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Light responsive azo-tz-PEDOT:PSS for cell interfacing

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Light responsive azo-tz-PEDOT:PSS for cell interfacing

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And for you, my dear reader. Results and ideas are meaningless if not revised, questioned, and reinterpreted. I thank you in advance for taking your time to try to understand my work, and I hope it gives you interesting insights.

*"e não se trata de querer voltar
ou de nunca ter saído
trata-se do esforço
de recordar dia
após dia que a vida
se faz de improviso
e que partir sempre
é outra maneira
de ficar"*
(Adriana Lisboa, em *Deriva*)

ABSTRACT

In recent years, non-genetic optical methods have emerged as a promising approach for cell stimulation. These techniques utilize photoactive conductive substrates to trigger a response in cells through localized heating or electric charge generation, offering spatio-temporal resolution and avoiding the need for invasive voltages or genetic modification (XU et al., 2023). Organic p-type semiconductors, particularly the conductive polymer poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), have earned substantial interest in the field due to their biocompatibility, flexibility, stability, and dual electronic and ionic conduction, which are crucial for effective integration with biological environments (PITSALIDIS et al., 2022). Despite the progress made with pristine PEDOT:PSS, its application in optoelectronic cell stimulation is limited by low absorption in the UV-visible spectrum. There is a critical need for materials that combine excellent biocompatibility, stability, and conductivity with enhanced optical properties to enable efficient and controllable light-based cell stimulation.

This study aims to characterize a novel light-responsive derivative, azo-tz-PEDOT:PSS, which incorporates photoresponsive azobenzene moieties into a PEDOT:PSS film (CORRADO et al., 2023). The goal is to evaluate its potential for organic optoelectronic cell interfacing by assessing the morphological, electrochemical, and photoelectronic properties. The morphology of the films was analyzed using scanning electron microscopy (SEM), atomic force microscopy (AFM) and contact angle measurements, revealing a surface roughness of 24.50 nm. The electrochemical properties and stability of the material were examined through cyclic voltammetry and electrochemical impedance spectroscopy in both saline (DPBS) and cell media, showing a cathodic charge storage capacity of 1.10 mC/cm² in DPBS and 1.36 mC/cm² in cell medium. The cut-off frequency analysis indicated minimal signal distortion for frequency components above 50.12 Hz in DPBS and 73.99 Hz in cell medium, suggesting effective signal transduction capabilities. Additionally, upon UV irradiation at 365 nm, the films generated a photocurrent without requiring an external voltage bias, demonstrating effective charge injection dependent on the electrolyte composition and light intensity. Finally, biocompatibility tests using L-929 and retinal precursor R28 cell lines confirmed negligible cytotoxicity, supporting the material's potential for safe long-term cell interfacing applications.

The promising results suggest that azo-tz-PEDOT:PSS films can be further developed to exploit the spatio-temporal precision of light for more controlled cell stimulation. Future research will focus on exploring the effects of the photogenerated current on cellular activity and optimizing the material to shift its absorption wavelength in order to increase

light penetration depth and enhance biocompatibility.

Keywords: Optoelectronic cell stimulation, conductive polymers, azobenzenes, organic bioelectronics

RESUMO

Nos últimos anos, métodos ópticos não genéticos surgiram como uma abordagem promissora para a estimulação celular. Essas técnicas utilizam substratos condutores fotoativos para desencadear uma resposta nas células por meio do aquecimento localizado ou da geração de carga elétrica, proporcionando uma resolução espaço-temporal e evitando a necessidade de tensões invasivas ou modificação genética (XU et al., 2023). Semicondutores orgânicos do tipo p, especialmente o polímero condutor poli(3,4-etilenodioxitiofeno) sulfonato de poliestireno (PEDOT:PSS), têm despertado grande interesse no campo de estudo de bioeletrônica devido à sua biocompatibilidade, flexibilidade, estabilidade e condução dupla (eletrônica e iônica), cruciais para a integração eficaz com ambientes biológicos (PITSALIDIS et al., 2022). Apesar do progresso alcançado com o PEDOT:PSS puro, sua aplicação na estimulação optoeletrônica de células é limitada pela baixa absorção no espectro UV-visível. Existe uma crescente necessidade de materiais que combinem excelente biocompatibilidade, estabilidade e condutividade com propriedades ópticas aprimoradas para possibilitar uma estimulação celular baseada em luz de maneira eficiente e controlável.

Este trabalho visa caracterizar um novo derivado de PEDOT:PSS fotossensível, azo-tz-PEDOT:PSS, que incorpora grupos fotoativos de azobenzeno em um filme de PEDOT:PSS (CORRADO et al., 2023). O objetivo é avaliar seu potencial para interface optoeletrônica orgânica com células, analisando suas propriedades morfológicas, eletroquímicas e fotoeletrônicas. A morfologia dos filmes foi analisada usando microscopia eletrônica de varredura (SEM), microscopia de força atômica (AFM) e medições de ângulo de contato, revelando uma rugosidade de superfície de 24,50 nm. As propriedades eletroquímicas e a estabilidade do material foram examinadas por meio de voltametria cíclica e espectroscopia de impedância eletroquímica em solução salina (DPBS) e em meio de cultura celular, revelando uma capacidade de armazenamento de carga catódica de 1,10 mC/cm² em DPBS e 1,36 mC/cm² em meio de cultura celular. A análise da frequência de corte indicou distorção mínima do sinal para componentes de frequência acima de 50,12 Hz em DPBS e 73,99 Hz em meio de cultura celular, sugerindo capacidade de transdução de sinais eficaz. Além disso, sob irradiação UV a 365 nm, os filmes geraram uma fotocorrente sem a necessidade de polarização externa de tensão, demonstrando uma injeção de carga eficaz dependente da composição do eletrólito e da intensidade da luz. Por fim, testes de biocompatibilidade utilizando as linhagens celulares L-929 e precursoras retinianas R28 confirmaram uma citotoxicidade negligenciável, evidenciando o potencial do material para aplicações seguras de interface com células a longo prazo.

Os resultados promissores sugerem que os filmes azo-tz-PEDOT:PSS podem ser desenvolvidos ainda mais para explorar a precisão espaço-temporal da luz, proporcionando uma estimulação celular mais controlada. Pesquisas futuras se concentrarão na exploração dos efeitos da corrente fotogerada na atividade celular e na otimização do material para alterar seu comprimento de onda de absorção, de forma a aumentar a profundidade de penetração da luz e a melhorar a biocompatibilidade.

Palavras-chave: Estimulação celular optoeletrônica, polímeros condutores, azobenzenos, bioeletrônica

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LIST OF ABBREVIATIONS AND ACRONYMS

AFM	atomic force microscopy
ATP	adenosine triphosphate
azo-alkyne	4-propargyloxyazobenzene
azo-tz-	(E)-1-((2,3-dihydrothieno[3,4-b][1,4]dioxin-2-yl)methyl)4-((4-
PEDOT:PSS	(phenyldiazenyl)phenoxy)methyl)-1H-1,2,3-triazole-PEDOT:PSS
BSE	backscattered electron
CE	counter electrode
CIC	charge injection capacity
CP	conductive polymer
CPE	constant phase element
CSC _c	cathodal charge storage capacity
CuAAC	copper(I)-catalyzed azide-alkyne cycloaddition
CV	cyclic voltammetry
DI-H ₂ O	deionized water
DMEM	Dulbecco's modified Eagle's medium
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
DPBS	Dulbecco's phosphate-buffered saline
EDL	electrical double layer
EDOT	3,4-ethylenedioxythiophene
EDOT-Cl	chloromethyl-EDOT
EIS	electrochemical impedance spectroscopy
EtOAc	ethyl acetate
FBS	fetal bovine serum
HOMO	highest occupied molecular orbital
IPA	isopropanol
ITO	indium tin oxide
LSV	linear sweep voltammetry
LUMO	lowest unoccupied molecular orbital
MEA	microelectrode array
MEM	minimum essential medium
N ₃ -EDOT	azidomethyl-EDOT
N ₃ - PEDOT:PSS	azidomethyl-PEDOT:PSS

NA	numerical aperture
OECT	organic electrochemical transistor
OFET	organic field effect transistor
P3HT	poly(3-hexylthiophene-2,5-diyl)
PEDOT	poly(3,4-ethylenedioxythiophene)
PEDOT:PSS	poly(3,4-ethylenedioxythiophene):poly(4-styrenesulfonate)
PEEK	polyether ether ketone
PSS	poly(styrene sulfonate)
PSSNa	poly(sodium 4-styrenesulfonate)
RE	reference electrode
RM-A	reference material A
RM-B	reference material B
RM-C	reference material C
RMS	root-mean-square
SE	secondary electron
SEM	scanning electron microscopy
SMU	source meter unit
THF	tetrahydrofuran
TLC	thin layer chromatography
WE	working electrode

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1 INTRODUCTION

1.1 PRINCIPLES OF BIOELECTRONICS

The multidisciplinary field of bioelectronics aims to bridge biological systems with electronic devices. This integration facilitates the monitoring and regulation of biological activities across various scales, from cellular membranes to entire organs. By establishing a seamless interface with the cellular environment, bioelectronics exploits established electronic technologies to send, retrieve and process signals to and from living organisms (SIMON et al., 2016).

Bioelectronic devices are particularly useful for understanding and manipulating the signaling pathways within biological systems, especially in *in vitro* models. Cells communicate via ions, which are essential for transmitting signals and maintaining physiological processes. In electrically excitable cells, such as neurons and cardiac cells, ions play a vital role in the propagation of action potentials through the processes of depolarization and repolarization of the cellular membrane (KANDEL, ERIC R. et al., 2014). To effectively record and stimulate cellular activities, it is crucial to transduce ionic movements into electrical signals with precision.

A wide range of *in vitro* platforms and devices have been developed throughout the years for applications such as electrophysiology recording and stimulation, biosensing, drug delivery, and tissue engineering. Each platform provides unique insights into the biological system from different perspectives. Patch-clamp analysis, for instance, enables high-resolution intra- and extracellular single-channel electrophysiology recording, providing details on the electrical properties of individual cells (LEHMANN-HORN et al., 2010). To understand more complex networks of electrogenic cells, high-density microelectrode arrays (MEAs) are a more suitable option, allowing for the monitoring and stimulation of multiple cells simultaneously with high spatial and temporal precision (BETTUCCI; MATRONE; SANTORO, 2022). Additionally, organic electrochemical transistors (OECTs) offer highly sensitive biosignal detection and amplification with low power consumption, as ions released by the cells diffuse through the transistor's channel and modulate the readout current (MARQUEZ; MCEVOY; PAKDEL, 2020).

1.2 ORGANIC BIOELECTRONICS

Traditional electronic devices are made of inorganic materials such as gold, platinum, and silicon. While the field of electronics is highly advanced, and these materials can be used with integrated circuitry technology to produce devices for biointerfacing

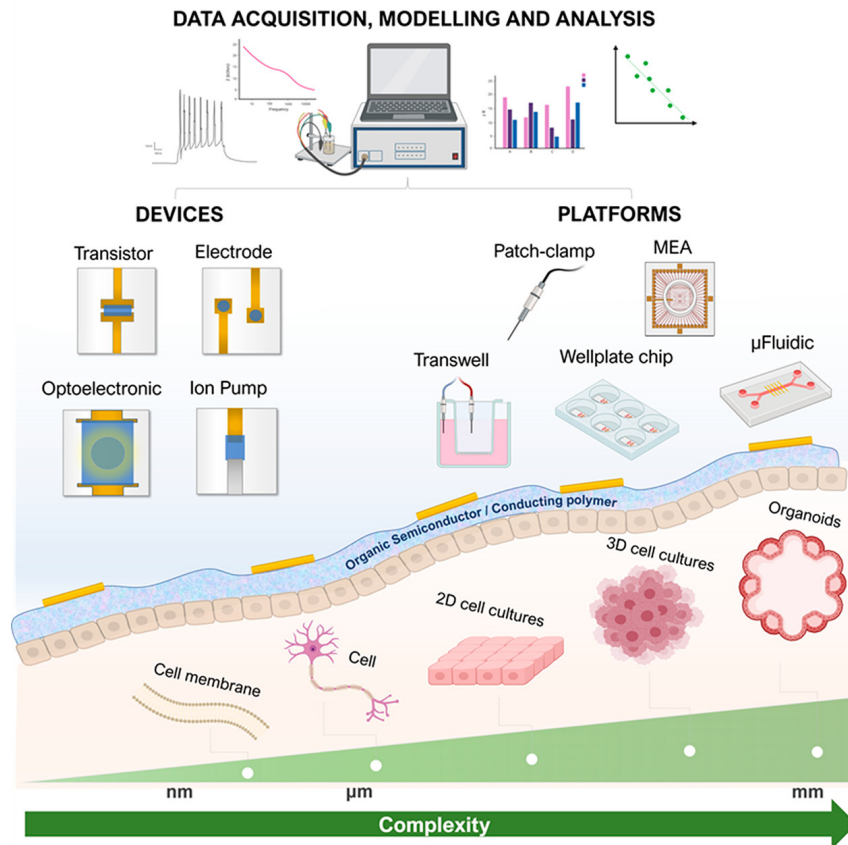


Figure 1 – Outline of *in vitro* bioelectronics, starting from biological systems, to interfacing with electronics and signal processing. Adapted from (PITSALIDIS et al., 2022).

with great electronic capabilities, they fail to achieve intrinsic coupling with cells and to mimic the *in vivo* environment. Inorganic materials are rigid and are primarily limited to electronic conduction. Achieving deeper integration with biological systems enhances signal acquisition and improves stimulation outcomes.

Organic electronic materials emerge as promising alternatives for interfacing with the living biological world. These materials not only share similar chemical structures with biological tissues but also exhibit mechanical and electrical properties that are more compatible with the cellular environment. Conductive polymers (CPs) are particularly interesting for their ability to conduct both ions and electrons. This mixed conductivity makes CPs intrinsic transducers, enhancing the quality of the transferred information between biological systems and electronic devices. As shown in Figure 2, hydrated cations penetrate the bulk of the organic material, in contrast to inorganic materials. Additionally, CPs can be synthetically tuned and mechanically tailored to achieve greater compliance with biological tissues. By exploring the unique properties of CPs, bioelectronics can achieve a more effective and natural interaction with biological systems, paving the way for advanced medical devices and therapies.

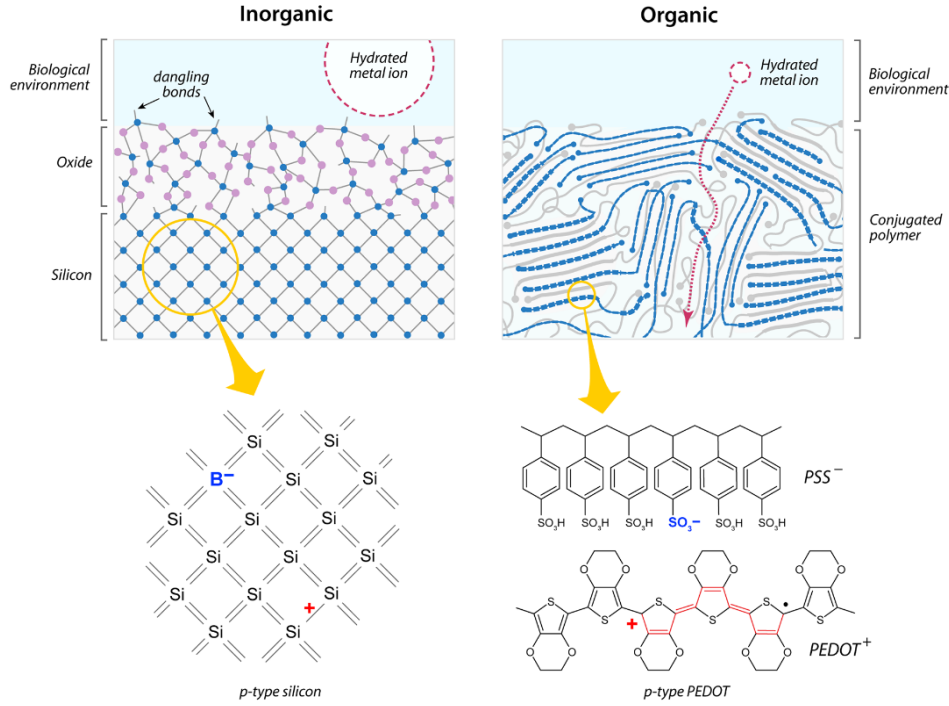


Figure 2 – Schematics of inorganic (silicon) and organic (PEDOT) p-type semiconductors in contact with a biologic environment. Adapted from (RIVNAY; OWENS; MALLIARAS, 2014).

In CPs, each carbon atom in the polymeric backbone forms three covalent σ -bonds, and one π -bond. The σ -bonds are the strongest covalent bonds, while π -bonds are weak covalent bonds with low electron density, formed by the overlap of unhybridized atomic orbitals. The electrons in π -bonds are delocalized, allowing them to move freely along the polymer backbone (MDLULI et al., 2022). This delocalization of π -electrons enables electrical conductivity in the polymer. Although CPs show lower conductivity values with respect to inorganic metals and semiconductors, their electrical properties can be enhanced upon electrochemical doping (INAL et al., 2018), which allows them to transport electrons (n-type) or holes (p-type). When in contact with an electrolyte, ions can penetrate the polymer bulk and alter its electrochemical properties through a process called electrochemical de-doping. In organic bioelectronics, p-type organic semiconductors are preferred due to their higher carrier mobility. The current CPs used in the field are depicted in Figure 3, and among them poly(3,4-ethylenedioxythiophene):poly(4-styrenesulfonate) (PEDOT:PSS) is the leading material due to its high conductivity and electrochemical stability (BOEHLER; AQRAWWE; ASPLUND, 2019).

PEDOT:PSS is composed of an 3,4-ethylenedioxythiophene (EDOT) polymer chain stabilized with negatively charged poly(styrene sulfonate) (PSS) to promote conduction. Despite the poor solubility of EDOT, the hydrophilicity of PSS enables the polymer to

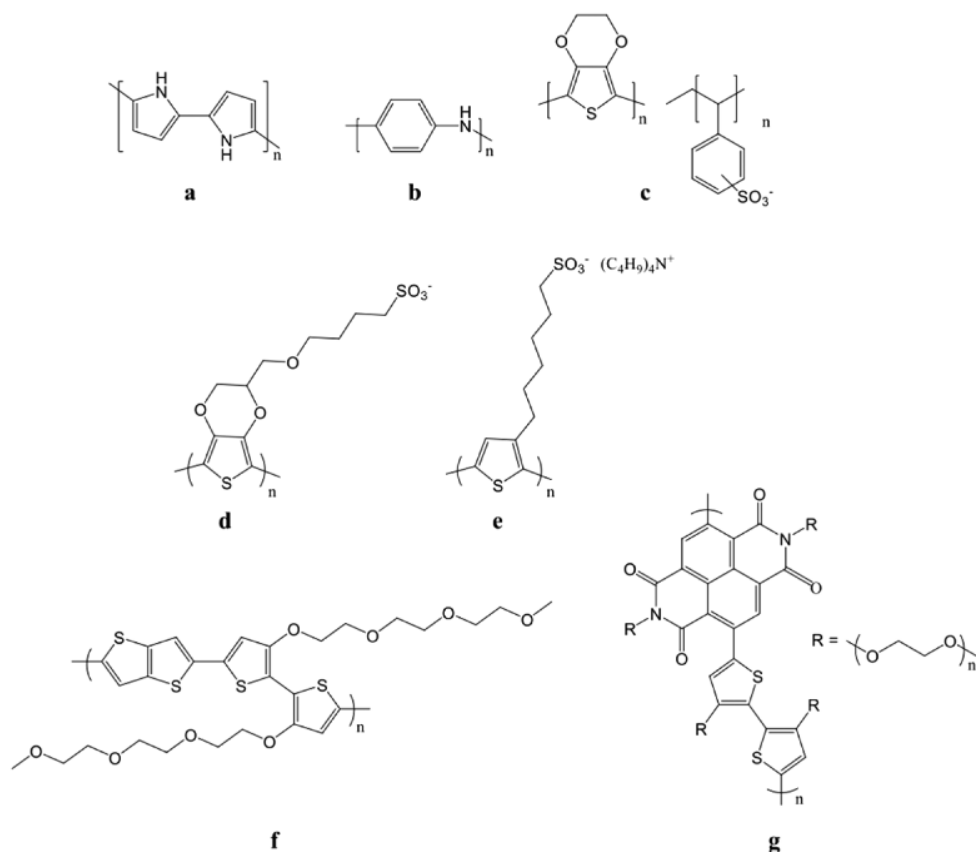


Figure 3 – Conductive polymers in organic bioelectronics: (a) polypyrrole (PPy), (b) polyaniline, (c) PEDOT:PSS, (d) PEDOT-S, (e) PTHS, (f) p(g2T-TT), (g) p(gNDI-g2T). Adapted from (INAL et al., 2018).

swell and achieve high ionic mobility within PEDOT:PSS matrix. De-doping occurs when cations from the electrolyte are injected into the polymer, which causes an electrostatic compensation of the fixed charges in the bulk of the polymeric film by these mobile ions (BERGGREN; MALLIARAS, 2019). This process leads to volumetric charging of the film, similar to the charging of capacitor electrodes, giving PEDOT:PSS its predominantly capacitive behavior. Upon reverse polarization, ions and water leave the bulk, causing PEDOT:PSS to shrink and return to its doped state.

Additionally, PEDOT:PSS is known for its excellent biocompatibility, exhibiting negligible cytotoxicity towards various cell types, making it suitable for biomedical applications (LIANG, Y. et al., 2021). Its stability in aqueous environments further enhances PEDOT:PSS applicability *in vitro* bioelectronics, where it maintains its structural integrity and conductive properties over prolonged periods (DIJK; RUTZ; MALLIARAS, 2020). This stability is crucial for applications involving interfacing with biological tissues, ensuring reliable performance and longevity of the polymer in physiological conditions.

1.3 CELL-ELECTRODE INTERFACING

1.3.1 Cell signaling

The neuron is one of the fundamental signaling cells of the body. It is composed of a cell body (soma), dendrites, an axon and pre-synaptic terminals, as depicted in Figure 4. The soma acts as the metabolic center of the cell, summing the activity of all input signals to produce a trigger signal. The axon, a long tubular branch extending from the soma, transmits electrical signals over long distances, ranging from 0.1 mm to 2 m. At the end of the neuron, the axon branches into pre-synaptic terminals, where the signal is propagated to other cells at communication sites called synapses. The post-synaptic targets are crucial for signal interpretation and pathway processing. Dendrites receive incoming signals from other neurons.

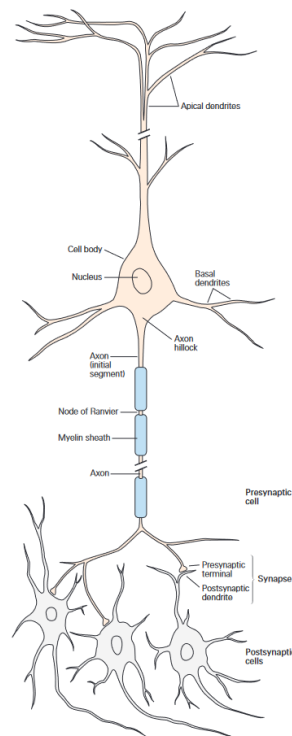


Figure 4 – Schematic representation of a neuron. Adapted from (KANDEL, ERIC R. et al., 2014).

All the information in the brain is received and transmitted through action potentials. These signals are very distinct, and their information content is not modulated, but interpreted based on the signal pathway throughout the brain to generate responses, such as sensations. Action potentials are initiated at the initial segment of the axon, if the sum of all incoming potentials reaches a threshold, and they propagate to the pre-synaptic terminals. The myelin sheath, an insulating lipid layer covering the axon and periodically

interrupted by nodes of Ranvier, ensures fast propagation. The sheath insulates parts of the axon, so the action potential is not continuously propagated but regenerated at each node of Ranvier.

Ion channels maintain a steady, negative resting membrane potential with zero net charge flow due to constant charge separation. Neurons have a resting membrane potential of about -70 mV. Sodium-potassium ion pumps (Na^+ - K^+ pumps) at the membrane actively maintain the concentration of Na^+ ions ten times lower inside the cell, while the concentration of K^+ ions is 20 times higher than outside. This concentration gradient drives K^+ ions out of the cell through ion channels highly permeable to K^+ ions, leaving the intracellular medium negatively charged. Conventionally, the direction of electric current is defined by the flow of positive charges (cations). When there is an increase in cations inside the membrane, such as during action potentials, the membrane potential becomes less negative, resulting in a depolarization. On the other hand, when the intracellular medium becomes more negative and the separation of charges across the membrane increases, hyperpolarization occurs.

In a typical neuron, an increase in potential above the threshold potential (-55 mV) changes locally the permeability of ion channels, allowing positively charged Na^+ ions to enter the cell, depolarizing the membrane and increasing its potential to about 40 mV. After 1 to 2 ms, the potential quickly drops to -90 mV, entering a refractory period before returning to the resting state. This refractory period ensures the unidirectional propagation of the action potential along the whole axon. The action potential signal is represented in Figure 5.

The propagation of action potentials happens in synapses, the junction between two cells where direct communication occurs. Synapses can be chemical, mediated by neurotransmitters, or electrical, where ions flow between gap junctions connecting cell membranes. Neuronal signaling typically occurs via chemical synapses. An initial stimulus activates specialized receptor proteins in the dendrites, opening ion channels in the membrane. The ionic flow disturbs the local resting membrane potential, driving it to a receptor or synaptic potential. These potentials can be excitatory, causing depolarization of the membrane, or inhibitory, causing hyperpolarization. The receptor (or synaptic) potential is transmitted along the axon. When the action potential reaches the pre-synaptic terminals, it releases neurotransmitters across the synaptic cleft. The neurotransmitters bind to receptor proteins on the post-synaptic neuron, opening ion channels and altering the membrane potential, driving it to a synaptic potential. The longer the receptor (or synaptic) potential lasts, the longer the train of action potentials, so the frequency of fired action potentials is related to the amplitude and duration of the stimulus (input signal).

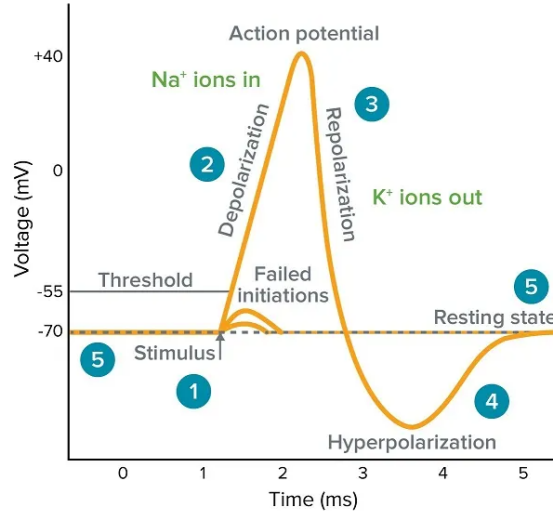


Figure 5 – Action potential stages: (1) incoming stimulus; (2) depolarization of the membrane in case of a sufficiently high potential, Na^+ ions enter the cell; (3) repolarization of the membrane, K^+ leave the cell; (4) hyperpolarization of the membrane (refractory period); (5) resting state. Adapted from (MOLECULAR DEVICES, 2024).

1.3.2 Electrical equivalent circuit

One important step in transducing neuronal signaling with electronics is modeling the cell-electrode interface. The equivalent circuit, the attenuation of the signal can be assessed and strategies to improve the acquired signal can be studied once the equivalent circuit of the interface is established. The point-contact model describes the electrical coupling, considering three major components: the cell membrane, the cell-electrode interface, and the electrode itself (MARIANO et al., 2022). Figure 6a depicts the electrical and biological counterparts of the interface.

As introduced in Section 1.3.1, the separation of cations and anions (positive and negative charges, respectively) across the cell membrane creates a potential difference between the extracellular and the intracellular environments, known as the membrane potential (V_M). The standard model for passive cell membranes consists of a resistance (R_M) in parallel to a capacitance (C_M). The resistance accounts for the ion flux across the membrane (i.e. due to ion channels), and the capacitance models the charge separation.

In a simplified cell-electrode interface model (SPIRA; HAI, 2013), the cleft between the cell and the electrode is modeled by a sealing resistance (R_{seal}), determining the quality of the recorded signal. R_{seal} is inversely proportional to the cleft height, representing the signal losses to the electrolyte.

In contact with an electrolyte (cell media), ions accumulate on the surface of the electrode and an electrical double layer (EDL) is formed. There are different theories

in literature regarding the structure of the EDL, i.e. Helmholtz, Gouy–Chapman, Stern, and Grahame models (SUNG; SHIN, 2020). According to the Stern model, the EDL is composed of the Stern layer, where ions are adsorbed onto the surface and the potential drop is linear, and the diffuse layer, where hydrated ions diffuse across the electrolyte and the potential decays exponentially. The ions in the Stern layer of the EDL and the electrically charged electrode behave as a parallel plate capacitor (C_{dl}), which is modeled in parallel to an impedance (Z_L) representing the faradaic processes occurring at the interface (BOEHLER et al., 2020).

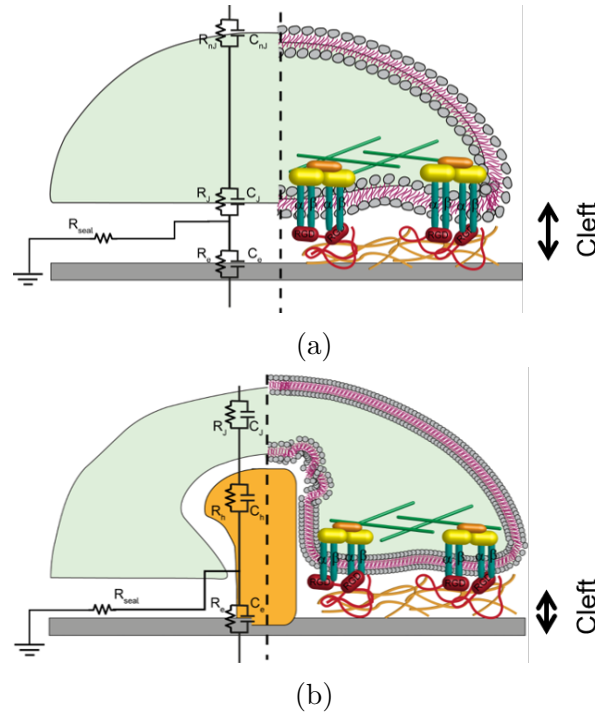


Figure 6 – Cell-electrode equivalent circuit in case of (a) planar electrode and (b) 2.5D electrode. Adapted from (MARIANO et al., 2022).

Effective coupling between the cell and the electrode is achieved by minimizing the separation between them, also known as the cleft. More realistically, the cleft and junctional membrane can be described as functions of time to better understand the dynamic coupling behavior (BRUNO; MARIANO; SANTORO, 2021). To enhance contact, MEAs have been designed with biomimetic 2.5D micro- and nanoprotusions that emulate the neuronal environment. These structures promote cell engulfment, improving the adherence and thus reducing the cleft. By optimizing the aspect ratio of these 2.5D structures, this approach stimulates cell growth and enhances extracellular recordings (MARIANO et al., 2022). Further improvements in cellular recording/stimulation capabilities can be achieved by coating the 2.5D electrodes with PEDOT:PSS (RUGGIERO et al., 2022).

1.3.3 Charge transfer mechanisms

Generated charges at the electrode interact electrochemically with the electrolyte and cells. Different mechanisms of charge transfer can occur at the interface, each playing a role in promoting cell polarization. For polymeric electrodes, the primary processes occurring at the interface are capacitive, faradaic, and thermal.

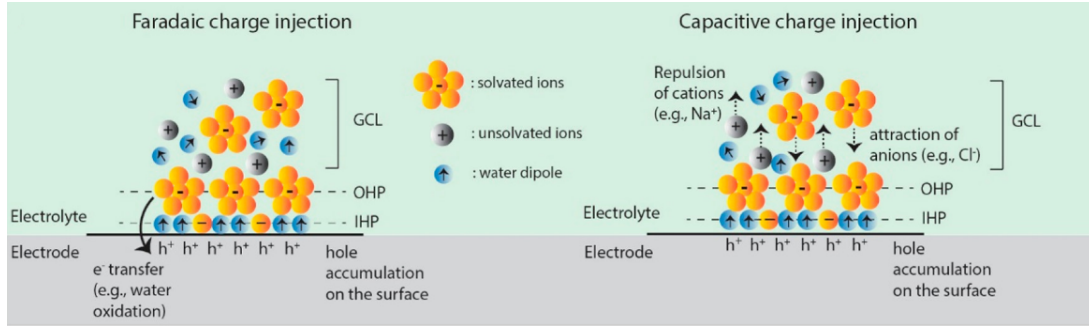


Figure 7 – Faradaic and capacitive charge transfer mechanisms in the case of hole accumulation on the surface. GCL is the Gouy-Chapman diffuse-charge layer, OHP is the outer Helmholtz plane and IHP is the inner Helmholtz plane. Adapted from (HAN; KARATUM; NIZAMOGLU, 2022).

1.3.3.1 Capacitive charge transfer

In capacitive charge transfer, there is a redistribution of charged species (cations and anions) in the electrolyte. No electron transfer occurs; instead, the change in net charge on the electrode is compensated by the attraction and repulsion of charges in the electrolyte. Two layers of opposing charges are created at the interface: electrons or holes in the electrode, and an ionic Stern layer in the electrolyte. This behavior is analogous to a capacitor, where the rearrangement of charges causes a local and transient electric field at the interface. Capacitive charge transfer allows for reversible charging and discharging depending on the polarity of the electrode.

1.3.3.2 Faradaic charge transfer

Faradaic charge transfer involves reduction and oxidation processes where electrons are transferred between the electrode and the electrolyte. Reduction occurs at a negatively charged electrode (cathode), which injects electrons into the electrolyte, generating a cathodic current that reduces the species. Oxidation, on the other hand, occurs at a positively charged electrode (anode), which receives electrons from the electrolyte, generating an anodic current that oxidizes the species (MERRILL, 2021). Faradaic redox reactions perturb ionic concentrations by creating chemical species, which can remain bound to

the electrode surface, stay in close proximity, or diffuse throughout the electrolyte. If the products diffuse too far into the liquid phase, they may not be recovered even with a reverse current, and the reaction is called irreversible. These free generated species can be harmful to both electrodes and tissues (BOEHLER et al., 2020). Faradaic processes of charge transfer are modeled by an impedance. When both capacitive and faradaic mechanisms occur, the corresponding circuitry is a parallel combination of a capacitor and a resistor.

1.3.3.3 Thermal charge transfer

Upon heating the polymer, a temperature gradient is formed at the interface. As bodies tend to reach thermodynamic equilibrium, there will be a thermal propagation until equilibrium is reached. Thermal charge transfer triggers temperature-responsive ion channels, decreases cell resistance, and alters physical properties of the cell membrane, which can be viewed as a change in capacitive reactance (HOPKINS et al., 2019).

1.4 OPTOELECTRONIC CELL STIMULATION

The discovery of light-sensitive proteins revolutionized the monitoring and manipulation of cellular activity. By genetically modifying neurons to express rhodopsins, which are responsible for phototransduction in retinal rod cells, it is possible to modulate neuronal behavior with high spatial precision. Upon light exposure, rhodopsins undergo a fast photoisomerization, triggering a cascade of reactions that lead to hyperpolarization of photoreceptor cells (INUKAI et al., 2023). Over time, optogenetics has advanced to incorporate a variety of light-sensitive ion channels and proteins, leading to the first successful application of optogenetic therapy in a human patient with retinitis pigmentosa to restore partial vision (LASSOUED et al., 2021). Although optogenetic tools are widely used in various fields of biology and medicine (XU et al., 2023; CHEN et al., 2021), they rely on genetic modification of cells prior stimulation. This dependency on genetic modification raises concerns about the safety of viral transfection, especially on the brain, and presents ethical aspects for *in vivo* applications.

Non-genetic optical methods exploit photoactive conductive substrates to indirectly stimulate cells. When these interfaces are illuminated, they generate localized heating or electric charge carriers, which in turn thermally or electrically stimulate the cells. Organic semiconductors are of particular interest for this application due to their biocompatibility, low mechanical stiffness, stability and mixed conduction properties. Additionally, they can also be chemically engineered to enhance specific characteristics, such as photo-

responsiveness. This approach allows for precise cell stimulation without the need for potentially harmful voltages or genetic modification of biological systems.

Unlike inorganic materials, which have energy bands, organic semiconductors have discrete energy levels associated with the energy of their molecular orbitals. According to molecular orbital theory, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are separated by a bandgap (E_g), and the energy required to excite an electron from the HOMO to the LUMO is determined by this gap. A parallel can be drawn with inorganic semiconductors, where electrons absorb incident energy and move from valence to conductive bands. In photo-excitable organic materials, incident photons with energy higher than E_g can generate an exciton, composed of an excited electron that hops to the LUMO and a hole in the HOMO that diffuses through the polymer.

Depending on the employed material, different types of photostimulation can be achieved based on the charge transfer mechanisms described in Section 1.3.3 (refer to Figure 8). In photothermal activation, light absorbed by the polymer is converted into thermal energy, which propagates to the cell and activates temperature-responsive ion channels. This mechanism typically requires higher illumination intensities and longer illumination durations to dominate over other mechanisms.

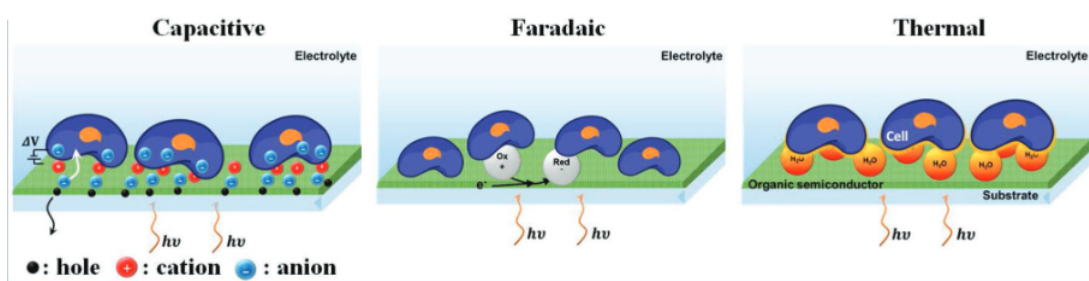


Figure 8 – Schematics of optocapacitive cell stimulation, faradaic photoelectrochemical stimulation and photothermal activation mechanisms. Adapted from (HOPKINS et al., 2019).

Faradaic photoelectrochemical stimulation involves the generation of a faradaic current flow upon illumination. The current can be either cathodic, when the excited electrons reduce the electrode by flowing into the bulk of the substrate, or anodic, when the excited electrons are injected into the electrolyte, oxidizing the electrode. The change in ionic concentration due to redox reactions at the electrode-electrolyte interface can polarize or depolarize the surrounding cell membrane, influencing cellular activity.

Optocapacitive stimulation modulates the charge density through illumination, allowing for precise control over cellular response. This mechanism is based on the migration of photoexcited electrons (or holes) to the interface, causing a redistribution of charges

in the electrolyte. Depending on the sign of charges generated by the photostimulation of the polymer, the cells within the electric field can have their membranes polarized or depolarized.

1.5 OPTOELECTRONIC ORGANIC DEVICES

1.5.1 Molecules and film-based optoelectronic materials

The organic photoactive polymer poly(3-hexylthiophene-2,5-diyl) (P3HT) is widely used for optoelectronic cell stimulation. As an electron donor material, its use has been reported in a variety of configurations: in heterojunctions and polymer blends (GHEZZI et al., 2011; MAYA-VETENCOURT et al., 2017), alone on top of a conductive layer (GHEZZI et al., 2013), as a coating for 2.5D electrodes (TULLII et al., 2019; MILOS et al., 2021), and in nanoparticles integrated into scaffolds (TANG et al., 2022) and fibers (WU et al., 2019). Although P3HT is well-known for its extensive use in photovoltaic applications, biocompatibility, and optoelectronic properties (VAQUERO et al., 2016), it is impaired by poor electrical conductivity, degradation upon oxygen exposure, difficult fabrication procedures, brittleness, and low flexibility (KANG, Y. H. et al., 2021).

Combining PEDOT:PSS with optoelectronics for cell stimulation would be highly advantageous due to its unique properties. However, pristine PEDOT:PSS exhibits very low absorbance in the UV to visible spectrum (SINGH; KUMAR, 2019). Through chemical engineering, CPs can acquire properties of the covalently bounded functional groups (FENOY et al., 2022). By linking photoresponsive molecules, poly(3,4-ethylenedioxythiophene) (PEDOT) can be modified to absorb light with smaller wavelengths and show optoelectronic behavior. This functionalization could potentially overcome the limitations of P3HT, providing a more robust and efficient material for optoelectronic cell stimulation.

Photoresponsive molecules respond physicochemically to light exposure (PURKAIT et al., 2018). Molecular photoswitches, in particular, change their chemical structure by absorbing specific wavelengths, in a process called photoisomerization¹. This mechanism is photochemically or thermally reversible, and can occur via *trans* to *cis* isomerization, 6π electrocyclization or a mix of both (WELLEMAN et al., 2020). The switching behavior depends highly on the light wavelength and intensity. Figure 9 displays several examples of molecular photoswitches and their absorption wavelengths. In bioelectronic applications, it is desired that photoresponsive molecules are stable in aqueous and cellular environments,

¹ Process in which the structure or configuration of the molecule changes, keeping its chemical composition. In the case of a photoisomerization, the modification is triggered by illumination at a specific wavelength.

are negligibly cytotoxic, and are resistant to photodegradation. Indeed, photoswitchers such as azobenzenes and spiropyrans are nowadays gaining an increasing attention for cell interfacing, exhibiting an improved and controlled cell adhesion (KEYVAN RAD; BALZADE; MAHDAVIAN, 2022).

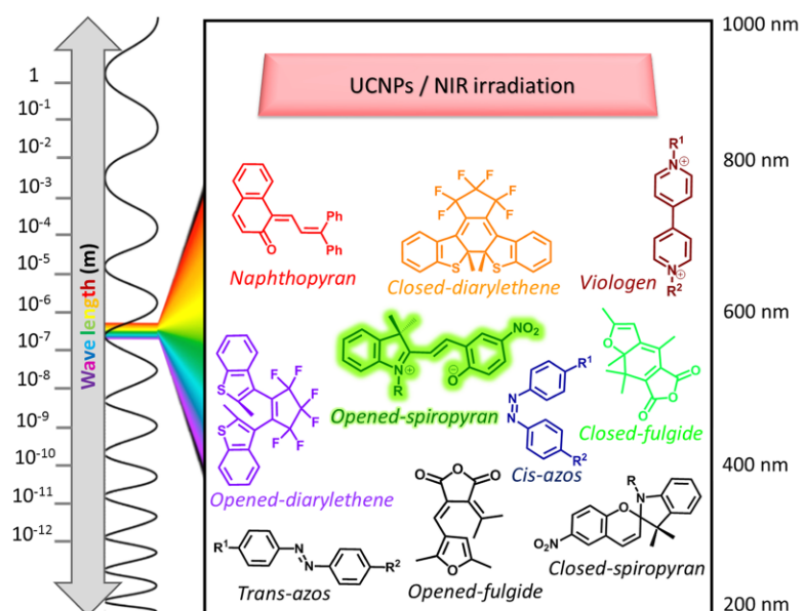


Figure 9 – Photoactive molecules and corresponding absorption wavelengths. Adapted from (KEYVAN RAD; BALZADE; MAHDAVIAN, 2022).

1.5.2 Conductive polymers functionalized with azobenzenes

Azobenzenes are popular photoswitches, composed of two phenyl rings linked by a characteristic $N = N$ (azo group). The photoisomerization causes a geometrical change in the structure of the molecule, as shown in Figure 10: the *trans* isomer has a straight shape with non-planar conformation with respect to the azo group, while the *cis* isomer has a bent configuration with phenyl rings in the same plane. The *trans* isomer is the most thermodynamically stable, requiring higher energy to undergo isomerization. The reverse interconversion (*cis* to *trans*) can be triggered both by light and temperature increase (JERCA, F. A.; JERCA, V. V.; HOOGENBOOM, 2021).

Figure 11a shows the absorption spectra of an azobenzene derivative illuminated with a 365 nm laser and monitored over time. The first and higher absorbance peak, at around 320 nm, is due to $\pi - \pi^*$ transition. It occurs with the excitation of an electron from the bonding π orbital (HOMO) to the anti-bonding π^* orbital (LUMO). At this wavelength, *trans* to *cis* isomerization takes place, and the absorbance decays with the

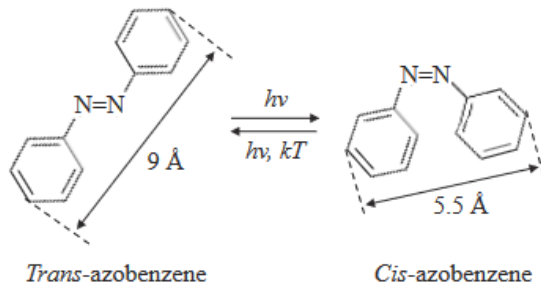


Figure 10 – Isomerization of azobenzenes. Adapted from (KANG, H. S.; YANG, 2022).

reduced amount of *trans* isomers. At around 440 nm, there's a second absorption peak due to $n - \pi^*$ transition. This transition occurs with the excitation of an electron from the non-bonding orbital (HOMO) to the anti-bonding π^* orbital (LUMO), requiring the lowest amount of energy (JERCA, F. A.; JERCA, V. V.; HOOGENBOOM, 2021).

The modification of azobenzenes with substituent groups shifts the energy of the transitions. This way, the isomerization can be triggered by different wavelengths, as shown by shifts in the absorption spectrum of various azobenzene derivatives in Figure 11b. This is particularly relevant for biomedical applications, once long exposure to UV light can be harmful to cells and tissues, and has low penetration depth (ZHANG, H. et al., 2021).

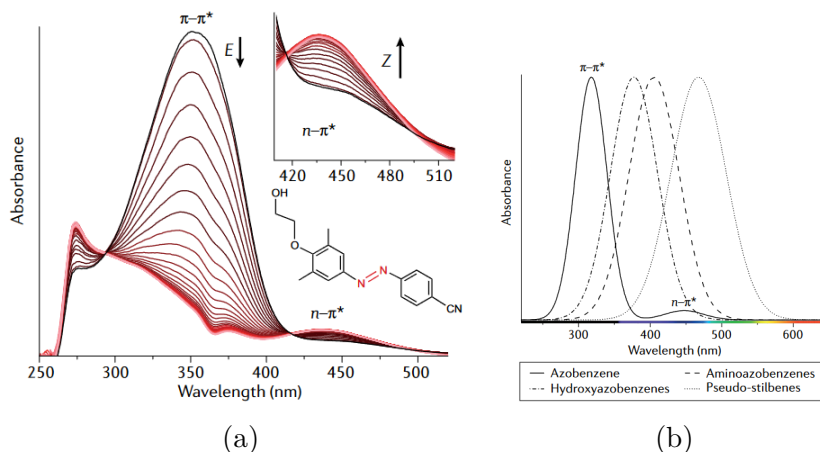


Figure 11 – (a) Absorbance spectra of azobenzene derivative illuminated with 365 nm laser (5 mW) and monitored with a fibre optic spectrometer over time. (b) Absorbance spectra of different azobenzene classes. Adapted from (JERCA, F. A.; JERCA, V. V.; HOOGENBOOM, 2021).

The incorporation of azobenzenes into CPs allows the reversible tuning of the polymer's electrical properties *via* light exposure. Optically tunable organic field effect transistors (OFETs) have been designed using a blend of azobenzene derivatives with polymers, employing them in both the gate (LEE et al., 2016) and the channel (TIAN, J.

et al., 2019, 2021).

A derivative of EDOT, azidomethyl-EDOT (N_3 -EDOT), has emerged as a promising building block for the functionalization of PEDOT:PSS due to the presence of an azide group (SHAVOKSHINA et al., 2021). Figure 12 illustrates two strategies for the functionalization of the N_3 -EDOT derivative. The monomer can be chemically linked to the functional group, followed by polymerization. This approach, seemingly simple, faces difficulties in the case of bulky radical groups and easily oxidized groups that hinder the polymerization. Another approach consists of a post-polymerization functionalization. A simple addition reaction, such as Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes, can be used to cleanly "click" the azide of azidomethyl-PEDOT:PSS (N_3 -PEDOT:PSS) to a variety of radical groups (BU et al., 2008).

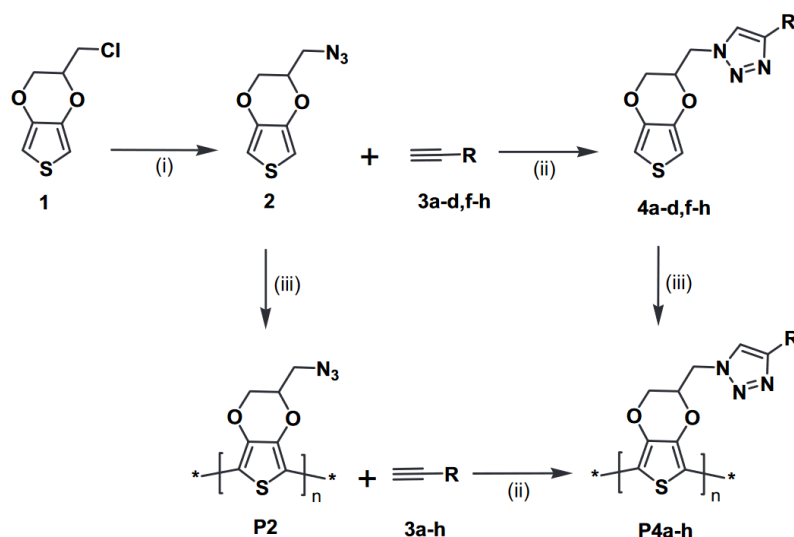


Figure 12 – Functionalization approaches: functionalization of the monomer (**4**) followed by polymerization (**P4**) or polymerization (**P2**) and subsequent post-functionalization (**P4**) with the radical group (**3**). Adapted from (BU et al., 2011).

Corrado et al. (2023) reported the first use of PEDOT:PSS functionalized with azobenzenes via click chemistry in the gate of an OECT to modulate the channel current with light (Figure 13a). This innovative approach combines the excellent electrical properties of PEDOT:PSS with the photoswitchable characteristics of azobenzenes, creating an (E)-1-((2,3-dihydrothieno[3,4-b][1,4]dioxin-2-yl)methyl)-4-((4-(phenyldiazenyl)phenoxy)methyl)-1H-1,2,3-triazole-PEDOT:PSS (azo-tz-PEDOT:PSS) film that generates current and has its conductivity enhanced upon UV illumination. Figure 13b shows the comparison between the absorption spectrum of PEDOT:PSS and azo-tz-PEDOT:PSS (with and without light exposure). By incorporating azo-tz moieties, the polymer's conductivity can be reversibly

increased upon exposure to specific wavelengths of light, since the energy bandgap of the *cis* isomer is smaller. Facilitated by the photoisomerization, the absorbed photons excite electrons from the HOMO of the azo-tz to the LUMO. As shown in Figure 13, since the energy gap to the HOMO of the PEDOT is lower, the excited electrons are transferred to the PEDOT backbone, resulting in a photo-induced charge transfer.

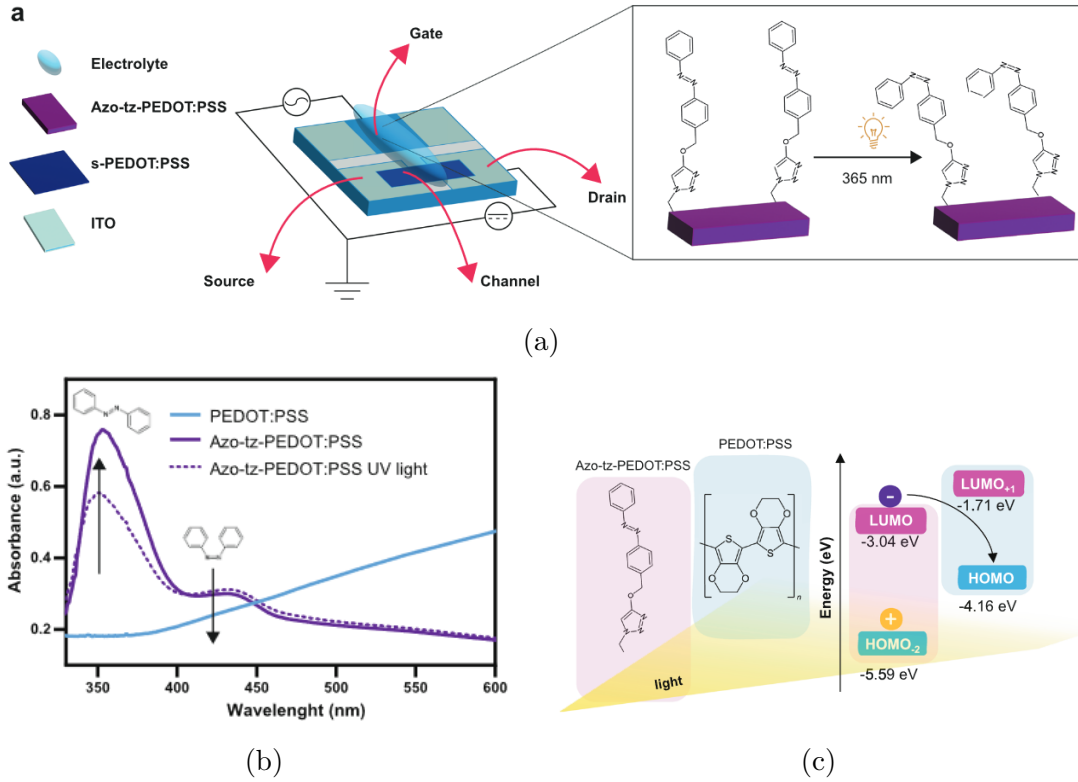


Figure 13 – (a) Proposed OEET with azo-tz-PEDOT:PSS gate and spin-coated PEDOT:PSS channel. (b) UV-visible spectrum of PEDOT:PSS (blue), azo-tz-PEDOT:PSS before and after light stimulation (purple solid and dotted lines, respectively). (c) Energy levels of *cis*-azo-tz-PEDOT:PSS and PEDOT:PSS calculated according to DFT/TD-DFT. Adapted from (CORRADO et al., 2023).

1.6 AIM OF THE PROJECT

The present work explored the potential of azo-tz-PEDOT:PSS films for optoelectronic cell stimulation. Non-genetic optical stimulation provides a non-invasive alternative to traditional electrical methods, offering precise spatio-temporal control of cellular activity through light-based modulation. The unique properties of PEDOT:PSS, which facilitate cell interfacing, combined with the light-responsiveness of azobenzenes, make azo-tz-PEDOT:PSS a promising material for the development of bioelectronic devices capable of targeted cell stimulation. To achieve this goal, the following objectives were

pursued:

1. Characterization of the morphology of azo-tz-PEDOT:PSS, N₃-PEDOT:PSS and PEDOT:PSS films: Measurements of thickness, contact angle, optical microscopy, scanning electron microscopy, and atomic force microscopy.
2. Analysis of the electrochemical characteristics and stability: The films were tested in two electrolytes, saline and cell media, to extract important figures of merit for neural stimulation and recording electrodes. This included measurements of charge storage capacity and impedance at 1 kHz using cyclic voltammetry and electrochemical impedance spectroscopy techniques.
3. Investigation of the photo-generated current and charge injection capacity: Light stimulation experiments were conducted to explore the photo-generated current and the charge injection capacity of the films.
4. Assessment of the material's biocompatibility: The toxicity of the films was tested on two cell lines, L-929 and R28, to ensure the material's suitability for neural applications.

2 MATERIALS AND METHODS

2.1 FABRICATION OF AZO-TZ-PEDOT:PSS FILMS

The fabrication procedure of azo-tz-PEDOT:PSS films consists of a series of steps, adapted from the protocol initially proposed by Corrado et al. (2023):

1. Synthesis of N₃-EDOT
2. Synthesis of 4-propargyloxyazobenzene (azo-alkyne)
3. ITO-coated glass cleaning
4. N₃-EDOT solution for deposition preparation
5. Electrodeposition of N₃-EDOT with poly(sodium 4-styrenesulfonate) (PSSNa)
6. Functionalization of N₃-PEDOT:PSS films with azo-alkyne

2.1.1 Synthetic Route

2.1.1.1 Synthesis of N₃-EDOT

For the synthesis of the N₃-EDOT, chloromethyl-EDOT (EDOT-Cl) (Merck KGaA, Germany) (200 mg, 0.262 mmol, 1 eq) was fully dissolved in 12 mL of N,N-dimethylformamide (DMF) (VWR Chemicals, Belgium) at room temperature. Sodium azide (TCI Chemicals, Germany) (136 mg, 0.524 mmol, 2 eq) was added, and the mixture was vigorously stirred at 120°C for 3 hours. Thin layer chromatography (TLC) was performed using silica gel Al foils (Sigma-Aldrich, Germany) to evaluate the reaction progress, using as eluant petroleum ether:ethyl acetate (EtOAc) = 10:1. The reaction solvent was evaporated under reduced pressure, and the crude mixture was extracted with EtOAc (Merck KGaA, Germany) and DI-H₂O. Sodium sulfate anhydrous (Merck KGaA, Germany) was added to the organic phase and then filtered. The EtOAc was evaporated under reduced pressure until the pure N₃-EDOT was obtained as an oil (192 mg, 0.243 mmol, 93%).

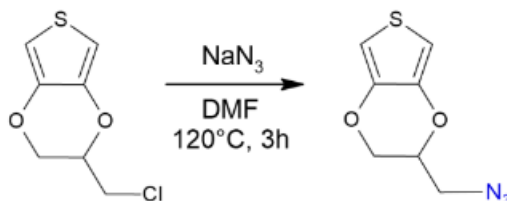


Figure 14 – Schematics of N₃-EDOT synthesis (CORRADO et al., 2023).

2.1.1.2 Synthesis of azo-alkyne

4-(Phenylazo)phenol (TCI chemicals, Belgium) (200 mg, 0.252 mmol, 1 eq) was dissolved in 12 mL of degassed acetone (Merck KGaA, Germany), at room temperature. Potassium carbonate (Merck KGaA, Germany) (696 mg, 1.26 mmol, 5 eq) was added, and the mixture was vigorously stirred at 56°C for 90 minutes. Propargyl bromide (TCI Chemicals, Germany) (600 mg, 1.26 mmol, 5 eq) was then added, and the mixture was refluxed overnight at 56°C. TLC was performed to evaluate the reaction progress. After the evaporation of acetone under vacuum, and the crude mixture was re-dissolved in EtOAc for liquid-liquid extraction with DI-H₂O. Sodium sulfate (Merck KGaA, Germany) was added to the organic phase and then filtered. An orange powder (226 mg, 0.239 mmol, 95%) was obtained after the evaporation under reduced pressure.

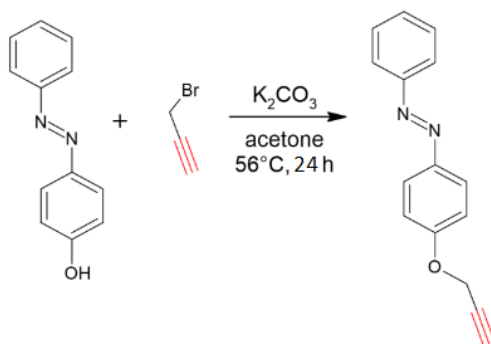


Figure 15 – Schematics of azo-alkyne synthesis (CORRADO et al., 2023).

2.1.2 N₃-EDOT Electrodeposition

2.1.2.1 Substrate preparation

Unpatterned ITO coated glass slides (25 mm x 25 mm, Ossila B. V., Germany) were used as substrates for the electrodeposition. Cleaning is a key step in the film fabrication. Glass slides were sonicated in an ultrasonic bath (HC – Series EMMI 40HC, EMAG) for ten minutes at 100% power and extensively rinsed with each of the following: 10 g/L Alconox (Merck KGaA, Germany) aqueous solution, DI-H₂O, acetone and isopropanol (IPA) (Merck KGaA, Germany).

2.1.2.2 N₃-EDOT solution

The solution for electrodeposition was prepared as follows. N₃-EDOT (synthesized as described in section 2.1.2.3) was first dissolved in acetone (10% w/v), transferred to a vial, and dried with a nitrogen spray gun, obtaining 39.44 mg. 150 mg (7.5 mg/mL)

PSSNa (Merck KGaA, Germany) was dissolved in 20 mL DI-H₂O and added to the vial containing N₃-EDOT, which results in a concentration of 10 mM. The aqueous suspension was stored in the fridge for at least 24 hours prior to the electrodeposition.

2.1.2.3 Electropolymerization via CV electrodeposition

Electrochemical polymerization, commonly referred to as electropolymerization, is a method for CP films synthesis. This technique involves the growth of polymer chains via redox reactions induced by an externally applied voltage or current. An EDOT monomer loses an electron to form a radical cation, which couples with other oxidized monomers to form cationic dimers, trimers, and oligomers (Figure 16). During the formation of a conjugated PEDOT chain, the counter anion present in the electrolyte act as a dopant, stabilizing charge imbalance.

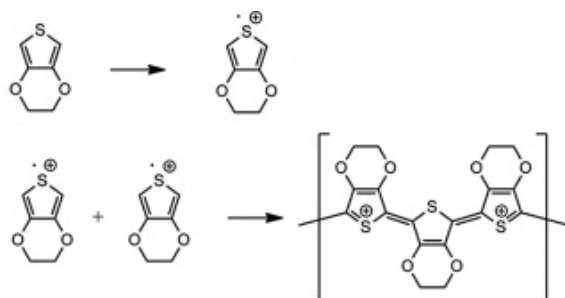


Figure 16 – Electropolymerization process of EDOT monomers. Adapted from (MUELLER et al., 2012).

The typical set-up consists of a three-electrode electrochemical cell, with a working electrode (WE), counter electrode (CE), and a reference electrode (RE) submerged in the electrolyte, as depicted in Figure 17. In the electrodeposition technique, the substrate acts as the WE, onto which the polymer is directly deposited. The CE is connected to the WE via a power source (potentiostat), closing the circuit, and the RE tunes the potential of the system. By applying a suitable bias, the oxidation of monomers present in the electrolyte solution and oligomerization occur at the electrode surface, initiating an *in situ* nuclei growth process. From these nucleation sites, polymer chains spread and grow, forming a film strongly adhered to the electrode surface. This controlled deposition, as well as the counter ion present in the electrolyte, enables precise tuning of the conductive polymer's properties by means of polymerization rate, applied voltage (or current), electrode substrate, and deposition mode.

The RE should have a stable and known potential with respect to the standard hydrogen electrode (basic reference with null potential across all temperatures) and high

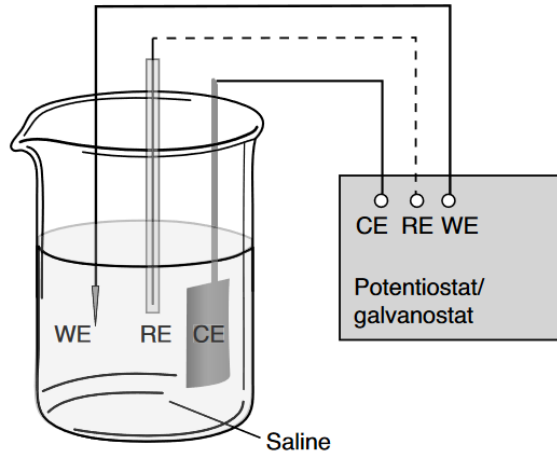


Figure 17 – Three-electrode electrochemical cell setup. Adapted from (BOEHLER et al., 2020).

impedance, which means zero net current flow through it. The most frequently used RE is the secondary Ag/AgCl electrode, consisting of a silver wire electrochemically covered with a thin silver chloride layer, immersed in a saturated or 3 M KCl solution. The WE potential is measured against the RE, and the potentiostat adjusts the current flow between WE and CE to maintain the set values in case of potential fluctuations.

The concentration of oxidized and reduced species is related to the electrode potential through the Nernst equation:

$$E = E^0 + \frac{RT}{zF} \ln \frac{[\text{Ox}]}{[\text{Red}]} \quad (1)$$

where E [V] is the cell potential, E^0 [V] is the standard cell potential, R [J K⁻¹ mol⁻¹] is the universal gas constant, T [K] the temperature, z is the number of electrons transferred in the reaction, F is the Faraday constant [C mol⁻¹], and [Ox] and [Red] are the concentrations of oxidized and reduced species, respectively. An increase in the cell potential leads to the oxidation of species at the WE, causing it to act as the anode in an electrolytic cell. On the contrary, a decrease in the potential elicits the reduction of species, making the WE behave as the cathode.

While a redox process is occurring at the WE, a counter reaction happens at the CE, so that the same absolute current I flows through in both electrodes (JERKIEWICZ, 2022). The current density (j) flowing through an electrode is described by the Butler-Volmer equation (Eq. (2)) as the rate of the electrochemical reaction occurring at the electrode's surface, inversely proportional to the electrochemically active surface area of the electrode (A_{ecsa}). To avoid that the kinetics of the reaction at the CE limits the rate of the process at the WE, $A_{\text{ecsa,CE}}$ should be larger than $A_{\text{ecsa,WE}}$ (TIAN, M. et al.,

2016). Platinum is the most commonly utilized material due to its inert nature and low overpotential (KLEIN; WALDVOGEL, 2022).

$$j = j_0 \cdot \left\{ \exp \left[\frac{a_a z F \eta}{RT} \right] - \exp \left[-\frac{a_c z F \eta}{RT} \right] \right\} = \frac{I}{A_{\text{electrode}}} \quad (2)$$

where j ($j = I/A_{\text{ecsa}}$) [A/m²] is the electrode's current density, j_0 [A/m²] is the exchange current density, a_a and a_c are the anodic and cathodic charge transfer coefficients, z is the number of electrons transferred in the reaction, F [C mol⁻¹] is the Faraday constant, η ($\eta = E - E_{\text{eq}}$) [V] is the activation overpotential, R [J K⁻¹ mol⁻¹] is the universal gas constant and T [K] the absolute temperature.

Electrodeposition can be conducted using several techniques, including galvanostatic, galvanodynamic, potentiostatic, or potentiodynamic methods. Each method controls different parameters to achieve the desired deposition properties. In galvanostatic deposition, a constant current density is applied, ensuring a steady deposition rate. In galvanodynamic deposition, on the other hand, the applied current varies over time. As for potentiostatic and potentiodynamic techniques, a controlled potential is applied. Potentiostatic depositions involve maintaining a constant potential at the WE, while potentiodynamic techniques involve sweeping the potential in linear or cyclic manner.

In linear sweep voltammetry (LSV), the voltage is scanned from an initial potential E_1 to a final potential E_2 , primarily oxidizing the species. A more comprehensive electrochemical technique is CV, in which both oxidation and reduction processes occur. Figure 18 illustrates the concept through the CV of a reversible ferrocenium (Fc^+) redox reaction. The potential at the WE is swept at a fixed rate as in LSV, but when the maximum potential is reached (point D), the scan is reversed, and the potential is swept back to the initial value (point G), reducing the species. According to the Nernst equation (see Eq. (1)), the positive potential sweep (anodic) increases the amount of oxidized species, while the negative sweep (cathodic) reduces the species at the WE.

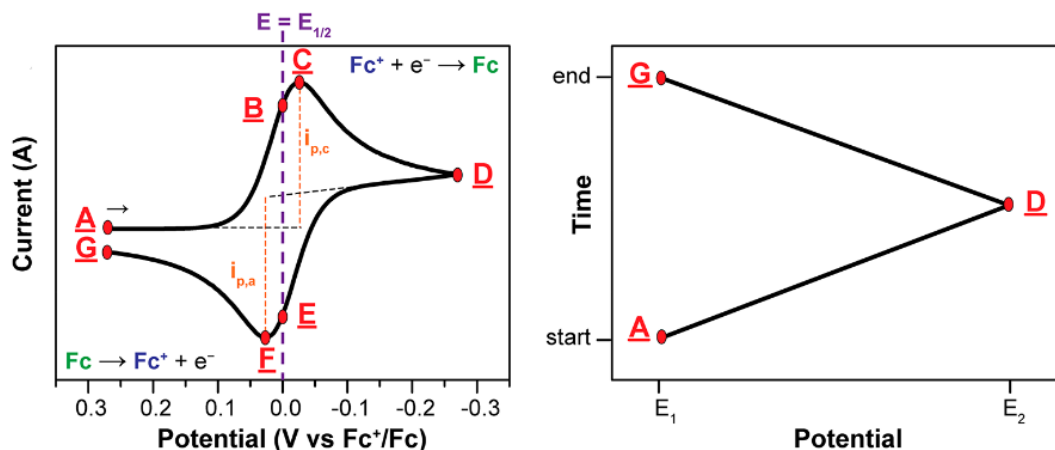


Figure 18 – CV of ferrocenium, with linear sweep from E_1 (point A) to E_2 (point D), and back to E_1 (point G). At point C occurs the oxidation peak ($i_{p,c}$), and at point F occurs the reduction peak ($i_{p,a}$). Adapted from (ELGRISHI et al., 2018).

In this work, CV technique was applied for a potentiodynamic film growth. Before the deposition, the solution prepared as described in Section 2.1.2.2 was vortexed for 30 seconds, sonicated in ultrasonic bath at 100% power for 10 minutes, and vigorously stirred for 30 minutes. A custom-made vertical electrodeposition setup was used (Figure 19a), with a platinum-covered titanium mesh (25 mm x 25 mm, Redox.me, Sweden) CE (Figure 19b), an unpatterned ITO coated glass slide (cleaned as described in Section 2.1.2.1) as WE and a Ag/AgCl 3M KCl (Redox.me, Sweden) as RE (Figure 19c). The electrodeposition was carried out using an EG&G Model 283 potentiostat/galvanostat (Princeton Applied Research/Ametek, USA) equipped with CorrWare (Scribner, USA) software. The potential was scanned from 0.0 V to 1.5 V or 1.2 V at a scan rate of 50 mV/s for 10 cycles. Between depositions, the aqueous dispersion was stirred for 5 minutes, and after the films were rinsed with DI- H_2O , dried with a nitrogen spray gun, and annealed on a hotplate at 120 °C for 1 hour.

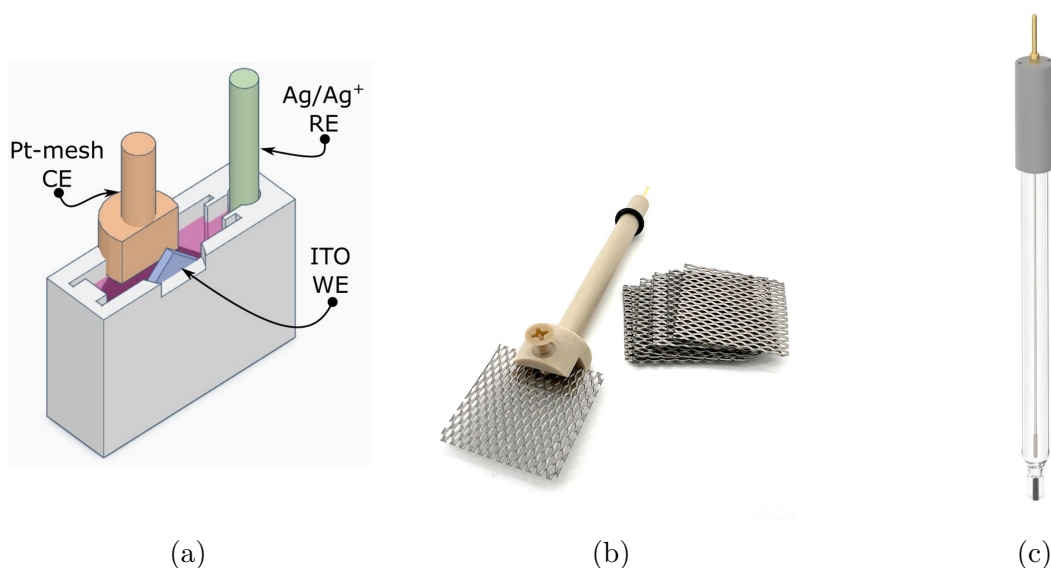


Figure 19 – (a) Electrodeposition setup, (b) counter electrode, (c) reference electrode.

2.1.3 Functionalization with azo-alkyne

The term *click chemistry* refers to a set of reactions designed to link simple building blocks for the synthesis of complex molecules, exploiting the reaction between an alkyne and an azide, leading to the formation of a triazole (KOLB; FINN; SHARPLESS, 2001). One of the most well-known click reactions is the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction (LIANG, L.; ASTRUC, 2011), which efficiently "clicks" together molecules with different functional groups. In this reaction, an active Cu(I) catalyst is generated using sodium ascorbate as a reducing agent for copper sulfate. A mixture of solvents is usually employed to solubilize both reactants and catalyst.

The following procedure describes the functionalization of N₃-PEDOT:PSS films with light-responsive azo-alkyne via click chemistry. Azo-alkyne (47 mg, 0.6 mmol, 1 eq) was dissolved in 10 mL of tetrahydrofuran (THF). Copper sulfate (50 mg, 0.64 mmol, 1.07 eq), and sodium ascorbate (40 mg 0.53 mmol, 0.89 eq) dissolved in 10 mL of DI-H₂O were then added, and the mixture was vortexed for 20 seconds. An in-house designed and manufactured polyether ether ketone (PEEK) setup with a stirring chamber was used for the functionalization, since the mixture needed to be constantly stirred. N₃-PEDOT:PSS films were carefully placed inside the setup and submerged in the mixture. After 24 hours of stirring, the functionalized azo-tz-PEDOT:PSS films were washed with THF and DI-H₂O, and dried with a nitrogen spray gun.

2.1.4 PEDOT:PSS electrodeposition

As for the control group, electrodeposited PEDOT:PSS films were also fabricated via CV. A solution of 100 mM EDOT (Merck KGaA, Germany) and 7.5 mg/mL PSSNa were mixed with DI-H₂O. Prior to the deposition, the aqueous suspension was vortexed for 20 seconds, sonicated in an ultrasonic bath for 10 minutes, and vigorously stirred for 30 minutes. The electropolymerization took place in a vertical 3-electrode setup as described for N₃-PEDOT:PSS, with a voltage sweep from 0 V to 1.0 V at 50 mV/s for 10 cycles. After deposition, films were rinsed with DI-H₂O, dried with a nitrogen spray gun and annealed in a hotplate at 120 °C for one hour.

2.2 MORPHOLOGICAL CHARACTERIZATION

2.2.1 Film Thickness

The thickness of the produced films was measured with a Stylus Profilometer (Bruker) equipped with Bruker's Vision64 software. On each sample, three scratches were made, and the profile was scanned along a line intersecting the transition from film to ITO substrate and back to film. The baseline was established by averaging a section of the film profile, and the film thickness was determined by measuring the step height. The thickness was averaged across all measurements, encompassing three samples for each material, resulting in a total of $N = 9$ measurements.

2.2.2 Contact Angle

The wettability of a material can be assessed through the angle between the tangents of the solid-liquid interface and of the liquid-gas interface, when a droplet of water of a defined volume is dispensed on the surface. Young's equation describes the relation of the interfacial tension (energy) of the three phases in equilibrium to the contact angle:

$$\cos \theta_C = \frac{\gamma_{SG} - \gamma_{SL}}{\gamma_{LG}} \quad (3)$$

where θ_C is the contact angle, γ_{SG} is the solid-gas interfacial tension, γ_{SL} is the solid-liquid interfacial tension and γ_{LG} is the liquid-gas interfacial tension. When surface attractive forces are higher than the surface tension of the liquid, the droplet spreads on the surface, resulting in a contact angle lower than 90°. In this case, the material is said to have good wettability. On the contrary, when the surface tension dominates and the contact angle is higher than 90°, the material is considered hydrophobic, showing poor wettability.

The static sessile drop technique was used to determine the contact angle. With an OCA 25 (DataPhysics Instruments, GmbH), 2 μL of Milli-Q water was disposed on the surface of the film and a photograph of the droplet was taken. The baseline was set, and the droplet was fitted and the contact angle was calculated based on the fitted tangents.

2.2.3 Optical Microscopy

Optical microscopy is a technique of magnification with visible light and a series of lenses. An optical microscope typically consists of a system with bright light source, condenser lens, objective lens, tube lens, and eyepiece lens (Figure 20). The condenser lens focuses the light from source onto the specimen, and the objective lens collects the light from the specimen, creating a magnified image. The tube and eyepiece lenses further magnify and focus the image for viewing.

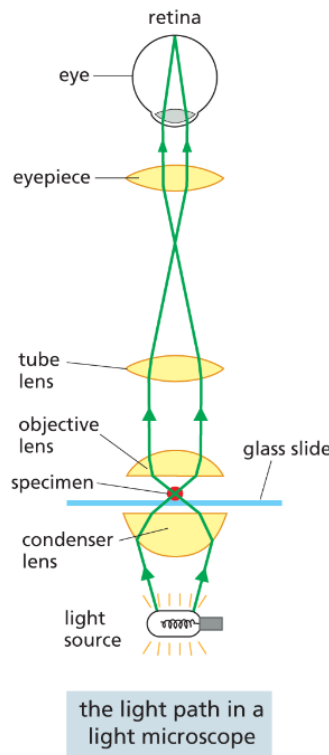


Figure 20 – Working principle of optical microscopy. Adapted from (ALBERTS, BRUCE et al., 2023).

The ability to distinguish two closely spaced points is called resolution. Abbe's theoretical diffraction limit (Eq. (4)) defines the minimum separation of the two points that can be resolved by the microscope. This limit is determined by the wavelength of bright light and the numerical aperture (NA) of the objective lens. The NA is proportional to the refractive index (n) of the medium and the sine of the half-angle (Θ) of the objective lens' aperture ($NA = n \sin \Theta$) (GOODWIN, 2014).

$$D = \frac{\lambda}{2NA} \quad (4)$$

where D is the diffraction limit, λ is the wavelength of input light, and NA is the numerical aperture. To increase resolution, the diffraction limit should be minimized by using light with a shorter wavelength or and objective lens with a higher NA . Optical microscopes can achieve magnifications up to 1000x - 2000x, allowing visualization of structures as small as 200 nm, which corresponds to the shortest wavelength of visible light with an ideal objective (ALBERTS, BRUCE et al., 2023). Images were aquired with a VK-X3000 3D Laser scanning Microscope (Keyence GmbH, Germany) and processed with the Keyence software “Multi-file Analysis Application”.

2.2.4 Scanning Electron Microscopy

Electron microscopy operates on principles similar to optical microscopy, but with a key difference in the illumination source. Instead of using visible light, electron microscopes utilize a focused beam of accelerated electrons to inspect the sample. Electrons have much shorter wavelengths compared to visible light, resulting in significantly reduced diffraction and thus higher resolution images.

Electron microscopes provide several advantages over optical microscopes, including higher magnification and superior resolution. They can achieve magnifications of up to 2.000.000x, allowing for the visualization of structures at the nanometer scale, compared to the micrometer scale limit of optical microscopes. Additionally, electron microscopes offer a larger depth of field and better depth of focus, making them particularly useful for imaging rough or uneven surfaces (inkson_2_2016).

According to the de Broglie principle, every particle exhibits wave-like properties. The wavelength of a propagating electron is inversely proportional to its momentum and, consequently, the accelerating voltage of the electron microscope, as described by the de Broglie equation (Eq. (5)).

$$\lambda_{\text{electron}} = \frac{h}{\sqrt{2meV}} \quad (5)$$

where h is Planck’s constant, m is the mass of the electron, e is the electron charge, and V is the accelerating voltage. As the accelerating voltage increases, the wavelength of the electrons decreases, enhancing the precision and resolution of the microscope. This enables electron microscopes to visualize features down to a few nanometers, far surpassing the capabilities of light microscopes.

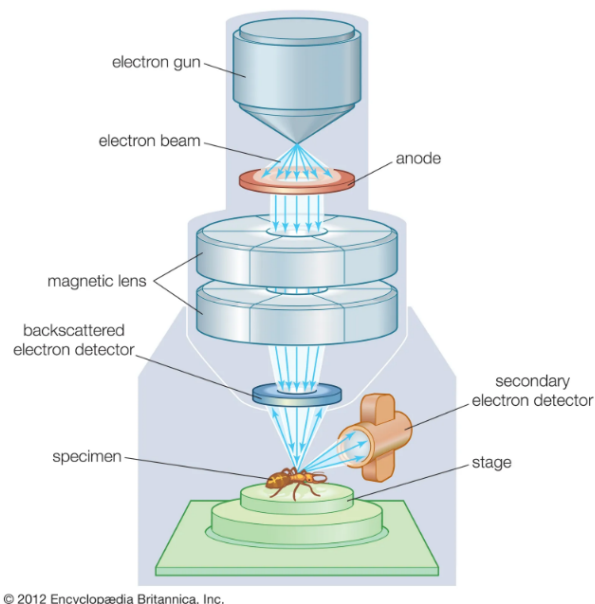


Figure 21 – Schematics of a SEM. Adapted from (BRADBURY, SAVILE; JOY, DAVID C.; FORD, BRIAN J., 2024).

The scanning electron microscopy (SEM) is a type of electron microscope that detects the scattered electrons from the sample to create detailed images. Figure 21 present the schematics of a SEM. Accelerated primary electrons are emitted from an electron gun and focused by magnetic field lenses and metal slits, scanning across the sample surface. To prevent overcharging, the sample surface must be electrically conductive. For non-conductive surfaces, such as polymers, the surface is typically sputter-coated with a thin layer of conductive material (metal or carbon) prior imaging.

The entire process requires a high vacuum environment to ensure that electrons can travel from the gun to the sample without interference from air molecules. As the electrons interact with the specimen, they emit many signals, which are collected by detectors and processed via software to form an image. These signals can be in the form of secondary electrons (SEs), backscattered electrons (BSEs), or X-rays (photons). For characterizing surface morphology and topography, SE are primarily used.

In this work, films were coated with a thin layer of platinum/palladium (2-3 nm) to reduce charging effects on the surface and loaded to the sample holder of the SEM. Images were acquired with a GeminiSEM 360 (Zeiss, Germany) on secondary electron mode, with 5 kV of accelerating voltage and magnitudes of 1,000 X, 10,000 X and 100,000 X.

2.2.5 Atomic Force Microscopy

To analyze surface roughness, atomic force microscopy (AFM) is a highly suited technique due to its high resolution and capability to visualize at nano and atomic scales. AFM consists of a flexible cantilever with a sharp tip at its end, which scans across the sample surface. As the tip interacts with the sample, short-range chemical forces (due to the overlap of sample and tip molecular orbitals) or long-range forces (such as van der Waals, electrostatic and magnetic forces) cause mechanical vibrations in the tip and bending of the cantilever (GIESSIBL, 2003). A laser beam shines on the cantilever, and its bending alters the path of the reflected light, which is captured by a photodetector. The signal is then processed to generate a detailed three-dimensional topographical image of the sample at nanoscale resolution.

AFM operates in different imaging modes depending on the distance of the tip from the sample. When the distance is smaller than 0.5 nm, it is said to be in contact mode (DE OLIVEIRA, R. R. L. et al., 2012). In this mode, the feedback loop maintains a constant cantilever deflection, and the height profile is obtained from vertical motion of the tip. Although contact mode allows for fast scanning, it can also damage the sample, especially in case of soft samples. In tapping mode, instead of constant contact, the tip periodically contacts ("taps") the surface due to vibration of the cantilever at its resonant frequency. The reduced contact time in tapping mode minimizes sample damage, making it more suitable for characterizing polymer surfaces. The tip-sample interactions change both amplitude and phase of the cantilever's oscillations, and a feedback loop compensates for these changes to keep the oscillations constant (see Fig. 22). This mode produces two types of images: amplitude and phase. Phase imaging provides information on material properties, such as chemical composition, while amplitude imaging reveals surface features. The root-mean-square (RMS) roughness of the film can be calculated with height values from the amplitude image (Equation (6)).

$$R_{rms} = \sqrt{\frac{1}{N} \sum Y_i^2} \quad (6)$$

where R_{rms} is the RMS roughness, N is the number of data points, and Y is the height value of each data point.

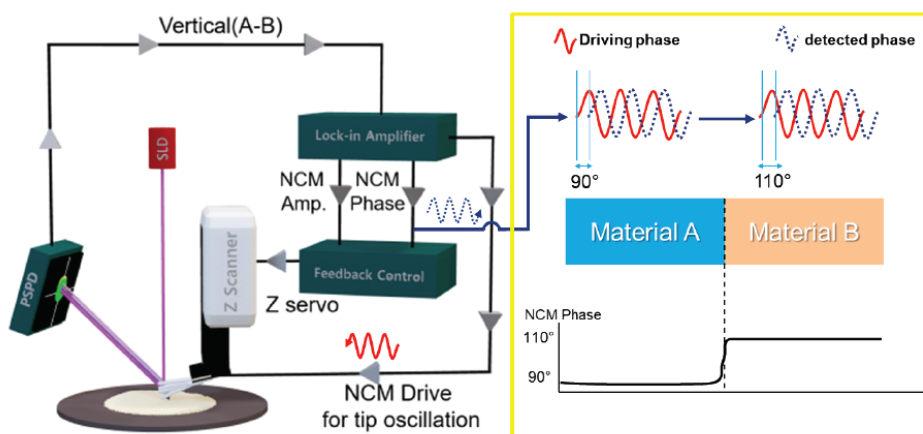


Figure 22 – AFM setup for tapping mode. Adapted from (PARK SYSTEMS, n.d.).

Polymer films were analyzed with AFM both before and after swelling. For the latter, films were soaked in DI-H₂O for one hour and then dried with a nitrogen spray gun. The measurements were performed using a Park NX20 (Park Systems, South Korea) in tapping mode, with scan rate of 0.6 Hz for 256 x 256 pixel images and gain optimized to reduce noise. The root-mean-square (rms) roughness of the surfaces was calculated with software Gwyddion (<http://gwyddion.net/>) from a 5 μm x 5 μm area.

2.3 ELECTROCHEMICAL CHARACTERIZATION

2.3.1 Cyclic Voltammetry

Electrochemical characterizations were performed in two electrolyte solutions, Dulbecco's phosphate-buffered saline (DPBS) (ThermoFischer Scientific, Germany) and cell medium, to understand the behavior of the film in conditions similar to cell culture. The medium was prepared by mixing 44.5 mL of Dulbecco's modified Eagle's medium (DMEM)/Nutrient Mixture F-12 Ham (Sigma-Aldrich, Germany) with 5 mL of fetal bovine serum (FBS) (Sigma-Aldrich, Germany) and 500 μL of Pen Strep (ThermoFischer Scientific, Germany).

Through CV technique, electrochemical reactions occurring at the test electrode when a potential is applied can be measured as current variations. The current response varies with sweep rate, area and roughness of the electrode, giving insights on the stability of the electrode and the amount of electroactive material. Stimulation electrodes are commonly characterized by their cathodal charge storage capacity (CSC_c) (Figure 23), which is estimated by calculating the time integral of the negative current in a CV curve, over a potential window within the electrolysis of water.

$$CSC_c = \frac{1}{v} \left| \int_{E_c}^{E_a} j_c(u) dU \right| \quad (7)$$

where CSC_c is the CSC_c [mC/cm^2], v is the sweep rate [mV/s], E_c and E_a are the cathodic and anodic potential limits of the water window respectively, $j_c(u)$ is the cathodal current density [mC/cm^2], and U is the potential with respect to the RE [V].

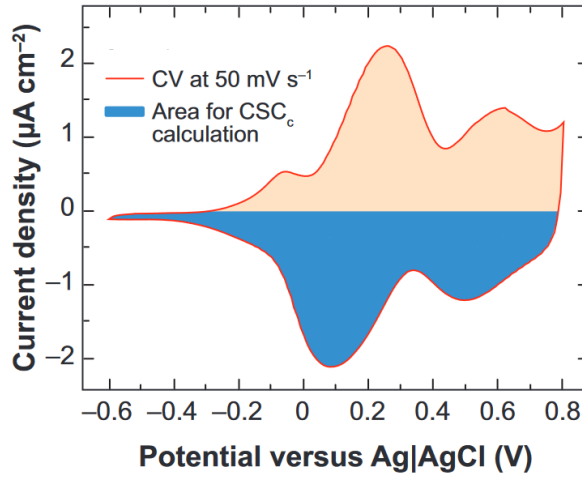


Figure 23 – CV of activated iridium oxide film, the blue region is the area used for CSC_c calculation. Adapted from (COGAN, 2008).

CV characterizations were performed with potentiostat/galvanostat as discussed in section 2.1.2.3. DPBS or cell medium was added and measurements were done directly after electrolyte addition, and after 30 minutes, 24 hours, 48 hours and 7 days at room temperature. Platinum wire (Redox.me, Sweden) was used as CE, electrodeposited films on ITO coated glass slides as WE and Ag/AgCl 3M KCl (Redox.me, Sweden) as RE. The CV was carried from - 0.6 V to 0.6 V, at a scan rate of 50 mV/s for 3 cycles, and the last cycle was recorded.

2.3.2 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) is an electrochemical technique to measure current-voltage dependencies at an electrode over a range of frequencies by applying a small sinusoidal perturbation. This method allows for the analysis of various processes occurring within the electrochemical system, each with different time behaviors: the charging and discharging of the EDL at the electrode/electrolyte interface, the kinetics of redox reactions, and the diffusion of species. In the frequency domain, both fast (high frequency) and slow (low frequency) processes can be analyzed. The results of EIS are commonly visualized using Nyquist and Bode plots. The Nyquist plot (Figure 24a) displays

the real part of impedance (Z') versus the imaginary part (Z''), enhancing the visualization of resistive and capacitive behaviors of different electrochemical processes. The Bode plot, on the other hand, shows the impedance amplitude and phase angle as functions of frequency (Figure 24b), providing insights into the frequency-dependent behavior of the system.

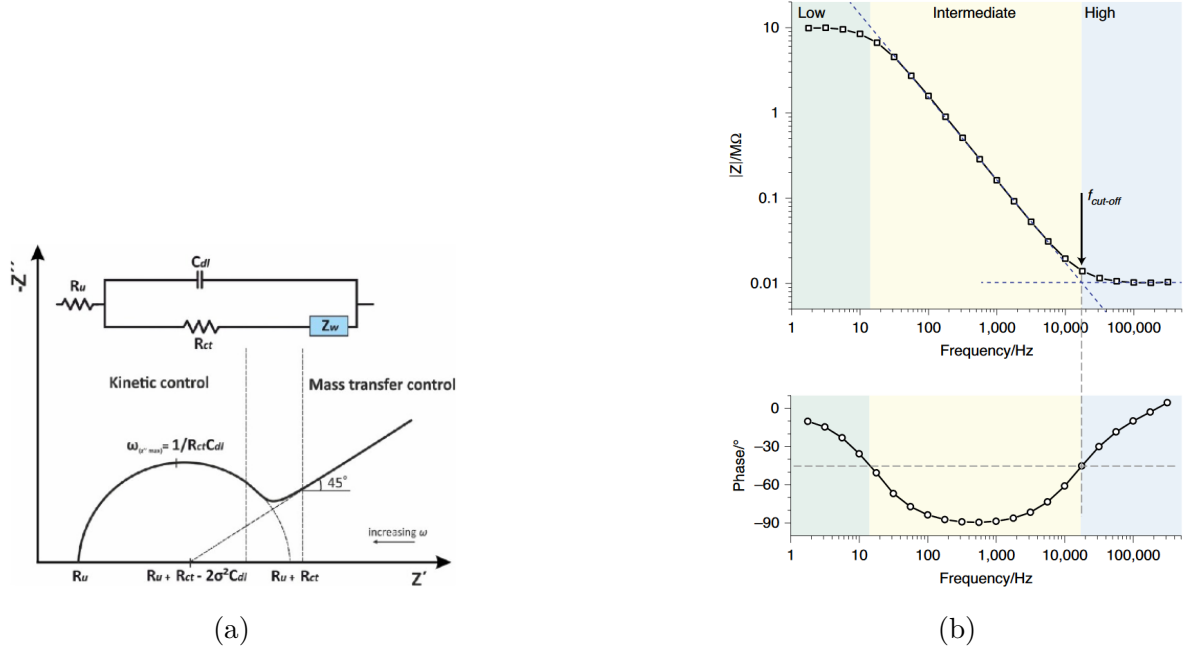


Figure 24 – Representative EIS plots: (a) Nyquist, adapted from (LAZANAS, ALEXANDROS CH.; PRODROMIDIS, MAMAS I., 2023), and (b) Bode, adapted from (BOEHLER et al., 2020).

To guarantee a linear system behavior, a small amplitude AC signal is applied since real electrochemical cells do not exhibit a linear current-voltage relationship. In a typical three-electrode electrochemical cell setup operating in potentiostatic mode, the impedance of the cell in the presence of an electrolyte can be modeled by the Randles equivalent circuit (Figure 25). This model takes into account both currents from the charging and discharging of the EDL (i_C) and from the faradaic process (i_F). The EDL behavior is approximated to a capacitance (C_{dl}), due to its charge storage capability. The impedance of the redox reaction (Z_F) is defined by a combination of both kinetics and diffusion processes.

$$Z_F = R_{ct} + Z_W \quad (8)$$

The charge transfer resistance (R_{ct}) represents the kinetics of the electrochemical reaction, while the Warburg impedance (Z_W) represents the mass transport of species to

the electrode. Z_W is often modeled as a constant phase element (CPE) with ideal phase of 45° .

$$Z' = R_u + \frac{R_{ct} + \sigma\omega^{-1/2}}{(\sigma\omega^{1/2}C_{dl} + 1)^2 + \omega^2 C_{dl}^2 (R_{ct} + \sigma\omega^{-1/2})^2} \quad (9)$$

$$-Z'' = \frac{\omega C_{dl}(R_{ct} + \sigma\omega^{-1/2})^2 + \sigma^2 C_{dl} + \sigma\omega^{-1/2}}{(\sigma\omega^{1/2}C_{dl} + 1)^2 + \omega^2 C_{dl}^2 (R_{ct} + \sigma\omega^{-1/2})^2} \quad (10)$$

At high frequencies, the total current flowing through the electrolyte charges C_{dl} . As $\omega \rightarrow \infty$, the kinetics of faradaic reactions dominate the system ($R_{ct} \gg Z_W$). Equations (9) and (10) can be simplified to:

$$Z' = R_u + \frac{R_{ct}}{1 + \omega^2 C_{dl}^2 R_{ct}^2} \quad (11)$$

$$-Z'' = \frac{\omega C_{dl} R_{ct}^2}{1 + \omega^2 C_{dl}^2 R_{ct}^2} \quad (12)$$

Substituting ω from equation (12) into (11), we get:

$$\left(Z' - R_u - \frac{R_{ct}}{2}\right)^2 + (Z'')^2 = \left(\frac{R_{ct}}{2}\right)^2 \quad (13)$$

Which is the equation of a circle with a radius of $R_{ct}/2$, centered at $Z' = R_u + R_{ct}/2$ and $Z'' = 0$.

At low frequencies, as $\omega \rightarrow 0$, the diffusion of species to the electrode surface governs the system's behavior. Capacitive components exhibit high reactance at low frequencies, impeding AC current flow, thus the total current will flow through R_{ct} and Z_W . Equations (9) and (10) can be simplified to:

$$Z' = R_u + R_{ct} + \sigma\omega^{-1/2} \quad (14)$$

$$-Z'' = \sigma\omega^{-1/2} + 2\sigma^2 C_{dl} \quad (15)$$

By substituting $\sigma\omega^{-1/2}$ in equation (15) into (14), we get:

$$-Z'' = Z' - R_u - R_{ct} + 2\sigma^2 C_{dl} \quad (16)$$

This equation represents a straight line with a 45° tilt, where $Z'(Z'' = 0) = R_u + R_{ct} - 2\sigma^2 C_{dl}$.

Bode plots, on the other hand, are particularly useful for presenting the behavior of the electrode/electrolyte interface over a range of frequencies, and for understanding

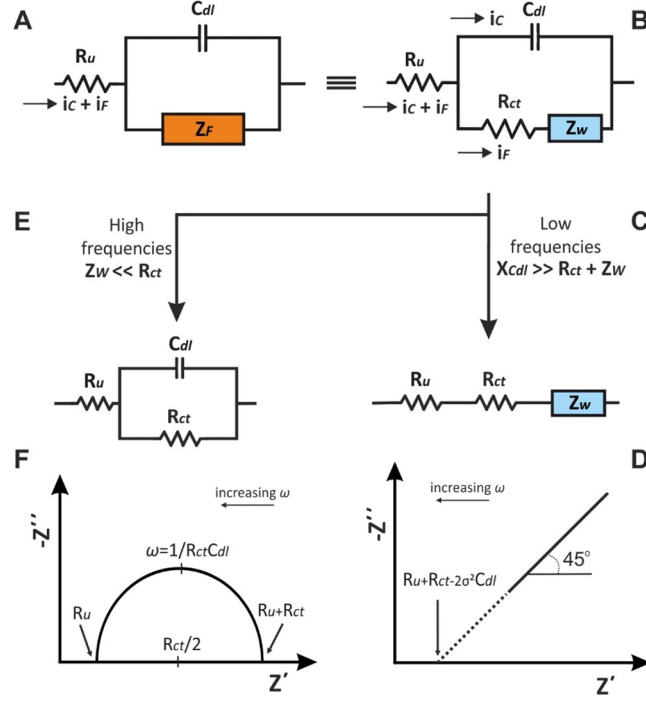


Figure 25 – Randles circuit behavior for low (C, D) and high (E, F) frequencies. Adapted from (LAZANAS, ALEXANDROS CH.; PRODRMIDIS, MAMAS I., 2023).

how well the input signal is transferred throughout the electrolyte due to the occurring processes. As shown in figure 24b, the typical behavior of an electrode in an electrolyte is analogous to a high-pass filter. This is in accordance with what was previously discussed. At very high frequencies, the main contribution is due to the electrolyte resistance (R_u), and the signal flows in a frequency- and phase-independent manner. At high to intermediate frequencies, the charging of the EDL (C_{dl}) attenuates the signal non-linearly and causes a 90° phase shift. At lower frequencies, almost no charge is transferred through the EDL (the reactance of C_{dl} is too high), and the main contribution is due to slow processes like the diffusion of species as described by the Warburg impedance.

For the characterization of electrodes for neural recording, the impedance at 1 kHz is mainly used as figure of merit (BOEHLER et al., 2020). The principal components of an action potential are located in this frequency region, but during depolarization and repolarization, higher frequency components also appear. A lower impedance in this region is favorable in order to increase the signal-to-noise ratio. As previously discussed (see Section 1.3.2), the distance between the neuron/tissue and the electrode decreases the recorded signal, hindering current flow. The electrode should be able to collect the maximum amount of signal possible.

EIS measurements were performed with same potentiostat/galvanostat of other electrical characterizations in a three-electrode electrochemical cell setup. DPBS (Ther-

moFischer Scientific, Germany) or cell medium (produced as described in Section 2.3.1) was added and measurements were done directly after electrolyte addition, and after 30 minutes, 24 hours, 48 hours and 7 days at room temperature. Platinum wire (Redox.me, Sweden) was used as CE, electrodeposited films in ITO coated glass slides as WE and Ag/AgCl pellet as RE. AC voltage of 10 mV amplitude were applied in a frequency range from 100 mHz to 10 MHz.

2.4 LIGHT STIMULATION

In order to further investigate the material response to UV light stimulation, a setup similar to what would be used for optoelectronic cell stimulation is proposed as shown in Figure 26 (FEYEN et al., 2016; GHEZZI et al., 2013). In each sample, two wells were glued and filled one with cell media and the other with DPBS one hour prior first measurement, and a ITO corner was coated with silver paste to improve the contact with the measuring probe. The measurements were performed with ARKEO multichannel (Cicci Research) with a two-channel source meter unit (SMU) equipped with a LED source (1.5 mW/cm^2). For each measurement channel, a three-electrode electrochemical cell (as discussed in section 2.1.2.3) was used, with a probe connected to silver paste coating on ITO as WE, and a platinum wire (CE) and an Ag/AgCl pellet electrode (RE) immersed in the electrolyte. Zero voltage bias was applied, and the current was measured simultaneously in both channels for 240 s. After 60 s, a 115 s far UV light (360 - 370 nm) pulse was applied. The measurement was repeated after 24 h, 48 h and 7 days. Light intensities of 0.3 mW/cm^2 , 0.9 mW/cm^2 and 1.5 mW/cm^2 were analysed, and N₃-PEDOT:PSS and PEDOT:PSS films were used as control groups.

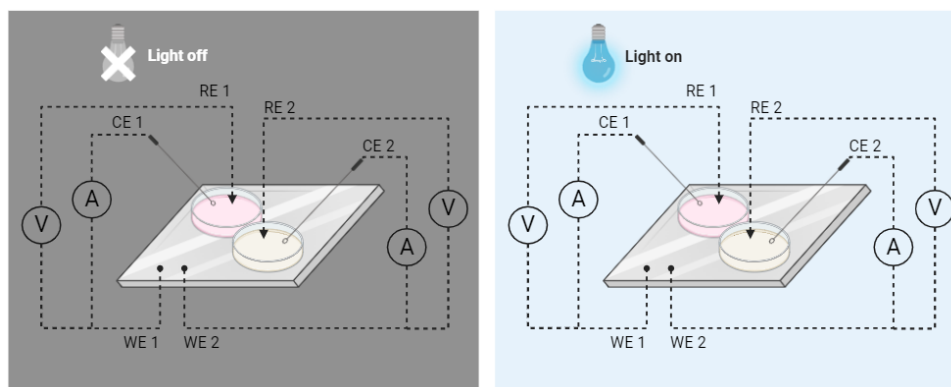


Figure 26 – Light stimulation (365 nm) and current measurement set-up, in a three-electrode electrochemical cell configuration and 0 V bias. Created with BioRender.com.

The performance of the photo-generated current is characterized by the amount of

charge injected in the system during the pulse. The charge injection capacity (CIC) of the films is calculated according to Equation (17) from the current measurements. As control, the experiment was repeated in triplicates of bare ITO, PEDOT:PSS and N₃-PEDOT:PSS films, with 0.9 mW/cm² light power.

$$CIC = \frac{1}{GSA} \int_{T_{ph}} i(t) dt \quad (17)$$

where GSA is the geometric surface area [cm²], T_{ph} is the phase period [s] and i is the photo-generated current [A].

2.5 BIOCOMPATIBILITY ASSAYS

The biocompatibility of materials for cell interfacing was assessed through direct and indirect growth assays using L-929 and R28 cell lines, in accordance with the ISO 10993 "biological evaluation of medical devices" standards, specifically part 5 (tests for *in vitro* cytotoxicity) and part 12 (sample preparation and reference materials). L-929 is a standard cell line for biocompatibility testing due to its high sensitivity, while R28 is a retinal precursor cell line. The experiments were conducted by the Department of Ophthalmology, University Hospital RWTH Aachen, as reported in (TIAN, M. et al., 2016; SCHAFFRATH et al., 2021).

Control materials, with known levels of toxicity and effects on cell growth, are essential for validating the results of biocompatibility assays. Positive controls are expected to show cytotoxicity, while negative controls should not affect cell growth. Both types of control are necessary to exclude false negatives and false positives. In this work, positive and negative reference materials (RM; Hatano Research Institute, Japan) were used as controls. reference material A (RM-A), a polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC), served as positive control due to its moderate cytotoxicity. reference material B (RM-B), containing 0.25% zinc dibutyldithiocarbamate (ZDBC), was used as another positive control with weaker cytotoxicity. reference material C (RM-C), a polyethylene film, was used as negative control. Additionally, glass wells were used as negative controls, and cell culture medium with 10% dimethyl sulfoxide (DMSO) served as another positive control.

Indirect contact tests were conducted to characterize cell-substrate interactions. The materials were incubated in cell culture medium at 37 °C for 72 hours. The resulting media were then diluted to various concentrations and added to pre-cultivated cells. After a 24-hour incubation period, cell viability was assessed.

PEDOT:PSS, N₃-PEDOT:PSS and azo-tz-PEDOT:PSS films were prepared as described in Section 2.1. To fit the films into the media chamber, samples were cut into 7 mm x 10 mm pieces with a diamond tip glass cutter. Control substrates were cut to the same size. Test materials were autoclaved and placed on 4-well Nunc™Lab-Tek™Chamber Slide System (Thermo Fischer Scientific, Germany). The chambers were then filled with 500 μ L of the respective medium and placed in the incubator for 72 hours. L-929 cell culture media consisted of minimum essential medium (MEM) Eagle (PAN-Biotech, Germany) with 50% FBS (Sigma-Aldrich, Germany), 1.1% L-glutamin (Biochrom, Switzerland) and 1.1% Pen/Strep (Lonza, Switzerland). R28 cell culture medium consisted of DMEM (PAN-Biotech, Germany) with 10% FBS, 1.1% 100x MEM vitamin solution (Biochrom, Switzerland), 1.1% 100x MEM non-essential aminoacids (Biochrom, Switzerland) and 1.1% Pen/Strep (Lonza, Switzerland).

After 72 hours of incubation, the medium was collected for indirect contact tests. L-929 cells (100,000 cells/well) and R28 cells (100,000 cells/well) were seeded in 96-well plate and incubated at 37 °C for 24 hours. The previously collected media were then diluted directly in the plate, at 1:1, 1:2 and 1:4 dilutions in fresh medium. Additionally, the same dilutions were performed in 10% DMSO cell medium as control. After 24 hours of incubation, cell viability was assessed using the CellTiter-Glo®Luminescent Cell Viability Assay (Promega, WI) according to the manufacturer's protocol. The luminescence produced in the assay is directly proportional to the number of metabolic active cells, which release adenosine triphosphate (ATP) and generate the measured luminescent signal.

3 RESULTS AND DISCUSSION

3.1 OPTIMIZATION OF ELECTRODEPOSITION POTENTIAL

3.1.1 Initial Parameters

N_3 -PEDOT:PSS and PEDOT:PSS films were electrodeposited via CV technique, as described in Section 2.1.2.3 and 2.1.4. Figure 27 shows the polymerization process of N_3 -EDOT in solution with PSSNa once a potential is applied. Initially, N_3 -PEDOT:PSS was electropolymerized in a potential range from 0 V to 1.5 V, as previously described in literature (CORRADO et al., 2023).

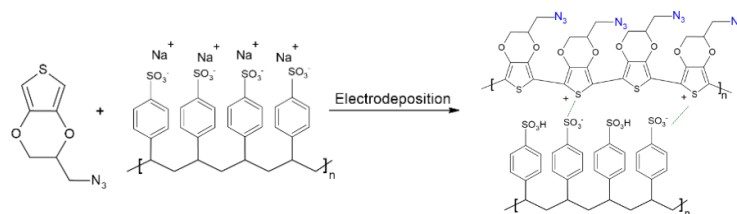


Figure 27 – Electropolymerization process of N_3 -EDOT with PSSNa.

The typical electrodeposition curves of both materials are shown in Figure 28. The first cycle of PEDOT:PSS voltammogram (Figure 28a) is characterized by a lower current peak and an intersection between the forward and the reverse current, called a "nucleation loop". During this first cycle, monomers are beginning to oxidize and couple, forming dimers, trimers and so on — intermediates in the oligomerization process. In parallel, these oligomers deposit on the ITO surface, forming nuclei. In the next cycles, the chain grows from these sites, reaching higher current peaks. The resulting PEDOT:PSS films have a thickness of 82.95 ± 6.28 nm, measured as described in Section 2.2.1.

Interestingly, N_3 -PEDOT:PSS curves (Figure 28b) show a different behavior: the peak current decreases with the scanning cycles. The higher current yields thicker films, of 218.09 ± 22.16 nm, and a color change can also be observed: while pristine PEDOT:PSS is light blue, N_3 -PEDOT:PSS films are black. After the functionalization (Section 2.1.3), films with 166.03 ± 8.85 nm were obtained, indicating an etching of the polymer during the reaction. To better understand the implications of these differences between the films, the surface and the electrochemical properties of the N_3 -PEDOT:PSS and azo-tz-PEDOT:PSS films were examined.

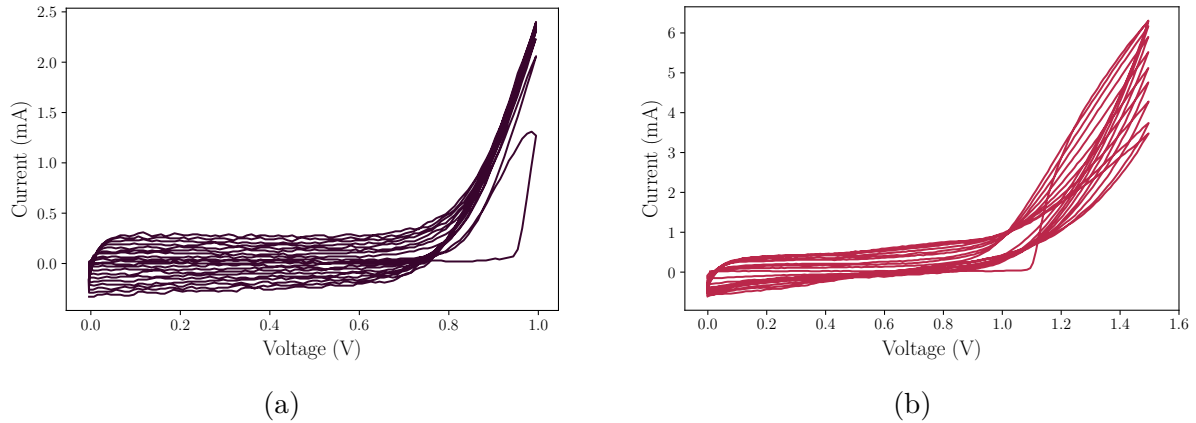


Figure 28 – Representative electrodeposition curves for (a) PEDOT:PSS and (b) N₃-PEDOT:PSS. The voltage is reported vs Ag/AgCl electrode.

3.1.2 Observations and Limitations

In addition to the shape difference of the electrodeposition curves, N₃-PEDOT:PSS films show darker, randomly distributed aggregation spots on their surface. Figure 29 shows the images obtained with an optical microscope, with two magnifications. Profilometer measurements evidenced that the darker spots are an accumulation of material, creating protruding structures across the surface.

Electrochemical techniques can give insights on underlying mechanisms occurring in the N₃-PEDOT:PSS and azo-tz-PEDOT:PSS films. CV measurements, as depicted in Figure 30, show low redox response and almost negligible conductivity after 24 hours in electrolyte. The lower integration area is perceived as a reduced areal capacitance (REN; ZHOU; ZHAI, 2021).

It can be seen from the EIS measurements (Figure 31) a time-dependent phenomena occurring after 24 hours in electrolyte, especially in intermediate frequencies. The Nyquist plots in Figure 32 show an increase in charge transfer resistance (R_{ct}) by the increase in semi-circle diameter, in accordance to the increase in impedance at medium frequencies in the Bode plots. ITO resembles an ideal polarizable electrode, where no faradaic reactions take place, leading its R_{ct} to approach infinity. The film adsorbed on the surface of the ITO reduces the resistance, therefore R_{ct} represents the extent of surface coverage (ZALKA et al., 2017). The observed increase in R_{ct} , displayed in Figures 31 and 32, presumably results from partial delamination of the films over time in DPBS, which exposes the underlying ITO substrate to the measuring system.

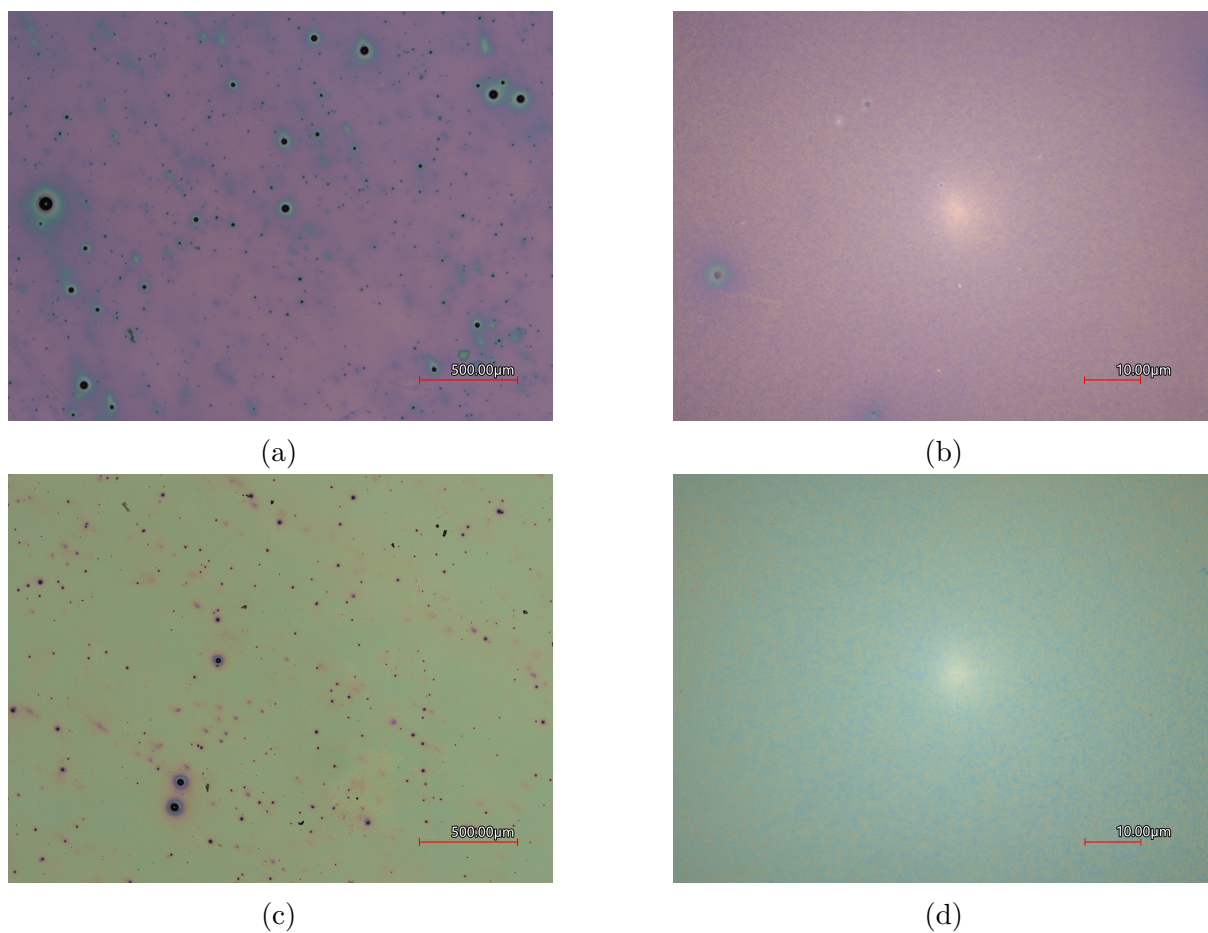


Figure 29 – Optical microscopy images of N_3 -PEDOT:PSS films with (a) x5 and (b) x150 magnification, and of azo-tz-PEDOT:PSS films with (c) x5 and (d) x150 magnification.

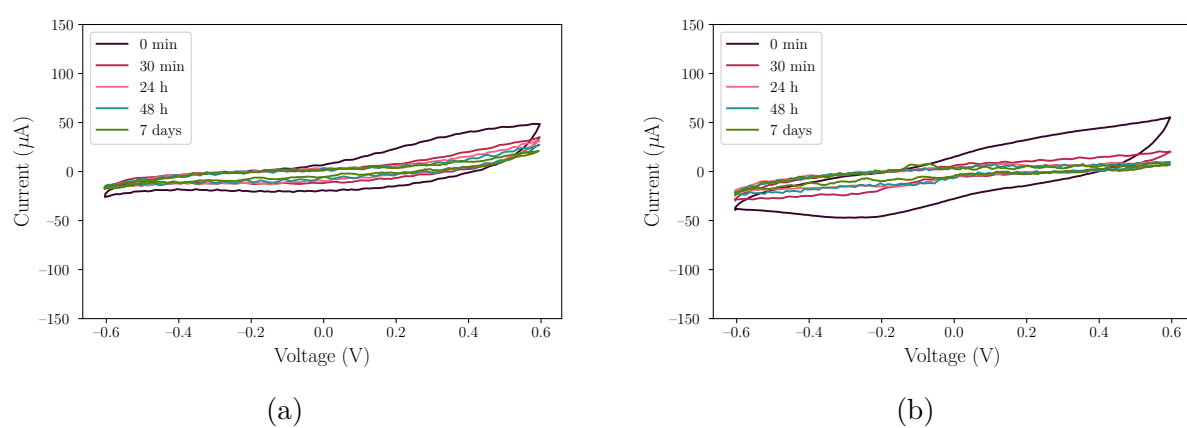
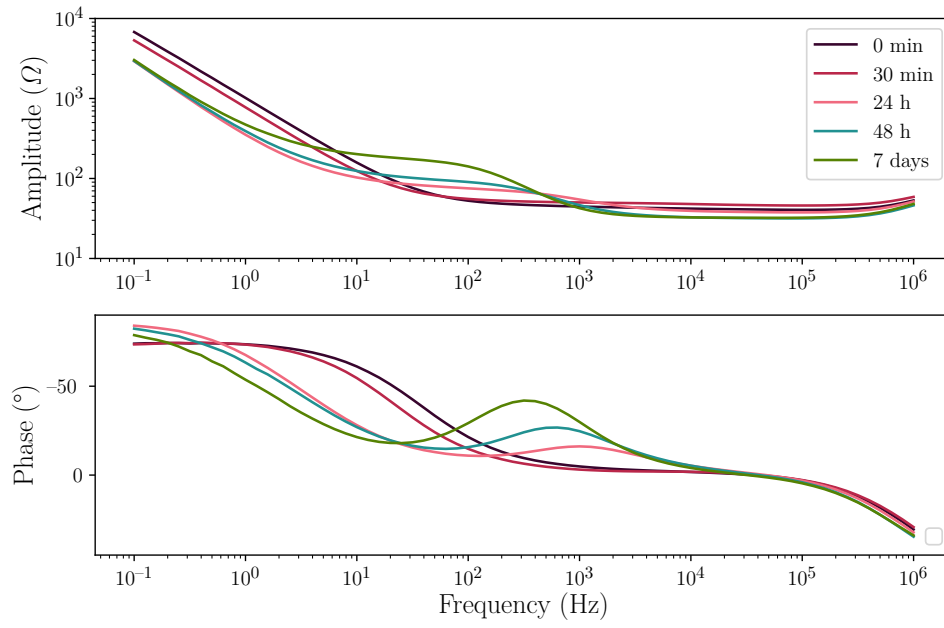
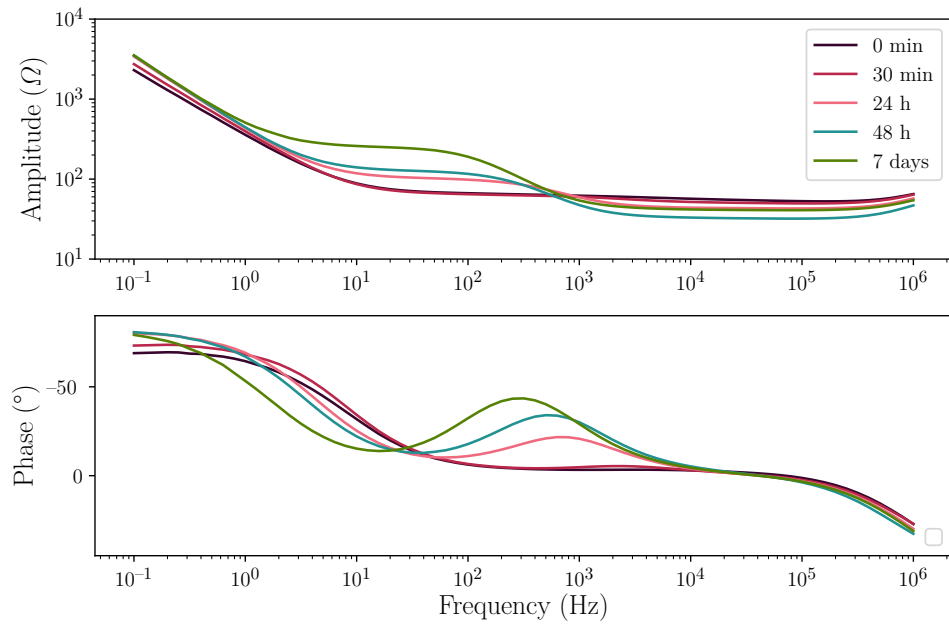


Figure 30 – Representative cyclic voltammetry curves for (a) N_3 -PEDOT:PSS and (b) azo-tz-PEDOT:PSS in DPBS. The voltage is reported vs Ag/AgCl electrode.



(a)



(b)

Figure 31 – Representative Bode plot of EIS measurements of (a) N_3 -PEDOT:PSS and (b) azo-tz-PEDOT:PSS in DPBS.

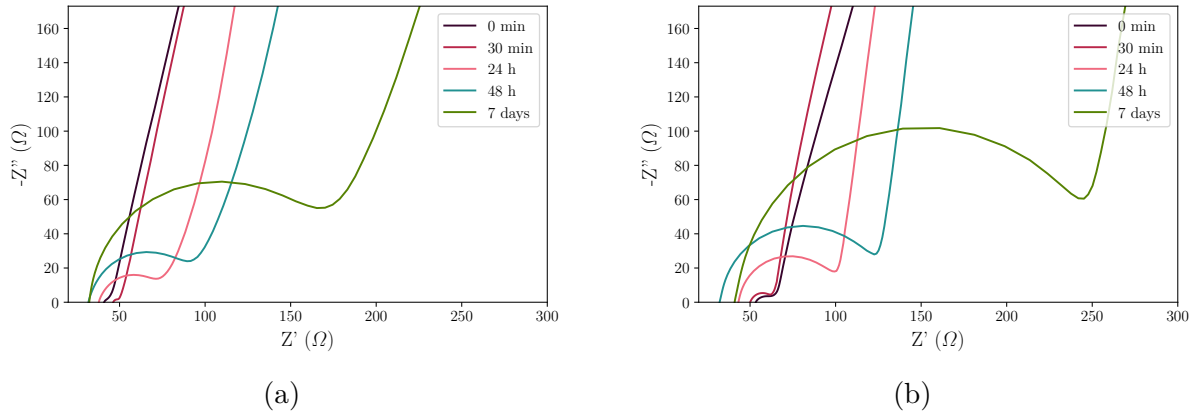


Figure 32 – Representative Nyquist plots for (a) N₃-PEDOT:PSS and (b) azo-tz-PEDOT:PSS in DPBS.

Overall, these observations suggest that the peak current decrease noted during the electrodeposition with increasing cycles (Figure 28b), along with decreasing capacitance and increasing charge transfer resistance over time, might be caused by a strong overoxidation of polymer films, as reported in literature (LÁNG et al., 2012). Overoxidation can be either chemical, i.e. due to the addition of chemical oxidants, or electrochemical, i.e. due to an applied potential, and it potentially degrades the polymer films by an increased internal stress. This can cause cracking, delamination and the release of degradation products. Nevertheless, overoxidized films can show unique sensing properties, and have been used for perchlorate (BENDIKOV; HARMON, 2005) and dopamine (LIN et al., 2014) sensing.

3.1.3 Voltage Reduction Rationale

Although not inherently negative, the results expose a decrease in performance due to overoxidation. A poor electrochemical activity and hindered charge transfer at the electrode-electrolyte interface is observed in both N₃-PEDOT:PSS and azo-tz-PEDOT:PSS. This could limit the application of the films for cell stimulation and recording. To test the hypothesis of overoxidation, the potential window of the N₃-EDOT CV electrodeposition was increased stepwise from 1.0 V to 1.5 V, determining, in this way, the minimum potential for the beginning of the nucleation process and the optimal potential range for deposition with best film performance and reliability.

With the custom set-up (detailed on Section 2.1.2.3), the most consistent results were found to appear in a voltage sweep from 0 V to 1.2 V. The obtained electrodeposition curve of N₃-PEDOT:PSS is presented in Figure 33, which exhibits the same behavior as PEDOT:PSS (Figure 28a). The growth is also in agreement with literature (SCAVETTA

et al., 2014). If not stated otherwise, the following experiments were conducted using films produced under this optimized condition.

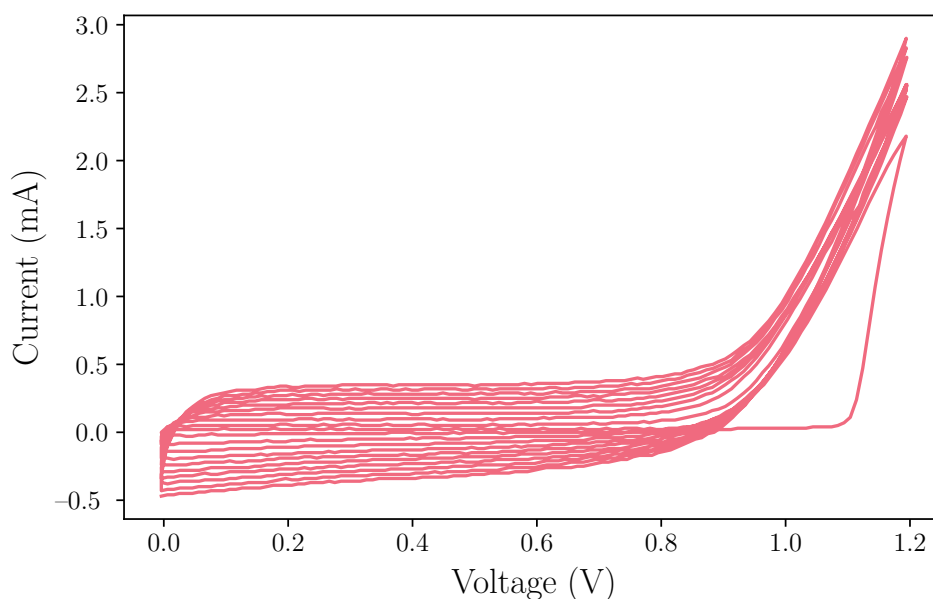


Figure 33 – Representative electrodeposition curve for N₃-PEDOT:PSS. The voltage is reported vs Ag/AgCl electrode.

3.2 MORPHOLOGICAL CHARACTERIZATION

3.2.1 Film thickness

The capacitance of polymeric thin films increases with film thickness due to the greater availability of charge sites for ion storage in thicker films (PROCTOR; RIVNAY; MALLIARAS, 2016). In order to compare the performance of different polymer films, it's relevant to consider the thickness of the films as it directly impacts the material's capacity for charge storage. PEDOT:PSS films showed an average thickness of 82.95 ± 6.28 nm, while N₃-PEDOT:PSS showed an average thickness of 108.80 ± 3.50 nm. In contrast to what was observed on overoxidized films, after the functionalization, azo-tz-PEDOT:PSS films became 60.94% thicker, with 175.10 ± 12.51 nm. Measurements were performed following the protocol described in Section 2.2.1, and are displayed in Table 1.

Table 1 – Thickness measurements

Material	Electrodeposition potential range [V]	Mean $\pm$ std [nm]
PEDOT:PSS	0 - 1.0	82.95 $\pm$ 6.28
N3-PEDOT:PSS	0 - 1.2	108.80 $\pm$ 3.50
N3-PEDOT:PSS	0 - 1.5	218.09 $\pm$ 22.16
Azo-tz-PEDOT:PSS	0 - 1.2	175.10 $\pm$ 12.51
Azo-tz-PEDOT:PSS	0 - 1.5	166.03 $\pm$ 8.85

3.2.2 Contact angle

The contact angle of the samples was calculated as described in Section 2.2.2, and Figure 34 depicts photographs of the droplets of water at the surface of the films. It is visible that the water drop spreads on N₃-PEDOT:PSS film (Figure 34a), while in the case of azo-tz-PEDOT:PSS, the surface tension is higher (Figure 34b). PEDOT:PSS films are very hydrophilic, with an average contact angle of $29.42^\circ \pm 4.98^\circ$, and N₃-PEDOT:PSS films have an average $58.28^\circ \pm 1.49^\circ$. Azo-tz-PEDOT:PSS films, on the other hand, are on the hydrophobic spectrum, with an average contact angle of $103.25^\circ \pm 2.94^\circ$.



Figure 34 – Images of DI-H₂O droplets on (a) N₃-PEDOT:PSS and (b) azo-tz-PEDOT:PSS films, used for contact angle calculations.

Table 3 – Contact angle

Material	Electrodeposition potential range [V]	Mean $\pm$ std [$^\circ$]
PEDOT:PSS	0 - 1.0	29.42 $\pm$ 4.98
N3-PEDOT:PSS	0 - 1.2	58.28 $\pm$ 1.49
Azo-tz-PEDOT:PSS	0 - 1.2	103.25 $\pm$ 2.94

3.2.3 Scanning Electron Microscopy

As described in Section 2.2.4, the films' surface was inspected via SEM imaging. Representative micrographs are presented in Figure 35, with three magnifications: 1,000 X, 10,000 X and 100,000 X. Both PEDOT:PSS and N₃-PEDOT:PSS with optimized electrodeposition (Figures 35a and 35b, respectively) show similar homogeneous surface. As anticipated in Section 3.1.2, the morphological changes observed in Figure 35c of the N₃-PEDOT:PSS film can also be attributed to the higher electrodeposition voltage, once overoxidation also increases the cristallinity of polymer films (TOMSIK et al., 2018). Although similar to the N₃-PEDOT:PSS precursor in lower magnifications, azo-tz-PEDOT:PSS at 100,000 X magnification show some filaments on the structure.

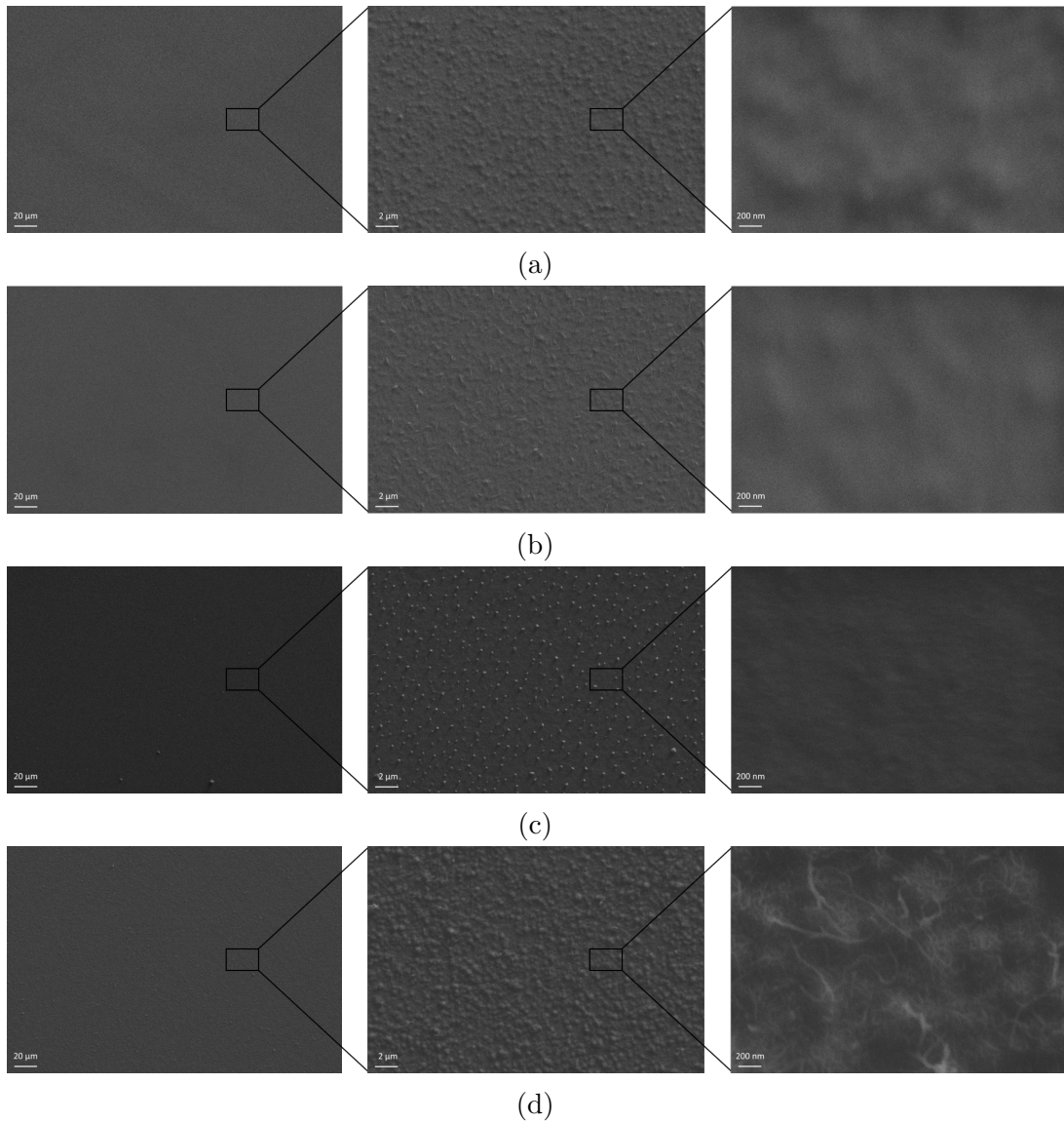


Figure 35 – SEM images of (a) PEDOT:PSS films, (c, d) N₃-PEDOT:PSS films electrodeposited with 1.2 V and 1.5 V, respectively, and (e) azo-tz-PEDOT:PSS films.

3.2.4 Atomic Force Microscopy

From AFM images, PEDOT-rich and PSS-rich domains can be distinguished, as illustrated in Figure 36a. PEDOT-dominant sites provide hole conductivity, while PSS-dominant sites provide ionic conductivity (VOLKOV et al., 2017). The phase image gives information on the surface topography and energy dissipation during scanning, with the contrast indicating different chemical compositions. PSS-dominant regions are more hydrophilic, being usually softer and swelling easily, providing darker (lower) phase signals and higher height signals (ZHANG, Y. et al., 2023). PEDOT-dominant regions, on the other hand, are harder surfaces with higher phase signals (perceived as brighter phase images).

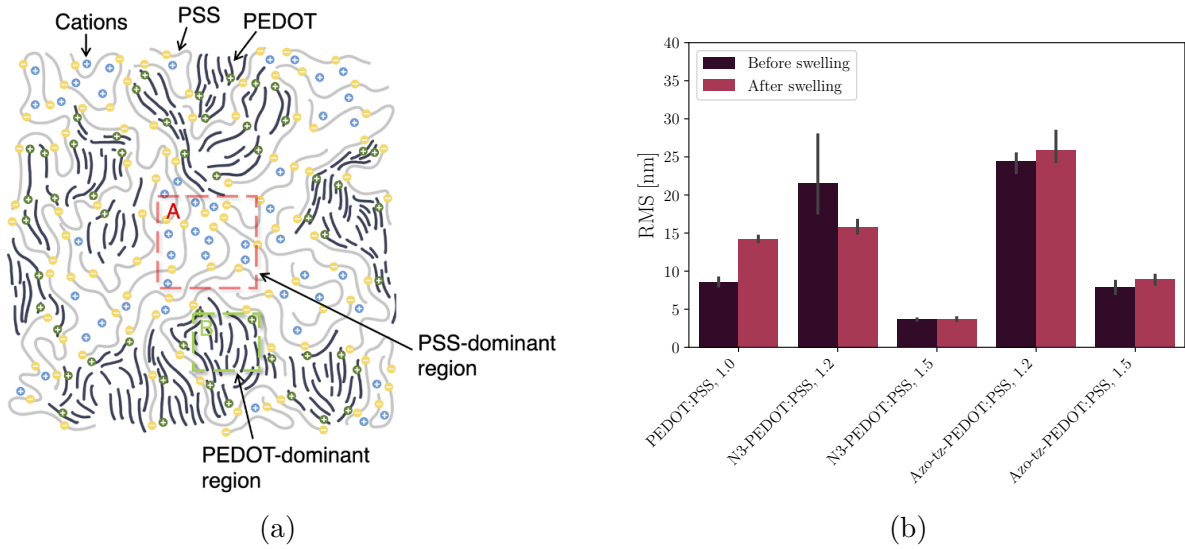


Figure 36 – (a) Schematics of the distribution of material in a PEDOT:PSS film surface, adapted from (ZHANG, Y. et al., 2023), (b) RMS roughness of the analysed films, with different electrodeposition potential ranges.

Figure 36b depicts the RMS roughness of the three materials, PEDOT:PSS, and N₃-PEDOT:PSS and azo-tz-PEDOT:PSS electrodeposited with both potential ranges (0 V to 1.2 V and to 1.5 V), all before and after one hour of swelling. Measurements are detailed in Section 2.2.5. A considerably lower roughness is observed in overoxidized films. As discussed previously in literature, upon overoxidation, the structure of PEDOT becomes more crystalline, resulting in a smoother surface (TOMSIK et al., 2018). Figures 37 and 38 show the height and phase images of PEDOT:PSS, N₃-PEDOT:PSS, and azo-tz-PEDOT:PSS films before and after swelling, respectively. Table 5 presents all calculated RMS roughness values and standard deviation.

Table 5 – Roughness measurements

Material	Electrodeposition potential range [V]	Roughness $\pm$ std [nm] - before swelling	Roughness $\pm$ std [nm] - after swelling
PEDOT:PSS	0 - 1.0	8.58 ± 0.61	14.15 ± 0.38
N ₃ -PEDOT:PSS	0 - 1.2	21.61 ± 5.50	15.79 ± 0.83
N ₃ -PEDOT:PSS	0 - 1.5	3.63 ± 0.08	3.64 ± 0.26
Azo-tz-PEDOT:PSS	0 - 1.2	24.50 ± 1.36	25.83 ± 2.21
Azo-tz-PEDOT:PSS	0 - 1.5	7.86 ± 0.95	8.90 ± 0.59

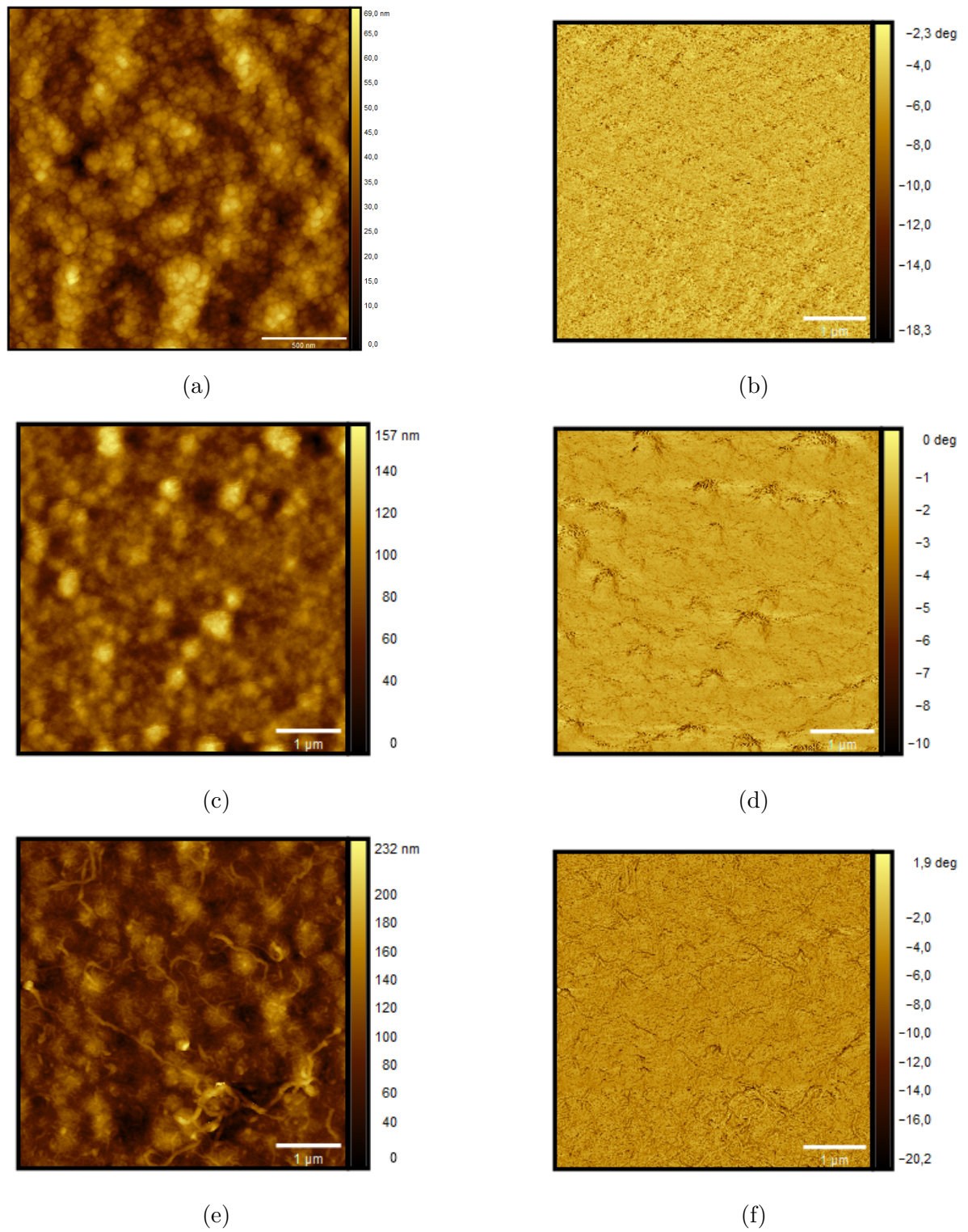


Figure 37 – AFM images of PEDOT:PSS films (a) height and (b) phase, N_3 -PEDOT:PSS films (c) height and (d) phase, and azo-tz-PEDOT:PSS films (e) height and (phase) before swelling.

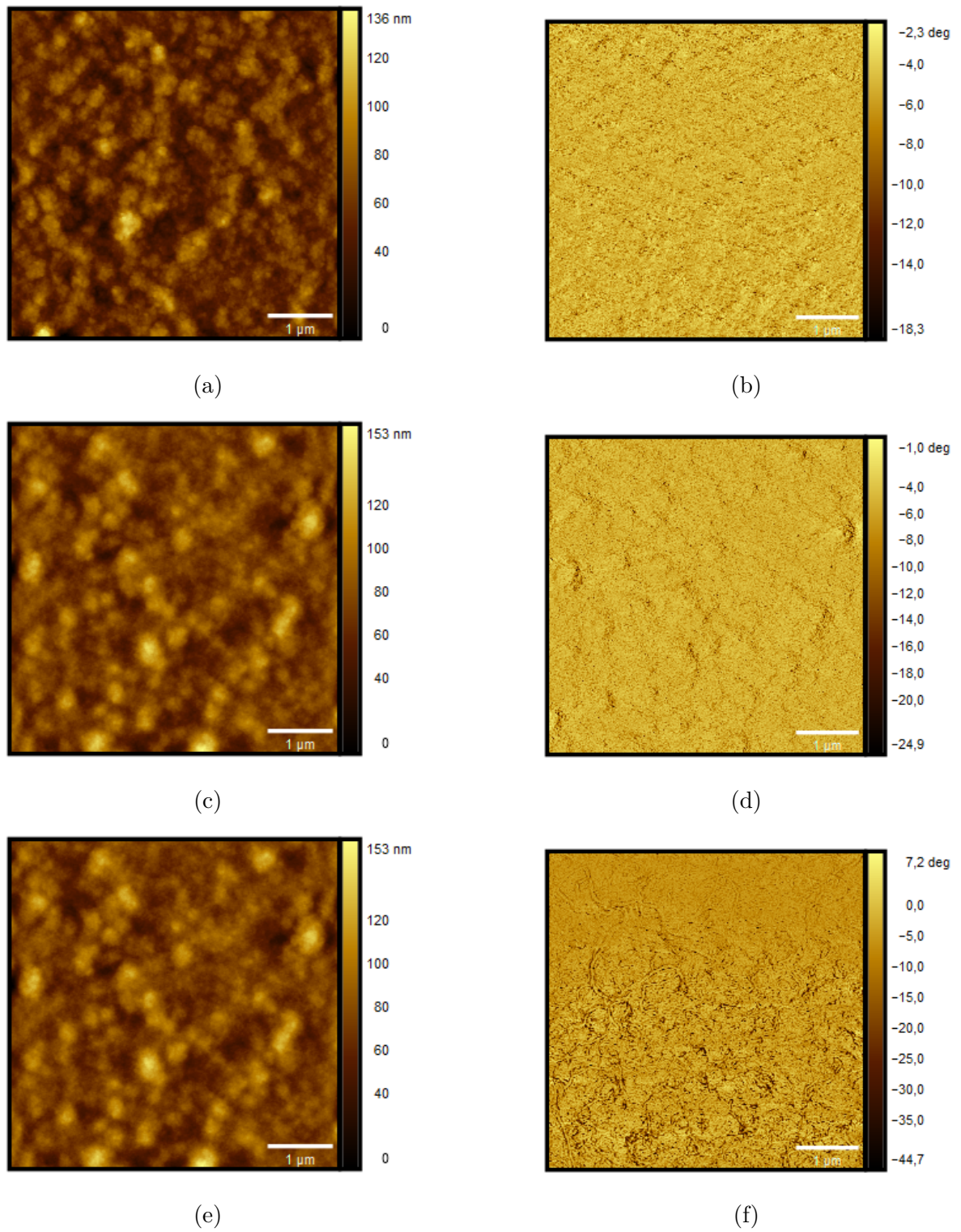


Figure 38 – AFM images of PEDOT:PSS films (a) height and (b) phase, N_3 -PEDOT:PSS films (c) height and (d) phase, and azo-tz-PEDOT:PSS films (e) height and (phase) after swelling.

3.3 ELECTROCHEMICAL CHARACTERIZATION

3.3.1 Cyclic Voltammetry

Charge transfer reactions at the test electrode in both DPBS and cell medium were inspected through CV technique, and are depicted in Figures 39 and 40, respectively. Capacitive charge transfer is responsible for steady current, while faradaic charge transfer causes voltage-dependent variations on the measured current. An increase in current is observed when the activation voltage for a reaction is reached, followed by a decrease when reactants at the surface are consumed. From the voltammograms in Figures 39a, 39b and 39c, no faradaic reactions at the electrode-electrolyte interface are detectable.

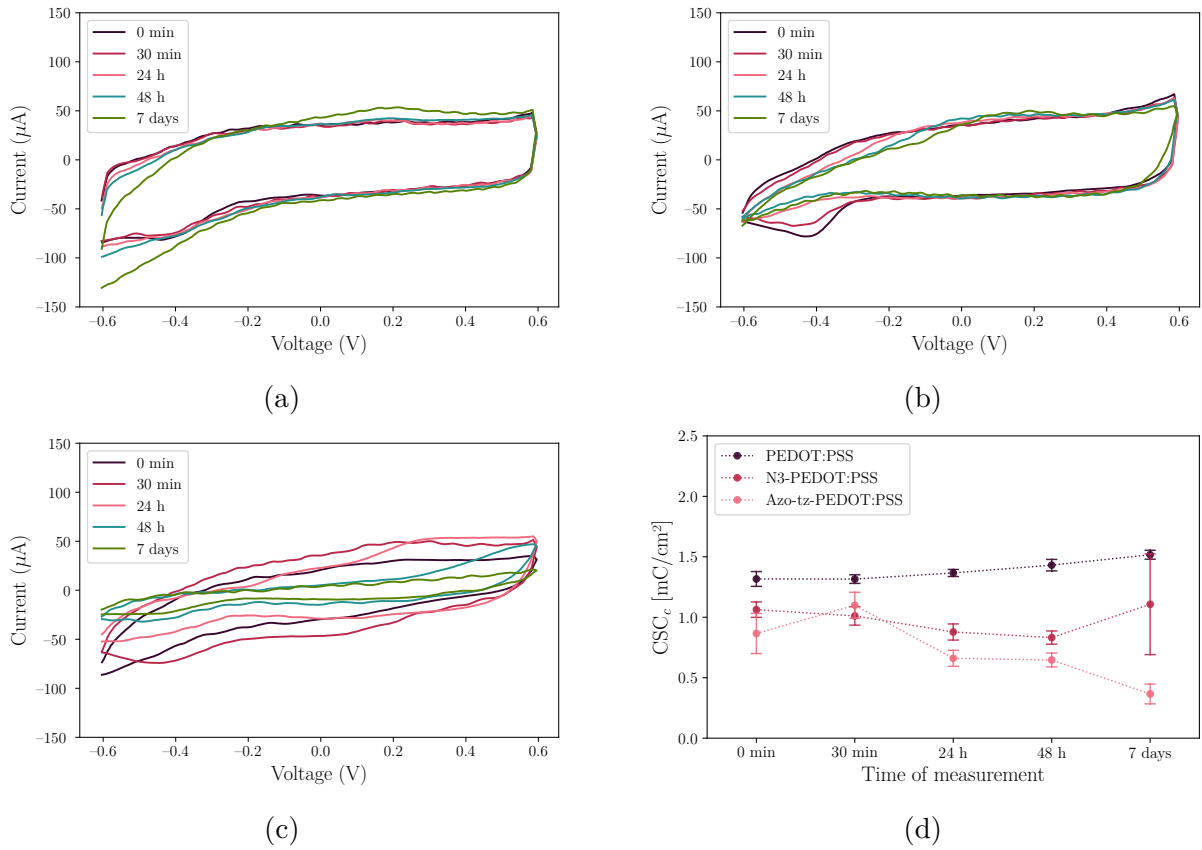


Figure 39 – Representative cyclic voltammetry curves for (a) PEDOT:PSS, (b) N3-PEDOT:PSS and (c) azo-tz-PEDOT:PSS in DPBS. The voltage is reported vs Ag/AgCl electrode. (d) CSC_c of the materials in DPBS.

CSC_c determines the total amount of charge available for stimulation within the voltage boundaries. It is significant to draw attention to the fact that the totality of charge available does not represent the actual injected charge during a stimulation pulse. The calculated CSC_c for the three materials over the course of seven days in DPBS is presented in Figure 39d. There's a decay tendency in the capacitance of N3-PEDOT:PSS and azo-

tz-PEDOT:PSS, and on the last day of measurement, the CSC_c of N₃-PEDOT:PSS films shows a great variability.

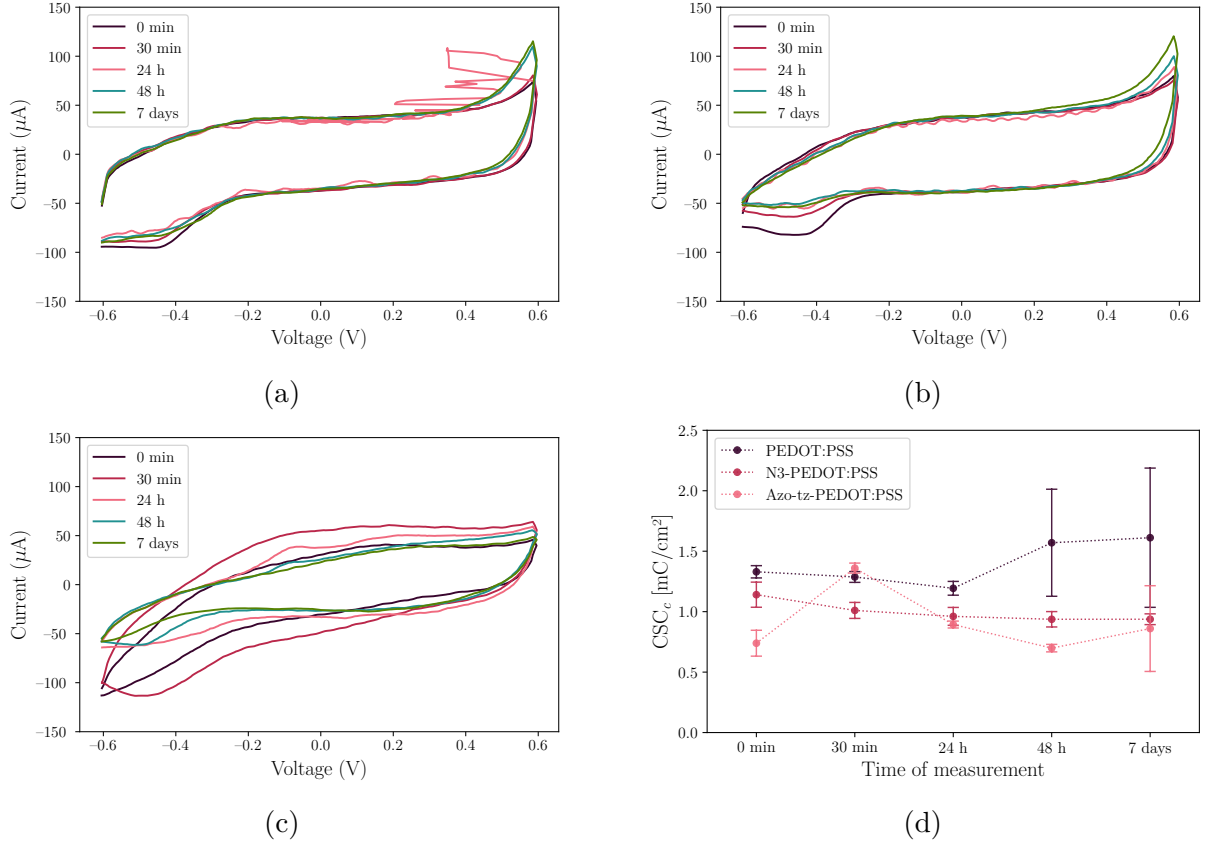


Figure 40 – Representative cyclic voltammetry curves for (a) PEDOT:PSS, (b) N₃-PEDOT:PSS and (c) azo-tz-PEDOT:PSS in DPBS. The voltage is reported vs Ag/AgCl electrode. (d) CSC_c of the materials in cell medium.

In cell medium, the overall behavior of the films is more stable throughout the days of measurement, but after a long period of stimulation PEDOT:PSS and azo-tz-PEDOT:PSS have increased variability. Representative CV curves are depicted in Figures 40a, 40b and 40c. Figure 40d presents the CSC_c values calculated from the CV curves. Table 7 comprises all CSC_c values for both DPBS and cell medium.

Table 7 – Cathodic charge storage capacity

Material	Time of measurement	DPBS - Mean $\pm$ std [mC cm ⁻²]	Cell medium - Mean $\pm$ std [mC cm ⁻²]
PEDOT:PSS	0 min	1.32 $\pm$ 0.06	1.33 $\pm$ 0.05
PEDOT:PSS	30 min	1.31 $\pm$ 0.04	1.29 $\pm$ 0.04
PEDOT:PSS	24 h	1.37 $\pm$ 0.03	1.19 $\pm$ 0.06
PEDOT:PSS	48 h	1.43 $\pm$ 0.05	1.57 $\pm$ 0.04
PEDOT:PSS	7 days	1.52 $\pm$ 0.04	1.61 $\pm$ 0.57
N ₃ -PEDOT:PSS	0 min	1.06 $\pm$ 0.06	1.14 $\pm$ 0.10
N ₃ -PEDOT:PSS	30 min	1.01 $\pm$ 0.08	1.01 $\pm$ 0.07
N ₃ -PEDOT:PSS	24 h	0.88 $\pm$ 0.07	0.96 $\pm$ 0.07
N ₃ -PEDOT:PSS	48 h	0.83 $\pm$ 0.05	0.94 $\pm$ 0.06
N ₃ -PEDOT:PSS	7 days	1.11 $\pm$ 0.42	0.94 $\pm$ 0.04
Azo-tz-PEDOT:PSS	0 min	0.87 $\pm$ 0.17	0.74 $\pm$ 0.11
Azo-tz-PEDOT:PSS	30 min	1.10 $\pm$ 0.11	1.36 $\pm$ 0.04
Azo-tz-PEDOT:PSS	24 h	0.66 $\pm$ 0.07	0.89 $\pm$ 0.03
Azo-tz-PEDOT:PSS	48 h	0.65 $\pm$ 0.06	0.70 $\pm$ 0.03
Azo-tz-PEDOT:PSS	7 days	0.36 $\pm$ 0.08	0.86 $\pm$ 0.35

3.3.2 Electrochemical Impedance Spectroscopy

The response of the system to perturbation in various frequencies is depicted in Figure 41. PEDOT:PSS and N₃-PEDOT:PSS films behave similarly, while azo-tz-PEDOT:PSS films have higher impedance and attenuate signals at higher frequencies. The impedance at 1 kHz, as explained in Section 2.3.2, gives an insight on how action potential signals will be received by the electrode. Figures 42c and 42d show how this impedance varies over time on the analysed materials. On the first day of measurement, azo-tz-PEDOT:PSS films have higher impedance than PEDOT:PSS and N₃-PEDOT:PSS films, but after 24 hours, the impedance settles to a value similar to the others. The electrolyte doesn't alter the overall behavior, solely the impedance values and variability — indeed, a higher impedance is observed for azo-tz-PEDOT:PSS films 0 and 30 minutes after addition of cell medium. This is in accordance with the previous contact angle measurements (refer to Section 3.2.2), which indicate a higher hydrophobicity of azo-tz-

PEDOT:PSS films in comparison to N₃-PEDOT:PSS and PEDOT:PSS.

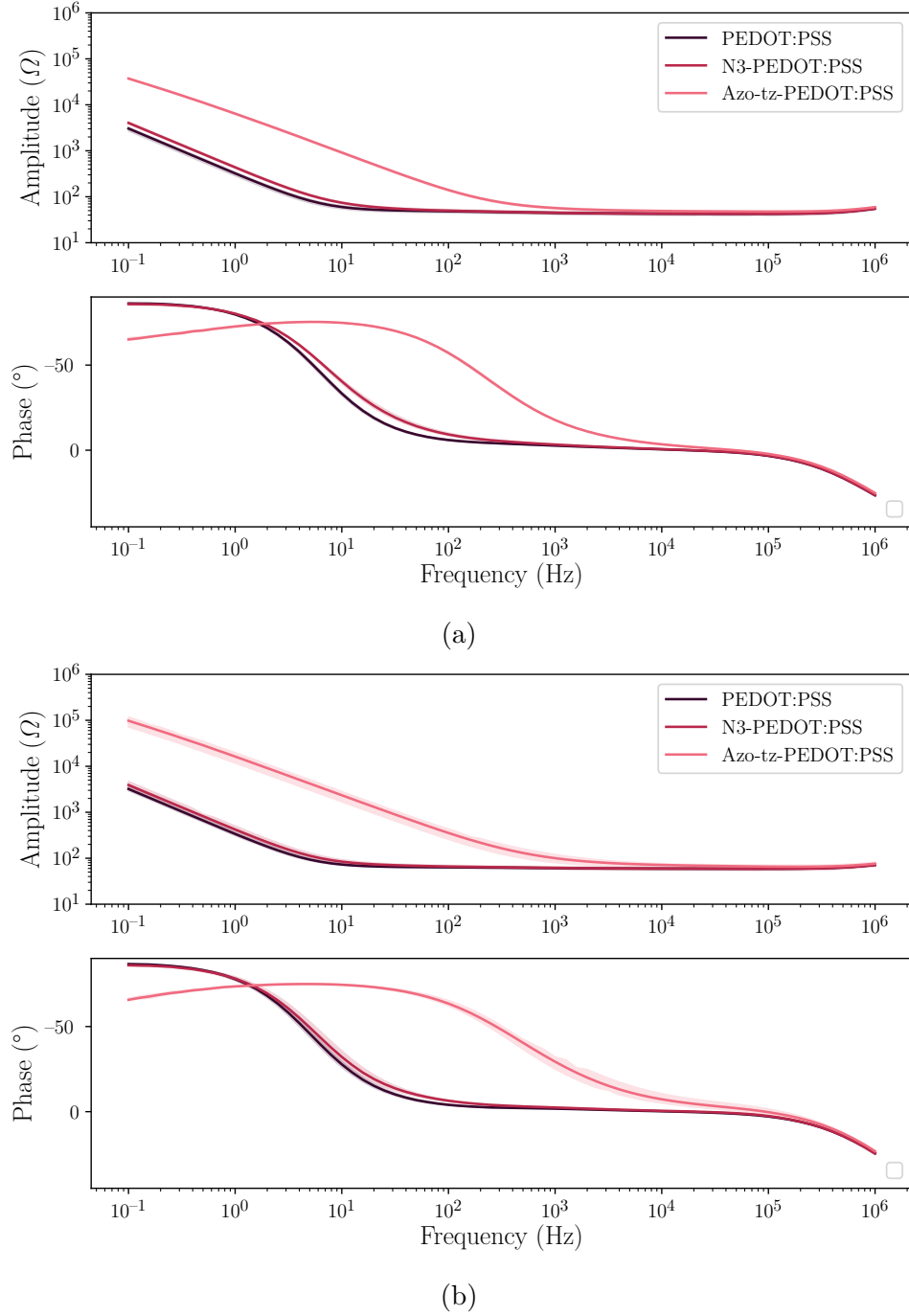


Figure 41 – Representative Bode plot of EIS measurements of PEDOT:PSS, N₃-PEDOT:PSS and azo-tz-PEDOT:PSS films after 30 minutes in (a) DPBS and (b) cell medium.

A more comprehensive description of the system includes the cut-off frequency ($f_{\text{cut-off}}$) of the electrode, which delimits the frequency that reduces the input signal by 3 dB, or 70.7%. Frequency components higher than $f_{\text{cut-off}}$ are accurately retrieved by the electrode, while lower frequencies are filtered or phase-shifted. Figures 42c and 42d evidence

how $f_{\text{cut-off}}$ of azo-tz-PEDOT:PSS films decrease greatly in the first day of measurement in both electrolytes, and after 24 hours the decay is hindered allowing current flow with minimal distortion above 50 Hz in DPBS, and above 75 Hz in cell medium. Figures 43, 44 and 45 present representative EIS curves of PEDOT:PSS, N₃-PEDOT:PSS, and azo-tz-PEDOT:PSS films, respectively, in DPBS and cell medium over the course of 7 days. Tables 9 and 11 contain the measured values of impedance at 1 kHz and cut-off frequency, respectively.

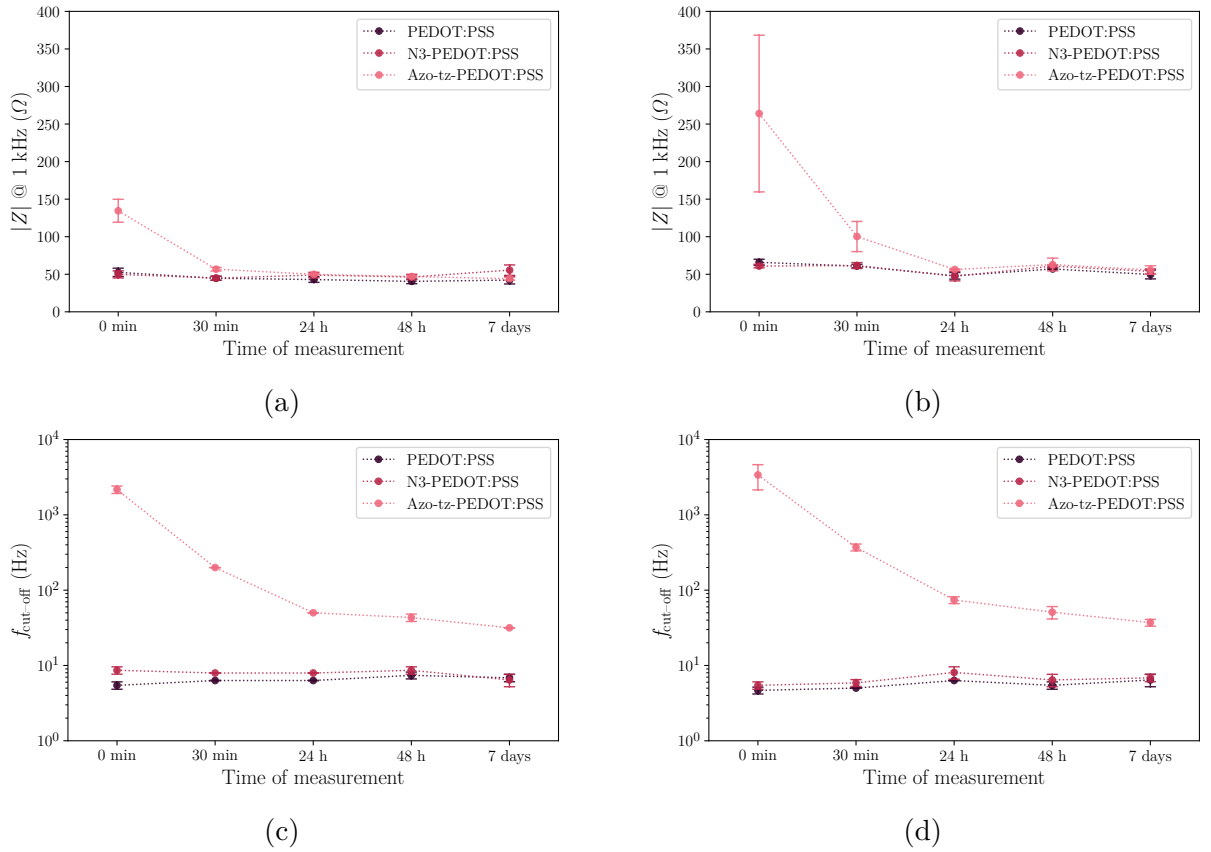
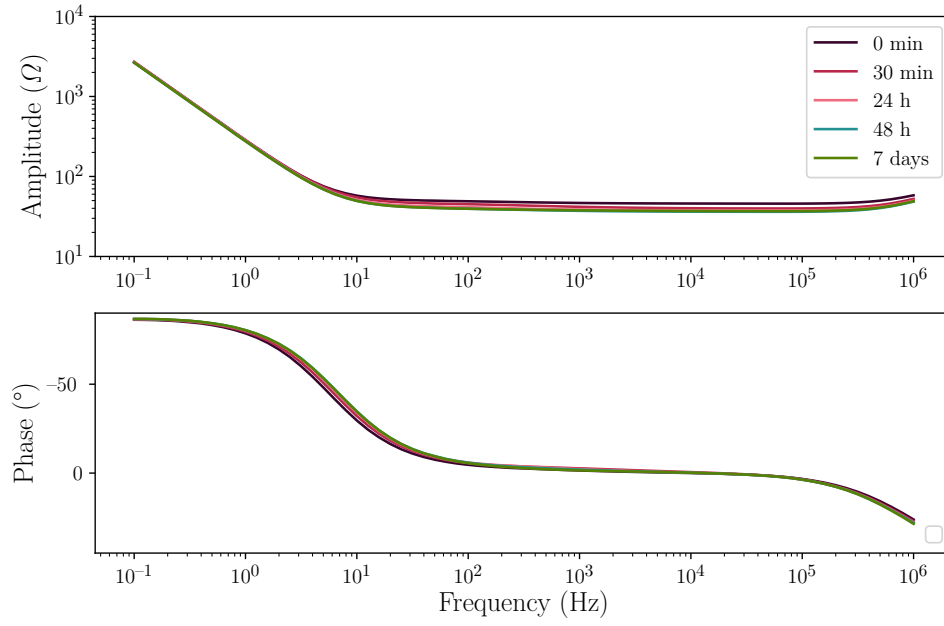
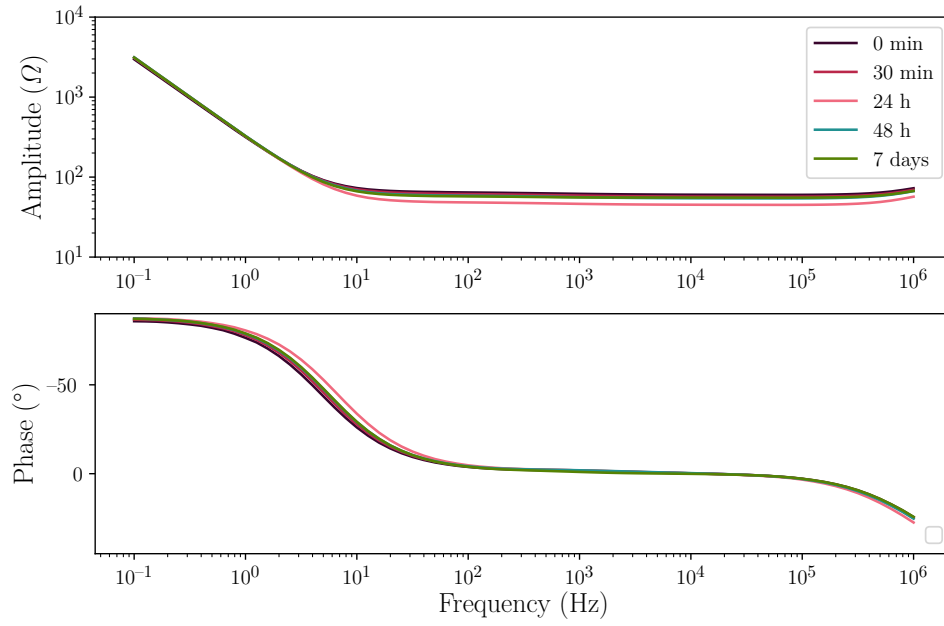


Figure 42 – Impedance of PEDOT:PSS, N₃-PEDOT:PSS and azo-tz-PEDOT:PSS films at 1 kHz in (a) DPBS and (b) cell medium, and cut-off frequency of the films in (c) DPBS and (d) cell medium.

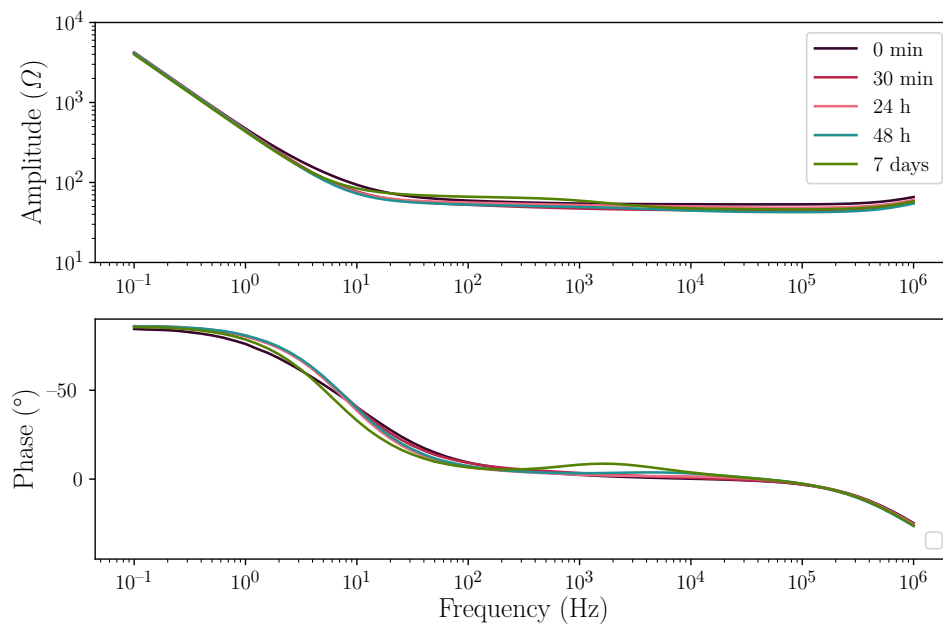


(a)

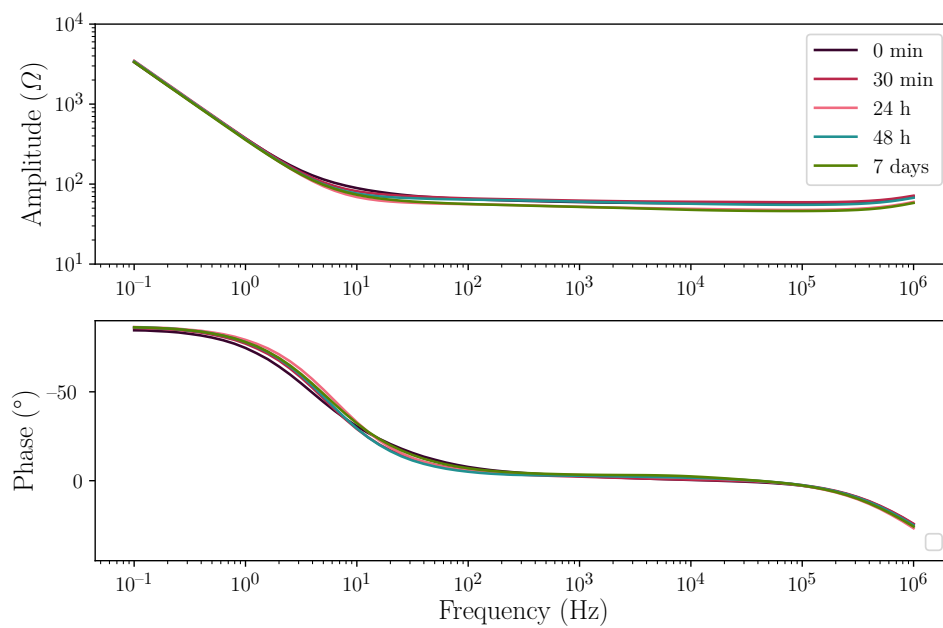


(b)

Figure 43 – Representative Bode plot of EIS measurements of PEDOT:PSS films in (a) DPBS and (b) cell medium.

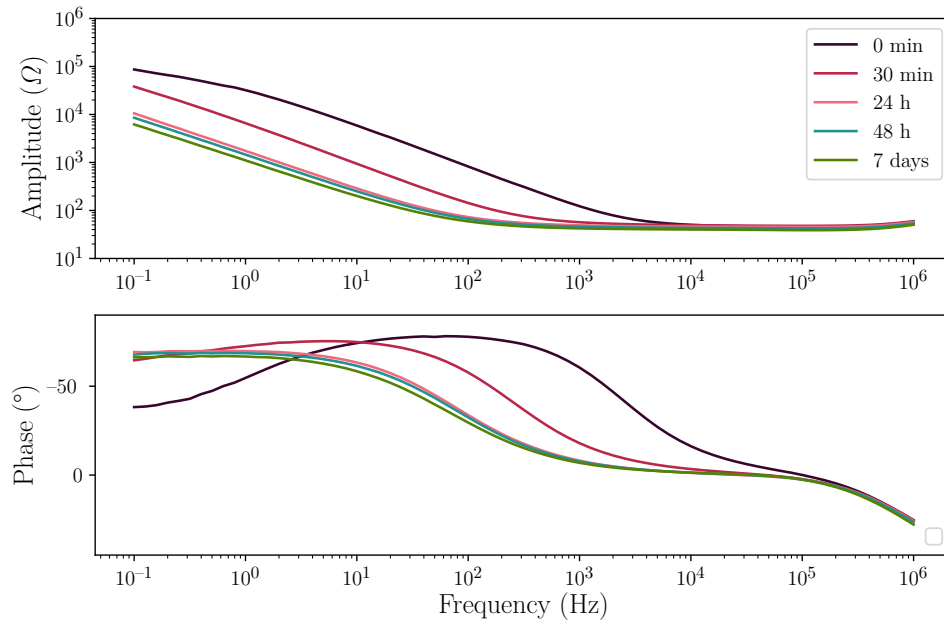


(a)

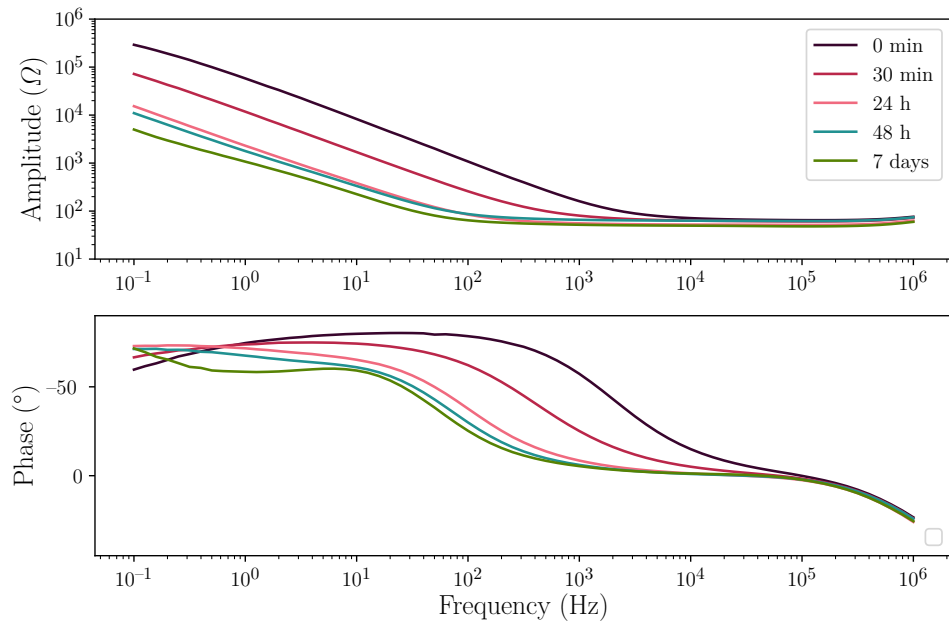


(b)

Figure 44 – Representative Bode plot of EIS measurements of N₃-PEDOT:PSS films in (a) DPBS and (b) cell medium.



(a)



(b)

Figure 45 – Representative Bode plot of EIS measurements of azo-tz-PEDOT:PSS films in (a) DPBS and (b) cell medium.

Table 9 – Impedance at 1 kHz

Material	Time of measurement	DPBS - Mean $\pm$ std [Ω]	Cell medium - Mean $\pm$ std [Ω]
PEDOT:PSS	0 min	52.53 $\pm$ 5.57	65.91 $\pm$ 4.0
PEDOT:PSS	30 min	44.66 $\pm$ 2.69	60.85 $\pm$ 2.37
PEDOT:PSS	24 h	42.96 $\pm$ 3.73	47.91 $\pm$ 4.39
PEDOT:PSS	48 h	40.55 $\pm$ 3.08	57.08 $\pm$ 1.20
PEDOT:PSS	7 days	42.24 $\pm$ 5.15	49.70 $\pm$ 5.87
N ₃ -PEDOT:PSS	0 min	49.81 $\pm$ 4.70	60.90 $\pm$ 2.31
N ₃ -PEDOT:PSS	30 min	44.92 $\pm$ 2.60	61.61 $\pm$ 3.93
N ₃ -PEDOT:PSS	24 h	48.93 $\pm$ 3.63	47.39 $\pm$ 6.18
N ₃ -PEDOT:PSS	48 h	46.32 $\pm$ 3.63	46.32 $\pm$ 3.63
N ₃ -PEDOT:PSS	7 days	55.48 $\pm$ 6.91	53.84 $\pm$ 3.29
Azo-tz-PEDOT:PSS	0 min	134.53 $\pm$ 15.25	263.96 $\pm$ 104.31
Azo-tz-PEDOT:PSS	30 min	56.76 $\pm$ 2.43	100.23 $\pm$ 20.08
Azo-tz-PEDOT:PSS	24 h	49.95 $\pm$ 1.06	56.38 $\pm$ 0.64
Azo-tz-PEDOT:PSS	48 h	46.97 $\pm$ 1.27	62.89 $\pm$ 8.53
Azo-tz-PEDOT:PSS	7 days	44.14 $\pm$ 2.00	62.89 $\pm$ 8.53

Table 11 – Cut-off frequency

Material	Time of measurement	DPBS - Mean $\pm$ std [Hz]	Cell medium - Mean $\pm$ std [Hz]
PEDOT:PSS	0 min	5.44 ± 0.61	4.67 ± 0.49
PEDOT:PSS	30 min	6.31 ± 0.00	5.01 ± 0.00
PEDOT:PSS	24 h	6.31 ± 0.00	6.31 ± 0.00
PEDOT:PSS	48 h	7.40 ± 0.77	5.44 ± 0.61
PEDOT:PSS	7 days	6.85 ± 0.77	6.42 ± 1.20
N ₃ -PEDOT:PSS	0 min	8.63 ± 0.97	5.44 ± 0.61
N ₃ -PEDOT:PSS	30 min	7.94 ± 0.00	5.88 ± 0.61
N ₃ -PEDOT:PSS	24 h	7.94 ± 0.00	8.08 ± 1.51
N ₃ -PEDOT:PSS	48 h	8.63 ± 0.97	6.42 ± 1.20
N ₃ -PEDOT:PSS	7 days	6.42 ± 1.20	6.85 ± 0.77
Azo-tz-PEDOT:PSS	0 min	2167.47 ± 244.11	3389.80 ± 1244.91
Azo-tz-PEDOT:PSS	30 min	199.52 ± 0.00	370.81 ± 38.69
Azo-tz-PEDOT:PSS	24 h	50.12 ± 0.00	73.99 ± 7.72
Azo-tz-PEDOT:PSS	48 h	43.25 ± 4.87	51.01 ± 9.55
Azo-tz-PEDOT:PSS	7 days	31.62 ± 0.00	37.08 ± 3.87

3.4 LIGHT STIMULATION

In order to understand the light-responsive behavior of azo-tz-PEDOT:PSS, the films were illuminated for 115 s with three light intensities (0.3, 0.9 and 1.5 mW/cm²) of 365 nm, as described in Section 2.4. Without applying any bias, the current was measured simultaneously in cell medium and DPBS in two different channels of a SMU.

The readout current over the course of seven days for light power of 0.9 mW/cm² is depicted in Figure 46. When light is switched on (light-blue background), a negative current flows, from the CE to the film (WE). As discussed in Section 1.5.2, the azobenzene moiety bonded to the polymer chain absorbs incident photons at 365 nm, generating an exciton. The measured current comes from the excited electrons that are promoted to a higher energy orbital (LUMO) and are collected by the ITO substrate. The positively charged polymer at the interface causes a movement of ions in the electrolyte, compensated at the CE. The photo-induced isomerization of the azobenzenes further assist the conductivity,

once the *cis* isomer requires less energy for electron flow from HOMO to LUMO due to $n - \pi^*$ transitions (JERCA, F. A.; JERCA, V. V.; HOOGENBOOM, 2021).

In the first day in DPBS, a sustained current is generated. From the second day on, the current shape changes to a more capacitive behavior, decaying over time (PARAMESWARAN et al., 2018). In a different manner, in cell medium the current preserves its shape, but its amplitude decreases over the days. Indeed, on the last day of measurement, it is completely attenuated. A possible cause is denaturation of proteins in FBS and vitamins in DMEM/F-12 due to UV light exposure, hindering the conduction. The same behavior was observed for samples illuminated with 0.3 and 1.5 mW/cm^2 .

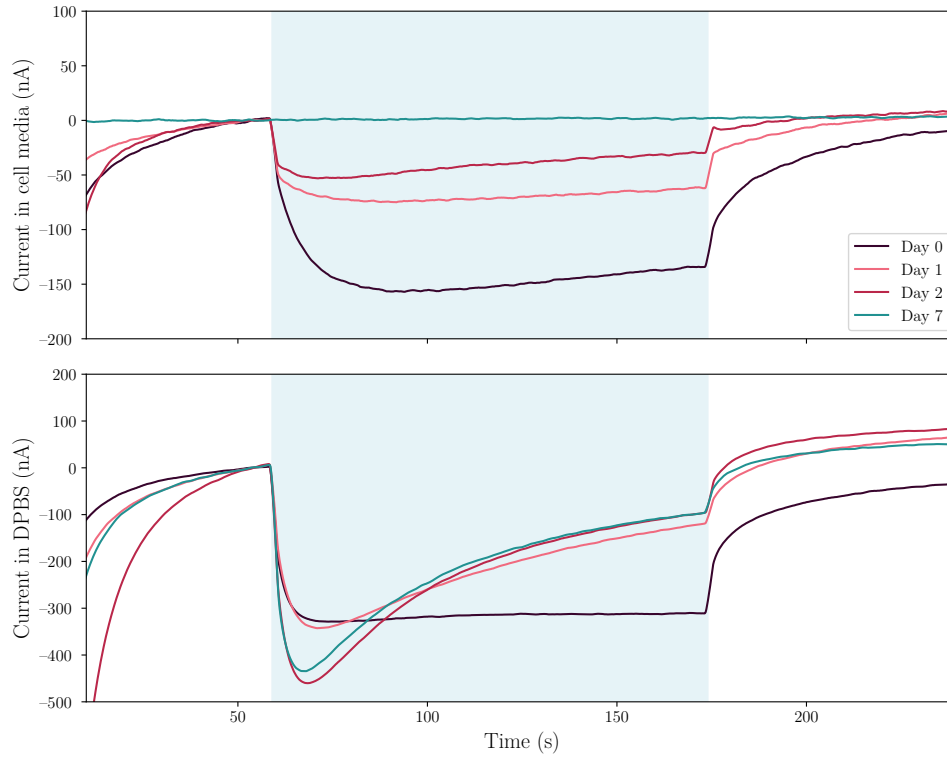


Figure 46 – Representative light stimulation curves of azo-tz-PEDOT:PSS films in cell medium (top) and in DPBS (bottom), with light power of 0.9 mW/cm^2 . The light-blue background represents the period of illumination.

The shape difference of the azo-tz-PEDOT:PSS curves in DPBS does not change the injected charge (Figure 47a). In fact, the CIC in DPBS remains stable throughout the stimulation days, only showing higher variability in the highest power case (1.5 mW/cm^2). The CIC of the control materials is almost negligible, and the measured current is probably generated by interferences on the measuring system, rather than the substrates themselves.

Azo-tz-PEDOT:PSS films in cell medium show reduced charge injection capabilities in comparison to DPBS. In addition, the CIC decreases considerably from the second

day of stimulation. This behavior contrasts with the comparable CSC_c observed in both electrolytes (refer to Section 3.3.1), even though the amount of available stored charge is not directly proportional to the actual charge injected during stimulation (GANJI et al., 2017). It is possible to presume that the phenol red present in cell medium might absorb part of the light, reducing charge transfer mechanisms at the polymer film. It is stated in literature that phenol red has low absorption at 365 nm, and two absorption peaks: a higher at 432 nm, and a lower at 560 nm (SUNDARAKRISHNAN et al., 2016). Nevertheless, it would be of interest to examine the effects of a medium without phenol red in the CIC.

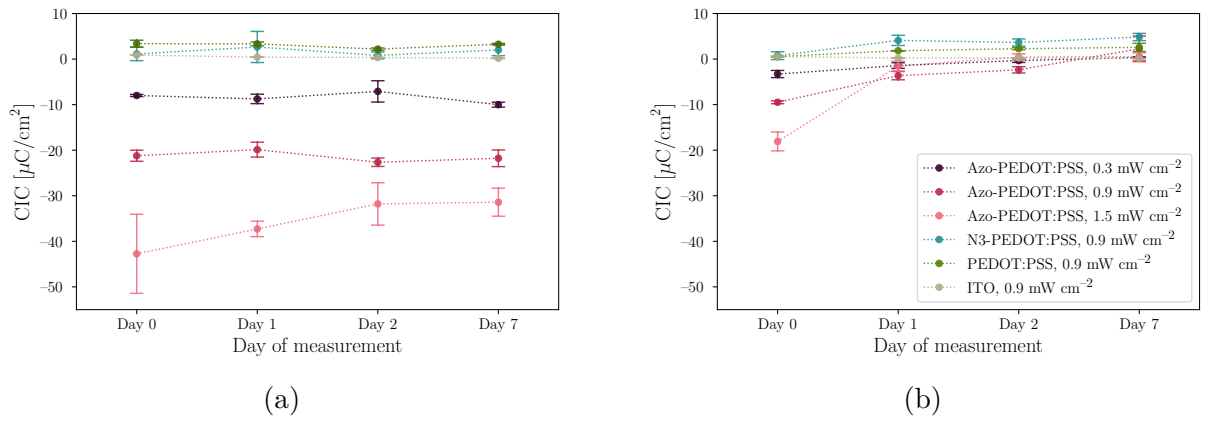


Figure 47 – Charge injection capacity of azo-tz-PEDOT:PSS (0.3 mW/cm^2 , 0.9 mW/cm^2 and 1.5 mW/cm^2), N3-PEDOT:PSS (0.9 mW/cm^2) and PEDOT:PSS (0.9 mW/cm^2) films, and bare ITO (0.9 mW/cm^2) in (a) DPBS and (b) cell medium.

In an effort to comprehend the underlying mechanism of progressive change in the measured current over time upon illumination, a new experimental approach has been proposed. Samples were prepared as before, however, instead of starting the measurement after one hour of electrolyte addition, the first light pulse was applied only in the following day. This way, the response of the film to periodic illumination is isolated from the effects of the electrolyte on the film over time. Figure 48 shows the measured current in both conditions, illumination since day 0 (in pink) and since day 1 in electrolyte (in turquoise).

Even without prior illumination, the current response on the first day of measurement in DPBS exhibits a noticeable decay, indicative of capacitive behavior likely due to charge accumulation at the electrode surface. Notably, the CIC from the initial light pulse on day 1 is 68.52% higher compared to the first light pulse on day 0, as depicted in Figure 49a. By day 2, the current responses in both scenarios coincide, exhibiting similar behavior.

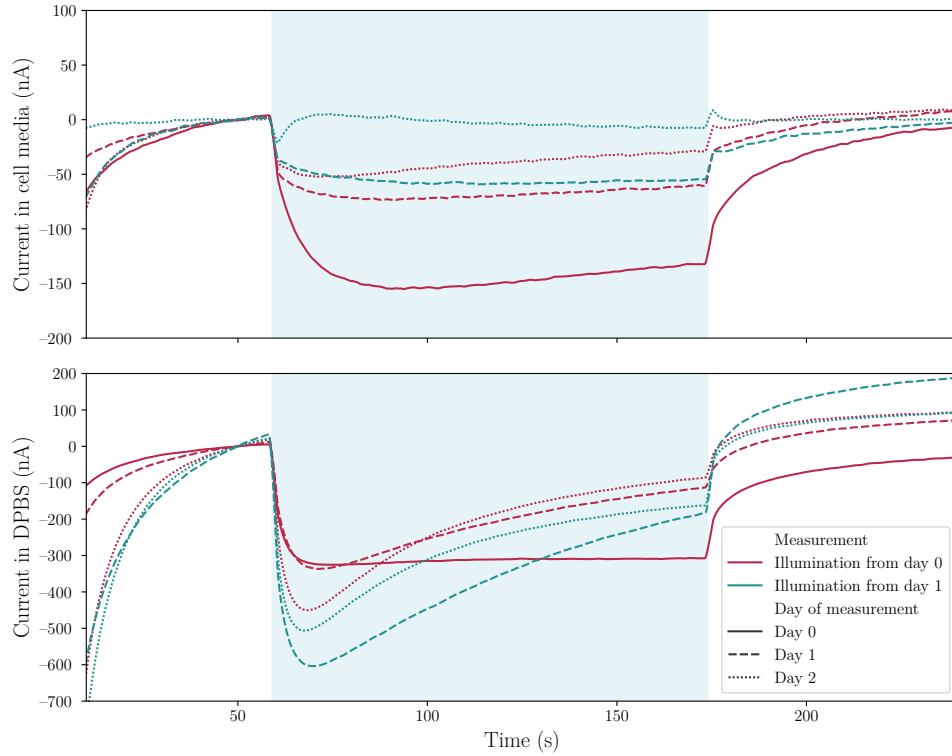


Figure 48 – Representative light stimulation curves of azo-tz-PEDOT:PSS films in cell medium (top) and in DPBS (bottom). Curves in pink are from samples illuminated on days 0, 1 and 2 in electrolyte, and curves in turquoise are from samples illuminated on days 1 and 2 in electrolyte. The light-blue background represents the period of illumination, with 0.9 mW/cm^2 of light power.

In contrast, when in cell media (see Figure 49b), the CIC on day 1 remains similar in both conditions, suggesting that the decay observed over subsequent measurement days, as illustrated in Figures 46 and 47b, is not primarily driven by illumination effects as previously considered. This indicates that the differences in behavior are more likely attributable to the intrinsic properties of the electrolytes and their effects on the films rather than the illumination conditions. Further experiments are in due course in order to elucidate the mechanism of current generation upon light exposure, and the effects of such illumination on the performance of azo-tz-PEDOT:PSS films.

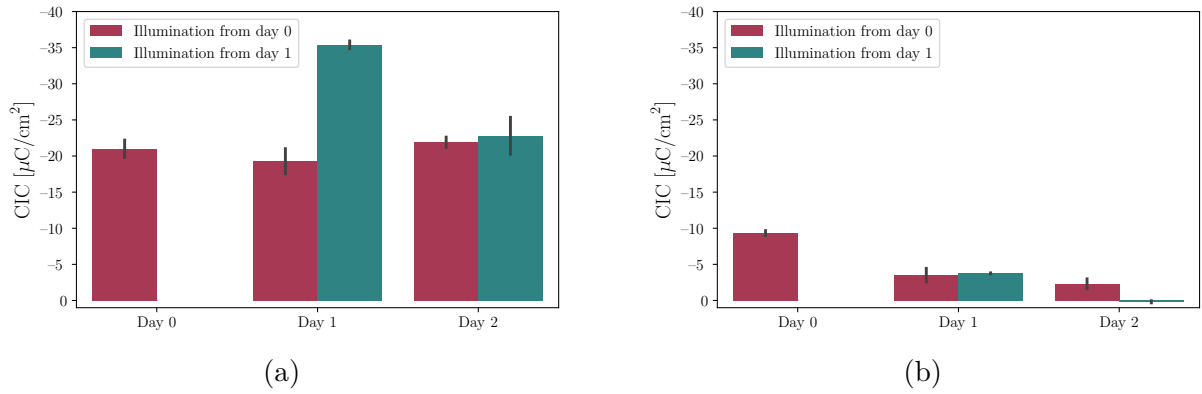


Figure 49 – Charge injection capacity of azo-tz-PEDOT:PSS films in (a) DPBS and (b) cell medium, with illumination of $0.9 \text{ mW}/\text{cm}^2$.

Table 13 – Charge injection capacity

Material	Day of measurement	DPBS - Mean $\pm$ std [$\mu\text{C cm}^{-2}$]	Cell medium - Mean $\pm$ std [$\mu\text{C cm}^{-2}$]
ITO	Day 0	0.87 ± 0.02	0.56 ± 0.06
ITO	Day 1	0.43 ± 0.05	0.23 ± 0.05
ITO	Day 2	0.35 ± 0.01	0.24 ± 0.00
ITO	Day 7	0.18 ± 0.10	0.14 ± 0.05
PEDOT:PSS	Day 0	3.39 ± 0.75	0.54 ± 0.09
PEDOT:PSS	Day 1	3.33 ± 0.42	1.83 ± 0.02
PEDOT:PSS	Day 2	2.19 ± 0.24	2.28 ± 0.23
PEDOT:PSS	Day 7	3.26 ± 0.18	2.56 ± 0.89
N ₃ -PEDOT:PSS	Day 0	1.13 ± 1.47	0.74 ± 0.86
N ₃ -PEDOT:PSS	Day 1	2.65 ± 3.41	4.10 ± 1.11
N ₃ -PEDOT:PSS	Day 2	0.80 ± 0.75	3.64 ± 0.78
N ₃ -PEDOT:PSS	Day 7	2.00 ± 1.25	4.84 ± 0.80

Table 15 – Charge injection capacity of azo-tz-PEDOT:PSS films

Light intensity [mW cm ⁻²]	Day of measurement	DPBS - Mean ± std [μC cm ⁻²]	Cell medium - Mean ± std [μC cm ⁻²]
0.3 mW cm ⁻²	Day 0	-7.99 ± 0.24	-3.28 ± 0.79
0.3 mW cm ⁻²	Day 1	-8.75 ± 1.03	-1.42 ± 0.61
0.3 mW cm ⁻²	Day 2	-7.10 ± 2.34	-0.32 ± 0.42
0.3 mW cm ⁻²	Day 7	-10.01 ± 0.54	0.35 ± 0.14
0.9 mW cm ⁻²	Day 0	-21.22 ± 1.20	-9.48 ± 0.33
0.9 mW cm ⁻²	Day 1	-19.87 ± 1.63	-3.64 ± 0.91
0.9 mW cm ⁻²	Day 2	-22.64 ± 0.92	-2.35 ± 0.69
0.9 mW cm ⁻²	Day 7	-21.77 ± 1.83	2.26 ± 2.80
1.5 mW cm ⁻²	Day 0	-42.74 ± 8.67	-18.01 ± 2.07
1.5 mW cm ⁻²	Day 1	-37.28 ± 1.69	-1.53 ± 1.08
1.5 mW cm ⁻²	Day 2	-31.79 ± 4.64	0.44 ± 0.72
1.5 mW cm ⁻²	Day 7	-31.41 ± 3.08	0.54 ± 0.82

Table 17 – Charge injection capacity of azo-tz-PEDOT:PSS films in different illumination conditions

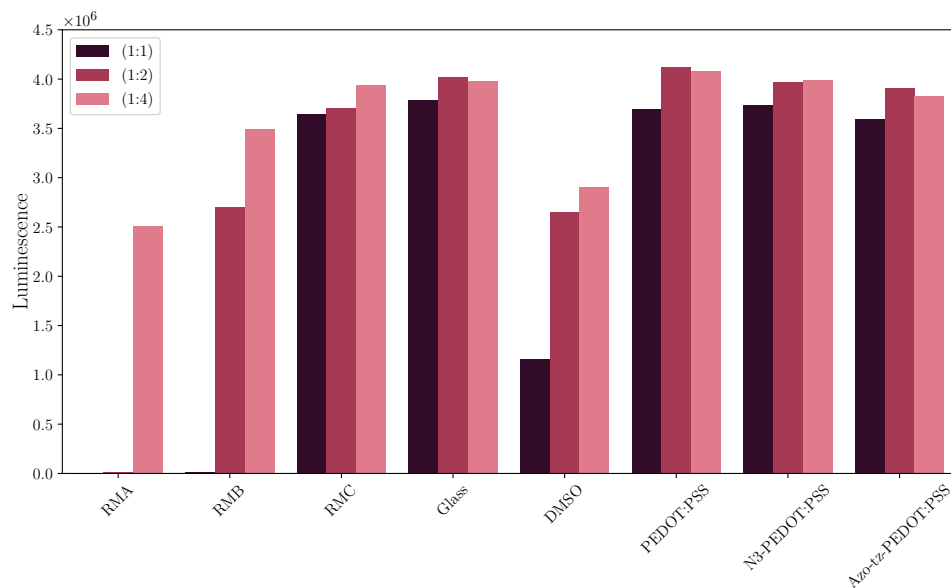
Start of illumination protocol [†]	Day of measurement	DPBS - Mean ± std [μC cm ⁻²]	Cell medium - Mean ± std [μC cm ⁻²]
Day 0	Day 0	-21.00 ± 1.20	-9.33 ± 0.34
Day 0	Day 1	-19.27 ± 1.74	-3.50 ± 0.94
Day 0	Day 2	-21.91 ± 0.71	-2.31 ± 0.68
Day 1	Day 0	0.00 ± 0.00	0.00 ± 0.00
Day 1	Day 1	-35.39 ± 0.54	-3.75 ± 0.06
Day 1	Day 2	-22.81 ± 2.54	0.18 ± 0.14

[†]With respect to the addition of electrolyte in day 0.

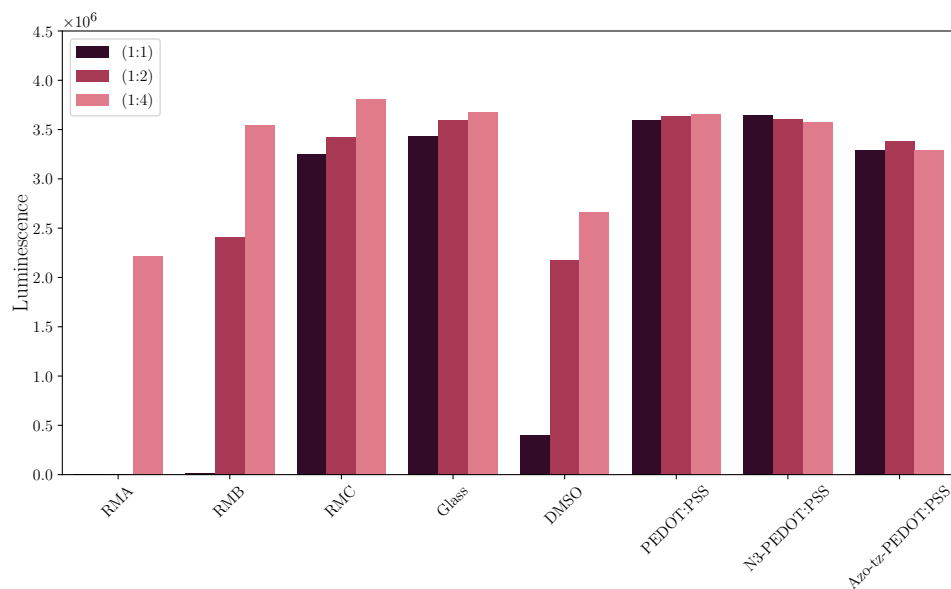
3.5 BIOCOMPATIBILITY ASSAYS

Indirect biocompatibility tests assess the potential release of toxic substances from substrates into cell medium during incubation. In this study, cells were cultured in media pre-incubated with the substrates under test, and then diluted to three different concentrations. The cell viability was evaluated after 24 hours of incubation by the luminescence generate by active cells. These results were compared to those obtained with materials of known cytotoxicity to establish a reference for toxicity levels.

As previously discussed in Section 2.5, the data presented in Figure 50 show that RM-A exhibits the highest level of toxicity among the tested controls. In the case of 1:1 dilutions, both RM-B and DMSO demonstrate substantial toxicity for the cell lines tested. Notably, both cell lines responded similarly to the various dilutions, with the exception of L-929 cells exhibiting reduced sensitivity to the higher concentration of DMSO compared to R28 cells.



(a)



(b)

Figure 50 – Relative luminescence measured of (a) L-929 and (b) R28 cells, cultured with cell medium incubated in each substrate in different dilutions, 1:1 (purple), 1:2 (dark pink) and 1:4 (bright pink).

The luminescence measurements reveal that the number of viable cells in PE-

DOT:PSS, N₃-PEDOT:PSS and azo-tz-PEDOT:PSS films is comparable to that on RM-C and glass substrates, which were used as negative controls. This consistent behavior across both L-929 and R28 cell lines suggests that the polymer films possess minimal cytotoxicity. These findings indicate that azo-tz-PEDOT:PSS exhibits biocompatible behavior, making it a promising candidate for cell interfacing applications. However, further studies are necessary to confirm its long-term biocompatibility and to thoroughly assess the cellular response to its stimulation.

Table 19 – Indirect biocompatibility tests - L-929 cell line

Substrate	Dilution	Luminescence [10 ⁶]
RMA	1:1	0.00
RMA	1:2	0.01
RMA	1:4	2.51
RMB	1:1	0.01
RMB	1:2	2.70
RMB	1:4	3.50
RMC	1:1	3.65
RMC	1:2	3.71
RMC	1:4	3.94
Glass	1:1	3.79
Glass	1:2	4.02
Glass	1:4	3.98
DMSO	1:1	1.16
DMSO	1:2	2.66
DMSO	1:4	2.90
PEDOT:PSS	1:1	3.70
PEDOT:PSS	1:2	4.13
PEDOT:PSS	1:4	4.08
N ₃ -PEDOT:PSS	1:1	3.74
N ₃ -PEDOT:PSS	1:2	3.97
N ₃ -PEDOT:PSS	1:4	3.99
Azo-tz-PEDOT:PSS	1:1	3.59
Azo-tz-PEDOT:PSS	1:2	3.91
Azo-tz-PEDOT:PSS	1:4	3.83

Table 21 – Indirect biocompatibility tests - R28 cell line

Substrate	Dilution	Luminescence [10^6]
RMA	1:1	0.01
RMA	1:2	0.01
RMA	1:4	2.22
RMB	1:1	0.01
RMB	1:2	2.41
RMB	1:4	3.55
RMC	1:1	3.25
RMC	1:2	3.43
RMC	1:4	3.81
Glass	1:1	3.43
Glass	1:2	3.60
Glass	1:4	3.67
DMSO	1:1	0.40
DMSO	1:2	2.17
DMSO	1:4	2.66
PEDOT:PSS	1:1	3.59
PEDOT:PSS	1:2	3.64
PEDOT:PSS	1:4	3.66
N ₃ -PEDOT:PSS	1:1	3.65
N ₃ -PEDOT:PSS	1:2	3.61
N ₃ -PEDOT:PSS	1:4	3.57
Azo-tz-PEDOT:PSS	1:1	3.29
Azo-tz-PEDOT:PSS	1:2	3.38
Azo-tz-PEDOT:PSS	1:4	3.29

4 CONCLUSION

This thesis aimed to explore the potential of polymer films for optoelectronic cell interfacing by evaluating the morphology, electrochemical properties, stability, light stimulation capabilities, and biocompatibility of the novel organic semiconductive material azo-tz-PEDOT:PSS. The revised fabrication protocol successfully optimized the material's electrochemical performance and stability, and increased the surface roughness from 7.86 nm to 24.50 nm. The final material thickness was approximately 175.10 nm.

In DPBS, after 30 minutes, a CSC_c of 1.10 mC/cm^2 was obtained, which is 16.03% lower than that of PEDOT:PSS. In cell medium, the CSC_c was 1.36 mC/cm^2 , slightly higher than PEDOT:PSS by 0.05% under the same conditions. These results suggest that initially, more charge is available in cell medium than in DPBS. Over the course of 7 days, the CSC_c in DPBS decreased by 67.27%, whereas in cell medium it decayed by 36.76%, indicating greater stability.

In both electrolytes, impedance measurements at 1 kHz showed higher values on the first day, likely due to the hydrophobicity of the azo-tz-PEDOT:PSS films, as indicated by a contact angle of 103.25° . After 24 hours, impedance values approached those of PEDOT:PSS and remained stable in subsequent days. The cut-off frequency decays considerably after 24 hours, allowing signals with frequency components higher than 50.12 Hz in DPBS and 73.99 Hz in cell medium to flow with minimal distortion.

Azo-tz-PEDOT:PSS films generated a current flow upon light exposure to 365 nm light, with the response dependent on light intensity. In DPBS, charge injection remained stable at 0.3 and 0.9 mW/cm^2 , but decreased by 26.51% on the last day when the power of the lamp was increased to 1.5 mW/cm^2 . In cell medium, the CIC is greatly hindered on the second day of illumination across all light intensities, highlighting a critical area for future research in optoelectronic cell stimulation with azo-tz-PEDOT:PSS.

Finally, indirect cell viability tests suggest that azo-tz-PEDOT:PSS films have potential for interfacing with biological systems, pointing towards their promise in future biomedical applications. Nonetheless, additional studies are needed to further investigate and confirm their long-term biocompatibility and effectiveness in biological environments.

4.1 PERSPECTIVES AND OUTLOOK

The versatility of click chemistry offers an exciting path for further exploration, particularly in the incorporation of different photoactive molecules into N_3 -PEDOT:PSS films. This strategy aims to shift the material's absorption band, and by that extending

its optical properties across a range of wavelengths. Such an approach enables the creation of custom-tailored responses that can be fine-tuned for specific bioelectronic applications. The potential to modify and control the optical characteristics of N₃-PEDOT:PSS opens up new possibilities for its use in applications requiring precise light manipulation.

Future research will also focus on optimizing the deposition of N₃-PEDOT:PSS on MEAs with micropillar structures to enhance cell adhesion and interface performance (RUGGIERO et al., 2022). The incorporation of photoactive molecules into these structures will pave the way for the development of 2.5D photoresponsive electrodes. We envision the development of advanced organic optoelectronic devices with superior cell-electrode integration, which could play a crucial role in the evolution of neural implants and bioelectronics, providing enhanced functionality and stability for both research and clinical applications.

Upcoming experiments will be dedicated to investigate the effects of photostimulation on azo-tz-PEDOT:PSS films and their impact on cell properties. By employing advanced techniques such as calcium imaging and patch-clamp recordings, we plan to explore how refined light stimulation protocols influence cellular behavior and action potential generation. Our ultimate goal is to design optimal light-based stimulation strategies that can precisely control cellular activities, opening new ways for non-invasive treatments and innovative bioengineering applications.

REFERENCES

ALBERTS, BRUCE; HOPKIN, KAREN; JOHNSON, ALEXANDER; MORGAN, DAVID; ROBERTS, KEITH. Cells: The Fundamental Units of Life. In: **ESSENTIAL Cell Biology**. 6th edition. [S.l.]: W W Norton & Co Inc, 2023.

<https://nerd.wwnorton.com/nerd/231302/r/goto/cfi/22!/4?control=control-assignment&demo=true>. ISBN 1-324-03335-5.

BENDIKOV, Tatyana A.; HARMON, Thomas C. Long-lived solid state perchlorate ion selective sensor based on doped poly(3,4-ethylenedioxythiophene) (PEDOT) films. **Analytica Chimica Acta**, v. 1-2, n. 551, p. 30–36, 2005. ISSN 0003-2670.

BERGGREN, Magnus; MALLIARAS, George G. How conducting polymer electrodes operate. **Science**, v. 364, n. 6437, p. 233–234, 19 Apr. 2019. Publisher: American Association for the Advancement of Science.

BETTUCCI, Ottavia; MATRONE, Giovanni Maria; SANTORO, Francesca. Conductive Polymer-Based Bioelectronic Platforms toward Sustainable and Biointegrated Devices: A Journey from Skin to Brain across Human Body Interfaces. **Advanced Materials Technologies**, v. 7, n. 2, p. 2100293, 2022. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/admt.202100293>. ISSN 2365-709X.

BOEHLER, Christian; AQRAWA, Zaid; ASPLUND, Maria. Applications of PEDOT in Bioelectronic Medicine. **Bioelectronics in Medicine**, v. 2, n. 2, p. 89–99, 1 June 2019. Publisher: Taylor & Francis __eprint: <https://doi.org/10.2217/bem-2019-0014>. ISSN 2059-1500.

BOEHLER, Christian; CARLI, Stefano; FADIGA, Luciano; STIEGLITZ, Thomas; ASPLUND, Maria. Tutorial: guidelines for standardized performance tests for electrodes intended for neural interfaces and bioelectronics. **Nature Protocols**, v. 15, n. 11, p. 3557–3578, Nov. 2020. Number: 11 Publisher: Nature Publishing Group. ISSN 1750-2799.

BRADBURY, SAVILE; JOY, DAVID C.; FORD, BRIAN J. **Scanning electron microscope**. Britannica. 2 May 2024. Available from: <https://www.britannica.com/technology/scanning-electron-microscope>.

BRUNO, Ugo; MARIANO, Anna; SANTORO, Francesca. A systems theory approach to describe dynamic coupling at the cell–electrode interface. **APL Materials**, v. 9, n. 1, p. 011103, 1 Jan. 2021. ISSN 2166-532X.

BU, Hang-Beom; GÖTZ, Günther; REINOLD, Egon; VOGT, Astrid; SCHMID, Sylvia; BLANCO, Raúl; SEGURA, Jose L.; BÄUERLE, Peter. “Click”-functionalization of conducting poly(3,4-ethylenedioxythiophene) (PEDOT). **Chemical Communications**, n. 11, p. 1320, 2008. ISSN 1359-7345, 1364-548X.

BU, Hang-Beom; GÖTZ, Günther; REINOLD, Egon; VOGT, Astrid; SCHMID, Sylvia; SEGURA, José L.; BLANCO, Raúl; GÓMEZ, Rafael; BÄUERLE, Peter. Efficient post-polymerization functionalization of conducting poly(3,4-ethylenedioxythiophene) (PEDOT) via ‘click’-reaction. **Tetrahedron**, v. 67, n. 6, p. 1114–1125, 11 Feb. 2011. ISSN 0040-4020.

CHEN, Ritchie; GORE, Felicity; NGUYEN, Quynh-Anh; RAMAKRISHNAN, Charu; PATEL, Sneha; KIM, Soo Hyun; RAFFIEE, Misha; KIM, Yoon Seok; HSUEH, Brian; KROOK-MAGNUSSON, Esther; SOLTESZ, Ivan; DEISSEROTH, Karl. Deep brain optogenetics without intracranial surgery. **Nature Biotechnology**, v. 39, n. 2, p. 161–164, Feb. 2021. Publisher: Nature Publishing Group. ISSN 1546-1696.

COGAN, Stuart F. Neural Stimulation and Recording Electrodes. **Annual Review of Biomedical Engineering**, v. 10, n. 1, p. 275–309, 2008. _eprint: <https://doi.org/10.1146/annurev.bioeng.10.061807.160518>.

CORRADO, Federica; BRUNO, Ugo; PRATO, Mirko; CARELLA, Antonio; CRISCUOLO, Valeria; MASSARO, Arianna; PAVONE, Michele; MUÑOZ-GARCÍA, Ana B.; FORTI, Stiven; COLETTI, Camilla; BETTUCCI, Ottavia; SANTORO, Francesca. Azobenzene-based optoelectronic transistors for neurohybrid building blocks. **Nature Communications**, v. 14, n. 1, p. 6760, 2 Nov. 2023. ISSN 2041-1723.

DE OLIVEIRA, R. R. L.; ALBUQUERQUE, D. A. C.; CRUZ, T. G. S.; YAMAJI, F. M.; LEITE, F. L. Chapter 7 - Measurement of the Nanoscale Roughness by Atomic Force Microscopy: Basic Principles and Applications. In: **ATOMIC Force Microscopy - Imaging**,

Measuring and Manipulating Surfaces at the Atomic Scale. [S.l.]: IntechOpen, 2012. P. 270. ISBN 978-953-51-0414-8.

DIJK, Gerwin; RUTZ, Alexandra L.; MALLIARAS, George G. Stability of PEDOT:PSS-Coated Gold Electrodes in Cell Culture Conditions. **Advanced Materials Technologies**, v. 5, n. 3, p. 1900662, 2020. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/admt.201900662>. ISSN 2365-709X.

ELGRISHI, Noémie; ROUNTREE, Kelley J.; MCCARTHY, Brian D.; ROUNTREE, Eric S.; EISENHART, Thomas T.; DEMPSEY, Jillian L. A Practical Beginner's Guide to Cyclic Voltammetry. **Journal of Chemical Education**, v. 95, n. 2, p. 197–206, 13 Feb. 2018. ISSN 0021-9584, 1938-1328.

FENOY, Gonzalo E.; HASLER, Roger; QUARTINELLO, Felice; MARMISOLLÉ, Waldemar A.; LORENZ, Christoph; AZZARONI, Omar; BÄUERLE, Peter; KNOLL, Wolfgang. “Clickable” Organic Electrochemical Transistors. **JACS Au**, v. 2, n. 12, p. 2778–2790, 26 Dec. 2022. Publisher: American Chemical Society.

FEYEN, Paul; COLOMBO, Elisabetta; ENDEMAN, Duco; NOVA, Mattia; LAUDATO, Lucia; MARTINO, Nicola; ANTOGNAZZA, Maria Rosa; LANZANI, Guglielmo; BENFENATI, Fabio; GHEZZI, Diego. Light-evoked hyperpolarization and silencing of neurons by conjugated polymers. **Scientific Reports**, v. 6, n. 1, p. 22718, 4 Mar. 2016. Publisher: Nature Publishing Group. ISSN 2045-2322.

GANJI, Mehran; TANAKA, Atsunori; GILJA, Vikash; HALGREN, Eric; DAYEH, Shadi A. Scaling Effects on the Electrochemical Stimulation Performance of Au, Pt, and PEDOT:PSS Electrocardiography Arrays. **Advanced Functional Materials**, v. 27, n. 42, p. 1703019, 2017. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adfm.201703019>. ISSN 1616-3028.

GHEZZI, Diego; ANTOGNAZZA, Maria Rosa; DAL MASCHIO, Marco; LANZARINI, Erica; BENFENATI, Fabio; LANZANI, Guglielmo. A hybrid bioorganic interface for neuronal photoactivation. **Nature Communications**, v. 2, n. 1, p. 166, 25 Jan. 2011. Publisher: Nature Publishing Group. ISSN 2041-1723.

GHEZZI, Diego; ANTOGNAZZA, Maria Rosa; MACCARONE, Rita; BELLANI, Sebastiano; LANZARINI, Erica; MARTINO, Nicola; METE, Maurizio; PERTILE, Grazia; BISTI, Silvia; LANZANI, Guglielmo; BENFENATI, Fabio. A polymer optoelectronic interface restores light sensitivity in blind rat retinas. **Nature Photonics**, v. 7, n. 5, p. 400–406, May 2013. Publisher: Nature Publishing Group. ISSN 1749-4893.

GIESSIBL, Franz J. Advances in atomic force microscopy. **Reviews of Modern Physics**, v. 75, n. 3, p. 949–983, 29 July 2003. ISSN 0034-6861, 1539-0756.

GOODWIN, Paul C. Chapter 10 - Quantitative deconvolution microscopy. In: WATERS, Jennifer C.; WITTMAN, Torsten (Eds.). **Methods in Cell Biology**. [S.l.]: Academic Press, 1 Jan. 2014. v. 123. (Quantitative Imaging in Cell Biology). P. 177–192.

HAN, Mertcan; KARATUM, Onuralp; NIZAMOGLU, Sedat. Optoelectronic Neural Interfaces Based on Quantum Dots. **ACS Applied Materials & Interfaces**, v. 14, n. 18, p. 20468–20490, 11 May 2022. Publisher: American Chemical Society. ISSN 1944-8244.

HOPKINS, Jonathan; TRAVAGLINI, Lorenzo; LAUTO, Antonio; CRAMER, Tobias; FRABONI, Beatrice; SEIDEL, Jan; MAWAD, Damia. Photoactive Organic Substrates for Cell Stimulation: Progress and Perspectives. **Advanced Materials Technologies**, v. 4, n. 5, p. 1800744, 2019. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/admt.201800744>. ISSN 2365-709X.

INAL, Sahika; RIVNAY, Jonathan; SUIU, Andreea-Otilia; MALLIARAS, George G.; MCCULLOCH, Iain. Conjugated Polymers in Bioelectronics. **Accounts of Chemical Research**, v. 51, n. 6, p. 1368–1376, 19 June 2018. Publisher: American Chemical Society. ISSN 0001-4842.

INUKAI, Shino; KATAYAMA, Kota; KOYANAGI, Mitsumasa; TERAOKITA, Akihisa; KANDORI, Hideki. Investigating the mechanism of photoisomerization in jellyfish rhodopsin with the counterion at an atypical position. **Journal of Biological Chemistry**, v. 299, n. 6, p. 104726, 1 June 2023. ISSN 0021-9258.

JERCA, Florica Adriana; JERCA, Valentin Victor; HOOGENBOOM, Richard. Advances and opportunities in the exciting world of azobenzenes. **Nature Reviews Chemistry**, v. 6, n. 1, p. 51–69, 18 Nov. 2021. ISSN 2397-3358.

JERKIEWICZ, Gregory. Applicability of Platinum as a Counter-Electrode Material in Electrocatalysis Research. **ACS Catalysis**, v. 12, n. 4, p. 2661–2670, 18 Feb. 2022. Publisher: American Chemical Society.

KANDEL, ERIC R.; SCHWARTZ, JAMES H.; JESSELL, THOMAS M.; SIEGELBAUM, STEVEN A.; HUDSPETH, A. J. **Principles of Neural Science**. Fifth Edition. New York, NY: McGraw-Hill Education, 2014.

KANG, Hong Suk; YANG, Shu. Photopatterning via photofluidization of azobenzene polymers. **Light: Advanced Manufacturing**, v. 3, n. 1, p. 1, 2022. ISSN 2831-4093.

KANG, Young Hun; KO, Seo-Jin; LEE, Min-Hye; LEE, Young Kuk; KIM, Bumjoon J.; CHO, Song Yun. Highly efficient and air stable thermoelectric devices of poly(3-hexylthiophene) by dual doping of Au metal precursors. **Nano Energy**, v. 82, p. 105681, 1 Apr. 2021. ISSN 2211-2855.

KEYVAN RAD, Jaber; BALZADE, Zahra; MAHDAVIAN, Ali Reza. Spiropyran-based advanced photoswitchable materials: A fascinating pathway to the future stimuli-responsive devices. **Journal of Photochemistry and Photobiology C: Photochemistry Reviews**, v. 51, p. 100487, 1 June 2022. ISSN 1389-5567.

KLEIN, Martin; WALDVOGEL, Siegfried R. Counter Electrode Reactions—Important Stumbling Blocks on the Way to a Working Electro-organic Synthesis. **Angewandte Chemie International Edition**, v. 61, n. 47, e202204140, 2022. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/anie.202204140>. ISSN 1521-3773.

KOLB, Hartmuth C.; FINN, M. G.; SHARPLESS, K. Barry. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. **Angewandte Chemie International Edition**, v. 40, n. 11, p. 2004–2021, 2001. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/1521-3773%2820010601%2940%3A11%3C2004%3A%3AAID-ANIE2004%3E3.0.CO%3B2-5>. ISSN 1521-3773.

LÁNG, G. G.; UJVÁRI, M.; BAZSÓ, F.; VESZTERGOM, S.; UJHELYI, F. In situ monitoring of the electrochemical degradation of polymer films on metals using the bending beam method and impedance spectroscopy. **Electrochimica Acta**, v. 73, p. 59–69, 1 July 2012. ISSN 0013-4686.

LASSOUED, Ayoub; ZHANG, Furu; KUROKAWA, Kazuhiro; LIU, Yan; BERNUCCI, Marcel T.; CROWELL, James A.; MILLER, Donald T. Cone photoreceptor dysfunction in retinitis pigmentosa revealed by optoretinography. **Proceedings of the National Academy of Sciences**, v. 118, n. 47, e2107444118, 23 Nov. 2021. Publisher: Proceedings of the National Academy of Sciences.

LAZANAS, ALEXANDROS CH.; PRODROMIDIS, MAMAS I. Electrochemical Impedance SpectroscopyA Tutorial. v. 3, n. 3, p. 162–193, 2023.

LEE, Kang Eun; LEE, Jea Uk; SEONG, Dong Gi; UM, Moon-Kwang; LEE, Wonoh. Highly Sensitive Ultraviolet Light Sensor Based on Photoactive Organic Gate Dielectrics with an Azobenzene Derivative. **The Journal of Physical Chemistry C**, v. 120, n. 40, p. 23172–23179, 13 Oct. 2016. Publisher: American Chemical Society. ISSN 1932-7447.

LEHMANN-HORN, Frank; FAULER, Michael; HOLZHERR, Boris; JURKAT-ROTT, Karin. Principles of Electrophysiological In Vitro Measurements. In: SIGG, Daniel C.; IAIZZO, Paul A.; XIAO, Yong-Fu; HE, Bin (Eds.). **Cardiac Electrophysiology Methods and Models**. Boston, MA: Springer US, 2010. P. 119–134. ISBN 978-1-4419-6658-2.

LIANG, Liyuan; ASTRUC, Didier. The copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) “click” reaction and its applications. An overview. **Coordination Chemistry Reviews**, v. 255, n. 23, p. 2933–2945, 1 Dec. 2011. ISSN 0010-8545.

LIANG, Yuanying; OFFENHÄUSSER, Andreas; INGEBRANDT, Sven; MAYER, Dirk. PEDOT:PSS-Based Bioelectronic Devices for Recording and Modulation of Electrophysiological and Biochemical Cell Signals. **Advanced Healthcare Materials**, v. 10, n. 11, p. 2100061, 2021. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adhm.202100061>. ISSN 2192-2659.

LIN, Jia-Min; SU, Ya-Ling; CHANG, Wei-Ting; SU, Wan-Yu; CHENG, Shu-Hua. Strong adsorption characteristics of a novel overoxidized poly(3,4-ethylenedioxythiophene) film and application for dopamine sensing. **Electrochimica Acta**, v. 149, p. 65–75, Dec. 2014. ISSN 00134686.

MARIANO, Anna; LUBRANO, Claudia; BRUNO, Ugo; AUSILIO, Chiara; DINGER, Nikita Bhupesh; SANTORO, Francesca. Advances in Cell-Conductive Polymer Biointerfaces and Role of the Plasma Membrane. **Chemical Reviews**, v. 122, n. 4, p. 4552–4580, 23 Feb. 2022. Publisher: American Chemical Society. ISSN 0009-2665.

MARQUEZ, Ariana Villarroel; MCEVOY, Niall; PAKDEL, Amir. Organic Electrochemical Transistors (OECTs) Toward Flexible and Wearable Bioelectronics. **Molecules**, v. 25, n. 22, p. 5288, Jan. 2020. Number: 22 Publisher: Multidisciplinary Digital Publishing Institute. ISSN 1420-3049.

MAYA-VETENCOURT, José Fernando; GHEZZI, Diego; ANTOGNAZZA, Maria Rosa; COLOMBO, Elisabetta; METE, Maurizio; FEYEN, Paul; DESII, Andrea; BUSCHIAZZO, Ambra; DI PAOLO, Mattia; DI MARCO, Stefano; TICCONI, Flavia; EMIONITE, Laura; SHMAL, Dmytro; MARINI, Cecilia; DONELLI, Ilaria; FREDDI, Giuliano; MACCARONE, Rita; BISTI, Silvia; SAMBUCETI, Gianmario; PERTILE, Grazia; LANZANI, Guglielmo; BENFENATI, Fabio. A fully organic retinal prosthesis restores vision in a rat model of degenerative blindness. **Nature Materials**, v. 16, n. 6, p. 681–689, June 2017. Publisher: Nature Publishing Group. ISSN 1476-4660.

MDLULI, Siyabonga B.; RAMOROKA, Morongwa E.; YUSSUF, Sodiq T.; MODIBANE, Kwen D.; JOHN-DENK, Vivian S.; IWUOHA, Emmanuel I. -Conjugated Polymers and Their Application in Organic and Hybrid Organic-Silicon Solar Cells. **Polymers**, v. 14, n. 4, p. 716, Jan. 2022. Number: 4 Publisher: Multidisciplinary Digital Publishing Institute. ISSN 2073-4360.

MERRILL, Daniel R. Chapter 3 - Electrodes and instrumentation for neurostimulation. In: GÜÇLÜ, Burak (Ed.). **Somatosensory Feedback for Neuroprosthetics**. [S.l.]: Academic Press, 1 Jan. 2021. P. 77–150. ISBN 978-0-12-822828-9.

MILOS, Frano; TULLII, Gabriele; GOBBO, Federico; LODOLA, Francesco; GALEOTTI, Francesco; VERPELLI, Chiara; MAYER, Dirk; MAYBECK, Vanessa;

OFFENHÄUSSER, Andreas; ANTOGNAZZA, Maria Rosa. High Aspect Ratio and Light-Sensitive Micropillars Based on a Semiconducting Polymer Optically Regulate Neuronal Growth. **ACS Applied Materials & Interfaces**, v. 13, n. 20, p. 23438–23451, 26 May 2021. ISSN 1944-8244, 1944-8252.

MOLECULAR DEVICES. **What is an action potential?** 2024. Available from: <https://www.moleculardevices.com/applications/patch-clamp-electrophysiology/what-action-potential>.

MUELLER, Michael; FABRETTO, Manrico; EVANS, Drew; HOJATI-TALEMI, Pejman; GRUBER, Cynthia; MURPHY, Peter. Vacuum vapour phase polymerization of high conductivity PEDOT: Role of PEG-PPG-PEG, the origin of water, and choice of oxidant. **Polymer**, v. 53, n. 11, p. 2146–2151, 9 May 2012. ISSN 0032-3861.

PARAMESWARAN, Ramya; CARVALHO-DE-SOUZA, João L.; JIANG, Yuanwen; BURKE, Michael J.; ZIMMERMAN, John F.; KOEHLER, Kelliann; PHILLIPS, Andrew W.; YI, Jaeseok; ADAMS, Erin J.; BEZANILLA, Francisco; TIAN, Bozhi. Photoelectrochemical modulation of neuronal activity with free-standing coaxial silicon nanowires. **Nature Nanotechnology**, v. 13, n. 3, p. 260–266, Mar. 2018. Publisher: Nature Publishing Group. ISSN 1748-3395.

PARK SYSTEMS. **Tapping mode**. Park Systems. Available from: <https://www.parksystems.com/park-spm-modes/91-standard-imaging-mode/2204-tapping-mode>.

PITSALIDIS, Charalampos; PAPPA, Anna-Maria; BOYS, Alexander J.; FU, Ying; MOYSIDOU, Chrysanthi-Maria; NIEKERK, Douglas van; SAEZ, Janire; SAVVA, Achilleas; IANDOLO, Donata; OWENS, Róisín M. Organic Bioelectronics for In Vitro Systems. **Chemical Reviews**, v. 122, n. 4, p. 4700–4790, 23 Feb. 2022. Publisher: American Chemical Society. ISSN 0009-2665.

PROCTOR, Christopher M.; RIVNAY, Jonathan; MALLIARAS, George G. Understanding volumetric capacitance in conducting polymers. **Journal of Polymer Science Part B: Polymer Physics**, v. 54, n. 15, p. 1433–1436, 2016. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/polb.24038>. ISSN 1099-0488.

PURKAIT, Mihir Kumar; SINHA, Manish Kumar; MONDAL, Piyal; SINGH, Randeep. Photoresponsive Membranes. In: *INTERFACE Science and Technology*. [S.l.]: Elsevier, 2018. v. 25. P. 115–144. ISBN 978-0-12-813961-5.

REN, Mengyao; ZHOU, Haihan; ZHAI, Hua-Jin. Obvious enhancement in electrochemical capacitive properties for poly(3,4-ethylenedioxythiophene) electrodes prepared under optimized conditions. **Journal of Materials Science: Materials in Electronics**, v. 32, n. 8, p. 10078–10088, 1 Apr. 2021. ISSN 1573-482X.

RIVNAY, Jonathan; OWENS, Róisín M.; MALLIARAS, George G. The Rise of Organic Bioelectronics. **Chemistry of Materials**, v. 26, n. 1, p. 679–685, 14 Jan. 2014. ISSN 0897-4756, 1520-5002.

RUGGIERO, Amedeo; CRISCUOLO, Valeria; GRASSELLI, Sara; BRUNO, Ugo; AUSILIO, Chiara; BOVIO, Claudia Latte; BETTUCCI, Ottavia; SANTORO, Francesca. Two-photon polymerization lithography enabling the fabrication of PEDOT:PSS 3D structures for bioelectronic applications. **Chemical Communications**, v. 58, n. 70, p. 9790–9793, 2022. ISSN 1359-7345, 1364-548X.

SCAVETTA, E.; MAZZONI, R.; MARIANI, F.; MARGUTTA, R. G.; BONFIGLIO, A.; DEMELAS, M.; FIORILLI, S.; MARZOCCHI, M.; FRABONI, B. Dopamine amperometric detection at a ferrocene clicked PEDOT:PSS coated electrode. **Journal of Materials Chemistry B**, v. 2, n. 19, p. 2861–2867, 16 Apr. 2014. Publisher: The Royal Society of Chemistry. ISSN 2050-7518.

SCHAFFRATH, Kim; LOHMANN, Tibor; SEIFERT, Jan; INGENSEP, Claudia; RAFFELBERG, Pascal; WASCHKOWSKI, Florian; VIGA, Reinhard; KOKOZINSKI, Rainer; MOKWA, Wilfried; JOHNEN, Sandra; WALTER, Peter. New epiretinal implant with integrated sensor chips for optical capturing shows a good biocompatibility profile in vitro and in vivo. **BioMedical Engineering OnLine**, v. 20, n. 1, p. 102, 12 Oct. 2021. ISSN 1475-925X.

SHAVOKSHINA, Vera A.; KOMKOVA, Maria A.; APARIN, Ilya O.; ZATSEPIN, Timofei S.; KARYAKIN, Arkady A.; ANDREEV, Egor A. Improved Electroactivity of Redox Probes onto Electropolymerized Azidomethyl-PEDOT: Enabling

Click Chemistry for Advanced (Bio)Sensors. **ACS Applied Polymer Materials**, v. 3, n. 3, p. 1518–1524, 12 Mar. 2021. Publisher: American Chemical Society.

SIMON, Daniel T.; GABRIELSSON, Erik O.; TYBRANDT, Klas;
BERGGREN, Magnus. Organic Bioelectronics: Bridging the Signaling Gap between Biology and Technology. **Chemical Reviews**, v. 116, n. 21, p. 13009–13041, 9 Nov. 2016. Publisher: American Chemical Society. ISSN 0009-2665.

SINGH, Vinamrita; KUMAR, Tanuj. Study of modified PEDOT:PSS for tuning the optical properties of its conductive thin films. **Journal of Science: Advanced Materials and Devices**, v. 4, n. 4, p. 538–543, 1 Dec. 2019. ISSN 2468-2179.

SPIRA, Micha E.; HAI, Aviad. Multi-electrode array technologies for neuroscience and cardiology. **Nature Nanotechnology**, v. 8, n. 2, p. 83–94, Feb. 2013. Publisher: Nature Publishing Group. ISSN 1748-3395.

SUNDARAKRISHNAN, Aswin; HERRERO ACERO, Enrique; COBURN, Jeannine;
CHWALEK, Karolina; PARTLOW, Benjamin; KAPLAN, David L. Phenol red-silk tyrosine cross-linked hydrogels. **Acta Biomaterialia**, v. 42, p. 102–113, 15 Sept. 2016. ISSN 1742-7061.

SUNG, Juho; SHIN, Changhwan. Recent Studies on Supercapacitors with Next-Generation Structures. **Micromachines**, v. 11, n. 12, p. 1125, Dec. 2020. Number: 12 Publisher: Multidisciplinary Digital Publishing Institute. ISSN 2072-666X.

TANG, Jiajia; WU, Chengheng; QIAO, Zi; PI, Jinkui; ZHANG, Yusheng; LUO, Fang;
SUN, Jing; DAN WEI; FAN, Hongsong. A photoelectric effect integrated scaffold for the wireless regulation of nerve cellular behavior. **Journal of Materials Chemistry B**, v. 10, n. 10, p. 1601–1611, 2022. ISSN 2050-750X, 2050-7518.

TIAN, Jianwu; FU, Lulu; LIU, Zitong; GENG, Hua; SUN, Yanan; LIN, Gaobo;
ZHANG, Xisha; ZHANG, Guanxin; ZHANG, Deqing. Optically Tunable Field Effect Transistors with Conjugated Polymer Entailing Azobenzene Groups in the Side Chains. **Advanced Functional Materials**, v. 29, n. 12, p. 1807176, 2019. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adfm.201807176>. ISSN 1616-3028.

TIAN, Jianwu; LIU, Zitong; WU, Changchun; JIANG, Wenlin; CHEN, Liangliang; SHI, Dandan; ZHANG, Xisha; ZHANG, Guanxin; ZHANG, Deqing. Simultaneous Incorporation of Two Types of Azo-Groups in the Side Chains of a Conjugated D–A Polymer for Logic Control of the Semiconducting Performance by Light Irradiation. **Advanced Materials**, v. 33, n. 8, p. 2005613, 2021. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adma.202005613>. ISSN 1521-4095.

TIAN, Min; COUSINS, Christine; BEAUCHEMIN, Diane; FURUYA, Yoshihisa; OHMA, Atsushi; JERKIEWICZ, Gregory. Influence of the Working and Counter Electrode Surface Area Ratios on the Dissolution of Platinum under Electrochemical Conditions. **ACS Catalysis**, v. 6, n. 8, p. 5108–5116, 5 Aug. 2016. Publisher: American Chemical Society.

TOMSIK, E.; KONEFAŁ, M.; KOHUT, O.; IVANKO, I.; HROMÁDKOVÁ, J.; ZHIGUNOV, A.; STEINHART, M. Phase Transitions of Polyaniline Induced by Electrochemical Treatment. **Macromolecular Chemistry and Physics**, v. 219, n. 7, p. 1700627, 2018. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/macp.201700627>. ISSN 1521-3935.

TULLII, Gabriele; GIONA, Federica; LODOLA, Francesco; BONFADINI, Silvio; BOSSIO, Caterina; VARO, Simone; DESII, Andrea; CRIANTE, Luigino; SALA, Carlo; PASINI, Mariacecilia; VERPELLI, Chiara; GALEOTTI, Francesco; ANTOGNAZZA, Maria Rosa. High-Aspect-Ratio Semiconducting Polymer Pillars for 3D Cell Cultures. **ACS Applied Materials & Interfaces**, v. 11, n. 31, p. 28125–28137, 7 Aug. 2019. Publisher: American Chemical Society. ISSN 1944-8244.

VAQUERO, Susana; BOSSIO, Caterina; BELLANI, Sebastiano; MARTINO, Nicola; ZUCCHETTI, Elena; LANZANI, Guglielmo; ANTOGNAZZA, Maria Rosa. Conjugated polymers for the optical control of the electrical activity of living cells. **Journal of Materials Chemistry B**, v. 4, n. 31, p. 5272–5283, 3 Aug. 2016. Publisher: The Royal Society of Chemistry. ISSN 2050-7518.

VOLKOV, Anton V.; WIJERATNE, Kosala; MITRAKA, Evangelia; AIL, Ujwala; ZHAO, Dan; TYBRANDT, Klas; ANDREASEN, Jens Wenzel; BERGGREN, Magnus; CRISPIN, Xavier; ZOZOULENKO, Igor V. Understanding the Capacitance of

PEDOT:PSS. **Advanced Functional Materials**, v. 27, n. 28, p. 1700329, 2017. __eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adfm.201700329>. ISSN 1616-3028.

WELLEMAN, Ilse M.; HOORENS, Mark W. H.; FERINGA, Ben L.;
BOERSMA, Hendrikus H.; SZYMAŃSKI, Wiktor. Photoresponsive molecular tools for
emerging applications of light in medicine. **Chemical Science**, v. 11, n. 43,
p. 11672–11691, 2020. ISSN 2041-6520.

WU, Yingjie; PENG, Yanfen; BOHRA, Hassan; ZOU, Jianping;
RANJAN, Vivek Damodar; ZHANG, Yilei; ZHANG, Qing; WANG, Mingfeng.
Photoconductive Micro/Nanoscale Interfaces of a Semiconducting Polymer for Wireless
Stimulation of Neuron-Like Cells. **ACS Applied Materials & Interfaces**, v. 11, n. 5,
p. 4833–4841, 6 Feb. 2019. Publisher: American Chemical Society. ISSN 1944-8244.

XU, Shumao; MOMIN, Marzia; AHMED, Salahuddin; HOSSAIN, Arafat;
VEERAMUTHU, Loganathan; PANDIYAN, Archana; KUO, Chi-Ching; ZHOU, Tao.
Illuminating the Brain: Advances and Perspectives in Optoelectronics for Neural Activity
Monitoring and Modulation. **Advanced Materials**, v. 35, n. 42, p. 2303267, 2023.
__eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adma.202303267>. ISSN
1521-4095.

ZALKA, D.; KOVÁCS, N.; SZEKERES, K.; UJVÁRI, M.; VESZTERGOM, S.;
ELISEEVA, S.; KONDRATIEV, V.; LÁNG, G. G. Determination of the charge transfer
resistance of poly(3,4-ethylenedioxythiophene)-modified electrodes immediately after
overoxidation. **Electrochimica Acta**, v. 247, p. 321–332, 1 Sept. 2017. ISSN 0013-4686.

ZHANG, Hao; ZHAO, Hangbo; ZHAO, Xingyue; XU, Chenkai; FRANKLIN, Daniel;
VÁZQUEZ-GUARDADO, Abraham; BAI, Wubin; ZHAO, Jeffrey; LI, Kan;
MONTI, Giuditta; LU, Wei; KOBEISSI, Aya; TIAN, Limei; NING, Xin; YU, Xinge;
MEHTA, Sunita; CHANDA, Debashis; HUANG, Yonggang; XU, Shuai;
PEREZ WHITE, Bethany E.; ROGERS, John A. Biocompatible Light Guide-Assisted
Wearable Devices for Enhanced UV Light Delivery in Deep Skin. **Advanced
Functional Materials**, v. 31, n. 23, p. 2100576, 2021. __eprint:
<https://onlinelibrary.wiley.com/doi/pdf/10.1002/adfm.202100576>. ISSN 1616-3028.

ZHANG, Yuanmin; CHEN, Yuqi; CONTERA, Sonia; COMPTON, Richard G.
Electrochemical and Nanostructural Characterization of
Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) Films as Coatings for Neural
Electrodes. **ACS Applied Polymer Materials**, v. 5, n. 7, p. 5555–5566, 14 July 2023.
ISSN 2637-6105, 2637-6105.

APPENDIX A – CHEMICALS AND MATERIALS

Chemicals	Catalog Number	Specification	Vendor
ITO coated glass substrates (Unpatterned)	S2006B1-100	25 x 25 mm ² , 20 Ω/cm^2	Ossila B.V.
DPBS	14190144	500 ml, w/o Ca Mg (1X)	ThermoFischer
Biphasic glue (Pico-dent, eco-sil)	1300 6100	-	Dental-Produktions- und Vertriebs-GmbH
Parafilm	9170002	PARAFILM®M	TH.Geyer
EDOT	483028	10 g	Merck
EDOT-Cl	802247		Merck
DMF	83691.320		VWR Chemicals
Sodium azide (NaN ₃)	S0489		TCI Chemicals
Silica gel Al foils	60800-20EA	silica gel matrix, L x W 10 cm x 20 cm, with fluorescent indicator 254 nm	Sigma-Aldrich
Ethyl acetate	33211		Merck
Sodium sulfate (Na ₂ SO ₄)	238597		Merck
4-(Phenylazo)phenol	P0149		TCI chemicals
Acetone	179124		TCI chemicals
Potassium carbonate (K ₂ CO ₃)	P5833		Merck
Propargyl bromide	P0484	stabilized with MgO	TCI chemicals
Alconox	Z742914		Merck
Isopropanol	190764		Merck
PSSNa	434574	average Mw 1,000,000	Merck
THF	87368		Merck

Copper sulfate (CuSO ₄)	23172.293	≥ 98,5%	VWR Chemicals
Sodium ascorbate (C ₆ H ₇ NaO ₆)	A0539		TCI Chemicals
PEEK			
MEM Eagle	P04-09500	w: EBSS, stable Glutamine, 2.2 g/L NaHCO ₃	PAN-Biotech
DMEM/Nutrient Mix- ture F-12 Ham	D6421	w: 15 mM HEPES and sodium bicar- bonate, without L-glutamine, liquid, sterile- filtered, suitable for cell culture	Sigma-Aldrich
Fetal Bovine Serum	F7524		Sigma-Aldrich
L-glutamin	K 0282		Biochrom
Pen Strep (Penicillin Streptomycin)	15140-122	10,000 U/mL	ThermoFischer Scientific
DMEM	P04 04510	w: 4.5 g/L Glucose, sta- ble Glutamine, Sodium pyru- vate, 3.7 g/L NaHCO ₃	PAN-Biotech
Nunc™Lab- Tek™Chamber Slide System	177399		ThermoFischer Scientific

APPENDIX B – INSTRUMENTS

Item	Specification	Company
Ultrasonic bath	EMMI 40HC	EMAG
Ag/AgCl 3M KCl		Redox.me
Ag/AgCl pellet	-	-
Pt-mesh with holder (for organic electrolytes)	71151000	Redox.me
Pt wire	-	-
Potentiostat	EG&G Potentiostat/Galvanostat 283	Princeton Applied Research/Ametek
Stylus Profilometer	DektakXT	Bruker
Optical contact angle measuring system	OCA25	DataPhysics
Laser Scanning Microscope	VK-X3000	Keyence
Scanning electron microscope		
Atomic force microscope	Park NX20	Park Systems
Arkeo	-	Cicci Research

APPENDIX C – EXPERIMENTAL DATA AND ANALYSIS

C.1 CSC_C CALCULATION

```

1  # Function definitions
2
3  def process_txt_file(file_path, data_list, columns, cycles):
4      # Define your logic for processing the .txt file here
5      data=pd.read_csv(file_path,names=columns,sep="\t" or " ",
6      ↪ encoding_errors='ignore',
7      skiprows=6,decimal=".")
8      directory, file_name_with_extension = os.path.split(file_path)
9      file_name = os.path.splitext(file_name_with_extension)[0]
10     dataalc = data[int(len(data.voltage)/3*2) :]
11     datapd = pd.DataFrame(dataalc, columns = columns)
12     data = datapd
13     filtere_current = data.current.values * 1e6
14
15     # Define the number of values and the range for the filtered voltage
16     num_values = len(filtere_current)
17     min_voltage = -0.6
18     max_voltage = 0.6
19
20     # Generate the filtered voltage array with 2406 values ranging from -0.6 to 0.6
21     #and back down to -0.6
22     positive_voltage = np.linspace(min_voltage, max_voltage, num_values // 2 + 1)
23     negative_voltage = np.linspace(max_voltage, min_voltage, num_values // 2)
24     filtered_voltage = np.concatenate((positive_voltage[:-1], negative_voltage))
25
26     #CSC calculation
27     act_csc = []
28     curr = filtere_current/1e3
29     volt = filtered_voltage
30
31     zero_crossings = np.where(np.diff(np.signbit(curr)))[0]
32     volt_indices = np.where(np.diff(np.sign(volt + 0.6)))[0]
33
34     if(curr[0]<0):
35         curr =
36         ↪ np.concatenate((curr[zero_crossings[0]+1:],curr[:zero_crossings[0]+1]))
37         volt =
38         ↪ np.concatenate((volt[zero_crossings[0]+1:],volt[:zero_crossings[0]+1]))
39
40     zero_crossings = np.where(np.diff(np.signbit(curr)))[0]
41     volt_indices = np.where(np.diff(np.sign(volt + 0.6)))[0]
42
43     if(zero_crossings.size>1):

```

```

41     start=zero_crossings[0]
42     end=zero_crossings[len(zero_crossings)-1]
43     turn=volt_indices[0]
44 else:
45     start=zero_crossings[0]
46     end=len(curr)
47     turn=volt_indices[0]
48
49     area1 = np.trapz(np.array(curr[start:turn]), np.array(volt[start:turn]))
50     area2 = np.trapz(np.array(curr[turn:end]), np.array(volt[turn:end]))
51     area = abs(area1)-abs(area2)
52     act_area=np.pi*0.5**2
53     scan_rate=0.05
54     act_csc = abs(area)/(act_area * scan_rate)
55
56     parts = file_name.split('_')
57
58     # Extracting information\n",
59     date = parts[0]
60     material = parts[1]
61     potential = parts[2]
62     n = parts[3]
63     time = parts[4]
64
65     data.current = moving_average_filter(data.current,5)
66
67     # Constructing a dictionary for the file and appending to the data list
68
69     file_data = {
70         "File Name": f"{file_name}",
71         "Date": date,
72         "Material": material,
73         "ED potential": potential,
74         "N sample": n,
75         "Time of measurement": time,
76         "Current" : data.current*1e6,
77         "Voltage" : data.voltage,
78         "CSC": act_csc,
79     }
80     data_list.append(file_data)
81
82     return data_list
83
84 def moving_average_filter(data, window_size):
85     """
86     Smooths the input data using a simple moving average filter.
87
88     Parameters:

```

```

89     data (array-like): The input data to be smoothed.
90     window_size (int): The number of data points to include in the moving
    ↪     average window.
91
92     Returns:
93         numpy.ndarray: The smoothed data.
94     """
95     if window_size < 1:
96         raise ValueError("Window size must be at least 1.")
97
98     # Ensure window_size is odd to keep the window centered around each data point
99     if window_size % 2 == 0:
100         window_size += 1
101
102     # Pad the data at the beginning and the end to handle the edges
103     pad_width = window_size // 2
104     padded_data = np.pad(data, pad_width, mode='edge')
105
106     # Initialize an empty array to hold the smoothed data
107     smoothed_data = np.empty_like(data, dtype=float)
108
109     # Apply the moving average filter
110     for i in range(len(data)):
111         smoothed_data[i] = np.mean(padded_data[i:i+window_size])
112
113     return smoothed_data
114
115 def find_and_process_txt_files(root_directory, cycles):
116     for root, dirs, files in os.walk(root_directory):
117         txt_files = glob.glob(os.path.join(root, '*.txt'))
118         for txt_file in txt_files:
119             #if "_cv" in txt_file:
120                 process_txt_file(txt_file, data_list, columns, cycles)
121
122 # LOADING and ORGANIZING DATA\n",
123
124     data_list=[]
125     cycles = 3
126
127 # RWTH potentiostat stores columnwise the data as follows
128     columns = ["voltage", "current"]
129
130     root_directory = r'C:\\Users\\...' # Main directory path
131     find_and_process_txt_files(root_directory, cycles)
132
133 # Creating a DataFrame from the data list and exploding it to make it compatible
    ↪ with seaborn plotting functions
134     df = pd.DataFrame(data_list)

```

```
135 df = df.explode(["Voltage","Current"])
```

C.2 $f_{\text{cut-off}}$ AND IMPEDANCE AT 1 KHZ CALCULATION

```

1  # Function definitions
2
3  def process_txt_file(file_path, data_list, columns):
4      # Define your logic for processing the .txt file here
5      data=pd.read_csv(file_path,names=columns,sep="\t" or " ",
6      ↪ encoding_errors='ignore',skiprows=7,decimal=",")
7      directory, file_name_with_extension = os.path.split(file_path)
8      file_name = os.path.splitext(file_name_with_extension)[0]
9      parts = file_name.split('_')
10
11     # Extracting information\n",
12     date = parts[0]
13     material = parts[1]
14     potential = parts[2]
15     n = parts[3]
16     time = parts[4]
17
18     # Temporary filter data to include only frequencies higher than 1 Hz for cut-off
19     ↪ frequency calculation
20     filtered_data = data[data['Freq'] > 1]
21
22     # Check if the filtered data is not empty
23     if not filtered_data.empty:
24         # Calculate the cut-off frequency where the phase angle (Theta) is closest
25         ↪ to -45°
26         cut_off_idx = (filtered_data['Theta'] + 45).abs().idxmin() # Change here to
27         ↪ target -45°
28         cut_off_frequency = filtered_data.loc[cut_off_idx, 'Freq']
29     else:
30         cut_off_frequency = np.nan # Assign NaN if no valid frequency found
31
32     #data.ZRe = np.round(data.ZRe,2)
33     #data.ZRe -= data.ZRe[0]
34
35     # Constructing a dictionary for the file and appending to the data list
36
37     file_data = {
38         "File Name": f"{file_name}",
39         "Date": date,
40         "Material": material,
41         "ED potential": potential,
42         "N sample": n,

```

```

39     "Time of measurement": time,
40     "Frequency" : data.Freq,
41     "Z Re" : data.ZRe,
42     "Z Im" : -(data.ZIm),
43     "Modulus" : data.Modulus,
44     "Theta" : data.Theta,
45     "Cut-off Frequency" : cut_off_frequency
46 }
47 data_list.append(file_data)
48
49 return data_list
50
51 def find_and_process_txt_files(root_directory):
52     for root, dirs, files in os.walk(root_directory):
53         txt_files = glob.glob(os.path.join(root, '*.txt'))
54         for txt_file in txt_files:
55             #if "_EIS" in txt_file:
56                 process_txt_file(txt_file, data_list, columns)
57
58 # LOADING and ORGANIZING DATA\n",
59
60 data_list=[]
61
62 # RWTH potentiostat stores columnwise the data as follows
63 columns = ["Pt", "Freq", "ZRe", "ZIm", "Frequ", "Modulus", "Theta"]
64
65 root_directory = r'C:\\Users\\...' # Main directory path
66 find_and_process_txt_files(root_directory)
67
68 # Creating a DataFrame from the data list and exploding it to make it compatible
69 ↪ with seaborn plotting functions
70 df = pd.DataFrame(data_list)
71 df = df.explode(['Frequency', 'Z Re', 'Z Im', 'Modulus', 'Theta'])
72
73 # Modulus at 1kHz
74
75 # Filter rows where Frequency_kHz is 1
76 filtered_df = df[df['Frequency'] == 1000]
77
78 # Select only the relevant columns, if needed
79 df_1kHz= filtered_df[["File Name", "Time of measurement", "Material", "ED
80 ↪ potential", "N sample", "Frequency", "Modulus", "Theta"]]

```

C.3 CIC CALCULATION

```

1  # Function definitions
2
3  def calculate_baseline(current_data, time_data):
4      baseline_indices = np.where((time_data >= 50) & (time_data <= 58))[0]
5      baseline_value = np.mean(current_data[baseline_indices])
6      return baseline_value, baseline_indices
7
8  def calc_mov_avg(data, window_size=10):
9      data = np.array(data)
10     cumsum = np.cumsum(data)
11     mov_sum = cumsum[window_size-1:] - np.concatenate([[0], cumsum[:-window_size]])
12     mov_avg = mov_sum / window_size
13     #Extend the moving average to have the same size as the data
14     extension = np.full(window_size - 1, mov_avg[-1])
15     mov_avg = np.concatenate((mov_avg, extension))
16     return mov_avg
17
18  def calculate_cic(current_data, time_data, baseline_value):
19     # Subtract baseline value
20     current_data -= baseline_value
21
22     # Find the indices corresponding to the start and end time
23     start_index = np.where(time_data >= 60)[0][0] # First index where time is
24     ↪ greater than or equal to 60s
25     end_index = np.where(time_data >= 115)[0][0] # First index where time is
26     ↪ greater than or equal to 115s
27
28     # Extract the portion of current and time corresponding to the specified range
29     pulse_current = current_data[start_index:end_index]
30     pulse_time = time_data[start_index:end_index]
31
32     # Surface area
33     surface_area = np.pi * 0.5**2
34
35     # Integrate current with respect to time to get charge
36     charge = np.trapz(pulse_current, x=pulse_time)
37
38     # Calculate charge injection capacity (charge divided by surface area)
39     cic = charge / surface_area
40
41     return cic
42
43  def process_txt_file(file_path, data_list, columns):
44     try:
45         # Define your logic for processing the .txt file here
46         data=pd.read_csv(file_path,names=columns,sep="\t" or " ",

```

```

45         encoding_errors='ignore',skiprows=37,decimal=".")
46     directory, file_name_with_extension = os.path.split(file_path)
47     file_name = os.path.splitext(file_name_with_extension)[0]
48     parts = file_name.split('_')
49
50     # Extracting information\n",
51     date = parts[0]
52     routine = parts[2]
53     name = parts[3]
54     material = parts[4]
55     potential = parts[5]
56     n = parts[6]
57     measurement = parts[7]
58     intensity = parts[8]
59     day = parts[9]
60
61     # Apply moving average filter to current data
62     data.current_SMU1=calc_mov_avg(data.current_SMU1)
63     data.current_SMU2=calc_mov_avg(data.current_SMU2)
64
65     # Calculate baseline for current
66     baseline_SMU1, baseline_indices_SMU1 = calculate_baseline(data.current_SMU1,
67     ↪ data.time)
68     baseline_SMU2, baseline_indices_SMU2 = calculate_baseline(data.current_SMU2,
69     ↪ data.time)
70
71     # Subtract the baseline from current_SMU1
72     adjusted_current_SMU1 = (data.current_SMU1 - baseline_SMU1) * 1e9
73     adjusted_current_SMU2 = (data.current_SMU2 - baseline_SMU2) * 1e9
74
75     # CIC calculation
76     cic_SMU1 = calculate_cic(data['current_SMU1'], data['time'], baseline_SMU1)
77     cic_SMU2 = calculate_cic(data['current_SMU2'], data['time'], baseline_SMU2)
78
79     # Constructing a dictionary for the file and appending to the data list
80     file_data = {
81         "File Name": f"{file_name}",
82         "Date": date,
83         "Routine": routine,
84         "Measurement": measurement,
85         "Name": name,
86         "Material": material,
87         "ED potential": potential,
88         "N sample": n,
89         "Light intensity": intensity,
90         "Day of measurement": day,
91         "Baseline SMU1": baseline_SMU1,
92         "Baseline SMU2": baseline_SMU2,

```

```

91         "Current SMU1" : adjusted_current_SMU1,
92         "Voltage SMU1": data.voltage_SMU1,
93         "Current SMU2" : adjusted_current_SMU2,
94         "Voltage SMU2": data.voltage_SMU2,
95         "Time" : data.time,
96         "CIC SMU1": cic_SMU1*1e6,
97         "CIC SMU2": cic_SMU2*1e6,
98     }
99     data_list.append(file_data)
100
101 except Exception as e:
102     print(f"Error processing file: {file_path}")
103     print(f"Error message: {str(e)}")
104     traceback.print_exc()
105
106     return data_list
107
108 def find_and_process_txt_files(root_directory):
109     for root, dirs, files in os.walk(root_directory):
110         txt_files = glob.glob(os.path.join(root, '*.txt'))
111         for txt_file in txt_files:
112             try:
113                 process_txt_file(txt_file, data_list, columns)
114             except ValueError as e:
115                 print(f"Error processing file: {txt_file}. Details: {str(e)}")
116                 continue
117
118 # LOADING and ORGANIZING DATA\n",
119
120     data_list=[]
121
122 # RWTH potentiostat stores columnwise the data as follows
123     columns = ["Pt", "Freq", "ZRe", "ZIm", "Frequ", "Modulus", "Theta"]
124
125     root_directory = r'C:\\Users\\...' # Main directory path
126     find_and_process_txt_files(root_directory)
127
128 # Creating a DataFrame from the data list and exploding it to make it compatible
129 ↪ with seaborn plotting functions
130     df = pd.DataFrame(data_list)
131     df = df.explode(["Time", "Voltage SMU1", "Current SMU1", "Voltage SMU2", "Current
132     ↪ SMU2"])

```