Mid-infrared photothermal spectroscopy in aqueous media

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Background – Photothermal spectroscopy (PTS) leverages site-specific changes in the refractive index upon the absorption of infrared (IR) radiation to analyse the chemical properties of a sample. Given that the refractive index of a material changes with temperature, sample molecules irradiated by a (mid-)IR pump beam will create a refractive index profile, which is detected by a second laser beam. The pump laser wavelengths exploit the strong absorption capacity of biological tissues, while the probe laser wavelength is usually in the visible (VIS) spectrum to achieve sub-micron spatial resolution.

The combination of high-resolution imaging and spectroscopy makes mid-IR PTS a particularly suitable tool for the examination of biological specimens. In addition, Quantum Cascade Lasers (QCLs) can be tuned to very narrow IR wavelength bands to target specific molecular vibrations. This allows for the highly sensitive and label-free detection of different cell constituents and offers a huge potential for identifying different cell and tissue types.

Photothermal experiments on biological specimens are rare, especially in aqueous media. Transmission imaging examples include single preadipocyte cells (cf. [2]) and cancer cells in saline solution (cf. [5]). Measurements in a backscattering configuration were performed on ovarian cancer cells (cf. [1]) as well as bacteria and fungal cells (cf. [4]).

As forward scattering becomes increasingly dominant with increasing size of the scatterer (cf. [3]) and sub-cellular structures are hundreds of nanometres long, a transmission geometry is expected to provide the strongest photothermal signal. Moreover, backscattering setups have made use of highly reflective substrates and are not deemed suitable for imaging of samples in aqueous environments.

Based on the above considerations, a mid-IR PTS instrument was developed and optimised for the broadband-IR measurement of sub-micron structures. The technique will be evaluated with regard to its potential for the analysis of samples in aqueous media or organ-on-a-chip platforms.

Methods – To optimise the mid-IR PT setup for broadband IR laser sources, simulations were carried out in python and FRED (Photon Engineering, https://photonengr.com/fred-software) to test critical components.

A schematic of the mid-IR PTS setup is illustrated in Fig. 1. The visible laser beam (light blue line) is produced by a 633 nm diode laser, while the infrared beam source (red line) is a 4-chip EC-QCL tuneable between 3.4 µm and 11.2 µm (covering marker bands for proteins, lipids, DNA/RNA and carbohydrates). Both beams are combined using a custom beam splitter (BS) and directed towards a reflective objective (Ealing, 36x, focal length: 5.4 mm, NA: 0.5). The sample holder has an IR-transparent window on its bottom and an IR-opaque window on its top to filter out remaining IR pump radiation. The photothermal signal is collected by a visible objective (Nikon CF IC Plan ELWD, focal

length: 5.4 mm, NA: 0.55). After passing a pinhole, the PT signal is read out by a silicon photodiode detector (Thorlabs) and processed by a lock-in amplifier (not shown).

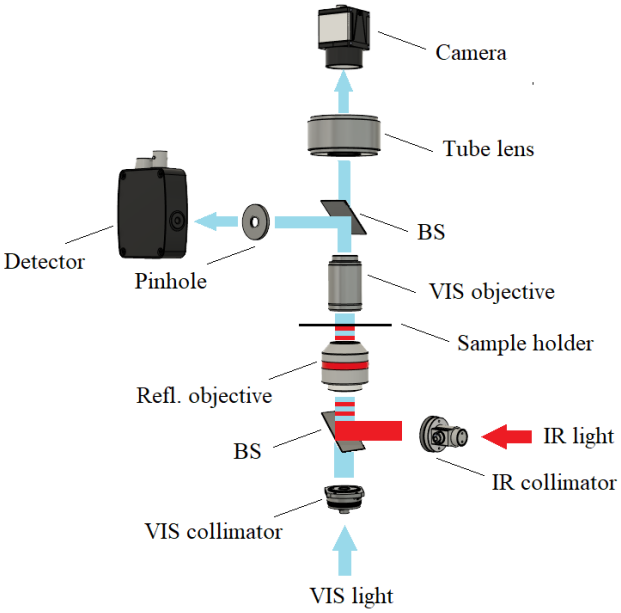

Fig. 1. Experimental PTS setup. The blue line illustrates the visible beam, the red line the IR beam. BS - beam splitter.

To test the sensitivity of the PTS setup and its ability to accurately reproduce absorption features, sub-micron-sized polystyrene beads will be analysed both with the PTS setup and a commercial FTIR microscope (Bruker Hyperion 3000). To test the suitability of mid-IR PTS for probing sub-micron structures in aqueous solutions, the beads will also be measured in saline before being inspected with the PTS setup.

Results and Conclusions – In this work, a PTS mid-IR chemical imaging setup is designed and optimised for the analysis of cells in liquid. In contrast to the conventional spectrometer, the PTS microscope does not only provide better resolution, but is also able to measure test beads when they are immersed in aqueous media (saline). Moreover, the experimental PTS setup covers a large fraction of the mid-IR spectral range.

References

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