

**Caracterización óptica del esparcimiento en modelos estables de
tejido infundidos en Intralipid usando sensores de imagen**

**Characterizing optical scattering of stable Intralipid infused tissue
phantoms using Imaging sensors**

by

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Dedicated to
*my 2017-self, for being brave
enough to make difficult
decisions. You made it.*

– Isabella Aguilera C.

ABSTRACT

Optical Tomography is a method that performs imaging through tissue using light. Contrary to typically used techniques, such as X-rays, light does not damage human tissue but it does face a highly significant barrier which is the fact that it is heavily scattered when passing through the tissue. For this reason, it is especially important to develop new and accessible techniques that are capable of separating heavily scattered light in order to gradually replace techniques that are as harmful to patients as X-rays. Through this research, we performed an optical characterization of long lived stable Intralipid infused tissue phantoms, which simulate optical properties of skin tissue, by measuring scattering properties of these phantoms in a set-up using lasers at five different wavelengths (408, 532, 632, 670 and 808 nm) and a DSLR camera as an imaging sensor. The images acquired by this sensor were processed in a MATLAB program that conducted data calculation using regression analysis and obtained Anisotropy Factors and Scattering Coefficients of each sample. From this, Reduced Scattering Coefficients for the samples were obtained and found to be similar to measured coefficients in real biological tissue. Furthermore, the results showed that the Henyey-Greenstein model and the method fit efficiently the propagation of light through turbid media, especially when considering the 632 and 670 nm wavelengths within the lasers used.

Keywords: Turbid media, Optical imaging, Scattering, Anisotropy, Tissue phantoms.

RESUMEN

La tomografía óptica es una técnica de imagenología que permite visualizar través de los tejidos utilizando luz. Contrario a técnicas utilizadas habitualmente, como los rayos X, la luz no es perjudicial para el tejido humano pero sí enfrenta una barrera muy importante como lo es el hecho de que se dispersa en gran medida al pasar a través del él. Por esta razón, es de crucial importancia desarrollar técnicas nuevas y accesibles que sean capaces de separar la luz altamente dispersada para gradualmente sustituir procedimientos nocivos e invasivos para los pacientes. A través de esta investigación, se realizó una caracterización óptica de modelos estables de tejido infundidos en Intralipid y de tiempo de vida extenso, los cuales simulan las propiedades ópticas del tejido de la piel, al medir las propiedades de dispersión de estos con ayuda de un montaje que utilizó láseres de cinco longitudes de onda diferentes (408, 532, 632, 670 y 808 nm) y una cámara DSLR como sensor de imagen. Las imágenes adquiridas por este sensor fueron procesadas en un código de MATLAB que realizó cálculos de datos mediante el análisis de la regresión con el método de estimación de mínimos cuadrados y obtuvo factores de anisotropía y coeficientes de dispersión de cada muestra. De esto, se calcularon los coeficientes de dispersión reducidos para cada una de ellas y se encontró que estos valores son similares a coeficientes medidos en tejido biológico real. Además, los resultados mostraron que el modelo de función de fase de Henyey-Greenstein y la técnica de medición utilizada ajustan eficientemente el fenómeno de la propagación de la luz en medios turbios, especialmente cuando se consideran las longitudes de onda de 632 y 670 nm dentro del rango de láseres usados.

Keywords: Medio Turbio, Imagenología Óptica, Dispersión, Anisotropía, Modelos de tejido.

Contents

1	Introduction	1
1.1	Problem statement	1
1.2	Objectives	3
1.2.1	General Objective	3
1.2.2	Specific Objectives	3
2	Literature Review	4
2.1	Theoretical Framework	4
2.1.1	Scattering, Absorption and Anisotropy Factor	4
2.1.2	Light propagation in turbid medium	5
2.2	Experimental Framework	7
2.2.1	Phantom Fabrication	7
2.2.2	Matlab program	10
2.2.2.1	Initialization and Processing	10
2.2.2.2	Center and Data Range Determination	11
2.2.2.3	Curve Fitting and Plotting	11
2.3	State of Art	12
3	Methodology and Experimental Measurements	14
3.1	Experimental set up	14
3.2	Phantom and Power Measurements	20
3.3	Optical Characterization of Phantoms	20
3.4	Image Processing	22
4	Results and Analysis	24
5	Conclusions	39
	References	41

List of Figures

2.1	Deflected photon after a scattering event [1].	4
2.2	Plots of one-term Henyey-Greenstein phase functions [2]	6
2.3	Shrink Agar in solid phantom [3].	8
2.4	Procedure for creating the solid phantoms [3].	9
2.5	Solid phantom encapsulated within PMMA layers [3].	10
3.1	Schematic set up for Image acquisition [3].	14
3.2	Schematic scattering phantom and camera arrangement [3].	15
3.3	Set up for the HeNe laser aligned to the camera at a 38,1 cm distance.	15
3.4	Set up for the laser pointers aligned to the camera at a 15,24 cm distance.	16
3.5	Spot marked on the sample for the light beam to pass through it.	16
3.6	First 3D print modeling the bayonet mount.	17
3.7	3D print holder fitting into the camera.	17
3.8	Image of the sample not completely parallel to the holder.	18
3.9	Safety goggles as a filter to obtain a NIR light beam.	19
3.10	Measured wavelength of the resulting NIR beam.	19
3.11	Camera LCD (liquid-crystal display) showing the live view of the sensor.	21
3.12	Live view shown on the laptop screen using the EOS Utility software.	21
3.13	Red channel extracted from a HeNe image and converted into grayscale.	22
4.1	Fit and residuals for violet laser 408 nm in Sample 1 using ISO 100 and ET 1/100.	25
4.2	Fit and residuals for green laser 532 nm in Sample 1 using ISO 100 and ET 1/1600.	25
4.3	Fit and residuals for red laser 670 nm in Sample 1 using ISO 100 and ET 1/160.	26
4.4	Fit and residuals for HeNe laser 632 nm in Sample 1 using ISO 100 and ET 1/125.	26
4.5	Fit and residuals for NIR beam 808 nm in Sample 1 using ISO 100 and ET 0.6.	27
4.6	Images corresponding to Green and Violet wavelengths using Sample 1.	28
4.7	Image corresponding to NIR light source in Sample 1.	28

4.8	Fit and residuals for violet laser 408 nm in Sample 2 using ISO 100 and ET 1/320.	29
4.9	Fit and residuals for green laser 532 nm. in Sample 2 using ISO 100 and ET 1/4000	30
4.10	Fit and residuals for red laser 670 nm in Sample 2 using ISO 100 and ET 1/640.	30
4.11	Fit and residuals for HeNe laser 632 nm in Sample 2 using ISO 100 and ET 1/160.	31
4.12	Fit and residuals for NIR beam 808 nm in Sample 2 using ISO 100 and ET 0.6.	31
4.13	Image corresponding to Violet light source in Sample 2.	32
4.14	Image corresponding to Green light source in Sample 2.	33
4.15	Image corresponding to NIR light source in Sample 2.	33
4.16	Fit and residuals for violet laser 408 nm in Sample 3 using ISO 100 and ET 1/100.	34
4.17	Fit and residuals for green laser 532 nm. in Sample 3 using ISO 100 and ET 1/4000	34
4.18	Fit and residuals for red laser 670 nm in Sample 3 using ISO 100 and ET 1/160.	35
4.19	Fit and residuals for HeNe laser 632 nm in Sample 3 using ISO 100 and ET 1/160.	35
4.20	Fit and residuals for NIR beam 808 nm in Sample 3 using ISO 200 and ET 0.6.	36
4.21	Image corresponding to violet light source in Sample 3.	37
4.22	Image corresponding to red light source in Sample 3.	37

List of Tables

3.1	Camera configurations used for each phantom and wavelength.	22
4.1	Average data obtained for each wavelength using Sample 1, SC and RSC are presented in $[cm^{-1}]$	27
4.2	Average data obtained for each wavelength using Sample 2, SC and RSC are presented in $[cm^{-1}]$	32
4.3	Average data obtained for each wavelength using Sample 3, SC and RSC are presented in $[cm^{-1}]$	36
5.1	Spectra of human tissues in vivo for μ_a and μ'_s as a function of wavelength measured using different spectroscopy methods [4].	45

List of Abbreviations

Abbreviation	Definition
AVRI	Average Intensity
DOT	Diffuse Optical Tomography
DSLR	Digital Single Lens Reflex
EOS	Electro-Optical System
ET	Exposure Time
HG	Henyey-Greenstein
ISO	Sensitivity to light as a numerical value
LCD	Liquid-Crystal Display
LCI	Low Coherence Interferometry
MRI	Magnetic Resonance Imaging
Nd:YAG	Neodymium-doped Yttrium Aluminum Garnet
NIR	Near-Infrared
NIRS	Near-Infrared Spectroscopy
OCT	Optical Coherence Tomography
OT	Optical Tomography
PG	Probability of g occurring
PMMA	Polymethyl methacrylate
REO	Research Electro-Optics (Company)
RGB	Red, Green and Blue
RSC	Reduced Scattering Coefficient
SC	Scattering Coefficient
SR	Scattering Ratio
UV	Ultraviolet

Chapter 1

Introduction

1.1 Problem statement

The interaction of light with biological matter is essentially determined by the reflection, absorption, scattering, and transmission of photons when they propagate through the medium. This interaction is highly studied and applied in the medicine field to develop non-invasive optical techniques that play a crucial role in detecting, diagnosing, and treating diseases at the micro (cellular) to macro (whole organ) levels.

When the biological system of study is particularly a tissue, the light-matter interaction occurs in *turbid* or *scattering* media. Tissue can be considered a system with random distributions of scattering centers, where the presence of its many different cellular components with different refraction indexes produce an inhomogeneous path and lead significant effects to take place in the propagation of light [5], creating great challenges in the Optical Imaging field by producing a substantial amount of background noise, which makes it difficult to obtain an accurate image.

Optical Imaging techniques face great difficulties that are mainly attributed to the fact that light is heavily scattered when it passes through a turbid medium. Ideally, all photons from a collimated beam would be transmitted and exit the tissue in the same direction as the incoming beam or within a small deviation angle, but most of them are likely to be scattered numerous times, losing useful information. However, if techniques can be developed to image through tissue, they would allow a minimally or non-invasive visualization of processes and structures inside the human body using ultraviolet, visible and infrared light, without the need to expose the patient to ionizing radiation as typical imaging techniques do, such as X-rays. Furthermore, the real-time, sensitive information obtained with Optical Imaging, along with the low cost it represents, provide this set of procedures with a huge importance in the imaging field and highlight the need to improve and develop methods

that are able to separate the slightly or un-scattered light from the scattered component, creating noise that is billions of times greater [6].

Among a wide variety of Optical Imaging techniques is Optical Tomography (OT), which uses Near Infrared light (NIR) to provide three dimensional images by projecting the light across the tissue in parallel beams to an array of photodetectors and repeating this process at various angles to perform a mathematical reconstruction ([7]). One of the most powerful and commonly used OT procedures, especially within ophthalmology applications is Optical Coherence Tomography (OCT). OCT works based on tissue backscattering properties and performs micrometer-scale and cross-sectional imaging using low-coherence optical sources at longer wavelengths, providing quality images with high resolution [8]. Another highly important OT modality is the Diffuse Optical Tomography (DOT), which allows 3-D quantitative imaging of optical properties by monitoring spatial-temporal variations in the light absorption and scattering of the tissue typically using NIR wavelengths and algorithms based on the approach of inverse problems [9]. Unfortunately, while these OT techniques provide great resolution, they are limited to very thin structures of few mm and their effectiveness and accuracy diminish with depth. Hence researchers are looking for new techniques that permit imaging through thicker samples.

In order to test imaging procedures to improve these methods, tissue phantoms are commonly used to mimic the optical properties of human tissue so that it is not necessary to use actual tissue, overcoming the inconveniences of having unstable characteristics in real biological matter and avoiding ethical protocol difficulties in these tests. To create a controllable and calibrated scattering medium that mimics human tissue, long lived, stable, solid tissue phantoms have been fabricated using Intralipid [10] infused Agar encapsulated and sealed within Poly(Methyl MethAcrylate) (PMMA) layers that serve as a barrier between the phantom and the external environment, which can be manipulated as other optical components [3].

Scattering properties of tissue phantoms are commonly determined by the angular distribution of photons after a single or multiple scattering events using a goniometer to experimentally measure the phase function of an optically thin tissue section [11]. However, the goniometer only allows to measure the light intensity values at selected angles relative to the laser beam, which is very time consuming and provides a short range of resulting measurements.

The research in this thesis presents a novel and rapid method to extract scattering coefficients with an optical set up using lasers ranging from 532 nm to 808 nm, and a

DSLR camera as the imaging sensor, with tissue phantoms as the target samples. When passed through the samples, light is captured by a high-end digital camera sensor that gives millions of measurements simultaneously over a $\pm 12^\circ$ range, which is sufficiently large data to carry out curve fitting methods, such as the nonlinear least squares one, to fit the data with the Henyey-Greenstein phase function and obtain scattering measurements with high statistical significance.

1.2 Objectives

The objectives of this work were set to be accomplished within a three-month period during the MITACS Globalink Research Internship (GRI) at Simon Fraser University Burnaby, Canada.

1.2.1 General Objective

Develop a novel method for extracting scattering coefficients with an optical setup using lasers and a DSLR camera as an imaging sensor, with tissue phantoms as target samples, with which it is possible to obtain scattering characteristics more rapidly and accurately.

1.2.2 Specific Objectives

- Use the classical optical hardware that was first developed in the project [3], which used a holder for the samples located as close as it could be to the camera, and extend this work by improving and constructing a experimental setup with a new 3D printed holder for the samples that fits directly on the camera and allows to measure scattering properties of test phantoms reducing possible error sources.
- Obtain the corresponding measurements of five lasers at different wavelengths within the Visible and NIR spectrum ranging from 532 nm to 808 nm approximately, when passed through the scattering test phantoms using a DSLR camera to capture the images.
- Process the acquired images using a statistical curve-fitting MATLAB program, which converts the picture images into data and performs the fit using regression analysis. Proceed to data calculation using this program to obtain Anisotropy Factors and Scattering Coefficients of test phantoms, and improve this previously created program throughout the internship for current and further measurements.

Chapter 2

Literature Review

2.1 Theoretical Framework

2.1.1 Scattering, Absorption and Anisotropy Factor

As light travels through a turbid medium, the interaction with particles causes the photons to be absorbed or scattered, causing the light to change the direction of its propagation. Among other factors, scattering and absorption depend highly on the relative refractive index of the scatterer and its surrounding medium, and the dimensions of the scattering particles relative to the wavelength of light [12]. This process can be either elastic or inelastic depending on the exchanged energy during the process, and its total extinction coefficient or attenuation coefficient (μ_t) describes the probability of the photon to be absorbed (μ_a) or scattered (μ_s) [cm^{-1}] in a new direction within a path length. Beer-Lambert Law describes the loss of light intensity along a traveled distance z due to absorption and scattering, considering $\mu_t = \mu_a + \mu_s$, as follows:

$$I(z) = I_0 e^{-(\mu_t)z} \quad (2.1)$$

When a light beam enters a turbid medium and impacts the scattering particles, the trajectory of the photons is deflected through angles θ ranging from $\pm 0^\circ$ to $\pm 180^\circ$, and rotated by an angle Ψ , as presented in figure 2.1:

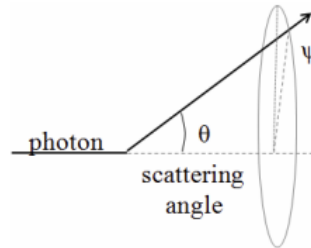


Figure 2.1: Deflected photon after a scattering event [1].

The $\cos(\theta)$ is the component of the new trajectory aligned in the forward or original trajectory. The average of this deflection angle and mean value of $\cos(\theta)$ is the anisotropy factor (g), which is dependent on the wavelength and absorption properties, and explains the amount of forward direction retained after a scattering event [13].

2.1.2 Light propagation in turbid medium

Light propagation in biological tissue is mainly governed by elastic scattering, which means that photons are re-emitted with the same energy as before encountering the scatterer, but with a probability of changing their path. In this type of turbid medium, forward scattering is the most likely to occur, but owing to the numerous presence of particles in tissue and their differences in morphology and biochemical structures, multiple scattering events take place in the propagation of light, which is taken into account by considering a reduced scattering coefficient (μ'_s), defined as:

$$\mu'_s = \mu_s(1 - g) \quad (2.2)$$

Where g is the anisotropy factor, which typically varies from 0.8 to 1 for tissue [5].

Scattering is often defined as a phase function ($p(\theta)$) that describes the angular distribution of photons after a single scattering event and the anisotropy factor follows [14]:

$$g = 2\pi \int_0^\pi p(\theta) \sin(\theta) \cos(\theta) \cdot d\theta \quad (2.3)$$

- If $g < 0 \rightarrow$ Backward scattering
- If $g = 0 \rightarrow$ Uniform or Isotropic scattering
- If $g > 0 \rightarrow$ Forward scattering

The Henyey-Greenstein (HG) function [15] is typically used as the phase function to describe the probability density that a photon will scatter with scattering cosine $\cos\theta$ according to:

$$p(\theta) = \frac{1}{2} \left(\frac{1 - g^2}{(1 + g^2 - 2g\cos(\theta))^{3/2}} \right) \quad (2.4)$$

Where g spans from -1 to 1. Although this function does not have a physical basis, it is often used to provide an empirical fit and it has been shown to have good agreement in applications where photons have undergone successive order, multiple scattering [2].

Plots of the one-term HG function are shown in figure 2.2, where the y axis represents the function depending on $\cos(\theta)$, ($p_{gh}(\cos(\theta))$), and the x axis represents θ in degrees.

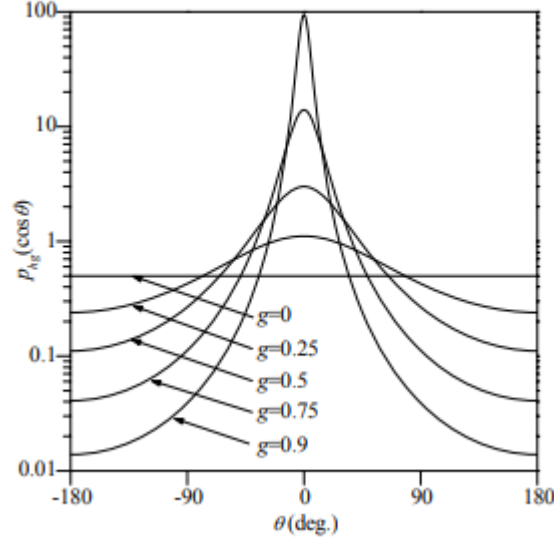


Figure 2.2: Plots of one-term Henyey-Greenstein phase functions [2]

However, considering the possibility that light may be scattered according to two different anisotropy factors within a medium, it was found [16] that the HG function often takes the form of a linear combination of weighted one-term HG phase functions as follows:

$$p(\theta) = \alpha \left(\frac{1 - (g_\alpha)^2}{(1 + (g_\alpha)^2 - 2g_\alpha \cos(\theta))^{3/2}} \right) + \beta \left(\frac{1 - (g_\beta)^2}{(1 + (g_\beta)^2 - 2g_\beta \cos(\theta))^{3/2}} \right) \quad (2.5)$$

Resulting the two-term HG phase function where α and β are the probabilities of scattering with anisotropy factors g_α and g_β respectively, and $\alpha + \beta = 1$.

Although the Henyey-Greenstein function provides an efficient approximation of scattering behavior in a wide range of turbid media, including biological tissue, it also faces some limitations such as the restricted applicability due to its simplicity, since it only depends on the anisotropy parameter and it is most adequate with relatively small and homogeneous scatterers, while in reality light propagation through tissue may have a much higher complexity considering the possibility of a spectral and spatial dependence, polarization effects, among others.

On the other hand, from the Beer-Lambert law (2.1) it is possible to calculate the Scattering Ratio (**SR**), which is the ratio between scattered photons and those whose original direction remains unchanged, called ballistic photons. Ballistic photons are unscattered and hence carry the full information of the internal structure. SR is defined as shown in equation (2.6):

$$SR = \frac{I_{in}}{I_{out}} - 1 \approx e^{(\mu_a + \mu_s')d} \quad (2.6)$$

Where I_{in} is the intensity of the incoming beam, I_{out} is the intensity of the ballistic photons leaving the sample, d is the depth of the medium, μ_a is the absorption coefficient and μ_s' is the reduced scattering coefficient.

When propagating in turbid media, light behavior is largely determined by these very important optical properties (μ_s, g) mainly related by equation 2.2, whose measurement and calculation can highly contribute to the development of optical imaging techniques and understanding of photons behavior in biological tissue.

2.2 Experimental Framework

2.2.1 Phantom Fabrication

Optical tissue phantoms are synthetic models that mimic the behavior of light when propagating through tissue and properties of the tissue itself. The creation of these phantoms has allowed the development, reproducibility and optimization of numerous optical techniques without the limitation of ethics requirements due to the fact that they do not use real biological matter.

Since these tissue phantoms are humanly-fabricated, it is possible to control the composition, geometry and optical properties of the sample such as the scattering (depending on the size, form and concentration of the scatterers in the tissue) and absorption (depending on the concentration of various chromophores) characteristics depending on the optical technique to be used and the type of tissue it is trying to imitate. Common elections for the scatterers in tissue phantoms include fat emulsions such as Intralipid [10] and Liposyn, milk, TiO₂, latex, and polystyrene microspheres. For absorbing media different types of inks (India ink, black ink, red ink) and dyes (blue dye, naphthol green dye, nigrosin, indocyanine green) are frequently used [17].

Although liquid phantoms are among the most commonly used phantoms in biomedical applications and allow a straightforward fabrication, solid phantoms provide the advantage of adapting to the geometry of the container where they are formed according to the experimental needs and can be easily manipulated in different orientations while maintaining their homogeneous characteristics.

A novel tissue phantom composition of Intralipid, ink solution and Agar was proposed by [18], where the addition of Agar to the liquid solution of Intralipid and ink allowed the sample to solidify with the advantage of Agar powders having very low absorption and turbidity characteristics. Based on this technique, long lived encapsulated Intralipid infused Agar tissue phantoms were created [1] and used as target samples for this project.

At first, the solid Intralipid infused Agar phantoms were elaborated in a disk shape with a 1% Agar and various Intralipid concentration solutions, which were diluted by using deionized water. Although the life time of the phantoms was extended from a few hours to around three days, these first trials were not completely successful due to environment factors, more specifically temperature and humidity effects that lead the samples to dry out into a thin sheet of material and shrink, as it can be observed in Figure 2.3:

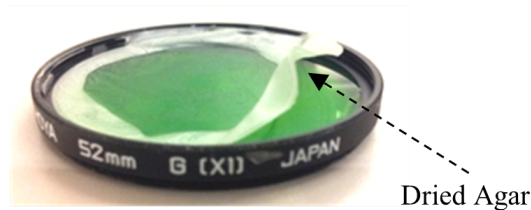


Figure 2.3: Shrink Agar in solid phantom [3].

Due to these inconveniences came up the solution of using a clear polymer to encapsulate and seal the solid intralipid-infused agar so that it could isolate the phantom from external environments and contamination, conserve the optical properties of the Intralipid and allow the sample to be orientated in any position according to the experimental requirements.

The clear polymer chosen to encapsulate the phantoms was Poly(Methyl MethAcrylate) (PMMA) given that it met the conditions named above and has a very low optical absorptivity in the Visible-Near IR wavelength range, with a known refractive index of 1.52. Furthermore, this technique allowed to fabricate more complex structures to better

replicate biological tissue by, for example, adding an autofluorescence layer to mimic the deteriorated collagen fibers in skin tissue. The elaboration of the phantoms followed the next steps: first, a bottom layer of PMMA was fabricated using a cup shape mold, which was coated so it could be easily released from the PMMA after hardening. Then, embedded structures such as resolution targets were added on top of the PMMA bottom layer. Afterwards, the intralipid infused agar mixture was poured into the structure and after 24 hours it was sealed with the top PMMA layer. Finally, a chemical polish was used on top and bottom of the sample to increase the transparency of the PMMA. A figure summarizing these steps is presented next (2.4):

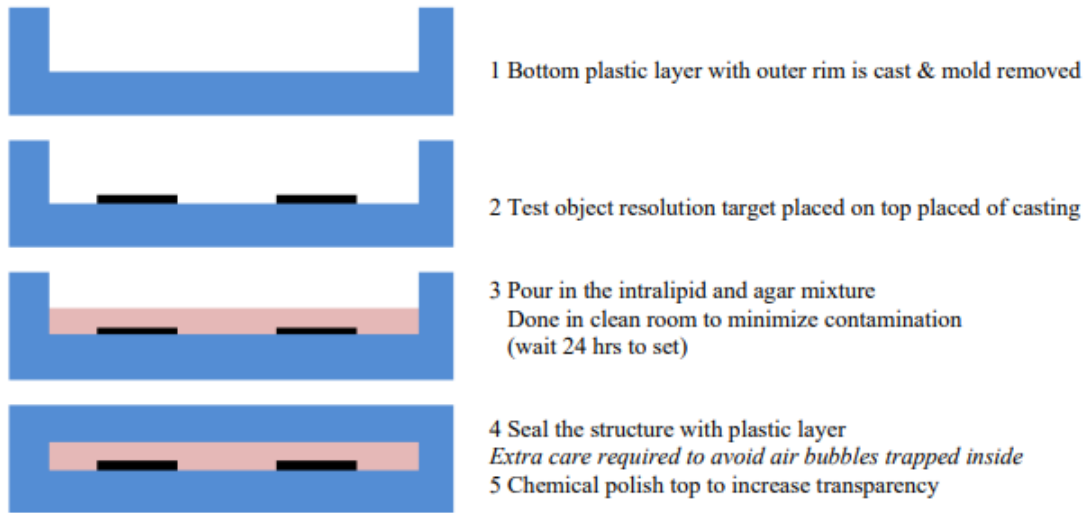


Figure 2.4: Procedure for creating the solid phantoms [3].

These various phantoms ranging from 1 mm to 8 mm thickness were successfully created and have been proven to survive for almost 10 years now, having their scattering coefficient and anisotropy factor experimentally measured every so often to verify these characteristics continue to be preserved. A picture of one of these phantoms is presented in Figure 2.5:

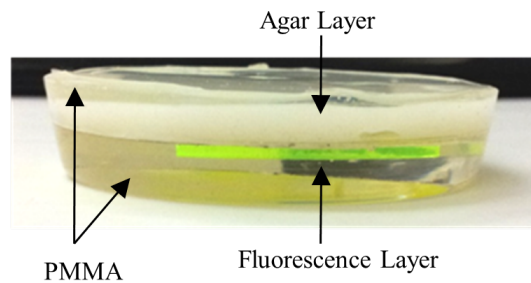


Figure 2.5: Solid phantom encapsulated within PMMA layers [3].

2.2.2 Matlab program

A Matlab code is used to process images captured with the DSLR camera, which corresponds to the data collected, and obtain the Anisotropy factors and Scattering coefficients of the tissue phantoms from them. This code was created and edited by students of the project Weijie Lin and Peter Le in 2016 and 2017 respectively, and operates according to the following structure:

2.2.2.1 Initialization and Processing

The code creates loops which iterate over a range of image values to process a sequence of images. For this purpose, a function is defined to receive seven inputs corresponding to:

1. Number or name of the first image that will be processed within a group taken using the same set up.
2. Number of images captured for this same setup.
3. Distance between the tissue phantom sample and camera sensor.
4. Thickness of the plastic layer that is closer to the sensor.
5. Thickness of agar in the sample.
6. power of the laser beam entering the sample.
7. Power of the laser beam exiting the sample.

And some variables important for further calculations are declared, such as the pixel size (**35/5616 [mm/pixel]**) and the refractive index of the plastic (**1.46**). Then, a number of matrices are generated and initialized with zeros to establish a starting point or initial state and ensure that all elements have a known value before calculations or assignments are

performed, allowing the values to be updated incrementally. These matrices correspond to variables such as *theta* (ranging from 0.0001 to 0.2518 rad in steps of 0.0001 rad) and *alpha* (calculated based on the refractive index and θ).

Furthermore, the Scattering Ratio is calculated with input variables 6 and 7 using the formula in 2.6.

2.2.2.2 Center and Data Range Determination

Images are converted to matrices with a floating-point data type that scales the pixel values to the range [0.0,1.0], where each element corresponds to a pixel in the image. Here, a threshold of **0.99** is set so that pixels with intensity values above it are set to white (1), and those below or equal to it are set to black (0), obtaining a binary representation of the image.

Then, the center of the beam is located by extracting the brightest pixels in the gray-scale image based on the set threshold and when a group of these pixels is found, the centroid is located by calculating the mean coordinates using the intensity values of the pixels. From this, the data range is extended within a circle with radius going from the center to the horizontal edge.

For noise reduction purposes, data within a circle of 234 pixels (1.5 mm) around the center is not used for further calculations due to the fact that these pixels correspond essentially to ballistic and quasi-ballistic photons. Additionally, the code performs a calculation of average intensity values for pixels with the same radius and filters out pixels with an intensity value of zero.

2.2.2.3 Curve Fitting and Plotting

The code creates the curve taking the angle and average intensity as input arguments and performs the fitting according to equation 2.5 using the nonlinear least-squares method. Along with this, it calculates the differences between the actual measured values and the fitting to plot the residuals, indicating the discrepancies between the observed values and the model predictions. A **P** value is also calculated for each processed image, which is the probability that the residuals are random. The lower this value is, the more it indicates the presence of non-random patterns or correlations in the residuals.

Finally, the program provides the plots with both the measured/fitted values and the residuals, and two excel files with the anisotropy factors and scattering coefficients for each of the processed images, but also as mean values for all the images taken using the same set up.

2.3 State of Art

Optical imaging techniques are of great importance in the biomedical research field since they allow to visualize biological structures and develop diagnose and therapeutic methods for several medical conditions. These powerful non-invasive techniques make possible to obtain detailed information using non-ionizing radiation (commonly within the Visible and NIR spectrum) and highly reducing the damaged caused to patients with typical imaging techniques such as X-Rays, or surgical procedures. These methods represent a versatile and relatively affordable option to conduct real-time and high resolution imaging and provide quantitative data, which can be used for analysis in numerous fields.

A very important optical imaging sub-field is the Optical Tomography, which allows to reconstruct 3D images of biological tissue with Visible or NIR light by collecting data from multiple angles around the tissue at various depths and using mathematical algorithms. It has a wide range of applications that include cancer detection, organ imaging and the study of molecular interactions. Some of the most common Optical Tomography modalities are Optical Coherence Tomography (OCT) and Diffuse Optical Tomography (DOT).

OCT is a non-invasive optical imaging technique that enables high resolution visualization of tissue by conducting cross-sectional imaging using low-coherence interferometry between a signal from a sample, which is the case of study, and a local reference signal [19]. The first in vitro OCT images were presented by a group of researchers from Tufts University and Massachusetts Institute of Technology using a coherence domain reflectometer device based on low coherence interferometry (LCI) [20]. Later, the same group presented studies on the clinical application of OCT in ophthalmic diagnostics and demonstrated the efficiency of this technique in the quantitative evaluation and monitoring of various diseases of the macula and the optic nerve head [21].

OCT has shown to be a powerful tool in imaging of macular diseases, the anterior eye, retinal imaging, and quantification of nerve fiber layer thickness. Recently, a group of researchers from Duke University developed a 3D Optical Coherence Refraction Tomography technique [22] that addressed limitations of typical OCT such as the difficulty to acquire high-resolution images over a wide field of view in all directions simultaneously, and high levels of random noise (speckle).

On the other hand, DOT is a technique based on measuring the intensity variation of diffusely transmitted light and reconstructing images and spatial maps of tissue properties such as total hemoglobin concentration, blood oxygen saturation and scattering, using

linearization, and nonlinear iterative approaches. DOT has been developed as a technique to overcome the limitations of conventional near-infrared spectroscopy (NIRS) and provide diagnostic optical imaging. Some of its most successful and recent applications include functional cerebral development in infants [23], breast cancer detection [24], physiological functions of muscle [25], and many more.

With DOT, it is possible to detect lesions without exogenous probes or contrast agents, it can be employed in neonates, and it is useful for planning of photodynamic therapy. Furthermore, it can be combined with other imaging techniques such as magnetic resonance imaging (MRI) or X-rays, to also provide structural information of tissue and improve the imaging quality [9].

These and many more optical techniques attend the need to separate the scattered from the unscattered or slightly scattered light coming from a beam and passing through biological tissue. When dealing with ballistic photons and thin tissue (less than a few millimeters) or microscopic imaging, techniques like OCT are most commonly used, while techniques like ODT deal with scattered photons themselves [26]. However, since both OCT and ODT are most effective for imaging within a few millimeters beneath the tissue surface, beyond that depth, the signal collected faces many limitations due to scattering and absorption phenomena. This reduces their utility for media with greater depth and trades off the very high resolution for only seeing through thin structures. To work on the development of these methods, tissue phantoms are commonly used to recreate optical properties of tissue and provide a resourceful way to conduct tests without the need of using actual biological samples.

The Quantum Optics Research Group at Universidad del Valle has been working around the tissue optics field since 2002 to study different optical properties on biological models [27] [28] [29] [30] and adipose cells [31]. Parallel to this, the research group working under the supervision of Prof. Dr. Glenn Chapman at Simon Fraser University has also obtained significant results around this field by creating and characterizing long lived tissue phantoms and developing the novel method presented in this thesis to measure scattering properties by using DSLR cameras as imaging sensors [32] [3] [1].

Chapter 3

Methodology and Experimental Measurements

3.1 Experimental set up

Typical techniques to obtain scattering properties of phantoms use a goniometer to measure light intensity values at certain angles, but this limits the results to a small range of data since measurements can only be made one angle at a time. A novel method was developed [3] replacing the goniometer with a camera system where a laser is held in front of a sample holder and a camera, and everything is confined to a two dimensional translational motion. The camera is connected to a computer so it can be controlled remotely through an EOS (Electro-Optical System) Utility Software and this provides a Live View mode of the sensor so the laser can be properly aligned. Outlines of the experimental set up are presented in Figures 3.1 and 3.2:

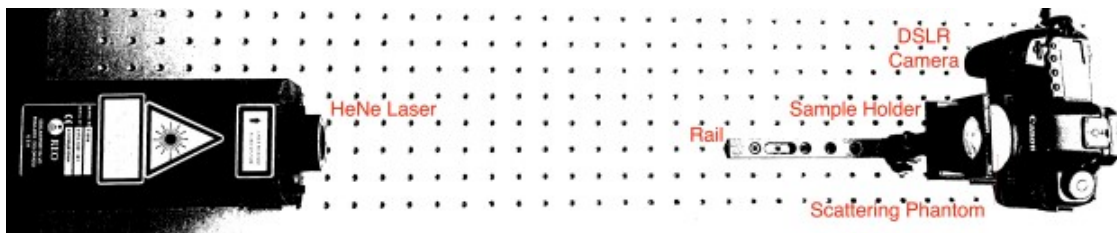


Figure 3.1: Schematic set up for Image acquisition [3].



Figure 3.2: Schematic scattering phantom and camera arrangement [3].

The experimental set up was placed in an optical breadboard using four different laser sources: a Helium-Neon (HeNe) REO laser of **632 nm** and laser pointers of wavelengths **670 nm** (Red), **532 nm** second harmonic Nd:YAG (neodymium-doped yttrium aluminium garnet) (Green) and **408 nm** (Violet). Each one of the lasers was aligned perpendicularly and held at a distance of 15,24 cm (6 in) from the scattering medium and the DSLR camera system, except for the HeNe laser which was held at a distance of 38,1 cm (15 in). The set up for the HeNe and pointer lasers is shown in Figures and 3.3 and 3.4:

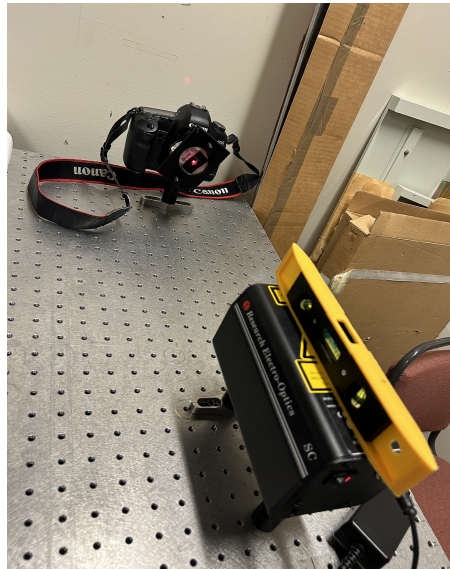


Figure 3.3: Set up for the HeNe laser aligned to the camera at a 38,1 cm distance.



Figure 3.4: Set up for the laser pointers aligned to the camera at a 15,24 cm distance.

This optical table allowed the set up to be stable enough for the measurements and all the optical components were properly leveled. Additionally, each of the samples had a mark for the light beam to pass due to the fact that throughout the years the Agar between the layers lost homogeneity over the sample and it was intended to measure each wavelength passing through the same spot:



Figure 3.5: Spot marked on the sample for the light beam to pass through it.

The traditional optical mounts shown in Figure 3.2 were previously used to hold the samples right in front of the camera body as close as they could get. As an improvement, the

Engineering Physics undergraduate student, Matthew Schilling, designed and constructed a 3D-printed optical mount that holds the samples and accommodates directly into the camera body using the bayonet lens shape so it fits just like standard lens, rigidly aligning the tissue phantom to the camera, and reducing environment effects on the measurements. In this cooperative work, tests of the set up were continuously performed to achieve iterative progress of the new optical set up. The first 3D-print obtained from this was used to verify the fit between the holder and the camera body, as shown in Figure 3.6.



Figure 3.6: First 3D print modeling the bayonet mount.

Subsequently, a second design was printed with the right fit to the camera body and the holder for the sample, as shown in Figure 3.7:



Figure 3.7: 3D print holder fitting into the camera.

However, some of the samples did not fully fit into the holder and could not be held entirely parallel to the mount, as it can be observed in Figure 3.8:



Figure 3.8: Image of the sample not completely parallel to the holder.

Despite this inconvenience, a first measurement trial was carried out with this mount since the distance angle from the sample to the holder was very small. Three samples with different Intralipid concentrations were used for this first trial.

One of the objectives of the research was to measure the phantom properties using a NIR source, which had not been done in previous work. To accomplish this, in addition to the four wavelengths mentioned above, a fifth **808 nm** light source was obtained by modifying the second-harmonic Nd:YAG laser. Second-harmonic generation is a nonlinear optical process in which photons with the same initial frequency interact with a nonlinear material, which in this case is an optical crystal, are "combined" and generate a new photon with twice the frequency of the initial photons [33]. Typical green laser pointers start with a 808 nm diode laser pumping a Nd:YAG crystal which outputs a 1064 nm beam. This beam passes through a second harmonic crystal to create the 532 nm green light. Based on this and considering that the green laser pointer used did not have an IR filter, as it is typically the case with cheap laser pointers, Argon Ion laser safety goggles were used as a filter for the light coming out of the laser (with both 532 and 808 nm wavelengths), which filtered all light shorter than 560 nm and allowed a beam of 808 nm to pass through and reach the target samples. In Figure 3.9 it is possible to see the NIR light through the goggles reaching the sample in the marked spot:

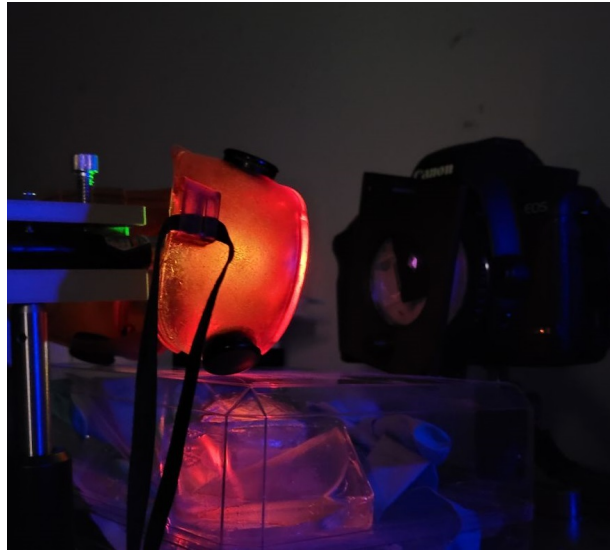


Figure 3.9: Safety goggles as a filter to obtain a NIR light beam.

The picture from Figure 3.9 was taken with a Samsung S20 which does not have an IR filter so the 808 nm beam can be seen as a whitish spot on the camera. The reason the spot is white is that the normal red, green and blue pixel filters in most color cameras pass all wavelengths longer than 770 nm, hence the camera sees this as all three colors equally illuminated and this shows up as a white spot. To be completely certain of the wavelength of this resulting beam, the undergraduate Electrical Engineering student, Xin Wei, measured the wavelength using a spectrometer and obtained the corresponding result shown in figure 3.10:

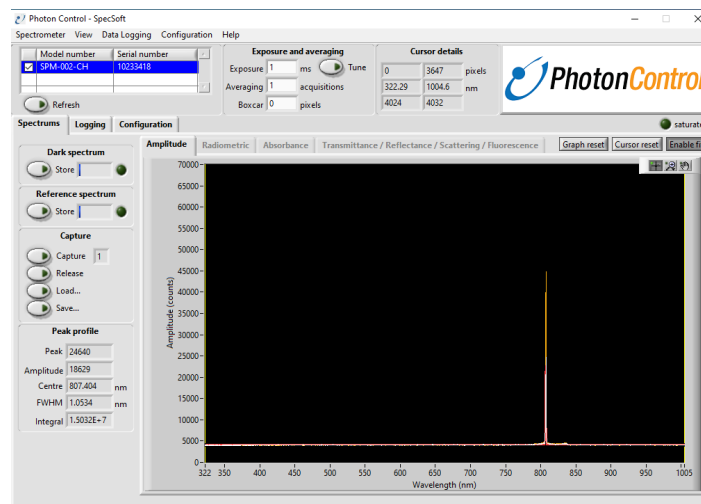


Figure 3.10: Measured wavelength of the resulting NIR beam.

After the first trial measurements, the holder was modified by grinding part of the material that was not allowing the sample to be entirely parallel to the mount and two more trials were performed with this modification.

3.2 Phantom and Power Measurements

Measurements of the phantoms and the light beams were taken to provide the inputs 3,4,5,6 and 7 of the MATLAB code (Section 2.2.2.1).

For this, the distances between the phantoms and the sensor, and the thicknesses of the Agar and plastic layer were measured using a digital vernier caliper with precision of 0.01 mm. Three measurements were taken along the circumference of the scattering sample and then averaged. An illustration of these distances is shown in Figure 3.2.

A power meter was used to measure the power of the laser beam going into and exiting the sample by placing the meter around 38 cm away from the HeNe laser and 15 cm away from the laser pointers to measure the power entering the target. Then, each sample was positioned in between the corresponding laser and the meter to measure the power exiting the target, which corresponds to the intensity of the ballistic photons after passing through the scattering medium.

3.3 Optical Characterization of Phantoms

To proceed with the acquisition of images, the DSLR camera in the setup was plugged into a laptop that worked with the EOS Utility Software and that allowed to have a Live View of the sensor on the laptop screen. The camera was set to manual mode and with the software it was possible to modify the ISO (sensitivity to light as a numerical value) and ET (Exposure Time) according to the needs of each setup, aiming to obtain a peak intensity within 40% and 60% for the interested channel in the RGB (Red, Green and Blue) histogram depending on which wavelength is being used. This is shown in Figures 3.11 and 3.12:

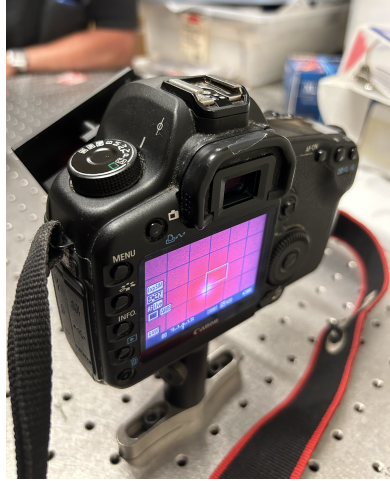


Figure 3.11: Camera LCD (liquid-crystal display) showing the live view of the sensor.

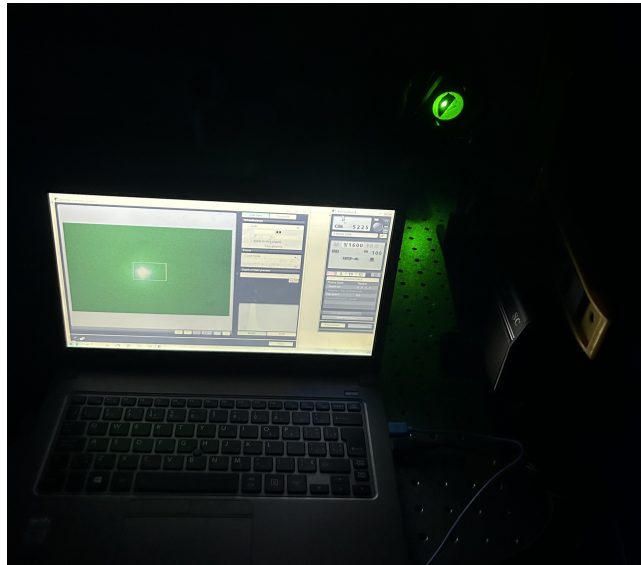


Figure 3.12: Live view shown on the laptop screen using the EOS Utility software.

Each of the laser beams passed through three tissue phantoms [1] which simulate optical properties of human tissue with 1% Agar concentration, various Intralipid concentrations and thicknesses of 5,54 mm, 3,64 mm and 4,23 mm. After a process of trial and error to obtain the most adequate ISO and ET for each setup, the following parameters were used for the final measurements:

	Violet		HeNe		Red		Green		IR	
Sample	ISO	ET	ISO	ET	ISO	ET	ISO	ET	ISO	ET
1	100	1/100	100	1/125	100	1/160	100	1/1600	100	0.6
2	100	1/320	100	1/160	100	1/640	100	1/4000	100	0.6
3	100	1/100	100	1/160	100	1/160	100	1/4000	200	0.6

Table 3.1: Camera configurations used for each phantom and wavelength.

For each sample-laser arrangement, ten images were taken every ten seconds in a darkroom, and the whole process was repeated for three trials. As it can be observed in Figure 3.12, there is a distribution of light that is brightest at the center of the image and fades as it gets closer to the edges. This is the $p(\theta)$ distribution (2.4).

3.4 Image Processing

To reduce possible noise effects, the captured images were processed through an Adobe Photoshop Automation program which uses the split channel function and separates the red, green, and blue channels. Depending on the wavelength of the laser used, one of these channels was extracted and exported as a grayscale .TIF file to be processed using the MATLAB code since it only reads images in grayscale. For the NIR, HeNe and red lasers, the red channel was extracted and for the green and violet lasers, the green and blue channels were extracted, respectively. Figure 3.13 shows an example of one of these images processed through Photoshop:

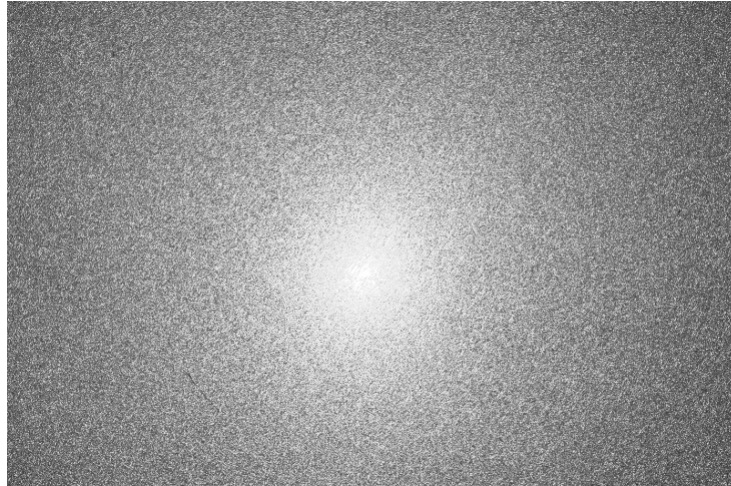


Figure 3.13: Red channel extracted from a HeNe image and converted into grayscale.

The images of scattered light were then processed in a MATLAB code (see Section 2.2.2) that was previously created by students of the project and it allowed to collect the raw

data extracted from the sensor and processed through the Photoshop Automation program, in order to proceed to data calculation and to finally obtain the Anisotropy factors and Scattering coefficients of the samples. This code considers the intensity of the light in the pixels of the image, relating them to the formula in equation 2.4 and performs the statistical fitting of the data by using the regression analysis or least squares method. With this, desired coefficients of g and μ_s from images of each phantom were extracted. For running the main function in the code, the following inputs were used to process each group of images during the first trial:

Sample 1

HeNe: main2(1101, 10, 57.52, 3.36, 5.54, 0.950, 0.0153)
Red: main2(1111, 10, 57.52, 3.36, 5.54, 1.950, 0.0471)
Blue: main2(1131, 10, 57.52, 3.36, 5.54, 3.06, 0.000386)
Violet: main2(1121, 10, 57.52, 3.36, 5.54, 4.430, 0.322)
IR: main2(1171, 10, 57.52, 3.36, 5.54, 3.43, 0.00425)

Sample 2

HeNe: main2(1301, 10, 56.29, 2.13, 3.64, 0.950, 0.0617)
Red: main2(1321, 10, 56.29, 2.13, 3.64, 1.950, 0.1309)
Green: main2(1331, 10, 56.29, 2.13, 3.64, 4.430, 0.571)
Violet: main2(1341, 10, 56.29, 2.13, 3.64, 3.06, 0.02026)
IR: main2(1371, 10, 56.29, 2.13, 3.64, 3.43, 0.00332)

Sample 3

HeNe: main2(1501, 10, 58.67, 4.51, 4.23, 0.950, 0.0114)
Red: main2(1511, 10, 58.67, 4.51, 4.23, 1.950, 0.0691)
Green: main2(1521, 10, 58.67, 4.51, 4.23, 4.430, 0.554)
Violet: main2(1531, 10, 58.67, 4.51, 4.23, 3.06, 0.000348)
IR: main2(1571, 10, 58.67, 4.51, 4.23, 3.43, 0.01241)

For the second and third trial, only the first parameter regarding the name of the image was changed starting the serial numbers with "2xxx" and "3xxx", respectively.

Chapter 4

Results and Analysis

Initially, several trials we carried out to find the best possible configuration of ISO and ET. Then, with these parameters chosen, three final trials were performed and a total of 570 images were acquired and analyzed. Between all of them, the best fitting results were selected and are presented in this research for each sample and wavelength.

Each graphic shows two plots: the upper one presenting the variation of the AVRI (average intensity of light) in the pixels according to the angle increase from the center (AVRI vs. Angle), and the lower one being the residuals, which represents the difference between the measured and the predicted values, according to the angle increase from the center (Residuals vs. Angle). In both cases the blue curve represents the experimental values, while the red curve represents the fit for the AVRI behavior given by equation (2.5), and the zero line for the residuals case.

A summary of the data obtained for the samples throughout the three trials is also presented showing the probability of g occurring (PG), mean g value, reduced scattering coefficient (RSC) and scattering coefficient (SC). Regarding the multiple scattering events and the possibility that light may be scattered according to two different anisotropy factors, for each wavelength the first column in the tables corresponds to alpha (α) values, which represent the Intralipid medium and the second one, to beta (β), which represents the Agar medium. Since light propagation in human tissue is highly forward scattered, the reduced scattering coefficient is a better representation of photon scattering behavior in this type of turbid medium, which is why we will focus more on analyzing this result rather than the scattering coefficient to conduct comparisons with real tissue measurements.

Sample 1

Figures 4.1 to 4.5 show the results for each wavelength used in Sample 1.

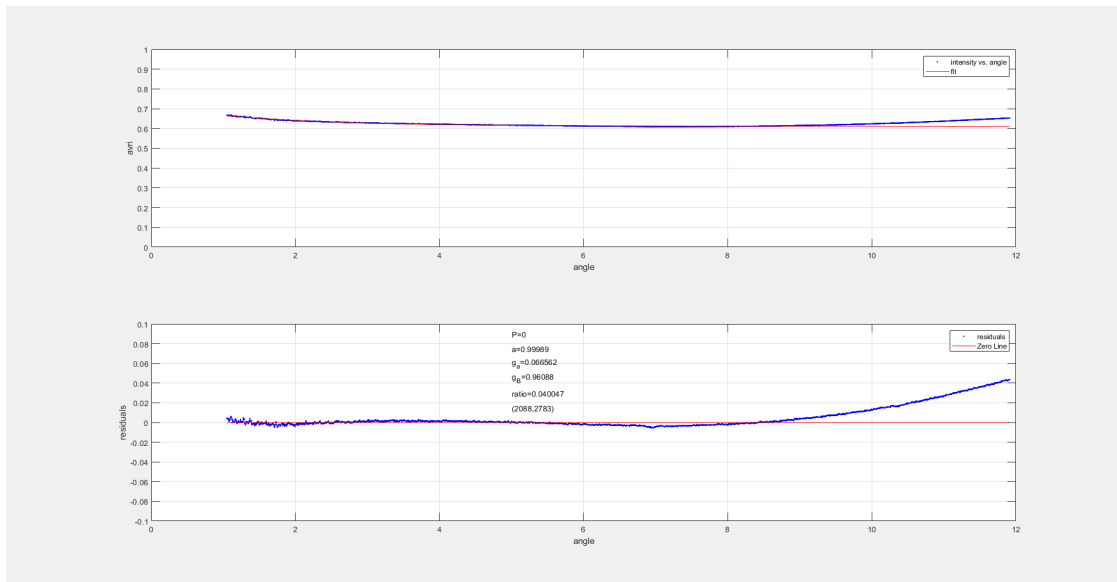


Figure 4.1: Fit and residuals for violet laser 408 nm in Sample 1 using ISO 100 and ET 1/100.

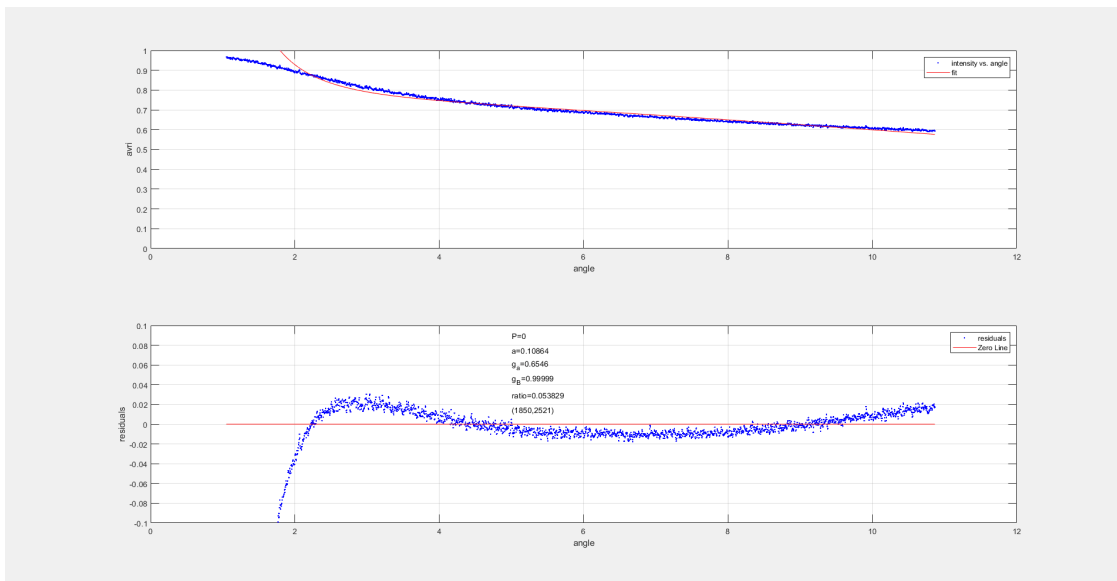


Figure 4.2: Fit and residuals for green laser 532 nm in Sample 1 using ISO 100 and ET 1/1600.

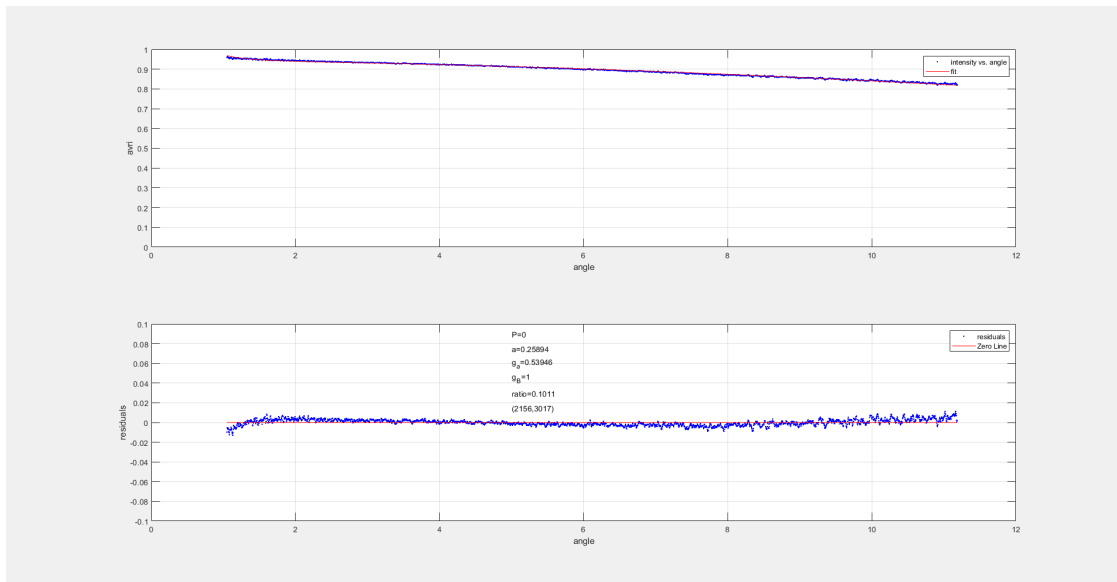


Figure 4.3: Fit and residuals for red laser 670 nm in Sample 1 using ISO 100 and ET 1/160.

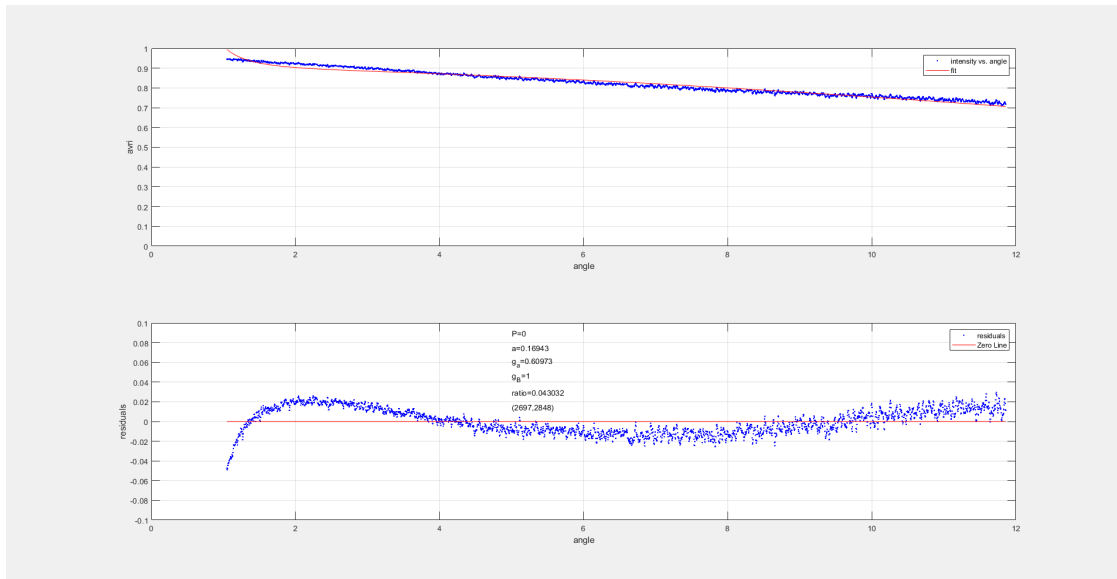


Figure 4.4: Fit and residuals for HeNe laser 632 nm in Sample 1 using ISO 100 and ET 1/125.

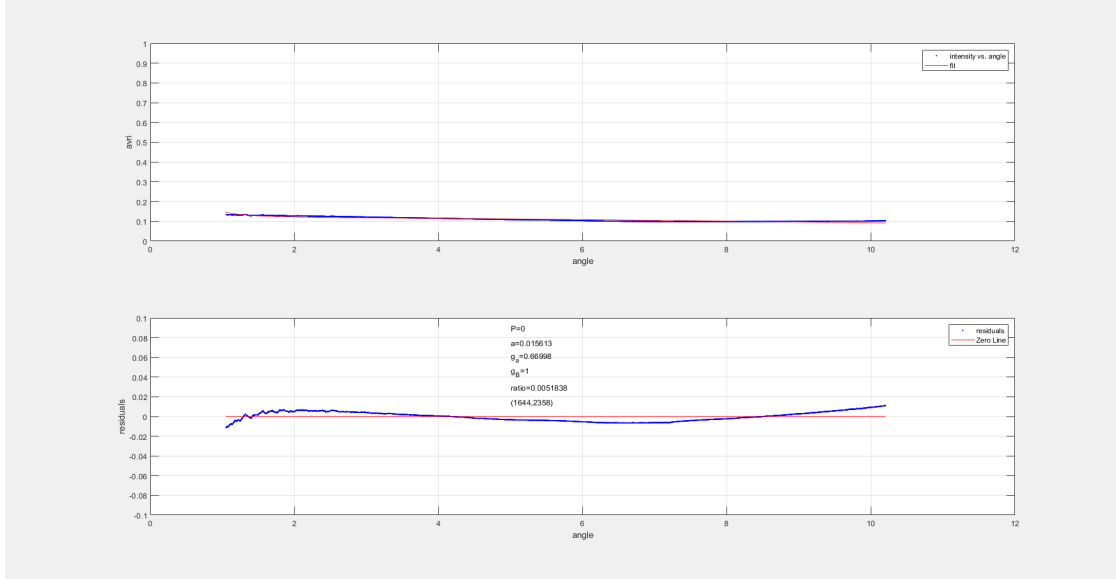


Figure 4.5: Fit and residuals for NIR beam **808 nm** in Sample 1 using ISO 100 and ET 0.6.

	HeNe 1/125		Red 1/160		Green 1/1600		Violet 1/100		NIR 0.6	
PG	0,098	0,902	0,260	0,740	0,137	0,863	1,000	0,000	0,014	0,986
Mean g	0,702	1,000	0,542	1,000	0,598	1,000	0,086	0,913	0,695	1,000
RSC	7,423	7,423	6,677	6,677	4,596	4,596	16,206	16,206	12,080	12,080
SC	85,103	4,95E+12	28,516	1,28E+14	27,572	4,41E+13	18,158	1,56E+04	115,250	8,40E+13

Table 4.1: Average data obtained for each wavelength using Sample 1, SC and RSC are presented in [cm^{-1}].

It is possible to see from the graphics that the best fits correspond to the higher wavelengths (**632, 670 and 808 nm**), more specifically the best one being the **670 nm** or red laser.

The green (**532 nm**) laser starts off with a high intensity and decreases relatively rapid while the angle increases. Meanwhile, the violet (**408 nm**) laser has a medium intensity that stays almost the same throughout the angle increase. This situation can also be observed in the pictures corresponding to the fits:

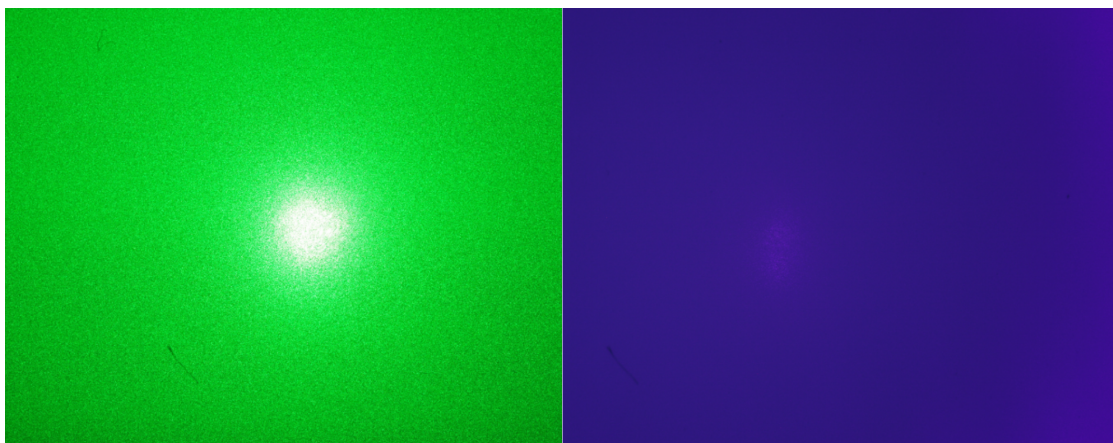


Figure 4.6: Images corresponding to Green and Violet wavelengths using Sample 1.

Where it is appreciable that the light is much more uniformly scattered in the sample when using the **408 nm** violet light beam. This can also be confirmed in Table 4.1, where the mean g value for the violet beam is much more closer to **0 (uniform or isotropic scattering)** than for the green or even for any other one.

On the other side, the decrease in intensity for the red and HeNe lasers is very similar and moderate, but the fitting curve adjusts much better the red measured values than the HeNe ones. Regarding the NIR light, as it is with the violet light, the decrease is very small and it is also clear in the pictures of this set up that there is no huge difference in the intensities as the angle increases:

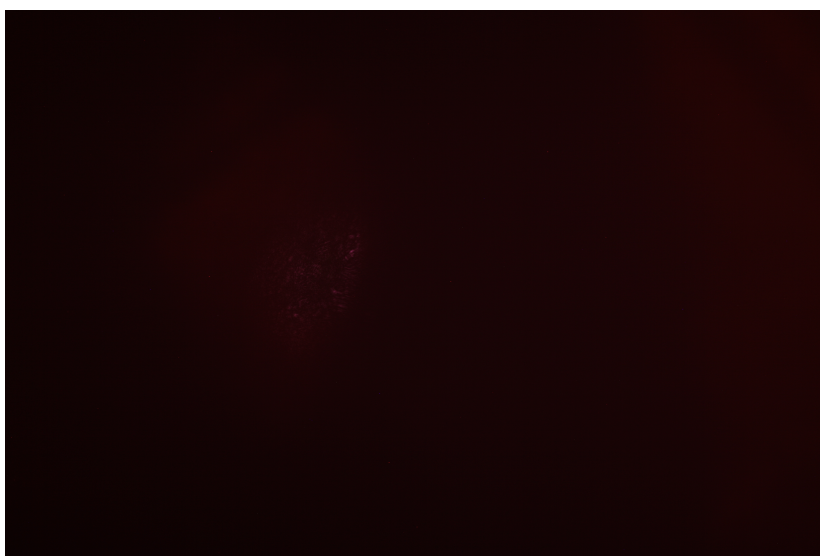


Figure 4.7: Image corresponding to NIR light source in Sample 1.

However, Table 4.1 shows a mean anisotropy factor for the NIR light much bigger than the one for the violet light and even bigger than most of the other beams. The anisotropy factor in this case does not represent an almost uniform scattering as it does with the violet light, but it can also be observed from Figure 4.5 that the initial intensity for the NIR is already very small compared to all the others initial intensities, so it makes sense that the decrease in it is also very small. One of the factors that may have contributed to this behavior is the fact that the NIR source was produced by using safety goggles as a filter to a green laser pointer, so the power of the NIR beam is already reduced when it reaches the sample. Despite this, the curve shows a good fit for this case.

Sample 2

Figures 4.8 to 4.12 show the results for each wavelength used in Sample 2.

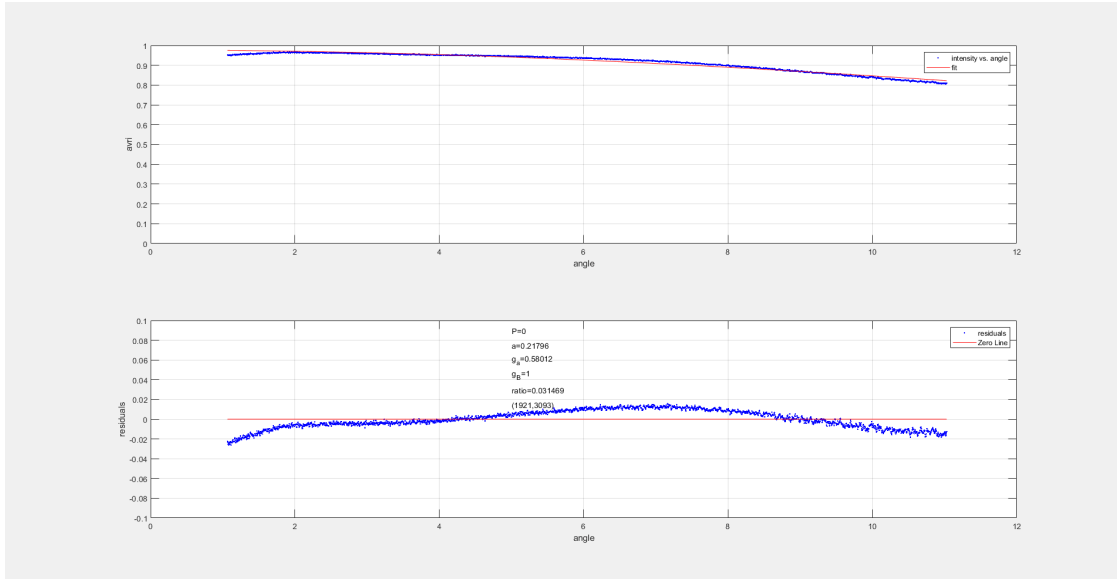


Figure 4.8: Fit and residuals for violet laser 408 nm in Sample 2 using ISO 100 and ET 1/320.

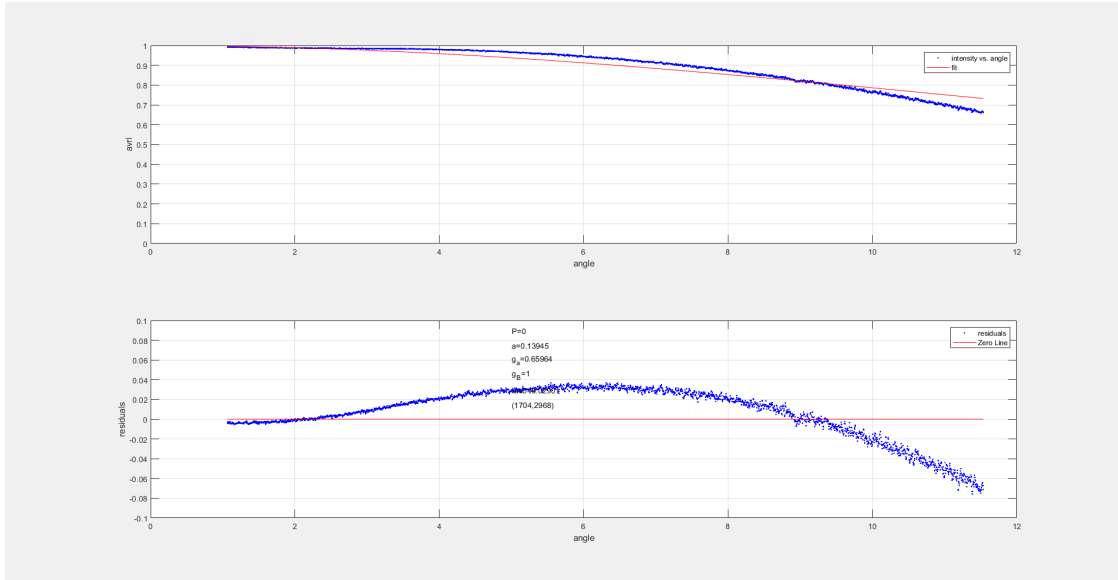


Figure 4.9: Fit and residuals for green laser **532 nm.** in Sample 2 using ISO 100 and ET 1/4000

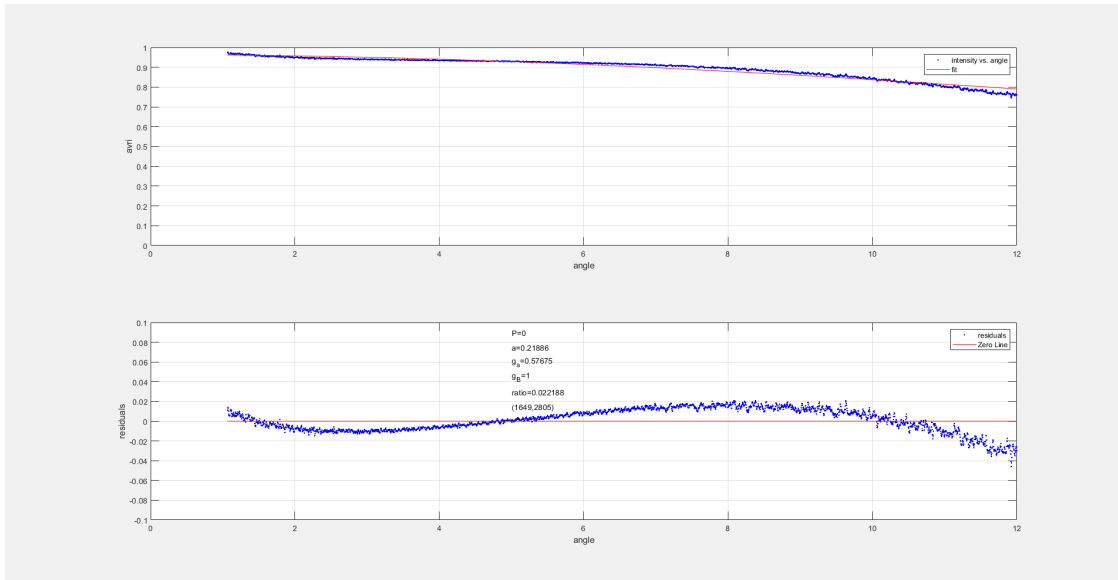


Figure 4.10: Fit and residuals for red laser **670 nm** in Sample 2 using ISO 100 and ET 1/640.

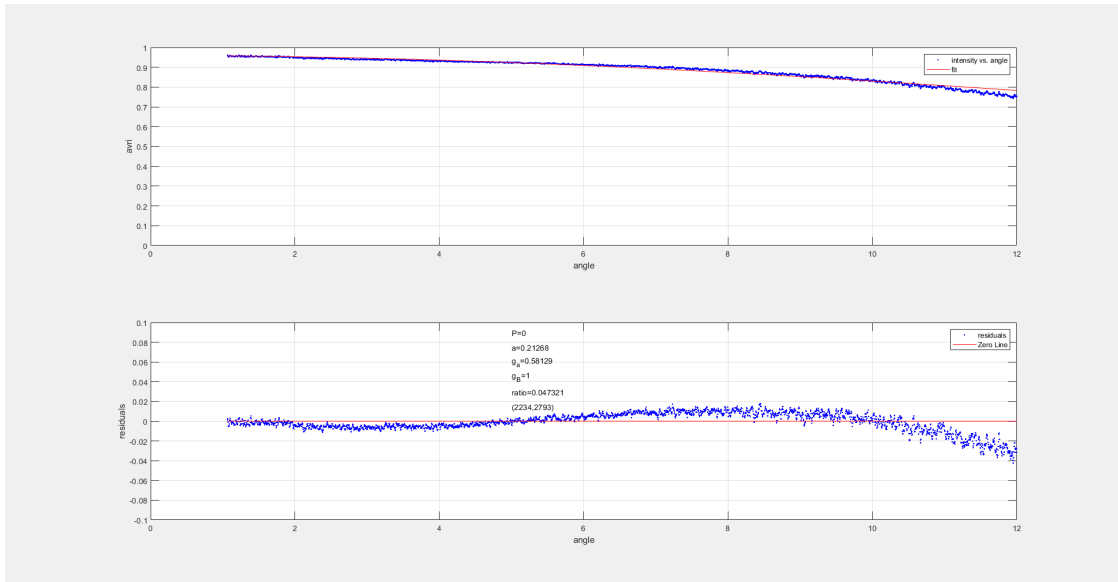


Figure 4.11: Fit and residuals for HeNe laser **632 nm** in Sample 2 using ISO 100 and ET 1/160.

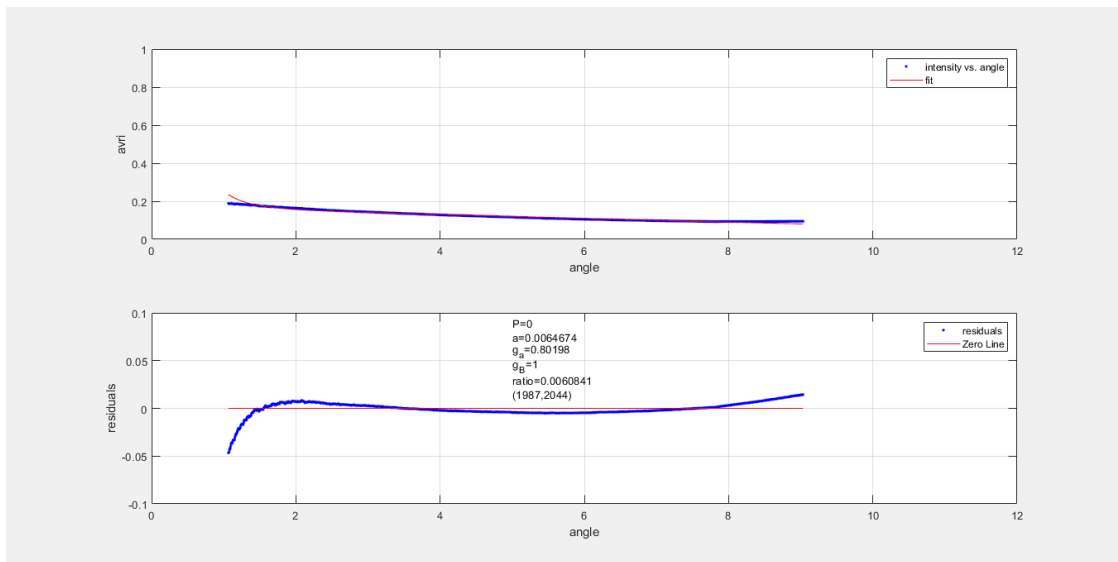


Figure 4.12: Fit and residuals for NIR beam **808 nm** in Sample 2 using ISO 100 and ET 0.6.

	HeNe 1/160		Red 1/640		Green 1/4000		Violet 1/320		NIR 0.6	
PG	0,312	0,688	0,116	0,884	0,427	0,573	0,173	0,827	0,006	0,994
Mean g	0,512	1,000	0,687	1,000	0,483	1,000	0,613	1,000	0,801	1,000
RSC	7,327	7,327	7,230	7,230	5,249	5,249	13,766	13,766	19,064	19,064
SC	27,656	1,57E+14	72,100	6,55E+04	24,662	6,55E+04	82,145	5,74E+14	439,653	6,90E+13

Table 4.2: Average data obtained for each wavelength using Sample 2, SC and RSC are presented in $[cm^{-1}]$.

Sample 2 results reveal that using the violet beam the decrease in light intensity is also very small, but not as much as it is for Sample 1 and the fit is relatively good. The anisotropy factor in this case is much bigger than for the first case and the pictures are coherent with this behavior, since they show a very noticeable peak intensity which starts decreasing with the angle and is not as uniform as with Sample 1, as Figure 4.15 shows:

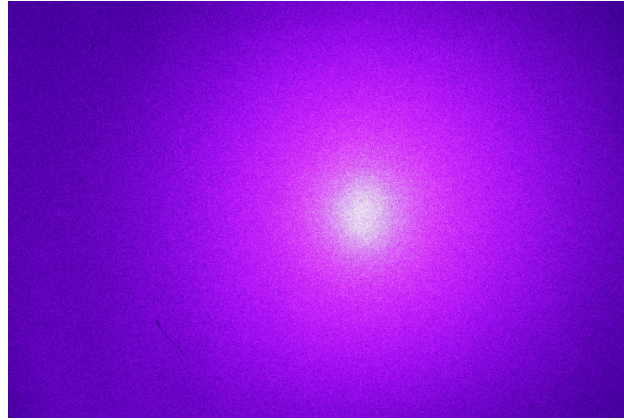


Figure 4.13: Image corresponding to Violet light source in Sample 2.

On the other side, the data for green light suggests that light intensity continues to decrease rapidly and in this situation the curve does not seem to fit very good. For this case, it was difficult to reduce the high brightness of the light in the sample even with small values of ISO and ET, as shown in Figure 4.15:



Figure 4.14: Image corresponding to Green light source in Sample 2.

The decrease in intensity for red and HeNe light continues to behave moderately and the fit for these two cases are the best ones overall, and very similar to one another. In the NIR case, the initial light intensity continues to be very small and its decrease too. Even so, it is slightly bigger than the case using Sample 1 and it can be easily observed in the pictures, where the intensity peak is more visible than in the first case.

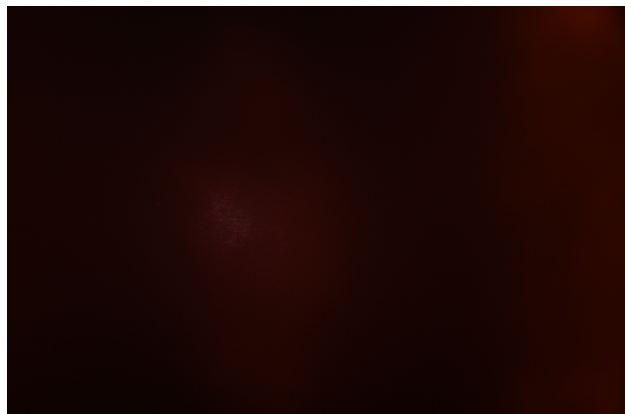


Figure 4.15: Image corresponding to NIR light source in Sample 2.

For all the cases using Sample 2 the anisotropy factors indicate essentially forward scattering.

Sample 3

Figures 4.16 to 4.20 show the results for each wavelength used in Sample 3.

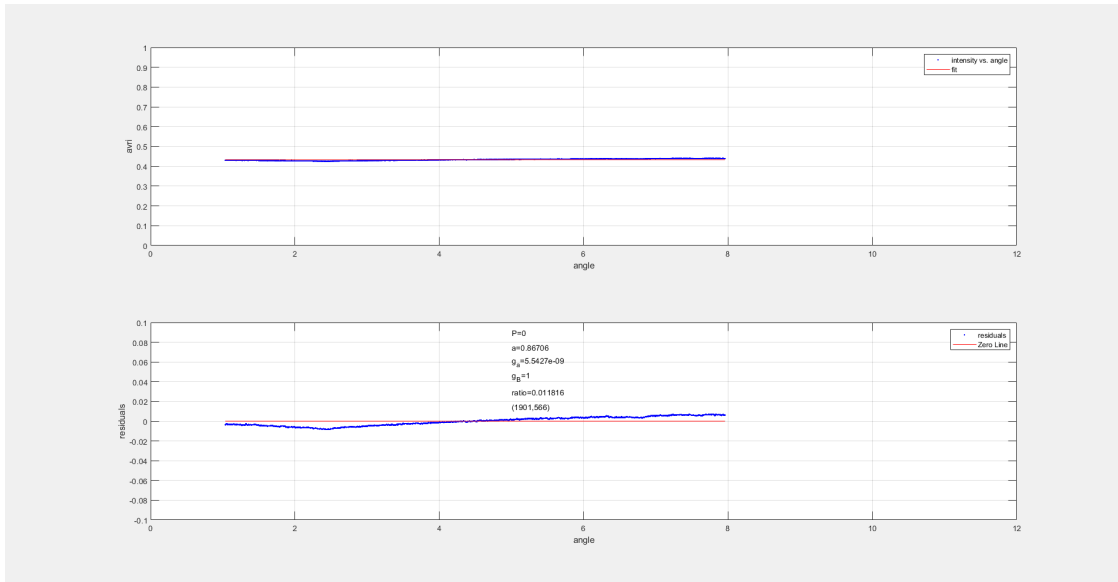


Figure 4.16: Fit and residuals for violet laser **408 nm** in Sample 3 using ISO 100 and ET 1/100.

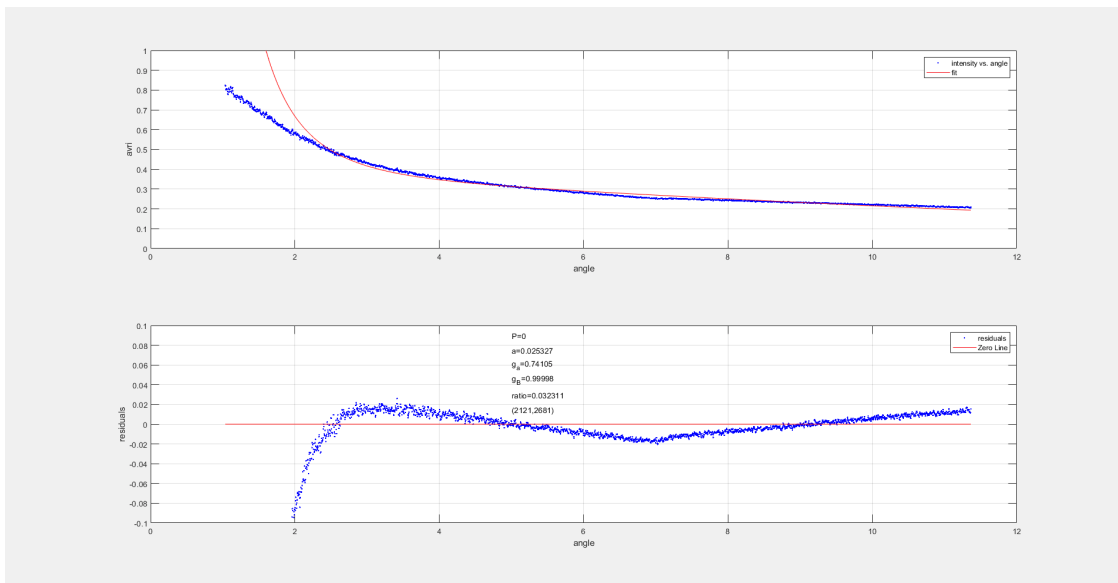


Figure 4.17: Fit and residuals for green laser **532 nm.** in Sample 3 using ISO 100 and ET 1/4000

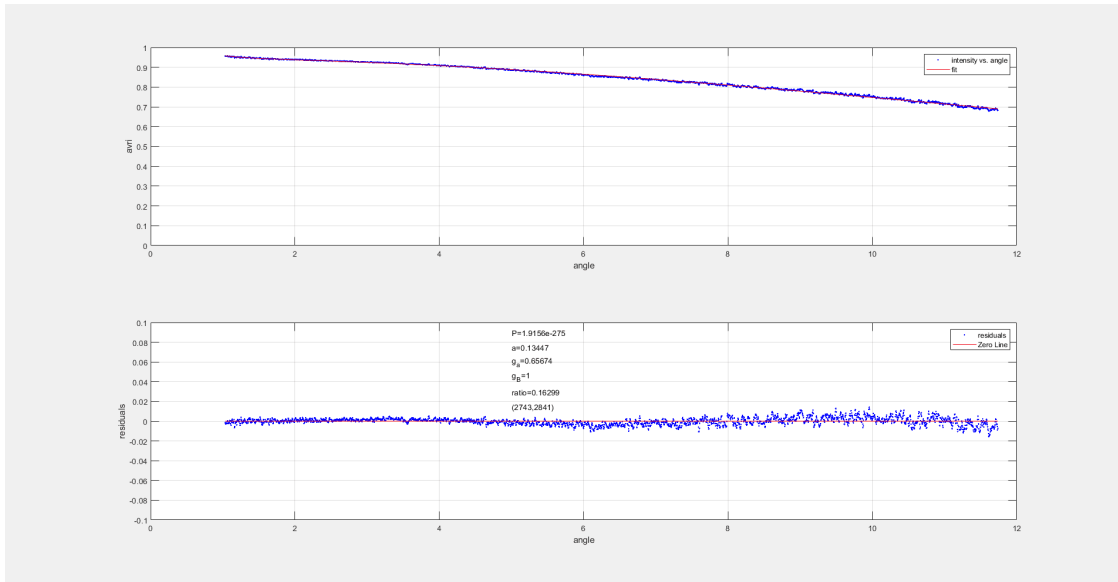


Figure 4.18: Fit and residuals for red laser **670 nm** in Sample 3 using ISO 100 and ET 1/160.

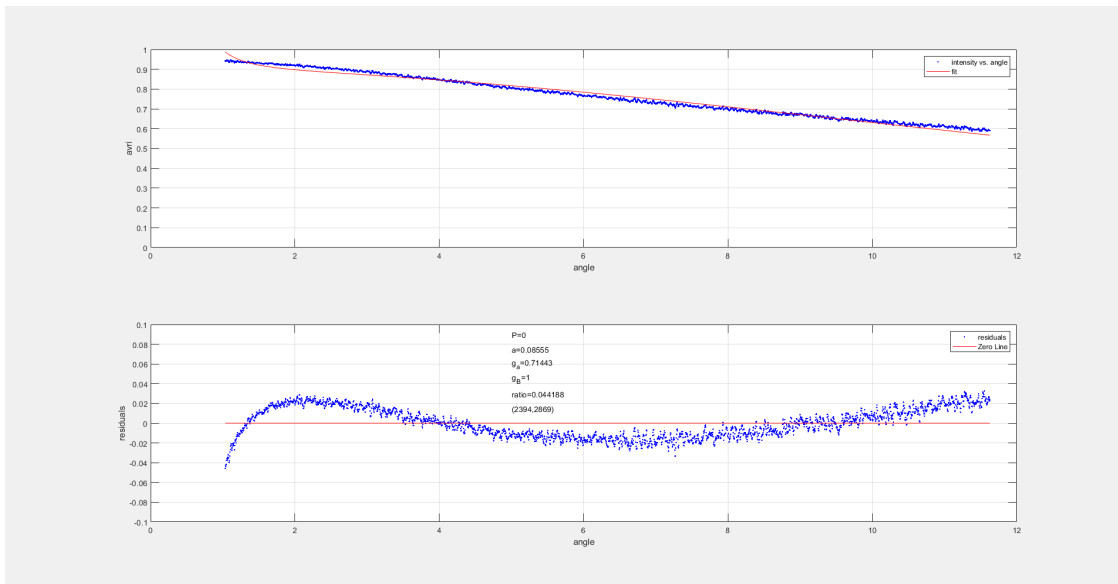


Figure 4.19: Fit and residuals for HeNe laser **632 nm** in Sample 3 using ISO 100 and ET 1/160.

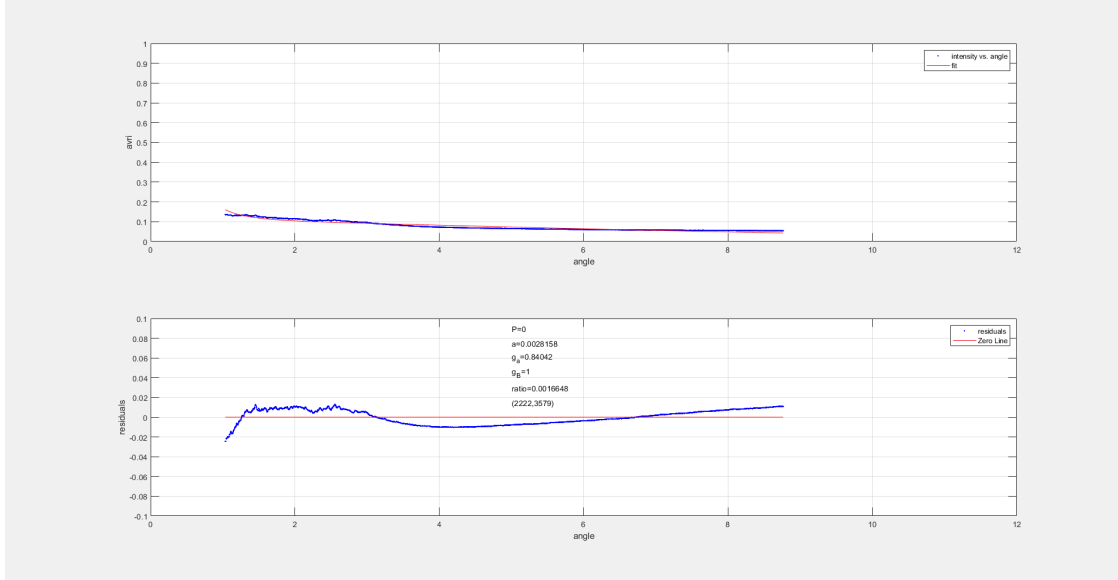


Figure 4.20: Fit and residuals for NIR beam 808 nm in Sample 3 using ISO 200 and ET 0.6.

	HeNe 1/160		Red 1/160		Green 1/4000		Violet 1/100		NIR 0.6	
PG	0,078	0,922	0,184	0,816	0,030	0,970	0,696	0,304	0,003	0,997
Mean g	0,725	1,000	0,608	1,000	0,708	0,998	0,156	1,000	0,707	1,000
RSC	10,427	10,427	7,811	7,811	4,599	4,599	21,470	21,470	13,282	13,282
SC	123,490	2,34E+13	46,603	3,75E+14	48,920	1,03E+10	32,295	3,29E+14	137,659	2,92E+12

Table 4.3: Average data obtained for each wavelength using Sample 3, SC and RSC are presented in $[cm^{-1}]$.

The intensity decrease with violet light in Sample 3 is very small and almost null, which is coherent with the anisotropy factor obtained for this case (**0,156**), it being much smaller than the anisotropy factor for all the other lasers and indicating a tendency of uniform scattering. This behavior is also shown in the pictures acquired for this set up:



Figure 4.21: Image corresponding to violet light source in Sample 3.

The decrease for green light continues to be very rapid and, in this case, even more than with the other samples. The model creates a good fit when the decrease starts to slow down. As for red and HeNe lasers, the fit is well behaved and the decreases are very moderate, being the red fit the best one overall. A picture of the red scattered light is shown in Figure 4.22:

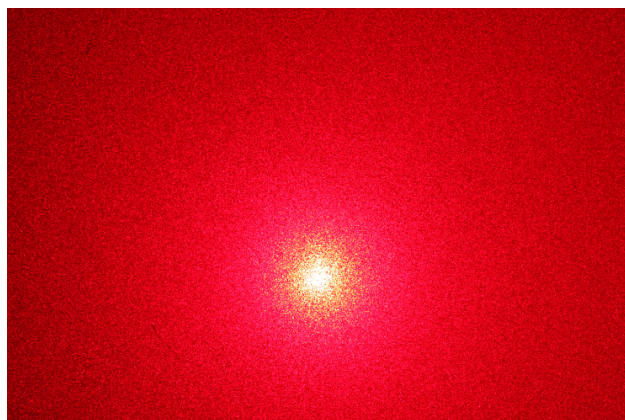


Figure 4.22: Image corresponding to red light source in Sample 3.

For NIR, the behavior shown in the first two cases continues to replicate: both the initial light intensity and the decrease are very small, but the anisotropy factor does not indicate an uniform scattering. The anisotropy factors for all of the lasers mostly indicate forward scattering, except for the case with the violet beam that, even though it is bigger than zero, it's still very small and shows a tendency for uniform scattering.

The RSC in all the cases lies between reasonable values if we take into consideration measured values in real human tissue (see Table 5.1). However, we cannot longer say

that all of these measured values of the phantoms correspond to specifically skin tissue mimicking and this may be due to variations on their optical properties throughout the years. Comparing the Reduced Scattering Coefficient in all the three samples it follows that:

- Sample 1: Violet > NIR > HeNe > Red > Green
- Sample 2: NIR > Violet > HeNe > Red > Green
- Sample 3: Violet > NIR > HeNe > Red > Green

From where we can observe that the three samples show a similar behavior regarding the scattering properties when illuminated with different laser wavelengths, being Sample 2 the only one that differs with the highest RSC, but still very similar to the others. In all of the cases it was also common that red light was the one best behaved regarding the moderate light decrease as the angle increases and showing the most adequate fit. Nevertheless, the fact that the NIR light has a high RSC in all of the samples does not correspond to the evolving literature around optical imaging since tissue is relatively transparent within this range. This may be due to an absorption effects of the Agar and variations of the optical characteristics of the phantoms, but possible causes of this phenomenon are being explored since this is the first time the method and the phantoms are tested with NIR light.

Still, considering the results for the scattering characteristics obtained compared to real measured values, and the residuals plotted for each graphic of the measured data and the predicted by the Henyey-Greenstein model, it is pertinent to say that the fitting method is adequate for the studied cases and the proposed procedure to collect the data is very efficient.

Chapter 5

Conclusions

This research presents a successful reproduction of the novel method to characterize tissue phantoms using a DSLR camera as an imaging sensor [3]. Three long livid tissue phantoms made from Intralipid infused Agar and encapsulated in PMMA layers were illuminated with five lasers at different wavelengths ranging from 532 nm to 808 nm, to study the propagation of light in these turbid media by calculating scattering and anisotropy coefficients of the phantoms.

The method with which the measurements were carried out allowed to collect a large amount of data in a very accessible way during a short period of time, taking into consideration possible human error and noise effects and providing good mechanisms to minimize these effects as much as possible. For the fitting and calculation of the coefficients, the Henyey-Greenstein model worked efficiently to predict the behavior of light intensity due to scattering effects through turbid media. Furthermore, the MATLAB code allowed to perform this fitting in a very rapid and competent way, highly reducing possible calculation errors through the automated and iterative process of the regression analysis or least squares method.

It was notable from the images captured and the data calculated that Violet light (**408 nm**) had the highest and most uniform scattering in two of the samples, while in the third one this corresponded to NIR light (**808 nm**). This variation may be due to the different concentrations of Intralipid and Agar on the samples and the scattering and absorption properties they had when interacting with each of the wavelengths. Since it is the first time a NIR source is tested with the phantoms using this novel method, it remains to explore more about this type of light propagation and to improve possible error sources such as the great decrease in light intensity when using the safety goggles to obtain NIR light, and so on. However, the results showed reasonable data compared to measured values in real

human tissue and from this it can be said that the method was effective.

In addition to the easy reproducibility of the method, it allowed measurements using a range of wavelengths from UV to NIR and the ease of handling of the phantoms could let them to be arranged in different configurations of experimental setups by varying, for example, the angle of incidence of the light to the sample, the distances between the optical components, the compositions of these phantoms, and many other parameters.

Regarding the tissue phantoms, it was found that some of their coefficients have varied over time and in some samples, inhomogeneities and air bubbles have appeared between the layers of plastic making it necessary to create marks at the specific points of the sample where the measurements are to be taken to guarantee that the propagation of light in each case is in a medium of equal conditions. However, it is important to highlight that these phantoms were created around ten years ago and still allow obtaining coefficients that, when compared to measurements made on real biological tissue, lead to the conclusion that said phantoms continue to effectively recreate the optical properties of tissue.

Since the presented method was proposed by Dr. Chapman's group [3] and has been tested repeatedly, future work related to this project includes conducting a detailed comparison between the data obtained in this occasion and the data obtained in past years regarding the variations of scattering coefficients and analyze possible causes that can be taken into account when elaborating tissue phantoms with the technique presented [1], considering the improvements done in both the optical set up and the image processing method throughout the years.

Furthermore, the fact that this was the first time the method was used to measure scattering characteristics on tissue phantoms using NIR light emerges the key perspective to develop and improve the optical set up proposed to measure with this source and analyze the possible causes for the results obtained.

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Appendix

Tissue	λ (nm)	μ_a (cm^{-1})	μ'_s (cm^{-1})
Bladder	532	0.27–0.71	1.28–3.30
	630	0.28–0.76	2.5–6.37
Bone	650	0.09–0.14	12.5–15.8
	420	0.01–3.51	18.75–55.83
Brain	532	0.02–3.84	0.10–46.3
	630	0.02–0.50	3.72–21.97
	780	0.078–0.089	8.42–9.16
Breast	660	0.037–0.110	11.4–13.5
	760	0.031–0.10	8.3–12.0
	530	0.60–0.86	28.0–32.1
Tumor	690	0.070–0.10	14.7–17.3
	895	0.068–0.102	12.4–13.1
Heart	630	0.03–1.55	17.56–75.06
	661	0.12–0.18	5.22–90.80
Liver	630	1.15–1.56	21.6–30.4
Lung	630	0.16–1.36	1.07–83.81
	661	0.49–0.88	21.14–22.52
	630	0.05–1.0	3.41–17.02
Prostate	672	0.09–0.72	7.1–25.0
	762	0.11–1.6	1.2–40.0
Skin	630	0.05–1.11	2.26–20.95
	661	0.51–0.64	2.24–5.77
	800	0.16–0.23	6.80–9.84

Table 5.1: Spectra of human tissues in vivo for μ_a and μ'_s as a function of wavelength measured using different spectroscopy methods [4].