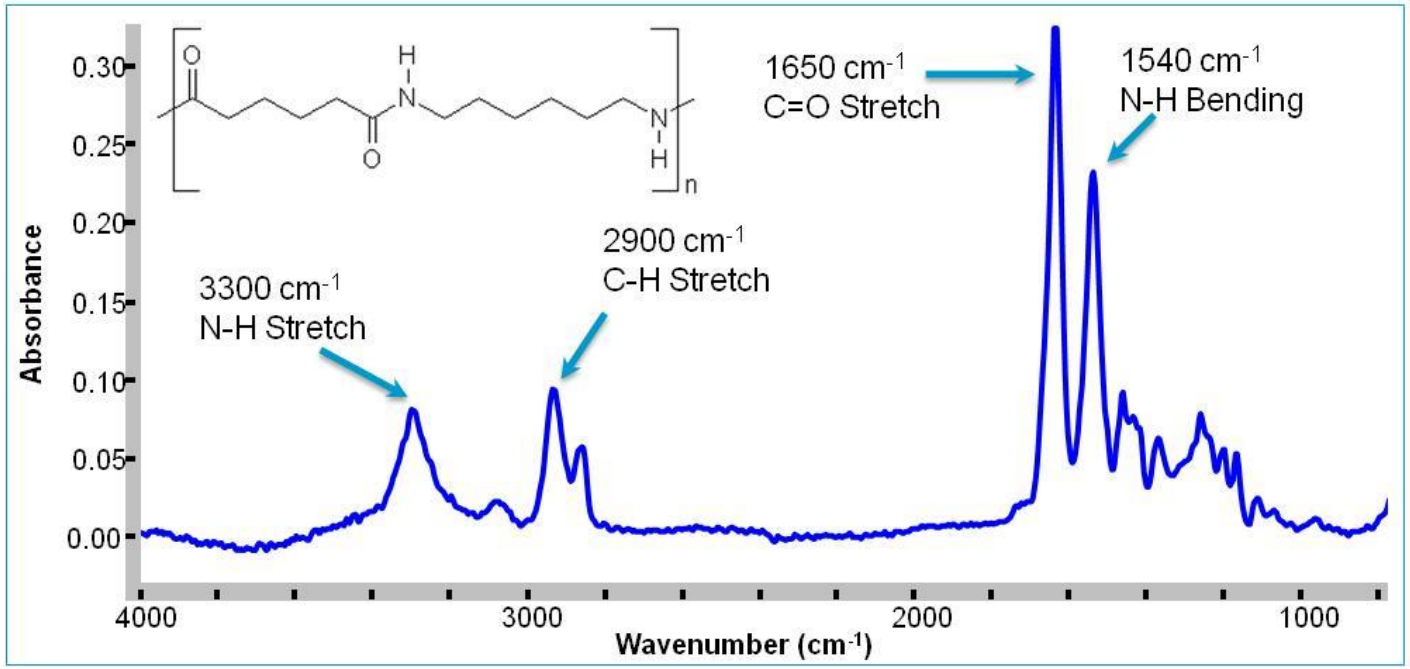
Identify Defects and Characterize in Minutes with FTIR Imaging Microscopy

Dr. Mustafa Kansiz
FTIR Imaging and Microscopy Product Manager

Agilent Technologies

FTIR Spectroscopy - What is it?

- Fourier Transform InfraRed Spectroscopy is the study of the interaction of infrared light with matter.
- The vibrations of bonds between atoms in a molecule are excited by IR light leading to absorbances that are specific to chemical structure specific



How can FTIR microscopy imaging help me?

An FTIR microscope has two essential purposes:

1. To allow users to visually see small (micron) sized samples
2. Collect accurate FTIR spectra from small samples

FTIR Imaging takes this to another level by providing spatial and spectral information from an area on your sample

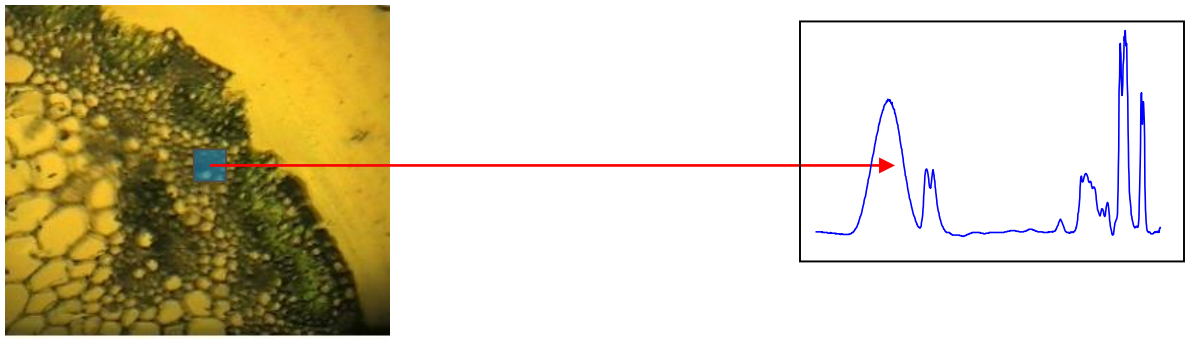
FTIR microscopy can collect data in four modes:

1. Single point
2. Single point mapping
3. Linear array mapping
4. 2-D Focal Plane Array (FPA) imaging

FTIR Microscope Measurement Modes

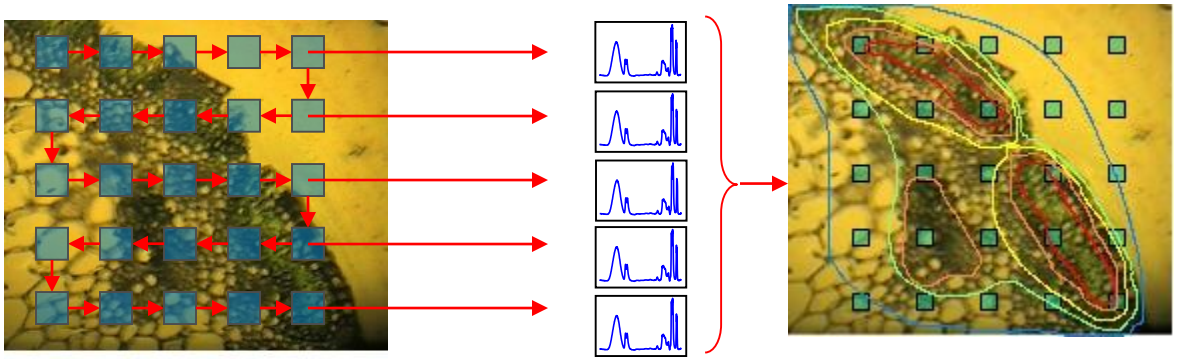
1 : Single Point

Single or multiple spectra of different zones of a sample



2: Single Point Mapping

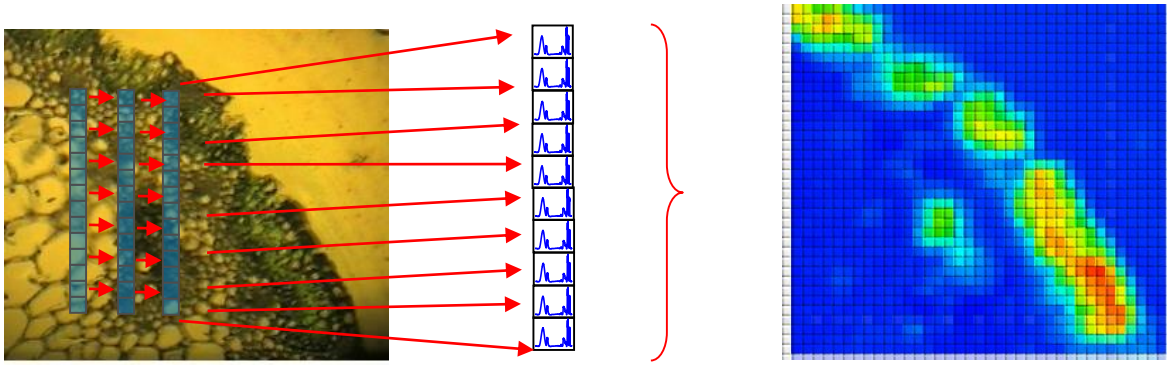
Automated acquisition of spectra (one by one) defined by a grid. A hundred points can take several hours.



FTIR Microscope Measurement Modes:

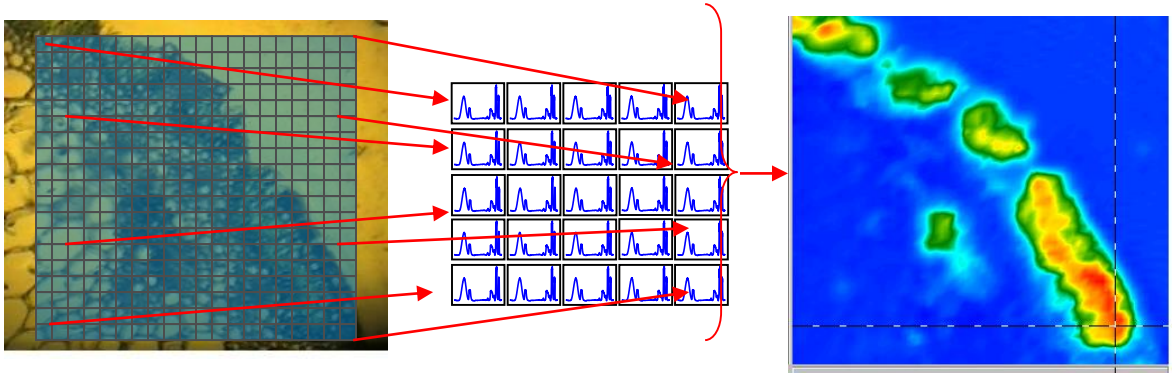
3: Linear array Mapping

Acquisition of spectra by a row (1x16) of detectors. Faster than single point mapping, but still much slower than FPA imaging



4: FPA Imaging

With an FPA detector, up to 16384 spectra can be recorded **simultaneously** in a single measurement.



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

Challenges facing the materials scientist/technician…

- Troubleshooting production samples (polymers, pharmaceuticals, 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)
  - Visualisation of chemical distribution at the “micro” scale, without damaging the sample, allowing later characterisation via other analytical techniques

FTIR Chemical Imaging and its applications

Materials/Polymer

Polymer laminates *(functional layer & adhesive identification)*
Defect analysis
Phase distribution
Composites

Biomedical/Biological Research

Early disease diagnosis (Cancers)
Study of diseases (Alzheimer's, kidney)
Plant/fungi tissue studies
Live cell chemical imaging (in water)
Microbial identification
Bone, teeth and cartilage

Forensics

Car paint layer & structure analysis
Trace evidence analysis

Art Conservation

Painting components & layer identification

Pharmaceuticals

Ingredient distribution
Coating analysis
Defect/particle analysis

Geology

Study of inclusions

Electronics

Defect analysis

Food/Cosmetics

Study of emulsions, eg cheese, mayonnaise, cream

A New Method of Magnification Enhancement

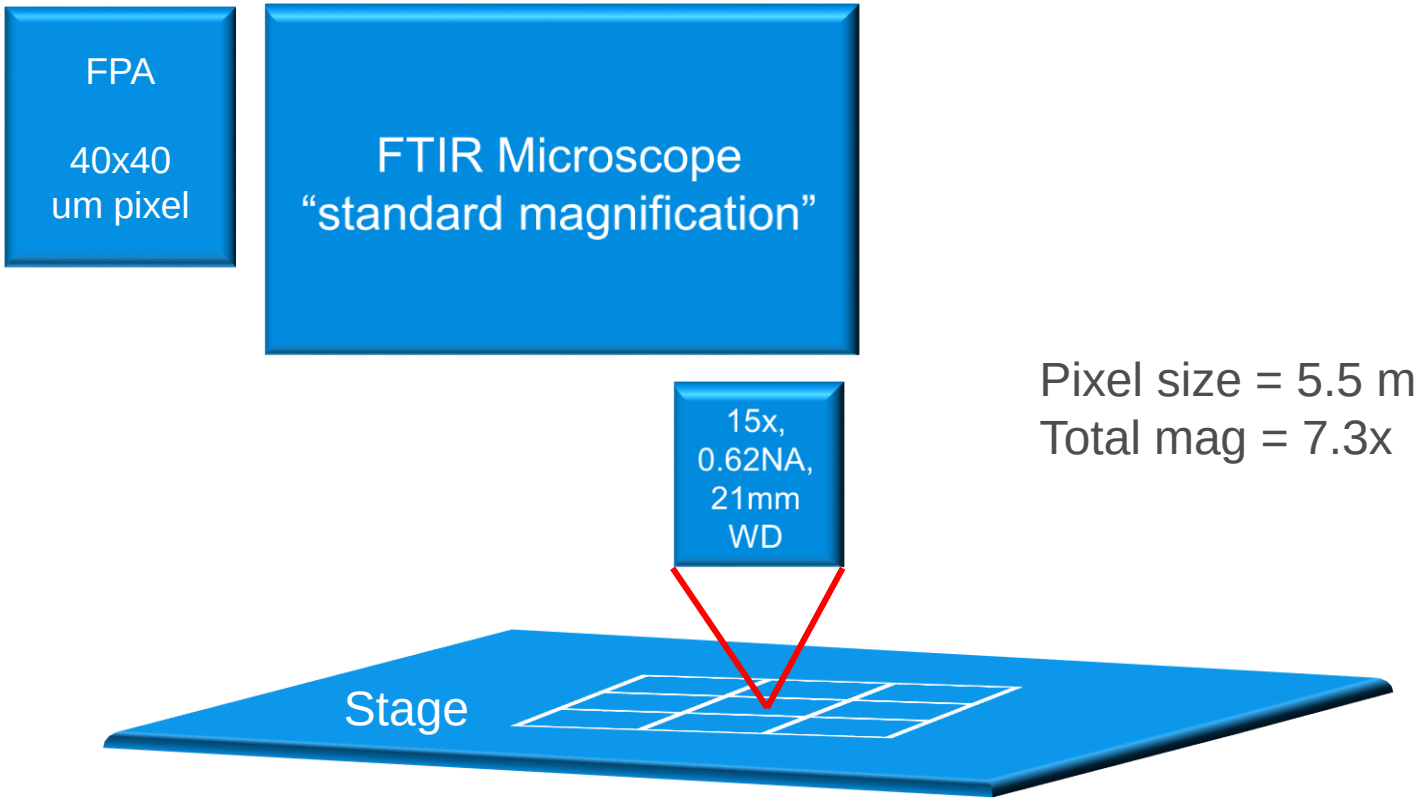
New Method of Magnification Enhancement

- 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

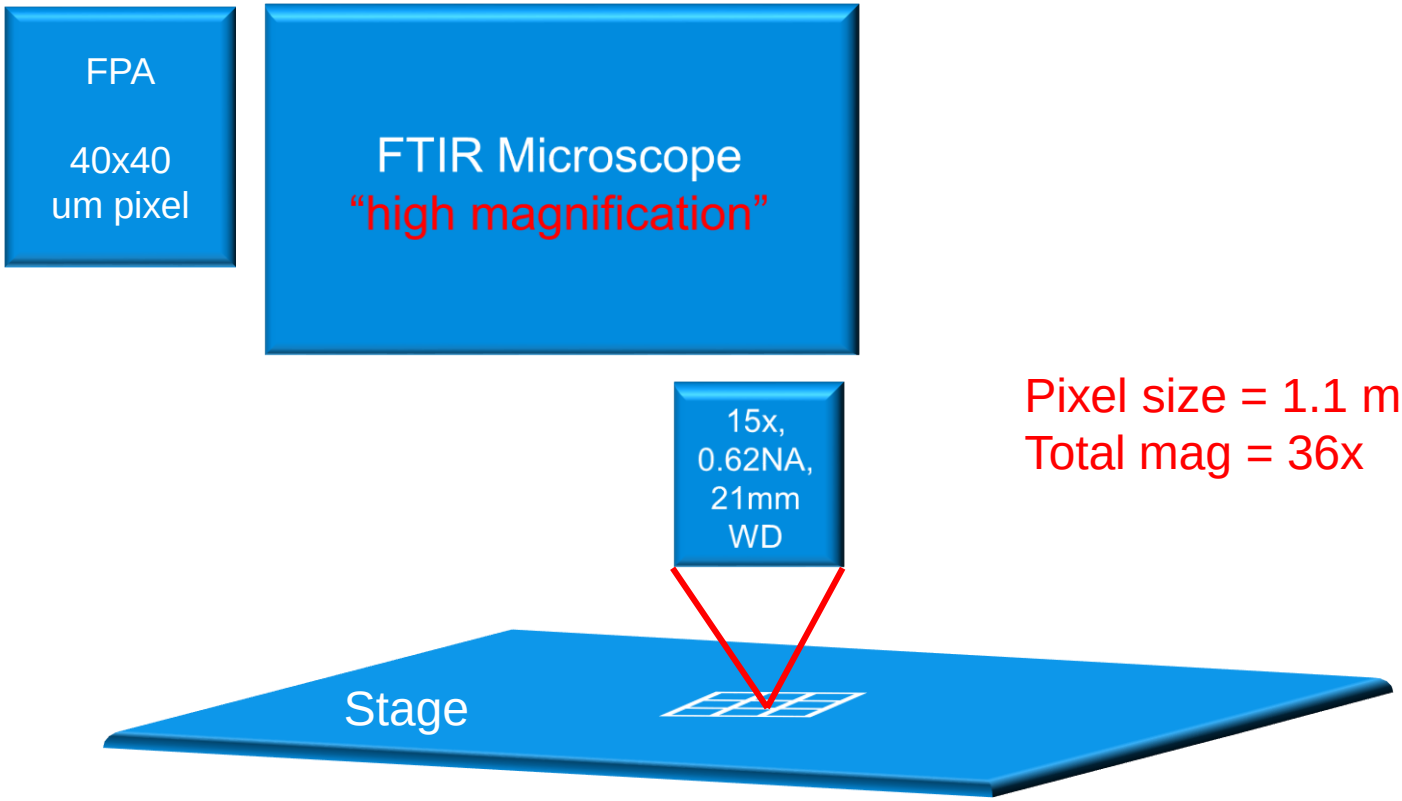
- It is important to note that, pixel resolution (total system magnification) is therefore NOT ONLY governed by the objective.

FTIR Microscope Magnification Schematic



Pixel size = 5.5 microns
Total mag = 7.3x

FTIR Microscope Magnification Schematic

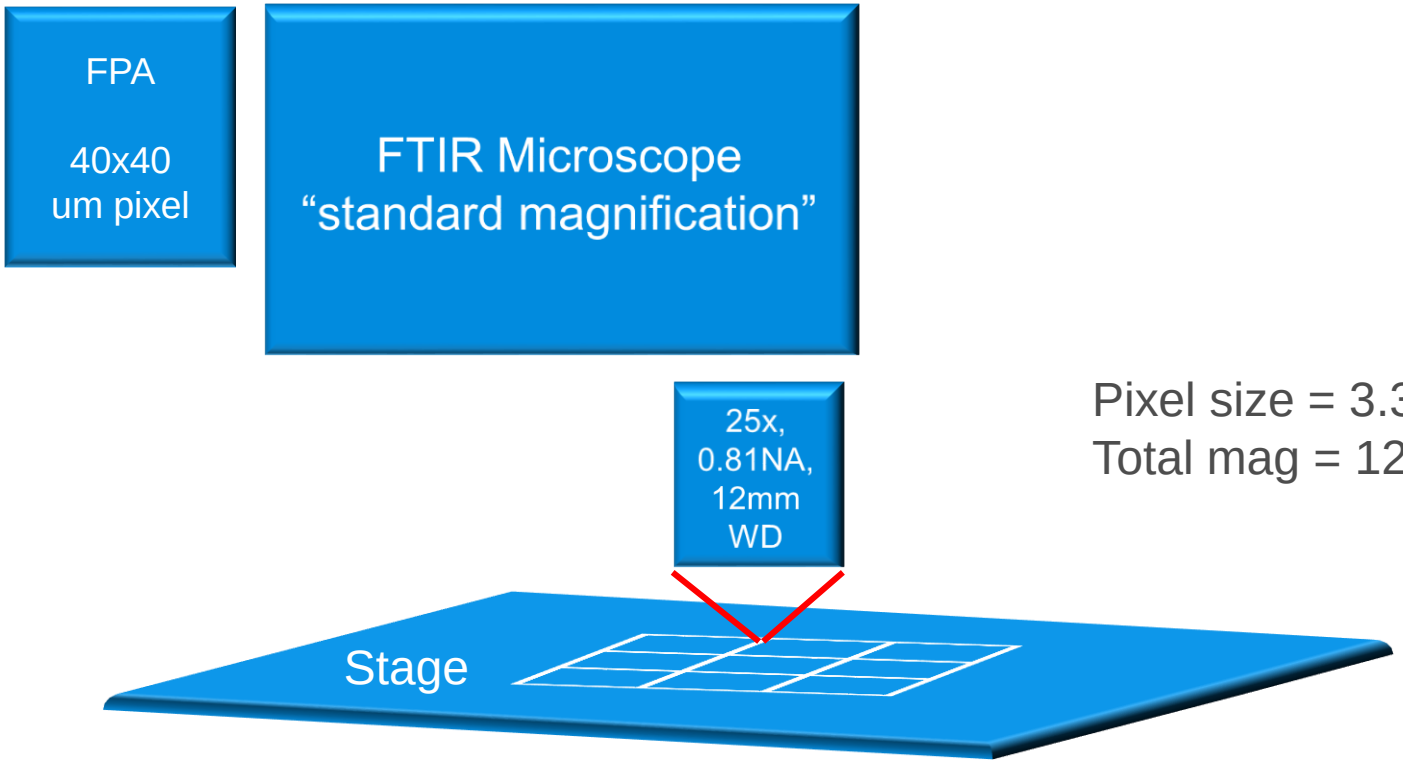


Pixel size = 1.1 microns
Total mag = 36x

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

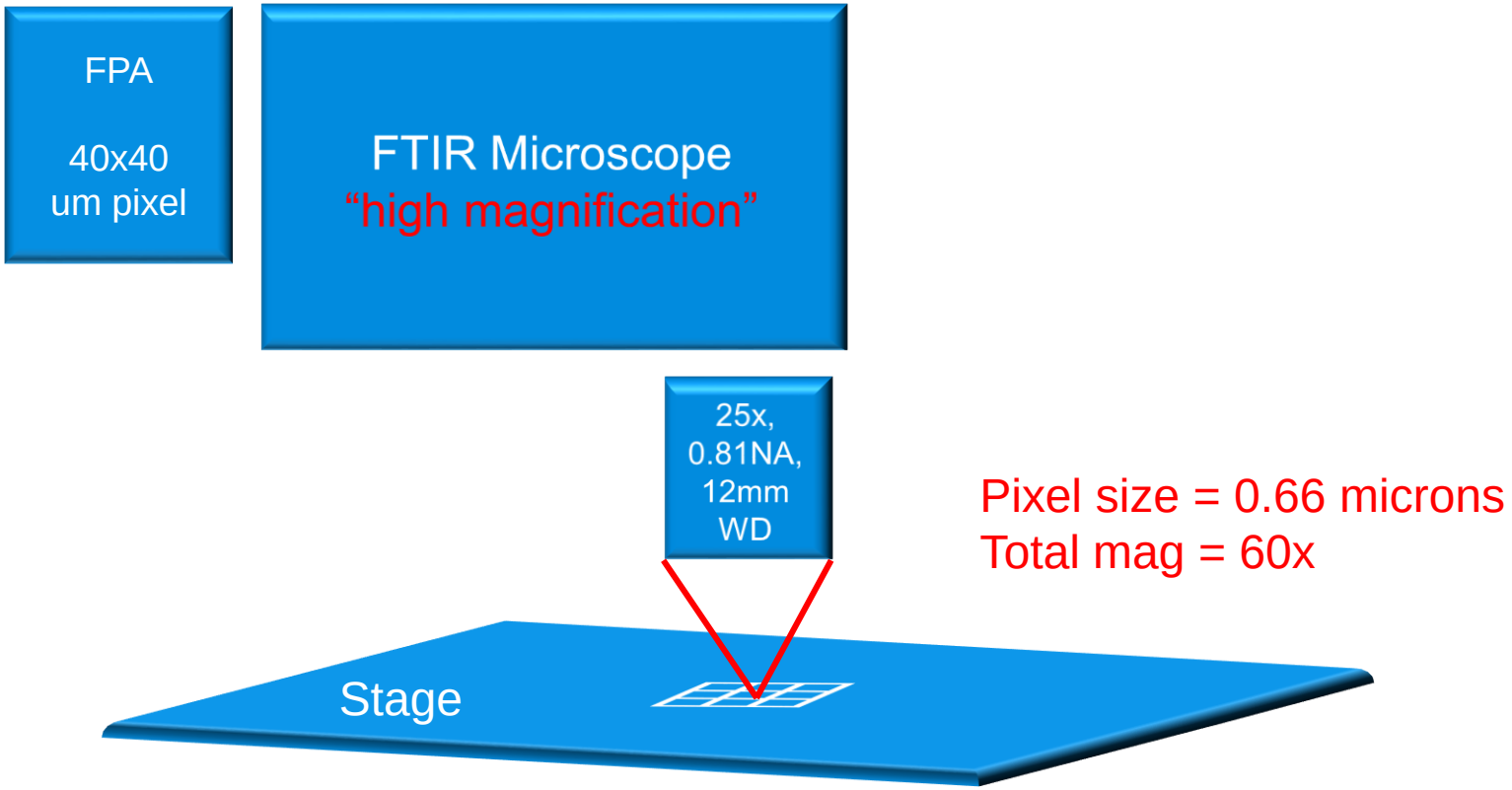
A big advantage of this approach is FULL PRESERVATION of the long objective working distance of 21mm, allowing a wide array of accessories and sample holders to be used

FTIR Microscope Magnification Schematic



Pixel size = 3.3microns
Total mag = 12x

FTIR Microscope Magnification Schematic



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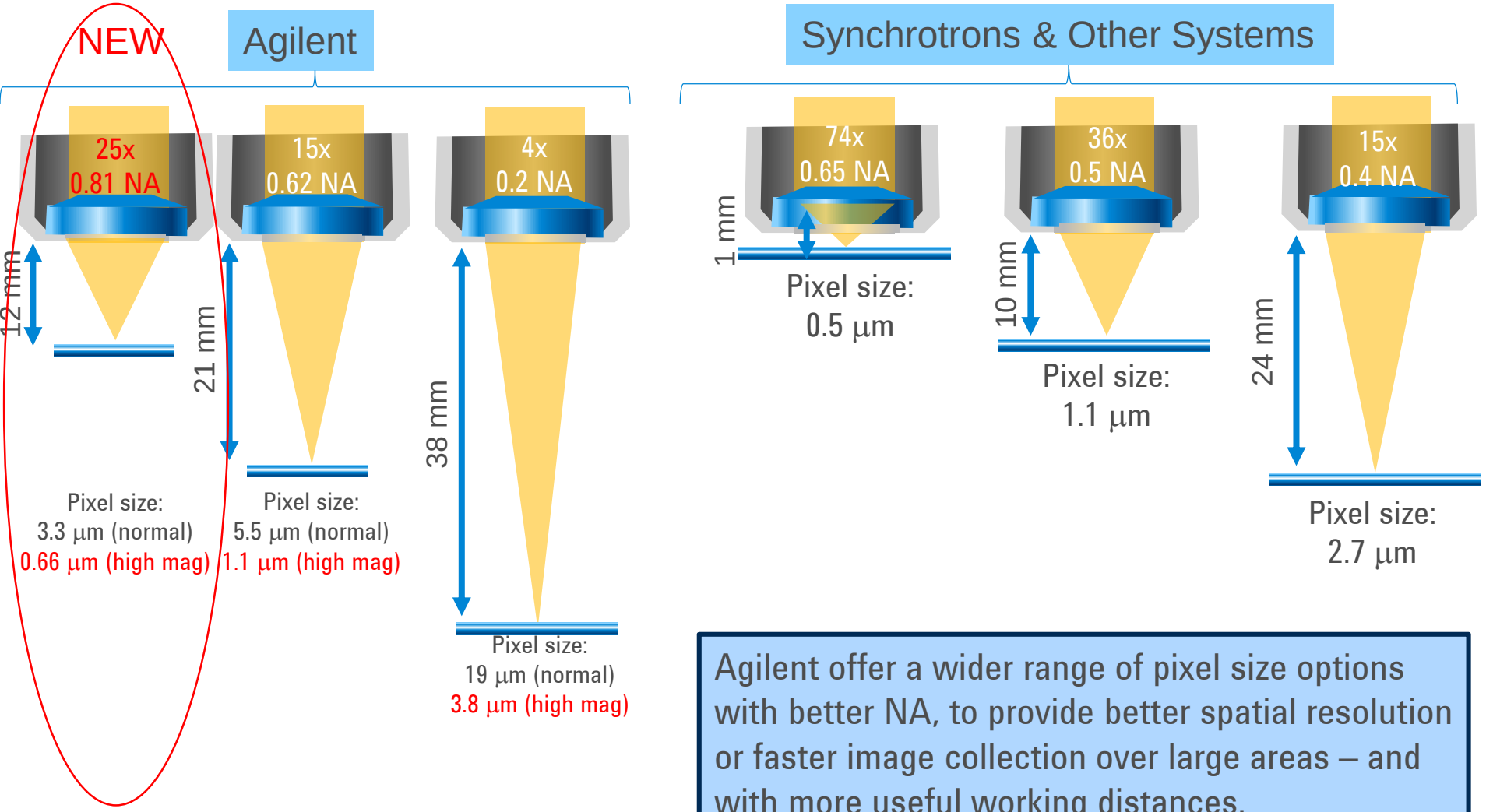
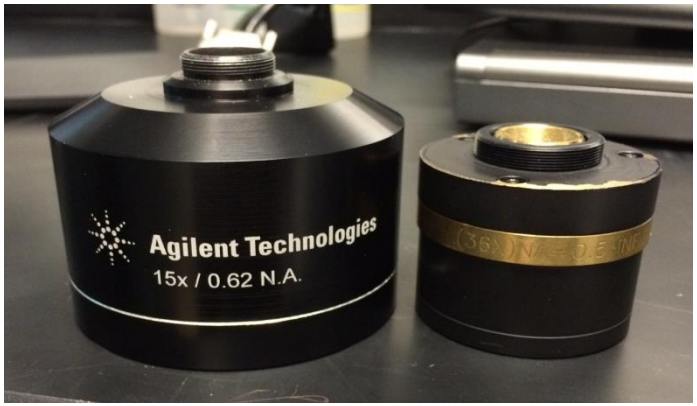
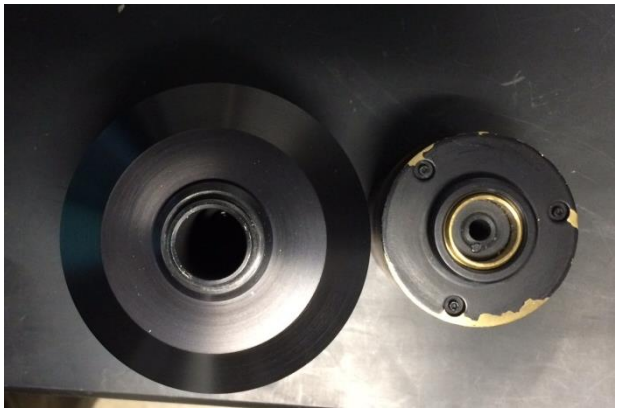
Microscope objectives: some comparisons

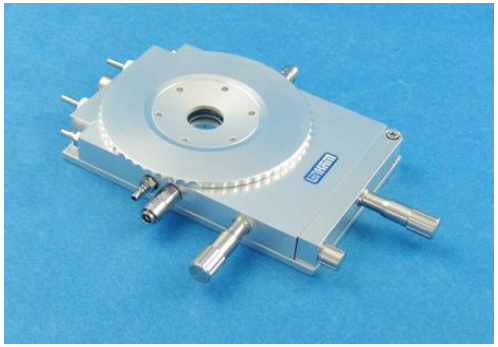


Agilent offer a wider range of pixel size options with better NA, to provide better spatial resolution or faster image collection over large areas – and with more useful working distances.

Objective comparisons



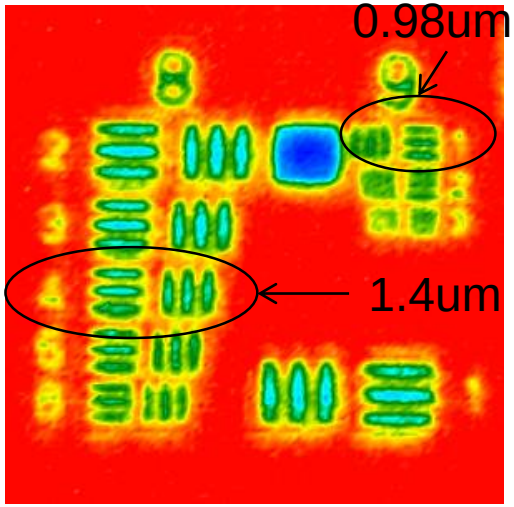


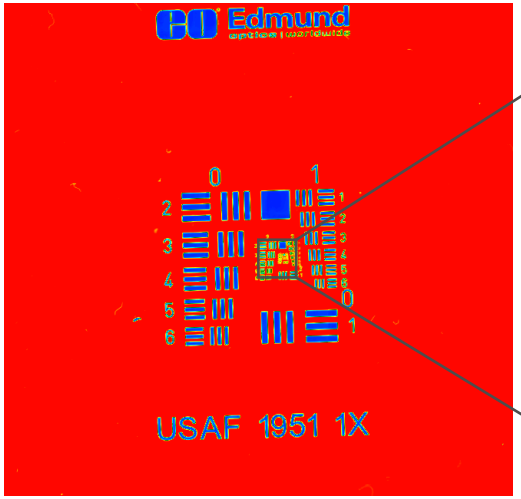


For the first time, one can do <1um pixel size (with high NA) Imaging with enough working distance to enable the use of accessories such as environmental (heating/cooling) stages like Linkam and liquid flow cells

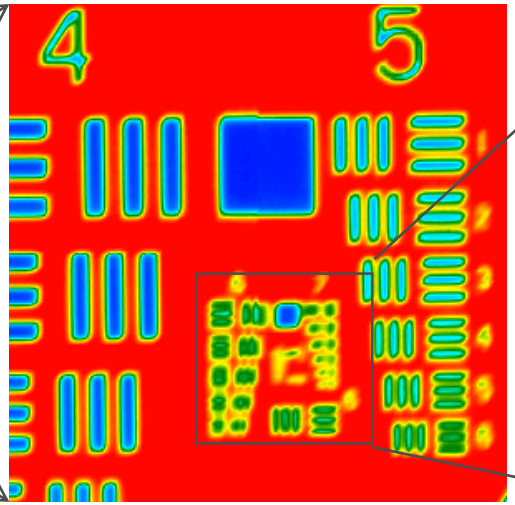
Achieved Spatial Resolution Summary

Pixel Size (obj/mode)	Achieved Spatial Resolution 3750 cm-1	Achieved Spatial Resolution 2500 cm-1	Single FPA tile FOV (with 128x128FPA)
3.3 um (25x, normal)	4.3 um	5.0 um	420x420 um
0.66 um (25x, high mag)	**1.4 um**	**1.7 um**	85x85 um
5.5 um (15x, normal)	6.9 um	7.6 um	700x700 um
1.1 um (15x, high mag)	2.4 um	3.0 um	140x140 um
19 um (4x IR, normal)	20.4 um	20.0 um	2400x2400 um

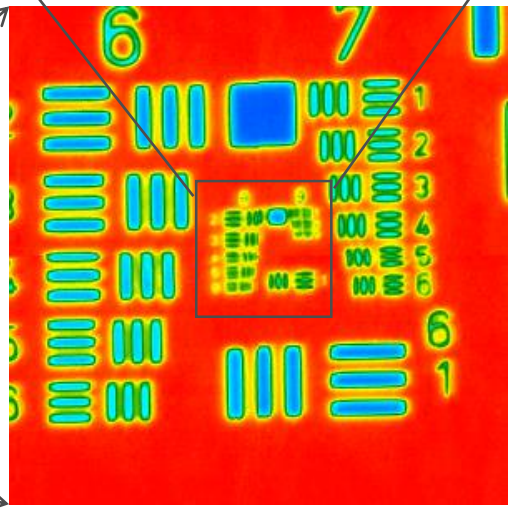




Entire 2"x2" (50x50mm) USAF target imaged at 19 um pixel resolution with 4xIR objective in 90 minutes
(21x21 tile mosaic with128FPA)



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 mosiac with 128FPA

Advancing FTIR Imaging with the Agilent Cary 620

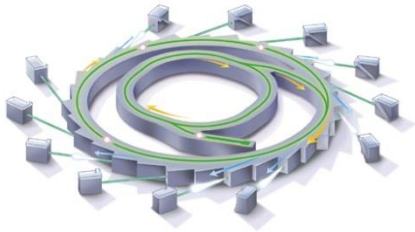
Highest spatial resolution

Largest Field of View

Superior signal-to-noise

Fastest data collection

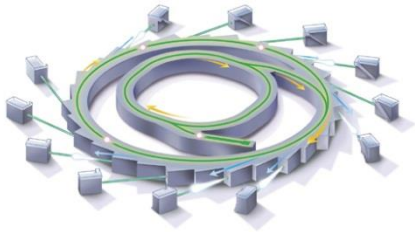
Synchrotron FTIR microscopy



- What is a synchrotron?
  - A synchrotron is a huge particle accelerator that energizes electrons to create bright beams of X-rays, infrared and ultraviolet light. Beams are taken off the main line and directed to detectors located on different nodes.

- Why use a synchrotron?
  - Great source of narrow intense beam of light (eg IR), which are great for single point microscopy with apertures <10 microns.

Synchrotron FTIR microscopy



- FTIR imaging on a synchrotron?
  - Synchrotrons are great for very small FOVs (<10um), but to image large FOVs (>hundreds of microns), requires multiple beam extractions and/or defocusing to spread the light out, removing all the brightness advantages of a synchrotron
  - For large area imaging, simple optics rules tell us that a large area source is required to illuminate a large area detector, such as an FPA
  - For this, the traditional FTIR globar is still the best option, as the large area globar is matched to the large area FPA detectors.

Synchrotron single point FTIR microscopy

10 um synchrotron source

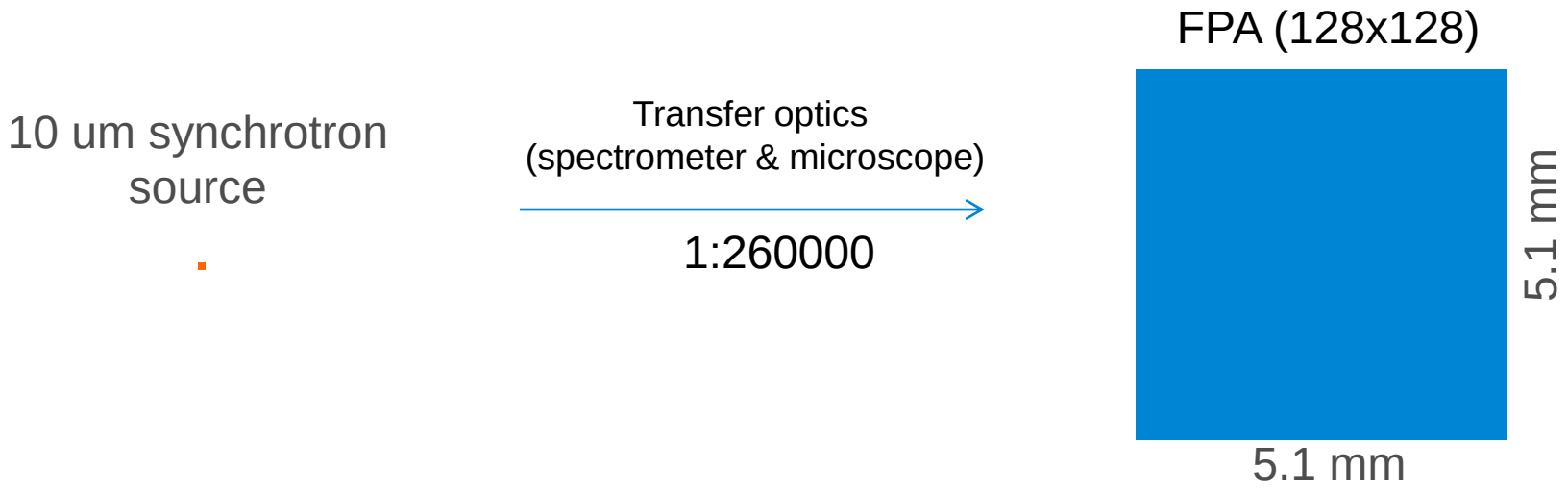
Transfer optics (spectrometer & microscope)

1:1

Single point MCT (100x100um)

In single point mode, beam does not need to illuminate full area of MCT detector. High brilliance of synchrotron beam is utilised

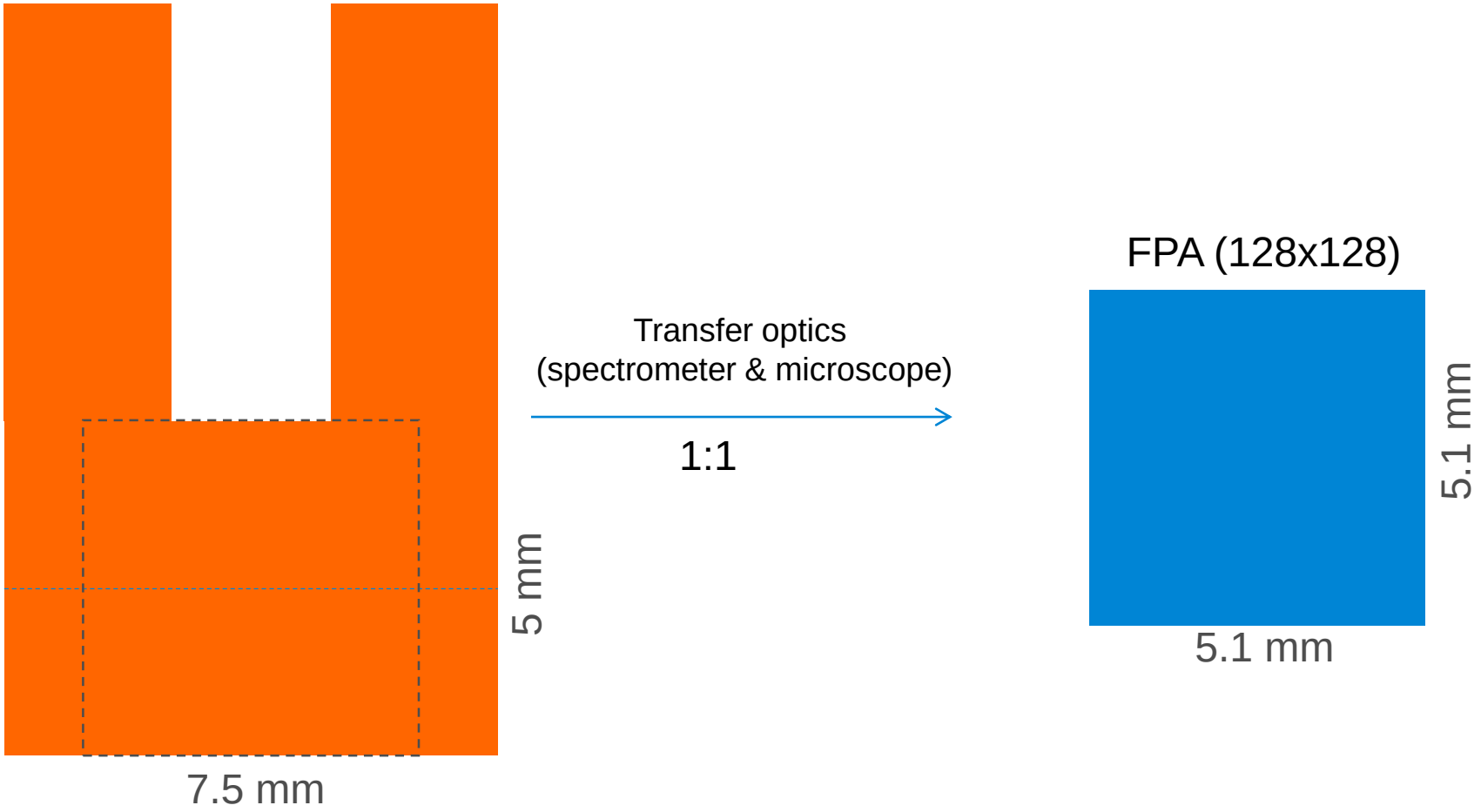
Synchrotron FPA FTIR Imaging



In imaging mode, beam must illuminate full area of FPA detector, this therefore requires a 260,000x defocussing, losing the synchrotron intensity advantage

Agilent's Thermal source FPA FTIR Imaging

Globar (double image with retroreflector source optics)



With a globar (image doubled with retroreflector), the hot spot is area is a 1:1 match to the FPA area, maximising intensity usage, resulting in better sensitivity

ADVANTAGES AND BENEFITS OF THE NEW FEATURES

Cary 620 top 4 advantages

Highest Spatial Resolution

- New high mag optics
- >400% IR energy
- Better than synchrotron IR imaging

Largest Field of View

- Proprietary 4x IR objective
- Measure cm x cm areas in minutes

Fastest analysis time

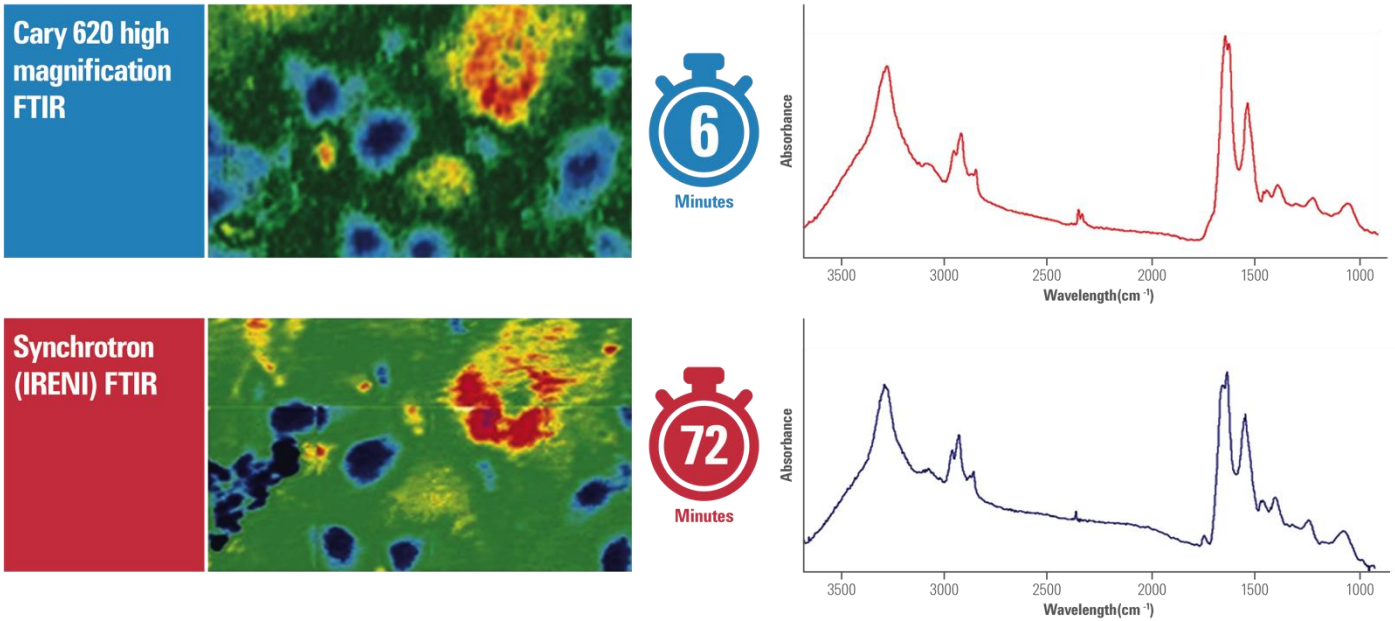
- >10x - other FPA's
- > 50x - linear array
- >100x - single point

Live FPA imaging

- Enhanced chemical contrast software mode
- Eliminate sample prep
- Avoid damaging samples

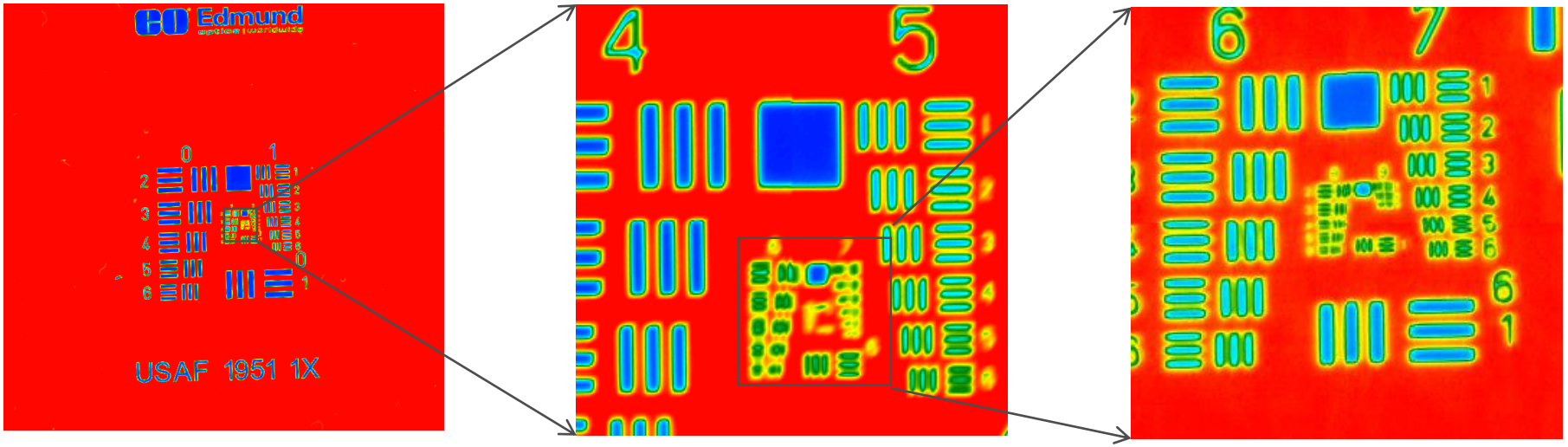
1. Highest spatial resolution

- New high magnification optics & ultra high NA (0.81) 25x objective provide pixel resolution down to 0.66 um in transmission/reflection
- Superior IR energy throughput from FTIR bench and new IR objectives ensures highest quality data when measuring in high mag mode
- Achieve data quality of high spatial resolution BETTER than that of a Synchrotron FTIR imaging system



2. Largest Field of View (FoV)

- Use proprietary 4x IR objective to measure samples cm x cm in area within minutes
- Change objectives in seconds to increase spatial resolution and zoom in on smaller areas of interest
- Switch to high magnification mode to resolve features as small as 1.4 um.



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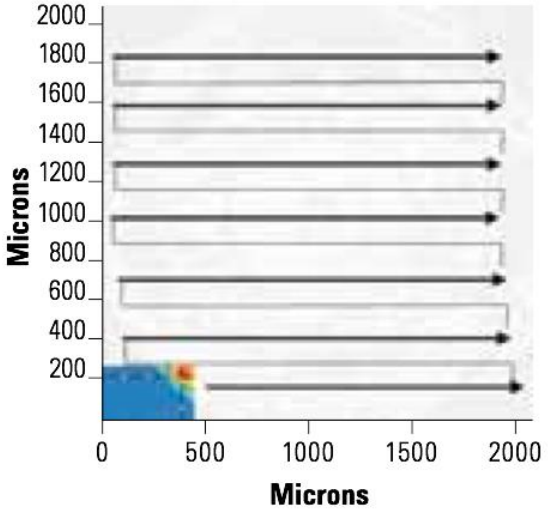
Single 128FPA tile

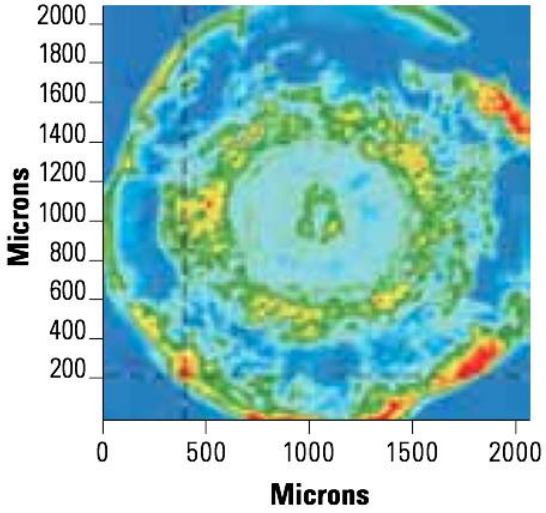
USAF target imaged (280x280um) at 1.1 um resolution (high mag mode) with 15x objective in 8 minutes.
2x2 tile mosiac with 128FPA

3. Fastest analysis time

- Unparalleled IR energy from the FTIR bench (>400%) coupled with increased IR throughput of new 15x objective (>40%) guarantees the highest quality data in the shortest period of time.
- Faster than any other FPA, Linear Array or single point detector available today
- Measure the largest samples at the highest resolution in the shortest period of time!

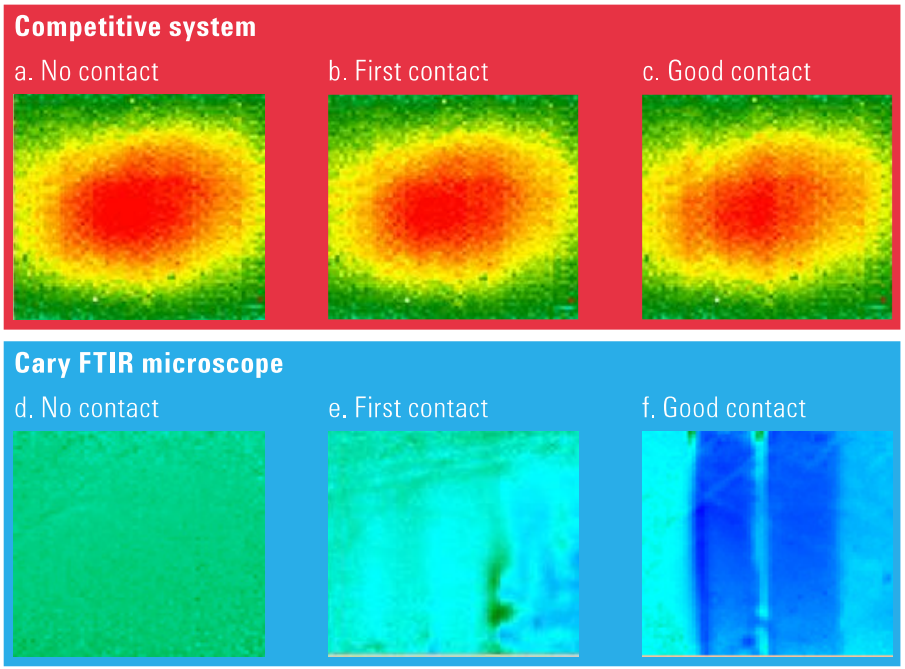


Linear array mapping – in 20 min, only 5% of image is collected.



Agilent FPA imaging – in 20 minutes 100% of image collected at 5.5um resolution

4. Live FPA Imaging with enhanced chemical contrast



Agilent's unique chemical contrast feature provides clear differences in live IR mages 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.

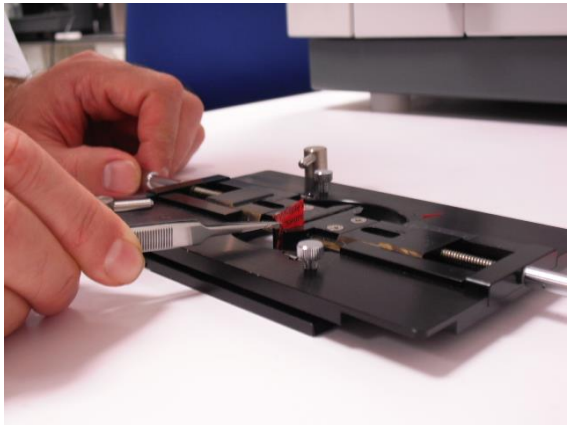
MARKETS AND APPLICATIONS

Polymer Film Laminate FTIR Imaging

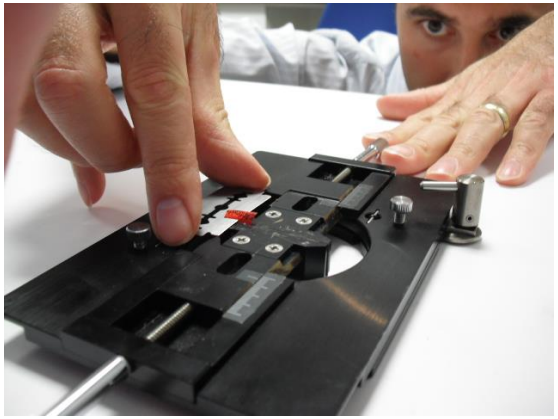
Sample Preparation Free FTIR Chemical Imaging of Polymer laminates & Films

Step 1. Cut out small piece

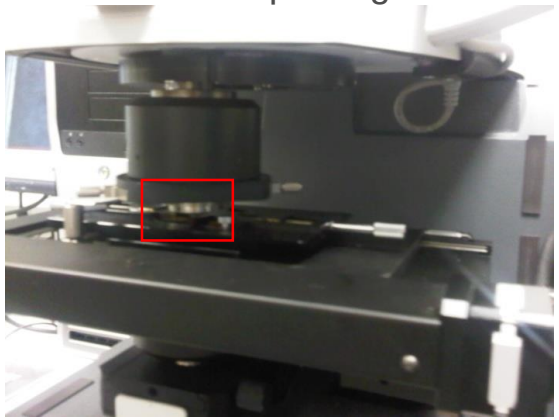
Step 2. Place cut-out piece in micro-vice.



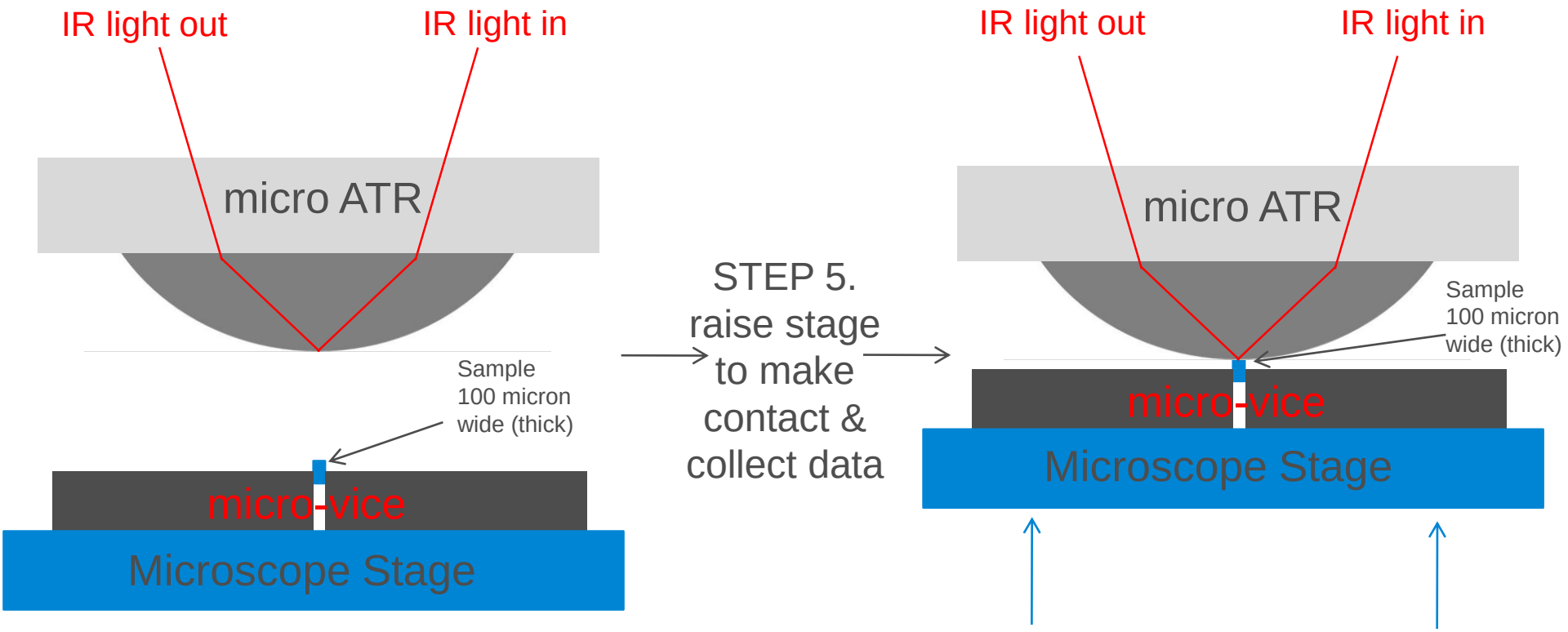
Step 3. Cross-section sample with razor



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

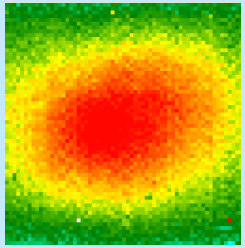


ATR Contact with sample



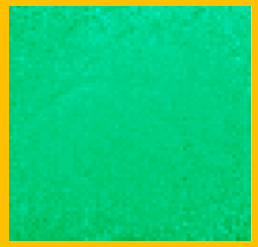
“Live/Real-Time” ATR contact monitoring

Standard Live ATR direct FPA IR Image – without correction



No Pressure
(before contact)

Live ATR direct FPA IR Image with Enhanced Chemical Contrast



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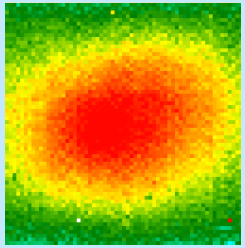
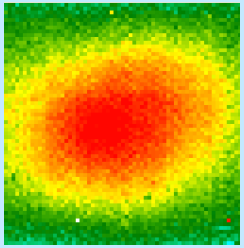


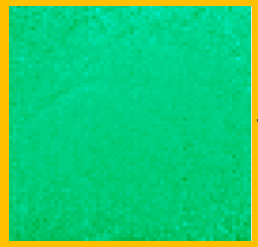
Stage is raised →



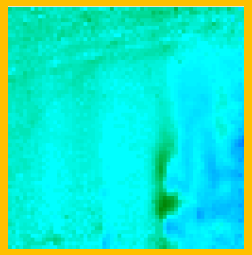
No Pressure (before contact)

First Contact

Live ATR direct FPA IR Image with Enhanced Chemical Contrast



Stage is raised →



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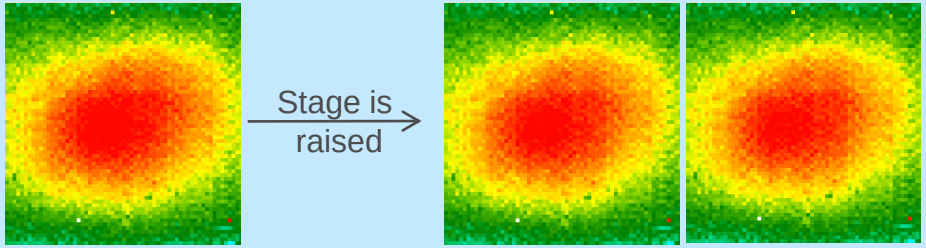
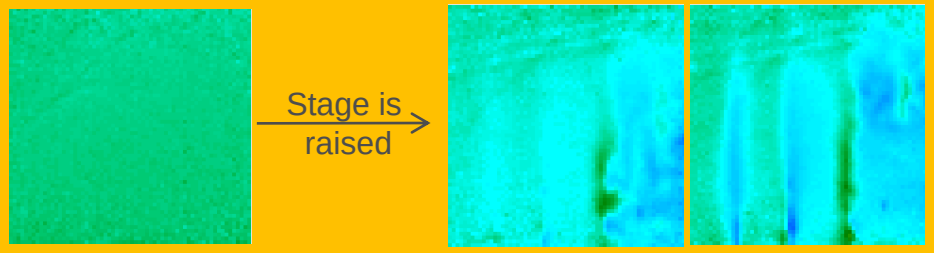


Live ATR direct FPA IR Image with Enhanced Chemical Contrast



“Live/Real-Time” ATR contact monitoring

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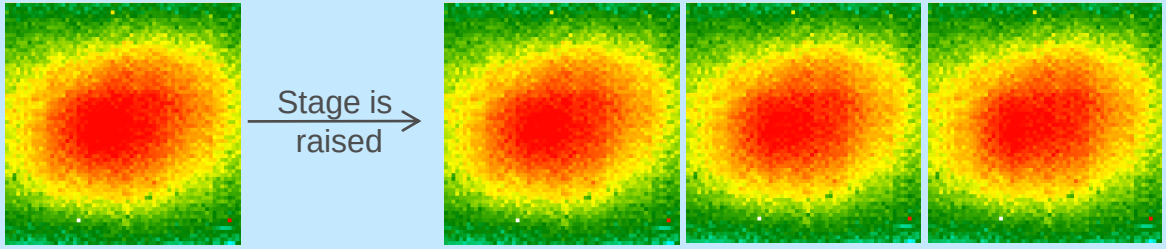

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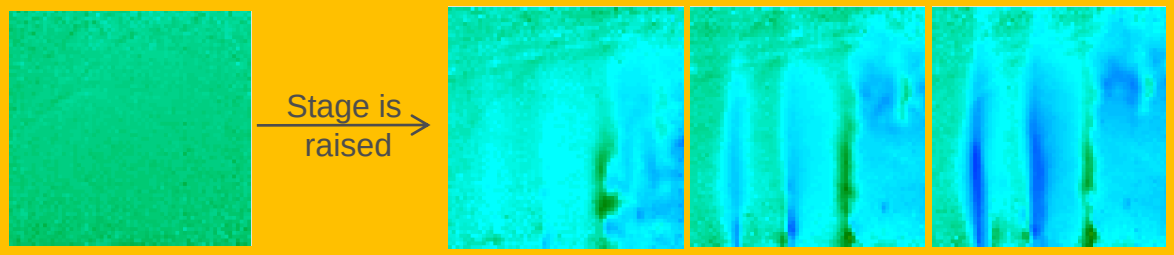
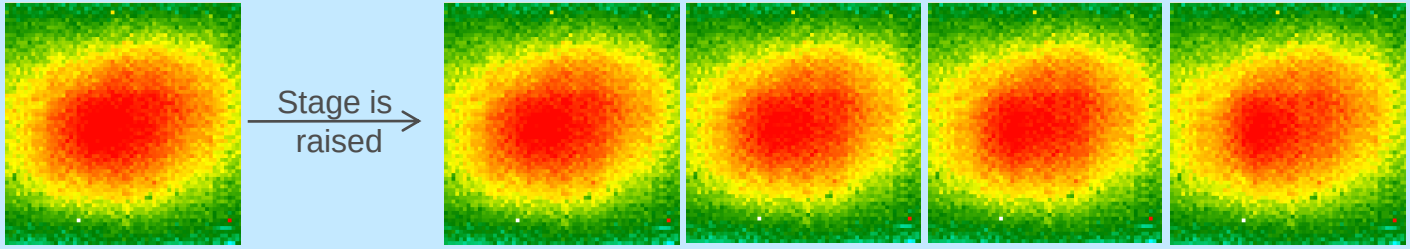
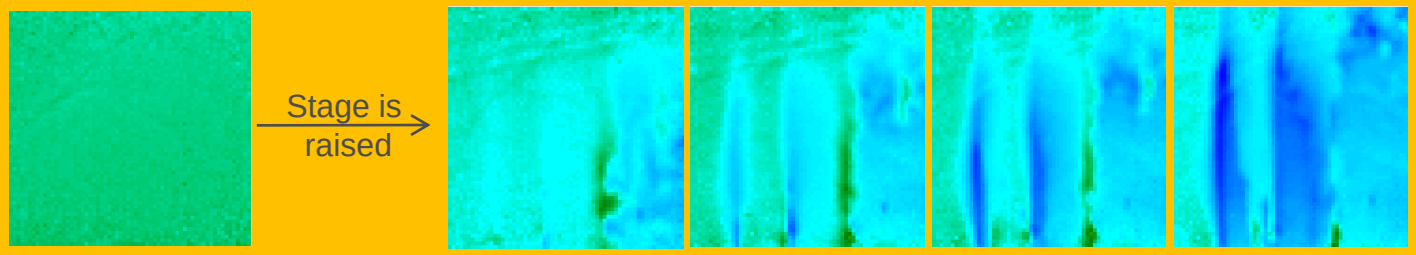


“Live/Real-Time” ATR contact monitoring

Standard Live ATR direct FPA IR Image – without correction

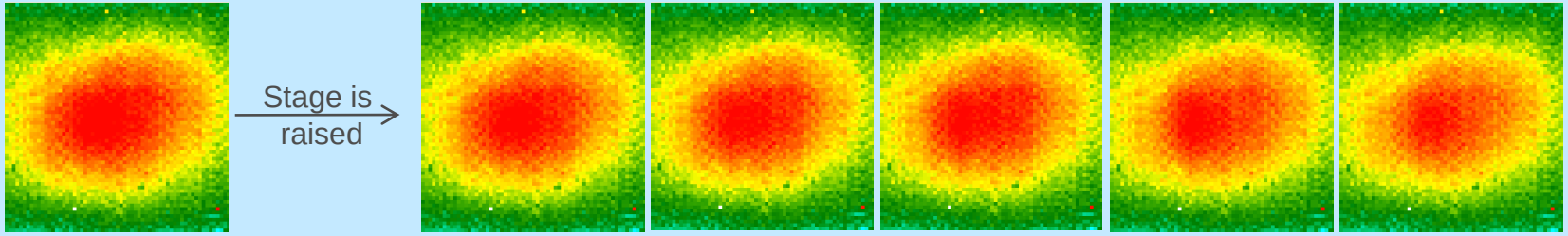


Live ATR direct FPA IR Image with Enhanced Chemical Contrast

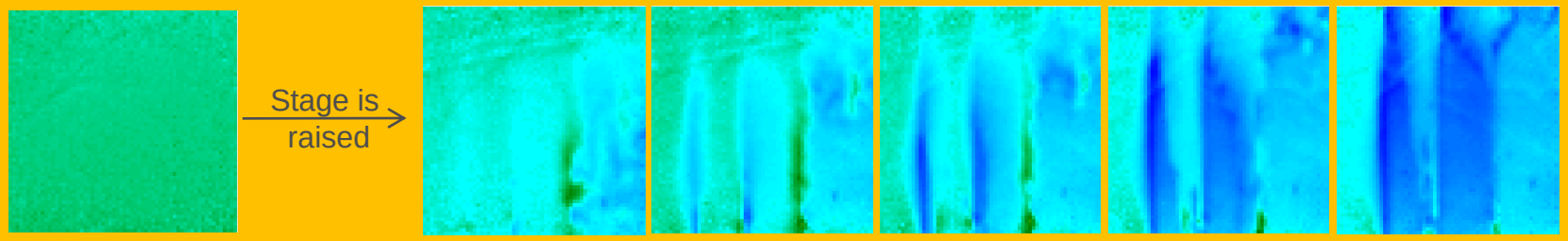


“Live/Real-Time” ATR contact monitoring

Standard Live ATR direct FPA IR Image – without correction

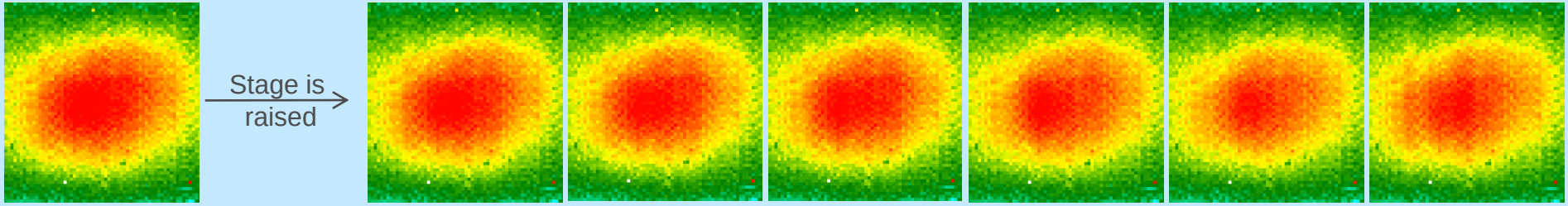


Live ATR direct FPA IR Image with Enhanced Chemical Contrast

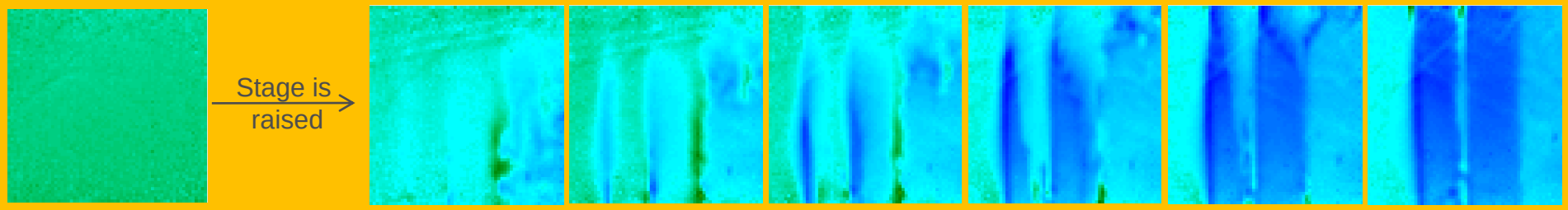


“Live/Real-Time” ATR contact monitoring

Standard Live ATR direct FPA IR Image – without correction



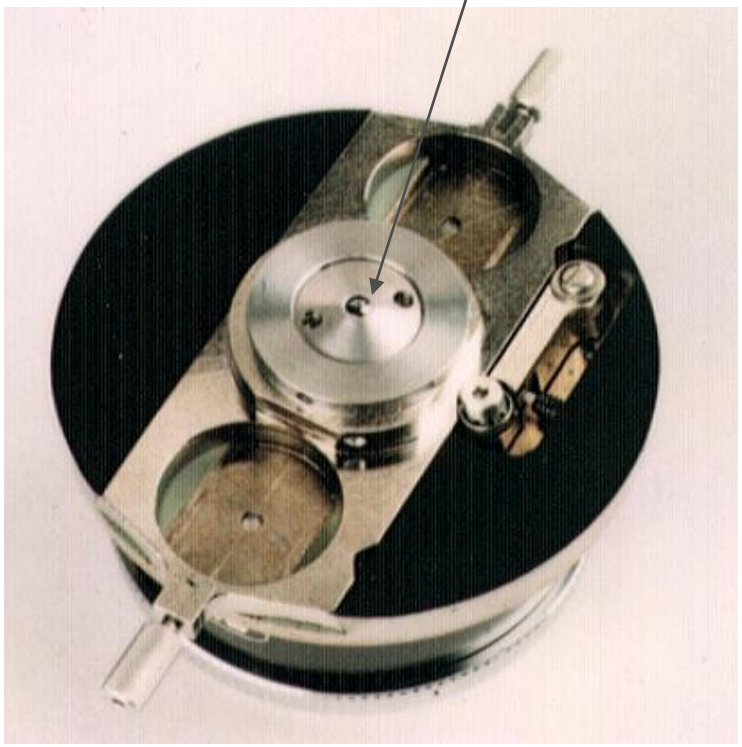
Live ATR direct FPA IR Image with Enhanced Chemical Contrast



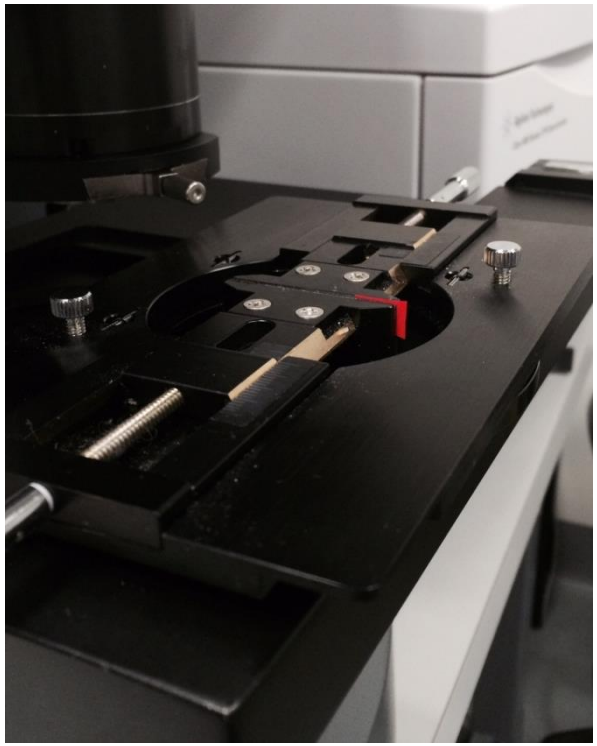
Agilent Slide-on ATR Accessory in 15x Cassegrain objective

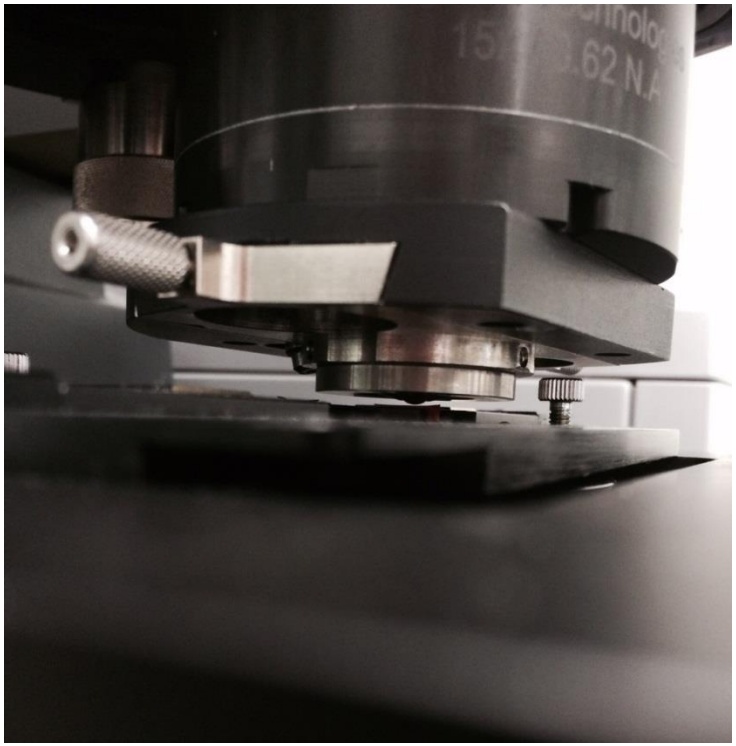
- Micro ATR
  - choice of Ge, Si or diamond crystals as IRE
- Low contact pressure required
- Easy to change between different crystals (eg Ge vs Diamond)
- No damage to the sample
- Easy to clean
- Does not require seperate objective
- High infrared throughput
- High S/N at the sample

Single reflection hemispherical internal reflection element (IRE)



Sample Presentation

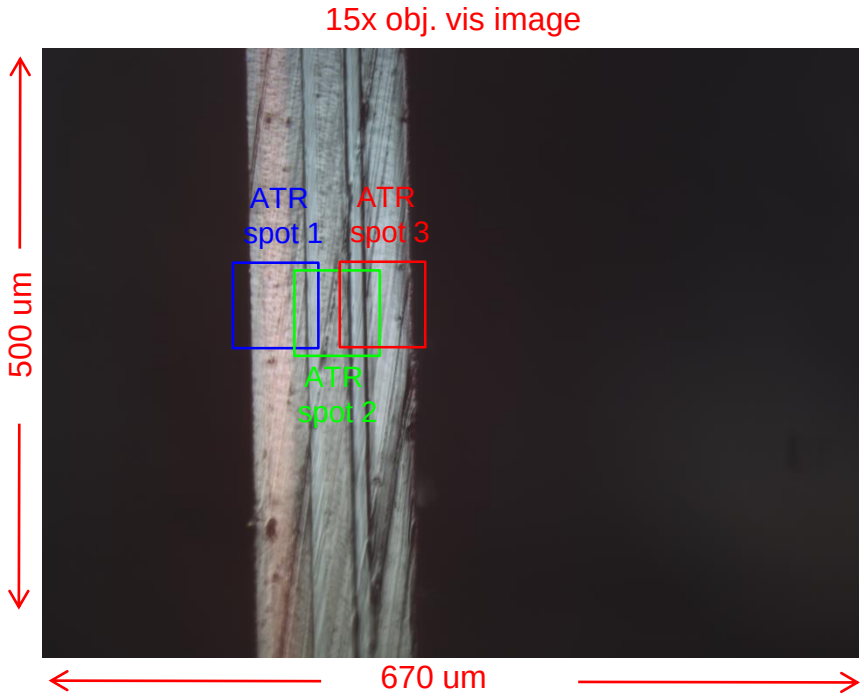




A small piece of the sample was cut out and place into a micro-vice sample holder where it was cut flat with a sharp razor

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

Sausage Packaging (Red): Visible images ATR Imaging Sampling Location



Collection Conditions:

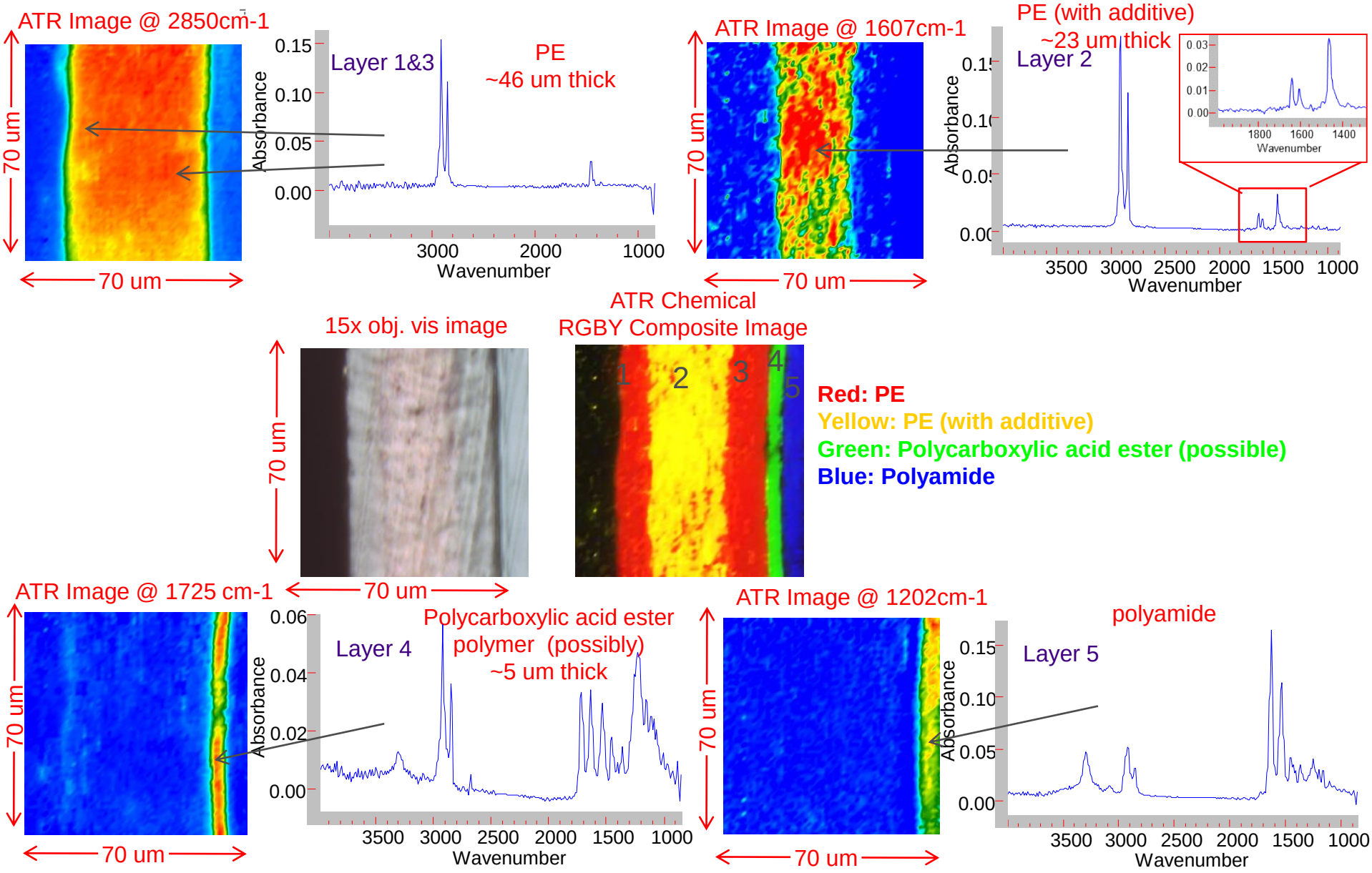
Resolution:	4 cm-1
Scans (time):	32 scans (~30 sec) per spot
Spectral Range:	4000 – 850 cm-1
Collection Mode:	Micro ATR & transmission (5 micron microtomed slices)
Pixel Size & FOV:	1.1 microns/pixel, 70x70 microns

Different collection mode comparison

1. Micro ATR (1.1 micron @ 70x70 micron FOV)
2. Transmission with 15x normal mag (5.5 micron pixel size & 700x700 micron FOV)
3. Transmission with 15x HIGH mag (1.1 micron pixel size & 140x140 micron FOV)
4. Transmission with NEW 25x normal mag (3.3 um pixel size & 420x420 um FOV)
5. Transmission with NEW 25x HIGH mag (0.66 um pixel size & 85x85 um FOV)

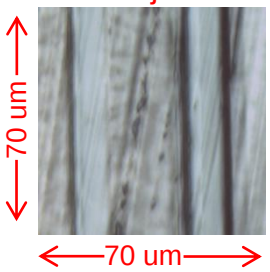
-

Sausage Packaging – ATR Chemical Images (Spot 1)

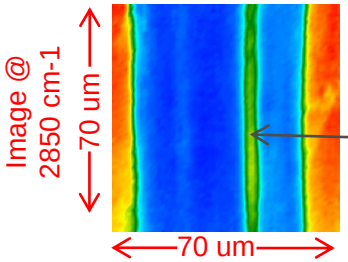


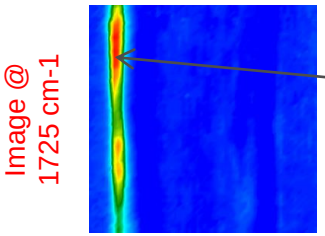
Sausage Packaging – ATR Chemical Images (Spot 2)

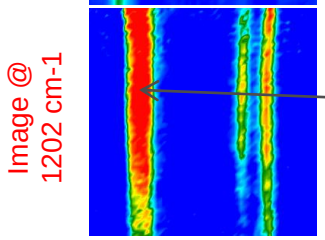
15x obj. vis image

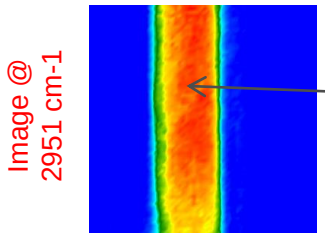


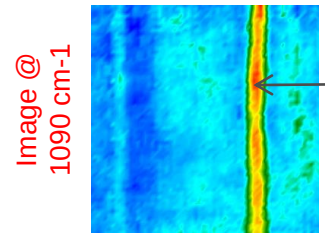
ATR Chemical Image

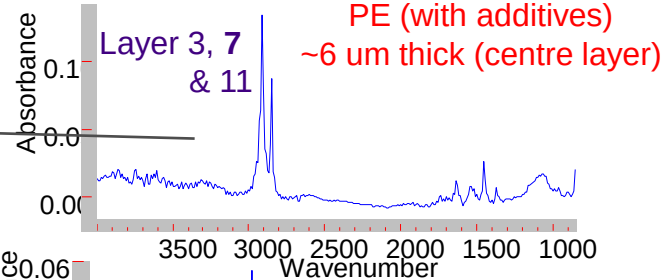




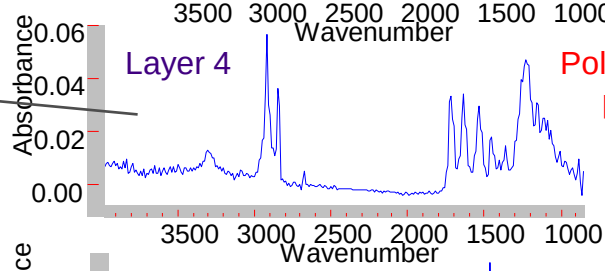




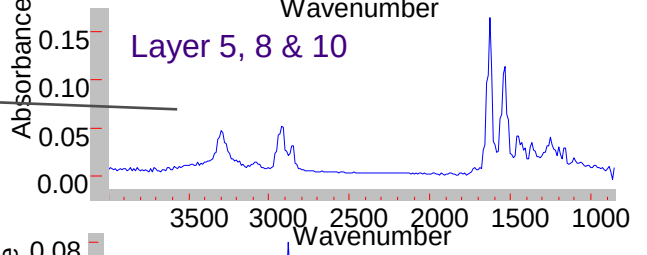




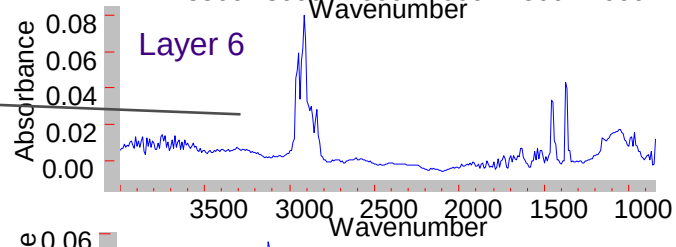
PE (with additives)
~6 um thick (centre layer)



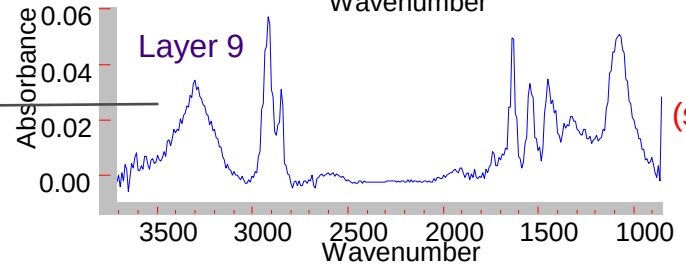
Polycarboxylic acid ester polymer (possibly)
~5 um thick



Polyamide
~7um (left)
~2um (centre)
~3um (right)



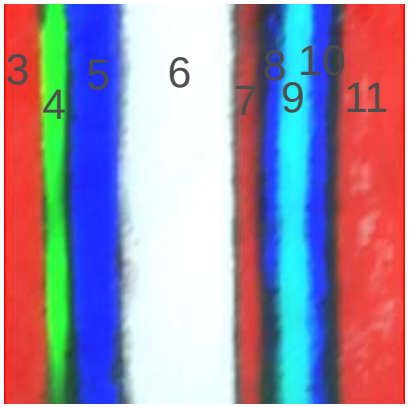
Polypropylene
~20um



EVOH
~5um
(spectral overlap from Polyamide present)

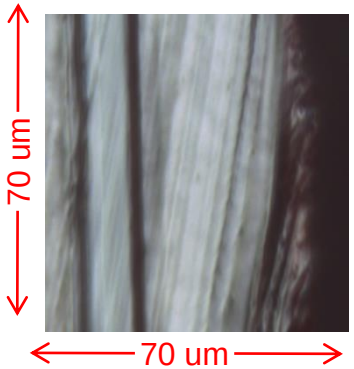
Red: PE
Green: Polycarboxylic acid polymer (possibly)
Blue: Polyamide
White: Polypropylene
Cyan: EVOH

RBGWC composite image

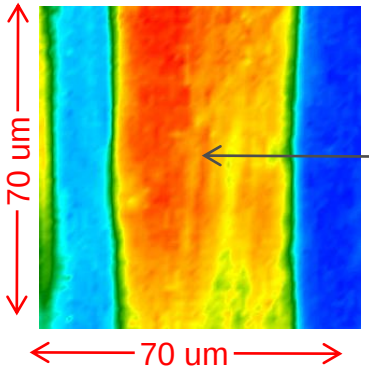


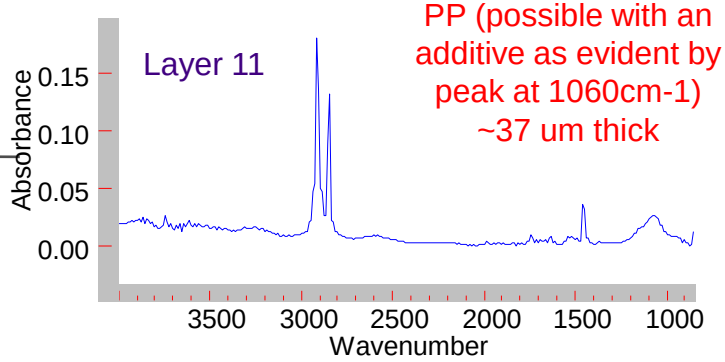
Sausage Packaging – ATR Chemical Images (Spot 3)

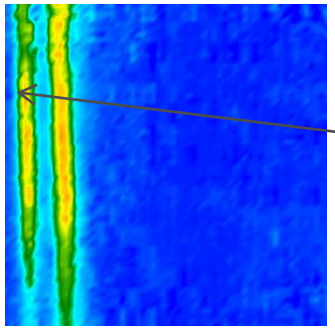
15x high mag. obj. vis image

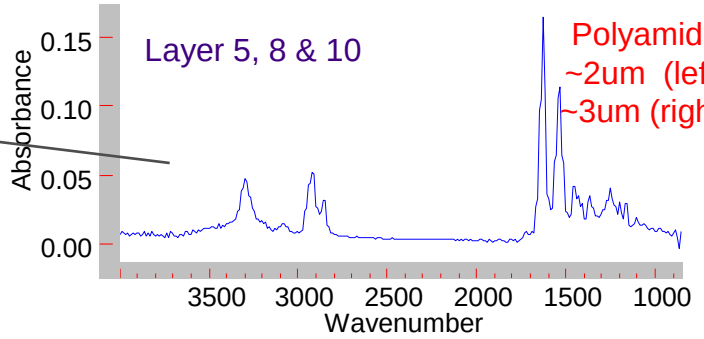


ATR Chemical Image



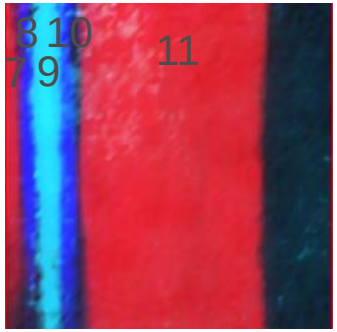


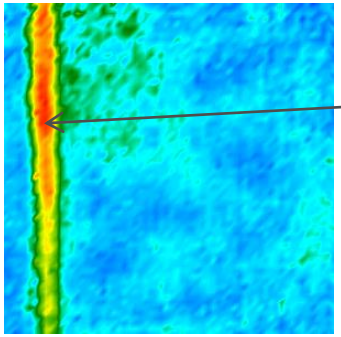


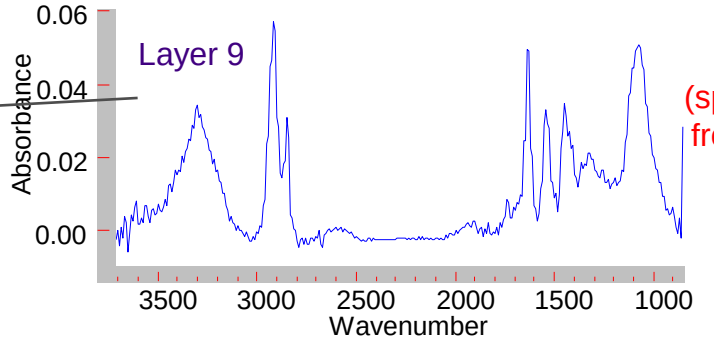


RBC composite image

Red: PE
Blue: Polyamide
Cyan: EVOH







EVOH
~5um thick
(spectral overlap from Polyamide present)

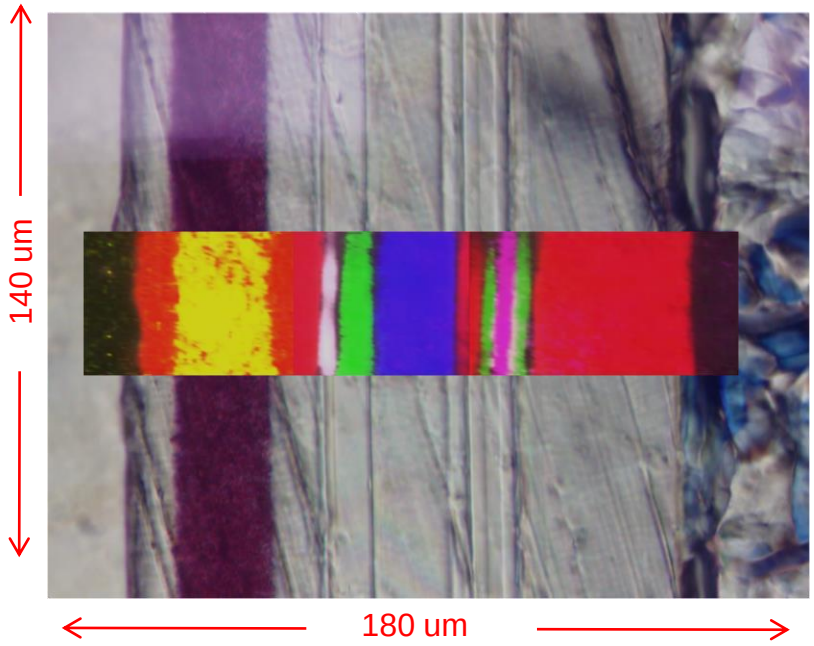
Sausage Packaging – ATR Chemical Images

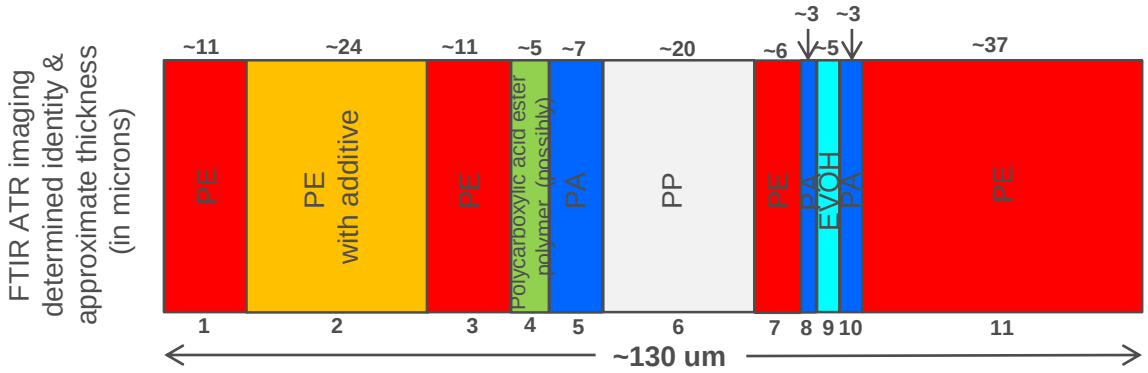
The sample was measured across its entire width with three slightly overlapping ATR measurements, with a total collection time of ~ 1.5 mins.

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.

40x obj. vis image (polarized)

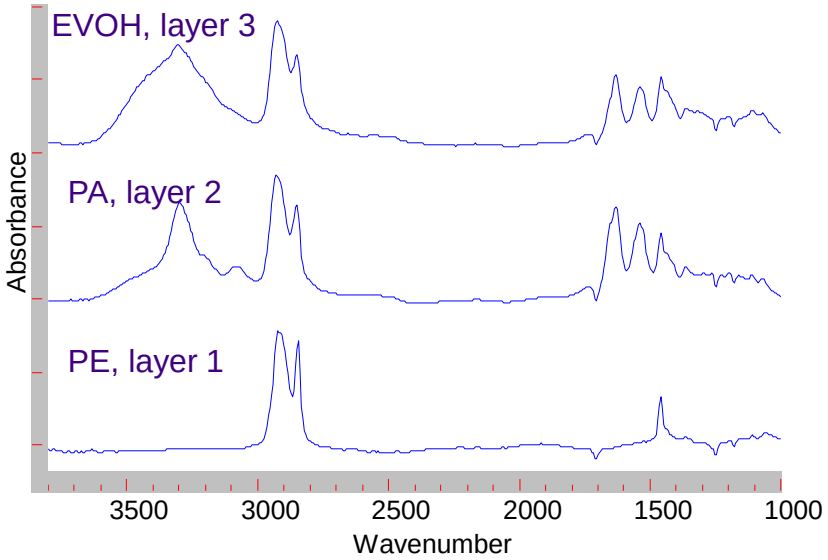




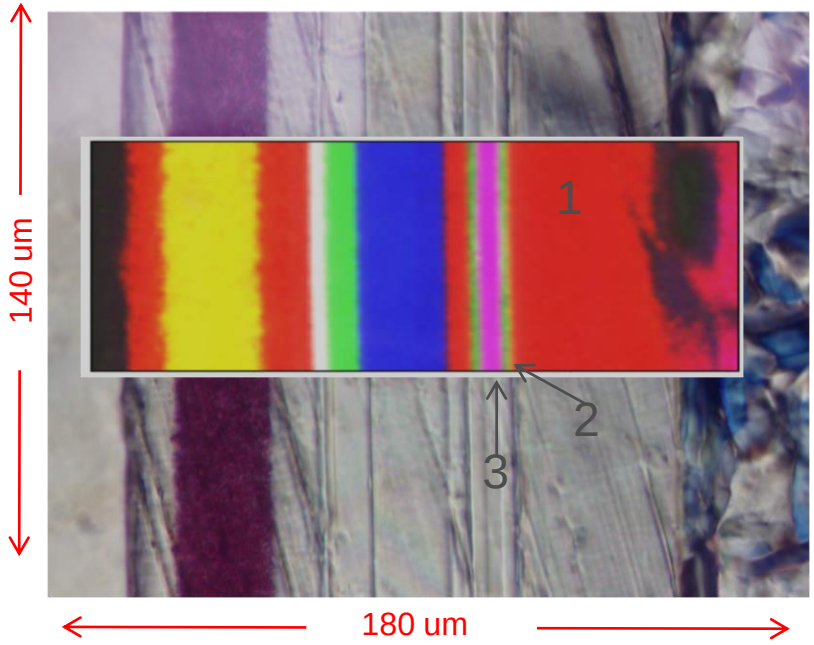
Pixel size:	1.1micron
Obj mag:	15x, 0.62NA
FOV:	70x70um
Total system mag:	36x

Sausage Packaging – 25x 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



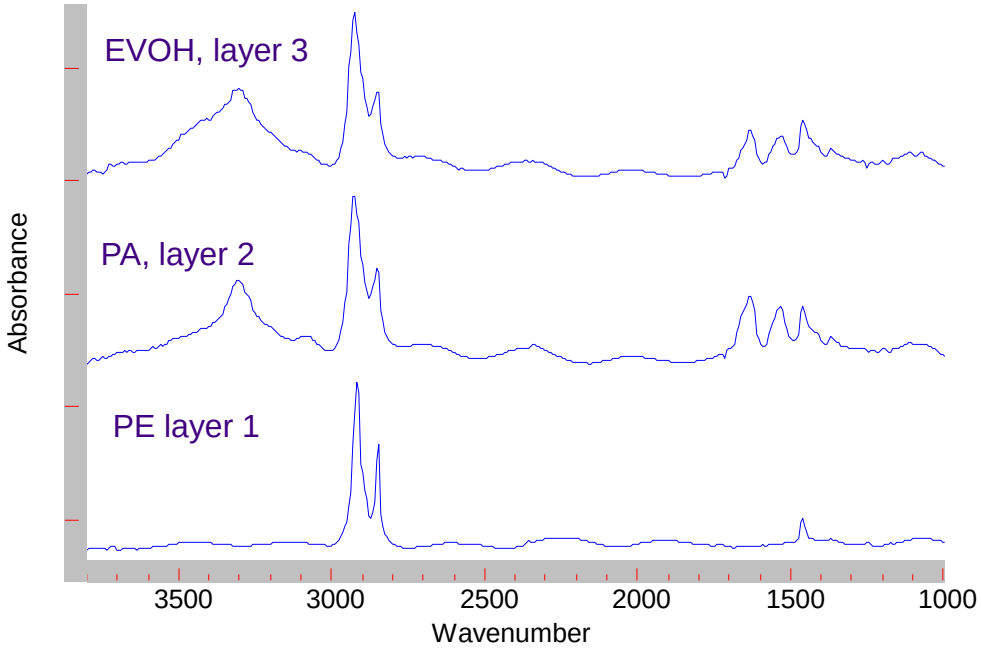
40x obj. vis image (polarized)



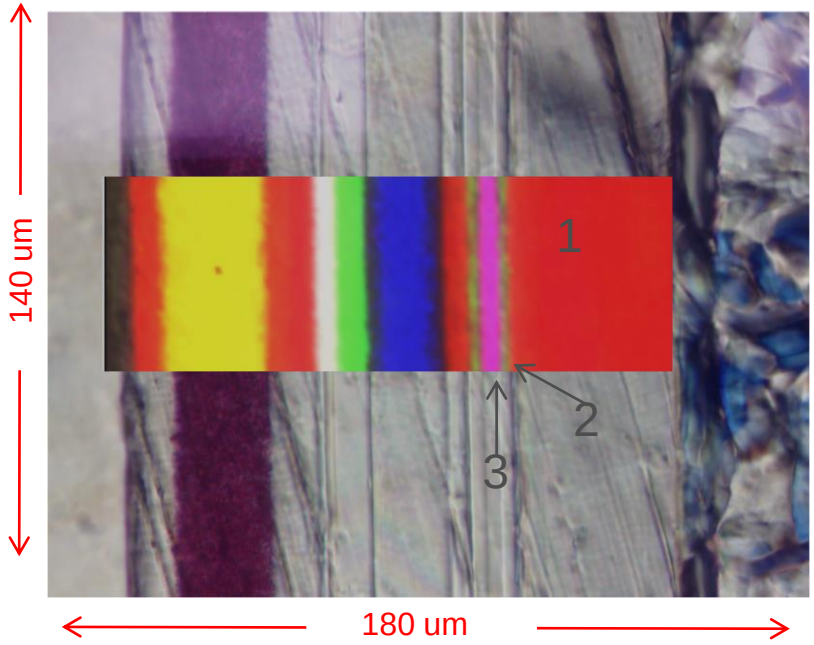
Pixel size:	0.66 micron
Obj mag:	25x, 0.81NA
High mag:	ON
FOV:	85x85um
Total system mag:	61x
Working distance:	12mm

Sausage Packaging – 15x High mag Chemical Images

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.



40x obj. vis image (polarized)

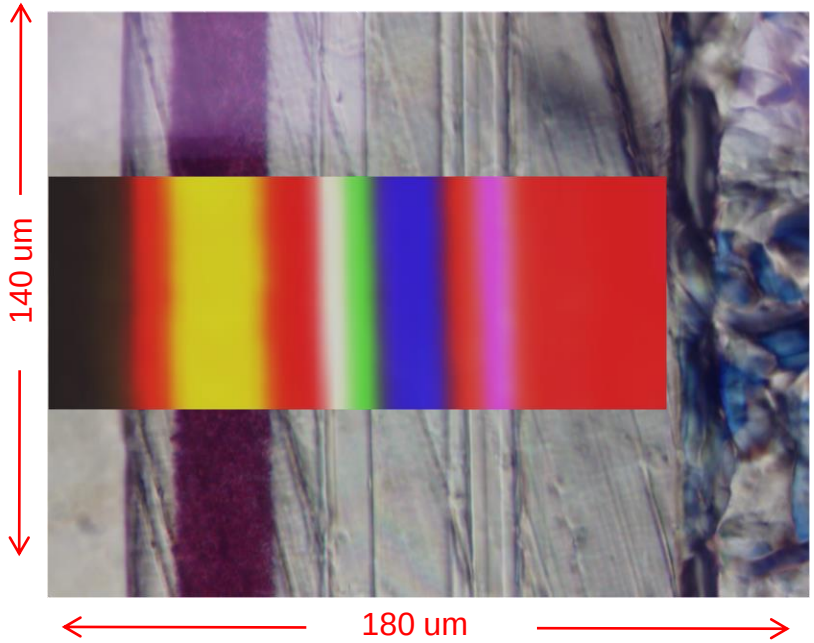


Pixel size:	1.1micron
Obj mag:	15x, 0.62NA
High mag:	ON
FOV:	140x140um
Total system mag:	36x
Working distance:	21mm

Sausage Packaging – 25x std mag Chemical Images

With the larger pixel size, now at 3.3um, even with the high
NA 25x objective, the ~3um PA layers cannot be spatially resolved

40x obj. vis image (polarized)

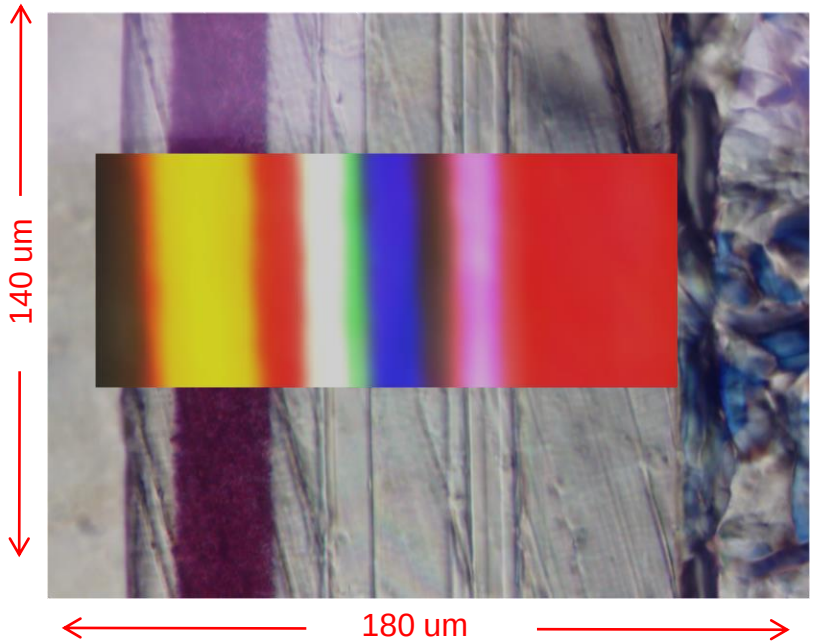


Pixel size:	3.3micron
Obj mag:	25x, 0.81NA
High mag:	OFF
FOV:	420x420um
Total system mag:	12x

Sausage Packaging – 15x std mag Chemical Images

As the pixel size gets larger, now at 5.5um, layers that are ~5 um now start to blur out

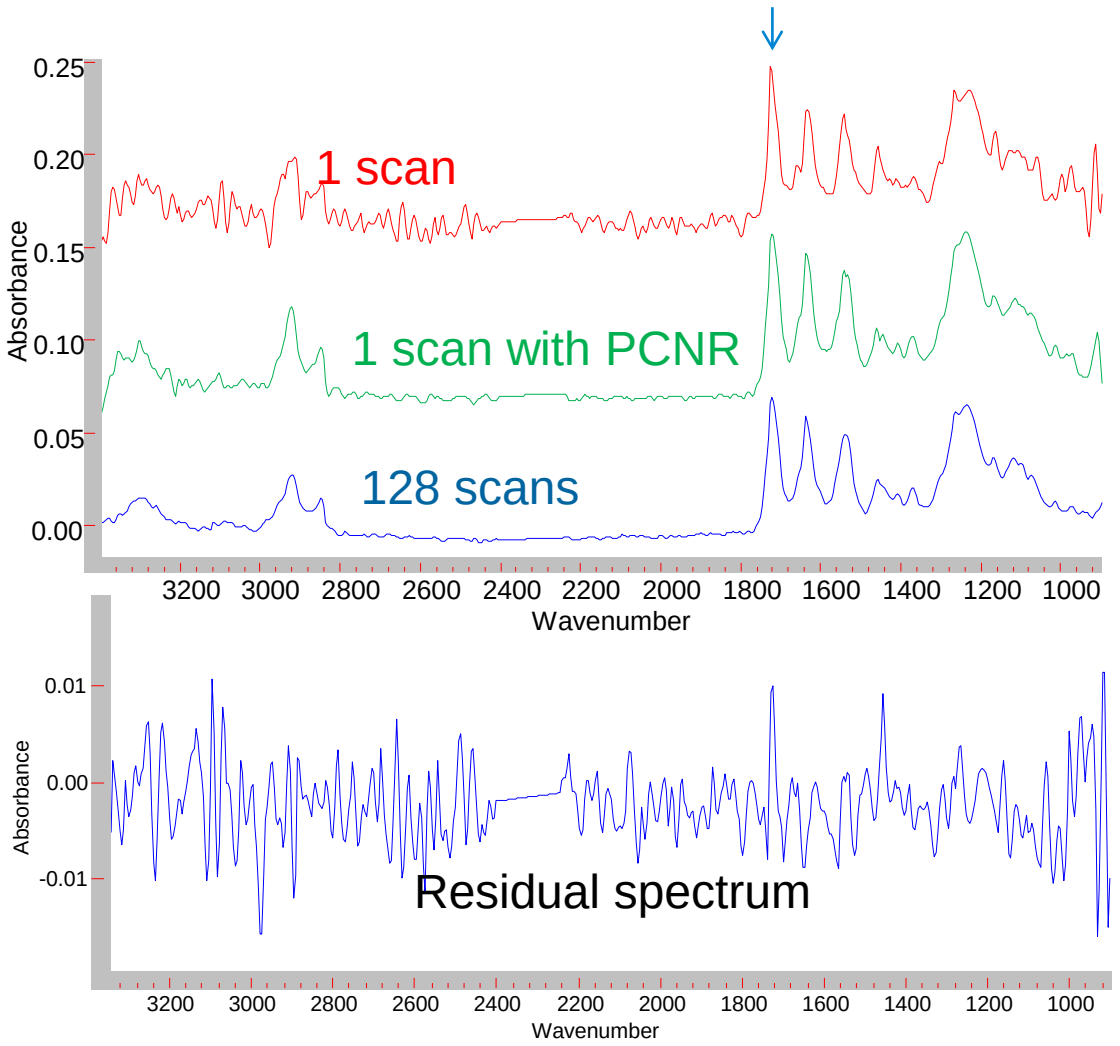
40x obj. vis image (polarized)



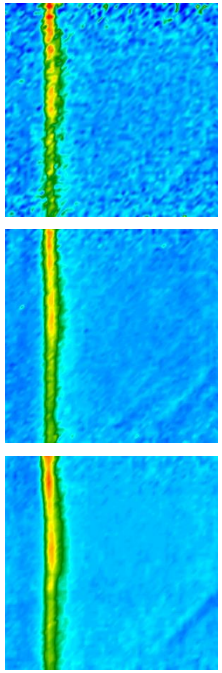
Pixel size:	5.5 micron
Obj mag:	5x, 0.62NA
High mag:	OFF
FOV:	700x700 um
Total system mag:	7.3x

Principal Component Noise Reduction (PCNR)

- Removes ONLY noise
- Typical S:N improvement of ~5-10x
- Corresponding time saving (for equivalent S:N) is 25-100x !
- Only FTIR imaging system to include PCNR

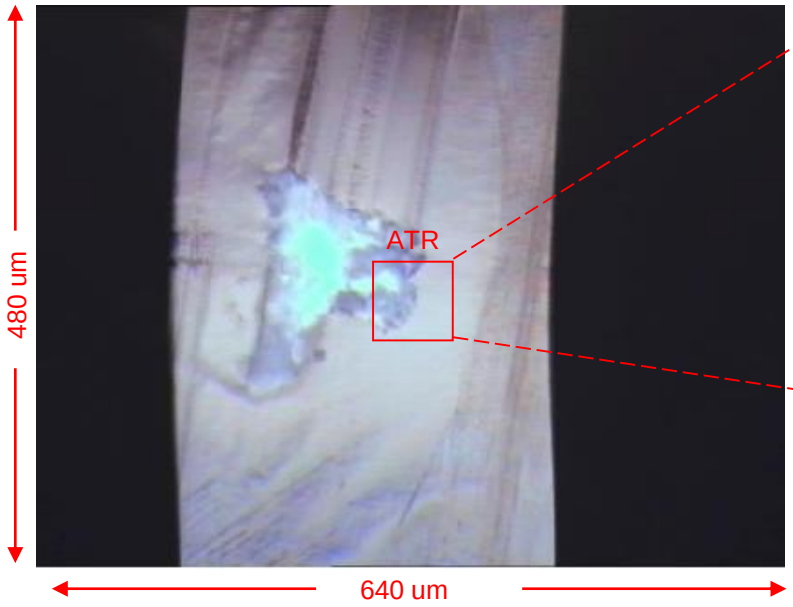


1720 cm-1

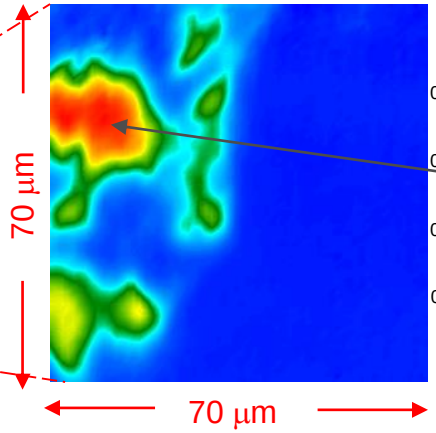


Polymer Film Defects - Visible Images & ATR Imaging Sampling Location

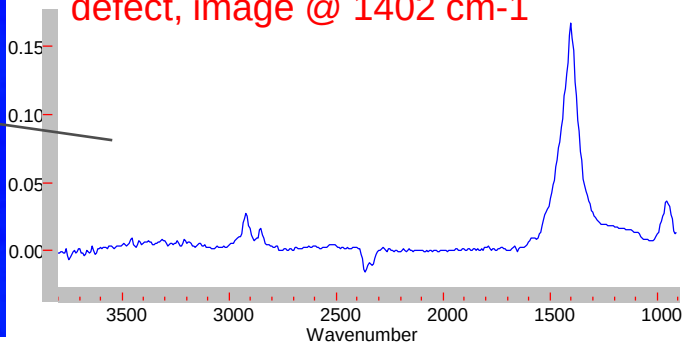
15x obj. vis image – cross-section view

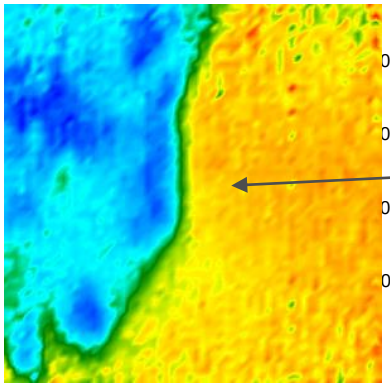


ATR Chemical Image

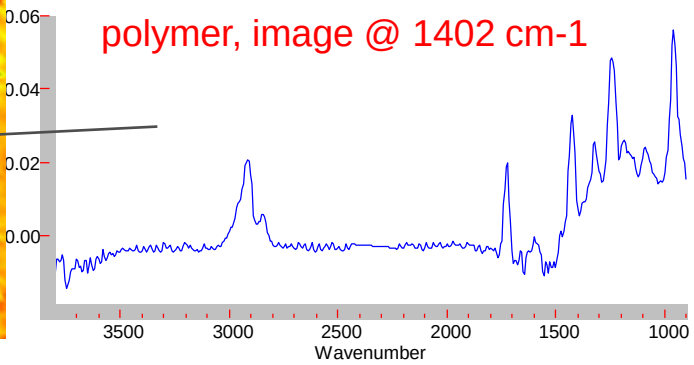


defect, image @ 1402 cm-1

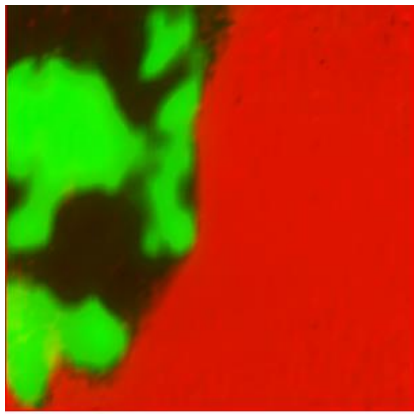




polymer, image @ 1402 cm-1



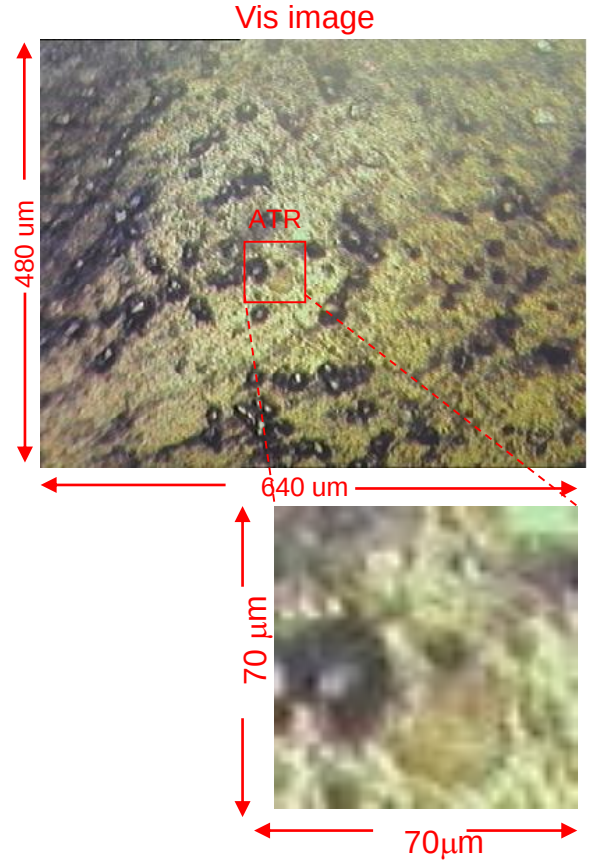
Composite (Green/Red) image

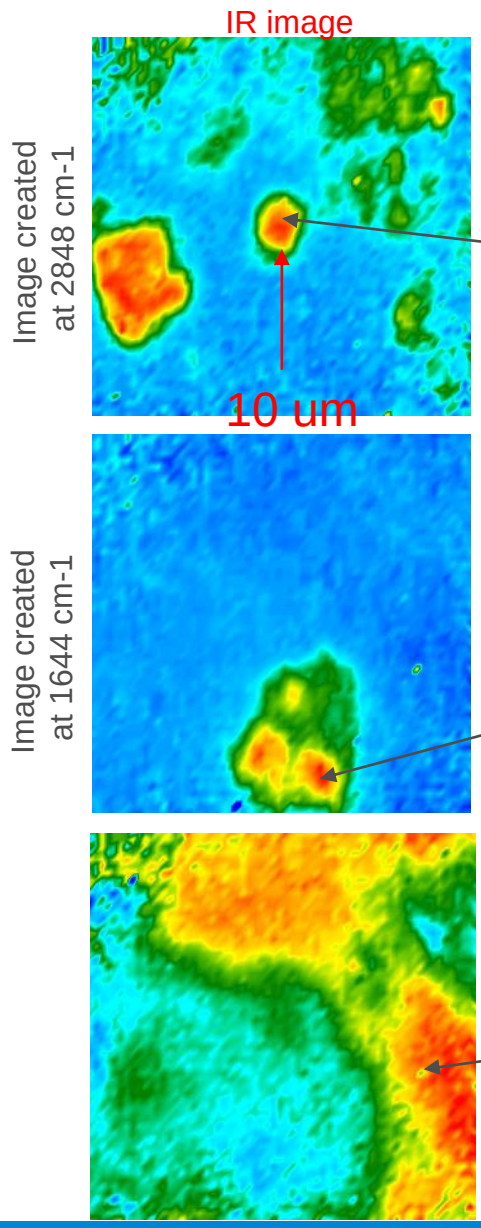


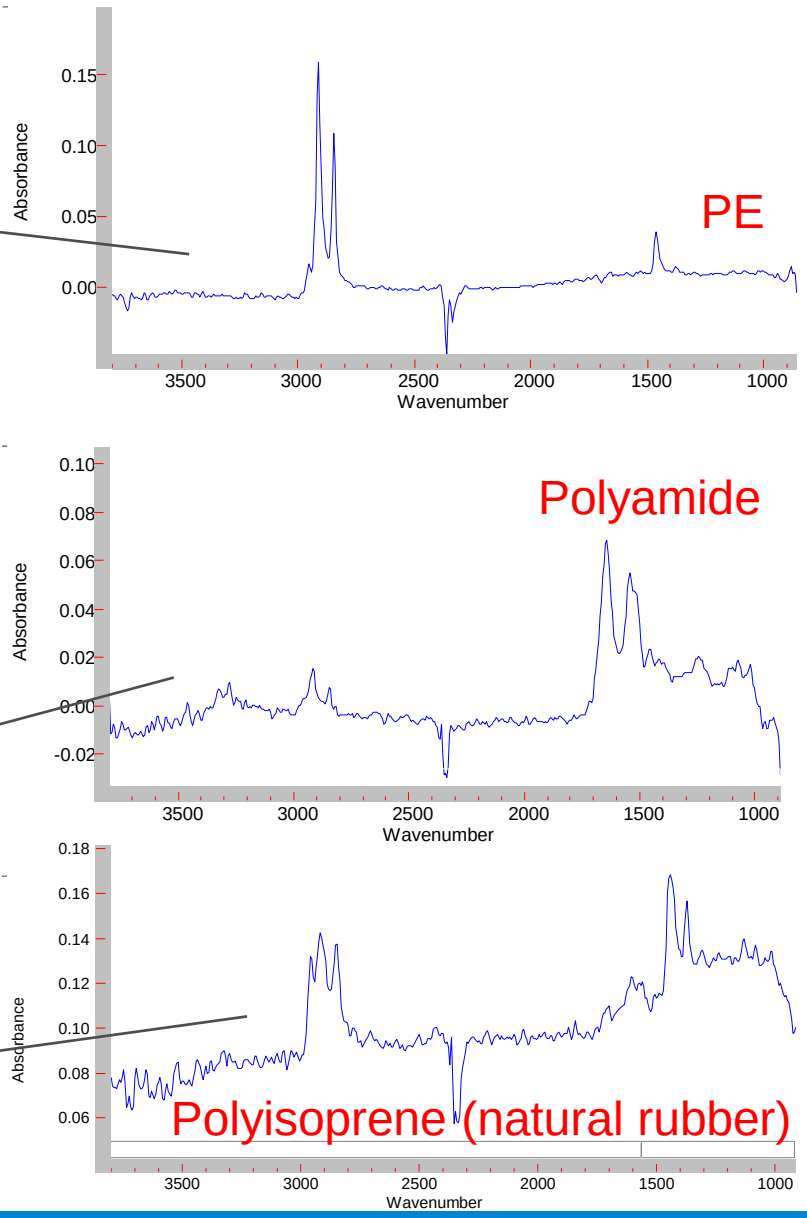
GREEN – DEFECT
RED - POLYMER

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

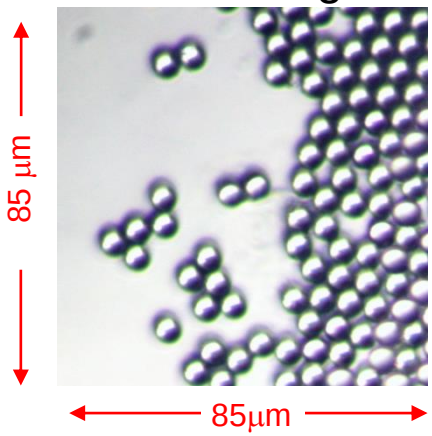




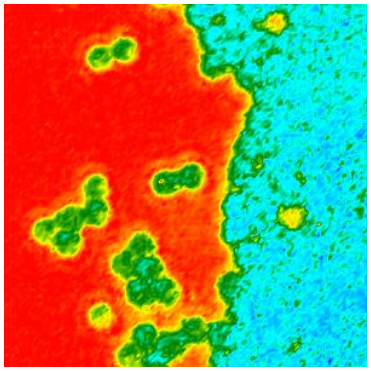


25x, 0.81 NA Objective in “high mag” mode for Polymer bead analysis

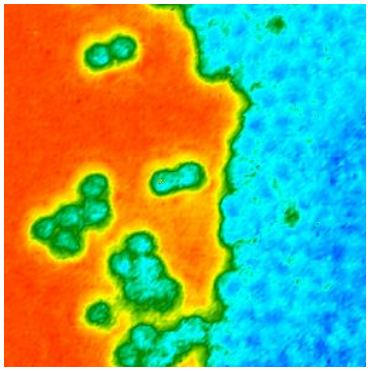
40x vis image



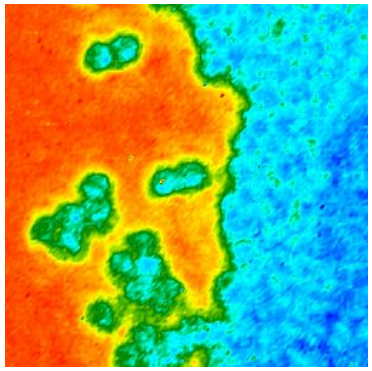
2915 cm-1
(3.4 microns)



1442 cm-1
(6.9 microns)



1118 cm-1
(8.9 microns)



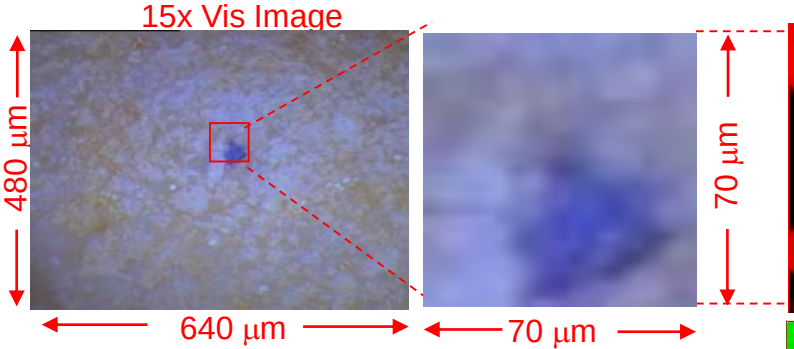
5 micron polystyrene beads on BaF2 substrate are clearly *chemically* distinguished.

Even at relatively long wavelengths (low wavenumbers), 5 micron beads are clearly resolved

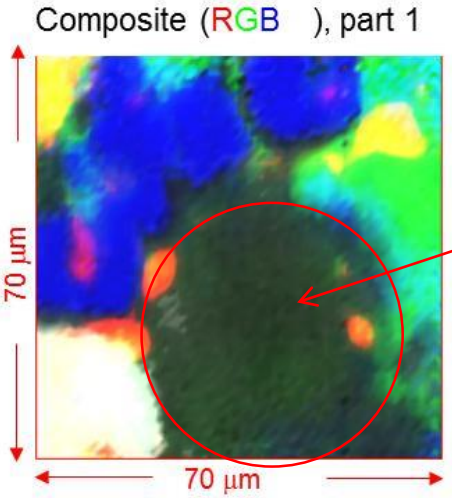
Pixel size:	0.66 micron
Obj mag:	25x, 0.81 NA
High mag:	ON
FOV:	85x85 um
Total system mag:	61x
Working Distance:	12 mm

Pharmaceutical FTIR Imaging

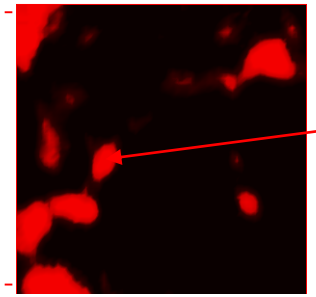
Tablet – Contamination Location

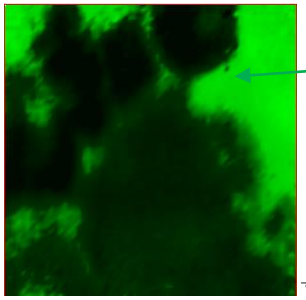


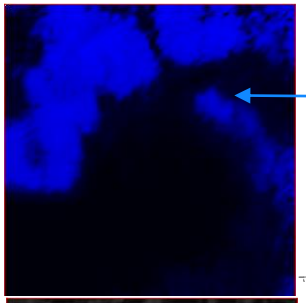
Clear and distinct domains from
4 constituents were detected:
- **Active,** compared to provided standard
- **Lactose**, identified from library search
- **Starch,** identified from library search
- **Cellulose** identified from library search

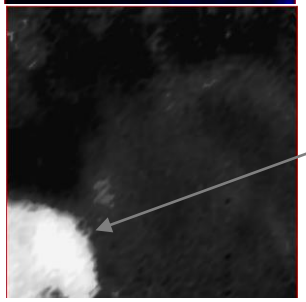


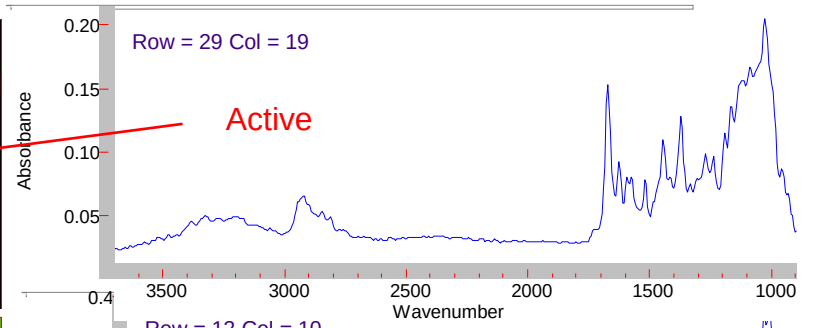
Defect
(next slide)

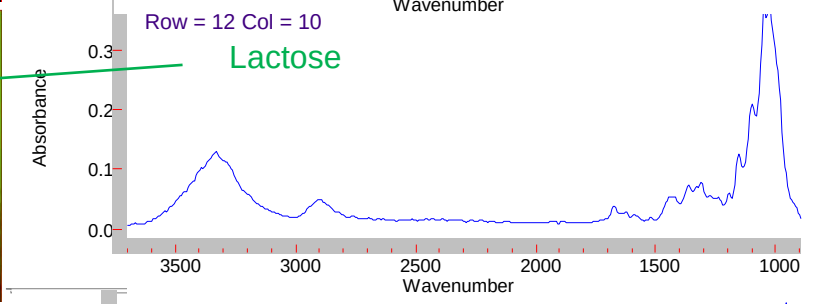


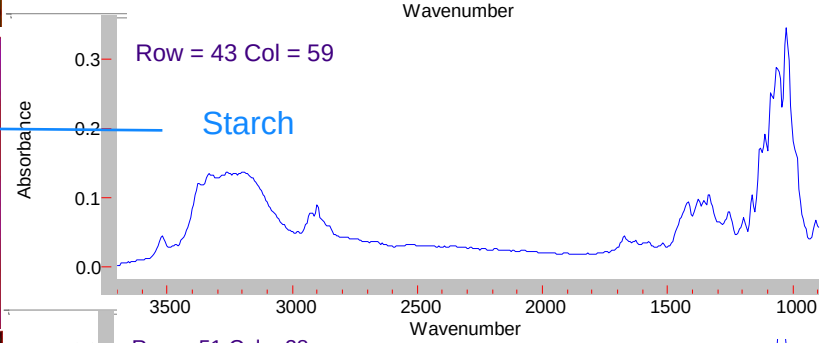


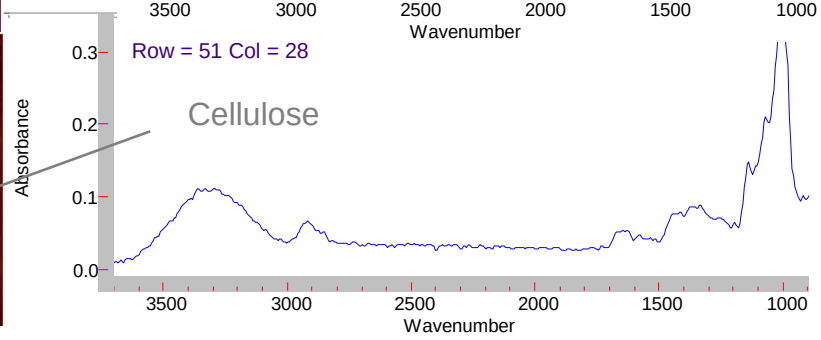




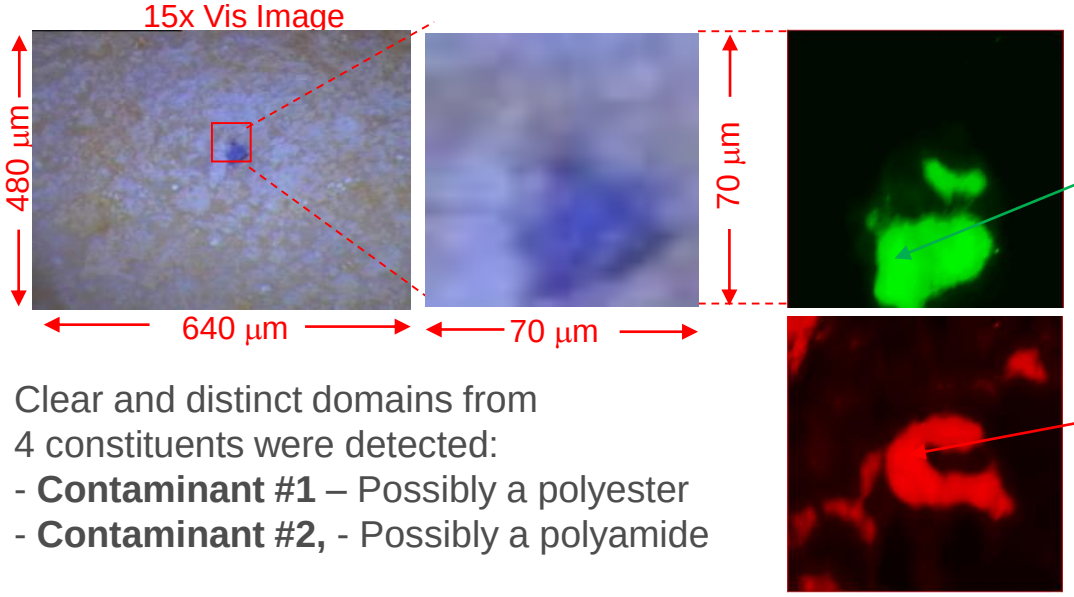


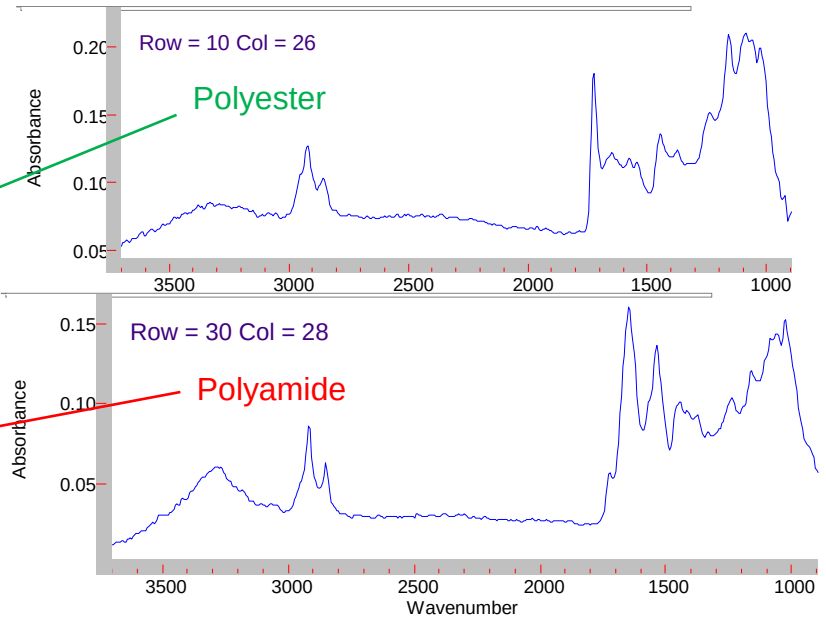






Tablet – Contamination Location

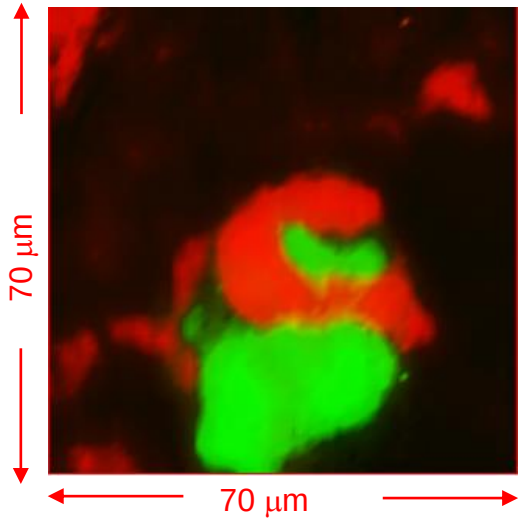




Clear and distinct domains from 4 constituents were detected:

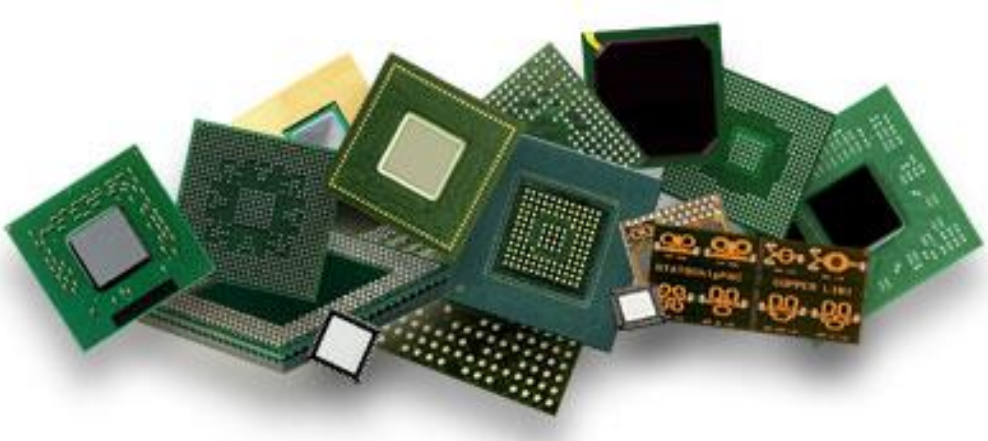
- **Contaminant #1** – Possibly a polyester
- **Contaminant #2,** - Possibly a polyamide

Contaminant Composite (RG), part 2

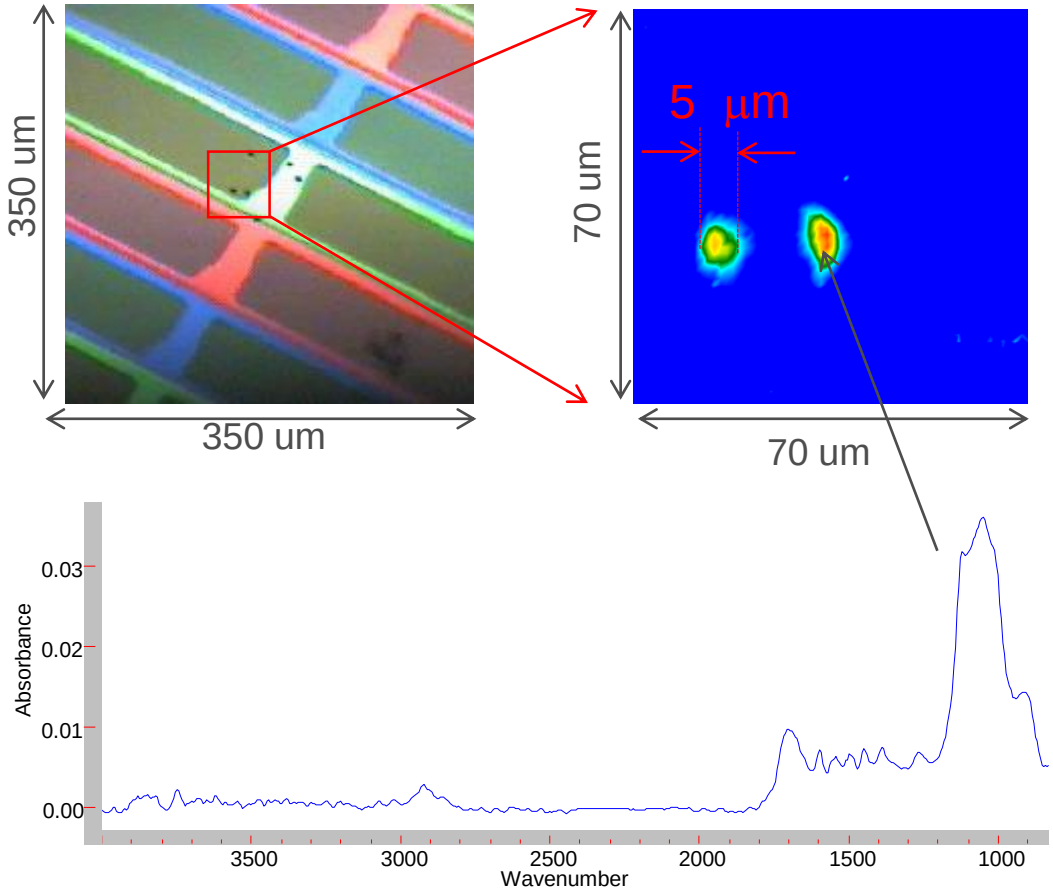


From a single FTIR ATR imaging measurement and without damaging the sample, 4 known constituents and 2 unknown contaminants were imaged in 1 min

Electronics/Semicon FTIR Imaging



Spacer contamination on LCD filter

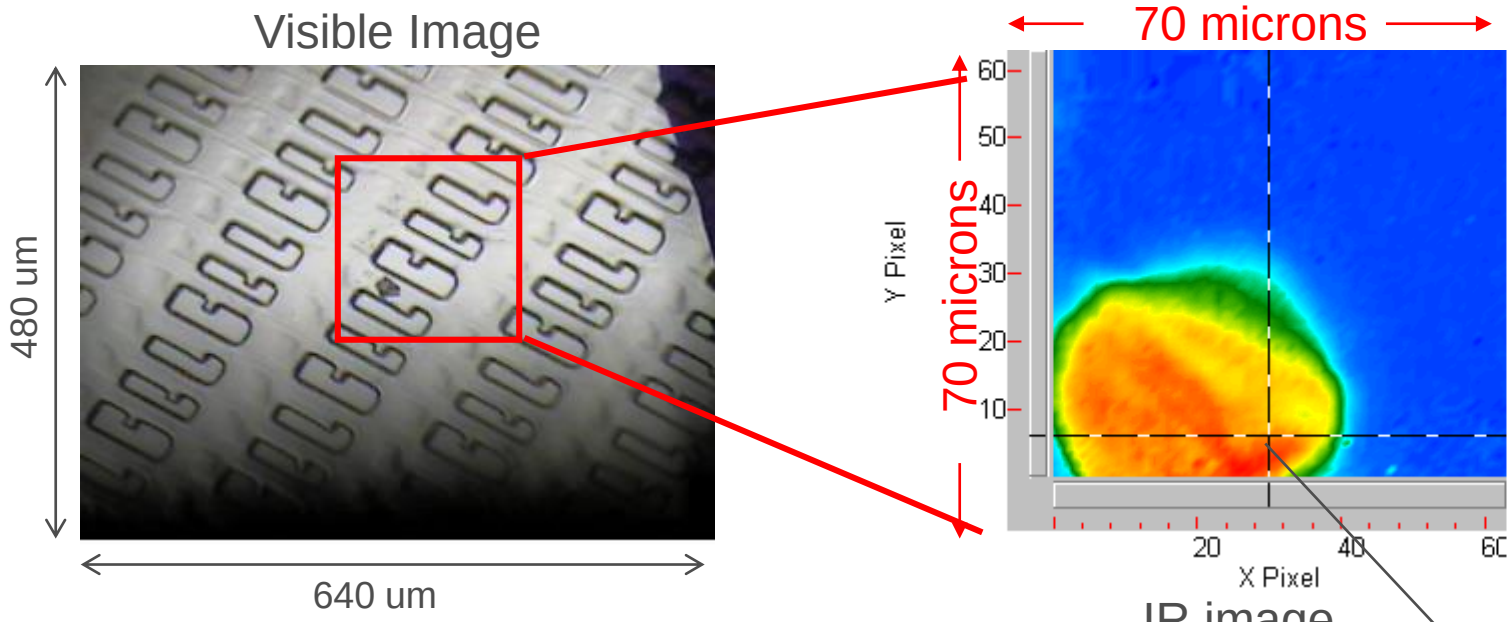


Total analysis time = 2 mins

Defects identified as dislodged Spacers

No sample prep and no sample damage

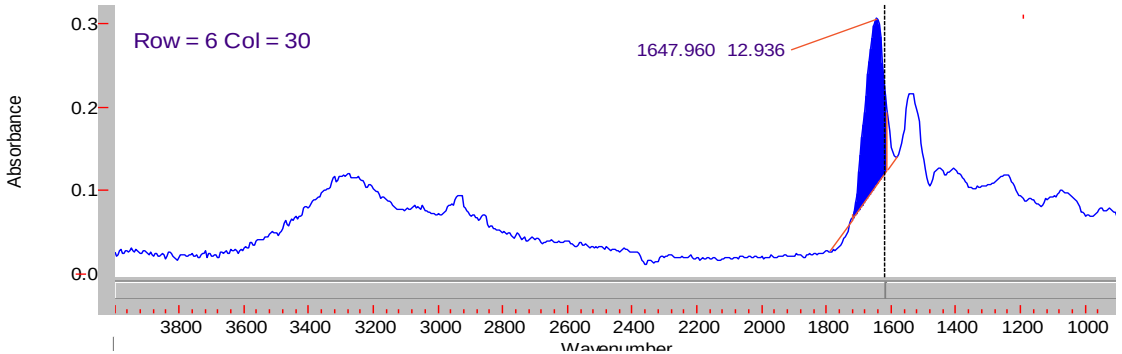
LCD Defect – Protein image (1647 cm-1)



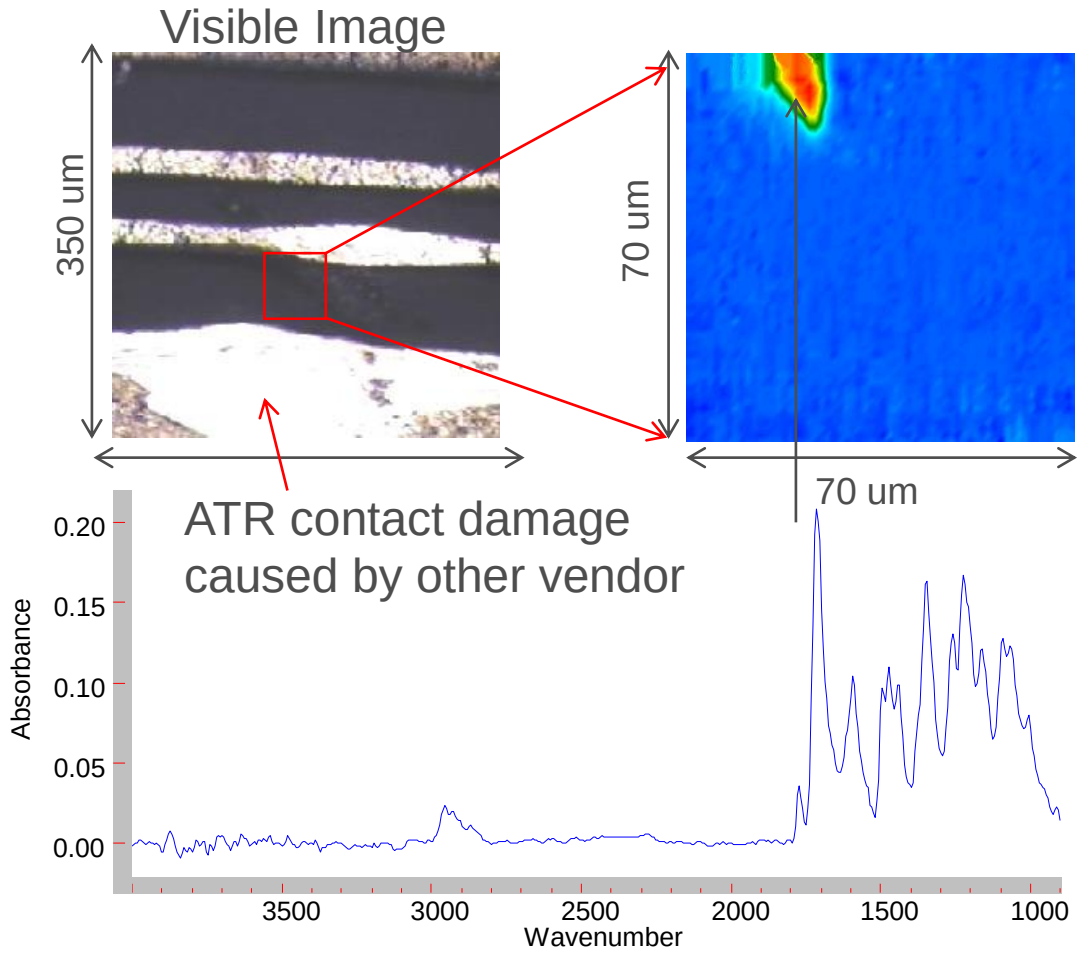
Total analysis time = 1 mins

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

Summary of Cary FTIR Imaging

Highest Spatial Resolution

with new 25x, 0.81NA obj.

Re-defines, what is possible with spatial resolution <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