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### *Ex vivo* and *in vivo* imaging of human brain tissue with different OCT systems

P. Strenge<sup>a\*</sup>, B. Lange<sup>a</sup>, C. Grill<sup>b</sup>, W. Draxinger<sup>b</sup>, V. Danicke<sup>a</sup>, D. Theisen-Kunde<sup>a</sup>, M. Bonsanto<sup>c</sup>, R. Huber<sup>b</sup>, R. Brinkmann<sup>a,b</sup>

<sup>a</sup>Medical Laser Center Lübeck, Peter-Monnik-Weg 4, 23562 Lübeck, Germany

<sup>b</sup>Institute of Biomedical Optics, University of Lübeck, Peter-Monnik-Weg 4, 23562 Lübeck, Germany

<sup>c</sup>Department of Neurosurgery, University Medical Center Schleswig-Holstein, Campus Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany

#### ABSTRACT

Optical coherence tomography (OCT) is a non-invasive imaging technique which is currently investigated for intraoperative detection of residual tumor during resection of human gliomas. Three different OCT systems were used for imaging of human glioblastoma *in vivo* (830nm spectral domain (SD) OCT integrated into a surgical microscope) and *ex vivo* (940nm SD-OCT and 1310nm swept-source MHz-OCT using a Fourier domain mode locked (FDML) laser). Before clinical data acquisition, the systems were characterized using a three-dimensional point-spread function phantom. To distinguish tumor from healthy brain tissue later on, attenuation coefficients of each pixel in OCT depth profiles are calculated. First examples from a clinical study show that the pixel-resolved calculation of the attenuation coefficient provides a good image contrast and confirm that white matter shows a higher signal and more homogeneous signal structure than tumorous tissue.

Keywords: Optical coherence tomography, OCT, FDML laser, MHz-OCT, glioblastoma, intraoperative imaging, brain imaging

#### **1. INTRODUCTION**

Glioblastoma brain tumors are highly invasive and lack a true border to the normal brain tissue [1]. Due to this diffuse growth pattern and a low inherent contrast of tumor, invaded and adjacent normal brain tissue, the detection of residual tumor during neurosurgical resections remains an important challenge, specifically because the extent of resection correlates with the survival expectations of patients [1]. At the moment different imaging modalities are used during the resection of the tumor, such as magnetic resonance imaging and fluorescence microscopy, but those techniques are not able to distinguish the glioblastoma with high certainty in the peripheral zone. Prior work has shown that OCT has the potential to detect glioblastoma by evaluating changes in tissue structure and / or optical properties compared to healthy brain tissue [2-5]. In this study three different OCT systems are characterized using a point-spread function phantom and evaluated in their ability to distinguish tumor from normal brain tissue, using in a first approach the attenuation coefficient as differentiation criterion and present here first results of an ongoing clinical study.

#### 2. METHODS

Three OCT systems were used for imaging of glioblastoma tissue during surgery (see Fig. 1 A), one for *in vivo* and two for *ex vivo* measurements (ethics application file No. 18-204). The *in vivo* acquisition was done with a SD-OCT system which is integrated into the surgical microscope and has an imaging wavelength of 830 nm (iOCT, Haag-Streit, Wedel, Germany). The two *ex vivo* OCT systems are a SD-OCT system (Callisto, Thorlabs, Dachau, Germany, imaging wavelength 930 nm) and a swept-source OCT system (OptoRes OMES, München, Germany) that uses a 1310 nm FDML laser, allowing an A-scan rate of 1.6 MHz [6]. Each system acquired a C-scan of the target tissue volume. For the *ex vivo* acquisition the brain tissue was embedded into a negative agar cuboid (4x4x2 mm or 5x5x3 mm, depending on the sample



*Figure 1* A) Extraction of a tissue sample with a Yasargil forceps; B) brain sample during ex vivo scanning (red rectangle is field of view of the Thorlabs Callisto OCT); C) ex vivo OCT B-scan of the Thorlabs Callisto; D) histological section of the brain tissue sample (H&E stained).

size), which gives the tissue a distinct shape (see Fig. 1 B). This step simplifies the histological analysis, which comes after the acquisition and the tissue fixation with 4.5% formalin. A neuropathologist creates H&E stained histological sections from the tissue sample (see Fig. 1 D), in the same orientation as the acquired B-scans (see Fig. 1 C). The OCT-scans will be segmented based on the histological sections (e.g. tumorous, non-tumorous, white matter, grey matter ...).

Before clinical use, spatially mapped point-spread function (PSF) measurements of all OCT systems were performed with a resolution validation phantom (polyurethane cylinder sparsely filled with FeO particles; National Physical Laboratory (NPL)). The particles are evenly spaced ( $30\mu$ m mean distance) and have a size of 300-800 nm, which is significantly smaller than the resolutions of the used OCT systems. Fig. 2 shows OCT B-scans acquired by each of the three systems. Note that the NPL phantom was tilted by four degrees in order to reduce backscattering artifacts caused by the phantom's surface. This allows the determination of the spatially variant PSF and the intensity distribution for each B-scan over the whole field of view (FOV). The PSF is characterized by h(x,z), which is fitted on to each visible particle in the B-scan [7].

$$h(x,z) = I_0 \exp\left(-\frac{(x-x_0)^2}{w_x^2} - \frac{(z-z_0)^2}{w_z^2}\right)$$
(1)

 $I_0$  is the maximum intensity of the particle signal,  $x_0$  and  $z_0$  are the center positions of the particle and  $w_{x/z}$  describes the  $e^{-1}$  radius of the Gaussian profile along each dimension. By transforming each  $w_{x/z}$  into the full width at half-maximum (FWHM) one retrieves the lateral  $\Delta x$  and axial resolution  $\Delta z$  [7]:

$$\Delta x = 2w_x \sqrt{\frac{ln2}{2}}$$
(2)  
$$\Delta z = 2w_z \sqrt{ln2}$$
(3)

Mapping the calculated intensities over the FOV allows the determination of  $h_c(z)$ , the axial confocal PSF, which is proportional to the intensity distribution along the z-axes [8].

$$h_c(z) = \left(\left(\frac{z - z_{cf}}{z_R}\right)^2 + 1\right)^{-1}$$
 (4)

The parameter  $z_{cf}$  is the position of the confocal gate and  $z_R$  is the Rayleigh length. In order to calculate h(x,z) and  $h_c(z)$  properly the following processes were carried out. The surface of the NPL phantom was detected by using a dynamic shortest path search algorithm proposed by Duan et al. [9]. The detected surface is then suppressed, which leads to an OCT B-scan, which only contains particle signals and noise. Each B-scan was then split into 100 equally sized sub-B-scans. For each sub-B-scan the particles were first roughly detected by using a maximum filter followed by the particle tracking method proposed by Rogers et al. [10], before Eq. (1) was fitted onto each particle. The fits of Eq. (1) and Eq. (4) were



Figure 2 Example B-scans of the NPL-Phantom acquired with the Thorlabs Callisto (left), Optores OMES (center) and iOCT (right).

based on the least-squares solution proposed by Guo et. al. [11]. The information gained through these step, can be used to improve further analysis, e.g. by deconvolution of the B-scans with the help of h(x,z) [7].

The imaging process during surgery was as follows: first an *in vivo* C-scan was taken with the Haag Streit iOCT system. Secondly a tissue sample was taken from the same area, which was then imaged by the two *ex vivo* OCT systems. In order to investigate whether the attenuation coefficient might be used to classify tumor from healthy brain tissue, the attenuation coefficient was calculated (see e.g. [3]). The depth resolved method proposed by Vermeer et al. was used to transform the intensity value *I* of each pixel *z* of an OCT A-scan into an attenuation value  $\mu$ , where  $s_z$  is the pixel spacing [12]:

$$\mu(z) = \frac{1}{2s_z} \log\left(1 + \frac{I(z)}{\sum_{i=1}^{\infty} I(z)}\right)$$
(5)

The method works under the assumption that the measured tissue attenuates almost all light along the axial direction and that the light reflected towards the photo detector is a fixed fraction of the attenuated light.

#### 3. RESULTS AND DISCUSSION

C-Scans of the NPL phantom were acquired with the three OCT systems in order to characterize each regarding the resolution and the focus parameters. It should be noted, that the refractive index for all calculations was set to 1 (refractive index of polyurethane for near infrared light is 1.49). The field of view (FOV) and the spot size (FWHM) of the three systems vary drastically: iOCT (FOV: 15.7x15.7x3.8 mm, spot size:  $17.5 \mu$ m), Thorlabs Callisto (FOV: 5.2x2x1.7 mm, spot size:  $4.7 \mu$ m) and OptoRes OMES (FOV: 6x6x5 mm, spot size:  $17.5 \mu$ m). It is expected, that NPL phantom due to its properties should give good results for Thorlabs Callisto, because the particle spacing is much higher, than the spot size. The spot size of the other two systems is too high for the given particle spacing of  $30\mu$ m in order to measure individual particles, which leads to speckles. For the analysis each B-scans of the phantom was divided into 100 sub-images and for each sub-image the five brightest particles were measured in regards of intensity and size.

Fig. 3 shows the linear interpolated values from the NPL phantom analysis of  $\Delta x$ ,  $\Delta z$  and  $I_0$  for one B-scan acquired by the Thorlabs Callisto (for example B-scan see Fig. 2). The interpolated values show interpolating errors on the left and right edges, which is why they are neglected from further analysis. The results for the lateral resolution show clearly the focus plane, where the lateral resolution has its minimum (measured:  $5.34 \pm 0.2 \mu$ m, theoretical:  $4.7 \mu$ m). The focus planes is chopped, because of the phantom tilt. The broadening of the lateral resolution with increasing distance to the focus plane due to a bigger spot size and dispersion is clearly visible and behaves as one would expect. The measured intensity behaves inversely proportional to the lateral resolution should be constant over the whole field of view, since it is independent from lens effects. The results show, that the measured axial resolution behaves almost constant to an imaging depth of 1.2 mm. Below this depth there are no more particle visible (see Fig. 2) in order to determine the axial resolution. The measured axial resolution behaves a distance to 7  $\mu$ m.



*Figure 3 Results from the NPL analyzation of the Thorlabs Callisto: lateral resolution*  $\Delta x$  (*left*), *axial resolution*  $\Delta z$  (*center*) *and intensity*  $I_0$  (*right*). *The results from the 100 subimages were linear interpolated over the FOV of the B-scan.* 

Fig. 4 shows the results of the NPL analyzation of the OptoRes OMES system (associated B-scan shown in Fig. 2). Here the measured values of the axial and lateral PSF size do not correlate with the resolution, they rather correlate with the speckle size since the average distance of the particles is similar to the spot diameter. This is why the results show no defined focus area. The measured axial and lateral PSF size are  $31.17 \pm 3.93 \,\mu\text{m}$  and  $21.77 \pm 1.99 \,\mu\text{m}$ . These values vary from the theoretical resolution values ( $\Delta x = 17.5 \,\mu\text{m}$ ,  $\Delta z = 15 \,\mu\text{m}$ ). For the results for the iOCT (see Fig. 5), the axial ( $11.06 \pm 0.58 \,\mu\text{m}$ ) and lateral PSF size ( $19.77 \pm 0.33 \,\mu\text{m}$ ) also do not represent the resolution ( $\Delta x = 17.5 \,\mu\text{m}$ ,  $\Delta z = 5 \,\mu\text{m}$ ). In contrast to the OptoRes OMES, the iOCT shows a clearly visible focus area. This leads to expected results of the distribution of the PSF size and intensity over the FOV. The results of the analyzation show, that the system parameters of Thorlabs Callisto can be determined with the help of the NPL phantom. For the other two systems the NPL phantom has not an appropriate particle spacing – suitable ones are under construction. After completion, results of the phantom measurements will be used for the calculation of the attenuation coefficient (including correction with respect to the focal position) and will be used to improve the image quality via spatially variant deconvolution [8].



*Figure 4* Results from the NPL analyzation of the OptoRes OMES: lateral PSF size  $\Delta x$  (left), axial PSF size  $\Delta z$  (center) and intensity  $I_0$  (right). The results from the 100 subimages were linear interpolated over the FOV of the B-scan.



*Figure 5 Results from the NPL analyzation of the iOCT: lateral PSF size*  $\Delta x$  (*left*), *axial PSF size*  $\Delta z$  (*center*) *and intensity*  $I_0$  (*right*). *The results from the 100 subimages were linear interpolated over the FOV of the B-scan.* 

B-scans of mainly white matter and tumorous brain tissue were acquired with the three systems. Each B-scan was processed with the algorithm proposed by Vermeer et al. (eq. (5), [12]). For the calculation of the attenuation coefficient the pixel spacing  $s_z$  was adjusted according to the valid refractive index ( $n_{air} = 1$ ,  $n_{tissue} = 1.34$ ), after the tissue surface was detected by the method proposed by Duan et al. [9]. The calculated values were not corrected for the roll-off of each system. The results are shown in the Figure 6 for the Thorlabs Callisto, in Fig. 7 for the OptoRes OMES and in Fig. 8 for the iOCT. Past studies e.g. by Yashin et al. have shown, that white matter has a higher attenuation coefficient, than tumor tissue [13]. The same behavior is also visible in the results presented in Fig. 6 and 7. It is also expected, that the calculated attenuation coefficients will differ from system to system, since the attenuation coefficient is dependent on the wavelength. The coefficient for white matter calculated for the three systems is as follows:  $\mu_{wm} = 5.04 \pm 1.26 \text{ mm}^{-1}$  (Thorlabs Callisto),  $\mu_{wm} = 3.38 \pm 1.29 \text{ mm}^{-1}$  (OptoRes OMES) and  $\mu_{wm} = 2.02 \pm 1.08 \text{ mm}^{-1}$  (iOCT). The attenuation coefficients for the tumor tissue have the following values:  $\mu_t = 2.43 \pm 0.64 \text{ mm}^{-1}$  (Thorlabs Callisto),  $\mu_t = 1.11 \pm 0.35 \text{ mm}^{-1}$ (OptoRes OMES) and  $\mu_t = 1.48 \pm 0.83 \text{ mm}^{-1}$  (iOCT).



*Figure 6* Calculated attenuation coefficient of ex vivo mainly white matter brain tissue (left) and tumor tissue (right) acquired by the Thorlabs Callisto (left). The displayed depth z is not corrected for the refractive index.



*Figure 7* Calculated attenuation coefficient of ex vivo mainly white matter brain tissue (left; tissue is in the range x~1.2mm to x~4.5mm; at the edges the agar is visible) and tumor tissue (right) acquired by the OptoRes OMES. The displayed depth z is not corrected for the refractive index.



*Figure 8* Calculated attenuation coefficient of in vivo subarachnoid space and cortex (left) and tumor tissue (right) acquired by the iOCT. The displayed depth z is not corrected for the refractive index.

The results show that the method proposed by Vermeer et al. is able to detect differences between different tissues and the values behave in the same way other groups already have shown (e.g. [14]). The disadvantage of the algorithm introduced by Vermeer et al. is the exponential increase of the calculated attenuation coefficient at the bottom of the B-scan. This results from the assumption, that all the light will be attenuated within the imaging depth [12]. There are already new methods, which try to compensate the exponential increase of the signal [15]. As described in the literature (e.g. [16]), one expects more heterogeneous signals in tumorous tissue than in white matter. This difference is qualitatively recognizable in Fig. 6-8 (right sides). Fig. 8 left shows a B-scan of the subarachnoid space taken *in vivo*. The vertical shadows are caused by the blood flow in the vessels below the arachnoidae.

#### 4. CONCLUSION AND OUTLOOK

Three OCT systems were evaluated for brain tissue imaging. One of them allows *in vivo* imaging (SD-OCT with 830nm, integrated into surgical microscope), whereas the swept-source OCT system used for *ex vivo* imaging has an A-scan acquisition rate of up to 1.6 MHz and a large imaging depth (FDML laser with 1300nm). All systems were characterized with a PSF phantom. Since the mean distance of the phantom's scattering particles is too small for two of the OCT systems to measure the PSF functions, and only suited for the SD-OCT with 940nm used in *ex vivo* scans, we will manufacture appropriate ones. In the future the results of those phantom measurements will used for the calculation of the attenuation coefficient and will be used to improve image quality via spatially variant deconvolution [8].

In an ongoing clinical study we started recording data from tumor and brain tissue from glioblastoma patients and we plan to evaluate supervised classification algorithms and neural networks to differentiate glioblastoma from white and grey matter. One approach is to look for changes in the optical attenuation. We presented here the use of a method to calculate the attenuation coefficient of each pixel in the OCT depth profile [12]. By providing localized per pixel values, this enables characterization of heterogeneous tissue without pre-segmentation.

OCT might have the potential for intraoperative tissue analysis and detection of residual tumor, which may be used for guidance of the neurosurgical resection, especially using an FDML laser for swept-source OCT with high imaging speeds.

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