Integration of Cytosurge FluidFM® functionality into BioAFM products

Microfluidic techniques for single cell manipulation

The use of microfluidics for single cell manipulation applications is a fast-growing field. Conventional techniques for cell manipulation that have led to valuable scientific findings feature micro-needle cell insertion [1], pipette pulling [2], cantilever-based cell scraping [3], and micropipette cell aspiration [4, 5]. However, most of these techniques are either technically challenging or not straightforward to interpret in terms of mechanical models. Single cell force spectroscopy applications with functionalized cantilevers are an excellent alternative for measuring adhesion forces [6, 7], but also require labor-intensive protocols. In recent years, the Cytosurge FluidFM technology has emerged as an excellent solution that features the best of both microfluidic and force microscopy solutions [8–12].

FluidFM technology

The FluidFM technology features FluidFM probes that have a force-sensing, micro-machined cantilever with an inner capillary channel and a fluidic pressure control system (Fig. 1), which enables both suction and precise localized injection [8, 11]. The FluidFM probes can be operated at a liquid flow rate down to <femto-liter/sec and with a software-controlled applied pressure range of -800 to 1000 mbar. Varying in size, stiffness, and type, the probes can be used in a range of applications such as single cell manipulation and adhesion measurements (FluidFM micropipettes), nanoprinting, spotting, and bacterial cell adhesion (FluidFM nanopipettes), as well as cell injection/extraction and basic imaging applications (FluidFM nanosyringes) (Fig. 2). The FluidFM ADD-ON (probe, AFM specific probe holder, pressure control, pump unit, and software) is available as an add-on for all Bruker BioAFMs including BioScope® Resolve. A fully integrated solution, where the FluidFM functionality is completely embedded in JPK BioAFMs, is also now available.

Fig. 1: Setup. *NanoWizard® 4 XP BioScience AFM with a Cytosurge FluidFM ADD-ON.*

FluidFM and JPK BioAFM family - a perfect match

JPK BioAFM systems provide the perfect capabilities to leverage the FluidFM technology to a maximum. Thanks to the best closed loop sensors on the market, JPK BioAFMs provide the most sensitive force control of the probe and highest accuracy for probe to sample positioning in xyz.

With our leading probe-scanning design, where the sample stays stationary during operation of the AFM, the user has an unrestricted optical view of the sample at all times.

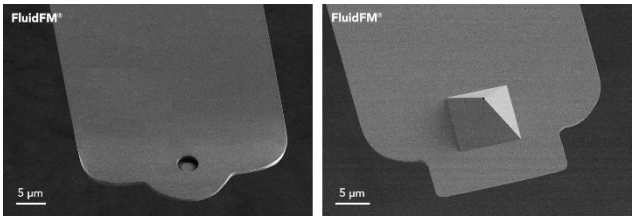

Fig. 2: Examples of FluidFM probe types*. FluidFM micropipette with a 4 µm aperture (left), and FluidFM nanopipette equipped with a pyramidal tip with a 300 nm aperture (right). Image courtesy of Cytosurge AG.*

The systems are compatible with the broadest range of inverted optical research microscopes. Using optical microscopy techniques, the user can identify a spot of interest on the sample where to apply FluidFM and has the FluidFM experiments under optical control. The unparalleled optical design of our AFM heads with a free optical path, enables typical transmission-based contrast methods such as DIC and optical phase contrast using standard optical microscope condensers. Here, one of the biggest advantages comes to play. Compared to classical micropipettes, FluidFM probes delivers far better optical access to the sample spot and probe position.

FluidFM can also be combined with fluorescence and advanced optical microscopy techniques (e.g. Confocal, FLIM, TIRF, FRET, FCS). In particular, super-resolution microscopy methods such as STED, SIM, and PALM/STORM can bring additional benefits to the FluidFM experiments and are, of course, perfectly compatible with JPKs unique probe-scanning BioAFM systems.

Overlaying AFM scanning space coordinates with the optical image coordinates can be challenging, particularly for higher NA objectives with higher magnification. The optical images are stretched by non-perfect optical lense systems in the objectives, resulting in an imprecise overlay of AFM images and optical images. To overcome this restriction, we developed the DirectOverlay™ mode for perfect calibration of the optics and AFM. Using the AFM probe as a ruler and the perfect AFM scanner motion (metrology-like) guided by the xyz sensor system, we can “de-stretch” the optical image and get a perfect overlay of the AFM and optical image. This works down to the single molecule level.

Bruker’s newly developed and patented DirectOverlay 2 feature enables easy, optically-guided navigation to reach positions with highest precision, and maximizes the benefit of simultaneous AFM and optical measurements. For enhanced usability, DirectOverlay 2 has a “1-click” calibration.

The contact free calibration method, based on Sader, allows a gentle, quick, and tip-protecting cantilever calibration. This approach can be applied to all of the FluidFM probes.

Other highlights are the HybridStage™ and Motorized Stage, which enable automated access to a large sample area. Travel ranges of 20 x 20 mm² and the optical tiling feature allow the user perform experiments over a very large area. In addition, the HybridStage has a long z-piezo scanner (>100 µm), which is a significant advantage for cell-adhesion measurements. Combined with the ExperimentPlanner™ software module, the user can seamlessly automate his experiments.

JPK BioAFMs provide not only perfect optical integration, but also come with their renown modularity and an extensive range of accessories, which fulfil specific requirements for, e.g., environmental control (temperature, atmosphere), automation, large x, y travel and enhanced z-ranges.

Compatibility

The FluidFM ADD-ON can be used as an add-on or fully integrated with the new NanoWizard® 4 XP AFM, a benchmark in correlative, high-performance BioAFM combined with advanced optical microscopy (Fig.1).

The FluidFM ADD-ON is also compatible with the entire JPK BioAFM family, such as CellHesion® 200, ForceRobot® 300, and the new NanoWizard UltraSpeed 2 system.

FluidFM ADD-ON

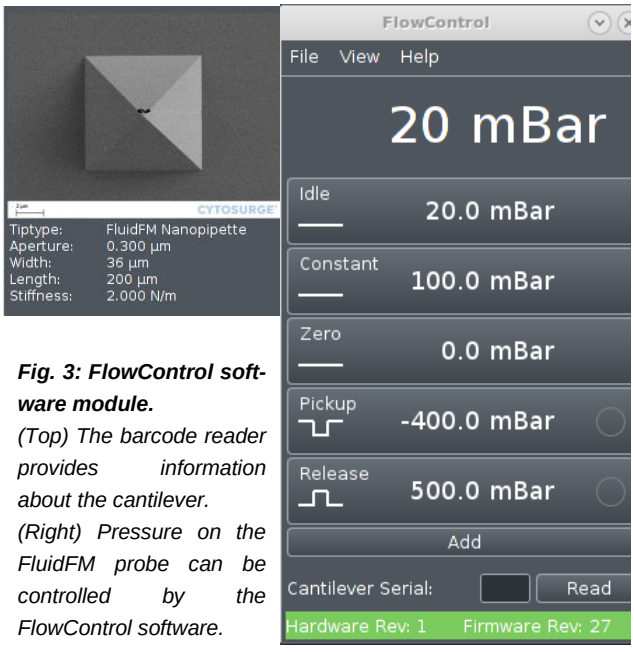

Fig. 3: FlowControl software module.
(Top) The barcode reader provides information about the cantilever.
(Right) Pressure on the FluidFM probe can be controlled by the FlowControl software.

The FluidFM ADD-ON can be controlled from the same PC as the JPK BioAFM. The newly developed JPK FlowControl software module allows the user to define and set constant pressure or apply pressure pulses of defined lengths (Fig. 3). The intuitive handling of pressure control enables users to run complex experiments easily.

The bar code reader supplied is of great help and provides all the relevant information about the probe package being used, including an SEM image of each FluidFM probe (Fig. 3). This makes quality control easy.

Complete implementation of FluidFM functionality into the new JPK V7 software

Users spend about 80% of their working-time in front of a screen. To improve the output, we developed with the new V7 version a workflow-based, user guidance software interface. The complete integration of the FluidFM functionality into our V7 SPM control software guaranties maximum productivity and enhanced performance. Equipped with modern user guidance and online help, users of all experience levels can quickly set up an experiment and obtain high-quality data.

The implementation enables the synchronisation of AFM and FluidFM operation and correlation with optical measurements. Using the JPK Advanced Force Spectroscopy module and its RampDesigner™ feature, force distance curves, including segments for pressure control, can be defined by the users. This enables the synchronized control of force, contact time, pressure changes and travel distances, etc. (Fig. 4).

The RampDesigner comes with predefined experiment schemes, e.g., for cell injection, spotting or pick-up. In addition, it provides experienced users with the flexibility to fine-tune experimental setups to their specific needs. In combination with the JPK ExperimentPlanner, customized experiments can be performed in a highly automated and efficient way. With all these ingredients, the new V7 software provides the basis for successful scientific work.

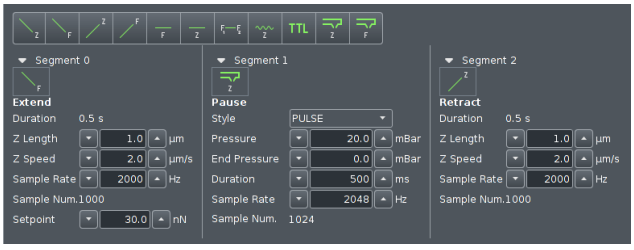

***Fig. 4: Integration of the FluidFM functionality in the RampDesigner**. Synchronisation of AFM and pressure adjustments*

Fields of application

With the FluidFM technology, positive and negative fluidic pressure can be precisely applied . Integrated into a JPK BioAFM with its ease-of-use, sensitive force control, and

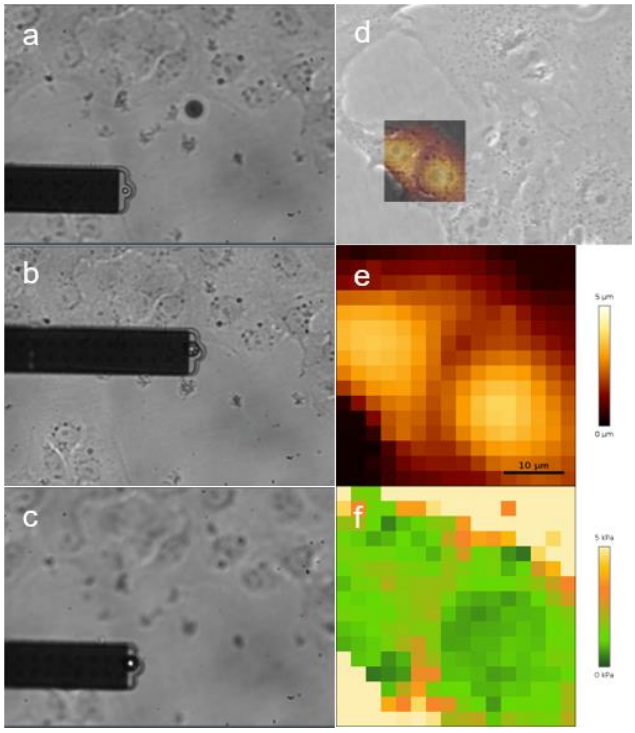

***Fig. 5: Pick-up of a bead for Force Mapping on living cells.** (a,b,c): Phase contrast images of living vero cells, the FluidFM micropipette and an 11 µm polystyrene bead in front of it (a), during (b) and after pickup (c). (d) Overlay of optical image with Height map using the polystyrene bead. (e, f) Height and apparent stiffness map of two cells.*

accurate positioning, and combined with perfect optical integration for observation and control, it can be used for a large range of applications such as:

- Single cell, bacteria or microbe adhesion
- Colloidal force spectroscopy
- Nanospotting, Nanoprinting
- Single cell picking and isolation
- Single cell injection or extraction

The accurate pressure control of the FluidFM system allows submicron precise deposition of an extremely small volume of a drug (< 1 fL). This can be used to trigger local changes in the cell network, which can be characterized with nanomechanical measurements using AFM.

Single cell / particle manipulation

A gentle suction can be applied to pick up an object, such as a cell, and to place it somewhere else. Alternatively, the suction can be maintained and the object itself can be used as a probe. This is a convenient way to reversibly attach a cell to the cantilever for cell adhesion measurements. This

method can also be used for rapid cyclic manipulation of probe cells, where one cell after another can be picked up. This can dramatically shorten the duration of the experiment.

FluidFM is also advantageous for objects where the traditional chemical attachment is challenging, e.g., bacteria, blood or yeast cells.

Another possibility is aspiration of spherical particles, such as certain colloids, to the cantilever (see Fig. 5). If the particle diameter is known, the contact area can be defined and used to reveal mechanical properties via Force Spectroscopy, Force Mapping or QI™ mode. Using the integrated fit models (e.g. Hertz or DMT), precise elasticity maps can be obtained (see Fig 5f).

By applying an overpressure, the sphere can be released, and the cantilever can immediately be re-used for the next experiment.

Cell injection

A further interesting possibility is to fill the FluidFM probe with a solution, which can then be injected, for example, into a cell (see Fig. 6). Here, the fluorescence marker propidium iodide was injected into individual living cells in order to stain the DNA and visualize the nucleus. It is also possible to use FluidFM for highly localized drug delivery. Individual cells can be specifically targeted.

Changes in the topography, elasticity and adhesion of the cells after injection can be determined using Force Mapping or QI™ mode. Using RampDesigner, user-defined force distance curves, including pressure changes, can be specified to perform high-precision injection experiments.

Cell extraction

The extraction of intracellular content from living cells is another application which demonstrates the benefits of the precise control of pressure, force and position provided by the FluidFM integration. Optical integration with the BioAFM allows not only the precise positioning of the probe, but also direct observation of the process in real time.

The sensitive force control of the BioAFM allows gentle penetration of the cell membrane, and the precise pressure control allows the extraction of predefined volumes down to < femto-liter [12].

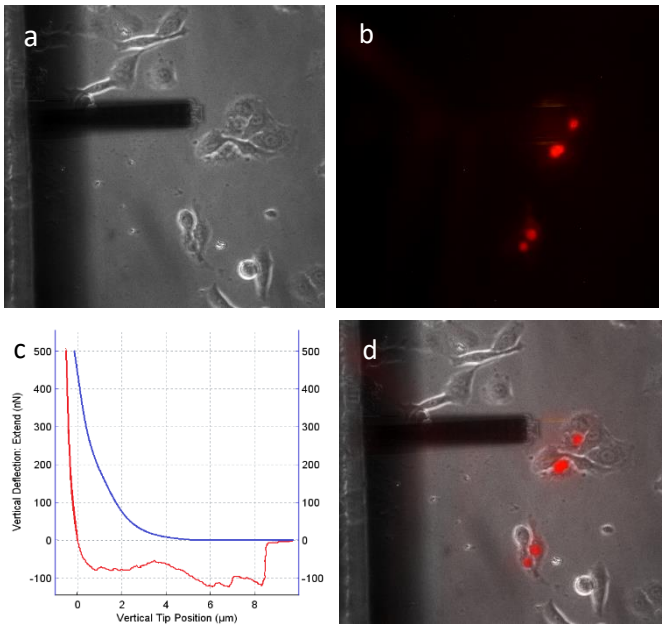

Fig. 6: Injection experiment *(a) Phase contrast image (b) Fluorescence image after injection of propidium iodide (c) Force distance curve during injection (d) Overlay of phase and fluorescence image to demonstrate nucleus position*

This stands in stark contrast to traditional micropipette methods, whose use are limited to large cells, nanoliter aspiration and poor force control, all of which have a detrimental impact on the cell survival rate.

Conclusion

The Cytosurge FluidFM ADD-ON is a highly flexible tool which can be used in a broad range of applications. It can be used for adhesion measurements or as a nano-syringe to extract cell material or inject specific target substances into cells or tissues. Precise sub-micron drug deposition and nanomanipulation are further capabilities of the technology.The combination of the FluidFM ADD-ON with JPK BioAFMs is the perfect solution, as it provides precise control over force, position and fluidic pressure. The easy-to-use V7 software and perfect optical integration enable any user set-up quickly and perform high-throughput experiments. The large range of flexible accessories for the BioAFMs allow further customization of advanced experiments.

FluidFM is available as a separate add-on (FluidFM ADD-ON Silver) or can be fully integrated into the systems of the JPK BioAFM family (FluidFM ADD-ON Gold and Platinum).

Benefits of FluidFM – JPK BioAFM combo

- Force sensitivity down to picoNewtons with Brukers lowest noise deflection detection
- Sub-nm positioning accuracy in xy and z as a result of market-leading closed-loop technology
- Combination with inverted optical microscope techniques, including phase contrast, fluorescence, super-resolution microscopy
- Purpose-built probe-holder for JPK BioAFMs
- Lowest liquid flow rate down to <fL/sec
- Pressure range -800 to 1000 mbar for highest flexibility in applications
- Two options: Either as add-on with FlowControl Software module OR full integration into JPK BioAFM systems via V7 software
- New DirectOverlay 2 feature for easy and precise optical navigation and combined information
- RampDesigner for synchronization of force, contact time, pressure and pressure duration

Acknowledgements

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