Shear force imaging of soft samples in liquid using a diving bell concept

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We present a reliable and easy-to-use system, with a perfect analogy to a diving bell, to perform tuning fork based shear force microscopy on soft cells in liquid. Using the diving bell concept the tuning fork vibrates in air, while the tip is immersed in solution. In this way Q factors of 200 and higher in liquid are routinely obtained. The force feedback is reliable and stable over hours requiring a minimum adjustment of the set-point during imaging. With this system, tip–sample interaction forces are kept below 350 pN, enabling us to image soft dendritic cells in a buffer solution. © 2003 American Institute of Physics. [DOI: 10.1063/1.1634385]

Near-field scanning optical microscopy (NSOM) is, until today, the only technique that combines subdiffraction limit optical resolution (<90 nm) with topographical information. To control the probe–sample distance, the use of shear force detection based on tuning fork feedback has proven to be an easy, cheap, sensitive, and reliable method.¹ However, despite the wide application for operation in air, the imaging of soft biological samples in liquid such as living cells, has witnessed a low success rate.

Live cell imaging requires the scanning system to be kept in force feedback with a small interaction force under liquid conditions. Several approaches to image soft biological samples in liquid have been reported based on either optical shear force detection,² home-built piezo feedback mechanisms,³ or tuning fork feedback.^{4–6} In all these cases, immersion in liquid results in a decrease in sensitivity of the feedback mechanism due to the liquid viscosity and drag. Recently, our group demonstrated a recovery of the Q factor up to 60 after full immersion of the tuning fork prongs, which allows stable imaging on hard samples.⁴ However, in this case the tip sample interaction force still exceeds 4 nN and, moreover, the tuning fork has to be coated to prevent an electrical shortcut in a buffer solution. Lee *et al.*⁵ reported Qfactors of 400 by immersing only the fiber tip, but in this case the liquid level is extremely critical, demanding an elaborated and impractical sample holder design. Höppener et al.⁶ use a tapping-mode-like distance control to image a nuclear envelope using a tuning fork as a force sensor. In this letter we present a diving bell concept in which the tuning fork is vibrating in air, while the tip is immersed in liquid. We apply the system to image the topography of dendritic cells immersed in a buffer solution and compare the results to tapping mode AFM at similar conditions.

The basic model for tuning fork dynamics was introduced by Karrai and Grober.¹ If the tip–sample distance is regulated to achieve a constant preset phase shift between the drive and the oscillator, the interaction force is given by⁷

$$F_{\text{interaction}} = \frac{kx_{\text{res}}}{Q} \Delta \varphi, \qquad (1)$$

where $\Delta \varphi$ is the phase shift caused by force interaction, k the spring constant of the tuning fork, x_{res} the tip amplitude at resonance, and Q the quality factor of the system. Clearly, the higher the Q, the lower the interaction force between tip and sample. Using a 32 kHz tuning fork in air we routinely operate at Q = 700 and $x_{res} = 0.1$ nm. The tuning fork in combination with our phase feedback circuit has a bandwidth of 300 Hz.8 The minimal phase set-point we can achieve with our electronics corresponds to the detection of 0.017 rad phase shift, which, together with a spring constant of 40 $kN m^{-1}$, gives an estimation of the maximum interaction force of 100 pN. We work with large area scanners (a range in z of 26 μ m), therefore the main contribution to the vertical vibration noise in our measurements is the electronic noise in our z scanner that corresponds to a height difference of 6.4 nm.

The diving bell consists of a small glass tube, carefully glued into an aluminum holder, as shown in Fig. 1. Through the airtight sealing of the aluminum holder, the glass tube will act as a diving bell for the tuning fork, keeping the air–liquid interface at a fixed level, at the bottom of the glass tube and independent of the amount of liquid used. The aluminum holder with the glass tube is positioned over a 32 kHz tuning fork, which is attached via a magnet to a dither piezo to excite it mechanically at its resonance frequency. We have chosen 32 kHz tuning forks instead of the 100 kHz reported in our previous work⁴ because of their larger physical dimen-



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FIG. 1. A schematic diagram of the design of the tuning fork diving bell.

5083

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FIG. 2. The frequency response of a 32.768 kHz tuning fork in PBS and in air. The tuning fork has a pulled glass fiber attached to one of its prongs. The first response curves in PBS is obtained directly after immersion of the diving bell; the second response curve after a time interval of 1 h.

sions, making them easier to handle. A pulled glass fiber (diameter 125 μ m) is attached to one of the prongs using superglue with the tip end protruding $\sim 500 \ \mu m$ from the prong's end. In this way the free end of the fiber can still be considered stiff with respect to the tuning fork. We checked the oscillation mode of the tuning fork and found that the two prongs move toward and away from each other as expected at resonance.⁴ The tuning fork, together with the glass tube holder, is connected to the NSOM head, which is used to position the tip in close proximity of the sample scanning stage. The wiring of the tuning fork and the NSOM fiber are fed through a tiny hole in the head. The hole is sealed on top using vaseline. By inserting small silicone disks between the different diving bell components we ensure an airtight sealing of the system. The length of the glass tube is chosen such that a fiber length of $\sim 200 \ \mu m$ is sticking outside the aperture plane of the glass tube. Because of the airtight sealing, once this system is immersed into the liquid, the surface of the liquid remains in the plane of the aperture. Lambelet and co-workers have proposed a similar scheme to control the immersion depth of a fiber tip in an optical shear force feedback system.² Essentially, there are no restrictions on the amount of solution to be used. Furthermore, buffer exchange is easily possible, even with the tip in close proximity to the sample. Using the diving bell concept we are be able to image in any liquid without further adaptations to the scanning system or the tuning fork while maintaining a high Qfactor.

To verify the performance of the diving bell we measured the frequency response of the system by driving the fork with the dither piezo and recording the piezoelectric response of the fork. In Fig. 2 the response curve of a 32.768 kHz tuning fork both in air and phosphate buffered saline (PBS) is shown. In air the resonance frequency f_0 is shifted to 33.595 kHz due to the stiffening effect of the glass fiber on the tuning fork prong.⁴ The *Q* factor (calculated as f_0 /FWHM) in air is 1460. We then immerse the system in liquid using the diving bell and record the frequency response, directly after immersion and after one hour. As can be seen from Fig. 2, after immersion the resonance frequency



FIG. 3. (a) and (b) show, respectively, the topography and the feedback error signal image of an immature dendritic cell in PBS obtained with tuning fork shear force feedback. The bottom images (c) and (d) show, respectively, the topography and the error signal image of the same cell type in water with tapping mode AFM. The white line in (a) indicates the position of the line trace in Fig. 4.

has slightly shifted and the Q factor is reduced to 1020, which to our knowledge is the largest reported Q factor for a tuning fork feedback system in liquid without the use of electronic Q enhancement.⁹ We also observed an extra damping in the recorded tip amplitude probably due to an effective reduction of the amplitude driving of the fork. Because part of the energy is dissipated into the liquid surrounding the glass tube, a similar driving amplitude as used in air will result in a less efficient excitation of the fork and smaller tip amplitude. This can be easily corrected by increasing the excitation amplitude. After one hour the resonance frequency has slightly shifted and the Q factor is still as high as 1010.

We verified the performance of several tip-tuning fork constructs and in each case the immersion of the system only resulted in a small Q factor reduction as expected when only the tip of the fiber is being immersed. Using the tuning fork diving bell concept we routinely obtain Q factors of 200 or higher, resulting in a maximal tip-sample interaction force of 350 pN. We have noticed that although the reduction of Q is small, the system is not yet fully stable, and over a period of hours the resonance frequency shifts at a pace of ~ 50 Hz/hour typically together with a small decrease in Q. This drift results in a very slow change in the phase difference that is an input for the feedback system. Because it takes less than 11 min to record an image, the total shift in frequency is less than 10 Hz. Typically, for a tuning fork with a Q of 200, the response remains linear for about 80 Hz. Therefore it is easy to correct for these small shifts by carefully readjusting the phase set point of the feedback during imaging.

To test whether the interaction forces are low enough to image soft biological material, a sample containing human immature dendritic cells (imDC) was prepared. In our immune system, these dendritic cells play a crucial role in cell signaling processes.¹⁰ The cells are prepared on microscope slides covered with poly-l-lysine. After stretching they are fixed using 1% paraformaldehyde and stored in a buffer so-

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FIG. 4. The line trace through a dendritic cell. The position of the line trace on the cell is indicated in Fig. 3(a).

lution with 1% paraformaldehyde. Just before imaging they are washed with PBS, mounted in our liquid cell and covered with 1 ml of PBS solution. In Fig. 3 the topography at the edge of a dendritic cell is shown. The tip used had a Q of 210 with a scanning speed of imaging of 8 μ m/s. The edges of the cell are flat, elevated 200 nm above the glass substrate. The small dendrites are clearly visible on the right side of the image. The shear force image is comparable to an image obtained using tapping mode AFM in liquid with a silicon nitride cantilever with a spring constant of 0.10 N/m and a resonance frequency of 38 kHz at a similar scanning speed. In both cases the interaction forces with the sample are low, preserving the smooth membrane structure. To demonstrate the ability of our system to follow the contours of the cell, we present a line trace through the cell in Fig. 4. Clearly, the interaction forces between the tip and sample are small enough to prevent sample damage.

In conclusion, we have demonstrated the reliable use of 32 kHz tuning forks as feedback sensors in buffer solutions.

The tuning fork diving bell keeps the tuning fork vibrating in air while only the fiber tip is immersed in liquid. We reproducibly obtain Q factors of at least 200, thus having a maximum tip-sample interaction force of 350 pN. These forces are low enough to image soft dendritic cells in PBS. Because of the simplicity of the diving bell and the minor adaptations that have to be made to the scanning system we expect the tuning fork diving bell to be an important ingredient for NSOM imaging of living cells.

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