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Title
Use of Sub-Cellular Fluctuation Imaging (SCFI) for real-time monitoring of antibiotic resistance in *N. gonorrhoeae*

Introduction
We have developed a phenotypic and label-free Antibiotic Susceptibility Test (AST) termed Sub-Cellular Fluctuation Imaging (SCFI) to address rising rates of incidence and resistance of *N. gonorrhoeae* infections [1-2].

SCFI is an advanced machine-learning enabled microscope that monitors real-time intracellular fluctuations of the bacteria 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 [3-7].

Here, we show that SCFI can accurately classify resistance profiles of clinical *N. gonorrhoeae* strains after only 20-minute incubation with front-line antibiotic Ceftriaxone. Results demonstrated categorical agreement for susceptibility: Accuracy 94%, Sensitivity 92%, Specificity 93%, NPV 92%, PPV 95% across all strains tested.

Materials and Methods
100mL 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 100mL of GC broth containing either a treated (with antibiotic) or untreated (without antibiotic) condition for 20 minutes. Images used to measure Mean Fluctuation Values 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 classification of *N. gonorrhoeae* reference strains ATCC19424, ATCC49226, FA1090) and 3 local clinical isolates (BG27, BG52, BG56) to determine sensitivity, specificity, PPV and NPV when compared to known disk diffusion and E-test techniques.

Results
For all experiments, when Mean Fluctuation Values were plotted as a function of time, significant changes (t-test, p value= 0.05) were observable within 20-30 minutes of antibiotic exposure.

All conditions were tested in triplicate (n=150 cells) and demonstrated 94% accuracy (Sens 92%, Spec 93%, PPV 95% and NPV 92%) when compared to known resistance profiles.

Discussion

In these experiments we have successful demonstrated accurate (≥90%) real-time classification of N. gonorrhoea resistance states. This data 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

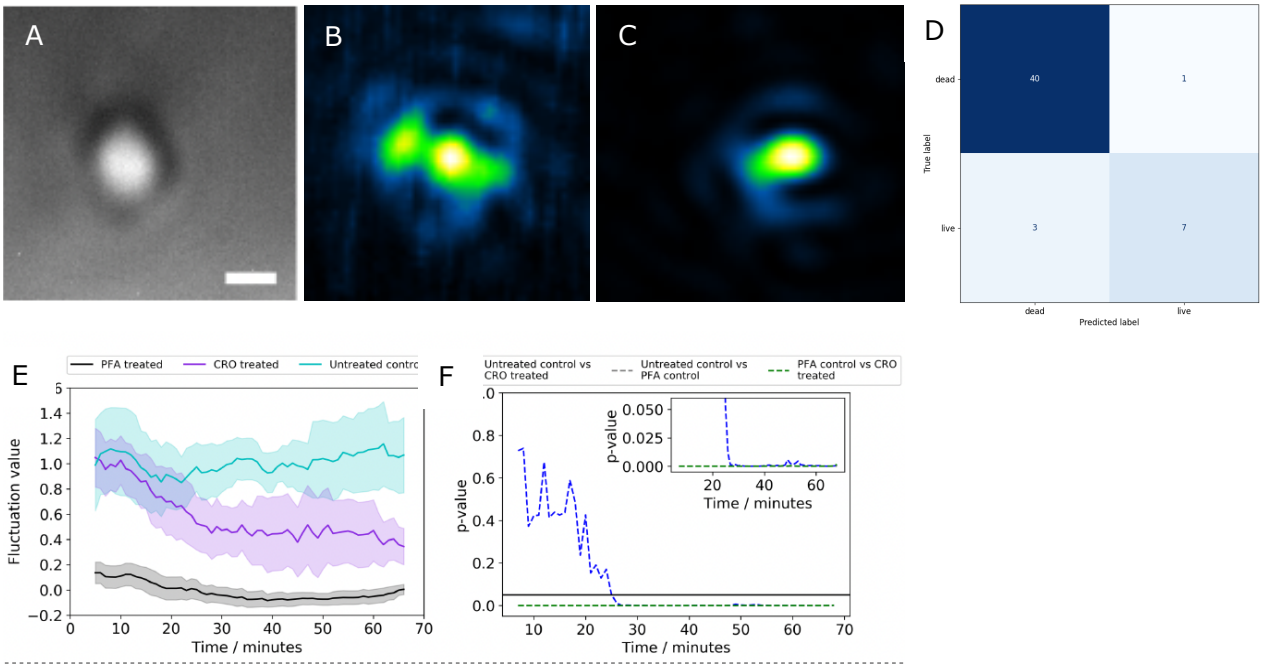

Figure 1: Example of SCFI images and analysis

A: Example of N. gonorrhoeae NCTC 8375 in brightfield microscopy. Scale bar 3μM

B: Maximum Z-stack of SCFI imaging of untreated (live) N. gonorrhoea

C: Maximum Z-stack of SCFI imaging of Ceftriaxone treated (dead) N. gonorrhoea

D: Confusion matrix obtained after classification of live and treated bacteria, showing accurate prediction of deep learning model.

E: Mean Fluctuation Values (MFV) measured over 60-minutes for PFA, Ceftriaxone and untreated N. gonorrhoea

F: Significance (t-test) of MFV measured over 60-minutes for PFA, Ceftriaxone and untreated N. gonorrhoea

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