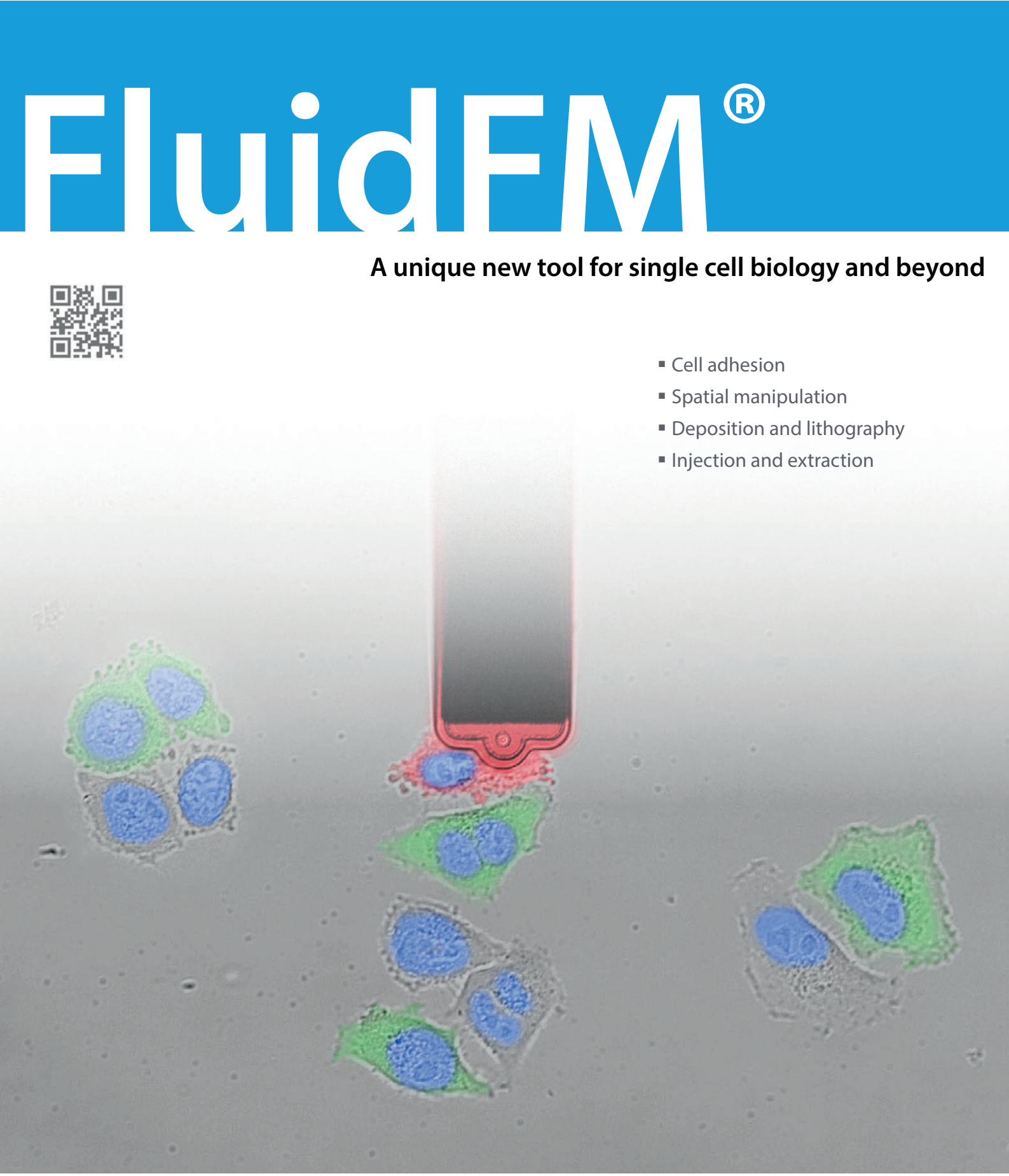
FluidFM®

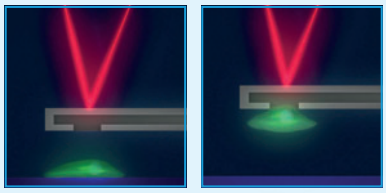
A unique new tool for single cell biology and beyond

- Cell adhesion
- Spatial manipulation
- Deposition and lithography
- Injection and extraction

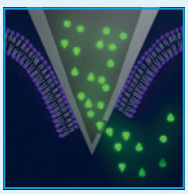


Nanosurf®

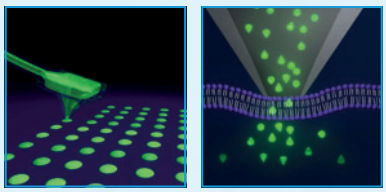
CYTOSURGE®



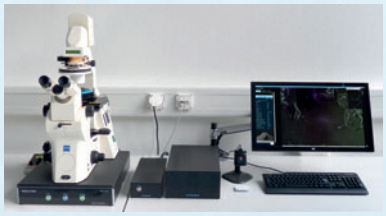
Cell adhesion / spatial manipulation. Precise micromanipulation of small objects. Adhesion force measurements on any type of surface and any type of object (cell, polystyrene beads, etc.); no need to coat the cantilever to promote adhesion. Quick re-use of the same cantilever; over and over.



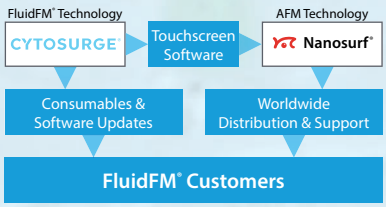
Injection and extraction. Injection or extraction under constant force control ensures minimal mechanical stress to the cell.



Deposition and lithography. Deposition of micro-sized patterns and volumes on surfaces (left). Local drug delivery to a cell via diffusion (right). Both can be performed under highly sensitive force control.



Example of a typical FluidFM® setup. From left to right: FlexAFM-NIR scan head on an inverted microscope and active vibration isolation table, FluidFM® pressure controller and pump, CytoClip QR-code reader and a probe package, touchscreen and keyboard.



Strong partners. FluidFM® technology and software development by CYTOSURGE. AFM technology and worldwide FluidFM® distribution by Nanosurf.

FluidFM®

A unique new tool for single cell biology and beyond

FluidFM® combines the unique possibilities of Cytosurge nanofluidics with the positional accuracy and force sensitivity of the Nanosurf FlexAFM to provide a whole new level of control and possibilities in single-cell biology and beyond.

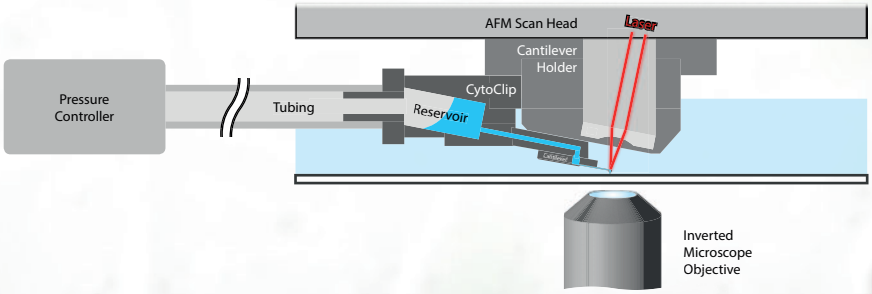
Main features

- Optical access to the sample and highly accurate pressure, force, and position control
- Optimized experimental workflows: (1) cell adhesion and spectroscopy mapping, (2) spatial manipulation, (3) deposition and lithography, (4) injection and extraction
- Complete system integration, operation, and handling via intuitive touchscreen control software

A multitude of new applications in the field of single cell biology, biosensors, biofunctionalization, nanopatterning, and nanolithography is now within reach!

Working principle

A hollow cantilever with fluid reservoir attached to a nanofluidics pressure control system and an AFM:

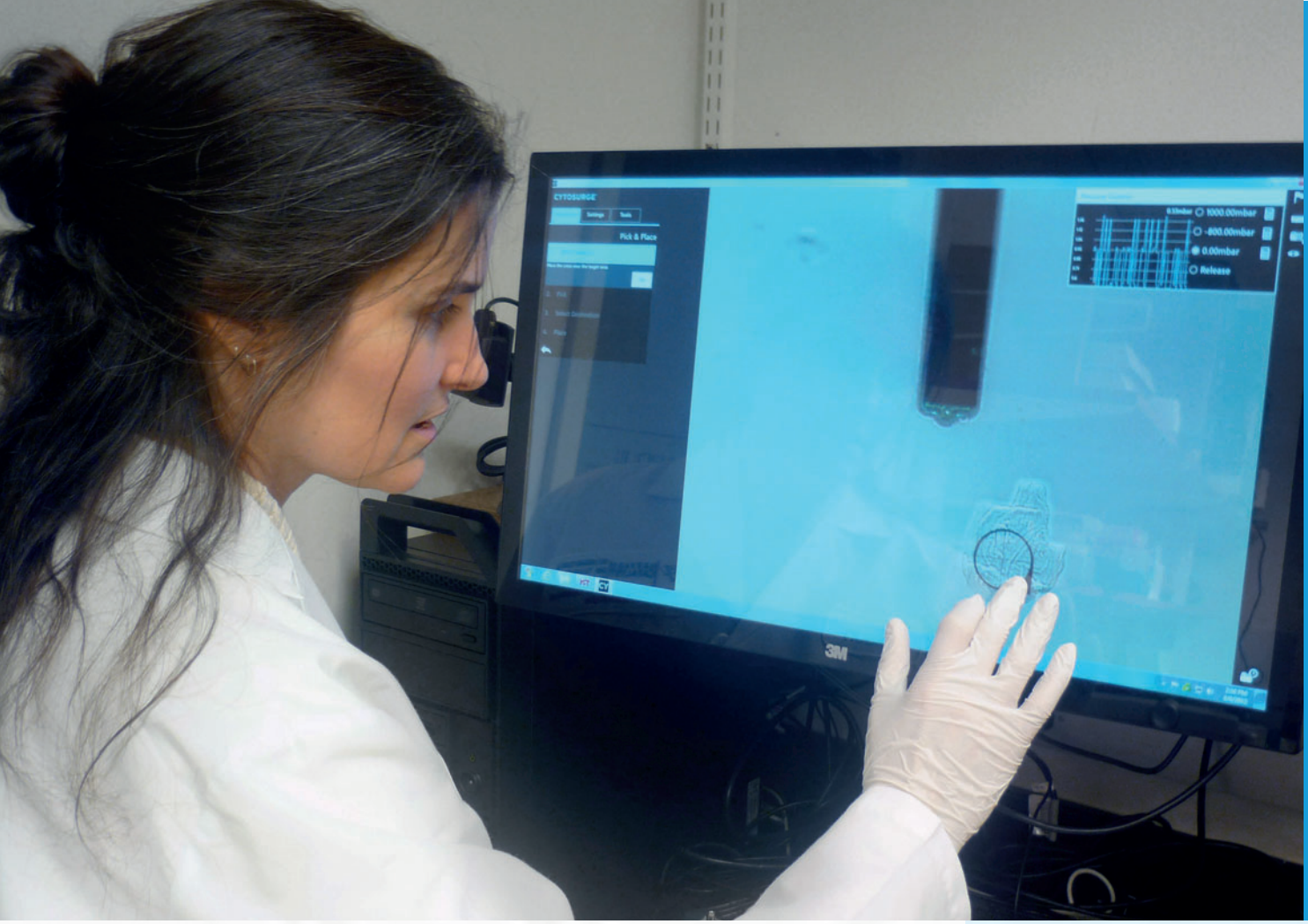


Components of the system:

- FlexAFM-NIR scan head and controller
- Motorized inverted microscope stage
- FluidFM® pressure controller and pump unit
- Dedicated, fully configured PC with touchscreen
- Pre-installed control software to drive all hardware components
- Optional application packages for individual FluidFM® workflows
- A range of FluidFM® CytoClip probes with hollow, microfabricated cantilevers

Required infrastructure:

- Inverted light microscope
  (full functionality supported for Zeiss Axio Observer.Z1)
- Vibration and optional acoustic isolation
- Incubator chamber for inverted microscope (depending on application)



Seamless workflows in a revolutionary touchscreen interface

Integration

FluidFM® uses a whole new approach to instrument handling and experimental design. The integrative nature of its touchscreen-based control software brings together optical, force, pressure, and position control in one place. The entire system is easy to use and allows objects and experimental settings to be manipulated via on-screen interactions. Moving a sample or indicating measurement positions has never been more intuitive.

Workflows

Experiments are performed via pre-defined and optimized experimental workflows, the parameters of which are also controlled and modified on-screen. The system guides the operator through all relevant steps and will then steer position, forces, microfluidics, and optics synchronously, thus putting their combined power at your fingertips.

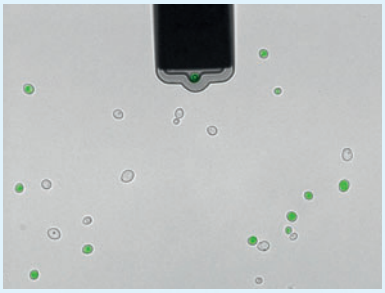
Media management and overlay tagging

Thanks to the unique storage concept of the control software, measurement data is indelibly mapped to the microscope's optical data in both position and time, providing full details for each measurement performed during the experiment. This data can be recalled, tagged, and overlaid at all times.

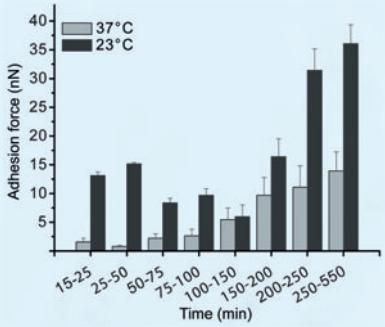
Cantilever identification and information. Mounted cantilever details are retrieved online after scanning the QR-code on the packaging material. This data is then used and stored with the experimental data by the software.

Intuitive touchscreen control. Dragging a finger across the screen moves the sample in real time via the motorized inverted microscope stage. The selected position follows your movements exactly. Other supported gestures include: focus, click/select, area select, magnification, etc.

Applications



Quick selection of cells. The use of fluorescent markers (here GFP expression in yeast cells) illustrates how the user can quickly discriminate between cells that should and should not be used during an experiment. Image is merged brightfield and green fluorescence.



Adhesion of *C. albicans* to moderately hydrophobic substrates. Time-dependent comparison of maximum adhesion forces at 23 and 37°C. At least 7 F–D curves were recorded per condition and time frame. Data represent the mean ± standard error. [doi:10.1371/journal.pone.0052712.g004]

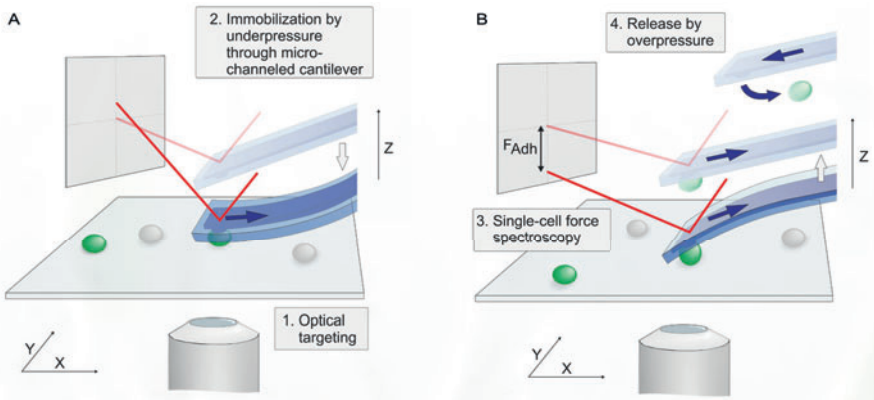
Cell type	Substrate	Mean ± SE
C. albicans	DDP	39 ± 7 nN
	DDP-OH	10 ± 3 nN
S. cerevisiae	DDP	5 ± 1 nN
	DDP-OH	2 ± 0.3 nN
HEK	Glass	33 ± 9 nN
	Fibronectin	53 ± 15 nN
HeLa	Glass	473 ± 127 nN
	Fibronectin	593 ± 70 nN

Comparison of the maximal adhesion forces of various cell types on different substrates. Results show the force and cell type range that FluidFM® can accomodate. [doi:10.1371/journal.pone.0052712.t001]

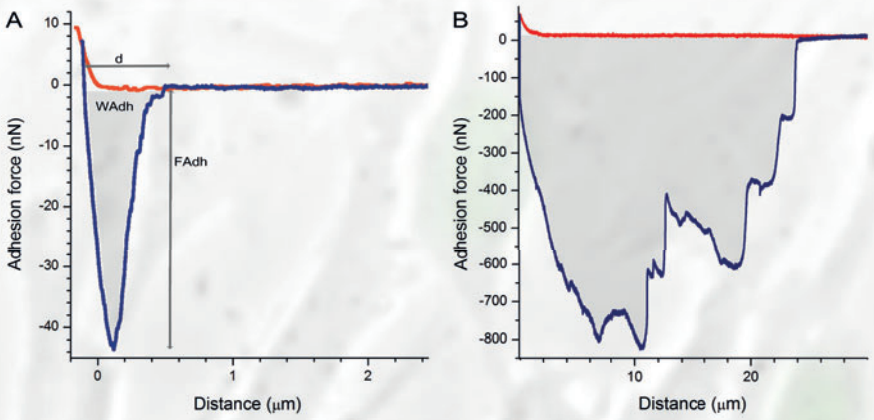
FluidFM®-based single-cell force spectroscopy (cell adhesion)

Cell adhesion to surfaces represents the basis for niche colonization, biofilm formation, and cell survival. Single-cell force-spectroscopy studies are, however, often hampered by the relatively slow pace at which a statistically relevant amount of data can be obtained. Potthoff *et al.* have recently shown that FluidFM® can dramatically increase the amount of data that can be recorded per day. By applying underpressure, FluidFM® cantilevers can attach to a selected cell within seconds. In addition, the probe can be immediately re-used after each adhesion experiment by releasing the detached cell from the probe through the application of overpressure.

The maximum cell adhesion forces measured in the study were in the range of 500 pN to 1600 nN. The latter value represents a one-order of magnitude increase compared to conventional AFM approaches.



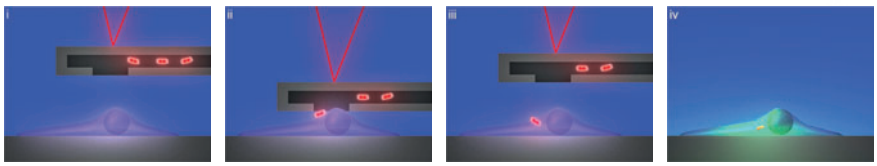
Schematic view of the experimental principle. (A) Cell targeting and immobilization to the cantilever through the application of underpressure. (B) Single-cell force spectroscopy and subsequent release of the measured cell. [doi: 10.1371/journal.pone.0052712.g001]



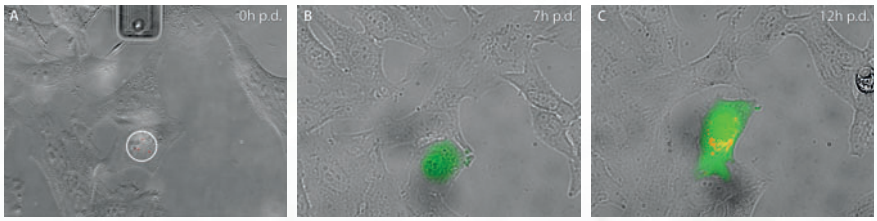
Representative example of the force-distance (F–D) curves that were obtained with a C. albicans cell on DDP (A) and a HeLa cell on fibronectin (B). The data show a force range between 20 and 800 nN (red: approach, blue: retraction curve). The maximal adhesion force was computed as the minimum force value (FAdh). The work performed by the Z-piezo during the detachment process (WAdh) was calculated as the area below the baseline (shaded area). The distance (d) is the distance required for the complete separation of the yeast cell from the substrate. [doi: 10.1371/journal.pone.0052712.g002]

FluidFM®-based virus infection of cells (virus deposition)

The mechanisms used by viruses to enter and replicate within host cells are subjects of intense investigation. Virus entry and infection are generally monitored by dispensing bulk virus suspensions on layers of cells without accounting for the fate of each virion. Stiefel *et al.* have used FluidFM® to deposit single vaccinia virions (VACV) onto individual cells in a controlled manner — a feat not possible with any other currently available method.



FluidFM® virus deposition procedure. (i) Positioning of the cantilever above a specific cell, (ii) force-controlled approach onto the cell and release of a virus by applying overpressure, (iii) retraction of the cantilever with pressure suspension to prevent unwanted virion delivery, and (iv) representation of virion entry and early gene expression. [doi: 10.1021/nl3018109.g003]



Single cell infection by a defined number of deposited virions. (A) Four mature pE/L-EGFP/mCherry-A5 vaccinia virions (red dots inside white circle) were placed onto a single HeLa cell. (B) Expression of late EGFP could be detected at 7 h p.d. (C) Visualization of newly assembled virus particles (red) at 12 h p.d. Images are merged brightfield, red fluorescence, and green fluorescence.

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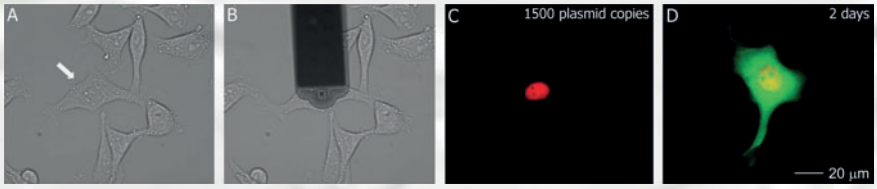
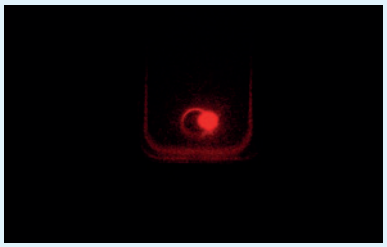
FluidFM®-based nuclear injection of DNA (subcellular injection)

Micro-injection of exogenous substances into cells emerged in the 70's and is a powerful tool to perturb intracellular processes by introducing proteins, peptides, nucleic acids, drugs, organelles or nanoparticles into living cells. The technology is however limited by its laborious handling, the relatively large size and unfavorable conical shape of glass micropipettes, and the lack of feedback to monitor probe insertion. Micro-injection into small cells or subcellular structures and the delivery of femtoliter volumes are particularly challenging. FluidFM® can overcome these limitations, as was impressively shown by Guillaume-Gentil *et al.*, who injected controlled amounts of DNA into cell nuclei.

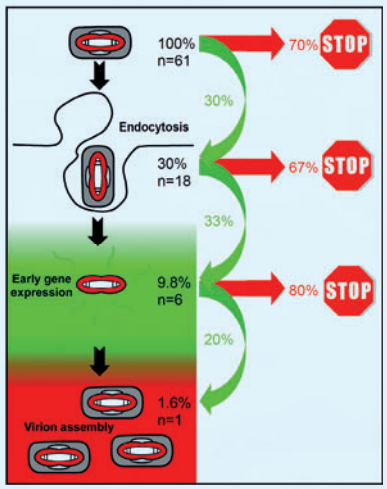


Transfection of a HeLa cell with pmaxGFP. The phase contrast images show the cell before (A) and during (B) injection. The fluorescence images show the cell immediately (C) and 2 days (D) after injection. DexTRITC (red) co-injected with the plasmid remained localized in the nucleus, and 2 days post-injection the cell was producing GFP (green). [doi:10.1002/smll.201202276.g003]

All figures reprinted with permission from Guillaume-Gentil *et al.*, Small 2012, doi: 10.1002/smll.201202276. Copyright © 2012 Wiley.

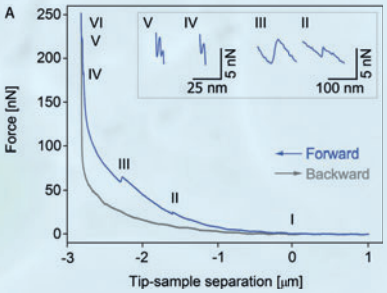


A fluorescently-labelled virion is leaving the FluidFM® cantilever in a controlled manner. [doi: 10.1021/nl3018109.g002]



The majority of VACV MVs are blocked early in the virus lifecycle. Seventy-three individual single-cell, single VACV MV infections were monitored for virus entry, early gene expression, and virus assembly. The percentage of virions successful for each stage, the percentage of particles blocked at each stage, and the overall percentage of blocked particles is shown. [doi: 10.1021/nl3018109.g006]

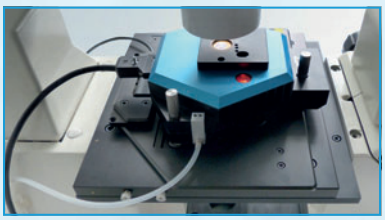
Cantilever design and injection principle. [doi: 10.1002/smll.201202276.g001]



Force spectroscopy. Insertion/withdrawal curve obtained in HeLa cells. (II) Upper cell membrane. (III) Upper nuclear membrane. [doi: 10.1002/smll.201202276.g002]

Components & specifications

FlexAFM-NIR scan head specifications	
Maximum Petri dish height (fluid level)	9 mm (6 mm)
Automatic approach range	1.1 mm
Maximum scan range	100 µm (1)
Maximum Z-range	10 µm (2)
Drive resolution in Z	0.6 pm (3)
Drive resolution in XY	6.0 pm (3)
XY-linearity mean error	< 0.1%
XY-flatness at maximum scan range	typ. 5 nm
Z-measurement noise level (RMS, dynamic mode in air)	typ. 30 pm
Scan head dimensions	143 × 158 × 53 mm
Scan head weight	1.25 kg
(1) Manufacturing tolerances ± 5% (2) Manufacturing tolerances ± 10% (3) Calculated by dividing the maximum range by 24 bits	

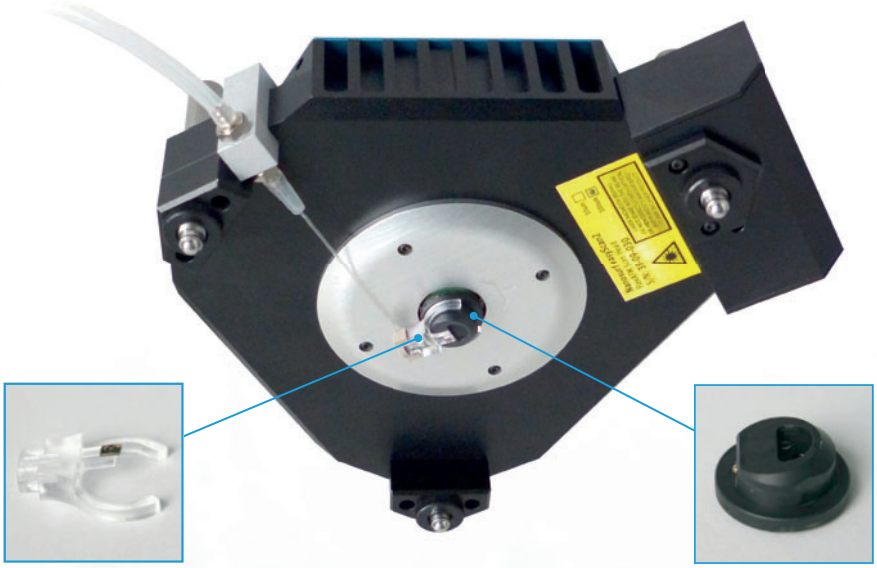


Inverted microscope integration.

Motorized inverted microscope stage specifications	
Closed loop positioning in all directions	
XY-range	12 mm
XY-resolution	1 µm
XY-repositioning accuracy	< 5 µm
XY-positioning	Stick-slip drives
Z-range	100 µm
Z-resolution	< 1 nm
Z-positioning	Piezo drive

FlexAFM-NIR scan head

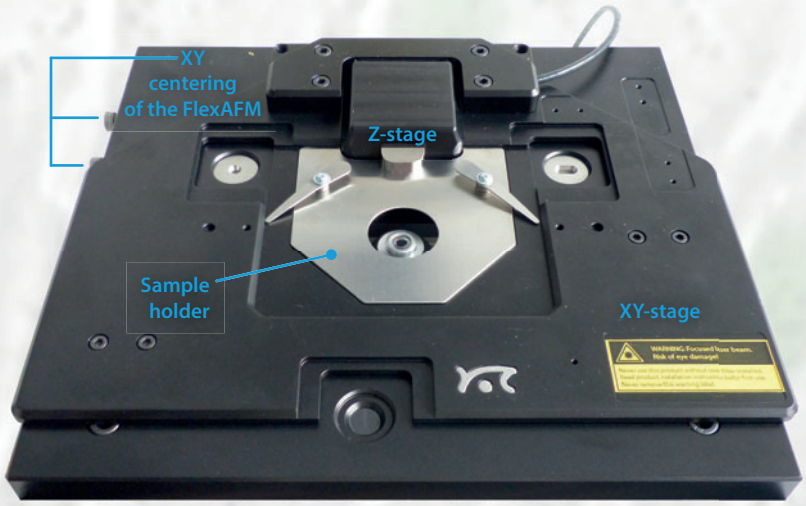
The Nanosurf FlexAFM-NIR scan head provides the force and positional control to manipulate your measurement samples. With its near-infrared laser it is compatible with most commonly-used fluorescent labels in cell biology.



As a central part of the FluidFM® detection system, the cantilever holder contains all optics related to Nanosurf's top and side view technology, and alignment structures for mounting the FluidFM® CytoClip probes with pre-mounted hollow cantilevers. The cantilever holder is magnetically attached to the scanner unit to allow quick removal from the scan head. A new CytoClip probe can be easily mounted and is quickly attached to the nanofluidics pressure control system.

Motorized inverted microscope stage

Replacing the default sample stage of your inverted microscope, the Nanosurf motorized inverted microscope stage is an integral part of the FluidFM® system, allowing measurement samples to be positioned and accessed through the FluidFM® control software.



The motorized inverted microscope stage consists of a universal sample stage with 12 mm motorized travel in XY and a microscope-specific stage adapter. Optionally, a 100-µm Z-stage can be installed to offer closed loop linearity and an extended range in Z, e.g. for adhesion force spectroscopy on mammalian cells. The stage's sample holder has room to accommodate microscope slides as well as a variety of differently sized Petri dishes.

FluidFM® pressure controller and pump

The Cytosurge pressure control system was optimized for FluidFM® applications. It consists of a fast and high-precision pressure controller and a pump unit.

Cytosurge FluidFM® pressure controller and pump unit. The pressure controller has been separated from the pump unit to mitigate unwanted system vibrations. The controller can be conveniently placed on the workbench, while the larger pump unit may be moved out of the way, for example underneath the bench, freeing up valuable bench space.

Pressure system specifications	
Pressure range	–800 to 1000 mbar
Pressure precision	better than 2.5% of full range
Min. output pressure step (< 0 mbar)	0.1% of max negative pressure range
Min. output pressure step (> 0 mbar)	0.1% of max positive pressure range
Power consumption	< 32 W

Available FluidFM® CytoClip probes / cantilever tips

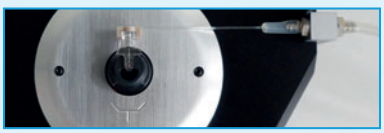
All FluidFM® CytoClip probes come in sterile packages containing an individual QR-code that allows the control software to automatically retrieve all relevant cantilever data online and directly use it during experiments.

Cantilever type	Hole diameter	Hole position	Spring const.	Tip shape
 Tipless	2, 4, and 8 µm	Cantilever end	0.3 or 3 N/m	None
 Apex opening	50–300 nm	Tip apex	0.3 or 3 N/m	Pyramidal
 Syringe	200–500 nm	Tip side	0.3 or 3 N/m	Pyramidal

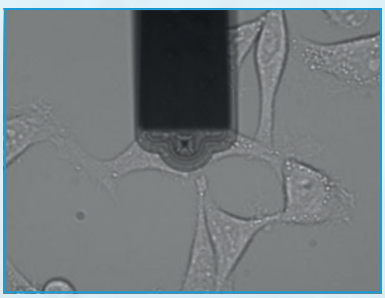
All cantilevers come pre-mounted as sterile and ready-to-use tip cartridges (FluidFM® CytoClip probes) with a 10-µl reservoir. With the Cantilever Holder FluidFM® and a new CytoClip, cantilever exchange in less than 30 seconds is possible.

Connections of the Cytosurge FluidFM® pressure controller and pump unit. Top: controller. Bottom: pump.

The oneLink™ pressure and electronics cable. This specially developed cable reliably connects the pressure controller to the pump unit.



Connections to the cantilever. A connector matching the reservoir end of all CytoClips models connects to the pressure controller via standard tubing.



Exact cantilever positioning. Due to the semi-transparent nature of the cantilevers of all FluidFM® CytoClip probes, the location of the tip can be easily determined and precisely placed over the location of interest. [doi: 10.1021/nl3018109.g003]

CytoClip package. A unique QR-code on each package allows clear identification and automatic retrieval of cantilever details.

FluidFM®

Nanosurf AG
Gräubernstrasse 12–14
4410 Liestal
Switzerland
+41 61 927 47 47 (phone)
+41 61 927 47 00 (fax)
www.nanosurf.com
info@nanosurf.com

Nanosurf GmbH
Rheinstrasse 5
63225 Langen
Germany
+49 6103 202 7163 (phone)
+49 6103 202 7182 (fax)
www.nanosurf.de
info@nanosurf.de

Nanosurf Inc.
300 Trade Center, Suite 5450
Woburn, MA 01801
United States of America
781 549 7361 (phone)
781 549 7366 (fax)
www.nanosurf.com
info@nanosurf.com

Nanosurf 中国中心
Nanosurf China
上海市天宝路578号 (200086)
飘鹰世纪大厦703室, 中国
+86 18621896399 (电话)
+86 21 5512 7698 (传真)
www.nanosurf.com
info@nanosurf.com