PerkinElmer
*Science with Purpose*

# **Illuminating the Invisible:** Microparticle Insights with FTIR Microscopy

International Particle Technology Forum
RSC-PCIG

Rhys Kelham | FT-IR Application Specialist

# The Innovators in IR and IR Microscopy

**1944:** First Commercially available Infrared IR spectrometer from PerkinElmer, The PerkinElmer model 12.

**1954:** First commercially available IR microscope developed by PerkinElmer, The PerkinElmer 85 Infrared microscope.

# IR Spectroscopy at PerkinElmer UK


All of Perkin Elmer's IR instruments are manufactured in Llantrisant, South Wales

The Global R&D team are based at our new Spectroscopy Centre of Excellence in High Wycombe along with our remote service technical support team

On site and remote support from our dedicated IR application specialist and our field customer service engineers

# FTIR Theory

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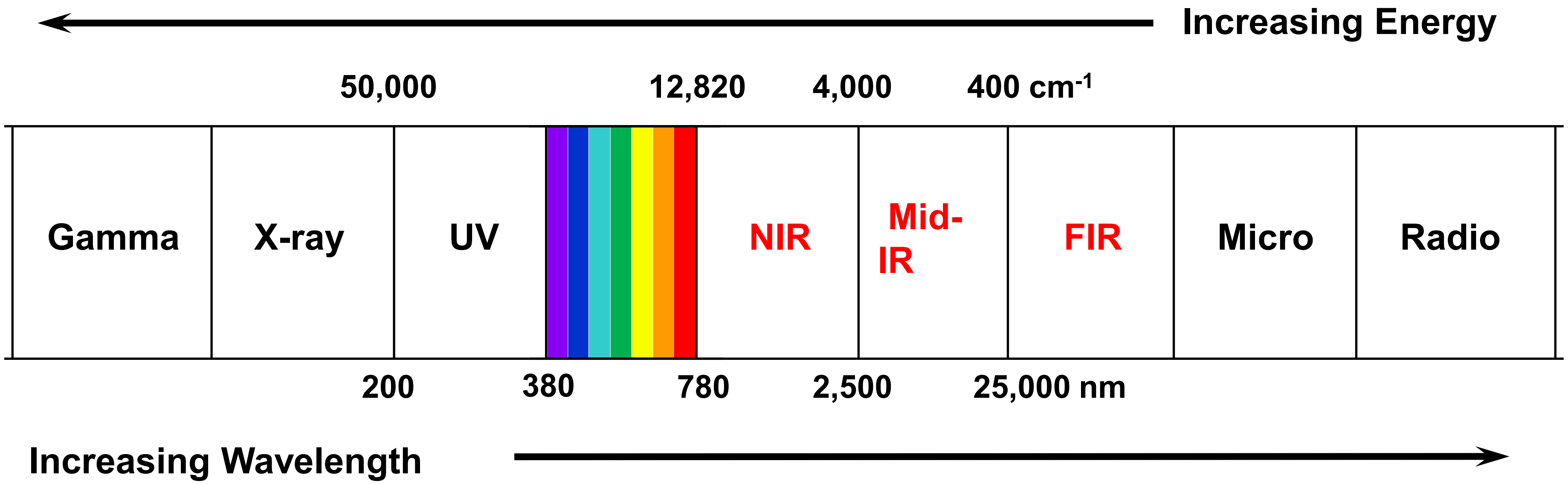
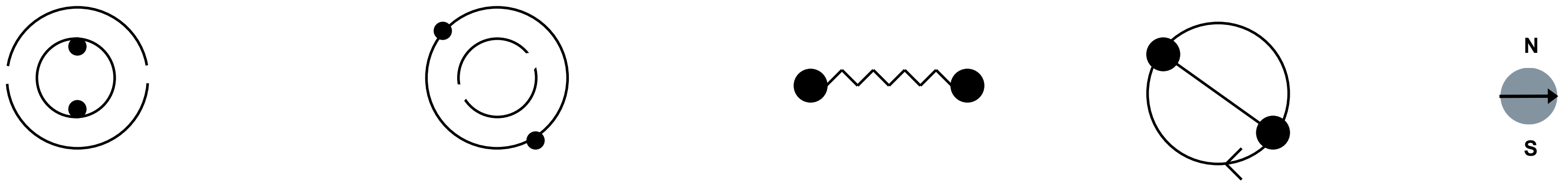
# Spectroscopy and The Electromagnetic Spectrum

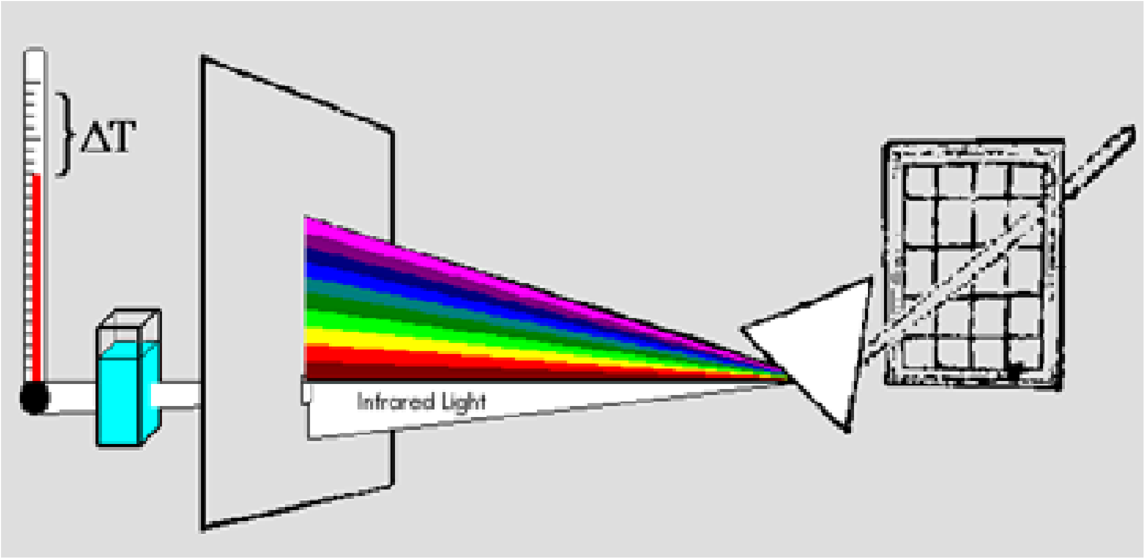
Spectroscopy the study of the interaction of light and matter

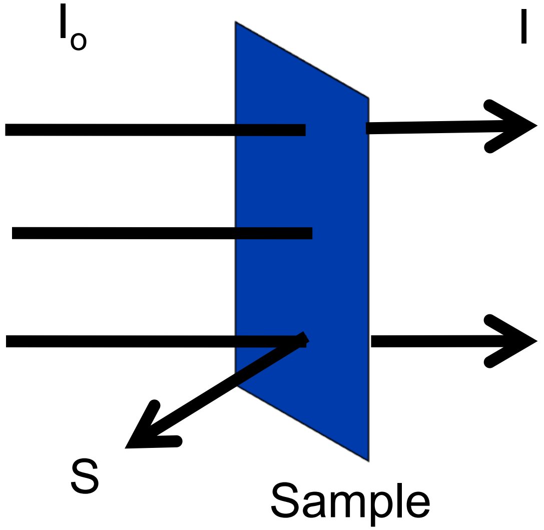


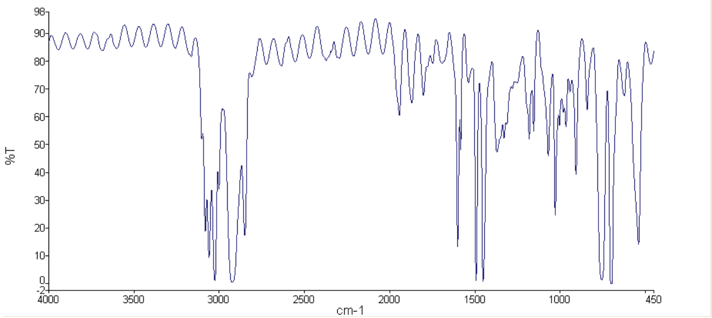

| K- and L-shell electrons | Valence electrons | Molecular vibrations | Molecular rotations | Nuclear spin transitions |
|---|---|---|---|---|
| XRD/XRF | UV-Vis, Fluorescence | FT-IR, Raman | FIR/MW | NMR |

# Just a refresher- Simplified IR theory



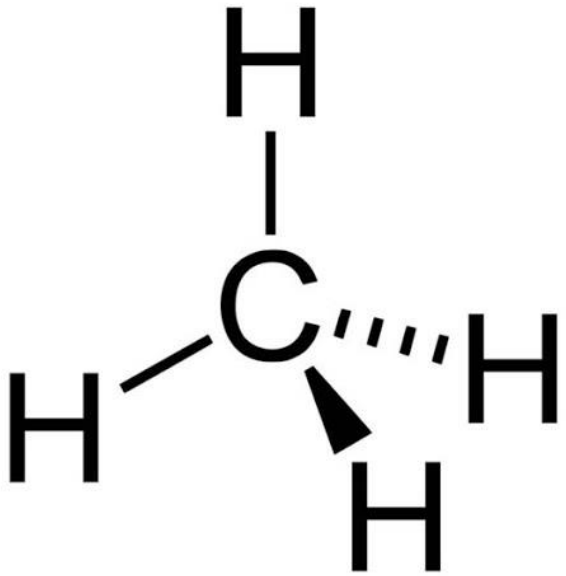

Absorption of IR energy occurs because the frequency of molecular vibrations is the same as IR radiation frequency



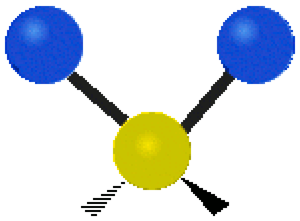
# What does IR measure?

- FT-IR measures vibrational and rotational transitions

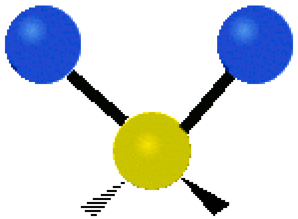
  - With proper sample handling, can measure solids, liquids and gases
  - Species must have covalent bonds
    - Cannot measure single atoms (e.g. NaCl) *
    - Primarily used for organic species containing C, H, O, N, B, Si, P, S, Cl, Br *
  - Qualitative (identification) and quantitative
  - Spectra aid in eludicating structure (functional group analysis)
  - Spectra can be compared with reference library for positive identification



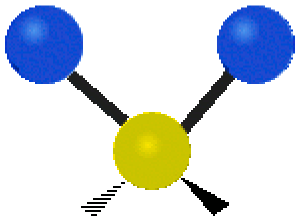
# Type of vibrations



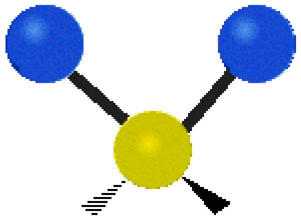
Asymmetric stretching



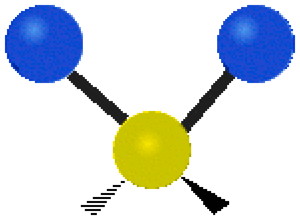
Symmetric stretching



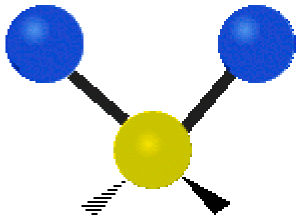
Scissoring (in-plane bending)



Twisting
(out-of-plane bending)



Wagging
(out-of-plane bending)



Rocking
(in-plane bending)

**In general, stretching a chemical bond requires more energy than bending**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# Do all bonds absorb IR radiation?

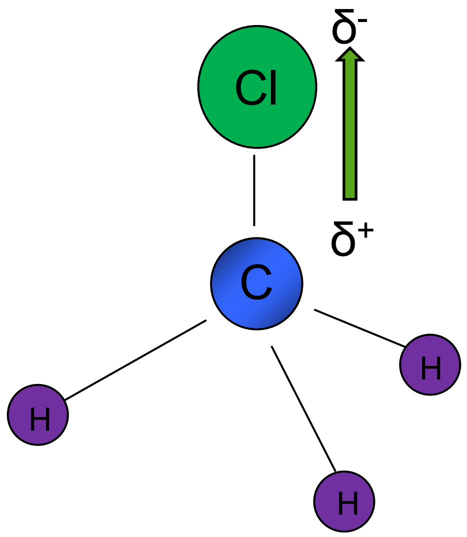
No!

There must be a change in dipole moment. Which really means that the bond must be asymmetric.

This is why simple molecules such as Hydrogen, Nitrogen, etc do not have an IR spectrum whereas Zinc Oxide does.

# Do All Bonds Absorb IR Radiation?

Example of molecules that will not absorb IR radiation?

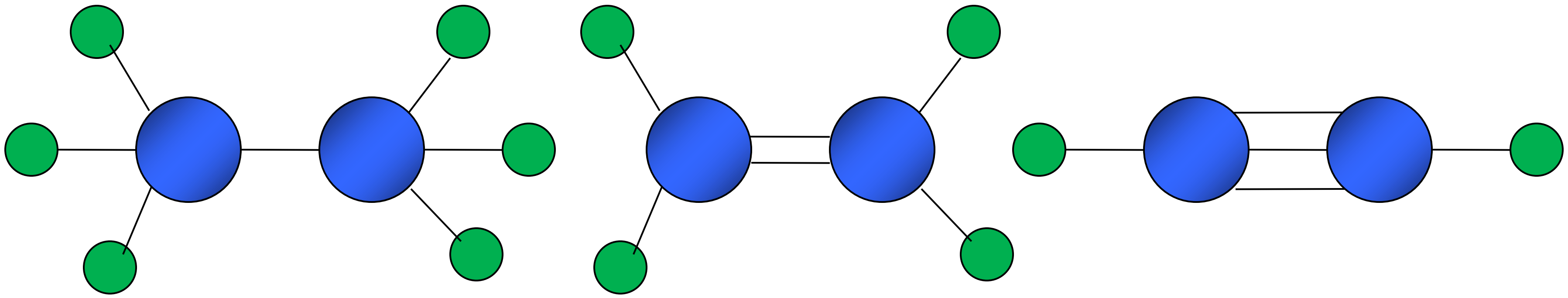



No!

There must be a change in dipole moment. Which really means that the bond must be asymmetric.

# What factors determine absorption frequency of a bond?

The strength of the bond.



The C--C single bond will absorb at a lower frequency (~1200cm$^{-1}$) than the C--C double bond (~1600cm$^{-1}$) than the C--C triple bond (~2150cm$^{-1}$).

# What factors determine absorption frequency of a bond?

The atoms that the bond links. The heavier the atoms the lower the absorption frequency.

C--F absorption = ~1100cm$^{-1}$

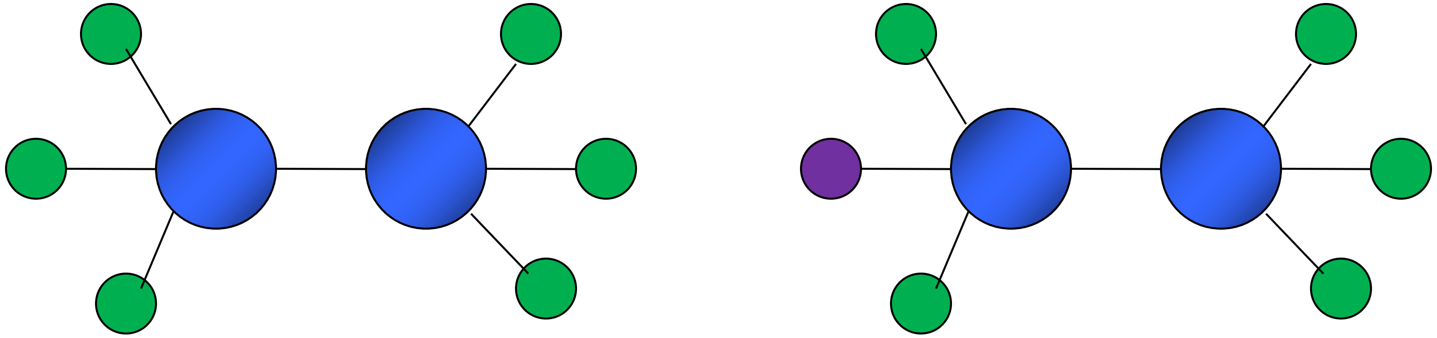
C--Cl absorption = ~750cm$^{-1}$

C--Br absorption = ~650cm$^{-1}$

C--I absorption = ~500cm$^{-1}$

# What factors determine absorption frequency of a bond?

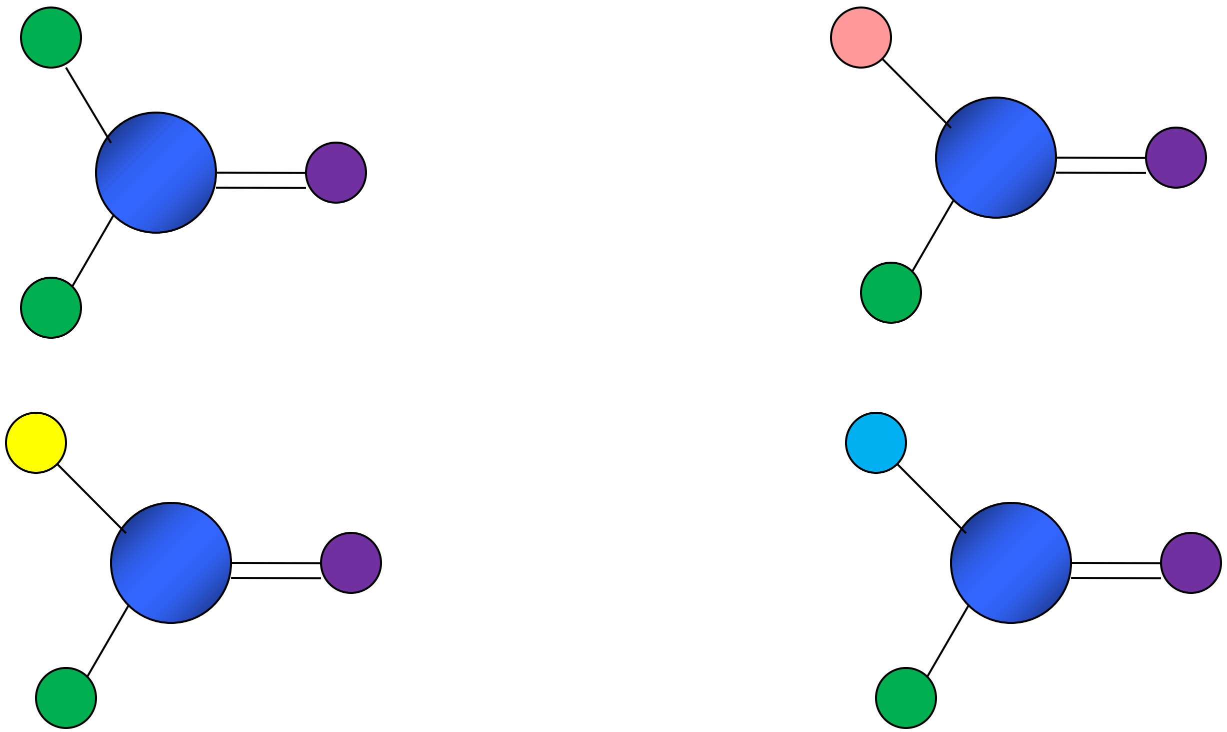
The “molecular environment” of the bond. In general this is a small but subtly important effect on the absorption frequency.



The C--C absorption frequency will be different in these molecules because of the replacement of one hydrogen by a fluorine atom.

# What factors determine absorption frequency of a bond?

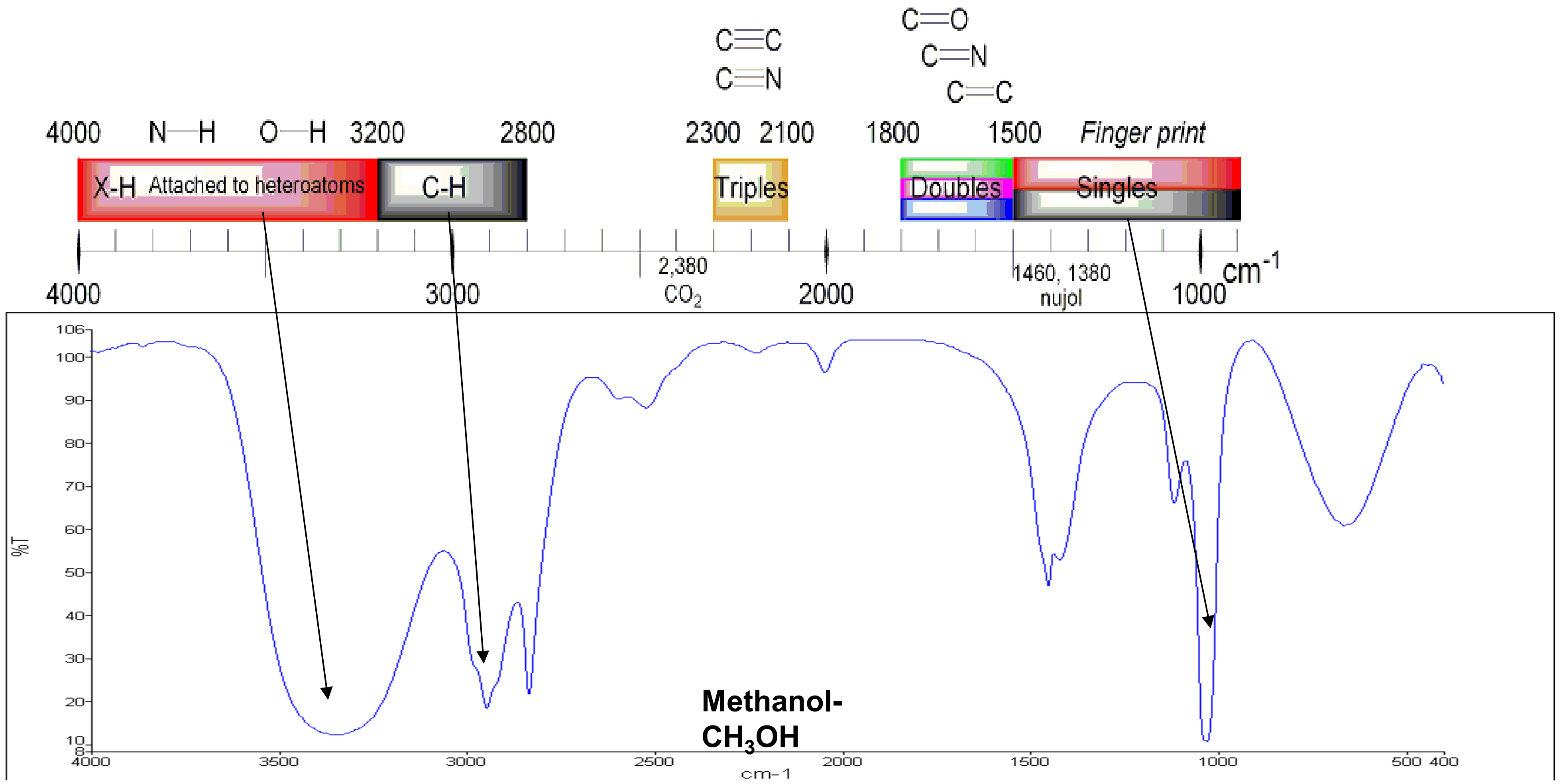
The "molecular environment"



It is possible to distinguish between ketones, aldehydes, carboxylic acids, amides simply because they have different carbonyl absorption frequencies.

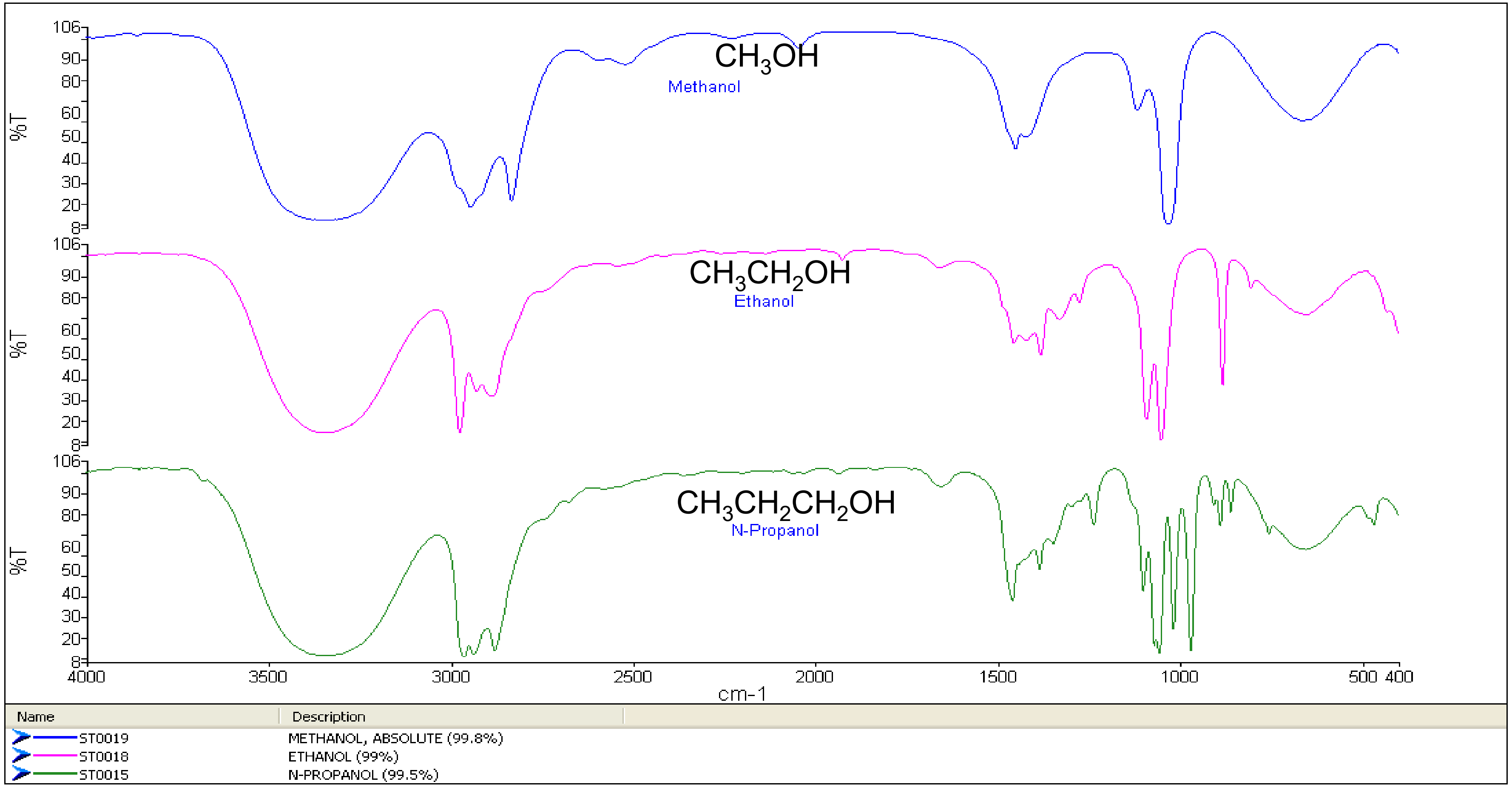
# Typical Infrared Spectrum

Functional groups within a molecule have characteristic peak positions




What happens as you add functional group?

# Molecular Fingerprint




Adding one or more carbons and hydrogens (methylene group) to the alcohol has a significant effect on the infrared spectrum

# IR Microanalysis and Sample Size

Smaller

**Macro IR**

Smallest

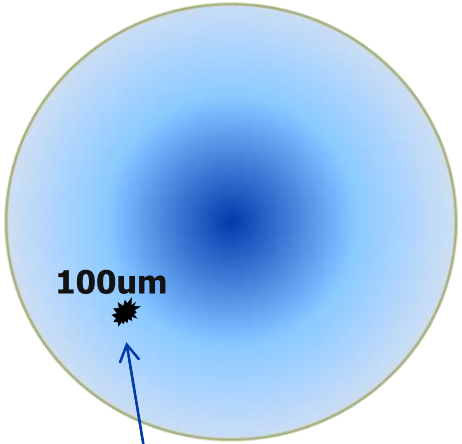
**Micro IR**

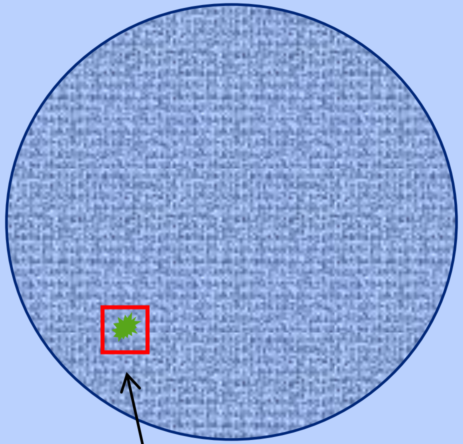
**Imaging IR**

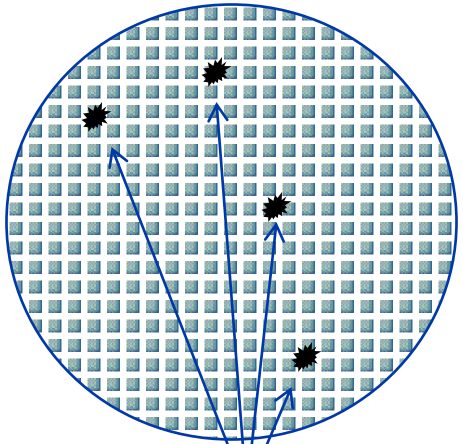
Beam size

1 cm

<1 mm





**Contaminant?**

You usually can't detect it | You need to see it first | It can find it for you

Spectrum 2, Spectrum 3
Isolated 100um - **Yes**
100um within 1cm - **No**

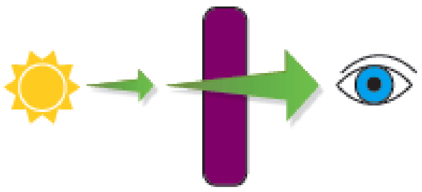
**Spotlight Aurora**

Spotlight Aurora-I

Application: Find/identify contaminants, measure v. small samples, characterise spatial distribution

# FTIR and FTIR Microscopy Sampling Modes

### Transmission

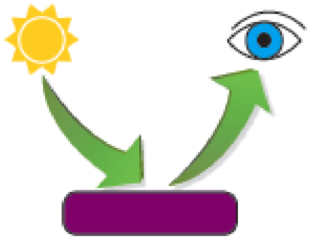


IR light passes through the sample and is absorbed dependent on its molecular structure

**Pros:**
- Most sensitive technique

**Cons:**
- IR light must pass through sample (<100um thickness)
- Can involve difficult sample preparation

### Reflectance



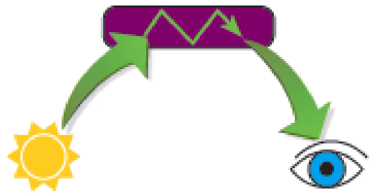
IR light is reflected off sample surface

Can be specular or diffuse reflectance which is dependent on sample surface

**Pros:**
- Minimal sample preparation
- No sample contact needed

**Cons:**
- Quality of data dependent on samples reflectivity
- Prone to reflectance artefacts

### Attenuated Total Reflectance (ATR)



IR light is internally reflected inside the ATR crystal

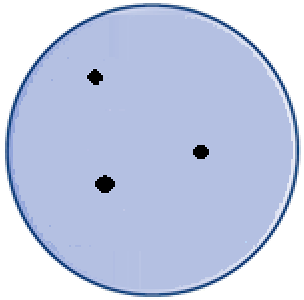
The attenuated reflection forms an evanescent wave which interacts with the sample surface. The IR energy absorbed is attenuated back down to the detector

**Pros:**
- Minimal sample preparation
- Analysis of samples in natural state

**Cons:**
- Only looks at surface of sample (1-3um depending on ATR crystal)

# FTIR Microscopy - Measurement Modes

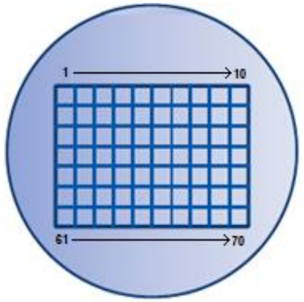
## Point Mode



Only measure points of interest.

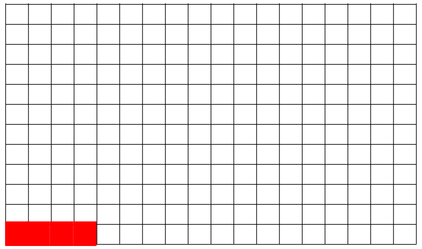
This can be individual particles or specific areas of a sample.

## Mapping



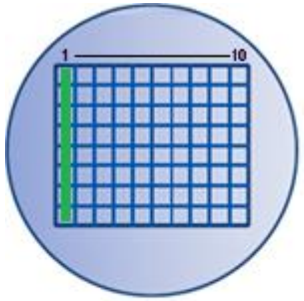

Scan-move-scan each point over large region.

Spectra collected at each point across a set X and Y distance



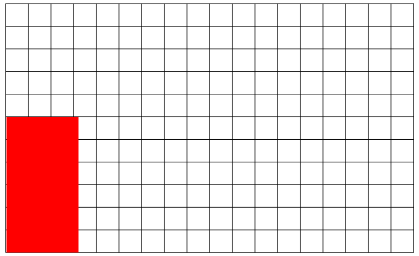
Point-by-point

## Linear Imaging




Multiple detector array measures multiple points simultaneously

Creates a digital image of sample with each pixel representing a spectrum



Linear array (pushbroom)

# Example: Microplastics in drinking water

# FTIR Analysis of Microplastics



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# Sample Preparation Methods

## Flotation - Density Separation

- Sodium Chloride  1.2 g cm-3
- Sodium Polytungstate 1.4
- Zinc Chloride 1.5-1.7
- Sodium Iodide 1.8

Plastics range in density from approximately 0.9 g $cm^3$ for polypropylene (PP) and low density polyethylene (LDPE) up to 1.4 g $cm^3$ for polyethylene terephthalate (PET) and polyvinyl chloride (PVC).

## Sample Digestion

- Acidic – HCl, $HNO_3$
- Basic – NaOH
- Peroxide – $H_2O_2$, Fentons Reagent
- Enzymatic – Proteinase K

  Proteinase K is stable over a wide pH range (4–12), with a pH optimum of pH 8.0.[5] An elevation of the reaction temperature from 37 °C to 50–60 °C may increase the activity several times


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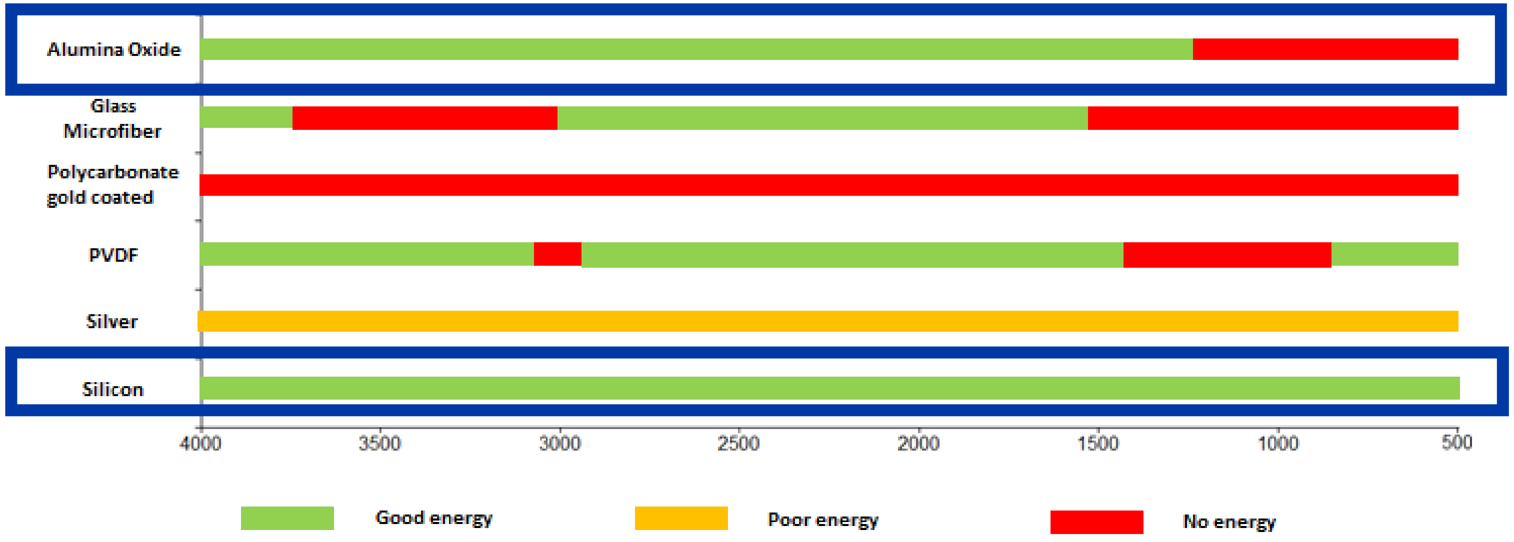
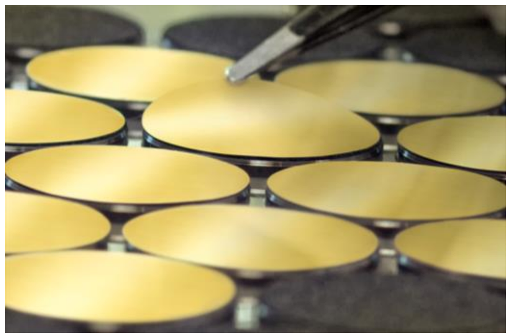
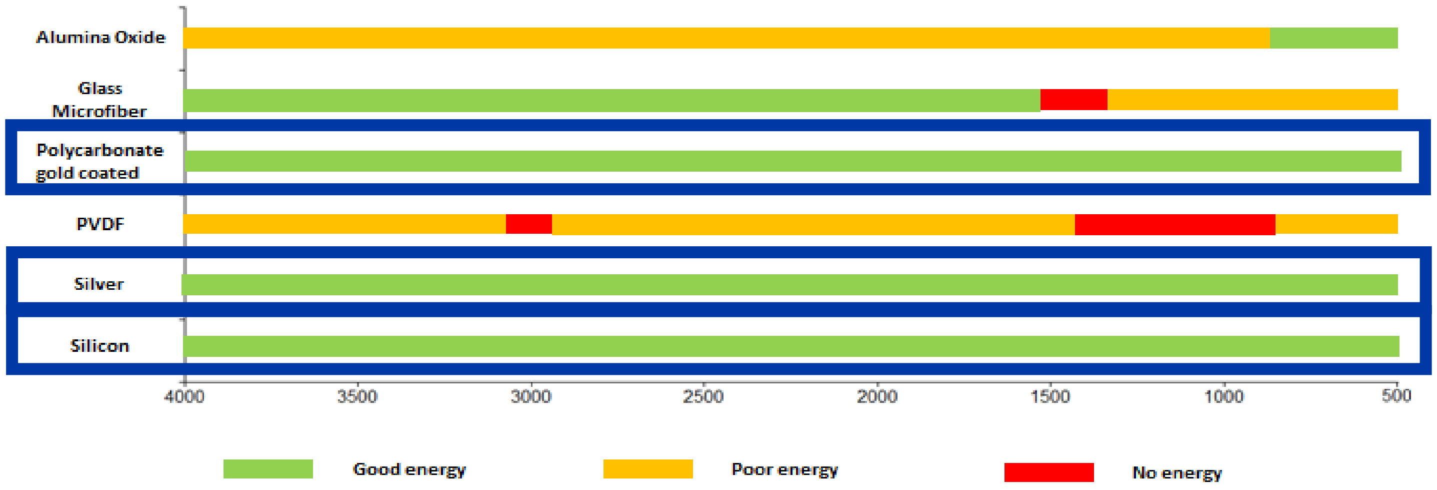
# IR Spectral Range of Filter Materials


## Transmission Range




## Reflectance Range





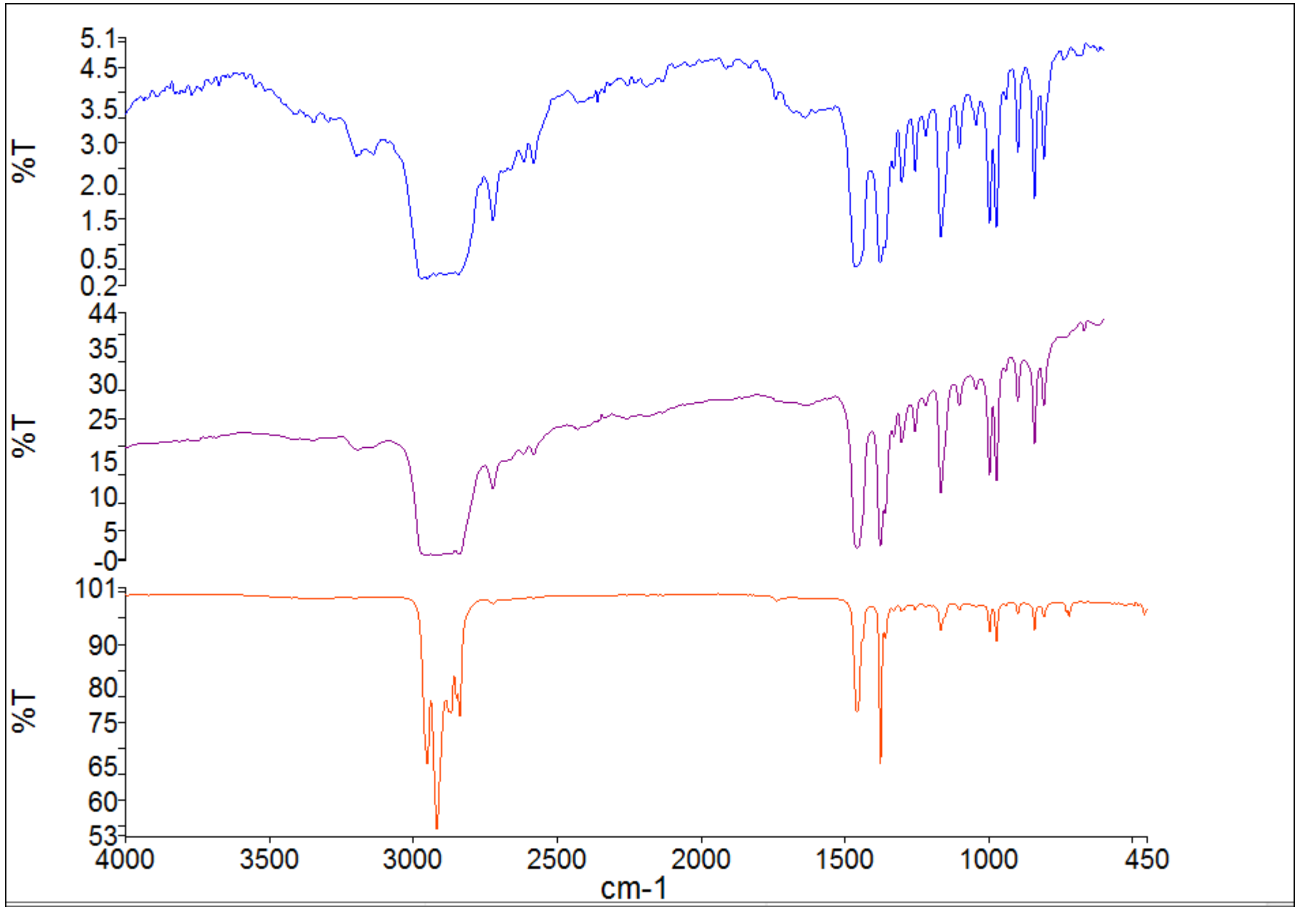

Other Considerations:

Filter material compatibility with digestion matrix

Pore size

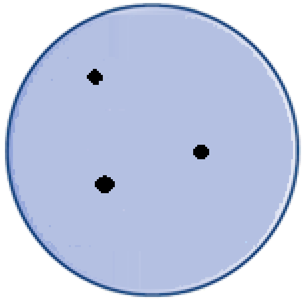
# FTIR and FTIR Microscopy Sampling Modes for Microplastics

- Reflectance
  - Fast and easy
  - Prone to reflectance artefacts
- Transmission
  - Fast and easy
  - Particles may be too thick leading to poor (too strong) spectra
- ATR
  - Best IR spectra
  - Particles may move under force
  - Particles may stick to ATR crystal



# FTIR Microscopy – Microparticle Measurement Modes

## Point Mode



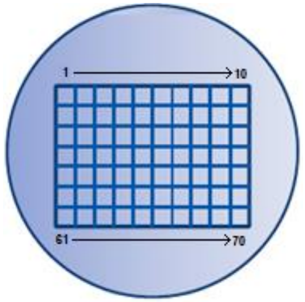
Only measure points of interest.

Fastest for small numbers of particles.

Use with particle detection for automation.

Relies on particle detection for finding particles of interest

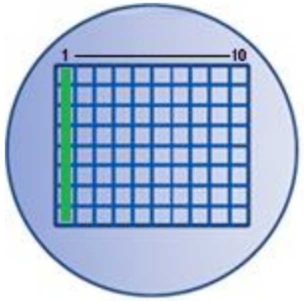
## Mapping




Scan-move-scan each point over large region.

Can be slow depending on size of the area of interest

## Linear Imaging




Multiple detector array measures multiple points simultaneously

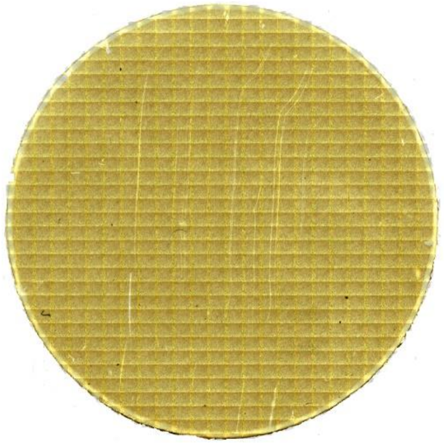
Fast for large regions

Higher Signal to Noise ratios at smaller pixel sizes

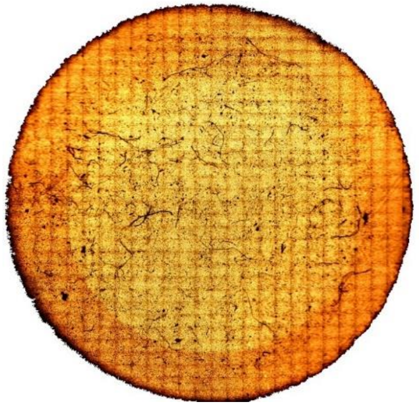
Finds particle of interest for you

# Infrared Microscopy Workflow for Microplastics

1. Collect Visible Image Survey
2. Select Point mode or Imaging
3. Perform IR Data Collection
   a) Point mode
   b) Imaging Mode
4. Analyse Data

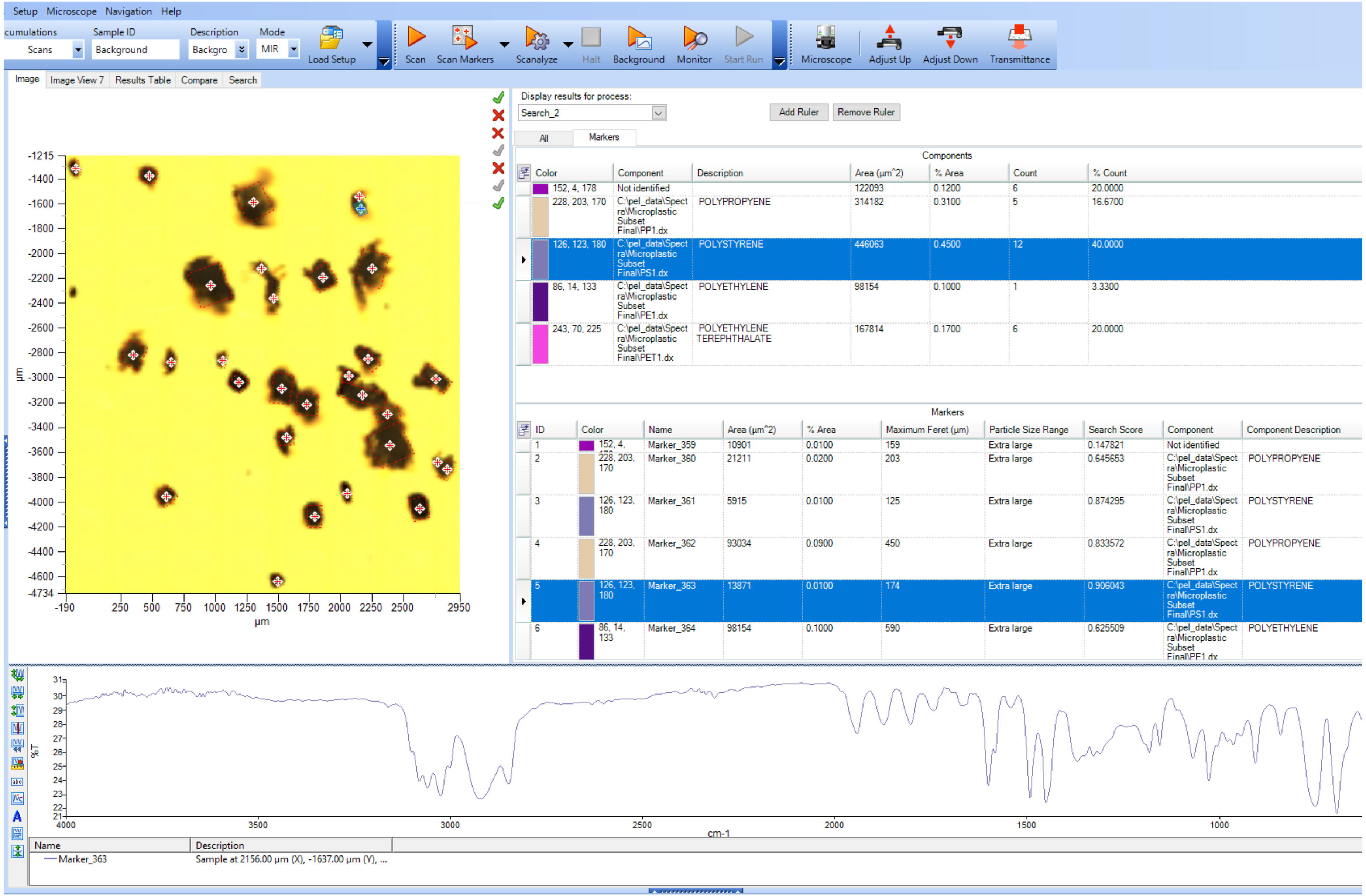


Brand “B” bottled water



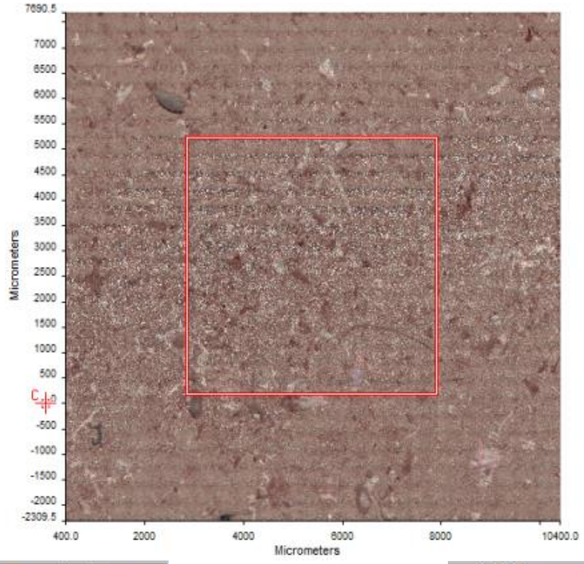
Laboratory tap water

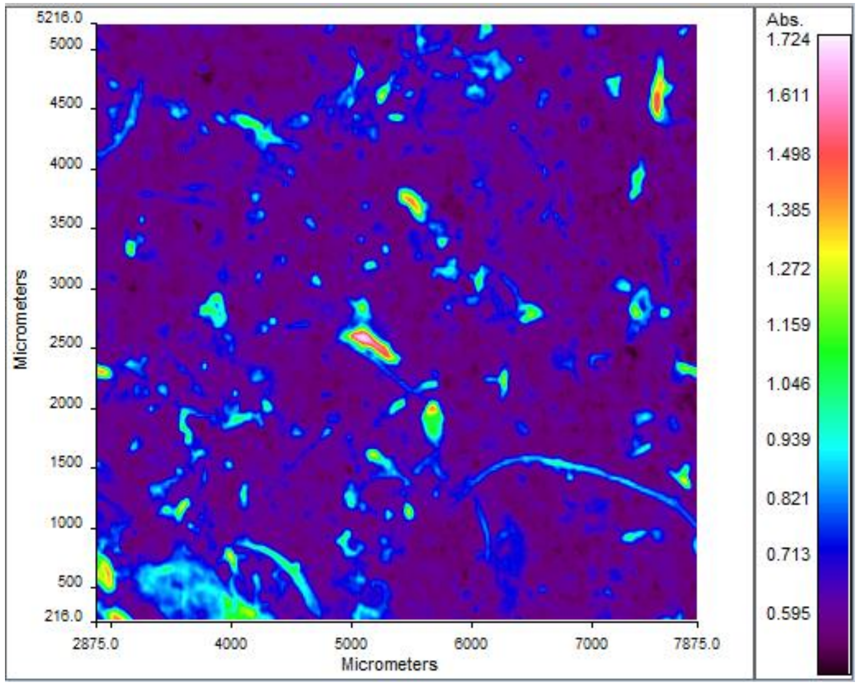
# A) Point mode - Automatic Detection of Particles



# B) IR Imaging of Microplastic Filters

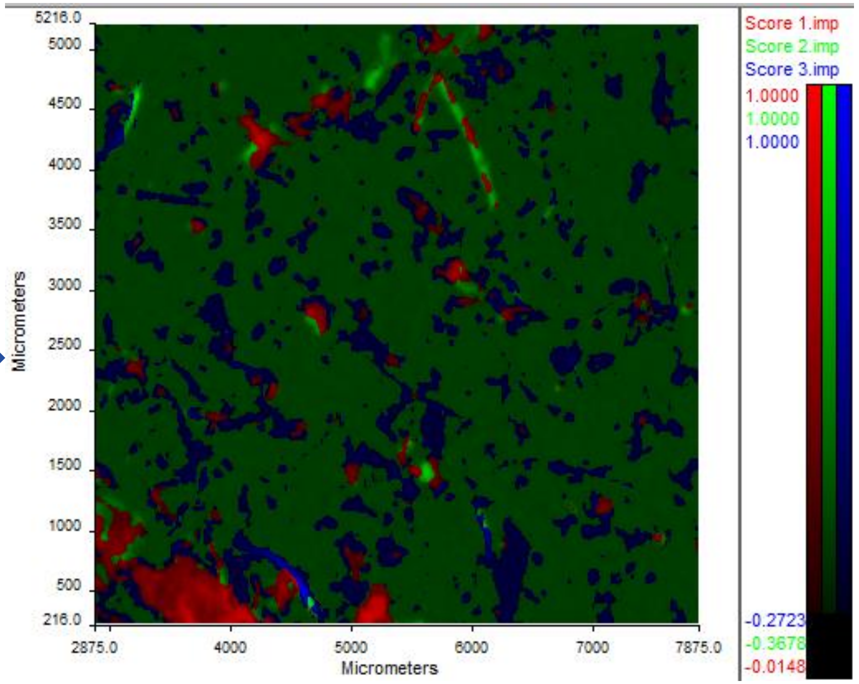
IR Image




Chemometric

Data Processing



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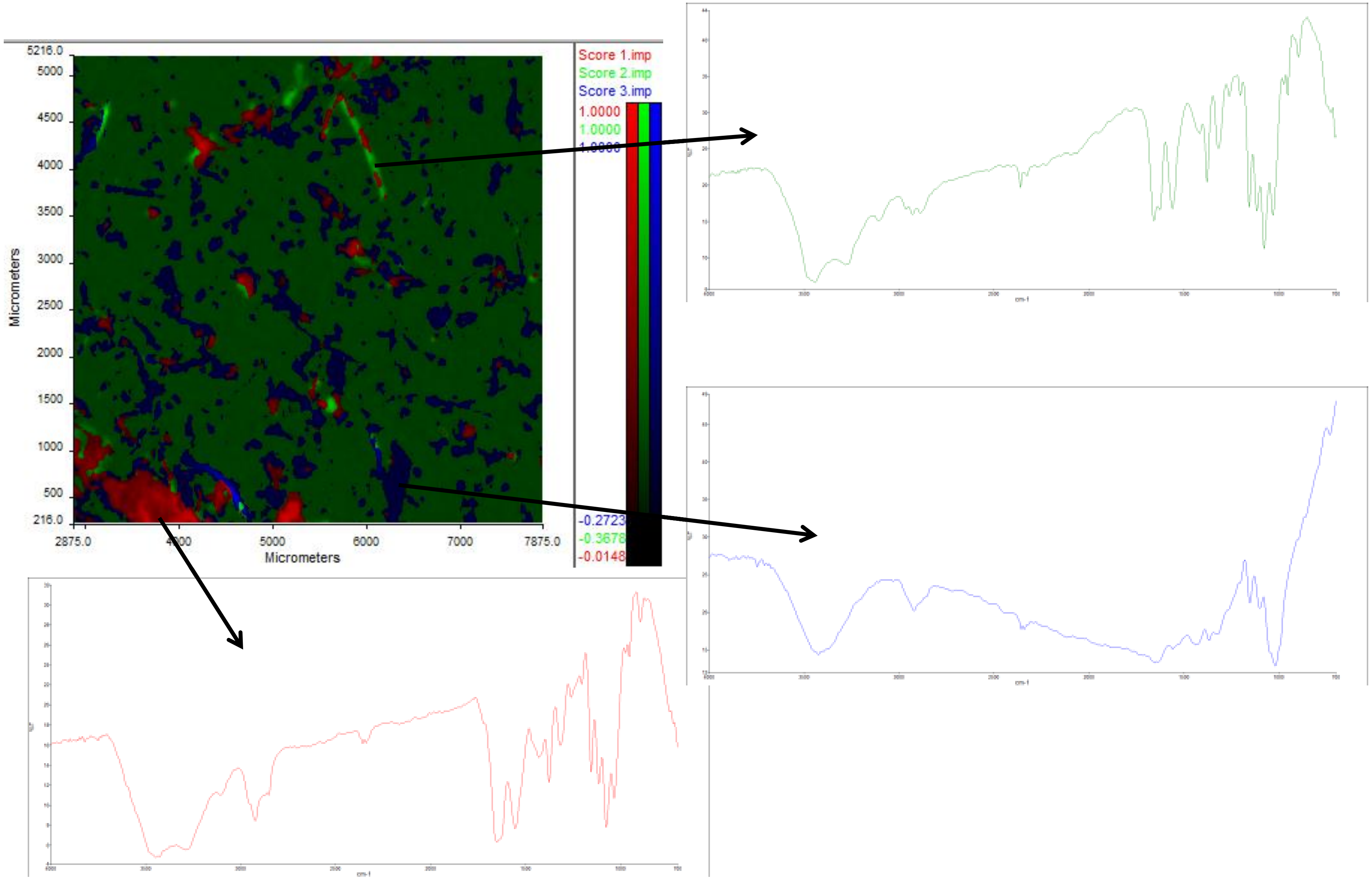
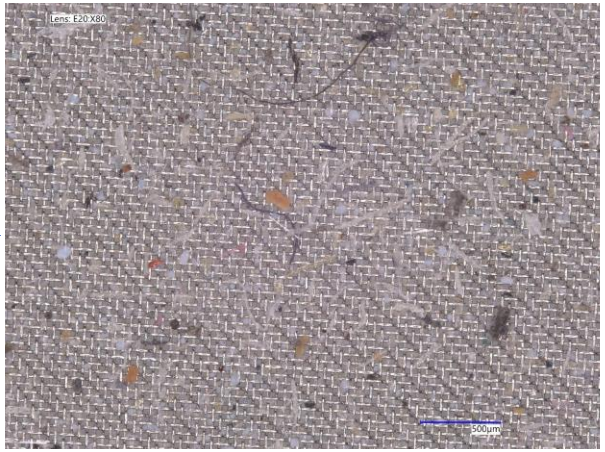
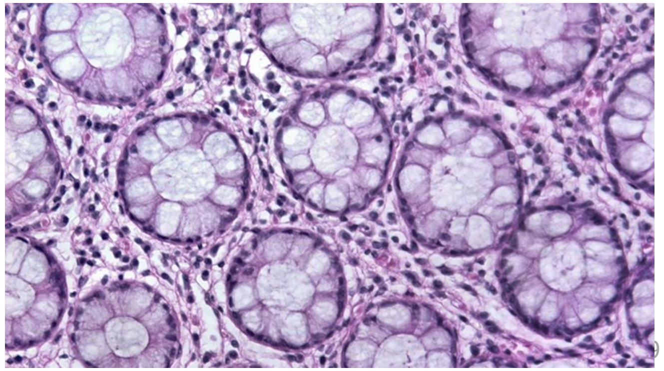


# What A Micro Nuisance! - Microparticles

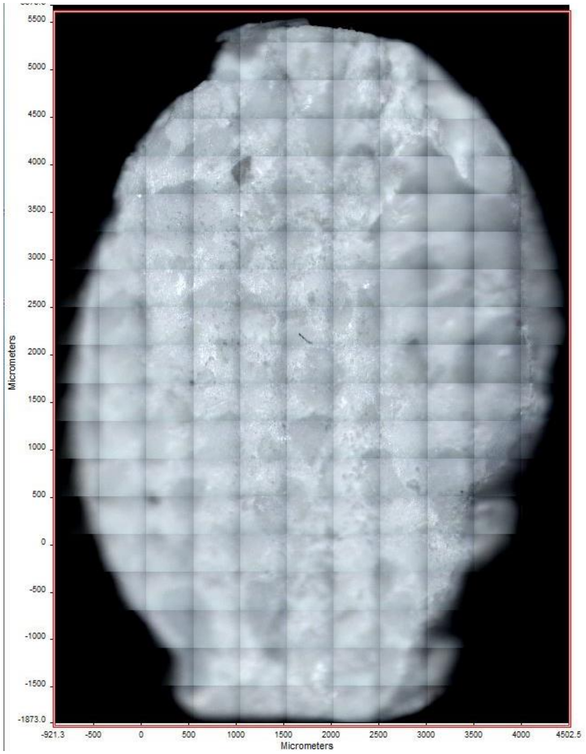


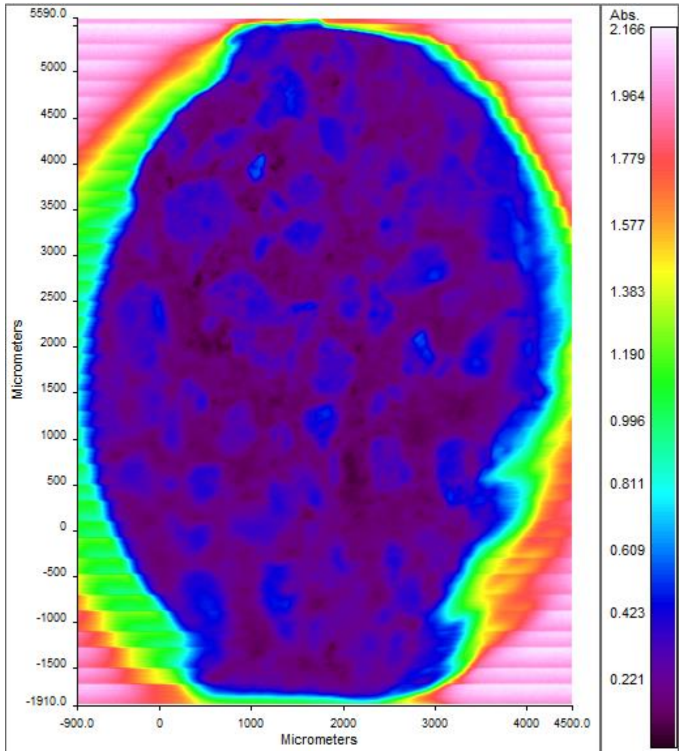


# Application Examples: Spatial Distribution

# Make Sure You’re Getting the Right Dose!

## NIR Imaging of Pharmaceutical Tablet




Tablet Cross Section :

Visible Image Survey (left)

Average IR Absorbance Image (Right)

## Spatial Distribution of Ingredients – Importance in Formulations

**QA/QC Analysis of Manufactured Products**

**- Test tablet for :**

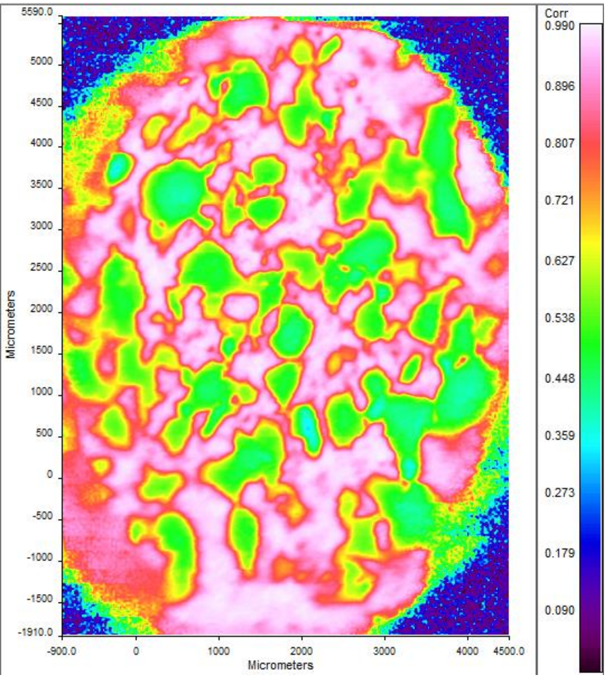
- Uniformity
- Active ingredient concentration
- Size distribution

**Ensuring the product performs as intended**

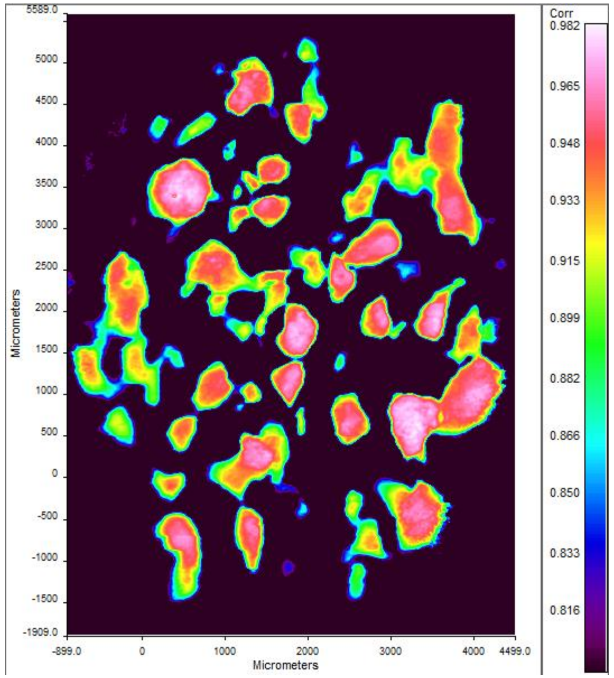
**NIR Imaging**

- Fast due to lower resolution measurements
- Direct reflectance measurements
- Non-contact measurement
- Non-destructive

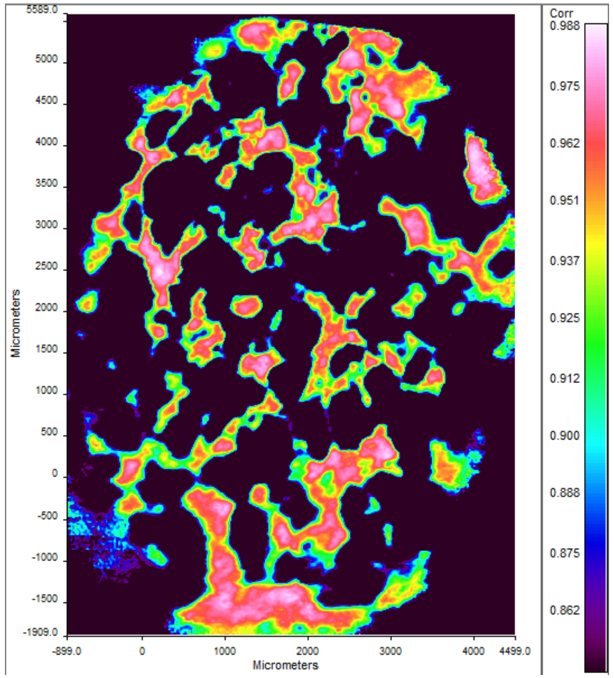
# Make Sure You're Getting the Right Dose!



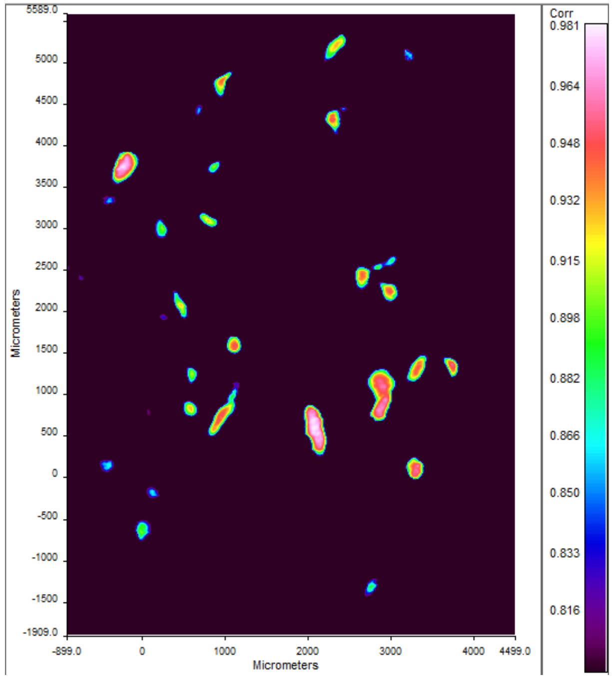

Average Absorbance




Acetyl Salicylic Acid

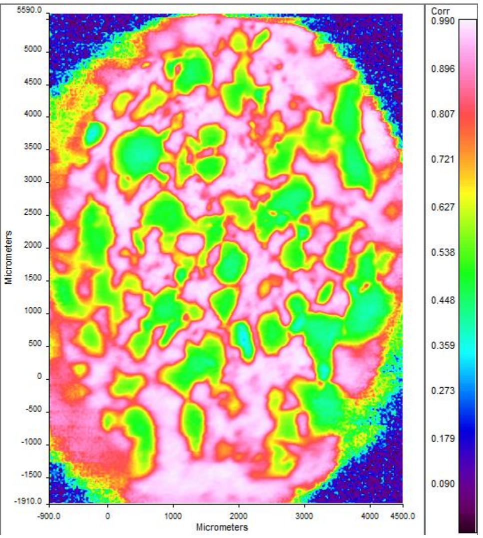



Paracetamol

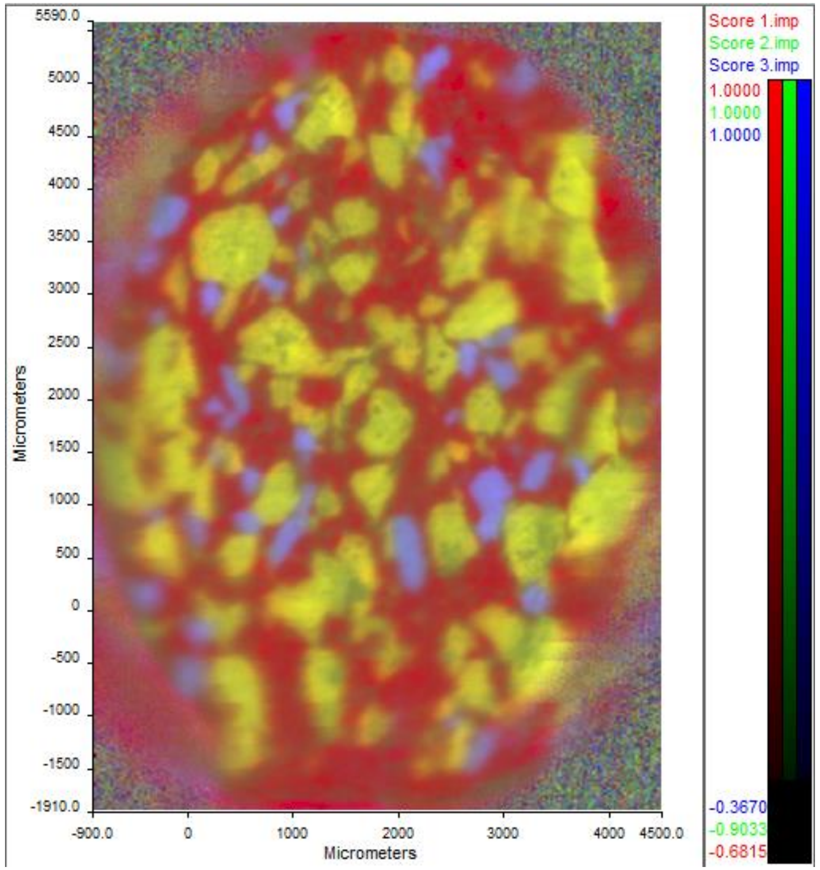



Caffeine

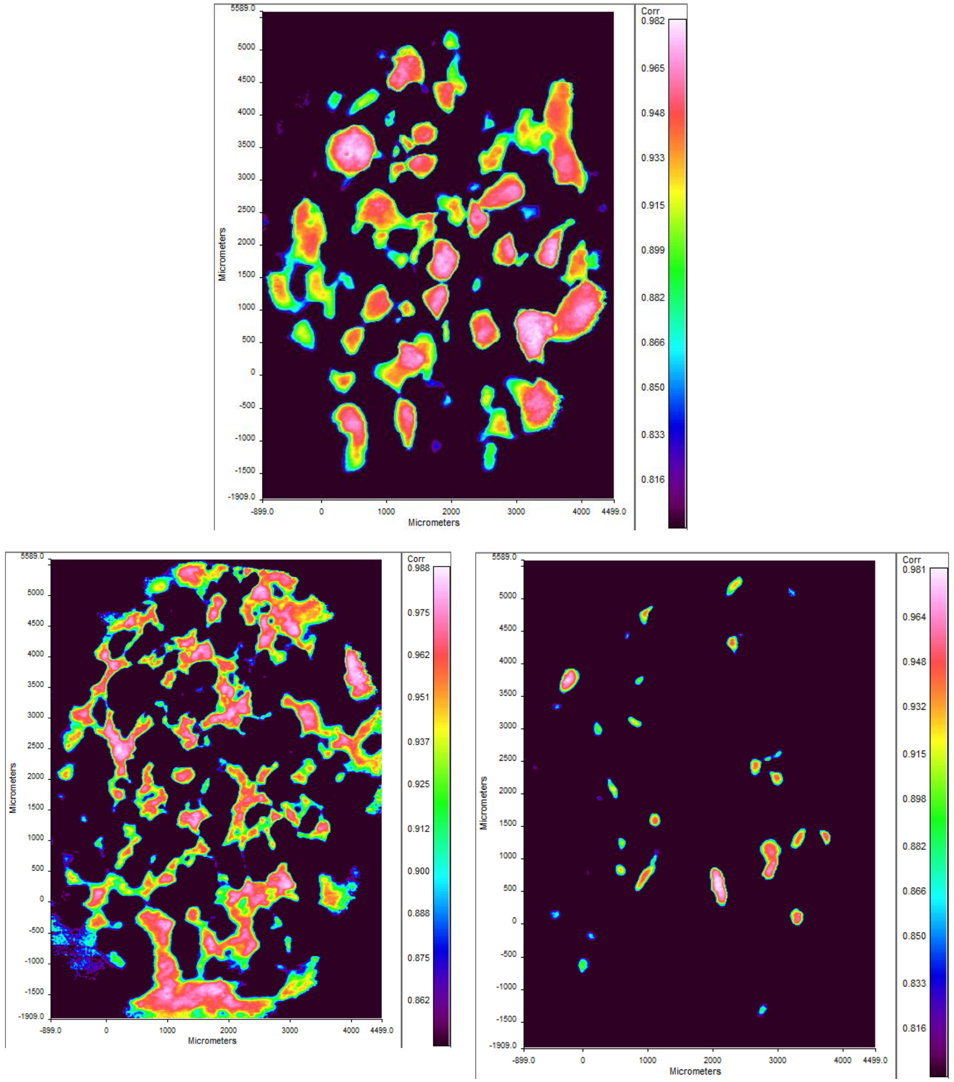
# Make Sure You're Getting the Right Dose!



Average Absorbance Image




Principal Component Analysis



Correlation Images

# FTIR Microscopy, your micro detective

perkinelmer.com
Rhys Kelham (rhys.kelham@perkinelmer.com)

FTIR Application Specialist

# Thank you!

PerkinElmer
*Science with Purpose*

# Spotlight Aurora

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Science with Purpose

# Spotlight Aurora


## IR Microscopy for All

*Find* – Best in class visible imaging system and stage allows for non-flat sample analysis. Easier, faster sample prep. and visible imaging.

*Analyse* – Spectrum IR assisted workflows means that all sample workflows can be automated for all users of any skill level.

*Optimise* – Multiple detector options including room temp MCT, MIR and NIR options plus compatibility with SP2 and SP3. The ultimate in flexibility.

*Solve* – tackle most demanding applications with research-level macro and micro infrared sensitivity and performance

# Next Gen FT-IR Microscopy

**Productivity**

- Find your region of interest faster with smarter, improved visable imaging
- Single click analyses – with customisable workflows
- Easy to use - built-in automatic image optimization

**Innovation**

- Dual camera system with multiple viewing options
- Multiple modes of operation, multiple range options – all software controlled
- Built-in image optimization brings faster insights

**Cost effective solutions**

- Flexible design, multiple detectors – Lower cost of ownership
- Single integrated electronics - Higher uptime
- Upgradeable and compatible with field Spectrum 2 and Spectrum 3