Use of a bacterial fluorescence imaging device: wound measurement, bacterial detection and targeted debridement

Objective: Diagnostics which provide objective information to facilitate evidence-based treatment decisions could improve the chance of wound healing. Accurate wound measurements, objective bacterial assessment, and the regular, consistent tracking of these parameters are important aspects of wound care. This study aimed to assess the accuracy, clinical incorporation and documentation capabilities of a handheld bacterial fluorescence imaging device (MolecuLight *i*:X).

Method: Benchtop wound models with known dimensions and clinical wound images were repeatedly measured by trained clinicians to quantify accuracy and intra/inter-user coefficients of variation (COV) of the imaging device measurement software. In a clinical trial of 50 wounds, wound dimensions were digitally measured and fluorescence images were acquired to assess for the presence of bacteria at moderate-to-heavy loads. Finally, fluorescence imaging was implemented into the routine assessment of 22 routine diabetic foot ulcers (DFU) to determine appropriate debridement level and location based on bacterial fluorescence signals.

Results: Wound measurement accuracy was >95% (COV <3%). In the clinical trial of 50 wounds, 72% of study wounds demonstrated positive bacterial fluorescence signals. Levine sampling of wounds was found to under-report bacterial loads relative to fluorescence-guided curettage samples. Furthermore, fluorescence documentation of bacterial presence and location(s) resulted in more aggressive, fluorescence-targeted debridement in 17/20 DFUs after standard of care debridement failed to eliminate bacterial fluorescence in 100% of DFU debridements.

Conclusion: The bacterial fluorescence imaging device can be readily implemented for objective, evidenced-based wound assessment and documentation at the bedside. Bedside localisation of regions with moderate-to-heavy bacterial loads facilitated improved sampling, debridement targeting and improved wound bed preparation.

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bacterial fluorescence imaging • MolecuLight • wound assessment • wound documentation • wound measurement

Despite advances over the past decades in topical antimicrobials, skin substitutes, negative pressure and other advanced therapies, the percentage of wounds that heal within 12 weeks remains at a disappointing 40%[1] and non-healing wounds continue to burden health-care systems worldwide.[2,3] An area of advancement that has lagged behind other medical fields is diagnostic imaging.[4] Imaging advances have the potential to revolutionise diagnosis in wound care, just as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning have done for the fields of cardiology and oncology. Diagnostic imaging augments patient assessment by providing objective evidence and documentation that aids clinicians in making improved and more timely decisions and interventions.

Wound care is conservatively estimated to cost more than $50 billion dollars in the US and £5 billion pounds in the UK, annually.[2,3] Much of this cost is due to hard-to-heal wounds, which become stalled in the normal healing cascade, require more provider visits and do not resolve.[5] Early identification of problematic, hard-to-heal wounds would enable a course correction to prevent worsening responses to treatments, escalation through the infection cascade,[6] amputations and other high cost procedures.[7,8] Wound care diagnostics that can provide objective information to identify hard-to-heal wounds and facilitate evidence-based treatment decisions could improve a wound's chance of healing.

Metrics which are highly predictive of poor or hard-to-heal wounds are:

- Wound area reduction (<25% within four weeks of treatment)[9]
- The presence of bacteria at loads of $\geq 10^4$ colony forming units (CFU)/g.[10]

Therefore, wound measurement and bacterial status are important for monitoring progression, informing treatment, and predicting wound healing. The MolecuLight *i*:X imaging device is a novel, handheld,

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point-of-care diagnostic imaging tool designed to accurately and digitally measure wound areas and to provide objective, real-time evidence on the presence and location of high bacterial loads (both planktonic and in biofilm.[11–16] This provides objective documentation, performed at the bedside, during which the device captures an image in a format compatible with most electronic medical records (EMR) systems. The device contains built-in digital wound measurement software and emits a safe violet light which is used for non-invasive, contrast agent free fluorescence imaging to identify regions with concerning levels of bacteria in real-time. Multisite clinical trials have established that red and cyan colours on the device's fluorescence images are highly predictive of moderate-to-heavy bacterial loads ($\geq 10^4$CFU/g).[14,17] These images serve as a visual biomarker of the presence and location of bacteria at loads which are known to delay wound healing.[10] Adding bacterial visualisation through fluorescence information to the standard of care has been shown to trigger a switch to a healing trajectory in a series of 12 previously hard-to-heal wounds.[18] A separate study found that average weekly wound area change was a 6% increase when bacterial fluorescence was present and switched to a 27% decrease in wound area once bacterial fluorescence was eradicated through targeted debridement and other antimicrobial strategies.[19] Fluorescence information in this study provided evidence-based documentation to establish the appropriate level of debridement.[19]

Validation is important to assure appropriate clinical implementation. In this study, accuracy, inter- and intra-user variability of the imaging device's digital wound measurement software was assessed through benchtop models and clinical images. Ease of wound measurement and fluorescence imaging implementation in the clinical setting was then assessed in a clinical trial of 50 wounds. This trial further documented the high prevalence of bacterial fluorescence in the general wound population, which is vastly underestimated by standard of care clinical signs and symptoms assessment.[15,20,21] Lastly, bacterial fluorescence imaging was used to assess pre- and post-debridement bioburden, and to inform and document when there is a need for additional and more targeted debridement to remove bacteria-burdened tissues. This compilation of work demonstrates that the bacterial fluorescence imaging device can be readily and reproducibly implemented for real-time, point-of-care wound assessment to improve wound documentation and targeted treatment in the clinical setting.

Methods

Verification of digital wound measurement software accuracy and repeatability

To statistically calculate the mean measurement error, the coefficient of variation for intra/inter-user repeatability and to verify that the accuracy and repeatability specifications for the measurement application were met, combinations of n number of wound models, m number of repeated measurements and k number of clinicians required were determined. This generated a list of statistically appropriate combinations of m, n and k. From that list, the exact combination used in this study was chosen to maximise the number of wound models/clinical wound images while using at least five clinicians, thus enabling a wider distribution of wound sizes and shapes to be tested and capturing the variability between wounds typically found in a clinical setting. Validation was a two-part process: firstly, using benchtop wound models for repeated measurements, and secondly evaluating device/user performance in documented clinical images, as described below. We recruited five clinicians who received training on use of the bacterial fluorescence imaging device and instructions for using wound measurement software before completing the study.

Benchtop wound models: 17 unique wound printouts with known dimensions (wound area range: 1.79–37.68cm^2) were generated (SolidWorks) and affixed to one of four surface types (flat surface, slanted surface, cylindrical surface, cylindrical slanted surface). Clinicians placed two WoundStickers (for calibration) beside each wound, according to the instructions for use, and used the range finder indicator light to place the device at the instructed distance to capture images (8–12cm). Each user measured each of the wound models three times in 'auto wound border trace' mode and three times in 'manual wound border trace' mode, yielding a total of 51 measurements for each mode. These measurements facilitated assessment of the accuracy, inter-rater repeatability and intra-rater repeatability for the wound measurement software in measuring area, maximum length and maximum width of wound shapes. Average measurement error and intra/inter-user coefficients of variation were calculated for manual and auto modes independently.

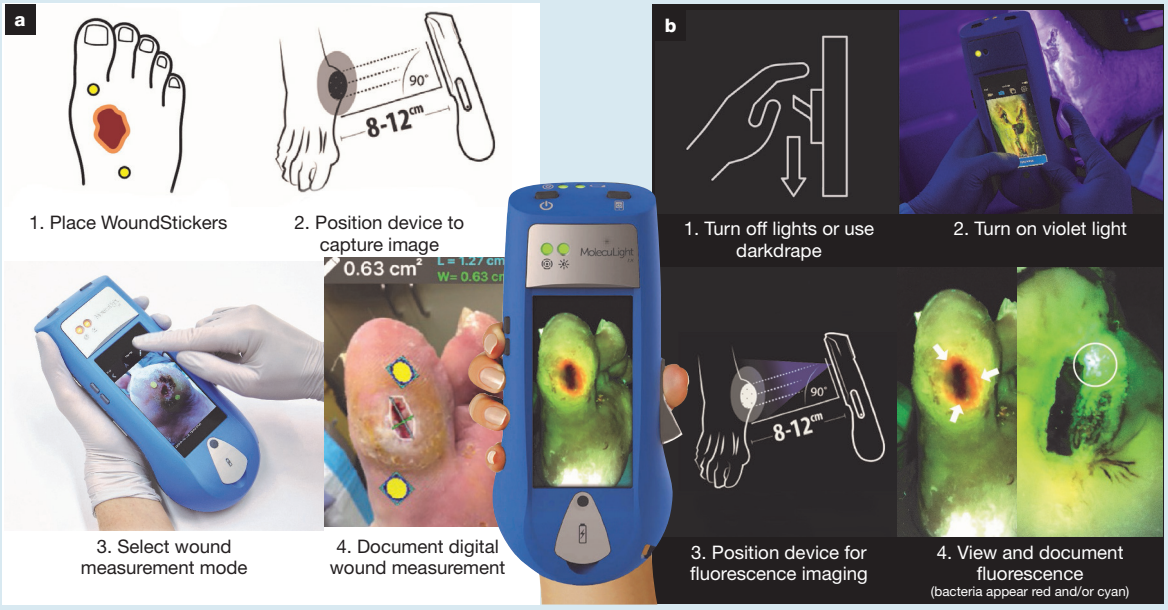
Clinical images

To evaluate clinical performance of the measurement software, the same five clinicians measured 17 clinical wound images (seven venous or arterial leg ulcers, four diabetic foot ulcers (DFU), two pressure ulcers (PU), four surgical wounds), which had been captured by a different wound clinician in their clinical setting, using two WoundStickers and the device's range finder, according to the instructions for use. Each wound image was measured by each clinician three times to assess intra/inter-rater repeatability for the measurement feature of the bacterial fluorescence imaging device and to calculate intra/inter-user coefficients of variation (COV) to further validate the repeatability when measuring images of real wounds taken in the clinical setting.

Clinical use of wound measurement software

Over five clinic days, 50 wounds (36 DFUs, four venous leg ulcers (VLU), three arterial leg ulcers, seven other) of unknown infection status were imaged from

Fig 1. Acquiring images for wound measurement and fluorescence detection. To measure wounds, two yellow WoundStickers were placed adjacent to the wound opposite one another for calibration (**a**). The patient and device were positioned such that the device was in the same plane as the wound (parallel) and at a distance of 8–12cm. This distance was indicated by the device's range finder light, which turns green only within the range. The device could automatically focus in the centre of the field of view, or the clinician could touch the screen to focus on a specific region, at which point an image was captured. From that image, the clinician would select to enter wound measurement mode. In this mode, the software automatically detects the WoundStickers and wound border, though the clinician could opt to manually outline wound border with a stylus, if they preferred. Confirmation of the wound border was required before the area, maximum length and maximum perpendicular width were generated and overlaid onto the image as documented. Fluorescence requires darkness, so the lights were switched off before imaging (**b**). Alternatively, a disposable DarkDrape accessory could be used. The violet light was turned on and the patient and device were positioned such that the device was in the same plane as the wound (parallel), at a distance of 8–12cm (optimal for fluorescence imaging). Fluorescence information instantly appears on the screen, localising regions with moderate-to-heavy bacterial loads (red and/or cyan colour on images) in real-time.[13,14] This was documented by the clinician by capturing an image or video. In the examples shown, a region of red fluorescence (arrows) was confirmed on cultures as *Proteus mirabilis* and cyan fluorescence (circled) was confirmed as *Pseudomonas aeruginosa*. Green fluorescence of tissue is from matrix components (e.g. collagen, fibrin)[13]



39 patients (79% male, 21% female) who had given informed, signed consent for photography release (Clinicaltrials.gov #NCT03754426). The bacterial fluorescence imaging procedure followed for this study for wound measurement and fluorescence imaging is depicted in Fig 1. Per the calibration protocol, two yellow WoundStickers were used in order to measure the wounds using the wound imaging system measurement application. Standard images were acquired under normal room light conditions. The measurement software recorded wound area (cm^2) as well as the maximum length and width of the wound (cm). Stickers were removed, the room was made dark, and the device's safe violet light (405nm) was used to excite fluorophores in the wound during fluorescence[13] to evaluate the presence of bacteria in or around the wound. Images were interpreted by a panel of experienced users who had received certified training in fluorescence image interpretation (e-learning course and certification quiz is openly available at https://learning.moleculight.com; passing grade of 80% or higher is required).

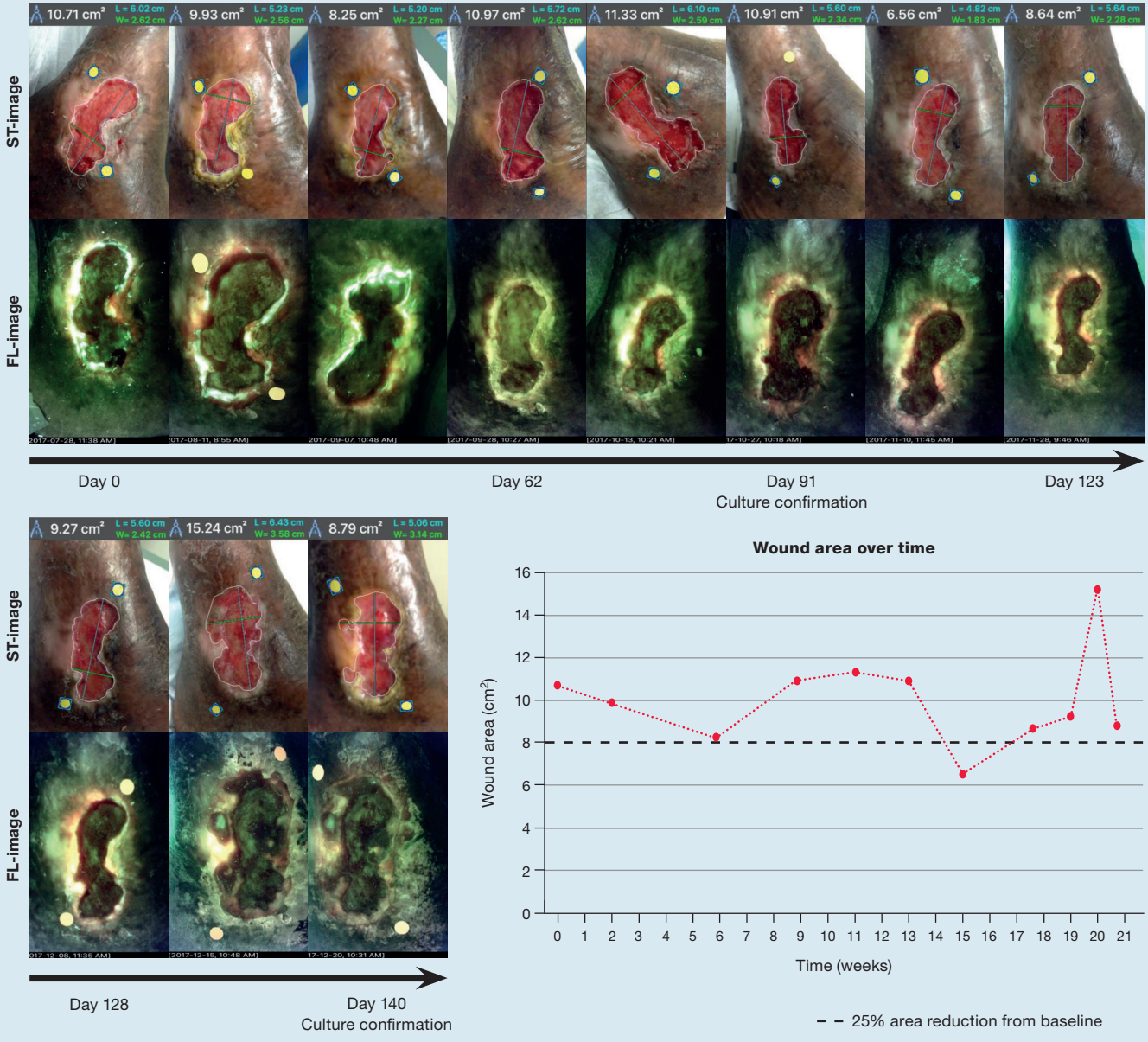
Validation of bacterial presence

Wounds exhibiting red/pink/blush or cyan fluorescence under the device's fluorescence imaging, were considered bacterial fluorescence positive. After fluorescence imaging, each wound was swabbed using the Levine technique and samples were sent for semi-quantitative culture analysis to determine the presence and load of bacterial species in the wound. Approximately 20% of the wounds (n=11) were sampled both with Levine technique in the wound bed and with fluorescence-guided curettage sampling, in which debridement scrapings from a fluorescence-positive (red or cyan) region were sent for culture analysis.

Consideration of patient skin tone

As melanin content can affect the green fluorescence hues of skin,[13] the skin tone of the subjects was assigned according to the Fitzpatrick scale, which is the gold standard for skin tone classification.[22] This scale classifies the lightest skin tone as 1 and the

Fig 2. Relationship between bacteria (red fluorescence) and lack of healing. A 74-year-old patient with a venous leg ulcer (VLU) whose treatment included debridement, infection management with antimicrobial cleansing and compression bandages. The wound area failed to decrease by 25% in four weeks (dashed line on graph) and red bacterial fluorescence persisted in the periwound, therefore the care plan was re-assessed. Tissue samples obtained at 91 and 140 days confirmed bacterial presence (moderate-to-heavy loads) in the regions of red fluorescence on the fluorescence (FL) images. The lack of progress of the wound between the 62–91 day time points was followed by another reassessment of the care plan, which rapidly decreased the size of the wound over a two week period. The wound increased in size dramatically at 140 days, again prompting a change in care, including sampling of microbiological load, which confirmed heavy bacterial loads in red fluorescing regions. A more aggressive antibacterial strategy was initiated and the wound again experienced a steep decrease in wound area. Note that in some cases, measurement calibration stickers were not removed before capturing a fluorescence image. This did not create an artefact or impede fluorescence detection in other regions of the wound. ST-image—standard image; FL-image—fluorescence image



darkest skin as tone 6. All skin tone Fitzpatrick scores were represented in the 50 wounds; the majority of wounds (58%) had skin tone Fitzpatrick score of 2 with 24% having a score of 3, and 14% with a score from 4 to 6.

Use of real-time bacterial fluorescence information in wound debridement

In a separate series of wounds, digital wound measurement and fluorescence imaging was incorporated into 22 routine wound assessments of

12 DFUs classified as 'healable' (some wounds imaged at multiple sessions). Patients provided signed informed consent for photography release. Based on clinical assessment, including an initial fluorescence scan, the clinician chose whether or not to sharply debride the wound. When chosen, initial sharp debridement was performed with a curette to aggressively remove hyperkeratotic tissue on and around the wound, according to current best practices. This initial debridement was not done under fluorescence guidance.

Fluorescence images were acquired after initial debridement to evaluate effectiveness of the initial debridement intervention. When deemed clinically appropriate, fluorescence information was used to target remaining regions of bioburden, after which a final fluorescence image was obtained and wound measurement was performed.

Results

Wound measurement accuracy and repeatability

Accuracy of both the automatic and manual trace wound measurement options was very high. Benchtop wound model measurements deviated <5.5% from the known dimensions for wound area, length and width for both the auto and manual border trace measurement modes. Measurement accuracy for length and width was ≥95.75% and measurement accuracy for wound area was ≥94.62%. The median intra/inter-user COV were <2.78% for all parameters in benchtop models. Clinical image results found intra-user coefficients of variation to be 5.11% (area) and 3.02% and 3.59% for length and width. Inter-user COV for wound area was 8.59% due to differing clinician opinions on wound boundaries. The slight difference in variability between clinical wound image measurements and the benchtop was expected as real wound edges are inherently less clear than models and rely more on user judgement.

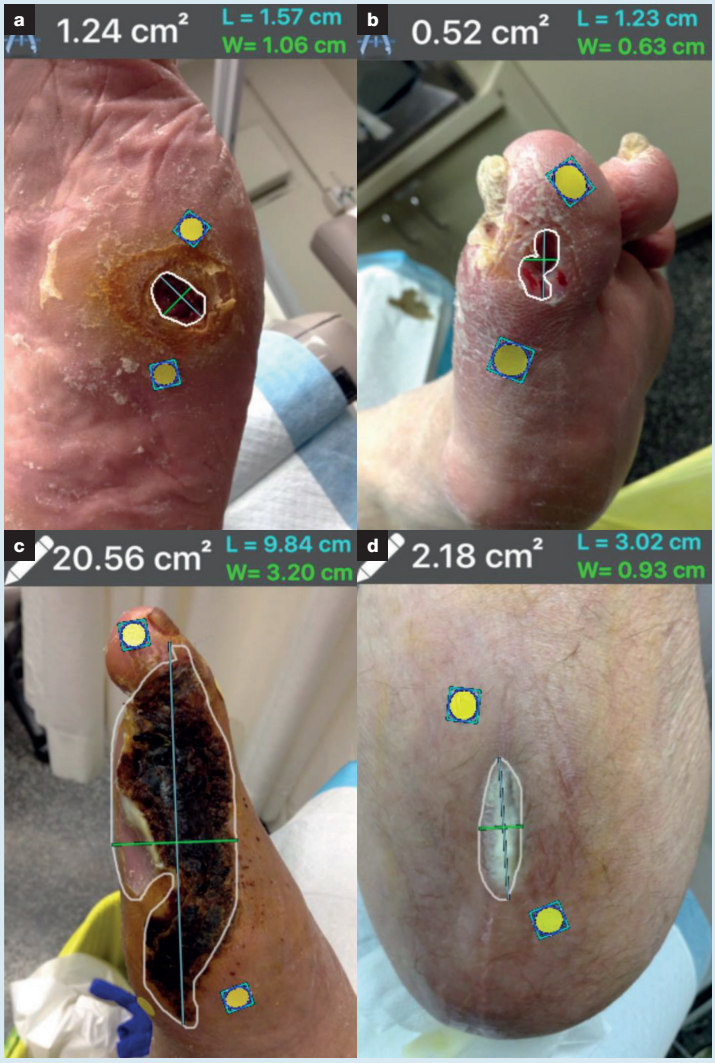
Clinical use of digital wound measurement software

The workflow for capturing images occurred during a single visit for this study, but could be repeated at each wound assessment to monitor wound progression over time (Fig 2).

Wound measurement

We measured 48/50 wounds (96%) using the measurement application (Fig 3). It was not possible to measure the remaining two wounds due to inappropriate sticker placement, which prevented sticker detection. Clinicians had the choice of automatic (Fig 3a and b) or manual (Fig 3c and d) wound tracing. The average wound area, based on wound circumference, was 5.1cm^2 (range: 0.3–43.4cm^2). The smallest wounds tended to be DFUs (average DFU area: 4.9cm^2; average other study wounds: 15.0cm^2). The median wound area was 0.96cm^2. All wounds were further assessed by conventional wound measurement practices, computing wound area based on maximum length and width (LxW) calculations. This approach resulted in an average overestimation of 31% (and up to 52%) compared with circumference-based measurements. This is grossly inferior to the digitally computed wound area for monitoring the true wound size and wound progression over time, as has been reported by other measurement studies.[23–25]

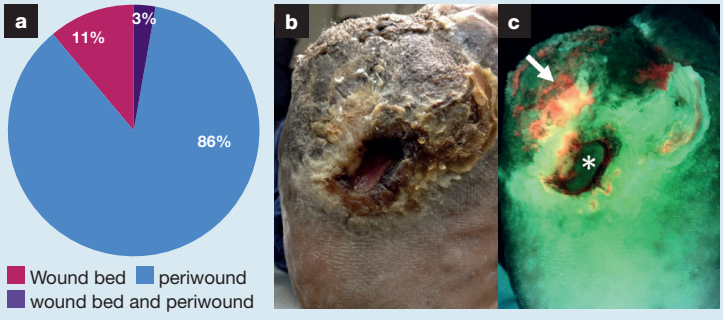
Fig 3. Digital wound measurement with automatic (a,b) and manual (c,d) border detection. If clinical opinion differed from the auto-generated boundaries, they could manually draw the boundaries using a stylus or their fingers on the device's touch screen. In the case of wound C, manual mode was selected to include the lighter pink region of the wound, in addition to the darker eschar. In wound C, using length x width to calculate area equates to 31.48cm^2, a % error of 53%



Fluorescence images and microbiological cultures

Of 50 wound images, 36 (72%) were positive for red/pink/blush or cyan fluorescence (Fig 4), only 11% were positive in the wound bed, 86% in the periwound tissue and 3% in both wound bed and periwound tissues (Fig 4a). These findings were consistent across wound types. For example, 75% of the 36 DFUs in the study were positive for fluorescence signals indicative of high levels

Fig 4. Bacterial fluorescence signals in a 50-patient clinical trial. In the majority of fluorescence-positive wounds, red and cyan emanated from periwound tissues (**a**). An example is shown, with standard image (**b**) and fluorescence image (**c**) exhibiting red fluorescence throughout the periwound and along the wound edge. A Levine swab from wound centre (*), which was fluorescence-negative, revealed only light growth of mixed bacteria; curettage sample from the periwound region of red fluorescence (arrow) revealed heavy growth of *Serratia marcesens* and heavy growth of mixed aerobic and anaerobic bacteria



of bacteria. These signals were present in the periwound in the vast majority of fluorescence-positive DFUs.

Levine swab samples were taken from the centre of the wound bed and sent for semi-quantitative culture analysis. Of 50 wounds, 35 (70%) had microbiology reporting some level of bacteria ranging from 'growth from broth' to heavy growth. However, only 10 wounds (20%) had one or more species with bacterial loads of moderate-to-heavy growth, which is the typical range of bacterial loads detected by the device.[14] This does not agree with the incidences of red/cyan fluorescence observed on the floursecent images in these patients when considering the wound as a whole (including periwound fluorescence), but it did closely agree with the presence or absence of bacterial fluorescence in the wound bed, the region which was sampled as per standard Levine technique. This indicates that the Levine sampling technique was under-representing the bacterial loads. The bacterial species that were most commonly observed were *Staphylococcus aureus* (37%), *Pseudomonas aeruginosa* (17%), *Staphylococcus epidermis* (14%) and general mixed bacteria (31%).

Curettage samples were acquired, in addition to Levine swabs, in ~20% of study wounds (n=11), eight of which were targeted to regions positive for bacterial (red or cyan) fluorescence. Wounds sampled by fluorescence-targeted curettage displayed, on average, higher bacterial loads, which aligned better with the fluorescence images. All wounds with moderate-to-heavy bacterial loads displayed areas of red fluorescence. Each wound was assessed individually to determine if and how the Levine swab and curettage sample results differed. In 55% of wounds, the curettage sample resulted in a heavier bacterial load and in 45% of wounds, additional bacterial species were detected. In three wounds where the Levine technique suggested 'light growth' curettage-cultures came back as 'heavy growth', as in Fig 4c, and one wound negative on Levine cultures had 'moderate growth' from fluorescence-targeted curettage. In the three

Fig 5. Fluorescence-guided diabetic foot ulcer (DFU) debridement. A 57-year-old male with DFU on left toe, who lacked offloading footwear and self-treated with an over the counter antibiotic ointment for two months before seeking specialist treatment. A thick callus was present upon initial assessment (**a**) and no bacterial fluorescence was evident. Initial curettage debridement to remove the callus was performed per standard of care, after which fluorescence images were acquired to assess initial debridement effectiveness (**b**). Bacterial (red) fluorescence observed throughout the periwound region (arrows) led the clinician to debride more aggressively, specifically targeting the red fluorescing regions. The wound was debrided under fluorescence guidance until red fluorescence was no longer observed (**c**)

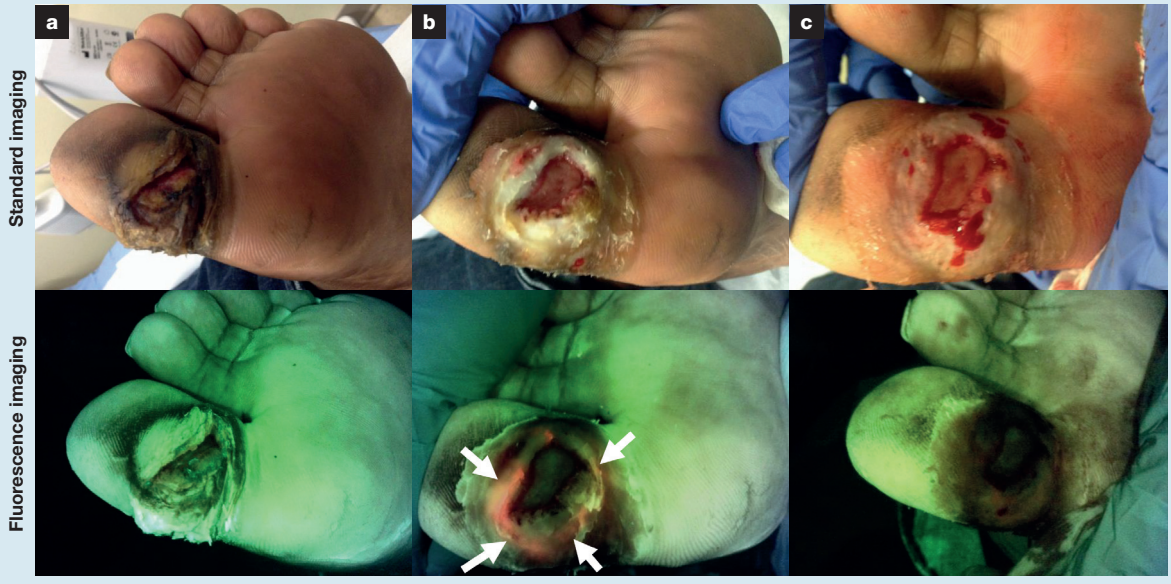
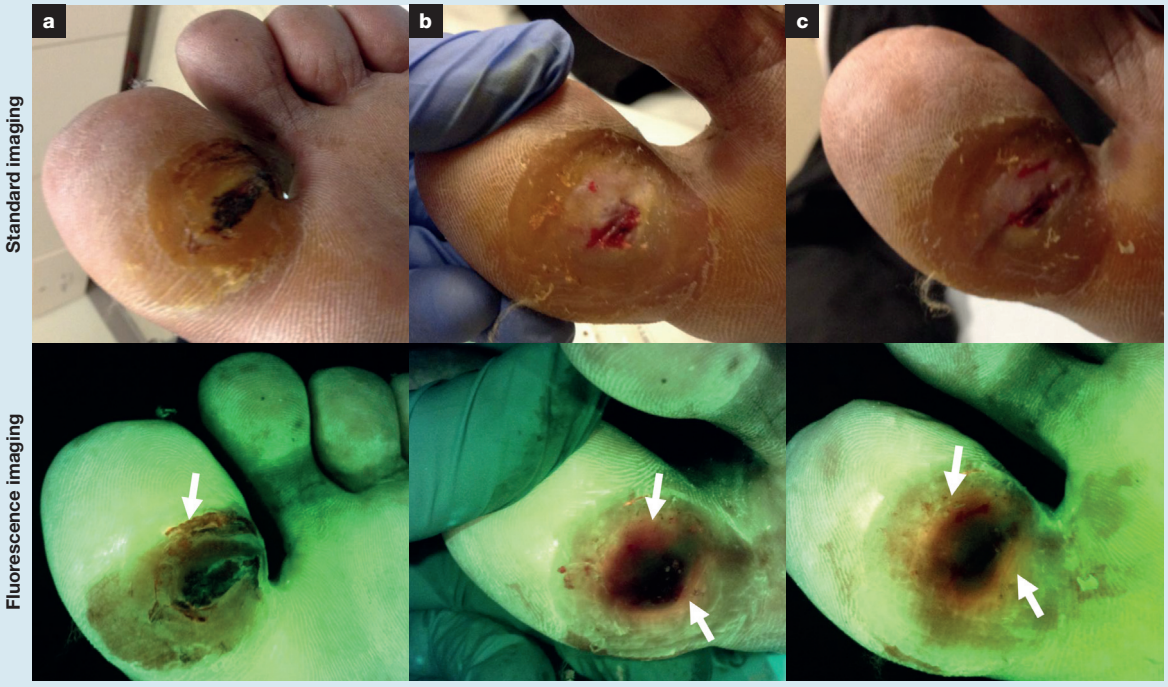


Fig 6. Fluorescence-targeted diabetic foot ulcer (DFU)-debridement. A 52-year-old male with small (0.3cm^2) DFU on left toe (**a**). DFU has repeatedly closed/reopened due to lack of proper offloading footwear. Bacterial fluorescence (red, arrows) was observed pre-debridement and after initial standard of care debridement. Red fluorescence persisted after additional fluorescence-targeted debridement (**b**). Based on the persistence of bioburden after aggressive debridement (**c**). The clinician determined that patient required more frequent debridement (weekly) in addition to antimicrobial dressings



curettage-sampled wounds deemed negative for bacterial fluorescence, all cultures confirmed, at most, only light bacterial growth.

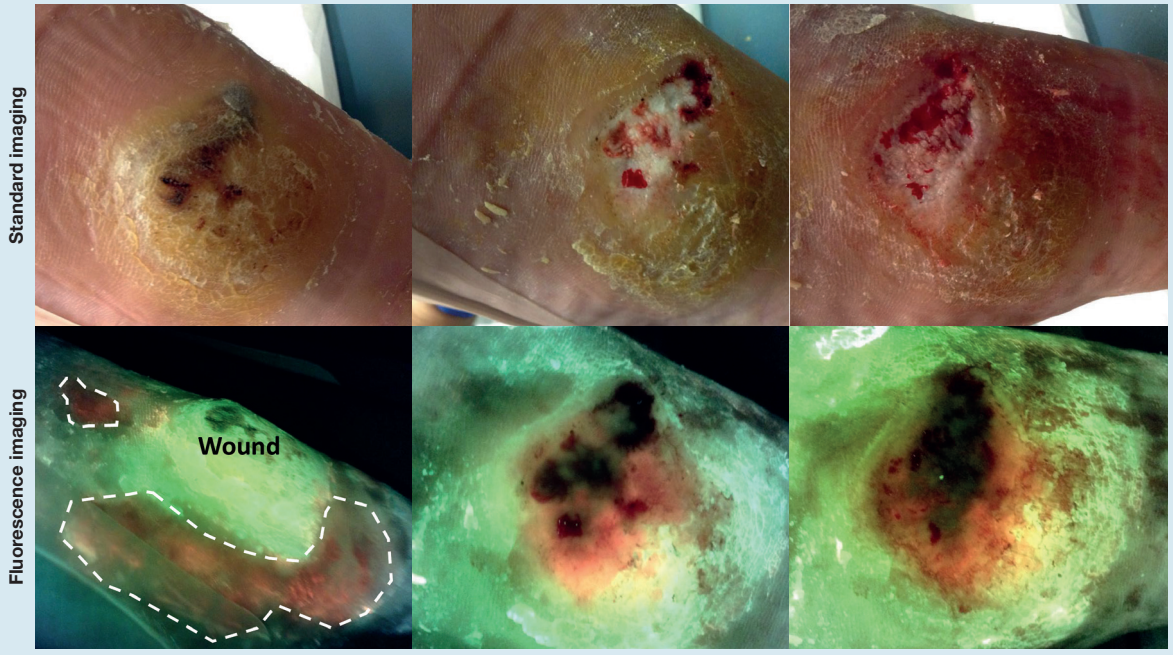
Use of real-time bacterial fluorescence information in wound debridement

Given the evidence above for fluorescence-targeted curettage to appropriately identify and facilitate removal of tissue with high bacterial loads, we next investigated a potential role for fluorescence in guiding curettage debridement to remove contaminated tissue in and around DFUs. Before any wound cleaning or debridement, bacterial (red) fluorescence was only observed in 11/22 DFU assessments (50%). However, fluorescence signal penetration can be achieved only up to a depth of 1.5mm with the imaging device[13] and many of the DFUs negative for fluorescence presented with heavy, thick calluses (Fig 5). The clinician chose to debride, based on routine standard of care in 20/22 DFU assessments. Following aggressive curettage debridement, 100% of debrided wounds revealed red fluorescence, indicating incomplete removal of bacterial burden. Based on this fluorescence information, additional curettage debridement was performed in 85% (17/20) of the wounds assessed under targeted-fluorescence, often to include a larger surface area and/or deeper tissues. Bacterial fluorescence lessened with additional, targeted debridement in each case, but could not always be entirely removed through debridement alone (Fig 6 and 7). In these cases, additional antimicrobial strategies were implemented. Of 20 wounds assessed, three (15%) were not further debrided, as in some cases more aggressive debridement would involve deeper tissue and cause uncontrollable bleeding, or the patient would not accept deeper wounds created by aggressive debridement, or there were time constraints of the visit, such as patient pick-up by pre-arranged services. Interestingly, off-site bacteria was also observed in three of the 22 wounds, such as in foot creases, prompting targeted cleaning.

Discussion

This work demonstrates that the bacterial-fluorescence imaging device can be readily and reproducibly implemented for real-time, point-of-care wound assessment to improve wound documentation and targeted treatment in the clinical setting. The handheld device immediately documented wound area, length and width, and this was achieved with an accuracy >95% for these measurements when overlaid on an image of the wound. In addition, presence and location(s) of bacterial fluorescence was assessed across diverse wound types with microbiological cultures confirming bacterial status approximately three days later.

Fig 7. Fluorescence-guided diabetic foot ulcer (DFU)-debridement. A 82-year-old male with plantar DFU, heavy callus build-up. Bacterial fluorescence (red) was observed surrounding the wound pre-debridement (circled), which prompted thorough cleaning of this region. Persistent bioburden after aggressive, targeted debridement of the wound demonstrated the need for more frequent debridement



Lack of effective documentation is costly not only to wound clinics, health-care systems, and payers,[3] it also places an economic burden onto wound care patients.[26] Data in this body of work highlights how documentation hurdles can be overcome with this bedside wound measurement and fluorescence imaging device:

- *Imprecise or inaccurate analogue wound measurement techniques*. Despite the availability of digital wound area measurement tools and applications, the most common method of measuring wounds is the ruler-based method, which measures the longest length top to bottom and the longest perpendicular width of a wound. This method is rapid and readily available, but it has been shown to grossly overestimate wound area (by >40%),[23–25] including in the current study and it is inconsistent in where the wound is measured from week to week and from clinician to clinician
- *Non-digital and/or time-consuming wound assessment data*. Hand-written documentation, including ruler measurements and notes on assessment of a wound's bacterial status, tends to be incomplete, is easily misplaced in a patient's file, and still requires incorporation into the EMR through dictation, manual transcription or scanning. Documentation should be completed as soon as possible after each patient encounter, preferably during or immediately after the visit, but often this happens at the end of the day or shift which inherently leads to errors and forgotten information. Wound area traces on digital photographs and digital planimetry wound measurement are more accurate[25] and typically are EMR compatible. However, they often require upload to a computer before the measurement can be made, a time-consuming and often clinically impractical step that removes wound assessment from the point-of-care. The ability to document wound measurement and bacterial status in an image format at the patient bedside reduces risk of errors and eliminates time consuming additional steps
- *Subjective assessment metrics*. The current standard of care for bacterial assessment of a wound is made from subjective assessment metrics, for example swelling, odour, redness, heat, pain, all of which are host responses to high bacterial loads that vary from patient to patient. These clinical signs and symptoms have a poor predictive value[20,21] and poor sensitivity[15,20,21] for the detection of high levels of bacteria and infection, yet they are relied upon routinely to guide decisions on where to sample, antimicrobial/antibiotic usage and the level and location of debridement. This problem is compounded in centres where patient wounds are treated by staff inexperienced with wound assessment and with little or insufficient training in complex wound care. Diagnostic tools can standardise care by providing objective assessment information between centres and between care providers. Multisite clinical trials with the bacterial-fluorescence imaging device, in combination with clinical signs and symptoms assessment, have increased the sensitivity for detection of moderate-to-heavy bacterial loads by three

to fourfold, across multiple wound types,[15,27] facilitating evidence-based treatment decisions.

Fluorescence-guided sampling versus Levine swabs

The ability to detect locations of high bacterial loads facilitates targeted treatment (cleaning debridement) as well as targeted sampling for speciation and antibiotic sensitivities. A pilot evaluation comparing standard Levine swab results (not fluorescence-guided) to fluorescence-guided curettage samples found that 36% of samples (4/11) obtained under Levine technique resulted in a false-negative laboratory report. Assuming a sampling cost of $136/sample (based on 2016 physician-billed test payment and sampling cost reported by Centers for Medicare and Medicaid Services), this equates to $544 of wasted expense and laboratory resources in this small cohort of 11 patients or $49.45/patient wasted on laboratory resources. Even more concerning is that under standard of care these misinforming light or no growth culture reports would have, inappropriately, led the clinician and patients down an incorrect care plan and the wound not given the best chance to heal. It is tempting to speculate that similar misinformation throughout the wound care field may be a root cause of the poor and stagnant healing rates widely observed.

Debridement

The goal of debridement intervention is to remove contaminated and necrotic tissues, break up biofilm, and ultimately increase both the ability to heal, and the rate of wound healing.[28] In this study, red (bacterial) fluorescence was present in 100% of DFUs after initial, aggressive, standard of care curettage debridement. This is especially concerning given that red fluorescence equates to a bacterial load of 10^4CFU/g or higher (moderate-to-heavy bacterial loads).[14] This is not the first study to report that sharp debridement leaves behind high levels of bacteria in wound tissues.[29–31] A recent prospective study of 25 hard-to-heal wounds by Moelleken et al. reported that a single round of curettage debridement, performed without fluorescence guidance, left behind 30% of the pre-debridement bacterial fluorescence signal.[31] Furthermore, a clinical trial of 36 hard-to-heal wounds (primarily DFUs) by Kim et al. showed that the reduction in bacterial load after aggressive sharp debridement, again debridement without fluorescence guidance, was <1 log ($6.7x10^4$ to $1.7x10^4$CFU/cm^2) when compared using qPCR.[29] A decrease in log(s) of bacteria is generally accepted as a standard for detection of a meaningful impact of an intervention. Thus, results of this study and others demonstrate that current best DFU debridement practices of visual inspection and clinician judgement (i.e. without fluorescence guidance):

- Fails to maximise removal of bioburden
- Leaves behind an unacceptably high bacterial load (≥10^4CFU/g) that is considered detrimental to wound healing[32]
- Fails to optimally prepare the wound for antimicrobial dressings/treatments. When wound bed preparation is not optimised, best practices and advanced therapies cannot be given their best chance for success.

In this study, bacterial fluorescence signals on images increased post-debridement in over half of the study wounds. This is likely due to subsurface bacteria becoming nearer to the surface and apparent on fluorescent images. Similarly, a prospective clinical study comparing fluorescence signatures pre- and post-debridement in 63 venous/lymphoedema ulcers reported that nearly half the wounds had bacterial fluorescence remaining post-debridement and that in 10% of wounds the bacterial fluorescence signal increased after debridement.[33] The author hypothesised that the subgroup with persistent bacterial fluorescence post-debridement was at increased risk of deep compartment infection and required more frequent debridement and/or antibiotics.[33] Increasing post-debridement bacterial fluorescence was also observed in DFUs by Kim et al. in a subset of study wounds.[29] Further studies are warranted to determine whether wounds harbouring deeper bacteria, remaining after initial debridement, on the fluorescence images, are indeed at increased risk of deep infection. Pilot studies suggest that fluorescence targeting of debridement can improve healing rates,[18,19] but controlled studies are required to determine the effect of this imaging device on wound area reduction rates in a larger population.

Limitations

As with any diagnostic tool, there are limitations of this device which warrant discussion. Visualisation of bacteria in and around a wound does not necessarily mean infection is present, though bacteria at loads above the detection threshold have been shown to delay healing.[10] Bacteria deeper than 1.5mm from the wound surface cannot be detected with the device due to inherent limitations of optical imaging.[13] Therefore this device does not replace the need for clinician judgement and assessment for infection-related signs and symptoms. The device also does not indicate which bacterial species are present nor does it provide bacterial antibiotic sensitivities; microbiological culture is still required if the clinician desires that information.

Fluorescence imaging must be performed under dark conditions. The device has an indicator light which informs when sufficient darkness has been achieved. This is not a problem in windowless rooms, in which lights can simply be turned off. However, the required darkness for capturing fluorescent images is a challenge in inpatient rooms with large windows. To overcome this challenge, a disposable drape attachment can be used, and work has demonstrated its effectiveness in achieving the required darkness.[16]

Conclusion

In summary, incorporation of bacterial fluorescence imaging into routine wound care in this study resulted in more aggressive debridement. This specifically ➤

Reflective questions

- What information do you currently use to determine appropriate degree of debridement for diabetic foot ulcers (DFU)? How would real-time information on bacterial load and location change your debridement practices?
- How confident are you in the current methods you use to measure wounds? What impact would increased accuracy of wound measurement have on your documentation practices?
- What sampling practices do you use to evaluate bacterial burden in wounds? How would targeted sampling based on fluorescence information change your treatment planning?
- How can bacterial fluorescence information be used to optimise wound bed preparation for advanced therapies?

targeted regions of bioburden, and avoided unburdened tissue, providing a more optimal state for healing. Results highlight the potential of bacterial fluorescence imaging to dramatically improve current debridement practices by enabling point-of-care, evidence-based decision-making on which tissue, and how much tissue, to selectively remove. Additionally, by identifying patients with bacteria deep in their wound tissues, who may be at higher risk of infection, it may be possible to more effectively tailor their wound management plan resulting in timely and improved healing outcomes. **JWC**

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