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Traditional IR vs Raman Spectroscopy — and how to carry out both simultaneously

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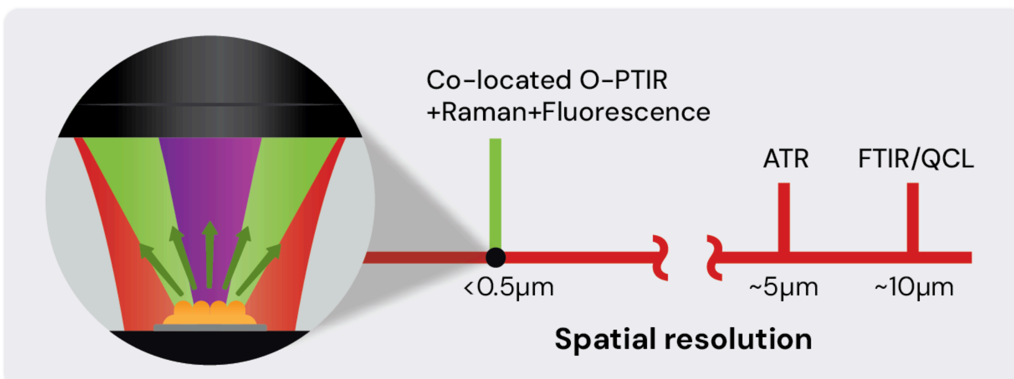

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Infrared (IR) and Raman microscopy are foundational systems in modern molecular analysis, each providing unique performance capabilities and insights into chemical structures. By merging these capabilities through Optical Photothermal Infrared (O-PTIR) technology, researchers can achieve simultaneous submicron IR and Raman spectroscopy, breaking through the constraints of *traditional systems, such as FTIR and QCL-based systems. This advancement empowers researchers to capture submicron chemical details with unparalleled precision. Let's explore the individual strengths of these methods and uncover the transformative potential of their integration.*

Understanding the Basics: Traditional FTIR and Raman Microscopy

Both FTIR and Raman microscopy provide molecular fingerprints of samples by analyzing vibrational modes, but they do so in fundamentally different ways:

- Infrared (IR): IR spectroscopy works by absorbing IR light when the vibration of a bond causes a change in the dipole moment of that bond. Thus, IR spectroscopy is especially sensitive to molecules with polar bonds. This process reveals information about functional groups and bond vibrations, making it highly effective for identifying organic compounds. However, traditional IR systems are IR diffraction-limited, with spatial resolutions constrained from several to ~15 microns.
- Raman: Raman spectroscopy likewise measures molecular vibrations but differs in that it measures the inelastic scattering of light caused by molecular vibrations that cause a change in molecular polarizability. Thus, conversely to IR, Raman in general is more sensitive to non-polar bonds. This fact is the basis for the complementarity of the two techniques. Raman furthermore excels in analyzing inorganic and symmetric molecules, offering high spatial resolution and compatibility with aqueous environments. Yet, Raman often suffers from poor spectral sensitivity and fluorescence interference, particularly when studying biological samples or complex materials.

The Key Differences between Traditional Infrared and Raman

Spatial Resolution:

- Traditional IR systems are diffraction-limited to several to ~15 microns, while Raman microscopy can achieve better resolutions, often approaching the submicron level.
- O-PTIR technology bridges this gap by enabling submicron IR analysis and overcoming diffraction limits through the detection of these photothermal effects with a short wavelength visible probe beam.

Sample Preparation:

- Traditional IR microscopy typically does not work well in reflection mode where it can generate spectral artefacts, thus this usually requires the preparation of either thin (<10 micron) samples for transmission mode measurement or requires the use of a contact accessory – Attenuated Total Reflectance (ATR), which is typically composed of a diamond or Germanium crystal that must be in contact with the samples. Such requirements for contact run the risk of sample and/or ATR crystal damage or cross-contamination from multiple contacts.
- Raman microscopy, on the other hand, often works in reflection mode and requires little to no sample preparation.

Complementary Strengths:

- IR is ideal for detecting the more polar functional groups, while Raman provides complementary insights into the more non-polar bonds.

Limitations:

- Traditional IR has poor spatial resolution, spectral artefacts, especially in reflection mode, and suffers from strong water absorbances, making it less suited to samples in an aqueous environment. The main issues with Raman are its extreme susceptibility to fluorescence interference and its relatively poor spectral sensitivity, which necessitates longer (slower) measurement times.

Overcoming Limitations with O-PTIR

Combining the strengths of IR and Raman in a single platform offers numerous benefits, including:

- Submicron Spatial Resolution: O-PTIR overcomes the IR diffraction limit by exciting the sample with an IR laser (QCL) but detecting the result of this excitement (local subtle heating) by a secondary, short wavelength visible probe beam, which then defines the diffraction limit, thus enabling IR analysis at submicron scales.
- Simultaneous IR and Raman Spectra: This technology allows researchers to collect IR and Raman data from the exact same point, at the same time with the same spatial resolution on a sample, delivering a comprehensive chemical profile without repositioning, alignment or registration issues.
- Non-Destructive Analysis: O-PTIR performs label-free and non-contact measurements, preserving the sample's native state while providing detailed molecular insights.

Applications of Combined IR and Raman Microscopy

1. Biopharmaceutical Research: Simultaneous IR and Raman analysis enables detailed characterization of protein structures, aggregates, and excipients. For example, O-PTIR can differentiate α-helix and β-sheet structures in therapeutic proteins, providing critical data for drug formulation.
2. Microplastics Detection: O-PTIR's ability to provide chemical ID for submicron particles makes it invaluable in identifying and mapping microplastics in environmental samples, enhancing efforts to monitor and mitigate pollution.
3. Materials Science: In polymer research, simultaneous IR and Raman spectra offer insights into both chemical composition and structural properties, aiding in developing advanced materials.
4. Cellular Imaging: O-PTIR's co-located fluorescence microscopy and vibrational spectroscopy allow researchers to study biochemical distributions within cells, enabling breakthroughs in disease research and drug development.

Why Simultaneous IR and Raman Matters

Traditionally, researchers had to choose between IR and Raman microscopy or use separate instruments, often resulting in misaligned data, incomplete analysis or even lost samples during movement between the two different instruments. The integration of these methods through O-PTIR eliminates this trade-off offering:

- Efficiency: Faster workflows by collecting complementary data in a single session.
- Accuracy: Co-located measurements ensure that IR and Raman spectra are acquired from the same region of interest.
- Versatility: Applicable across a wide range of industries and sample types, from biological tissues to synthetic materials.

Enhancing Molecular Analysis with O-PTIR

Combining submicron IR and Raman microscopy (https://www.photothermal.com/products/mlrage-r/) marks a significant leap forward in chemical imaging. Photothermal's O-PTIR technology is at the forefront of this innovation, enabling researchers to tackle complex challenges with unparalleled precision and efficiency. Whether you're developing life-saving drugs, studying environmental contaminants, or designing new materials, O-PTIR offers the tools you need to succeed.

Ready to see how simultaneous IR and Raman spectroscopy can transform your research? Contact us today to learn more about our groundbreaking solutions and how they can elevate your work.

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