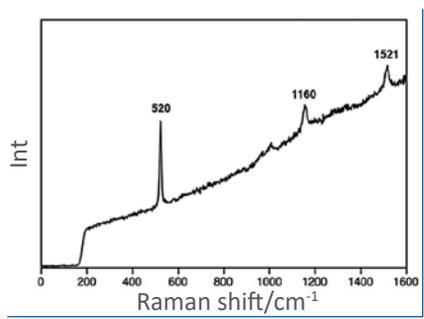


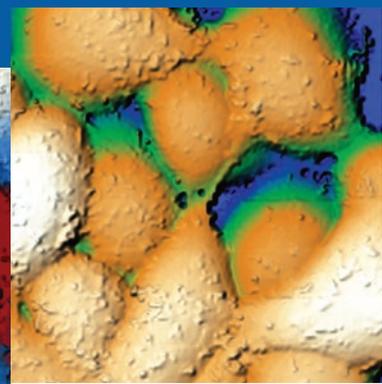
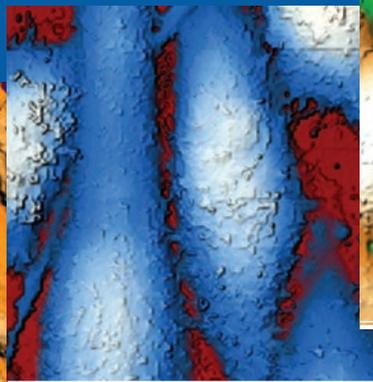
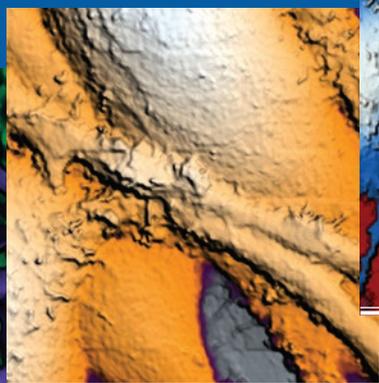
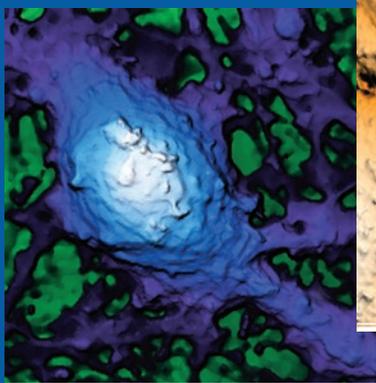


# NTEGRA Marlin

Cutting-edge AFM-RAMAN-SICM System  
for Biological Studies

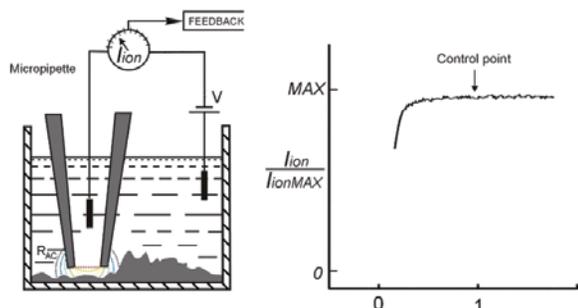


Project is supported by  
The Foundation for Assistance  
to Small Innovative Enterprises (FASIE)

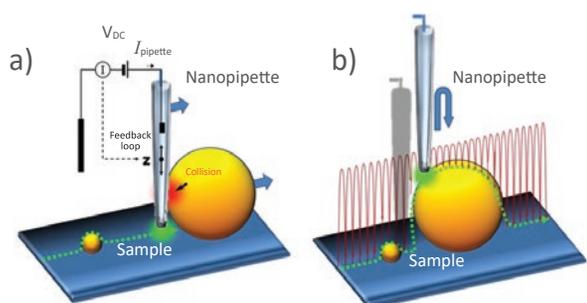


# NTEGRA Marlin – Cutting-edge AFM-SICM-RAMAN System for Biological Studies

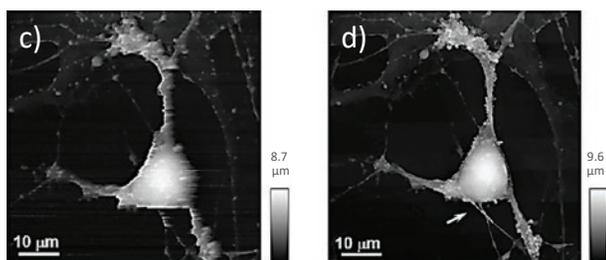
## SICM Principle



SICM (Scanning Ion Conductance Microscopy) is an SPM technique which uses nano-pipette (sharp glass electrode) for non-contact 3D surface mapping at high resolution. In SICM, the probe to sample distance is controlled via the decrease of ionic current flowing through the tip, as it approaches the sample surface. *Biophys.Journ.* 73, 653-658



(a) Illustration of a scanning nanopipette probe operating in continuous scan mode colliding with a spherical object possessing a steep vertical slope. (b) Illustration of the hopping mode used in HPICM showing how the pipette is withdrawn to a position well above the sample before approaching the surface.



(c,d) Topographical images of the same fixed hippocampal neuron obtained first with hopping mode (d) and then with continuous left-to-right raster scan mode (c), using the same nanopipette.

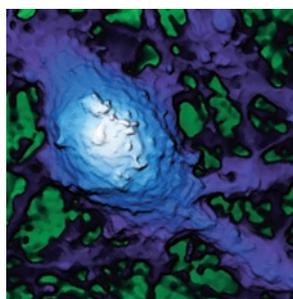
Hopping mode algorithm applied to SICM allows to image uneven and convoluted samples at high resolution ensuring that pipette always approaches from above rather than “dragging” along the surface. *Nature Meth.* (2009) 6: 279-281

Continuous and “Hopping” SICM images of highly corrugated neuron cell

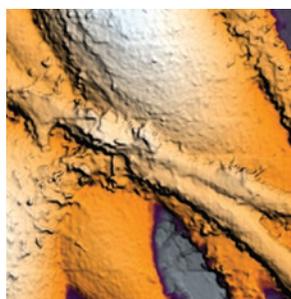
## SICM imaging of living cells

Noncontact algorithm of hopping SICM enables stable, fast and high-resolution imaging of soft and highly corrugated objects like **living cells** under physiologically relevant conditions.

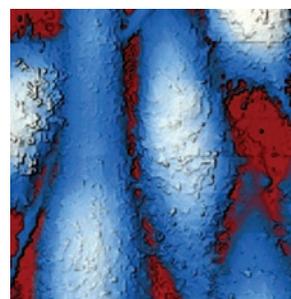
Scanning method ensures that the probe is always approaching the sample in vertical direction, thus, it becomes possible to visualize even those objects that are “suspended” in space.



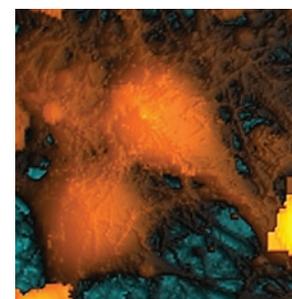
Neuron from mouse hippocamp 10×10×6,3 μm



B16 melanoma cells 25×25×5,4 μm

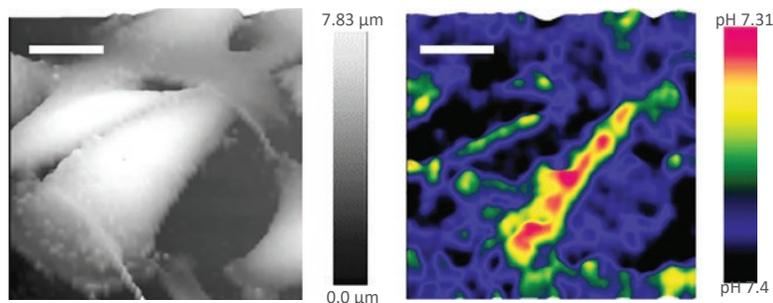


PC3 human prostatic carcinoma cells 40×40×6,8 μm



SICM image of live neuron from mouse hippocamp 40×40×13.3 μm

## Extracellular pH mapping of single living cells



**Extracellular pH mapping** of living cancer cells with high spatial resolution and sensitivity can be implemented by means of SICM utilizing double-barrel nanopipette. Morphology and pH-map of low-buffered living melanoma cells are shown from the left. Scale bars represent 20 μm.

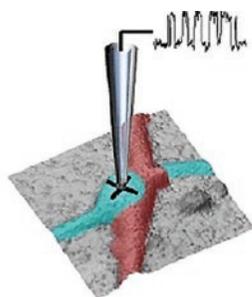
Nature Comm. (2019) **10**, 5610

## Smart patch clamp

**Smart patch-clamp** combines conventional patch-clamp and SICM.

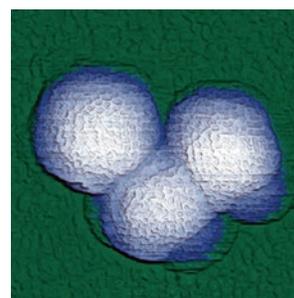
SICM generates a high-resolution topography followed by ion current recording in a specified location.

Neuron (2013) **79**, 1067-77.



*Nanoscale-Targeted Patch-Clamp Recordings of Functional Presynaptic Ion Channels*

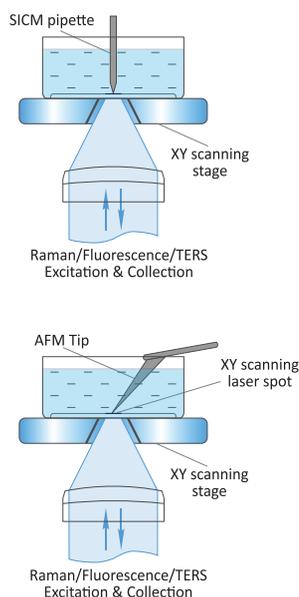
## High Resolution AFM



*200×200×100 nm high-resolution AFM topography of rhinovirus particles*

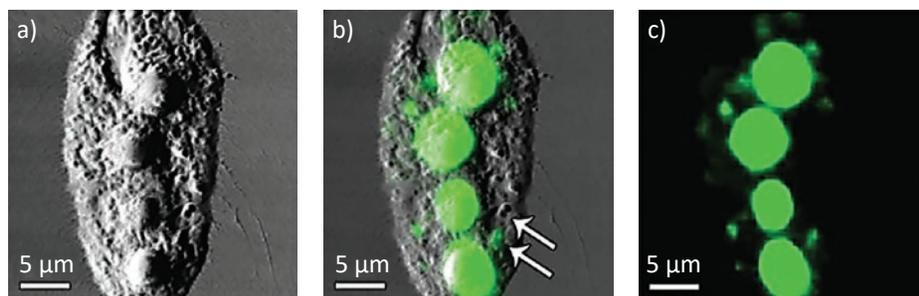
**High-resolution** AFM microscopy is available by means of Contact, Tapping and Hybrid modes and is empowered by lowest signal-to-noise ratio of OBD loop on the market down to 25 fm/VHz

## Correlative Imaging



Flawless hardware and software integration of AFM/SICM with confocal **Raman/fluorescence** microscopy provides the widest range of additional information about the sample. Simultaneously measured AFM/SICM and Raman/fluorescence maps of exactly the same sample area deliver complementary information about sample physical properties and chemical composition.

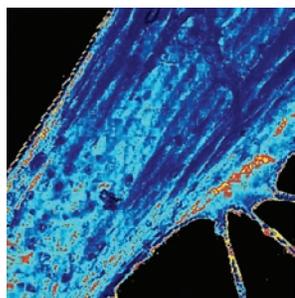
When equipped with specially prepared probes, which are working as “nanoantennas”, AFM/SICM-Raman combination allows to perform optical mapping with resolution less than diffraction limit and is called **TERS: Tip Enhanced Raman Scattering**.



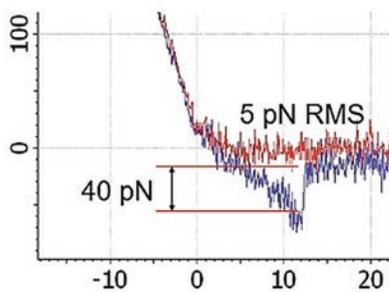
*Combined AFM-Raman microscopy studies of cyanobacterial film. AFM Phase map (a). Raman map showing the intensity distribution of the Raman band at 1521  $\text{cm}^{-1}$  corresponding to beta-carotene (c). Raman-AFM overlay (b). Typical Raman spectrum of the sample containing a band at 520  $\text{cm}^{-1}$  that is the Si-Si stretching mode of the silicon AFM tip and bands at 1160  $\text{cm}^{-1}$  and 1521  $\text{cm}^{-1}$  assignable to beta-carotene*

## Quantitative Nanomechanical Studies (QNM)

Combination of SICM and HybriD Mode™ AFM expands the boundaries of real-time quantitative nanomechanical mapping to 10 orders of elastic modulus keeping the possibility of single-point force

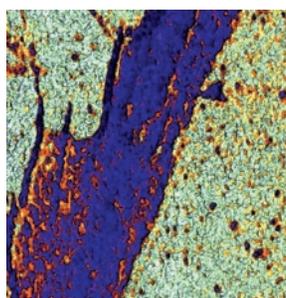


SICM E-Modulus map of live fibroblast cell. SICM E-modulus map of live fibroblast  
25×25 μm E = 2 Pa..3,4 MPa

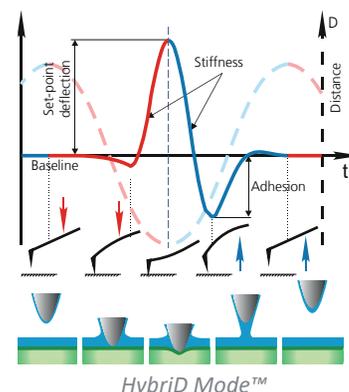


Streptavidin-biotin affinity single-molecule detection

spectroscopy experiments. Low- or noninvasive nature of tip-sample interaction allows to study delicate biological and jelly samples that are weakly attached to the substrate.



HybriD AFM E-modulus map of stem cell in HybriD Mode  
18×18 μm  
E = 200 kPa..50 MPa



## Specifications

### SICM

Position control: capacitive closed-loop sensors on X,Y,Z axes

XY travel range: 30×30 μm (optionally up to 100×100 μm)

Z travel range: 25 μm

Z position accuracy: 0.1 nm

Applications: SICM (Hopping mode), SECM, Smart Patch-Clamp, Microinjection, QNM, pH mapping

Typical image acquisition time: less than 5 minutes (depends on point number and sample roughness)

### Optical Spectroscopy

Confocal Raman/fluorescence/Rayleigh imaging runs simultaneously with AFM/SICM

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

Continuously variable ND filter with the range 1 - 0.001 for precise change of laser power

Motorized variable beam expander/collimator: adjusts diameter and collimation of the laser beam individually for each laser and each objective used

Fully automated switching between different lasers - with few mouse clicks

Full 3D (XYZ) confocal imaging with powerful image analysis

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 cm<sup>-1</sup>)

Up to 3 different detectors can be installed:  
- TE cooled (down to -100 °C) CCD/EMCCD cameras  
- APD in photon counting mode or FLIM detector  
- PMT for fast confocal laser (Rayleigh) imaging

Flexible motorized polarization optics in excitation and detection channels, crosspolarized Raman measurements

Low wavenumber/THz Raman spectroscopy: <10 cm<sup>-1</sup> with Bragg volume filters

Hyperspectral imaging (recording complete Raman spectrum in every point of 1D, 2D or 3D confocal scan) with further software analysis

Highest possible resolution optics is used simultaneously with AFM/SICM: up to 1.45 NA

Dual scan: scan by sample AND scan by laser spot (for Hot Spot mapping in TERS)

Closed-loop scanning mirrors for precise laser spot positioning to the tip (important for SNOM, TERS)

### AFM

XYZ closed-loop tip scanner 100×100×10 μm

High-performance low noise AFM: Z noise <0.1 nm (RMS in 10-1000 Hz bandwidth)

Measurements in gas and liquid environment

Applications: all standard AFM imaging modes (40+), Force-distance spectroscopy

Non-resonant oscillatory HybriD Mode™ allowing direct and fast force detection for QNM mapping (E-modulus, Adhesion, Deformation, etc.)