Optimizing Combined Laser Treatment for the Removal of Port Wine Stains and Cryogen Spray Cooling to Reduce Thermal Heating at the Skin Surface

BEE/MAE 4530: Computer Aided Engineering: Biomedical Processes Spring 2010

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Table of Contents

1.0 Executive Summary

Port wine stains (PWS) are birthmarks caused by the presence of dilated blood vessels, typically 15-55μm in diameter, located in the upper dermis of the skin. Currently, lasers in conjunction with cryogen cooling are the preferred treatment for PWS removal because they can selectively target PWS blood vessels while leaving the surrounding tissue unharmed.

In this project, we compared the effectiveness of various combinations of laser, pre-heating and cooling methods for PWS removal. In COMSOL, we implemented pulsed dye laser heating using a finite element model of light diffusion coupled with heat transfer. Our geometry was based on a two-dimensional histological cross-section from a PWS punch biopsy in order to more accurately mimic the vascular anatomy of a PWS. We compared two types of cooling methods, cryogen spray cooling and water contact cooling. In addition, we implemented a preheating step to achieve higher temperatures in deeper blood vessels. We determined effectiveness of the treatment using an Arrhenius thermal damage equation to calculate injury values over the course of the treatment.

For a single 2 ms laser pulse without cooling, the blood vessels reached a maximum temperature of 89°C. However, the skin surface temperature reached 65°C indicating that we would need to implement a cooling method in parallel with laser heating. We compared cryogen spray cooling with cold water therapy and found the cryogen spray to be more effective. Cryogen cooling for 100 ms before and during the laser treatment kept the post-laser epidermal temperature below 26°C, while water cooling only brought the post-laser temperature down to 34°C. Because cryogen was the more effective treatment, we used it as our preferred method of cooling for the remainder of our study. We then implemented this cooling method with a ten laser pulse treatment scheme, which elevated temperatures in the blood vessels but did not achieve coagulation temperatures in the deeper blood vessels. The addition of a 40 second preheating step at 60°C effectively increased the temperatures in the deeper blood vessels to desired levels, while keeping damage to the epidermal and dermal layers at a minimum.

Our model of light transport and heat generation in the epidermis, dermis and blood vessels verified that it is possible to target blood vessels with laser therapy while inflicting minimal damage to surrounding tissue. Current methods for treating PWS using pulsed dye lasers are limited in that they cannot target blood vessels deeper in the tissue. In our design, we showed that including a preheating step and multiple laser pulses can effectively target blood vessels deeper in the dermis. Our model can be used to select process parameters and different treatment combinations prior to experiments and clinical trials. Simulating laser treatments as well as various pre-cooling and pre-heating methods in COMSOL reduces the need for excess experimentation and potentially decreases the time before new designs are approved for use.

2.0 Introduction

Port wine stains (PWS) are congenital vascular malformations that occur in approximately 3 out of 1000 newborns [2]. These dark pink or purple birthmarks, which commonly appear on large areas of the face and upper torso, may make one feel self-conscious and contribute to emotional distress. Several approaches to treating PWS include cosmetic cover-up, skin grafting, radiation and cryosurgery. However, these methods are not ideal because they are short-lived or potentially damaging to the surrounding tissue [4]. Until the development of laser treatments for PWS, cosmetic cover-up was the only viable option [4].

Figure 1: 3D histological reconstruction of epidermis and PWS vessels [1].

Currently, pulsed dye laser (PDL) irradiation is the preferred treatment method for the removal of PWS. Other lasers, such as carbon dioxide lasers and argon lasers, lead to significant scarring [4]. PDLs are able to avoid scarring because they take advantage of selective absorption of laser radiation. Erythrocytes in the blood absorb the laser light, which in turn causes heating of the vessel walls and irreversible thermal damage. By choosing a wavelength of light that is strongly absorbed by hemoglobin and minimally absorbed by the surrounding tissue, damage to the skin layers can be reduced. However, a key issue in PDL treatment is the pigment melanin in the epidermal layer will also absorb light and may produce significant heating at the skin surface. Cooling methods such as cryogen spurts, cool air and water cooling have been used during and prior to PDL treatment to reduce the surface skin temperature in an effort to minimize thermal damage to the epidermis.

While PDL irradiation is the current gold standard for treating PWS, very few PWS are completely lightened. In fact, 20% of PDL-treated PWS show no improvement at all [4]. One possible explanation for this is that the laser light does not penetrate deep enough to damage the blood vessels further down in the dermis [4]. Pulsed dye lasers have traditionally been applied at 577nm, the peak absorption wavelength of hemoglobin, and a pulse width of 0.45 ms. Recently, it has been proposed that PDL treatments at higher wavelengths, higher powers and longer pulse durations could be used to treat these deeper blood vessels with larger diameters [3].

Our aim was to develop a treatment strategy that leads to the damage of blood vessels deeper in the dermis in order to improve the effectiveness of PDL removal of PWS. This was done by comparing the effectiveness of different cooling methods, laser powers, and number of laser pulses in treating a two-dimensional model of a PWS. Additionally, preheating with hot air to raise the pre-laser temperature of blood vessels was investigated to see if it aided in the treatment of deeper blood vessels [5].

2.1 Design Objectives

The goal of this project was to use COMSOL to model various combinations of pre-heating, PDL treatment and cooling for the removal of PWS. The effectiveness of the treatment was determined by modeling temperature changes in the blood vessels and surrounding tissues caused by absorption of laser light diffusing through the tissues. The model was then used to find optimal laser powers and pulse durations to cause coagulation of blood vessels while minimizing heating at the skin surface. We modeled the 2D temperature profile in the skin resulting from the absorption of the laser radiation and determined whether the epidermal and dermal tissue was damaged as a result. Damaged skin was roughly defined as the regions that sustained a temperature greater than 45°C following the treatment period, while blood vessel occlusion was designated to occur at 80°C [5]. More precise measurements of thermal damage were obtained by an Arrhenius thermal damage module. We also compared the effects of water cooling and cryogen cooling to prevent damage and pain to the superficial skin layers without compromising the ability of the laser to occlude the blood vessels. The pain temperature threshold for thermal nociceptors in the upper layers of the dermis of the skin is believed to be 43°C [6].

2.2 Schematic

Figure 2 shows a histological cross section of a PWS vascular malformation illustrating the arrangement of blood vessels. Notice that in the true to life cross-section, the majority of the vessels in the malformation are of varying diameters and are clustered into particular regions. Meanwhile, some of the vessels are detached from these clusters and exist separately. In our design, we tried to mimic these arrangements by forming similar clusters of vessels with varying diameters as well as by including an outlying detached vessel.

Figure 2: Histological Cross Section of Port Wine Stain Vascular Malformation. Blood vessels and epidermal layer are outlined [1].

Our computational domain was designed to represent a simplified two-dimensional cross-section of a PWS vascular malformation present near the surface of the skin. Figure 3 shows the geometry is composed of a single superficial epidermal layer and a deeper dermal layer in which an assortment of blood vessels are represented by circles. Blood vessel diameters ranged from 15μm to 55μm, with an epidermal thickness of 80 μm, and dermal thickness of 200 μm as per literature values [1]. This simplified representation was meant to convey the somewhat random arrangement of blood vessels seen in histological cross-sections of actual PWS vessels.

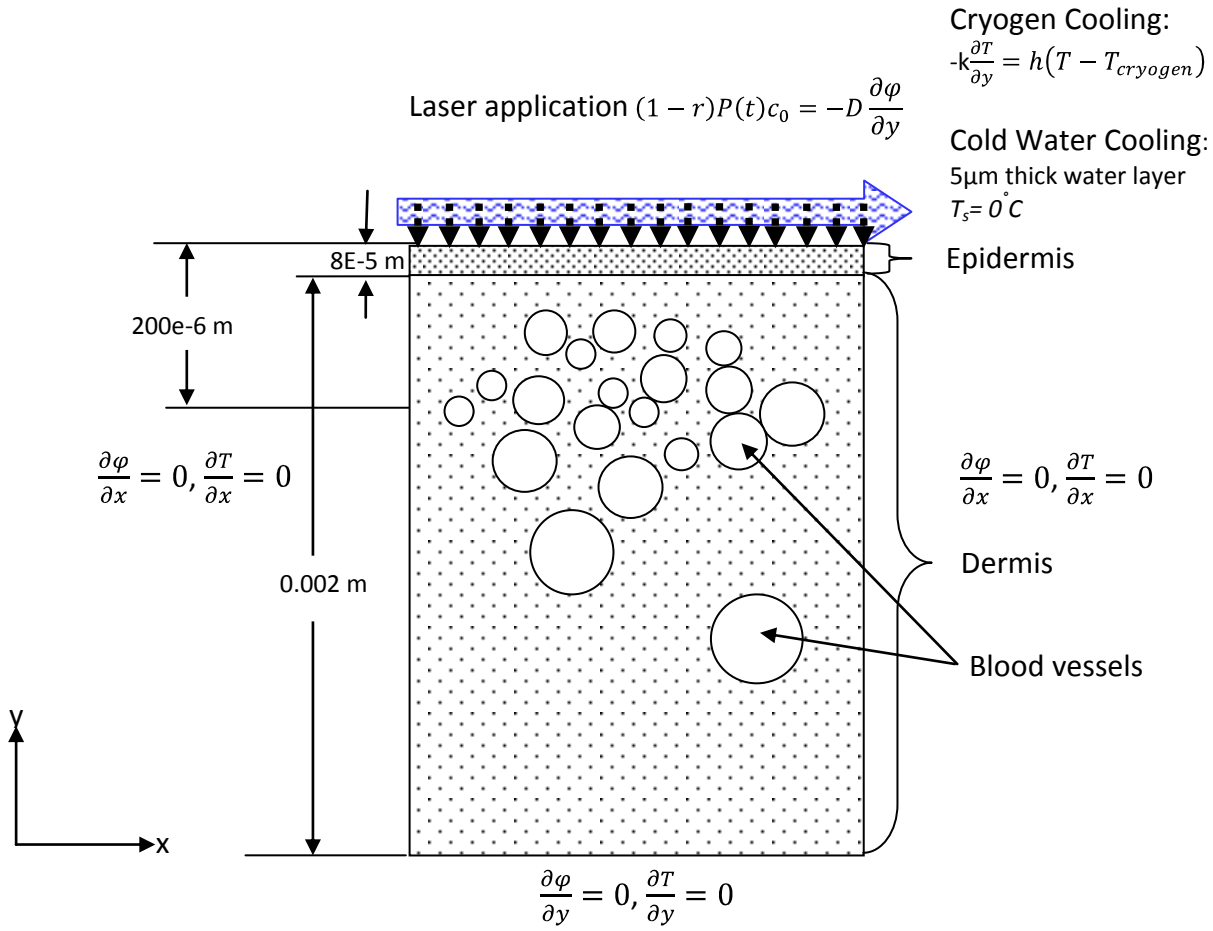

Figure 3: Schematic of the Port Wine Stain Skin Model.

3.0 Results and Discussion

3.1 Interpretation of Results

To provide a baseline, we modeled the heating of the PWS with a 2 ms laser pulse using no cooling methods to prevent skin damage. Figure 4 shows that with no cooling methods, the blood vessels reached a maximum temperature of around 89°C, past the threshold to achieve thermal damage. The vessels towards the surface of the skin heated up the most because as the pulsed dye laser diffused through the skin, those vessels absorbed the laser radiation and limited diffusion of the laser light to regions further below the surface. A major cause for concern in heating the vessels without any cooling methods is the epidermal skin layer reached a temperature of around 65°C, which would cause severe burn damage to this region.

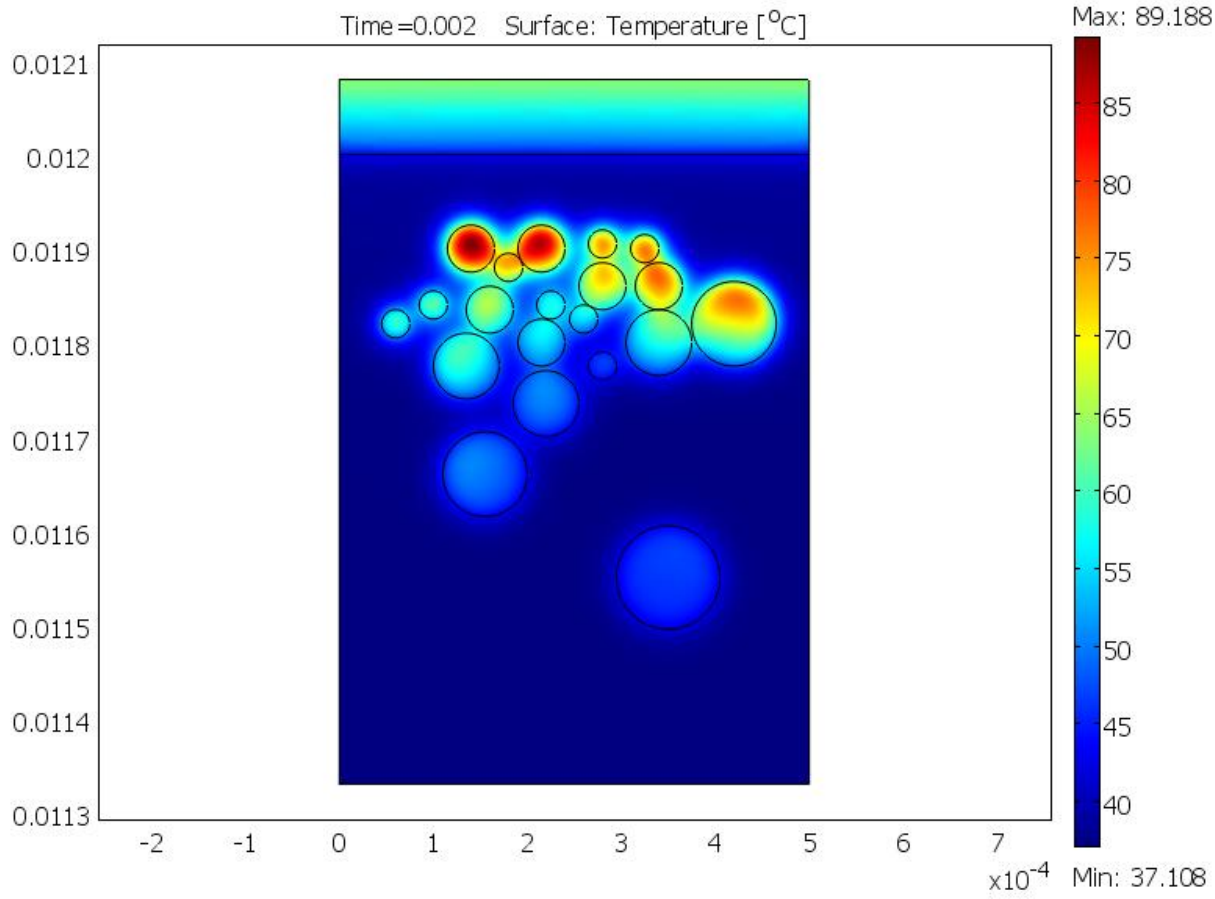

Figure 4: Surface plot of blood vessel and skin after 2 ms laser pulse (1300W/m^2).

Since it became obvious that we needed to implement some type of cooling in order to minimize thermal damage, we first looked at cryogen spray cooling. Information on this cooling method is readily available in the literature [5,8,9], making it easy to implement in our model. We first cooled the skin using a cryogen spurt for 100 ms and then applied a brief 2 ms laser pulse. Figure 5 shows that the surface temperature remained at about room temperature and the blood

vessels were able to achieve temperatures necessary for thermal damage. The vessels did reach a lower maximum temperature than the initial baseline modeling with no cooling, but this could be accounted for by the pre-cooling of the upper layer vessels by the cryogen spray prior to laser application.

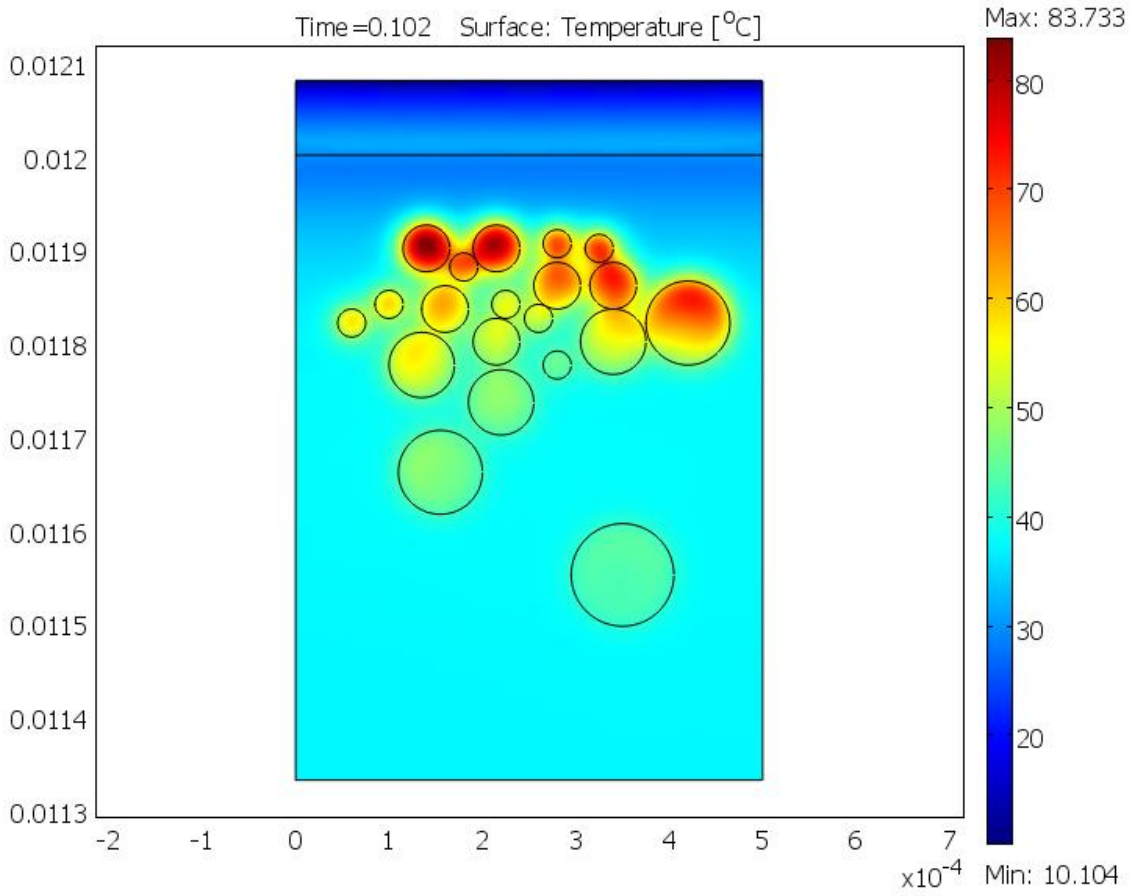

Figure 5: Surface plot of blood vessel and skin after 2 ms laser pulse (1300W/m^2) and 102 ms cryogen cooling (-44°C).

Next, we modeled an alternative cold water cooling method to alleviate heating of the epidermal layer of the skin. The cold water layer was modeled as a 5 μm thick domain with a boundary temperature of 0°C. We modeled the application of the laser through the layer of water and made sure to account for absorption and scattering of the laser due to the water. As with cryogen spray cooling, Figure 6 shows the cold water cooling method was able to prevent overheating of the epidermis, while simultaneously achieving temperatures necessary to coagulate the blood vessels.

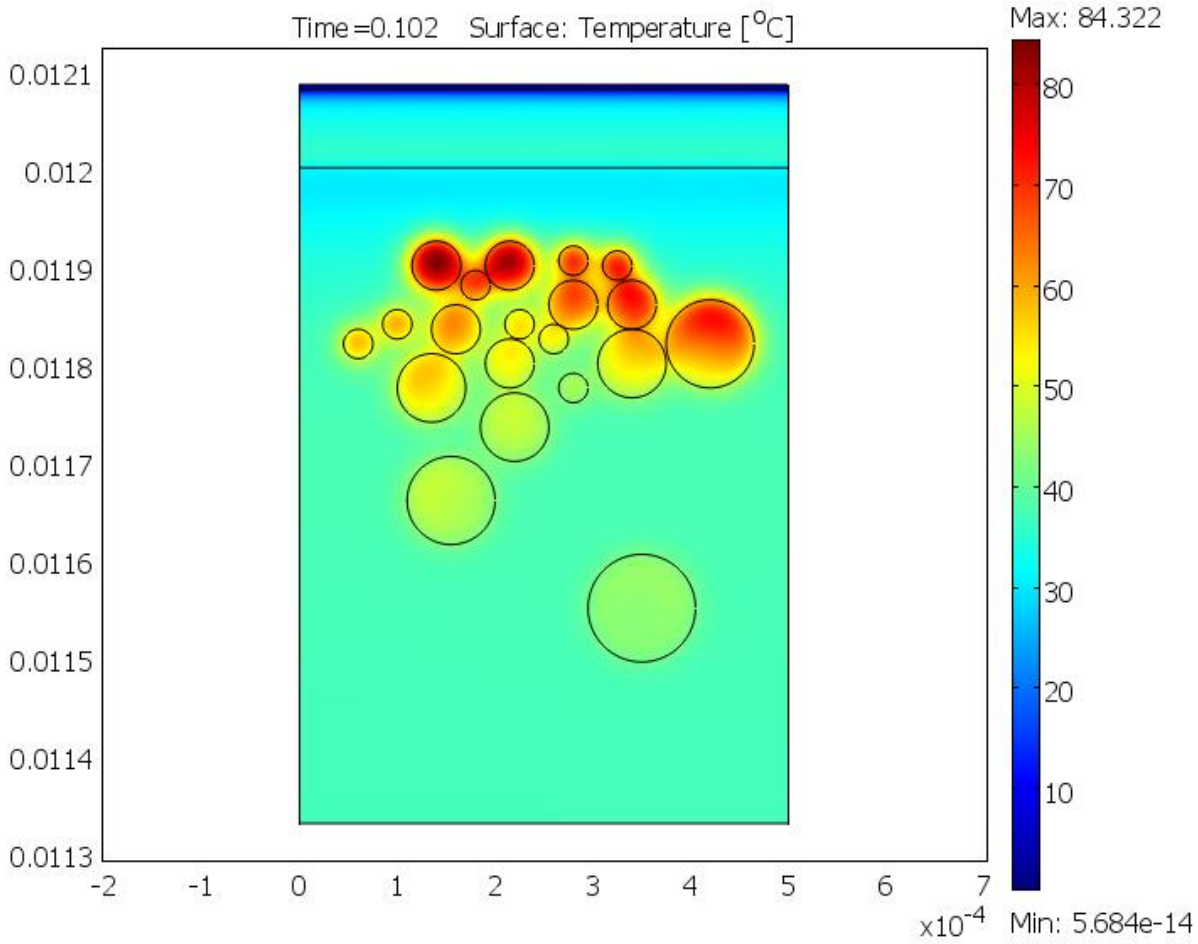

Figure 6: Surface plot of blood vessels and skin after 2 ms laser pulse (1300W/m^2) and 102 ms water cooling.

To further differentiate between the two cooling methods, we compared cryogen spray cooling and cold water cooling side by side to analyze their effectiveness. We determined that cryogen spray cooling was more effective at preventing thermal damage of the skin than cold water cooling. Both cooling methods reached maximum temperatures in the blood vessels of approximately 84°C and primarily differed in their ability to prevent overheating of the epidermal layer. Figure 7 shows cryogen spray cooling was able to bring the temperature of the epidermis to 7°C during the cooling period as compared to water cooling which only brought the temperature to 15°C. Thus, cryogen spray cooling is ideal for the treatment of PWS since the more efficient cooling of the skin with this method will allow for the implementation of more intensive heating methods, such as preheating and multiple laser pulses, without the fear of causing severe skin burns.

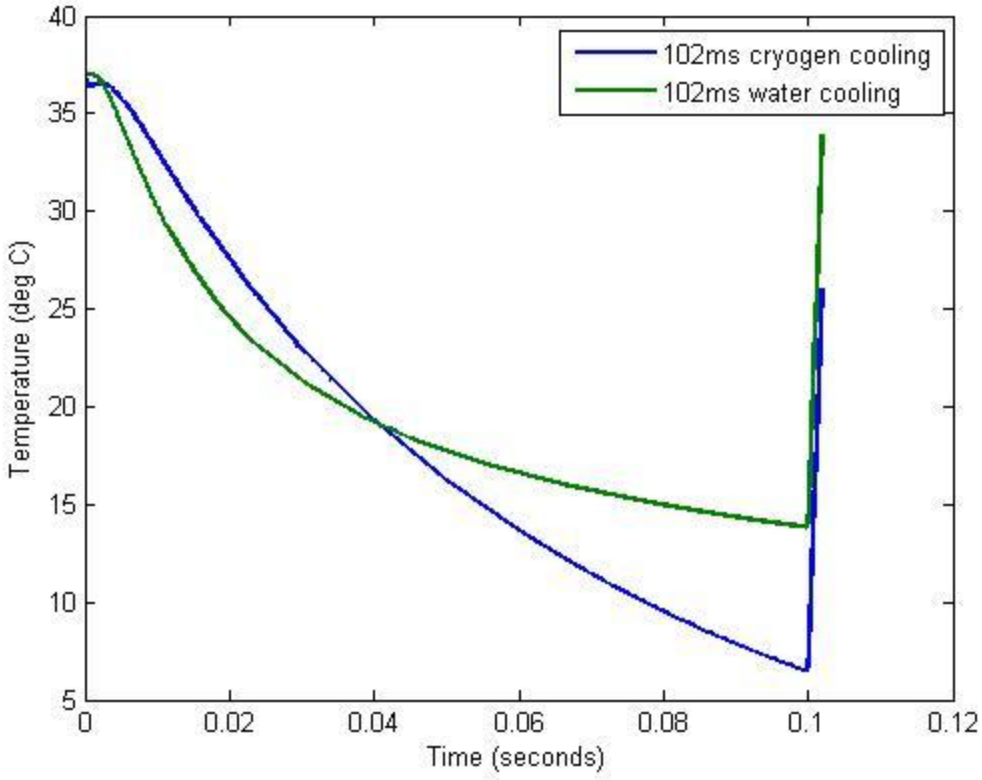

Figure 7: Comparison of cryogen cooling and water-cooling at a point in the epidermis after a 2 ms laser pulse (1300W/m^2) and 102 ms of the respective cooling methods.

Finally, we compared the effectiveness of the preheating method by running a pair of separate cryogen-cooled models, one with skin preheating using a 60°C hot air jet and another without. We also used this opportunity to incorporate multiple pulses in these models, a total of ten, in order to achieve enhanced heating of the deeper vessels. Figure C-1 in Appendix C shows brief 2 ms laser pulses with intermediate cooling periods kept the skin at normal temperatures while allowing heat to penetrate deeper into the skin and affect the more sub-epidermal vessels.

The comparison illustrated in Figure 8 shows that the preheating had significantly more heat penetration than the non-preheating method, and that the pulsed laser with intermittent cooling greatly limited the amount of damage to the epidermal layer without sacrificing heat within the blood vessels. More of the vessels reached coagulation temperatures in the preheated skin than in the non-preheated skin, while in both cases, the epidermal temperature stayed low enough to avoid thermal damage.

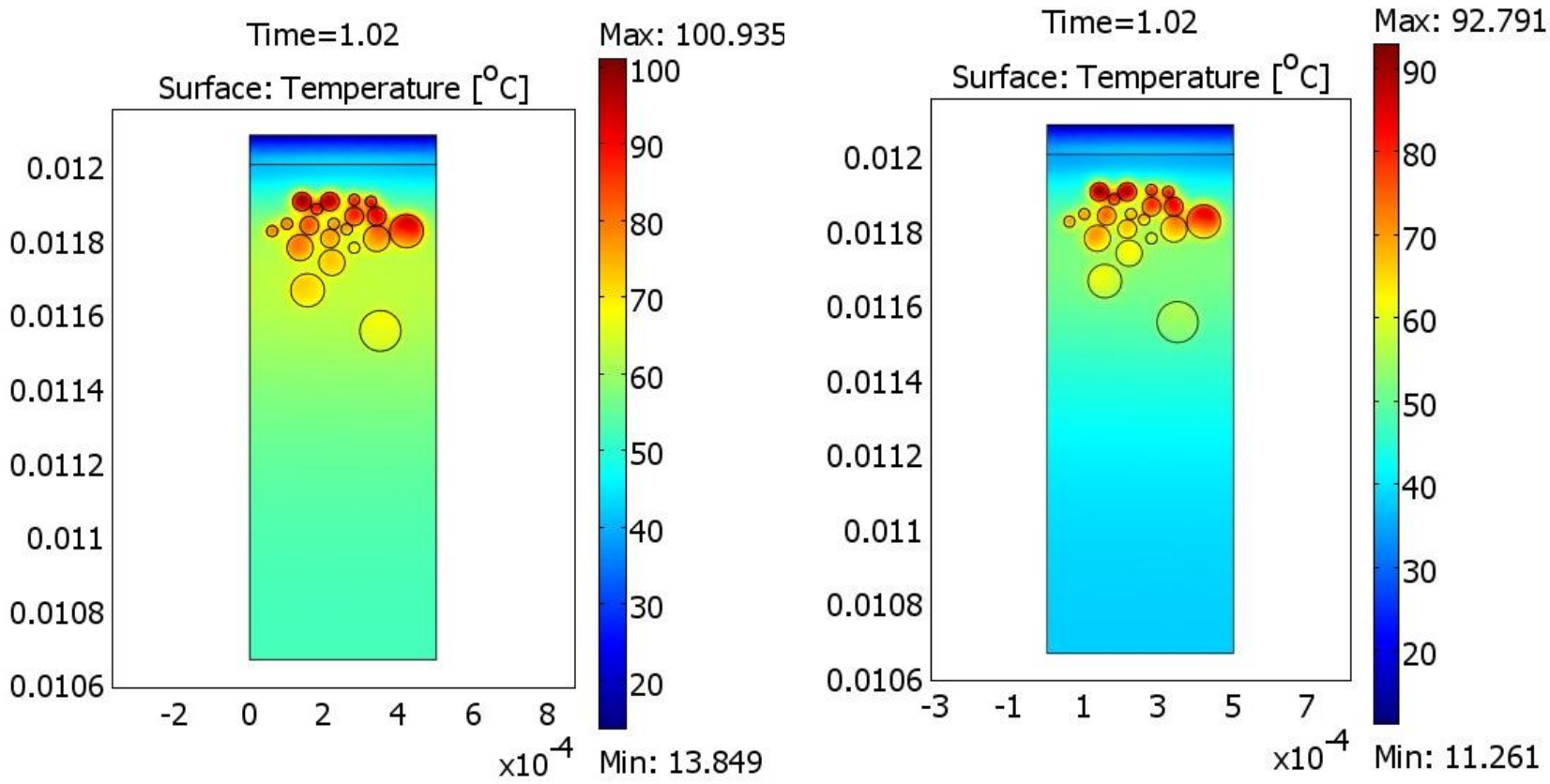

Figure 8: Side by side comparison of preheating (left) and non-preheating (right), both with a ten pulse laser. Dimensions are in meters. Temperature is in °C, and time is in seconds.

To obtain a more precise measurement of coagulation and skin damage, we calculated values for thermal damage for our optimal treatment of preheating, cryogen and 10 laser pulses. Figure 9 shows a surface plot of thermal damage values between $0 \leq \Omega \leq 1$ in the blood vessels and tissue. Coagulation of blood vessels is a function of temperature and time, and can be modeled by using an Arrhenius thermal damage equation and solving for Ω (See Appendix A). The threshold for irreversible coagulation is defined as $\Omega = 1$ [7] and the threshold for tissue damage is defined as $\Omega = 0.53$ [8]. The results of running our preheated, cryogen-cooled, ten-pulse treatment scheme can be seen in Figure 9, where areas in white correspond to $\Omega > 1$.

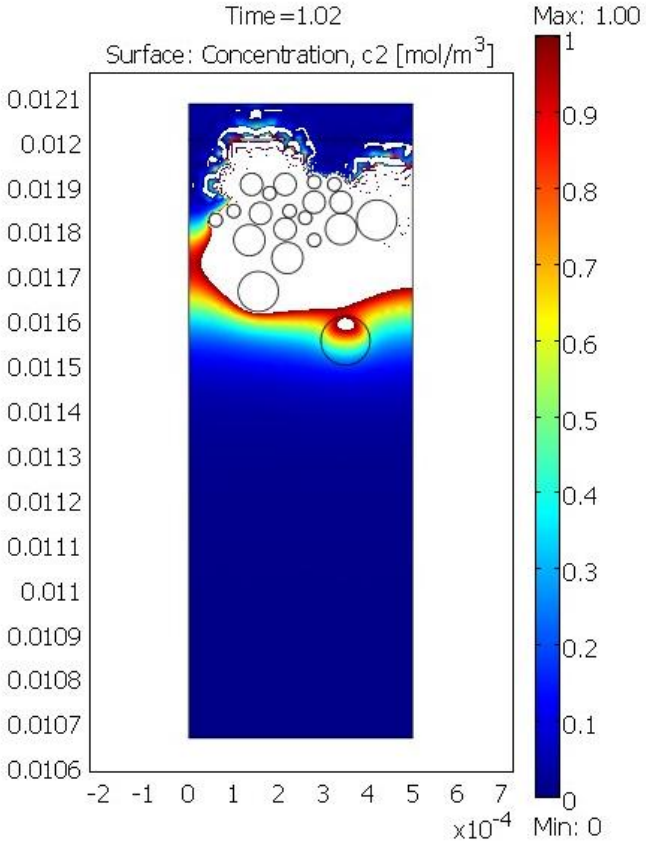

Figure 9: Surface plot of thermal damage after preheating, cryogen cooling spray and 10 laser pulses (1300 W/m^2). White space ($\Omega > 1$) corresponds to coagulated blood vessels and damaged tissue. $\Omega > 0.53$ is the thermal damage threshold for tissue.

The results in the above figure show the extent coagulation in the blood vessels and tissue damage in the dermis. The majority of the blood vessels were coagulated from the treatment, with damage even occurring in the deeper blood vessels. At the same time, there was minimal damage to the epidermis and a majority of the dermis. Those areas of the dermis that were damaged were not a cause for great concern because of the regenerative properties of skin tissue.

3.2 Sensitivity Analysis

For our sensitivity analysis, we varied the density, specific heat, thermal conductivity, optical anisotropy, absorptivity, and scattering coefficient of the epidermis, dermis and blood vessels as sensitivity analysis run with a baseline 2 ms laser pulse without any cooling or preheating.

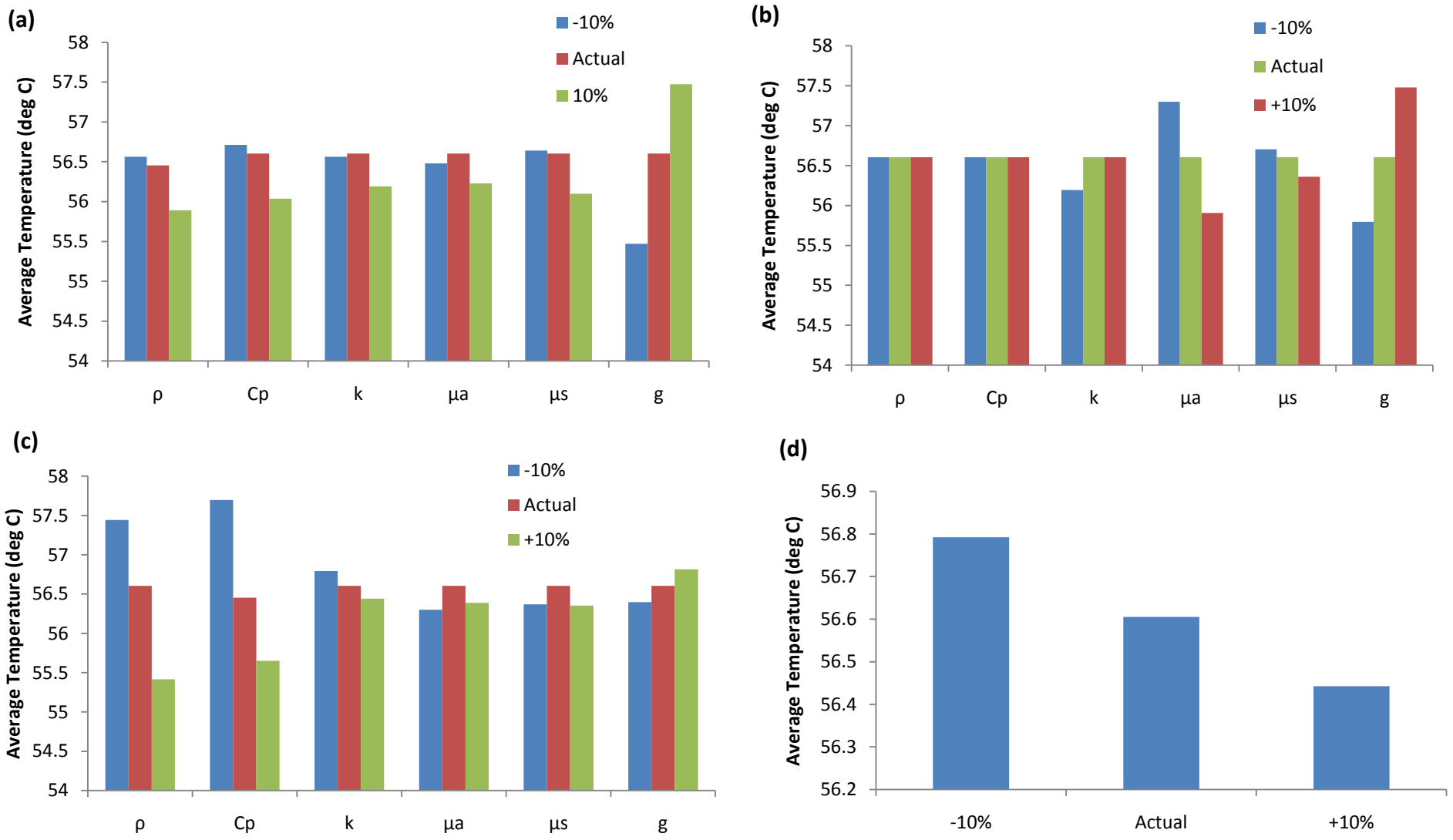

Figure 10: Sensitivity analysis of average temperature in the blood vessels for parameters in the (a) epidermis, (b) dermis, (c) blood vessels, and (d) variations in r. Parameters varied: density (ρ), specific heat(c_p), conductivity (k), absorptivity (μ_a), scattering(μ_s), optical anisotropy (g)

We found that average blood vessel temperature was particularly sensitive to changes in the blood vessel parameters, especially density and specific heat. This result can be attributed to the fact that the absorptivities are, on absolute terms, much higher for blood than for the epidermis or dermis. Thus, the largest energy deposits occurred in the blood vessels. Because the blood vessels have the most heat in our computational domain, slight variations in their thermal properties had a significant effect on average blood vessel temperature. In addition, the changes in optical parameters of the epidermal and dermal domains, such as absorptivity and optical anisotropy, also produced significant temperature variations. Modification of the directionally dependent parameter, optical anisotropy, can cause variations in the amount of laser radiation that passes through the epidermis and dermis. This would affect the amount of radiation that is absorbed by the blood vessels, which will directly influence average temperature. Furthermore, modifications in the absorptivity of the epidermis and dermis will affect the amount of radiation that is absorbed by these layers, which will also affect the amount of radiation that reaches the blood vessels. .

3.3 Accuracy Check

A comparison with literature data was used to determine the accuracy of this model. Figures 11 and 12 show the results of a post-laser pulse plot of temperature vs. skin depth performed by Jia *et al*. (2006) matched the results of our model run with the same laser power and laser pulse time. Both plots showed the characteristic plateau region towards the surface of the skin, and an initial dip below body temperature as the skin depth reaches the dermis. Both plots also peak at the same maximum temperature of about 120°C, and dip down to body temperature as distance into the dermis increased.

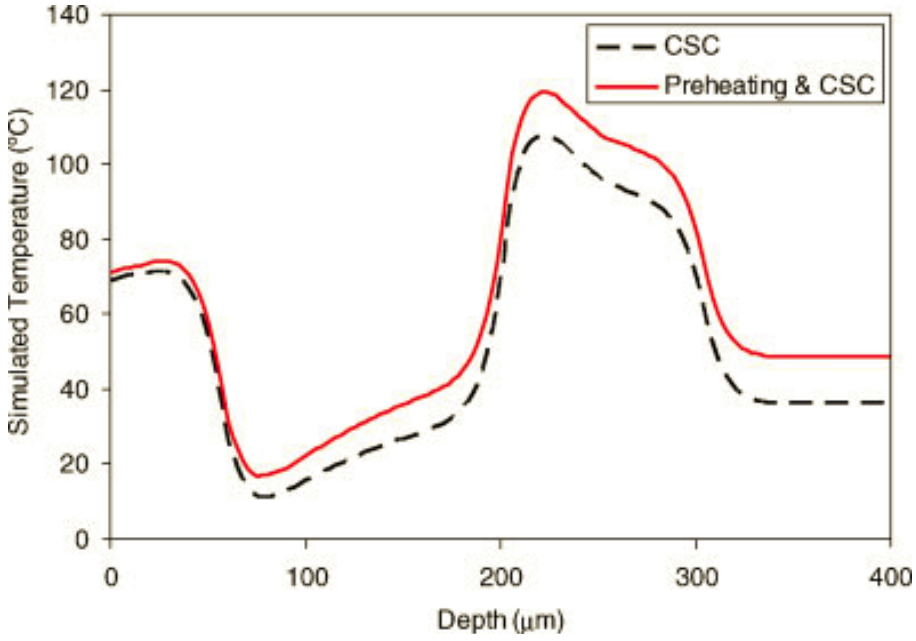

Figure 11: Temperature profile as a function of Depth (μm): Experimental temperature data measured with thermocouple [5].

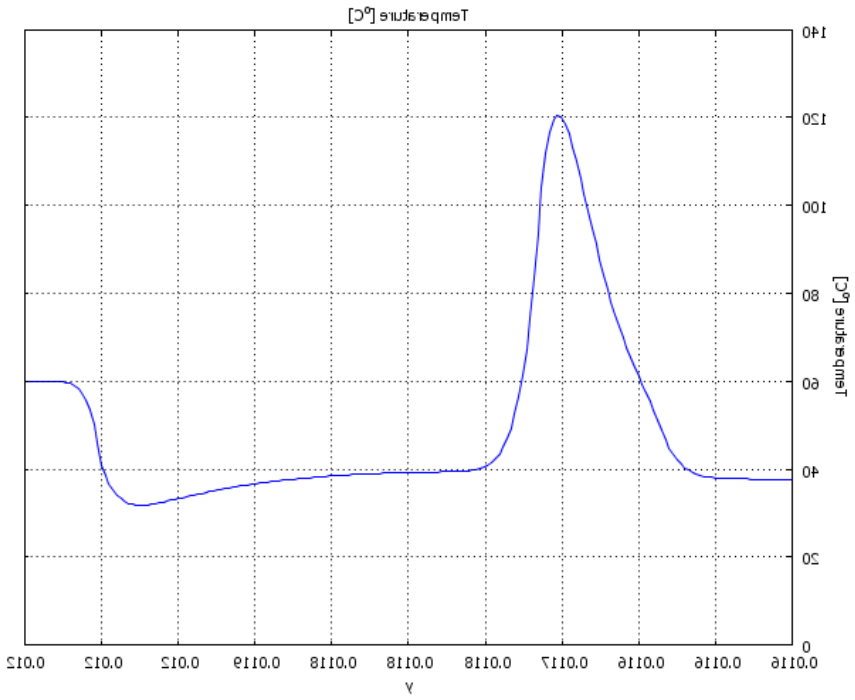

Figure 12: Temperature profile as a function of depth (m): COMSOL Multiphysics.

There are, however, clear differences between the two graphs. The plot from Jia *et al.* (2006) shows a broader peak at the depth of the blood vessel with a characteristic shoulder. Additionally, the initial temperature drop in their model is significantly greater than in our model. These differences can be reconciled by variations in computational domain geometry and disparities in the cooling methods implemented in each model. Jia *et al.* (2006) determined heat flux due to temperature change over time using experimental temperature data from a cryogen cooling experiment. This heat flux was then applied as the boundary condition to model the heat transfer resulting from the cryogen spray. Our model determined heat flux via a convective boundary condition, using a heat transfer coefficient and the cryogen ambient temperature to model the heat transfer effects of the cryogen spray. Because the Jia *et al.* (2006) experimental data includes heat loss due to evaporation of the cryogen on the skin, the temperature drop in their model is significantly greater than in our model. Additionally, their computational domain contains a row of evenly spaced, multiple vessels of varying diameters that lie at an exact depth of 200 μm. This resulted in a broader peak with a characteristic shoulder in the temperature profile from Jia *et al.* (2006), as the larger vessels in the domain created an uneven distribution of heat as depth increased. Our computational domain for this accuracy check contained a single vessel, which resulted in a more evenly distributed temperature profile and a single narrower peak. Thus, taking the explanations for these disparities into account, our model can be considered accurate based on the favorable comparison to the temperature profile available from literature.

4.0 Conclusions and Design Recommendations

4.1 Design Recommendations

Upon analysis of the skin after the sole use of laser heating, we determined that we would need to add some type of cooling to allow for successful blood coagulation without causing pain or damage to the patient. We investigated two possible methods for cooling the skin surface: (1) 100 ms cryogen spray cooling and (2) 100 ms contact water cooling. From our computational model, we found that cryogen cooling was more effective than water cooling at keeping the epidermis at a low temperature.

With the use of one 2 ms pulse alone, we were not achieving sufficient heating in the deeper blood vessels. This problem is very common for this procedure and is hypothesized to be a reason for the incomplete lightening of the skin during PDL treatment [4]. Possible improvements to target these deeper vessels include optimizing laser pulse energies, pulse durations, preheating and number of pulses. For our design, we focused our attention on modifying our heating methods by adding preheating and applying multiple pulses. After first implementing 10 laser pulses we still observed insufficient heating in the deeper blood vessels, which prompted us to incorporate preheating into our model. We determined that preheating the skin at 60°C for 40 seconds followed by a combination of cryogen cooling and 2 ms laser pulses at 100 ms intervals allowed for improved coagulation of the deeper vessels.

This design works well for PWS removal and meets safety concerns and economic constraints. The changes we made to the treatment are quite feasible since these are all processes which are currently implemented commercially. Therefore, FDA approval of the treatment method would be expedited. The process would also be cost effective to implement in a clinical setting. Most cryogen sprays are about $20-$30, pulsed dye lasers are about $20,000, and preheating equipment is around $35. The pulsed dye laser would be a one-time installation cost and therefore, on average, the practitioner would only need to cover the replacement costs of the laser and cryogen spray.

4.2 Conclusions

In this report, we investigated different treatment strategies for the removal of PWS. We were particularly concerned with developing a method to target blood vessels deeper in the dermis that are typically resistant to PDL treatment. After establishing a 2-dimensional model of PWS and implementing our treatment methods, we validated our model with a comparison to literature data. We found that our results were consistent with experimental results and determined that our model was reasonably accurate. To follow, we performed a sensitivity analysis to understand what parameters affect temperature. We realized that current laser methods fail to heat blood vessels deep in the dermis. Therefore, we modified the current design to heat deeper blood vessels without compromising patient comfort during the process. Using our model we found an optimal treatment that is able to coagulate deeper blood vessels by combining preheating, 100 ms cryogen cooling and 10 laser pulses.

Since we used COMSOL to model the PWS treatment, there is still the question of whether our results would match experimental data because the process was done via computation. However, in this case, computational modeling is still a more appropriate choice than direct experimental methods. COMSOL gives us the opportunity to quickly evaluate a variety of therapeutic combinations. By simply altering parameters, application times, etc., we were able to gauge whether a method would be successful without actually consuming the resources necessary for experimentation. Testing preliminary methods on patients would not be ethically safe since it could cause harm to the patients. In this way, COMSOL and preliminary computational modeling in general allows us to weed out potentially ineffective or harmful treatments. Granted, this is only the first step. If our method were to be actually implemented commercially, we would need to start an FDA approval process for clinical trials. Producing a computational model beforehand is advantageous in that it reduces the time and resources needed to conduct later-stage trials.

Appendix A: Mathematical Statement of the Problem

Governing Equations:

For our model, we will drop the convective terms as we will only be concerned with the heat transfer within the skin and into the blood vessel wall, both of which are solid regions that lack any sort of bulk flow. The Q term is the heat due to laser irradiation and will be determined through the relationship between the laser intensity, absorption and scattering of the particular wavelength of laser by the particular skin layer/blood vessel tissue, and the penetrative volume of the laser radiation. Blood perfusion is assumed to be negligible because of the short duration of the laser pulse.

Heat Equation

$$\rho C_P \frac{\partial T}{\partial t} = k\left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2}\right) + Q$$

Heat Source Term: $Q = \mu_a \varphi$

Light transport:

$$\frac{\partial \varphi}{\partial t} - D\nabla^2\varphi + c_*\mu_a\varphi = 0$$

where: φ = fluence [J/m^2]
D = optical diffusion coefficient = $c_*[3(\mu_a + (1-g)\mu_s]^{-1}$
c_* = speed of light in tissue [m/s] = c_0 / n
c_0 = speed of light in vacuum
n = index of refraction
μ_a = absorption coefficient of tissue [m^{-1}]
μ_s = scattering coefficient of tissue [m^{-1}]
g = optical anisotropy factor

Initial Conditions:
At time t= 0
$T_{epidermis} = T_{dermis} = 37°C$
$T_{blood} = 41°C$

Boundary Conditions:

- Boundary condition for laser radiation at skin surface: $(1-r)P(t)c_0 = -D\frac{\partial \varphi}{\partial y}$
  where r = ratio of reflected light to laser power output
  P(t) = power [W/m^2]
  c_o = speed of light in vacuum [m/s] = 2.998x10^8 m/s
  n = normal direction
- Boundary condition for cryogen cooling: $-k\frac{\partial T}{\partial y} = h_c(T - T_{cryogen})$
- Boundary condition for water cooling: $T_{water} = 0$ ºC

- Boundary condition for preheating: $-k\frac{\partial T}{\partial y} = h_p(T - T_{air\ jet})$

- Zero flux at bottom and sides

Thermal Damage

$$\frac{d\Omega}{dt} = Ae^{-\frac{\Delta E}{RT}}$$

Where Ω = thermal injury
A = frequency factor [s^{-1}]
ΔE = activation energy [W/m]
R = gas constant = 8.314 [J/molK]
T = temperature [K]

Parameters

	Thickness/ Diameter[μm]	**μ_a [m^{-1}]**	**μ_s [m^{-1}]**	**g**	**k [W/mC]**	**ρ [kg/m^3]**	**C_p [J/kgC]**	**n**
Epidermis	80	1800	47000	0.79	0.21	1200	3600	1.37
Dermis	200	240	12900	0.79	0.53	1200	3800	1.37
Blood	15-55	19100	46700	0.99	0.55	1100	3600	1.33
Water	5	0.08	2.7e-4	0.45	0.56	1000	4186	1.33

References: [10] and [11]

Thermal Damage [8]

Parameter	**Unit**	**Value**
Frequency Factor, A	s^{-1}	3×10^{98}
Activation energy, ΔE	J/mol	6.3×10^{8}
Threshold for coagulation, Ω	N/A	1.0
Threshold for injury, Ω	N/A	0.53

Process Parameters: Pulse Dye Laser

Parameter	**Unit**	**Value**
Power	W/m^2	1300
Pulse Duration	ms	2
Ratio of Reflected light	N/A	0.45

Process Parameters: Preheating

Parameter	**Unit**	**Value**
Temperature, $T_{air\ jet}$	C	60
Heat transfer coefficient, h_p	W/m	200

Process Parameters: Cryogen Spray Cooling

Parameter	**Unit**	**Value**
Temperature, $T_{cryogen}$	C	- 44
Pre-cooling Duration	ms	100
Heat Transfer Coefficient, h_c	W/m^2K	4000

Process Parameters: Water Cooling

Parameter	**Unit**	**Value**
Water Temperature, T_{water}	C	0
Thickness of water layer	μm	5

Appendix B: Solution Strategy

The solver settings used to run the COMSOL model were as follows:
Solver: direct UMFPACK
Time-step: preheating: 0.1 seconds, cryogen/water cooling/laser 0.0005 seconds
Relative tolerance: 0.001
Absolute tolerance 0.0001

Mesh Convergence

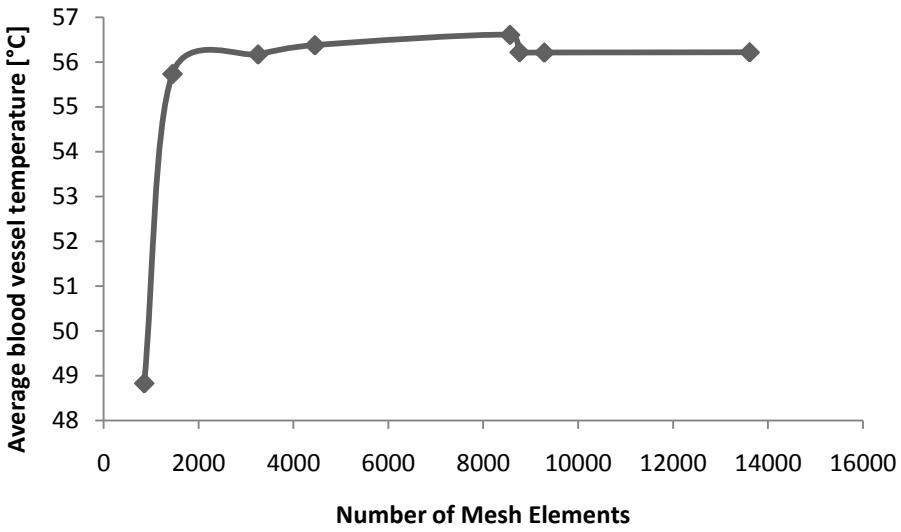

Figure B-1: Plot of average blood vessel temperature vs. number of mesh elements.

The above graph is a plot of maximum temperature at a point inside the blood vessel vs. number of mesh elements. The temperature converges for a mesh with 4451elements.

Final Mesh

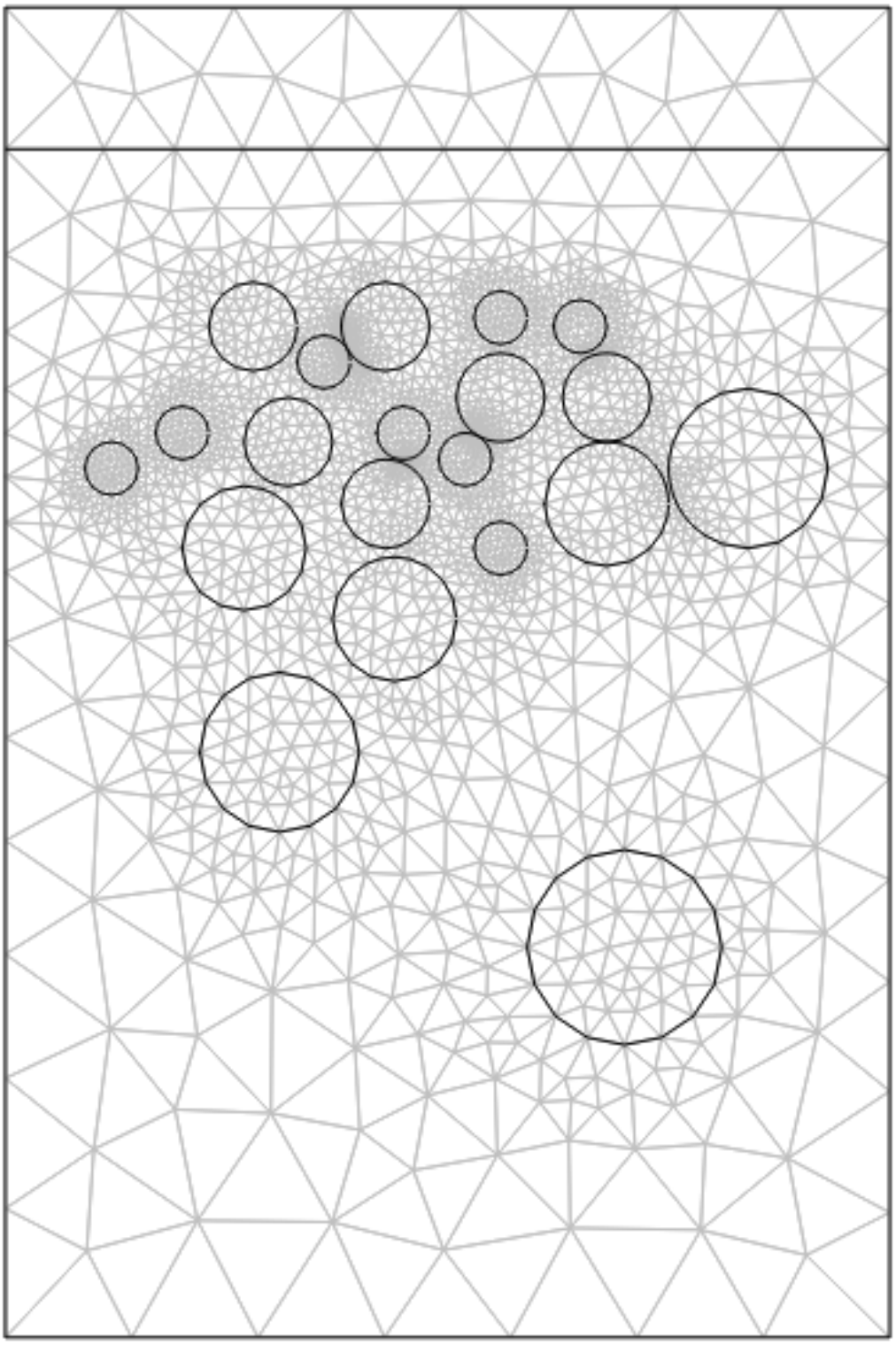

Figure B-2: Final Mesh with 4451 elements.

Appendix C: Additional Visuals

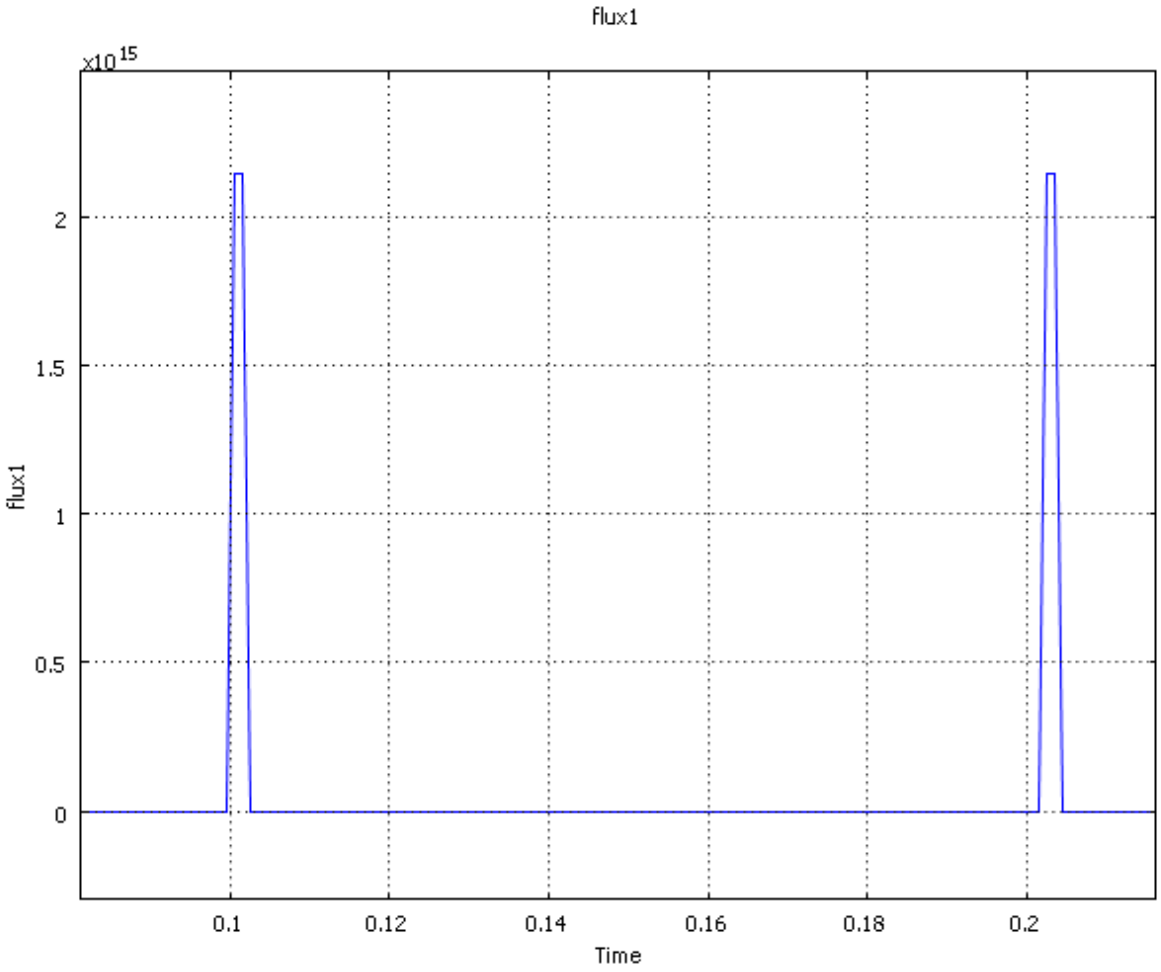

Figure C-1: A pair of laser pulses with a 100 ms cooling period in between. Flux1 corresponds to the light flux into the skin. Time is in seconds.

Appendix D: References

[1] Smithies, DJ et al. "Three-Dimensional Reconstruction of Port Wine Stain Vascular Anatomy from Serial Histological Sections" Physics in Medicine and Biology 42 (1997): 1843.

[2] Van der Horst, C. M. A. M. "Effect of the Timing of Treatment of Port-Wine Stains with the Flash-Lamp-Pumped Pulsed-Dye Laser" New England Journal of Medicine 338.15 (1998): 1028-1033.

[3] Lanigan, S. W., and S. M. Taibjee. "Recent Advances in Laser Treatment of Port-Wine Stains." The British Journal of Dermatology 151.3 (2004): 527-33.

[4] Jasim, Z. F., and J. M. Handley. "Treatment of Pulsed Dye Laser-Resistant Port Wine Stain Birthmarks" Journal of the American Academy of Dermatology 57.4 (2007): 677-82.

[5] Jia, W., et al. "Improvement of Port Wine Stain Laser Therapy by Skin Preheating Prior to Cryogen Spray Cooling: A Numerical Simulation " Lasers in Surgery and Medicine 38.2 (2006): 155-62.

[6.] Stoll, AM and LC Greene."Relationship between pain and tissue damage due to thermal radiation" J Appl Physiol 14 (1959): 373-382.

[7] Shafirstein, G., et al. "A New Mathematical Approach to the Diffusion Approximation Theory for Selective Photothermolysis Modeling and its Implication in Laser Treatment of Port-Wine Stains" Lasers in Surgery and Medicine 34.4 (2004): 335-47.

[8] Diller KR and LJ Hayes. "A finite element model of burn injury in blood-perfused skin" Journal of Biomechanical Engineering, 105 (1983): 300-307.

[9] Aguilar, G., et al. "Cryogen Spray Cooling Efficiency: Improvement of Port Wine Stain Laser Therapy through Multiple-Intermittent Cryogen Spurts and Laser Pulses" Lasers in Surgery and Medicine 31.1 (2002): 27-35.

[10] Pfefer, T. J, et al. "Bioheat Transfer Analysis of Cryogen Spray Cooling during Laser Treatment of Port Wine Stains" Lasers in Surgery and Medicine 26.2 (2000): 145-57.

[11] "Light Absorption & Scattering in Water Samples" D&A Instrument Company (2005): Accessed 4/16/2010 <http://www.d-a-instruments.com/light_absorption.html>.

[12] " For Sale CANDELA 2000 V-Beam Laser - Pulsed Dye" CANDELA (2010): Accessed 5/1/2010 <http://www.dotmed.com/listing/667293>.

[13] "Cryo Embedding/Cryo Spray" Ted Pella, Inc (2010): Accessed 5/1/2010 <http://www.tedpella.com/cryo_html/cryo2.htm>.

[14] “GPL36” Ushio (2010): Accessed 5/1/2010 <http://www.sciencelighting.com/servlet/the-1191/GPL36-Germicidal-%28AKA-254nm/Detail>.

[15] “Port Wine Stains (Red Birthmark” The Victorian Cosmetic Institute: Accessed 5/1/2010 <http://www.thevictoriancosmeticinstitute.com.au/red-birthmarks/>.