Clinical Evaluation of a Handheld Fluorescence Imaging Device for Real-time Wound Care Monitoring

by

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A thesis submitted in conformity with the requirements for the degree of Master of Health Science in Clinical Engineering

Institute of Biomaterials and Biomedical Engineering

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Abstract

Wound care is currently a major clinical challenge worldwide [1]. A major complication during wound healing is bacterial infection, which causes serious impediment to the healing process [2,3].

This thesis studied a hand-held autofluorescence-imaging platform called PRODIGi™ that is capable of detecting and quantifying bacterial load for point-of-care wound infection diagnosis and procedural guidance. The aim of this study is to extend the feasibility of PRODIGi™ to a clinical setting, validate its ability to detect and quantify bacteria in wound in real time thus guide interventions, and establish whether fluorescence-guided interventions improve healing of chronic wounds over time.

A murine skin model was first established to validate PRODIGi™ on tracking bacterial fluorescence. Then a clinical trial was completed monitoring 23 patients up to 8 months. Wounds were found to heal faster with than without PRODIGi™ guidance. Subsequently, a standard operating procedure was created for PRODIGi™ to be used in other clinical settings.
Acknowledgements

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List of Acronyms

PRODIGI = Portable Real-time Optical Detection Identification and Guide for Intervention

REB = Research Ethics Board

WL = White Light

FL = Fluorescence

JDRTC = Judy Dan Research & Treatment Centre

UWT = Ultrasound Wound Therapy

SOP = Standard Operating Procedure

PMH = Princess Margaret Hospital

UHN = University Health Network

BLI = Bioluminescence

PBS = Phosphate Buffer Saline

ITA = Investigational Testing Authorization

SLR = Single-Lens Reflex
Chapter 1
Introduction

1.1 Rationale

Wound care presents a huge burden to healthcare systems and is a major clinical challenge worldwide [1]. Care for an average chronic wound and an average acute wound cost Canadians $10,376 and $11,840, respectively [4]. Chronic diabetic foot ulcers alone cost the Canadian healthcare system over $150 million annually and are the leading cause of amputations [4]. The large costs come from long period of treatment required for the wounds to heal. During the wound healing, a number of key biological changes happen at the tissue and cellular levels, including inflammation and connective tissue remodeling in the dermis [2]. A major complication during wound healing is bacterial infection, which can cause serious impediment to the healing process, extending it from days to months [3]. A wound with bacterial infection contains a larger number of microorganisms than a contaminated or colonized wound [5,6] and it is important to note that, while a contaminated wound will heal (bacterial balance), an infected wound will not (bacterial imbalance).

Clinicians currently make the diagnosis of wound infection with the unaided eye under normal white light (WL) illumination, based on signs and symptoms caused by bacterial infection in and around the wound bed, such as pain, erythema, purulence and heat [2,3,7,8]. However, bacteria are invisible to the unaided eye, so that this standard of practice is highly subjective and neither quantitative nor easily standardized across different jurisdictions. Extensive research has been done to attempt to improve diagnosis based on wound assessments i.e. wound location, size, appearance, odor, etc. [9,10,11]. But studies showed that even experienced physicians were unable to accurately make the diagnoses of wound infection based on physical examination [12]. A better quantitative assessment method is needed to provide clinicians with the critical underlying information at the tissue and cellular level such as inflammation, matrix remodeling, and infection.

Fluorescence (FL) imaging (without contrast agents) can provide a powerful means for in situ visualization of bacteria and connective tissue that are invisible under normal WL [13]. It has been used in other clinical applications, such as gastroenterology to image both collagen and bacterial fluorescence [14,15]. But, there has been no fluorescence-imaging device used in wound care to obtain biologically relevant information at tissue and cellular levels in real-time in order to improve the wound-healing process through improved intervention. In this study, we introduce a new FL imaging technology to be used in the setting of clinical wound care.
1.2 Chronic Wounds

Chronic wounds are ulcers or sores on the skin that do not heal in an orderly set of stages and in a predictable time, as most wounds do [16]. Normally, wounds are considered as chronic if they do not heal in three months [17]. The long treatment times can be due to the unknown cause of the wound and the complications during the healing stages. There are different types of chronic wounds, including diabetic ulcers, venous ulcers and pressure ulcers. Among these, diabetic ulcers are the leading cause of amputations [18]. It is estimated that more than 4,000 Canadians with diabetes had a limb amputated in 2006 [18]. Diabetic ulcers are sores that usually happen when the patients lose feeling in their feet and injury happens without the patient being aware. This type of ulcer can easily get infected because the patients may not even know they have a sore [19,20].

1.3 Current Diagnosis

The current standard for infection diagnosis in chronic wounds involves visual assessment under WL, as explained earlier. Clinical signs and symptoms caused by bacterial infection in and around the wound bed, such as pain, swelling, erythema, purulence and heat, are assessed to make clinical decisions. The visual inspection of the wound provides a broad, qualitative idea of the wound status but does not provide any information on the types and locations of bacteria in the wound, the drugs to which the bacteria are resistant or any quantitative measures of bacterial load in the wound.

Thus, in addition, bacterial swabs are collected on suspicion of infection at the time of wound examination [21,22]. Bacterial swabs are normally analyzed in a lab where the samples are grown on a culture dish and identification of specific bacterial/microbial species and a semi-quantitatively measure of the bacterial load can be provided. The report usually shows the white blood cells count, the gram staining result (gram positive or gram negative), the type of bacteria found in the culture (e.g. mixed bacteria, Staphylococcus aureus, Pseudomonas aeruginosa etc.), the semi-quantitative growth of the bacteria found (occasional, light, moderate or heavy) and sometimes the susceptibilities to different drugs (e.g. Clindamycin, Erythromycin, Penicillin, etc.). Some species requires specific tests to be identified. For examples, *S. aureus* is differentiated from other staphylococci with a coagulase test, since *S. aureus* coagulates while the others species do not [23]. However, these specific tests take more time and are sometimes omitted depending on the technician who performed the testing procedure. The test report would then simply return as “mixed bacteria” without the specific bacteria types.
Moreover, the swabbed area must be specific and targeted in order to obtain accurate results [21]. Without the appropriate guidance, swabs are often collected randomly from the wound site and not targeted directly at the area with bacterial load. Some swabbing techniques may even spread the microorganisms around during the collection process, causing more harm to the healing process [22]. Most importantly, the results from microbial testing are usually not available until 2 to 4 days after the swabbing, thus significantly delaying diagnosis and treatment, not to mention that the bacteria types and loads may change over this period of time [24].

1.4 Common Pathogenic Bacteria Strains

There are many bacteria strains living around us. Some of the most common pathogens in hospital are Escherichia Coli (13·7%), Staphylococcus Aureus (11·2%), Enterococci (10·7%), and Pseudomonas Aeruginosa (10·1%) [25].

1. *Staphylococcus Aureus*

*Staphylococcus Aureus* is an anaerobic gram-positive coccocococcus bacterium. It appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies when grown on blood agar plates [26]. *Staphylococcus Aureus* is not always pathogenic and is frequently found in the human respiratory tract and on the skin, but can infect tissues when the skin or mucosal barriers are breached. *Staphylococcus Aureus* infections can spread through contact with pus from an infected wound, through direct skin-to-skin contact with an infected person or through indirect contact with objects used by an infected person (e.g. towels, sheets and clothing).

2. *Pseudomonas Aeruginosa*

*Pseudomonas Aeruginosa* is an aerobic gram-negative coccobacillus bacterium. It is often identified by its pearlescent appearance and grape-like or tortilla-like odor *in vitro* [26]. *Pseudomonas Aeruginosa* can thrive in normal atmosphere and hypoxic atmospheres. It uses a wide range of organic materials for food and can even colonize in medical equipment, including catheters. Studies have shown that *Pseudomonas Aeruginosa* is reduced at acidic pH [27,28] and acetic acid is commonly used topically to eliminate *Pseudomonas Aeruginosa* from soft tissue wounds [29].
3. *Escherichia Coli*

*E. Coli* is an anaerobic gram-negative bacterium. Most *E. Coli* strains are harmless and are part of the normal flora of the gut, benefiting the host by producing vitamin K2 and preventing pathogenic bacteria establishment [30]. However, *E. Coli* can cause serious infection when outside the normal habitat of the gut, such as in post-surgical wounds and the urinary tract [25]. *E. Coli* is widely used as the model organism in microbiology because it can be grown easily and inexpensively in a laboratory setting.

4. *Enterococci*

Enterococci are anaerobic gram-positive cocci. And the most common specie of Enterococci is *Enterococcus Faecalis*. Enterococci often occur in pairs or short chains and look very similar to streptococci [31]. Enterococci can survive in extreme temperature (10-45), pH (4.5-10.0) and salt concentrations [32]. Enterococci can cause life-threatening infections in humans, especially in hospital settings, due to the high resistance to antibiotics [33].

It is important to note that there are many types of bacteria existing in, on and around us, but not all are harmful. It is only certain bacteria species under certain conditions that cause bacterial infections. First of all, the location of the bacteria is an important factor. *S. Aureus* is part of normal nasopharyngeal, skin and GI flora but when it is found in a wound or inflammation site it can cause abscesses and wound infection [34]. Secondly, the host *versus* bacteria balance is another important factor. If the bacterial load is small and the host is strong enough to fight off the bacteria, there will be no infection; but if the bacterial load is too large for the host to fight off, then infection can occur. The term colonization describes a bacterial-balanced situation when the bacteria reside on the wound but do not cause an infection to the host, whereas the term infection describes a bacterial imbalanced situation when the bacteria grow out of control and cause complications to the wound.

1.5 **Current Treatments**

Common treatments available for patients with foot ulcers include surgical debridement under WL, topical antibacterial medications with dressings, and systemic antibiotics [35].

1.5.1 **Debridement**

As a first step, debridement can be performed to remove bacterial biofilm and dead tissues in and around the wound. A biofilm is a group of microorganisms that stick to each other on a surface, and when
bacteria form a biofilm in a wet wound this can provide an environment for more bacteria to grow and populate. The bacteria can slowly occupy larger wound areas and the wound bed keeps getting infected, preventing healing. Thus, it is necessary to remove the top layer of bacteria and necrotic tissue such that the healthy tissues underneath can grow to heal the wound [36]. When the healthy tissues are exposed after debridement, the wound turns into an acute state that can lead into a normal healing state. The rule of thumb for debridement at the Judy Dan Research & Treatment Centre (JDRTC) is to debride until the tissues bleed, because the fresh tissues are then exposed with good vasculature. To ensure effectiveness, the debridement should be performed frequently, usually on a weekly basis [37].

Conventionally, debridement is done using scalpel and forceps. Necrotic tissues are lifted up and cut using a scalpel. Scissors are often used as well to assist the debridement. At JDRTC, curette is another effective way to remove necrotic tissues and bacterial biofilms. The curette has a plastic handle with a sharp circular ring at the tip. When scraping the wound bed with the circular ring, the layer of biofilm is easily removed from the wound. These conventional debridement methods work well on wet wound beds where the watery biofilm and lacerated skin can be easily removed, but is not ideal for dry wounds because it is hard to judge the depth of the necrotic tissues. They rely on manual maneuvers and are highly dependent on the clinicians’ skill and experience.

An ultrasound wound therapy (UWT) debridement device was recently approved by Health Canada. This removes necrotic slough and biofilm with deep tissue penetration using ultrasonic energy [38]. It is capable of debriding diabetic foot ulcers, pressure ulcers, venous stasis ulcers, arterial ulcers etc. There are two types of UWT transducers on the market: piezoelectric and magnetostrictive [39]. In piezoelectric transducers, a voltage is applied across a piezoelectric ceramic (a single or double thick disc, typically Lead Zironate Titanate) and due to the changes in lattice structure of the piezoceramic the ceramic expands or contracts, depending on the polarity. This physical displacement generates a sound wave that propagates into the cleaning solution. On the other hand, in the magnetostrictive transducer, a current is supplied through a coil of wire to create a magnetic field, which causes the magnetostrictive material to contract or elongate, thereby generating the sound wave into the cleaning solution.

The Qoustic Wound Therapy System™ from Arobella Medical, LLC (Figure 1) is a magnetostrictive ultrasound device used at the JDRTC. It contains a dome-shaped Qoustic Qurette and an ultrasound generator. A current is supplied to the generator to create a magnetic field, which causes the magnetostrictive material to contract and elongate, and produces a sound wave at frequency of 35kHz (±2kHz) and maximum amplitude of 50-70µm into the saline solution [40]. During debridement, the
saline solution flows through the Qoustic Qurette and is targeted toward the wound site to break down the necrotic tissues at the high frequency. The saline would look like a mist during the debridement because of the high vibration frequency. The saline also helps to moisten the dry skin and soften hard necrotic tissue such that the unwanted tissues can be easily washed off.

![Qoustic Wound Therapy System](image)

*Figure 1: Qoustic Wound Therapy System™ from Arobella Medical, LLC [40]*

Four different types of modes can be used, depending on the wound type (*Figure 2*): both the basic treatment mode and the cavitation mode apply the face of the dome-shaped Qoustic Qurette toward the wound with light contact. While the basic treatment mode contacts the wound at the distal end of the Qurette allowing fluid to flow in a U turn and come out at the distal end, the cavitation mode contacts the wound at the lateral side of the Qurette [41]. The scooping and the edge modes apply the edge of the Qurette to the wound at 90° and 30°, respectively, to mechanical remove the non-viable tissue, slough, exudate, debris, fragments and other material.
Figure 2 Modes of debridement using the Qoustic Qurette [41]

One problem with both the conventional and ultrasound debridement is that the clinicians debride under WL, without knowing where the bacteria reside. In another words, they are debriding “blindly”. Clinicians tend to debride until the tissues bleed, such that they know that healthy tissues have been exposed. However, that may have already debrided too deeply, so that some healthy tissues were unnecessarily removed. There is not yet a quantitative measurement to guidance clinicians with debridement in real time.

1.5.2 Topical antibacterial medications

After debridement and during daily wound care, topical medications and dressing are usually applied to kill bacteria and promote wound healing. There are many different types of topical medications and dressings on the markets. Some of the common topical medications used at the JDRTC include:

- **Chlorohexidine (solution)** is an antiseptic effective on both gram-positive and gram-negative bacteria. It is used commonly for general wound cleansing.

- **Betadine (solution)** is a povidone-iodine topical antiseptic (10% povidone-iodine in water). It is one of the strongest antimicrobial agents and is used for infected wounds. It kills bacteria but does not help with wound healing. Sometimes Betadine dries healthy skins around wound, preventing speedy wound healing.
Iodosorb (paste) contains iodine and as iodosorb swells with fluid absorption, it slowly releases iodine to kill bacteria in infected wounds. However, unlike povidone-iodine, studies show Iodosorb may cause acceleration of healing in chronic wounds, due to an increase in epithermal regeneration and epithelialization [42].

Flamazine (cream) is an antiseptic with silver sulfadiazine and is also active against a broad range of organisms, including both gram-positive and gram-negative bacteria and some viruses and fungi. However, reviews have shown that there is insufficient evidence to determine if silver sulfadiazine promotes wound healing and have raised concerns about delayed healing [43].

Silvasorb (gel) is a hydrating gel for dry wounds and provides antimicrobial protection with microscopic particles of ionic silver.

Dressings are applied on wounds after debridement and/or topical medication to protect the wound and some dressings can provide antibacterial property to the wounds as well. Ideally, after debridement the wound bed should be kept moist but not wet. So, depending on the wound, the appropriate dressing should be chosen to achieve the moist environment. There are many types of dressings on the market. A table comparing the different types of dressings is shown in Table A1 in the Appendix. Some of the common silver dressings used at the JDRTC include the following.

Gauze is the basic clean material to protect the wound.

Foam is the basic thick dressing that can absorb pus from the wound. It is commonly used for wet and exudative wounds.

Aquacel is also a highly absorptive dressing. It is capable of retaining exudate and locks exudate in the dressing. The dressing creates a soft gel after absorbing fluid and aids autolytic debridement of the wound.

Inadine is a povidone-iodine based non-adherent dressing. The povidone polymer provides sustained release of iodine [44].

Anticoat Flex 3 is a flexible polyester layer coated with nanocrystalline silver. The high concentration of silver kills bacteria in the wound.

Silvercel is a non-adherent dressing containing a mixture of alginate, carboxymethylcellulose, and silver coated nylon fibers to manage exudate, provide antibacterial quality, and maximize protection of the wound bed due to its non-adherent property.

Promogran Prisma contains large amount of collagen and oxidized regenerated cellulose (ORC) to manage exudates and help wound healing. The dressing contains some silver to prevent infection.
Biotain Ibu contains ibuprofen, which is an anti-inflammatory agent and an active pain reliever. This dressing is mostly used for patients with painful wounds. Negative pressure wound dressing therapy is sometimes used to create a negative pressure at the local wound site by connecting the dressing to a vacuum pump. This drains exudate from the wound and is useful for some patients with deep ulcers [45]

1.5.3 Systemic Antibiotics

In addition to local topical medications and dressings, broad-spectrum antibiotics can be prescribed as well to treat deep soft tissue infections. However, caution should be taken before prescribing systemic antibiotics to patients. Microbiology reports must be consulted before starting antibiotics to reduce potential adverse events and unnecessary antibiotics should be avoided to prevent spread of multidrug-resistant bacteria, such as Methicillin-resistant Staphylococcus aureus (MRSA) [46].

One problem with choosing both the topical antibacterial medications and systemic antibiotics is that the clinicians do not know in real-time what kind of bacteria are in the wound and what bacterial load they are dealing with. Bacterial swab results do not come back until 2-4 days after sampling, so that the clinicians cannot make an accurate decision on the day of the patient visit. Topical medications are typically chosen only by visible signs and symptoms and often applied throughout the whole area instead of targeted to the areas with bacteria. Systemic antibiotics are usually prescribed according to the swab results of the previous visit, which may not be accurate since weeks may have passed since the last visit. There is not yet a quantitative measurement to guide clinicians choosing the topical antibacterial medications and the systemic antibiotics for patients in real time.

1.6 PRODIGi™

In 2008, Dr. DaCosta’s team invented a novel handheld FL-imaging platform called PRODIGi™ (Portable Real-time Optical Detection, Identification and Guidance for Intervention) for point-of-care wound infection diagnosis and procedural guidance (Figure 3). The device allows for collection of both WL and FL images to detect pathogenic bacteria on normal skin as well as within and around wounds, without the use of exogenous contrast agents. When illuminated with blue/violet light, endogenous collagen/elastin in connective tissue matrix emits a green fluorescence signal, whereas most pathogenic bacterial colonies (e.g. S. Aureus) emit a red fluorescence signal due to the production of endogenous
porphyrins [47,48,49,50]. Some bacterial species (e.g. *P. Aeruginosa*) produce a green fluorescence, due to siderophores/pyoverdins [51,52], which can be differentiated spectrally and textually from the fluorescence of connective tissue. Hence, this FL imaging technology (405±20 nm excitation, 500-550 nm and 590-670 nm emission, **Figure A1**) requires no exogenous contrast agents during imaging *in situ*, making it particularly appealing as a diagnostic imaging method for clinical use.

**Figure 3** Photograph of the handheld prototype PRODIGI™ imaging device and its real-time visualization of bacteria otherwise invisible under white-light.

Left. PRODIGI™ aimed at a diabetic foot ulcer. High-resolution fluorescence images are displayed in real-time on the LCD screen, indicating the location and extent of bacterial load in and round the wound. Dual broadband white-light and blue light emitting diodes (LED) arrays provide illumination of the wound, with the fluorescence emission filter placed in front of the CCD sensor. Middle. White-light photograph of the wound taken with PRODIGI™. Bacteria are not visible. Right. Fluorescence photograph of the wound taken with PRODIGI™, showing bacteria around the periphery of the wound and also at a distant location (red fluorescence).

PRODIGI™ spectrally separates fluorescence from different bacteria species by the multiband emission filter and display them on a composite RGB image without image processing or color-correction. The fluorescence imaging at $\lambda_{\text{exc}}=405$ nm and $\lambda_{\text{emiss}}=490-550$ nm and 690 nm can sample bacteria at approximately 1-1.5 mm of tissue depth [53,54]. The device sensitively detects fluorescence of *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *Candida*, *S. marcescens*, *S. viridans*, Diptheroid bacilli, *S. pyogenes*,...
Enterobacter, and Enterococci, as verified by bacterial swabs. S. aureus can be distinguished from P. aeruginosa in real-time, since S. aureus emits red fluorescence >600 nm while P. aeruginosa emits bright bluish-green fluorescence peaking at 480 nm (Figure 4).

**Figure 4 Fluorescence imaging of different bacteria species.**

A) WL image of a chronic wound on a non-diabetic patient’s right ankle. B) corresponding FL image distinguishing the red fluorescence from the endogenous porphyrins in S. aureus (smaller circle) and the green fluorescence from the siderophores/pyoverdins in P. aeruginosa (larger circle). C) corresponding point fluorescence spectra confirming that, while both species emit green fluorescence between 490-550 nm with 405 nm excitation, S. aureus emits red fluorescence >600 nm while P. aeruginosa emits bright bluish-green fluorescence peaking at 480 nm. Scale bars represent 1cm.

Preclinical studies have shown that PRODIGi™ is capable of collecting FL images of wounds and detecting the presence and relative changes in connective tissue content involved in wound healing. As well it can detect the presence and relative amounts of commensal and pathogenic bacteria within the wound based on FL alone, thus providing a measure of bacterial infection status. This could significantly impact clinical wound care by 1) reducing the complications associated with missed detection of bacterial infection under conventional practice, 2) facilitating image-guided wound sampling by targeted swabbing, and 3) monitoring wound healing and treatment response over time.
Two of the current handheld devices in the wound care market for assessing skin conditions are Aranz Medical that uses WL to record depth profiling of wounds and 3 Cert that uses magnifying lens with LEDs to visualize skin melanomas. Some of the other potential competitors are shown in Figure A2. However, there has yet to be a fluorescence device to visualize bacteria in real-time to guide procedures without contrast agents.
Chapter 2
Research problems, hypotheses, and objectives

During this project, I investigated whether FL image-guided interventions can reduce wound size and bacterial loads and thus improve the healing process. In order to do so, I investigate quantitatively the changing wound size and bacterial loads over time during the wound healing process, with a specific focus on the healing of diabetic foot ulcers.

The hypotheses to be tested were that: 1) FL imaging using PRODIGI™ can detect and quantify bacteria in wounds, even those that are invisible under standard WL examination, and can guide clinical interventions in real-time; 2a) FL-guided wound sampling can better target bacterial load in wounds compared with standard wound sampling; and 2b) FL-guided debridement and topical treatment can result in lower overall bio-burden compared with standard debridement and topical treatment, which can lead to improved healing in chronic wounds over time.

The objectives were:

1) To establish a murine skin wound model to test PRODIGI™ imaging in vivo.

2) To extend the feasibility testing to a clinical setting for real-time, non-invasive detection of pathogenic bacterial presence and interventions. And in the end create a standard operating procedure (SOP) for implementing the device into current workflow, and perform a clinical cost analysis with the implementation of the device.

3) To determine whether FL imaging provides better guidance to target bacterial swabs to specific areas of bacterial load.

4) To determine whether FL-guided debridement and topical treatment result in lower overall bio-burden and faster wound closure.
First of all, two devices were assembled from components, following which the devices were approved by the Canadian Standards Association (CSA) to be used in the clinic setting.

### 3.1 Device Assembly

For each device, the main components include: a Sony camera DSC-T900, a Sony memory card adapter, two 405 nm light-emitting diodes, two neutral white light-emitting diodes, two switches, four cooling fans, two excitation light source filters (Thorlabs), a detection dual bandpass filter (Chroma), a spare Sony Li-ion battery, an AC/DC power supply, a system shell (built in house), and a carrying case. The total purchase cost of components was approximately CAD$1600, excluding labor or additional materials. A picture of the components and a list of the components are shown in Figure 5.

![Figure 5 Components of PRODIGI™ and the costs of the main components.](image)

The electrical wiring lay out is shown in Figure 6. The device uses a power supply of 24 VDC (1.33A), and a main power switch after the connector controls the ON and OFF of the input power. Once the power switch is turned on, the current flows either to the white light source or the violet-blue light source, with
the second switch determining the path of the current flow. If the current is flowing to the white light source, the white light LEDs are powered and the corresponding cooling fans (connected in series) are switched on. Similarly, if the current is flowing to the violet-blue light source, the violet-blue LEDs will light up and so will the corresponding cooling fans. There are potentiometers connected to the violet-blue LEDs so that the LED output power can be adjusted.

![Electrical diagram of PRODIGI™](image)

Figure 6 Electrical diagram of PRODIGI™

### 3.2 CSA approval

Auditors from QPS Evaluation Services were invited to test the device on May 10, 2013 for CSA approval. All of the PRODIGI™ devices were displayed and how the devices work and the intended clinical uses were explained. The auditors tested the devices and took a few fluorescence images. The main concern was the power supplies. These are AC-DC converters (MENB1030A2403B01 or MW173KB2403B01: previous version of MENB1030A2403B01) purchased from Mouser Electronics,
which both have inputs of 100-240 V~, 50-60 Hz, 1.0 A, and output +24 V, 1.33 A (Figure 7). The auditors considered the voltage and current of the power supplies to be normal but were concerned whether the power supplies purchased were officially Underwriters Laboratories (UL) approved to be medical grade. All of the power supplies were purchased as medical grade equipment from Mouser Electronics, a well-recognized catalog distributor of electronic components (Figure A3) but the auditors could not find any approved medical grade power supplies under the models MENB1030A2403B01 or MW173KB2403B01, so that approval was withheld on the first visit.

![Figure 7 Power Supplies for PRODIGII™](image)

After the meeting with the auditors, an extensive search was done by going through each items on UL website, [www.ul.com](http://www.ul.com), and calling Mouser Electronics representatives. The power supplies were found to be medical grade under the Product category QQHM2, QQHM8 and under the Model/Type Reference BP(a)030(b)(c)(e)(f) and (a)ENB1030(b)(c)(d)(e)(f) (Figure 8). Since the power supplies were confirmed to be UL certified medical equipment, the auditor issued the CSA certificate.
### COVER PAGE FOR TEST REPORT

**Product Category:** Power Supplies, Medical and Dental  
**Product Category CCN:** QQHM2, QQHM8  
**Test Procedure:** Component Recognition  
**Product:** AC-DC Adaptor  
**Model/Type Reference:** BP(a)030(b)(c)(e)(f) and (a)ENB1030(b)(c)(d)(e)(f)  
(a) can be A to Z for family related designs.  
(b) can be S for single output in model BP(a)010 series and (b) can be A to Z for design revision changes in model (a)ENB1010 series.  
(c) can be 05, 06, 07, 09, 12, 15, 16, 18, 24 and 48 for output voltage.  
(d) can be can be 00 thru 99 for standards output cord options (*d* is not provided in model BP(a)010series).  
(e) can be F or N or Q or B or H or G or M or C for input plug type.  
F-Class I appliance inlet type: IEC60320-C14  
Q-Class II appliance inlet type: IEC60320-C18  
N-Class II appliance inlet type: IEC60320-C8  
B or C-Class I & Class II direct-plug-in for North America, China, Japan and Argentina (Changeable Direct-plug-in type is only used for Class II)  
H-Class I & Class II direct-plug-in for Australia (AS/NZS 3112)  
G-Class I & Class II direct-plug-in for British (BS 1364)  
M-Class I & Class II direct-plug-in for European (CEE /16]) & Korea.  
(f) can be 00 thru 99 for customer options.  
**Rating(s):**  
Rated Input; 100-240Vac, 50/60Hz, 1.0 A  
Rated Output; 5Vdc, 4.0A or 7.5Vdc, 3.0A or 9Vdc, 3.0A or 12Vdc, 2.5A or 14Vdc, 2.1A or 15Vdc, 2.0A or 18Vdc, 1.67A or 24Vdc, 1.33A or 48Vdc, 0.4A or 48Vdc, 0.67A  
(Rated output voltage is designated according to the model name designation system)  
**Standards:**  
CAN/CSA-C22.2 No. 601.1-M90, 2005 (Medical Electrical Equipment - Part 1: General Requirements for Safety)
The auditor also allowed cables to be extended from the power supplies, as this would not change the power adapter approved by UL. Prior to approval, the power supply cables were extended using extension cords, such that the device can reach a patient at a distance from a power plug. However, the extension cords were quite heavy and difficult to move around when imaging a patient. After the approval, I extended all cables on all PRODIGI™ systems such that the cords are longer and easier to use in the clinical settings.

Labels were also made for all devices and each device was labeled with the unique serial number from system A to system F. The date of the approval was specified on the label. For each device suitcase, lists of components were also made and placed in the top cover.
Chapter 4
Animal Studies

4.1 Objectives

Mouse experiments were used as first step to ensure that the PRODIGI™ device works as intended before using it in a clinical trial on humans. The skin wound model used has been reported previously to study wound status and healing [55]. Here, we established the model to validate the capability of the PRODIGI™ device to accurately detect bacterial loads in wounds in real-time and to track wound progress over the course of antibacterial treatment. The model was used to correlate the fluorescence signals to the absolute bacterial load, determined using bioluminescence imaging (BLI).

The objectives were:

1. To establish the mouse skin wound model.
2. To use PRODIGI™ for intervention guidance and to track wound status over the course of treatment
3. To correlate fluorescence signals with BLI signals and examine the effectiveness of fluorescence-guided interventions.

4.2 The Model

4.2.1 Method

Our first mouse skin wound model used phenotypically normal male mice (8-12 weeks, heterozygous for diabetes spontaneous mutation (Lepr<sup>db</sup>)). This is based on a previous Japanese model performed on rats [56]. Mice were first anesthetized under 5% isofluorane and cleaned with Povidone scrub and solution. Two skin wounds of equal size were then created on either side of the spine using a biopsy punch (6 mm diameter) and an O-ring (VWR) was sutured on the periphery of each wound. Tissue glue (3M) was applied around each of the O-rings to ensure a good seal. After the wounds were created, <i>S. Aureus</i> (10<sup>3</sup> or 10<sup>10</sup> colony forming units (CFU) in 0.5 mL phosphate buffer saline (PBS) was subcutaneously injected into one wound and 0.5mL PBS (control) was injected into the other wound and also spread on the surface of the wounds. Lastly, Tegaderm was applied across the two wounds 15 minutes after the injection to enclose the liquids in the wounds (Figure 9). All mice were monitored daily, and both WL images and FL images were taken of the wounds. The Tegaderm dressing was changed after each imaging procedure.
Our second mouse skin wound model used female nude mice aged 8-12 weeks (NCRNU-F Heterozygous). On the surgery day (day 0), mice were anesthetized under 5% isofluorane. Dorsal skin areas were shaved and cleaned with Povidone scrub and solution. Two circular full thickness wounds (6 mm diameter) were created on either side of the spine using a biopsy punch with the assistance of scissors and forceps. The test group mice received an injection of *S. aureus* (10^{10} CFU) suspended in 0.5 mL PBS in one wound and 0.5 mL PBS in the other wound. The control group mice received injections of 0.5 mL PBS in both wounds. All injections were performed subcutaneously. Solutions were injected until visual bubbles were formed on the wound surface, and the residue solutions were then spread on the surface of the wound. Gauze was used to cover the wounds after the surgery and Tegaderm was applied on top of the wounds (Figure 10). All mice were monitored daily and both WL images and FL images were taken of the wounds. Tegaderm was changed after each of the imaging procedure.
Our third mouse skin wound model used female nude mice aged 8-12 weeks (NCRNU-F Homozygous). The procedure was as in the second model, except that the homozygous mice are hairless and so do not require the shaving step, and Tegaderm was not changed until it was necessary for imaging.

### 4.2.2 Results

Three mouse skin wound models have been established and six experiments have been completed to date: two experiments with the first model (n=2 and 4), three with the second model (n=5, 1 and 5) and one with the third model (n=10). The experiments with the first model did not yield any detectable fluorescence. Red fluorescence was detected in the test groups with the second (experiments 3-5) and third (experiment 6) models. The lack of expected bacterial fluorescence in the first model could be due to the fact that the non-nude mice have functioning immune systems that were able to fight off the injected bacteria. For the nude mice experiments, two animals had serious cross contamination of the wounds and were sacrificed. In the remaining mice without cross contamination, red fluorescence was observed in the wounds where *S. aureus* was injected but not in the control wounds where PBS-only was injected. Hence, the heterozygous and homozygous nude mouse models could be successfully used to test fluorescence guided interventions and the accuracy of the PRODIGI™ fluorescence signals.

There were several optimization steps in moving from the first to the second and then to the third experiment. For example, it was found that O-rings were not necessary in establishing the wound
boundaries and did not affect the validity or usability of the model, so that the second and third models did not use O-rings. Secondly, the Tegaderm was not changed until necessary for imaging in the third model, the reason being that changing the dressing inevitably removes the top layer of the tissue, thereby potentially skewing the results. Lastly, the skin played an important role in the experiment procedure. Mice with hair required shaving and application of hair removal cream prior to making the wounds, and this can cause skin irritation. The use of nude mice eliminated these steps, so that wounds could be made directly on the smooth skin.

4.3 Tracking of bacterial load over time

4.3.1 Methods

With the mouse skin wound model established using nude mice, it is now possible to monitor the status of the mouse wound as the bacterial infection progresses. Experiment 3 was performed with the second model (female nude mice aged 8-12 weeks, NCRNU-F Heterozygous) (n=5). Both WL and FL images were taken of the wound every day as it became infected. Treatment was used when the fluorescence intensity peaked and the effect of the treatment was observed over time using PRODIGI™. After the initial inoculation of *S. Aureus* in the wound, bacteria were expected to colonize in the wound and infect the wound as time goes on. And the red fluorescence would be increased in intensity as the bacteria load increases over time. Once the fluorescence signal had reached its peak and started to decline, an antibacterial cream, Mupirocin was applied at the wound site (three times daily). WL and FL image acquisition of the wound were continued daily after the treatment. The wounds were monitored for a total of ten days and the mice were sacrificed after the ten days. Bacteria concentration from FL images and wound size from WL images were measured and quantified using MATLAB software. They were compared over time to determine the wound healing status.

4.3.2 Results

The red fluorescence signal started to be detectable in the FL images on day 3±1 (range 2-4) and peaked on day 5±1 (range 4-6). The WL, FL and BLI images over the 10d period for one mouse are shown in Figure 11. The bacteria were not visible during the first 2 days but, as the bacteria proliferate and colonize in the wound, the red fluorescence was picked up by PRODIGI™ on day 3 and increased over days 4-6, by which time and the infection could be visualized under WL. On day 7, the red fluorescence
intensity seemed to have peaked and started to drop. Mupirocin was applied onto the wound on day 7 and the bacterial load seen by the red fluorescence decreased significantly, to almost zero intensity on day 8. However, the red fluorescence was developed to the surface again after day 9, possibly from the deeper bacterial load in the wound. The mouse was sacrificed on day 10. This experiment confirmed that PRODIGI™ imaging can be used to track infected wounds over time.

Figure 11 Example of the second mouse skin wound model tracked over time.
A) Photos showing the two equal-sized wounds on both sides of the spine. The right wound was inoculated with S. Aureus in PBS and the left wound was inoculated with PBS (control). (Top row) white light images, (middle row) fluorescent images, (bottom row) MATLAB quantified images showing the bacterial areas and intensities. The fluorescence imaging data demonstrated significant increase in bacterial fluorescence intensity in the wound inoculated with S. Aureus, compared with the control wound, peaking on day 6. Mupirocin (day 7: red arrow) caused significant decrease in bacterial fluorescence on day 8 to almost zero, indicating the treatment effect. Bacteria regrowth is seen on days 9 and 10. B) Graph showing quantitative changes in bacterial load from fluorescence images obtained in panel A.
4.4 Correlating FL with BLI

4.4.1 Methods

BLI data have been shown to provide the most sensitive screening tool for determining bacterial load and so was used here to measure the absolute bacteria amounts in vivo [57]. BLI imaging collects the light emitted from the enzymatic reaction of luciferase and luciferin and so it does not require any excitation light. In this experiment, BLI imaging was used for comparison with the FL imaging. Since the BLI signal is orders of magnitude dimmer than the bacterial FL signal, it can only be detected using intensified-CCD cameras and so does not contribute significantly to the signal in consumer grade-CCD camera used in PRODIGI™.

Gram-positive bioluminescent (BLI) *Staphylococcus aureus*-Xen8.1 from the parental strain *S. aureus* 8325-4 (Caliper) was grown to mid-exponential phase the day before pathogen inoculation. Bacteria with the BLI cassette produce the luciferase enzyme and its substrate (luciferin), thereby emitting a 440-490 nm bioluminescent signal when metabolically active [58,59] (Figure 12). 10^{10} CFU of the bacteria were suspended in 0.5mL of PBS and injected into the mouse wound using the third mouse model (athymic nude mice). WL, FL images using PRODIGI™ as well as BLI images using the Xenogen IVIS Imaging System 100 were taken of the wound every day as it became infected. Bacterial amount from FL images was measured and quantified using ImageJ Version 1.45. RGB channels were separated and the average red channel intensities in the hand-drawn wound areas were recorded. The BLI images were captured using Living Image In Vivo Imaging software, with the total intensity counts recorded in a fixed circular area at all time points. The FL signals were correlated to the absolute bacteria amount measured from the BLI signals.
Figure 12 BLI imaging of bacteria.
A) Example of BLI imaging of S. aureus Xen8.1 in vitro on culture plates at different concentrations. Higher CFU counts give rise to higher BLI intensities (blue color). B) An example of a mouse inoculated with S. aureus Xen8.1 vs. a control mouse. The figure shows non-invasive longitudinal BLI imaging tracking of the bacteria over 5 days.

4.4.2 Results

7 mice were imaged in this experiment over 7 days. There was a strong positive correlation between the bacterial fluorescence captured by PRODIGI™ and the absolute bacterial load measured by the Xenogen imaging system: Pearson correlation coefficient 0.6889, p-value 0.04 (Figure 13). Both imaging modalities showed an increase in bacterial load from Day 0 to Day 4. This initial increase in bacterial load is likely due to the fact that bacteria were introduced into the hosts and the bacteria colonized and grew in numbers. After Day 4 both imaging modalities showed a drop in bacterial load. This may be partially caused by the removal of bacteria on the wound surface by the Tegaderm dressing change; it could also be due to the mouse immune system not being completely compromised. Overall, the FL intensity has a correlated trend to the BLI intensity (absolute bacterial load) as the bacteria load increases and decreases in wounds.

It is important to note that while fluorescence intensity increased by approximately 3 folds, the bioluminescence intensity increased by approximately $10^4$. This is a significant different in the scaling of the two intensities. BLI is still the standard for accurately detecting the absolute bacterial load in a wound, both superficial bacteria and underlying bacteria. When the BLI intensity increases, it means the bacteria load, both superficial and underlying, are increasing. On the other hand, FL imaging only detects the
superficial bacterial load (<1.5mm depth). FL imaging can provide a good prediction of the bacterial load on the surface of the wound, but may not predict the absolute bacterial load deep in the tissue.

At the beginning, a spike was observed in the BLI intensity after inoculation, but was not observed in the FL intensity. This can be explained by two factors: 1. Portion of the inoculated bacteria were deep under the skin and could not be detected by FL imaging, but BLI imaging could detect bacteria in deep tissue. So the intensity of the BLI had a large spike after inoculation but not the FL intensity. 2. *S. aureus* fluorescence green instead of red when in media and the fluorescence intensity shown here was calculated by filtering the red fluorescence only. Although the bacteria were suspended in PBS before inoculation, the FL intensity post inoculation could still be green after the inoculation, as seen in Figure 13. This could have contributed to the low red fluorescence intensity right after inoculation.

![Figure 13 Fluorescence intensity](image)

(A) as a function of time compared to Bioluminescence intensity (B) in the mouse skin wound model (n=7). The AF and the BLI signals were monitored from Day 0 (pre-inoculation and post-inoculation) to Day 7. Both intensities showed an increase from Day 0 to Day 4 and a drop after Day 4 to Day 7. Standard errors are shown in the graphs.

An example of a mouse tracked over time using WL imaging, FL imaging, and BLI imaging is shown in Figure 14. The wound on the right was inoculated with *S. Aureus* on Day 0 (bacteria appeared as green on FL image). The bacteria colonized the wound and grew in number after the inoculation. In this case, the FL detected surface bacteria peaking on Day 3 and the BLI detected all bacteria, both surface and deep tissue, peaking on Day 4. Both intensities dropped after Day 4.
Figure 14 Example of longitudinal monitoring of white light (top row), fluorescence (middle row) and bioluminescence (bottom row) from Day 0 (pre-inoculation) and post-inoculation to Day 7.

The FL signal increased from day 0 to day 3, while BLI continued to increase to day 4. Both signals dropped after day 4. The red arrows indicated the day that the Tegaderm dressing was changed, during which surface bacteria loads may be removed from the wound.

4.5 Summary

In conclusion, infected skin wounds were created using 3 different mouse models, of which the third model using female NCRNU-F homozygous nude mouse model worked best, followed by the second model. The third model eliminated the need for shaving hairs, used nude mice that lack immune systems against *S. aureus*, and had an optimized protocol such as the using of Tegaderm. The second model was used successfully to test PRODIGiTM’s ability to track bacterial load over time, before, during and after antibacterial treatment. Bacteria were visualized on fluorescence images in every day over the course of 10 days. Using the third model, the fluorescence intensity correlated with the absolute bacterial load as determined using the BLI signal over time and can be a good predictor of the superficial bacteria load in a wound.
5.1 Objectives

The clinical trial took place at the JDRTC, also known as the Ontario Wound Care Centre. The UHN Research Ethics Board (REB) approved the trial for using PRODIGITM on chronic wounds (REB # 12-5003) and the trial is also listed on clinicaltrials.gov (identifier NCT01651845). The main objectives were 1) to integrate PRODIGITM into the current clinical workflow; 2) to determine whether FL imaging provides a better guidance to target bacterial swabs to specific areas of bacterial load; and 3) to determine whether FL-guided debridement and topical treatment result in lower overall bio-burden and faster wound closure.

5.2 Investigational Testing Authorization renewal and amendment

The initial trial was approved in 2012, with annual renewal required. The information on the current trial was listed in the Investigational Testing Authorization (ITA) renewal, including the protocol, the patient recruitments and adverse events if any. The form was submitted and approved in June 2013 (Figure A4). There were also changes made to the trial protocol since its initial creation. An amendment was filed to accommodate the changes (Figure A5), which were as follows:

A) In the previous clinical trial protocol, area tracing was to be done by hand using a 1 mm tipped marker. To ensure better accuracy and a faster procedure, an electronic Samsung Galaxy Note 10.1 tablet was purchased with a fine tipped electronic 's' pen for area tracing. White light and fluorescence images are loaded into the tablet and the areas on the WL and FL images are traced using this electronic pen. Images are then processed using the MATLAB traceArea program.

B) The wound depth is not measured. (However, in future this capability will be built directly into the PRODIGITM device).

C) Bates-Jensen Wound Status Tool (BWAT) scores and Pressure Sore Status Tool (PSST) scores are not used at JDRTC, so this component was removed.

D) The previous protocol specified for one wound to be randomly selected from multiple wounds that may be found on a patient. However, here, each wound was a deemed an appropriate study wound.
E) In the previous protocol, the contralateral limb was used as a control for each patient. Here, the patient's wound is its own control. The "non-guided" period of assessment then acts as the control for the "guided intervention" period.

F) Previously, microbiology swabs were taken based on both the white light and fluorescence images. This was clarified for the present trial: clinicians will take swabs based on white light images, while scientific personnel will take additional swab(s) according to the fluorescence images.

G) The intervention period from the previous protocol was altered. There are now two periods defined: the non-guided control period and the fluorescence-guided interventions period. These two periods can be repeated. The guided intervention will examine the influence of the fluorescence images on the treatment regimen, while the non-guided periods serve as controls.

H) The case report form was updated.

### 5.3 Methods

23 patients (males and females) were enrolled in the first REB-approved clinical trial. Patients were included with the following criteria: 1) aged 18 years or older and consenting to the study; 2) presenting with chronic wound(s) on the lower limb(s), ideally presenting with diabetic foot ulcer(s); 3) not presented with contra-indication to routine wound care or monitoring.

Since PRODIGI™ is shown to be capable of detecting superficial bacteria in a wound and allowing clinicians to visualize the location(s) of the bacteria, it adds a new quantitative measurement to assess the bacterial load on a wound surface, and the standard procedure maybe altered because of this real-time information. Hence, there were two objectives to this trial: firstly, to determine whether FL imaging provides better guidance to target bacterial swabs to specific areas of bacterial load and, secondly, to determine whether FL-guided debridement and medications result in lower overall bio-burden and faster wound closure.

In collaboration with University Health Network (UHN) Biostatistics, analyses focus on confidence interval estimation of parameters. Hypothesis tests are interpreted with caution, as there was no formal pre-trial power calculation. A mixed-statistical model using SAS v9.2 was employed with the following study outcome measures obtained over time:

- wound size,
- WL-based decisions if wounds have clinically significant bacterial load,
• FL imaging-based decisions if wounds have clinically significant bacterial load,
• total bacterial load and amount of connective tissue in the wound bed,
• periphery and off-site based on FL images,
• microbiology results per swab (semi-quantitative growth scores, bacterial species, and Gram signing)

5.3.1 PRODIGI™ guided bacterial swabs

The use of fluorescence image guidance may change the way clinicians take microbiology swabs. The current standard of practice is to take swabs at the center of the wound when there is a suspected infection area. Swabs are sent to a microbiology lab (Gamma Dynacare) to be analyzed. A report is generated 3-5 days later, with the bacterial species identified and a semi-quantitative measure of the bacteria growth (occasional, light, moderate, or heavy). The report also provides the susceptibility result, if any, to give guidance to the clinician on which drugs should or should not be used on the wound. Some of the drugs tested at Gamma Dynacare are clindamycin, erythromycin, oxa/cloxacillin, penicillin, and trimethoprim/sulfa. However, if these swabs were always taken at the center of the wound without guidance, the microbiology results may not be accurate to treat the entire wound area. For instance, if the main bacterial load was on the peripheral of the wound but the swab was taken from the center of the wound, the report could come back as negative but would not reflect the condition of the entire wound area. The ideal way of swabbing is to target where the bacterial load is, in this example at the peripheral of the wound instead of the center. the concept is then that PRODIGI™ will guide the clinician to take swabs at the specific areas in the wound where the bacteria are located.

In this study, both WL images and FL images of selected wound sites were collected using PRODIGI™ once weekly at the JDRTC. Images were collected in 1-2 s from a distance of approximately 10 cm. The WL images were taken under room lighting and the FL images were taken by illuminating the wound site with violet-blue excitation. For each wound, the clinicians collected swabs according to the WL image, while the trial personnel independently collected swabs according to the FL image at the locations of red or green fluorescence. Areas of the swabs taken were marked on the corresponding WL or FL photographs. Results of the normal WL swabs and the FL-guided swabs were compared to determine if FL-guided swabs captured areas with bacteria that were different from the area of the normal WL swab. For example, there may be areas of swabbing due to the red fluorescence in the FL images but not noticed in the WL images. The types of bacteria, growth of bacteria, and susceptibility of bacteria were also
compared to determine if the FL-guided swabs provided clinicians with new information on the wound. Furthermore, the bacterial growths in the swab reports (occasional, light, moderate, and heavy) were correlated with the red fluorescence on the images. True positive, true negative, false positive and false negative findings on the FL images were assessed to determine the sensitivity and specificity of PRODIGI™ in detecting bacteria.

5.3.2 PRODIGI™ guided debridement and medications

The use of fluorescence imaging may also change the way clinicians debride and apply topical treatment to the wounds. The debridement removes the top layer of necrotic tissue and biofilm - ideally all of the bacteria in the wound bed - such that the wound can turn into an acute state with healthy tissue trying to recover to wound closure. However, commonly, not all bacteria are removed in debridement, so that topical antibacterial treatment is often applied to kill the residual bacteria. Currently, there is no measure on if and how much bacteria are present in the wound after debridement or where any residual bacteria are located. It is postulated that PRODIGI™ can help guide the debridement by generating a fluorescence image prior to debridement and then guide the topical treatment by fluorescence imaging post debridement. This would allow maximum bacteria removal during debridement and effective usage of antibacterial treatment targeted to the residual bacteria after debridement.

The trial was separated into a control period and a test period, performed on each of the patients enrolled. Both WL images and FL images were taken for both periods. During the control period of 2 months, clinicians performed debridement and topical treatment as per their standard procedures under WL without seeing the FL images. During the test period, FL images taken prior to debridement were shown to the clinician so that bacterial load in the wound could be targeted during the debridement. FL images taken after the debridement were also shown to the clinician so that the topical antibacterial treatment could be targeted to the areas of residue bacteria and appropriate systemic medications can be prescribed according to the bacterial load. The patients were tracked over a period of 4 months and two measurements were collected from the procedure: 1) wound size, measured by tracing the wound edge on the WL image (a ruler is visible in all wound pictures) and 2) bacterial load, quantified using MATLAB software and also confirmed by the bacterial swab reports. It would be expected that the FL image-guided patient group can lead to a faster and better wound healing process, with faster wound closure and lower bacterial loads over time.
5.4 Results

Patients were enrolled on a rolling basis, and 23 patients in total were enrolled. 7 patients have been tracked since Jan 30, 2013 (of which 2 withdrew from the study due to long commutes and other surgery), 5 patients have been tracked since Jan 31, 2013 (of which 2 withdrew due to physician transfer and personal reasons), 1 patient has been tracked since Feb 27, 2013, 1 patient has been tracked since Mar 27, 2013, 4 patients have been tracked since Apr 3, 2013, 2 patients have been tracked since Apr 17, 2013, 1 patient has been tracked since May 8, 2013, 1 patient has been tracked since May 15, 2013, and 1 patient has been tracked since July 17, 2013. Patients were followed with both WL and FL images before and after debridement.

5.4.1 PRODIGITM accurately detects bioburden in the wound bed, periphery and offsite, assisting bacterial swabbing

619 swabs were taken either by clinicians or research staff on 23 patients over the course of 8 months. Swabs were taken before and after each debridement session. In total, clinicians took 63 swabs and the researchers took 556 swabs. In the 63 swabs taken by clinicians, 62 were confirmed to have bacteria present and 1 was negative for bacteria. In the 556 swabs taken by researchers, 417 swabs were taken with fluorescence visible on the images and 139 swabs were taken without visible fluorescence. In the former, 414 swabs were confirmed to have bacteria present and 3 swabs were negative for bacteria. In the 139 swabs taken without fluorescence on the images, 132 swabs were confirmed to have bacteria present and 7 swabs were negative. The results are summarized in Table 1.

Table 1 The distribution of true positive, false positive, false negative and true negative from the swab results (556 swabs taken by researchers in total)

<table>
<thead>
<tr>
<th>True positive</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>414 swabs</td>
<td>3 swabs</td>
</tr>
<tr>
<td>74.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td>False negative</td>
<td>True negative</td>
</tr>
<tr>
<td>132 swabs</td>
<td>7 swabs</td>
</tr>
<tr>
<td>23.7%</td>
<td>1.3%</td>
</tr>
</tbody>
</table>
These results indicate that PRODIGI™ is capable of detecting bacterial loads and guiding swabs accurately in 99.3% (414/417) cases when fluorescence is seen on the FL image. This accuracy is comparable to that of conventional swabbing at 98.4% (62/63). However, of the 608 swabs confirmed to have bacterial presence, clinicians only took 62 swabs (10.2%) under standard WL evaluation and missed 89.8% of the positive swab results because they did not think that a swab was needed for the wound. On the other hand, 68.1% (414/608) of the swabs with positive microbiology findings were taken with positive FL indications of bacterial load. The clinicians took swabs in 43 patients visits. And in 153 visits when they did not take swabs, FL-guided swabs found positive results.

Moreover, the sensitivity (positive predictive value) and specificity (negative predictive value) of the PRODIGI™ guided swabs can be calculated from the formula below:

\[
\text{Sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}
\]

Hence, the sensitivity of the PRODIGI™ guided swabs = \(\frac{414}{546} = 75.8\%\).

\[
\text{Specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}
\]

Hence, the specificity of the PRODIGI™ guided swabs = \(\frac{7}{10} = 70\%\)

The scoring system was then implemented for each of the swabs. For each bacteria species found from the lab microbiology results, a number was assigned to the amount of growth: 0 for no bacteria, 1 for occasional growth, 2 for light growth, 3 for moderate growth and 4 for heavy growth. There could be more than one type of bacteria species growing in one swab, in which case the scores were added to get the final score. The average scores were comparable (p=0.17 one tailed distribution), 4.3 and 4.5 for the standard clinician swabs and the fluorescence-guided swabs, respectively. But a significantly larger numbers of fluorescence-guided swabs were taken as compared to the white light swabs and fluorescence-guided swabs targeted bacterial loads in different areas of the wounds while the conventional swabs mostly sampled only the center of the wound.

The locations of the swabs taken under fluorescence were then investigated. In particular, locations of the red fluorescence (e.g. S. Aureus) were compared to those of the green fluorescence (e.g. P. Aeruginosa). Three locations were analyzed for each of the two colors: center of the wound, periphery of the wound, and offsite of the wound. The results are summarized in Table 2.
### Table 2: Fluorescence locations in and around the wound bed (#of swabs)

<table>
<thead>
<tr>
<th></th>
<th>Center</th>
<th>Periphery</th>
<th>Offsite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red fluorescence</td>
<td>86</td>
<td>241</td>
<td>7</td>
</tr>
<tr>
<td>Green fluorescence</td>
<td>62</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

Thus, 72.2% of the red fluorescence found was at the wound periphery, while 71.3% of the green fluorescence was at the wound center, so that there was a clear trend, with bacteria such as *S. aureus* mostly residing in the periphery and bacteria such as *P. aeruginosa* mostly in the wound center. There is no obvious explanation for this novel finding, but possible factors include the different pH, moisture level and temperature. It was also observed that in some wounds the red fluorescence tended to close in with the wound, from the periphery to the center, as the wound closed, likely due to the change in wound microenvironment with healing.

### 5.4.2 Quantitative guidance of wound debridement and medication

Based on PRODIGI\textsuperscript{TM}'s ability to track the wound status over time, we investigated whether FL imaging could be used to guide wound treatment (debridement and topical/systemic antibiotics) over time to reduce the bioburden and help close the wounds faster than conventional management. Hence, wound areas were tracked in 12 patients (13 wounds) over approximately 6 months, comprising three contiguous 2-month periods, comparing the rate of wound closure and treatments delivered without FL imaging guidance (control) and those utilizing FL imaging guidance. While this pilot study was based on a relatively small sample size, the initial data suggest a possible clinical benefit of using fluorescence guidance to focus treatment on the area(s) with high bacterial load, which cannot be achieved with the conventional treatments.

As shown in **Figure 15**, there was a slight decrease in average wound size in the first control period (slope \(-0.003 \text{ cm}^2/\text{day}\)). However, when fluorescence imaging was used to target debridement procedures and antibiotic application, the slope increased (to \(-0.046 \text{ cm}^2/\text{day}\)) and this was statistically significant (p =
When image guidance was then not used in the second control period, we observed a slight increase in the average wound area over time (slope +0.007 cm$^2$/day) that was significantly different from the response during the fluorescence-guided period ($p = 0.010$). For comparison, from extrapolation of the average slopes, standard treatment had an average wound closure of 0.09 cm$^2$ per month during the first period and a increase of 0.21 cm$^2$ per month during the second period, whereas fluorescence-guided treatments had wound closure of 1.38 cm$^2$ per month over the same period. Hence, FL-guided treatments leads results in significantly faster wound healing as compared to conventional treatment.

**Figure 15** Wound closure in FL-guided treatment period compared with conventional WL treatment period.  
A) wound area measurements for 12 patients (13 wounds) as a function of time from the onset of the study over the first control period (blue), the FL image guided period (red), and the second control period (green). During the control periods, treatments were administered without real-time FL image guidance.  
B) plot of the average rate of change in all wounds over the course of the study estimated from the regression model. The slopes and $p$ values are shown for each study period.
Moreover, we investigated the fluorescence intensities before and after debridement during each of the visits. Debridement removes the top layer of biofilm and necrotic tissue on the wound surface such that healthy tissues underneath are exposed and given a chance to heal in an acute wound state. Currently, there is no standard as to when to stop the debridement and clinicians typically debride until the wound bleeds. If the debridement is not deep enough, residual bacteria can repopulate the wound, but if the debridement is too deep then healthy tissue can be unnecessarily removed. FL imaging reflects the amount of bacteria before and after debridement, as well as the locations of the bacteria in the wound. Although these images do not determine directly when to stop debridement, they do indicate whether there are more or less bacteria underneath the necrotic tissue as debridement proceeds. Based on this information, the clinician can decide to continue debridement or prescribe an appropriate medication (topical or systemic) to combat the remaining bacteria load.

Of the 13 wounds analyzed, 10 showed higher bacterial FL signal after debridement compared to before the debridement. This can be due to the fact that debridement removed the top layer of dead tissue and so revealed the underlying bacteria (given the limited penetration of the violet/blue excitation light). In the 3 wounds that showed lower bacterial FL signal after debridement, the bacteria may have been superficial to begin with, so that the debridement removed these. An example is shown in Figure 16 in which the fluorescence intensity was mostly higher after debridement.
**Figure 16** Example of wound monitored over 12 visits.

A) Top row shows WL images, middle row shows FL images, and bottom row shows bacterial quantification. Topical medication was already applied on day 7 before the image was taken post-debridement. B) Trend of fluorescence (before and after) over each visit.
Figure 17 shows another example of fluorescence intensities before and after debridement. After a thorough ultrasonic debridement on this patient’s left heel wound on the first visit (focused on the periphery where bacteria were seen under fluorescence image) and the application of heavy iodine focused on the area with residual bacteria, a marked decrease in bacterial load was seen on the second visit 21 days later. Hence, FL image guided debridement and topical medication helped this patient in removing bacteria in the wound effectively.

Figure 17 Wound example showing decreasing in wound size.
A) Photograph of the ultrasonic debridement of a diabetic foot ulcer on a 62-year-old male patient during the first visit. B,C) WL, FL, and quantified FL images taken before and after debridement, 3 weeks apart. Fluorescence imaging revealed presence of bacteria deep into devitalized tissue at the wound periphery (white arrow), invisible to WL visualization. d) Bacterial load comparison between the two visits, before and after debridement. *Scale bars represent 2cm.*
5.4.3 Detection of subsurface bacterial burden

There were instances where offsite or peripheral areas were swabbed under the FL imaging guidance that were missed by WL assessment and were later confirmed to have bacteria present.

**Case Report** A 50-year-old man presented with a diabetic foot ulcer on the sole of his right foot. Comorbidities included obesity, hypertension and Charcot foot. In 1995, this patient experienced a motorcycle accident, resulting in a fractured right femur and severe nerve damage in his lower right extremity, causing a drop foot. In 1997, he was diagnosed with type I diabetes, which has since been poorly maintained. In 1999, an ulcer on the mid-plantar surface of his right foot was discovered; surgery was performed in 2002 to remove this ulcer. Post-surgery, the patient injured himself in the midst of physiotherapy sessions, fracturing the tarsal, mid-tarsal and calcaneus of his right foot. An orthopedic surgeon recommended amputation of the right foot; however, after seeking a second opinion, he has been treated since 2007 at the JDRTC with hyperbaric oxygen therapy (HBOT), various debridement techniques and antibacterial medications. Additional surgeries were performed to clip the protruding bones and to reconstruct the foot. This wound has closed and re-opened numerous times. During the control period from January to March 2013, FL imaging detected the presence of bacteria below the actual wound at the mid-foot area that was missed under standard WL. Swabs were taken at this location and confirmed to have moderate growth of mixed bacteria (gram positive cocci) (**Figure 18**).
The patient presented at his weekly visit on Apr 10, 2013 with hyperkeratosis (6cm X 8cm), which was examined under standard white light (Figure 19A), based on which the clinician was uncertain if debridement was necessary. A fluorescence image (Figure 19B) was then taken, revealing an area of bright red fluorescence at the wound site below the fifth toe, indicating the presence of bacteria in the wound, based on which the clinician decided to debride with a scalpel and curette, exposing the underlying tissue. During the procedure, a second fluorescence image revealed a large area of red fluorescence medial to the primary wound site (Figure 19D), based on which debridement was continued and a secondary wound was revealed, which was also not visible under white light. A post-debridement image of the 6 mm X 2 mm wound was taken under both white light and fluorescence light (Figure 19E,F). The wound was dressed with iodine and gauze and the patient was prescribed 500mg of CIPRO®. Bacterial load was quantified (area in cm²) with in-house threshold-based software using MATLAB (Version 7.9.0; Figure 19C,G).
Figure 19 WL and FL images of the wound on Apr 10

(A) WL image (plantar view) before debridement, (B) FL image before debridement, (C) Quantitative FL images of bacterial load before debridement, (D) FL image during debridement, (E) WL image after debridement, (F) FL image after debridement (G) Quantitative FL images of bacterial load after debridement.
Chapter 6
Discussion

6.1 Clinical decision changes due to fluorescence imaging

From the results shown above, fluorescence imaging was shown to give clinicians the information needed to guide management that lead to faster wound healing. From the case report forms taken during the interventional period, treatments before seeing FL images and treatments after seeing FL images were compared for individual visits. For debridement, the clinicians usually debride the wound center and take off some of the dry skin around the wound before seeing the FL images, but after seeing the FL images they would focus the debridement on the areas with bacterial load. After debridement, if there was significant fluorescence visible, heavy antibacterial medication (e.g. iodine) would be used topically and systemic antibiotics would be considered if the clinical signs and symptoms suggested infection. On the other hand, if there were little fluorescence apparent, heavy antibacterial medication would be avoided as it can cause dry skin and prevent wound healing, and a less potent topical antibacterial medication would be used (e.g. a silver compound). During the 2 months of guided period, there were 38 out of 69 visits where the clinicians changed their intervention decision after FL images were presented to them. The changes can be the debridement method (scalpel vs. ultrasonic), the depth of debridement, the use of skin grafting, and the type of medication used. When the clinicians decided to keep the same interventions after seeing the FL image, the FL image helped confirming their initial decision.

Moreover, there were instances where areas of bacteria were totally missed during wound care but were detected using fluorescence imaging. Below are two examples of bacterial pockets hidden between the toes (Figure 20). Clinicians normally ignored these areas during routine wound cleaning since they are apart from the wound and the skin looks intact. However, these diabetic patients tend not to have feelings at the bottom of their feet and can easily get a wound on their feet. If there are already bacteria living on the skin it will be very easy for the wound to get infected. After seeing the fluorescence images, the clinicians can see the areas of bacteria and clean these areas during the cleaning routine to prevent contamination. These two examples were swabbed and confirmed to have heavy growth of bacteria.
In regards to the bacterial swabbing, FL guided swabs have found many areas with bacteria missed by WL swabs. Of the 608 swabs confirmed to have bacterial presence, only 10.2% would be taken using the conventional swabbing techniques and 89.8% of the positive swabs would have been missed without the guidance of fluorescence. The missing positive swabs (89.8%) were mostly on the peripheries of wounds or offsite of wounds, sometimes centers of wounds, as the conventional WL swabs take the centers of the wounds only. The locations of the swabs can significantly affect the results, as most *S. Aureus* findings were located on the peripheries and offsite of wounds whereas most *P. aeruginosa* findings were located in the centers of wounds. Thus if swabs are always taken in the centers of wounds, the findings will be skewed towards high *P. aeruginosa* findings and low *S. aureus* findings. After knowing the fluorescence images, clinicians can swab according to the areas with bacteria, instead of simply relying on clinician signs and symptoms in the centers of wounds.

### 6.2 Technical and procedural challenges

There were several limitations during the clinical trial data collection and analysis that could have affected the results and conclusions of the trial. Some were caused by the clinical workflow and some were caused by the technology of the imaging device.

In the general clinical workflow:
- Swabbing was difficult when the patient’s skin was dry and hard. So some swabs were taken from the superficial surface of the dry skin. This may not be as accurate as if the swab was taken in a wet wound with a large amount of discharge. Also centers of the wounds tend to be wetter than the peripheries of the
wounds, so this could have caused a lower bacterial count in the FL-guided swabs, as FL-guided swabs tend to swab more around the peripheries of the wound. Biopsy or tissue collection from the debridement procedure can be an alternative in the future studies.

- The depth of debridement was not always consistent. Physician will be deciding and controlling the depth of debridement during each visit. Although Dr. Linden was usually the person who does debridement, Rachel and Kim sometimes do debridement when the schedule is busy, he can differ debridement depth from day to day depending on his schedule and his own judgment that day. Other factors include patient’s pain tolerance and equipment availability etc. and these factors can cause some inconsistency to the result of the study.

- Routine cleaning procedures were variable. The patients were seen only at the clinic and only the cleaning procedures could be controlled at the clinic. Home care could not be controlled during this study but dependent on the patient or the home care provider. This could be a big factor since the patient only visited the clinic approximately once a week and the rest of the week was not cared by the clinicians. Future studies should involve both the clinic and the home care if possible.

Challenges with the technology:

- The room was kept dark during most of the imaging points with all doors closed. However, when there were two patients seen at the same time in the treatment room, some ambient lighting would exist due to the small lamplight from the other bed. Depending on the amount of background light the MATLAB program used for calculating bacterial red fluorescence does not quantify the actual red fluorescence perfectly. Sometimes the software could consider background red light as bacteria. In this case, manual cropping of pictures would be needed on images with the background light before the red fluorescence quantifications. The procedure for cropping the pictures is listed in the SOP and by only quantifying the wound area without the background eliminated the effect of background interference. A true fluorescence value could be obtained. In the future, when the trial extends to multi-centered visits, it will be important to keep room background as consistent as possible. One solution is to create a non-transparent cover to cover the wound such that all images are taken under the cover to ensure no background lighting exists inside the cover.
- There was also potential variability in calibrating of the wound areas on the images due to the curved tissue surface. Since the foot is round in shape, calibrating stickers were placed in a parallel plane to the camera as much as possible. Slight deviation, possibly 1mm, between pictures could occur if the sticker was slightly on an angle to the camera.
Chapter 7
Device Integration

One of the goals of this clinical engineering thesis project was to integrate the new imaging system into the wound care center. Current wound care clinic is not designed to have a fluorescence imaging system in the treatment room and the wound care workflow does not use a fluorescence imaging system during a treatment. So acquisition/making of new accessories, modification of old equipment, workflow planning with PRODIGi™, and maintenance of PRODIGi™ are necessary to ensure the proper integration into the wound clinic.

7.1 Current wound clinic

First of all, the current clinic layout and workflow are studied at the JDRTC. A sketch of the treatment room is shown in Figure 21. There are two treatment beds in the treatment room. The clinical team tries to take one patient in the treatment room at a time to allow patient privacy, but two patients are seen in the treatment room, when schedule is busy, with a curtain separating the two beds. One clinical cart is placed in the center of the room, shared between the two beds. Two magnifying lamps are mounted on either side of the clinical cart. A lot of medical equipment are placed on this cart, including gloves, tapes, alcohol swabs, gauze, tongue depressors, bacterial swabs, curettes, and the patient specific surgical equipment and topical medications.

Therefore, there is not a lot of free space to place the PRODIGi™ system on the clinical cart. And the PRODIGi™ does not have any dock to secure its location if placed on the cart. If the power cord gets pulled, it can be dragged and fall onto the ground. Moreover, there is no power outlet on the clinical cart so PRODIGi™ cannot be plugged in from the center of the room but from the walls of the room. Last but not least, there is no designated area for placing the PRODIGi™ case containing accessories, such as the power cord, battery charger, lens filters, screwdrivers etc. (Figure 22).
Figure 21 A sketch of the JDRTC Wound care room.

Figure 22 The device and its accessories do not have a good place to be placed in the treatment room.
7.2 Integration of PRODIGI™

7.2.1 PRODIGI™ Cart

So the first thing PRODIGI™ needs is a table or surface such that it can be placed without disturbing the normal clinical workflow. From observations of the clinical workflow, the clinicians perform procedures and gather medical supplies in the center of the room and around the two treatment beds such that there is a free area located on the left side of the room against the wall between the medical cabinet and the chair (Figure 23). Also it is ideal if this table can be wheeled around the room between the two different patients. A cart with four wheels was acquired to provide a designated area to place PRODIGI™. The cart is 45cm long X 50cm wide, approximately a third of the total width of the wall span and was able to fit comfortably between the cabinet and the chair against the wall. The cart is wider than the PRODIGI™ case, providing enough space to place the device and accessories. A power outlet is located just above the cart so the device can be easily plugged into the power outlet. And the power cord can hang along the wall to avoid tripping over the wires when walking across the room (Figure 23).
The cart has three levels in total to allow storage of the device accessories (Figure 24). The top surface can be used as a table, PRODIGI and notepad can be placed on the table to allow quick access to imaging and take notes. The second level is the space underneath the surface table and can be used as a temporary storage of consent forms, consent checklist, case report forms etc. The third level is a large compartment, which can be used to store the PRODIGI™ suitcase along with all the tools and accessories.
Secondly, it is convenient to have a docking station to hold PRODIGI™ on a flat surface so it is secure and placed in an upright position. As mentioned earlier, PRODIGI™ is not stable when simply lying on the table. If the device is lying with the LEDs facing down, the LEDs can get damaged and the hinge of the LEDs can be bent. If the device is lying with the LEDs facing upwards, then there are only three points of contact so it can be easily pulled off the cart by the cable. Furthermore, upright position reduces the footprint required on the table, providing more space to place notes and other medical equipment. Lastly, The dock allows the camera screen to be seen without touching the device. This is important for sterile situations where minimal contact with the device is needed.

A prototype docking station was designed and fabricated by machining hard Styrofoam such that PRODIGI™ sits tightly in the dock, as shown in Figure 25. The dock does the job well by holding PRODIGI™ in the upright position securely. However, this is a temporary solution, future designs can be machined with plastic or metal, which can provide more stability and enduring.
7.2.4 Samsung Tablet

In the previous trials in the lab, annotation of the areas swabbed can be very time consuming and labor intensive. Pictures had to be printed and marked by hand. The process required connecting the camera to a computer, selecting the images to print on the computer, walking to the printer at the front desk to collect the printouts, and annotating on the printed images. The process requires approximately 5 minutes to complete. This is a significant amount of time because each patient treatment appointment is usually scheduled for 15-20 minutes. This process can all be done electronically, which saves time and avoid excessive paper printing. Many tablets were compared to determine the most suitable device for annotating. Tablets reviewed include iPad, Toshiba, Nexus, Lenovo, and Samsung. Of which, Samsung Galaxy note 10.1 tablet is the only tablet with a fine stylus and a powerful Photoshop app for annotations, hence was chosen for this annotating images during the patient visits. Pictures taken on the camera can be easily loaded onto the tablet and the image can be annotated directly on the tablet with the fine stylus (Figure 26). On average, annotating on the Samsung Galaxy Note 10.1 tablet takes 1-2 minutes to complete, including connecting camera to the tablet, opening the Photoshop app, and annotating on the tablet. This significantly reduced the working time from the original 5 minutes to 1 minute. Please refer to the JDRTC Standard Operating Procedure to learn how to annotate images on the Samsung tablet.
7.2.5 Case Report Form (Survey Form)

A new case report form (survey form) was created to better assist note taking during the procedures. The previous form only had two main blocks for note the “White Light Imaging Findings” and the “Fluorescence Imaging/Spectroscopy Findings” (Figure 27). There was no space to describe the actual treatment and how PRODIGI™ helped guide treatment during the clinical workflow. The new case report form includes the treatment performed (before and after images included), dressings used (before and after images included), medications prescribed (before and after images included), swabs taken and temperature taken, in addition to the original WL and FL findings (Figure 28). This new form provides more information and includes comparison of treatment/dressing/medication when only WL was used and when FL imaging was added, such we can clearly see how FL imaging affects the decision making in the clinical workflow.
Case Report Form

Date of Examination: _____________________________ Visit number: _____________________________

Patient ID Number: ___________________________ Examiner’s Initials: ___________________________

Patient Age: _______ Sex: ________________

Wound Type: ___________________________ Appendage: ___________________________

White Light Imaging Findings:

Fluorescence Imaging/Spectroscopy Findings:

Bacteriology samples sent to lab.
(Reference number of swab/bx): ____________________________________________

Figure 27 Original Case Report Form
## Case Report Form
**JUDY DAN RESEARCH & TREATMENT CENTRE**

**Date:** ______________  **Patient I.D. #:** __________  **Sex:** M/F  **Visit Number:** __________

**Wound Type:** ______________________________________  **Examiner’s Initials:** ______________

### White Light Imaging Findings:

### Fluorescence Imaging Findings:

### Treatment (before seeing FL image):

### Treatment (after seeing FL image):

### Dressings (before seeing FL image):

### Dressings (after seeing FL image):

### Medications (before seeing FL image):

### Medications (after seeing FL image):

### Swabs:

<table>
<thead>
<tr>
<th>1) Before/After</th>
<th>Left/Right</th>
<th>Location:</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF/RF/NF</td>
<td></td>
<td></td>
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<table>
<thead>
<tr>
<th>2) Before/After</th>
<th>Left/Right</th>
<th>Location:</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF/RF/NF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3) Before/After</th>
<th>Left/Right</th>
<th>Location:</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF/RF/NF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Temperature:

| Before: ___ °C Location: ______; ___ °C Location: ______; ___ °C Location: ______ |
| After: ____ °C Location: ______; ____ °C Location: ______; ____ °C Location: ______ |

### Legend:
- GF = Green Fluorescence
- RF = Red Fluorescence
- NF = No Fluorescence

*Note: The individual patient swab results are filed in a separate folder, as well as electronically in an excel file entitled “JDRTC Patient Swab Results” located on the DaCosta shared drive.*

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**Figure 28 Updated Case Report Form**
**7.2.6 Calibration**

Calibration of the PRODIGI LEDs was initiated in July 2013. The procedure was developed for calibrating System B used at JDRTC, but the procedure can be extended for calibrations of all PRODIGI™ systems. Calibrations should be performed at least twice a year to ensure the system is operating with a consistent power output over time. If the power output is lower or higher than the system standard when illuminating the wound, the imaging result (fluorescence intensity) can be skewed. So it is important to keep the illuminating power consistent. For system B, it should be operating consistently at its maximum power output of 55mW ± 2mW. The calibration results should be recorded in the Research Equipment Calibration Log, stored in the JDRTC trial binder. New calibration log forms can be found on the shared drive under dacostaclinical on ‘RIS File services -> Clinical Trials DaCosta -> JDRTC.

A detailed step-by-step calibrating procedure with pictures is in the JDRTC Standard Operating Procedure. In brief, an analog power meter shown in Figure 29 as well as a circular sensor associated with the power meter are first gathered from the Princess Margaret Hospital 7th floor surgical suite. The sensor is then connected to the power meter. The power meter is turned on and set to measurement range of 100mW. The PRODIGI™ LED should be placed directly on the circular sensor such that there is no gap between the two and the LED is matched with the circular hole on the sensor. The value should be read on the analog dial (top scale of 10) to be approximately 55mW. If the value is different from the 55mW, open the handle using a screwdriver, and find the blue potentiometer at the bottom of the case (Figure 30). To increase the power output, turn the gold switch on the potentiometer clockwise. To decrease the power output, turn the gold switch on the potentiometer counterclockwise. Once the device is calibrated, close the handle cover and record the final value on the Research Equipment Calibration Log.
Figure 29 Testing the LED power output

Figure 30 Turn the potentiometer to adjust the power output
**Power output vs. Potentiometer turns**

An experiment was designed to find the adjustment curve of the PRODIGI™ System B power output in relation to the number of turns on the potentiometer. The power output of the system B was measured at every turn of the potentiometer inside the handle, and all values were plotted on the graph shown in **Figure 31** (power output in mW vs. number of turns on potentiometer). It was found that system B’s power output plateaus at 55mW ± 2mW and before the plateau, and the potentiometer adjustment has a linear relationship with the power output, $R^2 = 0.995$. Hence, turning the switch clockwise on the potentiometer gives a linear increase to the power output in PRODIGI™ system B used at JDRTC.

![LED Power Output vs. Potentiometer Turns](image)

*Figure 31 Linear relationship of the power output vs. the # of potentiometer turns*

The same experiment was performed on other devices by another clinical engineer in the lab (Danielle Starr) and linear relationships were found between the power output and potentiometer turns on both PRODIGI™ system A and system E (**Figure 32**).
The light source is approximately 1 cm² so that the maximum power density from the light of System B on the wound is 0.055 W/cm², which is within the range of international standards of 0.2 W/cm² formulated by the International Electrotechnical Commission (IEC) [60].

7.2.7 Sensitivity testing of PRODIGITM

A sensitivity test was performed to determine the lowest bacterial load detectable by fluorescence using PRODIGITM and bioluminescence using Xenogen. A titration was made in a 96 well plate using bacterial concentration of $10^{11}$, $10^{10}$, $10^9$, $10^8$, $10^7$, and $10^6$ CFU/mL. 200uL of each concentration was pipetted into the wells with triplets (row A, row C, and row E), highest concentration was pipetted in the far left column and every other column to the right contains a concentration one magnitude lower (Figure 33).

It was found that bioluminescence could detect signals at concentration as low as $10^{10}$ CFU/mL, which contains 2X$10^9$ CFU in one well. And fluorescence could detect signals at concentration as low as $10^{11}$ CFU/mL, which contains 2X$10^{10}$ CFU in one well.
7.3 Standard Operating Procedure and Manual

A SOP is created as part of the project, providing a detailed report on how PRODIGI™ was integrated into the clinical workflow at JDRTC. First of all, the SOP introduces the JDRTC, explains how PRODIGI™ works, and describes some of the tests performed on the device such as the power output calibration and the sensitivity of detecting bacterial loads. The calibration is important for the device to function consistently over time and the user should understand the sensitivity limit of the device when imaging. Then the SOP explains the step-by-step procedure of the clinical workflow and shows exactly when PRODIGI™ is used during the workflow. The procedure to annotate the wound on the new Samsung tablet is listed and the procedure to quantify wound area and bacterial load on the MATLAB software is described. Lastly, the limitations of PRODIGI™ and the possible failures of PRODIGI™ are explained. The SOP includes many pictures guiding anyone in the DaCosta Lab to use the system at JDRTC. The table of content is listed below:

Section 1  SUMMARY

Section 2  JUDY DAN RESEARCH & TREATMENT CENTRE

Section 3  PRODIGI™
Also a manual is created by multiple lab members to cover all uses of the device, including but not limited to the ALA trial and the wound care trial, in which the wound care clinical trial section was written as part of this thesis. The manual listed some of the features explained in the SOP, as well as some additional information such as how to operate the Sony camera by itself.

Figure 34 shows a simple diagram showing the step-by-step procedure that can be followed at a wound care clinic. It is included in both the SOP and the manual. It includes the steps of patient set up, white light imaging, fluorescence imaging, swabbing, debridement, dressings and medications, and sanitization of the instrument.
Step 1: Patient Set-Up

Step 2: Prepare PRODIGITM in the white light setting

Step 3: Take white light image

Step 4: Prepare PRODIGITM in the fluorescence setting

Step 5: Take fluorescence image

Is there autofluorescence detected?

Yes

Step 6a: Take swab in area of fluorescence as indicated by the PRODIGITM

Step 7a: Fluorescence guided debridement

Repeat steps 1-5

No

Step 6b: Take swab in areas clinician indicates is necessary

Step 7b: Debridement procedure according to the discretion of clinician

Is there autofluorescence detected?

Yes

Step 8a: Fluorescence guided application of dressings and prescription of medication

Step 9: Sanitization of the PRODIGITM

No

Step 8b: Application of dressings and prescription of medication to the discretion of clinician

Figure 34 Implementing PRODIGITM into the clinical workflow
7.4 Cost analysis

Currently, the average cost of care for a chronic in Canada is reported as approx. $10,000 [4]. We investigated the specific costs at JDRTC (Table 3).

Table 3 cost breakdowns for wound care at JDRTC

<table>
<thead>
<tr>
<th>Items</th>
<th>Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transportation</td>
<td>Toronto Transit Committee (TTC) Wheel Trans: $3 each way, $6 for round trip</td>
</tr>
<tr>
<td>Debridement visit (OHIP)</td>
<td>Minor assessment $21.70 + Tray fee $11.15 + Debridement fee ($20 if the patient has 1 wound, $30 if the patient has 2 wounds, $45 if the patient has 3 wounds, $60.00 if the patient has more than 4 wounds, and $17.45 if the patient needs a nail debridement)</td>
</tr>
<tr>
<td>Follow-up visit (OHIP)</td>
<td>Minor assessment $21.70</td>
</tr>
<tr>
<td>Hyperbaric treatment (OHIP)</td>
<td>Minor assessment $21.70 + Hyperbaric out of chamber (preparation) $71.85 + Hyperbaric treatment fee $35.90/15 minutes</td>
</tr>
<tr>
<td>Microbiology swab analysis</td>
<td>Approx. $28 each for analysis at Gamma Dynacare</td>
</tr>
</tbody>
</table>

- Each debridement visit would cost 6+21.70+11.15+20=$58.85 if the patient has 1 wound, and 6+21.70+11.15+60=$98.85 if the patient has more than 4 wounds. For one year, 52 weeks, the debridement cost lies between $3,060 and $5,140 assuming weekly debridement.
- Each hyperbaric treatment costs 6+21.70+71.85+35.90*7=$350.85 assuming a typical 105 minutes of treatment. For a standard treatment period of 30 sessions (5 times a week for 6 weeks), the hyperbaric treatment costs $10,525.
- Assuming one swab is taken per week for one year, 28*52=$1,456.

So, the total cost for one patient per year currently at JDRTC is approx. $17,121, so that for each day that the wound does not close the cost to the health care system is approx. $47.
Over the course of the study, the average wound closure rate for FL-guided care was -0.046 cm\(^2\)/day, which is significantly higher than the conventional-care rate (results from this particular study period) of +0.001 cm\(^2\)/day (average of -0.005 cm\(^2\)/day and +0.007 cm\(^2\)/day). Hence, hypothetically for a 1 cm\(^2\) wound, FL-guided care would heal the wound in 21.7 days whereas conventional care would not be able to heal the wound, which makes it difficult to compare the costs rigorously. However, assuming that a 1 cm\(^2\) wound requires double the time to heal with conventional care compared with FL-guided care (43 days), the saving (at $47 per day) is approx. $1000. For larger wounds the savings would be greater.
Chapter 8

Summary of Contributions

The work in this Master’s thesis comprised 4 main phases: specific skills training / project proposal, in vivo model studies, clinical trial, and device workflow / optimization.

The training phase included device handling trainings in the DaCosta lab, animal study trainings (UHN short course: wet lab, anesthesia, surgical procedures) and human clinical trial trainings (UHN good clinical research practice, UHN research safety, UHN fire safety).

For the in vivo mouse model studies, I developed the protocol for the mice skin wound model using 3 different methods, and thereby identified the optimal model, based on their suitability for tracking of bacteria over time throughout the treatment course (pre-intervention, intervention, and post-intervention). I also correlated the fluorescence signal to the BLI signal to determine the sensitivity of the PRODIGI™ device in detecting absolute bacterial loads.

In the clinical trial phase, I collected data over a period of 8 months on 23 consented patients. Images (WL and FL), bacterial swabs and case reports were collected on a weekly basis. I investigated the question of whether fluorescence imaging of the bacteria present in and around wounds can guide clinicians with appropriate interventions (debridement, topical and systemic medications) in real-time as well as the question of whether PRODIGI™-guided treatments leads to faster wound healing compared to conventional treatments.

In the device integration phase, I integrated PRODIGI™ into the current wound care workflow, including assessing the current clinic layout and workflow at JDRTC, integrating PRODIGI™ cart into the treatment room, making PRODIGI™ dock, optimizing the annotation process using Samsung tablet, creating a detailed survey form to be used at each patient visits, performing an initial calibration procedure for PRODIGI™, and testing the sensitivity of PRODIGI™ camera. All of these were captured in the standard operating procedure for PRODIGI™ to be used in JDRTC. Furthermore, I contributed to the development of the PRODIGI™ manual and completed a first cost analysis of the current wound care vs. wound care with the integration of PRODIGI™. Last but not least, I assembled two PRODIGI™ devices with the newest design and approved all PRODIGI™ devices for CSA licenses.
Specific accomplishments include:

1. I was the first author in the accepted and published paper to Journal of Biomedical Optics named “Autofluorescence imaging device for real-time detection and tracking of pathogenic bacteria in a mouse skin wound model: preclinical feasibility studies”. This paper included the experimental data collected in the preclinical studies on mice.

2. I was the first author in two other papers that were submitted but not published. The first one was the submission to CMAJ named “A hand-held Fluorescence-Imaging Device for Real-time Wound Assessment at the Point-of-Care”. This was an innovation paper describing the new technology: PRODIGI™, explaining PRODIGI™'s uses as well as its limitations. The second one was the case report submission to International Wound Journal named “Real-time wound monitoring using a handheld fluorescence-imaging device: a case report”. This case report describes one patient whose infection was missed by physician under white light but was detected by the red fluorescence using PRODIGI™. This case report is now included in the submission below.

3. I was a co-author on a manuscript “A Novel Point-of-Care Autofluorescence Imaging Device for Real-time Detection and Tracking of Pathogenic Bacteria in Chronic Wounds: First in-Human Results” submitted for peer-reviewed publication. For this, I provided 6 months of patient-tracking data, analyzing the results to compare the clinical outcomes for PRODIGI™-guided interventions versus non-guided interventions. I also generated the specific case report of PRODIGI™-detected subsurface bacterial burden missed by conventional examination, including the data (FL and WL) and the analysis for comparison of pre and post debridement.

4. I presented at the University of Toronto Institute of Biomaterials and Biomedical Engineering (IBBME) Scientific Day in May 2013 and won first place in the “Engineering in a Clinical Setting” competition. Moreover, I attended the international conference “Wound Care Beyond the Basics: Practical Pearls”, held in One King West Hotel Toronto on February 22, 2013.
The timeline of the work reported in this thesis is shown below:

**Figure 35 Thesis Timeline**
Chapter 9
Conclusions

The completion of this study demonstrated initial evidence that the novel FL imaging device, PRODIGi™, can detect pathogenic bacteria within chronic infected wounds that are invisible under standard WL examination. As a result, it can guide clinical decision making in real-time and improve wound healing to close wounds faster. Both measured wound sizes and bacterial loads were tracked over time to illustrate the healing status in the FL-guided period and the control period. The FL-guided cohort showed significantly faster wound healing as compared to the control period, suggesting PRODIGi™ provided necessary guidance leading to improved treatment management.

The instrument was also successfully integrated into a real-life wound care facility setting, through a Standard Operating Procedure, with a few modifications being identified and implemented to better fit with the clinical environment. The technique of fluorescence imaging of bacteria in and around wounds can be used in an ergonomic and efficient way to complement and supplement the current methods using clinical signs and symptoms for diagnosing bacterial infection, such as pain, swelling and heat. In addition, PRODIGi™ can provide clinicians with the critical underlying information, such as the bacterial load and location, when performing interventions and also post intervention to decide on the medications for the patient.

9.1 Future Studies / Proposed Modifications

This clinical study has showed a promising initial result, and a full multi-centered clinical trial will be needed to provide statistically significant evidence that PRODIGi™ guidance does in fact improve wound-healing rates. In the new trial, variations between clinics and between different physicians need to be considered. Firstly, the environment at different clinics will be different, for example the darkness of the room, which may cause discrepancies between images. One solution would be to use a disposable cover / drape to wrap around the foot and device. Secondly, different physicians have different workflow in treating wounds. Thus, when to take images and where to place the device may have to be varied.

Thirdly, the speed of image acquisition should be improved. The current PRODIGi™ device uses SONY DSC-T200 consumer grade digital camera. This camera is small and easy to use, however, it only has automatic settings such as exposure time and aperture. Also due to the sensor size of the camera, it requires a significant amount of time (approximately 1 to 2 seconds) to capture the image in a dark room. This can cause a blurry image if the camera is not held still or the patient twitches. In the future, singe-
lens reflex (SLR) camera can be a better alternative as it can provide a larger sensor to capture light faster as well as set the exposure time and aperture to be consistent from visit to visit. For example, Sony has the compact SLR NEX-5R, which as a big 16-megapixel sensor that can generate crisp images in challenging conditions such as the dark room.

Fourthly, an experiment with different known bacterial load and fluorescence intensities can be correlated. This can be done using the semi-quantitative bacterial growths scale or with full quantitative titrations of bacterial loads scale. And the corresponding fluorescence intensities can be printed onto a scale with occasional, light, moderate, and heavy growth for semi-quantitative measures and CFU amounts for full quantitative measures. Once this scale is placed beside the wound and a fluorescence image is taken of the wound, the bacteria load can be read instantly on the fluorescence image according to the scale.

If the next full clinical trials confirms the same results that fluorescence imaging is capable of assisting clinicians in deciding interventions to heal wounds faster, this may yield a new, non-invasive and cost-effective technology that will allow clinicians to 1) obtain critical underlying information within the wound as well as the healing status of the wound, 2) provide accurate and effective treatment to infected wounds earlier and heal wounds faster, and 3) measure the therapeutic efficacy to improve patients’ quality of life. Given that is it relatively low cost, simple and ergonomic to use, portable and rugged, PRODIGI™ can potentially have a global impact on wound care, not only in the specialist wound-care center setting but also in point-of-care settings.
## Appendices

### Table A1 Characteristics and Uses of wound dressing materials [61]

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
<th>Description</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>AlgiSite, Comfeel, Curasorb, Kaltogel, Kaltostat, Sorbsan, Tegagel</td>
<td>Alginate dressings are made of seaweed extract contains guluronic and mannuronic acids that provide tensile strength, and calcium and sodium alginates that have absorptive capacity. Some can leave fibers in the wound if they are not thoroughly irrigated. These dressings are secured with secondary coverage.</td>
<td>These dressings are highly absorbent and useful for wounds that have copious exudate. Alginate rope is particularly useful to pack exudative wound cavities or sinus tracts.</td>
</tr>
<tr>
<td>Hydrofiber</td>
<td>Aquacel, Aquacel-Ag, Versiva</td>
<td>An absorptive textile fiber pad, hydrofiber, is also available as a ribbon for packing of deep wounds. This material is covered with a secondary dressing. The hydrofiber combines with wound exudate to produce a hydrophilic gel. Aquacel-Ag contains 1.2% ionic silver that has strong antimicrobial properties against many organisms, including methicillin-resistant <em>Staphylococcus aureus</em> and vancomycin-resistant enterococci.</td>
<td>Hydrofiber absorbent dressings used for exudative wounds.</td>
</tr>
<tr>
<td>Debriding agents</td>
<td>Hypergel (hypertonic saline gel), Santyl (collagenase), Accuzyme (papain urea)</td>
<td>Various products provide some chemical or enzymatic debridement.</td>
<td>Debriding agents are useful for necrotic wounds as an adjunct to surgical debridement.</td>
</tr>
<tr>
<td>Foam</td>
<td>LYOfoam, Spyrosorb, Allevyn</td>
<td>Polyurethane foam has absorptive capacity.</td>
<td>These dressings are useful for cleaning granulating wounds with minimal exudate.</td>
</tr>
<tr>
<td>Hydrocolloid</td>
<td>CombiDERM, Comfeel, DuoDerm CGF Extra Thin, Granuflex, Tegasorb</td>
<td>Hydrocolloid dressings are made of microgranular suspensions of natural or synthetic polymers, such as gelatin or pectin, in an adhesive matrix. The granules change from a semi-hydrated state to a gel as the wound exudate is absorbed.</td>
<td>Hydrocolloid dressings are useful for dry necrotic wounds, wounds with minimal exudate and for clean granulating wounds.</td>
</tr>
<tr>
<td>Hydrogel</td>
<td>Aquasorb, DuoDerm, Intrasite Gel, Granulge, Normigel, Nu-Gel, Purilon Gel, KY Jelly</td>
<td>Hydrogel dressings are water-based or glycerin-based semipermeable hydrophilic polymers; cooling properties may decrease wound pain. These gels can lose or absorb water depending upon the state of hydration of the wound. They are secured with secondary covering.</td>
<td>These dressings are useful for dry, sloughing, necrotic wounds (eschar).</td>
</tr>
<tr>
<td>Low-adherence dressing</td>
<td>Mepore, Release, Skintact</td>
<td>Low-adherence dressings are made of various materials designed to remove easily without damaging underlying skin.</td>
<td>These dressings are useful for acute minor wounds such as skin tears, or as a final dressing for chronic wounds that have nearly healed.</td>
</tr>
<tr>
<td>Transparent film</td>
<td>OpSite, Skintact, Release, Tegaderm, Bioclusive</td>
<td>Transparent films are highly conformable acrylic adhesive films with no absorptive capacity and little hydrating ability. They may be vapor permeable or perforated.</td>
<td>These dressings are useful for clean, dry wounds with minimal exudate. They also are used to secure an underlying absorptive material, to protect high-friction areas and areas that are difficult to bandage (e.g., heels) and to secure intravenous catheters.</td>
</tr>
</tbody>
</table>
Figure A1 PRODIGI™ uses a band pass filter at 405±20 nm for excitation, and dual-band filters for the 500-550 nm (green) and 590-670 nm (red) emission. The image would show up with both red and green colors.
## Competitors

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Product</th>
<th>Features</th>
<th>Cost (USD)</th>
</tr>
</thead>
</table>
| AranzMedical:          | Silhouette Mobile           | • PDA Device that employs white light to record depth profiling of wounds  
                          | • Regulatory approval for Canada and Australia *only*.                                                                                                                                                    | $6,000-$10,000            |
| AstronClinica:         | Siascope                    | • Handheld device to measure concentration of melanin haemoglobin and collagen  
                          | • Permits analysis of skin melanoma, rashes                                                                                                                                                              | $5,600                    |
| Electro-Optical Sciences: | Melafind                   | • Handheld device provides multispectral analysis *(non-fluorescence)* to diagnose melanoma.  
                          | • In clinical trials- not commercially available.                                                                                                                                                       | Est. $7,500 + per patient use fee |
| 3 Cert:                | Dermiite                    | • Magnifying lens with LEDS  
                          | • Visualize skin melanomas                                                                                                                                                                             | $700 - $1,500             |
| Quest Medical:         | Artemis System              | • Handheld device *(cart-based endoscope)* provides white light and fluorescence image co-registration in real-time  
                          | • Neds exogenous contrast agents.                                                                                                                                                                        | $100,000-150,000          |

*Figure A2 Potential competitors of PRODIGI™ on the market.*
Figure A3 Power Supplies purchased from Mouser Electronics as medical grade equipment
Annual Review Form

UNIVERSITY HEALTH NETWORK
RESEARCH ETHICS BOARD

The Notice of Late Submission of the Annual Review Form must accompany this form if submitted less than two weeks before the expiry date or after the expiry date of the study.

<table>
<thead>
<tr>
<th>Principal Investigator (UHN): Dr. Ralph S. DaCosta</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHN REB Number:12-5003-A</td>
</tr>
<tr>
<td>Full Study Title: Evaluation of a 'Hand-held' Fluorescence Digital Imaging Device for Real-Time Advanced Wound Care Monitoring (JDRTC/UHN).</td>
</tr>
</tbody>
</table>

Review type requested: ☒ Delegated ☐ Full Board

If Full Board is requested, please explain:

**STUDY ENROLMENT STATUS**

- ☐ No enrolment to date at UHN. Reason for no enrolment:
  - For multicentre studies, have any participants been enrolled at other centres?
- ☒ Enrolling participants at UHN.
- ☐ Enrolment complete but study is ongoing at UHN (i.e. participants receiving intervention, data collection).
- ☐ Intervention & follow-up complete for all UHN participants; however, data clarification and/or data transfer ongoing.

If the study has been completed or needs to be terminated prematurely please complete the Study Termination Form instead.

**SUMMARY OF PARTICIPANTS AT UHN ONLY**

<table>
<thead>
<tr>
<th>Retrospective Chart Review and Tissue Studies ☒ N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of charts reviewed to determine eligibility</td>
</tr>
<tr>
<td>Number of participants included in retrospective chart review study</td>
</tr>
<tr>
<td>Number of tissue samples utilized during the study</td>
</tr>
</tbody>
</table>

Figure A4 ITA Annual Review Form
Research Ethics Board
Amendment Form

Please consult the UHN REB website for current submission procedures and requirements.

See the Guidelines for Submitting Proposed Amendments, Administrative Changes and Changes in Principal Investigator for more information, including definitions of capitalized terms.

[Note: The REB reminds you to update your research protocol any time that changes are proposed to the design or conduct of your study and attach them to your amendment submission.]

SECTION 1 – Study Identification
UHN REB Number: 12-5003
Study Title: Evaluation of a 'hand-held' fluorescence digital imaging device for real-time advanced wound care monitoring

SECTION 2 – Contact Information
NOTE: For a change in Principal Investigator, please use the “Change in Principal Investigator Form”

Principal Investigator: Ralph DaCosta
PI Telephone: 4165818645
PI Email Address: rdacosta@uhnresearch.ca

Name of Person Completing the Form: Charlie Wu
Telephone: 4169464501*5745
Email Address: yichaowu@uhnres.utoronto.ca

SECTION 3 – Review Background Information

1. Are you requesting review of this request at a 'Full Board' meeting (a convened meeting of the REB)?
   □ Yes  ☑ No
   If "Yes", please explain why:

2. Has this amendment already been implemented to eliminate an apparent immediate hazard to one or more study participants? □ Yes  ☑ No
   [If "Yes", please include details about this in your answer to #2 in Section 4.]

3. Will the number of study participants change due to this amendment? □ Yes  ☑ No
   [If “Yes”, please include details about this in your answer to #2 in Section 4.]

4. Is this study regulated by Health Canada? □ Yes  ☑ No
   a) If “Yes”, do these changes require authorization from Health Canada? □ Yes  ☑ No
   b) If “Yes”, has Health Canada authorization been received? □ Yes  ☑ No
Reference

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