



# Characterizing the Effect of Anticytoskeletal Drugs on Living Cells Using MIRO Software and the BioScope Catalyst AFM

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## INTRODUCTION

In the following study, we demonstrate the power of combining atomic force microscopy AFM and fluorescence microscopy techniques to probe real-time, in-situ effects of two highly specific drugs that are able to disrupt different cytoskeleton networks inside living cells. Using Veeco Multiple Image Registration Overlay (MIRO) software and the new Bioscope Catalyst Atomic Force Microscope (Veeco Instruments, Santa Barbara, CA), we realized unprecedented accuracy in terms of navigation and force curves quality, establishing this important new imaging technique as a foundation for many exciting applications in cancer research.

## BACKGROUND

Living cells can exhibit a very wide range of elasticity or compliance properties (from a few tens of Pa to 100 MPa) depending on their types and functions, which can be used to assess and correlate the effects of drug treatment, aging, or pathology.<sup>1-5</sup> The literature describes many various techniques that can be used to probe cell elasticity, such as cell poking, micropipette aspiration, biointerface probes, microplates, magnetic twisting, laser tracking microrheology, or acoustic microscopy.<sup>6-12</sup> However, AFM has emerged in the last decade as the technique of choice to study and quantifiably characterize this correlation.<sup>13</sup>

Cell mechanical properties are determined by their cytoskeleton networks. Though many proteins are involved in the organization of these networks, two highly conserved globular

proteins, actin and tubulin, are primarily responsible for cell architecture. Actin is a 42 kDa monomer that is responsible for cell shape and comprises microfilaments involved in many key processes, such as cell division, vesicle trafficking, cell contraction, cell motility, and cell signaling. Tubulin, on the other hand, is a 55 kDa monomer that assembles to form dimers of  $\alpha$ - or  $\beta$ -tubulin and regulates cell division, flagella motility and organelles trafficking. Since cell division and cell mechanics are two critical factors in the spread of cancer, tubulin and actin are important targets for anticancer drugs like taxol, vinblastin and vincristin.

Many AFM studies involving cytoskeleton disruption are carried out using cytochalasins that are known to inhibit actin polymerization and thus change cell morphology or inhibit cellular processes. In contrast, this application note discusses studies on two drugs that induce a rapid and direct disruption of cytoskeleton; first latrunculin, which destroys actin filaments, and secondly nocodazole, which is widely used in cell synchronization experiments and which targets tubulin cytoskeleton. The experiments were made possible by the use of new AFM technology that permits drug effects to be monitored in real time by a combination of epifluorescence imaging, AFM imaging, and AFM force measurements.

## SAMPLE PREPARATION

Experiments were carried out on fixed and living HeLa and CHO-K1 cells and on two osteosarcoma cell lines that stably express either red fluorescent actin or tubulin (U2-OS-FP635-Actin and

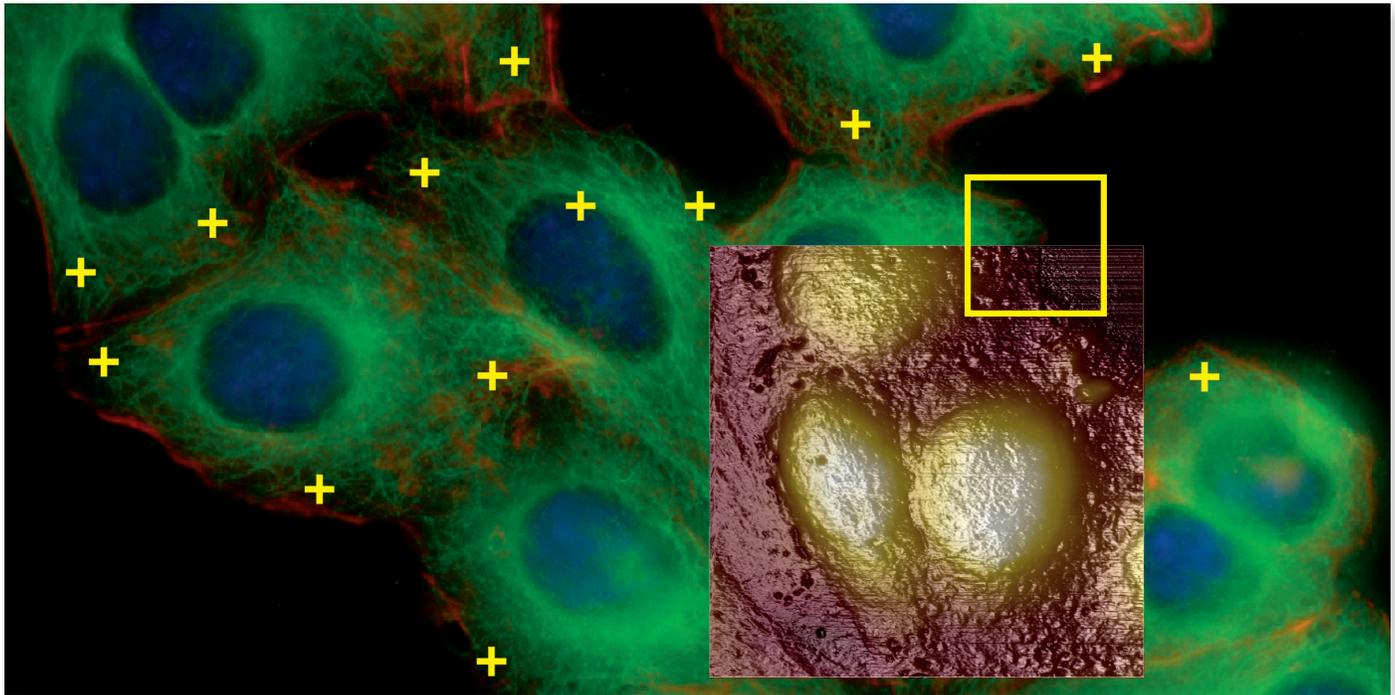


Figure 1. In the MIRO canvas, the fluorescence image is used as a background to target AFM measurements. The overlay is made in real time, pixel by pixel. At the end of the scan, the AFM image (height channel, 35x35µm) is automatically integrated into the optical image. The software can also be used to directly navigate the AFM probe to preferential locations for force curve acquisition (yellow crosses), AFM scanning, or force volume measurements (yellow frame).

U2-OS-Tag-RFP-Tubulin, Marinpharm, Luckenwalde, Germany), respectively. CHO-K1 cells were labeled with DAPI for nucleus and phalloidin-Alexa488 for actin. Hela cells were labeled with DAPI for nucleus, phalloidin-RITC for actin and anti- $\alpha$ tubulin conjugated with FITC for tubulin. Cells were grown on glass-bottom petri dishes (Willco), and treated directly inside the dishes with either nocodazole or latrunculin (50µM) for 30 minutes at room temperature. This protocol allowed the skipping of the permeabilization step, which can rapidly lead to cell death.

#### EXPERIMENT SETUP

Imaging and force measurements were performed with two separate BioScope™ Catalyst™ Atomic Force Microscope systems that were fully integrated with inverted optical microscopes. One system used a Leica DMI 6000 with a Hamamatsu ORCA camera, while the other used a Zeiss Axio Observer with an AxioCam camera. Several AFM probes (Veeco MLCT, SNL, MSNL) were utilized during the experiments; however, the same tip was used before and after drug delivery to avoid the influence of tip shape and spring constant when comparing the force curves.

Veeco's Microscope Image Registration and Overlay (MIRO) software provided a simple and quick way to navigate the tip to an area of choice to perform the AFM measurements. The registration can be done using either three points or fourth- or fifth-order polynomial calibration (for non-ideal optical microscopy conditions).

Though the primary advantage of MIRO consists in automatically overlaying optical and AFM images, in these studies we also utilized MIRO to target the best locations for force measurements (see figure 1). As AFM force measurements cannot be performed at the center of the cells, where they are too soft, the optical navigation allowed the direct selection of the best locations for force volume images and single force measurements. This allowed us also to decrease the time to data acquisition and preserve the sensitive samples and probes. In addition, the software gave us the ability to zoom, adjust color and contrast, and modify the opacity of AFM channels in the regions of interest to more perfectly match the optical images and observe before-and-after results. Finally, the vertical laser path in the Catalyst microscope head enabled much higher stability, lower noise and increased detection sensitivity.

#### EXPERIMENTAL RESULTS

Figure 1 shows the "MIRO canvas" during a typical experiment with fluorescence and AFM images overlaid, along with navigation markers for force curve measurements and force volume. Nuclei are labeled in blue, the tubulin network in green, and the actin network in red. The distribution of the red dye confirms that actin is located at the cell periphery, whereas tubulin is distributed more in the deeper cytosol. Typical areas of interest for AFM measurements are indicated by the yellow box (force volume) and yellow crosses (single force measurements). These experiments were done on fixed Hela and CHO-K1 cells.

Additional experiments investigating the effects of drug treatments were conducted on living Hela cells and U2-OS osteosarcoma cells. These cell lines stably express red fluorescent actin or tubulin, respectively, and offer the advantage of avoiding the plasmid transfection step.

The drug treatments led to different consequences in the optical images and AFM data (see table 1). The effects

of the two drugs could easily be seen, but not differentiated on the optical images. The green and red fluorescences totally vanished on all samples 30 minutes after incubation with nocodazole and latrunculin, respectively. However, the anticytoskeletal agents exhibited measurably different effects in the AFM images. After nocodazole treatment, the tracking was still good, whereas after latrunculin injection, the cells appeared much too soft for being scanned in imaging mode. This indicated that the treatments led to different changes in cell mechanical properties. Tubulin disruption caused only a slight change in cell elasticity, whereas actin disruption gave rise to a dramatic change in cell elasticity.

Figures 2C and 2F give a representative example of force curves recorded on osteosarcoma cells. (Very similar effects were observed on the HeLa cells as well.) Note that the AFM force measurements were performed on the edge of the cells to avoid indenting too much in the sample, and to ensure clear, reproducible force curves. It is very likely that the change in elasticity is even greater in the thickest parts of the cells. Taken together, the results suggest that the actin cytoskeleton is playing a much more important role in cell rigidity than the tubulin network.

## CONCLUSION

This study demonstrates how the use of a fully integrated BioScope Catalyst can enable enhanced investigation of drug effects on cells by combining results from fluorescence microscopy and AFM. The integration also enables better, more efficient control of the AFM to guide imaging and localized force measurements. This was clearly shown in the analysis of the effects of two different drugs on two distinct cytoskeleton networks in living cells. This type of experiment can be extended to many research fields involving cell elasticity measurements, including investigation of cardiovascular and infectious diseases, cancer research, and drug targeting.

	Fluorescence	AFM imaging (tracking)	Change in cell elasticity
Control	+++	+++	-
Nocodazole	∅	++	+
Latrunculin	∅	∅	+++

Table 1. Effects of nocodazole and latrunculin treatments in both optical and AFM experiments.

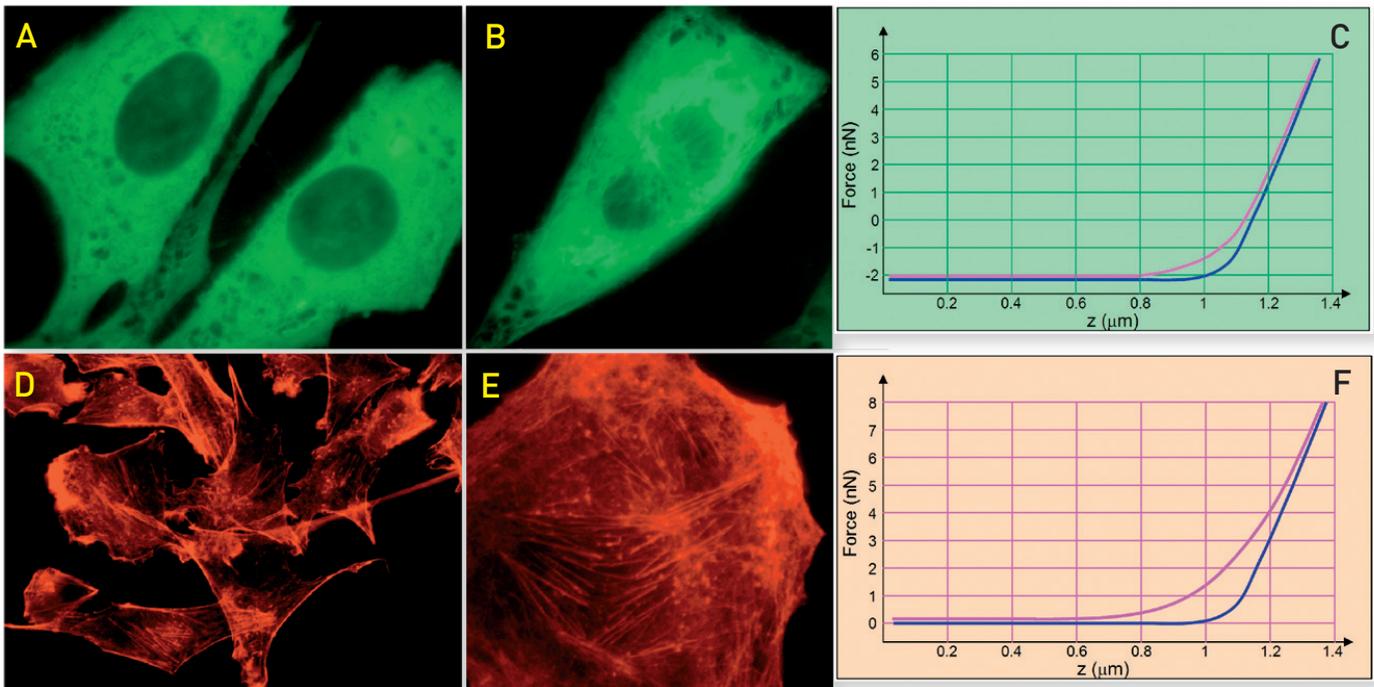


Figure 2: Nocodazole and latrunculin were incubated with living HeLa (A and D respectively) and osteosarcoma (B and E respectively) cells, and a minimum of 30 force volume images were collected on each type of sample. After drug addition, the red and green fluorescence disappeared (not shown here). C and F show representative “approaching” force curves recorded before (blue trace) and after (purple trace) drug treatment on osteosarcoma cells (results on HeLa cells were similar). The two agents showed dramatically different effects on cell elasticity. The tubulin network was only slightly disrupted, showing minimal effect on the global cell elasticity (note the two curves are only slightly displaced on C). In contrast, the actin network disruption dramatically altered cell elasticity (purple curve reflects a significant softening effect in F).

## REFERENCES

1. Wu, H.W., T. Kuhn, and V.T. Moy. "Mechanical properties of L929 cells measured by atomic force microscopy: effect of anticytoskeletal drugs and membrane crosslinking." *Scanning* 20: 389-97 (1998).
2. Roth, C., and M. Radmacher. "Drug-induced changes of cytoskeleton structure and mechanics in fibroblasts: an atomic force microscopy study." *Biophys. J.* 78: 520-35 (2000).
3. Berdyeva, T.K., C.D. Woodworth, and I. Sokolov. "Human epithelial cells increase their rigidity with ageing in vitro: direct measurements." *Phys. Med. Biol.* 50: 81-92 (2005).
4. Sokolov, I., S. Iyer, and C.D. Woodworth. "Recovery of elasticity of aged human epithelial cells in vitro." *Nanomedicine* 2: 31-36 (2006).
5. Dulinska, I., M. Targosz, W. Strojny, M. Lekka, P. Czuba, W. Balwierz, and M. Szymonski. "Stiffness of normal and pathological erythrocytes studied by means of atomic force microscopy." *J. Biochem. Biophys. Methods* 66: 1-3 (2006).
6. Petersen, N.O., W.B. McConnaughey, and E.L. Elson. "Dependence of locally measured cellular deformability on position on the cell, temperature, and cytochalasin B." *Proceed. Nat. Acad. Sci. USA* 79: 5327-31 (1982).
7. Hochmuth, R. M. "Micropipette aspiration of living cells." *J. Biomech.* 33: 15-22 (2000).
8. Evans, E., K. Ritchie, and R. Merkel. "Ultrasensitive force technique to probe molecular adhesion and structure at biological interfaces." *Biophys. J.* 68: 2580-87 (1995).
9. Thoumin, O., and A. Ott. "Time scale dependent viscoelastic and contractile regimes in fibroblasts probed by microplate manipulation." *J. Cell Sci.* 110: 2109-16 (1997).
10. Wang, N., J.P. Butler, and D.E. Ingber. "Mechanotransduction across the cell surface and through the cytoskeleton." *Science* 260: 1124-27 (1993).
11. Yamada, S., D. Wirtz, and S.C. Kuo. "Mechanics of living cells measured by laser tracking microrheology." *Biophys. J.* 78: 1736-47 (2000).
12. Kundu, T., J. Bereiter-Hahn, and I. Karl. "Cell property determination from the acoustic microscope generated voltage versus frequency curves." *Biophys. J.* 78: 2270-79 (2000).
13. Binnig, G., C.F. Quate, and G. Gerber. "Atomic force microscope." *Phys. Rev. Lett.* 56: 930-33 (1986).

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