Diagnosis of vulvar lesions by non-invasive optical analysis: a pilot study

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Abstract

A procedure that could allow an early *in vivo* and non-invasive detection of vulvar lesions would be extremely useful. We tested an innovative optical method (Optiprobe), which uses a harmless, visible light source for the *in vivo*, on-line detection of minimal alterations in the structure of vulvar epithelium. A group of 3 female volunteers without gynecological symptoms were first screened to evaluate optical properties of normal vulvar tissue. Next, a group of 16 patients undergoing gynecological examination for vulvar lesions was evaluated by the Optiprobe at suspected sites before these sites were biopsied for histological analysis. Adjacent, non-involved sites were also measured to provide internal controls. Histological analysis of the biopsies identified one case that did not show obvious alterations, 4 cases of high-grade vulvar intraepithelial neoplasia (VIN), 5 cases of vulvitis, and 6 cases of lichen sclerosis (LS).

The optical properties of the VIN cases were significantly different from those of controls, due to a decrease in the absorption spectra and an increase in the scattering spectra. In contrast, a significant increase in the absorption spectra and a decrease in the scattering spectra were observed in the cases of vulvitis. In the LS cases, the absorption spectra were as in controls, whereas the scattering spectra were significantly decreased. We conclude that the Optiprobe provides a useful tool for a rapid and non-invasive detection of vulvar alterations. The method should contribute to reduce the number of biopsies and to facilitate the long-term follow-up of vulvar lesions.

Introduction

Vulvar squamous cell carcinoma of the vulva (SCC) accounts for about 4% of all gynecological malignancies.[1] It has an incidence of about 1.8 per 100,000, which peaks at approximately 20 per 100,000 after the age of 75, making it as common as cervical carcinoma.[2,3] SCC is usually slow growing and begins as a pre-malignant lesion, referred to as vulvar intraepithelial neoplasia (VIN).[4] This lesion actually comprises a spectrum of epithelial changes that range from mild to severe dysplasia, which immediately precede invasive carcinoma. Although advanced VIN lesions may appear as variably pigmented patches under vulvoscopy investigation, they may be difficult to distinguish from a variety of benign diseases. This difficulty often leads to delayed diagnosis, until the obvious stage of invading squamous cell carcinoma.[5] Presently, the standard procedure for definitive diagnosis consists in performing a biopsy of all suspected areas, even though only a fraction of these are at risk of neoplastic transformation.[6] Because vulvar sampling is painful and bears infectious risks for the patient, an alternative method that would allow for a non-invasive diagnosis would be of great value in the screening programs of vulvar cancer.

Light-scattering spectroscopy is a recently developed optical method that relies on the *in situ* measurement of the light back-scattered (reflectance) from an illuminated tissue. The two underlying ideas are that light propagation, and thus reflectance, is modified under conditions altering the structure and/or composition of a tissue,[7-11] and that optical coefficients can be derived from reflectance values, which provide information on these alterations.[12-14] Thus, the absorption coefficient, which is thought to reflect the concentration of chromophores, and the scattering coefficient, which is thought to reflect the size and numerical density of cells and organelles, significantly differ in normal and pathological tissues.[15-18]

We have built an optical set-up, referred to as Optiprobe, which comprises a multi-fiber mini-probe to screen small tissue volumes of about 1 mm^3.[17,19] We have found that this equipment allows for an *in vivo*, real-time detection of minimal tissue lesions, like those expected at the beginning of inflammatory and cancerous alterations.[15,20] Here we have assessed whether the method has a sufficient sensitivity to identify alterations of vulvar tissues in patients undergoing vulvoscopy. We report that the Optiprobe method discriminated normal and pathological cases, with a sensitivity and specificity that compared well with those of histological analysis, while avoiding the need of invasive biopsies.

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Materials and Methods

Patient selection

The study was approved by the Committee for Ethics in Research of the University of Geneva Hospitals, and conducted according to the guidelines of this Committee. After informed consent, a first group of 3 healthy volunteers were screened with the Optiprobe technology to determine the optical properties of normal vulvar tissue. A second group of 16 patients, undergoing vulvoscopic examination for suspicion of vulvar lesions, were similarly evaluated.

The very same sites were then sampled for standard histological analysis. Sites of non-involved tissue from nearby regions were also

measured to provide an internal control. The optical-biopsy combined procedure did not imply additional discomfort for the patients, inasmuch as the fiber-optic probe was gently applied on vulvar surfaces for illumination with a harmless visible light source. On average, the duration of the combined vulvoscopic-optical-biopsy procedure was about 10 min longer than that of the standard vulvoscopic-biopsy examination.

Optical measurements

The optical mini-probe set-up, and the theory of the method used to collect reflectance signals and to derive optical coefficients, have been previously described.[17,19,21,22] Briefly, the tip of the hand-held optical probe was applied on the vulvar epithelium. Once in place, a 400 msec pulse of white-light (wavelength range 480-950 nm) provided by a halogen lamp (Ocean Optics LS-1) was delivered through the source fiber of the probe. Intensity of reflectance, i.e. light backscattered from the illuminated tissue, was simultaneously recorded at various distances from the source, using the 10 collecting fibers of the probe. Collected reflectance was brought to a spectroscope (Jobin-Yvon CP 200), focused on a cooled CCD camera (Hamamatsu C7041) during an exposition time controlled by a mechanical shutter, and computer digitized in 16 bits (National Instruments PCI-MIO-16E-4). Reflectance measurements were repeated 6-8 times per site and averaged. In each patient, at least one suspicious and one control site were probed before the surgical biopsy.

Once the gynecological examination was completed, the acquired reflectance spectra were analyzed using a Monte Carlo simulation and a modified form of the Henyey-Greenstein phase function to compute an absorption and a scattering coefficient. These optical parameters are thought to reflect the interaction of light with chromophores and the changes of light propagation in small tissue volumes, respectively.[17,19,21]

Histology

Biopsies of the vulvar tissue, taken at the very same sites that were probed optically, were fixed in 10% formol and processed for standard histological analysis by an expert pathologist. In this study, each sample was assigned to a normal, lichen sclerosis, VIN or a vulvitis group.

Statistics

Optical measurements from altered and control sites were compared using appropriate non-parametric statistics, as provided by the SPSS program (SPSS Inc., Chicago, USA). Sensitivity of optical analysis (hereafter referred to as tests) was estimated by dividing the number of true positive cases (i.e. cases showing positive tests in patients with abnormal histology) by the number of true plus false negative cases (i.e. cases showing normal tests in patients with abnormal histology). Specificity of tests was evaluated by dividing the number of true negative cases (i.e. cases showing normal tests in patients with normal histology) by the number of true negative cases plus false positive cases (i.e. cases showing altered tests in patients with normal histology).

Results

The optical spectra of normal vulvar tissue changes with hormonal status

The optical properties of normal vulvar tissue were first evaluated by recording the absorption and scattering spectra in 3 healthy volunteers, whose vulva was clinically normal. In each case, maximal light absorption was observed between 400 and 500 nm (Figure 1A), reflecting the normal presence of chromophores oxy and deoxy-hemoglobin within surface tissues. In the same volunteers, the scattering coefficient decreased as a function of increasing wavelength (Figure 1B), consistent with the light-scattering characteristics of surface tissues. Comparison between the recordings from the 3 volunteers revealed significant ($p<0.001$) differences in both absorption (Figure 1A) and scattering (Figure 1B) coefficients. These differences were represented by a variation coefficient varying from 25-140% for the absorption spectra (Figure 1C), and from 5-20% for the scattering spectra (Figure 1D).

Histology set the standard for the retrospective validation of the optical findings

Immediately after the optical measurements, a biopsy was taken at each of the 16 suspected sites that had been investigated. The histopathological scoring of these samples resulted in the identification of one site showing no detectable alteration, 4 sites showing high-grade VIN, 5 sites with vulvitis and 6 sites with lichen sclerosis.

Optical analysis distinguished normal and pathological vulvar epithelium

Absorption and scattering coefficients were determined for wavelengths between 400 and 700 nm, in 16 sites suspicious of vulvar lesion and in nearby sites that appeared phenotypically not involved and were used as controls. Non-parametric statistics confirmed that the optical properties of VIN sites were significantly ($p<0.001$) different from those of controls, due to a decrease in the absorption coefficient (Figure 2A) and an increase in the scattering coefficient (Figure 3A). Thus, on average, the absorption and scattering coefficients of VIN cases were 50% and 40% those of controls (Figures 2D and 3D). Taking these values as optical threshold for diagnosis, we calculated that the Optiprobe would detect VIN cases with a sensitivity of 75% (absorption) -

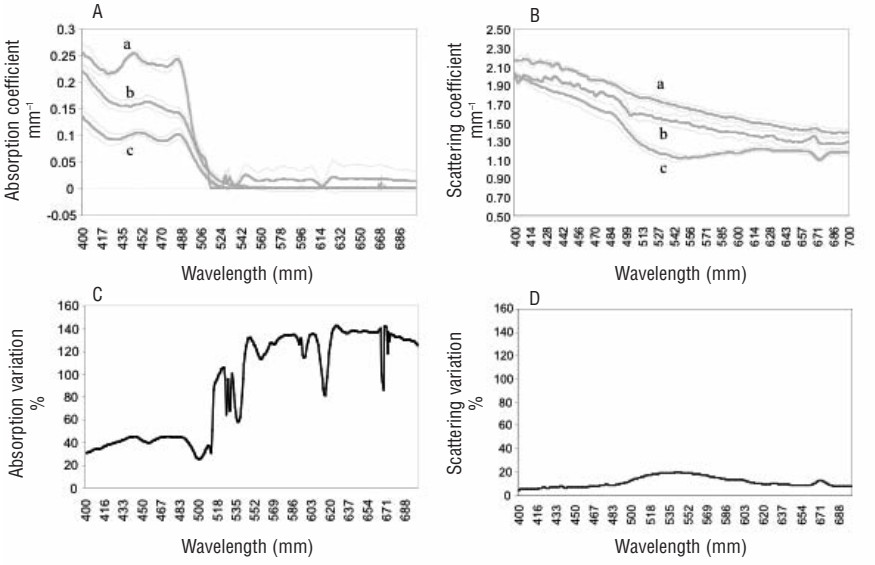

Figure 1. Optical spectra of normal vulvar tissue vary between healthy women. Mean (bold line) and standard deviations (thin lines) of the absorption (A) and scattering coefficients (B) measured on 3-4 adjacent sites in each healthy volunteer (a, b and c are recordings from the 52-, 48- and 34-year old women, respectively.

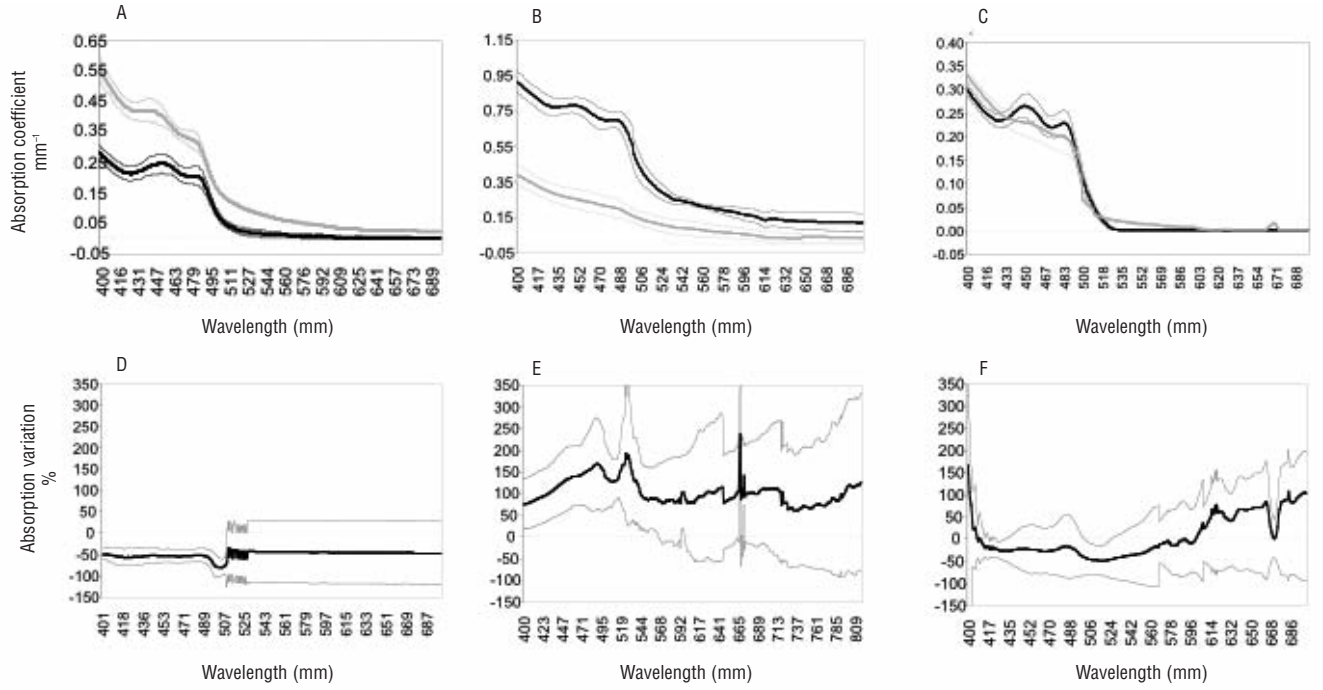

Figure 2. (A-C) Absorption analysis discriminated VIN and inflammatory lesions, but not lichen sclerosis from normal tissue. (A) Mean (bold line) and standard deviations (thin lines) of absorption spectra obtained at sites featuring histology characteristics of VIN (A), inflammation (B) and lichen sclerosis (C). Absorption spectra of VIN and vulvitis (black) were significantly (p<0.001) different from those of controls spectra (gray), for light wavelengths of 400-700 nm. In contrast, absorption spectra of lichen sclerosis (black) were not different from those of controls (gray). (D-F) Mean (bold line) and standard deviations (thin lines) of percentage of change in absorption spectra between pathological and control sites were calculated for the 4 VIN (D), the 5 vulvitis (E), and the 6 lichen sclerosis sites (F). Graphs show that VIN lesions induced a significant decrease of absorption between 400 nm and 500 nm, whereas a significant increase was observed at inflammatory sites. No significant change was observed at lichen sclerosis sites.

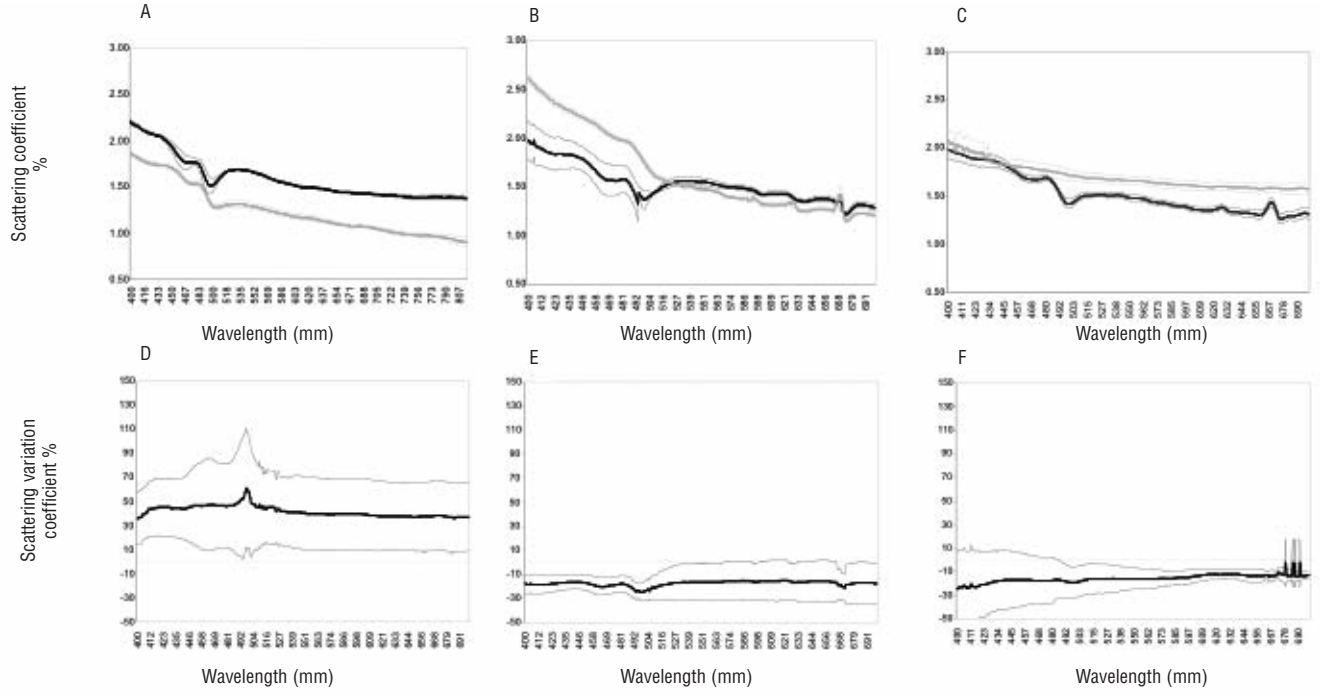

Figure 3. (A-C) Scattering analysis discriminated VIN, inflammatory and lichen sclerosis lesions from normal tissue. Mean (bold line) and standard deviations (thin lines) of scattering spectra obtained in women diagnosed with VIN (A), inflammation (B) and lichen sclerosis (C). Scattering spectra of VIN sites, vulvitis sites and lichen sclerosis sites (black) were significantly (p<0.001) different from controls spectra (gray) for light wavelengths of 400-700 nm, 400-500 nm, and 500-700 nm, respectively. (D-F) Mean (bold line) and standard deviations (thin lines) of percentage change over control was calculated for the 4 VIN (D), the 5 vulvitis (E) and the 6 lichen sclerosis sites (F). Graphs show significant scattering variation in VIN, vulvitis and lichen sclerosis cases, for light wavelengths of 400-700 nm, 400-510 nm and 500-700, nm respectively.

50% (scattering).

Compared to controls, sites of vulvitis featured significantly ($p<0.001$) increased absorption values (Figure 2B) and decreased scattering values (Figure 3B). When expressed as percentage of the corresponding control values, the average absorption and scattering values of vulvitis cases represented 100% and 20% of control values, respectively (Figures 2E and 3E). Using these values, the test discriminated normal from vulvitis cases with a sensitivity of 80% for both absorption and scattering coefficients.

The absorption coefficients evaluated at sites of lichen sclerosis were not significantly different from controls for all investigated light wavelengths (Figure 2 C,F). In contrast, a significant ($p<0.001$) decrease in scattering spectra was observed (Figure 3C), resulting, on average, in a 20% of variation from control values (Figure 3F). Using this scattering variation as threshold optical limit, the diagnostic test differentiated lichen sclerosis from normal cases with a sensitivity of 66%.

Discussion

We have developed a new optical procedure and equipment for a non-invasive, *in vivo* analysis of small tissue volumes. The procedure is based on the spectroscopic measurement of the reflectance of white light, combining wavelengths of 400-700 nm, from which various optical parameters can be derived.[28, 32,33] Among these parameters, scattering and absorption coefficients are of particular interest for medical diagnosis inasmuch as they are thought to provide information about changes in cell structure and organization within tissues, and in content of various molecules, respectively.[35-38] We have already shown that the determination of these optical coefficients enable minimal alterations of skin surface to be detected in an experimental murine model,[26] and can also correctly evaluate human mucosa in patients undergoing gastroscopy for suspected gastritis alterations.[20] Here, we have tested whether the procedure can also be used to detect alterations of the vulvar epithelium, including VIN, vulvitis and lichen sclerosis. We have further assessed whether the method has a sufficient sensitivity to become a useful complement of standard clinical diagnosis, while limiting the need for an invasive biopsy for histological diagnosis.

To address these questions, we have first studied the optical properties of normal vulvar tissue in 3 volunteer women. The major light absorption for 420-480 nm wavelengths featured the presence of hemoglobin, which corresponds to the main chromophore encountered by probing photons.[23,24] Scattering spectra revealed a slow decrease correlated to the increase of wavelengths, as is usually observed with surface biological tissues.[23,25] Although both absorption and scattering measurements had a comparable shape in all volunteers, we observed significant differences in their absolute values. These differences could not be attributed to variations of the optical set-up, which probed stable standards with a variation coefficient of less than 5% for absorption and scattering measurement. Thus, the variation coefficients observed for absorption (30-140%) and scattering (5-20%) measurements of normal vulvar tissue clearly resulted from inter-individual variation. While the reason of this variation remains to be determined (could this be due to hormonal status?) the data imply the necessity of having a proper internal control for each individual undergoing the Optiprobe analysis. We have followed this strategy to investigate a series of 16 patients undergoing vulvoscopy for evaluation of benign or pre-malignant vulvar lesions by comparing the measurements made at suspected sites to those of phenotypically not-involved sites that were used as internal controls. We found significant variations in scattering and/or absorption coefficients in all the three types of lesions that were investigated. While the nature of the biological parameters that affected the optical coefficients remains to be determined, this method provides a new tool for a rapid distinction between pathological and normal sites. The changes in absorption and scattering appeared specific for a given type of lesion, inasmuch as they were decreased or increased differentially in cases of VIN, vulvitis, and lichen sclerosis. The observation of these combined variations in a clinical setting should indicate the type of lesion. Our analysis of spectral variations between pathological sites and control counterparts also enabled us to determine the wavelengths allowing for the best detection of each type of lesion. The absorption and scattering coefficients estimated at these wavelengths determined a sensitivity of the approach of more than 75% for the detection of both VIN and vulvitis cases, and of 66% for the detection of lichen sclerosis. These values show that the Optiprobe instrument can detect a majority of vulvar lesions. Further refinements of the set-up, based on a combined analysis by spectroscopic and fluorescence methods or tissue imaging, should further improve its performance. Our analysis shows promising results for a future application of optical methods in the diagnosis of vulvar lesions. Improvement in the mathematical processing of the optical coefficients from the reflectance measurements may facilitate the routine, clinical use of the method.

To conclude, we report here on a novel optical method that efficiently detects vulvar lesions with a sensitivity that compares well with the standard biopsy-histology approach, while avoiding the tissue invasion required by the latter approach. This method should prove a useful complement to clinical diagnosis of vulvar lesion in directing the biopsy procedure, as well as in guiding invasive therapeutic acts (e.g. laser irradiation, excision) to only relevant areas of the vulvar surface.

References

1. Eifel P, Levenback C. Cancer of the lower genital tract. Ed. B.D.Inc London Hamilton 2001;1-8.
2. Silverberg E. Statistical and epidemiological information on gynecological cancer. American Cancer Society 1986;80:9.
3. Cramer D. Epidemiology of the gynaecologic cancers. Com Theor 1978;4:9-17.
4. Hart WR. Vulvar intraepithelial neoplasia: historical aspects and current status. Int J Gynecol Pathol 2001;20:16-30.
5. Joura EA. Epidemiology, diagnosis and treatment of vulvar intraepithelial neoplasia. Curr Opin Obstet Gynecol 2002;14:39-43
6. Girardi F, Pickel H, Joura EA, et al. Guidelines for diagnosis and therapy of intraepithelial neoplasia and early invasive carcinoma of the female lower genital system (cervix uteri, vagina, vulva) established by the AGK (Colposcopy Work Group in the OGGG) Gynakol Geburtshilfliche Rundsch 2001;41:197-200. German
7. Rolfe P. In vivo near-infrared spectroscopy. Annu Rev Biomed Eng 2000;2:715-54.
8. Wallace VP, Crawford DC, Mortimer PS, et al. Spectrophotometric assessment of pigmented skin lesions: methods and feature selection for evaluation of diagnostic performance. Phys Med Biol 2000;45:735-51.
9. Koenig F, Larne R, Enquist H, et al. Spectroscopic measurement of diffuse reflectance for enhanced detection of bladder carcinoma. Urology 1998;51:342-5.
10. Marchesini R, Brambilla M, Clemente C, et al. In vivo spectrophotometric evaluation of neoplastic and non-neoplastic skin pigmented lesions--I. Reflectance measurements. Photochem Photobiol 1991;53:77-84.
11. Mourant JR, Bigio IJ, Boyer J, et al. Elastic scattering spectroscopy as a diagnostic tool for differentiating pathologies in the gastrointestinal tract: preliminary testing. J Biomed Optics 1996;1:192-9.
12. Kienle A, Lilge L, Patterson MS, et al. Spatially resolved absolute diffuse reflectance measurements for noninvasive determination of the optical scattering and absorption coefficients of biological tissue. Appl Opt 2002;35:2304-14.

13. Bays R, Wagnières G, Robert R, et al. Clinical determination of tissue optical properties by endoscopic spatially resolved reflectometry. Appl. Opt 1996;35:1756-66.
14. Nair MS, Ghosh N, Raju NS, et al. Determination of optical parameters of human breast tissue from spatially resolved fluorescence: a diffusion theory model. Appl Opt 2002;41:4024-35.
15. Charvet I, Vermeulen B, Thueler P, et al. A new optical method for the non invasive detection of minimal tissue alterations. Phys Med Biol 2002;47:2095-108.
16. Tromberg BJ, Shah N, Lanning R, et al. Non-invasive in vivo characterization of breast tumors using photon migration spectroscopy. Neoplasia 2000;2:26-40.
17. Bevilacqua F, Dominique Piguet, Pierre Marquet, et al. In vivo local determination of tissue optical properties: applications to human brain. Applied Optics 1999;38: 4939-50.
18. Zonios G, Perelman L, Backman, et al. Diffuse reflectance spectroscopy of human adenomatous colon polyps in vivo. Applied Optics 1999;38:6628-37.
19. Bevilacqua F, Depeursinge C. Monte Carlo study of diffuse reflectance at source–detector separations close to one transport mean free path. J Opt Soc Am A 1999;16:2935-45.
20. Charvet I, Ory G, Thueler P, et al. Diagnosis and grading of gastritis by non invasive optical analysis. Eur J Gastro 2004;16:1089-98.
21. Thueler P, Charvet I. Bevilacqua F, et al. In vivo endoscopic tissue diagnostics based on spectroscopic absorption, scattering and phase function properties. J Biomed Opt 2003;8:495.
22. Thueler P, Ghislain M, CD Depeursinge. Optical local and superficial probing of tissues for in vivo diagnosis. Proceedings of SPIE 2003; 5141:266-71.
23. Dawson JB, Barker DJ, Ellis DJ, et al. A theoretical and experimental study of light absorption and scattering by in vivo skin Phys Med Biol 1980;25:695-709.
24. Anderson RR, Parrish JA. The optics of human skin. J Invest Dermatol 1981;77:13-19.
25. Mourant JR, Canpolat M, Brocker C, et al. Light scattering from cells: the contribution of the nucleus and the effects of proliferative status. J Biomed Opt 2000;5:131-7.
26. Nappi RE, Mancini M, Veneroni F, et al. Clitoral artery blood flow in healthy young women: preliminary report on menstrual cycle and hormonal contraception. J Sex Marital Ther 2002;28:187-93.
27. Nauth HF, Haas M. Cytologic and histologic observations on the sex hormone dependence of the vulva. J Reprod Med 1985;30:667-74.