# Fluorescence Spectroscopy and Imaging for Photodetection of Cervical Intraepithelial Neoplasia

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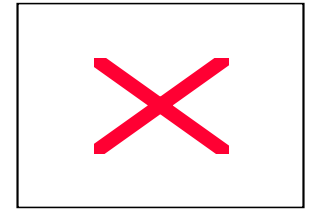
Presentation Plan:

- ⌘ Objectives: The utility of «Fluorescence spectroscopy» in Gynecology?

- ⌘ Principles of fluorescence spectroscopy
  - ⌃ What is fluorescence spectroscopy?
  - ⌃ How can fluorescence spectroscopy be useful for diagnosis in gynecology

- ⌘ Clinical applications:
  - ⌃ Single point measurements: Optical biopsies
  - ⌃ Imaging:
    - ⌧ Photodetection of CIN using ALA (h-ALA) induced PPIX
    - ⌧ Fluorescence lifetime imaging

- ⌘ Conclusions and future prospects

Need for improved CIN diagnosis methods

- ⌘ Significant decline in the incidence and mortality of cervical cancer due to large **screening programs** over the last 50 years in most rich countries.

- ⌘ Despite of **Papanicolaou smear**, cervical cancers remains an important health problem:
  - ☑ Second common malignancy in the word.
  - ☑ 16000 Women are diagnosed each year with invasive cervical cancer and 4800 women die from this disease in the US.

- ⌘ **Pap. Smear**: poor sensitivity (false negative rate of 20-30%).

- ⌘ **Colposcopy**: poor specificity (about 50%).
  - ☑ Need for biopsies: expensive, unpleasant, no "see and treat"

- ⌘ Pap. Smear and colposcopy require **high expertise**

The ideal diagnosis tool

- High selectivity
- High specificity
- "Pleasant for the patient"
- Low cost
- Easy: Does not require high expertise
- Immediate results "see and treat"

Fluorescence Spectroscopy ?

Fluorescence Spectroscopy and
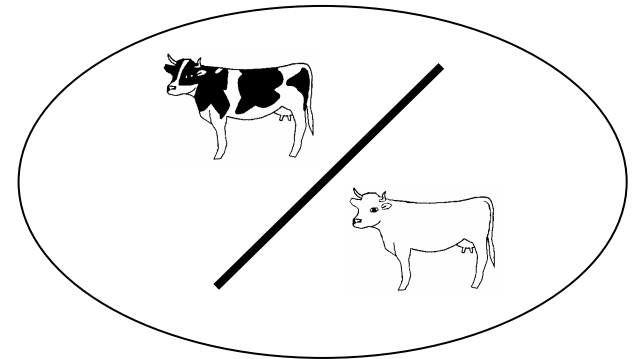Imaging in Gynecology

Why fluorescence spectroscopy?

**Contrast between
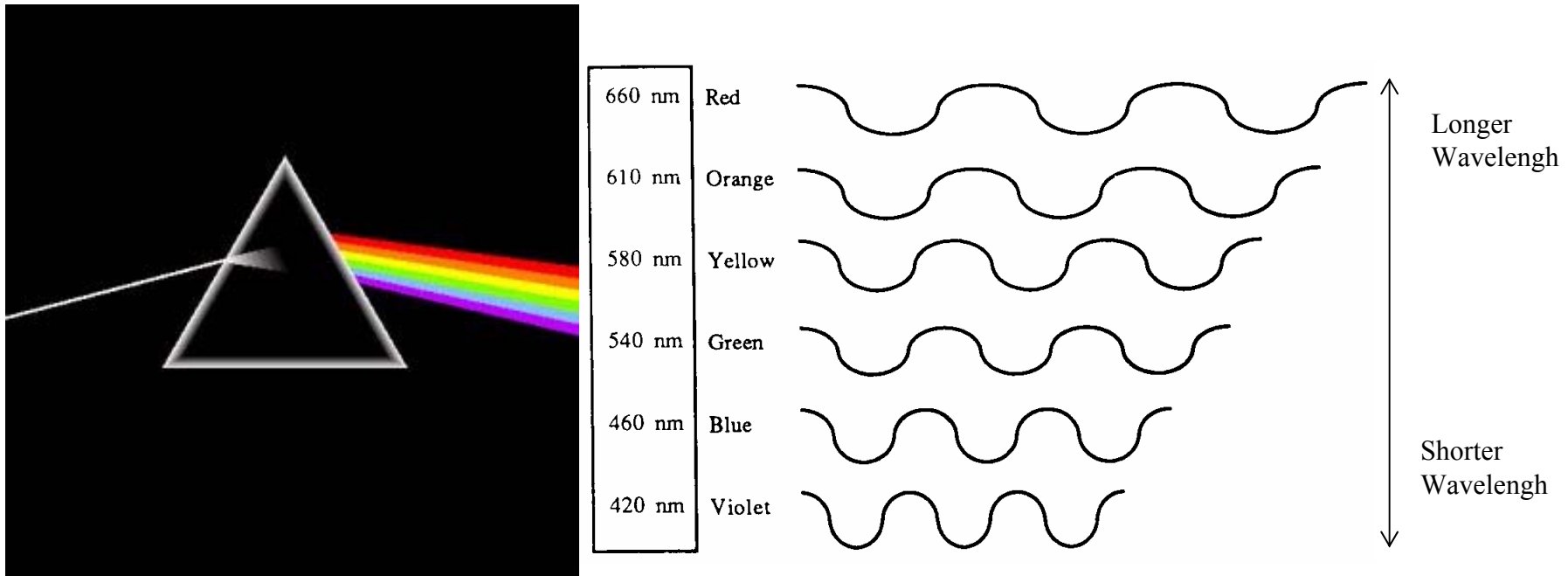healthy tissue / early lesion**

1) What is fluorescence?

2) What is fluorescence spectroscopy?

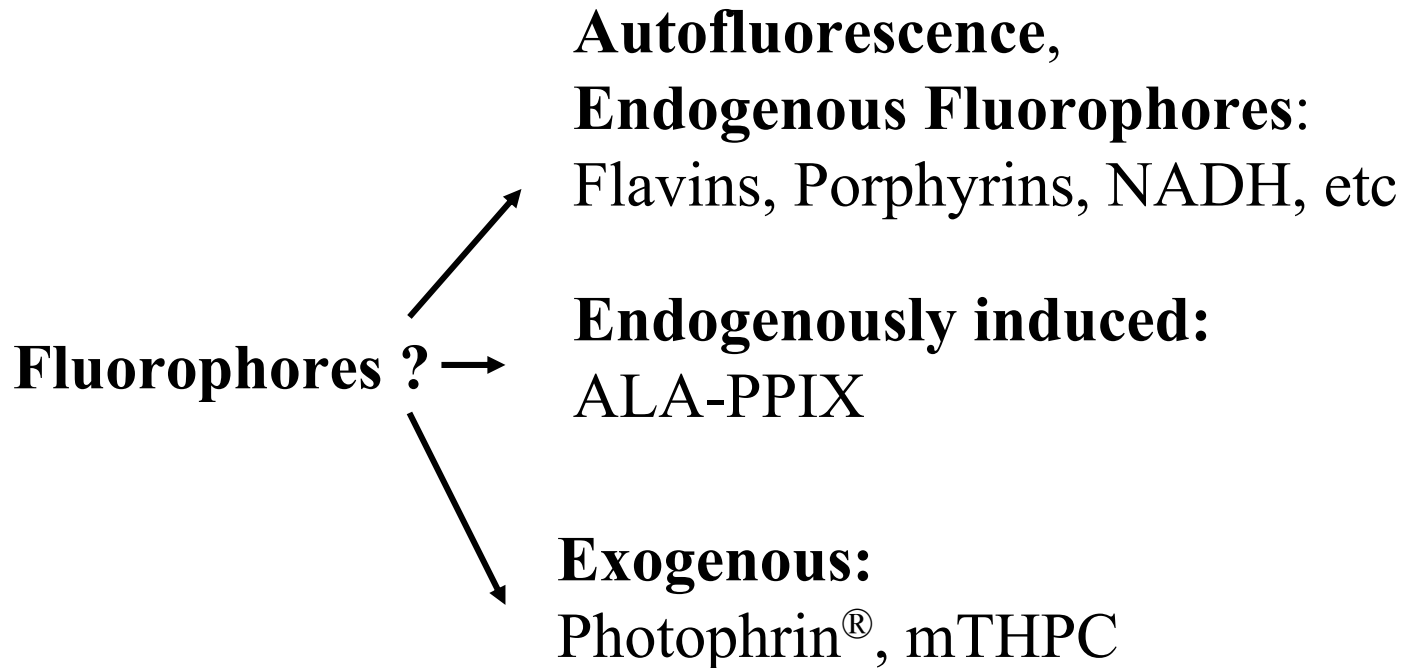3) Can Fluorescence spectroscopy be useful to induce a contrast between healthy tissue / early lesion?

Spectral range of visible light

660 nm	Red
610 nm	Orange
580 nm	Yellow
540 nm	Green
460 nm	Blue
420 nm	Violet

Longer Wavelengh

Shorter Wavelengh

All colours have a specific wavelength

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

Fluorescence contrast

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

Fluorophores ?

Endogenously induced:
ALA-PPIX

Exogenous:
Photophrin®, mTHPC

Definitions: Selectivity & Sensitivity

Table 1. Definition of terms in estimating the diagnostic sensitivity and specificity of fluorescence diagnosis (*e.g.* lesion detection)*

Detection of lesion by fluorescence	Gold standard assessment of abnormality	
	Yes	No
Yes	True positive TP *Easy*	False positive FP *Easy*
No	False negative FN *Very difficult*	True negative TN *Difficult*
Sensitivity	TP/(TP + FN)	
Specificity	TN/(TN + FP)	
Accuracy	(TP + TN)/(TP + TN + FP + FN)	

*The descriptors in *italics* suggest the degree of difficulty in obtaining absolute values for the various parameters in clinical trials. For example, TP values are straightforward to determine, because this involves simply scoring lesions detected as positive according to some pre-set criteria, whereas FN values are generally very difficult to measure, because the rate of unseen lesions is not known.

Comparison of SIL Detection Methods

Presence vs absence of SILs	HG vs LG SILs
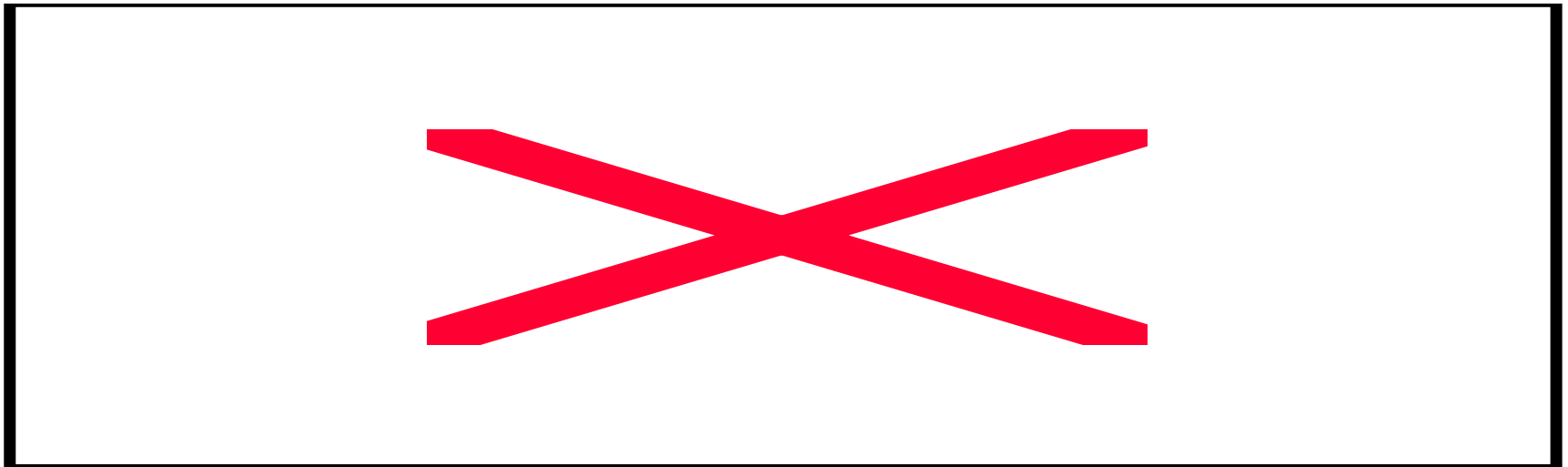
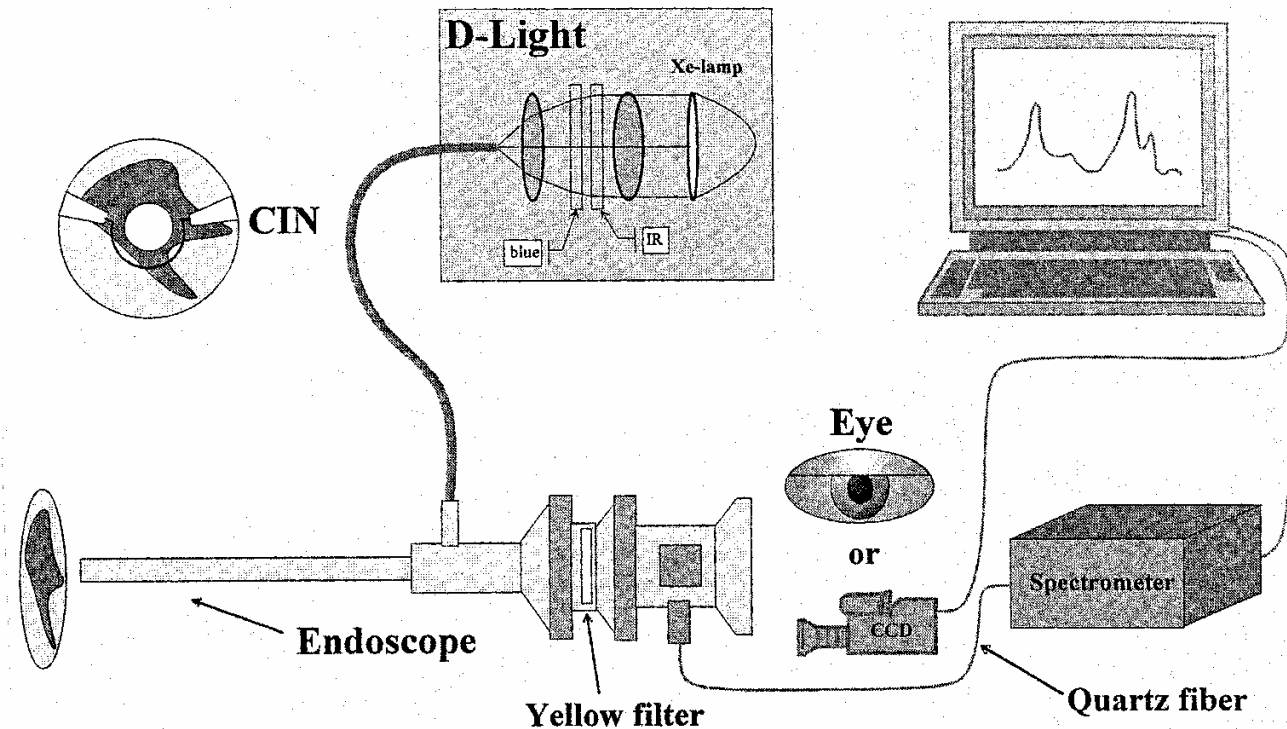1) Hillemanns & al. Photodetection of Intraepithelial Neoplasia, Cancer 88, 2275-92 (2000)

2) Cantor & al. Cost-Effectiveness Analysis of Diagnosis and Management of Cervical squamous Intraepithelial lesions, Obstetrics and gynecology 91, 270-277 (1998)

Topical application of ALA or h-ALA

- A solution of ALA of 1% is applied topically on the cervix with help of a gauze sponge
- The examination is performed after a given time interval (typically 1 hrs)

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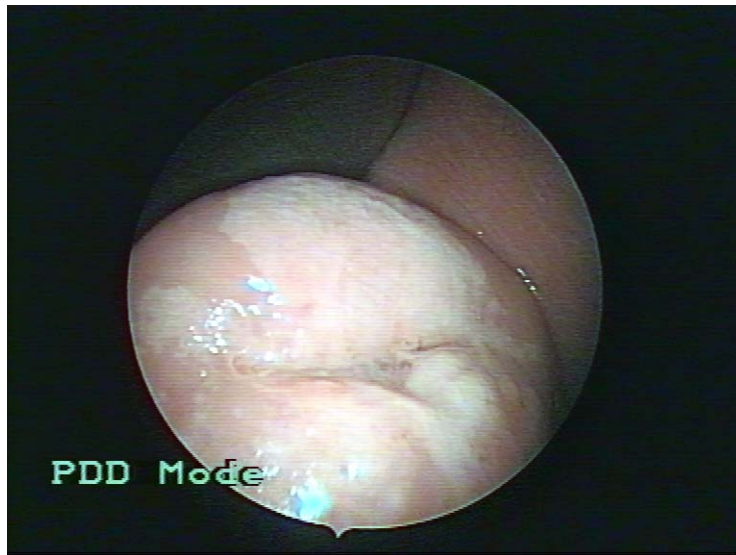

Hillemanns & al. Photodetection of Intraepithelial Neoplasia, Cancer 88, 2275-92 (2000)
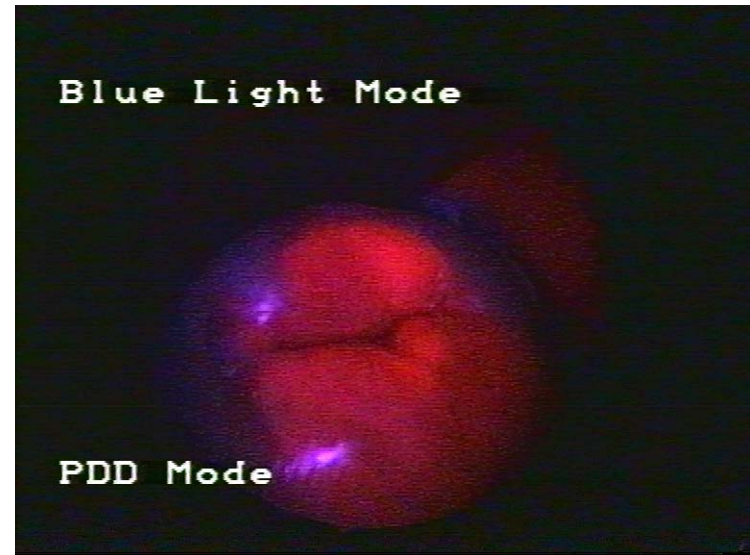
Fluorescence image of the cervix after h-ALA application
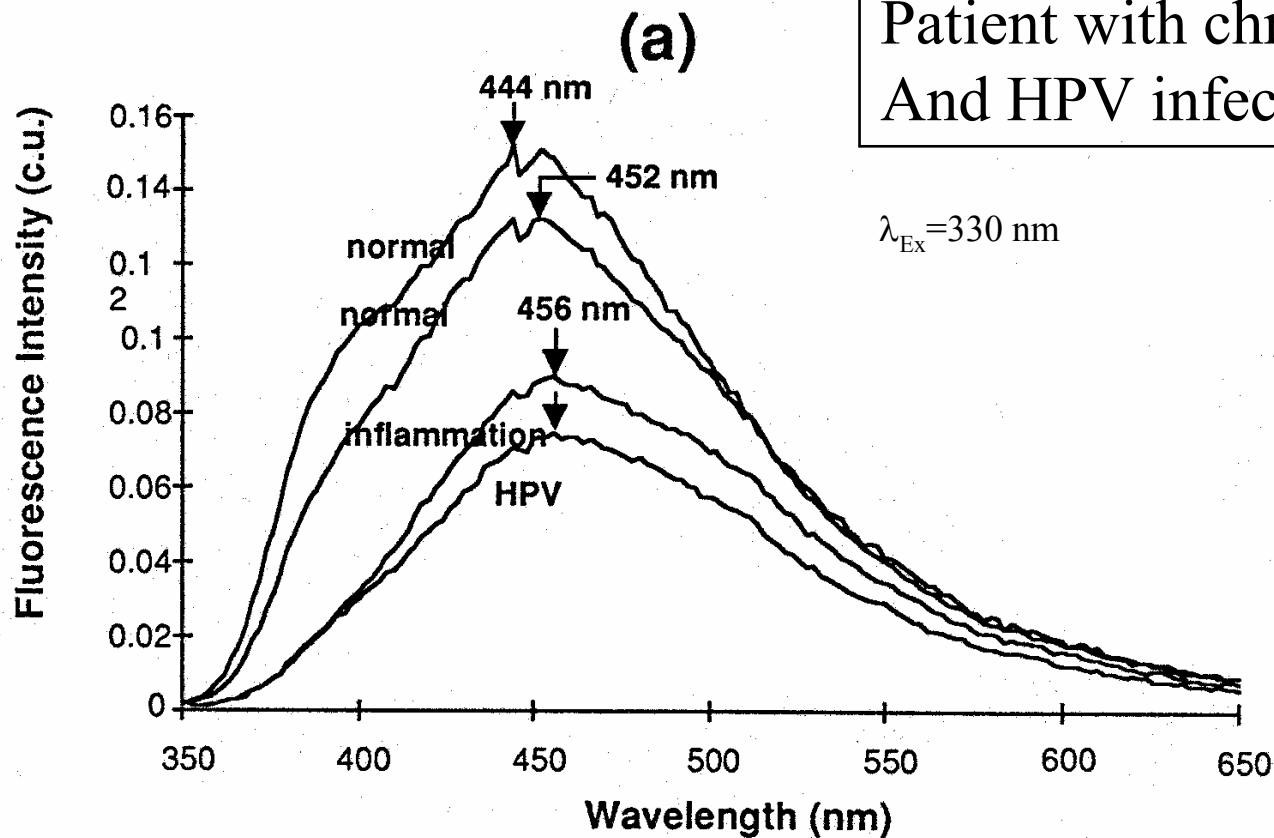
White light

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. (courtesy of Nora Dögnitz)

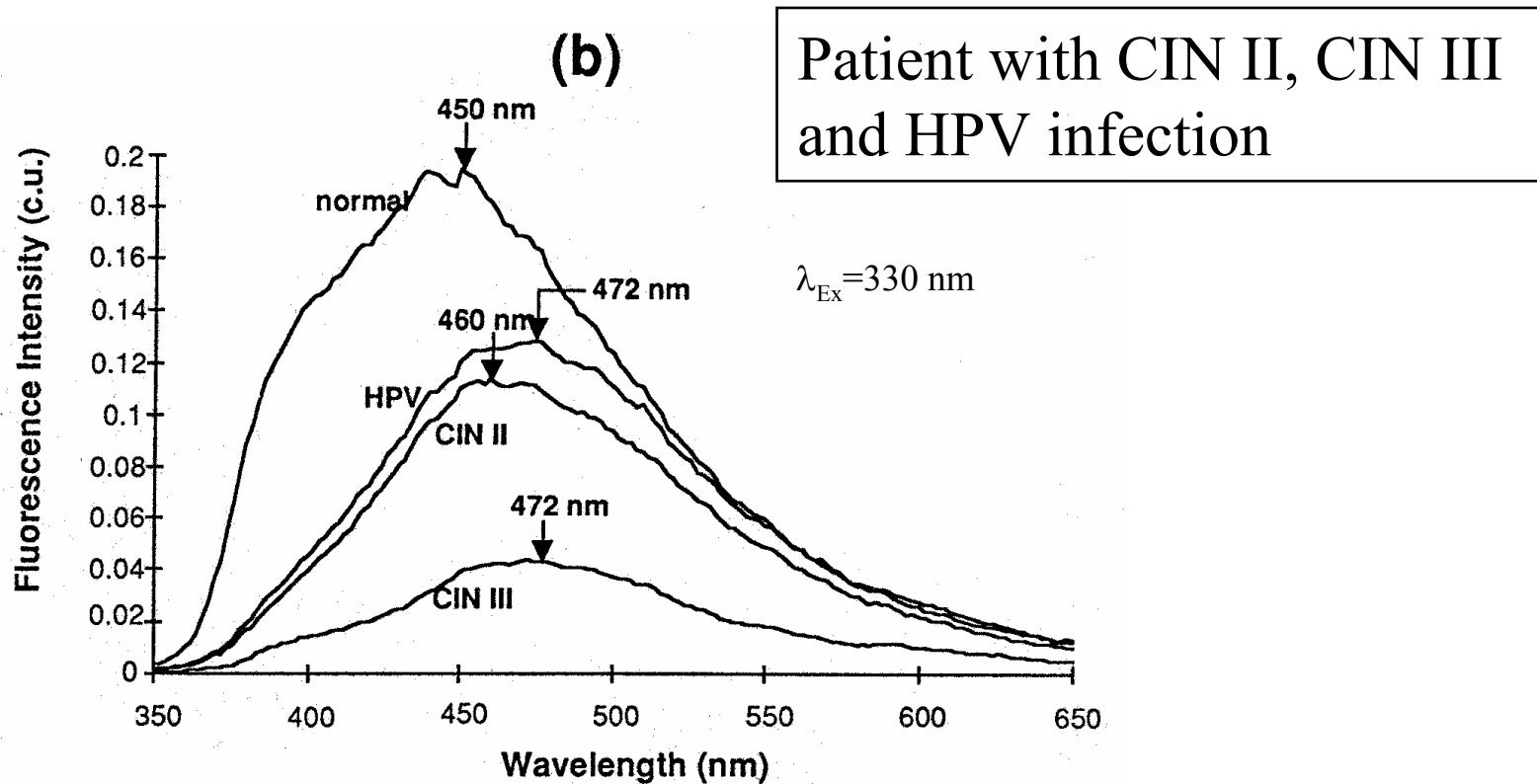# Fluorescence emission spectra of cervical tissues

(a)

Patient with chronic inflammation And HPV infection

$\lambda_{Ex}=330$ nm

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

Fluorescence Spectroscopy and Imaging in Gynecology

Fluorescence emission spectra of cervical tissues

(b)

Patient with CIN II, CIN III and HPV infection

λ_{Ex}=330 nm

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

Average peak intensity from colposcopically normal and histologically abnormal tissues
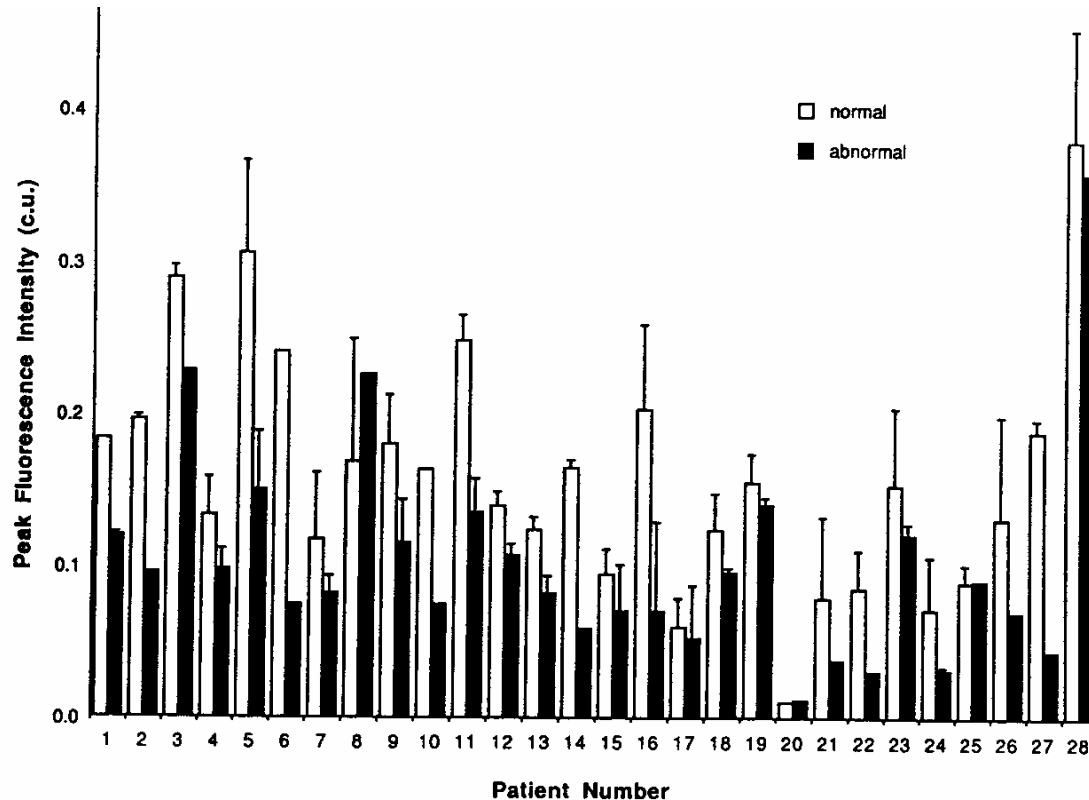

FIG. 3. Bar chart displaying average peak fluorescence intensities of spectra from colposcopically normal (white) and histologically abnormal (black) tissues from 28 patients. The error bars represent one standard deviation. Fluorescence intensity is reported in calibrated units.

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

Relative peak intensity versus the slope over 420 to 440 nm of colposcopically normal (white) and histologically abnormal (black) spectra
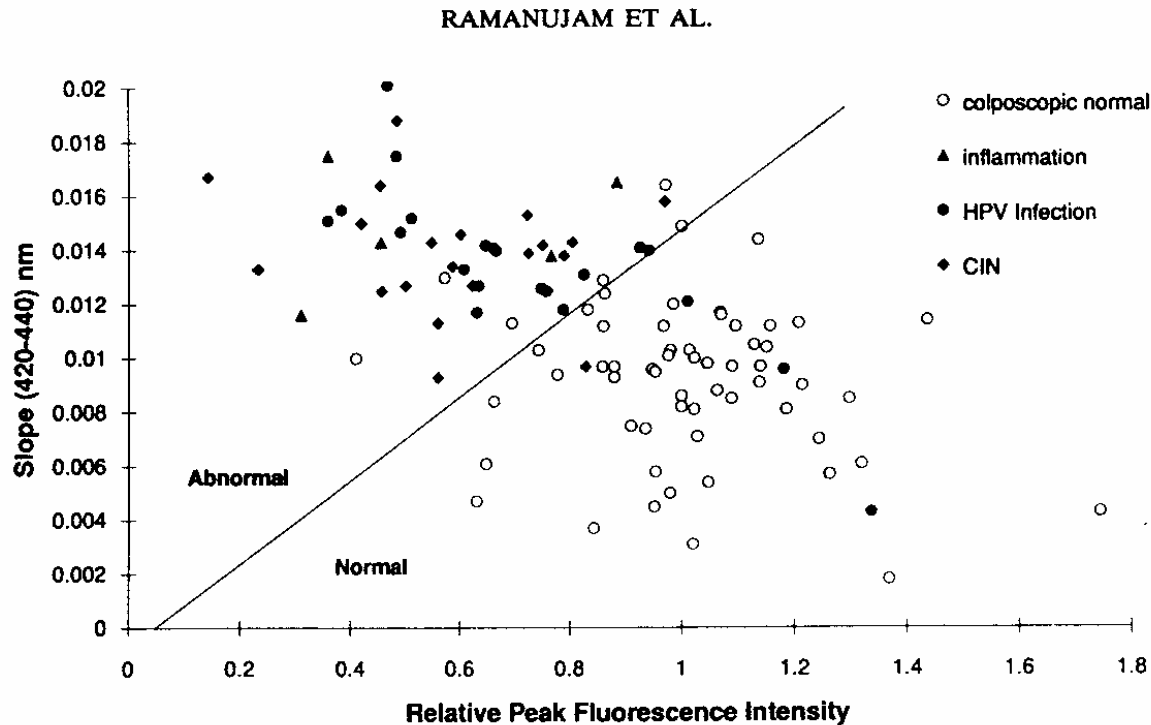

RAMANUJAM ET AL.

FIG. 4. Two-dimensional scatter plot of the relative peak fluorescence intensity versus the slope over 420 to 440 nm of colposcopically normal (white) and histologically abnormal (black) spectra. The peak fluorescence intensities of all spectra are normalized to the average peak intensity of colposcopically normal spectra from the same patient. The straight line represents the decision surface chosen to minimize the number of misclassified samples.

Why is there a difference between neoplasic lesions vs. non neoplasic lesion/ 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 ...

SQUAMOUS METAPLASIA   DYSPLASIA   CARCINOMA IN SITU
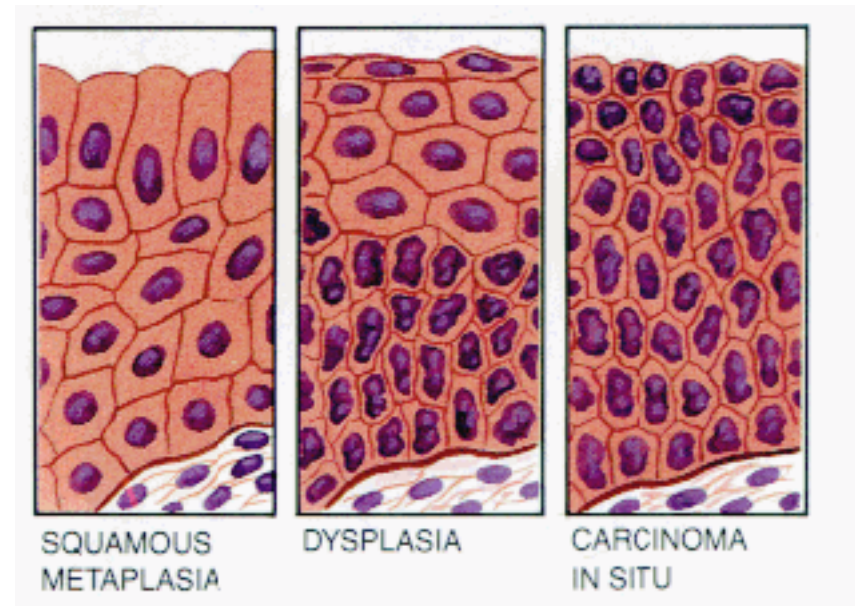
Conclusions

Fluorescence is useful to induce a contrast!

It could become a good complement to white light colposcopy

Further studies are still necessary to assess the precision of the method

Fluorescence Spectroscopy Imaging in Gynecology

People working on the project

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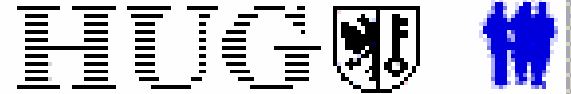FÉDÉRALE DE LAUSANNE

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