

**Atomic Force Microscopy
Confocal Raman / Fluorescence Microscopy
Scanning Near-Field Optical Microscopy
Optimized for Tip Enhanced Raman Scattering**



Time – and – Space Correlated Measurements on a Single Integrated System

NTEGRA
S P E C T R A

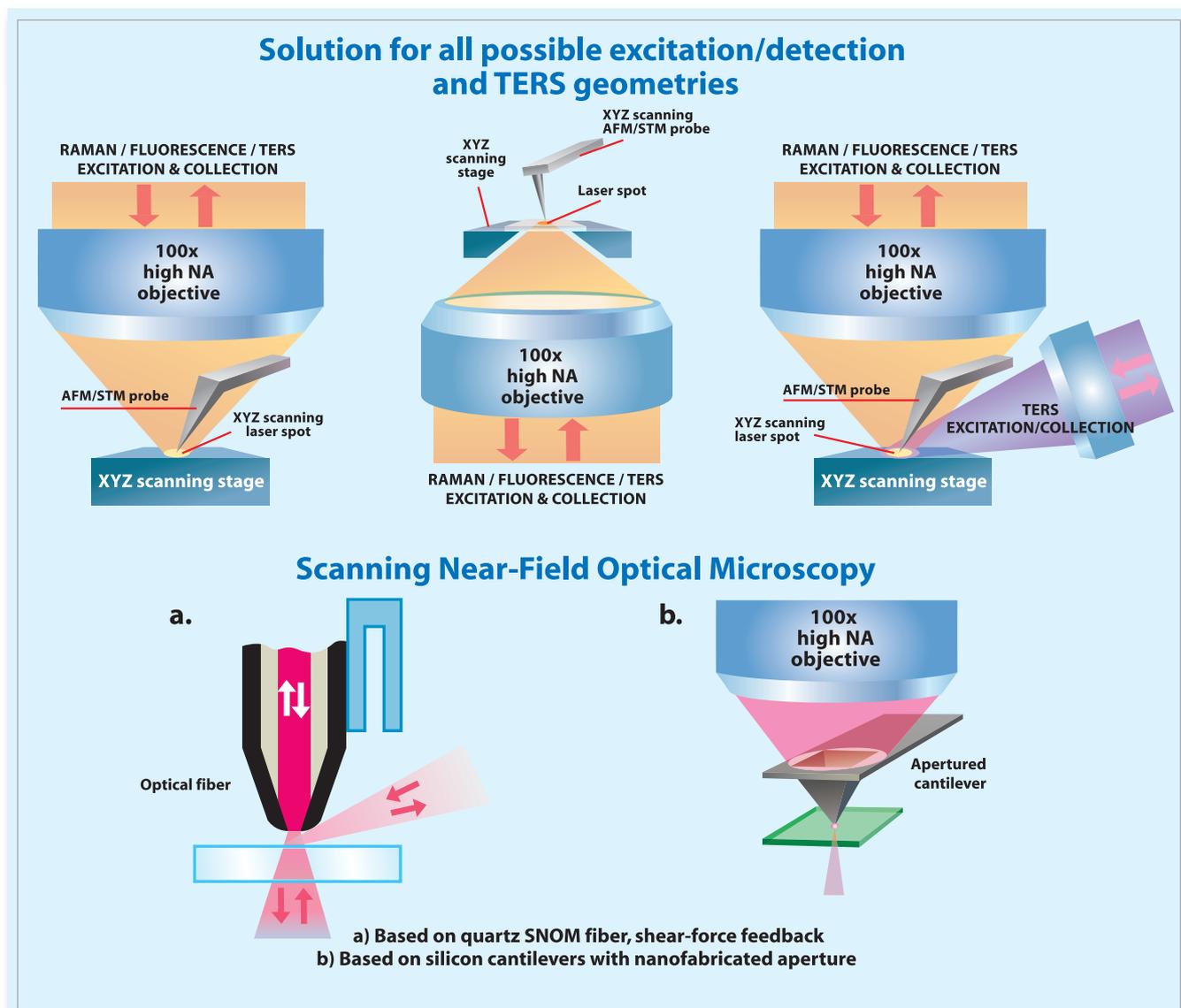




Award Winner 2006



- ❑ Atomic Force Microscopy (> 30 modes)
- ❑ Confocal Raman / Fluorescence / Rayleigh Microscopy
- ❑ Scanning Near-Field Optical Microscopy (SNOM)
- ❑ Optimized for Tip Enhanced Raman and Fluorescence (TERS, TEFS, TERFS) and scattering SNOM (s-SNOM)



Integration: The key to the new sciences

Change happens at interfaces and today's most exciting changes in microscopy are happening where multiple technologies are interfaced together. **NTEGRA Spectra** is a prime example, uniting the full power of atomic force microscopy (AFM), confocal Raman and fluorescence microscopy and scanning near-field optical microscopy (SNOM) in one platform.

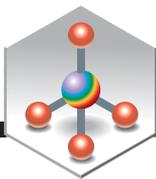
Simultaneous AFM and confocal Raman / Fluorescence imaging

NTEGRA Spectra supports most of the existing AFM modes (more than 30) providing comprehensive information about physical properties of the sample with nanometer scale resolution: local stiffness, elasticity, conductivity, capacitance, magnetization, surface potential and work function, friction, piezo response etc. *Simultaneously* with AFM, confocal Fluorescence and Raman measurements, taken from exactly the same sample area, provide

information about sample chemical composition, crystal structure and its orientation, presence of impurities and defects, macromolecular conformation, and so on. Measurements can be performed either through upright or inverted light excitation geometries. The sample can be in a controlled atmosphere or in a liquid environment, all under controlled temperature. Complete Raman/fluorescence spectrum is recorded in each point of 2D/3D scan with further powerful software analysis. Due to the excellent microscopy performance of the **NTEGRA Spectra**, 3D spectral distribution can be studied with the spatial resolution reaching the theoretical limit.

Microspectroscopy at the molecular scale

Diffraction limited spatial resolution and weakness of Raman signal are the two major challenges in Raman microscopy. When using visible light, resolution of classical confocal microscopy does not go below 200 nm. The Raman signal is often only 1/millionth of the strength of a fluorescence



signal. The new world of nanotechnology has disclosed a fascinating phenomenon: the electromagnetic field can be strongly enhanced near nanometer-scale metal asperities (“nano-antennas”). The resulting effects are called Surface Enhanced Raman Scattering (SERS) and, when done in conjunction with an SPM tip, one can get Tip-Enhanced Raman Scattering (TERS). By using a specially prepared sharp needle tip, **NTEGRA Spectra** can multiply the Raman signal strength by a few orders of magnitude from a precisely scanned, localized spot on the surface several nanometers in diameter. Even single molecules can be detected and recognized by their spectra. Lateral resolution of Raman (TERS) and fluorescence maps is no longer limited by light diffraction and can be less than 15 nm.

■ A laser for every purpose

NTEGRA Spectra is built to offer you maximum flexibility. As with many microscopy parameters, Raman presents trade-offs. The intensity of Raman scattering is inversely proportional to the fourth order of the excitation wavelength. Therefore, to obtain the maximum signal, the experiment dictates the use of the shortest possible wavelengths. However, longer wavelengths penetrate deeper into the

sample, produce less fluorescence background and are less harmful to delicate preparations, especially biological samples. **NTEGRA Spectra** can be configured with several different software selectable lasers. Simply choose the one that fits your needs best and change the laser by a mouse click.

■ One master Nova™ software program makes the complex simple

Truly great engineering makes complex processes transparent to the user. **NTEGRA Spectra** is a prime example of NT-MDT’s brilliant engineering. Taken piece by piece, **NTEGRA Spectra** can be overwhelming: there are multiple lasers, a spectrometer, a confocal laser system, polarizers, pinholes, photomultipliers and other detectors, and of course, the scanning probe microscope. All of these have to be individually controlled and seamlessly integrated. Not to worry. One can manage them easily through the fully integrated AFM – Raman/fluorescence system software. Specify the pinhole size on the confocal system, choose the appropriate laser & polarization, adjust the spectrometer, find cantilever resonance and optimal scanning parameters – all with using a few clicks with the mouse.



▣ Inverted setup:

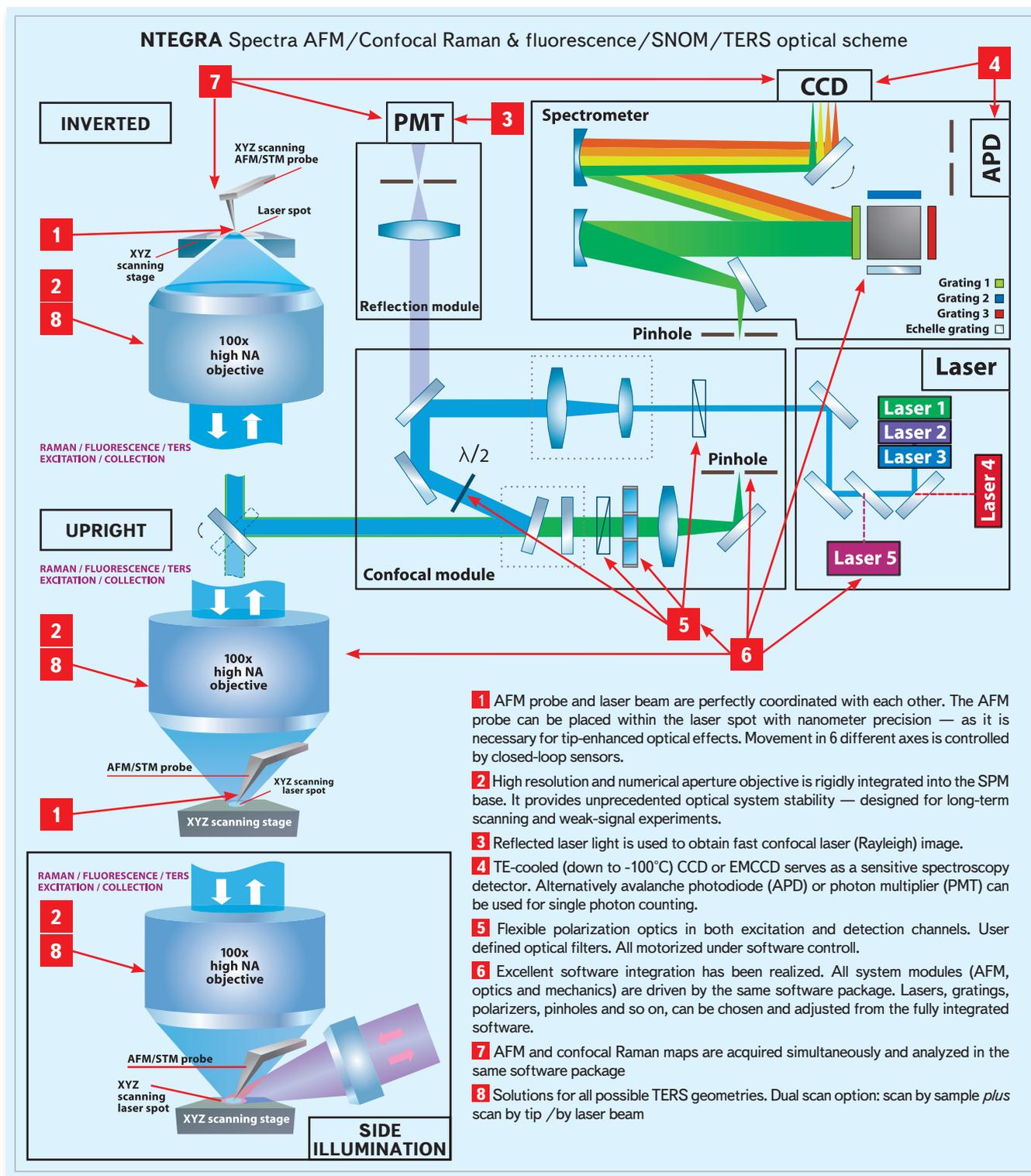
- Optimized for transparent samples
- Highest optical resolution achievable (<200 nm) *simultaneously* with AFM
- Highest efficiency of Raman/fluorescence photon collection (with immersion optics) *simultaneously* with AFM
- Probe scanning in addition to sample scanning (important for TERS)
- Equipped with heating stage, temperature controlled liquid cell and environmental chamber
- Fits most commercial inverted microscopes, supporting advanced imaging modes

Work both with cantilevers (contact, intermittent contact and other modes: more than 30) and with metal tips (STM mode, shear force mode, normal force mode)



▣ Upright setup:

- Optimized for opaque samples
- Highest optical resolution (280–400 nm) *simultaneously* with AFM
- Highest efficiency of Raman/Fluorescence photon collection *simultaneously* with AFM
- Beam scanning in addition to sample scanning (necessary for TERS)
- Equipped with heating stage, environmental chamber

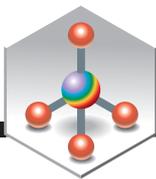


Modes:

- AFM (mechanical, electrical, magnetic properties, nanomanipulation etc.)
- White Light Microscopy and Confocal Laser (Rayleigh) Imaging
- Confocal Raman Imaging and Spectroscopy
- Confocal Fluorescence Imaging and Spectroscopy
- Scanning Near-Field Optical Microscopy (SNOM)
- Tip Enhanced Raman and Fluorescence Microscopy (TERS, TEFS, TERFS)

Controlled environment:

- Temperature
- Humidity
- Gases
- Liquid
- Electrochemical environment
- External magnetic field



Atomic force microscopy:
mechanical, electrical, magnetic
properties and nanomanipulations

Confocal fluorescence:
imaging and spectroscopy

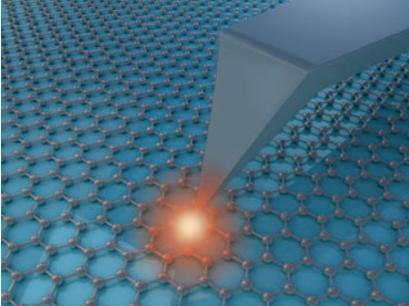
Confocal Raman:
imaging and spectroscopy

**Tip enhanced Raman and
fluorescence microscopy**

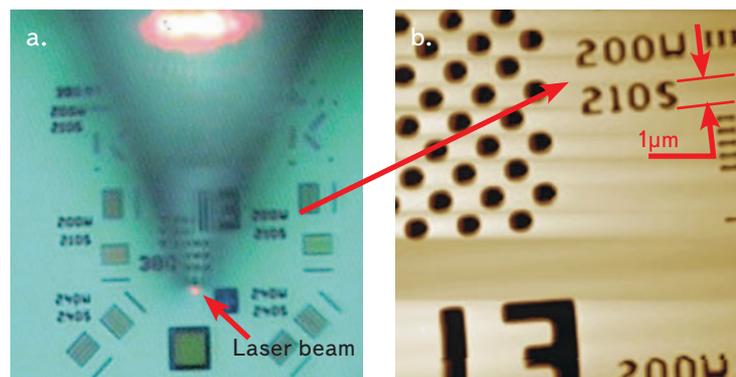
**Near-field optical
microscopy**

**Light microscopy and
reflected laser confocal
imaging**

All techniques can be applied to the same sample

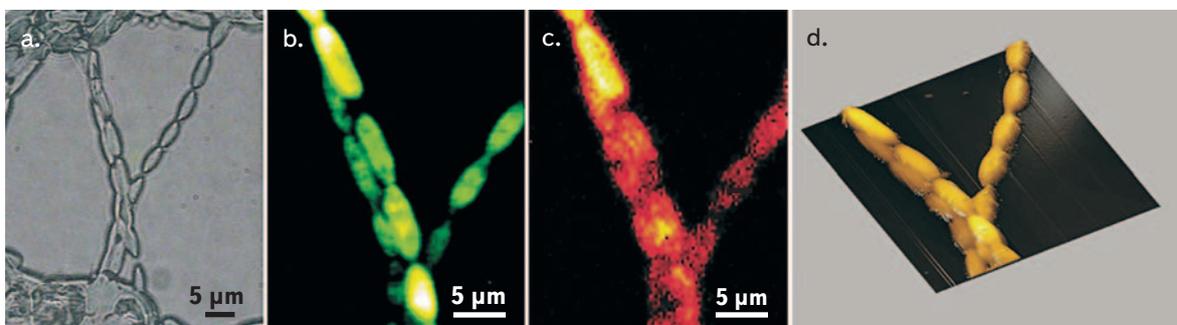


■ AFM working simultaneously with 400 nm resolution upright optics



“AFM + confocal microscope” with high magnification optics in upright configuration. Note extremely high imaging resolution of 100x objective as seen on 1 μm height characters on Si substrate a). Due to the high numerical aperture (0.7) of the objective, opaque silicon AFM probe looks “transparent” on the image. The very end of the tip can be seen. AFM scanning b) can be obtained simultaneously with both white light and confocal Raman/fluorescence imaging. Thanks to the additional beam scanning option, a tightly focused laser spot can be positioned exactly at the apex of the AFM probe — as required for TERS experiments.

■ Comprehensive analysis of biological structures



Algal cells visualization by different techniques. a) Bright field overview. b) Confocal Raman map at 1524 cm⁻¹ (beta-carotene line). c) Confocal image of autofluorescence at 492–513 nm. d) AFM image.

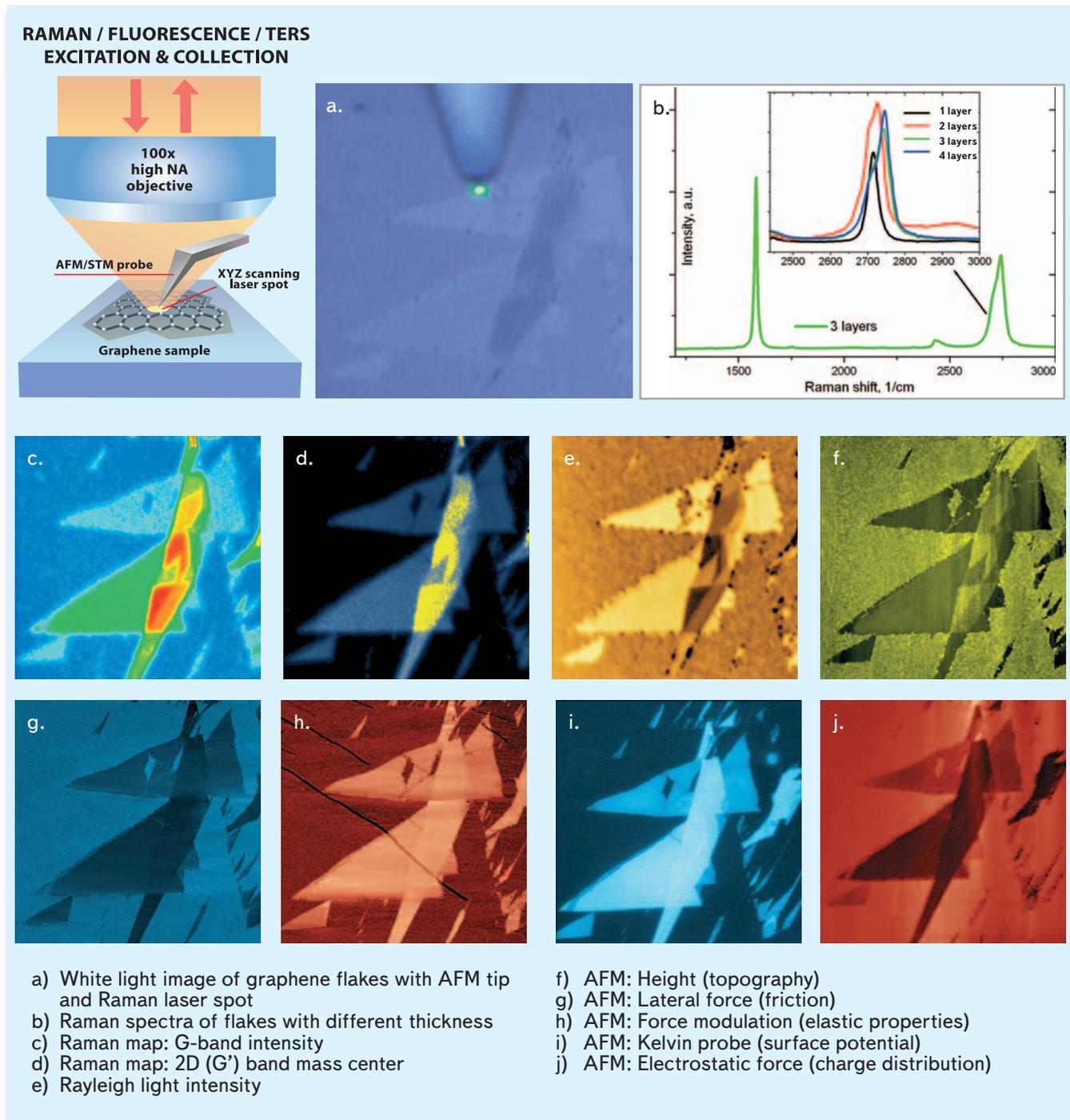
Sample courtesy of Don McNaughton, Monash University, Victoria, Australia

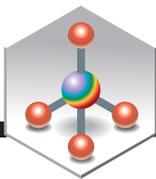
Graphene studied by various optical, AFM and spectroscopy techniques

Combination of AFM, confocal Raman / Fluorescence / Rayleigh microscopy and Scanning Near-Field Optical Microscopy provides unique opportunities for graphene investigation. Different AFM techniques allow studying of mechanical, electrical, magnetic and even elastic properties of graphene flakes. Studies of local work function, conductivity, capacitance, piezoresponse and many other surface properties are available. At the same time, Raman microscopy (available simultaneously with AFM) provides information about flake thickness, structural uniformity,

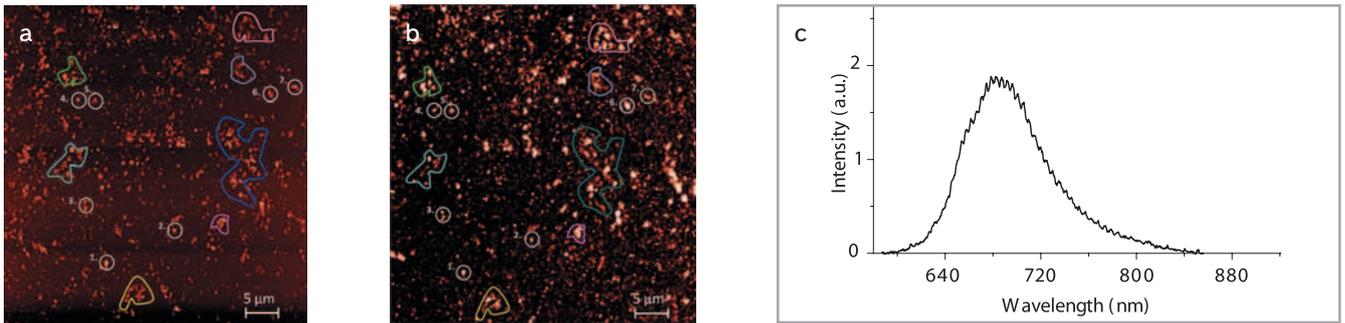
presence of impurities and defects etc. Additionally, Rayleigh imaging and SNOM measure local optical properties of the sample providing further information about flake structure.

Importantly, most of the measurements can be performed under environmental control: at variable humidity and temperature, in controlled atmosphere, in liquid and even (in some configurations) in electrochemical environment and with external magnetic field.





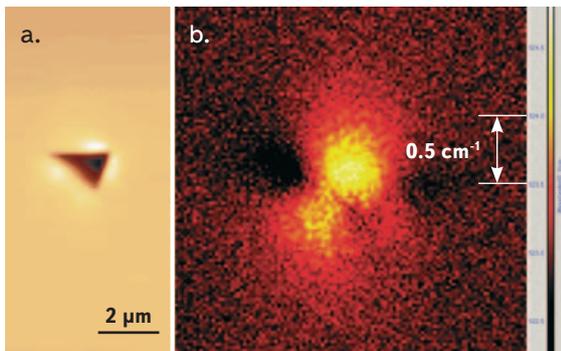
■ Nitrogen-vacancy (NV) color centers in nanodiamonds



Observation of nitrogen-vacancy (NV) color centers in discrete detonation nanodiamonds. a) AFM topography image; smallest particles observed are discrete isolated nanodiamonds of ~ 5 nm size. b) Confocal fluorescence map of the same sample area; nitrogen-vacancy luminescence from isolated nanodiamonds is clearly seen. c) Luminescence spectrum of individual NV center in a 5 nm crystal host.

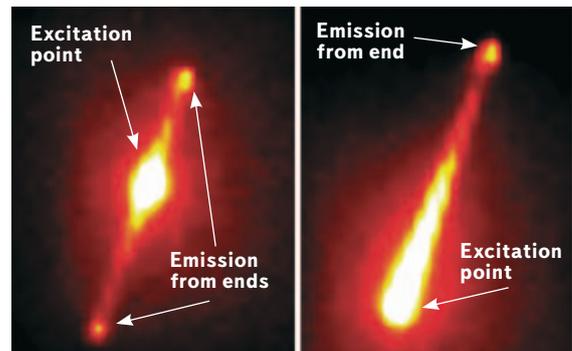
Image Credit: A/Prof. James Rabeau, Quantum Materials and Applications group, Department of Physics and Astronomy, Macquarie University (Sydney, Australia). For more details see: C. Bradac et al., Nature Nanotechnology 5, 345 - 349 (2010)

■ Stress mapping in silicon structures



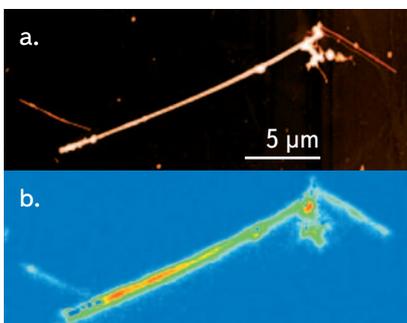
a) AFM topography of indentation in silicon substrate. b) Center of mass shift of 520 cm^{-1} silicon Raman band is showing stress distribution around the indentation. Spectral resolution is better than 0.1 cm^{-1}

■ Light transport in nanostructures

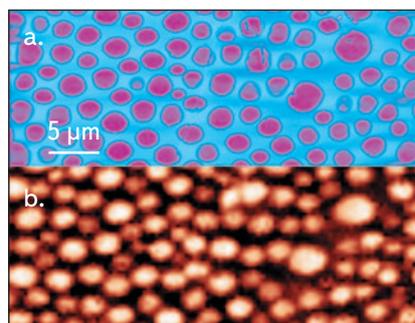


Fluorescent GaN nanowire is excited by 488 nm light at the body (left image) and at the left bottom end (right image). Excitation light is completely cut off from the image by two edge filters (with 10^{-6} transmission). Part of the fluorescence light emitted from nanowire ($>10\%$) is transmitted through it and is emitted from nanowire ends

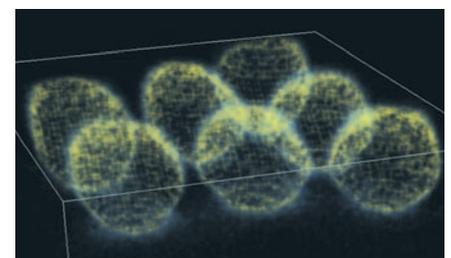
■ More applications



a) AFM topography and b) confocal Raman map (spectral shift of 520 cm^{-1} Si band) of individual silicon nanowire

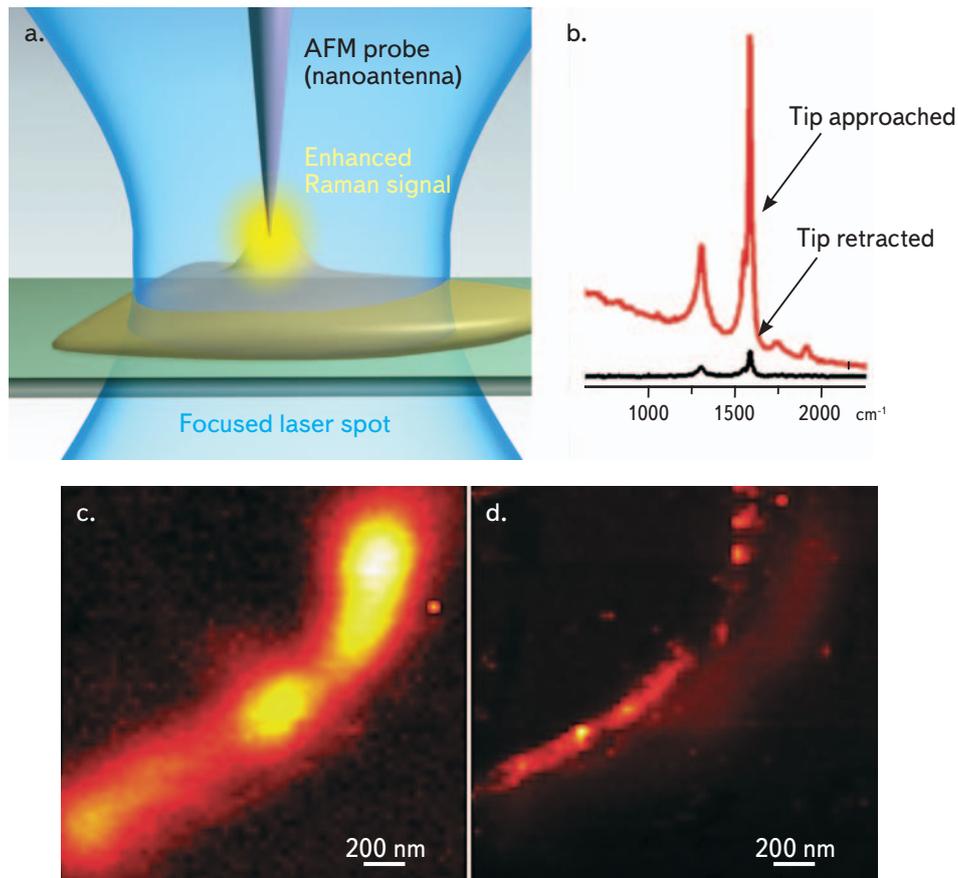


Co-localized AFM (a) and Raman (b) images of block copolymer



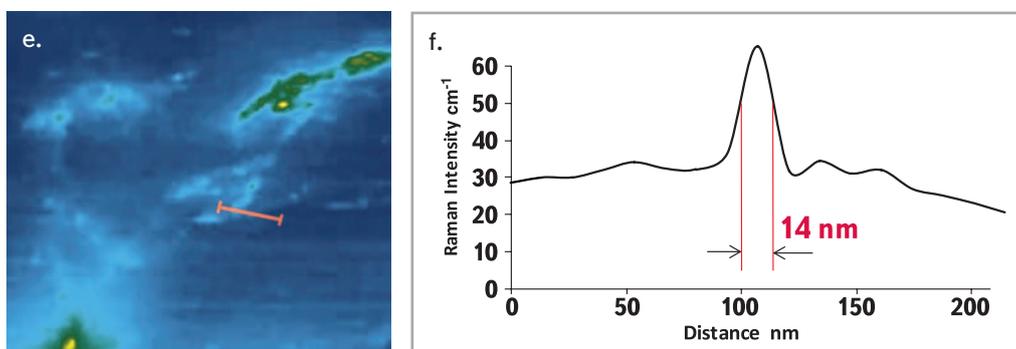
3D confocal Raman image of polystyrene microspheres. Scan size: $10 \times 10 \times 14\ \mu\text{m}$. Full Raman spectrum was recorded in each point of 3D map, further software analysis allowed to build 3D Raman maps based on any selected Raman band.

Raman microscopy with ultra-high spatial resolution (TERS)



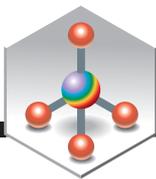
a) A specially prepared AFM probe (metal coated cantilever or etched metal wire) is precisely positioned inside a tightly focused laser spot. b) Intensity of carbon nanotube G- and D- Raman bands increases by several orders of magnitude when the special AFM probe is landed and positioned over a small (5 nm height) nanotube bundle — the effect of Tip Enhanced Raman Scattering (TERS). c) “Conventional” confocal Raman image of the nanotube bundle, the observed width of the bundle is ~ 250 nm (diffraction limit of confocal microscopy, laser wavelength — 633 nm). d) TERS image of the same bundle — now the observed width is ~ 50 nm. Note, in this example, TERS provides more than 4-times better spatial resolution as compared to confocal microscopy.

Measurements are done with NTEGRA Spectra in Inverted configuration. Data courtesy of Dr. S. Kharintsev, Dr. J. Loos, Dr. G. Hoffmann, Prof. G. de With, TUE, the Netherlands and Dr. P. Dorozhkin, NT-MDT. For more information see: Nanotechnology 18, 315502 (2007).

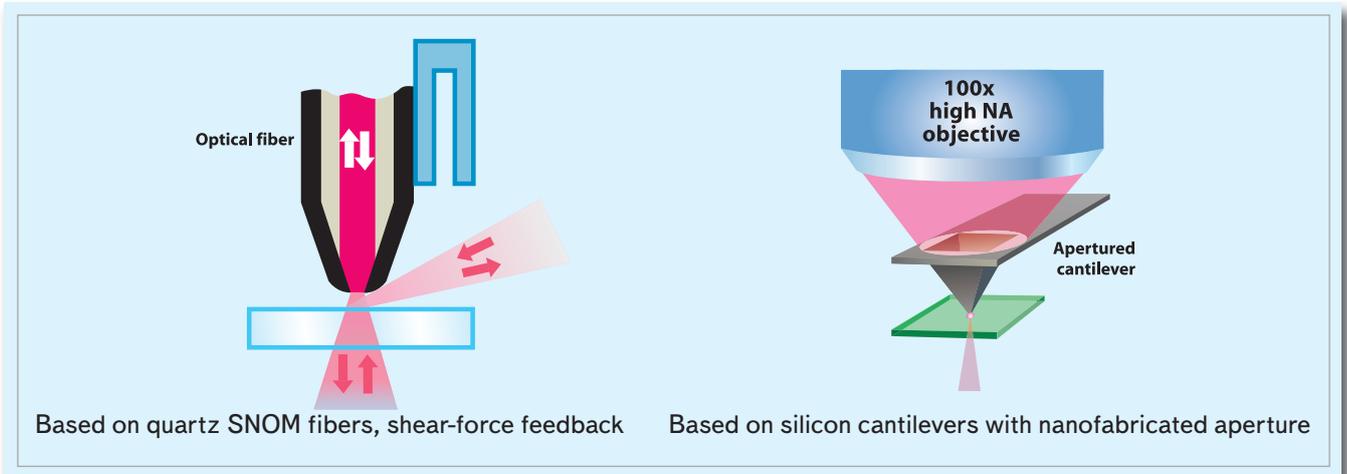


e) “Nano-Raman” (TERS) image of carbon nanotubes with corresponding line cross-section f) showing 14 nm spatial resolution.

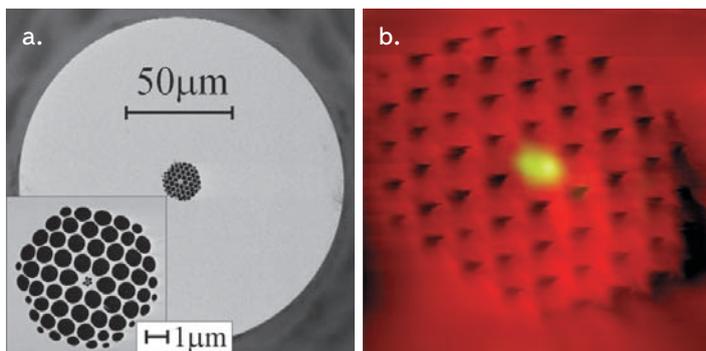
For more information see: Chan K.L., Kazarian S.G., “Finding a needle in a chemical haystack: tip-enhanced Raman scattering for studying carbon nanotubes mixtures”, Nanotechnology 21, 445704 (2010).



Scanning Near-field Optical Microscopy (SNOM)



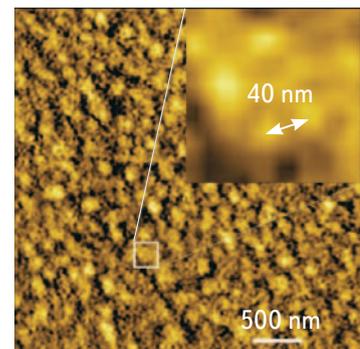
Photonic crystal fibers



(a) SEM image of the optical fiber cross-section, showing photonic crystal structure in the fiber core. (b) Overlay of topography map (red palette) and light intensity (SNOM collection) image (green palette) taken from the fiber section. Light propagating in the fiber is perfectly localized in the center of the photonic crystal structure.

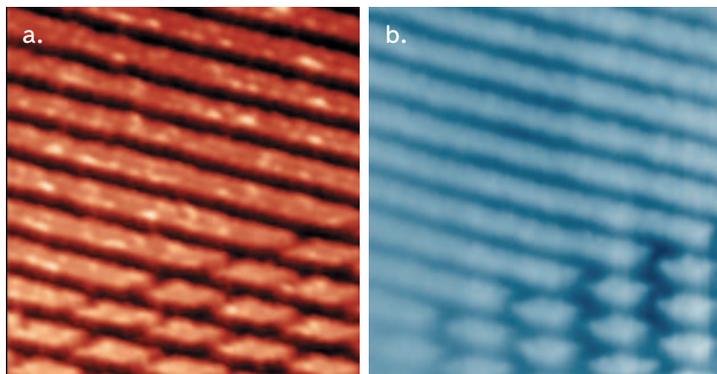
Data courtesy: Yinlan Ruan, Heike Ebendorff-Heidepriem, Tanya M. Monro. Centre of Expertise in Photonics, School of Chemistry & Physics, University of Adelaide

Polymers



SNOM reflection image of polymer with granular structure. Two grains separated by about 40 nm (enlarged inset) show excellent spatial resolution of the technique.

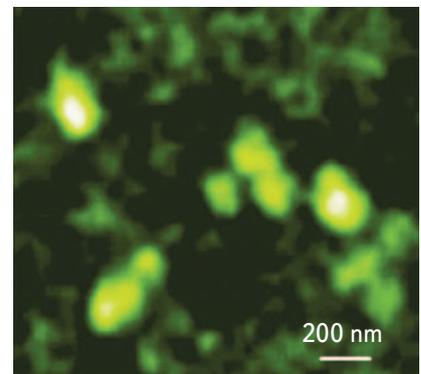
SERS substrate – Au nanodiamond array on quartz



(a) AFM topography and (b) SNOM transmission image. Period of the structure: 200 nm. Resolution of SNOM image: ~50 nm

Sample courtesy: Dr. Henrik Schneidewind, Institute of Photonic Technology (IPHT Jena), Germany

Biological objects



SNOM image of mitochondria dyed with FITC-labeled antibodies.

☒ Confocal Raman / fluorescence microscopy
Confocal Raman / fluorescence / Rayleigh imaging runs simultaneously with AFM (during one sample scan)
Diffraction limited spatial resolution: <200 nm in XY, <500 nm in Z (with immersion objective)
True confocality; motorized confocal pinhole for optimal signal and confocality
Motorized variable beam expander / collimator: adjusts diameter and collimation of the laser beam individually for each laser and each objective used
Full 3D (XYZ) confocal imaging with powerful image analysis
Hyperspectral imaging (recording complete Raman spectrum in every point of 1D, 2D or 3D confocal scan) with further software analysis
Optical lithography (vector, raster)
☒ AFM/STM: Integration with spectroscopy
Upright and inverted optical AFM configurations (optimized for opaque and transparent samples correspondingly); side illumination option
Highest possible resolution (numerical aperture) optics is used simultaneously with AFM: 0.7 NA for Upright, 1.3–1.4 NA for Inverted
AFM/STM and confocal Raman / Fluorescence images are obtained simultaneously (during one scan)
All standard SPM imaging modes are supported (>30 modes) — combined with confocal Raman / Fluorescence
Low noise AFM / STM (atomic resolution)
Vibrations and thermal drifts originating from optical microscope body are minimized due to special design of optical AFM heads
Focus track feature: sample always stays in focus due to AFM Z-feedback; high quality confocal images of very rough or inclined samples can be obtained
☒ Software
Seamless integration of AFM and Raman; all AFM / Raman / SNOM experiment and further data analysis is performed in one and the same software
Powerful analysis of 1D, 2D and 3D hyperspectral images
Powerful export to other software (Excel, MatLab, Cytospec etc.)

☒ Spectroscopy*
Extremely high efficiency 520 mm length spectrometer with 4 motorized gratings
Visible, UV and IR spectral ranges available
Echelle grating with ultrahigh dispersion; spectral resolution: 0.007 nm (< 0.1 1/cm)**
Up to 3 different detectors can be installed: <ul style="list-style-type: none"> - TE cooled (down to -100°C) CCD camera. EMCCD camera is optional — <i>for ultrafast imaging</i> - Photon multiplier (PMT) or avalanche photodiode (APD) in photon counting mode - Photon multiplier for fast confocal laser (Rayleigh) imaging
Flexible motorized polarization optics in excitation and detection channels, cross-polarized Raman measurements
Fully automated switching between different lasers — with a few mouse clicks
☒ Scanning Near Field Optical Microscopy (SNOM)
Two major SNOM techniques supported: (I) based on quartz fiber probes, (II) based on silicon cantilever probes
All modes are supported: Transmission, Collection, Reflection
All SNOM signals are detected: laser intensity, fluorescence intensity, spectroscopy
SNOM lithography (vector, raster)
☒ Optimized for Tip Enhanced Raman Scattering (TERS) and other tip-related optical techniques (S-SNOM, TEFS, STM-LE etc.)
All existing TERS geometries are available: illumination/collection from bottom, from top or from side
Different SPM techniques and TERS probes can be used: STM, AFM cantilever, quartz tuning fork in tapping and shear force modes
Dual scan (for Hot Point Mapping in TERS): scan by sample AND scan by tip / by laser spot
Motorized polarization optics to produce optimal polarization for TERS

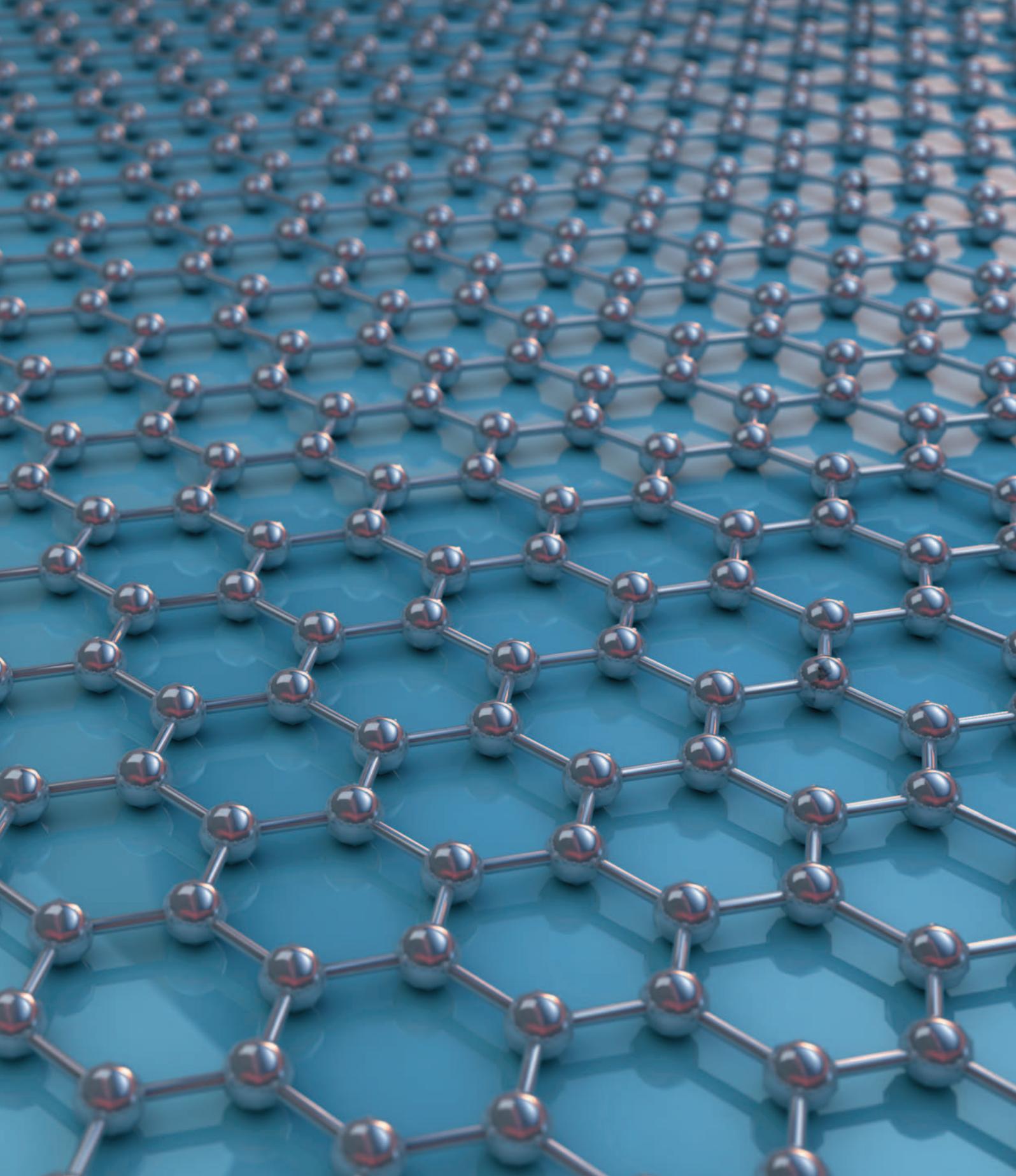
■ **AFM-Raman measurements can be run in air, in a controlled atmosphere or in liquid — all with variable temperature**

Some features listed are *optional* — not included into basic system configuration

* NT-MDT AFM can be integrated with Renishaw inVia or with NT-MDT spectrometer. Specifications are given for the latter.

Renishaw specifications can be found at www.renishaw.com/AFM-Raman

**Exact value of spectral resolution highly depends on how “resolution” is defined



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