From sample structure to optical properties and back
A theoretical framework for quantitative OCT and its clinical application
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CHAPTER 5

Pilot feasibility study of *in vivo* intraoperative quantitative optical coherence tomography of human brain tissue during glioma resection

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M. Almasian, L.S. Wilk, P.R. Bloemen, T.G. van Leeuwen, M. ter Laan, M.C.G. Aalders, *Pilot feasibility study of in vivo intraoperative quantitative optical coherence tomography of human brain tissue during glioma resection*
ABSTRACT

High resolution detection of glioma tissue during brain tumor surgery may allow precise and thorough tumor resection while preserving functional brain areas, and improving overall survival. Currently a tool for high resolution detection of glioma tissue is not clinically available. In an ex vivo study on fresh brain samples promising results were obtained for discriminating cancer from normal brain tissue by means of quantitative optical coherence tomography (OCT). The optical attenuation ($\mu_{\text{OCT}}$) was found to be significantly lower in glioma tissue compared to normal white matter. In addition to $\mu_{\text{OCT}}$, analysis of speckle distribution in OCT images has yielded promising results towards tissue characterization, providing for added contrast in rat liver and brain based on tissue heterogeneity. In this pilot study we will investigate the feasibility of in vivo quantitative OCT of brain tissue during glioma resection surgery. Therefore, in vivo 3D OCT data sets were collected during standard surgical procedure, before resection (cortical (n=5)) and after partial resection of the tumor (sub-cortical (n=3)), both from glioma tissue and normal parenchyma. These areas were labelled by the neurosurgeon by visually inspecting the brain tissue as well as pre-operative MRI images. Subsequently, $\mu_{\text{OCT}}$ and speckle contrast were extracted from the OCT data sets using an automated and validated algorithm. An average $\mu_{\text{OCT}}$ of $3.7 \pm 1.5 \text{ mm}^{-1}$ for normal cortical tissue and $3.9 \pm 1.4 \text{ mm}^{-1}$ for glioma tissue was found in patients with high grade glioma (n=3). In patients with low grade glioma (n=2) an average $\mu_{\text{OCT}}$ of $4.0 \pm 1.1 \text{ mm}^{-1}$ for normal cortical tissue and $3.1 \pm 0.5 \text{ mm}^{-1}$ for glioma tissue was found. The subcortical scans yielded an average $\mu_{\text{OCT}}$ of $6.0 \pm 1.1 \text{ mm}^{-1}$ and $4.5 \pm 1.0 \text{ mm}^{-1}$ for the normal brain and high grade glioma (n=3), respectively. The obtained $\mu_{\text{OCT}}$ values for the subcortical (white matter) scans are in good agreement with the values previously reported ex vivo study. An average speckle contrast value of $0.54 \pm 0.18$ and $0.55 \pm 0.20$ for cortical and subcortical data sets was obtained, respectively. No difference in speckle contrast between glioma and normal brain tissue was found. We used the determined speckle contrast values to select the $\mu_{\text{OCT}}$ results from more or less homogenous tissue areas, corresponding to speckle contrast values between $0.47$ and $0.57$. From the results of this pilot study we conclude, that the proposed method for quantitative in vivo OCT of the brain cortex is feasible during glioma resection surgery.
INTRODUCTION

The recurrence and survival benefit for glioma patients can be increased by more effective tumor resection.\(^1,2\) The gold standard for glioma diagnosis is histological and immunohistochemical analyses. In daily practice pre-operative MRI is used to image the patient’s brain as a visual guidance for the surgeon. However, due to the limited resolution of MRI (~ 1 – 1.5 mm), there is a need for a high resolution intraoperative imaging modality, which has led to the development and study of various imaging techniques for surgical navigation, such as: intraoperative CT and MRI, fluorescence imaging, Raman spectroscopy and ultrasound.\(^3\)

Optical Coherence Tomography (OCT) allows non-contact label-free volumetric visualization of tissue with a resolution of \(\sim 10-20\) micrometer and a depth of view of 1-2 mm, for conventional systems. OCT has previously been applied to image ex vivo and in vivo tissues in various human organs, including the retina, coronary artery, gastric tract, urinary tract, prostate, breast, and also the human brain.\(^3–9\) The label-free and real-time imaging capabilities make OCT a suitable tool for intraoperative use.\(^10,11\)

In addition to tissue visualization, extraction of quantitative parameters from the OCT signal is proposed as a tool to discriminate between healthy and diseased tissue. The micro-structure and organization of tissue is altered due to disease and reflected in its optical properties\(^12\), which are accessible through quantitative OCT parameters. A frequently used quantitative OCT parameter is the optical attenuation coefficient (\(\mu_{OCT}\)). At commonly used OCT wavelengths, at which absorption in most biological tissues is much lower that the scattering, \(\mu_{OCT}\) estimates the scattering properties of tissue. Within the single scattering approximation, \(\mu_{OCT}\) can be determined by fitting a single exponential model to the OCT data after correction for system dependent parameters.\(^13\) Multiple studies have shown promising results for the use of \(\mu_{OCT}\) in order to enhance contrast between healthy and diseased tissue for visual guidance.\(^14–19\) In addition to \(\mu_{OCT}\), the speckle distribution of OCT images has been shown to contain information on sub-resolution scattering particles and is studied as a quantitative parameter for tissue characterization.\(^20–22\) In a study on rat liver and brain tissue, speckle distribution analysis of the OCT images has shown to provide for added contrast based on tissue heterogeneity.\(^21\) In general, the speckle of the OCT signal is expected to follow a Rayleigh distribution for static homogenous tissues.\(^22,23\) Tissue inhomogeneity may therefore be detectable through OCT speckle analyses.\(^21\)

Imaging of human brain tissue using OCT has been investigated in multiple studies.\(^3–9\) Most of these studies have examined the difference between cancerous and non-cancerous ex vivo brain samples.\(^3–6,8,9\) Assayag et al.\(^6\) show that brain tissue microstructures are clearly identified using full-field OCT with a high lateral resolution of 1 \(\mu\)m in ex vivo fresh samples of human brain. In their study, OCT images of brain tissue morphol-
ogy were matched with histology slides as closely as possible, from which the authors conclude that high grade glioma, but not low-grade glioma, could be distinguished in the OCT images. However, a disadvantage of high resolution OCT is the limited imaging depth (from 1-2 mm for conventional resolution systems to ~200 μm for 1 μm resolution systems) and the increase in acquisition time and data volume.

Quantitative analysis of conventional resolution OCT images may provide real-time visual guidance by discriminating normal from glioma brain tissue based on the difference in optical properties. The main contribution to scattering by brain tissue is considered to be caused by myelin. Due to its lower myelin content gray matter is expected to have lower scattering compared to white matter. This finding is substantiated by quantitative OCT studies which found a lower \( \mu_{OCT} \) for gray matter compared to white matter in fixed\(^7\) and fresh ex vivo samples of human brain tissue.\(^3\) The difference in \( \mu_{OCT} \) of normal brain tissue and glioma (high- as well as low-grade) of fresh ex vivo samples was studied by Kut et al in 32 patients.\(^3\) A lower \( \mu_{OCT} \) was found for glioma brain tissue compared to normal brain white matter, especially in the infiltrative zones of the tumors. The decrease of \( \mu_{OCT} \) in glioma compared to normal brain tissue, attributed to the degradation of myelin, is also reported in a qualitative OCT study on human brain tissue by Böhringer et al.\(^5\) The results presented by Kut et al. are promising for the application of \( \mu_{OCT} \) in the differentiation of normal brain tissue from glioma and therefore serve as motivation for continued investigations, particularly in in vivo and intraoperative settings.

The only in vivo OCT study on the human brain to our knowledge (Böhringer et al.\(^5\)) reports on OCT images in 9 patients (all with high-grade gliomas) during brain tumor resection (in vivo and ex vivo fresh tissue samples) using a TD-OCT system. After semi-quantitative analysis, the authors conclude that analysis of visible microstructure and light attenuation in OCT images discriminates between normal brain tissue, infiltrative zone, solid cancer and necrosis. However, the optical attenuation in the OCT images is not quantitatively assessed and system dependent parameters were not corrected.

Motivated by the previously reported findings in literature on quantitative OCT in order to differentiate glioma from normal brain tissue, this pilot study was set up to investigate the feasibility of in vivo quantitative OCT of brain tissue during glioma resection surgery. Intraoperative measurements on in vivo tissue introduces challenges of time pressure, motion artifacts caused by heartbeat and respiration, unequal tissue edges, accumulation of liquid and blood on top of the imaged tissue. These factors make the collection of high quality OCT images and the quantitative analysis of the OCT images challenging. Moreover, automated data analysis is needed for these large data sets, to objectify the analysis as much as possible and enable real time visual guidance during surgery.

Here, we present the results of the pilot feasibility study of in vivo quantitative OCT during brain surgery in 5 patients. 3D OCT data sets were recorded during the standard surgery protocol, before (cortical) and after partial resection (subcortical) of the brain
tumor from areas labelled as cancer and non-cancer. These areas were labelled by the neurosurgeon by visually inspecting the brain tissue as well as pre-operative MRI images. Afterwards, the collected OCT data sets were quantitatively analyzed by extracting $\mu_{\text{OCT}}$ and speckle contrast from the OCT data sets.

**METHODS**

**Study design**

OCT data sets were recorded during the standard procedure of glioma resection surgery in 5 patients ($n=5$) at the Radboud University (Nijmegen, Netherlands) according to the Declaration of Helsinki for experiments involving humans. Patients were included only if the tumor was expected to be visible at the cortex upon opening of the dura, as judged based on pre-operative MRI scan. An informed consent was obtained from each patient and the privacy of the patients was ensured by anonymization of the data. After craniotomy and opening of the dura, OCT images were recorded cortically (gray matter) before resection and subcortically (white matter) after partial resection of the tumor. The OCT data sets were collected from tissue areas labelled as glioma and normal brain based on visual inspection of the brain tissue as well as MRI based neuro-navigation by the neurosurgeon. 3D OCT data sets of ($x,y,z$) 1024, 1024, 600 pixels corresponding to 10 mm, 10 mm, 3.75 mm, respectively, were collected using a commercial 50 kHz Santec IVS 2000 swept source OCT systems, operating at a center wavelength of $\sim$1300 nm with an axial and lateral resolution, experimentally determined as $\sim$13$\mu$m and $\sim$25$\mu$m FWHM, respectively. All distances in depth throughout this paper are given in physical length (mm), using a refractive index of 1.4 for brain tissue. In accordance to standard surgical procedure, a sample of the tumor was collected for histopathological analysis. Based on the histopathological outcome we have grouped the patients as low grade (grade 2) and high grade (grade 4).

**Data selection for quantitative analysis**

A total of 19 cortical 3D OCT data sets were recorded in 5 patients and a total of 8 subcortical 3D data sets were recorded in 3 patients (Figure 1). Due to the surgical settings and time restrictions we were not able to record subcortical scans in 2 patients.

After visual inspection a region of approximately 2.4 mm by 2.4 mm suitable for quantitative analyses was selected from each OCT data set. This selection was made in order to exclude parts of the scans with low quality or image artifacts caused by out of focus, mirror images, large vessels and accumulation of blood and fluids on the tissue surface, from quantitative analysis. 9 cortical and 3 subcortical OCT data sets were fully excluded, due to low quality and image artifacts. An overview of patient and data inclusion is shown in figure 1.
Quantitative analysis

Automated quantitative analysis of the OCT data included determination of the OCT attenuation coefficient and OCT speckle contrast. The OCT attenuation coefficient was determined by a non-linear least squares (NLS) fit using the following model in:

$$\langle A(z) \rangle = t(z) \cdot h(z) \cdot A \cdot \exp(-\mu_{\text{OCT}}(z-z_0)) + \text{noise}$$  \hspace{1cm} (1)

Where, $\langle A(z) \rangle$ is the averaged OCT amplitude in depth, $z$ is the position in depth, $z_0$ is the position of the tissue boundary, $A$ and $\mu_{\text{OCT}}$ are free running parameters (amplitude and attenuation coefficient, respectively). The system-dependent parameter $t(z)$ (describing the point spread function) is defined as: $t(z) = \frac{1}{\sqrt{(z-z_f)^2 + 1}}$, where $z_f$ is the position of the focus in depth, $Z_{R0}$ is the Rayleigh length, and $n$ is the refractive index of the medium. The sensitivity roll-off $h(z)$ is defined as: $h(z) = \text{sinc} \left( \frac{\pi \cdot z}{Z_{\text{max}}} \right) \cdot \exp \left( -\frac{\pi^2 s^2}{16 \cdot \ln(2)} \cdot \left( \frac{z}{Z_{\text{max}}} \right)^2 \right)$. The contribution of the point spread function and the sensitivity roll-off were determined by fitting the equation for $\langle A(z) \rangle$ to the OCT amplitude of a sample with a low concentration of Intralipid (0.003%), for which an scattering coefficient of 0.15 mm$^{-1}$ was assumed, and $Z_{R0}$ and $s$ were the free running parameters.

Speckle contrast

The speckle contrast ($C$), which is the ratio of standard deviation over mean of the OCT amplitude values, was calculated from each ROI:

$$C = \frac{\sigma_A}{\langle A \rangle}$$  \hspace{1cm} (2)
Edge detection and ROI selection

Quantitative analysis of the OCT data was performed using custom-written code (Matlab 7.11.0 R2010b, The Mathworks Inc., Natick, MA, USA). Prior to edge detection, the uppermost 28 pixels of all A-lines were removed to exclude system-related signal artefacts. Detection of the brain tissue surface in the dB-scale data was achieved by first excluding low SNR regions by means of a pixel value threshold after which we applied a 3x3 median filter and then a vertical edge filter. The thus detected tissue edge was then smoothed further by carrying out local regression using weighted linear least-squares and a 2nd degree polynomial model. Selecting all data below this smoothed edge and converting it to linear scale then yielded raw OCT amplitude data of the brain tissue. Finally, this data was straightened and then divided into laterally overlapping regions of interest (ROIs): 10 x 10 x 30 pixels (0.1 mm x 0.1 mm x 0.2 mm) for the determination of μOCT and 10 x 10 x 5 pixels (0.1 mm x 0.1 mm x 0.03 mm) for the determination of speckle contrast. These ROIs were positioned below the detected tissue edge to exclude structures on the tissue surface from the fit analysis. In the cortical scans the ROIs were set between 10 and 30 pixels below the tissue surface while for the subcortical scans the ROIs were positioned 10 pixels below the tissue edge. The A-lines of the individual ROIs for the μOCT determination were then averaged, yielding a single average A-line for each ROI. Subsequently, a ROI-specific μOCT per pixel was determined by non-linear least squares fitting equation 1 to this average A-line. The noise was defined as the average of the last 20 pixels of the average A-line. Speckle contrast was calculated from the un-averaged OCT amplitude data from the speckle contrast ROIs, for which the depth of the ROI was limited to 5 pixels (0.03 mm) in order to avoid influence of attenuation of the OCT signal in depth on the speckle distribution within the ROI.

Inclusion criteria for μOCT based on fit statistics and speckle contrast

The residue and R² of the NLS fits were calculated to serve as a threshold to only include the outcome of fits for which the fitted curve has a good overlap with the experimental data. The thresholds (residue > 0.4, R² > 0.8) were chosen based on visual inspection of the overlap between model-based fitted curves and experimental data from a selection of the data sets. In addition, only the outcome of fits on experimental data with speckle contrast between 0.47 and 0.57 were included, which is assumed to correspond to a homogenous tissue layer. Finally, μOCT values for each included ROI were obtained after correction of the depth axis for the refractive index of brain tissue, 1.43, yielding values per mm. A flow chart of the analysis steps is given below in Figure 2.
Validation of analysis software

Intralipid is often used as a calibration sample in scattering-based experiments and frequently reported in literature. To validate the custom-written automated algorithm used in this paper, $\mu_{OCT}$ and speckle contrast were extracted from OCT data of a concentration series of Intralipid (Figure 3). The obtained $\mu_{OCT}$ values using our custom-written software were in good agreement with literature.26

Histopathology

The outcomes of the histopathological determination of cancer type and grade are shown in table 1. In summary, patient 1 and 4 were diagnosed with a low grade tumor and patient 2, 3 and 5 with a high grade tumor.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Histological outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt1</td>
<td>astrocytoma (grade 2)</td>
</tr>
<tr>
<td>Pt2</td>
<td>glioblastoma (grade 4)</td>
</tr>
<tr>
<td>Pt3</td>
<td>glioblastoma (grade 4)</td>
</tr>
<tr>
<td>Pt4</td>
<td>oligodendroglioma (grade 2)</td>
</tr>
<tr>
<td>Pt5</td>
<td>glioblastoma (grade 4)</td>
</tr>
</tbody>
</table>

Table 1. Histopathological results per patient.
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Qualitative analysis of OCT data sets

3D OCT data sets were successfully collected from cortical brain tissue in 5 patients and from subcortical brain tissue in 3 patients (Fig. 1). Cross sections (B-scans) from normal brain and glioma areas of cortical and subcortical brain tissue are shown in figure 4. In the cortical scans superficial blood vessels were clearly visible next to the structure of the arachnoid space. No visual features could be identified to differentiate between glioma and normal brain tissue. Figure 5 shows a volumetric view of a subcortical OCT data set together with a cross-sectional view at two locations on the y-plane.

![Figure 4](image1.png)

**Figure 4.** Cortical and subcortical grey scale cross sectional OCT scans of glioma and normal brain tissue. a) arachnoid space, b) blood vessel c) shadowing due to blood vessel d) blood or fluid pool. The red squares show a typical region selected for quantitative analyses.

![Figure 5](image2.png)

**Figure 5.** Volumetric view of a subcortical OCT data set (a) together with a cross-sectional view at two locations (b,c) on the y-plane.

Quantitative analysis of OCT data sets

The regions selected for quantitative analysis were analyzed using our custom-written automated software to extract $\mu_{\text{OCT}}$ and speckle contrast from the OCT data. The obtained $\mu_{\text{OCT}}$ and speckle contrast per scan are shown in a color-coded map in figure 6 and 7 for the cortical and subcortical data sets, respectively. For the cortically collected OCT data sets we found an average $\mu_{\text{OCT}}$ of 3.7 ± 1.5 mm$^{-1}$ for normal cortical tissue and 3.9 ±
1.4 mm$^{-1}$ for glioma tissue in patients with high grade glioma ($n=3$). In patients with low grade glioma ($n=2$) we found an average $\mu_{\text{OCT}}$ of $4.0 \pm 1.1 \text{ mm}^{-1}$ for normal cortical tissue and $3.1 \pm 0.5 \text{ mm}^{-1}$ for glioma tissue.

In the subcortical data sets (all high grade tumours) an average $\mu_{\text{OCT}}$ of $6.0 \pm 1.1 \text{ mm}^{-1}$ and $4.5 \pm 1.0 \text{ mm}^{-1}$ was found in the as normal and glioma labeled tissue areas, respectively. We found an average speckle contrast of $0.54 \pm 0.18$ and $0.55 \pm 0.20$ for cortical and subcortical data sets, respectively. Moreover, no difference was found in speckle contrast between the as glioma and as normal labeled locations.

![Figure 6. Color map of $\mu_{\text{OCT}}$ and speckle contrast of the cortical OCT data sets. The $\mu_{\text{OCT}}$ color scale bar runs from 0 mm$^{-1}$ to 10 mm$^{-1}$ and the color scale bar of the speckle contrast runs from 0 to 1. The $\mu_{\text{OCT}}$ average and standard deviation are given below the color maps.](image-url)
Our study has demonstrated the feasibility of the proposed method for in vivo quantitative OCT of brain tissue during glioma resection surgery (n=5). OCT data sets of cortical and subcortical brain tissue were successfully recorded in 5 and 3 patients, respectively. 15 out of 27 of these OCT data sets were suitable for automated quantitative analyses enabling successful extraction of $\mu_{OCT}$ and speckle contrast from the OCT data. The collection of subcortical OCT data sets was challenging due to the dimensions of the OCT probe compared to the size of the resection site. The collection and quality of the OCT images would strongly benefit from a dedicated probe design and scanning protocol. To our knowledge, this is the second study to obtain in vivo OCT images of human brain tissue. Böhringer et al. report OCT images recorded in 9 patients during glioma resection (in vivo and ex vivo fresh tissue on ice) and conclude that qualitative analysis of the imaged microstructure and optical attenuation of the OCT images discriminates between normal brain tissue, infiltrated brain tissue, solid cancer and necrosis. The imaged pathological microstructures described by Böhringer et al. were not clearly visible in the OCT images in our study. Moreover, the optical attenuation of the OCT imaged was

**DISCUSSION**

![Figure 7. Color map of speckle contrast and $\mu_{OCT}$ of the subcortical OCT data sets. The $\mu_{OCT}$ color scale bar runs from 0 mm$^{-1}$ to 10 mm$^{-1}$ and the color scale bar of the speckle contrast runs from 0 to 1. The $\mu_{OCT}$ average and standard deviation are given below the color maps.](image-url)
only assessed qualitatively by Böhringer et al. without correction for system-dependent parameters and not quantified, which hampers comparison to our results.

We found an average $\mu_{\text{OCT}}$ of $3.8 \pm 1.1 \text{ mm}^{-1}$ and $6.0 \pm 1.1 \text{ mm}^{-1}$ for normal gray matter (cortical) and white matter (subcortical), respectively. The lower $\mu_{\text{OCT}}$ for gray matter is in agreement with literature and is attributed to the lower myelin content in gray matter compared to white matter.\textsuperscript{3,6,7} For the cortically collected OCT data sets in patients with high grade glioma (n=3) we found an average $\mu_{\text{OCT}}$ of $3.7 \pm 1.5 \text{ mm}^{-1}$ for normal cortical tissue and $3.9 \pm 1.4 \text{ mm}^{-1}$ for glioma tissue. In patients with low grade glioma (n=2) we found an average $\mu_{\text{OCT}}$ of $4.0 \pm 1.1 \text{ mm}^{-1}$ for normal cortical tissue and $3.1 \pm 0.5 \text{ mm}^{-1}$ for glioma tissue. The results reported by Kut et al. show an increase in $\mu_{\text{OCT}}$ for glioma tissue compared to normal cortical tissue for both high and low grade glioma. We do observe the same trend in the high grade gliomas, however for the low grade gliomas we found lower $\mu_{\text{OCT}}$ values for the glioma tissue compared to normal cortical tissue. The possible difference for low grade gliomas can be very interesting for further research: Whereas for high grade gliomas differentiation with normal tissue is easier both visually and with fluorescence-based techniques, discrimination of low-grade glioma and normal brain tissue is challenging.\textsuperscript{1} For the subcortical (white matter) data sets we found an average $\mu_{\text{OCT}}$ of $6.0 \pm 1.1 \text{ mm}^{-1}$ and of $4.5 \pm 1.0 \text{ mm}^{-1}$ for tissues labelled as normal and glioma, respectively. All subcortical data sets included were from patients diagnosed with a high grade cancer based on histopathology (table 1). The trend of lower $\mu_{\text{OCT}}$ for glioma tissue compared to normal white matter is in agreement with literature.\textsuperscript{3,5} The found $\mu_{\text{OCT}}$ values are close to $\mu_{\text{OCT}}$ values reported by Kut et al. (6.2 ± 0.8 mm$^{-1}$ and 3.9 ± 1.6 mm$^{-1}$ for subcortical normal white matter and glioma, respectively). The differences in $\mu_{\text{OCT}}$ values between the present study and the study by Kut et al. can be attributed to differences between \textit{in vivo} and \textit{ex vivo} tissue optical properties and the absence of precise spatial matching between OCT images and histology in this study. In the cortical data sets, areas of normal brain tissue were easier to select in comparison to the subcortical data sets. Because the subcortical data sets of normal subcortical tissue are collected from the resection edge, these data sets have a high possibility of containing tumor infiltrative zone. Contrary, the subcortical data sets of glioma tissue are collected from the tumor core and therefore expected to be more reliable compared to the cortical glioma data sets, in which the depth of the quantitative analysis might form a limitation causing the data sets to contain tumor infiltrative zone.

We found average speckle contrast values of $0.54 \pm 0.18$ and $0.55 \pm 0.20$ for cortical and subcortical data sets, respectively. For both cortical and subcortical data sets, the average speckle contrast for the normal brain locations was equal to the average speckle contrast for glioma. The distribution of OCT speckle is expected to be Rayleigh distributed for a homogenous distribution of scattering particles.\textsuperscript{22,23} For such Rayleigh distribution, a speckle contrast of 0.52 is expected. In our study, the homogeneity within
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a ROI, and thereby the speckle contrast within the ROI, is influenced by both tissue inhomogeneities and the detected location of the tissue edge. In case of incorrect edge detection, the speckle contrast is expected to deviate from 0.52. We applied the speckle contrast gated (0.47 and 0.57) as a means to only include the outcome of fits on ROIs containing more or less homogenous tissue and correct edge detection, hence outcomes of fits from a ROI with speckle contrast lower than 0.47 and higher than 0.57 were excluded.

Limitations

Per-operatively tumor tissue was identified using neuronavigation on pre-operative MRI scans. Identified areas were part of the bigger tissue sample that was sent for histopathological examination and all were positive for glioma. However, obviously, normal brain was not sent for histopathological examination, so we cannot rule out tumor infiltration in the areas that were labeled as normal brain.

Moreover, the intraoperative setting (e.g. time limitation, movement caused by heartbeat and respiration, accessibility of the resection site) limits the quality of the recorded OCT images. As a result of the hard to image resection site, no subcortical OCT data sets were recorded in 2 patients. Furthermore, a total of 12 out of the 27 recorded OCT data sets were excluded from further quantitative analyses due to poor data quality (e.g. out of focus, mirror images, large vessels and accumulation of blood and fluids on the tissue surface). The quality of the OCT images would improve by a smaller scanning area and by the use of a OCT probe design dedicated to brain tumor resection surgery.3

To extract reliable $\mu_{OCT}$ values from OCT data we have fitted a well-studied and validated model of the OCT signal to the data. In this model system dependent parameters are taken into account. Moreover, we have used fit statistics to only include the outcome of the NLS fits that are a good representation of the data. However, the model has limitation, which we describe here. The model described the OCT signal based in the assumption of single scattering from a homogeneous region of tissue. Moreover, blood vessels in the region of interest will influence the extracted $\mu_{OCT}$: the strong scattering by red blood cells will result in a higher $\mu_{OCT}$.27 We have selected areas for quantitative analysis (250 by 250 pixels) without large blood vessel. In literature, image post processing is suggested to excluded blood vessels.28 However, the attenuation of the OCT signal will still be influenced by sub resolution vasculature. The resolution and depth at which the quantitative parameters are extracted is limited compared to the imaging resolution and depth. For the ROIs a lateral area of approximately 10 by 10 $\mu$m was averaged and the depth of the ROIs reached a depth, varying per dataset, of ~0.2-0.47 mm from the detected edge, varying per data set. We assumed that the depth of our quantitative analysis was limited to the upper tissue layer. Alternatively, the software can be adjusted to extract quantitative parameters from multiple ROIs in depth. To be applied as a visual
guide during surgery, real time extraction of quantitative parameters is needed. Adjustments of the to the software, as suggested by Yuan et al.\textsuperscript{8} are possible to allow real time quantitative analyses of the OCT data. However, irregular tissue structures present in \textit{in vivo} collected OCT data complicate analysis steps such as edge detection and ROI selection, which at the moment require supervision. Additional segmentation and image analysis steps could be added to provide fully unsupervised analysis of \textit{in vivo} OCT data.

**CONCLUSION**

Quantitative OCT has been suggested in literature for intraoperative imaging to discriminate glioma from normal brain tissue as a visual guide during brain tumor resection. This pilot study investigates the feasibility of \textit{in vivo} OCT imaging during glioma resection surgery (n=5) in combination with automated quantitative analysis by extracting $\mu_{OCT}$ and speckle contrast from the OCT data.

Cortical and subcortical scans were successfully recorded in respectively 5 and 3 patients. 12 out of 27 recorded OCT data sets were suitable for quantitative analysis. For the cortically collected OCT data sets in patients with high grade glioma (n=3) we found an average $\mu_{OCT}$ of 3.7 $\pm$ 1.5 mm$^{-1}$ for normal cortical tissue and 3.9 $\pm$ 1.4 mm$^{-1}$ for glioma tissue. In patients with low grade glioma (n=2) we found an average $\mu_{OCT}$ of 4.0 $\pm$ 1.1 mm$^{-1}$ for normal cortical tissue and 3.1 $\pm$ 0.5 mm$^{-1}$ for glioma tissue. The subcortical scans yielded an average $\mu_{OCT}$ of 6.0 $\pm$ 1.1 mm$^{-1}$ and 4.5 $\pm$ 1.0 mm$^{-1}$ for normal and glioma tissue, respectively. From the results of this pilot study we conclude, that the proposed method for quantitative \textit{in vivo} OCT of the brain cortex is feasible during glioma resection surgery. Applicability for subcortical areas is limited due to surgical restrictions and current probe size, which has resulted in low data quality. For the quantitative analysis, we propose a validated and automated approach to extract $\mu_{OCT}$ and speckle contrast values from the OCT data. While the found $\mu_{OCT}$ values are close to the previously reported study on \textit{ex vivo} brain tissue samples, further studies in which \textit{in vivo} OCT scans and gold standard histology are matched as close as possible are needed to investigate the use of $\mu_{OCT}$ as a tool to discriminate between glioma and normal brain tissue.
REFERENCES