

Visualization of cell structure by atomic force microscopy

Abstract

Cell structure has been extensively studied by light and electron microscopy. However, there are relatively few papers on the study of internal cell structure with the atomic force microscope. In this mini review, efforts to visualize and analyze inner cell structure with the atomic force microscope are here presented, using the technique for sample preparation derived from the standard transmission electron microscopy. Semithin sections of epon embedded samples mounted on glass slides are scanned with a microscope working on contact or intermittent modes. The surface of sections revealed internal cell structure. Animal and plant cells show structures as nuclei, nucleoli, chromatin, cell wall, mitochondria. These works opens up the perspective to analyze these organelles and structures at a nanoscale under liquid physiological conditions.

Keywords: atomic force microscopy, cell structure, nanoscale

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María de Lourdes Segura-Valdez,¹ Alma Zamora-Cura,² Luis F Jiménez-García¹

¹Department of Cell Biology, Universidad Nacional Autónoma de México (UNAM), México

²National Institute of Genomic Medicine, México

Correspondence: Luis F Jiménez García, Laboratorio de Nanobiología Celular Departamento de Biología Celular, Universidad Nacional Autónoma de México (UNAM), Circuito Exterior, C.U., 04510, Cd. Mx., México, Tel (55)56224988, Fax (55)56224828, Email luisfelipe_jimenez@ciencias.unam.mx

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Introduction

Since its invention in 1986¹ atomic force microscopy (AFM) has been used to study properties of surface of materials at the nanoscale. In biological sciences, AFM has mainly been used for the study of isolated molecules and their interactions, even in real time²⁻⁴ and recently at high resolution and high speed.⁵ However, the use of AFM to analyze inner cell structure is scarce. The inner structure of the cell was extensively described by optical and electron microscopy ever since the discovery of cells in cork and is extensively displayed in many articles and texts.⁶ AFM offers the opportunity of analyze several properties of the inner structure of the cell at nanoscale, included topographic characterization. In this mini-review, we revised briefly several efforts intended to visualize inner structure of cells by AFM.

The atomic force microscope is a topographic technique that would allow us to analyze entire cells, revealing only the external side of the membrane. Therefore, using sections of material processed for transmission electron microscopy was an approach that produced results. Although initial efforts were made producing images,⁷ material processed using epoxy resins and semithin sections stained with toluidine blue or even unstained sections, produced images with better Z resolution.⁸⁻¹⁷

The samples are processed by fixation with glutaraldehyde, post fixation with osmium tetroxide, dehydration with graded concentration of ethanol and embedded in epoxy resin. Semithin sections of 250nm width are attached or mounted on glass slides. Surfaces of sections are observed with an AFM working in contact or tapping mode.

Results obtained over the years include samples of plant and animal cell as well as samples from unicellular parasites as *Giardia lamblia* and *Entamoeba Histolitica*. For example, the plant *Lacandonia schismatica*, a species with the sex organs inverted, was used as a model to test the technique, producing images similar to those obtained with the light and electron microscopes.^{8-10,13,16} The cell nucleus and nucleolus were recognized, as well as compact chromatin. In fact, compact chromatin visualized by AFM was used as an additional criterion for the diagnosis of reticulated chromatin in *Ginkgo biloba*.¹⁸

In plants, cell wall was a marker to recognize plant cell, and structure within. However, animal cell were more difficult to visualize. Using tapping mode on Hep2 cells, images of cell nuclei were obtained and nuclear structures as interchromatin granule clusters were resolved.¹⁷ Previously, nuclear particles named *Lacandonia* granules¹⁹ were observed individually,¹³ opening the possibility to analyze gene expression at the nanoscale *in situ*.^{20,21} It is interesting to mention that aspects of cell biology as mitosis have been also studied.¹²

Figure 1 shows an image of a human Hep2 cell prepared for transmission electron microscopy. Semithin section was placed onto a glass slide and scanned with the AFM in tapping mode. Several internal structures are observed as cell nucleus, nucleolus, chromatin, mitochondria.

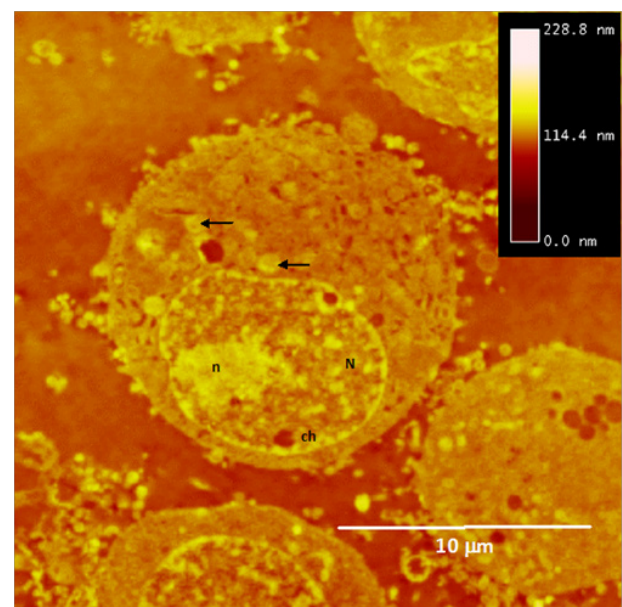


Figure 1 Atomic force micrograph of Hep2 cells. The cell in the center shows a large nucleus (N) and nucleolus (n) and clumps of compact chromatin (ch). In the cytoplasm, mitochondria are seen (arrows).

Discussion

Microscopes produce images of cell that may further be analyzed. While the light and electron microscopes use lenses and a source of light or electrons, atomic force microscopes use a very fine tip that scan the sample, producing a high resolution image of the surface. Therefore, with light and electron microscopes, internal structure of cells has been possible to analyze. On the opposite, the atomic force microscope is a surface instrument. The approach mentioned in this mini review reveals also internal structure since scanning the surface of a sections, the texture correlates to cell organelles. Each section may be analyzed and every section then act as a part of internal structure. So, internal *in situ* cell structure can be studied by AFM. In fact, this microscope analyze the surface of the sample, therefore analysis of internal structure is initially not possible. An approach using sections of biological material and using each section as a surface, would reveal the structure present in each sections. The structure of the surface of each sections probed to be identical to the cell structure, when compared to light and electron micrographs of similar samples. Combining this approach with an AFM working in tapping mode, the resolution increases. Images are obtained similarly even if samples are observed under water or using different resins. Many biological samples can be observed now and eventually, the *in situ* analysis at nanoscale of processes under liquid as ribosome biogenesis, gene expression and others will be possible.

Current research mainly is focused on the understanding of biological processes at the nanoscale, as recognition of molecules and their properties with no labeling and the interaction among them.^{22–24} This mini review revealed that only few papers deals with inner cell structure *in situ*.

Conclusion

Internal cell structure as nuclear structures can be studied *in situ* with the atomic force microscope using semithin sections mounted on glass slides, from biological material as plant, animal and fungi cells and also unicellular parasites as *Giardia lamblia* processed for standard transmission electron microscopy.

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Conflict of interest

Author declares that there is no conflict of interest.

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