NT-MDT
Molecular Deuices and Tools for NanoTechnology

Raman spectroscopy, confocal microscopy and SPM for interdisciplinary science at the molecular level

NTEGRA SPECTRA

R&D 100

Award Winner
2006

NTEGRA
SPECTRA

Integration: The key to the new sciences

Change happens at interfaces and today's most exciting changes in microscopy are happening where multiple technologies interface. **NTEGRA** Spectra is a prime example, uniting the full power of confocal microscopy, scanning probe microscopy, and Raman spectroscopy in one platform.

Simultaneous Confocal imaging and chemical mapping

NTEGRA Spectra can map chemical properties of the sample. Show as subtle changes in spectra reflect changes in strain, polarizability, or macromolecular conformation. Measurements can be performed either through upright or inverted viewing mode.

The sample can be either in air or in liquid environment. **NTEGRA** Spectra provides two separate detection channels: one for acquiring the laser confocal (reflected) signal and the second for simultaneous but independent collection of the delicate Raman map that reveals the local chemical composition. The second channel can also be used for fluorescence spectroscopy or direct fluorescence imaging. Due to excellent microscopy performance 3D spectral distribution can be studied with the spatial resolution close to the theoretical limit.

Microspectroscopy at the molecular scale

Signal strength is a major challenge in Raman measurements. The Raman signal is often only 1/ millionth the strength of a fluorescence signal. The new world of nanotechnology has disclosed a fascinating phenomenon: the electromagnetic field is strongly enhanced near nanometer-scale asperities. The resulting effects are called Surface Enhanced Raman Scattering (SERS) and, when done in conjunction with an SPM tip, Tip-Enhanced Raman Scattering (TERS). By using a specially prepared sharp needle tip, **NTEGRA** Spectra can multiply the Raman signal strength by factors of hundreds, thousands and even millions from a precisely scanned, localized spot on the surface several nanometers in diameter. Even single molecules can be detected and recognized by their spectra.

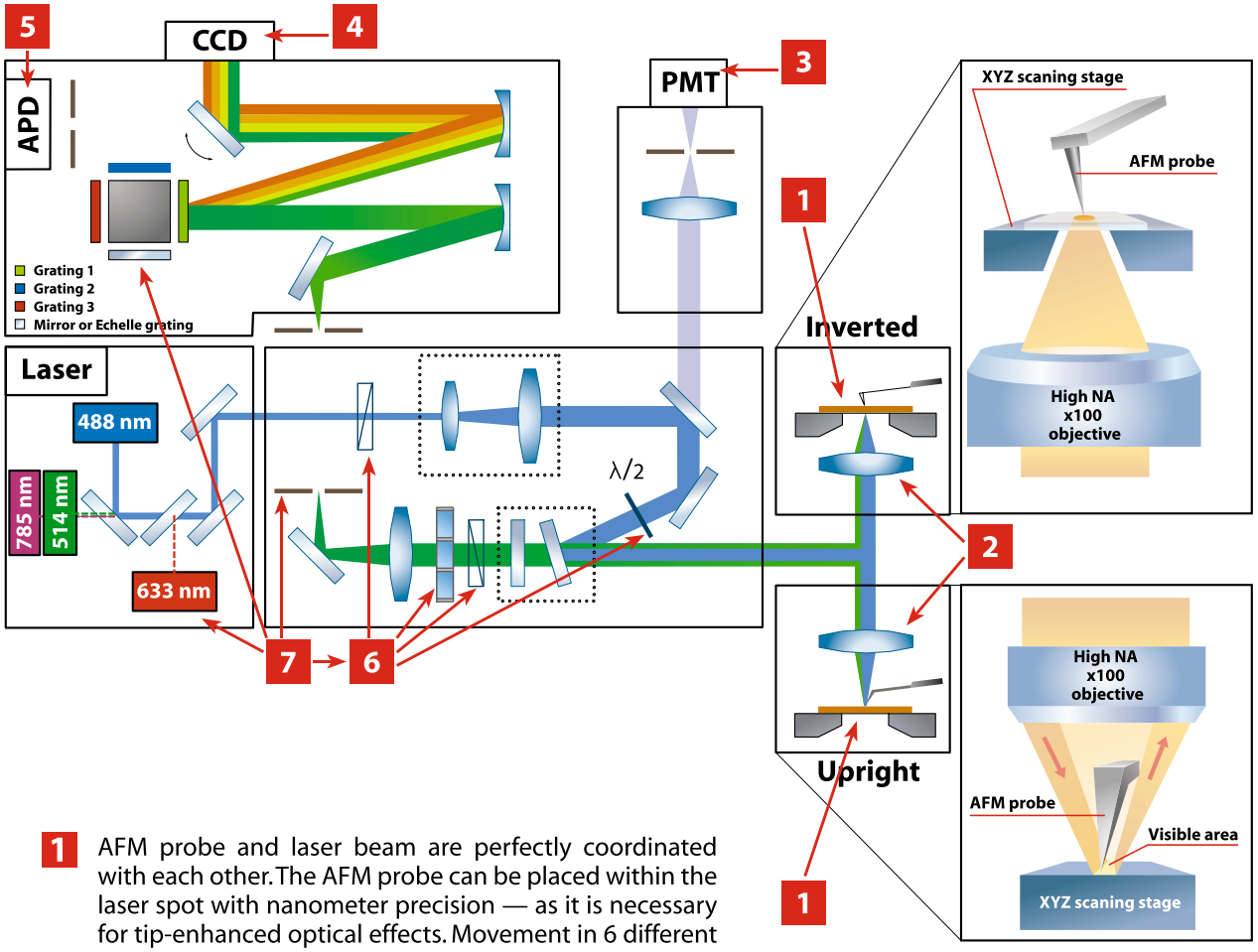
A laser for every purpose

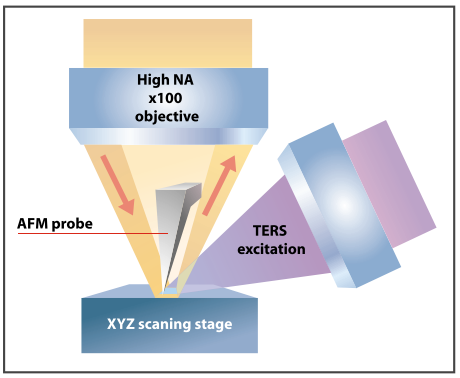
NTEGRA Spectra is built to offer you maximum flexibility. As with many microscopy parameters, Raman presents trade-offs. The intensity of Raman scattering is inversely proportional to the fourth order of the excitation wavelength. Therefore, to obtain maximum signal, the experiment dictates the use of the shortest possible wavelengths. However, longer wavelengths penetrate deeper into the sample and are less harmful to delicate preparations, especially biological samples. To optimize your experimental design, **NTEGRA** Spectra can be configured with three different software selectable lasers. Simply choose the one that best fits your needs.

One master software program makes the complex simple

Truly great engineering makes complex processes transparent to the user. **NTEGRA** Spectra is prime example of NT-MDT's brilliant engineering. Taken piece by piece, 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 have to be individually controlled and seamlessly integrated. Not to worry. Manage them easily through the fully integrated system software. Specify the pinhole diameter on the confocal system, choose the appropriate laser, adjust the spectrometer… all with the click of your mouse.

NTEGRA Spectra optical scheme





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1 AFM probe and laser beam are perfectly coordinated with each other. The AFM probe can be placed within the laser spot with nanometer precision — as it is necessary for tip-enhanced optical effects. Movement in 6 different axes is controlled by closed-loop sensors.

2 High NA objective is rigidly integrated into the SPM base. It provides unprecedented optical system stability — designed for long-term and weak-signal experiments.

3 Reflected laser light is used to obtain confocal laser image.

4 TE-cooled (-70°C) CCD serves as a sensitive spectroscopy detector.

5 Alternatively avalanche photodiode can be used for photon counting.

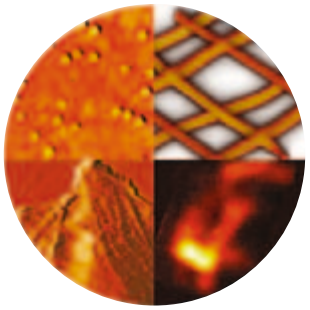
6 Flexible polarization optics in both excitation and detection channels. User defined optical filters.

7 Excellent software integration has been realized. All system modules (AFM, optics and mechanics) are driven by the same software package. Lasers, gratings, pinholes etc, can be chosen and adjusted from the program.

8 NT-MDT provides solutions for all possible TERS geometries

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

**Confocal fluorescence:
imaging and spectroscopy**



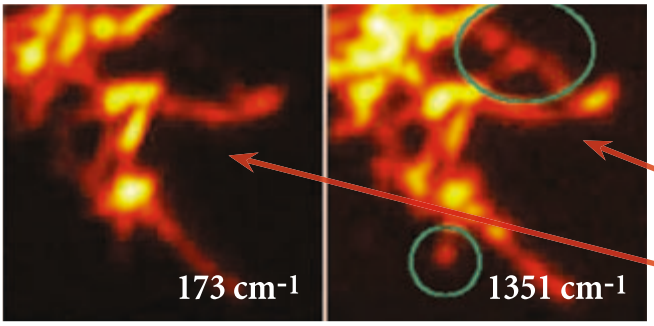
**Near-field optical
microscopy**

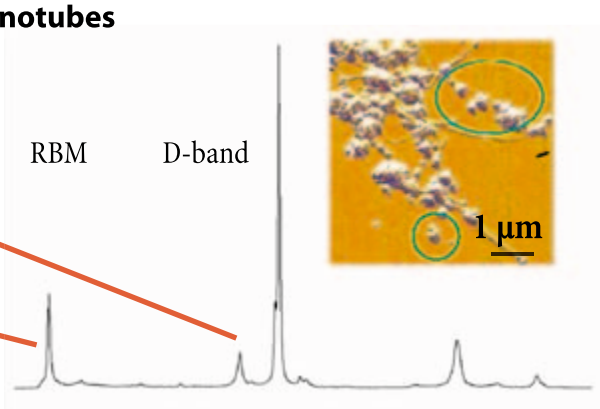
**Confocal Raman:
imaging and spectroscopy**

**Conventional microscopy
and reflected laser confocal
imaging**

All techniques can be applied to the same object

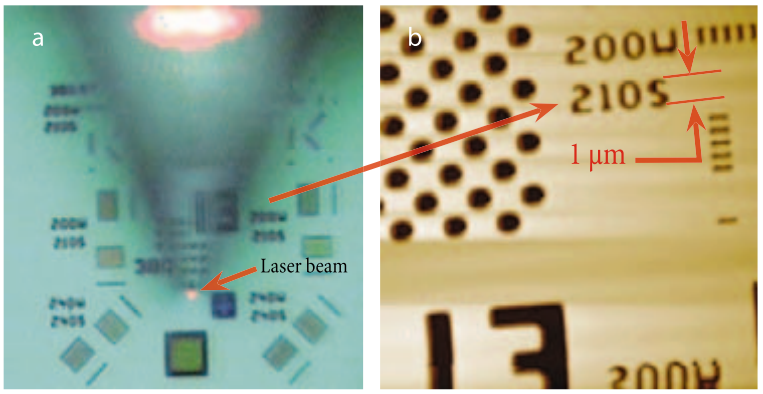
Carbon nanotubes





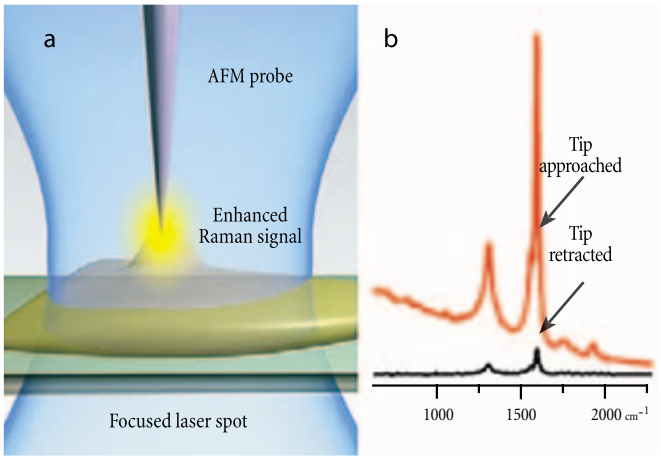
AFM image, Raman spectra and 2D Raman maps of single-walled carbon nanotube material. Amorphous carbon is visualized in D-band (1351 cm^{-1}) while well structured nanotubes are present in RBM-band (173 cm^{-1}). Raman images size 5x5 µm. Images courtesy of Dr. Kudryashov, TII, Tokyo, Japan.

AFM working simultaneously with 400 nm resolution optics

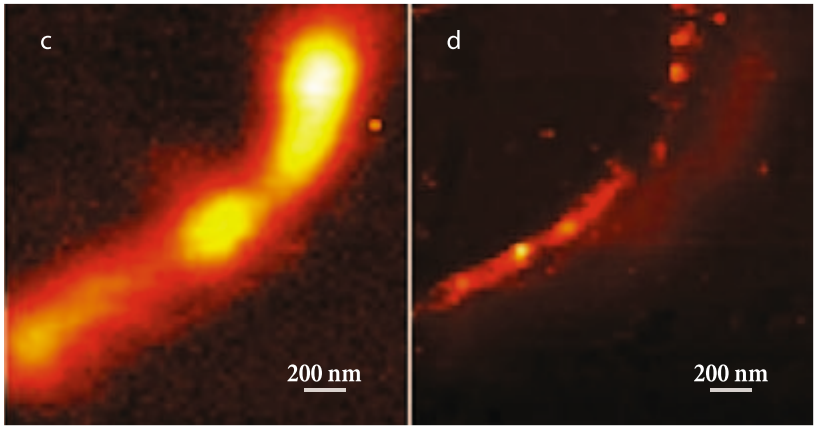


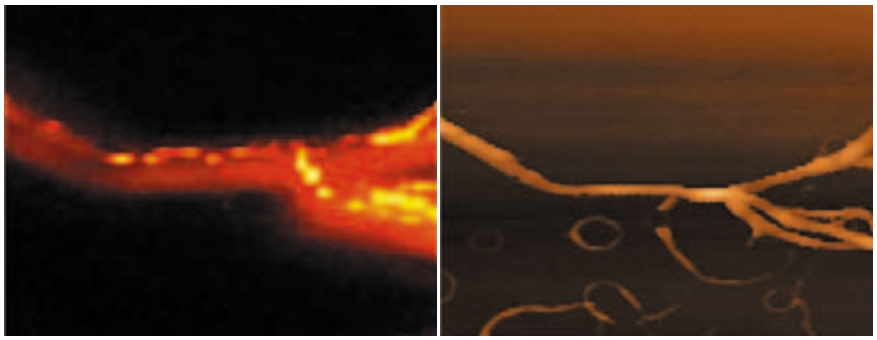
"AFM + confocal microscope" with high magnification optics in upright configuration. Note extremely high imaging resolution of 100x objective as seen on 1 µm height characters on Si substrate (a). Due to the high-numerical aperture (0.7) of the objective, opaque silicon AFM probe looks "transparent" on the image. AFM scanning can be obtained simultaneously (b) with both direct and confocal optical images. 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.

Raman microscopy with ultra-high spatial resolution



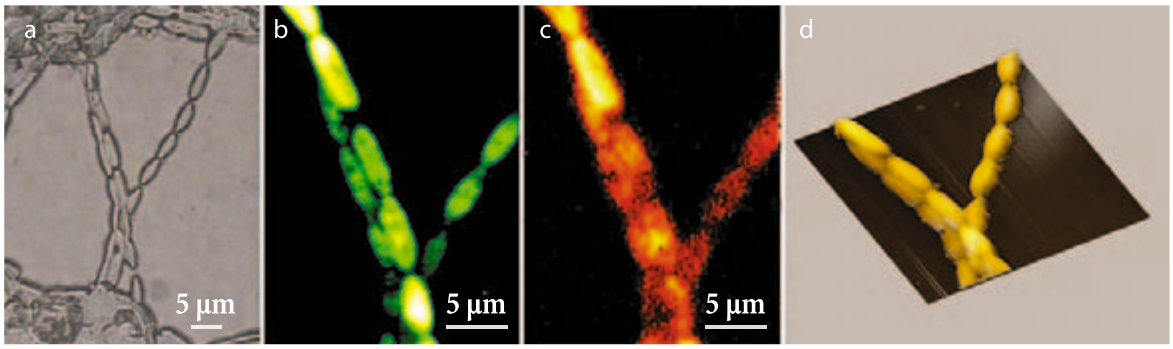
A — a specially prepared AFM probe (metal coated cantilever or etched metal wire) is precisely positioned inside a tightly focused laser spot. b — intensity of carbon nanotube G- and D- Raman bands increases by several orders of magnitude when the special AFM probe is landed and positioned over a small (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 ~70 nm. Note, in this example, TERS provides more than 4-times better spatial resolution as compared to confocal microscopy. Resolution down to 10 nm and less is theoretically possible. 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 Co.





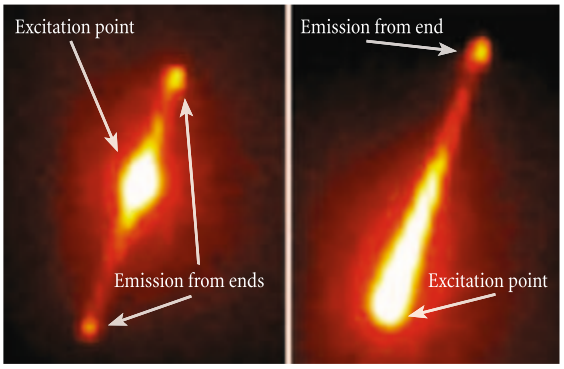
TERS imaging provides almost the same resolution as SPM one: branching points of carbon nanotube bundle are clearly seen on both AFM (right) and TERS (left) images. Image courtesy of Prof. R. Zenobi, ETH Zurich, Switzerland, Dr. G. Hoffman, Dr. J. Loos, TUE, the Netherlands, and Dr. P. Dorozhkin, NT-MDT.

Comprehensive analysis of biological structures



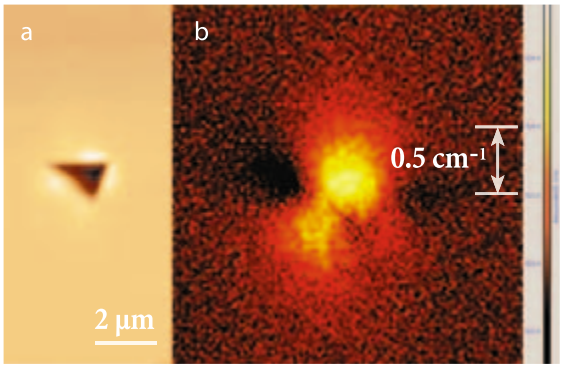
Algal cells visualization by different techniques. a — bright field overview, b — confocal Raman map at 1524 cm^{-1} (beta-carotene line), c — confocal image of autofluorescence at 492-513 nm, d — AFM image.
Sample courtesy of Don McNaughton, Monash University, Victoria, Australia.

Light transport in nanostructures



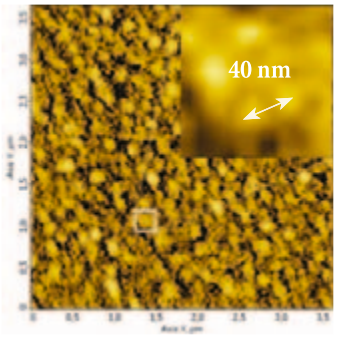
Fluorescent nanowire is excited by 488 nm light at the body (left image) and at the left end (right image). Excitation light is completely cut off from the image by two edge filters (with 10^{-6} transmission).
Partof the fluorescence light emitted from nanowire (>10%) is transmitted through the nanowire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

Stress mapping in silicon structures

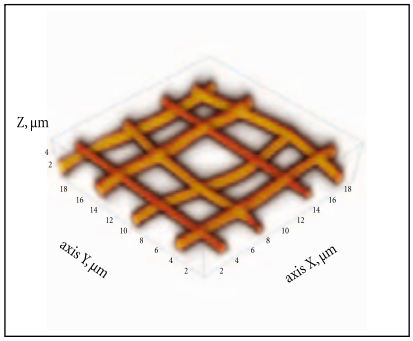


A — AFM topography of indentation in silicon substrate.
b — Center of mass position shift of 520 cm^{-1} silicon line - proportional to stress distribution around the indentation.
Spectral resolution: better then 0.1 cm^{-1}

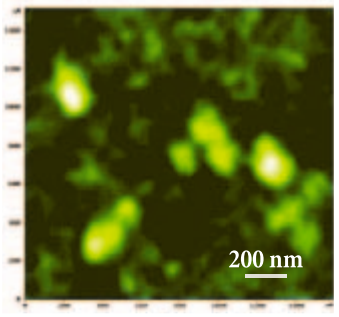
More applications



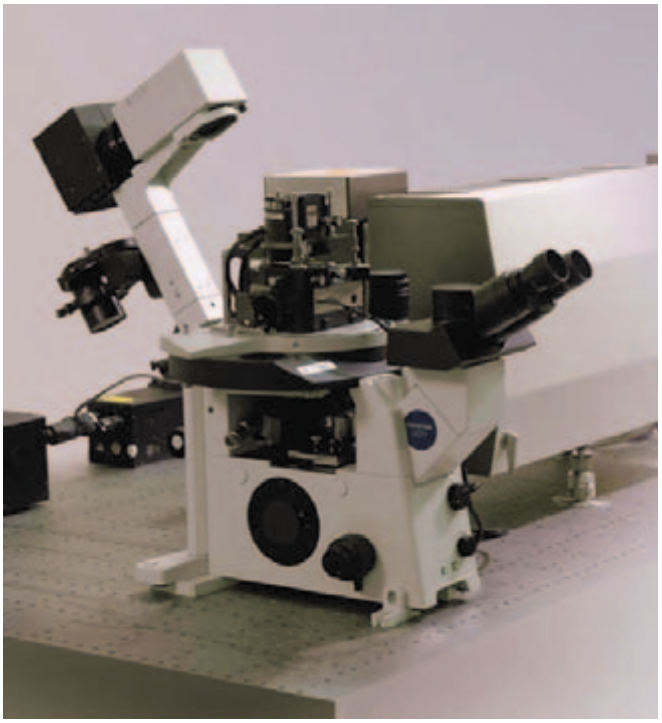
Scanning near-field optical image (SNOM) of polymer with granular structure. Two grains separated by about 40 nm (enlarged inlet) show excellent spatial resolution of the technique.



3D confocal image of optically distinct network structures made by femtosecond laser pulses within transparent polymer sample.
Image courtesy of Dr.Kudryashov, TII, Tokyo, Japan.

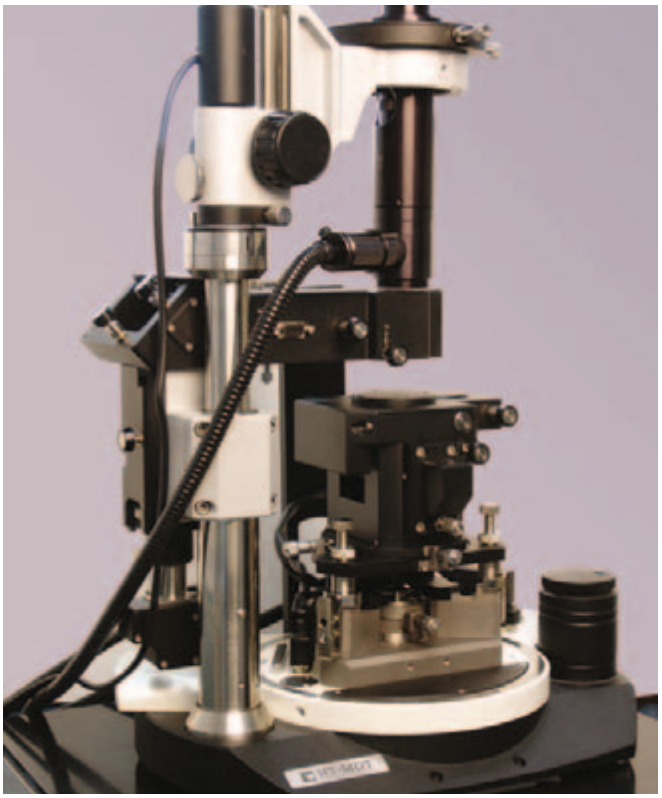


SNOM image of mitochondria dyed with FITC-labeled antibodies. Note XY resolution beyond the diffraction limit.



NTEGRA Spectra inverted setup:

- Designed for transparent samples
- Highest optical resolution achievable
- Highest efficiency of Raman photon collecting
- Operates in liquid
- SNOM
- Probe scanning in addition to sample scanning (important for TERS)
- Fits most commercial inverted microscopes and spectrometers



NTEGRA Spectra Epi setup:

- Designed for opaque samples
- Highest optical resolution simultaneously with AFM
- Highest efficiency of Raman signal collecting simultaneously with AFM
- Beam scanning in addition to sample scanning (important for TERS)
- Open design (fits almost any commercial spectrometer)

Nanolaboratory advantages

Due to conceptual versatility the SPM module of the NTEGRA Spectra can be re-specialized into:

- SPM with advanced environmental control (e.g. vacuum/artificial atmosphere and temperature control);
- the most powerful SPM for characterization of electric and magnetic properties;
- AFM tomography system (serial AFM imaging followed with 3D reconstruction);
- other highly specialized SPM-based systems.

Inverted or upright optical microscopy

Registration system	CCD or video camera, monitor
Standard techniques	bright field, fluorescence
Optional techniques (in inverted setup)	dark field, phase contrast, TIRF

Confocal microscopy

Resolution	XY	< 200 nm
	Z	< 500 nm
Optical sectioning	2D in selected plane (XY, YZ or XZ)	
	3D with reconstruction*	
Wavelength range	440-1050 nm (IR and UV are also available as option)	
Confocal pinhole	adjustable, 1-1500 μm	

Near-field scanning optical microscopy

Resolution	<100 nm
Standard mode	transmission
Optional modes	Reflection , luminescence

Atomic force microscopy

Modes	AFM (contact + semi-contact + non-contact), Lateral Force, Phase Imaging, Force Modulation, Adhesion Force Imaging, Magnetic Force, Electrostatic Force, Capacitance Microscopy, Kelvin Probe Microscopy, Spreading Resistance Imaging, Acoustic Microscopy (AFAM), Lithography (force, current)	
Noise level (RMS with closed loop on)**	XY	< 0.07 nm
Environmental control	air, liquids	
Temperature control	-30... +170°C	

Spectroscopy

Focal length		520 mm
Gratings(up to four in one system)		100, 150, 300, 600, 1200, 1800, 2400, 3600 lines/mm, Echelle
Spectral resolution	**1800 lines/mm grating**	<0.025 nm
	Echelle grating (optional)	<0.007 nm
Standard detectors		CCD, PMT
Optional detectors		avalanche photodiode, photon counting PMT, IR PMT and others

* Software for 3D reconstruction is already included in standard package.
** Spatial resolution in NTEGRA-based AFM is limited only by the probe. For example, super sharp DLC probes provide XY resolution of 1 nm and better.

Distributors world-wide

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