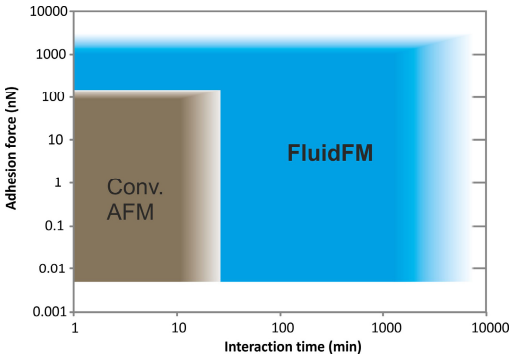
Single Cell Force Spectroscopy

Advantages of FluidFM compared to classical AFM methods

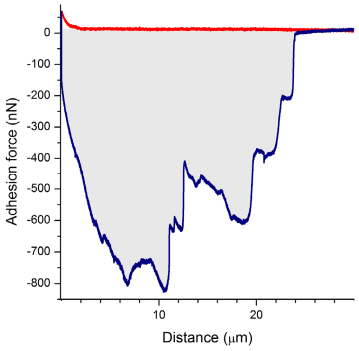
Cell adhesion to surfaces represents the basis for niche colonization and cell survival. Single-cell force-spectroscopy studies, however, are often hampered by the relatively slow pace at which a statistically relevant amount of data can be obtained. Potthoff *et al.* [1] have recently shown that FluidFM® can dramatically increase the amount of data that can be recorded in a single day. By applying underpressure to the system, FluidFM® cantilevers can attach to a selected cell within seconds. Likewise, 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.

Advantages of FluidFM

- Higher cell-substrate adhesion forces can be measured — one order of magnitude
- Longer cell-substrate interaction times can be studied — orders of magnitude
- Much shorter measurement time / Many more adhesion measurements — factor 20



FluidFM extends accessible cell–substrate interaction time and interaction force by orders of magnitude, allowing experiments not feasible with conventional AFM methods to be performed.



Force–distance (F–D) curve obtained using FluidFM on a HeLa cell grown on fibronectin [1]

Conventional AFM methods

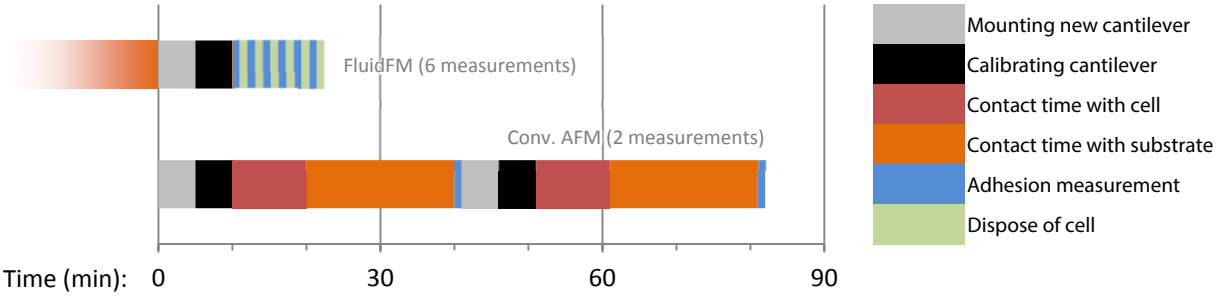
- 10 min for proper cell–cantilever interaction
- Contact time of cell with substrate only up to 20 min; any longer and the adhesion forces will be too high to actually lift the cell from the surface. It will stay attached.

FluidFM

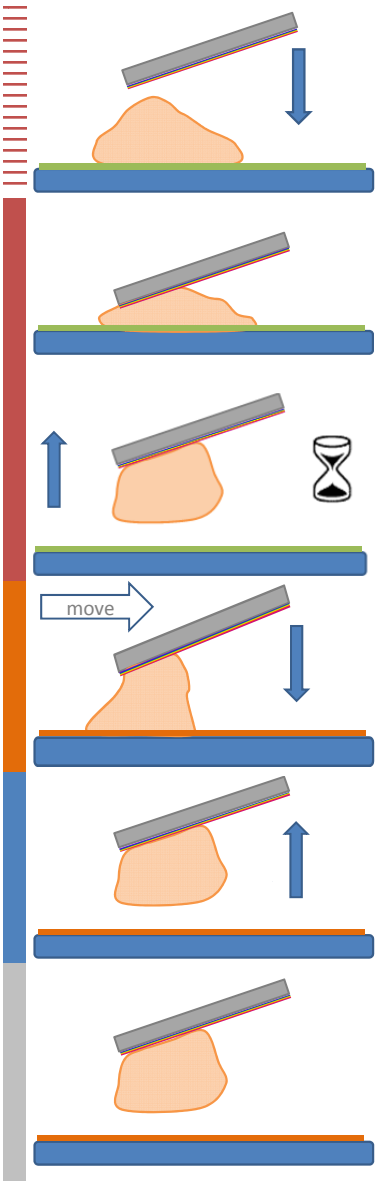
- 5 sec underpressure for cell–cantilever contact
- Statistically relavent numbers of cells (30+) can now — for the first time — be readily measured within a single day, allowing real cell biology experiments to be performed
- Contact time of cell with substrate before picking up is virtually unlimited, since very high forces can be overcome. The convenient overnight seeding of cells becomes possible!

[1] Potthoff E, Guillaume-Gentil O, Ossola D, Polesel-Maris J, et al. (2012) Rapid and Serial Quantification of Adhesion Forces of Yeast and Mammalian Cells. PLoS ONE 7(12): e52712. doi:10.1371/journal.pone.0052712

Typical spectroscopy series with one adhesion experiment per cell*. FluidFM and conventional AFM compared. Many more measurements (blue) can be performed repeatedly with FluidFM — and in much less time for each!



Conventional AFM method



Only possible with cells grown on substrate with (partially) non-adherent coating (green layer) for cell removal

10 sec for first interaction!

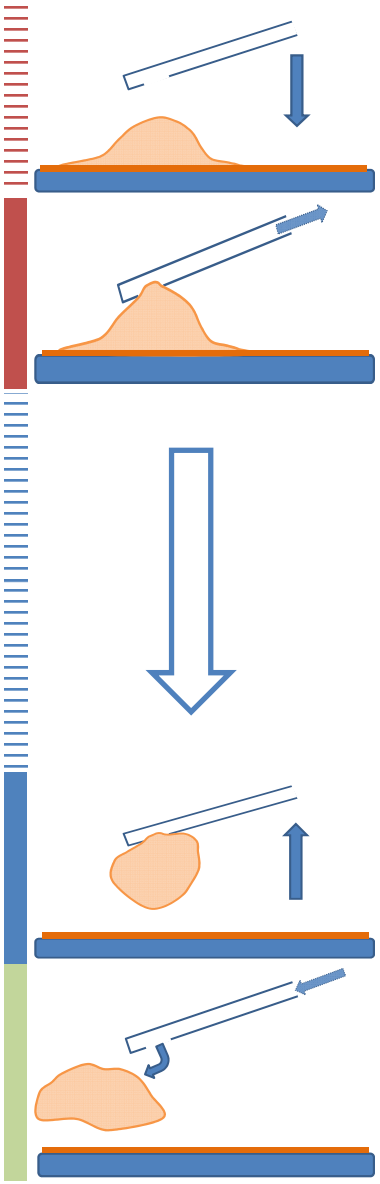
Experiments can only be performed after 10 min or more cell–cantilever interaction

Interaction time between cell and substrate (orange layer) under continuous cantilever pressure (up to 20 min)

Lifting of cells uncertain due to weak interaction between cantilever and cell: Measurement done?

To repeat the experiment with a new cell, the cantilever needs to be exchanged for another previously prepared cantilever (10-20 min)

FluidFM



All cells, all substrates

Underpressure and 5 seconds of interaction is sufficient!

Experiments can be performed immediately!

Reliable lifting of cells: Measurement done!

Discard the old cell and immediately repeat the experiment with a newly selected cell;
as often as you like!

* The above schematic represents a simplification of the steps actually performed for each method. They may vary with the goals for each experiment and method. Although not ideal scientifically, conventional AFM may be conducted differently to overcome some of the inherent limitations of the method, and FluidFM could be used in a more comparable "classical" way. With mammalian cells, a prior coating of the FluidFM cantilever is recommended to avoid aspecific binding. All this notwithstanding, strongly adherent cells remain inaccessible to conventional AFM, and FluidFM will always provide more experimental freedom and faster performance.