# Design of Optoelectrodes for the Remote Imaging of Cells and in situ Electrochemical Detection of Neurosecretory Events

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**ABSTRACT:**

Optical fibers have opened avenues for remote imaging, bioanalyses and recently optogenetics. Besides, miniaturized electrochemical sensors have offered new opportunities in sensing directly redox neurotransmitters. The combination of both optical and electrochemical approaches was usually performed on the platform of microscopes or within microsystems. In this work, we developed optoelectrodes which features merge the advantages of both optical fibers and microelectrodes. Optical fiber bundles were modified at one of their extremity by a transparent ITO deposit. The electrochemical responses of these ITO-modified bundles were characterized for the detection of dopamine, epinephrine and norepinephrine. The analytical performances of the optoelectrodes were equivalent to the ones reported for carbon microelectrodes. The remote imaging of model neurosecretory PC12 cells by optoelectrodes was performed upon cell-staining with common fluorescent dyes: acridine orange and calcein-AM. An optoelectrode placed by micromanipulation at a few micrometers-distance from the cells offered remote images with single cell resolution. Finally, in situ electrochemical sensing was demonstrated by additions of $K^+$-secretagogue solutions near PC12 cells under observation, leading to exocytotic events detected as amperometric spikes at the ITO surface. Such dual sensors should pave the way for in vivo remote imaging, optogenetic stimulation, and simultaneous detection of neurosecretory activities.

**KEYWORDS** : optoelectrode; electrochemistry; catecholamine; ITO; imaging; optogenetics.

## 1. INTRODUCTION

Optoelectrodes, quoted also as optrodes (sometimes optodes), are sensors constituted of two types of transducing elements: 1) an optical element to transport light and induce photochemical or photobiological processes, and 2) an electrical conductor to achieve electrical or electrochemical processes.[1-7] Optical fibers (OF; named as well as optic fibers) have been widely used as light carriers to induce/capture events at distance from the photosensor (CCD, microscope, etc.) and have found multiple sensing applications.[4, 8-10] Their interests range from remote imaging for in vivo observations, remote light delivery as for optogenetics, and detection of chemical or biological reactions producing light.[4, 9, 11-18] Moreover, optical fibers can be fabricated of very diverse types of doped silica and glasses, used as single light conducting objects or organized in arrays such as in OF bundles. Their physical dimensions can be modulated at will in length (meters to mm) and diameter (centimeters to sub-micrometers) as function of their fabrication and etching (heating) techniques. Furthermore, OF are used as platforms, which surfaces can be currently grafted with any types of biological (antibodies, DNA, proteins, etc.) or chemical elements (dyes, nanoparticles, polymers, etc.) in particular for bioanalytical applications.[4, 9, 11, 12, 19] Striking examples concerning single molecule detections, ultrasensitive immunosensors, surface-enhanced Raman scattering (SERS) platforms, or as near-field probe arrays, have been reported.[19-25] More particularly, OF have raised a fast interest in neurosciences over the last two decades for first, brain imaging by fluorescence and the development of micro-endoscopes, and second for the achievement of optogenetics.[16, 26, 27] Single or bundles of OF are reported as minimally invasive and can be used in living and freely-moving animals.[28, 29] Imaging of brain zones or layers and specific neurons in rodents or humans was reported, as well as the monitoring of diverse brain activities such as calcium fluxes and handling or the electrical activity by using voltage-sensitive dye. Besides, optogenetics rely on the photo-actuation of a biological process (delivery, enzymatic activity, etc.) following animal genetic manipulations.[30] A localized and in situ photoactivation in the brain tissue with excellent time and spatial resolutions is mandatory to activate or inhibit a sub-population of neurons under study.[6, 26]

Among their multiple applications in neurosciences, electrodes have been used either for electrical measurements and electric pulse applications or as electrochemical sensors of neuroactive molecules. In this context, electrical and electrochemical sensors can be made of multiple types of bare conductors (metals, carbon, etc.) or semi-conducting layers and of any size (tens of nanometers and larger). Electric neural probes are mostly constituted of bare metal electrodes (tungsten, platinum, etc.) or metal deposit area on a multisensory support of micrometric dimensions. Of note, electrochemical sensors for neurobiology are devoted to the monitoring of neurosecretory events due to neurotransmitters (dopamine, glutamate, epinephrine, etc.) or neuromodulators (serotonin, nitric oxide, hydrogen peroxide, etc.). Some of these species are electroactive and can be directly detected on most of electrode surfaces, including the catecholamines (dopamine, epinephrine, etc.)[31-33] or serotonin, but some

others are not (peptide hormones, GABA, etc.) and necessitate the development of catalytic surfaces, in particular biosensors, such as for glutamate.[34-37] A major breakthrough of electrochemistry in neurosciences was the detection of secretory events at the membrane of single cells (neurons, adrenal chromaffin cells, mastocytes, etc.) and the ensuing deciphering of the steps of the exocytosis process.[38-42] In this case, exocytosis was studied with quantitative and time resolutions reaching zeptomoles and tens of microseconds, respectively (corresponding locally to detected fluxes of about 1000 molecules/ms).

Consequently, there is an evident and strong interest in developing analytical objects which possess optical and electrical / electrochemical conducting features, namely the optoelectrodes. The design of optoelectrodes has often, until recently, been proposed as an assembly or juxtaposition of an optical waveguide, an optical fiber or a glass capillary, with a metal wire or a carbon fiber[43]. This configuration leads to robust sensors, but with often a different localization of both sensing surfaces and thus to a loss of resolution, at least the spatial one. Therefore, such an optoelectrode allows one to provide light locally in a neuronal plane while the electric or electrochemical method is applied within the depth of the tissue. Electrochemical sensing with optoelectrodes was performed either on carbon microelectrodes and on surfaces designed by micro- and nanotechnologies. An alternative design is to coat the optical fiber end with a transparent or semi-transparent conductive material.[1, 44, 45] Neuronal probes may thus integrate areas for optical light delivery and capture, and some other micrometric windows for sensing of different neuromediators. Some recent designs also integrate microleds for light delivery at specific wavelengths, on which electroactive surfaces are added, for instance diamond and PEDOT:PSS layers.[46, 47] These whole integrated sensors demand multi-steps and multi-techniques developments for fabrication and thus often reported as engineering challenges.

We therefore propose in this work to use conventional OF bundles, extensively used as waveguides in imaging and widely transposed to bioanalytics over the last two decades. The distal end surface of OF bundles is modified by a transparent and semi-conductor Indium Tin Oxide (ITO) deposit providing the object with the desired dual sensing ability: optical and electrochemical detections. This design keeps the imaging properties of the optoelectrode and provides a colocalization of the electrochemical and optical responses. The shape, size and design of the final object is defined by the one of the OF bundle, as function of its composition in OF number, the glass properties, the separating cladding features and the whole dimensions (length, diameter). Herein, we used bundles of 300 µm diameter including about 6000 individual OFs (or cores) organized in a coherent pattern. The optical and electrochemical properties of these optoelectrodes, i.e. ITO-covered OF bundles, were characterized for the remote monitoring of neurosecretory cells. Thus, model cells originating from a pheochromocytoma of the adrenal medulla, PC12 cells, were both observed by bright field and fluorescence microscopy in remote mode through the optoelectrode. A single cell-imaging resolution (~ 5 µm) was achieved, demonstrating that such OF bundles offer, when used as optoelectrodes, a resolved imaging and accuracy for their

placement next to cells. The electrochemical detection of catecholamines (dopamine, norepinephrine, epinephrine) was evidenced in vitro and demonstrated on PC12 cells activated by a secretagogue (potassium solution injection). Amperometric peaks recorded with the optoelectrode are due to vesicular secretion via the exocytosis process and were similar to the ones usually measured with carbon fiber microelectrodes. Finally, our results pave the way for the development of optoelectrodes from existing optical objects via a 3-step process, and of further applications in combined optogenetics, fluorescence imaging and electrochemical sensing in neurobiology.

< Figure 1 here >

## 2. MATERIALS AND METHODS

**2.1. Preparation of ITO modified optic fiber bundles.** Coherent OF bundles with a total diameter of 300 µm comprising 6000 individually cladded 3 µm diameter cores were purchased from Sumitomo Electric Industries (IGN-035/06).[48] The imaging area and the numerical aperture (NA) of the OF bundle were 270 µm and 0.35, respectively. The black insulating jacket of the bundle was removed with dichloromethane and sonicated in water for 30 s to remove any residuals. Both faces of the bundle were cleaved before use. The distal end and the sides of the bundles were covered by ITO (100-150 nm thickness; 70-140 ohm.square$^{-1}$) deposited through a radio-frequency magnetron sputtering process.[49] The ITO-coated bundle was electrically connected to a copper wire with a silver paste and mechanically insulated on the sides with a commercial nail varnish. The only electroactive surface was then the distal surface of the OF bundle.

**2.2. Electrochemical experiments**. Cyclic voltammetry analyses were performed using a three-electrode electrochemical cell composed of the optoectrode or an ITO-coated glass (both prepared simultaneously) as working electrode, an AgCl-coated-Ag wire as reference electrode (250 µm diameter) and a Pt wire as counter electrode (500 µm diameter). All electrochemical measurements were carried out using a bipotentiostat (BioLogic, VSP-300, EC-Lab software) equipped with low current modules and kept in a home-made Faraday cage.

**2.3. Cell culture and sample preparation.** PC12 cells were purchased from the American Type Culture Collection (Manassas, VA). The cells were maintained in RPMI-1640 medium, supplemented with 10% heated inactivated horse serum (Life technologies), 5% heated inactivated fetal bovine serum (Life technologies) and 1% penicillin streptomycin solution (Life technologies) in a 5% $CO_2$, 100% humidity atmosphere at 37 °C. Cells were grown on cell culture flask with filter cap and were sub-cultured approximately every 4-5 days or when confluency reached almost 100%. Throughout the cell culture's lifetime, the medium was refreshed every 2 days. PC12 cells were then grown on human placenta collagen (Bornstein and Traub type IV)-coated 50 mm glass bottom dish (MatTek Corporation, Ashland,

USA) for 48 h in complete growth medium. For imaging purposes, PC12 cells in a dish were first washed with PBS and incubated either in RPMI 1640 medium (serum free) with 5 µM calcein-AM (5 mM stock solution in DMSO) for 30 min at 37 °C, or with a 5 µM solution of acridine orange in PBS for 30 min at 37 °C. Then, cells were washed three times with PBS and observed by epifluorescence microscopy (Leica DMI6000B) with the right filters. Before secretory measurements with the optoelectrodes, PC12 cells were loaded by L-DOPA. L-DOPA was dissolved and kept at 10 mM in a buffer stock solution, composed of 11.2 mL of 1 M D-Glucose, 1.68 mL of 2.5 M KCl, 20 mL of 0.5 M HEPES (pH 7.5) and 35 mL of 0.02 M $MgCl_2$. 5.13 mL of 3 M NaCl solution were then added to 10 mL of the buffer stock solution, and the whole solution was subsequently diluted to 100 mL using water. Cells were incubated with 100 µM L-DOPA in bath solution for at least 60 minutes at 37°C before secretory stimulation and recording. All chemical and reagents are from Sigma-Aldrich Co., France, except when noted.

**2.4. Optoelectrode measurements on a cell population.** Electrochemical recording of exocytosis from PC12 cells were performed on an inverted microscope (Observer D1, Carl Zeiss AG) inside a Faraday cage. Before the experiment, the cells were rinsed three times with PBS and were maintained in HEPES physiological saline (150 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 5 mM Glucose, 10 mM HEPES and 2 mM $CaCl_2$) throughout the experiment at room temperature. The working optoelectrode was positioned by a micromanipulator (Model MHW-103, Narishige Co., London, UK) in contact with a population of PC12 cells (distance of less than 0.5 µm). During the experiment, the electrode was held at a constant potential of +600 mV vs. Ag/AgCl using a low-current potentiostat (model AMU-130, Radiometer Analytical Instruments, Copenhagen, Denmark). The output was digitized at 40 kHz and the current was recorded as a function of time. A glass microcapillary containing the HEPES physiological saline medium supplemented with a $K^+$ 100 mM (KCl) secretagogue (osmolality is kept constant by adapting the NaCl concentration) was positioned by another micromanipulator under the optoelectrode in order to inject the secretagogue at less than 50 µm from the PC12 cells population. The amperograms were collected from cells stimulated by a 60 s-duration $K^+$ injection.

**2.5. Data analysis.** Each amperometric trace recorded during cell secretion was visually inspected and false positive peaks (artefacts, superimposed and kinetically distorted events) were manually rejected. The peaks with their maximum current 3 times higher than the fluctuation (~1 pA) of the baseline current were identified as exocytotic spikes and were collected from amperograms. Each spike characteristics, i.e., the maximum oxidation current $I_{max}$ (pA), the full width at half maximum $t_{1/2}$ (ms), the total electrical charge Q (fC) were analyzed by a lab-made software. Values were reported as the mean ± SEM of the data.

< Figure 2 here>

## 3. Results and discussion

### 3.1. Optoelectrode fabrication.

The first part of this work was devoted to the technical development of optoelectrodes with dual detection abilities, i.e. optical and electrochemical. The current protocol derives from a previously reported one[50], aimed at designing gold microelectrodes possessing arrays of nanotips on their surface, and their use for spectro-electrochemical characterization of surfaces (physical, biological) by e.g. combined SECM (Scanning Electrochemical Microscopy)[51] and SERS (Surface Enhanced Raman Spectroscopy).[21, 52, 53] In this work, an optical fiber bundle of 300 µm diameter, composed of about 6000 individual multi-modal optical fibers (3 µm mean diameter each), was covered by an electrically-conductive surface (Au layer) providing the sensor with the ability to perform electrochemistry on one its surface (Fig. 2). However, the Au layer prevents the use of the modified OF bundle for imaging purposes. In this work, the distal end of the whole bundle was covered by a layer of ITO (middle picture in Fig. 2) thanks to magnetron sputtering technique. The absorbance of the ITO layer (100-150 nm thickness) was measured by spectrophotometry and was not higher than 8 % within the 380-800 nm wavelengths window, in comparison with a non-modified glass coverslip (Fig. S1). ITO absorbance rose at wavelengths below 350 nm (UV region). Then, in order to transform the ITO modified bundle into an electrode, the side surface of the bundle cylinder was covered with a layer of an insulating varnish. The process was performed with caution to keep the end-surface of the bundle free from resin and create an effective disk electrode surface (right picture in Fig. 2). An electrical connection between a wire and the ITO layer was made on sides of the bundle (far from the tip) and insulated. These sensors were sufficiently stable mechanically to be manipulated (bending, positioning, placed in a holder, etc.), such like for a usual OF bundle or microelectrode, and could be used for at least 10 experiments, without observing net delamination of the resin or of the ITO deposits over. These analytical objects were then characterized as efficient optoelectrodes for neurosecretory cell measurements.

### 3.2. Electrochemical characterization of the optoelectrodes.

The electrochemical activity of the ITO-covered optical bundles was tested towards several catecholamine moieties – dopamine, epinephrine (quoted also as adrenaline) and norepinephrine (quoted also as noradrenaline), since these are the main catecholamine species to be released by different types of neuronal or neuroendocrine cells. The optoelectrode response was tested first by cyclic voltammetry (CV) to assess on the oxidation potentials of species and electrode kinetics of ITO surfaces. As shown on Fig. 3, voltammograms recorded at low scan rate (10 mV $s^{-1}$) display typical pseudo-stationary and irreversible oxidation waves peaking at +258, +337 and +320 mV vs Ag/AgCl for dopamine, epinephrine and norepinephrine, respectively. These values are typical for the oxidation of catecholamines detected usually on glassy carbon or carbon fiber electrodes[32, 54, 55], and

demonstrate that sufficiently fast transfer kinetics occur at thin film ITO surfaces prepared herein.[56, 57]

< Figure 3 here >

ITO optoelectrodes were then analyzed by amperometry for their response versus concentration variations of the catecholamine species (Fig. 4A); dopamine, epinephrine and norepinephrine are the main redox species released by neuroendocrine cells such as PC12 cells used further for the detection of a secretory activity. Bolus of 2 µM catecholamine solutions, each independently, were progressively injected to a PBS buffer solution and detected at optoelectrodes (Fig. 4A, 4B and 4C for dopamine, epinephrine and norepinephrine, respectively). The current variations, i.e. the increases between plateau values, were plotted versus the catecholamine concentration in solution (plot for dopamine in Fig. 4D shown as an example). The optoelectrode responses appeared perfectly linear ($R^2 = 0.99$) with concentrations of each catecholamine in this usual working range (up to 100 µM). A limit of detection (LOD) of 0.5 µM (current variation equals to 3 times the mean noise value) could be detected. In addition, the current rises due to dopamine injections were fast enough (essentially hampered by the injection and mixing times *in vitro*) to use such sensors for the monitoring of secretory events on cells. The question about the selectivity of such catecholamine measurements versus interferent species may arise as for any electrochemical sensor. As widely discussed in literature, such concern specially applies for the measurement of steady-state concentrations of neurotransmitters, herein catecholamines, in body fluids including the whole blood, blood plasma, cerebrospinal fluid, etc..[58-60] However, in present work, the aim is to detect secretory events by exocytosis at living cells. Such extracellular release events lead to transient concentration variations and thus transient current increases. These faradaic transients are less susceptible to the baseline current level, possibly modified as function of the concentration of oxidizable interferents (typically, ascorbic acid, uric acid, etc.) that are present *in vivo*. Overall, the electrochemical features of the ITO optoelectrodes afforded the necessary requirements for a dual sensor design. The imaging properties of the optoelectrodes were then studied to assess for their ability to observe cells with enough resolution and sensitivity for further *ex vivo* analyses (and potent future *in vivo* applications).

< Figure 4 here >

**3.3. Imaging of neurosecretory cells with the optoelectrodes.**

Model neurosecretory PC12 cells were used for optical imaging experiments because of their ease of culture, widely reported catecholamine exocytosis features, as well as of their small size (5-10 µm diameter, displaying a rather round shape in absence of differentiation factors) equivalent to neuronal cells. PC12 cells were pre-loaded with L-DOPA under a standard protocol used to increase their secretory vesicle content and quantal catecholamine release. Cells were cultured in Petri dishes and observed first with an inverted optical microscope in bright field and by fluorescence (Fig. 5A) following two types of staining: the addition of acridine orange, known to stain acidic compartments such as secretory vesicles, and of calcein-AM to stain active cells.

< Figure 5 here >

The optoelectrodes were then used to image PC12 cells in remote mode as compared to direct observations. The experimental requirements to perform such optical fiber-based imaging are the following: each bundle face must be perfectly flat and clean; the unmodified and freshly cleaved face (i.e. proximal end) is positioned in front of the microscope objective, at its focal plane to inject light (and collect back) in the optical fibers composing the bundle; the other ITO-modified face of the bundle (i.e. distal end) is positioned with a micromanipulator in the Petri dish (filled with buffer solution) where cells are present. Excitation light is injected in the proximal end of the optoelectrode and guided in each core until the distal end where it excites fluorescence. The optical signals are transmitted back through the coherent bundle to the objective and CCD camera of the microscope for analysis of the images. To obtain well-focused images, the distal end of the optoelectrode is gently and progressively displaced towards cells. The whole image through the optoelectrode first appears as blurred and unfocused. However, when the distance between cells and the ITO surface reaches the focal distance (2-3 µm) of the fibers in the bundle, cells located below are observed and well-resolved. As shown on Fig. 5B, islets and individual cells are observed with good resolution with both fluorescent dyes. Bright field observations were more difficult to achieve, though not impossible, because of a lack of optical contrast for cell observation.

Obviously, as with any coherent assembly of optical fibers, the working distance was much shorter than with a classical objective. Such optical sensors are indeed dedicated to *in situ* observations and delivery of light, very near the location where cells must be imaged or photo-activated (optogenetics). Despite this experimental difficulty of positioning at micrometric distance from the cell layer, the optoelectrode was sufficiently mechanically robust to be manipulated and to keep constant properties.

**3.4. Electrochemical detection of cell secretion with optoelectrodes.**

The aforementioned very low distance, few micrometers at most, between the electrode surface (ITO covered bundle face herein) and cells is actually a pre-requisite for electrochemical detection of

exocytotic events at cell membranes. Indeed, several groups have studied in details the cell-electrode distance effect on the quantitative and kinetic features of amperometric spikes related to exocytosis.[61-64] Quantal exocytotic releases are fast (milliseconds timescale, see below) events related to minute amounts of neurotransmitters secretion. Consequently, any increase of the distance leads to a loss of response-time and collection efficiency, which impairs the detection of such single biological events due to single vesicle releases. The micrometric range of distances between the optoelectrode surface and PC12 cells might thus be a positive condition to confine cell secretion events and possibly observe them. In other words, this micrometric distance fulfills both optical and electrochemical criteria.

< Figure 6 here >

Fig. 6A displays a typical experiment where PC12 cells in culture in a Petri dish are observed directly through the microscope objective and the optoelectrode is positioned next to the cell layer. Then, a micro-injection pipette is inserted in between the sensor and cells in order to inject locally a secretagogue, potassium herein to induce membrane depolarization and vesicular exocytosis. As shown in Fig. 6B, when a $K^+$ solution (100 mM KCl in buffer) was injected, well-resolved amperometric spikes were detected within seconds following the secretion activation. Control experiments show that in the absence of secretagogue injections or cell mechanical stimulation that may happen during the optoelectrode positioning, the amperometric response was very stable and no spikes were observed (Fig. 6C). A careful analysis of the detected amperometric spikes provided the following quantitative and kinetic parameters (n=26 from 3 experiments): $I_{max}$ = 10.3 ± 1.1 pA; Q = 167 ± 16 fC; $t_{1/2}$ = 13.2 ± 0.7 ms. Such values are similar to those usually reported for PC12 cells,[65-67] which correspond to minute amounts of detected catecholamines (N ≈ 2 attomoles/spike, though cells were previously loaded with L-DOPA), fast releases ($t_{1/2}$ = 2-20 ms) and various maximum fluxes ($I_{max}$ = 3-32 pA), mostly related to the secretory vesicle features (diameter, mode of exocytosis). Of note, much less-defined peaks of low amplitude and slower kinetics could also be detected in these experiments but could not be analyzed properly. These signals certainly arose from cell secretions occurring at higher distances from the ITO optoelectrode surface, either from cells located on the outskirt of the optoelectrode or when the surface is positioned at higher distances from cells below. Such a situation may happen because of an imprecise optoelectrode positioning, due a tilt of the ITO surface versus the one of the dish or due to a distance not enough close from cells. However, when using the optoelectrode as the electrochemical sensor and simultaneously as the remote optical sensor, the right distance would be similar for both the imaging (right focal plane) and maximum collection efficiency (electrochemical oxidation) of cell exocytotic events. Consequently, in operando conditions would benefit both detection strategies.

## 4. Conclusions

In this work, we developed optoelectrodes providing dual optical and electrochemical sensing features for neurobiological applications. We demonstrated that optoelectrodes based on optical fiber bundles allowed to image remotely individual neurosecretory cells (i.e. PC12 cells) and simultaneously detect their secretion of catecholamines following a stimulation. The electrochemical performances of the reported optoelectrodes were similar to the ones of carbon microelectrodes, which are classically used for such experiments. Amperometric peaks corresponding to single exocytotic events at the level of stimulated PC12 cells were resolved at the optoelectrode surface. Such sensors could as well be used to inject light through in order to perform optogenetic activations. The current optoelectrode was about 300 µm in diameter, which should be reduced for future in vivo insertion and lowering damages. However, optical fiber bundles with a lower number of individual element (i.e. core) are commercialized with typical size in the range of few tens of micrometer. In addition, the starting optical fiber bundles could possibly be pulled down[68] since these are based on doped silica, and henceforth lower diameters of the final sensor should be reached, typically few tens of micrometers. This will improve all manipulation aspects of the optoelectrode and collection efficiency of secretory events but decrease the size of the area imaged remotely. Also, the ITO surface could be modified by other sensing, more specific layers including enzymes to gain better bioanalytical performances. Such developments will constitute a further step toward fully operational optoelectrodes for *in vivo* applications.

## Credit authorship contribution statement

SG, PG and LG were involved in the design, fabrication and optic, electrochemical characterizations of the optoelectrodes. SG, JP and MGC performed cell cultures and microscopy observations. MGC, JP, FL and SA performed electrochemical measurements on cells and data treatments. NS and SA conducted the project funding acquisition, administration and scientific supervision. SA, NS, MGC, and FL wrote the manuscript. All authors have given approval to the final version of the manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A.** Supporting information. The supplementary data associated with this article can be found at the end of this manuscript and further in the online version.

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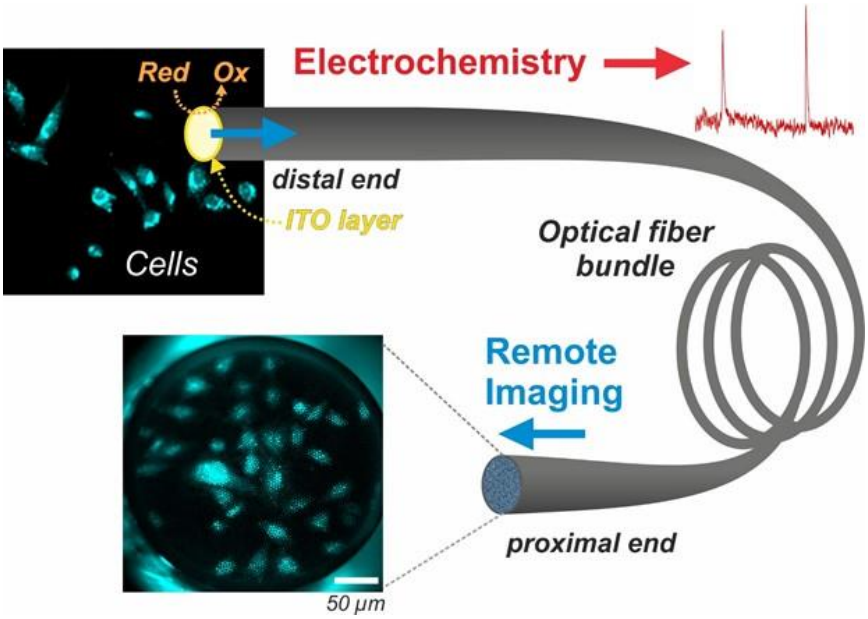


**Figure 1.** Sketch of the features of optoelectrodes developed in this work, allowing to image remotely single neurosecretory cells and detect in situ by electrochemistry their release of neurotransmitters. Optoelectrodes have their distal end modified by a transparent and conductive ITO deposit used as the working electrode.

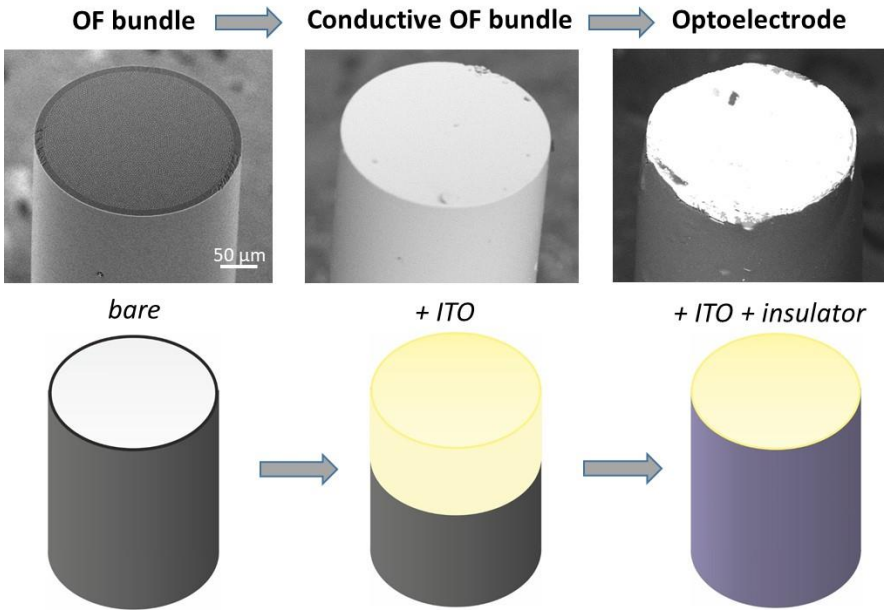


**Figure 2.** Major steps in the fabrication of optoelectrodes. They are based on the modification of distal face of the OF bundles (300 µm-diameter including ~ 6000 individual fibers of 3 µm-mean diameter) with thin semi-transparent and conductive ITO layer and ensuing lateral coverage of the bundle body by an electrically and optically insulator (e.g. nail varnish).

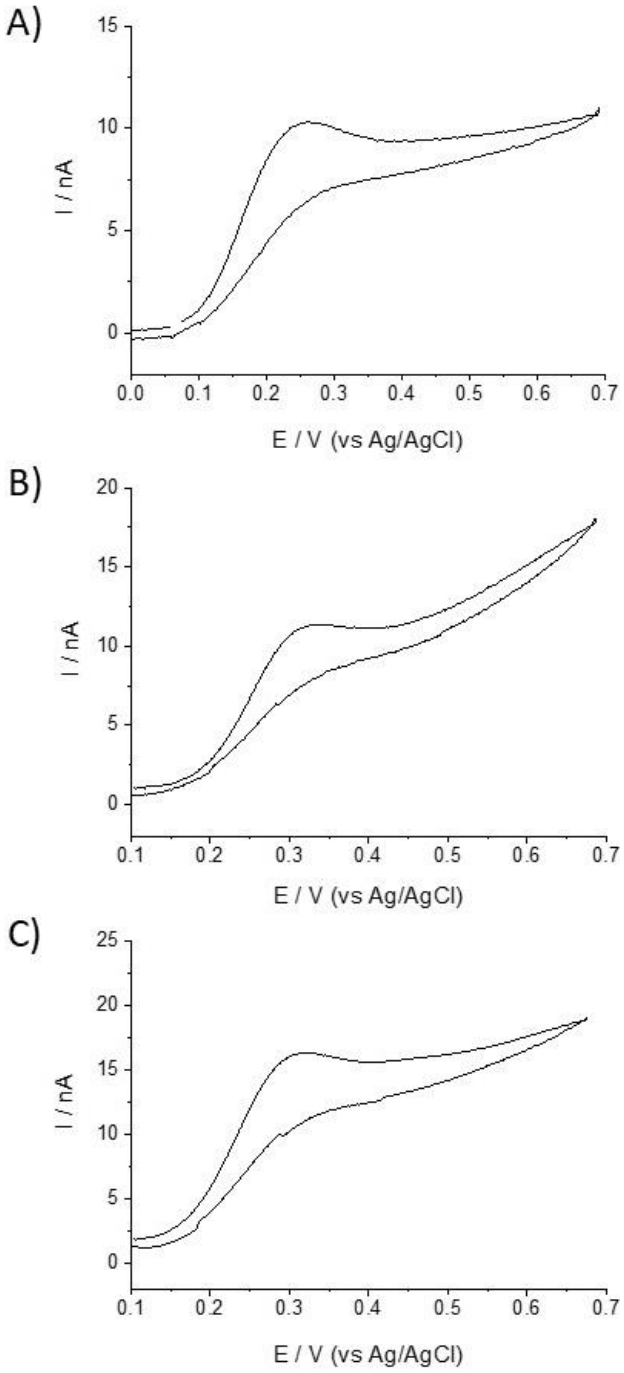


**Figure 3.** Cyclic voltammograms performed with an ITO optoelectrode in solutions of A) dopamine, B) epinephrine and C) norepinephrine, each at 50 µM in PBS (10 mM, 330 mOsm, pH 7.4). Curves were subtracted from the background response in PBS. Scan rate: 10 $mV.s^{-1}$.

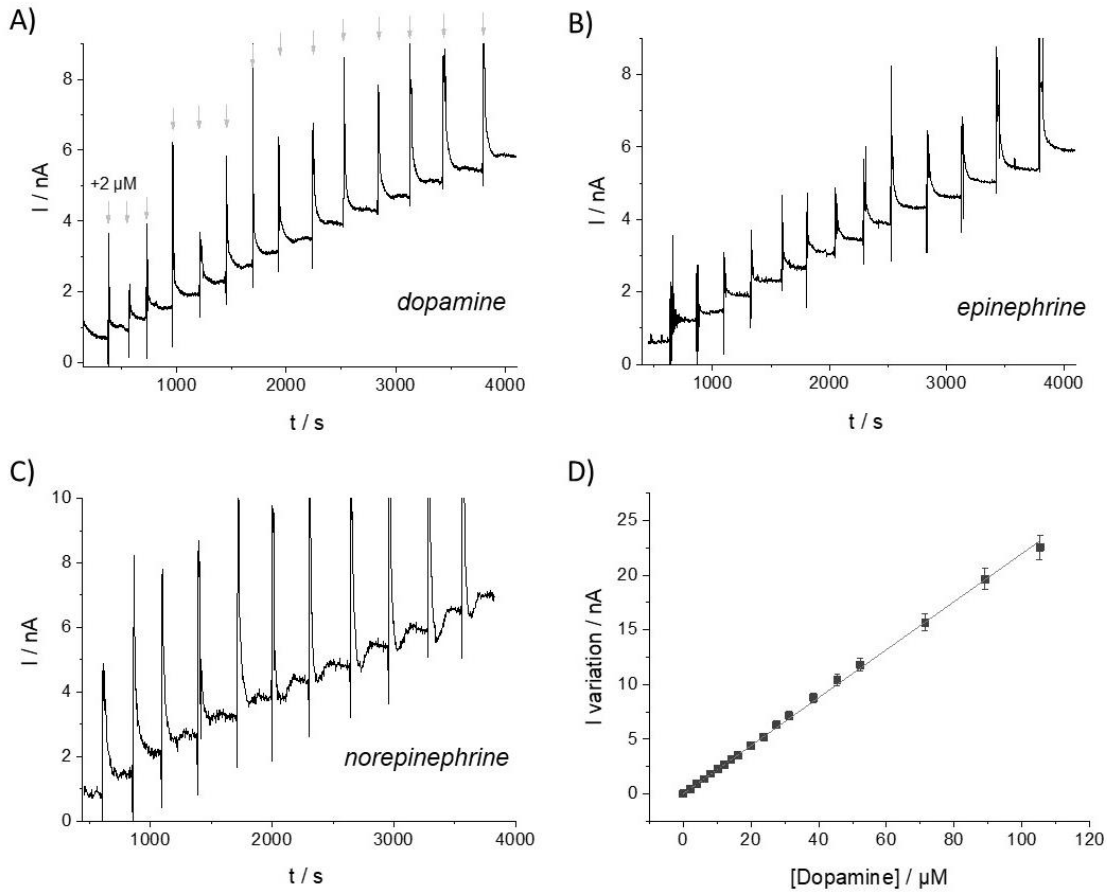


**Figure 4.** Faradaic current responses of the ITO optoelectrodes versus the concentration of catecholamines in solution (PBS, pH 7.4). Current variations were detected by amperometry at +600 mV vs Ag/AgCl (3 M NaCl) following several injections of 2 µM solutions of each catecholamine: A) dopamine, B) epinephrine and C) norepinephrine. Artifacts on curves are due to injections and rapid mixing of the solution to reach a homogenous concentration. D) Plot of the current increase as function of the total dopamine concentration in solution, shown as example. Slopes of the response linear fits ($R^2$ = 0.99) are 0.26 nA $\mu M^{-1}$, 0.35 nA $\mu M^{-1}$ and 0.39 nA $\mu M^{-1}$ for dopamine, epinephrine and norepinephrine, respectively; values are the means (± SD in graph) from 3 experiments with different optoelectrodes.

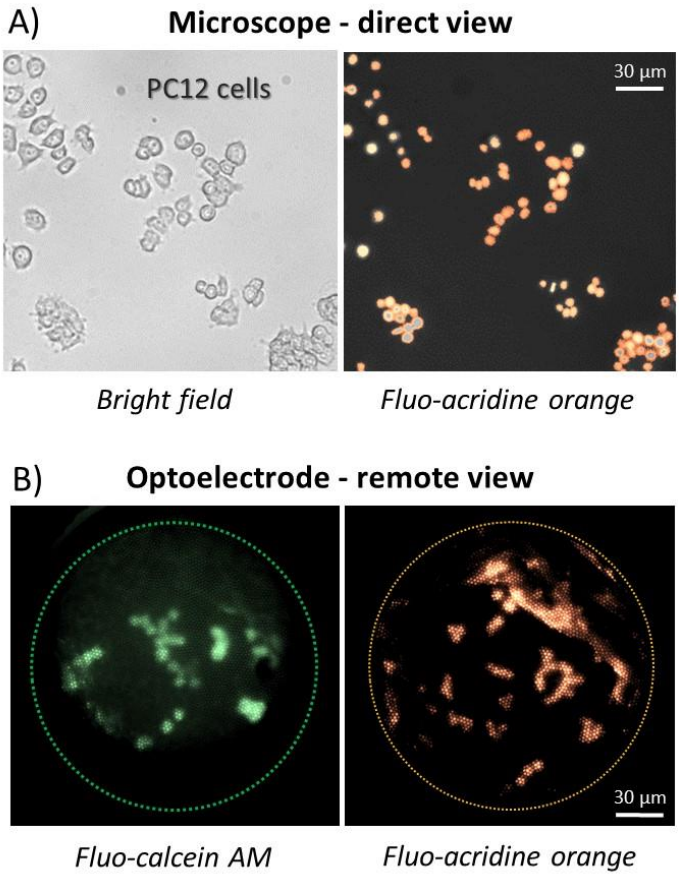


**Figure 5.** Microscope imaging of PC12 cells cultured in a Petri dish, either (A) in direct mode of observation (inverted microscope) or (B) in remote mode through an optoelectrode. Cells were observed in transmitted light or by fluorescence, owing to two different dyes accumulating in acidic compartments (acridine orange) or in the cytocol of metabolically active cells (calcein-AM). The optoelectrode flat faces were placed by micro-manipulation on one side (ITO surface) in the Petri dish at few microns-distance from cells, and on the other side at the focal plane and parallel to the objective (x20) to achieve remote imaging.

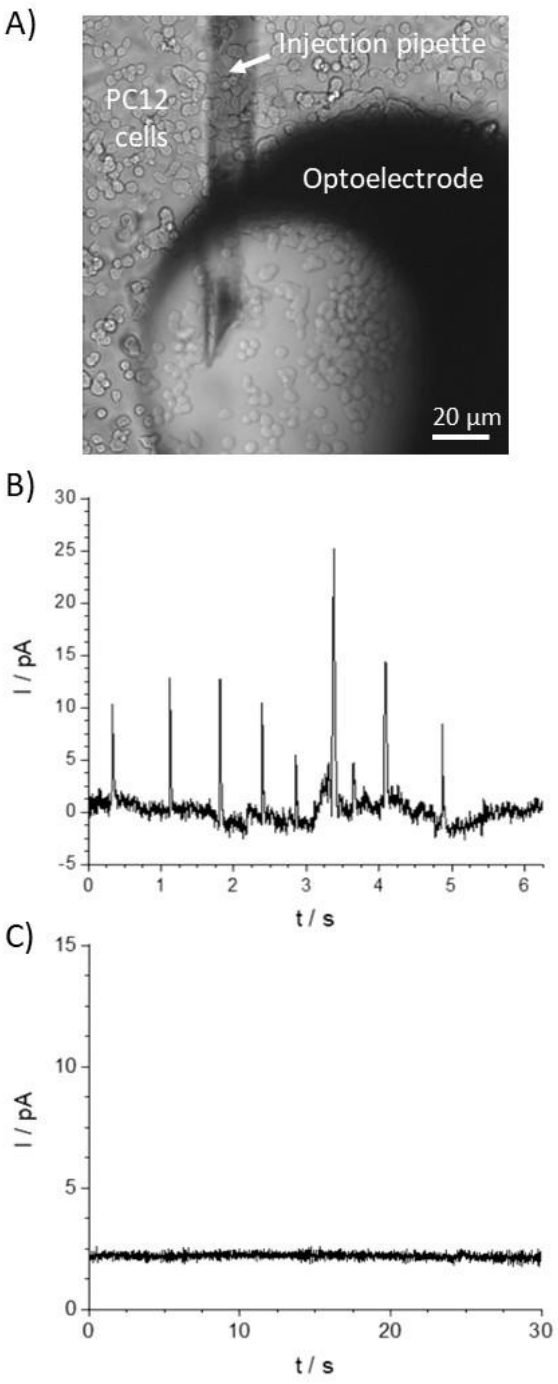


**Figure 6.** Electrochemical detection of catecholamine secretion by PC12 cells with an ITO optoelectrode. (A) Microscope imaging of the cells and optoelectrode tip placement next to them, while the tip of a microcapillary is placed in between to inject locally a $K^+$ 100 mM-secretagogue solution. (B) Amperometric response (at +600 mV vs Ag/AgCl) detected with the optoelectrode and displaying exocytotic peaks few seconds after the secretagogue injection. (C) Control experiment, same conditions as in (B) without the secretagogue injection nor optoelectrode movement during detection.

**APPENDIX A : Supplementary Information**

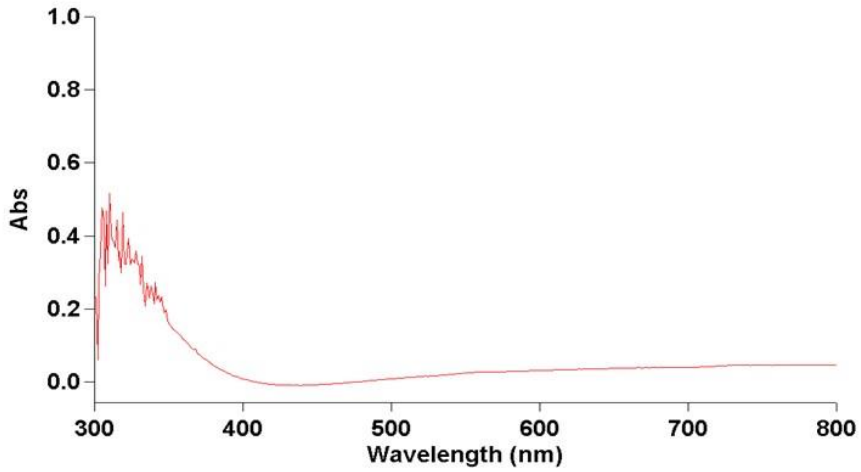


**Figure S1.** Absorption spectrum in the range of visible wavelengths of the ITO deposit (subtracted from the support glass slide contribution) used to prepare optoelectrodes from modified OF bundles.