Combined Scanning Electrochemical and Fluorescence Microscopies using a tetrazine as a single redox and luminescent (electrofluorochromic) probe.

L. Guerret-Legras, J.F. Audibert, G.V. Dubacheva, and F. Miomandre *

Supplementary information

1. Experimental section

Chemicals. Chloromethoxy-s-tetrazine (purchased from Crime Science Technology) was investigated in a 2 mM solution of acetonitrile (Spectrosol, Carlo Erba) in addition with tetrabutylammonium hexafluorophosphate (TCI, electrochemical grade) 0.1 M as the supporting electrolyte. Before the experiments the solution was purged with argon for about 10 minutes to remove dissolved oxygen, then kept flushing at low flow rate during the measurements.

Electrochemical measurements. All electrochemical and SECM measurements were performed at room temperature using a homemade three-electrode electrochemical cell on top of an inverted optical microscope (see below). A platinum wire and a Ag/AgCl wire were used as a counter and reference electrode respectively. Glass coverslips (purchased from VWR, 1 mm thickness), ITO (purchased from Solems, 25-35 ohm., 80 nm thickness) and gold (see coating details below) were used as the substrate. A 20 μ m platinum UME (purchased from Ametek) was used as the SECM tip and positioned above the substrate thanks to a mechanic arm connected to a piezo positioner (VersaSCAN motion controller) which enables to control the tip position under VersaSCAN Software (purchased from Ametek). For all cyclic voltammetry and chronoamperometry experiments, UME and substrate potentials were controlled simultaneously by a bipotentiostat (VersaSTAT 3F setting as floating mode for the tip and VersaSTAT 4 for the substrate, Ametek) under the VersaStudio software.

Negative feedback electrochemical approach curves were obtained by polarizing the platinum disk electrode at -0.8 V vs Ag|AgCl. For positive feedback approach curves, the tip and substrate potentials were stepped to -0.8 V and 1 V vs Ag|AgCl respectively when the substrate was ITO, and to -0.8 V and 0 V vs Ag|AgCl with the gold substrate. All approach curves were carried out under control by the VersaSCAN software with a step size of 5 μ m at 10 μ m/s.

Au coating on ITO: Au deposition was performed using EMITECH K650 sputter coater. Before Au sputtering, ITO surfaces were cleaned by immersing in isopropanol solution for 20 min, followed by isopropanol rinsing and drying. The following conditions were applied to achieve homogeneous thin Au layer on ITO: Ar atmosphere (0.1 mbar), 60 mA applied for 4 min. The thickness and homogeneity of the Au coating was verified by AFM. AFM images were recorded in tapping mode (Thermo Microscope, Veeco) using silicon cantilevers (PPD-NCHR-50,

Nanosensors). The data show that Au layer is represented by a homogeneous grain-like film with an average thickness of 11 ± 2 nm and average RMS roughness of 2.0 ± 0.3 nm, the latter being slightly higher than that of the underlying ITO (typically between 0.5 and 1.0 nm, see Fig. S0).



Fig. S0. Representative AFM image of Au-ITO (A) with line analysis; inset: bare ITO surface. Representative AFM image of Au layer deposited on glass surface followed by scratch (B). The samples (A) and (B) were prepared in parallel under the same Au spattering conditions. AFM images are obtained in tapping mode using Thermo Microscope (Veeco) and silicon cantilevers (PPD-NCHR-50, Nanosensors). Image and line analysis yielded RMS roughness and thickness of 2.0 ± 0.3 and 11 ± 2 nm, respectively (number of analyzed images = 3 in each case).

Optical measurements. Optical measurements were performed on an inverted microscope (Ti Eclipse Nikon) with a x40 (X1.5) NA 0.75 objective in a wide field epi-illumination. The tetrazine solution was excited by the association of a Hg lamp (Intensilight Nikon) with a band pass excitation filter (BP 482 nm/35nm) and FITC dichroic (506 nm). Emitted light is collected through a long pass emission filter (LP 520 nm). Acquisition time-lapses are carried out using a CCD Pixel Fly camera from PCO at 10 images/s under the μ Manager open source software. Fluorescence intensity over time is reported as the mean intensity inside a square region of interest (94 x 94 pixels) of the total image centered on the platinum tip. $I_{max}(z)$ was obtained by averaging the signal coming from this ROI with a scanning of 5 μ m step in absence of electrochemical reaction.

Emission spectra are recorded with an Ocean Optics spectrometer coupled to the microscope with a UV-VIS (400 μ m diameter) optical fiber plus collimator placed in an intermediate image plane, with a 60 μ m MFD (Mode Field Diameter) in the sample plane centered around the tip. Spectra are corrected from optical chain sensitivity and background.

Acquisition procedure. First the contact of the tip with the substrate was determined optically (position zero) and centered in the camera field. Then a 500 μ m depth approach curve was recorded. Then the tip was positioned close to the substrate at a distance spotted on the approach curve and raised step by step (typically by 5 μ m steps). At each tip/substrate distance, the same spectroelectrochemical measurement was carried out.

2. Modelling

The geometry we have considered for the modelling is described in Scheme S1.



Scheme S1: Geometry of the tip-substrate half-space used for the simulation. The region of interest (ROI) for fluorescence measurement is indicated in yellow.

The axial symmetry of our experiment has enabled us to carry out 2D calculation in cylindrical coordinates. Our modelling relies on a disk shaped microelectrode of radius a sealed in an insulator sheath of radius R and immersed in a solution of an EF molecule in its oxidized and luminescent form at concentration C_{0x}^{bulk} . The microdisk is located at a distance d of the substrate. The cuvette dimensions have been chosen large enough to avoid any edge effects (*i.e.* $R_{\infty} \gg a$ and $|Z_{\infty}| \gg a$).

The differential equation of diffusion mass transfer in solution (convection and migration are neglected) (1) is solved numerically in the steady-state case using finite element analysis, for various tip-substrate distances :

$$\frac{\partial^2 C}{\partial r^2} + \frac{1}{r} \frac{\partial C}{\partial r} + \frac{\partial^2 C}{\partial z^2} = 0 \quad (1)$$

Boundary conditions have been chosen in order to simulate the reduction of the oxidized form at the tip and to model the behavior of the conducting or insulating substrate. The EF reduction at the tip and its reoxidation on the conducting substrate have been considered to be limited only by mass transfer, which might be a relatively drastic approximation especially for a semiconductor like ITO[1].

The boundary conditions selected are the following :

• On insulating substrate $(r \ge 0 \text{ and } z = d)$:

$$\left(\frac{\partial C_{0x}}{\partial z}\right)_{z=d} = 0 \text{ and } \left(\frac{\partial C_{Red}}{\partial z}\right)_{z=d} = 0 (2a)$$

• On conducting substrate $(r \ge 0 \text{ and } z = d)$:

$$C_{Red} = 0$$
 and $C_{Ox} = C_{Ox}^{bulk}$ (2b)

• At the tip $(0 \le r \le a \text{ and } z = 0)$:

$$C_{0x} = 0$$
 and $C_{Red} = C_{0x}^{bulk}$ (3)

• At the insulating sheath :

for $a \le r \le R$ and z = 0:

$$\left(\frac{\partial C_{Ox}}{\partial z}\right)_{z=0} = 0 \text{ and } \left(\frac{\partial C_{Red}}{\partial z}\right)_{z=0} = 0 \quad (4a)$$

for r = R and $z \le 0$:

$$\left(\frac{\partial C_{Ox}}{\partial r}\right)_{r=R} = 0 \quad and \quad \left(\frac{\partial C_{Red}}{\partial r}\right)_{r=R} = 0 \quad (4b)$$

• On the other boundaries :

for $r = R_{\infty}$ and $Z_{\infty} \le z \le 0$ or $R \le r \le R_{\infty}$ and $z = Z_{\infty}$

$$C_{0x} = C_{0x}^{bulk} \qquad (5)$$

The mesh has been chosen very tight around the boundaries with the more likely pronounced concentration gradients, that is to say : the microdisk $(z = 0 \text{ and } 0 \le r \le a)$, the substrate $(z = d \text{ and } r \ge 0)$ and the symmetry axis between the microdisk and the substrate $(r = 0 \text{ and } 0 \le z \le d)$.

Such calculations give a spatial concentration profile for the oxidized species $C_{0x}(r,z)$ from which fluorescence intensity emitted in a particular region can be deduced by considering it directly proportional to the amount of oxidized form in the same region. Under this assumption $C_{0x}(r,z)$ simply has to be spatially integrated on the volume of the region of interest (ROI) to get fluorescence intensity.

The simulated ROI must be chosen as representative as possible of the experimental one. Given the linear relationship between I_{max} and the tip-substrate distance experimentally observed (see figure S3), the influence of the field depth can be neglected and the experimentally collected fluorescence can be considered as coming equally from all the emitters in the volume delimited by the red square on the one hand and by the tip-substrate distance on the other hand. Thus a theoretical ROI must necessarily begin at z = d and finish at z = 0.

Under all these assumptions, a, R, R_{∞} and Z_{∞} values have been fixed to 10 µm, 110 µm, 1 cm and -0.3 cm respectively. C_{0x}^{bulk} has been chosen equal to 2 mM as in our experiments. Results are reported for the ROI formed by the symmetry axis between the microdisk (tip) and the substrate (r = 0 and $0 \le z \le d$), with d varying from 5 to 500 µm by 5 µm steps. $I_{min}(d)$ and $I_{max}(d)$, corresponding to the fluorescence intensity in the ROI respectively in absence and in presence of the electrochemical reaction, can be calculated according to the following equations:

$$I_{min}(d) \propto \int_{z=0}^{d} C_{0x}(r=0,z) dz \qquad (6a)$$
$$I_{max}(d) \propto C_{0x}^{bulk} \int_{z=0}^{d} dz = d \times C_{0x}^{bulk} \qquad (6b)$$

Finally, the normalized amplitude is given by :

$$\frac{I_{max}(d) - I_{min}(d)}{I_{max}(d)} = 1 - \frac{\int_{z=0}^{d} C_{0x}(r=0,z) dz}{d \times C_{0x}^{bulk}} \quad (6c)$$

Reference :

[1] Neufeld, A. K.; O'Mullane, A. P., Effect of the mediator in feedback mode-based SECM interrogation of indium tin-oxide and boron-doped diamond electrodes. *Journal of Solid State Electrochemistry* **2006**, *10*, 808-816.



Figure S1

CVs recorded for the tetrazine compound on the Pt tip (blue trace), on ITO substrate (orange trace) and on ITO substrate coated with a thin layer of Au (yellow trace) before (full line) and at the end (dashed line) of the experiment.



Figure S2: A) Tip current vs. Tip position (approach curve) on ITO. The red dot shows the position used for the generation-collection mode. B) CV on the tip (scan rate: 100 mV/s) recorded at the position represented by the red dot in A) when the substrate is kept polarized at 0 V (green trace, bottom) or -0.8 V (red trace, top).



Figure S3: Experimental variation (red) and linear fit (blue, $r^2 = 0.92$) of $I_{max}(z) / I_{max}(0)$ vs. z on insulating substrate. The red shading denotes the standard deviation of the experimental signal.