into tissue oxygen saturation and total hemoglobin parameters, which can provide valuable information about blood volume and oxygenation differences in viable vs. nonviable injuries. In this study, imaging and point devices could differentiate burn wounds based on the changes in the reflected light. A superficial burn shows increased oxygen saturation and total hemoglobin at the site of injury. The opposite occurs with full-thickness burns in which there was no oxygenation or blood flow.

This study represents our preliminary efforts to build devices that could be used in a clinical setting. The devices, therefore, were first tested on known clinical entities (superficial and full-thickness burns) and in patients with small total body surface area burns. Histology was not performed to corroborate the clinical and NIR diagnosis and is a limitation of this work. Biopsies within the area of interest would have added strength to the study and helped to validate the results as they would have been compared with a “gold” standard. In addition, the number of patients included in each group was small. This does not reflect on the clinical applicability of the devices but instead is a reflection of the type and number of patients referred to our burn center. As the regional burn center for the province of Ontario, we generally see larger TBSA burns and superficial burns are not referred to a regional burn center and tend to be seasonal.

Measurements using the devices took place at different time points for the two clinical groups with full thickness burn data acquired on average 5.5 days greater than superficial injuries. This could be a confounding variable in this study as a burn wound has the capacity to progress over time and therefore it is possible that some of the full-thickness injuries may have been deep partial thickness wounds at the time of injury. However, for the majority of the full-thickness burn patients, the timing of presentation to the burn center was 2 days or less postburn injury (62.5%, n=5), which is comparable with the superficial group. The capacity of a burn wound to change over time is a challenge to any technology designed to assess depth and may necessitate that multiple measures be acquired to monitor this progression. Although it is not clinically useful to assess a burn wound at 7 days, it was necessary in this study to collect data from burn wounds that were full thickness. This means that the spectra of human full-thickness burn wounds are now known and that the mathematical algorithm is developed to differentiate burn injuries at earlier time points.

The results of this study have also shown that the surface of a full-thickness burn is nonuniform. This caused some variability with respect to NIR point spectroscopy as evidenced by the large
error bars at detector position 1 and shown in Figure 2. At this position, light is primarily interrogating into the epidermis, which is destroyed in full-thickness injuries and replaced by a nonviable thick eschar. As deeper more consistent tissue was sampled, the variability was reduced and the burn injuries were discernible. Future work in this area will require modifications in the design of the probe with a longer source collector.

**Figure 4. Near Infrared Imaging Spectroscopy: Full thickness burn.** Digital photograph and gray-scale tissue hemodynamic images of a full thickness burn of the lower leg. A) Digital photograph. B) Oxygen saturation image showing a decrease in oxygen saturation as represented by black within the burn region (0%). C) Total hemoglobin images that show a decrease in total hemoglobin within the burn region as represented by dark grey (0.01mMcm⁻²). Marked areas (shown by arrows) represent registration markers and are not associated with the full thickness burn.

separation at detector position number 1. This variability may also be a reflection of the different anatomical sites of the burn wounds in our sample population. The thickness of the skin varies with anatomical location and this was not taken into account in this work.

Tissue on its own exhibits diffuse reflectance, whereby the incident light is scattered in all directions. Acquiring spectroscopic images on a highly reflective surface is confounded by specular reflectance. If the tissue is wet or shiny (skin with ointments), it acts like a mirror. This changes the angle of incidence so that light is directly reflected back to the camera and produces artifacts in the image and renders some of the pixels nonmeaningful. All optical technologies are affected by specular reflectance and clinical prototypes have to account for this loss of data secondary to this phenomenon. This is a limitation of this first prototype and necessitated that wounds be free of all creams and ointments during data collection.

In this study, there are several limitations with respect to the study design and the technology. However, we hope that the limitations do not detract from the potential scientific and medical information that this technology can provide based on a simple noninvasive reflectance of light. Our group is defining the mathematical algorithms necessary for the spectral extraction and analysis of human burn wounds. The purpose of this study is to identify these devices as a new technology and highlight the advantages that NIR spectroscopy and imaging have over other technologies trialed in the arena of burn-depth assessment.

The biggest advantage that NIR has over other technologies to date is its capacity to obtain the concentrations of physiologic parameters within the burn wounds with accuracy. In this study, we limited the concentrations to oxyand deoxy-hemoglobin in this study but other parameters such as water content can be measured with ease. Other technologies used to assess burn depth rely on changes in temperature, measures of blood flow, or a physical assessment of depth. Thermography uses an infrared camera to determine the surface burn wound temperature and has not been utilized as it is grossly affected by environmental temperatures and has not been shown to be clinically useful in predicting patient outcome. Measures of blood flow include vital dyes and laser Doppler imaging. The injection of vital dyes is invasive, impractical, and has associated risks postadministration of the dye. Laser Doppler measures blood flow through a frequency shift between stationary and moving blood cells within a sample of tissue and the results are presented as perfusion units (PU) or a flux value. Blood flow suggests that the microvasculature of the tissue is intact but does not provide information about the oxygenation of the tissue or the carrying capacity of hemoglobin. In addition, studies performed using laser Doppler are difficult to compare as they report differences in blood flow units (PU’s vs. volts) and the cutoff values to define burn depth categories vary from study to study. Other technologies utilize a physical assessment of depth to determine burn wound classification. High-frequency ultrasound detects medium changes in which a sound wave is reflected in tissue and an interface is created. The interpretation of this interface can be difficult and it is hard to discern whether the deeper signals are deep dermal plexus blood flow or in fact the dermal–fat interface. An acoustic interface in burn tissue suggests that there is a certain “cutoff” level within the skin whereby everything above a level is nonviable and below is viable. This concept is difficult to apply to burn wounds as they are dynamic with significant variability in all levels of the tissue.

Light is attenuated by melanin and it is critical for optical technologies such as NIR to control for this loss of light. In the trauma literature, where NIR spectroscopy has become popular, very few of the NIR technologies described account for melanin content in the sample population. Our devices were specifically designed to account for our multicultural population and an offset term is added to all our mathematical algorithms, which improves the intensity of the signal and the clarity of the spectra obtained. Eschar and interstitial edema have made it difficult for other technologies, such as laser Doppler and ultrasound, to assess burn wound depth accurately. NIR can penetrate deep into the tissue and can reach depths from 2 to 10cm, which is appropriate for the interrogation of skin. In addition, NIR is able to measure the water concentration and is therefore not affected by edema.

NIR is not sensitive to motion artifacts and any gross patient movements are controlled through the use of registration markers. This has been a criticism of both laser doppler and optical coherence tomography as patient movement, breathing, and any inadvertent perturbation of the sensor are serious confounding factors leading to variation in measurements from different sites in the same subject and from one site in a single individual at intervals of minutes, hours, and days. NIR imaging does require that the patient remains within the field of view of the camera but movement does not directly affect the results.

NIR spectroscopy is portable, noninvasive, and can be used for any anatomical location. Data collection is short and the devices were comfortable for the majority of patients. NIR was easily incorporated into routine dressing changes and did not interfere with nursing duties. In this study, environmental conditions such as temperature were not measured at the time.
of data collection. Environmental conditions can affect local perfusion but there have been no studies to date that have assessed the effect of temperature on NIR results in burn patients. The measurement of body systemic and room temperature should be incorporated into all future studies where blood flow and tissue hemodynamics are being assessed.

In conclusion, this study represents our preliminary work using NIR spectroscopy to determine burn depth. The transition from the bench to the bedside has enabled us to develop clinical mathematical algorithms for superficial and full-thickness injuries and to extract hemodynamic information from a simple reflectance of light. Obtaining physiologic information from an acute burn injury in a clinical setting is exciting and the possibility of this technology to assess and differentiate partial-thickness injuries is the objective of our future work.

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