Optical pre-screening for laryngeal cancer using reflectance spectroscopy of the buccal mucosa

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Abstract: A new approach in early cancer detection focuses on detecting field cancerization (FC) instead of the tumor itself. The aim of the current study is to investigate whether reflectance spectroscopy can detect FC in the buccal mucosa of patients with laryngeal cancer. The optical properties of the buccal mucosa of patients were measured with multidiameter single-fiber reflectance spectroscopy. The blood oxygen saturation and blood volume fraction were significantly lower in the buccal mucosa of laryngeal cancer patients than in non-oncologic controls. The data of these two parameters were combined to form a single ‘biomarker a’, which optimally discriminates these two groups. Alpha was lower in the laryngeal cancer group (0.28) than the control group (0.30, p = 0.007). Alpha could identify oncologic patients with a sensitivity of 78% and a specificity of 74%. These results might be the first step toward optical pre-screening for laryngeal cancer.

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Introduction

Head and neck cancer (lip, oral cavity, nasopharynx, other pharynx and larynx) is the 7th most frequent type of cancer worldwide. There were an estimated 686,000 new cases and 404,000 associated mortalities in 2012. Males are more often affected than females with a ratio of 3:1 [1]. The epidemiology of head and neck cancer varies greatly depending on geographic region and level of exposure to risk factors [1, 2]. The main risk factors are smoking and alcohol use, which cause physiological and mutagenic effects on the exposed mucosa of the upper aerodigestive tract (UADT). They have a synergetic effect and account for about 75% of all head and neck tumors [2, 3].

One of the most important prognostic factors for head and neck cancer is TNM-stage [4–6]. Early tumors have a significant better disease specific survival rate than advanced tumors [7]. However, the majority of head and neck squamous cell carcinomas (HNSCC) are discovered at late stages of tumor progression. This highlights the need of a reliable detection method to facilitate early HNSCC detection [8]. Head and neck cancer appears ideally suited to screening because of (a) the significant morbidity and mortality associated with the disease, (b) the survival advantage of early diagnosis, (c) the association of identifiable risk factors, and (d) the ability to diagnose early tumors with a clinical examination [9]. All HNSCC population (pre-)screening methods so far have relied on visual and manual examination of patients and have focused mainly on oral cavity tumors and lymph node metastases [10–13]. Although these studies show promising results, their approaches are time consuming and have a low sensitivity for early stage tumors and for tumors beyond the oral cavity, i.e., the pharynx and larynx. This leaves us, at present, without a reliable and practical screening method for HNSCC.
A promising new approach to cancer screening is focused on detecting field cancerization (FC). Field cancerization is the notion that a multitude of physiological and nanoscale architectural alterations affect an entire organ or tract before ultimately resulting in a focal neoplasm in one area of the organ [14]. These mucosal changes occur superficially in the epithelial layer, the basal membrane, and the vascularized papillary layer of the lamina propria. There is evidence that FC of head and neck, lung and esophageal cancers encompasses the entire UADT [15]. Accurate detection of FC in an easy accessible location of the UADT such as the buccal mucosa could potentially be used to screen for distant HNSCC [15, 16]. There are several tissue alterations related to FC. Specifically, alterations in cells due to changes in their microvasculature and nanoscale architecture have been linked to FC. Optical techniques, such as reflectance spectroscopy, have the potential to be sensitive to these sub-diffractional length scale alterations caused by FC [17, 18].

Reflectance spectroscopy enables the measurement of both concentration of tissue chromophores and ultrastructural information related to scattering of the tissue [19]. Optical spectra acquired from tissue contain the combined effects of all tissue optical properties and are also dependent on illumination and detection geometry. The measurement of individual tissue optical properties and tissue components are still a major challenge. Single-fiber reflectance (SFR) spectroscopy can be used to address this challenge. In SFR, the illumination and detection are performed by the same optical fiber. This results in measurements of shallow tissue depths on the order of the fiber diameter. This shallow measuring depth is well matched with dimensions of the mucosa in which the FC changes occur [20, 21]. Additionally, measurements are also sensitive to the scattering phase function [22]. As such, SFR spectroscopy may be well suited for detection of ultrastructural changes of FC.

It has been shown that the tissue absorption coefficient \( \mu_a \) [mm\(^{-1}\)] (in this article, all wavelength-dependent variables are presented in boldface) can be accurately quantified from an SFR measurement without prior knowledge of the tissue scattering properties [23]. \( \mu_a \) can then be further decomposed into the constituent absorption spectra of known tissue chromophores. This enables accurate measurement of the concentration of chromophores and of physiological parameters such as microvascular blood oxygen saturation, blood volume fraction and mean vessel diameter. These parameters could be used to differentiate between tissue with and without FC [24].

SFR is also sensitive to the scattering phase function. We previously showed that it is possible to quantify the reduced scattering coefficient \( \mu_s' = \mu_s \cdot (1 - g_1) \) [mm\(^{-1}\)], and the phase function parameter, \( \gamma = (1 - g_2) / (1 - g_1) \), by acquiring multiple SFR measurements with different fiber diameters [25, 26]. Quantitative measurements of \( \mu_s' \) and \( \gamma \) could provide insight into the tissue ultrastructure because the scattering phase function is directly related to the tissue refractive index correlation function [27]. However, sequential placement of multiple optical fibers to conduct multi-diameter single fiber reflectance (MDSFR) spectroscopy is known to cause errors. To solve this problem, our group has previously demonstrated a MDSFR device, which uses a 19-core fiber bundle. This allows for multiple single-fiber measurement of different diameters without moving the fiber tip [19].

Recently, the use of MDSFR spectroscopy to detect FC in the buccal mucosa of patients with esophageal cancer was investigated [28]. The objective was to differentiate between patients with esophageal cancer and non-oncologic controls based on the MDSFR measurements at a distant, but accessible site. The median value of the optical biomarker \( \sigma \), a combination of two scattering parameters, was increased in patients with esophageal squamous cell carcinoma compared to control patients (2.07 vs. 1.8, \( p = 0.022 \)). This finding might be the first step towards non-invasive, optical buccal mucosa screening for esophageal cancer using FC detection. In similar approaches progress has been made with the use of low-coherence enhanced backscattering (LEBS) spectroscopy. This has been applied to detect FC of colorectal, pancreatic, and lung tumors [29–31]. For patients with lung cancer, LEBS
spectroscopy of the buccal mucosa was able to identify patients with tumors with a promising sensitivity of 79% and specificity of 83% [29].

The present study describes the first attempt to develop a non-invasive, easy-to-use pre-screening method for laryngeal cancer based on detecting FC with the use of fiber-optic spectroscopy. Our aim is to assess whether detection of FC in the buccal mucosa using MDSFR spectroscopy is feasible to identify patients with laryngeal cancer. If proven feasible, this technology could serve as a basis for the development of a patient-friendly pre-screening tool of a selected high-risk population.

Materials and methods

Subjects and examination procedure

The Medical Ethics Committee of the Erasmus Medical Center approved this case-control study (MEC-2015-356). Patients were recruited from the outpatient clinic of the department of Otorhinolaryngology and Head and Neck Surgery of the Erasmus Medical Center Cancer Institute between January 2016 and February 2017. Clinical parameters such as sex, age, medical history, smoking (pack years), and TNM-stage of tumor were recorded using the electronic medical record (CSC-iSOFT, Virginia, USA). Patients were divided into an oncologic group and a non-oncologic control group. The oncologic group consisted of patients with primary and untreated laryngeal SCC. They were referred to our clinic for diagnosis and treatment of their tumor. Tumors of all TNM-stages were included. The head and neck squamous cell carcinomas were confirmed by laryngoscopy, CT-scan and histopathology. The non-oncologic control group consisted of patients with chronic rhinosinusitis with or without nasal polyps and patients treated for cholesteatoma. The absence of an occult, unexpected HNSCC was confirmed by laryngoscopy. All included patients were smokers or ex-smokers. Patients with a medical history of lung or esophageal cancer were excluded from both groups. The study size estimation was based on the optical buccal mucosa differences between lung cancer and control patients of a previous study [14]. All patients signed an informed consent form before inclusion in this study.

Optical measurements of the buccal mucosa were performed at the outpatients clinic. All measurements were done by a single investigator (OB). The probe tip was gently placed in contact with the buccal mucosa, after disinfection of the fiber bundle with Tristel Trio (Tristel Solutions Ltd, Snailwell, UK). Five consecutive MDSFR spectra were acquired per patient at a single site without moving the probe tip, with a total duration of forty seconds (Fig. 1). Measurements were performed in the exact same way in the oncologic and non-oncologic group.
Fig. 1. Multidiameter single-fiber reflectance spectroscopy measurement. Subject is a volunteer who gave written consent to use and publish her image. A) Overview of setup with patient, probe, and laptop for data storage. B) Placement of probe tip on the buccal mucosa. C) Close-up of probe tip.

MDSFR model for extraction of optical properties

We have previously described the MDSFR model for extraction of optical properties in detail [19]. In summary, our group has developed semi-empirical models for the collected SFR in the absence of absorption, \( R_{SF}^0 \ [%\] , and the effective photon path length for SFR, \( \langle L_{SFR} \rangle \) [mm], based on experimentally validated Monte Carlo simulations [20, 32, 33]. The tissue absorption coefficient can be determined from a single SFR measurement using a modified Beer–Lambert law relationship

\[ R_{SF} = R_{SF}^0 e^{-\mu R_{SFR}} , \]  

with the model for effective SFR path length

\[ \langle L_{SFR} \rangle d_t = \left( \mu \right)^{p_4} \left( \mu d_t \right)^{p_5} \frac{C_{PF, p_2}}{p_3 + \left( \mu d_t \right)^{p_4}} , \]  

and using the model for \( R_{SF}^0 \),

\[ R_{SF}^0 = \eta_{lim} \left( 1 + p_6 e^{-\mu d_t} \right) \frac{\mu d_t^{p_5}}{p_4 + \left( \mu d_t \right)^{p_6}} , \]  

with a background scattering model [34]. In the above equations, \( \eta_{lim} \) is the collection efficiency at the diffusion limit. This is given as 2.7% for a fiber numerical aperture of 0.22 in a medium of refractive index 1.38 [35]. The parameters \([C_{PF, p_1, p_2, p_3}]\) and \([p_4, p_5, p_6]\) are fitted parameters. The fitted parameters have been previously determined by Monte Carlo.
simulations of the SFR covering the parameter ranges of \( d_f = [0.2-1.0 \text{mm}] \), \( \mu_s' = [0.3-3.6 \text{mm}^{-1}] \), and \( \mu_a = [0-3.0 \text{mm}^{-1}] \) using modified and unmodified Henyey–Greenstein phase functions with \( g_1 = [0.8, 0.9, 0.95] \) and \( \gamma = [1.4-1.9] \) [20, 22]. The values \([0.944, 1.54, 0.18, 0.64]\) for \([C_{PF}, p_1, p_2, p_3]\) and the values \([6.82, 0.969, 1.55]\) for \([p_4, p_5, p_6]\) were found to minimize the residual error between the SFR model and the simulations [20, 22].

Recently, we have demonstrated that \([p_4, p_5, p_6]\) are specifically sensitive to the phase function parameter, \( \gamma \), where \([p_4, p_5, p_6] = [2.31\gamma^2, 0.57\gamma^2, 0.631\gamma^2]\) [32]. As a result, successive SFR measurements with at least two fiber diameters enable simultaneous solution of Eq. (3) for absolute quantification of \( \mu_s' \) and \( \gamma \) over the measured wavelength range [25, 26].

**Design of the MDSFR device**

The optical properties of the buccal mucosa were measured using a custom made MDSFR device, which has been described in detail in a previous paper [19]. In short, MDSFR uses a 19-core fiber bundle of individual 200 \( \mu \text{m} \) fibers for both light delivery and collection (Fig. 2). At the proximal end of the fiber bundle each fiber is trifurcated to be connected to a) a fiber delivering light from a halogen lamp in the visible to near-infrared wavelength range (400-900 nm), b) a fiber delivering light from a 365 nm and 405 nm LED and c) a fiber collecting light returning from the tissue and delivering it to the spectrometer. At the distal end of the fiber bundle, which is placed in contact with the buccal mucosa, the fibers are bundled into three concentric groups comprised of one, six and twelve fibers. They are polished at an angle of 15 degrees to minimize the collection of specular reflections. The most distal part of the fiber bundle was encased in a 12 mm diameter curved metal housing for the optimal application on buccal mucosa (Fig. 1). A series of fiber optic interconnects and three computer-controlled shutters enables illumination and spectroscopic detection of the center fiber, the middle ring, and the outer ring of fibers, independently. In this way, a sequential coaxial single fiber reflectance (SFR) measurement of 200, 600 and 1000 \( \mu \text{m} \) can be made without moving the probe. The entire device is installed on a portable medical cart that is approved to be used in the outpatients clinic.

![Fig. 2. A schematic representation of the MDSFR device with numbering of the fiber cores, trifurcation at the proximal end of each fiber in the bundle, and the fiber tree. A photograph of the fiber tree is added on the right.](image)

**System calibration**

MDSFR system calibration is important for quantitative estimation of optical properties. The calibration procedure to account for the spectral illumination, transmission and detection efficiencies of the measurement system was described in detail previously [19]. In short, the MDSFR system merges spectra from different fibers and spectrometers to create measurements with varying effective fiber diameters. Correct merging of spectrometer
channels requires correction for differences in spectral sensitivity and transmission efficiency between channels. This can be achieved by comparing the spectra measured by each channel under uniform illumination. This is accomplished by inserting the probe into an integrating sphere and illuminating the sphere with the halogen lamp through a side port. The spectra acquired from the center fiber \( I_{\text{center}} \) [counts/s] and middle ring of fibers \( I_{\text{middle}} \) are then normalized by the spectrum acquired from the outer ring of fibers \( I_{\text{outer}} \). These spectra are used to arrive at wavelength-dependent weighting coefficients \( W_{\text{center}} \) [-] and \( W_{\text{middle}} \) for the two innermost channels:

\[
W_{\text{center}} = \frac{1}{12} \frac{I_{\text{int.sphere center}}}{I_{\text{int.sphere outer}}} \tag{4}
\]

and

\[
W_{\text{center}} = \frac{6}{12} \frac{I_{\text{int.sphere center}}}{I_{\text{int.sphere middle}}} \tag{5}
\]

The weighting coefficients are calculated relative to the outer channel because this channel has the largest detection area and thus the largest signal-to-noise ratio. During an MDSFR measurement, the spectra from each channel are combined using these weighting coefficients into effective single-fiber spectra with three different effective diameters, where

\[
I_{\text{eff small \ center}} = I_{\text{center}} \tag{6}
\]

\[
I_{\text{eff med \ center + middle}} = W_{\text{center}} I_{\text{center}} + W_{\text{middle}} I_{\text{middle}} \tag{7}
\]

and

\[
I_{\text{eff large \ center + middle + outer}} = W_{\text{center}} I_{\text{center}} + W_{\text{middle}} I_{\text{middle}} + I_{\text{outer}} \tag{8}
\]

These spectra are then calibrated to account for the spectral illumination and transmission efficiencies and the spectrometer sensitivity for each effective fiber diameter. This calibration is achieved by acquiring MDSFR spectra from a water sample and from an Intralipid based scattering optical phantom. The spectrum acquired from the water sample \( I_{\text{eff water}} \) originates from back reflections within the system and is subtracted from every measurement, while the spectra acquired from the Intralipid scattering phantom \( I_{\text{cal}} \) is compared with the absolute reflectance for this phantom \( \Phi_{\text{cal}} \), which has been simulated for each effective fiber diameter using a Monte Carlo model. The resulting measurement is calibrated into absolute reflectance \( \Phi_{\text{SF}} \), where

\[
\Phi_{\text{SF}} = \Phi_{\text{cal}} \frac{I_{\text{eff meas}} - I_{\text{eff water}}}{I_{\text{cal}} - I_{\text{eff water}}} \tag{9}
\]

Equation (9) is used to calibrate the reflectance spectra from each effective fiber diameter independently, where the measured spectra, \( I_{\text{eff}} \), are given by Eqs. (6), (7), or (8), depending on the effective diameter.

A system validation was also performed to prove that the merged spectra acquired from the individual fibers are equivalent to the SFR spectra measured by a single solid-core fiber [19].
Spectral analysis

The complete analysis of spectra is well described in previous papers [24, 36, 37]. In short, the SFR spectra were analyzed using a mathematical model that describes the wavelength-dependent effects of scattering and absorption on the reflectance intensity collected by the device following Eqs. (1-3). For the background scattering model we use a power law dependence, with fitted parameters describing the scattering amplitude ($\alpha_1$) and scattering slope ($\alpha_2$). For the tissue absorption we assume the presence of blood and bilirubin. The summed contribution of the chromophores oxygenated and deoxygenated hemoglobin (HbO2 and Hb, respectively) and albumin-bound bilirubin is given as follows:

$$\mu_{\text{tissue}} = \rho C_v \left( \text{StO}_2 \cdot \mu_{\text{HbO2}} + \left( 1 - \text{StO}_2 \right) \cdot \mu_{\text{Hb}} \right) + \mu_{\text{bil}} \cdot [\text{BIL}]_{\text{tis}}. \quad (10)$$

Here $\rho$ is the blood volume fraction (BVF), $\text{StO}_2$ is the microvascular saturation, $\mu_{\text{HbO2}}$ is the specific absorption coefficient of oxygenated hemoglobin, $\mu_{\text{Hb}}$ is the specific absorption coefficient of deoxygenated hemoglobin, and $\mu_{\text{bil}}$ and $[\text{BIL}]_{\text{tis}}$ are the specific absorption coefficient and concentration of albumin-bound bilirubin, respectively. The basis spectra for these 3 chromophores were reported in [37, 38]. Within tissue, blood (and in turn Hb and HbO2) is located within the vasculature. This heterogeneous distribution affects the spectral shape of the absorption detected by reflectance spectroscopy; an effect that is characterized by the $C_v$ term, which is given as:

$$C_v = \frac{\left( 1 - \exp \left( - \left( \text{StO}_2 \cdot \mu_{\text{HbO2}} + \left( 1 - \text{StO}_2 \right) \cdot \mu_{\text{Hb}} \right) / D_v \right) \right)}{\left( \text{StO}_2 \cdot \mu_{\text{HbO2}} + \left( 1 - \text{StO}_2 \right) \cdot \mu_{\text{Hb}} \right) / D_v}. \quad (11)$$

and includes an estimate of the average vessel diameter ($D_v$).

Statistical analysis

Outcome parameters were calculated by taking an average of the five buccal mucosa measurements taken per patient weighted by the individual confidence intervals of the fitted parameters. Ten result parameters were analyzed: $\text{StO}_2$, BVF, VD, $[\text{BIL}]_{\text{tis}}$, $\mu_s'$ at 450 nm, $\mu_s'$ at 800 nm, $\mu_s'$ power law scattering parameter, $\gamma$ at 450 nm, $\gamma$ at 800 nm, and average $\gamma$. Our quantitative variables were not normally distributed due to the relative small groups. We thus report our results as median value and interquartile range (IQR). Differences between the oncologic and non-oncologic group were analyzed using the Mann-Whitney U test. Qualitative data were reported as counts and frequencies, and differences between groups were analyzed using the $\chi^2$-test. To optimally identify HNSCC patients, a linear discriminant analysis was used in SPSS to create biomarker $\alpha$. We included all significantly different parameters with the 'stepwise method'. This analysis shows the relative contribution of these parameters to the differentiation between cancer patients and controls, which allows the most optimal merge into combined biomarker $\alpha$. A ROC-curve of $\alpha$ was created to perform a sensitivity and specificity analysis. There were no missing data. Statistical analysis was performed using SPSS version 21 (IBM Co., Armonk, NY, USA) and the cut off point for significance was a p-value < 0.05.

Results

Forty-six patients were included in this study: 23 head and neck squamous cell carcinoma patients and 23 non-oncologic control patients (Table 1). The male to female ratio in the HNSCC group was 6.7:1 and 3.6:1 in the control group ($p = 0.437$). The median age at time of measurement was the same for HNSCC group and the control group (68.2 [IQR 56.7-73.1] vs. 67.8 [61.9-70.1], $p = 0.575$). The median amount of pack years was 35.0 (20.0-45.0) in the HSNCC and 30 (17.5-50.0) in the control group ($p = 0.895$).
Table 1. Patient demographics

|                           | HNSCC group (n = 23) | Control group (n = 23) | P-value |
|---------------------------|----------------------|------------------------|---------|
| Male sex (%)              | 86.9                 | 78.3                   | 0.437   |
| Age, year (median [IQR])  | 68.2 (56.7-73.1)     | 67.8 (61.9-70.1)       | 0.575   |
| Smoking, PY (median [IQR])| 35.0 (20.0-45.0)     | 30.0 (17.5-50.0)       | 0.895   |

HNSCC = head and neck squamous cell carcinoma, PY = pack year, IQR = interquartile range. P-values calculated with χ² test (sex) and Mann-Whitney U test (age and smoking).

The HNSCC group consisted of 18 patients with a glottic tumor and five with a supraglottic tumor. Five tumors were carcinomas in situ. Eight tumors were staged T1, three T2, seven T3, and zero T4. The majority of the tumors (20 [87.0%]) had not metastasized. Three tumors had metastasized to regional lymph nodes. They were all staged N1. There were no patients with distant metastases in this cohort.

The intra-patient variation of the five consecutive measurements varied between 3 and 22% deviation from the mean for the ten parameters. The variations the absorption outcome parameters StO₂, BVF, VD and [BIL]ₜis were 6, 21, 18, and 22% respectively. In the scattering parameters intra-patient variations ranged from 7 to 15% in the μₛ parameters and from 3 to 5% in the γ parameters.

Table 2. Results of MDSFR physiological parameters

| Parameter (median [IQR]) | HNSCC group (n = 23) | Control group (n = 23) | P-value |
|--------------------------|----------------------|------------------------|---------|
| StO₂ (%)                 | 73.1 (72.0-79.1)     | 78.4 (72.9-81.7)       | 0.038*  |
| BVF (%)                  | 2.61 (2.00-3.40)     | 3.19 (2.80-3.62)       | 0.024*  |
| VD (mm)                  | 0.05 (0.04-0.07)     | 0.06 (0.04-0.07)       | 0.410   |
| [BIL]ₜis (μmol/L)        | 6.96 (4.10-8.42)     | 6.20 (4.48-8.05)       | 0.913   |

IQR = interquartile range, HNSCC = head and neck squamous cell carcinoma, StO₂ = blood oxygen saturation, BVF = blood volume fraction, VD = vessel diameter, [BIL]ₜis = tissue bilirubin concentration. * = p-value < 0.05. P-values calculated with Mann-Whitney U test.
Fig. 3. Example of spectra acquired from the buccal mucosa of A) a patient with a laryngeal tumor and B) a non-oncologic control. The three lines per patient represent the spectra derived from the effective 200, 600 and 1000 µm fiber diameters. Patient A features a lower absorption coefficient in the wavelength region from 500 to 600 nm (indicated by vertical dashed lines). The spectrum from patient A correlates with a lower blood oxygen saturation and blood volume fraction and consequently a lower value of biomarker \( \alpha \) than patient B.

Two physiological parameters, StO\(_2\) and BVF, recovered from MDSFR measurements on the buccal mucosa, were significantly different between the HNSCC and the control group. Table 2 shows the group values of all four physiological parameters. The remaining 6 parameters, all based on scattering contrast, were not significantly different between cancer and control groups and are therefore not shown in Table 2. Figure 3 shows representative MDSFR spectra from the three fiber diameters of (A) an HNSCC patient and (B) a control patient. The median StO\(_2\) was lower in the HNSCC group (73.3% [72.0-79.1]) than the control group (79.4% [72.9-81.7], \( p = 0.030 \)). The same was true for the median value of BVF (2.6% [2.0-3.4] vs. 3.2% [2.8-3.6], \( p = 0.020 \)). This decrease was more pronounced in patients with tumors of higher T-stages (Fig. 4). The scattering parameters were not able to differentiate between the HNSCC group and the control group.

Based on a linear discriminant analysis of all the parameters, the StO\(_2\) and BVF parameters were combined into biomarker \( \alpha \). Alpha was significantly lower in the HNSCC group than in the control group (0.28 [0.27-0.29] vs. 0.30 [0.28-0.33], \( p = 0.007 \)). Biomarker \( \alpha \) had the potential to distinguish patients with a HNSCC from healthy controls with a sensitivity of 78.3%, a specificity of 73.9%, and an area under the curve of 73.0% (Fig. 5).
Discussion

In this study we have, for the first time, demonstrated that the optical properties of the buccal mucosa of patients with laryngeal cancer were significantly different than controls. The differences in optical properties were related to differences in the microvascular blood oxygen saturation and blood volume, possibly related to field cancerization. Measurements were performed with an easy-to-use, non-invasive fiber-optic technology: multidiameter single-fiber reflectance spectroscopy.

Our findings represent a first step towards the use of MDSFR spectroscopy on the buccal mucosa as a pre-screening tool for HNSCC. MDSFR spectroscopy has the potential to be used in a large scale community based testing of high risk patients. In such a scenario, patients with a positive test will be referred to a head and neck oncology center to undergo additional tests (e.g., complete clinical examination, laryngoscopy, and CT-scan) to confirm a HNSCC.

![Fig. 4. Values of BVF split up per T-stage versus non-oncologic control group. Median values are plotted in squares. Error bars represent interquartile range. * = p-value <0.05. P-values calculated with Mann-Whitney U test. Cis = carcinoma in situ, T1-3 = T-stage 1-3.](image)

![Fig. 5. ROC curve of biomarker α (composed of BVF and StO₂). AUC = area under the curve.](image)
In a recent study, we investigated if the current approach of performing MDSFR spectroscopy of the buccal mucosa could also be used in patients with esophageal squamous cell carcinoma cancer [28]. Interestingly, the parameters that were found to be discriminative differed between laryngeal and esophageal cancer patients. The present study found that the physiological parameters (blood oxygen saturation and blood volume fraction) were altered, while $\mu_s'$, a scattering parameter, was altered in the buccal mucosa of esophageal cancer patients. The mechanisms behind this difference are not yet fully understood. A possibility is that the FC has a distinct physiological and architectural signature between different types of distant malignancy. However, this hypothesis has to be tested.

The sensitivity and specificity of MDSFR pre-screening for laryngeal cancer (78% and 74%) found in the present study are similar to those of a recent optical screening study for lung cancer (79% and 83%) [29]. This study investigated whether low-coherence enhanced backscattering spectroscopy of the buccal mucosa could predict the presence of a distal lung tumor. Despite the use of reflectance spectroscopy in both studies, the discriminatory parameters were also of different optical origin. Similar to our esophagus study, LEBS screening for lung cancer found differences in light scattering, while the present study found differences in light absorption: the microvascular StO$_2$ and BVF were lowered in patients with HNSCC. Interestingly, these results suggest a decrease in microvasculature in FC. This contradicts the general notion that a local increase of microvasculature occurs in a tumor.

While we have not yet fully elucidated the mechanisms underlying the altered physiological parameters in the buccal mucosa of HNSCC patients, it is interesting to speculate on potential reasons of the lower values of StO$_2$ and BVF. The decrease in BVF could be caused by hemolysis in the HNSCC that leads to heme accumulation and upregulation of heme oxygenase (HO). In this scenario a local increase will also increase circulating levels of HO. Activity of HO will then increase circulating levels of vasoactive metabolites such as carbon monoxide. Heme itself also has a major impact on vascular tone via multiple mechanisms (including NOS, COX, CYP450, and sGC) [39]. The combination of both pathways has complex effects on the vascular tone. This could, in principle, lead to buccal vasoconstriction and reduced BVF in patients with HNSCC. An additional result of our study supports this hypothesis: the decreased BVF was more pronounced in advanced tumors than early stage tumors. A final possibility is that the actual BVF is not decreased but that we measure a lower value due to an increased thickness of the epithelial layer. Buccal mucosa is a multi-layered tissue; if the non-vascularized epithelial layer is thickened it is possible that MDSFR spectroscopy will interrogate a smaller volume of the vascularized lamina propria, thus resulting in an apparently low BVF. The low StO$_2$ values may be an indication of oxidative stress in the buccal mucosa of HNSCC patients.

A possible limitation of this study is the relatively small number of patients per group. This prevented us from splitting our cohort in a training a validation set to test the discriminative power of biomarker $\alpha$. It could also cause an underestimation of p-values and a less ‘smooth’ ROC-curve. Another potential issue is that we did not account for all patients characteristics that could potentially have an influence on the optical properties of the buccal mucosa. While we did account for the most important risk factor, smoking, we did not do so for alcohol use.

The HNSCC group in this study consisted of laryngeal tumors. This subsite was chosen since it is the most distant from the buccal mucosa. The aim of this study was, however, to demonstrate the feasibility of a screening method for all HNSCC locations. We will further investigate this in a following study. We also plan to investigate whether the approach described in this study is applicable to screen for lung cancer. All tumors of the UADT have a similar etiology and share smoking and alcohol use as their two major risk factors, which can be explained by their shared embryologic origin [40]. Previously reported analogous studies have shown that reflectance spectroscopy has the potential to screen for lung and esophageal cancer [29, 30].


Conclusion

In conclusion, our results demonstrate that the buccal mucosa of patients with laryngeal cancer is altered, possibly by field carcinization. Multidiameter single-fiber reflectance spectroscopy measurements showed that the blood oxygen saturation and blood volume fraction was decreased in HNSCC patients. Our biomarker $\alpha$ was able to differentiate between HNSCC patients and controls with a sensitivity of 78% and a specificity of 74%. This shows promise for the use of MDSFR spectroscopy of the buccal mucosa to pre-screen high risk patients. Diagnosing non-symptomatic, early stage tumors could significantly decrease the associated morbidity and improve the survival and quality of life of HNSCC patients.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

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