Hochaufgelöstes konfokales Raman-Imaging

Neue Einblicke in der Polymeranalytik

AK Polymeranalytik, Darmstadt 2019
Dr. Jan Englert, WITec GmbH
WITec Headquarters – Partners & Network
Cutting-Edge Technologies in Microscopy & Spectroscopy

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**
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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

Raman
AFM
SNOM

Download Brochure:
WITec alpha300 Microscope
Products & Solutions
Flexible Beampath – Accepts new challenges by design

- **VIDEO-CAMERA COUPLER**
  - able to house different camera models

- **AFM BEAM-DEFLECTION UNIT**
  - for cantilever-based topography and near-field optical measurements

- **MICROSCOPE TURRET**
  - up to 6 objectives
  - WITec SPM objective

- **STEPPER MOTOR**
  - for 2-movements, focusing, depth profiling, 3D stacks

- **POSITIONING & SCAN STAGES**
  - cooperative scanning of piezo-driven and/or motorized stages

- **LASER INPUT COUPLERS**
  - Single & triple wavelength modules
  - combinable and stackable for more wavelengths

- **KÖHLER WHITE-LIGHT ILLUMINATION**
  - with adjustable LED intensity

- **OUTPUT COUPLER**
  - additional spectrometers

Download Brochure:
WITec alpha300 Microscope
Products & Solutions
When benchtop and upgrade capabilities count – WITec *alpha ACCESS*

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

Download Brochure: WITec alpha300 access
Products & Solutions
Chemical and Nanoscale Structural Imaging in one System – alpha300RA

Download Brochure:
WI Tec 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

An inverted beam path allows liquid samples to be placed on the fixed plane of the stage for quick and repeatable measurements.

Bulky samples that would be challenging to investigate underneath a conventional microscope objective turret can be accommodated by placing them on the stage of the alpha300 Ri.

The motorized sample stage also facilitates the mounting of environmental enclosures and other accessories.

Download Brochure:
WITec alpha300 Ri Microscope

Video Link:
WITec Product release video @ Analytica Munich 2018
Products & Solutions
Correlative Microscopy with Raman integrated in SEM – RISE Microscopy
Multimodal Imaging – Raman & SEM
Integration of a fully confocal Raman Microscope inside a SEM

Aragonite

Vaterite
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

Benefits of confocal microscopy:

- Less background which causes "blurring"
- 3D information by slicing the sample optically
- Enhanced resolution ...
Basics of Confocal Raman Imaging
Resolution in optical microscopy – lateral resolution

Resolution limit of an optical microscope?

Ernst Abbé (1840-1905): Diffraction Theory

\[ \Delta x_{\text{min}} \approx \frac{\lambda_{\text{exc}}}{\text{NA}} \]

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

FWHM ≈ 300 nm

FWHM ≈ 830 nm
Basics of Confocal Raman Imaging
3D Reconstruction – getting an inside view
High resolution Raman Imaging

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Confocal Raman Imaging

1. Confocal microscopy
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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
Basics of Raman Spectroscopy
What can be measured spectrally - Nitrogen

Raman effect:

- **non-resonant excitation** of vibrational quantum states (vibs) – comparable to IR
- \( N_{\text{vibs}} = 3N_{\text{atoms}} - 3v_{\text{transl}} - 3v_{\text{rot}} \).

- 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.)

\[ \nu_{\text{Raman}} = \nu_{\text{laser}} \pm \nu_{\text{vib}}. \]
Basics of Raman Spectroscopy
What can be measured spectrally – organic molecules

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

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

\[ C_9H_8O_4 \rightarrow 57 \text{ Eigenvibrations} \]
High resolution Raman Imaging

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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

Objective
Coupling Element & Filter
Focusing Element
Pinhole / Slit
Fibers / Mirrors
Spectrograph & Grating
CCD Camera

Objective
Fibers/Mirrors
CCD-Detectors

Transmission [%]
Transmission [%]
Transmission [%]

Wavelength [nm]
Wavelength [nm]
Wavelength [nm]
Products & Solutions
Challenges for Imaging – Optimization of Beampath Efficiency

Mirrors vs. Optical Fibers:
Reflectivity of Fresh Aluminum

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

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Source: thorlabs.de
**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!**

**Alternative Technology**

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

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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 FL- 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: 532 nm, 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)

CH-stretching Intensity with 4.2 µm/pixel

CH-stretching Intensity with 0.25 µm/pixel

Good intrinsic S/N but high background

Very good S/N with little background (after targeted averaging)

- 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 \( \mu \text{m}^2 \) (180x180 pixel)
Scan Range Z: 30 \( \mu \text{m} \) (32 layers (~1\( \mu \text{m} \) step)
Total No. of spectra: 1.04 Mio.

Integration 500ms /spectrum (comparison)
\[ \Rightarrow 1 \text{ image 4.5 hours, 32 images = 6 days} \]

WITec system: Integration 1 ms /spectrum
\[ \Rightarrow 1 \text{ image 32s , 32 images = 18 minutes} \]
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)
Fast Raman Imaging: Depth Scans
Packing Technology: Optically deconstructing juice containers

inner coating of a fruit juice container
multilayer polymer film

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

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)

Intensity at 2950/cm

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

delta (ord-disord.)

disordered

ordered

polypropylene
Fast Raman Imaging
Generating Chemical Contrast – Pushing Resolution Limits

White light image of sample with measurement are marked in red

Color coded Raman images according to the identified spectra on the right

Depth scan (X/Z) along the line to resolve 2 phases

CCD cts

800 1600 2400 3200
rel. 1/cm

Samples: University Bayreuth
Fast Raman Imaging
Generating Chemical Contrast – Pushing Resolution Limits

Image Scan:
Points per Line: 120
Lines per Image: 120
Scan Width [μm]: 10
Scan Height [μm]: 10
Integration Time [s]: 0.1

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
Multimodal Imaging – AFM & Raman
Correlating Topography and Chemistry

PET

PMMA

A 20x20x0.2 μm³ scan area

B 20x20 μm² scan area, 150x150 pixel
• 0.05 s integration time
• total 22500 spectra
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
Fast Raman Imaging
Generating Chemical Contrast – Confocality and Rough Surfaces

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

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
WITec ParticleScout – Saves your time!
Find, Classify and Identify Particles

Find

Bright Field

Dark Field

200 μm
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

Database Search with WITec TrueMatch
WITec ParticleScout – Saves your time!
Find, Classify and Identify Particles

Corresponding spectra

Anatase (TiO$_2$)  
Boron Nitride  
Oil

Raman image

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.)