Raman Microscopy and Imaging

DR. CARLOS MORILLO

JASCO (Nihon Bunko)
R&D and Manufacturing, Hachioji, Japan

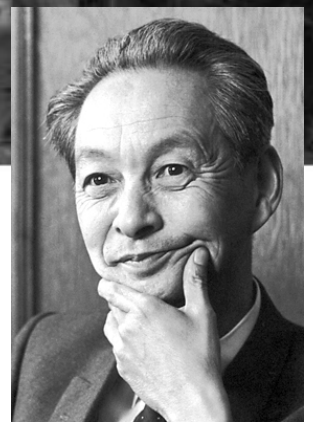
Founding Members

Established 1958 at the Optical Research Institute at Tsukuba University, Tokyo

Founding members include:

- World famous physicist Yoshio Fujioka
- Nobel Prize winner Shinichiro Tomonaga (1965 - Physics for QED with Richard Feynman)

JASCO in the USA, first incorporated in 1972.



Dr. Tomonaga

JASCO: Our Products

Presentation Overview

Raman Spectrometer Schematic

Why Micro Raman?

JASCO Raman Products

Observation Modes

- Bright, Dark and Mix
- Polarization
- DIC

Quick **R**aman **I**maging (**QRI** - Fast Mapping)

- Hardware and Software
- Data processing to create images

Surface **S**canning **I**mage (**SSI –** Tilted or Uneven surfaces)

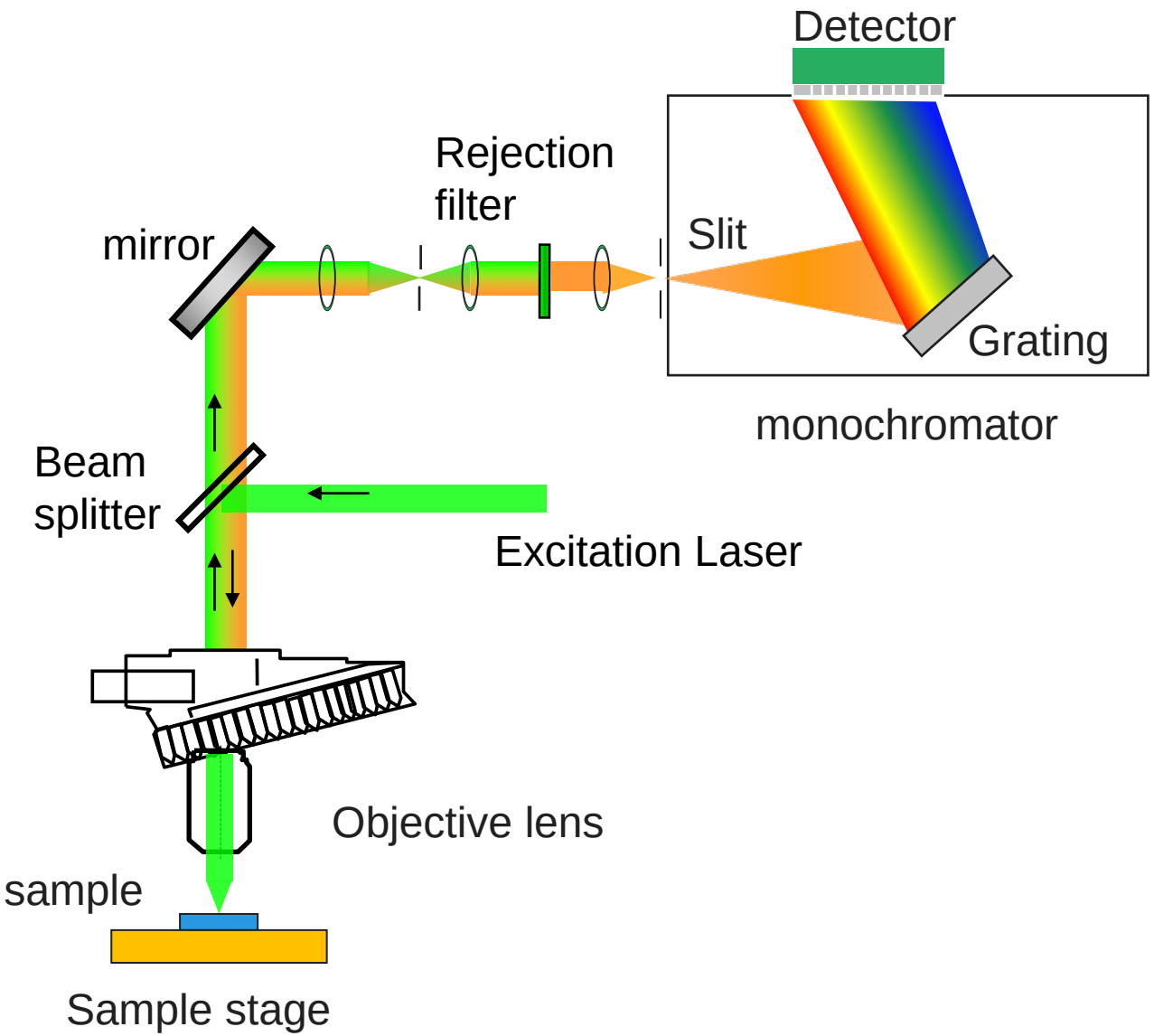
JASCO Imaging Features

- Auto Focus, Image NAV, Search Engine, **IQ Frame**

Applications

- Pharmaceutical tablet mapping, carbon materials, semiconductor contamination,

Raman Spectrometer Schematic

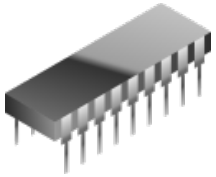


Why Micro-Raman?

- Raman is inherently a micro-technique; wherever the laser is focused, light scattering will occur
- Raman microscopes are based on the microscope optics defining the sampling area and spatial resolution
- Requires less sample and generates much less fluorescence
- A confocal microscope can provide depth profiling capability

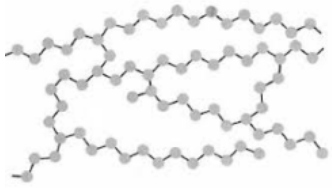
Applications of Laser Raman Spectrometer

- **Semiconductor**



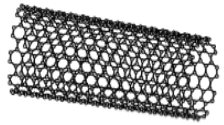
- Stress, Impurity, Study of super lattice structure, Heterostructure, Doping effect, Superconductor, photoluminescence, etc.

- **Polymer**



- Identification of polymorphic form, Distribution condition of blend polymer, Analysis of monomer and isomer, Crystallinity, Orientation, Multi-layer construction, Monitoring of polymerization reaction, Composite material analysis, Purity and fault, etc

- **Carbon material**



- Diamond Like Carbon (DLC), Characterization of fullerene and nanotube, Diamond, Graphite, Quality analysis of carbon film, HDD coating, etc

- **Pharmaceuticals**

- Crystal polymorphism, Imaging of medicinal ingredient, etc.

- **Geology Mineralogy**

- Included gas/liquid in mineral, Temperature behavior in extreme condition, crystal structure, etc.

JASCO Laser Raman Spectrometers

Portable Raman
Raman Probe

RMP-500

Standard model

NRS-4500

High-end models

NRS-5500

Flagship models

NRS-7500

QRI High speed imaging system

Focal length

20cm — **30cm** — **50cm**

One detector CCD/EMCCD

Quality control environment
Academia

Microplastics, multilayer materials, carbon materials, impurities , biological

Dual detector CCD/EMCCD or InGaAs

Physical property evaluation（R&D）

High spectral resolution
Low wave number measurement

Stress and impurities in semiconductor

How to Create High Definition Raman Images

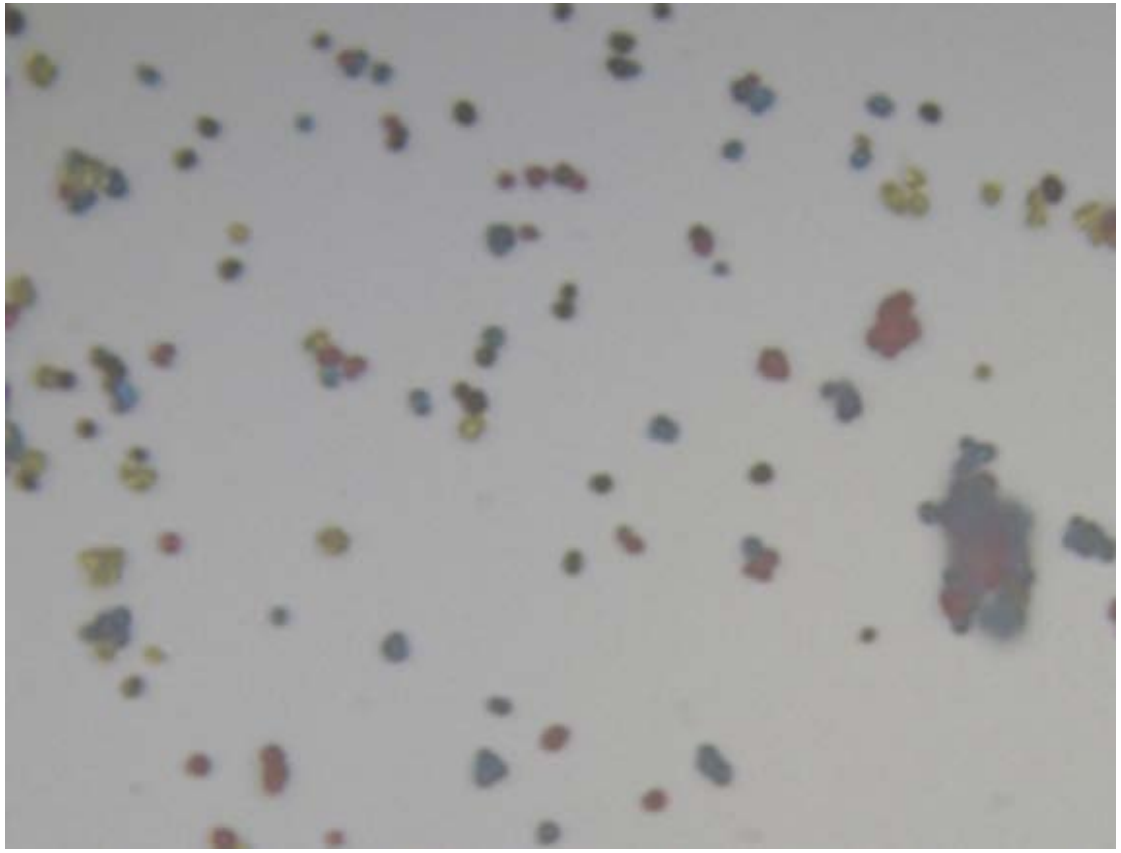
- Different observation modes
- High-definition visible image using a **high pixel camera**
- Imaging measurement not only in XY directions but also in the Z direction (**Auto Stage**)
- **Q**uick **R**aman **I**maging (**QRI**) high-speed imaging function enables wide-area imaging measurement
- **S**urface **S**canning **I**mage (**SSI**) for tilted or uneven surface
- Easy analysis of imaging data using multivariate analysis and new **data processing method**

Microscopy Observation Modes

Microscopy Observation Modes - Bright Field

Most common microscopy technique, illuminate straight from the above or below of the objective lens.

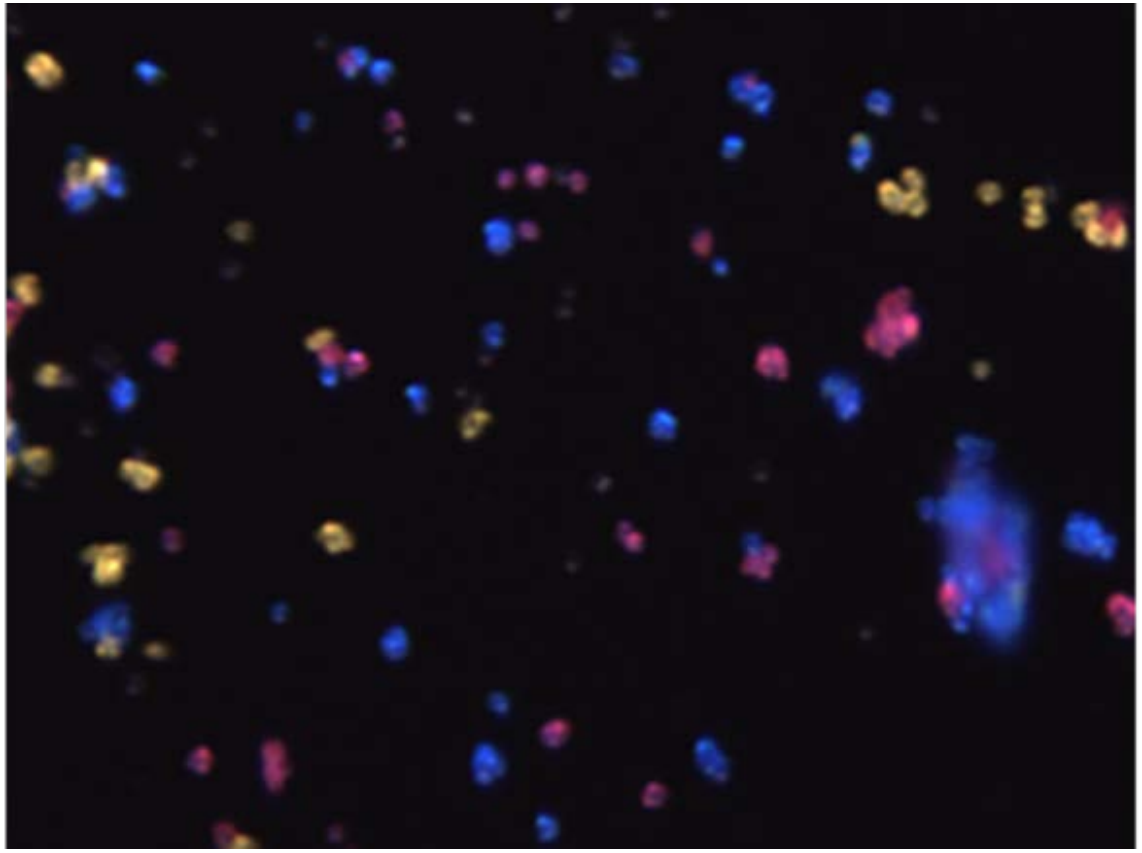
Observe the contrast due to the reflectance and transmittance of the sample.



Bright field image of toner

Microscopy Observation Modes - Dark Field

- It illuminates obliquely so that the illumination light does not enter the objective lens, and observes the scattered light.
- Good color reproducibility and enables observation of minute irregularities (several tens of nm)

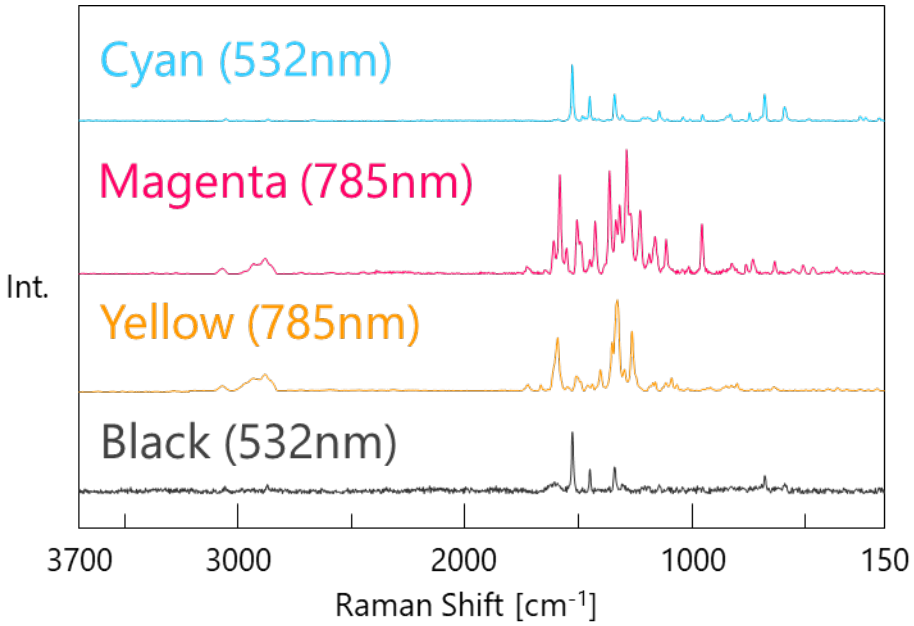


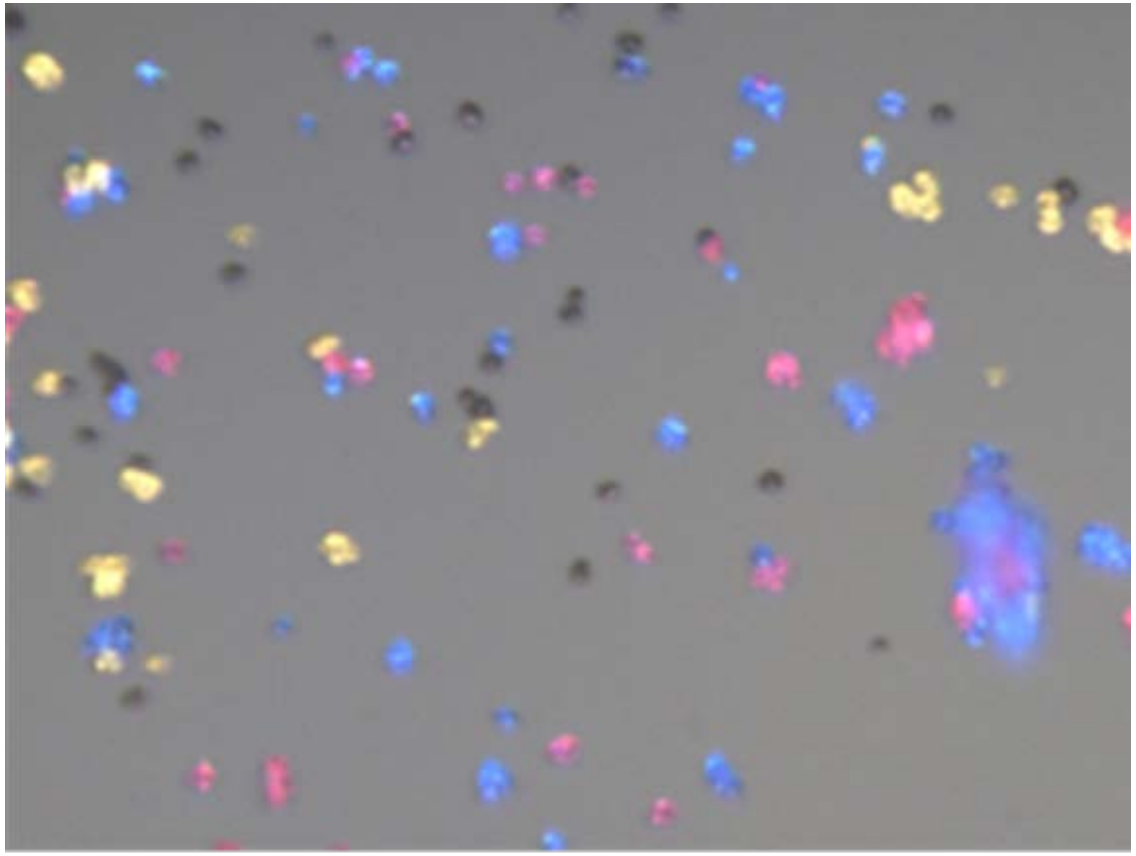
Dark field image of toner

Microscopy Observation Modes - Mix

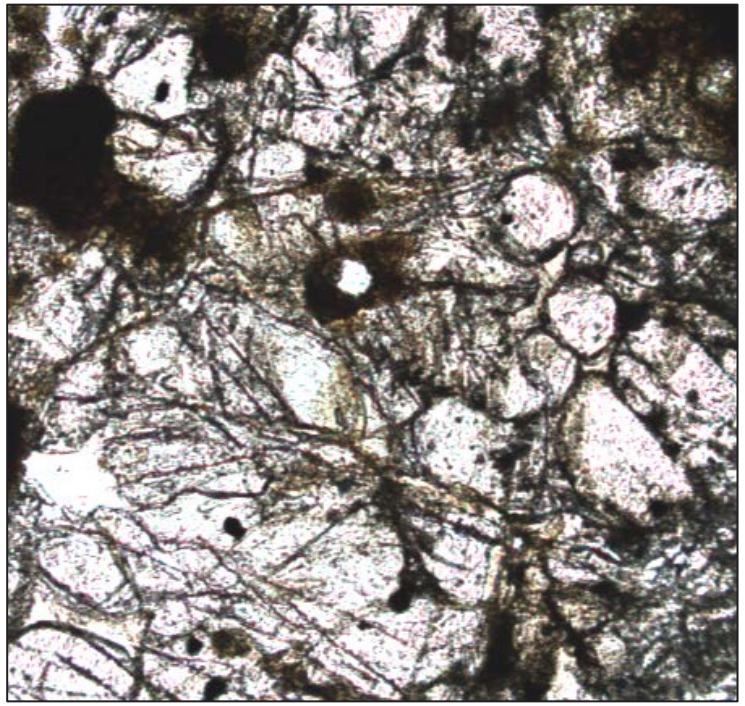
Bright field and dark field can be used at the same time.

Allows observation of dark objects

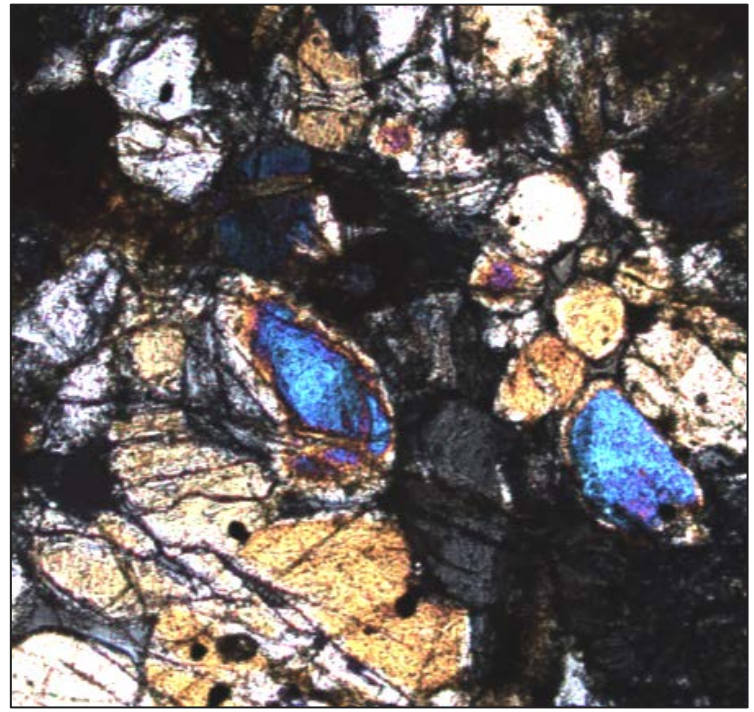




Microscopy Observation Modes - Polarization



Parallel Nicol

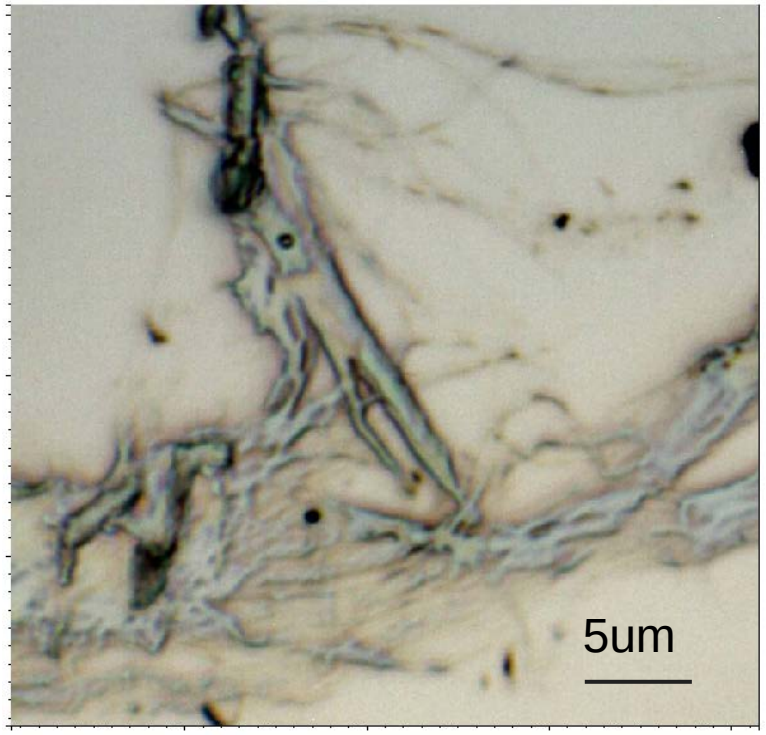


Crossed Nicol

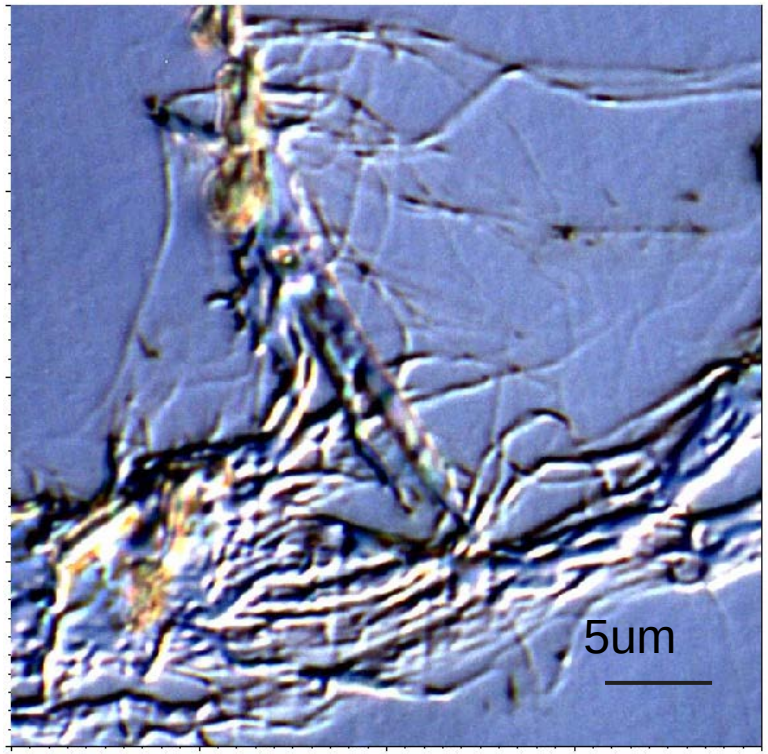
Sliced rock sample

Microscopy Observation Modes - Differential Interference Contrast (DIC)

- When illuminated, the sample image is contrasted and observed using the phase difference that occurs in the sample gradient and steps.
- Allows observation of minute irregularities (several nm)



Bright field



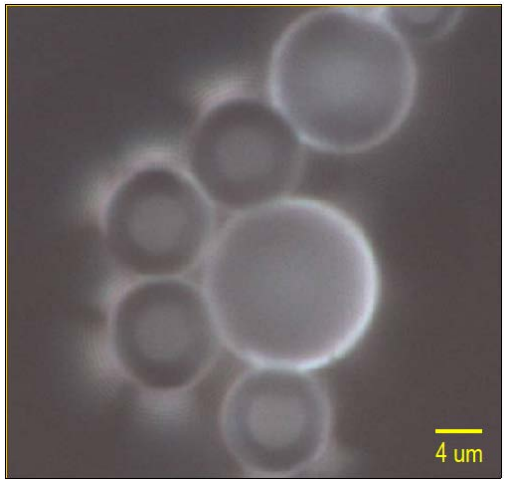
DIC

Carbon nano tube

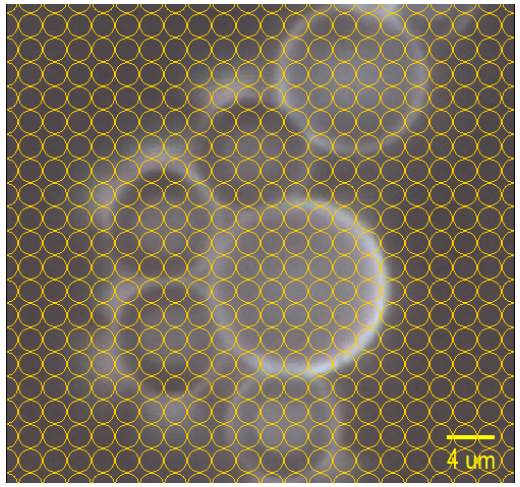
Spectroscopic Imaging

Visualization of molecular distribution using key spectral bands

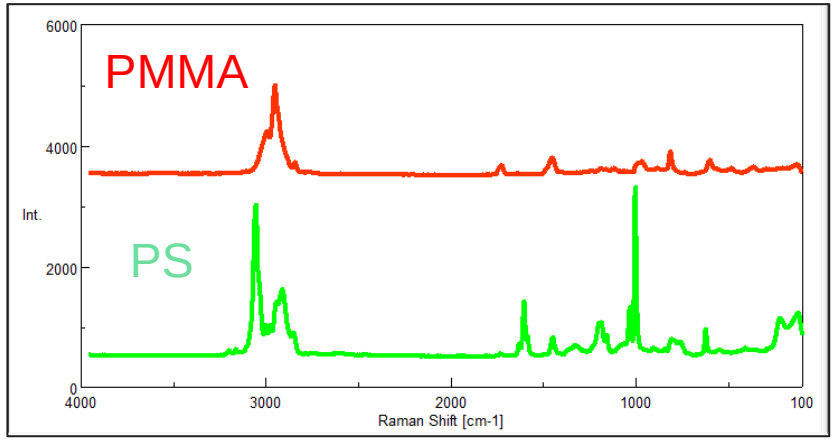
Useful for visualizing of detailed chemical composition in a structure



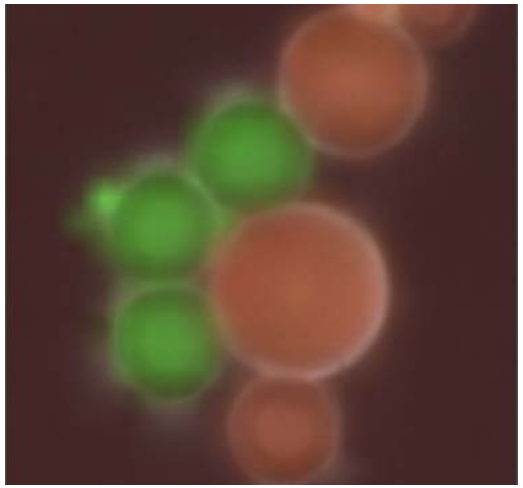
Optical image
(bright field)



Array is set up
Raman spectrum
is collected at
each circle



Raman spectra are measured

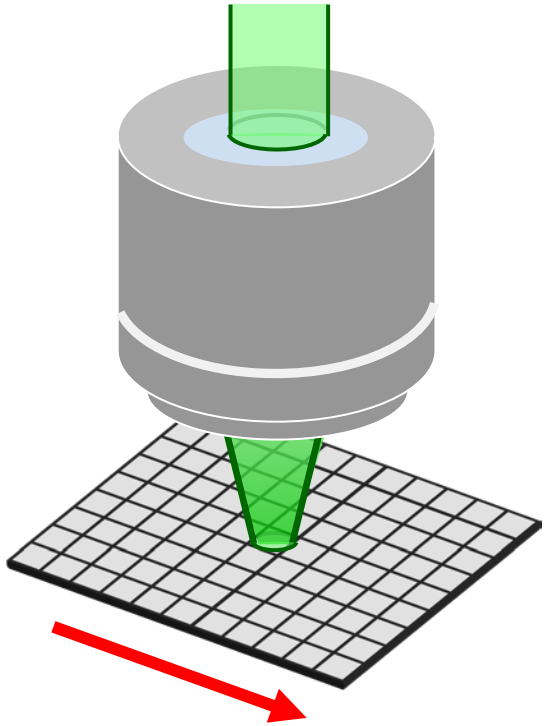


Raman image is
created based on
specific vibration
bands

QRI (Quick Raman Imaging)

FOR HIGH SPEED MAPPING AND IMAGING

High Speed Raman Imaging



Stage moving type

QRI : Quick Raman Imaging

Models: **NRS-4500**
NRS-5500/5600
NRS-7500/7600

QRI System Configuration

Auto Imaging system

Laser Raman spectroscopy
NRS-4000/5000/7000

\+

High speed stage
for NRS-5000/7000
NRS-4500

\+

QRI option

1. HIGH SPEED IMAGING PROGRAM
2. DIGITAL FILTER
3. SPECTRUM AVERAGING

\+

Detector option

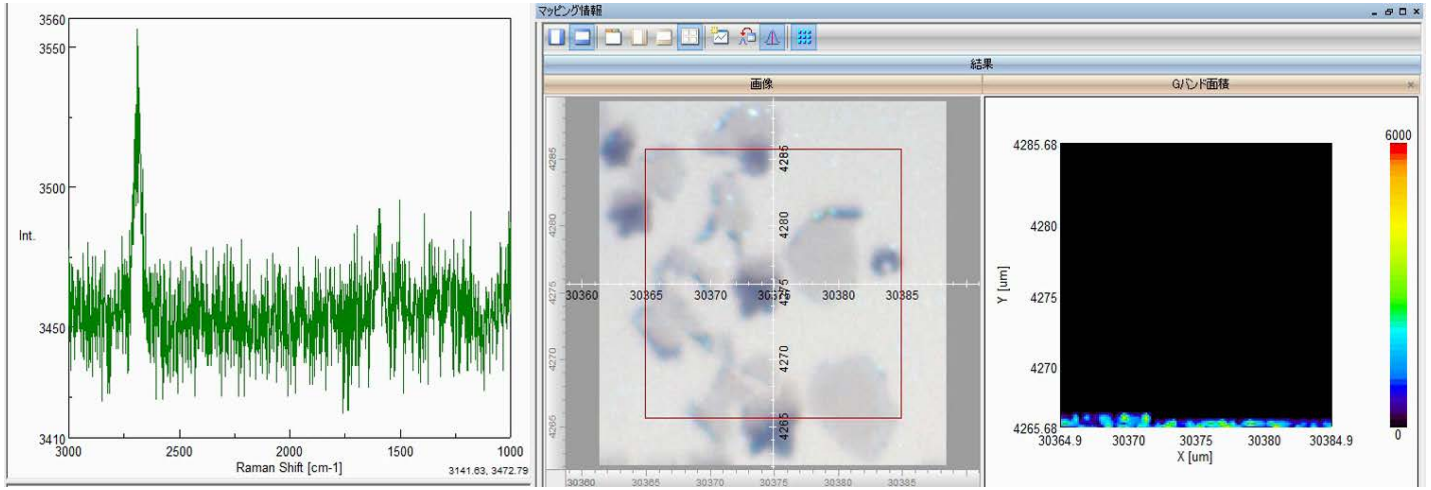
EMCCD **exposure < 50ms**

Imaging Speed Comparison

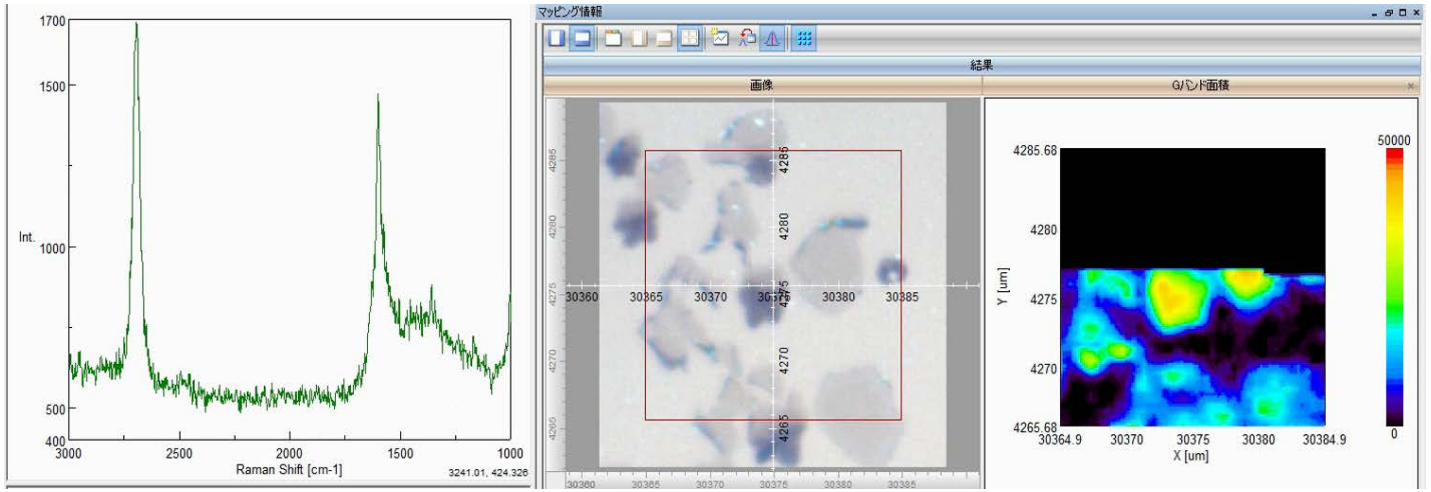
Conventional Imaging vs QRI , Sample: Graphene

Using the same measurement conditions, the imaging speed of QRI is x 50 faster than conventional mapping

Conventional imaging

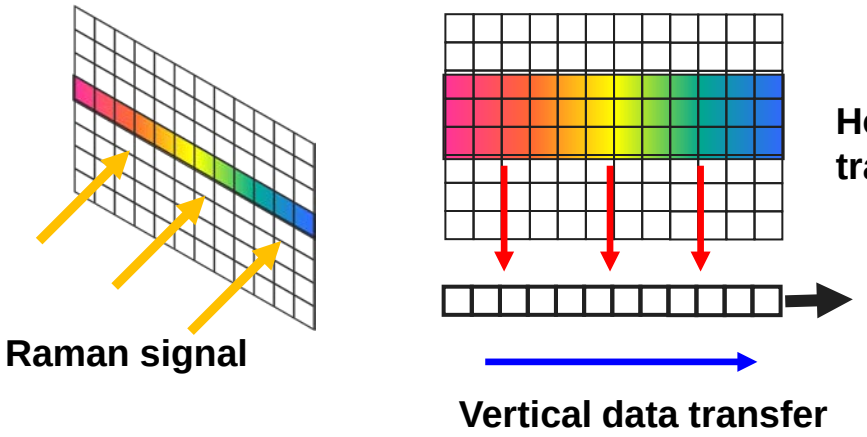


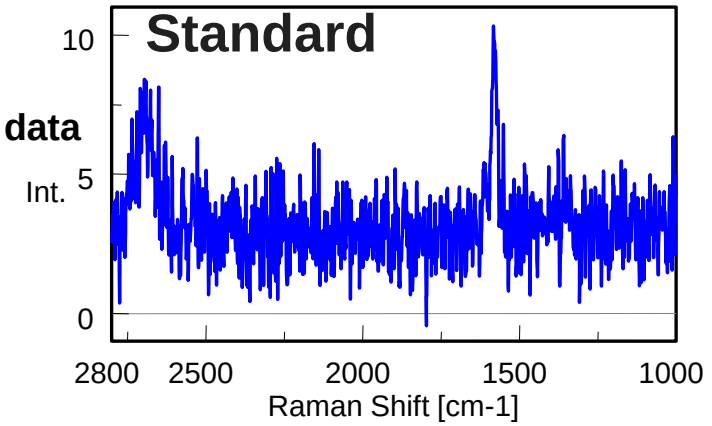
QRI



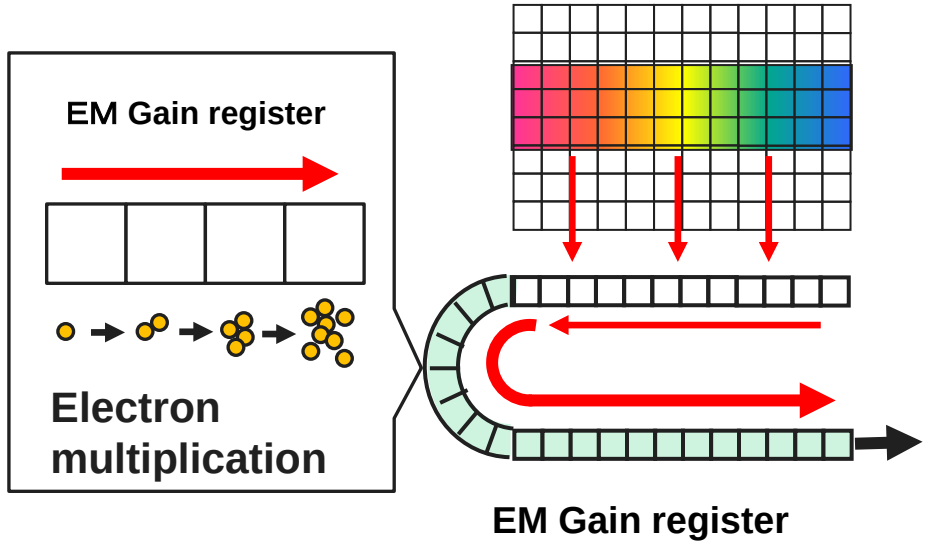
EMCCD Detector Modes

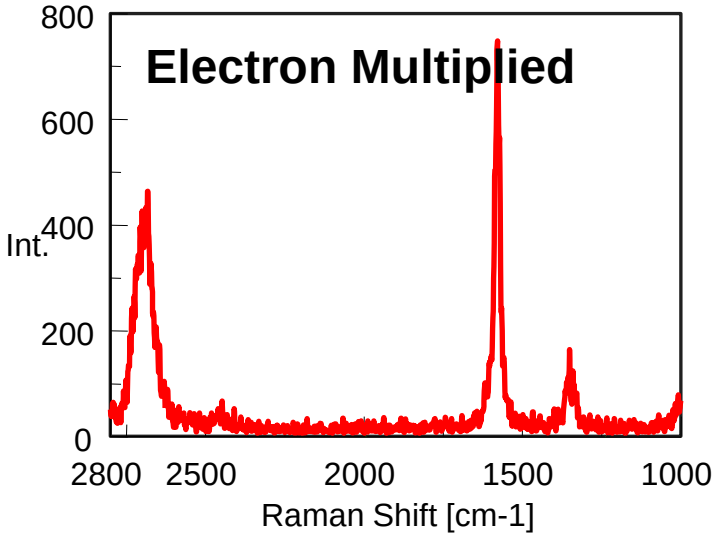
CCD mode



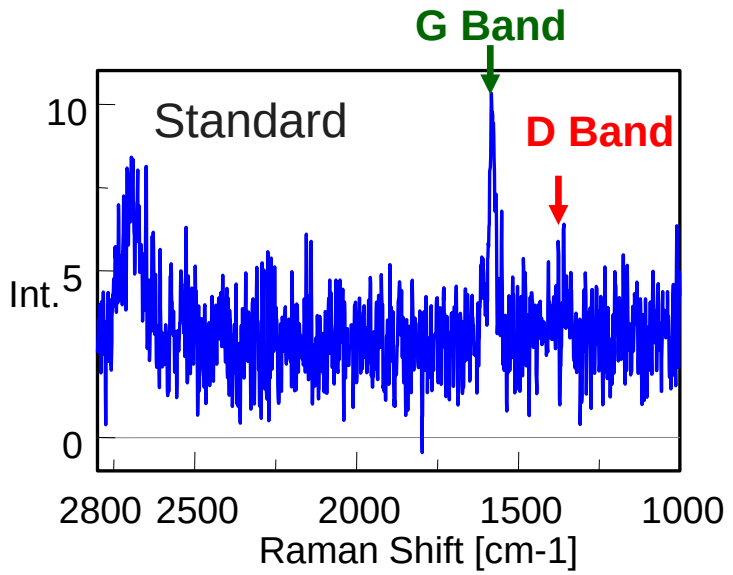


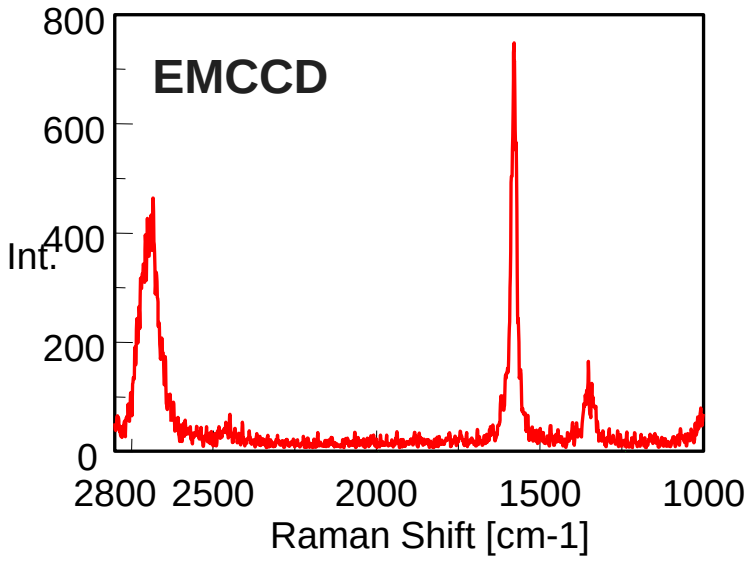
EMCCD mode

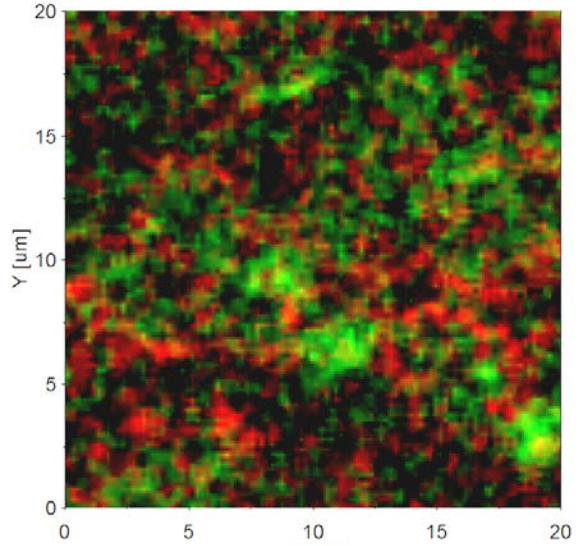


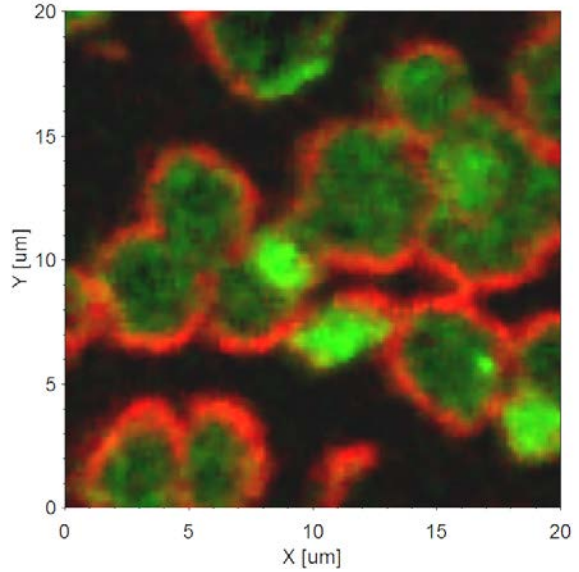


Imaging Detectors - Standard CCD vs EMCCD







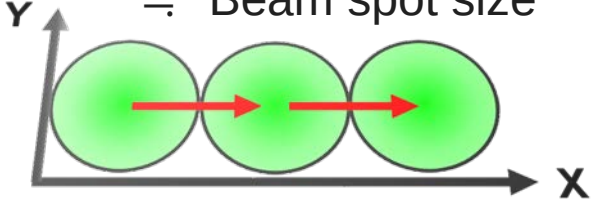


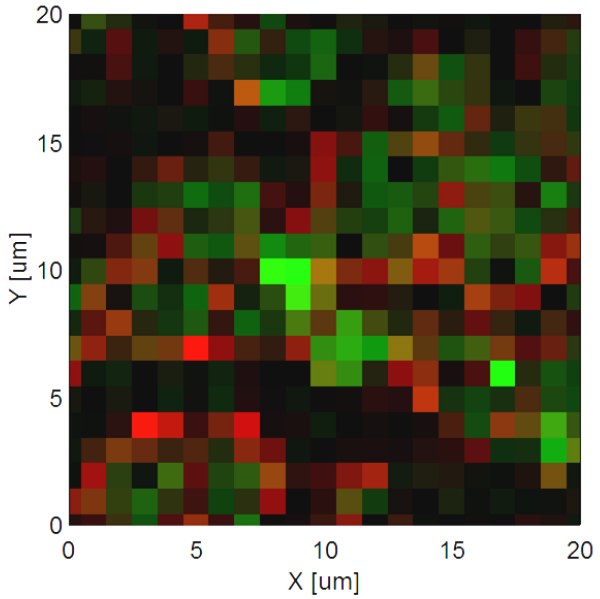
Red: D Band

Green: G Band

High-definition, High-speed Imaging

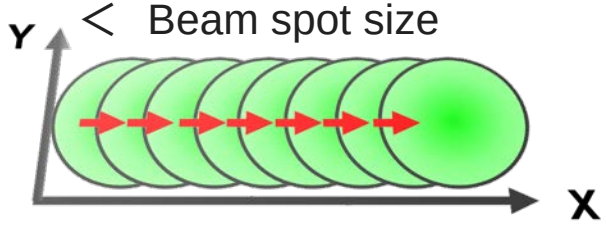
Measurement interval
≒ Beam spot size

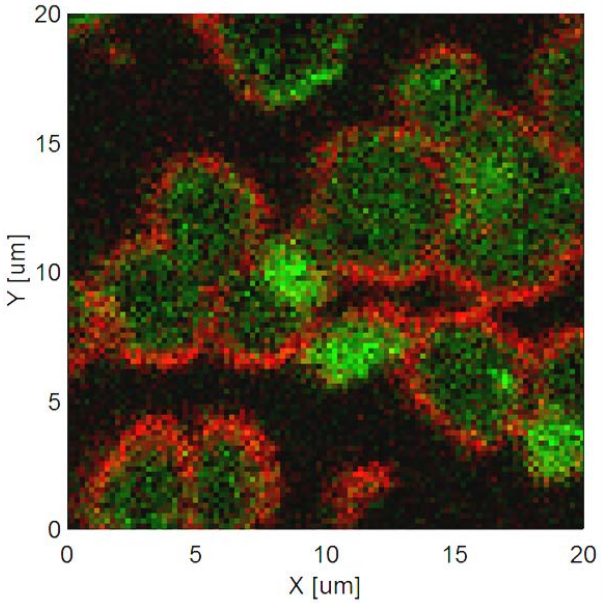




Previous Image
1um step

Measurement interval
< Beam spot size





High definition Image
0.1um step

Further improvement

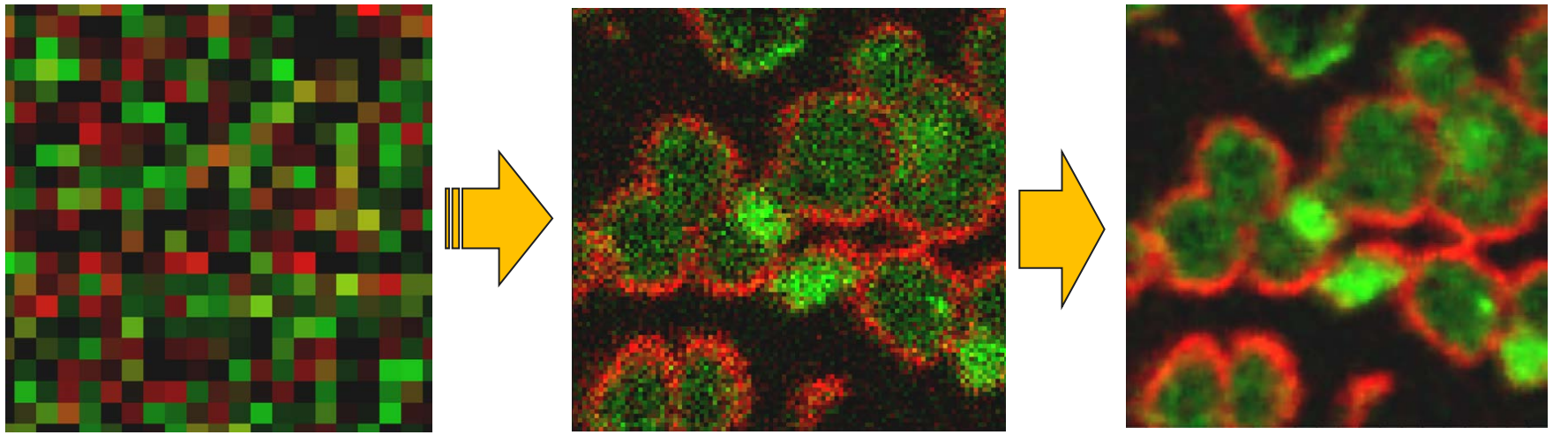
Digital Filter

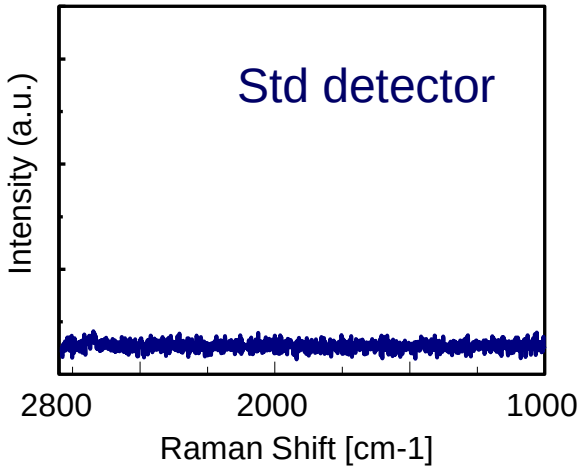
High Speed Imaging with QRI

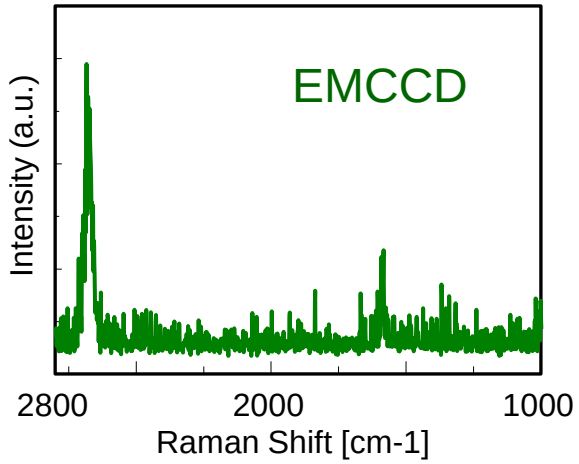
Previous

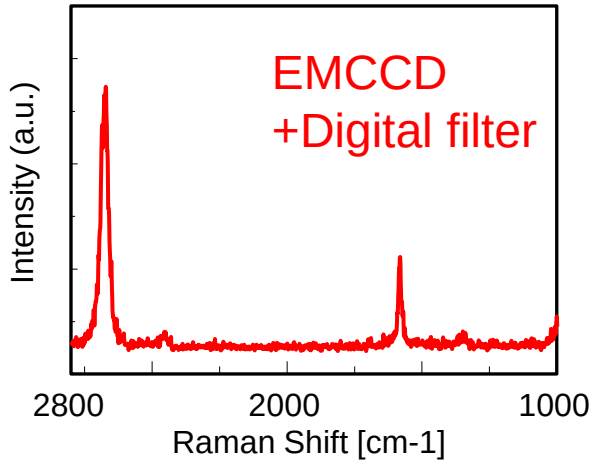
QRI+EMCCD

QRI+EMCCD+Filter

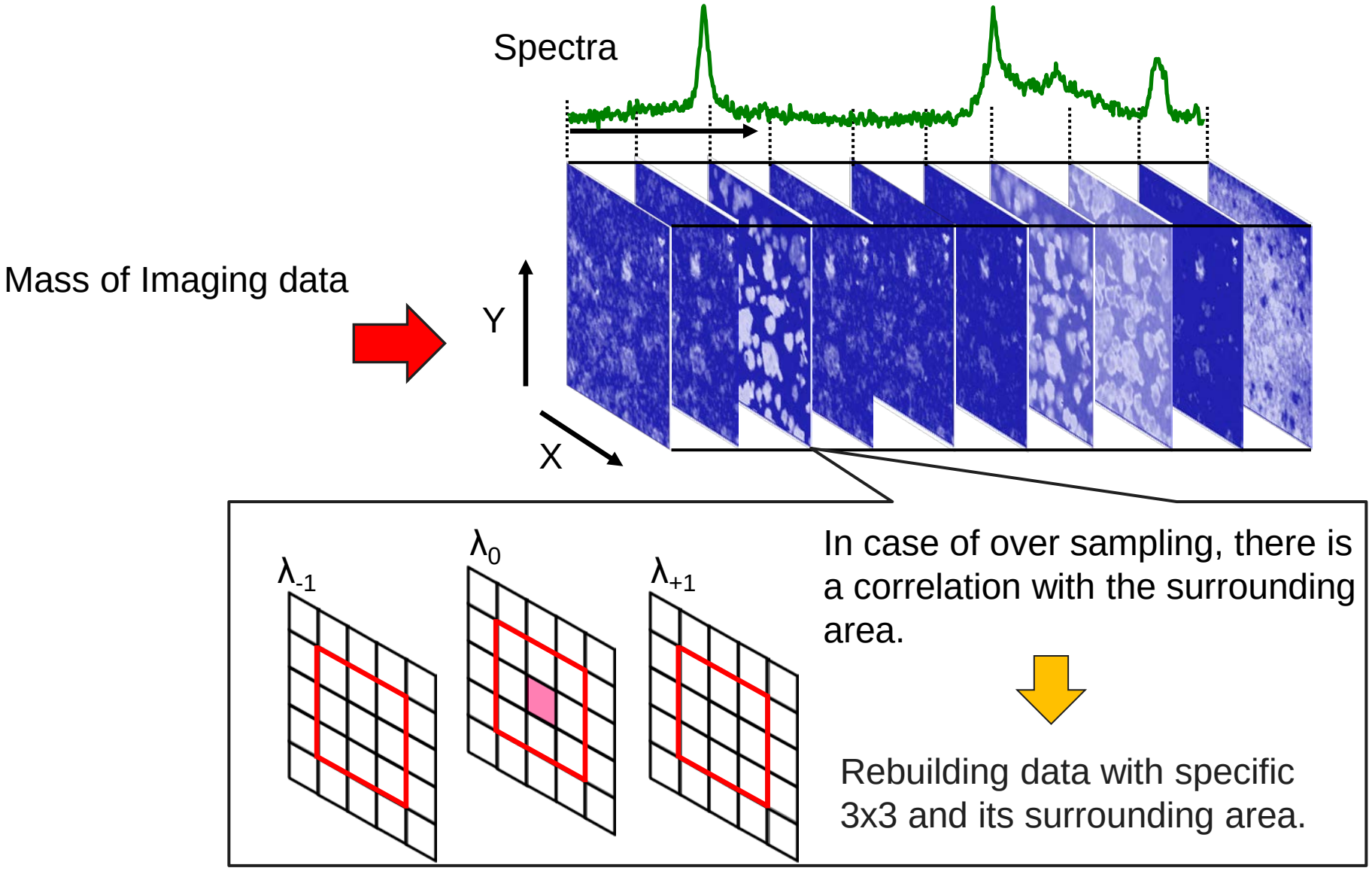




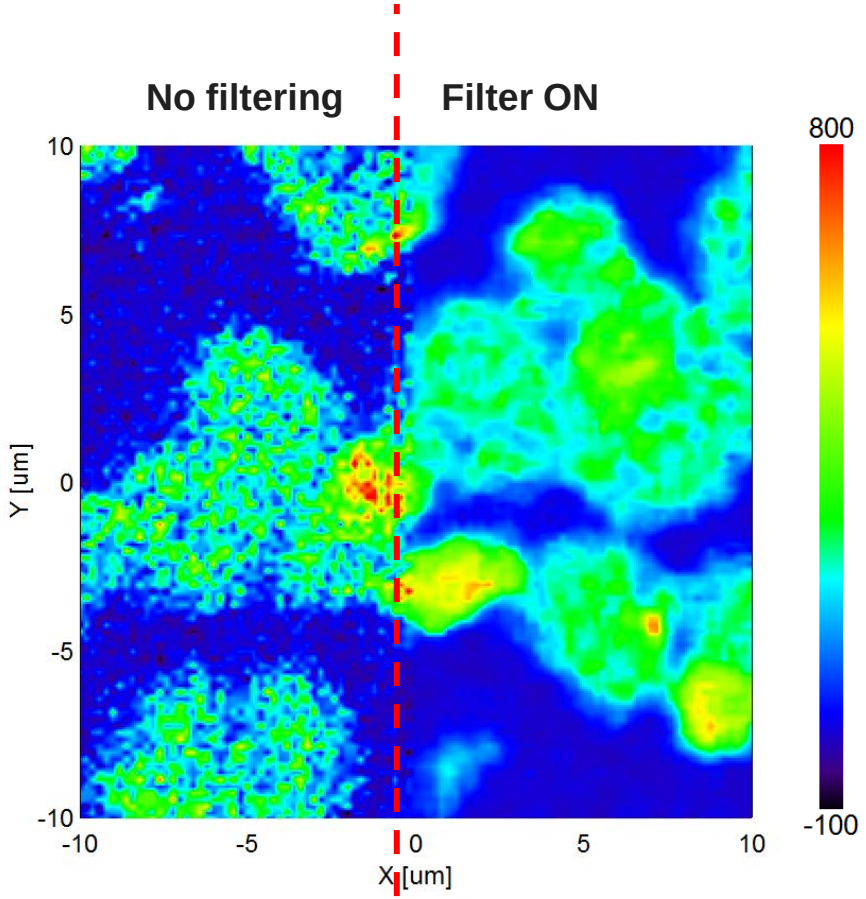


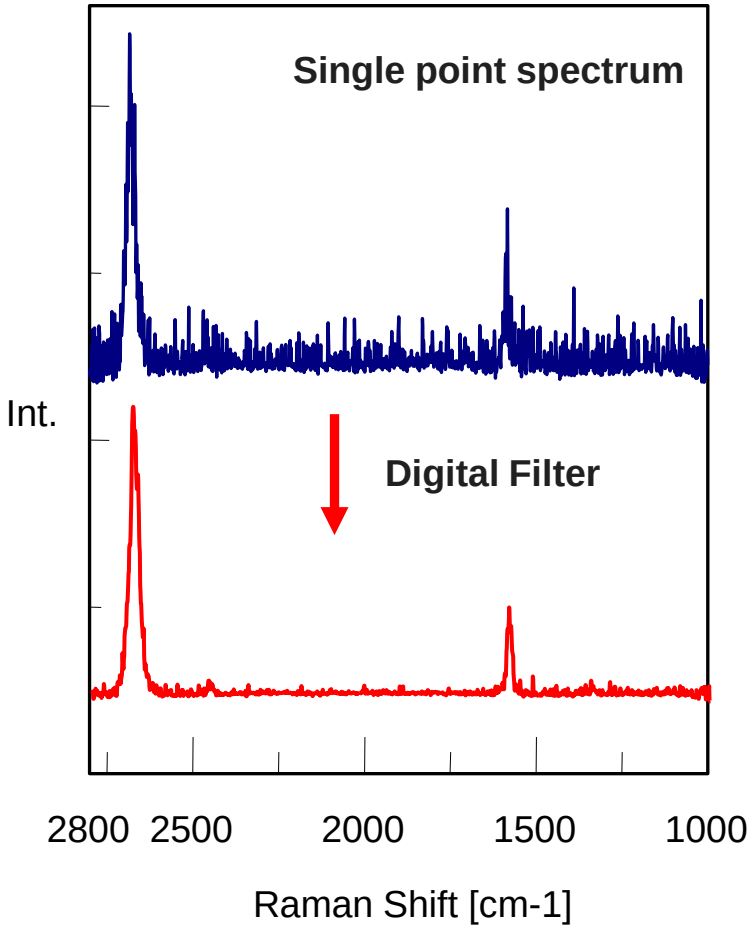


Digital Filtration



Improvement with Digital Filter

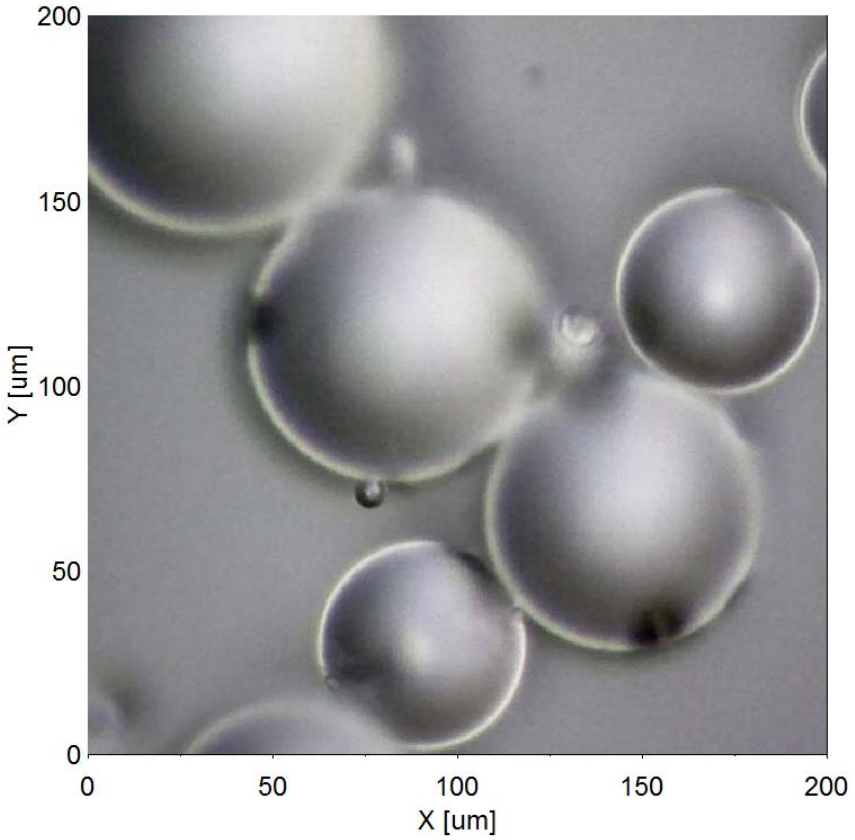




Measurement: 1ms exposure /point

Spectrum Averaging (1)

PMMA : poly methyl-methacrylate



Step 1: Objective view

NRS-4500
- 532nm laser 20mW
- 900gr/mm
- BS
- Objective x20
- Standard detector iVac DR 324

Area : 200um x 200um

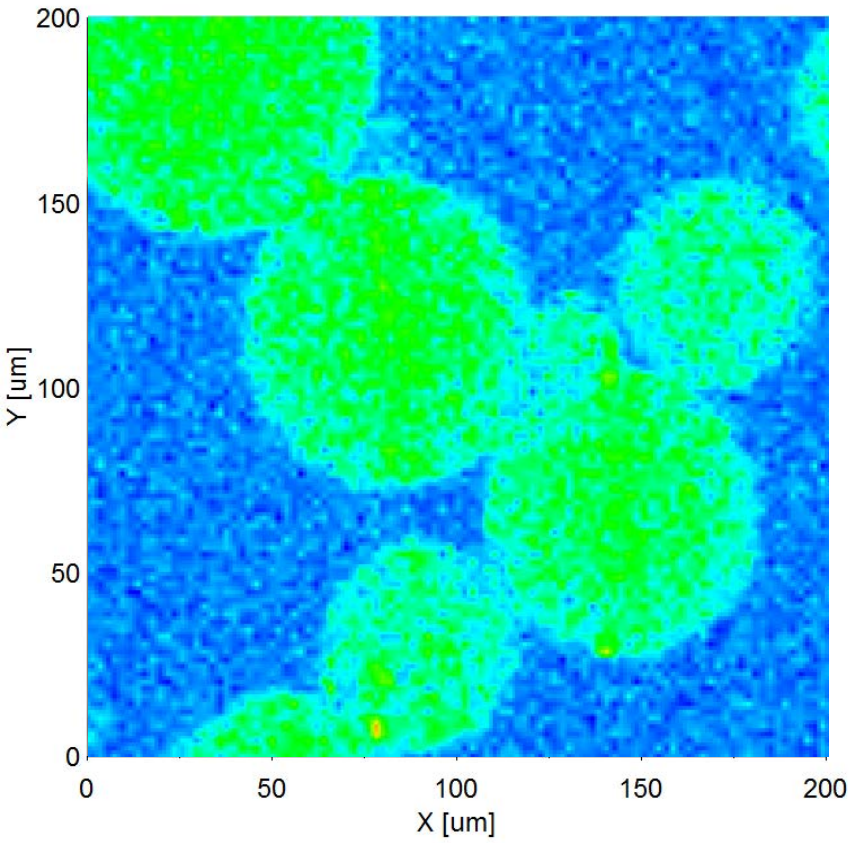
Imaging step : 2um

Exposure : 1ms

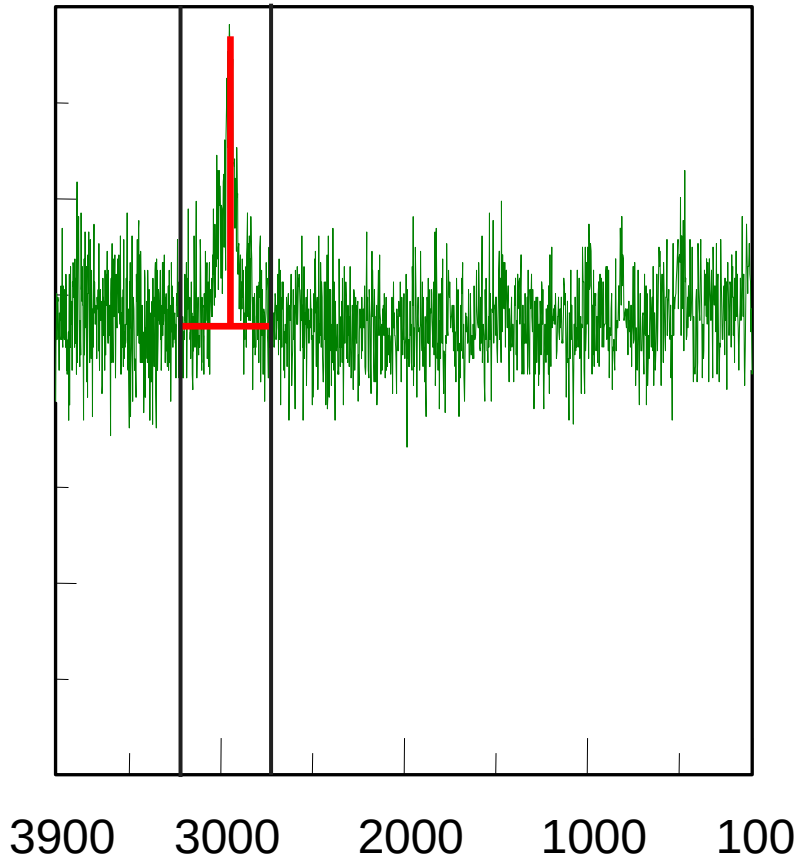
Measurement of PMMA micro spheres without using EMCCD.

Spectrum Averaging (2)

PMMA



Peak height

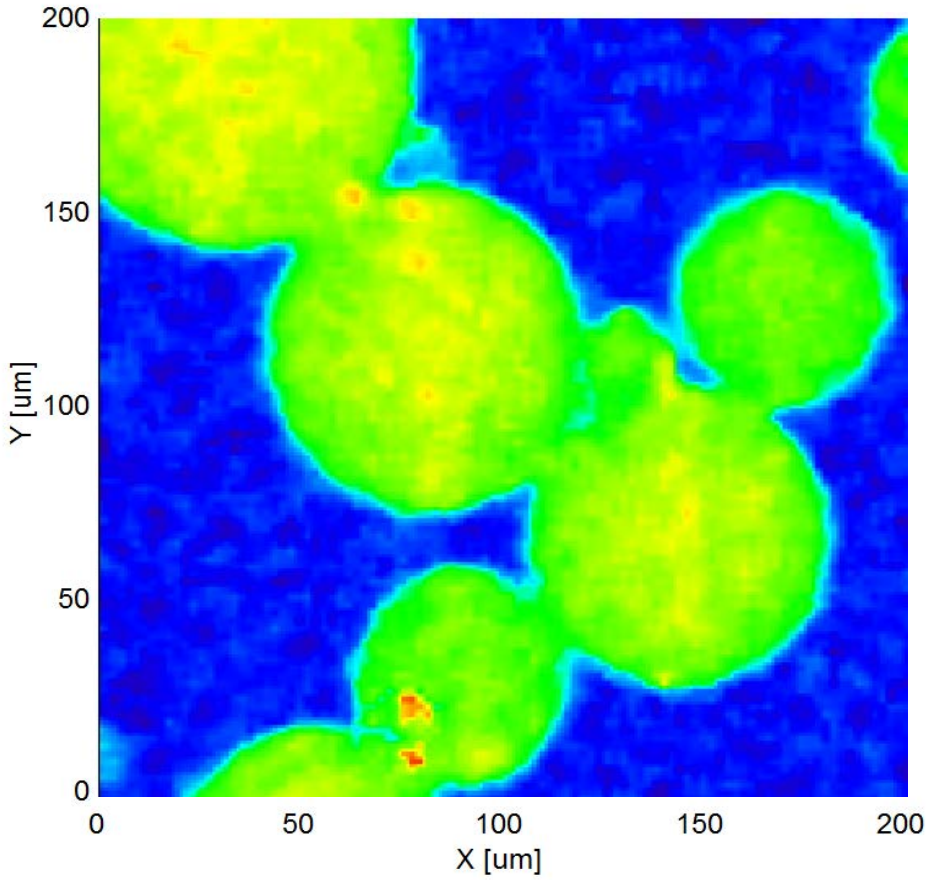


Step 2: Imaging view

As exposure time is quite short - 1ms, the first spectrum is very noisy and only shows the CH bond at 3000cm-1.

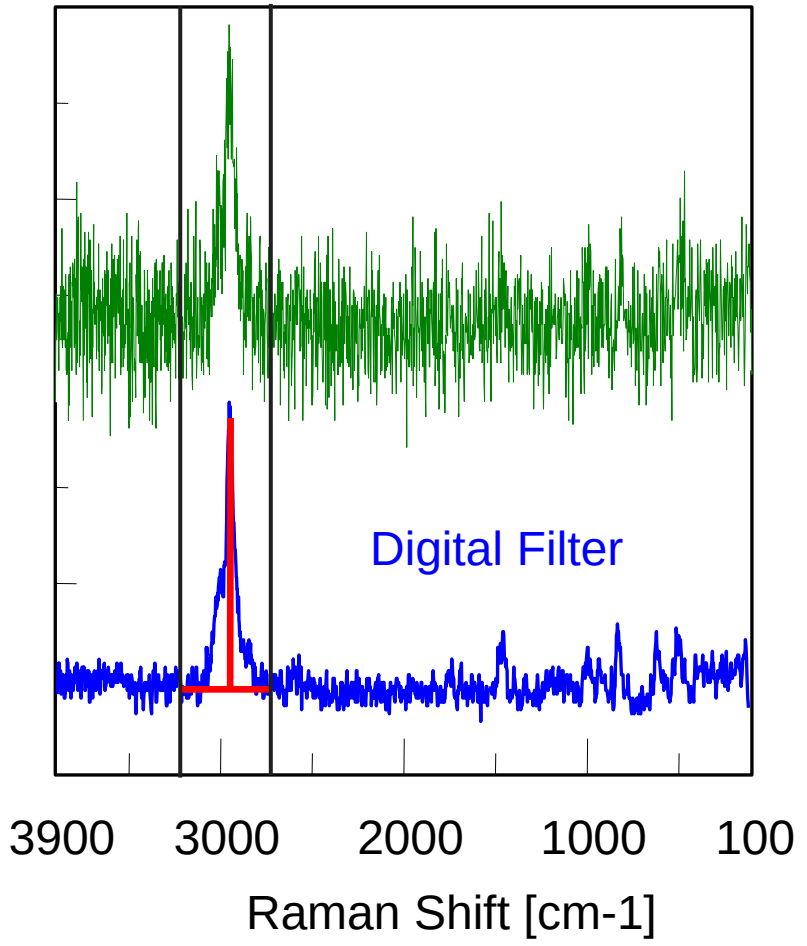
Spectrum Averaging (3)

PMMA



Step 3: Digital filtration

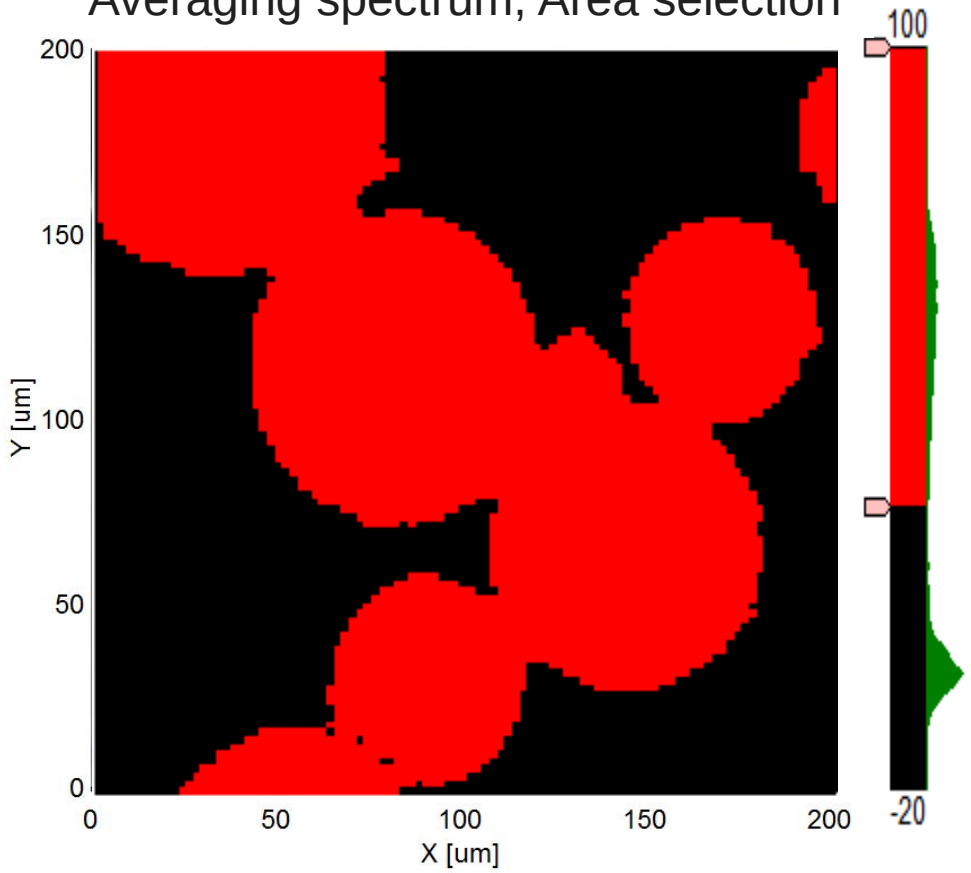
Peak height



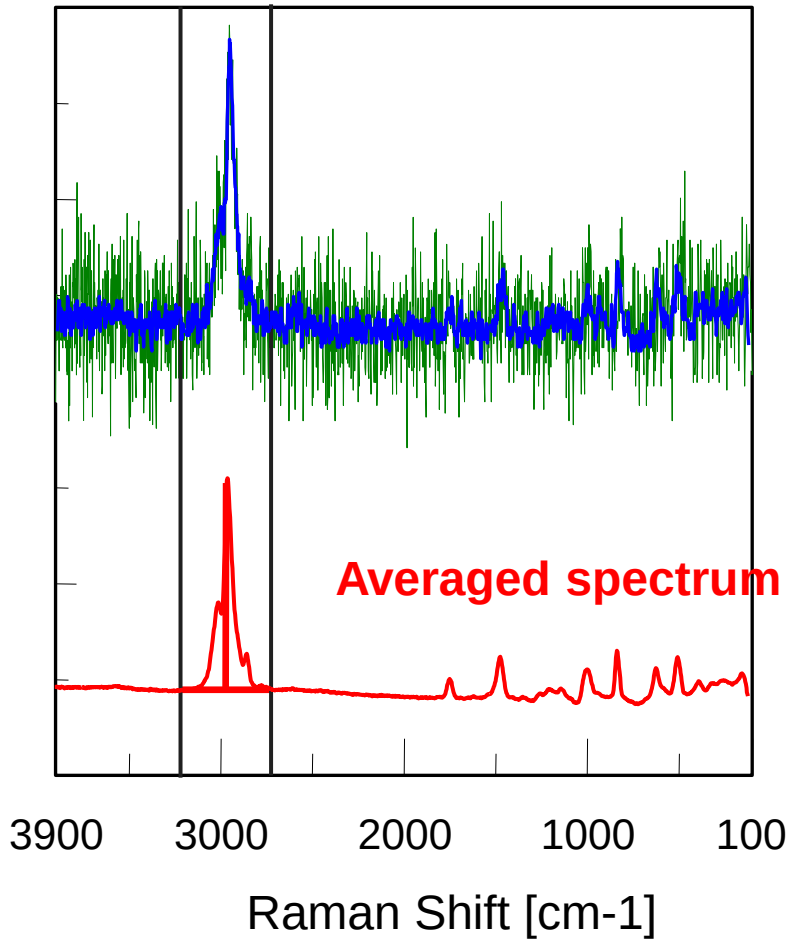
Digital filtration provides a better peak image.

Spectrum Averaging (4)

Averaging spectrum, Area selection



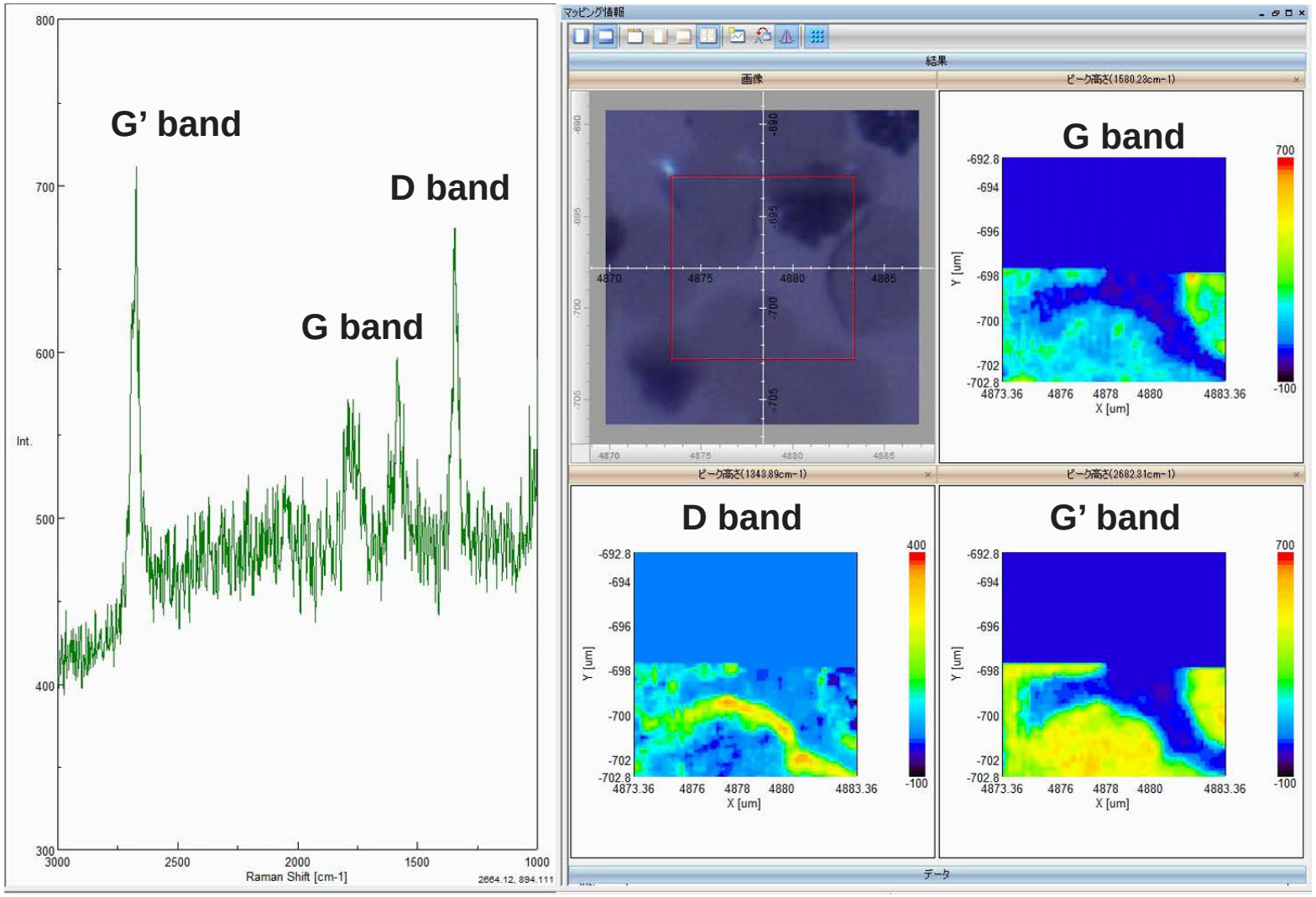
Peak height



Step 4: Averaged spectrum

We selected the peak region of CH bond, execute Averaging Spectrum function.
This improved the S/N of the spectrum. QRI has incorporates this function.

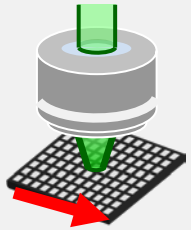
QRI High Speed Imaging Example

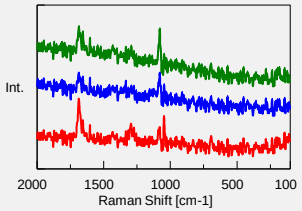


Flow Chart from Imaging Process to Data Analysis

Measurement

QRI

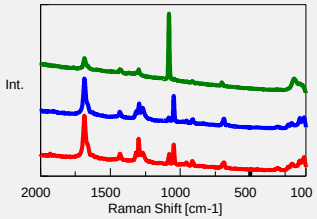


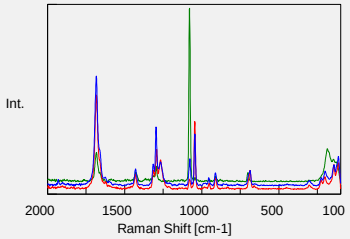


Data Processing

Digital filtration
(when over sampling)

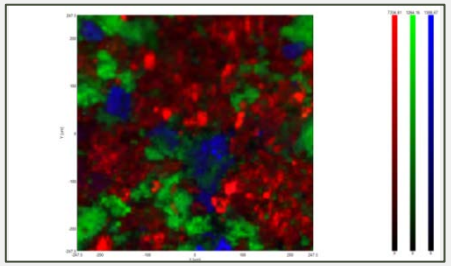
PCA noise removal
(when many spectra)





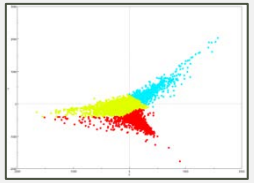
Bulk
fluorescence
correction

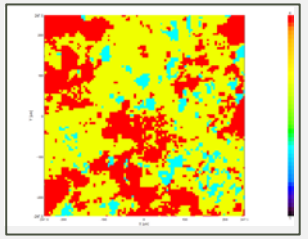
Color-coded diagram



Multivariate Curve Resolution (MCR)
Qualitative / Semi-quantitative

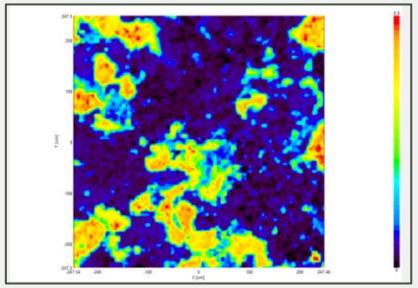
Principle Component Analysis





Grouping

Spectral
correlation

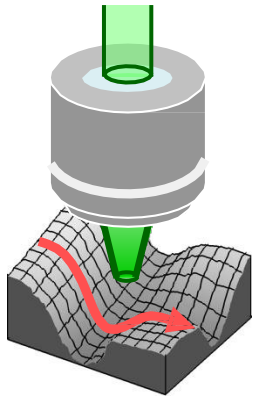


Color-coded by correlation coefficient

Surface Scanning Image (SSI)

TILTED AND UNEVEN SURFACES

Surface Scan Imaging - SSI

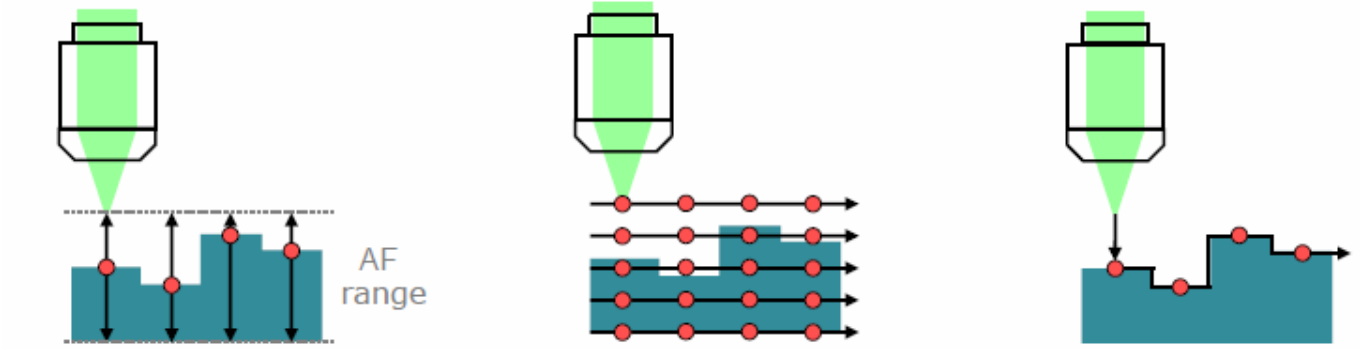


NRS-4500/5500/7500

- Scan the stage to XYZ based on All-in-focus image.
- Provide solutions for rough/inclined surface samples

Uneven (Rough) surface sample measurement

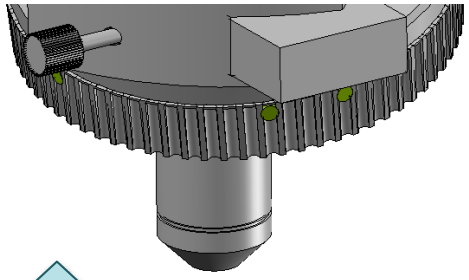
①AF at each point ②3D imaging ③ SSI

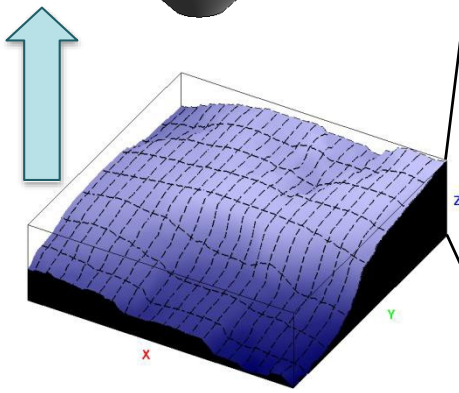


All-in-focus Image

Image shooting

▶Take pictures continuously moving Z stage



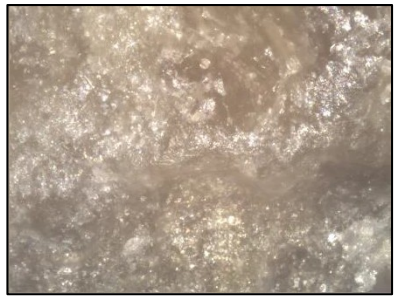


Composition

Depth composition

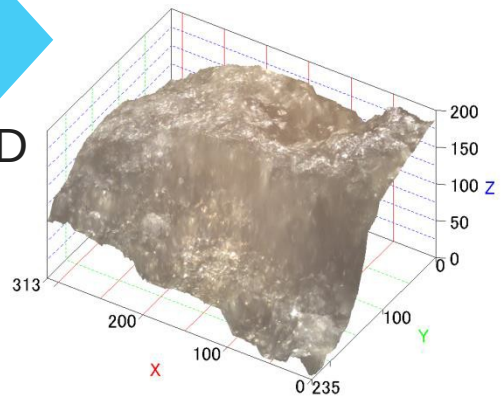
▶Compose All-in-focus image, focused on each points.

2 D



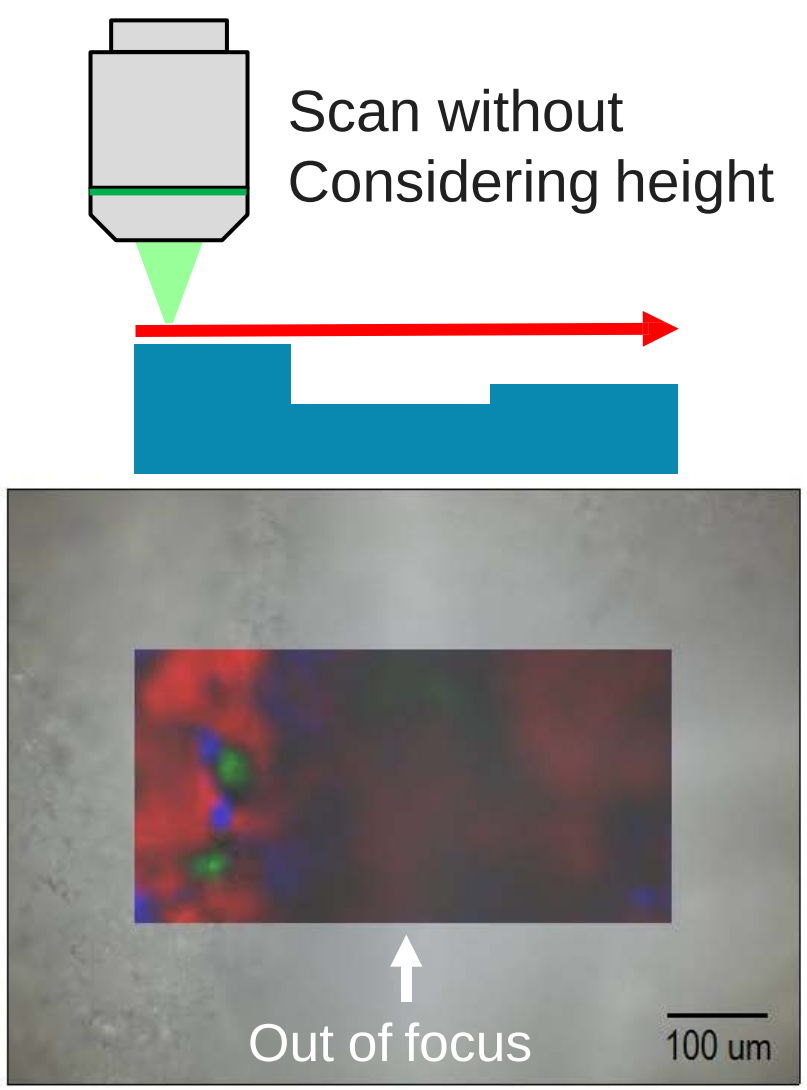
All-in-focus image

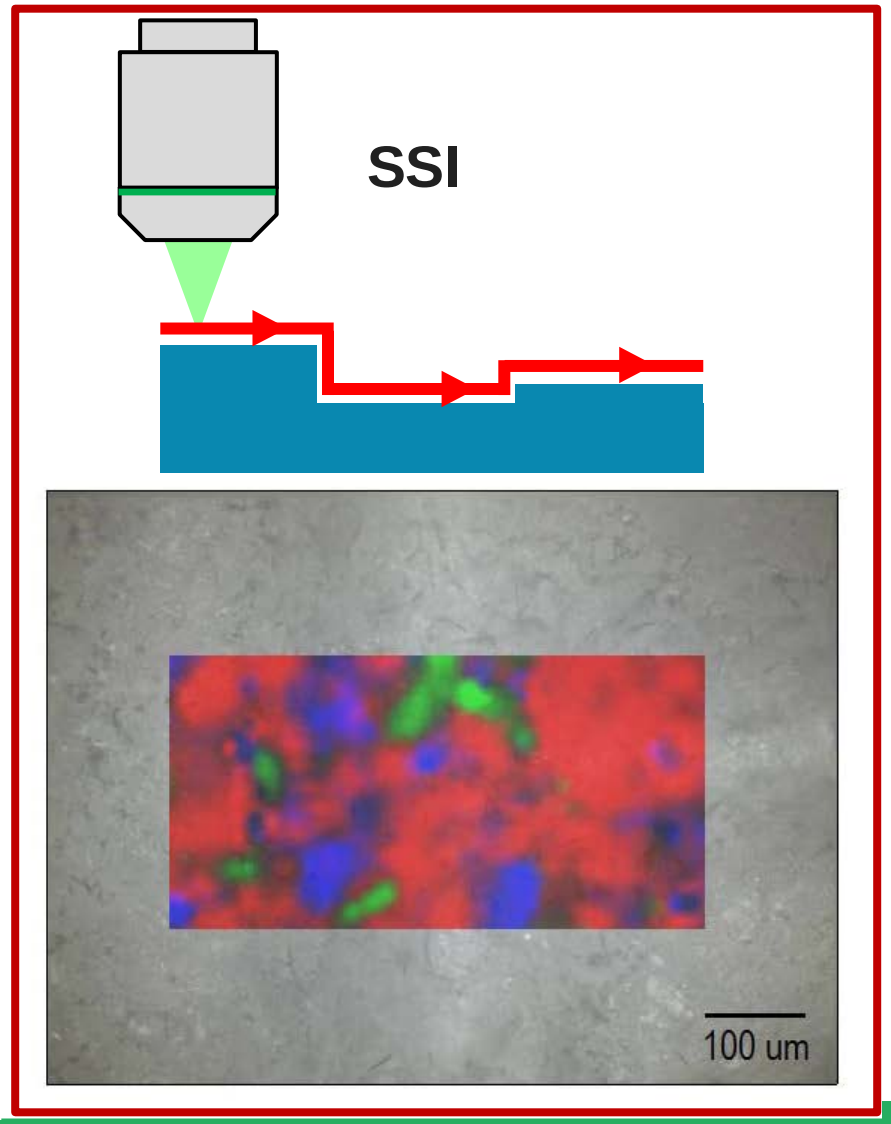
3 D



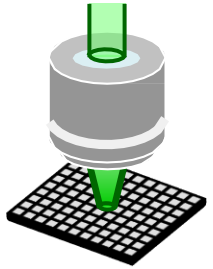
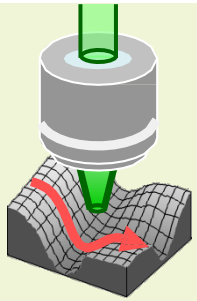
Surface shape information

Comparison of Scanning Methods





Comparison between Surface Scanning Modes

Method	Advantage
 **Stage scan: QRI** Stage moves	◎Measurement range is wide :μm ~ mm ◎High speed measurement
 **Surface scan: SSI** All-in-focus image.	◎Effective when roughness of surface is bigger than spatial resolution for Z direction

JASCO Imaging Features

IQ RAMAN NAV

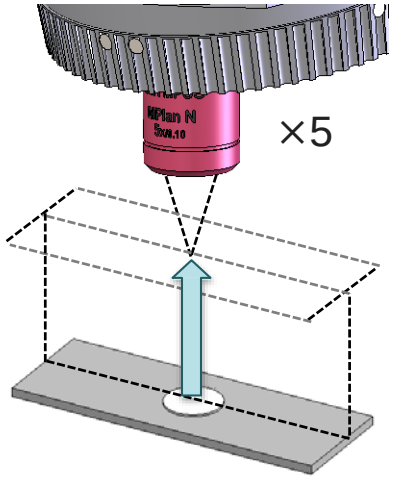
Focus NAV – Autofocus

Once set sample on stage, software can adjust focus and light intensity automatically.

It is possible to focus from maximum 30 mm.

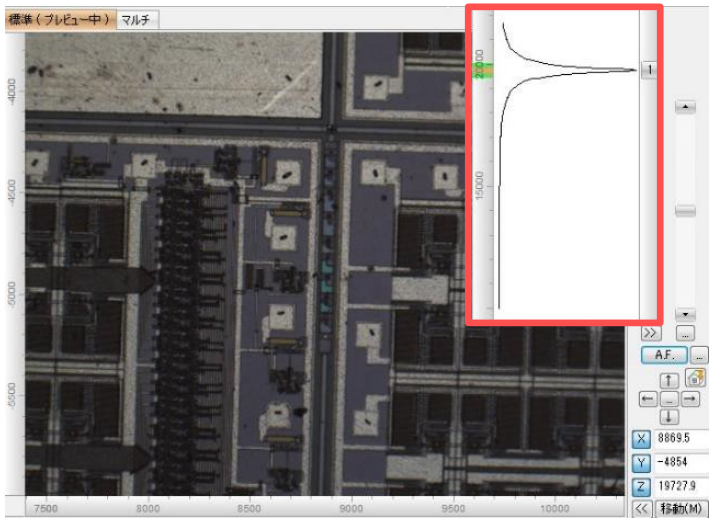
Set sample

Focus NAV



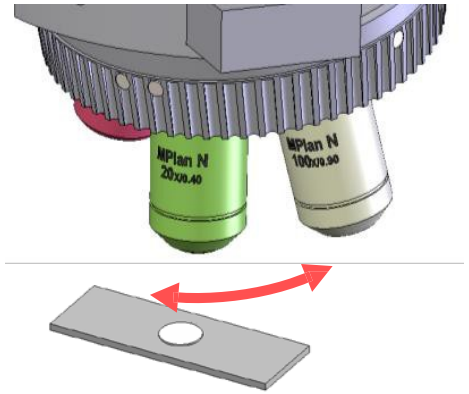
Max. 30 mm

AF signal

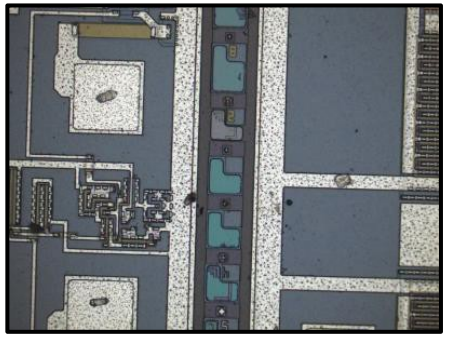


View NAV – Auto Light Adjustment

When switching objective lens, setting parameter of camera can be optimized automatically.

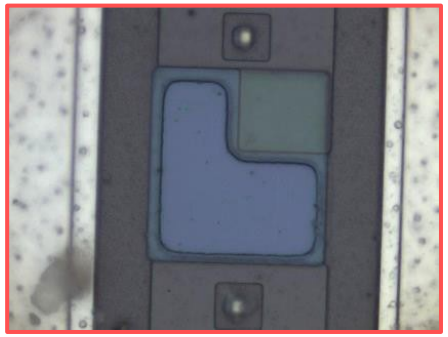


20



100

Automated optimization

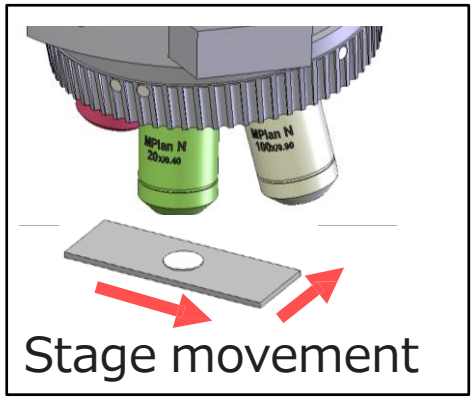


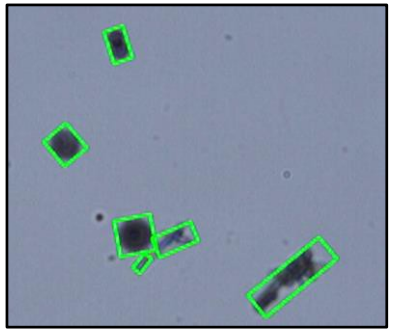
Search NAV – Sample Search Function

Upgrade the “Sample Search” Function.

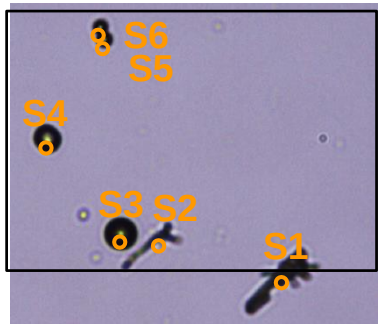
- Real time recognition of measurement candidate points, and registration of the measurement points by one click.
  - Upgrade the detection algorithm
    Search refinement is available (size, shape and contrast) 1 point measurement or imaging measurement

- Perform the measurement and qualitative analysis simultaneously, and the result of qualitative analysis is displayed.

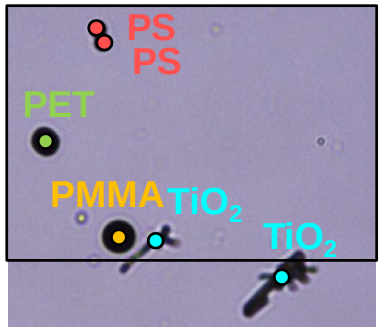




Real time recognition



Automated registration with shape information of sample.

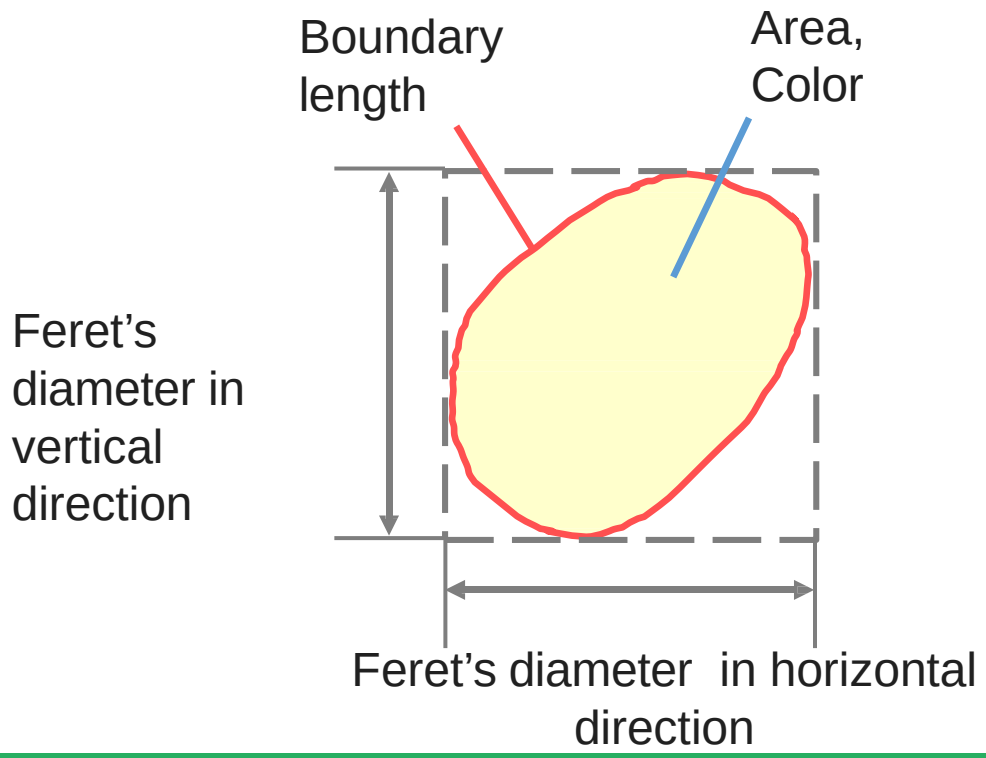


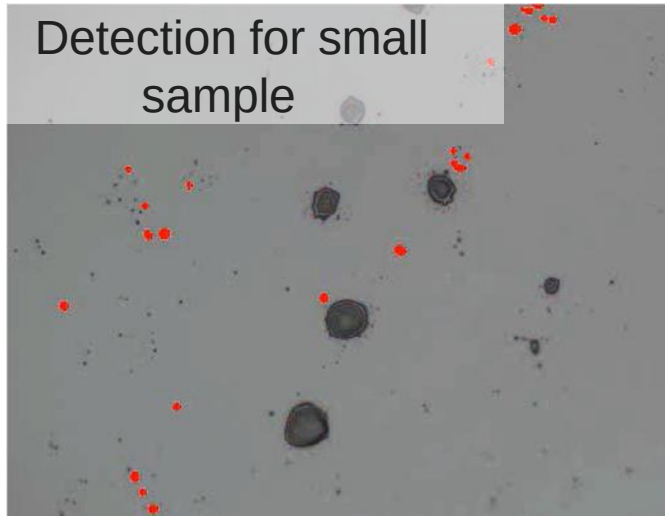
Measurement and a qualitative analysis are performed simultaneously.

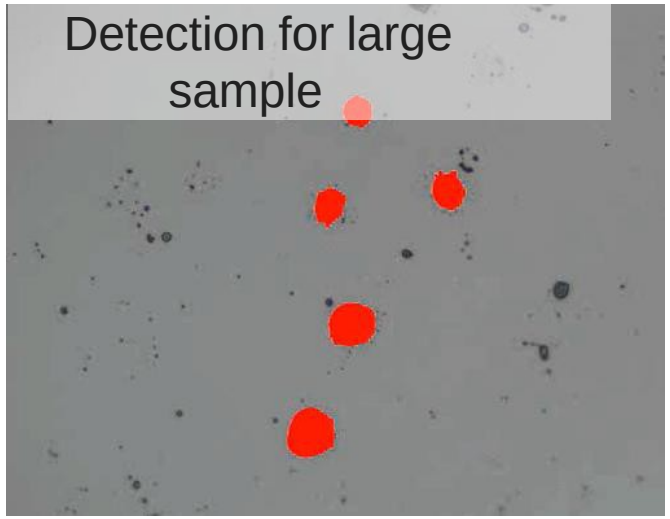
Search NAV – Particle Parameters

Search refinement

- Size (area, Feret's diameter)
- Circularity
- Color (R, G, B, brightness)







IQ Frame – Location of Measurement

FTIR Microscope

Sample Holder
Relative coordinates

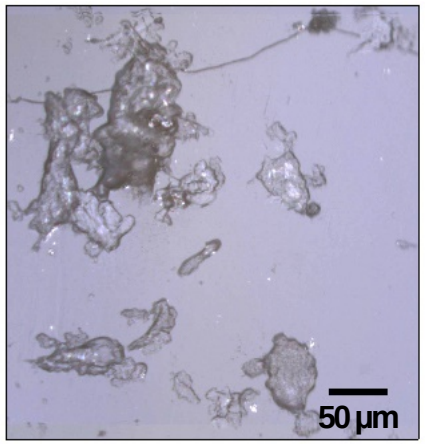
Measure the same location within the same or different instrument

Possibility to remeasure same sample at same location (micrometer) different time

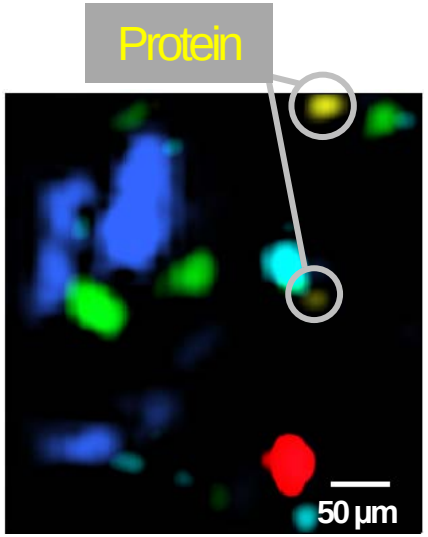
Raman

IR and Raman Combined Analysis

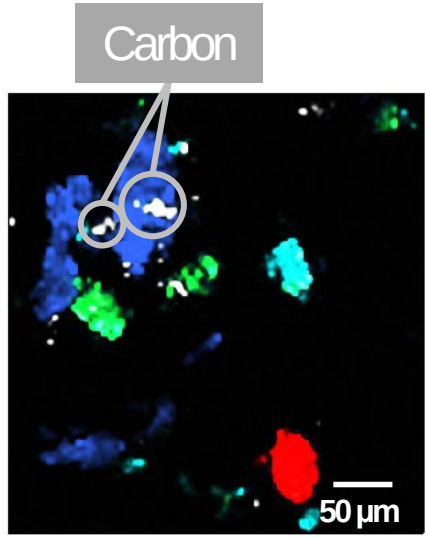
Plastic particles



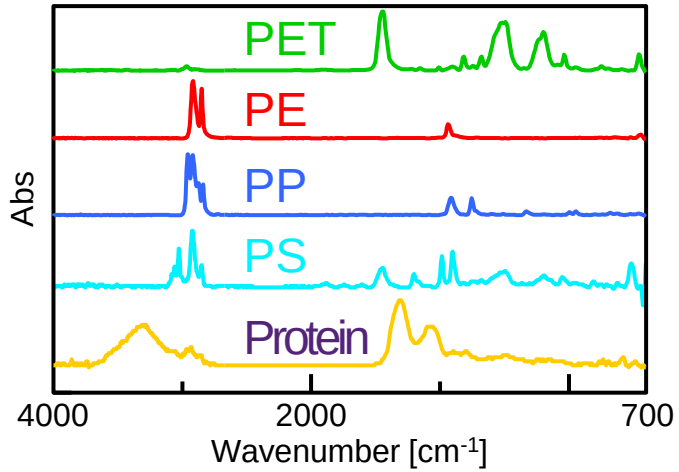
Observation image

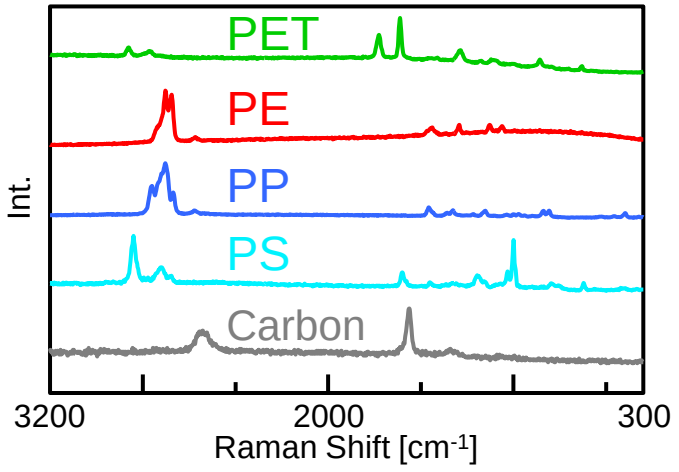


IR Chemical image



Raman Chemical image

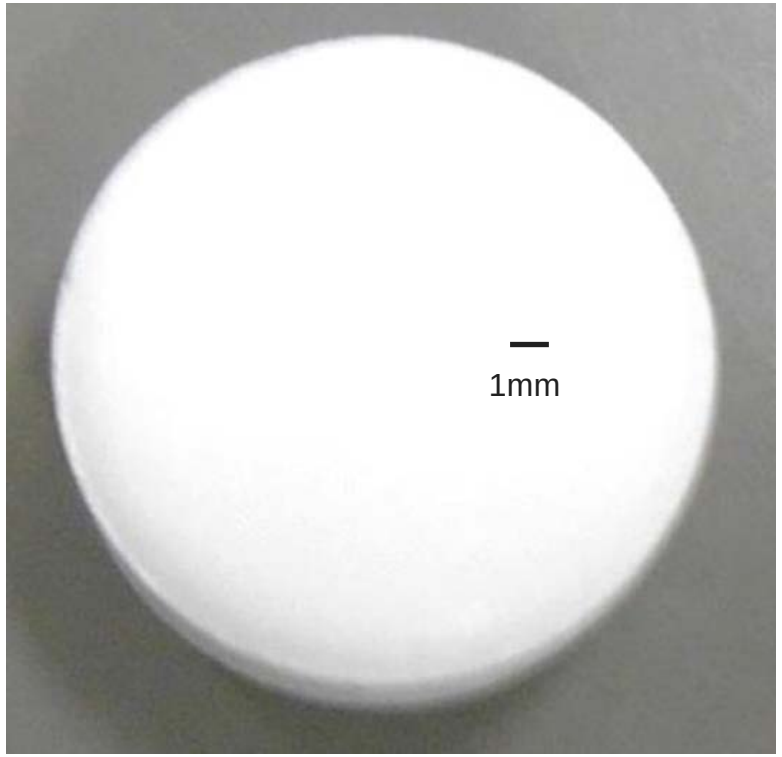




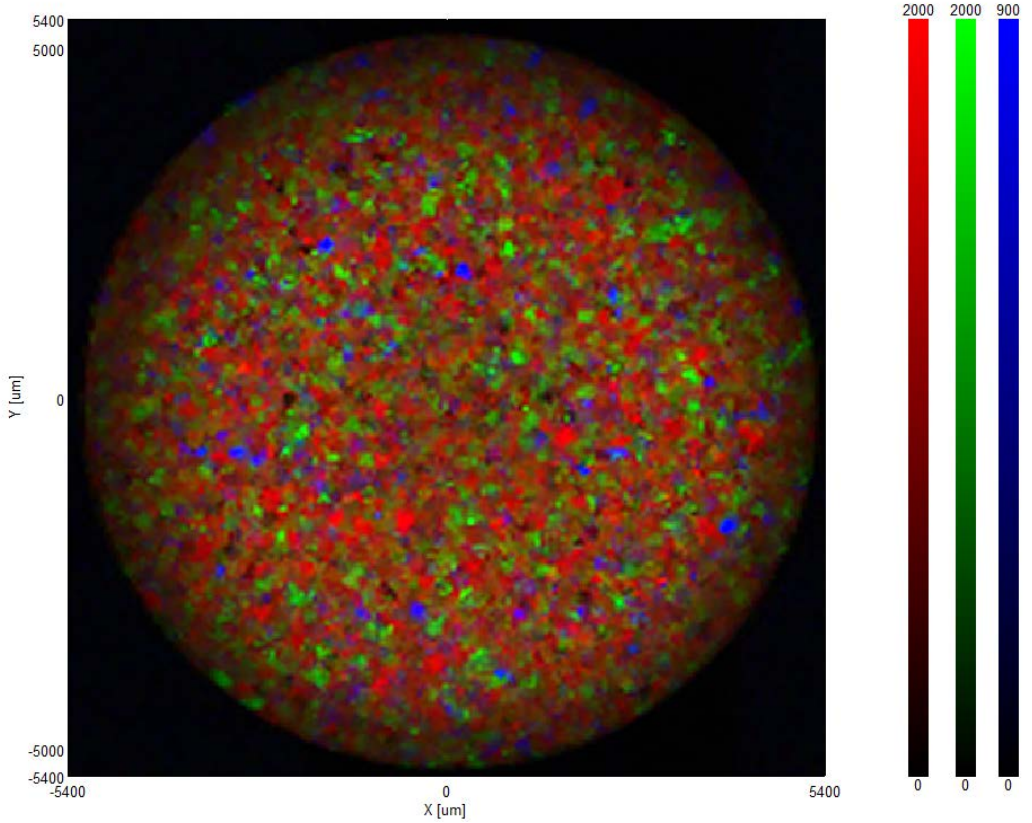
Raman Imaging

APPLICATIONS

Wide Area Imaging of Pharmaceutical Tablet



Visible Image

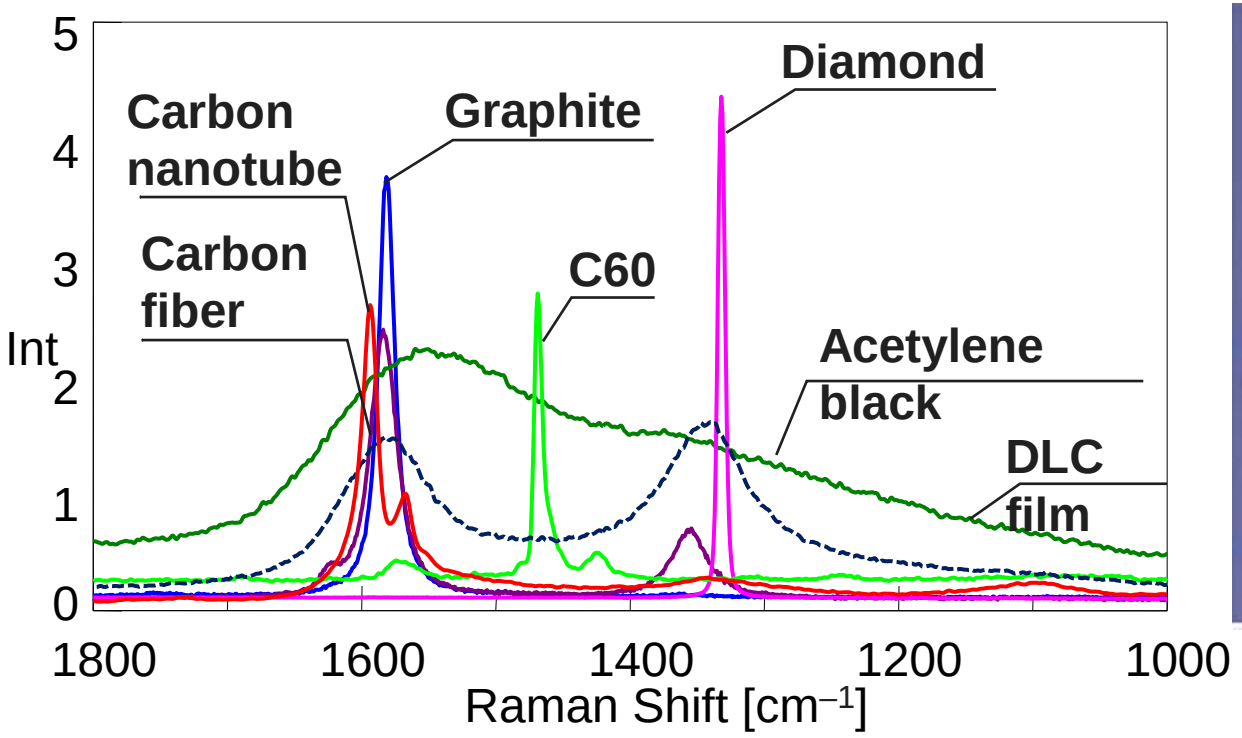


Distribution map of each component
（Etenzamide, Acetaminophen, Caffeine）

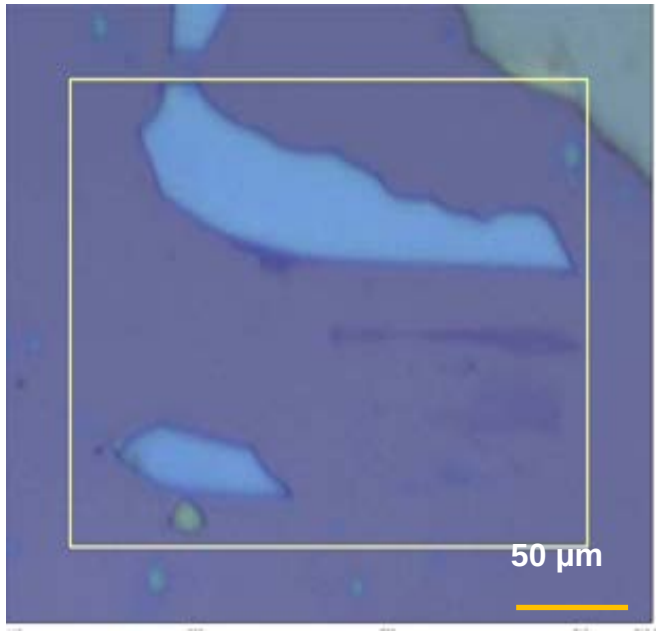
Ultra-high speed Image with excellent distribution map and spectra is achieved.

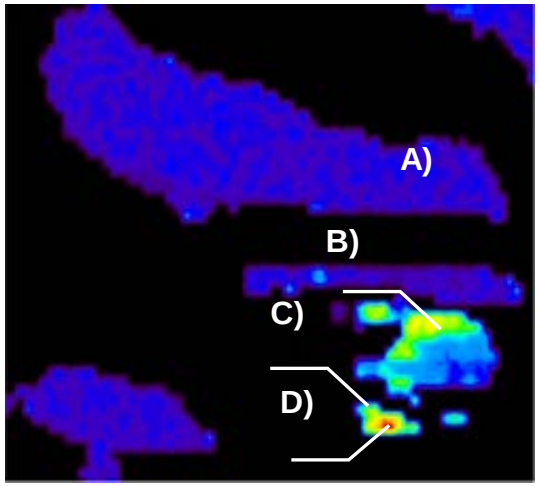
Imaging of Carbon Materials - Graphene

Raman spectroscopy can measure the characteristics of carbon materials.
In this measurement, the layer structure of graphene is evaluated using Raman imaging.



Raman spectra of carbon materials



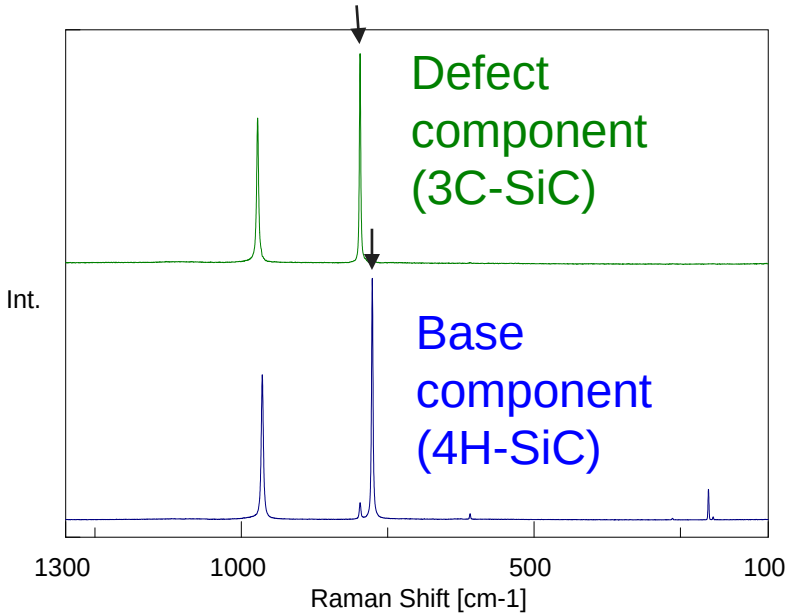


It is shown that the 2D/G of monolayer graphene is greater than 4 times.

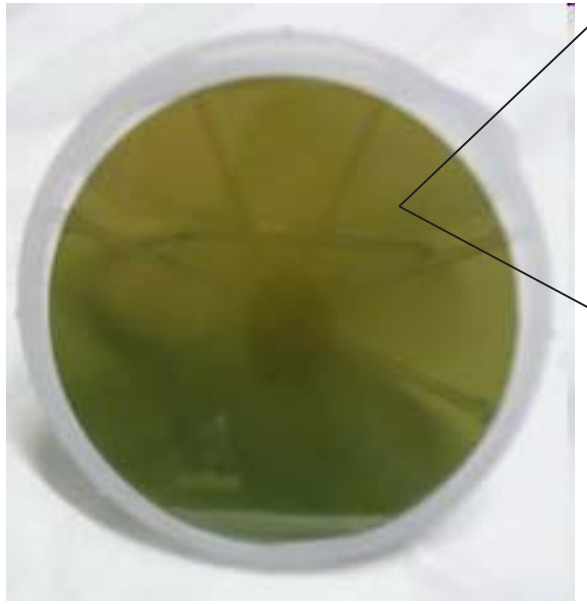
Evaluation of Crystal Features in SiC Using Raman Imaging

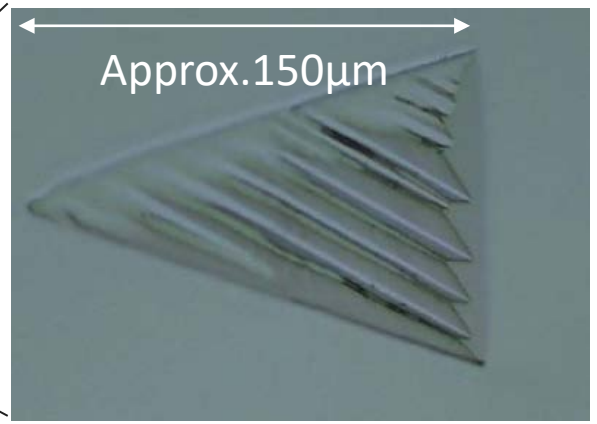
SiC has attracted attention as a power semiconductor. SiC has more than 200 crystal polymorphs. So the evaluation of polymorphism is very important.

In this measurement, crystal structure are evaluated using Raman imaging.

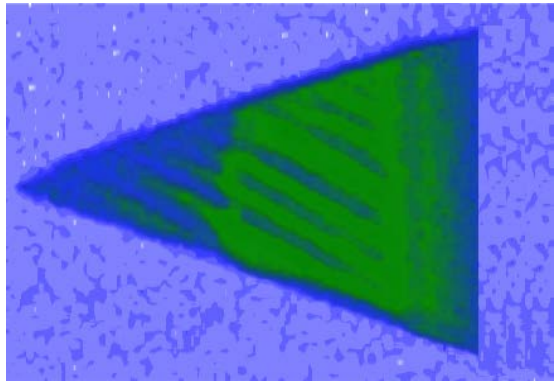


Raman imaging was made using each key-band.



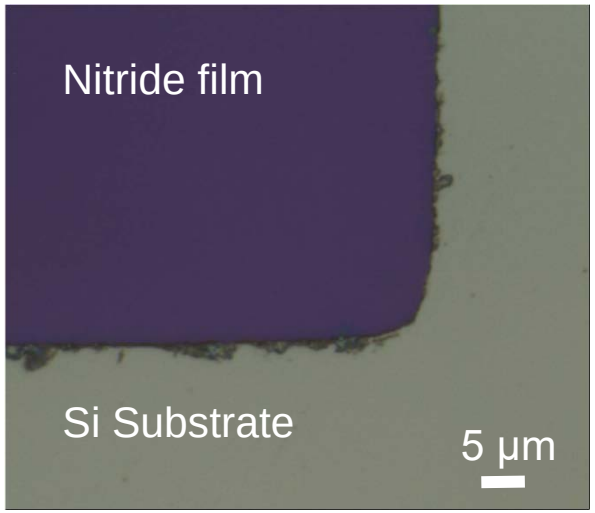


Defects in 4H-SiC wafer

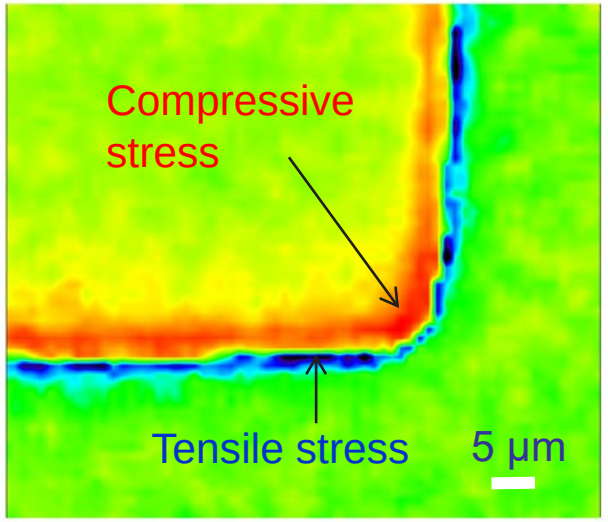


3C-SiC 4H-SiC

Stress Evaluation by Raman Imaging



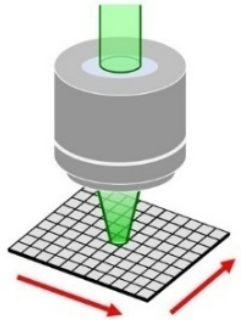
X100 Objective view



Color diagram of peak shit

+0.2 cm^{-1}
(Compressive stress 50MPa)

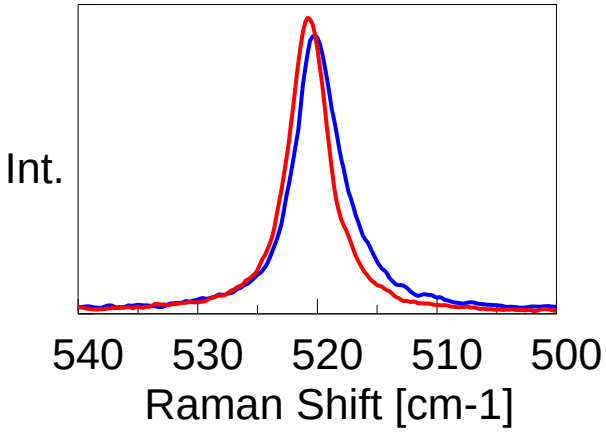
-0.2 cm^{-1}
(Tensile stress 50MPa)



QRI

Compressive stress
Tensile stress

60μm square area
Approx. 3800 points
Approx. 7 minutes

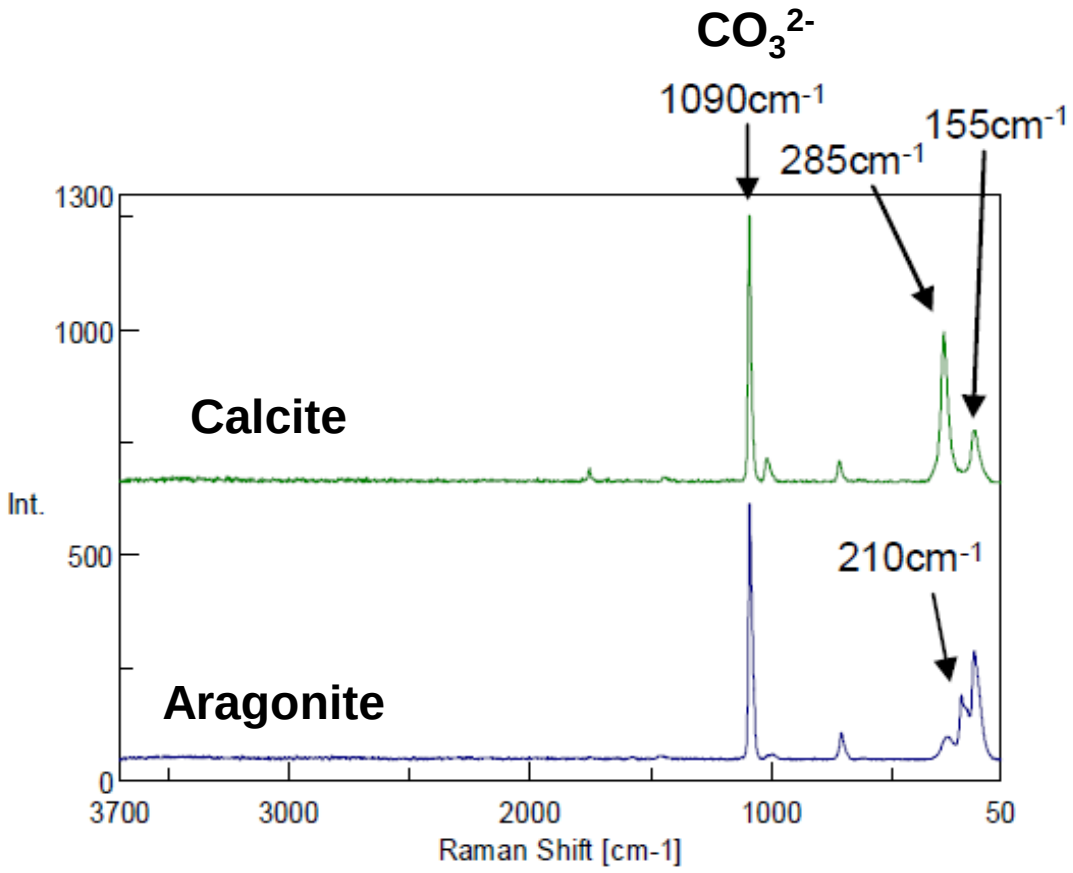


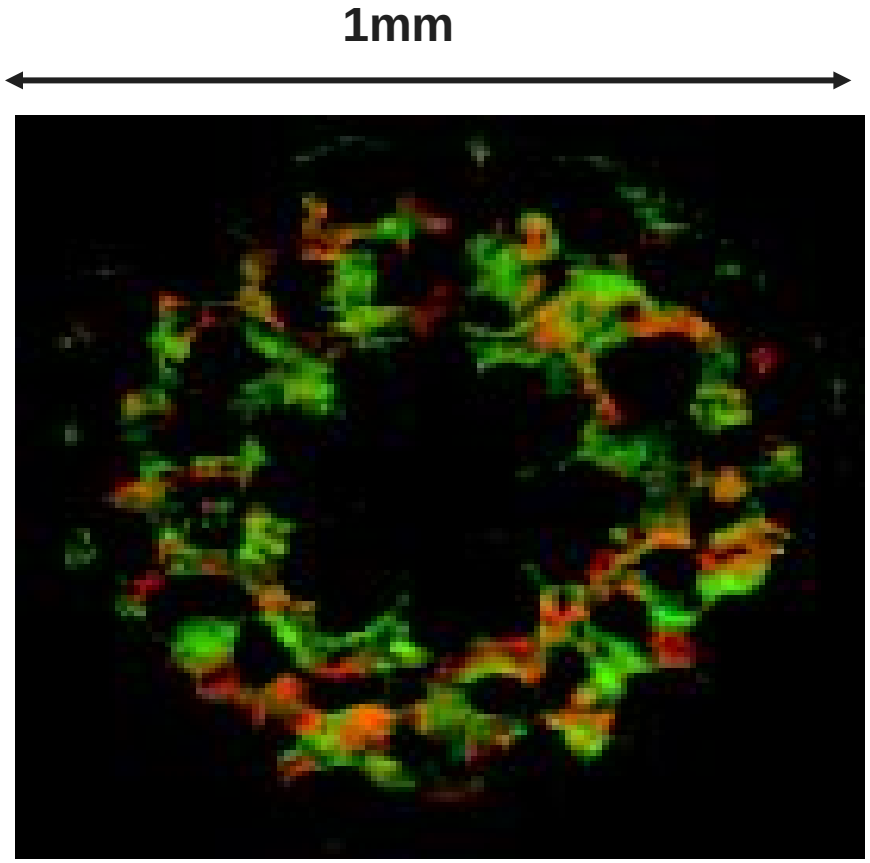
NRS-7000 series

(f=50cm, grating 2400 gr/mm, CCD：2048ch)

Biological Sample Imaging

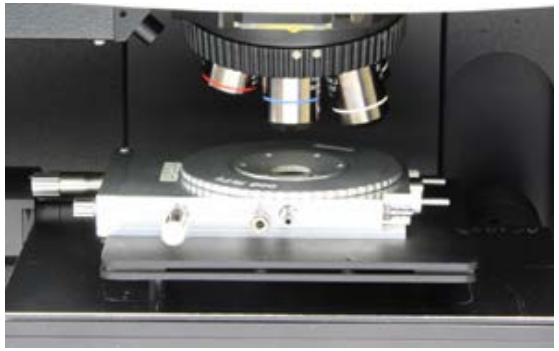
Visualization of Coral Crystal Polymorph Distributions



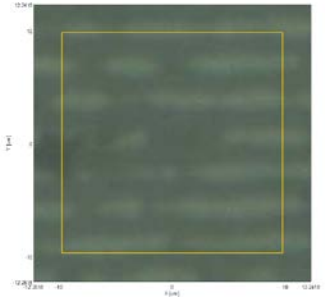


Red : Aragonite **Green: Calcite**

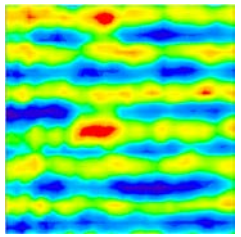
Sample Heating Imaging Analysis



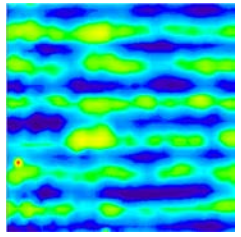
Heating/cooling stage



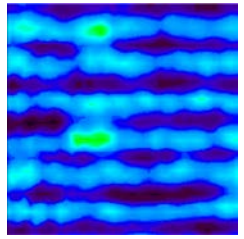
Objective view x50L



30℃

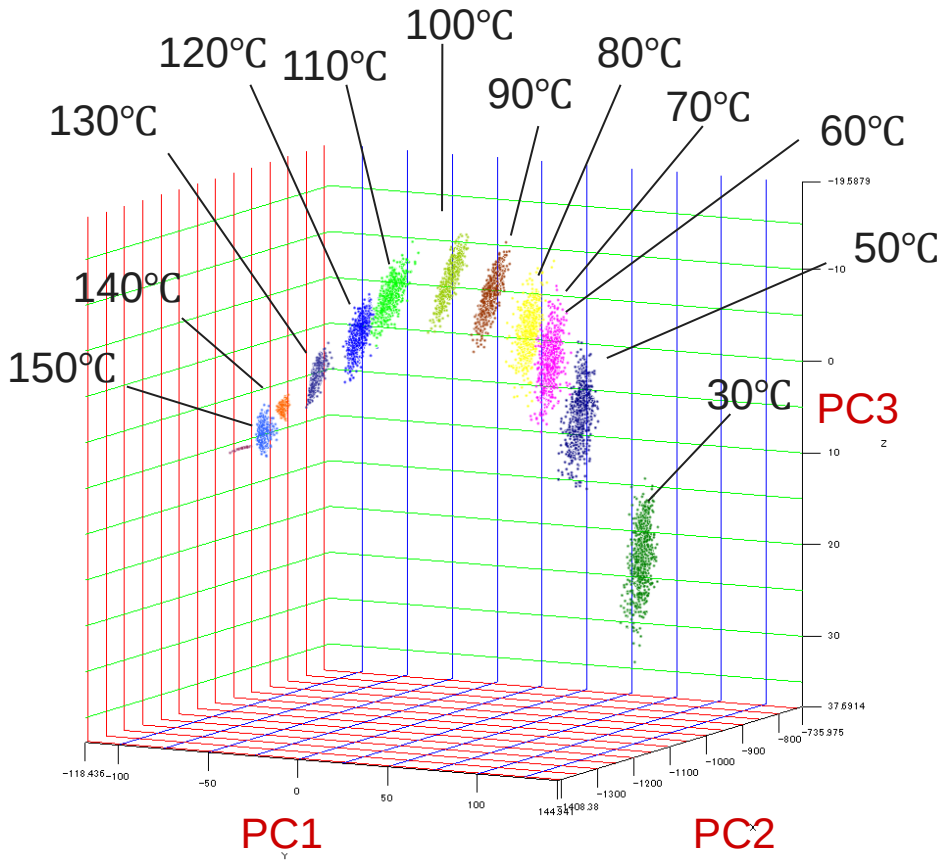


100℃



150℃

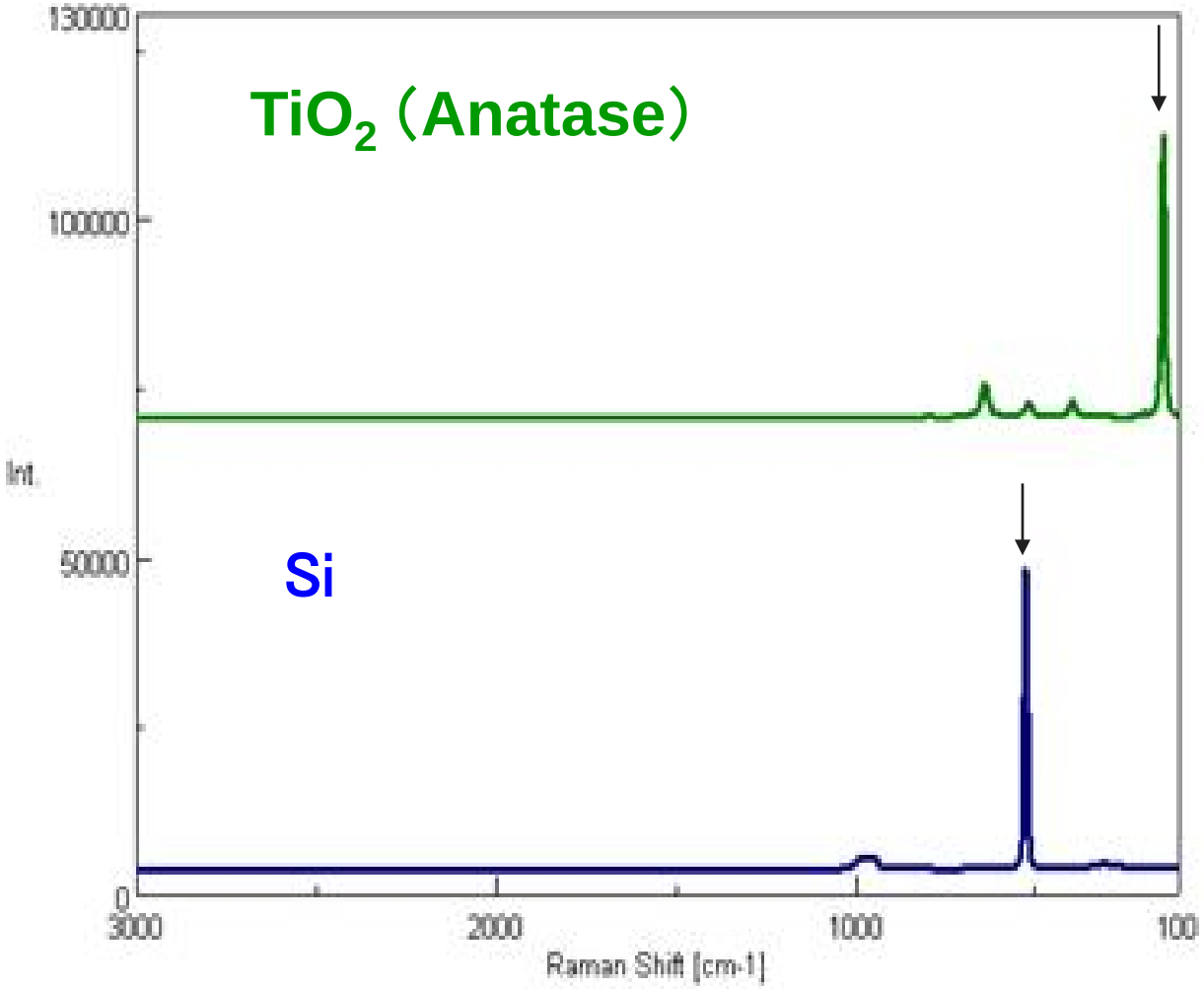
Laminated ceramic capacitor



Result of PCA analysis

Embedded Foreign Materials - 3D Imaging

Sample: Particles embedded in polymer

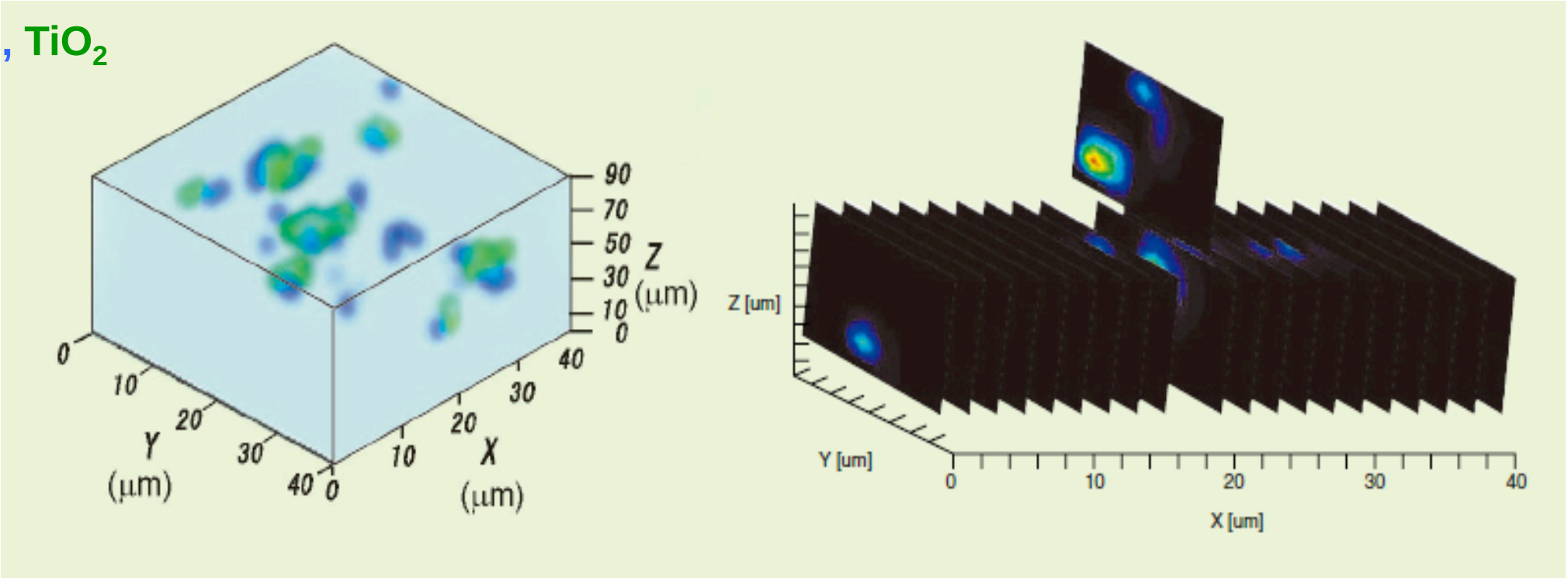


3-D Raman Imaging

The system acquires depth imaging data from a sample using confocal profiling of the Raman spectrometer and creates a 3-D image from the Raman intensity data.

Multilayer sample analysis is also possible using this feature.

Si, TiO_2

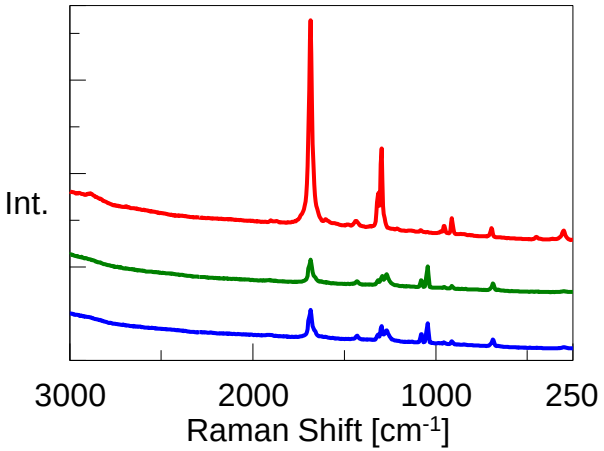


3-D Raman image display

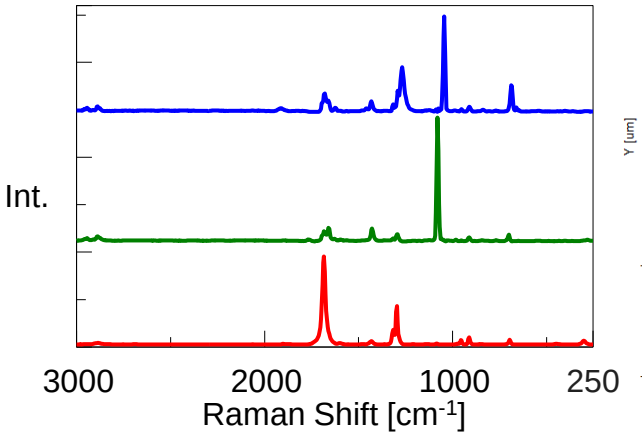
3-D image slice display

Analysis of Imaging Data by MCR Method

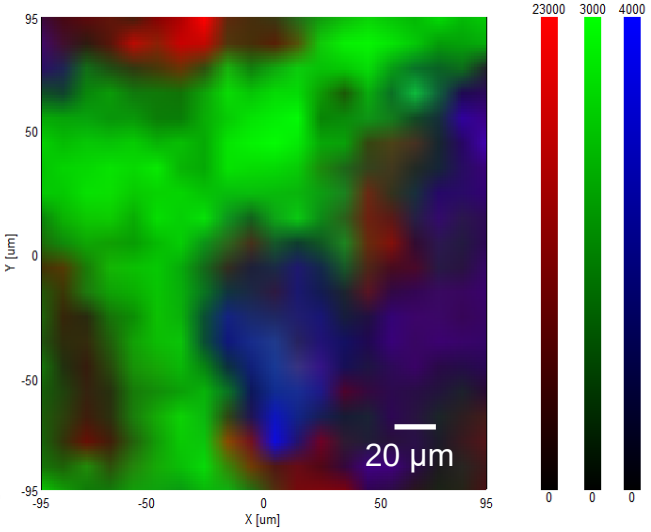
Bath Salt Tablet



Raw spectra at each point



Each principal component spectra



Distribution map of each component score

- Principal component spectra are extracted from entire measured points of imaging data, and usually similar to pure spectra, so they are applicable for database search.
- Based on score (contribution value) of each principal component spectrum, semi-quantification is possible and distribution map can be drawn.

JASCO Educational Resources

Upcoming Webinars:

- SFC Theory and Applications
- FTIR Microscopy

E-books and/or Tips and Tricks Posters

- Raman
- Fluorescence
- FTIR
- CD

KnowledgeBase

ResearchGate

- Fundamental theory and application of circular dichroism spectroscopy

NEXT WEBINAR WILL BE ON
SFC Theory and Applications

Mr. DJ Tognarelly
TUESDAY MAY 19TH AT 2:00 PM EDT

Thanks for joining us!!
Questions?