Atomic Force Microscopy of Microbial Cells

By: Y. F. Dufrêne

Introduction

Microbial cells (bacteria, yeasts and fungal spores) are surrounded by fairly rigid cell walls which play several vital functions: protecting the cytoplasm from the outer environment, providing the cell with a rigid structure, determining the cell shape and controlling cell adhesion phenomena. Microbial adhesion processes have major consequences in natural environments (symbiotic interactions, biofouling), medicine (infections), and biotechnology (bioremediation, immobilized cells in bioreactors). Therefore, understanding the structure, properties and functions of microbial cell surfaces is of great significance for both fundamental and applied research.

Light and electron microscopies have long been recognized as the key techniques to look at microbial cells. Light microscopy is useful for counting and identifying the cells as well as for determining their general morphological details; but because the resolution is limited to the wavelength of the light source, information at the nanometer level is not accessible.

High-resolution images of microbial samples can be obtained by electron microscopy, which uses high energy electrons instead of light as the incident beam. The use of freeze-fracture and surface replica in transmission electron microscopy (TEM) makes it possible to visualize cell surface structures at high resolution. However, these approaches are limited by the requirement of vacuum conditions during the analysis, i.e., living cells cannot be directly investigated in their native environments. In addition, electron microscopy cannot provide information on the biophysical properties of the cell surface.

Atomic force microscopy (AFM) has opened exciting new avenues in microbiology and biophysics for probing microbial cells. The unprecedented capabilities of AFM can be summarized as follows: i) imaging surface topography with nanometer lateral resolution and under physiological conditions; ii) measuring local physical properties such as adhesion forces and elasticity. This application note describes a convenient sample preparation
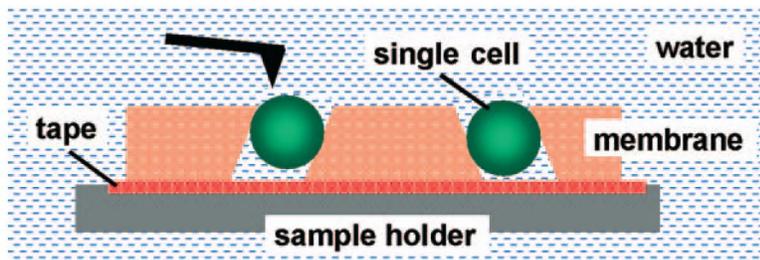

Figure 1. Immobilization method used for investigating single microbial cell under physiological conditions. Living cells are trapped in a porous polymer membrane with a pore size comparable to the cell dimension.

procedure for AFM of native microbial cells and presents a series of examples to highlight the various applications of the technique in this field. All of the data in this application note was obtained using a Digital Instruments MultiMode® atomic force microscope (AFM) equipped with a fluid cell.

Sample preparation

Cells were among the first biological samples to be investigated by AFM[1]. While the potential of the instrument for probing living (non-fixed) animal cells was recognized quite early[2], attempts to perform such measurements on microbial cells have been more limited, due in part to the difficulties associated with the sample preparation. As opposed to animal cells, microbial cells have a well-defined shape and have no tendency to spread over substrates. As a result, the contact area between cells and substrate is very small, often leading to cell detachment by the scanning probe. A second issue is the vertical motion of the sample (or probe), typically limited to a few micrometers, which makes it difficult to image the surface of large objects such as microbial cells. To circumvent these problems, a convenient immobilization procedure is to trap the cells in a porous filter membrane[3,4].

As shown in Figure 1, a concentrated cell suspension is gently sucked through a polymer membrane with a pore size slightly smaller than the cell size. After cutting the filter (1cm x 1cm), the lower part is quickly dried on a sheet of tissue and the specimen is then attached to the sample holder using a small piece of adhesive tape. The main advantage of this procedure is that it does not involve chemical treatments which could cause rearrangement/ denaturation of the surface molecules. Examples of AFM height images showing single cells trapped in a pore of the membrane are shown in Figures 2 and 3, for the fungus *Aspergillus oryzae* and the bacterium *Lactococcus lactis*, respectively. The cells can be imaged in an aqueous solution repeatedly without cell detachment or surface damage.

Applications

Imaging

AFM can provide high-resolution images of the surface structure (cell wall layers, appendages) of microbial cells[5]. Both height and deflection images are often useful because they yield complementary information. Height images (Figures 2 and 3) provide quantitative height measurements, thus allowing an accurate measurement of cell dimensions and of the surface roughness. Deflection images do not reflect true height variations, but because they are more sensitive to fine surface details they are useful for revealing the surface ultrastructure of curved, rough samples such as cells. An example of high-resolution deflection image is shown in Figure 4 for the surface of *A. oryzae* spores in the dormant state. The surface is covered with patterns of nanoscale rodlets, presumably proteins, which are assembled in parallel to form supramolecular fascicles[6]. Although such structures were previously observed by electron microscopy, they can now be visualized directly in aqueous solution.
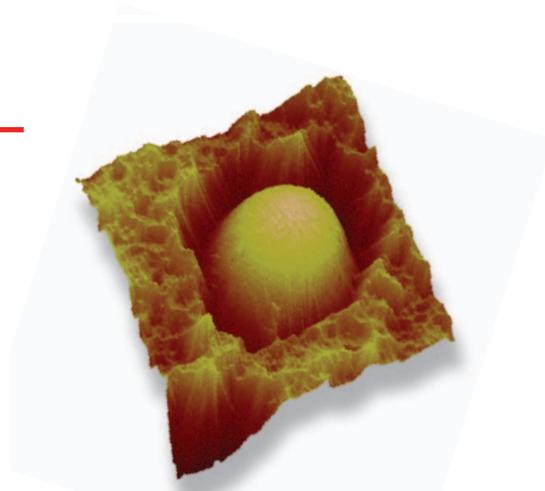

Figure 2. 3D topography showing fungal spore (Aspergillus oryzae) trapped into a pore of the membrane. 10µm x 10µm.
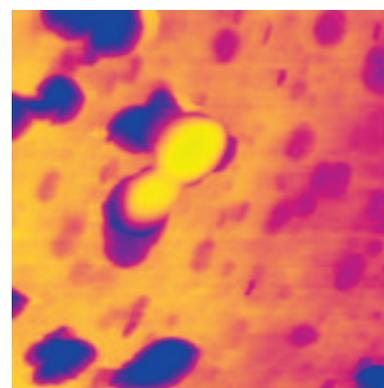

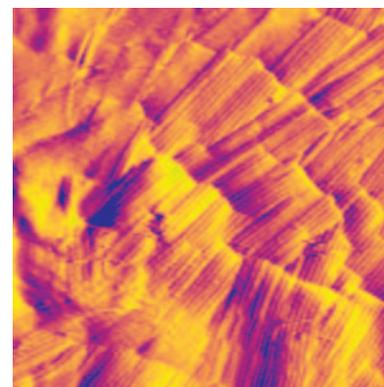Figure 3. Topography of two dividing bacterial cells (Lactococcus lactis). 5µm x 5µm.

Figure 4. Visualizing cell surface nanostructures. High-resolution deflection image in aqueous solution of the surface of a dormant spore (A. oryzae). The surface is covered with well-ordered nanostructures, called "rodlets," that are 10nm in diameter and assembled in parallel to form fascicles interlaced in different orientations. 750nm x 750nm.

Appendages (fimbriae, fibrils, flagella) is another class of surface structures often found on bacterial cells. Here again, AFM can be used to image these structures directly in the native state. For instance, fibrils about 200nm in length could be detected on the surface of the bacterium *Streptococcus salivarius HB*[7]. Note, however, that due to the soft and fragile nature of the fibrils, the image quality is not excellent and the contrast mechanism unclear, emphasizing the need to use alternative immobilization procedures and imaging modes.

Of particular interest for microbiologists is the ability to study physiological processes such as cell growth and germination. Figure 3 shows two dividing daughter cells, indicating that AFM could be used to follow division processes in real time. Another example is the germination of fungal spores, a process which is important both in natural and biotechnological situations. Figures 5 shows a 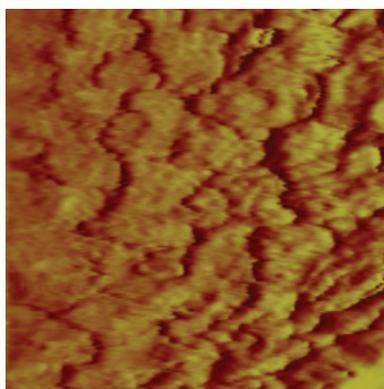deflection image of the surface of *A. oryzae* after a few hours of germination. Clearly, spore germination causes dramatic changes of the surface structure: the crystalline rodlet layer (Figure 4) changes into a layer of soft, deformable material attributed to polysaccharides[6].

Force Measurements

AFM is actually much more than a topographic imaging tool in that it also enables physical properties to be measured. Force-distance curves can be used to probe long-range surface forces (e.g., van der Waals and electrostatic forces) and to map adhesion forces on microbial cell surfaces, thereby providing new insight into the molecular basis of biological events such as cell adhesion. As an example, Figure 6 shows a set of data obtained for the surface of a germinating spore of the fungus *Phanerochaete chrysosporium*[4,5]. The heterogeneous surface morphology (Figure 6a) is directly correlated with differences in adhesion forces as revealed by retraction force curves. While no adhesion forces are sensed between the probe and the granular material (top curve, Figure 6c), strong adhesion forces of 9 ± 2 nN magnitude are measured on the smooth zone (bottom curve, Figure 6d).

Force-volume imaging, which consists in recording arrays (typically, 64 x 64) of force curves in the x, y plane, provides spatially resolved force measurements on biological samples[8]. The power of this approach in microbiology is illustrated in Figure 6.

Figure 5. Probing physiological changes. Deflection image showing the dramatic change of the spore surface morphology upon germination. 2μm x 2μm.
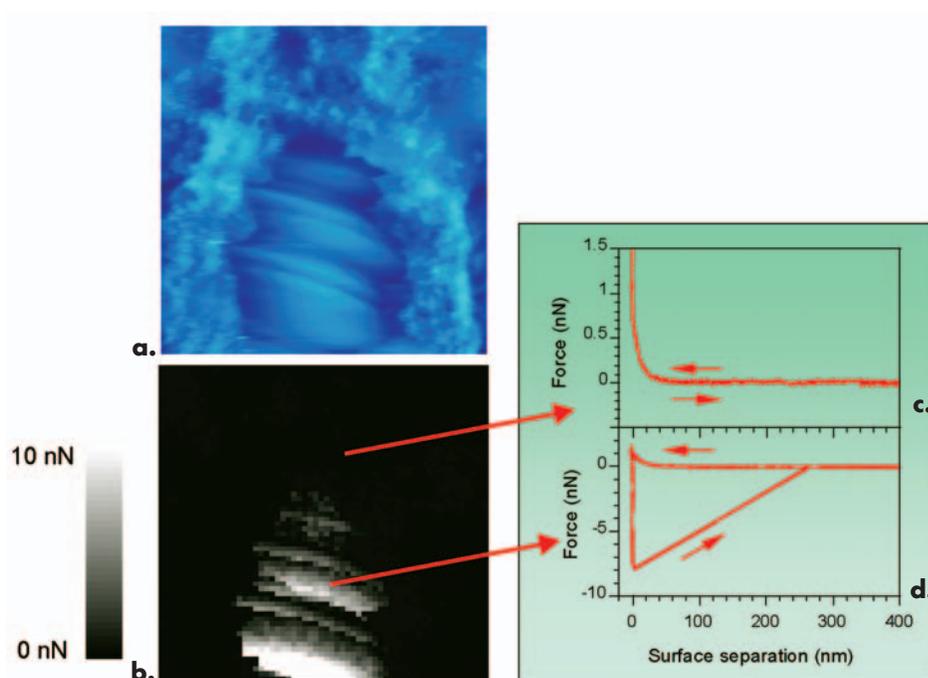

Figure 6. Spatially resolved force measurements using force-volume imaging. Topographic image (a) and adhesion map (b) acquired on the same area of a germinating spore of *P. chrysosporium*. The adhesion map is obtained by recording 64 x 64 force-distance curves, calculating the adhesion force for each force curve and displaying adhesion force values as grey levels. The smoother area in the center of the image shows strong adhesion forces, which are thought to play a central role in spore aggregation. Representative force-distance curves are shown on the right (c). 2μm x 2μm scan.

Differences in adhesion forces across the spore surface yield a highly contrasted adhesion map (Figure 6b), which is correlated with the heterogeneous surface morphology (Figure 6a). The measured adhesion forces may be of great biological significance in that they may be responsible for cell aggregation observed during germination.

In addition to measuring non-specific forces, the AFM probe can be functionalized with various molecules to measure specific interactions. For instance, OH- and CH$_3$-terminated probes were used to quantitatively map the surface hydrophobicity (surface energy) of *P. chrysosporium* spores[9].

With the AFM probe, one can also push locally onto cell wall components and surface appendages to measure their mechanical properties quantitatively. These properties play an important role in controlling events such as cell growth, cell division and

cell adhesion. Force-distance curves, recorded in buffer solution, were used to determine the effective compressibility of the cell wall of the magnetic bacterium *Magnetospirillum gryphiswaldense*[10], and to measure the surface softness of the fibril layer at the surface of the bacterium *Streptococcus salivarius HB*[7].

Finally, AFM can be used to pull on macromolecules to learn about their elastic properties. Single molecule force spectroscopy experiments have provided new insight into the nanomechanical properties of single DNA, protein and polysaccharide molecules[11]. Stretching single molecules directly on living cells remains very challenging due to the complex and dynamic nature of the surface. However, data indicates that this goal could soon be achieved. As seen in Figure 7, retraction force curves recorded on germinating spores of *A. oryzae* showed attractive forces of 400 ± 100pN magnitude, along with characteristic elongation forces

and rupture lengths of up to 500nm. Elongation forces were well-fitted with an extended freely jointed chain (FJC+) model from statistical mechanics, using fitting parameters that were consistent with the stretching of individual dextran polysaccharide molecules. This observation, together with the topographic information (Figure 5) and with biochemical data, suggest that the measured forces may reflect the stretching of long, flexible cell surface polysaccharides.
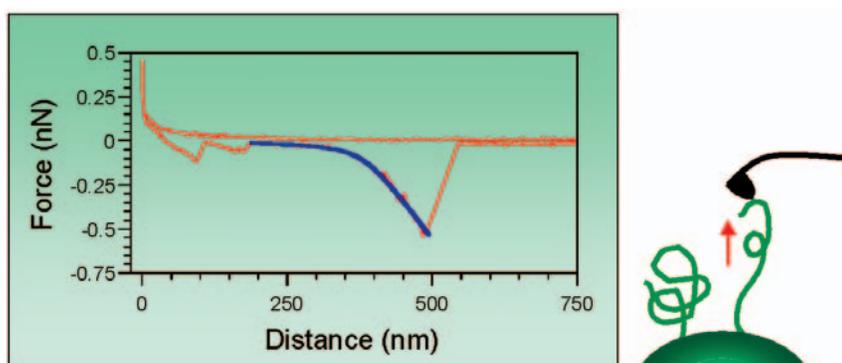

Figure 7. Stretching cell surface macromolecules. Typical force-distance curve recorded on germinating spores of *A. oryzae*. Retraction curves are well-fitted with an extended FJC model (blue line), with parameters consistent with values reported for the elastic deformation of single dextran and amylose polysaccharides.

Summary

AFM provides exciting possibilities for probing the structural and physical properties of living microbial cells. Using topographic imaging, cell surface nanostructures (rodlets, appendages) can be directly visualized and the changes of cell surface morphology occurring during physiological processes (germination, division) can be determined. Force-distance curves provide complementary information on surface forces, adhesion and nanomechanics, yielding new insight into the mechanisms of biological events such as cell adhesion and aggregation. Improvements in sample preparation techniques, instrumentation and recording conditions may bring subnanometer resolution to these living cells for monitoring molecular conformational changes, as is already the case with reconstituted microbial surface layers[12].

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