



# In Vitro Studies of Microtubule Structures Using MAC Mode AFM

## Application Note

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### Introduction

Microtubules are long, hollow, stiff polymers that extend throughout the cell cytoplasm. They are involved in diverse functions that range from governing the location of membrane-bounded organelles to chromosome separation during mitosis. The basic structural unit of a microtubule is tubulin, which is a heterodimer consisting of two closely related and tightly linked globular polypeptides called  $\alpha$ - and  $\beta$ -tubulin. Alternating  $\alpha$ - and  $\beta$ -tubulin subunits form protofilaments, thirteen of which bundle around a central core to form a microtubule. The detailed structures of cytoplasmic microtubules have been studied extensively using various electron microscopy techniques (see Figure 1).

As microtubules are dynamic structures in constant transition between growing and shrinking phases, it would be extremely interesting to investigate the structural organization of the subunit tubulin molecules in a buffer close to physiological conditions. With its high resolution and ability to image in

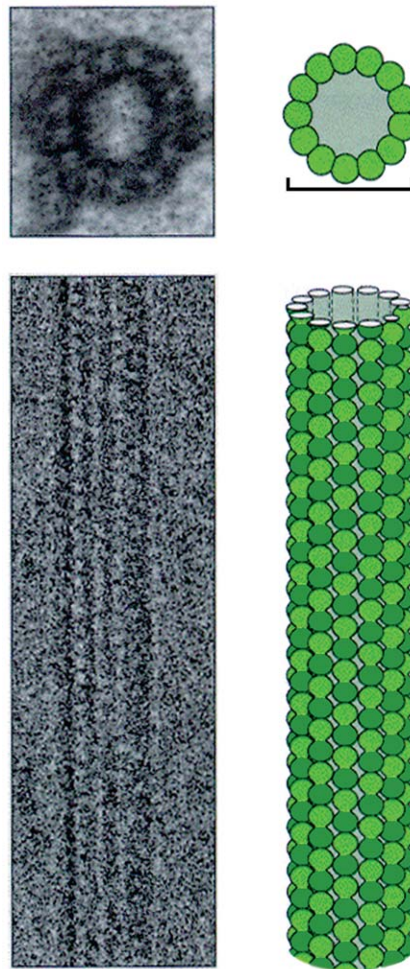


Figure 1. Cytoplasmic microtubules.

fluid, MAC Mode (magnetic AC mode) atomic force microscopy (AFM) makes it possible to study biological structures in their native environments. Previous AFM studies (1, 2) have revealed the surface striations of microtubules in ambient conditions or after glutaraldehyde fixation; however, the individual tubulins were not visible.

### MAC Mode AFM

MAC Mode is a patented AFM imaging technology (3). Similar to other AC techniques, the MAC Mode cantilever oscillates as it scans a sample. The change in oscillation amplitude is monitored to track surface contours. However, instead of driving the cantilever with an external piezo, MAC Mode uses a solenoid to directly oscillate the AFM cantilever, which is coated with a paramagnetic material. Because the cantilever's or AFM tip's oscillation amplitude changes predictably on contact, a MAC Mode AFM tip is gentle on samples and is thus ideal for imaging soft biological structures in fluid. Here we report the use of MAC Mode to study microtubule structures in buffer.



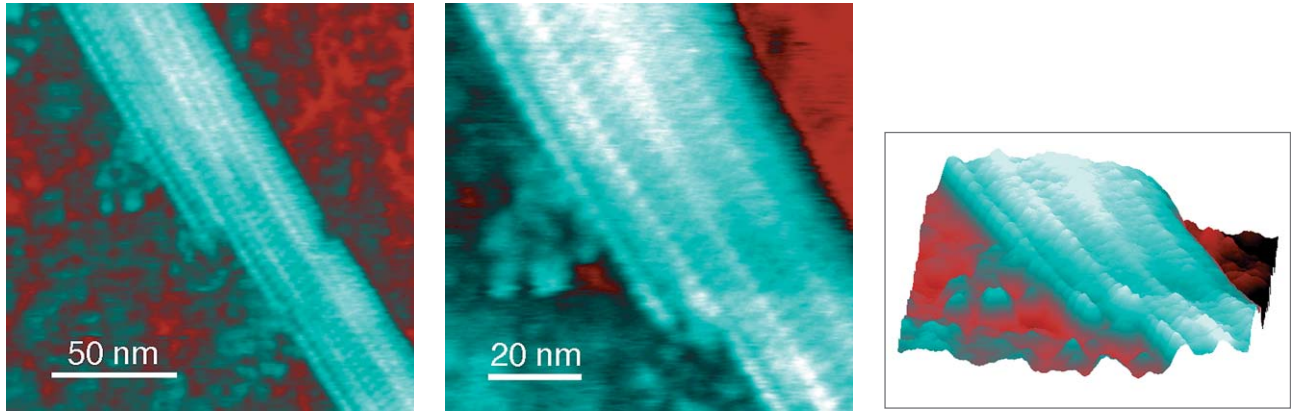


Figure 3. Portion of a microtubule molecule. (a) Scan size = 163 nm. (b) Scan size = 88 nm. (c) 3D view.

### Microtubules

Bovine brain tubulin (15 mM) was mixed with an equal volume of polymerization mix (containing GTP) and incubated at 37 °C for 1 hour. The reaction was terminated with 20 μM taxol. The tubulin concentration in the reaction mixture was measured to be around 15 μM. Since microtubules have a negative surface charge, aminopropyltriethoxy silane (APTES) treated mica (4) was used as a substrate for AFM imaging. 40 μl of the sample was deposited on APTES mica and left stand for 10-15 minutes. The surface was then rinsed with the same buffer and imaged immediately in 500 μl of the buffer.

Figure 3 shows part of a microtubule molecule at (a) 163-nm and (b) 88-nm scan size, respectively. Individual tubulin subunits,  $\alpha$  and  $\beta$ , can be clearly identified in Figure 3(b). Twenty microtubule subunits spanned a horizontal distance of 80 nm; therefore, the size of each repeating unit was 4 nm, which is in good agreement with previous electron microscopy results.

Tubulins of one protofilament seemed to interconnect with tubulins of neighboring protofilaments. Figure 3(c) provides a 3D view of the area, showing protofilaments as ridges separated by 4-nm-wide valleys. In a different area (Figure 4, scan size = 147 nm), before the microtubules seemed to form bundles.

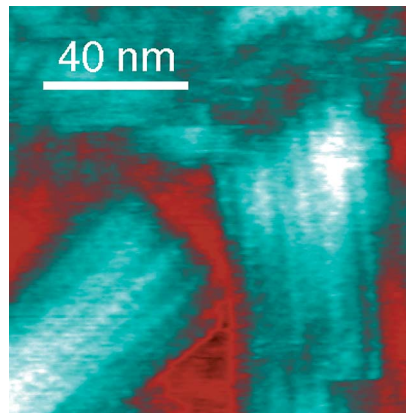


Figure 4. Portion of a microtubule molecule. Scan size = 147 nm.

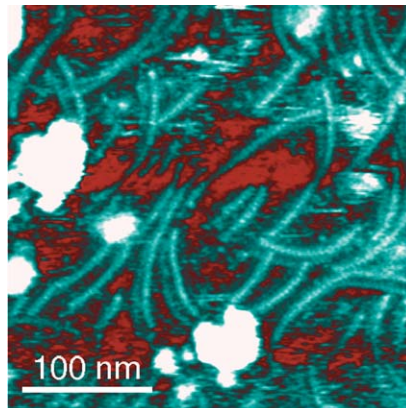


Figure 5. Portion of a microtubule molecule. Scan size = 315 nm.

### Protofilaments

When the polymerized tubulin mixture was imaged at a relatively high concentration (1- to 100-fold dilution, instead of 1000-fold dilution), only short protofilaments could be seen (Figure 5, scan size = 315 nm). The protofilaments measured 30-100 nm in length and the repeating tubulin subunits were once again identified in most molecules.

The fact that MAC Mode AFM results were dependent on the dilution factor of the sample seemed puzzling. Preliminary efforts to separate microtubules and protofilaments by ultracentrifugation in the presence of a sucrose gradient failed to do so. Therefore, it is suggested that (i) microtubules and protofilaments may coexist under the conditions used in this study and (ii) smaller protofilaments with higher surface diffusion rates may compete strongly with microtubules to bind with the substrate.

### Microtubule Severing by Katanin

Katanin is a microtubule-severing proteins that catalyzes the disassembly of microtubules. Katanin-regulated microtubule severing is speculated to be part of the microtubule cytoskeleton reorganization *in vivo*. The mechanism of severing is still under investigation, although it is known that the process utilizes energy from ATP hydrolysis.

Figure 6 (scan size = 1  $\mu\text{m}$ ) is a MAC Mode AFM image of the p60 subunit, which is the active katanin subunit purified from sea urchin eggs. The image was taken in BRB12 buffer on mica. Ring-like structures with diameters of 15-20 nm can be seen, in agreement with a previous electron microscopy study.

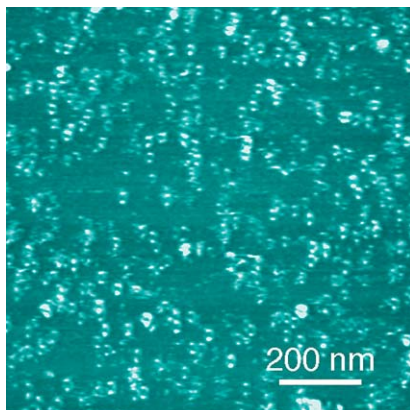


Figure 6. The p60 subunit. Scan size = 1  $\mu\text{m}$ .

When microtubules were mixed with katanin prior to AFM imaging, the structures disassembled quickly on the surface of APTES mica. Figure 7 (scan size = 153 nm) shows two images taken 5 minutes apart, with the second image appearing much more disrupted than the first. Although the AFM tip can contribute partly to the observed effect even when the oscillation amplitude is minimal, katanin is likely to be the main factor in inducing the disassembly of the microtubules. In fact, when no katanin was present in the mixture, as in Figure 3, the microtubule could be scanned repeatedly without damaging the structure.

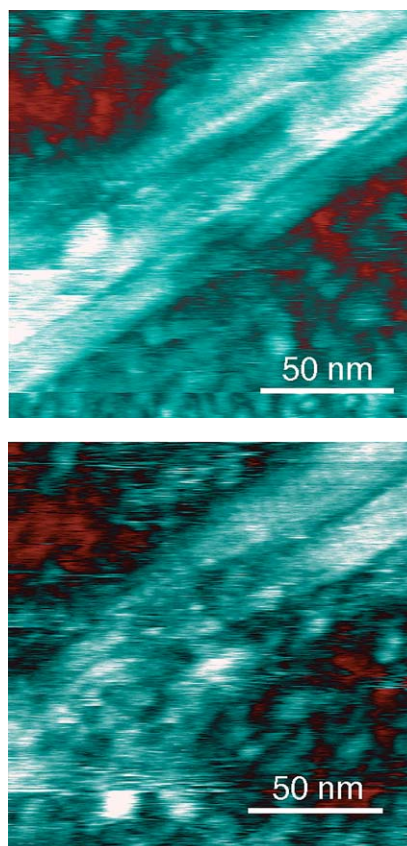


Figure 7. (7A & 7B) Katanin-induced disassembly of microtubules. Scan size = 153 nm.

### Conclusions

We were able to clearly identify the tubulin subunits in AFM images of microtubules in buffer. The high resolution of the images presented here show MAC Mode to be a powerful tool in studying biological structures *in vitro*. With such resolution, it is feasible to design many future experiments involving microtubule and protofilament systems. When combined with the precision environmental and temperature control provided by complementary Agilent AFM products, MAC Mode can create time-lapse images of biological samples in physiologically relevant conditions.

### References

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