**STAND-ALONE ATOMIC FORCE MICROSCOPE FEATURING LARGE SCAN,
FRICTION MEASUREMENT, ATOMIC RESOLUTION AND CAPABILITY OF LIQUID OPERATION.**

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ABSTRACT.

We have developed a stand-alone atomic force microscope featuring large scan, friction measurement, atomic resolution and capability of in liquid operation. Cantilever displacements are detected with optical beam deflection. Cantilever and laser diode are both attached at the piezo tube and thus scanned simultaneously. As a direct consequence the maximum scan range, $25 \times 25 \mu\text{m}^2$, is solely determined by the characteristics of the piezo tube and not by the dimensions of the cantilever and/or the waist of the laser beam. The stand-alone atomic force microscope is suitable to be combined with any inverted optical microscope (including the confocal laser scanning microscope), as is illustrated with fluorescence and height images of K562-cells. Results on thin films consisting of a mixture of polymers show the strength of measuring friction and height simultaneously. Images of mica show that atomic resolution can be obtained both in height and friction mode.

1. INTRODUCTION.

One of the drawbacks of the standard layout of the atomic force microscopes (AFM) most widely used is the fact that the samples to be investigated are restricted in size, because the sample is being scanned with the piezo tube. One is not able to investigate the surface of large objects e.g. mirrors, without cutting the sample. The same goes for standard microscope slides ($75 \times 25 \text{ mm}^2$) used in biology. In the set-up in our laboratory featuring an integrated optical microscope¹ it is possible to use standard microscope slides which are attached by a small vacuum chamber on the piezo tube². This demands samples or sample supports which have a reasonably flat bottom surface. These problems related to sample properties can be solved by designing a stand-alone AFM in which the tip and not the sample is scanned.

A further reason to come to a new design lies in the fact that in our existing design the integrated optical microscope has only a numerical aperture of 0.25¹, barely sufficient to perform fluorescence microscopy. In our view there were two options to increase the quality of our optical system: 1) improve the integrated optical microscope or 2) design an AFM which can be combined with a commercially available optical microscope. To improve the optical quality of the integrated optical microscope, objectives with higher numerical apertures have to be used. The diameter of such an objective, however, is significantly larger than the 8 mm of the lens currently positioned inside the hollow piezo tube; standard microscope objectives have a diameter around 25 mm. To accommodate the larger objectives, the inner diameter of the piezo tube has to be increased³. As a direct result the scan range of the piezo tube decreases dramatically³. We therefore chose to follow the second route and design an AFM which can be combined with existing inverted optical microscopes.

Several groups have designed stand-alone AFMs: Sarid et al.⁴, Hipp et al.⁵ and Putman et al.⁶. These designs all have their specific drawbacks. Because the design by Sarid uses laser diode interferometry to sense the cantilever deflections, friction cannot be monitored in real time. Furthermore, it is hard to operate this stand-alone AFM under liquid. The designs by Hipp⁵ and Putman⁶ use optical beam deflection for force detection and therefore are in principle capable of measuring friction and height simultaneously⁷. Operation under liquid is also possible, as is shown by Putman imaging Langmuir Blodgett films under liquid⁶. The stand-alone AFM of Putman et al. has also been combined with a fluorescence microscope. However, the designs by Hipp et al. and Putman et al. use laser beams which have a fixed position. The cantilever is then scanned in the broad waist of the laser beam. As a direct result the scan range is limited, because exertions of the cantilever beyond a certain point from the center of the laser beam give large variations in optical power incident on the detector and consequently the feedback loop will introduce large variations in the loading force. The scan range in both setups is about $5 \times 5 \mu\text{m}^2$.

Here we present a design of a stand-alone atomic force microscope which is capable of measuring friction and height simultaneously. It can be combined with an inverted optical microscope with high numerical aperture objectives (with oil-immersion objectives numerical apertures of 1.25 and higher are attainable) and operated under liquid, which makes it ideally suitable for biological applications. The scan range, $25 \times 25 \mu\text{m}^2$, is determined by the characteristics of the piezo tube only.

2. DESIGN OF THE STAND-ALONE ATOMIC FORCE MICROSCOPE.

In figure 1 a schematic drawing of the stand-alone AFM is shown. In figure 1a the overall layout is given; in figure 1b a close-up of the piezo tube-laser diode-cantilever assembly (piezo tube purchased from Staveley Sensors Inc., Hartford CT, USA; cantilevers with integrated pyramidal tips from Park Scientific Instruments, Sunnyvale, USA; laser diode was a kind gift from Philips, Netherlands). The frame of the AFM is formed by a brass box fixed to a base plate. The brass box contains the piezo tube-laser diode-cantilever assembly, the pre-amplifier electronics and an adjustable beam steering mirror. The base plate supports the quadrant detector and three micrometer screws. The two coarse approach micrometer screws are positioned in hinges (#7 in figure 1a) to get the cantilever in the field of view of the optical microscope or to select a new scan area. The tripod construction gives a geometrical reduction factor of 15, which allows for a gentle manual fine approach.

The laser diode and the focusing lens are positioned on an adjustable plate as shown in figure 1b. The laser beam can be manipulated at the cantilever by adjusting two screws with small knobs on top of the AFM (#1 in figure 1a). The connection between the knobs and the screws formed by flexible rods (diameter 2 mm) has no influence on the performance of the AFM, not even on an atomic scale. The laser diode and cantilever are rigidly coupled; the laser beam stays on the cantilever while scanning and consequently the maximum scan range is solely determined by the characteristics of the piezo tube. The maximum scan range of the piezo tube used is $25 \times 25 \mu\text{m}^2$ at a driving voltage of around 200 V. The laser beam reflected off the backside of the cantilever, held down by a steel wire, goes via an adjustable beam steering mirror to the quadrant detector. The scan direction is perpendicular to the length axis of the cantilever. This allows us to monitor the height and friction simultaneously, as was first shown by Marti et al.⁸ using microfabricated cantilevers.

Figure 1. A schematic diagram of the stand-alone AFM. a) Overall layout: 1) adjustment knobs for manipulation of the laser beam, 2) pre-amplifier electronics, 3) beam-steering mirror, 4) adjustment knobs for the beam-steering mirror, 5) fine approach, 6) adjustment knobs to change the position of the cantilever, 7) coarse approach, 8) cantilever holder, 9) piezo tube, 10) quadrant detector. b) Close-up of the piezo tube-laser diode-cantilever holder assembly: 11) laser diode, 12) focusing lens, 13) adjustable plate, 14) cantilever.

The stand-alone AFM can be combined with any commercially available inverted optical microscope if there is sufficient space for the AFM (length x width x height: $110 \times 80 \times 80 \text{ mm}^3$). A small adaption of the object stage, a V-groove and a hole at the positions of the coarse approach micrometer screws, suffices. By attaching a small piece of plexiglass to the cantilever holder serving as an optical window for the laser beam, the AFM can also be operated under liquid.

From the layout of the AFM (figure 1) it is clear that a displacement of the cantilever in the scanning plane gives about the same displacement of the laser beam at the detector. These displacements give artificial friction and height signals. This behavior has several consequences for the operation of the AFM. First, over a scan of $20 \text{ }\mu\text{m}$ a pseudo height of 15 or 30 nm (depending whether $100 \text{ }\mu\text{m}$ or $200 \text{ }\mu\text{m}$ cantilevers are used) will be included in the height information. This corresponds with artificial tilt angles of less than 0.1° . In practical imaging the induced tilt angle is negligible to the real non-parallelism between sample surface and scanning plane. Second, more important than the extra tilt are the variations in applied force -ranging from 1 to 5 nN- which will be introduced because the feedback loop reacts to the pseudo height. These effects can be minimized by electronically adding a signal to the height signal to cancel the pseudo-height contribution.

3. RESULTS AND DISCUSSION.

As already indicated in the previous section, the stand-alone AFM can be combined with an inverted optical microscope. In a preliminary study we have studied

dried cells and conjugates of cells with a set-up combining the stand-alone AFM and a confocal laser scanning microscope (CLSM)⁹. The CLSM is equipped with an inverted fluorescence microscope (Leica Fluovert FU) and an air-cooled argon-krypton laser used for excitation. In figure 2a a fluorescence image recorded with the CLSM of a K562-cell (predecessor of erythroblasts) stained with an F-actin-specific probe is shown. An edge of this cell is also imaged with AFM (figure 2b shows only the height image). The holes in the cell membrane are most probably due to drying. Recording both images gives a more complete view of the cell under investigation: the CLSM gives information on the internal and membrane structure and the AFM gives high-resolution information on the cell membrane. Imaging dried cells has a negative influence on the quality of the CLSM images. Experiments on living cells are currently performed and the results will be presented at a later stage.

Figure 2. Images of a K562-cell stained with BODIPY 581/591 Phalloidin with the (a) CLSM and (b) AFM. Image areas: (a) $40 \times 40 \mu\text{m}^2$, (b) $24 \times 24 \mu\text{m}^2$.

Thin films of optically active polymers, used for efficient second harmonic generation^{10,11}, were investigated with the stand-alone AFM. These thin films consist of a mixture of an optically active polymer and polymethylmethacrylate (PMMA). In figure 3 the height (*top*) and the friction (*bottom*) images, back and forth scans, are shown. In the height images domains with an average diameter of about 150 nm are visible. They protrude 1 to 2 nm out of the surface. Probably these domains consist of the optically active polymer because the ratio of mixture was 1:3 (optically active polymer/PMMA). The height images, however, do not give conclusive evidence whether the chemical composition of the protruding domains differs from the surrounding material. Overney et al. recently showed that friction measurements are extremely suitable to observe phase separation in thin films¹². The friction images in figure 3 (*bottom*) show a reversal of contrast between back and forth scans. This conclusively demonstrates that the chemical composition of the domains differs from the surrounding film. Thus it is possible to obtain friction information on a

submicrometer scale.

Figure 3. Images of polymer films recorded with the stand-alone AFM. *Top*: height, back and forth, *bottom*: friction, back and forth. Image area: $1.5 \times 1.5 \mu\text{m}^2$.

Figure 4. Images of mica recorded with the AFM. (a) height, (b) friction. Image area: $6 \times 6 \text{ nm}^2$.

Both in height and friction atomic resolution images can be obtained (figure 4). The hexagonal packing of the molecules in mica is clearly visible. Changing the loading force resulted frequently in large variations in contrast, both in height and in friction. In our opinion this indicates that at the apex of the 30 to 50 nm

pyramidal tip small protruding nanotips exist. Changing the loading force changes the interaction site between tip and surface. Furthermore, sometimes the difference in contrast between back and forth scans is very large, again indicating the presence of nanotips. This implies that the interaction is localized on a subnanometer scale¹³ and that the contrast is not due to an averaging effect of contributing interactions between the large pyramidal tip and the surface.

4. CONCLUSIONS.

The stand-alone AFM as presented is ideally suitable to be combined with an inverted optical microscope. Measuring height and friction simultaneously allows one to discriminate objects not only on basis of topography but also on the basis of the chemical composition. Atomic resolution can be obtained both in height and friction. Operation under liquid is also possible (not shown here).

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