Summary in English

In the introduction (chapter 1), the **clinical question addressed in this thesis and the general approach** are described. For the treatment of malignant lesions in the oral cavity, early detection is essential. Unfortunately, it is impossible to visually distinguish early malignant lesions from some more common benign lesions. Therefore, the common procedure includes performing a biopsy. Sometimes it is difficult to determine the optimal location for performing this biopsy, which may cause underdiagnosis or create the need for repeated biopsies. Optical spectroscopy is a potential method for non-invasive detection and classification of oral lesions. Autofluorescence spectroscopy is a promising, relatively inexpensive and easily applicable example. In the introduction, the basis on which the technique relies is described, as well as the subquestions that will be addressed in this thesis.

Chapter 2 concentrates on the **influence of anatomical location** on the autofluorescence characteristics. The oral cavity is lined with a rich variety of mucosal types. For example, the tongue is of a different form and structure than the mucosa of the cheek. The resulting influences on autofluorescence characteristics might be large and possibly have to be taken into account when performing lesion diagnostics. For this study, 97 volunteers with no oral lesions were measured. Autofluorescence measurements were performed at thirteen different locations (dorsal side of the tongue, lateral border of the tongue, lip mucosa, gingiva, cheek mucosa etc.) that were expected to produce different autofluorescence spectra on the basis of tissue type. Spectra were recorded using seven excitation wavelengths and compared using principal components analysis (PCA). Of the thirteen locations, eleven turned out to be very similar, i.e. the variations related to anatomical variation were smaller than spontaneous spectral variations within the separate locations. The only exceptions were the vermilion border of the lip and the dorsal side of the tongue. Spectra recorded from the vermilion border of the lip showed more blood absorption and therefore displayed a lower fluorescence intensity. Ninety-four percent of the spectra recorded from the dorsal side of the tongue were characterized by a porphyrin-like peak for at least one of the excitation wavelengths. Porphyrins are produced by micro-organisms, that may find a natural habitat in papillary cavities like those at the dorsal side of the tongue. In the literature, this porphyrin-like peak has been suggested to be helpful in cancer detection, but since it frequently is found in healthy mucosa – not only at the dorsal side of the tongue, but at all other locations – it cannot be used for reliable diagnostics. It is concluded that autofluorescence spectra from the dorsal side of the tongue and the vermilion border of the lip have to be analyzed separately, but the eleven remaining locations can be grouped together.

In chapter 3, the **influence of individual characteristics** at the autofluorescence spectrum is studied using the volunteer population from chapter 2. PCA and multivariate linear regression techniques were applied to determine the influences of individual characteristics on the spectrum. The studied characteristics were age, sexe, smoking habits, alcohol consumption, skin colour and the wearing of dental prostheses. Except for age, all factors turned out to have a statistically significant influence on the relative importance of the constituent spectra (PCs). For volunteers drinking more than five alcoholic units per day, the porphyrin component was more present in the spectra, possibly due to a shifted microbial oral balance. Smokers also showed more porphyrin fluorescence and next to that they had a different bulk autofluorescence contribution. The greatest differences were found between heavy smokers (more than 20 cigarettes per day) and non-smokers. No significant differences were found between non-smokers and former smokers, which

means that the influence of smoking on the autofluorescence spectra is reversible. The effects of wearing dentures were mainly visible in a less strongly present blood absorption component. Possibly, this can be explained by a thickening of the mucosa that reduces the influence of blood absorption. However, the effects did not seem very dependent on the anatomical location from which the spectra were recorded. Women showed less blood absorption than men, for which we have no satisfying explanation. Of all components examined, skin colour had the greatest influence on the autofluorescence spectra. By racial pigmentation, a large part of the produced autofluorescence could be absorbed. When performing lesion diagnostics, this has to be taken into account.

For chapter 4 we measured and compared autofluorescence spectra from 172 benign, dysplastic and malignant lesions to examine the possibilities for **diagnostics**. The healthy volunteer spectra from chapter 2 were added to the dataset. Spectra were recorded under six different excitation wavelengths at the center, the border and the surroundings of the lesion, as well as at the contralateral position of the lesion, which appeared healthy to the eye. Spectra were compared using PCA, artificial neural networks (ANN) and four fluorescence intensity ratios as have been applied in the literature. The scores were compared using ROC-curves, which are less dependent on a chosen cut-off value than the more regularly used sensitivity and specificity. Three clinical questions were addressed:

1. Can malignant tumours be distinguished from healthy oral mucosa?
2. Can lesions in general be distinguished from healthy oral mucosa?
3. Can malignant and dysplastic lesions be distinguished from benign lesions?

The answer to the first two questions was affirmative (maximal areas under the ROC-curve: 0.97 and 0.88). However, malignant and dysplastic lesions could not be distinguished from benign lesions (ROC-area <0.65).

In chapter 5 the value of **diffuse reflectance spectra** for diagnostics of lesions of the oral mucosa is examined. Autofluorescence and diffuse reflectance spectra recorded from 172 lesions and 70 healthy volunteers were analyzed using PCA. The three clinical questions from chapter 4 were addressed again, but now for diffuse reflectance spectra and autofluorescence spectra that had been corrected in first order for blood absorption artifacts. The results were comparable to those from chapter 5. Classification of malignant versus healthy oral mucosa gave ROC-areas up to 0.98 for both diffuse reflectance and corrected autofluorescence spectra, which means that the classification was excellent. For lesions versus healthy tissue, classification was good for both types of spectra (ROC-areas up to 0.90). However, for the distinction between benign and (pre-)malignant lesions, both types of spectra proved to be unsuitable. Combining autofluorescence- and diffuse reflectance spectra hardly improved the results. The relevant information for the first two clinical questions therefore appeared to be contained in both diffuse reflectance and autofluorescence spectra. The third and clinically most relevant question could not be answered by any of the techniques.

In chapter 6, a **microscopic study** is performed. Autofluorescence images and spectra under two excitation wavelengths as well as Raman spectra were recorded from freeze-dried tissue section. The 37 tissue sections were biopsied from lesions from which autofluorescence spectra had also been recorded *in vivo* before performing the biopsy. Because of the influence of freeze-drying and long-term storage, the *in vivo* measurements could not be compared to the *ex vivo* measure-

ments. Autofluorescence images showed high intensities from keratin and connective tissue layers, but hardly any intensity from the epithelium. All spectra showed maximum intensities around 520 nm and did not show any specific features like porphyrin-like peaks or blood absorption dips. Using cluster analysis, no relationship could be found between spectra on the one hand and lesion type or cell layer from which the spectra had been recorded on the other hand. Raman spectra from different cell layers could be distinguished, but in this study no relationship with lesion type could be established.

In chapter 7, the **relevant literature on autofluorescence spectroscopy and imaging in the oral cavity** is discussed. Next to that, the results from the literature are compared with those from this thesis. Some important conclusions could be drawn. Both autofluorescence spectroscopy and imaging give good results for distinguishing lesions from healthy oral mucosa. In the case of imaging, this could be useful for the detection of new lesions or invisible extensions of known lesions. Since it has been noticed that especially early, flat lesions are easily distinguished using autofluorescence imaging, the potential for finding these early tissue alterations is large. Autofluorescence spectroscopy, however, is unsuitable for the detection of new lesions because of the small tissue area that can be investigated in one measurement. Since it is infeasible to scan the complete oral cavity using point measurements, the probe will in practice be placed on a lesion that has already become visible or that was classified as suspicious for maligny using another method. The relevant clinical question then is whether the lesion is benign or (pre-)malignant, since this is crucial for the treatment planning. If autofluorescence spectroscopy is able to anser this question, the method can be used to find the most dysplastic part of a lesion and thus guide the clinician to the optimal location for performing a biopsy. Unfortunately, from the literature and this thesis it was concluded that autofluorescence spectroscopy cannot answer this clinical question. Therefore, the final conclusion is that only autofluorescence imaging can potentially be used as an easily applicable, inexpensive en sensitive methode for the detection of oral lesions. Further research is required to establish this potential.