

The Single Cell Force Testing Solution for Cell Adhesion and Elasticity Studies

CELLHESION[®] 200



Innovative platform for adhesion and cell mechanics experiments

Characterization of cell/cell or cell/substrate interaction, cell elasticity, tether formation, adhesion, and cellular response

Quantitative measurements from single molecules to entire cells and tissue

Integrates with advanced optical imaging (DIC, phase contrast, confocal microscopy, TIRF, FRET etc.)

JPK
Instruments

we have joined Bruker



QUANTIFYING CELLULAR ADHESION AND RESPONSE TO EXTERNAL MECHANICAL STRESS WITH SINGLE-MOLECULE PRECISION

FORCES IN BIOLOGY

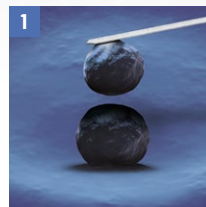
The measurement of cellular adhesion and cell elasticity is a primary focus of research activity in the life sciences. Many different disciplines have been interested in the interaction of cells with other cells or substrates. This is especially true for biophysics, biochemistry, cellular and medical research in cell migration, implant research, wound healing, developmental biology, stem cell research, infection biology and immune response.

Up to now, cellular adhesion has been studied with fluorescence microscopy, capillary techniques or mechanical methods such as rotation assays and flow chambers. All these methods have their limitations. Either they provide qualitative, difficult to interpret results or are difficult to operate. The need for quantitative and statistically reliable measurements has proven elusive – until now. With the new CellHesion® 200, JPK Instruments has created an integrated system for measuring cell-cell and cell-substrate interactions. The system can also be used to quantify cell elasticity and cellular response to external mechanical stress.

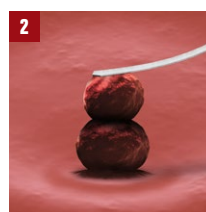
Providing reproducible quantitative results for single cells with precision down to the single-molecule level – the innovative CellHesion® methodology opens up new paths for the study of cellular interactions delivering data of unprecedented quality. The software automatically determines the important parameters involved in cellular adhesion from each dataset, including maximum adhesion force, single unbinding events, tether characteristics and work of removal.

CellHesion® 200 can also be used to determine cyto-mechanical characteristics such as cell elasticity or stiffness by probing different spots of the cell or the tissue surface with defined indentation forces. The user can extract Young's modulus from the software.

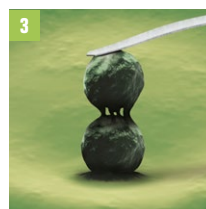
CELL-CELL ADHESION OPERATING PRINCIPLE



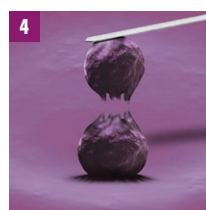
A single living cell is chemically bound to the cantilever sensor (e.g., through a fibronectin coating) under optical control.



This cell is brought with a defined force into contact with the binding target (molecular layer, implant surface, single cell, confluent monolayer) on the substrate (slide, coverslip or Petri dish).



After a user-defined reaction time the cell on the cantilever is separated from the substrate cell by retracting the cantilever in vertical direction (z-axis) through a piezo actuator.

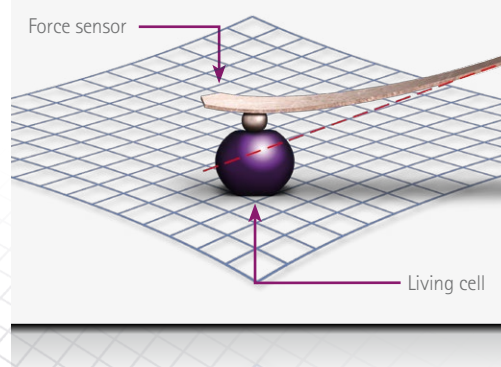


The cell resists the attempt of removing it from the surface if it adheres to the target. Therefore the cantilever bends noticeably, which is measured by a detector.

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. 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.

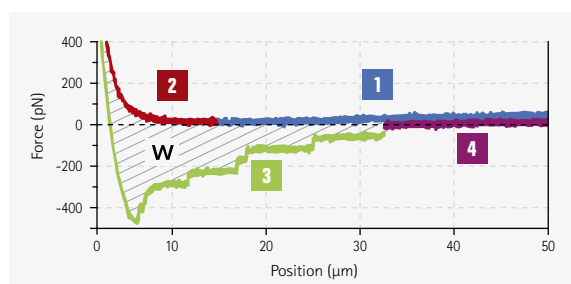
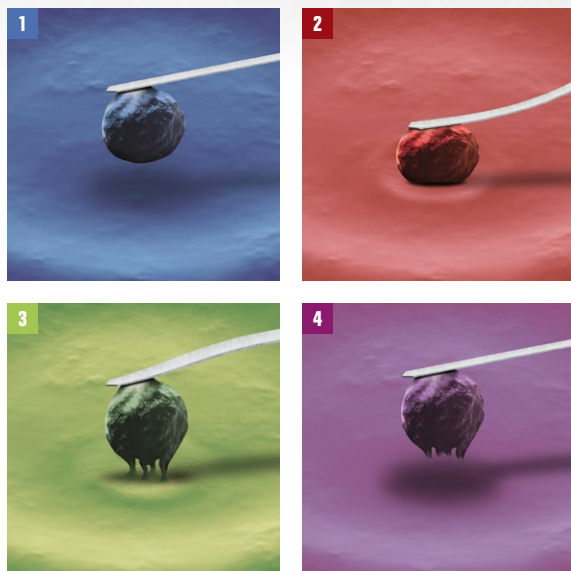
A living cell is locally probed with a cantilever sensor to measure the mechanical response (indentation) to an external force to quantify cellular elasticity and stiffness

CELL ELASTICITY & STIFFNESS PROBING



PERFECT OPTICAL INTEGRATION AND ENVIRONMENTAL CONTROL SOLUTIONS

CELL-SUBSTRATE ADHESION OPERATING PRINCIPLE



The result of a single measurement cycle is a force vs. distance curve, which allows to determine single molecule events, the "work of removal" W , tether formation, the maximum adhesion force and viscoelastic parameters.

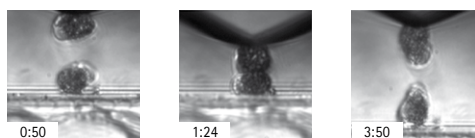
MADE FOR OPTICAL INTEGRATION

The easy and complete integration of the CellHesion® 200 into inverted research microscopes from leading manufacturers such as Carl Zeiss, Leica, Nikon and Olympus makes it a powerful combination. Techniques such as epifluorescence or confocal microscopy can be used simultaneously with the CellHesion® system. Observation of target cells with fluorescence

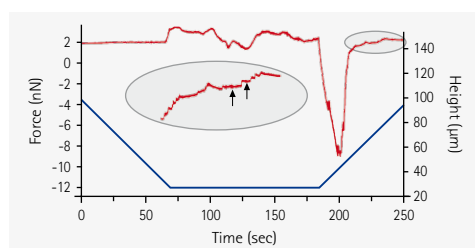
techniques such as TIRF, CLSM, FRAP or Ca^{2+} imaging parallel to CellHesion® experiments gives insight about the molecular mechanisms involved in adhesion processes or cytoskeleton dynamics. All modes of optical transmission illumination techniques such as DIC or phase contrast can be used simultaneously. This is an important feature when transferring cells to the force sensor (cantilever) or to check the condition of the specimen. Structural information derived by these optical methods can be overlaid with functional data that is determined by force measurements.

ENVIRONMENTAL CONTROL

CellHesion® 200 is tailor-made for the specialized requirements of working with living cells. The use of standard substrates such as 35mm Petri dishes with or without glass bottom or round coverslips makes cell cultivation and handling easy. Temperature control from 15°C to 60°C, fluid exchange and ports for CO_2 control are integrated in the JPK PetriDishHeater™ or in the JPK BioCell™ designed for coverslips. As an option, the CellHesion® 200 can be integrated in existing incubators (ask for model). All parts of the setup which are in contact with the sample can be sterilized. CellHesion® 200 offers enough vertical-axis travel range to handle large cells and separate even well-adhering cells from their substrates.



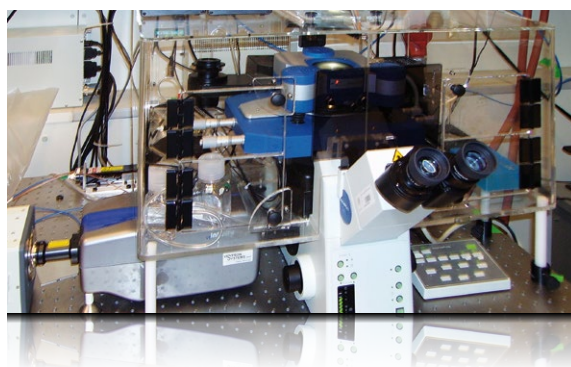
Living *Xenopus laevis* cells. Side view images, showing approach (0:50 min.), contact (1:24 min.) and retract phase (3:50 min.).



Piezo height (blue) and force curve (red) showing membrane tethers (arrows).

Data courtesy of C. Gonnemann, Dr. D. Stamov and Dr. C. Franz, KIT Germany

CellHesion® 200 mounted on Olympus base with incubator



CELLHESION® 200 SOFTWARE

Easy-to-use software is the key to handling large quantities of data. Our new user interface architecture enables the operator to quickly and easily control the whole system. The CellHesion® software includes also settings for advanced force spectroscopy and mapping modes with an easy-to-use scripting tool (ExperimentPlanner™) for user-defined experiments. With powerful batch processing of data, the user can analyze and quantify datasets with the push of a button.

SPECIFICATIONS FOR THE CELLHESION® 200

- Innovative platform for cell adhesion/cell mechanics research for measurements from single-molecules to entire cells and tissue
- Compatible with inverted microscopes for combined experiments, e.g., Zeiss Axio Observer, Axiovert A1, Nikon Ti/TE 2000, Olympus IX series and Leica DMI series
- Compatible with all major light microscopy techniques such as DIC and phase contrast or fluorescence and confocal techniques such as TIRF, FRAP, LSM and others
- Compatible with upright microscopes, such as Zeiss AxioZoom and Leica Z16
- Automated range adaption in z direction for strong corrugated surfaces
- Cantilever sensor lifting system with >110µm travel range with closed-loop control through high-speed capacitive sensor feedback
- Equipped with motorized precision stage, in particular for tissue probing, within 20x20mm range
- Living cell studies in native environment with temperature control, perfusion and gas flow (CO₂) on coverslips with the JPK BioCell™ or 35mm Petri dishes with or without glass bottom from Wilco, BD, WPI with the JPK PetriDishHeater™
- All parts which came in contact with the sample are easy to clean
- Compatible with standard incubators (specify model)
- High-throughput determination of cellular interaction parameters through JPK software batch processing
- Single-molecule events, work of removal, maximum adhesion forces, number of unbinding events and viscoelastic parameters such as Young's modulus in one system



CellHesion® 200 setup on Zeiss Axio Observer

LITERATURE

- **Gonnermann et al. (2015):** Quantitating membrane bleb stiffness using AFM force spectroscopy and an optical side-view setup. *Integr. Biol.*, doi:10.1039/C4IB00282B
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- **Pagliara et al. (2014):** Auxetic nuclei in embryonic stem cells exiting pluripotency. *Nature Materials* 13, 638–644, doi:10.1038/nmat3943
- **Siamantouras et al. (2014):** Quantitative investigation of calcimimetic R568 on beta cell adhesion and mechanics using AFM single-cell force spectroscopy. *FEBS Letters* 588(7), 1178–1183, doi:10.1016/j.febslet.2014.02.058
- **Schubert et al. (2014):** Assay for characterizing the recovery of vertebrate cells for adhesion measurements by single-cell force spectroscopy. *FEBS* 588(19), 3639–48, doi:10.1016/j.febslet.2014.06.012
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Temperature control with JPK PetriDishHeater™

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