I, Shona A Burkes, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences/Biopharmaceutics.

It is entitled:
Quantitative Multimodal Skin Imaging in Pediatric Health Care: Infantile Hemangiomas and Hypertrophic Burn Scars

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Quantitative Multimodal Skin Imaging in Pediatric Health Care: Infantile Hemangiomas and Hypertrophic Burn Scars

A dissertation submitted to the
Graduate School
of the University of Cincinnati
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

In the Department of Pharmaceutical Sciences
of the College of Pharmacy

by

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Texas A&M University, 2003

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ABSTRACT

The traditional approach to evaluating infantile hemangiomas (IHs) and hypertrophic burn scars (HTS) in clinical pediatrics relies on subjective visual assessment by experienced clinicians. This method is often associated with high variability and lacks quantitation, thereby making it more challenging to determine the extent of treatment response over time and compare treatment modalities. IHs and HTS (benign cutaneous tumors) represent two skin conditions that develop from aberrant physiological processes, culminating in excessive growth and can be associated with mortality and morbidity. Reliable methods that can assess progression and detect early treatment effects are needed to optimize therapy individually.

The development and application of multimodal skin imaging methods including, three-dimensional (3D) laser scanning for skin height, high-resolution color imaging, and biomechanical measurements of skin elasticity were used to assess treatment response in 17 burn scar patients with 27 HTS undergoing combination pulsed-dye laser and compression therapy (PDL+CT). This protocol was extended and the application of 3D surface scanning, high-resolution color imaging, and infrared (IR) thermography in static mode and active dynamic mode following 30 second cold stress were used to objectively characterize changes of height, color, temperature, area of involvement, and perfusion in 119 subjects with 134 IHs (35 superficial, 97 mixed) in the natural ontogeny (observation/untreated) and in response to treatment (oral propranolol, topical timolol). Additionally, a custom graphical user interface (GUI) was developed for co-registration of high-resolution color and IR thermal images and automated image analysis algorithms.
to objectively quantify and map relevant physiological information. Both multimodal protocols and methods for analysis of the data were designed to quantify the specific characteristics for each condition in comparison to the conventional subjective clinical evaluation.

Overall, significant reductions in redness after 2 treatments and height and elasticity after 3 treatments were detected with the objective modalities in PDL+CT compared to CT alone (p < 0.05). Visual assessment of the scar by therapists, revealed significant changes in vascularity, pigmentation, pliability, and height in response to PDL+CT treatment than for CT alone after three treatments. IHs treated with propranolol had significant reductions over time in height, redness, temperature, a greater reduction in rewarming behavior and significant increases in yellowness and lightness (approaching normal tissue) compared to untreated and timolol treated hemangiomas. Significant decreases in tumor vascularity were detected using dynamic IR thermography between 10 - 18 and after 36 months of age compared to the first 6 months of age. Mixed IHs had significantly higher rewarming responses compared to superficial IHs. The data generated demonstrate that multimodal skin imaging is feasible for evaluating the ontogeny of IHs over time and differentiating various treatment techniques in HTS and IHs. Active dynamic infrared thermography shows great promise in discerning tumor vascularity. These methodologies may permit more standardized assessment of disease progression and treatment response, and have in comparing treatment modalities. Quantitative multimodal skin imaging can also be applied to other skin conditions, such as wounds, pressure ulcers, irritant dermatitis, and atopic dermatitis.
ACKNOWLEDGEMENTS

“I can do all things through Christ who strengthens me.” Philippians 4:13. First, I have to give thanks and praise to God for guiding me and providing continual strength through this journey. To my parents, Philip and Rosemary Burkes, thank you for your love, support, and encouragement as “we” obtained our Ph.D. degree. I could not have done this without you…I love you! To Ajiri, thank you for your patience and support. You have been a source of light in my life. To Dr. Julie Todaro, thank you for being a great mentor and friend. You have been tremendously helpful throughout this journey and a true source of inspiration. I would like to thank Jennifer Alexander and Lola Kelly-Smalls, for being great examples of successful African-American women in our industry and always providing words of encouragement. To my friends, and extended family, thank you for your love and support. To my Alma Mater, Texas A&M University, thank you for giving me a solid educational foundation.

This thesis would not have been possible without the help, support and patience of my principle advisor, Dr. Marty Visscher, not to mention her advice and unsurpassed knowledge of skin research. Thank you for always being optimistic and believing in me to complete the hemangioma research. Your contributions to my work, my growth, and my future are undeniable. To Dr. Randall Wickett, thank you for accepting me into the Cosmetic Science program, and for providing me with so many unexpected opportunities. Your good advice and support as my co-advisor have been invaluable, for which I am extremely grateful. It is an honor to be your omega Ph.D. student. I would like to thank Dr. Denise Adams for your medical expertise, for entrusting me with your Hemangioma
and Vascular Malformation Center (HVMC) families and for always reminding me of the strength of our data. To Dr. Donna Jones, thank you for your 3D expertise, help with practical issues, and a very enjoyable work environment. To Dr. Gerald Kasting, thank you for contributions to my projects and for being available for discussions and recommendations. Also, a special thank you for your support and words of encouragement during my transition from a Master’s to Ph.D. student. I am forever grateful. To Dr. Kevin Li, thank you for lending your expertise in Pharmaceutical Sciences, your recommendations for funding and encouraging me throughout this experience.

My deepest gratefulness goes to the HVMC patients and families participating in the imaging research, knowing it wouldn’t be a direct benefit to them, but to help others in the future. I’m grateful to Dr. Adrienne Hammill and Carol Chute, ANP, of the HVMC staff, for your medical expertise and for being available for discussions and with suggestions. I would also like to thank Dr. Kevin Bailey for your burn expertise and support, for which I am grateful. My thanks also goes to Dr. Kenneth Eaton for your invaluable help and technical expertise with image analysis in Matlab and Dr. Jeffery Welge for your statistical expertise.

Further, I would like to thank College of Pharmacy faculty and staff, especially Karen Henry and Monica Brown, and my fellow Ph.D. students/friends Dr. Terri LaCount, Allison Rush, JàNay Woolridge and Sudhir Baswan.
I would like to acknowledge the financial, academic and technical support of the University of Cincinnati, Cincinnati Children’s Hospital Medical Center, Shriners Hospitals for Children - Cincinnati, Society of Pediatric Dermatology, American Foundation of Pharmaceutical Education, Yates Foundation, Skin Sciences Program, and Center for Clinical & Translational Science & Training (CCTST). Lastly, I also thank the College of Pharmacy for their support and assistance since the start of graduate work in 2008.
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ΔTcool</td>
<td>Change in temperature during cooling</td>
</tr>
<tr>
<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>α*</td>
<td>Redness</td>
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<tr>
<td>AUCrw</td>
<td>Area under the curve during rewarming</td>
</tr>
<tr>
<td>b*</td>
<td>Yellowness</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>CCHMC</td>
<td>Cincinnati children’s hospital medical center</td>
</tr>
<tr>
<td>Color</td>
<td>High resolution color</td>
</tr>
<tr>
<td>CON</td>
<td>Control</td>
</tr>
<tr>
<td>CT</td>
<td>Compression therapy</td>
</tr>
<tr>
<td>CTM</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DIR</td>
<td>Digital infrared</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear models</td>
</tr>
<tr>
<td>GLUT1</td>
<td>Glucose transporter 1</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical user interface</td>
</tr>
<tr>
<td>IH</td>
<td>Infantile hemangioma</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>HTS</td>
<td>Hypertrophic burn scars</td>
</tr>
<tr>
<td>HVMC</td>
<td>Hemangioma and vascular malformation center</td>
</tr>
<tr>
<td>L*</td>
<td>Lightness</td>
</tr>
<tr>
<td>LDI</td>
<td>Laser doppler imaging</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant differences</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>PDL</td>
<td>Pulsed-dye laser</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>RP</td>
<td>Raynaud’s phenomenon</td>
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<tr>
<td>tmax</td>
<td>Time to maximum temperature</td>
</tr>
<tr>
<td>TIMP1</td>
<td>Tissue inhibitor of metalloproteinases-1</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<tr>
<td>VSS</td>
<td>Vancouver scar scale</td>
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<td>US</td>
<td>Ultrasound</td>
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CHAPTER 1

Introduction
1.1. Infantile Hemangiomas

Infantile hemangiomas (IHs) are the most common tumors of infancy, occurring in up to 12% of infants. IHs occur more commonly in Caucasians, females (F:M=3:1) and premature infants. The most common locations are on the head and neck (60%). IHs are benign vascular neoplasms that are usually not present at birth, but instead are noticed several weeks after birth. It is assumed that birth triggers IH development. All IHs have a very typical and consistent natural growth course including presentation after birth, an initial proliferative phase, with rapid growth of the tumor due to endothelial cell proliferation for the first year of life (proliferative phase), followed by a plateau period between 9 – 18 months of life (stable phase), and then a slow spontaneous resolution with diminishing cellular activity and fibrous fatty deposition occurring between 12 – 18 months of life and lasting for up to 5-7 years (involution phase).

Histopathological evaluations of hemangiomas reveal characteristic findings in proliferating and involuting clinical phases. In the proliferative phase, the lesions show masses of plump, rapidly dividing endothelial cells and pericytes with few capillary lumens. Electron microscopy revealed multi-laminated basement membrane underlying the endothelium in proliferating hemangiomas. Histological features of IHs dramatically change from proliferation to involution, but there is no clear cut delineations between the two stages as both processes occur simultaneously. The stabilization period occurs when neither proliferation nor involution dominates. The onset of involution coincides with cellular apoptosis. During involution, the capillary lumens enlarge, endothelial cells flatten diminishing their activity, and fibrous fatty tissue is deposited (Figure 1.1).
IH development is caused by endothelial cell dysfunction, resulting in an abnormal angiogenesis and/or vasculogenesis. The normal regulation of angiogenesis is governed by a balance between factors that induce the formation of blood vessels (i.e., proangiogenic factors) and those that halt or inhibit the process (i.e., antiangiogenic factors). During IH formation, this balance is destroyed resulting in excessive angiogenesis which causes increased blood vessel formation involving endothelial cell hyperplasia. It is unknown when and how these angiogenic factors are regulated. Increased expressions of proangiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and proliferating cell nuclear antigen (PCNA) induce the abnormal proliferation and migration of the immature cells to initiate the excessive formation of the capillary network for hemangioma development. As the endothelial cells and pericytes differentiate, an influx of mast cells and tissue inhibitor of metalloproteinase (TIMP 1) occurs changing the growth pattern and initiating involution. All stages of IH can be distinguished from other
benign vascular anomalies by their strong immunopositivity for biological markers, glucose transporter protein isoform 1 (GLUT1), FcγRII, Lewis Y antigen, and merosin.5,15,16

1.1.1. Clinical Appearance

The clinical appearance of proliferating IHs exhibits wide heterogeneity and depends on their dermal involvement (Figure 1.2).17

- Superficial IHs develop in the papillary dermis and are bright red in color, slightly elevated or flat patches, warmer than normal surrounding tissue, and non-compressible reaching maximal size by 6-8 months of age.
- Deep IHs develop in the reticular dermis or subcutaneous fat and are softer, warm, slightly bluish and may proliferate for 12-14 months, or in rare cases up to 2 years. The overlying epidermis appears normal, although dilating veins radiating from the lesion are visible.
- Most IHs are mixed, exhibiting features of both superficial and deep.

![Figure 1.2. Clinical appearance of (A) superficial, (B) deep, and (C) mixed hemangiomas.](A) (B) (C)
Although, superficial, deep and mixed hemangiomas vary in physical appearance and depth, they follow an identical clinical course (i.e., proliferation, stabilization and involution). Regression of a superficial IH is indicated by changes in color from bright red to greying beginning centrally then extending to the periphery of the lesion. Superficial portions of mixed IH can show signs of involution, while deeper components are still proliferating. As involution progresses, the lesion decreases in size, has more laxity and is cooler. Table 1.1 shows the characteristics and visual appearance of a hemangioma during the proliferation, stable and involution stages.

The newer classification of localized, segmental or indeterminate is used to help predict complications and/or need for treatment, e.g., segmental IHs are larger with a higher incidence of complications and developmental anomalies of the brain, cerebrovasculature, cardiovasculature, eyes, and chest wall.
Table 1.1. Hemangioma status characteristics.

<table>
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<th>Stage</th>
<th>Pathophysiology</th>
<th>Characteristics</th>
<th>Examples</th>
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<tr>
<td>Proliferation (growth)</td>
<td>• Excessive Angiogenesis and/or Vasculogenesis</td>
<td>• Rapid growth</td>
<td><img src="image1.png" alt="Image 1" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Firm to palpation</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Red (Superficial) or Blue (Deep)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Warm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High blood flow</td>
<td></td>
</tr>
<tr>
<td>Stabilization (plateau)</td>
<td>• Balance between Proliferation and Involution</td>
<td>• Very little change in IH appearance</td>
<td><img src="image2.png" alt="Image 2" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No change in size</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• skin is less shiny</td>
<td></td>
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<tr>
<td>Involution (shrinking)</td>
<td>• Apoptosis of endothelial cells</td>
<td>• Shrinking</td>
<td><img src="image3.png" alt="Image 3" /></td>
</tr>
<tr>
<td></td>
<td>• Fibrous fatty tissue deposited</td>
<td>• Softer/more compressible</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Lightens, grayish</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Lower Temperature</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduced blood flow</td>
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1.1.2. Diagnosis

A key decision in patient care is determining the stage of the IH, because treatment is strongly dictated by stage. Pharmacotherapy should be administered during the proliferative phase and a more conservative “wait-and-see” approach is taken for stable and involuting IHs, as these IHs are naturally undergoing involutional changes. IHs diagnosis has been traditionally based on patient medical history and physical examination of size, color, growth stage, depth, tactile characteristics, and morphologic subtype. The patient’s medical history is crucial for diagnosing a hemangioma and
two questions that are commonly asked are: Was the lesion present at birth and did proportional or disproportional growth of the lesion occur after birth?\textsuperscript{24} The presence of the lesion at birth and proportional growth supports a diagnosis of a vascular malformation or congenital hemangioma, not an infantile hemangioma. In contrast to infantile hemangioma, both noninvoluting congenital hemangiomas and rapidly involuting congenital hemangiomas proliferate in utero and are fully developed at birth or undergoing involution.\textsuperscript{25} Additionally, congenital hemangiomas are histologically different from IHs and lack GLUT1 surface markers, and thus are not considered to be a variant of an infantile hemangioma.\textsuperscript{5,25} For deep infantile hemangiomas that are usually more difficult to assess fully on clinical criteria alone, magnetic resonance imaging (MRI), Doppler ultrasound (US) and computed tomography (CTM) are used, but the cost is prohibitive for widespread clinical application and these procedures carry higher risk for children due to the sedation. In addition, the diagnostic success depends significantly on the radiologist’s expertise in vascular anomalies.

\subsection{1.1.3. Management}

Most IHs are not clinically significant, but up to 24% develop life- or function-threatening complications that require treatment by multi-disciplinary specialist teams.\textsuperscript{2} Complications include ulcerations, bleeding, functional compromise (vision, airway, feeding), and permanent disfigurement.\textsuperscript{2,3} Half reach maximum involution by age 5 years and 90\% by age 9 years.\textsuperscript{15} Nevertheless, in 40-50\% of all affected children, significant cosmetic effects including telangiectasia, hyperpigmentation, hypopigmentation, fibrofatty tissue masses, epidermal atrophy, and/or scarring can be observed as a residue
of the lesions. IHs can also have significant psychological impact in children and adolescents, particularly with facial IHs (cosmetic sensitive areas) as these lesions can leave residual scarring or structural deformities.

There is no FDA approved therapy and there is a lack of randomized controlled trials to support the effectiveness of IH interventions, thus physicians rely on clinical judgment to determine optimal treatment of infants with hemangiomas. The goal of treatment is to have (1) stabilization of growth or (2) accelerated involution. Current guidelines recommend treatment for (1) life- and function-threatening hemangiomas, (2) large, disfiguring facial hemangiomas, (3) hemangiomas in locations that may lead to permanent scarring or deformity, and (4) ulcerated hemangiomas. Management includes propranolol, topical timolol, interferon, chemotherapeutic agents, lasers, and surgical removal.

1.1.3.1. Propranolol

Propranolol, a non-selective beta blocker, is currently the first line of treatment for severe IHs. Its mechanism of action for IHs is unknown, but has been hypothesized to regulate growth through vasoconstriction, inhibition of angiogenesis, induction of endothelial cell apoptosis, or the inhibition of matrix metalloproteinase. Propranolol is commonly administered at an initial dosage of 0.5 mg/kg/day with increasing increments of 0.5 mg/kg each day up to a maximum of 2 mg/kg/day. The maximum dosage is maintained until the lesion involutes or the child is 12 months old. Researchers have shown rapid growth arrest, color decrease, softening of lesion, and fewer adverse effects than
In 2008, Léauté-Labrèze et al.\textsuperscript{28} were the first group to document the effectiveness of propranolol in IHs. They treated 11 infants with hemangiomas (2 in case reports and 9 in study) with propranolol and demonstrated changes in color from red to purple and softening of the lesion within 24 hours after the initiation of treatment. After these initial changes, the lesions continued to improve until they were nearly flat. No regrowth was noted for any of the infants. Manunza et al.\textsuperscript{31} reported treating 30 infants with complicated IHs with propranolol. In majority of the infants (26/30) the hemangiomas stopped growing and became paler and softer within 1 week and the remainder responded within 1 month. The most common adverse effects associated with propranolol use include hypoglycemia, hypotension, and bradycardia.\textsuperscript{21} The appropriate administration of these therapies presupposes reliable and reproducible methods both for the detection of IHs stages and also the assessment of therapeutic efficacy.

1.1.3.2. Timolol

Timolol maleate is a non-selective topical beta blocker that has been used for the treatment of IHs.\textsuperscript{32-35} Timolol maleate 0.5\% gel is applied topically (1 drop) twice per day and massaged into the hemangioma. This dosage provides an estimated 0.5 mg of timolol per day.\textsuperscript{32} Several recent non-controlled studies have associated topical timolol effectiveness with reductions in size, redness and thickness in IHs within 2 to 4 post treatment initiation.\textsuperscript{34,35} Chambers et al.\textsuperscript{36} compared topical 0.25\% timolol maleate gel with placebo group and found significant improvement in size, color and thickness based on photographic documentation after 8 weeks of treatment. Similarly, Chan et al.\textsuperscript{32} determined that timolol maleate 0.5\% gel provided therapeutic onset after 12 to 16
weeks, and significantly higher proportion of IHs decreasing in size by ≥ 5% after 8 weeks of treatment compared to an increase in the placebo control group.

1.1.4. Rationale for Multimodal Imaging in IHs

Randomized clinical trials will be important in determining infantile hemangioma treatment efficacies. Typically, IHs are assessed by clinical judgment (visual and palpation) and photography (not standardized for lighting, color or position). These methods have low reliability, low reproducibility, contain significant variability, can be convoluted by other physical attributes and therefore, are limited for discerning treatment effectiveness and mechanisms of action. Quantitation of multiple tumor characteristics over time may reduce the limitations of the current classification systems. Standardized digital photography and three-dimensional reconstruction have been shown to increase clinician efficiency and overcome the limitations of recall.\(^{37}\)

Currently, there are no prospective randomized controlled studies in the literature that apply multimodal skin imaging methods to characterize treatment response and treatment effectiveness for infantile hemangiomas. In addition, quantitative information about differences in growth patterns between hemangioma stages are lacking.\(^{26}\) Multimodal imaging utilizes the different physical information for the assessment of the tissue parameters with respect to morphology (i.e. color, shape, texture) and function (i.e. perfusion and temperature) providing a more complete infantile hemangioma characteristic database, which allows for better differentiation between stages. Multimodal imaging translates the morphological and functional parameters into
mathematical terms (i.e. quantitative values). This provides the quantitative characteristics of significant features of skin lesions, introducing objectivity and reproducibility in the classification and treatment efficacy processes.

1.2. Quantitation of Cutaneous Hemangioma Characteristics

Standardized digital color imaging and analysis, three-dimensional surface scanning, and thermal imaging (static and dynamic modes), are non-invasive methods that provide objective, physiological measures of skin color, volume/size, surface temperature, and skin perfusion, respectively. There are a limited number of studies utilizing objective methods to investigate treatment response in hemangiomas.38-40

1.2.1. High Resolution Color Imaging

Color photographs for documentation are commonly used by plastic surgeons and dermatologist,41,42 they are generally not taken with standardized positioning making the comparison of images over time difficult. IHs change color from darker, bright red during proliferation to lighter, grey color during involution. Port wine stain color differences before and after treatment were measured using images and L*a*b* color analysis and correlated with clinical grades. Color imaging techniques have been used to improve objectivity, quantify features (e.g., erythema, dispigmentation, abrasion)43-45 and quantify wounds, lesions, photoaging, blanching, skin atrophy, and disease (e.g., psoriasis).44,46-50
1.2.2. Three-Dimensional (3D) Imaging

The changes in volume/height are widely used as a measure of IH growth and regression. Hermans et al. recently noted the usability and clinical relevance of 3D phototechnology as an objective measure of volume changes in IHs. Hemangiomas are often raised or large during proliferation and flat and smaller with involution. IH volume is commonly approximated via two tape measurements, perpendicular to each other across the surface of the IH, i.e., “hemispheric measurement”. Researchers expressed potential measurement bias using the hemispheric volume method, and the need for a more sensitive system to measure height/depth of IH. Radiologic imaging, such as MRI and CTM are not practical for measuring IH volume due to cost and the need for sedation and general anesthesia for young children. Three-dimensional imaging is a non-invasive quantitative method of objectively measuring height, shape, volume, size and roughness. It is used in the clinical setting for craniofacial treatment planning, to quantify facial swelling, and quantify volume and response to treatment in keloid scars.

1.2.3. Infrared (IR) Thermography

IR thermography has emerged as a reliable, noninvasive, technique to evaluate vascular function through skin temperature. Thermal IR imaging records the natural infrared radiation emitted by the skin surface in the 0.8 µm – 1.0 mm frequency and provides a thermal map of the skin surface distribution. IHs vary in temperature from increased microcirculation during proliferation and reduced circulation with involution.
Infrared thermography found temperature increase of 3.0 ± 0.4°C for proliferative IHs and a 2.5 °F (1.4 °C) decrease in IH temperature with the treatment of a systemic beta blocker. Both studies concluded that thermography is a valuable non-invasive way to identify clinical states and an effective diagnostic tool in the pediatric population. Infrared thermal imaging has been used to evaluate malignant breast lesions and burns, and the assessment of inflammatory changes associated with localized scleroderma in children.

1.2.4. Perfusion Imaging

Hemangiomas are dense arterial-venous network tumors with fast-flow properties during the proliferative phase and reduced flow during the involution phase. During proliferation, the lesion displays high vessel density (>5 vessels/cm²), high Doppler shifts (> 2kHz), low resistance flow. Verity et al. showed the blood flow rates from feeding vessels in periocular capillary hemangiomas to be up to three-fold higher than normal capillaries. During involution, the vasculature is reduced and the lesion exhibits flow characteristics similar to normal tissue. Although, laser doppler imaging (LDI), MRI, CTM, and color Doppler ultrasound (US) have been used to assess vascular behavior in IHs, these imaging techniques are rarely used clinically due to cost, sedation risks, movement artefacts, and operator dependent limitations. Dynamic IR thermography involves the application of a thermal stress to the tissue, which alters the structures beneath the surface of the skin. The subsurface structures, e.g., vasculature, respond by restoring the skin to an equilibrium state. The response patterns, e.g., time to achieve pre-stress temperature, provides additional functional information about the
tissue. Investigators and researchers have used dynamic IR thermography with a cold challenge for the detection and evaluation of primary Raynaud’s phenomenon (RP), system sclerosis, malignant melanoma and muscular lesions, but this approach has not been applied in IHs.
1.3. Hypertrophic Burn Scars

Hypertrophic burn scars (HTS) are characterized as erythematous, firm, pruritic, raised fibrous masses that remain within the boundaries of the original wound and may regress over time. They are the most common type of scars following surgical and traumatic injuries, such as burns and skin grafting, particularly in children. McDonald and Deitch reported the prevalence of HTS is as high as 75% for grafted skin in children (Figure 1.3). A hypertrophic scar can cause significant morbidity and mortality in terms of the psychological, cosmetic, functional outcome and overall quality of life issues. Scarring mostly develops in anatomical locations with high tension, such as shoulders, neck, and extremities.

![Typical hypertrophic scar post skin grafting](image)

**Figure 1.3. Typical hypertrophic scar post skin grafting**

Hypertrophic scars result from deviation from the normal wound-healing processes. The wound-healing process consists of a series of complex biological events that proceed in three overlapping phases: inflammation, proliferation, and remodeling. In the normal wound healing process each phase proceeds in a timely and predictable manner leaving a
barely noticeable scar. For severe burn injuries there is an exaggerated inflammatory response consisting of an increased influx of inflammatory cells, growth factors and cytokines known to stimulate fibroblasts migration and proliferation leading to excess amounts of collagen and extracellular matrix deposition and ultimately the formation of an hypertrophic scar.\textsuperscript{78} Other processes involved in hypertrophic scar formation include prolonged reepithelialization, increased neovascularization, atypical extracellular matrix remodeling and reduced fibroblast apoptosis.\textsuperscript{79} Histologically, HTS are fine, well-organized type III collagen bundles, containing of fibroblasts, numerous myofibroblasts, endothelial cells and higher density of microvessssels arranged in nodules.\textsuperscript{77,80,81}

Numerous treatment methods have been recommended for hypertrophic scarring including the application of physical pressure (compression), laser therapy, occupational/physical therapy, topical silicone gel sheeting, steroid injection, and reconstructive surgery.\textsuperscript{82} Although these treatments may be beneficial, there still is no universal consensus regarding the best treatment for hypertrophic scar formation due to a lack of evidence-based research to support their effectiveness.\textsuperscript{83-85}

1.3.1. Compression Therapy (CT)

Pressure (compression) therapy is the gold standard for severe burn injuries and post-operative treatment of skin grafts at the Shriners Burn Hospitals.\textsuperscript{86,87} Patients are fitted with customized pressure garments to obtain between 24-40 mmHg of compression which must be worn for at least 23 hours a day.\textsuperscript{88} The exact mechanism of how compression therapy improves hypertrophic scarring is not completely understood.
However it is widely believed that compression therapy decreases blood flow causing excessive hypoxia resulting in fibroblast degeneration and collagen degradation.  

1.3.2. Pulsed-Dye Laser (PDL)

The 585 nm Pulsed-Dye laser (PDL) treatment is considered one of the more promising therapies for treating hypertrophic scars. There is no universal consensus on the mechanism by which the PDL improves the appearance of scars, however most believe laser therapy for scar reduction is based on the principle that vascular proliferation plays a key role in scar formation. The spectral absorption characteristic of light through human skin is well established. Figure 1.4 shows the response curve for melanin and oxyhemoglobin in relation to the PDL. The 585-nm PDL uses selective photothermolysis that targets hemoglobin (oxyhemoglobin absorption peak is 542 nm) resulting in microvascular ablation at a penetration depth between 1 – 1.5 mm, without damaging surrounding tissue. The destruction of scar microvasculature leads to thrombosis resulting in a reduction of collagen in the scar. Histology shows the PDL decreases TGF-beta expression, fibroblast cell proliferation, collagen Type III deposition and increase in MMP-13 activity. The most common side effect of PDL treatment is purpura, a slightly bruised appearance lasting 7 – 10 days. Hyperpigmentation has also been reported, mainly in dark skinned individuals.
Figure 1.4. Absorption spectra of the principal skin chromophores: melanin and oxygenated hemoglobin.

The use of the PDL for treating HTS has been well documented. However, the effectiveness of the PDL for the treatment and prevention of hypertrophic burn scars remains controversial. Various studies have evaluated the PDL and have shown it to be effective in improving the erythema, texture, pliability, height and overall cosmetic appearance of hypertrophic scars. One of the earliest PDL studies in 14 patients with erythematous and/or hypertrophic scars treated with the 585nm PDL demonstrated a 57% to 83% improvement in color, thickness and surface texture in the scars after one to two PDL treatments, respectively. McCraw et al. showed the benefit of early use of the PDL in preventing HTS formation. They treated 106 patients with 171 HTS with the 585-nm PDL and demonstrated faster resolution in scar erythema, stiffness and color uniformity of the scars when treated with the laser within the first 2 weeks after surgery. Alter and Nanni treated 16 patients with 40 HTS and showed improvement in scar
texture, color and pliability with minimal side effects after an average of 2.75 laser sessions.

More recently there have been contradictory results concerning the efficacy of the PDL in scar improvement. In a prospective, randomized controlled study of 20 patients with HTS did not show significant differences between treatment with PDL or silicone gel sheets compared with the control group. Allison et al. conducted a study to examine the effectiveness of the 585-nm PDL on both adult and children with mature and new hypertrophic burn scars in 38 patients. They concluded that PDL provided symptomatic improvement in pruritis, but there were no significant reductions in scar redness and texture between the treatment and control. However, this study had a heterogeneous population of patient age and maturity of scars. Likewise, in another study 56 Chinese patients with a total of 71 hypertrophic scars using the PDL reported that while the laser significantly decreased pruritis, it did not have an effect on scar thickness and elasticity compared to the control. The author suggests the use of the PDL should not be considered standard of practice. In this study the scars were from surgical incision, not burns, and did not include multiple skin types.

1.3.3. Rationale for Multimodal Imaging in HTS

As demonstrated above, there is a paucity of published PDL treatment efficacy studies that use validated objective measures of scar assessment. These studies measured outcomes differently; some were not randomized and lacked a control group without PDL treatment. To date, there has not been a prospective randomized controlled
clinical study including multiple skin types and using multiple quantitative objective methods to examine the effectiveness of the pulsed-dye laser treatment. It is important that the efficacy of this treatment be clearly established, so patients are not exposed to potential risk (i.e. purpura and hyperpigmentation) without any benefit.
CHAPTER 2

Hypotheses and Specific Aims
2.1 Objective

The purpose of our research is to determine whether multiple quantitative skin imaging modalities can effectively (1) determine treatment response in HTS patients, (2) differentiate the characteristics of IHs and if these specific imaging modalities can (3) demonstrate the physiological changes over time in IH patients requiring medical treatment and without treatment. The multimodal skin imaging will provide objective measures that clarify the ontogeny, expand the diagnostic capabilities and permit effective measurement of disease response for further therapeutic protocols.

2.2 Hypotheses:

1. The combination of Pulsed-Dye Laser (PDL) plus Compression Therapy (CT) treatment will provide better outcomes in reduced scar erythema, increased elasticity, and decreased scar height relative to CT alone over time after a series of PDL treatments.

2. The application of quantitative methods will show differences in the skin conditions outcome earlier than subjective methods (clinical assessment).

3. Infantile hemangiomas treated with propranolol will have a lesser degree of disease involvement (i.e. reduced volume/height and normalized skin color, mechanical properties, temperature and perfusion) relative to the untreated patients.
2.3 Specific Aims:

1. Determine the effects of the Pulsed-Dye Laser in pediatric burn scars

2. Evaluate the utility of quantitative measures of color, shape, tissue perfusion, infrared thermography and viscoelasticity to effectively differentiate the clinically relevant characteristics of IHs during their ontogeny

3. Determine the effects of Propranolol for the treatment of cutaneous IHs
CHAPTER 3

Multimodal Quantitative Analysis of Early Pulsed-Dye Laser Treatment of Scars at a Pediatric Burn Hospital

3.1 Introduction

Survival from thermal injury has improved markedly in recent history but a concomitant improvement in cosmetic outcomes is lagging.\textsuperscript{102,103} Multiple modalities have been used for scar management, including glucocorticoid injections, retinoic acid, application of silicone gels, pressure garments, and simple massage.\textsuperscript{104-109} There has been no clearly superior method to prevent or treat scars related to burn injury. The Pulsed-Dye Laser (585 and 595nm) offered the potential improvement of hypertrophic scars, including scars related to burn injury.\textsuperscript{95,97,110,111}

Published studies indicated that the Pulsed-Dye Laser (PDL) might reduce erythema, lighten the skin and improve subjective appearance, vascularity and pliability.\textsuperscript{95,97,110,112} A comprehensive review of laser treatment of hypertrophic scars identified the studies using pulsed-dye lasers of on scars older than 6 months. Improvement was low for 585 nm and moderate for 595 nm lasers.\textsuperscript{113} However, the utility of PDL for the treatment of scars in patients treated at a pediatric burn facility has not been reported in the context of a controlled trial. Furthermore, we wanted to objectively confirm our clinical judgment that the use of the PDL improved the outcomes of scars in pediatric burn patients.

We conducted a randomized controlled study to evaluate the effects of PDL on scar at the seams of newly healed skin grafts placed for reconstruction after burn injury. One half of the seam was treated repetitively with the PDL and both halves received conventional compression therapy. Standard clinical assessments (expert blinded observer) and
objective measures of scar color, height, and biomechanical properties were used to evaluate the scars.

### 3.2 Materials and Methods

Patients scheduled to undergo burn scar revision at Shriners Hospitals for Children in Cincinnati, Ohio were enrolled. Eligible subjects were ≥ 9 years old, had scars on the extremities (excluding the hand) and could return for evaluation and treatment. Pregnant patients were excluded. Initial evaluations and initiation of treatment were to begin 2-4 weeks after engraftment. The research was approved by the Institutional Review Board of the University of Cincinnati (♯08-06-24-01). Subjects ≥ 18 years provided written informed consent. Parents or guardians provided written informed consent for patients < 18. Patients older than 14 years provided informed assent.

Prior to each treatment, scar seams and graft were assessed for vascularity, pigmentation, pliability and height by clinical experts (therapists) using the Vancouver Scar Scale (VSS). The areas were evaluated using standardized high resolution digital photographs, three-dimensional (3D) surface topography, and biomechanical properties. Adjacent, uninvolved skin sites served as “normal skin” controls. The seam/graft area was measured along its longest axis and divided in half. Treatments were randomly assigned (SAS Institute Incorporated, Cary, NC). One half of the seam was treated with the Pulsed-Dye laser (PDL) and the other was untreated. The entire graft received standard compression therapy. Prior to PDL treatment, 4% lidocaine (LMX4®, Ferndale Laboratories, Inc., Ferndale, MI) was applied topically to the entire graft.
The same surgeon (JKB) marked the graft and performed all treatments with a Vbeam® system (pulsed-dye laser, 595nm wavelength, Candela Corporation, Wayland, MA). A 10 mm laser spot size and 0.45 ms pulse duration were the same for each treatment. The dynamic cooling setting was 30ms/20ms. Fluency was initially set at 5.0 J/cm² with an increase of 0.25 – 0.5 J/cm² with each successive treatment up to a maximum of 6.75J/cm². PDL sites were treated to produce either transient or persistent purpura with increases in fluency adjusted accordingly. A photograph of the ideal purpura target was viewed prior to each treatment. Subsequent evaluations and treatments took place at approximately 6 week intervals. Compression garments were fabricated with compression levels of 10-20 mmHg from powernet (spandex and nylon, Darlington Fabrics, Westerly, RI) or tricot (spandex, Spandex House, Inc., New York, NY) based on patient tolerance. PDL treatments continued until maximal improvement occurred, i.e., standard clinical endpoint of a thin, flat, pale, and pliable scar, on either side of the graft.

The high resolution images were acquired under standardized conditions with a Nikon D-90 camera, a Micro-Nikkor 60mm lens and a Nikon R1 wireless close-up flash system. The medial and lateral portions represent the PDL plus compression therapy (PDL + CT) and compression therapy along (CT alone) treatment regions. The center third was excluded to eliminate any potential for laser treatment overlap. The images were processed to determine erythema (a*) using Image J (NIH, Bethesda, MD) as described previously. A threshold was established and used to separate the graft area from the surrounding skin. The scar seam was extracted by reducing the shape of selection (−4.8 mm) and subtracting this region from the entire graft area. The “graft area” was taken
as the total minus the seam. A 1 cm$^2$ uninvolved skin area adjacent to the graft with the lowest a* value served as the ”normal skin” control.

Quantitative skin erythema, referred to as excess erythema, was defined as the percentage of red pixels in the a* image (seam, graft) with redness higher than the mean value plus one standard deviation as previously described (ImageJ). Three dimensional (3D) topography was obtained with a Cyberware Rapid 3D Digitizer laser scanner (Cyberware, Inc., Monterrey, CA). Scar height was determined by subtracting the 3D surfaces for each scan from the scan taken prior to the first treatment. Biomechanical properties including elasticity (mm), elasticity percent, elastic deformation (mm) and stiffness (mmHg/mm) were measured at a 1 cm$^2$ area on each seam and in an adjacent ”normal skin” site using the BTC-2000™ (SRLI Technologies, Nashville, TN) through two cycles at 10mmHg/second for 15 seconds with 5 seconds of relaxation between cycles.

Outcome measures were analyzed using linear mixed models repeated measures procedures with visit as the repeat (F statistic, p < 0.05) (SPSS, SPSS, Inc., Chicago, IL). The covariance type was diagonal. Treatment comparisons were made with the LSD method (p < 0.05). Covariates included starting skin condition to account for variations in the pre-operative condition for scar halves. VSS scores were compared as change from baseline. Treatment effects were determined as the difference treatment and control for the objective measures.
3.3 Results

The demographic characteristics are shown in Table 3.1. Only the data from subjects who completed at least two treatments were included in the analyses.

Comparison of the two scar halves indicated significant differences (p < 0.05) at baseline, i.e., prior to treatment, for PDL + CT versus CT alone for VSS height (0.4 ± 0.1 and 0.1 ± 0.1, respectively), seam excess erythema (0.9 ± 0.1 and 0.8 ± 0.1, respectively) and interior graft excess erythema (0.8 ± 0.1 and 0.7 ± 0.1, respectively).

Figure 3.1 provides an example of the observed clinical changes after two laser treatments. The VSS for vascularity, pigmentation, pliability and height were lower for PDL + CT versus CT alone after three treatments (p < 0.05) (Figure 3.2a, b, and c). Treatment of burn scars with PDL + CT resulted in lower excess erythema of both the seam and skin graft than CT alone (p < 0.05) (Figure 3.3). Reduction in excess erythema was found for PDL + CT versus CT after two treatments and further reduction occurred with additional therapy. Similarly, greater reduction in excess erythema also occurred for the inner portion of the graft for PDL + CT versus CT alone (p < 0.05) (Figure 3.4).
Table 3.1. Demographic information for subjects who completed at least one laser treatment and two study visits (start, post treatment 1)

<table>
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<th>Age (mean, range)</th>
<th>15.2 (9-19) yrs</th>
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<tbody>
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<td>Gender</td>
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<td>Female</td>
<td>6 (46%)</td>
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<td>Male</td>
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<td>Fitzpatrick Type IV</td>
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<td>Scar Location</td>
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<tr>
<td>Forearm</td>
<td>5 (24%)</td>
</tr>
<tr>
<td>Foot</td>
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</tr>
<tr>
<td>Wrist</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>Thigh</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Antecubital</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Mean Graft Size (cm²)</td>
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</tr>
<tr>
<td>Mean Laser Fluency (J/cm²)</td>
<td></td>
</tr>
<tr>
<td>Treatment 1</td>
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<td>Time: Original Injury to Revision</td>
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<tr>
<td>No. Sites (scars)</td>
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<td>Number with Least 2 Treatments</td>
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<tr>
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<tr>
<td>Post Treatment 5</td>
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</table>
Figure 3.1. Visual clinical changes were observed at baseline and after two treatments. The arrows indicate regions along the graft seam. The top row are the pulsed-dye laser plus compression therapy (CT) site before (left) and after two laser treatments (right). The bottom are the CT alone site at the same time points.

Skin elasticity was higher (more elastic) for the PDL + CT versus CT alone after three treatments ($p < 0.05$) (Figure 3.4). Scar height was lower for PDL + CT versus CT alone after three treatments with values of $0.07 \pm 0.04$ mm and $0.20 \pm 0.04$ mm, respectively ($p < 0.05$) but could only be determined over multiple treatments for five patients due to difficulties in reproducibly positioning the subjects (data not shown).
Figure 3.2. A, B, and C: Clinical assessment of the treated areas according to the Vancouver Scar Scale. The clinical evaluations for vascularity, pigmentation, pliability, and height were significantly lower for pulsed-dye laser plus compression therapy (CT) than for CT alone after three treatments (p < 0.05).
**Figure 3.3.** Excess erythema of the seam over the treatment course. Treatment with pulsed-dye laser (PDL) plus compression therapy (CT) resulted in lower excess erythema than CT alone after two treatments ($p < 0.05$). Reduction in scar erythema continued with additional PDL treatments.

**Figure 3.4.** Elasticity of the seam over treatment course. Skin elasticity measured instrumentally was higher (more elastic) for the pulsed-dye laser plus compression therapy (CT) than for CT alone after three treatments ($p < 0.05$).
3.4 Discussion

We have demonstrated an improvement over compression therapy alone with repeated PDL treatment soon after surgery. The concurrent use of compression therapy was deemed necessary to create equipoise, as our institutional practice involves the aggressive use of compression therapy. The data do not permit comment on the effectiveness of the PDL therapy alone versus compression therapy alone. Further studies evaluating the variables associated with the application of compression therapy (e.g., compression forces, time in garment) are needed to make these clinical decisions.

Our findings are similar to Nouri et al.\textsuperscript{123} who treated linear operative scars three times beginning on the day of suture removal and found improvement in VSS scores. The relatively subjective VSS clinical assessment is used historically, but it has unacceptable reliability.\textsuperscript{84,124} We would highlight the subjective nature of the VSS based on the observer’s judgment of appreciated elements of color and pliability. Instrumental measures, including reflectance spectroscopy and laser Doppler perfusion, can be used to objectify the assessment of each of the VSS elements.\textsuperscript{83,125} Computer analysis of digital images offers potential as refinements in standardization of digital photography and analysis become more widely accepted. We have shown that systematic image analysis offers the potential to quantify changes in scar color and to detect changes earlier. Such changes may go unappreciated by the human eye (e.g. Chan’s discovery of the effect on “lightness” but not “redness”).\textsuperscript{100} We used standardized software that is widely available, flexible, and economical in application. The potential advantages of this technique
include not only more precise assignment of color changes, but the potential to detect smaller increments of change. This increased sensitivity could help discriminate potentially effective treatments (e.g., if only intensity were to be changed) from truly fruitless efforts.

One study limitation was the low subject retention, related in part to restrictive inclusion criteria. It was important to include only older children, who would not require sedation in order to improve patient compliance and improve safety. The attrition was probably related to the long travel distances for patients. The study construct allowed laser treatment to become optional when the compression garments were discontinued. This resulted in a small sample size (n = 6) beyond treatments that, in turn, limited the statistical power of the study. The failure to return and the low request for continued PDL laser treatment might suggest that the relative cost of time and travel may have outweighed any further cosmetic improvement. This highlights the need to include patient perception of treatment value in future studies.

The PDL laser “dose” (power, number of passes) was selected to achieve purpura and individualize treatment. Consequently, it was a variable in the study. There was a significant negative correlation (r = -0.47, p < 0.001) between cumulative laser power over the treatment period and outcome, i.e., change in erythema as a* from the color image. Application of higher cumulative laser power achieved a greater reduction in scar erythema, suggesting that outcomes may be further enhanced by adjusting the power. Studies to determine the optimum settings are warranted.
3.5 Conclusion

The study supports the efficacy of PDL therapy in combination with compression therapy for improving scar outcomes in burn patients. It demonstrates the potential for further improvement by optimizing laser selection and operation. The study also supports the augmentation of clinical evaluation (VSS) with objective measures of skin properties. This comprehensive approach offers the potential to not only confirm clinical judgment, but also allow detection of small changes in outcome that could help point the way for more effective treatment strategies.
CHAPTER 4

Method Development and Validation for Infantile Hemangioma Studies
4.1 Introduction

Infantile hemangiomas (IHs) are benign vascular neoplasms that develop based on endothelial cell hyperplasia. Development begins with a proliferation phase, during which time the IH grows rapidly, is firm to touch, has a red or blue coloration, is warm and has high blood flow. This is followed by a short stabilization phase, then an involution phase where the size of the decreases, is more compressible, color changes to grayish or purple color, with reduced temperature and blood flow.

Clinicians typically evaluate IH status by visual inspection and photography for color, size, shape, and palpation for temperature and tactile characteristics. Visual assessment, although the gold standard, often has low reproducibility, increased variability, and low reliability; and lacks quantitation, thereby limiting the clinicians’ ability to determine the extent of treatment response overtime and make treatment efficacy comparisons.

Accurate quantitative assessment of IH status can provide important information for appropriate staging (i.e. disease activity) and responses to new treatment modalities in IHs. We have used multimodal quantitative imaging and image analysis techniques to objectively quantify the structural (i.e. surface color, size, shape) and functional (i.e. temperature and perfusion) characteristics in infantile hemangiomas that aimed to support clinical evaluation of IH status and monitoring treatment efficacies in IHs. Herein we describe the method development for the three-dimensional surface scanning technology, high resolution color imaging, infrared thermal imaging for temperature and perfusion, and the approach to co-register, then map and extract relevant color and thermal...
information to support clinical assessment, along with the experiments to validate the objective techniques.

4.2 Material and Methods

4.2.1 Subjects

One hundred and nineteen (n=119) patients from the Hemangioma and Vascular Malformation Clinic (HVMC) at Cincinnati Children’s Hospital Medical Center (CCHMC) were recruited to participate in the study. The HVMC is one of the largest multidisciplinary centers in the country for vascular anomalies. Subject exclusion criteria included: 1) older than 5 years of age, 2) diagnosis other vascular anomaly, and/or congenital hemangioma, 3) IH location on the lip, nasal tip, eyelid, or genitals. Deep IHs (limited number), and ulcerated/bleeding IHs confounded the data set and were later excluded. The Institutional Review Board of the CCHMC (#2011-0443) approved the research protocol and parents or guardians provided written informed consent for study participation.

4.2.2 Study Design

Subject visits were conducted during the HVMC clinic visits. When possible, subjects were enrolled at the start of treatment or the first clinic visit. Patients acclimated to the room temperature and humidity for least 15 minutes prior to imaging. Non-involved areas, typically contralateral to the IH, served as the within-subject control sites. Characteristics of the infantile hemangioma and a non-involved site were measured with each imaging technique in the following order: (1) Color image, (2) Thermograph, (3) 3D
scan, and (4) Dynamic response for perfusion. Demographic data included age, sex, IH location, depth, morphology, treatment type (propranolol, timolol, untreated), dosage, age at start of treatment, room temperature and relative humidity.

4.2.3 Clinical Assessment

The HVMC clinicians evaluated the IHs for the characteristics of color, temperature, tactile deformity, dimensionality, depth of involvement and tissue morphology using the scheme shown in Table 4.1, then assigned a growth stage as: 1 (Proliferating), 2 (Stable), or 3 (Involuting). The clinicians were unaware of the objective findings.

Table 4.1. Clinician Assessment of Infantile Hemangioma

<table>
<thead>
<tr>
<th></th>
<th>Bright Red</th>
<th>Faint Red</th>
<th>Blue</th>
<th>Faint Purple</th>
<th>Grey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>Very Warm</td>
<td>Warm</td>
<td>Slightly Warm</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Tactile Deformity</td>
<td>Firm, Not Compressible</td>
<td>Deformable, Some Elasticity</td>
<td>Fully Deformable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimensionality</td>
<td>Raised</td>
<td>Slightly Raised</td>
<td>Flat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth of Involvement</td>
<td>Superficial</td>
<td>Deep</td>
<td>Mixed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue Morphology</td>
<td>Localized</td>
<td></td>
<td>Segmental</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The clinical assessment was then assigned to a three-point scale (0-2) and four-point scale (0-3), using the following scale:

- Color (Red and Blue), Red: 0 (Normal), 1 (Grey), 2 (Faint Red), 3(Bright Red); Blue: 0 (Normal), 1(Faint Purple), 2 (Blue)
- Temperature: 0 (Normal), 1 (Slight Warm), 2 (Warm), 3 (Very Warm)
- Tactile Deformity: 0 (Fully Deformable), 1 (Deformable, Some Elasticity), 2 (Firm, Not Compressible)
- Dimensionality: 0 (Flat), 1 (Slightly Raised), 2 (Raised)

Half-point increments were used for intermediate conditions.

4.2.4 Three-Dimensional (3D) Surface Scanning

Three-dimensional (3D) surface volume/height data were captured with the Artec MHT™ 3D scanner (Artec Group, San Jose, CA, USA) and acquired using the Artec 3D Studio v0.7 software. The scanner operates on the principle of white light fringe projection. A unique white light grid is projected onto the surface of the object being scanned. The distortions in the grid pattern due to the surface shape of the object are detected and translated into 3D contours scans. The scanner collects surface data with a 0.5 resolution, 0.1 mm accuracy and a rate of 15 frames/s with an acquisition speed up to 500,000 points/s. The specifications of the scanner make it ideal for capturing surface data from children. Scans of the IH and control sites were captured approximately 2 feet from the subject.

The scans were first processed in Artec 3D Studio v0.7 software to create a 3D model of the object involving the following steps: 1) removal of background and confounding regions using the “Erase” tool, 2) semi-automatic alignment of scans using “Fine Serial Registration”, 3) global data registration using the “Global Registration” function, followed by the fusion the scans into a single 3D model using the “Fusion” function.126
The fused scan was exported from Artec software using file extensions “.obj” files and imported into 3dMD Vultus software (3dMD, Atlanta, GA). The *.obj files were converted to *.tsb file, proprietary format for 3dMD, for volume/shape analysis in Vultus software. A mirrored image was created to serve as the within-subject control site. The original and the mirrored 3D scans were aligned with using a complex surface registration algorithm. To perform this registration, on both the original and mirrored scans areas on the forehead, glabella, and temple were selected. The quality of the registration was quantified by the RMS error over registration surface area value. An error of less than 2.0mm is good, less than 1.0 mm is optimal. Soft tissue landmarks were used to create the region of interest (ROI) and applied for all evaluations. The elevated height (mm) measurements were calculated relative to the within-subject control site.
Figure 4.1. Volume/Height Quantification Scheme. 3D scan entered into Vultus. A mirrored image of the scan is created to serve as the control. The two scans are registered using an automated surface registration algorithm. The region of interest is determined based on anatomical landmarks and used to calculate the increased volume relative to the control.
4.2.5 High Resolution Digital Images

High-resolution color (Color) images were captured in RGB (red, green, blue) format using a Nikon D90 SLR digital camera (12.2M; 4288 x 2848 pixels) and 60-mm f/2.8D AF Micro Nikkor lens with a Nikon R1 Wireless Close-Up flash system (Nikon Inc, Melville, NY, USA). The lens and flash are cross-polarized, so photons that reflect off the surface of the skin are filtered. Photons that penetrate into the skin are depolarized and can pass through the lens filter providing information regarding the sub-epidermal microvasculature. A customized scale bar containing color correction chips was mounted to the base of the flash system to allow for consistent data capture at distance of 1ft and perpendicular to the site. Images were adjusted for white balance, color corrected, compressed at a ratio of 4:1 (1072 x 712 pixels) and converted to TIFF format, using ImageJ® software v 1.47G (National Institute of Health, Washington D.C., USA).

4.2.6 Infrared Thermography

Infrared (IR) images were acquired using the FLIR T400 camera (FLIR Systems Inc, Wilsonville, OR, USA). The camera is equipped with an uncooled microbolometer Focal Plan Array (FPA) detector, 320 x 240 infrared resolution, ± 2°C (<2%) accuracy, and <0.05°C @ 30°C sensitivity, providing temperature distributions within the infrared spectral range from 7.5 – 13 μm. The emissivity value was set to 0.98, and images were obtained from a distance of approximately 2ft.
4.2.6.1 Static Infrared Thermography

The static images were captured with the camera set to “Simultaneous” mode setting, which captures and stores a digital image (DIR) (3.1M; 2048 x 1536 pixels; JPG) and infrared image simultaneously. IR images were exported as TIFF (32-bit floating point) format using the FLIR ExaminIR MAX software.

4.2.6.2 Dynamic Infrared Thermography

Following the static image acquisition, a cold stress (disposable Jack Frost™ insulated cold pack, Cardinal Health, McGaw Park, IL) with a temperature 18 ± 0.2°C, was applied to the hemangioma and surrounding uninvolved skin for 30 seconds. The cooling duration was determined by patient comfort and demonstrated acceptable cooling and rewarming. A thermal video (7 frames/sec) was captured immediately on removal of the cold stress for 30 seconds (rewarming phase).

The region of interest (ROI) was created using the “Area Tool” from ExaminIR software, and taken to be the IH area of thermal involvement (excluding IH feeding and draining vessels and other anatomical locations with increased temperature, e.g., eyes, nose and inner ear) from the frame captured at the end of rewarming (30 sec) (ExaminIR software, FLIR). Data files were imported into Matlab® (R2012a, Mathworks, Natick, MA, USA) and processed to create thermal distributions (maps of area and thermal intensity). Thermal features of the surrounding uninvolved tissue were removed by selecting a threshold. The distribution of the highest 10% of the temperature, after cooling was selected as the threshold value because it identifies the highest thermal activity within the
IH and segments IH features from surrounding uninvolved skin. This provides a better signal to noise ratio compared with thresholds based on the control value. The rewarming curves were generated from 5-second intervals for further analysis. Outcome measures included the difference of mean temperatures at baseline and immediately after cooling (ΔT\text{cool}), the area under the curve during 30 sec rewarming (AUC\text{rw}), and the time to maximum temperature (t\text{max}). The maps represent the mean temperatures and area distribution above the threshold for thermal behaviors at baseline and during rewarming.

4.2.7 Color and Thermal Co-registration and Image Analysis

Accurate assessment of cutaneous color characteristics and vascular behavior is important for appropriate staging and treatment in IHs. We have developed a custom graphical user interface (GUI) for co-registration of high-resolution color and infrared thermal images using landmark-based registration and image analysis algorithms to objectively extract relevant physiological information. Our system was designed to provide an overlay image and maps of the color and thermal attributes of the hemangioma allowing the clinicians to visualize areas of proliferation and involution within the cutaneous IH and to quantify outcomes to assess changes in growth and monitor the effectiveness of treatment.

The co-registration and image analysis GUI developed in this work was created using Matlab® v7.14 (R2012a, MathWorks, Natick, MA, USA). The co-registration codes were written by Kenneth Eaton, PhD from the Imaging Research Center at CCHMC in
collaboration with SAB and the image analysis algorithms (Appendix B1) were written by SAB. The program consists of 7 windows that were completed in consecutive order, which include the Subject Overview, Hemangioma IR to DIR registration, Hemangioma DIR to Color registration, Control IR to DIR registration, Control DIR to Color registration, Control to Hemangioma Color registration, and Post-Processing. The Subject Overview window was used to load the subject data. The next 5 windows provided tools for co-registering all the collected images using a landmark-based algorithm. Since the region of thermal activity (intensity) extends beyond the visible borders of the IH a landmark-based registration was selected. At least 3 control points (e.g. an anatomical landmarks, hemangioma features and/or interface point between flank and diaper, or ear and background), that are easily identifiable in both images were placed to facilitate image co-registration. Each registration window has a similar layout, where the reference (fixed) image is displayed on the top left panel and the sensed (moving) image is displayed on the bottom left panel. The overlay of the two co-registered images is displayed on the right panel and served to check the accuracy of the registration. An affine transformation was applied at each registration step, and combined to align all the images to the Hemangioma Color image space (base reference image space). After the registration was completed, the Post-Processing window was used to select the region of interest (ROI) for the hemangioma and control, threshold the hemangioma activity from surrounding tissue, and quantify the outcomes of the hemangioma relative to the control skin. The ROI was created using a square selection surrounding the IH, similar to the “Area Tool” selector from ExaminIR software as previously described, and taken to be the IH area of thermal involvement (excluding
feeding and draining vessels and other anatomical locations with increased temperature, e.g., eyes, nose and inner ear, surrounding the IH). The procedures discussed above are illustrated in Figure 4.2.

The protocol for co-registering the high resolution color image with the thermal image includes the following steps:

1. Load the Hemangioma and Control high-resolution color images (Color), thermal (IR) images, and simultaneous low-resolution digital (DIR) images. (Figure 4.2A)
2. Orient the control images to match the direction of the hemangioma images by selecting the “apply left-right flip” at the base of the window, if needed.
3. Co-register the Hemangioma IR (moving) image to the Hemangioma DIR (fixed) image using anatomical landmarks and/or interface points (Figure 4.2B)
4. Co-register the Hemangioma DIR (moving) image to the Hemangioma Color (fixed) image using IH anatomical features (Figure 4.2C)
5. Co-register the Control IR (moving) image to the Control DIR (fixed) image using anatomical landmarks and/or interface points (Figure 4.2D)
6. Co-register the Control DIR (moving) image to the Control Color (fixed) image using anatomical landmarks (Figure 4.2E)
7. Co-register the Control Color (moving) image to the Hemangioma Color (fixed) image using anatomical landmarks (Figure 4.2F)
8. Select the color and thermal ROI based on the thermal area of involvement (Figure 4.2G)
9. Quantify and map the hemangioma color and temperature feature.
Figure 4.2. Image co-registration scheme. (A) Subject Overview to load the images, (B-F) Registration steps between images with final alignment to the to Hemangioma Color image, and (G) Post-Processing to select the region of interest and quantify and map the hemangioma features.
The RGB images were converted to L*a*b* color space and separated into three distinct images, as previously described. The L* describes the reflected light intensity and ranges from 0 (black) to 100 (white); a* describes the green-red color saturation and ranges from -60 to +60, respectively; and b* describes the blue-yellow color saturation and ranges from -60 to +60, respectively. The L*a*b* system is widely used to objectively measure human skin color. The a*, b* and L* represent the degree of skin redness, yellow coloration, and darkness to lightness, respectively. For a* image, the brighter a pixel appeared, the more intense the redness; and for b*, the darker a pixel appeared in the image, indicates more intense blue (less yellow) coloration (Figure 4.3).

Figure 4.3 Converted L*a*b* image separated into distinct channels (L*, a*, b*). The L*, a*, b* images were used for image analysis of Lightness, redness, and blue-yellow coloration, respectively.
To segment the hemangioma from the surrounding uninvolved control skin, a threshold was applied for the color (L*,a*,b*) and thermal (IR) features. Threshold values for the L*, a*, b* and IR images were selected based upon histogram distributions of the uninvolved skin as the values above the mean (µ) and multiple standard deviations (σ). The method of using the number of pixel values greater than the µ + σ, referred to as “excess erythema” was used previously to quantify erythema in health care workers,\textsuperscript{117} irritant contact dermatitis,\textsuperscript{118} and burn scars.\textsuperscript{138} Higher standard deviations were chosen for this study so that only strong edges are detected. Pixels with L* values less than µ-4σ, were used to represent the darkness-lightness of the IH, pixels with a* values higher than µ+4σ, were used to represent the redness of the IH, pixels with b* values less than µ-3.5σ, were used to represent the yellow coloration of the IH, and pixels with IR values higher than µ+3σ, were used to represent the temperature of the IH. Following the segmentation algorithms, the pixels beyond the thresholds were used to quantify and map the features of the IH including:

- Total ROI from thermal area (pixels): Total area of IH thermal involvement
- Lightness Intensity (L*): Mean value of lightness (L*) pixels above the threshold – threshold value
- Lightness Area (pixels): Number of lightness pixels below the threshold
- Red Color Intensity (a*): Mean value of red (a*) pixels above the threshold – threshold value
- Red Color Area (pixels): Number of red (a*) pixels above the threshold
• Yellow Color Intensity (b*): Mean value of yellow (b*) pixels below the threshold – threshold value
• Yellow Color Area (pixels): Number of yellow (b*) pixels below the threshold
• Temperature Intensity (°C): Mean value of temperature pixels above the threshold – threshold
• Thermal area (pixels): Number of thermal pixels above the threshold
• RedYellow Percent Overlap: The number of red (a*) and yellow (b*) pixels above the threshold that overlap with each other/IR Thermal Area
• RedIR Percent Overlap: The number of red pixels and thermal pixels above the threshold that overlap with each other/ IR thermal Area
• YellowIR Percent Overlap: The number of blue-yellow pixels and thermal pixels above the threshold that overlap with each other / IR thermal Area

4.2.8 Statistical Analysis

The assessment of reproducibility in image capturing and processing methods were calculated using the coefficient of variation (%CV = σ/µ *100). The differences between the hemangioma and control sites before and after cooling were compared with Paired-Samples t-test with significance (p < 0.05). The normal skin behavior between adults and infants were compared using univariate general linear models procedures (GLM). Analyses were performed using SPSS v12 (SPSS, Inc., Chicago, IL).
4.3 Results/Discussion

4.3.1 Clinical Assessments

Thirteen (13) HVMC clinicians participated in the study. A total of 344 evaluations were performed and 155 evaluations (45%) with at least 2 physicians participated. Out of the 155 multiple clinician evaluations, the clinicians disagreed 61 times on stage (39%), 51 times on depth (33%), and 17 times on morphology (11%). Most discrepancies in staging were between identifying stable and involuting (28; 46%), followed by proliferating and stable (16; 26%) and proliferating and involuting (7; 11%). There were 9 (15%) instances where multiple stages (proliferating/stable or stable/involuting) were identified by a single clinician. Most discrepancies in depth were between superficial and mixed (30; 59%), followed by mixed and deep (19; 37%), and then superficial and deep (2; 4%). The point scale data were also evaluated and reveal 74 discrepancies in the red category (49%), 28 in the blue category (18%), 76 in the temperature category (51%), 39 in the deformity category (26%) and 38 in the dimensionality category (28%).

Treatment and patient care decisions of hemangiomas are strongly dependent on the staging and depth of involvement of the lesion. Response to treatment has been shown to be more pronounced in patients that received propranolol treatment in the proliferative phase (≤ 6 months of age) compared to older patients. Additionally, topical timolol and laser therapy are not recommended treatment options for deeper IHs (deep or mixed) during the proliferative phase due to the lack of therapeutic penetration to the deeper skin layers. Systemic propranolol, corticosteroids, or surgical therapy have been suggested as treatment options for deeper component IHs.
4.3.2 Variability between Color and Thermal Images

The reproducibility of color (L*,a*,b*) from the high-resolution digital camera and temperature of the infrared camera were verified by imaging the hemangioma and contralateral control side for 8 subjects. Two to three imaging sets per subject were taken (5 minutes apart). Each subject was repositioned between each imaging set. The assessment of variability was calculated using the coefficient of variation (%CV = σ/µ *100) of the mean value of L*, a*, b* and temperature. From the measurements, the %CV did not exceed 1% for each of the 8 subjects (Table 4.2).

Table 4.2. Variability between color and thermal images

<table>
<thead>
<tr>
<th>Sub#</th>
<th>Lightness (L*)</th>
<th>Redness (a*)</th>
<th>Blue-Yellow (b*)</th>
<th>Thermal (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Mean Value</td>
<td>%CV</td>
<td>Average Mean Value</td>
<td>%CV</td>
</tr>
<tr>
<td>1</td>
<td>126.0 ± 0.16</td>
<td>0.13</td>
<td>151.3 ± 0.27</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>155.2 ± 0.61</td>
<td>0.40</td>
<td>149.2 ± 0.14</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>196.6 ± 0.07</td>
<td>0.04</td>
<td>144.9 ± 0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>187.2 ± 0.99</td>
<td>0.53</td>
<td>148.5 ± 0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>5</td>
<td>167.6 ± 0.37</td>
<td>0.22</td>
<td>146.7 ± 0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>166.3 ± 0.08</td>
<td>0.05</td>
<td>145.4 ± 0.53</td>
<td>0.37</td>
</tr>
<tr>
<td>7</td>
<td>173.4 ± 0.35</td>
<td>0.21</td>
<td>142.0 ± 0.74</td>
<td>0.52</td>
</tr>
<tr>
<td>8</td>
<td>157.6 ± 0.30</td>
<td>0.19</td>
<td>147.1 ± 0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values presented as mean ± SD

Similar evaluations were conducted at a later visit for 5 subjects and produced similar results (Table 4.3). The %CV did not exceed 1% for each of the 5 subjects at the later evaluation.
Table 4.3. Variability color and thermal images over time

<table>
<thead>
<tr>
<th>Sub#</th>
<th>Lightness (L*) Initial Eval</th>
<th>Follow up</th>
<th>Redness (a*) Initial Eval</th>
<th>Follow up</th>
<th>Blueness (b*) Initial Eval</th>
<th>Follow up</th>
<th>Thermal (°C) Initial Eval</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.44</td>
<td>0.11</td>
<td>0.18</td>
<td>0.32</td>
<td>0.05</td>
<td>0.02</td>
<td>0.04</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>0.53</td>
<td>0.73</td>
<td>0.14</td>
<td>0.22</td>
<td>0.18</td>
<td>0.01</td>
<td>0.34</td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>0.22</td>
<td>0.94</td>
<td>0.04</td>
<td>0.12</td>
<td>0.00</td>
<td>0.01</td>
<td>0.28</td>
<td>0.09</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
<td>0.05</td>
<td>0.39</td>
<td>0.37</td>
<td>0.09</td>
<td>0.01</td>
<td>0.07</td>
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</tr>
<tr>
<td>5</td>
<td>0.14</td>
<td>0.60</td>
<td>0.05</td>
<td>0.03</td>
<td>0.31</td>
<td>0.08</td>
<td>0.09</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Canning et al. used a similar method to test the reproducibility of their digital imaging system, and found percentage of uncertainty values (calculated as σ/μ *100) less than 1%, as acceptable. These data provide evidence that our techniques are capable of producing reproducible measures from a single point and over time. Therefore, spatial and temporal heterogeneity is the main contributor to variability in color and thermal readings.

4.3.3 Effects of Distance on Thermal Camera

The effect of distance (1.5ft, 2ft, and 2.5ft) on the temperature values were independently investigated by imaging the hemangioma in 6 subjects. The ROI was determined, as described above, within each image, and the mean values were used to determine the %CV. From the measurements, the %CV did not exceed 1% for each of the 6 subjects (Table 4.4). These data provide evidence that there is minimal to no variation in temperature within the range of 1.5-2.5ft. Our imaging protocol measured temperature at a distance of 2ft.
### Table 4.4. Effects of distance on temperature values

<table>
<thead>
<tr>
<th>Sub#</th>
<th>Mean Temp (°C) 1.5 ft</th>
<th>Mean Temp (°C) 2 ft</th>
<th>Mean Temp (°C) 2.5 ft</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.6</td>
<td>33.5</td>
<td>33.4</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>36.4</td>
<td>36.4</td>
<td>36.4</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>36.4</td>
<td>36.5</td>
<td>36.4</td>
<td>0.12</td>
</tr>
<tr>
<td>4</td>
<td>37.0</td>
<td>37.1</td>
<td>36.7</td>
<td>0.59</td>
</tr>
<tr>
<td>5</td>
<td>37.2</td>
<td>37.0</td>
<td>36.9</td>
<td>0.43</td>
</tr>
<tr>
<td>6</td>
<td>35.9</td>
<td>35.9</td>
<td>36.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Variation in temperature values as a result of variations in distance was negligible compared to the much larger variations observed between hemangioma and control skin.

#### 4.3.4 Effects of Matlab and Upsampling in Matlab

IR images were captured using a FLIR camera and exported from FLIR ExaminIR processing software into Matlab for image processing and analysis. The co-registration method involves upsampling the IR image (320 X 240) to match the size of the color image (1072 X 712), by a factor of $9.92[(712/240)*(1072/320)]$. A bilinear interpolation is used to calculate the pixel intensity values located between existing pixel values by linearly weighting the 4 closest neighboring values. In a successful resampling, the image intensities between the upsampled and reference images should be similar. The effects of using Matlab for image processing, and effects of upsampling the IR images from ExaminIR were investigated using single visit data from 3 subjects with 4 IHs. Two tests per subject were used to compare the reference hemangioma and control images from ExaminIR to Matlab (Effects of Matlab) in order to determine the processing variation; and to the upsampled hemangioma and control images from Matlab (Effects of
Upsampling) in order to find the interpolation variation in during upsampling. Figure 4.4 illustrates the ROI selection for both image processing methods.

In this experiment, the variables consisted of the hemangioma ROI selection and control ROI selection. The ROI for the hemangioma and control were selected with ExaminIR. The raw IH and control images (*.tif) (320 X 240) were uploaded into Matlab and the ROI for both sides were selected. The %CV for the IH and Control mean values were minimal and ranged from 0.02 – 0.06% and 0.00 – 0.06%, respectively (Table 4.5). The %Area above the threshold variation was also minimal and ranged from 0.0 – 6.4%.
In this experiment, the variables consisted of resizing the IH and control images, and ROI selections for the IH and control. The ROI for the hemangioma and control were selected with ExaminIR. The raw IH and control images (*.tif) were uploaded into Matlab, increased in size from (320 X 240) to (1072 X 712), and the ROI for both sides were selected. The %CV for the IH and Control mean values were minimal and ranged from 0.02 – 0.13% and 0.00 – 0.08%, respectively (Table 4.6). The %Area above the threshold variation was also minimal and ranged from 0.0 – 7.9%.

These data provide evidence there is minimal to no variation in temperature values as a result of processing and upsampling the images in Matlab.
Table 4.5. Effects of Matlab processing on temperature values

<table>
<thead>
<tr>
<th>Site#</th>
<th>ExaminIR Hem Mean (°C)</th>
<th>Matlab Hem Mean (°C)</th>
<th>Hem %CV</th>
<th>ExaminIR Con Mean (°C)</th>
<th>Matlab Con Mean (°C)</th>
<th>Con %CV</th>
<th>ExaminIR %Area above Threshold</th>
<th>Matlab %Area above Threshold</th>
<th>Area %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36.3</td>
<td>36.3</td>
<td>0.02</td>
<td>33.0</td>
<td>33.0</td>
<td>0.06</td>
<td>100.0</td>
<td>100.0</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>36.1</td>
<td>36.1</td>
<td>0.02</td>
<td>34.9</td>
<td>34.9</td>
<td>0.02</td>
<td>41.0</td>
<td>43.6</td>
<td>4.22</td>
</tr>
<tr>
<td>3</td>
<td>36.2</td>
<td>36.2</td>
<td>0.04</td>
<td>35.5</td>
<td>35.6</td>
<td>0.01</td>
<td>33.8</td>
<td>37.1</td>
<td>6.40</td>
</tr>
<tr>
<td>4</td>
<td>35.9</td>
<td>35.9</td>
<td>0.06</td>
<td>35.3</td>
<td>35.3</td>
<td>0.00</td>
<td>52.4</td>
<td>55.0</td>
<td>3.36</td>
</tr>
</tbody>
</table>

Table 4.6. Effects of upsampling in Matlab

<table>
<thead>
<tr>
<th>Site#</th>
<th>ExaminIR Hem Mean (°C)</th>
<th>Upsampled Hem Mean (°C)</th>
<th>Hem %CV</th>
<th>ExaminIR Con Mean (°C)</th>
<th>Upsampled Con Mean (°C)</th>
<th>Con %CV</th>
<th>ExaminIR %Area above Threshold</th>
<th>Upsampled %Area above Threshold</th>
<th>Area %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36.3</td>
<td>36.4</td>
<td>0.11</td>
<td>33.0</td>
<td>33.0</td>
<td>0.08</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
<td>2</td>
<td>36.1</td>
<td>36.2</td>
<td>0.02</td>
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<td>0.02</td>
<td>41.0</td>
<td>41.2</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>36.2</td>
<td>36.3</td>
<td>0.10</td>
<td>35.6</td>
<td>35.6</td>
<td>0.01</td>
<td>33.8</td>
<td>37.8</td>
<td>7.87</td>
</tr>
<tr>
<td>4</td>
<td>35.9</td>
<td>36.0</td>
<td>0.13</td>
<td>35.3</td>
<td>35.3</td>
<td>0.00</td>
<td>52.4</td>
<td>54.5</td>
<td>2.66</td>
</tr>
</tbody>
</table>
4.3.5 Variability of Cold Packs

The reproducibility of the cold packs was verified using 6 different cold packs. The cold packs were activated, 1 paper towel was placed over the pack (protective effect for the skin), and the temperature was measured for 30s (video mode) at a distance of 2ft using the infrared camera. During this experiment, room temperature and relative humidity were measured as 23°C and 31%, respectively. These conditions are consistent with the HVMC unit room conditions. The average temperature between the cold packs ranged between 17.5 to 18.5°C (mean ± SD: 18 ± 0.2°C, %CV: 0.03) (Table 4.7). These data provide evidence that the average temperature applied to the skin is approximately 18°C, and the variability within each cold pack and between packs during exposure are minimal.

<table>
<thead>
<tr>
<th>Cold Pack#</th>
<th>Mean Temp over time, 30s (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.68</td>
</tr>
<tr>
<td>2</td>
<td>17.48</td>
</tr>
<tr>
<td>3</td>
<td>18.48</td>
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<tr>
<td>4</td>
<td>18.25</td>
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<td>5</td>
<td>18.14</td>
</tr>
<tr>
<td>6</td>
<td>18.76</td>
</tr>
<tr>
<td>Average</td>
<td>18.1</td>
</tr>
</tbody>
</table>
4.3.6  Variability of Cooling Method

The reproducibility of the cooling method was verified by imaging the forearms of two adult male control subjects. The day-to-day variations in temperature were studied during three consecutive days. Each subject acclimated to the room conditions at least 15 minutes, room temperature and relative humidity range from 22 - 23°C and 43 – 45%, respectively. These conditions are consistent with the HVMC unit conditions. Before the experiment, the position of the forearm used for the measurement was marked, and the measurement was repeated at the same position for subsequent evaluations. Baseline static images were captured, then the cold packs were activated, a towel was placed over the pack (protective effect for the skin), and the temperature was measured for 30s (video mode) at a distance of 2ft using the infrared camera. The day-to-day variation of mean temperature difference between pre- and post-cooling for both subjects was (%CV: 7.1 and 11.6) (Table 4.8).

Table 4.8 Variability in cooling method

<table>
<thead>
<tr>
<th>Sub#</th>
<th>Day</th>
<th>Mean Temp (°C) pre-cool</th>
<th>Mean Temp (°C) post-cool</th>
<th>Diff (°C) (pre-post)</th>
<th>Average Diff (°C)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>33.5</td>
<td>29.6</td>
<td>3.9</td>
<td>3.79</td>
<td>0.27</td>
<td>7.09</td>
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<tr>
<td>1</td>
<td>2</td>
<td>33.2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>32.5</td>
<td>28.5</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>33.3</td>
<td>29.1</td>
<td>4.2</td>
<td>4.87</td>
<td>0.56</td>
<td>11.57</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>33.8</td>
<td>28.7</td>
<td>5.1</td>
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<td>26.9</td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3.7 Effects of Cooling & Rewarming on Control Skin in Adults and Infants

To assess the effect of the cooling and rewarming on control skin, we compared the cooling and rewarming behavior on the forearms in 10 healthy adults (5F: 5M) to the control skin behavior in 6 subjects in 24 evaluations. Imaging was conducted in a controlled environment for the adult subjects, using the protocol as previously described (Variability in Cooling Method) and in the HVMC clinic area for the infant subjects, using the study protocol. Adult room conditions ranged from 22 - 23°C for temperature and 25 – 43% for relative humidity, which were consistent with the HVMC unit conditions. The parameters evaluated included mean temperature difference between pre-and post-cooling, rate of cooling, mean temperature difference between post-cooling and end of rewarming (30s), and rate of rewarming. There were no significant differences in the cooling and rewarming behavior between adult and infant skin. The temperature cooled in adult skin was -4.02°C ± 0.63 and in infant skin was -4.5°C ± 0.406, corresponding to a cooling rate of -0.13 ± 0.02 °C/sec and -0.15 ± 0.02 °C/sec, respectively (p = 0.474). The rewarming temperature in adult skin was 1.5°C, with a rate of 0.05 °C/sec compared to an increase of 1.8°C, with a rate of 0.05 °C/sec in infant skin. Changes in cooling and rewarming values in adult skin were similar regardless of age or gender.

4.3.8 Control Selection for Dynamic Thermal

To identify a control site for the dynamic thermal cooling, we compared the static temperature of an uninvolved area adjacent to the edge of the thermal ROI (cooled control area) to an area on the contralateral side in 11 subjects with 24 evaluations. There
there was no significant difference between the threshold temperatures (mean + 3SD) in the uninvolved areas (p = 0.83, Figure 4.5). These data provide evidence that the uninvolved area adjacent to the thermal ROI was suitable for an uninvolved control region for the evaluation of dynamic images.

![Figure 4.5 Effect of Control site on the threshold temperatures for dynamic thermal cooling. Data presented as mean ± SEM](image)

### 4.4 Conclusion

Here we have described and validated a protocol that combines three-dimensional surface scanning, high resolution color imaging and infrared thermography for assessment of disease progression and response to treatment in IHs. We have demonstrated the variability introduced by image capture and processing is low. The skin temperature and response to thermal stress between adults and infants are similar.
CHAPTER 5

Quantitative Skin Imaging Differentiates Treatment Effects in Infantile Hemangiomas
5.1 Introduction

Infantile hemangiomas (IHs) are benign vascular neoplasms that undergo rapid endothelial cell proliferation via excessive angiogenesis and/or vasculogenesis shortly after birth,\textsuperscript{7,9} stabilize, and slowly involute with diminishing cellular activity and fibrous fatty deposition for up to 5 – 7 years.\textsuperscript{4,140} IHs most commonly occur in females and in the head and neck. They are classified as superficial, deep, or mixed based on the depth of dermal involvement.

Clinicians typically evaluate IH status from visual inspection and photography for color, size, and shape, and by palpation for temperature and deformability. Magnetic resonance imaging and computed tomography are rarely used to evaluate treatment response due to cost and risks.\textsuperscript{55,67} Physiologically-based objective measurements are important for determining the effectiveness of new treatments. Standardized digital photography, infrared thermography and three-dimensional imaging have been used for assessing IHs.\textsuperscript{38-40,51,141} Infrared (IR) thermography is a reliable, noninvasive, low-cost technique to evaluate vascular function.\textsuperscript{38,39,131} IR temperature distribution at the skin surface is primarily affected by tumor depth, size, and internal temperature from metabolism and blood perfusion.\textsuperscript{142-144} We have previously used quantitative skin imaging to quantify cutaneous conditions including burn scars and irritant dermatitis.\textsuperscript{117,118,120,138}

We describe the application of multiple skin imaging techniques, including three dimensional (3D) scanning, high resolution color imaging, and infrared thermography, and demonstrate their utility in characterizing the ontogeny and treatment response
among patients with IHs. These techniques parallel the aspects of clinical assessment and aim to supplement them.

5.2 Patients and Methods

5.2.1 Subjects

A prospective observation clinical cohort study was conducted among patients from the Hemangioma and Vascular Malformation Center (HVMC) at Cincinnati Children’s Hospital Medical Center (CCHMC). Eligible patients had superficial or mixed IH and were less than 5 years of age. Patients with IH on the lip, nasal tip, eyelid, or genitals were excluded because these locations confounded assessments in this validation study. Deep IHs (limited number), ulcerated and small (< 30 mm²) IHs were later excluded. The Institutional Review Board approved the protocol (CCHMC #2011-0443) and parents/guardians provided written informed consent.

5.2.2 Study Design

Skin images were collected at enrollment and each clinic visit between October 2011 and February 2013. An uninvolved skin site, typically contralateral to the IH, served as the within-subject control. Subjects acclimated to the room conditions for least 15 minutes prior to imaging. CCHMC practice standards were followed to determine treatment, resulting in three groups: oral propranolol, topical timolol or observational (untreated). Propranolol dosing ranged of 1-2 mg/kg per day divided 2 times daily and timolol dosing was one drop of Timolol Maleate 0.5% gel spread on the lesion twice per day, for 0.5 mg
of timolol per day. Age, gender, IH location, depth, morphology, treatment type, dosage, and age at start of treatment were recorded.

5.2.3 Skin Imaging and Analysis

5.2.3.1 Three-Dimensional Surface Scanning

Three-dimensional imaging was used to quantify IH height and surface scans were acquired with the light-based Artec MHT 3D scanner (Artec Group, San Diego, CA, USA) (0.5 mm resolution, 0.1 mm accuracy, at a distance of 60 cm). Soft tissue landmarks created the area of interest for quantitation with the 3dMD Vultus software (3dMD, Atlanta, GA). Height (mm) was calculated for the IH versus the control using a distance map.

5.2.3.2 High Resolution Color Imaging

High-resolution images to quantify IH size and color were taken at 30 cm distance, perpendicular to the site with a Nikon D90 12.3 megapixel camera, Micro Nikkor 60-mm lens using cross polarization, and a Nikon R1 Wireless Close-Up flash system (Nikon Inc, Nikon Corporation, Tokyo, Japan). The images were color corrected (ImageJ, NIH, Washington, DC, USA), converted to CIELab color space, and separated into three channels: L*-image (dark-light), a*-image (green-red) and b*-image (blue-yellow).
5.2.3.3 Infrared (IR) Thermography

Infrared thermography was used to measure temperature intensity and distribution for the ROI with a FLIR T400 camera at a perpendicular distance of 60 cm (FLIR Systems Inc, Wilsonville, OR, USA) (resolution of 320 X 240 pixels, ± 2°C (<2%) accuracy, <0.05°C @ 30°C thermal sensitivity, emissivity of 0.9860).

5.2.3.4 Image Analysis

Color and IR images were co-registered and analyzed with a custom graphical user interface (GUI) consisting of landmark-based registration and image analysis algorithms written in MATLAB® (v7.14 (R2012a), MathWorks, Natick, MA, USA). Quantitative outputs and maps are created for visualization of IH thermal and color activity. Since the thermal activity extends beyond the IH visible borders, the ROI was determined from the IR image and used to analyze color outcomes, as previously described in section 4.2.7. The features of the surrounding uninvolved skin were removed by applying color and thermal thresholds to identify features attributable to the IH color only. Thresholds, were typically three to four standard deviations above the control for red color (a*) and IR temperature and below the control for lightness (L*) and yellow color (b*).

5.2.4 Outcome Measures

Quantitative outcomes were height, total region of interest (ROI) from the thermal image, size from the color image, lightness intensity, lightness area, red intensity, red area, yellow intensity, yellow area, thermal intensity, and thermal area (Table 5.1). Maps representing the intensity and area distribution for lightness, red color, yellow color and
thermal parameters were generated. The relative change in IH color and thermal intensity were measured as the difference from a threshold selected to separate contributions from the unaffected skin.

**Table 5.1: Quantitative outcomes**

<table>
<thead>
<tr>
<th>Outcome (Units)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (mm)</td>
<td>Maximum height above control skin surface</td>
</tr>
<tr>
<td>Total ROI from thermal area (pixels)</td>
<td>Total area of IH infrared radiation (temperature) greater than uninvolved skin</td>
</tr>
<tr>
<td>Size from the color area (mm²)</td>
<td>Total area of the color intensity greater than uninvolved skin</td>
</tr>
<tr>
<td>Lightness Intensity</td>
<td>Mean lightness (L*) value below (darker than) the threshold defined as control L* – 4 x SD (standard deviation of control region)</td>
</tr>
<tr>
<td>Lightness Area (pixels)</td>
<td>Number (L*) pixels below the threshold</td>
</tr>
<tr>
<td>Red Intensity</td>
<td>Mean red (a*) value above (more red) the threshold defined as control a* + 4 x SD (standard deviation of control region)</td>
</tr>
<tr>
<td>Red Area (pixels)</td>
<td>Number (a*) pixels above the threshold</td>
</tr>
<tr>
<td>Yellow Intensity</td>
<td>Mean yellow (b*) value below (more blue) the threshold defined as control b* – 3.5 x SD (standard deviation of control region)</td>
</tr>
<tr>
<td>Yellow Area (pixels)</td>
<td>Number (b*) pixels below the threshold</td>
</tr>
<tr>
<td>IR Thermal Intensity (°C)</td>
<td>Mean temperature value above (warmer than) the threshold defined as control temperature + 3 x SD (standard deviation of control region)</td>
</tr>
<tr>
<td>IR Thermal area (pixels)</td>
<td>Number of thermal pixels above the threshold</td>
</tr>
</tbody>
</table>
5.2.5 Statistical Analysis

The effects of time on IH by treatment group were analyzed using two-way repeated-measures analysis of variance (ANOVA). The between-subjects factor was treatment and the repeated factor was time since start of treatment (or first observation for untreated hemangiomas), which was discretized into 2-4-month intervals. The residual covariance structure was compound-symmetric. Post hoc t-type comparisons were made among treatment groups when significant (p< 0.05) treatment-by-time interactions were observed. Baseline measurements and age were examined as covariates to account for varying baseline levels between each treatment group. For thermal measures, room temperature and humidity were included as additional covariates in the analysis. Variation among treatments in baseline characteristics was evaluated using one-way ANOVA followed by pairwise post-hoc tests. Changes over time for each treatment were evaluated using univariate general linear models procedures (GLM) using subject id as a random factor. The hemangioma and control sites were compared with paired-samples t-tests. Analyses were performed using SAS v9.3 (SAS Institute, Cary, NC) and SPSS v12 (SPSS, Inc., Chicago, IL).

5.3 Results

5.3.1 Quantitative Skin Imaging

An example of the co-registered color and thermal images is shown in Figure 5.1. The thermal ROI extends beyond the IH visible borders and is approximately 2 °C higher than the surrounding tissue. Imaging outcomes over time are shown in Figure 5.2: (A) before and at (B) one, (C) two and (D) six months of propranolol treatment. At one month
(Figure 5.2B), red area, red intensity and temperature had increased, and yellow intensity decreased. Height, dark-light area and intensity and thermal area decreased. Between months one and two (Figures 5.2B and 5.2C), IH height, intensities and areas for lightness, red color and temperature decreased and yellow intensity increased, which continued after 6 months of treatment (Figure 5.2D), suggesting that the IH was approaching normal skin in color, temperature and height. Note that at 6 months of treatment (Figure 2D), the IR intensity and area were zero (i.e. no different from control).

Figure 5.1. Co-registered High-resolution Color and Thermal Images
Figure 5.2. Case study of IH (A) before treatment and after (B) 1 mo, (C) 2 mo, and (D) 6 mo of propranolol treatment. The IH becomes more normalized in color, temperature and height.

5.3.2 Comparison with Uninvolved Control Skin

Significant differences in all color and temperature outcomes were noted for IH versus control sites ($P < 0.001$). IHs had higher temperature and red intensity, lower yellow intensity (i.e., more blue) and were darker (i.e., lower L values) than the controls. The within image variability was significantly higher in the IH versus control indicating greater heterogeneity (Table 5.2)
Table 5.2. Hemangioma vs Contralateral Control Intensity Outcomes

<table>
<thead>
<tr>
<th>Attribute</th>
<th>N</th>
<th>Hemangioma Mean ± SD</th>
<th>Control Mean ± SD</th>
<th>Difference between locations (95% CI of the difference)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lightness Intensity (L*)</td>
<td>64</td>
<td>163.7 ± 11.2</td>
<td>199.4 ± 4.4</td>
<td>-35.7 (-38.1 to -33.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Red Intensity (a*)</td>
<td>64</td>
<td>148.3 ± 3.0</td>
<td>139 ± 1.0</td>
<td>9.2 (8.5 – 10.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Yellow Intensity (b*)</td>
<td>64</td>
<td>133.9 ± 1.3</td>
<td>138.7 ± 0.9</td>
<td>-4.8 (-5.2 to -4.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IR Thermal Intensity (°C)</td>
<td>62</td>
<td>35.9 ± 0.4</td>
<td>33.9 ± 0.3</td>
<td>2.0 (1.7 – 2.3)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

5.3.3 Ontogeny and Treatment Response

From the 119 subjects enrolled, 56 subjects with 64 IHs were included in this analysis. Thirty-eight were treated with propranolol, 13 with timolol and 14 were untreated. The median age at initiation for propranolol and timolol were similar, 3.6 months and 3.2 months, respectively, and 6.7 months for untreated IHs (time from birth).

5.3.3.1 Baseline Characteristics

IH ranged in size from 31 – 1770 mm$^2$ (average 292 mm$^2$) in full color, which was substantially smaller than thermal area of involvement which ranged from 95 – 3031 mm$^2$ (average 753 mm$^2$) the mean ratio was 0.35. There were significant differences prior to treatment among the three groups, which may reflect the differences in treatment strategy. Subjects in the untreated group were older (mean ± SEM: 12.6 ± 3.4) compared to propranolol (6.7 ± 1.4) and timolol (4.2 ± 0.6) patients (P=0.04). IHs in the propranolol group were larger overall, i.e., greater area, for red, yellow, dark-light and...
temperature versus timolol and untreated groups. The height was greater for the propranolol (7.1 ± 0.6) versus timolol (3.8 ± 1.0) and untreated (6.3 ± 1.1) (P = 0.02). Yellow intensity was lower (i.e. higher blue color) for propranolol (-2.0 ± 0.1) than timolol (-1.2 ± 0.2) and untreated (-1.5 ± 0.3) (P = 0.004).

5.3.3.2 Ontogeny and Treatment Response

The changes over time and treatment comparisons over the 15 month period are shown in Figures 5.3-5.5. Decreases in height for propranolol and untreated IHs were significant over time (P = 0.000 and P = 0.003, respectively), but not for timolol (Figure 5.3). Timolol and untreated IHs increased in height initially then decreased after two months, with timolol returning to baseline values at 9 months. Lightness intensity increased over time for propranolol and untreated IHs (P < 0.001 and P = 0.008, respectively) while lightness intensity did not change over time for timolol (Figure 5.4A). Lightness area in propranolol IHs decreased after treatment initiation and continued over time (P=0.001) (Figure 5.4B). For timolol and untreated, lightness area increased initially, then decreased after two months, with significant reductions for untreated IHs (P=0.005). Red intensity decreased over time for propranolol (P<0.001) and untreated IHs (P=0.04), but not for timolol (Figure 5.4C). Red area decreased over time for propranolol and untreated IHs (Figure 5.4D). In contrast, red area increased and remained elevated for timolol. Yellow intensity increased for propranolol IHs (P=0.03), but not for timolol and untreated over time (Figure 5.4E). The yellow area decreased for propranolol IHs
(P=0.001), did not differ over time for timolol and untreated (Figure 5.4F). IR temperature decreased for propranolol (P<0.001) and untreated (P=0.008) IHs over time; however, no differences were observed for timolol (Figure 5.4A).

Figure 5.4A. Propranolol and timolol were significantly different for IH height (p = 0.006) (Figure 5.3) due to a mean decrease for propranolol and an increase for timolol. Propranolol and untreated IHs were not different in height. IHs treated with propranolol were lighter versus timolol (P = 0.006) (Figure 5.4A) but did not reach significance between timolol and untreated IHs (P=0.06). Lightness area differences among the three treatments did not reach significance. Red intensity was not different among the three groups. The red area decreased for propranolol (P=0.004) and untreated IHs (P=0.04) (Figure 5.4D), compared to timolol IHs. No statistically significant differences in intensities or areas for yellow color and temperature were found among treatment groups.
Figure 5.4. Lightness, Red Color, Yellow Color: Ontogeny and Treatment Effects
5.4 Discussion

We used three skin imaging techniques: three-dimensional (3D) surface scanning, color imaging, and infrared thermography to quantify the ontogeny and treatment responses of infantile hemangiomas. We have demonstrated that these real-time imaging technologies can (1) quantitatively measure ontogeny/disease progression over time (Figure 5.2), (2) differentiate treatment effects between propranolol and timolol (Figures 5.3-5.5), and (3) show clinicians the regions of change/response within the IH via co-registered maps (Figure 5.1). The thermal area of hemangioma involvement extends beyond the visible tumor margins. Structural characteristics (i.e. size, color, topography) can be assessed visually by an experienced clinician, but functional characteristics, such as thermal area of involvement, had never been reported in the literature because it cannot be assessed by current clinical standards. Baseline characteristics between the color and thermal ratio revealed that only 35% of the IH involvement can be seen through subjective visual assessment.
Proangiogenic factors (vascular endothelial growth factor and basic fibroblast growth factor) and matrix metalloproteinases are increased during proliferation causing excessive vasculature.\textsuperscript{13,23,147} This leads to increases in height, red color, temperature, lightness area, red area, yellow area, and thermal area and decreases in yellow color (i.e. more bluish color) and lightness (i.e. darker IH). During involution, the endothelial cellular activity diminishes, capillaries disappear and adipose tissue begins to separate the blood vessels within the tumor.\textsuperscript{4,13} We would expect to see decreases height, color (i.e. reductions in area and intensity for red, lightness area, and yellow area, and increases in yellow and lightness intensities) and temperature (i.e. decreases in thermal intensity and area), as the IH approaches more normal like tissue.

The addition of standardization (lighting, positioning, color correction) increases the utility of the digital photograph commonly used clinically to evaluate IH progression. The skin imaging outcomes reveal information related to the underlying IH status and, thereby, extend the capability of clinicians. Previous studies have demonstrated that propranolol treatment resulted in significant improvement and decrease in color and size using visual scoring.\textsuperscript{139,148} This finding is supported by our study, in which we quantified hemangiomas treated with propranolol had greater reductions in red intensity, area measures of lightness, red color, yellow color, and increases in yellow intensity and lightness intensity compared to untreated and timolol treated hemangiomas (Figures 5.2, 5.4). These changes reflect propranolol treated tissue approaching more normative tissue, indicating the color changes reflect tumor involution. Researchers have reported lightening of color as an important factor used to evaluate IH progression and response to
treatment, but controversies have been stated regarding its difficulty to quantify.\textsuperscript{23,28} Our findings indicate the use of standardized digital imaging provide quantitative measures of IH lightening, which was significantly reduced with propranolol treatment.

The changes in volume/height are widely used as a measure of IH growth and regression.\textsuperscript{40,51-53} Hermans et al.\textsuperscript{40} recently noted the usability and clinical relevance of 3D phototechnology as an objective measure of volume changes in IHs. Increasing tumor volume has been associated with a continued proliferative phase. Treatment with propranolol resulted in a height decrease after treatment initiation (Figures 5.2-5.3), with possible mechanisms of actions including vasoconstriction, inhibition of angiogenesis, and induction of apoptosis.\textsuperscript{29} This is consistent with Hogeling et al.\textsuperscript{54} who found propranolol stopped IH growth and reduced volume by week 8 compared to an increase in size for the placebo group. Several recent controlled studies have associated topical timolol effectiveness with reductions in size in IHs after 8 to 16 weeks post treatment compared to the placebo controlled group using photographic document,\textsuperscript{36} and the hemispheric estimate.\textsuperscript{32} Researchers expressed potential measurement bias using the hemispheric volume method,\textsuperscript{54} and the need for a more sensitive system to measure height/depth of IH.\textsuperscript{32} In this study we showed similar increases in height for timolol treated IHs, perhaps suggesting that topical timolol has limited effectiveness on IH volume/height relative to the untreated control (Figure 5.3). The consistency of the younger age for the timolol treated IHs, in conjunction with the increases in the outcome parameters after treatment was initiated is indicative of a tissue that may still be undergoing proliferation. Chantasart et al.\textsuperscript{33} noted that more lipophilic β-blockers, such
as propranolol, are more permeable across the lipophilic stratum corneum than timolol, indicating that propranolol would be a better topical agent than timolol.

In this study, the temperature in the hemangiomas was an average of 1.8 °C higher than the contralateral control skin. In patients treated with propranolol, there was a 0.6°C decrease in thermal intensity, assessed by IR thermography, over time. Previous studies have reported higher decreases in temperature 2.5 °F (~1.4 °C) following treatment with a systemic beta blocker.\textsuperscript{39} In addition, we did not find significant differences over time or between treatments for thermal area. This lack of changes in thermal area and lesser reduction in temperature may be ascribed to the IH depth: as the IH decreases in size, its depth within the tissue (heating source) possibly approaches the skin’s surface resulting in increased temperature and changes to the distribution pattern.\textsuperscript{142,143}

This study documents the natural ontogeny of IHs with quantitative measures of volume/height, color and temperature. The majority of IH growth occurs between 2 – 5 months of age.\textsuperscript{23,149} Our initial visit averaged 12 months of age for untreated IHs. Unfortunately, the rapid growth for these parameters had largely passed by the time of our initial visit, making it difficult to capture early changes.\textsuperscript{149} Despite this, the methods detected changes over time for untreated IHs.

There were no differences in outcomes between propranolol and untreated IHs. The lack of difference may be a reflection of the significant age differences between the two groups, and not the absence of clinically relevant information. We observed that the
parameters for the propranolol group, despite being significantly younger in age, showed improvements that paralleled the untreated group over time, suggesting that propranolol gets hemangiomas to involution faster than the natural ontogeny, as suggested in Figure 5.2.

Visual assessment and volume estimation\textsuperscript{51} are used historically, but are limited by lack of standardization and poor inter- and intra-observer agreement.\textsuperscript{39,40,150} We have shown that the use of standardized, physiologically-based imaging and analysis techniques can be used to quantify changes in IH over time and in response to treatments. The combination of these imaging techniques provide clinicians with non-invasive tools (1) to “visualize” the underlying biology and its relationship to the surface characteristics (2) are more sensitive to physiological changes than visual assessment,\textsuperscript{138} (3) and allowed us to comprehensively characterize different physical information (i.e. morphology – color/shape/size and function-temperature) associated with IH growth and regression to assess tissue status as depicted in Figure 5.2.

5.4.1 Study Limitations

Some of specific features of the present study are noteworthy, for they both emphasize the utility and potential limitations of the results. Our institution is one of the largest multidisciplinary centers in the country for vascular anomalies. Consequently, our subject population consisted of higher risk lesions (i.e. larger size and facial) that more commonly require systemic treatment, reflecting a non-randomized comparative study and a referral bias. IH growth and regression can last up to 7 years or longer, so several
years are needed to determine all aspects of change. In addition, subjects treated with timolol and untreated IHs did not return to clinic as often as the propranolol treated group resulting in smaller sample size for return visits.

5.5 Conclusions

The present study demonstrates that multimodal skin imaging can be used clinically to examine structural and functional characteristics of IHs. This objective approach may permit more standardized assessment of disease progression and treatment response. Thus these modalities have utility in measuring and comparing treatment effectiveness in larger multicenter controlled clinical trials as new treatment modalities emerge. We are exploring the development of a multicenter image database for sharing understanding regarding the natural ontogeny and treatment response. This information can be used to determine optimal treatment dosing and duration of therapy and provide additional evidence for diagnosing rebound in IHs (i.e. increased temperature and height).
CHAPTER 6

Infantile Hemangioma Status: Use of Dynamic Infrared Thermography
6.1 Introduction

Infantile hemangiomas (IHs) are benign rapidly proliferating vascular neoplasms occurring in up to 12% of births\(^1\) and more frequently in Caucasians, females, and premature infants.\(^2,3\) They exhibit increased angiogenesis and/or vasculogenesis, disorganized structure, increased perfusion and resultant higher temperatures than uninvolved tissue.\(^7,8,151,152\) They may be superficial, deep or mixed based on the dermal involvement. IHs have high blood flow until 6-10 months, stabilize and then slowly involute with reduced blood flow and fat deposition for 7-10 years.\(^4,140\)

Temperature distribution at the skin surface depends upon blood perfusion, metabolism, thermal conductivity, drug effects, sympathetic nervous system activity, and environmental conditions.\(^60,143,153\) Surface temperature over a tumor is determined by tumor size, i.e., higher with increasing size, and depth, i.e., inversely with distance of the vasculature from the skin surface.\(^142-144\) As a result, measurement of surface temperature distribution may be useful in determining the physiological status, disease progression and treatment response of IHs.

Infrared (IR) thermography records emitted radiation 0.8 \(\mu\)m – 1.0 mm and provides a thermal intensity map to visualize the temperature distribution transmitted to the skin’s surface.\(^60\) It is a reliable, noninvasive, low-cost technique to evaluate vascular function.\(^38,39,131\) IR thermography provides physiological information about lesions or disease rather than structural information obtained via magnetic resonance imaging (MRI) and computed tomography (CT) methods.\(^153\) Static IR thermography captures the
steady-state conditions at a point in time. In the dynamic IR thermographic mode, a stress is applied to the tissue, which alters the structures beneath the surface of the skin. The subsurface structures, e.g., vasculature, respond by restoring the skin to an equilibrium state. The response patterns, e.g., time to achieve pre-stress temperature, provides additional functional information about the tissue. This method has an advantage in that it eliminates environmental effects on tissue response. Dynamic IR with a cold stress has been used for evaluating melanoma wherein lesions exhibited higher temperatures during recovery compared to benign lesions and normal skin. This methodology has not yet been reported for the evaluation of IHs.

We determined the disease status as a function of time, i.e., age, by measuring the dynamic thermal responses to a cold stress among subjects with mixed or superficial infantile hemangiomas.

6.2 Methods

6.2.1 Subjects

A prospective within control observational study was conducted among patients from the Hemangioma and Vascular Malformation Clinic (HVMC) from October 2011 to February 2013. Eligible subjects had superficial or mixed IH confirmed by HVMC physicians. Patients with IHs on the lip, nasal tip, eyelid, or genitals were excluded as application of the cold stress was not feasible. Subjects were either treated or observed (untreated) as determined by HVMC practice standards. The Institutional Review Board
approved the protocol (CCHMC #2011-0443) and parents/guardians provided written informed consent.

6.2.2 Study Protocol

Patients acclimated to the room temperature and humidity for least 15 minutes. High-resolution digital images were taken to quantify IH size and color at 30cm, perpendicular to the site with a Nikon D90 digital camera, Micro Nikkor 60-mm lens with a Nikon R1 Wireless Close-Up flash system (Nikon Inc., Nikon Corporation, Tokyo, Japan) as previously described. Standardized IR images of IHs, including adjacent regions, and uninvolved contralateral control sites, were taken at a perpendicular distance of 60 cm and an emissivity of 0.98 with a FLIR T400 (FLIR Systems Inc., Wilsonville, OR, USA; range 7.5 – 13 μm; uncooled microbolometer focal plan array detector, 320 X 240 pixels resolution; sensitivity < 0.05°C at 30°C; accuracy ± 2°C (<2%)). Static thermal images were acquired and later were co-registered with the color images, as previously described, so regions of thermal activity could be identified clinically. A cold stress was applied at 18 ± 0.2°C for 30 sec (Jack Frost™ insulated cold pack, Cardinal Health, McGaw Park, IL). The 30 second cooling phase was consistent and reproducible (subject % coefficient of variation (%CV) of 7.1% - 11.6% and cool pack %CV of 2.59%). The rewarming behavior was recorded in the video mode (7 frames/sec) for 30 sec after removal of the cold stress. The thermal response of an area nearby to the thermal ROI (within the region of cool stress application) and the uninvolved skin contralateral to the IH were similar (n = 24, cooled: 32.5° ± 1.4°C, contralateral: 32.7° ±
1.2°C; p = 0.36). Therefore, we used adjacent skin sites as the control region to minimize the number of cooling procedures.\textsuperscript{71,154}

6.2.3 Dynamic IR Thermography Image Analysis

The region of interest (ROI) was the IH area of thermal involvement above the temperature of uninvolved tissue after 30 seconds of rewarming and applied to all other time points (ExaminIR software, FLIR), as previously described in 4.2.6.2. Thermal distributions were determined using Matlab\textsuperscript{®} (R2012a, Mathworks, Natick, MA, USA). Effects of the surrounding uninvolved tissue were removed by selecting a threshold of the highest 10\% of the temperature,\textsuperscript{129-131} immediately after cooling. This procedure isolates the highest thermal activity within the IH and segments IH from surrounding uninvolved skin. This procedure provides a better signal to noise ratio compared with thresholds based on the control value. Rewarming curves were generated from frames from baseline and at 5-second intervals for 30 seconds (Figure 6.1).\textsuperscript{132} Outcomes were: (1) temperature change with cooling (temperature after cooling – static temperature), (2) area under the curve during rewarming (AUCrw), and (3) time to maximum temperature (tmax). The maps (Figure 6.1) show the temperature distribution (thermal intensity (°C) and area (pixels)) of the IR pixels above the threshold at baseline (static), after cooling (t0) and at intervals during rewarming.
Figure 6.1. Thermal images and Maps of an IH at Baseline, Cooling and Rewarming phases. Representative (mixed IH) example of the (A) Co-registered high resolution color image and series of thermal maps showing increases in temperature and area at (B) Before cooling (static), (C) 0 sec, (D) 5 sec, and (E) 30 sec post cold challenge. (F) Time profiles of cutaneous hemangioma and control in a representative Mixed IH for each phase (static, cooling and rewarming). Illustrates (i) deeper portion of IH and (ii) superficial portion of IH and how parameters $\Delta T_{cool}$, $t_{max}$ and AUCrw are calculated from data.
6.2.4 Statistical Analysis

Hemangioma thermal characteristics over time were analyzed using univariate general linear models procedures (GLM) with treatment as a covariate. The post-hoc least significant difference (LSD) method was used for pairwise comparisons with a p < 0.05 significance level. The IH and uninvolved sites before versus after cooling were compared with paired-samples t-test. The effects of IH depth over time were analyzed using two-way repeated-measures analysis of variance (ANOVA) with age group as the repeat factor (SPSS v12, SPSS, Inc., Chicago, IL). The residual covariance structure was compound-symmetric. Treatment effects between the first and last evaluations for propranolol versus untreated were analyzed for a subgroup with multiple visits using GLM with age as a covariate and site as a random factor. Data are reported as mean ± SEM.

6.3 Results

Dynamic thermal imaging was conducted on fifty-seven subjects with 41 mixed and 19 superficial IHs. There were 42 IHs on girls and 18 on boys, ranging in age from 6 weeks to 12 years (superficial: 10.8 ± 5.6 mo, mixed: 16.2 ± 3.8 mo). Twenty-five took part in more than one imaging sessions over time. The impact of time (age) was evaluated by grouping the individual data by age (months) at evaluation for ≤ 3, 4-6, 7-9, 10-18, 19-36 and > 36.

Figure 6.1 shows the dynamic IR results for a mixed IH at 9 months of age that has been co-registered with the color image. The static IR image hemangioma show the
temperature distribution where the center is 36.5 – 37°C and the edges are lower, 35 – 35.5°C (Figure 6.1B). The IH is 2.1°C above the control temperature for the static image. After the cold stress, the temperature pattern changes and appears to delineate the thermal margins between the superficial and mixed components (Figures 6.1C-6.1E). The region of greatest thermal activity is identified immediately after the cold pack is removed (t0) (Figure 6.1C). As shown in Figure 6.1D, there was a rapid temperature increase five seconds later (t5), corresponding to the deeper regions. After 30 sec (t30) (Figure 6.1E) the average temperature is 36.4°C, but increased above baseline temperatures in the deeper portion of the IH (Temp > 37°C) due to an exaggerated temperature response to the cold stress, suggesting this region may still be undergoing proliferation. This patient later discontinued treatment because of lack of efficacy and the IH was excised. The histology results confirmed that the superficial area was in early involution and deeper region in proliferation.

6.3.1 Static versus Dynamic

As expected the temperature before versus after cooling for 30 sec were significantly different for the IH and uninvolved control skin (Figure 6.1F). The difference between IH and uninvolved skin was significantly greater for the temperature after cooling 3.59 ± 0.2°C versus static temperature 1.00 ± 0.07°C, P < 0.001).
6.3.2 Dynamic Thermal Response Over Time

For mixed IHs, there were significant reductions in area under the curve during rewarming (AUCrw) and the temperature decrease with cooling (ΔTcool) with time as subject age (P = 0.008 and P = 0.04, respectively) with significant reductions by the 10-18 month period (Figures 6.2A and 6.2B). There was an increase in the time to maximum temperature (tmax) indicating a slower rate of recovery over time and was significant for the comparison of ≤ 3 months versus 10-18 months (P < 0.05) (Figure 6.2C).

Figure 6.2. Changes in Mixed IHs (A) AUCrw, (B) ΔTcool, and (C) tmax over time.

*P < 0.05 vs ≤ 3 mo; ‡P < 0.05 vs 4 – 6 mo

Figure 6.3 depicts the quantitative functional images and the co-registered color image at several time points (baseline, t0, t5, and t30) during (A) proliferation (age 3 mo) and (B) involution (age 14 mo) for the same subject with a mixed IH. The variations in AUCrw, ΔTcool, and tmax responses during rewarming time (t0-30) reflect the changes in vasculature of the IH. During proliferation (Figure 6.3A), the dynamic IR responses indicates an increased recovery in temperature, faster rate of recovery, and minimal temperature decrease to cooling in areas of increased vasculature (i.e. proliferation).
During involution (Figure 6.3B), we observed a slower recovery to the thermal stress, which was indicated by reduced temperature (AUCrw) and ΔTcool, and increased tmax (Figures 6.3C-6.3E).

**Figure 3.** Thermal maps of the same IH during (A) Proliferation and (B) Involution. Showing representative examples of rapid increase in temperature, time to maximum temperature and minimal reduction in during cooling of a proliferating IH (A) and reduced temperature, extended rate of recovery and increasing area for an involuting IH (B).
6.3.3 Thermal Response Characteristics by IH Depth

We examined the dynamic thermal responses for superficial and mixed IHs given the differences in depth and dermal involvement between these types. We used data for subjects less than 18 months since the age distributions were similar. The AUC during rewarming and temperature reduction with cooling decreased over time for both types approaching normal skin conditions, but the values were consistently higher for mixed IHs (Figures 6.4A and 6.4B). Mixed IHs were significantly different in AUC during rewarming ($P = 0.001$) and temperature reduction with cooling ($P = 0.016$) compared to superficial IHs. The rate of recovery slowed with increasing age for both types (Figure 6.4C). Mixed IHs had recovered more quickly versus superficial IHs, but was not significant ($P = 0.6$). Treatment effects and height were not significant in the model.

![Figure 6.4](image-url)

**Figure 6.4.** Depth comparison in (A) AUCrw, (B) $\Delta$Tcool, and (C) $t_{max}$ over time. $P$-value between depth types, Mixed vs Superficial
6.3.4 Treatment Effects

The dynamic thermal responses were evaluated in a small subset with mixed hemangiomas with data from multiple visits over time. Seven received propranolol and six were observed (untreated). The AUC during rewarming was significantly higher for propranolol compared to untreated IHs (526.6 ± 6.1°C/s and 506.6 ± 6.0°C/s, P = 0.04, respectively). There was a general decrease in AUCrw values over time for both treatment groups during follow-up. The overall $R^2$ is 0.684 and adjusted $R^2$ is 0.623. The two regression equations are as follows:

Response (Propranolol) = -1.20*Age + 556.3

Response (Untreated) = -0.72*Age + 543.6

The rate of AUCrw changes over time for (A) propranolol treated and (B) untreated groups are presented graphically in Figure 6.5.

![Graph showing temperature responses over age for Propranolol and Untreated groups.](image)

**Figure 6.5.** Treatment Effects between (A) Propranolol and (B) Untreated Mixed IHs
6.4 Discussion

To our knowledge, this is the first study that used active dynamic infrared thermography as a potential measure to assess infantile hemangioma vasculature (i.e. status). We applied a reliable, non-invasive technique used to assess vascular function\textsuperscript{71,73,131,155-157} to quantify the thermal response of IHs, which provided physiological information about the tissue, allowing us to determine the status over time and depth characteristics of IHs in a clinical setting.

It has been proposed that deeper IHs have a prolonged growth compared to superficial IHs, due to their increased size and vasculature.\textsuperscript{15,158} This has been supported by recent research highlighting the growth rate characteristics in IHs,\textsuperscript{23} and growth patterns between superficial and deeper (deep and mixed) periocular hemangiomas.\textsuperscript{159} Fong et al.\textsuperscript{160} recently investigated the vasculature of IHs using diffuse optical imaging. Their preliminary results revealed increased concentrations of oxy- and deoxy-hemoglobin in mixed IHs compared to superficial IHs, which suggest more blood supply in deeper content (deep and mixed) hemangiomas. This study demonstrated consistent findings with the previous studies. Mixed IHs had a more rapid thermal response (higher AUCrw, and lower ΔTcool) compared to superficial IHs indicating increased vasculature, blood supply and perfusion.

IH tissue responses following a cold stress are easily identifiable using dynamic IR thermography as illustrated in Figures 6.1 and 6.3. Our results demonstrated that IH response changes with age (Figures 6.2 and 6.3) and the thermal maps provide the
capability to visualize the underlying biology identifying specific regions where proliferation (increased temperature) continued and areas that have begun involution. Proliferating IHs are characterized by rapid endothelial cellular growth and high expressions of proangiogenic factors (vascular endothelial grown factor and basic fibroblast growth factor) and matrix metalloproteinases resulting in increased vasculature with compressed vessel lumens. These lesions contains high vessel density (>5 vessels/cm²), high Doppler shifts (> 2kHz), low resistance flow, and measure a 2 to 3-fold increase in blood velocity compared to normal capillaries. Thermograms display higher temperatures for actively growing tumors due to increased angiogenesis, blood perfusion and proliferating metabolic activity. In contrast, during involution, endothelial cellular activity is significantly reduced, vascular channels dilate, and fibrofatty tissue is deposited into the lesion. The temperature area under the curve during rewarming (AUCrw) and temperature difference of cooling (ΔTcool) are an indication of efficiency of heating and cooling in the IH, respectively. The thermal response of normal tissue to cooling is characterized by rapid vasoconstriction, followed by vasodilation of the blood vessels. Proliferating IHs lack the effect to vasoconstriction due to the tightly packed endothelial cellular matrix in the tissue and increased vascularization resulting in a rapid thermal response. Conversely, involuting IHs have lesser IR detectable radiation, suggesting decreased vasculature, proliferation and perfusion. This study revealed a rapid onset and exaggerated temperature recovery for the IHs before 6 months of age compared to IHs between 10 – 18, and greater than 36 months of age, suggesting IHs under the age of 6 months are still undergoing proliferation (Figures 6.2A and 6.3A). Similar profile was observed for ΔTcool (Figure
Data are not shown, but the thermal response at 10 – 18 months (decrease in temperature and recovery rate and increased cooling temperature) is determined by propranolol group and 19 months and above are populated by untreated IHs. As involution progressed, > 36 months of age, the IH rewarming patterns significantly decreased and responded similarly to the control tissue. Our findings are consistent with Chang et al.\textsuperscript{23} found that 93% of overall growth (proliferation) was on average completed by 6 months of age. The findings from this study suggest that rapid rewarming of IHs after a cold challenge is a good indicator of increased vascularization and proliferation within the IH.

We demonstrated in a subset of subjects that propranolol treated subjects have a more overall exaggerated temperature compared to untreated IHs, however, the rewarming behavior in the propranolol group decreases at a faster rate in comparison to the untreated IHs (-1.20 vs -0.7, respectively) (Figure 5). Bingham et al.\textsuperscript{162} reported reductions in lesion volume and vessel density with propranolol treatment. Talaat et al.\textsuperscript{163} used the resisting index as an indicator of vascular activity and found ~50% increase (less activity) with propranolol treatment. Both studies objectively measured changes with color Doppler US, however did not use controls (untreated IHs) to compare propranolol effects to ensure changes were associated with treatment and not the natural course of the IH. This limitation was acknowledged.\textsuperscript{162}

Dynamic IR assessments can provide valuable clinical information including characterizing the vasculature and metabolic activity in IHs and discovering the
anatomical extent of involvement. Such information could be useful in intraoperative detection,\textsuperscript{164,165} and treatment planning of IHs, since dramatic reductions in lesion temperature and perfusion are indicators of involutional changes and therapeutic benefits may be minimal.

For clinicians, a key decision in patient care is determining the stage of the IH (e.g. “Where are patients are on the hemangioma curve?”), because treatment is strongly dictated by stage. Although, laser Doppler imaging, MRI, CT, and Doppler US have been used to assess vascular behavior in IHs, these imaging techniques are rarely used clinically due to cost, sedation risks, movement artefacts, and operator dependent limitations.\textsuperscript{55,67-70} Dynamic IR thermography may represent a reliable, noninvasive, low-cost alternative to evaluate vascular characteristics and IH status.\textsuperscript{38,39,131} Furthermore, IR has great advantage in mapping IHs on the face (where other methods of investigation are difficult to use without sedation).\textsuperscript{38}

### 6.4.1 Study Limitations

The limitations of our study stem from the relatively small sample size and limited follow-up. Statistical differences in all parameters evaluated may have been observed with the larger sample size, and the slow regressive nature of IHs requires years to determine all aspects of change.
6.5 Conclusion

The dynamics of rewarming described by its rate and pattern are easily recorded with infrared thermography. Quantitative analysis of the pattern of cooling and rewarming of IHs revealed differences between IHs before 6 months of age and after 10 months of age and showed distinct characteristics between mixed and superficial IHs. This technique shows promise in monitoring functional changes in IH activity to evaluate treatment efficacies for current and new modalities in prospective randomized-controlled trials.
CHAPTER 7

Summary and Future Directions
7.1 Summary

In this thesis, multimodal methods for assessing treatment effects in both HTS and IHs studies have been established. The quantitative methods for both studies included high resolution color imaging, and three-dimensional imaging. The IH study incorporated infrared imaging, which is a novel technique for examining temperature and tissue perfusion in infantile hemangiomas. The main focus of this thesis has been to characterize IH and evaluate treatment response; however the HTS study was performed initially to demonstrate the feasibility of multimodal skin imaging methods for clinical assessment.

In Chapter 3, the combination PDL + CT therapy resulted in significant reductions in scar erythema, scar height, and increased elasticity compared to CT alone. Significant differences in the seam excess erythema were observed after 2 treatments and continued reduction occurred with additional laser treatments. The VSS also saw reductions in vascularity, pigmentation, pliability and height after 3 treatments. These findings support the first hypothesis that the combination of PDL plus compression therapy (CT) treatment would provide better outcomes in reduced scar erythema, decreased scar height and increased elasticity relative to CT alone, which was substantiated with quantitative methods, and the second hypothesis as the quantitative methods were more sensitive providing more detailed subtle differences earlier than the subjective methods.
The positive results for this work demonstrated the feasibility of multimodal skin imaging methods for clinical assessment and established the basis for the infantile hemangioma studies.

In Chapter 4, the established protocol was extended with infrared thermography, co-registration and automated image analysis algorithms. The new methods revealed that the techniques used to capture and process the images were reproducible and spatial and temporal heterogeneity is the main contributor to variability in color and thermal readings. Univariate GLM analysis found similar cooling and rewarming behaviors between adult and infant skin.

In Chapter 5, the methods were applied to clinical IH study to evaluate the progression for three treatment groups. The methods found several key findings. 1) The thermal area of hemangioma involvement extends beyond the visible tumor margins. Structural characteristics (i.e. size, color, topography) can be assessed visually by an experienced clinician, but functional characteristics, such as thermal area of involvement, had never been reported in the literature because it cannot be assessed by current clinical standards. 2) Significant differences in baseline characteristics, such as increased height and less yellow coloration (i.e. more blueness) may attribute to the recommendation of propranolol treatment versus timolol or observational treatment. 3) Taking, those baseline differences into account, the two-way ANOVA analysis found subjects treated with propranolol resulted in significant reductions in height, skin color and temperature over time compared to untreated and timolol treated hemangiomas. In addition, timolol
patients showed increases in height, suggesting topical timolol has limited effectiveness on IH volume/height.

In Chapter 6, active dynamic infrared thermography was used to show how vascular characteristics within hemangiomas change with age. The AUCrw, an indication of perfusion, was measured after cooling stress and showed significant rewarming/reperfusion differences between depth types, the vascular behavior reduced after 10 months of age and 36 months of age for all patients, and in propranolol treated subjects perfusion had greater decrease over time in comparison to untreated IHs.

The findings from Chapters 5 & 6 support the third hypothesis that IHs treated with propranolol will have a lesser degree of disease involvement (i.e. reduced volume/height and normalized skin color, mechanical properties, temperature and perfusion) relative to the untreated patients.

*Overall, the multimodal skin imaging methods, three-dimensional (3D) surface scanning, high-resolution color imaging, and infrared thermal imaging, used in this thesis can measure relevant physiological changes in infantile hemangiomas and detect treatment effects.*
7.2 Future Directions

Future research strategies are as follows:

- Conduct randomized placebo-controlled studies to monitor changes in height, color, temperature and perfusion during systemic propranolol and topical propranolol to determine comparative effects of treatment. Conduct follow-up studies for both treatment types to determine if patients rebound after the treatment is stopped. From this information criteria can be established on characteristics of a rebound patient and duration of dosing.

- Conduct randomized placebo-controlled multi-duration multicenter study of severe IHs required systemic propranolol to determine optimal treatment duration.

- Conduct randomized placebo-controlled multi-dose multicenter study of severe IHs required systemic propranolol to determine optimal treatment dosage.

- Conduct larger studies using the imaging methods and comparing to current clinical assessments to validate the methods
  - Use size parameter in the Hemangioma Severity Scale\(^{166}\) to compare with quantitative height/volume
  - Use histology and ultrasound to compare with the quantitative color, temperature and perfusion.
REFERENCES


Appendix A
(cf CHAPTER 3)

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Appendix B

(cf CHAPTER 4)

B1: Color and Thermal Image Analysis Algorithms
   B1.1. Summary of Post-processing Codes for Hemangioma Study
   B1.2. GUIAnalysis Code (Generate Results)
   B1.3. Thermal Analysis
   B1.4. Color Analysis
   B1.5. Overlay Analysis
   B1.6. Results Coding
1. **GUIAnalysis** is the main code used for the analysis. It includes the prefix for the filenames, the thermal inputs, and color/thermal analysis, overlay mask, and results. After each major section, the work completed is saved as a mat file.

   a. **Thermal Analysis** contains multiple codes to quantify and map the area of pixels above thresholds for the thermal data.

      i. **IRMSDPlots** – within this code: 1) histogram is created for the hemangioma and control ROIs; 2) the quantities for the hemangioma and control are determined; 3) Maps of the hemangioma above the control thresholds are created.

      ii. **IRIHConSurfPlots** – creates surface plots of the hemangioma and control on the same graph. This requires external codes: cbfreeze and freezeColors (both extracted from Matlab exchange)

      iii. **IRDiffPlots** – creates difference surface plots of the hemangioma – control

   b. **Color Analysis** contains multiple codes to convert the color images from RGBtoLab, then quantify and map the area of pixels above thresholds for the color data. **HVMCColormaps** is a workspace that contains customized colormaps: bluetoyellowcmap and hotcmap that have been created for this section.
i. The **exploreLab** is an external code, used to display the hemangioma and control color ROI in different views/channels: color (RGB), grayscale, lightness, red-green, and blue-yellow.

ii. The images are converted from RGB to Lab color space using the external code: **RGB2Lab**, then separated into different channels for the analysis.

iii. **aMSDPlots, bMSDPlots and LMSDPlots** – within these codes 1) histogram is created for the hemangioma and control ROIs; 2) the quantities for the hemangioma and control are determined 3) Maps of the hemangioma above different control thresholds are created for each channel.

iv. **aDiffPlots, bDiffPlots, and LDiffPlots** - creates difference surface plots of the hemangioma – control at threshold for each channel.

c. **Overlay Analysis** creates masks for the red and blue color and thermal data at their thresholds; and counts the area of overlap. In addition, this section determines the size of the hemangioma based on the color images.

d. **Results** saves all of the quantified color/thermal/overlap data into an excel spreadsheet titled “Stats.xlsx”. It also saves the data into excel spreadsheet titled “Data.xlsx”.

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B.1.2. GUIAnalysis Code (generate results)

```matlab
function generate_results(~, ~)
    % Function for generating all the post-processing output.
    fnhandle = get(hPrefix, 'String');
    Hem_IR = double(cropHIR);
    Hem_RGB = cropHRGB;
    Con_IR = double(cropCIR);
    Con_RGB = cropCRGB;
    ROI = subjectData.cropping.points;
    ROI = cell2mat(get(ROI(1:4), 'XData', 'YData'));

    % Save RGB images of cropped High Resolution color Hem and Con images
    filename = [fnhandle 'H_ColorRoi.tif'];
    imwrite(Hem_RGB, fullfile(dataPath, filename));
    filename = [fnhandle 'C_ColorRoi.tif'];
    imwrite(Con_RGB, fullfile(dataPath, filename));

    % Thermal IR inputs
    LL = str2double(get(hLL, 'String'));
    UL = str2double(get(hUL, 'String'));
    DiffUL = str2double(get(hDiffUL, 'String'));

    post_proc(Hem_IR, Hem_RGB, Con_IR, Con_RGB, fnhandle, LL, UL, ...
               DiffUL, dataPath, ROI, overlayImageH, underlayImageH, ...
               overlayImageC, underlayImageC);
end
```
function post_proc(Hem_IR, Hem_RGB, Con_IR, Con_RGB, fnhandle, ...
   LL, UL, DiffUL, currentPath, ROI, overlayImageH, underlayImageH,
   overlayImageC, underlayImageC)

oldPath = pwd();
cd(currentPath);

% ------------------------------------------------------------------------
% Set negative numbers to 'empty'
Hem_IR(Hem_IR < 27) = NaN; Hem_IR(Hem_IR > 42) = NaN;
Con_IR(Con_IR < 27) = NaN; Con_IR(Con_IR > 38) = NaN;

% Subplot of ROIs for IR and High Res Color
figure(); subplot(2, 2, 1);
imshow(Hem_IR, []);
title('Cropped Hem IR');
colormap(jet(15)); caxis([min(Hem_IR(:)), max(Hem_IR(:))]);
originalSize = get(gca, 'Position');
colorbar
set(gca, 'Position', originalSize);

subplot(2, 2, 2);
imshow(Hem_RGB, []);
title('Cropped Hem Color');
colormap(jet(15)); caxis([min(Hem_IR(:)), max(Hem_IR(:))]);
originalSize2 = get(gca, 'Position');
colorbar
set(gca, 'Position', originalSize2);
```matlab
subplot(2, 2, 4);
imshow(Con_RGB, []);
title('Cropped Con Color');
filename = [fnhandle 'ROI selection subplots.jpg'];
saveas(gcf, filename);

% Subplot of ROI in full Color and Thermal Images
hrect = [ROI(1) ROI(5) ROI(3)-ROI(1) ROI(7)-ROI(5)];
crect = [ROI(2) ROI(6) ROI(4)-ROI(2) ROI(8)-ROI(6)];
figure();
subplot(2, 2, 1);
imshow(overlayImageH, []);
rectangle('Position', hrect, 'LineWidth', 2, 'EdgeColor', 'w');
title('Hem IR'); colormap(jet(15)); caxis([30, max(overlayImageH(:))]);
originalSize = get(gca, 'Position');
colorbar
set(gca, 'Position', originalSize);
subplot(2, 2, 2);
imshow(underlayImageH, []);
rectangle('Position', hrect, 'LineWidth', 2, 'EdgeColor', 'w');
title('Hem Color');
originalSize2 = get(gca, 'Position');
colorbar
set(gca, 'Position', originalSize2);
```
subplot(2, 2, 4);

imshow(underlayImageC, []);

rectangle('Position', rect, 'LineWidth', 2, 'EdgeColor', 'w');

title('Con Color');

filename = [fnhandle 'ROI selection subplots FULL.jpg'];

saveas(gcf, filename);

%----------------------------------------------------------------------

FnHeader_IR = strcat(fnhandle, ['H_IR', ' C_IR']);

HistogramHeader_IR = ['Count', strcat(fnhandle, ['H_IR', ' C_IR'])];

FnHeader_Color = strcat(fnhandle, ['H_a_IRRoi', ' C_a_IRRoi', 'H_b_IRRoi', ' C_b_IRRoi', 'H_L_IRRoi', ' C_L_IRRoi']);

HistogramHeader_Color = ['Count', strcat(fnhandle, ['H_L_IRRoi', ' C_L_IRRoi', 'H_a_IRRoi', ' C_a_IRRoi', 'H_b_IRRoi', ' C_b_IRRoi'])];

%----------------------------------------------------------------------

limit_IR = [LL, UL];

levels_IR = 0.2; inc = LL:levels_IR:UL;

Maps = (UL - LL)/levels_IR; cmap_IR = jet(Maps); cmap_IR1 = jet(Maps); cmap_IR2 = gray(Maps);

Difflimit_IR = [0, DiffUL];

Diffinc = 0:levels_IR:DiffUL;

DiffMaps = (DiffUL/levels_IR); Diffcmap_IR = jet(DiffMaps);

% Level for FULL HEMANGIOMA and FULL CONTROL IR SURFACE PLOTS

Full_levels = 0.5;
% Get IR Data and Maps

**Go to B1.3: Thermal Analysis**

IRMSDPlots
IRIHConSurfPlots
IRDiffPlots
IRImagePlots

% Save workspace

MatFilename = [fnhandle 'Thermal.mat'];
save(MatFilename);

close all

%-----------------------------------------------------------------------------

% Color Colorbar Ranges

load('HVMCColormaps')

limit_a = [140, 170]; cmap_a = hotcmap; levels_a = 2;
limit_b = [120, 150]; cmap_b = bluetoyellowcmap; levels_b = 1;
limit_L = [110, 190]; cmap_L = copper(8); levels_L = 5;

%% Look at the image with imtool

exploreLab(Hem_RGB);
filename = [fnhandle 'ExploreLab_H.tif'];
saveas(gcf, filename);

exploreLab(Con_RGB);
filename = [fnhandle 'ExploreLab_C.tif'];
saveas(gcf,filename);

%%% Convert from RGB to Lab
Hem_Lab = RGB2Lab(Hem_RGB);
Con_Lab = RGB2Lab(Con_RGB);

%%% Separate Lab image into L, a*, b* images
Hem_L = Hem_Lab(:,:,1); Hem_a = Hem_Lab(:,:,2); Hem_b = Hem_Lab(:,:,3);
Con_L = Con_Lab(:,:,1); Con_a = Con_Lab(:,:,2); Con_b = Con_Lab(:,:,3);

%%% Get separate L, a*, b* plots for Mean +/- SD values

Go to B1.4: Color Analysis

aMSDPlots
LMSDPlots
bMSDPlots
abLImagePlots

%%% Get separate L, a*, b* plots for Difference relative to Control (IH - Control)

aDiffPlots
LDiffPlots
bDiffPlots

%%% Save Workspace
filename = [fnhandle 'Color.mat'];
save(filename);

close all

--------------------------------------------------------
B1.3: Thermal Analysis

IRMSDPlots

%% Show Histogram in thermal image difference
% Rename the IR Images for the Histogram data
Hem_IRHist = Hem_IR;
Con_IRHist = Con_IR;

% Resize control image to match hemangioma image
[M_IR, N_IR] = size(Hem_IRHist);
Con_IRHistRS = imresize(Con_IRHist,[M_IR N_IR]);

bins = [20:0.1:40].';
HemHist = histc(Hem_IRHist,bins);
ConHist = histc(Con_IRHist,bins);
HemHist_tst = transpose(sum(transpose(HemHist)));
ConHist_tst = transpose(sum(transpose(ConHist)));

ConHistRS = histc(Con_IRHistRS,bins);
ConHistRS_tst = transpose(sum(transpose(ConHistRS)));

figure
bar1 = bar(bins, HemHist_tst, 'FaceColor', 'r');
hold on;
bar2 = bar(bins, ConHist_tst, 'FaceColor', [.78 0.7 0.6], 'EdgeColor', [.78 0.7 0.6], 'BarWidth', 0.8);
hold off;
legend('Hemangioma','Control')
Chartname = ['Histogram of IR Temperature (°C) images'];
% Scale x axis manually. *grayLevels(end)
xlim([25 40]); % Scale x axis manually. *grayLevels(end)
xlabel('Temperature Intensity (°C)'); ylabel('Number of pixels')
MSDFilename = [fnhandle 'Histogram_IR.jpg'];
saveas(gcf,MSDFilename);

%%% Get Stats Data for Hemangioma and Control Thermal ROI
HemMean_IR = nanmean(Hem_IR(:));
HemSD_IR = nanstd(Hem_IR(:));
Hemmin_IR = nanmin(Hem_IR(:));
Hemmax_IR = nanmax(Hem_IR(:));
HemMed_IR = nanmedian(Hem_IR(:));

ConMean_IR = nanmean(Con_IR(:));
ConSD_IR = nanstd(Con_IR(:));
Conmin_IR = nanmin(Con_IR(:));
Conmax_IR = nanmax(Con_IR(:));
ConMed_IR = nanmedian(Con_IR(:));

ConMSD_IR = ConMean_IR + ConSD_IR;
IHMax_ConMSD_IR = (Hemmax_IR - ConMSD_IR);

[rows columns] = size(Hem_IR);
AreaHem = Hem_IR > Hemmin_IR-1;
HemTotalArea_IR = sum(sum(AreaHem));
% HemTotalArea_IR = rows * columns;

[Conrows Concolumns] = size(Con_IR);
AreaCon = Con_IR > Conmin_IR-1;
ConTotalArea_IR = sum(sum(AreaCon));

% ConTotalArea_IR = Conrows * Concolumns;

%%% Get Excess Thermal Data

% mean + 3SD of Control image

threshold3SD_IR = ConMean_IR + (3*ConSD_IR);

Hem3SD_IR = Hem_IR > threshold3SD_IR;
HemArea3SD_IR = nansum(nansum(Hem3SD_IR));
HemPercent3SD_IR = 100.0 * (HemArea3SD_IR / HemTotalArea_IR);
HemMean3SD_IR = nanmean(Hem_IR(Hem3SD_IR));
HemSD3SD_IR = nanstd(Hem_IR(Hem3SD_IR));
HemMedian3SD_IR = nanmedian(Hem_IR(Hem3SD_IR));

% mean + 4SD of Control image

threshold4SD_IR = ConMean_IR + (4*ConSD_IR);

Hem4SD_IR = Hem_IR > threshold4SD_IR;
HemArea4SD_IR = nansum(nansum(Hem4SD_IR));
HemPercent4SD_IR = 100.0 * (HemArea4SD_IR / HemTotalArea_IR);
HemMean4SD_IR = nanmean(Hem_IR(Hem4SD_IR));
HemSD4SD_IR = nanstd(Hem_IR(Hem4SD_IR));
HemMedian4SD_IR = nanmedian(Hem_IR(Hem4SD_IR));

% 90th Percentile

threshold90_HIR = prctile(Hem_IR(:,90);
Hem90_IR = Hem_IR > threshold90_HIR;
HemArea90_IR = nansum(nansum(Hem90_IR));
HemMean90_IR = nanmean(Hem_IR(Hem90_IR));
HemSD90_IR = nanstd(Hem_IR(Hem90_IR));
HemMedian90_IR = nanmedian(Hem_IR(Hem90_IR));
HemMin90_IR = nanmin(Hem_IR(Hem90_IR));

threshold90_CIR = prctile(Con_IR(:,90));
Con90_IR = Con_IR > threshold90_CIR;
ConArea90_IR = nansum(nansum(Con90_IR));
ConMean90_IR = nanmean(Con_IR(Con90_IR));
ConSD90_IR = nanstd(Con_IR(Con90_IR));
ConMedian90_IR = nanmedian(Con_IR(Con90_IR));
ConMin90_IR = nanmin(Con_IR(Con90_IR));

%%% HEMANGIOMA ABOVE MEAN + 3 STD DEV OF CONTROL
%HemIR = imresize(Hem_IR,[54 46]);
%axis([1 46 1 54]); axis equal; axis([1 46 1 54]);
%surf(HemIR,'edgecolor','none');figure(gcf)

if Hemmax_IR > threshold3SD_IR
    figure
    surf(Hem_IR,'edgecolor','none');figure(gcf)
    set(gca,'YDir','reverse', 'ZLim', [threshold3SD_IR, Hemmax_IR], 'CLim', limit_IR);
    colormap(cmap_IR);
    cb_IR = colorbar; set(cb_IR, 'YTick',inc); ylabel(cb_IR,'Temperature (°C)')
    %xlabel('X horizontal'); ylabel('Y horizontal');
zlabel('Temperature (°C) IH above Control Mean + 3SD');
axis([1 N_IR 1 M_IR]); axis equal; axis([1 N_IR 1 M_IR]);
view([0 90]); box('on');

MSDFilename = [fnhandle 'IR3SD.jpg'];
saveas(gcf,MSDFilename);
end

%%% HEMANGIOMA ABOVE 90th Percentile
if Hemmax_IR > threshold90_HIR
    figure
    surf(Hem_IR,'edgecolor','none');figure(gcf)
    set(gca,'YDir','reverse','ZLim',[threshold90_HIR, Hemmax_IR], 'CLim', limit_IR);
    colormap(cmap_IR);
    cb_IR = colorbar; set(cb_IR,'YTick',inc); ylabel(cb_IR,'Temperature (°C)');
    zlabel('Temperature (°C) IH of Highest 10%');
    axis([1 N_IR 1 M_IR]); axis equal; axis([1 N_IR 1 M_IR]);
    view([0 90]); box('on');

    MSDFilename = [fnhandle 'IR90.jpg'];
saveas(gcf,MSDFilename);
end

IRIHConSurfPlots

%%% FULL HEMANGIOMA and FULL CONTROL SURFACE PLOTS
Full_LL = floor(Conmin_IR); Full_UL = ceil(Hemmax_IR); FullRange_IR = [Full_LL,Full_UL];
Full_nbands = (Full_UL-Full_LL)/Full_levels; cmap_HIR = jet(Full_nbands); cmap_CIR = gray(Full_nbands);
Full_Inc = Full_LL:Full_levels:Full_UL;

%%% Resize control image to match hemangioma image
[M_IR, N_IR] = size(Hem_IR);
Con_IRresize = imresize(Con_IR,[M_IR N_IR]);

%%% figure
h(1) = surf(Hem_IR,'DisplayName','IR Hemangioma');figure(gcf)
set(gca,'YDir','reverse','CLim', FullRange_IR, 'ZLim', FullRange_IR);
colormap(cmap_HIR); freezeColors;
cb_HIR = cbfreeze(colorbar); cblabel('IH Temperature, °C');
hold on;

h(2)= surf(Con_IRresize,'DisplayName','IR Control');figure(gcf)
set(gca,'YDir','reverse');
colormap(cmap_CIR); freezeColors;

cb_CIR = cbfreeze(colorbar); cblabel('Control Temperature, °C');
hold off;

set (h,'EdgeColor','none');
axis([1 N_IR 1 M_IR]);
set(cb_HIR,'YTickLabel',Full_Inc,'YTick',Full_Inc);
set(cb_CIR,'YTickLabel',Full_Inc,'YTick',Full_Inc);

view([-22 14]);
xlabel('X horizontal')
ylabel('Y horizontal')
zlabel('Hemangioma and Control Temperature °C')

MSDFilename = [fnhandle 'IR Con and IH.jpg'];
saveas(gcf,MSDFilename);
%
% MSDFilename = [fnhandle 'IR Con and IH.fig'];
% saveas(gcf,MSDFilename);

view([90 0]);
MSDFilename = [fnhandle 'IR Con and IH_viewYZ.jpg'];
saveas(gcf,MSDFilename);

% % HEMANGIOMA ABOVE MEAN + 3 STD DEV OF CONTROL
if Hemmax_IR > threshold3SD_IR
figure
h(1) = surf(Hem_IR,'DisplayName','IR Hemangioma');figure(gcf)
set(gca,'YDir','reverse','CLim', limit_IR, 'ZLim', [threshold3SD_IR, Hemmax_IR]);
colormap(cmap_IR1);freezeColors;
cb_HIR = cbfreeze(colorbar); cblabel('IH Temperature, °C'); %,'Rotation',0)
hold on;

h(2)= surf(Con_IRresize,'DisplayName','IR Control');figure(gcf)
set(gca,'YDir','reverse');
colormap(cmap_IR2);freezeColors;

cb_CIR = cbfreeze(colorbar); cblabel('Control Temperature, °C');
hold off;

axis([1 N_IR 1 M_IR]);
set(cb_HIR,'YTickLabel',inc,'YTick',inc);
set(cb_CIR,'YTickLabel',inc,'YTick',inc);

view([-22 14]);
set(h,'EdgeColor','none');
xlabel('X horizontal'); ylabel('Y horizontal'); zlabel('Temperature °C IH above Control Mean + 3SD')

MSDFilename = [fnhandle 'IR Con3SD and IH.jpg'];
saveas(gcf,MSDFilename);

view([-90 0]);
MSDFilename = [fnhandle 'IR Con3SD and IH_view90.jpg'];
saveas(gcf,MSDFilename);
end

IRDiffPlots

%%% Difference from CONTROL MEAN + STD DEV --------Change:
ImageDiffMSD_IR/DiffMSDmax_IR2/Mean+SD

% Mean + 3 SD
if Hemmax_IR > threshold3SD_IR
    Dif3SD_IR = (Hem_IR - threshold3SD_IR);
    Dif3SDMax_IR = round((max(Dif3SD_IR(:))+levels_IR)/levels_IR)*levels_IR;
end

figure();
surf(Diff3SD_IR,'edgcolor','none');figure(gcf)
set(gca,'YDir','reverse', 'ZLim', [0, Diff3SDMax_IR], 'CLim', Difflimit_IR);
colormap(Diffcmap_IR); cb_IR = colorbar;
set(cb_IR, 'YTick',Diffinc); ylabel(cb_IR,'Temperature (°C) Difference Hemangioma - Control Mean+3SD')
xlabel('X horizontal'); ylabel('Y horizontal'); zlabel('Temperature (°C) Difference IH - Control Mean+3SD')

axis([1 N_IR 1 M_IR]); axis equal; axis([1 N_IR 1 M_IR]); view([0 90]); box('on');
MSDFilename = [fnhandle 'IR IH-Con3SD.jpg'];
saveas(gcf,MSDFilename);
end
B1.4: Color Analysis

aMSDPlots

%%% Resize control image to match hemangioma image

[M, N] = size(Hem_a);
Con_aresize = imresize(Con_a,[M N]);

%%% Convert images to uint8 for Histogram Hemmax_a

Hem_aHist = uint8(Hem_a);
Con_aHist = uint8(Con_a);

% Get Histogram Data from Hemangioma and Control Redness (a*) Images

%Histogram information needs to be in unit8!!

[HempixelCount_a grayLevels] = imhist(Hem_aHist);
[ConpixelCount_a grayLevels] = imhist(Con_aHist);

figure
bar(HempixelCount_a, 'FaceColor', 'r');
hold on;
bar(ConpixelCount_a, 'FaceColor', [.78 0.7 0.6], 'EdgeColor', [.78 0.7 0.6]);
hold off;
legend('Hemangioma','Control')
Chartname = ['Histogram of Redness (a*) images'];
xlim([115 190]); % Scale x axis manually.
xlabel(['Pixel values'; 'More redness -------------------------->']);
ylabel('Intensity');
saveas(gcf,'fnhandle Histogram_a.jpg');
%% Get Stats Data for Hemangioma and Control Redness (a*) Images

HemMean_a = nanmean(Hem_a(:));
HemSD_a = nanstd(Hem_a(:));
Hemmin_a = nanmin(Hem_a(:));
Hemmax_a = nanmax(Hem_a(:));
HemMed_a = nanmedian(Hem_a(:));

ConMean_a = nanmean(Con_a(:));
ConSD_a = nanstd(Con_a(:));
Conmin_a = nanmin(Con_a(:));
Conmax_a = nanmax(Con_a(:));
ConMed_a = nanmedian(Con_a(:));

ConMSD_a = ConMean_a + ConSD_a;
IHMax_ConMSD_a = (Hemmax_a - ConMSD_a);

[rows columns] = size(Hem_a);
HemTotalArea = rows * columns;
[Conrows Concolumns] = size(Con_a);
ConTotalArea = Conrows * Concolumns;

%% Get Excess Erythema Data

% Mean + 4 SD
threshold4SD_a = ConMean_a + (4*ConSD_a);

Hem4SD_a = Hem_a > threshold4SD_a;
HemArea4SD_a = sum(sum(Hem4SD_a));
HemPercent4SD_a = 100.0 * (HemArea4SD_a / HemTotalArea);
HemMean4SD_a = mean(Hem_a(Hem4SD_a));
HemSD4SD_a = std(Hem_a(Hem4SD_a));
HemMedian4SD_a = median(Hem_a(Hem4SD_a));

% Mean + 7 SD
threshold7SD_a = ConMean_a + (7*ConSD_a);

Hem7SD_a = Hem_a > threshold7SD_a;
HemArea7SD_a = sum(sum(Hem7SD_a));
HemPercent7SD_a = 100.0 * (HemArea7SD_a / HemTotalArea);
HemMean7SD_a = mean(Hem_a(Hem7SD_a));
HemSD7SD_a = std(Hem_a(Hem7SD_a));
HemMedian7SD_a = median(Hem_a(Hem7SD_a));

%%% HEMANGIOMA ABOVE MEAN + 4* STD DEV OF CONTROL
if Hemmax_a > threshold4SD_a
    figure
    surf(Hem_a,'edgecolor','none');figure(gcf)
    set(gca,'YDir','reverse', 'ZLim', [threshold4SD_a, Hemmax_a], 'CLim', limit_a);
    colormap(cmap_a); cb_a = colorbar;
    axis([0 N 0 M]); axis equal; axis([0 N 0 M]);
    ylabel(cb_a,'Quantitative Redness (a*) IH above Control Mean+4SD')
    view([0 90]); box('on');
    %xlabel('X horizontal'); ylabel('Y horizontal')
    filename = [fnhandle 'a4SD.jpg'];
    saveas(gcf,filename);
end
bMSDPlots

%%% Resize control image to match hemangioma image

[M, N] = size(Hem_b);
Con_bresize = imresize(Con_b,[M N]);

%%% Convert images to uint8 for Histogram info

Hem_bHist = uint8(Hem_b);
Con_bHist = uint8(Con_b);

% Get Histogram Data from Hemangioma and Control Blueness (b*) Images
%Histogram information needs to be in unit8!!

[HempixelCount_b grayLevels] = imhist(Hem_bHist);
[ConpixelCount_b grayLevels] = imhist(Con_bHist);

figure
bar(HempixelCount_b, 'FaceColor', 'b');
hold on;
bar(ConpixelCount_b, 'FaceColor', [.78 0.7 0.6], 'EdgeColor', [.78 0.7 0.6]);
hold off;
legend('Hemangioma','Control')
Chartname = [Chartname,'Histogram_b.jpg'];
filename = [fnhandle 'Histogram_b.jpg'];
saveas(gcf,filename);
%% Get Stats Data for Hemangioma and Control Blueness (b*) Images

HemMean_b = nanmean(Hem_b(:));
HemSD_b = nanstd(Hem_b(:));
Hemmin_b = nanmin(Hem_b(:));
Hemmax_b = nanmax(Hem_b(:));
HemMed_b = nanmedian(Hem_b(:));

ConMean_b = nanmean(Con_b(:));
ConSD_b = nanstd(Con_b(:));
Conmin_b = nanmin(Con_b(:));
Conmax_b = nanmax(Con_b(:));
ConMed_b = nanmedian(Con_b(:));

ConMSD_b = ConMean_b - ConSD_b;
IHMax_ConMSD_b = (ConMSD_b - Hemmin_b);

[rows columns] = size(Hem_b);
HemTotalArea = rows * columns;
[Conrows Concolumns] = size(Con_b);
ConTotalArea = Conrows * Concolumns;

%% Get Excess Blueness Data

% Mean - 3.5 SD
threshold35SD_b = ConMean_b - (3.5*ConSD_b);

Hem35SD_b = Hem_b < threshold35SD_b;
HemArea35SD_b = sum(sum(Hem35SD_b));
HemPercent35SD_b = 100.0 * (HemArea35SD_b / HemTotalArea);
HemMean35SD_b = mean(Hem_b(Hem35SD_b));
HemSD35SD_b = std(Hem_b(Hem35SD_b));
HemMedian35SD_b = median(Hem_b(Hem35SD_b));

% Mean - 5 SD
threshold5SD_b = ConMean_b - (5*ConSD_b);

Hem5SD_b = Hem_b < threshold5SD_b;
HemArea5SD_b = sum(sum(Hem5SD_b));
HemPercent5SD_b = 100.0 * (HemArea5SD_b / HemTotalArea);
HemMean5SD_b = mean(Hem_b(Hem5SD_b));
HemSD5SD_b = std(Hem_b(Hem5SD_b));
HemMedian5SD_b = median(Hem_b(Hem5SD_b));

%% HEMANGIOMA Below MEAN - 3.5 STD DEV OF CONTROL
if Hemmin_b < threshold35SD_b
    figure
    surf(Hem_b,'edgcolor','none');figure(gcf)
    set(gca,'YDir','reverse','ZLim', [Hemmin_b, threshold35SD_b], 'CLim', limit_b);
    colormap(cmap_b); cb_b = colorbar;
    axis([0 N 0 M]); axis equal; axis([0 N 0 M]);
    ylabel(cb_b,'Quantitative Blue-Yellow (b*) IH below Control Mean-3.5SD')
    view([0 90]); box('on');
    xlabel('X horizontal'); ylabel('Y horizontal');
    filename = [fnhandle 'bM3.5SD.jpg'];
    saveas(gcf,filename);
end
%% Resize control image to match hemangioma image

[M, N] = size(Hem_L);
Con_Lresize = imresize(Con_L,[M N]);

%% Convert images to uint8 for Histogram info
adjL = 0;

Hem_LHist = uint8(Hem_L);
Con_LHist = uint8(Con_L);
Con_LHist = Con_LHist - adjL;

% Get Histogram Data from Hemangioma and Control Lightness (L) Images
%Histogram information needs to be in unit8!!

[HempixelCount_L grayLevels] = imhist(Hem_LHist);
[ConpixelCount_L grayLevels] = imhist(Con_LHist);

figure
bar(HempixelCount_L, 'FaceColor', 'k');
hold on;
bar(ConpixelCount_L, 'FaceColor', [.78 0.7 0.6], 'EdgeColor', [.78 0.7 0.6]);
hold off;
legend('Hemangioma', 'Control')
Chartname = ['Histogram of Lightness (L) images'];
xlim([80 grayLevels(end)]); % Scale x axis manually.
xlabel('Pixel values'; '---------- Darker');
ylabel('Intensity')
Filename = [fnhandle 'Histogram_L.jpg'];
saveas(gcf,Filename);

%%% Get Stats Data for Hemangioma and Control Lightness (L) Images

HemMean_L = nanmean(Hem_L(:));
HemSD_L = nanstd(Hem_L(:));
Hemmin_L = nanmin(Hem_L(:));
Hemmax_L = nanmax(Hem_L(:));
HemMed_L = nanmedian(Hem_L(:));

ConMean_L = nanmean(Con_L(:));
ConSD_L = nanstd(Con_L(:));
Conmin_L = nanmin(Con_L(:));
Conmax_L = nanmax(Con_L(:));
ConMed_L = nanmedian(Con_L(:));

ConMSD_L = ConMean_L - ConSD_L;
IHMax_ConMSD_L = (ConMSD_L - Hemmin_L);

[rows columns] = size(Hem_L);
HemTotalArea = rows * columns;
[Conrows Concolumns] = size(Con_L);
ConTotalArea = Conrows * Concolumns;

%%% Get Excess Darkness Data

% Mean - 2SD
threshold2SD_L = ConMean_L - (2*ConSD_L);

Hem2SD_L = Hem_L < threshold2SD_L;
HemArea2SD_L = sum(sum(Hem2SD_L));
HemPercent2SD_L = 100.0 * (HemArea2SD_L / HemTotalArea);
HemMean2SD_L = mean(Hem_L(Hem2SD_L));
HemSD2SD_L = std(Hem_L(Hem2SD_L));
HemMedian2SD_L = median(Hem_L(Hem2SD_L));

% Mean - 4SD
threshold4SD_L = ConMean_L - (4*ConSD_L);

Hem4SD_L = Hem_L < threshold4SD_L;
HemArea4SD_L = sum(sum(Hem4SD_L));
HemPercent4SD_L = 100.0 * (HemArea4SD_L / HemTotalArea);
HemMean4SD_L = mean(Hem_L(Hem4SD_L));
HemSD4SD_L = std(Hem_L(Hem4SD_L));
HemMedian4SD_L = median(Hem_L(Hem4SD_L));

%%% HEMANGIOMA Below MEAN - 4 STD DEV OF CONTROL
if Hemmin_L < threshold4SD_L
    figure
    surf(Hem_L,'edgecolor','none');figure(gcf)
    set(gca, 'YDir','reverse','ZLim', [Hemmin_L, threshold4SD_L],'CLim', limit_L);
    colormap(cmap_L); cb_L = colorbar;
    axis([0 N 0 M]); axis equal; axis([0 N 0 M]);
    ylabel(cb_L,'Quantitative Lightness (L*) IH below Control Mean-4SD');
    view([0 90]); box('on');
    xlabel('X horizontal'); ylabel('Y horizontal');
    filename = [fnhandle 'L4SD.jpg'];
    saveas(gcf, filename);
end
**aDiffPlots**

%% Difference from CONTROL MEAN + 4* STD DEV

if Hemmax_a > threshold4SD_a

Dif4SD_a = (Hem_a - threshold4SD_a);

Dif4SDMax_a = ceil((max(Dif4SD_a(:)) + levels_a)/levels_a)*levels_a;

DiffMaps_a = abs(Dif4SDMax_a/levels_a);

Diffcmap_a = flipud(autumn(DiffMaps_a));

Diffinc_a = 0:levels_a:Dif4SDMax_a;

figure();
surf(Diff4SD_a,'edgecolor','none');figure(gcf)

set(gca,'YDir','reverse','ZLim', [0, Diff4SDMax_a], 'CLim', [0, Diff4SDMax_a]);
colormap(Diffcmap_a); cb_a = colorbar;

set(cb_a, 'YTick',Diffinc_a); ylabel(cb_a,'Redness (a*) Difference Hemangioma - Control Mean+4SD')
xlabel('X horizontal'); ylabel('Y horizontal'); zlabel('Quantitative Redness (a*) IH - Control Mean+4SD')

axis([1 N 1 M]); axis equal; axis([1 N 1 M]); view([0 90]); box('on');

MSDFilename = ['fnhandle ' a IH-Con4SD.jpg'];
saveas(gcf,MSDFilename);

end

**bDiffPlots**

%% Difference from CONTROL MEAN + 3.5 STD DEV

if Hemmin_b < threshold35SD_b

Diff35SD_b = (Hem_b - threshold35SD_b);
Diff35SDMin_b = ceil((min(Diff35SD_b(:))-levels_b)/levels_b)*levels_b;

DiffMaps_b = abs(Diff35SDMin_b/levels_b);

v = linspace(1,0,DiffMaps_b); vmap = [v v flipud(v)];

Diffcmap_b = flipud(vmap); Diffinc_b = Diff35SDMin_b:levels_b:0;

if Diff35SDMin_b < 0;
    figure();
    surf(Diff35SD_b,'edgecolor','none');figure(gcf)
    set(gca,'YDir','reverse', 'ZLim', [Diff35SDMin_b, 0], 'CLim', [Diff35SDMin_b, 0]);
    colormap(Diffcmap_b); cb_b = colorbar;
    set(cb_b,'YTick',Diffinc_b); ylabel(cb_b,'Blue-Yellow (b*) Difference Hemangioma - Control Mean-3.5SD')
    xlabel('X horizontal'); ylabel('Y horizontal'); zlabel('Quantitative Blueness (b*) IH - Control Mean-3.5SD')

    axis([1 N 1 M]); axis equal; axis([1 N 1 M]); view([0 90]); box('on');
    MSDFilename = [fnhandle 'b IH-ConM3.5SD.jpg'];
    saveas(gcf,MSDFilename);
end

LDiffPlots

%% Difference from CONTROL MEAN - 4 STD DEV
if Hemmin_L < threshold4SD_L
    Diff4SD_L = (Hem_L - threshold4SD_L);
    Diff4SDMin_L = ceil((min(Diff4SD_L(:))-levels_L)/levels_L)*levels_L;
DiffMaps_L = abs(Diff4SDMin_L/levels_L); Diffcmap_L = copper(DiffMaps_L); Diffinc_L = Diff4SDMin_L:levels_L:0;

if Diff4SDMin_L < 0
    figure();
    surf(Diff4SD_L,'edgecolor','none');figure(gcf)
    set(gca,'YDir','reverse','ZLim', [Diff4SDMin_L, 0], 'CLim', [Diff4SDMin_L, 0]);
    colormap(Diffcmap_L); cb_L = colorbar;
    set(cb_L, 'YTick',Diffinc_L); ylabel(cb_L,'Lightness (L*) Difference Hemangioma - Control Mean-4SD')
    xlabel('X horizontal'); ylabel('Y horizontal'); zlabel('Quantitative Lightness (L) IH - Control Mean-4SD')
    axis([1 N 1 M]); axis equal; axis([1 N 1 M]); view([0 90]); box('on');
    MSDFilename = [fnhandle 'L IH-Con4SD.jpg'];
    saveas(gcf,MSDFilename);
end
end
B.1.5. Overlay Analysis

%% Resize the IR Image to match Color image
Hem_IRRS = imresize(Hem_IR,[M N]);

%% Redness 4SD and Thermal 3SD
figure();
newimg = zeros(M, N, 3);
aMask = Hem_a > threshold4SD_a; newimg(:,:,1) = aMask;
IRMask = Hem_IRRS > threshold3SD_IR; newimg(:,:,2) = IRMask;
imshow(newimg);
Red4SDIR3SDOverlap = nnz(aMask & IRMask);
filename = [fnhandle, 'Red4SD and IR3SD Mask.jpg'];
saveas(gcf, filename);

%% Redness 4SD and Thermal 90th
figure();
newimg = zeros(M, N, 3);
aMask = Hem_a > threshold4SD_a; newimg(:,:,1) = aMask;
IRMask = Hem_IRRS > threshold90_HIR; newimg(:,:,2) = IRMask;
imshow(newimg);
Red4SDIR90Overlap = nnz(aMask & IRMask);
filename = [fnhandle, 'Red4SD and IR90 Mask.jpg'];
saveas(gcf, filename);

%% Redness 4SD and Thermal 90th
figure();
newimg = zeros(M, N, 3);
aMask = Hem_a > threshold4SD_a; newimg(:,:,1) = aMask;
IRMask = Hem_IRRS > threshold4SD_IR; newimg(:,:,2) = IRMask;
imshow(newimg);
Red4SDIR4SDOverlap = nnz(aMask & IRMask);

%% Blue-Yellow 3.5SD and Thermal 3SD
figure();
newimg = zeros(M, N, 3);
bMask = Hem_b < threshold35SD_b; newimg(:, :, 3) = bMask;
IRMask = Hem_IRRS > threshold3SD_IR; newimg(:, :, 2) = IRMask;
imshow(newimg);
Blue35SDIR3SDOverlap = nnz(bMask & IRMask);
filename = [fnhandle, 'Blue3.5SD and IR3SD Mask.jpg'];
saveas(gcf, filename);

%% Blue-Yellow 3.5SD and Thermal 90th
figure();
newimg = zeros(M, N, 3);
bMask = Hem_b < threshold35SD_b; newimg(:, :, 3) = bMask;
IRMask = Hem_IRRS > threshold90_HIR; newimg(:, :, 2) = IRMask;
imshow(newimg);
Blue35SDIR90Overlap = nnz(bMask & IRMask);
filename = [fnhandle, 'Blue3.5SD and IR90 Mask.jpg'];
saveas(gcf, filename);

%% Blue-Yellow 3.5SD and Thermal 3SD
figure();
newimg = zeros(M, N, 3);
bMask = Hem_b < threshold35SD_b; newimg(:, :, 3) = bMask;
IRMask = Hem_IRRS > threshold4SD_IR; newimg(:, :, 2) = IRMask;
imshow(newimg);
Blue35SDIR4SDOverlap = nnz(bMask & IRMask);

%% Redness 4SD and Blue-Yellow 3.5SD
figure();
newimg = zeros(M, N, 3);
aMask = Hem_a > threshold4SD_a; newimg(:,:,1) = aMask;
bMask = Hem_b < threshold35SD_b; newimg(:,:,3) = bMask;
imshow(newimg);
Ren4SDBlue35SDOverlap = nnz(aMask & bMask);
filename = [fnhandle, 'Red4SD and Blue3.5SD Mask.jpg'];
saveas(gcf, filename);

%%% Create Overlay of Color and Thermal
figure();
imgGray = repmat(rgb2gray(Hem_RGB), [1 1 3]); % Create the grayscale underlay
imagesc(imgGray); % Plot the image
hold on; % Add to the existing plot
hOverlay = imagesc(Hem_IRRS); % Plot the overlay image
set(gca, 'CLim', limit_IR);
colormap(cmap_IR); % Create the colormap
cb_IR = colorbar;
set(cb_IR, 'YTick', inc);
axis([0 N 0 M]);
axis image;
set(hOverlay, 'AlphaData', 0.3); % Make the overlay transparent
filename = [fnhandle, 'Color and Thermal Overlay.jpg'];
saveas(gcf, filename);

%%% Calculate the Area of the hemangioma
aMask = Hem_a > threshold4SD_a;
bMask = Hem_b < threshold35SD_b;
LMask = Hem_L < threshold4SD_L;
data = regionprops(aMask | bMask, 'Area', 'PixelIdxList');
[~, index] = max([data.Area]);
newMask = false(size(aMask));
newMask(data(index).PixelIdxList) = true;
newMask = imclose(newMask, strel('disk', 7));
figure; imshow(newMask);
saveas(gcf, [fnhandle 'HemArea.jpg']);
HemArea = nnz(newMask);

close all

%--------------------------------------------------------------------------
B.1.6. Results

%% Results to Excel Files

warning off
MATLAB:xlswrite:AddSheet

Statsfilename = [fnhandle 'Stats.xlsx'];

% COLOR RESULTS

ResultsHeader_Color = ['Filename', 'Mean', 'Std Dev', 'Median', 'Min', 'Max', 'Total Area', 'Threshold (a4SD, b3.5SD, L2SD)', '
#pixels (a4SD, b3.5SD, L2SD)', '
%pixels (a4SD, b3.5SD, L2SD)', 'Mean (a4SD, b3.5SD, L2SD)', '
StdDev (a4SD, b3.5SD, L2SD)', 'Median (a4SD, b3.5SD, L2SD)', 'Threshold (a7SD, b5SD, L4SD)', '
#pixels (a7SD, b5SD, L4SD)', '
%pixels (a7SD, b5SD, L4SD)', 'Mean (a7SD, b5SD, L4SD)', 'StdDev (a7SD, b5SD, L4SD)', 'Median (a7SD, b5SD, L4SD)', 'HemArea from Color', 'Red4SDBlue3.5SD Overlap', '(a4SD, b3.5SD) IR3SD Overlap', '(a4SD, b3.5SD) IR4SD Overlap'];

HemResult_a = [HemMean_a HemSD_a HemMed_a Hemmin_a Hemmax_a HemTotalArea
threshold4SD_a HemArea4SD_a HemPercent4SD_a HemMean4SD_a HemSD4SD_a HemMedian4SD_a
threshold7SD_a HemArea7SD_a HemPercent7SD_a HemMean7SD_a HemSD7SD_a HemMedian7SD_a
HemArea Red4SDBlue35SDOverlap Red4SDIR3SDOverlap Red4SDIR4SDOverlap];
ConResult_a = [ConMean_a ConSD_a ConMed_a Conmin_a Conmax_a ConTotalArea];

HemResult_b = [HemMean_b HemSD_b HemMed_b Hemmin_b Hemmax_b HemTotalArea
threshold35SD_b HemArea35SD_b HemPercent35SD_b HemMean35SD_b HemSD35SD_b
HemMedian35SD_b threshold5SD_b HemArea5SD_b HemPercent5SD_b HemMean5SD_b
HemSD5SD_b HemMedian5SD_b HemArea Red4SDBlue35SDOverlap Blue35SDIR3SDOverlap
Blue35SDIR4SDOverlap];
ConResult_b = [ConMean_b ConSD_b ConMed_b Conmin_b Conmax_b ConTotalArea];

HemResult_L = [HemMean_L HemSD_L HemMed_L Hemmin_L Hemmax_L HemTotalArea
threshold2SD_L HemArea2SD_L HemPercent2SD_L HemMean2SD_L HemSD2SD_L]
HemMedian2SD_L threshold4SD_L HemArea4SD_L HemPercent4SD_L HemMean4SD_L HemSD4SD_L HemMedian4SD_L HemArea Red4SDBlue35SDOverlap;
ConResult_L = [ConMean_L ConSD_L ConMed_L Conmin_L Conmax_L ConTotalArea];

xlswrite(Statsfilename,ResultsHeader_Color, 'Color Results', 'A1');
xlswrite(Statsfilename,FnHeader_Color,'Color Results','A2');
xlswrite(Statsfilename,HemResult_a, 'Color Results', 'B2');
xlswrite(Statsfilename,ConResult_a, 'Color Results', 'B3');
xlswrite(Statsfilename,HemResult_b, 'Color Results', 'B4');
xlswrite(Statsfilename,ConResult_b, 'Color Results', 'B5');
xlswrite(Statsfilename,HemResult_L, 'Color Results', 'B6');
xlswrite(Statsfilename,ConResult_L, 'Color Results', 'B7');

Hist2 = [grayLevels HempixelCount_L ConpixelCount_L HempixelCount_a ConpixelCount_a HempixelCount_b ConpixelCount_b];
xlswrite(Statsfilename,HistogramHeader_Color, 'Color Histogram', 'A1');
xlswrite(Statsfilename,Hist2, 'Color Histogram', 'A2');

% THERMAL RESULTS

ResultsHeader_IR = ['Filename', 'Mean', 'Std Dev', 'Median', 'Min', 'Max', 'Total Area', 'Threshold90', '#pixels90', 'Mean90', 'StdDev90', 'Median90', 'Min90', 'Threshold3SD', '#pixels3SD', '%pixels3SD', 'Mean3SD', 'StdDev3SD', 'Median3SD', 'Threshold4SD', '#pixels4SD', '%pixels4SD', 'Mean4SD', 'StdDev4SD', 'Median4SD'];

HemResult_IR = [HemMean_IR HemSD_IR HemMed_IR Hemmin_IR Hemmax_IR HemTotalArea_IR threshold90_HIR HemArea90_IR HemMean90_IR HemSD90_IR HemMedian90_IR HemMin90_IR threshold3SD_IR HemArea3SD_IR HemPercent3SD_IR HemMean3SD_IR HemSD3SD_IR];
HemMedian3SD_IR; threshold4SD_IR; HemArea4SD_IR; HemPercent4SD_IR; HemMean4SD_IR; 
HemSD4SD_IR; HemMedian4SD_IR;

ConResult_IR = [ConMean_IR; ConSD_IR; ConMed_IR; Conmin_IR; Conmax_IR; ConTotalArea_IR; 
threshold90_CIR; ConArea90_IR; ConMean90_IR; ConSD90_IR; ConMedian90_IR; ConMin90_IR];

xlswrite(Statsfilename,ResultsHeader_IR, 'IR Results', 'A1');  
xlswrite(Statsfilename,FnHeader_IR, 'IR Results', 'A2');  
 xlswrite(Statsfilename,HemResult_IR, 'IR Results', 'B2');  
 xlswrite(Statsfilename,ConResult_IR, 'IR Results', 'B3');

Hist2 = [bins HemHist_tst ConHist_tst];

xlswrite(Statsfilename,HistogramHeader_IR, 'IR Histogram', 'A1');

xlswrite(Statsfilename,Hist2, 'IR Histogram', 'A2');

% Data Files

DataFilename = [fnhandle 'Data.xlsx'];

xlswrite(DataFilename, Hem файла, 'Hemangioma_IR', 'A1');

xlswrite(DataFilename, Con файла, 'Control_IR', 'A1');

xlswrite(DataFilename, Hem_a, 'Hemangioma_a', 'A1');

xlswrite(DataFilename, Con_a, 'Control_a', 'A1');

xlswrite(DataFilename, Hem_b, 'Hemangioma_b', 'A1');

xlswrite(DataFilename, Con_b, 'Control_b', 'A1');

xlswrite(DataFilename, Hem_L, 'Hemangioma_L', 'A1');

xlswrite(DataFilename, Con_L, 'Control_L', 'A1');

warning off MATLAB:xlswrite:AddSheet
close all

MatFilename = [finhandle 'EntireAnalysis.mat'];
save(MatFilename);

%cd(oldPath);