



Research article

Non-invasive three-dimensional thickness analysis of oral epithelium based on optical coherence tomography—development and diagnostic performance

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ABSTRACT

Objectives: Evaluating structural changes in oral epithelium can assist with the diagnosis of cancerous lesions. Two-dimensional (2D) non-invasive optical coherence tomography (OCT) is an established technique for this purpose. The objective of this study was to develop and test the diagnostic accuracy of a three-dimensional (3D) evaluation method.**Methods:** The oral lip mucosa of 10 healthy volunteers was scanned using an 870-nm spectral-domain OCT device (SD-OCT) with enhanced depth imaging (EDI). Four raters semi-automatically segmented the epithelial layer twice. Thus, eighty 3D datasets were created and analyzed for epithelial thickness. To provide a reference standard for comparison, the raters took cross-sectional 2D measurements at representative sites. The correlation between the 2D and 3D measurements, as well as intra- and inter-rater reliability, were analyzed using intraclass correlation coefficients (ICC).**Results:** Mean epithelial thickness was $280 \pm 64 \mu\text{m}$ (range 178–500 μm) and $268 \pm 49 \mu\text{m}$ (range 163–425 μm) for the 2D and 3D analysis, respectively. The inter-modality correlation of the thickness values was good (ICC: 0.76 [0.626–0.846]), indicating that 3D analysis of epithelial thickness provides valid results. Intra-rater and inter-rater reliability were good (3D analysis) and excellent (2D analysis), suggesting high reproducibility.**Conclusions:** Diagnostic accuracy was high for the developed 3D analysis of oral epithelia using non-invasive, radiation-free OCT imaging.**Clinical significance:** This new 3D technique could potentially be used to improve time-efficiency and quality in the diagnosis of epithelial lesions compared with the 2D reference standard.

1. Introduction

Evaluating structural changes in oral epithelia can help with the detection of cancerous lesions [1,2,3]. Monitoring epithelial changes might also help to improve cancer treatment [1,4] and manage therapeutic side effects, such as mucositis [5,6,7]. Optical coherence tomography (OCT) is a radiation-free, high-resolution method to quantify epithelial thickness in vivo [8,9,10]. A good correlation has been confirmed between OCT and histopathological measurements of epithelial thickness [11]. OCT enables non-invasive imaging of multiple sections and can thus be considered an optical biopsy [12].

Currently, two-dimensional (2D) OCT cross-sections are measured at several sites in multiple sections to gain a representative overview of epithelial thickness [1,4,13]. The logical next step is to analyze the entire scan on a three-dimensional (3D) basis. Compared with the 2D approach, a 3D method offers two key advantages. First, it enables time-efficient 3D visualization of the entire scanned area. Second, it allows for advanced geometric analysis, such as volumetric evaluation of objects across multiple 2D sections.

The aim of the present study was to investigate the prerequisites for 3D analysis of epithelial scans. More specifically, the objective was to develop an efficient and reproducible 3D segmentation and analysis

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procedure, and to compare its diagnostic performance with that of the current reference standard, the traditional cross-sectional 2D approach.

2. Materials and methods

The oral lip mucosa of 10 healthy volunteers was scanned by use of a modified, commercially available 870-nm SD-OCT device with EDI (HRA + OCT Spectralis, Heidelberg Engineering) (Figure 1). Informed consent was obtained from all volunteers, and all procedures were performed in compliance with relevant laws and institutional guidelines (ethical approval: S-370/2015). To adapt the optics for mucosal imaging, the front lens was removed, and the intermediate image plane was imaged onto the sample by means of a one-to-one telescope, as described previously [14]. The volume scanning mode was used to acquire multiple (~49) cross-sectional B-scans over a 15-mm region on the inner side of the lower lip. The device used a broadband super-luminescent diode (center wavelength: 870 nm) as a low coherent light source. The axial and lateral optical resolution was 7 μm and 14 μm , respectively [15]. Imaging was performed using a proprietary software package (Heidelberg Eye Explorer, Version 1.9.13.0; Heidelberg Engineering). OCT measurement results were corrected by the refractive index of the substrate, human gingiva [16].

The OCT scans were exported as DICOM datasets (Figure 2). Using opensource software (3D Slicer), semi-automatic segmentation of the epithelial layer was performed by four raters with different levels of experience in dental imaging (1–8 years). The semi-automatic segmentation tool “segmentation based on local seeds” was used, which is implemented in 3D slicer. The four raters segmented the scans during two

separate sessions, yielding a total of 80 non-invasively acquired 3D maps of human epithelium. The reconstructions were exported as STL data and imported into computer-aided design software (Geomagic, Design X). To remove segmentation artifacts, volume surfaces were re-meshed with a triangle edge length of 0.02 mm. The volume (V) was then calculated. Mean epithelial thickness (t) was calculated using the formula $t_{\text{mean}} = V/A$, in which A is the area of the OCT scan ($A = 6.4769 \text{ mm}^2$). In addition to this 3D evaluation, the four raters measured epithelial thickness by means of the traditional cross-sectional 2D approach. They were able to freely scroll through the stacks of OCT scans, measuring epithelial thickness at five representative sites chosen at random [4]. This 2D measurement was repeated for a second round. For the 2D reference approach, mean epithelial thickness was calculated from the two rounds of measurement for every OCT scan. Inter-modality correlation and intra- and inter-rater reproducibility were assessed using intraclass correlation coefficients (ICC) and interpreted according to Koo and Li [17].

3. Results

To ensure a time-efficient and intuitive assessment of the entire scanned surface, an automated calculation of epithelial thickness plots was programmed (Figure 2). The distance between the upper and lower side of the epithelial layer was calculated in relation to a base plate. These thickness plots depict local epithelial thickening and thinning in different colors, thus enabling any abnormalities to be easily detected.

The traditional 2D measurements resulted in a mean epithelial thickness for the scanned lip mucosa of $280 \pm 64 \mu\text{m}$ (range 178–500 μm). For the 3D analysis, mean epithelial thickness was $268 \pm 49 \mu\text{m}$

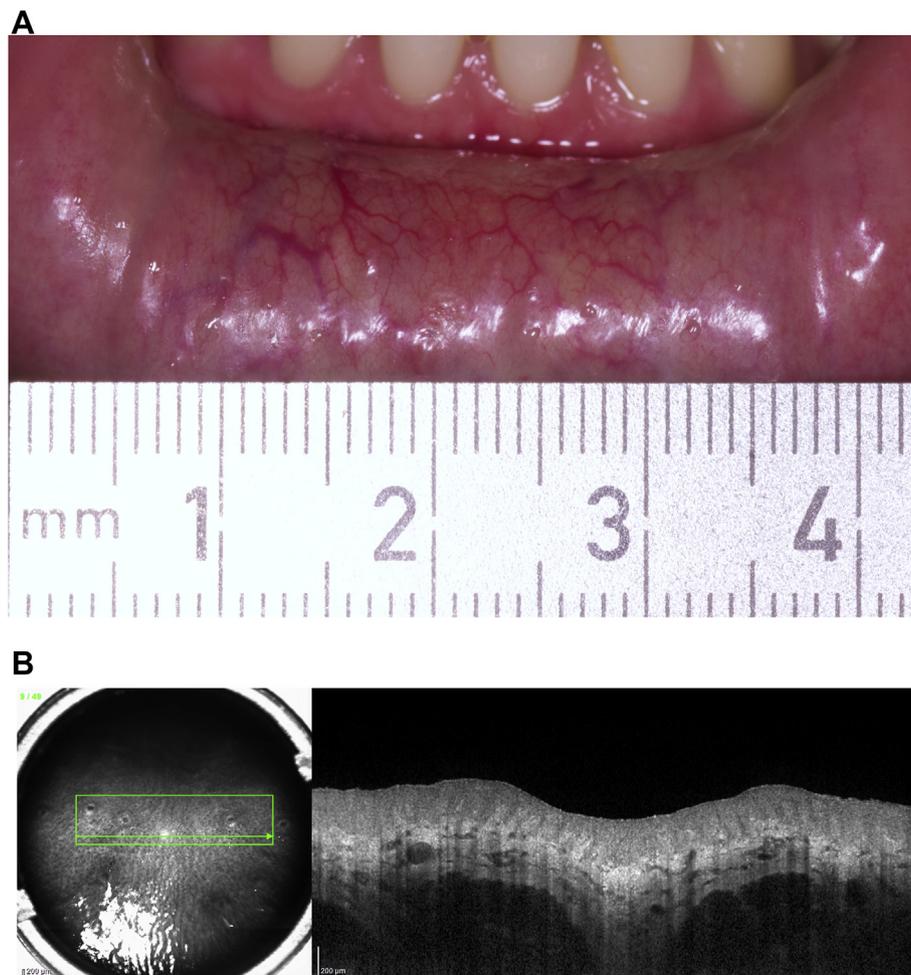


Figure 1. A. Lower lip mucosa of healthy volunteer. B. OCT surface image (left) and OCT scan (right) of oral epithelium.

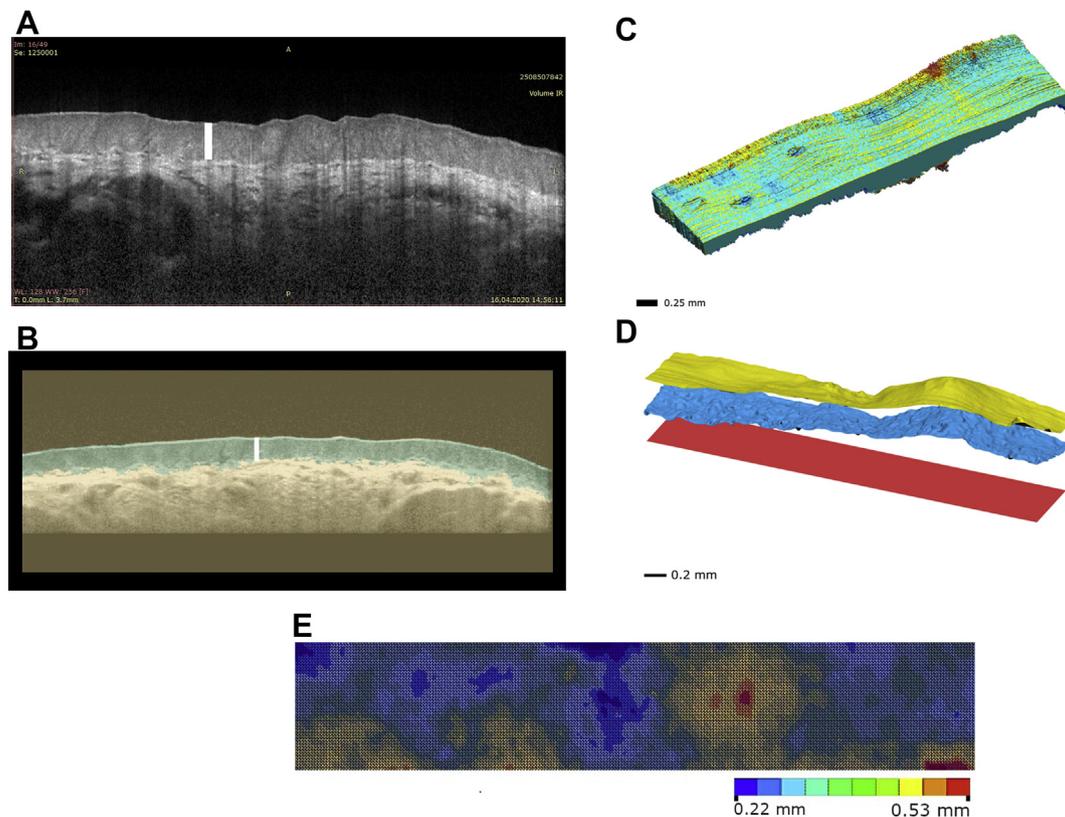


Figure 2. A. Oral lip mucosa was scanned using optical coherence tomography. The white bar indicates epithelial thickness. B. Semi-automatic segmentation was performed. Green: detected epithelium; brown: no epithelium. C. 3D volumes were exported (STL format). D. Re-meshed surfaces of the 3D file for the upper (yellow) and lower (blue) side of the volume, as well as the projection area (red). E. Thickness plot for distributed distance measurements perpendicular to the projection area, visualized in a color-coded image. Local epithelial thickening (red) and thinning (blue) can be subjected to closer examination. The mean thickness for such an evaluation is given by $t_{\text{mean}} = V/A$.

(range 163–425 μm). The inter-modality correlation of thickness values was good, with an ICC value of 0.76 (0.626–0.846). This indicates that 3D analysis of epithelial thickness produces valid results. Mean values for intra- and inter-rater reliability were higher for the 2D than for the 3D analysis (Figure 3, Table 1), whose reliability was excellent and good, respectively. These findings indicate that 2D and 3D analysis both provide reproducible measurements, but that the 2D method is superior in this regard.

4. Discussion

Structural changes in oral epithelia can be caused both by oral pathologies and their treatment. Abnormal cell proliferation is a fundamental mechanism of oral squamous cell carcinogenesis [18]. Radio- and chemotherapy can also cause oral mucositis, which has been associated with mucosal atrophy, i.e., the loss of the rapidly proliferating epithelial cells [19,20]. Measurement of epithelial thickness can, therefore, be used to objectively classify side effects of cancer therapy even before their clinical manifestation [5,6,7]. In the field of radiotherapy, OCT monitoring during irradiation could potentially be used to adapt radiotherapy treatment plans [21].

The epithelial thickness of lip mucosa found in this study (280 and 268 μm for the 2D and 3D analysis, respectively) is consistent with that in the literature: Prestin et al. reported a value of 294 μm for buccal mucosa [13]. The correlation between the 3D and 2D analysis was good, with high values for intra- and inter-rater reliability. This means the 3D segmentation and analysis method developed was reproducible. The reproducibility of the 2D evaluation of only five representative sites was even higher than that of the 3D approach. This is probably because only healthy volunteers were evaluated. The epithelial thickness of the

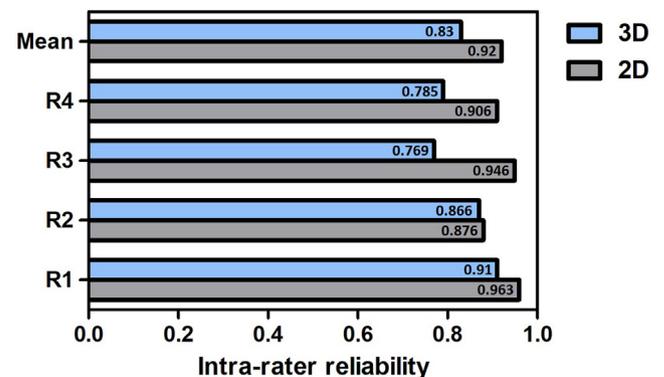


Figure 3. Intra-rater reliability for the three-dimensional (3D) and two-dimensional (2D) analysis. Intraclass correlations were calculated for the two rounds of measurement for each of four raters (R1–R4).

participants was relatively consistent, without any pronounced local changes. In such cases, 2D evaluation of a small number of scan sections is sufficient to reliably evaluate epithelial thickness, without the risk of introducing any errors during an additional segmentation step. The 2D approach might be less favourable in a clinical setting, however, where local changes in thickness could be overlooked on the basis of only a few sections. In contrast, 3D mapping provides a quick overview of the complete dataset.

Several limitations of the presented technique must be discussed. First, image quality is strongly user-dependent [22]. More specifically, even slight changes in the orientation of the 870-nm SD-OCT device can

Table 1. Inter-rater reliability. Intraclass correlations were calculated for the raters.

Mean inter-rater	3D	2D
1 + 2	0.8 (0–0.957)	0.919 (0.644–0.980)
1 + 3	0.884 (0–0.979)	0.862 (0.447–0.966)
1 + 4	0.918 (0.723–0.979)	0.925 (0.722–0.981)
2 + 3	0.938 (0.772–0.984)	0.913 (0.706–0.977)
2 + 4	0.733 (0–0.945)	0.929 (0.743–0.982)
3 + 4	0.746 (0–0.941)	0.828 (0.472–0.954)
Mean	0.84	0.90

The values in parentheses indicate the upper and lower bound of the 95% confidence interval.

lead to blurring or to greater measurement distances, depending on the angle of the light [23]. This limits the use of the 3D technique in general practice. Second, the 870-nm SD-OCT device used was selected because it is a clinically approved commercial device that is modifiable for dental imaging [14,24]. However, its size is optimized for ophthalmology, not for intraoral purposes, which makes examining the oral cavity difficult. New devices have recently been developed that include pens for intraoral use [25,26]. Lastly, the inside of the lower lip was investigated in this study because it is more accessible than the buccal plane or tongue, which are more susceptible to movement artifacts. Validity and reliability might therefore be lower for oral epithelium in other areas of the mouth.

5. Conclusions

Diagnostic accuracy was high for the developed 3D analysis of oral epithelia using non-invasive, radiation-free OCT imaging. This might be useful for increasing time-efficiency and quality in the diagnosis of epithelial lesions compared with the 2D assessment standard.

Declarations

Author contribution statement

Charlotte Theresa Trebing and Franz Sebastian Schwindling: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sinan Sen: Performed the experiments.

Stefan Rues, Christopher Herpel and Maria Schöllhorn: Analyzed and interpreted the data.

Christopher J. Lux and Peter Rammelsberg: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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