

Inhomogeneity in optical properties of rat brain: a study for LLLT dosimetry

Marcelo V. P. Sousa^{*a}, Renato Prates^b, Ilka T Kato^b, Caetano P. Sabino^b, Tania M. Yoshimura^b, Luis C. Suzuki^b, Ana C Magalhães^a, Elisabeth M. Yoshimura^a, Martha S. Ribeiro^b.

^aInstitute of Physics, University of São Paulo, São Paulo, Brazil; ^bCenter for Laser and Applications, IPEN-CNEN/SP, Brazil

ABSTRACT

Over the last few years, low-level light therapy (LLLT) has shown an incredible suitability for a wide range of applications for central nervous system (CNS) related diseases. In this therapeutic modality light dosimetry is extremely critical so the study of light propagation through the CNS organs is of great importance. To better understand how light intensity is delivered to the most relevant neural sites we evaluated optical transmission through slices of rat brain point by point. We experimented red ($\lambda = 660$ nm) and near infrared ($\lambda = 808$ nm) diode laser light analyzing the light penetration and distribution in the whole brain. A fresh Wistar rat (*Rattus norvegicus*) brain was cut in sagittal slices and illuminated with a broad light beam. A high-resolution digital camera was employed to acquire data of transmitted light. Spatial profiles of the light transmitted through the sample were obtained from the images. Peaks and valleys in the profiles show sites where light was less or more attenuated. The peak intensities provide information about total attenuation and the peak widths are correlated to the scattering coefficient at that individual portion of the sample. The outcomes of this study provide remarkable information for LLLT dose-dependent studies involving CNS and highlight the importance of LLLT dosimetry in CNS organs for large range of applications in animal and human diseases.

Keywords: low-level light therapy dosimetry, low-level light therapy in central nervous system, scattering and absorption coefficients, light interaction with brain, brains diseases, traumatic brain injury, rat brain.

1. INTRODUCTION

Nowadays LLLT is being used in branches of medicine that require inflammation reduction, pain relief, healing, tissue regeneration or tissue death prevention [1]. It is broadly accepted that LLLT promotes DNA and RNA synthesis, leading to protein production. The most accepted hypothesis to explain the effectiveness of photobiomodulation in almost all tissues is the increase of production of ATP in the mitochondria after photon absorption by cytochrome-c-oxidase (COX). The absorption spectra of COX has a broad peak at near infrared (NIR) region, approximately 830 nm, and another peak near 665 nm (red). These absorption peaks are in a region of light spectra known as optical window because light is not strongly attenuated by biological tissues in this region [2].

Remarkable results have been found in Neurology, especially with Transcranial LLLT, a noninvasive treatment for serious brain diseases or injuries [3]. Transcranial LLLT improves motor recovery after strokes in rats [4] and in humans [5]; reduces significantly recovery time in Traumatic Brain Injury (TBI) [6] with little evidence of side effects [7]. Encouraging results of transcranial LLLT were found in some degenerative CNS diseases as familial amyotrophic lateral sclerosis [8], Parkinson's, and Alzheimer's diseases [9, 10]. Single neuron light stimulation [11] is connected to pain relief.

Measurements of optical properties of rat brain can expand the knowledge of mechanisms of light based therapies, like LLLT and photodynamic therapy (PDT), as one can better estimate the light fluence in a specific region of the rat brain. The knowledge of optical properties of specific regions of the brain can also support data obtained by light based diagnostic tools like optical coherence tomography (OCT) and optoacoustic tomography (OAT) [12].

The light-tissue interaction gives rise to a large number of effects. Some of them are used to diagnose like optothermal and optoacoustic effects and loss of coherence. On the other hand, photochemical reactions are responsible for Biochemical changes which lead to photobiomodulation,

*contact: marcelovictor@usp.br

phosphorescence, fluorescence and so on. Despite this myriad of effects light crossing the interior of biological tissue interacts, basically, in two ways: absorption and scattering [13].

The absorption occurs when a photon interacts with an atom or molecule and the entire energy of the photon is transferred to the atom or molecule. Molecules have specific absorption spectra, which depend on the chemical bounds. The absorption spectrum of a given tissue depends of absorption spectrum of every molecule which compound the tissue. Absorption is quantified by the absorption coefficient (μ_a), which is related to the probability of this interaction in a unit of length.

The scattering interactions can change both direction and energy of photons (inelastic scattering), or only the direction (elastic scattering). Visible and NIR light interacting with biological tissue give rise mainly to elastic scattering. The scattering depends on size, shape and refraction index of the scattering center and on the wavelength of the incident light. To quantify elastic scattering two parameters are necessary: the scattering coefficient (μ_s), which expresses the probability that scattering occurs, and the anisotropy factor (g), which is defined as the average cosine of the scattering angle. The total attenuation coefficient is $\mu_t = \mu_a + \mu_s$ [14].

Knowledge of the penetration and distribution of light inside biological tissues is an important task to improve LLLT dosimetry. Unfortunately it is a hard problem since absorption and scattering depend on wavelength, tissue biochemistry and anatomy [15].

Historically there are two approaches to the problem of multiple scattering: the analytical approach and the radiation transport theory (RTT). The analytical approach starts from Maxwell's equations and the properties of absorption and scattering of particles to obtain statistical quantities such as average intensities. In another way the RTT deals heuristically with the transport of energy by a turbid medium. A differential equation to describe RTT is equivalent to the Boltzman equation used in the kinetic theory of gases [16].

$$\frac{dI(\vec{r}, \hat{s})}{ds} = -\rho\sigma_t I(\vec{r}, \hat{s}) + \frac{\rho\sigma_t}{4\pi} \int_{4\pi} p(\hat{s}, \hat{s}') I(\vec{r}, \hat{s}') d\Omega' \quad (1)$$

This equation can be solved exactly for simple geometries and under certain ranges of concentrations of scatterers and absorbers. The numerical solution of this equation is the basis for software based on Monte Carlo simulation calculating distribution of light in turbid media.

Here we present an experimental study, which show images of samples of specific parts of rat brain illuminated with red ($\lambda = 660$ nm) and near infrared ($\lambda = 808$ nm). The differences among optical properties like absorption, and scattering gives rise to differences in light transmission at substructures of the brain. We carried out experiments to determine light radiance through rat brain slices [17].

2. MATERIALS AND METHODS

Two diode laser sources were used in this experiment ($\lambda = 808$ nm e $\lambda = 660$ nm), and the main characteristics of these sources are presented in table 1. The angular apertures of the beams were 30° producing a broad beam (figure 1). A neutral variable filter was used to regulate light intensity, and a high resolution camera (14.7 Mega pixels, 56 pixels/mm) was used to capture photons transmitted through rat brain slices. The camera lenses were adjusted to focus on the surface of the sample.

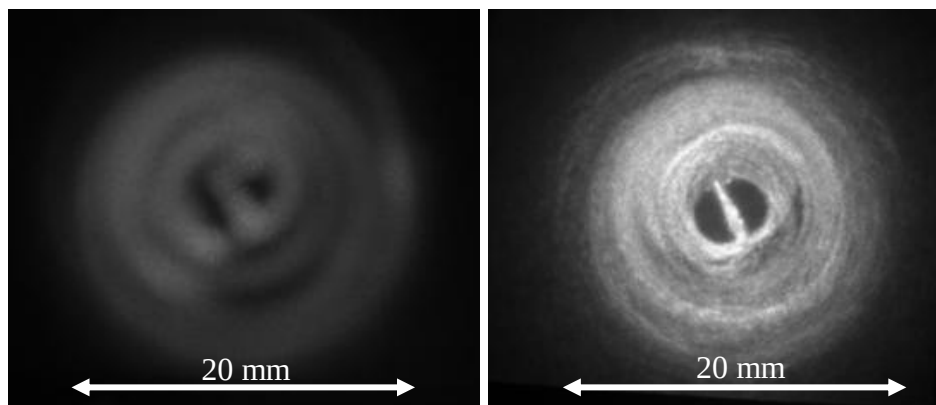


Figure 1: Image of the broad beam without sample, 660 nm (left), 808 nm (right).

Table 1: Characteristics of laser sources.

Source	λ (nm)	Power (mW)	Angular aperture of the beam (°)
Red	660	30	30
NIR	808	30	30

Adult Wistar rat (*Rattus Novergicus*) were anesthetized euthanized, decapitated and had their brains removed. The brains were sectioned by sagittal cut producing two slices with 2.0 mm thickness (figure 2). Each slice is considered a parallel plane of turbid material. The slice A is the part of the brain delimited by middle plane and a plane 2.0 mm away from that. And the slice B is delimited by planes 2.0 and 4.0 mm away from the middle

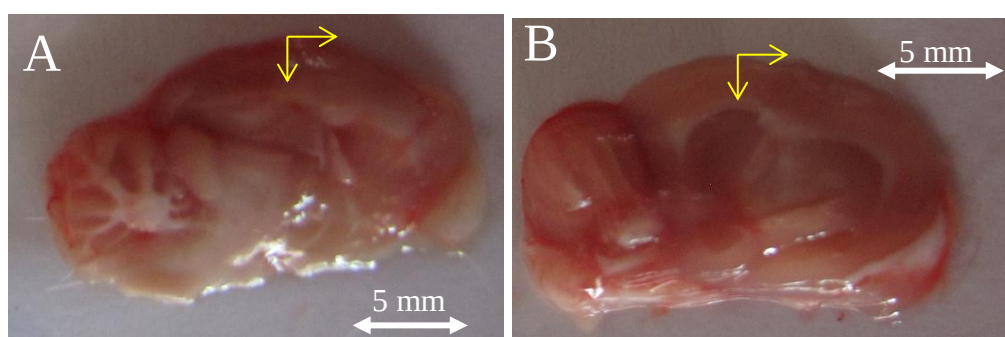


Figure 2: The sample, slice A (left), slice B (right).

Each sample was throughout illuminated by the broad light beam and the light transmitted through the slices was acquired by the camera positioned in the geometry shown in figure 3. The images produced by the transmitted light and captured by the camera were analyzed with the software ImageJ 1.44. Profiles of the gray levels in the image were traced. The signal was obtained from images in 8-bit format - 256 gray-levels (gl) from black to white [18]. As the sensitivity of the camera is very low for NIR exposure time was 4.0 s for NIR light and 10 ms for red light.

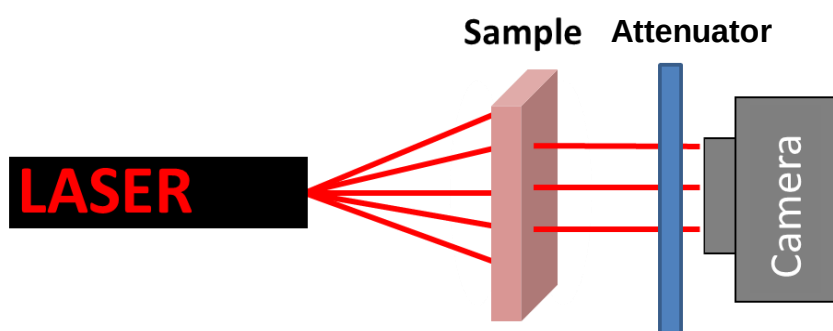


Figure 3: Setup of the experiment, laser illuminates the sample and transmitted light is captured by the camera.

3. RESULTS

The images shown in figures 4 to 7 correspond to the transmitted light of both lasers NIR ($\lambda = 808$ nm) and red ($\lambda = 660$ nm) through the slices A and B. The tissue boundaries and the differences of attenuation inside the brain are qualitatively clear in the pictures. Since the light incident on the samples was not uniform and the multiple scattering makes some blur at the image we cannot ensure that the intensity at a given point (x, y) obeys Lambert-Beer equation (2). On the other hand, differences on transmitted light can qualitatively ensure that there are strong differences among sub-structures of rat brain optical properties.

$$I(z) = I_0 e^{-\mu_t z} \quad (2)$$

Where $I(z)$ is the intensity in a deep z , I_0 is the intensity at the surface ($z = 0$) and μ_t is the attenuation coefficient.

The light captured by the camera, at (x, y) , is proportional to the intensity, $I(x, y)$, shown in gray-level (gl) scale. We traced profiles of intensity changing x position and keeping y constant. We did it for four y positions (2, 4, 6, 8 mm away from the top of the sample) in each picture. We do not show the boundaries in the profiles since the thickness of these regions are not uniform.

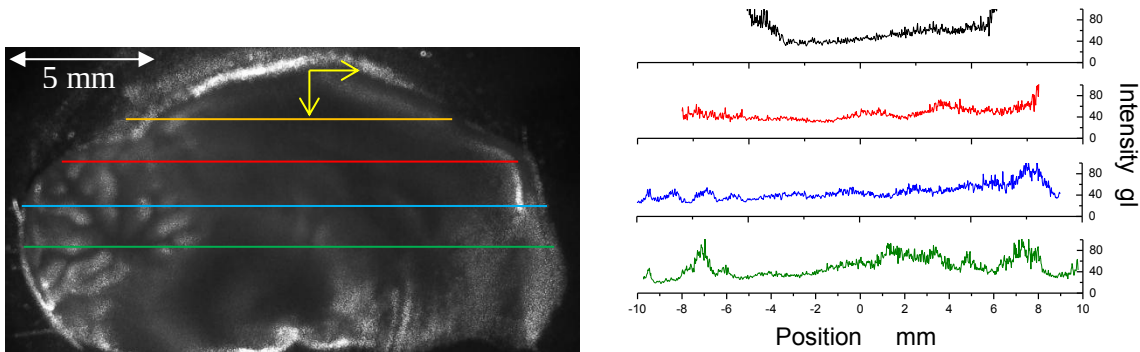


Figure 4: Red (660 nm) laser light transmitted through slice A (left). Profiles of intensity in the x direction in the positions $y = 2, 4, 6, 8$ mm from the top of the sample (right).

Figure 4 shows an image generated by red light transmitted through the slice A. We can see clearly that intensity differs for different regions of the brain crossed by the light. In particular in the cerebellum image there are abrupt variations of transmitted intensity point by point. These differences are evidenced with peaks in the profiles of intensity shown.

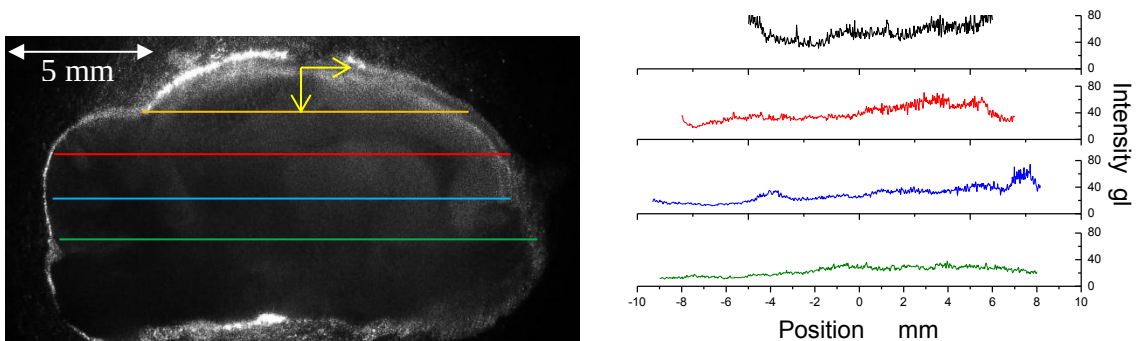


Figure 5: Red (660 nm) laser light transmitted through slice B (left). Profiles of intensity in the x direction in the positions $y = 2, 4, 6, 8$ mm from the top of the sample (right).

The transmitted intensity is smaller in slice B than in slice A. We can see it because the image in figure 5 is darker than the image in the figure 4. In the profile of slice B ($x = 8 \text{ mm}$, $y = 6 \text{ mm}$) we can see a peak meaning low attenuation at this region.

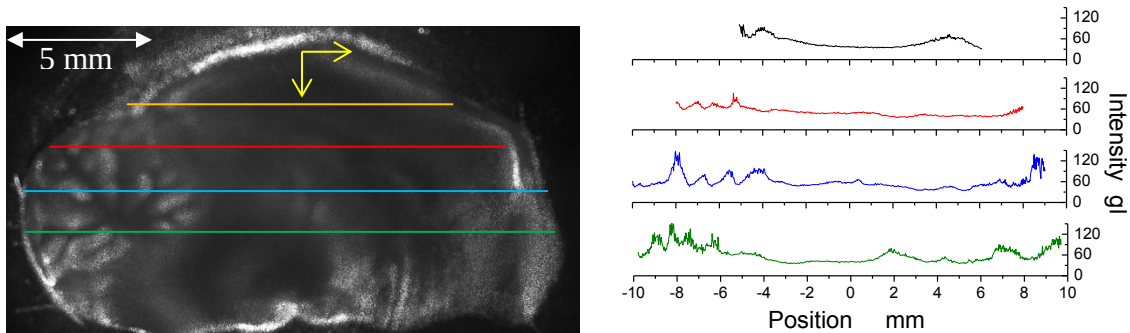


Figure 6: Infrared (808 nm) laser light transmitted through slice A (left). Profiles of intensity in the x direction in the positions $y = 2, 4, 6, 8 \text{ mm}$ from the top of the sample (right).

Figure 6 shows a image generated by infrared light transmitted through the slice A. Comparing the profiles of light transmitted through slice A we can see clearly that infrared light is less attenuated than red light. We can see that both present peaks in same points, nevertheless typical values of peaks for NIR light are two times more intense.

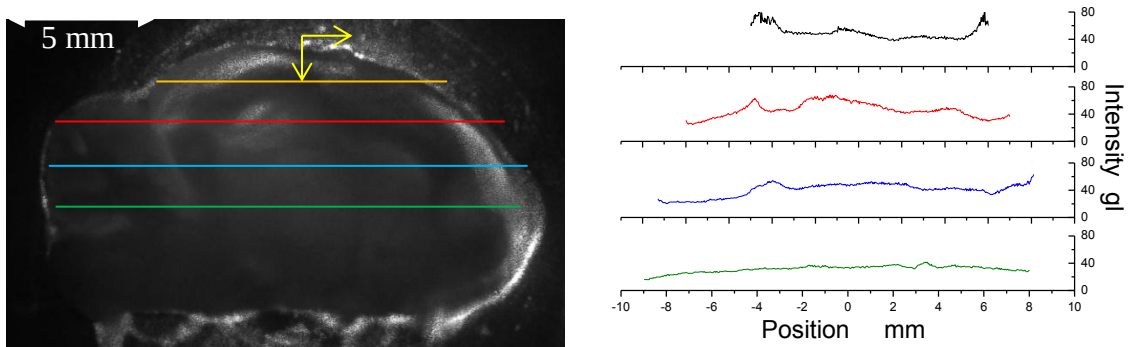


Figure 7: Infrared (808 nm) laser light transmitted through slice B (left). Profiles of intensity in the x direction in the positions $y = 2, 4, 6, 8 \text{ mm}$ from the top of the sample (right).

In the profile of slice B ($y = 2 \text{ mm}$) the intensity is too low when compared with other regions. The illumination by the top of the head is common in experiments for transcranial rat brain LLLT. Depending of the targeted area, it would be advisable the illumination by other region in order to avoid the strong attenuation at this area.

4. CONCLUSIONS

Studying the images and the profiles we can conclude that sub-structures of brain have different attenuation coefficient and NIR light is less attenuated in the brain than red light. The knowledge of attenuation properties of the whole brain can provide useful information to improve transcranial LLLT dosimetry.

In order to obtain a better contrast of images and differentiate better the brain's sub-structures we have to study thinner slices. To increase resolution we have to use a more uniform source of light. Analyze intensity profiles of images from tissues is a simple and powerful method to study quantitatively and qualitatively optical properties.

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