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Abstract. Vulvar squamous cell carcinoma (VSCC) is a gynecological cancer with an incidence of two to three per 100,000 women. VSCC arises from vulvar intraepithelial neoplasia (VIN), which is diagnosed through painful punch biopsy. In this study, optical coherence tomography (OCT) is used to differentiate between normal and VIN tissue. We hypothesize that (a) epidermal layer thickness measured in OCT images is different in normal tissue and VIN, and (b) quantitative analysis of the attenuation coefficient (μ_{oct}) extracted from OCT data differentiates VIN from normal vulvar tissue. Twenty lesions from 16 patients are imaged with OCT. Directly after data acquisition, a biopsy is performed. Epidermal thickness is measured and values of μ_{oct} are extracted from 200 OCT scans of normal and VIN tissue. For both methods, statistical analysis is performed using Paired Mann–Whitney-test. Correlation between the two methods is tested using a Spearman-correlation test. Both epidermal layer thickness as well as the μ_{oct} are different between normal vulvar tissue and VIN lesions ($p < 0.0001$). Moreover, no correlation is found between the epidermal layer thickness and μ_{oct}. This study demonstrates that both the epidermal thickness and the attenuation coefficient of vulvar epithelial tissue containing VIN are different from that of normal vulvar tissue. [DOI: 10.1117/1.JBO.17.11.116022]

Keywords: optical coherence tomography; vulvar intraepithelial neoplasia; imaging.

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1 Introduction

In the last 30 years, the incidence of vulvar intraepithelial neoplasia (VIN)—a premalignant skin disorder that often causes pruritus, pain, and psychosexual dysfunction—has increased more than 400% to approximately 2.5 cases per 100,000 women in the United States.[1] In the Netherlands, the incidence of VIN was 2.2 per 100,000 women a year in 2005.[2] VIN was previously graded into VIN 1 to 3. Recently, a new classification was adapted, which divides VIN into differentiated-type and usual-type VIN (dVIN and uVIN).[3] dVIN is associated with lichen sclerosis, and uVIN is caused by a persistent infection of human papillomavirus (HPV).[4,5] Both types may progress into invasive vulvar squamous cell carcinoma (VSCC). The incidence of VSCC has risen by 20%, making it the fourth most common gynecological type of cancer, with an incidence of 2.2 cases per 100,000 women annually in the United States.[1] The progression rate of VIN into VSCC is about 9% in untreated patients, and 3.3% in patients after treatment.[6] Overall, the rise in incidence of VIN and VSCC is mainly seen in women younger than 50 years.[1,7–9]

In case of a VIN lesion, treatment consists of conservative surgical excision, laser vaporization or medical therapy. However, every attempt is made to avoid vulvar mutilation that may possibly lead to psychosexual distress.[1,10,11] Recently, two medical treatments were studied in VIN.[12,13] In 2008, a randomized controlled trial demonstrated that imiquimod 5% cream (Aldara, 3M Pharmaceuticals) was successful in the treatment of VIN, although it is not yet approved by the U.S. Food and Drug Administration for this purpose.[12] In 2009 a prospective study with therapeutic vaccination was also successful in treating VIN.[13] Nevertheless, even with imiquimod 5% cream or therapeutic vaccines, there is a chance of occult invasion and of recurrence of VIN after treatment. Therefore, patients are regularly examined to foresee occult invasion and check for possible new VIN lesions.[6] However, the only way to obtain definite diagnosis in case of a vulvar lesion of uncertain significance is by taking a punch biopsy, which can be painful.

Thus both diagnosis and follow-up after treatment express the urgent need for a fast, effective diagnostic tool for noninvasive assessment of VIN lesions. Optical coherence tomography (OCT) might be such a tool. OCT image formation is equivalent to ultrasonography, except that back-scattered light instead of back-reflected sound waves is used to produce cross-sectional images. The micro meter-scale resolution images range to approximately 2 mm in depth: a limitation mainly due to light scattering, which causes a decrease of OCT signal magnitude with increasing depth.

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The first clinical application of OCT was in ophthalmology two decades ago to obtain *in vivo* cross-sections of the anterior[14] and posterior segment,[15,16] to diagnose glaucoma and corneal diseases, resp. retinal diseases.[17] Nowadays OCT is commercially available and is widely used in ophthalmology. Besides ophthalmology, OCT is gaining momentum in other fields of specialties, such as cardiology and oncology.[18–22] In gynecology, OCT is not yet established in the clinic, though several clinical studies have been performed. In one of these studies, OCT images of normal cervical tissue and cervical intraepithelial neoplasia (CIN) lesions were compared with histology reports. All images of normal cervix exhibited a repetitive pattern that presented normal squamous epithelium, contrary to the images of tissue that contained CIN II,III-lesions. Those images showed an unstructured homogeneous highly backscattering region with fast attenuation of the signal in 89% of the patients. In the same study, three patients with Paget's disease of the vulva (a potential premalignant lesion) were imaged with OCT. When studying the images, the authors observed clear irregularities in the epithelial layer. Moreover, the basement membrane was no longer present in the microstructure.[23] During transition from VIN to invasive carcinoma, the basement membrane is interrupted and becomes discontinuous or absent.[24,25] In addition, in VIN, cells grow, change, and the epithelial layer thickens.[26] This layer thickness can be measured from OCT images,[23] though it does not provide information about the architectural and cellular changes that occur in the layer itself during carcinogenesis. These changes can be elucidated from the light scattering properties[27] that are measured from the signal decrease with depth from OCT images, which is quantified by the attenuation coefficient μ_{oct}. Studies have shown that quantitative measurement of μ_{oct} allows *in vivo* differentiation between different tissue types; for example, atherosclerotic plaque components.[28–30] In the kidney, it was shown OCT can distinguish between normal renal tissue and renal cell carcinoma.[31,32]

We therefore hypothesize that OCT can be used as an optical imaging tool to differentiate between VIN lesions and healthy vulvar tissue, enabling the gynecologist real-time measurement of suspicious lesions and reducing the need to perform a physical biopsy. The optical imaging consists of qualitative assessment of OCT volumetric imaging, quantification of the epidermal layer thickness through direct measurement from the OCT images, and attenuation coefficient measurement to determine cellular organization in the epidermal layer.

2 Materials and Methods

2.1 *Data Collection*

From August 2010 until June 2011, we performed a prospective study in patients with clinical suspicion of VIN from whom a punch biopsy or a local excision had to be taken in the outpatient clinic or in the operation room of the Netherlands Cancer Institute in Amsterdam, the Netherlands. Patient characteristics are given in Table 1. This study was approved by the Medical Ethical Committee of our institute. Written informed consent was obtained from all patients included. In total, 16 consecutive patients with a total of 20 suspicious lesions were included.

2.2 *OCT Imaging and Analysis*

OCT images were made with a commercially available 50 kHz swept source OCT system (Santec Inner Vision 2000) with a depth resolution of ~10 μm and lateral resolution of ~20 μm (in tissue) operating at wavelengths of 1300 ± 60 nm. All scans were stored to be analyzed at a later date by one investigator (RW) blinded for the pathology report. From each patient, five OCT scans per suspicious site were recorded as well as five scans from a contralateral site, which was judged as normal (by one gynecologist). After OCT imaging, biopsy of the suspicious lesion was taken. When excision instead of biopsy took place, either an extra biopsy of the excised tissue was taken or a suture was used to mark the imaged tissue region to ensure that the pathologist would analyze the same tissue-part as imaged.

Table 1 Patient characteristics.

$n = 16$	
Age	56 (range 42 to 67)
Previous surgical treatments of the vulva	2 (median; range 0 to 16)
Menopausal status	Premenopausal: 1
	Postmenopausal: 15

In total, 200 OCT scans were analyzed. Our analysis is illustrated in Fig. 1. First, the thickness of the epithelial layer was determined by careful analysis of the OCT image by the investigator. The epidermal layer appeared as a dark gray homogenous band within this image. This layer thickness could be determined with 10 μm uncertainty (corresponding to the OCT depth resolution). Second, quantitative analysis of the OCT data, i.e., to determine the decrease of light intensity per millimeter (attenuation coefficient, μ_{oct} [mm^{-1}]), was performed as described before[33] using custom written software (LabVIEW

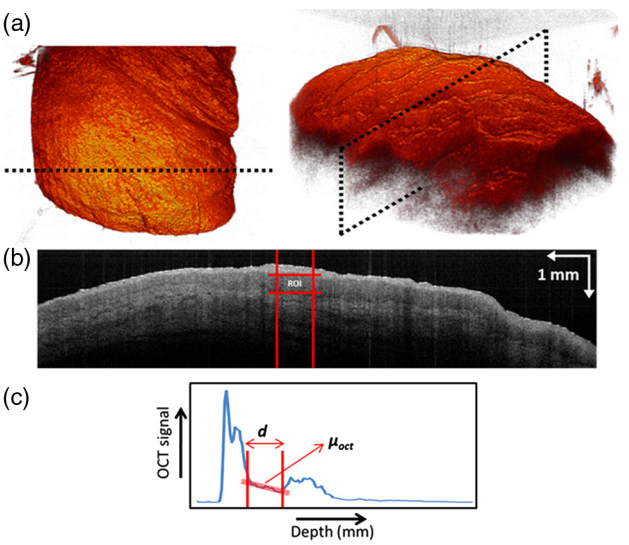

Fig. 1 (a) Three-dimensional (3-D) representation of 15 by 15 by 3 mm OCT scan; (b) two-dimensional (2-D) cross-sectional image with the region of interest (ROI) depicted in red. The epithelial layer is shown as the second dark gray layer in the cross-sectional image; (c) average A-scan obtained from the ROI in the 2-D OCT scan. The thickness of the epithelial layer is measured in this graph and is represented as *d*. Attenuation fit (μ_{oct}) is represented by the slope of the OCT signal shown in transparent red.

2011, National Instruments, Austin TX, USA). For this analysis, the data was fitted with a single exponential decay model after careful calibration of the total OCT system that includes specific definition of the point-spread-function of the sample arm optics and the roll-off of the OCT system.[33,34] In short, the investigator selected the epidermal region of interest (ROI) in the OCT image. A suspected lesion was clearly discoverable due to visible structural differences from normal epidermal OCT images and was therefore selected by the investigator for the analysis.

2.3 *Statistical Analysis*

Standard pathological report was considered gold standard for comparison. All stained sections were reviewed by one gynecological pathologist (HvB). From the OCT data, multiple values of epithelial layer thickness and epidermal μ_{oct} were available per patient for both normal and suspicious tissue. The mean thickness and mean epidermal $\mu_{oct} \pm$ respective SD for each imaged site was calculated and grouped according to the histopathology report.

All data were collected and analysed in R version 2.12 (The R foundation for statistical computing, Vienna, Austria). In this study, we focused on the use of OCT in differentiating between normal tissue and VIN. In accordance, we concentrated on the OCT data of the sixteen lesions that contained VIN.

The difference in mean epithelial layer thickness (per site) and mean epidermal attenuation coefficient μ_{oct} between normal vulvar tissue and VIN lesions per patient was tested using Mann-Whitney paired tests. Differences were considered statistically significant if the two-sided p-value was <0.05. Receiver operating characteristic (ROC) curves were constructed to determine the optimal threshold [using the closest-to-(0,1) criterion] maximizing sensitivity and specificity. The ROC area under the curve (ROC-AUC) was calculated, and a bootstrap was used to determine the 95% confidence interval. Spearman correlations were used to compare mean epithelial layer thickness and mean epidermal attenuation coefficients for VIN and mean healthy tissue separately.

3 Results

Sixteen consecutive patients with a total of 20 suspicious lesions were included. The mean age was 56 years (range 42 to 67). Fifteen patients were postmenopausal; one patient was premenopausal. Patients underwent a median of two (range 0 to 16) surgical interventions previous to this study (Table 1). Of the measured lesions, 10% were located periclitorial, 5% on the labia minora, 35% on the labia majora, and 20% were located perianal. Most of the lesions were white (70%), a few were pink (15%), fewer lesions were brown (10%) or red (5%). The histology report showed 16 lesions contained VIN, two contained hyperplastic tissue, one lesion appeared to be VSCC, and one lesion was normal vulvar skin.

Figures 2 and 3 present OCT images of two lesions that contained VIN, including the histopathology slide and the epidermal layers pointed out. Layers in OCT images showed close resemblance to the layers in the pathology slides. The cross-sectional OCT images are depicted with the corresponding histology from approximately the same site. These images show the thickened horny layer, which is sometimes present in VIN, and the thickened epidermal layer. Furthermore small arterioles might be present, shown as dark spots in the images.

Figure 4(a) presents the mean epidermal layer thickness for normal and suspected tissue per patient. The within-patient

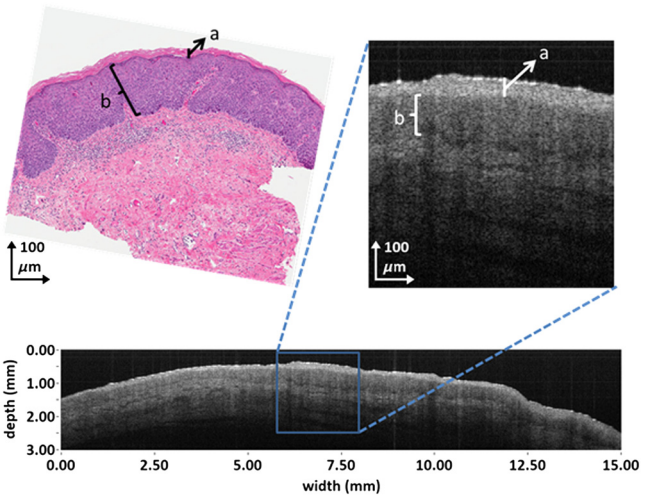

Fig. 2 Cross-sectional OCT image versus histology corresponding from the approximately same site: (a) shows the thickened horny layer that is sometimes present in VIN; (b) shows the thickened epidermal layer.

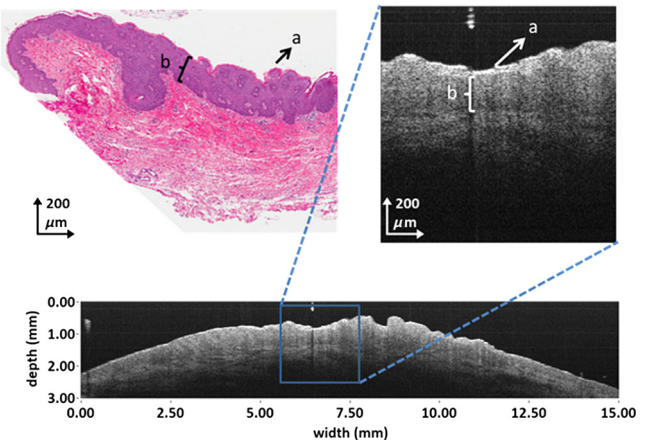

Fig. 3 Cross-sectional OCT image vs histology corresponding from the approximately same site: (a) shows the thickened horny layer that is sometimes present in VIN; (b) shows the thickened epidermal layer.

difference in mean epidermal layer thickness was significant, with VIN tissue being thicker ($p < 0.0001$). Averaged over all patients, the mean epidermal layer thickness in normal vulvar tissue was 0.19 ± 0.04 mm, while VIN tissue had a mean epidermal layer thickness of 0.56 ± 0.22 mm [Fig. 4(b) boxplots]. Being perfectly separated, both the sensitivity and specificity was 100% for thresholds between 0.24 and 0.26 mm.

Figure 5(a) presents the mean epidermal μ_{oct} of normal and suspicious tissue per patient. The attenuation coefficient in VIN tissue was higher than in normal tissue ($p < 0.0001$). There was one outlier (second bar). The healthy skin of this patient had a μ_{oct} of 6.7 mm^{-1} and the μ_{oct} of the VIN lesions was 8.7 mm^{-1}. This patient appeared to have an erythema of the vulvar skin, a later diagnosed contact allergy. Averaged over all 16 patients, the VIN lesions had a mean μ_{oct} of 6.2 ± 2.1 mm^{-1} and all imaged normal tissue sites had a μ_{oct} of 2.1 ± 1.4 mm^{-1} [Fig. 5(b) boxplots]. In addition, sensitivity (95% confidence interval) of the μ_{oct} was 88% (62 to 98%), and specificity 94% (70 to 100%) when using a threshold of 2.9 mm^{-1}. The ROC-AUC was 0.95 (95% confidence interval: 0.86 to 1.00).

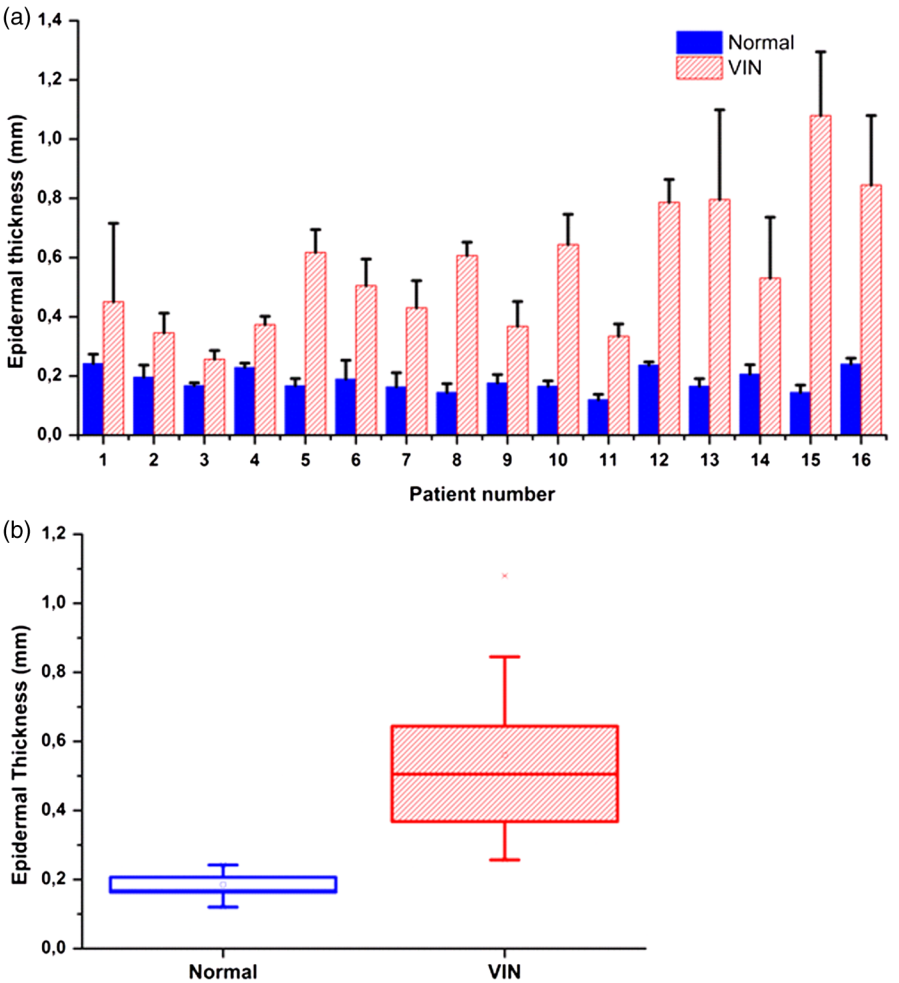

Fig. 4 (a) Individual measurements and boxplots; (b) of epidermal thickness in healthy tissue and VIN tissue. Mean value for the normal group is 0.19 ± 0.04 (IQR = 0.05). Mean value for the VIN group is 0.56 ± 0.22 (IQR = 0.34).

In normal tissue as well as VIN tissue, epidermal layer thickness and attenuation coefficient were not correlated ($p = 0.49$ and 0.23, respectively).

4 Discussion and Conclusion

In this study, normal vulvar tissue and suspicious lesions of the vulva were imaged *in vivo* with OCT. In the qualitative analysis of the OCT images, the main similarity between OCT images and pathology slides were the structural layers in the tissue. Quantitative analysis of these OCT images demonstrates that normal tissue and VIN lesions have a significant difference in both epidermal layer thickness and the attenuation coefficient.

To the best of our knowledge, this is the first study that images VIN lesions *in vivo* using OCT and quantifies image features related to morphological changes occurring during carcinogenesis (e.g., epithelial layer thickness and attenuation coefficient). The application of OCT to vulvar disease was partially studied when 47 patients with premalignant lesions of the cervix and three patients with Paget's disease of the vulva were imaged.[23] As in our study, qualitative comparison between OCT images of tissue structure and histology was performed.

Our study provides unique quantification of VIN morphology. It is well known that VIN leads to thickening of the epithelial layer.[26] We hypothesized that epithelial layer thickness could thus be used as a marker for the presence of VIN. Our findings confirm this hypothesis, albeit in a modest group of 16 patients and only to differentiate VIN from normal tissue. Clearly, other factors such as inflammation may also lead to epithelial thickening, reducing the specificity of these measurements. Moreover, 15 out of 16 patients in this study were postmenopausal. Postmenopausal vulvar skin tends to be atrophic and thinner, compared with premenopausal vulvar skin.[35] Thicker layers, as long as they are within the maximum measurement depth of OCT, create more reliable attenuation coefficients compared to thinner layers.[36] As premenopausal women might have a thicker epithelial layer, we can expect an even more reliable attenuation coefficient determination.

Light scattering measurements are sensitive to variations in tissue morphology (density) at subwavelength scales.[27] Our OCT measurements are sensitive to variations on length scales of around $\lambda/2 \approx 650$ nm; e.g., on the scale of organelles and cells.[37] Which processes and changes during carcinogenesis are responsible for the measured differences as in our paper yet remain to be resolved, but possible mechanisms may be identified. For example, cancers are characterized by a high proportion of dividing cells[38] during which the cells increase their DNA fraction. The refractive index of the nucleus, governing light scattering properties, increases during the cell cycle when cells increase their DNA[39] leading to changes in scattering properties compared with normal cells. In dysplastic cells, like the cells in premalignant epithelial lesions such as VIN, DNA

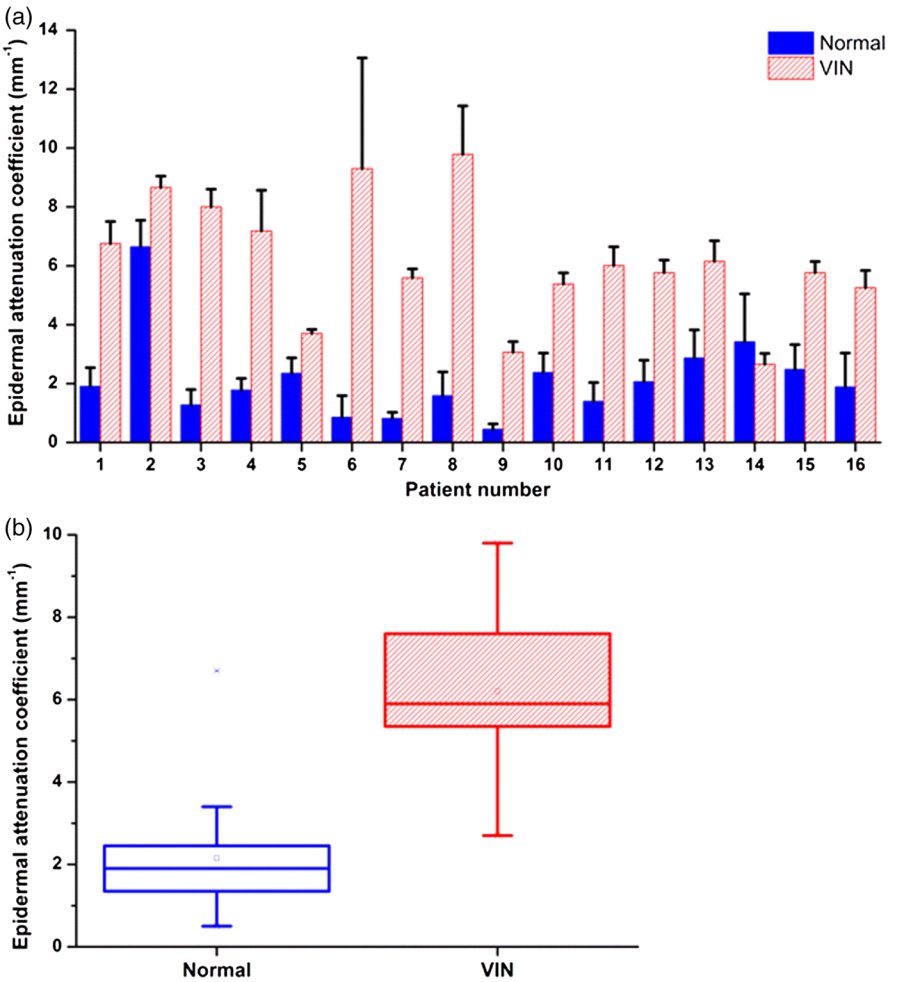

Fig. 5 (a) Individual measurements and boxplots; (b) of the attenuation coefficient in healthy tissue and VIN tissue. Mean value for the normal group is 2.1 ± 1.4 (IQR = 1.1). Mean value for the VIN group is 6.2 ± 2.1 (IQR = 2.3).

replication takes place as well[40] so that changes in scattering properties may be anticipated. Our findings confirm these differences between VIN and normal vulvar skin (as quantified through the epidermal attenuation coefficient) albeit under the same restrictions as the epidermal thickness measurements. For example, lesion number two in Fig. 5(a) shows an increased attenuation coefficient for normal skin. The patient was later diagnosed with contact allergy, in which a complete cascade of signals lead to recruitment of cells in the skin, changing the light scattering and absorption compared to normal, healthy skin.[41]

The measurements of epidermal layer thickness and attenuation coefficient per lesion exhibited minimal correlation for both normal skin and VIN tissue. This finding suggests that both measurements can be used as markers for VIN and that possibly different mechanisms underlie their difference with normal values. For example, epithelial thickening caused by more but morphologically identical cells will not yield differences in μ_{oct}, while changes in intracellular refractive index will not cause large changes in layer thickness but can yield pronounced changes in μ_{oct}. More importantly, a combination of the two measurements is likely to increase diagnostic accuracy when the analysis groups are expended beyond normal versus VIN only.

It is necessary to confirm our preliminary results in a larger study population and investigate other vulvar diseases as well. In the Netherlands, the incidence of VIN is 2.2 patients per 100,000 women.[2] Due to the low incidence of VIN, patients available for research are limited. Until now, patients with a suspicious lesion of the vulva undergo a punch biopsy to get histological diagnosis. Besides the fact that histological diagnosis cannot be performed *in vivo*, histological grading of VIN is difficult and pathologists have a high inter-observer variability.[42] Unlike pathology, OCT and OCT analysis can be performed noninvasively, *in vivo* and in real time. Like pathology, our present OCT analysis relies on the experience of the investigator, specifically in selecting layer boundaries for thickness/attenuation measurements. A study that elucidates both inter- and intra-observer variability for OCT analysis is currently being conducted in our institute.

The present study is a first step in using OCT to distinguish between VIN and normal vulvar tissue. Several reasons make this technique desirable. First, nowadays medical treatment becomes successful in treating VIN and biopsies will become more important in the outpatient clinic.[12,13] Second, VIN recurs frequently, and patients have to be followed for the rest of their lives. In the gynecological clinic, OCT could therefore improve diagnostic opportunities and reduce the number of biopsies needed.

In conclusion, this study shows that the epidermal thickness and attenuation coefficient of vulvar epithelial tissue containing VIN is different from normal vulvar tissue. Successful optical imaging without the need for puncturing the patient might become possible in the gynecological outpatient clinic.

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