Normal tissue and diseased tissue differ in their overall molecular composition. Raman spectra are a representation of this molecular composition and can therefore be used to investigate the changes that accompany disease and to discriminate between normal and diseased tissue. The facts that the technique is non-destructive and does not require any sample pre-treatment make it very suitable for in vivo application. Development of dedicated miniaturized fiber optical probes paves the way for Raman- guidance of clinical procedures, such as taking biopsies and surgery, and in vivo diagnosis of disease.

Towards Raman-guided clinical procedures

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Imaging techniques such as CT-scans, MRI, ultrasound-imaging provide detailed 3D images of the body, which can be used in the diagnosis of disease and e.g. the preparation of surgical procedures. However, the spatial resolution of these techniques is significantly lower than that of optical techniques (microns-range), precluding e.g. the detection of epithelial dysplasias, or the detailed investigation of atherosclerotic plaque. It is also not possible to use these techniques in a practical way during clinical procedures. This is e.g. a problem in neurosurgery, where movement or swelling of tissue during a procedure reduces the possibility to rely on images made before the procedure for navigation. Below a typical problem is described, which neurosurgeons are confronted with, namely to sample representative brain-tumor tissue for diagnostics purposes by means of a stereotactic procedure. Raman-guidance of stereotactic procedures may solve this problem.

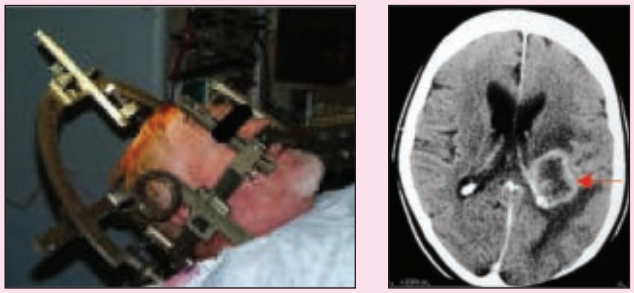

Figure 1. *Left: Stereotactic frame Right: Typical MRI image of glioblastoma (right side). A representative tumor sample should be taken in the small area (arrow) between the dark necrotic center of the tumor and the bright ring of vital tumor.*

Distinguishing vital glioblastoma from necrosis

Glioblastoma is the most malignant brain tumor. The diagnosis is based upon histopathological evaluation of tumor tissue samples, usually obtained by CT/MRI guided stereotactic surgery (Figure 1). It is difficult to sample representative tissue because tumor tissue is heterogeneous, while stereotactic biopsy samples are small. Moreover, precise targeting of representative tumor areas based on CT/MRI images is affected by brain shift, which commonly occurs during neurosurgical procedures.

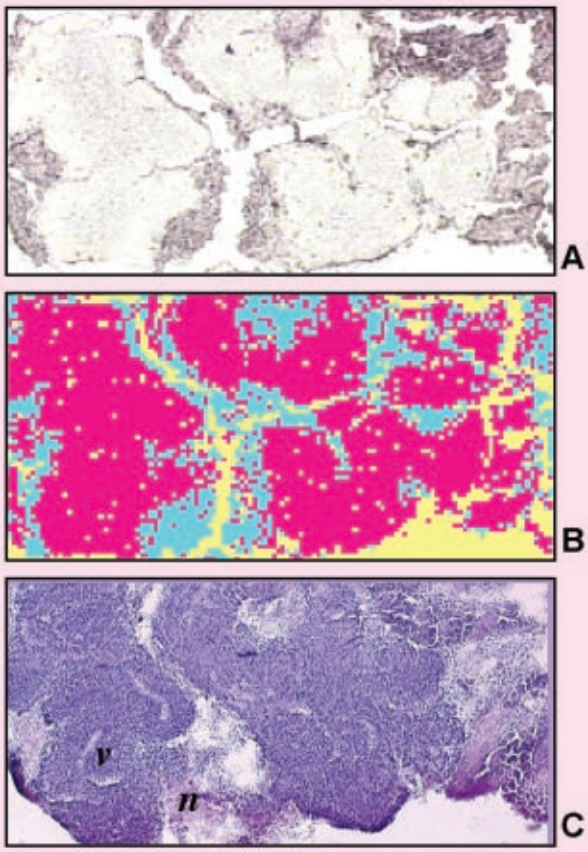

Figure 2. *Discriminating vital glioblastoma tissue from necrosis by Raman spectroscopy. A: unstained tissue section of glioblastoma. B: Raman prediction map. The spectra obtained from each pixel are classified as vital (red) or necrotic (blue) by prediction model. C: Stained (adjacent) section showing vital tumor (v) and necrosis (n). Areas of necrosis are correctly identified by Raman spectroscopy.*

Tumor necrosis is an important parameter for establishing a diagnosis of glioblastoma. The tumor grade is underestimated when no necrotic tissue is sampled. On the other hand, histological diagnosis cannot be made when only necrotic tissue is present in the tissue sample. Raman maps of glioblastoma tissue sections were made in order to determine if Raman spectra enable the discrimination between vital tumor and necrosis, which would be the basis for the development of an ***in vivo*** Raman method for real-time intra-operative brain biopsy guidance.
Raman data from 11 patients were used to create a classification model for discrimination between vital and necrotic tumor tissue based on Linear Discriminant Analysis. The model was evaluated using independent Raman data obtained from 9 other patients and yielded 100% accuracy. Figure 2 is an illustration in which the Raman map (2B) is the result of classification of each pixel as necrotic (blue) or vital (red) on the basis of a Linear Discriminant Analysis model.

Fiber optic probes

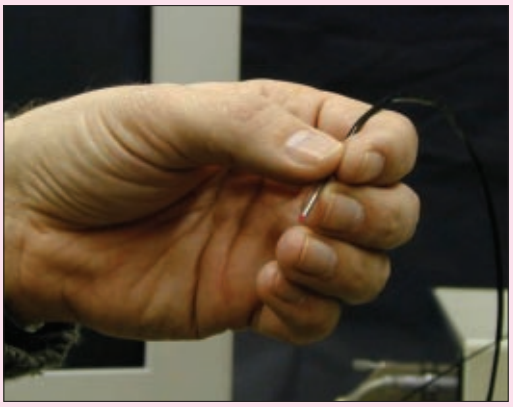

Fig 3. *Fiber optic Raman probe*

Miniaturized fiber optic probes have been constructed (Figure 3). They are a necessary step in the development of the ***in vivo*** application of Raman spectroscopy in neuro-surgical and other clinical procedures. In their simplest form these probes consist of a single optical fiber, through which laser light is guided to the tissue. The fiber also collects Raman scattered light from the tissue which is then guided back to a spectrometer for analysis. Signal collection times in the order of 1 second or less are feasible and the capability of on-line signal analysis (tissue identification) has been demonstrated2. Work in progress shows that it is possible to discriminate between different structures in the brain, which is of great importance for stereotactic procedures (Koljenovi_ ***et al***. unpublished). Their small size will make it possible to integrate these fiber probes in clinical instruments for guidance of procedures (oncological surgery, neurosurgery, laparoscopy, biopsy).

Classification of independent spectra using a Linear Discriminant Analysis model

		Pathology			
		HGD	LGD	Normal	Total
Raman	HGD	10	0	0	10
	LGD	0	7	2	9
	Normal	0	2	17	19
	Total	10	9	19	38

Table 1. *Classification results for independent 10 s acquisition spectra: LGD = Low Grade Dysplasia, HGD = High Grade Dysplasia.*

In vivo discrimination of dysplastic tissue

Using Raman spectroscopy through fiber optic probes, it is in principle possible to perform ***in vivo*** pathology. We tested the feasibility to discriminate ***in vivo*** between normal tissue, dysplastic tissue and tumor tissue, using an existing animal model for studying oral cancer3 (figure 4). High quality spectra of normal and dysplastic rat palate tissue of in total 10 rats were obtained in vivo, using (still relatively long) signal collection times of 100 s per spectrum.
These spectra were used to create a Linear Discriminant Analysis model to distinguish normal tissue from low grade dysplasia (LGD), and high grade dysplasia/carcinoma in situ (HGD).
This model was tested with an independent set of in vivo spectra, obtained with signal collection times of 10 s. This independent test was classified with a selectivity of 0.93 and a sensitivity of 0.78 for detecting low grade dysplasia and a specificity of 1 and a sensitivity of 1 for detecting high grade dysplasia/carcinoma in situ (Table 1). These results show that it is feasible to use Raman spectroscopy as a (pre-) screening tool to detect dysplasia in an early stage and to increase the number of informative biopsies and reduce the chance of missing aberrant areas.

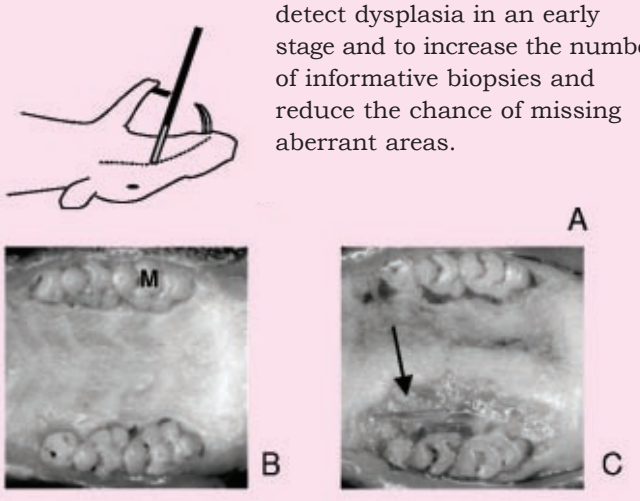

Figure 4. *A: probe placement, B: normal rat palatum, C: palatum with high grade dysplastic / carcinoma in situ (arrow).*