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Neural Network-Based Quantification of Microbial Surface Coverage on Implant Materials

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Introduction

Fluorescence microscopy is widely used to analyze co-culture and *in situ* grown biofilms. However, quantification of image-derived parameters is challenging because of overlapping signals from microorganisms, human cells, and background noise. This study developed a neural network-based workflow to measure microbial surface coverage on titanium implant materials in such images.

Methods

Confocal images were obtained from an *in vitro* co-culture model and an *in situ* splint study. After automatic preprocessing of the green fluorescence channel, images were analyzed using one of three routes depending on image quality: thresholding alone, thresholding after manual subtraction of interfering regions, or combined segmentation with two custom Cellpose models (CP_I for individual bacteria and CP_M for microcolony detection). Segmentation outputs were manually corrected and used to calculate microbial surface coverage.

Results

CP_I achieved a best test accuracy of 0.68, outperforming the best pretrained Cellpose model (0.15). In the *in vitro* model, estimated microbial surface coverage was 3.47% on top surfaces versus 0.05% on lateral implant surfaces. In the *in situ* dataset, 176 images were analyzed: 14 by thresholding alone, 79 by thresholding plus subtraction, and 83 by the combined dual-model workflow. CP_I reached an accuracy of 0.63 for small regions, and CP_M reached 0.74 for larger microcolonies. Mean microbial coverage increased from 27.81% on day 2 to 71.88% on day 8, and the workflow reduced manual correction time by 70.13%.

Conclusion

This study presents a practical semi-automated workflow for quantifying microorganisms in images containing co-localized bacteria and human cells, where classical methods perform poorly. The approach supports more efficient analysis of implant-associated biofilms and related co-culture systems, although additional high-quality training images and optimized microscopy may further improve accuracy and facilitate fully automated analysis.