

# Application of fluorescence imaging in identifying wound bed infections

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## Research

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## Abstract

Infection of the wound bed is the 2nd most common cause of Graft loss following split skin graft, next to hematoma. Wound bed infections increase the duration of hospital stay, increased cost of treatment, prolonged treatment and treatment failure. Hence identifying wound bed infection and treating it becomes the primary duty of the surgeon before applying skin graft for any wounds. In the current setting, wound biopsy and culture is the important Modality through which wound infection is confirmed. Though Wound biopsy is gold standard in identifying wound infection, it is time consuming which further delays the management of the wound. In this article we would like to report about the use of a real time fluorescent imaging in rapid identification of bacterial infections in wound bed.

**Key Words:** Fluorescence, Imaging, Wound, Infections.

## Introduction

Infection of the wound bed is the 2nd most common cause of graft loss following split skin graft, next to hematoma<sup>1</sup>. Wound bed infections increase the duration of hospital stay, increased cost of treatment, prolonged treatment and treatment failure. Hence identifying wound bed infection and treating it becomes the primary duty of the surgeon before applying skin graft for any wounds. In the current setting, wound biopsy and culture is the important Modality through which wound infection is confirmed. Though Wound biopsy is gold standard in identifying wound infection, it is time consuming which further delays the

management of the wound<sup>2</sup>. In this article we would like to report about the use of a real time fluorescent imaging in rapid identification of bacterial infections in wound bed.

## Materials and methods

The study was conducted in the Department of Plastic Surgery in a tertiary care center. The patient was a 35 year old male with no known comorbidities, with raw area over the left leg following electrical burn injury (Figure 1). The wound was initially treated with serial minimal debridements and negative pressure wound therapy. Clinically wound had healthy granulation tissue with no active exudation and no signs of infection but to confirm whether infection is present or not fluorescence technology (Illuminate™ equipment) was used (Figure 2) as wound culture takes 2 to 3 days to confirm infection. Wound culture was also sent to correlate & confirm findings of fluorescent imaging.

Illuminate™ by Adiuvo diagnostics, Chennai, India is a novel first of its kind screening device that uses Multispectral auto fluorescence imaging combined with machine learning to detect and classify pathogens on wounds along with automated wound measurements in under 2 minutes when compared to gold standard culture method. This device takes advantage of auto fluorescent property of microorganisms to accurately classify the organisms and locate their area of concentration. This device detects microorganisms if any, provides gram type of bacteria & visual data about burden of infection in the wound and even helps to detect biofilm in 2 minutes. Pointed to the region of interest, the device captures 16 serial images at different excitation emissions and produces a clinical image overlaid with the superimposed infection, if any, marking the presence of gram-positive bacteria in red, gram-negative in green, and fungus in blue.

Before use of this device, wound of interest is chosen and wound is washed with 0.9% normal saline. Wound and its surrounding is cleaned and dabbed to make it dry. Patient is either taken to a dark room or dark drapes are used to provide dark environment around the wound. The fluorescent imaging device is kept at a distance of 10cms from the wound and area of interest is focused and images are taken. The device takes 16 serial images at different excitation emissions and produces a final image. Infection if any would be highlighted red in presence of gram positive

infection, green in case of gram negative infection and blue in case of fungal infection.

## Results

In our patient, the wound showed presence of polymicrobial infection with gram negative predominance (Figure 3).

Wound culture which was sent also confirmed presence of infection & hence plan of skin grafting was deferred and patient was started on antibiotics according to the culture & sensitivity report. This shows that fluorescence technology can detect infection far earlier in 2 minutes compare to wound culture which takes 2 to 5 days,

## Discussion

Auto fluorescence property exhibited by pathogens is due to the presence of autofluorescing markers like NADH and flavin and infectious markers like porphyrin and pyoverdin. NADH has strong emission peak linkages in producing blue auto fluorescence, while flavin and porphyrin peak at around 525 nm and 620 nm corresponding to green and red fluorescence, respectively. Gram-negative organisms have a higher intensity of NADH and flavin than gram-positive organisms. This helps in differentiating gram positive and negative organisms from each other. Pseudomonas possesses pyoverdin, a unique marker having blue-green color enabling its detection. Similarly fungus has characteristic blue fluorescence due to the presence of chitin and dityrosine linkages. On further analyzing amount of autofluorescence, it is also possible to differentiate the organisms up to genus level if not species level. It is noted that autofluorescence will be able to detect as little as 10<sup>3</sup> colony forming units/gram which will be helpful in providing early intervention for wounds before clinically significant infection occurs<sup>3</sup>. In a study conducted by daCosta et al in 2015 showed that autofluorescence is superior in identifying clinically significant bioburden in wound bed as well as in surroundings compared to examination under white light<sup>4</sup>. It is also possible to quantify the amount of bio burden in the wound bed based on the intensity of autofluorescence. It can be an alternative non-invasive method for bacterial detection in the wound. Instead of blind biopsies, fluorescence guided biopsy can be taken which would increase the yield and also be preventing inadequate sampling in case of polymicrobial infections of same wound. Debridement and wound wash can also be guided by autofluorescence thereby increasing its efficacy. Moreover it can be used as a preliminary guide in providing appropriate antibiotic treatment there by helps in at least partly following appropriate antibiotic

stewardship practices which in turn will help in decreasing antibiotic resistance<sup>5</sup>. Though autofluorescence has its applications in broad area of identification of wound infection and its management it cannot detect deep seated infections and possibly differentiate between pathogenic and commensal organisms. Though it can approximately quantify bacterial load, for accurate calculation, tissue biopsy is required. And also it becomes inevitable for tissue biopsy to choose appropriate antibiotics for diagnosis in the era of drug resistance. The limitation of this study is that it is a single case report & single institute study. It requires multicentric randomized control study to validate the reports.

## Conclusion

Our case report describes about the use of fluorescent imaging in identifying wound bed infection in otherwise clinically healthy wound, there by helping in timely management of the wound. This proves that fluorescent imaging is superior to clinical examination under white light for identifying infections and more time saving compared to conventional wound culture. Fluorescent imaging has a wide range of scope that can be effectively implemented in wound management.

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### Figures



Figure 1: Clinical photograph of the wound in left leg.

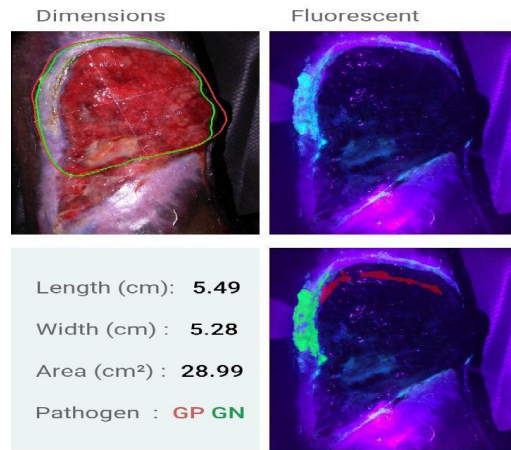


Figure 3: IlluminateTM fluorescent imaging report showing presence of infection.



Figure 2: IlluminateTM equipment.