PHOTOTHERMAL
SPECTROSCOPY CORP

Widefield O-PTIR™

Widefield fluorescence-detected sub-500µm Infrared multimodal microscopy

Widefield O-PTIR chemical and simultaneous fluorescence measurement offers:

- Imaging of fluorescently labeled, and autofluorescent samples
- O-PTIR imaging of unlabeled & IR tagged samples
- Single wavelength chemical images in seconds and hyperspectral arrays in minutes
- Dynamic chemical imaging up to 5 frames per second
- Co-located fluorescence microscopy and sub-micron IR spectroscopy

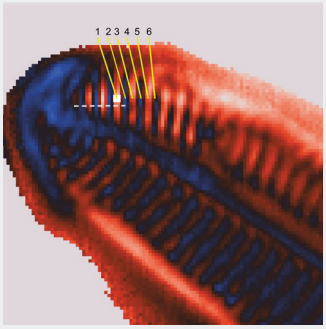

Fig. 2 Diatom Image: Image ratio at 1200/1072 cm-1 with a 80 cm-1 peak integration width. White box shows size of 260 nm 2x2 pixel ROI used to calculate spectra in figure 5. Dashed line shows approximate location of cross-section measurement in figure 3.

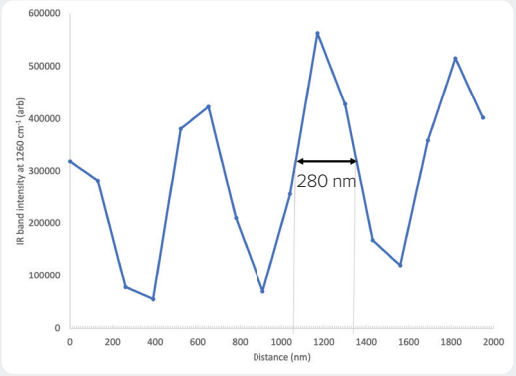

Fig. 3: Demonstration of sub-500 nm spatial resolution. Plot shows intensity of the white dashed line figure 2.

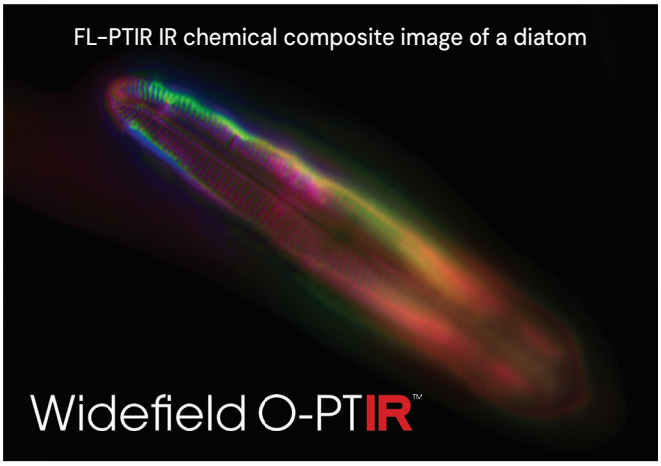

Fig. 1: MCR-ALS composite image created in CytoSpec. Diatom courtesy of Prof. K Gough, University of Manitoba. Refer to figure 4 for spectra.

Widefield fluorescent-infrared microscopy with sub-micron O-PTIR spatial resolution

Widefield O-PTIR is powered by fluorescence-detected photothermal infrared (FL-PTIR), a patented1 novel widefield chemical IR imaging approach based on a combination of Optical Photothermal Infrared (O-PTIR) and fluorescence microscopy. FL-PTIR uses changes in fluorescent emission intensity from a sample to detect localized, chemically specific IR absorption at over 260,000 spatially defined locations (pixels) on a sample instantaneously while providing simultaneous and co-located widefield fluorescence imaging. This combination leverages the advantages of both techniques for a wide range of cellular and tissue imaging applications.

Widefield O-PTIR™ is a new sub-micron IR, high-speed widefield chemical imaging mode for measurement of a wide range of labelled and auto fluorescent samples covering cellular, bacterial and tissue applications.

Autofluorescent, label-free chemical imaging

Autofluorescence has gone from being a major interferent to a major enabler and sensitivity enhancer. Samples with high autofluorescence are ideal, as they can support rapid Widefield O-PTIR chemical imaging without adding exogenous fluorophores (e.g., fluorescent dyes/proteins).

Label-free, fluorescently labeled, autofluorescent, IR tags

The possibilities for life science measurements are endless. Widefield O-PTIR with its multi-modal capabilities, (O-PTIR, FL-PTIR, Raman, & Epifluorescence), enables measurement across a wide range of life science sample types, whether fluorescently labeled, unlabeled or autoflourescent samples. Additionally with new Hyperspectra QCL laser developments in the silent region (2300-2000 cm-1), Widefield O-PTIR can also exploit the power of small molecule IR labels or tags, further broadening the capabilities for multi-modal vibrational spectroscopy in life science research.

The most comprehensive vibrational spectroscopy platform for life science research

The mIRage-LS with Widefield O-PTIR offers unique vibrational spectroscopy and microscopy capabilities with O-PTIR, integrated fluorescence microscopy, and optional Raman spectroscopy. Both high speed widefield IR chemical imaging and high speed single spectra capabilities are enabled in an easy-to-use optical microscope-based platform.

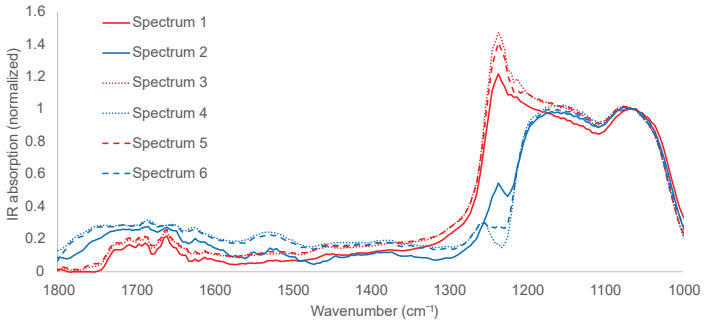

Fig. 4: IR absorption spectra from 260 nm regions of interest with positions indicated in figure 2. Note both the substantial spectral variation over 260 nm length scales and spectral similarity in the alternating regions of the periodic structures (striae) in the diatom frustule. Spectra are normalized to 1072 cm-1.

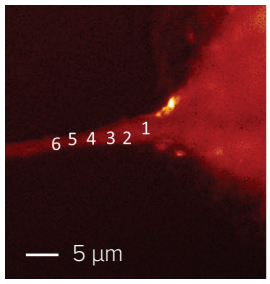

Fig. 5: Collagen fibril, FL-PTIR autofluorescence image. Sample courtesy of K. Gough. University of Manitoba.

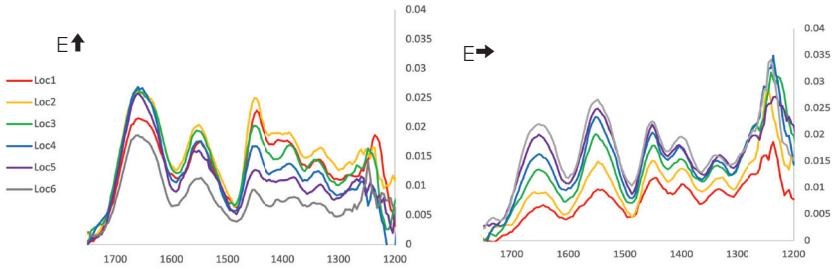

Fig. 6: FL-PTIR spectra extracted from along the collagen fibril in figure 5. Spectra were collected with vertical (top) and horizontal (bottom) IR polarization orientations.

System specifications

Widefield imaging area:	Standard Range 65 µm x 65 µm (with 50x, 0.8NA objective). 262,144 total pixels (512x512) at 130 nm pixel size. Note that pixel size and field of view are objective magnification dependent.
Imaging speed:	Typical imaging speeds are application dependent, with single wavelength imaging speeds in seconds, resulting hyperspectral images created in minutes. Single IR wavelength dynamic imaging up to 5 FPS.

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