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The Clinical Utility of Autofluorescence Imaging for Bacterial Detection in Wounds: A Systematic Review

Sadie Badrie[1] | Zena Moore[2,3,4,5,6,7,8] | Declan Patton[2,8,9] | Tom O'Connor[2,7,8] | Rosemarie Derwin[3,10]

[1]Royal College of Surgeons Ireland, University of Medicine and Health Sciences, Dublin, Ireland | [2]School of Nursing and Midwifery, Royal College of Surgeons in Ireland (RCSI), University of Medicine and Health Sciences, Dublin, Ireland | [3]Cardiff University, Cardiff, UK | [4]School of Nursing & Midwifery, Griffith University, Gold Coast, Queensland, Australia | [5]School of Health Sciences, Faculty of Life and Health Sciences Ulster University, Coleraine, UK | [6]Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium | [7]Lida Institute, Shanghai, China | [8]Department of Nursing, Fakeeh College for Medical Sciences, Jeddah, Saudi Arabia | [9]Fakeeh College of Health Sciences, Jeddah, Saudi Arabia | [10]School of Nursing & Midwifery, Trinity College Dublin, Dublin, Ireland

Correspondence: Sadie Badrie (sadiebadrie20@rcsi.ie) | Rosemarie Derwin (derwinro@tcd.ie)

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ABSTRACT

This systematic review evaluated the clinical utility and diagnostic accuracy of autofluorescence imaging in detecting bacterial presence in wounds. A literature search was conducted in January 2025 across PubMed, Scopus, Cochrane, and EMBASE databases. Eligible studies included clinical trials and observational studies assessing autofluorescence imaging for wound bacterial detection. Seventeen studies were included; sixteen assessed the MolecuLight i:X device, and one evaluated PRODIGI. Autofluorescence imaging demonstrated higher accuracy than White Light and Clinical Signs and Symptoms-based assessment in detecting bacterial burden. Five studies highlighted its role in enhancing swabbing techniques, with fluorescence-guided sampling yielding higher bacterial counts than conventional methods. Ten studies reported significant bacterial reduction with autofluorescence-guided debridement. Six studies emphasized its role in refining treatment decisions and accelerating wound healing. Quality appraisal was undertaken using Evidence-Based Librarianship criteria, which deemed 10 studies valid, while 7 had limitations related to population representation. In conclusion, autofluorescence imaging enhances wound assessment by improving bacterial detection and may support more targeted clinical interventions. However, further research is needed to clarify its impact on infection control and long-term healing outcomes.

1 | Introduction

A wound arises when the skin's cutaneous barrier is compromised [1]. Acute wounds result from sudden skin integrity disruptions and typically follow a well-defined healing process [1, 2]. In contrast, non-healing wounds fail to progress through the normal healing stages and remain open longer [1]. A critical factor that can disrupt wound healing is infection, which occurs when harmful microorganisms release toxins and enzymes that damage the tissue [3]. These non-healing wounds impose a significant burden on patients and healthcare systems, increasing hospitalisations, antimicrobial use, and treatment costs [4–7]. When infection is not promptly detected and managed, it can delay wound healing and result in complications such as inflammation, biofilm formation, and increased exudate production [8, 9]. Consequently, early and accurate identification of bacterial burden is essential to optimising wound care and reducing infection-related complications [10–12].

Traditionally, bacterial detection in wounds relies on clinical judgement based on observable signs and microbiological testing such as swabs or tissue biopsies [13]. However, these methods have significant limitations. Clinical signs of infection can be subjective and nonspecific, leading to delayed diagnosis [14, 15]. Microbiological

Summary

- Autofluorescence imaging improves the detection of clinically significant bacterial loads in wounds.
- Fluorescence-guided wound assessment enhances swabbing accuracy, targeted debridement, and treatment decision-making.
- Autofluorescence imaging shows higher diagnostic accuracy than conventional white light and clinical signs-based assessment.
- Further research is needed to validate the impact of autofluroescence imaging on long-term wound healing and infection control.

testing, while useful, requires sample collection and laboratory processing, making it time-consuming, invasive, and costly [16]. Standard swabbing techniques may also underestimate bacterial burden, particularly in biofilm-containing wounds where bacteria are unevenly distributed. Despite its importance, the process can be cumbersome and may only identify some present microbial species [15–17]. Furthermore, the overuse of antibiotics has increased antibiotic-resistant strains, emphasising the need for careful diagnosis and judicious antimicrobial intervention [16–19].

Given the limitations of conventional methods, there is an increasing demand for precise, non-invasive, and cost-effective diagnostic tools. A promising advancement is auto-fluorescence imaging (AFI), a real-time imaging technique that detects endogenous fluorescence emitted by bacterial metabolites. Such technology allows for non-contact, high-resolution visualisation of bacterial burden, facilitating more objective and reliable wound assessment, which is crucial for planning timely and effective interventions [14, 18]. Incorporating objective methods into wound assessment has the potential to predict healing outcomes and alert the clinician to the early onset of infection [9, 20–24].

AFI has shown promise in enhancing the sensitivity of bacterial detection in wounds, as demonstrated in the literature [15, 24]. By providing real-time visual cues, it improves surgical site monitoring, refines clinical decision-making, and potentially reduces infection rates. Clinicians experienced in interpreting AFI may be better equipped to optimise treatment strategies and improve wound management outcomes [25, 26] With the ability to detect moderate to heavy bacterial loads non-invasively, AFI has emerged as a valuable tool in clinical practice [22]. It enables more precise wound assessments, guiding clinicians towards personalised and effective treatment approaches [14, 24]. Thus, AFI holds the potential to shift infection management from a reactive to a proactive strategy [18]. Despite increasing research in this area, no systematic review has synthesised the evidence on AFI for bacterial detection in wounds, highlighting a critical gap in the literature. This review evaluates the diagnostic accuracy and clinical application of AFI in detecting bacterial burden in chronic wounds.

2 | Aim and Research Question

This systematic review aimed to determine the clinical utility of AFI devices in detecting the presence of bacteria in wounds. This systematic review explored the research question: 'What is the efficacy and diagnostic accuracy of AFI compared to traditional methods in detecting the presence of moderate to heavy loads of bacterial growth in wounds?' The research question was structured using the PICO framework, details for which can be found in Table S1.

3 | Methods

This systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [27]. The protocol for this systematic review was registered with PROSPERO (CRD42022340236) to ensure adherence to pre-specified objectives and methodology.

3.1 | Inclusion and Exclusion Criteria

Inclusion criteria, studies:

- Of patients in any healthcare setting with an infected or non-healing wound.
- Employing a quantitative research design.
- Exploring the use of an AFI device for bacterial detection, wound assessment, or clinical decision-making.
- Published in the English language.

Exclusion criteria:

- Post hoc analyses derived from datasets from already included studies (to prevent potential data duplication bias).
- Retrospective studies (due to potential methodological limitations and selection bias).
- Animal studies.
- Written in a language other than English.

3.2 | Outcomes Measured

The primary outcome was the ability of AFI to detect bacterial ($\geq 10^4$ CFU/g) presence in chronic wounds.

Secondary outcomes included:

- To evaluate whether AFI-guided microbiological sampling improves bacterial detection rates compared to standard sampling methods.
- To assess whether AFI enhances the precision and effectiveness of debridement by identifying bacterial loads and guiding clinical interventions

- To determine whether AFI enhances treatment decisions and outcomes by improving the detection of bacterial burden in chronic wounds.

3.3 | Electronic Sources and Search Strategy

A systematic search was conducted in January 2025 across the following electronic databases:

- Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library) (latest issue);
- Ovid MEDLINE (1946 to January 2025);
- Ovid MEDLINE (In-Process & other Non-indexed Citations) (latest issue);
- Ovid EMBASE (1974 to January 2025);
- EBSCO CINAHL Plus (1937 to January 2025);
- Scopus.

The reference lists of all included studies and relevant systematic reviews were scanned to identify further published, unpublished, and ongoing studies. The detailed search strategy, including Boolean operators, keywords, and filters, is available in Appendix A.

3.4 | Study Selection

The study selection process involved a two-stage screening procedure conducted independently by two reviewers. In the first stage, the titles and abstracts of all articles identified through the search strategy were screened independently by two reviewers using the Rayyan. AI platform, which enabled blinding and unbiased screening. In the second stage, the two reviewers independently reviewed the full texts of potentially relevant articles. Discrepancies at any stage were resolved through discussion, referencing the inclusion and exclusion criteria outlined.

3.5 | Data Extraction

Data extraction was conducted independently by two reviewers using a standardised data extraction table in Microsoft Excel with the following headings: author and year of publication, country, setting, sample group (number and type of wound), study design, data collection period, intervention, comparison, outcomes, results, limitations, and quality appraisal score. Missing or unclear data were addressed by contacting study authors where possible. If data remained unavailable, the study was only included in a descriptive capacity.

3.6 | Risk of Bias Assessment

All the studies included were appraised using the Evidence-Based Literature (EBL) checklist [25]. This tool is designed to evaluate a study's validity, applicability, and pertinence by examining critical components of the research process: Population, Data collection, Study design, and Results. By the EBL criteria, a study is considered valid if its overall validity score (Yes/Total) is $\geq 75\%$, or the combined ratio of (No + Unclear)/Total is $\leq 25\%$ [28].

3.7 | Data Analysis

Data from the selected studies were entered into a standardised extraction table for analysis. Due to substantial heterogeneity in study design, sample sizes, wound types, AFI devices used, and outcome measures, a meta-analysis was deemed inappropriate. Instead, a structured narrative synthesis was conducted, with findings tabulated in summary tables comparing study characteristics, interventions, and key outcomes. Missing summary statistics were explicitly noted, and available data were reported transparently. Heterogeneity was explored qualitatively by comparing study designs, outcome measures, and sample characteristics, but no subgroup analyses or meta-analysis were conducted due to the variability in study designs. Furthermore, no formal sensitivity analyses were performed, given the narrative synthesis approach. This synthesis provided a comprehensive interpretation of both primary and secondary outcomes.

3.8 | Reporting Bias Assessment

Due to the heterogeneity of included studies and the narrative synthesis approach, formal assessment of publication bias (e.g., funnel plot) was not feasible. However, reporting bias was considered by evaluating study funding sources, selective outcome reporting, and conflicts of interest. No major discrepancies were found between reported results and expected outcomes based on study protocols. Most studies did not disclose funding sources, and no clear evidence of funding bias was identified.

3.9 | Certainty of Evidence

Certainty of evidence was not formally assessed using GRADE due to the narrative synthesis approach. However, study quality was evaluated using the EBL checklist, which identified moderate methodological limitations in some studies. Certainty of findings varied across study designs, with higher confidence in prospective trials and lower confidence in retrospective and case report studies.

4 | Results

4.1 | Overview of Included Studies

A total of 265 records were identified through database searches (Figure 1). During the initial screening of titles and abstracts, 229 records were excluded. Among these, three studies [24, 29, 30] were identified as topically relevant to AFI but were excluded as they comprised post hoc or retrospective analyses utilising datasets already included in other studies within this review. Of the remaining 36 articles, 13 were not retrievable. The full-text review of the remaining 23 articles resulted in the exclusion of a further six studies [31–36] for the following reasons: non-eligible population ($n = 2$), inappropriate outcomes ($n = 1$), foreign language publication ($n = 1$), and unsuitable study design ($n = 5$) (Table 1). Ultimately, 17 studies met the predefined inclusion criteria and were included in this systematic review [37–53].

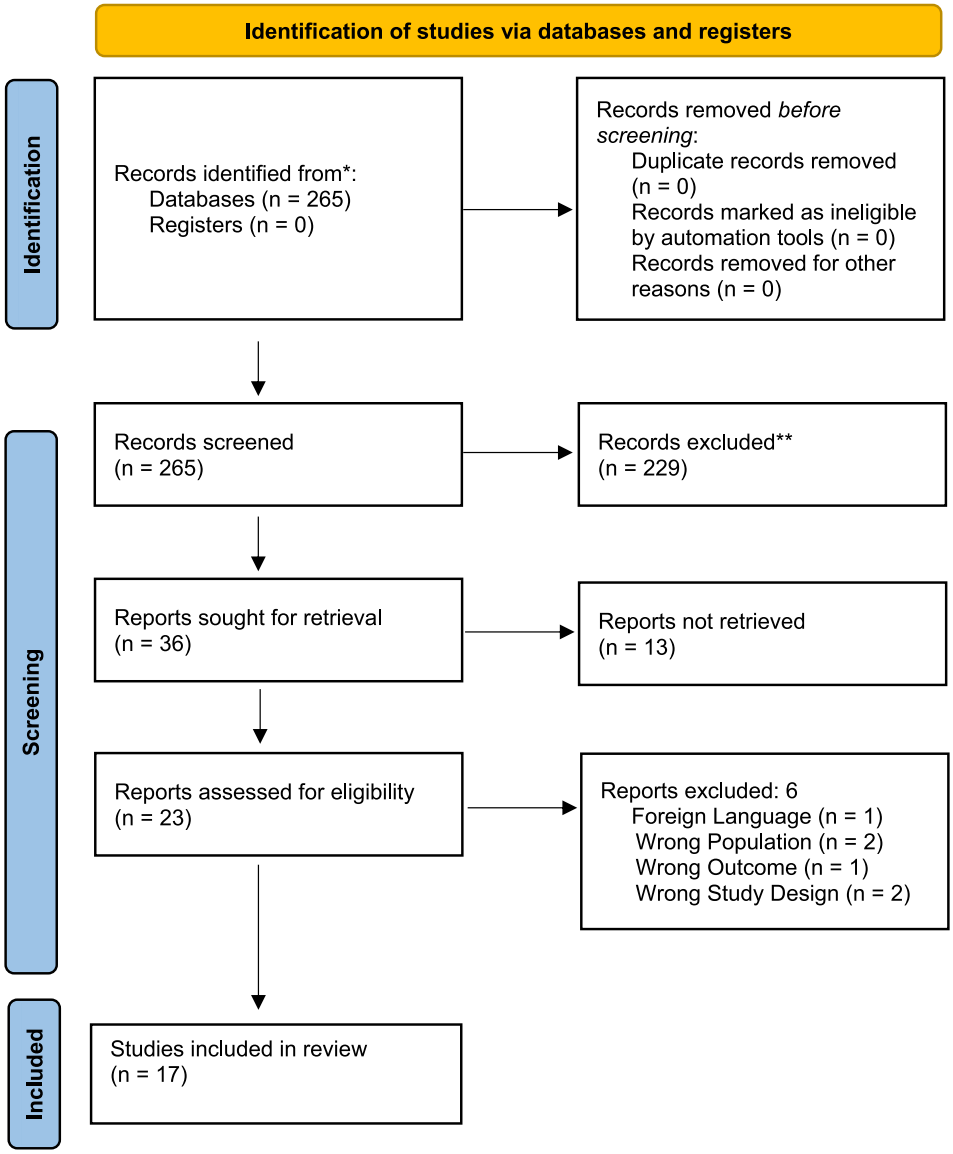

FIGURE 1 | PRISMA flow chart showing the screening process for studies to be included in this review.

4.2 | Characteristics of Studies

Table 2 provides an overview of the included studies.

4.2.1 | Study Design

The study designs of the included studies comprised clinical trials ($n = 7$) [9, 37–42], case reports ($n = 3$) [43–45], observational studies ($n = 6$) [46–51], and cross-sectional studies ($n = 1$) [52].

4.2.2 | Geographical Location

The studies were conducted between 2015 and 2023. Six studies were conducted in Canada [9, 37–40], and four studies in the UK [42, 43, 46, 49]. Countries with one study each included Ireland [47], Netherlands [48], USA [52], Italy [41], Taiwan [50], Japan [44], Poland [45] and South Korea [51].

4.2.3 | Study Settings

The studies were primarily conducted in wound care centres, hospitals, or outpatient healthcare settings [9, 37, 38, 40–43, 45–52]. However, two studies did not specify the study setting [39, 44].

4.2.4 | Sample Size and Wound Type

The mean sample size of the studies was 77 wounds, ranging from 2 [45] to 350 [52] wounds. The most frequently reported wound types included diabetic foot ulcers, venous leg ulcers, and burn wounds.

4.2.5 | Intervention/Description of Devices

Two AFI devices were evaluated across the studies:

TABLE 1 | Excluded studies.

Study	Study	Reason for exclusion
Armstrong et al. [29]	Point-of-care fluorescence imaging reveals extent of bacterial load in diabetic foot ulcers	Post hoc analysis of a multicentre clinical trial; does not meet inclusion criteria.
Johnson et al. [24]	Skin pigmentation impacts the clinical diagnosis of wound infection: Imaging of bacterial burden to overcome diagnostic limitations	Post hoc study focusing on skin pigmentation effects, not AFI effectiveness in bacterial detection.
Jacob et al. [30]	Lights, fluorescence, action—Influencing wound treatment plans including debridement of bacteria and biofilms	Retrospective analysis, does not fit the prospective study inclusion criteria.
Wu et al. [31]	Auto fluorescence imaging device for real-time detection and tracking of pathogenic bacteria in a mouse skin wound model: Preclinical feasibility studies.	Relates to an animal study
Rennie et al. [32]	Understanding real-time fluorescence signals from bacteria and wound tissues observed with the Moleculight I:X.	Relates to a tutorial on how to use the Moleculight i:X device
Alawi et al. [33]	Imaging of bacteria in burn wounds treated with split-thickness grafts in Meek/Mesh Technique: A pilot study with first experiences in clinical wound evaluation with autofluorescence.	Study written in a foreign language
Serena [34]	Incorporating point-of-care bacterial fluorescence into a wound clinic antimicrobial stewardship program.	Does not answer the primary outcome
Serena et al. [35]	Are semi-quantitative clinical cultures inadequate? Comparison to quantitative analysis of 1053 bacterial isolates from 350 wounds.	Relates to the wrong study design
Lopez et al. [36]	Detection of bacterial fluorescence from in vivo wound biofilms using a point-of-care fluorescence imaging device.	Relates to an in vivo study

- MolecuLight i:X ($n = 16$ studies) [9, 38–52]: This device provides real-time identification of bacterial fluorescence, particularly at bacterial loads of clinical concern ($\geq 10^4$ CFU/g). It captures fluorescence from tissues and other fluorescent elements within its field of view. Clinicians interpret the diverse colours and features displayed on the device's fluorescence images. Cyan and red fluorescence signals indicate potentially pathogenic bacteria at moderate to heavy bacterial loads. Red fluorescence signals represent Gram-positive/negative bacteria ($> 10^4$ CFU/g) such as *Staphylococcus aureus, E. coli*, and *Proteus*. Blush red or yellow fluorescence suggests sub-surface bacteria, while cyan fluorescence indicates *Pseudomonas aeruginosa*. Wounds displaying red/pink/blush or cyan fluorescence are considered bacterial fluorescence-positive.
- PRODIGI ($n = 1$ study) [37]: The PRODIGI device, as described by DaCosta et al., is a prototype equipped with a high-resolution camera capable of capturing both white light (WL) and AFI. The device utilises broad-spectrum white LEDs for WL imaging and specific violet-blue LED arrays for fluorescence light imaging to detect bacteria.

4.2.6 | Comparators Included

- White light (WL) inspection
- Clinical signs and symptoms (CSS) assessment
- Microbiological culture or qPCR reference standards

4.3 | Primary Outcome: Diagnostic Accuracy of AFI for Bacterial Detection in Chronic Wounds

This systematic review assessed the diagnostic accuracy of autofluorescence imaging (AFI) in detecting bacterial loads $\geq 10^4$ CFU/g in chronic wounds. Seventeen studies evaluated AFI across various wound types, including diabetic foot ulcers (DFUs), venous leg ulcers (VLUs), burn wounds, and surgical wounds. AFI performance was compared to clinical signs and symptoms (CSS) assessment and white light (WL) inspection, using microbiological culture or qPCR as reference standards. The primary diagnostic parameters assessed included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR). The quantitative significance of the seventeen included studies is summarized below (see Table 4).

Across all studies, AFI exhibited sensitivity ranging from 45% [38] to 100% [47] and specificity between 55.6% [51] and 92.3% [50] for detecting bacterial burden in wounds. PPV varied from 64.0% [48] to 95.4% [47], while NPV ranged from 35.3% [50] to 100% [47]. Four studies [9, 37, 40, 50] found AFI to be significantly more accurate than CSS and WL in detecting bacterial loads, while one study did not report statistical significance. Compared to standard assessment methods such as white light inspection and clinical signs and symptoms (CSS), AFI generally exhibited higher sensitivity in detecting bacterial presence, particularly in wounds without overt clinical signs

TABLE 2 | Overall characteristics of the 17 studies included in the systematic review.

Study	Country	Study design	Setting	No. of patients	No. of swabs	Sample group (number of patients and type)	Intervention	Comparison
DaCosta et al. [37]	Canada	Two-phase I Single centre, non-randomised clinical trial	Hospital	28	490	$N=61$ Part 1: $N=48$ • 26 Diabetic foot ulcers, • 22 non-diabetic wounds Part 2: $N=13$ • 11 Diabetic foot ulcers • 2 non-diabetic wounds	PRODIGI	Microbiology (Swabs)
Rennie et al. [38]	Canada	Non-randomised single blind clinical trial	Health Centre	60	Not specified	$N=60$ • 47 diabetic foot ulcers, • 12 venous leg ulcers • 1 amputation	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Swabs)
Ottolino-Perry et al. [8]	Canada	Non-randomised clinical trial	Hospital	29	128	$N=33$ Diabetic foot ulcers	Handheld K2 imaging device (provided by MolecuLight i:X, Toronto, ON, Canada).	CSS; Microbiology (Swabs)
Blumenthal et al. [43]	UK	Case Report (4 patients)	Burn Centre	20	16	$N=20$ Burn wounds	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Swabs)
Blackshaw and Jeffery [46]	UK	Prospective observational study	Hospital	14	17	$N=17$ • 4 BCC • 1 malignant melanoma excision • 1 hematoma removal • 1 Gorlin syndrome • 1 Alkaline burn • 1 skin graft site • 1 fasciotomy wound. • 1 ulcer • 2 tissue damage-RTA • 1 abscess • 1 electric burn	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Swabs)

(Continues)

TABLE 2 | (Continued)

Study	Country	Study design	Setting	No. of patients	No. of swabs	Sample group (number of patients and type)	Intervention	Comparison
Hurley et al. [47]	Ireland	Prospective observational study	Hospital	33	43	*N* = 32 • 21 Lower limb • 2 thighs • 2 upper limbs • 2 sacrum • 2 scalps • 2 chest walls • 1 natal cleft	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Swabs)
Pijpe et al. [48]	Netherlands	Observational study	Burn Centre, Hospital	14	Not specified	*N* = 20 Burn wounds	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Swabs)
Raizman et al. [39]	Canada	Clinical trial	Not specified	39	Not specified	*N* = 50 • 36 DFUs • 4 VLUs • 3 ALU • 7 other wound types	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Swabs)
Farhan and Jeffery [49]	UK	Single-centre observational study	Burn Centre	10	Not specified	*N* = 15 Burn wounds • 8 upper extremities • 5 lower limbs • • 2 trunks	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Swabs)
Raizman et al. [40]	Canada	Non-randomised, single centre clinical study	Health Centre	28	28	*N* = 28 • 17 VLU • 4 DFU • 2 Surgical sites • 1 Pressure ulcer • 1 Lymphedema • 1 Rheumatoid wound • 2 other	MolecuLight i:X, Toronto, ON, Canada.	CSS
Le et al. [52]	USA	Prospective, single-blind, multicentre cross-sectional study	Health Centre	350	367	*N* = 350 • 138 DFUs • 106 VLUs • 60 surgical sits • 22 pressure ulcers • 24 others	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Punch Biopsies)

(Continues)

TABLE 2 | (Continued)

Study	Country	Study design	Setting	No. of patients	No. of swabs	Sample group (number of patients and type)	Intervention	Comparison
Janowska et al. [41]	Italy	Clinical prospective study	Hospital	43	Not specified	$N=43$ • 21 Venous ulcers • 3 arterial ulcers • 4 vasculitis • 7 pyoderma gangrenosum • 7 traumatic ulcers • 1 neoplastic ulcer.	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (Punch Biopsies); CSS
Wu et al. [50]	Taiwan	Observational study	Hospital	53	Not specified	$N=60$ • 19 Diabetic • 11 Arterial insufficiency • 4 venous ulcers • 5 pressure injury • 19 trauma • 2 others	MolecuLight i:X, Toronto, ON, Canada.	Modified wound blotting with Alcian blue grading
Rahma et al. [42]	UK	Single centre prospective pilot randomised controlled trial	Hospital	56	Not specified	$N=56$ Diabetic foot ulcers	MolecuLight i:X, Toronto, ON, Canada.	CSS
Kurokami et al. [44]	Japan	Case reports	Not specified	20	Not specified	$N=20$ Skin ulcers	MolecuLight i:X, Toronto, ON, Canada.	Not specified
Mościcka et al. [45]	Poland	case reports	Hospital	2	Not specified	$N=2$ Venous leg ulcers	MolecuLight i:X, Toronto, ON, Canada.	Not specified
Koo et al. [51]	South Korea	Observational, single-centre study	Diabetic wound centre, Korea University Guro Hospital	35	Not specified	$N=48$ Diabetic foot ulcer	MolecuLight i:X, Toronto, ON, Canada.	Microbiology (tissue biopsy)

as reported in three studies [39, 43, 46]. Additionally, AFI showed high accuracy in detecting *Pseudomonas aeruginosa*, with a sensitivity of 66.7% and specificity of 87.2% ($p < 0.001$) [51]. However, variations in microbiological reference standards and fluorescence interpretation influenced diagnostic accuracy across studies.

4.4 | Summary of Individual Study Findings

DaCosta et al. [37] used the PRODIGI autofluorescence (AF) device to detect bacterial presence in 28 patients with 48 wounds. Results were verified via swab tests. The results demonstrated that autofluorescence accurately detects bioburden in the wound bed, periphery, and off-site. AF correctly detected 74.5% of wounds with clinically significant bioburden compared with 52.5% for standard practice ($p = 0.003$). This was statistically significant. Additionally, AF imaging identified bacteria in the wound periphery 82.4% of the time ($p < 0.001$) and 67.1% in other areas ($p = 0.006$) which would have been overlooked using standard methods.

In Rennie et al. [38], a total of 60 patients (45 males, 15 females) with chronic wounds, including 47 diabetic foot ulcers (DFUs), 12 venous leg ulcers (VLUs), and 1 amputation wound, were evaluated using fluorescence imaging to detect bacterial presence. Regions exhibiting red fluorescence were sampled for bacterial analysis. Of the 30 wounds sampled via biopsy in areas of red fluorescence, all tested positive for bacterial presence using quantitative PCR (qPCR) and microbiological culture, confirming a 100% positive predictive value (PPV) with no false positives. Bacterial loads ranged from 10^4 to 10^8 CFU/g, with 87% of samples exceeding $\geq 10^5$ CFU/g, thresholds associated with delayed wound healing. These findings underscore the reliability of red fluorescence as a real-time, non-invasive indicator of clinically significant bacterial burden in chronic wounds.

Ottolino-Perry et al. [9] examined the accuracy of autofluorescence (AF) imaging in identifying the bacterial burden in diabetic foot ulcers (DFUs) compared to traditional clinical signs and symptoms (CSS) assessment. The study included 33 DFUs from 29 patients, with 128 swabs collected for analysis. The AF imaging significantly outperformed CSS in terms of specificity (78% vs. 38%, $p = 0.0043$) and accuracy (78% vs. 52%, $p = 0.048$) in predicting the presence of moderate or heavy bacterial growth.

Blumenthal and Jeffery [43] utilised autofluorescence imaging (AFI) to detect bacterial burden in 20 patients with burn wounds. Burn wounds were photographed under standard light and violet light illumination to compare presentations of obvious infection signs and symptoms. Microbiology swab samples were obtained to correlate any bacterial presence to the images. The device successfully identified high bacterial levels in 16 patients who also demonstrated positive microbiology swabs during admission. Whereas four patients showed negative microbiological growth on swabs and no high bacterial levels according to MolecuLight imaging. Interestingly, two patients did not display clinical signs and symptoms (CSS), while the images and swabs proved positive for bacterial presence. One patient showed CSS, while the images and swabs proved negative.

Blackshaw et al. [46] included 14 patients with 17 wounds. An auto-fluorescence imaging device was utilised to detect bacterial presence. Of the 17 samples, nine were negative for microbiological growth on swabs taken during the dressing clinic; their results came back as 'no growth' or 'mixed skin organisms. Of these nine, the Moleculight i:X device was unable to detect significant levels of bacteria in eight of them. In one patient, AFI detected cyan fluorescence, yet the swab results came back as "mixed skin organisms"'. The patient did not show CSS, and their swab result was negative, yet AFI captured cyan fluorescence, indicating the presence of high levels of *Pseudomonas aeruginosa*. The device was notably effective in cases where clinical signs and symptoms were absent, yet bacterial growth was present.

Hurley et al. [47] included 33 patients (64% male, 36% female) with wounds varying in location and bacterial presence. Utilising a fluorescence imaging device, all wounds showed bacteria presence, which was confirmed with a positive predictive value of 95.4% (41 out of 43) of swabs showing bacterial growth. Seven patients displayed signs of infection, including erythema, pain, tenderness, and malodour. All of these patients exhibited a red fluorescence when imaged using the wound imaging device, which indicates potentially pathogenic bacteria. FL-imaging had a sensitivity and specificity of 100% in detecting Pseudomonas species, with overall sensitivity and specificity of 100% and 78%, respectively.

Pijpe et al. [48] included 14 patients with 20 wounds to assess the effectiveness of a bacterial fluorescence imaging device in detecting bacterial burden in wounds. Cohen's kappa of 1.00 ($p < 0.000$) indicated perfect agreement between microbiological test results of high-fluorescent areas and standard swabs. The sensitivity of the device was 78% (CI: 40%–79%), and the specificity was 64% (CI: 31%–89%). The device demonstrated a positive predictive value of 64% (CI: 42% to 80%) and a negative predictive value of 78% (CI: 49%–93%). For *Pseudomonas aeruginosa* (PA), sensitivity, specificity, positive predictive value, and negative predictive value were 100%, 70%, 44%, and 100%, respectively.

Raizman et al. [39] included 39 patients with 50 diabetic wounds. The MolceuLight i:X imaging device was used for wound assessment and bacterial detection. AFI demonstrated a high degree of accuracy in identifying bacteria in wounds. Of 50 wounds examined using fluorescence imaging, 36 (72%) were positive for red/pink/blush or cyan fluorescence; only 11% were positive in the wound bed, 86% in the peri-wound tissue, and 3% in both wound bed and peri-wound tissues. These findings were consistent across all wound types.

Farhan et al. [49] examined the use of AFI technology for bacterial detection in paediatric burn wounds. This involved case studies of five patients with seven wounds, where AFI demonstrated the capacity to identify bacterial presence that was missed by routine assessment and spectrally discern *Pseudomonas aeruginosa* by its cyan fluorescence signal. Despite some cases of bacterial detection inconsistencies between AFI and conventional culture results, overall, the application of AFI appeared beneficial in supporting clinical decision-making.

Raizman et al. [40] included 28 patients. The MolecuLight i:X device indicated the presence of *Pseudomonas aeruginosa* (PA) in 26 wounds, whereas under WL conditions, one wound portrayed CSS. Culture analysis confirmed the presence of PA in 26/28 wounds. The bacterial load was moderate-to-heavy in most cases, and PA was observed to be polymicrobial in 92.3% of wounds. Eighteen (64.3%) wounds had heavy growth of PA, corresponding to cyan-positive regions, while in six (21.4%) wounds, moderate growth of PA, corresponding to cyan fluorescence, was observed. In two wounds, light growth of PA was observed, while in two wounds, semi-quantitative analysis revealed scant or no growth of PA from the cyan-positive curettage sample.

Le et al. [52] included 350 wounds to evaluate the MolecuLight i:X device for detecting bacterial burden in wounds. Results indicated that adding FL to CSS assessments significantly improved sensitivity four-fold in identifying wounds with bacterial loads $>10^4$ CFU/g ($p<0.001$). Specificity was similarly high (84.1%) for CSS + FL, comparable to CSS only. FL also improved Diagnostic Odds Ratio (DOR), with CSS + FL at 8.3, 3.1-fold higher than CSS alone. Additionally, the NPV and accuracy of CSS + FL were significantly increased compared to CSS alone ($p<0.001$). The device also guided clinicians in identifying bacterial presence outside the wound bed in 42.4% of wounds negative for CSS.

Janowska et al. [41] examined 51 different areas of various ulcers, aiming to detect bacterial burden through AFI. Certain fluorescence areas demonstrated a negative correlation between pH and Wound Bed Score (WBS), such as red (−0.97) and light green (−0.16), suggesting that higher pH corresponded to lower WBS. The Wilcoxon tests revealed statistically significant differences in pH between cyan and light green areas ($p=0.02$), and in WBS between cyan and dark red–purple–black areas ($p=0.03$). These results emphasise the potential of AFI in detecting bacterial burden in ulcers.

Wu et al. [50] investigated the utility of a modified wound blotting technique with Alcian Blue Grading for assessing bacterial burden in wounds against the MolecuLight i:X imaging device. The findings suggested that wounds initially showed grade-zero staining, indicative of a lower bacterial burden (p-values ranging from 0.018 to 0.002). Both techniques demonstrated the ability to detect bacterial presence in wounds, with the modified wound blotting grading between 90-day healed and unhealed groups: $p<0.001$ (statistically significant); and the MolecuLight i:X results between 90-day healed and unhealed groups: $p<0.001$ (statistically significant).

Rahma et al. [42] randomly assigned 56 patients with diabetic foot ulcers (DFUs) to either a control group or an FL-imaging group. A sensitivity analysis was performed; 13 ulcers (45%, CI 26%–64%) had healed in the AFI arm, compared to 10 (37%, 95% CI 19%–58%) in the control arm. This showed the ability of AFI to detect bacteria and support wound management.

In Kurokami et al. [44], the MolecuLight i:X device was utilised for autofluorescence imaging in a study involving 56 patients with diabetic foot ulcers (DFUs). The tool identified positive autofluorescence, indicating bacterial presence, in 55.2% of the patients in the treatment group.

Mościcka et al. [45] present two cases of patients with chronic wound ulcers, both examined with a fluorescence imaging device to determine bacterial burden. In the first case, a 68-year-old female exhibited ulceration on her left lower limb. Fluorescence imaging indicated areas with increased red and cyan fluorescence, suggesting bacterial presence. In the second case, a 32-year-old male had a similar wound on his left lower limb. Fluorescence imaging revealed increased red colour emission, indicative of bacterial load. In both cases, a sample for microbiological testing was obtained with swab sticks, revealing the presence of gram-negative bacteria. These results suggest that fluorescence imaging can potentially identify areas of increased bacterial load in chronic wounds.

Koo et al. [51] investigated the diagnostic accuracy of the MolecuLight i:X autofluorescence imaging (AFI) device in detecting bacterial presence in diabetic foot ulcers (DFUs). The study included 35 patients with 48 wounds, utilising swab cultures and tissue cultures as the reference standard for bacterial identification. The AFI device demonstrated a sensitivity of 64.1% and a specificity of 55.6% for detecting bacterial presence. The positive predictive value (PPV) was 86.2%, while the negative predictive value (NPV) was 26.3%, resulting in an overall diagnostic accuracy of 62.5%. When analysing bacterial subtypes, the AFI device showed a sensitivity of 66.7% and specificity of 87.2% for *Pseudomonas aeruginosa*, with a PPV of 54.6% and an NPV of 91.9%. In contrast, for non-Pseudomonas bacteria, the sensitivity and specificity were 43.8% and 62.5%, respectively, with a PPV of 70.0% and an NPV of 35.7%. The statistical significance of AFI in detecting *Pseudomonas aeruginosa* was $p<0.001$ for both sensitivity and specificity, while the PPV was not statistically significant ($p=0.3671$), and the NPV was significant ($p=0.0198$). The study highlights the varying diagnostic performance of AFI in detecting different bacterial species in DFUs, with higher accuracy for *Pseudomonas aeruginosa* compared to non-Pseudomonas species.

4.5 | Heterogeneity and Justification for Absence of Meta-Analysis

Significant variability was observed across the included studies, making a meta-analysis unfeasible. Differences in study design (e.g., clinical trials, observational studies, and case reports), sample sizes, and wound types contributed to methodological heterogeneity. While most studies assessed the MolecuLight i:X device, one study evaluated PRODIGI, introducing potential variation in imaging protocols and fluorescence interpretation.

A key limitation was the lack of standardised diagnostic thresholds for bacterial fluorescence positivity, which varied across studies. The microbiological reference standards also differed, with some studies employing quantitative PCR (qPCR) while others used semi-quantitative culture methods, leading to inconsistencies in sensitivity and specificity. Further variability in outcome reporting was noted. Some studies reported only sensitivity and specificity, while others included positive predictive

value (PPV), negative predictive value (NPV), diagnostic odds ratios (DOR), or Cohen's kappa values. This lack of a uniform statistical framework for defining and reporting AFI diagnostic accuracy prevented direct data pooling. Given these methodological and analytical differences, a qualitative narrative synthesis was performed instead of a meta-analysis to provide a structured interpretation of the findings (Tables 3 and 4).

4.6 | Secondary Outcome: Effectiveness of AFI in Wound Swabbing Accuracy and Targeted Bacterial Sampling

Five studies [9, 40, 42 51, 52] investigated the influence of AFI on swabbing techniques. Overall, the studies report that swab collection was guided more effectively with AFI, providing more accurate sampling and leading to higher detection rates compared to traditional swabbing methods (see Table 5). Ottolino-Perry et al. [9] and Raizman et al. [39] indicated that the Levine sampling technique was under-representing the bacterial loads as the Levine technique is typically taken from the centre of the wound. In contrast, higher bacteria levels were frequently identified in the peri-wound area. In contrast, AFI was more effective at detecting bacterial presence in wound peripheries [37]. Overall, the studies emphasised AFI's potential role in informing clinicians about where to sample [9, 37]. Cultures from fluorescence-targeted curettage exhibited higher bacterial loads, aligning better with fluorescence images.

4.7 | Secondary Outcome: Impact of Autofluorescence Imaging on the Effectiveness of Debridement in Wounds

Nine studies [37–40, 42–44, 50, 52] investigated the impact of AFI on debridement. They found that standard debridement may be insufficient, and AFI could enhance the detection of surface bacteria, reducing the amount of bacteria after debridement guided by AFI (see Table 6). All studies indicated that post-debridement fluorescence imaging depicted a notable bacterial reduction [37–40, 42–44, 50, 52], emphasising the role of debridement in infection prevention. As a result, clinicians were prompted to adopt more extensive debridement methods, with some procedures revealing sub-surface fluorescence after initial cleaning, necessitating further interventions. Le et al. [52] and Wu et al. [50] highlighted the incorporation of fluorescence data influencing cleaner choice and advanced therapeutic applications. Overall, AFI identified bacterial loads and influenced clinical interventions, prompting more comprehensive debridement, and potentially improving wound care outcomes.

4.8 | Secondary Outcome: Effect of AFI on Treatment Decisions and Outcomes in Chronic Wound Care

Five studies [37, 39, 40, 42, 52] investigated the impact of AFI on treatment. The studies consistently report AFI's capacity to inform clinicians about bacterial burden in the wound, leading to refinements in treatment plans that promote effective wound closure (see Table 7). Overall, AFI not only aided in detecting bacterial burdens but also served as a pivotal tool in enhancing the precision and effectiveness of wound treatment strategies.

DaCosta et al. [37] demonstrated statistically significant wound area reductions when treatments were guided by AFI. Raizman et al. [39, 40] pinpointed the precision AFI brings, particularly in bacterial control, and its capability in tailoring treatments upon detecting specific bacteria, like *Pseudomonas aeruginosa*. Le et al. [52] highlighted changes in diagnoses and treatment decisions influenced by AFI, mainly related to tissue management and infection control. Rahma et al. [42] reported marked wound area reductions in the AFI group.

4.9 | EBL Checklist Critical Appraisal Analysis

The EBL Appraisal Checklist was used to assess the methodological quality of included studies across four domains: population, data collection, study design, and results. Table 8 summarises validity scores and identified methodological limitations. The mean validity score was 74% (range: 63%–83%), with seven studies scoring below 75%, indicating moderate risk of bias due to small sample sizes, lack of randomisation, and potential bias sources. Study design heterogeneity, particularly between RCTs and observational studies, further impacted interpretation. Common concerns included population representation, randomisation, and sample size issues, while blinding procedures and ethical approvals were inconsistently reported. In the results domain, confounding variables were often uncontrolled, and external validity assessments were lacking.

4.10 | Reporting Potential Bias

A formal assessment of publication bias was not conducted, as a meta-analysis was not performed. However, all included studies reported positive findings regarding AFI, with no studies presenting negative or inconclusive results. This raises the possibility of selective reporting, where studies demonstrating AFI efficacy may have been more likely to be published.

Several studies disclosed industry funding, particularly for the MolecuLight i:X device, introducing the potential for bias in study design and reporting. However, the extent of this influence remains unclear due to the absence of comparative analyses between industry-funded and independently funded studies.

The lack of large, independent randomised controlled trials (RCTs) further limits the ability to confirm AFI's diagnostic accuracy without potential funding-related bias. Given these factors, publication bias should be acknowledged as a potential limitation. Future research should prioritise independently funded studies and pre-registered protocols to enhance transparency and minimise bias.

4.11 | Certainty of Evidence and Strength of Findings

A GRADE framework assessment was not conducted. The certainty of evidence was affected by methodological limitations,

TABLE 3 | Results for primary outcome: Ability of auto-fluorescence imaging devices to detect bacterial presence in wounds.

Study	Intervention	Did the device detect bacteria? (Y/N)	Parameter	Results
DaCosta et al. [37]	PRODIGI	Yes	AF detecting bioburden in wound bed vs. WL inspection	$p = 0.003$
			PRODIGI detecting bioburden in wound periphery/other areas vs. standard	$p < 0.001$
			Overall accuracy of AF vs. CSS with WL	AF: 74.5% CSS with WL: 35.5% ($p < 0.001$)
			AF accuracy in different locations	Wound bed: 74.5%
				Wound periphery: 82.4%
				Off-site areas: 67.1%
			WL accuracy in different locations	Wound bed: 52.5%
				Wound periphery: 17.6%
				Off-site areas: 32.9%
			PPV	CSS with WL: 94.5% AF-Imaging: 74.5%
Rennie et al. [38]	MolecuLight i:X, Toronto, ON, Canada.	Yes	PPV of red FL for bacterial detection	100%
			False positive results	0%
Ottolino-Perry et al. [8]	Handheld K2 imaging device (provided by MolecuLight i:X, Toronto, ON, Canada).	Yes	Specificity of AF > CSS technique	($p = 0.0043$)
			Diagnostic Accuracy ($p = 0.048$)	CSS Sensitivity: 73%; CSS Specificity: 38%
				AFI Sensitivity: 78%; AFI Specificity: 78%
			Diagnostic Odds Ratio ($p = 0.00022$)	CSS: 3.07 (95% CI: 0.93–10.14)
				AFI: 7.67 (95% CI: 2.6–22.6)
Blumenthal et al. [43]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Patients with significant bacterial loads with AFI	80%
Blackshaw and Jeffery [46]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Patients negative for microbiological growth with swabs	52.9%
			Patients negative for microbiological growth with AFI	47.1%

(Continues)

TABLE 3 | (Continued)

Study	Intervention	Did the device detect bacteria? (Y/N)	Parameter	Results
Hurley et al. [47]	MolecuLight i:X, Toronto, ON, Canada.	Yes	AFI Sensitivity for identifying pathological bacteria	Sensitivity: 100%
			AFI Specificity for identifying pathological bacteria	Specificity: 78%
			PPV of AFI	95.4%
			NPV of AFI	100%
			AFI Sensitivity and Specificity for *Pseudomonas* species	100%
Pijpe et al. [48]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Specificity of AF	64% (CI: 31% to 89%)
			Sensitivity of AFI	78% (CI: 40% to 79%)
			Positive predictive value (PPV) of AFI	64% (CI: 42% to 80%)
			Negative predictive value (NPV) of AFI	78% (CI: 49% to 93%)
			Agreement between high-fluorescent area results and standard swabs	$p < 0.0001$
Raizman et al. [39]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Positive fluorescence images	36/50 (72%)
			Positive fluorescence in wound bed	11%
			Positive fluorescence in peri wound tissue	86%
			Positive fluorescence in both	3%
			DFUs positive for fluorescence signals	75% of the 36 DFUs
			Wounds with some level of bacteria	35/50 (70%)
			Wounds with moderate-to-heavy bacterial loads	10/50 (20%)

(Continues)

TABLE 3 | (Continued)

Study	Intervention	Did the device detect bacteria? (Y/N)	Parameter	Results
Farhan and Jeffery [49]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Wounds with clinically significant bacterial fluorescence	50%
			AFI consistency with CSS	87.50%
			Detection of FL in absence of CSS	12.5%
			AFI consistency with culture analysis	81%
Raizman et al. [40]	MolecuLight i:X, Toronto, ON, Canada.	Yes	PPV of cyan FL in detecting *pseudomonas aeruginosa*	92.90%
Le et al. [52]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Addition of FL to CSS improved sensitivity	Improved by 4-fold ($p < 0.001$)
			Specificity for CSS + FL	84.1% (95% CI, 75.1%–93.2%)
			DOR (Diagnostic Odds Ratio) of CSS + FL	8.3 (95% CI, 4.1–17.0)
			NPV of CSS + FL	$P < 0.001$
			PPV of FL to CSS alone	Comparable
Janowska et al. [41]	MolecuLight i:X, Toronto, ON, Canada.	Yes	AFI Positive wounds	79%
			AFI Negative wounds	21%
			CSS Positive wounds	37%
			CSS Negative wounds	63%
Wu et al. [50]	MolecuLight i:X, Toronto, ON, Canada.	Yes	AFI accuracy	$p = 0.184$
			Sensitivity of AFI	45.0%
			Specificity of AF	92.3%
			PPV of AFI	94.7%
			NPV of AFI	35.3%
Rahma et al. [42]	MolecuLight i:X, Toronto, ON, Canada.	Yes	AFI sensitivity analysis	13 (45%, 95% CL 26%–64%)
			Control sensitivity analysis	10 (37%, 95% CL 19%–58%)

(Continues)

TABLE 3 | (Continued)

Study	Intervention	Did the device detect bacteria? (Y/N)	Parameter	Results
Kurokami et al. [44]	MolecuLight i:X, Toronto, ON, Canada.	Yes	FL+and Culture+	14
			FL+and Culture−	4
			FL− and Culture+	5
			FL− and Culture−	12
Mościcka et al. [45]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Qualitative analysis	FL imaging complemented the microbiological diagnostics of chronic wounds.
Koo et al. [51]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Sensitivity of AFI	64.1%
			Specificity of AFI	55.6%
			PPV of AFI	86.2%
			NPV of AFI	26.3%
			AFI Accuracy	62.5%
			Sensitivity for *Pseudomonas aeruginosa*	66.7%
			Specificity for *Pseudomonas aeruginosa*	87.2%
			PPV for *Pseudomonas aeruginosa*	54.6%
			NPV for *Pseudomonas aeruginosa*	91.9%
			Sensitivity for non-Pseudomonas bacteria	43.8%
			Specificity for non-Pseudomonas bacteria	62.5%
			PPV for non-Pseudomonas bacteria	70.0%
			NPV for non-Pseudomonas bacteria	35.7%
			Accuracy for non-Pseudomonas bacteria	50.0%

Abbreviations: AFI, autofluorescence imaging; CSS, clinical signs and symptoms; FL, fluorescence; WL, white light.

TABLE 4 | Quantitative significance of AFI.

Study	Intervention	Detected bacteria?	Quantitative analysis–parameter	*p*
DaCosta et al. [37]	PRODIGI	Yes	AF detecting bioburden in wound bed vs. WL inspection	0.003
			PRODIGI detecting bioburden in wound periphery/other areas vs. standard	< 0.001
			Overall accuracy of AF vs. CSS with WL	< 0.001
Ottolino-Perry et al. [8]	Handheld K2 imaging device (provided by MolecuLight i:X)	Yes	Specificity of AF vs. CSS technique	0.0043
			Diagnostic accuracy sensitivity of CSS	0.048
			Diagnostic accuracy sensitivity of AFI	
			Diagnostic odds ratio	0.00022
Pijpe et al. [48]	MolecuLight i:X	Yes	Cohen's kappa (agreement between AFI and microbiological analysis)	< 0.000
Le et al. [52]	MolecuLight i:X	Yes	Addition of FL to CSS improved sensitivity	< 0.001
			NPV of CSS + FL	< 0.001
Wu et al. [50]	MolecuLight i:X	Yes	AFI Accuracy	0.184
Koo et al. [51]	MolecuLight i:X	Yes	Sensitivity for *Pseudomonas aeruginosa*	< 0.001
			Specificity for *Pseudomonas aeruginosa*	< 0.001
			PPV for *Pseudomonas aeruginosa*	0.3671
			NPV for *Pseudomonas aeruginosa*	0.0198

Abbreviations: AFI, autofluorescence imaging; CSS, clinical signs and symptoms; FL, fluorescence; NPV, negative predictive value; WL, white light.

including a limited number of randomised controlled trials (RCTs) and inconsistencies in study design. Some studies incorporated blinded assessments and standardised microbiological reference standards, but variability across methodologies reduced overall evidence strength.

Sample sizes varied significantly, ranging from 2 to 990 wounds, with a mean of 77 wounds per study. Smaller sample sizes may limit generalisability and contribute to variability in AFI diagnostic performance. Differences in microbiological reference standards, including culture-based methods and qPCR, further introduced inconsistencies in bacterial detection and fluorescence interpretation. The absence of standardised bacterial load thresholds also hindered direct comparisons across studies.

These limitations suggest that the certainty of evidence supporting AFI for detecting bacterial presence in chronic wounds remains moderate to low. Further high-quality RCTs with larger sample sizes and standardised diagnostic criteria are needed to strengthen the evidence base.

5 | Discussion

High bacterial loads have been identified as a major contributor to impaired wound healing [13]. The application of auto-fluorescence imaging (AFI) in chronic wounds has the potential to improve the standardisation of current wound assessment and treatment plans [13]. The primary objective of this systematic review was to determine the ability of auto-fluorescence imaging to detect the presence of moderate to heavy bacterial loads in chronic wounds. This review synthesised the findings of 17 studies [37–51]. Across the 17 reviewed studies, the AFI devices (PRODIGI and MolecuLight i:X) showed high sensitivity and specificity figures for the detection of bacteria ($\geq 10^4$ CFU/g) in wounds. A recurring theme across the studies was the device's effectiveness in scenarios where clinical signs were absent, yet bacterial growth was present [37–51]. Overall, the findings of this systematic review suggest that auto-fluorescence imaging devices offer a reliable, efficient, and possibly superior method for detecting bacterial presence in wounds, outpacing many traditional assessment techniques. Data consistent with these observations were also reported by Serena et al. [52] and Anghel et al. [53]. Additionally, AFI demonstrates the potential to transform wound management, particularly in the realms of swabbing, debridement, and treatment.

Across five studies [9, 37, 42–44], AFI's ability to guide swab collection more effectively is significant, considering the limitations of conventional swabbing techniques. Traditional methods, such as the Levine technique, have been shown to underrepresent bacterial loads primarily because they focus on the centre of the wound [9]. In contrast, AFI has proven more effective at detecting bacterial presence in wound peripheries, areas often missed by conventional methods. This capability is particularly important, given that the peri-wound area can

TABLE 5 | Effectiveness of autofluorescence imaging in wound swabbing accuracy and targeted bacterial sampling.

Study	Intervention	Method	Did auto-fluorescence influence swab technique? Y/N	Results
DaCosta et al. [37]	PRODIGI	Swab collection was guided either by WL or AF imaging.	Yes	• AF correctly detected 74.5% of wounds with clinically significant bioburden compared with 52.5% for WL inspection ($p = 0.003$). • AF imaging detected clinically significant bacterial load in 85% of wound peripheries missed by conventional methods ($p < 0.001$) • AF imaging helped clinicians decide if and where wound margins require sampling.
Ottolino-Perry et al. [8]	Handheld K2 imaging device (provided by MolecuLight i:X, Toronto, ON, Canada).	Swab collection was guided either by WL (using Levine Technique) CSS examination or AF imaging.	Yes	• AF identified specific areas with red AF positive signals, indicative of potential bacterial presence in 52% of wounds. • A targeted swabbing approach facilitated more accurate sampling of the wound, leading to higher bacterial detection rate compared to traditional methods.
Pijpe et al. [48]	MolecuLight i:X, Toronto, ON, Canada.	Standard Swab collection was guided either by WL examination or AF imaging. Standard swab = swabbing open wounds twice a week, regardless of signs of infection.	Yes	• The device guided swabs to areas of bacterial colonisation missed by traditional methods. • AFI missed some pathogens detected by standard swabs.
Raizman et al. [39]	MolecuLight i:X, Toronto, ON, Canada.	Swab collection was guided either by WL (using levine technique in the wound bed or AF imaging). Curettage samples were taken guided by AF.	Yes	• Guided the best locations to swab for microbial cultures. • Fluorescence-targeted curettage identified areas with high bacterial loads. • The device highlighted bacterial presence in the wound bed, peri wound tissues and wound edge.
Farhan and Jeffery [49]	MolecuLight i:X, Toronto, ON, Canada.	Swab collection was guided either by WL (using Levine Technique) CSS examination or AF imaging.	Yes	• In Patient 1, fluorescence images showed a red signal in wound. This was used to guide a targeted Levine swab. • In Patient 2, the fluorescence images displayed a characteristic cyan colour (indicating the presence of *P. aeruginosa*) and red fluorescence (indicating other bacteria), which guided where to sample, • In Patient 3, fluorescence images again revealed red fluorescent signals in both wounds, which led to the decision to obtain targeted swabs from those areas. However, it's also worth noting that there were instances where conventional non-targeted swabbing was performed regardless of fluorescence signals, particularly, when clinical signs and symptoms indicated potential infection.

Abbreviations: CSS, clinical signs and symptoms; WL, white light.

TABLE 6 | Effectiveness of auto-fluorescence imaging in guiding targeted debridement for chronic wounds.

Study	Intervention	Effectiveness of auto-fluorescence in debridement?	
		Y/N	Results
DaCosta et al. [37]	PRODIGI	Yes	Real-time quantitative FL visualisation of clinically-significant bacterial load (moderate-to-heavy growth) provided localization of bacteria in wound bed and periphery earlier guiding debridement. The rate of wounds closure with FL- guided debridement 1.38 cm2 (decrease) compared to standard treatment: 0.09 cm2 (decrease).
Rennie et al. [38]	MolecuLight i:X, Toronto, ON, Canada.	Yes	With a PPV of 100%, localization of bacterial burden can enable debridement specifically targeted regions of bacterial burden, sparing areas where burden is not detected.
Blumenthal et al. [43]	MolecuLight i:X, Toronto, ON, Canada.	Yes	After a shower and debridement of the burn, the fluorescence image showed a reduced amount of bacteria, showing how invaluable debridement is in preventing infection. AF identified areas needing debridement and targeting treatment.
Raizman et al. [39]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Fluorescence images were acquired after initial debridement to evaluate effectiveness of the initial debridement intervention. Fluorescence information was used to target remaining bioburden.
Raizman et al. [40]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Fluorescence images revealed the presence of bright white cyan fluorescence in the peri wound region; this promoted additional debridement of the wound.
Le et al. [52]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Information on location of bacterial burden at point-of-care was impactful for debridement.
Wu et al. [50]	MolecuLight i:X, Toronto, ON, Canada.	Yes	MolecuLight i:X showed bacterial distribution over the wound bed and peri wound region that can be used for targeting wound debridement.
Rahma et al. [42]	MolecuLight i:X, Toronto, ON, Canada.	Yes	AFI was utilised to guide and inform the debridement process, identifying bacterial presence that required wound debridement
Kurokami et al. [44]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Identified the site for targeted debridement based on fluorescence images.

harbour high bacterial loads that contribute to an infection and hinder healing [29].

The effectiveness of AFI in guiding debridement is supported by ten studies reviewed [37–40, 42–44, 50, 52, 54], with all reporting a notable reduction in bacterial load post-debridement when guided by fluorescence imaging. By enabling the visualisation of bacterial distribution across the wound bed and its periphery, AFI facilitates targeted debridement. This targeted approach spares areas free of significant bacterial load, thereby preserving healthy tissue and focusing interventions where they are most needed. Cole and Coe [54] supported these findings, demonstrating that AFI assisted in determining the location and extent of debridement required.

Moreover, the quantitative and qualitative enhancements in wound care practices brought about by AFI usage extend beyond mere identification and reduction of bacterial load. The technology's influence on clinical decision-making processes, as highlighted by Le et al. and Wu et al., suggests that fluorescence data can inform the selection of cleaning agents and advanced therapeutic strategies. Such applications of AFI not only contribute to more effective debridement but also to the optimisation of overall wound management protocols.

Furthermore, the influence of Autofluorescence Imaging (AFI) on the treatment of chronic wounds, as evidenced by six studies [31, 34, 35, 37, 41, 47], is significant and multifaceted. Similarly, Price et al. [53] found the use of AFI caused a decrease in the number of antimicrobial dressings used to treat bacterial wounds, thereby decreasing the economic burden on patients and the healthcare system. Cole and Coe [54] also noted an average reduction in wound area of 27.7% per week ($p < 0.05$), indicative of a healing trajectory, supporting the findings of this

TABLE 7 | Effectiveness of auto-fluorescence imaging in guiding treatment choices for chronic wounds.

		Effectiveness of auto-fluorescence in guiding treatment (antibiotics, dressing or change of treatment)			
Study	**Intervention**	**Y/N**	**Methods**	**Wound changes-Intervention group (with FL-guidance)**	**Wound changes-control group**
DaCosta et al. [37]	PRODIGI	Yes	In part 2, wound area was tracked in a separate group of 12 patients over approx. 6 months-3 contiguous 2 month periods, comparing treatment delivered with control and with FL-guidance.	Wound area changes ($N=12$ patients, 6 months): $-0.046\,cm^2$/day (significant decrease, $p=0.017$) Rate of wound closure over 30 days: $1.38\,cm^2$ (decrease)	Wound area changes ($N=12$ patients, 6 months): $-0.005\,cm^2$/day (insignificant decrease, $p=0.579$) Rate of wound closure over 30 days: $0.09\,cm^2$ (decrease)
Raizman et al. [39]	MolecuLight i:X, Toronto, ON, Canada.	Yes	The device also identified off-site bacteria, prompting precise cleaning actions. Additional antimicrobial treatments.	More targeted debridement in 85% of assessed wounds	Not specified
Raizman et al. [40]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Management plan changed according to AF	Not specified	
Le et al. [52]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Management plan changed according to AF in 68.9% of wounds. AFI-guided wound bed preparation in 84.6% of wounds	Improvements in patient care due to FL: 90.00% Change in diagnosis due to FL: 52.30% Treatment plan changes due to FL: 68.90%	Not specified
Rahma et al. [42]	MolecuLight i:X, Toronto, ON, Canada.	Yes	Management plan changed according to AF Enhanced wound debridement dressings changed to antimicrobial dressings	The median (%) wound-area reduction at 4-weeks: 40.7% At 12-weeks after randomization, the median (%): 91.3%	The median (%) wound-area reduction at 4-weeks: 38.6% At 12-weeks after randomization, the median (%): 72.8%

TABLE 8 | EBL checklist for critical appraisal analysis.

	Validity (%) not reported/unclear issues identified in each domain				
Study	**Population**	**Data collection**	**Design**	**Results**	**Overall validity**
DaCosta et al. [37]	83% (representative of population, randomization)	83% (blinding)	80% (face validity)	83% (confounding variables)	79% (Valid)
Rennie et al. [38]	83% (representative of population, randomization)	83% (blinding)	60% (face validity, ethics)	67% (confounding variables, external validity)	71% (Invalid)
Ottolino-Perry et al. [8]	83% (representative of population, randomization)	83% (blinding)	80% (face validity)	67% (confounding variables, external validity)	75% (Valid)
Blumenthal et al. [43]	67% (representative of population, randomization, sample size)	83% (blinding)	60% (face validity, ethics)	50% (confounding variables, external validity, future research)	63% (Invalid)
Blackshaw and Jeffery [46]	67% (representative of population, randomization, sample size)	83% (blinding)	60% (face validity, ethics)	50% (confounding variables, external validity, future research)	63% (Invalid)
Hurley et al. [47]	83% (representative of population, randomization)	83% (blinding)	80% (face validity)	67% (confounding variables, external validity)	75% (Valid)
Pijpe et al. [48]	83% (representative of population, randomization)	83% (blinding)	80% (face validity)	67% (confounding variables, external validity)	75% (Valid)
Raizman et al. [39]	67% (representative of population, randomization, sample size)	100%	60% (face validity, ethics)	50% (confounding variables, external validity, future research)	67% (Invalid)
Farhan and Jeffery [49]	67% (representative of population, randomization, sample size)	100%	60% (face validity, ethics)	67% (confounding variables, external validity)	71% (Invalid)
Raizman et al. [40]	67% (representative of population, randomization, sample size)	100%	80% (face validity)	67% (confounding variables, external validity)	75% (Valid)
Le et al. [52]	100%	100%	80% (face validity)	67% (confounding variables, external validity)	83% (Valid)
Rahma et al. [42]	83% (representative of population, sample size)	83% (blinding)	80% (face validity)	67% (confounding variables, external validity)	79% (Valid)
Janowska et al. [41]	83% (representative of population, randomization)	83% (blinding)	80% (face validity)	67% (confounding variables, external validity)	79% (Valid)
Wu et al. [50]	100%	100%	80% (face validity)	67% (confounding variables, external validity)	83% (Valid)
Kurokami et al. [44]	67% (representative of population, randomization, consent)	100%	60% (face validity, ethics)	50% (confounding variables, external validity, future research)	67% (Invalid)
Mościcka et al. [45]	67% (representative of population, randomization, consent)	100%	60% (face validity, ethics)	50% (confounding variables, external validity, future research)	67% (Invalid)
Koo et al. [51]	67% (representative population, inclusion/exclusion criteria stated, but sample size is relatively small for precise estimates)	83% (blinding)	80% (replication feasilbility)	67% (confounding variables, external validity)	74% (Invalid)

study. The core takeaway from these findings is that AFI serves as a critical tool in enhancing the precision of clinical interventions, leading to improved wound care outcomes. AFI's primary impact is its capacity to inform clinicians about the presence and extent of bacterial burden, thereby refining treatment plans to promote more effective wound closure.

AFI-guided treatment adjustments include targeted debridement, precise cleaning actions, and the selection of appropriate antimicrobial treatments. For instance, the capacity to detect specific bacteria, such as *Pseudomonas aeruginosa*, enables clinicians to tailor treatments to the pathogens present, which is a considerable advantage over traditional methods that may not identify the bacterial species involved [35]. Moreover, the technology has been shown to influence significant adjustments in antimicrobial decision-making, with changes in both the use of topical agents and the prescription of systemic antibiotics [55, 56]. Jacob et al. [57] supported these findings, stating that of 1000 chronic wounds recorded for analysis, 528 wound treatments were altered after AFI, resulting in improved bacterial infection management. These findings collectively suggest that AFI's contribution to wound treatment not only aids in the detection of bacterial burdens but also significantly impacts the strategic planning and execution of wound care interventions, leading to more effective treatments and better patient outcomes.

5.1 | Limitations and Challenges in Clinical Application

Despite the evidence presented, several limitations must be acknowledged. One significant limitation is the heterogeneity of the included studies, which resulted in considerable variability in methodologies, study aims, and designs. This diversity made direct comparisons challenging and complicated the interpretation of results, thereby affecting the overall consistency and reliability of findings. Additionally, the lack of comprehensive statistical analysis in some studies: Blumenthal and Jeffery [43] and Farhan et al. [49] did not provide explicit *p*-values or significance measures, limiting the robustness and generalisability of the findings. Raizman et al. [39] also suggested the possibility of under-representing bacterial loads when relying solely on traditional swab techniques, thereby potentially underestimating the true bacterial burden in wounds. Another notable limitation is small sample sizes in a number of included studies [43, 45, 49], which might introduce bias and reduce the reliability of their outcomes. Furthermore, challenges in clinical application were reported by the studies included in this review. The absence of large-scale, multicenter trials limits the applicability of these findings across different clinical settings. The most frequent limitations encountered included Depth limitations –AFI devices could only detect bacterial presence up to 1.5 mm; Need for darkness–AFI devices required complete darkness for usage; Training required for correct device usage and interpretation of results. Furthermore, this review did not include a cost-effectiveness analysis, which is a critical factor in determining the feasibility of integrating AFI into routine wound assessment protocols. While AFI provides real-time bacterial detection, its high equipment costs and training requirements could pose challenges for widespread clinical adoption.

5.2 | Recommendations for Future Research and Policy Implications

Given the findings and limitations, future research should aim to enhance our understanding of AF imaging's clinical utility to enhance wound outcomes. More comprehensive studies investigating the influence of AFI on wound swabbing and sampling techniques for microbiological analysis, guided debridement of chronic wounds, and treatment choices should be conducted to promote faster wound healing. Future research should also explore whether AFI reduces overall healthcare costs by minimising unnecessary antibiotic prescriptions and hospitalisations. Additionally, workflow integration studies should assess whether AFI speeds up diagnosis and intervention in real-world wound care settings.

Developing and implementing extensive training programmes for healthcare providers is imperative for the technology's wider clinical adoption. This would ensure a uniform understanding and interpretation of AFI images, leading to more consistent clinical decisions. Lastly, larger, multi-centre trials involving diverse patient populations would enhance the robustness and generalisability of the findings, providing a more comprehensive view of the clinical utility and impact of AF imaging in chronic wound care. These recommendations, if pursued, could substantially contribute to advancing knowledge and practice in using auto-fluorescence imaging for chronic wound management.

In conclusion, this systematic review reveals the potential clinical utility of AFI in chronic wound management, particularly in its utility in guided swabbing, debridement, and treatment choices. The collective evidence from 17 studies supports the effectiveness of AFI devices in detecting bacterial burden in wounds. However, the variability in outcomes across studies [31–47] highlights the need for larger, more rigorous trials to determine the effectiveness and applicability of this technology in different clinical settings conclusively. Given the promising potential of auto-fluorescence imaging, there is a clear need for more effective strategies in managing wound infections Caputo et al. [26]. Overall, auto-fluorescence imaging demonstrates promise as a tool in chronic wound care, contributing to our understanding of wound microbiology and potentially guiding debridement. As this field continues to develop, ensuring the technology is used to its full potential while addressing the inherent challenges will be crucial to realise the benefits for patients carefully.

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The authors have nothing to report.

Ethics Statement

This study is a systematic review and does not involve primary data collection or direct interaction with human or animal subjects. Therefore, ethical approval was not required.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available within the article and its supplementary materials. Further data inquiries can be directed to the corresponding authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Appendix A

Systematic Review Search Strategy

PICO Table

Population	Patients with chronic or non-healing wounds
Intervention	Auto-fluorescence imaging devices-MolecuLight i:X device, fluorescence-guided imaging
Control	Conventional methods (e.g., wound swabs, biopsies, clinical signs).
Outcomes	Diagnostic accuracy (sensitivity, specificity), microbiological yield, clinical utility in wound care decisions

Search Strings Used in Key Databases

Database	Search String
PubMed	('Wounds and Injuries'[MeSH] OR 'Diabetic ulcer' OR 'chronic wound' OR 'Pressure Ulcer'[MeSH] OR 'infected wound*' OR 'non-healing wound*' OR 'chronic wound*') AND ('Autofluorescence Imaging' OR 'fluorescence imaging' OR 'fluorescence-guided imaging' OR 'MolecuLight' OR 'point-of-care imaging' OR 'optical imaging' OR 'PRODIGI') AND ('Bacteria'[MeSH] OR 'Bacterial Infection' OR 'microbial detection' OR 'bacterial burden' OR 'colony-forming unit*') AND ('sensitivity' OR 'specificity' OR 'diagnostic accuracy' OR 'positive predictive value' OR 'negative predictive value')
EMBASE	('wound'/exp. OR 'chronic wound'/exp. OR 'diabetic ulcer'/exp. OR 'venous ulcer'/exp. OR 'pressure ulcer'/exp. OR 'infected wound*' OR 'non-healing wound*') AND ('autofluorescence imaging'/exp. OR 'fluorescence imaging'/exp. OR 'moleculight' OR 'point-of-care imaging' OR 'optical imaging'/exp. OR 'prodigi') AND ('bacterium'/exp. OR 'bacterial infection'/exp. OR 'bacterial burden'/exp. OR 'colony-forming unit*') AND ('sensitivity'/exp. OR 'specificity'/exp. OR 'diagnostic accuracy'/exp. OR 'positive predictive value'/exp. OR 'negative predictive value'/exp)
Cochrane CENTRAL	('Wound*' AND ('autofluorescence' OR 'fluorescence imaging' OR 'MolecuLight' OR 'optical imaging') AND('bacteria' OR 'bacterial detection' OR 'bacterial burden'))
EBSCOhost (CINAHL Plus)	('Wound*' OR 'chronic wound*' OR 'diabetic ulcer*' OR 'pressure ulcer*' OR 'non-healing wound*' OR 'infected wound*') AND ('autofluorescence imaging' OR 'fluorescence imaging' OR 'MolecuLight' OR 'point-of-care imaging' OR 'optical imaging') AND ('bacteria' OR 'bacterial burden' OR 'microbial detection' OR 'colony-forming unit*') AND ('sensitivity' OR 'specificity' OR 'diagnostic accuracy' OR 'positive predictive value' OR 'negative predictive value')
Scopus	TITLE-ABS-KEY ('wound*' OR 'chronic wound*' OR 'diabetic ulcer*' OR 'pressure ulcer*' OR 'infected wound*' OR 'non-healing wound*') AND ('autofluorescence imaging' OR 'fluorescence imaging' OR 'MolecuLight' OR 'point-of-care imaging' OR 'optical imaging') AND ('bacteria' OR 'bacterial burden' OR 'bacterial detection' OR 'colony-forming unit*') AND ('sensitivity' OR 'specificity' OR 'diagnostic accuracy' OR 'positive predictive value' OR 'negative predictive value')
Google Scholar	'autofluorescence imaging' OR 'fluorescence imaging' AND 'bacterial detection' filetype:pdf

Number of Articles Retrieved per Database

Table showing the number of articles identified on each database.

Database	Number of articles retrieved
PubMed	14
EMBASE	22
Cochrane CENTRAL	11
EBSCOhost (CINAHL Plus)	20
Scopus	51
ClinicalTrials.gov	4
ProQuest	28
Google scholar	115
Total	265