Scanning Force Microscopy Jumping and Tapping modes in liquids

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In this work theoretical considerations on 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 as compared to air while the situation for Tapping mode should become worse. In order to confirm this statement 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 to image soft samples under liquids having similar or even better performances than those exhibited by Tapping, but without the complex experimental requirements of this mode.

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 one is particularly relevant since SFM can be used as an unique technique to resolve biological structures at the molecular level[7]. Ohnesorge and Binnig[8] studied the possibilities of SFM in liquid environment and obtained true atomic resolution images of a calcite sample immersed in water. As the authors discussed in this work true atomic resolution is only possible due to the small tip-sample interaction (forces as small as 10 pN are reported in this work) present in liquids, for example; van der Waals forces are screened roughly by a factor of ten under water and the adhesion force is almost negligible. As a consequence force vs. distance plots in general exhibit a smooth and continuous trace without the typical jump to contact and jump-off 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 the scanning motion causes irreversible damage in soft materials and therefore static contact mode can not be used to image delicate biological samples in liquids. The obvious solution was to use Dynamic Scanning Force Microscopy (DSFM) commonly known as Tapping Mode (TM)[9, 10, 11].

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	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
Applied force (pN)	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 set-up[c)]	Yes[b)]	Yes	No	No

[a)] Best images obtained without contact [b)] More sophisticated than in air [c)] Respect to contact mode

Table 1. Summary of the relevant parameters of Dynamic Scanning Force Microscopy and Jumping Mode.

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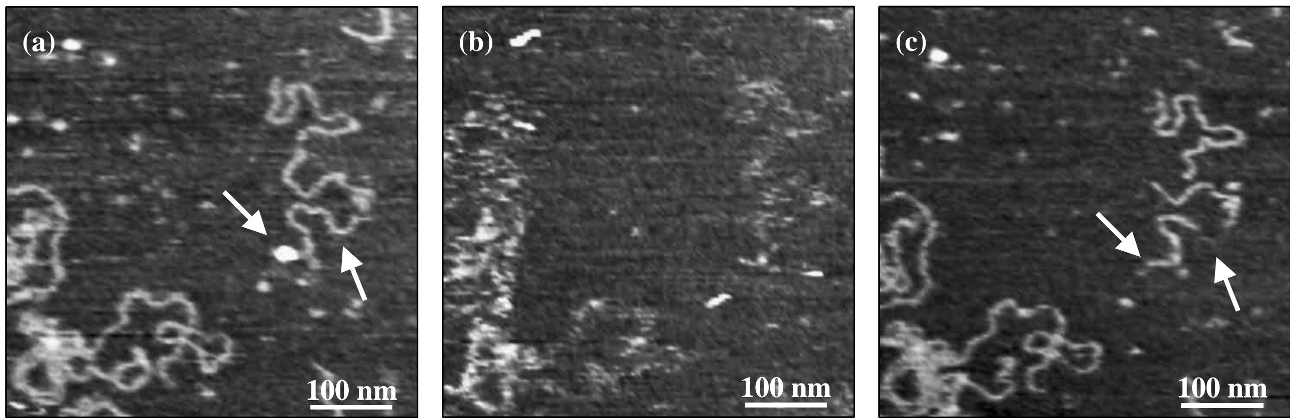

Figure 1. Sequence of SFM images of DNA molecules under water imaged using Jumping Mode (JM) (A), Contact Mode (CM) (B) and Jumping Mode (C) scanned on the same area. Since JM images are reproducible and good quality images CM ones present a poor quality and distortion of the molecules. This effect is clear when the same area is scanned again in JM (see arrows).

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 are used as the feedback signal. In DSFM contact as well as non-contact operation is possible. In solid-vacuum interfaces, non-contact DSFM has shown atomically resolved images comparable to those obtained with Scanning Tunnelling Microscopy[2 4 12 13]. In gas environments the relatively high Q-factor of the system (?100) significantly improves its sensitivity and therefore DSFM can be used as a non-contact technique suitable to measure soft samples. When DSFM is used in liquids, the high viscosity of the medium dramatically reduces the Q factor, (?10) producing a parallel reduction on the sensitivity of the method; this problem can be partially solved by modifying electronically 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 the drag of the surrounding liquid. This effects leads to slower scan rates. Finally, non-contact operation under liquids is more difficult than in air ambient due to the weak van der Waals interaction. Therefore, the theoretical performance of DSFM in liquids is reduced as compared to operation in air ambient. In addition to drawbacks of 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 the excitation of the liquid cell. This problem can be avoided by using a magnetic field to drive the cantilever[15], but then cantilevers have to be coated with a magnetic material producing 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 can not be used. Finally, magnetically covered cantilevers are expensive and difficult to purchase. In the present work we would like to introduce the Jumping Mode (JM) as a suitable technique to image soft samples in liquids but without the technical problems related to Tapping 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 the digital signal processor memory. However, the presence of high adhesion forces in air ambient conditions (?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, 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 occurs always 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

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conditions large z displacements (>200 nm), are needed to withdraw tip and sample due to the high adhesion force. Therefore the tip-sample separation and approach step take a relatively long time requiring 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 the attractive and adhesion forces are very weak, hence small tip excursions are enough to separate tip and sample allowing 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 vs. distance plots are continuous and smooth it is possible to work using extremely low loading forces by selecting low force constant cantilevers. The operation in liquids therefore improves for JM and becomes less favorable for DSFM as compared with the operation in air. Table 1 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 being much easier to implement than DSFM. In fact, JM is just a software method in the digital signal processor that controls the microscope.

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In light of this, we have carried out experiments to compare both modes, JM and DSFM. The experimental set up includes a commercial SFM from Nanotec Electronica™ with a liquid cell. For DSFM a force modulation unit and a homemade coil is used. The coil, attached to the microscope head, drives the cantilever oscillation. DSFM experiments were carried out with Olympus type cantilever 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 WSxM; this software allows 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 as a 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% volume of APTES for 15 min. Then, rinsed with 2-propanol and ultrapure water and dried with nitrogen. The substrates prepared this way are positively charged. A drop with the DNA solution is placed on the treated mica and allowed to bind for an hour. Then the sample is again rinsed with water and dried with nitrogen.

Figure 1a shows a 256x256 points topographical image taken in JM of ?-DNA molecules adsorbed on mica in a water environment. The acquisition time of the image was about 3 minutes, the typical jumping conditions are given in Table I. From the images a height of the DNA molecules of 1.4?0.3 nm is obtained. Figure 1b shows a subsequent image of the same region but now in contact mode with the same force set point. Finally, figure 1c was taken again in JM image on 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 (see arrows). Consecutive JM images of this region show a good repetitively with any further modification. Figure 2 is a 256x256 points DSFM image of the same sample but in a different region (note that we use cantilevers with different force constant for DSFM and Jumping modes), the acquisition time of this image was about 3 minutes, as in JM. The height of the molecules is 1.1?0.3 nm, a little lower than in the case of JM. The two values agree within the 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.

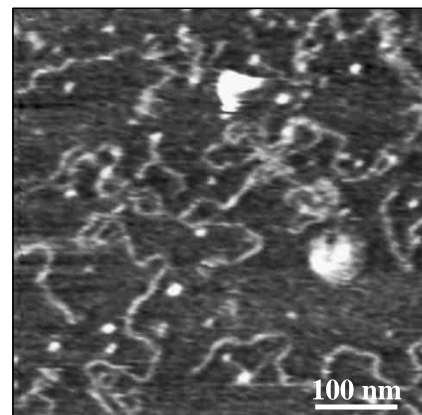

Figure 2. SFM image of DNA molecules under water imaged using Tapping Mode (TM). Although Jumping Mode (JM) and TM images present a comparable quality, the height of the DNA molecules is lower when imaged using TM than using JM suggesting that JM is a less intrusive technique than TM.

We have performed JM and Tapping Mode images under liquid on microtubules samples with similar findings. In particular, high resolution images of microtubules have been obtained. 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 to image soft samples under liquids, with results comparable to those obtained with DSFM. Several clear advantages of JM vs. DSFM should be remarked: 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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[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 non-contact modes.

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