Hochaufgelöstes konfokales Raman-Imaging

Neue Einblicke in der Polymeranalytik

AK Polymeranalytik, Darmstadt 2019
Dr. Jan Englert, *WITec* GmbH

made
in
Germany

WITec Headquaters – Partners & Network

Cutting-Edge Technologies in Microscopy & Spectroscopy

Headquarters Germany

WITec Spain

WITec USA

WITec China

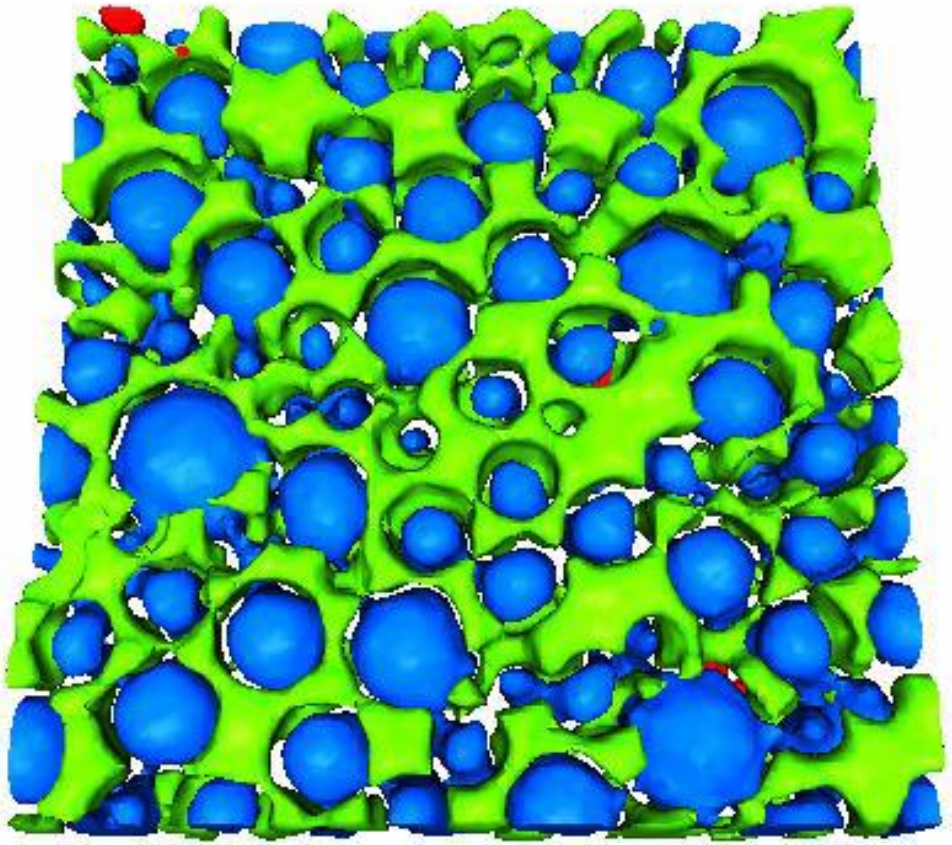
WITec Japan

WITec Asian Pacific

made in Germany

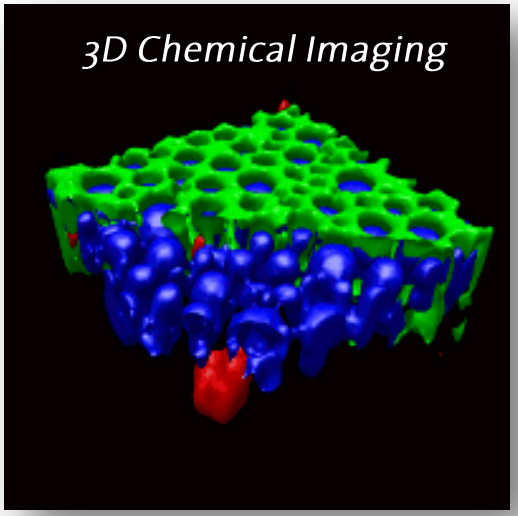
What can you expect?

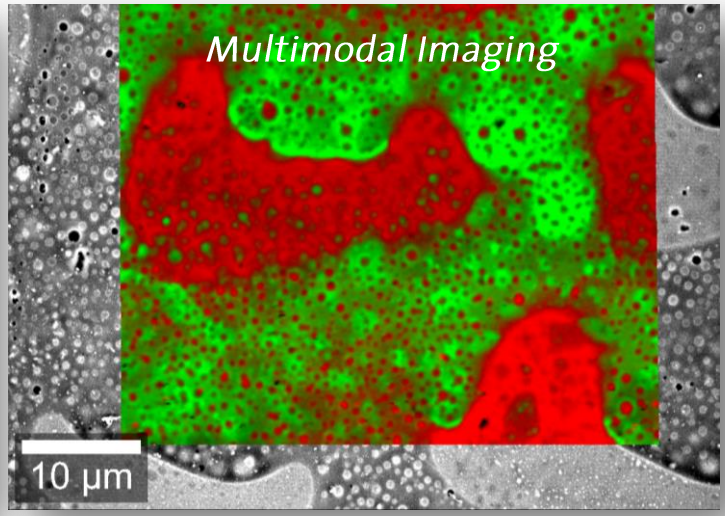
3D High-Resolution Chemical Imaging



Products & Solutions

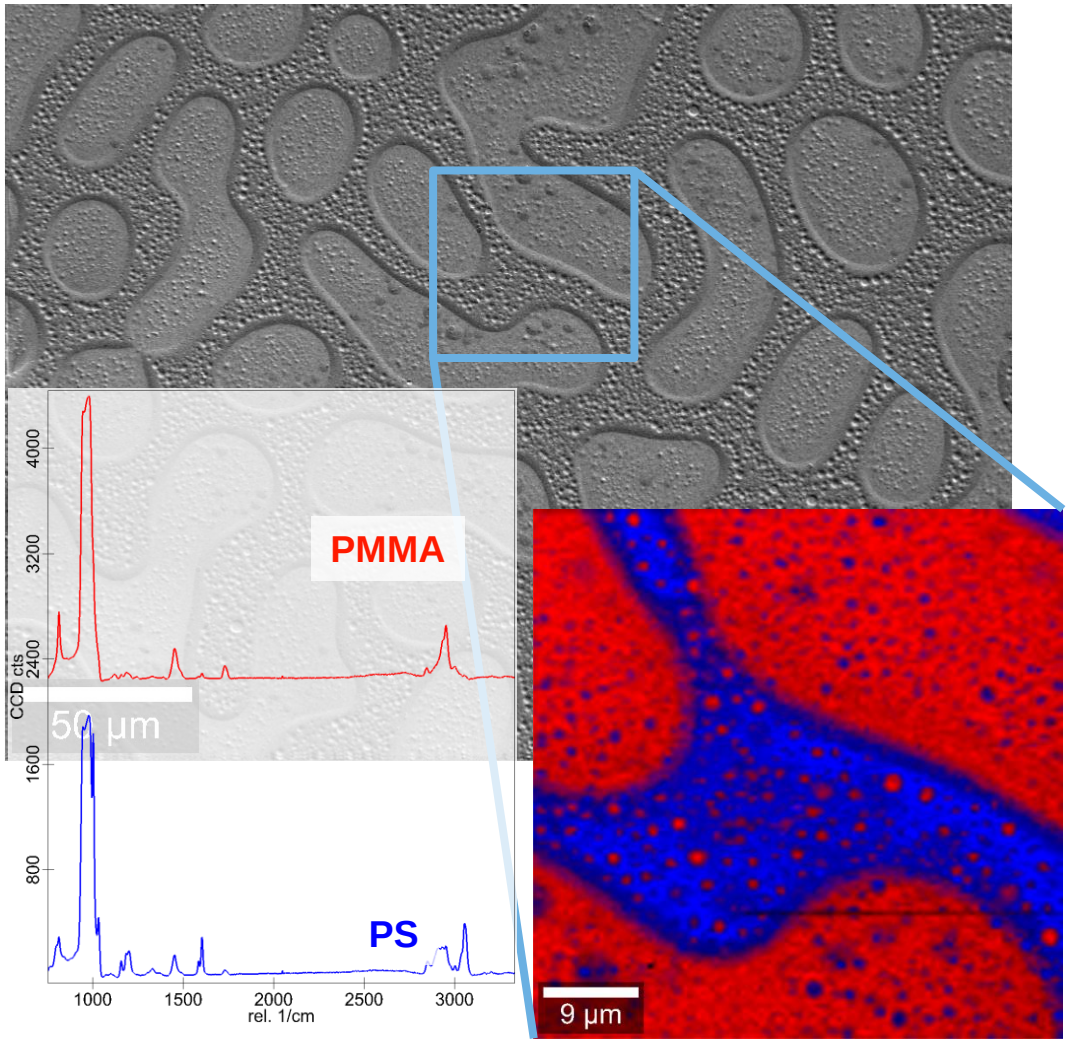
Cutting-Edge Technologies in Microscopy & Spectroscopy





Products & Solutions

Performance without compromises – Every component is vital



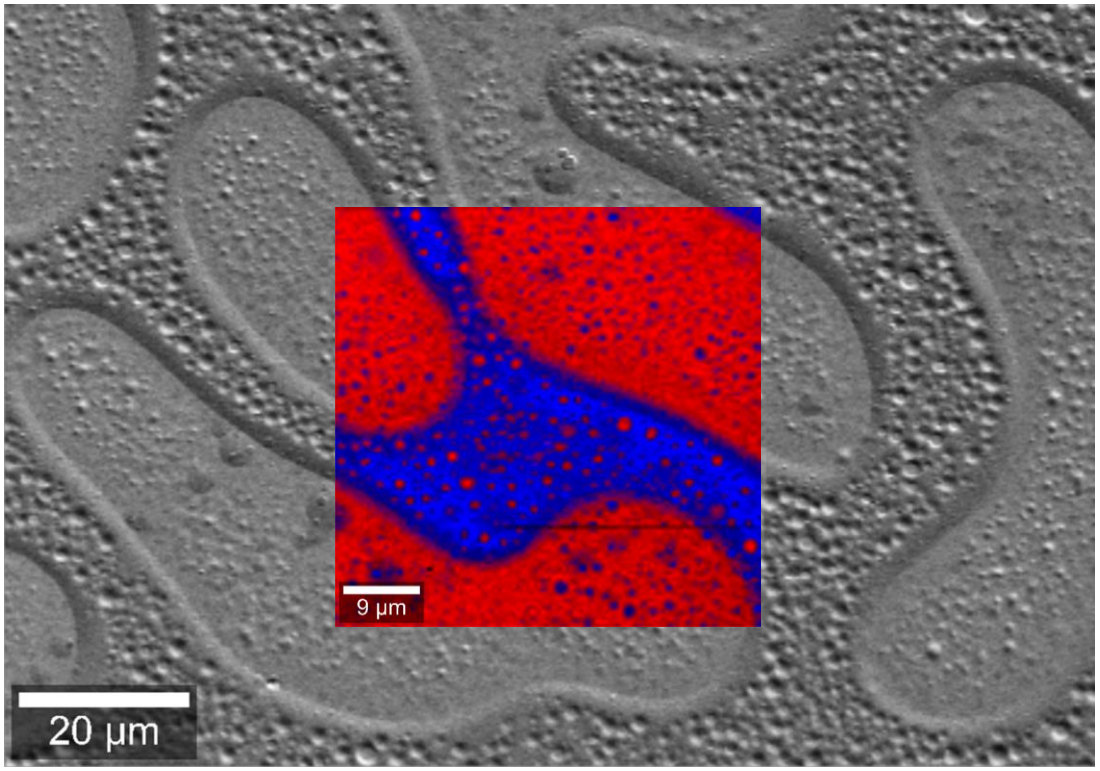
Measurement Workflow:

- Image Acquisition
  - High Resolution AOI
  - Large field of view
- Raman Acquisition
  - 50 mW 532nm Laser
  - 50 x 50 µm² scan area
  - 100 nm pixel size
  - **0.25 Mio. spectra**
  - **2 ms** Integration time/pixel
  - **~ 9 min.** total integration time
- Spectral Analysis WITec SuiteFIVE
  - Software supported identification of components
  - Generation of component's maps
- Superimposition of color coded maps on SEM image

Download Brochure:
WITec RISE Microscopy

Products & Solutions

Performance without compromises – Every component is vital



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Web Link Raman on Polymers:
www.witec.de/applications/polymers/

Download Brochure:
WITec RISE Microscopy

Products & Solutions

Integration of SPM and Optical Microscopy without compromise

alpha300 Series

The Instruments

alpha300 R

Raman

alpha300 RA

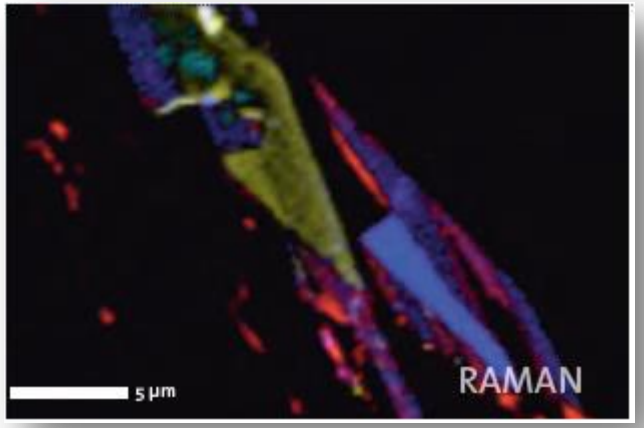
alpha300 RS

alpha300 A

alpha300 S

SNOM

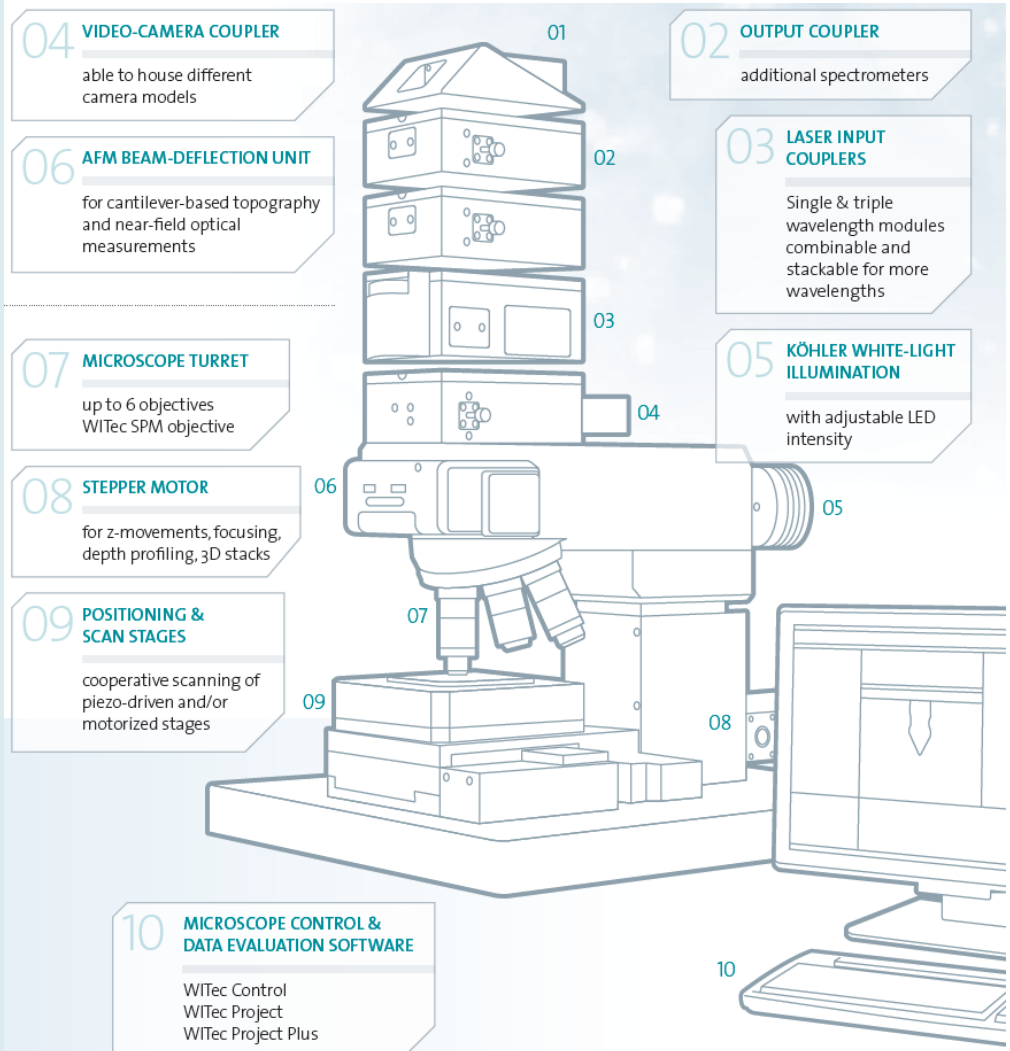
AFM

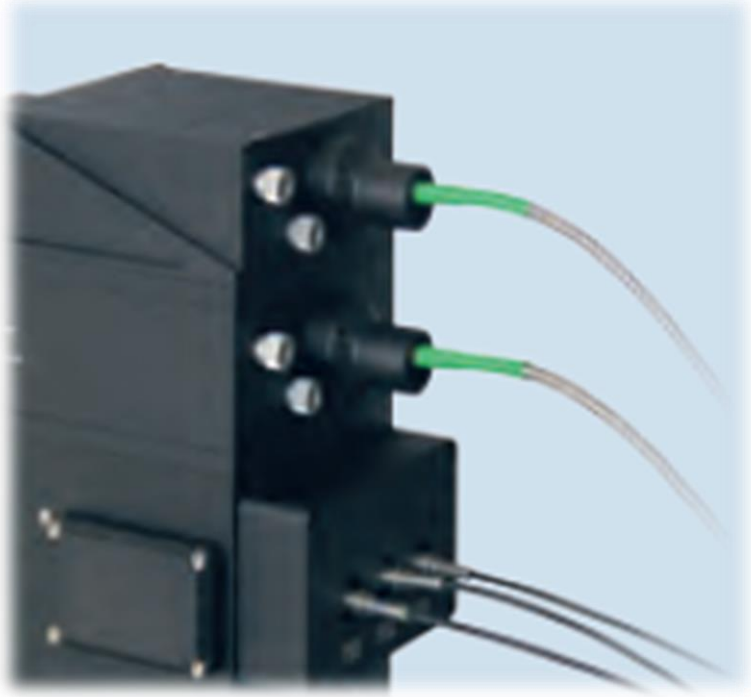


Download Brochure:
WITec alpha300 Microscope

Products & Solutions

Flexible Beampath – Accepts new challenges by design

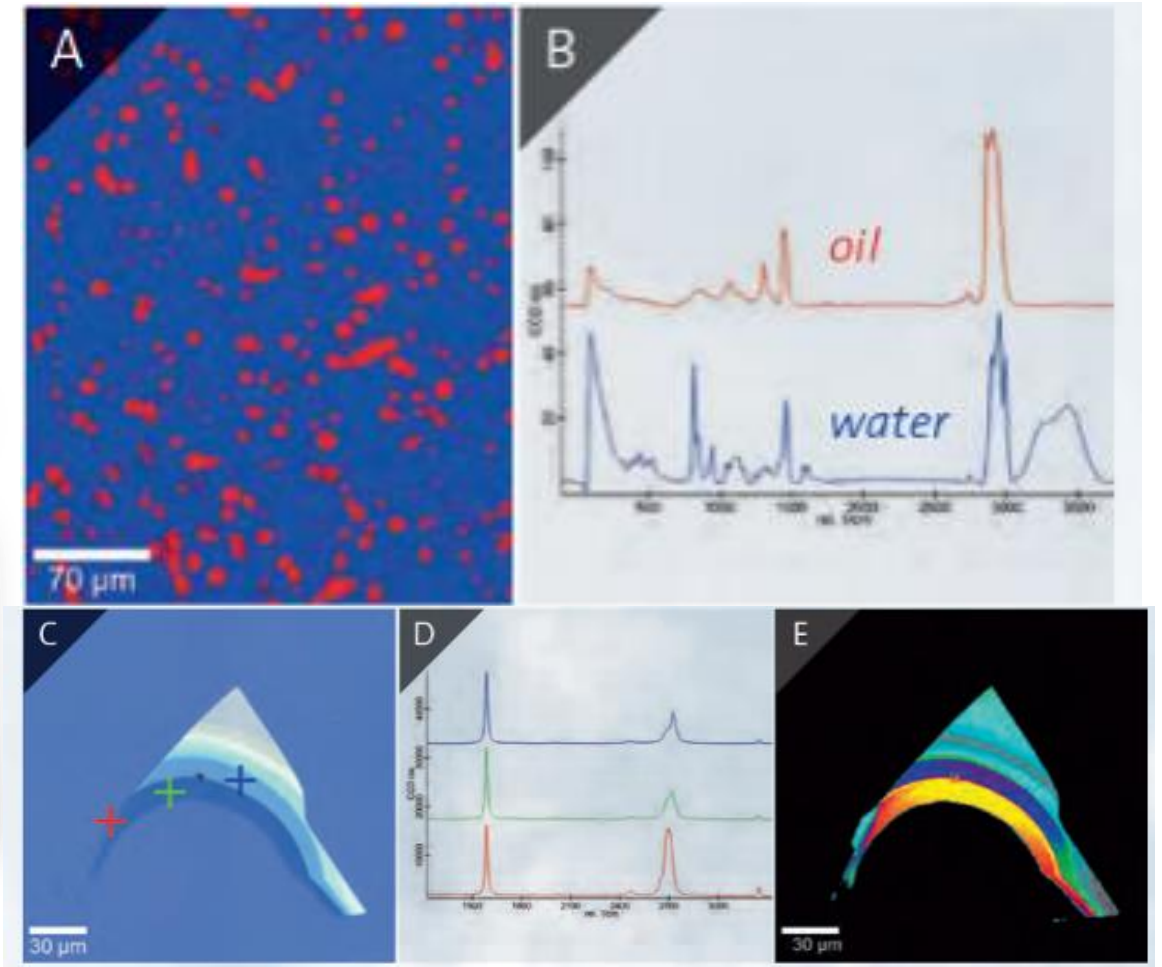




Download Brochure:
WITec alpha300 Microscope

Products & Solutions

When benchtop and upgrade capabilities count – WITec *alpha ACCESS*

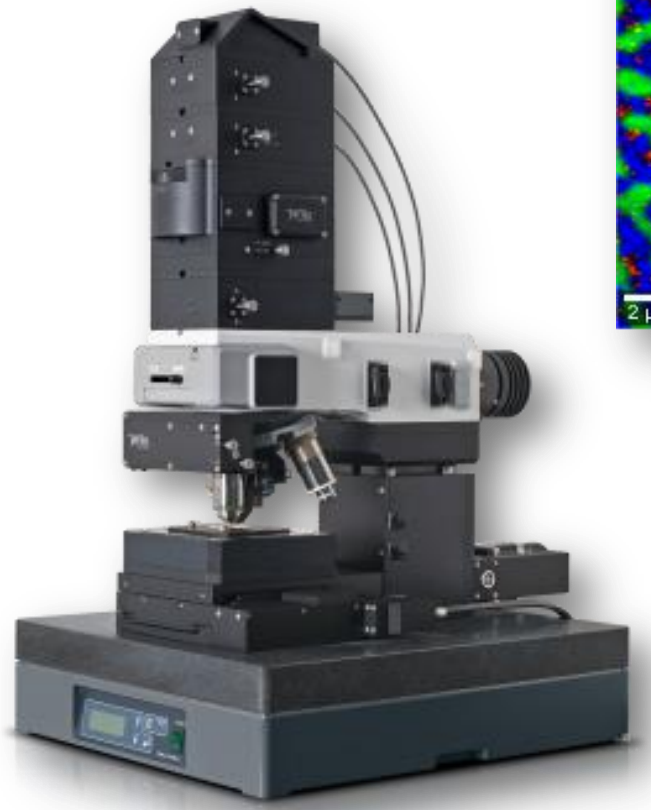


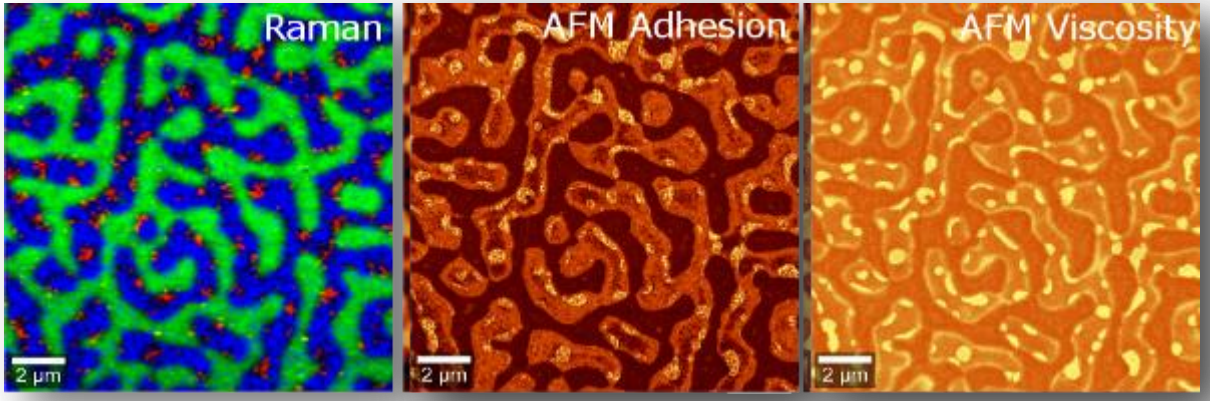
Download Brochure:
WITec alpha300 access

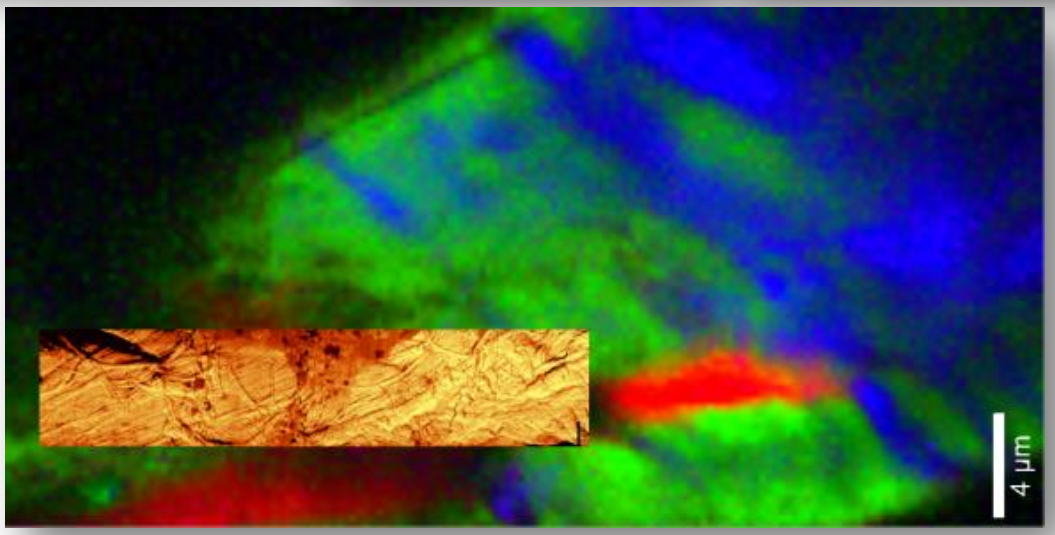
Gain entrance to the Raman world today –
Stay flexible for emerging challenges tomorrow

Products & Solutions

Chemical and Nanoscale Structural Imaging in one System – *alpha300RA*







Download Brochure:
WITec alpha300 Microscope

Web Link Raman on Polymers:
www.witec.de/applications/polymers/

Products & Solutions

When simplicity meets performance – WITec *apyron* Microscope

Laser wavelength selection from UV to NIR

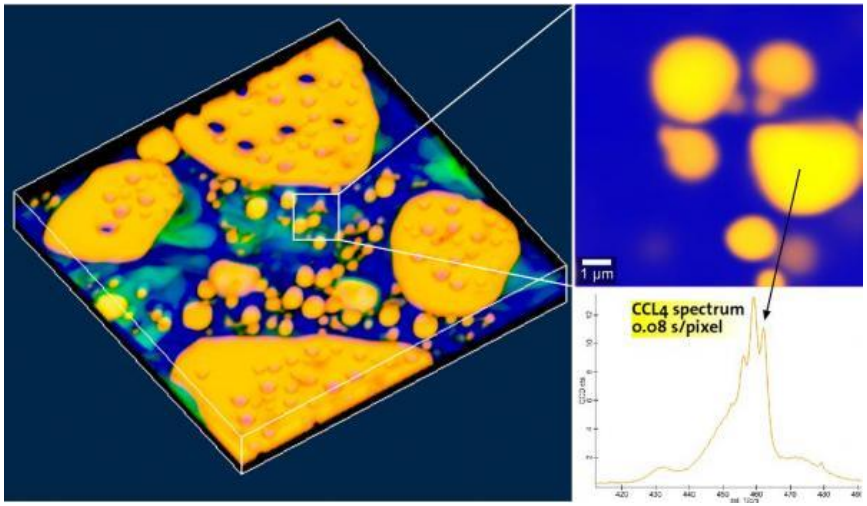
Automated adjustment and calibration of spectrometer and microscope components, including filters, gratings and cameras

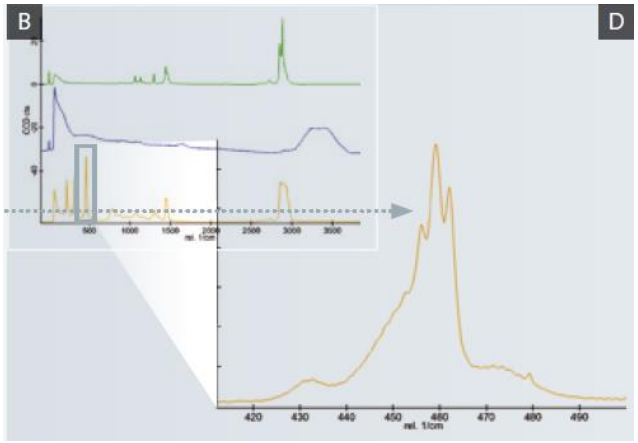
BENEFITS:
- User-friendly laser selection with a mouse click
- Consistently optimized system performance

Focus stabilization

Automated routine that employs a user selectable reference point to optimize the Raman signal and compensates for thermal and mechanical variations during long-term measurements

BENEFITS:
- Stable focus for sharp Raman images
- Lab environment-independent results





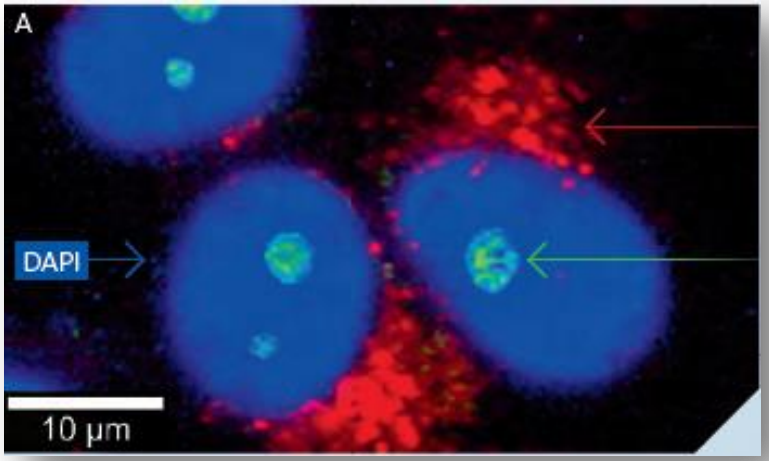
Class-leading Performance: Automatically

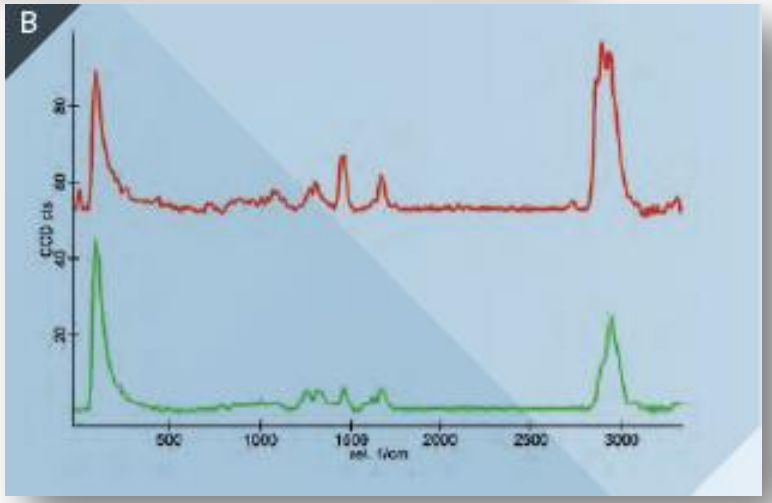
Download Brochure:
WITec apyron Microscope

Video Link:
WITec apyron Microscope

Products & Solutions

Looking to your samples at a new angle – WITec inverted *alpha300 Ri*



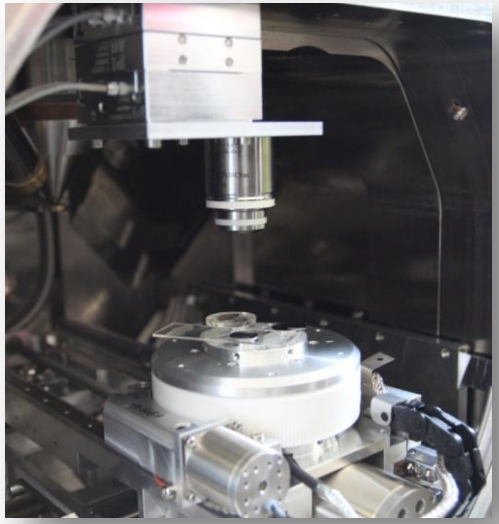


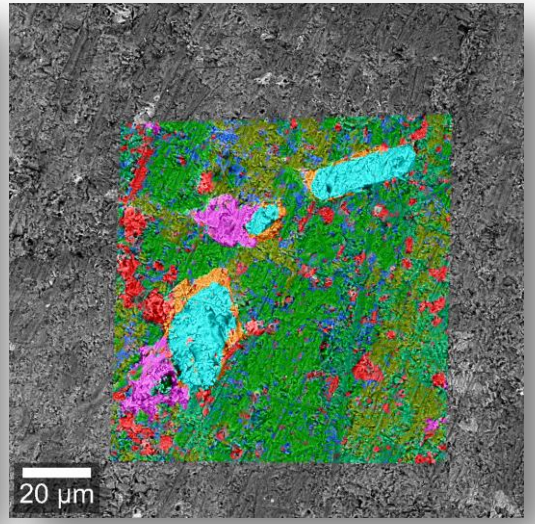
Download Brochure:
WITec alpha300 R*i* Microscope

Video Link:
WITec Product release video @ Analytica Munich 2018

Products & Solutions

Correlative Microscopy with Raman integrated in SEM – RISE Microscopy





SEVENTH FRAMEWORK PROGRAMME

univ sem
universal scanning electron microscope

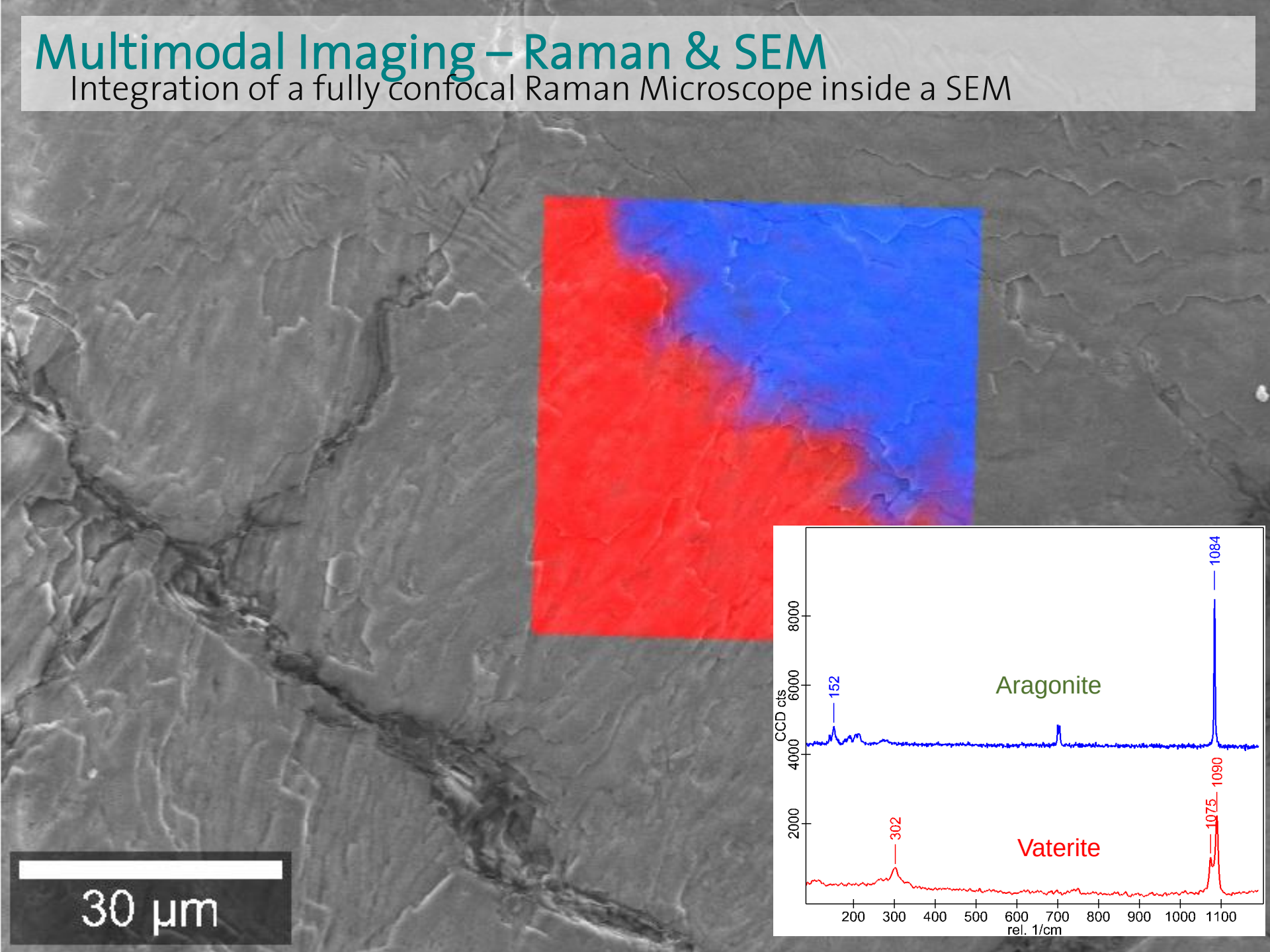
Download Brochure:
WITec RISE Microscopy

Download AppNote:
One the RISE

Video Link:
RISE Extension on ZEISS Sigma300

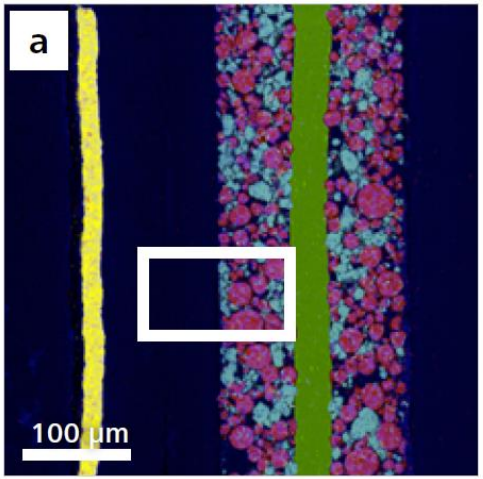
Multimodal Imaging – Raman & SEM

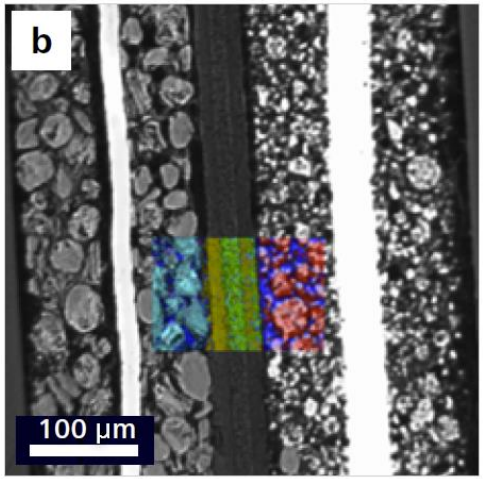
Integration of a fully confocal Raman Microscope inside a SEM

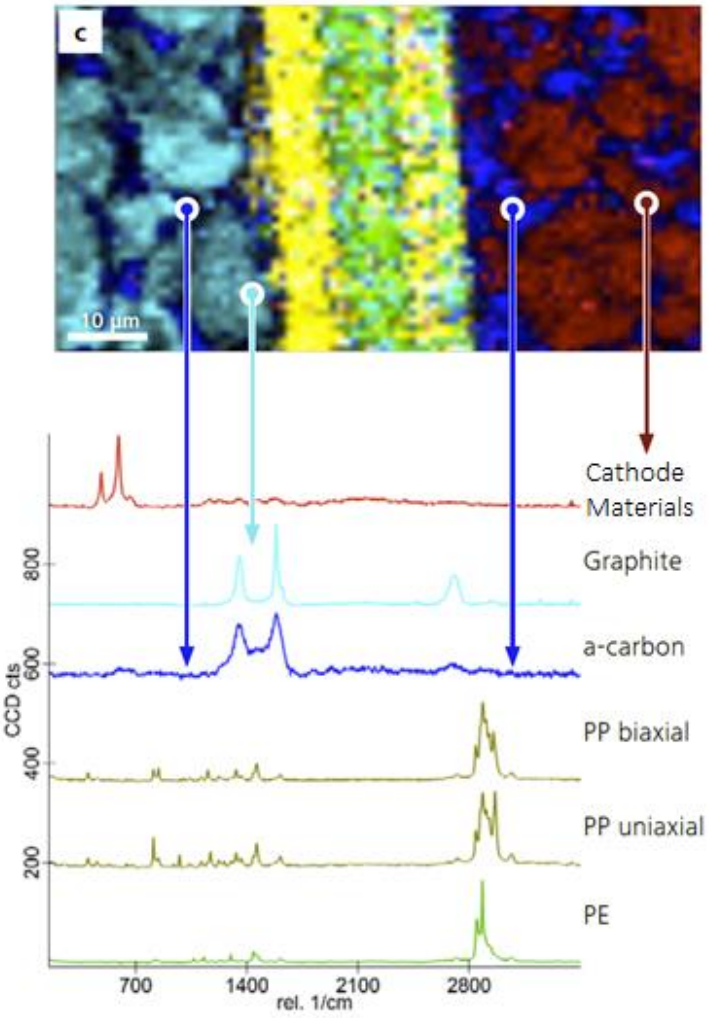


Application Example

Battery Separator (SEM/EDS/RISE)







- Commercial 18650 battery was cross-sectioned and imaged using correlative SEM, EDS and confocal Raman capabilities

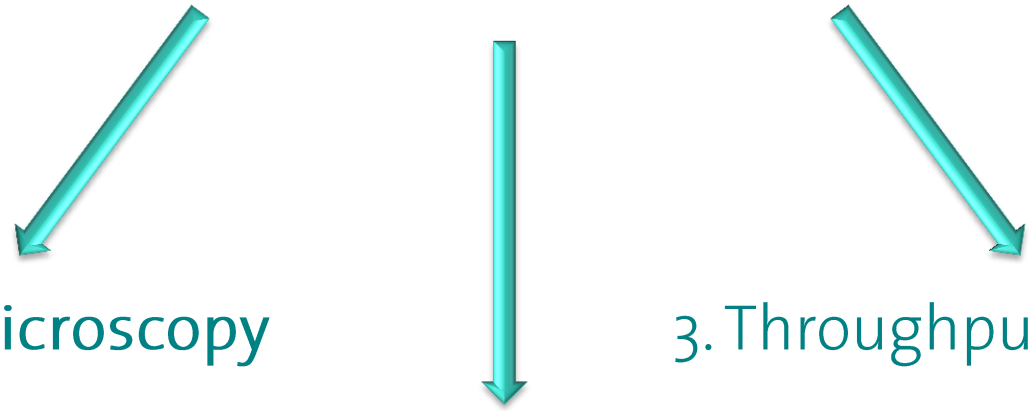
- Results reviewed the multi-phase nature of the separator, including:
  - Uniaxial/Biaxial PP
  - PE

Web Link Battery Failure analysis:
www.zeiss.com/microscopy/

High resolution Raman Imaging

Outline

Confocal Raman Imaging



1. Confocal microscopy

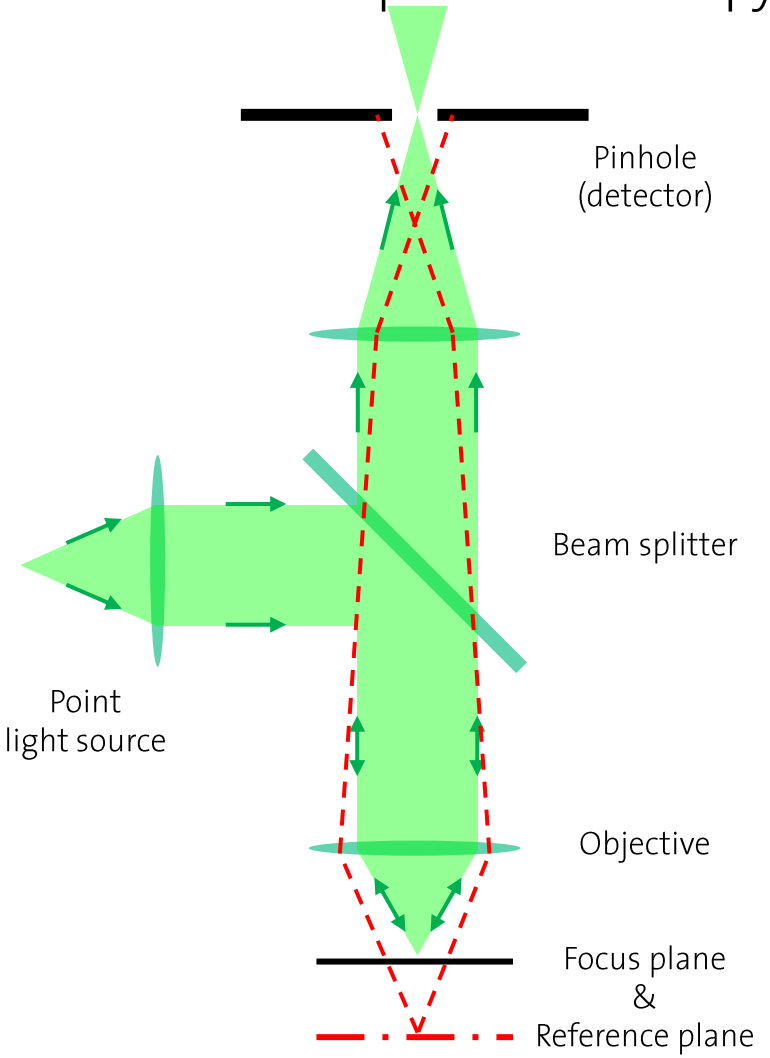
2. Basics of Raman spectroscopy

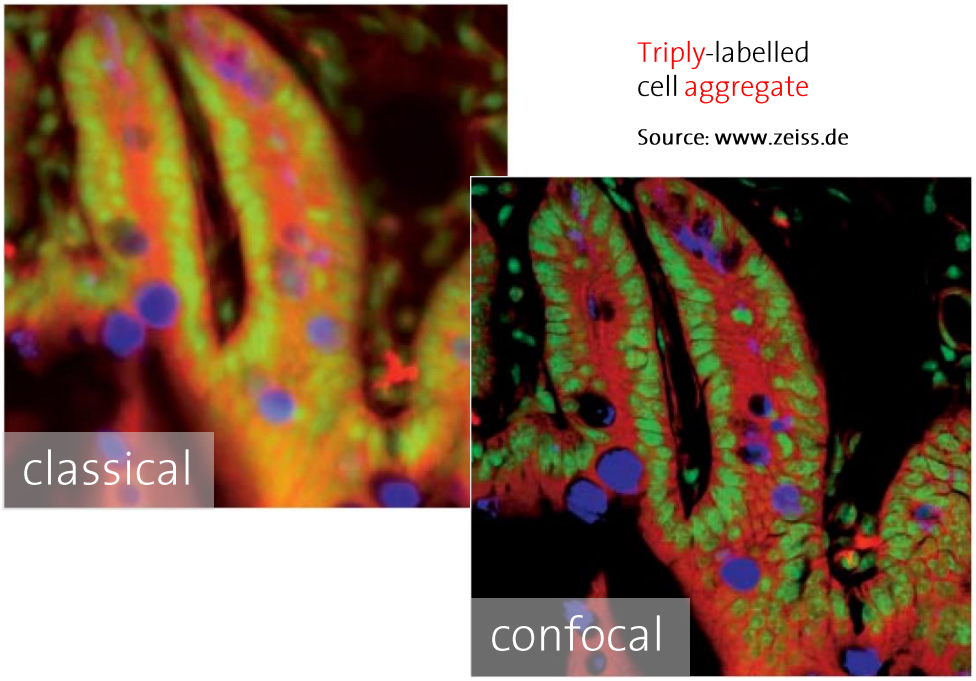
3. Throughput optimization

Diffraction limited Microscopy with chemical contrast at ultimate integration times

Basics of Confocal Raman Imaging

Resolution in optical microscopy





Triply-labelled
cell aggregate

Source: www.zeiss.de

Benefits of confocal microscopy:

- Less background which causes “blurring”
- 3D information by slicing the sample optically
- Enhanced resolution …

Copyright WITec GmbH

Basics of Confocal Raman Imaging

Resolution in optical microscopy – lateral resolution

NA = n * sin(α)

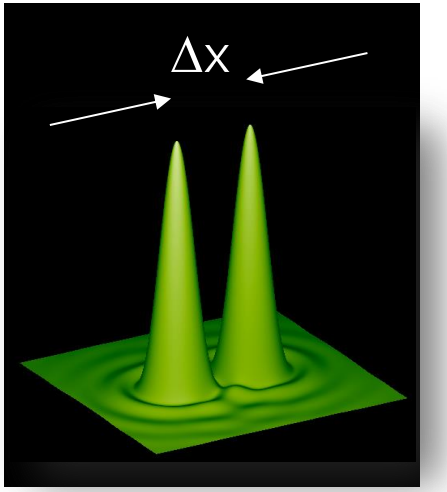
Resolution limit of an optical microscope?

Ernst Abbé (1840-1905): Diffraction Theory

$$\Delta x_{\min} \approx \lambda_{exc} / NA$$

NA is an inherent property of the used objective:

- defines collection angle/collection efficiency
- inverse proportional to the working distance

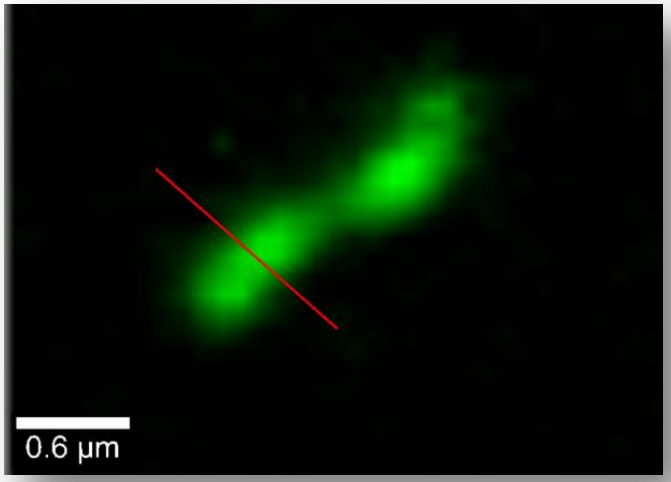


High resolution requires high NA, not high magnification

Basics of Confocal Raman Imaging

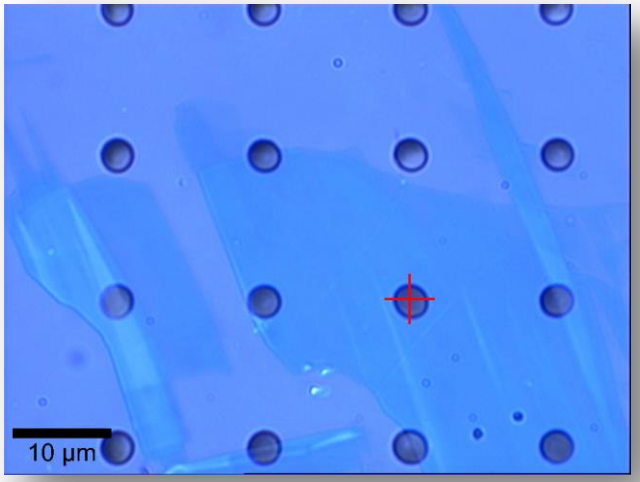
How can resolution of a confocal optical microscope be determined?

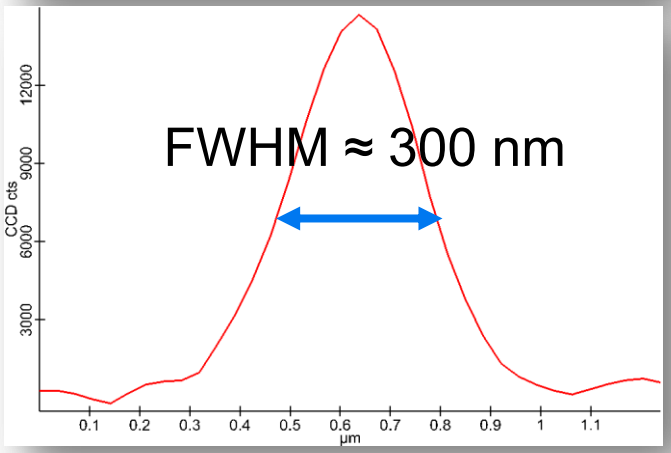
Isolated CNTs & suspended graphene – ideal system to measure resolution

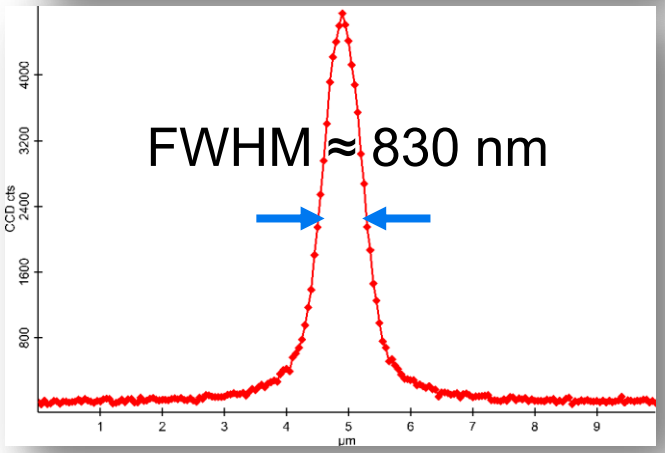


Excitation Laser:
532nm

Objective:
100x/0.90NA







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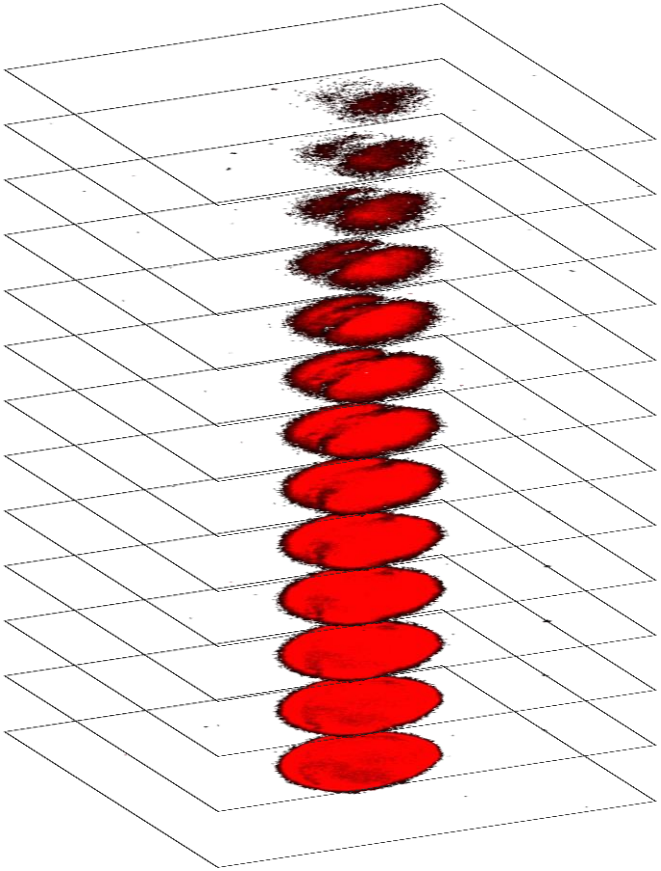
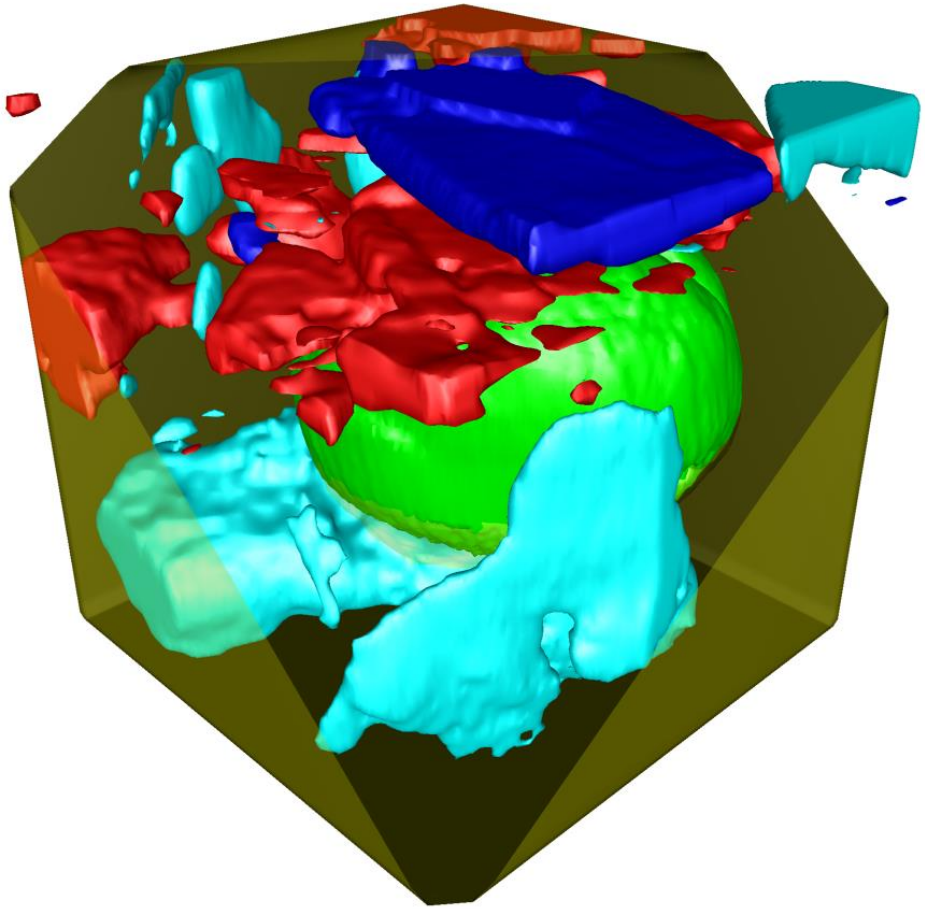
Basics of Confocal Raman Imaging

3D Reconstruction – getting an inside view

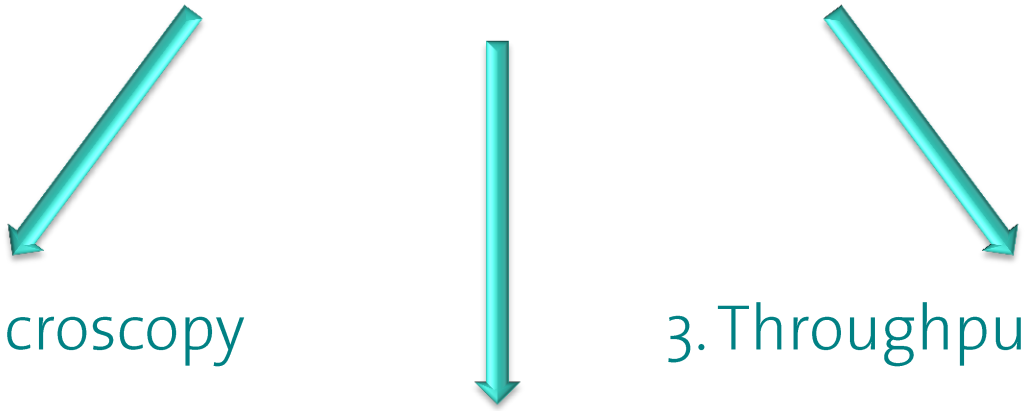




High resolution Raman Imaging

Outline

Confocal Raman Imaging



1. Confocal microscopy

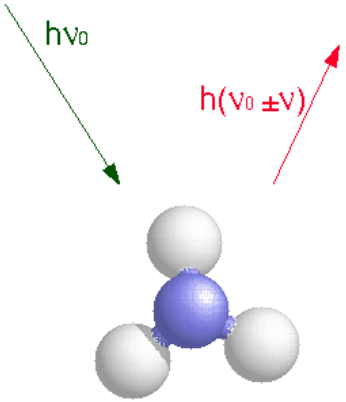
2. Basics of Raman spectroscopy

3. Throughput optimization

Diffraction limited Microscopy with chemical contrast at ultimate integration times

Basics of Raman Spectroscopy

Where do we come from



- non-resonant excitation (Stokes) or annihilation (Anti-Stokes) of a vibrational quantum
- energy shift between the exciting and scattered photon is characteristic for the molecules involved in the scattering process

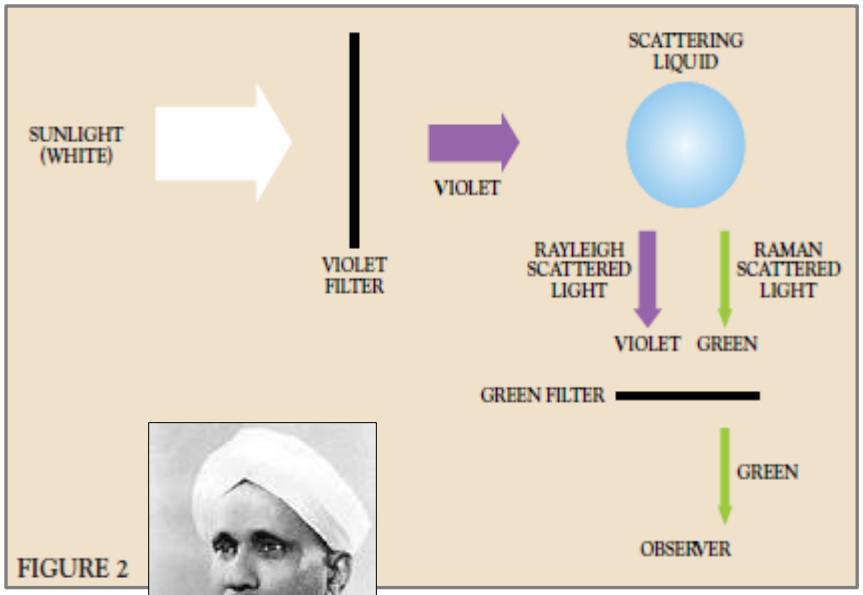
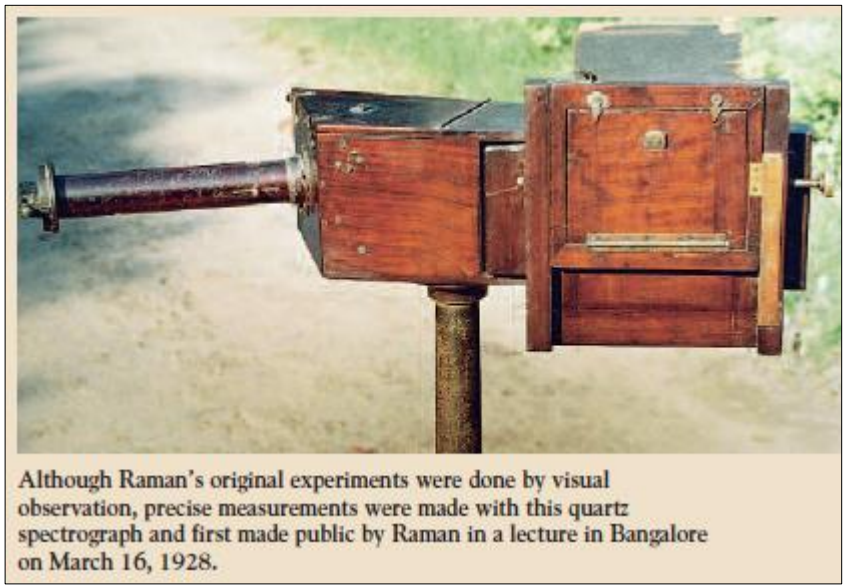


Image Courtesy:
The Raman Effect
American Chemical Society
The Indian Association for the Cultivation of Science

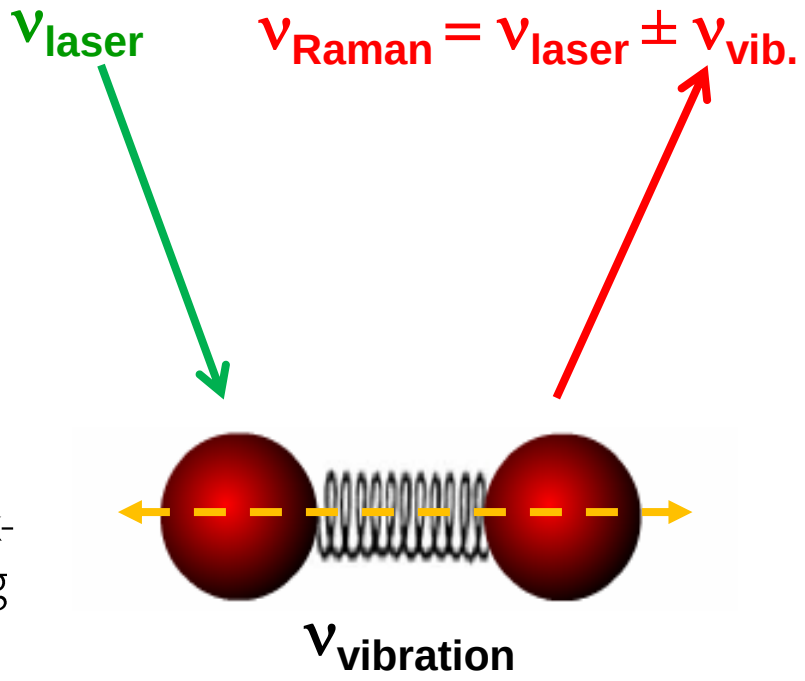


Although Raman's original experiments were done by visual observation, precise measurements were made with this quartz spectrograph and first made public by Raman in a lecture in Bangalore on March 16, 1928.

Image Courtesy:
The Raman Effect
American Chemical Society
The Indian Association for the Cultivation of Science

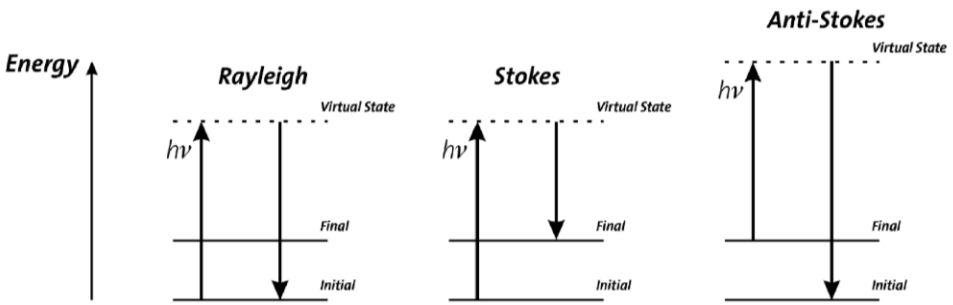
Basics of Raman Spectroscopy

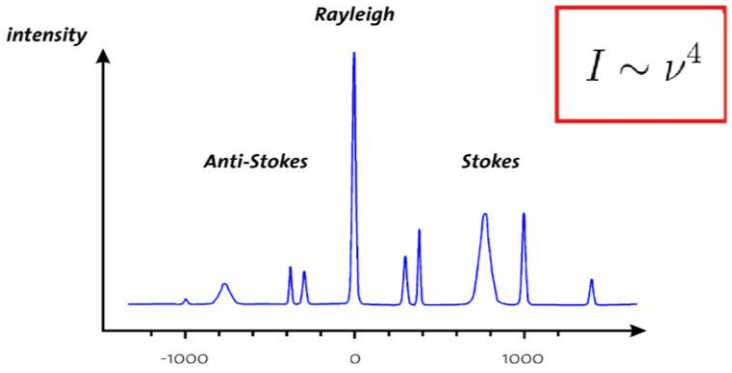
What can be measured spectrally - Nitrogen



Raman effect:

- non-resonant excitation of vibrational quantum states (vibs) – comparable to IR
- $N_{vibs} = 3N_{atoms} - 3vibs_{transl.} - 3vibs_{rot.}$
- energy shift between the excitation and scattered photon is characteristic for the back-driving force and hence the nature of bonding within the target (C-C vs. C=C or C=O etc.)

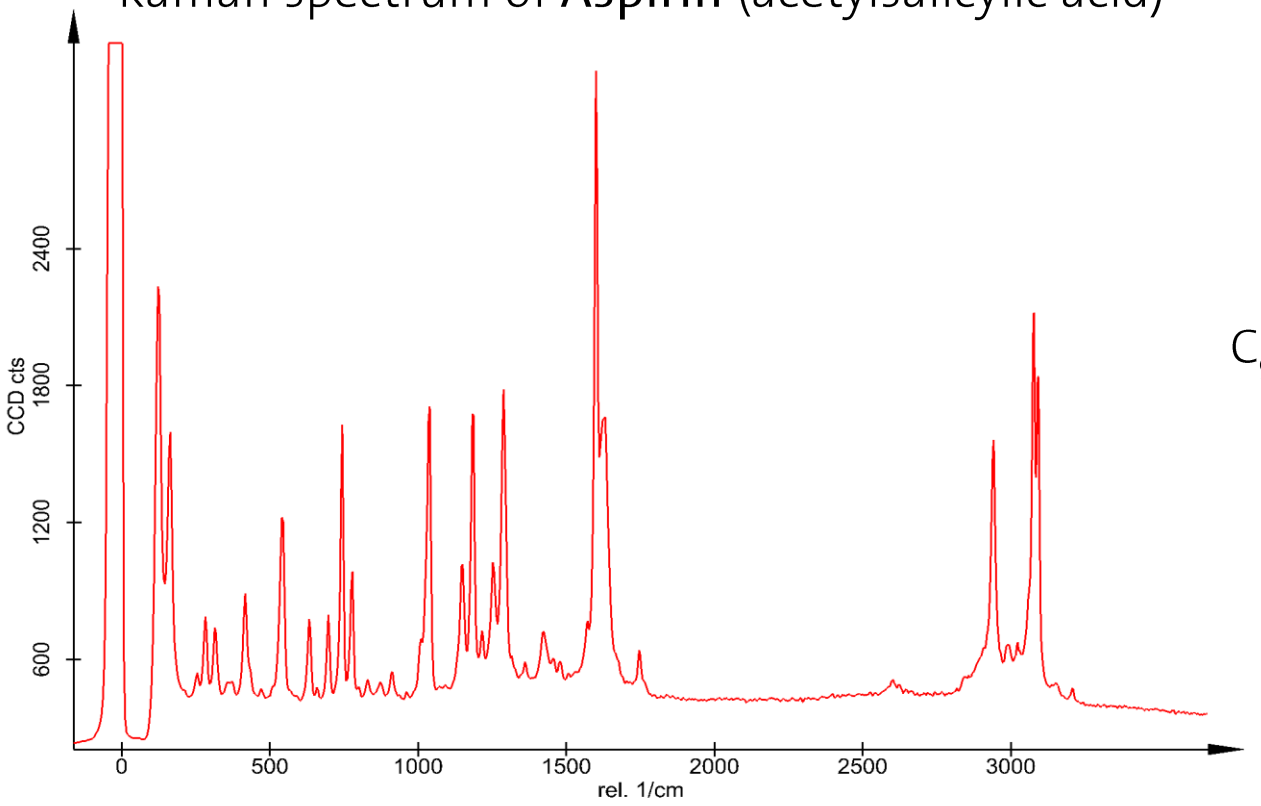


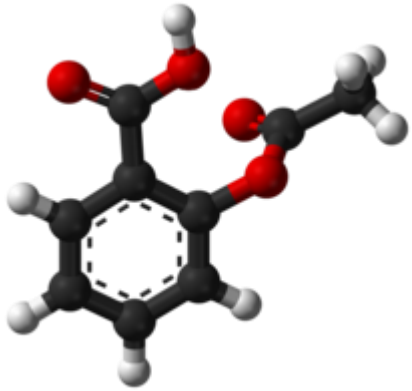


Basics of Raman Spectroscopy

What can be measured spectrally – organic molecules

Raman spectrum of **Aspirin** (acetylsalicylic acid)





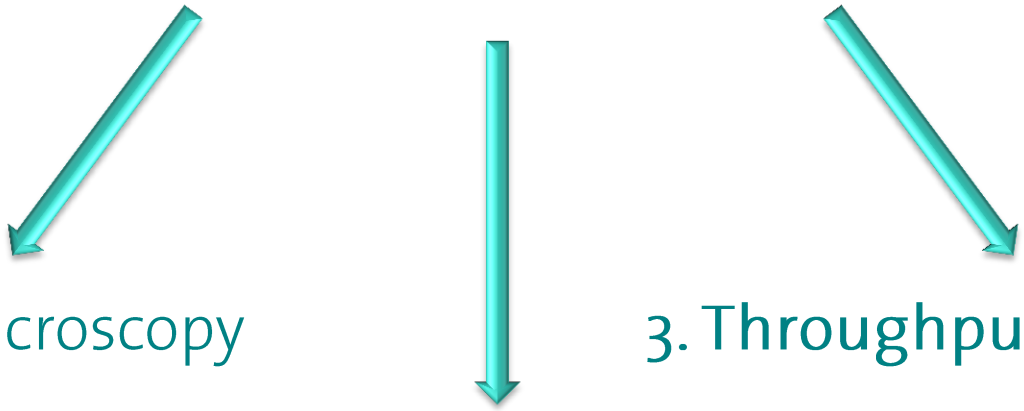
$C_9H_8O_4$ ➔ 57 Eigenvibrations

- theoretical prediction 1923 by A. Smekal
- experimentally discovered 1928 by Sir Chandrasekhara Raman, Nobel Prize 1930

High resolution Raman Imaging

Outline

Confocal Raman Imaging



1. Confocal microscopy

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Diffraction limited Microscopy with chemical contrast at ultimate integration times

Basics of Raman Spectroscopy

Hurdles and Challenges for Imaging – Low Signal Intensity

Confocal Microscope

Low photon throughput (Pinhole)

Raman Process

Low quantum yield ($10^{-3} – 10^{-4}$)

long acquisition/integration times can be anticipated

Standard Setup (Step-by-Step Mapping)

Range: 100 x 100 µm² @ 250nm steps

Resolution: 400 x 400 px. = 160k px.

Pixel integration time: 500 ms

Total integration time: ~22 hours

Optimized Setup (Continuous Imaging)

Range: 100 x 100 µm² @ 250nm steps

Resolution: 400 x 400 px. = 160k px.

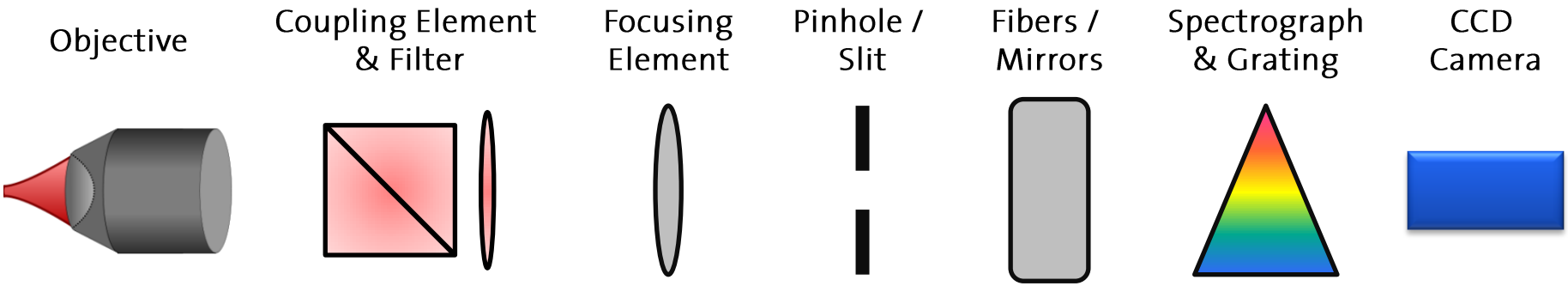
Pixel integration time: 2 ms

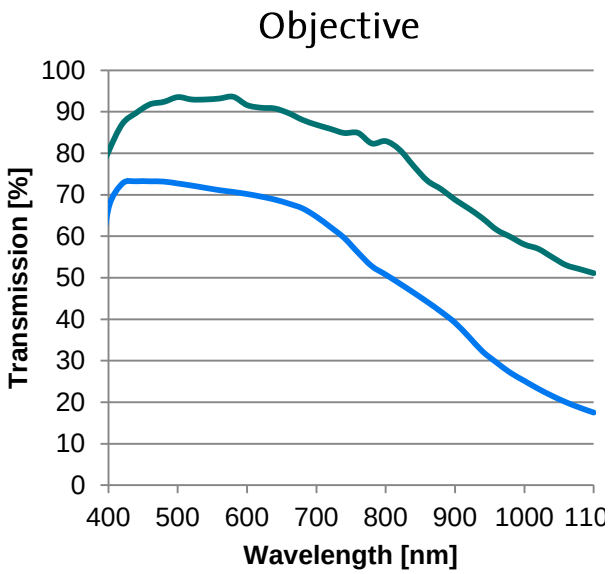
Total integration time: ~6 minutes

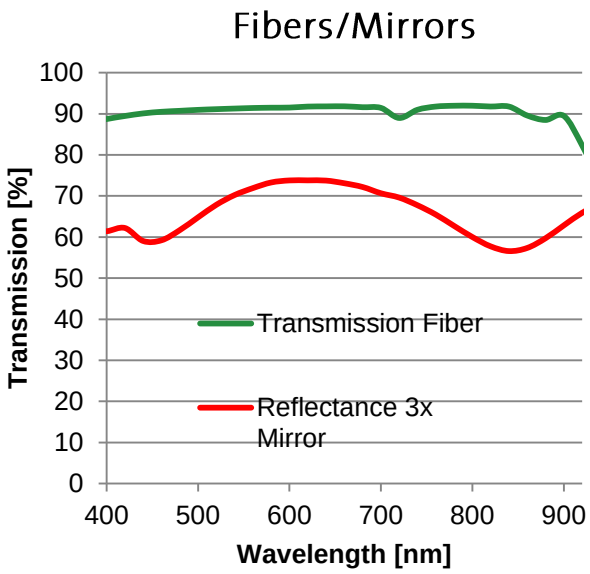
- Short integration times needed (max. 100 ms / pixel)
  - System must have highest efficiency possible

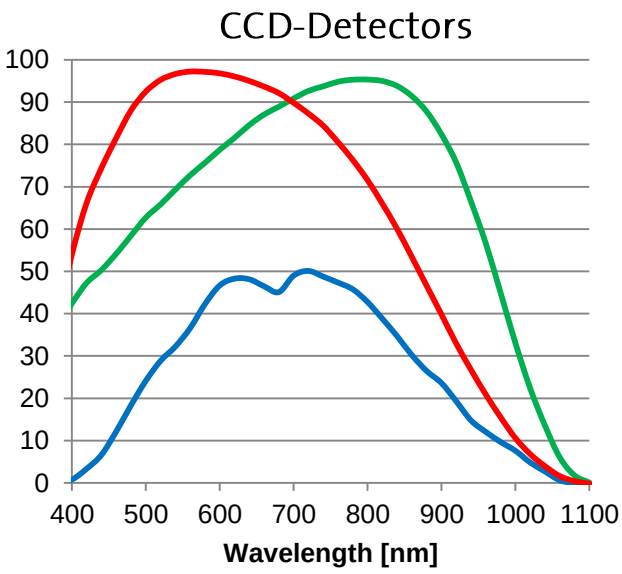
Basics of Raman Spectroscopy

Challenges for Imaging – Optimization of Beampath Efficiency







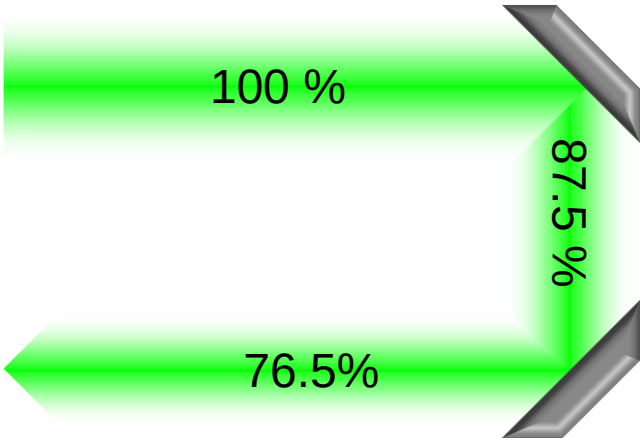


Products & Solutions

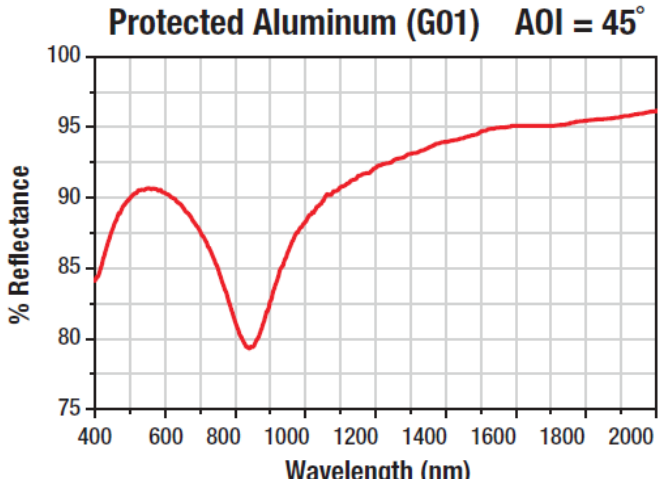
Challenges for Imaging – Optimization of Beampath Efficiency

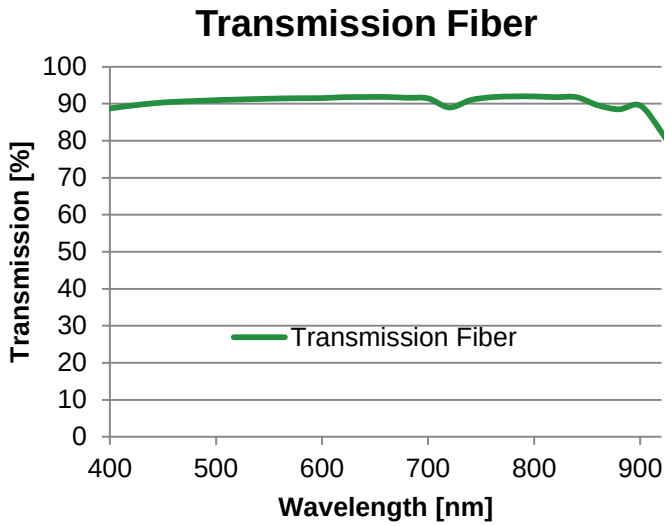
Mirrors vs. Optical Fibers:

Reflectivity of Fresh Aluminum



Source: thorlabs.de





Further benefits:

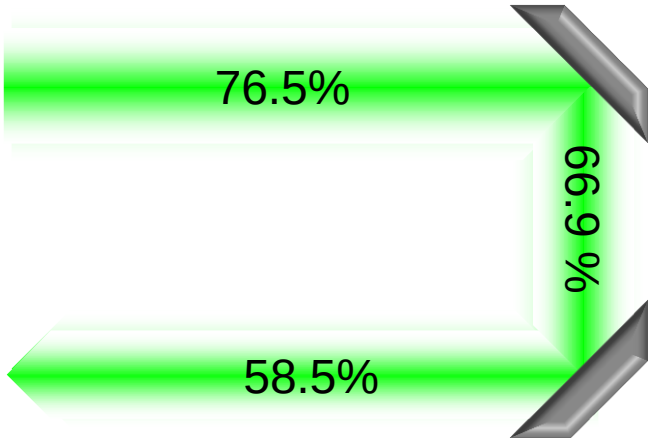
- no additional pinhole
- no additional entrance slit at spectrometer
- no additional optics for spectrometer aperture matching
- no additional optical table needed
- no restrictions in placing the optical periphery

Products & Solutions

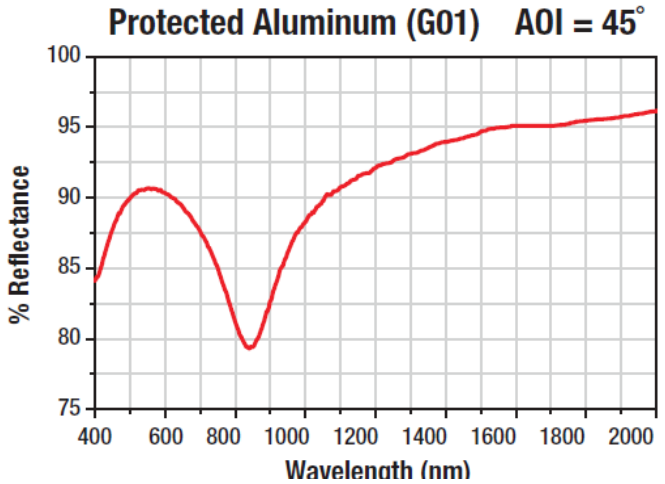
Challenges for Imaging – Optimization of Beampath Efficiency

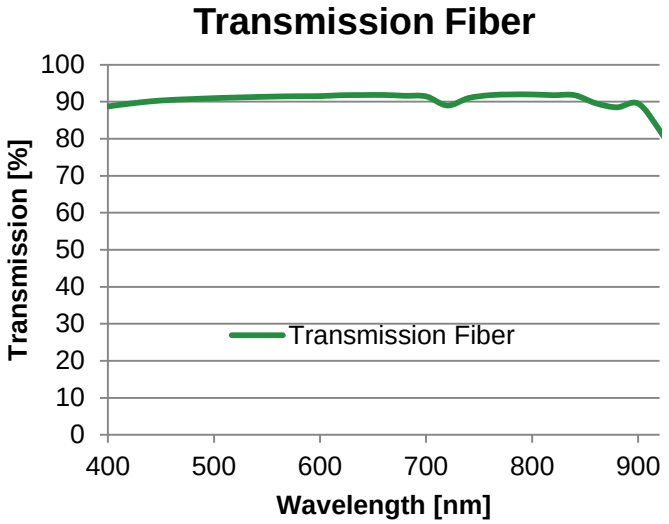
Mirrors vs. Optical Fibers:

Reflectivity of Fresh Aluminum



Source: thorlabs.de





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Products & Solutions

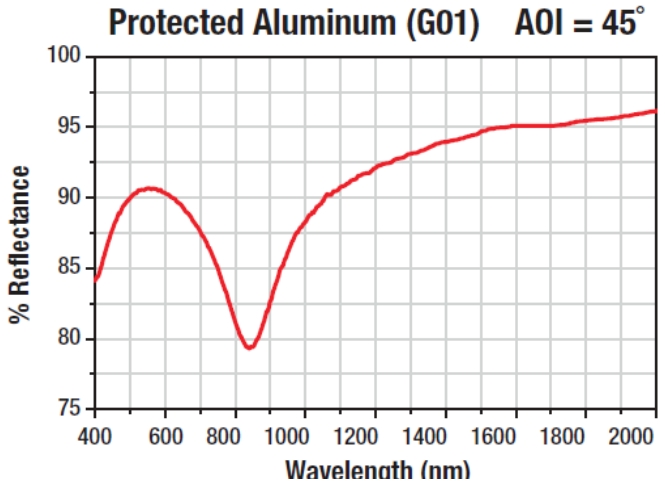
Challenges for Imaging – Optimization of Beampath Efficiency

Mirrors vs. Optical Fibers:
Reflectivity of Fresh Aluminum

Already 5 mirrors with 87.5% reflectivity

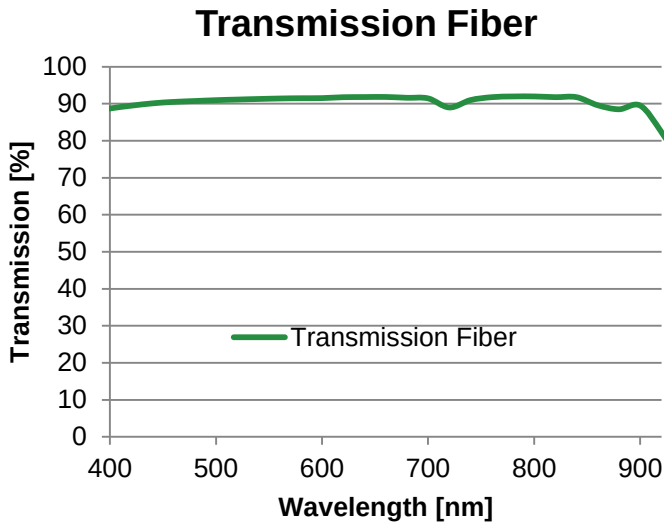
Lead to ~50% of signal loss!

Source: thorlabs.de



Alternative Technology

Optical fiber >75% total transmission
Including losses due to coupling/damping



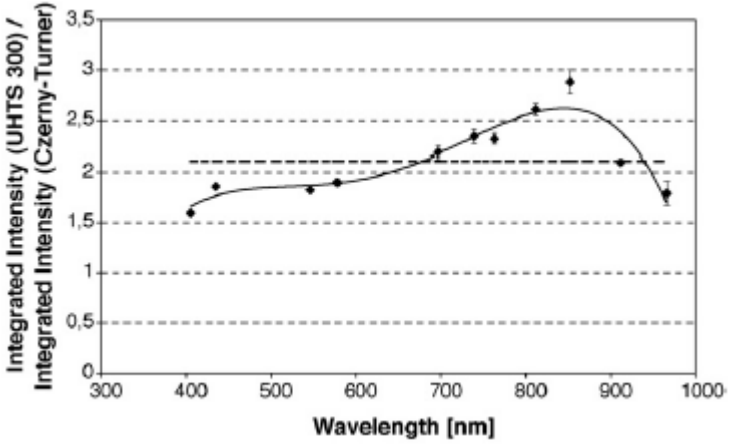
Further benefits:

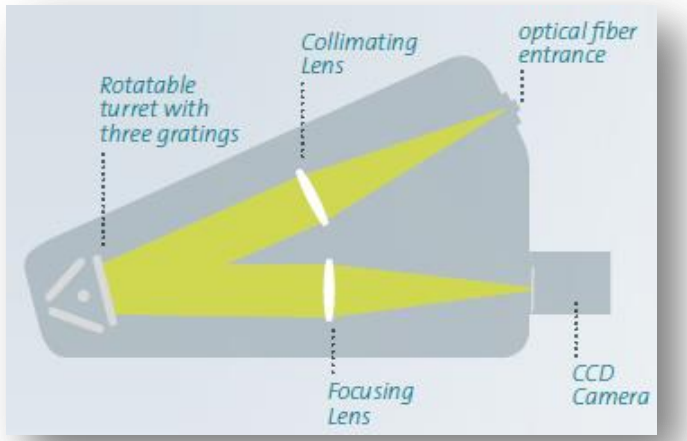
- no additional pinhole
- no additional entrance slit at spectrometer
- no additional optics for spectrometer aperture matching
- no additional optical table needed
- no restrictions in placing the optical periphery

Spectroscopic System

Ultra-High Throughput Spectrometers (UHTS)

- ✓ Lens-based imaging spectrometers
- ✓ Specifically designed for low light intensities
- ✓ Peak throughput >70%
- ✓ Symmetric peak shape (coma/astigmatism free)
- ✓ FC/APC optical fibre port
- ✓ Automatic triple-grating turret
- ✓ Can be fitted with FI- and BI-CCDs
- ✓ Fully Software controlled





Best spectrometer available for Raman Imaging

Download Brochure:
WITec Spectroscopy Solutions

Fast Raman Imaging

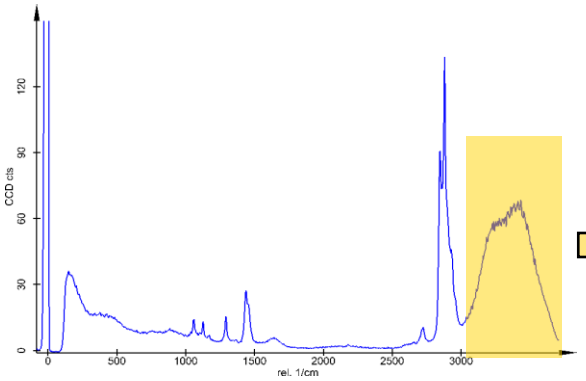
From Spectroscopy to Fast Imaging

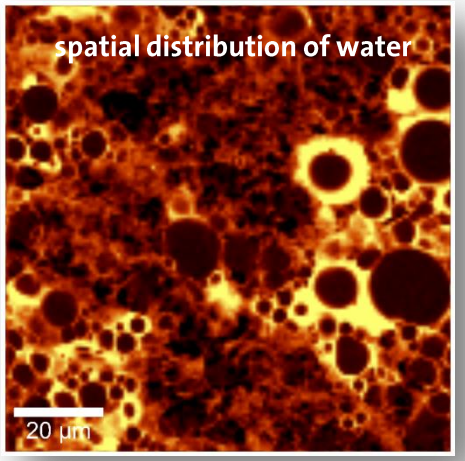
Sample: 2 immiscible phases

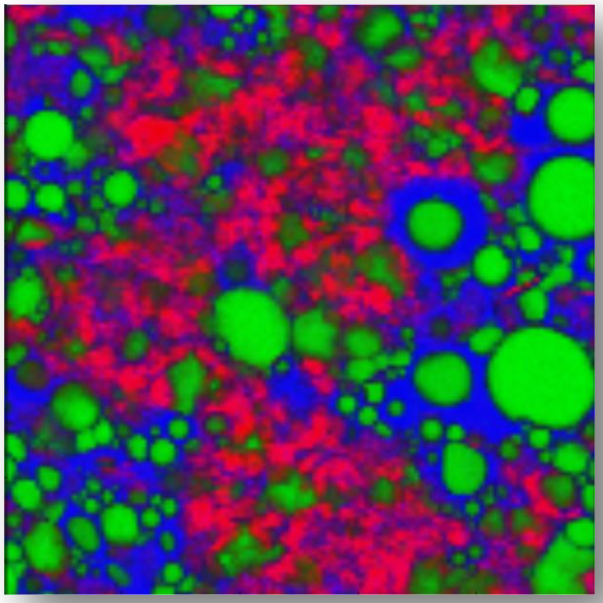
Excitation: 532nm, 2 mW

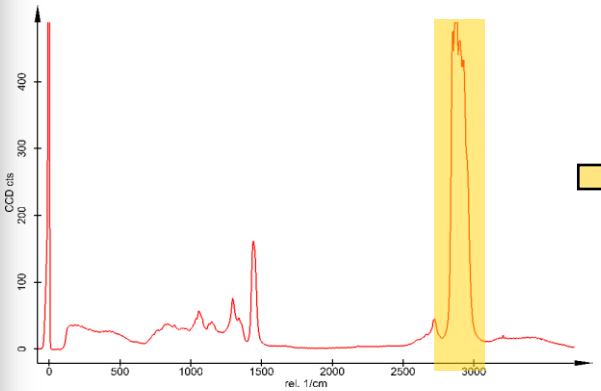
Range: 100 x 100 µm²

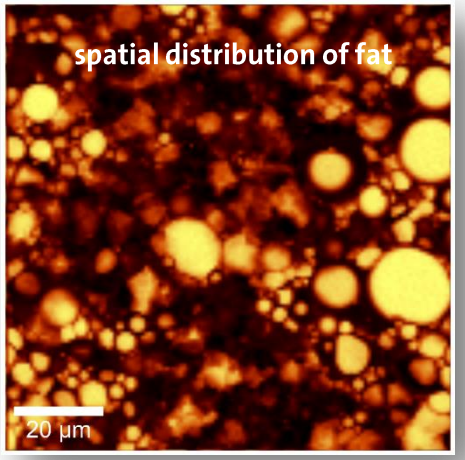
Resolution: 180 x 180 spectra





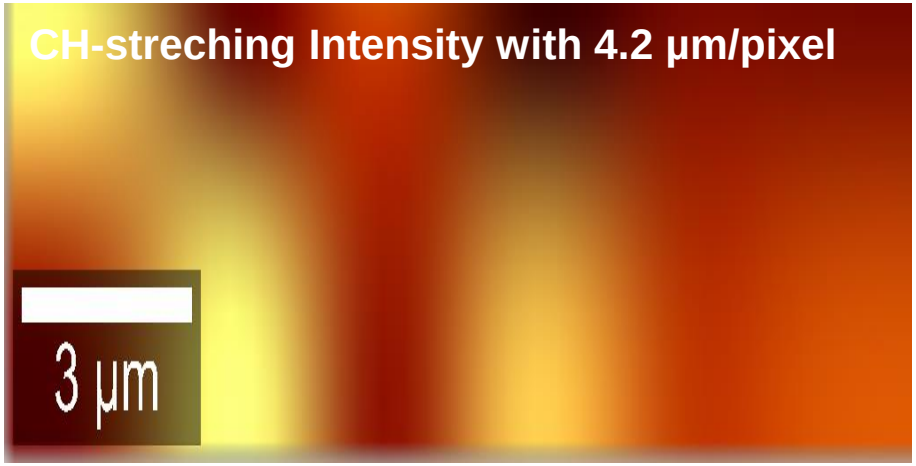


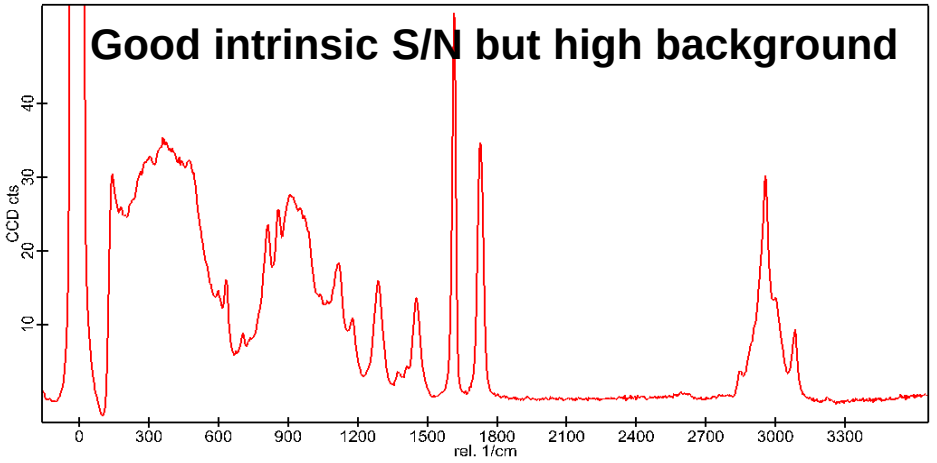


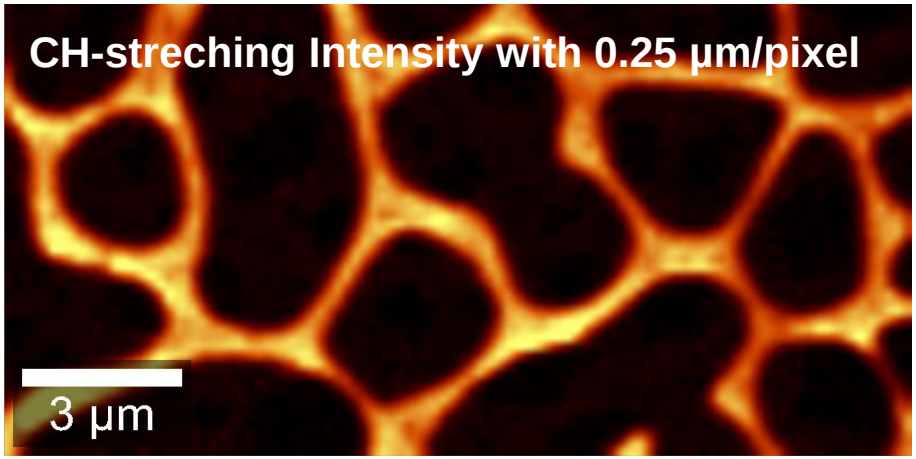


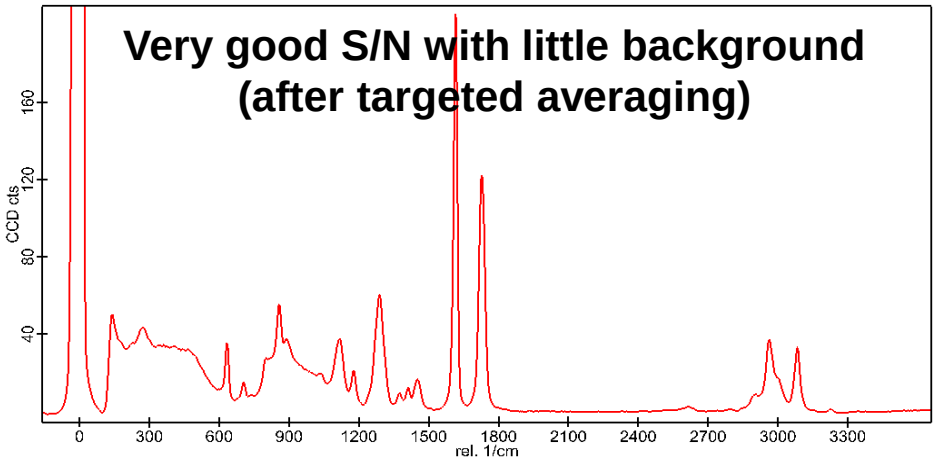
Fast Raman Imaging

The benefits of high resolution with fast scanning times (PET/PMMA blend)





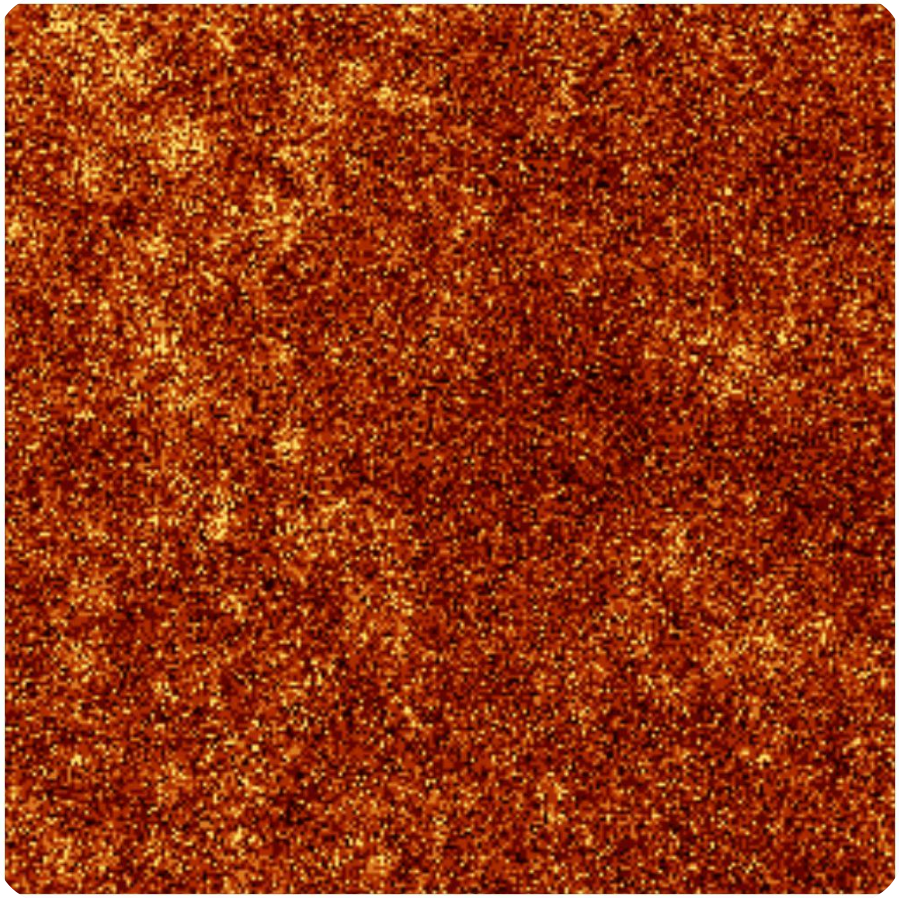




- Starting at low spatial resolution with high individual S/N (few spectra) spatial information cannot be recovered
- Starting at high spatial resolution with low individual S/N (many spectra) spectral averaging can recover Signal

Fast Raman Imaging

Fast Imaging: Confocal & Layer by Layer

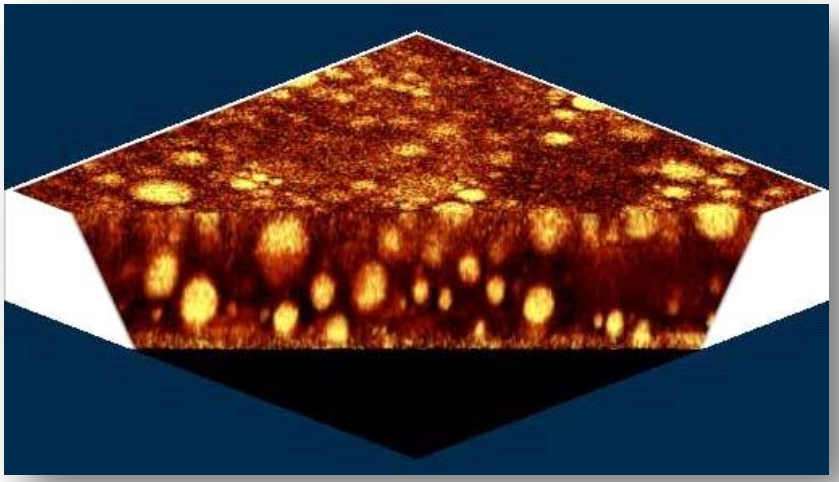


Evaluated band: CH-stretching (3D Reconstruction)

Scan Range XY: 100 x 100 µm² (180x180 pixel)
Scan Range Z: 30 µm (32 layers (~1µm step)
Total No. of spectra: 1.04 Mio.

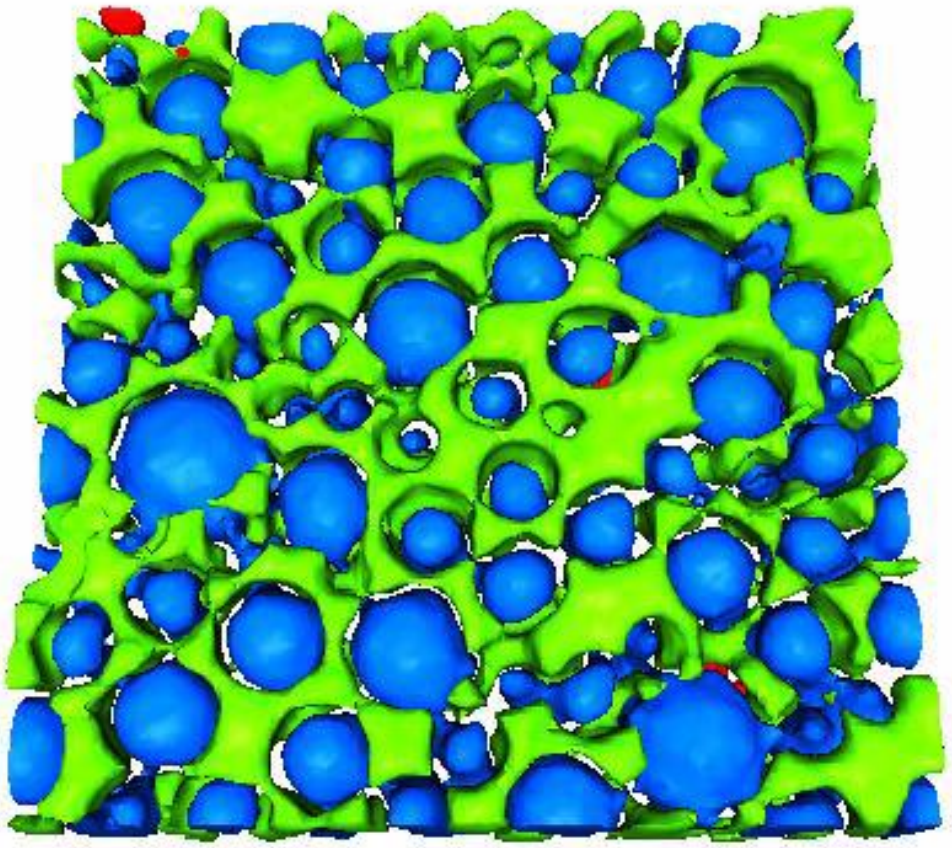
Integration 500ms /spectrum (comparison)
⇒ 1 image 4.5 hours, 32 images = 6 days

WITec system: Integration 1 ms /spectrum
⇒1 image 32s , 32 images = 18 minutes



Fast Raman Imaging

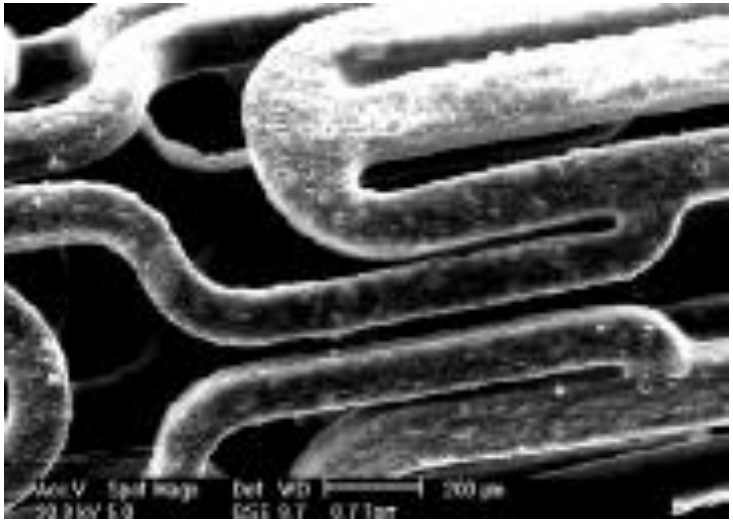
Fast Imaging: Confocal & Layer by Layer

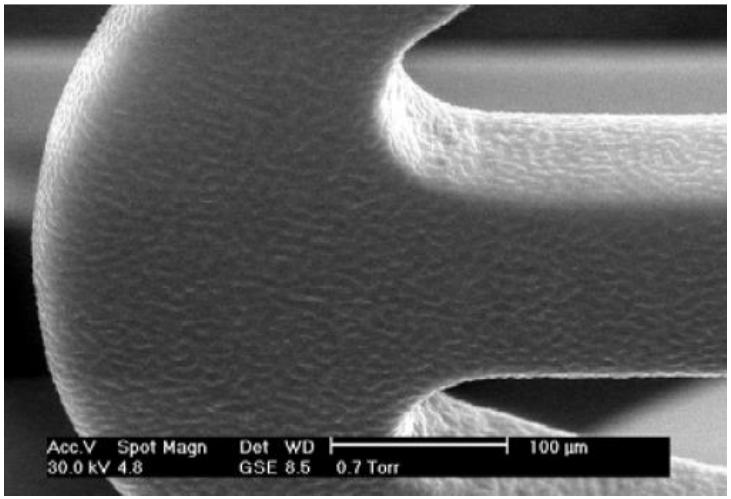


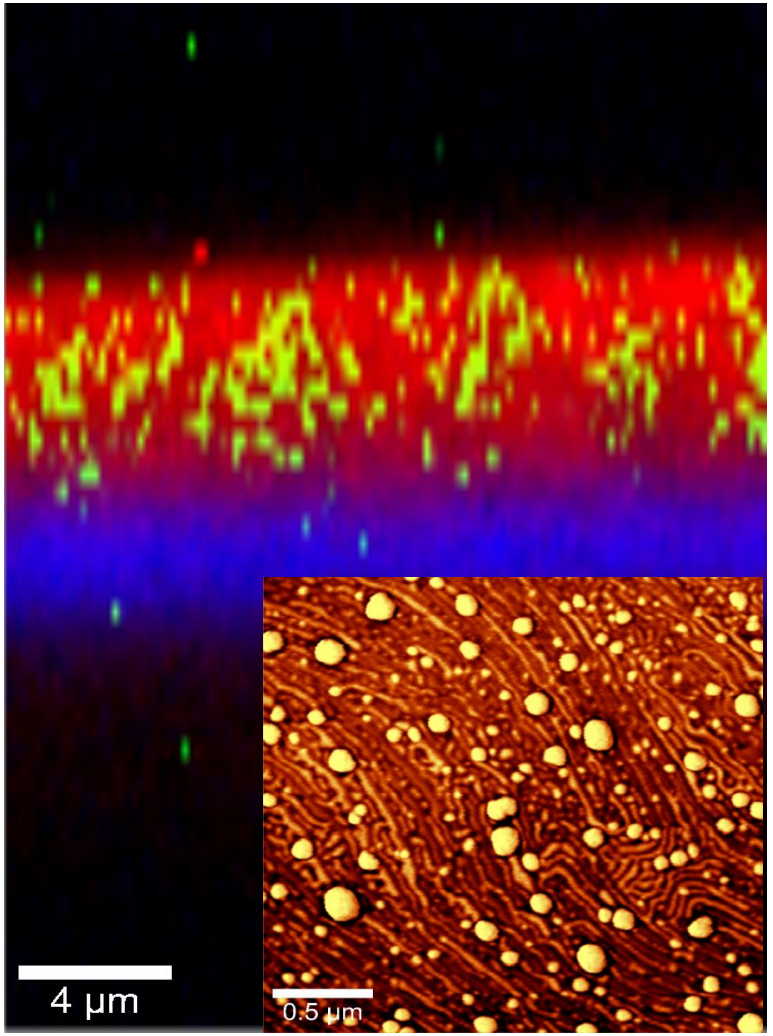
Video Link: WITec Large Area Confocal Imaging

Fast Raman Imaging: Depth Scans

Imaging molecular loading within polymers (drug eluting stents)







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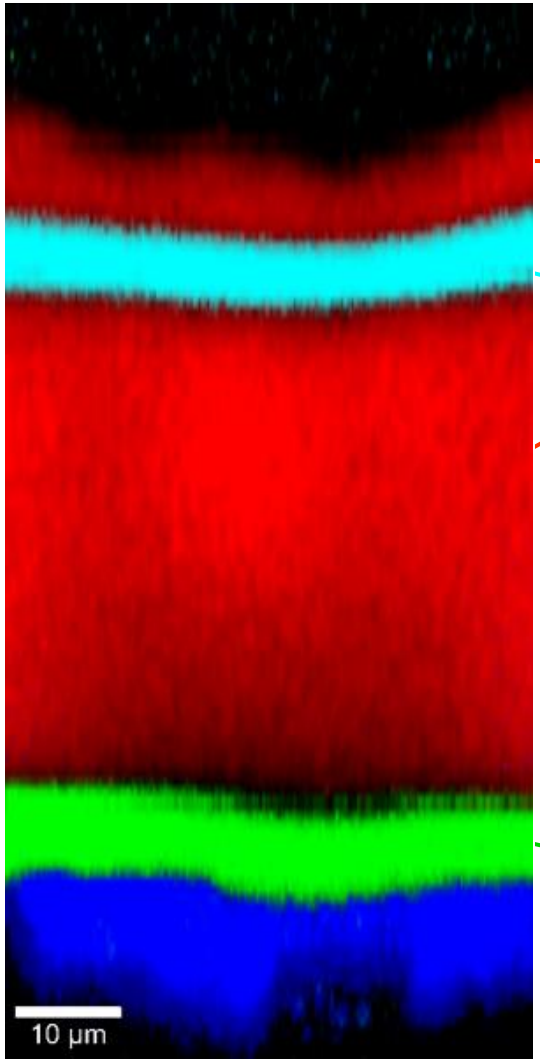
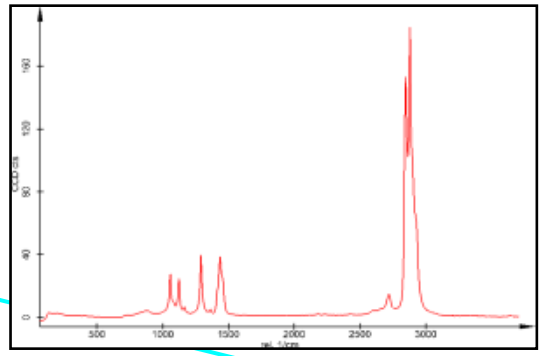
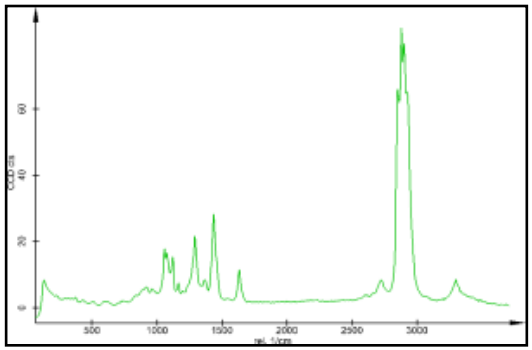
Fast Raman Imaging: Depth Scans

Packing Technology: Optically deconstructing juice containers



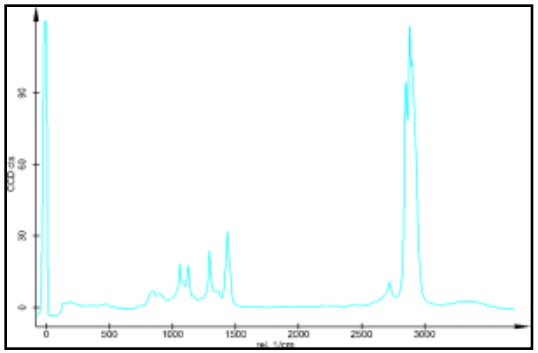


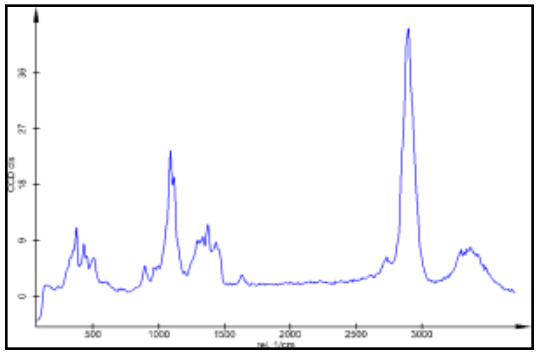
50µm x 100 µm² depth scan,
120 x 200 = 24000 spectra, 0.05 s.
100x objective (NA= 1.25), 532nm



inner coating of a fruit juice container

multilayer polymer film

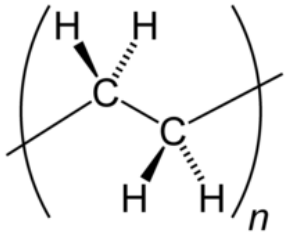




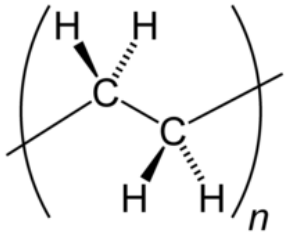
Download AppNote Polymers:
Correlative Raman Microscopy on Polymers

Fast Raman Imaging: Depth Scans

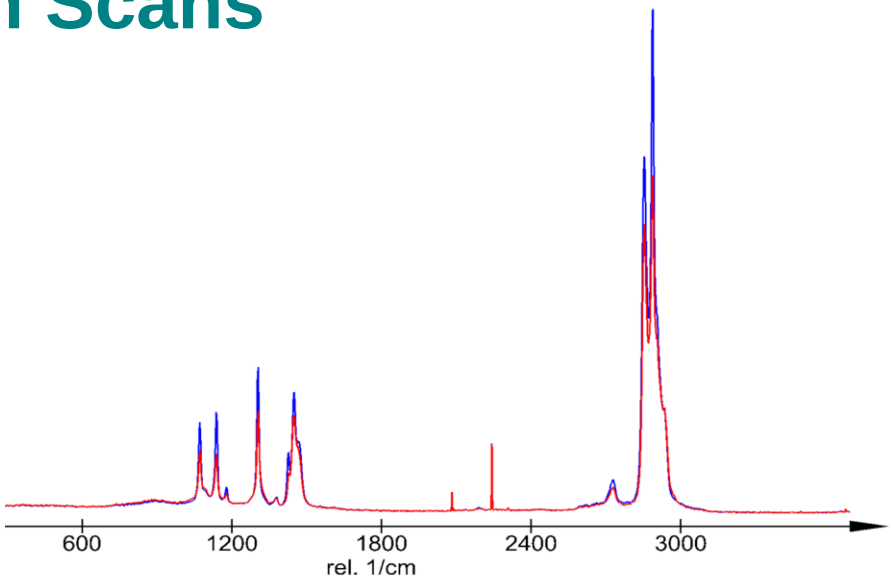
Imaging minimal chemical differences

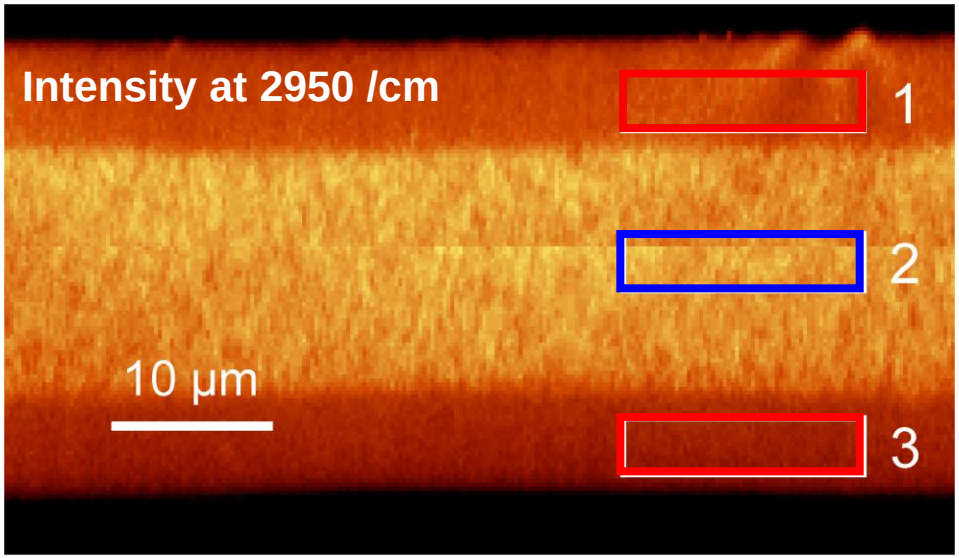


High density PE (HDPE)



Linear low density PE (LLDPE)



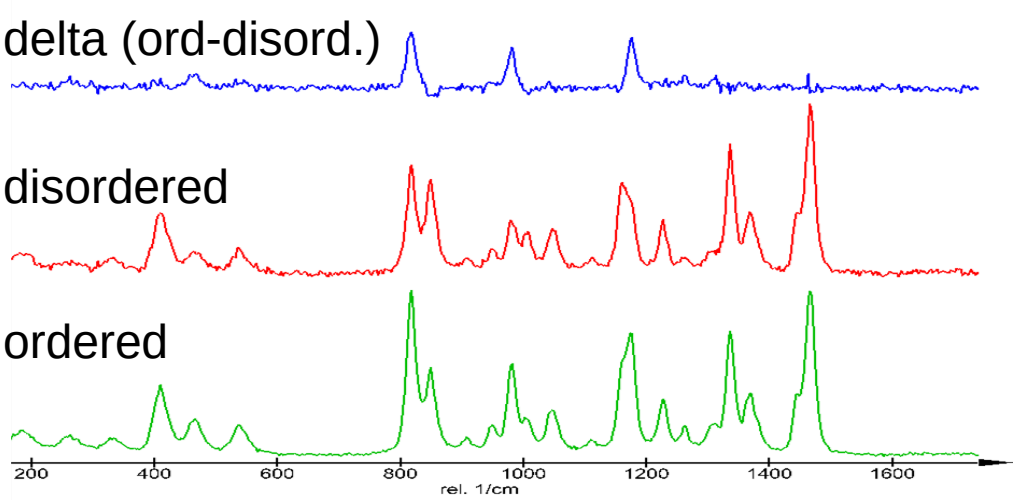


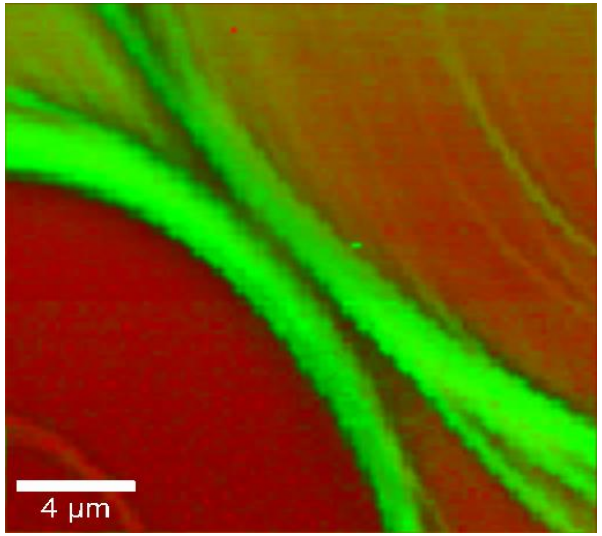
Raman Spectral Imaging

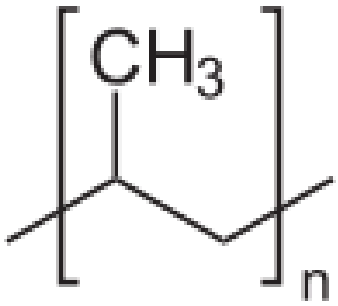
Depth profiling
60 x 35 micrometer x-z scan
240 x 140 pixel (= 33 600 spectra)
100 ms per spectrum
10 mW @532 nm

Fast Raman Imaging: Molecular Orientations

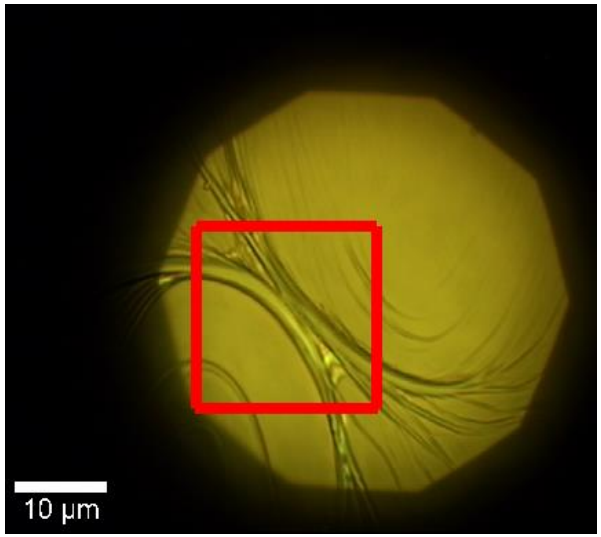
Imaging differences polymer orientation

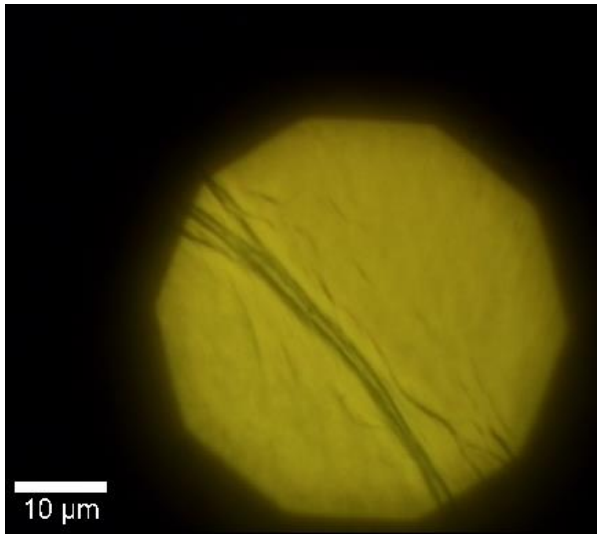






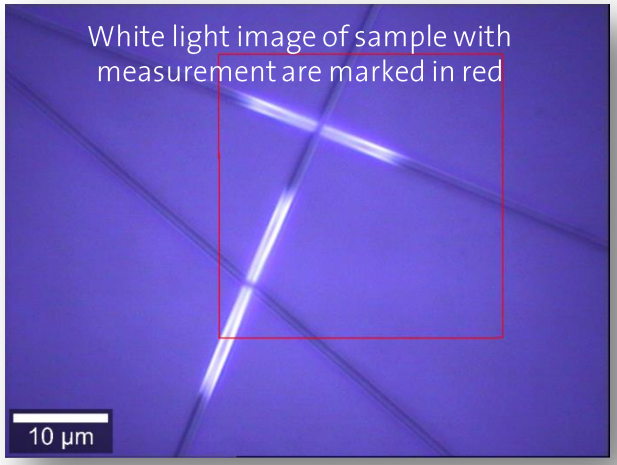
polypropylene

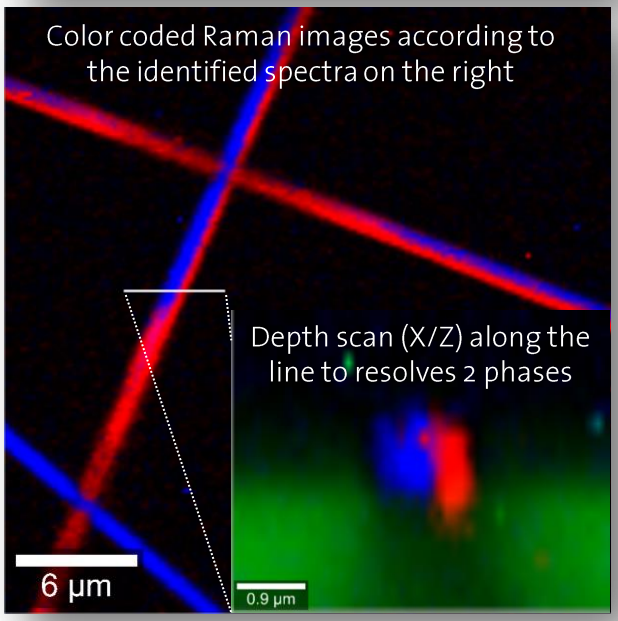


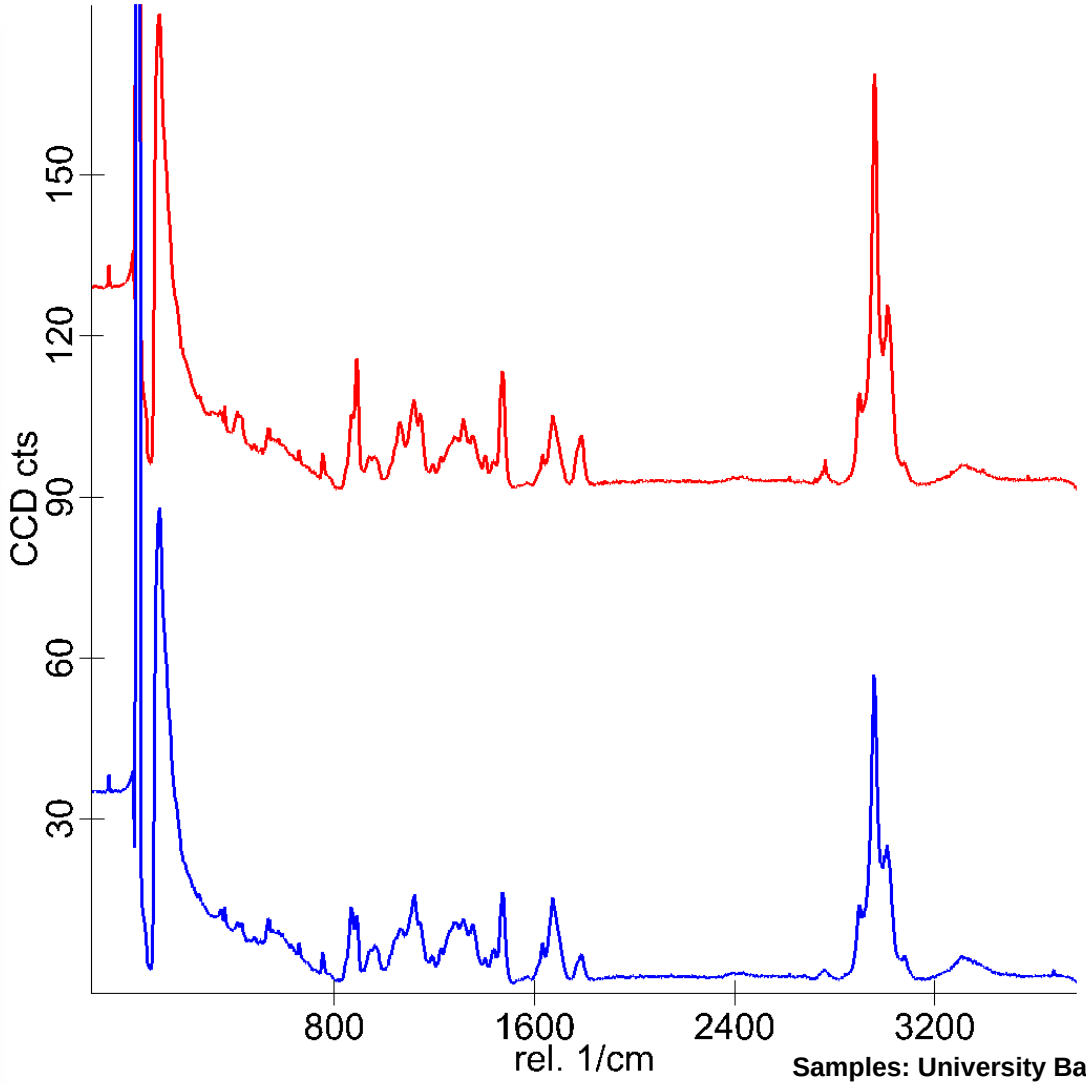


Fast Raman Imaging

Generating Chemical Contrast – Pushing Resolution Limits



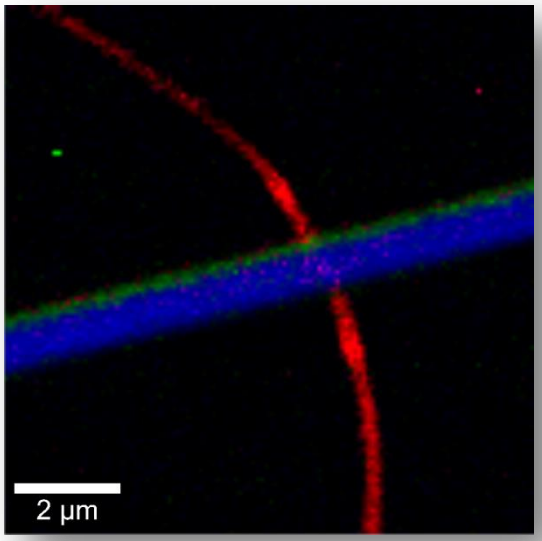


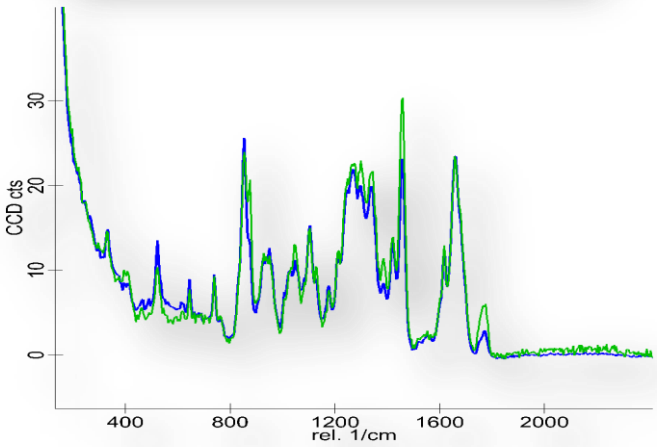


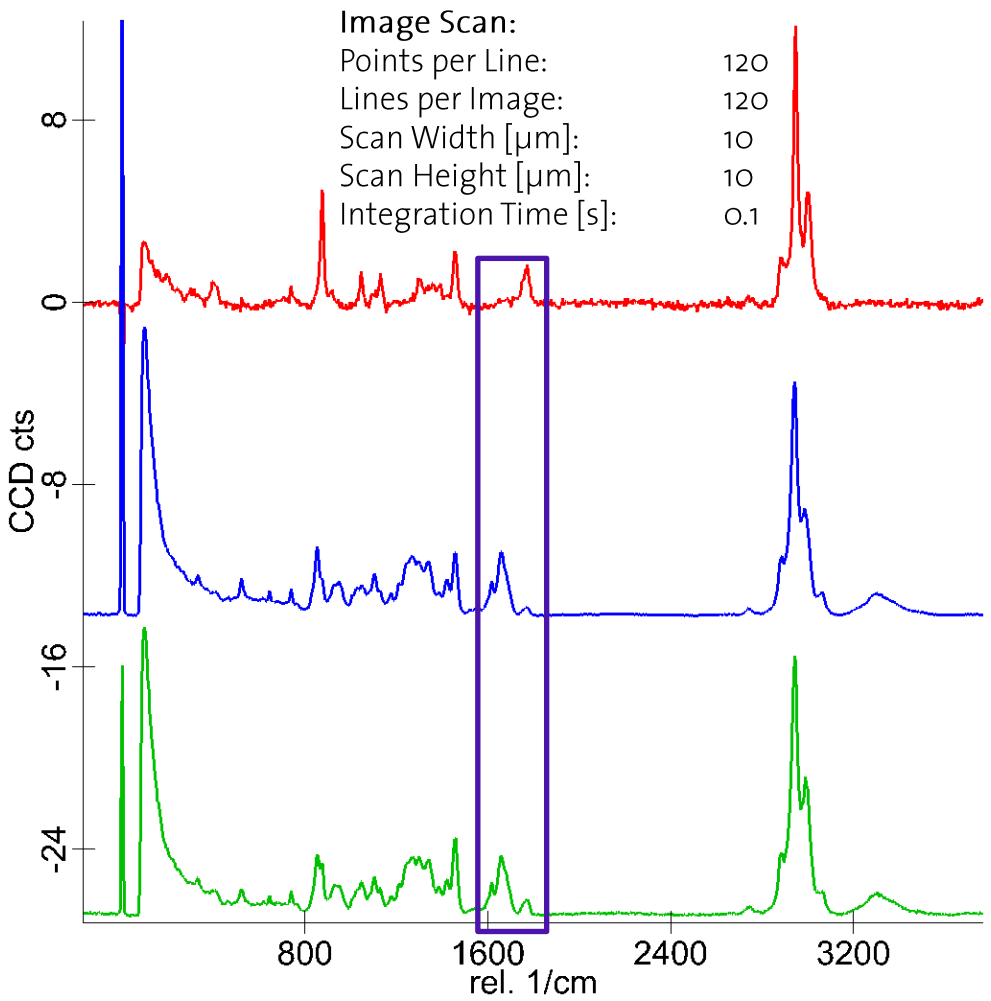
Samples: University Bayreuth

Fast Raman Imaging

Generating Chemical Contrast – Pushing Resolution Limits







Samples: University Bayreuth

Multimodal Imaging – AFM & Raman

Correlating Topography and Chemistry

Confocal Raman Microscopy

- identification of chemical phases
- lateral resolution ≈ 200-300 nm
- depth resolution up to 500 nm
- sensitivity: < 30nm layer thickness in milliseconds

AFM

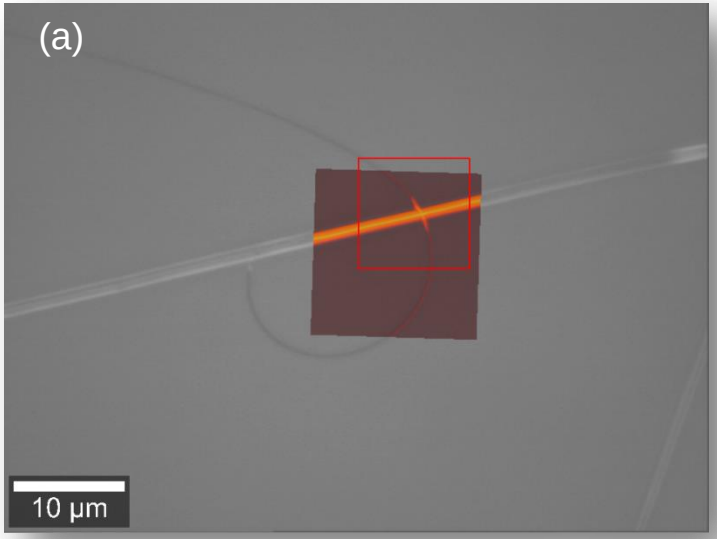
- high resolution topographic imaging
- mechanical properties on the nm scale (stiffness, adhesion, viscosity..)

Switching between the measurements methods upon a turn of the turret.

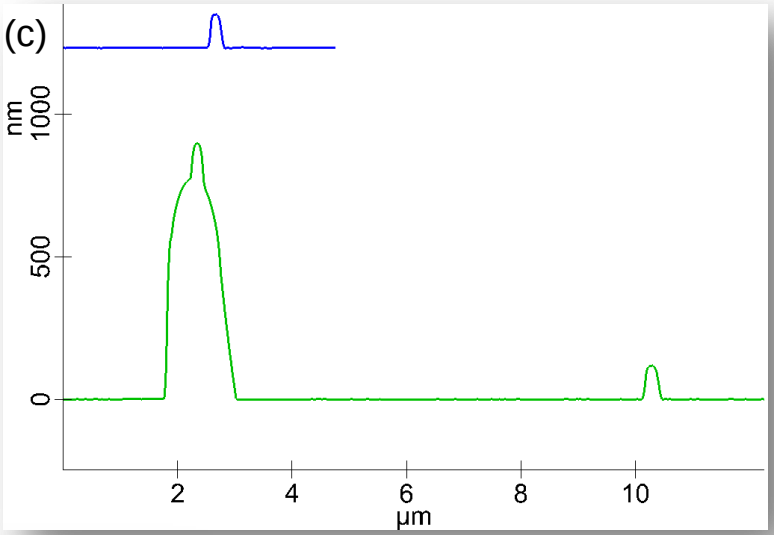
Download Brochure:
WITec Atomic Force Microscopy
WITec alpha300 Multimodal Imaging Platform

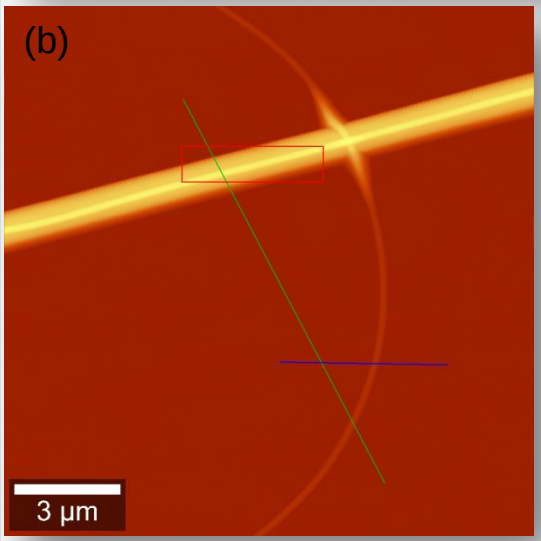
Fast Raman Imaging

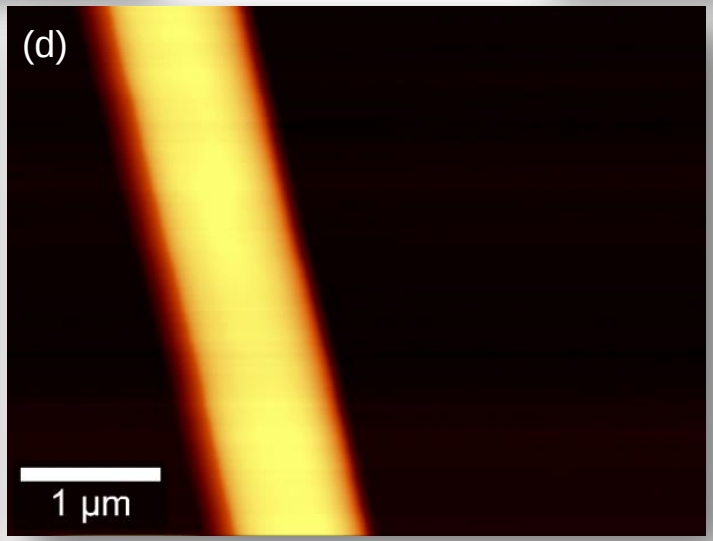
Generating Chemical Contrast – Pushing Resolution Limits



(a) White-light Microscopy image of target fibers overlain with AFM topography
(b) AFM with cross sections indication
(c) Color coded cross sections with absolute topography
(d) AFM scan along a homogenous fiber without small fiber attached







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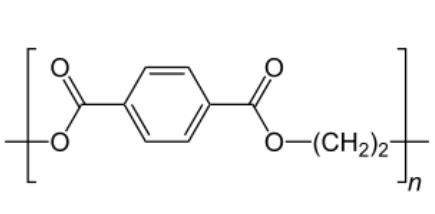
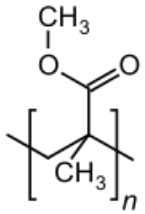
Multimodal Imaging – AFM & Raman

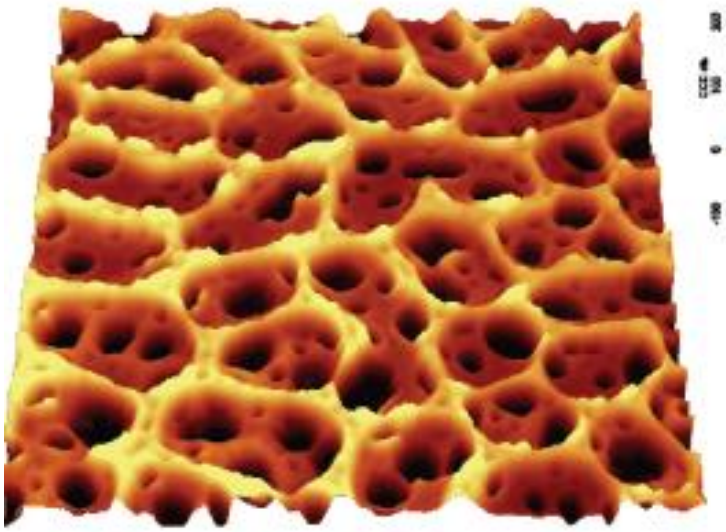
Correlating Topography and Chemistry



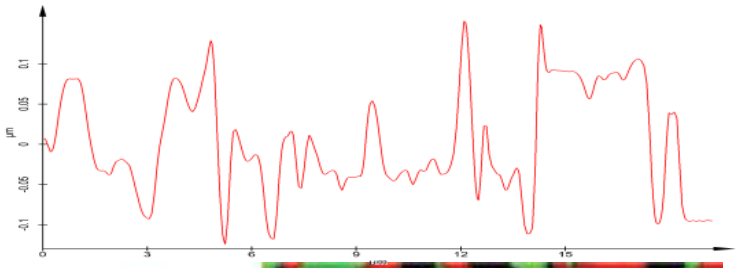
PET

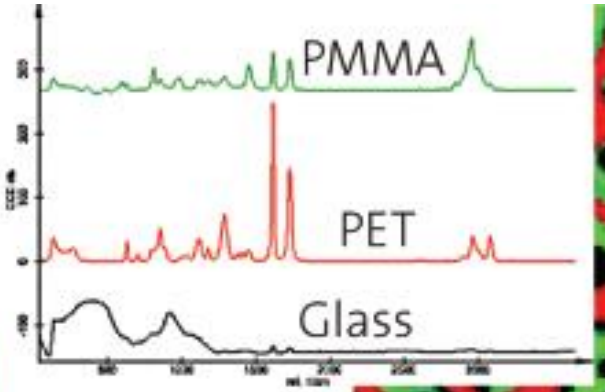


PMMA

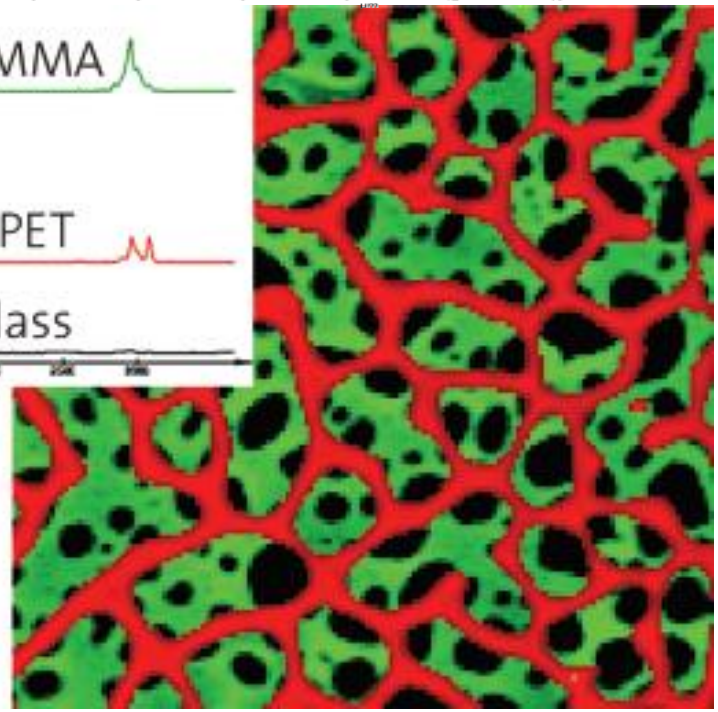


A 20x20x0.2 µm³ scan area





C



B

20x20 µm² scan area, 150x150 pixel

- 0.05 s integration time
- total 22500 spectra

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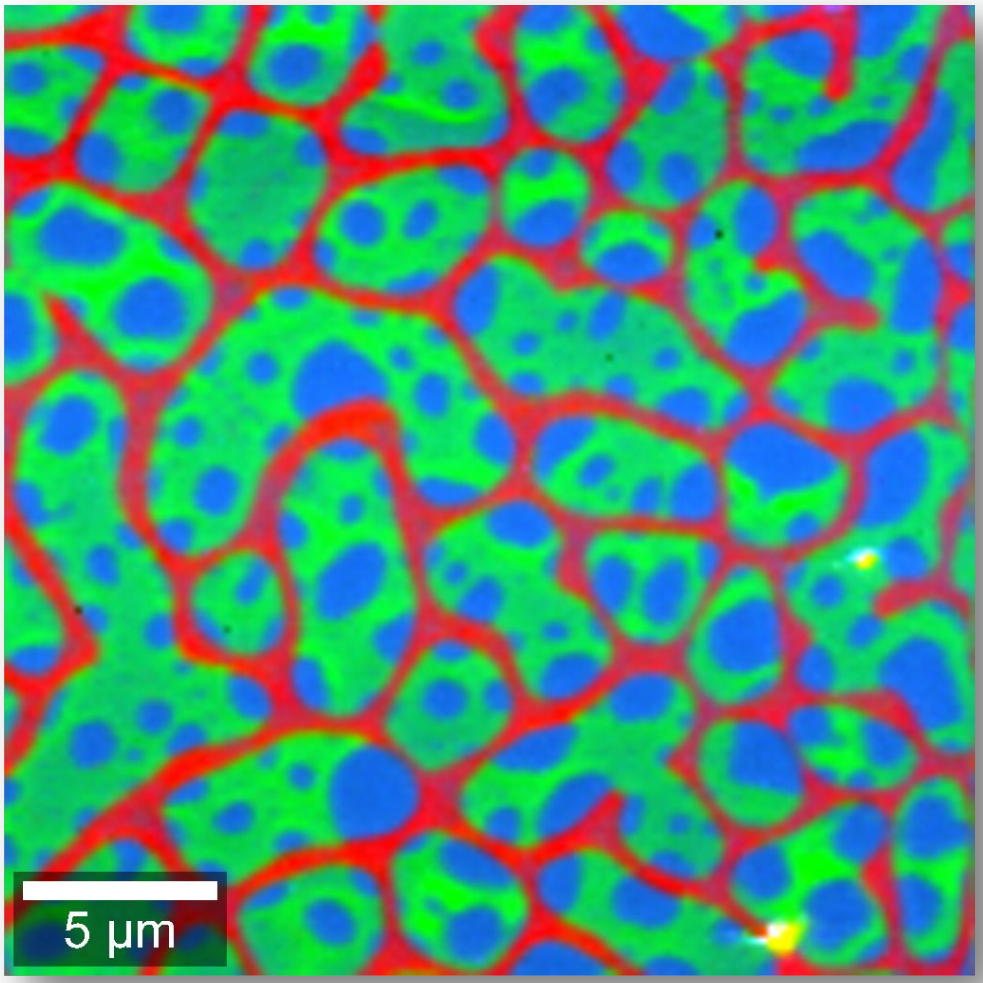
Multimodal Imaging – AFM & Raman

Correlating Topography and Chemistry



WITec SuiteFIVE

TrueComponent Analysis

Live Demo
during coffee break ...

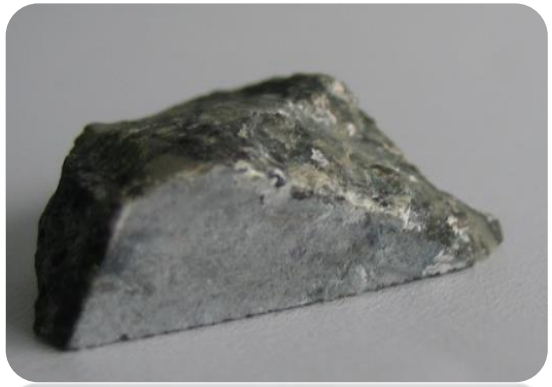
Video Link: WITec SuiteFIVE – Revolutionizing Raman Imaging

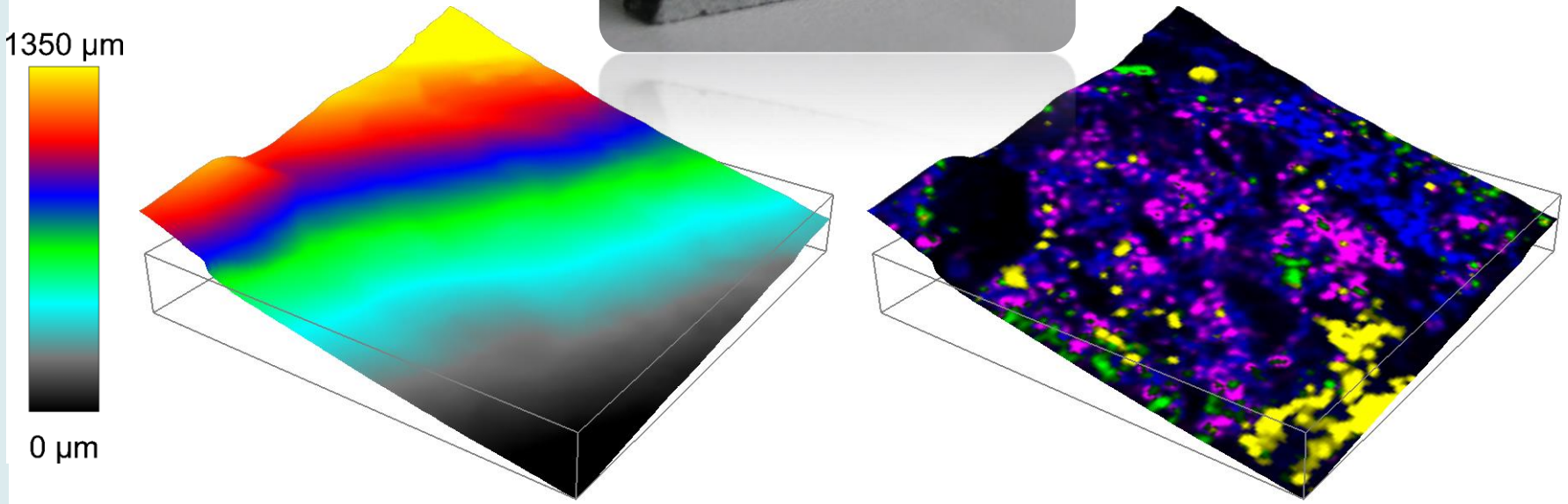
Download AppNote: SuiteFIVE

Sample from the Josefsdal Chert 99SA07
Sample courtesy of Frances Westall, CNRS Orleans, France

Fast Raman Imaging

Generating Chemical Contrast – Confocality and Rough Surfaces



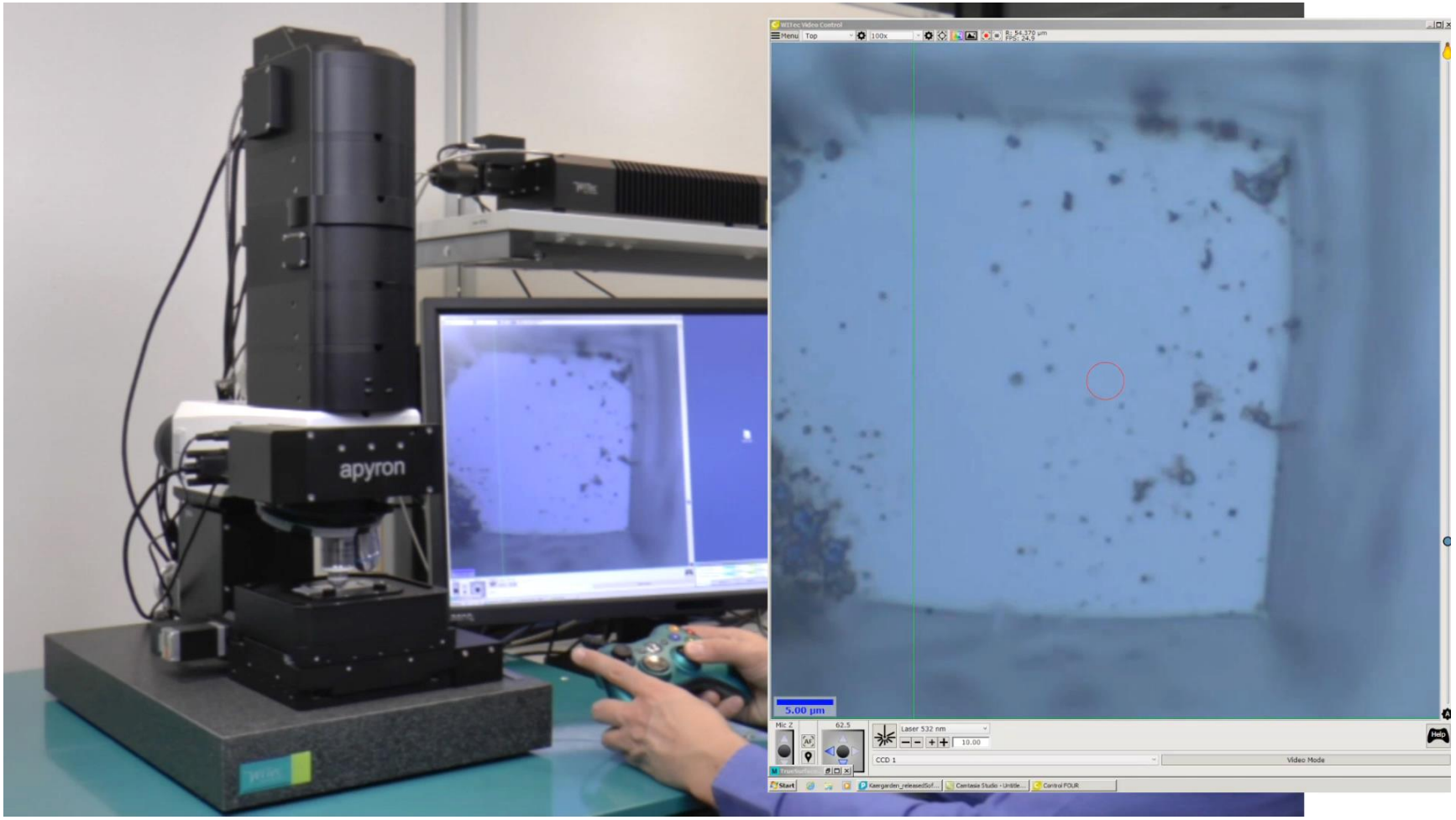


Topography

Confocal Raman Image overlaid on the topography

WITec TrueSurface – Get all the information

From the inventors of topography corrected confocal Raman



Video Link: TrueSurface Microscopy - Performance Redefined

Download AppNote: TrueSurface

WITec ParticleScout – Saves your time!

Find, Classify and Identify Particles

Video Link: ParticleScout Video Introduction

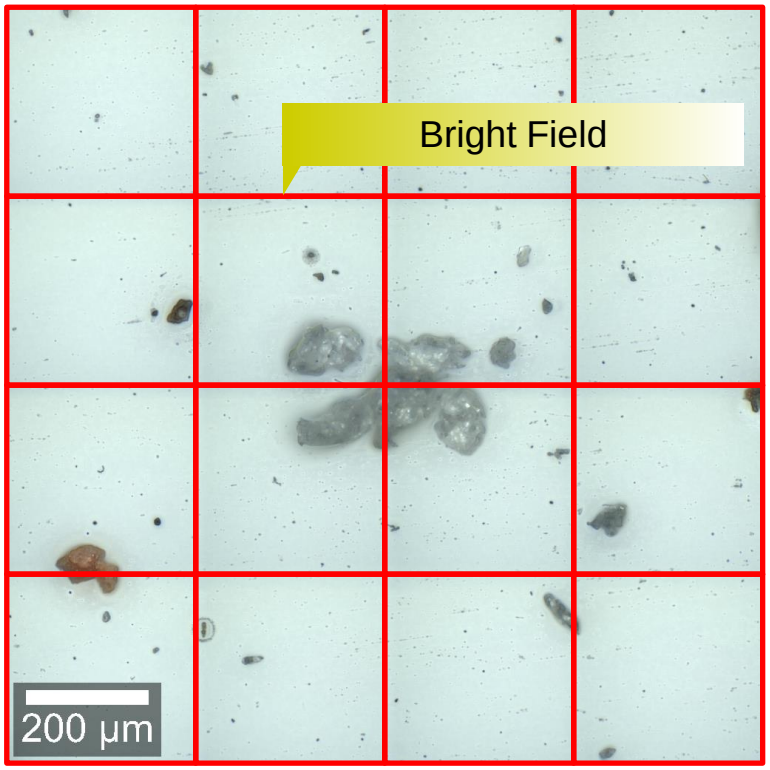
Download AppNote: ParticleScout

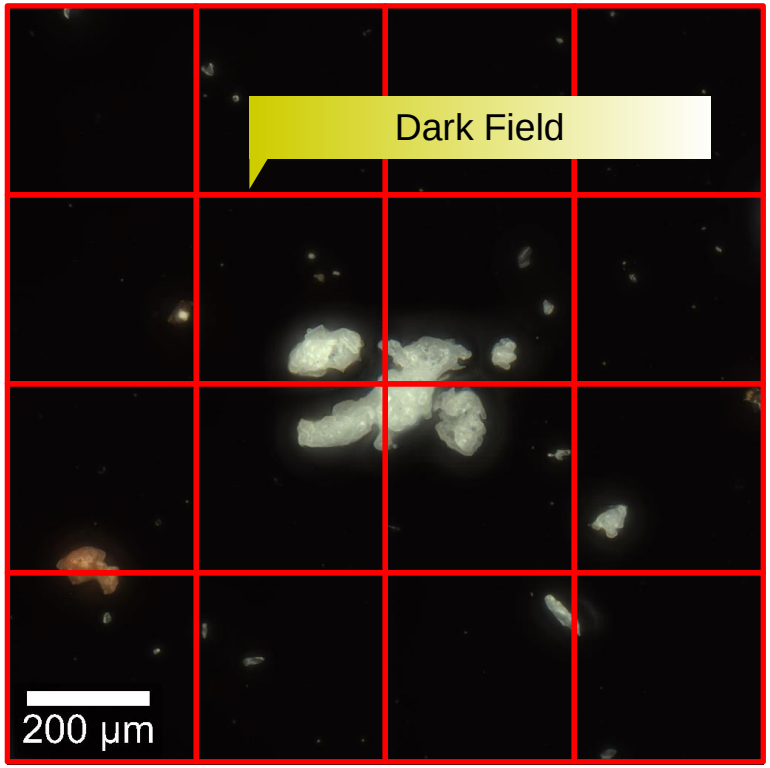
NEW

WITec ParticleScout – Saves your time!

Find, Classify and Identify Particles

Find



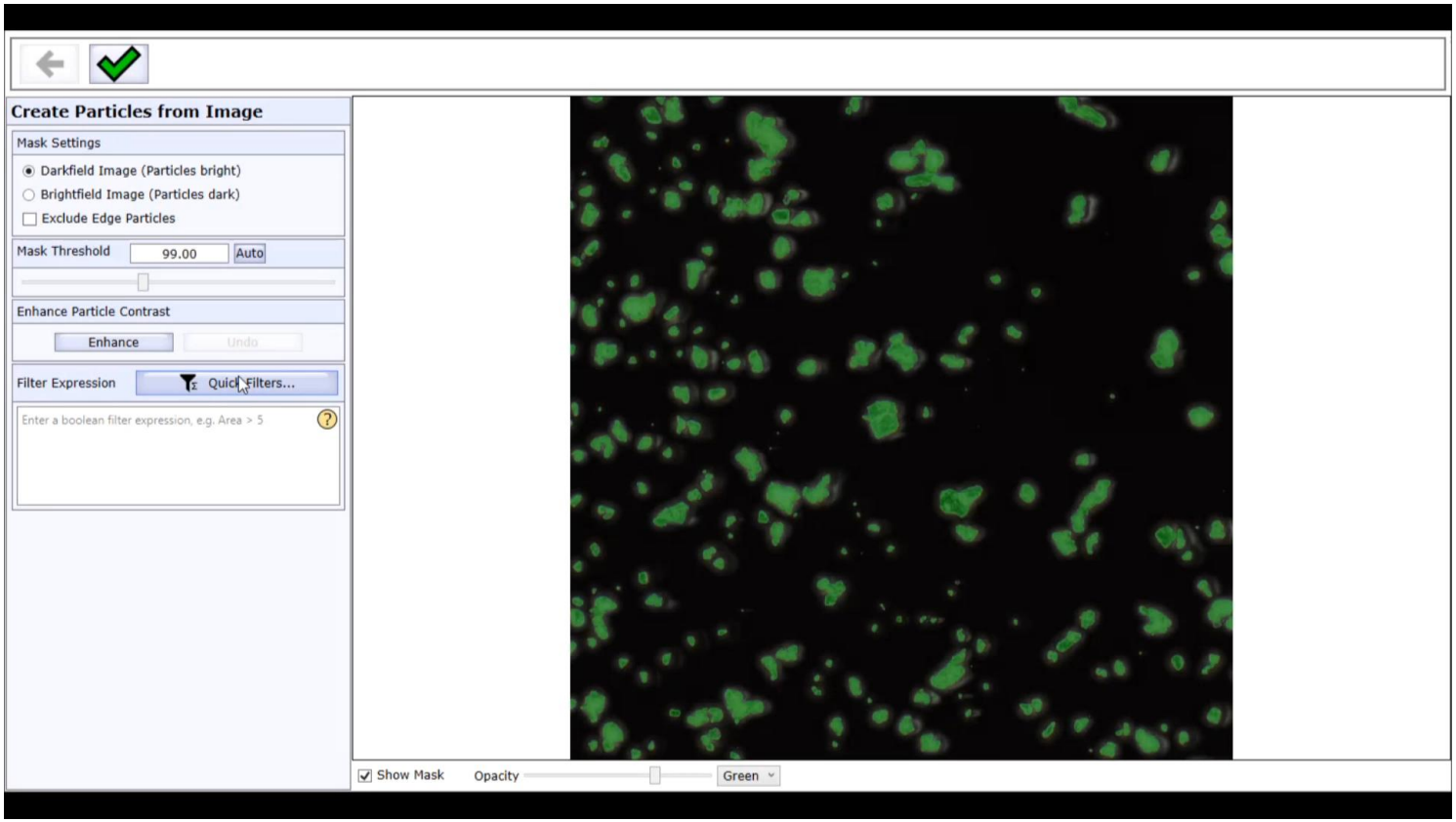


WITec ParticleScout – Saves your time!

Find, Classify and Identify Particles

Classify

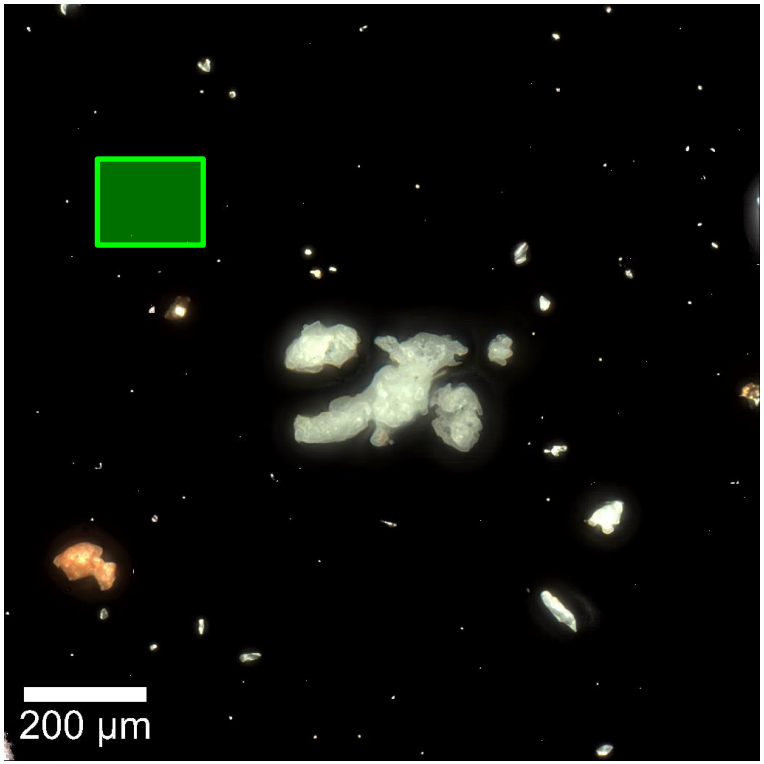
Generate Mask

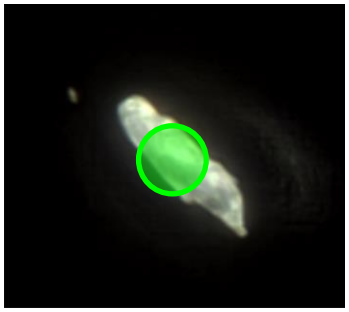


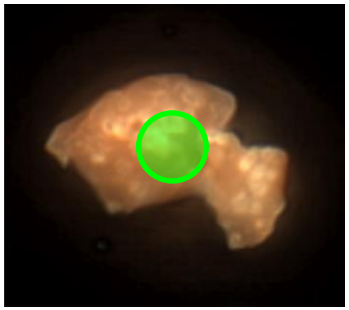
WITec ParticleScout – Saves your time!

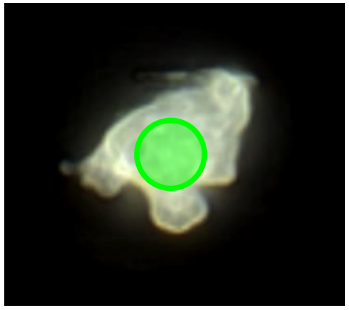
Find, Classify and Identify Particles

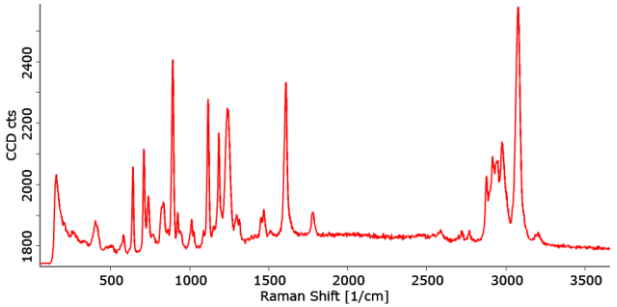
Raman spectral acquisition at each particle

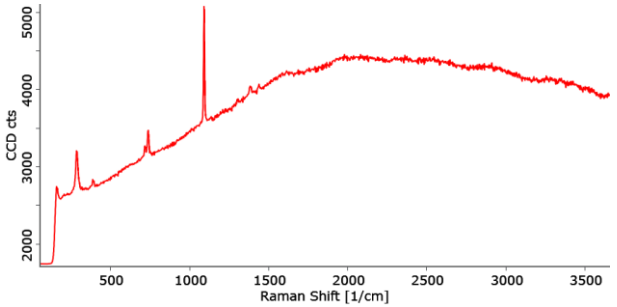


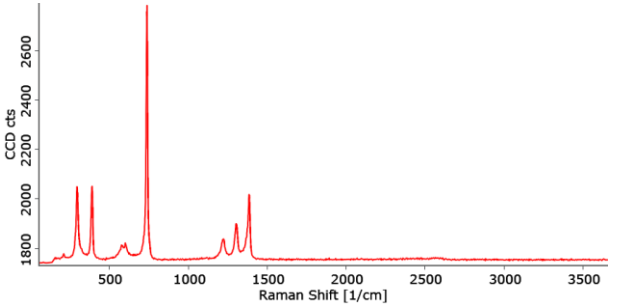










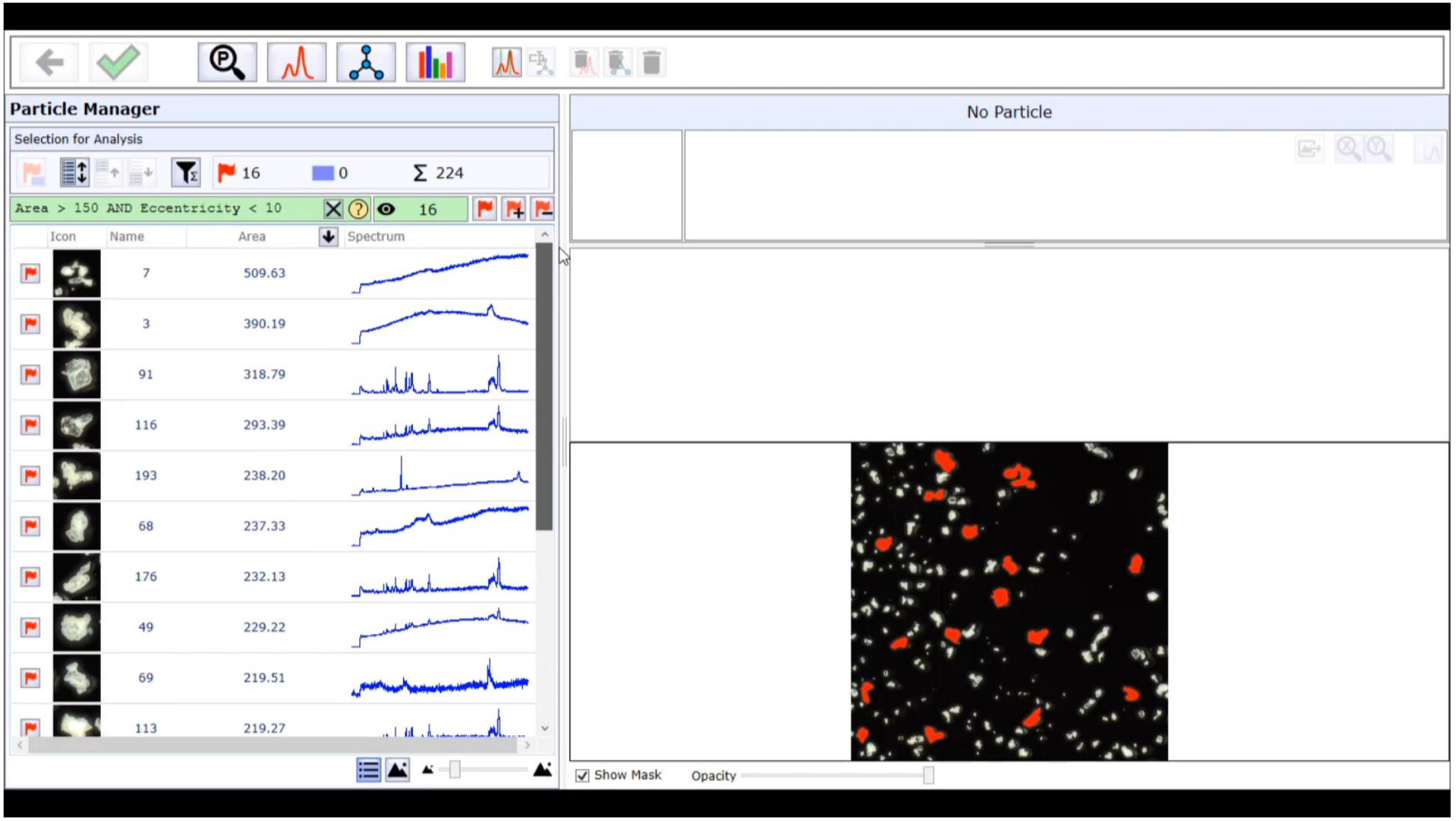


WITec ParticleScout – Saves your time!

Find, Classify and Identify Particles

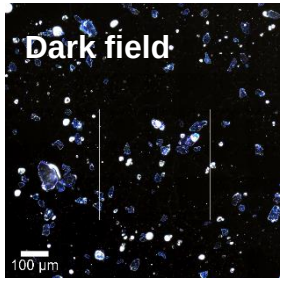
Identify

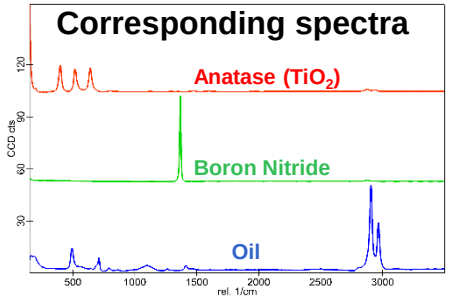
Database Search with WITec TrueMatch

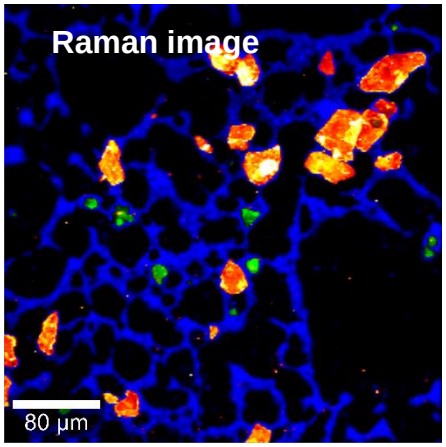


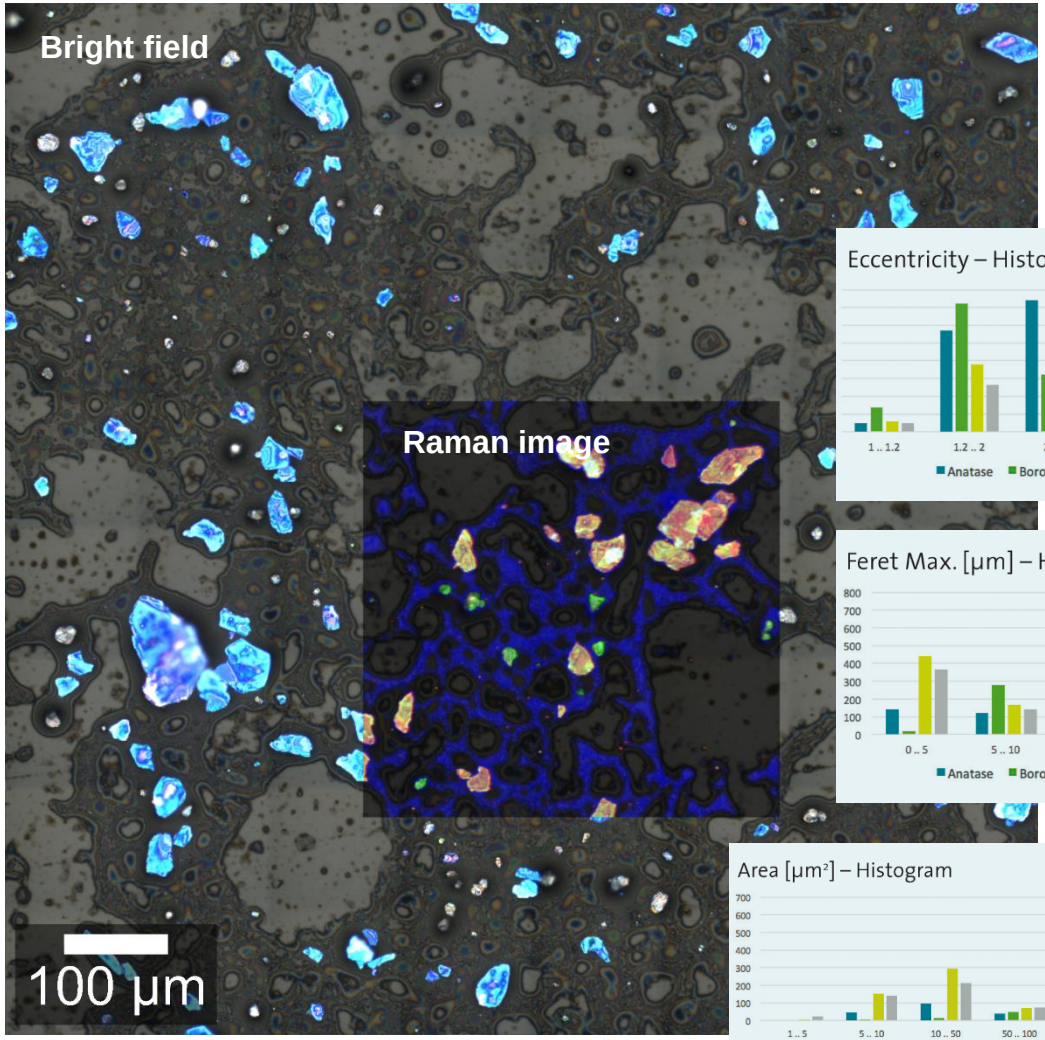
WITec ParticleScout – Saves your time!

Find, Classify and Identify Particles

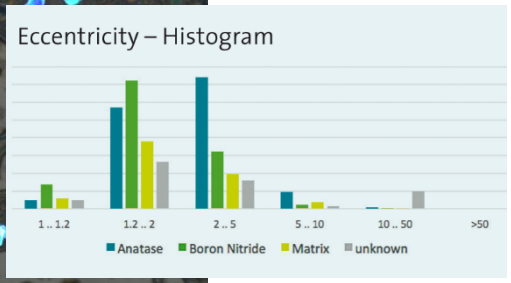


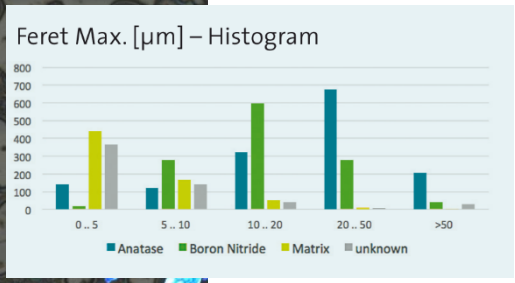


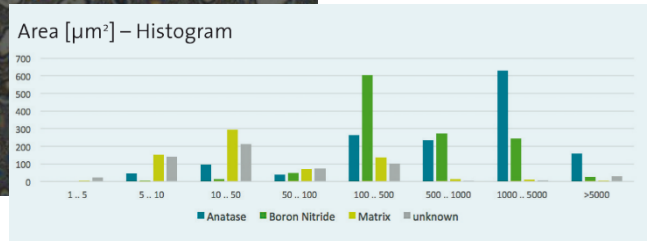




Report







Cosmetic Peeling Cream (3941 particles were analyzed)

Thank you for your kind attention

Further examples and information
can be found in:

„Confocal Raman Microscopy“
by T.Dieing, O.Hollricher & J.Toporski (eds.)