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

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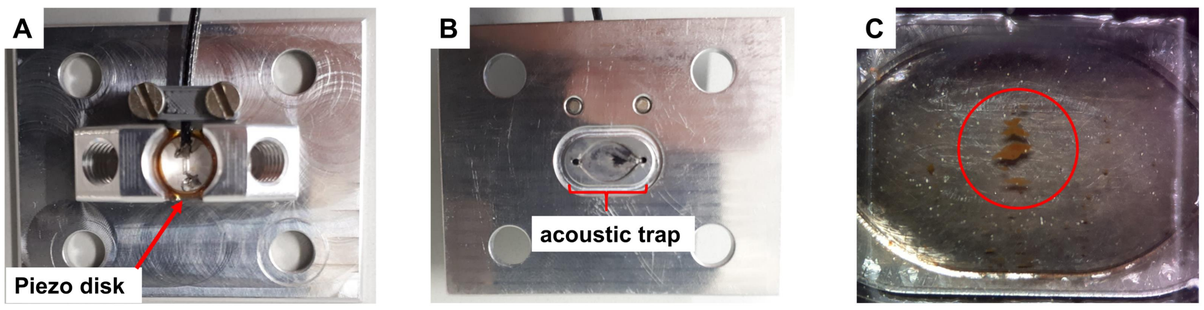
Introduction

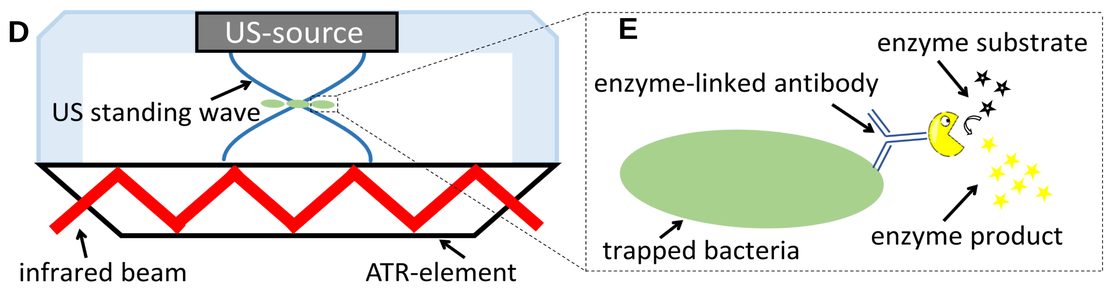
- In this work, we present our efforts towards the combination of an acoustic trap [1,2] and attenuated total reflection (ATR) Fourier-transform infrared (FTIR) spectroscopy [3] to perform an ultrasound (US) enhanced assay for the rapid detection of bacteria in drinking water.
- By mounting an acoustic 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 beads, encircled 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 a custom built ATR fixture, holding a multibounce zinc sulfide ATR element (17 x 10 x 1 mm^3 ,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.



Schematic of the acoustic trap on top of the multibounce ATR element (D), Principle of the assay performed with acoustically trapped bacteria (E).

- TRIS buffered saline pH 7.6 containing 0.5 % non-fat dry milk and 0.02 % Tween 20 was used as carrier. Antibody was diluted in the same buffer.
- 50 mM *p*-nitrophenylphosphate in 2 M diethanolamine buffer pH 9.8 was used as substrate.
- Bacteria were trapped and retained in the cell due to acoustic radiation forces. Subsequently, a solution containing an antibody labelled with the enzyme alkaline phosphatase is pumped into the cell.

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 pave the way for ultrasound enhanced assays.
- Future efforts will focus on optimizing the overall assay procedure to measure lower concentrations of bacteria.

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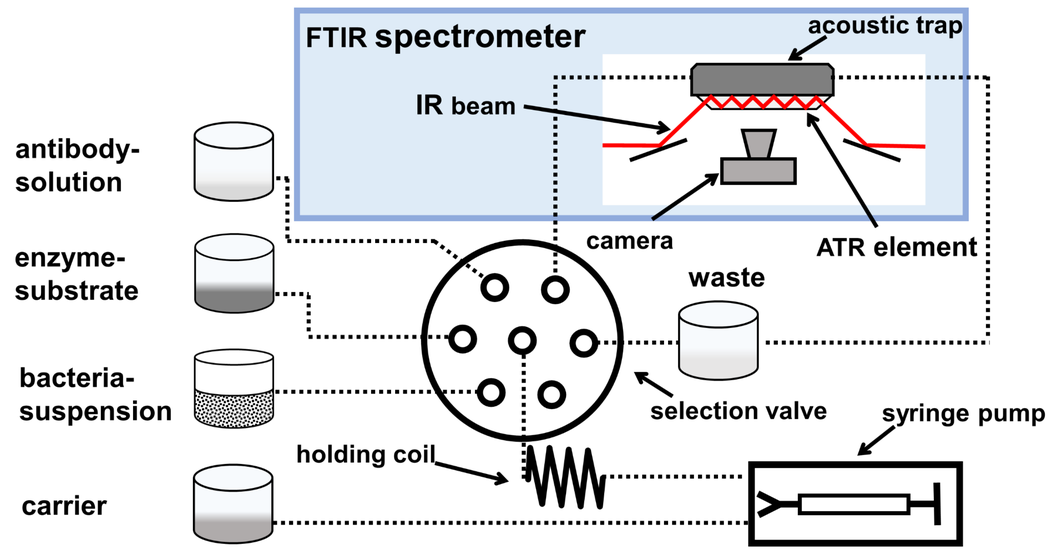
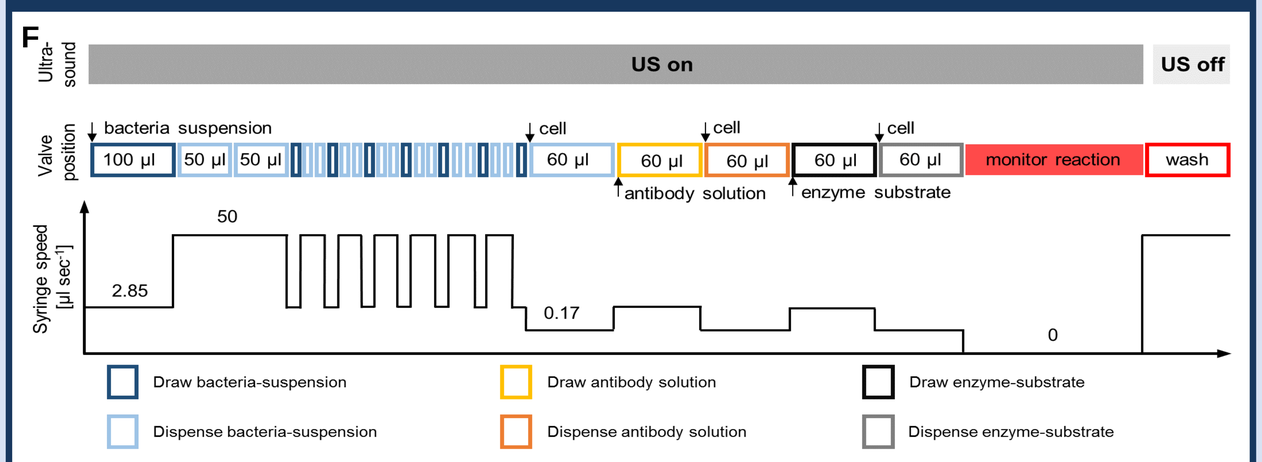
Experimental Setup



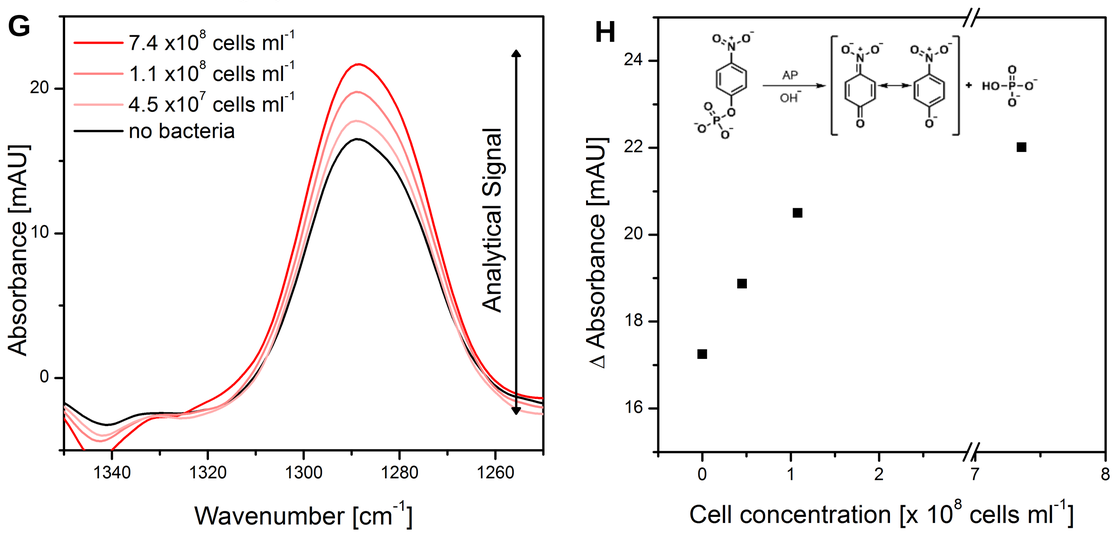
- ATR measurements were performed by guiding the IR beam of a FTIR spectrometer (Vertex 70v, Bruker Optics) through a custom-made multibounce ATR setup with the acoustic trap on top.
- Automated liquid handling is performed via a sequential injection analysis manifold consisting of a selection valve and a syringe pump.

Results



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 inhomogeneity 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.



Mid-IR band of the antibody induced enzymatic conversion by different bacteria suspensions originating from the NO_2 moiety 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.

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[2] Freitag, S., Baumgartner, B., Tauber, S., Gasser, C., Radel, S., Schwaighofer, A. and Lendl, B. Analytical Chemistry **91**, 7672-7678 (2019).

[3] Ramer, G. and Lendl, B., Attenuated total reflection Fourier transform infrared spectroscopy, John Wiley & Sons, Ltd., (2013).