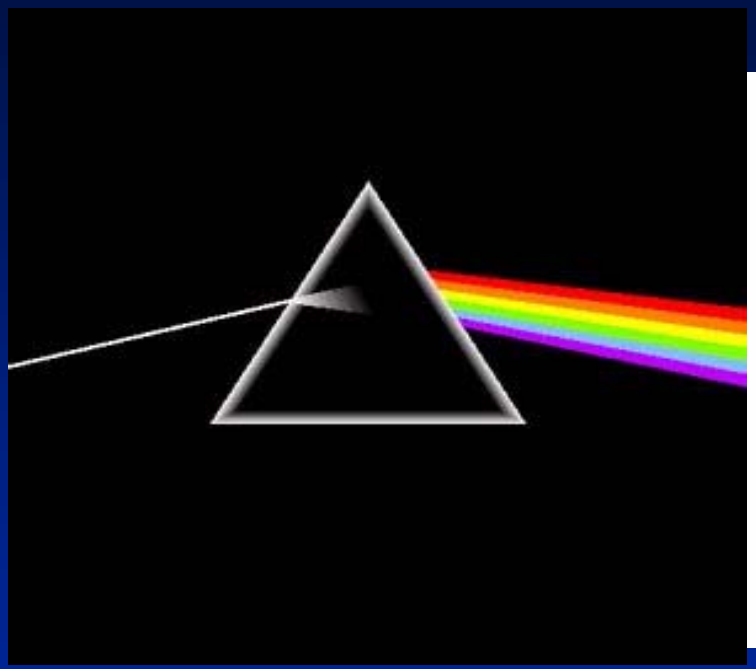


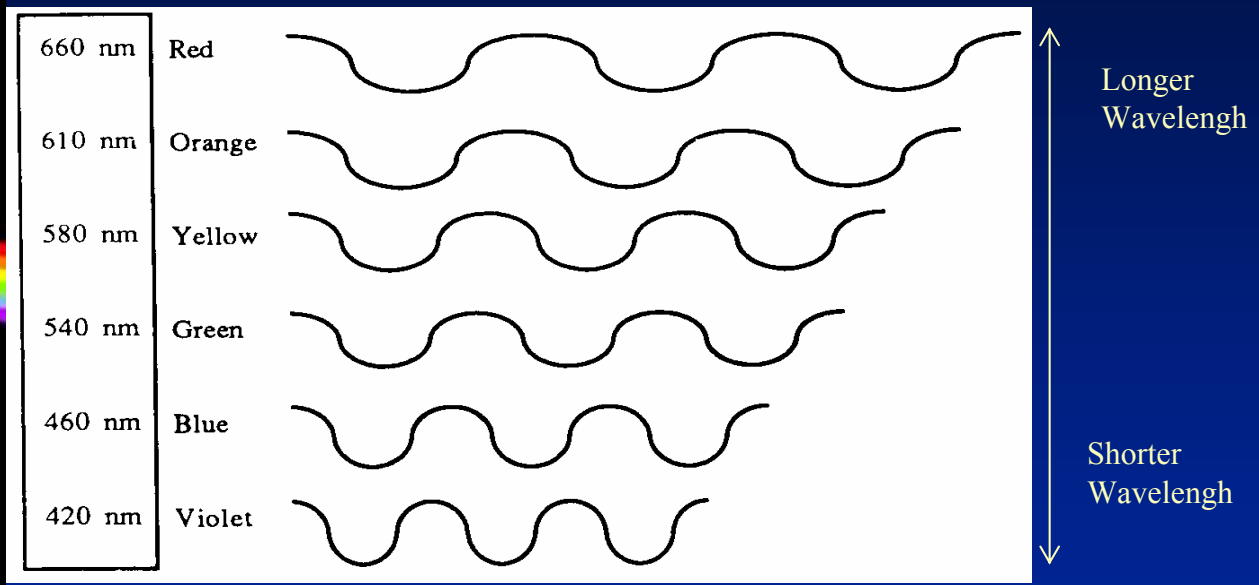
Fig. 10.1 Common detector types - absolute responsivity, unfiltered.

Luminescent Sources



Spectral range of visible light

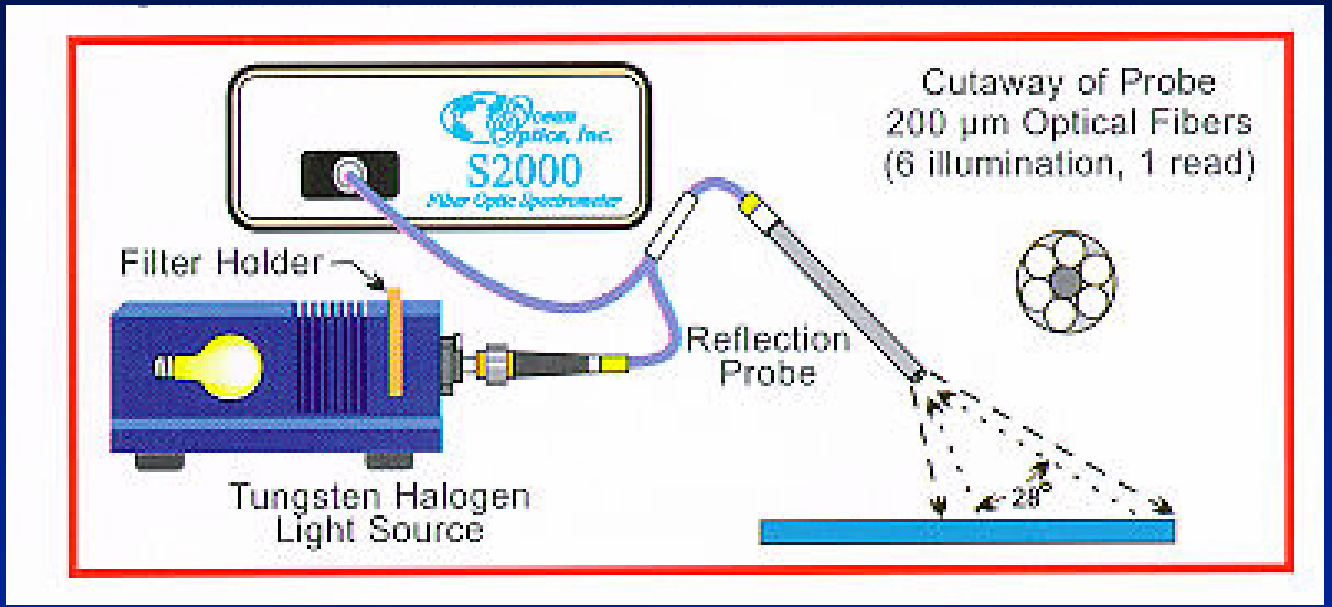




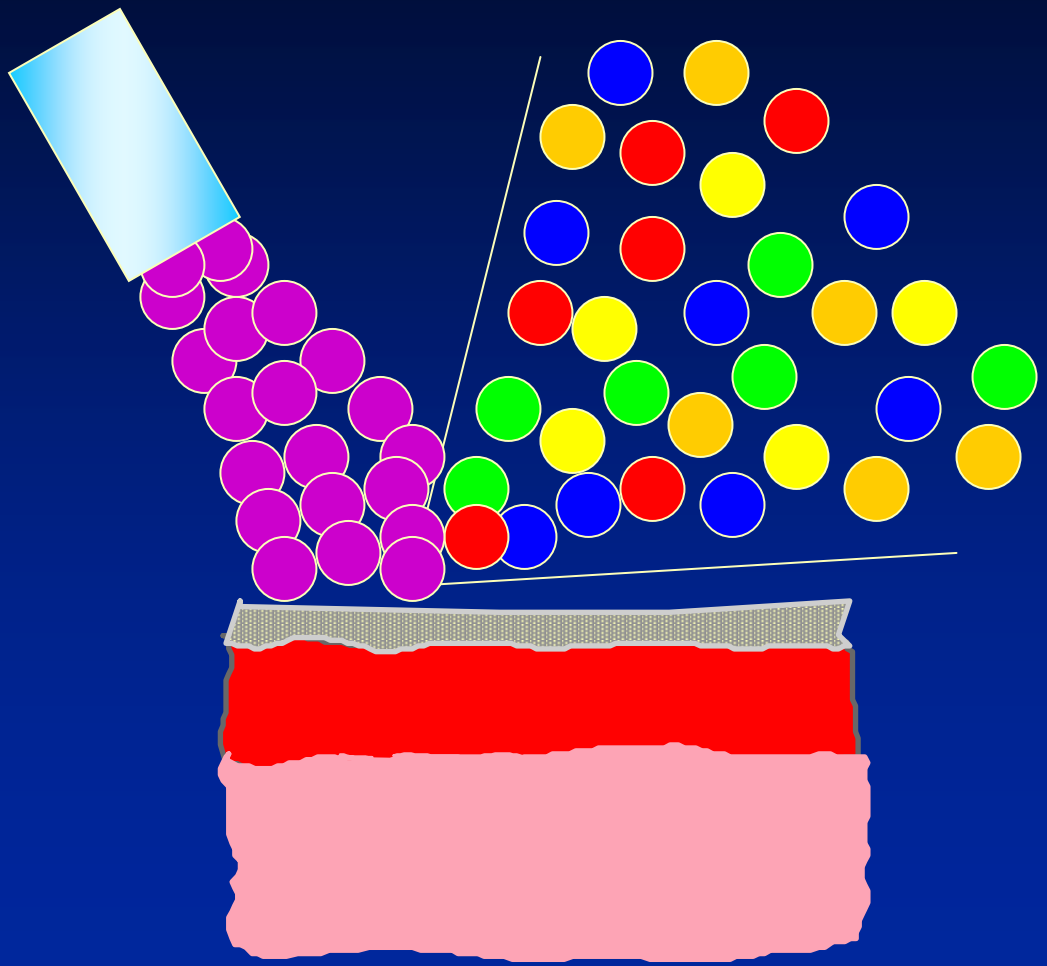
All colours have a specific wavelength

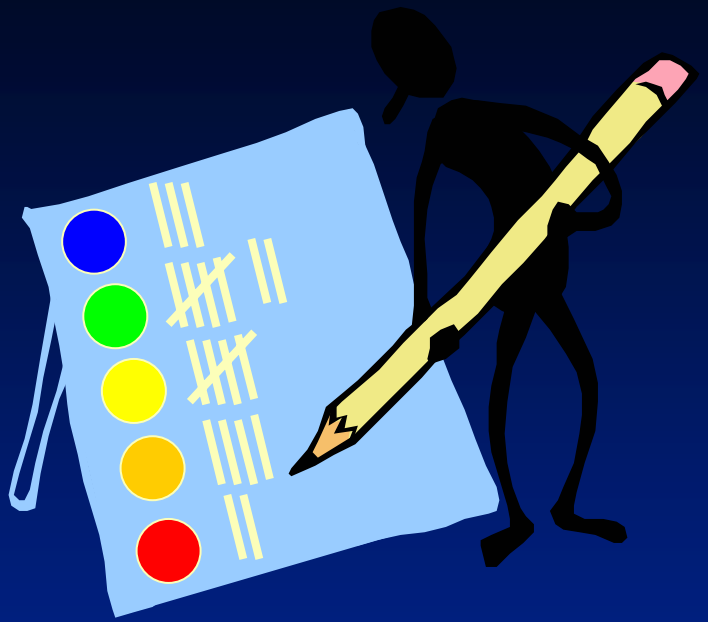
The wavelength increases as the colours approach the red end of the spectrum

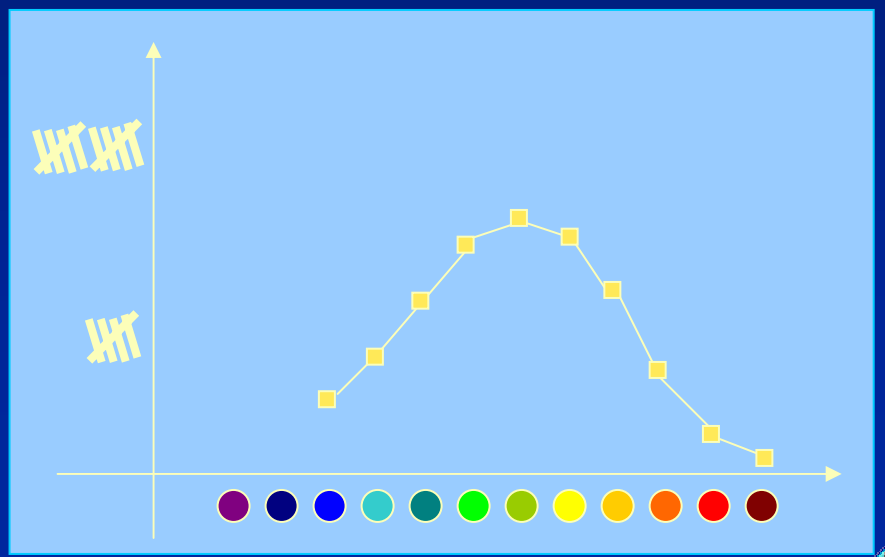
Spectroscope setup



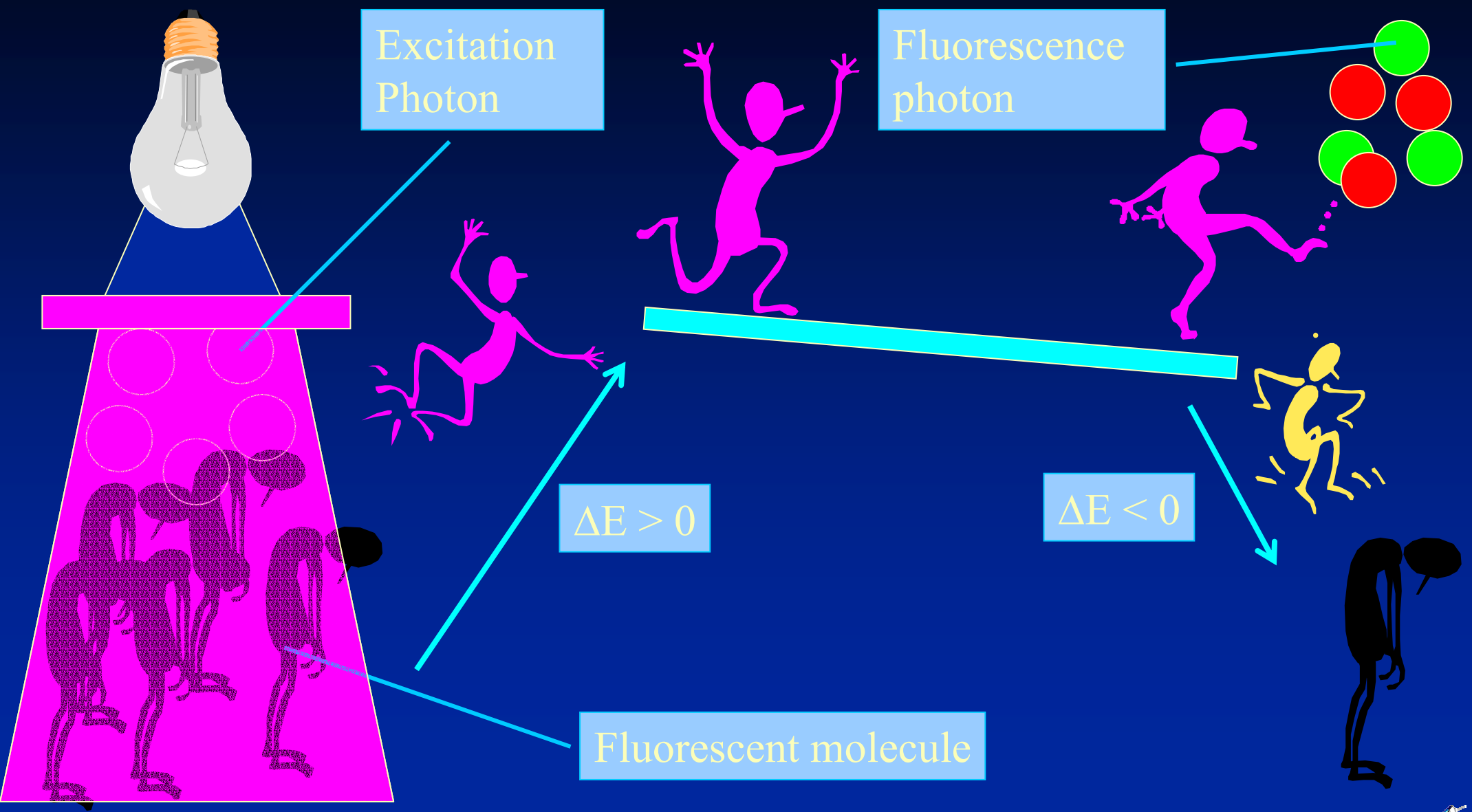
Principle of fluorescence spectroscopy

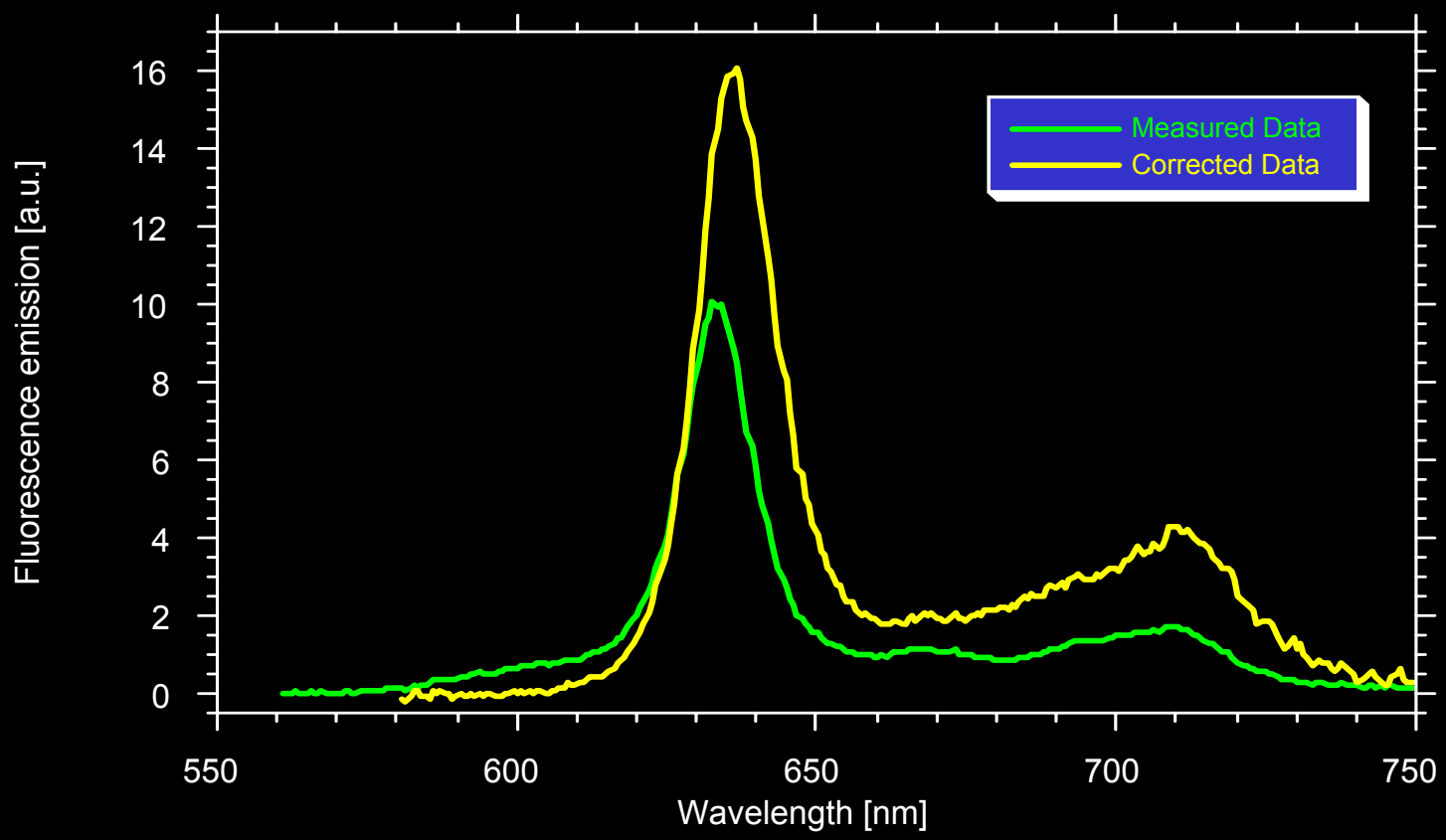




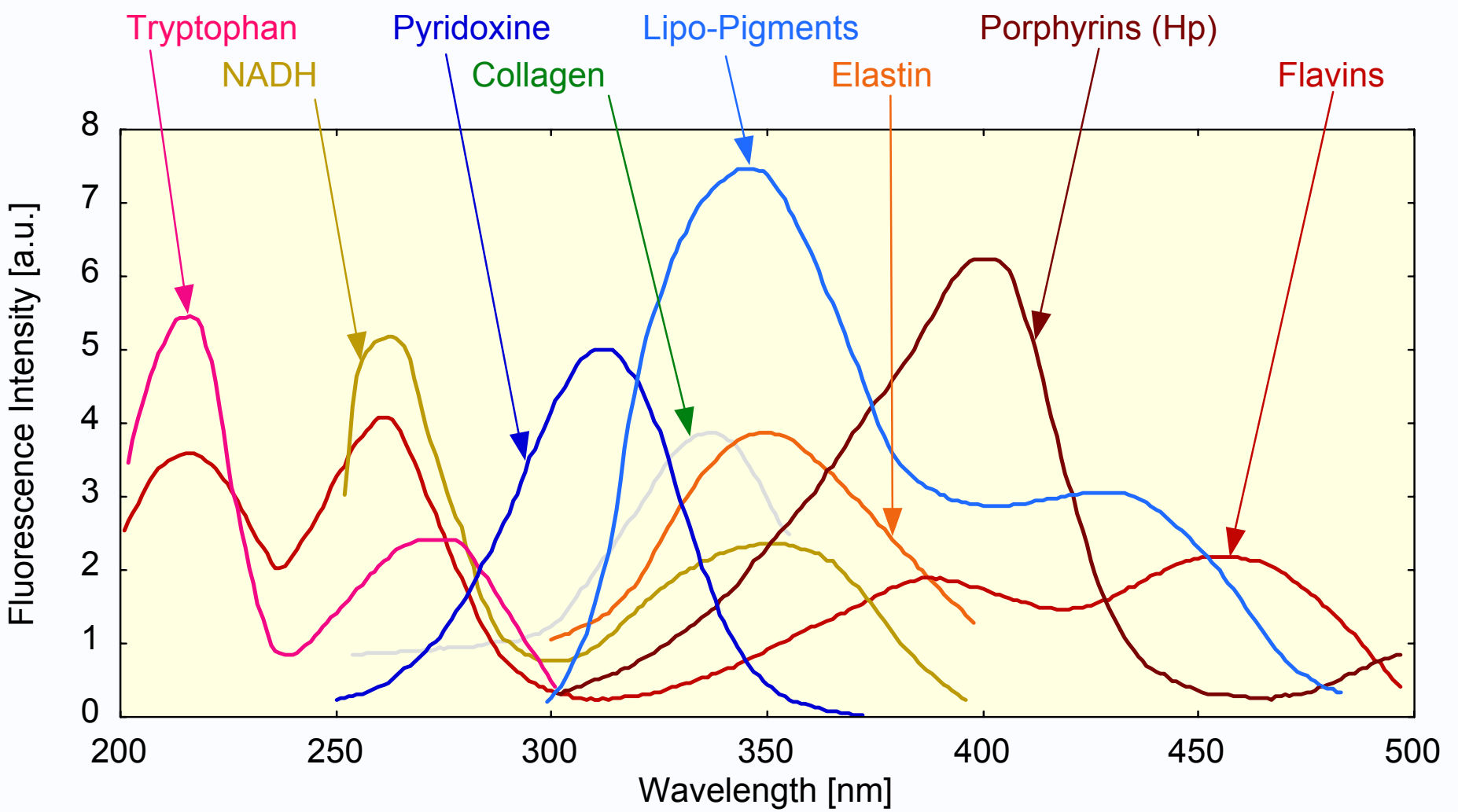


Principle of fluorescence

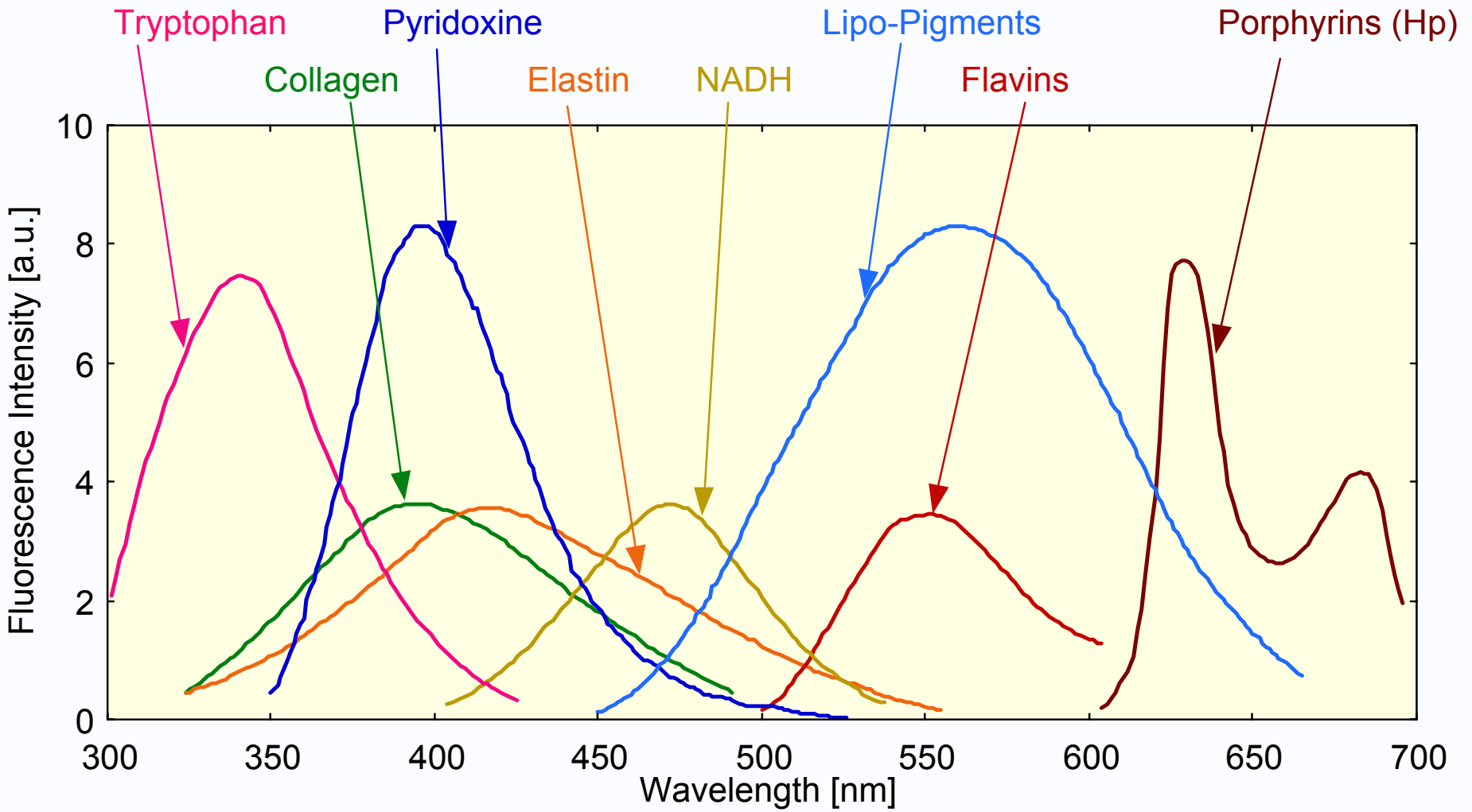



Measured Data
Corrected Data
Fluorescence emission [a.u.]
Wavelength [nm]
0
2
4
6
8
10
12
14
16
550
600
650
700
750

Excitation Spectra of some Bio-molecules

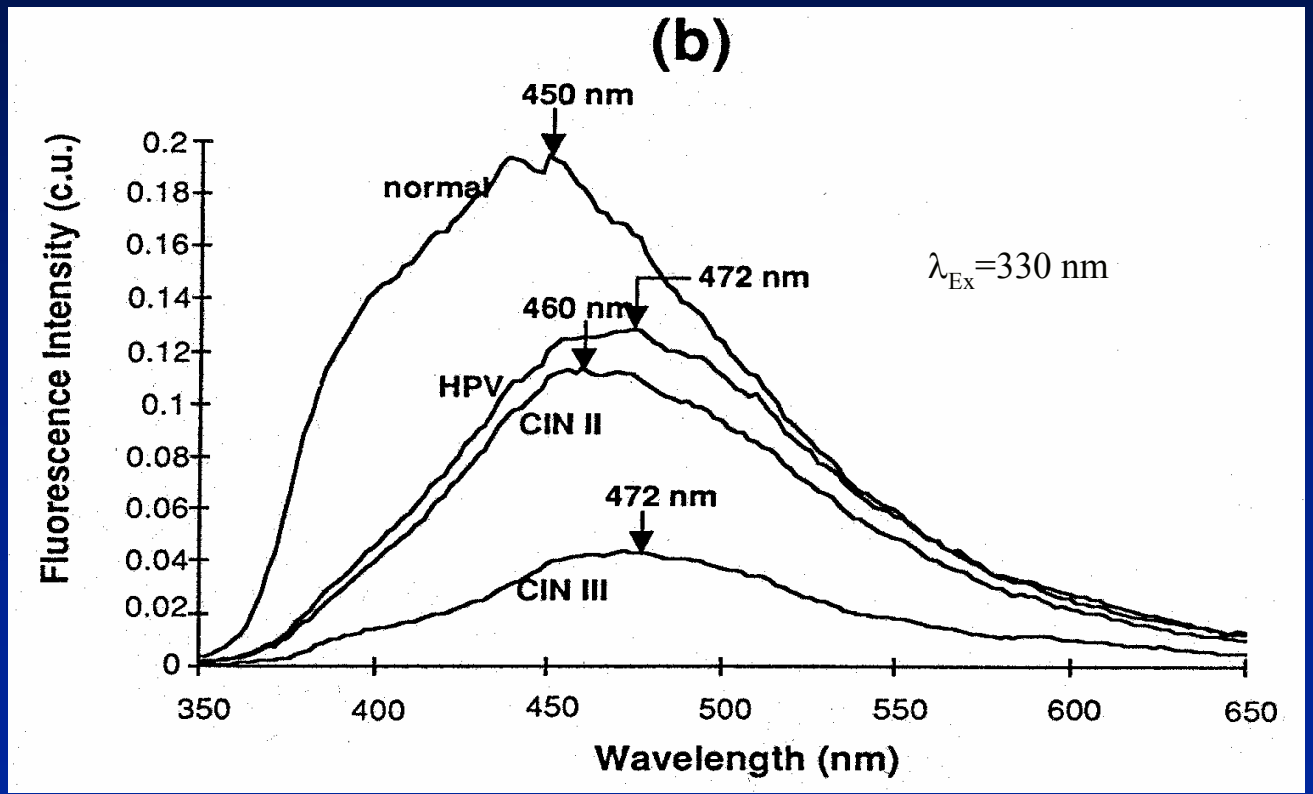


Emission Spectra of some Bio-molecules



Fluorescence emission spectra of cervical tissues

Patient with CIN II, CIN III and HPV infection



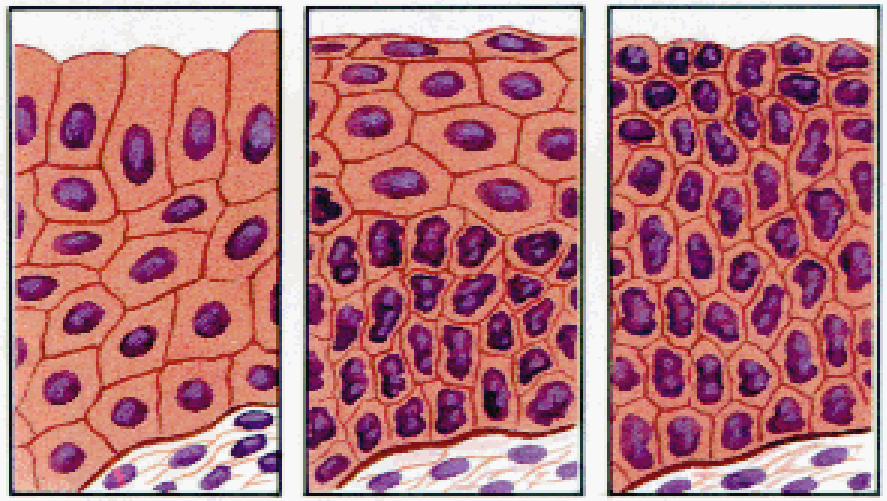
Ramanujam & al. Spectroscopic diagnosis of CIN, Gynecologic Oncology 52, 31-38 (1994)

Why is there a difference between neoplasic lesions vs. healthy tissue

- The diagnostic basis of spectroscopy is not yet understood at the biochemical level

Possible explanations:

- Attenuators
  - Increase in Oxy-haemoglobin attenuation
- Relative contribution of tissue fluorophores
  - Decrease in contribution of collagen fluorescence
  - Increase in the contribution of NADH
- Architectural effect
- Other ...

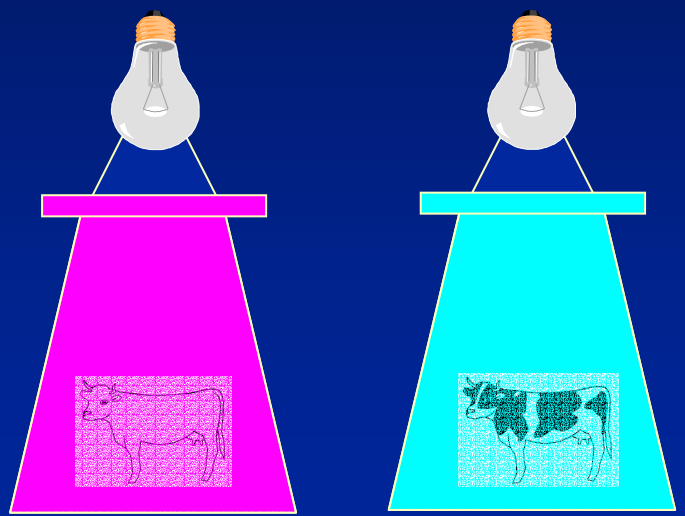


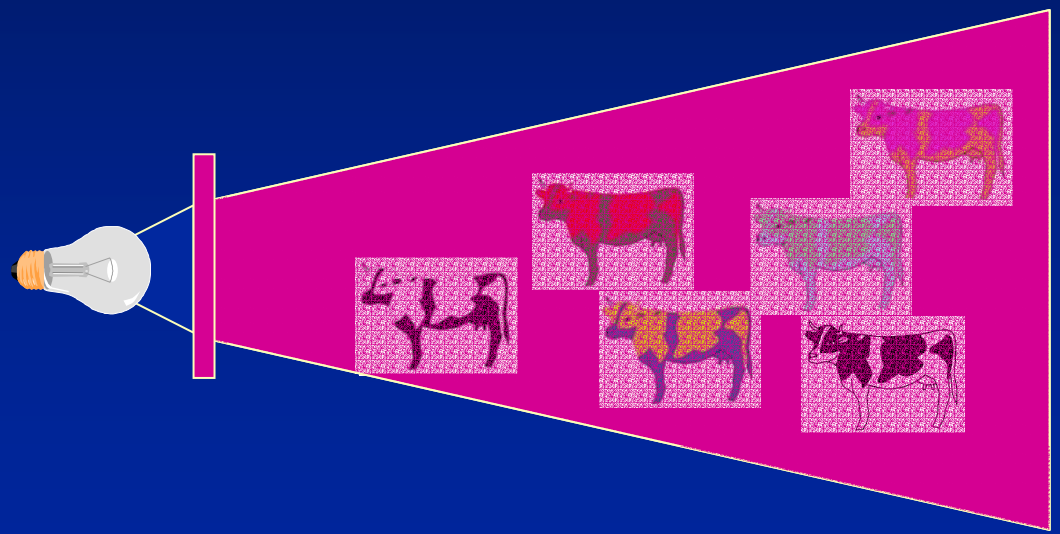
Parameters of fluorescence spectroscopy

Fluorescence Spectroscopy

Excitation wavelength?

Color of fluorescence photons ?

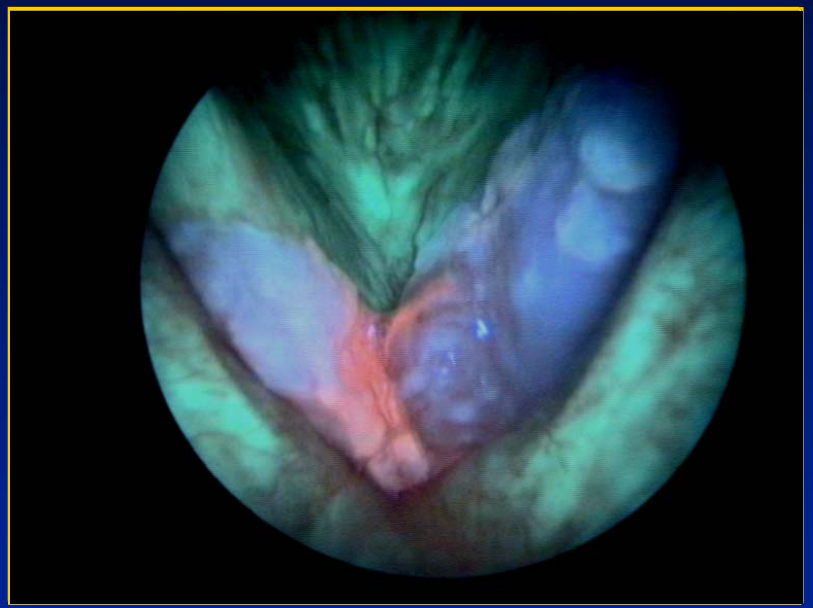




Autofluorescence (ENT)

Early Tumor Detection

<u>without</u> Marker Substance

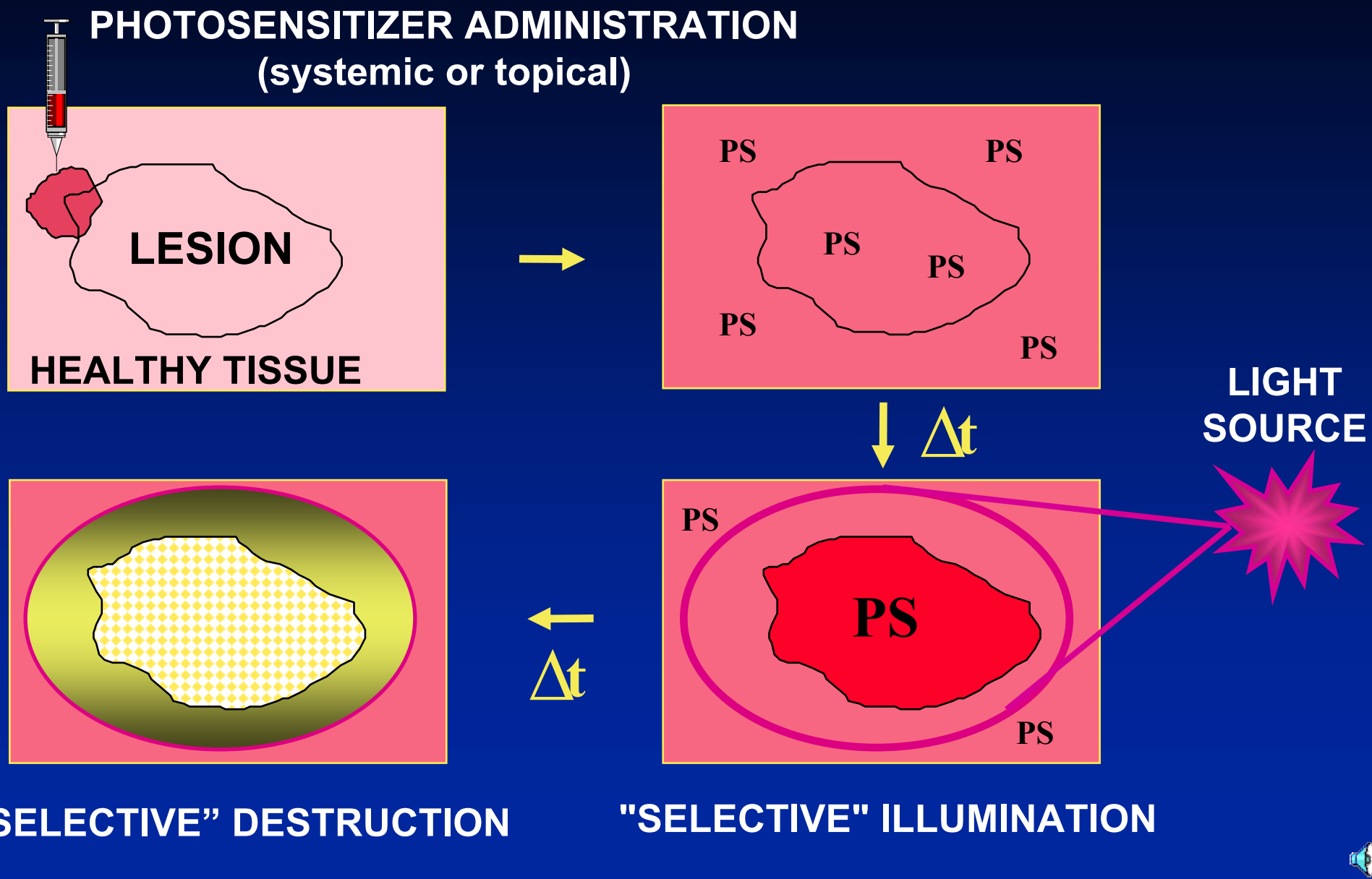


Carcinoma of the left vocal cord,
precancerous lesion right vocal cord
(bacterial growth)

K. Malzahn, C. Arens, H. Glanz

Justus-Liebig-University Giessen

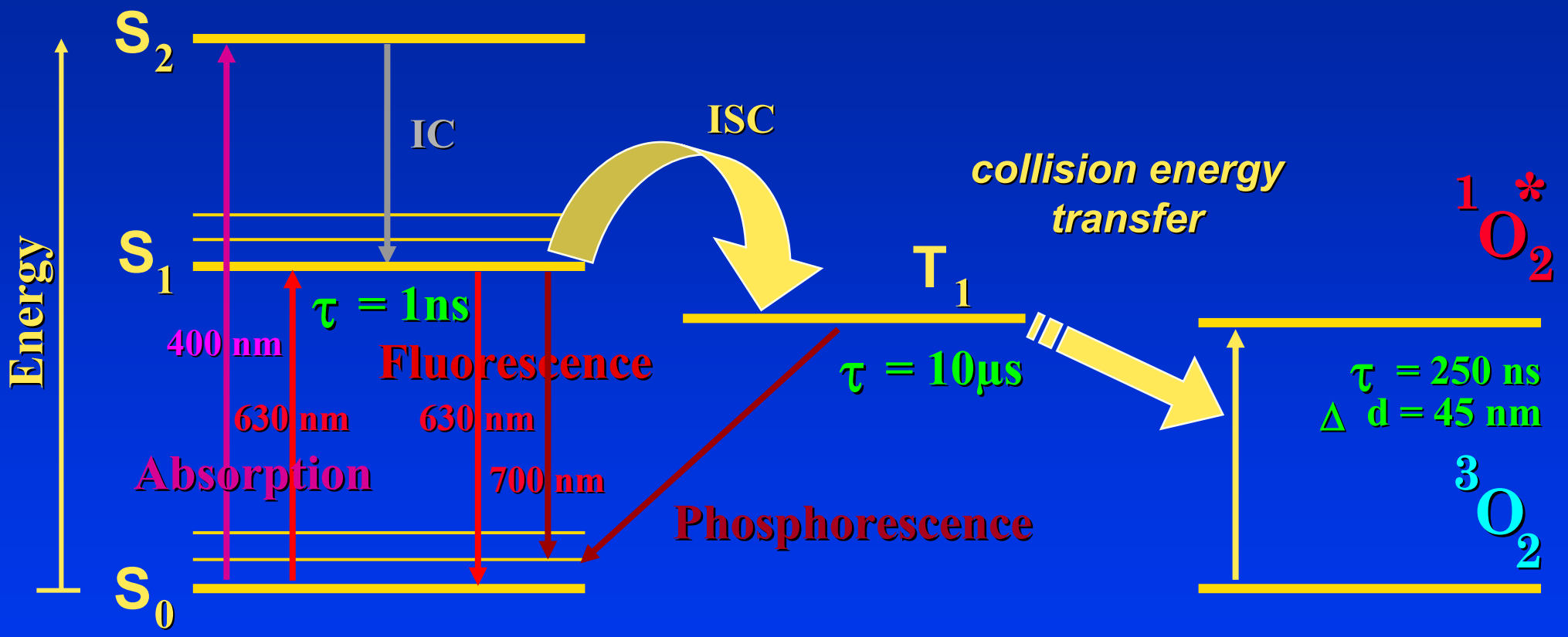
Principle of PDT



Photophysical Processes in:

Fluorescence detection

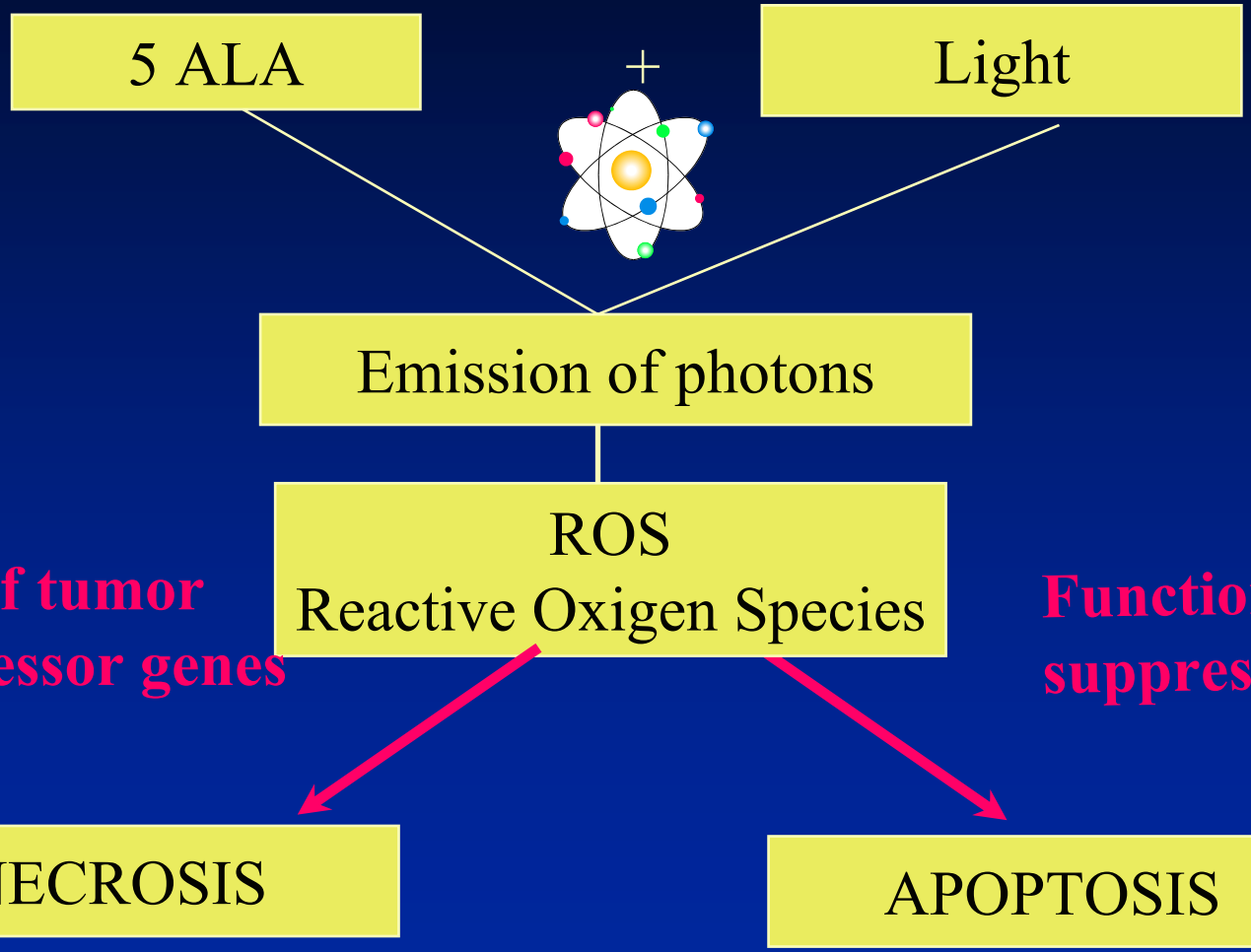
Photodynamic Therapy



Porphyrins spectroscopy

Singlet Oxygen production

Possible Effects of PDT



Mechanisms of Selective Tumor Uptake and Localization of Exogenous Photosensitizers

- **The Properties of the Tumor**
  - Leaky Vasculature
  - Compromised Lymphatic drainage
  - Large interstitial space
  - Decreased pH value

    (reduces solubility of porphyrins

    ⇓

    aggregation + protein association)

Mechanisms of Selective Tumor Uptake and Localization of Exogenous Photosensitizers

- **The Properties of the Tumor (Cont.)**
  - Elevated numbers of low-density protein receptors
  - Presence of macrophages
    (take up large amounts of HPD)
  - High amount of newly synthesized collagen
    (that binds porphyrins)
  - High amount of Lipid
    (that has a high affinity for lipophilic dyes)
  - Membrane potentials of malignant cells

PDT with second generation PS

5 - ALA / PpIX

- Absorption at 635 nm (Blue light also used)
- Dose 30 - 60 mg/kg orally (20% for topical administrations)
- D / L interval 4 - 18 h
- Light dose 10 - 150 J/cm2
- Skin photosensitization 24 - 48 h

Fluorescence contrast

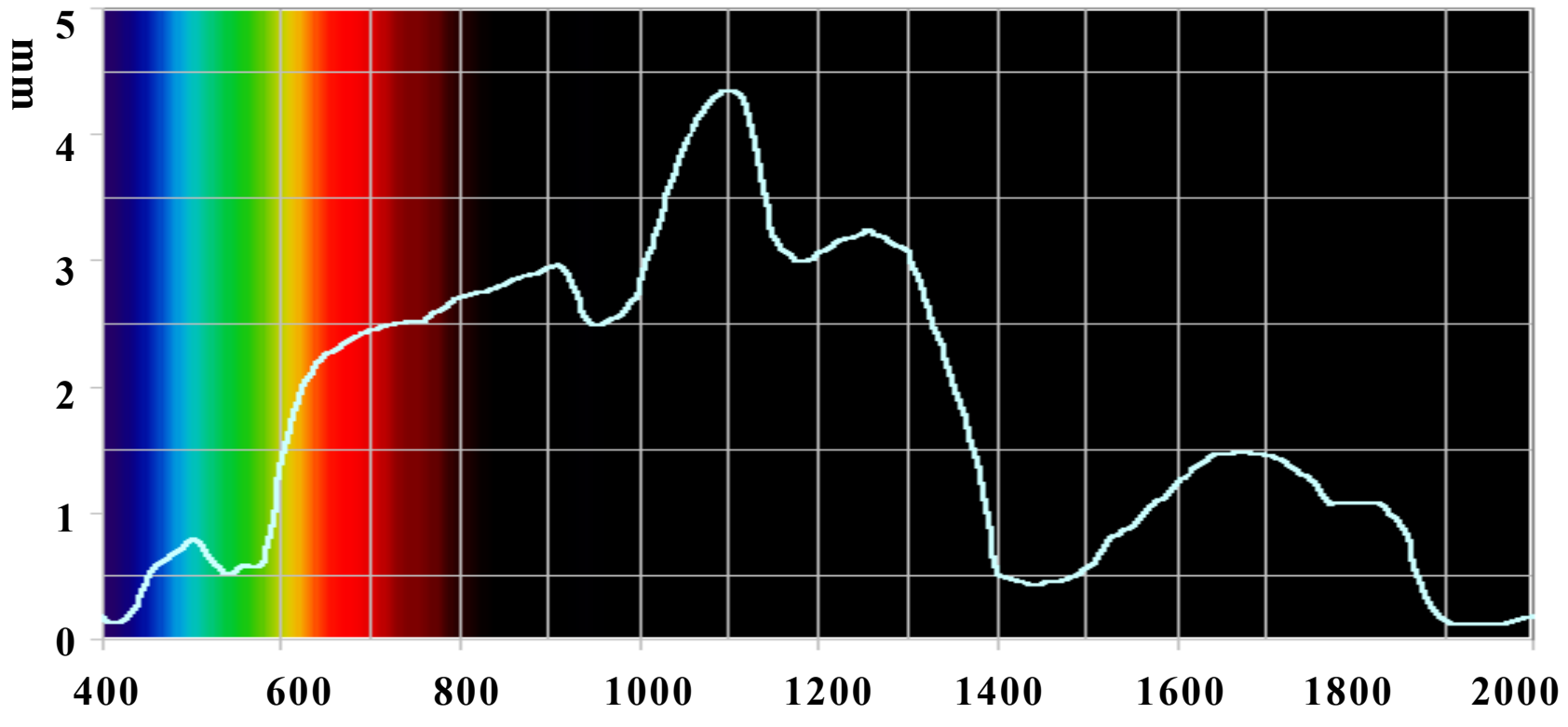
Fluorophores ?

Autofluorescence,
Endogenous Fluorophores:
Flavins, Porphyrins, NADH, etc

Endogenously induced:
ALA-PPIX

Exogenous:
Photophrin®, mTHPC

Penetration depth of light in tissue in relation to the wavelength



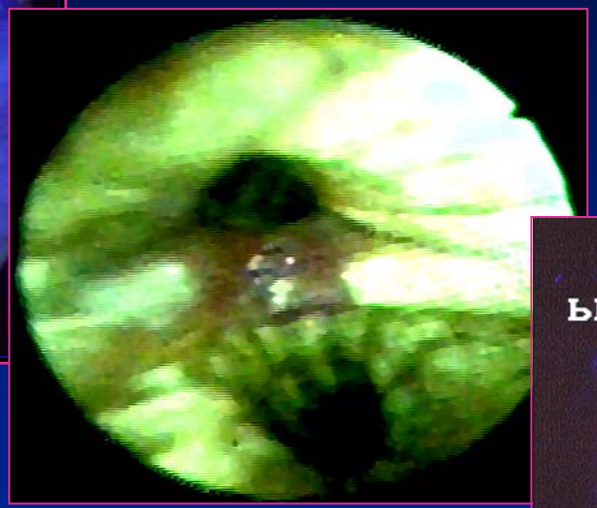
Photodynamic Therapy

Light parameters

- Wavelength
- Drug - light interval
- Irradiance
- Duration of irradiation
- Total light dose

Early Tumor Detection
PDD / AF

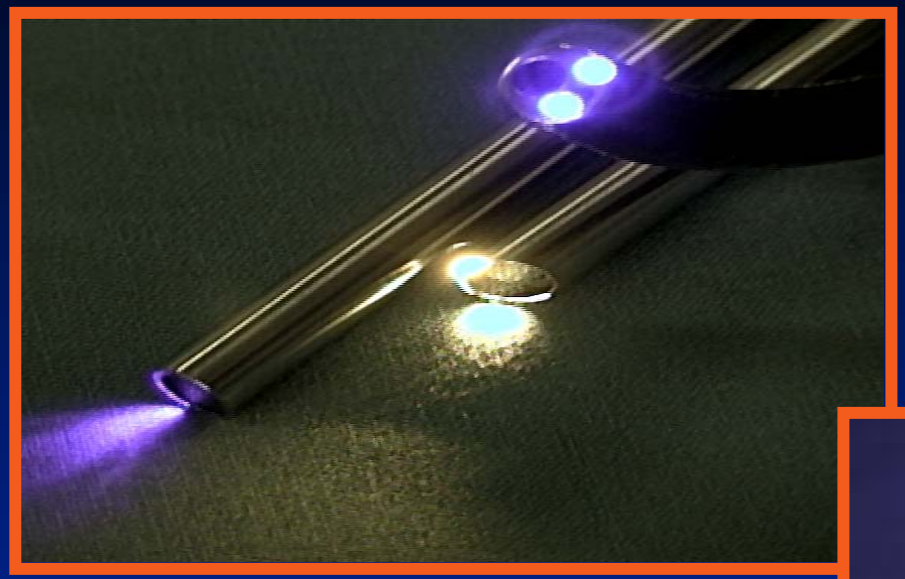
Urology



Pneumology

Neurosurgery

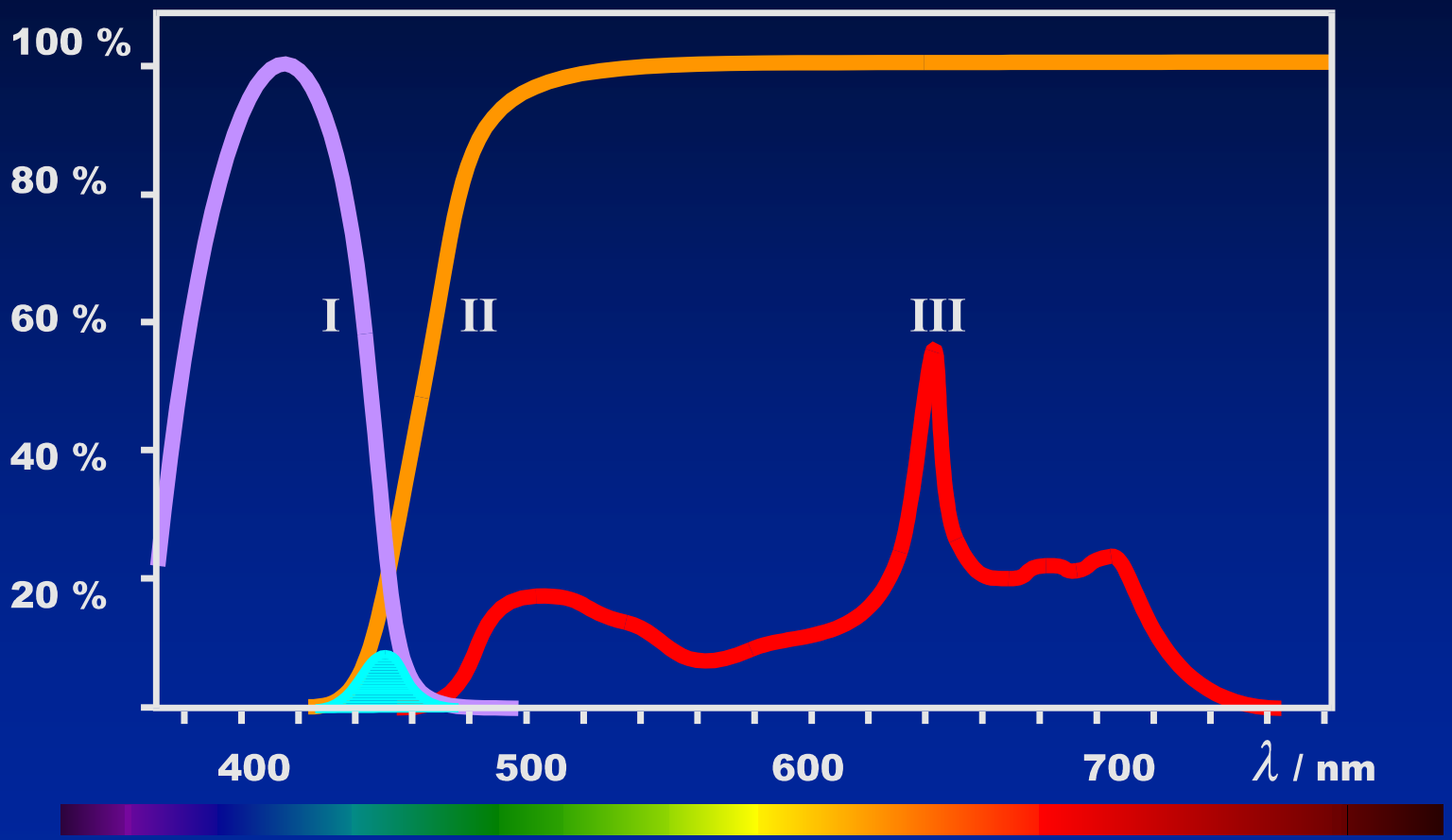
Combined Diagnosis System



- Rigid Telescopes
- Fiberscopes
- OP - Microscopes

- White Light
- ALA-Mode
- Autofluorescence-Mode

Fluorescence Observation (PPIX)



Neurosurgery

Special Fluorescence
Microscope
Storz AG/ **Carl Zeiss**

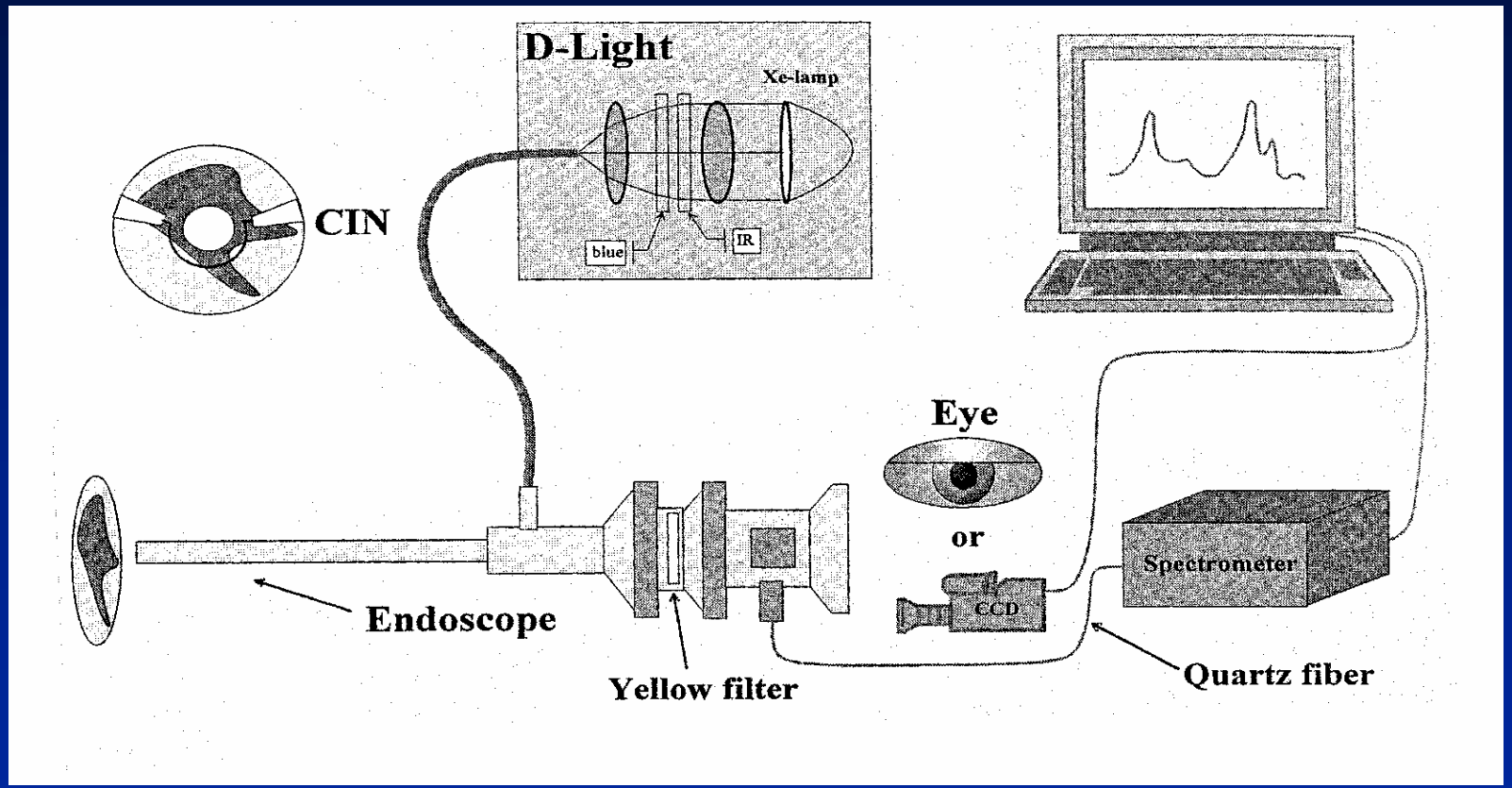
Early Tumor Detection

<u>with</u> Marker Substance

High grade Glioma

Stummer, Reulen
Munich-Großhadern

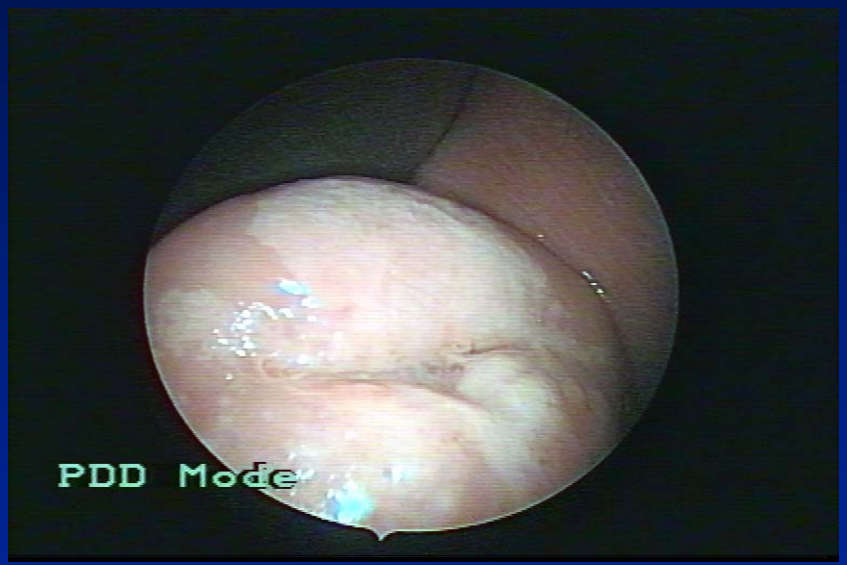
Fluorescence imaging and spectroscopic system used for fluorescence photodetection of cervical lesions after topical application of 5-ALA or h-ALA



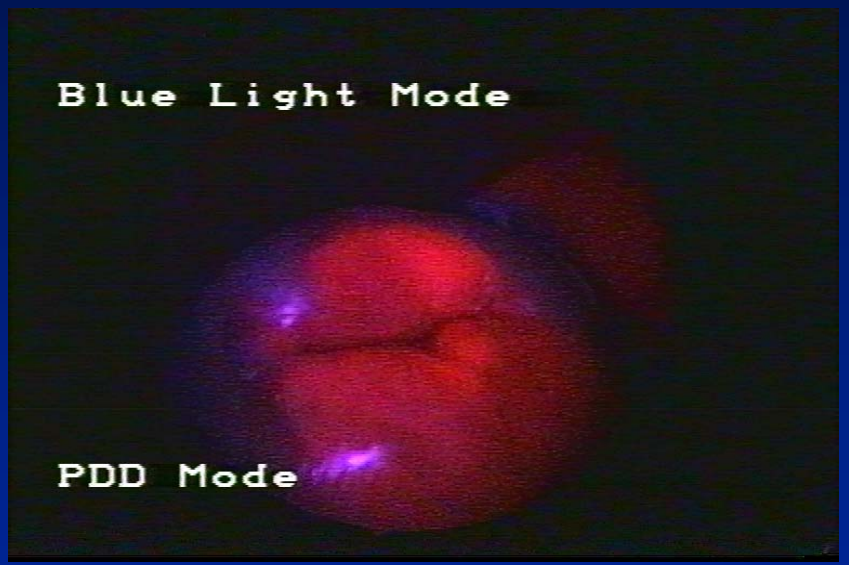
Hillemanns & al. Photodetection of Intraepithelial Neoplasia, Cancer 88, 2275-92 (2000)

Fluorescence image of the cervix after h-ALA application

White light



Fluorescence



Fluorescence image and white light image of the cervix uteri after the application of 3% acetic acid. Application of 10mg h-ALA in 10ml 0.9% NaCl solution on the cervix during 3 hrs.

Conclusion

- Existing instrumentation in clinical practice
- In certain disciplines may need adaptation or development
- Much clinical development and research is done in the field of photosensitizers
- Autofluorescence diagnostic procedures become more interesting