



## ● Raman Imaging: Unlocking Solid Dosage Form Evaluation

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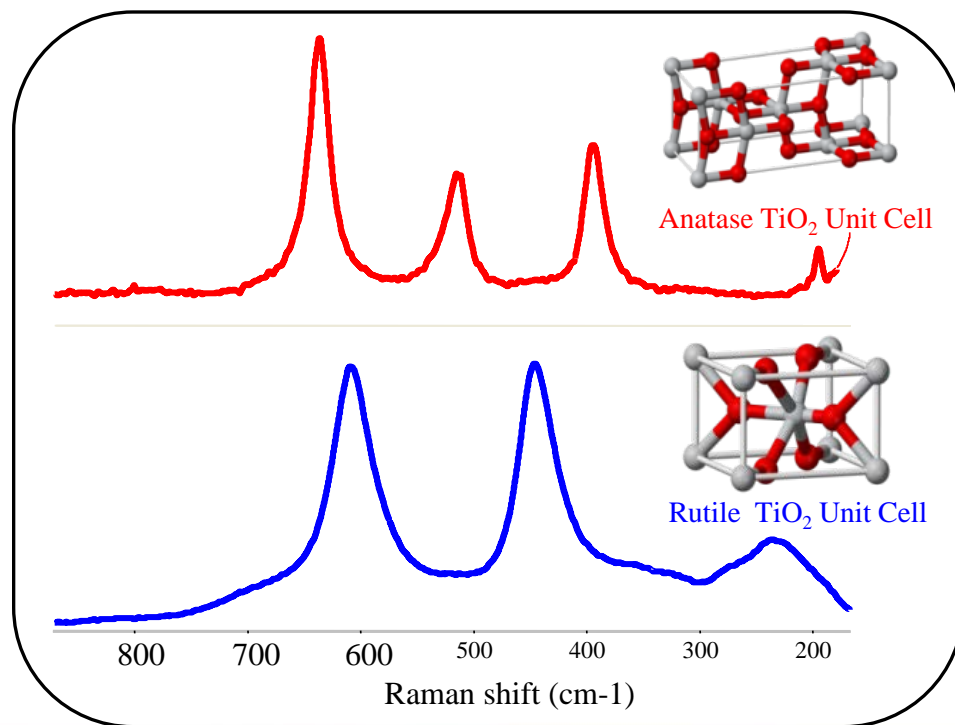
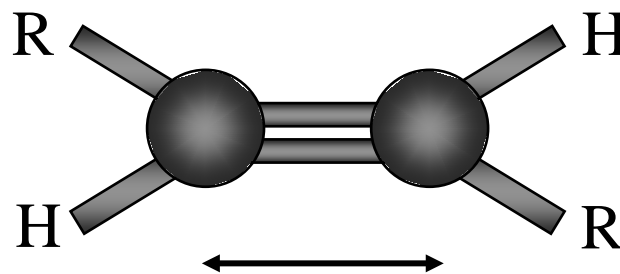
# Agenda

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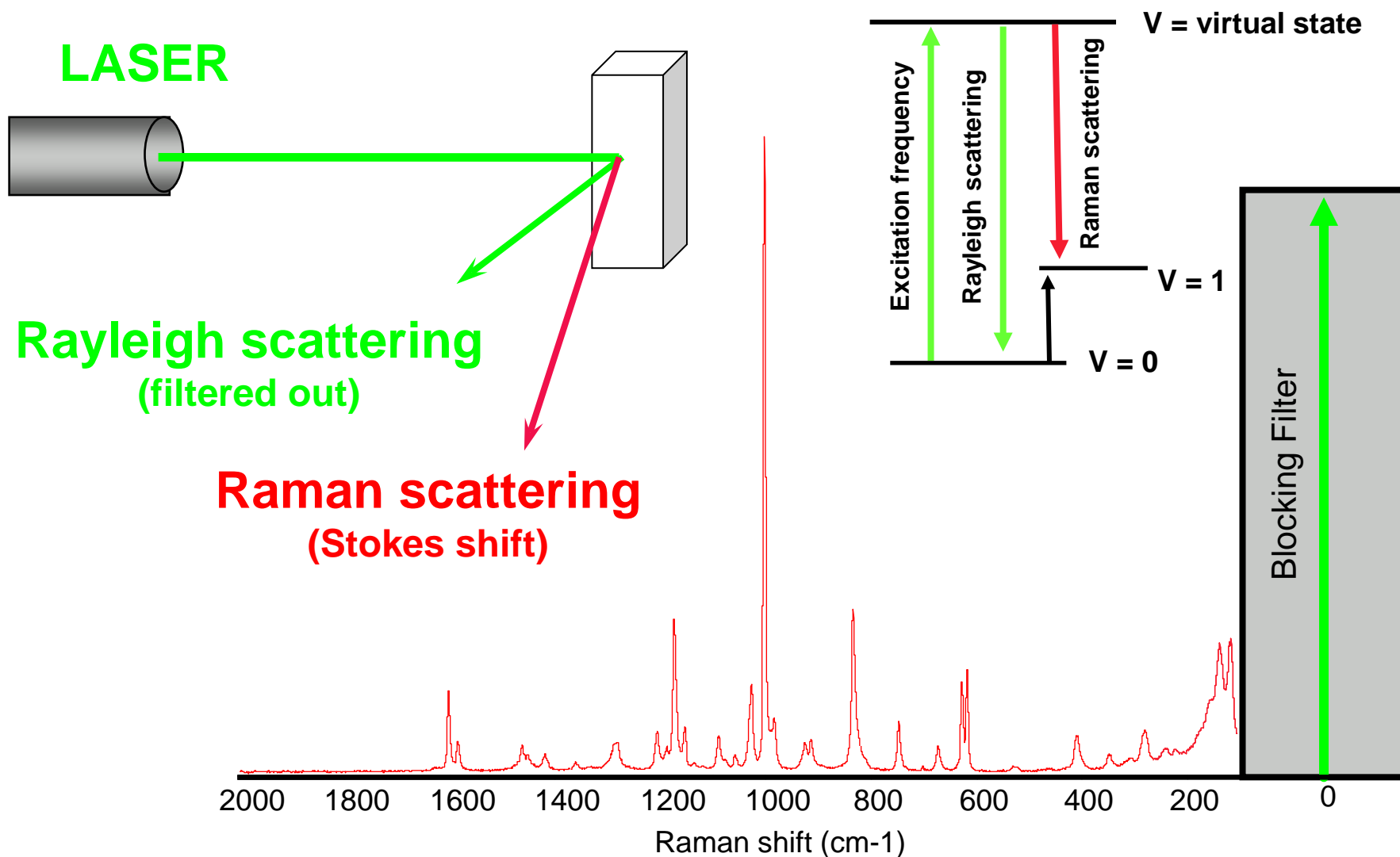
- Brief overview of Raman spectroscopy & Raman imaging
- Introducing the Thermo Scientific™ DXR™xi Raman imaging microscope
- Raman imaging for pharmaceutical products
  - Examples
    - Pharmaceutical Tablet – homogeneity and content uniformity
    - Low Dose Tablet – distribution of polymorphs
    - Hot Melt Extruder Products – component characterization

# What is Raman Spectroscopy?

- Complementary technique to infrared (IR) spectroscopy
- Uses light to probe covalent chemical bonds by looking at vibrations
- Provides detailed molecular information: sensitive to even slight changes in bond angle or strength
- Useful for identifying unknown solids and liquids, including both inorganic and organic materials
- Can also detect sensitive changes in structure, morphology, and even temperature!

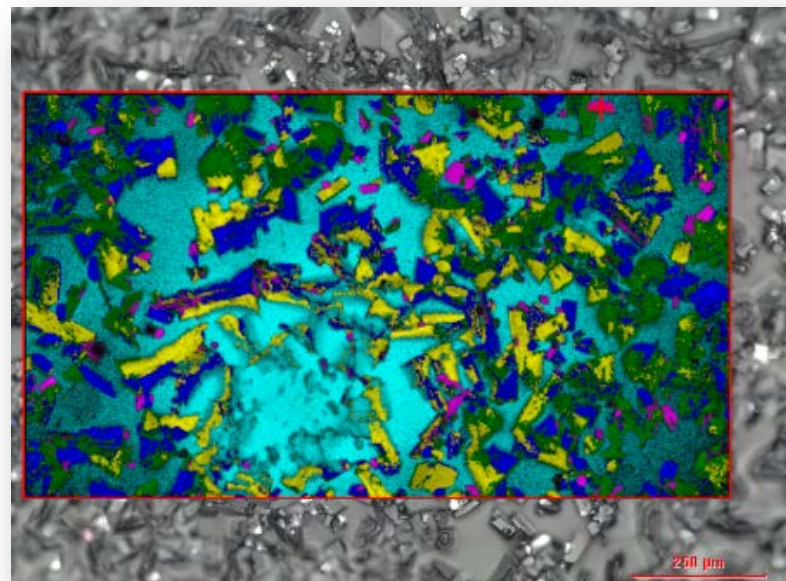


# Raman Spectroscopy – The Raman Effect



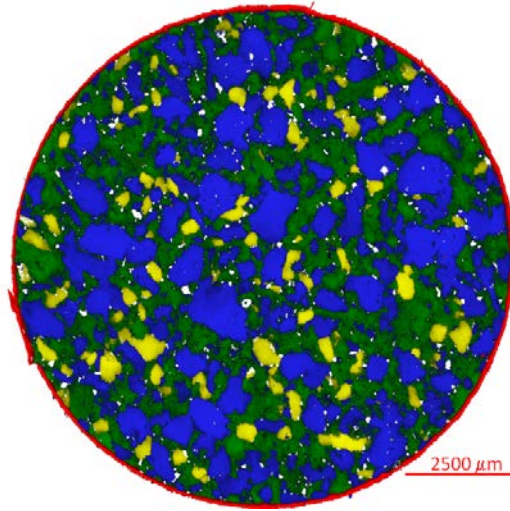
# What Can Raman Imaging Do?

- Extends the advantages of Raman analysis across the sample
- Rapid collection of vast amounts of spectroscopic data
- Provides visual images depicting differences in molecular structure and chemical environment
- Raman images provide views of the samples that are not always apparent in the visual images



# Application Areas for Raman Imaging

## Pharmaceuticals



### Other Application Areas

Polymers and Packaging

Semiconductors and Thin Films

Carbon Nanomaterials

Geology / Mineralogy

Life Sciences

# Introducing the Thermo Scientific DXRxi Raman Imaging Microscope

A total imaging system: hardware and software integration combines **powerful performance** with **image-centric** analysis and **ease of use**

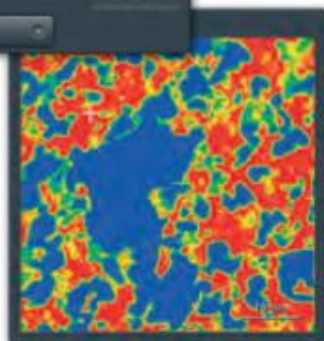
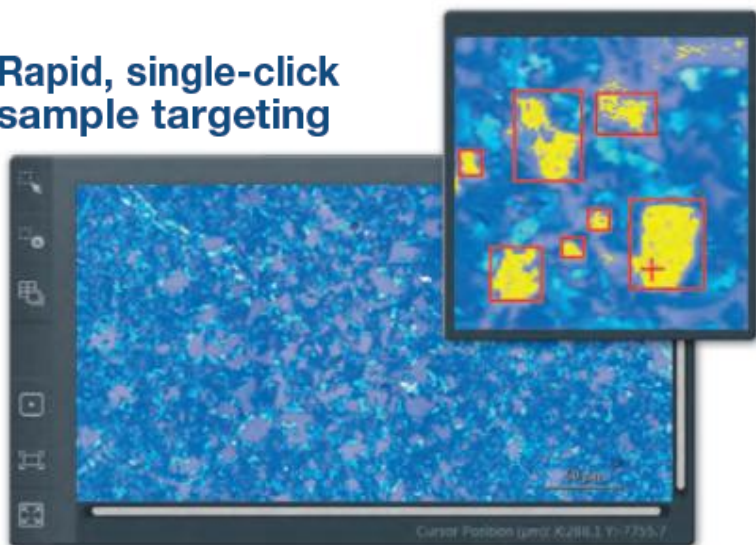


*A completely **new approach** to Raman imaging!*



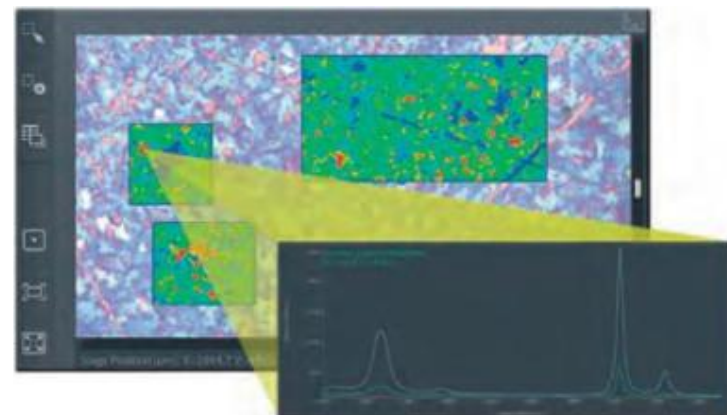
# Intelligent Workflow with Excellent Flexibility

**1** Rapid, single-click sample targeting

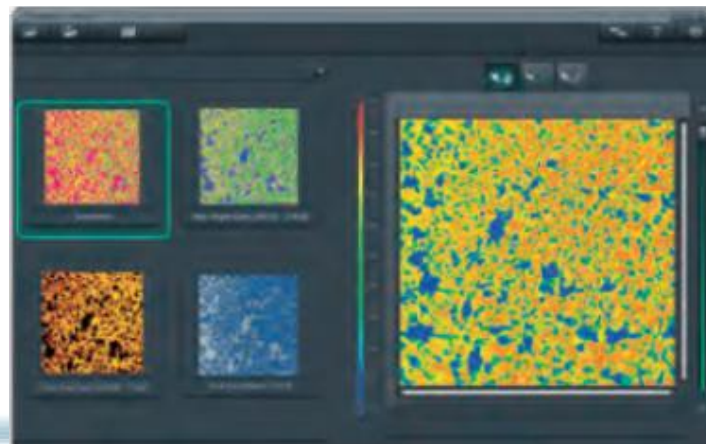


**2** Confidently optimize settings with intuitive controls

**3** Quickly prioritize multiple regions of interest and run



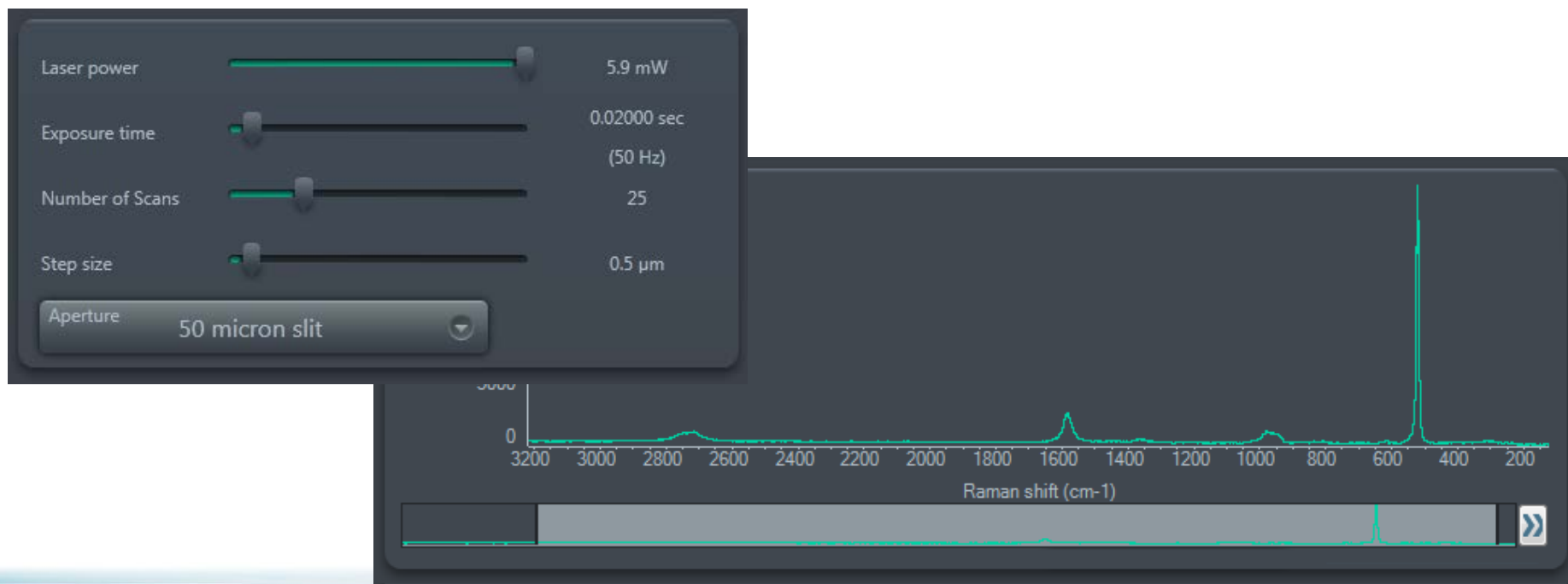
**4** Information-rich images reveal a multitude of material characteristics





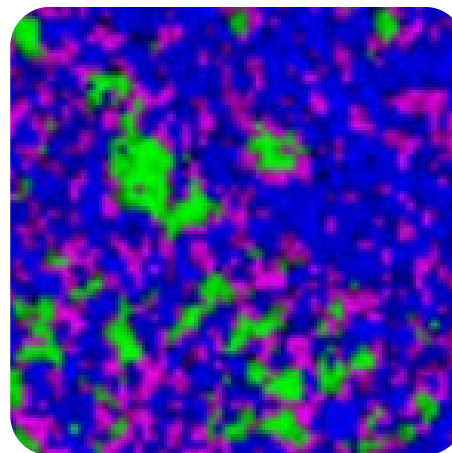
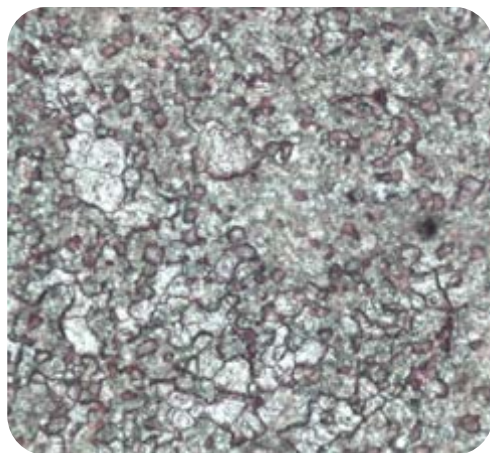
# No Raman Expertise Required to get the **Best** Results

- Visual controls and instantaneous, continuous visual feedback
  - NO lengthy trial and error
  - NO guesswork
  - You can see when parameters are optimal
  - Focus quickly on the problem, not the technique



# Raman Image Preview

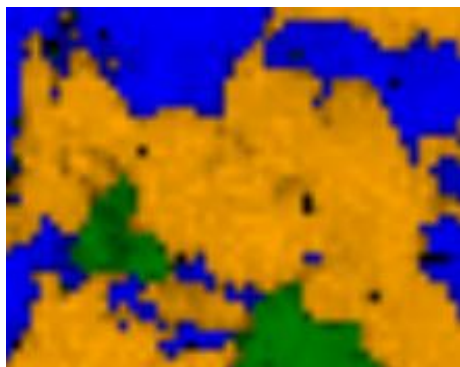
- Just like using an visual image to inspect the sample now it is possible to use a Raman image preview of the sample.
- Don't waste time guessing at your region of interest
- Don't wait until an image is collected to learn if parameters were ideal
- Rapidly see and identify constituents and domains without the wait



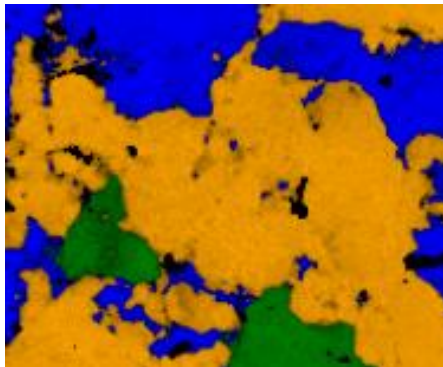
Preview in seconds!

# Get There Faster By Getting Just What You Need

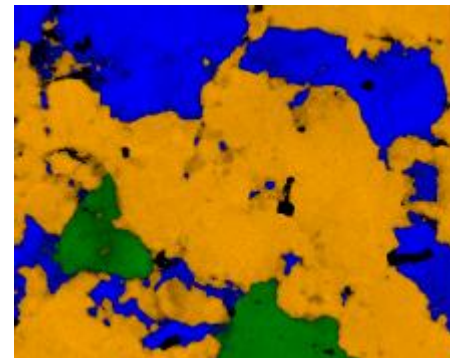
- Optimize **image** collection, not individual spectra
  - Quickly and visually balance image collection time with necessary detail level
  - Remove unanticipated results somewhere in an image
  - Stop any time if results are good enough rather than wait for multiple scans of each point in entire image to finish, one at a time



20 micron image pixels  
1900 spectra, 2 scans  
1 minute



5 micron image pixels  
30,000 spectra , 2 scans  
4 minutes



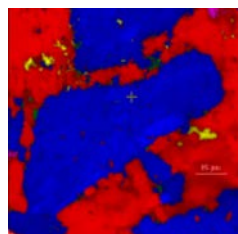
1 micron image pixels  
191,000 spectra , 2 scans  
25 minutes

# Image-centric vs. spectral-centric at same “spectral speed”

100 x 100 microns 1 micron spacing (10,000 spectra); 500 Hz data collection; 10 “co-adds”

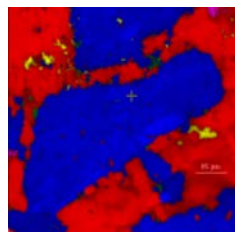
**DXRxi**, rastering entire images to desired quality level, like other microscopes

20 seconds



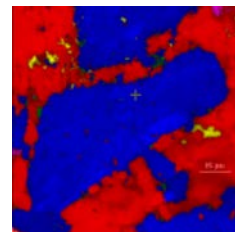
Single scan of entire image with MCR, 10,000 spectra

1 minute



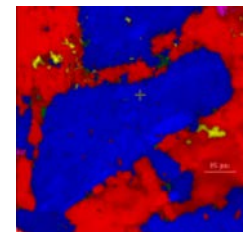
3 scans of entire image with MCR

200 seconds



10 scans of entire image with MCR

Some Time Later....



**Other Raman Imaging Systems**, building images one spectrum at a time



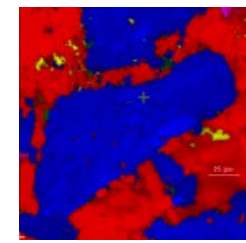
1000 co-added spectra, no useful image information



3000 co-added spectra, no useful image information



10,000 spectra collected

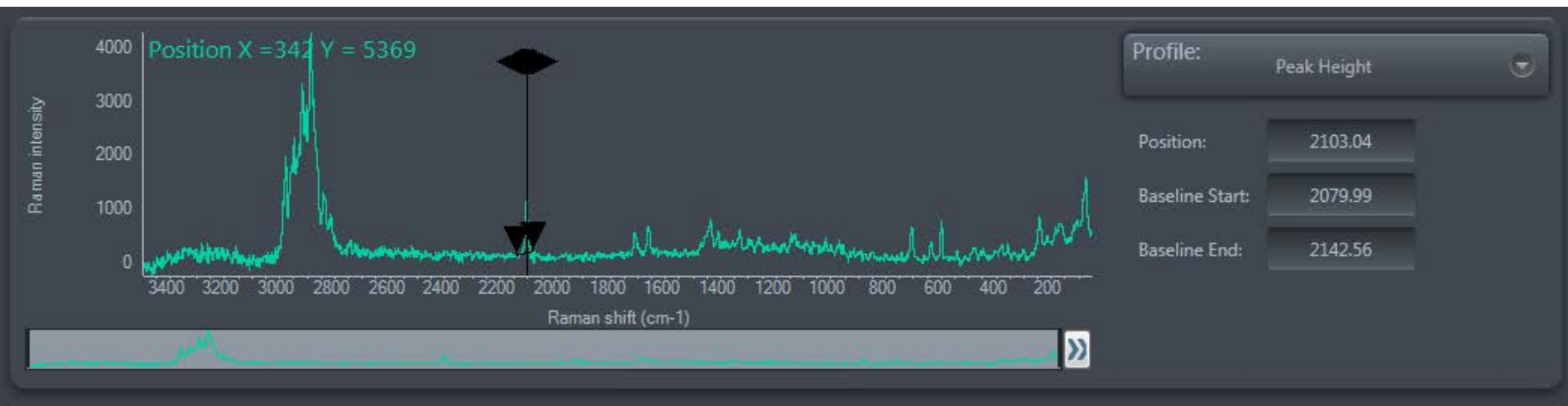
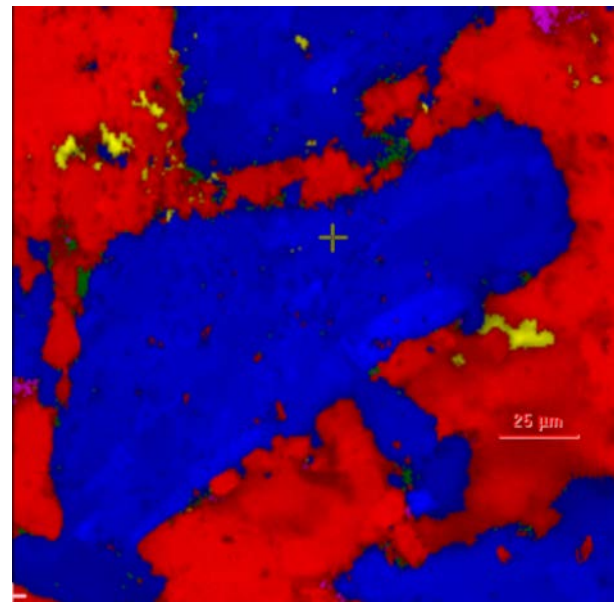


MCR Processed Map

# Built-In Expertise: Profiles and MCR (Multivariate Curve Resolution)

- Standard profiles (correlation, chemigram, peak area, peak height, peak ratios, peak shift) applied immediately via graphical interface
- Component analysis calculated *in real-time*

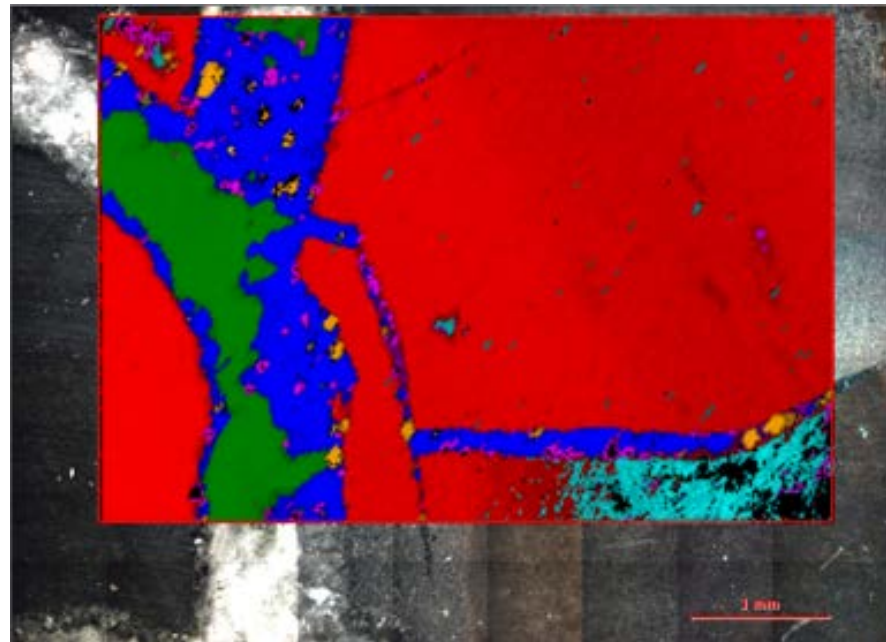
***One-click application of image profiles is a unique concept – adds value at every step of the workflow***





# Data Processing Concurrent With Data Collection

- Raman images with component identification are created in real time
  - Without configuring a spectroscopic method
  - Without prior operator knowledge of what's in the sample
  - Without waiting for an entire image data set to be collected
- Instant and obvious interpretation even if you don't know what you're looking for





# Integration and Cross-Compatibility

- Access to raw data
  - All data (chemical image, video image, spectra) can be quickly exported using a full array of formats
  - HDF5 provides open-source solution for compatibility with third party packages
  - Send data to OMNIC and Spectra with a single click!



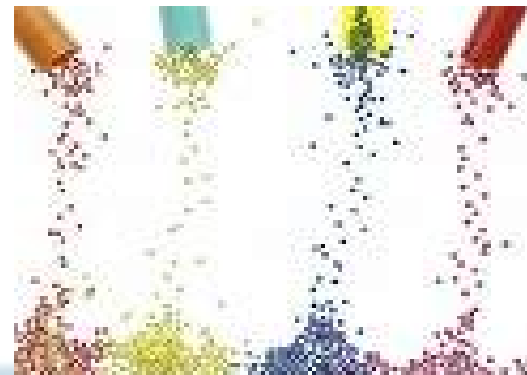
# Microscopy Options

- Supports a wide variety of sample measurement options, including:
  - Single and dual microscope slide holders
  - Heating and cooling stages – even during imaging!
  - Rotating stage insert
  - Industry standard wafer holders and SEM accessories
  - ‘Breadboard’-style holder for custom configurations
  - Holder for the Thermo Scientific K-Alpha XPS!
- Integrated Olympus research grade optics for peak performance and stability:
  - High NA and long working distance objectives
  - Optional brightfield and darkfield optics
  - DIC and visual polarizers for more challenging samples
  - Available with transmission illumination

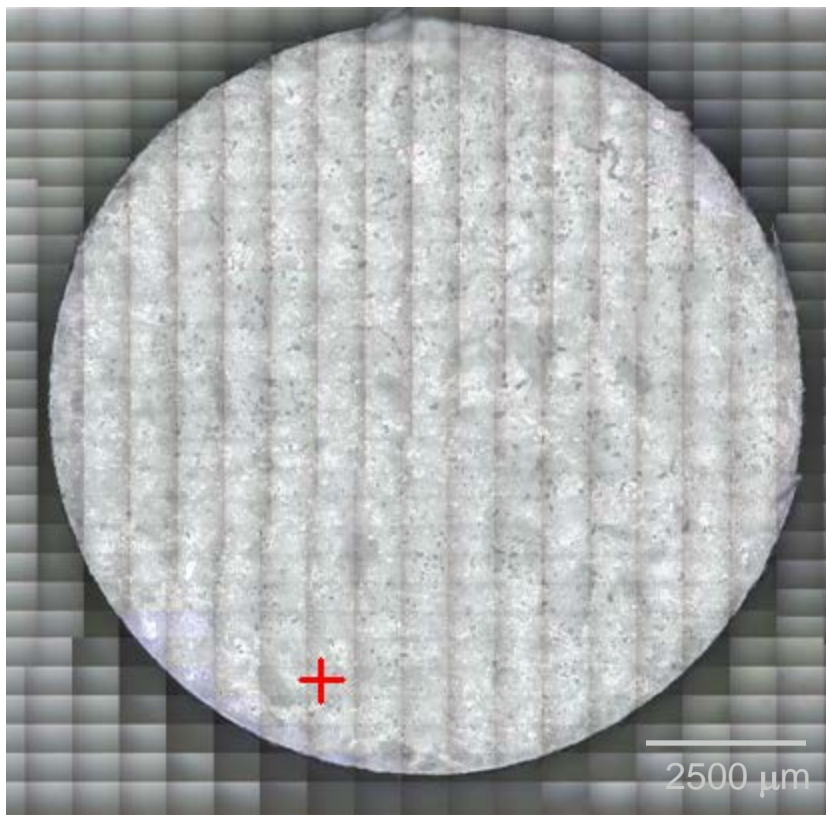


# Pharmaceutical Formulations

- Typically complex multi-component mixtures
- Need to identify and verify components
  - Known components
  - Impurities
  - Identify changes in components during processing
- Distribution of components
  - Homogeneity
  - Particle size
  - Content uniformity



# Tablet Imaging Example



Video Mosaic Image  
(10X objective, 100X total magnification)

## Migraine Relief Tablet

11 mm diameter, 676 mg

### APIs

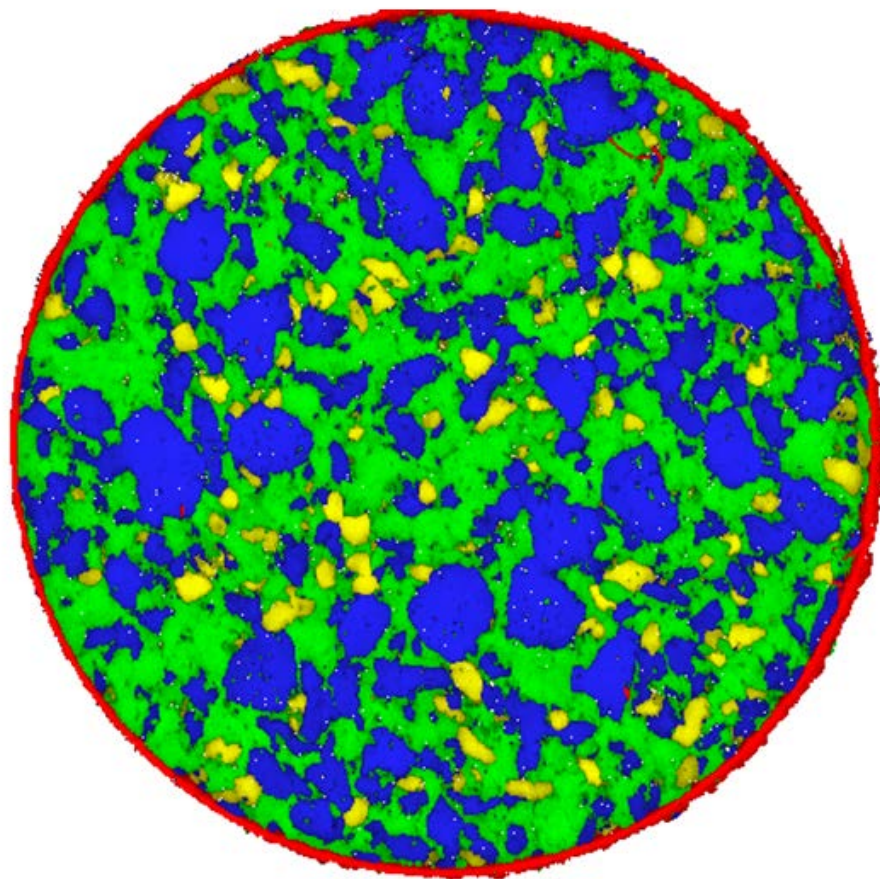
Acetaminophen	250 mg (37%)
Aspirin	250 mg (37%)
Caffeine	65 mg (9.6 %)

### Inactive

corn starch, microcrystalline cellulose,  
sodium lauryl sulfate, sodium starch,  
glycolate, crospovidone, polyethylene glycol,  
polyvinyl alcohol, povidone, stearic acid, talc,  
titanium dioxide

# Imaging the Whole Tablet

Raman MCR Image



Area Imaged - 11 x 11 mm<sup>2</sup>

10X objective

Image Pixel Size - 25 μm

226,000 spectra

Exposure Time 1.8 ms (550 spectra per s)

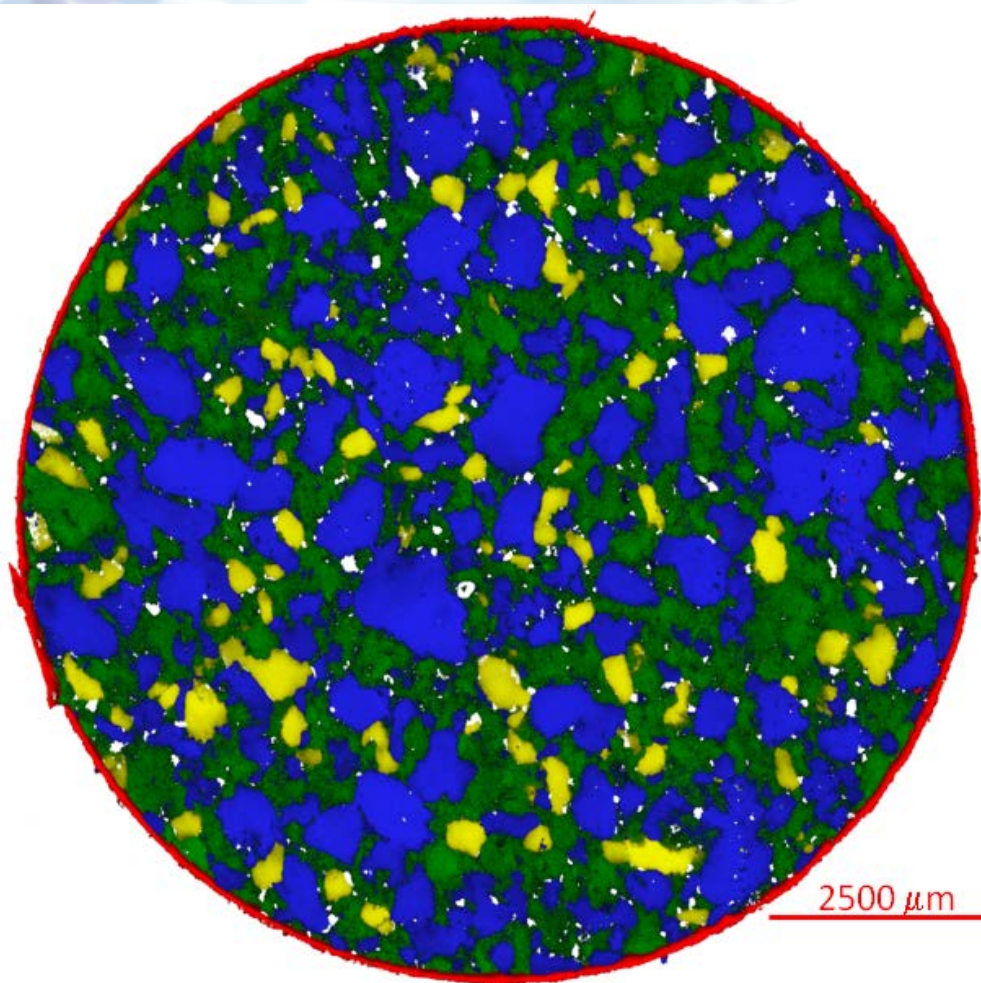
532 nm laser,

**8 minute collect time!!**

■ Aspirin ■ Acetaminophen ■ Caffeine ■ Titanium Dioxide



# Higher Resolution Image – Whole Tablet



Area Imaged - 11 x 11 mm<sup>2</sup>

10X objective

Image Pixel Size - 5 μm

5.4 million spectra

Exposure Time 1.8 ms (550 spectra per s)

532 nm laser

36 GB file

Size only computer limited (128 GB RAM)

6 hour collection time (3 hr estimated)

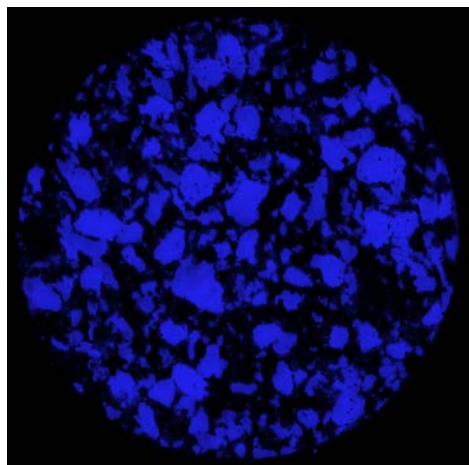
## Image Analysis % Area of Particles

Component	Calculated % (Surface Area)	Reported %
Aspirin	38.6	37
Acetaminophen	35.4	37
Caffeine	7.7	9.6

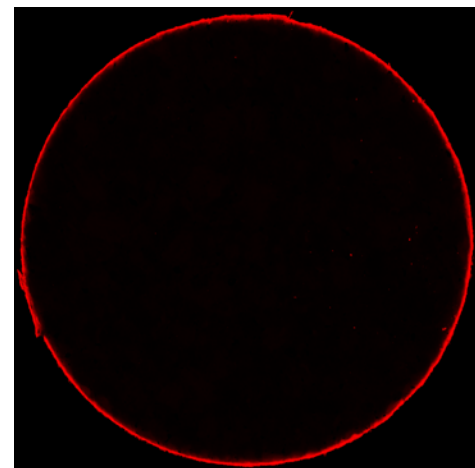
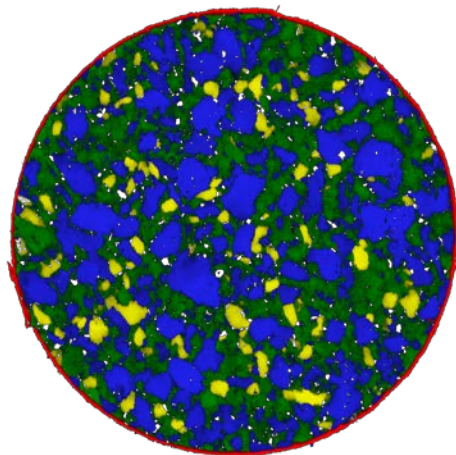
■ Aspirin ■ Acetaminophen ■ Caffeine ■ Titanium Dioxide



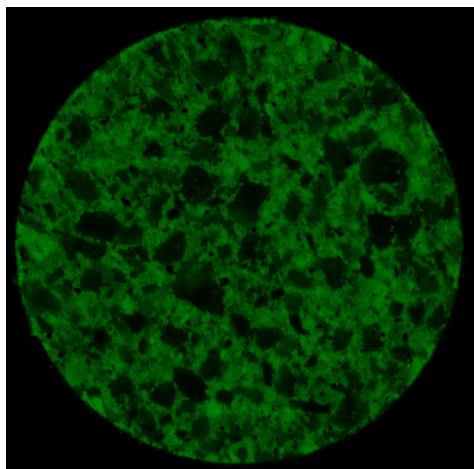
# Tablets Components – From Multivariate Curve Resolution (MCR)



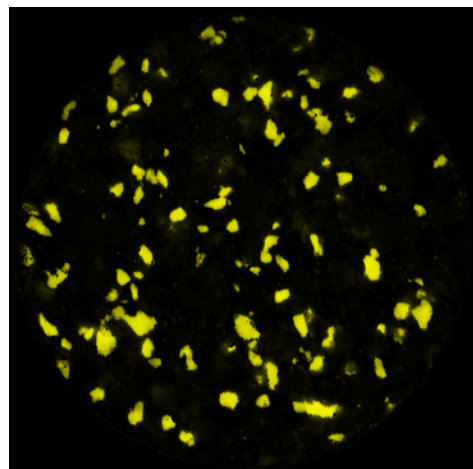
Aspirin



Titanium Dioxide

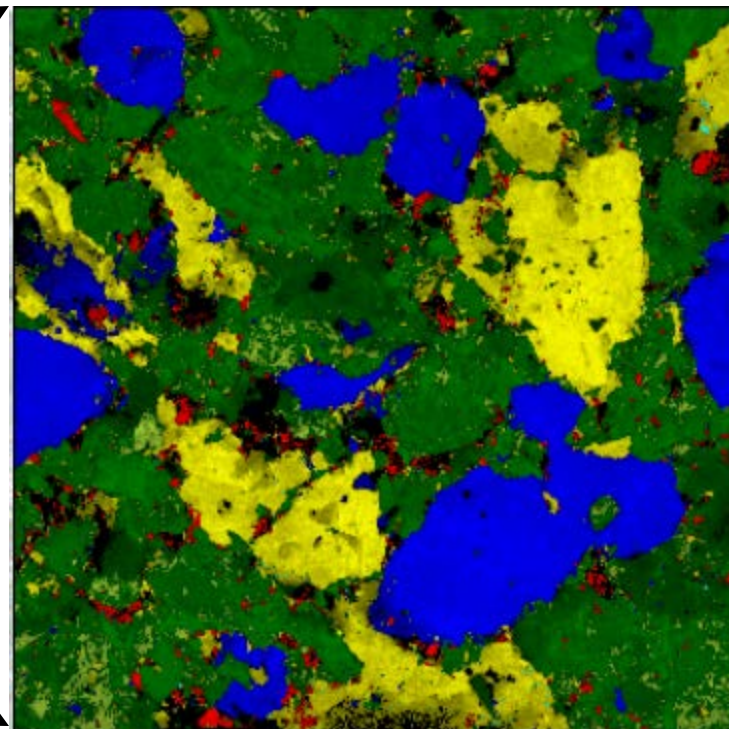
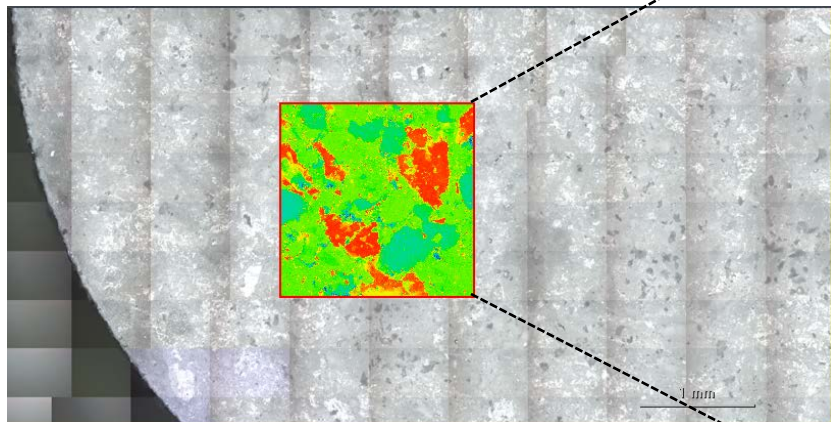


Acetaminophen



Caffeine

# Identify Area of Interest – Longer Exposure Time



1.6 x 1.7 mm<sup>2</sup>

50X Objective

5 micron image pixel size

116000 spectra

Exposure time 5 ms (200 spectra per s)

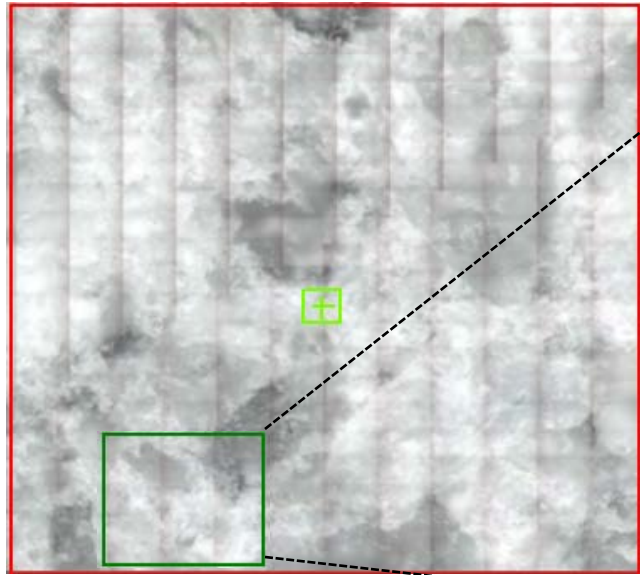
532 nm laser

5 averaged scans, 55 minutes

500 μm

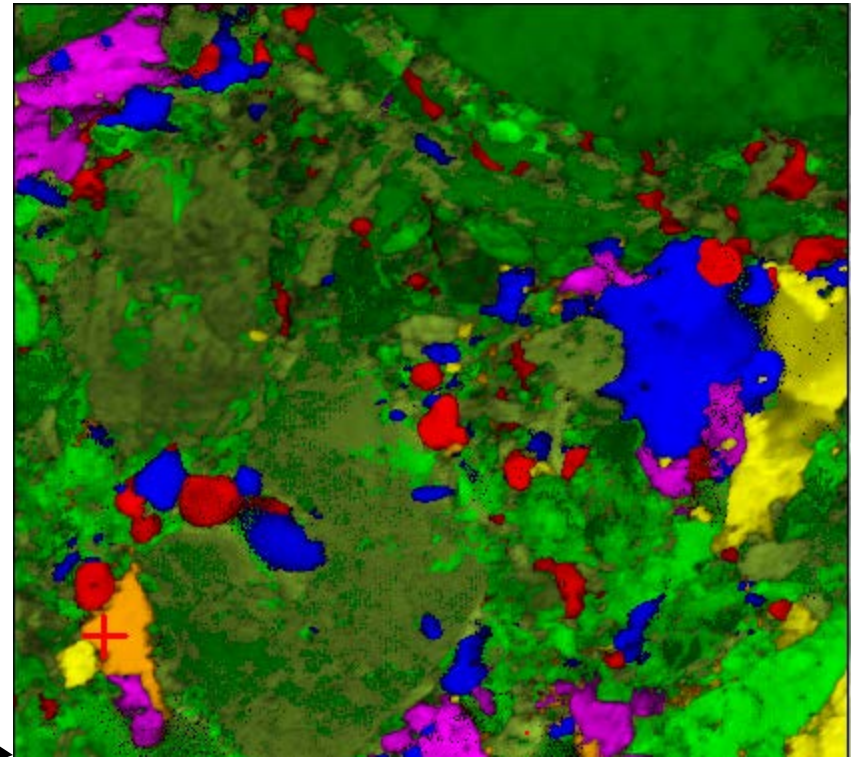
**Blue** – Caffeine,  
**Green** - Acetaminophen,  
**Yellow** – Aspirin, and **Red** – starch

# Define Area Further, Higher Resolution, Longer Exposure Time



500  $\mu\text{m}$

225 x 250  $\mu\text{m}^2$   
100X Objective  
0.5 micron image pixel size  
229000 spectra  
Exposure time 10 ms (100 spectra per s)  
532 nm laser  
5 averaged scans, 3 hr collect



100  $\mu\text{m}$

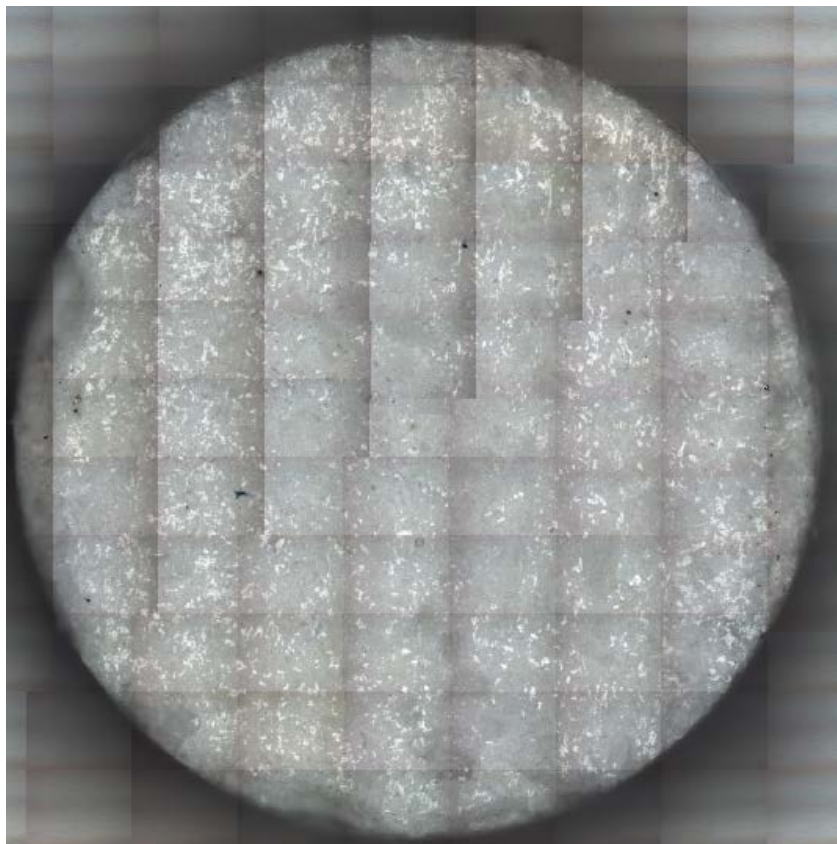
**Blue** – Aspirin, **Green** – Acetaminophen,  
**Yellow** – Caffiene, **Red** – starch,  
**Fuchsia** – microcrystalline cellulose,  
**Orange** – sodium lauryl sulfate.



# Summary of Tablet Imaging

- Possible to Image an entire 11 mm diameter tablet in 8 minutes
- Higher resolution images on whole tablets are possible but may not be necessary and there are other alternatives to imaging the whole tablet (select regions, multiple regions)
- Raman imaging can give spatial distribution of components including particle size estimates and relative percentages based on areas occupied by different components
- APIs tend to be strong Raman scatterers. Weaker excipients may require longer exposure times and possibly better spatial resolution to differentiate them.

# Low Dose Tablet Example



Video Mosaic Image  
(10X objective, 100X total magnification)

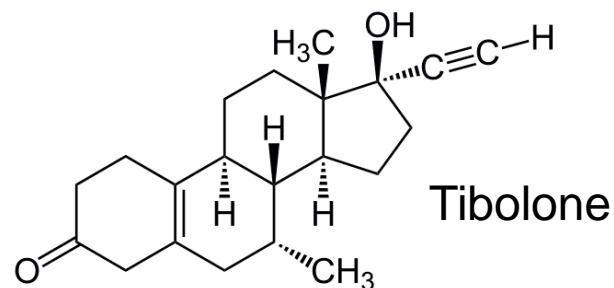
## Tibolone Tablet

3% Tibolone

A synthetic steroid used in hormone replacement therapy

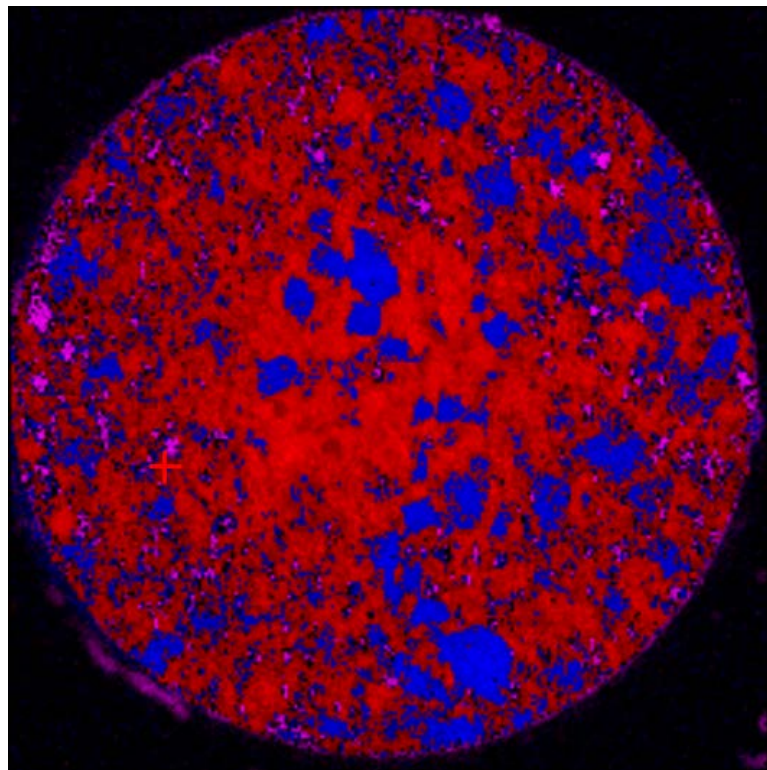
6 mm diameter

Polymorphs (monoclinic, triclinic)



# Raman MCR Image of Low Dose Tablet

## Raman MCR Image



5.7 x 5.7 mm<sup>2</sup> area

10X objective

25 μm image pixel size

52000 spectra

Exposure Time 20 ms (50 spectra per s)

532 nm laser

10 averaged scans

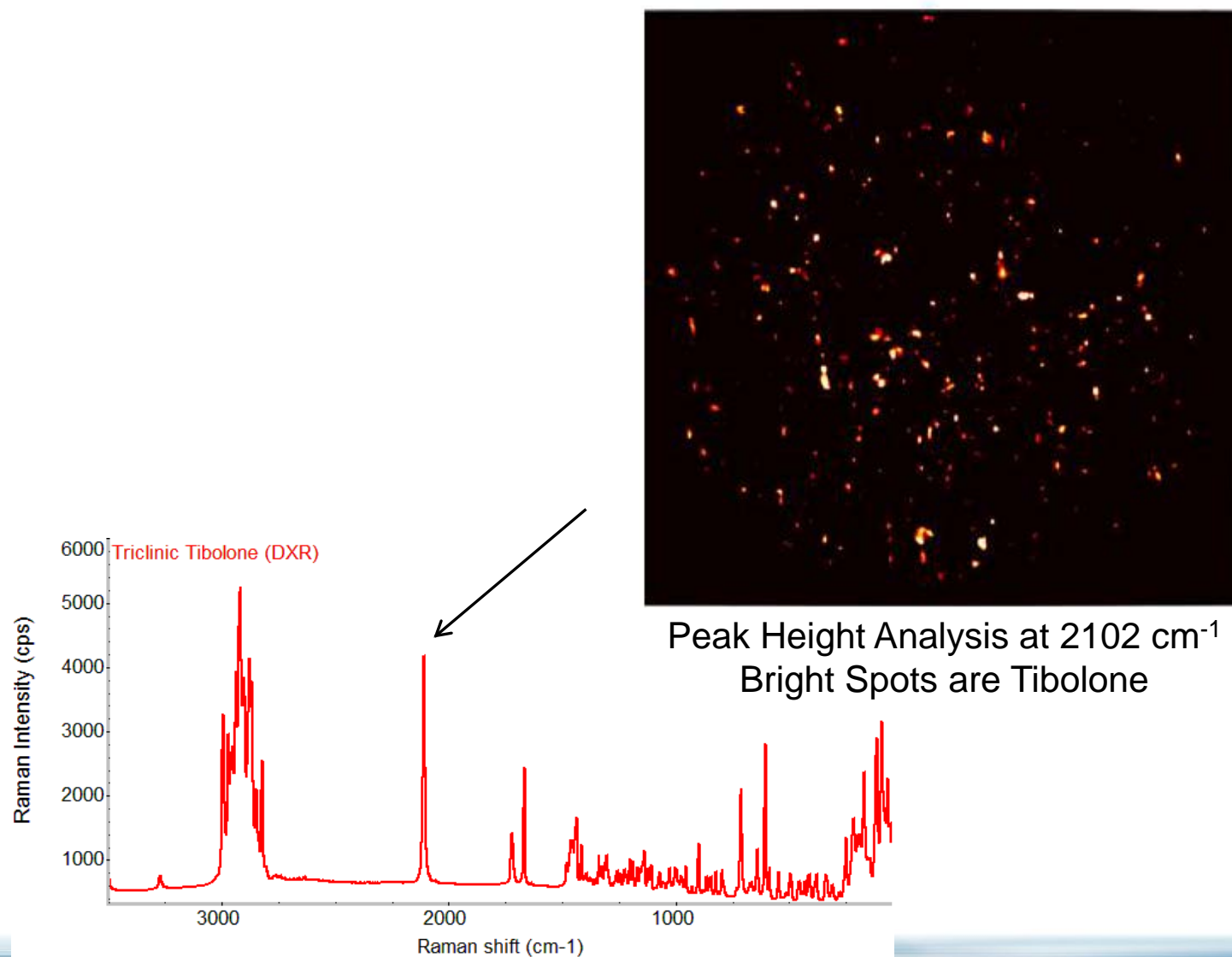
3 hr collect

Tibolone was not readily differentiated  
using MCR

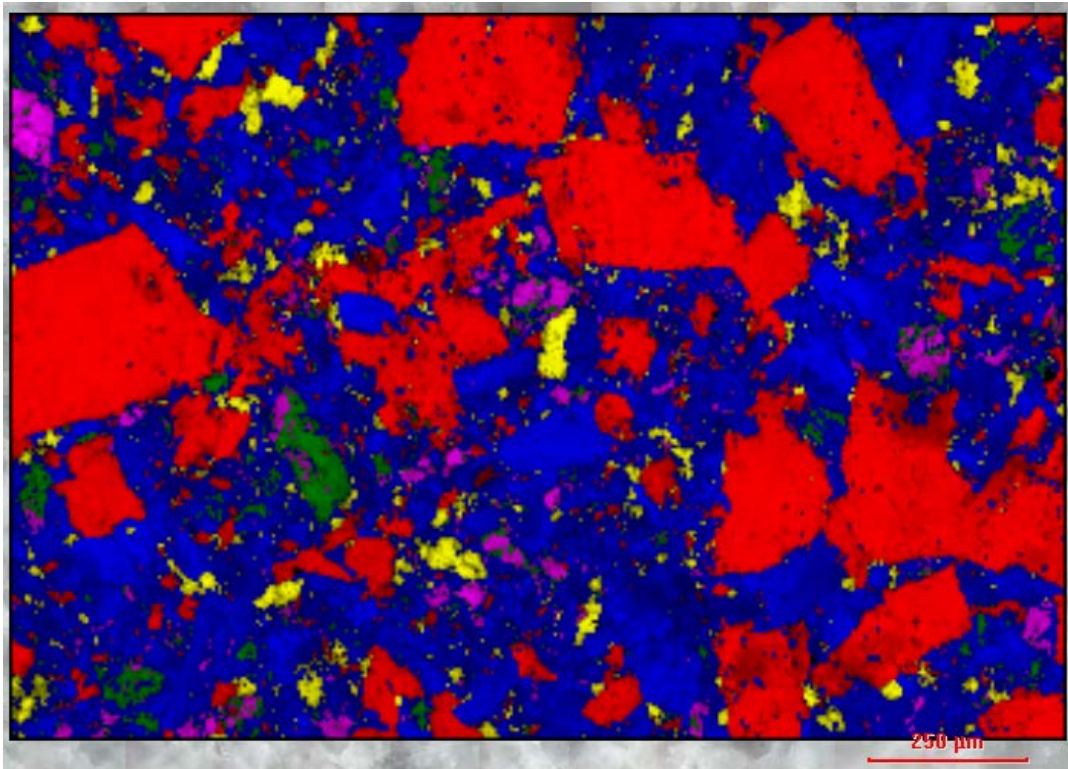
■ Starch ■ Lactose ■ Fluorescent Compound



# A Peak Height Profile Readily Shows the Location of the Tibolone



# Defined Area of Interest, Higher Resolution, More Scans



■ Tibolone ■ Starch ■ & ■ Lactose ■ Fluorescent Compound

1.1 x 1.6 mm<sup>2</sup> area

10X objective

5 μm image pixel size

75000 spectra

Exposure Time 20 ms (50 spectra / s)

532 nm laser

25 averaged scans

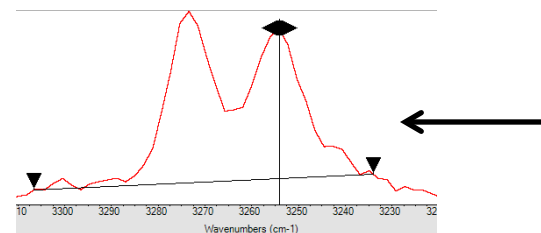
10 hr collect

Tibolone is one of the components defined by the MCR analysis

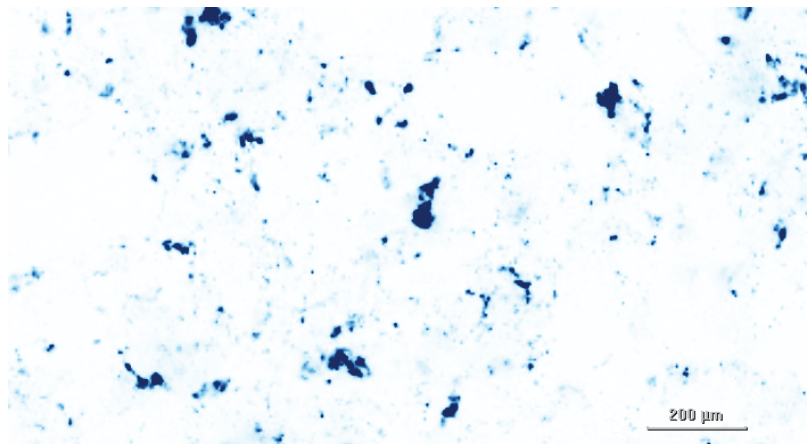
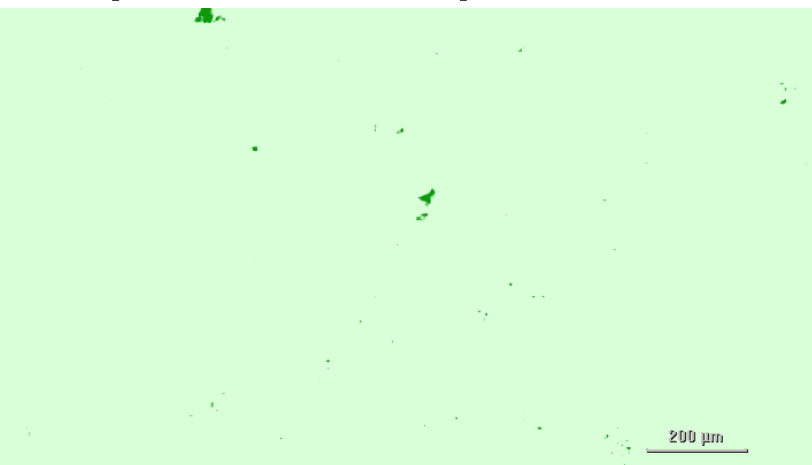
# Distribution of Polymorphs of Tibolone

All Tibolone (peak at  $2102\text{ cm}^{-1}$ )

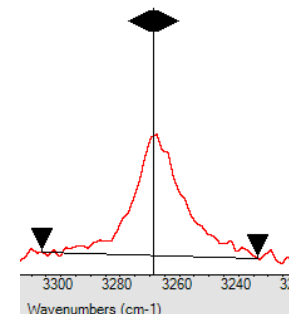
$3273, 3254\text{ cm}^{-1}$



**B (monoclinic)**



$3267\text{ cm}^{-1}$



**C (triclinic)**



# Summary of Low Dose Tablet Imaging

- Lower dose tablets are commonly encounter pharmaceutical products
- Other profile options (peak height etc.) might be better choices than MCR for displaying the spatial distribution of low concentration components.
- Multiple options are available in the OMNICxi software for generating different types of Raman images based on different aspects of the spectra.
- Whole tablet imaging is possible
- Evaluation of the presence and distribution of polymorphs is possible.

# Raman Imaging of Hot Melt Extruder (HME) Samples

Hot melt extrusion is a novel way of formulating solid dosage pharmaceutical products (tablets, granules, pellets, and transdermal films)

Has been used extensively for a long time in the plastics industry

API and other components are combined with a pharmaceutically approved thermoplastic polymer (usually at higher temperatures).

Screw threads control the mixing and transport properties at various stages.

Final form depends on the die and post extruder processing.



# HME General Processing Advantages

- Continuous process – inline monitoring and control
- Establish stable solid solutions
  - Increase the availability of poorly soluble ingredients
- Flexibility to easily produce different dosage products
- Availability of time release forms
- Taste masking
- Special dosage form designs (films, rods, etc...)
  - Die change provides different shapes for special applications
- Reduce the consumption of solvents
  - Compared to wet granulation process

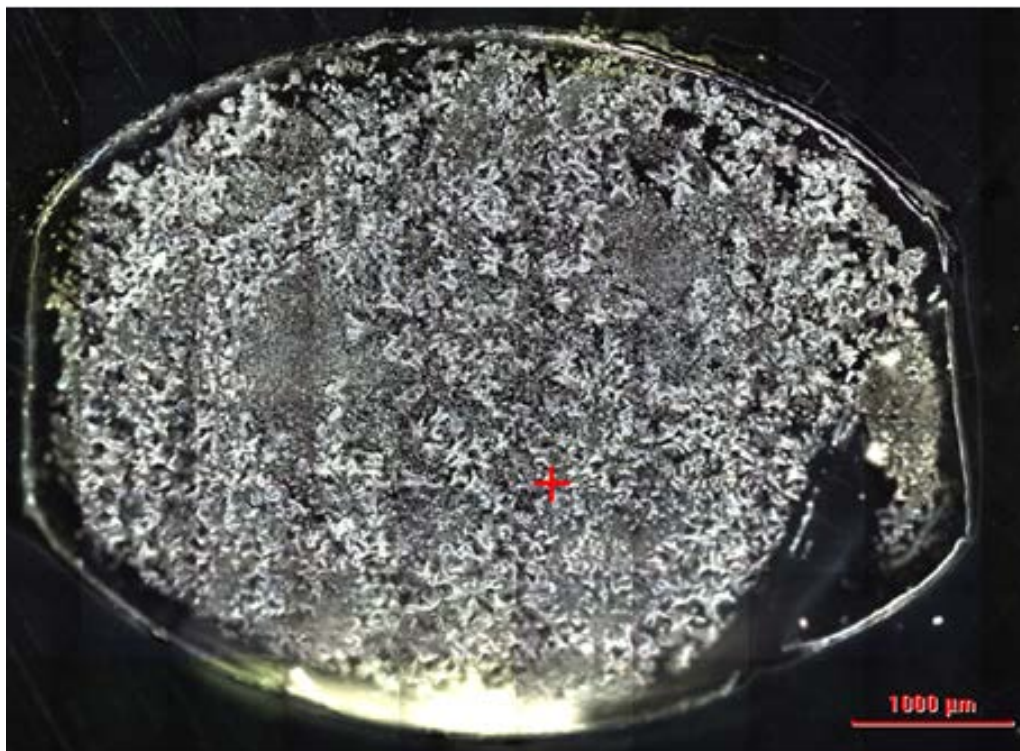


# Raman Imaging of HME Products

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- Hot melt extrusion produces new forms using new processes
- Processing can effect the components
- Monitoring components
  - Changes in molecular structure
- Spatial distribution and particle size of components
- Identification of unknowns (impurities and defects)

# Example of Raman Imaging of HME Products\*



Video Mosaic Image  
(10X objective, 100X total magnification)  
Darkfield Illumination

HPMCAS (hydroxypropyl methyl cellulose acetate succinate or hypromellose acetate succinate) polymer carrier

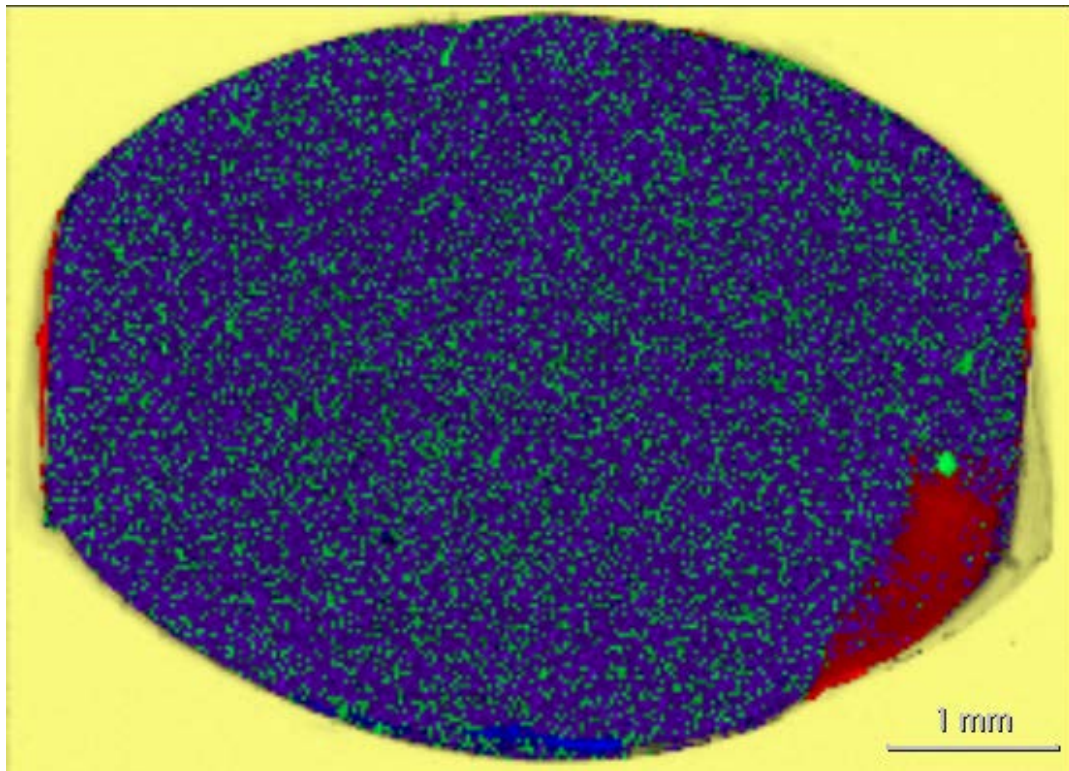
Ibuprofen (25-33%) and ibuprofen (25-33%) + D-mannitol (7-15%).

Cross-sections mounted in epoxy for analysis

To evaluate the spatial distribution of components and to look for any unforeseen changes caused by processing conditions

**\* HME Samples Provided by Dr. Adrian Kelly, School of Engineering, Design and Technology, Bradford University, UK**

# Raman Image of HME Product



6.35 x 4.6 mm<sup>2</sup> area

10X objective

25 μm image pixel size

46736 spectra

Exposure Time 10 ms (100 spectra /s)

780 nm laser

100 averaged scans

## Image Analysis % Area of Particles

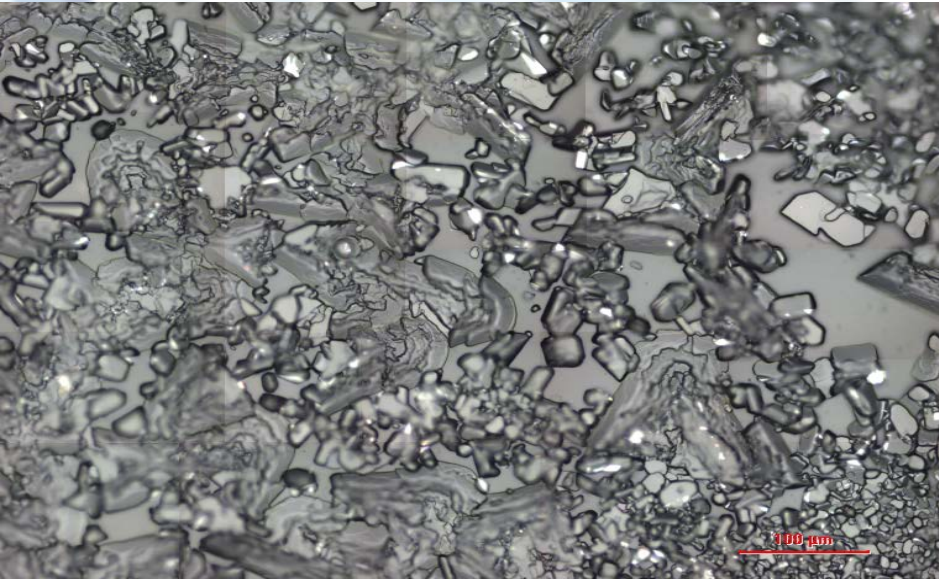
Component	Calculated % (Surface Area)	Reported %
ibuprofen	23	25

## Raman MCR Image

**purple** – HPMCAS, **green** – ibuprofen, **yellow** – epoxy,  
**red** – cyanoacrylate, **blue** – inorganic impurity

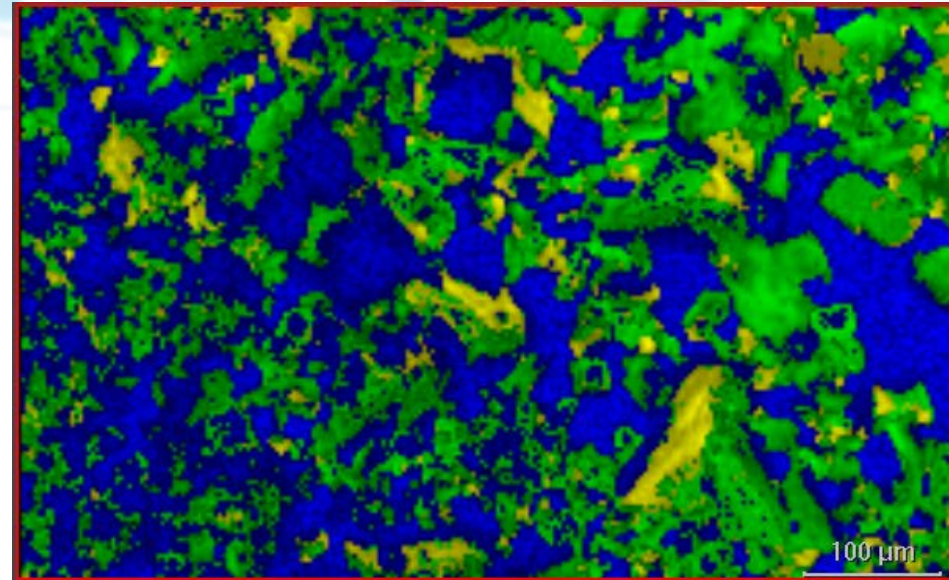


# Smaller Area, Higher Resolution, Raman Imaging



Video Image

(50X objective - brightfield illumination)

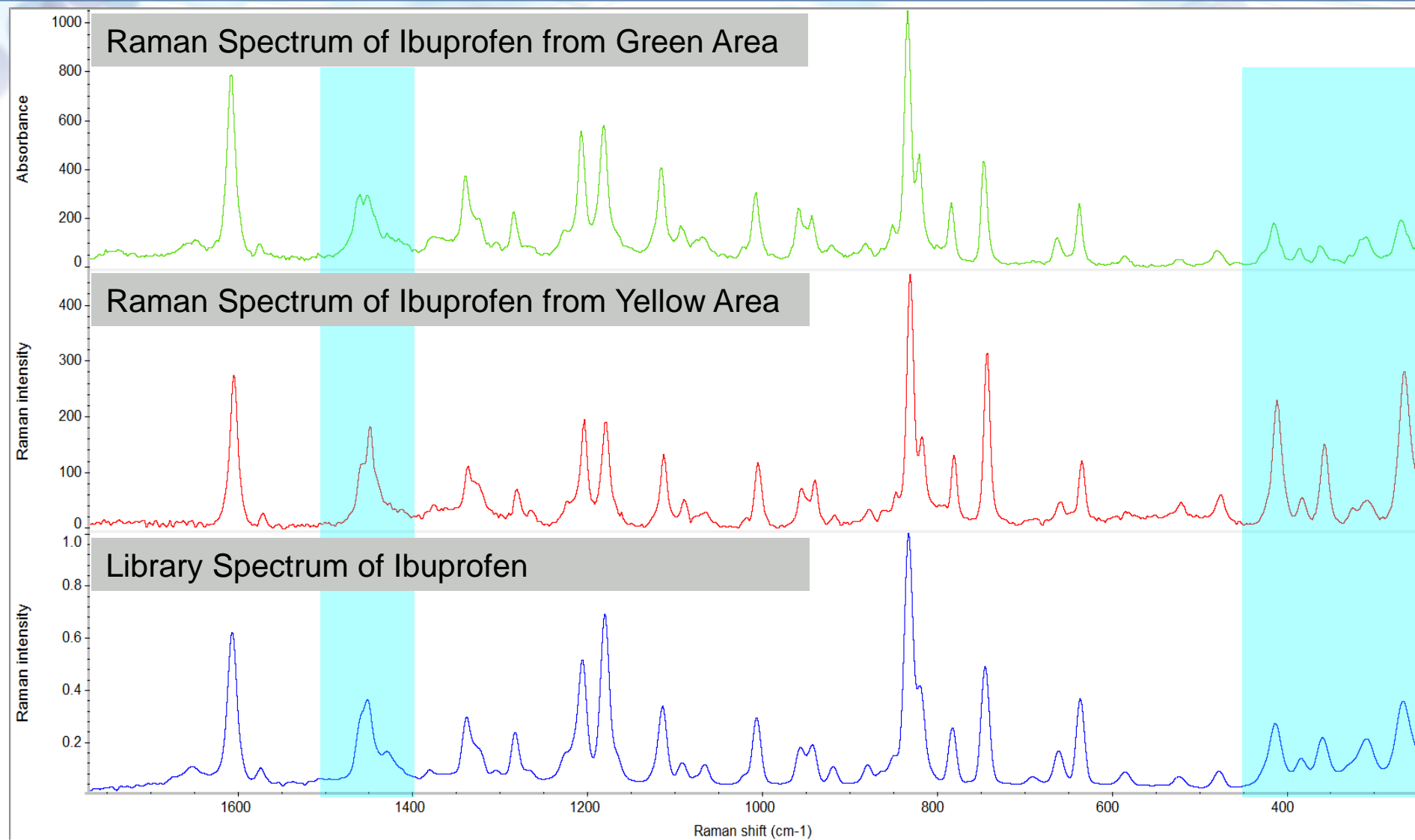


Raman MCR Image

blue – HPMCAS, green – ibuprofen, yellow – ibuprofen

50X objective, 780 nm laser, 24 mW, 687 x 423  $\mu\text{m}$  area, 3.0  $\mu\text{m}$  image pixel size, 32289 spectra, 0.0100 s exposure time, 100 scans

# Subtle Differences in Ibuprofen Spectra

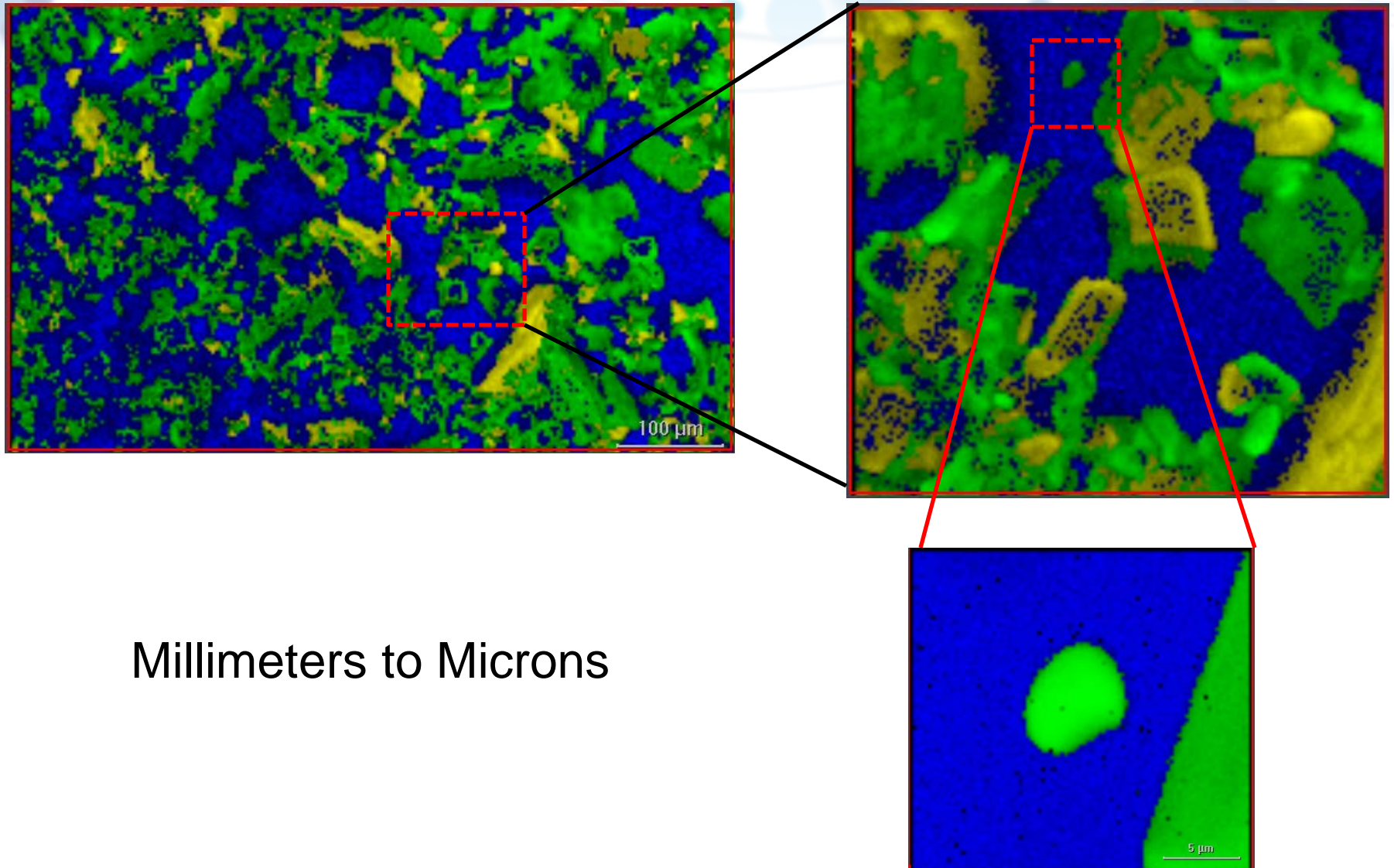




# Differences in the Raman Spectra of Ibuprofen

- **Ibuprofen is a mixture of stereoisomers**
  - Not distinguishable with this type of experiment (ROA required)
- **The active form is S (+) ibuprofen**
- **Different polymorphs of ibuprofen have been reported**
  - Phase I (thermodynamically stable) & Phase II (metastable)
- **Degree of crystallinity effects the Raman spectra**
  - Crystallinity versus amorphous
- **Co-crystallization of ibuprofen with other components can alter the Raman spectrum**
- **Ibuprofen association with other carriers (polyvinyl pyrrolidone (PVP) can cause slight differences in Raman spectra**
- **The observed differences in the ibuprofen spectra in these products does not match with any of these effects**
  - However it does illustrate how sensitive Raman imaging can be

# Raman Imaging – From Whole Samples to Small Particles

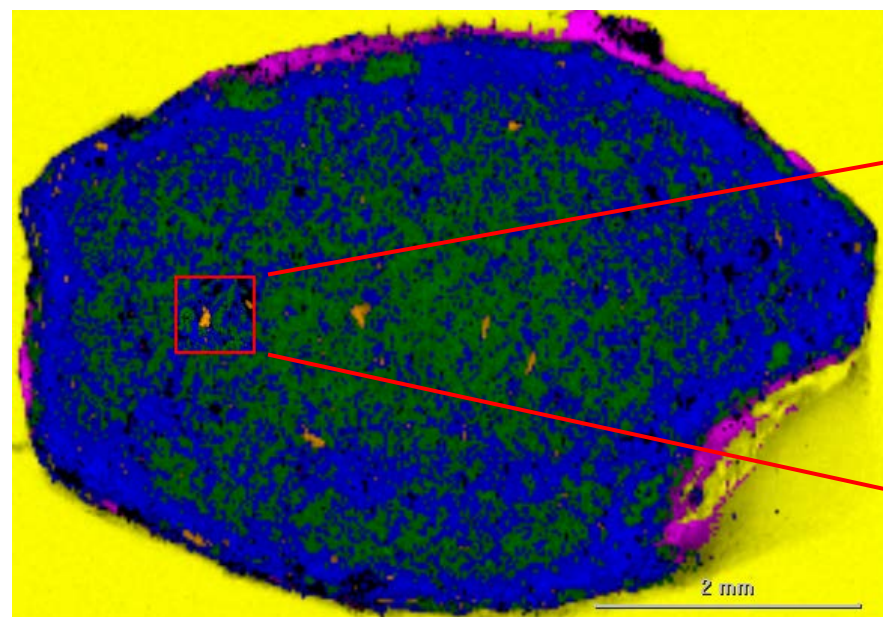


Millimeters to Microns

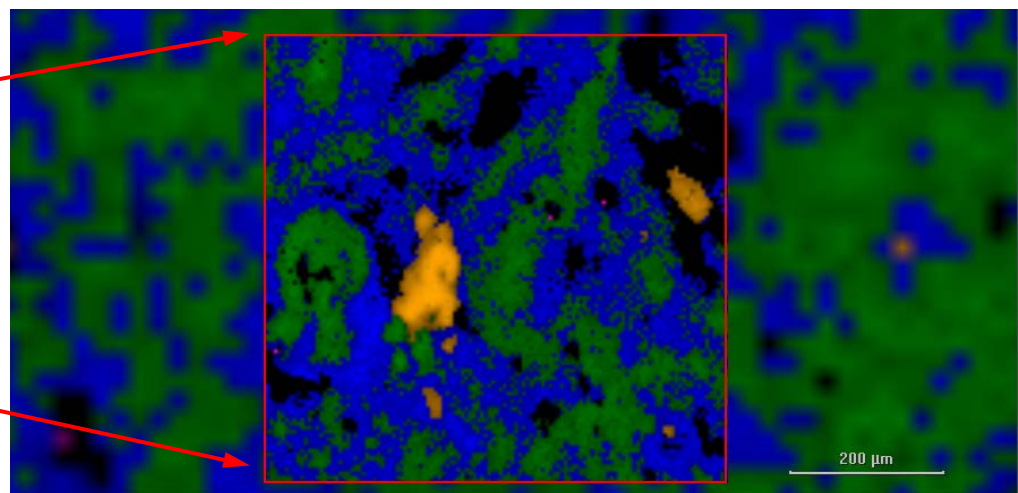
# Hot Melt Extruder Product – Ibuprofen & D-Mannitol

## Raman MCR Image

**blue** - HPMCAS, **green** –ibuprofen, **orange** – mannitol,  
**yellow** – epoxy, **fuschia** – cyanoacrylate



10X objective, 780 nm laser, 24 mW,  
6.7 x 4.7 mm area, 25  $\mu\text{m}$  image pixel size, 49476  
spectra, 0.0100 s exposure time, 100 scans



50X objective, 780 nm laser, 24 mW,  
600 x 580  $\mu\text{m}$  area, 5  $\mu\text{m}$  image pixel size, 13930  
spectra, 0.0100 s exposure time, 100 scans

# Summary of Raman Imaging of Hot Melt Extruder Products

- Raman imaging allows evaluation of the spatial distribution of components in hot melt extruder products. This is not generally available with the typical inline monitoring of the products.
- Raman can be used to monitor any changes in molecular structure and chemical environment including molecular associations that might be induced during the HME processing.
- There are many options for Raman imaging from imaging whole samples down to small particles (millimeters to microns). This is important for these types of samples where there can be a significant range in particle sizes.



# Let the Power of Raman Imaging Work for You

- Raman imaging is clearly a very useful analytical tool for evaluating pharmaceutical products
- Raman imaging extends the power of Raman spectroscopy across greater areas and further expands the utility of Raman spectroscopy
- Raman imaging gives you the ability to identify materials and to assess subtle differences in molecular structure and chemical environment
- The DXRxi Raman imaging microscope will help you:
  - Get results and solve problems quickly with exceptional performance
  - Allow more people to solve problems without the need for a central expert
  - Walk up and use the system within minutes, anytime, without a significant learning curve





# Thank you!

- Thank you for attending!
- Visit **[thermoscientific.com/DXRxi](https://thermoscientific.com/DXRxi)** for more information on the Thermo Scientific DXRxi Raman imaging microscope

Accelerate your work

**Visualize your answers**

