



Nanonics,

the leading provider of cuttingedge AFM technology, is proud to present its newest breakthrough:

The Hydra BioAFM[™] featuring **VISTA**[™] (Vivid Imaging Soft Touch AFM) mode

This innovation offers ultrasensitive single pN force mapping combined with the benefits of super-resolution optical/fluorescent imaging.

Imaging of live cells with <100nm optical resolution and direct correlation with AFM topography is now possible. The finest structures, such as microvilli, can now be analyzed with on-line mechanics.

The Hydra BioAFM[™], with its single or multiprobe capability, allows versatile optical integration with dual microscopes & side illumination.

The system enables Super-resolution methods like STED, PALM, STORM, and live cell NSOM with any available dye.



Imaging Live Cells

The Hydra BioAFM features VISTA[™] (Vivid Imaging Soft Touch AFM) – *enabling the imaging of live* cells with the highest resolution. Combine highest resolution force mapping with controlled and known point of contact.

VISTA Offers:

- Imaging of *live cells* with probes exhibiting Q factors in the thousands even in liquid
- Single pN force measurement the highest force resolution of any commercial AFM
- Force spectroscopy no Jump to Contact, allowing for investigating surface forces to the last nm from the membrane surface
- Ultralong extended probe tips with large aspect ratio and ideal angle for minimal cell perturbation
- 90µm Z range the largest of any commercial AFM for imaging biological samples





AFM topography of living MDCK cells





Topography of living MDCK cells imaged with Nanonics VISTA showing microvilli



DNA decorated with quantum dots

Corresponding modulus map, showing stiffer submembrane regions and softer microvilli

True Optical Integration

The Hydra BioAFM is designed for all forms of optical microscopy. The Hydra is the only BioAFM to integrate with either upright or inverted microscopes and dual (4pi) microscopes. Furthermore, side illumination for light sheet microscopy is available. The Hydra BioAFM has rendered obsolete solutions such as shuttling a sample from your AFM back and forth to an upright microscope. All of this is achieved with no compromise in force sensitivity and resolution.

The Largest Versatility in Optical Configuration:

- Upright
- Inverted
- Light sheet side illumination
- Combined 4 pi illumination (dual upright and inverted microscope)
- Brightfield & darkfield
- Phase contrast
- Epifluorescence
- TIRF
- Confocal fluorescence
- Raman and all forms of spectral Imaging
- Non linear two, three photon, SHG and STED imaging

See the Whole Picture

- Highly scattering tissue
- Bacterial cultures in Agar
- Opaque samples, such as microfluidic networks

Hydra MultiProbe with two AFM probes integrated with Nanonics Dual Microscope — combined upright and inverted microscopes



Extended transparent glass probe with high Q Factor



AFM with online water immersion objective for the highest optical resolution and purity



Optical Overlay

Biological research requires a comprehensive view of the sample; by combining multiple perspectives a deeper understanding emerges.

The Hydra BioAFM features a correlated view:

- AFM overlaid with real-time CCD optical imaging
- Complete optical access combined with glass AFM probes provides for an unobstructed view
- Quick and easy probe-sample placement
- Quick selection of the area of interest for AFM imaging within the CCD image
- Generate beautiful 3D collages, correlating optical view with topographical and force information



AFM Optical Raman imaging of wood cell



AFM/Raman collage image of the marked area on the CCD picture. (The cellulose Raman band is presented by colors overlaid on the 3D topography image of the cell walls.)

Configurations

The Nanonics BioAFM platform offers a state of the art solution for biological imaging. Featuring high stability and unparalleled sensitivity, the system easily produces images of the most challenging biological samples with the highest resolution. Nanonics offers two BioAFM models in multiple configurations in order to address a wide range of budgets and experimental requirements:

MultiView 2000 BioAFM



- Compact design
- *Versatile optical configurations*
- XYZ range: 180μm
- Largest Z range of any commercial AFM



Hydra Multiprobe BioAFM



- Multiple AFM probes for combined SPM measurements
- Compatible with standard biological *µManipulators*
- Keep your patch clamping pipette in place while imaging with AFM

Super-Resolution Optical Imaging

Over the last decade, a new powerful set of farfield optical tools has emerged for optical imaging of biological samples below the diffraction limit. These Super-resolution techniques have opened new horizons in the understanding of key biological phenomena. The Hydra BioAFM is compatible with all optical modes of Super-resolution. It permits the integration of a dual microscope for STED. PALM and STORM with total internal reflection prisms and an upright microscope are readily allowed.

The Hydra BioAFM can combine AFM characterization with the following Super-resolution techniques:

- STED
- PALM
- STORM



Linear Confocal, Non-linear Two Photon and Second Harmonic and Super-resolution Absorption NSOM Imaging of Living MDCK Cells With Dark Dots Indicating MicroVili Staining

Super-Resolution Techniques Comparison

| | Imaging Methods | Resolution | Characteristics | Limitations | | |
|------------|--|-------------------------------|---|---|--|--|
| | Standard Far-field Imaging | XY: 500 nm Z: 1.6 microns | Absorption Fluorescence | No Super-resolution No 3D Topography Out-of-focus Light | | |
| | Confocal | XY: 250 nm Z: 0.7 microns | Fluorescence | No Super-resolution No 3D Topography Reduced Out-of-focus Light | | |
| Far-Field | STED | XY: <100 nm Z: 0.7 microns | Fluorescence | Super-resolution No 3D Topography Reduced Out-of-focus Light Special Dyes High Light Intensities* : (104-109W/cm2) Photobleaching* Largely Fixed Cells* | | |
| - | STORM PALM | XY: <100 nm Z: >1 micron | Fluorescence of Special Dyes | Super-resolution No 3D Topography Reduced Out-of-focus Light Special Dyes High Light Intensities* : (103-104W/cm2) Photobleaching* Largely Fixed Cells* | | |
| Near-Field | NSOM | XY: <50 nm Z: 0.01 microns | Absorption Fluorescence Illumination | Super-resolution Pixel by Pixel Correlated 3D Topography Low Intensity Highly Local Photobleaching | | |
| | *E. Betzig, Angew. Chem. Int. Ed. 2015, 54, 2 – 22 | | | | | |

Opening A New Frontier Live Cell Imaging With NSOM

Near-field Scanning Optical Microscopy (NSOM) marks a paradigm shift in Super-resolution imaging. Live cell imaging with this powerful technique has been compromised in the past by stiff AFM cantilevers. Today Nanonics, with VISTA[™], achieves the potential of live cell imaging with NSOM.

Open New Frontiers of Super-resolution Optical Imaging (<100nm) that:

- Permits absorption and fluorescence
- Avoids the need for special dyes
- Correlates all optical contrast methods with 3D topography
- Allows highly confined zeptoliter illumination for fluorescence correlation spectroscopy (FCS) with physiologically relevant dye concentrations
- Enables AFM correlated FRET and dynamic FRAP with time resolutions previously unachievable
- Opens the door for the study of near-membrane phenomena with highly confined Z illumination



An NSOM probe is seen approaching yeast cells A. Seen from above with an upright microscope B. AFM image of the cells C. Fluorescence NSOM D. Transmission absorption NSOM

Specifications

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| User friendly software with video tutorials and on-line support by our experts Data analysis and processing software | Hybrid optical/AFM mode for control and synchronization of hybrid imaging techniques | | | | |
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