Combination of Hand-Held Probe and Microscopy for Fluorescence Guided Surgery in the Brain Tumor Marginal Zone

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Highlights
A hand-held probe (HHF-probe) connected to a fluorescence spectroscopy system, and a fluorescence-based surgical microscope (FGR-microscope) were evaluated for fluorescence detection in the tumor marginal zone in 18 brain tumor surgeries. The HHF-probe was superior to the FGR-microscope in sensitivity. The threshold for the analysis of the quantitative HHF-probe data can be adjusted to achieve a compromise between sensitivity and specificity. The combination of the HHF-probe and the FGR-microscope was beneficial during surgery.

Abstract

BACKGROUND: Visualization of the tumor is crucial for differentiating malignant tissue from healthy brain during surgery, especially in the tumor marginal zone. The aim of the study was to introduce a fluorescence spectroscopy-based hand-held probe (HHF-probe) for tumor identification in combination with the fluorescence guided resection surgical microscope (FGR-microscope), and evaluate them in terms of diagnostic performance and practical aspects of fluorescence detection.

METHODS: Eighteen operations were performed on 16 patients with suspected high-grade glioma. The HHF-probe and the FGR-microscope were used for detection of protoporphyrin (PpIX) fluorescence induced by 5-aminolevulinic acid (5-ALA) and evaluated against histopathological analysis and visual grading done through the FGR-microscope by the surgeon. A ratio of PpIX fluorescence intensity to the autofluorescence intensity (fluorescence ratio) was used to quantify the spectra detected by the probe.

RESULTS: Fluorescence ratio medians (range 0-40) measured by the probe were related to the intensity of the fluorescence in the FGR-microscope, categorized as “none” (0.3, n =131), “weak” (1.6, n =34) and “strong” (5.4, n =28). Of 131 “none” points in the FGR-microscope, 88 (67%) exhibited fluorescence with the HHF-probe. For the tumor marginal zone, the area under the receiver operator characteristics (ROC) curve was 0.49 for the FGR-microscope and 0.65 for the HHF-probe.

CONCLUSIONS: The probe was integrated in the established routine of tumor resection using the FGR-microscopes. The HHF-probe was superior to the FGR-microscope in sensitivity; it detected tumor remnants after debulking under the FGR-microscope. The combination of the HHF-probe and the FGR-microscope was beneficial especially in the tumor marginal zone.
Keywords: High-grade glioma, Fluorescence guided resection (FGR), 5-Aminolaevulinic acid (5-ALA), Fluorescence spectroscopy, Protoporphyrin (PpIX)
Introduction

The basic treatment concept for high-grade gliomas proposes cytoreductive surgery in order to enable optimized oncological treatment [1]. A higher extent of resection, over 78% and even stepwise improvements from 95% to 98% and to 100%, have been associated with a higher survival advantage [2]. The introduction of the FGR-microscopy technique into neurosurgery, based on the pioneering work of Stummer and his team [3, 4], has been of substantial benefit to patients with malignant gliomas. The technique has become an integrated routine of the standard operation settings in many European neurosurgical centers [5-7]. The visual discrimination of tumor tissue with fluorescence from healthy brain tissue without fluorescence allows for more extensive resection of the tumors [5] even beyond the MRI contrast enhancement [4, 8]. The technique has therefore increased precision in the surgical treatment of these tumors and improved preconditions for the oncologic therapies, thus contributed to prolonged progression free survival [3, 9] as well as to prolonged overall survival [5, 9].

Generally, the intention of surgical treatment of malignant gliomas within the cytoreductive concept is the so-called gross total resection (GTR), defined by the absence of contrast media enhancing tumor tissue on the postoperative magnetic resonance images [10, 11]. However, the problem remains that primary brain tumors have no membranous limitation distinguishing them from healthy brain tissue. The surgical challenge is to delineate the malignant tissue and to preserve functional brain which is mainly achieved by visual means. In addition to the FGR- microscope, the 5-ALA induced PpIX in the tumor can be visualized by other fluorescence measurement techniques e.g. by means of fiber-optic based spectroscopy [12, 13]. 5-ALA naturally exists in the heme biosynthesis cycle where its transformation to PpIX in the cells and binding of PpIX to iron produces heme. The externally applied 5-ALA reaches the tumor cells through the disrupted blood brain barrier and accumulates in the mitochondria as PpIX due to the altered enzyme levels in the tumor cells [14-18] and additional factors including pH and temperature [16]. PpIX is a known fluorophore with an emission spectrum in a wavelength range of 600-750 nm and an absorption spectrum in a wavelength range of 250-650 nm. As the maximum absorption of PpIX occurs at approximately 400 nm, it is preferred to use blue light for the excitation of PpIX for diagnostic purposes whereas the excitation light at approximately 635 nm is commonly used for therapy purposes [19] and longer wavelengths for multiphoton excitation [20].
The conventional FGR-microscope allows for safe resection of the viable tumor bulk, which in the operational field is perceived by the surgeon as “strong” fluorescence, whereas “weak” fluorescence areas represent tissue with an uncertain degree of tumor infiltration. It has been stated that the “weak” areas should not be considered for resection [21]. However, this transitional zone, where stronger signals gradually or irregularly shift into weaker signals, remains poorly defined and dependent on the surgeon’s subjective eyesight. Data published on the relation between 5-ALA induced PpIX fluorescence and the actual degree of infiltration in areas with “weak” fluorescence is limited and retrieved by means of the FGR-microscopy method only [7, 22-24]. Moreover, there is no practical procedure that takes into account fluorescence attenuating factors including photobleaching which inevitably occurs due to light exposure from various sources in the operating room (OR), including the FGR-microscope.

At Linköping University Hospital, the FGR-microscope and an in-house HHF-probe have been implemented as stand-alone systems in the clinical practice during tumor resection. The HHF-probe has been used as a hand-held device [12] and in combination with ultrasound based navigation [13]. Fluorescence intensity in the tumor and marginal zone have been measured using a lower 5-ALA dose (5 mg/kg) with the HHF-probe. This lower 5-ALA dose showed comparable diagnostic performance to the conventional dose of 20 mg/kg used for FGR-microscopy [25]. In the present study, integration of the FGR-microscope and the HHF-probe was evaluated in the process of intraoperative tumor tissue identification. Of special interest were the systems’ diagnostic performances in the tumor marginal zone, and the practical aspects of the detection techniques.

Material and Methods

Patients

Sixteen patients (7 males and 9 females, median age of 62 years, range 18-82 years) with suspected glioblastoma (GBM) were included in the study. Fifteen patients at the Department of Neurosurgery at Linköping University Hospital and one patient at Norrland University Hospital in Umeå. Patients were randomly included from referrals with clinical and radiological signs of suspected high-grade glioma or with a known recurrence, on the basis of standard criteria for surgery in these categories. Most of the surgical procedures (n = 13) were performed by the study surgeon (JR), as he was well acquainted with both the FGR-microscope and the HHF-probe techniques. Postoperative
MRI-scans within 72 hrs. were used to verify GTR. Written informed consent was obtained from the patients. The protocol was approved by the local ethical board (Project No: M139-072012/333-32). In total 18 operations were performed, two of them on recurrent tumors both of which were included in the study for the primary tumor operation as well.

**HHF-probe and FGR-microscope**

The FGR-microscope (M720 OH5, Leica GmbH, Germany) was used with the FL400 fluorescence module (blue light). The FGR-microscope’s light source is a 300 W xenon-arc lamp (irradiance of 40 mW/cm²), the field of view is 125×143 mm. The in-house built probe based system [12, 26] consisted of an excitation laser (Oxxius, Lannion, France) with a wavelength of 405 nm. A spectrometer (EPP2000, Stellarnet, Tampa, FL) operating in the wavelength range of 240–850 nm with a spectral resolution of 3 nm, measured dynamic range of 3900 (maximum scale of 8190 a.u.) and signal to noise ratio (SNR) of ≤ 620 collected the data for further processing and real-time presentation in the OR. The measurement probe (lprobe = 12 cm, Ø = 2 mm, lcable > 4 m) had one excitation and several collecting fibers connected to the laser and the spectrometer, respectively. The collecting fibers were placed adjacent to the excitation fiber, center-center distance = 450 µm [12] (Fig. 1). The surface of the probe tip was flat but the outer circumference was rounded to avoid tissue damage. The fluorescence spectroscopy system could be used in a pulsed or in a continuous mode. The system’s pulsed mode was designed for omitting the ambient light the details of which have been published earlier [12]. Specific in-house software was developed in LabVIEW (National Instruments, Inc., TX, USA), which made it possible to control the laser output, pulse length and number of spectra captured for each measurement session. In the current study, the laser output was set to 10 mW (irradiance of 3.5 W/cm²), the pulse length to 400 ms (800 ms for each measurement) and the number of spectra captured to three (single spot measurement). The measurements could also be performed over a longer time, up to 160 s, by increasing the number of spectra to 200. The tip of the probe was then moved over the tissue surface in order to capture the varying spectra along the timeline by waiting for at least 800 ms at each measurement point (Supplemental Video 1). The long time measurements enabled the surgeon to define a line of demarcation along the tumor’s marginal zone. An example of the HHF-probe placement on the measurement site is shown in Fig. 1.
The spectra captured with the HHF-probe were analyzed postoperatively in MATLAB® (The MathWorks, Inc., Natick, MA, USA). A ratio of the PpIX fluorescence intensity at 635 nm to the autofluorescence (AF) maximum at 510 nm in the same signal was calculated. Using this ratio, the PpIX fluorescence signals recorded during surgery were normalized [12, 27]. Examples of fluorescence spectra with the wavelengths marked for calculation of the fluorescence ratio are given in Fig. 2. The fluorescence ratio considered the fluorescence intensity at 635 nm (I635) minus the estimated IAF at the same wavelength (I_{AF(635)}) which gave fluorescence intensity of PpIX at 635 nm (I_{PpIX}) divided by the maximum of I_{AF} at 510 nm [12]. If (SNR_{AF(510nm)} > 0 and SNR_{PpIX (635nm)} = 0) the signal was assigned to be fluorescence negative. The presence of blood in front of the probe could be identified from the absorption pattern of oxygenated hemoglobin in the autofluorescence [28].

**Surgical procedure and measurements**

The standard dose of 5-ALA (Gliolan®, Medac GmbH, Germany) 20 mg/kg bodyweight (20.3 ± 4.5) was dissolved in water and given to the patients orally 2-3 hours prior to the operation. The operations were performed according to the routine procedure for resection along the cytoreductive concept by debulking as established at the Neurosurgical Departments involved. This included white and blue light FGR-microscopy and ultrasound-based navigation (Sonowand 1.4, Trondheim, Norway) which both gave an intraoperative image of the tumor [13]. The OR was darkened during the procedure, as a standard precaution for adjustment of the surgeon’s eyesight during the blue light vision. With a fixed setting of the FGR-microscope at an optical focus of 300 mm, the blue light vision provided the intended conditions for resection of “strong” fluorescing tumor tissue.

During the measurements the surgeon started by grading the fluorescence under the blue light mode of the FGR-microscope (Fig. 3a) to “none”, “weak”, “strong”. At the next step, the HHF-probe was used to record the fluorescence spectra on the tissue surface or at a small distance (~1 mm) above the surface (Fig. 3b). Thereafter, a biopsy was taken at the measurement sites as precisely as possible and the suspected tumor parts were resected without basing the decision on the fluorescence detected by the HHF-probe. The procedure was documented for each measurement. Of special interest were the spots with “weak” fluorescence as seen through the surgical microscope. In such regions the
resection was performed until either no more residual tumor tissue could be identified, with or without the FGR-microscope, or until it was decided that further resection would cause lesions with possible subsequent neurological impairment of the patient. It is clearly seen in Fig. 3b that the HHF-probe enhances the fluorescence of the tissue. The fluorescence spectrum in the left corner corresponds to the PpIX spectrum visible on a separate monitor during surgery in the OR. In some areas of the resection cavity the HHF-probe was used for a continuous measurement. On such occasions the HHF-probe was moved just above the surface (~1 mm) and spectra were recorded every 400 ms (Supplemental Videos 2-3).

Biopsies were taken from areas presenting fluorescence with any of the systems, i.e., the FGR-microscope or the HHF-probe. The majority of the biopsies, however, were harvested from the marginal zone where the FGR-microscope had shown “weak” fluorescence before resection and if the HHF-probe still registered fluorescence after resection. Due to ethical reasons, no biopsies were taken from tissue outside of the suspected marginal zone.

**Histopathological classification**

Each resection specimen and biopsy were separately fixed in 4% buffered paraformaldehyde and paraffin embedded. Routinely, 4 μm sections were mounted and hematoxylin and eosin stained. All samples from a patient were used for the clinical diagnosis and graded according to WHO [29] by an experienced neuropathologist (MH). The neuropathologist was blinded to the fluorescence measurements. To allow the fluorescence at each site to be correlated to the tumor growth, each biopsy was separately graded as described.

The biopsy samples (n = 80) were categorized depending on the neuropathology diagnosis into one of the groups of non-malignant brain tissue, low grade tumor (LGT, corresponding to WHO grade II), high-grade tumor (HGT, corresponding to WHO grades III and IV), necrosis containing tumor and the intermediate inflammatory zone which was further divided in two categories, namely ‘area containing gliotic tissue with histologically identified tumor cells’ and ‘area containing gliotic tissue with no histologically identified tumor cells’. The LGT group is the tissue within the high grade tumor as the tumor is usually a mixture of different malignancy grades and the final pathological diagnosis is placed based on the highest detected WHO malignancy grade.
Data and statistical analysis

The number of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) were defined based on the histopathology diagnosis to calculate the diagnostic performance parameters [30]. True positives included the low and high-grade tumor tissue, intermediate inflammatory zone with tumor cells and necrotic tissue with tumor cells. True negatives included intermediate inflammatory zone without tumor cells; the reverse was valid for the false positives and false negatives. Two diagnostic performance parameters were calculated as defined by sensitivity, TP / (TP+FN) and specificity, TN / (TN+FP). Within the FGR-microscopy data, any visible fluorescence, i.e. “weak” and “strong”, were grouped as tumor positive. For the statistical analysis, values of 0, 1 and 2 were assigned to “none”, “weak” and “strong” fluorescence.

The fluorescence ratios calculated from the spectra measured by the HHF-probe were first compared against the grades of fluorescence (“none”, “weak”, “strong”) seen through the FGR-microscope (n = 193). Thereafter, for data associated with a biopsy (HHF-probe n = 79 and FGR-microscope n = 70) were compared against histopathologic diagnosis. Signals affected by blood (SNR_{AF} ≥ 0) were included in the comparison of the HHF-probe and the FGR-microscope. However, the signals totally blocked by blood (SNR_{AF} = 0 and SNR_{PpIX} = 0) were removed from the comparison with histopathology.

MATLAB® (2015, The MathWorks, Inc., USA) was used for the data analysis and statistical tests. Linear Pearson correlation was implemented to investigate goodness of fit (R^2) and p-value between the calculated fluorescence ratios measured by the HHF-probe and the grades of fluorescence seen by the surgeon through the FGR-microscope. Median values were calculated and boxplots were used as the datasets were non-normally distributed. Boxplots as defined by MATLAB® illustrate the Q_1 (25 %) to Q_3 (75 %) percentiles with a box and the median values (50 %) with a line in the box for each data set. Whiskers are drawn up/down to the closest outliers. Outliers were by default defined to be larger than Q_3 + w (Q_3 - Q_1) and smaller than Q_1 - w (Q_3 - Q_1) where w is 1.5, which corresponds to 99.3 % of the confidence interval. The receiver operating characteristics (ROC) curve was plotted for comparing the classification efficiencies of the FGR-microscopy vs. spectroscopy considering the values positive if greater than or equal to a certain threshold. The data points and the area under the ROC curve (AUC) were calculated using SPSS® (Version 22, IBM, USA) statistical software.
Results
The two systems were easily integrated in the OR. The HHF-probe’s blue light excited a higher fluorescence emission on the irradiated spot which enhanced the fluorescence seen under the FGR-microscope. Measurements by the HHF-probe could be performed without any disturbance in the blue light mode of the FGR-microscope. The histopathology analysis confirmed twelve GBM WHO grade IV, five oligodendroglioma grade III and one high-grade glioma of unknown type.

The fluorescence ratios calculated from the measured spectra (n = 193) with the HHF-probe were in a total range of 0-40. When comparing the detection performance of the HHF-probe and the FGR-microscope, the median fluorescence ratios corresponding to the “none”, “weak” and “strong” fluorescence seen in the microscope were 0.3 (n =131), 1.6 (n = 34) and 5.4 (n = 28), respectively (Fig. 4a). The median of the fluorescence ratios measured with the HHF-probe on single spots correlated well (R² = 0.92, p > 0.05) with the grades of fluorescence seen through the FGR-microscope, however, the point-to-point correlation was low (R² = 0.1, p < 0.05).

Using the HHF-probe, PpIX fluorescence was seen in 88 spots out of 131 (67 %) that were below the recognizable fluorescence level under the FGR-microscope. Some of the outliers with a high fluorescence ratio were located at tissue walls or with an angle to the surface where they were possibly not well excited with the light from the FGR-microscope. Of the 62 points positive for tumor tissue under the FGR-microscope (“weak” and “strong”) only 7 (11 %) were negative with the HHF-probe. This was suspected to be due to the attenuation of light transmission at the tip of the probe by blood.

The HHF-probe measurements were well related with the pathological analysis (Fig. 4b). Within the tumor groups, 50 % in the LGT group and 91 % in the HGT group exhibited PpIX fluorescence (Table 1). Within the groups of gliotic tissue without and with tumor cells, 66 % and 77 % of the samples presented PpIX fluorescence, respectively. Within the HGT group 91 % showed fluorescence with the HHF-probe and only 25 % showed fluorescence through the FGR-microscope.

Fig. 5 shows an ROC curve for comparing the two fluorescence measurement techniques diagnostic performance. The thresholds for positivity of fluorescence ratio and the fluorescence grade seen through the FGR-microscope are labeled in the graph. For fluorescence ratios ≥ 0.05, the sensitivity of the HHF-probe was considerably higher than
that of the microscope (0.79 vs. 0.29). For the same threshold the specificity of the HHF-probe was lower than that of the FGR-microscope as it was capable of detecting the “weak” signals from the inflammatory zone without tumor cells (0.33 vs 0.66), which according to the categorization in this paper were not considered malignant. For fluorescence ratios ≥ 0.6 the specificity of the HHF-probe increased to that of the FGR-microscope (0.66). When the fluorescence ratio threshold was set to 7.6, the sensitivity of the HHF-probe equaled that of the FGR-microscope (0.29). For fluorescence ratios ≥ 12.4 the specificity of HHF-probe equaled that of the microscope (0.97) if the threshold for resection using the FGR-microscope was set to “strong”. The area under the ROC curve for the tumor marginal zone was 0.49 for the FGR-microscope and 0.65 for the HHF-probe.
Discussion
The 5-ALA induced PpIX fluorescence in the marginal zone of the highly malignant brain tumors was studied using a FGR-microscope and an HHF-probe. The FGR-microscope enabled resection of intensely fluorescing tumor parts by providing a wide-field view, while the HHF-probe detected the fluorescence with a higher sensitivity in the “weak” or non-fluorescing field seen under the microscope. As a complementary feature, the HHF-probe could be used for quantification of the fluorescence based on objective criteria. This initial study showed that the combination of the two fluorescence detection techniques enhanced the surgeon’s capability in exploring the fluorescence in the tumor marginal zone.

Practical aspects
The main advantage of the HHF-probe is its sensitivity, i.e. the evaluation system that can deliver objective data from the signal intensity and indicate tumor infiltration in the tissue. One further advantage of the HHF-probe, not investigated in this work, is that it can be inserted in the tumor to investigate the tumor extent beyond the surface of the resection cavity, especially in combination with a navigation system [13]. On the other hand, the HHF-probe seems to be more easily affected by blood than the microscope, as blood can attach to the probe tip. Therefore, the blood on the surface should often be rinsed. As an alternative technical solution, the blood interference can be reduced by using a red light excitation (635 nm) [31, 32] in the spectroscopic system. However, the red light excitation light is more beneficial in the stand-alone applications of the HHF-probe rather than simultaneous use with the FGR-microscope as the red light reflection can be perceived as fluorescence in the blue light mode of the FGR-microscope.

During the course of the survey two minor, yet possibly interesting phenomena concerning the practical handling of the HHF-probe were observed. When held at some distance to the tissue (~1 mm) it brought the tumor infiltrated tissue to emit fluorescence that could again be seen in the FGR-microscope in an approximately 3 mm diameter spot. The intensity seen was less than what has been called “strong” fluorescence, but stronger than most of the so called “weak” fluorescent areas. This helped the surgeon to trace the tumor margin, in similarity to a flashlight (Fig. 3b). Moreover, pulsation of the spectroscopy system was found unnecessary when the HHF-probe was used in combination with the FGR-microscope in the fluorescence mode (blue light), as there was
no disturbance from the blue light in the recorded signals.

**Quantification of spectral data**

Normalizing the fluorescence as a ratio might facilitate comparison among systems. The results reported by Eljamel et al. state a comparable fluorescence ratio range (0-27) [33] compared to the 0-40 range presented in this study. However, details of the system are not released in [33], therefore, an accurate technical comparison is not possible. Of future interest is a universal measurement standard, e.g., phantoms [34, 35] to allow for comparison of the fluorescence intensity measurements with spectroscopic systems [36]. Several types of minor errors may have occurred in the quantification and analysis of the fluorescence spectra. This includes the effect of blood in calculating the fluorescence ratio, e.g., when the spectrum was slightly affected (n = 15). One other source of possible error is photobleaching induced by the excitation light of the HHF-probe and by the light sources in the FGR-microscope on the HHF-probe measurements. The photobleaching induced by the HHF-probe is less than 10% of the initial fluorescence intensity when the measurements are performed using a total of 1 s exposure [37, 38].

As the surrounding OR light sources except for the FGR-microscope are off or extremely low, the fluorescing tumor tissue is only exposed to the blue and white light of the FGR-microscope that may cause photobleaching in the PpIX [39]. The surgeon can apply a certain type of “roughing” technique in the superficial layers of the resection cavity in order to revive the fluorescence from underneath the exposed surface of the tissue, where the light exposure has not yet caused photobleaching. Nevertheless, the photobleaching process induced by the FGR-microscope is slow (63% of fluorescence decays in approximately 30 min. for the blue light excitation and 90 min. for the white light excitation) [39], and is expected not to have had a significant effect on the measured signals by the HHF-probe as the measurements were performed shortly (less than one minute) after the new tumor surface was exposed to the blue light of the FGR-microscope. Any effect of possible photobleaching should not have significantly affected the comparison of the two fluorescence detection techniques. However, some photobleaching might have affected the comparison of fluorescence intensity levels with histopathology.

**Histopathology categorization and ROC curve**

The higher sensitivity of spectroscopic methods for fluorescence detection has been
reported by peer groups [33, 40-42]. As explained by Stummer [40] the specificity of the fluorescence detection using FGR-microscopy cannot be reliably determined in absolute values since biopsies cannot be taken from suspected functional brain tissue for ethical reasons. The same issue is encountered by the HHF-probe technique. Moreover, as histopathology references are categorized differently by various researchers [7, 22, 40, 43-45], a direct comparison of the diagnostic parameters is not always suitable across the groups. In this work, the sensitivity and specificity values were calculated mainly for the tumor marginal zone and only aim at comparing the two detection techniques.

As HHF-probe measurements allow for quantification of spectra, threshold adjustment in the ROC curve (Fig. 5) is possible to define a compromise of sensitivity and specificity. So far, the data collected within this cohort does not support a reliable definition of such a threshold i.e. an absolute ratio for the degree of infiltration. The dataset included in this study supports the estimation that fluorescence ratios $\geq 12.4$ correspond to the specificity of “strong” fluorescence where resection is recommended (Fig. 5). Fluorescence ratios in the range of 0.6-12.4 correspond to the specificity of “weak” fluorescence as seen in the FGR-microscope. One concern regarding defining a malignancy threshold is that several tissue types (e.g. gliotic tissue and LGT and sometimes HGT) can have fluorescence values at the same intensity level. The finding that fluorescence is often detected in the immediate adjacent gliotic tissue has been described previously [7, 22, 23, 46]. The reason for this observation is not clearly known, however, it likely depends on the fact that multiple factors contribute to the accumulation and increased PpIX production [47, 48]. Furthermore, localization of the tumor is reported to play a role in the availability of PpIX in the adjacent normal tissue [49].

**Complementary techniques**

Like any new instrument the HHF-probe can open new opportunities, but it may also evoke new questions. It is a basic fact, that malignant gliomas cannot be cured by surgical means, but until this day the surgical resection is in most of the cases a precondition for optimized oncological therapy when a GTR is carried out. In theory, to achieve GTR, visualization of all of the tumor tissue would be ideal. On the other hand, since the tumor tissue infiltrates healthy and functional brain tissue, surgical resection of all of the tumor tissue would most certainly cause lesions in the healthy tissue. Therefore, it is necessary to limit the resection. Neurophysiological recording is of distinguishable usefulness for
limiting the resection [50] by monitoring the functional tissue. Other conventional preoperative and intraoperative imaging methods such as tractography or navigation can also be valuable aids, although they illustrate the morphological and not the functional tissue.

**Clinical aspects**

The two different fluorescence detection techniques were not evaluated against each other in terms of achieving GTR as the study was not designed for such a comparison. It could therefore not be determined whether GTR or a higher extent of resection would have been achieved more reliably with one or the other system. Future investigations including more patients could allow for such a follow up and investigate whether a greater area of resection can be achieved in a higher percentage than is reported in the literature for patients operated with FGR-microscopy only [5]. Neurological evaluation of the patients’ status postoperatively could not show any greater risk for impairment, but it could also not show any greater benefit, since no long-term follow up more than 72 hours was performed or intended. The main advantage of the HHF-probe is that it can both be a stand-alone device possible to couple to any intraoperative navigation system, and also be used as a complement to the FGR-microscope.

**Conclusions**

The HHF-probe detected tumor tissue with “weak” fluorescence signals with a higher sensitivity as seen under the FGR-microscope, resulting in additional guidance in the tumor marginal zone. The equipment delivered quantitative data by which the fluorescence available in different tissue types could be categorized in an objective manner. The HHF-probe can be used as a stand-alone system for tumor resection guidance; however, combination of the HHF-probe and the FGR-microscope was conveniently implemented and found to be beneficial.
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Short title: Fluorescence Guided Brain Tumor Resection

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References


Figures Captions

Figure 1. Placement of the HHF-probe on a measurement site in the brain. The configuration of the fibers in the probe are shown at the top left of the image.

Figure 2. Typical recorded fluorescence spectra. The PpIX fluorescence intensity ($I_{PpIX}$) at 635 nm, the AF at 635 nm and its maximum at approximately 510 nm are used for quantification and normalization of the PpIX fluorescence signals. a) Signal measured on confirmed GBM (HGT), b) signal measured on a confirmed tumor intermediate zone as gliosis with tumor cells and c) intermediate zone without any tumor cells. More recorded spectra can be found in the Suppl. Videos 1 and 3.

Figure 3. a) The FGR-microscope fluorescence image with “weak” fluorescence seen in pink. b) Image of the fluorescence seen in the FGR-microscope in combination with the HHF-probe measurement. The fluorescence is enhanced in pink around the probe tip. A fluorescence spectrum is presented to the left. Image in (b) is from a different site than (a).

Figure 4. a) Comparison of the calculated fluorescence ratios measured with the HHF-probe and seen through the FGR-microscope. Median fluorescence ratios of 0.3, 1.6 and 5.4 correspond to the “none”, “weak” and “strong” fluorescence seen through the FGR-microscope, respectively.
b) The HHF-probe and biopsy results: The values obtained on normal tissue, gliosis without tumor cell infiltration, gliosis with tumor cell infiltration, low-grade tumor (LGT), high-grade tumor (HGT) and necrotic tissue mixed with HGT.

Figure 5. The ROC curve for the FGR-microscopy and HHF-probe in the tumor marginal zone. Several fluorescence thresholds for the calculated sensitivity and specificity of both systems are labeled in the graph. For fluorescence ratios $\geq 0.6$ the specificity of the probe equals that of the FGR-microscope (0.66) and for fluorescence ratios $\geq 7.6$, the sensitivity of the spectroscopy system equals that of the FGR-microscope (0.29). ROC: Receiver operating characteristic, AUC: Area under the ROC curve. 0.05 is the first value.
Tables
Table 1. The number of the fluorescence negative and positive points detected by the HHF-probe and the FGR-microscope as confirmed by the histopathology examination. Both for the HHF-probe and FGR-microscope any fluorescence > 0 is considered tumor positive.

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