

Tracking cell footprints: Modern microscopy methods visualize bio-nanotubes

Introduction

During cell movement across surfaces, some cells release tube-like structures, so-called cell traces. These tubes can be seen as a molecular fingerprint of their parent cells. Observing them by simultaneous use of optical and atomic force microscopy gives information about functional and structural details of cell traces. This is an important requirement for the application of these systems in cell analytics [1-3].

Cell motion plays an important role in biology. The whole area of tissue and organ development, including growth and differentiation is determined by cell mobility. It is also important for wound healing and the response of the immune system.

Cell traces: "wear debris" during cell motion

Until now, little attention has been drawn to the release of regularly structured, branched and highly ordered cell membrane filaments by adherent animal cells. The length of cell traces can reach some hundred microns and show a diameter of 100 nm. They are covered by a lipid membrane containing cytoplasm, RNA, cytoplasmatic proteins, and, perhaps, also cell organelles. There is evidence from immunofluorescence data showing cellular surface receptors that the cell traces can be seen as a fingerprint of their parent cell. Therefore, the cell traces play an important role for cell analytics.

Though modern optical microscopy techniques allow the analysis of the morphology of the cell traces and distribution of protein units by fluorescent markers, structural information can only be studied by AFM due to the cell traces proportions. Combining both optical and force microscopy methods give a deeper insight into these systems.

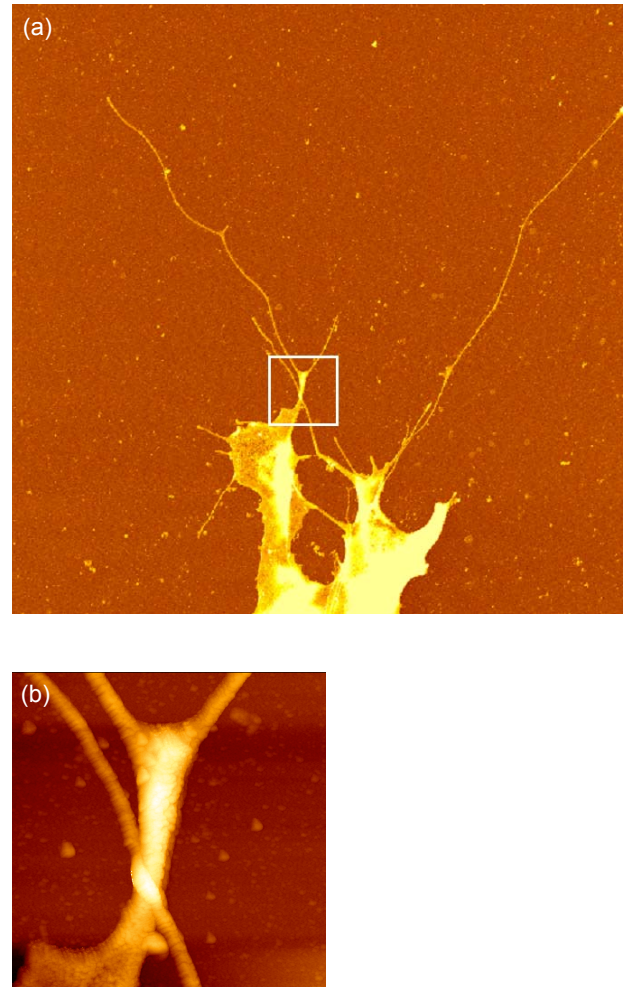


Fig. 1 (a) AFM overview image (topography) of dehydrated mouse fibroblast cell traces. Image size $35.3 \times 35.3 \mu\text{m}^2$, height range 600 nm. (b) True zoom AFM image, corresponding to the marked region in (a). Image size $3.1 \times 3.1 \mu\text{m}^2$, height range 440 nm.

Mouse fibroblasts as a model system

L929 mouse fibroblasts have been fixed and rinsed with buffer. Figure 1 shows intermittent contact mode AFM images of dehydrated samples. Figure 1 (a) shows an AFM overview image. A part of the cell body can be recognized at the bottom of the image. Cell traces, 10 to 20 μm long, spreading in a V-shape across the image, were left behind during the migration of the cell. A bifurcated area next to the center of the image has been selected for a zoom. At this position a new image, Figure 1 (b), was obtained, showing the branch and a second cell trace crossing the first.

Combination of AFM with IRM

Figure 2 is an optical IRM (interference reflection microscopy) image, showing a net of cell traces. The IRM image was obtained with an Olympus IX 71. The JPK NanoWizard[®] system was mounted on the Olympus inverted optical microscope, so that during the experiment, the AFM cantilever could be observed simultaneously with the cell traces. The scan process was started right above the middle of the image to obtain the AFM image shown in Figure 3. The cell traces seen in Figure 3 are expanding to a long and rectangular structure which is surrounded by a convex rim. This structure is in the sub-micron range and can not be identified in the optical microscopy image.

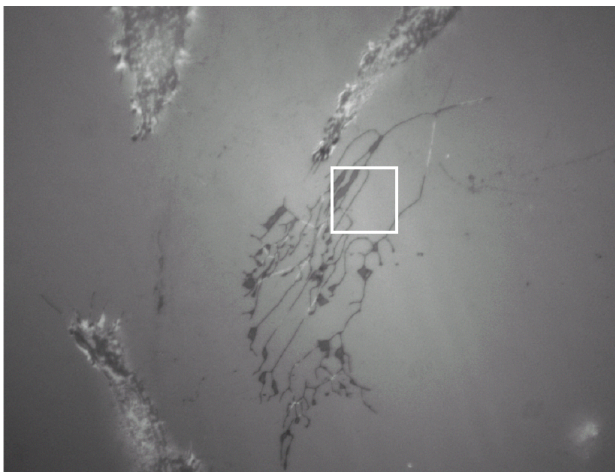


Fig. 2 IRM image showing mouse fibroblast cell traces in buffered solution.

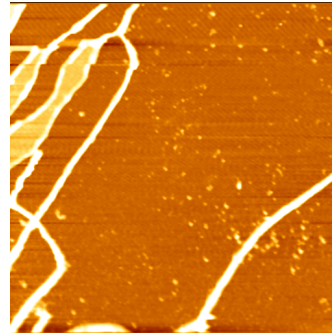


Fig. 3 AFM image, corresponding with the box marked in the optical image in Figure 2. Image size 15 x 15 μm^2 , height range 150 nm.

In this study, it has been shown that AFM is a suitable microscopy technique to image cell traces with high resolution. Distortion-free images were obtained both with dehydrated samples and with samples in buffered solution. Combining AFM with IRM allows a fast overview examination of the sample with IRM in order to place the AFM tip at a suitable position.

Authors

Ines Westphal
Fraunhofer Institut für Biomedizinische Technik IBMT
Invalidenstr. 42
10115 Berlin
ines.westphal@ibmt.fraunhofer.de

Hauke Clausen-Schaumann
Fraunhofer Institut für Biomedizinische Technik IBMT
Invalidenstr. 42
10115 Berlin
hauke.clausen-schaumann@ibmt.fhg.de

Christian Löbbe
JPK Instruments AG
Bouchéstr. 12
12435 Berlin
loebbe@jpk.com

Günter Fuhr

Fraunhofer Institut für Biomedizinische Technik IBMT
Ensheimer Str. 48
66386 St. Ingbert
guenter.fuhr@ibmt.fraunhofer.de

This report has been published in German in the April 2004 edition of "Zellbiologie" journal (p. 30 f.), GIT-Verlag Darmstadt, Germany.

Literature

- [1] Fuhr G., Richter E., Zimmermann H., Hitzler H., Niehus H. and Hagedorn R. "Cell traces - footprints of individual cells during locomotion and adhesion". Biol. Chem. 379, 1161-1173 (1998).
- [2] Zimmermann H., Hagedorn R., Richter E. and Fuhr G. "Topography of cell traces studied by atomic force microscopy". Eur. Biophys. J. 28, 516-525 (1999).
- [3] Zimmermann H., Richter E., Reichle C., Westphal I., Geggier P., Rehn U., Rogaschewski S., Bleiss W. and Fuhr G.R. "Mammalian cell traces – morphology, molecular composition, artificial guidance and biotechnological relevance as a new type of „bionanotube“". Appl. Phys. A 73, 11-26 (2001).