Multi-modal Submicron IR (O-PTIR) Spectroscopy and Imaging

PHOTOTHERMAL SPECTROSCOPY CORP

Advances in Spatial Resolution, Sensitivity & Label Free Multi-modality (IR, Raman & Fluorescence)

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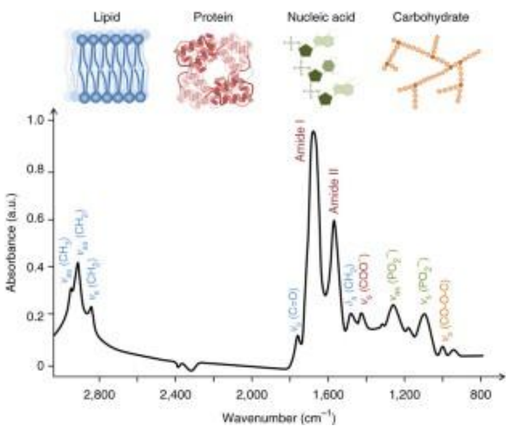
Abstract

- Vibrational spectroscopic techniques such as Infrared (IR) and Raman spectroscopy hold great promise as label free imaging and broad chemical characterizing techniques
- Sub-micron IR and simultaneous Raman now allows for true complementarity of these two techniques: Same Spot, Same Resolution, Same Time
- Now with integrated widefield epi Fluoresence for co-located Fluoresence and OPTIR with no image registration issues – be guided by the FL image
- New "Counter-Propogating" modes have significnalty improved IR spatial resolution to <500nm and sensitivity

What does IR spectroscopy bring to life science research?



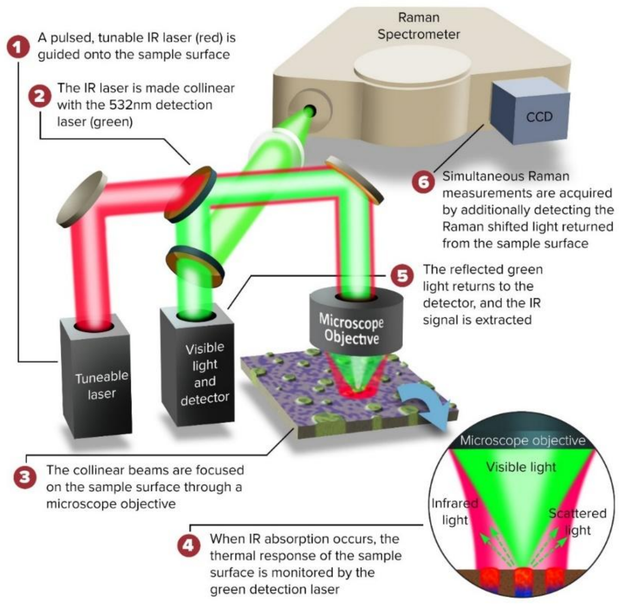
- Spatial distribution of biochemistry

(e.g macromolecules such as proteins, lipids, Nucleic Acids, Carbohydrates as well as metabolites and drugs)

- Relative concentration information, spatially
- Non-destructive analysis, down to the single bacterial cell, subcellular level (<500nm)

Submicron simultaneous IR and Raman – A world first

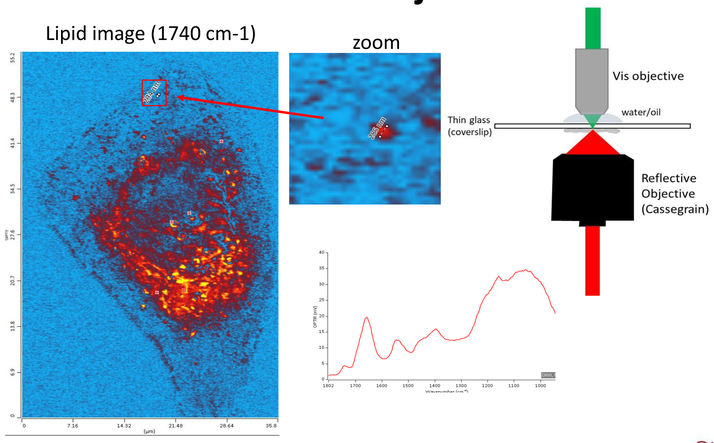
IR+Raman
- Same spot (co-registration)
- Same time (simultaneous)
- Same resolution (submicron)



O-PTIR advantages vs traditional FTIR/QCL

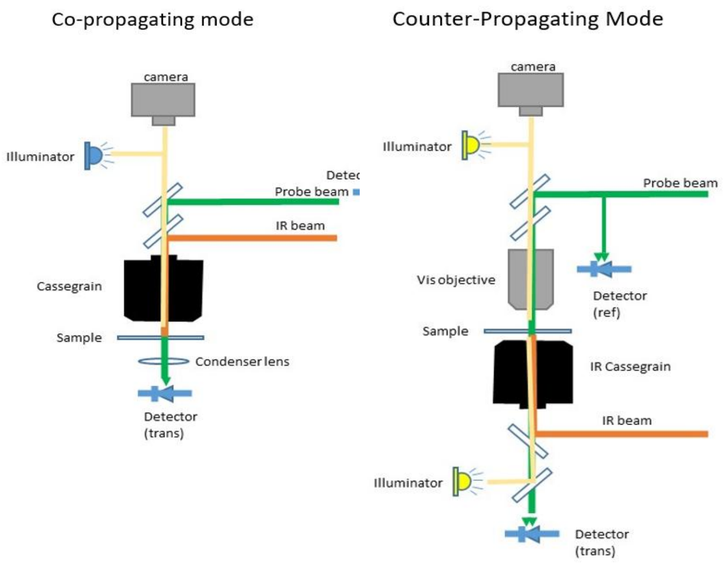
- Sub-micron IR spectroscopy and imaging
- Non-contact reflection-based IR measurement with FTIR transmission-like spectral quality
- No IR spectral artifacts like Mie/diffuse scattering or specular reflection
- No sample thickness requirements
- Measure in live cells in water

O-PTIR of fixed cell with oil immersion obj.



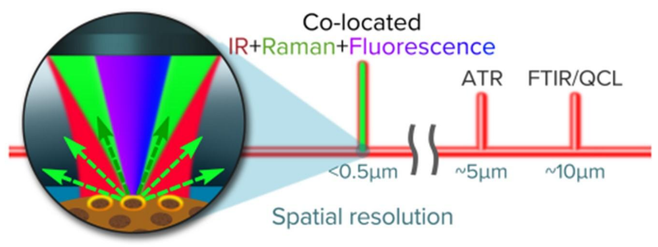
- Cheek cells on glass coverslip (170nm)
- Oil immersion objective, 100x, NA 1.30
- Spectra collected in seconds
- Images in minutes
- Images collected in single freq mode with 50nm steps size
- Probe beam detected in reflection mode
- Spatial resolution of ~285nm

New Optical Modes



- Allows use of high quality high power refractive (glass) objectives for data collection (inc. IR)
  - 100x, 0.9NA dry
  - Water/oil immersion objectives (NA up to 1.4)
  - Water dipping objective
  - LWD objectives for use with 1mm thick glass slides
- Better spatial resolution
- Better SNR
- Better imaging

mIRage-LS: New 500nm IR with simultaneous Raman and co-located Fluorescence



mIRage®-LS

- Co-located Fluorescence microscopy and sub-micron IR spectroscopy
- <500nm IR and Raman spatial resolution
- Simultaneous IR and Raman spectroscopy
- Non-contact reflection-based IR measurement with FTIR transmission-like spectral quality
- No IR spectral artifacts like Mie/diffuse scattering or specular reflection
- Hydrated cell imaging (IR, Raman and Fluorescence)

Live cell imaging of isotopically labelled lipids in water

Spatiotemporal Heterogeneity of De Novo Lipogenesis in Fixed and Living Single Cells

Published as part of The Journal of Physical Chemistry virtual special issue "Early-Career and Emerging Researchers in Physical Chemistry Volume 2".

Sydney O. Shuster, Michael J. Burke, and Caitlin M. Davis*

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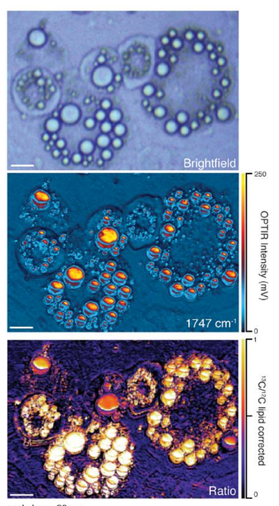


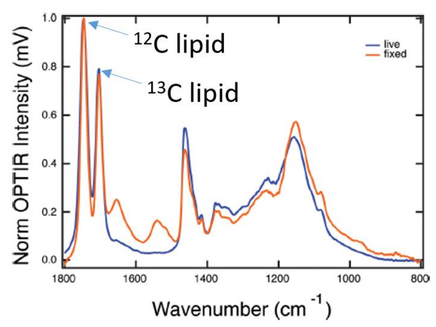
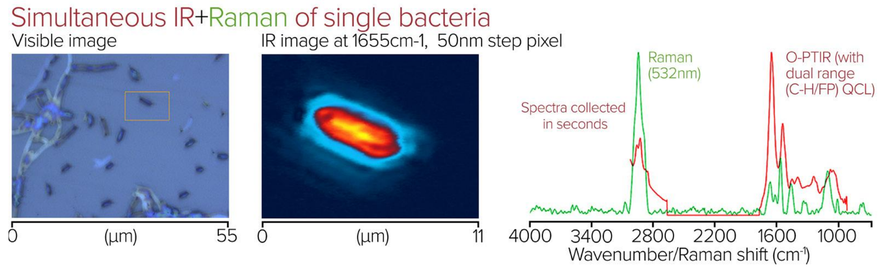


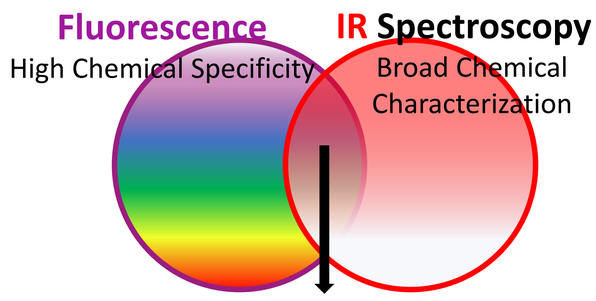
Figure 7. Representative spectra from live and fixed cells. Spectra from live (blue) and fixed (orange) differentiated 3T3-L1 adipocytes 72 h after feeding with ^{13}C glucose. Data normalized to the ^{12}C lipid ester carbonyl.

- Do novo lipogenesis is a critical metabolomic process, that is difficult follow with traditional tools, because lipids are challenging to label with FL
- Labelled (13C) glucose was fed to monitor uptake and conversion to triglycerides and incorporation into lipid droplets
- Traditional IR (FTIR/QCL) cannot measure in water, with O-PTIR the water background is limited with better SNR than Raman

Simultaneous IR+Raman of single bacteria

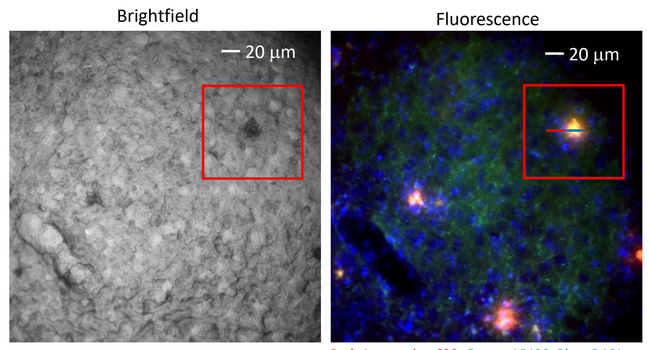


Co-located Fluorescence & sub-micron O-PTIR

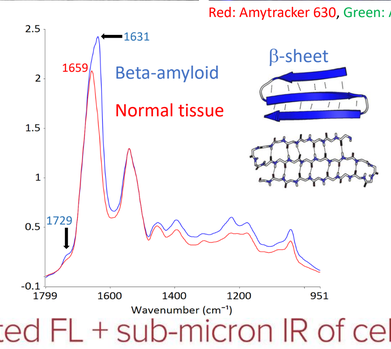


Co-located Fluorescence and sub-micron O-PTIR

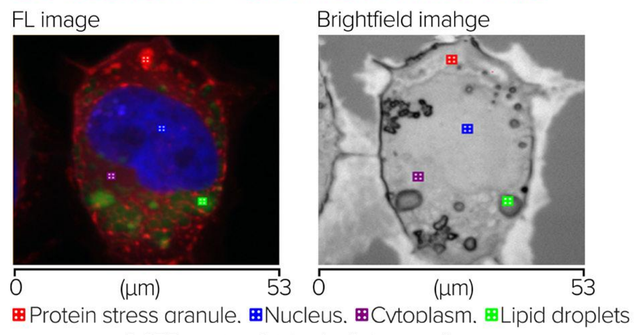
Using fluorescence to localize O-PTIR measurements



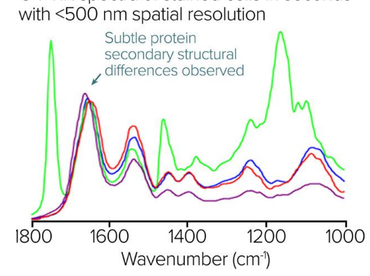
Red: Amytracker 630, Green: AF488, Blue: DAPI



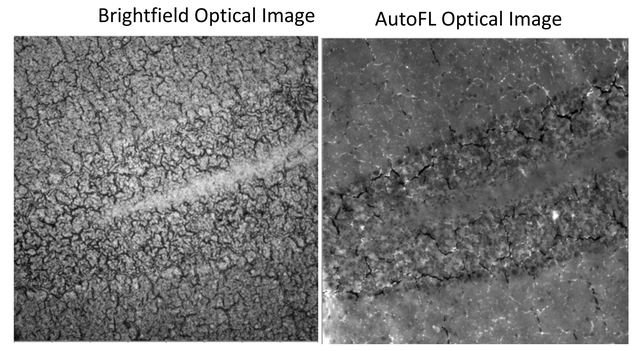
Co-located FL + sub-micron IR of cells



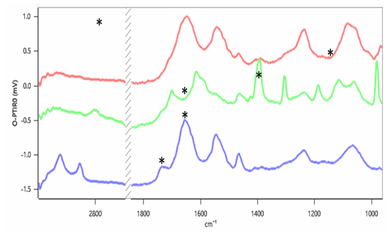
O-PTIR spectra of stained cells in seconds with <500 nm spatial resolution



Mouse Brain tissue section - unlabelled



Red: Nucleic acid (1080/1140cm^{-1}), Green: Creatine (1404/1655cm^{-1})
Blue lipid (1738/1655cm^{-1})



- Limited brightfield and AutoFL contrast
- Detailed, label free biochemical contrast images and spectroscopy collected in seconds and minutes

Summary

- O-PTIR takes IR microscopy beyond known traditional and accepted limits, essentially combining the best of IR and Raman and Fluorescence into a single platform
  - Co-located Fluorescence and sub-micron IR spectroscopy
  - Particle shape/size independent IR spectra for more accurate ID
  - Non-contact (reflection mode) measurement
  - Glass substrate suitable
- IR+Raman - Submicron simultaneous IR and Raman
  - Same spot
  - Same time
  - Same spatial resolution