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Quantitative assessment of graded burn wounds using a commercial and research grade laser speckle imaging (LSI) system

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ABSTRACT

Burn wounds are often characterized by injury depth, which then dictates wound management strategy. While most superficial burns and full thickness burns can be diagnosed through visual inspection, clinicians experience difficulty with accurate diagnosis of burns that fall between these extremes. Accurately diagnosing burn severity in a timely manner is critical for starting the appropriate treatment plan at the earliest time points to improve patient outcomes. To address this challenge, research groups have studied the use of commercial laser Doppler imaging (LDI) systems to provide objective characterization of burn-wound severity. Despite initial promising findings, LDI systems are not commonplace in part due to long acquisition times that can suffer from artifacts in moving patients. Commercial LDI systems are being phased out in favor of laser speckle imaging (LSI) systems that can provide similar information with faster acquisition speeds. To better understand the accuracy and usefulness of commercial LSI systems in burn-oriented research, we studied the performance of a commercial LSI system in three different sample systems and compared its results to a research-grade LSI system in the same environments. The first sample system involved laboratory measurements of intralipid (1%) flowing through a tissue simulating phantom, the second preclinical measurements in a controlled burn study in which wounds of graded severity were created on a Yorkshire pig, and the third clinical measurements involving a small sample of clinical patients. In addition to the commercial LSI system, a research grade LSI system that was designed and fabricated in our labs was used to quantitatively compare the performance of both systems and also to better understand the "Perfusion Unit" output of commercial systems.

Keywords: Burns, perfusion, laser speckle imaging

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1. INTRODUCTION

On an annual basis in the United States, 500,000 will require treatment for a burn injury and almost 10% of that number will be admitted into a specialized burn center[1]. The most difficult task for physicians in these burn units is to accurately categorize the burn injury. Accurate stratification is important because the treatment plan for a patient is directly related to the initial diagnosis. A burn that is fairly superficial will not require any surgical intervention. A full thickness burn where significant damage is done requires surgical intervention. It is not difficult for clinicians to categorize burn wounds in their extreme state, but it is difficult to categorize the partial thickness burns that are in between the extreme categories. The accuracy of diagnosing these partial thickness burns by clinicians is typically 60-80% when the diagnosis is done several days after the burn injury[2]. While early intervention has been shown to be beneficial for burn patients, clinical assessments are more accurate if clinicians wait to make a diagnosis[2]. New technologies that could aid clinicians in categorizing wound severity shortly after injury would provide great benefit to patients.

Recently we reviewed technologies that have been employed to assess burn severity[3]. One of the technologies that have found some acceptance in burn centers and appears to offer the best data-supported estimates of burn severity to date is Laser Doppler Imaging (LDI). LDI allows a clinician to visualize spatial maps of perfusion, a key indicator of burn depths and eventual healing times. One of the issues associated with LDI is long acquisition times because the laser is mechanically scanned over the patient for up to 5 minutes per image, resulting in a considerable risk of movement artifact and long data collection times for burns that cover a large area. An alternative technology under study is Laser speckle imaging (LSI), which is a similar noninvasive technique that also quantifies blood flow by examining the

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fluctuations in a speckle pattern caused by the movement of red blood cells. While LDI and LSI both report measurements of blood flow, LSI has the ability to capture an entire field of view at a single time point and can avoid artifacts due to movements.

To better understand the accuracy and usefulness of commercial LSI systems in burn-oriented research, we studied the performance of a commercial LSI system in three different environments and compared its results to a research-grade LSI system[4] in the same sample systems. The first sample system involved laboratory measurements of intralipid (1%) flowing through a tissue simulating phantom. The second system involved preclinical measurements in a controlled burn study in which wounds of graded severity were created on a Yorkshire pig (UCI IACUC # 2015-3154-0). Finally, we look at performance in a clinical setting involving a small sample of patients (UCI IRB #2009-7322).

2. METHODS

2.1 Commercial laser speckle imaging system

The PeriCam PSI (Perimed AB, Sweden) is an imager that utilizes laser speckle contrast analysis to provide real time analysis of tissue perfusion. It utilizes a single-exposure speckle algorithm with background correction and is calibrated with a Brownian motion standard. Speckle contrast values are calculated at each pixel using the equation below:

$$K = \frac{\sigma(I)}{\langle I \rangle} \quad (1)$$

Where <I> is the mean intensity around a square area of pixels and σ(I) is the standard deviation of the intensity around those pixels. This speckle contrast value is converted to a "perfusion unit" measurement that is recorded and displayed to the end user using the equation below.

$$P = G\left(\frac{1}{K}\right) - 1 \quad (2)$$

Where G is a gain factor reported by the software.

2.2 Research grade laser speckle imaging system

The research-grade LSI instrument, which was developed at BLI, consisted of three main hardware components: a laser source, CCD camera, and computer. The laser source was a continuous-wave laser light (λ = 808 nm, 150 mW, Ondax Inc., Monrovia, CA). The CCD camera was a thermoelectrically-cooled Retiga 2000R (QImaging, Burnaby, BC, Canada) with a pixel resolution of 1600 x 1200 (1.92 megapixels, each pixel having dimensions of 7.4 µm x 7.4 µm). The laptop (Sager P170EM, City of Industry, CA) was equipped with a GTX650 Graphics Processing Unit (GPU) (NVIDIA, Santa Clara, CA). The speckle pattern generated by the laser was captured with the CCD camera and processed using custom LabVIEW (Version 8.0, National Instruments, Austin, TX) software, integrating CUDA GPU code to perform real time processing as described previously [5]. Blood flow is quantified by a metric known as Speckle Flow Index (SFI), which we have described previously[6, 7] using the equation below:

$$SFI = \frac{1}{2T\langle K^2 \rangle} \quad (3)$$

Where T is the exposure time of the camera and K is the speckle contrast value.

2.3 Flow Phantoms

A flow phantom designed to approximate human tissue was constructed out of silicone (PDMS) using India ink as the absorbing agent and titanium dioxide as the scattering agent. An acrylic tube with dimensions 13 mm x 13 mm was embedded in the phantom to act as a flow channel. The tube was approximately 500 µm beneath the surface of the silicone. A syringe pump (NE-1000, New Era Pump Systems, Farmingdale, NY) was connected to the tubing and 1% intralipid was pumped through the setup. The pumping speed was increased from 0 – 5 mm/s at increments of 1 mm/s with a 60s wait time in between increases. This range was chosen to approximate a range of red blood cell velocities encountered in microcirculation[8].

2.4 Porcine Studies

All experiments were performed in accordance with the UC Irvine Institutional Animal Care and Use Committee (IACUC protocol #2015-3154). Three Yorkshire pigs (55 kg average weight) were used in this experiment. Animals were allowed to acclimate to their individual housing for 7 days prior to the experiment. An intramuscular injection of tiletamine-zolazepam (Telazol, 6 mg/kg) was used to sedate the animals before being intubated so that isoflurane (2%) could be used to maintain sedation. Controlled, graded burn wounds were created using a custom-made device setup described previously[9]. Briefly, custom made cylindrical brass probes (3 cm diameter) with stainless steel posts were fabricated along with a spring loaded device to hold the probes. The brass probes were heated in a warm bath incubator to 100°C and held in place by a custom made aluminum block. 16 burns were created along the dorsal side approximately 1 cm from the spine and approximately 3 cm away from each other. 8 wounds were created on each side of the spine, with contact times of 5, 10, 15, 20, 25, 30, 35, and 40 seconds. A non-adherent gauze (Telfa, Tyco Healthcare, Mansfield, MA) was placed on top of the wound and held in place with Ioban™ (3M, St. Paul, MN).

2.5 Preliminary Clinical Imaging

University of California Irvine (UCI) IRB approved protocol (HS #2009-7322) was used to enroll nine subjects into the study. Subjects were recruited from patients seen in the burn and surgical intensive care unit at UCI Medical Center by the attending physician (N.B.). Subjects who agreed to participate signed an informed consent form. Once informed consent was obtained, target thermal burn wounds of indeterminate depth were selected and imaged using the Pericam PSI system. Dressings, if present, were removed and standard wound care was provided by the health care provider. The physician remained blinded to the perfusion images to prevent any potential bias during visual assessment. Patients were checked for progress of treatments typically ten days after their admitted date.

2.6 Statistical Analysis

For flow phantom measurements a 1 cm x 5 cm region of interest was selected within the boundary of the flow channel and relative perfusion values at different speeds were calculated from baseline values for both systems. A paired t-test was performed on the two sets of data, with a 95% confidence interval. A p-value less than 0.05 was considered statistically significant for this study. Sensitivity measurements were calculated for each system using the equation below:

$$S = \frac{(P_{k+1} - P_k) + (P_k - P_{k-1})}{2 * P_k} \tag{4}$$

Where P_k is the measured perfusion at a given speed k.

For the porcine studies a 1 cm^2 ROI was selected within each burned section for both systems. The perfusion values were averaged across burns of the same severity over all animals. A paired t-test was performed on the two sets of data, with a 95% confidence interval. A p-value less than 0.05 was considered statistically significant for this study.

For the clinical measurements a 1 cm^2 ROI selection was chosen based on areas of interest pointed out by the attending physician. The perfusion values were then averaged within each ROI and compared.

3. RESULTS

3.1 Flow Phantoms

The PeriCam PSI and research-grade LSI system use separate units to express perfusion, so relative flow measurments were used to associate the two. The relative flow measurements for each system were determined by referencing the flow at each speed to the baseline measurement of 0 mm/s, when intralipid is stagnant within the flow phantom as illustrated in Fig 1. Comparing the trend of this percent change of a flow measurement from baseline indicated that both systems registered an increase in perfusion as the pump increased flow. Overall, the PeriCam PSI system expressed a higher percent change from baseline than the research-grade system, for each of the measured flow speeds. The sensitivity of each system to varying speeds was determined by averaging the percent change between each flow speed for all five measured speeds. The sensitivity for the PeriCam PSI system was 0.23, whereas the sensitivity for the research grade LSI system was 0.17.

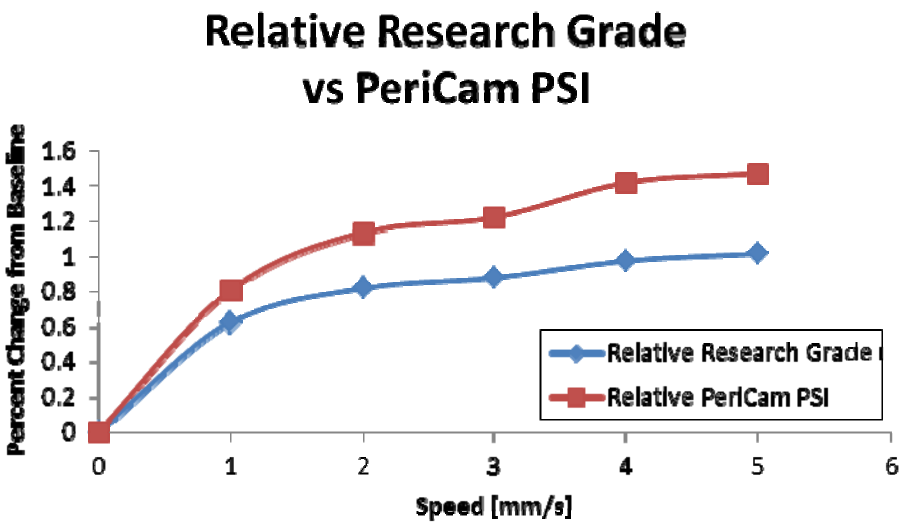

Figure 1. Graph of relative flow values for multiple speeds of intralipid through a silicon phantom. Sensitivity of individual systems were determined by dividing the change in perfusion by the baseline, averaged across all phantom flow speeds.

3.2 Porcine Studies

The day 1 color, research grade LSI, and PeriCam PSI measurements of the shortest and longest burn times are seen in Fig 2a. The research grade LSI system indicated that both the 5 second (superficial partial thickness) and 40 second (full thickness) burns experienced lower perfusion when compared to the surrounding, uninjured skin. While lower than in the surrounding tissue, the 5 second burn did display higher perfusion than the 40 second burn, suggesting that there was less damage to the underlying vasculature. Lower perfusion in the 40 second burn region was also reported by the PeriCam PSI device. However, in the PeriCam PSI system, the 5 second burn had a slight increase in perfusion.

The relative values averaged for each burn time compared to the baseline is shown for each system in Fig 2b. The PeriCam PSI system (red) indicates an increase in flow compared to baseline, for the superficial 5 second burn, but a decrease in perfusion for the longer burn times. The research grade LSI (blue) system only showed a decrease in perfusion with increasing burn time.

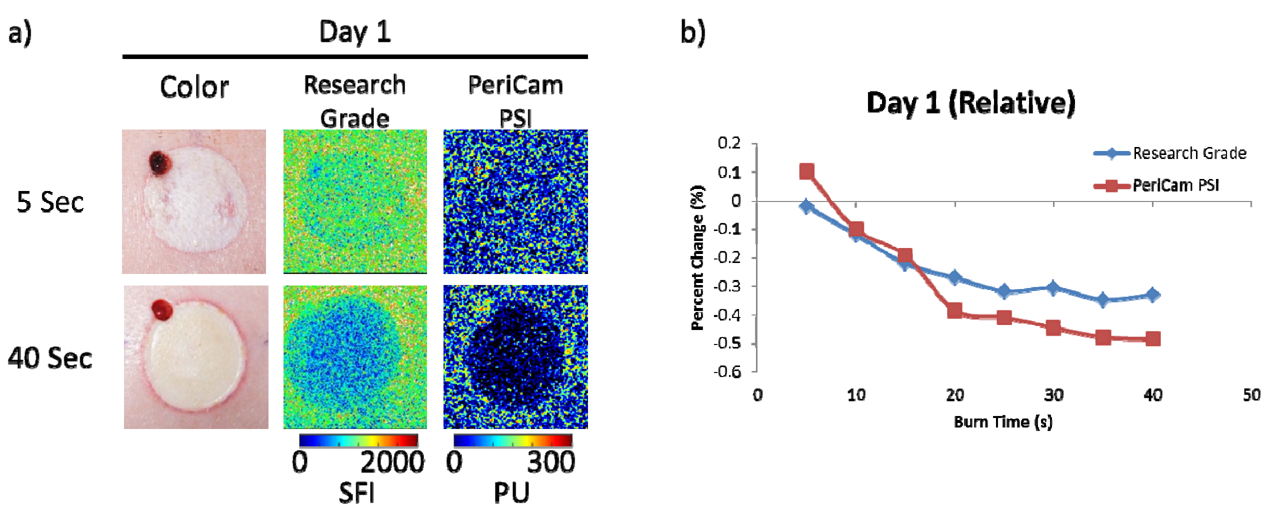

Figure 2. a) RGB color, research grade LSI, and PeriCam PSI images of a 5 second and 40 second burn wound at day 1. b) Graph of relative flow values for both the PeriCam PSI and research grade LSI systems for all burn times at day 1.

By day 4, both systems show lower perfusion in the 5 second burns compared to the surrounding tissue, as seen in Fig. 3a. This phenomenon is repeated by each system to a greater extent in the 40 second burns. Unlike the day 1 burns, there is a visible area of high perfusion surrounding the burn, and seen in each burn time through each system.

As seen in Fig 3b., the 5, 10 and 15 second burns taken with the PeriCam PSI system (red), and the 5 and 10 second burns taken with the research grade LSI system (blue) have increased perfusion relative to the baseline on day 4. All other burns continue to have decreasing perfusion with increasing burn times.

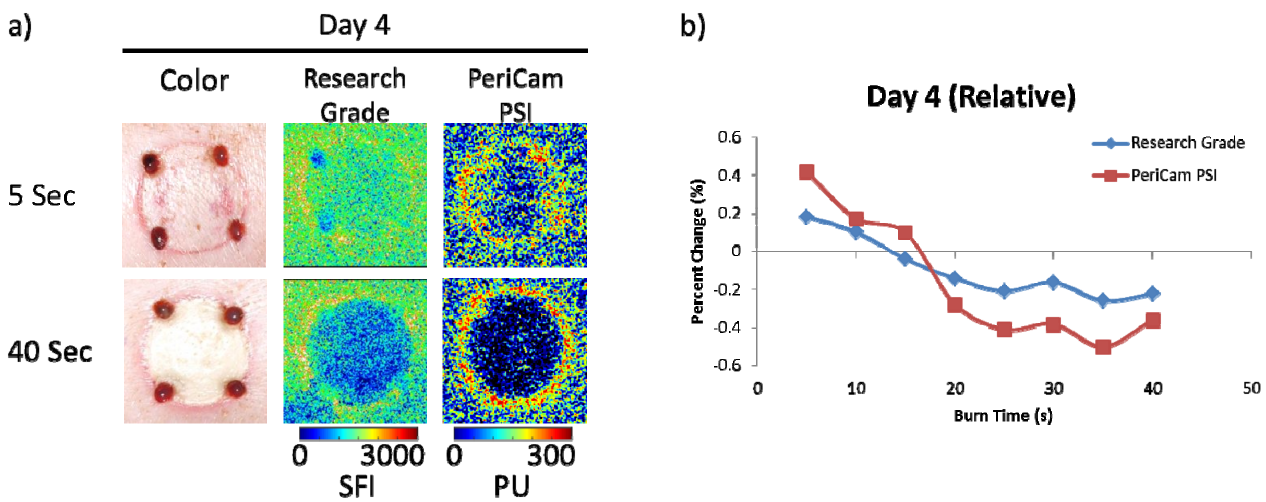

Figure 3. a) RGB color, research grade LSI, and PeriCam PSI images of a 5 second and 40 second burn wound at day 4. b) Graph of relative flow values for both the PeriCam PSI and research grade LSI systems for all burn times at day 4.

The relative values in Fig 2b. and Fig 3b. were determined, using average baseline measurements from each respective pig. A paired t-test was performed on the two sets of data, with a 95% confidence interval. A p-value less than 0.05 was considered statistically significant for this study. There were no statistically significant differences between the systems for each burn time on either day.

PeriCam PSI Relative Values								
Burn Times	5	10	15	20	25	30	35	40
Day 1	0.104493	-0.10131	-0.19076	-0.38369	-0.40927	-0.44387	-0.47709	-0.48379
Day 4	0.418729	0.174014	0.09948	-0.27731	-0.40815	-0.38059	-0.50035	-0.35877

Table 1. Relative perfusion values measured by the PeriCam PSI system for all burn times, for 1 and 4 days post-burn.

Research Grade LSI Perfusion Values								
Burn Times	5	10	15	20	25	30	35	40
Day 1	-0.01802	-0.11927	-0.21858	-0.26871	-0.31609	-0.30571	-0.34576	-0.32845
Day 4	0.181701	0.104315	-0.03969	-0.14149	-0.20611	-0.16147	-0.25794	-0.22239

Table 2. Relative perfusion values measured by the research grade LSI system for all burn times, fo 1 and 4 days post-burn.

3.3 Preliminary Clinical Imaging

Fig 4a shows RGB color images of a scald burn taken on day 2 (when the patient presented to the Burn unit) and day 10 (standard of care follow-up) post-burn. Perfusion images were collected on day 2 using the commercial PeriCam PSI system. Four regions of interest were chosen from the perfusion maps and further analyzed. The first region of interest, indicated in the figure by a dark blue box, shows lower perfusion with values similar to that of the uninjured control region (red). This area was evaluated by clinicians on day 2 and the determination was to wait and see if it would heal on its own. By day 6 it was determined it would not heal and a graft would be necessary. Another region of interest was chosen in the surrounding burn area (light blue box), showed significantly higher perfusion values than both the grafted and control regions. This region was evaluated by clinicians on day 2 and the determination was it would likely heal on its own and not require surgery. The region represented by the yellow box showed perfusion levels slightly higher than the control values, but this area was not grafted. This region was evaluated by clinicians on day 2 and the determination was it would likely heal on its own and not require surgery. The day 10 color image shows that this same region is not healing as quickly as the surrounding burn tissue.

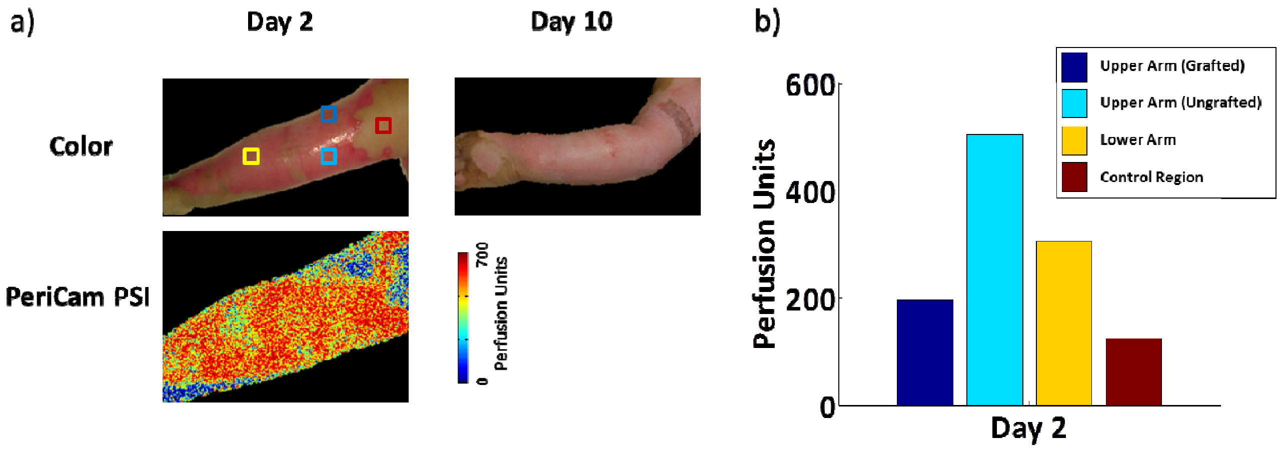

Figure 4. a) RGB color and PeriCam PSI image of a scald burn on an arm. Regions of interest are indicated on the RGB image. b) Avergae perfusion values measured by the PeriCam PSI system over the four regions of interest indicated.

4. DISCUSSION

The flow phantom data showed similar sensitivity between the research grade LSI system and the PeriCam PSI device. Despite different units for measuring perfusion from both systems, there was no significant difference in the relative measurements of the systems at any of the speeds tested in the intralipid flow phantom. Similarly, there were no significant differences between the two systems when measuring the relative changes in blood flow of our graded severity burn model. We were able to detect changes in relative blood flow with both systems that directly correlated with the duration of the burn. These changes were more apparent by day 4, but could still be seen in day 1. The success at the day 1 time point is likely due to the controlled environment that these burns were created. This flow information could be used to distinguish superficial partial thickness burns from deep partial thickness burns as we have shown previously [10]. For clinical measurements we see a practical example where assessing blood flow information could help assess which regions require skin grafts. The PeriCam PSI system highlighted two areas with reduced blood flow compared to the surrounding tissue on day 2 after the injury. Only one of these areas was ultimately chosen by the attending physician on day 6 as requiring a skin graft. By day 10 the grafted area is healing successfully, but the second area pointed out by the system is not and will require a graft. In addition to successfully predicting which areas require a graft, the PeriCam (PSI) system, was also able to do this on day 2 after the injury while the clinical assessment was not confirmed until day 6.

Here we studied the performance of a commercial PeriCam PSI system in a controlled environment using 1% intralipid flowing through a tissue simulating phantom and found it performed similarly to a research grade LSI system over a physiologically relevant range of flow rates. This is particularly interesting to us as the Choi lab develops new LSI based technologies and this provides some reassurance that these devices perform similarly to a commercial device. We also applied the device in a controlled burn study in which wounds of graded severity were created on a Yorkshire pig and again found it performed similarly to research grade LSI system. This provides us some baseline understanding of the performance of commercial LSI within a burn context. This is important because we are investigating other optical technologies such as spatial frequency domain imaging, within the context of burn wound evaluation, and there is interest on the part of clinicians as to how the predictive performance (in terms of burn severity and time to heal) of new devices compares to commercial LSI. Finally, we have demonstrated the potential benefits of a speckle imaging system within the context of clinical burns by showing the ability to identify regions that require intervention through grafting earlier than clinical assessment.

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