

MEASUREMENTS OF THERMAL DAMAGE IN BIOLOGICAL TISSUE USING POLARIZED LIGHT SIMULATION

Jong-In Youn, Ph.D. *

Department of Biomedical Engineering, College of Medical Science,
Catholic University of Daegu, Gyeongan 712-702, South Korea
jyoun@cu.ac.kr

Abstract- Recently, thermal treatment has been used for collagen tightening and tissue contour enhancement. It is important to monitor the condition of collagenous tissue during and immediately after thermal treatment. When collagen is denatured, birefringence is reduced and a change in polarization state occurs. In this study, Monte Carlo simulation for polarized light was used to calculate the degree of linear polarization (DOLP) of backscattered light from thermally damaged porcine skins and the results were evaluated by comparison with the Mueller matrix. We observed a decrease in the DOLP and a rapid change in the colored regions of the Mueller matrix at temperatures ranging from 55 to 65°C. This could be attributed to the reduction of birefringence from thermal denaturation in the tissue. The DOLP method has a potential implementation in a real-time closed-loop feedback system with various thermal treatment methods by measuring the birefringence change in target tissue.

Keywords- Monte Carlo simulation, polarized light, birefringence, collagen denaturation.

I. INTRODUCTION

Collagen, a common constituent of many biological tissues, plays an important role in maintaining the structure and determining the function of tissue [1, 2]. Recently, laser-, light-, and radiofrequency- (RF) based technologies have been used for collagen tightening and tissue contour enhancement by thermally inducing collagen contraction and the subsequent remodeling [3, 4]. A variety of thermal treatment modalities exist, such as thermal capsulorrhaphy, thermokeratoplasty, and the thermal treatment of cruciate ligament, knee, and skin [5-7]. Continuous thermal stimulation of a target tissue can raise the temperature of the tissue excessively, which results in the structural transition of collagen in the tissue from ordered helix structures to a random-coil conformation. Thus, collagen can be irreversibly shrunk and denatured [8, 9]. Therefore, it is very important to assess the condition of the collagenous tissue during and immediately after thermal treatment.

To determine the extent of the thermal denaturation of collagen, various methods have been introduced such as differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR), and light scattering measurement systems [10]. However, these methods require a post-processing procedure involving the extraction and solubilization of a specific protein, which means that it is difficult to investigate complex tissues [11]. Thus, the observation of structural transition changes in collagenous tissues is difficult to conduct in real-time. An alternative method is to measure the surface temperature of a treatment region. However, collagen denaturation is not only affected by temperature, but also depends strongly on time, according to the Arrhenius equation

[12]. Therefore, collagen denaturation occurs at various combinations of time and temperature, rather than only at a specific temperature.

Over the last few years, polarized light-based techniques have been adopted for non-invasive diagnosis of biological tissues, utilizing intrinsic properties of collagen such as birefringence [13]. Birefringence resulting from a highly organized collagen fiber is an intrinsic optical property of tissue, and has a higher refractive index along the length of the fibers than along their cross section [14, 15]. Götzinger et al. [16] observed changes in the cornea's birefringence properties using polarization-sensitive coherence tomography (PS-OCT), which indicates corneal abnormalities such as keratoconus. Wood et al. [17] demonstrated the ability of a polarized light-based birefringence measurement to characterize myocardial infarction by measuring a decrease in birefringence in infarcted cardiac muscle. In addition, numerous theoretical studies have also been conducted to analyze polarized light propagation in biological tissues [18-20]. For example, Wang et al. [18] examined the propagation of polarized light in birefringence turbid media using a Monte Carlo simulation. Similarly, Ambrirajan et al. [19] used a backward Monte Carlo simulation to calculate the degree of polarization (DOP) of diffuse reflected light from turbid media, while Cameron et al. [20] calculated the diffuse backscattering Mueller matrix from the suspension of polystyrene spheres using a numerical method.

The destruction of molecular composition caused by thermal damage can directly affect changes in birefringence on a target tissue [21-23]. This phenomenon changes the polarization state, which can be observed by analysis of the backscattered polarized light. In this paper, we have calculated the degree of linear polarization (DOLP) of backscattered light from thermally damaged porcine skins using a Monte Carlo simulation. Since the DOLP provides the polarization degree of the measured light, we can estimate the birefringence level of the target tissue. Also, the DOLP can be calculated in real time, so we believe that the DOLP measurement may help to indicate the condition of the collagenous tissue during and immediately after thermal treatment in real situations. The DOLP results were evaluated by comparison with the Mueller matrix, which is another method for analyzing the polarization-based characterization of tissue.

II. MATERIALS AND METHODS

A. Optical property measurements

We conducted an *in vitro* measurement using thermally damaged porcine skins to derive the optical properties applied in the Monte Carlo simulation. Fresh porcine skins were obtained

from a local abattoir and all samples were stored in a phosphate-buffered saline solution to maintain freshness. Six pieces of porcine skin tissues were placed in a temperature-controlled water bath providing six different temperature levels (25°C, 35°C, 45°C, 55°C, 65°C, and 75°C) for one minute. Thermocoupling was used to monitor the temperature inside the water bath. The optical properties were taken using an integrating sphere system as illustrated in Figure 1.

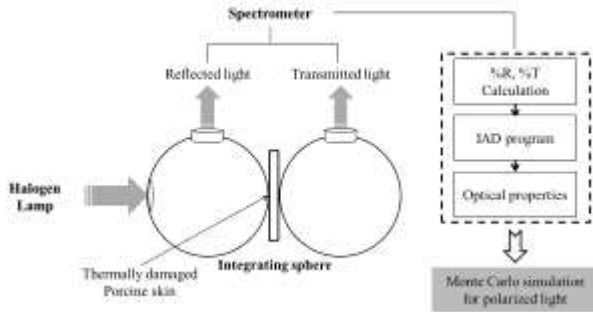


Figure 1. Integrating sphere system.

The light from a tungsten halogen lamp (HL-2000, Ocean Optics) was incident upon a small aperture in the integrating sphere (AvaSphere-30-IRRAD, Avantes), and the reflectance and transmittance lights were collected by a visible light spectrometer (USB4000, Ocean Optics). These measured parameters were then entered into the IAD-program and optical properties including absorption coefficient, μ_a , scattering

coefficient, μ_s , and anisotropic factor, g , were derived from the program. The optical properties of the heated skin tissue are summarized in Table 1.

	25°C	35°C	45°C	55°C	65°C	75°C	
Porcine skin tissue	μ_a [cm^{-1}]	0.63 54	0.61 84	0.60 74	0.61 83	0.60 28	0.59 38
	μ_s [cm^{-1}]	2.6	2.56 7	3.06 4	4.03 4	4.64 6	4.77 9
	g^*	0.81 84	0.79 43	0.76 67	0.73 36	0.64 49	0.57 92

Table 1. Optical properties of thermally heated skin tissues obtained by the integrating sphere system.

B. Monte Carlo simulation for polarized light

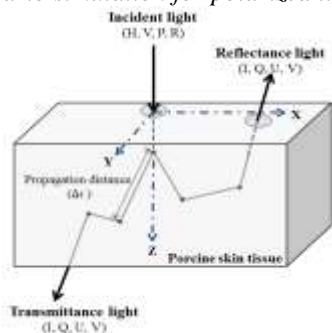


Figure 2. Model geometry of the Monte Carlo simulation.

A publicly available polarized light Monte Carlo simulation developed by Ramella-Roman et al [24, 25] was used to simulate the photon trajectories of the polarized light as it travelled through a medium. The geometry of the Monte Carlo simulation is shown in Fig. 2. The results from the DOLP analysis and the Mueller

1) Monte Carlo algorithm for polarized light

The first step is to launch one of several million photons of polarized light along the z-axis direction of the medium and to set up the optical properties derived using the integrating sphere system. In this study, the number of launched photons was 10^6

at $\lambda = 632.8 \text{ nm}$. We conducted the simulation on four different

polarization states (H (at 0°), V (at 90°), P (at 45°), and R (right circular)). The polarization state of the photons is described by the Stokes vector. During each scattering event, a fraction of the photon weight is absorbed by the interaction site and the Stokes vector of the photon in the new coordinate system is then modified. Launched photons are repeatedly scattered in the media. When the photon reaches the boundaries of the media or its weight value falls below a threshold (typically 0.001), the photons' lives are ended and recorded. Using this simulation, transmittance and reflectance light intensity were measured simultaneously for each of the four input polarizations (H, V, P, and R).

2) Degree of linear polarization (DOLP)

Each of the light beams can be represented by the Stokes vector $S = [I \ Q \ U \ V]^T$. In the Stokes vector, the first

variable, I , represents the intensity of the collected light, Q represents the difference in intensities between linear polarization at 0° and 90° , the third term, U , is the difference in intensities between the linear polarization at 45° and 135° , and finally, V is the difference in intensities between the right and left circular polarizations.

We then calculate the DOLP of the backscattered light from the porcine skin tissues using the Stokes vector components resulting from the Monte Carlo simulation

$$DOLP = \frac{\sqrt{(Q^2 + U^2)}}{I}, \tag{1}$$

The DOLP values have a range of 0 to 1. A value of 0 corresponds to light with no linear polarization characteristics, whereas a value of 1 corresponds to complete linear polarization [26].

3) Mueller matrix

To verify the DOLP result, the 4X4 Mueller matrix was determined. This is another mathematical tool used to describe the polarization-altering properties of a material/tissue [27, 28]. Thus, the Mueller matrix was used to observe the change in polarization-altering properties in the porcine skin tissue as a result of thermal damage. The elements of the matrix can be obtained by calculating various combinations of input and output polarization states generated by the Monte Carlo simulation and the Mueller matrix patterns images can then be obtained. All the elements of the 4X4 matrix appeared with the same color map

(from -1 to 1) and each element was constructed with 100 x 100 pixels. Positive values were rendered using red colors, whereas negative values were represented by blue colors. Also, the number of pixels in relation to temperature level was calculated in order to observe the changes in the Mueller matrix patterns in accordance with collagen denaturation.

III. RESULTS AND DISCUSSION

The DOLP of backscattered light from skin tissues at a depth of 0.5 mm and at various temperature levels is shown in Fig. 3. The DOLP value showed a tendency to decrease with a rise in temperature. Most notably, a rapid decrease in the DOLP value was observed at temperatures ranging from 55 to 65 °C. This reduction ratio is more than three times higher than other ranges. This means that collagen denaturation, which transforms the alpha helix to a random coil conformation and leads to a reduction in birefringence, occurred between 55–65°C, and hence it affected the polarization state of the light.

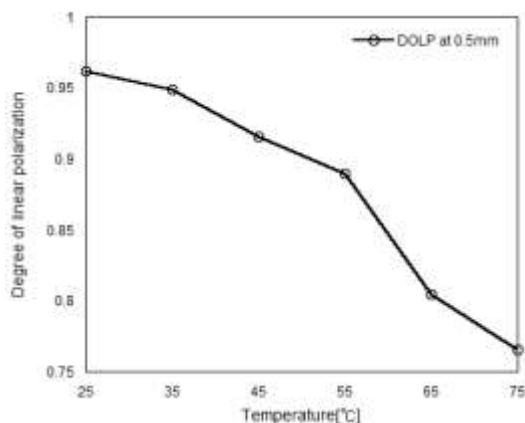


Figure 3. Degree of linear polarization (DOLP) of backscattered light from thermally damaged porcine skin tissues at various temperatures levels.

We will now compare this result with previously published studies. De Boer et al. [29] also investigated the effect of temperature on collagen birefringence and found that, at 60°C, a reduction in the birefringence and shrinkage of porcine tendon can be observed. In addition, Pierce et al. [30] noted that birefringence is significantly lower in burned human skin compared to normal skin. These studies have demonstrated that collagen denaturation and reduction of birefringence mainly occur between 55–65°C.

Also, we found a decrease in the anisotropic factor, g , in six porcine tissues using data recorded by the integrating sphere system (Table 1). In general, birefringence is also considered to be anisotropic because structural birefringence strongly depends on molecular anisotropy. The effects of the anisotropic factor on the polarization state have also been investigated. Ramella-Roman et al. [25] applied the Monte Carlo method to four different poly-disperse solutions of a micro-sphere. They observed that the DOLP value decreased with the decreasing anisotropy parameter, g , of the micro-sphere. Gomes et al. [31] observed that polarized light was preserved for longer paths for higher anisotropy parameters. These studies also support our result that the decrease in birefringence in the porcine skin at

65°C, caused by denaturation of the collagen fibers, leads to a decrease in the DOLP value at temperatures between 55–65°C.

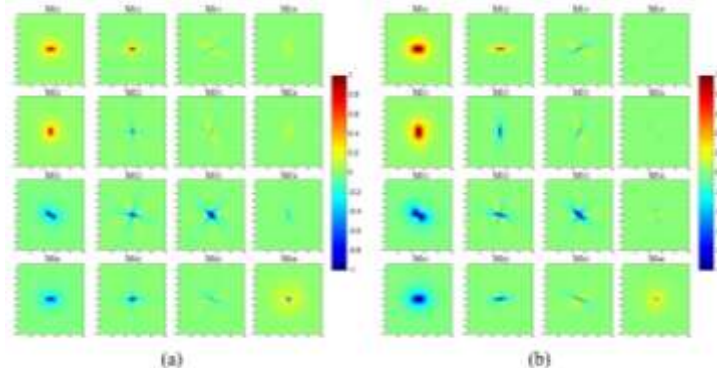


Figure 4. The Mueller matrix patterns of backscattered light from porcine skin at (a) 55°C and (b) 65°C.

The Mueller matrix was used to evaluate the DOLP result. The Mueller matrix pattern can represent various polarization characteristics of the porcine skin tissues. Figure 4 shows the Mueller matrix patterns of backscattered light from porcine skin at 55°C (Fig. 4(a)) and at 65°C (Fig. 4(b)). Each element of the matrix shows a different pattern as given by the calculated values. The main characteristics of the Mueller matrix in Fig. 4(a) are similar to those of Fig. 4(b), including both the patterns and the distribution of positive and negative values. When we compare Figs. 4(a) and (b), it can be seen that the color region of the porcine skin at 65°C is wider than that of the porcine skin at 55°C. This implies that the loss of collagen birefringence due to thermal damage can directly affect the Mueller matrix. The change of color region is distinct in the first column, which mainly involves linear polarization.

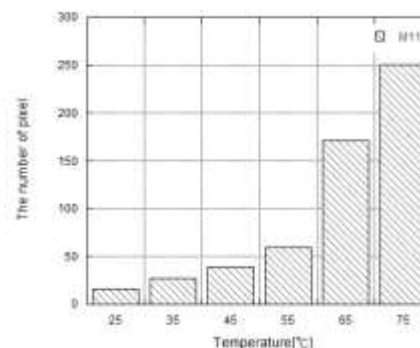


Figure 5. The number of pixels above 0.5 for M11 at various temperature levels.

Figure 5 shows the number of pixels above 0.5 of M11 from the Mueller matrix, which shows a distinct change of the color region as a response to temperature variation. The result shows a rapid increase in the number of pixels when the heating temperature reached the 55–65°C region. This tendency was similarly observed in the M21, M31, and M41. This result is in line with a previous study in which Wang et al. [18] compared the Monte Carlo simulated Mueller matrices of birefringent anisotropic media with those of non-birefringent isotropic media. They observed that the shape and contrast in the patterns of the Mueller matrix were highly associated with the linear birefringence in the media [18]. Therefore, the wide colored

region in Fig. 4(b) and the rapid increases in the number of pixels at temperatures ranging from 55 to 65°C can be understood, as the birefringence decreases due to thermal damage. These changes are also consistent with the results of the DOLP calculation.

In this study, we have observed certain changes in thermally damaged porcine skins at temperatures ranging from 55 to 65°C, in both the DOLP and the Mueller matrix, using a Monte Carlo simulation for polarized light. We expect that the DOLP calculation using the Stokes vector will be a simple and convenient method to observe collagen denaturation due to thermal damage on tissue in real time, with high precision, and which corresponds to the Mueller matrix result. While the Mueller matrix is not appropriate for use in real-time feedback applications because of its complex post-processing requirement, the DOLP method can be implemented in an effective closed-loop feedback system with various thermal treatment methods by measuring the birefringence change in target tissue.

IV. CONCLUSION

In the present study, the DOLP and Mueller matrix were obtained from thermally heated porcine skins under various temperature conditions using a Monte Carlo simulation for polarized light. At temperatures ranging from 55 to 65°C, we observed a decrease in the DOLP and a rapid change in the colored regions in the Mueller matrix. This can be attributed to the reduction of birefringence by thermal denaturation. The DOLP method can be easily used with varying incident polarization angles and the calculation process is simple compared to the Mueller matrix. Our results indicate that rapid decreases in the DOLP value at certain temperatures directly reflect the birefringence change in the collagenous tissue, and this can be useful for determining collagen denaturation during and immediately after thermal treatment.

ACKNOWLEDGMENT

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012R1A1A2039274).

REFERENCES

- [1] O. S. Rabotyagova, P. Cebe, and D. L. Kaplan, "Collagen structural hierarchy and susceptibility to degradation by ultraviolet radiation," *Mater. Sci. Eng. C*, **28**, 1420-1429 (2008).
- [2] N. T. Wright and J. D. Humphrey, "Denaturation of collagen via heating: an irreversible rate process," *Annu. Rev. Biomed. Eng.*, **4**, 109-128 (2002).
- [3] N. Sadick, "Tissue tightening technologies: Fact or fiction," *Aesthet. Surg. J.*, **28**, 180-188 (2008).
- [4] R. E. Fitzpatrick, E. F. Rostan, and N. Marchell, "Collagen tightening induced by carbon dioxide laser versus erbium:YAG laser," *Lasers Surg. Med.*, **27**, 395-403 (2000).
- [5] A. Miniaci and J. McBirmie, "Thermal capsular shrinkage for treatment of multidirectional instability of the shoulder," *J. Bone Joint Surg. Am.*, **85**, 2283-2287 (2003).
- [6] R. Brinkmann, B. Radt, C. Flamm, J. Kampmeier, N. Koop, and R. Birngruber, "Influence of temperature and time on thermally induced forces in corneal collagen and the effect on laser thermokeratoplasty," *J. Cataract Refract. Surg.*, **26**, 744-754 (2000).

- [7] T. Kuo, M. T. Speyer, W. R. Ries, and L. Reinisch, "Collagen thermal damage and collagen synthesis after cutaneous laser resurfacing," *Lasers Surg. Med.*, **23**, 66-71 (1998).
- [8] E. Buxbaum, *Fundamentals of Protein Structure and Function* (Springer, New York, USA, 2007).
- [9] T. Stylianopoulos, A. Aksan, and V. H. Barocas, "A Structural, Kinetic Model of Soft Tissue Thermomechanics," *Biophys. J.*, **94**, 717-725 (2008).
- [10] C. Mu, D. Li, W. Lin, Y. Ding, and G. Zhang, "Temperature induced denaturation of collagen in acidic solution," *Biopolymers*, **86**, 282-287 (2007).
- [11] D.A. Bruggemann, J. Brewer, J. Risbo, and L. Bagatolli, "Second harmonic generation microscopy: A tool for spatially and temporally resolved studies of heat induced structural changes in meat," *Food Biophysics*, **5**, 1-8 (2010).
- [12] M. S. Si, T. E. Milner, B. Anvari, and J. S. Nelson, "Dynamic heat capacity changes of laser-irradiated Type I collagen films," *Lasers Surg. Med.*, **19**, 17-22 (1996).
- [13] S. Alali, M. Ahmad, A. Kim, N. Vurgun, M. F. G. Wood, and I. A. Vitkin, "Quantitative correlation between light depolarization and transport albedo of various porcine tissues," *J. Biomed. Opt.*, **17**, 045004 (2012).
- [14] X. Wang and L. V. Wang, "Propagation of polarized light in birefringent turbid media: time-resolved simulations," *Opt. Express*, **9**, 254-259 (2001).
- [15] M. C. van Turnhout, S. Kranenbarg, and J. L. van Leeuwen, "Modeling optical behavior of birefringent biological tissues for evaluation of quantitative polarized light microscopy," *J. Biomed. Opt.*, **14**, 054018 (2009).
- [16] E. Götzinger, M. Pircher, I. Dejaco-Ruhswurm, S. Kaminski, C. Skorpik, and C. K. Hitzenberger, "Imaging of birefringent properties of keratoconus corneas by polarization-sensitive optical coherence tomography," *Invest. Ophthalmol. Vis. Sci.*, **48**, 3551-3558 (2007).
- [17] M. F. Wood, N. Ghosh, M. A. Wallenburg, S. H. Li, R. D. Weisel, B. C. Wilson, R. K. Li, and I. A. Vitkin, "Polarization birefringence measurements for characterizing the myocardium, including healthy, infarcted, and stem-cell-regenerated tissues," *J. Biomed. Opt.*, **15**, 047009 (2010).
- [18] X. Wang and L. V. Wang, "Propagation of polarized light in birefringent turbid media: A Monte Carlo study," *J. Biomed. Opt.*, **7**, 279-290 (2002).
- [19] A. Ambirajan and D. C. Look, "A backward Monte Carlo study of the multiple scattering of a polarized laser beam," *J. Quant. Spectrosc. Radiat. Transfer*, **58**, 171-192 (1997).
- [20] B. D. Cameron, M. J. Rakovic, M. Mehrubeoglu, G. W. Kattawar, S. Rastegar, L. V. Wang, and G. L. Cote, "Measurement and calculation of the two-dimensional backscattering Mueller matrix of a turbid medium," *Opt. Lett.*, **23**, 485-487 (1998).
- [21] S. Bosman, "Heat-induced structural alterations in myocardium in relation to changing optical properties," *Appl. Opt.*, **32**, 461-463 (1993).
- [22] L. X. Chin, X. J. Yang, R. A. McLaughlin, P. B. Noble, and D. D. Sampson, "En face parametric imaging of tissue birefringence using polarization-sensitive optical coherence tomography," *J. Biomed. Opt.*, **18**, 066005 (2013).
- [23] D. J. Maitland and J. T. Walsh Jr., "Quantitative measurements of linear birefringence during heating of native collagen," *Lasers Surg. Med.*, **20**, 310-318 (1997).
- [24] J. C. Ramella-Roman, S. A. Prahl, and S. L. Jacques, "Three Monte Carlo programs of polarized light transport into scattering media: part I," *Opt. Express*, **13**, 4420-4438 (2005).
- [25] J. C. Ramella-Roman, S. A. Prahl, and S. L. Jacques, "Three Monte Carlo programs of polarized light transport into scattering media: part II," *Opt. Express*, **13**, 10392-10405 (2005).
- [26] P. J. Wu and J. T. Walsh Jr., "Stokes polarimetry imaging of rat tail

- tissue in a turbid medium: degree of linear polarization image maps using incident linearly polarized light,” *J. Biomed. Opt.* **11**, 014031 (2006).
- [27] R. A. Chipman, "Polarimetry," in *the Handbook of Optics*, 2nd ed., M. Bass ed. (McGraw-Hill, New York, NY, USA, 1995), Chapter 22.
- [28] S.-Y. Lu and R. A. Chipman, "Mueller matrices and the degree of polarization,” *Opt. Commun.* **146**, 11–14 (1998).
- [29] J. F. De Boer, S. M. Srinivas, A. Malekafzali, Z. P. Chen, and J. S. Nelson, "Imaging thermally damaged tissue by polarization sensitive optical coherence tomography,” *Opt. Express.* **3**, 212–218 (1998).
- [30] M. C. Pierce, R. L. Sheridan, B. H. Park, B. Cense, and J.F. de Boer, "Collagen denaturation can be quantified in burned human skin using polarization-sensitive optical coherence tomography," *Burns.* **30**, 511-517 (2004).
- [31] A. J. Gomes, V. Turzhitsky, S. Ruderman, and V. Backman, "Monte Carlo model of the penetration depth for polarization gating spectroscopy: influence of illumination-collection geometry and sample optical properties,” *Appl. Opt.* **51**, 4627–4637 (2012).