UNIVERSITY OF EASTERN FINLAND

Identifying oral cancer biomarkers from saliva and oral soft tissue using near-infrared spectroscopy

Nordic Network for Diversity in Physics, 04.11.2019

Miia Hurskainen

UEF // University of Eastern Finland

Contents

- Introduction
  - Oral health
  - What is near-infrared spectroscopy (NIRS)
  - Why use NIRS to measure biological samples
- Materials and methods
  - Measuring L-fucose and L-proline solutions and saliva
  - Measuring pig enamel
- Results
- Discussion

Introduction

Oral health

- Oral health is important factor in both general health and well-being according to the World Health Organization (WHO)
- Oral cancer, oral infectious diseases and hereditary lesions are three of the six most frequent oral diseases worldwide
- Oral diseases or irritation cause abnormal changes in oral mucosal tissue that cause symptoms that might not be visible for human eye
- Oral diseases and cancers are detected using palpation and visual inspection, conventional oral examination (COE). The findings of COE vary between observers. Lesions may be confused with cancer and benign tumors might be confused with malignant tumors

Introduction

What is near-infrared spectroscopy (NIRS)

- Non-ionizing optical imaging method
- Measures transitions in vibrational levels through the IR-absorption
- Infrared (IR) light can be divided into three regions: near-IR (680 nm-2500 nm), mid-IR (2500 nm-50 000 nm) and far-IR (>50 000 nm)
- NIR light is divided into short and long NIR wavelengths, 680-1000 nm and 1100-2500 nm, respectively
- Overtones and combination bands of molecular vibrations of C-H, N-H and O-H groups absorb long NIR wavelengths

Introduction

Why use NIRS to measure biological samples

- NIR light scatters more extensively than it absorbs into the tissue, which makes tissues transparent to NIR
- NIR radiation penetrates deep into the organic tissues
- NIRS gathers information about tissue compositions
  - Spectrum quantifies proteins, carbohydrates, water, fats, and other organic and biological substances
  - Each organic substance has a “fingerprint region” on their spectrum

Introduction

Study of oral mucosa and saliva

- NIRS probe is in contact with the tissue in intra-oral measurements
  - Measurement is noninvasive as the probe does not penetrate in to the tissue
- Measurements should cover diseased area and healthy control area of the oral cavity
- NIRS measurements *in vivo* need to take into account saliva
  - High water content and strong NIR absorption of water
  - Oral epithelial and cancer cells shed into saliva
  - Saliva contains locally expressed proteins and substances

Materials and methods

Measuring L-fucose and L-proline solutions and saliva

- L-fucose and L-proline biomarkers that are found from saliva of oral cancer patients
- Solutions of fucose and proline (1:1, 1:10, 1:100 & 1:1000) and saliva of oral cancer patients were measured by placing a 20 µl drop to a reflective material
- NIRS probe was brought in contact with the drop and spectrum was measured
- Pure fucose and proline tablets (3 mm thick) were measured for reference
- Each solution (6 drops) was measured 6 times, tablets and saliva samples 3 times (3 drops/tabletsa) each

Materials and methods

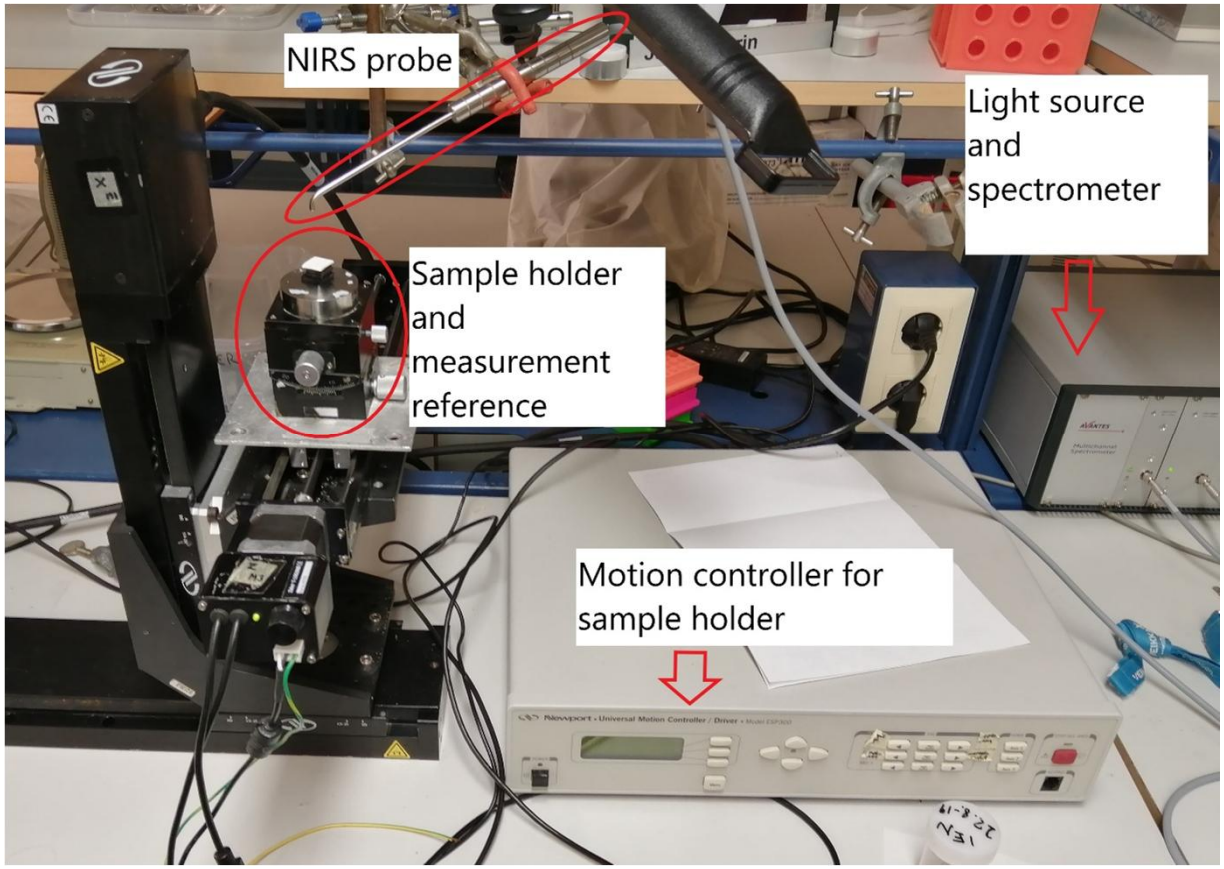
Measuring pig enamel

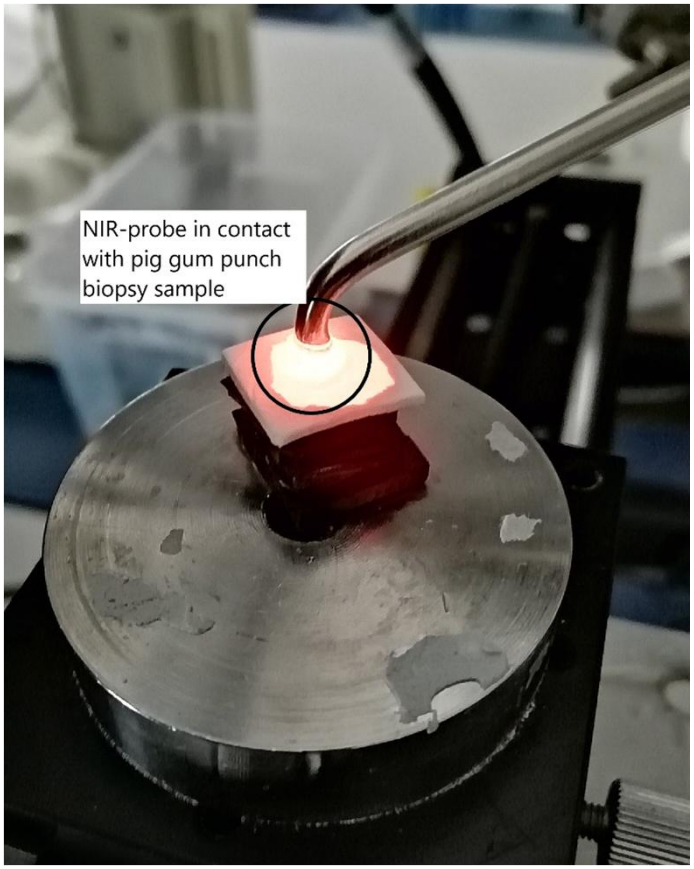
- 12 pig enamel punch biopsy samples *ex vivo*
- Samples were stored in 4 % saline solution
- Enamel sample was placed on a reflective material and probe was brought in contact with the sample

Measurement set up

- Two spectrometers: AvaSpec-ULS2048L and AvaSpec-NIR256-2.5-HSC, Avantes BV, Apeldoorn, The Netherlands
- A light source AvaLight-HAL-(S)-Mini, Avantes BV
- Customized arthroscopic optical fiber probe

Materials and methods



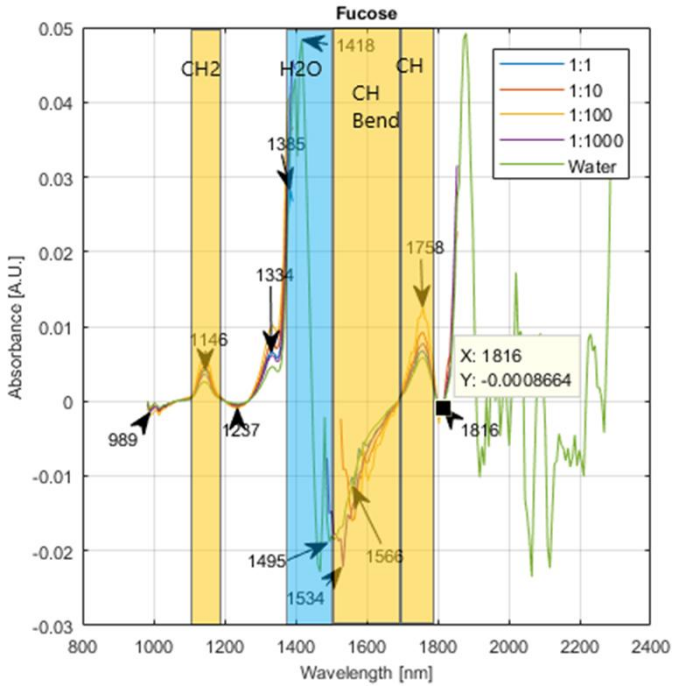


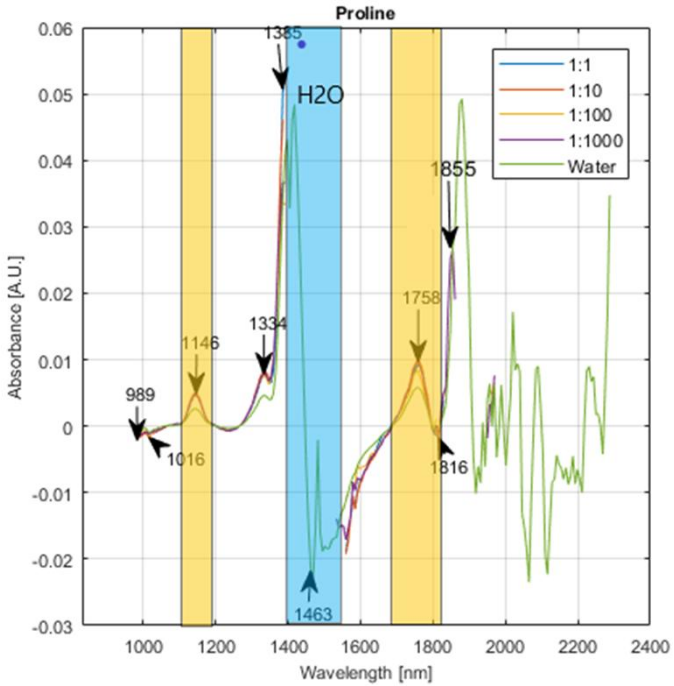
Materials and methods

Data analysis

- Absorbance was calculated using Beer-Lambert law $A = -log_{10}\frac{(S-D)}{(W-D)}$, where A is absorbance, S is sample spectrum, D and W are dark and white reference spectra, respectively
- Differential spectrum was computed for each sample using Savitzky-Golay algorithm in MATLAB
- The aim of the study of liquids was to see if it is possible to discriminate solutions with different concentrations from each other and water
- The aim of the study of pig enamel is to optimize oral tissue measurements with arthroscopic probe

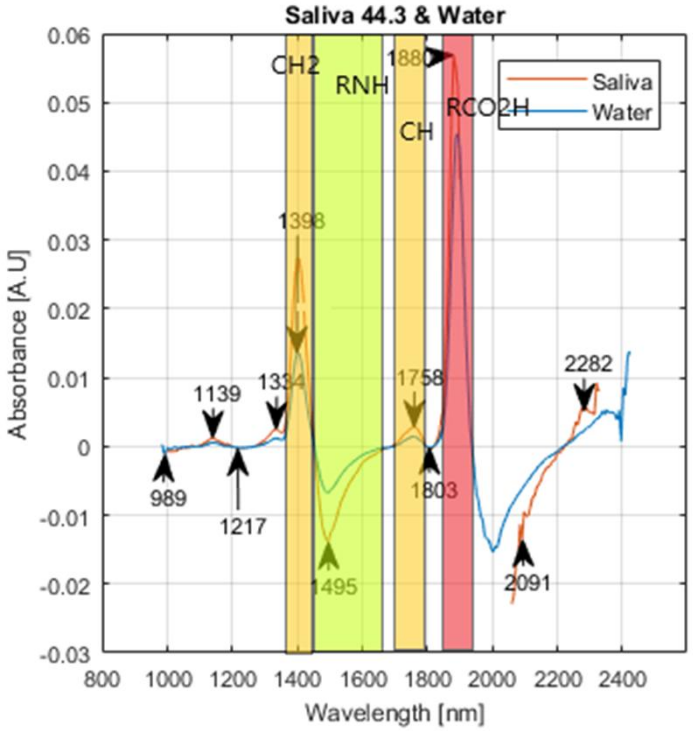
Results

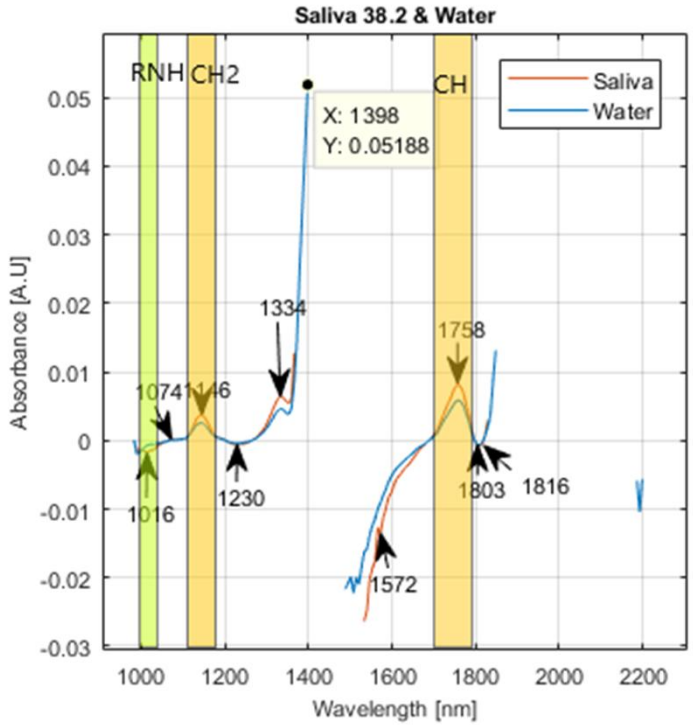




Water absorbance overlaps the absorbance peaks of proline and fucose in the water solutions. Some peaks were not assigned to water, but those peaks could not be assigned to fucose or proline either.

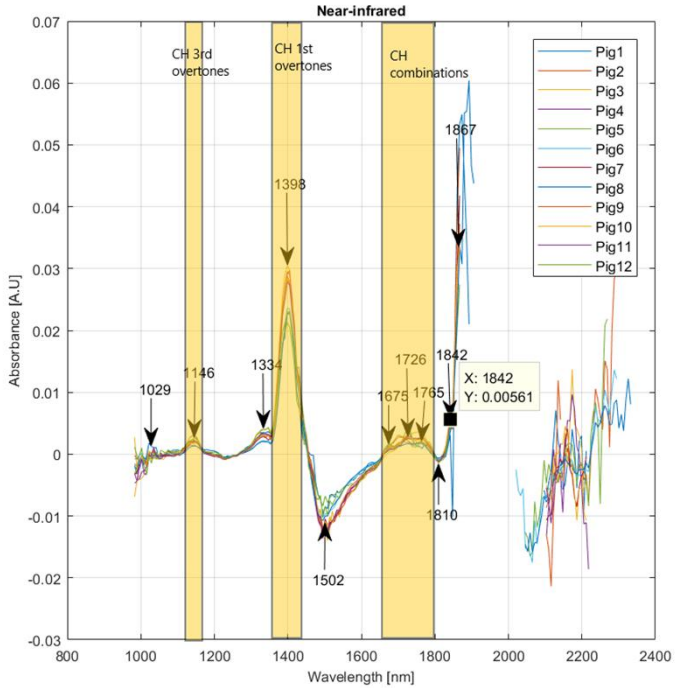
Results

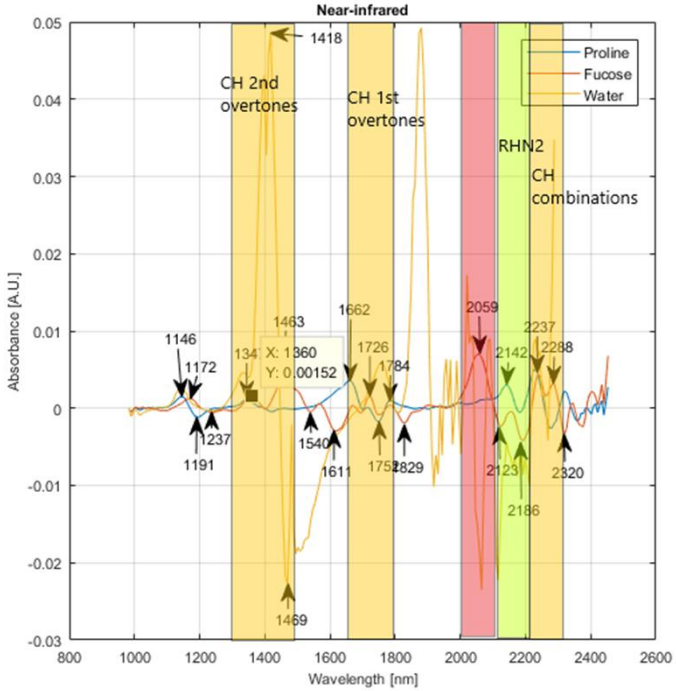




Saliva seems to have stronger absorption than water but the peaks are the same.

Results





Pig enamal differential spectra has similar with that of water. Pure proline and fucose have different shape on their differential spectra compared to water.

Results

- The custom made NIR probe can measure liquid samples but the results are qualitative as the distance from the reference material to liquid surface varies
- The absorbance of water is overbearing making it hard to distinct other substances in the solution
- Saliva has stronger peaks than pure water
- Pig enamel in saline solution shows similar differential spectrum as water

Discussion

- Water is overbearing spectra
  - The effect of water
- Saliva samples should be dried for measuring
  - How to ensure that drop dries out evenly and what is the best possible sample holder not affecting the results?
- Measurements done in oral cavity should take saliva into account => Area of interest could be dried but that might cause discomfort to patients and possibly irritate tissues affecting the results
  - Irritation might be confused with diseased or inflammated tissue
- More work is needed to optimize the measurements:
  - Tissue samples measured with PBS buffering
    - How does that affect the tissue?

Thank you!

uef.fi