



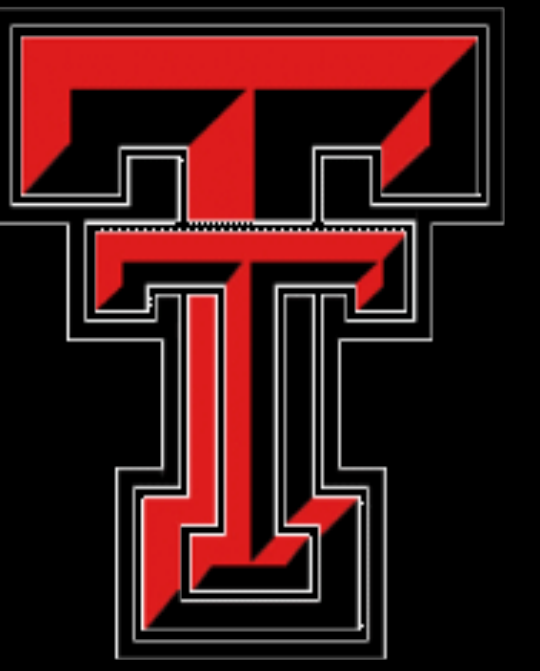
In vivo detection of polymicrobial bacterial biofilm with real-time fluorescence imaging

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ABSTRACT

Chronic wounds are a significant cause of patient morbidity and mortality in the US annually, and commonly harbor polymicrobial biofilms, generating infections that are difficult to characterize and to treat. Current diagnostics methods require long periods of testing for microbial identification, but often provide little information on the complex characteristics of the chronic wound environment. An essential step to the healing process for a patient with a complex wound is accurate diagnosis to better provide personalized care. Bacterial fluorescence imaging with the handheld MolecuLight *i:X* device uses safe violet light to detect auto fluorescent properties of most clinically-relevant species of bacteria, allowing detection of relative bacterial bioburden in a wound in real time. In this study, we assessed the ability of the MolecuLight *i:X* device to detect autofluorescent properties of a polymicrobial bacterial biofilm utilizing an established chronic wound murine model and were able to demonstrate that bacteria encased within the extracellular matrix of the biofilm still exhibit detectable autofluorescence. These data demonstrate that the device has the ability to detect bacteria encased within a biofilm, which further validates the MolecuLight *i:X* device for patient use.

INTRODUCTION

- Chronic wounds commonly harbor bacteria. These bacteria can exist as polymicrobial biofilms, resulting in more aggressive infections.
- Confirmation of bacterial presence is clinically confirmed via microbiological testing. Culture-based diagnostics identify dominant microorganisms as well as their antimicrobial susceptibility, however the lag time to obtain those results (3 days – 4 weeks) significantly impacts wound care and treatment.
- The MolecuLight *i:X* imaging device visualizes fluorescence from wound **tissues (green)** and **bacteria (red)**, enabling point-of-care localization of regions with moderate-to-heavy bacterial loads.
- The device illuminates a wound with safe violet light (405 nm) and visualizes resulting endogenous fluorescence from bacterial porphyrins in real-time. This allows physicians to direct specimen sampling to the area with the heaviest bioburden, improving diagnostic capabilities and to direct bacterial targeted treatments.
- Prior work demonstrated MolecuLight *i:X* detection of planktonic monomicrobial bacteria *in vivo* and *in vitro* from the most common wound pathogens (e.g. *Staphylococcus*, *Enterobacter*, *Klebsiella*, *Proteus*, *Acinetobacter*, *Bacteroides*, *Serratia spp.*).
- This study investigated the device's capability to detect **biofilm** using our polymicrobial *in vitro* biofilm model, which is representative of the chronic wound environment.

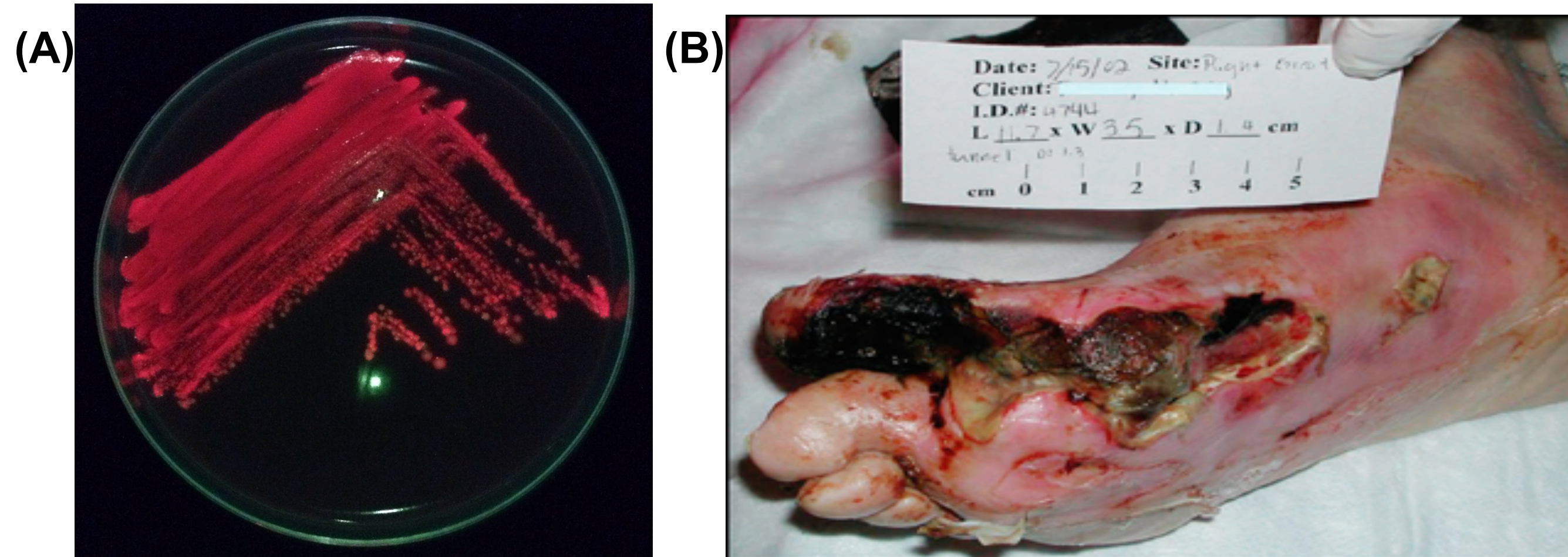


Figure 1. (A) Red fluorescence of the porphyrin-positive *S. aureus* at 40 hr (Porphyrin Test Agar) **(B)** Diabetic Foot Ulcer, photo courtesy of Dr. Randall Wolcott, MD, Southwest Regional Wound Care Center, Lubbock, TX

BIOFILM-ASSOCIATED INFECTIONS

- Chronic wounds commonly harbor biofilm-associated polymicrobial infections. Biofilms are polymicrobial communities of microorganisms encased in a self-produced extracellular matrix (EPS).
- EPS blocks penetration of immune system mechanisms and antimicrobial agents. Up to 1000X the antimicrobial concentration is required to eliminate a biofilm infection, compared to planktonic (free-living) associated infection.
- Chronic wound biofilms can contain 100 different microbial species [1], which can synergize their activities, leading to more aggressive and virulent infections [2].
- Our *in vitro* wound-like model contains physiologically representative conditions and has been previously validated to support the growth of polymicrobial biofilms [2]
- Three common chronic wound pathogens were selected: *Staphylococcus aureus* (ATCC ® 25923), *Enterobacter cloacae* (ATCC ® 13047), and *Escherichia coli* (ATCC ® 25922).

MOLECULIGHT *i:X* IMAGING DEVICE

- The MolecuLight *i:X* fluorescence imaging device detects most medically-relevant species of bacteria via endogenous red fluorescence of porphyrins [3].
- Porphyrin production in bacteria requires δ -aminolaevulinic acid (ALA), an intermediate in heme synthesis.
- The device illuminates a wound with violet light and visualizes resultant bacterial fluorescence in real-time, indicated by bright red on images [3]. This allows physicians to follow empiric therapy for treatment as well as target sampling of the wound for culture-based diagnostics.

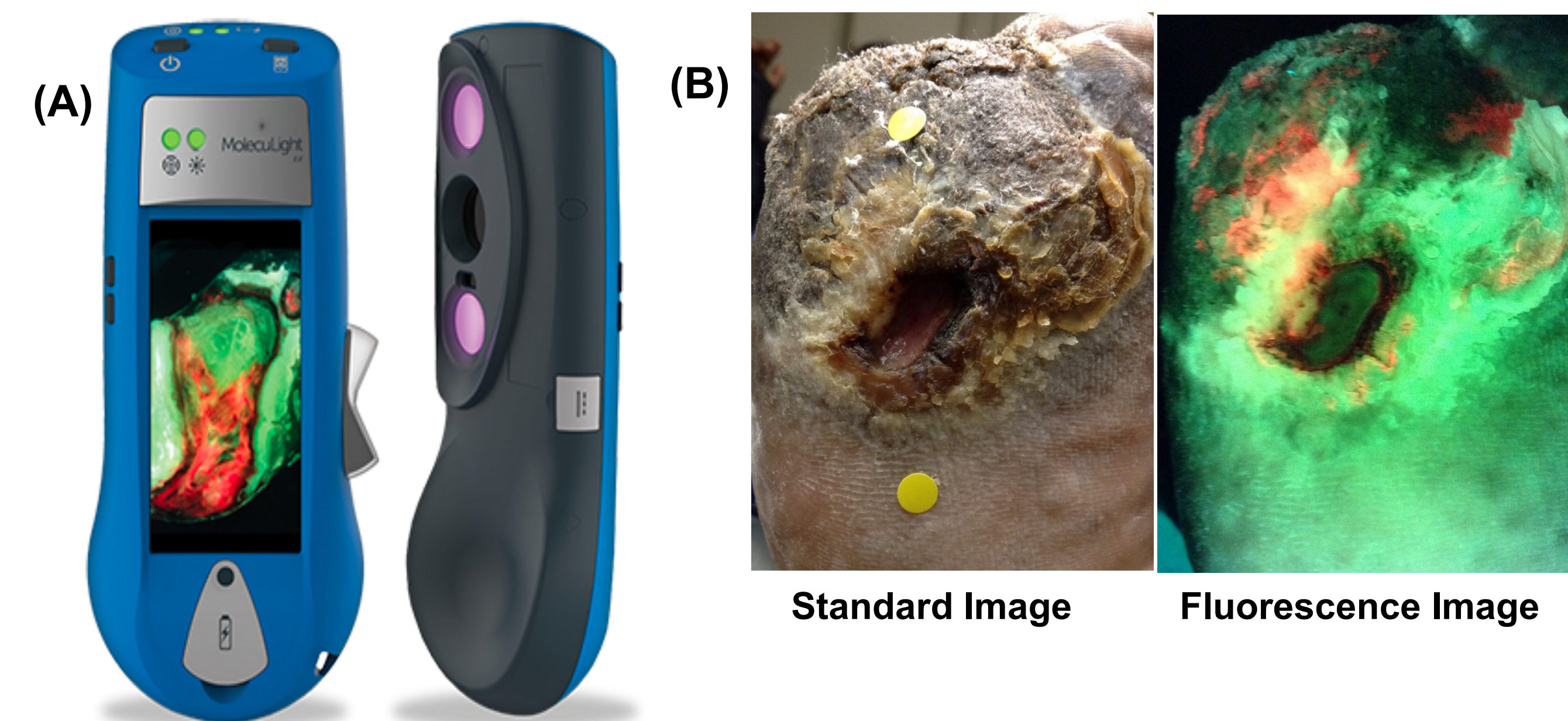
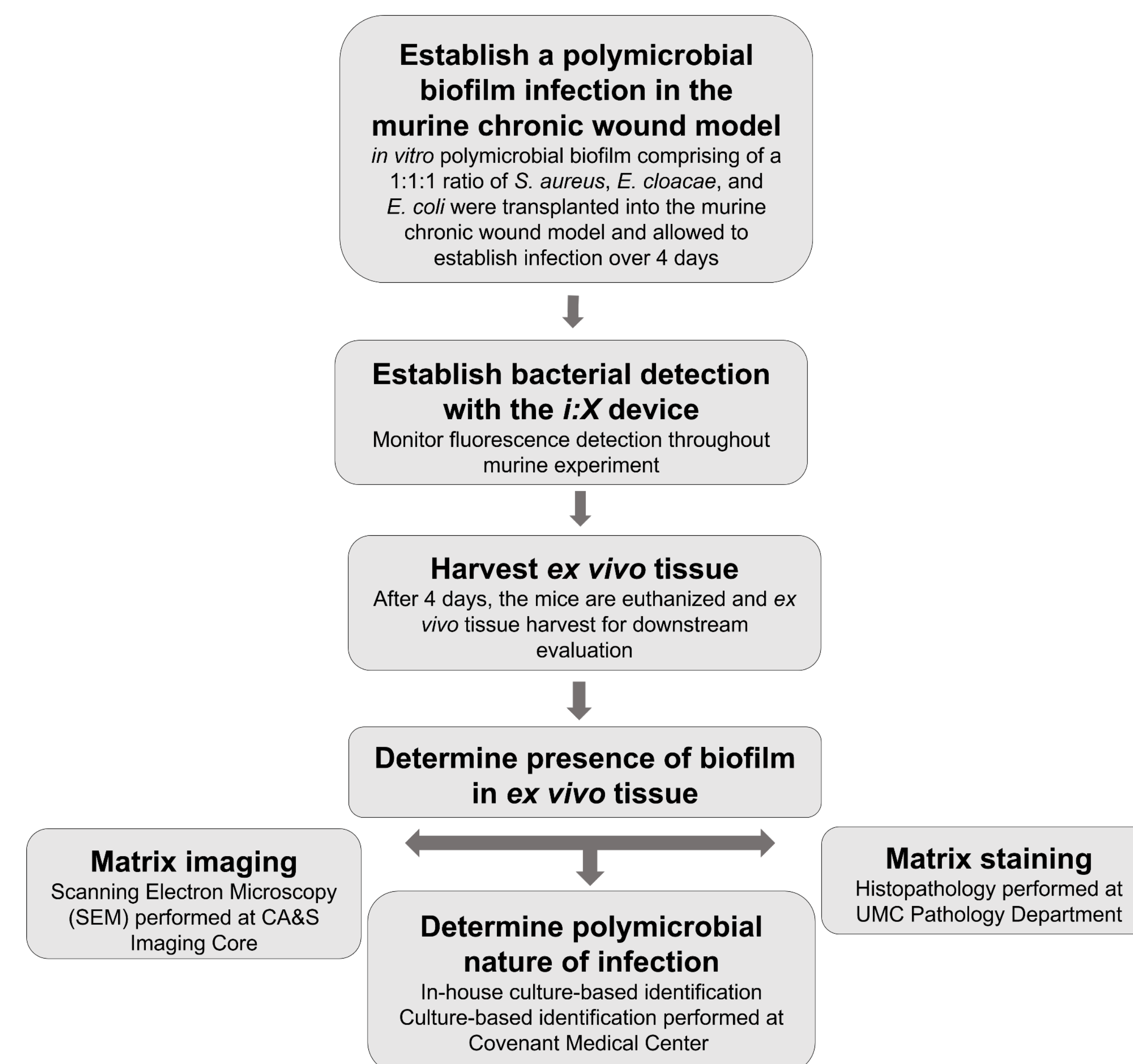


Figure 2: MolecuLight *i:X* Imaging Device (A) Hand-held device and **(B)** images of a chronic foot ulcer wound. Sampled regions of red fluorescence were positive for heavy growth of *Serratia marcescens* [3].

PURPOSE

To validate that the MolecuLight *i:X* device can detect porphyrin-producing species of bacteria encased within a biofilm matrix *in vivo*

EXPERIMENTAL DESIGN



Acknowledgments

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RESULTS

To confirm presence of all three species within the biofilm/mouse *ex vivo* tissue, in house culture-based identification was done. Samples were also taken to Covenant Medical Center to validate our in-house results. Data not shown

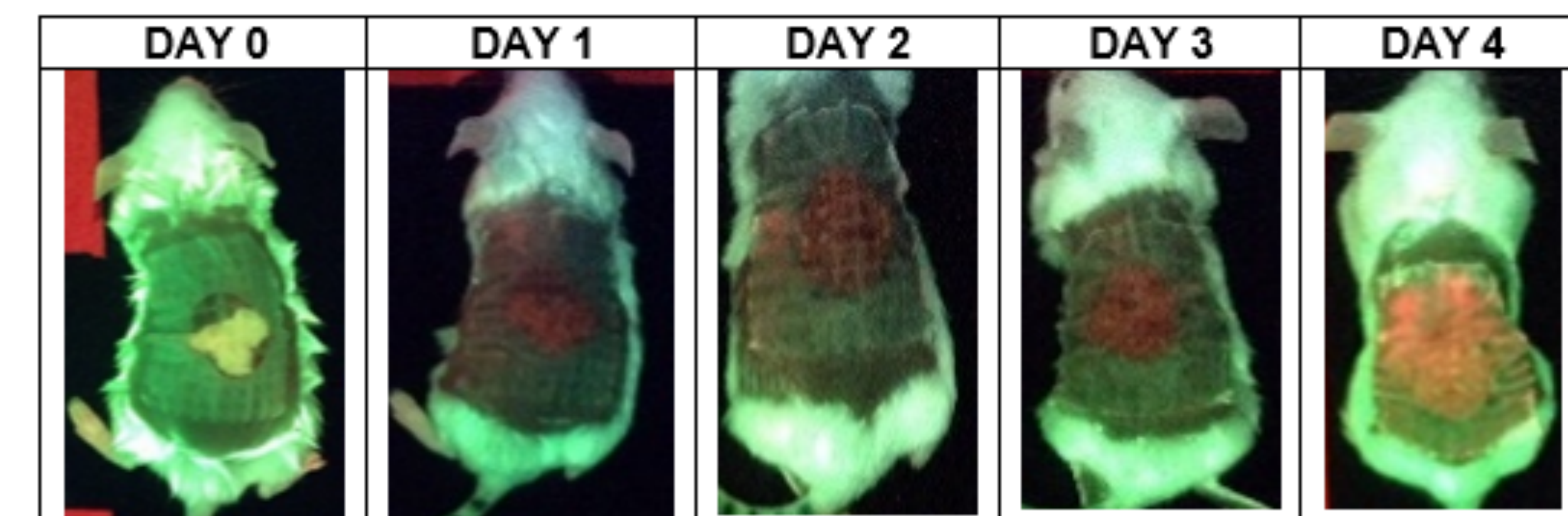


Figure 3: Induction of red fluorescence over 4 day polymicrobial biofilm infection. Representative images shown. n=24

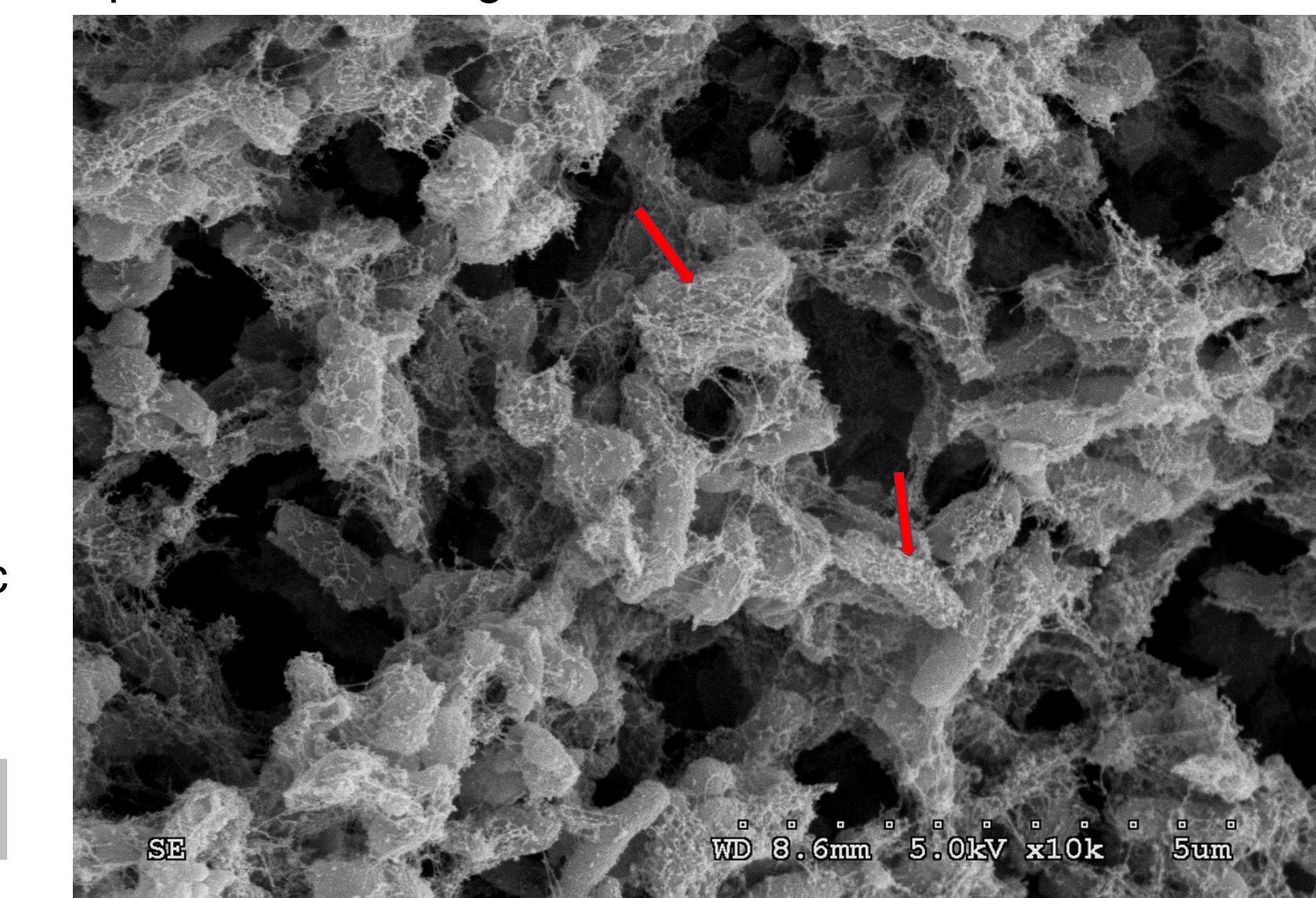


Figure 4: Scanning Electron Microscopy (SEM) of ex vivo tissue samples. Red arrows note regions of close association between the extracellular matrix and the bacterial cells, with the assumption that cell-associated matrix may be bacterial derived versus host derived, and therefore indicative of biofilm. SEM conducted at the College of Arts and Sciences Microscopy Core.

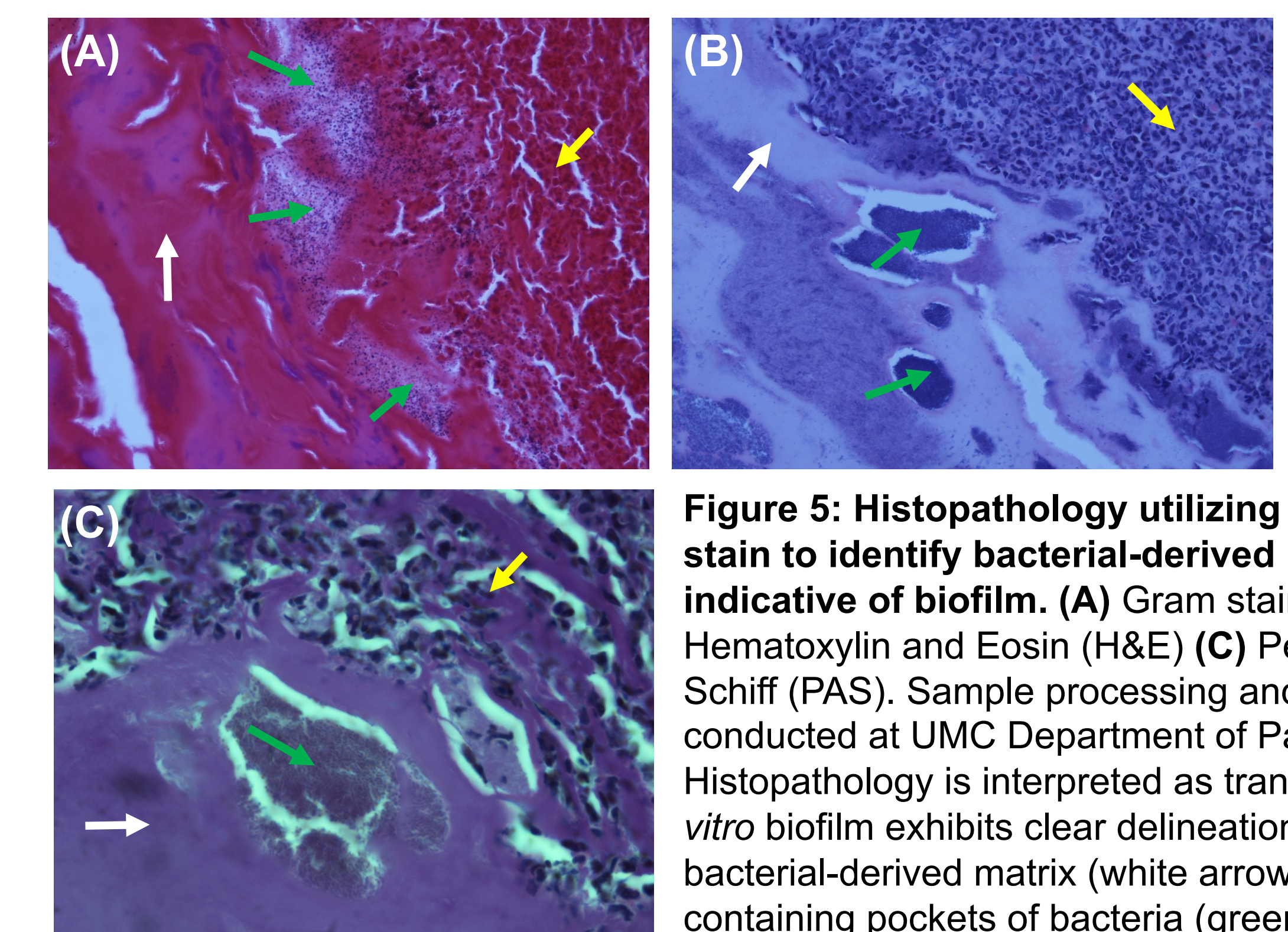


Figure 5: Histopathology utilizing matrix stain to identify bacterial-derived matrix indicative of biofilm. (A) Gram stain **(B)** Hematoxylin and Eosin (H&E) **(C)** Periodic Acid Schiff (PAS). Sample processing and staining conducted at UMC Department of Pathology. Histopathology is interpreted as transplanted *in vitro* biofilm exhibits clear delineation between bacterial-derived matrix (white arrows) containing pockets of bacteria (green arrows) and host-derived matrix (yellow arrows) denoted by infiltration of immune system cells.

DISCUSSION

Taken together, our data demonstrate that the device can detect fluorescence signal from polymicrobial, biofilm-associated infections. Detection with the device was validated by characteristic red fluorescence signatures from the murine chronic wound model. The polymicrobial nature of the infection was confirmed by in-house and send-out culture-based microbiology. The *ex vivo* tissue was found to be biofilm-associated via histopathology staining for matrix and scanning electron microscopy (SEM). This work further validates the use of the MolecuLight *i:X* device clinically for the real-time detection of wound pathogens in complex chronic wound infections.

Works Cited

- [1] Dowd *et al.* 2008. Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. *BMC Microbiology*. 8: 43
- [2] DeLeon *et al.* 2014. Synergistic Interactions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an *In Vitro* Wound Model. *Infection and Immunity*. 82(11): 4718-4728.
- [3] Rennie *et al.* 2019. Understanding Real-Time Fluorescence Signals from Bacteria and Wound Tissues Observed with the MolecuLight *i:X*. *Diagnostics*. 9(1).