



Comparison of Wavelength-Dependent Penetration Depth of 532 nm and 660 nm Lasers in Different Tissue Types

Ali A. Aldalawi^{1,2}, Nursakinah Suardi^{1*}, Naser Mahmoud Ahmed¹, Mahdi A. S. Al-Farawn³, Mohammed Ali Dheyab^{1,4}, Wisam I. Jebur², Faisal J. Kadhim²

¹School of Physics, Universiti Sains Malaysia, Gelugor Penang, Malaysia

²Department of Vocational, Ministry of Education, Hilla, Iraq

³Department of Dentistry, Al-Amal University College for Medical Sciences, Karbala, Iraq

⁴Department of Physics, College of Science, University of Anbar, Ramadi, Iraq

*Correspondence to

Nursakinah Suardi,
Email: nsakinahsuardi@usm.my

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Abstract

Introduction: The depth of laser light penetration into tissue is a critical factor in determining the effectiveness of photodynamic therapy (PDT). However, the optimal laser light penetration depth necessary for achieving maximum therapeutic outcomes in PDT remains unclear. This study aimed to assess the effectiveness of laser light penetration depth at two specific wavelengths, 532 nm and 660 nm.

Methods: Chicken and beef of different thicknesses (1, 3, 5, 10, and 20 mm ± 0.2 mm) were used as in vitro tissue models. The samples were subjected to irradiation by a low-level laser diode of 532 and 660 nm in continuous mode for 10 minutes, with power densities of 167 and 142 J/cm², respectively. Laser light transmission through the tissue was measured using a power meter.

Results: For beef samples, the 660 nm wavelength achieved a maximum transmission intensity of 30.7% at 1 cm thickness, while the 532 nm laser had a transmission intensity of 6.5%. Similarly, in chicken breast samples, the maximum transmission occurred at 1 cm thickness with 68.1% for the 660 nm wavelength and 18.2% for the 532 nm laser.

Conclusion: Results consistently demonstrated a significant correlation ($P < 0.05$) between tissue thickness and laser light penetration. Thicker tissues exhibited faster declines in light transmission intensity compared to thinner tissues within 10 minutes. These findings highlight the importance of further research to enhance light delivery in thicker tissues and improve the efficacy of PDT in various medical conditions.

Keywords: Photodynamic therapy; Laser light penetration; Tissue thickness; Chicken breast; Optical properties.



Introduction

Photodynamic therapy (PDT) is a promising and unique method that uses a photosensitizer (PS) combined with a specific wavelength of visible light to produce reactive oxygen species (ROS) and eliminate both cancerous and non-cancerous diseases.¹ Human skin is the primary interface between humans and their surroundings, and it is the organ most exposed to medical laser applications such as PDT, as it provides the highest relative proportion of incident light energy to a given volume of tissue.^{2,3} When photons enter tissues, they are quickly absorbed into the mitochondria and the cell membrane, where they are converted into chemical energy in the form of adenosine triphosphate (ATP), which in turn restores cell function, eases pain, and so on.^{3,4} Several limitations have been identified in photomedicine applications involving living tissues, including challenges in accurately predicting the delivery of light dose to the

skin due to variations in tissue chromophores, resulting in lower energy density as the optical depth increases.^{3,5} Additionally, the penetration depth of laser light into biological tissues is influenced by numerous factors, such as laser beam properties, tissue composition, and data interpretation. However, there is significant variability in this information across different studies, making it difficult to compare penetration depths.⁶ On the basis of these considerations, a simulation study was deemed crucial for accurately assessing the role of penetration depth and light transmission in different tissue pathologies, thereby facilitating the development and standardization of PDT in clinical practice. Moreover, it should be noted that higher doses of laser light can impact tissues and induce various biological effects, influenced by the absorption spectrum of both endogenous and exogenous cutaneous chromophores, as well as indirect DNA damage through the generation

of ROS.⁷ Since different tissue types exhibit varying penetration depths for different wavelengths of light, understanding the optimal wavelength for specific tumors or lesions becomes essential.⁸ As a result, the objective of this study is to investigate how lasers with wavelengths of 532 nm and 660 nm interact with different types of tissue to determine the ideal wavelength for treating specific cancers or lesions. With this newfound knowledge, medical professionals and researchers will be better equipped to determine the most effective light source and treatment parameters for PDT, ultimately leading to enhanced treatment efficacy and improved patient outcomes.

Materials and Methods

Details of the tests performed to measure optical penetration depth for this study are shown in Figure 1.

Different meat samples, specifically chicken breast and beef muscle, were employed as representative models of different types of human tissues. The appearance of these samples can be observed in Figure 2. It is important to note that the tissue layers consist of diverse components, including proteins, lipids, fibroblasts, cells, fluids, blood vessels, and other constituents.

Chicken breast tissue exhibits similarities to connective tissue found between bones and muscles. Its composition closely resembles that of human tissue, containing significant amounts of lipids, proteins, fibroblasts, fluids, and other components.⁹ On the other hand, beef tissue is similar to human epithelial tissue, the muscle tissue that covers the edges of the body from the outside and defines its cavities from the inside. The cells within these tissues are densely packed, adhered closely to one another, and abundant in fats, proteins, collagen fibers, and various fluids.^{10,11} For each of these tissue types, five thicknesses were replicated in triplicate, ranging from 1 to 20 mm

(1 mm, 3 mm, 5 mm, 10 mm, and 20 mm $\pm$ 0.2 mm), to investigate the impact of their biological properties on absorption and laser penetration depth.

To assess laser beam transmission, the laser source was positioned perpendicular to the surface of the tissue layers. To ensure controlled conditions and minimize optical scattering or reflection, a custom-made dark aluminum box was utilized.¹² Inside the box is a wooden sampler platform with baffles for tuning the laser beam and a slot at the bottom for the laser power meter probe sensor (Figure 3). The power meter automatically measured multiple laser powers, such as 40 μ W, 400 μ W, 4 mW, and 40 mW. Correction factors were applied to the power meter readings for each wavelength ranging from 400 nm to 1100 nm. The correction factor for the 660 nm readout was 0.95, while the correction factor for the 530 nm readout was 1.71. These correction factors represented the power generated by the laser radiation,¹³ as indicated in equation 1.

$$\text{Conversion readings (W)} = \text{Reading (W)} \times \text{Correction coefficient} \quad (1)$$

Each tissue thickness was exposed to two different single-mode diode laser sources operating at either 532 nm or 660 nm, with output powers of 34 mW and 28 mW. These lasers were selected because they emitted coherent light and had consistent beam angles and diameters across all sources.^{12,14}

In this study, the Beer-Lambert rule was employed to determine the optical penetration depth, which describes the relationship between light attenuation in a substance or tissue.^{15,16} This rule allows the calculation of transmittance (T) by evaluating the ratio of transmitted light intensity (I) to the incident light intensity (I_0) within the tissue. The incident light intensity (I_0) is directly proportional to both

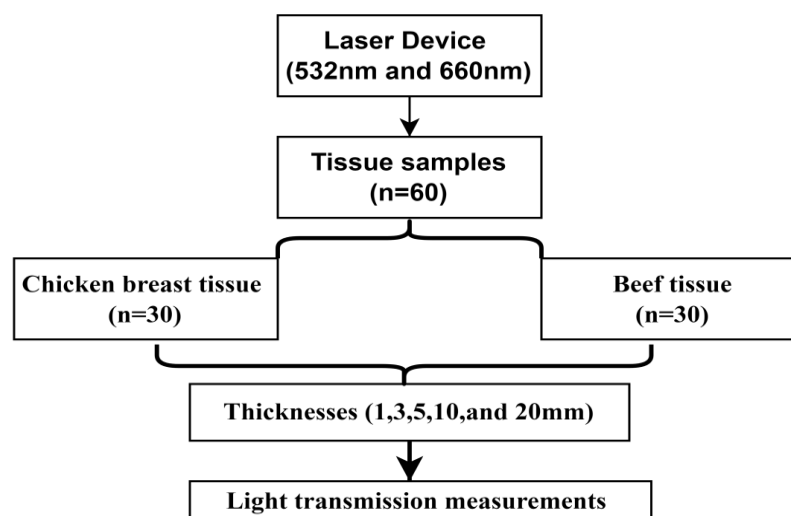


Figure 1. Experimental Setup of Light Transmittance Measurements

the tissue thickness and the absorption coefficient. This relationship is expressed by equation 2:

$$T = I/I_0 = e^{-\mu d} \quad (2)$$

Where (T) is the light transmission, (I) is the transmission intensity (W/cm^2) at the surface of the tissue, (I_0) is the intensity of the incident or transmitted through the tissue samples, μ is expressed as the attenuation modulus, and d is the thickness of the tissue sample. In this study, the transmitted light intensity through the tissue was recorded using a power meter at different thicknesses. The transmittance percentage of light was then calculated using equation 3:

$$T = I/I_0 \times 100 \% \quad (3)$$



(a) Beef tissue

(b) Chicken tissue

Figure 2. Tissue Samples Used in This Study

Results

The results presented in Figure 4 illustrate the measured transmission spectra of two visible wavelength bands (660 nm and 532 nm) across varying thicknesses of beef tissue, ranging from 1 mm to 20 mm. Statistical analysis revealed significant differences ($P < 0.05$) in the average optical transmission values among the different penetration depths. Additionally, Figure 5 compares the average optical transmission of different chicken breast tissue thicknesses, showing statistically significant differences ($P < 0.05$) between the two laser wavelengths. These findings highlight notable variations in optical transmission and penetration depths of laser beams within both beef and chicken breast tissues. The observed statistical significance emphasizes the importance of considering tissue thickness and its impact on the

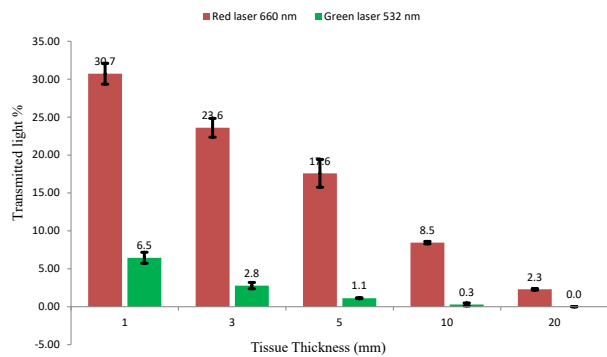


Figure 4. The Transmittance Rates of 532 and 660 nm Laser Light of Beef Tissue at Different Depths

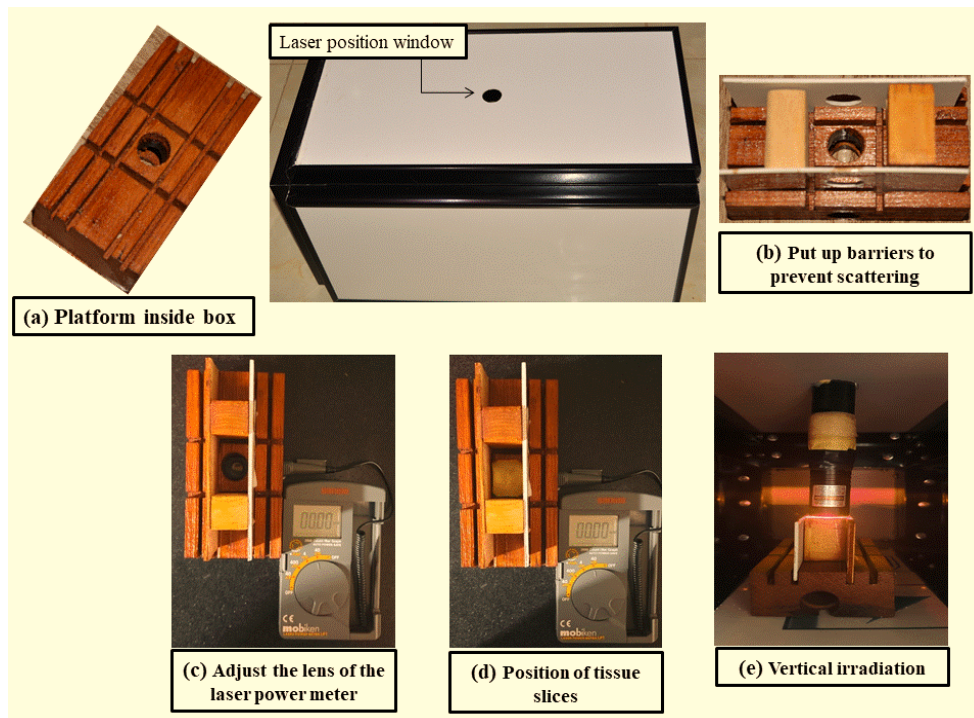


Figure 3. Experimental Setup Box

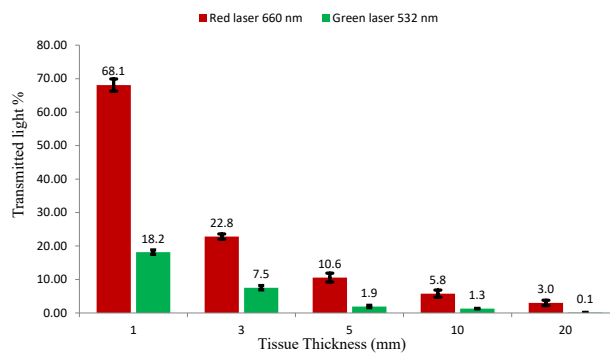


Figure 5. The Transmittance Rates of 532 and 660 nm Laser Light of Chicken Breast Tissue at Different Depths

effectiveness of laser penetration during PDT.

Discussion

The penetration depth results are influenced by both the characteristics of the laser beam and the composition of the tissue. The observed differences in penetration depth are directly linked to tissue composition and wavelength.^{6,17} In the case of chicken breast tissue, the 660 nm laser exhibited deeper penetration compared to the 532 nm laser. Similarly, in beef muscle tissue, the penetration depth of the 660 nm laser was greater than that of the 532 nm laser. The deeper penetration achieved with the 660 nm laser can be attributed to its wavelength which is near the infrared wavelength. Studies have shown that near-infrared light can penetrate biological tissues more effectively than visible light.^{18,19} Existing interpretations suggest that light energy emitted by laser therapy devices is predominantly absorbed within the first 10 mm of biological tissue. This finding aligns with previous research indicating that laser light can penetrate several millimeters into biological tissues.^{6,20,21}

The penetration of laser light into biological tissues is influenced by various factors, including laser beam parameters, tissue sample preparation, and data processing. The substantial variation in these characteristics across different studies makes it challenging to directly compare penetration depths.⁶ Additionally, the ability of light to pass through tissues is primarily determined by scattering and reflection phenomena. Some photons are absorbed by the tissues, while others undergo scattering and may be detected at a certain distance from the initial light source.²² Many studies investigating penetration depths have overlooked the losses caused by reflection.^{23,24}

Understanding the interactions between lasers and optics in PDT is crucial for assessing the relationship between the numerous spectral measurements that are anticipated to be obtained.²⁵ While PDT is an effective treatment method, the evaluation of penetration depth has broader applications across both clinical and non-clinical settings. This knowledge allows for the repeated utilization of the therapy at the same location, enabling

targeted treatment of tissues within the body with minimal or no damage.²⁶ By comprehending the intricacies of laser-optical interactions, the benefits of PDT can be maximized and harnessed for various therapeutic purposes.

Conclusion

In conclusion, this study highlights the critical role of laser light penetration depth in PDT. The choice of laser wavelength has a significant impact on the depth of penetration into biological tissues during PDT. Our results clearly demonstrate that the 660 nm laser achieves much greater penetration depths (up to 20.0 mm) compared to the 532 nm laser (up to 10.0 mm) in both chicken and beef tissue examined. This finding suggests that the 660 nm laser may be more effective for treating deep-seated tumors, while the 532 nm laser could be better suited for superficial lesions. However, further investigation is required to gain a comprehensive understanding of the factors influencing laser light penetration depth in biological tissues and to optimize PDT as a therapeutic modality. Continued research efforts will contribute to the development and refinement of PDT techniques, enhancing their efficacy and expanding their applications in clinical practice.

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Authors' Contribution

Conceptualization: Nursakinah Suardi, Ali A. Aldalawi, Naser Mahmoud Ahmed.

Data curation: Nursakinah Suardi, Ali A. Aldalawi.

Funding acquisition: Faisal J. Kadhim.

Investigation: Nursakinah Suardi, Ali A. Aldalawi.

Methodology: Ali A. Aldalawi, Nursakinah Suardi, Mahdi A. S. Al-Farawn.

Project administration: Nursakinah Suardi, Mahdi A. S. Al-Farawn.

Resources: Wisam I. Jebur, Faisal J. Kadhim.

Supervision: Nursakinah Suardi, Mahdi A. S. Al-Farawn.

Validation: Naser Mahmoud Ahmed, Mahdi A. S. Al-Farawn.

Visualization: Faisal J. Kadhim, Mohammed Ali Dheyab.

Writing—original draft: Nursakinah Suardi, Ali A. Aldalawi.

Writing—review & editing: Ali A. Aldalawi, Nursakinah Suardi, Mohammed Ali Dheyab.

Competing Interests

The authors stress that there is no conflict of interest or other benefit.

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References

1. Honda N, Ishii K, Terada T, Nanjo T, Awazu K. Determination of the tumor tissue optical properties during and after photodynamic therapy using inverse Monte Carlo method and double integrating sphere between 350 and 1000 nm. *J Biomed Opt.* 2011;16(5):058003. doi: [10.1117/1.3581111](https://doi.org/10.1117/1.3581111).
2. Avci P, Gupta A, Sadasivam M, Vecchio D, Pam Z, Pam N, et

- al. Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. *Semin Cutan Med Surg.* 2013;32(1):41-52.
3. Ash C, Dubec M, Donne K, Bashford T. Effect of wavelength and beam width on penetration in light-tissue interaction using computational methods. *Lasers Med Sci.* 2017;32(8):1909-18. doi: [10.1007/s10103-017-2317-4](https://doi.org/10.1007/s10103-017-2317-4).
4. Tuchin VV. Tissue optics and photonics: light-tissue interaction. *J Biomed Photonics Eng.* 2015;1(2):98-134.
5. Mustafa FH, Jaafar MS. Comparison of wavelength-dependent penetration depths of lasers in different types of skin in photodynamic therapy. *Indian J Phys.* 2013;87(3):203-9. doi: [10.1007/s12648-012-0213-0](https://doi.org/10.1007/s12648-012-0213-0).
6. Kaub L, Schmitz C. More than ninety percent of the light energy emitted by near-infrared laser therapy devices used to treat musculoskeletal disorders is absorbed within the first ten millimeters of biological tissue. *Biomedicines.* 2022;10(12):3204. doi: [10.3390/biomedicines10123204](https://doi.org/10.3390/biomedicines10123204).
7. Mahmoud BH, Hessel CL, Hamzavi IH, Lim HW. Effects of visible light on the skin. *Photochem Photobiol.* 2008;84(2):450-62. doi: [10.1111/j.1751-1097.2007.00286.x](https://doi.org/10.1111/j.1751-1097.2007.00286.x).
8. Mallidi S, Anbil S, Bulin AL, Obaid G, Ichikawa M, Hasan T. Beyond the barriers of light penetration: strategies, perspectives and possibilities for photodynamic therapy. *Theranostics.* 2016;6(13):2458-87. doi: [10.7150/thno.16183](https://doi.org/10.7150/thno.16183).
9. Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmoulière A, Varga J, et al. Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *Am J Pathol.* 2012;180(4):1340-55. doi: [10.1016/j.ajpath.2012.02.004](https://doi.org/10.1016/j.ajpath.2012.02.004).
10. Sureka CS, Armpilia C. *Radiation Biology for Medical Physicists.* CRC Press; 2017.
11. Reece WO, Rowe EW. *Functional Anatomy and Physiology of Domestic Animals.* John Wiley & Sons; 2017.
12. Kim MM, Darafsheh A. Light sources and dosimetry techniques for photodynamic therapy. *Photochem Photobiol.* 2020;96(2):280-94. doi: [10.1111/php.13219](https://doi.org/10.1111/php.13219).
13. Jada N, Shamkhi I. Determination of 650nm wavelength for rapid detection of sickle cell anemia. *Res Sq [Preprint].* March 17, 2020. Available from: <https://www.researchsquare.com/article/rs-17409/v1>.
14. Dawood MS. The effects of ultrasound and alternating current on the laser penetration in the tissue. *Lasers Med Sci.* 2016;31(5):955-64. doi: [10.1007/s10103-016-1937-4](https://doi.org/10.1007/s10103-016-1937-4).
15. Măntele W, Deniz E. UV-VIS absorption spectroscopy: Lambert-Beer reloaded. *Spectrochim Acta A Mol Biomol Spectrosc.* 2017;173:965-8. doi: [10.1016/j.saa.2016.09.037](https://doi.org/10.1016/j.saa.2016.09.037).
16. Wang S, Wang J, Yin Q, Wang Y. Light extinction method for solubility measurement. *Chin Opt Lett.* 2005;3(3):149-51.
17. Souza-Barros L, Dhaidan G, Maunula M, Solomon V, Gabison S, Lilge L, et al. Skin color and tissue thickness effects on transmittance, reflectance, and skin temperature when using 635 and 808nm lasers in low intensity therapeutics. *Lasers Surg Med.* 2018;50(4):291-301. doi: [10.1002/lsm.22760](https://doi.org/10.1002/lsm.22760).
18. Baser Keklikci H, Yagci A, Yay AH, Goktepe O. Effects of 405-, 532-, 650-, and 940-nm wavelengths of low-level laser therapies on orthodontic tooth movement in rats. *Prog Orthod.* 2020;21(1):43. doi: [10.1186/s40510-020-00343-3](https://doi.org/10.1186/s40510-020-00343-3).
19. Cios A, Cieplak M, Szymański Ł, Lewicka A, Cierniak S, Stankiewicz W, et al. Effect of different wavelengths of laser irradiation on the skin cells. *Int J Mol Sci.* 2021;22(5):2437. doi: [10.3390/ijms22052437](https://doi.org/10.3390/ijms22052437).
20. Nasouri B, Murphy TE, Berberoglu H. Near infrared laser penetration and absorption in human skin. In: *Mechanisms for Low-Light Therapy IX.* Vol 8932. California, United States: SPIE; 2014. p. 67-78. doi: [10.1117/12.2040337](https://doi.org/10.1117/12.2040337).
21. Nussbaum EL, Van Zuylen J, Jing F. Transmission of light through human skin folds during phototherapy: effects of physical characteristics, irradiation wavelength, and skin-diode coupling. *Physiother Can.* 2007;59(3):194-207. doi: [10.3138/ptc.59.3.194](https://doi.org/10.3138/ptc.59.3.194).
22. Kozłowska TI, Kolisnik PF, Titova NV, Pavlov VS, Wójcik W, et al. Physical-mathematical model of optical radiation interaction with biological tissues. In: *Photonics Applications in Astronomy, Communications, Industry, and High Energy Physics Experiments.* Vol 10445. Wilga, Poland: SPIE; 2017. p. 1047-53. doi: [10.1117/12.2280928](https://doi.org/10.1117/12.2280928).
23. Arslan H, Dolukan YB. Optical penetration depths and fluence distributions in chicken breast and liver tissues. *Opt Spectrosc.* 2019;127(4):763-8. doi: [10.1134/s0030400x19100035](https://doi.org/10.1134/s0030400x19100035).
24. Hudson DE, Hudson DO, Wininger JM, Richardson BD. Penetration of laser light at 808 and 980 nm in bovine tissue samples. *Photomed Laser Surg.* 2013;31(4):163-8. doi: [10.1089/pho.2012.3284](https://doi.org/10.1089/pho.2012.3284).
25. Zam A. Laser-tissue interaction. In: Stübinger S, Klämpfl F, Schmidt M, Zeilhofer HF, ed. *Lasers in Oral and Maxillofacial Surgery.* Cham: Springer; 2020. p. 25-34. doi: [10.1007/978-3-030-29604-9_3](https://doi.org/10.1007/978-3-030-29604-9_3).
26. Niculescu AG, Grumezescu AM. Photodynamic therapy—an up-to-date review. *Appl Sci.* 2021;11(8):3626. doi: [10.3390/app11083626](https://doi.org/10.3390/app11083626).