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# Microfluidic Impedance Cytometry as a novel technique for rapid detection/identification of pathogens causing microbial keratitis

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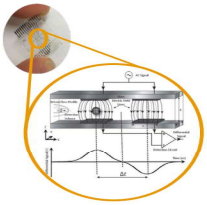
## ABSTRACT

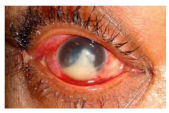
Microbial keratitis is a sight-threatening acute or chronic infection of the cornea. Prompt diagnosis is crucial to prevent permanent visual loss and minimise structural damage of the cornea. Culture and corneal tissue sampling for direct microscopic detection of pathogens are the current gold standards for microbial keratitis diagnosis. Unfortunately, corneal scraping is an unpleasant procedure that may cause ocular discomfort and, in most cases, ocular pathogens are non-culturable, hampering precise diagnosis.

## AIM

Development of a real time Microfluidic Impedance Cytometry assay as a high-throughput single-cell analytical method that is non-invasive, label-free and which does not require bacterial culture.

### Impedance Cytometry technology

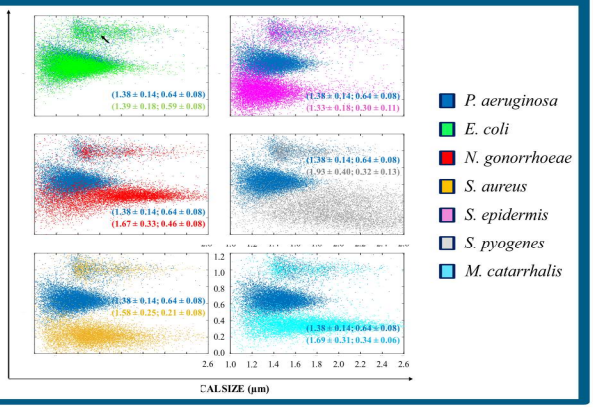




✓ Non-invasive
✓ Label free

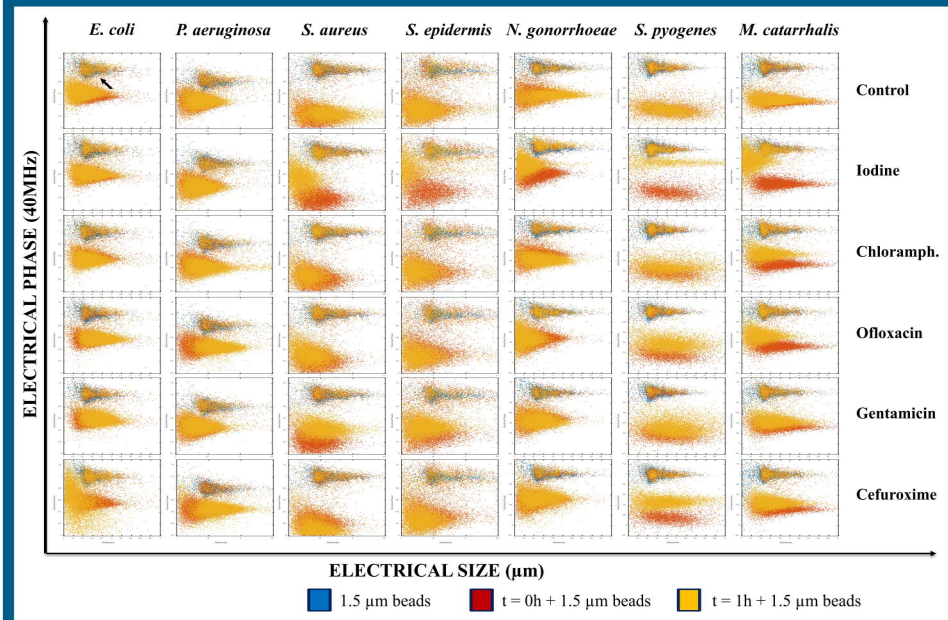
Two sets of parallel facing electrodes are fabricated inside a microfluidic channel. Cells suspended in an electrolyte are driven through the channel by pressure driven flow. An AC voltage is applied to the top two electrodes and the difference in current flowing through the bottom two electrodes is measured using a custom detection circuit.

### Electrical profile of ocular pathogens



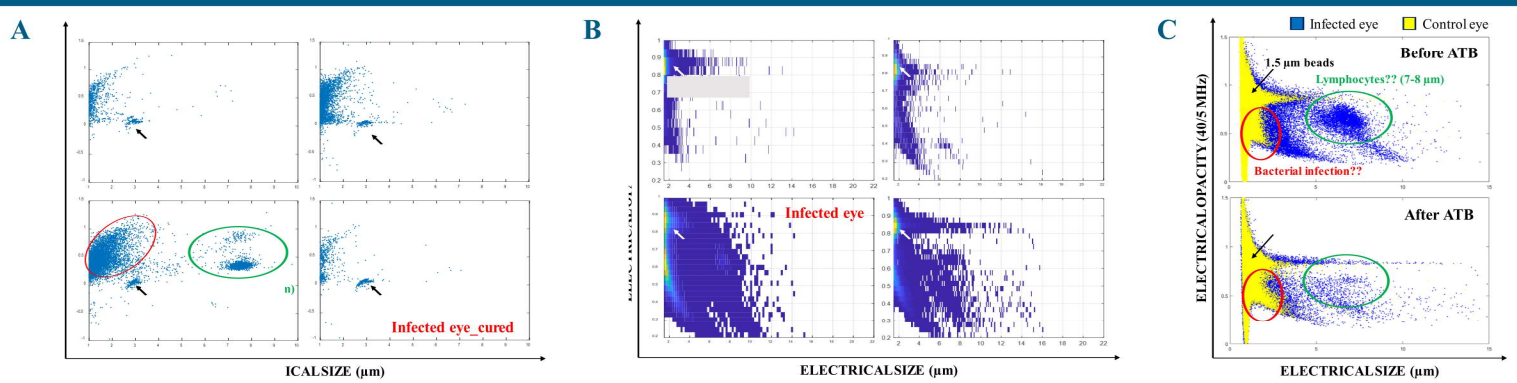

## RESULTS

### Antimicrobials affect the electrical properties of bacteria




Bacterial ocular pathogens were grown to log-phase and treated with different antimicrobials (clinical eye drops) for 1 hour. 1.5 µm polystyrene beads (identified with an arrow in the first panel as an example) were used as an external reference to normalize all plots at x = 1.5 µm and y = 1. The electrical phase (40 MHz) was measured before and after treatment and compared. Bacterial viability was also assed (data not shown).

### Analysis of tear samples from patients with microbial keratitis by Microfluidic Impedance Cytometry




Tear samples were collected from patients diagnosed with microbial keratitis before and after ATB treatment. The non-infected eye was used as a negative control ('healthy'). Polystyrene beads (identified with an arrow) were used as external reference to normalize all scatter plots. Scatter plots on panel B are coloured by density. The electrical phase (40 MHz) and opacity (40/5 MHz) were measured before and after treatment and compared. Panels B and C correspond to the same patient.

## CONCLUSION

**This study provides a proof of concept that highlights Microfluidic Impedance Cytometry as a novel technique with the potential for accurate sensing and diagnosis, thereby granting a more efficient and targeted use of antibiotics.**

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