FTIR Microscopy

DR. JAMES BURGESS

About JASCO

- Founded in 1958 by Yoshio Fujioka and **Shinichiro Tomonaga** (Nobel Prize winner, QED).

- US Application Scientists:
  - 6 PhD Scientists
  - 2 Vibrational Spectroscopists

DS-301 Infrared Spectrophotometer

JASCO Spectroscopy Expertise

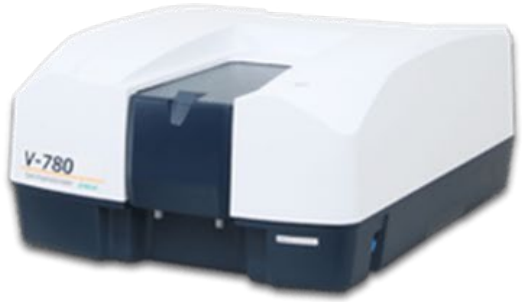
FT/IR

Raman Microscopy

ECD/VCD

IR Microscopy

UV-Vis



Fluorimetry

FTIR Microscopes: IRT-5000/7000 Series

FT/IR-6700 with IRT-5200

IRT 5100 – RT detector
IRT 5200 – MCT detector
IRT 7100 – MCT Detector
IRT 7200 – Linear Array Detector

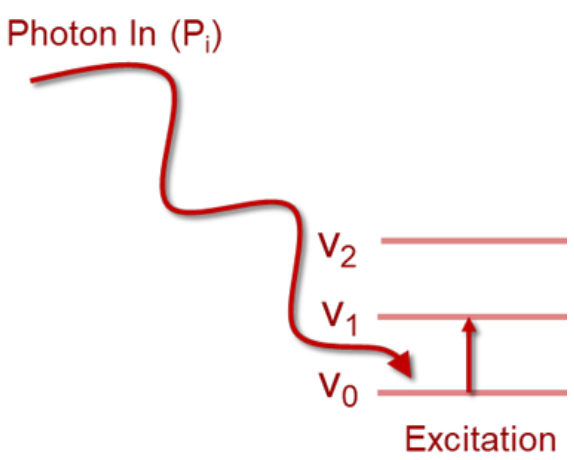
Outline

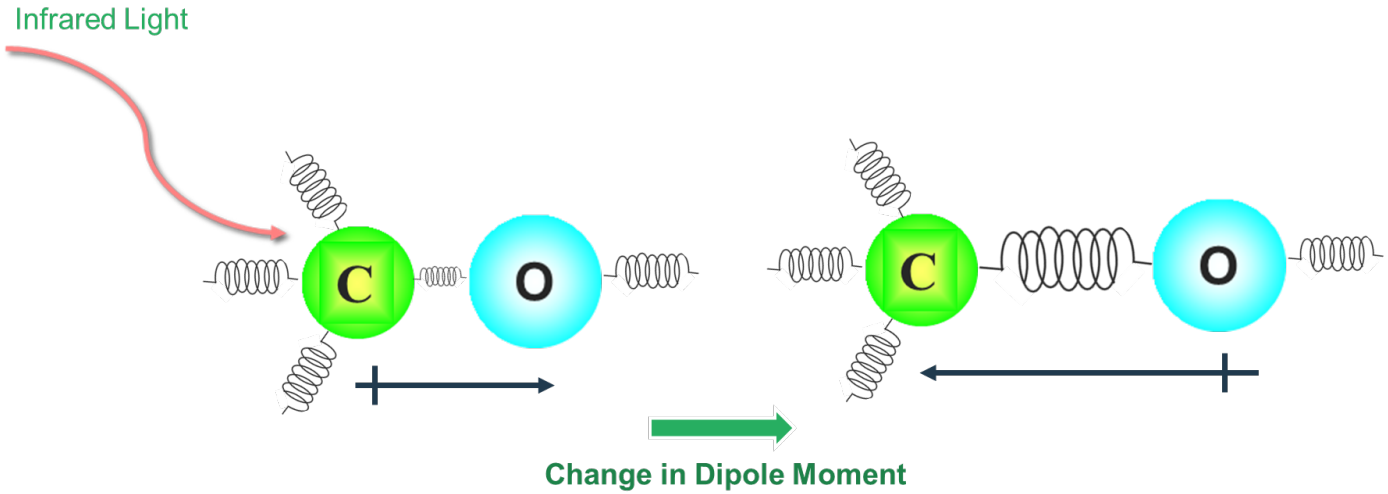
- Introduction
- Theory and Instrumentation
- Sample Preparation
- Techniques
- Complementary Microscopic Techniques
- Imaging Software

FTIR Theory and Instrumentation

Infrared Transitions

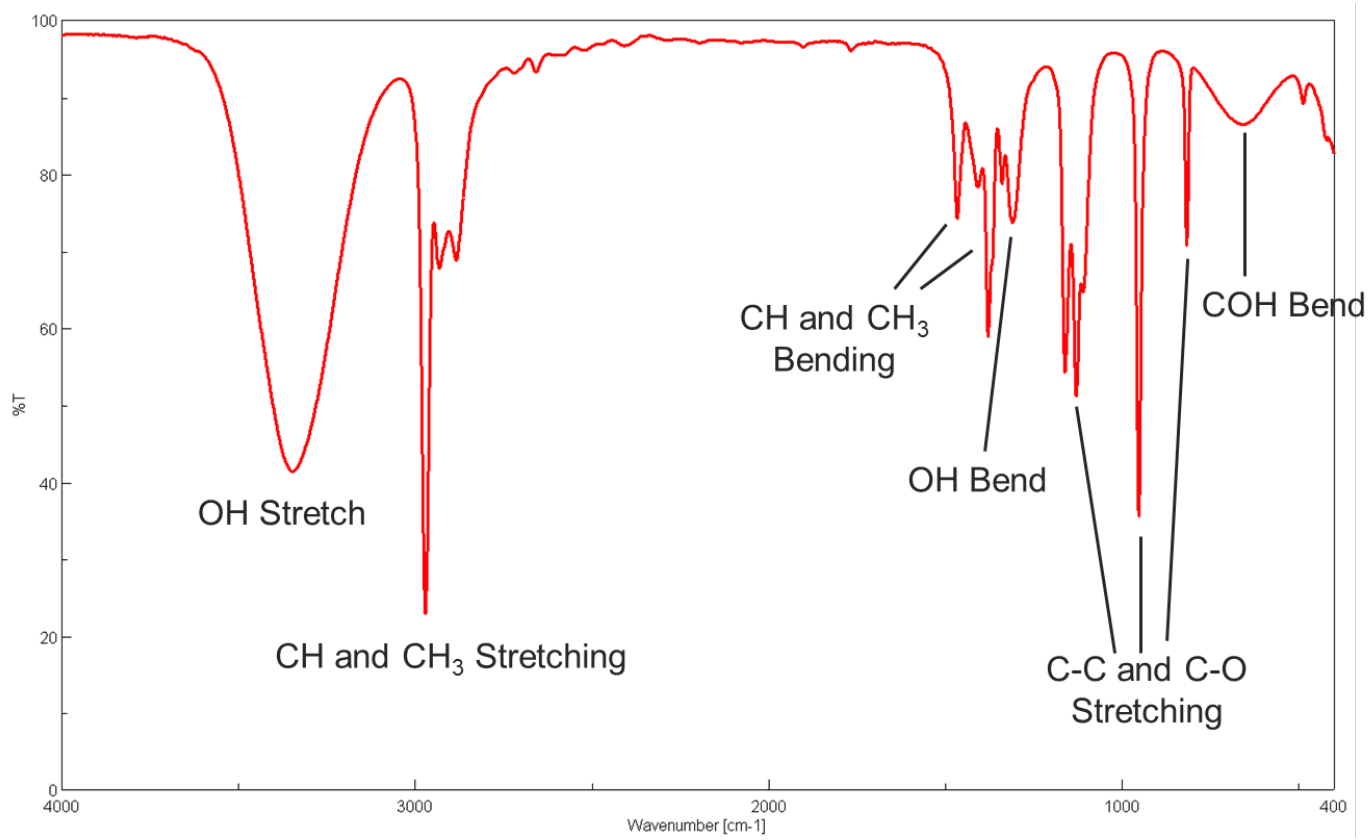
Infrared photons excite molecules through absorption. When a molecule absorbs infrared light, a change in dipole moment occurs causing a vibration within the molecule.



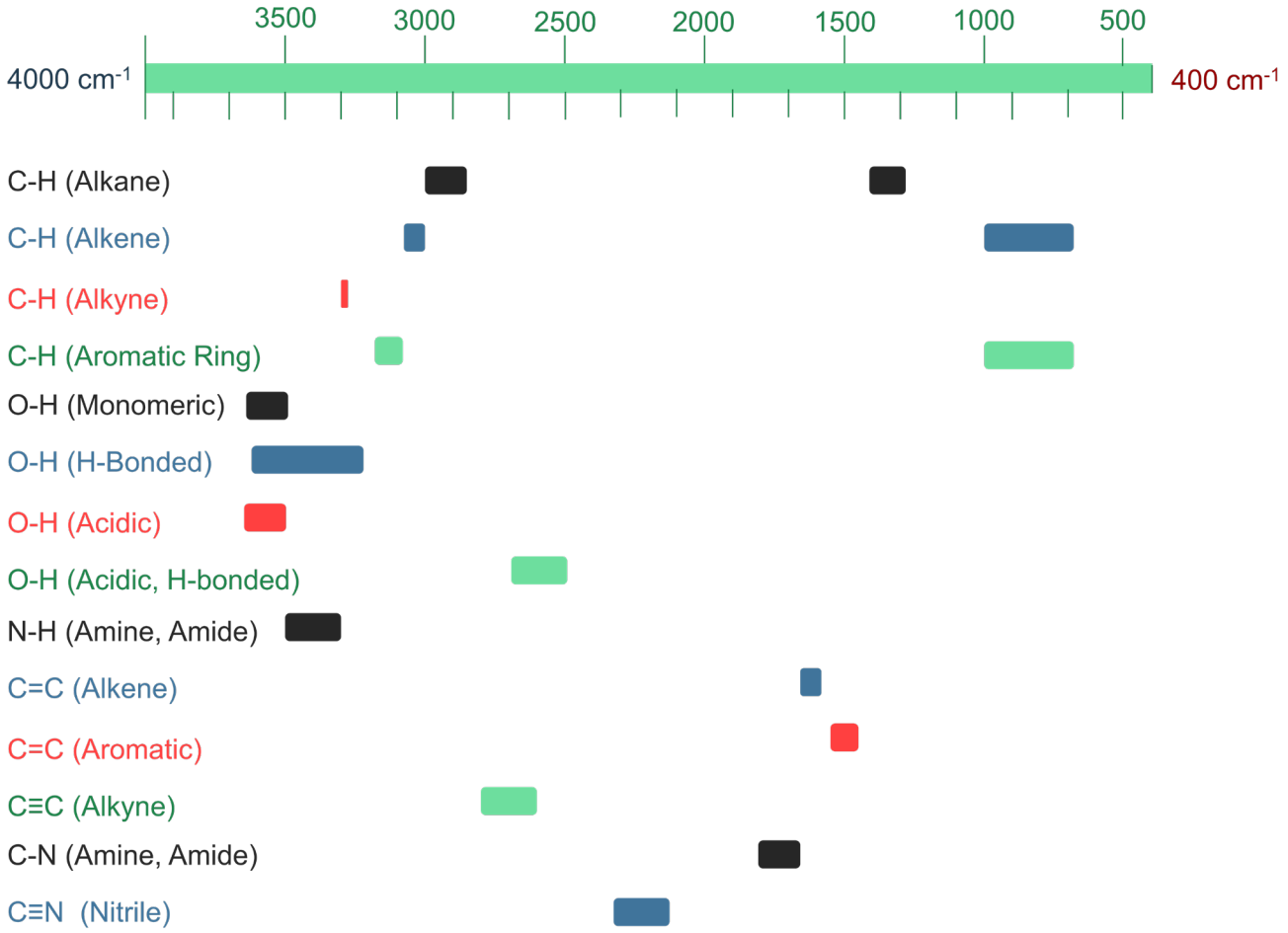


Number of vibrations in a non-linear molecule = 3N-6

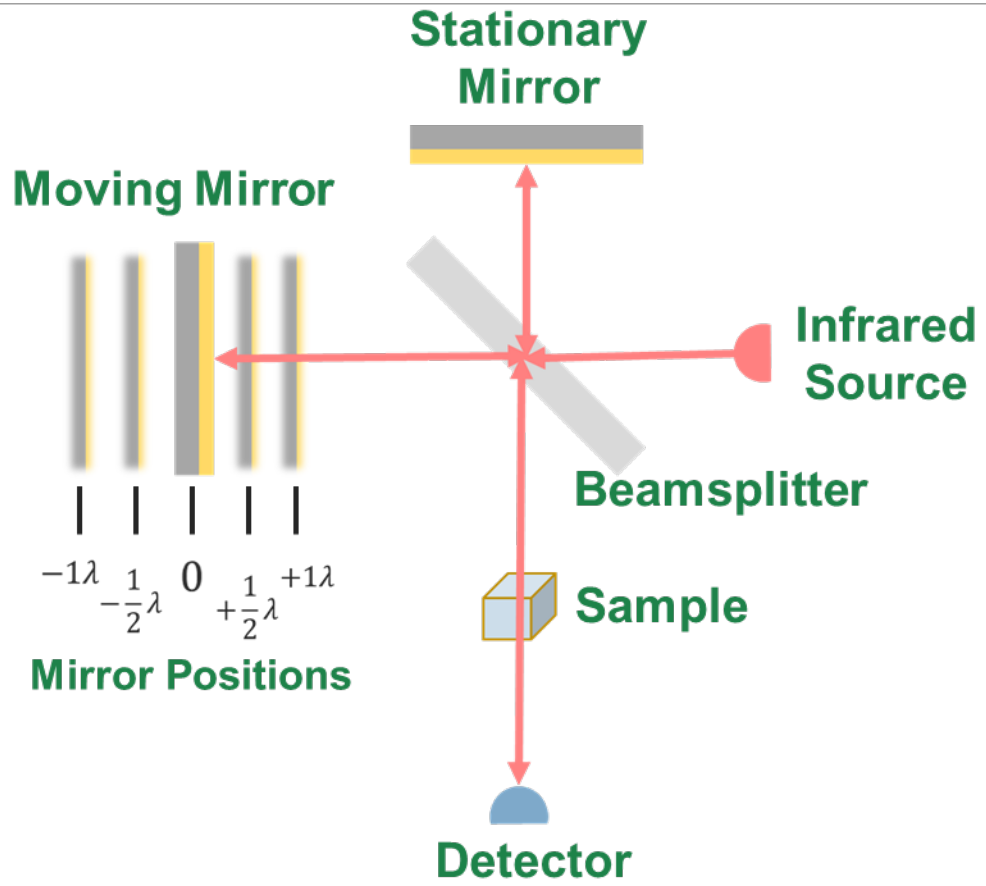
Infrared Spectrum of Isopronanol

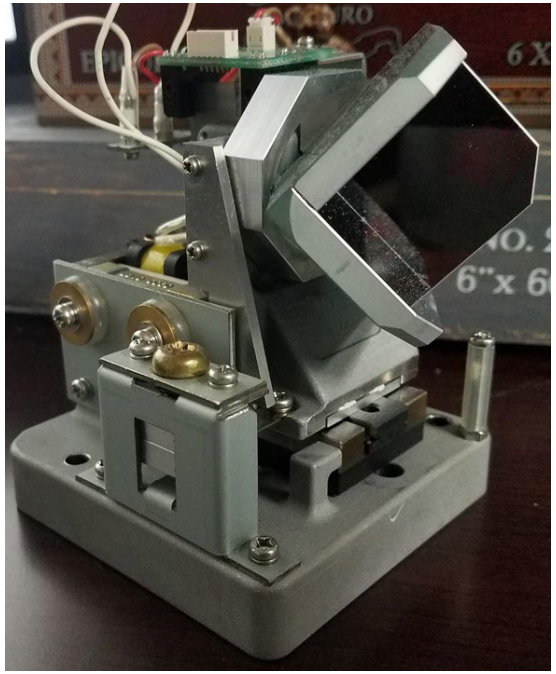


Correlation Chart



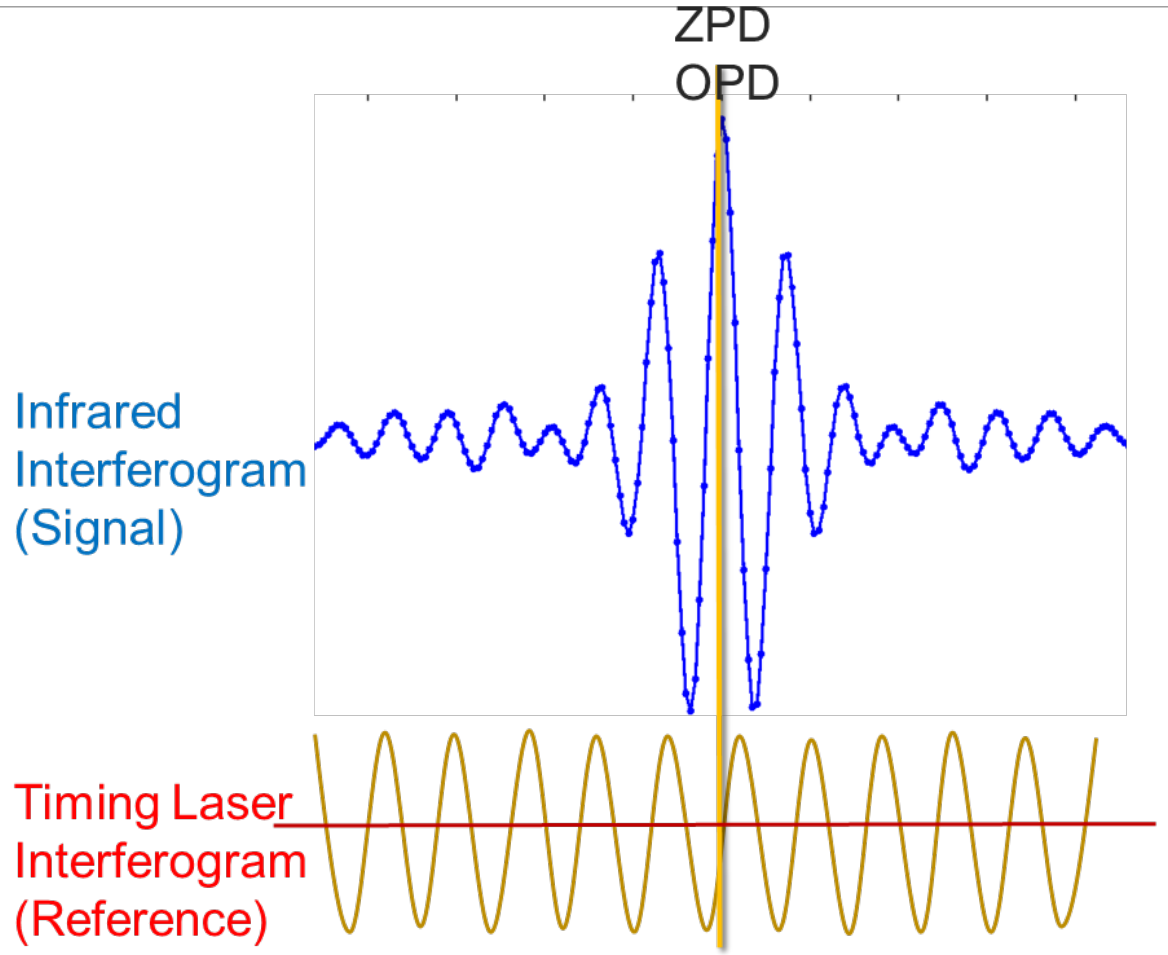
Michelson Interferometer





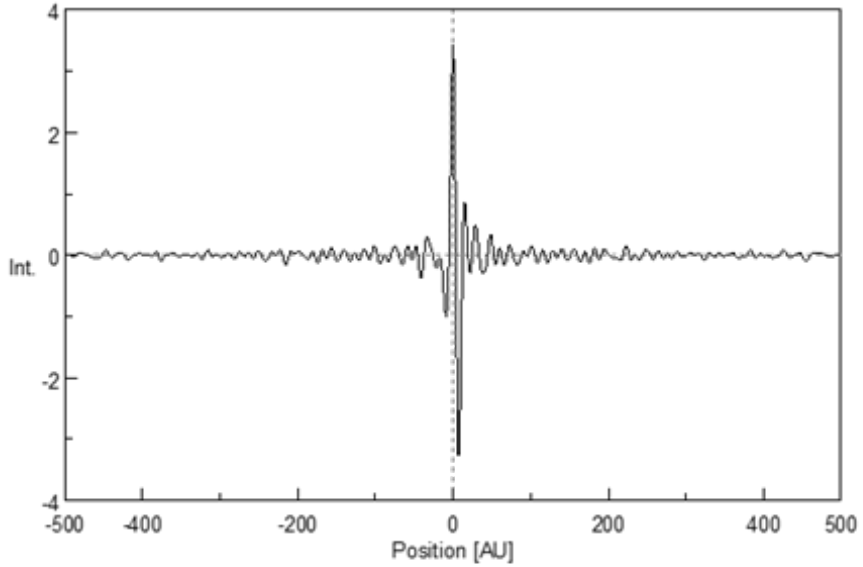
Moving mirror with cube-cornered optics from a JASCO FTIR

Mirror Position Monitoring



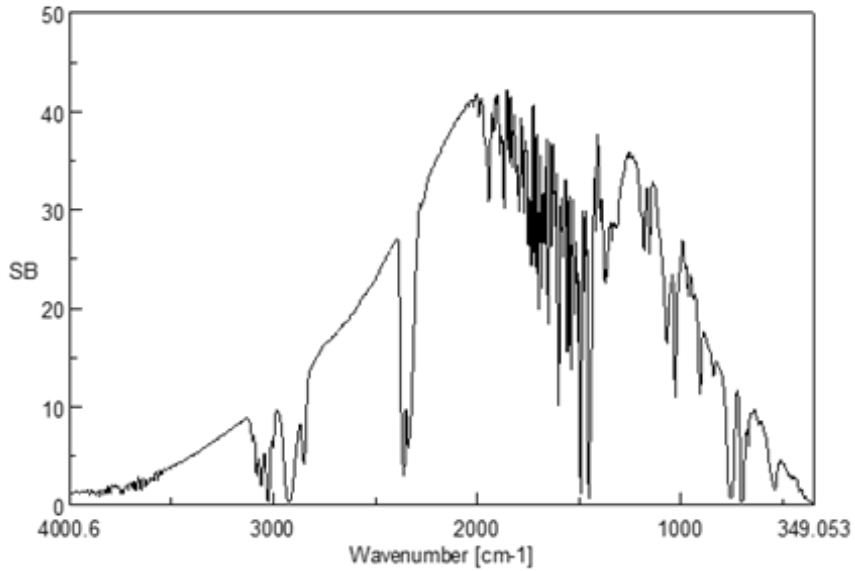
Fourier Transform

Interferogram
(Time Domain)

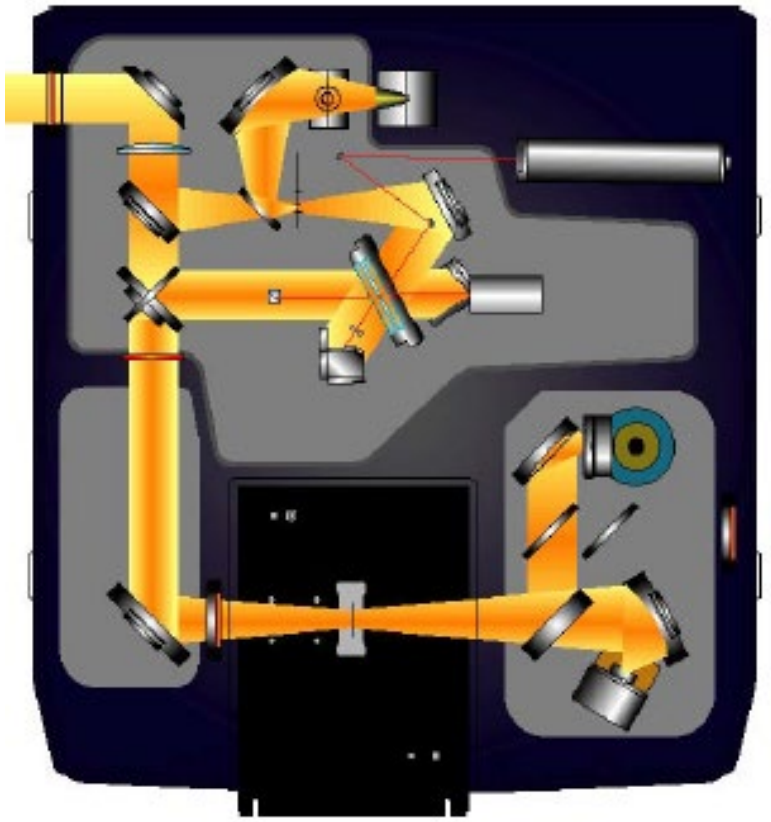


Fourier
Transform

Single Beam Spectrum
(Frequency Domain)



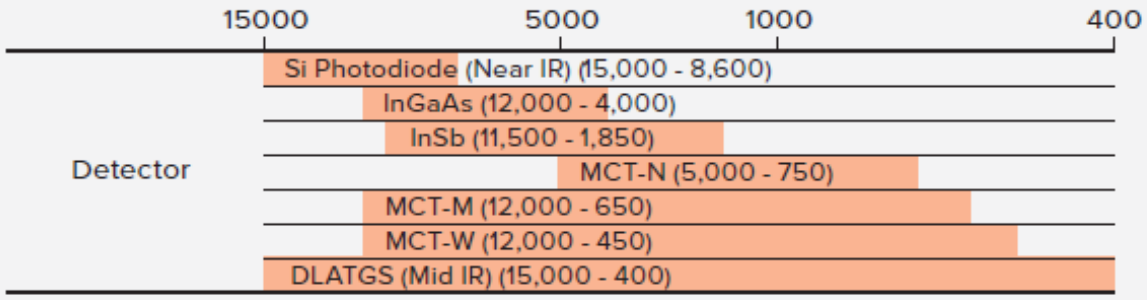
Light Path for Microscopy



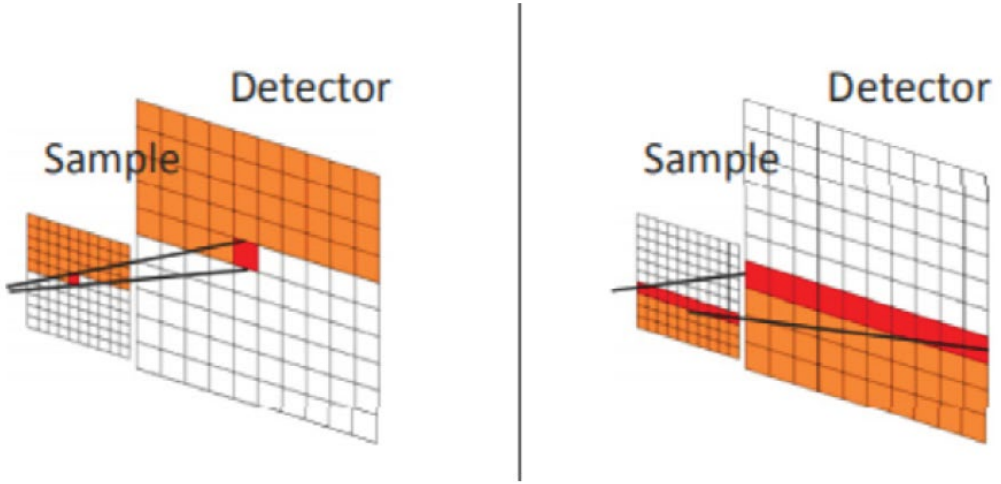
FT/IR-6000 Series Optical design

Single Point Detectors

Detectors

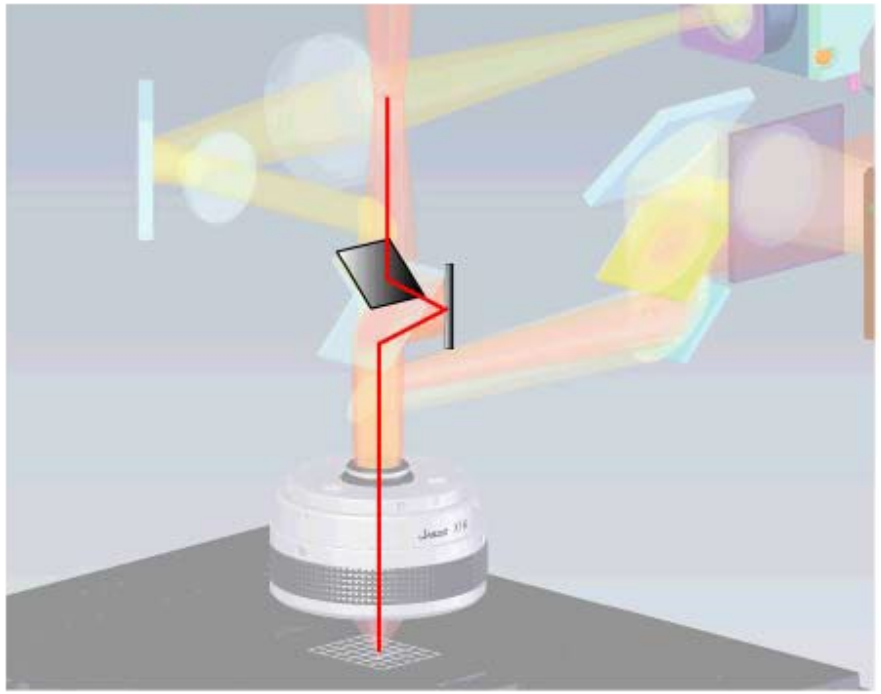


Linear Array Detectors



- Linear array of MCT (IR) or InGaAs (NIR) detector elements.
- 16 points as opposed to single point.
- Faster scanning, better spatial resolution.

IQ Mapping



- Uses a scanning mirror in the IR beam path.
- Allows for the measurement of multiple points without moving the stage.
- Useful for samples that may deform or shift on contact with the ATR.

Automated XYZ Sample Stage

Environmental Stage

Linkam Stage and Temperature Controller

- Temperatures from -196 to 600 °C

Purge and Vacuum Stages

Purged Stage

Evacuated Stage and Vacuum FTIR

Sample Preparation

KBr Pellet – Transmission Microscopy

- Powders
  - 3% sample in KBr.
  - Ground together in fine powder.
  - Pressed with high pressure until KBr is crystalline.
- Liquid
  - Must be non aqueous
  - Drop placed on KBr window
  - Z axis just above the KBr window.

Diamond Compression Cell

- Diamond anvil cell makes sample prep simple.
  - Sample is placed between two diamond windows
  - The sample is compressed using thumbscrews until IR transparent.

Sample Mounting

- Double sided tape may be used for thin, flat samples.
- Epoxy resin can be used for cross sections.
- Silly putty or rubber-tak are good for stabilizing and flattening larger, irregular samples.

Slicemaster

HS-1 Vertical Slicer

Easily cuts samples vertically for cross-section observation

HK-1 Angled Slicer

Observe wide cross-sections of samples with thin layers such as coatings.
(Approximately 4 times as wide as a vertical cross-section area)

HW-1 Multi-Angle Slicer

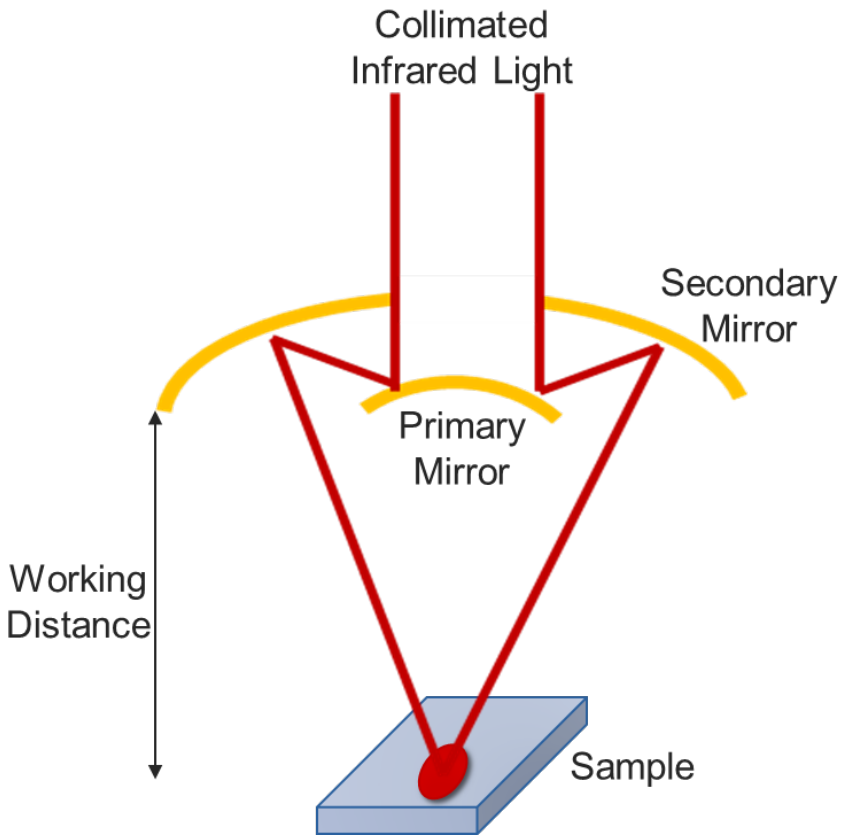
Variable cutting angle for cross-section observation of small samples

TS-1 Tablet Slicer

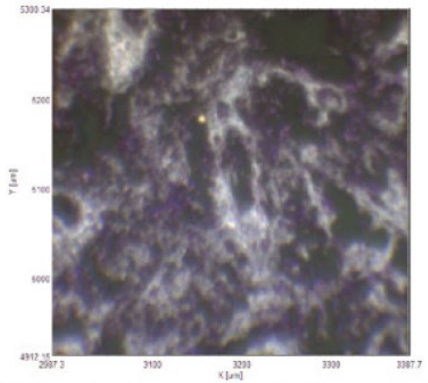
For clean cutting fragile samples (such as tablets, cereal grains etc.).
Variable angle cutting

Techniques

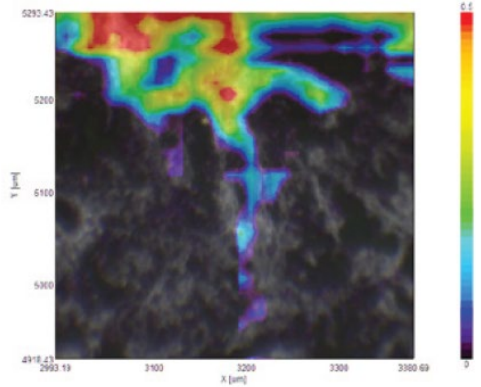
Reflection


Collimated
Infrared Light
Secondary
Mirror
Primary
Mirror
Working
Distance
Sample

Application Example: Cancerous Human Tissue

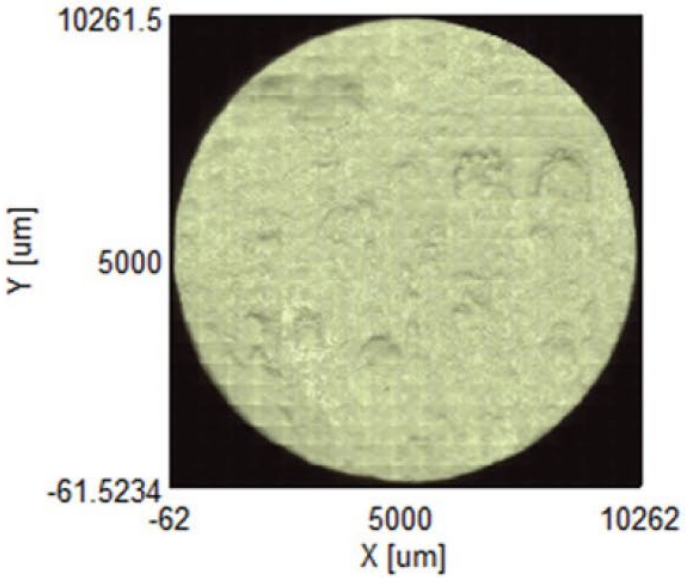


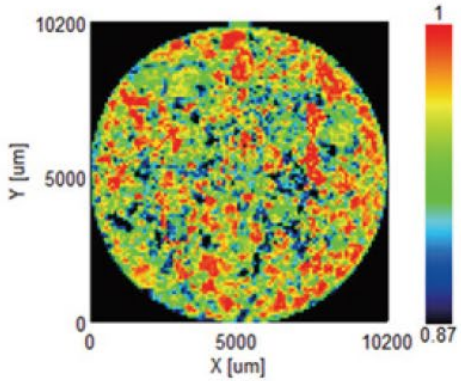
IQ Mapping™ - Imaging collection, reflection mode, of a malignant human tissue

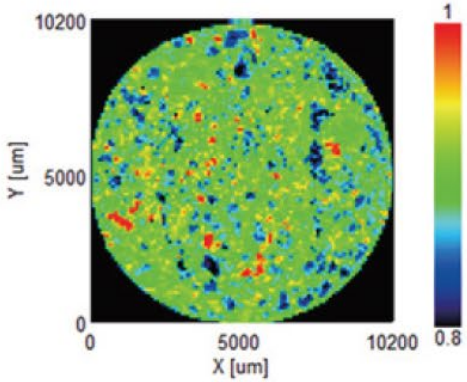


Intensity plot of protein amide band, 1,647 cm^{-1}, overlaid with visual image of cancerous tissue

Application Example: Tablet

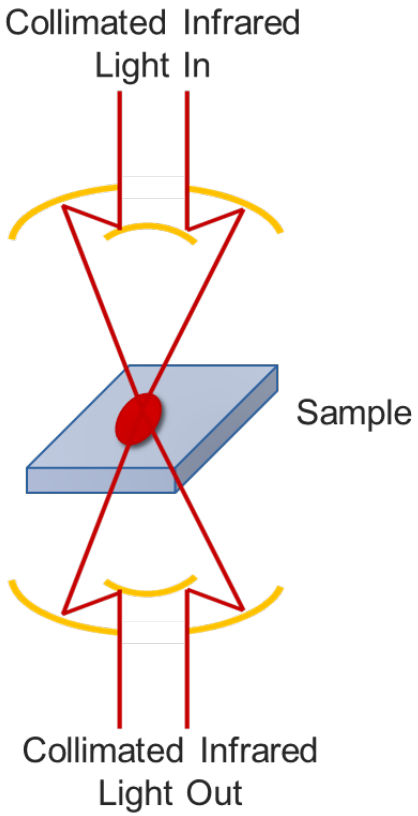






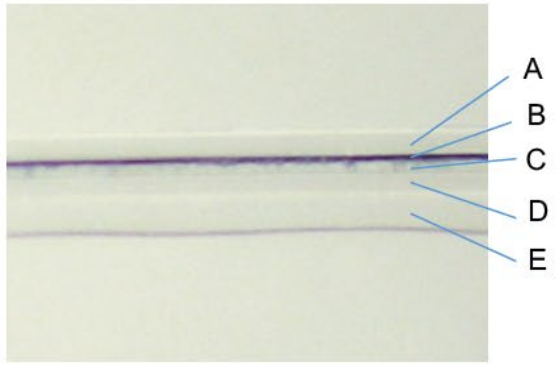
Left: Observation view
Center: Distribution of medicinal component
Right: Distribution of additive components

Transmission

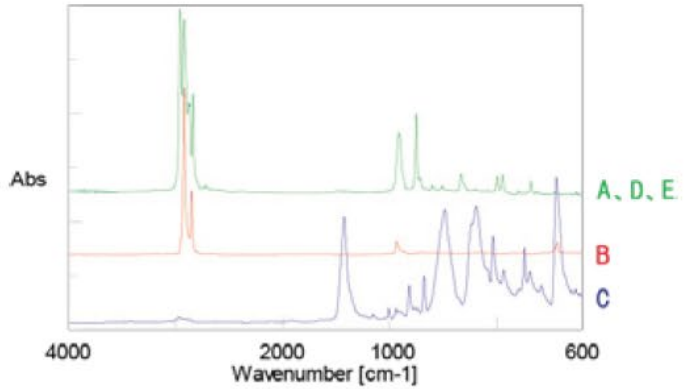


Application Example: Cross Section of Chip Bag

Food packaging bag (multi-layer film)

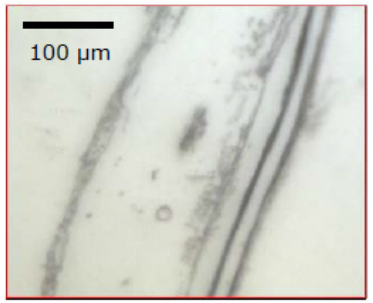


Cross-section

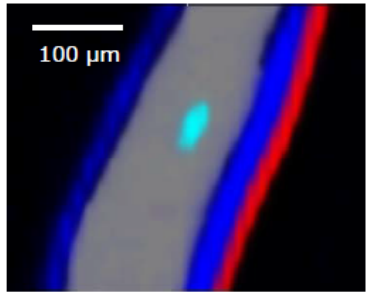


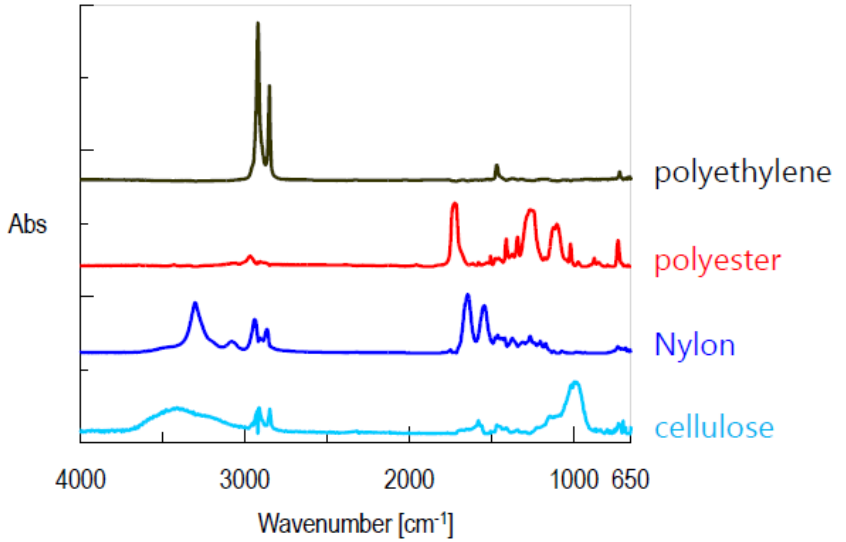
Transmission spectrum of each layer

Application Example: Multi-layered Film



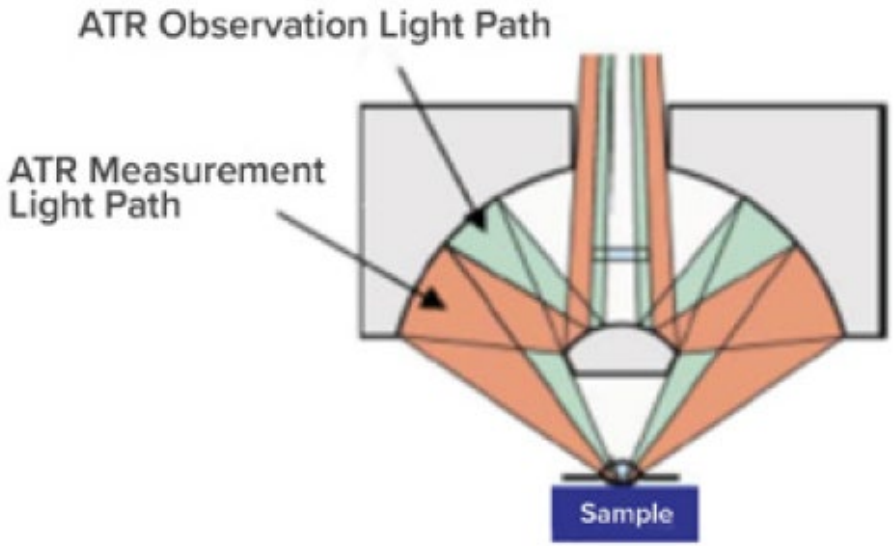
Observation image





Spectra of main component

Attenuated Total Reflectance (ATR)



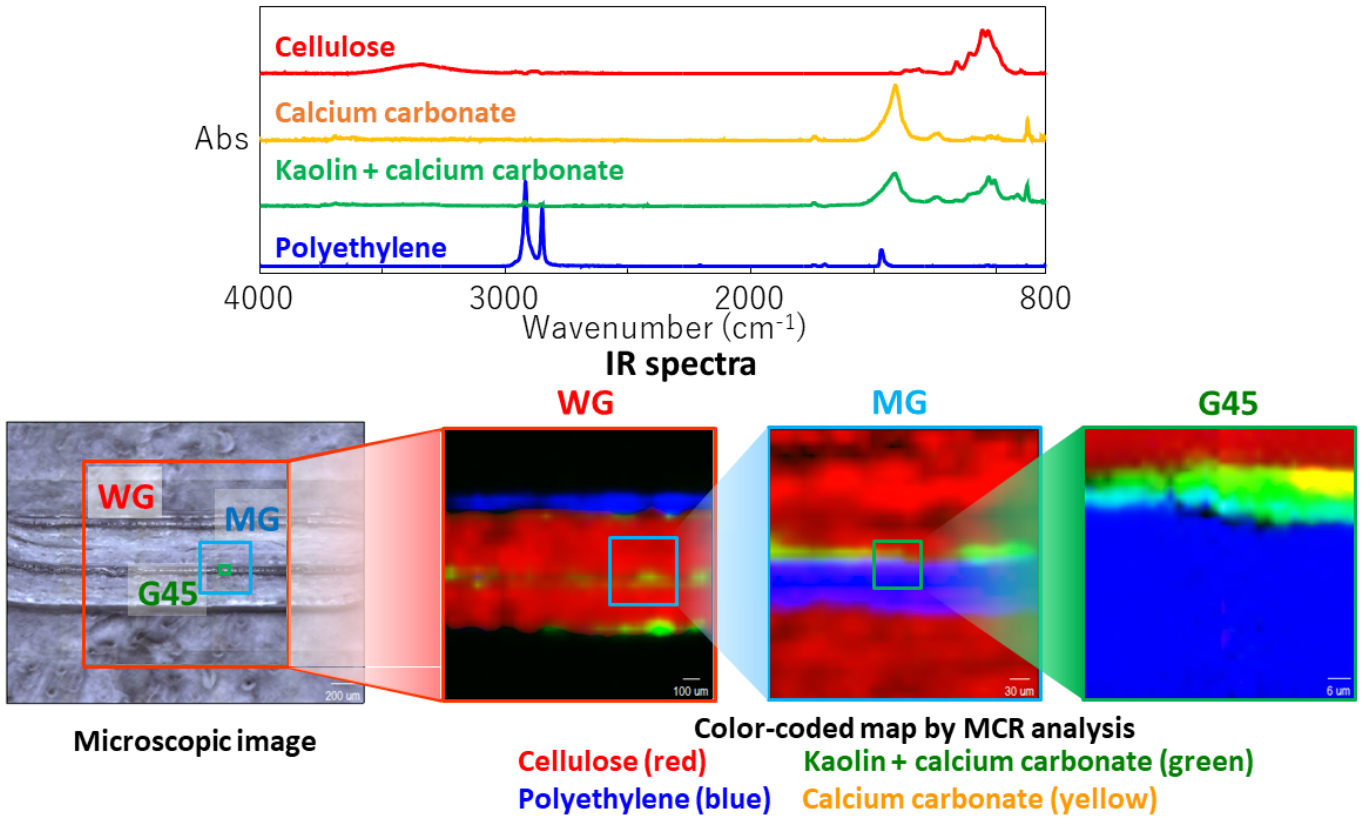
ATR Objectives

Specifications

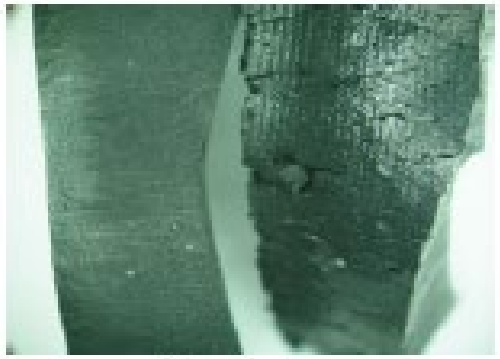


		ATR-5000-SD	ATR-5000-SS	ATR-5000-SG
Applicable Sample Refractive Index		1.0-1.5	1.0-1.5	1.0-2.7
Wavelength Range (cm^{-1})		7,000-2,500 1,600-700	7,000-700	5,200-650
Magnification	Crystal in Raised (View) Position	16		
	Crystal and Sample Contact (Sample Position)	35.2		64
ATR Crystal Element	Material	Diamond	ZnS	Ge
	Refractive Index (@ 1000 cm^{-1})	2.4	2.2	4.0
	Area in Contact with Sample	Φ 500 µm		Φ 250 µm
	Number of Internal Reflections	1		
Simultaneous Sample View when Crystal is in Contact with the Sample Surface		Possible		Impossible
IQ Mapping Area (µm)		180 x 180		100 x 100

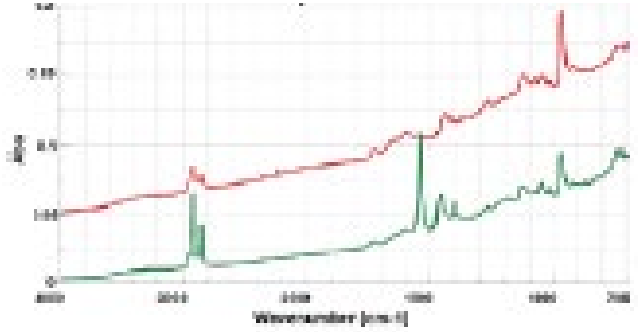
ATR Microscopy – Laminate Layers



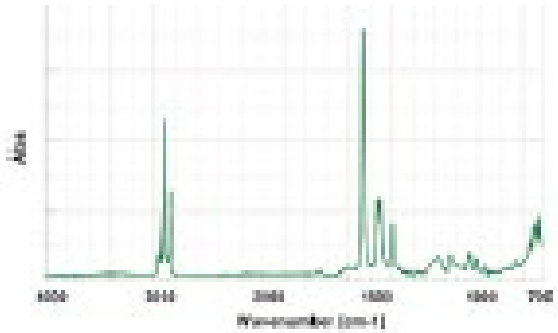
Application Example: Rubber Samples



Sample view
(Left: Normal rubber, Right: Deteriorated rubber)



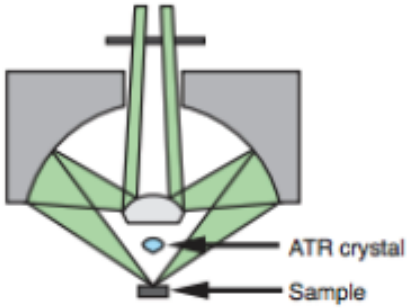
Spectrum of rubbers
(Green: Normal rubber, Red: Deteriorated rubber)



Subtraction spectrum

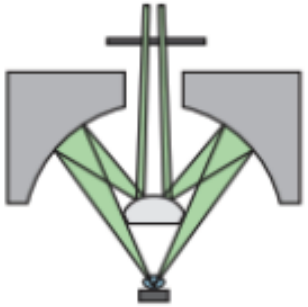
ClearView™ – View Through ATR

Normal sample view with the crystal element in the raised position



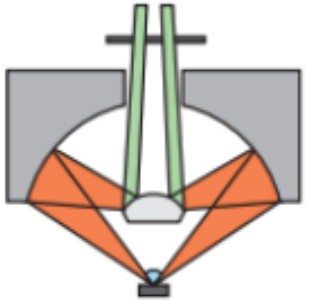
The ATR-5000-SD/SS/SG enables sample viewing by setting the ATR crystal in the raised position.

Sample viewing after crystal contact with the sample area



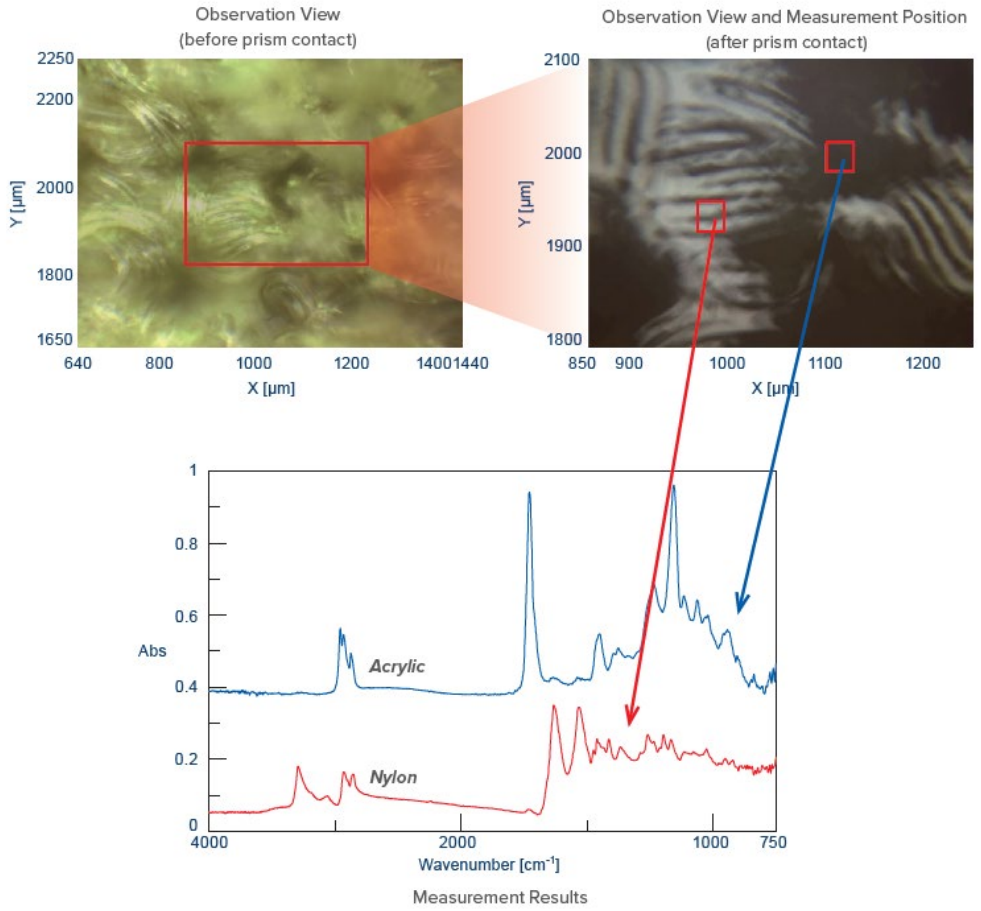
The ATR-5000-SD and SS enable sample viewing through the ATR crystal after contact with the sample surface.

ATR measurement and simultaneous sample viewing

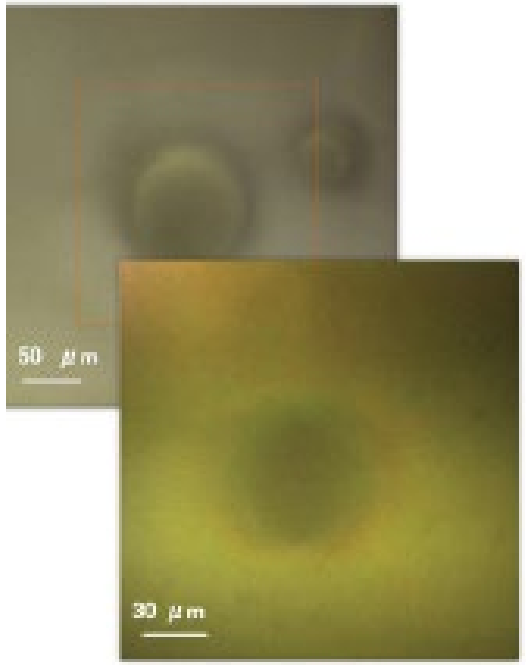


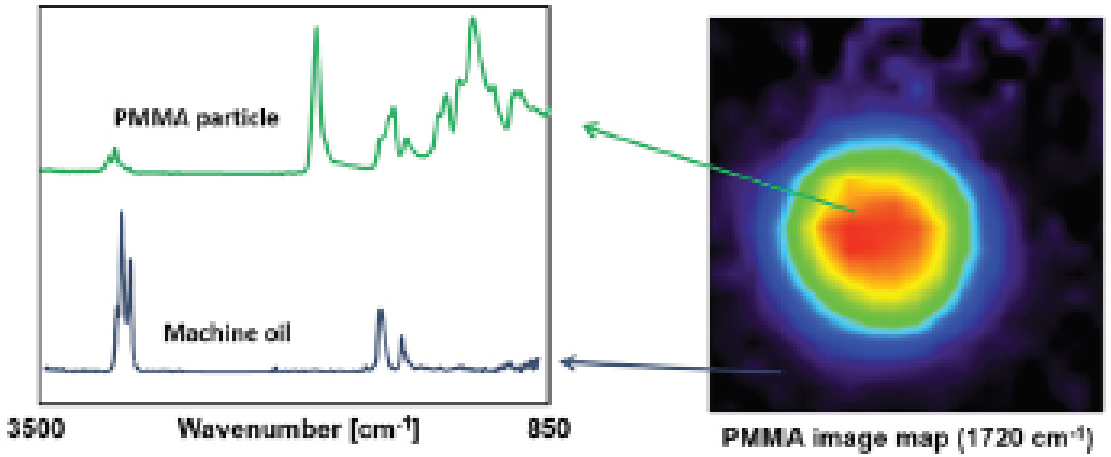
The ATR-5000-SD and SS provide simultaneous sample view during ATR data collection.

Application Example: Thread



Application Example: Bead in Machine Oil



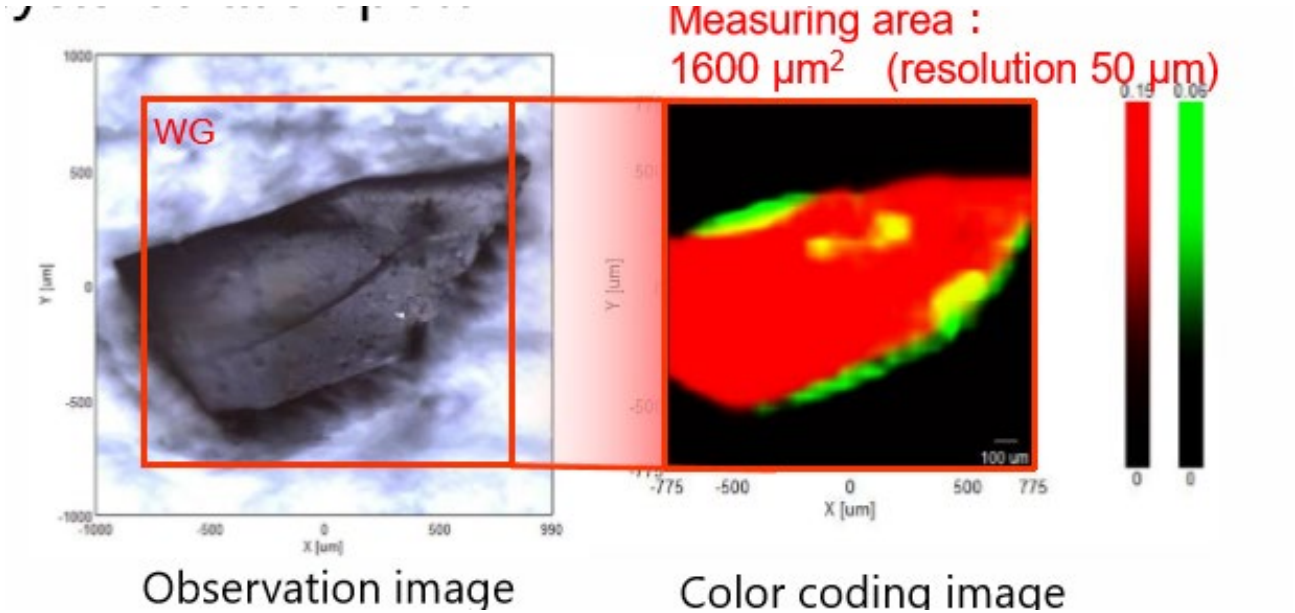


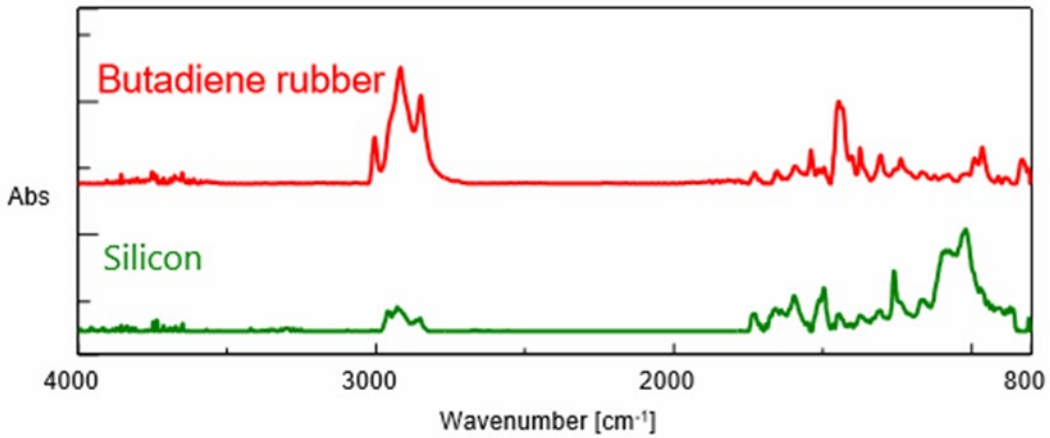
WG Objective

400 x 400 μm Sampling area

Combined with IQ mapping can cover a large area without repositioning crystal.

Application Example: ATR-5000-WG

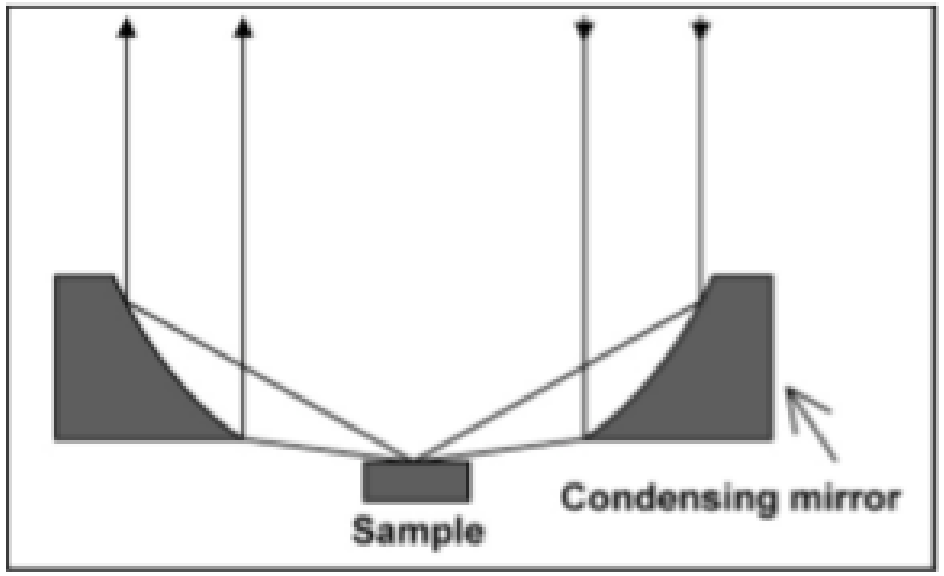




Sensor Plate

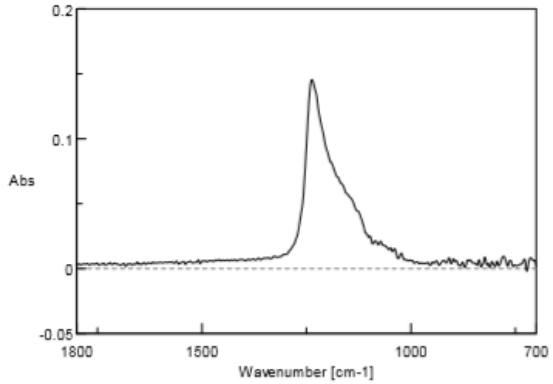
Pressure Monitor Sensor

Grazing Angle

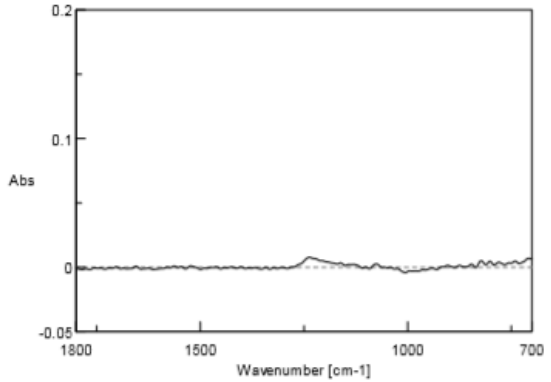


- 70° incidence angle
- Need samples on a metal substrate
- Can see films <2.5 μm
- Linear Array or MCT detecor

Application Example: Silicon Film



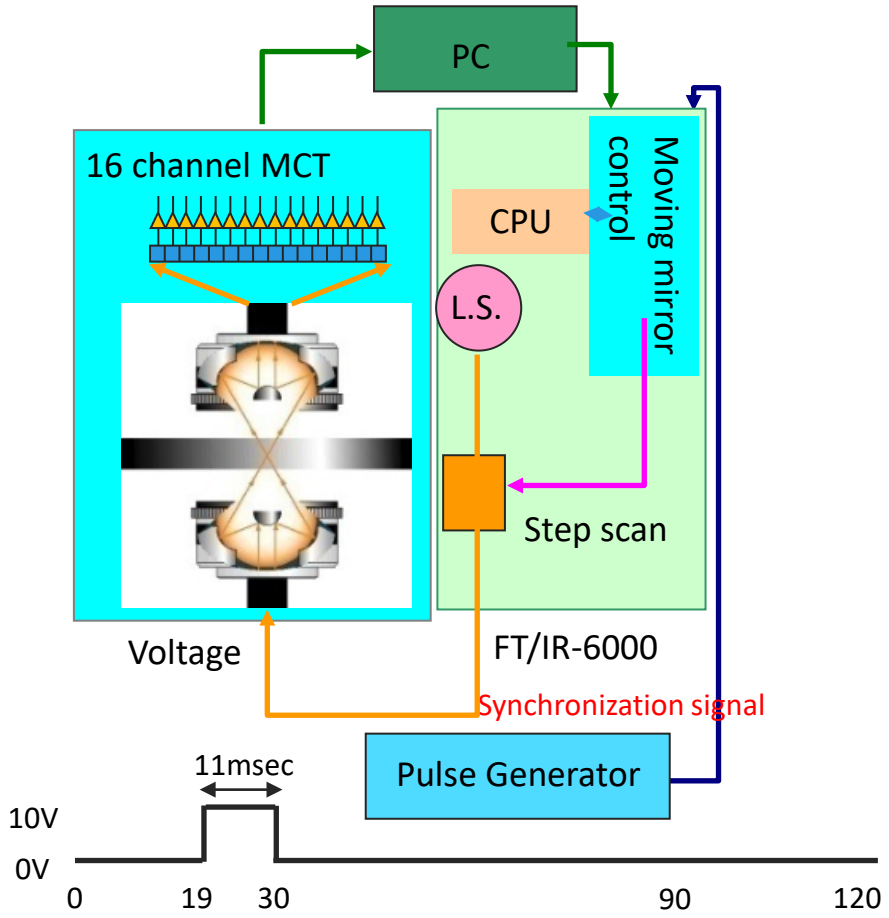
0° polarized

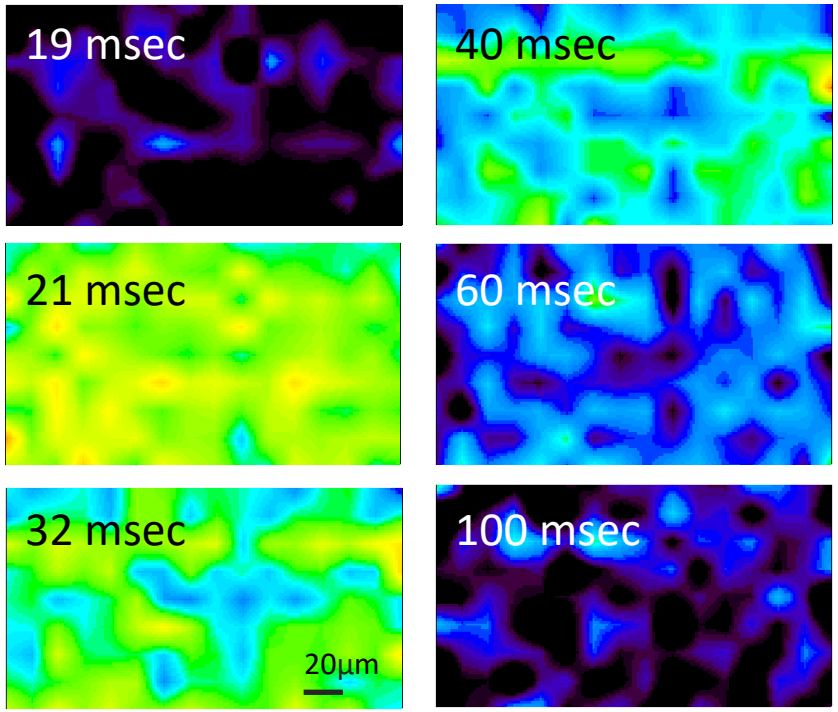


90° polarized

Figure 6.1 SiO_2 Spectra using RAS-5000.

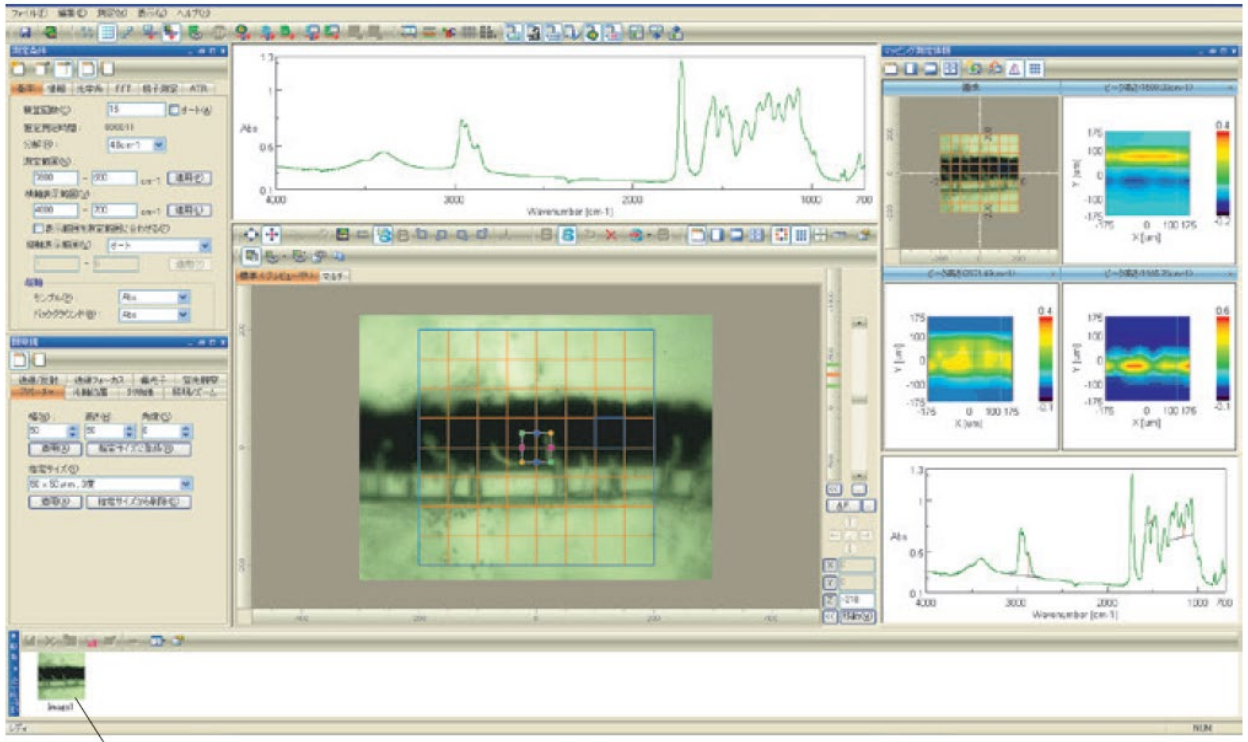
Time Resolved Spectroscopy





Software for Imaging

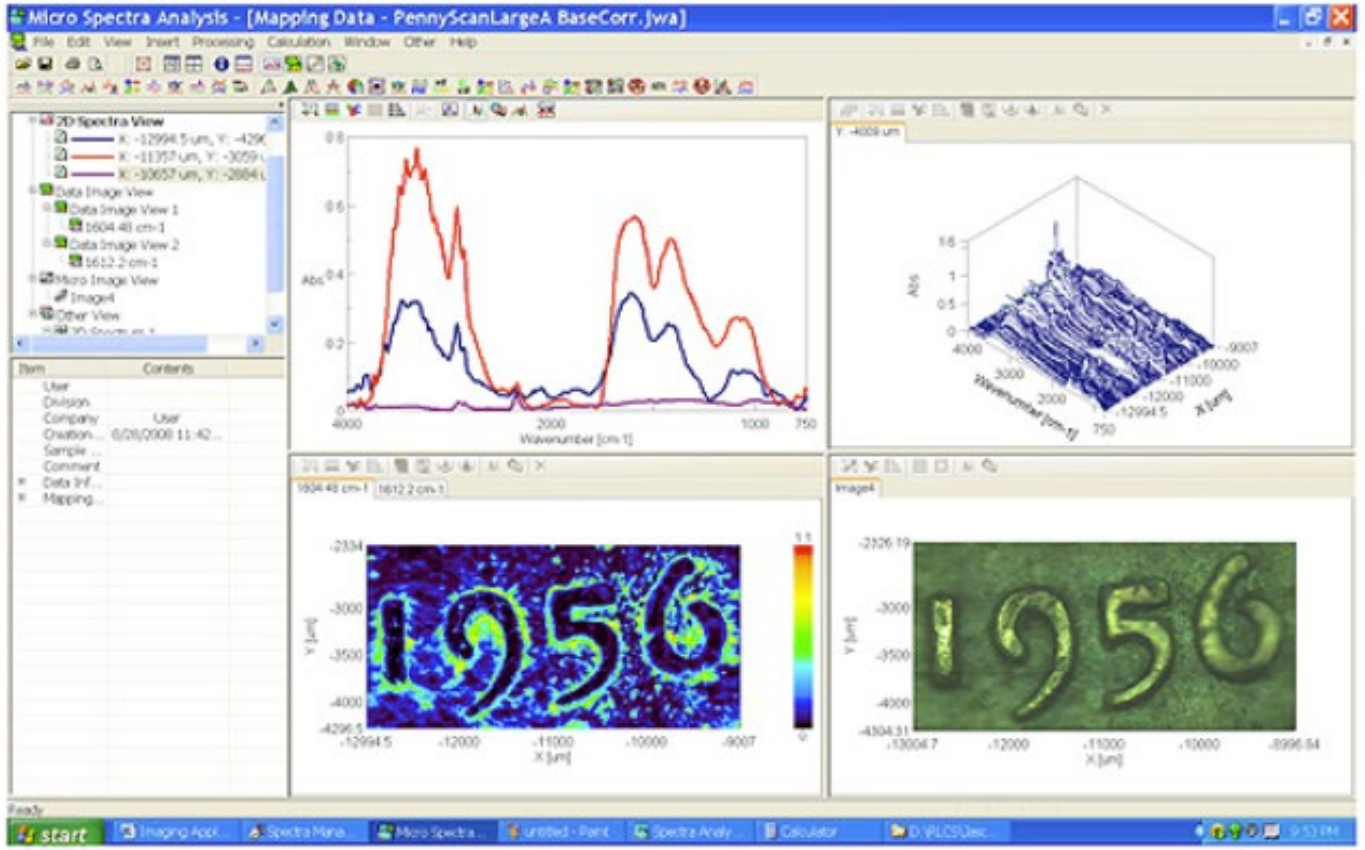
Spectrum Manager Imaging Software



Thumbnail

Spectra Manager Micro
Imaging Analysis

Analysis Software



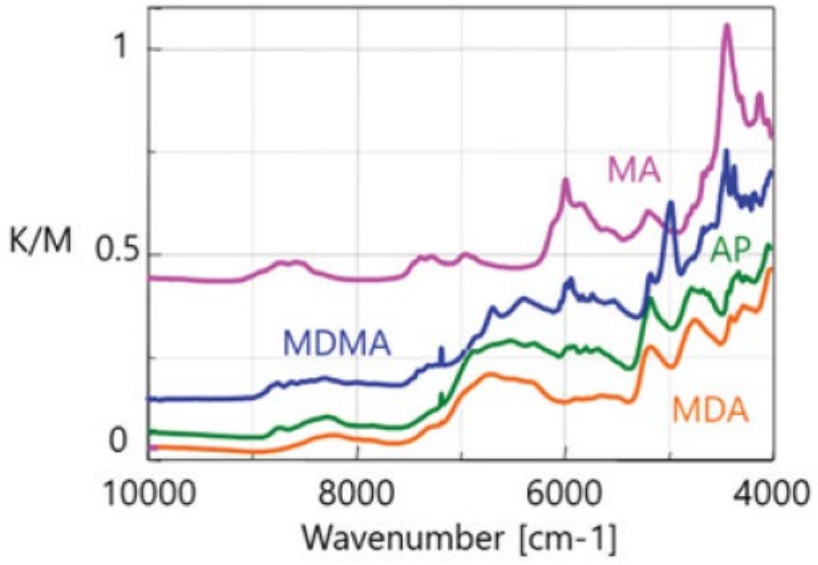
Beer’s Law Variants

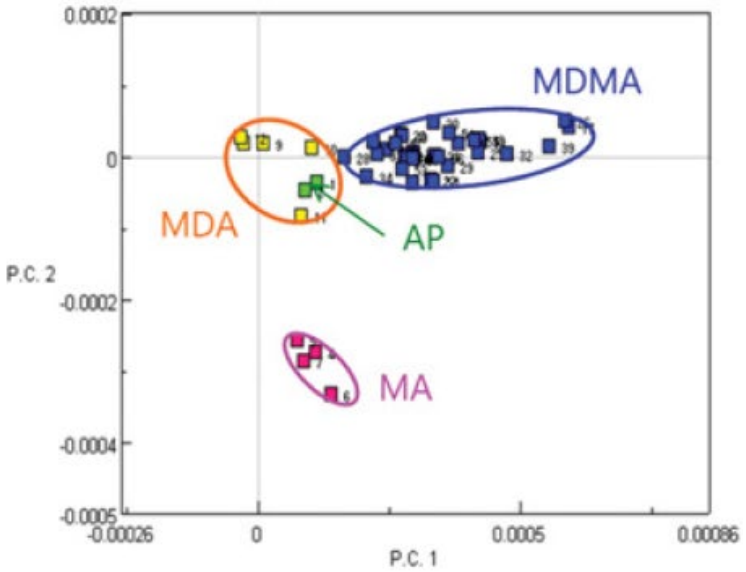
- Beer’s Law $A = \varepsilon bc$
- Classical Least Squares – Compares the absorptivity to one of a known concentration.
- Inverse Least Squares - Absorbance of multiple spectral features.

Principal Component Analysis

- Principal Components Analysis (PCA) – Reduces the variables to principle components and scores and groups the principle components.

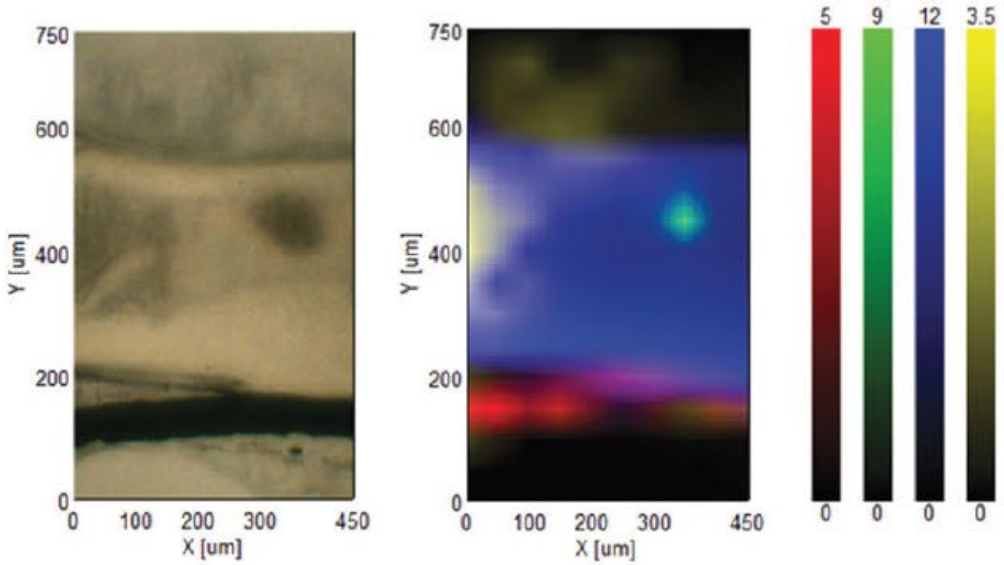
- Principal Component Regression (PCR) – Combines PCA and ILS by grouping components then forming a regression on the result.

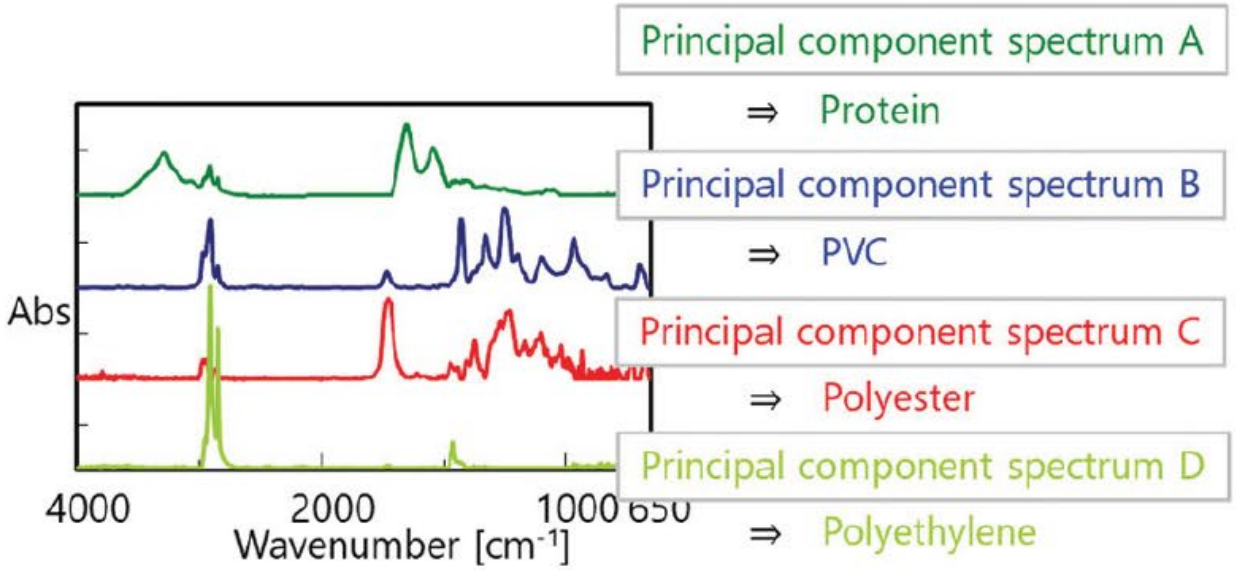




Multivariate Curve Resolution (MCR)

- In the case of multivariate curve resolution, the standard sample spectra are not known, only the matrix of spectra. Therefore, PCA is performed on multiple spectra and principal component spectra are then calculated. Different components can be found in an unknown sample.

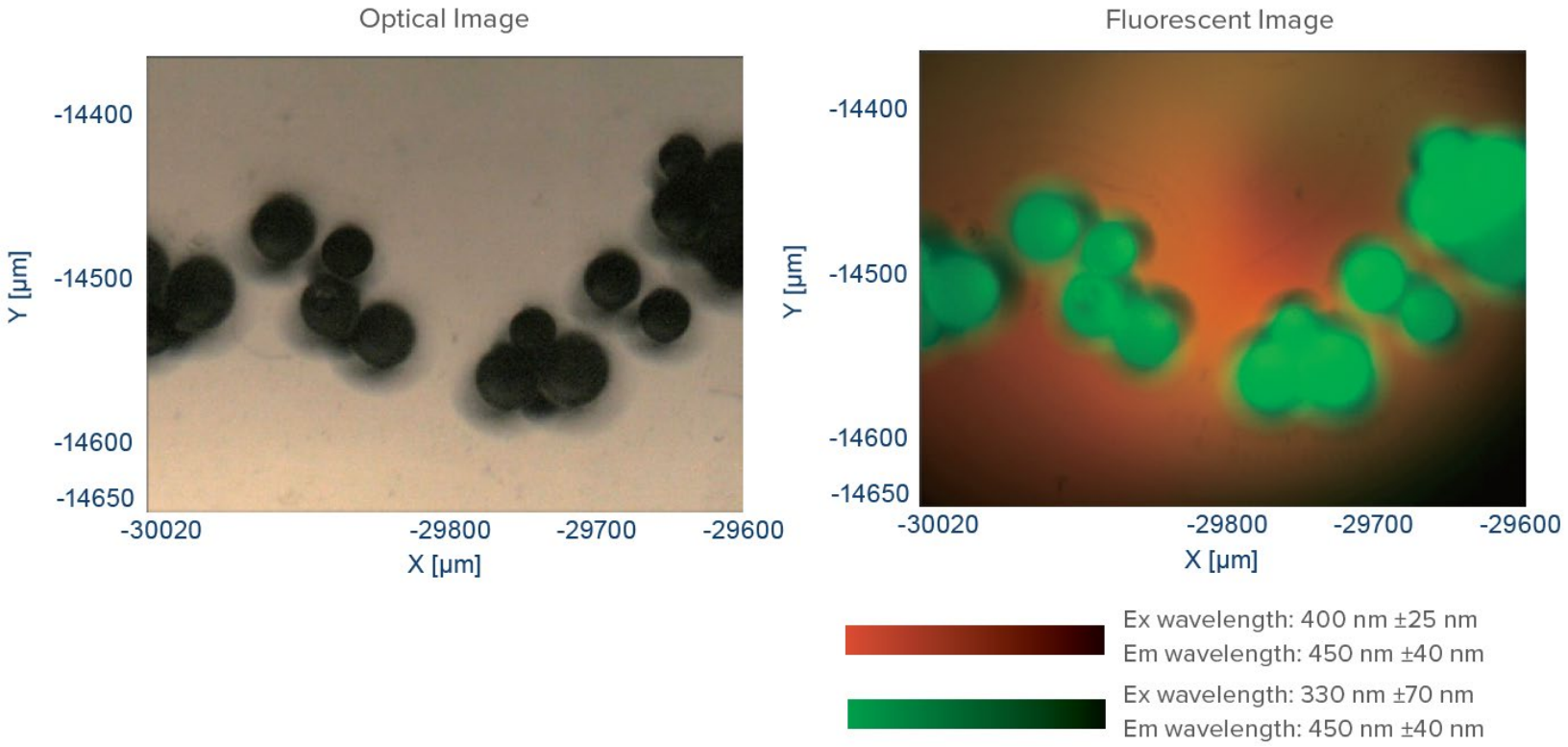




Complementary Microscopic Techniques

Fluorescence Observation

For better view of fluorescing samples



Differential Interference Contrast (DIC)

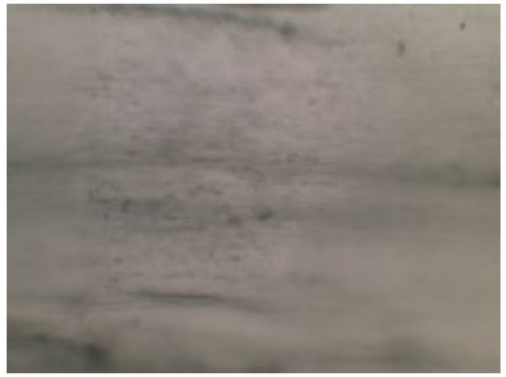
For samples that are clear or of low contrast

Optical Image

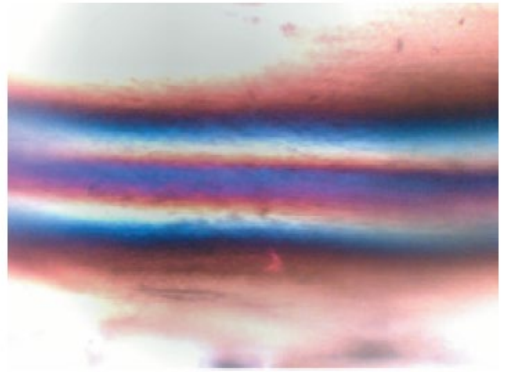
Differential Interference Contrast Image

Visible Polarization

For samples that are visually indistinguishable.



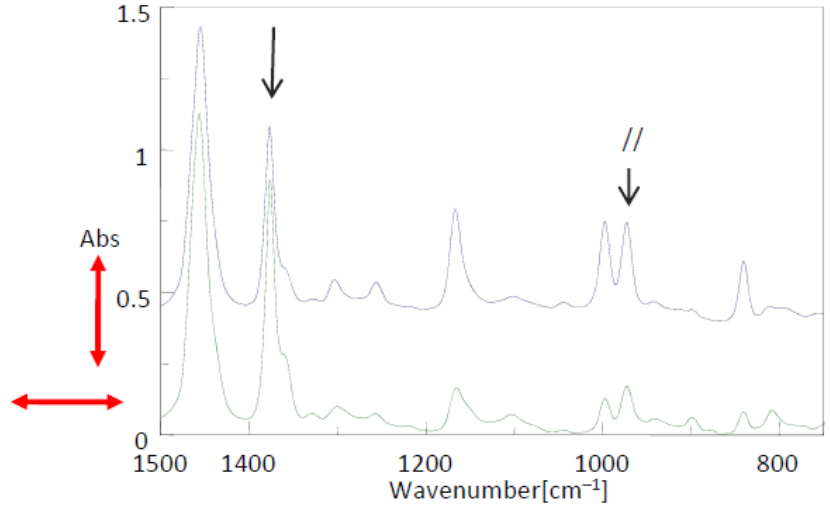
Optical Image

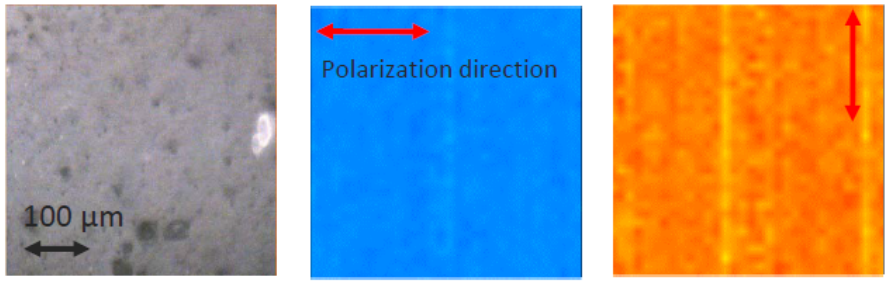


Polarization Image

IR Polarizer

[Result and discussion]





Visible image

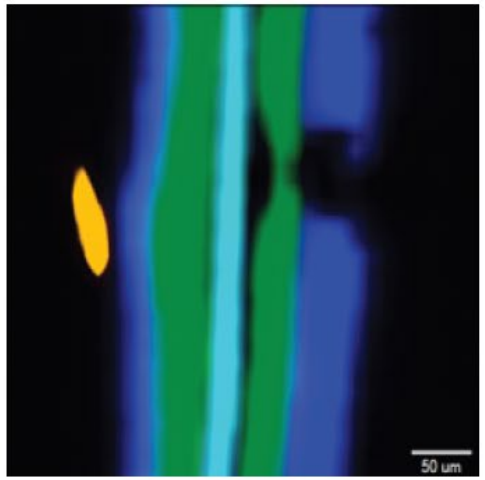
973cm^{-1}(//) /1378 cm^{-1}(standard)

IQ Frame

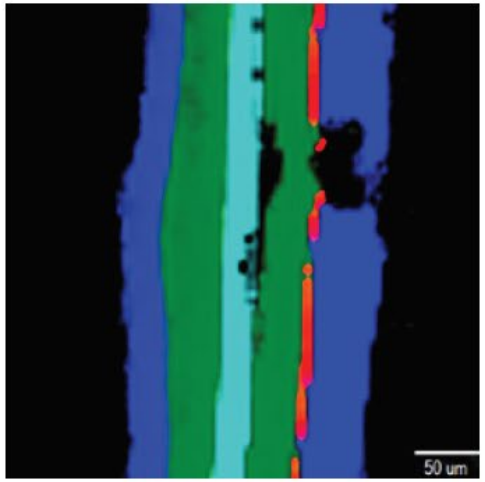
Raman Microscopy

- Raman microscopy is complementary to FTIR
- Good for:
- Samples containing water
- Inorganic compounds
- Carbon materials

FTIR



Raman



Chemical Imaging
(Left: from IR data, Right: from Raman data)

Green: Polyethylene
Aqua: PET
Blue: Polypropylene
Yellow: Cellulose
Red: TiO2

Summary

- FTIR spectroscopy is a valuable tool to identify and quantify compounds.
- FTIR Microscopy is useful for multilayered films, surface composition, inclusions, and compositional analysis.
- FTIR objectives include reflection, transmission, a variety of ATR choices, and grazing angle.
- The Clearview ATR objective along with IQ Mapping allow for a large area to be scanned without moving the stage/objective.
- The common detectors are DLaTGS, MCT, and linear array.
- Depending on sample, DIC, visible polarization, or fluorescence may be useful to visualize the sample.
- Chemometrics are a valuable tool to determine the composition of a sample.

JASCO Educational Resources

- Upcoming Webinar
  - HPLC, June 2nd
- Past Webinars:
  - Vibrational Circular Dichroism
  - FTIR Theory, Instrumentation, and Techniques
  - Circular Dichroism
  - FTIR Microscopy
  - Raman Microscopy and Imaging
  - SFC
  - Fluorescence
- E-books:
  - Raman
  - Raman Tips & Tricks
  - Fluorescence
  - FTIR
- KnowledgeBase