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Visualizing, Characterizing, and Analyzing Pharmaceutical Constituents with Raman Imaging

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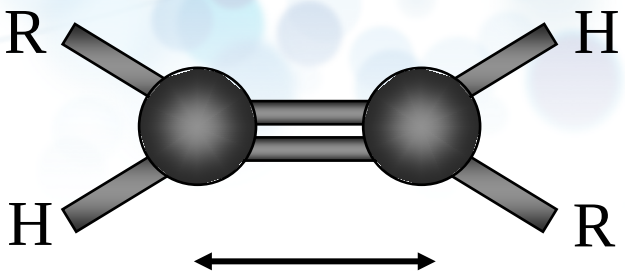
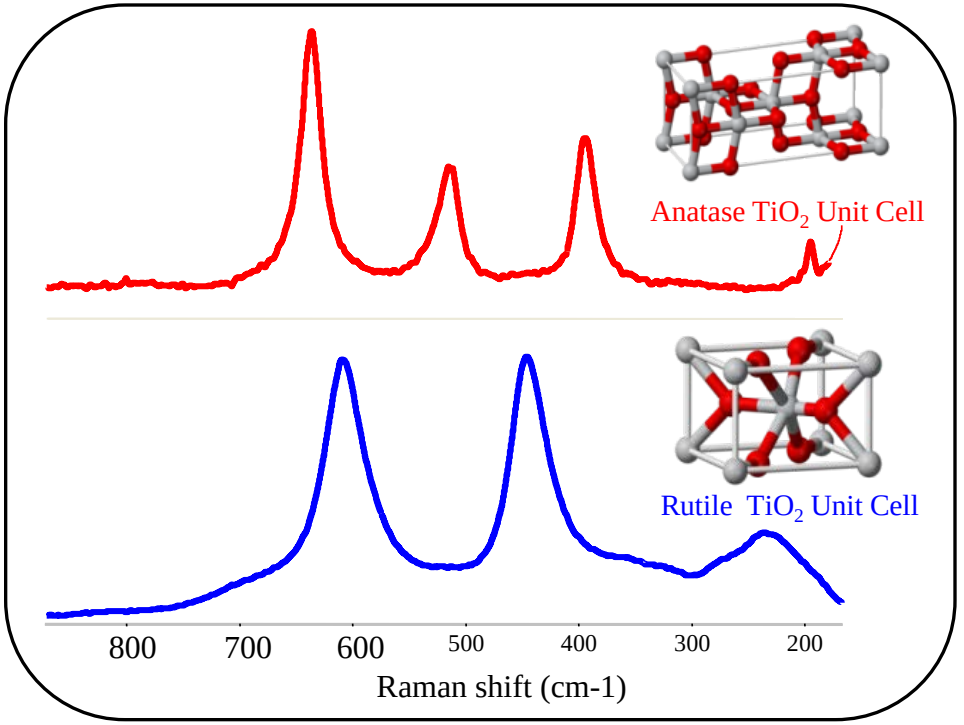
Agenda

- 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!





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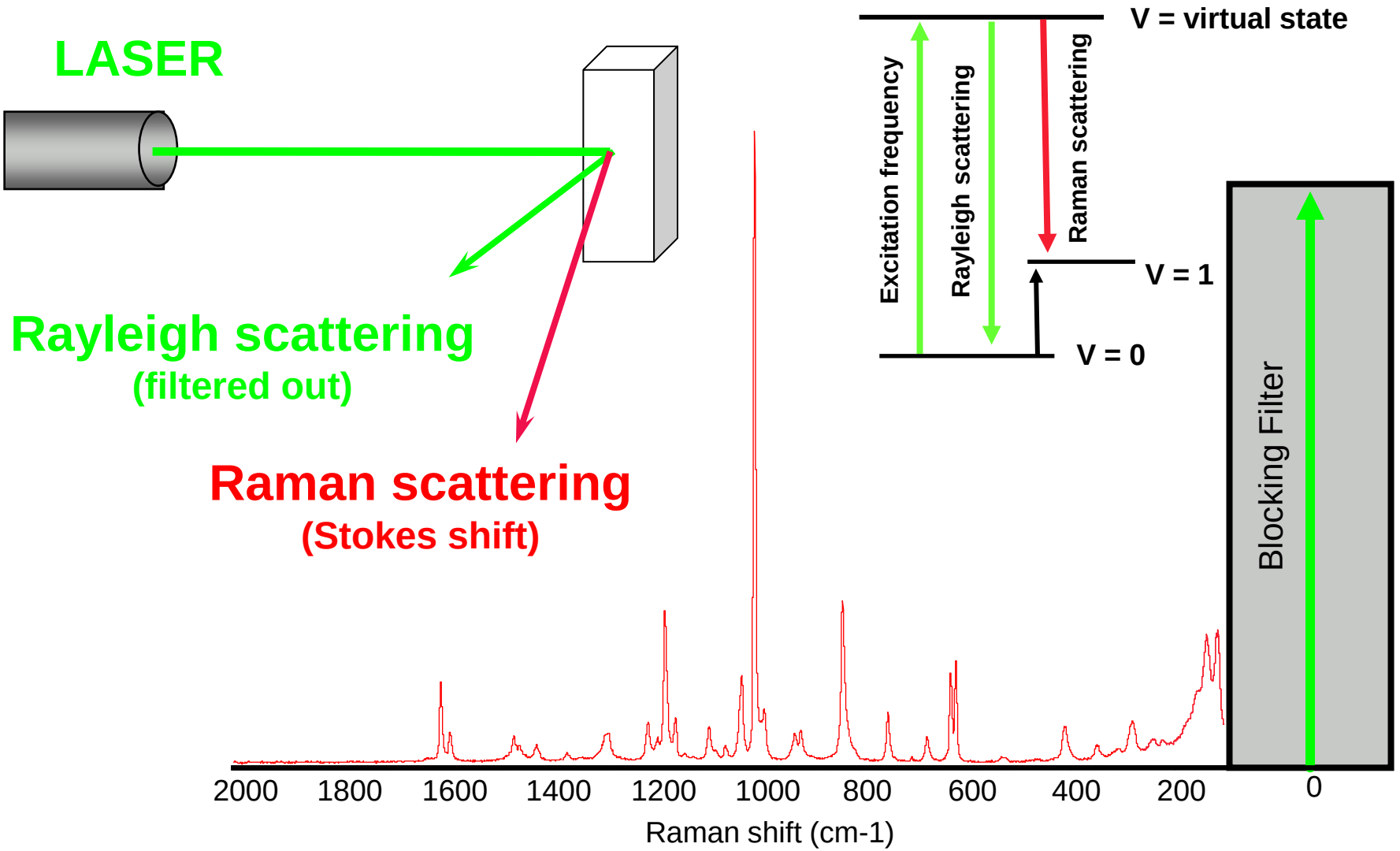
Raman Spectroscopy – The Raman Effect



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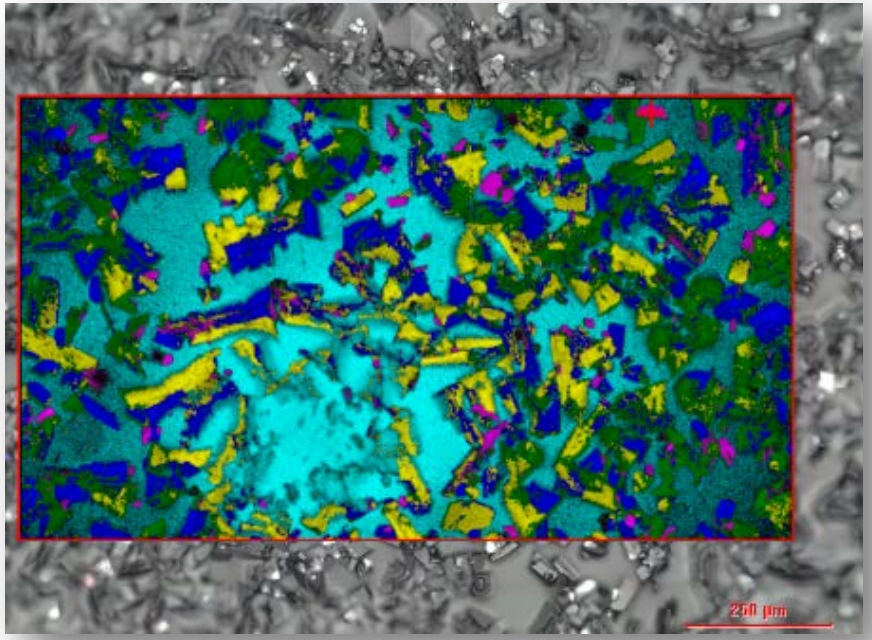
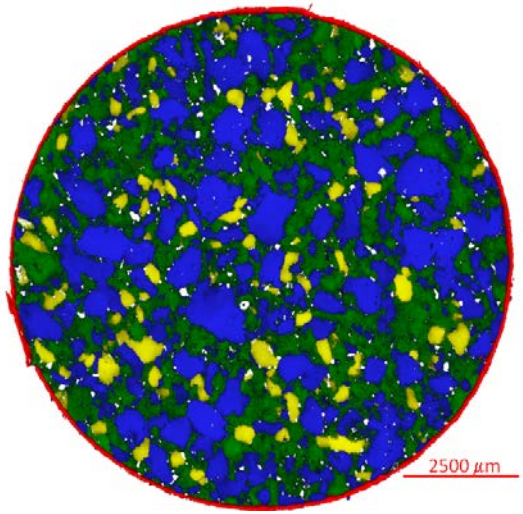
What Can Raman Imaging Do?

- 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



<u>Other Application Areas</u>

Polymers and Packaging
Semiconductors and Thin Films
Carbon Nanomaterials
Geology / Mineralogy
Life Sciences

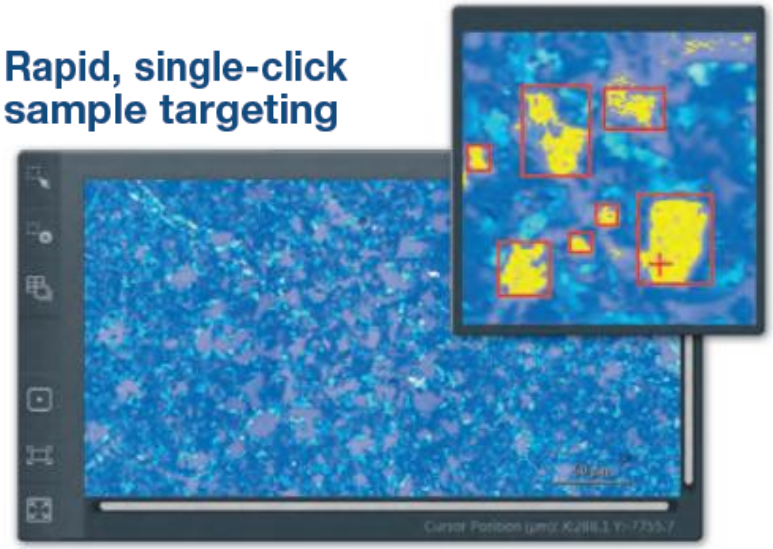
Introducing the Thermo Scientific DXRxi Raman Imaging Microscope

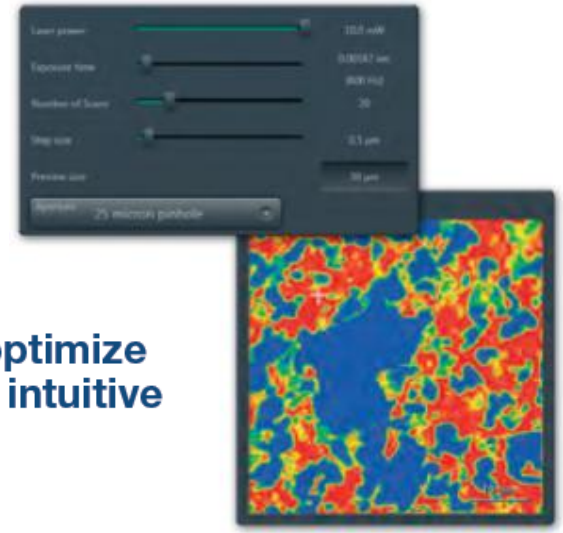
A total imaging system: hardware and software integration combines **powerful performance** with **image-centric** analysis and **ease of use**

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

Intelligent Workflow with Excellent Flexibility

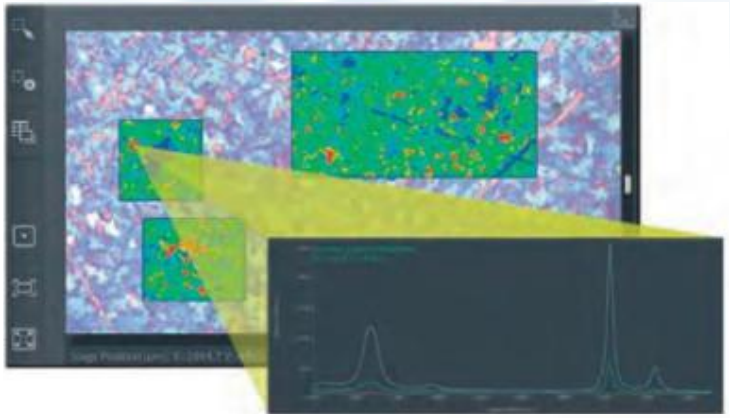
1 Rapid, single-click sample targeting



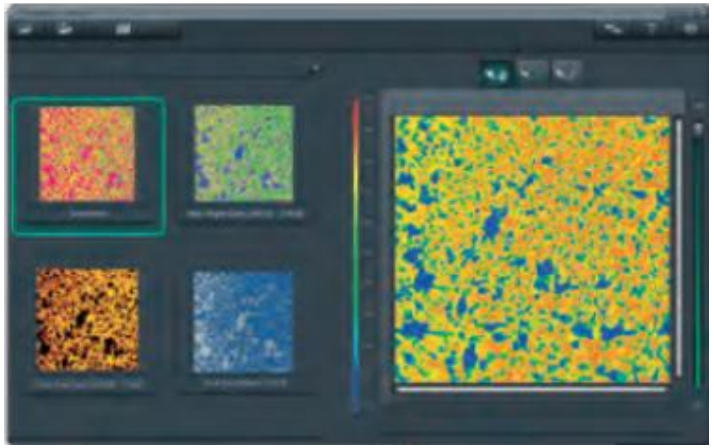


2 Confidently optimize settings with intuitive controls

3 Quickly prioritize multiple regions of interest and run

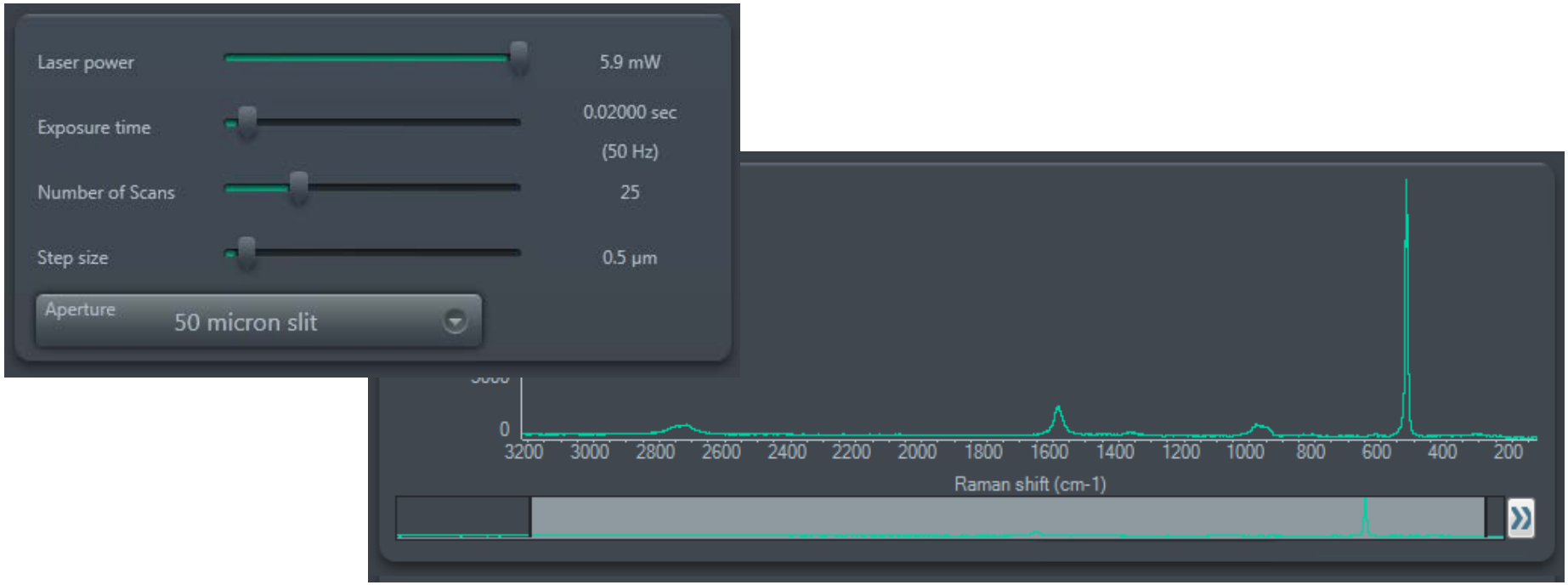


4 Information-rich images reveal a multitude of material characteristics



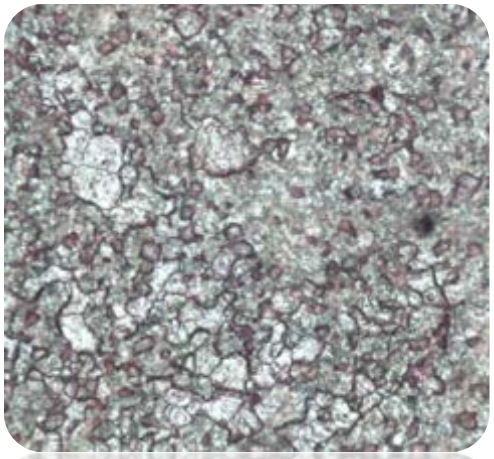
No Raman Expertise Required to get the ***Best*** Results

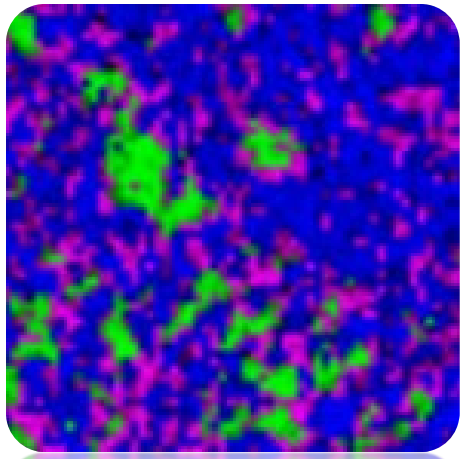
- Visual controls and instantaneous, continuous visual feedback
  - NO lengthy trial and error
  - NO guesswork
  - You can see when parameters are optimal
  - Focus quickly on the problem, not the technique



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

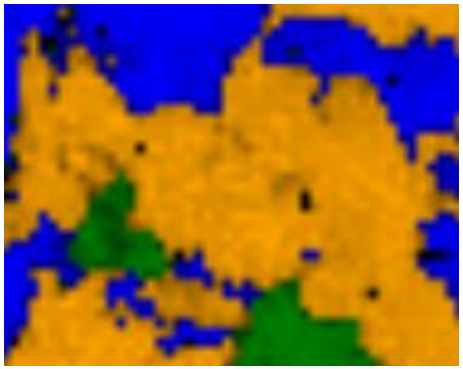




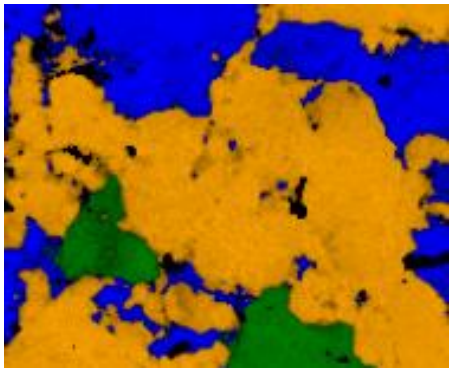
Preview in seconds!

Get There Faster By Getting Just What You Need

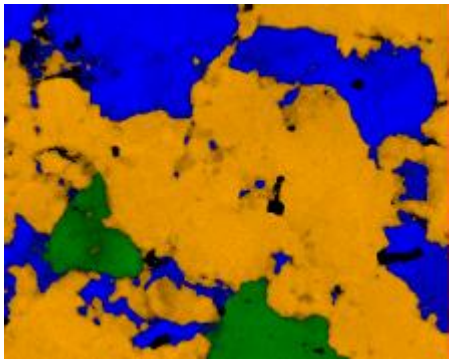
- Optimize ***image*** collection, not individual spectra
  - Quickly and visually balance image collection time with necessary detail level
  - Remove unanticipated results somewhere in an image
  - Stop any time if results are good enough rather than wait for multiple scans of each point in entire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

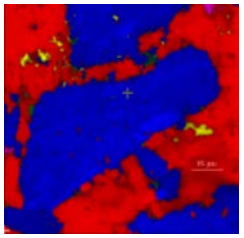
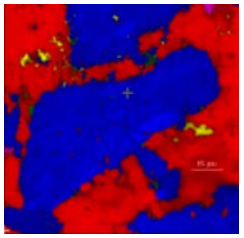
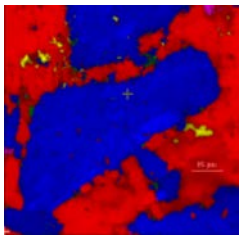
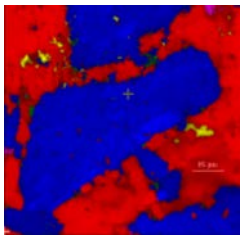


1 micron image pixels
191,000 spectra , 2 scans
25 minutes

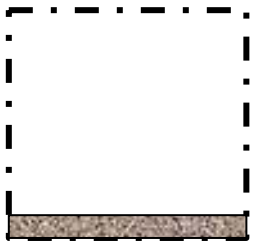
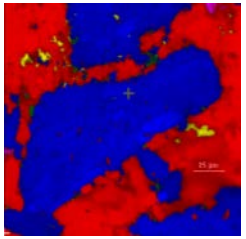
Image-centric vs. spectral-centric at same “spectral speed”

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	1 minute	200 seconds	Some Time Later….
			
Single scan of entire image with MCR, 10,000 spectra	3 scans of entire image with MCR	10 scans of entire image with MCR	

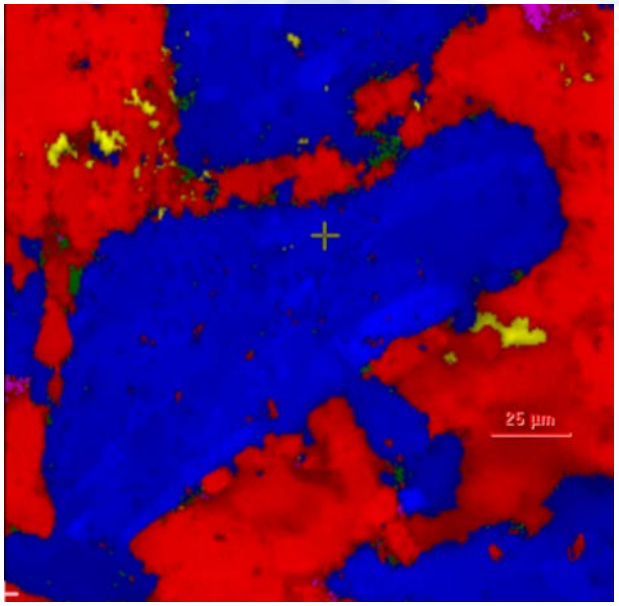
Other Raman Imaging Systems, building images one spectrum at a time

			
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



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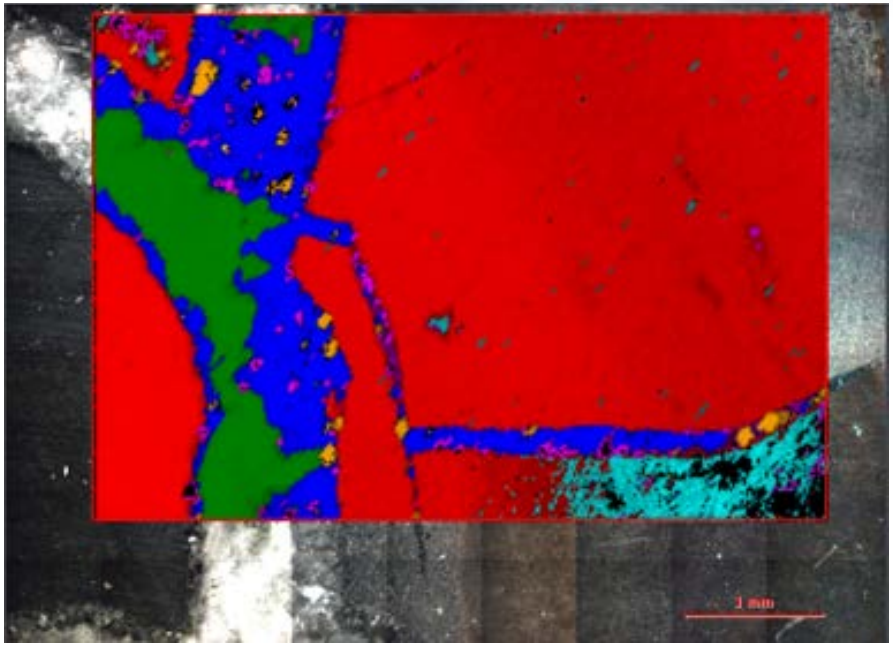
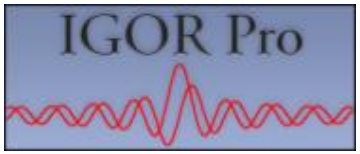
Data Processing Concurrent With Data Collection

- 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 Specta with a single click!

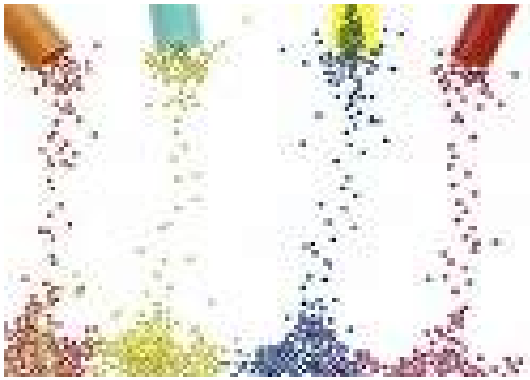


Microscopy Options

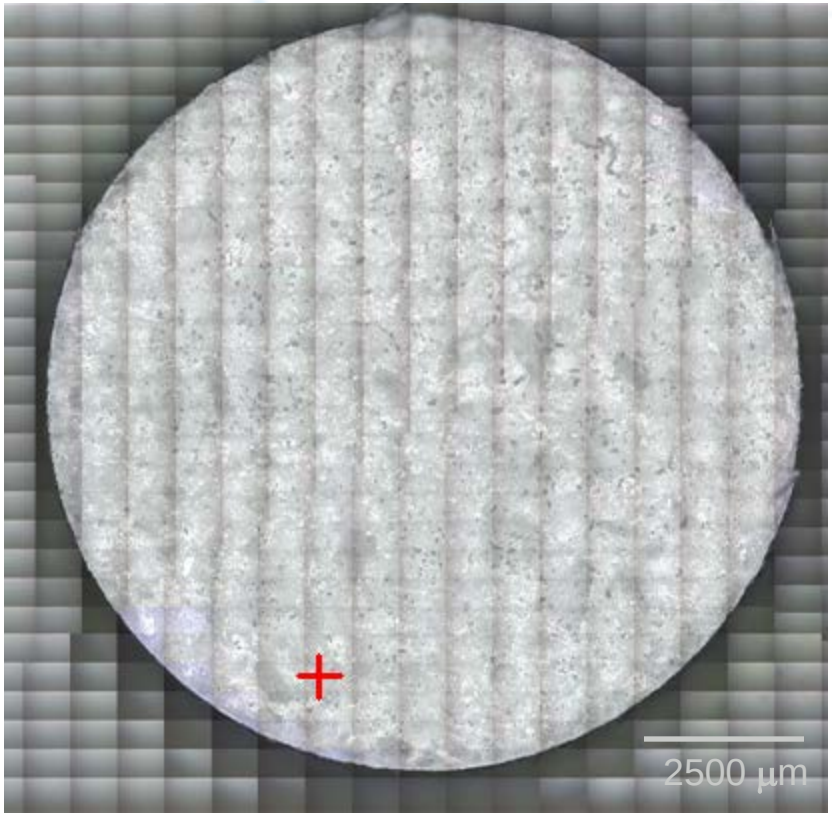
- Supports a wide variety of sample measurement options, including:
  - Single and dual microscope slide holders
  - Heating and cooling stages – even during imaging!
  - Rotating stage insert
  - Industry standard wafer holders and SEM accessories
  - ‘Breadboard’-style holder for custom configurations
  - 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

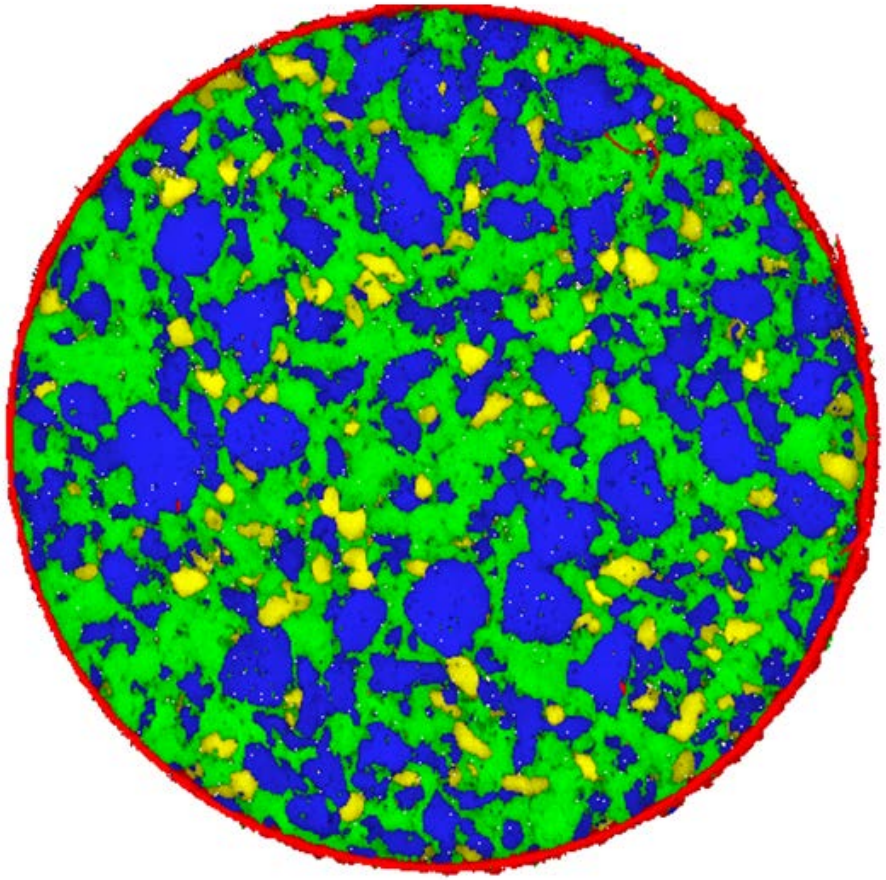
Acetaminophen	250 mg (37%)
Aspirin	250 mg (37%)
Caffeine	65 mg (9.6 %)

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

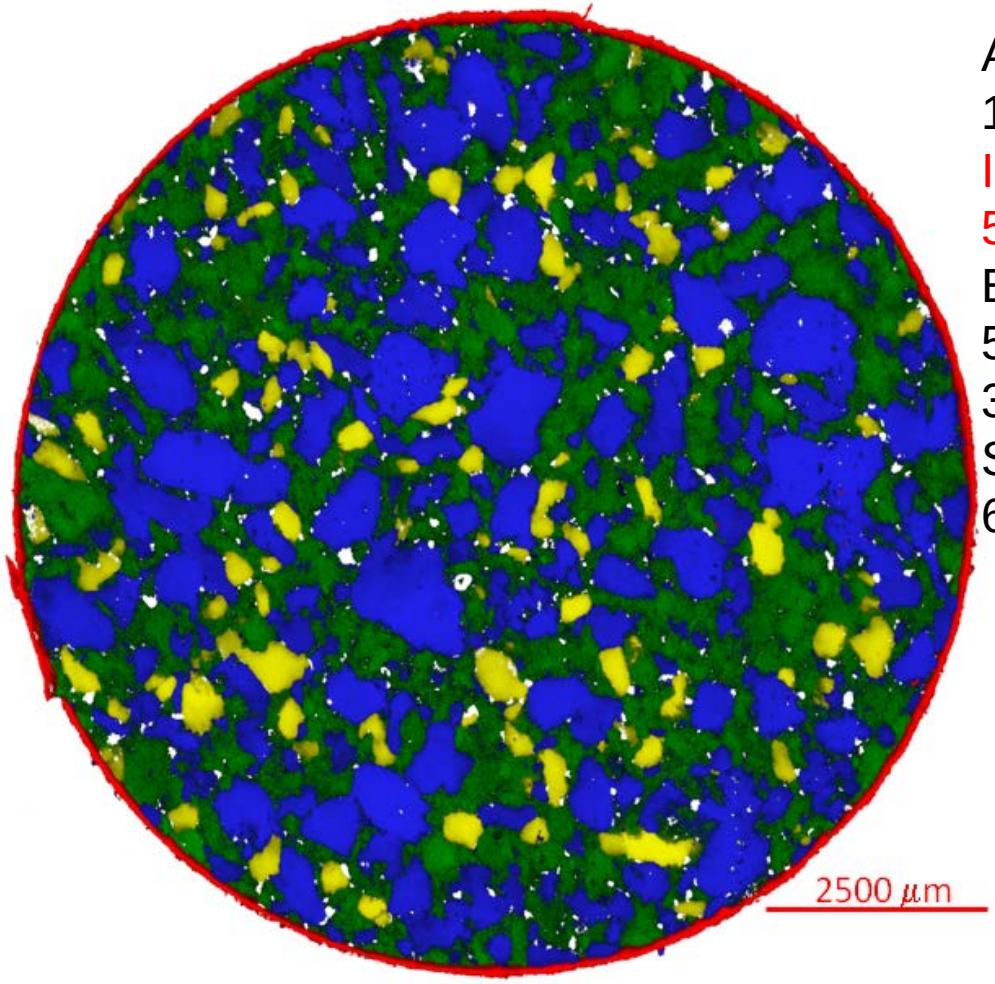


Aspirin Acetaminophen Caffeine Titanium Dioxide

Area Imaged - 11 x 11 mm^2
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!!

Higher Resolution Image – Whole Tablet



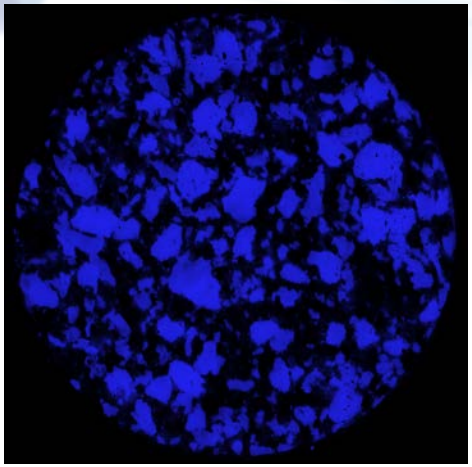
Aspirin Acetaminophen Caffeine Titanium Dioxide

Area Imaged - 11 x 11 mm^2
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)

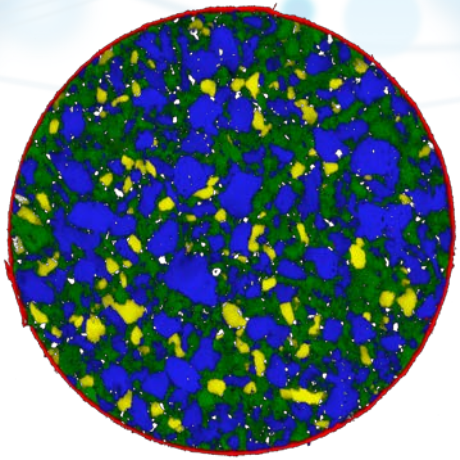
Image Analysis % Area of Particles

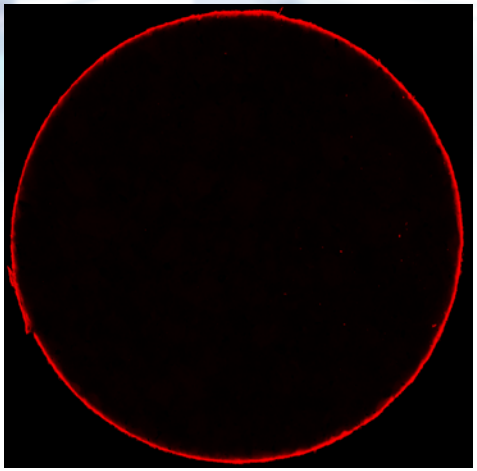
Component	Calculated % (Surface Area)	Reported %
Aspirin	38.6	37
Acetaminophen	35.4	37
Caffeine	7.7	9.6

Tablets Components – From Multivariate Curve Resolution (MCR)

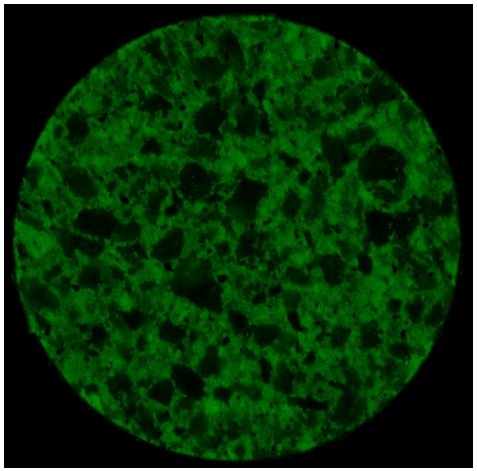


Aspirin

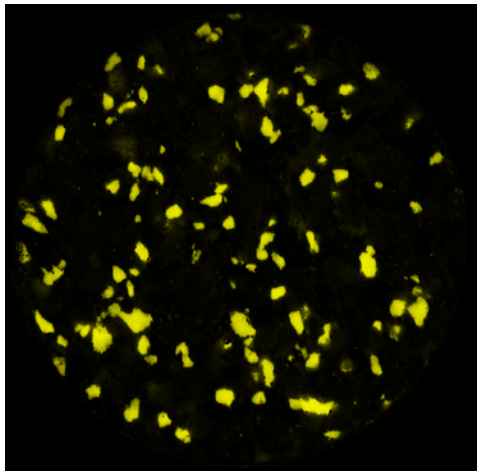




Titanium Dioxide

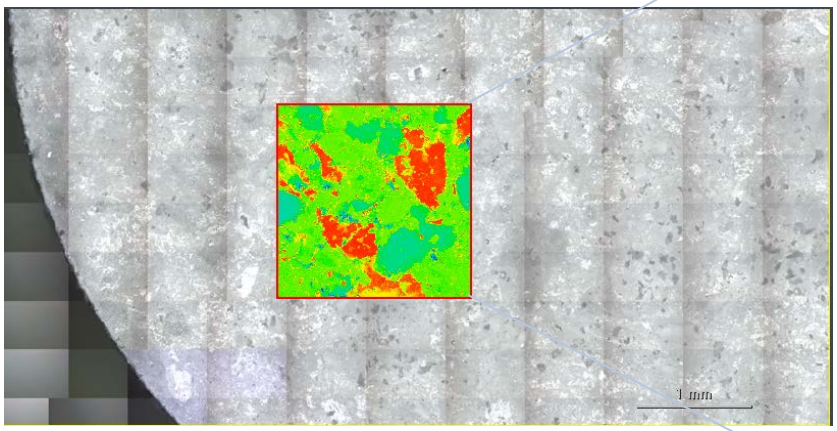


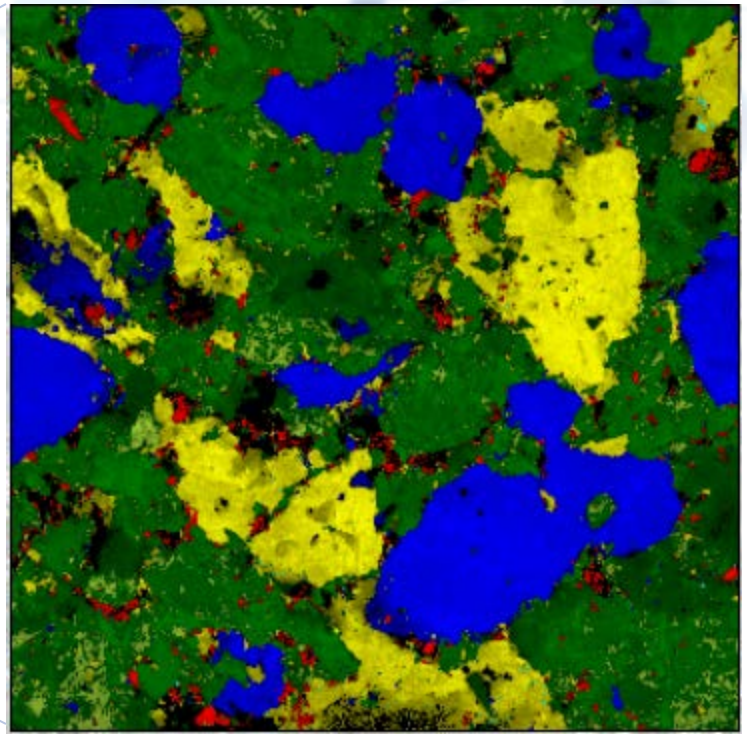
Acetaminophen



Caffeine

Small Area – Longer Exposure Time



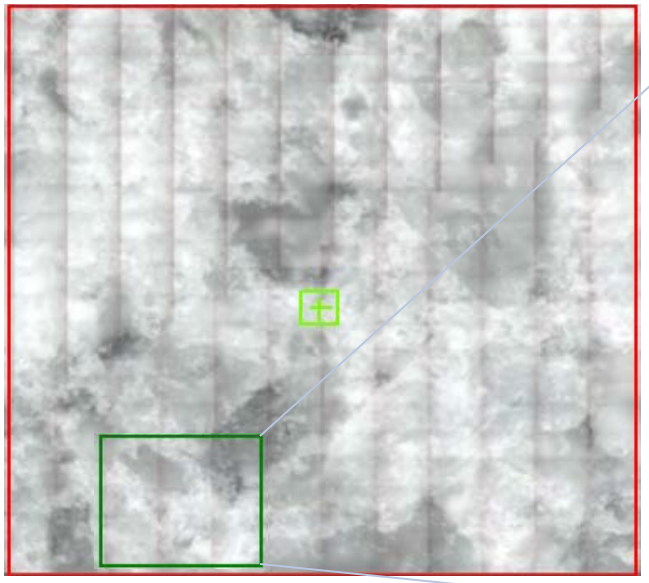


500 µm

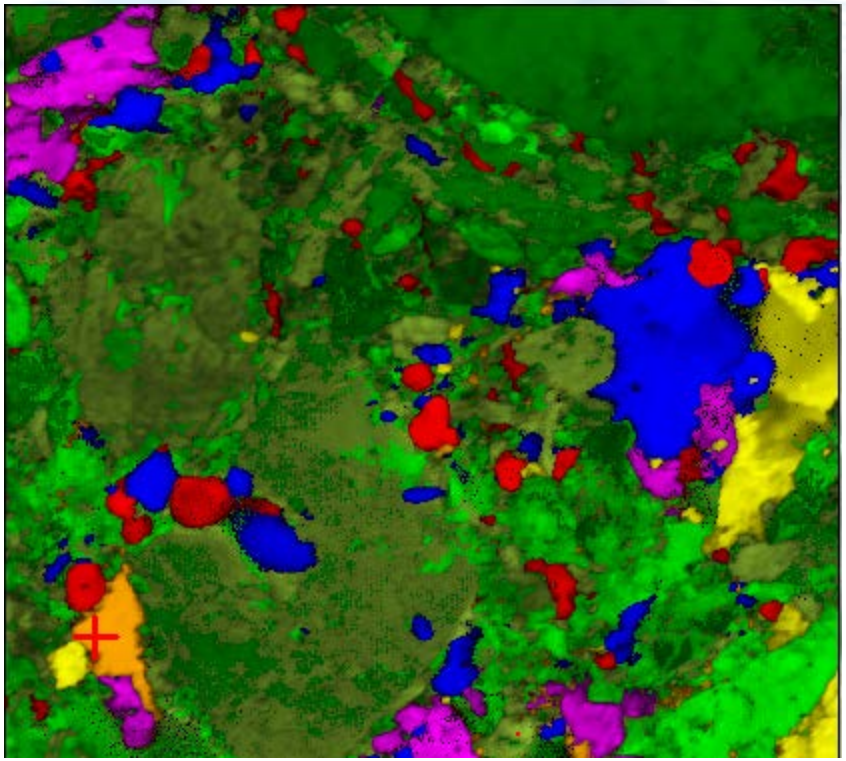
1.6 x 1.7 mm^2
50X Objective
5 micron image pixel size
116000 spectra
Exposure time 5 ms (200 spectra per s)
532 nm laser
5 averaged scans
55 minutes

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

Smaller Area, Higher Resolution, Longer Exposure Time



500 μm



100 μm

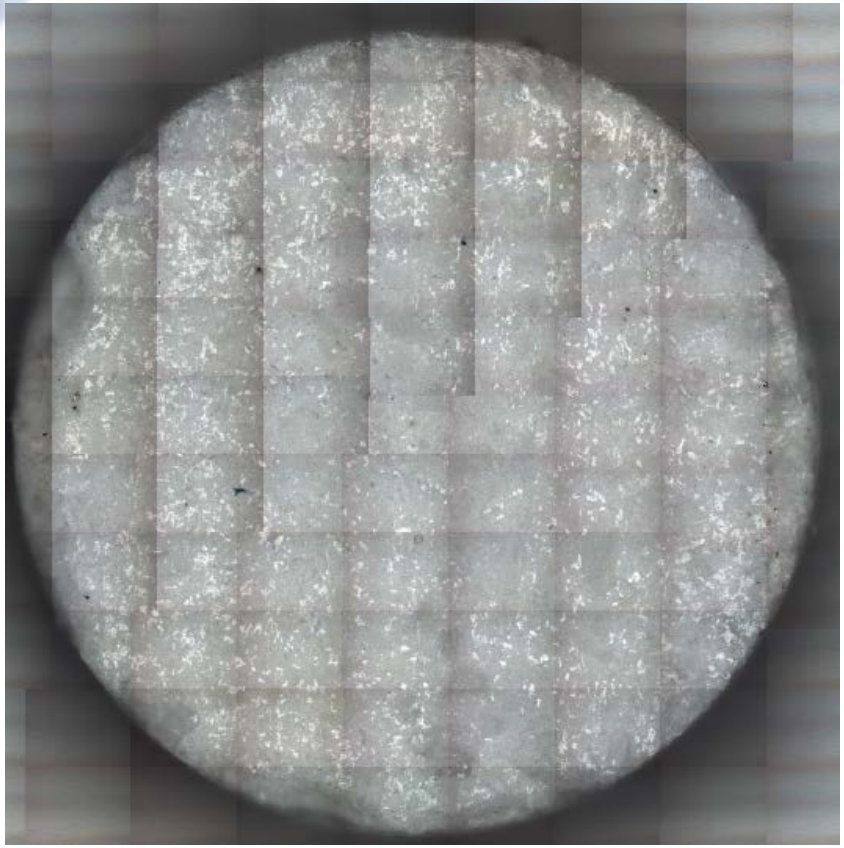
225 x 250 μm^2
100X Objective
0.5 micron image pixel size
229000 spectra
Exposure time 10 ms (100 spectra per s)
532 nm laser
5 averaged scans
3 hr collect

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)

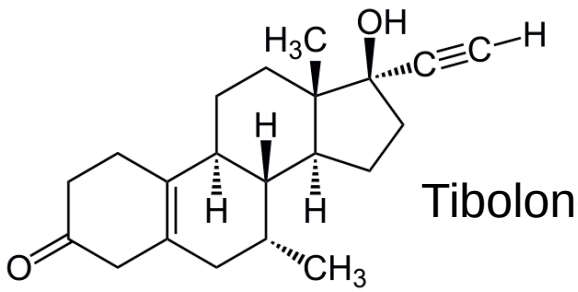
Tibolone Tablet

3% Tibolone

A synthetic steroid used in hormone replacement therapy

6 mm diameter

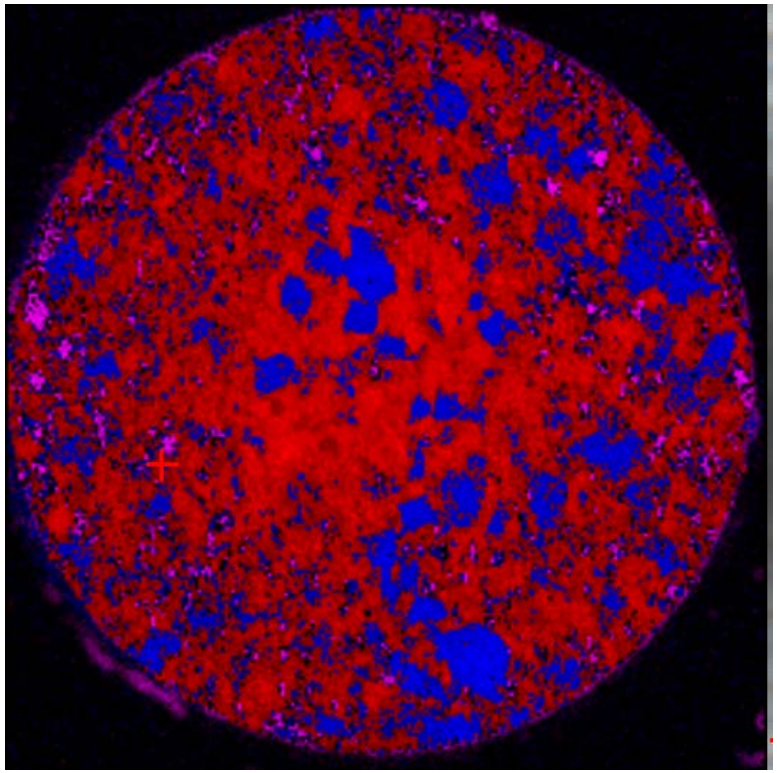
Polymorphs (monoclinic, triclinic)



Tibolone

Raman MCR Image of Low Dose Tablet

Raman MCR Image

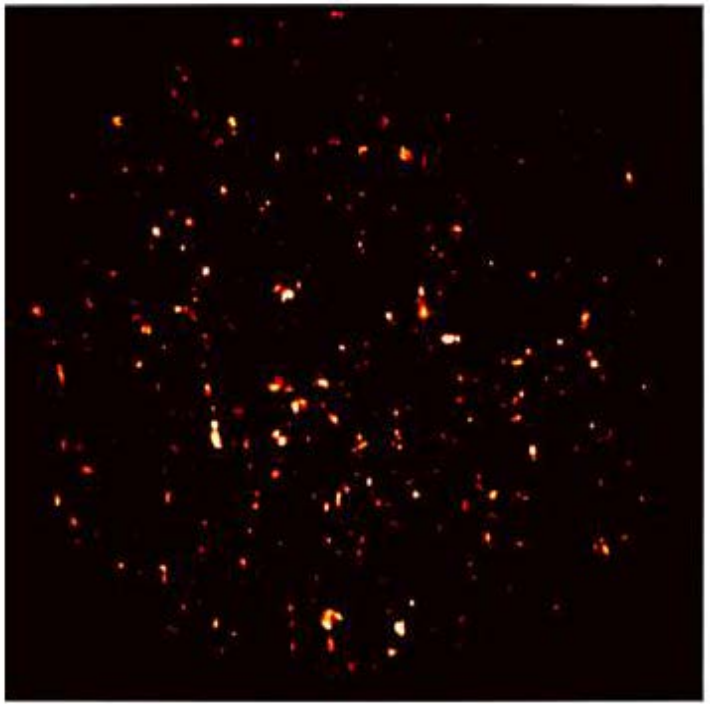


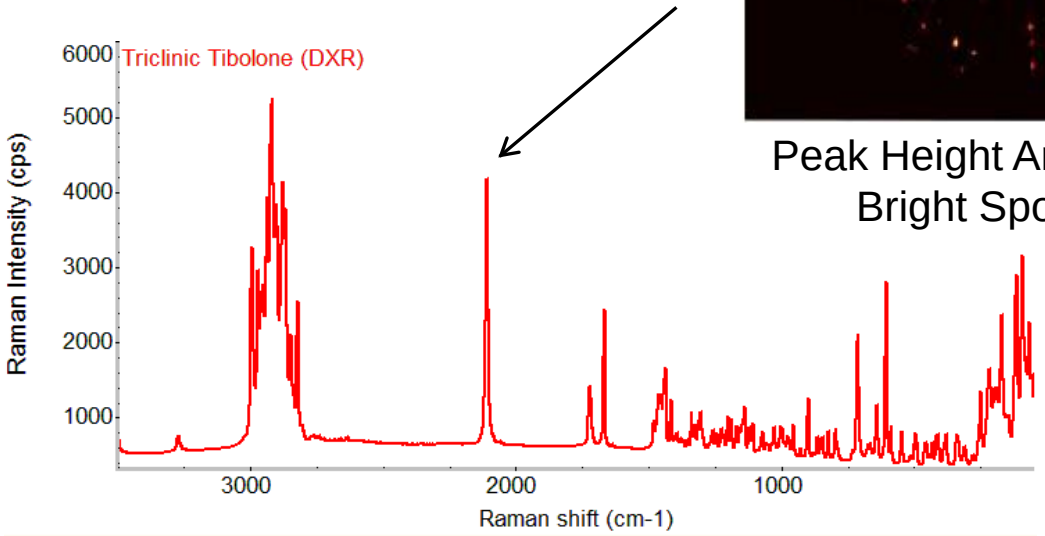
Starch Lactose Fluorescent Compound

5.7 x 5.7 mm^2 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

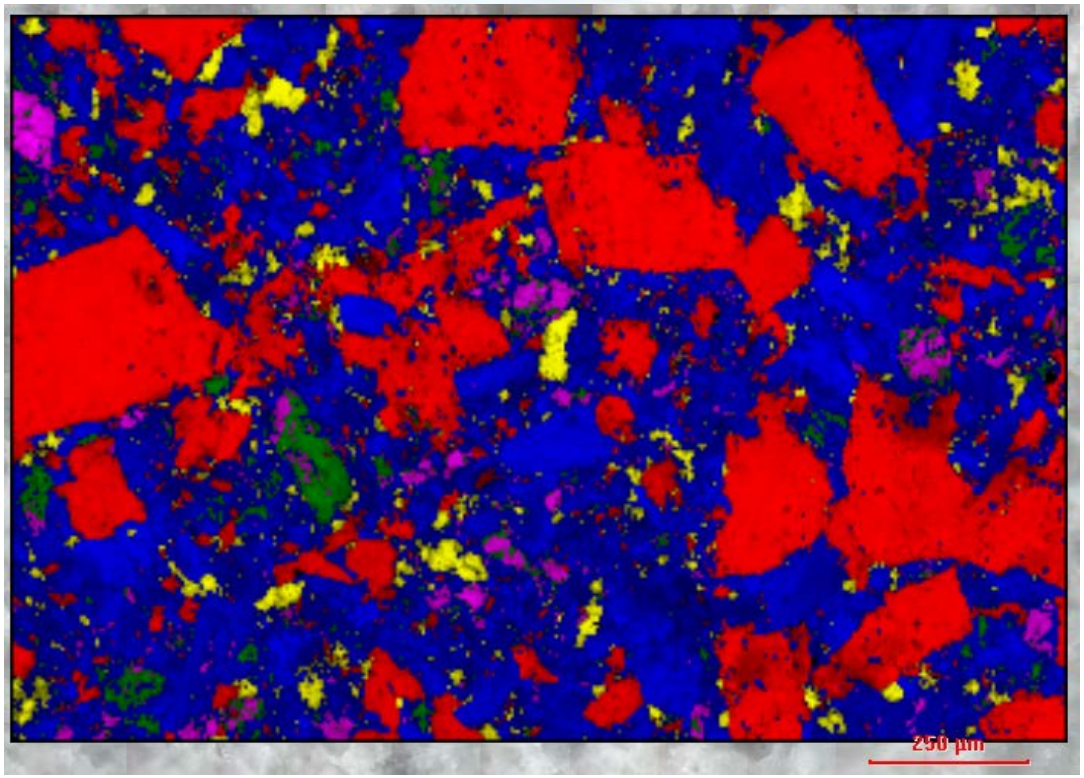
A Peak Height Profile Readily Shows the Location of the Tibolone





Peak Height Analysis at 2102 cm^{-1}
Bright Spots are Tibolone

Smaller Area, Higher Resolution, More Scans



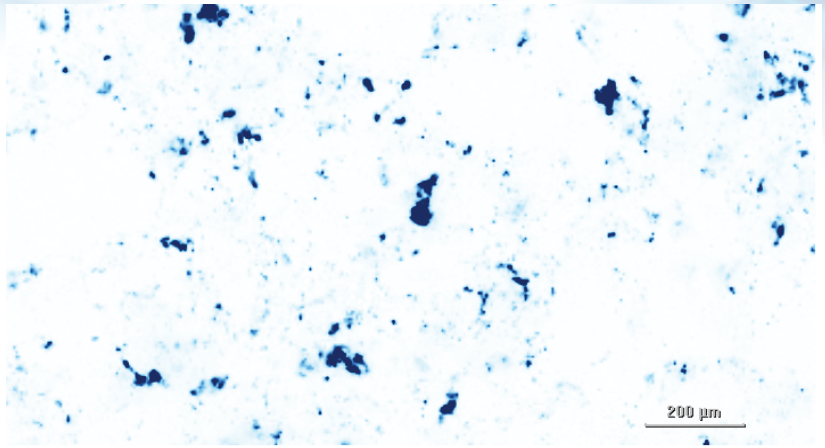
Tibolone Starch & Lactose Fluorescent Compound

1.1 x 1.6 mm^2 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

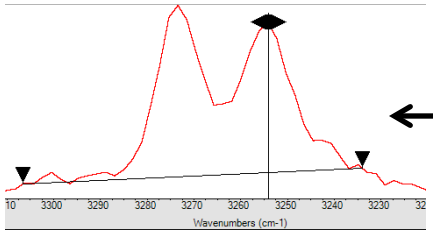
Tibolone is one of the components defined by the MCR analysis

Distribution of Polymorphs of Tibolone

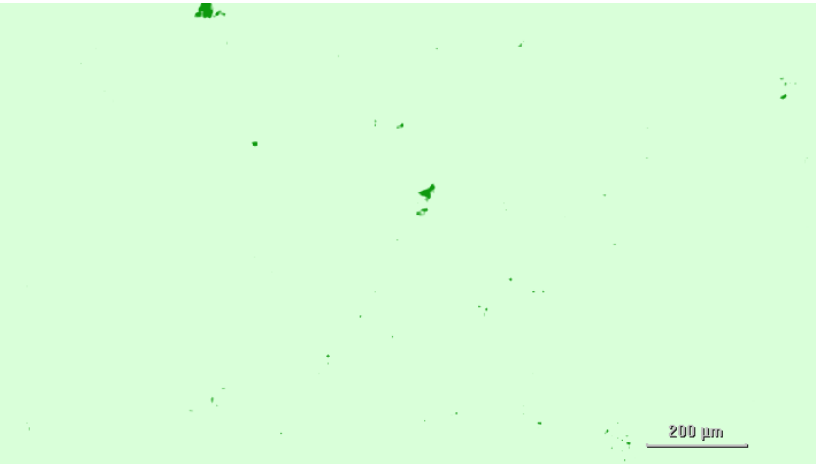
All Tibolone (peak at 2102 cm^{-1})



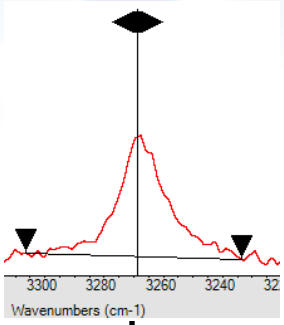
3273, 3254 cm^{-1}



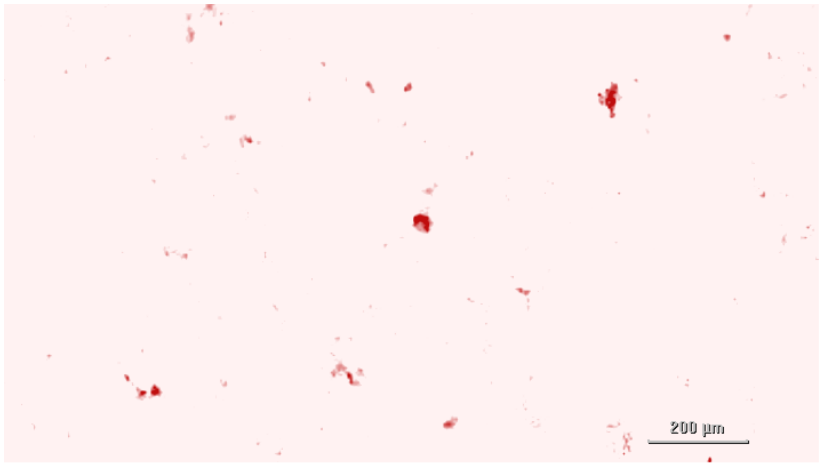
B (monoclinic)



3267cm^{-1}



C (triclinic)



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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.

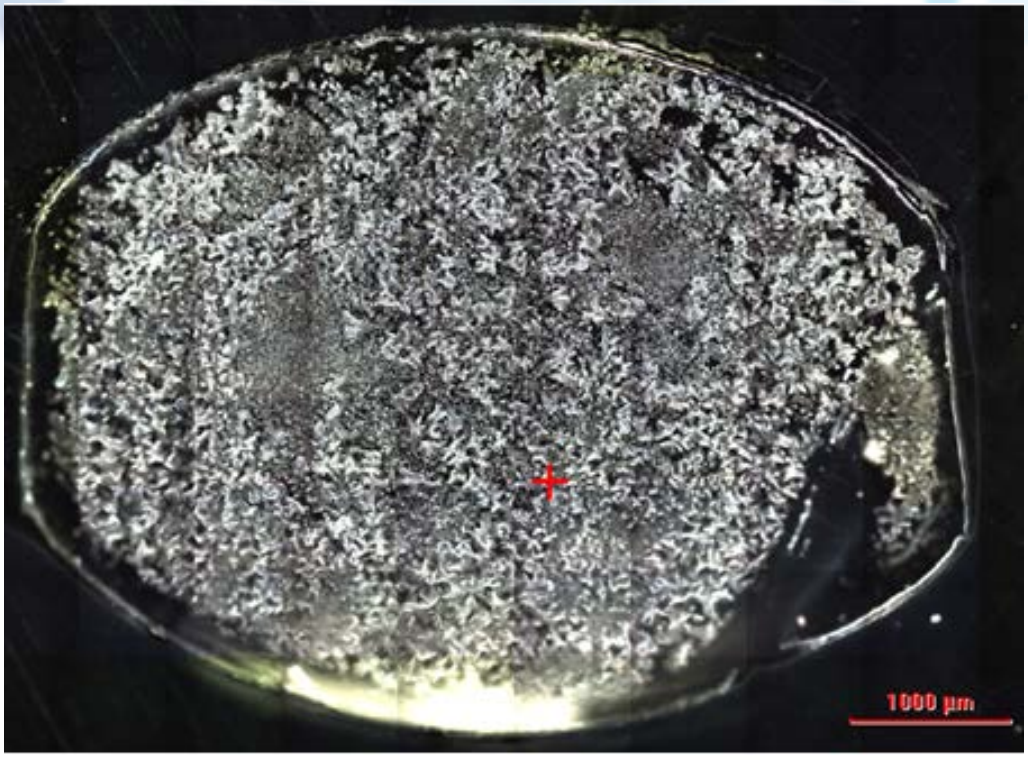
HME General Processing Advantages

- 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
  - Compare to wet granulation process

Raman Imaging of HME Products

- 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

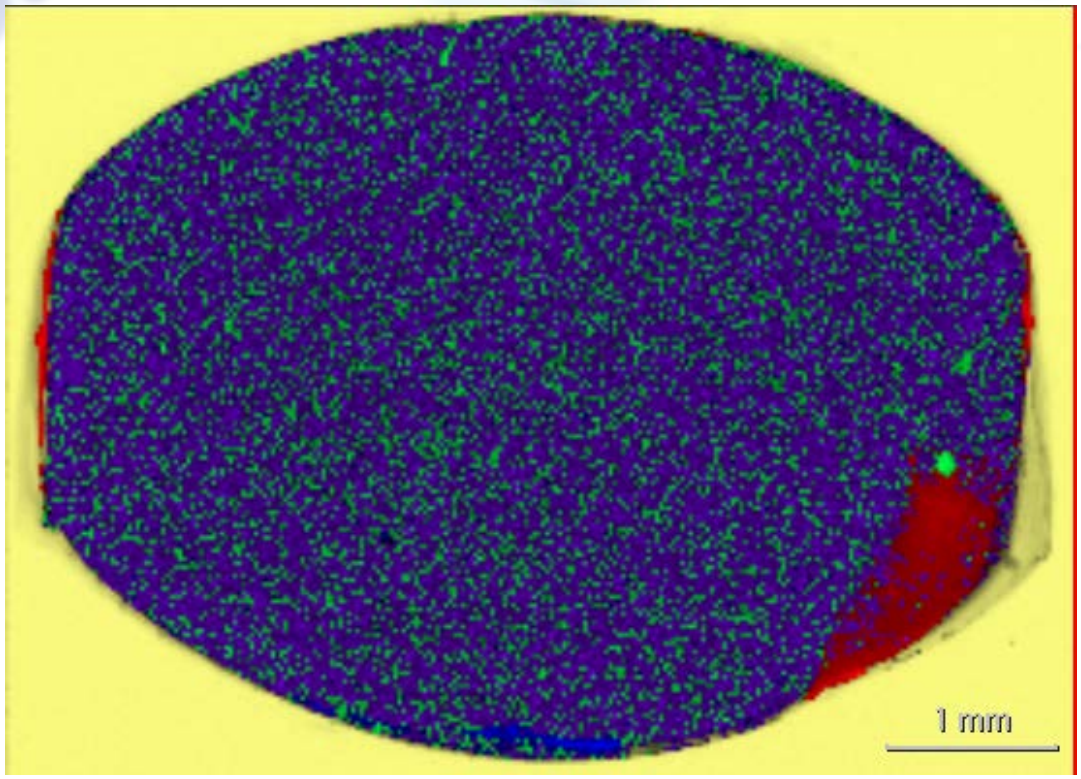
Ibuprofen (25-33%) and ibuprofen (25-33%) + D-mannitol (7-15%).

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



Raman MCR Image

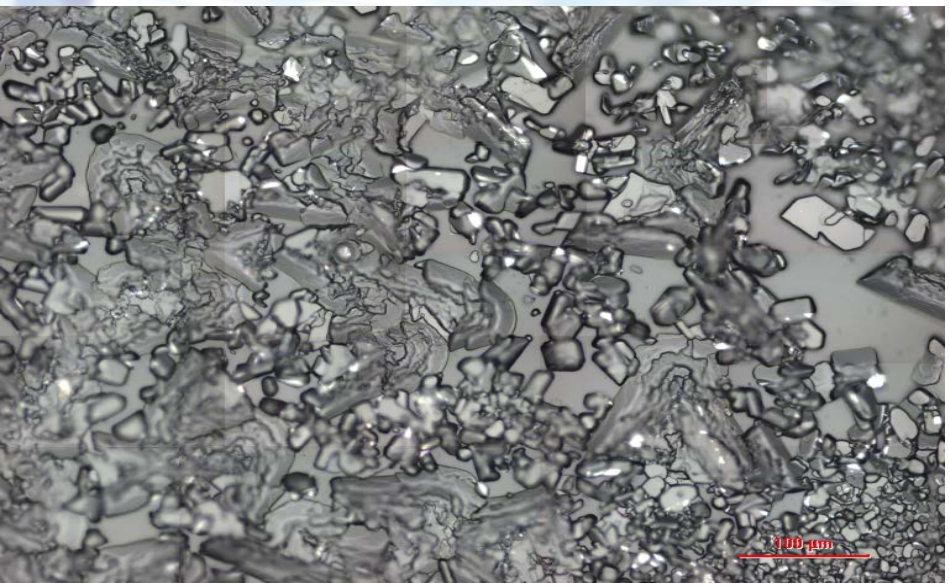
purple – HPMCAS, green –ibuprofen, yellow – epoxy,
red – cyanoacrylate, blue – inorganic impurity

6.35 x 4.6 mm^2 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

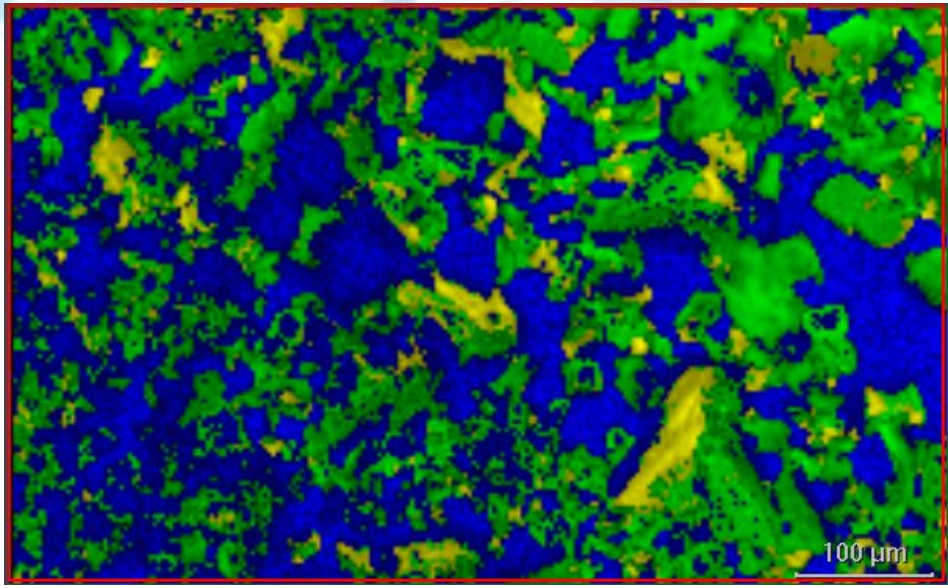
Component	Calculated % (Surface Area)	Reported %
ibuprofen	23	25

Smaller Area, Higher Resolution, Raman Imaging



Video Image

(50X objective - brightfield illumination)

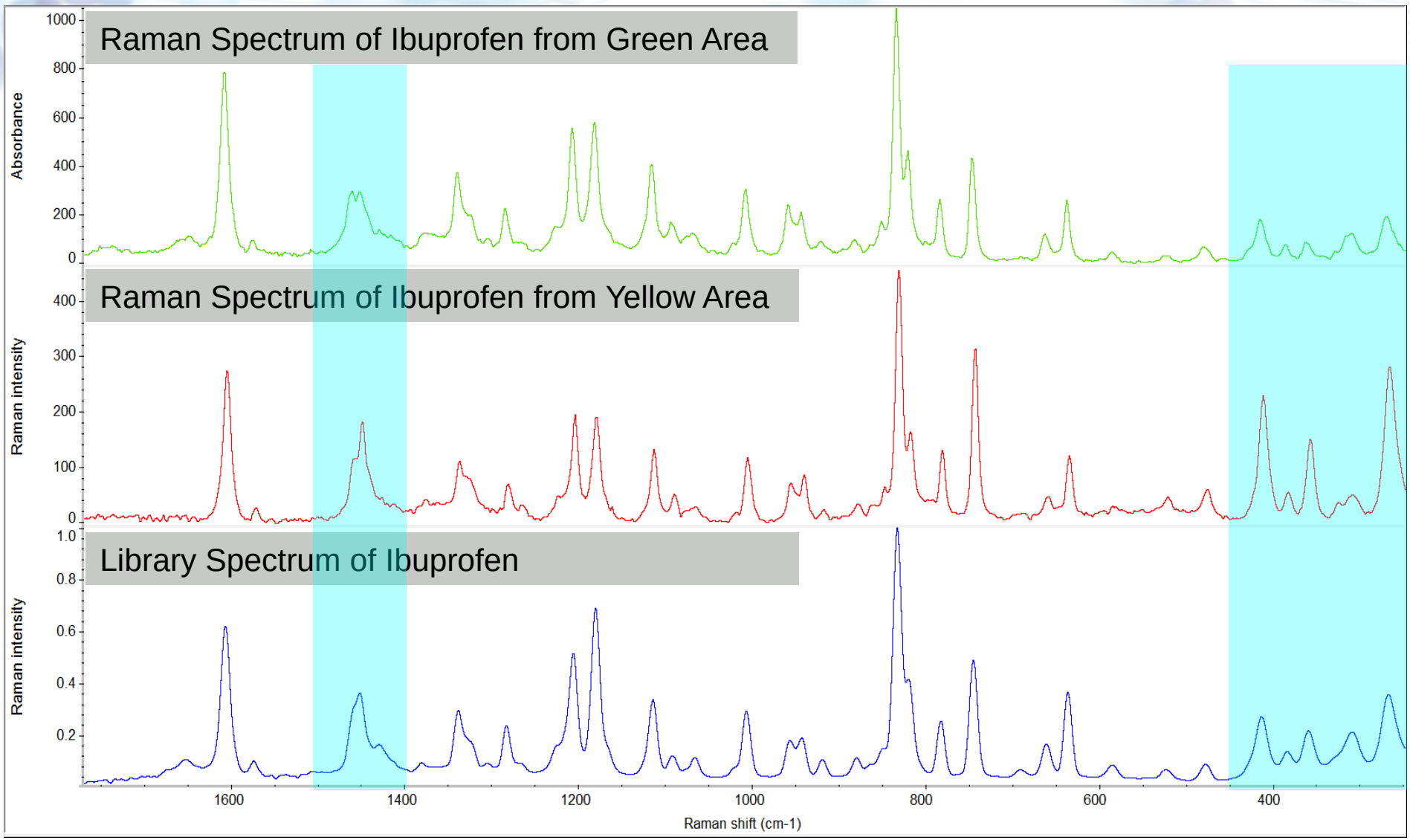


Raman MCR Image

blue – HPMCAS, green –ibuprofen , yellow – ibuprofen

50X objective, 780 nm laser, 24 mW, 687 x 423 µm area, 3.0 µm image pixel size, 32289 spectra, 0.0100 s exposure time, 100 scans

Subtle Differences in Ibuprofen Spectra



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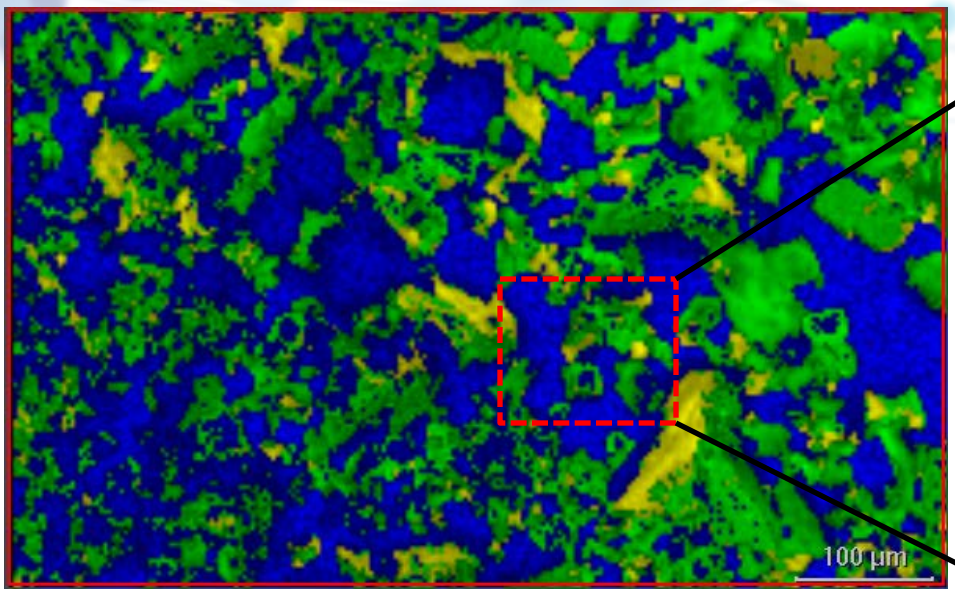
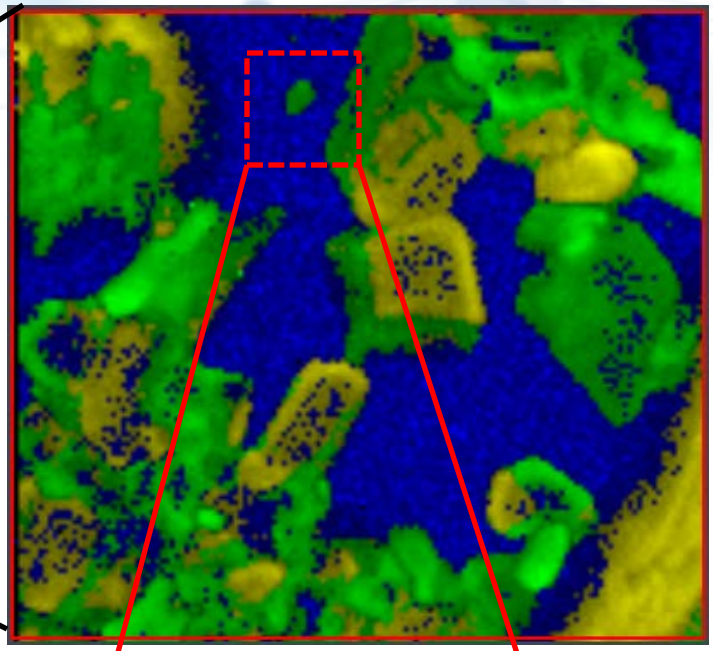
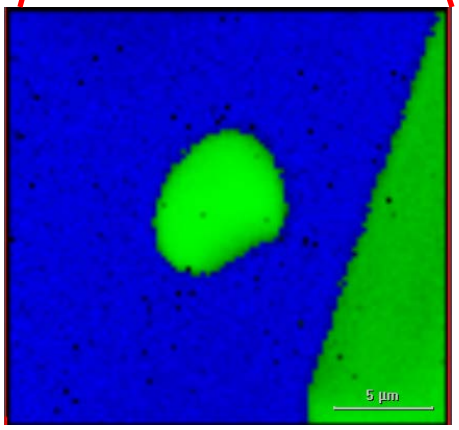
Differences in the Raman Spectra of Ibuprofen

- **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

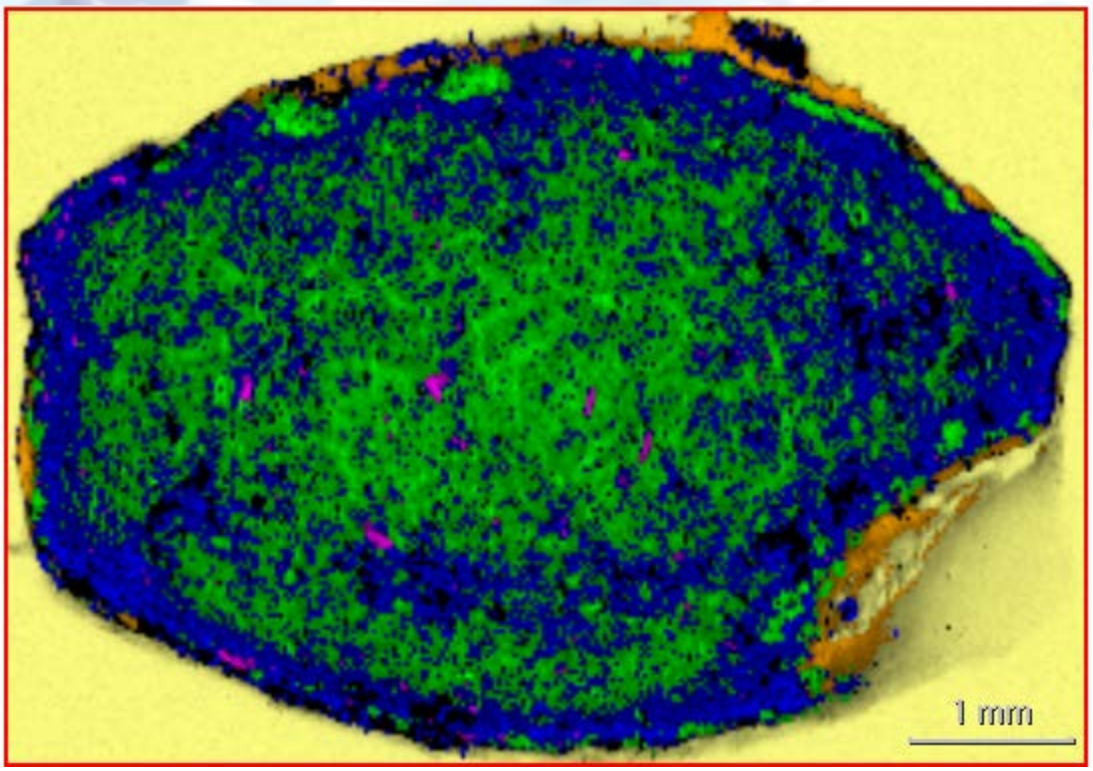






Millimeters to Microns

Hot Melt Extruder Product – Ibuprofen & D-Mannitol



Raman MCR Image

blue – HPMCAS, green –ibuprofen, yellow – epoxy,
orange – cyanoacrylate, fuchsia – D-mannitol

6.65 x 4.65 mm^2 area
10X objective
25 μm image pixel size
49476 spectra
Exposure Time 10 ms (100 spectra /s)
780 nm laser
100 averaged 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

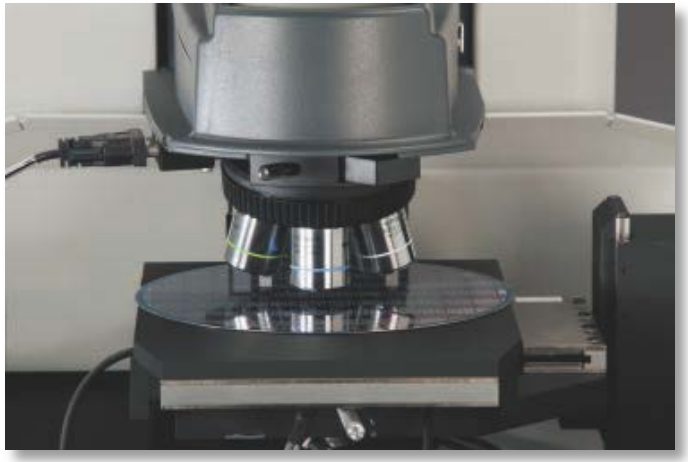
- Raman imaging is clearly a very useful analytical tool for evaluating pharmaceutical products
- Raman imaging extends the power of Raman spectroscopy across greater areas and further expands the utility of Raman spectroscopy
- Raman imaging gives you the ability to identify materials and to assess 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** for more information on the Thermo Scientific DXRxi Raman imaging microscope

Accelerate your work

Visualize your answers



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