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 world.
  - 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?
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

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
- 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)*

<table>
<thead>
<tr>
<th>Detection of lesion by fluorescence</th>
<th>Gold standard assessment of abnormality</th>
</tr>
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<tbody>
<tr>
<td>Yes</td>
<td>True positive</td>
</tr>
<tr>
<td></td>
<td>False positive</td>
</tr>
<tr>
<td></td>
<td>TP</td>
</tr>
<tr>
<td></td>
<td>FP</td>
</tr>
<tr>
<td>Easy</td>
<td>Easy</td>
</tr>
<tr>
<td>No</td>
<td>False negative</td>
</tr>
<tr>
<td></td>
<td>True negative</td>
</tr>
<tr>
<td></td>
<td>FN</td>
</tr>
<tr>
<td></td>
<td>TN</td>
</tr>
<tr>
<td>Very difficult</td>
<td>Difficult</td>
</tr>
</tbody>
</table>

Sensitivity: \( \frac{TP}{TP + FN} \)

Specificity: \( \frac{TN}{TN + FP} \)

Accuracy: \( \frac{(TP + TN)}{(TP + TN + FP + FN)} \)

*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


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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


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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

Patient with chronic inflammation
And HPV infection

λ_{Ex}=330 nm

Fluorescence emission spectra of cervical tissues

Patient with CIN II, CIN III and HPV infection

\( \lambda_{\text{ex}} = 330 \text{ nm} \)

Average peak intensity from colposcopically normal and histologically abnormal tissues

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

**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 neoplastic lesions vs. non neoplastic 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 ...**
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
People working on the project

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Attila Major
Frank Lüdicke
Hélène Fältin-Traub

Norbert Lange

The nurse team

Georges Wagnières Didier Goujon

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