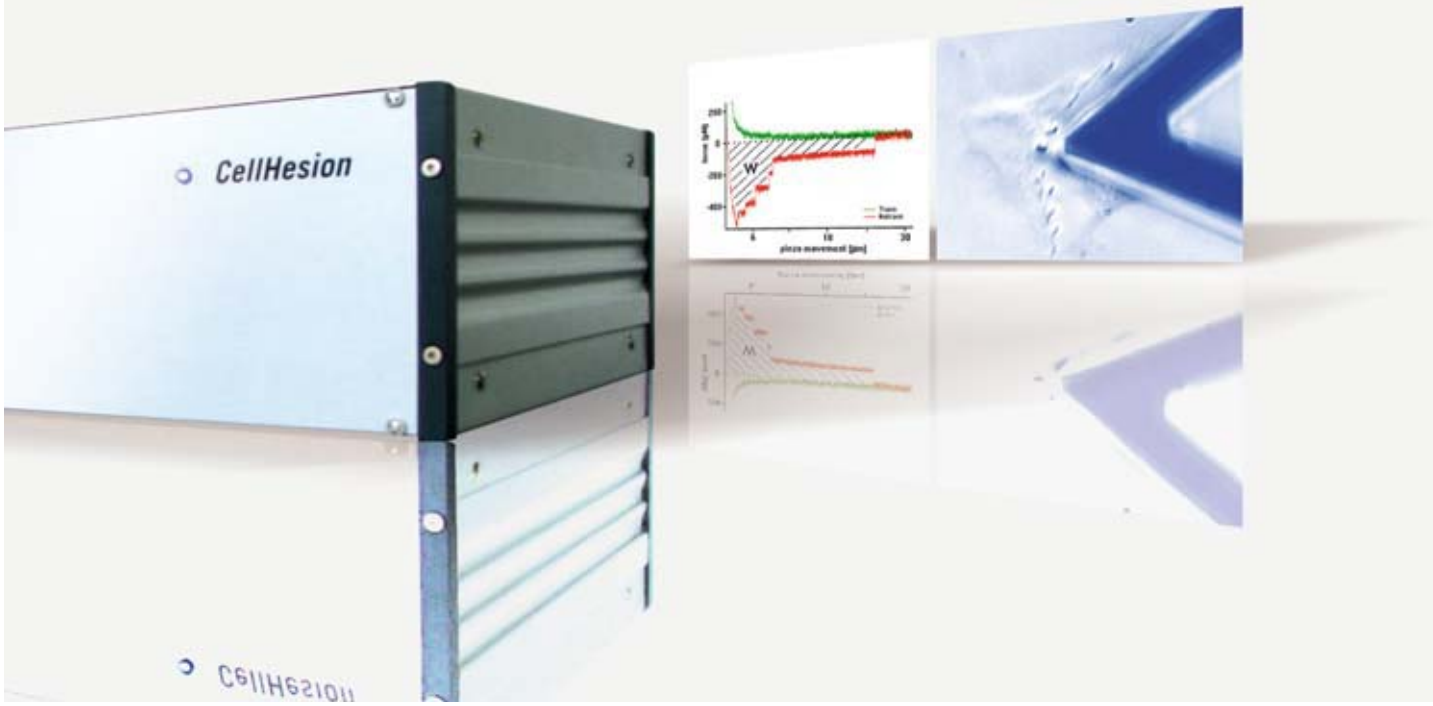
CellHesion®. The Ultimate AFM Based Solution for Quantitative Measurements of Cell Adhesion and Cell Mechanics at the nm-Scale.



JPK Instruments

Nanotechnology for Life Science

CellHesion®. Research in Cel

New Dimension. And a New

The measurement of cell adhesion and cell mechanics phenomena has been for years a primary focus of research activity in the life sciences. Many different disciplines have been interested in the interaction of cells with other cells or substrates. This is especially true for biophysics, biochemistry, cellular and medical research in cell migration, implant research, wound healing, developmental biology, or even in bioengineering for the development of bacteriophobic materials (e.g., long-term catheters). Up to now, adhesion phenomena have been studied with fluorescence microscopy, capillary techniques, or mechanical methods such as rotation assays.

All these methods have their limitations, however: either they provide qualitative results only, or they are imprecise, not to mention difficult to operate and interpret. The need for quantitative and statistically reliable measurements has proved elusive – until now. With its new CellHesion® module for the BioAFM NanoWizard®, JPK Instruments has created an integrated system for measuring cell-cell and cell-substrate interactions.

100 µm in z: CellHesion® expands the AFM spectrum

Quantitative results for single cells, precision down to the picoNewton (pN) level, total reproducibility – the innovative CellHesion® methodology opens up new paths for the study of cellular interactions – and a completely new level of quality in the results. The CellHesion® module expands the vertical travel range of the NanoWizard® AFM to more than 100 µm by integrating a precise sample lift mechanism into the AFM stage. The flexibility of the optical

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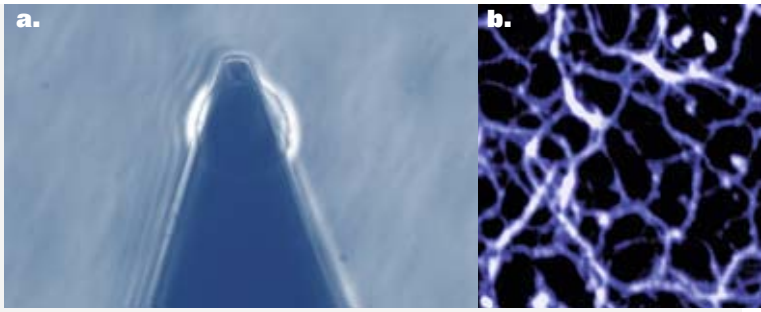
Quality in Scientific Results.

setup is enhanced by a PIFOC® focus tracking system that allows the user to keep the plane of interest in focus at all times during the experiments. The fully integrated CellHesion® development kit offers enough room in the vertical direction to handle even large cells and separate even well adhering cells from their substrates. The NanoWizard® platform ensures that the full range of modern AFM technology is available – from high resolution imaging to single molecule force spectroscopy, nanomanipulation or nanolithography – while allowing complete integration into inverted research microscopy such as fluorescence or confocal microscopy.

All modes of optical transmission illumination techniques such as DIC or phase contrast can be used simultaneously – an important feature when transferring cells to the cantilever, or for observing cells with fluorescence techniques such as TIRF, CLSM, FRAP or Ca^{2+} imaging parallel to CellHesion® experiments.

From imaging to elasticity: a myriad of measurement possibilities

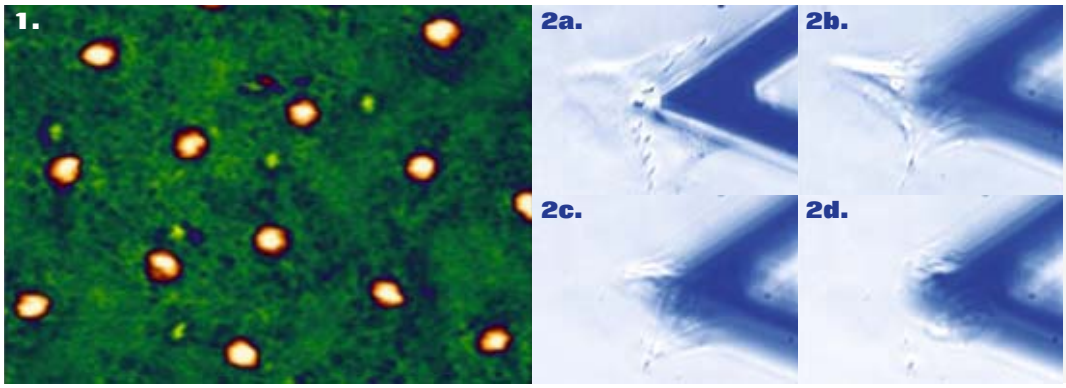
CellHesion® provides precisely quantifiable and reliably reproducible measurements of the adhesive and stiffness characteristics of cells or other microscopic objects. The system delivers precise information about single molecule behavior. Additionally, quantifiable and reproducible data can be produced for a number of important parameters involved in cell adhesion, including single bond partner interactions. CellHesion® can also be used to determine mechanical characteristics, such as cell elasticity or



stiffness – information that is important for the understanding of cell adhesion and cell motility since cell stiffness influences the contact surface as well as the number of possible bond partners on the molecular scale. With the CellHesion®, the mechanical characteristics of a cytoskeleton while varying temperature, pH values, ion concentration, or active agents can be tested while the cell response is simultaneously observed by optical methods.

Precision, performance, stability: German engineering down to the smallest detail

Outstanding piezo linearity and precise positioning – the capabilities of the CellHesion® system are based on piezoelectric elements from PI (Physik Instrumente) that have been specifically designed for JPK. PI is the global leader in parallel kinematic technology with friction-free flexures. Its systems lead the market in terms of dynamics, resolution, reproducibility and control accuracy. All axes have integrated capacitive sensors for closed-loop control – guaranteeing absolute positioning precision. The entire system is designed to provide utmost stability for the most demanding applications.



1. AFM image of gold clusters imaged in buffer. Scan area 375 nm x 500 nm.

2. A living cell is slowly detached from a gold nanopattern substrate through a chemically functionalized cantilever (phase contrast images). In 2d. the cell is completely detached from the surface.

Courtesy of C. Selhuber and J. P. Spatz, Heidelberg University, Germany

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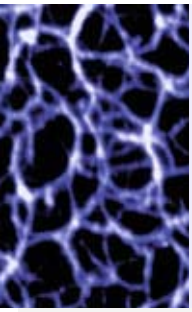
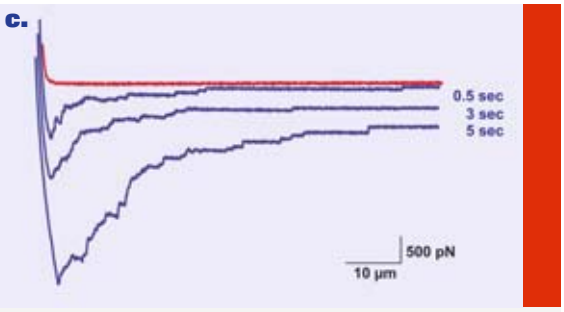
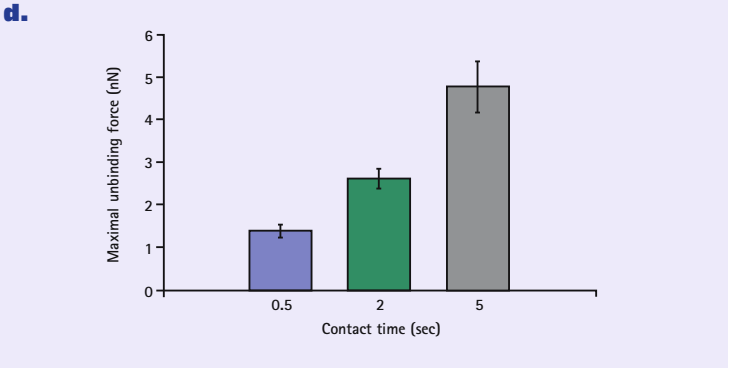


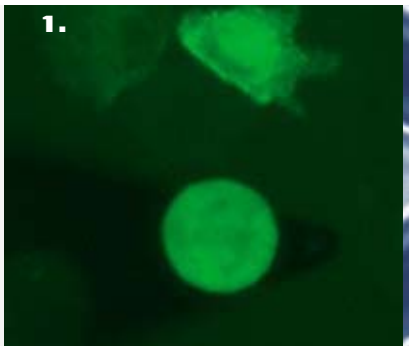


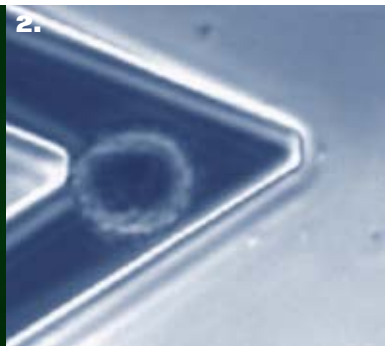
a. Phase contrast image of a SAOS cell (expressing the A2 integrin subunit) attached to the AFM force sensor (cantilever).
b. AFM image of collagen type I micro-fibrils on glass, imaged in buffer. Scan area 600 nm x 960 nm.
c. Force distance curves of the interaction between SAOS cells and collagen exhibiting 67 nm periodicity at different contact times.
d. Quantification of the maximal unbinding force for SAOS-A2 – collagen binding.

A critical point in combining AFM and fluorescence is the cross coupling from the AFM beam deflection laser with the fluorescence signal. The JPK infrared laser system used for cantilever deflection detection and special filters in the NanoWizard® AFM head, ensure not only fluorescence without cross coupling, but also avoid interference artifacts in force spectroscopy.

The unique coupling of the lift mechanism of the sample with a simultaneous focus tracking is essential for pulling experiments over a large travel range. The plane of interest is tracked via a PIFOC® objective stage controlled via the JPK software. The CellHesion® software includes advanced settings for force spectroscopy, mapping and an easy-to-use scripting tool for user defined experiments. With powerful batch processing of the data, the user can analyse and quantify datasets with the push of a button.







Complete or modular: the CellHesion® development kit

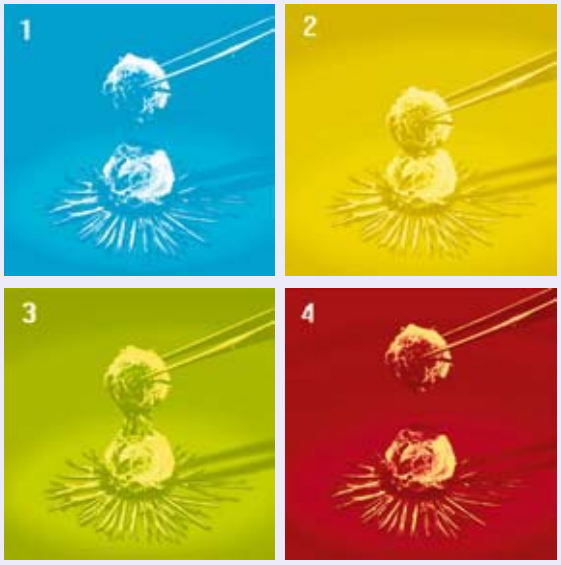
Temperature range from 15°C to 60°C, liquids, and simultaneous operation of optical contrast-enhancing transmission illumination techniques – CellHesion® is tailor-made for the specialized requirements of working with biological materials. Based on high-end BioAFM NanoWizard® technology and integrated into inverted microscopes, CellHesion® is a complete system for todays research needs. As an extra module, it can also be added easily to an existing NanoWizard® AFM platform.

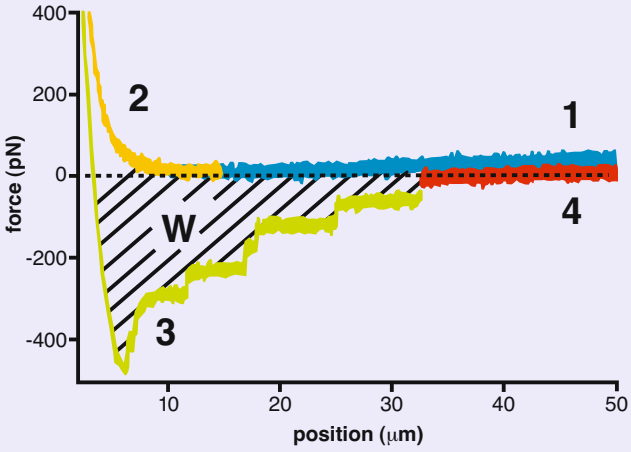
Because JPK Instruments specializes in life science and soft matter applications, it also offers a broad spectrum of accessories, so an existing research platform can be extended to meet individual needs. The BioCell™, for example. This patent pending mini-incubator for coverslip substrates can handle 400 µL of buffer solution and operating temperatures of 15°C to 60°C. Or the JPK CoverslipHolder: a similar design as the BioCell™, with perfusion but without the temperature control. Another alternative: the JPK SmallCell™, a closed fluid cell for small volumes below 70 µL.

All components are designed to work seamlessly with optical methods that are particularly useful in adhesion studies like Reflection Interference Contrast or TIRF. Structural information derived by these optical methods can be overlaid with functional data that is determined by the force measurements.

1. Fluorescence image of REF52 fibroblast attached to the cantilever and in contact with the surface next to the cantilever cell is a fluorescent cell that has adhered to the substrate.

2. Phase contrast image of a melanoma cell attached to the AFM force sensor (cantilever).





The result (see curve):
- *Single molecule events*
- *The "work of removal" (W)*
- *The maximum adhesion force*
- *Viscoelastic parameters*

CellHesion® at a glance

- Innovative platform for cell adhesion/cell mechanics research integrated into inverted research microscopes
- Measurements from single molecules to entire cells
- 3 axes tip scanning AFM system with 100 µm x 100 µm x 15 µm travel range with closed-loop control through high-speed capacitive sensor feedback
- 100 µm additional z-range with capacitive sensors for sub-nm precision
- 100 µm focus tracking by PI closed-loop PIFOC® system keeps the plane of interest in focus during force measurements (substrate or cell surface)
- Compatible with inverted microscopes for combined experiments, e.g., Carl Zeiss Axiovert 200
- Quantitative determination of cellular interaction parameters through JPK software batch processing
- Single molecule events, work of removal, maximum adhesion forces, number of events and viscoelasticity parameters such as Young's modulus in one system
- Living cell studies in native environment with temperature control, perfusion and gas flow (CO_2) on coverslips, slides or Petri dishes
- Proven NanoWizard® AFM technology for high resolution imaging and force measurements

PIFOC® is registered trademark of
Physik Instrumente (PI) GmbH & Co. KG

Precision pure and simple: the operating principle of CellHesion®

1. A single living cell is chemically bound to the cantilever (e.g., through a fibronectin coating) under optical control
2. This cell is brought into contact with the binding target (molecular layer, implant surface, single cell, confluent monolayer) on the substrate (slide, coverslip or Petri dish)
3. After a user defined reaction time the cell on the cantilever is separated from the substrate cell by retracting the vertical piezo (z-axis).
4. The cell resists the attempt of removing it from the surface if it adheres to the target. Therefore the cantilever bends noticeably, which is measured by a detector.
5. Because, in physical terms, a cantilever is a leaf spring, the actual adhesive forces and energies can be derived from the measured bending. This allows the identification of single molecule binding events that contribute to the adhesion.
6. The experiment is repeated many times with the same cell, with different cells, on different targets and with different conditions to gain statistically relevant information.

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