Innovative “Raman – needle” fiber optic probe for tumour delineation in the oral cavity

Oral cavity cancer is a major public health issue, with annually 300.000 new cases.
Surgery is the mainstay of treatment, but adequate tumour removal is not achieved in 85% of the cases. This affects survival and leads to additional treatment of patients by radiotherapy or chemotherapy. There is a clinical need to assess the resection margins of tongue cancer specimens, intraoperatively. In frames of RaSure, the European project funded by Eurostars, a fully automated Raman spectroscopic device is under development to achieve complete tumour removal in oral cancer surgeries.
Raman spectroscopy is a non-destructive optical technique that will be used to determine the water concentration in the tissue that is removed by the surgeon during oral cancer surgery. Precisely, the technology will enable inspection of the resected tissue to determine if a tumour has been completely removed.
The product will consist of a dedicated Raman-based tissue inspection device, the operational system and disposable fiber-optic needle probes.
The consortium includes three partners: ***art photonics GmbH***, ***RiverD International BV*** and ***Erasmus MC.*** The role of ***art photonics GmbH*** is the development of the unique disposable Raman-needle fiber probe that can be inserted into the tissue multiple times and measure without loss of quality of the results.

Evaluation of the Raman needle probes was performed in Charité Clinic in the operational theatre for oral cancer surgery.

Sample Preparation

The fresh resected human tongue samples, consisted of tumour tissue surrounded by healthy tissue were taken from patients who underwent surgery in the ***Klinik für Hals-, Nasen-, Ohrenheilkunde, Charité Universitätsmedizin Berlin***. Each point of tissue was measured in 3 points for 3 times. Multivariate data analysis including principle component analysis (PCA) was performed using Interval Selection Toolbox for MatlabTM (MathWorks, Natick, MA, USA).

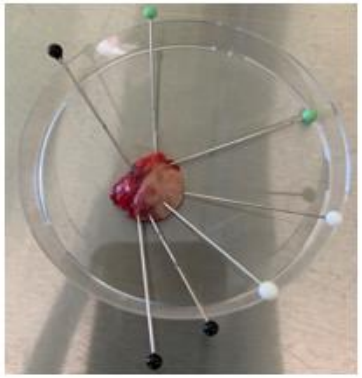

Figure 1: Left: Human tongue tissue samples: cancer tissue marked with the red pins, surrounded by healthy tissue marked with the green pins. Right: "Raman – needle" fiber optic probe.

All Raman experiments were carried out using the pilot version of "proof-of-principle" Raman system provided by project partner RiverD with laser excitation equal 680nm on the tissue biopsies. Measurements were conducted with different Raman needle probes. (Ocean Optics) coupled to fiber optic probes. One single fiber was used as the excitation channel and the detection channel simultaneously for spectra obtained in the high-wavenumber region of 2600–3600 cm^{-1}. Each point of tissue was measured in 3 points for 3 times.

Middle-infrared absorption (MIR) measurements

MIR measurements were performed using an IR Fourier spectrometer FT-805 (*Simex LLC*) spectrometer equipped with a mercury-cadmium-telluride (MCT) detector cooled by liquid nitrogen. Spectra were acquired in contact with the tissue using a standard ATR probe, based on two polycrystalline (PIR) fibers with an outer diameter of 100 µm and at the probe head a Si-ATR element (*art photonics GmbH*) optimized for the fingerprint region. Air was used as a background (to obtain the reference spectrum). MIR spectra were obtained at 64 scans with a resolution of 8 cm^{-1}.

Near-infrared diffuse reflectance measurements (NIR)

Diffuse reflectance measurements were performed in a spectral range from 900 to 1700 nm with a portable fiber optic NIRQuest512 spectrometer (*Ocean Insight*) with an indium gallium arsenide detector with a resolution of about 3.1 nm. For spectral collection, a fiber-optic probe with seven collecting and one emitting 400 mm fibers (*art photonics GmbH*) was used. The tip of the probe was in contact with the tissue during the measurements. As a light source, an LS-1 tungsten halogen lamp (*Ocean Insight*) was used. Before data measurements on the tissue, a reference spectrum on a Spectralon white diffuse reflectance standard (Labsphere Inc.) and a dark current spectrum by covering the slit of the spectrometer were collected. To ensure a proper data calibration, all tissue spectra were subsequently divided by this reference spectrum, after previous subtraction of the dark current spectrum from both.

Results and Discussion

The best determination of cancer and non-cancer tissues were gained with the use of Raman HWN technique. Raman spectroscopic measurements with the needle probes allowed to get clear signal and to highlight the differences in spectra of malignant and healthy tissues correlated to ratio of lipids (~2900 cm^{-1}) and water (~3400 cm^{-1}). The exemplary spectra of two types of tissues: tumour (red) and non-tumour (green) are presented in the Figure 2 (left). Multivariate data analysis including principle component analysis (PCA) was performed by means of segmented cross-validation using the data in unique measurement positions as segments. The score plots are presented on the Figure 2 (right).

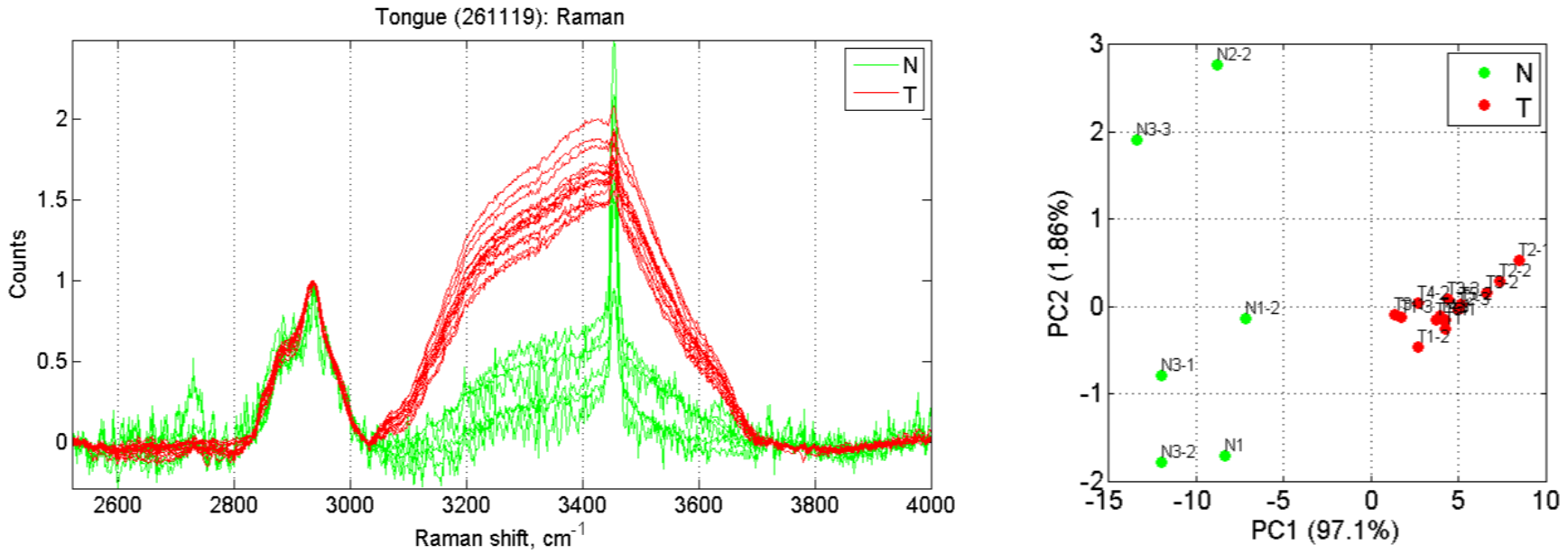

Figure 2: Left: Raman spectra of human tongue tissue samples, including tumour (red), normal (green), measured by "proof-of-principle" Raman-System, provided by RiverD. Right: Principal Components Analysis score plots for Raman Spectra.

PCA analysis proved the clear separation of spectral characteristics of the cancer and non-cancer tissues, and, therefore, the effectiveness of the proposed method and spectral system.

MIR absorption allows to obtain reliable results on cancer and non-cancer separation as well. MIR spectra of tongue tissues (Figure 3, left) demonstrate contributions mostly from proteins, especially in the Amide I (1650 cm^{-1}), Amide II (1560 cm^{-1}) and Amide III (1300 cm^{-1}) bands. The peak high of these bands contribute to characteristic signals of cancer tissue: The Amide II band is more pronounced in the spectra obtained from the tumour samples.

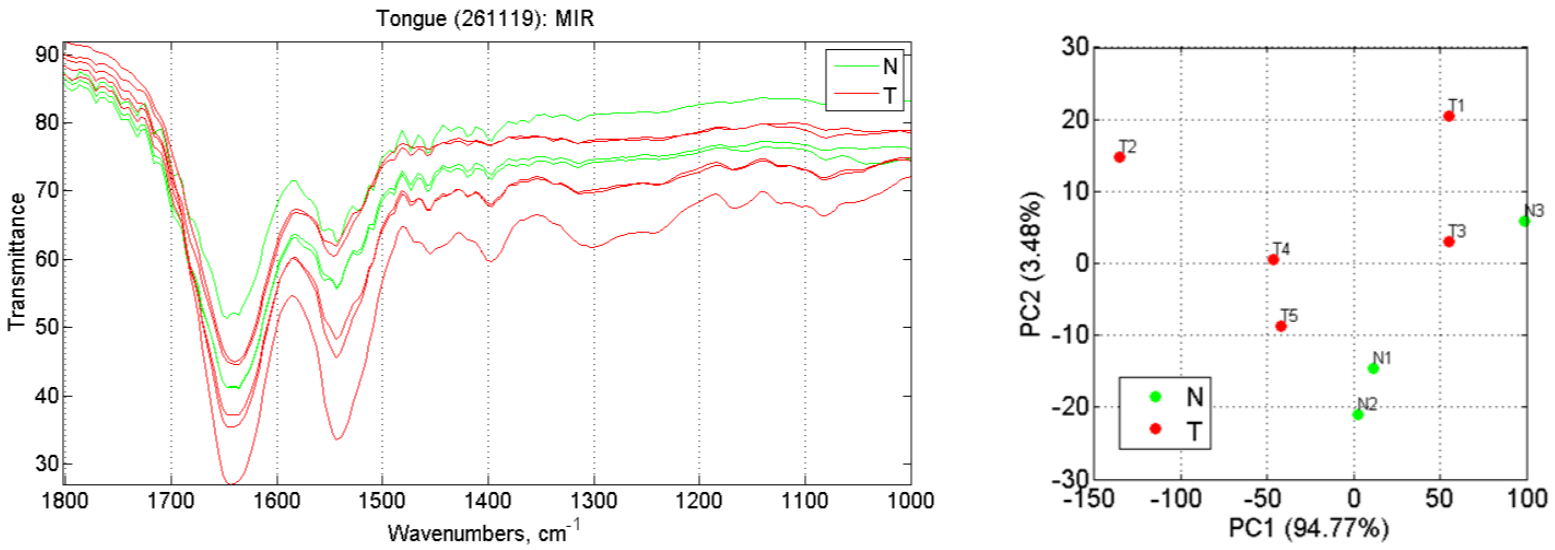

Figure 3: left: MIR absorption spectra of human tongue tissue samples, including tumour (red), normal (green). Right: Principal Components Analysis score plots for MIR absorption Spectra.

The spectral differences in NIR reflection can display variation of composition between tumor and normal tissues. The largest spectral differences were seen in the interval characteristic for the water second overtone (approx. 1000 nm), CH second overtone (approx. 1200 nm) associated to glycoproteins, glycolipids, an OH first overtone (approx. 1600 nm) associated to carbohydrates.

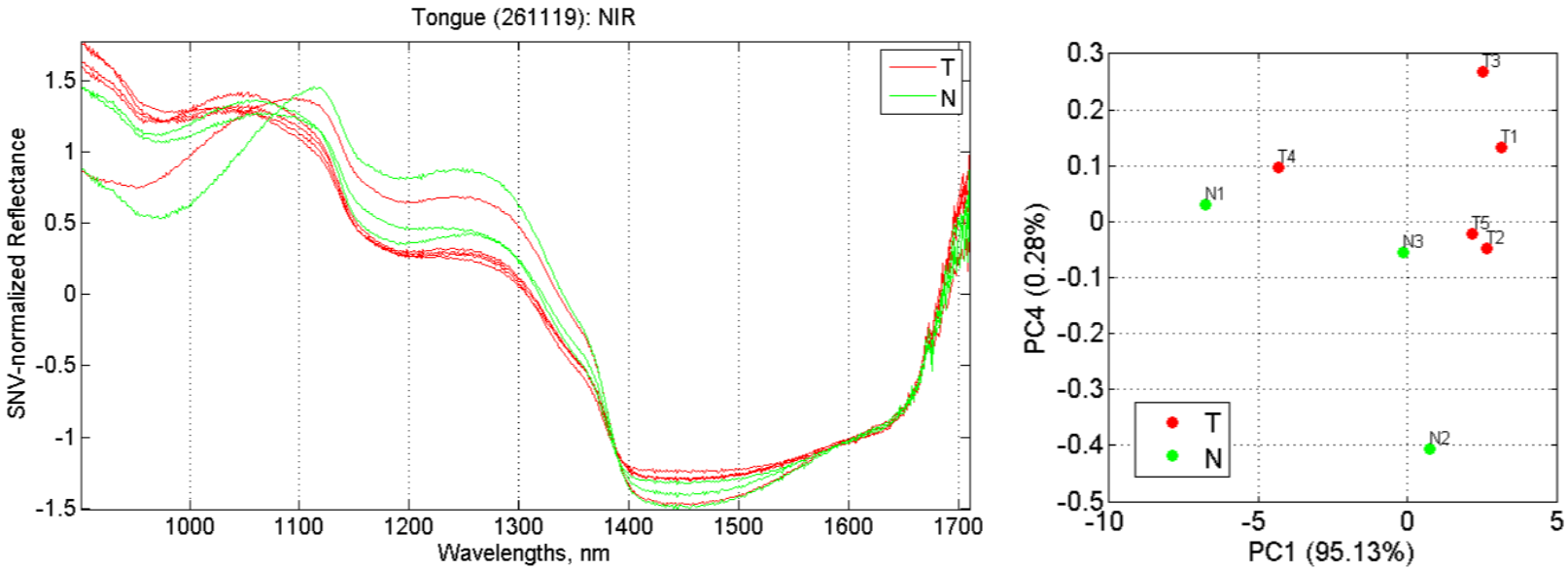

Figure 4: left: NIR absorption spectra of human tongue tissue samples, including tumour (red), normal (green). Right: Principal Components Analysis score plots for MIR absorption Spectra

Conclusion:

All presented spectroscopic methods were able to distinguish tumour from non-tumour tissues with a good accuracy. Raman HWN spectroscopy presented the most promising result. The differentiation between non-cancer and cancer samples appeared to be very straightforward based on Raman spectroscopy. Raman fiber optic spectroscopic device with needle probes has a strong potential for intra- operative assessment of tumor resection margins of tongue tumour. This may optimize surgery, as the entire resection surface can be scanned rapidly and the results can be readily available.