Elasticity measurements with colloidal probes

Advantages of FluidFM compared to classical AFM methods

AFM can be used to determine the mechanical properties of cells, tissue, and substrates. These mechanical properties have shown to be important for fundamental cellular processes like migration [1] and differentiation [2], as well as in the characterization and understanding of cancer [3].

Although colloidal probes are commercially available, it is still common practice to glue beads on the cantilever. This is a precision job and can only be done one bead at a time. Coating has to be applied with the glued bead on the cantilever. With FluidFM, gluing of beads can be circumvented [4]: pre-coated beads are mounted to the hollow cantilever shortly before the experiment by application of underpressure. Upon contamination, a bead can be immediately exchanged and measurements can continue with the same cantilever.

Conventional AFM methods

Preparation:

- Time-consuming and complex gluing routine needs preparation and experience
- Coating procedure on complete cantilever, cumbersome to parallelize

Experiment:

- A simple replacement of the bead after contamination or for experiments using differently coated beads is impossible

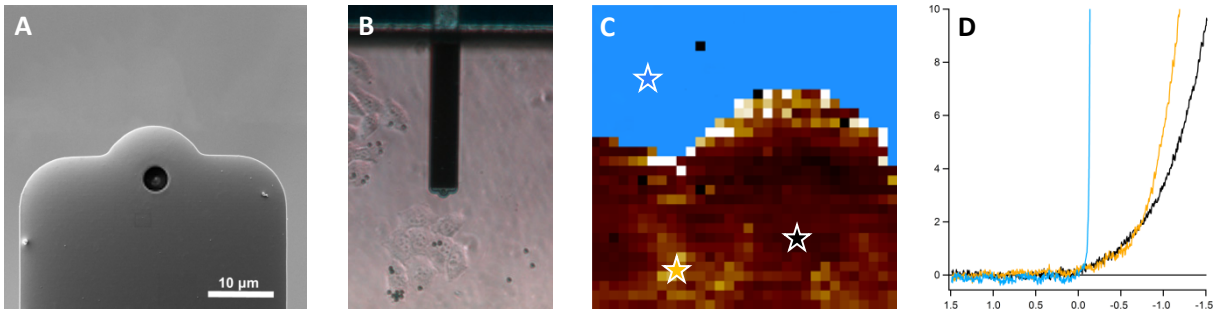
FluidFM

Preparation:

- Pre-coating of beads in solution on excess numbers of beads
- Reversible mounting within seconds by application of underpressure

Experiment:

- Easy exchange of beads by simply switching from under- to overpressure



Colloidal spectroscopy of HeLa cells using FluidFM. (A) SEM image of cantilever. (B) Optical view of cantilever, colloidal beads, and cells in the Cytosurge control software. (C) Map of Young's modulus (scan size: 60 µm; blue area is glass). (D) Approach curves (force in nN versus tip–sample distance in µm) for the locations indicated with a star in C: glass (blue), harder cell areas (orange) and softer cell areas (black).

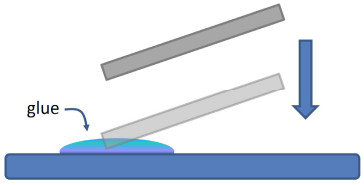
Advantages of FluidFM

- Handling — Pre-coated beads and easy mounting
- Experiment design — Same cantilever and different beads
- Time — Fast mounting and bead exchange
- Easy — Intuitive touchscreen-based workflow environment

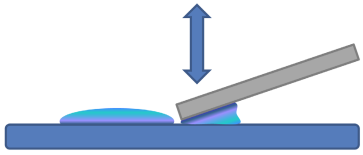
[1] Park et al. 2005, *Cell Motility and Local Viscoelasticity of Fibroblasts*, Biophys. J. 89, 4330–4342
[2] Engler et al. 2006, *Matrix Elasticity Directs Stem Cell Lineage Specification*, Cell 126, 677–689
[3] Plodinec et al. 2012, *The nanomechanical signature of breast cancer*, Nat. Nanotechn. 7, 757–765
[4] Dörig et al. 2013, *Exchangable colloidal AFM probes for the quantification of irreversible & long-term interactions*, Biophys. J. 105, 463–472

Typical elasticity measurement series with self-attached micro-spheres*.
FluidFM and conventional AFM compared.

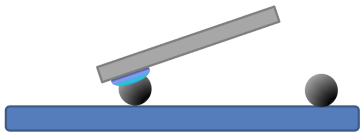
Preparation: Conventional AFM method



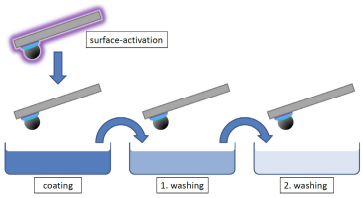
Glue needs to be deposited on substrate in a defined way, followed by force curve to pick it up



To remove surplus glue at the cantilever tip, 5–10 force curves must be performed on clean substrate

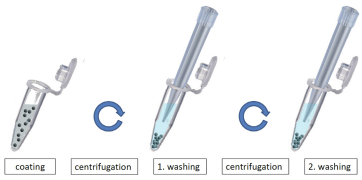


Move to 2nd glass slide, slowly approach bead to not displace it via induced flow (curing time: several minutes for standard UV-curable glue)



Each cantilever needs to be cleaned and activated, followed by incubation in coating solution

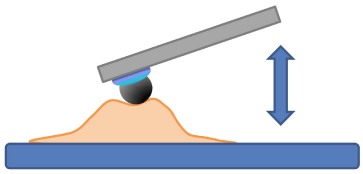
FluidFM



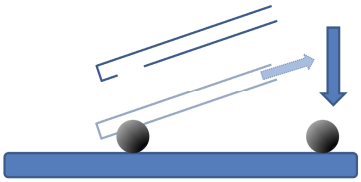
Batch processing of multiple beads: a large reservoir of equally coated beads is accessible.

Due to the pre-coated and ready-to-use beads and the lack of a glue routine, experiments can be performed immediately and even on the same glass-slide!

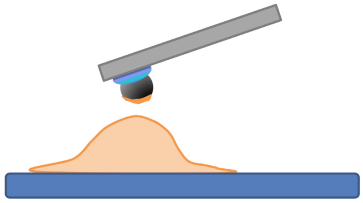
Experiment:



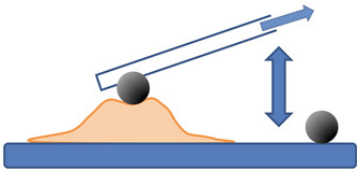
Significance of measurements uncertain: Is the bead coated with glue? Where is the exact position of the bead?



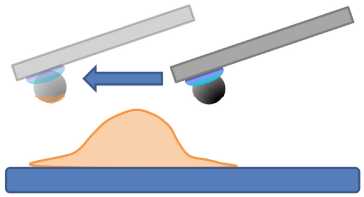
Underpressure prevents bead from being displaced by induced flow.



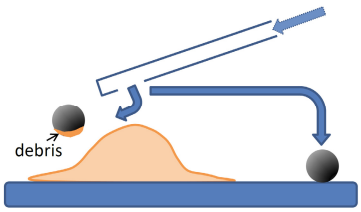
No possibility to get rid of a contaminated bead instantly



Repeatable measurements with clean beads at a precisely defined position!



To repeat the experiment, the cantilever needs to be exchanged for another previously prepared cantilever (10–20 min)



Discard the old bead and immediately repeat the experiment with a newly selected one; even with differently coated beads!

* 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. Nevertheless, changing the bead without changing the cantilever remains inaccessible to conventional AFM, and FluidFM will always provide more experimental freedom and faster performance.