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## Enhanced Tri-modal Optical-Photothermal Infrared (O-PTIR) Spectroscopy – Advances in Spatial Resolution, Sensitivity & Tri-modality (IR, Raman & Fluorescence)

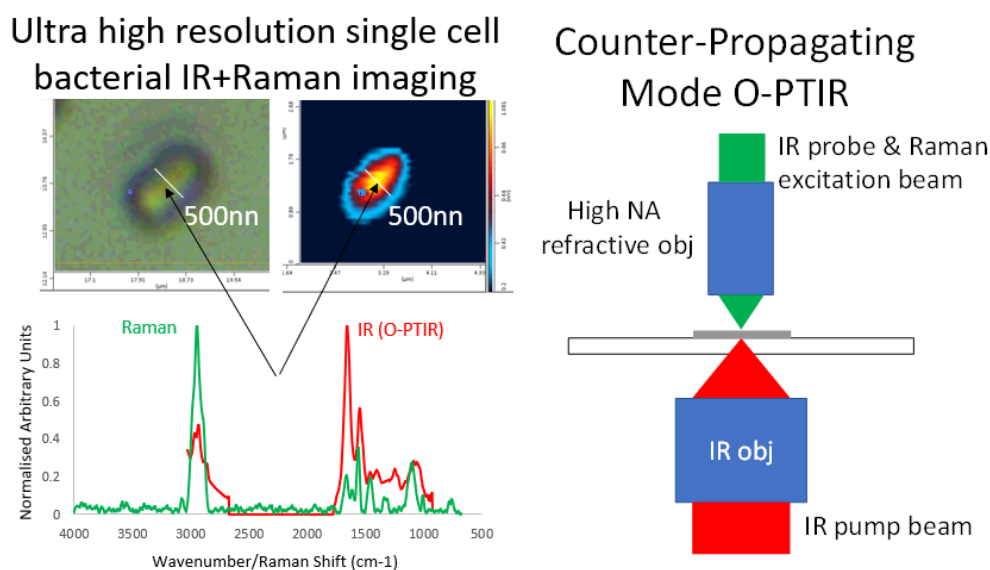
Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a cutting edge vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopic tools, providing submicron simultaneous IR+Raman microscopy, in non-contact mode with high sensitivity. The ability to collect, for the first-time submicron IR spectroscopic data in an optical microscope has enabled new research outcomes across a range of application fields, such as life sciences (cells, tissues, bacteria), polymers, cultural heritage and microplastics.

A new modality, “counter-propagating” has been engineered to provide for enhanced IR (and Raman) spatial resolution and sensitivity, through decoupling the need for a reflective objective. The IR pump beam can now be directed to the sample via the underside, thus allowing the collection objective for the visible probe (and Raman excitation beam) to be a high-NA refractive objective. This improves spatial resolution to ~300nm for both IR and Raman, whilst improving sensitivity, image quality and facilitating immersion objective studies.

To further integrate vibrational spectroscopic tools into life science workflows, we coupled widefield epifluorescence to facilitate a novel concept – fluorescence guided (or fluorescence co-located) O-PTIR microspectroscopy. Rather than, or in addition to the visible image, the fluorescence image can now be used to guide the user to the region of interest, thus combining the well-established specificity of fluorescence imaging with the broad macromolecular profiling capabilities of IR spectroscopy

Several life sciences examples from bacteria, cells and tissues will be provided to demonstrate these new capabilities and how they can enable new experiments and research findings..

### Figures



**Figure 1:** Left: Single E.Coli cell imaged in counter-propagating mode with 50nm pixel/step size and submicron simultaneous IR+Raman spectra from centre of bacterial cell. Right: Schematic of counter-propagating layout.