

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

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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 μ m.

B Time slices

3 examples of time slices (y-axis through time) of the ROI in A). Scale bars: 0.5 μ m, 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 μ m, 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%

Metrics per images

True label	dead	exponential	stationary
dead	4812	585	203
exponential	129	2845	326
stationary	222	324	3154

Metrics per cells

True label	dead	exponential	stationary
dead	53	2	1
exponential	0	30	3
stationary	0	1	36

F

Kanamycin on E.coli

	Sensitivity	Specificity	Accuracy
	100%	100%	100%

Metrics per images

True label	Resistant	Susceptible
Resistant	3692	108
Susceptible	109	3591

Metrics per cells

True label	Resistant	Susceptible
Resistant	38	0
Susceptible	0	37

Trimethoprim on E.coli

	Sensitivity	Specificity	Accuracy
	88%	88%	88%

Metrics per images

True label	Untreated	Treated
Untreated	3472	828
Treated	760	2540

Metrics per cells

True label	Untreated	Treated
Untreated	38	5
Treated	4	29

Methicilin on S.aureus

	Sensitivity	Specificity	Accuracy
	81%	89%	86%

Metrics per images

True label	Resistant	Susceptible
Resistant	3446	954
Susceptible	794	2406

Metrics per cells

True label	Resistant	Susceptible
Resistant	39	5
Susceptible	6	26

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

1

Sample

2

Incubated with/without antibiotic

3

Immobilisation on coverslip

4

Identification of bacteria location

5

Bright field & SCFI imaging

6

SCFI Principle

The diagram illustrates the SCFI principle, showing a petri dish with flow channels, a sample stage, an objective lens, adjustable mirrors, a laser, a returning beam, an immersion oil, a glass coverslip, a cell membrane, ribosomes, DNA, and an evanescent field. It also shows the resulting bright field and SCFI images.

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.

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