

## Article type: Letter

**Title:** Spectral attenuation of brain and retina tissues in the near-infrared range measured using a fiber-based supercontinuum device

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## Abstract

A novel setup for the efficient constant optical measurements of biological tissues in the near infrared is presented. The system combines the use of a fiber-based supercontinuum source with a simple optics fiber collimator. This configuration allows a wide spectral range of measurement and, at the same time, can efficiently filter the straightforward transmitted light while avoiding scattered light. As a performance example, the optical characterization of rat brain and retina tissues are shown. The attenuation coefficient for both tissues in the near infrared region is also obtained. This technique could be applied in clinical research as a noninvasive method with several potential practical applications.

**Short title:** JE Saldaña-Díaz et al.: Spectral attenuation measured using a fiber-based supercontinuum device.

**Graphical Abstract:** A fiber-based supercontinuum source has been configured for near infrared biological tissues imaging, and this configuration allows performing straightforward

transmitted light measurements while avoiding scattered light. Spectral attenuation from 1100 nm to 2240 nm has been analyzed for both normal rat brain and retina tissues.

## **Introduction**

The study of light propagation in biological tissues is essential to many biomedical applications since, as the light propagates through the tissue, its power is attenuated by both absorption and scattering. A strong attenuation shortens the optical penetration depth and limits the application of *in vivo* and non-invasive techniques. While the absorption is due to the presence of chromophores in the tissue, the scattering is produced by the discontinuities in the refractive index at the microscopic level [1]. Scattering at shorter wavelengths, which is mainly from 400 nm to 1300 nm, limits the imaging depth [2], and recent efforts have been focused on developing methods of measurement using longer wavelengths for a better penetration depth. Biological samples have a high water content with the rodent brain being greater than 70% water, and results have been partially mitigated by this strong water absorption. A wavelength window near 1700 nm has been described as having deeper tissue penetration when both scattering and absorption are considered [3]. The knowledge of light's behavior in biological materials can create a basis for potential forthcoming applications. Currently, several devices that operate at approximately 1300 nm for both *in vivo* and *ex vivo* have been studied [4-6]. Both optical coherence tomography (OCT) using a supercontinuum light source [7] and three-photon microscopy [3] at 1700 nm are currently used to study structures in a non-invasive way in the living mouse brain. Although the main characteristics of longer wavelength OCTs for deep imaging in biological soft tissue remain, to the best of our knowledge, unpublished.

Development of new biophotonic techniques requires the use of optical sources with a wide spectral range and requires determining spectral zones with low optical attenuation. There are three primary possibilities for the study of attenuation in a very wide spectral range as follows: halogen lamps, tunable lasers or supercontinuum sources. As biological tissues can

induce a strong attenuation, the optical source must emit a high spectral power to obtain accurate values of the transmitted power. Halogen lamps commonly have considerably wide spectrum although they emit a low spectral power. Tunable lasers have a high spectral power, but they offer a short tuning range. In contrast, supercontinuum sources combine a very wide spectrum (typically over 1000 nm) with a high spectral power.

In addition, an accurate measurement of the attenuation coefficient requires avoiding scattered light. Typically, if a 10% of scattered light reaches the photodetector, a relative error approximately 30% is found in the attenuation coefficient. In this work, we present a cost-effective alternative based on a fiber collimator, which only accepts straightforward light because it acts as an optical filter significantly eliminating scattered light.

In this paper, we describe a fiber-based supercontinuum device for soft tissue measurements combining a wide range of measurements with properly scattered light filtering. The spectral attenuation coefficients were measured between 1100 nm and 2250 nm for two mouse tissues (brain and retina, respectively) demonstrating that this novel method is suitable for this type of measurements. By comparing the signal attenuation versus the tissue depth at different wavelengths, we quantitatively demonstrate the best range for deep brain and retina infrared imaging. To the best of our knowledge, this is the first study aimed to evaluate these tissues in this infrared spectrum zone.

## **Material and methods**

Rats used in the experiments were obtained from Dr. M. LaVail (University of California, San Francisco, CA, USA) and bred in a colony at the University of Zaragoza, Zaragoza, Spain. Animals were housed and handled with the authorization and supervision of the Institutional Animal Care and Use Committee from the University of Zaragoza. Procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Two 6-month-old Long Evans normal rats were injected with a mixture of ketamine (41.7 mg/kg) and xylazine (2.5 mg/kg). The depth of anesthesia was verified

using tail and toe pinch reflex of lower extremities, and the animals were sacrificed by administering a lethal dose of pentobarbital. The brain and retina were removed, fixed in 4% paraformaldehyde for 1 hour at room temperature, washed in phosphate buffer and sequentially cryoprotected in 15% sucrose for 1 hour and 30% sucrose overnight at 4°. The tissues were sliced at different thicknesses (from 15  $\mu\text{m}$  to 100  $\mu\text{m}$ ) using a cryostat (Leica Biosystems Nussloch GmbH, Nussloch, Germany).

The light transmission spectra were acquired by means of a custom-made spectroscopic system. The near-infrared power was generated from 1100 nm to 2250 nm in a highly nonlinear silica fiber, NL-1550-Zero type (Yangtze Optical Fibre and Cable Company Ltd., Wuhan, Hubei, China) pumped by a periodic train of ultrashort pulses. The nominal dispersion of this nonlinear fiber at 1550 nm is null with a dispersion slope of less than 0.025 ps nm<sup>-2</sup> Km<sup>-1</sup>. The nonlinear coefficient is greater 10 W<sup>-1</sup> Km<sup>-1</sup>, and the Raman gain coefficient is greater than 4.8 W<sup>-1</sup> Km<sup>-1</sup>. The train of ultrashort pulses was generated by means of a passive mode-locked ring laser based on the nonlinear polarization rotation (NPR) effect. The laser cavity consists of an erbium doped fiber amplifier (EDFA) operating in the C-band (Highwave Optical Technologies SA, Lannion, Brittany, France, model C20-G20-H-FC/APC-BTO 3.0, saturation output power of 20 dBm) as an amplifier medium, an optical coupler extracting 10% of the confined power, and an optical modulator based on the NPR effect, which is formed by a linear polarizer placed between two polarization controllers, model PLC-003-M-25 (General Photonics, Chino, CA, USA). The output laser pulses were amplified using a second EDFA operating in the C-band (Manlight SAS, Lannion, Brittany, France, model HWT-EDFA-GM-SC-BO-C26, saturation output power of 26 dBm). A more complete description of this laser system can be found in previous papers [8, 9].

The output power was collimated by means of a gradient-index lens obtaining a beam diameter of 0.5 mm. After propagating through the biological tissue, the transmitted power

was again coupled into a single-mode optical fiber using a second gradient-index lens. As the acceptance angle of the gradient-index lens is very small (0.15 deg), only straightforward light is coupled into the output fiber eliminating almost all the diffuse light (it has been experimentally verified that only a 0.1% of diffuse light is coupled). Therefore, this fiber device allows us to measure the attenuation spectral coefficient (due to both scattering and absorption) of biological tissues.

Spectra were measured from 1100 nm to 1700 nm with a spectral resolution of 1 nm by means of an optical spectrum analyzer (Agilent Technologies, Santa Clara, CA, USA, OSA model 86142B). From 1700 nm to 2250 nm, the spectra were measured with a spectral resolution of 4 nm by means of a monochromator (Horiba Jobin Yvon Inc, Edison, NJ, USA, model SPEX 340E) with a diffraction grating with 300 grooves per millimeter and a blaze wavelength of 2000 nm (Horiba Scientific, Edison, NJ, USA) and a PbS photoconductor with spectral sensitivity from 1000 nm up to 2750 nm (Thorlabs Inc., Newton, NJ, USA, model FDPS3X3).

## **Results and discussion**

Before determining the attenuation coefficients, the background offset and sensitivity roll-off were individually corrected for each detection system (OSA and PbS photodetector). The attenuation coefficients include effects of both scattering and absorption, which is mainly due to the water absorption in the infrared zone.

Figure 1 shows the transmitted experimental power (dB) measured for the brain and retina tissues from 1100 nm up to 2240 nm. Two thicknesses (25  $\mu\text{m}$  and 50  $\mu\text{m}$ ) of each sample are plotted as along with their respective reference measurements. Considering the measurements for tissues with a thickness of 50  $\mu\text{m}$ , we can observe that the mean losses in the studied region were 16 dB (transmission factor  $T = 2.5\%$ ) for brain and 12 dB ( $T = 6.3\%$ ) for retina compared to the reference. For longer wavelengths, the spectral attenuation fell off quickly for both tissues. For a shorter wavelength, the brain tissue showed a lower transmittance than the retina tissue although both tissues showed similar transmittance at longer wavelengths. Brain

tissue attenuated approximately 21 dB ( $T = 0.8\%$ ) at 1200 nm and 10.5 dB ( $T = 8.9\%$ ) at 2000 nm, and the retina tissue lost 16 dB ( $T = 2.5\%$ ) at 1200 nm and 9.5 dB ( $T = 11.2\%$ ) at 2000 nm (compared always to reference). For wavelengths greater than 2150 nm, both tissues showed an attenuation approximately 9 dB ( $T = 12.6\%$ ). Similar qualitative behaviors have been observed for other depths of tissue.

To study the dependence of the attenuation coefficients on the tissue depth, Figure 2 gathers the transmittance of several thicknesses of both tissues (brain: 25  $\mu\text{m}$ , 50  $\mu\text{m}$ , and 83  $\mu\text{m}$ ; retina: 15  $\mu\text{m}$ , 25  $\mu\text{m}$ , 50  $\mu\text{m}$ , and 100  $\mu\text{m}$ ) obtaining the attenuation spectral coefficients (absorption and scattering) for both tissue type. The absorbance of both tissues fell off at longer wavelengths achieving similar values at 2050 nm. This behavior demonstrates that the spectral attenuation was mainly due to scattering, while absorption had a low influence. Neither spectral holes nor peaks were found in the attenuation spectrum. Therefore, long wavelengths are more suitable to improve the penetration depth.

Figure 3 plots the transmitted light (logarithm units) as a function of the tissue thickness for a single wavelength (1540 nm). As expected, the measurements fit a linear dependence, since the transmitted signal decays exponentially along the tissue. The absorption coefficient can be determined by linear fitting with a good agreement between the experimental and linear fit.

In summary, we have presented a novel technique for the measurement of biological tissues while avoiding non-direct transmitted light. In addition, it should be noted that, since diseased tissues commonly show different optical performances compared with healthy tissues, our technique could be useful for distinguishing between healthy and diseased tissues. There are some other techniques for reducing the presence of scattered light and light absorption, such as optical clearing in *ex vivo* tissues [10]. However, the results of our proposed method are suitable for clinical applications where noninvasive methods are required for the determination of the optical properties of tissues.

## Conclusion

A fiber-based supercontinuum device has been introduced, allowing the measurement of spectral attenuation of biological tissues in a very wide spectral range in the near infrared region. The setup combines supercontinuum emission with the use of optical fiber collimators. With this configuration, the system can efficiently filter the scattered light, leading to an accurate measurement of attenuation values. This technique can be directly applied in *ex vivo* tissues without optical clearing, allowing potential clinical applications.

A spectral attenuation from 1100 nm to 2240 nm has been measured for both rat brain and retina tissues. The absorption mechanism has a low influence on the attenuation coefficient, which is mainly due to scattering. The scattering coefficient decreases for longer wavelengths in the near-infrared region. Therefore, longer wavelengths are preferable to improve the performance of biophotonic devices, allowing deeper penetration.

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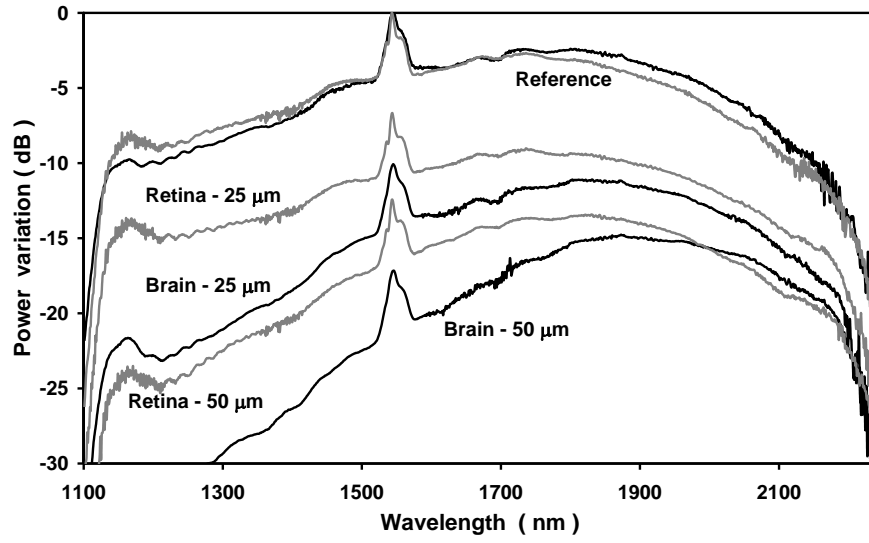


Figure 1. Spectra of the transmitted power through the brain (black lines) and retina (gray lines) samples. The thicknesses of the samples were 25  $\mu\text{m}$  and 50  $\mu\text{m}$ .

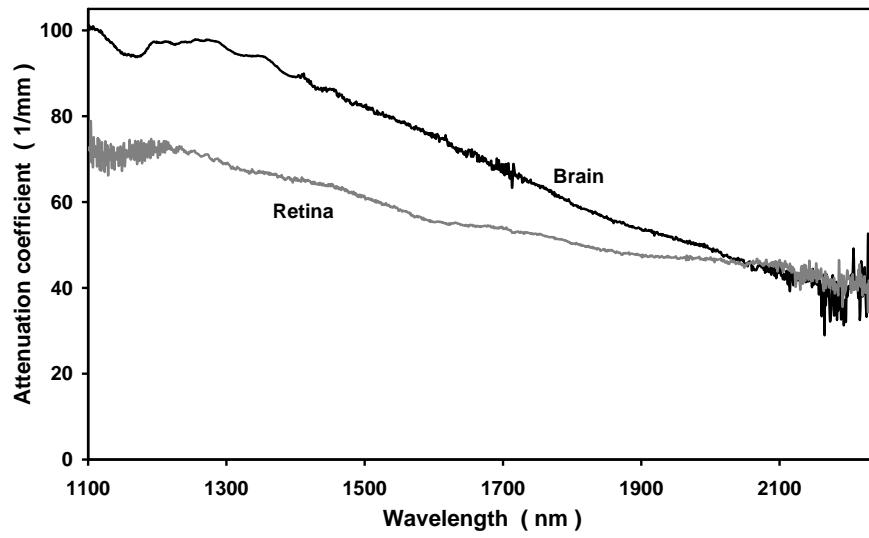


Figure 2. Attenuation spectral coefficients of the brain and retina tissues.

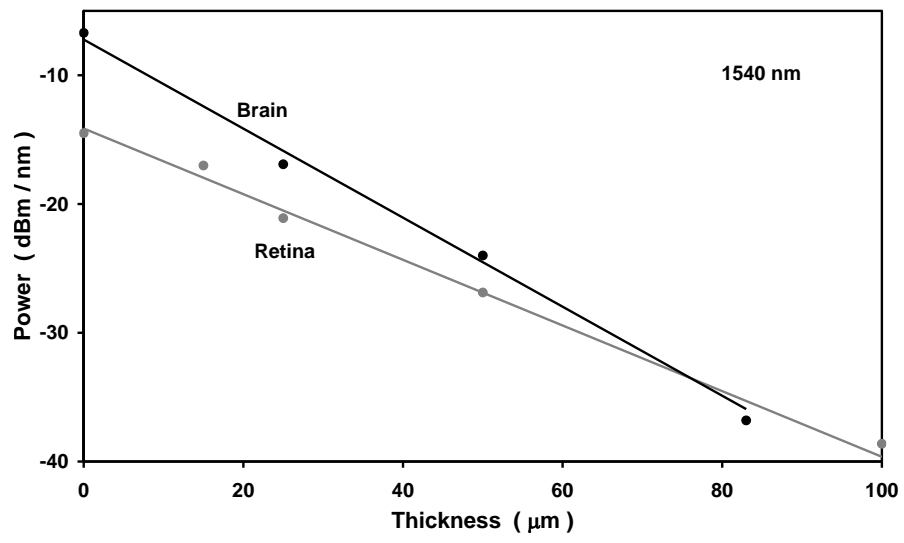


Figure 3. Linear fitting of the transmitted power (in logarithm units) versus the sample thickness (black line: brain; gray line: retina). The wavelength was 1540 nm.