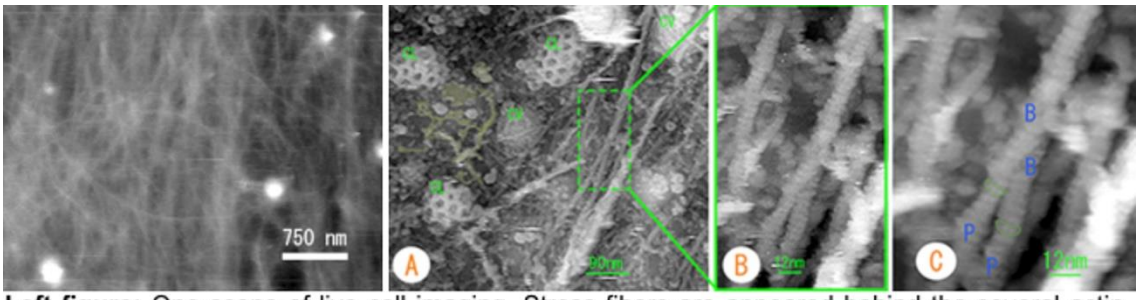
Cutting Edge of Atomic Force Microscopy (AFM) of the Cell: Live Cell imaging and Structure Analysis of Cytoskeletal Actin Filaments at High Resolution

Usukura, E.[1], Narita, A.[1], Yagi, A.[2], Sakai, N.[2], Uekusa, Y.[2], Imaoka, Y.[2], Ito, S.[2] and Usukura, J.[1]

[1] Nagoya University, Japan, [2] Olympus Corporation, Japan

Increasing in scanning speed and mechanical stability improved in AFM facilitated live cell imaging and high-resolution structural analysis of organelles in cells.AFM also is an important tool for observing cells in physiological condition or PBS at higher resolution than light microscope, and therefore enable to observe fine structures containing water. In addition, images obtained by scanning needle with touching in AFM is basically different in quality from those in light and electron microscopes, in which images are formed by scattering, absorption or phase contrast of electron or photon. High speed AFM developed recently succeeded to arrest the movement of actin filaments near cell membrane. Although stress fibers showed low mobility, actin filaments forming complicated meshwork beneath the cell membrane moved well. Under careful observation, actin filaments formed focal points even in cytoplasm so that many actin filaments gathered at the focal contact on cell membrane. In order to view actin filaments and organelle more in detail, apical cell membrane was unroofed mechanically with customized low power sonication. All actin filaments showed short periodicity with about 5~6 nm derived from G-actin linked in beads form, and sometimes long periodicity of helical characteristic. Furthermore, the polarity of actin filaments was determined based on the shape of a periodic striation without any decoration or labeling. However, unroofing technique is effective only in culture cells, but not in tissues. We tried to solve this problem by cryosectioning. Because the cantilever could access all parts in cryosection of a tissue sample after the embedding medium (sucrose) has been replaced with phosphate-buffered saline. The use of cryosectioning enabled the morphological analysis and immunocytochemistry of cells in tissues in atomic force microscopy (AFM). In retinal tissue, the AFM images showed disks stacked and enveloped by the cell membrane in rod photoreceptor outer segments at EM resolution. In addition, three-dimensional architecture of synaptic ribbons, and the surface of the post-synaptic membrane facing the active site were revealed, which were not apparent using thin-section EM. AFM could depict the molecular binding of anti-opsin antibodies conjugated to a secondary fluorescent antibody bound to the disk membrane. The specific localization of the anti-opsin binding sites was verified through correlation with immunofluorescence signals in AFM combined with confocal fluorescence microscope.



Left figure: One scene of live cell imaging. Stress fibers are appeared behind the several actin filaments meshwork showing high mobility

Right figure: AFM image of cytoplasmic surface of cell membrane exposed by unroofing (A). Actin filaments extend along the inside surface of the cell membrane. CL: clathrin coat, CV: caveolae. B: High magnification image of boxed area. All actin filaments show short periodic striation. C: Averaged image of 10 consecutive raw scanning images of the same area in image B. Periodic elements appear to be slightly curved (highlighted with green lines). p : pointed end. b: barbed end.