# The application of the NanoWizard® ULTRA Speed A atomic force microscope to study dynamics of living KPG7 fibroblasts

## Introduction

The last three decades have seen the rise of the atomic force microscope (AFM) as an indispensable tool for high-resolution structural analysis of specimens ranging from single molecules [1] to complex biological systems [2]. Unlike other high-resolution imaging techniques, such as advanced electron microscopy, as well as super-resolution optical microscopy, AFM remains the only tool that currently offers premium resolution of the analyzed biological systems (proteins, cells, etc.) while being able to simultaneously acquire information about the sample's mechanical properties at near physiological/native sample conditions. By default it also does not demand any sample modification and does not introduce preparation artefacts.

Studying single macromolecule dynamics and the function of complex biological systems, such as individual living cells, requires a tool that can meet the requirements for both high spatial and temporal resolution [3]. Developments in the last 10-15 years have paved the way towards the application of ultra-small cantilevers, piezoactuator-based sample scanners, and optical beam deflection (OBD) detectors for studying of high-speed single molecule processes [4–6]. Such high-speed developments however are hardly applicable for living cells due to the significantly reduced XY-scan (a few micrometres) and Z-scan size (less than a micrometre) [7]. The very recent fast-speed developments in tip-scanning AFMs make possible the successful structural analysis of a multitude of dynamic processes in cells such as exocytosis, vesicle transport, cytoskeleton reorganization, cell migration, taking place on the timescale of seconds. Structurally resolving morphological surface changes and the above-mentioned cellular events is no longer fundamentally limited by the conventional optical diffraction limit, but rather combined with other advantages of optical/fluorescence detection systems, as seen for correlative atomic force microscopy [8].

## ULTRA Speed imaging of cells in liquid

The conventional AFM imaging of live cells in intermittent contact (and contact) mode is typically rather challenging due to the rather slow image acquisition times and relatively slow feedback being unable to cope with the rather soft and topographically inhomogeneous samples. The fast-scanning NanoWizard® ULTRA Speed A atomic force microscope by JPK Instruments AG can be applied for studying weak and rapidly changing signals, such as dynamic cellular processes at near physiological liquid conditions.

**Figure 1**. Typical setup of a NanoWizard® ULTRA Speed A atomic force microscope on an inverted optical Zeiss microscope with motorized precision stage (20x20 $mm^2$ travel range). The AFM is further compatible with specific inverted models from Zeiss, Olympus, Nikon and Leica, commercial confocal microscopes, as well as the JPK TopViewOptics™ for opaque samples.

This is further made possible by the latest enhancements at one of the lowest noise scanners, very fast feedback algorithm and detection systems, delivering the most accurate force control on delicate samples such as living cells, as well as soft single molecules.

The example in Fig. 2 is given for a living KPG7 fibroblast, acquired in DMEM/F-12 cell culture medium with temperature control of 37 °C, and immediately resolves the filamentous structure of the underlying cytoskeleton. Ultra-short cantilever (NanoWorld) with resonant frequency ~ 0.3 MHz and spring constant ~ 0.3 N/m was used for all of the measurements, carried out in a JPK PetriDishHeater™. The same imaging conditions were applied to the rest of experimental examples as well.

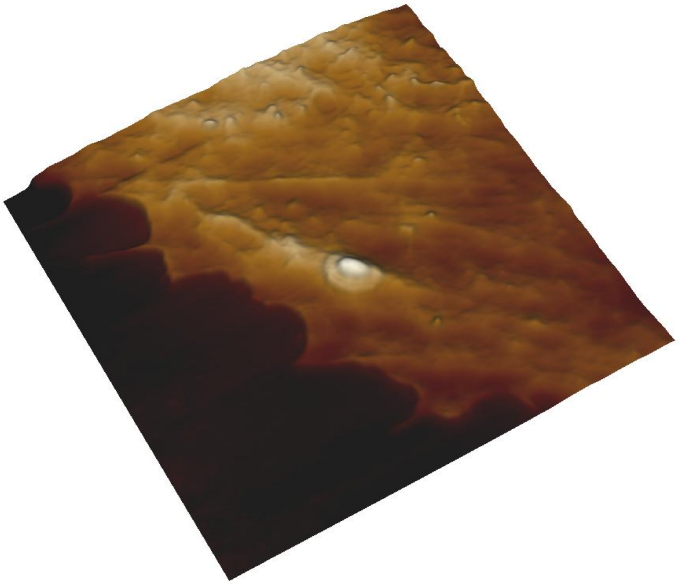

**Figure 2.** 3D Height image of a live KPG7 fibroblast. Scan size is 15x15 µm, Z-height is 125 nm.

## Real-time cytoskeleton reorganisation

Successful dynamical measurements on living cells require a significant reduction in image acquisition times. In the example given below, the cell was imaged with a line-rate of 45 Hz and at resolution of 256x256 pixels, which results in near 5.7 s per single frame. Figures 3A-C show the phase channel of three consecutive frames that clearly depict the cytoskeletal reorganization taking place under the cell membrane of the quite active fibroblasts. The cell was imaged for about an hour at different speeds and scan sizes to verify that the tip-sample interaction is not leading to a specific cell retraction or introduction of further morphological artefacts.

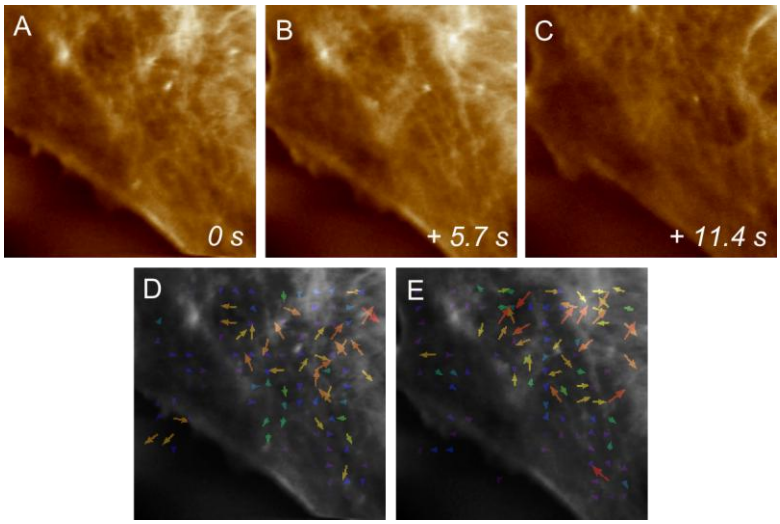


**Figure 3**. Realtime dynamics of cytoskeletal reorganization changes close to the cell periphery of living KPG7 fibroblasts. (A-C) Consecutive phase images recorded with a temporal resolution of ~ 5.7 s per frame. (D) and (E) show superimposed overlays of (A) and (B) with the particle image velocimetry (PIV)-calculated displacement fields from (A/B) and (B/C) respectively (PIV and FTTC plugins are courtesy of Qingzong Tseng, and force fields are only given for representative purposes). Image scan size is 5x5 µm.

In the case of rather complex macromolecular networks it is often difficult to draw conclusions about the effective changes in the underlying cell cytoskeleton, or any associated migration events. By applying an external plugin for PIV it is possible to roughly visualize the displacements/force fields between the different images (Fig. 3D,E). This can be rather helpful with the visualization of dynamic contractile processes arising from the filamentous actin reorganization changes, as well as formation of focal adhesion points, as seen above.

## Cell membrane dynamics

Another example of the application of fast AFM for studying of biological systems with high temporal resolution is the direct observation of cell membrane dynamics. Living cells are constantly interacting with their surrounding and exchange a number of molecules, and signals. This is mostly associated with membrane turnover for e.g. membrane ruffling, or vesiculation e.g. with internalization of external molecules and vesicles

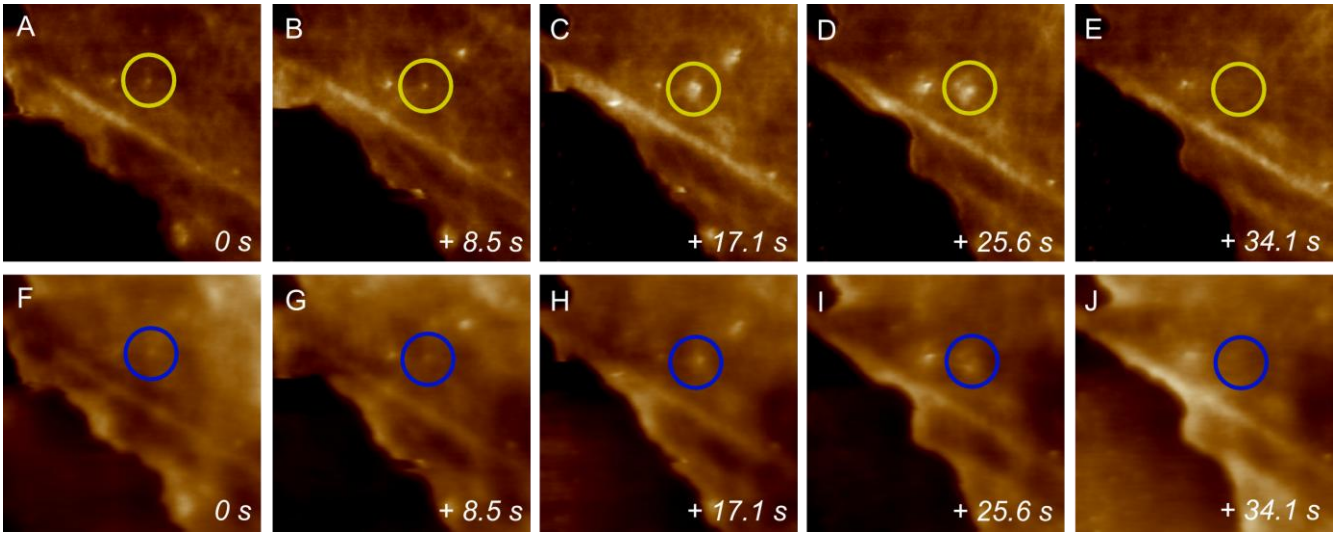


**Figure 4.** Studying dynamic morphological changes on the KPG7 fibroblast surface, associated with plausible exocytic events. (A-E) Phase and (F-J) Height images acquired with a temporal resolution of ~ 8.5 s per frame. Marked areas in yellow and blue colour focus on the particular budding event discussed above. Scan size is 5x5 µm, Z-height in (F-J) is 100 nm.

(endocytosis), as well as release of metabolitic degradation products, or signaling molecules to other cells (exocytosis). The timescale of most of these processes can depend on the type of membrane fusion or secretion, and normally ranges for seconds to minutes [9], [10]. The example below shows a plausible exocytosis event on the timescale of 30-40 seconds which is associated with a morphological change happening directly on the cell surface (Fig. 4).

The budding vesicle can be clearly resolved in the Phase images due to the much higher sensitivity of the phase channel to the rapidly changing and weak signals from the morphological differences in the cell surface. The same features can be clearly resolved in the Height channel as well, which corroborates to the very good force control applied to study even such soft membrane structures. The height of the budding area gradually increased from 10 to about 25 nm.

## Conclusions

NanoWizard® ULTRA A atomic force microscope was successfully applied to monitor two examples of dynamic biological processes in living KPG7 fibroblasts in nearly physiological buffer conditions. Both cytoskeletal reorganisation, as well as cell membrane protrusion events were studied on the time scale of 0.1-0.2 frames per second (fps). Such events are typically impossible to observe with conventional AFM due to the rather low temporal resolution, as well as slow feedback unable to resolve the rather heterogeneous structural features of rapidly changing and very active living cells. The imaging of soft biological matter now makes possible to get beyond a single snapshot of cellular events, and gain additional information about the interaction between not only single cells, but also organelles and macromolecular protein complexes. The full compatibility of the system with high-resolution optical techniques for correlative microscopy would open new possibilities to combine its native high spatiotemporal resolution with the additional molecular information available from various molecular or immunostains. Future examples and studies will demonstrate the ability of the system to image dynamic biopolymerization processes that underlie the formation of a number of key macromolecules taking place both *in vivo* and *in vitro*.

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