Diffuse reflectance spectroscopy for margin assessment in breast tumor surgery
Luuk Buijs, MSc. (Applied Physics, Delft University of Technology)
Institute: Netherlands Cancer Institute (NKI)
Group: Surgical Oncology

Introduction
Patients with early-stage breast cancer can be treated with breast-conserving surgery (BCS) in combination with adjuvant radiation therapy. This results in long-term survival comparable to mastectomies, with the advantage of a better cosmetic outcome and a better quality of life (1). A strict condition for successful BCS is the removal of the entire tumor with a rim of benign tissue, hence, the absence of positive resection margins (see Figure 1) (2; 3). Unfortunately, BCS is often inadequate; 10–60% of patients treated with BCS require additional surgery to obtain negative margins (4; 5; 6).

To date, there are no robust methods to help the surgeon identify tumor tissue at the resection margin during surgery. Intra-operative margin assessment by imprint cytology and frozen section analysis can reduce the number of re-excisions (7), but both techniques experience major disadvantages. They require additional operation time and a skilled on-site pathologist to correctly interpret the results. In addition, the reliability of imprint cytology is hampered by drying, cautery, and irregularity of the tissue surface; while frozen section analysis damages the resection specimen and is prone to artifacts and sampling errors (8).

Diffuse Reflectance Spectroscopy
A possible solution for in vivo margin assessment may be the use of optically based techniques. One of these techniques is diffuse reflectance spectroscopy (DRS), which measures the amount of diffusely reflected light after it has undergone multiple scattering and absorption events within the tissue.

Within the visible wavelength region, oxygen saturation, b-carotene, and light scattering have proved to be able to discriminate tumor tissue from healthy tissue (11; 12; 13). However, in this wavelength range, blood is one of the predominant absorbers. The blood that is present at the tissue surface during surgery can hamper the reliability of intra-operative in vivo DRS measurements based on the visible wavelength region. In the extended NIR wavelengths (*1000–1600 nm), absorption of light by the blood is negligible, making this region more robust for an in vivo surgical application.
Previous research
In an earlier study that was performed in our group by Lisanne de Boer et al., the effects of inter-patient variation and tissue status (in vivo versus ex vivo) on the performance of several DRS parameters were investigated (10). In vivo and ex vivo measurements of 45 breast cancer patients were obtained using a 2 mm fiber distance optical probe and quantified with an analytical diffusion model to acquire the optical parameters. The optical parameter representing the ratio between fat and water provided the best discrimination between normal and tumor tissue, with an area under the receiver operating characteristic curve of 0.94.

Current activities
We incorporated fiber-optic DRS technology into a surgical probe that is able to measure DRS spectra in (near) real-time (Figure 3). The probe incorporates one optical source fiber and 6 receiving fibers (400 µm, 3 mm distance between fibers). The spectrometer setup consists of a spectrograph for the visible (350-1100nm) and near-infrared (900-1700nm) parts of the spectrum. A study protocol that aims to investigate the surgical feasibility of the probe setup is currently being composed. The study will involve several surgeons testing the probe instrument in vivo, in order to evaluate its convenience of use.

Meanwhile, we are working on the adaptation of an analytical diffusion theory-based formula, that is used to estimate fat/water ratios. The formula is based on reflectance values at isosbestic points in the absorption spectrum of tissues in which the main absorbers are fat and water. Utilizing the reflection values in a narrow spectral band near an isosbestic point is convenient because there, the penetration depth is independent of the tissue composition (Figure 3). In other words, the measurements should prove to be relatively insensitive to tissue inhomogeneity. The original formula was derived for a broad incident beam (camera applications) and is being altered for two-fiber purposes.

Figure 3: in-house DRS probe and initial phantom measurements. (Pictures courtesy of our group)

Figure 4: Simulated effective penetration depth of samples with various fat/water ratios
In a follow-up study, measurements will be performed on ex vivo resection specimens immediately after resection. By marking the measurement location, a robust correlation can be made between the optical measurements and histopathology. Accordingly, a classification algorithm will be developed to predict positive resection margins. Contrary to the aforementioned study, development of this classifier will utilize reflectance values at all available wavelengths.

We will test the classification algorithm in an in vivo setting. In patients undergoing BCS, the surgeon will be asked to perform tissue measurements at several different locations that - according to his judgement - are most at risk for a positive resection margin. In addition, 2 tissue areas that are most likely benign are measured. Measurement locations will be marked and compared to histopathology. In this way an in vivo data set is acquired, that can be used to validate and refine the classification algorithm.

References