Introduction

Oral cavity cancer is a major public health issue, with 300,000 new cases per year worldwide (1). Most oral cancers arise from the epithelium of the mucosal surface and are referred to as oral cavity squamous cell carcinoma (OCCSCC). OCCSCC mortality is high, with a 5-year survival rate of around 50% and 145,000 deaths per year worldwide (1, 2). Despite advances in treatment modalities (surgery, radiotherapy, and chemotherapy), these numbers have not shown significant improvement over the last decades (3, 4). Important determinants of the clinical outcome of patients with OCCSCC are tumor subsite, tumor–node–metastasis (TNM) classification, age, comorbidity, and tumor histologic characteristics (3–7). Surgery is the mainstay of treatment for OCCSCC. Adequate tumor resection with acceptable remaining function and physical appearance is the main goal. At our institute, we follow the guidelines of the Royal College of Pathologists (United Kingdom). The distance between tumor and the nearest resection surface (DBTNRS) determines the adequacy of the surgical procedure. This distance is histologically measured in mm. A resection margin can be classified as clear (>5 mm of DBTNRS), close (1–5 mm of DBTNRS), and positive (<1 mm of DBTNRS; ref. 8). Clear margins are regarded as adequate and close and positive margins as inadequate. Adequate resection margins are crucial for disease control and survival (8–14). Patients with inadequate resection margins often receive adjuvant therapy (chemotherapy and/or radiation) or re-resection. However, these can have a negative effect on patient morbidity.

Achieving adequate resection margins is challenging. The lack of reliable intraoperative guidance and the proximity of tumors to vital structures are the common causes of inadequate tumor resection. Despite comprehensive preoperative imaging of the tumor (by CT scan, MRI, etc.), the surgeon decides where to cut, based on visual inspection and palpation of the tumor during the operation. Earlier, we have reported the surgical results obtained in two Dutch centers (Erasmus Medical Center Rotterdam and Leiden University Medical Center). For OCCSCC surgery, adequate resection margins were obtained in only 15% of the cases (9). A similar result was recently reported by the Harborview Medical Center and the University of Washington Medical Center in Seattle (11). Clearly, visual inspection and palpation of the tumor and surrounding tissue by the surgeon are insufficient to warrant adequate tumor resection.

Intraoperative assessment of resection margins by means of a frozen section procedure can be used (15). This procedure, in which the pathologist performs microscopic evaluation of a piece...
of suspicious tissue, is currently the gold standard of intraoperative diagnostics (15–17). The main limitation of the frozen section procedure is that only a fraction of the resection margins can be investigated. The method is prone to sampling error, which often leads to false-negative results (9, 18). As a result, the frozen section procedure is not very effective in improving surgical success rate. Ideally, the entire resection surface should be evaluated intraoperatively, which requires an objective and fast technology.

Intraoperative assessment of resection margins on the resection specimen (i.e., specimen-driven approach) has been reported to be superior to assessment of the wound bed (i.e., defect-driven approach) by different groups. Specimen-driven intraoperative assessment of resection margins leads to a higher surgical success rate and increase of patient survival than defect-driven or no intraoperative assessment at all (11, 17–19). Various techniques like ultrasonography, imprint cytology, and various optical techniques are being explored for intraoperative use in surgical oncology (20–28). Some of these techniques are being applied for OCSCC, which were recently reviewed by Ravi and colleagues (26). Optical techniques like high-resolution microendoscopy (HRME), optical coherence tomography (OCT), fluorescence spectroscopy, elastic light scattering spectroscopy, and Raman spectroscopy are promising because of their ease of use, relatively low cost, and high speed in screening large tissue areas (20–28).

Raman spectroscopy is an optical technique that is being investigated for intraoperative evaluation of the surgical margins. Raman spectroscopy can be applied to assess the mucosa, as well as, the deep soft tissue layers (29–34). It is an objective technique based on inelastic scattering of monochromatic light that provides detailed quantitative and qualitative information about the molecular composition of tissue. The technique is nondestructive, and there is no need for reagents or labeling, which promotes easier translation to the clinics (35, 36).

The goal of our research is to develop a Raman spectroscopic technique for objective intraoperative assessment of the entire resection surface, with the ultimate goal to improve the success rate of OCSCC surgery. In a first pilot study, we have demonstrated that Raman spectra of resection specimen discriminated tumor from healthy surrounding tissue with a sensitivity of 99% and a specificity of 92% (37). The primary discriminating factor of the Raman spectra proved to be the water concentration in the tissue. Raman spectroscopy is very suitable for rapid quantitative determination of the water concentration in tissue, as has been demonstrated by our group (38–40). The objectives of the current study were to investigate how the change in water concentration correlates with the border between tumor and surrounding healthy tissue and, consequently, to verify if this information can be used to assess resection margins.

Materials and Methods

Medical ethical approval

This study was approved by the Medical Ethics Committee (MEC-2013-345) of the Erasmus MC Cancer Institute, University Medical Center Rotterdam. Prior to the operation, informed consent was obtained from the patients. Measurements were conducted ex vivo on resection specimen of patients undergoing surgery for OCSCC. The allowed time for the experiments was 60 minutes, after which the resection specimen was put in formalin for routine histopathologic evaluation.

Tissue samples and handling

Immediately after resection, the surgeon brought the specimen to the cutting room of the pathology department, which is in close proximity to the operating room. A dedicated pathologist and surgeon inspected the specimen together. This process included labeling of the anatomic sites and documentation of the specimen with diagrams and digital images (Fig. 1A). After orienting and defining the resection margins, the pathologist and the surgeon surveyed all resection planes by visual inspection and palpation. After this, the pathologist cut the specimen in 3 to 5 cross-sections (with a thickness of about 5–10 mm), perpendicular to the resection margin plane (Fig. 1B). For specimens comprising bone (i.e., mandibular resection specimens in patients with OCSCC invading the bone), the soft tissue was cut till the bone. The pathologist measured the distance between tumor and resection surface. Often, this macroscopic assessment only was sufficient to decide on the further course of the operation without the need for frozen sections. In case of an unclear tumor border, the pathologist may decide to further refine the information by microscopic examination of frozen sections. Provided with this intraoperative information regarding inadequate margins, the surgeon continues to harvest more tissue from the wound bed (e.g., immediate re-resection) to achieve an adequate surgical result.

After this intraoperative diagnostic procedure, one of the specimens cross-sections was chosen for Raman experiments (further called "Raman tissue section"). The cross-section was regarded suitable when containing tumor and >5 mm of healthy looking surrounding tissue (Fig. 1B). The remaining specimen cross-sections were immersed in formalin.

Blood was rinsed from the Raman tissue section using physiological salt solution (0.9% NaCl) and gently patted dry with gauze. The area of interest (i.e., tumor and >5 mm of surrounding healthy tissue) was macroscopically chosen by the pathologist. The Raman tissue section was inserted in a closed cartridge to avoid drying of the tissue. The upper side of the cartridge consists of a fused silica window. This cartridge allows the scanning of a 3 × 3 cm² tissue area. The Raman tissue section was placed in the cartridge with the surface to be measured in contact with the fused silica window. Digital images of all handling steps were made, including images for the macroscopic representation of the tissue area measured (Fig. 1C).

After the experiment, the Raman tissue section was removed from the cartridge and immersed in formalin, together with the rest of the specimen to follow the routine procedure for final pathological processing.

Raman instrumentation and mapping experiments

Raman ex vivo mapping experiments were performed using a confocal Raman microscope (CRM), built in-house. The equipment was placed in a laboratory close to the operating room. The setup, as explained in our previous work (37), comprised a multichannel Raman Module (HPRM 2500, RiverD International BV), a 671-nm laser (CrystaLaser, CL671-150-SO), and a charge-coupled device (CCD) camera fitted with a back-illuminated deep depletion CDD-chip (Andor iDus 401, DU401A BR-DD, Andor Technology Ltd.). A microscope (Leica DM RXA2, Leica Microsystems Wetzlar GmbH) and a computer-controlled sample stage...
Figure 1.
Overview of the experimental protocol. **A,** immediately after surgical resection, the specimen (excision of tongue SCC) was transferred to the pathology room and orientation was digitally recorded (anterior (A), posterior (P), and medial (M)). **B,** specimen was cut perpendicular to the resection surface in three sections for intraoperative assessment of the resection margins. Thereafter, a tissue section was chosen for the Raman experiment. **C,** Raman tissue section was inserted into a cartridge. The area to be measured was defined by the pathologist, containing tumor and >5 mm of surrounding healthy tissue, at least in one direction. **D,** Raman mapping experiments were performed on a grid. The water concentration for each measured point was calculated. A 2D image was obtained by using a nonlinear color scale to represent the water concentrations. **E,** after Raman measurement, the specimen was routinely processed. H&E-stained slide was made from the whole Raman tissue section, within which, pathologists identified the tissue area that was measured. The histopathologic annotation of the tumor (T), healthy tissue (H), and of the tumor border (red line) was performed. **F,** on the basis of the annotated tumor border in the H&E image (red line), the position of the adequate surgical margin (>5 mm of distance to the tumor border) was determined within the water map (green line).
(Leica DM STC) were coupled with the Raman Module. Eighty mW of laser light was focused in the tissue by means of a microscope objective (0.4 numerical aperture) with a free working distance of 1.1 mm (N PLAN 11566026, Leica Microsystems BV). The depth resolution was 40 μm, experimentally determined. Spectral information was collected in the wavenumber range 2,500 to 4,000 cm⁻¹ with a resolution <5 cm⁻¹.

For each measurement, the cartridge with the tissue section was fixed on the microscope stage. The selected area was measured point-by-point using a grid. The grid cell size was between 300 μm per 300 μm to 1,000 μm per 1,000 μm, depending on the size of the tissue section and on the allowed time of 60 minutes to perform the experiment. In some cases, more than one map per specimen was measured depending on the size of the tissue section and on the allowed time. The acquisition time per spectrum was 1 second. Laser light was focused in the tissue at about 50 μm below the fused silica window surface.

Calibration and processing of spectra

All spectra were calibrated on the relative wavenumber axis and corrected for the wavelength-dependent detection efficiency of the setup, according to instructions of the spectrometer supplier (RiverD International BV). Preprocessing of the spectral data was performed by removal of cosmic ray events and subtraction of the signal background generated in the optical path of the setup itself (39). MATLAB R2014b was used for data processing and data visualization.

The tissue Raman spectra showed varying levels of background signal originating from tissue autofluorescence. For the calculation of tissue water concentrations, the autofluorescent background signal was estimated by a third-order polynomial and subtracted from the measured spectra.

Spectra with a relative intensity lower than 5% of the average intensity of all spectra measured from the sample were discarded. Intensity of the spectra was determined for the range 2,700 to 3,100 cm⁻¹, in which almost all spectral signatures from lipids and proteins are localized. Low signal intensities were encountered in cases where the tissue was locally not fully in contact with the measurement window.

The ratio of the Raman bands at 3,390 cm⁻¹ and 2,935 cm⁻¹ was used to determine the concentration of water per spectrum according to the method developed by Caspers and colleagues (40) and described in detail in our previous study (38, 40).

Raman water maps

Raman water maps were created by plotting the water concentration as a 2D map using pseudo colors to represent the water concentration range. A convolution of the water map with a 3 x 3 averaging filter was applied, as shown in Fig. 1D, to obtain values that are more representative of the local water concentration (reducing noise in the image), and for better visualization of the difference in water concentration between tumor and the surgical margins (41).

Histopathology

Histopathologic evaluation of the measured areas was performed by two dedicated pathologists on routine hematoxylin and eosin (H&E)-stained thin tissue sections. Subsequently, the H&E-stained section was digitized and the pathologists delineated healthy tissue, tumor, and tumor border (Fig. 1E).

Data analysis

On the basis of the projection of the tumor border in the H&E image (red line) onto the Raman water map, each pixel was labeled as tumor border, tumor, or healthy (Fig. 1F). The precision with which the individual pixels could be annotated in this way is limited by the much lower resolution of the Raman map compared with the microscopic image. The error was estimated to be half of the Raman map pixel size. Thereafter, the minimal Euclidean distance between each Raman map pixel and the tumor border was calculated. On the basis of these distances, the position of the adequate surgical margin (all pixels with distance >5 mm to the tumor border) was obtained (Fig. 1F).

For each map, the average and SD of the water concentration were separately calculated for tumor, for the inadequate margin

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Gender</th>
<th>Maps</th>
<th>Primary tumor location</th>
<th>pTNM</th>
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<tbody>
<tr>
<td>1</td>
<td>71</td>
<td>F</td>
<td>1</td>
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<tr>
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<td>72</td>
<td>M</td>
<td>1</td>
<td>Floor of mouth</td>
<td>T2N2bM0</td>
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<tr>
<td>3</td>
<td>52</td>
<td>F</td>
<td>1</td>
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<td>T3N2bM0</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>F</td>
<td>1</td>
<td>Lateral side of tongue</td>
<td>T1N0M0</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>M</td>
<td>1</td>
<td>Lateral side of tongue</td>
<td>T1N0M0</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>M</td>
<td>1</td>
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<td>T1N0M0</td>
</tr>
<tr>
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<td>59</td>
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</tr>
<tr>
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<tr>
<td>9</td>
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<td>F</td>
<td>1</td>
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<td>T1N0M0</td>
</tr>
<tr>
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<td>42</td>
<td>F</td>
<td>1</td>
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<tr>
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<td>67</td>
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<td>2</td>
<td>Inferior alveolar process</td>
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</tr>
<tr>
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<td>60</td>
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<td>1</td>
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<td>TINOM0</td>
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<tr>
<td>13</td>
<td>69</td>
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</tr>
<tr>
<td>14</td>
<td>61</td>
<td>M</td>
<td>1</td>
<td>Lateral side of tongue</td>
<td>T1N0M0</td>
</tr>
<tr>
<td>15</td>
<td>68</td>
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<td>1</td>
<td>Lateral side of tongue</td>
<td>TINOM0</td>
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<tr>
<td>16</td>
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<tr>
<td>17</td>
<td>68</td>
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</tr>
<tr>
<td>18</td>
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<td>F</td>
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<td>Tongue and floor of the mouth</td>
<td>T3N1M0</td>
</tr>
<tr>
<td>19</td>
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<td>1</td>
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<td>20</td>
<td>61</td>
<td>F</td>
<td>1</td>
<td>Lateral side of tongue</td>
<td>T2N0M0</td>
</tr>
</tbody>
</table>

NOTE: Number of maps measured per patient (Maps). Primary tumor location and pathologic TNM classification (pTNM) of malignant tumors (42). Tumor size varied from less than 1 cm (T1) to more than 4 cm. In some patients, tumor had extended into the mandible (T4a). N stage varied from no regional metastasis in lymph nodes to multiple lymph nodes with metastasis of 6 cm or less in greatest dimension (N0-N2b). Distant metastasis was not encountered (M0).
(i.e., distance from tumor border $\leq 5$ mm), and for the adequate margin.

The Mann–Whitney U test was used to determine whether the distribution of the water concentrations in tumor, in inadequate margins, and in adequate margins are significantly different from each other.

Next, we calculated the average water concentration of the tissue as a function of the distance to the tumor border. This was done by calculating the mean water concentration of pixels falling within a 0.5-mm distance interval and moving this interval from $-15$ mm (inside the tumor) to $+10$ mm (in the healthy tissue). Likewise, the SD in the water concentration was calculated as function of distance to the tumor border.

**Results**

Twenty-five ex vivo Raman mapping experiments were performed on fresh resection specimens from 20 patients treated by surgery for OCSCC. Table 1 shows patient and tumor characteristics.

Each map had an average of 406 spectra (range comprehended between 97 and 1,250 spectra) and an average area of 240 mm$^2$ (from 18.9 to 624 mm$^2$). The average tumor area per map was 84 mm$^2$ (range was between 13 and 390 mm$^2$), the average inadequate margin area per map was 85 mm$^2$ (minimum value was 27.9 mm$^2$ and maximum value was 237 mm$^2$), and the average adequate margin area per map was 71 mm$^2$ (minimum and maximum values were, respectively, 4 mm$^2$ and 379.2 mm$^2$).

In total, 3,526 Raman spectra from tumor were obtained. From the surrounding healthy tissue, 3,620 spectra were obtained at a distance of less than 5 mm from the tumor border (i.e., within the area of inadequate margin) and 3,001 spectra were obtained at a distance greater than 5 mm from the tumor border (i.e., from the area of adequate margins).

As an example, the results for 3 experiments performed on fresh resection specimens from 3 patients are shown in Fig. 2. The macroscopic images of the measured areas are shown in column A. Column B shows the water concentration maps. These maps were interpolated to a pixel size of 300 $\mu$m, which was the smallest step size used for mapping. In column C, the averaged water maps after interpolation to the same pixel size are presented. Column D shows the annotated H&E-stained sections. Column E shows the average water concentration (blue line) and SD (black line).

For each map, the mean and SD of the water concentration for tumor, inadequate, and adequate margins were calculated (Table 2). The average water concentration in tumor is 76% ± 8%, in the inadequate margin it is 59% ± 24%, and in the adequate margin it is 54% ± 24%.

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**Figure 2.**

Rows 1–3, examples of the data obtained by means of mapping experiments on three Raman tissue sections from three patients. A, photograph of the measured fresh tissue surface. B, Raman water map with indication of tumor border (red; based on final histopathology shown in panels of column D) and adequate surgical margin (green). C, averaged Raman water map with indication of tumor border (red; based on final histopathology shown in panels of column D) and adequate surgical margin (green). D, H&E-stained section obtained from the measured tissue surface, with tumor border (red), tumor (T), healthy surrounding tissue (H) indicated by pathologist. E, graphs showing water concentration as function of the distance to the tumor border. Blue line, average water concentration calculated per 0.5-mm distance interval. Black line, SD of the water concentration, per 0.5-mm distance interval. The red line at 0 mm represents the tumor border, and the green line represents a distance of 5 mm from tumor border.
Mann-Whitney U tests show that these difference in water concentration between tumor, inadequate margin, and adequate margin are all significantly different with \( P < 0.0001 \).

In Fig. 3, the water concentration (blue line) is shown, calculated as the mean and SD over all experiments, as a function of distance to the tumor border, using 0.5-mm distance intervals. From this figure, it is clear that the water concentration in tumor is much higher than in the surrounding healthy tissue. The figure also shows that the decrease in water concentration coincides with the tumor border. The water concentration starts to decrease inside the tumor mass, close to the tumor border and continues to decrease steeply until about 4 mm into the surrounding healthy tissue. From there, the decline in water concentration continues with a smaller gradient. Interestingly, the SD in water concentration also shows that the decrease in water concentration coincides with the SD in water concentration being more than 15% just outside the tumor.

**Discussion**

The aim of our research is the development of a clinical tool for intraoperative guidance of surgical oncological procedures motivated by the main goal of surgery: adequate tumor resection and preservation of function and physical appearance. Of the many factors that affect the clinical outcome of patients with OCSCC, only the resection margins can be influenced by the surgeon and pathologist. The objective intraoperative assessment of resection margins is the key to increase the number of adequate resections in surgical oncology, therefore, an objective tool for assessment and guidance is needed.

Multiple techniques are being explored for intraoperative use in surgical oncology (20–28). Until now, fluorescence spectroscopy (20), diffuse reflectance spectroscopy (21), elastic light spectroscopy (22), HRME (23), and OCT (24) have explored in vivo delineation of the tumor at the mucosal surface, prior to surgery. However, 87% of inadequate margins are found in the deeper (submucosal) soft tissue layers (43). Therefore, the design of these studies is not perfect to be applied at the submucosal layers of soft tissue, which is where the majority of inadequate margins are found.

OCT is a promising technique that has been used to investigate OCSCC resection margins. A recent study published by Hamdoon and colleagues (44) concluded that OCT is a valuable tool in the assessment of surgical margins. This study reported that the diagnostic accuracy was about 85%. However, they mentioned that the use of OCT technology is limited because the created image can be affected by the lack of normal tissue perfusion. Therefore, the resolution and contrast of the OCT images are influenced by the "ex vivo nature" of the approach (44, 45). Moreover, not only OCT but also HRME has as disadvantage that it requires complicated subjective image interpretation (23, 24, 44, 45).

Raman spectroscopy has proved to be a reliable technique that can be applied to assess mucosa as well as the deep soft tissue layers (31, 36–38). This objective and nondestructive technique was used in our first study, where it showed to be accurate in discriminating OCSCC from the surrounding healthy tissue. In this previous study, we showed, by means of high-wavenumber Raman spectroscopy, that water concentration within the tumor (OCSCC) is significantly higher than in the surrounding healthy tissue enabling discrimination between tumor and healthy tissue with 98% accuracy (37). The notion that certain tumors contain more water than surrounding healthy tissue was not new; already in 1971, water content was described as one of the discriminators with 98% accuracy (37). The notion that certain tumors contain more water than surrounding healthy tissue was not new; already in 1971, water content was described as one of the discriminators with 98% accuracy (37).
normal and malignant tissues to generate contrast between the two (46).

In the current study, we investigated how the water concentration changes from inside the tumor toward the adequate surgical margin. The results show a clear correlation between the tumor border and the change in water concentration. The transition from a high water concentration inside the tumor to a lower water concentration in the surrounding tissue takes place as a negative gradient over a distance of about 4 to 6 mm across the border of the tumor. By analyzing this negative water concentration gradient (Fig. 3), we observed that the decrease in water concentration from tumor toward the adequate margin is accompanied by an increase in the SD of the water concentration, that is, the heterogeneity increases. Inside the tumor, the water concentration was higher than 69%, with a relatively low SD of less than 15%. This low SD indicates that OCSCC is homogeneous concerning water concentration, regardless of pTNM classification (Table 2). Inside the tumor, at about 1.5 mm distance to the tumor border, the water concentration of the tumor starts to decrease and the SD starts to increase (Fig. 3). The average precision with which the Raman image could be annotated with the image of the H&E-stained section was ±0.38 mm (from ±0.15 to ±0.5 mm) and was determined by the resolution of the Raman measurements as explained in Materials and Methods. The increase in the SD can indicate that close to the tumor border, the water concentration heterogeneity increases, possibly explained by the presence of stroma, blood vessels, and lymphatic vessels (47). Another interesting finding is that at approximately 4 mm beyond the tumor border, the SD of the water concentration levels off at about 26%. This high variance of the water concentration in the surrounding healthy tissue is due to the heterogeneity in these areas comprising fat tissue, muscle (M), and vessels (Fig. 4).

In this study, we show the water concentration distribution across the tumor border. The shape of the water profile from inside the tumor toward the adequate margin for OCSCC is a new

**Figure 3.** Water concentration profile from inside the tumor toward adequate margin. All individual water concentration percentages of the 25 maps were averaged per interval to calculate the mean (blue) and SD (black) of the water concentration as a function of the distance to the tumor border. The red line at 0 mm indicates the tumor border. The green line at 5 mm indicates the beginning of the adequate surgical margin.

**Figure 4.** H&E-stained section obtained from a measured tissue surface, with tumor border (red line), tumor (T), and healthy surrounding tissue (H) indicated by pathologist. A representative region of the adequate margin was enlarged and the tissue structures annotated. Tissue structures present are muscle (M), adipose tissue (A), and blood vessels (B).
finding, as well as the increase in water concentration heterogeneity at the tumor border.

We are currently devising fiber optic probe configurations and fiber optic probe measurement strategies to capture this information in a way that can be implemented for rapid intraoperative assessment of resection specimens.

We believe that Raman spectroscopy is a promising candidate for comprehensive intraoperative inspection of the surgical margins for OCSCC resection specimens, which will fit in the surgical workflow and can help to significantly improve the percentage of adequate resections.

We expect that water concentration analysis will prove equally useful in localizing the tumor border in other locations of the body and plan to expand this line of investigation accordingly.

Disclosure of Potential Conflicts of Interest

P.J. Caspers is employed with RiverD International BV as a senior applicant and R&D scientist. V. Noordhoek Hegt, S. Koljenović, and G.J. Puppels have ownership interest (including patents) in RiverD International BV. R.J. Baatenburg de Jong reports receiving other commercial research support from shareholder River D. T.C. Bakker Schut is an employee of RiverD International BV. G.J. Puppels is employed with RiverD International BV as a CTO & Managing Director. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.M. Barroso, C.G.F. van Lanschot, P.J. Caspers, A. Sewnaik, V. Noordhoek Hegt, T.C. Bakker Schut, S. Koljenović, G.J. Puppels

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Water Concentration Analysis by Raman Spectroscopy to Determine the Location of the Tumor Border in Oral Cancer Surgery


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