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Melanoma cell elasticity measurements by means of multicantilever atomic force microscopy

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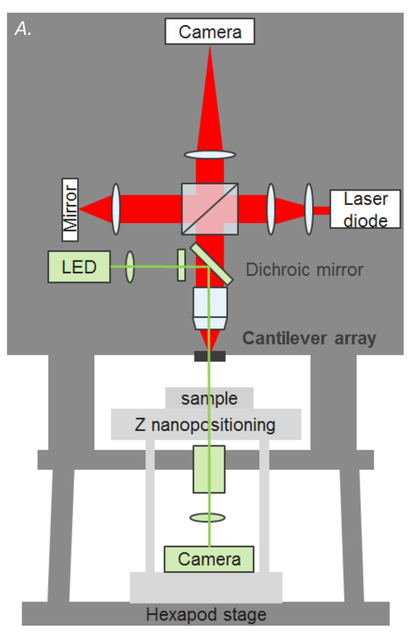
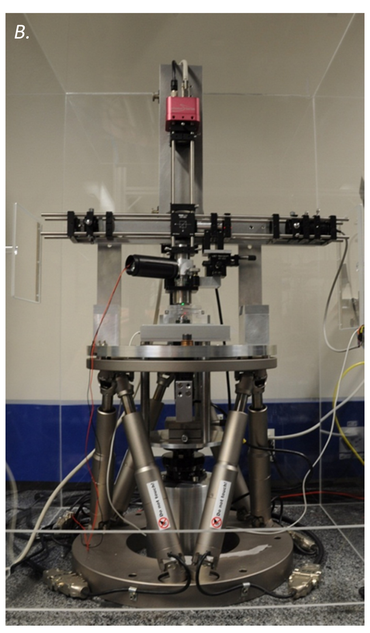
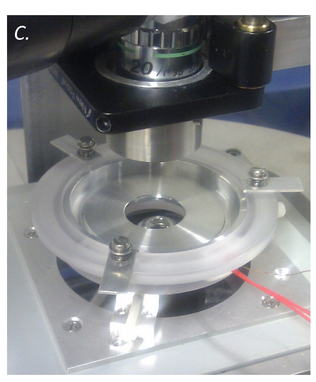
Atomic force microscopy (AFM) investigations of single living cells provide new information in both biology and medicine. For example, recent studies have shown differences in elasticity between cancerous and healthy cells. Slow cell dynamics and the need for statistically significant sample sizes mean that data collection can be an extremely lengthy process. We address this problem by parallelizing AFM experiments using a two-dimensional cantilever array instead of a single cantilever. The final goal is to understand if cell elasticity of melanoma cells varies during tumor progression.

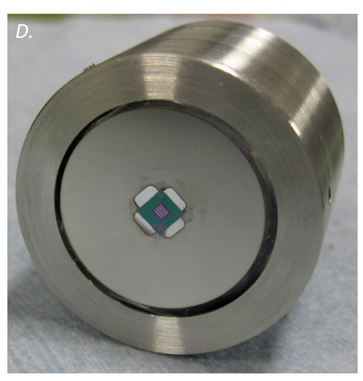
Parallel AFM Force Spectroscopy

Force spectroscopy is inherently a single cell technique. Nanomechanical experiments are very time consuming and the determination of values to get reasonable statistics remains a major challenge. To increase the measurement throughput, we are developing an AFM platform that allows the measurement of multiple cells in parallel. The platform includes the optical read-out of the cantilever deflections, micro- and nanopositioning stages, phase contrast microscope as well as a fluid chamber suited for standard petri dishes to keep the cells in a liquid environment at 37°C. The deflections of all cantilevers in the array are measured in parallel using interferometry. An interferogram captured with a CMOS camera is analyzed using dedicated software to determine the deflection of each cantilever.

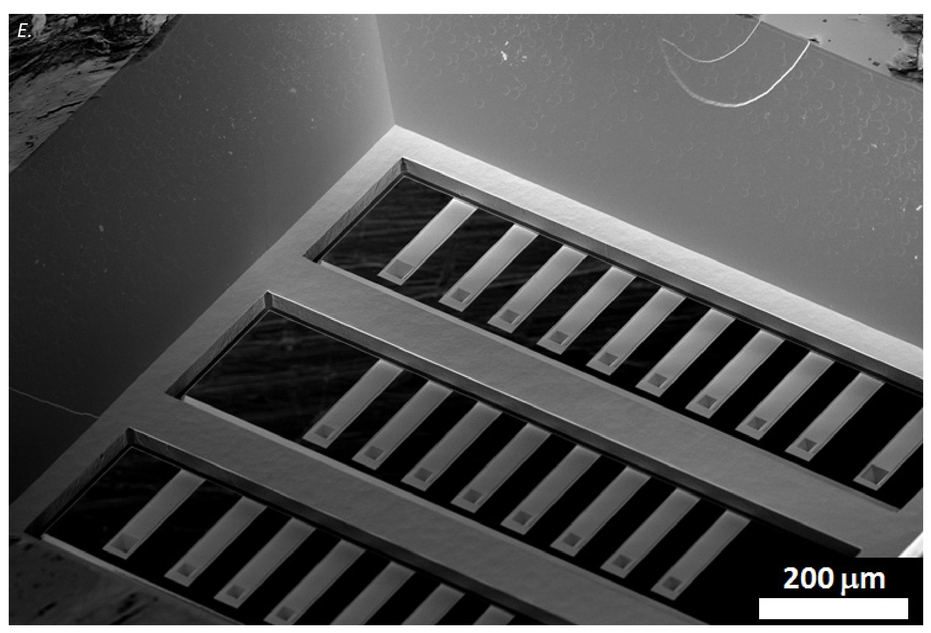








(A) Schema of the AFM array measurement platform including the interferometric cantilever read-out (red) and the phase contrast microscope (green).
(B) Picture of the platform
(C) Enlarged picture of the heatable fluidic chamber.
(D) AFM probe array chip mounted on its holder.
(E) Scanning electron microscopy image showing the detail of the AFM probe array



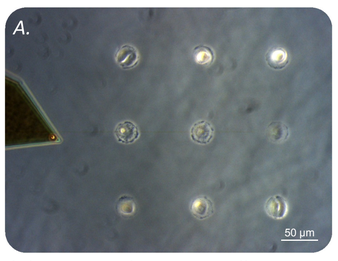
Results

Cell arrays

Cell arrays with a cell pitch compatible with the AFM probe arrays are a key element of parallel AFM studies to guarantee that each cantilever of the probe arrays addresses an individual cell. Two types of cell array substrates were developed for parallel force spectroscopy, one for adherent cells, and second for non-adherent cells.

Cell positioning of adherent cells

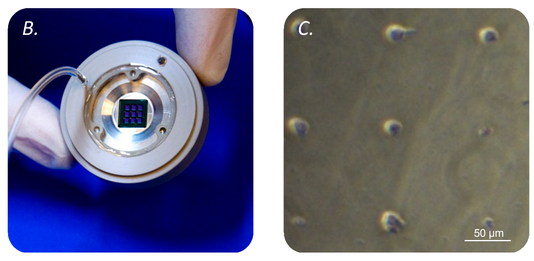
Adherent cells are positioned on a biochemically patterned surface using cytophilic fibronectin spots surrounded by an cytophobic matrix (CYTOO®). After sedimentation, the cells adhere only on the fibronectin spots



(A) Positioned WM239 cells on spots functionalized with fibronectin.

Cell positioning of non-adherent cells

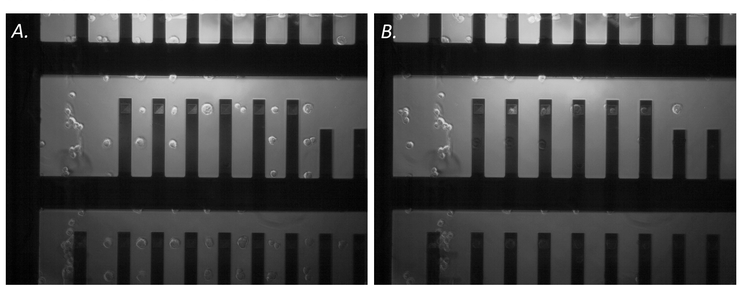
Alternatively, cells in suspension are immobilized by hydrodynamic drag on ultrathin silicon nitride microporous membranes. The cells can be instantaneously released to be transferred onto the probe array for further measurements.



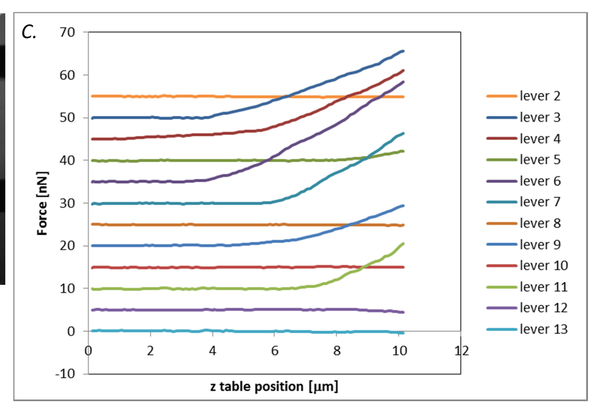
(B) Picture of a microporous membrane mounted in a fluidic chamber and (C) immobilized WM239 cells.

Parallel Force Spectroscopy on living cells

The system was tested by parallel force spectroscopy on living cells in liquid environment. The phase contrast microscope implemented on the platform allows a good optical visualization of the living cells in order to properly align the probe array regarding the cell array. Before to start a force spectroscopy experiment, the probe array is adjusted to be parallel with the sample surface using the micropositioning Hexapod stage (six degrees of freedom) by making contact with the sample surface between the cells.

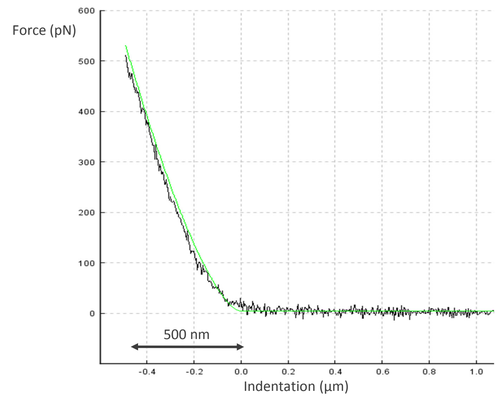


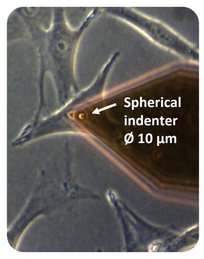
Optical picture (A) before and (B) after the alignment between the cell array (living metastatic WM239 melanoma) and the cantilever array. (C) Proof of concept has been demonstrated by performing parallel force spectroscopy on living WM239 cells. Not all probes entered in contact with the cells.



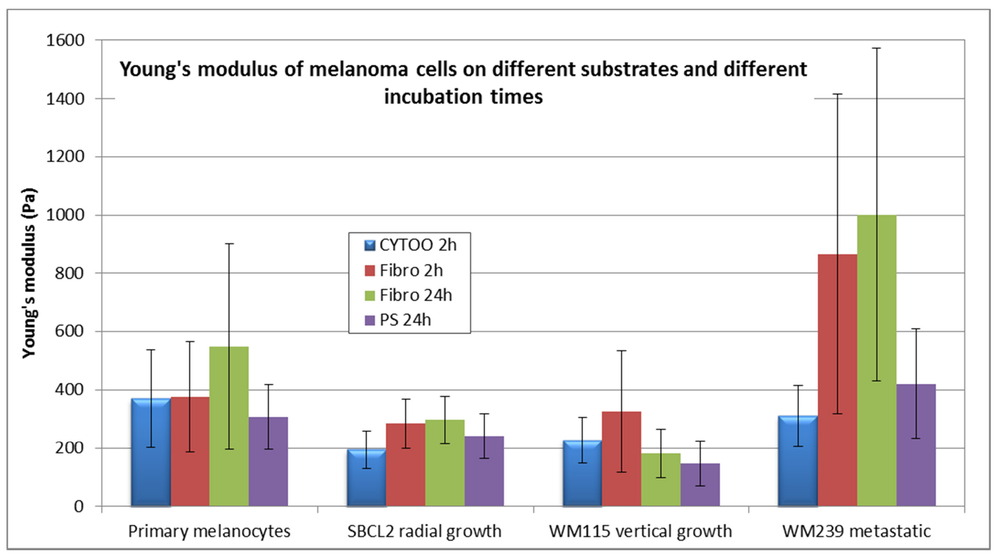
Serial Force Spectroscopy

In order to establish references for parallel force spectroscopy investigations, initial experiments were performed using single cell force spectroscopy on human melanoma cell lines representing the different cancerous development phases: radial growth phase (SBCL2), vertical growth phase (WM115) and metastatic (WM239), and on primary melanocyte as reference. The cells were grown in different conditions to analyze the influencing parameters on the cell elasticity.





Example of a force indentation curve. The green line represents the fit (Hertz model) to obtain the Young's modulus.



Young's moduli of the different cell types grown in different conditions. The influencing parameters are the cell morphology (cell spreading on fibronectin spots on the CYTOO chips versus on plain fibronectin), the incubation time (cell incubated on plain fibronectin for 2h versus 24h), and the surface chemistry (plain fibronectin versus plain polystyrene).

Conclusion

Currently cancer research and diagnosis is mainly based on molecular biology and biochemical assays to detect specific biomolecular markers. It has been shown recently that the stiffness of cancer cells affects the way they spread in the body. The availability of an easy-to-use method to measure cell stiffness and adhesion will greatly contribute to our understanding of cancer. For that reason, we are developing a method to increase the speed of single cell force spectroscopy based on the use of AFM cantilevers. Readout instrumentation, software, microfabricated cantilevers arrays, microscopy and cell arrays are key elements of this approach to investigate living cells under physiological conditions. The use of the resulting instrument is expected to bring force spectroscopy on cells to the next level and to make an important contribution in the field of cancer research: elasticity data will be available in sufficient quantities by parallel operation.

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