Industrial Applications of FTIR Imaging Microscopy

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Outline

1. History of FTIR Microscopy
2. Chemical Imaging
   • Mapping
   • Spatial Resolution and Pixel Size
3. Applications
4. Summary
1. BRIEF HISTORY
1. First Instruments

Prototype Model 12 (1943)

• PE, Beckman (National Technical Laboratories), and Baird (dual beam) made IRs to characterize gases used to manufacture rubber for defense purposes AND for penicillin manufacture during WWII

• Early Instruments were prism-based
1944 – First Commercial IR
PE IR12
The Early (IR) Microscope

• Provided reflective objective design solutions to optimize image reproduction

*R.Barer et al.(Nature, 163: 198 (1949))*
• Coupled a Burch design reflecting microscope to a PE recording IR

Discussed performance characteristics of IR Microscopes

*V.J Coates et al. (J.Opt. Soc. Am. 43: 984-989 (1953))*
Perkin-Elmer Model 85 - First commercial IR Microscope
PE Model 85 (1953)
2. Development of Modern Instruments
First patent for “Interference Technique and Spectral Analysis”

• Issued to Block Engineering (Digilab) in 1966
  Brought together the interferometry (old), Spectroscopy (old), Computers (sort of old but minicomputers had just come on the market) in combination (new) for IR spectroscopy

• First Commercial FTIR: The Digilab FTS-14 (1969)
Digilab FTS-14 FTIR (1969)
FTIR and Microscopy: The Perfect Match

• FT: High throughput and Multiplex Advantage
  • Excellent for microscopic analysis
    • Microscope is a beam condenser (reduce beam to match size of sample)
    • Aperture limits energy impinging on sample (need high throughput)
    • Need to bombard sample with a lot of photons (need high throughput)
    • Small sample size – not a lot of signal, requires detector that is sensitive to low-light levels (high throughput and multiplex)
  • Many scans to improve S:N (multiplex)
First Commercially Available FTIR Microscope: Digilab UMA 100 (1980-81)
1985-1995

- Automated Stages
- Automated Apertures
- Powerful computers
  - Making possible mapping experiments, programmed scanning, etc.
1995 Chemical Imaging

• Chemical Imaging

• Using a 2-D MCT Array detector
  • Neil Lewis, NIH
  • Curt Marcott et al., Procter and Gamble
  • Biorad (formally Digilab)

• Take an Infrared “Snapshot” of a sample area
Technology Convergence

• As with the development of FTIR, Imaging was also a convergence of technology: FTIR (old/established) + FTIR microscopy (established) + IR Array detectors (established) = FTIR Imaging (NEW)…………….
The Bio-Rad Stingray System (1997)

- Digital MCT Array Detector
- Single PC for Array Detector and FT-IR
- Contains Digital Frame Grabber
- FTS 6000 Spectrometer
- UMA 500 FT-IR Microscope
• Infrared Imaging with an Infrared Camera, referred to as a *Focal Plane Array (FPA)*

FPA-FTIR detectors provide the ability to acquire a grid of spectra in the same amount of time that it takes single point detectors to acquire one spectrum.

Sample is imaged onto a FPA detector \((n \times n\) pixel array) \(\rightarrow n^2\) spatially resolved spectra are collected simultaneously (e.g., 16 \(\times\) 16 array \(\rightarrow\) 256 spectra)

One pixel (~5.5\(\mu\)m) is an entire spectrum.
Early FPA Imaging Systems

• Used Javelin FPA; intended for single use
• Heat seeking antitank missiles
• Not built for Analytical applications (intended for military applications) – slow data acquisition rates
• Required step-scan benches to slow experiment down for data acquisition/processing

• Not all pixel responsive – rejects by the DoD
Current Technology

• FPAs are designed (new Lancer) for spectroscopy
• All pixels responsive
• Fast data acquisition rates – can operate in rapid-scan mode

Cary 620 Imaging System
2. CHEMICAL IMAGING
Approaches to FT-IR imaging

- Point-by-point Mapping
- Linear array (Hybrid)
- FPA
Point-by-point Mapping Disadvantages

• Time Consuming (hours/days)

• Use aperture to define area for analysis
  • for finer spatial detail, aperture closed down
  • S:N reduced; increase number of scans
  • Diffraction increases spectral crosstalk (contamination)

• Automated stage moves sample, point by point
Hybrid Imaging/Mapping

- A row of data from each pixel in the array (usually 1 x 16, 2 x 8, 2 x 16) collected simultaneously.

- Move the sample with a motorized stage and collect multiple scans.

- Build up a Map of the sample by “stitching” linear array scan together.
Advantages of FTIR FPA Imaging

• By use of multi-channel detector “FPA”
• Needs no aperture.
• Needs no sample scanning (raster scanning to produce an image).
• Whole area at the same time.
• Measuring time can be reduced dramatically.
• Provides high-fidelity chemical images
Why use FPA chemical imaging?

Two reasons:

1. Provides rapid high spatial resolution chemical distribution – the where (spatial) and the what (spectral)

2. Allows for the measurement of defects as small as a ~2 microns
SPATIAL RESOLUTION
Spatial Resolution

Ability to detect the presence of two objects next to one another

Distance between those objects given by Rayleigh criteria:

$$\Delta d = \frac{0.61\lambda}{NA} = \frac{0.61\lambda}{n \sin \theta}$$

Slide Courtesy of Dr. Andy Sommer, Miami University
Spatial Resolution

Rayleigh Criterion (FWHM)

\[ \Delta d = 0.61\lambda / NA = 0.61\lambda / n \sin \theta \]

\( \Delta d \) = the minimum resolvable separation
\( \lambda \) = source wavelength
\( n \) = refractive index
\( \theta \) = half-angle of the maximum cone of light

objective has \( NA = 0.61 \), so
\[ d = 1.02 \lambda \]

objective has \( NA = 0.81 \), so
\[ d = 0.75 \lambda \]

with Ge ATR (refractive index = 4): \( n \sin \theta = 4 \times 0.61 \): \( d = 0.25\lambda \)
Spatial Resolution

Therefore, for objectives with an NA ~ 0.61, the theoretical spatial resolution will be equivalent to the wavelength ($\lambda$).

Example:
At 5 microns (2000 cm$^{-1}$), the Rayleigh criterion determined spatial resolution for different NA's is as follows:

- **0.4NA**: $0.61 \times 5 / 0.4 = 7.6$ micron
- **0.5NA**: $0.61 \times 5 / 0.5 = 6.1$ micron
- **0.6NA**: $0.61 \times 5 / 0.6 = 5.1$ micron
- **0.7NA**: $0.61 \times 5 / 0.7 = 4.4$ micron
- **0.8NA**: $0.61 \times 5 / 0.8 = 3.8$ micron

**In summary:**

If NA = 0.61, theoretical spatial resolution = wavelength ($\lambda$)
If NA < 0.61, theoretical spatial resolution > wavelength ($\lambda$)
If NA > 0.61, theoretical spatial resolution < wavelength ($\lambda$)
PIXEL SIZE
Pixel size (magnification) and its role in achievable spatial resolution

• Fundamental factors that affect spatial resolution
  - Probe wavelength
  - Numerical aperture

• Effect of Pixel size
  - Size > theoretical spatial resolution, then pixel size is the limiting factor
  - Size < theoretical spatial resolution (ideally about ¼ size of theoretical spatial resolution), then optimal sampling for achieving theoretical spatial resolution

• Trade off between field of view and pixel size
  - Smaller pixels, smaller FOV
New Method of Magnification Enhancement

- The pixel size at the sample plane (pixel resolution) is a combination of:
  - Native FPA detector element size
  - Objective magnification
  - Intermediate optics magnification

- It is important to note that, pixel resolution (total system magnification) is therefore NOT ONLY governed by the objective.
FTIR Microscope Magnification Schematic

It’s the “total magnification” that matters, which together with the native FPA pixel size, equates to final pixel size at the sample plane. Objective magnification alone, is only a factor in the overall “total magnification” equation.

A big advantage of this approach is FULL PRESERVATION of the long objective working distance of 12mm, allowing a wide array of accessories and sample holders to be used.
Microscope objectives: some comparisons

Agilent offer a wider range of pixel size options with better NA, to provide better spatial resolution or faster image collection over large areas – and with more useful working distances.
## Achieved Spatial Resolution/Pixel Size Summary

<table>
<thead>
<tr>
<th>Pixel Size (obj mag, NA, mode)</th>
<th>Achieved Spatial Resolution 3750 cm⁻¹</th>
<th>Achieved Spatial Resolution 2500 cm⁻¹</th>
<th>Single FPA tile FOV (with 128x128FPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3 um (25x, 0.81NA, std mag)</td>
<td>4.3 um</td>
<td>5.0 um</td>
<td>420x420 um</td>
</tr>
<tr>
<td>0.66 um (25x, 0.81NA, high mag)</td>
<td>1.4 um</td>
<td>1.7 um</td>
<td>85x85 um</td>
</tr>
<tr>
<td>5.5 um (15x, 0.62NA, std mag)</td>
<td>6.9 um</td>
<td>7.6 um</td>
<td>700x700 um</td>
</tr>
<tr>
<td>1.1 um (15x, 0.62NA, high mag)</td>
<td>2.4 um</td>
<td>3.0 um</td>
<td>140x140 um</td>
</tr>
<tr>
<td>19 um (4x IR, 0.2NA, std mag)</td>
<td>20.4 um</td>
<td>20.0 um</td>
<td>2400x2400 um</td>
</tr>
</tbody>
</table>

**Entire 2"x2" (50x50mm) USAF target imaged at 19 um pixel resolution with 4xIR objective in 90 minutes (21x21 tile mosaic with 128FPA)**

**USAF target (700x700um) imaged at 5.5 um pixel resolution (normal mag. mode) with 15x objective in 2 minutes Single 128FPA tile**

**USAF target imaged (280x280um) at 1.1 um resolution (high mag mode) with 15x objective in 8 minutes 2x2 tile mosaic with 128FPA**
Cary 620 top 4 advantages

**Highest Spatial Resolution**
- New high mag optics
- >400% IR energy

**Largest Field of View**
- Proprietary 4x IR objective
- Measure cm x cm areas in minutes

**Fastest analysis time**
- >10x - other FPA’s
- > 50x - linear array
- >100x - single point

**Live FPA imaging**
- Enhanced chemical contrast software mode
- Eliminate sample prep
- Avoid damaging samples
4. APPLICATIONS
Challenges facing the industrial scientist/technician…

• Troubleshooting production samples (polymers, electronics, etc.)
  - Defects, contaminants, degradation etc...

• Reverse engineering competitor samples
  - Identification of laminate layer and tie (adhesive) composition

• General research (polymers, art conservation, pharmaceuticals, etc.)
  - Visualization of chemical distribution at the “micro” scale, without damaging the sample, allowing later characterization via other analytical techniques
Polymer Film Laminate FTIR Imaging
Agilent Slide-on ATR Accessory in 15x Cassegrain objective

- Micro ATR with Ge IRE
- Low contact pressure required
- Easy to change between different crystals
- No damage to the sample
- Easy to clean
- Does not require separate objective
- High infrared throughput
- High S/N at the sample

Single reflection hemispherical internal reflection element (IRE)
Sample Preparation Free FTIR Chemical Imaging of Polymer laminates & Films

Step 1. Cut out small piece

Step 2. Place cut-out piece in micro-vice.

Step 3. Cross-section sample with razor

Step 4. Place micro-vice (with sample) on microscope stage & touch ATR
ATR Contact with sample

STEP 5. raise stage to make contact & collect data

- ATR improves spatial resolution

with Ge ATR (refractive index = 4): 

\[ n \sin \theta = 4 \times 0.61: d = 0.25\lambda \]
A small piece of the sample was cut out and placed into a micro-vice sample holder where it was cut flat with a sharp razor.

The micro-vice is placed in a dedicated insert on the motorised stage and contact is made with the ATR crystal, using Agilent’s unique “Live ATR contact” method, without any need for resin embedding.
“Live/Real-Time” ATR contact monitoring

Standard Live ATR direct FPA IR Image – without correction

No Pressure (before contact) → First Contact → Increasing Pressure

Live ATR direct FPA IR Image with Enhanced Chemical Contrast

No Pressure (before contact) → First Contact → Increasing Pressure → Complete Contact
Sausage Packaging (Red): Visible images ATR Imaging
Sampling Location

Collection Conditions:

Resolution: 4 cm\(^{-1}\)
Scans (time): 32 scans (~30 sec) per spot
Spectral Range: 4000 – 850 cm\(^{-1}\)
Collection Mode: Micro ATR & transmission (5 micron microtomed slices)
Pixel Size & FOV: 1.1 microns/pixel, 70x70 microns
Sausage Packaging – ATR Chemical Images (Spot 1)

1. ATR Image @ 2850 cm⁻¹
   PE
   ~46 um thick

2. ATR Image @ 1607 cm⁻¹
   PE (with additive)
   ~23 um thick

3. ATR Image @ 1725 cm⁻¹
   Polycarboxylic acid ester (possible)
   ~5 um thick

4. ATR Image @ 1202 cm⁻¹
   Polyamide
   Layer 1&3

ATR Chemical RGBY Composite Image
Red: PE
Yellow: PE (with additive)
Green: Polycarboxylic acid ester (possible)
Blue: Polyamide
Sausage Packaging – ATR Chemical Images (Spot 2)

Red: PE
Green: Polycarboxylic acid ester polymer (possibly)
Blue: Polyamide
White: Polypropylene
Cyan: EVOH

RBGWC composite image

PE (with additives) ~6 um thick (centre layer)
Polycarboxylic acid ester polymer (possibly) ~5 um thick
Polyamide ~7um (left) ~2um (centre) ~3um (right)
Polypropylene ~20um
EVOH ~5um (spectral overlap from Polyamide present)
Sausage Packaging – ATR Chemical Images (Spot 3)

15x high mag. obj. vis image

ATR Chemical Image

PP (possible with an additive as evident by peak at 1060cm\(^{-1}\))
~37 um thick

RBC composite image

Red: PE
Blue: Polyamide
Cyan: EVOH

Polyamide
~2um (left)
~3um (right)

EVOH
~5um thick
(spectral overlap from Polyamide present)
Sausage Packaging – ATR Chemical Images

The sample was measured across its entire width with three slightly overlapping ATR measurements, with a total collection time of ~1.5 mins.

As extremely low pressures are applied, there is no sample preparation (via Agilent’s “live ATR imaging” method) with samples being measured “as is” and no risk of sample surface deformation, which might otherwise make for sequential side-by-side, or slightly overlapping measurements impossible. There was also no evidence of sample carryover between the measurements.

With the excellent signal-to-noise data collected, quite a complex sample with at least 11 layers were revealed with spectral library searches assisting in layer identification.

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FTIR ATR imaging determined identity & approximate thickness (in microns)

PE ~11
PE with additive ~24
PE ~11
Polyacrylic acid ester ~5
PA ~7
PP ~20
PE ~6
PA ~3
EVOH ~5
PA ~3
PE ~37

Pixel size: 1.1micron
Obj mag: 15x, 0.62NA
FOV: 70x70um
Total system mag: 36x
Sausage Packaging – 25x Transmission High mag Chemical Images

- Even in transmission mode, the ultra high NA and very small pixel size has allowed for the resolution of the 3 micron PA layer, hence rivalling the spatial resolution of Ge micro ATR
- Most books and papers still talk of ~10um spatial resolution for transmission imaging!
- The 25x, 0.81NA is a revolution in objective design

![Image](image_url)

- Pixel size: 0.66 micron
- Obj mag: 25x, 0.81NA
- High mag: ON
- FOV: 85x85um
- Total system mag: 61x
- Working distance: 12mm
The ~3 micron PA layers are only just resolved, but the spectral differences between PA and EVOH are now much less, owing to the lower NA, and hence resolving power, of the 15x objective compared to the 25x objective.
With the larger pixel size, now at 3.3um, even with the high NA 25x objective, the ~3um PA layers cannot be spatially resolved.
As the pixel size gets larger, now at 5.5um, layers that are ~5 um now start to blur out.

Pixel size: 5.5 micron
Obj mag: 5x, 0.62NA
High mag: OFF
FOV: 700x700 um
Total system mag: 7.3x
At initial analysis, it appears that the defect is likely to be an Inorganic material, most probably a carbonate, or a carbonate containing mixture.
Micro ATR (FPA) imaging of defects in black rubber sample

Image created at 1644 cm\(^{-1}\)

Image created at 2848 cm\(^{-1}\)

70 μm

480 μm

840 μm

70 μm

10 μm

Polyamide

PE (Polyethylene)

Polyisoprene (natural rubber)
Electronics/Semicon FTIR Imaging
Spacer contamination on LCD filter

Total analysis time = 2 mins

Defects identified as dislodged Spacers

No sample prep and no sample damage
LCD Defect – Protein image (1647 cm⁻¹)

Total analysis time = 1 mins

Defects identified as protein, most probably flake of dead skin

No sample prep and no sample damage
Contaminated Circuit Board – FTIR ATR Imaging

Total analysis time = 2 mins

Spectra library search reveals contaminant to be polyetherimide

ATR contact damage caused by other vendor
Summary of Cary FTIR Imaging

Highest Spatial Resolution
with new 25x, 0.81NA obj.
Re-defines, what is possible with actual spatial resolution of <1.5 microns possible in transmission mode!

Largest Field of View
Measure up to 2.4x2.4mm in a single shot

Fastest analysis time
Reduce analysis times by >100x, with higher light throughput & PCNR

Live FPA Imaging
Removes need for complex, time consuming sample prep & allows for delicate analysis