Atomic Force Microscopy

Biomedical Methods and Applications

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Atomic Force Microscopy

Biomedical Methods and Applications

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Preface

The natural, biological, medical, and related sciences would not be what they are today without the microscope. After the introduction of the optical microscope, a second breakthrough in morphostructural surface analysis occurred in the 1940s with the development of the scanning electron microscope (SEM), which, instead of light (i.e., photons) and glass lenses, uses electrons and electromagnetic lenses (magnetic coils). Optical and scanning (or transmission) electron microscopes are called “far-field microscopes” because of the long distance between the sample and the point at which the image is obtained in comparison with the wavelengths of the photons or electrons involved. In this case, the image is a diffraction pattern and its resolution is wavelength limited.

In 1986, a completely new type of microscopy was proposed, which, without the use of lenses, photons, or electrons, directly explores the sample surface by means of mechanical scanning, thus opening up unexpected possibilities for the morphostructural and mechanical analysis of biological specimens. These new scanning probe microscopes are based on the concept of near-field microscopy, which overcomes the problem of the limited diffraction-related resolution inherent in conventional microscopes. Located in the immediate vicinity of the sample itself (usually within a few nanometers), the probe records the intensity, rather than the interference signal, thus significantly improving resolution. Since the most well-known microscopes of this type operate using atomic forces, they are frequently referred to as atomic force microscopes (AFMs). Given the progressive spread of commercial atomic force microscopes, their biomedical applications have increased greatly, and they are now used by researchers in many different fields.

Pioneering AFM studies were carried out in the natural sciences and biological and medical fields in the 1990s. Nevertheless, although the literature in this area is steadily increasing, the vast majority of biomedical researchers still know little about the possibilities of AFM. This is probably because of its particular technical background, which is more familiar to physicists or engineers, but unusual to biologists or physicians.

In comparison with the more familiar, simpler, and in some cases more intuitive forms of microscopy used by researchers in the life sciences, AFM offers a number of special features: very high magnification with very high resolution; minimal tissue or cell preparation (no dyes, as in optical microscopy; no vacuum, critical point, or gold sputtering, as in scanning electron microscopy); the ability to obtain different views of the sample from a single data collection; the ability to work in an aqueous environment in real time using a nonintrusive local probe, thus making possible a study of the dynamic phenomena of live cells in their biofluid environment and under near physiological conditions with nanometer resolution; and finally (a breakthrough in the field of microscopy), the possibility of using the exploring probe in order to interact with the microscopic biological sample when measuring its electromagnetic and mechanical properties (stiffness, viscosity, elasticity, etc.). The limitations of the technique are still the relatively small scan size, the low scanning speeds, and the difficulties in imaging very soft biological samples.

A number of commercial instruments are on the market and various techniques have now been standardized. Although the number of articles in this field is increasing exponentially, they are mainly found in technical rather than more general biomedical journals, which is why this book is aimed at scientists beginning to use these techniques for the first time and with no prior knowledge.

The purpose of *Atomic Force Microscopy: Biomedical Methods and Applications* is to equip researchers in the life sciences with hands-on knowledge and to offer "recipes" like a cookbook to show the many-sided possibilities of different biomedical applications. As a volume of the Methods in Molecular Biology series, this book describes detailed practical procedures, accompanied by extensive practical details (how to do it). Each protocol is enhanced by Notes that help identify the problems that may be encountered and how they can be overcome. Each chapter in this volume is written in such a way that a competent scientist unfamiliar with the method can carry out the technique successfully at the first attempt by simply following the detailed descriptions of the practical procedures. Though biologists and physicians not yet working in this field may think the technical methodology complicated at first sight, careful reading of the various sections of this book and their schematic materials and methods should soon enable them to understand the basic approaches.

We decided to bring together a wide range of applications in order to provide examples of different subjects in different fields to first stimulate curiosity and then the interest of researchers in the life sciences in applying ingenuity to their specific fields, thus broadening and opening up new perspectives in ultrastructural biomedicine. We hope that this volume will help researchers approach this new microscopic world, find novel ideas and applications, and use AFM to add significant originality to their studies.

We gratefully acknowledge the contributions by our colleagues each of whom donated their experience in order to help us catalyze the development of this new and fascinating technology.

Pier Carlo Braga
Davide Ricci

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Part IV Functional Investigations With AFM

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