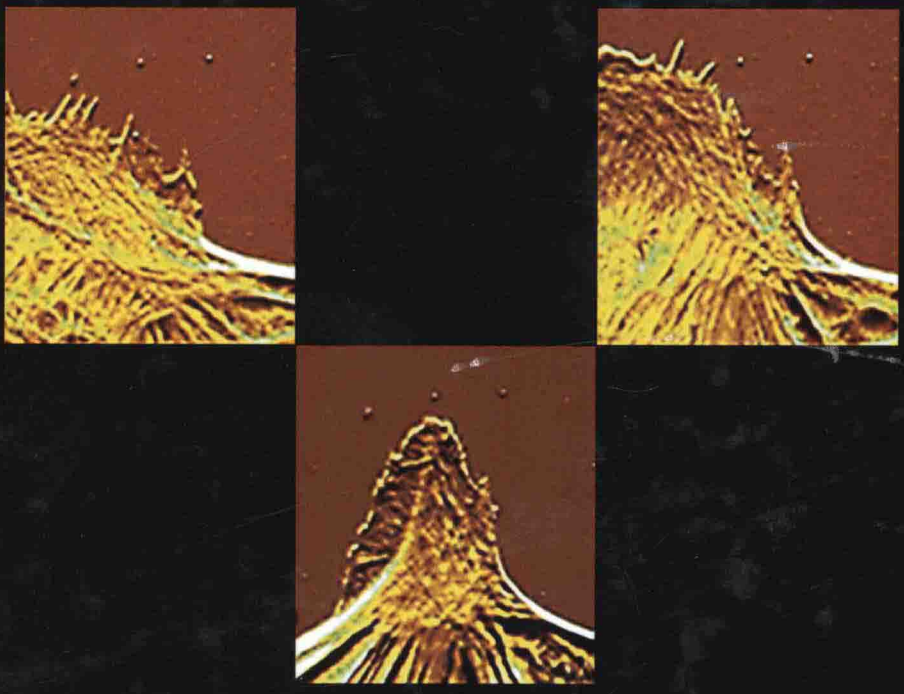
# ATOMIC FORCE MICROSCOPY



# IN NANOBIOLOGY

edited by
Kunio Takeyasu

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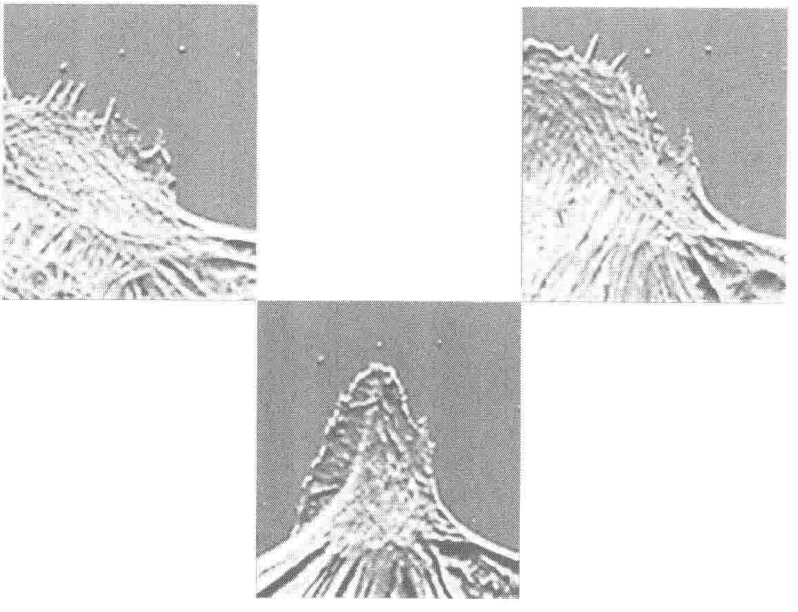

# Preface

Understanding the "events" around us through visualization and observation has always been a fundamental part of our philosophical activity, and the microscope has played a central role in these processes within the biological sciences. Since the early days of the invention of the light microscopes in the 17th century, vast numbers of pioneering discoveries, including the cell theory of Schleiden (1838) and Schwann (1839), have been made by using this key research tool [1–3]. More recent modifications of the light microscope in relation to oil emission, fluorescence, and phase contrast have enabled it to maintain its role as an invaluable research tool in modern biology. However, a major limitation associated with the light microscope is that its resolution is half the wavelength of light. This is the reason scientists and engineers are devoted to the development of new instruments with higher and better resolution.

The invention of the electron microscope (EM) in the 1920s to the 1930s has enabled the attainment of a hundred times' greater resolution than the light microscope and has opened a new era in our ability to "see" biological materials at the nanometer scale [4]. The EM requires special specimen preparation and operation techniques such as coating the sample in a fine layer of gold and observation in vacuum. These limitations were challenged in the 1980s by the invention of "scanning tunneling microscopy" (STM) [5] and "atomic force microscopy" (AFM) [6]—members of scanning probe microscopy (SPM)—giving rise to a new generation of microscopes that rely on a physical interaction between a sharp probe and the sample surface instead of a difference of wavelengths.

The application of AFM to biological samples dates back to the late 1980s. The significance of this microscopy is the achievement of

high spatial resolutions similar to the EM and of lesser requirements for sample preparation, allowing living matter to be monitored under physiological conditions [7–11]. The observation of deoxyribonucleic acid (DNA) strands by AFM was the first application of this technique to a biological sample [12]. The first notable application was made in the early 1990s for the observation of double-stranded DNA [13–15]. This achievement greatly encouraged many biological researchers to jump into the nanoworld in the late 1990s (see Chapters 5, 6, and 9–11). When AFM was invented, the scientists' immediate thought was that it was a potential tool that could be a bridge between light microscopy and X-ray crystallography, that is, to visualize working molecules under physiological conditions. Unfortunately, the slow imaging speed of the device at that time made it impossible to directly visualize the molecules in action. An extraordinary improvement in the device was made by Ando's group at Kanazawa University in 2001 [16]. The increased temporal resolution of several frames per second (fps) in the newly developed "fast-scanning AFM" allows the action scenes of biological molecules to be monitored more closely in the subsecond time scale (see Chapters 8, 12, and 13).

In addition to molecular imaging capability, AFM has another capability, "force measurement," to measure the elasticity of living cells [17–21] (see Chapters 14–17). When an AFM cantilever approaches and pushes against the cell surface, a large indentation in the cell and its surface is usually observed when the probe first contacts the cell surface. This indentation can be plotted against the force of the cantilever and fitted to the Hertz model equation [22] to estimate Young's modulus, which describes the elasticity of the sample. The actin network may be responsible for the elasticity of the cell [23]. Elasticity measurements have shown that both the plasma membrane and the nuclear envelope are "flexible" enough to absorb a large deformation formed by an atomic force microscope probe. Penetration of the plasma membrane and the nuclear envelope is possible when a probe with a sharp tip (tip angle of $\sim 25^{\circ}$) deeply indents the cell membrane, causing the membrane to come close to a hard glass surface [21]. These types of experiments will provide useful information for the development of single-cell manipulation techniques that are

applicable to the evaluation of cell properties under physiological and pathological conditions. The recent development of recognition imaging using the topography and recognition (TREC)™ mode has enabled identification of a specific molecule in the AFM image [24–27]. It is possible to simultaneously obtain a topographic image and also the position of a specific interaction caused by attractive forces between the specimen and the protein-coupled (e.g., specific antibody) cantilever (see Chapters 7 and 14).

Recent development of AFM has been accomplished by various technical and instrumental innovations including high-resolution imaging technology in solution, fast-scanning AFM, and general methods for cantilever modification and force measurement. These modern AFM technologies have made it possible to conduct biological studies under physiological conditions. Application of the recognitionimaging mode that can simultaneously obtain a topographic image together with a recognition signal is now successful by using a protein-(antibody)coupled cantilever and has revealed the specific proteinbindings sites on the chromatin. This monograph provides an overview of all these modern AFM technologies: basic AFM protocols in part I, newly developed technologies in part II, and most recent applications of AFM technologies to biological sciences in parts III and IV. Much effort will be made to put together most recent research activities toward establishing a new basic science field, "nanobiology." Many friends who contributed chapters in this monograph are highly acknowledged for their kind and serious efforts to describe their most recent progress in AFM research. They are leading scientists in the nanobiology field.

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