Critical Review of Noninvasive Optical Technologies for Wound Imaging

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Significance: Noninvasive imaging approaches can provide greater information about a wound than visual inspection during the wound healing and treatment process. This review article focuses on various optical imaging techniques developed to image different wound types (more specifically ulcers).

Recent Advances: The noninvasive optical imaging approaches in this review include hyperspectral imaging, multispectral imaging, near-infrared spectroscopy (NIRS), diffuse reflectance spectroscopy, optical coherence tomography, laser Doppler imaging, laser speckle imaging, spatial frequency domain imaging, and fluorescence imaging. The various wounds imaged using these techniques include open wounds, chronic wounds, diabetic foot ulcers, decubitus ulcers, venous leg ulcers, and burns. Preliminary work in the development and implementation of a near-infrared optical scanner for wound imaging as a noncontact hand-held device is briefly described. The technology is based on NIRS and has demonstrated its potential to differentiate a healing from non-healing wound region.

Critical Issues: While most of the optical imaging techniques can penetrate few hundred microns to a 1–2 mm from the wound surface, NIRS has the potential to penetrate deeper, demonstrating the potential to image internal wounds.

Future Directions: All the technologies are currently at various stages of translational efforts to the clinic, with NIRS holding a greater promise for physiological assessment of the wounds internal, beyond the gold-standard visual assessment.

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SCOPE AND SIGNIFICANCE

NONINVASIVE IMAGING APPROACHES can provide greater information about a wound than visual inspection during the wound healing and treatment process. This review article focuses on various noninvasive optical imaging techniques developed to image different wound types (more specifically ulcers). The work performed by different research groups in applying the optical imaging approaches to understand the physiology and structural information of ulcers (including pressure ulcers, venous skin ulcers, arte-

rial leg ulcers, and diabetic foot ulcers [DFUs]) is described.

TRANSLATIONAL RELEVANCE

The noninvasive optical imaging approaches developed by researchers for wound imaging are making efforts to translate the technology to the clinic. Imaging will assist the clinicians by providing them an objective quantitative assessment of the wounds from the different optical measurements. This will complement the gold-standard visual assessment

of wounds that is employed by clinicians worldwide during the wound healing and treatment process. There is an effort to make these imaging systems low cost, portable, and robust such that they can be made available at the clinician's office instead of an imaging or diagnostic center.

CLINICAL RELEVANCE

According to the Wound Healing Society, the accepted standard of improvement is 4 weeks, by which a reduction of the wound size by 40% is expected from the treatment. If the wound does not heal beyond 4 weeks, reevaluation of wound and other treatment options are considered.¹⁻³ The gold-standard clinical wound monitoring approach during its treatment process is by visual inspection of changes in the wound site, along with physical measurements of the wound size and depth. The underlying changes in the blood oxygenation or wound microstructure can differ with the wound's healing or nonhealing nature. These changes can be quantitatively recorded when applying optical imaging approaches to monitor wounds.

OVERVIEW

Current clinical approaches to assess and treat any type of wound are conducive to normal healing, although with its own set of limitations. Histology currently remains the standard of evaluation for wound healing. However, an alternative modality to the conventional methods may be optical imaging techniques. Herein, the various optical imaging techniques applied for wound healing assessment studies are introduced along with the description of advances in research in wound imaging using these techniques.

A major function of the skin is to act as a protective barrier against various pathogens and infections. In addition, the skin serves as a means of acquiring information about the environmental conditions. Loss of the integrity of large portions of the skin due to injury, illness, or infection may lead to major disease, disability, or even death.⁴ A wound is a disturbance of the skin that can be classified into acute and chronic wounds. Acute wounds heal in a timely manner, while a chronic wound does not heal in a timely manner, that is, within the typical stages of wound healing.⁴ The typical stages of wound healing include inflammation, proliferation, and maturation. Wounds can be further categorized as burns, which are caused by heat, chemicals, electricity or radiation damage to the skin, and ulcers.⁵ This review will concentrate on ulcers, which are usually a sore on the skin

accompanied by disintegration of tissue. Ulcers are subcategorized into pressure ulcers (decubitus ulcer), venous skin ulcers, arterial leg ulcers, and DFUs.^{4,6} Pressure ulcers occur in regions of unrelieved pressure over a defined area, usually over a bony prominence, resulting in ischemia, cell death, and tissue necrosis. Venous skin ulcer is a shallow wound that occurs when the leg veins do not return blood back toward the heart. Arterial ulcers are due to a reduced arterial blood supply to the lower limb. Last, DFUs affect patients with diabetes, where the foot is affected by ulceration that is associated with neuropathy and/or peripheral arterial disease of the lower limb.^{6,7}

Standard clinical methods include modern wound-healing adjuncts, such as bioengineered tissue, topical growth factors, and negative pressure wound therapy.^{4-6,8} Other conventional methods include biopsy and chamber-embedding, which are used to extract the wound tissue to perform histological analysis as well as measurement of the tensile strength or electrical impedance of the wound. Moreover, the evaluation of wounds can also be based on observations such as wound size, color, odor, and level of pain.^{3,6,8,9} These methods have their limitations in terms of effectiveness due to invasiveness and disruption because they introduce a new wound that interferes with the assessment of the wound healing process. This prevents the precise assessment of wound to heal over time and the evaluation of recurrent wounds where patients have high risk of infection.^{4,8} Hence, new optical imaging techniques can be used by the clinician to assess underlying processes within the tissue noninvasively, while at the same time, overcoming the subjectivity to determine and evaluate if a particular wound is healing and appropriately provide the required treatment.^{5,8,9}

DISCUSSION

Optical imaging techniques

The various noninvasive optical imaging techniques applied for wound imaging applications include hyperspectral imaging (HSI), multispectral imaging (MSI), near-infrared spectroscopy (NIRS), diffuse reflectance spectroscopy (DRS), optical coherence tomography (OCT), laser Doppler imaging (LDI), laser speckle imaging (LSI), spatial frequency domain imaging (SFDI), fluorescence imaging, and digital camera imaging. A summary of these imaging techniques, their applications in the area of wound imaging (in particular to ulcers), and the details of the typical light sources and detectors employed, along with populations it was assessed on are given in Table 1.

Table 1. Summary of literature of the optical imaging techniques developed for imaging different wound types

Modality	Applications	Typical Sources	Typical Detectors	Measured Parameters	Imaging Populations
HSI	Open wound (Calin <i>et al.</i> ¹⁰), Burns (Calin <i>et al.</i> ¹¹), DFU (Khoadhiar <i>et al.</i> ¹² , Yudovsky <i>et al.</i> ^{13,14} , Yudovsky <i>et al.</i> 2010, ⁷ Nouvong <i>et al.</i> ¹⁵), Venous leg ulcers (Denstedt <i>et al.</i> ¹⁶)	Halogen lamps (400–1,000 nm), LEDs (500–700 nm)	CCD camera, hyperspectral camera.	HbO, HbR, ISO ₂ , melanin concentrations, burn depth, epidermal thickness	One animal study on dog (hind leg), ¹⁰ and rest human subjects with wounds. ^{11–16}
MSI	Miscellaneous wounds/ulcers (Basiri <i>et al.</i> ¹⁷)	Halogen lamp (460–886 nm)	Digital CCD camera	ISO ₂	Human subjects with different wounds ¹⁷
NIRS	Decubitus ulcers (Moza <i>et al.</i> ²⁶), DFU (Weingarten <i>et al.</i> 2012, ¹⁸ Weingarten <i>et al.</i> 2010, ³ Weingarten <i>et al.</i> 2005, ¹⁹ Papazoglou <i>et al.</i> ^{20,21}), Chronic wounds (Papazoglou <i>et al.</i> ^{22–24})	LEDs (650–875 nm), Laser Diodes (685, 690, 780, 795, 830, 950 nm)	CMOS sensor, avalanche photodiodes	Diffuse reflected intensity, HbO, HbR, Water, ISO ₂	Four studies on diabetic hairless rats ^{19,22–24} and the rest were human subjects with DFU. ^{3,26,18–21}
DRS	DFU (Anand <i>et al.</i> ²⁵)	Tungsten halogen source (400 and 800 nm)	CCD Spectrometer	HbO, HbR, HbT, ISO ₂	Human subjects with DFU ²⁵
OCT and PS-OCT	Chronic wounds (Cobb <i>et al.</i> ²⁷ Singer <i>et al.</i> ²⁸ Oh <i>et al.</i> ²⁹), Burns (Pierce <i>et al.</i> ³⁰ Srinivas <i>et al.</i> ³¹ Park <i>et al.</i> ³²)	7- to 8-fs Ti:sapphire laser, superluminescent diode, semiconductor optical amplifier, low-coherence source	Photodetectors	Wound size, epidermal migration, collagen birefringence, collagen denaturation, burn depth,	Diabetic mice, ²⁷ pigs, ²⁸ white rabbits, ²⁹ Sprague-Dawley rats, ^{31,32} skin sites. ³⁰
LDI	Burns (Hoeksema <i>et al.</i> ³³ Holland <i>et al.</i> ³⁴ Droog <i>et al.</i> ³⁵ Kloppenberg <i>et al.</i> ³⁶ Pape <i>et al.</i> ³⁷)	Helium-neon laser (630–635 nm)	Photodetector	Burn depth	Human subjects with burns ^{33–37}
LSI	Burns (Ponticono <i>et al.</i> ³⁸ Stewart <i>et al.</i> ³⁹)	Helium neon laser (633 nm)	CCD camera	Burn scar perfusion	Porcine model ³⁸ and human subject ³⁹
SFDI	Burns (Ramella-Roman <i>et al.</i> ⁴⁰ Burnmeister <i>et al.</i> ⁴¹ Mazhar <i>et al.</i> ⁴² Ponticono <i>et al.</i> ³⁸ Lin <i>et al.</i> ⁴³ Nguyen <i>et al.</i> ^{44,45} Yafi <i>et al.</i> ⁴⁶)	LEDs (650–850 nm); NIR (650–950 nm); tungsten lamp	CCD camera or NIR camera	HbO, HbR, HbT, ISO ₂ , absorption coefficient	Animal models (rats, ^{40,44–46} swine ^{38,41,42} and mouse ⁴³)
Fluorescence	DFU (DaCosta <i>et al.</i> ⁴⁷ Wu <i>et al.</i> ⁴⁸)	White light and LED (blue-violet light)	CMOS	Autofluorescence signals	Human subjects ^{47,48}
Visual	Chronic wounds (Papazoglou <i>et al.</i> ⁴⁹), DFU (Bowling <i>et al.</i> ^{50,51})	Flashlight or no source	Digital camera	Wound area and wound size	Diabetic rats ⁴⁹ and diabetic human subjects with foot ulcers ^{49–51}

CCD, charge-coupled device; CMOS, complementary metal oxide semiconductor; DFU, diabetic foot ulcer; DRS, diffuse reflectance spectroscopy; HbT, total hemoglobin; HbS, hyperspectral imaging; LDI, laser Doppler imaging; LSI, laser speckle imaging; MSI, multispectral imaging; NIRS, near-infrared spectroscopy; OCT, optical coherence tomography; PS-OCT, polarization sensitive-optical coherence tomography; SFDI, spatial frequency domain imaging.

Figure 1. Schematic of hyperspectral imaging setup. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Hyperspectral imaging

HSI is an effective noncontact optical imaging technique that has the ability to capture an image and create a three-dimensional data cube. These data are in turn used to obtain a two-dimensional (2D) map of cutaneous oxygenation.^{7,8–15} HSI usually consists of a broadband light source within visible and near-infrared (NIR) wavelengths that is used to illuminate the skin and a camera with a tunable optical filter to capture images.^{8,9} The tunable filter scans a series of wavelengths, while a stack of images are gathered by the camera. Appropriate software is used to ensure that the images are spatially aligned, and a charge-coupled device (CCD) camera can analyze the intensity values registered at each pixel and for each wavelength.^{7,10–16} HSI deals with imaging narrow spectral bands over a continuous spectral range and producing the spectra of all pixels in the scene.^{8,9} A schematic of a typical HSI system is shown in Fig. 1.

Various research groups have employed HSI in imaging for wounds, including open wounds, burns, DFUs, and venous leg ulcers.^{7,10–16} Typically halogen lamps or LEDs are used as light sources and CCD or hyperspectral camera are used as detectors for no-contact HSI of wounds. Tissue oxygenation in terms of oxyhemoglobin (HbO), deoxyhemoglobin (HbR), and saturated oxygenation (ISO₂) are evaluated using HSI. In some cases, the concentration of melanin, burn depth (in burn-related wounds), and epidermal thickness are evaluated. The tissue oxygenation and microvascular abnormalities determined from HSI have shown to predict DFUs, monitor neuropathy, or map skin perfusions to date.

Multispectral imaging

MSI is an optical imaging technique that deals with images at discrete narrow bands. MSI typically have many bands covering the spectrum from the visible to infrared. These images do not produce

the full spectrum of an object.¹⁷ The minor difference between HSI and MSI is that MSI captures image data at specific frequencies across the electromagnetic spectrum.^{8,17} The wavelengths may be separated by filters or by the use of instruments that are sensitive to particular wavelengths, including light from frequencies beyond the visible light range, such as infrared.¹⁷ Only one research group has employed MSI on 22 different wounds/ulcers to quantify skin ISO₂.¹⁷ This model has the advantage of being less prone to artifacts, which is typical of more complex models that rely on the curves of absorption and scattering of the skin's most common chromophores (solar ultraviolet radiation absorbing endogenous chromophores), such as amino acids and melanin.¹⁷ MSI, like HSI, employs halogen lamps and CCD cameras during noncontact imaging of wounds.

Near-infrared spectroscopy

The basis of NIRS relies on two principles: (a) that tissue is relatively transparent to near-infrared light, and (b) there are compounds in tissue in which absorption of light is dependent on the oxygenation status of the tissue.^{3,5,9,18–24} The propagation of light in tissue depends on the combination of absorption, scattering, and reflection properties of photons. Absorption and scatter in tissue is dependent on the wavelength. Scatter decreases with increasing wavelengths; thereby favoring the transmission of NIR light (650–1,100 nm) compared to visible light.^{5,8,9} HbR has a maximum absorption of 760 nm, whereas absorption of HbO has a maximum at 900 nm. NIRS is able to differentiate between oxygenated and deoxygenated blood, which confers a distinct theoretical advantage to this modality.^{3,5,9,18–24} A general schematic of an NIRS system is shown in Fig. 2.

Two research groups have employed NIRS for imaging of decubitus ulcers,²⁶ DFUs,^{3,18–21} and chronic wounds.^{22–24} These multiwavelength NIRS

Figure 2. Schematic of near-infrared spectroscopy setup. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

systems typically employ LEDs or laser diodes between 650 and 900 nm to illuminate the tissues, and a complementary metal oxide semiconductor (CMOS) or avalanche photodiodes are used to detect the diffuse reflected signals. The measured parameters using NIRS are diffuse reflectance intensity, HbO, HbR, water concentration, and/or ISO₂ (saturated oxygen). Both animal studies on diabetic hairless rats and human subjects have been carried out using NIRS. NIRS has shown to provide information about blood perfusion (based on oxygenation),²⁶ monitor chronic wound in rats,^{22–24} and monitor wound healing by differentiating healing from nonhealing DFUs in human subjects.^{3,18}

Diffuse reflectance spectroscopy

DRS is an optical imaging technique that relies on the focused projection of the spectrometer beam

(of wavelengths varying across visible, infrared, etc.) into the sample where it is reflected, scattered, and then transmitted through the sample material.^{9,25} The scattered light that is back-reflected is then collected by the accessory and directed to the detector optics. The beam that is scattered within a sample and then returned to the surface is known as diffuse reflection.⁹ NIRS also acquires diffuse reflected signals from the tissue, but at a narrow wavelength band in the NIR region. There has been only one study using DRS for imaging of DFUs.²⁵ The DRS device employed a broadband, tungsten halogen light source between 400 and 800 nm and a CCD spectrometer detector. Tissue ISO₂, HbO, HbR, and total hemoglobin (HbT) were evaluated to demonstrate that DRS is capable of monitoring changes in oxygen dynamics of DFUs.

Optical coherence tomography

OCT uses low-coherence NIR light to produce a 2D image of optical scattering from tissues, effectively creating an “optical ultrasound,” but at a much higher resolution.^{8,9,27–32} The echo time delay of reflected light is measured using low-coherence interferometry, and the reflections from the target are correlated with light that travels a reference path.^{8,9} OCT has shown results in serial imaging of induced thermal injury and healing effects and provides structural information in larger tissue regions.^{8,9} A general schematic of an OCT system for tissue imaging is shown in Fig. 3. OCT images tissue with its larger field of view allowing for data on the structural level, as opposed to the cellular level. OCT imaging is completely noninvasive and requires no contact to be made between

Figure 3. Schematic of optical coherence tomography setup. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

the skin and the optical system, although some OCT systems do require light contact to be made between the skin and a window at the end of the probe.^{27–32}

Various research groups have employed OCT to image chronic wounds^{27–29} and burns.^{30–32} These OCT systems employ Ti:Sapphire lasers, superluminescent diodes, or other low-coherent sources, and photodetectors for detection. Most studies related to wounds have been on animals (*e.g.*, pigs, whit rabbits, diabetic mice, and Sprague-Dawley rats),^{27–29,31,32} apart from some human subject studies.³⁰ OCT provides structural information of the tissues by measuring wound size, collagen birefringence, collagen denaturation,³⁰ and epidermal migration and/or burn depth.^{31,32} Studies have demonstrated that OCT is a viable tool for non-invasively monitoring the structural hallmarks of wound healing *in vivo*²⁷ to detect the presence or absence of the epidermal layer of skin, allowing noninvasive tracking of wound reepithelization.²⁸ In the case of burn imaging, OCT provides physicians with a high resolution “optical biopsy” of the burn and also assists to distinguish burns of varying severity from its high resolution.

Laser Doppler imaging

LDI is based on the Doppler principle, which states that light particles which hit moving blood cells undergo a change in wavelength/frequency (a Doppler shift), while light particles which encounter static structures return unchanged. In this study, laser light penetrates the tissue and encounters scattering as well as absorption. Some of the scattered light returns to the tissue surface and is registered by a photo detector. The detected signal is processed to extract information about the microcirculatory blood flow. The perfusion can be calculated since the magnitude and frequency distribution of the Doppler-shifted light are directly related to the number and velocity of blood cells, but unrelated to their direction of movement.

LDI has been predominantly applied for burn imaging^{33–37} on human subjects. The technology uses helium neon laser in the red wavelengths (630–635 nm) as light source and a photodetector for detection. The LDI system provides an objective real-time evaluation of burn wound depth; however, it requires informed interpretation of the scan for reliable diagnosis of burn depth.

Laser speckle imaging

When laser light illuminated on an object, the backscattered light will form a random interference pattern consisting of dark and bright areas, which is called speckle pattern. Comparing to a

static object whose speckle pattern is stationary, the object with movement such as red blood cells in a tissue will show a speckle pattern that changes over time. Typically, a CCD camera is capable of capturing these changes in the speckle pattern. Depending on the degree of movement in the imaged area, the level of blurring will differ. The level of blurring is quantified by the speckle contrast, which is found to correlate with blood flow and blood perfusion measurements. The speckle contrast is defined as the ratio between the standard deviation of the intensity and the mean of the intensity. LSI has also been predominantly applied for imaging of burns, similar to LDI,^{38,39} by employing a helium neon laser (633 nm) and CCD camera for source and detector, respectively. The speckle contrast can assist in determining the burn scar perfusion as observed from human subject studies.³⁹ The LSI instrument is comparable with the established LDI instrument, yielding similar results.

Spatial frequency domain imaging

The SFDI imaging is an extension of NIR imaging (or NIRS), where the imaging physics remains the same, except the postprocessing of the recorded multiwavelength diffuse reflected signals. During NIR imaging, spatially modulated illuminations are projected onto the region of interest over a range of NIR wavelengths (650–950 nm). Diffusely reflected light is recorded using a CCD camera and then demodulated to extract the diffuse reflectance at each wavelength and spatial frequency. This is further reduced into absorption (μ_a) and reduced scattering (μ_s) coefficients by fitting to a known forward light propagation model. However, derivation of optical scattering and absorption parameters in SFDI is usually either performed by look-up-table methods in connection with Monte Carlo simulations or by relying on the diffusion approximation to radiative transfer.

SFDI has also been predominantly applied for imaging of burns in animal models (*e.g.*, rats, swines).^{40–46} The absorption and scattering coefficients from multiwavelength detector are in turn used to determine the HbO, HbR, and ISO₂ concentrations. Diffuse reflectance signal is processed as a function of where the sources and detectors are similar to NIR imaging (*i.e.*, LEDs or NIR tungsten lamp). Researchers state that SFDI-derived data may be useful for early quantitative noninvasive assessment of burn wound severity.

Fluorescence imaging

In fluorescence imaging, the illuminated light source at a particular wavelength excites the

Table 2. Comparison of the features of the different optical imaging techniques applied for wound imaging

Feature	HSI, MSI	OCT	NIRS	DRS	Fluorescence	LDI	LSI	SFDI
Noncontact imaging	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Benchtop	Yes	Yes	Yes (hand-held)	Yes	Yes	Yes	Yes	Yes
Image depth of wounds	<2 μm	~500 μm	mm-cm	100–300 μm	~15 μm	1–2mm	~700 μm	3–4nm
Hemodynamic data	Yes	No	Yes	Yes	No	No	No	Yes
Image whole wound area	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Resolution	7 nm (spectral)	3–5 μm (axial)	Limited by fiber density	Limited by fiber density	~15 μm (axial)	100 μm (axial)	Higher than LDI	~1 mm (lateral)

fluorescent molecules in the tissue, causing them to emit a fluorescence signal at a higher wavelength. The fluorescence signal is filtered from the attenuated diffuse signal (at source wavelength) before being detected at the tissue surface. The fluorescence signal is unique and has a higher optical contrast than diffuse reflectance signal, as it originates from the region of interest that contains the fluorescence molecules, unlike any of the other optical imaging techniques. There have been few studies using fluorescence imaging for DFUs in human subjects.^{47,48} These imaging systems employed white light or LED (in the blue-violet wavelength region) as sources and CMOS camera as detectors. There was no external fluorescence contrast agents used in this study, but the illumination wavelength lead to autofluorescence, which was detected by the camera. Fluorescence imaging was performed rapidly and safely before and during debridement to detect clinically relevant bacterial load at the wound site.

Digital camera

A digital camera is a relatively easy-to-use inexpensive camera linked to telemedicine computer software and provides information that is secure, easily stored, rapidly transferred, and clinically relevant.^{49–51} It also provides accurate and high-resolution wound measurements. It is possible to use the equipment in nearly any situation, so it is suitable for routine practice as well as for clinical trials of new treatments and dressings, case study analysis, and audit.^{49–51}

Digital cameras have been used to image chronic wounds⁴⁹ and DFUs^{50,51} on animal models (e.g., hairless rats) or human subjects. The visible light digital images were used to determine the surface wound size, wound boundary/area, or remotely monitor wounds. Although the digital images can provide reliable monitoring of wounds over time,^{49–51} images in the visible wavelength do not yield oxygenation or birefringence-related characteristics as in the other optical methods.

Comparison of the different optical imaging techniques for wound imaging

A comparison of the different noninvasive optical imaging approaches and their capabilities is given in Table 2. All these noninvasive optical imaging techniques image the wounds through contact (e.g., NIRS, DRS) or no-contact mode (e.g., HSI, MSI, OCT, SFDI, LDI, LSI, fluorescence imaging). In contact-based imaging (i.e., NIRS), optical fibers are typically used to illuminate and detect the optical signal, by contacting the wound/tissue. Based on the density of these fibers on the wound site, the resolution of the image gets impacted, unlike in noncontact imaging where wide-field illumination and detection are performed. Thus, the resolution of all optical imaging techniques, except NIRS or DRS, is higher from the use of high-resolution cameras.

All the noninvasive optical imaging techniques described so far have been applied to different wound types, including burns, open and chronic wounds, DFUs, venous leg ulcers, and decubitus ulcers. While HSI, MSI, NIRS, DRS, and SFDI provide the tissue oxygenation details in terms of HbO, HbR, and saturation oxygen concentration, OCT provides the micromorphology of the wounds based on backscattered intensity and birefringence. Studies have been done, to date, on both animals and humans to determine the effectiveness of all these techniques on clinical wounds. While most of the optical imaging techniques are limited in depth of the wounds imaged (microns to mm), NIRS has the potential to image deeper (mm-cm) based on the chosen source–detector distance during spectroscopic imaging. When the detected light signal is diffused and brings information of deeper tissues unlike surface structural information, the spatial resolution is compensated. In an attempt to image deeper and with improved resolution, a noncontact NIRS system has been developed at the Optical Imaging Laboratory, employing wide-field illumination and detection as described in the following section.

Figure 4. Schematic of near-infrared optical scanner (NIROS) setup in the clinic for wound imaging. NIROS is positioned on an articulating arm for stability during imaging. An endoscopic digital camera is used to obtain the white light images. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

These optical imaging techniques are currently at a clinical translational stage, with potential to serve as a quantitative and objective wound assessment tool during its healing and treatment process in the future. Based on the focus of what parameters are to be extracted or determined from the wounds and from what depth and with what resolution, the appropriate optical imaging technique is employed.

WOUND IMAGING STUDIES AT OPTICAL IMAGING LABORATORY

A hand-held noncontact-based area imaging near-infrared optical scanner (NIROS) has been developed in our Optical Imaging Laboratory. The NIROS has been recently used to demonstrate real-time dynamic imaging of the changes in blood flow in

the dorsal of the hand during venous occlusion^{52–54} and as a contact device for transilluminated breast imaging *in-vivo* to detect for absorption-contrasted targets (simulating tumors).⁵⁵ Herein, NIROS is implemented in monitoring the differences between healing/nonhealing foot ulcers from contralateral normal foot in diabetic subjects by noncontact area imaging at dual NIR wavelengths. This study is a preliminary step for periodic multiweek wound healing monitoring studies in the future.

NIROS uses NIRS to image body tissues without the use of optical fibers over large areas ($>12\text{ cm}^2$). The device consists of an NIR sensitive detector, LED source (710 and 830 nm, and max. 50 mW each), and source driver. The LED driver and the camera, which are USB powered, are synchronized for automated data acquisition. Continuous-wave

Figure 5. White light (a, d) and NIRS reflectance images (b, c, e, f) of DFUs. The first row (a–c) shows a nonhealing DFU, and the second row (d–f) shows a healing ulcer. The NIR images are acquired at 710 nm (b, e) and 830 nm (c, f), for both cases. In the healing DFU (d), differences in the healing and nonhealing regions are obvious from the increased reflectance from healing regions and decreased reflectance from nonhealing regions at both the NIR wavelengths. DFU, diabetic foot ulcer. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

diffuse reflectance imaging is carried out sequentially at both the source wavelengths. The diffuse-reflected signal is collected by the camera after passing through a 645-nm long-pass optical filter. An endoscopic camera is used to capture digital images of the tissues in approximately the same field of view as the images captured by NIROS. The schematic of the experimental setup is shown below in Fig. 4. Data processing is carried out using custom-developed Matlab software.

Clinical studies are currently being conducted at two clinical sites on diabetic subjects with DFUs or with venous leg ulcers. Subjects with multiple wounds (up to three wounds each) have been imaged to date, periodically during each visit. In this IRB-approved study, both the white light and NIR images of the wound at two wavelengths (710 and 830 nm) were obtained without contacting the wound. Figure 5 shows a sample white light and diffuse reflectance NIR image (at both wavelengths) of a nonhealing and healing DFU. From the diffuse reflectance images of the nonhealing DFU (Fig. 5a–c), the wound region shows less diffuse reflected light (or greater absorption of NIR light) than its surroundings. From the NIR reflectance images of the healing DFU (Fig. 5d–f), the wound region has both increased and decreased reflectance. The increased reflectance (or decreased absorption) corresponds to the healed/healing regions of the wound and the decreased reflectance (or increased absorption) corresponds to the nonhealed regions of the wound, when compared to the white light images. Past studies have demonstrated that nonhealing wounds have higher hemoglobin concentrations (or increased absorption) that does not decrease with time, in contrary to the healing wounds.^{2,3} The greater absorption observed in the nonhealing wound regions is possibly due to this increased hemoglobin concentrations at the wound site. This qualitative comparison states that NIR imaging of wounds can differentiate healing and nonhealing regions of the wound.⁵⁶ Currently, extensive imaging studies are ongoing to develop quantitative metrics to differentiate healing and nonhealing regions of the wound from background using NIR as well as hemodynamic information.

SUMMARY

The gold-standard assessment of wound healing is by visual inspection of the wound for surface epithelization and reduction in the wound size with time. An objective imaging intervention of the wound can complement the subjective clinical as-

essment. A review of the various noninvasive optical imaging approaches developed and applied for imaging of different wound types has been described and compared. Based on the focus of what parameters are to be extracted or determined from the wounds, from what depth, and with what resolution, the appropriate optical imaging technique is employed. While most of the optical imaging techniques are limited in depth of the wounds imaged (microns to mm), NIRS has the potential to image deeper (mm-cm) and also provide physiological (or hemodynamic) assessment of the wound. A noncontact NIRS device (NIROS) developed in our laboratory has shown to image the entire wound region in real time and differentiate healing from nonhealing DFUs. Translational efforts are ongoing for each of these technologies, with none commercially available to date.

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AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist.

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The gold-standard clinical approach to assess wound healing is by visual inspection and physical measurement of wound size reduction. Noninvasive optical imaging techniques can provide structural or physiological information of the wound, to better assess its healing. NIRS offers an added advantage of not only providing physiological information of the wound but also image deeper (mm-cm range) over other optical imaging techniques.

various agencies to date, including National Institutes of Health, Florida Department of Health, Department of Defense, American Cancer Society, and W.H. Coulter Foundation.

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Abbreviations and Acronyms

CCD	= charge-coupled device
CMOS	= complementary metal oxide semiconductor
DFU	= diabetic foot ulcer
DRS	= diffuse reflectance spectroscopy
HbO	= oxyhemoglobin
HbR	= deoxyhemoglobin
HbT	= total hemoglobin concentration
HSI	= hyperspectral imaging
ISO ₂	= oxygen saturation
LDI	= laser Doppler imaging
LSI	= Laser speckle imaging
MSI	= multispectral imaging
NIR	= near-infrared
NIRS	= near-infrared spectroscopy
NIROS	= near-infrared optical scanner
OCT	= optical coherence tomography
PS-OCT	= polarization sensitive-optical coherence tomography
SFDI	= spatial frequency domain imaging