

Efficacy of a bacterial fluorescence imaging device in an outpatient wound care clinic: a pilot study

***Ciaran M. Hurley**,¹ Senior House Officer; **Pat McClusky**,² Advanced Nurse Practitioner; **Ryan M. Sugrue**,¹ Senior House Officer; **James A. Clover**,¹ Consultant Plastic Surgeon; **Jason E. Kelly**,¹ Consultant Plastic Surgeon

*Corresponding author email: ciaranmartinhurley@gmail.com

1 Department of Plastic and Reconstructive Surgery, Cork University Hospital, Cork, Ireland.

2 Department of Wound Care, Cork University Hospital, Cork, Ireland.

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Objective: Subsurface bacterial burden can be missed during standard wound examination protocols. The real-time bacterial fluorescence imaging device, MolecuLight i:X, visualises the presence of potentially harmful levels of bacteria through endogenous autofluorescence, without the need for contrast agents or contact with the patient. The intended use of the imaging device is to assist with the management of patients with wounds by enabling real-time visualisation of potentially harmful bacteria. The aim of this study was to establish the accuracy of the wound imaging device at detecting pathogenic bacteria in wounds.

Methods: A single-centre, prospective observational study was conducted in Cork University Hospital in an outpatient plastic surgery wound care clinic. Patients had their wounds photographed under white and autofluorescent light with the imaging device. Auto-fluorescent images were compared with the microbiological swab results.

Results: A total of 33 patients and 43 swabs were included, of which

95.3% (n=41) were positive for bacteria growth. *Staphylococcus aureus* was the most common bacterial species identified. The imaging device had a sensitivity of 100% and specificity of 78% at identifying pathological bacteria presence in wounds on fluorescent light imaging. The positive predictive value (PPV) was 95.4%. The negative predictive value (NPV) was 100%. It demonstrated a sensitivity and specificity of 100% at detecting the presence of *Pseudomonas* spp.

Conclusion: The imaging device used could be a safe, effective, accurate and easy-to-use autofluorescent device to improve the assessment of wounds in the outpatient clinic setting. In conjunction with best clinical practice, the device can be used to guide clinicians use of antibiotics and specialised dressings.

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autofluorescent imaging • microbiological sampling • molecuLight • wound care

Acute and chronic wounds are a major burden to patients worldwide.¹ The cost per annum of treating patients with non-healing wounds is increasing. However a growing volume of evidence demonstrates that strategies focusing on accurate diagnosis and improving wound healing rates is of benefit to patients and economically.² The UK's National Health Service (NHS) annually manages an estimated 2.2 million patients with a wound, approximately 4.5% of the adult population.³

Wound infection is detrimental to wound healing, and the diagnosis of infection is controversial as it can vary between clinicians.⁴ Current practice in the outpatient setting for diagnosing wound infections is limited to clinical assessment of signs and symptoms of localised infection such as pain, heat, oedema, erythema, malodour, delayed healing and purulent exudate.⁵ However, wound healing may also be delayed in the absence of typical clinical features of infection.

Subsurface bacterial burden can be missed during standard wound examination protocols and can be led by the clinician's level of experience of diagnosing wound infection.⁶ This can lead to wound chronicity and patient morbidity.

The reference standard for the diagnosis of infection of a chronic wound is a deep tissue biopsy culture.^{7,8} This is often painful and invasive for patients in the outpatient setting, with microbiological swabs more commonly used. The best sampling technique for taking a swab has not yet been identified and validated, but the Levine technique is the preferred method.⁹ Furthermore, processing wound swabs is laborious and requires considerable financial resources.⁹ Mounting evidence suggests that wound swabs are commonly taken when they are not clinically indicated, and typically can take days for results to be available.¹⁰ To address these limitations in the outpatient setting, the bacterial fluorescence imaging device, MolecuLight i:X (MolecuLight Incorporated, Canada), has been developed. This is a handheld, non-invasive, autofluorescent imaging device.

The imaging device visualises the presence of potentially harmful levels of bacteria through endogenous autofluorescence, without the need for contrast agents or contact with the patient. The intended use of the device is to assist with the management of

*Ciaran M. Hurley,¹ Senior House Officer; Pat McClusky,² Advanced Nurse Practitioner; Ryan M. Sugrue,¹ Senior House Officer; James A. Clover,¹ Consultant Plastic Surgeon; Jason E. Kelly,¹ Consultant Plastic Surgeon

*Corresponding author email: ciaranmartinhurley@gmail.com

1 Department of Plastic and Reconstructive Surgery, Cork University Hospital, Cork, Ireland. **2** Department of Wound Care, Cork University Hospital, Cork, Ireland.

Table 1. Colour indicators for interpretation of fluorescence images

Colour	Indicator
Red	Potentially pathogenic bacteria
Green	Connective tissue
Dark/black	Blood, highly vascularised tissues, necrotic tissue, pigmented lesions
Cyan	<i>Pseudomonas aeruginosa</i>

patients with wounds by enabling real-time visualisation of potentially harmful bacteria. Under violet light illumination, the imaging device can capture and document images or videos of wounds and surrounding areas where fluorescent bacteria may be present. The bacterial fluorescence signals detected by the device provide a visual indication of bacterial presence, load and location, within and around wounds. When wounds are illuminated by violet light, endogenous collagens in the connective tissue matrix emit a green coloured fluorescent signal. Some bacteria, such as *Staphylococcus aureus*, emit a red coloured fluorescence signal due to the production of endogenous porphyrins, and others, such as *Pseudomonas aeruginosa*, emit a cyan coloured fluorescence signal due to the production of endogenous pyoverdine.^{11,12} The imaging device simultaneously captures fluorescence from both bacteria and tissues and creates a composite image on the high-resolution colour LCD (liquid crystal display) screen. This information can possibly be used to guide selection and application, and response to wound therapies and treatment.¹³ The aim of this study was to establish the accuracy and ease of use of the wound imaging device at detecting pathogenic bacteria in wounds.

Method

Study design and participants

A prospective observational study was conducted in a single centre. Ethical approval was granted by a local

Table 2. Bacteria identified on microbiological swab cultures of fluorescence positive images

Bacteria	n
<i>Staphylococcus aureus</i>	23
<i>Streptococcus dysgalactiae</i>	5
Proteus spp.	2
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	2
<i>Pseudomonas</i>	3
<i>Staphylococcus lugdunensis</i>	1
Mixed Gram-negative bacilli	5
<i>Enterococcus faecalis</i>	6
Coliform	3
No growth	2

ethics committee. All data was collected in the outpatient wound care clinic setting of Cork University Hospital over an eight week period. The clinics are primarily plastic surgery based and patients attending the clinics present with a mixture of wounds which can include postoperative wounds, chronic wounds, burns, skin grafts and trauma. The clinics are nurse and surgeon led.

All patients over the age of 18 years were included in the study regardless of mechanism of injury, gender, wound site, shape or size. Patients with wounds that demonstrated no change in wound healing in clinic review two weeks before the trial starting were included. This was based on clinical assessment and included wounds that were slow to heal, stagnant in decreasing dimensions and signs of potential infection.

Participants were excluded if they were taking antibiotics for a wound infection, had any contraindication to routine wound care (allergies to routine dressings) or were unable to provide consent. Written consent was obtained from all participants, including for the use of photographs.

Procedure

The images were captured using the handheld imaging device. The procedure was explained to all participants before imaging. All wound dressings were removed. Wounds were assessed by an advanced nurse practitioner, observing signs and symptoms of infection, including pain, tenderness, heat, swelling, erythema, purulent exudate and malodour.

The device-pulsed, laser-based range finder sensor was used to determine the distance between the device and the wound, 8–12cm away from the wound at a 90 degree plane. A white light (normal) photograph was taken. The clinic room lights were then dimmed and a fluorescent image was obtained. Using the device, real-time visualisation of the presence and distribution of bacteria in the wounds was assessed. Areas of red or cyan fluorescence were swabbed. Microbial swabs were taken and sent to the hospital microbiology laboratory for culture and sensitivity testing to assess bacterial growth, species and sensitivities. A standard Levine technique was used for swabbing the wounds. All images were stored in separate files on the device.

Data analysis

All anonymous images were transferred to an encrypted desktop computer for interpretation and analysis. Real-time visualisation of the images were interpreted according to the device’s user manual (Table 1). All wound swab results were collected from the online hospital system. All patient data was collected and stored anonymously in an encrypted database in Microsoft Excel, version 2016 (Microsoft Corp., US). To allow for statistical analysis, the anonymised data was transferred to GraphPad Prism Version 6.0 (GraphPad Software Inc.,US).

Results

Patient demographics

A total of 33 patients were identified for inclusion in the study. Each patient had a single wound and 64% were male (n=21) and 36% were female (n=12). All patients were Caucasian (n=33). Cohort mean age was 62.2 years (range: 30–89 years). The majority of wounds assessed were on the lower limb (n=21). Other wound positions included the thigh (n=2), upper limb (n=2), sacrum (n=2), scalp (n=2), chest wall (n=2), natal cleft (n=1) and abdomen (n=1). All 33 wounds assessed were positive for bacteria under fluorescent light.

A total of 43 swabs were taken on 33 first clinic appointments. All swabs were taken from the wound bed. A single swab was taken from 23 wounds, and two swabs were taken from 10 wounds, which were of a larger wound diameter, in different wound bed areas. Of the swabs taken, 95.4% (n=41) were positive for bacteria growth and nine different species of bacteria were identified (Table 2). *Staphylococcus aureus* was the most common bacterial species identified. Positive swabs for *Pseudomonas aeruginosa* were found in three patients. Under fluorescence imaging, three wounds were cyan, which correlated with the results of *Pseudomonas* from the microbiological swab culture. *Pseudomonas* was not detected as a secondary bacteria in any fluorescing red swab culture.

Overt signs of infection, including erythema, pain, tenderness and malodour were identified in seven patients. Of these patients, all exhibited a red fluorescence when imaged using the wound imaging device, which is a positive indication for potentially pathogenic bacteria. All seven patients commenced on an appropriate course of antibiotic therapy for one week. After two weeks, these seven wounds were reassessed in clinic using the imaging device and wound swabs were taken. All were fluorescence negative and the microbiological swabs were also negative, exhibiting no pathogenic bacterial growth.

The imaging device had a sensitivity of 100% and specificity of 78% (Table 3) at identifying pathological bacteria presence in wounds using fluorescent light (FL) imaging. The positive predictive value was 95.4%. The negative predictive value was 100%. It demonstrated a sensitivity and specificity of 100% at detecting the presence of *Pseudomonas* species on fluorescent light imaging.

Case 1

A 67-year-old male with a chronic lower leg wound secondary to a failed split-thickness skin graft (STSG; size: 5.4x4.1cm) of eight weeks' duration. This patient did not demonstrate the typical features of wound infection. Fig 1a demonstrates the white-light image captured by the wound imaging device. The yellow stickers allow the camera to correctly adjust its distance calculator. Fig 1b demonstrates the areas of red fluorescence suggesting the presence of potentially pathogenic bacteria; four wound swabs taken from the targeted area confirmed the presence of *Staphylococcus aureus*.

Table 3. Definition of 'true positive', 'true negative', 'false positive' and 'false negative'

	Fluorescent-light (FL) imaging	Microbiology result	n
True positive	Red FL positive	Pathogenic swab culture	41
False positive	Red FL positive	No growth	2
True negative	Red FL negative (postantibiotics)	No growth	7
False negative*	Red FL negative (postantibiotics)	Pathogenic swab culture	0

*Used in study

Fig 1. Case 1, a 67-year-old male with a chronic lower leg wound secondary to a failed STSG (size: 5.4x4.1 cm) of eight weeks' duration. White light image of a wound on the lower limb (a); autofluorescent image of wound showing red fluorescence suggesting presence of potentially pathogenic bacteria (b)

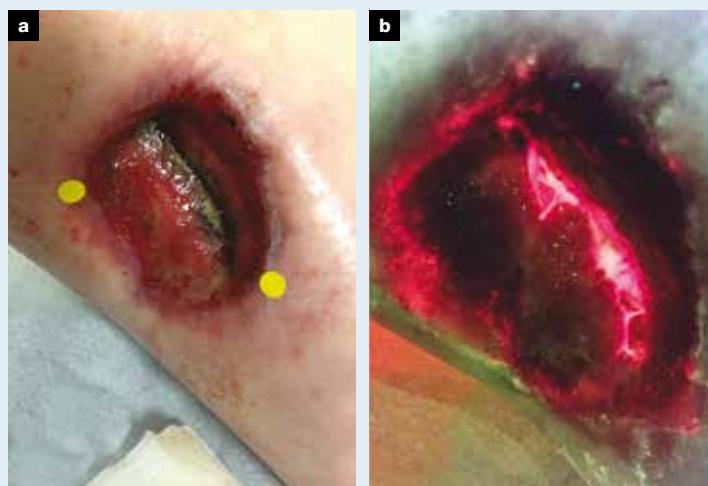
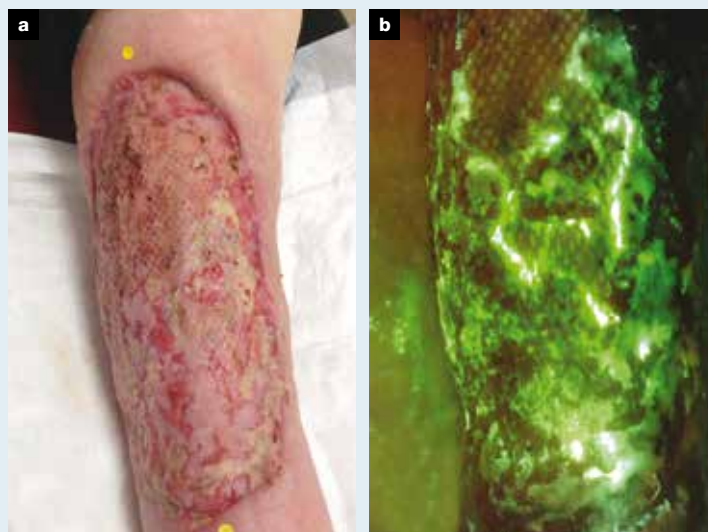


Fig 2. Case 2, a 58-year-old male, 12 days after a split-thickness skin graft (STSG) to the lower limb (24.3x6.2cm). White light image of the STSG (a); autofluorescent imaging demonstrating cyan in the wound bed (b). The patient was immediately started on antibiotics



Case 2

A 58-year-old male, 12 days post-STSG to the lower limb (size: 24.3x6.2cm). The wound bed was malodorous but did not demonstrate other typical signs of infection. Autofluorescent imaging, shown in Fig 2b, clearly demonstrates cyan fluorescence. The patient was immediately started on appropriate antibiotics for *Pseudomonas aeruginosa*. After four days, wound swab results formally demonstrated *Pseudomonas aeruginosa* in three separate swabs.

Case 3

A 61-year-old female with a chronic sacral pressure ulcer (PU) of nine months' duration (size: 6.5x5.8cm). Fig 3 demonstrates the built-in wound bed size estimator in the camera of the device. This was used to document wound progression by nursing staff. This chronic sacral PU was regressing over a number of weeks. The autofluorescent imaging was used after bedside debridement. Red fluorescence was identified, indicating the presence of potentially pathogenic bacteria. Wound swabs confirmed the presence of *Staphylococcus aureus*, and the patient was commenced on antibiotics and appropriate dressings to decrease the bacterial load.

Discussion

Using a imaging device has a number of advantages in the outpatient wound care clinic setting. It is simple to use, requiring little training and can be used by all health professionals. It has been demonstrated to be quick, with its procedure taking no more than a minute per patient longer than conventional clinical assessment.

As demonstrated by Blackshaw et al., results are shown in real-time with a decision on treatment being made at the bedside.¹⁴ Wu et al. accurately describes the use of autofluorescence imaging as an aid during bedside debridement to detect potentially pathogenic bacteria

below otherwise unremarkable wound beds, altering the clinician's decision-making process with the provision of antimicrobial dressings and the prescription of antibiotics.¹⁵

Our case studies demonstrate various aspects of the devices practicalities. Case 1 demonstrates the need for objective wound swab sampling methods. The bacterial fluorescence imaging allowed targeted sampling of the wound bed, which may otherwise have led to a false negative swab result. Case 2 exhibits determination of the presence of *Pseudomonas aeruginosa*. We found the device's positive predictive value of detecting *Pseudomonas aeruginosa* particularly useful in the plastic surgery clinic, due to this bacterium's potential to contaminate skin grafts, resulting in partial or complete graft loss. The same is also applicable for *Staphylococcus aureus* which was the most common bacteria detected in our study.¹⁶ The wound measuring tools used in Case 3 were useful in documenting the progression or regression of chronic wounds. Its measurements were instant and accurate. There were two false positive cases in our study (Table 3). The first was a 69-year old male, day 12 post-STSG to the lateral aspect of the leg. The second case was a 49-year old female, day eight post-STSG to the leg. Both were red FL-positive but grew no microorganism in the swab culture. This is likely due to poor swabbing technique. However, the fluorescent light may be identifying subsurface fluorescent bacteria that swabs fail to. This may be overcome with wound bed curettage.

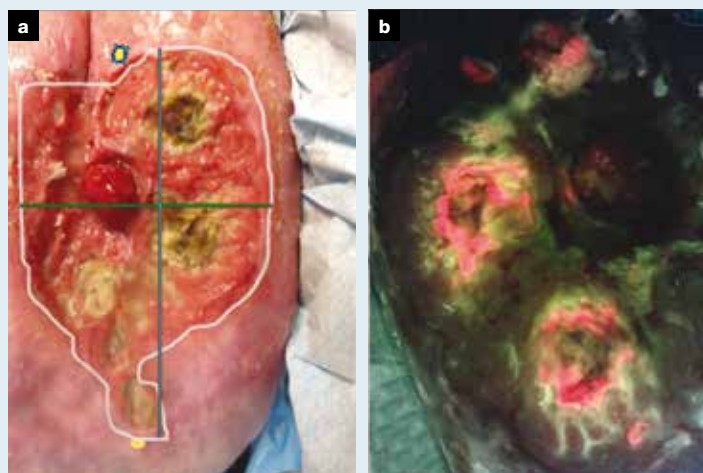
The efficacy of the imaging device has been proven previously in smaller trials.^{14,15,17,18} Its high sensitivity and specificity for detecting subclinical bacterial wound infections demonstrates its capacity. The fluorescent imaging prompted the discovery of secondary wound infection below otherwise normal skin, prompting the timely delivery of antibiotics. All seven of our patients who were started on antibiotic treatment had negative swabs upon their return visit to the clinic, demonstrating the validity our intervention.

The device was a useful adjunct in the outpatient wound care setting. Other investigators have demonstrated its effectiveness in the evaluation and management of burns, and even in the military and trauma setting.^{17,18} Moving forward with the device, we aim to assess its use preoperatively and perioperatively as a surgical tool.

Limitations

Despite our success with the imaging device, it has limitations in practical use. Blood and highly vascularised tissue are demonstrated as black on the fluorescent light photographs. Often, we encountered wounds with minimal active bleeding, which rendered the device incompatible. This was overcome with copious irrigation at the bedside with limited success. We therefore consider active bleeding or visible vascularised tissue as a relative contraindication to use of the device.

Fig 3. Case 3, a 61-year-old female with a chronic sacral pressure ulcer (PU) of nine months' duration (size: 6.5x5.8cm). White light image of PU (a); autofluorescent imaging of PU demonstrating red fluorescence suggesting the presence of potentially pathogenic bacteria (b)



Dressings containing silver, a potent antimicrobial, also rendered the photograph black. This was a major limitation when applied in our outpatient burns clinic, as the majority of these patients have various silver-based dressings applied for their antimicrobial properties.¹⁹

Darkness was needed for the device to produce accurate and quality autofluorescent images. This was overcome by the use of the imaging device accessory product DarkDrape which is made of high density polyethylene with an adjustable drawstring to ensure appropriate lighting conditions are met precisely. The accessory device is single use only, which is not practical in everyday clinic use.

The cost of the imaging device will be a major determinant of accessibility and practicality for use in the outpatient department clinic.

Conclusions

This imaging device could be a safe, effective, accurate and easy-to-use autofluorescent device, which improves the assessment of wounds in the outpatient clinic

Reflective questions

- What is the reference standard for the diagnosis of infection in a chronic wound bed?
- Explain how fluorescent light-imaging devices visualise bacteria?
- What compound does *Staphylococcus* produce to illuminate red under violet light?

setting. The device can accurately differentiate between *Pseudomonas aeruginosa* and *Staphylococcus aureus*, both clinically devastating species of bacteria, at the bedside. In conjunction with best clinical practice, the device can be used to guide clinicians' decision-making on the use of antibiotics and specialised dressings. Further research should be directed to its application in other environments, including preoperative and perioperative applications as a surgical assessment tool. **JWC**

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