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INSTITUTO DE FÍSICA DE SÃO CARLOS**

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**Theoretical evaluation of the light distribution and PDT
dose for a multi-wavelength light source**

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**Theoretical evaluation of the light distribution and PDT
dose for a multi-wavelength light source**

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Advisor: Prof. Dr. Sebastião Pratavieira

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*To Brazilian citizens,
whose taxes support the public University.*

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“Sometimes it’s more important to be persistent than a genius”

Mat. German Rojas - EPN

ABSTRACT

SANCHEZ GUACHAMIN, V. J. **Theoretical evaluation of the light distribution and PDT dose for a multi-wavelength light source.** 2019. 107p. Dissertation (Master in Science) - Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, 2019.

The broad absorption spectrum of Protoporphyrin IX (PpIX) allows being activated by a source with an emission spectrum in the visible region. Also, PpIX can be activated simultaneously by two or more sources whose emission spectrum overlaps with its absorption spectrum. Sources with a wide spectrum of illumination, such as lamps and Sun, have a different light-tissue interaction, and the constant monitoring of the dose of light and the total damage caused by photodynamic therapy (PDT) becomes difficult. The main objectives of this study were to simulate the distribution of light in the tissue for a multi-wavelength source and determine the total theoretical photodynamic dose. This study is composed of three parts. First, photobleaching experiments of PpIX using different light sources and the development of a mathematical model was used to explain the change in PpIX concentration. Second, Monte Carlo simulation using MCX (Monte Carlo eXtreme) was performed to know the light distribution through in a human skin model. Finally, the theoretical photodynamic dose was determined using the two steps mentioned previously. The experimental results show that the decrease in the concentration of PpIX is mainly dependent on the dose of photons absorbed. Therefore, for a multi-wavelength source, the total damage is calculated by partial damages caused by each wavelength that active the PpIX. The simulation of the light distribution in the human skin phantom demonstrated that the energy fluence rate decrease as a function of the depth. The mathematical model estimates that the efficacy of PDT, where it is guaranteed there is necrosis, has a diameter and depth of about 0.3 and 0.2 mm respectively. This model can be expanded to other biological media, other photosensitizers and even to any source of illumination.

Keywords: Photodynamic Therapy. PpIX. Multi-wavelength source. Mathematical modeling. Photodynamic dose.

RESUMO

SANCHEZ GUACHAMIN, V. J. **Avaliação teórica da distribuição de luz e dose da TFD para uma fonte de luz com múltiplos comprimentos de onda.** 2019. 107p. Dissertação (Mestrado em Ciências) - Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, 2019.

O amplo espectro de absorção da Protoporfirina IX (PpIX) permite sua ativação por uma fonte com um espectro de emissão amplo na região visível. Além disso, a PpIX pode ser ativada simultaneamente por duas ou mais fontes de luz, cujo espectro de emissão se sobrepõe ao seu espectro de absorção. Fontes com um amplo espectro de emissão, como lâmpadas e Sol, têm uma interação luz-tecido biológico diferente, e o monitoramento constante da dose de luz e o dano total causado pela Terapia Fotodinâmica (TFD) se tornam difíceis. Os principais objetivos deste trabalho foram simular a distribuição de luz no tecido para uma fonte de múltiplos comprimentos de onda e determinar a dose fotodinâmica teórica total. Este trabalho é composto de três partes. Primeiro, experimentos de fotodegradação da PpIX usando diferentes fontes de luz e o desenvolvimento de um modelo matemático foram usados para explicar a mudança na concentração de PpIX. Em segundo lugar, uma simulação de Monte Carlo usando MCX (Monte Carlo eXtreme) foi realizada para obter a distribuição de luz, de múltiplos comprimentos de onda, em um modelo de pele humana. Finalmente, a dose fotodinâmica teórica foi determinada usando as duas etapas mencionadas anteriormente. Os resultados experimentais mostram que a diminuição na concentração de PpIX é principalmente dependente da dose de fótons absorvidos. Portanto, para uma fonte de multi-comprimentos de onda, o dano total é calculado por danos parciais causados por cada comprimento de onda que ativa a PpIX. A simulação da distribuição de luz na pele humana demonstrou que a taxa de fluência de energia diminui em função da profundidade. O modelo matemático estima que a eficácia da TFD, onde é garantida a existência de necrose, tenha diâmetro e profundidade de aproximadamente 0.3 e 0.2 mm respectivamente. Este modelo pode ser expandido para outros meios biológicos, outros fotossensibilizadores e até mesmo para qualquer fonte de iluminação.

Palavras-chave: Terapia Fotodinâmica. PpIX. Fonte de multi-comprimentos de onda. Modelo matemático. Dose fotodinâmica

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LIST OF ABBREVIATIONS AND ACRONYMS

ALA	Acid δ -aminolevulinic
CFL	Compact fluorescent lamp
DMSO	Dimethyl sulfoxide
Dose 1O_2	Singlet oxygen doses
EMW	Electromagnetic wave
GPU	Graphics Processing Units
LASER	Light amplification by stimulated emission of radiation
LED	Light emitting diode
MCX	Monte Carlo eXtreme
PDT	Photodynamic Therapy
PDT dose	Dose in photodynamic therapy
PpIX	Protoporphyrin IX
PS	Photosensitizer

LIST OF SYMBOLS

μ_a	Absorption coefficient
g	Anisotropy term
1O_2	Excited state singlet to oxygen
S_0	Ground state singlet to PS
3O_2	Ground state triplet to oxygen
μ'_s	Reduced scattering coefficient
η	Refractive index
μ_s	Scattering coefficient

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1 INTRODUCTION

The photodynamic therapy (PDT) is a medical procedure for the treatment of several diseases of the skin, mainly non-melanoma skin cancer. PDT also can be used in other tissues/organs and to inactivate microorganisms.¹⁻⁴ This technique principle of operation is based on the combination of light, a photosensitizing agent (PS) and molecular oxygen (O_2), this combination produces damage in target cells.⁴ The death of the cell can be by necrosis or apoptosis. PDT is a highly selective process since only cells that have absorbed PS are affected. For example, the tumor cells have accelerated metabolism, this process facilitates the accumulation of PS within them.³⁻⁵

Generally, light sources used on PDT are monochromatic sources as LASER (Light amplification by stimulated emission of radiation) or LED (Light emitting diode) type.⁶ The interaction of light with the biological medium will depend on several factors, such as wavelength, irradiance, irradiation time, irradiation beam size, optical and thermal properties of the tissue.⁶⁻⁸ Regarding the optical properties, when light interacts with the tissue can be reflected, refracted, dispersed, absorbed and transmitted, these properties are characteristic of each medium. Biological media are highly scattering of light. Therefore, biological media are treated as turbid media. The skin has three main layers, epidermis, dermis and subcutaneous tissue, each of these layers are constituted by sub-layers.⁹

PS molecules are distributed spatially in the layers of the skin. PS absorb a fraction of photons that get into tissue. The absorbed photon produce to change in the electronic energy level of PS and consequently, there will be photochemical reactions.⁸ If the medium has molecular oxygen there is a probability that PS molecules in excited state are deactivated by quenching, i.e., the PS in excited triplet state transfer energy to a ground state of oxygen. The oxygen uses the energy to generate an excited electronic state known as singlet oxygen. The singlet oxygen is highly reactive (cytotoxic) the interaction with biological molecules results in the death of the cell. Also, there is a probability that singlet oxygen interacts with PS molecules. If the medium has molecular oxygen but low concentrations, the PS in the excited triplet in addition to performing quenching, it also interacts with neighbor PS molecules.¹⁰ For any one of the possible photochemical reactions, the PS concentration decreases and increases the formation of new species called photoproduct.¹¹

There is a variety of research related to clinical cases where the efficiency of PDT has been demonstrated. Moreover, there are researches *in vitro* and *in vivo* for use of new PS and light sources.¹²⁻¹⁵ Nowadays, theoretical and simulation researches have been developed to estimate the efficiency of PDT.¹⁶⁻¹⁸

In this study, the concept of the photon dose is addressed. We assumed that the total photodynamic effect is the sum of fractionated effects. That means, for the same photon dose, the total effect of PDT with a multi-wavelength source is equal to sum photodynamic effects caused by each wavelength that active the PS. To check this assumption, $1 \times 10^{19} [Photons/cm^3]$ was the photon dose and, photobleaching experiments were developed for the protoporphyrin IX (PpIX) by the illumination of three sources, blue, red and combination blue, and red. The experimental results check the supposition. Also, a mathematical model based on electronic transitions between the different electronic levels of PpIX was proposed, this model explains the temporal change in PpIX concentration. On the other hand, a 3D computerized phantom was developed to simulate the light distribution, in this phantom, the normal skin optical properties of epidermis and dermis were considered. The Monte Carlo eXtreme software was used to simulate the photon migration for a multi-wavelength source. Finally, with the mathematical model and light distribution, the PDT dose was evaluated. In addition, the cell survival fraction was evaluated considering $300 \mu M$ as the threshold of PDT.

1.1 Photophysics and photochemistry of photodynamic therapy

1.1.1 Light

The electromagnetic waves (EMW) transport energy and include radio waves, microwaves, light waves (infrared, visible and ultraviolet), x-rays and gamma-rays (Figure 1). The EMW are characterized by the range of wavelengths, frequencies and energy. The ranges of visible light go from 380 nm to 780 nm (Figure 2 (a)). The EMW can be created by the oscillation of an electric and magnetic fields, black body emission or luminescence process and propagates with a speed of light, in the vacuum the speed of light is $c = 3 \times 10^8 m s^{-1}$.⁵

Classically, the magnetic and electric fields are perpendicular at the direction of the propagation of EMW (Figure 2 (b)). The flux of energy density is represented by the Poynting vector ($\vec{S}$).

$$\vec{S} = \vec{E} \times \vec{H} \quad (1.1)$$

$$= \frac{1}{\mu} \vec{E} \times \vec{B} \quad (1.2)$$

where $\vec{E}$ is the electric field, $\vec{H}$ is the intensity of the magnetic field, $\vec{B}$ is the magnetic induction field and μ is the magnetic permeability of the medium. The $\vec{S}$ represents the energy density of a beam passing through an arbitrary surface per unit of time. The dimensions of the Poynting vector are $J m^{-2} s^{-1}$ or $W m^{-2}$. The module of $\vec{S}$ is known as irradiance.

In quantum mechanics, the particles associated with EMW are the photons. The photons are the carriers of energy. The Einstein relation for photon energy predicts that

Figure 1 – Electromagnetic spectrum for different EMW and relation with wavelength, frequency and energy.

Source: Adapted of MATTSON et. al.¹⁹

	Colour	Interval (nm)
	Red	618 - 780
	Orange	581 - 618
	Yellow	570 - 581
	Green	497 - 570
	Cyan	476 - 497
	Blue	427 - 476
	Violet	380 - 427

(a)

(b)

Figure 2 – (a) Intervals for visible spectrum. (b) The relation between the direction of the propagation of EMW with the electric and magnetic field.

Source: By the author.

energy is proportional at the frequency and inversely proportional at the wavelength of the photon, mathematically expressed as:

$$E_{\text{photon}} = h\nu = \frac{hc}{\lambda} \quad (1.3)$$

where, h is the Planck constant, ν , and λ are the frequency and wavelength respectively. Photons can be generated by the transitions of atoms or molecules from an excited state (high energy) to the ground state (minimum energy). Also, the energy of a photon can activate atoms and molecules.

Nowadays, the traditionally light sources for PDT are LASERs and LEDs, other alternatives sources can be sunlight and lamps.²⁰ The choice of a light source depends on the PS and the characteristic of the medical treatment.²¹

1.1.2 Protoporphyrin IX

The acid δ -aminolevulinic (ALA) is widely used in clinical applications in PDT. ALA induces inside the cells the production of endogenous porphyrins, mainly PpIX.⁵ The PpIX is the PS molecule i.e, absorbs the energy of photons incident and produces a change in the energy electronic level. The spectrum of absorption and emission of PpIX is described in figure 3 (b). Figure 3 (a) shows the ring formed by hydrogen and nitrogen atoms, it's characteristic of the porphyrins.

Figure 3 – (a) Characteristic ring of porphyrins formed by hydrogen and nitrogen atoms. (b) Absorbance and fluorescence spectrum to PpIX. The black line is the absorbance spectrum and red line is the emission of fluorescence spectrum.

Source: Adapted of ROLLAKANTI et al.²²

The absorption spectrum of PpIX is described as a strongly absorbent in the violet-blue region, this region is known as the Soret band. The next absorption region is composed of four absorption bands, called Q bands. The origin of absorption bands is related to molecular geometry. On the other hand, the emission or fluorescence spectrum has only two bands.

The ground state to PpIX is a singlet state (S_0), the excited states are singlet and triplet. For the incident photon with energies formed at wavelength violet-blue region, the PpIX molecule absorb the photon and generate the Soret band, where electronic transitions of S_0 to S_2 take place, here S_2 is an excited singlet state. If the energy of the photon is over the violet-blue region and less of infrared, the Q-bands are formed by electronic transitions of S_0 to S_1 , S_1 has less energy than S_2 , S_1 is an excited singlet state.

The formation of excited state S_1 and S_2 produce photochemical process by interaction with other molecules. In PDT is requested that the PpIX interacts with

molecular oxygen.

1.1.3 Oxygen

The basic state of oxygen or molecular oxygen is a triplet state, ${}^3\Sigma_g^-$. As shown the Figure 4, by molecular orbital theory, the excited state has two singlet state, ${}^1\Sigma_g^+$ and ${}^1\Delta_g$. Here, ${}^1\Delta_g$ is the first excited state. The energy level to ${}^1\Delta_g$ and ${}^1\Sigma_g^+$ are respectively 94.29 KJ/mol and 157 KJ/mol.^{4,10} ${}^1\Delta_g$ is more stable of the two excited states. On this work ${}^3\Sigma_g^-$ is the triplet oxygen (3O_2) and, ${}^1\Delta_g$ is the singlet oxygen (1O_2).

Figure 4 – The electrons distribution in molecular orbital to ground and excited state of oxygen

Source: By the author.

In PDT, the 1O_2 is generated by transfer energy from triplet state of PS to 3O_2 . That means that the 1O_2 is generated only if the energy transfer is greater than 94.29 KJ/mol. Furthermore, the 1O_2 is highly cytotoxic, with diffusion constant $1 \times 10^{-5} \text{ cm}^2/\text{s}$ and lifetime $1 \times 10^{-7} \text{ s}$ into cytoplasm.¹⁰

1.2 Optical properties

The optical properties in this work are described by: absorption coefficient, $\mu_a(\lambda)$, scattering coefficient, $\mu_s(\lambda)$, anisotropy parameter, g , and refractive index, $\eta(\lambda)$. Except for g , all other terms are a function of the wavelength.

The absorption and scattering coefficients express respectively the probability that an incident photon into tissue will be absorbed or scattered in an infinitesimal distance, the unit of measure is m^{-1} , although cm^{-1} or mm^{-1} are normally used. The reduced scattering coefficient is defined as $\mu'_s(\lambda) = (1 - g)\mu_s(\lambda)$, with equal units that $\mu_s(\lambda)$. The anisotropy parameter involves the angle with which a photon is deviated after having experienced a dispersion. The refractive index describes how fast light propagates through the material. g and $\eta(\lambda)$ are dimensionless number.^{8–10}

These optical properties were obtained of the literature and were used in the Monte Carlo simulation for light distribution.

1.2.1 Absorbance

The absorbance measures how much the radiant power is attenuated when crossing a material (Figure 5), it is defined mathematically as: ⁷

$$A(\lambda) = \log_{10} \left(\frac{I_0(\lambda)}{I(\lambda)} \right) = -\log_{10} T(\lambda) \quad (1.4)$$

where, $A(\lambda)$ is the absorbance that depend of wavelength λ , $I_0(\lambda)$ and $I(\lambda)$ are respectively the radiant power or radiant energy per unit time received and transmitted, and $T(\lambda)$ is the transmittance as function of λ . The absorption coefficient (μ_a) is related with absorbance by the next equation.

$$A(\lambda) = \int_0^l \mu_a(\lambda, z) dz \quad (1.5)$$

here, l is the thickness of the material. The absorption coefficient depends on λ and depth. If $\mu_a(\lambda, z)$ is uniform along the path, i.e. $\mu_a = \mu_a(\lambda)$, then,

$$A(\lambda) = \mu_a(\lambda) l \quad (1.6)$$

1.2.2 Beer-Lambert law

Figure 5 show to change in the intensity between the radiant power received and transmitted. The decrease in the radiant power is caused as consequence of the optical properties of the medium and of the thickness of the material. Another way to write the

Figure 5 – Schematic representation for Beer-Lambert law. I_0 is the radiant power received and I is the radiant power transmitted. z is the thickness of the material.

Source: By the author.

absorbance is by the next equation

$$A(\lambda) = \epsilon(\lambda) C z \quad (1.7)$$

since,

$$\epsilon(\lambda) = \mu_a(\lambda)/C \quad (1.8)$$

In eq. (1.7), z is the thickness of the material, C is the molar concentration of solution inside of the cuvette, $\epsilon(\lambda)$ is the molar attenuation coefficient as function of λ .

Matching eq. (1.4) with eq. (1.7), the logarithmic term can be converted to an exponential form.

$$I(\lambda) = I_0(\lambda)10^{-\epsilon(\lambda)Cz}, \quad (1.9)$$

or, by eq. (1.8)

$$I(\lambda) = I_0(\lambda)10^{-\mu_a(\lambda)z} \quad (1.10)$$

The last equation is the Beer-Lambert law. This expression describes the change in radiant power, where only the absorption coefficient was considered.

1.3 Monte Carlo method to light distribution

The photon migration in 3D turbid media can be simulated with a good approximation to reality by Monte Carlo simulation. Monte Carlo eXtreme or MCX is a Monte Carlo simulation software, it is one of the faster simulators because uses the massive number of parallel threads of a Graphics Processing Units (GPU), in the Figure 6 can be found a block diagram of how MCX works.

MCX is a free access software.²³ In an article published by Fang et. al. (2009)²⁴ there is more information about MCX. The version of MCX for MATLAB is the MCXLAB. A MEX function include in MCXLAB provides an interface between MATLAB and MCX codes and it can be called directly inside MATLAB.

MCXLAB is the specific Monte Carlo method used in the present dissertation and, it will be discussed with more details in Chapter 3.

Figure 6 – Block diagram for Monte Carlo eXtreme. The main difference is to simulate thousands of photons simultaneously, this is achieved by the use of NVIDIA graphics processing units (GPUs). MCX is one of the fastest and most accurate photon modeling tools.

Source: Adapted of FANG; BOAS.²⁴

1.4 Jablonski diagram for PDT

The next Jablonski diagram (Figure 7) describe electronic and vibrational energy levels for a PS after the absorption of an incident photon, also the energy transfer with molecular oxygen and possible interactions with the biological substrate.

When the PS molecule in the ground state (S_0) absorbs the energy of an incident photon, it goes into an excited state (S_n). The PS in S_n has a short lifetime, thus the PS performs a non-radiative process (vibrational process) to one state of lower energy (S_1), but of higher energy in relation to the state S_0 . In S_1 the PS can interact minimally with the biological substrate, the next process for the PS in S_1 is the return to the ground state by the emission of a fluorescence photon or by a vibrational process. The states S_0 , S_1 and S_n are singlet states, i.e., stays the spin orientation of the electron.

There is a process with less probability of occurrence, which implies a change in the spin orientation of the electron causing the PS to move to an excited triplet state

Figure 7 – Jablonski diagram for PDT. Thick horizontal lines represent electronic states, whereas thin horizontal lines are vibrational states. VR is the vibrational relaxation. IC is internal conversion. ISC is the inter cross-system from S_1 to T . The biological substrate is eliminated by type I (charge transfer) and II (singlet oxygen oxidation) mechanism.

Source: By the author.

(T). The lifetime of T is relatively higher than S_1 , thus allowing to PS in triplet state to undergo chemical reactions with other molecules in the environment. From the state T , the PS molecule can return to S_0 by emission of phosphorescence photon or, by transferring the energy of triplet state to the basic state of oxygen. The basic state of O_2 is in a state triplet (3O_2), in this case, the 3O_2 will use the energy transfer to move to an excited state known particularly as singlet oxygen (1O_2). In this state the oxygen is highly reactive, therefore, it interacts with all the molecules around it.

The biological substrate can be eliminate by charge transfer with PS and direct interaction with singlet oxygen. The first and second processes are the mechanisms type I and II of PDT respectively.

1.5 Mechanisms type I and II

A good PS has a high likelihood to produce a triplet state. The interaction of the triplet state with the ground state of PS, biological substrate and triplet oxygen result in different photochemical reactions.¹⁰ Two mechanisms of action of the PS are recognized.

The photochemical reactions *Type-I* are produced by transfer of electrons (or

protons) for O_2 or biological substrate to form a radical anion or cation, respectively.

where, $[S_0]^{-*}$ and $[S_0]^{+*}$ are respectively anion and cation species of PS. The superoxide radical $[O_2]^{-*}$ is a moderately reactive free radical. After, take place secondaries photochemical reactions. Note that the terms in brackets refer to the concentration of molecules in a given state.

The half-life of O_2^{-*} is 2-4 μs , can not cross biological membranes and is readily dissociated from hydrogen peroxide (H_2O_2). H_2O_2 is moderately reactive, has a long cell half-life (1 ms) and has the ability to diffuse freely through the membranes. The O_2^{-*} can react with (H_2O_2) to form the very reactive hydroxyl radical (OH^{-*}). The OH^{-*} can potentially react with all biological molecules like DNA, proteins, lipids and virtually any cellular component. As cells lack enzymatic mechanisms to eliminate this highly reactive species, their accumulation can lead to cell death.

The second photochemical reaction is called *Type-II*, here the state T transfers energy to 3O_2 .

The reactive oxygen species (ROS) found inside the cells are O_2^{-*} , H_2O_2 , OH^{-*} and 1O_2 . The reaction cytotoxic or oxidation of substrate is caused by ROS and $Substrate^{+*}$.

In an environment with high oxygen concentration, the main cytotoxic agent is the 1O_2 . However, the type I and II mechanisms occur simultaneously.²⁵ It's logical to think that in low concentrations of O_2 , the type I mechanism is the dominant, with little probability to produce 1O_2 . For the opposite case, high concentration of O_2 , the dominant mechanism will be type II.²⁶ Moreover, it's known that during the treatment with PDT the concentration of O_2 decreases, the time in which the tissue becomes a hypoxic medium will depend on several factors, such as initial concentration of O_2 , irradiance and absorption spectrum of the PS.

Additionally, the type I mechanism operates exclusively in intramolecular and, it's more promising in organized media than in homogeneous solution, while type II consists of two components, an intramolecular path and an intermolecular.²⁷

1.6 Typical mathematical model for PDT

The typical mathematical model in PDT is based on the Jablonski diagram, where only are considered the first excited states of PS. The charge transfer between S_1 and the biological substrate may be despised since the lifetime of S_1 is very short. The next set of differential equations describes the rate of the concentration molar of PS, oxygen and the biological substrate for a given state. The symbology used is described in Table 1.

$$\frac{d[S_0]}{dt} = -\frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda)[S_0] + k_f[S_1] + k_p[T] + k_{ot}[T][^3O_2] - k_{os}([S_0] + \delta)[^1O_2], \quad (1.19)$$

$$\frac{d[S_1]}{dt} = \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda)[S_0] - k_f[S_1] - k_{isc}[S_1], \quad (1.20)$$

$$\frac{d[T]}{dt} = k_{isc}[S_1] - k_p[T] - k_{ot}[T][^3O_2] - k_{ta}[T][A], \quad (1.21)$$

$$\frac{d[^3O_2]}{dt} = -S_\Delta k_{ot}[T][^3O_2] + k_d[^1O_2] + \Pi_{oxy}, \quad (1.22)$$

$$\frac{d[^1O_2]}{dt} = S_\Delta k_{ot}[T][^3O_2] - k_d[^1O_2] - k_{oa}[A][^1O_2] - k_{os}([S_0] + \delta)[^1O_2], \quad (1.23)$$

$$\frac{d[A]}{dt} = -k_{ta}[T][A] - k_{oa}[A][^1O_2] \quad (1.24)$$

In order to model the photo-physical-chemical reactions in PDT is common to use these differential equations to describe PS transitions, oxygen interactions and cell kill.^{16-18,28} The term with positive or negative sign means respectively that, the rate of the molar concentration increase or decrease by some process of energy absorption or release.

The first parameter to the right of the equation (1.19) is the absorption rate photons for PS concentration, $\sigma_a(\lambda)$ is the absorption cross section of PS, hc/λ is the activation energy of the ground state of PS, this energy is coming from the incident photons and $F_{ex}(\vec{r}, t, \lambda)$ is the energy fluence rate. This equation rates and activation mechanism is valid for linear optics (a low energy fluence rate). S_Δ in equation (1.22) and (1.23) is the fraction of molecular deactivation between triplet states of PS and oxygen, here we work with $S_\Delta = 0.5$. Π_{oxy} is the oxygen supply term, $\Pi_{oxy} = g_{oxy}(1 - [^3O_2](t)/[^3O_2]_0)$,¹⁷ $[^3O_2]_0$ is the initial oxygen concentration, g_{oxy} is the maximum oxygen supply rate when there is no oxygen gradient.

The decrease in PS concentration by interaction with 1O_2 is considered in equations (1.19) and (1.23). After the generation of 1O_2 and before to return at ground state, there is the probability of interact with PS molecules, if the PS concentration is low, 1O_2 interact with the molecule of PS that generated it. The parameter δ is a correction term

to introduce this process, it can be calculated by $\delta = \frac{1}{d^3 N_A}$ with N_A Avogadro's number and $d = \sqrt{6D\tau_\Delta}$, here D and τ_Δ are respectively the diffusion coefficient and the lifetime for 1O_2 into biological medium. In this study, δ is calculated by adjust with experimental data.

Table 1 – Definitions of the notations used in the mathematical model.

Symbol	Definition	Units
$[S_0]$	PS concentration in ground state	μM
$[S_1]$	PS concentration in singlet excited state	μM
$[T]$	PS concentration in triplet excited state	μM
$[^3O_2]$	Oxygen concentration in ground state	μM
$[^1O_2]$	Oxygen concentration in excited state	μM
$[A]$	Concentration of acceptor	μM
δ	Low PS concentration correction term	μM
Π_{oxy}	Oxygen supply term	$\mu M s^{-1}$
k_f	Monomolecular PS decay rate of the excited to ground state with fluorescence emission	s^{-1}
k_p	Monomolecular PS decay rate of the excited to ground state by phosphorescence emission	s^{-1}
k_{isc}	PS intersystem crossing rate of $[S_1]$ to $[T]$	s^{-1}
k_d	Monomolecular decay rate of singlet oxygen to the ground state	s^{-1}
k_{ot}	Bimolecular rate of triplet PS quenching by ground state oxygen	$M^{-1} s^{-1}$
k_{os}	Bimolecular rate of reaction between the singlet oxygen and the ground state of PS	$M^{-1} s^{-1}$
k_{oa}	Bimolecular rate of reaction between singlet oxygen and the acceptor	$M^{-1} s^{-1}$
k_{ta}	Bimolecular rate of reaction between the PS triplet and acceptor	$M^{-1} s^{-1}$
S_Δ	Fraction of quenching of PS triplets by oxygen resulting in the formation of singlet oxygen	
$\sigma_a(\lambda)$	PS absorption cross section as a function of λ	cm^2
hc/λ	PS activation energy as a function of λ	J
$F_{ex}(\vec{r}, t, \lambda)$	Energy fluence rate as a function of position, time and λ	$mW cm^{-2}$

Source: By the author.

1.6.1 Steady state

The lifetime of the excited states S_1 , T and 1O_2 are smaller than ground states S_0 , 3O_2 and A . Therefore, S_1 , T and 1O_2 can be treat as a instantaneous steady state and consequently, only S_0 , 3O_2 and A are altered temporally.

Thus, the equations (1.20), (1.21) and (1.23) are equalized to zero. The instantaneous

formations of $[S_1]$, $[T]$ and $[^1O_2]$ are respectively the equations (1.25), (1.26) and (1.27).

$$[S_1] = \frac{1}{k_f + k_{isc}} \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda) [S_0], \quad (1.25)$$

$$[T] = \frac{k_{isc}[S_1]}{k_p + k_{ot}[^3O_2] + k_{ta}[A]}, \quad (1.26)$$

$$[^1O_2] = \frac{S_\Delta k_{ot}[T][^3O_2]}{k_d + k_{oa}[A] + k_{os}([S_0] + \delta)} \quad (1.27)$$

The term $k_{oa}[A] + k_{os}([S_0] + \delta)$ in the denominator of the equation (1.27) can be reduced to $k_{oa}[A]$ since $k_{os}([S_0] + \delta) \ll k_{oa}[A]$. Then,

$$[^1O_2] \approx \frac{S_\Delta k_{ot}[T][^3O_2]}{k_d + k_{oa}[A]} \quad (1.28)$$

After some algebraic manipulations, the new expression of the equation (1.19) is

$$\frac{d[S_0]}{dt} = -k_{ta}[T][A] - k_{os}([S_0] + \delta)[^1O_2] \quad (1.29)$$

This expression shows that the PS concentration is affected temporally by type I and II mechanisms.

Now, using equations (1.25), (1.26) and (1.28) in equations (1.29), (1.22) and (1.24).

$$\begin{aligned} \frac{d[S_0]}{dt} = & -\phi_t \left(\frac{k_{ta}[A]}{k_p + k_{ta}[A] + k_{ot}[^3O_2]} \right) \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda) [S_0] \\ & - S_\Delta \phi_t \left(\frac{k_{os}}{k_d + k_{oa}[A]} \right) \left(\frac{k_{ot}[^3O_2]}{k_p + k_{ta}[A] + k_{ot}[^3O_2]} \right) \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda) ([S_0] + \delta) [S_0] \end{aligned} \quad (1.30)$$

$$\frac{d[^3O_2]}{dt} = -S_\Delta \phi_t \left(\frac{k_{oa}[A]}{k_d + k_{oa}[A]} \right) \left(\frac{k_{ot}[^3O_2]}{k_p + k_{ta}[A] + k_{ot}[^3O_2]} \right) \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda) [S_0] + \Pi_{oxy} \quad (1.31)$$

$$\begin{aligned} \frac{d[A]}{dt} = & -\phi_t \left(\frac{k_{ta}[A]}{k_p + k_{ta}[A] + k_{ot}[^3O_2]} \right) \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda) [S_0] \\ & - S_\Delta \phi_t \left(\frac{k_{oa}[A]}{k_d + k_{oa}[A]} \right) \left(\frac{k_{ot}[^3O_2]}{k_p + k_{ta}[A] + k_{ot}[^3O_2]} \right) \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda) [S_0] \end{aligned} \quad (1.32)$$

with $\phi_t = \left(\frac{k_{isc}}{k_f + k_{isc}} \right)$. To reduce term, the equations (1.30), (1.31) and (1.32) are simplified using the equations (1.33), (1.34), (1.35) and (1.36). Where, the parameters μ , $\xi(\lambda)$ and β are respectively the specific photobleaching ratio, the specific oxygen consumption rate and the oxygen quenching threshold concentration, with units μM^{-1} , $cm^2 mW^{-1} s^{-1}$ and μM respectively.

$$\mu = \frac{k_{os}}{k_{oa}[A]}, \quad (1.33)$$

$$\xi(\lambda) = S_{\Delta} \phi_t \frac{k_{oa}[A]}{k_d + k_{oa}[A]} \frac{\sigma_a(\lambda)}{hc/\lambda}, \quad (1.34)$$

$$\beta = \frac{k_p}{k_{ot}}, \quad (1.35)$$

$$\gamma = \frac{k_{ta}[A]}{k_{ot}} \quad (1.36)$$

The next equations describe the rate of the concentration molar of PS, ground-state of oxygen and biomolecules.

$$\begin{aligned} \frac{d[S_0]}{dt} = & - \left(\frac{\phi_t \gamma}{\beta + \gamma + [^3O_2]} \right) \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda) [S_0] \\ & - \mu \xi(\lambda) F_{ex}(\vec{r}, t, \lambda) \left(\frac{[^3O_2]}{\beta + \gamma + [^3O_2]} \right) ([S_0] + \delta) [S_0], \end{aligned} \quad (1.37)$$

$$\frac{d[^3O_2]}{dt} = -\xi(\lambda) F_{ex}(\vec{r}, t, \lambda) \left(\frac{[^3O_2]}{\beta + \gamma + [^3O_2]} \right) [S_0] + \Pi_{oxy} \quad (1.38)$$

$$\begin{aligned} \frac{d[A]}{dt} = & - \left(\frac{\phi_t \gamma}{\beta + \gamma + [^3O_2]} \right) \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(\vec{r}, t, \lambda) [S_0] - \xi(\lambda) F_{ex}(\vec{r}, t, \lambda) \left(\frac{[^3O_2]}{\beta + \gamma + [^3O_2]} \right) [S_0] \end{aligned} \quad (1.39)$$

1.7 Photoproduct of PpIX

As was described in the previous sections, the chemical structure and consequently the ground state of PpIX concentration is altered by type I and II mechanisms of PDT. The decrease in PpIX concentration results in the formation of a new chemical species called photoproduct (Pp). The photophysical and photochemical characteristics of the PpIX are altered by the photoproduct, since it could absorb energy in the same region of the PpIX. As an example, a kind of Pp of PpIX reported by Gudgin et. al. (1995)²⁹, Ma et. al. (2001)³⁰ and Brancalion et. al. (2004)¹¹ is the chlorin photoprotoporphyrin (Ppp) with an absorption spectrum around 670 nm.

The exact formation of the photoproduct is not completely known. It is believed that it could be by the interaction of 1O_2 with PS, nevertheless, have been reported Pp formed in low concentrations of oxygen.³¹ Depending on the organic solvent and the binding sites within the cell, the PS will generate different types of Pp. Furthermore, if the absorption spectrum of Pp is in the same region that the emission spectrum of the light source, the photoproduct can proceed as PS.¹¹

If the Pp is formed directly by the type I and II mechanisms, the next photochemical interactions could be used to describe its formation.

where $[P]$ is the concentration of Pp, $[SO]$ and $[SA]$ are respectively the photoproducts generated by the PpIX reactions with $[^1O_2]$ and with biological molecule.

Although the photoproduct is formed from the excitation of the PS and subsequent reactions, the excitation of the Pp does not result in the formation of PS molecule, thus, it's not a reversible process.³⁰

1.8 Dosimetry in photodynamic therapy

The tissue damage caused by PDT depends on the interaction between light, PS and 3O_2 concentrations. In conditions of abundant oxygen, the singlet oxygen is accepted as the main cytotoxic agent (type II mechanism) for the majority PSs, including PpIX.³² Therefore, the measure of the concentration of singlet oxygen generated during PDT can be a good approach for dosimetry.³³ In clinical applications, the dosimetry in PDT is monitored by the loss of fluorescence of PS and light exposure dose (energy fluence rate by treatment time). However, the latter may not be as accurate in determining tissue damage.

The way to apply PDT is quite simple, combine PS and light in an environment with O_2 . On the other hand, it's quite a challenge to obtain correct dosimetry, since the concentrations of PS and O_2 change spatially and temporally. The intensity of the light in the tissue is not uniform, there is variation in depth and wavelength.

In order to define the PDT dose requires a good understanding of the components involved: light exposure, PS and oxygen concentrations. There are three ways approaches of PDT dosimetry: explicit, implicit and direct dosimetry.

Explicit dosimetry aims to measure the main component of PDT, PS, O_2 concentrations and light distribution. It's important to know these components since the production of reactive oxygen species is related to this combination. *Implicit dosimetry* objective is to monitor the fluorescence (or absorbance) of PS, since the fluorescence/absorbance are proportional to the PS concentration. Then knowing the temporal change in these parameters, it's possible to obtain information about the production of 1O_2 . This assumption is based on the fact that the 1O_2 after being generated attacks the PS molecule resulting in the decrease in PS concentration and photoproduct formation. If the PS decreases only by

1O_2 , the modeling of this reaction is given by the following differential equation.

$$\frac{d[S_0]}{dt} = -k_{os}([S_0] + \delta)[^1O_2] \quad (1.43)$$

where k_{os} is biomolecular rate constant for reaction of 1O_2 with S_0 (units $M^{-1}s^{-1}$), and δ is the correction parameter entered by Dysart et. al. (2005).³⁴ In conditions of abundant O_2 this dosimetry is valid. For a hypoxic tissue, this dosimetry is altered by the presence of reactive oxygen species. *Direct dosimetry* measures the phosphorescence of the singlet oxygen generated. This dosimetry is also known as singlet oxygen luminescence dosimetry (SOLD).

1.9 Literature review

Photodynamic therapy is an emerging treatment modality, it's used in medicine to treat several diseases, mainly related to the skin.¹⁻³ Taking into consideration the depth of the photodynamic effect, sources with the emitting spectrum on the red or infra-red region are chosen, since these sources have the best penetration into tissue.⁷⁻⁹ However, for diseases generate in superficial tissue, for example, the actinic keratosis (AK)⁵ is possible to use not sources traditional.

Photodynamic therapy with daylight is an alternative to the conventional method for the treatment of AK.³⁵ Some of the advantages are optimization in the time because several people could be treated simultaneously, a greater area of light exposure and a significant reduction of pain during PDT.³⁶ Despite these advantages, exist an important consideration for an effective PDT, the threshold dose.³⁷ On the assumption that the dominant mechanism in the PDT is type II,²⁵ it will exist a threshold of singlet oxygen.³⁸ To superated the threshold dose, effectiveness photosensitizer concentration and light exposure are necessities.

A proposal to compare and determine the efficiency of PDF using different light sources is through mathematical modeling. For example, a modeling and simulation of treatment of AK with 5-ALA using three light sources (red light dose, $37 J/cm^2$, $75 mW/cm^2$, 500 s; blue light dose, $10 J/cm^2$, $10 mW/cm^2$, 1000 s; and daylight dose, 900 s) was comparated by Vignion et. al. (2015).³⁹ The results of this investigation showed that although all doses of light are efficient at the deepest part of the AK, the blue light produces a greater photodynamic dose. In this study, the parameter of comparison was focused on the standard protocols of light dose.

A study *in vivo* comparing different light sources was developed by Marra et. al. (2018).³⁶ In this case, the light sources were blue LED, white lamp and sunlight. The 5-ALA and normal mouse skin were used. In this study, the parameter of comparison was the PpIX-weighted irradiance. This term was calculated by multiplication between the normalized absorption spectrum of PpIX and the absolute spectral irradiance of each

light source, with $20 J_{eff}/cm^2$ over approximately 2 hours. Their results show that blue light was slightly inferior to other sources, but the white lamp and sunlight have the same results.

A current investigation made by LaRochelle et. al. (2019)⁴⁰ expand to the use of PpIX-weighted irradiance by introducing Monte Carlo simulation for estimating the effective fluence rate inside of tissue. Also, the PpIX concentration was estimated considering pro-drug incubation. The light sources use by LaRochelle have multiple broadband (Sun, halogen lamp, etc) and narrowband (blue, red). The medium was a normal skin with 7 layer considering optical properties. Their results define a threshold photodynamic dose of $0.035 (\mu M J_{eff}/cm^2)$, it was determined by the combination of an effective threshold fluence value of $0.7 J_{eff}/cm^2$ with a threshold PpIX concentration ($50 nM$).

Table 2 shows several light sources used by Vignion et. al. (2015), Marra et. al. (2018) and LaRochelle et. al. (2019). Also, there is an introduction to photon dose, this parameter will be discussed with more details in Chapter 3 of the present study.

Table 2 – Light dose in several studies. The value of $20 J_{eff}/cm^2$ is obtained by the multiplication between the normalized absorption spectrum of PpIX and absolute spectral irradiance of each light sources. * represents the effective fluence, with units mW_{eff}/cm^2 , this term is calculated by weighting the fluence rate by the PpIX absorption spectrum. The photon dose was calculated considering $5 \mu M$ as PpIX concentration.

Reference	Light source	Fluence rate ($mWcm^{-2}$)	Light dose (Jcm^{-2})	Treatment time (min)	Photon dose ($Phot.cm^{-3}$)
Vignion et. al. (2015) ³⁹	Red	75	37	8.33	1.7×10^{18}
	Blue	10	10	16.67	7.9×10^{18}
	Sunlight			150	
Marra et. al. (2018) ³⁶	Blue, Sun white lamp		20^a	120	
LaRochelle et. al. (2019) ⁴⁰	Sun	4.0*	7.2, 28.8	30, 120	
	Halogen lamp	0.9*	1.6, 6.5	30, 120	
	CFL	1.6*	2.88, 11.5	30, 120	
	Blue	73.4*	132	30	2.4×10^{18}
		73.4*	529	120	9.6×10^{18}
	Red	1.7*	3.1	30	5.9×10^{18}
		1.7*	12.2	120	2.4×10^{19}

Source: By the author.

2 OBJECTIVES

2.1 General objective

Evaluate the light distribution and PDT Dose inside of a 3D computerized phantom taking into account the optical properties of the epidermis and dermis for a broad spectrum.

2.2 Specific objectives

- Associate the emission spectrum of the light source with the absorption spectrum of PpIX.
- Study experimentally the photobleaching of PpIX for different light sources.
- Use a mathematical model of PDT to predict the temporal change of PpIX concentration.
- Simulate the light distribution inside 3D computerized phantom for a heterogeneous two-layer model considering the optical properties of the mentioned tissue.
- Determine the theoretical PDT dose and cell survival fraction for any location inside of the 3D computerized phantom of the heterogeneous two-layer model.

3 MATERIALS AND METHODS

3.1 Light sources

The most common way to characterize a light source is by measuring the spectral irradiance. This expression with units ($W/m^2/nm$) gives the power density at a particular wavelength. The integration of the spectral irradiance over wavelength to specific depth is the irradiance with units (W/m^2). The irradiances were measured with a spectroradiometer (calibrated USB 2000, Ocean Optics, USA). The spectroradiometer was calibrated using a National Institute of Standards Technology (NIST) traceable light source (DH-3plus-BAL-CAL, Ocean Optics, USA).

The energy fluence, with units (Jm^{-2}), is the quotient of the radiant energy incident on a sphere of cross-sectional area dS . The energy fluence rate or only intensity is the increment of the energy fluence in the time interval with units (Wm^{-2}) or ($Js^{-1}m^{-2}$). The irradiance and energy fluence rate have information of light propagation into the biological environment. For experiment with only absorption media, the irradiance and intensity can be modeled by Beer-Lambert's law. In this condition, $F_{ex}(\vec{r}, t, \lambda)$ is time constant and only the depth is considered, therefore, $F_{ex}(\vec{r}, t, \lambda) = F_{ex}(z, \lambda)$.

$$F_{ex}(z, \lambda) = F_{ex}(0, \lambda)10^{-A(\lambda)} \quad (3.1)$$

$$= F_{ex}(0, \lambda)10^{-\mu_a(\lambda)z} \quad (3.2)$$

where, $A(\lambda)$ is the absorbance, $\mu_a(\lambda)$ is the absorption coefficient, $F_{ex}(z, \lambda)$ is the radiant energy per unit time and area or energy fluence rate transmitted and, $F_{ex}(0, \lambda)$ is the radiant energy per unit time and area at depth equal to zero or energy fluence rate received (or only irradiance).⁴

(a) Lince MMOptics

(b) Spectral irradiance

Figure 8 – Spectral irradiance for blue and red LED of Lince MMOptics.

Source: By the author.

The equipment Lince (MMOptics, São Carlos, Brazil) was used as the light source, it is composed of two LED-based devices to deliver light in violet-blue ($\lambda_{max} = 407 \text{ nm}$, to easy the notation, in this work only describe as blue and not violet-blue) and red ($\lambda_{max} = 639 \text{ nm}$) region (Figure 8 (a)). This equipment is used for screening and treatment of non-melanoma skin lesions.^{3,41} The value of spectral irradiance was measured for each LED (Figure 8 (b)) using a spectral radiometer.

The emission of the spectrum of Sun was measure at 10:40 AM on 13th of March, 2018 in São Carlos - Brasil, with the wavelength between $[350, 700] \text{ nm}$ (Figure 9).

Figure 9 – Spectral irradiance for Sunlight.

Source: By the author.

At depth equal to zero, the total irradiance can be calculated by integration of spectral irradiance over all wavelength or using the sum in wavelength of the equation (3.3).

$$F_{ex}(0, \lambda) = (\lambda_{i+1} - \lambda_i) \left(\frac{E_{i+1} + E_i}{2} \right) \quad (3.3)$$

where λ_i is the wavelength and E_i is the value measure of the spectral irradiance in λ_i , $i \in [350, 700]$. λ_i and E_i are discrete values. The units of $F_{ex}(0, \lambda)$ are (Wm^{-2}) .

The number of emitted photons per unit of time and area can be estimated by quotient $F_{ex}(0, \lambda)$ and the energy of emitted photon, equation (3.4). The units are $(Phot.s^{-1}cm^{-2})$. The eq. 3.4 was used for each of the light sources.

$$Emitted\ Photons(\lambda) = \frac{F_{ex}(0, \lambda)}{hc/\lambda} \quad (3.4)$$

The number of absorbed photons per unit of time and volume is obtained by multiplication of the emitted photons of the light source and the absorption coefficient of PS, the units are $(Phot.s^{-1}cm^{-3})$.

$$Absorbed\ Photons(\lambda) = \mu_a(\lambda)Emitted\ Photons(\lambda) \quad (3.5)$$

After a period of irradiation will be a total amount of absorbed photons for PS. This amount is associated with a "Volumetric dose of photons", or simply "Dose of photons"

equivalent to "*Photon dose*", with units (*Photons.cm⁻³*). In this work, the *number of photons absorbed* is another way to refer to *photon dose*.

Patterson et al. (1990)⁴² investigated the photodynamic threshold dose in normal rat liver. They worked with aluminum chlorosulphonated phthalocyanene (AISPC) as photosensitizer at several concentrations. For the light source, the excitation wavelengths were 610, 640 and 665 nm. Their results showed that in these conditions, the threshold to be $(3.8 \pm 0.2) \times 10^{19}$ (*Photons.cm⁻³*) as a dose of absorbed photons, regardless of the PS concentration and wavelength.

In this study, 1×10^{19} (*Photons.cm⁻³*) will be used as photon dose. With this value of treatment time of each light source will be estimated, as it is established by the equation (3.6).

$$Treatment\ time = \frac{1 \times 10^{19}}{Absorbed\ Photons(\lambda)} \quad (3.6)$$

3.2 Absorbance analysis of PpIX

The PS in this study is the Protoporphyrin IX (PpIX). The PpIX has five absorption bands in the visible spectrum. The Soret band is most prominent than the four Q-bands (Figure 10). The PpIX can suffer a photobleaching effect and, this causes a photochemical alteration that produces breaking of covalent bonds of the PpIX molecule. It is an irreversible process that results in a decrease of fluorescence and absorbance spectrum.

One purpose of this study was to analyze the changes in the PS concentration caused by a certain amount of absorbed photons. The fluorescence and absorbance are proportional to the concentration of PS. Thus, to study the PpIX concentration the change in absorbance will be used.

Figure 10 – Absorbance spectrum of PpIX.

Source: By the author.

Three macro experiments were performed (Figure 11). Each macro experiment

consists of three experiments, corresponding to irradiation with blue light (experiment 1), red light (experiment 2) and together blue with red light (experiment 3). With these experiments, it's expected to understand the photobleaching of the PpIX when irradiated with different sources of light at same photon dose. In addition, it is expected to establish a relationship between the decrease in PpIX concentration as a function of the dose of photons.

With configuration 1, the first two macro experiments were performed. The first macro experiment was based on delivering approximately the same dose of photons in each experiment ($1 \times 10^{19} \text{ Phot.cm}^{-3}$). In contrast, the second macro experiment was based on delivering small doses of photons, that is, delivering a fractionated dose of photons at equivalent times between experiments. Only for the second macro experiment was analyzed by means of graphs and numerical values the behavior of the absorbance for the same fraction of doses of photons. On the other hand, the third macro experiment was carried out with configuration 2. This macro experiment conserves the dose of photons, as in the previous cases. The difference was in the treatment time, where for experiments 1 and 2 this value was equalized.

Figure 11 – Representation of the three macro experiments carried out for the study of photobleaching of PpIX with two experimental configurations and three light sources: blue, red, and combination blue and red.

Source: By the author.

Two Lince MMOptics were used to make the experiments of photobleaching of PpIX. The figure 12 shows the two configurations developed. Accordingly, the photobleaching of PpIX is carried out by three experiments using configuration 1 and 2, in each of the experiments there is a solution of $5 \mu M$ of PpIX in DMSO. Each solution is irradiated only with a blue LED or, a red LED or a combination of blue and red LED. Each experiment was done in triplicate. The intensity of each experimental configuration, treatment time and others important vales are listed in Table 3 and Table 4

(a) Configuration 1

(b) Configuration 2

Figure 12 – Experimental configurations.

Source: By the author.

Table 3 – Measures in experimental configuration 1 for the macro experiments 1 and 2.

Sources	Intensity ($mWcm^{-2}$)	Emitted photons ($Phot.s^{-1}cm^{-2}$)	Absorbed photons ($Phot.s^{-1}cm^{-3}$)	Dose of photons ($Phot.cm^{-3}$)	Time (min)
Blue 1	72.2	1.47×10^{17}	$(6.4 \pm 0.2) \times 10^{16}$	1×10^{19}	2.61
Red	170.8	5.45×10^{17}	$(4.0 \pm 0.8) \times 10^{15}$	1×10^{19}	41.61
Blue 1 and Red	243.0	7.05×10^{17}	$(6.8 \pm 0.3) \times 10^{16}$	1×10^{19}	2.45

Source: By the author.

Table 4 – Measures in experimental configuration 2 for the macro experiment 3

Sources	Intensity ($mWcm^{-2}$)	Emitted photons ($Phot.s^{-1}cm^{-2}$)	Absorbed photons ($Phot.s^{-1}cm^{-3}$)	Dose of photons ($Phot.cm^{-3}$)	Time (min)
Blue 2	4.75	9.79×10^{15}	$(4.1 \pm 0.1) \times 10^{15}$	1×10^{19}	40.39
Red	170.8	5.45×10^{17}	$(4.0 \pm 0.8) \times 10^{15}$	1×10^{19}	41.61
Blue 2 and Red	175.53	5.55×10^{17}	$(8.1 \pm 0.9) \times 10^{15}$	1×10^{19}	20.49

Source: By the author.

Figure 13 – Schematic representation for overlap between emission spectra of the light sources and the absorption spectrum of PpIX. For better visualization, each spectrum is normalized by the division of its respective maximum peak.

Source: By the author.

Each light source interacts differently with the PpIX. The blue LED active the Soret band (Figure 13 (a)). The red LED active the last Q-band (Figure 13 (b)). The combination blue and red LED activates the Soret and Q-band (Figure 13 (c)). The consequence of these interactions were observed in the treatment time.

Table 5 – Control on the measure of absorbance for PpIX using different light sources.

Absorbance	Blue	Red	Blue and red
A_0	t_0	t_0	t_0
A_1	$t_{1,b}$	$t_{1,r}$	$t_{1,br}$
A_2	$t_{2,b}$	$t_{2,r}$	$t_{2,br}$
A_3	$t_{3,b}$	$t_{3,r}$	$t_{3,br}$
A_4	$t_{4,b}$	$t_{4,r}$	$t_{4,br}$
A_5	$t_{5,b}$	$t_{5,r}$	$t_{5,br}$
A_6	$t_{6,b}$	$t_{6,r}$	$t_{6,br}$

Source: By the author.

Table 5 was used to control the measurement of absorbance. Here, $A_0, A_1, \dots, A_6$ are the measurement of absorbance at a specific time. The time t_0 corresponds to the

value of the absorbance without irradiation and, the other terms are treatment time calculated to each light source, with 1×10^{19} ($Phot.cm^{-3}$) as the maximum *Photon Dose*. The absorbance was measured after each irradiation time using the spectrophotometer CARY 50 UV-VIS (Varian, Australia). In all experiments, a 4 ml quartz cuvette was used.

3.3 Temporal change of PpIX, ground-state of oxygen concentrations and PDT dose

The main difference in the typical model of PDF, as was shown in the *Introduction Chapter*, was not to consider a fixed wavelength. Since part of this study was to theoretically evaluation the use of a multi-wavelength source for PDT. Therefore, in relation to the PDT model, for a homogeneous distribution of concentrations of PpIX and oxygen at a specific depth, there are temporal and wavelength dependence.

This study seeks to check the hypothesis that the total damage to biological tissue can be a sum of several photodynamic effects. Since the PpIX has a broad absorption spectrum and activation on of any band absorption produces a photodynamic effect. Accordingly, to resolve the equations (1.37) and (1.38) is necessary integrate over time and sum over wavelength.

$$[S_0] = \int \left(\sum_{\lambda} \frac{d[S_0](t, \lambda)}{dt} \right) dt, \quad (3.7)$$

$$[^3O_2] = \int \left(\sum_{\lambda} \frac{d[^3O_2](t, \lambda)}{dt} \right) dt \quad (3.8)$$

In this study, the total photodynamic therapy dose (PDT dose) is the accumulated $[T]$ and $[^1O_2]$ killing cell targets (mechanisms type I and II). Thus, PDT dose can be estimated by integration from $t = 0$ to $t = T$ of equation (1.39) and sum of each wavelength that contribute in the photodynamic effect.

$$PDT \text{ dose} = - \int_0^T \left(\sum_{\lambda} \frac{d[A]}{dt} \right) dt \quad (3.9)$$

The absorption cross-section, the energy of the incident photon and the spectral irradiance of the light source were measured experimentally. The absorption cross section was calculated implicit for the experimental measure of absorbance of PS. Since the absorbance is proportional to absorption coefficient.

$$\mu_a(\lambda) = \frac{\text{Absorbance}}{\text{Length of the light path}} \quad (3.10)$$

and, the absorption cross-section, $\sigma_a(\lambda)$ is

$$\sigma_a(\lambda) = \frac{\mu_a(\lambda)}{N} \quad (3.11)$$

here, $\mu_a(\lambda)$ is the absorption coefficient, N is the atomic number density ($N = \frac{\rho N_A}{M}$), ρ is the density, N_A is the Avogadro's number and M is the molar mass. A specific example is the PpIX, with $\rho_{PpIX} = 1.27g/cm^3$ and $M_{PpIX} = 562.6581g/mol$.

The total absorbed photons rate on the surface can be estimated by overlap between absorption cross-section of PpIX and $Emitted\ photons(\lambda)$, as is shown in equation 3.12. The units are ($Phot.s^{-1}$).

$$\sum_{\lambda} \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(0, \lambda) \quad (3.12)$$

In summary, our mathematical model is composed by temporary evolution for PS and oxygen, equations (3.7) and (3.8) respectively, and PDT dose (equation 3.9) together the total absorbed photons rate. The simulation of these differential equations was carried out in Wolfram Mathematica 11.3 version (Wolfram Research, USA) software.

3.4 Light propagation into tissue using Monte Carlo eXtreme

This study consider two layers of skin, epidermis and dermis. Every layer has their optical properties and a specific depth, 200 and 4800 μm for epidermis and dermis respectively. The two layers were placed in a 3D computerized phantom or simply optical phantom (OP). The Figure 14 show the epidermis and dermis together with the photon migration and corresponding value in units of mm. The build of the OP was make using MCXLAB with a resolution voxel of $1 \times 1 \times 1\ mm^3$. This structure is a cube of Cartesian dimensions $(x, y, z) = (101, 101, 50)$, in which non air layer was considered. The illumination source was located outside the epidermis, and it was chosen a disk beam as light pattern centered at $(51, 51, 0)$ and radius 0.2 mm.

Figure 14 – Geometry description for epidermis and dermis (heterogeneous two-layer model) in the 3D computerized phantom.

Source: By the author.

Numerical values of optical properties of the tissues are listed in Table 6 and 7. These values were obtained by different authors. The criterion was only considering *in vivo* experiments. The absorption coefficients were obtained by Meglinski et. al. (2002).⁴³ The reduced-scattering coefficients were obtained by Zonios et. al. (2006)⁴⁴. The anisotropy parameter for epidermis and dermis was considered constant, $g=0.9$.^{44–46} The refractive index to epidermis and dermis were obtained from graphical representation of Ding et. al. (2006)⁴⁷. The Figure 15 shown the curves of the optical properties versus wavelength for epidermis and dermis.

The simulation was carried out with a number equally at 5×10^7 photons together with the optical properties for epidermis and dermis (Figure 15). The interval was of $[400, 700]$ nm with an increment of 10 nm. The result was a cubic matrix for $flux$, units ($Phot.s^{-1}mm^{-2}$), for each wavelength. Therefore, there were 31 cubic matrix, each one with dimension (101,101,50) and voxel resolution of $1 \times 1 \times 1 \text{ mm}^3$.

Figure 15 – Optical properties to epidermis and dermis in the region 400 to 700 nm.

Source: By the author.

With the simulation of the distribution of light in the OP, the $flux$ was calculated for any location and wavelength within of the cubic. Afterwards, the intensity was obtained by multiplication of energy incident photon with $flux$ (equation 3.13). This process is

repeat for each cubic matrix.

$$Intensity = \left(\frac{hc}{\lambda} \right) flux(\lambda) \quad (3.13)$$

To simulate the real distribution of a light source within our OP is necessary to make a weighting. The weighting can be carried out using any real measure of spectrum emission of the source of light. The weighting coefficients were obtained by the division of the intensities of the spectrum measured experimentally with $I_{\lambda_{400}}$ fixed. Each of these coefficients (α_{λ}) were multiplied by its respectively cubic matrix.

$$\frac{I_{\lambda_{410}}}{I_{\lambda_{400}}} = \alpha_{410}; \frac{I_{\lambda_{420}}}{I_{\lambda_{400}}} = \alpha_{420}; \dots; \frac{I_{\lambda_{700}}}{I_{\lambda_{400}}} = \alpha_{700} \quad (3.14)$$

here, I_{λ} represent the intensity at specific λ .

Table 6 – The numerical values of the optical properties of the epidermis were obtained from Meglinski et al (2002), Zonios et. al. (2006) and Ding et. al. (2006). Only in vivo experiments were considered. The anisotropy parameter was 0.9

λ (nm)	Absorption coefficient (mm^{-1})	Reduced-Scattering coefficient (mm^{-1})	Refractive index
400	0.42	2.18	1.46
410	0.39	2.17	1.46
420	0.36	2.15	1.45
430	0.33	2.13	1.45
440	0.31	2.12	1.45
450	0.29	2.10	1.45
460	0.27	2.08	1.44
470	0.25	2.07	1.44
480	0.23	2.05	1.44
490	0.22	2.03	1.44
500	0.20	2.02	1.44
510	0.19	2.00	1.44
520	0.18	1.98	1.44
530	0.17	1.97	1.44
540	0.16	1.95	1.44
550	0.15	1.93	1.43
560	0.14	1.92	1.43
570	0.13	1.90	1.43
580	0.13	1.88	1.43
590	0.12	1.87	1.43
600	0.11	1.85	1.43
610	0.11	1.83	1.43
620	0.10	1.82	1.43
630	0.10	1.80	1.43
640	0.09	1.78	1.43
650	0.09	1.77	1.43
660	0.08	1.75	1.43
670	0.08	1.73	1.43
680	0.08	1.72	1.43
690	0.07	1.70	1.43
700	0.07	1.68	1.43

Source: By the author.

Table 7 – The numerical values of the optical properties of the dermis were obtained from Meglinski et al (2002), Zonios et. al. (2006) and Ding et. al. (2006). Only in vivo experiments were considered. The anisotropy parameter was 0.9

λ (nm)	Absorption coefficient (mm^{-1})	Reduced-Scattering coefficient (mm^{-1})	Refractive index
400	1.78	2.18	1.39
410	2.88	2.17	1.39
420	3.23	2.15	1.39
430	2.57	2.13	1.39
440	1.62	2.12	1.39
450	0.566	2.10	1.38
460	0.258	2.08	1.38
470	0.189	2.07	1.38
480	0.156	2.05	1.38
490	0.149	2.03	1.38
500	0.150	2.02	1.38
510	0.160	2.00	1.38
520	0.194	1.98	1.38
530	0.283	1.97	1.38
540	0.362	1.95	1.38
550	0.338	1.93	1.38
560	0.294	1.92	1.38
570	0.320	1.90	1.38
580	0.321	1.88	1.38
590	0.143	1.87	1.38
600	0.056	1.85	1.38
610	0.034	1.83	1.38
620	0.023	1.82	1.38
630	0.018	1.80	1.37
640	0.015	1.78	1.37
650	0.013	1.77	1.37
660	0.011	1.75	1.37
670	0.010	1.73	1.37
680	0.008	1.72	1.37
690	0.007	1.70	1.37
700	0.007	1.68	1.37

Source: By the author.

3.5 Numerical approximation of PDT dose and cell survival fraction into tissue

With the simulation of the distribution of light in the tissue, the availability of photons at any position within the OP was determined. As a specific example, the Sun spectrum was the source of light. Thus, the weighting was carried out with the sunlight spectrum. In addition, within each voxel of the OP was placed a certain concentration of PpIX and oxygen. A homogeneous distribution was assumed over these concentrations, that is, the same values of PpIX and oxygen for each voxel.

The Sun spectrum includes the activation of all absorbed bands of PpIX. Therefore, for numerical approximation of PDT dose, the availability of photons together with the equations (3.7), (3.8) and (3.9) were resolved.

The PDT dose is the quantification of all tissue damage. To ensure that the damage is effective, the PDT dose must reach at least a minimum concentration of cytotoxic products. This means a low probability of survival of cell target. The equation 3.15 expresses the relationship between PDT dose and individual cell survival probability *in vitro*.³⁸

$$SF = e^{-[^1O_2]/[^1O_2]_{rx0}} \quad (3.15)$$

where $[^1O_2]_{rx0}$ is the reacted 1O_2 when the cell survival is dropped to $1/e$, SF is the cell survival fraction of each individual cell. When $SF = 1$ all cell survive, if $SF = 0.1$ means 1-in-10-cells survival. In this study, the PDT dose is determined by the reactions of mechanism type I and 1O_2 .

Quantitatively, the approach to analyze in this part was classified into three main points, (a) Simulation of light distribution in a broad spectrum. (b) Classical modeling for PDT with the assumption that PS decrease is not dependent on the light source, but depends on the number of photons absorbed. (c) Finally, a calculus of theoretical PDT dose and estimation of cell survival fraction considering items (a) and (b). Figure 16 shows the idea of this part. All algorithms were written in Wolfram Mathematica 11.3 version (Wolfram Research, USA) software.

Figure 16 – Schematic representation of flux, intensity, weighting, overlap, PDT dose and cell survival fraction by voxel.

Source: By the author.

4 RESULTS AND DISCUSSION

4.1 Light sources and absorption spectrum for PpIX

The emission spectrum of three light sources, two LEDs (Blue and Red) and Sunlight were measured experimentally. Figure 17 shows the real irradiance for the light sources for a broad spectrum of emission in the visible region. Also, the total irradiance and emitted photons were calculated for each light source.

Figure 17 – An experimental measure of irradiance for the light sources

Source: By the author.

Figure 18 shows the absorption coefficient spectrum of PpIX. This Figure is the result of the division of the absorbance spectrum with the length of the cuvette, 1 [cm]. The absorbance spectrum was measured using 5 [μ M] of PpIX in DMSO.

Figure 18 – Absorption spectrum of PpIX

Source: By the author.

From the data of the Figures 17 and 18, the emitted photons, the absorbed photons, treatment time and the doses of light were determined. A summary of these results is

shown in Table 8. Where, the total irradiance or intensity is the area of the figures 17 (a) and (b) for each light source (evaluation of equation 3.3). The emitted and absorbed photons were evaluated respectively by equations 3.4 and 3.5. The dose of photons to produce necrosis into tissue was 1×10^{19} ($Phot.cm^{-3}$). The treatment time was calculated by equation 3.5 and, the dose of light was determined by the multiplication of the intensity with treatment time.

The efficiency of different light sources was compared using as a fixed parameter the dose of photons, another way to compare is using the effective dose of light. This light dose can be estimated by multiplication of the normalized spectrum of PpIX with emitted spectrum of the light source, with units ($J_{eff}.cm^{-2}$).^{36,40} These values were located in the last column in of Table 8.

Table 8 – Experimental data for absorbed and emitted photons. Blue 1 and Blue 2 corresponding respectively to experimental configurations 1 and 2. It's applicable for Blue 1 and Red/Blue 2 and Red. Instead, the conditions for the red source were the same in the two configurations.

Sources	Intensity ($mW.cm^{-2}$)	Emitted photons ($Phot.s^{-1}.cm^{-2}$)	Absorbed photons ($Phot.s^{-1}.cm^{-3}$)	Dose of photons ($Phot.cm^{-3}$)	Time (min)	Dose of light ($J.cm^{-2}$)	Effec. dose of light ($J_{eff}.cm^{-2}$)
Blue 1	72.2	1.47×10^{17}	$(6.4 \pm 0.2) \times 10^{16}$	1×10^{19}	2.61	11.45	9.8
Red	170.8	5.45×10^{17}	$(4.0 \pm 0.8) \times 10^{15}$	1×10^{19}	41.61	426.4	6.4
Blue 1 and Red	243.0	7.05×10^{17}	$(6.8 \pm 0.3) \times 10^{16}$	1×10^{19}	2.45	36.9	9.6
Blue 2	4.75	9.79×10^{15}	$(4.1 \pm 0.1) \times 10^{15}$	1×10^{19}	40.39	11.45	9.8
Blue 2 and Red	175.53	5.55×10^{17}	$(8.1 \pm 0.9) \times 10^{15}$	1×10^{19}	20.49	215.88	8.1
Sunlight	62.81	1.69×10^{17}	$(7.7 \pm 0.4) \times 10^{15}$	1×10^{19}	21.52	156.167	9.3

Source: By the author.

Table 9 – Total absorbed photons rate for each light source and two experimental configurations. These values corresponding to evaluation of the equation (3.12)

Sources	Total absorbed photons rate ($Phot.s^{-1}$)
Blue 1	$(8.6 \pm 0.3) \times 10^6$
Red	$(0.5 \pm 0.1) \times 10^6$
Blue 1 and Red	$(9.1 \pm 0.4) \times 10^6$
Blue 2	$(0.56 \pm 0.02) \times 10^6$
Blue 2 and Red	$(1.0 \pm 0.1) \times 10^6$
Sunlight	$(1.02 \pm 0.06) \times 10^6$

Source: By the author.

In the first experimental configuration (Blue 1, Red, Blue 1 and Red), the treatment time was very distinct, 2.61 and 41.61 minutes for blue 1 and red sources respectively.

This was due to the difference between the absorbed photons, $(6.4 \pm 0.2) \times 10^{16}$ for blue 1 and $(4.0 \pm 0.8) \times 10^{15}$ for red. On the other hand, the treatment time for the second experimental configuration (Blue 2, Red, Blue 2 and Red) was the same for blue 2 and red sources. Although, when the two sources are together, the time was half that the previous. Also, in this second configuration, the magnitude of absorbed photons for blue 2 and red source was approximately the same, 4.0×10^{15} ($Phot./s * cm^3$).

The values calculated for the dose of light were totally different between experimental configurations. Also, it's observed that the lowest calculated value for the effective dose of light is for the red source.

Table 9 shows the total absorbed photons rate for the three sources in the two experimental configurations. The data exhibit that the total absorbed photons rate have the same magnitude, 10^6 ($Phot.s^{-1}$). This quantities were used to solve the differential equations (3.7), (3.8) and the PDT dose.

4.2 Photobleaching of PpIX

4.2.1 Experimental measure of absorbance

Figure 19 shows the results of the three experiments. In all experiments, the photobleaching effect was observed through of decrease in absorbance of PpIX. The measurement of the absorbance was made after the time established in the right side of each figure. Furthermore, in each of the experiments the appearance of a absorption band in 653 nm was observed.

The absorption band in 653 nm was related to the formation of photoproduct. The data in Figure 19 indicates that the red source produces less photoproduct than other sources. We believe that this difference is related to the non-delivery of equals of fractionated doses of photons. That is, the dose of photons delivered at 30 seconds of irradiation in experiments 1 and 3 is not equal to the 2 minutes of irradiation in experiment 2. Therefore, the second macro experiment was made, considering equally fractionated dose of photons. The results are visible in the figure 20.

Figure 20 exhibits the photobleaching for three experiments. The treatment time for the red source was the same that the previous macro experiment. Instead, for experiments 1 and 3, the time is the equivalent for experiment 2 delivered the same fractionated dose of photons.

(a) Blue 1

(b) Red

(c) Blue 1 and Red

Figure 19 – PpIX absorbance measurements for different irradiation times. Using source (a) Blue 1, (b) Red, (c) Blue 1 and Red for macro experiment 1.

Source: By the author.

(a) Blue 1

(b) Red

(c) Blue 1 and Red

Figure 20 – PpIX absorbance measurements for different irradiation times and a fractionated dose of photons. Using source (a) Blue 1, (b) Red, (c) Blue 1 and Red for macro experiment 2.

Source: By the author.

In order to analyze the fractionated dose of photons, the temporal change in the

absorbance was included implicitly in the equation 3.6. With this modification, the next equation was used to calculate the dose of photons for any treatment time.

$$\text{Fractionated dose photons}(\lambda, t) = (\text{Treatment time})(\text{Absorbed Photons}(\lambda, t)) \quad (4.1)$$

$$\text{Fractionated dose photons}(\lambda, t) = t \left(\mu_a(\lambda, t) \frac{F_{ex}(0, \lambda)}{hc/\lambda} \right) \quad (4.2)$$

The Table 10 was build using the equation 4.2 and measurement of absorbance from Figure 20. Here, the error in the photon dose was calculated by three experimental measurements and, the error in the time, in the experiments 1 and 3, was considered constant, ± 0.4 seconds.

Table 10 – Fractionated dose of photons. Here A_0 , A_1 , A_2 , A_3 , A_4 and A_5 are the absorbances measure at different treatment time.

	Blue 1			Red		Blue 1 and Red	
	Time	Dose Photons	Time	Dose Photons	Time	Dose Photons	
	sec.	$\times 10^{19}$ Phot.cm $^{-3}$	min.	$\times 10^{19}$ Phot.cm $^{-3}$	sec.	$\times 10^{19}$ Phot.cm $^{-3}$	
A_0	0	0	0	0	0	0	
A_1	7.6 ± 0.4	0.043 ± 0.001	2	0.06 ± 0.03	7.1 ± 0.4	0.045 ± 0.002	
A_2	37.9 ± 0.4	0.208 ± 0.007	10	0.3 ± 0.1	35.6 ± 0.4	0.213 ± 0.005	
A_3	75.7 ± 0.4	0.39 ± 0.01	20	0.6 ± 0.3	71.2 ± 0.4	0.41 ± 0.01	
A_4	113.5 ± 0.4	0.55 ± 0.02	30	0.8 ± 0.4	106.8 ± 0.4	0.58 ± 0.02	
A_5	170.3 ± 0.4	0.78 ± 0.04	45	1.2 ± 0.6	160.2 ± 0.4	0.83 ± 0.04	

Source: By the author.

Figure 21 shows the comparison of the normalized absorbance for the three experiments and approximately the same fractionated dose of photons. It's noted equally compartment for Soret band and Q-bands for each one normalized absorbance. Nevertheless, the formation of photoproduct is different, the red source produces less photoproduct that other sources.

In order to explain the difference of the in the photoproduct formation between the three experiments was considered the same dose of photons delivered in equivalent times. It's important to note that to determine this dose of photons it's necessary to have measured the absorbance spectrum. This spectrum only is measured after a time of irradiation. Consequently, the fractionated dose of photons is approximately equal between experiments. The data in the Table 10 corroborate this statement. Here, the dose of photons is very similar for the experiments 1 and 3, and different for experiment 2. This result raises another question, what is the real contribution of the red source in the third experiment?

Figure 21 – Absorbance measurements for fractionated dose of photons. Using different time of irradiation. The absorbance amplitudes are normalized to the initial absorbance amplitude.

Source: By the author.

In order to have the same conditions for the blue and red sources, it was made a second experimental configuration (Figure 12). By modifications on the blue source, the treatment time and the absorbed photons were equalized in experiments 1 and 2 (Table 8). This was the origin for the last macro experiment.

Figure 22 shows the results of tree photobleaching experiments. Note that having the two sources together, the treatment time was reduced by half, 20 minutes.

Figure 22 – PpIX absorbance measurements for different irradiation times and same dose of photons. Using source (a) Blue 2, (b) Blue 2 and Red for macro experiment 3.

Source: By the author.

The temporal change in PpIX concentration was studied on all the absorption bands for each of the macro experiments. More detail are explain in the next subsection.

4.2.2 Analysis of Soret and Q-bands as function of time

Figures 23, 24 and 25 are the graphic representation for temporal change of normalized PpIX absorbance for three macro experiment. The maximum absorption peaks of all absorption bands were considered. The mean value and the standard deviation were calculated by three experimental measurements.

Figure 23 – Normalized PpIX absorbance decrease as a function of time of irradiation to Soret band and Q-bands using Blue 1, Red and Blue 1 and Red. Error bars are standard deviations from three experimental measurements. These results were obtained from Figure 19.

Source: By the author.

Figure 24 – Normalized PpIX absorbance decrease as a function of time of irradiation to Soret band and Q-bands using Blue 1, Red and Blue 1 and Red. Error bars are standard deviations from three experimental measurements. These results were obtained from Figure 20.

Source: By the author.

In order to compare the photobleaching of PpIX with the blue, red and combination blue and red sources, the three experiments were plotted in the same figure. It was observed that the normalized PpIX absorbance decreases with increasing exposure time. This is the expected behavior in photobleaching experiments.⁴⁸ Since, the absorbance peak amplitudes are related with the PpIX concentration. Furthermore, the variation in the absorbance amplitude can be associated with a change in the optical characteristics of the PpIX.

Figure 25 – Normalized PpIX absorbance decrease as a function of time of irradiation to Soret and Q-bands using Blue 2, Red and Blue 2 and Red. Error bars are standard deviations from three experimental measurements. These results were obtained from Figure 22.

Source: By the author.

Based on the observed data, for a constant concentration of oxygen, the experimental points can be fitted using an exponential decay function. It's possible by the assumption that the rate of PS is proportional to the instantaneous photosensitizer concentration.^{28,48} The equation 4.3 was used to fit the experimental data.

$$[S_0](t) = [S_0](0)e^{-\alpha \cdot t} \quad (4.3)$$

where, $[S_0](0)$ is the initial normalized concentration of PpIX and $\alpha = 1/\tau$, τ is the characteristic decay time. This decay time depends on wavelength, the energy fluence rate and the oxygen concentration. Values for α are shown in the Tab. 11, Tab. 12 and Tab. 13.

For the sum of two values of α , the error was calculated using $\sqrt{\sigma_a^2 + \sigma_b^2}$, where σ_a and σ_b are respectively the errors for α_a and α_b .

In order to know the dependence α with wavelength, the energy fluence rate and the oxygen concentration, the equation (1.29) was expanded with the assumption that the $[^3O_2]$ is constant.

$$\frac{d[S_0]}{dt} = -k_{ta}[T][A] - k_{os}([S_0] + \delta)[^1O_2] \quad (4.4)$$

using the eq. 1.28

$$\frac{d[S_0]}{dt} = -k_{ta}[T][A] - k_{os}([S_0] + \delta) \left(\frac{S_\Delta k_{ot}[T][^3O_2]}{k_d + k_{oa}[A]} \right) \quad (4.5)$$

$$= - \left(k_{ta}[A] + \frac{S_\Delta k_{os} k_{ot}[^3O_2]}{k_d + k_{oa}[A]} ([S_0] + \delta) \right) [T] \quad (4.6)$$

using the eqs. 1.26, 1.25 and algebraic manipulation

$$\frac{d[S_0]}{dt} = - \left(k_{ta}[A] + \frac{S_\Delta k_{os} k_{ot}[^3O_2]}{k_d + k_{oa}[A]} ([S_0] + \delta) \right) \frac{1}{k_{ot} \frac{k_p}{k_{ot}} + [^3O_2] + \frac{k_{ta}[A]}{k_{ot}}} \frac{\phi_t}{hc/\lambda} \frac{\sigma_a(\lambda)}{F_{ex}(z, \lambda)} [S_0] \quad (4.7)$$

using the eqs. 1.35 and 1.36

$$\frac{d[S_0]}{dt} = - \left(\gamma + \frac{S_\Delta k_{os}[^3O_2]}{k_d + k_{oa}[A]} ([S_0] + \delta) \right) \frac{\phi_t}{\beta + \gamma + [^3O_2]} \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(z, \lambda) [S_0] \quad (4.8)$$

$$= -((\gamma + C_2\delta) + C_2[S_0])C_1[S_0] \quad (4.9)$$

where,

$$C_1 = \frac{\phi_t}{\beta + \gamma + [^3O_2]} \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(z, \lambda) \quad (4.10)$$

and

$$C_2 = \frac{S_\Delta k_{os}[^3O_2]}{k_d + k_{oa}[A]} \quad (4.11)$$

finally

$$\frac{d[S_0]}{dt} = -(1 + \frac{C_2}{(\gamma + C_2\delta)} [S_0])(\gamma + C_2\delta)C_1[S_0] \quad (4.12)$$

Now, for $\delta > 0$, we assume that $\frac{C_2}{(\gamma + C_2\delta)} \ll 1$. Therefore, $(1 + \frac{C_2}{(\gamma + C_2\delta)} [S_0]) \approx 1$. In that way, the above equation can be written as

$$\frac{d[S_0]}{dt} = -(\gamma + C_2\delta)C_1[S_0] \quad (4.13)$$

This last equation is easy to integrate.

$$[S_0](t) = [S_0](0)e^{-(\gamma + C_2\delta)C_1 t} \quad (4.14)$$

By comparison between eq. 4.3 with eq. 4.14, the dependence of α with wavelength, the energy fluence rate and the oxygen concentration was determined.

$$\alpha = (\gamma + C_2\delta)C_1 \quad (4.15)$$

$$\alpha = \left(\gamma + \frac{S_\Delta k_{os} [^3O_2]}{k_d + k_{oa}[A]} \delta \right) \frac{\phi_t}{\beta + \gamma + [^3O_2]} \frac{\sigma_a(\lambda)}{hc/\lambda} F_{ex}(z, \lambda) \quad (4.16)$$

The experimental values for α are shown in Tables 11, 12 and 13. Here each table corresponding at a macro experiments. α_{blue} , α_{red} and α_{br} are associated with blue, red and combination blue and red sources.

Table 11 – α values for Soret band and Q-bands considering the experimental measure of Figure 23.

Time decay $\times 10^{-3}(s^{-1})$	408 nm	506 nm	540 nm	575 nm	630 nm
α_{blue}	3.7 ± 0.7	2.45 ± 0.04	3.6 ± 0.3	3.5 ± 0.7	2.9 ± 0.9
α_{red}	0.26 ± 0.05	0.21 ± 0.05	0.26 ± 0.06	0.26 ± 0.06	0.32 ± 0.05
α_{br}	4.3 ± 0.2	2.6 ± 0.7	3.6 ± 0.6	3.8 ± 0.7	4.0 ± 1.0
$\alpha_{blue} + \alpha_{red}$	3.9 ± 0.7	2.66 ± 0.06	3.8 ± 0.3	3.8 ± 0.7	3.2 ± 0.9

Source: By the author.

Table 12 – α values for Soret band and Q-bands considering the experimental measure of Figure 24.

Time decay $\times 10^{-3}(s^{-1})$	408 nm	506 nm	540 nm	575 nm	630 nm
α_{blue}	3.2 ± 0.4	2.1 ± 0.8	2.5 ± 0.9	2.0 ± 1.0	1.0 ± 1.0
α_{red}	0.25 ± 0.04	0.20 ± 0.05	0.25 ± 0.07	0.25 ± 0.06	0.32 ± 0.1
α_{br}	3.5 ± 0.1	2.5 ± 0.3	3.4 ± 0.5	3.2 ± 0.6	3.0 ± 0.8
$\alpha_{blue} + \alpha_{red}$	3.5 ± 0.4	2.3 ± 0.8	2.8 ± 0.9	3.0 ± 1.0	1.0 ± 1.0

Source: By the author.

Table 13 – α values for Soret band and Q-bands considering the experimental measure of Figure 25.

Time decay $\times 10^{-4}(s^{-1})$	408 nm	506 nm	540 nm	575 nm	630 nm
α_{blue}	2.0 ± 1.0	1.9 ± 0.3	2.4 ± 0.5	2.2 ± 0.7	2.6 ± 0.7
α_{red}	2.97 ± 0.02	2.5 ± 0.1	3.0 ± 0.2	2.9 ± 0.3	4.2 ± 0.3
α_{br}	5.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	5.0 ± 1.0
$\alpha_{blue} + \alpha_{red}$	5.0 ± 1.0	4.4 ± 0.4	5.4 ± 0.5	5.1 ± 0.7	6.8 ± 0.8

Source: By the author.

For Soret band and Q-bands in the first two macro experiments (Table 11 and Table 12), it's verified numerically that $\alpha_{br} \geq \alpha_{blue}$ and $\alpha_{blue} > \alpha_{red}$. This is also visible

in the Figures 23 and 24. Since, to get the dose of photons from $1 \times 10^{19} \text{ Phot.cm}^{-3}$, experiments 2 and 3 need a short treatment time. Instead, Table 13 shows that $\alpha_{br} > \alpha_{red}$ and $\alpha_{red} > \alpha_{blue}$.

In order to analyze the photobleaching effect on PpIX by the light sources blue, red and together blue plus red, Figure 26 describes graphically the relation between α_{br} and $\alpha_{blue} + \alpha_{red}$. These figures were constructed by the α values of the preceding tables.

Figure 26 – Comparison between α_{br} with $\alpha_{blue} + \alpha_{red}$ for macro experiment (a) 1, (b) 2 and (c) 3.

Source: By the author.

Figure 26 shows in all experiments that α_{br} is equal or approximately equal to $\alpha_{blue} + \alpha_{red}$. This result suggests that the decrease in PpIX concentration caused by the two sources together have the same effect caused by the sum of the two sources. Therefore, for a multi-wavelength source is expected that all the emission bands that activate the Soret band and Q-bands it contribute to the decrease of the PpIX concentration. In this sense, the use of sunlight or a white light source is justified for the realization of PDT with PpIX.

4.2.3 Analysis of Soret and Q-bands as function of dose of photons

Beyond knowing the change in PpIX concentration using different light sources, we seek to understand the relationship between photobleaching of PpIX with the number of absorbed photons (photon dose). It follows an analysis for the Soret band and Q-bands for all macro experiments.

Figure 27 – Normalized PpIX absorbance decrease as a function of photon dose for Soret and Q-bands using Blue 1, Red and combination Blue 1 and Red. Error bars are standard deviations from three experimental measurements. These results were obtained from Figure 19.

Source: By the author.

Figure 28 – Normalized PpIX absorbance decrease as a function of photon dose for Soret and Q-bands using Blue 1, Red and combination Blue 1 and Red. Error bars are standard deviations from three experimental measurements. These results were obtained from Figure 20.

Source: By the author.

For each of the macro experiments, the photon dose was determined, this dose was expressed on the x-axis as the number of photons absorbed, with units of Phot.cm^{-3} .

It's observed that in macro experiments 1 and 2, the red source has a higher photon dose. This is not a surprise, since as was determined in the Table 8, to get $1 \times 10^{19} \text{ Phot.cm}^{-3}$, treatment times of 2.61, 41.61 and 4.45 minutes are required for experiments 1, 2 and

3 respectively. The experiments did not exactly follow these treatment times. On the other hand, in the macro experiment 3, the treatment times are corrected, it complies approximately with $1 \times 10^{19} \text{ Phot.cm}^{-3}$ as photon dose.

Figure 29 – Normalized PpIX absorbance decrease as a function of photon dose for Soret and Q-bands using Blue 2, Red and combination Blue 2 and Red. Error bars are standard deviations from three experimental measurements. These results were obtained from Figure 22.

Source: By the author.

In all experiments, it's observed that PpIX concentration decrease as a function of the number of photons absorbed and, it does not depend on the light source. Therefore,

for a light source that overlaps with the absorption spectrum of PpIX, the photobleaching effect will depend on the number of photons absorbed.

4.2.4 Analysis of formation of photoproduct

In this subsection was discussed the photoproduct absorbance amplitude. It follows an analysis of the absorption band in 653 nm as a function of the treatment time and the number of photons absorbed.

Figure 30 – Photoproduct absorbance amplitude as a function of (a) time and (b) photon dose. These results were obtained from Figure 19.

Source: By the author.

Figure 31 – Photoproduct absorbance amplitude as a function of (a) time and (b) photon dose. These results were obtained from Figure 20.

Source: By the author.

Figure 32 – Photoprodut absorbance amplitude as a function of (a) time and (b) photon dose. These results were obtained from Figure 22.

Source: By the author.

Figures 30, 31 and 32 were obtained from the macro experiments 1, 2 and 3 respectively. The maximum peak is in $\lambda = 653 \text{ nm}$ and, the error bars are standard deviations from three experimental measurements. All figures show that the experiments 1 (Blue) and 3 (Blue and Red) produce more Pp than experiment 2 (Red). This compartment was observed in relation to the exposure time and the number of absorbed photons.

The macro experiment 3 (Figure 32) has the less separation of photoprodut absorbance amplitude between the experiments 1, 2 and 3. This means that the photoprodut absorbance amplitude depends on the exposure time. Consequently, it depends on absorbed photons (see Tables 8 and 9).

Despite the difference in the photoprodut absorbance amplitude in the macro experiments, the amount of photoprodut generated does not significantly affect the decrease of the PpIX concentration.

4.3 Experimental and theoretical change in the PpIX concentration

An adjustment was carried out considering only the Soret band of macro experiment 1. The equation (3.7) and the experimental data of the subfigure 23 (a) were used. The equation (3.7) with total absorbed photon rate can be used to predict the temporal change of PpIX concentration. Also, it's possible to predict the change of PpIX concentration as a function of photon dose, since the fractionated dose photons are evaluated by treatment time. On the other hand, to obtain the decrease of PpIX concentration for the red source and combination blue and red sources, only the equation 3.12 is altered. The specific values for this equation were listed in the table 9.

The equation (3.7) was resolved considering a constant concentration of oxygen, $40 \mu\text{M}$. The PpIX and δ concentrations were 5 and $100 \mu\text{M}$ respectively. The adjustment

values for specific constants are: $\phi_t = 0.831$,¹⁸ $\mu = 9.1 \times 10^{-5} (\mu M)^{-1}$,¹⁷ $\beta = 11.7 (\mu M)$,¹⁷ $\gamma = 5.26 \times 10^{-10} (\mu M)$ and $S_{\Delta} \phi_t \frac{k_{oa}[A]}{k_d + k_{oa}[A]} = 6.27 \times 10^{-8}$. The mathematical expressions for μ , β and γ are described respectively by the equations (1.33), (1.35) and (1.36), where the transitions constant were listed in table 1. Additionally, γ , $S_{\Delta} \phi_t \frac{k_{oa}[A]}{k_d + k_{oa}[A]}$ and δ were adjusted in this study. The simulation was resolved using the software Wolfram Mathematica 11.3 version, in a personal computer. Processor Intel(R) Core(TM) i5-4460, CPU 3.20 GHz. Memory 8.00 GB. 64-bit operating system.

Figure 33 shows the prediction and measures of photobleaching experiments. The theoretical results indicate a reasonable approximation to reality. In relation to the number of photons absorbed, the simulation shows the same behavior for the three sources. Therefore, the photobleaching effect does not depend on the light source, it depends on the number of absorbed photons.

Figure 33 – Prediction of the normalized PpIX absorbance for the macro experiments (a) 1, (b) 2 and (c) 3. The decrease of PpIX is plotted as a function of the exposure time and the photon dose. The points are the experiment measurements at $\lambda = 408 \text{ nm}$. The lines are the theoretical predictions. The error bars are standard deviations from measurements on three experiments.

Source: By the author.

4.4 Light distribution into epidermis and dermis

The simulation of the light distribution into tissue was carried out using MXCLAB. The results offer information about the flux of photons in any position within the volume of OP. The next subfigures show the spatial migration of photons for a multi-wavelength. The energy for these photons was associated with wavelengths between 400 to 700 nm.

Continue

Continuation

Figure 34 – Results of Monte Carlo simulation for light distribution into tissue using a broad spectrum.

Source: By the author.

Figure 34 shows the light distribution in the plane XZ . Where the coordinate z is the depth. The horizontal line in $z = 0.2$ mm is the border between epidermis and dermis. The results in all subfigures indicated symmetry around a vertical imaginary line in 5 mm over the axis x . In relation to the flux of photons, the photons with $\lambda = 400, 410, 420, 430$, and 440 nm has the less penetration into tissue. On the other hand, photons with $\lambda > 600$ nm has the best spatial distribution, this means that it is more likely to find a photon of this wavelength in a region deeper in the tissue.

With the results of Figure 34 was performed the next steps: to convert the flux in intensity and make the weighting with the Sun spectrum (Figure 35). To achieve it, a program was developed for each voxel using again the software Wolfram Mathematica 11.3 version. This part was explained in the last section of the methodology. It is important to mention that sunlight is a specific example of a light source. With the results of Figure 34 is possible to use other spectrum.

Figure 35 – (a) Real measure of Sun spectrum. (b) Monte Carlo simulation of Sun spectrum at position $(x,y,z)=(5,5,0.1)$ mm.

Source: By the author.

Figure 36 shows the intensity or irradiance for arbitrary positions within the volume of OP. The choice depths were 0.2, 1.0 and 3.0 mm.

Figure 36 – Some examples of the construction of the spectrum of sunlight for arbitrary positions within the volume of OP.

Source: By the author.

The high intensity was in the position (5,5,0.1) mm. It is reasonable, because, the position of illumination source for Monte Carlo simulation was (5,5,0) mm. The results demonstrate that irradiance decrease as a function of the position. In addition, far from the location of the source, the irradiance with λ between 600 to 700 nm was greater than other wavelengths.

4.5 3D PDT dose and cell survival fraction

In this section, the PDT dose and the cell survival fraction were simulated using the light distribution into OP and the mathematical model. Now, the oxygen concentration was not considered constant. Therefore, the equations (3.7), (3.8) and (3.9) were resolved considering the same adjustment values for ϕ_t , μ , β , γ and $S_{\Delta}\phi_t \frac{k_{oa}[A]}{k_d + k_{oa}[A]}$, see Table 14.

Table 14 – Initial conditions for the simulation.

Parameters	Value	Units	Reference
$[S_0]$	5	μM	Liu et al (2010)
$[^3O_2]$	40	μM	Sánchez et al (2017)
δ	100	μM	In this study
ϕ_t	0.831	–	Liu et al (2010)
μ	9.1×10^{-5}	μM^{-1}	Wang et al (2010)
β	11.7	μM	Wang et al (2010)
γ	5.26×10^{-10}	μM	In this study
$S_{\Delta} \phi_t \frac{k_{oa}[A]}{k_d + k_{oa}[A]}$	6.27×10^{-8}	–	In this study

Source: By the author.

Inside of the OP is placed to a known amount of PS and oxygen with distributions homogeneous. This means, the same PpIX and O_2 concentrations by voxel, 5 and 40 μM respectively. A schematic representation was described in the last section of the methodology.

Figure 37 – Visualization of theoretical PDT dose for the planes (a) XY and (b) XZ. The PDT dose in this work considers the mechanism type I y II.

Source: By the author.

Figures 37 and 38 are respectively the graphical representation of the evaluation of the equations (3.9) and (3.15) for the last treatment time (30 minutes). This treatment time is different from that shown in Table 8, since it was estimated with the simulated source, under the same criteria, to deliver $1 \times 10^{19} Phot.cm^{-3}$. This Dose of photons was calculated just for the voxel at the position (5,5,0.1) mm. On the other hand, for the cell survival fraction, 300 μM was the value for $[^1O_2]_{rx0}$. This value is a specific threshold for the singlet oxygen.³⁸

Sub Figures 37 (a) and (b) show that the maximum of PDT dose is greater than $400 \mu M$. This means, that the theoretical results overcome the threshold of singlet oxygen. Also, for the plane XY, the maximum value of PDT dose, where it is guaranteed there is necrosis to have a diameter of about 0.3 mm. Instead, for the plane XZ, the maximum depth of necrosis is about 0.2 mm.

Figure 38 – Visualization of theoretical Cell survival fraction for the planes (a) XY and (b) XZ.

Source: By the author.

Sub Figures 38 (a) and (b) show that considering the threshold of $300 \mu M$, there is 20 percent that of cell survival in the region near of the light source (blue region). If 20 percent is enough to guaranteed necrosis, then, the diameter and depth where it occurs are about 0.3 mm and 0.2 mm respectively.

In order to compare the theoretical results obtained for PDT dose and cell survival fraction, it continues a comparison with similar studies that worked with a light source of a broad spectrum.

Unlike the study of Vignion et. al. (2015)³⁹ that compare the photodynamic effect for different light sources by standard protocols of light dose. This study strives to provide an equivalent photon dose with the hypothesis that the total photodynamic effect depends on the number of photons that activate the PpIX. On the other hand, the effective fluence (with units J_{eff}/cm^2) used by Marra et. al. (2018)³⁶ and LaRochelle et. al. (2019)⁴⁰ was calculated by the multiplication of normalized absorption spectrum of PpIX with the emission spectrum of the light source. In this study, of number of photons absorbed or photon dose was calculated by multiplication between the treatment time, absorption spectrum of PpIX and emitted photons of the light source (with units $Photons/cm^3$), it was shown in equation 4.2. The main difference between effective fluence and photon dose

is the absorption spectrum of the PpIX. Since, Marra and LaRochelle worked with the normalized absorption spectrum of PpIX.

In the present study, we show that the photodynamic effect for a multi-wavelength can be estimated by a photon dose. Our results offer 3D visualization for damaged tissue. Considering a threshold of $300\ \mu M$ and 20 % as a good prediction of cellular death, the maximum depth for 30 minutes of treatment and with a sunlight source is about 0.2 mm. This depth is 6 times less than reported by LaRochelle et. al.⁴⁰ It's likely that this difference is because the dimensions of the 3D computerized phantom, the radius of the simulated source and optical properties aren't equally.

5 CONCLUSIONS

In this study, we evaluated the light distribution and PDT dose inside of a 3D computerized phantom. The methodological approach was based on three main points: photobleaching experiments for PpIX, a mathematical model for PDT and Monte Carlo simulation for photon migration. Furthermore, with the combination of the mathematical model and the distribution of the light inside of OP was possible to estimate the PDT dose and cell survival fraction for a multi-wavelength source.

The photobleaching experiments indicated that for $1 \times 10^{19} [Phot.cm^{-3}]$ as photon dose, the decrease in PpIX concentration follows an additive property. Since by exciting the Soret band (408 nm) and the last Q-band (630 nm) with respectively a blue and red sources, the parameter associated with decay time, α , showed in all experiments that α_{br} is equal or approximately equal to $\alpha_b + \alpha_r$. This means that the decrease of the PpIX concentration produced with the combination of blue and red sources is equivalent to have blue and red sources separate.

Once more, for equal photon dose, the experimental results proved that the decrease in the PpIX concentration is independent of the wavelength that activates the PpIX. Consequently, the decrease in PpIX concentration depends on the number of absorbed photons. On the other hand, the experimental results showed that the amplitude of the formation of the photoproduct in 653 nm depends on the rate of absorbed photons.

A mathematical model was developed to simulate the change of PpIX concentration as a function of treatment time and photon dose. This model incorporates the two mechanisms of the PDT. Also, the overlap between the spectrum of absorption and emission of the PpIX and the light source respectively was taken into account. As an approximation, the oxygen concentration was considered constant. In this approximation was possible to obtain an expression for α as a function of the wavelength, the energy fluence rate and the oxygen concentration. The simulated results showing that the model generates a reasonable prediction for analysis of the absorbance of PpIX using blue, red and combination blue and red light sources.

The light distribution considering optical properties of epidermis and dermis was simulated for a broad spectrum, $\lambda \in [400, 700] nm$. A weighting with a real measure of the solar spectrum was developed. With these results was possible build the sunlight irradiance for any location inside of 3D computerized phantom. For depth of 0.1 mm, the necessary time to obtain $1 \times 10^{19} [Phot.cm^{-3}]$ as photon dose was about 30 minutes. Also, the results showed that wavelengths higher than 600 nm have better penetration into tissue. This is reasonable due to the low absorption coefficient for epidermis and dermis in this region.

On the other hand, for surface treatments with PDT, it is possible to use wavelengths less than 600 nm, since, as demonstrated in the experiments of photobleaching of the PpIX, for equal photon dose, the photodynamic effect is the same.

The mathematical model was extended for estimate the theoretical PDT dose and cell survival fraction inside of a 3D computerized phantom. These parameters were determined considering the simulation of the light distribution into the tissue, 30 minutes as treatment time and $300\mu M$ as PDT threshold. The model estimate a PDT dose upper $400\mu M$ with 20 % of probability of cell survival fraction in the proximity of the source with the biological tissue. Therefore, we estimate that the efficacy of PDT, where it is guaranteed there is necrosis, has a diameter and depth of about 0.3 and 0.2 mm respectively.

Moving forward, this mathematical model could be extended to include PpIX production, an skin phantom model with more layers, optical properties of some lesion, and the light propagation of other sources.

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Appendix

APPENDIX A – PPIX PHOTBLEACHING SIMULATION CONSIDERING CONSTANT OXYGEN CONCENTRATION

A.1 Simulation of PpIX concentration for different light sources

Absorption coefficient of PpIX

```

λb2 = Import["PE/abs_02/abs02_blue.txt", "Table"][[All, 1]];
      |importa                                |tudo
Abt02 = Import["PE/abs_02/abs02_blue.txt", "Table"][[All, 2]];
      |importa                                |tudo
μb02 = Interpolation[Join[Table[{λb2[[1]], Abt02[[1]] / 1}, {1, 1, 500}]]]; (*1/cm*)
      |interpolação      |junta |tabela
μb0 = μb02;

```

Different light sources

```

(*Red LED*)
λRB = Import["RedBlue/Red/160R.txt", "Table"][[All, 1]];
      |importa                                |tudo
Red160 = Import["RedBlue/Red/160R.txt", "Table"][[All, 2]] ; (*uW/cm^2/nm*)
      |importa                                |tudo
Red168 = Import["RedBlue/Red/168R.txt", "Table"][[All, 2]] ;
      |importa                                |tudo
Red177 = Import["RedBlue/Red/177R.txt", "Table"][[All, 2]] ;
      |importa                                |tudo
ReduW150 = Table[Mean[{Red160[[i]], Red168[[i]], Red177[[i]]}], {i, 1, 3647}];
      |tabela |média
(*Blue 1 LED*)
λB1 = Import["RedBlue/LedBR.txt", "Table"][[All, 1]];
      |importa                                |tudo
BlueuW = Import["RedBlue/LedBR.txt", "Table"][[All, 3]] ; (*uW/cm^2/nm*)
      |importa                                |tudo
(*Blue 2 LED*)
λB2 = Import["RedBlue/B0011A.txt", "Table"][[All, 1]];
      |importa                                |tudo
b2AuW = Import["RedBlue/B0011A.txt", "Table"][[All, 2]];
      |importa                                |tudo
(*Sun*)
λsun = Import["TESTE_SOL_10h40_Capture27_10-42-23-056.txt", "Table"][[All, 1]]; (*nm*)
      |importa                                |tudo
iuWs = Import["TESTE_SOL_10h40_Capture27_10-42-23-056.txt", "Table"][[All, 2]];
      |importa                                |tudo
(*uW/cm^2/nm*)
(*Spectral irradiance*)
RedmW150 = ReduW150 / (1000); (*mW/cm^2/nm*)
BlueuW = BlueuW / (1000); (*mW/cm^2/nm*)
bluemW = b2AuW / (1000); (*mW/cm^2/nm*)
ImWs = iuWs / (1000); (*mW/cm^2/nm*)

```

```

(*Red LED*)
IredmW150 = Table[( $\lambda_{RB}[1+1] - \lambda_{RB}[1]$ )(RedmW150[1+1] + RedmW150[1])/2),
  {1, 1, 3646}];
ired150 = Interpolation[Join[Table[{ $\lambda_{RB}[1]$ , IredmW150[1]}], {1, 1, 3646}]]];
(*mW/cm^2*)

(*Blue 1 LED*)(*OBS. Don't run Blue 1 and Blue 2 together*)
(**)
IbluemW = Table[( $\lambda_{B1}[1+1] - \lambda_{B1}[1]$ )(BluemW[1+1] + BluemW[1])/2),
  {1, 1, 3646}];
ibblue = Interpolation[Join[Table[{ $\lambda_{B1}[1]$ , IbluemW[1]}], {1, 1, 3646}]]];
(*mW/cm^2*)

(*Blue 2 LED*)
(*)
IbluemW2A = Table[( $\lambda_{B2}[1+1] - \lambda_{B2}[1]$ )(bluemW[1+1] + bluemW[1])/2),
  {1, 1, 3646}];
ibblue = Interpolation[Join[Table[{ $\lambda_{B2}[1]$ , IbluemW2A[1]}], {1, 1, 3646}]]];
(*mW/cm^2*)
*)

(*Sun*)
Isunmw = Table[( $\lambda_{sun}[1+1] - \lambda_{sun}[1]$ )(ImWs[1+1] + ImWs[1])/2),
  {1, 1, 3646}];
isun = Interpolation[Join[Table[{ $\lambda_{sun}[1]$ , Isunmw[1]}], {1, 1, 3646}]]];
(*mW/cm^2*)

```

Determination of the constants

```

MPpIX = 562.6581; (*g/mol*)
ρPpIX = 1.27; (*g/cm³*)
NA = 6.022*1023; (*molec/mol*)

NPpIX = (ρPpIX NA) / MPpIX (*molec/cm³*)
Kf = 1*109; (*1.0*109/N*)(*s-1*)
Kp = 3.9*103; (*3.9+0.4*103 s-1*)
Kisc = 8.0*108; (*s-1*)
Kd = (1) 0.4*106; (*s-1*)
Kot = (1) 1.9*103; (*uM-1s-1*)(*1.9+0.2*103 M-1s-1*)
Kos = (1) 10; (*uM-1s-1*)
Koa = 132.53; (*uM-1s-1*)
KoaA = 1.1*105; (*1/s*)
KtaA = (1*10-5) 0.1; (*s-1*)

```

```

IBLUE = Total[Table[(i) (μb0[i]) iblue[i] Exp[-μb0[i]] (1 / (h c NPpIX)),
                    |total |tabela |exponencial
                    {i, 350, 700}]];
IRED = Total[Table[(i) (μb0[i]) ired150[i] Exp[-μb0[i]] (1 / (h c NPpIX)),
                    |total |tabela |exponencial
                    {i, 350, 700}]];
IBR = Total[Table[(i) (μb0[i]) (iblue[i] + ired150[i]) Exp[-μb0[i]] (1 / (h c NPpIX)),
                    |total |tabela |exponencial
                    {i, 350, 700}]];

(**)
SΔ = 0.5;
φt = (1.87) Kisc / (Kf + Kisc) (*sin unidades*)
μ = Kos / (KoaA) (*1/uM*)
β = (5.7) Kp / Kot (*uM*)
ξ = (0.7*^6) (1*^-12) (SΔ φt) (KoaA / (Kd + KoaA)) (*IBR*)
γ = KtaA / Kot (*6*^-9*)

```

Solution of differential equation

```

(*Initial conditions*)
δr = 100; (*uM*)
OxyR = 40; (*uM*)
S0R = 5; (*uM*)

(*Differential equations*)
SoluBlue =
NDSolve[
|resolve numericamente equação diferencial
{SB'[t] == -((φt γ) / (β + γ + OxyR)) (IBLUE) SB[t] -
μ ξ (IBLUE) (OxyR / (β + γ + OxyR)) (SB[t] + δr) SB[t], SB[0] == S0R}, SB,
{t, 0, TimeB}];

SoluRed =
NDSolve[
|resolve numericamente equação diferencial
{SRed'[t] == -((φt γ) / (β + γ + OxyR)) (IRED) SRed[t] -
μ ξ (IRED) (OxyR / (β + γ + OxyR)) (SRed[t] + δr) SRed[t], SRed[0] == S0R},
SRed, {t, 0, TimeR}];

SoluRB =
NDSolve[
|resolve numericamente equação diferencial
{SRB'[t] == -((φt γ) / (β + γ + OxyR)) (IBR) SRB[t] -
μ ξ (IBR) (OxyR / (β + γ + OxyR)) (SRB[t] + δr) SRB[t], SRB[0] == S0R},
SRB, {t, 0, TimeRB}];

```

Plot of results

(*PpIX and red source*)

```
PSR150 = Plot[Evaluate[ (SRed[t] / S0R) /. SoluRed], {t, 0, TimeR}, Frame → True,
  |gráf... |calcula |quadro |verdadeiro
  PlotRange → {{0, TimeR}, {0, 1}}, LabelStyle → {GrayLevel[0], Bold},
  |intervalo do gráfico |estilo de etiqueta |tons de cinzas |negrito
  FrameLabel → {"Time [s]", "PpIX concentration [μM]"}, PlotStyle → RGBColor[1, 0, 0]];
  |legenda do quadro |estilo do gráfico |cores do sistema RGB
```

(*PpIX and blue 1 source*)

```
PSBlue = Plot[Evaluate[ (SB[t] / S0R) /. SoluBlue], {t, 0, TimeB}, Frame → True,
  |gráf... |calcula |quadro |verdadeiro
  PlotRange → {{0, TimeR}, {0, 1}}, LabelStyle → {GrayLevel[0], Bold},
  |intervalo do gráfico |estilo de etiqueta |tons de cinzas |negrito
  FrameLabel → {"Time [s]", "PpIX concentration [μM]"}, PlotStyle → RGBColor[0, 0, 1]];
  |legenda do quadro |estilo do gráfico |cores do sistema RGB
```

(*PpIX and combination blue1 and red*)

```
PSRedBlue = Plot[Evaluate[ (SRB[t] / S0R) /. SoluRB], {t, 0, TimeRB}, Frame → True,
  |gráf... |calcula |quadro |verdadeiro
  PlotRange → {{0, TimeR}, All}, LabelStyle → {GrayLevel[0], Bold},
  |intervalo do gráfico |tudo |estilo de etiqueta |tons de cinzas |negrito
  FrameLabel → {"Time [s]", "PpIX concentration [μM]"}, PlotStyle → Green];
  |legenda do quadro |estilo do gráfico |verde
```

(**)

```
RedBlueD2 = Show[PSR150, PSRedBlue, PSBlue]
  |mostra
```

APPENDIX B – LIGHT DISTRIBUTION THROUGH OF 3D COMPUTARIZED PHANTON

B.1 Monte Carlo simulation for a multi-wavelength using MCXLAB

```

clear;
close;

%% preparing the input data
% set seed to make the simulation repeatible
cfg.seed=hex2dec('623F9A9E');

cfg.nphoton=5e7;

% define a 2 layer structure
cfg.vol=ones(101,101,50);
cfg.vol(:,:,3:end)=2;
% cfg.vol=ones(51,51,25);
% cfg.vol(:,:,10:end)=2;
cfg.vol=uint8(cfg.vol);

cfg.srctype='disk';
cfg.srcparam1=[2 0 0];

%%
cfg.unitinmm=0.1; %mm

% define the source position
cfg.srcpos=[51,51,0];
cfg.srkdir=[0 0 1];

cfg.prop=[0 0 1 1 %400 Epidermis/Dermis
0.42 21.8 0.9 1.463877877
1.78 21.8 0.9 1.39517757];

% cfg.prop=[0 0 1 1 %410
% 0.39 21.7 0.9 1.460456241
% 2.88 21.7 0.9 1.394433819];

% cfg.prop=[0 0 1 1 %420
% 0.36 21.5 0.9 1.458477174
% 3.23 21.5 0.9 1.392755488];

% cfg.prop=[0 0 1 1 %430
% 0.33 21.3 0.9 1.455956986
% 2.57 21.3 0.9 1.391450554];

% cfg.prop=[0 0 1 1 %440
% 0.31 21.2 0.9 1.45397834
% 1.62 21.2 0.9 1.390332971];

% cfg.prop=[0 0 1 1 %450
% 0.29 21 0.9 1.451277778
% 0.566 21 0.9 1.389588785];

% cfg.prop=[0 0 1 1 %460
% 0.27 20.8 0.9 1.449479085
% 0.258 20.8 0.9 1.388658118];

% cfg.prop=[0 0 1 1 %470
% 0.25 20.7 0.9 1.448402308
% 0.189 20.7 0.9 1.387353619];

```

```
% cfg.prop=[0 0 1 1 %480
% 0.23      20.5  0.9   1.447685859
% 0.156     20.5  0.9   1.386796349];

% cfg.prop=[0 0 1 1 %490
% 0.22      20.3  0.9   1.447149783
% 0.149     20.3  0.9   1.386239513];

% cfg.prop=[0 0 1 1 %500
% 0.2 20.2  0.9   1.444990763
% 0.15      20.2  0.9   1.385869159];

% cfg.prop=[0 0 1 1 %510
% 0.19      20    0.9   1.444093939
% 0.16      20    0.9   1.385312323];

% cfg.prop=[0 0 1 1 %520
% 0.18      19.8  0.9   1.443016742
% 0.194     19.8  0.9   1.384381656];

% cfg.prop=[0 0 1 1 %530
% 0.17      19.7  0.9   1.440857722
% 0.283     19.7  0.9   1.38363747];

% cfg.prop=[0 0 1 1 %540
% 0.16      19.5  0.9   1.440321646
% 0.362     19.5  0.9   1.382893719];

% cfg.prop=[0 0 1 1 %550
% 0.15      19.3  0.9   1.439424822
% 0.338     19.3  0.9   1.382523364];

% time-domain simulation parameters
cfg.tstart=0;
cfg.tend=5e-9;
cfg.tstep=5e-9;

% GPU thread configuration
cfg.autopilot=1;
cfg.gpuid=1;

%% running simulation with boundary reflection enabled
cfg.isreflect=1; % enable reflection at exterior boundary
cfg.isrefint=1; % enable reflection at interior boundary too
cfg.isnormalized=1;%normalizacao
tic;
[f2]=mcxlab(cfg);
toc;

%%Para imprimir los datos
aux=f2.data(:,:,:)
save('teste400.mat','aux');
```

APPENDIX C – SIMULATION OF PDT DOSE AND CELL SURVIVAL FRACTION THROUGH OF 3D COMPUTARIZED PHANTOM

C.1 Theoretical evaluation of PDT dose and cell survival fraction for a multi-wavelength

```

h = 6.626*^-34; (*Constante de Planck [Js]*)
c = 3*^8; (*Velocidad de la luz [m/s]*)
λTot = Table[1, {1, 400, 700, 10}]; (*[nm]*)
ET = Table[(1*^5) (1*^9) (h c) / λTot[[i]], {i, 1, 31}]; (*[J] → [mW/cm²]*)

f = 320; (*Adjustment constant*)
SPon = f * Table[isun[1 + 10] / isun[400], {1, 400, 690, 10}];

```

Monte Carlo results for a multi-wavelength and weighting with sunlight

```

DMF400 = Import["Datos_Matlab/teste400.mat"]; df400R = ET[[1]] * DMF400[[1]];
DMF410 = Import["Datos_Matlab/teste410.mat"]; df410R = SPon[[1]] * ET[[2]] * DMF410[[1]];
DMF420 = Import["Datos_Matlab/teste420.mat"]; df420R = SPon[[2]] * ET[[3]] * DMF420[[1]];
DMF430 = Import["Datos_Matlab/teste430.mat"]; df430R = SPon[[3]] * ET[[4]] * DMF430[[1]];
DMF440 = Import["Datos_Matlab/teste440.mat"]; df440R = SPon[[4]] * ET[[5]] * DMF440[[1]];
DMF450 = Import["Datos_Matlab/teste450.mat"]; df450R = SPon[[5]] * ET[[6]] * DMF450[[1]];
DMF460 = Import["Datos_Matlab/teste460.mat"]; df460R = SPon[[6]] * ET[[7]] * DMF460[[1]];
DMF470 = Import["Datos_Matlab/teste470.mat"]; df470R = SPon[[7]] * ET[[8]] * DMF470[[1]];
DMF480 = Import["Datos_Matlab/teste480.mat"]; df480R = SPon[[8]] * ET[[9]] * DMF480[[1]];
DMF490 = Import["Datos_Matlab/teste490.mat"]; df490R = SPon[[9]] * ET[[10]] * DMF490[[1]];

```



```
(**)  
DMF500 = Import ["Datos_Matlab/teste500.mat"]; df500R = SPon[ [10]] × ET[ [11]] × DMF500[ [1]];  
|importa  
DMF510 = Import ["Datos_Matlab/teste510.mat"]; df510R = SPon[ [11]] × ET[ [12]] × DMF510[ [1]];  
|importa  
DMF520 = Import ["Datos_Matlab/teste520.mat"]; df520R = SPon[ [12]] × ET[ [13]] × DMF520[ [1]];  
|importa  
DMF530 = Import ["Datos_Matlab/teste530.mat"]; df530R = SPon[ [13]] × ET[ [14]] × DMF530[ [1]];  
|importa  
DMF540 = Import ["Datos_Matlab/teste540.mat"]; df540R = SPon[ [14]] × ET[ [15]] × DMF540[ [1]];  
|importa  
DMF550 = Import ["Datos_Matlab/teste550.mat"]; df550R = SPon[ [15]] × ET[ [16]] × DMF550[ [1]];  
|importa  
DMF560 = Import ["Datos_Matlab/teste560.mat"]; df560R = SPon[ [16]] × ET[ [17]] × DMF560[ [1]];  
|importa  
DMF570 = Import ["Datos_Matlab/teste570.mat"]; df570R = SPon[ [17]] × ET[ [18]] × DMF570[ [1]];  
|importa  
DMF580 = Import ["Datos_Matlab/teste580.mat"]; df580R = SPon[ [18]] × ET[ [19]] × DMF580[ [1]];  
|importa  
DMF590 = Import ["Datos_Matlab/teste590.mat"]; df590R = SPon[ [19]] × ET[ [20]] × DMF590[ [1]];  
|importa  
(**)  
DMF600 = Import ["Datos_Matlab/teste600.mat"]; df600R = SPon[ [20]] × ET[ [21]] × DMF600[ [1]];  
|importa  
DMF610 = Import ["Datos_Matlab/teste610.mat"]; df610R = SPon[ [21]] × ET[ [22]] × DMF610[ [1]];  
|importa  
DMF620 = Import ["Datos_Matlab/teste620.mat"]; df620R = SPon[ [22]] × ET[ [23]] × DMF620[ [1]];  
|importa  
DMF630 = Import ["Datos_Matlab/teste630.mat"]; df630R = SPon[ [23]] × ET[ [24]] × DMF630[ [1]];  
|importa  
DMF640 = Import ["Datos_Matlab/teste640.mat"]; df640R = SPon[ [24]] × ET[ [25]] × DMF640[ [1]];  
|importa  
DMF650 = Import ["Datos_Matlab/teste650.mat"]; df650R = SPon[ [25]] × ET[ [26]] × DMF650[ [1]];  
|importa  
DMF660 = Import ["Datos_Matlab/teste660.mat"]; df660R = SPon[ [26]] × ET[ [27]] × DMF660[ [1]];  
|importa  
DMF670 = Import ["Datos_Matlab/teste670.mat"]; df670R = SPon[ [27]] × ET[ [28]] × DMF670[ [1]];  
|importa  
DMF680 = Import ["Datos_Matlab/teste680.mat"]; df680R = SPon[ [28]] × ET[ [29]] × DMF680[ [1]];  
|importa  
DMF690 = Import ["Datos_Matlab/teste690.mat"]; df690R = SPon[ [29]] × ET[ [30]] × DMF690[ [1]];  
|importa  
DMF700 = Import ["Datos_Matlab/teste700.mat"]; df700R = SPon[ [30]] × ET[ [31]] × DMF700[ [1]];  
|importa
```


Matrix construction

```
(*Dimensiones, area (X0 y Z0)*)
X0 = Flatten[Table[i, {j, 1, 50}, {i, 1, 101}]]; (*mm*)
      |achatar |tabela
Z0 = Flatten[Table[i, {i, 1, 50}, {j, 1, 101}]]; (*mm*)
      |achatar |tabela
Y = 51;

(*-----Matriz {101,50}-----*)
MDZ400 = Flatten[Table[df400R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ410 = Flatten[Table[df410R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ420 = Flatten[Table[df420R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ430 = Flatten[Table[df430R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ440 = Flatten[Table[df440R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ450 = Flatten[Table[df450R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ460 = Flatten[Table[df460R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ470 = Flatten[Table[df470R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ480 = Flatten[Table[df480R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ490 = Flatten[Table[df490R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
MDZ500 = Flatten[Table[df500R[ [z7, Y, x7] ], {z7, 1, 50}, {x7, 1, 101}]];
      |achatar |tabela
```

```
MDZ510 = Flatten[Table[df510R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ520 = Flatten[Table[df520R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ530 = Flatten[Table[df530R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ540 = Flatten[Table[df540R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ550 = Flatten[Table[df550R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ560 = Flatten[Table[df560R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ570 = Flatten[Table[df570R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ580 = Flatten[Table[df580R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ590 = Flatten[Table[df590R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ600 = Flatten[Table[df600R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ610 = Flatten[Table[df610R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ620 = Flatten[Table[df620R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ630 = Flatten[Table[df630R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ640 = Flatten[Table[df640R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ650 = Flatten[Table[df650R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ660 = Flatten[Table[df660R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ670 = Flatten[Table[df670R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ680 = Flatten[Table[df680R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ690 = Flatten[Table[df690R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
MDZ700 = Flatten[Table[df700R[{z7, Y, x7}], {z7, 1, 50}, {x7, 1, 101}]];
|achatar |tabela
```

Interpolation

```

FZ400 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ400[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ410 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ410[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ420 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ420[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ430 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ430[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ440 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ440[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ450 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ450[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ460 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ460[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ470 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ470[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ480 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ480[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ490 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ490[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ500 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ500[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela

(**)
FZ510 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ510[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ520 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ520[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ530 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ530[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ540 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ540[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ550 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ550[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ560 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ560[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ570 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ570[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ580 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ580[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ590 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ590[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela
FZ600 = Interpolation[Join[Table[{Z0[1]}, X0[1], MDZ600[1]}], {1, 1, 5050}]]];
|interpolação      |junta |tabela

```

```

(**)
FZ610 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ610[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ620 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ620[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ630 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ630[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ640 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ640[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ650 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ650[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ660 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ660[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ670 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ670[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ680 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ680[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ690 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ690[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela
FZ700 = Interpolation[Join[Table[{Z0[[1]], X0[[1]], MDZ700[[1]]}, {1, 1, 5050}]]];
      |interpolação   |junta |tabela

```

Data manipulation

```

EuT = 50 Table[{1 / (h * c * NpPIX)} 1 μb0[[1]], {1, 400, 700, 10}];
      |tabela

FexTZ2 =
Table[{EuT[[1]] * FZ400[i, j], EuT[[2]] * FZ410[i, j], EuT[[3]] * FZ420[i, j],
      |tabela
      EuT[[4]] * FZ430[i, j], EuT[[5]] * FZ440[i, j], EuT[[6]] * FZ450[i, j],
      EuT[[7]] * FZ460[i, j], EuT[[8]] * FZ470[i, j], EuT[[9]] * FZ480[i, j],
      EuT[[10]] * FZ490[i, j], EuT[[11]] * FZ500[i, j], EuT[[12]] * FZ510[i, j],
      EuT[[13]] * FZ520[i, j], EuT[[14]] * FZ530[i, j], EuT[[15]] * FZ540[i, j],
      EuT[[16]] * FZ550[i, j], EuT[[17]] * FZ560[i, j], EuT[[18]] * FZ570[i, j],
      EuT[[19]] * FZ580[i, j], EuT[[20]] * FZ590[i, j], EuT[[21]] * FZ600[i, j],
      EuT[[22]] * FZ610[i, j], EuT[[23]] * FZ620[i, j], EuT[[24]] * FZ630[i, j],
      EuT[[25]] * FZ640[i, j], EuT[[26]] * FZ650[i, j], EuT[[27]] * FZ660[i, j],
      EuT[[28]] * FZ670[i, j], EuT[[29]] * FZ680[i, j], EuT[[30]] * FZ690[i, j],
      EuT[[31]] * FZ700[i, j]}, {i, 1, 50}, {j, 1, 101}];
(*mW/cm²*)

```

Solution of differential equations

```
 $\delta r = 100; (*uM*)$ 
OxyR = 40; (*uM*)
S0R = 5; (*uM*)
TTN = TSun(*3600*); (*2 horas*)
```

SoluSun =

```
Table[
|tabela
NDSolve[
|resolve numericamente equação diferencial
{PS'[t] == - ((phi t gamma) / (beta + gamma + O2S[t])) (ISTZ3[[i, j]]) PS[t] -
mu xi (ISTZ3[[i, j]]) (O2S[t] / (beta + gamma + O2S[t])) (PS[t] + delta r) PS[t],
O2S'[t] == - xi (ISTZ3[[i, j]]) (O2S[t] / (beta + gamma + O2S[t])) PS[t] +
goxy (1 - (O2S[t] / OxyR)),
OxS'[t] == ((phi t gamma) / (beta + gamma + O2S[t])) (ISTZ3[[i, j]]) PS[t] +
xi (ISTZ3[[i, j]]) (O2S[t] / (beta + gamma + O2S[t])) PS[t], PS[0] == S0R,
O2S[0] == OxyR, OxS[0] == 0}, {PS, O2S, OxS}, {t, 0, TTN}], {i, 1, 50}, {j, 1, 101}];
```

Data export

```
OrT = 300;
```

```
(**)
```

```
OxT = Table[Evaluate[OxS[TTN]] /. SoluSun[{m, n, 1, 3}], {m, 1, 50}, {n, 1, 101}];
|tabela |calcula
```

```
TT1 = Table[OxT[[i]], {i, 1, 50}];
|tabela
```

```
Export["TT1.xlsx", %];
|exporta
```

```
(**)
```

```
SVT = Table[Exp[-OxT[[i, j]] / OrT], {i, 1, 50}, {j, 1, 101}];
|tabela |exponencial
```

```
TT2 = Table[SVT[[i]], {i, 1, 50}];
|tabela
```

```
Export["TT2.xlsx", %];
|exporta
```