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Title

Development of Deep Learning Models for Sub-Cellular Fluctuation Imaging (SCFI): a 30-minute antibiotic susceptibility test

Introduction

We have developed a phenotypic and label-free Antibiotic Susceptibility Test (AST) termed Sub-Cellular Fluctuation Imaging (SCFI) to address rising rates of antimicrobial resistance [1].

SCFI is an advanced machine-learning enabled microscope that monitors real-time fluctuations of bacterial cell membrane in response to antibiotics. By quantifying changes in magnitude and location of light scattering caused by subcellular movement, we can detect metabolic changes that occur when bacteria are challenged with antibiotics [2-6].

Here, we show that improvements to SCFI's Deep-Learning models can correctly classify bactericidal and bacteriostatic antibiotics for Gram positive and Gram-negative organisms.

Method

10μL per sample is introduced to microfluidic flow chambers and immobilised using a species-specific antibody coating for 10 minutes. The bacterial suspension is removed, washed (to minimise non-bound cells) and incubated with 200μL of GC broth containing either a treated (with antibiotic) or untreated (without antibiotic) condition for 30 minutes. Images are captured at a laser intensity of 20Hz for 20 seconds, for ≥50 individual bacterial cells per test.

Convolutional Neural Networks were developed to enable classifications of bactericidal (Kanamycin) and bacteriostatic (Trimethoprim and Methicillin) on *E. coli* and *S. aureus* species to determine their respective performance metrics (sensitivity, specificity, PPV and NPV).

Results

CNN models were developed to successfully classify treated, untreated, resistant and susceptible bacterial conditions. All conditions were tested in triplicate and demonstrated ≥90% accuracy (Sens 91-98%, Spec 96-99%, PPV 96-99% and NPV 91-98%) when compared to conventional disk diffusion assays.

Discussion

In these experiments we have successful demonstrated rapid (30 minutes) and accurate (≥90%) classification of bacterial resistance states by Deep Learning Techniques. This

data also continues to support existing literature that SCFI is an AST that is agnostic to the antibiotic class and bacterial species used.

This system is undergoing product development and will be translated into a bespoke hardware system for clinical and antibiotic research applications.

Figures

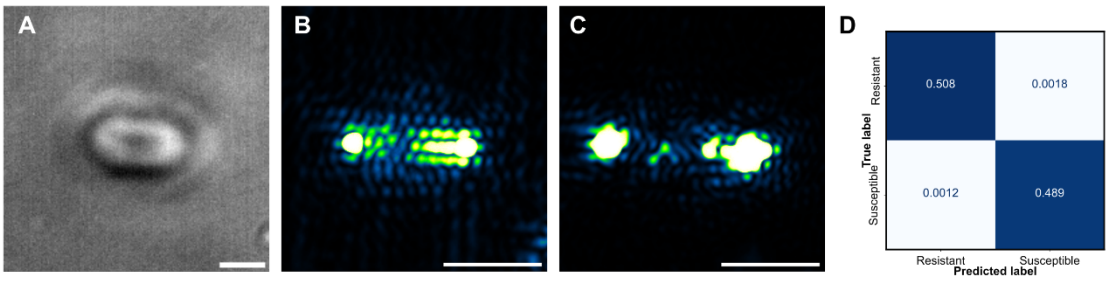

Figure 1: Example of SCFI recording and classification through deep learning model.
A: Example of E.coli bacteria in bright field micrscopy. Scale bar: 3 um.
B: Maximum Z-stack of SCFI imaging of a resistant E.coli bacteria treated with Kanamycin 20 ug/ml
C: Maximum Z-stack of SCFI imaging of a susceptible E.coli bacteria treated with Kanamycin 20 ug/ml
D: Confusion matrix obtained after classification of resistant vs suceptible bacteria, showing accurate prediction of deep learning model. Counts were normalised to the total number of images.

References

1. Global Burden of Antimicrobial Resistance in 2019: a systematic analysis. Murray et al. 2022. The Lancet. https://doi.org/10.1016/S0140-6736(21)02724-0
2. METHOD AND APPARATUS FOR BACTERIAL ANALYSIS- WO2019025771-A1
3. Imaging of sub-cellular fluctuations provides a rapid way to observe bacterial viability and response to antibiotics. Bermingham et al 2018. BioRxiv. https://doi.org/10.1101/460139
4. Towards a Fully Automated Scanning Probe Microscope for Biomedical Applications. Szeremeta W et al 2021. Sensors. https://doi.org/10.3390/s21093027
5. Longo G, Alonso-Sarduy L, Rio LM, et al. Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors. Nat Nanotechnol. 2013;8(7):522-526. https://doi:10.1038/nnano.2013.120
6. Bermingham CR, Murillo I, Payot ADJ, et al. Imaging of sub-cellular fluctuations provides a rapid way to observe bacterial viability and response to antibiotics. Published online November 2, 2018:460139. doi: https://doi.org/10.1101/460139