Towards an Ultrasound Enhanced Assay Using Attenuated Total Reflection Infrared Spectroscopy for Detection of Bacteria in Drinking Water

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Introduction

- In this work, we present our efforts towards the combination of an acoustic trap [1,2] and attenuated total reflection (ATR) Fourier-transform infrared (FTIR) spectroscopy [3] to perform an ultrasound (US) enhanced assay for the rapid detection of bacteria in drinking water.
- By mounting an acoustic trap on top of a custom-built ATR setup, we were able to trap bacteria by relying on so-called ultrasonic radiation forces, without the need of mechanical retention elements.
- To showcase the potential of the presented setup for sensing microbial pollution in water, we monitored Escherichia coli suspensions at different concentrations.
- Experiments were performed by retaining bacteria in the acoustic trap followed by an enzyme-labeled antibody solution pumped into the cell. In the end, the bacteria-antibody conglomerate was supplied with enzyme substrate and the conversion was monitored via ATR-FTIR spectroscopy.
- Throughout the whole liquid-handling sequence bacteria were stably retained in the cell. In contrast to common direct enzyme-linked immunosorbent assays (ELISA), no immobilization of E. coli on a surface is needed.

Ultrasound Enhanced Assay

Pictures of the top view of the assembled acoustic trap (A), the bottom side (B) and trapped bacteria, enclosed in red (C).
- The acoustic trap is made of aluminum. The liquid compartment has a volume of approximately 20 µL and a height of 500 µm. Above the sample compartment a 8 mm piezo disc (US-source) is directly glued to the aluminum body.
- The assembled acoustic trap was mounted on top of the custom built ATR fixture, holding a multibounce zinc selenide ATR element (17 x 10 x 1 mm³, 45°).
- The acoustic trap was operated using a sonic amp (USEPAT, Wien, Austria) US driver, set to a US frequency of 2.20 MHz and a gain of 65%. Prior to liquid handling, the US was turned on for 30 min to allow the system to thermally stabilize.

Conclusions & Outlook

- We introduced the combination of an acoustic trap with ATR-FTIR spectroscopy, for monitoring bacterial load in water.
- The overall assay time could be reduced to 60 min and different bacteria concentrations could be distinguished.
- The presented results paves the way for ultrasound enhanced assays.
- Future efforts will focus on optimizing the overall assay procedure to measure lower concentrations of bacteria.

Experimental Setup

Schematic of the assay sequence for bacteria trapping and liquid handling (F).
- Bacteria suspension was aspirated and returned to the reservoir eight times via the syringe pump (blue) to prevent inhomogeneously in the suspension.
- 60 µL of the bacteria suspension was injected into the acoustic trap (blue).
- In the next step, 60 µL antibody-conjugate solution was injected into the cell (yellow).
- In the end, enzyme substrate was pumped into the acoustic trap and the enzymatic conversion was monitored by recording 30 consecutive mid-IR spectra.
- After successfully monitoring the reaction, the US was switched off and the acoustic trap was rinsed thoroughly with carrier solution.

Results

Mid-IR band of the antibody induced enzymatic conversion by different bacteria suspensions originating from the NO2-enrichment that shifts upon cleavage of the enzyme (G), band height used as analytical signal vs bacteria concentration (H).

- Combining the acoustic trap with a dedicated fully-automated liquid handling procedure lead to a overall assay time of approximately 60 min.
- By only relying on US radiation forces for bacteria manipulation, it was possible to distinguish between different bacteria concentrations.

References