Surface Enhanced Raman Spectroscopy detection of p-coumaric acid from cell supernatant using gold-capped silicon nanopillar substrates

Morelli, Lidia; Jendresen, Christian Bille; Burger, Robert; Rindzevicius, Tomas; Nielsen, Alex Toftgaard; Boisen, Anja

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The purpose of the project is to use Surface Enhanced Raman Spectroscopy (SERS) to discriminate between two different bacterial populations, based on their p-coumaric acid (pHCA) production. The pHCA concentration is measured in a droplet of diluted supernatant dried on SERS substrates, using a Raman microscope. By analyzing the SERS signal of pHCA from the supernatant, considering the peak height at the characteristic frequency (1169 cm\(^{-1}\)) it is possible to distinguish between a producing and control strain, as also confirmed by HPLC analysis.

## Aim of the Project

**SERS: fabrication and working principle**

1. **RIE Etching**
2. **O\(_{2}\) Cleaning**
3. **Au Deposition**

**Fig. 1:** Process steps for Au capped nanopillars (NPs) fabrication (courtesy of Kaiyu Wu) [1]: (a) silicon NPs are fabricated by maskless reactive ion etching (RIE); (b) \(\text{O}_2\) cleaning removes the Si RIE contaminants from the Si surface; (c) a Au metal film is deposited by e-beam evaporation. (d) When a droplet of solution is dried on the substrate, the surface tension tends to pull the Au NPs together, forming irreversible clusters and trapping the analyte molecules between the NPs. Furthermore, E-field hotspots are created when two NPs lean close to each other. (e) SEM picture of leaning pillars.

## Bacterial cultures and measurements

- **Inoculation of GM E. coli in M9 medium**
- **Incubation at 30 °C, 250 rpm**
- **Centrifugation and sterile filtration**
- **1 µL of 10-fold diluted supernatant on the substrate**
- **Droplets drying and pillars leaning**

**Fig. 2:** Bacterial culture, supernatant extraction and SERS evaluation: (a) and (b) genetically modified E. coli are cultured in triplicates [2]; (c) aliquots of bacterial solution are extracted at 0, 3, 24 and 48 h, centrifuged and sterile filtered to extract cell supernatant; (d) and (e) 1 µL of supernatant diluted 10-fold with water is dried on SERS substrates. The SEM pictures show that the pillars stand vertically before wetting, and they lean towards each other after drying. (f) 5x5 maps are acquired on the droplet area with a DDX Raman microscope (Thermo Fisher Scientific Inc.) at 780 nm, 1 mW, 10x objective, 25 µm slit.

## Salt dilution

**Fig. 3:** 100 µM pHCA spiked in culture medium diluted with M9GQ water in different ratios (1:2, 1:5, 1:10, 1:20). The signal increases with medium dilution, as higher salt concentration clogs up the active surface and decreases the signal. 1:10 dilution was chosen as a compromise between signal intensity and dilution for measurements in the supernatant.

## Validation with HPLC

**Fig. 4:** (a) Concentration of pHCA in cell supernatant for producing (P, black) and control (C, red) strains measured with HPLC. Each point is the average of 3 measurements, each one from a strain. (b) SERS signal at 1169 cm\(^{-1}\) after baseline correction. Each point in the graph is the average of 3 maps of 25 points, whereas the error bars represent the standard error of the mean, calculated on the 3 average values.

## Outlook and conclusions

In this work we demonstrated that SERS is a rapid and effective tool for qualitative screening of bacterial strains, based on the amount of synthesized secondary metabolites (e.g. pHCA). These results open up new possibilities for high-throughput quantitative analysis. Currently we are focusing on improving sensitivity by extracting pHCA in organic solvent and on integration of the assays on automated and high-throughput microfluidic platforms, such as lab-on-a-discs.

## References