In vivo optical imaging is probably the oldest technique in diagnostic medicine. Even today, the eye is every clinicians indispensable tool. Developments in modern optics and electronics, however, have made it possible look with more than just the naked eye and visualise many phenomena previously invisible. As a result, in vivo optical imaging has great potential as a screening tool for early detection of superficial cancer.

In vivo optical imaging for detection and localisation of cancer

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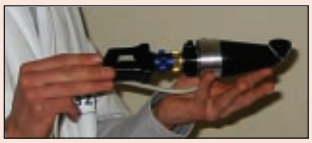

Figure 1. *(b) The prototype fluorescence imaging device developed for imaging in the oral cavity*

Our research activities in the field of optical imaging are focussed on applications in the field of cancer, both for diagnostic problems as well as for monitorring of cancer treatment. A wide range of different optical techniques is being investigated, as for the different clinical applications different biological parameters have to be measured. Moreover, different clinical boundary conditions require different approaches. In general, optical imaging gives information on a superficial layer of a few milimeter and in a few special cases up to several centimeters. This fundamental limitation renders optical imaging excellently suitable for imaging of superficial tissues, either on the outside (skin) or the inside of the body, such as the oral cavity. The purpose of our efforts to develop in vivo optical imaging is twofold. First, new in vivo imaging techniques enable us to look beyond present boundaries. This enables the study of fundamental processes such as occurring during cancer development or during treatment of cancer in great detail ***in vivo***. Even more importantly, the noninvasive nature of optical imaging allows us to do so, not just in animal models, but in real patients with real tumours, receiving real treatments. Second, the basic knowledge on the specific interaction between light and tissue of various pathological backgrounds obtained in this way enables us to identify potentially viable clinical applications of this new technology, thus generating an effective platform for challenging a variety of new clinical problems.

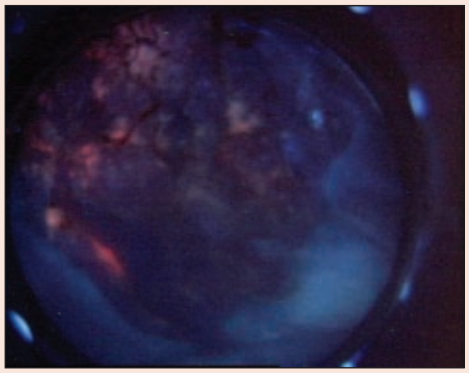

Figure 1. *(a) Fluorescence image of a squamous cell carcinoma in the oral cavity. The normal tissue (lower right) shows a regular pattern of bright bluish fluorescence while the tumour presents both red fluorescence caused by porphyrins and a decrease in blue fluorescence. In addition, the fluorescence in the tumour shows a much more irregular pattern.*

Detection of cancer in the oral cavity

Oral cancer is a disease that occurs in approximately 2500 new patients in the Netherlands each year. Most of these patients have a history of heavy smoking, often in combination with the frequent use of alcoholic drinks, especially strong liquors. More than 90 % of these cancer patients suffer from oral squamous cell carcinomas which arise from the upper layer of the tissue: the oral mucosa. It is this mucosa that has been exposed to tobacco and alcohol, and is therefore at risk for developing invasive tumours. During the process of the so-called 'field cancerisation', multiple areas of the oral mucosa undergo carcinogenic changes. This is an important feature of oral carcinogenesis and it can explain why the occurrence of second primary tumours in patients is so often seen. In fact, 28% of the patients diagnosed with a squamous cell carcinoma (SCC) of the oral mucosa will develop a second primary tumour within ten years. As with all cancers, the prospects for the patient are better when the malignancy is found in an early stage. Early treatment strongly improves the survival rates and results in a lower morbidity. For example, an early premalignant lesion can be removed with CO2 laser therapy (a mildly invasive treatment), while advanced squamous cell carcinoma's generally require extensive surgery and/or radiotherapy which may profoundly affect certain essential functions like swallowing and speech. Furthermore, these advanced carcinomas can metastasise, which significantly reduces

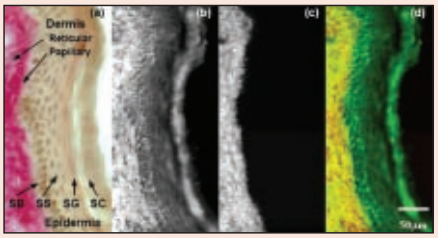

Figure 2. *A series of different microscopic images taken from a normal human skin section. The left image (a) is an image stained chemically which is the classical approach. Here a collagen stain is used so the collagen in the lower layer of the skin, the dermis, shows a bright red colour. The brown layers show the morphology of the different outer layers of the skin. Images (b) and (c) display the two-photon fluorescence (TPF) and the second harmonic generation (SHG) of the same section without staining and (d) a digital 2-color composite image of (b) and (c). This example shows that with nonlinear imaging morphological information can be obtained without staining the tissue which can only be performed on excised tissue.*

the survival chances. For this reason, patients who are at high risk for developing oral cancer, i.e. patients that have been treated for oral cancer, are submitted to a strict screening protocol based on visual inspection by a specialized oral and maxillofacial surgeon (oral oncologist). For earlier detection, the challenge is to trace these lesions before they can be detected visually.

Fluorescence imaging

In a previous study we have extensively characterised the fluorescent spectrsocopic properties of different malignant and premalignant lesions and normal oral mucosa. This study showed that on the basis of fluorescence spectral information it is excellently possible to distinguish visible lesions from normal healthy mucosa. In itself this has no great clinical value, as these lesions are already visible. An important question that arises is whether the lesions that are not yet visible also present this different fluorescent properties. If that is the case we can use fluorescence imaging to detect these lesions earlier. On the basis of the information aquired in the previous study we have developed a fluorescence imaging approach that is currently being evaluated and a typical result is shown in figure 1. The general impression is that autofluorescence is very sensitive to early changes, but has a very low specificity; i.e. many other nonmalignant changes invisible to the naked eye also become visible on a fluorescence image. We are now studying several approaches to improve this by combining imaging with spectroscopic measurements. (see also the paper by Amelink et al.).

Nonlinear imaging

At very high peakpowers the interaction between light and tissue presents nonlinearities that are characteristic for the specific molecules involved in the interaction as well as their particular shape. Many biomolecules naturally present in living tissue display non-linear phenomena such as second harmonic generation (SHG) and two-photon fluorescence (TPF) when exposed to high peak power femtosecond laser pulses. In close collaboration with the Department of Molecular BioPhysics of the University of Utrecht we are studying the non linear optical properties of tissue, presently in tissue samples, but eventually in vivo. The example given in figure 2 shows that nonlinear imaging can yield information on important tissue constituents such as collagen. An important role of collagen is to keep the tissue together and to give tissue its mechanical strength. We have shown that denaturation of the collagen induces a strong decrease in SHG. This may be of relevance for staging of cancer, as invasive tumours have a way of 'dissolving' the collagen matrix of the tissue enzymatically before they can grow invasively.

Optical contrast agents

For imaging in patients with a high risk of having cancer, or where cancer has already been diagnosed, but where we do not know the extent or the severeness of the disease, the slightly more invasive approach of using an optical contrast agent is feasible. An optical contrast agent is a drug that has a specific optical behavior, such as fluorescence, and localises more or less selectively in diseased tissue. An example of the great potential for this approach is given in figure 3. Here, as optical contrast agent we applied 5 aminolevulinic acid (ALA) to the cervix of women who had an abnormal pap-smear. One hour after application the ALA has diffused into the cervical mucosa and has been transferred into the fluorescent protoporphyrin IX (PpIX) by enzymes inside the cells. The amount of fluorescence at suspect areas indicated by the clinician appears to correllate excellently with the histopathological findings from the tissue sample taken at the same location. The different levels of PpIX fluorescence are caused mainly by the fact that the layer containing PpIX increases in thickness with progression of the disease. This technique may assist in finding the best spot for taking a biopsy and may contribute to a more accurate staging of this disease. The latter is of great relevance to the patient. An overestimation of the stage of the disease leads to unnecessarily severe surgery. In the present application of the cervix this is known to lead to infertility problems, which may present a mayor problem, especially to younger patients. On the other hand underestimating the stage of the disease may lead to insufficient treatment resulting in a spread of the cancer and death. Sofar, optical contrast agents used have been discovered as a byproduct of the search for new photosensitisers for Photodynamic Therapy. However, molecular biologists around the world are now focussed on developing new contrast agents that target specific disease related molecules. When this has become a clinical reality Optical Molecular Imaging will become a very powerfull tool for diagnosing disease such as cancer.

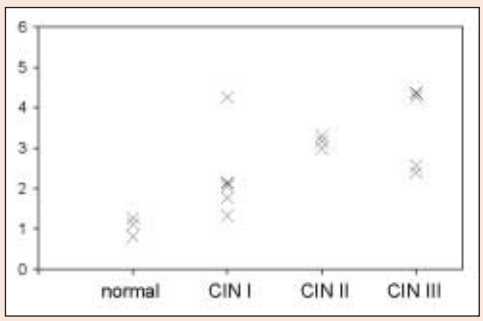

Figure 3. *Results from a fluorescence imaging study on the cervix of women with an abnormal pap smear. There is a strong correlation between the amount of contrast agent and the histopatological classification of the lesion*