3D optical coherence tomography of cervical intraepithelial neoplasia - early experience and some pitfalls

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Summary

Objectives: To compare two different systems for optical coherence tomography for the diagnosis of cervical dysplasia and to assess potential benefits of three-dimensional imaging. *Materials and Methods*: OCT images were taken from unsuspicious and suspicious areas of fresh conisation specimens using two different imaging systems, one with the capability to produce three-dimensional images. All OCT images were separately evaluated by two blinded investigators based on a 6-grade classification (normal, inflammation, CIN 1, CIN 2, CIN 3, squamous carcinoma) and later compared to the corresponding histology. Sensitivity and specificity of OCT in detecting cervical dysplasia were determined. *Results:* OCT images using both OCT systems were taken from 46 sites in ten conisation specimens and later compared to the corresponding histology. CIN lesions were diagnosed correctly by the two-dimensional OCT system with a sensitivity and specificity of 91% and 78% accordingly. Using the three-dimensional system sensitivity and specificity were 82% and 86% accordingly. *Conclusions:* Both OCT systems used were highly sensitive in identifying cervical intraepithelial neoplasia. Despite technical problems experienced in the present series, we believe that three-dimensional imaging has the potential to further improve the accuracy of optical coherence tomography.

Key words: Optical coherence tomography; OCT; Colposcopy; Intraepithelial cervical dysplasia; CIN.

Introduction

Approximately 500,000 women worldwide are annually diagnosed with invasive cervical carcinoma (ICC) and about 230,000 women die from the disease [1]. Although the incidence of ICC has declined over the last decade, the incidence of cervical intraepithelial neoplasia (CIN) has increased, especially in younger women. If untreated, 15-20% of these women develop severe dysplasia and 5-10% invasive carcinoma [1-3]. In industrialized countries the implementation of screening programs has led to a decline in the number of cervical cancer related deaths. However, this requires a complex diagnostic infrastructure providing cytology, HPV testing, colposcopy and histology [4, 5]. The present screening programs are associated with overall high costs and not feasible for most countries. Therefore, the implementation of new imaging techniques that allow cheap, rapid and non-invasive evaluation of the cervix would be a vast improvement in the prevention of ICC.

Optical coherence tomography (OCT) is a non-invasive high-resolution imaging technique that uses near-infrared light interferometry to visualize the microstructure of tissues. The technique is analogous to B-mode ultrasound imaging with the difference that it uses light as opposed to ultrasound waves. In ultrasound the time delay for an ultrasonic wave to be reflected back to the probe is used to generate an image of the tissue structure. As the speed of light is much greater than the speed of sound, time delay measurements with OCT necessitate a correlation technique known as low coherence interferometry. By providing cross-sectional images in real time with as much as 2 mm penetration depth and high spatial resolution optical coherence tomography fills an important gap between existing imaging modalities [6-8].

In two previous studies we demonstrated that OCT can achieve high-resolution images of cervical epithelium and is highly sensitive in identifying pre-invasive and invasive cancer of the uterine cervix [9, 10]. However, with the current available OCT devices the differentiation between low- and high grade dysplasia is difficult. The purpose of this study was to evaluate the feasibility of a new OCT system providing three-dimensional images and to compare the results with the two-dimensional images of our present device.

Materials and Methods

We present a prospective single-institution, institutional review board-approved, ex-vivo study comparing two different OCT systems. Images were taken from 46 sites in ten loop electrosurgical excision procedure (LEEP) specimens and later compared to the corresponding histology. All images were anonymized to preclude identification. Two investigators blinded for the final histological diagnosis evaluated the OCT images using a 6-grade classification (normal, inflammation, CIN 1, CIN 2, CIN 3, squamous carcinoma) as described before [9, 10].

OCT imaging was carried out using two different devices: The Niris imaging system (Imalux Corporation, Cleveland, OH) is an optical fiber-based interferometer with a superluminescence diode (SLD), providing a low-coherent broadband, near infrared (NIR) light. The reusable fiber-optic probe with a diameter of 2.7 mm provides a depth scanning range of ≤ 1.5 mm and a lateral scanning range of 1.6-2.4 mm. It is used in direct contact with the tissue. The system acquires real-time two-dimensional images of 200 x 200 pixels. The Niris imaging

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system has been approved by the Food and Drug Administration (FDA) and the European Community (EC).

The Vivosight OCT Scanner (Michelson Diagnostics, Orpington, Kent, UK) uses a swept source laser (HSL-2000-12-MDL, Santec Corporation, Ohkusa-Nenjyozaka, Komaki, Japan) with a wave length of 1305 nm. The reusable probe provides a depth scanning range of ≤ 2.0 mm and a lateral scanning range of 5 mm. Direct contact with the tissue is not necessary. Due to its multislice function the system acquires both two- and three-dimensional images with a lateral resolution better than 7.5 m and an axial resolution better than 10 m. The Vivosight scanner has been approved by the FDA and the EC.

Sensitivity and specificity were calculated as TP/(TP+FN) and TN/(FP+TN), respectively. The inter-investigator agreement was assessed by applying Cohen's Kappa statistics.

Table 1. — *By comparing 46 histological results with corresponding OCT findings and defining a threshold at CIN2, there were 21 (22) true positive, 18 (18) true negative, 2 (1) false negative and 5 (5) false positive results. The sensitivity calculates to 91% (96%), the specificity to 78% (78%).*

		Histology						
		normal	Inflam.	CIN 1	CIN 2	CIN 3	Ca	Total
OCT	normal	9 (9)		1				10 (9)
Invest. 1	Inflam.		(1)					(1)
Invest. 2 ( )	CIN1		(1)	8 (7)	1	1 (1)		10 (9)
	CIN2		2	3 (5)	8 (9)			13 (14)
	CIN3				2 (2)	11 (11)		13 (13)
	Ca							
	Total	9	2	12	11	12		46

Results

Forty-six OCT images for each system and corresponding histologies were taken from ten LEEP specimens within the first postoperative hour. The patients mean age was 34.1 years (27-44 years) and all women were premenopausal. Indication for conisation was a Pap III in one case, a Pap IIID in four cases and a Pap IVA in five cases according to the Munich nomenclature. All women were HPV high-risk positive.

All images of the Niris system were evaluated by two investigators working independently and being blinded for the final histology. The correlation between OCT images and histology is shown in Table 1. Thirty-six (second investigator: 37) of 46 sites were correctly diagnosed by OCT. All biopsy sites with histologically no dysplasia were correctly interpreted. Two (1) inflammatory changes were misinterpreted as CIN lesions. By comparing the 46 histological results with the corresponding OCT findings and defining a threshold at CIN 2, there were 21 (22) true positive, 18 (18) true negative, two (1) false negative and five (5) false positive results. The sensitivity calculates to 91% (96%) and the specificity to 78% (78%). Unweighted Kappa from a dichotomous classification with the threshold at CIN 2 was 0.65 (0.95 confidence interval: 0.42/0.87) indicating substantial agreement between both investigators as specified by Malpica *et al*. [11].

Two-dimensional (2D) and 3D images of the Vivosight system were evaluated by both investigators together as these images were far more difficult to interpret without previous experience. Twenty-seven of 46 sites were correctly diagnosed by OCT. Again, all biopsy sites with histologically no dysplasia were correctly interpreted. Eight image sets were of poor quality due to technical problems and could not be assessed. By including the remaining 38 images and defining the threshold at CIN 2, there were 14 true positive, 18 true negative, three false negative and three false positive results leading to a sensitivity of 82% and a specificity of 86%.

Figure 1 shows Niris and Vivosight images of normal epithelium, CIN 3 and inflammation obtained from identical areas. *Image a* demonstrates the high resolution of the Vivosight scanner with a well recognizable three-layer architecture and a sharp interface between epithelium and stroma optically representing the basement membrane. *Image b* shows a CIN 3 lesion with the typical irregularity of the epithelial layer and an increasing intensity of the stromal layer. Optically, the stroma seems to push its way towards the surface as vertical columns. However, this is caused by scattering and should not elude the fact that the intraepithelial changes originate from the basal membrane. *Image c* is typical for an inflammation with swelling of the epithelium and edema of the stromal layer. The basement membrane as an optical interface between epithelium and stroma is existent but less clear. Figure 2 gives two examples of 3D images generated with the Vivosight scanner. *Image a* exemplifies a well recognizable three-layer architecture with a sharp interface between epithelium and stroma. In addition, a plain epithelial surface is visualized. *Image b* shows a CIN 3 lesion with the typical irregularity of the epithelial layer and the loss of a sharp interface between epithelium and stroma.

Figure 3 (a and b) reveals technical problems. As the Vivosight probe has no contact with the epithelium uneven surfaces are difficult to scan with a loss of quality in deeper layers (a). In contrast, the probe of the Niris system has contact with the epithelium and flattens the surface. Another common problem were artefacts caused by reflections of the probe sheath impeding the evaluation (b).

Discussion

This study was carried out in order to compare two different OCT systems with the chance to evaluate 3D imaging of cervical epithelium. We had to choose an ex-vivo setting as the probe of the Vivosight scanner available at the time of our experiments was not appropriate for in-vivo imaging. Meanwhile the company has developed a new soft tissue probe which allows in-vivo imaging of cervical tissue and provides the same imaging properties as the topical probe.

The Vivosight scanner provides both 2D and 3D images over a 5 mm x 5 mm area producing up to 2000

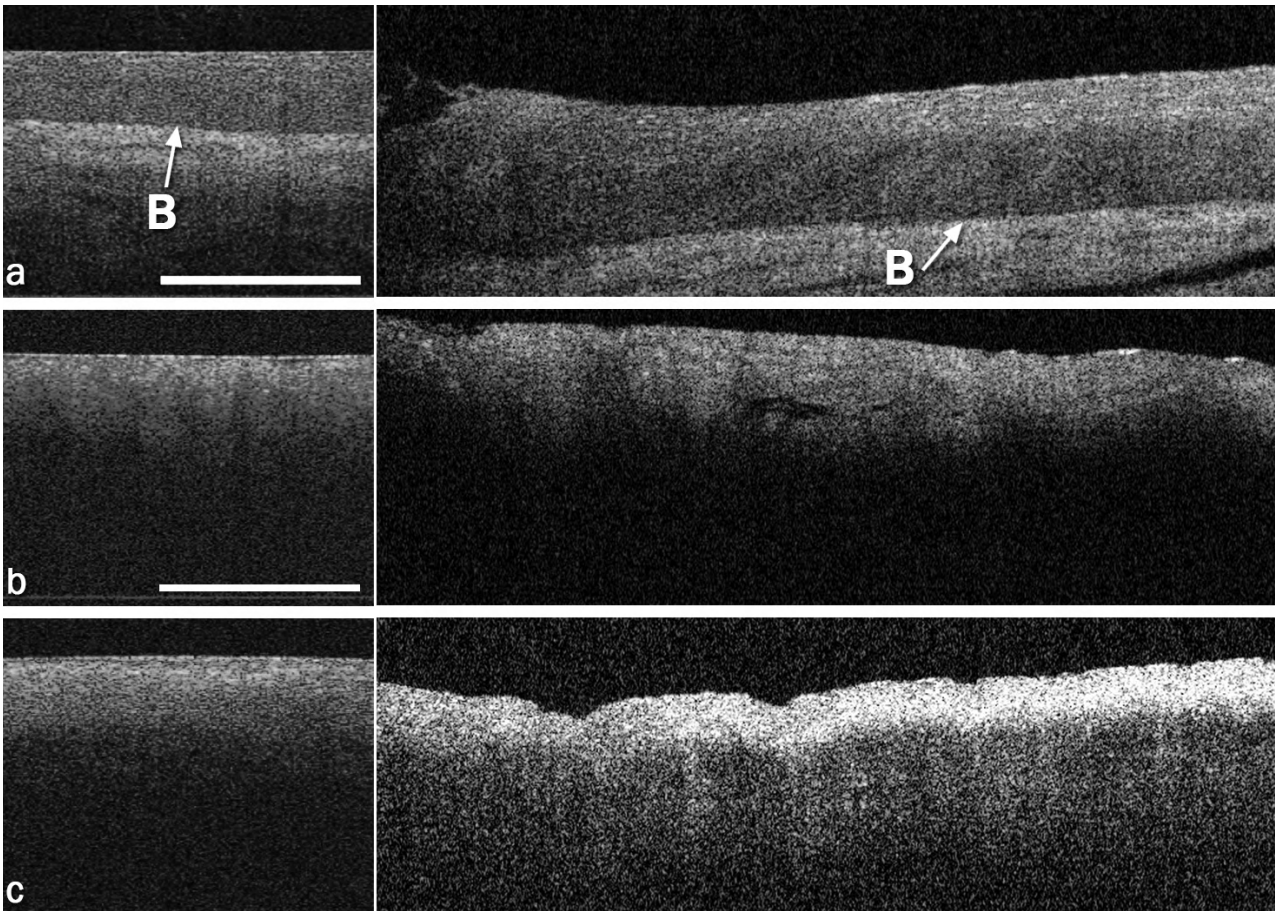


Figure 1. — Niris (left) and Vivosight images (right) of normal epithelium, CIN 3 and inflammation obtained from identical areas. (a) a well recognizable three-layer architecture and a sharp interface between epithelium and stroma optically representing the basement membrane (B). (b) a CIN 3 lesion with the typical irregularity of the epithelial layer. (c) Inflammation. The basement membrane as an optical interface between epithelium and the stroma is existent but less clear. The Vivosight image shows a much brighter epithelium as a correlate for inflammation.

cross sections. As in our series image margins frequently showed shadowing effects we reduced the format to 3 mm x 3 mm. Furthermore, we reduced the number of cross sections to 100/mm in order to minimize scan time and file size, to avoid oversampling and to utilize a lateral resolution of better than 7.5 μm.

Due to the higher resolution of the Vivosight scanner we expected more detailed OCT images in comparison to the Niris system. In fact, we obtained excellent results in a number of cases but we were not able to maintain this high standard throughout the study. The probe we were using represented the main cause for difficulties. As even minor movements caused artefacts and superpositions, it became necessary to fix the probe in a support frame. Finding the right distance between probe and epithelium to achieve a satisfactory depth scanning range, to exactly scan the area of interest and to scan the tissue in a right angle represented other problems. We assume that the new soft tissue probe available now will eliminate these difficulties.

The Volume Viewer plugin of the Image J software program (Wayne Rasband 2009) allows slices and volume visualization to be displayed using different interpolation and rendering techniques. The viewing position and the orientation and position of slices or volumes can be arbitrarily chosen. Therefore, 3D images provide not only additional information regarding the epithelial surface but can also visualize layers of interest such as the basement membrane. Figure 4 shows a sagittal (a) and a horizontal (b) plane of a CIN3 lesion. The level of the horizontal plane was chosen close to the basement membrane displaying an uneven column-like appearance as a typical feature of high-grade cervical dysplasia. However, to what extent 3D imaging improves the interpretation of cervical dysplasia has to be evaluated in future studies.

The Niris OCT system which we have routinely used in our out-patient clinic for more than two years produces reliable pictures and the results presented in this study are similar to those reported earlier [9, 10]. The system is

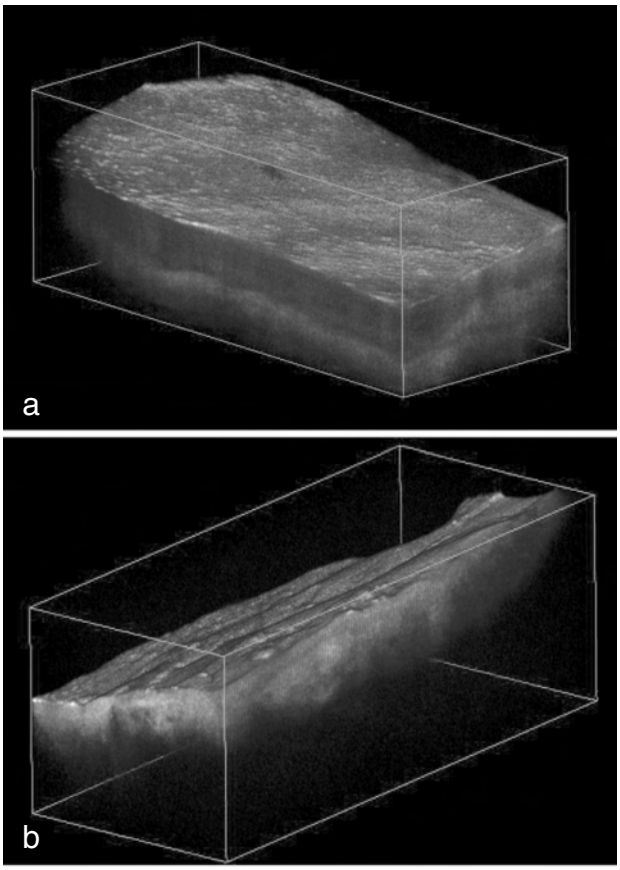
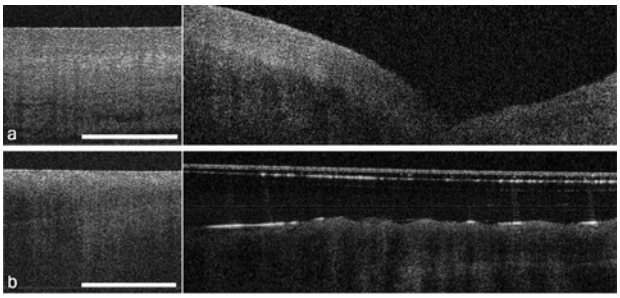


Figure 2. — 3D images generated with the Vivosight scanner. (a) normal epithelium showing a well recognizable three-layer architecture with a sharp interface between epithelium and stroma. In addition, a plain epithelial surface is visualized. (b) CIN 3.



Figures 3. — (a) As the Vivosight probe has no contact with the epithelium uneven surfaces are difficult to scan with a loss of quality in deeper layers (right). In contrast, the probe of the Niris system has contact with the epithelium and flattens the surface (left). (b) Another common problem were artefacts caused by reflections of the probe sheath impeding the evaluation (right).

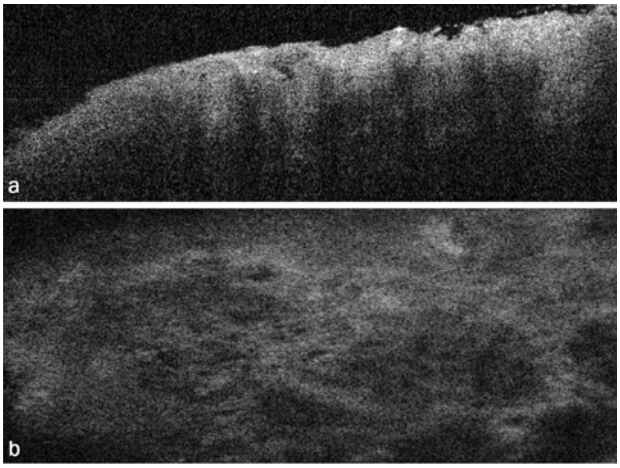


Figure 4. — Sagittal (a) and horizontal (b) plane of a CIN3 lesion. The level of the horizontal plane was chosen close to the basement membrane displaying an uneven column like appearance.

highly sensitive in identifying precancerous lesions, but is not as precise as requested in distinguishing between different CIN grades. A succeeding model with higher resolution and a new light source and optic will be available in 2011 and may improve the specificity as well as the differentiation of cervical dysplasia.

With only 46 specimens included, the calculation of sensitivity and specificity must be judged with caution. Furthermore, there is a bias in our study design as we gained the results from a very selected group of patients. All conisations were carried out to further assess suspicious Pap smears and colposcopy findings.

This study comprises just a small number of patients and one might ask why we present the data at all. Despite our problems with the Vivosight scanner and a large number of inadequate images, the successful images in the series clearly show the great potential of optical coherence tomography. As technical difficulties can be resolved, we are convinced that the future generation of OCT systems represents a substantial progress towards the identification and clinical management of precancerous and cancerous cervical lesions.

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