High Spatial Resolution Imaging with Near-Field Scanning Optical Microscopy in Liquids

Lynn F. Lee, Richard D. Schaller, Louis H. Haber, and Richard J. Saykally*

Department of Chemistry, University of California, Berkeley, California 94720-1460

The mechanism of tuning fork-based shear-force near-field scanning optical microscopy is investigated to determine optimal experimental conditions for imaging soft samples immersed in liquid. High feedback sensitivity and stability are obtained when only the fiber probe, i.e., excluding the tuning fork prongs, is immersed in solution, which also avoids electrical shorting in conductive (i.e., buffer) solutions. Images of MEH-PPV were obtained with comparable spatial resolution in both air and water. High optical resolution (~160 nm fwhm) was observed.

Near-field scanning optical microscopy (NSOM) has emerged as a versatile analytical tool that combines the high resolution of scanning probe microscopy with the noninvasiveness of optical microscopy.1 Despite rapid advances in NSOM for subwavelength imaging of a wide variety of samples, it has not yet been applied with routine success for imaging live biological samples. Imaging of hydrated cells avoids morphology perturbations due to the drying or freezing of the samples required by other imaging techniques, e.g., transmission electron microscopy (TEM), and also allows for the exciting possibility of measuring dynamic processes in real time. Unfortunately, imaging soft samples immersed in solution with noncontact NSOM is not trivial. Several problems, including compensating for evaporation to maintain a stable feedback and accommodating the decrease in sensitivity of the feedback mechanism due to the liquid viscosity and drag, must be addressed before NSOM imaging in solution can be performed with the same high spatial resolution that can be achieved in air or vacuum.2,3

In the past few years, several groups have explored the NSOM design using shear force feedback, wherein tip vibrations are monitored as a function of tip-sample separation, to image samples in a liquid environment.4–9 To date, all experiments have produced low resolution, unstable feedback, and low feedback sensitivity, which is exhibited by the significant decrease (>40%) in the quality factors when the mechanical detector is immersed in liquid.10 Large piezoelements are routinely used for mechanical detection but exhibit multiple resonance frequencies with relatively low Q-factor values (<200) in air. Even upon shallow immersion in liquid, this method suffers from a large decrease in the mechanical detector’s sensitivity.4–7 Karrai and Grober developed a shear-force feedback mechanism using a low resonance frequency quartz tuning fork (~33 kHz) with an optical fiber probe mounted to one of the prongs.8 This technique provides a single resonance frequency and produces high topographical resolution at low tip amplitudes (<1 nm) in air but also exhibits significantly decreased feedback sensitivity when immersed in liquid.

Recently, Rensen et al.9 employed a high resonance frequency tuning fork (~97 kHz) for liquid NSOM, which provides greater sensitivity and more stable feedback than a 33-kHz quartz tuning fork.9,11 In their work, the Q-factors decreased as a function of tuning fork immersion depth, but after immersing the prongs more than halfway up the tuning fork tines, the Q-factor was observed to increase. Based on this observed Q-factor recovery, they suggest complete immersion of the prongs to achieve optimal conditions for scanning in water. Moreover, with the prongs immersed completely, the solution level does not need to be absolutely constant, and as a result, no special considerations were needed to accommodate evaporation.

Here we present an alternative method of performing NSOM in liquids, demonstrating that optimal scanning and higher spatial resolution in solution are achieved with only the fiber probe, which is mounted on a high-frequency tuning fork, immersed in liquid. A sample cell (Figure 1) was constructed to maintain a constant liquid level throughout the scanning period, and we modified a chemically etched, uncoated SiO2 fiber-optic tip to overhang the fork prong edge ~3× more (~60 μm) than a commercial (Thermomicroscopes) tip (~200 μm, Figure 2). These modifications allowed us to obtain resonance frequencies higher than 90 kHz and high Q-factors (~400) when only the fiber-optic probe was immersed. Keeping the tuning fork dry also facilitated scanning in a greater variety of liquids, including buffer solutions and organics, which was not possible with the method of Rensen et al. due to electrical shorting problems.
To analytically calculate the physical parameters of the tuning fork system upon immersion in water, salt, and phosphate buffer solutions, we use an externally driven, damped, simple harmonic oscillator model. The equation of motion is

\[ \ddot{x} + \beta \dot{x} + \omega_0^2 x = F_0 \cos \omega t \tag{1} \]

where \( x \) is the displacement of the probe in the \( x \)-direction during oscillation, \( \omega_0 \) is the resonance frequency in air, the cosine term describes the driving force, and \( \beta \) is the damping coefficient, defined as

\[ \beta = \frac{b}{2m} \tag{2} \]

Here, \( b \) is the damping constant and \( m \) is the mass of the tuning fork and probe. These measurements were performed far from the sample surface, and \( \beta \) represents only the liquid contribution to the damping of tip vibrations. \( Q \) is the quality factor, which is a measure of the damping effects on the probe and defines the sensitivity of the system, and is given by

\[ Q = \frac{\omega_0}{2\beta} \approx \frac{\omega_0}{\Delta \omega} \tag{3} \]

where \( \omega_0 \) is the resonance frequency in the liquid environment and \( \Delta \omega \) is the fwhm of the resonance curve. The approximation in eq 3 is valid if the amplitudes are small; in our system, although \( x_0 \) (the tip amplitude in air) is not directly measured, its amplitude is always less than 1 nm. In all calculations here, \( x_0 \) is taken to be 0.1 nm. The damping force is given by

\[ F_D = k_0 x_0 / Q \sqrt{3} \tag{4} \]

where \( k_0 \) is the spring constant of the tuning fork in air. The damping force includes the liquid contributions to the tip damping and the drag effects due to the scanning motion.

**EXPERIMENTAL SECTION**

**Sample Cell.** To produce a stable liquid level over the sample, a glass sample cell was fabricated. A 5-mm-thick glass slide was milled to a depth of \( \sim 2.5 \) mm and the sample placed on an island that is located in the center of the well. An acrylic O-ring is used to create a negative meniscus for a thinner solution layer, and an inlet and outlet controls the flow of liquid into the cell. The liquid level is controlled by an HPLC pump which typically operated at a flow rate of \( \sim 50 \mu L/min \) to account for evaporation loss.

**Dipping Experiments.** A commercial NSOM system (Ther-momicroscopes, Lumina) equipped with the above nonoptical feedback system was employed for all measurements. Tip resonance frequency characterization experiments in air, water, sodium chloride, and phosphate buffer solutions were performed with an EG&G 7220 lock-in amplifier. Frequency scans were performed at different immersion depths of the tuning fork and probe as diagrammed in Figure 2. Lock-in frequencies were scanned between 80 and 100 kHz, and only one vibrational resonance was observed for each tuning fork. Tuning forks were allowed to dry for more than 10 min between different measure-
ments. For the salt and buffer solutions, salt residue was washed from the tuning fork with deionized water before drying.

**Imaging.** For topographical imaging, the polymer poly[2-methoxy-5-(2'-ethylhexyloxy)-1,4-phenylene vinylene] (MEH-PPV) was spin-cast onto glass slides.\(^{13}\) The uncoated tip is dithered 5–10 nm from the sample surface during the topography and optical image scans. Instead of raster scanning, forward and reverse motions of the sample were collected as separate images and used as a check of image reproducibility.

Linear reflectivity experiments\(^ {15}\) were performed on MEH-PPV films spin-cast from a 1% w/v solution in chlorobenzene with a Melles Griot HeNe laser (632.8 nm, \(\sim 50 \mu W\)). Although there have been several studies utilizing NSOM to investigate the properties of this conjugated polymer,\(^ {13,16–18}\) none have studied MEH-PPV in a liquid environment. Because the polymer forms mesoscopic physical domains of a few hundred nanometers in diameter from the spin-cast process,\(^{13}\) this sample was used to test the NSOM optical resolution in liquid.

### RESULTS AND DISCUSSION

Resonance frequencies and quality factors were determined via lock-in frequency scans at a fixed set of immersion depths (Figure 2) in water, salt, and buffer solutions. Values of the resonance frequencies (\(\omega_R\)) and Q-factors (Q) are summarized in Table 1. A Q-factor value of 464 was obtained for the tuning fork in air with a resonance frequency of 94.7 kHz. With only the fiber probe immersed in water, the Q-factors ranged from 434 (Table 1, Figure 3). In contrast, immersion of the fork prongs decreased the Q-factor by nearly an order of magnitude to values of 60, 41, and 36 as the water level increased to depth 4–6, respectively. Accordingly, it was also observed that the resonance frequency curve dramatically shifted to less than 90 kHz upon immersion of the tuning fork (Table 1, Figure 3).

In contrast to the work of Rensen et al.,\(^5\) we did not observe any recovery of the Q-factor when the prongs were completely immersed. Although complete immersion of the prongs would indeed facilitate a simplified experimental setup in that the exact liquid level is less crucial, we believe that the large Q-factor decrease, and hence the greatly decreased sensitivity of the feedback mechanism, reduces the image quality too much for this approach to be useful. By stabilizing the liquid level with our experimental design, the high Q-factor values that are obtained when only the probe is immersed indicates that optimal scanning conditions are achieved by this method.

Good feedback in ionic solution is also essential because in vivo imaging of biological samples requires immersion in a buffer solution due to osmotic pressure otherwise experienced by the cells. When the prongs are immersed in 0.2 M NaCl, 1.0 M NaCl, and phosphate buffer saline (PBS) solutions, the feedback current is destabilized and feedback cannot be obtained due to electrical shorting. Rensen et al. did not observe the shorting problem because they were working exclusively in (nonconductive) water. In all subsequent measurements in salt and buffer solutions, only the probe was immersed (Table 2). Table 2 shows that the damping force increases as the probe was immersed deeper into the liquid, and the greatest increase of damping force is \(\sim 43\%\) when the probe is completely immersed in PBS buffer. Although the damping force increases in ionic solutions, we were still able to maintain relatively high Q-factor values (\(\Delta Q <30\%\) in PBS).

### Table 1. Effects of Water on \(\omega_R\) and Q-Factor at Different Immersion Depths

<table>
<thead>
<tr>
<th>depth</th>
<th>(\omega_R) (Hz)</th>
<th>Q-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>947 34</td>
<td>464</td>
</tr>
<tr>
<td>1</td>
<td>946 90</td>
<td>439</td>
</tr>
<tr>
<td>2</td>
<td>946 86</td>
<td>417</td>
</tr>
<tr>
<td>3</td>
<td>946 71</td>
<td>377</td>
</tr>
<tr>
<td>4</td>
<td>893 24</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>852 14</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>850 68</td>
<td>37</td>
</tr>
</tbody>
</table>


buffer), which are greater than previous reports performed in water.4-10 Significantly, this is a smaller decrease than those observed with the large piezoelements used for mechanical detection, which exhibit \( Q \)-factor degradation by >40% with shallow immersion in water.4-7

Figure 4 shows the topography scans of the same sample area in air and water at a depth of <200 \( \mu \)m. To obtain greater feedback stability, we used a modified tip where the chemically etched fiber probe is mounted on a bare tuning fork with an overhanging length of 600 \( \mu \)m, 3\( \times \)longer than those commercially available. This helped in maintaining good feedback and achieving better image resolution. Good reproducibility is obtained in both forward and reverse scans, and the topographical spatial resolution in water is comparable to that obtained in air (<120 nm). Optical reflectivity images in water shown in Figure 5 also exhibit good reproducibility with a resolution of ~160 nm fwhm (~\( \lambda / 4 \)). Scans imaged in liquid in both Figures 4 and 5 show some “underwater”

distortion, which can be avoided by decreasing the scan speed. We note that maintenance of a stable solution level is an absolute necessity for performing experiments that transmit optical fields through the solution. If the liquid level is unstable during scanning, the parallax of the excitation beam will also vary as the liquid level decreases. This will result in misalignment of the beam to the tip of the probe as parallax is dependent on the length of the beam path traversed in air before reaching the air—liquid interface. Thus, an unstable liquid level setup is limited to only transmission—collection, transmission—illumination, and collection—illumination NSOM modes.20

In summary, we have described an approach to implement NSOM in a liquid environment without great sacrifice of feedback sensitivity and stability. These results demonstrate that NSOM scanning in solution does not necessarily imply resolution degradation and indicates that NSOM imaging of biological samples in vivo is possible. Moreover, we have studied the solvatochromic shifts of MEH-PPV photoluminescence spectra in a variety of solvents21 and are presently exploring the use of this new design for nonlinear near-field optical imaging of various samples in solution.

ACKNOWLEDGMENT

This work was supported by the Experimental Physical Chemistry Division of the National Science Foundation. We also acknowledge the Keck Foundation for supporting the UCB/UCLA Joint Institute for Chemical Imaging Ultramicroscopy. We thank Thuc-Quyen Nguyen and Benjamin Schwartz of UCLA for providing the MEH-PPV samples.

Received for review July 17, 2001. Accepted September 11, 2001.

AC010803K


(22) Tuning fork diagram was taken from the TopoMetrix User Manual for the Lumina System.