Accelerated Articles

Scanning Optical Microscopy with an Electrogenerated Chemiluminescent Light Source at a Nanometer Tip

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Electrogenerated chemiluminescence at electrodes with effective diameters down to 155 nm was used as a stable light source for near-field scanning optical microscopy imaging of an interdigitated array and a submicrometer size test substrate. Light was generated in a thin (~500 μm) layer of an aqueous solution of 15 mM Ru(bpy)$_3^{2+}$ and 100 mM tri-*n*-propylamine in a pH 7.5 buffer. The resolution obtained was compared to that found with a micrometer size electrode. The shear force from the tip attached to a quartz tuning fork was used to monitor and control the tip-to-substrate separation within the near field regime.

The use of electrogenerated chemiluminescence (ECL) at an ultramicroelectrode (UME)[1] as a light source for scanning optical microscopy has been previously proposed.[2] In these previous studies, the resolution was limited by the tip size and tip−substrate spacing. We discuss here an approach to resolution of ~100 nm with much improved stability of the ECL intensity with time, which is important in imaging applications. In the current study, the ECL behavior of different sized UMEs (from 1 μm to less than 100 nm effective diameter) was examined in an aqueous coreactant ECL solution, i.e., the Ru(bpy)$_3^{2+}$/tri-*n*-propylamine (TPrA) system. The ECL signal was very stable with time. As demonstrated by the images obtained using the UME tips, the resolution was mainly determined by the tip size. When the effective diameter of the tip was reduced to a size smaller than 200 nm, and the scan was carried out in a near-field regime, a resolution close to that of the near-field scanning optical microscope (NSOM)[3] was achieved. Compared to the typical metal-coated fiber-optic probe used in

NSOM, the preparation of the ECL tip is easier, and the use of a sharp metal tip avoids the fundamental resolution limitation caused by the finite skin depth of the metal coating. In addition, since this method does not require a laser, there is no heating of the sample or tip from absorption of light in the metal coating. This approach is thus a promising one for near-field optics in solution.

The ECL mechanism of Ru(bpy)$_3^{2+}$/TPrA system has been discussed previously.[4] At higher concentrations of Ru(bpy)$_3^{2+}$ (>0.1 mM), the catalytic oxidation of TPrA by electrogenerated Ru(bpy)$_3^{3+}$ is the dominant process for ECL. In this case, the ECL route can be expressed as follows:
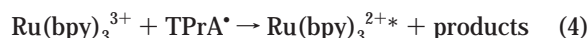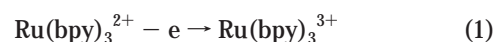
$$\text{Ru(bpy)}_3^{2+} - \text{e} \rightarrow \text{Ru(bpy)}_3^{3+} \qquad (1)$$

$$\text{Ru(bpy)}_3^{3+} + \text{TPrA} \rightarrow \text{Ru(bpy)}_3^{2+} + \text{TPrA}^+ \qquad (2)$$

$$\text{TPrA}^+ \rightarrow \text{TPrA}^\bullet + \text{H}^+ \qquad (3)$$

$$\text{Ru(bpy)}_3^{3+} + \text{TPrA}^\bullet \rightarrow \text{Ru(bpy)}_3^{2+*} + \text{products} \qquad (4)$$

$$\text{Ru(bpy)}_3^{2+*} \rightarrow \text{Ru(bpy)}_3^{2+} + h\nu \qquad (5)$$

Figure 1 shows the voltammetry and ECL curves at a 25-μm-diameter Pt disk electrode in 1 mM Ru(bpy)$_3^{2+}$/0.1 M TPrA/0.15 M phosphate buffer solution (pH 7.5). The oxidation of Ru(bpy)$_3^{2+}$ and catalytic oxidation of TPrA exhibited steady-state current. ECL occurred at ~1.0 V, and its intensity increased rapidly accompanying the formation of Ru(bpy)$_3^{3+}$. The ECL intensity reached a

(1) For a review of ECL, see: (a) Faulkner, L. R.; Bard, A. J. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1977; Vol. 10, p 1. (b) Knight A. W.; Greenway, G. M. *Analyst* **1994**, *119*, 879.

(2) (a) Fan, F.-R. F.; Cliffel D.; Bard, A. J. *Anal. Chem.* **1998**, *70*, 2941. (b) Maus, R. G.; McDonald, E. M.; Wightman, R. M. *Anal. Chem.* **1999**, *71*, 4944.
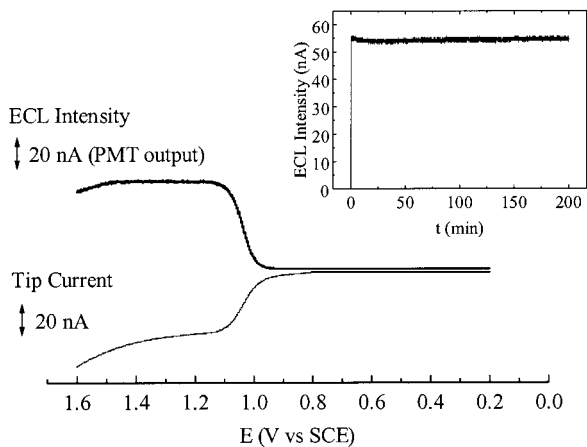
(3) (a) Betzig, E.; Trautman, J. K. *Science* **1992**, *257*, 189. (b) Dunn, R. C. *Chem. Rev.* **1999**, *99*, 2891.

(4) (a) Zu, Y. B.; Bard, A. J. *Anal. Chem.* **2000**, *72*, 3223. (b) Kanoufi, F.; Zu, Y.; Bard, A. J. *J. Phys. Chem. B* **2001**, *105*, 210.

(5) (a) Bard, A. J.; Fan, F.-R. F.; Kwak, J.; Lev, O. *Anal. Chem.* **1989**, *61*, 1, 132. (b) Bard, A. J.; Fan, F.-R. F.; Mirkin, M. V. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1994; Vol. 18, pp 243−373. (c) *Scanning Electrochemical Microscopy,* Bard, A. J., Mirkin, M. V., Eds.; Marcel Dekker: New York, 2001.

Figure 1. Voltammogram and ECL curve at a 25-μm-diameter Pt ultramicroelectrode in 0.15 M phosphate buffer solution (pH 7.5) containing 100 mM TPrA and 1 mM Ru(bpy)$_3{}^{2+}$. Potential scan rate, 2 mV/s. The inset shows the stability of the ECL signal when the UME potential was held at 1.2 V.

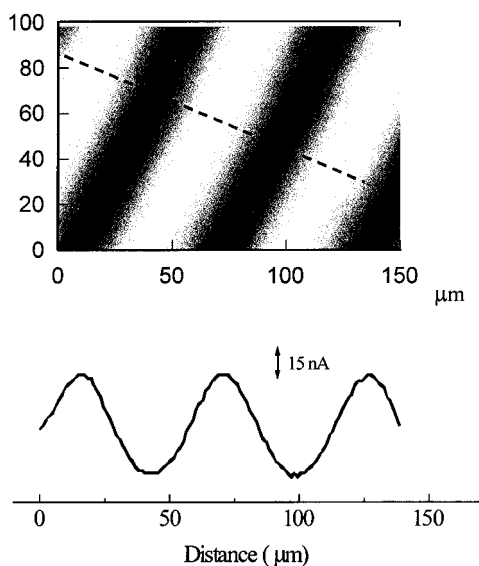**Figure 2.** ECL image and its cross section of an IDA consisting of Au bands (30 μm wide) spaced 25 μm apart deposited on a glass substrate. Tip size, 25-μm diameter. Tip-to-substrate distance, 5 μm. Data acquisition time, ~10 min.

constant level over the potential region between 1.1 and 1.5 V. When the electrode potential was held at 1.2 V, very stable light emission for periods of at least 200 min was observed. This contrasts to the ECL signal of the Ru(bpy)$_3{}^{2+}$/TPrA system in acetonitrile solution described previously,[2b] which is unstable, probably due to the formation of surface film during the ECL process. In aqueous solution, however, the ECL signal exhibited very good stability, which makes tip-generated ECL a suitable light source for scanning optical microscopy.

As a demonstration, this Pt UME was used as an ECL probe to image optically an interdigitated array (IDA), consisting of Au bands (~30 μm wide) spaced 25 μm apart deposited on a glass substrate. Figure 2 shows the ECL image of the IDA with a 25-μm-diameter Pt disk electrode. The image was obtained with a laboratory-built scanning electrochemical microscope (SECM)[5] with the distance between tip and substrate adjusted using SECM feedback in 1 mM Ru(NH$_3$)$_6{}^{3+}$ solution; this was replaced by 1

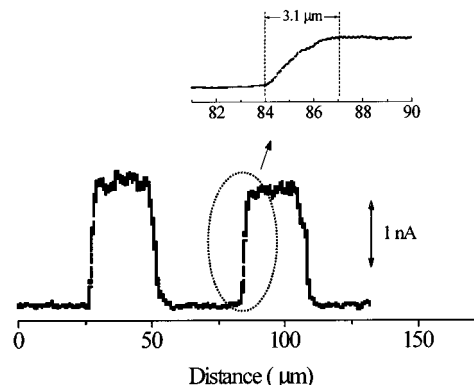**Figure 3.** Cross section of an ECL image of the IDA. Tip size, 2.1-μm diameter. Tip-to-substrate distance, 1 μm.
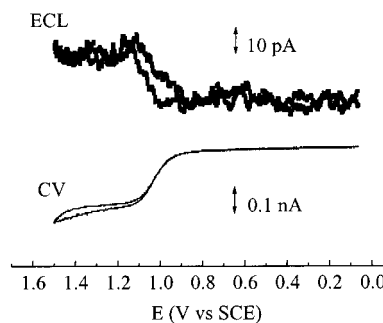

Figure 4. Cyclic voltammogram and ECL curve at a 60-nm-diameter (effective) Pt ultramicroelectrode in 0.15 M phosphate buffer solution (pH 7.5) containing 100 mM TPrA and 15 mM Ru(bpy)$_3{}^{2+}$. Potential scan rate, 50 mV/s.

mM Ru(bpy)$_3{}^{2+}$/0.1 M TPrA phosphate buffer solution before imaging. During the scan, the tip was held at a constant height (~5 μm) above the substrate and the ECL intensity was measured with a photomultiplier tube (PMT) (type R928, Hamamatsu Corp., Middlesex, NJ) located underneath the substrate. The ECL signal reflected the regular structure of the substrate. Generally, the edge sharpness of the signal obtained can be used to evaluate the resolution of an optical microscope. The resolution obtained in Figure 2 is quite low due to the large size of the tip. When a 2.1-μm-diameter UME was used as the probe and the tip-to-substrate distance was decreased and held at 1 μm, the ECL image resolution increased significantly, as shown in Figure 3. To improve the resolution further to the submicrometer range and bring it closer to the NSOM level (usually 50−150 nm), both the tip size and the tip-to-substrate distance need to be reduced greatly. However, the preparation of a conventional disk UME with a diameter smaller than 0.5 μm and with a small RG value (the ratio of glass insulator to metal diameter at the tip) is difficult. In addition, accurate control of the distance between tip and substrate during scanning is required to avoid the possible damage to the sharp tip by a tip crash. This requires maintaining a tip−substrate distance with submicrometer resolution; such accuracy is difficult to achieve by SECM feedback in the ECL system due to the consumption of the Ru(bpy)$_3{}^{2+}$ by the homogeneous catalytic reaction (eq 2).

A simple method was used to prepare electrodes with effective diameters from several nanometers to several hundred nanom-
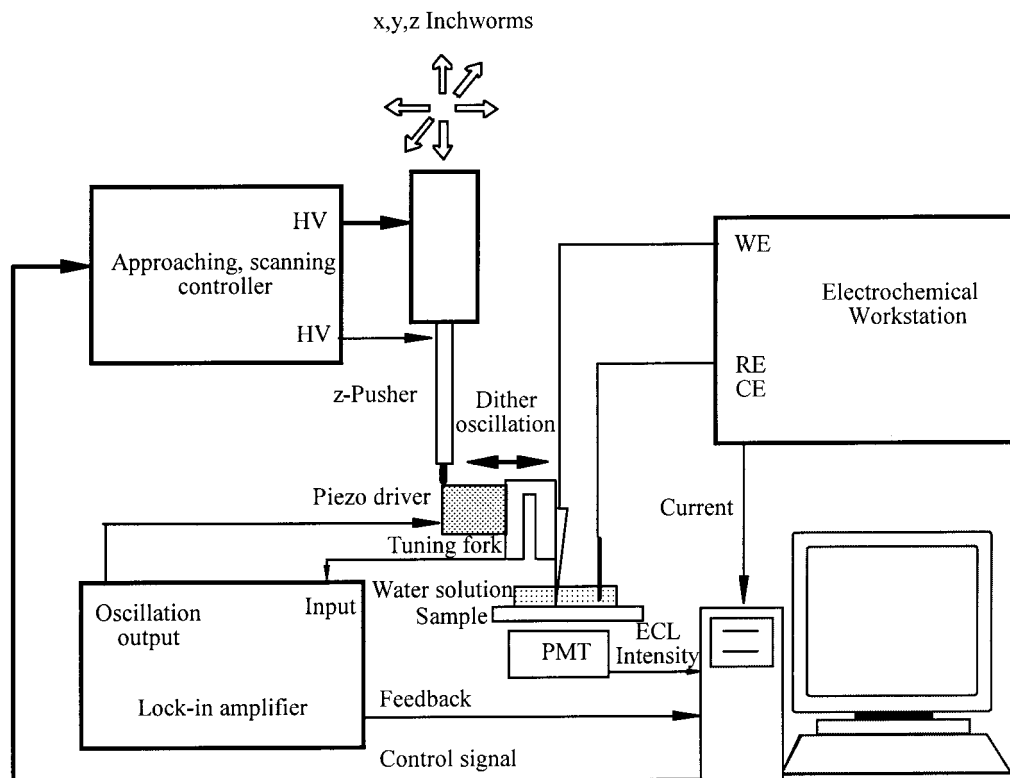
Figure 5. Block diagram of apparatus used in the ECL imaging experiments.

eters following the procedure reported previously.[6] The tips prepared by this method are conical. In the following, we define "effective diameter" as that obtained from the steady-state limiting current in a CV, calculated as if the electrodes had a hemispherical shape. A fine (250- or 25-μm) Pt wire was electrochemically etched and coated with electrophoretic paint anodically. After heating at ~160 °C, a conical-shaped sharp apex was exposed. Figure 4 shows the CV and ECL response at a ~60-nm-diameter (effective) UME in a 15 mM $Ru(bpy)_3^{2+}$/100 mM TPrA solution. A signal-to-noise ratio of ~4 was obtained for ECL detection. Considering the tip as a hemispherical microelectrode, the specific diffusion length (reaction layer thickness) of $Ru(bpy)_3^{2+}$ was determined by[7]

$$\mu = [r_o^{-1} + (k_{cat}/D)^{1/2}]^{-1} \qquad (6)$$

where r_o is the radius of the electrode, k_{cat} is the rate constant of reaction 2, and D is the diffusion coefficient of $Ru(bpy)_3^{2+/3+}$. As obtained in our previous studies, k_{cat} is 1.3×10^7 M^{-1} s^{-1} and D is 5.9×10^{-6} cm^2 s^{-1}. Therefore, for a tip with diameter of 100 nm, the thickness of the $Ru(bpy)_3^{2+}$ diffusion layer is ~15 nm in the $Ru(bpy)_3^{2+}$/TPrA system. In other words, the ECL reaction only occurs in a very thin layer around the electrode surface and is not significantly broadened by the diffusion of reactants away from the tip. This makes the size of the ECL light source very close to that of a typical NSOM probe.

The apparatus used in this experiment is shown in Figure 5. To bring the ECL tip close to the substrate surface within the near-field regime, the shear force from a quartz tuning fork (32.768 kHz) was used to monitor and control the tip-to-substrate separation.[8] Briefly, the tuning fork is caused to oscillate (is dithered) at its resonant frequency by an attached piezoelement and the maximum amplitude of oscillation is noted. Interaction of a tip attached to the tuning fork with a surface (i.e., the onset of lateral shear forces on the tip) causes a decrease in the amplitude of oscillation. This signals when the tip "touches" the surface. The tip was glued to one prong of the tuning fork and extended beyond the end of the tuning fork by ~1 mm. A thin-layer (~500 μm) aqueous solution containing 15 mM $Ru(bpy)_3^{2+}$ and 100 mM TPrA was spread onto a substrate. In the experiment, only the tip was immersed in the solution, while the tuning fork remained in air to maintain its sensitivity. A LabVIEW (National Instruments, Austin, TX) program was used to control the approach and scanning of the tip over a substrate and to acquire the data. To position the tip in a reproducible manner for each point in an $x-y$ scan and avoid problems with lack of coplanarity between the substrate and the plane of the $x-y$ scan, the following procedure was adopted. The potential of the tip was adjusted to the potential where ECL occurred. The tip approached the substrate until a shear force was detected (~60 ms). The tip was then withdrawn 24 nm (<1 ms). The ECL signal was then recorded for a time of 1 ms at this distance. The procedure of approaching to establish shear-force feedback, withdrawing 24 nm, and then recording the

(6) (a) Slevin, C. J.; Gray, N. J.; Macpherson, J. V.; Webb, M. A.; Unwin, P. R. *Electrochem. Commun.* **1999**, *1*, 282. (b) Conyers, J. L.; White, H. S. *Anal. Chem.* **2000**, *72*, 4441.
(7) (a) Delmastro, J. R.; Smith, D. E. *J. Phys. Chem.* **1967**, *71*, 2138. (b) Diao, G. W.; Zhang, Z. X. *J. Electroanal. Chem.* **1997**, *429*, 67.

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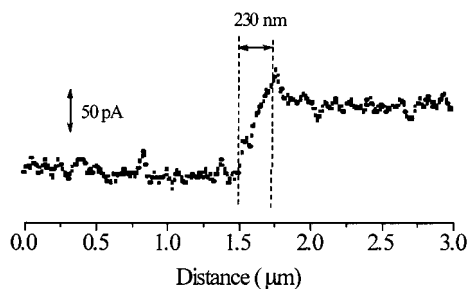
Figure 6. Cross section of an ECL near-field image of the IDA. Tip size, 155-nm diameter (effective). Data acquisition time, ~25 s.

ECL signal was followed for each point during the scan. As shown previously,[2b] when a disk UME was used as a SECM/ECL probe to approach a conducting or insulating transparent substrate, a significant decrease of ECL signal intensity was observed due to blocking of the reactants' access to the UME by the substrate. In the current study, however, this effect was not obvious due to the conical shape of the tip and the 24-nm withdrawal might also aid the mass transfer of the reactants.

Figure 6 shows the cross section of the ECL image of one edge in the IDA structure using a 155-nm-diameter (effective) tip. The resolution was dramatically increased compared to that obtained using the micrometer-sized tips (Figures 2 and 3). The width of the ECL signal across the edge of glass/gold bands was ~230 nm, close to one-third of the wavelength of the ECL emission in the $Ru(bpy)_3^{2+}$/TPrA system (the emission wavelength maximum is 645 nm). Figure 7 is an ECL image of a test substrate using a 172-nm-diameter (effective) electrode. The test substrate was made by spin coating polystyrene Latex spheres (from Ted Pella, Inc., Redding, CA, diameter ~0.482 μm) on a glass slide and then depositing by vacuum evaporation a 100-nm-thick layer of aluminum. The distribution of the beads was random. Some beads were isolated from one another while others clumped together. After the beads were removed by sonicating in dichlo-
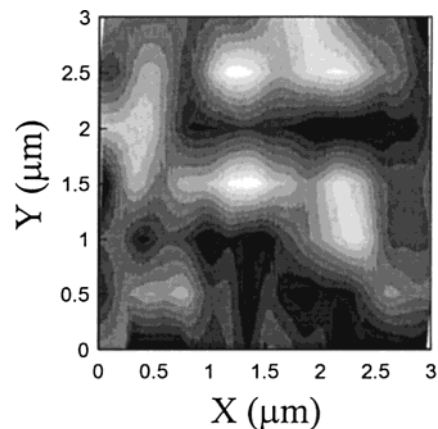

Figure 7. ECL near-field image of a test sample containing submicrometer holes. Tip size, 172-nm diameter (effective). Data acquisition time, ~8 min.

romethane, submicrometer-sized transparent holes were formed. In Figure 7, these holes were imaged optically with submicrometer resolution. For the images shown here, tips with effective diameters of ~150 nm were used to achieve good-quality ECL signals. To improve the image resolution even further with smaller tips, a more sensitive and low-background photon detector will be required.