# Scanning force microscopy jumping and tapping modes in liquids

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In this work theoretical considerations of the performance of scanning force microscopy jumping mode and tapping mode in liquids are discussed. *A priori*, jumping mode should improve in a liquid environment compared to in air while the situation for tapping mode should become worse. In order to confirm this we present jumping and tapping mode images of DNA molecules absorbed on a mica substrate immersed in water. The experiments demonstrate that jumping mode is a suitable scanning force microscopy method by which to image soft samples in liquid and that it has similar or even better performance than those exhibited by tapping, but without the complex experimental requirements of this mode. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1509856]

One of the most outstanding features of Scanning Force microscopy (SFM)[1] is its capability to image surfaces in different environments with nanometer resolution. During the last 15 years SFM has been used to study solid–vacuum,[2] solid–gas (typically air ambient)[1] and solid–liquid interfaces.[3] While the first two are important in different fields like surface science,[4] magnetic technologies,[5] materials science,[6] etc., the third is particularly relevant since SFM can be used as a technique by which to resolve biological structures at the molecular level.[7] Ohnesorge and Binnig[8] studied the possibilities of SFM in a liquid environment and obtained true atomic resolution images of a calcite sample immersed in water. As the authors discussed in their work true atomic resolution is only possible due to the small tip–sample interaction present in liquids (forces as small as 10 pN are reported in their work); for example, van der Waals forces are screened roughly by a factor of 10 under water and adhesion force is almost negligible. As a consequence force versus distance plots in general exhibit a smooth continuous trace without the typical jump to contact and jumpoff present in air ambient conditions. However, in spite of the small normal force exerted by the tip (10 pN), the presence of shear forces produced by scanning motion causes irreversible damage in soft materials and therefore static contact mode cannot be used to image delicate biological samples in liquids. The obvious solution was to use dynamic SFM (DSFM) commonly known as tapping mode (TM).[9–11] While in static contact mode the deflection of the cantilever is directly used as the feedback signal, in DSFM the tip is oscillated at its resonance frequency and the reduction of the oscillation amplitude, phase change or frequency shift is used as the feedback signal. In DSFM, contact as well as noncontact operation is possible. In solid–vacuum interfaces, noncontact DSFM has shown atomically resolved images comparable to those obtained with scanning tunneling microscopy.[2,4,12,13] In gas environments the relatively high $Q$ factor of the system ($\sim 100$) significantly improves its sensitivity and therefore DSFM can be used as a noncontact technique suitable for measuring soft samples. When DSFM is used in liquids, the high viscosity of the medium dramatically reduces the $Q$ factor, ($\sim 10$) producing a parallel reduction in the sensitivity of the method; this problem can be partially solved by electronically modifying the $Q$ of the system.[14] Besides, the resonance frequency of the cantilever is also reduced significantly since the effective mass of the cantilever increases due to drag of the surrounding liquid. These effects lead to slower scan rates. Finally, noncontact operation in liquids is more difficult than in air ambient due to weak van der Waals interaction. Therefore, the theoretical performance of DSFM in liquids is reduced compared to operation in air ambient. In addition to drawbacks in its basic nature, DSFM in liquids is a technique much more difficult to implement than in air. First of all, cantilever oscillation driven by a small piezoelectric attached to the cantilever chip produces a frequency spectrum with many spurious high amplitude resonances due to excitation of the liquid cell. This problem can be avoided by using a magnetic field to drive the cantilever,[15] but then the cantilevers have to be coated with a magnetic material, which could produce contamination problems in some cases. Also, if the cantilever is very small the force applied by the magnetic field is too weak and this method cannot be used. Finally, magnetically covered cantilevers are expensive and difficult to purchase. In the present work we would like to introduce jumping mode (JM) as a suitable technique with which to image soft samples in liquids but without the technical problems related to tapping mode.

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TABLE I. Summary of the relevant parameters of dynamic scanning force microscopy and jumping mode.

| | DSFM | | JM | |
|---|---|---|---|---|
| | Liquid | Air | Liquid | Air |
| Force constant (N/m) | 0.75 N/m | 0.75 N/m | 0.06 N/m | 0.75 N/m |
| Resonance frequency (kHz) | 25 kHz | 80 kHz | Does not apply | Does not apply |
| *Q* | 10 | 100 | Does not apply | Does not apply |
| Amplitude (nm) | 10 nm | 10 nm | 10 nm | 100 nm |
| Contact | Probably yes | Depending on conditions[a] | Yes | Yes |
| Contact time (ms) | Not clear | Depending on conditions[a] | 0.5 ms | 1.3 ms |
| Force applied (nN) | Not clear | Depending on conditions[a] | 0.1 nN | 5 nN |
| Lateral displacement out of contact | Not clear | Not clear | Yes | Yes |
| Time/point (ms) | 2 ms | 2 ms | 3 ms | 6 ms |
| Taps/point | 50 | 150 | 1 | 1 |
| Image time (s) | 120 s | 120 s | 180 s | $>360$ s |
| Additional experimental setup[c] | Yes[b] | Yes | No | No |

[a]Best images obtained without contact.
[b]More sophisticated than in air.
[c]With respect to contact mode.

JM,[16] which in its working principle is very similar to pulsed force microscopy[17] (PFM), was originally developed as a scanning mode to minimize shear forces. The difference between PFM and JM is that PFM is implemented electronically whereas JM is just a software method running in digital signal processor memory. However, the presence of high adhesion forces in air ambient conditions ($\sim 5-100$ nN), mainly caused by van der Waals and capillary forces may produce irreversible damage of soft samples and hence while it is less intrusive than contact mode it is more intrusive than DSFM.[18] JM mode operation can be described as a cycle repeated at each image point with the following steps: (i) tip–sample separation, (ii) lateral tip motion at the furthest tip–sample distance, (iii) tip–sample approach, and (iv) feedback, which is generally performed on the cantilever deflection. From this cycle one of the most relevant features of JM is that lateral motion always occurs when the tip is not in contact with the sample in order to avoid shear forces. Steps (i) and (iii) determine the scanning speed. In ambient conditions large *z* displacements ($>200$ nm) are needed to withdraw the tip and sample due to high adhesion force. Therefore the tip–sample separation and approach step take a relatively long time and require a rather low scanning speed. For all these reasons DSFM is the best choice to image soft samples in air ambient. As pointed out before, in liquids attractive and adhesion forces are very weak, hence small tip excursions are enough to separate the tip and sample, allowing one to use a much faster scanning speed. In fact, the tip excursion used for JM is similar to the oscillation amplitude applied in tapping mode (of the order of 10 nm). Moreover, since the force versus distance plots are continuous and smooth it is possible to work using extremely low loading forces by selecting low force constant cantilevers. Operation in liquids therefore improves for JM and becomes less favorable for DSFM compared with the operation in air. Table I summarizes several relevant parameters of both scanning modes. An important feature of JM that we would like to stress here is that the hardware requirements for this mode are the same as for regular contact mode and thus, it is much easier to implement than DSFM.

In light of this, we have carried out experiments to compare both modes, JM and DSFM. The experimental setup includes a commercial SFM from Nanotec Electronica™ with a liquid cell. For DSFM a force modulation unit and a homemade coil are used. The coil, attached to the microscope head, drives the cantilever oscillation. DSFM experiments were carried out with Olympus type cantilevers with a nominal force constant of 0.75 N/m. In order to respond to the magnetic field these cantilevers were covered with cobalt. JM experiments were carried out with both Olympus type (0.05 N/m) and Nanosensors cantilevers (0.06 N/m). The system was controlled with WS×M; this software al-

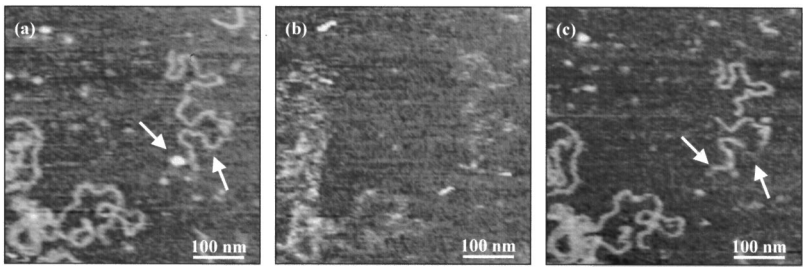


FIG. 1. Sequence of SFM images of DNA molecules in water imaged using jumping mode (JM) (a), contact mode (CM) (b) and jumping mode (c) scanned on the same area. JM images are reproducible and good quality images whereas CM ones present poor quality and distortion of the molecules. This effect is clear when the same area is scanned again in JM (shown by arrows).

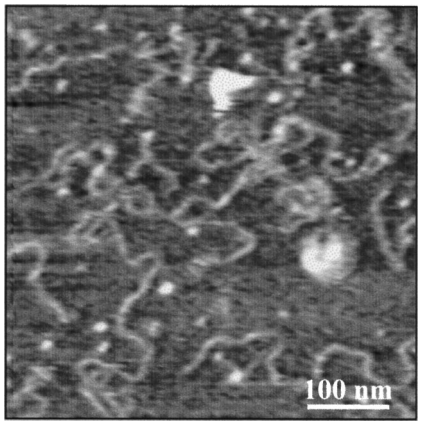


FIG. 2. SFM image of DNA molecules in water imaged using tapping mode (TM). Although JM and TM images are of comparable quality, the height of the DNA molecules is lower when imaged using TM than using JM, suggesting that JM is a less intrusive technique than TM.

lows one to perform images in both jumping and DSFM scanning modes.

In order to test the performance of these two scanning modes we have chosen DNA adsorbed on a mica substrate, which can be considered standard soft biological material. The sample preparation is as follows: mica substrates were pretreated with 3-aminopropyltriethoxysilane (APTES) by immersing them in a 0.1 Vol % of APTES for 15 min. Then, they were rinsed with 2-propanol and ultrapure water and dried with nitrogen. Substrates prepared in this way are positively charged. A drop of DNA solution is placed on the treated mica and allowed to bind for 1 h. Then the sample is again rinsed with water and never allowed to dry.

Figure 1(a) shows a $256\times256$ point topographical image taken in JM of λ-DNA molecules adsorbed on mica in a water environment. The acquisition time for the image was about 3 min, and the typical jumping conditions are given in Table I. From the images a DNA molecule height of $1.4\pm0.3$ nm is obtained. Figure 1(b) shows a subsequent image of the same region but now in contact mode with the same force set point. Figure 1(c) was again taken in JM in the same region. Two clear features can be seen in the contact image: first, the quality of the image is poor and, second, the sample is modified due to the shear forces (shown by arrows). Consecutive JM images of this region show good repeatability with no further modification. Figure 2 is a $256\times256$ point DSFM image of the same sample but in a different region (note that we use cantilevers with different force constants for DSFM and jumping modes), and the acquisition time for this image was about 3 min, the same as in JM. The height of the molecules is $1.1\pm0.3$ nm, a little lower than in the case of JM. The two values agree within experimental error and with the height typically reported by other authors using DSFM.[19,20] The slightly higher mean value measured in JM might suggest that JM is less intrusive that DSFM.

We have obtained JM and tapping mode images in liquid on microtubule samples, with similar findings. This molecule has a nominal height of 25 nm and JM images give this height value. Again DSFM yields a significantly smaller value.[21]

In summary, both theoretical considerations as well as experimental images support that JM is a suitable technique by which to image soft samples in liquids, with the results comparable to those obtained with DSFM. Several clear advantages of JM versus DSFM should be noted: The maximum normal force in jumping mode is known, lateral motion is always performed out of contact and, finally, from a technical point of view, JM is much easier to implement than DSFM.

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[1] G. Binnig, C. F. Quate, and C. Gerber, Phys. Rev. Lett. **56**, 930 (1986).
[2] F. J. Giessibl, Science **267**, 68 (1995).
[3] O. Marti, B. Drake, and P. K. Hansma, Appl. Phys. Lett. **51**, 484 (1987).
[4] S. Kitamura and M. Iwatsuki, Jpn. Appl. Phys., Part 2 **34**, L145 (1995).
[5] J. J. Sáénz, N. García, P. Grütter, E. Meyer, H. Heinzelmann, R. Wiesendanger, L. Rosenthaler, H. R. Hidber, and H. J. Güntherodt, J. Appl. Phys. **62**, 4293 (1987).
[6] P. J. de Pablo, C. Gómez-Navarro, J. Colchero, P. A. Serena, J. Gómez-Herrero, and A. M. Baró, Phys. Rev. Lett. **88**, 036804 (2002).
[7] C. Bustamante, C. Rivetti, and D. J. Keller, Curr. Opin. Struct. Biol. **7**, 709 (1997).
[8] F. Onhesorge and G. Binnig, Science **260**, 1451 (1993).
[9] A. J. Putman, K. O. van der Werf, B. de Grooth, N. F. van Hulst, and J. Greve, Appl. Phys. Lett. **72**, 1911 (1994).
[10] P. K. Hansma, J. P. Cleveland, M. Radmacher, D. A. Walters, P. E. Hillner, M. Bezanilla, M. Fritz, D. Vie, H. G. Hansma, C. B. Prater, J. Massie, L. Fukunaga, J. Gurley, and V. Elings, Appl. Phys. Lett. **64**, 1738 (1994).
[11] In this context, we would like to clarify that we use the term TM for contact DSFM. DSFM is therefore more general than TM, since it includes contact as well as noncontact modes.
[12] H. Veyana, M. Ohta, Y. Sugawara, and S. Morita, Jpn. J. Appl. Phys., Part 2 **34**, L1086 (1995).
[13] Y. Sugawara, M. Ohta, H. Veyana, and S. Morita, Science **210**, 1646 (1995).
[14] J. Tamayo, A. D. Humphris, R. J. Owen, and M. J. Miles, Biophys. J. **81**, 526 (2001).
[15] W. Han and S. M. Lindsay, Appl. Phys. Lett. **69**, 4111 (1996).
[16] P. J. de Pablo, J. Colchero, J. Gómez-Herrero, and A. M. Baró, Appl. Phys. Lett. **73**, 3300 (1998).
[17] A. Rosa-Zeise, E. Weilandt, S. Hild, and O. Marti, Meas. Sci. Technol. **8**, 1333 (1997).
[18] F. Moreno-Herrero, P. J. de Pablo, J. Colchero, J. Gómez-Herrero, and A. M. Baró, Surf. Sci. **453**, 152 (2000).
[19] N. H. Thomson, S. Kasas, B. Smith, H. G. Hansma, and P. K. Hansma, Langmuir **12**, 5905 (1996).
[20] F. Moreno-Herrero, J. Colchero, and A. M. Baró (unpublished).
[21] C. M. Kacher, I. M. Weiss, R. J. Stewart, C. F. Schmidt, P. K. Hansma, M. Radmacher, and M. Fritz, Eur. Biophys. J. **28**, 611 (2000).