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Aspects of photodetection in cervical and ovarian neoplasia

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Chapter 2

An introduction to Photodetection techniques in gynecology

Abstract

Gynecologic tumors form an important cause of death, despite major improvements in early detection methods for this group of tumors. It is therefore necessary to continue efforts in the development of more accurate and sensitive diagnostic techniques. We have explored the use of photodetection in gynecologic oncology. Photodetection is particularly suitable for the identification of superficial (pre)cancerous tissue. We focused on its use for the detection of cervical neoplasms and abdominal metastases of ovarian tumors. In this chapter, some general concepts of photodetection and its historical background are described.

Introduction

The prognosis of gynecologic cancers is directly related to the stage at which the disease is diagnosed. It is therefore important to have diagnostic techniques available that are capable of accurate early detection, preferably in a routine screening setting. Besides early detection, diagnostic techniques capable of accurate determination of the invasiveness and precise size of a diagnosed lesion (staging) may significantly increase the success of the available treatment methods.

After identification of ovarian tumor, the patient receives cytoreductive surgery. The amount of residual tumor after this procedure is directly related to the prognosis of the patient. It is therefore of great importance to localize small implants.

The use of non-invasive optical techniques for diagnostic purposes has greatly expanded over the last few decades. In addition to traditional visual (white light) inspection, technologically highly advanced techniques are available for use or are being developed for the detection of suspected tissue. This group of optical techniques comprises Raman spectroscopy¹⁻⁴, optical coherence tomography⁵⁻¹⁰, color doppler¹¹⁻¹⁴, confocal microscopy^{15,16}, transillumination¹⁷, near infrared spectroscopy^{18,19}, fluorescence spectroscopy²⁰⁻²⁸, and fluorescence imaging²⁹⁻³². Each technique exploits specific differences between properties of tumor and normal tissue. This article focuses on the development and current status of fluorescence diagnostic techniques and its applications in gynecologic cancer.

Fluorescence diagnostics, or photodetection (PD) has been widely used to identify neoplastic tissue. The technique is based on concentration differences of a fluorescent dye (the photosensitizer) in normal versus neoplastic tissue. The neoplastic areas can be made visible when illuminated by a light source of a specific wavelength leading to excitation of the accumulated dye. When the excited molecules return to the ground state this is accompanied by emission of fluorescence, which can be detected on the tissue surface. The distribution of the fluorescence can be used to demarcate the area of pathologic cells. Variations in fluorescence intensity are, besides on the differences in concentration of fluorophores, also influenced by the tissue optical properties.

The techniques that are used to correct for these artifacts will be discussed in chapters 4 and 7. Either naturally occurring fluorescent tissue elements or exogenously administered photosensitizers can be used for photodetection.

A few concepts in photodetection are explained below.

Autofluorescence

When intrinsic tissue fluorescence is used (autofluorescence), fluorescence is emitted by naturally occurring tissue elements like NADH, porphyrins, flavins and collagen. Variations in concentration of these elements may be used to characterize tissue. Autofluorescence is usually induced with wavelengths in the UV part of the spectrum (<400 nm). Information is only obtained from a very superficial tissue layer, as the penetration of UV/blue light in most tissue types is limited (<500 μm). This is one of the disadvantages of using autofluorescence. Autofluorescence imaging requires sensitive equipment, which was only recently developed. Commercial devices are currently available for the detection of early stage cancer of the GI tract, ENT and lung (e.g. the Xillix Life[™] systems). Interpretation of the autofluorescence images is difficult due to the large influence of tissue optical properties, particularly scattering, and imaging geometry. This increases the amount of false positive readings, reducing the specificity of this diagnostic tool. Autofluorescence for the detection of cervical cancer is currently being studied by the group of Richards-Kortum^{20-22,33,34}.

Xeno fluorescence

Another way to perform photodetection is by administering a fluorescent dye to the patient, which will then accumulate preferentially in neoplastic tissue. The success of photodetection with exogenously administered photosensitizers depends on the differential gradient of the concentration of the dye in tumor versus host tissue. The dye should be tumor selective, with a rapid clearance from the body after the diagnostic procedure, and minimal side effects, like photosensitization of the skin.

Photosensitization

Most dyes that are used in photodetection also possess the ability to photosensitize tissue. Illumination of the tissue will then result in tissue damage. This property is used in Photodynamic therapy (PDT) for the destruction of neoplastic tissue but has to be avoided during photodetection. The photosensitizing effect depends on the tissue type, type and concentration of the dye, on the wavelength and fluence received for its activation, the tissue oxygenation level etc.

Fluorescent dyes.

The first photosensitizers applied in PD were hematoporphyrin derivative (HPD) and its

more purified form Photofrin®. These dyes have relatively low tumor specificity and cause a prolonged skin photosensitization in the patient. Despite these disadvantages, photofrin is still widely used in clinical PDT. Second generation photosensitizers are now also available, of which the most commonly used is Aminolevulinic acid (ALA). ALA is a precursor of Protoporphyrin IX (PpIX) in the heme synthesis, and it is naturally available in the human body in small concentrations. Adding excess amounts of (exogenous) ALA leads to much higher concentrations of PpIX, which selectively accumulate in neoplastic tissue. This selective accumulation of PpIX is thought to be due to an altered activity of heme biosynthetic pathway enzymes in such tissue. Excess PpIX production after administration of ALA also occurs in normal tissue, with a preference for mucosa e.g. in stomach, intestines, skin and bladder. Despite the concurrent accumulation of PpIX in normal tissue, useful tumor to normal concentration ratios are obtained with most investigated tissue types (e.g. bladder, skin, cervix, esophagus, brain).

The tumor selectivity, short half life (< 24 hrs) and mild photosensitization at lower dosages makes ALA an attractive fluorescent dye for PD applications.

New developments concentrate on the synthesis of ALA esters, aiming for better penetration of the skin and the introduction of other, non-phototoxic but still highly fluorescent dyes.

Fluorescence imaging

Fluorescence imaging techniques using red-light sensitive cameras e.g. in endoscopes, aim to accurately detect tumor borders and the extent and spread of disease to adjacent tissues. It may be used for the optimization of localized treatments of solid tumors e.g. in brain surgery. The amount of fluorescence detected at a certain position is related to the concentration of fluorescent particles in the tissue. The location and extent of the tumor can be determined from the concentration distribution in case of sufficient contrast of fluorescence between tumor and normal tissue.

Current research focuses on improvement of the technical devices in order to optimize sensitivity and specificity of PD, and on the wider application of application of these devices in various tumor types. An example of a commercially available imaging system using an exogenous fluorescent dye is that developed by Baumgartner *et al.* for detection of neoplastic tissue in the urinary bladder. Baumgartner's group claim a sensitivity of 96.9% for ALA based fluorescence detection of bladder cancer versus 72.7% for regular white light cystoscopy.

Fluorescence spectroscopy

Every molecule in the tissue that emits fluorescence light contributes to the total measured fluorescence signal. When these molecules have distinct fluorescence spectra, it is possible to extract their contribution by spectral analysis of the total measured fluorescence. In this way discrimination between tissue types is possible. Wagnieres *et al.* have demonstrated the feasibility of using this technique to discriminate between normal and diseased tissue³⁵. However, the components that produce autofluorescence usually have a broadband fluorescence spectrum, without distinct narrow peaks. The extraction of information from the measured fluorescence spectrum is therefore difficult. An appropriate spectral pattern-recognition algorithm is of crucial importance deciding whether an investigated site is normal or abnormal. The use of artificial neural networks for this purpose has been investigated for autofluorescence spectroscopy in the detection of vascular disease during angioplasty³⁶.

Fluorescence spectroscopy can also be used on tissue after administration of exogenous administration of a photosensitizer, the technique may then be used to correct for tissue optical properties, autofluorescence and geometrical factors.

Clinical history of photodetection in oncology.

The possible use of photodetection for the localization of tumors was apparent when Policard observed red tumor autofluorescence in experimental rat sarcomas after exposure to near ultraviolet light from a Woods lamp.³⁷ The first photodetection with an exogenously administered dye was reported in 1948 by Figue *et al.*, who observed increased fluorescence in tumors and tumor positive lymph nodes after injection of hematoporphyrin³⁸. Hematoporphyrin was the first photosensitizer to be exploited both in research settings and clinically, primarily for photodynamic therapy of skin and bladder tumors. Disadvantages of this dye are the low tumor specificity and long duration of photosensitization of the skin, which causes blistering after exposure to sunlight.

A step forward for Photodetection came in the sixties with the introduction of an improved photosensitizer Hematoporphyrin Derivative (HpD). HpD is synthesized by selection of the tumor localizing fractions from Hematoporphyrin to improve tumor localization. One of the pioneers was Lipson who performed a series of clinical studies on photodetection and photodynamic therapy of skin, bladder, endobronchial and gynecological tumors with this dye³⁹. In gynecology, cervical cancer was most frequently studied because of the possibility to apply the photosensitizer topically, the recognizable

pre-cancerous phase of the disease and the possibility to measure or monitor the fluorescence distribution non-invasively. In 1968 Lipson reported positively on the possibility to detect and treat cervical cancer²⁹. In the same year Gregorie Jr. published on 226 patients, of whom biopsies were taken of squamous cell and adenocarcinomas of the cervix. In 75-85% of patients histology correlated to fluorescence findings. However 23% of benign lesions appeared to be false positives. Lack of specificity as generally shown in literature made the clinical application of PD using HPD of little value^{40,41-44}. In the years thereafter more specific and less toxic photosensitizers and more appropriate light sources were developed. In 1984 Dougherty *et al.* published on the use of dehematoporphyrin esters (DHE) (fractionated HPD) (commercial name Photofrin®). Photofrin® had a better tumor selectivity compared to HPD but still kept the disadvantage of prolonged photosensitization of the skin⁴⁵. As Photofrin® is the drug that obtained FDA approval for the treatment (PDT) of several tumors it is clinically still used.

The photosensitizer that that is currently most used in research is Aminolevulinic acid (ALA). As explained before ALA is not the fluorescent dye itself but is converted in the cells to Protoporphyrin IX. In 1990 and 1992, Kennedy and Pottier published on the use of ALA for photodetection and photodynamic therapy^{41,46}. They found a tissue specific fluorescence (skin, topically applied ALA), which provided the basis for photodynamic therapy of basal cell carcinomas.

The feasibility to use Photodetection with ALA for the detection of cervical neoplasms was investigated by Pahernik *et al.*⁴⁷ They concluded that ALA photodetection as a diagnostic tool may allow the early detection of tumors. A selective accumulation of PpIX was found, particularly in CIN 3 lesions. The optimal time interval between ALA application and fluorescence detection was found to be between 150 and 250 minutes and a tumor to tissue selectivity of up to 3:1 was observed.

Baumgartner *et al.*⁴⁸ also studied the detection of cervical neoplasms. Optimal fluorescence contrast between tumor and normal tissue was obtained at approximately 90 minutes after application of ALA to the cervix in these studies. A combination of conventional colposcopy with photodetection to increase the sensitivity of the screening procedure was suggested. More recently, extensive work has been done in this field by Hillemanns *et al.*⁴⁹ who published a large study in which conventional colposcopy was compared with fluorescence imaging and fluorescence spectroscopy for detection of cervical intraepithelial neoplasms in patients. The performance of fluorescence imaging

equals that of colposcopy. When imaging is combined with spectral measurements, the specificity increases from 50 to 75 %.

We have studied the possibility of using photodetection to grade CIN using double ratio fluorescence imaging of ALA induced PpIX of the cervix. The study is described in more detail in chapter 8. The value of the double ratio, as determined at the site of biopsy, correlated significantly with the histopathologically determined stage of the disease. This suggests that non-invasive staging of CIN using this technique is feasible. We believe that the results of this study, if confirmed on a larger scale, justify the development of a dedicated device combining regular white light colposcopy with double ratio ALA-mediated fluorescence imaging.

The first pre-clinical articles on the use of photodetection techniques to identify ovarian cancer metastases in the abdominal cavity have recently been published⁵⁰⁻⁵². In 1996, Major *et al.* reported the results of a rat study demonstrating the possibility of localizing metastases in the abdominal cavity⁵². They found good tumor/normal fluorescence ratios at three hours after administration of 100 mg/kg ALA intravenously. Fluorescence levels after administration of 25 mg/kg were also suitable for photodetection. The results suggested that this technique could be used to detect small volume ovarian tumor metastases. Similar results were obtained by Hornung *et al.*⁵⁰ in 1998. They found that fluorescence detection of small (<0.5 mm) cancer nodules on the rat peritoneum was feasible after intravenous injection of 100 mg/kg ALA. The optimal time interval between drug injection and diagnosis of metastases was found to be three hours. This finding was confirmed by experiments done in our group (see chapter 4) in which we expanded the exploration of the technique to multiple ALA doses. Significant differences were found in the amount of fluorescence in tumor compared to other tissue types in the abdominal tissue at 2-3 hours after systemic administration of low (5mg/kg) and higher doses of ALA (25 and 100 mg/kg).

The next step is to move to clinical studies. Two studies on fluorescence diagnosis of endometriosis using ALA have been performed, which may be also be used to determine the optimal parameters for photodetection of ovarian cancer metastases in the abdominal cavity. In the first study³⁷ patients received 30 mg ALA/kg body weight orally 10 to 14 hours prior to surgery. The sensitivity of the fluorescence diagnosis in detecting endometriosis in non-pigmented areas and normal-looking peritoneum was 100%, with a specificity of 75%. Diagnosis by simple visualization under white illumination has a

sensitivity of only 69% and a specificity of 70%. Occult areas of endometriosis were discovered using fluorescence diagnosis. In the second study⁴⁹, 1 or 10 mg/kg ALA was administered orally to fifteen women referred for laparoscopy because of suspected endometriosis. Using the higher dose level (10 mg/kg) and application intervals of >3 hours significantly higher porphyrin fluorescence was observed in areas of active peritoneal endometriosis than in adjacent normal peritoneum. The parameters that were used in these studies may be helpful for the introduction of fluorescence diagnostics for the detection of ovarian cancer metastases in the abdominal cavity.

Technical improvements in detection and data processing equipment have provided the basis for renewed interest in the use of **intrinsic tissue fluorescence** for the characterization of tissue. Studies on autofluorescence diagnostics in gynecological cancers were published in 1991 by Glassman *et al.*⁵³ Emphasis was on the development of accurate algorithms and neural networks for the extraction of typical features for the different tissue types⁵⁴. Consistent differences were found between normal and diseased tissue after excitation with 300-320 nm light. The development of appropriate algorithms and the optimization of the excitation wavelength are the main topics of current research^{55,56,57}. Koumantakis also investigated the use of autofluorescence detection for abdominal cancers⁵⁷. Ex-vivo measurements on biopsies demonstrated a good discrimination between tumor and normal tissues using a new algorithm. Recently, Brookner *et al.* published on autofluorescence patterns in short term cultures of cervical tissue from cancer patients. They performed imaging and spectroscopy on the different layers of the cervix. The technique seems to be feasible for the detection of abdominal tumors although there was a large influence of patients' age on the fluorescence spectra. The group of Richards-Kortum^{22,58-61} has also worked extensively in this field. Their studies emphasize chemical recognition of the fluorophores and quantification of the amount of fluorophores in the tissue.

Discussion

There has been a rising interest in fluorescence diagnostics in the last few years. In a number of applications, photodetection has been shown to improve the accuracy of staging and localization of tumors. This is mainly due to the increased availability of dedicated light sources and detection equipment. The decreased systemic sensitization and increased tumor selectivity of the more recent photosensitizers also makes photodetection more attractive for clinical use. Ongoing phase I clinical studies are aiming at optimization of the technique in dose finding and toxicity studies. The feasibility of

photodetection of small intra-abdominal ovarian and cervical cancers has already been demonstrated as described in this chapter.

Future technical research will focus on further improvements in light sources. For example small and powerful diode lasers that emit blue light for excitation of the dye are now becoming available. The second technical goal in research must be the improvement of the cameras used for fluorescence imaging, since the detection of autofluorescence requires sensitive equipment. The detection of even smaller tumors may be possible with more sensitive cameras. A combination of small light sources and miniature cameras enables the development of new probes, which may greatly expand the possible applications of photodetection.

The development of the tumor localizing fluorescent dye is also important to increase the amount of possible applications for photodetection. Gynecological cancers comprise a wide variety of different cell types and locations which all require a specific approach and a photosensitizer with dedicated properties. For some applications it is important to look into the deeper layers within the tissue. A fluorescent dye that requires long excitation wavelengths will then be preferred in order to achieve adequate penetration.

Other gynecological applications not yet explored include the detection of vulvar neoplasia. Early detection is very important in this disease since more advanced, invasive squamous cell carcinoma of the vulva is usually treated with radical vulvectomy, which has a high morbidity

Another application of abdominal photodetection may be the screening of woman with inherited mutations in the BRCA1 or BRCA2 genes, which is associated with a greatly increased lifetime risk of developing ovarian cancer (63% and 27% respectively⁶²), breast cancer and a modestly increased risk of several other cancer types. Early detection of neoplasia in ovarian surface tissue may be of great importance for this group.

In conclusion, Photodetection is a diagnostic tool with potential in several oncological applications. Although a great deal of preclinical and clinical research remains to be done³⁵, rapid developments and large scale clinical introduction in several fields in gynecological cancers can be expected within the next decade.

References

1. R. Manoharan, K. Shafer, L. Perelman, J. Wu, K. Chen, G. Deinum, J. Myles, J. Crowe, R.R. Dasari and M.S. Feld, Raman spectroscopy and fluorescence photon migration for breast cancer diagnosis and imaging. *Photochem. Photobiol.*, 1998, **67**, 15-22.
2. T.R. Hata, T.A. Scholz, I.V. Ermakov, R.W. McClane, F. Khachik, W. Gellermann and L.K. Pershing, Non-invasive raman spectroscopic detection of carotenoids in human skin. *Journal of Investigative Dermatology*, 2000, **115**, 441-448.
3. E.B. Hanlon, R. Manoharan, T.W. Koo, K.E. Shafer, J.T. Motz, M. Fitzmaurice, J.R. Kramer, I. Itzkan, R.R. Dasari and M.S. Feld, Prospects for in vivo Raman spectroscopy, *Phys.Med. Biol.*, 2000, **45**, 51-59.
4. C.J. Frank, R.L. McCreery and D.C. Redd, Raman spectroscopy of normal and diseased human breast tissues. *Anal. Chem.*, 1995, **67**, 777-783.
5. A.V. D'Amico, M. Weinstein, X. Li, J.P. Richie and J. Fujimoto, Optical coherence tomography as a method for identifying benign and malignant microscopic structures in the prostate gland. *Urology*, 2000, **55** 783-787.
6. C.A. Jesser, S.A. Boppart, C. Pitris, D.L. Stamper, G.P. Nielsen, M.E. Brezinski and J.G. Fujimoto, High resolution imaging of transitional cell carcinoma with optical coherence tomography: feasibility for the evaluation of bladder pathology, *Br. J. Radiol.* , 1999, **72**, 1170-1176.
7. M. Wallace and J. Van Dam, Enhanced gastrointestinal diagnosis: light-scattering spectroscopy and optical coherence tomography, *Gastroint. End. Clin.N. Am.*, 2000, **10**, 71-80.
8. C. Pitris, A. Goodman, S.A. Boppart, J.J. Libus, J.G. Fujimoto and M.E. Brezinski, High-resolution imaging of gynecologic neoplasms using optical coherence tomography. *Obstet. Gynecol.* , 1999, **93** 135-139.
9. S.A. Boppart, M.E. Brezinski, C. Pitris and J.G. Fujimoto, Optical coherence tomography for neurosurgical imaging of human intracortical melanoma, *Neurosurgery*, 1998, **43**, 834-841.
10. U. Schaudig, A. Hassenstein, A. Bernd, A. Walter and G. Richard, Limitations of imaging choroidal tumors in vivo by optical coherence tomography. *Graefes Arch. Clin. Exp. Ophthal.*, 1998, **236**, 588-592.
11. M. Kawai, F. Kikkawa, H. Ishikawa, K. Tamakoshi, O. Maeda, N. Hasegawa, K. Mizuna, A. Suzuki, A. Itakura, N. Nakashima and Y. Tomoda, Differential diagnosis of ovarian tumors by transvaginal color-pulse doppler sonography. *Gynecol. Oncol*, 1993, **54**, 209-214.
12. A. Kurjak, M. Predanic, S. Kupesic-Urek and S. Jukic, Transvaginal color and pulsed doppler assessment of adnexal tumor vascularity. *Gynecol. Oncol.*, 1993, **50**, 3-9.
13. C.-C. Wu, C.-N. Lee, T.-M. Chen, M.-K. shyu, C.-Y. Hsieh, H.-Y. Chen and F.-J. Hsieh, Incremental angiogenesis assessed by color doppler ultrasound in the tumorigenesis of ovarium neoplasms, *Cancer*, 1993, **73** 1251-1256.
14. J. Carter, A. Saltzman, E. Hartenbach, J. Fowler, L. Carson and L.B. Twiggs, Flow characteristics in benign and malignant gynecologic tumors using transvaginal color flow doppler. *Obstet. Gynecol*, 1994, **83** 125-130.
15. D. Aghassi, R.R. Anderson and S. Gonzalez, Confocal laser microscopic imaging of actinic keratoses in vivo: a preliminary report. *J. Am. Acad. Derm.*, 2000, **43**, 42-48.

16. R.A. Drezek, T. Collier, C.K. Brookner, A. Malpica, R. Lotan, K. Richards and M. Follen, Laser scanning confocal microscopy of cervical tissue before and after application of acetic acid. *American Journal of Obst. Gynecol.*, 2000, **182**, 1135-1139.
17. M. Cutler, Transillumination as an aid in the diagnosis of breast lesions. *Surg. Gynecol. Obstet.*, 1929, **48** 721-729.
18. K. Licha, B. Riefke, V. Ntziachristos, A. Becker, B. Chance and W. Semmler, Hydrophilic cyanine dyes as contrast agents for near-infrared tumor imaging: synthesis, photophysical properties and spectroscopic in vivo characterization. *Photoch. Photobiol.*, 2000, **72** 392-398.
19. Q. Zhu, E. Conant and B. Chance, Optical imaging as an adjunct to sonograph in differentiating benign from malignant breast lesions. *J. Biomed. Opt.*, 2000, **5**, 229-236.
20. N. Ramanujam, M.F. Mitchell, A. Mahadevan, S. Thomsen, E. Silva and R. Richards-Kortum, Fluorescence spectroscopy: a diagnostic tool for cervical intraepithelial neoplasia (CIN). *Gynecol. Oncol.*, 1994, **52**, 31-38.
21. N. Ramanujam, M.F. Mitchell, A. Mahadevan, S. Warren, S. Thomsen, E. Silva and R. Richards-Kortum, In vivo diagnosis of cervical intraepithelial neoplasia using 337-nm-excited laser-induced fluorescence. *Proc. Nat. Acad. Scie. USA*, 1994, **91**, 10193-10197.
22. R. Richards-Kortum, M.F. Mitchell, A. Mahadevan and S. Thomsen, In vivo fluorescence spectroscopy: potential for non-invasive, automated diagnosis of cervical intraepithelial neoplasia and use as a surrogate endpoint biomarker., *J. Cel. Biochem. - Supp.*, 1994, **19**, 111-119.
23. H.J.C.M. Sterenborg, A.E. Saarnak, K.M. Hebeda and F.W. v.d. Meulen, In vivo tumor detectie met behulp van fluorescentie. *Klin. Fys.*, 1994, 273-277.
24. H.J. Sterenborg, S. Thomsen, S.L. Jacques and M. Motamedi, In vivo autofluorescence of an unpigmented melanoma in mice. Correlation of spectroscopic properties to microscopic structure. *Melanoma Res.*, 1995, **5**, 211-216.
25. A. Gillenwater, R. Jacob, R. Ganeshappa, B. Kemp, A.K. El-Naggar, J.L. Palmer, G. Clayman, M.F. Mitchell and K. Richards, Noninvasive diagnosis of oral neoplasia based on fluorescence spectroscopy and native tissue autofluorescence. *Arch. Otolaryngol. -- Head & Neck Surg.*, 1998, **124**, 1251-1258.
26. Saarnak, A. E. Evaluation of fluorescence measurement techniques for tumor detection in vivo. 1-12-1999. Thesis
27. E.W.J. van der Breggen, A.I. Rem, M.M. Christian, C.J. Yang, K.H. Calhoun, H.J.C.M. Sterenborg and M. Motamedi, Spectroscopic detection of oral and skin tissue transformation in a model for squamous cell carcinoma: Autofluorescence versus systemic Aminolevulinic Acid-induced fluorescence, *IEEE J. Sel. Top. Quant. Elec.*, 1999, **2** 997-1007.
28. C.K. Brookner, M. Follen, I. Boiko, J. Galvan, S. Thomsen, A. Malpica, R. Lotan and R. Richards-Kortum, Autofluorescence patterns in short-term cultures of normal cervical tissue, *Photochem. Photobiol.*, 2000, **71**, 730-736.
29. M.J. Gray, R. Lipson, J.V. Maeck, L. Parker and D. Romeyn, Use of hematoporphyrin derivative in detection and management of cervical cancer. *Am. J. Obstet. Gynecol.*, 1967, **99** 766-771.
30. O.J. Balchum, A.E. Profio, D.R. Doiron and G.C. Huth, Imaging fluorescence bronchoscopy for localizing early bronchial cancer and carcinoma in situ. *Progr. Clin. Biol. Res.*, 1984, **170** 847-861.

31. H. Kato, K. Aizawa, J. Ono, C. Konaka, N. Kawate, K. Yoneyama, K. Kinoshita, K. Nishimiya, H. Sakai and M. Noguchi, Clinical measurement of tumor fluorescence using a new diagnostic system with hematoporphyrin derivative, laser photoradiation, and a spectroscope. *Las. Surg. Med.*, 1984, **4**, 49-58.
32. B.W. Chwirot, S. Chwirot, J. Redzinski and Z. Michniewicz, Detection of melanomas by digital imaging of spectrally resolved ultraviolet light-induced autofluorescence of human skin, *Eur. J. Cancer*, 1998, **34** 1730-1734.
33. E.N. Atkinson, M.F. Mitchell, N. Ramanujam and R. Richards-Kortum, Statistical techniques for diagnosing CIN using fluorescence spectroscopy: SVD and CART. *J. Cell. Biochem. - Suppl.*, 1995, **23**, 125-130.
34. N. Ramanujam, M.F. Mitchell, A. Mahadevan-Jansen, S.L. Thomsen, G. Staerkel, A. Malpica, T. Wright, N. Atkinson and R. Richards-Kortum, Cervical precancer detection using a multivariate statistical algorithm based on laser-induced fluorescence spectra at multiple excitation wavelengths, *Photochem. Photobiol.*, 1996, **64** 720-735.
35. G.A. Wagnieres, W.M. Star and B.C. Wilson, In vivo fluorescence spectroscopy and imaging for oncological applications, *Photochem. Photobiol.*, 1998, **68**, 603-632.
36. G.R. Gindi, C.J. Darken, K.M. O'Brien, M.L. Stetz, and L.I. Deckelbaum Neural network and conventional classifiers for fluorescence-guided laser angioplasty. *IEEE Trans. Biomed. Eng.*, 1991, **38**, 246-252.
37. A. Policard, Etudes sur les aspects offerts par des tumeurs experimentales a la lumiere de woods. *C.R.Soc.Biol.*, 1924, **91**, 1423-1425.
38. R.H. Figge, Near infrared light rays and fluorescence phenomena as aids to discovery and diagnosis in medicine. *Univ. Md. Med. Bull.*, 1942, **26**, 165-168.
39. B.W. Henderson and T.J. Dougherty, Photodynamic therapy, Marcel Dekker INC, New York, 1992,
40. G.D. Wilbanks and R.M. Richart, Fluorescence of cervical intraepithelial neoplasia induced by tetracycline and acridine orange, *Am. J. Obstet. Gynecol.*, 1970, **106**, 726-730.
41. J.C. Kennedy and R.H. Pottier, Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy. *J Photochem Photobiol B: Biol*, 1992, **14** 275-292.
42. G.A. Kyriazis, H. Balin and R.L. Lipson, Hematoporphyrin-derivative-fluorescence test colposcopy and colpophotography in the diagnosis of atypical metaplasia, dysplasia, and carcinoma in situ of the cervix uteri, *Am. J. Obstet. Gynecol.*, 1973, **117**, 375-380.
43. B.T. Mossman, M.J. Gray, L. Silberman and R.L. Lipson, Identification of neoplastic versus normal cells in human cervical cell culture, *Obstet. Gynecol.*, 1974, **43**, 635-639.
44. J.S. McCaughan, H.F. Schellhas, J. Lomano and B.H. Bethel, Photodynamic therapy of gynecologic neoplasms after presensitization with hematoporphyrin derivative, *Las. Surg. Med.*, 1985, **5** 491-498.
45. J.P. Rovers, Fundamentals of Photodynamic Therapy. In "Photodynamic therapy for colorectal liver metastases: a preclinical study", 2001, thesis, pp. 17-30.
46. J.C. Kennedy, R.H. Pottier and D.C. Pross, Photodynamic therapy with endogenous protoporphyrin IX: basic principles and present clinical experience. *J.Photochem.Photobiol.*, 1990, **B8** 143-148.
47. S.A. Pahernik, A. Botzlar, P. Hillemanns, M. Dellian, M. Kirschstein, C. Abels, M. Korell, J. Mueller-Hoecker, M. Untch and A.E. Goetz, Pharmacokinetics and selectivity of aminolevulinic acid-induced porphyrin synthesis in patients with cervical intra-epithelial neoplasia. *Int.J.Cancer*, 1998, **78** 310-314.

48. R. Baumgartner and H. Stepp, Photodynamic diagnosis using 5-aminolevulinic acid (5-ALA) for minimally-invasive treatment of cancer. *Min. Invas. Ther. Allied Techn.*, 1998, **7(6)** 495-501.
49. P. Hillemans, H. Weingandt, R. Baumgartner, J. Diebold, W. Xiang and H. Stepp, Photodetection of cervical intraepithelial neoplasia using 5-aminolevulinic acid-induced porphyrin fluorescence. *Cancer*, 2000, **88**, 2275-2282.
50. R. Hornung, A.L. Major, M. McHale, L.H.L. Liaw, L.A. Sabiniano, B.J. Tromberg, M.W. Berns and Y. Tadir, In vivo detection of metastatic ovarian cancer by means of 5-aminolevulinic acid-induced fluorescence in a rat model. *J.AM.Assoc.Gynecol.Laparosc.*, 1998, **5**, 141-148.
51. M.C.G. Aalders, H.J.C.M. Sterenberg, F.A. Stewart and N. van der Vange, Photodetection with 5-aminolevulinic acid induced protoporphyrin ix in the rat abdominal cavity: drug dose dependent fluorescence kinetics. *Photochem. Photobiol.*, 2000, **72(4)**, 521-525.
52. A.L. Major, G.S. Rose, C.F. Chapman, J.C. Hirserodt, B.J. Tromberg, T.B. Krasieva, Y. Tadir, U. Haller, P.J. DiSaia and M.W. Berns, In vivo fluorescence detection of ovarian cancer in the NuTu-19 epithelial ovarian cancer animal model using 5-aminolevulinic acid (ALA). *Gynecol. Oncol.*, 1996, **66** 122-132.
53. W.S. Glassman, C.H. Liu, G.C. Tang, S. Lubicz and R.R. Alfano, Ultraviolet excited fluorescence spectra from non-malignant and malignant tissues of the gynecological tract. *Las. Life Scie.*, 1991, **5** 49-58.
54. M.F. Mitchell, S.B. Cantor, N. Ramanujam, G. Tortolero-Luna and R. Richards-Kortum, Fluorescence spectroscopy for diagnosis of squamous intraepithelial lesions in the cervix, *Obstet gynecol.* 1999, **93** 462-470.
55. M.F. Mitchell, S.B. Cantor, C. Brookner, U. Utzinger, D. Schottenfeld and R. Richards-Kortum, Screening for squamous intraepithelial lesions with fluorescence spectroscopy. *Obstet. Gynecol.*, 1999, **94** 889-896.
56. N. Ramanujam, M.F. Mitchell, A. Mahadevan, S. Thomsen, A. Malpica, T. Wright, N. Atkinson and R. Richards-Kortum, Development of a multivariate statistical algorithm to analyze human cervical tissue fluorescence spectra acquired in vivo, *Las. Surg. Med.*, 1996, **19** 46-62.
57. E. Koumantakis, A. Vasileiou, A. Makrigiannakis, E. Unsold and T.G. Papazoglou, Spectral variations of laser-induced tissue emission during in vivo detection of malignancies in the female genital tract. *J. Photochem. Photobiol.*, 1997, **B40** 183-186.
58. R. Richards-Kortum and E. Sevick-Muraca, Quantitative optical spectroscopy for tissue diagnosis. *Ann. Rev. Phys. Chem.*, 1996, **47** 555-606.
59. A. Agrawal, U. Utzinger, C. Brookner, C. Pitris, M.F. Mitchell and R. Richards-Kortum, Fluorescence spectroscopy of the cervix: influence of acetic acid, cervical mucus, and vaginal medications. *Las. Surg. Med.*, 1999, **25**, 237-249.
60. D.L. Heintzelman, R. Lotan and K. Richards, Characterization of the autofluorescence of polymorphonuclear leukocytes, mononuclear leukocytes and cervical epithelial cancer cells for improved spectroscopic discrimination of inflammation from dysplasia, *Photochem. Photobiol.*, 2000, **71**, 327-332.
61. M. Follen and R. Richards-Kortum, Emerging technologies and cervical cancer, *J. Nat.Canc. Inst.*, 2000, **92** 363-365.
62. Lancaster JM, Carney ME, Futreal PA., BRCA 1 and 2--A Genetic Link to Familial Breast and Ovarian Cancer., *Med. Wom. Health* 1997 **2(2)**, 7.