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

Rama S[1], Antognozzi M[2], Szeremeta W[2], Phonrat K[2], Bermingham C[2], Frapwell C[2], KyriakidesM[1], Newman H[1], Bonney-Bhandal K[1], Preece J[1], Dorh N[1], and Dorh J[1]

[1] FluoretiQ Limited, Unit DX, St Philips Central, Albert Road, Bristol BS2 0XJ
[2] School of Physics, University of Bristol, Tyndall Avenue, Bristol, BS8 1TL

Abstract

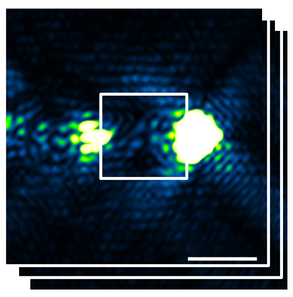
We have developed a phenotypic and label-free Antibiotic Susceptibility Test (AST) termed Sub-Cellular Fluctuation Imaging (SCFI) to address rising rates of antimicrobial resistance.

SCFI is an advanced machine-learning enabled microscope that monitors real-time changes of intracellular activity 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 exposed to antibiotics.

Here, we show that SCFI can correctly classify the effects of bactericidal and bacteriostatic antibiotics for Gram positive and Gram-negative organisms.

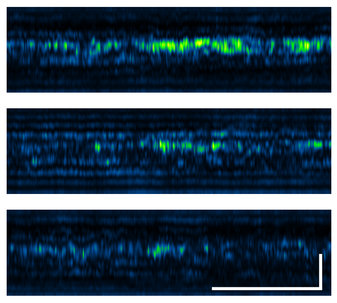
Results

A SCFI Stack acquisition



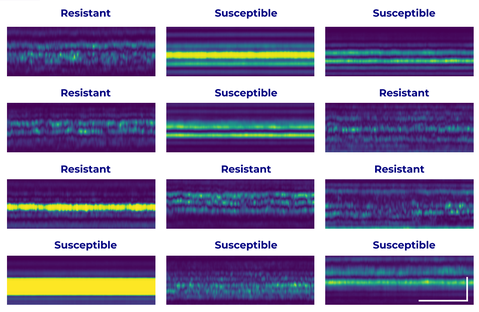
Example of SCFI stack acquisition of an E.coli bacteria. White square: ROI chosen for preprocessing and classification. Scale bar: 1 um.

B Time slices



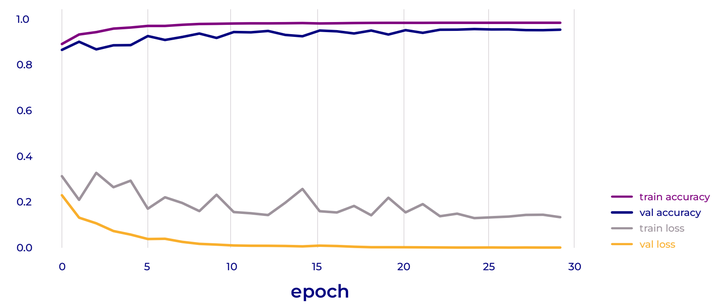
3 examples of time slices (y-axis through time) of the ROI in A). Scale bars: 0.5 um, 5 s.

C Dataset



12 images, example of a full dataset containing around 50 cells per each condition (Resistant or Susceptible bacteria). Scale bars: 0.5 um, 5 s.

D Training

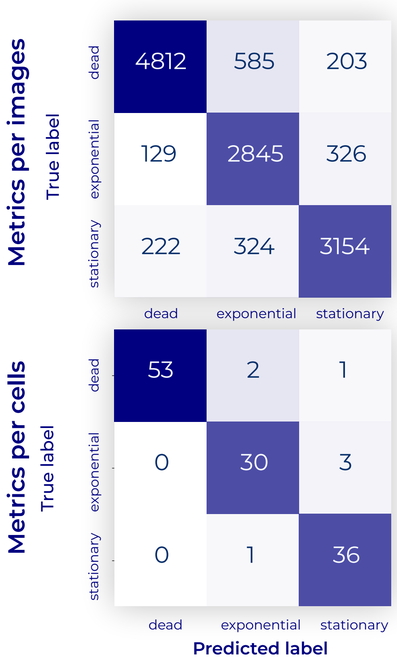


Example of learning curves when training a Convolution Neural Network on the preprocessed dataset. Loss values show slow decrease, accuracy grows in both train and validation datasets.

E

Classification of E.coli states

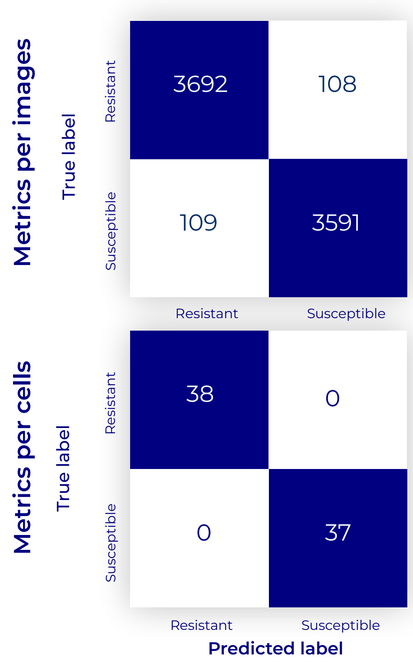
Sensitivity	Specificity	Accuracy
94%	97%	96%



F

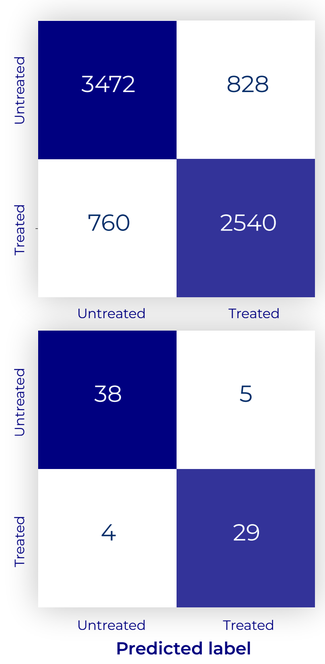
Kanamycin on E.coli

Sensitivity	Specificity	Accuracy
100%	100%	100%



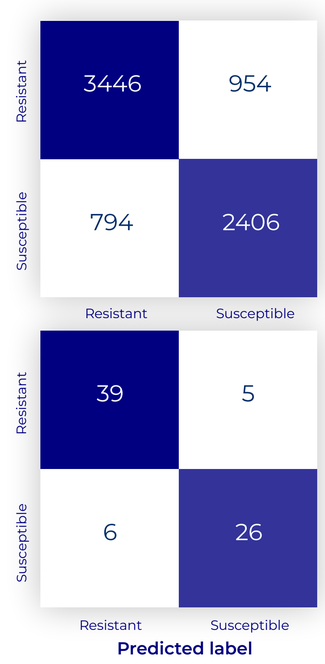
Trimethoprim on E.coli

Sensitivity	Specificity	Accuracy
88%	88%	88%



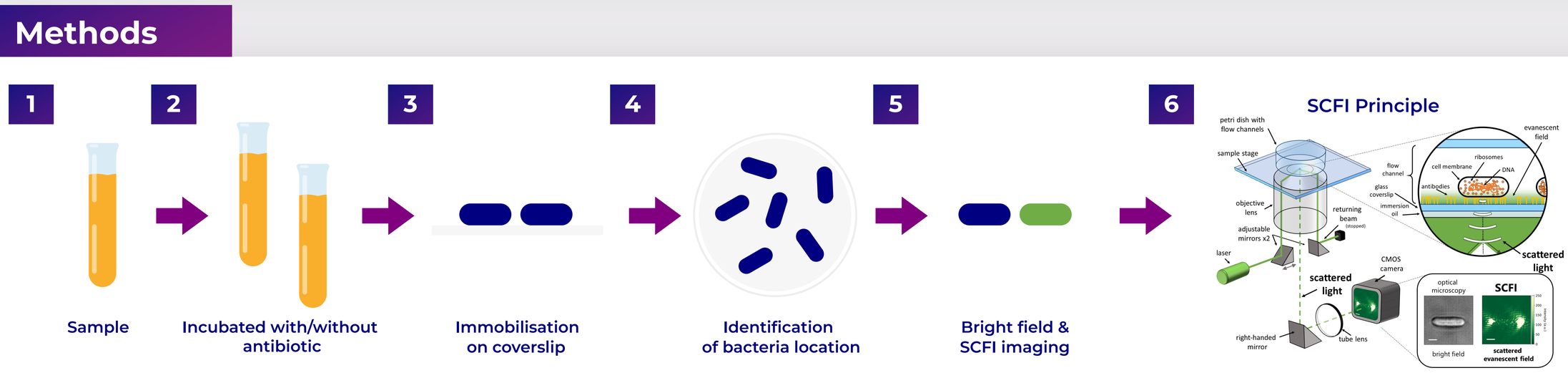
Methicilin on S.aureus

Sensitivity	Specificity	Accuracy
81%	89%	86%



E) Confusion matrixes obtained when classifying the different states (dead, exponential, stationary) of E.coli.
F) Confusion matrixes obtained for models classifying between resistant & susceptible bacteria (Kanamycin, Methicilin) or between treated and untreated bacteria (Trimethoprim).

Methods



Samples were incubated with antibiotics for 30 minutes at 37 degrees Celsius and then antibody-immobilised on glass coverslips for 10 minutes. Coverslips were set into SCFI rig, bacteria were visually identified and 50 of them recorded in SCFI imaging for 20 seconds each.

References

1. Global Burden of Antimicrobial Resistance in 2019: a systematic analysis. Murray et al. 2022. The Lancet.
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. Bermingam et al 2018. BioRxiv.
4. Towards a Fully Automated Scanning Probe Microscope for Biomedical Applications. Szeremeta W et al 2021. Sensors.
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.
6. Bermingam 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

7th AMR Conference
Novel Antimicrobials & AMR Diagnostics