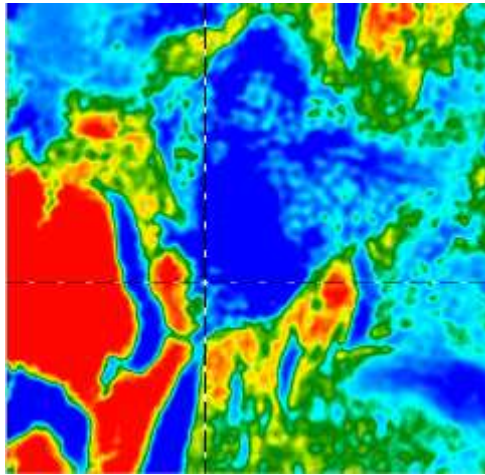


FTIR Imaging: The Benefits of Fine Spatial Detail

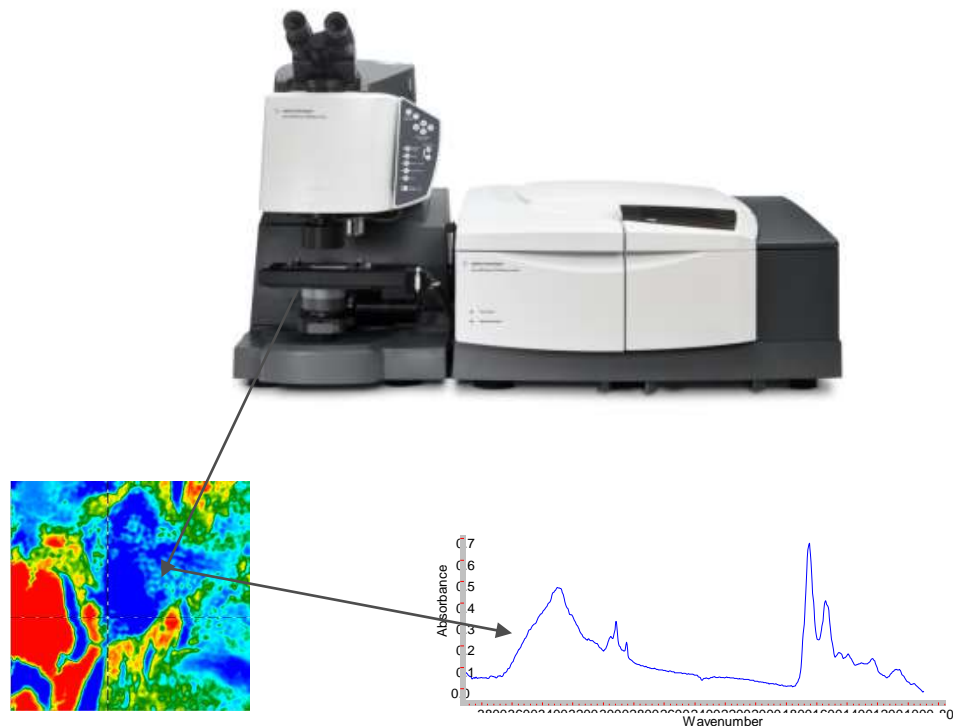


*Louis G. Tisinger, Ph.D.
FTIR Application Engineer*

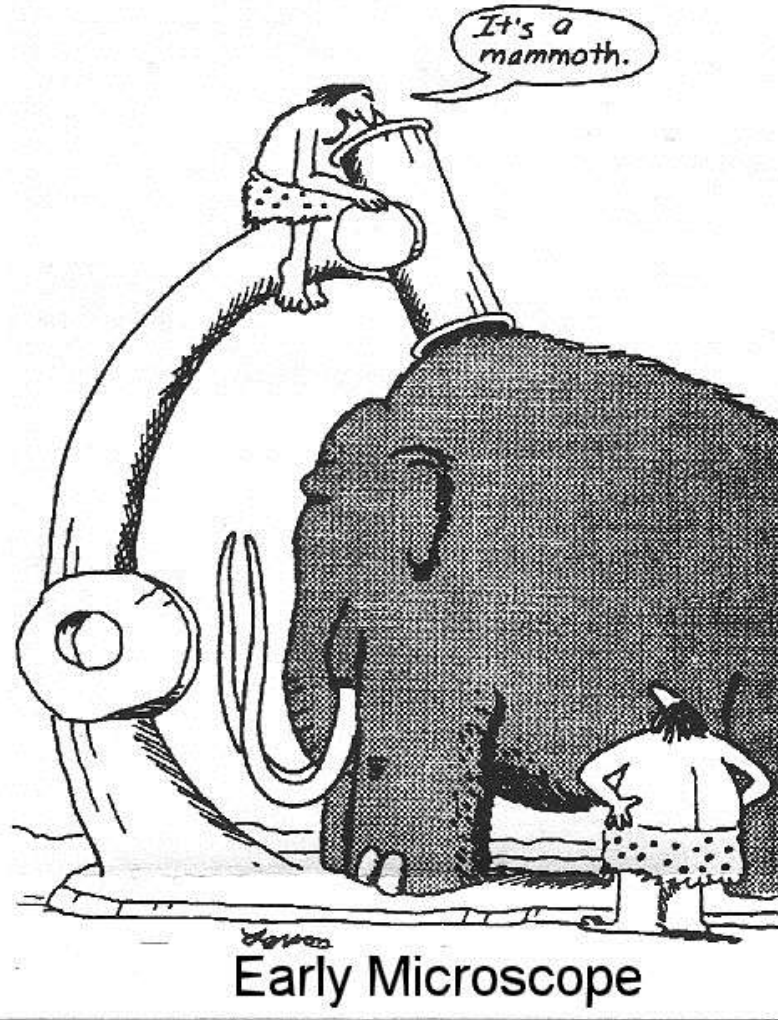
Agilent Technologies

Outline

1. Background and history
2. Spatial Resolution
3. Typical Applications
 1. Laminate
 2. Defect
 3. Biological
 4. Pharma

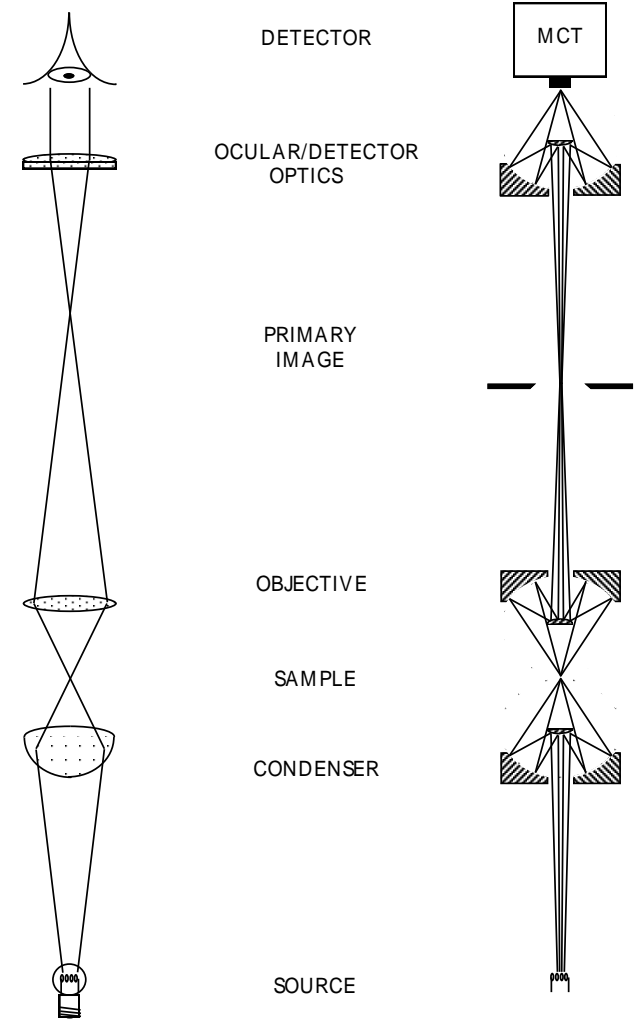


1. Background and History

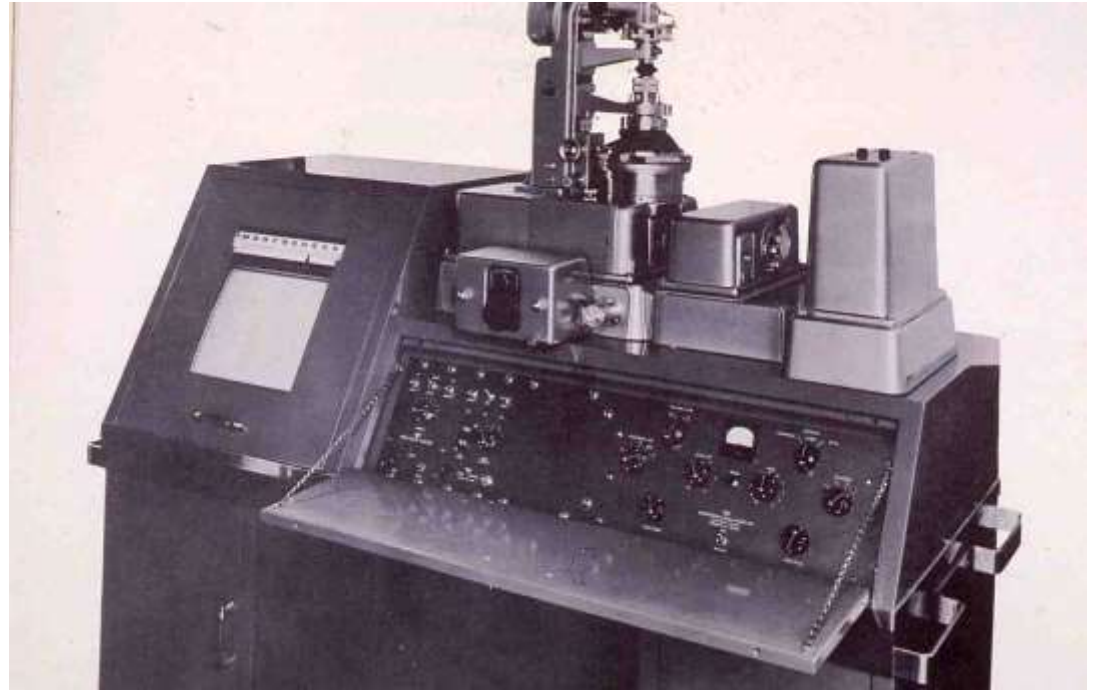


Visible and Infrared Microscopes

	Visible Microscope	IR Microscope
Region	Visible	IR
Optics	Refractive (lenses)	Reflective (mirrors)
Detector	Human Eye or Video Camera image on eye is magnified	IR sensitive elements e.g., single point and focal plane arrays detector



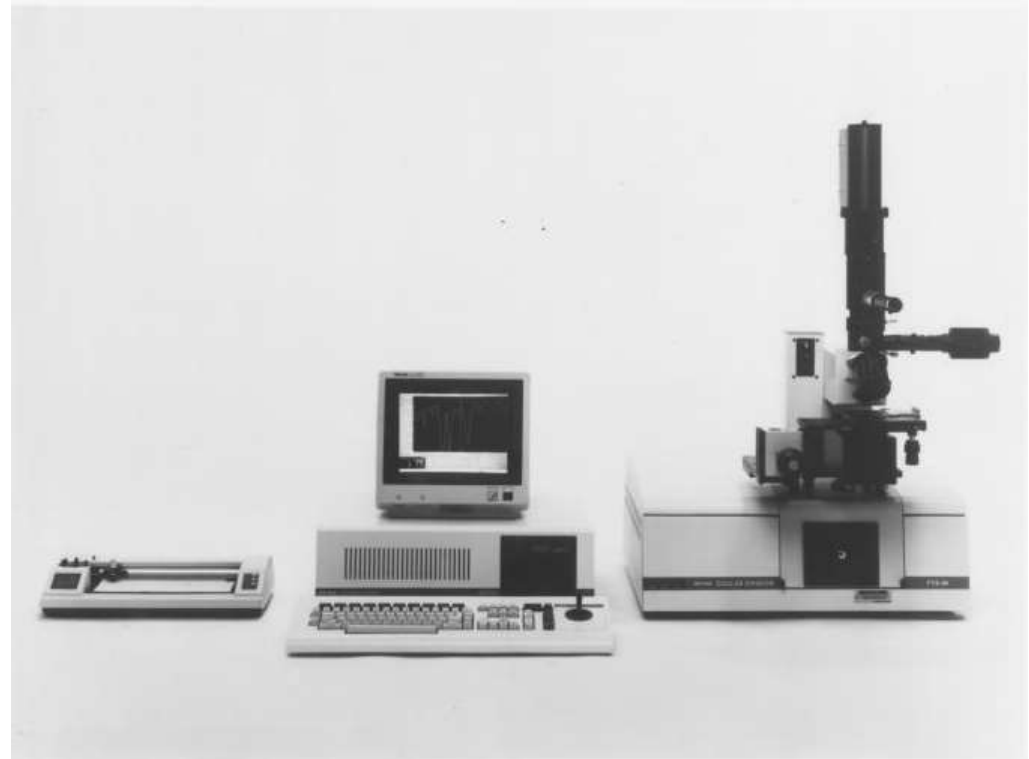
PE Model 85 (1953)



Digilab FTS-14 FTIR (1969)



First Commercially Available FTIR Microscope: Digilab UMA 100 (1980-81)



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



Agilent Technologies

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

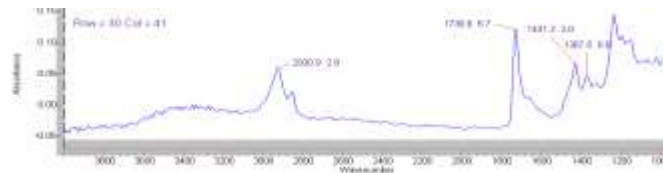
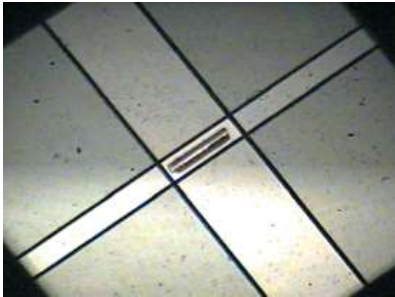


FTIR Microscopy/imaging measurement modes:

Single point: CARY 610: One detector element collects 1 spectrum per scan

To achieve the spatial resolution on the sample:

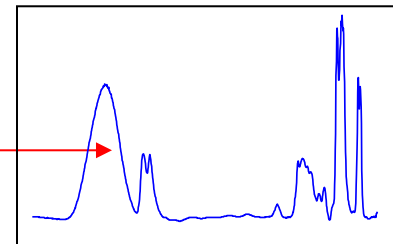
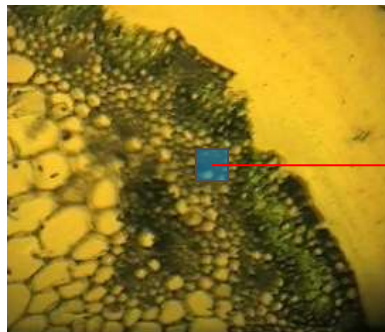
- aperture around the area of interest to eliminate spectral interference from the surrounding area.
- Typical achievable spatial resolution is 10-20 microns



Single-point Measurements

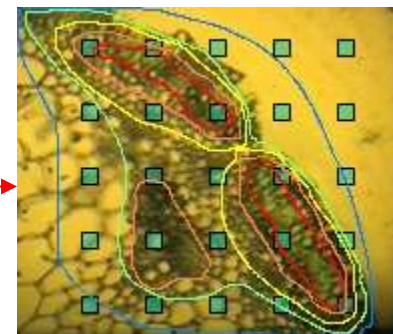
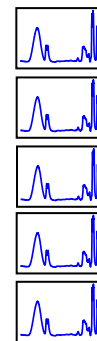
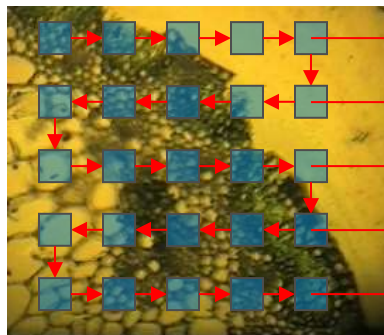
1 : Single Point

Single or multiple spectra of different zones of a sample



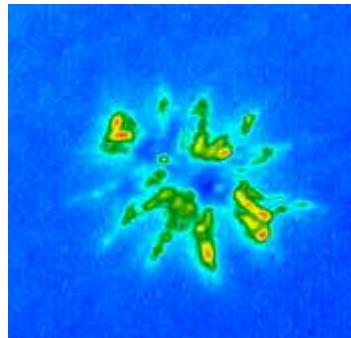
2: Single Point Mapping

Automated acquisition of spectra (one by one) defined by a grid. A hundred points can take several hours.



FTIR Imaging

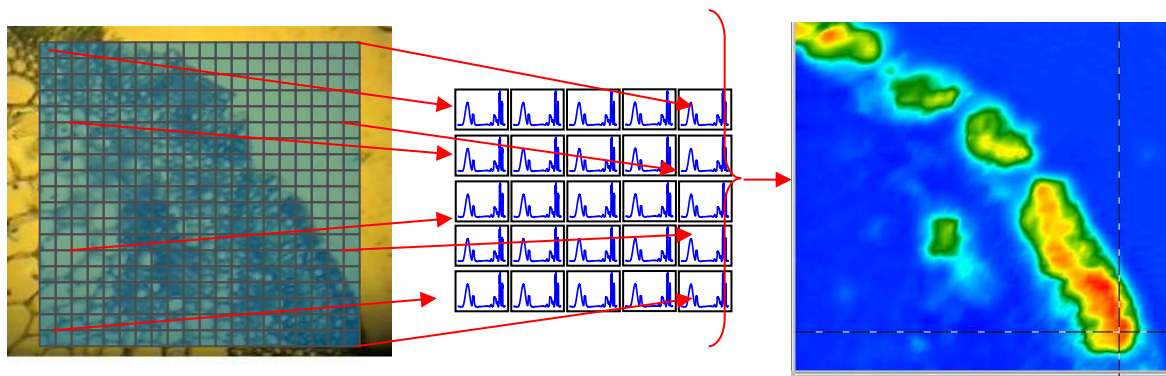
- By use of multi-channel detector, “Focal Plane Array (FPA)”
- Needs no aperture.
- Needs no sample scanning (raster scanning to produce an image).
- Whole area at the same time.
- Measuring time can be reduced dramatically.
- Provides high-fidelity chemical images



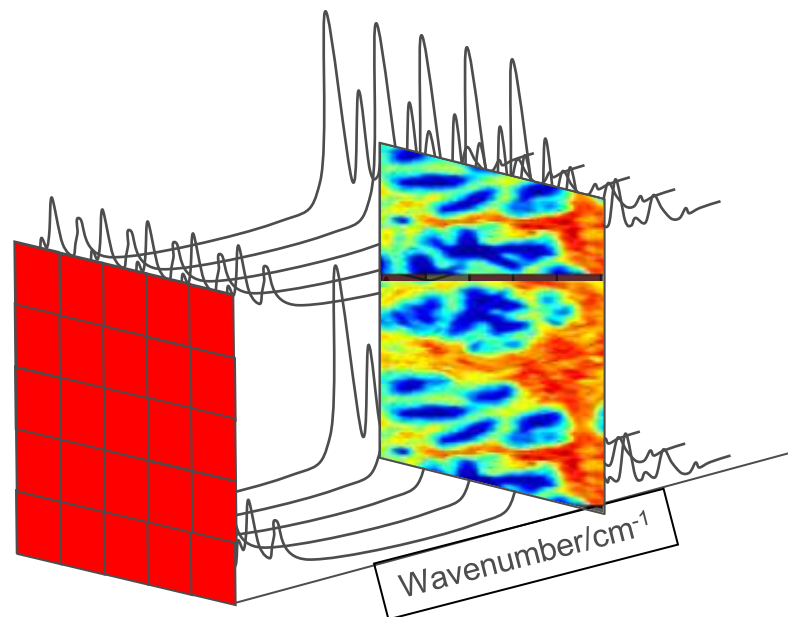
FTIR Microscopy/imaging measurement modes:

FPA Imaging

With an FPA detector (128x128), up to **16384** spectra simultaneously in a single measurement



Each pixel = 0.66 μm / 3.3 μm (25x objective)
1.1 μm / 5.5 μm (15x objective)
19 μm (4x objective)



System magnification

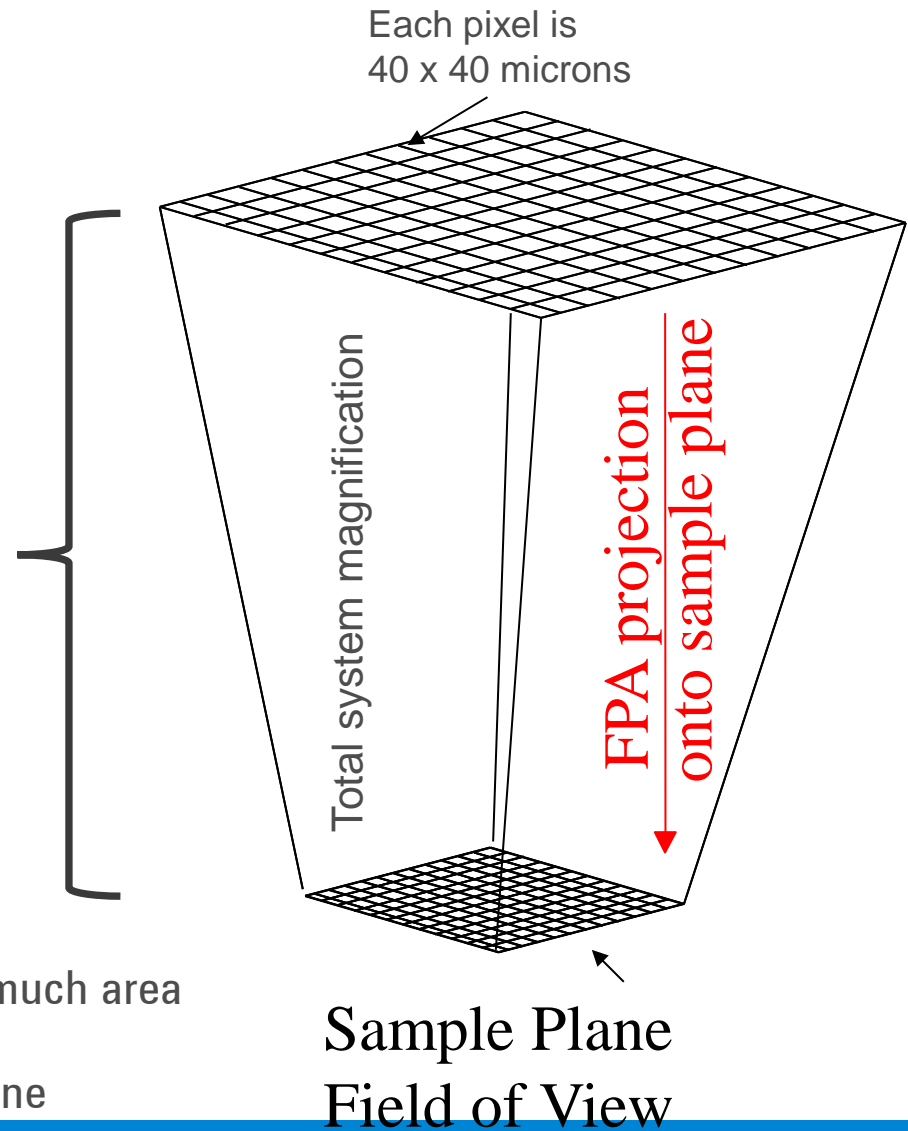
FPA

Focal Plane Array Detector

**Microscope
Optic**

FOV
Field Of View

FOV means how much area
the FPA can view
on the sample plane



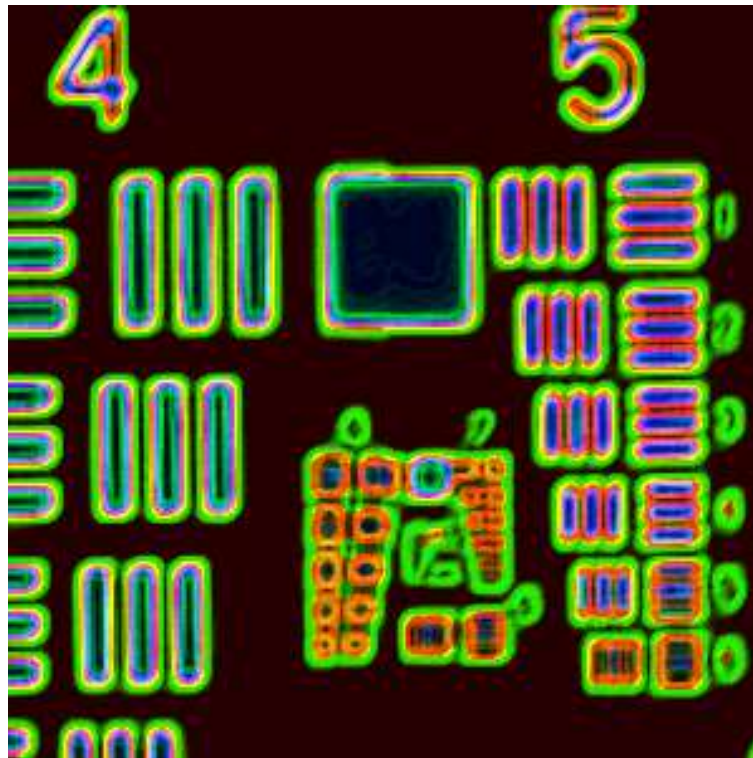
Agilent Technologies

Why use FPA chemical imaging?

1. Provides rapid high spatial resolution chemical distribution – the where (spatial) and the what (spectral)
2. Allows for the measurement of features as small as a ~2 microns



2. SPATIAL RESOLUTION



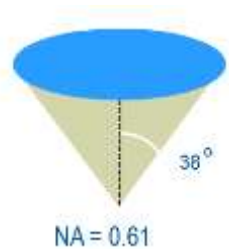
Spatial Resolution

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

Distance between those objects given by Rayleigh criteria:

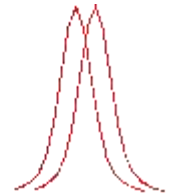
$$\Delta d = 1.22\lambda / 2(\text{NA}) = 0.61\lambda / n\sin\theta$$

If $\text{NA} = 0.61$, theoretical spatial resolution = wavelength (λ)



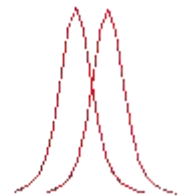
$$d < < \frac{0.61\lambda}{\text{N.A.}}$$

Unresolved



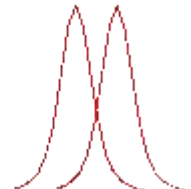
$$d < \frac{0.61\lambda}{\text{N.A.}}$$

Sparrow



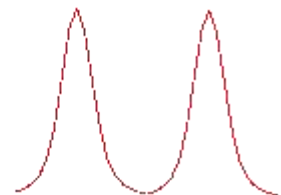
$$d = \frac{0.61\lambda}{\text{N.A.}}$$

Rayleigh

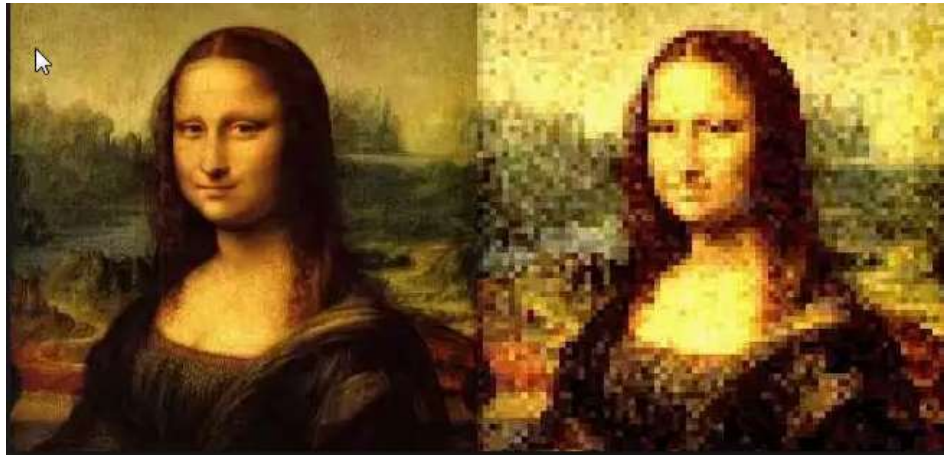


$$d > > \frac{0.61\lambda}{\text{N.A.}}$$

Resolved



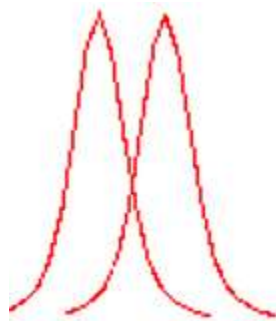
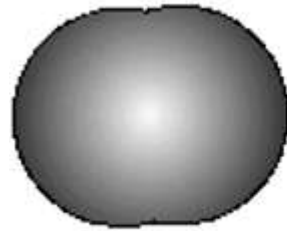
PIXEL SIZE



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

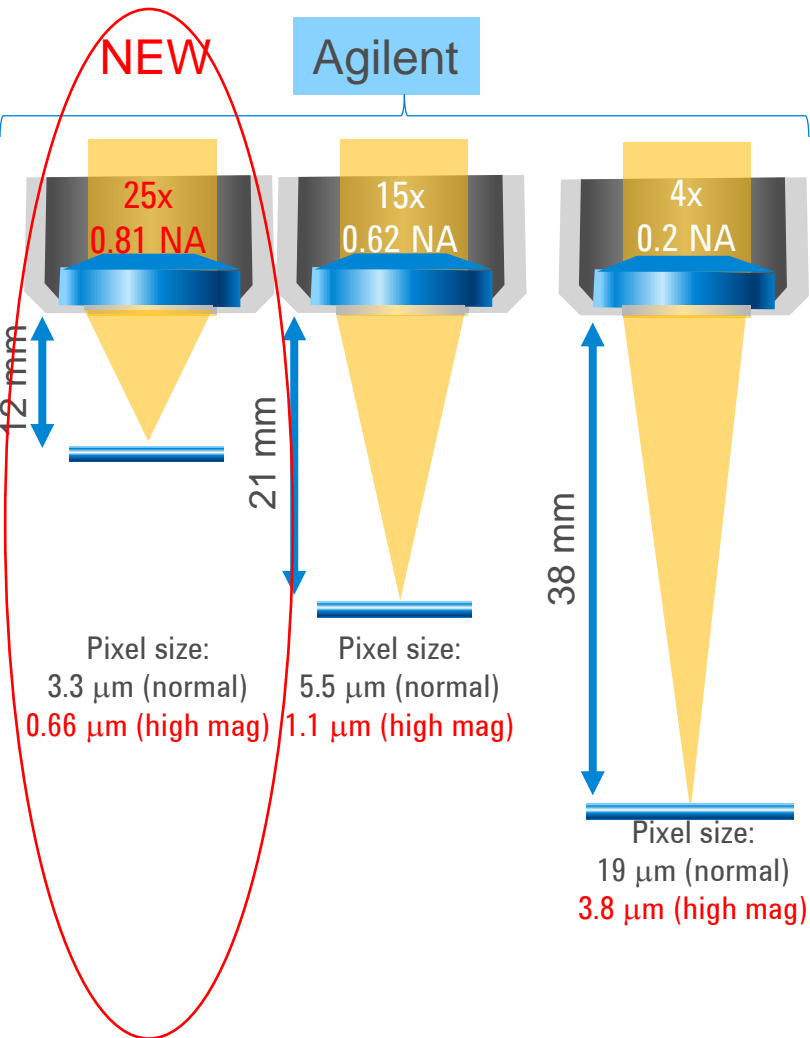
Summary of Spatial Resolution



Pixel size (magnification) and its role in achievable spatial resolution

- Spatial resolution depends on the objective NA and the wavelength (λ)
- Other Factor:
 - pixel size (function of microscope magnification)
 - pixel size > theoretical spatial resolution (Rayleigh Criterion), then pixels become the limiting factor for spatial resolution.
 - pixel size < the theoretical spatial resolution (ideally $\frac{1}{4}$), then this provides optimal sampling to achieve theoretical spatial resolution.
 - smaller pixels (high magnification) = Reduced FOV
- Agilent's system's high mag mode provides pixel size that is $\frac{1}{4}$ of theoretical spatial resolution

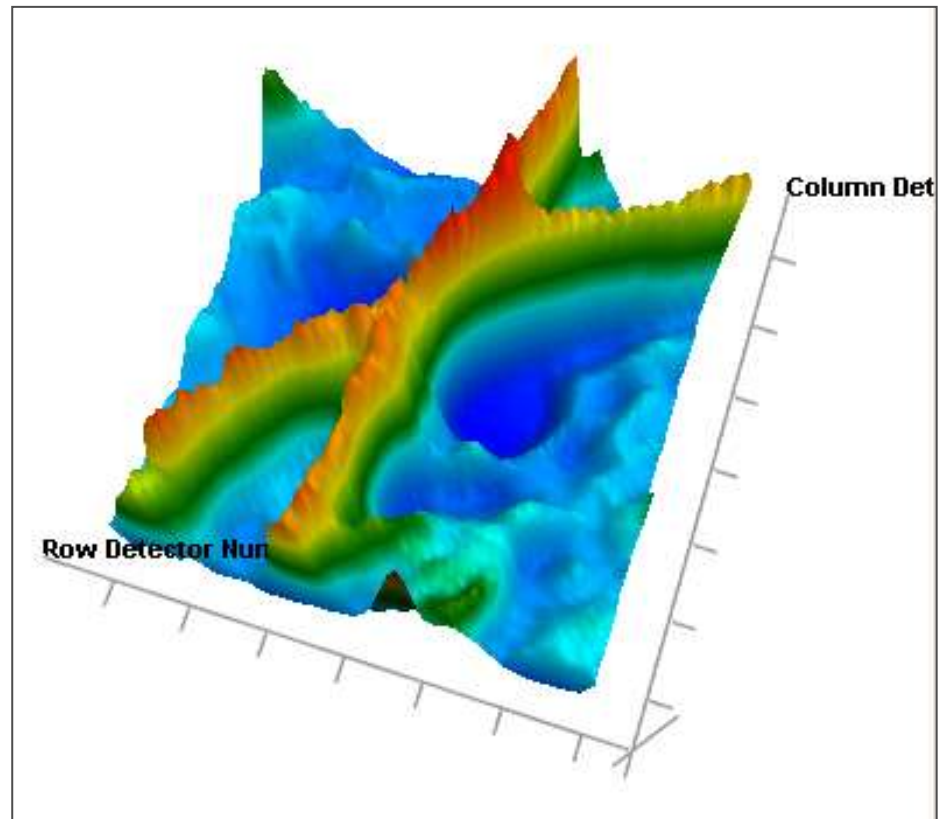
Microscope objectives: some comparisons



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.



3. APPLICATIONS



Polymer Film Laminate FTIR Imaging



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.



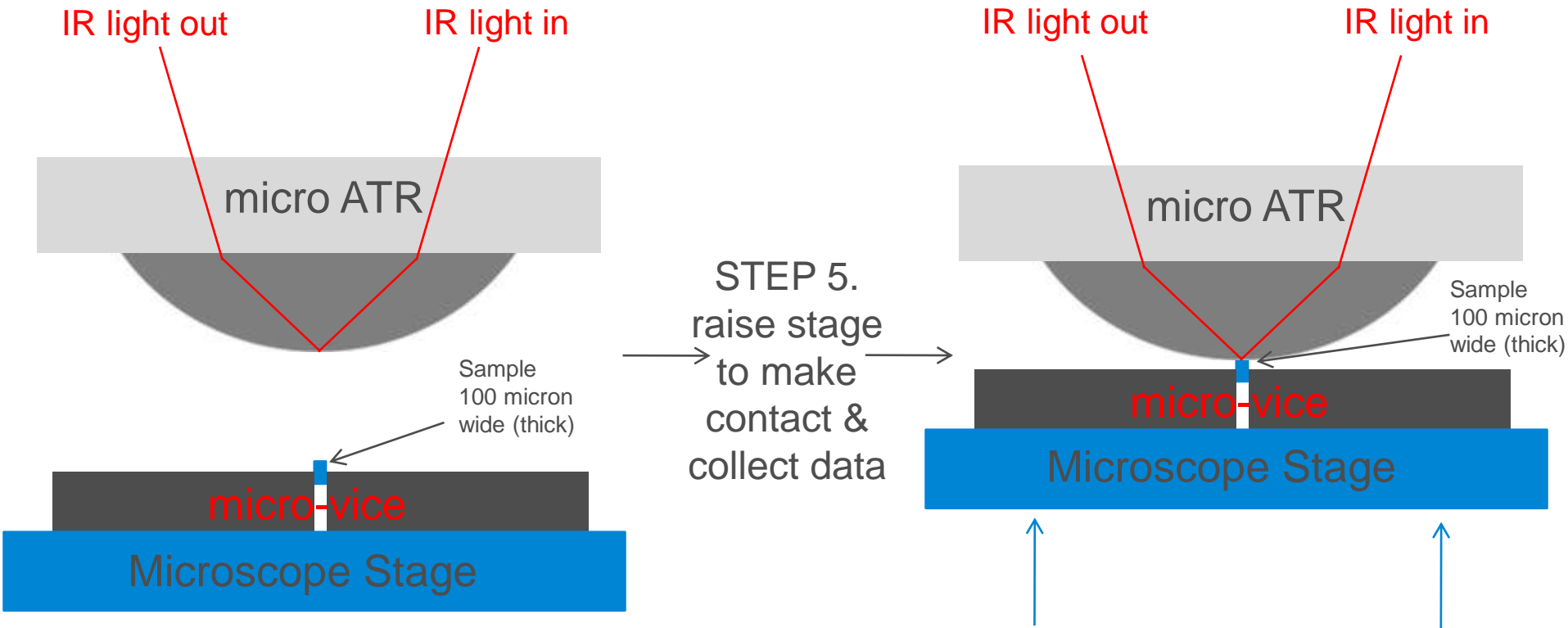
Step 3. Cross-section sample with razor



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



ATR Contact with sample



- ATR improves spatial resolution

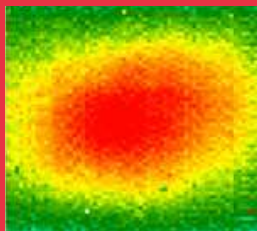
with Ge ATR (refractive index = 4): $n \sin \theta = 4 \times 0.61$: $d = 0.25\lambda$



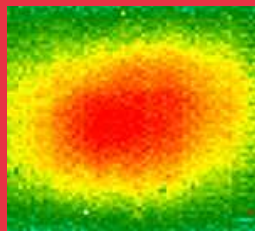
Live FPA Imaging with enhanced chemical contrast

Competitive system

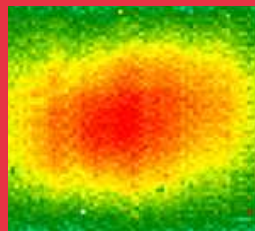
a. No contact



b. First contact



c. Good contact



Cary FTIR microscope

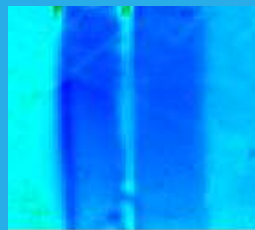
d. No contact



e. First contact



f. Good contact



Agilent's unique chemical contrast feature provides clear differences in live IR images between no contact being made with the sample vs good ATR contact.

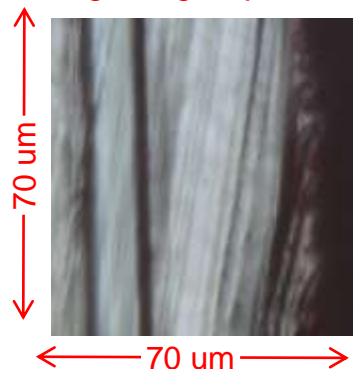
This provides a feedback mechanism on when to stop applying pressure to the sample, avoiding excess pressure that can damage the sample.

The benefit is removing time consuming resin embedding sample preparation to increase productivity, as well as avoiding applying too much pressure to your sample that can cause damage.

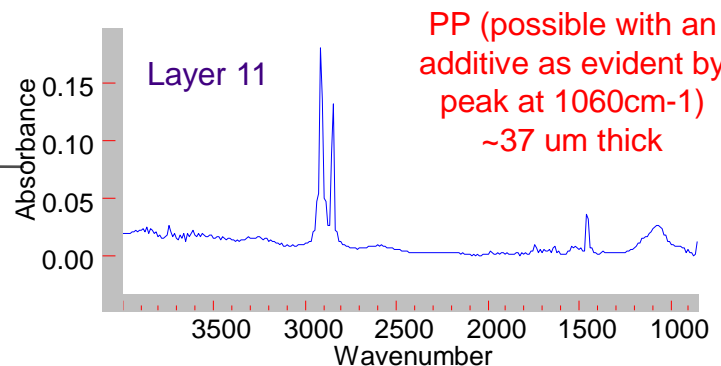
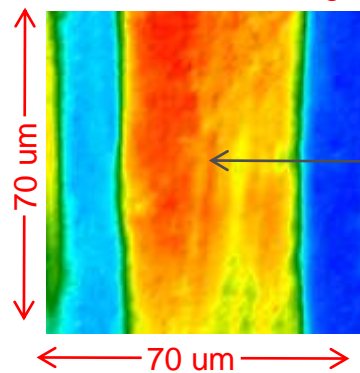


Sausage Packaging – ATR Chemical Images (Spot 3)

15x high mag. obj. vis image

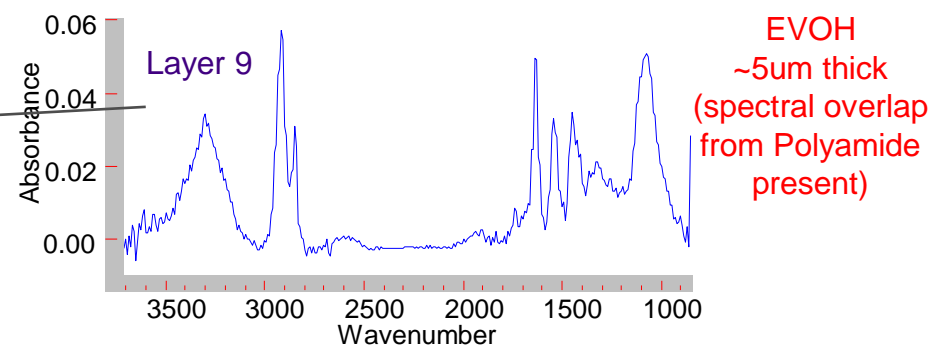
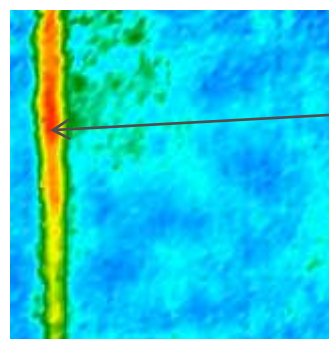
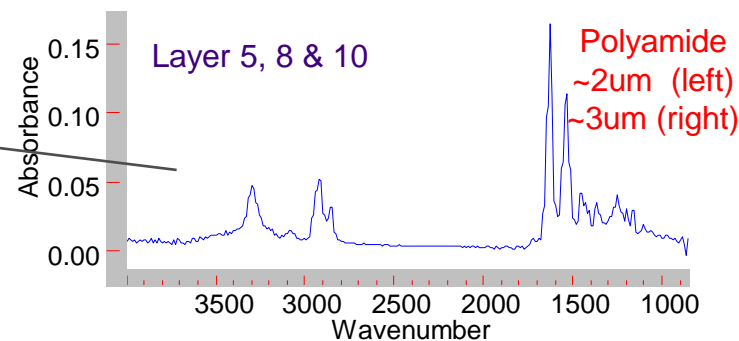
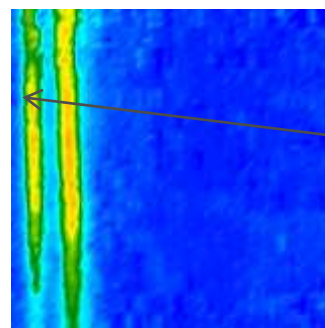
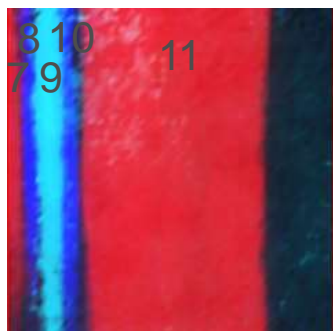


ATR Chemical Image

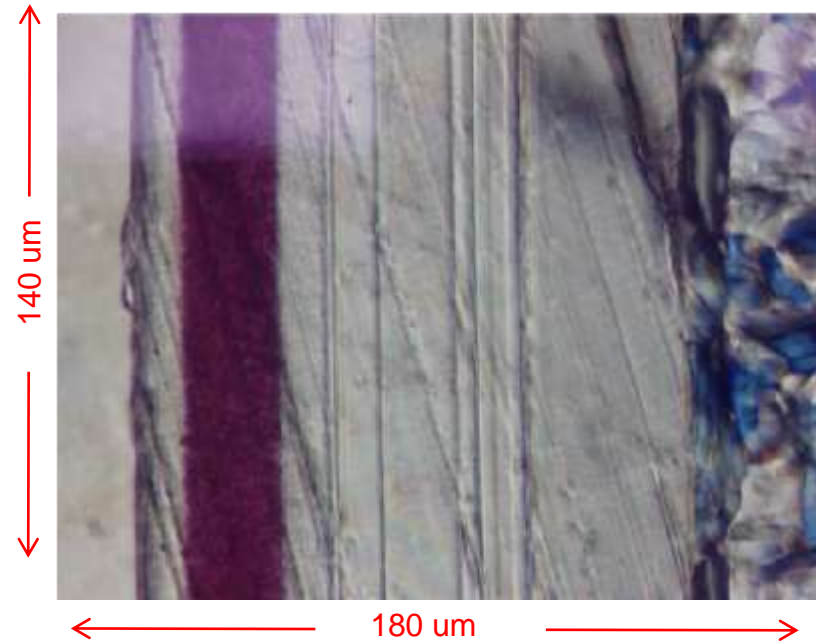


RBC composite image

Red: PE
Blue: Polyamide
Cyan: EVOH



Polymer Laminate comparisons



Micro Ge ATR
1.1um pixel size



25x, "high mag" trans,
0.66um pixel size



15x, "high mag" trans,
1.1um pixel size



25x, "std mag" trans,
3.3um pixel size



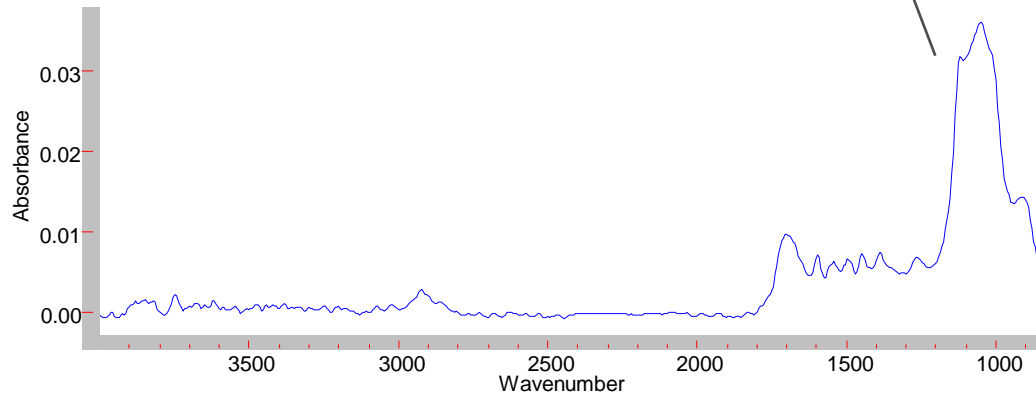
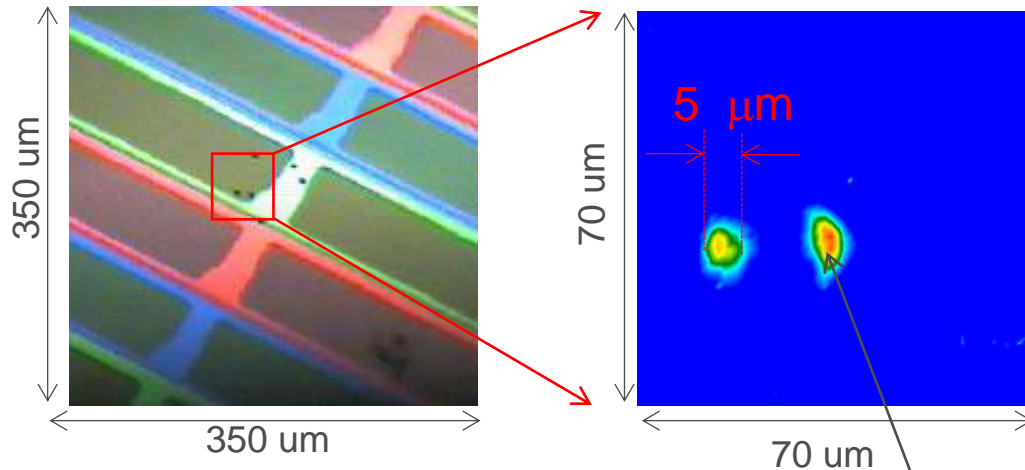
15x, "std mag" trans,
5.5um pixel size



Electronics/Semicon FTIR Imaging



Spacer contamination on LCD filter



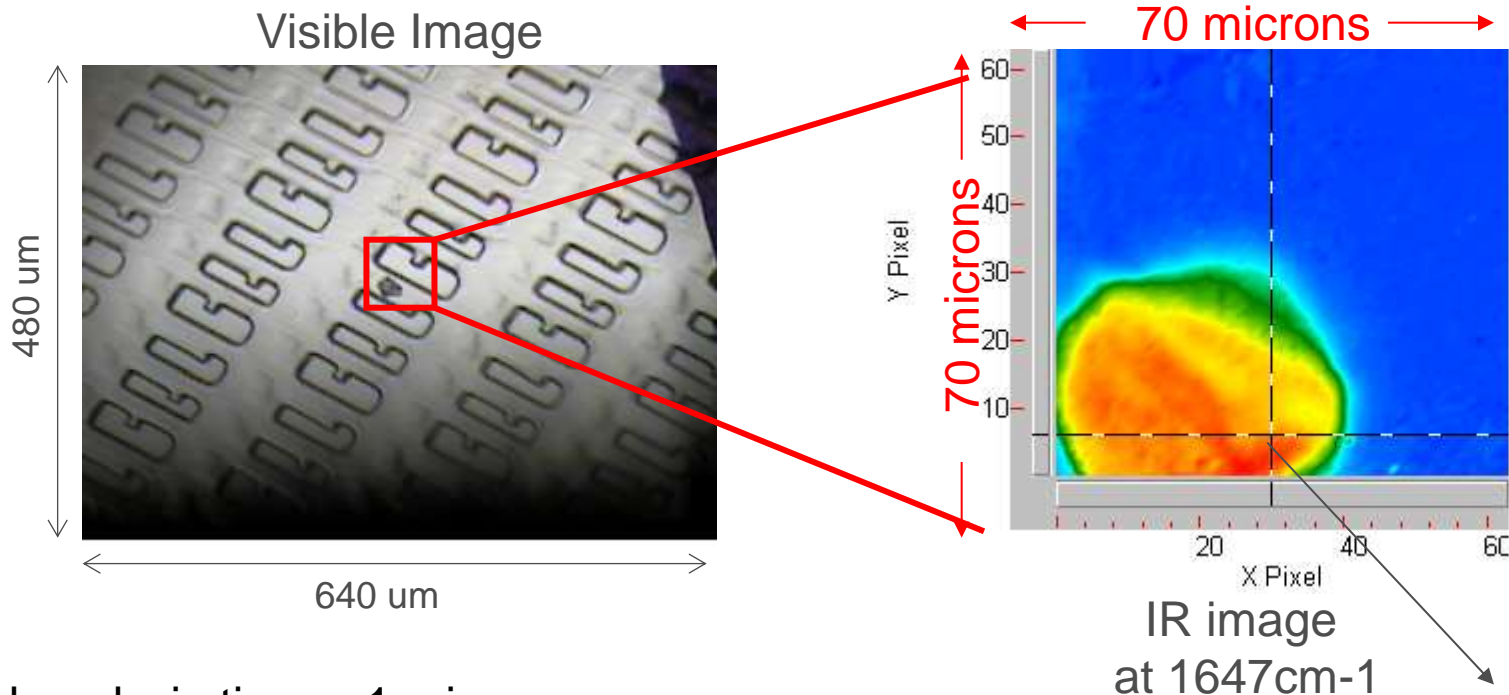
Total analysis time = 2 mins

Defects identified as dislodged Spacers

No sample prep and no sample damage



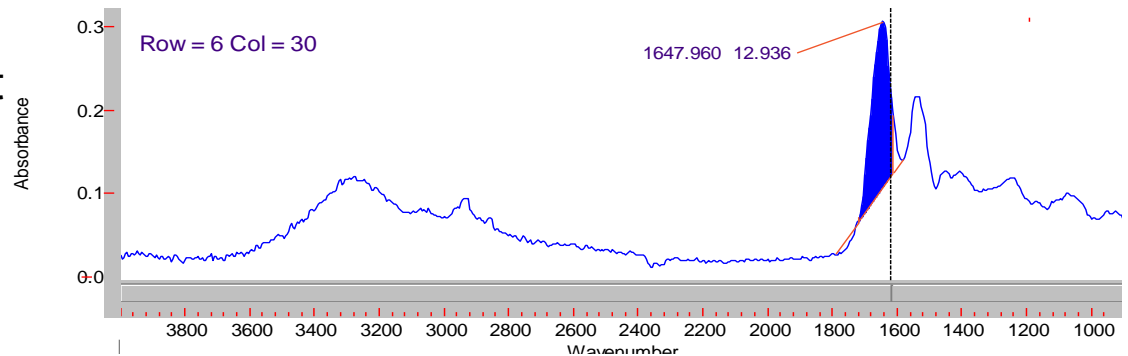
LCD Defect – Protein image (1647 cm⁻¹)



Total analysis time = 1 mins

Defects identified as protein, most probably flake of dead skin

No sample prep and no sample damage



Agilent Technologies

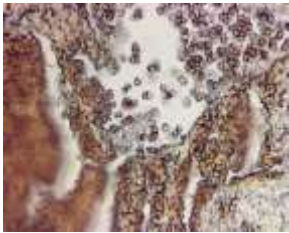
Biomedical & Biological FTIR Imaging



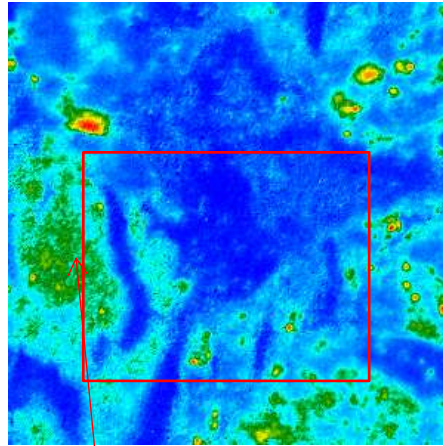
Breast Tissue imaging at different spatial resolution

Total IR FOV = 280x280 μm (2x2 mosaic), **pixel size = 1.1 μm** , total data collect time ~ 12 mins, (65,536 spectra!)

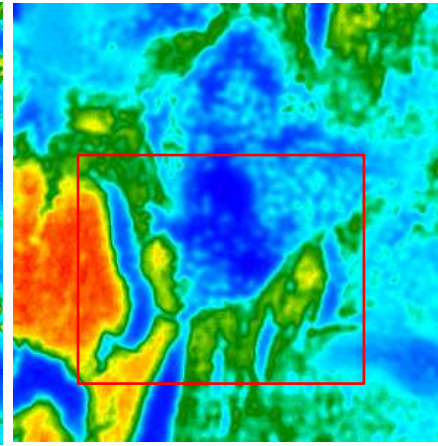
40x obj vis image



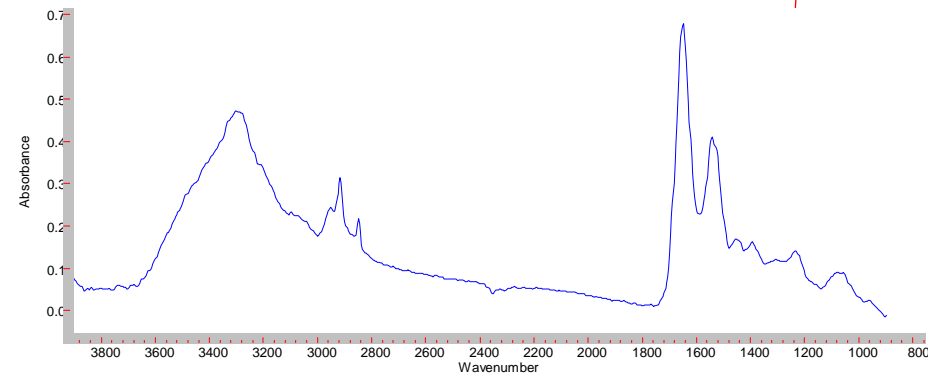
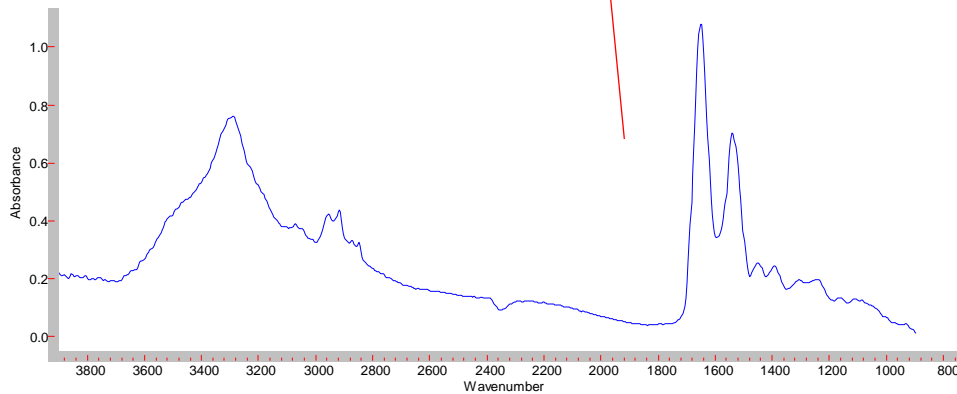
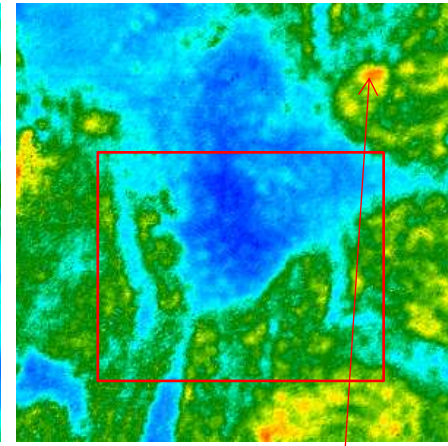
CH image (2943-2893 cm^{-1})



Amide I image

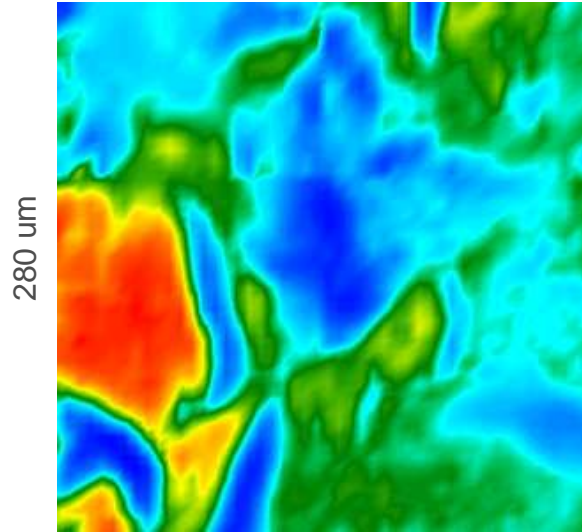


1233 cm^{-1} image

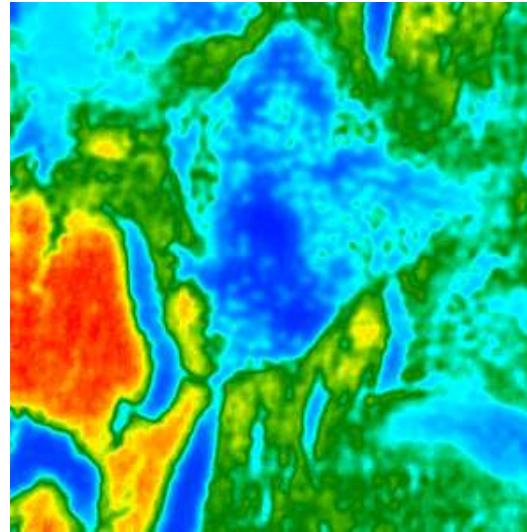


Protein images (Amide I): Normal, High & Ultra high magnification

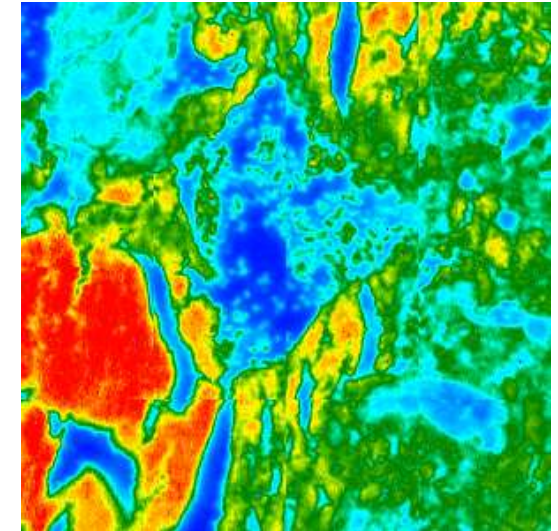
Normal Mag (5.5um)



High Mag (1.1um)

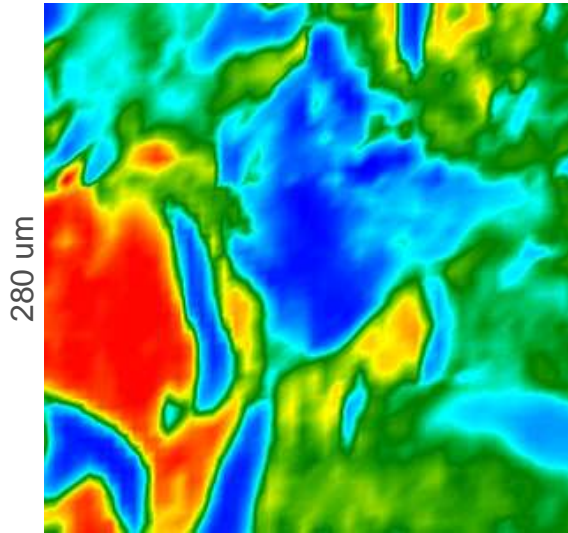


Ultra High Mag (0.66um)

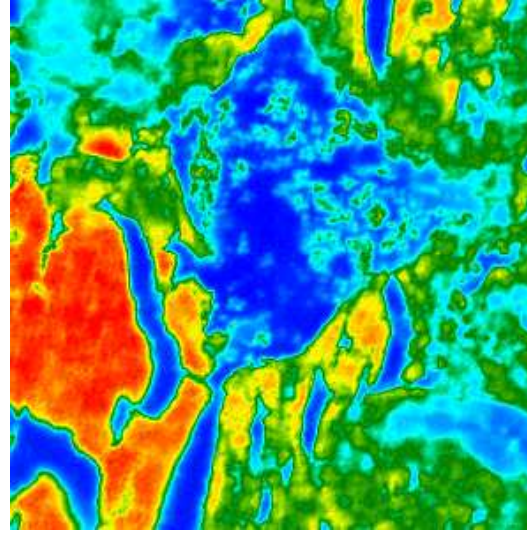


Protein images (OH/NH): Normal, High & Ultra high magnification

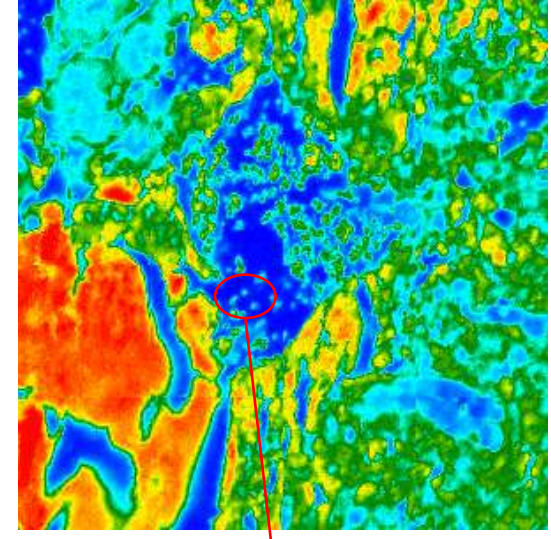
Normal Mag (5.5um)



High Mag (1.1um)



Ultra High Mag (0.66um)



40x obj vis image



The features (cells) within this oval shape are each about 1.5-2.0 um in diameter!

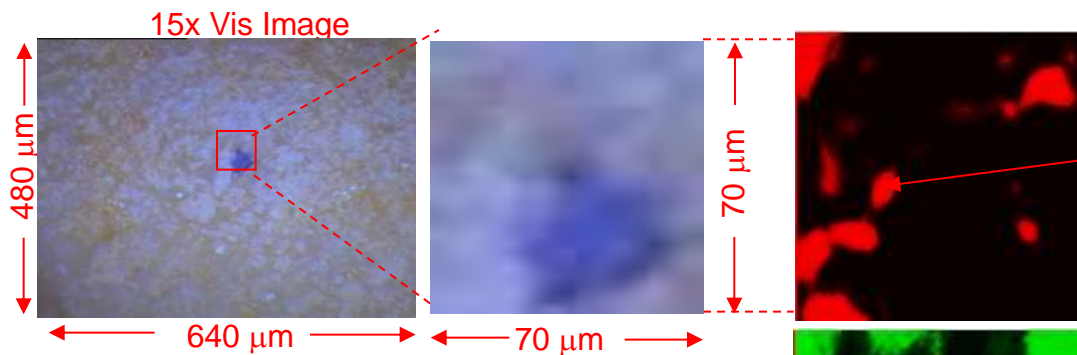


Agilent Technologies

Pharmaceutical FTIR Imaging



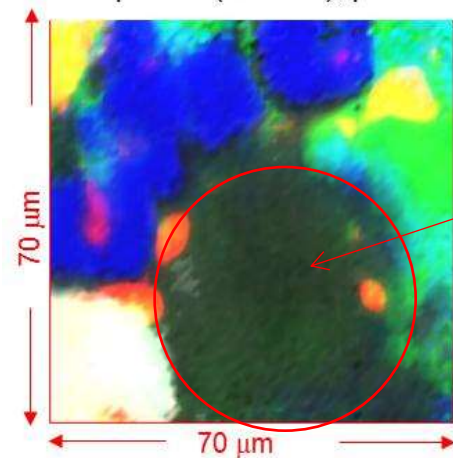
Tablet – Contamination Location



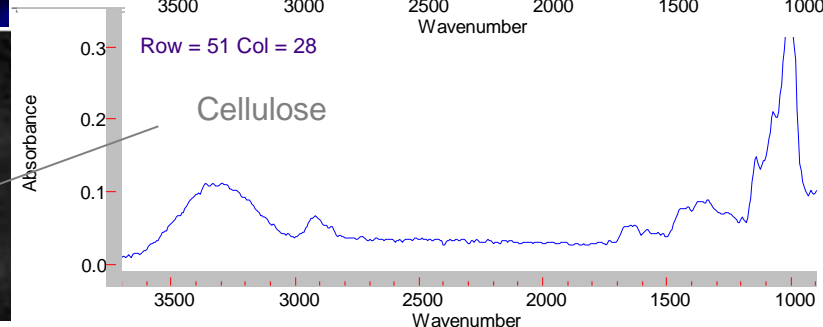
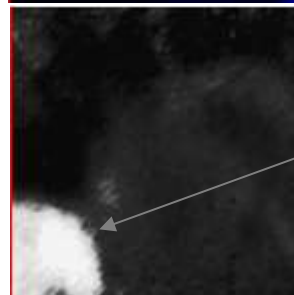
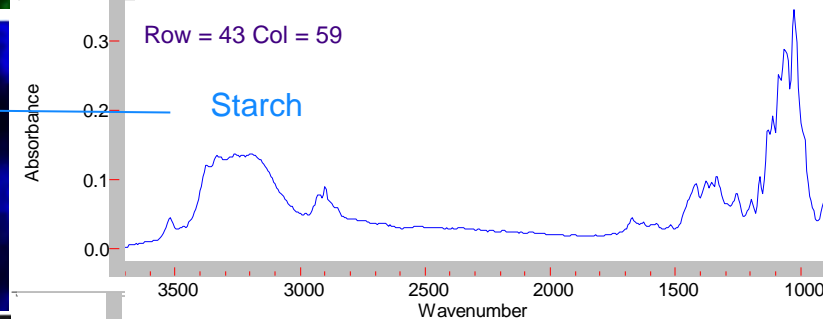
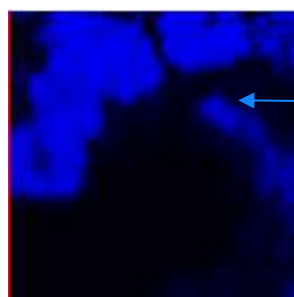
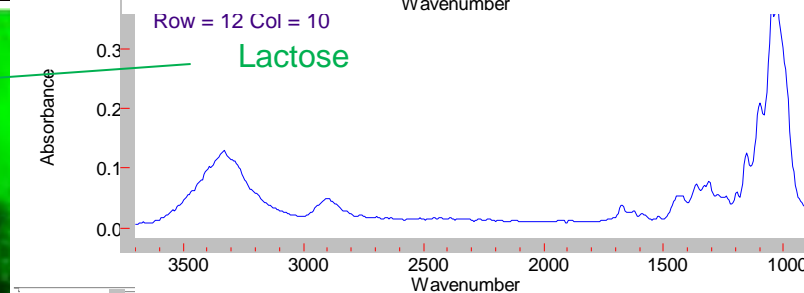
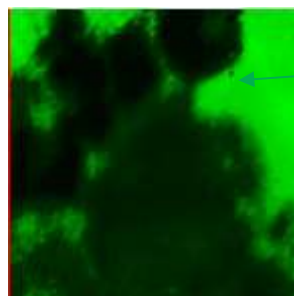
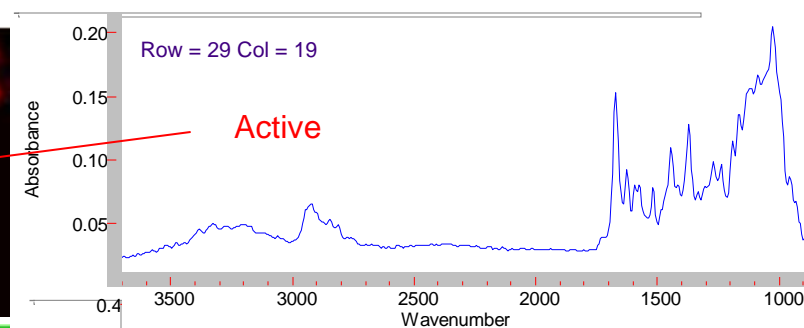
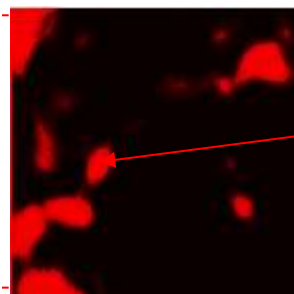
Clear and distinct domains from 4 constituents were detected:

- **Active**, compared to provided standard
- **Lactose**, identified from library search
- **Starch**, identified from library search
- **Cellulose** identified from library search

Composite (RGB), part 1

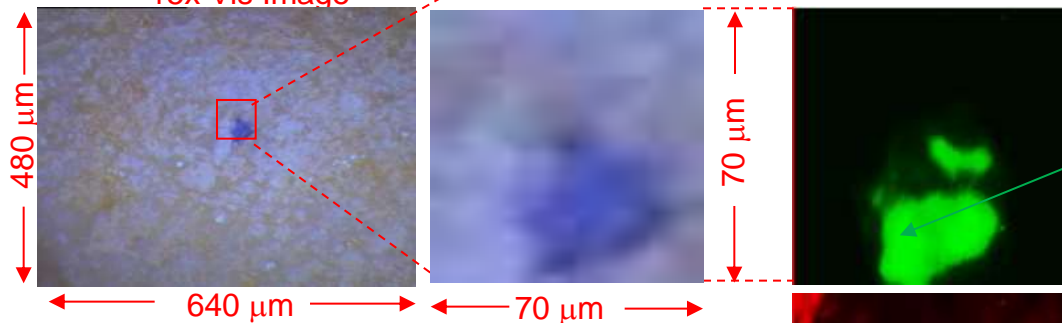


Defect
(next slide)



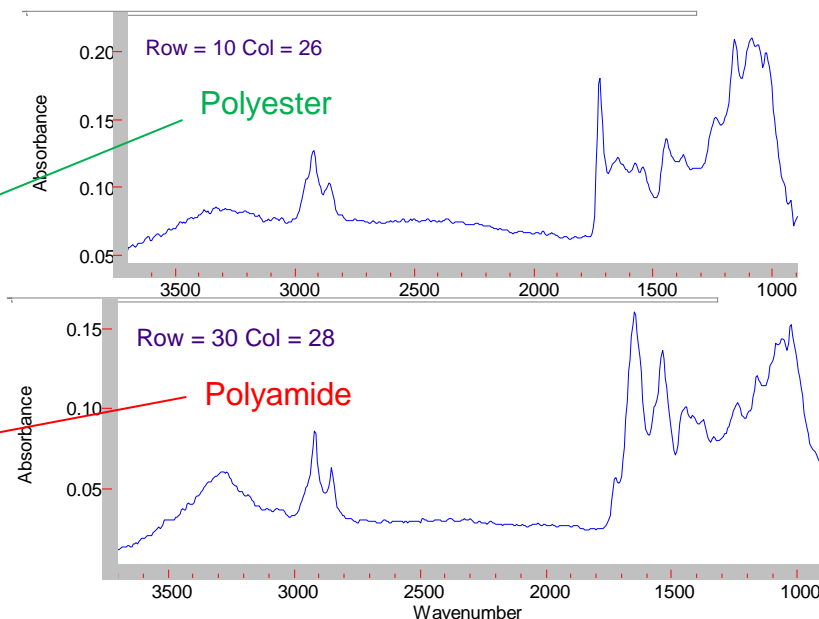
Tablet – Contamination Location

15x Vis Image

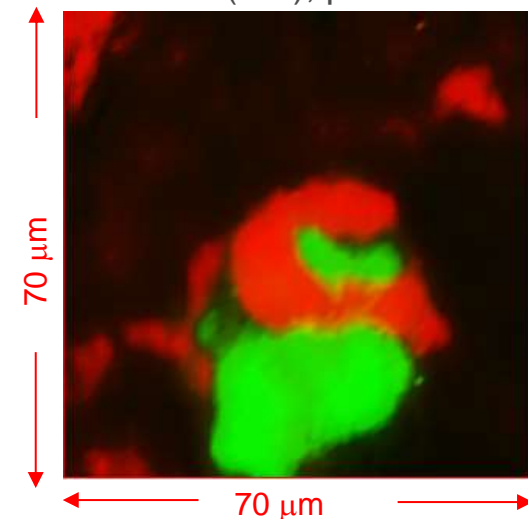


Clear and distinct domains from 4 constituents were detected:

- **Contaminant #1** – Possibly a polyester
- **Contaminant #2**, - Possibly a polyamide



Contaminant Composite (RG), part 2



From a single FTIR ATR imaging measurement and without damaging the sample, 4 known constituents and 2 unknown contaminants were imaged in 1 min

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

