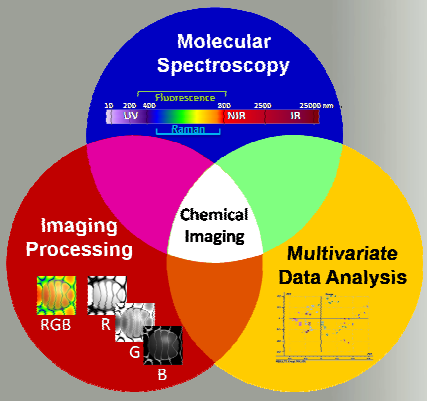
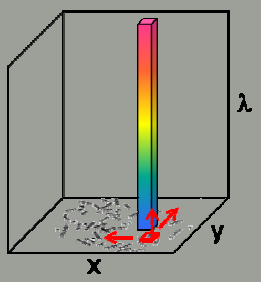


Chemical Imaging

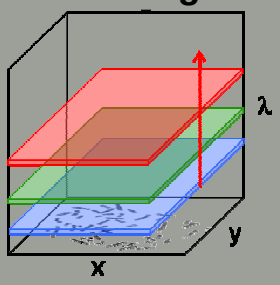
Chemical Imaging (CI) combines different technologies like optical microscopy, digital imaging and molecular spectroscopy in combination with multivariate data analysis methods. CI systems can be classified in *Whiskbroom*- (Mapping), *Staring*- (2D wavelength scanning) and *Pushbroom*- (line scanning with spectral dispersion) imagers. The concept can be applied over a wide spectroscopic range from UV-VIS-NIR, fluorescence, IR and Raman.

Whiskbroom



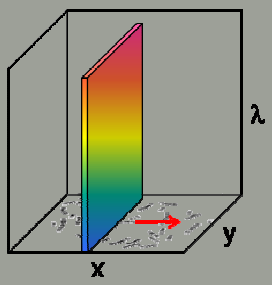
- **high spectral resolution**
- **high discrimination power**
- **high flexibility**

Staring



- **high lateral resolution**
- **easy to implement**
- **high information density**
- **stop motion method**

Pushbroom



- **good spectral & spatial resolution**
- **no motion stop**
- **real online and inline application**

Whiskbroom Imaging

Whiskbroom Imaging means that each single point of the sample is spectrally recorded with a single detector. Such systems have a broad flexibility in terms of sample size, raster width, spectral ranges and implementation in optical methods. This mode leads to a band-interleaved-pixel (BIP) data format. Whiskbroom Imaging is typical for IR, Raman and confocal microscopes.

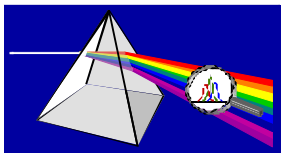
Staring Imaging

Staring Imaging means that a sequence of two-dimensional images of a fixed sample at different wavelengths is acquired. The spectrum of any Pixel can be obtained through a cut over the lambda stack by plotting the intensity vs. wavelength. The wavelength selection can be realized either by a rotating wheel with fixed band pass filters, acousto-optical-tunable filter (AOTF), liquid crystal tunable filters (LCTF) or by monochromatic illumination. This mode leads to a band-sequential imaging (BSQ) data format.

Pushbroom Imaging

This system uses a matrix detector together with a spectrograph and a lens system. Images contain the full spectral information along one line across the sample. One dimension of the detector corresponds to a spatial line image and the other dimension corresponds to the spectrum. The band-interleaved-by-line (BIL) format is a compromise for both spatial and spectral information.

Prof. Dr. Rudolf W. Kessler
Department of Process Analysis
Reutlingen Research Institute - Reutlingen University
Alteburgstraße 150, 72762 Reutlingen
Rudolf.Kessler@Reutlingen-University.de
Tel. / Fax ++49 (0) 7121 / 271 2010 / 2013

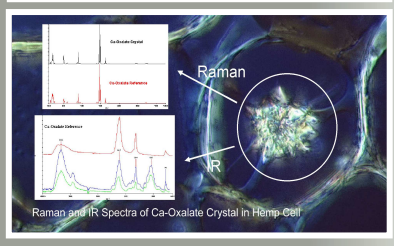


Chemical Imaging

FT-NIR/IR Imaging

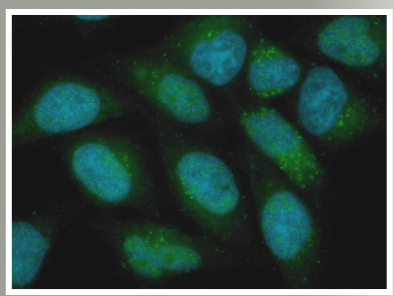
NIR and IR spectroscopy can distinguish specific changes in chemistry and morphology. It is often used for the identification and distribution of organic ingredients in a sample. Microscopic NIR and IR images can be measured with the Perkin Elmer AUTOIMAGE FT-NIR/IR Microscope. The spectra are mapped point-by-point in transmission, reflectance or ATR-mode. The microscope specifications are: 75 mm x 50 mm sample size, < 10 µm resolution, 6x Objective with 0.6 NA, 10000 to 700 cm^{-1} with S/N ratio of 4000:1.

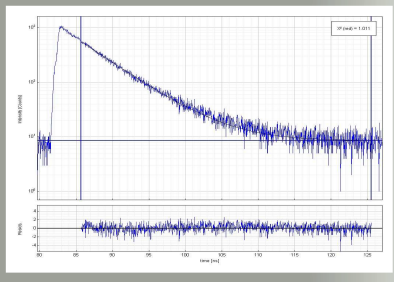
Raman Imaging



Raman Imaging provides intrinsic contrast without the necessity of dying, staining or complex sample preparation. The Renishaw semi-confocal Raman microscope is particularly suitable for measurements of Raman scattering in microscopic dimensions. Due to the confocal aperture, a layer discrimination in z- direction can be realized. The microscope specifications are: 114 mm x 70 mm sample size, < 300 nm resolution, 20/50/100x Objective, 250 to 4000 cm^{-1} Raman shift at 633 nm or 785 nm excitation.

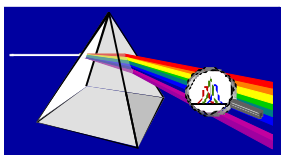
Fluorescence and Fluorescence-Lifetime-Imaging (FLIM)





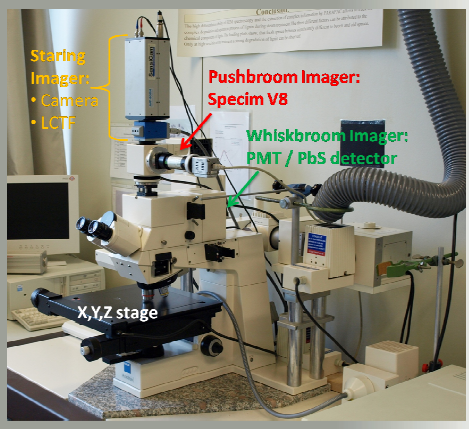
FLIM allows to identify the chromophor as well as the analysis of the lifetime. Anisotropy and FRET measurements allow the characterisation within the nanometer range. The TimeHarp 200 with 375nm and 485 nm excitation and three SPC from 200 nm – 820nm can be combined with the nearfield or farfield microscope.

Prof. Dr. Rudolf W. Kessler
Department of Process Analysis
Reutlingen Research Institute - Reutlingen University
Alteburgstraße 150, 72762 Reutlingen
Rudolf.Kessler@Reutlingen-University.de
Tel. / Fax ++49 (0) 7121 / 271 2010 / 2013



Chemical Imaging

Multi-Modal UV-VIS-NIR System

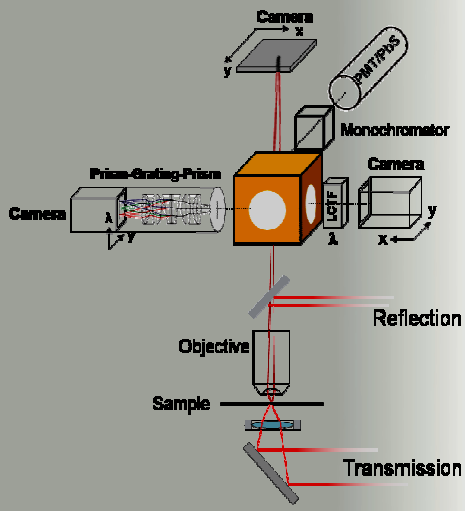


Spectroscopic features

- Zeiss Microspectrometer MPM 800
- UV-VIS-NIR detectors: PMT, PbS
- UV-VIS-NIR cameras
- UV-VIS-NIR imaging spectrograhps

Microscopic features

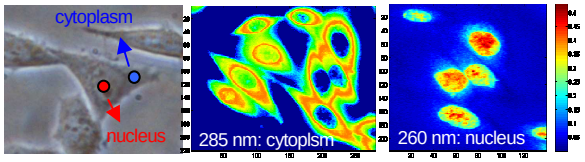
- Transmitted & reflected light
- Brightfield & dakfield
- Fluorescence (excitation / emission)
- Phase contrast
- Polarization
- Differential interference contrast



Our system is based on an optimized Microspectrometer of Zeiss MPM 800. The improved optical setup provides a six times higher S/N ratio in the UV range compared to the original equipment. Thus it is also possible to map and image two dimensional excitation and emission spectra to separate similar chemical species. Mapping and imaging is also possible in transmission, diffuse and specular reflectance and polarisation in a wide spectral range from 230 to 2300 nm. Some examples of marker-free characterizations are shown below.

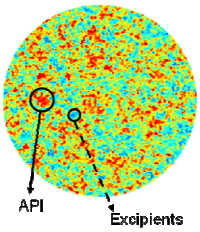
Bioimaging

The images show CHO cells at two different wavelengths. It is possible to separate different cell areas like cytoplsm and nucleus. The Whiskbroom technique provides a high spatial and spectral resolution.



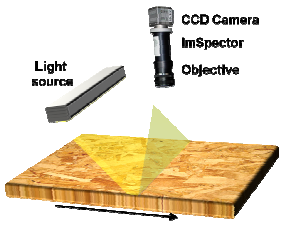
Tablet Imaging

The image shows an Aspirin tablet at 1660 nm. It is possible to visualize the active pharmaceutical increment and the excipient. The Staring technique provides images with a high spatial resolution.

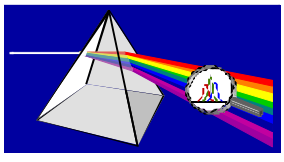


Online Pushbromm Imaging

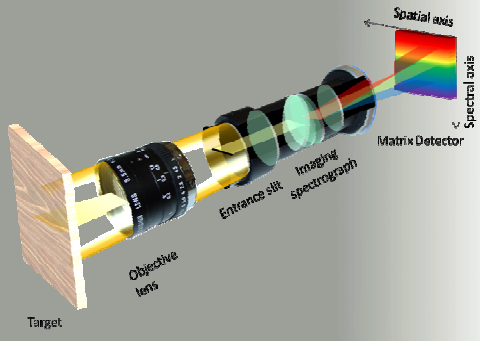
By scanning over the object or recording a moving target, a 2D spectral image can be formed. The collection of sequences of 2D images leads to a continuous multidimensional space consisting of time (or one spatial direction), space, wavelength and intensity.



Prof. Dr. Rudolf W. Kessler
Department of Process Analysis
Reutlingen Research Institute - Reutlingen University
Alteburgstraße 150, 72762 Reutlingen
Rudolf.Kessler@Reutlingen-University.de
Tel. / Fax ++49 (0) 7121 / 271 2010 / 2013



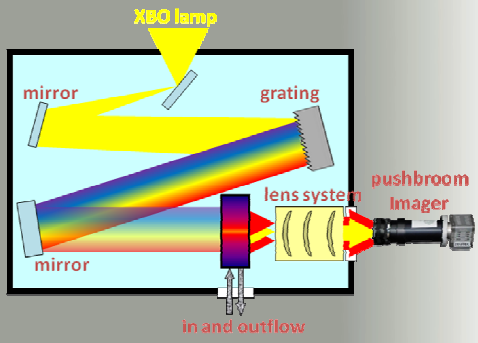
The pushbroom imager



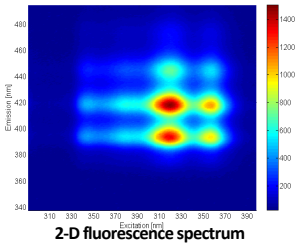
Our pushbroom imager system consists of a lens or fiber optics, a dispersive element (ImSpector spectrograph, Specim) and a matrix detector (PixelFly Vis camera and Zeutec NIR camera).

The camera captures a line image of a target and disperses light from each line pixel to a spectrum, with a spectral resolution of 2 nm and over a wide spectral range (380 – 780 nm, 900 – 2500 nm).

2D Fluorescence spectroscopy

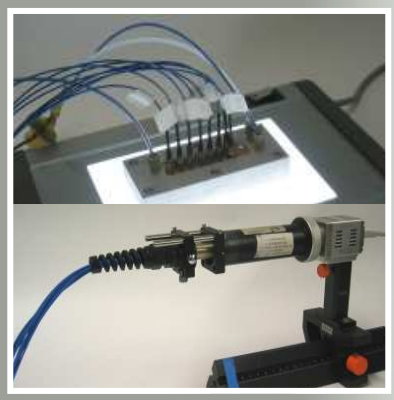


The combination of the pushbroom imager with a fluorescence system allows the real-time collection of 2D fluorescence spectra for the identification and characterization of chemical species in fast reactions.



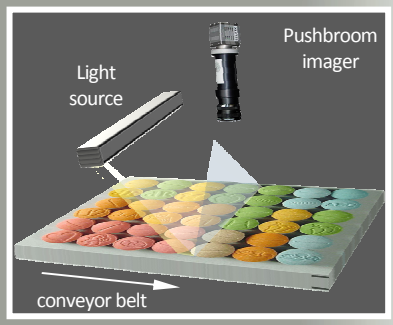
2-D fluorescence spectrum of anthracene

The spectrometer specifications are: 2 nm spectral resolution, 200 – 700 nm excitation wavelength, with simultaneous range of ca. 150 nm, 380 – 780 nm emission wavelength, up to 24 fps frequency of image acquisition.



Multiarray spectrometer

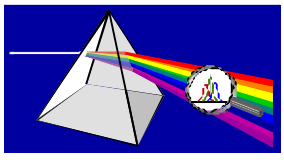
When coupled with multichannel fiber optics, the pushbroom imager converts the camera in a multiarray spectrometer for the simultaneous sampling of several discrete points (up to 100). Applications include for example continuous monitoring of chemical reactions in microreactors.



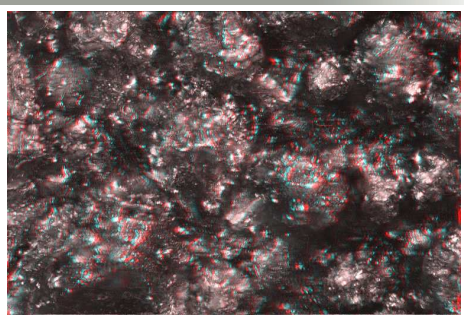
Online process monitoring and optimization

By scanning over the object or recording a moving target, a 2D spectral image can be formed. Real-time multidimensional data imaging, combined with efficient data extraction and analysis, can be used not only to monitor and control a process at a high level, but also to optimize its performance in a wide variety of industrial applications.

Prof. Dr. Rudolf W. Kessler
Department of Process Analysis
Reutlingen Research Institute - Reutlingen University
Alteburgstraße 150, 72762 Reutlingen
Rudolf.Kessler@Reutlingen-University.de
Tel. / Fax ++49 (0) 7121 / 271 2010 / 2013



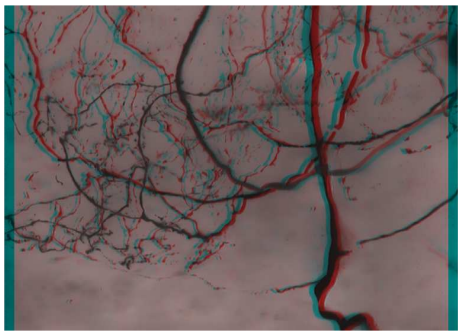
Microscopic Structures



Metal, 3D Image (Red-Green)

Image Analysis

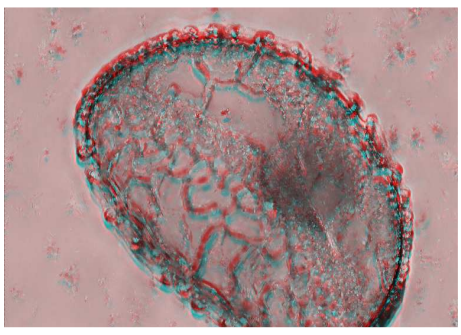
The microscopic methods range from dark field / bright field microscopy through polarisation- and inverse microscopy to techniques like differential interference contrast (DIC) or circular polarisation (CP). The integrated software quantifies the morphological structures. Thus the computer calculates, for example, distributions or fineness of fibre bundles or areas of corrosion.



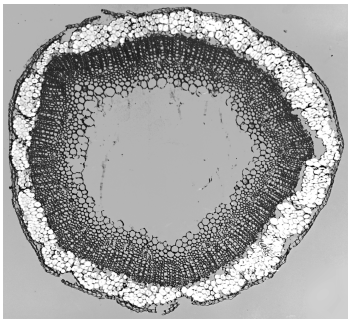
Neurons

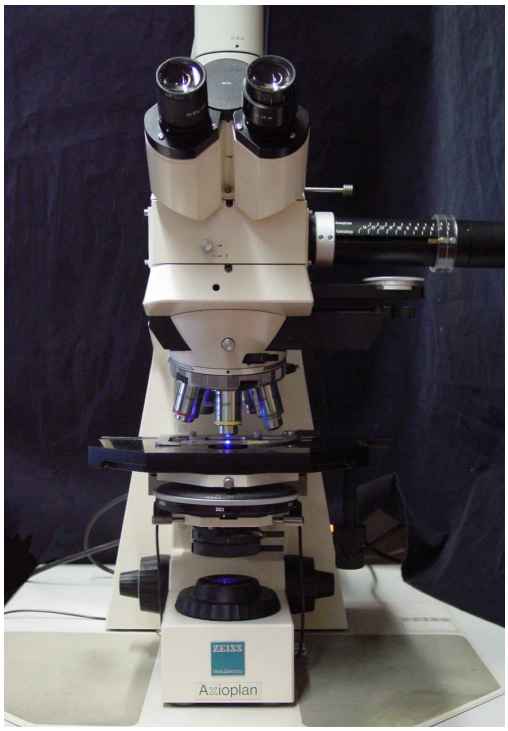
Digital Optical Microscopy (DOM)

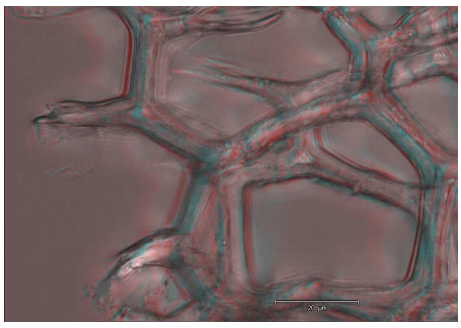
The basic technique is to extract sharp image information from several virtual layers of the object – like in confocal microscopy, but with the advantage to use all microscopic standard techniques. The result is visualised as 3 dimensional images. It is also possible to overlay two or more images, e. g. produced by different techniques, like DIC (differential interference contrast) combined with fluorescence. Magnifications over 2000 can be achieved.



Pollen



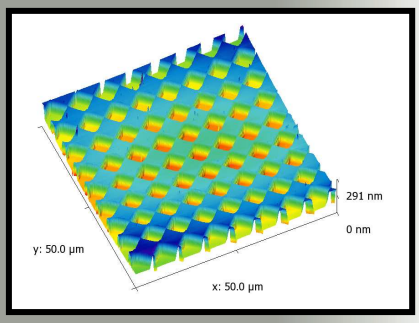




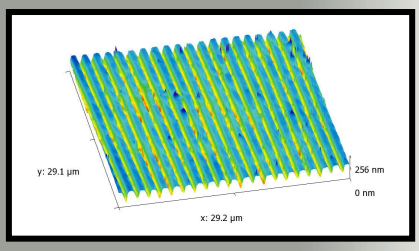
Prof. Dr. Rudolf W. Kessler
Department of Process Analysis
Reutlingen Research Institute - Reutlingen University
Alteburgstraße 150, 72762 Reutlingen
Rudolf.Kessler@Reutlingen-University.de
Tel. / Fax ++49 (0) 7121 / 271 2010 / 2013

Nanoscopic Analysis

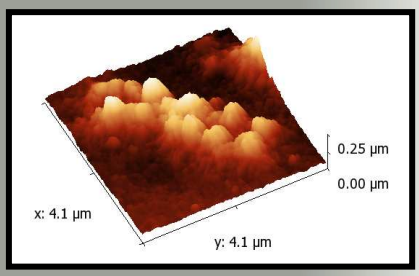
Atomic Force Microscope by Digital Instruments



Test grating



CD groves



Chromosome

Scanning Probe Microscopy (SPM)

Scanning probe microscopy measures the distance dependent interaction between the sample surface and a sensor tip. In this way, the topography of conducting and isolating samples on an atomic scale can be analysed. Depending on the sensor tip different measuring modes can be applied.

Features:

- AFM, MFM
- Tapping, Contact and Phase Imaging
- 125 x 125 µm x 5 µm scanning range
- ~7 nm x ~7 nm x ~0.2 nm resolution limit
- electrochemical cell
- flow cell

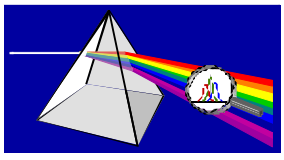
Atomic Force Microscopy (AFM)
The AFM is the most common tool for imaging, measuring and manipulating materials on a nanoscale. The topography of a surface can be imaged by scanning mechanically a silicon tip over the surface. Different surface forces lead to a deflection signal of a cantilever. The deflection is measured by a reflecting laser spot from the cantilever surface into a four quadrant diode.

Tender, fragile, particulate or adhesive surfaces are scanned in phase imaging or tapping mode with an oscillating probe tip. In this way, neither the tip nor the sample can be contaminated during scanning, and no force is exerted on to the surface. Thus, material contrasts are visible.

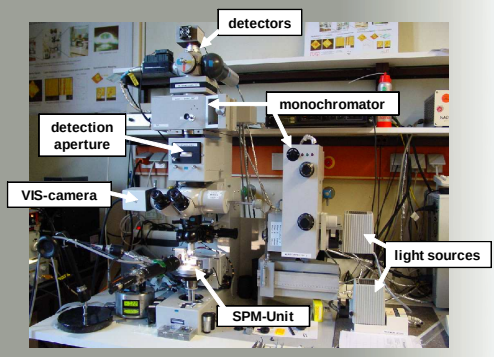
In addition, an electrochemical measuring mode can provide information of the electrochemical properties combined with topographic information.

Magnetic Force Microscopy (MFM)
The silicon tip is replaced by a permanent magnet, so a change in the magnetic interaction can be imaged.

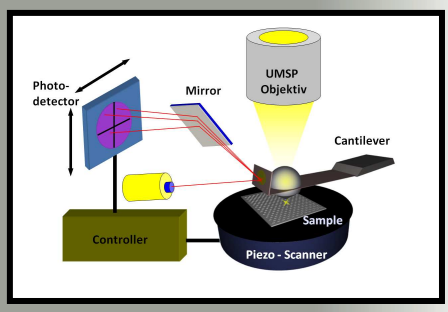
Prof. Dr. Rudolf W. Kessler
Department of Process Analysis
Reutlingen Research Institute - Reutlingen University
Alteburgstraße 150, 72762 Reutlingen
Rudolf.Kessler@Reutlingen-University.de
Tel. / Fax ++49 (0) 7121 / 271 2010 / 2013



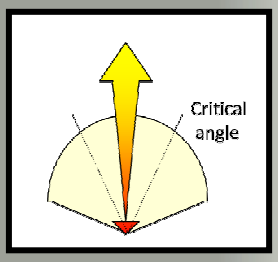
Nanoscopic Analysis



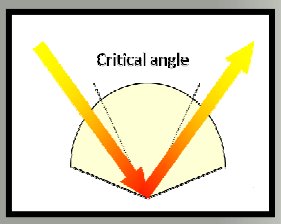
Near field spectrometer UMSP 80 Zeiss



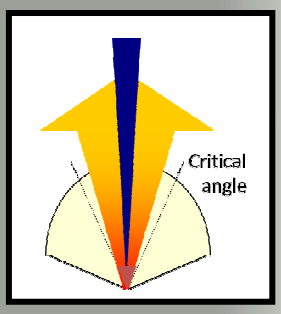
SIL-SNOM



Reflectance-SNOM



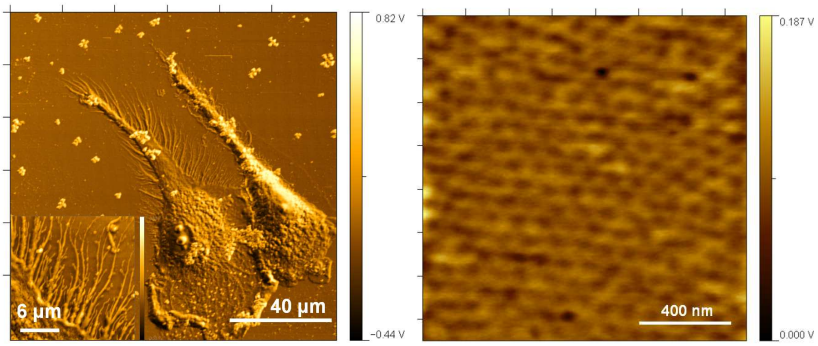
Photon-tunnelling SNOM



Fluorescence - SNOM

Scanning Near Field Optical Microscopy (SNOM) and Near Field Spectroscopy (SNOS)

The diffraction limit of wide-field microscopy prevents resolving features smaller than half of a wavelength. Near field optical microscopy is able to extend the range of optical measurements beyond the diffraction limit. During the last years, Scanning Probe Microscopy has developed as a valuable tool to image different surface interactions even in the nanoscale. The combination of this high resolution technique and the chemical information of spectroscopy makes scanning near-field optical microscopy attractive for the characterization of the morphology and the chemistry of surfaces and cell structures.



Cellstructures Aluminium oxide membrane

Topography and optical information can be acquired simultaneously by scanning near field optical microscopy (SNOM). For this purpose, a cantilever based solid immersion lens is combined with the UV/VIS microscope spectral photometer (Zeiss UMSP 80) or Raman spectrometer (Renishaw). An optical lateral resolution of 30 nm can be realized.

- Reflection-, Photon tunnelling-, Fluorescence contrast
- Fluorescence Lifetime Measurements
- 125 x 125 µm x 5 µm scanning range
- ~30 nm x ~30 nm x ~0.2 nm resolution limit
- VIS-, Raman Spectroscopy

Prof. Dr. Rudolf W. Kessler
Department of Process Analysis
Reutlingen Research Institute - Reutlingen University
Alteburgstraße 150, 72762 Reutlingen
Rudolf.Kessler@Reutlingen-University.de
Tel. / Fax ++49 (0) 7121 / 271 2010 / 2013