RESULTS

Of the 212 patients admitted to the burn unit during the study period, 22 patients fit the inclusion and exclusion criteria. Of these 22 patients, the data from 16 patients were of sufficient quality to analyze. All patients had to have sufficient data, from both the point spectroscopy and imaging system. If one device yielded insufficient data then the results from both of the devices were excluded. The demographic profile of the study group is summarized in Table 1a. The anatomical locations of the burn wounds were sampled from a variety of areas as summarized in Table 1b.

### NIR point spectroscopy

The results from the pairwise comparisons of hemodynamic values between superficial burns and control sites at the four detector positions are reported in Tables 2 and 3. Oxygen saturation values for the superficial burns demonstrated a moderate increase in relation to the control sites for each of the detector positions. The S\textsubscript{a}O\textsubscript{2} increase ranged between 0.53\% for detector position 1 (p<0.0095) and 4.3\% for detector position 4 (p<0.0001). A substantial increase in total hemoglobin was observed at all four detector positions ranging from 45.22\% (p<0.0001) at detector 1 to 101.52\% (p<0.0001) at detector position 4.

The opposite was observed with the full-thickness burns as reported in Tables 4 and 5. The S\textsubscript{a}O\textsubscript{2} values decreased considerably in contrast to the control sites (p=0.0001) for detector positions 2-4, which ranged between 90.03 and 97.19\. Moreover, the total hemoglobin values also reported a decrease (p<0.0001) from 72.16\% (p<0.0147) to 92.62\% (p<0.0001). Statistical significance, however, was not achieved at detector position 1 for both oxygen saturation and total hemoglobin.

The difference in the responses between superficial and full-thickness burns is given in Figure 1. Analysis of the wounds, taking the control site into account (burn site-control site), indicates a significant change in S\textsubscript{a}O\textsubscript{2} between burn depths at three of the four detector positions (p<0.01) but not a significant difference at detector position 1 (p=0.187). Conversely, the total hemoglobin values showed a significant difference at all four of the detector positions (p<0.0001). The control sites utilized in superficial and full-thickness injuries were also compared with no statistically significant difference at any of the detector positions (p>0.05).

### NIR imaging spectroscopy

The imaging technology takes a NIR digital image of the burned region. Each pixel of the image is analyzed for physiologic information and is displayed as a grayscale map of the burn wound. Figure 2 displays a digital grayscale map of a burn wound showing the differences in oxygen saturation between the burned and control sites.
photograph, NIR oxygen saturation, and total hemoglobin images of a control site showing uniform tissue oxygenation and total hemoglobin values. The superficial burn in Figure 3 shows an increase in oxygen saturation and total hemoglobin in comparison with the control site values. The full-thickness burn, as depicted in Figure 4, shows the opposite with a decline in SO\textsubscript{2} and tHb in comparison with the control site. These images clearly illustrate the contrast between superficial and full-thickness injuries. The four circular regions (highlighted by arrows) in both Figures 3 and 4 are not associated with the variables of interest and instead are regions corresponding to registration markers that were placed on and around the injury.

Patient feedback
Feedback from 22 patients provided information about the comfort and length of time of the investigation. The length of time for the procedure was satisfactory in the majority of patients (86.4%); however, three patients (13.6%) felt that the procedure was lengthy. Overall, none of the patients complained of pain during the imaging procedure, but four patients (18%) experienced discomfort with the probe. It is reasonable to expect that the patients who were uncomfortable also felt that the procedure was lengthy. Two patients had burns in the sensitive nipple areola complex and one patient was assessed in an uncomfortable position. One patient was an elderly female who could only receive small increments of fentanyl for pain control secondary to her multiple medical problems. None of these four patients described their discomfort as being severe.

DISCUSSION
NIR became popular after a publication by Jobsis in 1977, which showed its potential to monitor cerebral

| Table 3. Total hemoglobin (mMcm\(^1\)) superficial burns using NIR point spectroscopy (n=58) |
|------------------|------------------|------------------|------------------|------------------|
|                  | Control          | Burn             |                  |
|                  | Mean             | SD               | Mean             | SD               | Difference       | p-value         |
| SC 1             | 0.454905         | 0.277453         | 0.660624         | 0.299832         | 0.205720         | < 0.0001        |
| SC 2             | 0.263935         | 0.173380         | 0.445510         | 0.183139         | 0.181575         | < 0.0001        |
| SC 3             | 0.212011         | 0.187387         | 0.422831         | 0.185358         | 0.210820         | < 0.0001        |
| SC 4             | 0.222432         | 0.196849         | 0.448247         | 0.208177         | 0.225815         | < 0.0001        |

T-test for dependent samples significant at p < 0.05. SC, source collector; NIR, near infrared.

| Table 4. Oxygen saturation (%) full thickness burns using NIR point spectroscopy (n=58) |
|------------------|------------------|------------------|------------------|------------------|
|                  | Control          | Burn             |                  |
|                  | Mean             | SD               | Mean             | SD               | Difference       | p-value         |
| SC 1             | 0.993755         | 0.005441         | 0.825806         | 0.336092         | 0.167949         | 0.199160        |
| SC 2             | 0.977521         | 0.005504         | 0.097422         | 0.084110         | 0.880098         | < 0.0001        |
| SC 3             | 0.950771         | 0.018562         | 0.027068         | 0.051695         | 0.923703         | < 0.0001        |
| SC 4             | 0.907747         | 0.036551         | 0.025502         | 0.035319         | 0.882245         | < 0.0001        |

T-test for dependent samples significant at p < 0.05. SC, source collectors; NIR, near infrared.
t-test for dependent samples significant at p < 0.0500. SC, source-collector position; NIR, near infrared.

Figure 1. Near Infrared Point Spectroscopy: Oxygen Saturation and Total Hemoglobin of Superficial and Full thickness burns.

Burn hemodynamics as a function of source-collector separation. The hemodynamic parameters represent mean percent differences between a burn site and matched control site for superficial and full thickness injuries. The four source-collector separations, numbered 1 to 4, correspond to probe separation distances of 1.5, 3, 4.5, and 6 mm respectively. A) Mean percent difference in tissue hemoglobin oxygen saturation (StO$_2$, %). B) Mean percent difference in total hemoglobin concentration (tHb, mMcm$^{-1}$).

Since then, NIR has been used as an investigative tool to assess cerebral and muscle perfusion in neonates and adults along with the determination of end points of resuscitation in trauma patients. Its capacity to accurately determine changes in blood volume, oxygenation, and hydration that occur deep within the tissue as a result of impaired circulation or ischemia has been well documented.

To date, very little work has been performed using NIR spectroscopy to determine burn depth. Afromowitz et al. used a multispectral camera to evaluate burn depth using an imaging burn depth indicator (IBDI). This technology imaged patients through a filter that incorporated visible and NIR light at four different wavelengths. The authors used a Kubelka–Munk model to attempt to describe the propagation of light through the tissue. The simplicity of the Kubelka–Munk model has made it a popular method for measuring the optical properties of a scattering sample by diffuse reflectance. Unfortunately, the assumptions of isotropic scattering, matched boundaries, and diffuse irradiance are not typical of the interaction of light in tissue. Also, the Kubelka–Munk model cannot predict the spatial distribution of light due to scattering, as the model assumes that scattered light occurs in only two directions. Moreover, the authors used ratios of reflected intensities to predict burn wound healing in a temporal fashion. Ratio techniques did not provide any information about the physiologic status of the tissue and are difficult to apply beyond a sample population. Eisenbeiss et al. used a similar spectral technology to assess burn depth. The data processing method used was a Fuzzy C-means cluster algorithm used to look for patterns within the spectra and then these patterns are used to classify the burn depth. This algorithm is prone to error as the initial optimization of the classification is based on clinical evaluation of the burn, which has a high level of uncertainty. Therefore, the results obtained are not related to burn depth directly but rather the degree to which objects satisfy imprecisely defined observations. In burn injury, the cluster population will be very different which could influence the clustering results. A small cluster can be very important but it is often not found because the larger clusters determine the overall clustering result. Finally, interpretation of the results from a physiology point of view may be difficult with this approach.

Our technology differs significantly from the two previous studies as the wavelengths of interest are predominantly in the NIR region and consist of more sampled wavelengths (650–1050nm). Using the Beer–Lambert relationship, the absorption and reflectance of light are used to determine the concentration of oxy-hemoglobin and deoxy-hemoglobin. These variables are then translated.
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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%, n55), 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. This separation at detector position 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 deoxyhemoglobin 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 blood flow accurately. NIR can penetrate deep into the tissue and can reach depths from 2 to 10 cm, 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 NIRS 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 NIRS 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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REFERENCES