Impedance Flow Cytometry for Electrogenic Microorganisms

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This study expands upon recent discoveries highlighting the important role of electrogenic gut bacteria [1]. The main objective is to develop and demonstrate impedance-based flow cytometry that can detect electrogenic microorganisms based on their electrical properties in real time and without markers with high precision. For this purpose, a microfluidic system was designed and manufactured. The system consists of a microchannel with three microelectrodes allowing differential measurements. The microchannel was structured on a glass wafer together with a thin PDMS layer using femtosecond laser ablation techniques, while co-planar microelectrodes were fabricated separately using gold and titanium via sputtering and photolithography. Both parts of the system were then aligned and sealed with a previously spin-coated PDMS layer (**Fig. Ia,c,d**). The impedance measurements were conducted using two microorganisms: *Escherichia coli*, a non-electrogenic model organism, and *Shewanella oneidensis*, a well-known electrogenic microorganism at 1MHz using HF2LI lock-in amplifier (from Zürich Instruments, Zürich, Switzerland). When single microorganisms pass through the detection zone, they change the impedance, which serves as a measurable signal corresponding to their electrical behavior (**Fig. IIa**).

Analysis at 1 MHz revealed different behavior between *S. oneidensis* and *E. coli*. Both bacteria contain a membrane that acts as a capacitor in the corresponding electrical circuit (**Fig. IIb**). An increase in the current value for *S. oneidensis* indicates a lower impedance of the cytoplasm compared to the surrounding physiological medium which, suggesting a short-circuited membrane (**Fig. IIc**). *E. coli*, on the other hand, showed a decrease in current at the same frequency (**Fig. IId**) which means that the total impedance is still higher than that of the surrounding medium. This comparison suggests that the membrane of the electroactive microorganism is short-circuited at lower frequency than that of *E. coli* due to extracellular electron transfer (EET).

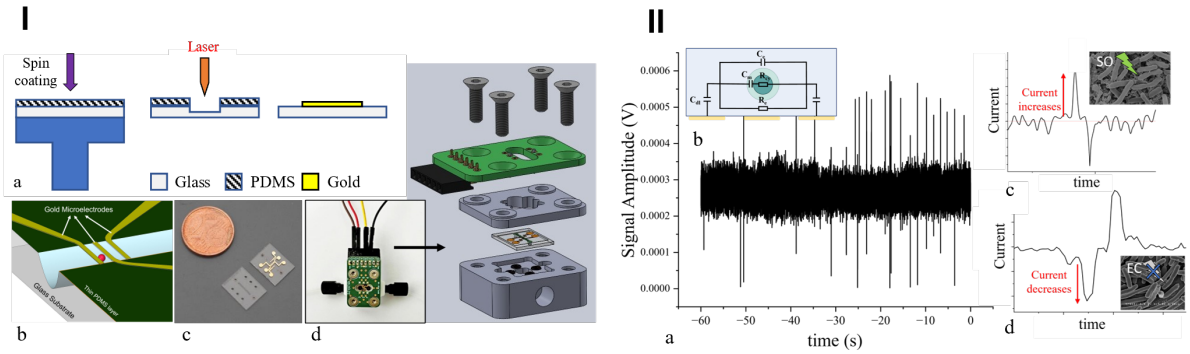

Figure: Ia) Fabrication of the microchannel and microelectrodes using femtosecond laser ablation and photolithography. **Ib)** Concept of impedance flow cytometer with the created sensing areas. **Ic)** Two separated parts of the microfluidic system and microelectrodes. **Id)** Assembled system with electric and fluidic connections. **IIa)** Signal variation showing the detection of microorganisms in an Impedance Flow Cytometer. **IIb)** Equivalent electrical circuit modelling the bacteria with membrane as a capacitor. **IIc)** Differential signal of single cell indicating the increase in the current in electrogenic *S. oneidensis* (SO) detection. **IId)** Differential signal of single cell indicating a decrease in the current in *E. coli* (EC).

References:

[1] Naradasu D, Miran W, Sakamoto M (2018). Frontiers in Microbiology 9, 3267.