Critical Review of Burn Depth Assessment Techniques: Part II. Review of Laser Doppler Technology

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The judgment of which wounds are expected to heal within 21 days is one of the most difficult and important tasks of the burn surgeon. The quoted accuracy of 64 to 76% by senior burn surgeons underscores the importance of an adjunct technology to help make this determination. A plethora of techniques have been developed in the last 70 years. Laser Doppler imaging (LDI) is one of the most recent and widely studied of these techniques. The technology provides an estimate of perfusion through the burn wound, the assumption being that a lower perfusion correlates with a deeper wound and, therefore, a longer time to heal. Although some reports suggest accuracy between 96 and 100% and that it does this 2 days ahead of clinical judgment, others have questioned its applicability to clinical practice. This article, the second of a two-part series, has two objectives: 1) a review of the Doppler principle and how the LDI uses it to estimate perfusion; and 2) a critical assessment of the burn literature on the LDI. Part I provides a historical perspective of the different technologies used through the last 70 years to assist in the determination of burn depth. Laser Doppler has brought technology closer to provide a reliable adjuvant to the clinical prediction of healing, yet, caution is warranted. A clear understanding of the limitations of LDI is needed to put the current research in perspective to find the right clinical application for LDI. (J Burn Care Res 2010;31:151–157)
arises why has this technology not gained more widespread use? Some have questioned the validity of the findings in relation to the clinical practice of burns, leading to the conclusion that the technology is best suited as a research tool and not yet ready for clinical practice. Part I of this two-part series provided a historical review of the other techniques used in the last 70 years, thus providing a framework for this study. The purpose of this article is to explain LD technology, to understand what it is capable of and what its limitations are, and then, to critically review the current burn literature.

Laser Doppler Technology

The Doppler effect is a change of frequency in an impinging waveform induced by a moving object. In LD, laser light at a singular frequency \( f \) strikes a moving object and it is scattered into a new direction at a different frequency \( f' \). The relationship between incident and reflected light is shown in Eq. (1), where \( v \) is the relative velocity of the moving object and \( c \) is the speed of light.

\[
f' - f = \Delta f = \frac{2v}{c - v} f \quad (1)
\]

The factor of 2 in this equation is because of the motion of the object relatively to both the source and the detector; if the moving object was also the source of the wave, then this factor would not be necessary. It is also important to note that only the component of the velocity vector directed toward or away from the detector contributes to the Doppler frequency.

Because the velocity of the laser light is much larger than the object’s velocity, \( c \gg v \), then Eq. (1) can be simplified to the following expression:

\[
\Delta f' = 2\frac{v}{c} f' \quad (2)
\]

By substituting the relationship between source wavelength \( \lambda \) and speed of light \( c = \lambda f \) in Eq. (2), we obtain the relationship between object velocity and frequency change, also known as Doppler shift, as follows:

\[
v = \frac{\lambda}{2} \Delta f' \quad (3)
\]

Finally, let us consider a practical situation where the laser beam impinges on a red blood cell at an angle \( \phi \) to the normal of the direction of the moving flow. Its scattered component is then collected at a different angle, giving us a general equation of the form:

\[
v = \frac{\lambda}{2\sin(\phi)\cos(\theta)} \Delta f' \quad (4)
\]

Equations (1)–(4) describe the Doppler principle for a single moving object where \( \theta \) is the angle between the direction of motion and the bisector of the angle determined by the incident beam direction and the collection direction. This is not the case when dealing with skin perfusion where superficial vasculature is a mixture of different sizes of capillaries, arterioles, and venules, as well as different red blood cell velocities. The result is a spread of Doppler-shifted frequencies about an average value and the generation of a homodyne signal generally used to investigate Brownian motion, as well as a heterodyne signal caused by the moving blood.

The quantity of perfusion obtained through a typical LD can be defined as the product of the magnitude of the velocity vector and the concentration of moving blood cells in the probed area. This depends on the tissue optical properties and the used wavelength. The fact that the probing depth changes for different tissues and different individuals is one of the major drawbacks in LD and contributes to the lack of absolute units for this technique. Light penetration depth, and consequently flow-probing depth, depends on the source wavelength. In the skin, short visible wavelengths have a shallower penetration than longer near-infrared wavelengths. Typical penetration values are between 1 and 2 mm. Some work is being conducted using multiple wavelengths and probes with several fibers appropriately spaced, but much work remains to be performed to standardize this technique.

An important concept when using LD to assess perfusion is the “biological zero.” This refers to the signal that remains in an area following vascular occlusion with a cuff to a suprasystolic pressure. Despite the fact that the main source of the signal has been eliminated, a spurious continuous signal remains. There are several possible sources for this persistent signal, among them: 1) collateral circulation through bone, 2) fluid and cellular movement within the interstitium, 3) movement within the intravascular compartment that is unrelated to flow (ie, red cell aggregation), 4) residual vasomotion, and 5) Brownian motion of macromolecules. Although a clear understanding of the biological zero is still lacking, and there is debate on how to best approximate it mathematically, there is evidence that it changes with edema formation in vivo and in skin hyperemic conditions. Understanding that there are many conditions that may alter the biological zero, some have suggested that it should be measured under ev-
LDI uses the Doppler principle for generating images of flow and perfusion in a dynamic biological media. Different from the more common fiber-based systems (i.e., LD flowmetry as described by Stern), LDI systems do not need contact with the skin. This is a great advantage when dealing with lesions such as those seen after thermal injury where pain and risk on infection from probe contact are important considerations.

Two types of LDI are commercially available. One type of LDI uses a scanning laser point source across a region of interest and a silicon detector to capture the Doppler shift at different points in the skin. This type of system is able to generate images of an area up to 500 mm × 500 mm in few minutes (reported values are up to 6 minutes). A typical instrumentation layout for a LDI system is shown in the Figure 1.

This system consists of a laser source (Helium Neon lasers or laser diodes at 633 and 780 nm are typical), a detection assembly (silicon photodiodes and lenses) connected to a Doppler signal processing system, and a scanning mirror. The mirror is connected to a galvanometer-based scanner (or other motorized scanning system), and its function is to redirect the source laser light into a desired pattern and area (Figure 1). Another function of the mirror is to redirect the light backscattered from the skin toward the lenses and finally to the photodiodes. The mirror’s motion is computer controlled.

This technique is similar to its fiber-based counterpart in the sense that the Doppler shift is observed in one point at a time. Several of these points are assembled to create an image. The drawback is that when the laser is scanning, any movement of the object will result in image artifacts. A technique based on snapshot imaging is hence preferable.

The second type of LDI system uses the fluctuation of speckle patterns on the skin as a contrast mechanism for blood perfusion. This type of imaging is often called speckle contrast imaging. Speckle contrast imaging produces images of superficial vasculature average blood flow.

A typical speckle-based LDI system layout is shown in Figure 2. It consists of a laser source and a computer-controlled camera. When laser light hits the skin, a speckle pattern is formed at the detector because of the coherence of the source and the microstructure of the skin. The speckle moves because of blood and tissue movements. The contrast is determined by the standard variation of the intensity fluctuation to the mean intensity. Any type of movement will cause averaging of the speckle pattern, a characteristic that is both useful when determining perfusion and problematic because dealing with live subjects. Important parameters in speckle-contrast LDI are the exposure time, which needs to be in the order of the correlation time of the speckle, and the pixels/speckle ratio. Too few pixels per speckle will result in reduced accuracy, whereas too many pixels per speckle will cause low spatial resolution. A number between five and seven pixels per speckle has shown to produce reliable results.

Burn Literature

In his original description of the LD technology, Stern cautioned about some of its limitations. He stated that the measurements were very labile and being affected by the room’s temperature, the patient’s position, respiratory rate, and even emotional stimulation. Some of these findings have been echoed by others more recently.

By using LD flowmetry, Waxman et al studied the effect of wound temperature on the readings obtained. He scanned unhealed burn wounds and found that at a lower temperature (35°C), specificity was

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**Figure 1.** Laser Doppler scanning imager.

**Figure 2.** Layout of a speckle-based laser Doppler imaging system.
very high (100%) but there was a poor sensitivity; however, at a temperature of 44°C sensitivity improved to 93%, but specificity decreased. Barachini et al assessed perfusion in healed burn wounds (wounds had healed either with skin grafts or on their own) and found that they behaved differently than open wounds. He found no difference in the perfusion units (PU) when changing the skin temperature between 36°C and 41°C. Both of these studies were performed with LD flowmetry. Different researchers working with LD scanning have maintained the room temperature between 22°C and 25°C, but the actual wound temperature is not detailed in these studies. A study assessing the effect of wound temperature on PU using LD scanning is lacking.

One of the advantages that LD scanning has over the older LD flowmetry is the fact that the wound can be assessed at a distance. This is more comfortable for the patient who suffers less pain, allows for the study of a larger area, and, in theory, should help limit wound infection rate as there is no contact with the tissue. This brings several questions. First, which is the ideal distance from the wound? In their original description, Niazi et al scanned from a distance of 1.6 meters. Other researchers have used 20 cm, 50 cm, variable distances of 30 to 40 cm, or 40 to 70 cm, whereas, in other reports, the distance was not documented. Often, as is the case with research, the results of one study were used as the basis for the next study. If there is no consistent distance from which to scan, the question that arises is what is the effect of changing this distance?

In an initial study assessing the effect of increasing the distance between target and scanner, it was found that this increased the PU. A follow-up report, using a different brand of LD, however, found the opposite, increasing the distance decreased the PU. The difference could be attributed to different equipment using different ways of “normalizing” the perfusion signal; however, this raises concern as it makes results from one study difficult to reproduce, let alone apply to clinical practice.

Another question that comes up when scanning from a distance is what is the effect of changing the scanning angle, or more practical, what is the effect of scanning over a curvilinear surface. Two studies have looked at this and found a decrease of up to 50% in perfusion when scanning over curvilinear surfaces, attributed simply to the curvature and not a true decrease in perfusion.

There are wound-specific factors that have been found to play a role in the readings obtained, for example, the “wetness” of the wound decreases PU between 13 and 33%. In an attempt to limit the time that a wound is uncovered and open to air, some researchers have scanned through a transparent dressing. Holland et al assessed the effect of the burn wound dressing on the LD scanning readings, particularly if different clear, or transparent, dressings would have an effect when scanning through them, rather than having to remove them. For their study, they used a healthy volunteer whose arm was subjected to pressure for 2 minutes. They found that different types of dressing and different ways of applying them affected the output measurement.

The questions about the effect of temperature, distance, angle, wound dressing, and dryness/wetness in the wound are all methodological questions that can, and should, be standardized to improve the power and applicability of the work being conducted. A more basic question is what information does the LD scanner telling us? The answer, at its root, is simple; it tells us how much movement there is in the skin (the product of average blood cell speed and concentration of moving blood cells). This has been equated with perfusion under the assumption that in the absence of movement because of respiration or shivering, the only moving particles in the area in question are blood cells, although at first glance, and under certain conditions, this statement might be true, this is often not the case with burn wounds.

A burn wound is a very dynamic environment, particularly in the first 24 to 48 hours. There is initially an inflammatory reaction, which is characterized, among other things, by a hyperemic response and by the formation of edema. Edema is formed by fluid particles moving out of the capillaries and into the interstitium. Similarly, at some point, this edema fluid will be reabsorbed into the capillaries and lymphatics, representing more movement of particles. Although the set “biological zero” can accommodate for some of this fluid-shift phenomenon, the effect in the recorded PU is in question. Furthermore, the biological zero is not a fixed number; it is a dynamic concept, which changes given different clinical scenarios. The fact that there is edema formation and interstitial cell movement following a burn injury suggests that the biological zero should be assessed and subtracted with every flow measurement, such as recommended by Kernick et al.

In a series of patients scanned every 4 hours during the first 48 hours of resuscitation, Jaskille and coworkers noted that the measured PU (in non-burned skin) did not follow a linear pattern, rather there were peaks and valleys, which corresponded clinically to changes in base deficit and wound pH. The significance of this is that particles other than red blood cells within the capillaries have a measurable
effect on the measured PU. The implication, thus, is that the timing at which the readings are obtained is very important and could have an effect on how reliably they could predict healing. Mileski et al.\textsuperscript{37} and Riordan et al.\textsuperscript{38} in independent studies, found that accuracy of prediction increases with serial or sequential scans over a period of time, rather than relying on a single scan.

Finally, another very important aspect of the laser Doppler imager is the output measurement of PU. Several researchers have given guidelines as to what wounds are expected to heal based on the PU obtained from the scan. These numbers vary widely. Early work using 4-point LD flowmetry suggested a scale between 0 and 1000 and stated that a cutoff point of 80 PU marks the determination of going to heal vs not going to heal,\textsuperscript{37} whereas others have established this cutoff at 250 PU.\textsuperscript{12} Some researchers have focused the attention on the depth of the wound, a different outcome altogether, and stated that 150 PU signifies a full-thickness wound, using this as the cutoff for the determination that these wounds are not expected to heal,\textsuperscript{14,30} while other researchers did not use PU as the output measurement, but rather volts.\textsuperscript{10} Finally, there are some that have used a scale between 0 and 256 and have not mentioned what numerical value was used to mark the determination of healing vs not healing, referring simply to high-flow and low-flow areas.\textsuperscript{9,11} The fact that different output measurements and scales are used makes comparisons between studies virtually impossible.

One important point that arises from the studies that used the same output measurement and the same scale is the fact that they provide different numbers for those wounds expected and not expected to heal. Not mentioned in any of those studies is the fact that different areas in the body have a different perfusion and what is the potential effect of this baseline differential perfusion on the PU obtained after a burn. In a study of normal volunteers, Allely et al\textsuperscript{39} found that the PU in different areas in the face vary widely from a mean of 280 PU in the forehead to a mean of 525 in the nose. What effect, if any, this difference can play in a burn patient is unknown. Furthermore, a word of caution about using measurements on normal volunteers and trying to predict what could happen in burn wounds is derived from the studies of Waxman et al\textsuperscript{28}

### Table 1. Summary of advantages and disadvantages of burn wound assessment tools

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactive isotopes</td>
<td>Radioactive phosphorus (\textsuperscript{32}P) taken up by the skin</td>
<td>Invasive, too cumbersome, poorly reproducible</td>
</tr>
<tr>
<td>Nonfluorescent dyes</td>
<td>Differentiate necrotic from living tissue on the surface</td>
<td>No determination of depth of necrosis; many dyes not approved for clinical use</td>
</tr>
<tr>
<td>Fluorescent dyes</td>
<td>Approved for clinical use</td>
<td>Invasive; marks necrosis at a fixed distance in millimeters, not accounting for thickness of the skin; large variability</td>
</tr>
<tr>
<td>Thermography</td>
<td>Noninvasive, fast assessment</td>
<td>Many false positives and false negatives based on evaporative cooling and presence of blisters; each center needs to validate its own values</td>
</tr>
<tr>
<td>Photometry</td>
<td>Portable, noninvasive, fast assessment, validated against senior burn surgeons, and color palette was developed</td>
<td>Single institution experience; expensive?</td>
</tr>
<tr>
<td>Liquid crystal film</td>
<td>Inexpensive</td>
<td>Contact with tissue required, unreliable readings</td>
</tr>
<tr>
<td>Nuclear magnetic resonance</td>
<td>Water content in tissue differentiates partial- from full-thickness wounds</td>
<td>In vitro assessment only, expensive, time consuming</td>
</tr>
<tr>
<td>Nuclear imaging</td>
<td>\textsuperscript{99m}Tc shows areas of deeper injury</td>
<td>Expensive, very time consuming, not readily available, and invasive</td>
</tr>
<tr>
<td>Pulse-echo ultrasound</td>
<td>Noninvasive, easily available</td>
<td>Underestimates depth of injury, operator dependent, and requires contact with tissue</td>
</tr>
<tr>
<td>Doppler ultrasound</td>
<td>Noncontact technology available, provides morphologic and flow information</td>
<td>Operator dependent, not as reliable as laser Doppler</td>
</tr>
<tr>
<td>Laser Doppler imaging</td>
<td>Noninvasive and noncontact technology, fast assessment, large body of experience in multiple centers, and very accurate prediction in small wounds in stable patients</td>
<td>Readings affected by temperature, distance from wound, wound humidity, angle of recordings, extent of tissue edema, and presence of shock; different versions of the technology available make extrapolation of results difficult</td>
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and Barachini et al\textsuperscript{29} on the effects of temperature. In their studies, open wounds and healed wounds had different reactions to changes in temperature, so, an extrapolation cannot be reliably performed.

**DISCUSSION**

The prediction of which burn wounds are likely to heal is one of the most important and difficult tasks facing the burn surgeon. A variety of techniques have evolved over the past 70 years to aid in this determination, ranging from the injection of radioactive isotopes and vital dyes to the use of thermography, photometry, nuclear imaging, and ultrasound.\textsuperscript{7} One of the more recent techniques, and perhaps the most studied, is LDI. Combining laser technology with the Doppler principle, it aims to provide an estimation of the burn wound perfusion, using this as a predictor of healing.

Many authors have suggested that the technique has a very high positive- and negative-predictive value\textsuperscript{10,11} and that it can provide an accurate estimate of healing up to 48 hours earlier than clinical judgment.\textsuperscript{14} Most of the studies to date, however, have looked at a specific patient population, and many have had a very limited number of subjects (less than 20 subjects in most studies), making broad conclusions difficult. Similarly, some of the limitations of the technique have not been directly discussed. Another difficulty in trying to draw conclusions from the current literature is that most clinicians have used a different methodology, such as using different makes of the machine, different scanning distances, and scanning different body areas, which makes extrapolation between studies virtually impossible. The first step in addressing some of these problems is to understand the technology. Only after a thorough understanding of the science behind the technology, and its limitations, can a more systematic study be undertaken.

Different tissues in the body have a different thickness; similarly, the skin thickness will vary across individuals. These factors are not accounted for by the currently available LD scanners, and this contributes to the lack of uniform, absolute units. Similarly, several other factors affect the measurement, such as the subject’s movement, the wound temperature and moisture, the incident angle of the laser, the presence or absence of shock, the extent of tissue edema, and whether the scan is being performed directly on the tissue or through a transparent dressing. This lack of uniformity makes providing guidelines difficult.

Some of these drawbacks notwithstanding, LD scanning has circumvented some of the pitfalls of previous techniques\textsuperscript{7} (Table 1). It is not as cumbersome, more reliable than the injection of radioactive isotopes, provides a more objective measurement, is less invasive than the injection of vital dyes, and is less operator-dependant than pulse-echo and Doppler ultrasound. No comparative study has been performed between the different adjuvant techniques, and a comparative study between LD scanning and some of the newer thermography techniques, such as active dynamic thermography\textsuperscript{40} or infrared thermography\textsuperscript{41} would provide an interesting discussion. Although more uniform and systematic research is needed, LD has brought technology closer to provide a reliable adjuvant to the clinical prediction of healing. The final answer probably lies in a tool, which combines different techniques.

**REFERENCES**

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