Supporting Information

Infrared Photoinduced Electrochemiluminescence Microscopy of Single Cells

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1. Experimental part

1.1. Reagents

Acetone (MOS electronic grade, Erbatron from Carlo Erba) and anhydrous ethanol (RSE electronic grade, Erbatron from Carlo Erba) were used without further purification. Tri-n-propylamine (TPrA), tris(2,2'-bipyridine)dichlororuthenium-(II) hexahydrate ($[Ru(bpy)_3]Cl_2$), sulfuric acid, hydrogen peroxide, disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$), sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), paraformaldehyde (PFA), calcein-AM and streptavidin from *Streptomyces avidinii* were from Sigma-Aldrich and used without any further purification. Biotin X was purchased from Fisher Scientific. DMSO was purchased from Invitrogen. Phosphate buffer solution (PBS) 0.65 M (pH 7.4) was obtained by mixing 0.05 M of $NaH_2PO_4 \cdot H_2O$ and 0.60 M of $Na_2HPO_4 \cdot 7H_2O$. The pH of the PBS solution was adjusted to 7.4 by adding concentrated sulfuric acid. PBS 1× and trypsin were from Gibco. Chinese ovary cells (CHO-K1) cells were from Public Health England (HPA) Culture Collections and supplied by Sigma (85051005). Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum and penicillin/ streptomycin 100 U/mL were used to grow cells in an incubator.

1.2. Surface preparation

All vials and tweezers used for cleaning silicon were previously decontaminated in 3/1 v/v concentrated H_2SO_4/30% H_2O_2 at 105 °C for 30 min, followed by copious rinsing with ultrapure water. *Caution: the concentrated aqueous H_2SO_4/H_2O_2 (piranha) solution is very dangerous, particularly in contact with organic materials, and should be handled extremely carefully.* The *n*-type silicon wafers (0.3-0.7 Ω cm resistivity, phosphorus-doped, single side polished, 475-525 μm) (100) and the p^{++}-type silicon wafers (0.001-0.005 Ω cm resistivity, boron-doped, single side polished, 490-510 μm) (100) were purchased from University Wafers. All the Si surfaces were degreased by sonication in acetone, ethanol, and ultrapure water for 10 min respectively. The Si surfaces were then decontaminated and oxidized in piranha solution at 105 °C for 30 min, followed by rinsing with copious amounts of ultrapure water and dried under Ar flow. The 2 nm of Ir films were deposited on the clean p^{++}-Si/SiO_x *n*-Si/SiO_x surfaces by sputtering with a Leica EM ACE600 coating system (Ir target purity: 99.95%, Neyco). The thickness of the film was controlled *in-situ* using a quartz crystal microbalance. Then the coated surfaces Si surfaces (1.3 x 1.3 cm^2) were processed to fabricate the electrodes. An Ohmic contact was done on the backside of Si wafer by scratching the surface with a diamond glass cutter; then a droplet of InGa eutectic (Sigma Aldrich, 99.99%, metals basis) and a copper tape was applied on the scratched part. A thin layer of silver paste (Electron Microscopy Sciences) was painted to cover the InGa eutectic contact as well as a part of the copper tape. After the drying of the paste, Kapton tape was deposited to shield the backside for the protection of ohmic contact.[1]

1.3. Cells

1.3.1. Cell culture

The CHO-K1 cells where plated on the surface on the iridium side and incubated for 48 h with culture medium at 37 °C, 5% CO_2.

1.3.2. Labeling with SA@Ru for PECL+

The SA@Ru labels were prepared with a solution of 100 μL of ruthenium complex (10 mg/mL) in anhydrous DMSO, 100 μL of streptavidin (1 mg/mL in PBS), and 800 μL PBS. The solution was vortexed for 4 h at 4 °C and dialyzed overnight under stirring at 4 °C. For the labeling, the cells were fixed on the surface for 10 min with PFA 4%, permeabilized for 10 min with Triton X-100 0.1%, labeled first with biotin X 11 μM for 1h and then with streptavidin-ruthenium complex solution, SA@$[Ru(bpy)_3]^{2+}$ (0.1 mg/mL) for 45 min.[2]

1.3.3. Labeling with calcein for PECL-

The cells were labeled next with calcein-AM (1 µM in culture medium) for 15 min in incubator and then fixed 10 min with PFA 4%.[3]

1.4. PECL imaging

1.4.1. Electrochemical and microscopy experiments

The PECL cell was a 3-electrode system adapted on a C-type chamber from the Idylle company (www.idylle-labs.com) with a working electrode (*n*-Si/SiO_x/Ir or p^{++}-Si/SiO_x/Ir), a platinum wire (Pt) as the counter electrode and Ag/AgCl/KCl 3 M electrode as the reference electrode. The experiments were performed using a PalmSens4 potentiostat while the surface was illuminated on the back (on the *n*-Si side) by a LED at 1050 nm (Thorlabs, M1050L4). The PECL emission were measured with a spectrometer (Spectra pro 2300i from Princeton Instrument). The fluorescence and PECL images were recorded on the iridium side of the surface using an epifluorescence inverted microscope (Leica DMI8) and an Electron Multiplying Charge Coupled Device (EM-CCD 9100-13) Camera from Hamamatsu (see Figure S1). A 40x objective (Leica HC APO; water immersion) with a 0.8 numerical aperture was used for the PECL and fluorescence experiments. The ECL imaging was performed as a control experiment in the same conditions but using a p^{++}-Si/SiO_x/Ir surface as working electrode and without the need of illumination by the infra-red LED. The images were treated and analyzed by the ImageJ-Fiji software (open source). Green (PL or FL) and red (ECL, PECL+ or PECL-) are false colours coding the luminescence intensity.

1.4.2. Imaging

1.4.2.1. PECL+

The PL images of the cells labeled with the SA@Ru were captured using a longpass FITC filter (λ_{exc} = 470 ± 20 nm; λ_{DM} = 510 nm; λ_{Suppr} = LP 515 nm) with an exposure time (t_{expo}) of 0.5 s and a camera CCD in normal mode. The ECL and PECL+ images of SA@Ru were captured with t_{expo} =10 s and t_{expo} = 20 s respectively, using a shortpass IR filter (λ_{Suppr} = SP 750 nm) and with an EMCCD camera (sensitivity gain = 255; gain = 1).

1.4.2.2. PECL-

The fluorescence (FL) images of the cells labeled with calcein-AM were captured using a FITC filter (λ_{exc} = 480 ± 20 nm; λ_{DM} = 505 nm; λ_{Suppr} = 527 ± 15 nm) with a t_{expo} = 1 s and a camera CCD in normal mode. The ECL and PECL- images of the cells were captured in a solution containing the freely diffusing $[Ru(bpy)_3]^{2+}$ with t_{expo} =5 s and t_{expo} = 10 s respectively, using a shortpass IR filter (λ_{Suppr} = SP 750 nm) and with an EMCCD camera (sensitivity gain = 255; gain = 1).

2. Supplementary figures

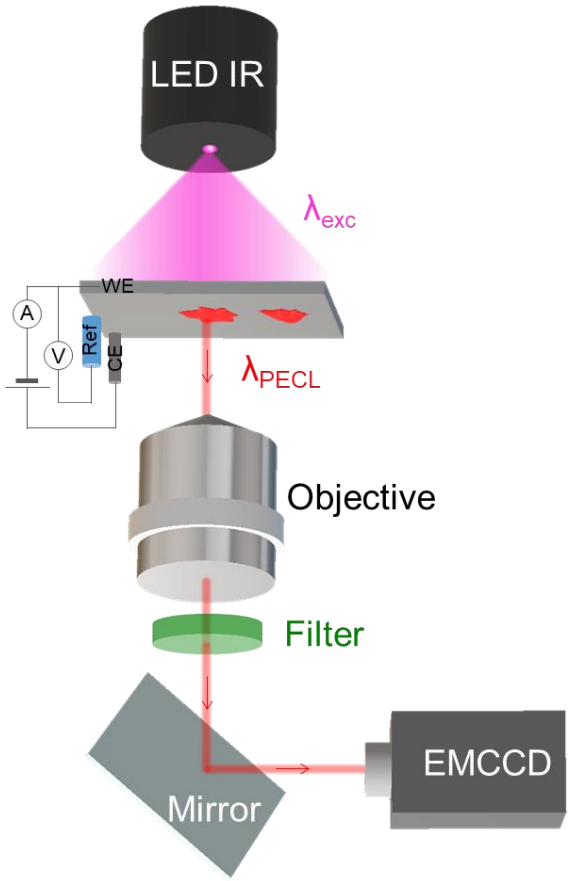

Figure S1. Scheme of the PECL set-up on an inverted epi-fluorescence microscope using a near-infrared LED in back-illumination.

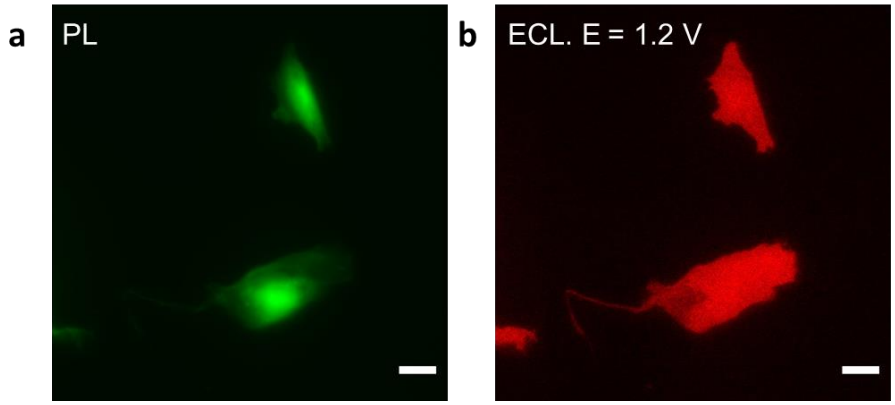

Figure S2. Microscopy images of CHO-K1 cells labeled with SA@Ru in a) PL and in b) ECL at E = 1.2 V (vs Ag/AgCl) in ProCell solution on p^{++}-Si/SiO_x/Ir electrode. Scale bar: 20 µm. Same region of interest (i.e. same cells) as Figure 4a,b.

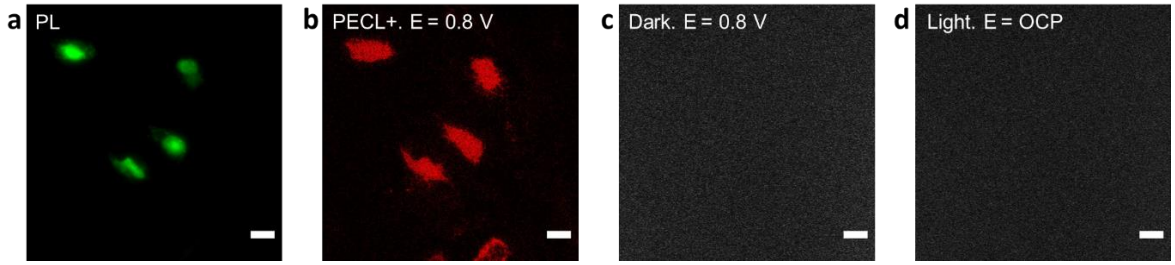

Figure S3. Microscopy images of CHO-K1 cells labeled with SA@Ru in a) PL, b) in PECL+ at 0.8 V in ProCell solution on *n*-Si/SiO_x/Ir electrode under infrared light. Control experiment showing the images recorded c) in the dark at 0.8 V and d) under infrared light at open circuit potential (OCP). Scale bar = 20 µm.

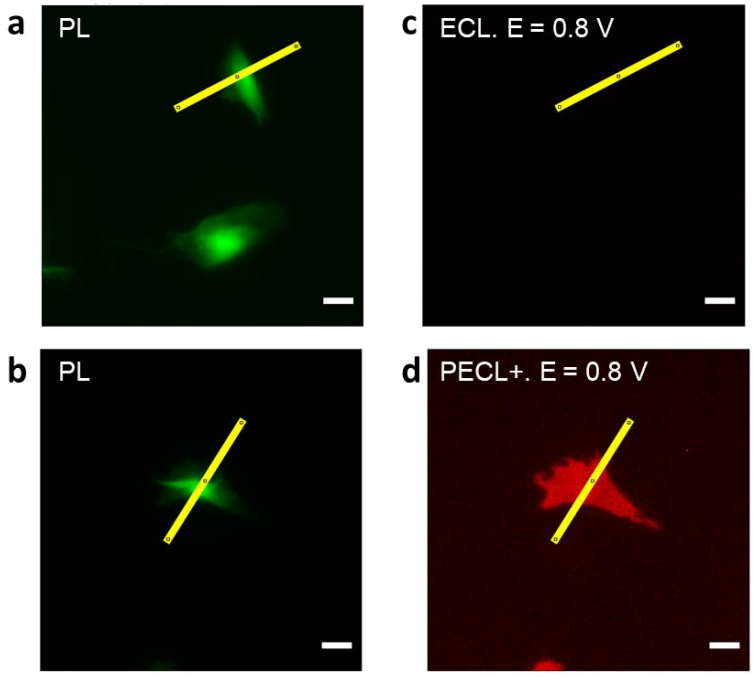

Figure S4. a,b) PL, c) ECL and d) PECL+ images of CHO-K1 cells labeled with SA@Ru at 0.8 V in ProCell solution. The luminescence intensity profiles were extracted along the yellow line. Same region of interest (i.e. same cells) as Figure 3. Scale bar: 20 µm.

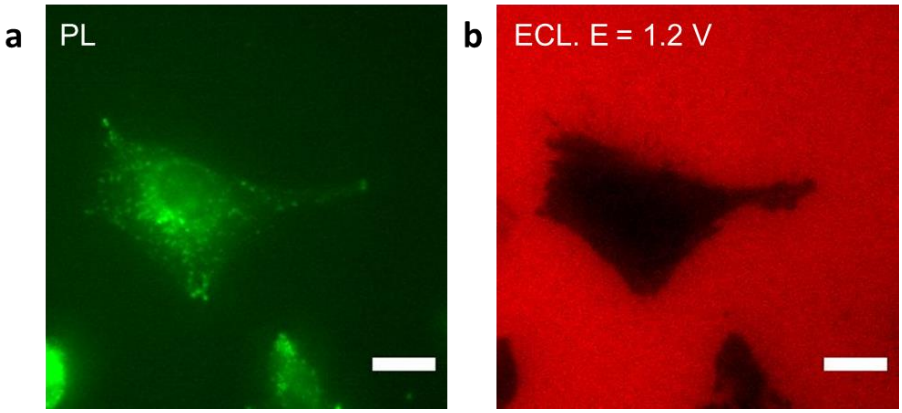

Figure S5. Microscopy pictures of CHO-K1 cells labeled with calcein-AM in a) FL and in b) ECL at 1.2 V recorded in PBS with 30 µM $[Ru(bpy)_3]^{2+}$ and 0.1 M TPrA on p^{++}-Si/SiO_x/Ir electrode. Same region of interest (i.e. same cells) as Figure 5a,b. Scale bar: 20 µm.

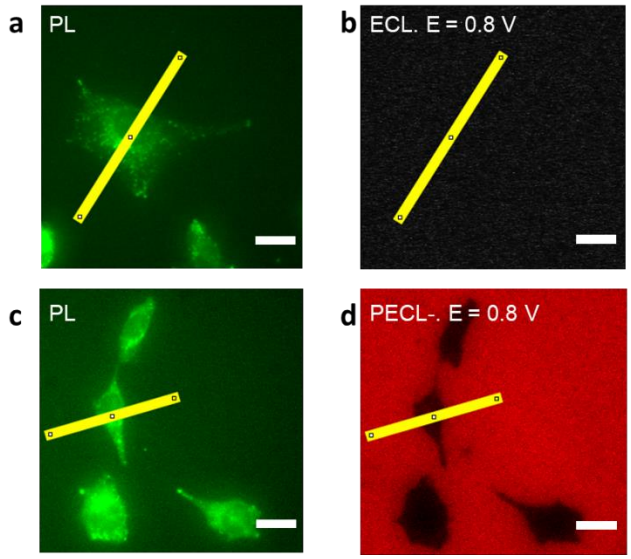

Figure S6. a,c) FL, b) ECL and d) PECL- images of CHO-K1 cells labeled with calcein-AM at 0.8 V in PBS containing 30 µM $[Ru(bpy)_3]^{2+}$ and 0.1 M TPrA. The luminescence intensity profiles were extracted along the yellow line. Same region of interest (i.e. same cells) as Figure 5. Scale bar = 20 µm.

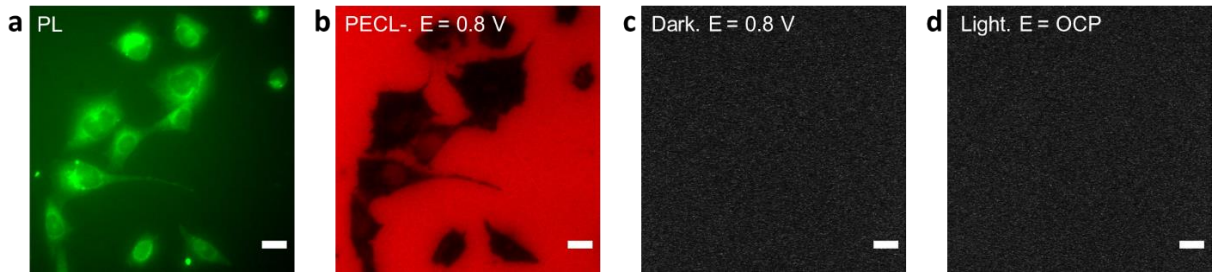

Figure S7. Microscopy pictures of CHO-K1 cells labeled with calcein-AM in a) FL, b) PECL- at 0.8 V in PBS containing 30 µM $[Ru(bpy)_3]^{2+}$ and 0.1 M TPrA on *n*-Si/SiO_x/Ir electrode under infrared light. Control experiment showing the images recorded c) in the dark at 0.8 V and d) under infrared light at open circuit potential (OCP). Scale bar = 20 µm.

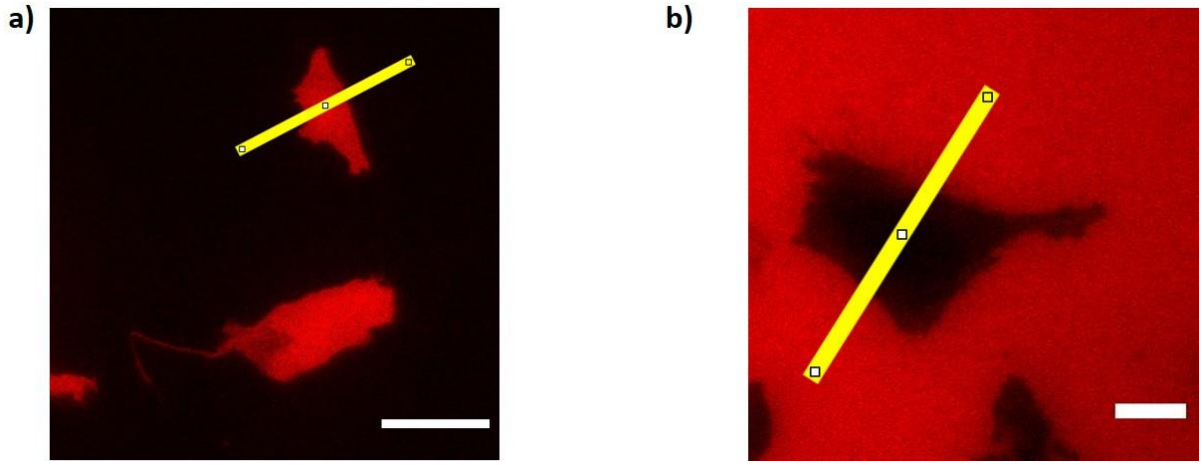

Figure S8. Luminescence intensity profiles along the yellow line of a) ECL+ with the same region of interest as in Figure S2 (scale bar: 50 µm) and of b) ECL- with the same region of interest as in Figure S5(scale bar: 20 µm).

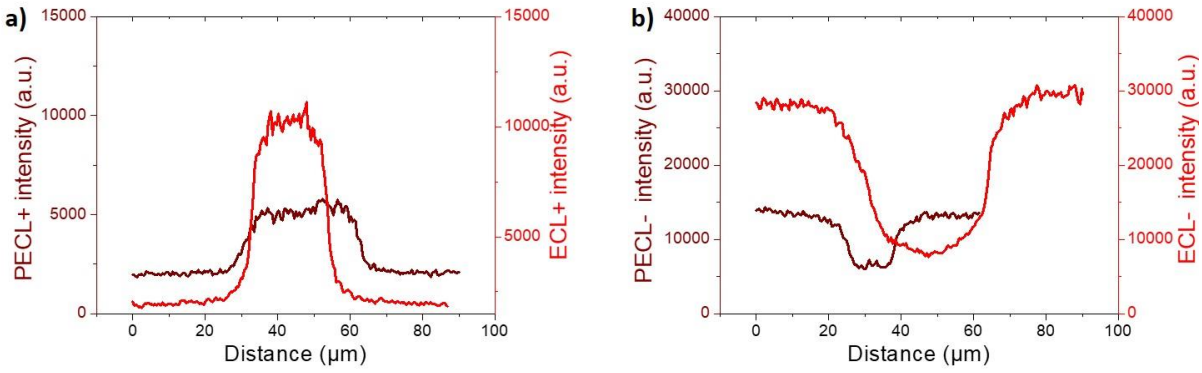

Figure S9. Comparison of the luminescence intensity profiles of the cells a) in ECL+ and in PECL+ and b) in ECL- and in PECL-. The axis along which the profiles were extracted are shown in Figure S4 for the PECL+, in Figure S6 for the PECL- and in Figure S8 for the ECL+/-.

3. References

1 Y. Zhao, J. Descamps, S. Ababou-Girard, J.-F. Bergamini, L. Santinacci, Y. Léger, N. Sojic and G. Loget, Ang*ew. Chem. Int. Ed.*, 2022, **61**, e202201865.

2 S. Voci, B. Goudeau, G. Valenti, A. Lesch, M. Jović, S. Rapino, F. Paolucci, S. Arbault and N. Sojic, *J. Am. Chem. Soc.*, 2018, **140**, 14753–14760.

3 J. Descamps, C. Colin, G. Tessier, S. Arbault and N. Sojic, *Angew. Chem. Int. Ed.*, 2023, **135**, e202218574.