Changes in the optical properties of tissue may reflect interesting morphological and physiological changes, such as those that occur in the (pre)cancerous transformation of normal epithelial tissue. Therefore, the development of non-invasive techniques which can reveal relevant changes in the local optical properties of tissues is of great importance.

Optical spectroscopy for early detection of lung tumours

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Early detection of lung cancer

Lung cancer is by far the commonest cancer in the Western world. It has a very high mortality rate due to the late stage at which lung tumours are usually diagnosed (rougly 8500 deaths per year in the Netherlands). Usually, by the time these tumours become symptomatic and the patiens seek medical attention, these cancers have spread to various parts of the lungs or beyond. In spite of the impressive advances in cancer treatment made in the last decade we can only temporarily alleviate the symptoms of these patients but usually cannot cure them. Early detection is expected to significantly increase the success rate of the therapy and extend the life expectancy of these patients. Research on this matter has been going on for several decades now. Conventional white light bronchoscopy has been shown to have a low diagnostic accuracy for early disease. More recently, fluorescence bronchoscopy was introduced. Fluorescence bronchoscopy shows an excellent sensitivity but too many false positives, i.e. a low specificity (a sensitivity of 90% or more, but a specificity of less than 20%, i.e. many false positives). To be able to fully exploit the excellent sensitivity of fluorescence bronchoscopy, we face the challenge to dramatically improve the specificity of the diagnostic procedure. The approach we use is by combining optical imaging with optical spectroscopy.

Optics of tissue

Measurement of the optical properties of tissue has been an important challenge in biomedical science for nearly two decades. Optical properties of tissue are important input parameters for light dosimetry, which is essential for better understanding and optimization of various therapies employing light (e.g. UV light therapies, laser surgery, photodynamic therapy). Additionally, optical properties of tissue may serve to characterize the tissue and can hence be instrumental for the development of low-cost, non-invasive medical diagnostic techniques. The optical properties of tissue are usually expressed in terms of two quantities, the scattering coefficient μ_s) and the absorption coefficient (μ_a), based on two different interaction mechanisms of light with tissue (figure 1). When light enters tissue it is scattered whenever it encounters refractive index variations. The fact that membranes, nuclei, mitochondria and other organelles all have a different refractive index from the surrounding cytoplasm makes tissue a highly scattering medium. The average distance that a photon travels before it is scattered is equal to the inverse of the scattering coefficient μ_s [mm^{-1}]. The scattering coefficient is wavelength dependent and biological variations are large, but in general the scattering coefficient of tissue ranges from 10 to 100 mm^{-1}

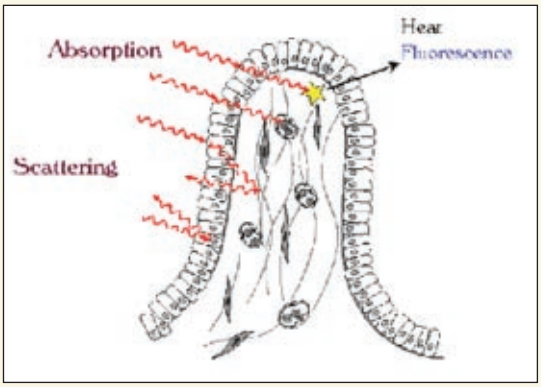

Figure 1. *Interaction mechanisms of light with tissue: scattering and absorption, possibly followed by fluorescence.*

in the visible/NIR wavelength range (350-1000 nm). The second interaction mechanism is absorption; molecules like water, fat, proteins and blood each have different absorption characteristics. Especially blood is an effective absorber in the visible wavelength range due to the presence of oxy- and deoxyhemoglobin, which both possess very distinctive absorption characteristics. The average distance that a photon travels before it is absorbed is equal to the inverse of the absorption coefficient μ_a [mm^{-1}]. The absorption coefficient is wavelength dependent and depends on the concentration of absorbing molecules, but in general the absorption coefficient of tissue ranges from 0 to 2 mm^{-1} in the visible/NIR wavelength range. The third interaction mechanism is fluorescence. When a molecule absorbs a photon, it is transferred to a higher energetic state. The excited molecule will quickly decay back to the lowest energetic state, mostly by heating its environment but sometimes by emitting another photon (fluorescence). Molecules in tissue that are known for their fluorescence properties are a.o. collagen, elastin, NADH and FAD.

Optical diagnostics

Changes in cell morphology will cause changes in the scattering pattern of the tissue. Furthermore, an increase in blood volume (possibly due to angiogenesis) causes the amount of absorbing hemoglobin molecules to increase, while an increased cell metabolism causes the relative amounts of oxy- and deoxyhemoglobin to change as well. These changes will be reflected in changes in the scattering and absorption coefficients. Additionally, an increased mucosal blood content will lead to less fluorescence from the submucosal collagen. Furthermore, the relative amounts of different fluorophores may change during cancerous transformations. Thus by measuring scattering, absorption and fluorescence spectra, one can detect subtle morphological, physiological and biochemical changes which occur during cancerous transformations. In the next section some examples of optical spectroscopic diagnostic measurements are given.

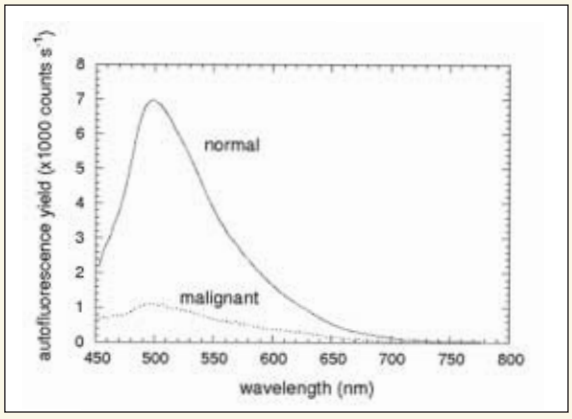

Figure 2. *Typical fluorescence spectra measured in the bronchial tree.*

Fluorescence spectroscopy

Figure 2 shows typical fluorescence spectra measured in vivo during bronchoscopy on normal and malignant bronchial tissue. The malignant tissue shows less fluorescence than the normal tissue, most likely due to a higher mucosal blood content in the malignant tissue. However, biological variations in fluorescence intensity are very large, and although it is possible to distinguish normal tissue from malignant tissue accurately, it has proven to be difficult to distinguish a benign lesion from a malignant lesion using fluorescence spectroscopy alone. Therefore, fluorescence spectroscopy is usually combined with white-light reflectance spectroscopy to improve the classification accuracy.

Diffuse reflectance spectroscopy

Several methods exist to determine the optical properties of tissue-like turbid media, including time-resolved, frequency-domain and spatially-resolved continuous wave systems. These systems require large source-detector separations to satisfy the validity of the diffusion approximation. As a consequence, the detected photons have traveled a long distance through the sample, and the extracted optical properties represent average values over a relatively large tissue volume. However, an important aspect in the detection of precancerous lesions is the fact that the relevant morphological and physiological changes typically occur in or just below the mucosa, a superficial tissue layer with a thickness of only a few hundred microns. To facilitate the determination of biologically relevant parameters such as neovascularization with formation of abnormal vessels and the microvascular oxygenation, a diagnostic tool which is sensitive to the optical properties of the most superficial layer of tissue is required. Therefore, we have recently developed a novel diagnostic technique (Differential Path-length Spectroscopy, DPS), which is sensitive to the optical properties in the most superficial layer of tissue.

Differential path-length spectroscopy

Figure 3 shows a schematic diagram of the DPS setup. Essentially, a DPS-setup contains a halogen light source, a 2-channel spectrometer, a computer and some dedicated fiber optics. The fiberoptic probe is manufactured in our own lab, while the other components are commercially available. We have found that unique properties of DPS are:

- The path-length of the photons contributing to the signal is very small (only a few hundred microns). Hence, only information on the most superficial tissue layer is obtained, which is essential for the detection of premalignant morphological and physiological changes.

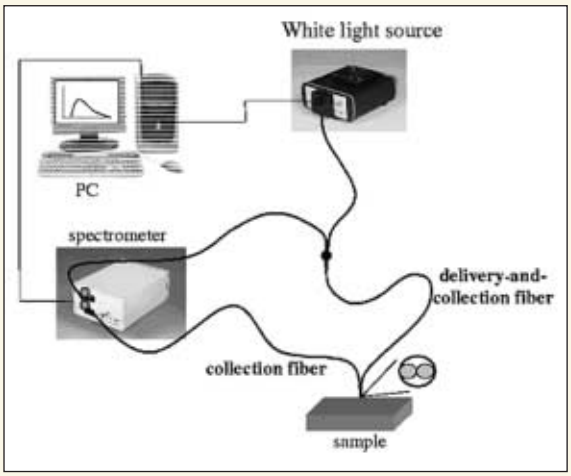

Figure 3. *Schematic diagram of the DPS-setup.*

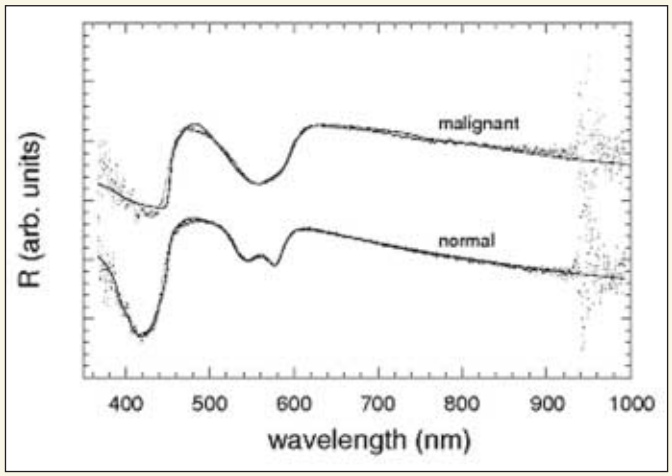

Figure 4. *Typical DPS-spectra and fits of normal and malignant bronchial tissue.*

- Photon path-lengths are constant (i.e. independent of the optical properties of the tissue). Hence, absolute concentrations of absorbing molecules can be measured. As oxyhemoglobin and deoxyhemoglobin have strong absorption bands in the visible wavelength region, DPS is particularly suitable for non-invasive in vivo measurements of capillary blood amount, saturation and vascular diameter, which are parameters we believe to be relevant for the detection of premalignant physiological changes.
- Photon path-lengths are adjustable (by adjusting the diameter of the fibers of the probe). Hence, the fiber-optic probe can be engineered to match the physiologically relevant dimensions of the tissue investigated.
- DPS measures superficial scattering properties. As the information obtained is from a superficial layer, the Mie scattering parameters obtained describe properties of the mucosa. Hence, changes in scattering properties observed can be directly related to morphological changes in the mucosa.

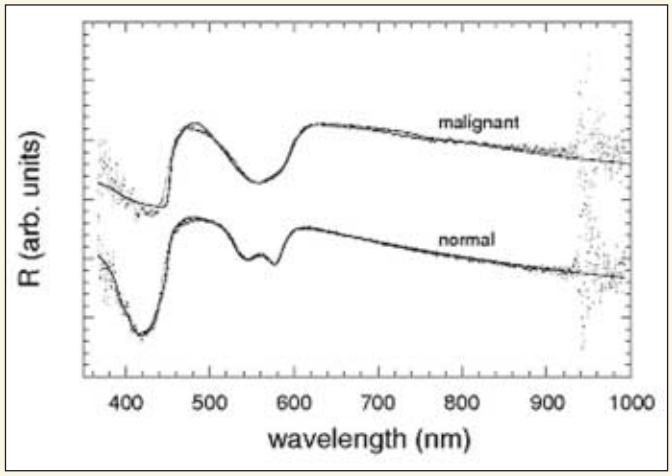

Figure 5. *Average microvascular saturation and relative standard deviations in saturation calculated from multiple DPS measurements on clinically suspicious lesions.*

We have recently shown that in the range of parameters relevant for biological tissue, the differential reflectance signal can be accurately modeled by

$$R(\lambda) = C_1 \lambda^{-b} \exp(-0.32\, C_{cor}(\lambda)\, k\, [StO_2\, \mu_a^{HbO2}(\lambda) + (1\text{-}StO_2)\, \mu_a^{Hb}(\lambda)])$$

with k the blood volume fraction, StO_2 the microvascular blood oxygenation, $\mu_a^{HbO2}(l)$ the absorption coefficient of fully oxygenated whole blood, $\mu_a^{Hb}(l)$ the absorption coefficient of fully deoxygenated whole blood, C_1 a proportionality constant and C_{cor} the correction factor which accounts for the inhomogeneous distribution of blood in tissue. Figure 4 shows examples of spectra and fits for normal and malignant bronchial mucosa. Striking differences are observed between the two spectra in the wavelength range [500,600] nm. In this wavelength region blood absorption dominates the spectral shape of the differential reflectance signal.

Comparing the spectra of figure 4 with the absorption spectra of Hb and HbO_2 it is clear that the malignant lesion has a much lower saturation than the normal bronchial mucosa, which is typical for malignant tissue. Thus, we have shown the feasibility of DPS to determine the local capillary oxygenation, blood volume fraction, blood vessel size and wavelength dependence of the scattering coefficient in vivo. Since all these parameters may be related to local morphological and physiological changes occurring during early malignant transformations, DPS may be used as a tool to discriminate premalignant lesions from normal mucosa. A prospective study using the same methodology in a large patient group is currently under way. Preliminary results depicted in figure 5 are very encouraging, indicating that a combination of optical imaging and optical spectroscopy may be helpful in finding suspicious lesions and selecting the ones that need biopsy.