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ORIGINAL ARTICLE

Modified optical coefficient measurement system for bulk tissue using an optical fiber insertion with varying field of view and depth at the fiber tip

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Abstract
To measure the few millimeter–scale macroscopic optical properties of biological tissue, including the scattering coefficient, while avoiding the instability that originates from sample slicing preparation processes, we performed propagated light intensity measurements through an optical fiber that punctures the bulk tissue while varying the fiber tip depth and the field of view (FOV) at the tip; the results were analyzed using the inverse Monte Carlo method. We realized FOV changes at the fiber tip in the bulk tissue using a variable aperture that was located outside the bulk tissue through a short high-numerical aperture (high-NA) multimode fiber with a quasi-straight shape. Using a homogeneous optical model solution, we verified the principle and operation of the constructed experimental system. A 200-μm-core-diameter silica fiber with NA of 0.5 and length of 1 m installed in a 21G needle was used. The detection fiber's shape was maintained over a radius of curvature of 30 cm. The dependences of the detected light intensity on the FOV and the depth showed better than 1.4% accuracy versus calculated dependences based on the measured optical properties of the solution. Adaptation of the method for use with complex structured biological tissue, particularly in the presence of a thick fascia, was not completely resolved. However, we believe that our specific fiber puncture–based measurement method for use in bulk tissue based on variation of the FOV with inverse Monte Carlo method-based analysis will be useful in obtaining optical coefficients while avoiding sample preparation–related instabilities.

Keywords Optical coefficient measurement · Bulk tissue · Field of view · Ray tracing simulation · Inverse Monte Carlo method

Introduction

The optical properties of biological tissue, including the absorption coefficient μ_a, the scattering coefficient μ_s, and the anisotropic parameter g, are used to calculate optical propagation in that tissue. The accuracy of calculations of light propagation in a particular tissue is ultimately dependent on how well the optical properties of that specific tissue are known [1]. Various experimental techniques have been developed to measure the optical scattering and absorption properties of biological tissues [2–6]. The ex vivo methods that are currently used to measure optical coefficients are classified into two types: measurements using optically thin tissue samples (with sample thickness of ≈100–1000 μm) and those performed using optically thick tissue samples (sample thickness of ≈few cm). Measurements using thin tissue samples present several optical problems in terms of tissue preparation [7–9]. First, thin tissue samples tend to dehydrate rapidly, which causes them to shrink and effuse intracellular fluid onto the sample surface [10]. Second, because these thin tissue samples must be sandwiched between glass slides to provide mechanical support, sample deformation may cause changes in the sample's optical characteristics, and air bubbles also commonly exist between the slide glass and the sample [11]. Apparent refractive index changes and fluctuations in the optical properties of tissues have both been induced by the

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procedures described above [10]. Biological tissue is structured in several thin layers and has heterogeneity. Macroscopic optical properties of the order of less than 1 cm are required to perform calculations for therapy and diagnosis purposes rather than the corresponding localized properties [10]. Therefore, to avoid the problems associated with thin tissue preparation, we propose use of an optically thick bulk tissue that can be punctured using an optical fiber to measure the light intensity. Methods for light intensity measurements in bulk tissue when punctured using an optical fiber have been reported since the 1980s [12]. Displacement of an optical fiber in the depth direction could be used to obtain the attenuation coefficient $\mu_{\mathrm{eff},}$ but separation of the absorption coefficient and the scattering coefficient was not achieved because of a lack of scattering information in the measurements. To improve this process, we devised a method to change the field of view (FOV) at the optical fiber tip to allow scattering information to be obtained. We propose that the FOV can be varied using a variable aperture, which is located outside the bulk tissue, to restrict the angle at which the detected light is emitted from the external fiber tip toward a sensor. The absorption coefficient, the scattering coefficient, and the anisotropic parameter were adjusted in our calculations to fit the measured light intensity at the tip as a function of both the depth and the FOV. We verified the principle and the operation of the constructed experimental system using a homogeneous optical model solution.

Materials and methods

Preparation of homogeneous optical model solution

We prepared an optical model solution using a blue edible pigment (Tartrazine 19140, Daiwa Kasei, Japan) as an absorption chromophore and Intralipos® (Intralipos Injection 10%, Otsuka Pharmaceutical Co, Japan) acting as a scattering chromophore in water. The weight ratio of the pigment relative to the Intralipos® and to the water was 1:465:30. This weight ratio was predetermined to obtain the required transmission for a 1-cm-long optical cell.

Measurement of optical coefficients using homogeneous optical model solution with the proposed method

A schematic illustration of the experimental transmission light measurement system for variation of the depth and the FOV at the detection tip of the silica optical fiber (core diameter: 200 μm; numerical aperture (NA): 0.5) used in the optical model solution is shown in Fig. 1. The experimental system is a modified device that can be used to add an FOV change capability to the conventional bulk puncture method [12]. An acrylic bath containing the optical solution was placed on an acrylic base. The bottom plate of the bath was 0.8 mm thick. Approximate optical matching can be established at the boundary between the optical solution and the bottom plate of the bath. A red diode laser operating at a wavelength of 664 nm was used to irradiate the solution upward from the base. The output aperture of this red laser was located 3.5 cm below the acrylic base. The emitted NA of the red laser beam was 0.22 and the laser's cross-sectional distribution was measured using a beam profiler (BGP-USB-L11059, Ophir Optronics Solutions, Israel) to be near-Gaussian. The operating wavelength was selected to fit the absorption band of talaporfin sodium, a second-generation photosensitizer that is used for photodynamic cancer therapy [13]. The silica detection optical fiber was installed in a 21G needle and was then immersed down to the surface of the model solution to measure the light intensity within the solution. We must be particularly careful to align the optical axis of the red diode laser beam with the detection fiber. The radius of curvature of the fiber path was maintained at over 30 cm during the measurements. The needle was moved vertically using an automated stage (location reproducibility: 1 μm). By moving the needle upward ranging from 1.5 to 0.3 mm, the light intensity could be measured at the output tip of the detection fiber using a silicon photodiode for the visible range (OP-2 VIS, Coherent Japan, Inc., Japan). The sensing diameter of the silicon photodiode was 7.9 mm. A fiber holder holding the end of the detection fiber, a variable aperture, and the silicon photodiode were placed on the same optical rail. The NA of the silicon photodiode could be varied from 0.1 to 0.5 by changing the aperture and the silicon photodiode location. The output light beam from the fiber end can be detected completely using the silicon photodiode for all NA values. This detection fiber, which was capable of multi-mode transmission, was relatively short (1 m long) and was maintained at a large radius of curvature on the fiber path. The correspondence relationship between the FOV at the detection optical fiber tip on the measurement side and the output NA of the detection fiber was then measured. Using the measured calibration curve, the interior FOV was obtained. As a result, we were able to vary the detection FOV in the solution. The FOV can also be varied at each tip depth over the range from 11.5 to 60°. The attenuation coefficients were calculated for each FOV via exponential fitting of the measured light intensity data in the depth direction.

Measurement of optical coefficients using homogeneous optical model solution with conventional inverse adding-doubling method

A spectrophotometer with an integrating sphere (UV-3600; Shimadzu Co., Kyoto, Japan) was used to measure the total transmittance and the total reflectance of the optical solution at a wavelength of 664 nm. The optical coefficients were then

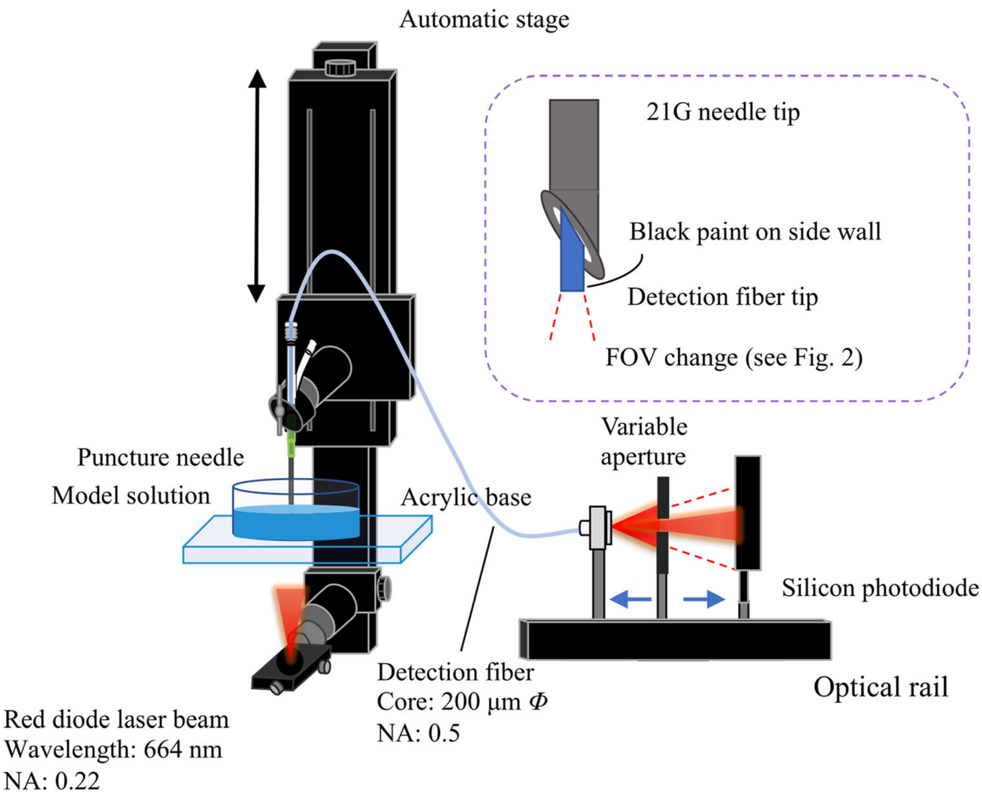


Fig. 1 Schematic illustration of experimental measurement system for variation of depth and FOV at optical fiber tip during light intensity measurements in a model solution

calculated using an inverse adding-doubling (IAD) method analysis [5, 14].

Ray tracing simulation (inverse Monte Carlo simulation)

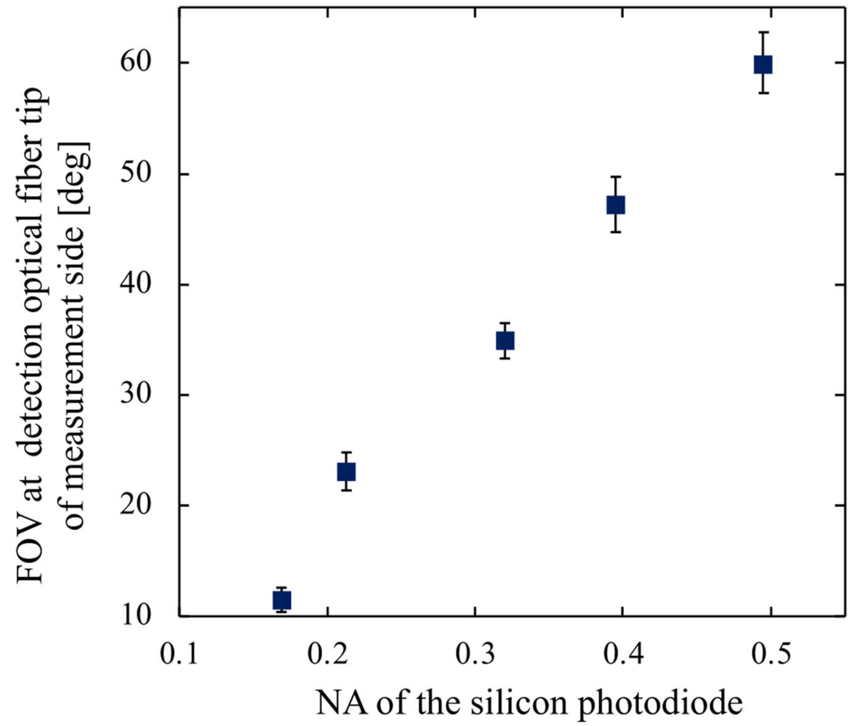
The Optis Works® (Optis Japan) ray tracing simulator was used to determine the optical coefficients from the experimental data via the inverse Monte Carlo method. A mortar-shaped cup with an open bottom with black matter attached to the detection fiber model was installed to limit the detection light angle and thus reproduce the FOV change in the calculation model. To mimic the irradiation laser, the beam shape was set to be Gaussian, and the output beam's NA was set to be 0.22. During this simulation, one million light rays were emitted. The absorption coefficient and the scattering coefficient were varied to be inputted to the calculations. Because the biological tissue has strong forward scattering characteristics, the anisotropic parameter value is approximately 0.9 [15]. Therefore, because the intention of this study was to provide proof of the principle of the proposed method, the anisotropic parameter was set at 0.9. We then varied both the absorption coefficient and the scattering coefficient within a range of ± 2.0 mm^{-1} of the values that were obtained via the IAD method in the "Measurement of optical coefficients using homogeneous optical model solution with conventional IAD method" section. To determine the optical coefficients of the model solution using the proposed method, we input the parameters manually into the ray tracing simulator to minimize the squared errors of the obtained FOV dependence of the measured attenuation coefficient, i.e., an inverse Monte Carlo method analysis was performed. The Henyey-Greenstein phase function was used in our calculations because this function is generally used in calculations of optical propagation in biological tissue [16].

Results

Measurement of optical coefficients using homogeneous optical model solution with the proposed method

The correspondence relationship between the FOV inside the sample and the NA of the silicon photodiode was investigated. The results are plotted in Fig. 2. The FOV at the tip and NA of the silicon photodiode show a stable linear relationship. Typical results for the light intensity that were measured in the optical solution while varying the FOV and the depth at the tip are shown in Fig. 3. The dotted lines show the results of exponential approximations, and the coefficients of determination that were used in these cases were in the 0.98–0.99 range. The measured attenuation coefficients obtained from the fitting results shown in Fig. 3 were then plotted in Fig. 4 as a function of the FOV. The attenuation coefficient decreased monotonically by 27% as the FOV increased from 11.5 to 60.0°.

Fig. 2 Correspondence relationship between the FOV at the detection optical fiber tip on the measurement side and the NA of the silicon photodiode ($N = 5$)



Triangular dots indicate the results of the inverse ray tracing simulations described in the "Ray tracing simulation (inverse Monte Carlo simulation)" section.

Measurement of optical coefficients using homogeneous optical model solution with conventional IAD method

The transmittance and reflectance of the optical model solution obtained using the spectrophotometer were $6.62 \pm 0.14\%$ and $6.04 \pm 0.12\%$, respectively. The absorption coefficients and the scattering coefficients were calculated using the IAD method from the transmittance and reflectance characteristics and had values of 0.252 ± 0.005 mm^{-1} and 2.52 ± 0.04 mm^{-1}, respectively.

Ray tracing simulation (inverse Monte Carlo simulation)

The attenuation coefficients were obtained via ray tracing calculations for absorption coefficients of 0.15, 0.25, and 0.35 mm^{-1} and scattering coefficients of 1.5, 2.5, and

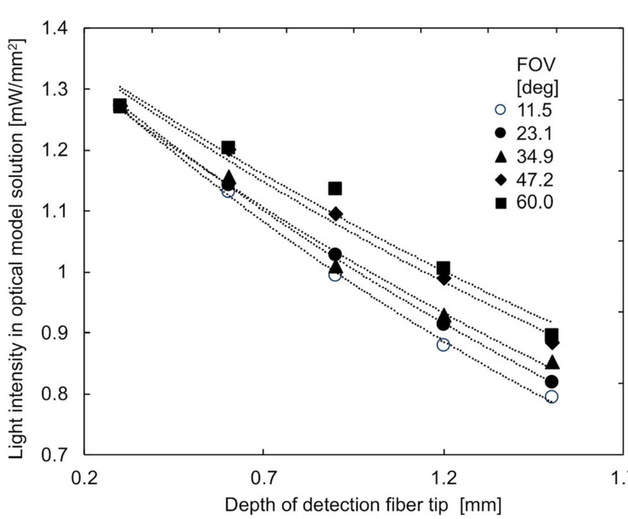


Fig. 3 Dependence of detection fiber tip depth on light intensity at various detection FOVs

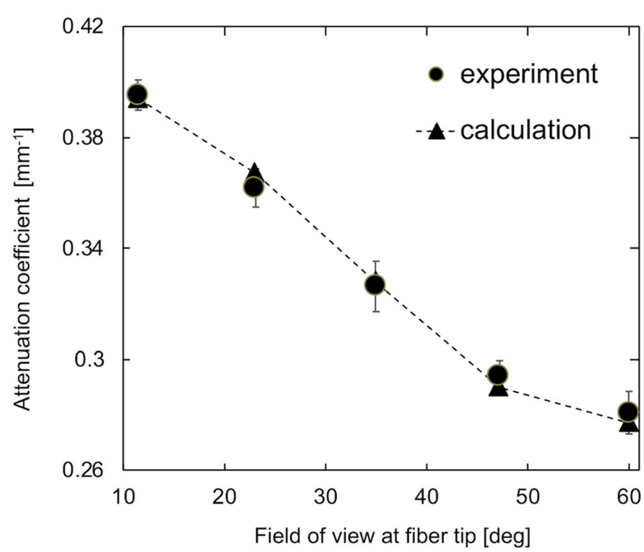


Fig. 4 Measured attenuation coefficients of the optical model solution at various FOVs obtained via experimental measurements ($N = 4$)

3.5 mm^{-1}. Because the aim of this study was to demonstrate the effectiveness of the proposed methodology, we performed calculations using nine parameter combinations. The sum of the squared errors between the experimental results and the calculated results for the attenuation coefficient for each FOV is plotted as shown in Fig. 5. The sum of the squared errors was minimized when the absorption coefficient was 0.25 mm^{-1} and the scattering coefficient was 2.5 mm^{-1}. We then used these coefficients to calculate the dependence of the FOV on the attenuation coefficient and plotted the results in Fig. 4. As the figure shows, the calculated FOV dependence was in good agreement with the experimental attenuation coefficient, with a maximum error of 1.4%. The optical coefficients of the optical model solution that were obtained via the calculations agreed almost perfectly with the optical coefficients that were obtained using the IAD method described above.

Discussion

The method of using an optical fiber to puncture bulk tissue has been reported previously [12]. When using this method, it is not necessary to slice biological tissue samples and thus the optical effects on the measured optical coefficients related to the sample preparation process can be minimized. Only the attenuation coefficient μ_{eff} could be obtained using this measurement technique because the light intensity was measured in the depth direction only at a fixed FOV. Despite the usefulness of the method described above, we have not found any reported improvements on this puncturing method for several decades [12]. To determine both the absorption coefficient and the scattering coefficient, we intended to increase the amount of optical information that could be gathered by incorporating a method to change the FOV into the experimental measurements in addition to the conventional puncture–based method. Because it is very difficult to install FOV-changing equipment at the tip of a thin fiber that has been inserted into bulk tissue, we designed our setup to perform the FOV change outside the bulk tissue via a change in the detection aperture. We used a multi-mode fiber with a 200-μm core diameter, NA of 0.5, a short length of 1 m, and a gently curved shape with a radius of more than 30 cm. In this situation, light propagates in the short fiber in a manner similar to a parallel plate waveguide, despite the occurrence of small high-order mode transfer phenomena due to the bending [17]. Therefore, the NA of a beam incident into this fiber is almost preserved at the fiber output beam. As shown in Fig. 2, the correspondence between the FOV at the detection optical fiber tip on the measurement side and the output NA of the detection fiber was in fact a stable and almost linear relationship. We have thus successfully demonstrated the use of the FOV change method in combination with a conventional fiber puncture method to increase the scattering information that can be gathered. The proposed method is considered to be effective for relatively homogeneous human organs such as the myocardium, the liver, and the kidney. In other tissues that include many nonuniform parts, such as fascias, it will be necessary to combine the proposed method with polarimetry measurements or other image analysis techniques to recognize the inhomogeneous membrane positions and locate each divided uniform compartment.

As the first limitation of our study, we did not organize a program that minimized the sum of the squared errors from the measured values in the calculations performed using the inverse Monte Carlo method. The anisotropic parameter was fixed and the other optical parameters were entered manually into the simulator. Because the aim of this study was to demonstrate the proposed methodology, we performed calculations using only nine parameter combinations. Use of a faster inverse Monte Carlo simulator will allow us to adjust the parameters in greater detail and thus determine more accurate optical coefficients. In this manner, the accuracy of proposed methodology was not determined in this study. As a second limitation, the calibration curve between the detection FOV inside the sample and the NA of the silicon photodiode may vary,

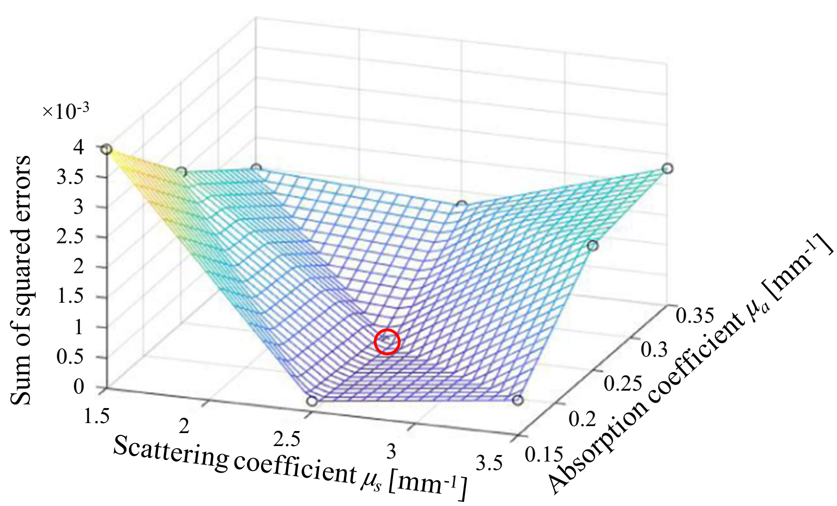


Fig. 5 Sum of squared errors between the experimental results and the calculated results for the attenuation coefficient for each FOV

depending on the shape of the multi-mode fiber waveguide. To date, we have not yet studied how the correspondence of the detection FOV inside the sample with the NA of the silicon photodiode varies with changes in the fiber shape.

Conclusions

To measure the macroscopic optical properties of biological tissues while avoiding the instability that originates from sample slicing–based preparation methods, we proposed a method of light intensity measurement through an optical fiber that had punctured the bulk tissue by varying the tip depth of the fiber and thus the FOV in combination with analysis based on the inverse Monte Carlo method. To obtain increased amounts of scattering information, we established a measurement method that can vary the FOV at the fiber tip inside the bulk tissue using an aperture that is located outside the bulk tissue. Using a homogeneous optical model solution, we successfully demonstrated that the proposed FOV change method on the outside increased the available scattering information and we also confirmed the accuracy of the constructed experimental system. We believe that the proposed fiber puncture–based measurement technique for bulk tissue using a varying FOV with inverse Monte Carlo method analysis will be useful in obtaining the absorption coefficients and reduced scattering coefficients of biological tissues.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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