Rapid determination of bacterial susceptibility to antibiotics combining Raman spectroscopy and Dielectrophoresis

G. Barzan[ab*], A. Sacco[ab], L. Mandrile[a], A. M. Giovannozzi[a], C. Portesi[a] and A. M. Rossi[a]

*contact: g.barzan@inrim.it

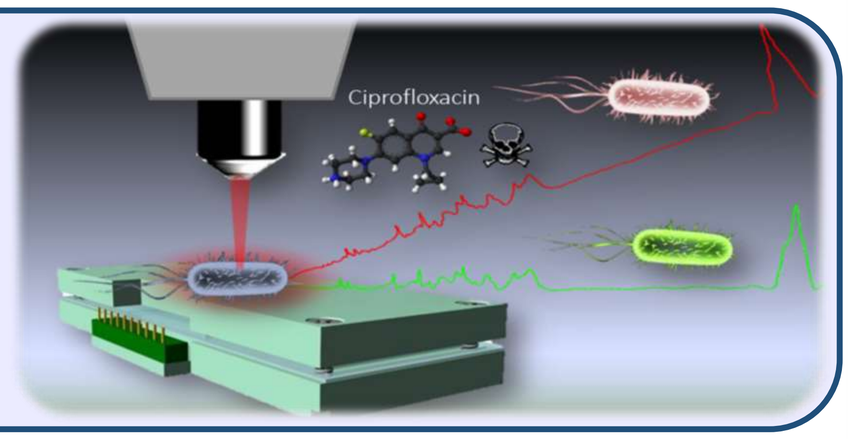
a) Quantum Metrology and Nano Technologies division, Istituto Nazionale di Ricerca Metrologica (INRiM), Strada delle Cacce, 91, 10135 Turin, Italy

b) Departement of Electronics and Telecommunications , Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129 Turin, Italy

The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

Introduction

The development of rapid, sensitive and specific methods to determine antibiotic susceptibility of bacteria is required to help reduce the widespread misuse of antibiotics and the growing multidrug-resistance problem [1]. This study presents a combined Raman spectroscopic and dielectrophoretic (DEP) approach to obtain direct, real-time measurements of a suspension of planktonic bacteria without the need of any labelling or other time-consuming sample preparation processes. Thanks to the spatial non-uniform DEP fields, bacteria are easily captured and concentrated under the laser spot of the Raman apparatus, increasing the sensitivity of the Raman technique [2]. Optimizing the setup conditions we were able to characterize different bacterial strands with high specificity. Using our Raman-DEP device, we demonstrated the susceptibility of E. coli towards the commonly prescribed second-generation fluoroquinolone [3] ciprofloxacin (CP), after only one hour of treatment, by monitoring spectral changes in the chemical fingerprint of the bacteria, which are related to the mode of action of the drug. Comparison between treated and untreated samples were performed at the MIC (minimum inhibitory concentration) and sub-MIC levels for different time points over a 3 hour span, and the Raman data were processed by supervised multivariate tools, such as PLS-Discriminant Analysis and PLS-Regression, for the calibration of descriptive models of cellular modifications. The models were validated using the cross-validation strategy. Simultaneously, standard microbiological assays based on cell viability, turbidity test and fluorescence microscopy, were carried out as reference methods to correlate the observed Raman response and to build strong predictive models.

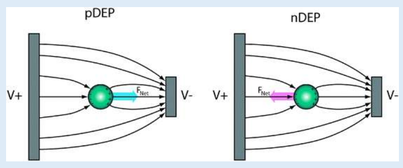


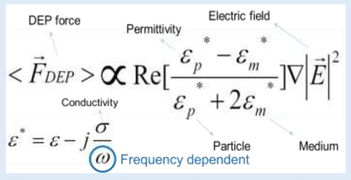
The DEP device for Raman analysis of bacteria

Dielectrophoresis (DEP)

Dielectric particles in a non-uniform electric field ⟹ **Net force**
Magnitude of the force depending on permittivity of both particle and medium
Can be positive or negative depending on which electrical permittivity is higher

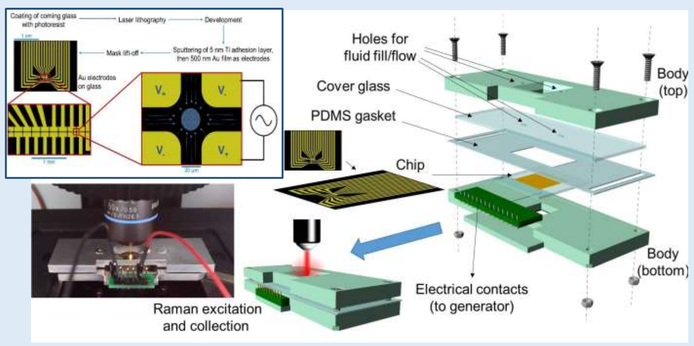
Polarity of the electric field is irrelevant! Variable (in time) electric field (AC)



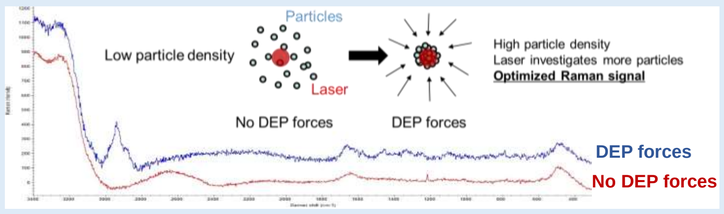


A dielectric particle placed in an electric field becomes electrically polarized as a result of partial charge separation, which leads to an induced dipole moment. The dipole moment is a consequence of the generation of equal and apposite charges at the boundary of the particle. In a non-uniform electric field, the particle experiences a net dielectrophoretic force. The magnitude of the induced dipole depends on the polarizability of the particle with respect to that of the medium. Applying the correct voltage and frequency, dielectric particles, such as bacteria, can be manipulated into clusters to increase their local concentration.

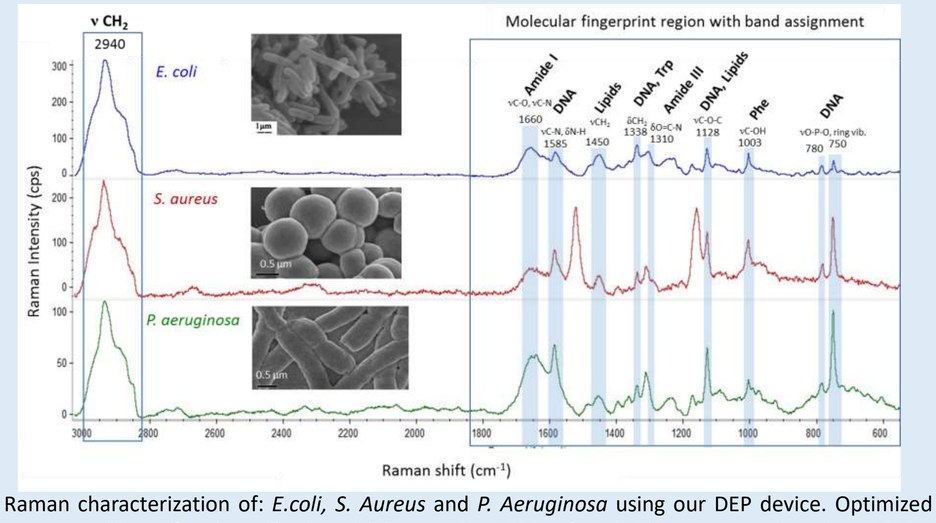
Raman-DEP device



Bacteria can be manipulated into clusters in order to maximize the signal



Raman spectra of Bacteria



Raman characterization of: *E.coli, S. Aureus* and *P. Aeruginosa* using our DEP device. Optimized setup conditions for the cell: sinusoidal wave V = 5 Vpp, f = 800 kHz, aggregation time: 360 s. Optimized setup conditions for Raman microscope: laser line: 532 nm, laser power: 10 mW, objective: 60x water immersion, integration time: 2.5 s for 24 scans (1 min per spectrum).

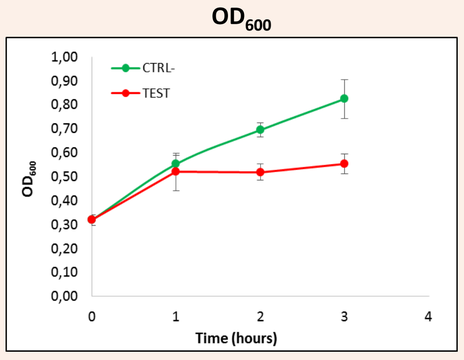
E. coli susceptibility to ciprofloxacin measured by Raman-DEP

Calibration of the susceptibility test method

Experimental setup: Bacteria were treated with 1 µg/ml of ciprofloxacin in the middle of their exponential growth phase, when their OD_{600nm} was 0.3, during which they are more sensitive to the antibiotic.

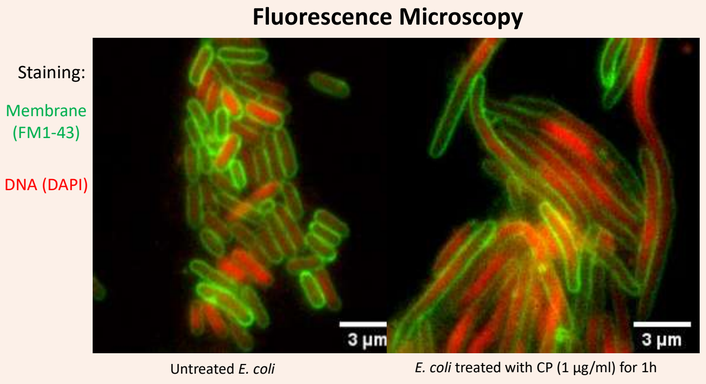
MIC of CP in E.coli MG1655 (OD_{600nm} = 0.3): **0.5 µg/ml**
MBC of CP in E.coli MG1655 (OD_{600nm} = 0.3): **1 µg/ml**

OD_{600}



E. Coli MG1655 untreated and treated with 1 µg/ml ciprofloxacin (CP) over a 3 hour span.

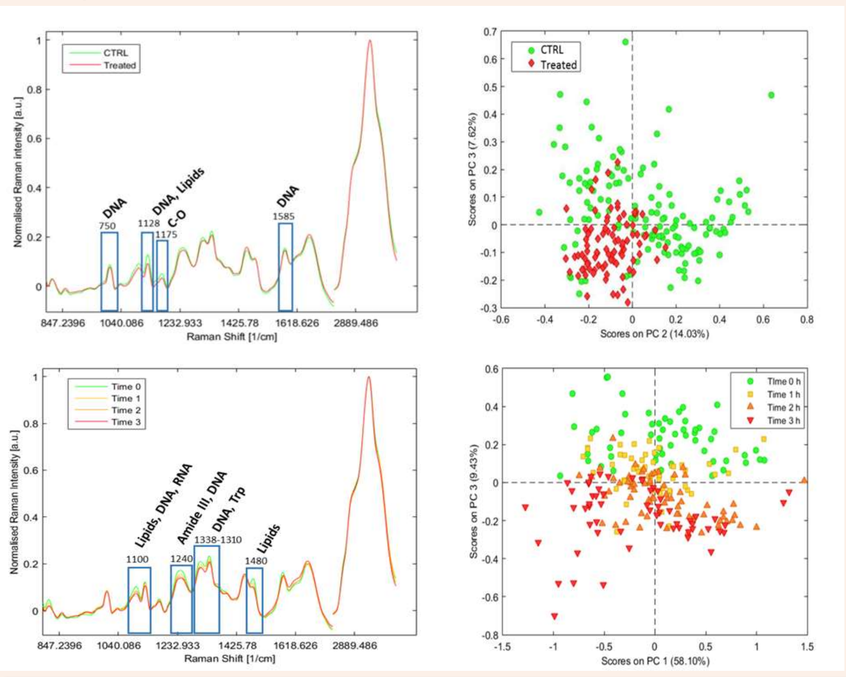
Fluorescence Microscopy



Untreated *E. coli* — *E. coli* treated with CP (1 µg/ml) for 1h

Morphological changes of E. coli due to the effects of the CP treatment are already visible after 1 hour.

Raman spectra of CTRL and treated bacteria collected over time with the DEP device



Principal Components Analysis (PCA) helps in the visualization of non random variation in spectral data of CTRL and Treated bacteria. The spectra of *E. coli* treated with CP are grouped in the PCA scores plot.

PCA also revealed that Raman spectra captured changes related to the passage of time in both CTRL and Treated samples.

This effect was taken into consideration for subsequent classification analysis.

PREDICTIVE CLASSIFICATION METHOD:
Partial least square-Discriminant Analysis (PLS-DA) model was used to classify the spectra of bacteria according to their response to antibiotic treatment.

	1 h	2 h	3 h
Sensitivity (Prediction)	0.67	1.00	1.00
Specificity (Prediction)	1.00	0.50	0.33
Class. Error (Prediction)	0.17	0.25	0.33

PLS-DA model after 1 hour of CP treatment (1µg/ml)

Six independent experiments were conducted for the construction of the model, five were used for the training and one for the validation.

Conclusions

The Raman-DEP device here described allows to characterize different bacterial strains with high specificity and to follow dynamic interactions of the bacteria with antibiotics.
Raman data processed with supervised multivariate data analysis are able to detect subtle spectral differences at a molecular level between treated or untreated bacterial cells after only 1 hour of treatment.
This Raman-DEP method could open the way to rapid bacterial antibiotic susceptibility test without the necessity of time consuming sample preparation and overnight incubation required by classical microbiological techniques.

Validations and applications of the method

1. Prediction of the susceptibility of *E. coli* at a CP concentration <<MIC (0.015 µg/ml)

2. Prediction of the susceptibility of *E. coli* PSA-I (modified to be resistant to CP)

Raman spectra of the E. coli treated with CP at sub-MIC or supposed to be resistant to the drug are all classified by the model as controls: viable bacteria, with high sensitivity.

3. Prediction of the susceptibility of E. coli treated with CP at MIC (0.5µg/ml) and triclosan (TCS) at sub-MIC (0.2µg/ml), known to induce antibiotic resistance

CFU assay

Untreated *E. coli*

E. coli + CP (0.5µg/ml)

E. coli + CP (0.5µg/ml) + TCS (0.2 µg/ml)

Fluorescence Microscopy

Untreated *E. coli* — *E. coli* treated with CP (0.5 µg/ml) for 1h — *E. coli* treated with 0.5 µg/ml CP for 1h after pre-treatment with 0.2 µg/ml TCS

Vitality assay performed by counting the CFU (Colony Forming Units) and the fluorescence microscopy confirmed that bacteria pre-treated with triclosan at sub-MIC gained a sort of resistance towards the treatment with ciprofloxacin at MIC already after 1 hour.

References

[1] World-Health-Organisation in Antimicrobial resistance, Vol. 2014, 2014.
[2] U.-C. Schröder et al. , *J. Biophotonics* 10, 1547–1557, 2017.
[3] O. Fasugba, A. Gardner, B. G. Mitchell, G. Mnatzaganian, *BMC Infect Dis* 15, 2015.