Organic Light Emitting Diodes (OLEDs) for Neuro-optoelectronics

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À Denise et Marc.
DECLARATION

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I declare that contents of this thesis are my own work, and that contributions from other sources are appropriately acknowledged and referenced.

Bruno Matarèse
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Figure 57 | Organic LED for two-dimensional voltage sensitive dye imaging. (a.) the activation map averaged over the time period of interest (black line in b.) following the presentation of a visual stimulus (red line: period of stimulus presentation). Time course of the VSD signal (b.) averaged inside the black ROI in a.
Preamble

Project aim

Fluorescence imaging (e.g. Fluorescence imaging (e.g. fluorescent proteins), optical detection (e.g. biosensors), and opsin based neuromodulation (e.g. optogenetics) are the major techniques that have changed the practice of neuroscience. All these techniques use arc lamps, Light Emitting Diodes (LEDs) and lasers as conventional light sources. However, these light sources are not biocompatible and are excessively bulky, which limits their effectiveness as brain implants. Many improvements are necessary, such as the development of appropriate alternative light sources integrated with biological cells to ensure their compatibility with established techniques in biological laboratories.

Organic Light Emitting Diodes (OLEDs) combine optical and electrical properties with known advantages of customized materials providing appropriate color tunability, lightness, and low-cost solution processing. In addition, mechanical flexibility combined with the high elastic modulus and biological inertness of carbon-based polymers and nanomaterials confer major advantages for use in non-conformal body cavities. These attractive and innovative qualities of OLEDs make them an alternative and better light source for organic neuro-optoelectronics. However, engineering optoelectronic devices for operation in liquid environments requires a comprehensive understanding of the consequences that may arise.

This research is innovative in designing and engineering stable and biocompatible OLEDs for incorporation into living tissues. As a result, organic LEDs do not have to alter cell morphology and electrophysiological integrity/function. The principal challenge is ensuring they can operate in a highly saline and biologically active aqueous environment. Finding materials that are inherently stable in the environment in which they function is key to device optimization. The research will investigate the stability and biocompatibility of commonly used OLED materials. Key electrodes, indium, tin, oxide, gold, aluminum and silver are characterized to examine their suitability for an aqueous environment. [1,2] Treating organic light emitting polymers with laminin has shown enhanced cell adhesion and biocompatibility. Prolonged immersion in cell culture medium highlights the importance of cross-linkable polymers maintaining electrical, optical and morphological properties. These properties are critical to device performance. Insulator materials play complementary and necessary roles at the interface of the OLED/bio environment – ensuring the protection of the device against oxidative or reductive processes. Insulators are critical for extending the lifetime of devices in aqueous operation. They must be encapsulated with hydrophobic
polymers for protection against neuron damage from electrical stimulation. Following from the above it becomes possible to design both a highly biocompatible prototype OLED device, which is suitable for fluorescence microscopy, and patch-clamp technique for electrophysiological investigations. This would be based on opsin activation and would also include the investigation of critical design parameters for efficient OLED-opsin coupling stimulation. Additionally, OLED devices have been investigated for in vivo optical imaging of functional cortical architecture and dynamics.

**Thesis outline**

This thesis is organized in eight chapters.

**Chapter one** explains the background to organic Neuro-optoelectronics and how Organic Light Emitting Diodes (OLEDs) may be applied to the field.

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**Figure 1** | Schematic representation of simple Organic Light Emitting Diode structure at interface with cells in a bioenvironment. *Anode and Cathode are the electrodes sandwiching the active layer which is emissive.*
Chapter two begins the investigation of light emitting polymers (LEP) that are the central active layer of organic LEDs (see Figure 1). These ideally remain physically isolated from the biological environment and we seek to evaluate the effect of such exposure. While the opto-electronically active materials reside at the core of the device, chemical degradation of insulators, and/or mechanical wearing of devices means there is an inherent risk of the underlying materials becoming exposed to the biological environment over time. This chapter aims to establish a protocol to investigate the suitability of the organic semiconductor (OSC) materials at the interface with living cells. It does this by investigating their functional stability in bio-environments and their biocompatibility in retaining cell morphological integrity and their electrophysiological function both in darkness as well as in an irradiated condition.

This work will create an understanding of the requirements for insulation of devices to make them mechanically stable over long periods of time in bio-environmental conditions.

Chapter three highlights the performance of a cross-linkable light emitting polymer (LEP) within an OLED and demonstrates the diverse uses in various biological applications. It has higher optical and morphological stability in various media than un-crosslinked LEPs. Cross-linkable light emitting polymer (LEP) shows its potential uses in biological media and its potential in the stacking of multiple layer applications. The successful fabrication of a robust light emitting polymer sheet that can be freely suspended has highlighted the efficacy of the cross-linkability of O.S.C. and sustainability in prolonged immersion in biological media. By applying this cross-linkable material in Organic Light Emitting Diodes (OLEDs), the work showed how this could be used for indirect contact with biology.

Chapter four shows the investigation of the stability of the electro-optical properties of several commonly used electrode materials for use in organic LEDs. The aim was to identify the advantages and drawbacks of their use in solid/liquid devices as well as the benefits of an adhesion layer for applications requiring long-term aqueous immersion. An alternative adhesion layer, formed from a thin layer of the negative-tone photoresist SU-8, offers key advantages over other types of adhesion such as chromium. The adhesion layer SU-8 has superior biocompatibility and improved stability in biological culturing media. Various treatment protocols for the SU-8 were investigated with a view to attaining high transparency and good mechanical and biochemical stability. Thermal annealing is used to induce partial cross-linking of the SU-8 film prior to gold deposition. Further annealing after deposition to complete cross-linking, was found to yield the optimum electrode properties. The optimized glass/SU8-Au electrodes displayed improved
stability in biological culturing medium with respect to conventional glass/Cr-Au electrodes, and offered the further advantage of enabling facile electrode patterning via selective UV-illumination.

In **chapter five**, polymeric insulators protecting organic LEDs were investigated to protect cells from direct contact interaction of putative photochemical stress as already described in **chapters two** and **three**. In Chapter **six**, operational ohmic diodes, protected by polymeric insulators, were investigated for the protection of cells against charge leakages from devices. Using the techniques of good mechanical bio SU8-Au electrodes described in **chapter four** and suitable active material evaluated in **chapter two** and **chapter three** enabled the fabrication of fully-biocompatible and freestanding OLEDs with robust protection against direct contact of the organic semiconductor with cell applications.

The focus of **chapters six** and **seven** is to investigate the limits of OLEDs for their optical power and their optimal use in optogenetics (in **chapter six**) and bioimagery (in **chapter seven**).

In **chapter six**, conventional solution processing OLEDs were pushed to their operating limits: assessing how light delivered at pulse rates from 10 kHz to continuous illumination affected the optical and thermal character of the devices. It became clear that the pulsing mode of operation effectively enhances performance stability at high voltage operation and lowers operating temperature – allowing for safe and controlled photo-stimulation of neurons expressing microbial opsins.

**Chapter seven** examines applying OLEDs as a light source for optical bio-imagery used in two *in vivo* optical imaging techniques, intrinsic and extrinsic, for understanding functional cortical architecture and its dynamics.

Finally, **chapter eight** summarizes the main findings of the work and the possible strategies for further development of the initial results.
Chapter 1: Relevant background of Organic Neuro-optoelectronics

Organic electronics offers flexibility with a different multi-functional structure allowing tailoring for specific applications such as: stereochemistry, average molecular weight, photo-physical and electrical properties. Organic electronics aids, development of soft, flexible, transparent, large, ultra-thin and highly biocompatible light sources. Organic semiconducting materials have key advantages over their inorganic counterparts, including lower Young’s modulus (0.1 GPa - 20 GPa for organic [3,4] and about 50 GPa - 300 GPa for inorganic [5-10]), high strain resistance [11,12] and a wide color tunability [13-18]. Organic materials combine the mechanical properties of “softness and flexibility” with electrical and optical properties suitable for stimulation and the reading-out of bioelectrical events. The intrinsic multi-functionality of organic materials, such as light emitting polymers, can be exploited to generate electroluminescence [17]. The use of these emitting materials also allows the stimulation of bioelectrical activity of neurons and glial cells in vitro and in vivo. The fundamental electronic and photo-physical properties of organic materials make them suitable for the development of organic light sources (OLS) for neuroscience.

1.1 Organic semiconductors

Organic semiconductors with semiconducting characteristics are composed mainly of carbon and hydrogen atoms. First time electroluminescence of organic acridine orange and quinacrine was reported by Bernanose and co-workers in the early 1950s by applying a high alternating electric field of up to 2000 V across these materials [19]. An OLED prototype described by Tang and Van Slyke in 1987, was the fundamental basis of the field, achieving an efficient green electroluminescence by sandwiching a thin film made of two organic materials, tris-(8- hydroxyquinoline), aluminium (Alq3) and aromatic diamine, between a transparent anode and cathode electrodes [20].

High conductivity in oxidized iodine-doped polypyrrole was observed by Weiss and co-workers in 1963. High conductivity in polyacetylene was obtained by iodine, fluorine and bromine oxidation and doping with halogens. The electroluminescence from a conjugated polymer material was reported by Burroughes et al. in 1990 – poly (p-phenylene vinylene) (PPV) contained between two metallic electrodes. The ease of fabrication enabled first applications of conjugated polymer-based LEDs to reach the market quickly.
1.2 \textit{π-Conjugated organic semiconductor}

The conductivity of \(\pi\)-conjugated semiconductors (polymer and small molecules) is influenced by their conjugation \([21-25]\). Organic chemistry is able to tailor targeted emission of color \([15-18]\); high quantum yield of fluorescence (phosphorescence), high carrier mobilities \([22,26-29]\), stability \([30,31]\), purity \([32-35]\), facility of processability \([25,36-43]\) and mechanical characteristics \([44-52]\) and furthermore, the length of \(\pi\)-conjugation and energy band gap and electron affinity characteristics for adequate device structure \([53-58]\).

1.2.1 Bonding

A carbon atom has a ground state that possesses six electrons arranged in a \(1s^22s^22p^1\) configuration: a \(2s\)-orbital and a \(2p\)-orbital that leads to three equivalent \(sp^2\) hybrid atomic orbitals lying in a plane at 120° to each other and a residual unhybridized \(2p_z\)-orbital lying above and below the plane. Molecules or chains are formed when a carbon atom bonds with an adjacent \(sp^2\) hybridized carbon atom. Linear overlapping of atomic orbitals enables the formation of three \(\sigma\)-bonds from the \(sp^2\) hybridized orbitals. Sideways overlapping enables the formation of weaker \(\pi\)-bonds from the remaining \(p_z\)-orbitals. For instance, ethene (\(CH_2=CH_2\)) is the simplest molecule formed by this process where each carbon atom that bonds with hydrogen atoms possesses two hybridized \(sp^2\) orbitals. (see fig 2)

![Diagram of \(sp^2\)-\(sp^2\) \(\sigma\)-bond and \(p\)-\(p\) \(\pi\)-bond](image)

\textbf{Figure 2} | Schematic diagram of the simplest \(\pi\)-electron system in ethene showing the formation of the \(sp^2\)-\(sp^2\) \(\sigma\)-bond and the \(p\)-\(p\) \(\pi\)-bond. On the left, one \(2p\)-orbital lies above and below the plane and three \(sp^2\) orbitals lie in a plane at 120° to each other. On the right, \(\sigma\)-bond formed by two \(sp^2\) orbitals, and a \(\pi\)-bond formed by the remaining \(p_z\) orbitals.
Figure 3 | Schematic diagram of the delocalized π-electron cloud.

An σ-bond is formed by overlapping with a third hybridized sp² orbital of another carbon atom, while a π-bond is formed by overlapping the remaining p_z orbitals of two carbon atoms. A carbon-carbon double bond (C=C) corresponds to the combination of one π-bond and one σ-bond. The π-bonds are weaker than the σ-bonds and they are spatially delocalized over the entire conjugated segment. The σ-bonds are highly localized in space and they guide the formation of the shape and structure of the molecule and do not allow charge carriers to be mobile.

Conjugated polymers are specific to the electrons – delocalized along the π orbital responsible for their electronic properties. The electronic environment from any elementary excitation of the π-bonds and structural relaxations of the surrounding atoms influences the charge mobilities of the conjugated polymers. Bonding and anti-bonding molecular orbitals are the result of the overlapping of two atomic orbitals. (See fig 3)

1.2.2 Electronic energy structure

In conjugated polymers, the pz electrons are delocalized along the polymer chain with alternation of sequence between single, double and/or triple bonds. The Highest Occupied Molecular Orbital (HOMO) is the highest electron occupied molecular orbital with electron accumulating in the ground state. The Lowest Unoccupied Molecular Orbital (LUMO) is the lowest unoccupied electron π* (anti-bonding) orbital. The difference of energy between the HOMO and LUMO levels corresponds to the energy gap – in the range of visible spectrum from 1.7 to 3.2 eV for typical polymers [39]. Conjugated polymers are excitable optically and/or electrically and can be used for diverse applications (e.g. OLEDs [17,60] and OPVs [61,62]. Absorbing incident light directly or injecting and removing charges through contacts with opposite electrode enables the excitation of the conjugated polymers.
An electron polaron, is a negative charged state that relaxes when the molecule adds an electron. A hole polaron is formed when the molecule removes an electron from its neutral state. A naturally forbidden electron energy gap may be forced with the appearance of new electronic states that happens when electrons add to a neutral molecule. A bipolaron is a molecule containing a bound state of spinless two electron polarons or two hole polarons.

![Energy level band diagrams](image)

**Figure 4 | Energy level band diagrams** for a neutral material, an electron-polaron state (containing an electron and its accompanying polarization field) and an electron-bipolaron state (with spinless two close-by polarons).

The energy difference (electron binding energy ($E_B$)) between the excited state and the ground state is lower than the difference between the ionization potential and the electron affinity due to the attraction of the electron and hole, and the structural relaxation. The particle exciton is the result of the formation of a bond occurring in excited state due to the coulomb attraction of an electron and a hole. Charge-transfer excitons may be formed between neighboring chains. The relaxation orientation of two unpaired particles is determined by two spin states of excitons. In the first, the exciton has three symmetrical spin compartments (in phase S=1) called a triplet. In the second, the exciton has an anti-symmetric spin component (out of phase S=0) called a singlet.

Exciton reaches an excited state when energy is transferred from an excited donor to an acceptor species. Förster energy transfer is a singlet-singlet process due to of the conservation of the spin state of donor and acceptor. Dexter transfer allows singlet-singlet and triplet-triplet transitions and refers to the exchange of electrons excited from the donor in the excited state to the acceptor in the ground-state. (see fig 5). Radiative transfer consists in the emitted light from one molecule (donor having a high quantum efficiency) being absorbed by a second molecule (acceptor having high absorption coefficient). An oscillating dipole in the acceptor molecule is induced by the electric field.
1.2.3. Absorbance and luminescence

Absorption and emission transitions represented in the configuration diagram show the ground state $S_0$ and the first excited state $S_1$. Each electronic state has vibrational levels (check Figure 6 and 7) with absorption from the lowest vibrational levels of $S_0$ towards the vibrational levels of $S_1$ (arrows Figure 6 and Figure 7). Internal conversion occurs when excitons relax to the 0 level of $S_1$ during excited states. This is a thermal relaxation leading to phonon emission with the exciton decaying to vibrational levels of $S_0$. However, non-radiative decay during the relaxation of the exciton returning to the ground state emits photons. The energy of the vibrational levels in the $S_0$ and $S_1$ states determine the emission wavelengths (represented in the Frank-Condon principal in Figure 7). The space between the ground state and excited state configurations is related to the vibrational levels. The oscillatory structure of the photoluminescence spectrum is explained by the excited state relaxing to a lower energy state before emission.
Figure 6 | Jablonski energy diagram. Singlet state Sn (here n= from 0 to 2) and triplet state Tn (here n= from 1 to 3). Absorption of photon represented in blue arrow, the fluorescence and phosphorescence represented in red arrows. The Internal conversion (ic), Vibrational relaxation (Vr) and Intersystem crossing (Isc) represented with green arrows.

The Jablonski diagram (Figure 6) represents the processes of absorption, internal conversion, fluorescence, inter-system crossing and phosphorescence. Upon electrical doping, 25% of exciton is formed in S1 state and 75% of triplet exciton may be formed in T1 state. Spin selection rules allow the transition $S_0 \rightarrow S_1$ with lifetime of the $S_1$ state being very short (~ ns). The transition $S_0 \rightarrow T_1$ with a lifetime of several orders of magnitude larger (~ μs or ms) is forbidden.

Upon optical and electrical excitations, the singlet states $S_n$ are excited ($S_0 \rightarrow S_n$) and relax to the lowest excited singlet state $S_1$ (non-radiative internal conversion). Fluorescent emission or thermal relaxations (internal conversion) occurs when the excited singlet state $S_1$ relaxes to the ground state ($S_1 \rightarrow S_0$). Intersystem crossing (conversion of an $S_1$ singlet to a $T_1$ triplet) or direct electrical excitation leads the triplet state $T_1$ to be populated with electron. Phosphorescence emission occurs when spin-orbit coupling or a mixture of singlet and triplet states allows the triplet state emission with radiative transition $T_1 \rightarrow S_0$. The incorporation of heavy atoms into a molecule enhances spin-orbit coupling leading to intersystem crossing. Heavy organo-metallic complexes based on iridium (Ir) or platinum (Pt) are often used in phosphorescent OLEDs [63–65].
**Figure 7 | Schematic representation of Frank-Condon principal.** Absorption (in blue) and Emission (in red) of photon. $S_0$ represent the ground state and $S_1$ the excited state. $V_n$ (here $n$ from 0 to 2) represent the vibrational modes.

### 1.3 Device physics and operation

In this section the basics of an OLED device are described and the different materials used in this thesis for the investigation of organic LEDs suitability for bioenvironment are introduced.

#### 1.3.1 Device physics and operation

A brief description of device operation is essential to understand its electrical and photo-physical processes.

An OLED is a light-emitting diode (LED) in which the emissive electroluminescent layer is a film of organic compound that emits light in response to an electric current $^{[66,67]}$ (fig 8). This layer of organic semiconductor is situated between two electrodes. The operation of organic light-emitting diodes (OLEDs) involves a light generation process with charge injection and transport, and charge recombination and optimization of the light outcoupling. A typical multilayer of OLEDs has hole injection from the anode into HOMO of the hole transport layer (HTL) and the electron injection from the cathode into LUMO of the
electron transporting layer (ETL). The injected holes and electrons each migrate towards the oppositely charged electrode. The charge jumps from molecule to molecule following a hopping transport regime. The drift and diffusion of charge carriers can lead to recombination of opposite charges to give an exciton. This is a localized electron-hole pair having an excited energy state through the Coulomb capture process. The radiative decay of the exciton emits light and a transparent electrode enables the escape of photons. The challenge of designing efficient OLEDs relies on charge injection from the electrodes with a well-balanced number of holes and electrons within the emitting layer to achieve high quantum efficiency for electroluminescence (EL) [66–69].

**Figure 8 | Schematic representation of typical Organic Light Emitting Diode structure with exciton formation and exciton decay in OLEDs.** Anode and Cathode are the electrodes sandwiching the hole injection layer (HIL), the hole transport layer (HTL), the active layer which is emissive, the electron transport layer (ETL) and the electron injection layer (EIL). Electrons (e\(^-\)) and holes (h\(^+\)) are injected into the active organic film under forward biases and migrate towards each other until they are close enough to recombine as excitons. The excitons can then decay radiatively to produce light.

OLEDs are flexible [76], stretchable [45,71], lightweight and thin [72] and can be produced in large sheets [73,74]. An OLED measures < 0.2 micrometer (μm) of thickness (see Figure 8) and consists of charge transfer layers (ETL and HTL), the electron and hole blocking layer (EBL and HBL) and the emission layer (EML) – each of about 100-200 nm thickness [72]. This is smaller than the size of typical neurons from 4 to 100 micrometer (μm) of diameter [75,76]. However, when the OLED is encapsulated with its necessary substrate, e.g. glass or PET, a device can measure a few millimeters thick. Various thin film encapsulation materials are under intensive research [77–84].
The innovative focus of this research is to design and engineer stable and biocompatible OLEDs of simple structure for incorporation into living tissues so that their operation does not alter cell morphology and electrophysiological integrity/function. One major challenge here is to overcome operating in a highly saline and biologically active aqueous environment. The aim is to find materials that are inherently stable in these environments by investigating the stability and biocompatibility of commonly used OLED materials. To exploit the maximum external quantum efficiency of high optical power devices at low voltages, the distance between the cells and emitting layers must be minimized. While the opto-electronically active materials reside at the core of the device, chemical degradation of insulators and/or mechanical wearing of devices imply an inherent risk of the underlying materials becoming exposed to the biological environment over time. It is important to investigate the suitability and biocompatibility of various types of material in the development of OLEDs, leading to design and fabrication of appropriate devices.

1.3.2 Suitable organic light-emitting materials

There are three main types of emissive material. First, fluorescent light-emitting polymers LEPs (e.g. (fluorene) PF-type, (thiophene) PT-type, (phenylene vinylene) PPV-type) that are the main emissive materials investigated in this thesis (fig 9). PT-type is used in chapter 2 as control material having very small light emissive properties. Light emitting polymers (LEPs) are chosen for their high fluorescence emission to investigate their suitability at their interface with cells in chapter 2. They are used in organic LEDs for stimulation of neurons Chapter 6 and for bio-imagery techniques chapter 7.

Figure 9 | The electroluminescent π-conjugated polymers types used in this thesis: polythiophenes (PTs); poly(p-phenylenevinylene) (PPV) and polythiophene (PT).

Second, phosphorescent metal complex-based polymers and small molecules, e.g. platinum and iridium \[^{63-65,85}\], that have short triplet lifetime in an unquenched excited state. In the fluorescent devices using light emitting polymer LEPs, only singlet excitons generate photons, while triplet excitons remain unused, non-radiatively relaxing as heat. \[^{86}\] By introducing a heavy metal into the organic materials,
phosphorescent OLEDs can enable the conversion of triplet excitons into photons, which can improve the internal quantum efficiency $\text{IQE}_{\text{oled}}$ to 100%. [87–89] This type of emissive material are widely used for cell imaging [90–95] and photodynamic cancer therapy. [96–100] Highly efficient solution-processed, phosphorescent organic light-emitting diodes require multilayer films based on small-molecule hosts [63]. This type of emissive material is used in Chapter 6 for the characterization of a series of blue and red solution processed phosphorescent OLEDs for the stimulation of neurons. Efficient solution processing phosphorescent thin film used in chapter 6 and the introduction of the investigation of their suitability for their interface with biological cells is discussed in Appendix 3 related to the chapter 2.

For the above two types of materials, the fabrication of a nanometric thin film of emissive small molecules is possible by thermal evaporation under high vacuum. The second type of material is difficult to form as a film by solution processing but can be blended in the host conductive matrix (Poly{9-vinylcarbazole} PVK, as doped with iridium emissive chromophores. Polymeric film can easily be fabricated via solution processing (spin coating, inject-printing and spray coating). Furthermore, inexpensive solution processing of LEPs for fabrication of PLED devices has been proved to perform efficiently in organic devices [14,28,35]. Light emitting materials must have electrical conductivity and high photoluminescence to perform efficiently. However, LEPs for LED applications are preferable to small organic molecules as they give greater mechanical flexibility [70] and are easier to process to make large area pixels. [73,74].

Third, new types of light emitting materials not covered in this thesis are based on Thermally Activated Delayed Fluorescence (TADF) [65,103–111] and Aggregation-induced delayed fluorescence (AIDF) [112–115] with low driving voltage of operation while providing highly efficient electroluminescent devices that would provide a safe interface with biological tissues. Furthermore, they can be tune to emit near infrared light [117,118] for optical bio imagery requirements. [119–125]

1.3.3 Suitable bio-electrodes

The conventional bottom-emission OLEDs are fabricated on a substrate coated with a transparent conducting electrode (TCE) [126–128] where emitted light passes through it. A typical bilayer electrode is composed of a thin layer of metallic polymer deposed onto a patterned indium tin oxide (ITO) anode. These transparent conducting electrodes are brittle. [129–131] The next generation of flexible organic LEDs include electrodes made of four categories (conducting polymer132–134, silver nanowire [17,135–139], carbon nanotube [73,133,140] and grapheme [141–146]) of materials that are not investigated in this thesis in order to
not add any complexity and focus on basic design approaches for biomedical suitability. In fact, before finding a best candidate electrode it is important to understand in chapter 4 the stability and biocompatibility of commonly used metal electrodes made of Gold (Au), Silver (Ag), Aluminum (Al) and Indium-Tin-Oxide (ITO) when immersed in physiological media. The material optimization aims to find materials that are inherently stable in their functional environment. The operation of Organic light emitting diodes (OLEDs) is fundamentally dependent on keeping the balance of the anode and cathode electrodes’ work functions $^{[1,2,147,148]}$ and high conductivity of the electrodes $^{[1,2,149]}$.

In such media, the strong polarizability of water gives rise to many forms of electrochemical activity, the latter being amplified by the rich ionic composition of biological media. This creates an overall environment where metals are very prone to corrosion and oxidation. For example, Ag$^+$ reduces readily in presences of Cl$^-$ ions – a prevalent component of biological media. Aluminum and silver are very sensitive to reduction by aqueous chloride $^{[1,2]}$ and, given their low work function, they are prone to easy and rapid oxidation in the presence of singlet oxygen. Similarly, it has been shown that gold may be dissolved by thiol-containing biological molecules like cysteine and glutathione $^{[1,2]}$. The metals commonly used in bioelectronics applications (Ag, Al and Au), especially the ones with lower work-function, when immersed in culturing medium are subject to strong electrical degradation in terms of their work function and conductivity. Furthermore, they are sometimes subject to delamination during long-time experiments $^{[1,2]}$. To solve this last issue the feasibility of using a polymeric adhesion layer is investigated in chapter 4. Similar interfacial adhesion layer is studied for their suitability as encapsulation material for organic LEDs in chapter 5.

1.3.4 Suitable materials for device encapsulation

The encapsulation of organic LEDs demands materials with low dielectric constants, low dissipation factors, insulation from oxygen and water (evaluated by the Water Vapor Transmission rate (WVTR) or the Oxygen Transmission Rate (OTR) , and good thermal stability. $^{[150-154]}$ They must be transparent to allow the light to escape the device, mechanically robust to sustain long lasting lifetime in bioenvironment and flexible $^{[155,156]}$ for use in vivo. Insulator materials play complementary and necessary roles at the interface of OLED/bio environment, ensuring the protection of the device against oxidative or reductive processes. Encapsulation insulates the electrodes from external harsh environments and protects the device from direct contact with the biological environment.
Organic LEDs must be encapsulated with water barrier polymers for protection against neuron damage from electrical stimulation. Encapsulation material must not alter cell adhesion and function and it is necessary to design a highly biocompatible prototype OLED device which is suitable for fluorescence microscopy and patch-clamp technique for electrophysiological investigations based on opsin activation or various other in vivo applications.

Inorganic materials (e.g. metal-oxides such as Si, Al2O3, SiO2, MgO) as encapsulant have acceptable durability and its efficacy as gas barrier is dependent on processing. Nevertheless, they are brittle and do not protect the device against water. On the other hand, polymers present a lower negative impact on the environment and may be partially hydrophobic— which provides a greater barrier to water than inorganic materials. However, the partially porous nature of polymers is favorable to the diffusion of oxygen.

Glass was used as the encapsulation material of the organic LEDs described in chapter 6 and chapter 7 to effectively insulate and protect the diodes from oxygen and the saline and redox environment. In turn, this protected cells from leak currents and the highly reactive calcium used for efficient electron injection to the active layer of OLEDs. Accordingly, commonly employed polymeric insulators were investigated for the fabrication of freestanding organic LEDs for their ability to adhere to cells, and hence their applicability as final barriers in OLED devices (see chapter 5).

The first type of polymeric encapsulation materials are thermoplastics which are plastic materials that melt to liquid form on heating and can be remolded and remelted which found its uses in plastic molding processes. They may freeze to a brittle, glassy state. They have low tensile moduli, low density value and transparency that is ideal for medical applications. Flexible OLEDs usually use the thermoplastic polyethylene terephthalate (PET) as encapsulation and substrate to support the multi-stack of layers of the device and has desirable mechanical strength and durability for biomedical application. Polystyrene (PS) is commonly used for Petri dishes test tubes and microplates in biomedical applications. Polyvinylchloride (PVC) is widely used in biomedical such as surgical and examination gloves; containers for blood, urine continence and ostomy products; Heart and lung bypass sets, tubing for dialysis, endotracheal, feeding and pressure monitoring; and blister packs for pills and tablets. Poly (methyl 2-methylpropenoate) (PMMA) is often used for false denture base materials and hard contact lenses. PMMA and poly(dimethylsiloxane) (PDMS) has several biomedical applications using microfluidic lab-on-a-chip devices. For instance, PDMS
microfluidics channels are used for cell separation by electrosmosis \textsuperscript{[235,236]}. PDMS has many methyl groups and by nature is hydrophobic – impermeable to water that would protect organic LEDs from the water environment \textsuperscript{[237–240]}. Finally, Parylene-C has uses in medial application as substrate and insulator layer \textsuperscript{[241–245]}. The second type of polymeric encapsulation materials are the various thermoset plastics \textsuperscript{[246]} which do not melt at high temperatures but can be softened on heating. They become irreversibly hard polymer network on cooling once catalyzed from a prepolymer in a soft solid or viscous state. Their cure may be effected through heat, through a chemical reaction, or irradiation such as electron beam processing \textsuperscript{[185,247–252]}. The three-dimensional network of bonds (cross-linking) of thermosetting plastics (e.g. polyester resins; vinyl ester resins; epoxy resins) and their tailored elasticity with excellent resistance to fatigue strength; corrosives and solvents; high temperature and excellent adhesion making them stronger than thermoplastic materials. Silicone plastic has the lowest heat capacity among thermoset plastics and using silicon as the backbone rather than the carbon of organic polymers. In addition, it has a fairly high density and a fairly high dielectric strength \textsuperscript{[25]}). However, epoxy resins has moderately low density and a young’s and tensile modulus of about 2.4 GPa \textsuperscript{[254]}. Epoxy resins in general are hydrophilic (contain OH-groups) but thick layers make an efficient barrier to water \textsuperscript{[254–263]}. Various biological applications use bis-benzocyclobutene (BCB)-based polymers do not emit any volatiles during the curing process and the products are nonpolar hydrocarbon moieties \textsuperscript{[264–269]}. Ormostamp (from Microresist Technology) is investigated for its high transparency in near UV and visible light and its high thermal and mechanical stability \textsuperscript{[270–272]}. Last but not least, particular attention has been focused on the negative-tone photoresist SU8 \textsuperscript{[273]}. This material has previously been used as an alternative adhesion layer to chromium since it adheres well to multiple substrate materials and (once cross-linked) is chemically inert \textsuperscript{[274,275]}. SU8 has the further advantage of being photo-patternable, allowing patterned electrodes with feature sizes down to a few microns to be readily fabricated using a simple two-step expose/develop procedure \textsuperscript{[276]}. SU8 has been used as adhesive layer for gold electrodes in chapter 4 and as insulating layer of freestanding organic LEDs in chapter 5 and as control insulation of organic semiconductors OSC in chapter 2.

1.4 Bio-applications using organic semiconductors (OSC)

Organic optoelectronics is ideally suited for biomedical applications due to its chemical similarities to biological systems (carbon, oxygen, nitrogen-based composition). Furthermore, organic semiconductor
properties explained in section 1.2 plays an important role in diverse mechanisms such as transduction, photo-thermal and charge transfer occurring at their interface with cells.

1.4.1 Cellular background and requirements

Cells have electrical properties resulting from the dielectric behavior of the plasma membrane that separates the internal and external cellular ionic solutions. The ions have different permeability coefficients enabling them to pass through trans-membrane proteins positioned at the surface of the membrane. These proteins have pores that are permeable and/or impermeable to the ions as a result of chemical control of the size of their membrane structure. Stimuli (e.g. electrical or chemical) may open the pores of these proteins and directly or indirectly transduce intracellular signals.

For Neurons, an action potential is possible from the inversion of polarity of the cell membrane potential from the accumulation of ionic charges at the soma of a neuron, leading to a capacitive current at the membrane and an ionic conductance across the membrane. These actions can be controlled via external stimuli. In fact, electrical, thermal, chemical and mechanical changes stimulate the cell and may lead to action potentials. The OSC have been used intensively as coatings of conventional electrodes in direct neuronal interfaces for recording and stimulating neuronal activity. For example organic conducting polymers such as poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) \[^{277–287}\]. In particular, stimulation of neurons has been shown to enable the restoration of hearing by cochlear implants \[^{288,289}\]; retinal prosthetics can restore light sensitivity to blind eyes; and deep brain stimulation helps to control epilepsy and Parkinson’s disease \[^{290–294}\].

Hence, there is a general requirement for new tools capable of reading, monitoring and controlling the actuation and inhibition of the electrical activities of these cells. The electrical, thermal, chemical, mechanical and optical organic semiconductor properties can address these requirements.

1.4.2 OSCs for neuronal stimulation

Control of the modulation of neuronal activity has been extensively examined in the literature \[^{295}\]. A starting option is to use external materials or molecules placed near the cell. An electrical, mechanical, chemical, or thermal stimulus from an organic semiconductor (in film or nanoparticle form) interfacing with the cell may modulate the neuron’s activity \[^{296}\]. A further option is to use the properties of endogenously expressed light-sensitive proteins present in cells to directly stimulate neurons with photons. An organic
semiconductor may be applied in a light emitting device and can be used to modulate the neuron action potential by applying optogenetics \cite{297,298} or use of the of natural or genetically modified photo-sensitive cells can be used to modulate the neurons’ activity \cite{299,300}.

The use of organic semiconductors for direct contact photo-stimulation of neurons has led to an understanding of the mechanism of such a bioenvironmental interface. Thermal stimulation is a fundamental property of the plasma membranes and leads to briefly depolarizing any eukaryotic. The temperature changes affect the physical and electrical properties of cells via the thermal conductance of ion channels \cite{301}. The effects on membrane properties and ion channel kinetics can be used to inhibit the activity of a neuron. Photovoltaic stimulation has been reported using photocurrent generation as a stimulus. Cells grown on a photovoltaic structure in contact with P3HT coated on ITO (ITO/P3HT) generate photocurrent and by capacitive effect (electron accumulating and charge separating) lead to the activation of cell activities. Photothermal stimulation has been reported using prolonged pulses of light (<100ms), having a biphasic effect on cell membranes \cite{302} leading to thermal inhibition of neural activity. The advantage of such technology is that it presents an opportunity to not necessarily modify cells genetically and it can have useful applications for retinal prosthetics \cite{303}.

One innovative aspect of this research is its aim to establish a protocol to investigate the suitability of organic semiconductor (OSC) materials at the interface with living cells. This can be done by investigating their functional stability in a bio-environment and their biocompatibility both in darkness and in an irradiated condition. A mechanism of cellular responses upon irradiation of their interface with OSC is established and confirmed to occur in thin films and nanoparticles. Furthermore, this work leads to an understanding of the requirements for the encapsulation of organic LEDs that must remain mechanically robust over long periods of time in bio-environmental conditions.

1.4.3 OLED for neurostimulation

In a different approach, Optogenetics, is a combination of techniques from optics and genetics which uses light for non-invasive cell-specific neuronal stimulation to control and monitor the activity of individual neurons in living tissue. This technique represents a considerable advance on previous methods, such as electrical stimulation that indiscriminately impacts all cells near the electrode tip. The recent development of neural photosensitization tools – genetically encoded switches such as channelrhodopsin-2 (ChR2) or a halorhodopsine (NpHR) pump – allows neurons to be turned on and off \cite{304-310}. Following illumination with
blue light (activation maximum 470 nm), ChR2 allows the entry of cations (mostly Na+ and very low levels of Ca2+) into the cell. NpHR is activated by yellow light illumination (activation maximum 580 nm) and allows the entry of Cl- anions \[^{311}\]. The use of these proteins offers new opportunities for non-invasive, cell-specific neuronal stimulation in culture, and also in the brains of live animals, and has established the research field of optogenetics \[^{312}\]. Consequentially, neuroscientists have rapidly adopted these neural photosensitization tools to investigate brain activity. Whilst important results have been obtained in this relatively new field of optogenetics, a significant problem facing researchers is how to provide light to specified deep brain areas of interest. To date, this technique has been achieved using light-emitting diodes (LEDs) or lasers as hardware (e.g. integrated fiber-optic and solid-state light sources) that are able to deliver light with a precise wavelength to specific areas of a mouse brain at high temporal control. These inorganic devices are brittle which limits their effectiveness as brain implants. Many improvements are necessary, such as the development of appropriate light sources that could provide two-dimensional stimulation with biocompatibility, sufficient irradiance \[^{311}\], appropriate color tunability, a large active area \[^{73,74}\], mechanical flexibility and low-cost processing \[^{314,315}\].

The innovation of the proposed research is to use organic light-emitting diodes (OLEDs), which are a promising alternative to conventional light-emitting diodes (LEDs), as light sources in optogenetics. The research topics will be focused on the development of biocompatible organic light-emitting diodes (OLEDs) for incorporation into living tissues, particularly for controlled photo-stimulation of neurons.

1.4.4 OLEDs for bio-imagery

Optical imaging, in neuroscience, aims at both visualizing brain function in vivo so as to study cortical development or its organization and functions and enabling at revealing the mechanism of processing of information. Intrinsic and extrinsic optical imaging are two complementary techniques of imaging widely used in neuroscience giving unique advantages.

First, intrinsic optical imagery has a slow intrinsic change enabling visualization of active cortical regions at high spatial resolution (50 µm). The optical properties of the active brain may be imaged by (1) changes in physical properties of tissues that are affecting the scattering of a light excitation or (2) changes in absorption and fluorescence of intrinsic molecules.

Second, extrinsic optical imaging uses. For instance, Voltage Sensor Dye Imagery (VSDI) in the form of a molecular transducer that transforms the voltage change in membrane potential into an optical signal that
needs to be excited by an appropriate light source. The advantage of VSDI is that it relies on its real-time sensing of activities, enabling the direct visualization of brain function and cortical dynamics.

Both, intrinsic and extrinsic optical imaging requires an excitation light source. For intrinsic, the tissues scatter/reflect the excitation light back differently depending on the activation of the area. For extrinsic, when absorbing an excitation light, the dye emits light back when bound to the surface of a brain active area. Alternative light sources that could provide a large homogeneous emissive area with biocompatibility, sufficient irradiance, appropriate color tunability and mechanical flexibility properties would enable fully integrated emitting devices to be placed in an optical chamber leaving a subject animal free to move during recording. Organic light-emitting diodes (OLEDs) have been investigated in Chapter 7 as a potential alternative light source for intrinsic and extrinsic in vivo optical imaging for their unique properties such as homogeneity, color tunability, mechanical flexibility and transparency.

1.5 Summary

The requirements for suitable OSC for direct and indirect interface with the bio-environment play an important role in finding adequate applications for such materials. Both direct and indirect contact of OSC with cell requires being biocompatible with stability, whether it be mechanical, chemical, optical or electronic.
Chapter 2: Reversible and irreversible cellular photo stimulation at the interface with organic light emitting semiconductors

The aim of this chapter is to establish a protocol to investigate the suitability of organic light-emitting semiconductors for their application in bioelectronics. The optical and morphological stability and photo-electrochemical interface between cells and light emitting polymer is investigating. The research is relevant for long-term implanted organic LEDs that may break up letting exposed to cells such initially encapsulated light emitting materials. Alternatively, applications for direct interface of light emitting semiconductors with biological cell are discussed.

2.1. Introduction

Many applications use Organic Light Emitting semiconductors in solid state devices e.g. Organic Light Emitting Diodes (OLEDs) [316]; Organic Light Emitting Electrochemical Cells (OLECs) [317]; Sensors [318–320]; or for the production of energy with hydrogen evolution [321,322]. Those applications operate in various environments which have effects on the optical, electrical and morphological material properties with irreversible consequences on their uses. Great progress has been made in understanding the degradation pathways of polymers, with the roles of oxygen, water and impurities leading to a need to protect the materials with adequate insulators against indirect contact with the bio-environment [323]. Other applications require direct contact with an aqueous interface between materials and the bio-environment to provide photo-stimulation of biological cells [303].

Here we aimed at a systematic approach to assess the suitability of the polymer for bioelectronics and/or biomedical applications with cellular and environmental imaging, photo-stimulation, and photo-ablation. We therefore chose to investigate different types of conjugated polymer (fluorene) PF-type, (thiophene) PT-type, (phenylene vinylene) PPV-type and a non-conjugated polymer PVK often used as a host matrix for phosphorescent organometallic dopant materials. Their optical, chemical and morphological stability was studied under various environmental conditions ranging from air to complex cell culture medium, and over wide time spans of exposure. We observed that oxygen was a primary source of film degradation, a rule generalizable across the investigated polymers. Utilizing both, the human embryonic kidney 293 cell line and the primary hippocampal neuron cultures, we understand the basic processes present at organic semiconductor (OSC) and cellular interface in saline settings, drawing out critical conclusions for suitable use of organic semiconductor material in biomedical devices. We then fabricated
and mixed nanoparticles (NPs) of organic light emitting semiconductors with cell culture media to investigate the photodynamic therapy applications.

### 2.2 Stability of organic semiconductors (OSC)

Here we investigated the parameters of degradation of materials in bio-environments.

#### 2.2.1 Bioenvironmental effects on OSC

Polymers are sensitive to temperature, pH, viscosity, and solvent polarity, photo-bleaching and quenching processes which cause their degradation [324,325]. The environmental effect on the conjugated polymer is an inter-molecular factor causing the degradation of the material. It has an effect on static and dynamic quenching on polymers. There are different types of quencher mechanism of photoluminescence: the dynamic mechanism quenching with Dexter electron transfer (ET) occurring when photo-induced excited electrons are transferred from a donor to an acceptor [326]; and Förster resonance energy transfer (FRET) with energy transfer between chromophores of the light emitting organic semiconductors and a donor through non-radiative dipole-dipole coupling [327]. Förster and Dexter energy transfer does not affect the shapes of the absorption and photoluminescence spectra of the materials but affects the non-radioactive lifetime. A third mechanism is attributed to exciplex interaction which is an excited state complex that is formed during photo-induction, and has the possibility of charge-transfer (CT) with its environment, with radical ions and unpaired electrons that can spin-pair to form covalent bonds and lower the energy of the charge transfer state [328].

On the other hand, the static quenching affects its ground state by forming fluorophores-quencher complexes e.g. the accumulation of external quenchers to minimize contact with water due to hydrophobicity forces. This static quenching reduces the number of unaffected fluorophores that emit but do not affect their excited state properties. It is possible to track static quenching when a linear decrease of fluorescence intensity and a non-degradation of the radiative lifetime are observed. Polymers are sensitive to temperature, pH, viscosity, and solvent polarity, photo-bleaching and quenching processes which cause their degradation [324,325]. The environmental effect on the conjugated polymer is an inter-molecular factor causing the degradation of the material. It has an effect on static and dynamic quenching on polymers. There are different types of quencher mechanism of photoluminescence: the dynamic mechanism quenching with Dexter electron transfer (ET) occurring when photo-induced excited electrons are transferred from a donor to an acceptor [326]; and Förster resonance energy transfer (FRET) with energy
Figure 10 | Optical degradation of conjugated polymers (PFO, F8BT, SY-PPV, SO-PPV, MEH-PPV, CN-PPV and P3HT) under prolonged soaking in bio-environment. (a.) On left, the absorbance (Abs) and photoluminescence (PL) before (0 hours) and after (672 hours) soaked in air (gray dash), di-water (color dash) and cell culture media (full line). (b.) In middle, the average radiative lifetime before (0 hours) and after (672 hours) soaked in air (gray dash), di-water (color dash) and cell culture media (full line). (c.) On the right, the absorbance (Abs in blue), photoluminescence (PL in red), average radiative lifetime (lifetime in black) and the quantum yield (QY in green) for each conjugated polymer after 672 hours AIR, DI-WATER, MEDIA (bare) and protected with dielectric SU8 and soaked in media.
transfer between chromophores of the light emitting organic semiconductors and a donor through non-radiative dipole-dipole coupling \([327]\). Förster and Dexter energy transfer does not affect the shapes of the absorption and photoluminescence spectra of the materials but affects the non-radioactive lifetime. A third mechanism is attributed to exciplex interaction which is an excited state complex that is formed during photo-induction, and has the possibility of charge-transfer (CT) with its environment, with radical ions and unpaired electrons that can spin-pair to form covalent bonds and lower the energy of the charge transfer state \([328]\).

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The stability of organic light emitting materials soaked in two different media was investigated. The selection of conjugated polymers were (PPV)-types (F8BT and PFO); PF-type: (SY-PPV; SO-PPV; MEH-PPV and CN-PPV); PT-type (P3HT) (Figure 10). The samples were prepared under equal spin and annealing settings, from solutions of equal concentration by weight (1 mg/ml) for all polymers tested. (See method section). The materials were sterilized with a 1-hour thermal treatment at 120-degree C and then immersed bare in Di-water (DI) and Dulbecco’s Modified Eagle’s Media (Medium) without addition of serum. In addition, there was a control of the material exposed bare in air and a control with the material encapsulated with the photoresist SU8 of 1 µm thick chosen for its dielectric properties, chemical inertness and its natural impermeability to water in order to avoid direct contact of organic semiconductors with the medium. Figure 10 shows the instability of the optical properties, namely absorption (Abs), photoluminescence (PL), lifetime (LT) and quantum yield (QY) of conjugated polymers. Atmospheric impurities in air and impurities such as essential ions, peptides, lipids, enzymes, growth factors and other additives and products of metabolism of the cell line in cell culture media has been discovered to play an important role in static quenching \([329-332]\).

We observed in all materials a reduction of the optical properties after exposure for 672 hours in air, Di-water and cell-culture media. There were no observable significant changes of shape either of absorbance (Figure 10a left side) or photoluminescence spectrum (Figure 10a right side). There was a strong diminution in photoluminescence intensity showing a sign of oxygen quenching. After further
examination, it was found that we could separate the materials into two groups. Group one is composed of the most stable material exposed in cell culture media after 672 hours: P3HT; SY-PPV; SO-PPV and MEH-PPV. The most stable of the two groups is P3HT (Figure 10-a, b, c) with decrease from baseline as deposited of 0.64% of its absorbance (Abs), 56% of its photoluminescence (PL), its average lifetime (LT) reducing from 0.42 to 0.35 nanoseconds and its quantum yield (QY) values from 5% to 2%, while the least stable of group one is MEH-PPV with decrease of abs 58 %, PL 42%, life time reducing from 0.2 to 0.1 nanoseconds and quantum yield values from 26% to 12%. SY-PPV and SO-PPV show very similar strong stability with a decrease of about 62% of its absorbance (Abs) and 52% of its photoluminescence (PL). Group two is composed of the least stable material exposed in medium after 672 hours: CN-PPV; PFO and F8BT. It is interesting to note that group two possess the lowest value of the Highest Occupied Molecular Orbital HOMO values (of -5.8 eV for PFO $^{[333]}$ -5.9 eV for F8BT $^{[336]}$ and -5.9 eV for CN-PPV) than group one (of -5.3 eV for SY-PPV -5.3 eV for MEH-PPV $^{[334]}$ and -4.8 eV for P3HT. This HOMO level corresponds to the energy necessary to remove electrons from their neutral atoms. However, PFO possess the largest value (-2.5 eV) $^{[335]}$ of Lowest Unoccupied Molecular Orbital (LUMO) energy level that corresponds to the released or spent amount of energy when the electron is added to a neutral molecule. It is then evident by comparing their energy levels that the materials in group two (PFO, F8BT and CN-PPV) may be easily ionized (oxidized) at their surface from bio-environmental contacts compared to other polymers studied. All organic semiconductor encapsulated with SU8 were more stable than the bare ones and this might be because the hydrophobicity and chemical inertness of SU8 protects the conjugated polymer against direct contact impurities.

We further investigated the different types of degradation that can occur during bio-treatments or operational uses with long-term immersion in media and photo-degradation. (Figure 11). Two well-known bio-functionalization treatments for cell culture growth were applied to the conjugated polymers in order to investigate the degradation of the optical properties by those treatments. The first one, named here BF1, used a coating of poly-L-Lysine, and horse serum on the surface of the polymers. In the second, named BF2, we coated the thin films with the protein laminin, a principal component of the brain’s extracellular matrix. Bio-functionalization (BF1 and BF2) seem to degrade the optical properties of all polymers within a range of 20% compared to non-functionalized (NO-BF) exposed in media for the same periods of time (Figure 11a).
Figure 11 | Variation of luminescence $F/F_o$ (a-d) and lifetime (e-h) for bio-functionalization (BF1 & BF2) compared to non-bio-functionalized (NO-BF) exposed in media for same period of time and control measurements (Ctrl) before BF1, BF2 AND NO-BF time (a.) and (e.), immersion in media (b.) and (f.), irradiation time at 0.1 mW/mm$^2$ (c.) and (g.) and intensity (d.) and (h.) on conjugated polymers in cell culture media. Full color lines for PF-types, dash lines for PPV-types and dot lines for PT-types.

However, this degradation is more marked during irradiation compared to soaking and bio-functionalization. During excitation, the dynamic quenching corresponds to collisional oxygen with fluorophores. This collisional quenching leads to different types of photochemical reaction with energy transfers previously discussed in the first paragraph of this section and in the chapter 1 introduction. Collision of atoms of oxygen with excited fluorophores will facilitate non-radiative transition to the ground. It is a diffusive process and marked by a linear decrease of the fluorescence intensity; life time and quantum yield over time. The first observation on irradiation time of the samples in Figure 11 shows a drastic decrease for the second group of materials (group two: CN-PPV; F8BT and PFO) within the first five hours at 0.1 mW/mm$^2$ power (Figure 11) while a more stable linear degradation for the first group of materials (group one: P3HT; SY-PPV and SO-PPV). The initial quantum yield measured of materials in group one (17 % for SY-PPV; 19 % for SO-PPV and 5 % for P3HT) is considerably lower than in group two (55 % for PFO; 50% for F8BT and 35 % for CN-PPV) and the first group is able to absorb more photons at their
respective peak of absorbance. Prolonged irradiation at the same optical power leads to a slow decrease of photoluminescence towards their extinction.

More information can be deduced from intensities of power of irradiation provided up to 5 mW/mm² (Figure 11d). Group one is still the most stable when exposed during two hours of irradiation at 0.1 mW/mm² with a decrease of photoluminescence of 9% for P3HT; 11% for SY-PPV; 22% for SO-PPV and 33% for MEH-PPV. Group two has a decrease of PL of 54% for F8BT; 58% for PFO and 61% for CN-PPV. The difference in degradation in group one and two can clearly be observed in Figure 11c at low optical power at 0.1mW/mm2. Below this irradiation range, material in group two seems to be saturated and their photoluminescence decreased at faster rates. The other notable difference occurring during photo excitation is that CN-PPV has a very long radiative average lifetime of 5.6 ns in comparison to all other materials under study (from 0.5 ns for P3HT to 1.7 ns for F8BT) and due to its long triplet lifetime state CN-PPV is likely to react with oxygen with triplet states of appropriate energies to allow for efficient energy transfer to ground state oxygen [336].

In view of the above, the degradation of the absorbance, photoluminescence, lifetime and quantum yield shows a static and dynamic quenching behavior occurring during the ageing of the material. It can be explained by the bio-environment heterogeneous system that leads to decrease of amplitude of the average lifetime (*excited state) and accounts for the irreversible chemical process that may lose the mechanical properties of the conjugated polymers.

2.2.2 Chemical and morphological degradation of OSC

Photolysis causes direct chemical reaction (chain scission, cross linking, oxidation) by absorbing photons [337]. It may initiate indirect formation of undesirable oxidation products. It is important to investigate the suitability of materials for their specific applications. Fourier transform infrared spectroscopy (FTIR) is a powerful tool to gain comprehensive information about chemical degradation of PPVs-types [338]; PF-types [339]; PT-types [324] which has been well covered in the literature in the case of exposure to oxygen. Here our primary aim is to study the effect of bio-environment on the chemical and morphological degradation of the conjugated polymers. The photo-bleaching of polymers (reported in section 2.2.1) leads to reduction of their conjugated length causing significant modification to the IR spectrum. The side-chain of a polymer can interact with some photo-products and impurities in the bio-environment influencing the formation of radical and the degree of the degradation of the polymer.
backbone \cite{340}. The Appendix A1 shows the carbonylic region (at 1600 – 1800 cm\(^{-1}\)) and C-H aliphatic region (at 3000 – 2750 cm\(^{-1}\)) absorbance bands appearing for all aged conjugated polymers soaked for two hours and for 672 hours in cell-culture media; a result that is accentuated when the samples are irradiated for two hours. The phototoxicity in certain buffer is well known \cite{341,342} Here we used a media free from phenol-red to avoid the phototoxicity effects, and in order to only focus on understanding the phototoxic coming from light emitting polymer investigated.

First of all, the carbonylic structure absorbance peak increases, forming aromatics at 1600–1700 cm\(^{-1}\), with a strong oxidation of quinoid structures and aliphatic ketones forming at 1700 cm\(^{-1}\). The general increase observed at this region is due to the fixing of oxygen molecules and leads to the main chain oxidation. The appearance of new bands at the C-H aliphatic region from 3000 to 2750 cm\(^{-1}\) was observed for all aged conjugated polymers soaked for 672 hours and the bands increased are accentuated after irradiations for two hours. The bands refer to hydroperoxides; alcohols and carboxylic acids formation \cite{343}.

The role of singlet oxygen in the photo-oxidation process has been much described in the literature as a mechanism of conjugated polymer degradation \cite{344-346}. Singlet oxygen is extremely reactive and can easily oxidize the excited state of the fluorophores and quench them by energy transfer with activation of excited singlet-triplet states and formation of singlet oxygen which is the precursor of photochemical reactions. This singlet oxygen can react with double bonds and produce unstable end-peroxide, leading to the formation of aromatic aldehydes and chain scission. Those consecutive processes may initiate the formation of other radicals. Other alternative mechanisms relying on light-induced oxidation degradation with the fixing of oxygen leading to hydrogen abstraction and double bond saturation may explain our findings \cite{344,347}. This leads to the formation of primary hydroperoxides that are photo and thermo-unstable which give the saturated carboxylic acid here observed at 1732 cm\(^{-1}\). These consecutive processes have been described in the literature as propagating the oxidation onto the main chain to form alkoxy radicals and hydroxyl radicals \cite{348,349}. Double bond saturation via hydrogen abstraction may form alcohol (shown with star symbols in Appendix A1 at 3460 cm\(^{-1}\) cm\(^{-1}\)); and cage reaction forming aromatic ketone (shown with triangles in Appendix A1 at 1680 cm\(^{-1}\)). The alkoxy radicals and hydroxyl radicals may directly add on the double bonds leading to its saturation and the formation of new type of radicals e.g. peroxy radical; hydroperoxide followed by macroalkoxy radical and hydroxyl radical \cite{350,351}. Other process can occur without oxygen and involve rearrangement (photo-fries norrish process; chain scissions and cross-linking) with direct absorption of light by chromophores of the aromatic polymers that engage various reactions.
Different types of morphological degradation of the conjugated polymers were observed under the microscope and SEM spectroscopy.

**Figure 12** | Morphological changes by bio-functionalization and under prolonged soakage in bio-environments. (a.) micrograph images, (e.) SEM image of morphological changes after laminin biofunctionalization BF-1. (d.) micrograph images, (f.) (g.) and (h.) SEM image of morphological changes after 672 hrs immersion of SY-PPV in media. (b.) micrograph images before immersion (0 hrs.) for SY-PPV in media. (b.) micrograph images after 24hrs immersion for SY-PPV in media. Scale bars denote 50 μm for micrograph image and 5 μm for SEM image.

We can see in **Figure 12a** micrograph image, before and after bio-functionalization of SY-PPV that there is clearly a change of appearance in the bio-functionalized (BF2) area compared to the non-bio-functionalized (no-BF) area. By looking at the SEM image **Figure 12e**, it is clear that a porous structure has developed (see **Figure 12b**) that may aid growth of cells by a reduction of hydrophobicity from 90° before to 35° after (laminin) BF-1 treatment. The **Figure 12d** shows the degradation of SY-PPV immersed in cell culture media for 672hrs. The polymer film developed distorted folds and detachments from the glass, suggesting a need for sticking layers or cross-linkability to improve the morphological stability. A closer observation of **Figure 12f** shows a three-dimensional porous structure with dense degradation of the initially amorphous material (**Figure 12b**), other areas of same sample show to have irregular nano fiber composite forming (**Figure 12g**), with some cracks and with powder forms we believed to be part of the polymer degraded in **figure 12h**. We show for example for SY-PPV the morphological changes that it can be

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observed that other light emitting polymers have similar change of appearance after long period of immersion. We also observed the formation of spherical agglomerate salt crystal deposited on the surface from non-well rinsed samples which is simply an artifact of process and some dispersion of dendrite due to the crystallization of salt after removing the material from the media.

For the purpose of understanding the application of solution processing phosphorescent film, the PVK as host blended with the metallo-organic phosphorescent dopant Firpic were shown not to be destroyed at 24 hours. However, the insoluble particles highlighted here with a black arrow, in Appendix A2, are assumed to be released from the host PVK and diffused toward the aqueous solution to accumulate at the surface. We then primarily focus on conjugated polymer to understand the interface interaction material/cells.

2.2.3 Electrochemical behavior upon light

In this section we aimed to explaining the photo-electrochemical behavior of light emitting conjugated polymers having different mechanisms of charge transfer at the interface with cell culture media.

The study of photo-electrochemical properties of conjugated polymers at the interface with cell culture leads to understanding the mechanism of charge transfer by the type of charged species generated that may have kinetic influences on cell responses. By using a three-electrode configuration, measurements were executed without any cells but using the cell culture media, silver chloride Ag/AgCl as a reference electrode and platinum as a counter-electrode. We measured cyclic voltammetry (CV), chrono-voltammetry (at open circuit potential) and chrono-amperometry (at open circuit potential) in darkness as well as irradiated to mimic how the conjugated polymer would behave in direct contact with neurons.

At negative potential, in darkness (CV in black Figure 13a), the electrons are injected into the film creating a negative current that leads to the reduction of the polymer. A high electron uptake occurs at the interface of the bulk polymer with the cell culture media. The cyclic voltammetry (CV in colors Figure 13a) measured under irradiation at 0.1 mW/mm² possess at negative potential a stronger reduction of all the materials with a large generation of negative photocurrent for the conjugated polymers under study. However, at positive potential (the oxidation state) in darkness, holes are injected into the film, leading to a positive current (Figure 13a). The irradiation of the surface of the polymer creates the appearance of very large positive waves of photocurrent (for SY-PPV; SO-PPV; MEH-PPV and PFO) at positive potential, while P3HT, F8BT and CN-PPV obtain weaker positive photocurrent.
Figure 13 | Photo-electrochemical properties of the light emitting polymers on ITO electrodes (pixel size 75 mm$^2$) immersed in cell culture media: (a.) Cyclic voltammetry (CV) in darkness (black) and irradiated (color) and with the black arrow indicating the CV for CN-PPV and CN-PPV-DPD at light; (b.) chronovoltammetry at open circuit potential (OCP) with the darkness time (black arrow) and the irradiated time (red arrow). (c.); (e.) and (f.) circles and (d.) bars empty at 0.1 mW/mm$^2$ and fully colored circles at 5 mW/mm$^2$. The black arrow represents the shift direction of the OCP under irradiation for (c.) Chronoamperometry (at open circuit potential) and (d.) Open circuit potential OCP shift values. (e.) The time rise on to attain saturation upon irradiation and (f.) the time rise off to recover to the dark state electrochemical behavior.

In order to understand the behavior of a material, without bias applied (to mimic biological conditions) we investigated the photo-current and potential shifts at open-circuit potential (equilibrium potential). The degree of neutrality of organic semiconductors may influence the alignment of energy at the interface with
cell culture media. There is a noticeable difference of direction of the photo-current (Figure 13c) and photo-voltage (Figure 13b and 13d) due to the conductive nature of the conjugated polymers at open circuit potential. For example, at open circuit potential, under illumination, we can observe that SY-PPV, PFO; SO-PPV; MEH-PPV; P3HT and F8BT; generate negative density of photo current of -0.31e-2, -2.9e-2, -0.12e-2, -0.84e-3, -0.41e-4 and -0.36e3 mA/cm² at saturation, while CN-PPV generates positive photocurrent of +0.2e-3 mA/cm² at saturation for an irradiation of 0.1 mW/mm². The photocurrent intensifies in the same direction at higher power (5 mW/mm²) and we can note that SO-PPV outperforming PFO with its negative photocurrent generated increasing of -0.37e-2 mA/cm² compared to an increase of -0.13e-2 mA/cm² for PFO. It is due to the fact that SO-PPV from first group of materials is able to absorb more photons than PFO from second group of materials at their peak of absorbance.

However, the addition of DPD unit on CN-PPV-DPD shifts the direction of photocurrent to positive at open-circuit potential. CN-PPV-DPD is naturally (in contact with cell culture media) at the limit between producing a negative and a positive photocurrent under light. The change of direction of potential is attributed to a preferential exchange type of carrier occurring at a redox state of the polymer.

Furthermore, the rise time of the shift of open circuit potential under irradiation has been recorded in order to get more understanding about the kinetic responses of charge transfer with cells. The black arrows in Figure 13b show, upon illumination of light intensity of 0.1 mW/mm², a sharp increase of potential until achieving a plateau of saturation. Fast rise time (~10s) occurs for PFO, CN-PPV and CN-PPV-DPD but very slow rise time for F8BT (~500s) and P3HT (~200s). While having a rapid time rise, CN-PPV and CN-PPV-DPD seems to recover very rapidly (~10s). The rise time on and off seems to be intensified at higher optical power (See Figure 13e and 13f). PFO has a very sharp shift of potential with an increase followed by a recovery when the irradiation is stopped, while F8BT has a very slow process of increase to saturation or recovery. (Figure 13e and 13f). This observable difference in kinetic might play an important role in photo-induced cell responses. Longer irradiation time on CN-PPV was observed to leads to an irreversible increase of photocurrent. The positive/negative photocurrent indicates that there is a directionality charge separation and transfer of electron/holes in the electrolyte and these may strongly influence potential behavior of cellular membrane with irreversible consequences. We know the material to degrade and may produce radicals in darkness and under irradiation. We observed electrochemical, optical, chemical and mechanical changes and they might influence the interaction with cells.
2.3 Interface with cells

2.3.1 Biocompatibility in darkness

**Figure 14 | Patch Clamp set-up with cells grown on organic semiconductors**

First a simple adhesion assay of HEK293T cell (The human embryonic kidney 293 cell line with T antigen for transfection) on spin-coated thin films of light emitting polymers was carried out (Figure 14). As expected from their chemical structures, F8BT and PFO with their extended aliphatic side-chains and inherently hydrophobic character, showed the lowest adhesion levels (Figure 15a, untreated), measured at 24 hours after cell plating. The other polymers, namely CN-PPV and MEH-PPV, displayed stronger cell adhesion levels on their surfaces.

**Figure 15 | Darkness biocompatibility of HEK293T cell grown on semiconducting polymers.** (a.) Representative image (Scale bars denote 50 μm.) of HEK293-T cell grown on bio-functionalized F8BT and the inset representative image (Scale bars denote 50 μm.) non-biofunctionalized F8BT. (b.) The resting membrane potential RMP of the HEK293-T cell grown on SY-PPV; F8BT; PFO; SO-PPV; MEH-PPV and CN-PPV coated on glass with polystyrene on glass as control. (c.) the %Area covered of the HEK293T cell grown on untreated and laminin bio-functionalized SY-PPV; F8BT; PFO; SO-PPV; MEH-PPV and CN-PPV.
We next sought to enhance the adhesion performance of the materials by surface modification. To this end, we bio-functionalized the polymers with BF2 described above in Section 2.2.2 by coating the thin films with the protein laminin, a principal component of a brain’s extracellular matrix. An overnight incubation at 10 M/μ dramatically and significantly increased adhesion of all the investigated conjugated polymers (Figure 15c); (uncoated versus laminin coated, p =0.03; 0.01;0.01; 0.02; 0.03;0.04 t-test two tailed, for F8BT, PFO, SO-PPV, SY-PPV, MEH-PPV, and CN-PPV respectively). While promising, the adhesion capability of (HEK293T) is not a reliable indication of cell viability. To assess more accurately the health of the cells we measured the resting membrane potential via patch clamp (Figure 15).

![Figure 15](image_url)

**Figure 15** | ** Darkness biocompatibility of neurons grown on semiconducting polymers.** (a.) Representative image (Scale bars denote 50 μm.) of neuron grown on bio-functionalized F8BT acquired at two-week (14DIV); (b.) Bar charts showing the membrane resistance; (c.) the capacitance and (d.) the resting membrane potential RMP for neuron grown on SY-PPV; F8BT; PFO; SO-PPV; MEH-PPV and CN-PPV coated on glass with uncoated glass as control. Number of cell patched N= 14 for each substrate material.
This revealed that following surface modification by laminin, cells were unperturbed by the presence of the light emitting materials and comparable to those plated on control polystyrene substrates (Figure 16b; p = 0.9; one-way ANOVA).

To further assess the biocompatibility of the light emitting materials, we moved to more sensitive primary hippocampal neuron cultures obtained from Sprague Dawley rats (Figure 16a) Following the bio-functionalization procedure BF1 described above with the coating of the polymer by poly-L-Lysine, and horse serum, neurons were plated and allowed to develop for 12 days. From 12-14 days in vitro (DIV), we selectively patched pyramidal neurons (see example Figure 16, N= 11, 10, 9, 8, 9 & 8, for glass, CN, MEH-PPV, F8BT, SO-PPV, SY-PPV, and PFO respectively) and assessed primary membrane properties. No difference was found for any of the materials investigated in terms of cell resting membrane potential (Figure 16d; p = 0.97; one-way ANOVA), cell capacitance Figure 16c; p = 0.95; one-way ANOVA), or cell membrane resistance (Figure 16b p = 0.96; one-way ANOVA).

2.3.2 Suitability of materials interaction with cells upon photo-excitation

Various types of conjugated polymers possess different chemical, optical and electrical properties that may lead to different effects of photo-stimulation on cellular membrane.

![Patch Clamp set-up with cells grown on OSC for photo-induces cell response recording](image)

We have previously shown that for HEK293T cells plated onto PT-type (P3HT) thin films, a light stimulus causes a biphasic effect on the membrane potential \[^{302}\]. The response is characterized by an initial depolarization which is followed by a sustained membrane hyperpolarization and was attributed to photothermal stimulation \[^{303}\]. This multiphasic response is fully attributable to thermal stimulation following non-radiative recombination of photo-excited electrons.
Figure 18 | Plurality of response Types of cellular membrane potentials of HEK293T following photon absorption in adjacent semiconducting Polymers. (a. and c.) Group one (SY-PPV (14mW/mm2 in yellow); SO-PPV(14mW/mm2 in orange); P3HT(14mW/mm2 in brown) and MEH-PPV(21mW/mm2 in purple) and, 8.65mW/mm2 in gray and 2.3mW/mm2 in black, for all of them and (b. and d.) group two (PFO (9 mW/mm2 in blue); F8BT (in green); CN-PPV (in green) and CN-PPV-DPD (in green) at 14mW/mm2 and, 8.65mW/mm2 in gray and 2.3mW/mm2 in black for all of them and (a. and b.) in response of short illumination of 500 ms (show in mV) and (c. and d.) prolonged illumination of 1 min (shown normalized, where 0 = the resting membrane potential, and 1 = 0mV)

However, based on the degradation study of conjugated polymers, which are exposed to cell culture media and irradiated, the FTIR results show the presence of directly or downstream generated radical
species on various conjugated polymers including P3HT. However, based on photo-electrochemical characteristics, the nature of oxidation states of polymer in direct contact with bio-environment influence the direction of charges. Therefore, it is important to understand if the formation of these radicals and the directional photocurrent influences the response of cells. The effect of photostimulation of the active substrate on HEK293T cells was assessed by measuring the light-induced changes in the plasma membrane potential with a standard patch-clamp setup. (Fig 17) Whole-cell recordings were carried out in current-clamp (I = 0) configuration. The conjugated polymers were excited at their respective peak of absorbance. Figure 18 shows the mean cellular responses recorded for each respective material at different light intensities (each trace represents a minimum of three recorded cells). The top row in Figure 18a represents the responses to a short illumination of 500ms. It is readily evident that two broad types of responses are observed in response to 500ms of illumination; Type one consists of reversible multiphasic stimulations [302] have previously ascribed to be a photo-thermal effects for material group one at short illumination. SY-PPV, P3HT, SO-PPV and MEH-PPV all present this response. Type two consists of irreversible depolarization which extends beyond the light period, and is obtained on materials of group two CN-PPV, F8BT, and PFO.

For types one and two, the amplitude of the effects intensifies at higher light irradiation (see power values in Figure 18 captions). For type one, this may be due to an increasing thermal signal as the light intensity increases, whereas in type two, the observation may be understood by an increased rate of reactive radical species generation consequent to intersystem crossing energy transfer pathways. In response to prolonged illumination, one minute (Figure 18c and 18d), the nature of the responses for materials in group one, MEH-PPV, SY-PPV, SO-PPV and P3HT changes dramatically. A characteristic depolarization occurring after the short first millisecond of illumination that is followed by a characteristic hyperpolarization that is attributed to thermal stimulation is then followed by the membrane potentials reverting again towards a prolonged and sustained irreversible depolarization. Such effects are particularly pronounced at prolonged high irradiation powers (Figure 18c, responses in colors). Cells recorded on materials relating to group 2 instead show sustained depolarization at any irradiation power, which rapidly reach equilibrium (0 mV).

For the purpose of understanding the application of solution processing phosphorescent organic LEDs, the active layer based with PVK as the host polymer blended with metallo-organic phosphorescent dopant were shown in Appendix A3, to respond with different interface interaction material/cells during irradiation.
2.3.3 Changes in cellular membrane integrity upon photo-excitation.

We further investigate MEH-PPV to represent type one (with reversible responses at low power of irradiation and slow irreversible depolarization at high power) and F8BT to represent type two of responses (with rapid and irreversible depolarization). We can see in the images Figure 19 a clear change of cell morphology in cells grown on F8BT that increase of size indicating that they are swelling during and after light irradiation at 0.02 mW/mm². However, no change of morphology observed before, during and after high intensity of light of 20 mW/mm² for cells grown on MEH-PPV.

![Microscope images of HEK293T cells grown on F8BT and MEH-PPV. Before, during and after irradiation of 0.02 mW/mm² for F8BT and 20mW/mm² for MEH-PPV. Scale bars denote 50 μm.](image)

We recorded the membrane capacitance in darkness and illumination for the two different materials. Both materials were recorded under two different light illumination powers for MEH-PPV (type-one) 14 mW/mm² and 30 mW/mm² and for F8BT (type-two) at a low irradiation of 0.8 mW/mm² and at a high irradiation of 14mW/mm² during 10 seconds.
Figure 20 | Electrophysiological response types are characterized by differential contributions of reversible and non-reversible effects on membrane capacitance of HEK293T cells. (a. and c.) The capacitance of HEK293T cells grown on F8BT and MEH was measured before during and after light exposure. Whereas photo-excitation of MEH leads to a reversible increase of HEK293T membrane capacitance, which scales with increasing light intensity, photo-stimulation of F8BT leads to irreversible changes in capacitance. At low illumination power (0.8 mW/mm² in black), capacitance increases slightly, whereas increasing light intensity (14 mW/mm² in red) leads to a breakdown of membrane dielectric as reflected in the loss of capacitance. (b.) The temperature measured for type one materials at depolarization and hyperpolarization cell response. (c.) Cell response, depolarization for Fresh, old F8BT and fresh F8BT with NaN₃ conditions (as a scavenger of singlet oxygen generation).

As expected, cells recorded on MEH-PPV show a reversible modulation of their membrane capacitance (in red Figure 20a at 14 mW/mm²), in correspondence with our previous work on photothermal stimulations of HEK293T cells on P3HT. The amplitude of this modulation of cellular capacitance increases, but remains reversible, as the light intensifies (in blue Figure 20a at 30mW/mm²), and thereby, the heat stimulus is increased (Figure 20b). In contrast, the capacitance of cells recorded on F8BT show increases at
low illumination power (in black Figure 20c at 0.8mW/mm²), which do not relax back to baseline upon light offset. At higher intensity, the capacitance instead sharply drops during illumination (in red Figure 20b). These effects could be interpreted on the basis of a lipid peroxidation mechanism [351,352]; at low illumination, chemical oxidation of membrane lipids leads to the formation of small holes, or membrane ‘pores’, and ions to be exchanged freely. This could result in cellular swelling and therefore an increased membrane surface area, and in increased capacitance. At higher powers, the amount of pores of the cellular membrane leads to a full breakdown of the membrane dielectric, resulting in the loss of capacitance, and an equilibrium (0 mV) for the ions recorded across a membrane with extreme low ionic resistance generated by transmembrane chemical poration.

We further included three different controls: sodium azide as scavenger (trap) of singlet oxygen generation; a sample F8BT aged and extensively quenched (> 6 months exposition in air) to yield reduced photochemical rates and a protective layer SU8 coated on the conjugated polymers (Figure 20c). While the response to light is fully blocked on cells plated on SU8, the aged samples, and the recording carried out under NaN₃ perfusion, both show decreased rates of the effect. The response increased for cell grown on F8BT in the presence of sodium azide; when aged and extensively quenched and when having SU8 coated on top.

The robust irreversible depolarization suggests the possibility to apply cellular response type two for controlling the excitation of electrically active cell types, or neurons. The depolarization may drive a cell to firing threshold. To investigate this further, we exposed neurons cultured on F8BT and PFO to different light intensities (for PFO (Figure 21b) and F8BT (Figure 21C) consecutive light pulses of 1 Mw/mm², 2.8 mW/mm² and 6.18 mW/mm² for 500 ms each at their respective peak of absorption.

As can be seen in the example traces, single pulses of light intensities 1 mW/mm² effectively depolarize neurons on PFO. However, more energy is required to drive neurons on F8BT to their firing potential. The photo-electrochemistry kinetics shows in Section 2.2.3 that PFO has a very sharp shift of potential when irradiation is stopped, while F8BT has a very slow process of increase to saturation or recovery. Therefore, this observable difference in kinetic in photo-electrochemical behavior of polymers may predict important information on photo-induced cell firing responses.

Light stimulation has no effect on the neurons grown on glass. While the experiments show that the electrical excitability of neurons can be controlled by the response type 2 eliciting materials as F8BT and PFO, the temporal precision of such a control seems limited, both in its onset, and offset kinetics, thereby
limiting applicability. Furthermore, and more importantly, the effects did not reverse during the observed time. Along with the observation of damage both by imaging (Figure 19), and capacitance (Figure 20) measures, it seems such platforms are more suited for targeted ablation applications.

Figure 21 | Electrophysiological response types for neurons grown on (a.) glass, (b.) PFO and (c.) F8BT.

2.4 Generation of free radicals and scavenging the cytotoxic effect

The radical oxidative stress production was measured upon irradiation of the films at 1 mW/mm² for 10 minutes. Singlet oxygen sensor green reagent (excited at 504 nm and emitting at 525 nm in presence of singlet oxygen); ROS sensors (excited at 490 nm and emitting at 525 nm in presence of radicals) and superoxide detection (excited at 550 nm and emitting at 620 nm in presence of superoxide) were able to confirm the oxidative species formation as causing cellular membrane damage.

Singlet oxygen and a radical oxidative sensor ROS formation under illumination of PFO, CN-PPV, CN-PPV-DPD, SO-PPV, P3HT and MEH-PPV films were studied but it was impossible to investigate F8BT and SY-PPV because of their photoluminescence spectrum matching with the emission of the sensor of these radicals. However, it was possible to use superoxide reagent in order to detect the formation of superoxide. Films of PFO, CN-PPV, CN-PPV-DPD, SO-PPV, P3HT and MEH-PPV were able to produce singlet oxygen and ROS with PFO and CN-PPV producing the highest amount, (Figure 22) with about 40% to 50% of increase of photoluminescence of singlet oxygen reagent and 25% to 40% of increase of photoluminescence of the ROS detection at 1 mW/mm² for 10 minutes. It is interesting to note a lower production for CN-PPV-DPD with about 20% of increase of photoluminescence of singlet oxygen and 20% of increase of photoluminescence.
of ROS at 1 mW/mm\(^2\) for 10 minutes. F8BT and SY-PPV films produced a weak superoxide sensor signal. (Figure 22) It seems that the F8BT was able to produce a greater amount of accumulated radicals (Figure 22b compared to Figure 22e) and this larger intensity confirms the ease of oxidation of F8BT compared to the highly stable SY-PPV.

**Figure 22** | Detection of singlet oxygen, superoxides and other oxidative stress on the different organic conjugated polymers. For single oxygen detection using bare material and with SU8 and uses of scavenger of radicals

For PFO, CN-PPV, CN-PPV-DPD, the addition of sodium azide (NaN\(_3\)) scavenger into the cell culture media provided weaker detection with about 18% to 20% of increase of photoluminescence of singlet oxygen reagent in the presence of the scavenger compared to 40-50% increase of photoluminescence of singlet oxygen reagent at 1 mW/mm\(^2\) for 10 minutes.

For all conjugated polymers protected with SU8 resin and at contact with the cell culture media, none of the radicals under detection were observable. It shows the efficacy of such dielectric polymer to protect organic semiconductors and any organic devices.
We were further interested in investigating what implications there would be in such photo-stimulation in conjugated nanoparticles which are frequently exploited for imaging and photo-stimulation applications both in thin film form and in as nanoparticles.

2.5 Discussion.

In this chapter, a protocol that investigates the suitability of organic light-emitting semiconductors for their application in bioelectronics has been developed. Specifically, the long-term optical and morphological degradation of conjugated light emitting polymers was investigated under bio-functionalization (BF2 & BF2), immersion in cell culture media, irradiation time and various light intensities. The interaction of the materials with cell responses was intensively studied in darkness and under irradiation. It was found that the irradiation of type-II: F8BT, CN-PPV and PFO with cells grown on top evokes a specific irreversible depolarization of cells at low optical power of exposure.

However, similar irreversible depolarization was observed only for type-I: P3HT, SY-PPV, SO-PPV and MEH-PPV at longer exposure while having reversible depolarization at short pulse of high optical power of irradiation. Those type-I materials investigated here are safe to be used with cells at low and short optical power. Further research is necessary for progress in synthesizing new types of materials providing safer and lower photo-inducing electrochemical cellular responses while keeping attractive optical and electrochemical properties for their uses in organic LEDs. The use of such light emitting materials in organic LEDs for applications in bioelectronics raises concern for long term use in vivo where defective devices may lead to such active material being in direct contact with cells; the prospect of multi-stack materials of organic LEDs with morphological instabilities due to stress caused by motion or chemical degradation in the bioenvironment may lead to exposure of active layers in contact with cells. Morphological and electrochemical stability and biocompatibility of materials in darkness and under light exposure is required to be suitable for applying organic LEDs in neuro-optoelectronics. The solutions to this problem may be using adhesive material with safe electrochemical properties or adding anti-oxidant in the culture media and/or blended with materials or operating in anoxic condition may protect against strong radical formation harmful for both devices and cells.

Photovoltaic retinal prosthetics applications using type-I of conjugated polymer P3HT, SY-PPV, MEH-PPV and SO-PPV may have little effect on cells at low powers, but it does not mean that radicals are not being generated at lower volumes and that those radical species are not likely to act as secondary
messengers in cell pathway communications. In fact, it is important to avoid high optical input to avoid further damage to patients’ eyes. While it is not the focus of this thesis it seems from initial experiments that the nanoparticles of type-II OSCs (CN pure, CN-PEG-PLGA, F8BT-PEG-PLGA) are reactive at the cellular membrane, leading to depolarization and swelling of the cell under illumination. The depolarization is evident in HEK293T (see Appendix A9), N2A (see Appendix A8), and neurons (see Appendix A9). In all cases the stimulation does not reverse in the recorded time window. From the HEK293T we can see that the amplitude of the depolarization is dependent on both light intensity and on nanoparticle concentrations. In neurons, stimulation leads to intense action potential firing. The amplitude of the action potentials decreases over the illumination period, before firing of the cell is silenced. This is also reflected over entire neural networks. While cellular swelling was observed by the patch clamp operator during recordings, a more direct evidence of this is presented in subpanel F of electrophysiology Appendix A9. Appendix A10 shows Multi Electrode Arrays (MEAs) recording before and after the illumination of neuron culture incubated with CN-PPV nanoparticles. A large amount of silencing of neural activity after illumination (shown by absence of spiking activity in each respective recorded channel) can be observed. The fact that all three cells types, N2a cell, HEK293T cell, and neurons, display similar electrophysiological responses, suggests that the mechanism is widely generalizable across cell types, indicating that the observed phenomena are likely mediated in large part by modulation of fundamental electrical properties of the cell membrane (i.e. like lipid peroxidation, which is known to generate ion and water conducting pores in plasma membrane). Lastly, while for some applications nanoparticles should remain intact over long periods of time (such as neural prosthetic applications), for live cell imaging, where long term and stable operation of a neural interface system is desired. In other cases, such as photodynamic therapy, the general conception is that the nanoparticles should be biodegradable (e.g. once the photoexcitation task is carried out and the nanoparticle is no longer needed in the human body, the cell, organ, and body containing the nanoparticle should ideally be able to process and expel it). As such, an investigation of nanoparticle stability can reveal some clues on degradability of the nanoparticles in aqueous systems.

2.6. Methods

Absorption Spectra. Absorption spectra in the range 400 nm to 1200 nm were measured with a Shimadzu UV-3101 NIR-Vis-NUV spectrophotometer. The samples in cell culture medium were washed by rapid immersion in DI water prior to measurement.
**Emission Spectra.** Emission spectra in the range 400 nm to 1200 nm were measured with a FLS980 photoluminescence spectrophotometer. The samples in cell culture medium were washed by rapid immersion in DI water prior to measurement.

**Fluorescence lifetime.** Time-resolved PL lifetime was performed with a TCSPC module SPC830 (Becker & Hickl, Germany). The module was synchronized to a Ti-Sapphire pulsed laser source (680–1080 nm, 80 MHz, 140 fs, Chameleon Vision II, Coherent Inc., Germany). Data were analyzed using a three-exponential decay and the goodness of fit ($\chi^2$) values provided the corresponding lifetime significance.

**Imaging.** Optical images in reflection and transmission mode were obtained using an optical microscope (Olympus BX51) fitted with a digital 9 MP camera (Conrad DP-M17). Unpolarised white light illumination was provided by a white light-emitting diode (Thorlabs LIU-PS). Scanning electron microscopy (SEM) micrograph have been acquired using the Tescan, Vega II.

**FTIR Spectra.** The vibrational modes in the light emitting polymers are recorded by FT-IR 6700 Nicolet™ FT-IR spectrometer from Thermo Electron Corporation.

**Measurement of ROS content.** All products were used when received. Tawed at room temperature, gently hand-mixed and briefly centrifuged. The oxidative stress detection reagent was reconstituted in 60 µL anhydrous DMF (yield 5 mM stock solution). The superoxide detection reagent was reconstituted in 60 µL anhydrous DMF (yielding 5 mM stock solution). Singlet oxygen sensor reagent stock solution prepared in methanol (100 µg in 33 µL of methanol yielding 5mM). Film or nanoparticles of organic semiconductors were immersed in Di-water in presence or absence of the reagents. The samples were exposed to irradiation of light followed by photoluminescence spectrometry measure in order to observe reagent photoluminescence.

**Electrophysiology.** Whole-cell patch-clamp recordings of cultured neurons were performed at room temperature using patch pipettes (4–8 M Ω), after attaining GΩ patch seals. Unless otherwise specified, traces were acquired in current-clamp mode using HEKA EPC10 amplifier and digitizer, and Patchmaster software (HEKA). The extracellular solution contained NaCl (135 mM), KCl (5.4 mM), MgCl2 (1 mM), CaCl2 (1.8 mM), HEPES (5 mM) and glucose (10 mM), and was adjusted to pH 7.4 with NaOH. The intracellular solution contained K-gluconate (126 mM), KCl (4 mM), MgSO4 (1 mM), CaCl2 (0.02 mM), BAPTA (0.1 mM), glucose (15 mM), HEPES (5 mM), ATP (3 mM) and GTP (0.1 mM), and was adjusted to pH 7.3 with KOH. Responses were amplified, low-pass-filtered at 10 kHz, digitized at 50 kHz, stored, and analyzed with
Matlab (Mathworks).

**Cell Cultures.** Human Embryo Kidney (HEK293T) cells, obtained from ATCC, were cultured in Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 10 % fetal calf serum, 2 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (Life Technologies). The samples thermally sterilized at 120 degrees Celsius, prior to overnight incubation in 0.1% PLL solution for HEK293T experiments. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO2. Primary cultures of hippocampal neurons were prepared from embryonic 18-day rat embryos (Charles River). Briefly, hippocampi were dissociated by a 15-min incubation with 0.25 % trypsin at 37 °C and cells were plated on poly-L-lysine-coated substrates (0.1% PLL in Borax solution overnight) in Neurobasal supplemented with 2 mM L-glutamine, 2% B27, 100 μg/ml penicillin and 100 μg/ml streptomycin, and with 10 % horse serum (Life Technologies) in the first 4 h of plating.

**Irradiation procedure.** Illumination of HEK293T cells and neurons was carried out on a Nikon FN1 upright microscope (Nikon Instruments) by using Spectra X LED system (Lumencor) to target the respective absorption spectrum maxima of the targeted polymer via dichroic mirror and 16x water immersion objective. Power densities (mW/mm²) employed LED are described at accompanying graphs. LED employed had peak emission at 390nm (PFO, CN-PPV), 435 (CN-PPV-DPD, SY-PPV) 473nm (F8BT, SO-PPV), 548nm (P3HT, MEH-PPV) for thin film experiments. Unless otherwise stated, nanoparticles were targeted by 435nm excitation. Gate timing was controlled by digital outputs of HEKA patch clamp amplifier.

The photo-electrochemical characterization was performed using Dulbecco’s Modified Eagles Medium (DMEM). A cyclic voltammetry was performed at a sweep rate of 0.5 mV/s between -0.6 to 0.6 V, chronoamperemeter and chrono-voltammeter was performed at open-circuit potential during 600 seconds.

**Material for nanoparticles.** Phenyl alkoxyphenyl PPV copolymer conjugated polymers Livilux™ PDO-124 (SO-PPV) and Livilux™ PDY-132 (SY) were supplied by Merck, Germany. Conjugated polymers poly(2,5-di(hexyloxy)cyanoterephthalylidene) (CN-PPV) and poly(9,9-diocytfluorene-2,1,3-benzothiadiazole) (F8BT); and the diblock copolymer poly(ethylene glycol) methyl ether-block-poly (lactide-co-glycolide) with 50:50 ratio of lactide/glycolide (PEG5K-PLGA55K); tetrahydrofuran (THF ReagentPlus®, ≥99.0%, catalogue # 178810) were supplied by Sigma-Aldrich Corporation (St. Louis, MO, USA).

**Nanoparticle Manufacture.** CPNs were prepared by solvent displacement technique, with a ratio of 1:10...
(conjugated polymer:PEG5K-PLGA55K). A total of 4 mL of THF solution (0.9 mg mL⁻¹ polymers) was added drop wise to 20 mL of water at room temperature, which was stirred until complete THF evaporation. Formulations containing 100% PEG5K-PLGA55K and 100% CN-PPV were also prepared with same polymer concentration. The final product presented 0.2 mg mL⁻¹ total solids, which was concentrated to a minimum of 1.0 mg mL⁻¹ using Amicon® Ultra-15 100K centrifugal filter devices.

**Instrumentation.** Hydrodynamic diameters were assessed by dynamic light scattering (DLS) using a Zetasizer NanoZS (Malvern Instruments Ltd, UK) at 25°C with a scattering angle of 173° and 50 μg/mL final polymer concentration. The zeta potential at 25°C, after sample dilution in NaCl 10 mM at final polymer concentration of 20 μg/mL, was measured in a Zetasizer NanoZS (Malvern Instruments Ltd, UK). The product yield and spectroscopic characterization were performed according to previously described for similar nanoparticles^353^. Briefly, the absorbance spectra of the conjugated polymers in THF solution (concentration range 0.8–25.0 μg mL⁻¹) were acquired using a UV spectrometer (Lambda 35, Perkin Elmer Inc., USA). The PL spectrum of conjugated polymers dissolved in THF and nanoparticles diluted in water at 10.0 μg mL⁻¹ CN-PPV and 0.8 μg mL⁻¹ SO, SY and F8BT were measured in a luminescence spectrometer (LS50B, Perkin Elmer Inc., USA) with the settings: excitation at 442 nm (SY), 461 nm (SO), 460 nm (F8BT) and 460 (CN-PPV), emission slit width of 4 nm, excitation slit width of 5 nm and emission scan from 350 to 800 nm.
Chapter 3: Novel application of cross-linkable light emitting polymer in bio-electronics

This chapter highlights the performance of a cross-linkable light emitting polymer (LEP) in order to demonstrate its sustainable uses in diverse biological applications. The stability of the adhesion of light emitting cross-linked thin film bonded to cell culture glasses is evaluated for direct bio-platform interface with cell (e.g. photo-inducing cell therapy). The fabrication of freestanding light emitting polymer sheets using chlorobenzene is examined in order to evaluate their stability when exposed to such solvents and determine their suitability to adhere to glass and to demonstrate solution-based multilayer facility for organic LEDs.

3.1. Introduction

Organic light emitting polymers for bio-electronics are required to be biocompatible with an optical and morphological stability of operation in biological media. Cross-linking the material with improved adhesion to substrate or surrounded material answers the requirements for optimal morphological stability. It is equally important for direct and indirect contact with cells. The applications are various: sustainability of solid-state light emitting devices applied with an indirect contact with the bio-environment (e.g. Organic Light Emitting Diodes (OLEDs); Organic Light Emitting Transistor OLETs and Organic Light Electrochemical Cells (OLECs) for bio-sensor \[^{354}\] and optogenetics \[^{355,356}\]); the second type having a direct interface with the bio-environment (e.g. Photo-induced cell therapy \[^{357}\]; Photo stimulation of cells \[^{302,303}\]).

A useful cross-linkable material would facilitate the possibility to fabricate multi-layer structures and sustain morphologically robust layers of the material by itself without any need of a substrate. Traditional OLEDs need many layers to be efficient, for example an emissive layer sandwiched between a hole and an electron transporting/blocking material \[^{66,67}\]. However, there is a problem using solution processing as the applied solution may dissolve the layers beneath it \[^{358}\]. Several fabrication strategies in the literature have been proposed to overcome these issues. Solution-based multilayer devices based on the formation of stacks of films from similar solvents without any modification of the organic semi-conductor can be formed by blade coating \[^{359}\]; liquid buffer layer \[^{358}\] electrospray deposition \[^{360}\] transfer printing \[^{361}\] and lamination \[^{362}\]. A alternative solution is to use orthogonal solvent systems- the use of solvents unable to dissolve other layers like polar solvents \[^{362}\] fluorinated solvents \[^{362}\] and hybrid approaches \[^{362}\] but some restriction of designs structure may occur \[^{358}\].
However, processed layers may become insoluble by cross-linking the material by modifying the organic semiconductor by chemical reaction. The addition of reactive groups e.g. siloxanes, styrene, acrylates, trifluorovylethers, cinnamates, oxetanes allows chemical reaction with cross-linking to occur after solution processing. Adhesion promoters [363] and coupling agents [364] can be made from alkoxy silanes with the labile nature of the alkoxy group makes the silanes very reactive to pendant hydroxyl groups. The alkoxy groups will hydrolyse forming hydroxysilanes when reacting with water. They are useful as cross-linking agents [365] and moisture scavengers.

There is a challenge of incorporating such reactive groups onto the polymer backbone by introducing alkoxy silanes onto conjugated polymer systems. Controlling the size of Polyfluorenes with pendant triethoxysilanes which are light-emitting conjugated polymer nanoparticles [366] was possible to cross-link in basic condition. The alkoxysilane post-polymerisation incorporated into hydroxyl-terminated side chains enables the modification.

We proposed cross-linkable materials with adhesive properties as a solution for organic semiconductors for long-term in-vivo and in-vitro applications and more particularly cross-linked light emitting polymers offering morphologically robust and efficient electroluminescent organic LEDs. The improve adhesion to smart cell culture glass substrate is required for application in-vitro (e.g. photo-induced cell ablation [302,303,357], photo-induced stimulation [367] applications and light emitting bio-platform for optical imagery [368-372] and freestanding light emitting polymer for diverse in-vivo applications. The modification occurs in a very simple one-step process, post-polymerisation. The only requirement for the modification is the presence of the FBT unit, a popular monomer used in OLEDs, no modification of monomers prior to polymerisation is required. The trimethoxysilane group hydrolyses and self-condenses forming an insoluble cross-linked polymer (Appendix A11 scheme 1-b) without the need for additives, external stimuli or exposure to air. (See Appendix A11 for further details).

The process is greatly accelerated by the presence of heat (at 120°C) during cross-linkability and/or aqueous environment, making it suitable for cell-culture environment.

3.2. Biocompatibility of cross-linked polymer

Biocompatibility of the active layer under study is important for its suitable application in bioelectronics or biomedical devices. Indeed, the risk that any material becomes exposed due to corrosion or physical cracking of the overlying metal or insulating layer in organic LED devices means that they should ideally
remain physically isolated from the biological environment. To evaluate the potential effects of such exposure, we plated acutely prepared hippocampal neurons obtained from Sprague Dawley rats onto (i) bare glass—(ii) glass coated with 350 nm fully cross-linked p(F8-FBT) and (iii) glass coated non-modified p(F8-FBT). Samples for neuronal use were incubated for 24 hrs in culture medium at 37 °C, and again overnight in aqueous PLL [Poly-L-Lysine] coating solution at 37 °C. Samples were sterilized and washed with deionized (DI) water prior to cell plating.

Neurons grown directly on glass, as well as on glass coated with the two polymers, were investigated using Patch-clamp recordings [373]. We evaluated the functional state of the neurons by quantifying their resting trans-membrane potentials. (Fig. 23a) Neurons grown on PF8FBT-CL; PF8FBT-NON-CL and glass had similar resting membrane potentials (Figure 23a; p = 0.98; one-way ANOVA); cell capacitance Figure 26b; p = 0.92; one-way ANOVA) and cell membrane resistance (Figure 23.c; p = 0.95; one-way ANOVA).

![Image of neuron on bio-functionalized F8BT-CL](image)

**Figure 23 | Electrophysiological characterization of primary neurons on glass, cross-linked F8FBT-CL and non-crosslinked F8FBT test-substrates.** (a.) Representative image (Scale bars denote 50 μm.) of neuron grown on bio-functionalized F8BT-CL acquired at two-week (14DIV); (b.) Bar charts showing the resting membrane potential RMP; (c.) the capacitance and (d.) membrane resistance for neuron grown on glass, cross-linked F8FBT-CL and non-crosslinked F8FBT test-substrates. Number of cell patched N= 14 for each substrate material.

Given the similar behavior of neurons on bare glass and PF8FBT-CL and PF8FBT-non-CL coated on glass, the two polymers appear to be promising candidates for bio-applications.

### 3.3. Cross-linkable polymer for optical and morphological stability in medium.

The stability of F8FBT-CL in various media from corrosive cell-culture media to chlorobenzene was investigated to understand the efficacy of cross-linkability and its adhesiveness to glass. *In vitro* bioelectronics investigations are typically carried out in cell culturing media [303,374,375] and it is necessary for light emitting polymeric sheets to remain operational over many weeks. In order to determine the
suitability of cross-linkable material for use in biology, PF8FBT-CL and PF8FBT-non-CL coated on glass were immersed in cell culture medium [Dulbecco’s Modified Eagles Medium (DMEM)]. A second set, PF8FBT-CL and PF8FBT-non-CL coated on glass were immersed in chlorobenzene in order to evaluate their stability when exposed to such solvents during solution based multi-layer organic OLEDs.

<table>
<thead>
<tr>
<th>a.)</th>
<th>p(F8FBT) - Crosslinked</th>
<th>p(F8FBT) - Uncrosslinked</th>
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<tr>
<td><img src="image1" alt="Graph" /></td>
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**Figure 24 | Optical characterization of p(F8-FBT) cross-linked and p(F8-FBT) uncross-linked before(t=0) and after (t= 672 hrs) immersion in different aqueous media.** (a.) Plots of the absorption versus wavelength in orange dash lines and plots of the photoluminescence versus wavelength in red dash lines when exposed in cell culture medium and measure at 0 hrs; 24 hrs; 48 hrs; 168 hrs; 336 hrs and 672 hrs. Plots of the absorption versus wavelength in clear green dash lines and plots of the photoluminescence versus wavelength in dark green dash lines when exposed in cell culture medium and measure at 0 hrs; 2hrs; 48 hrs; 168 hrs and 336 hrs. (b.) and (c.) The micrograph image acquired at t = 0 (inset) and t = 672 hrs (main images), showing cracks in PF8FBT uncrosslinked and an intact film for PF8FBT crosslinked exposed to cell culture medium and the micrograph image acquired at t = 0 (inset) and t = 336 hrs (main images), showing PF8FBT and an partially intact film for PF8FBT crosslinked, and at t = 0 (inset) and t = 336 hrs (main images), showing PF8FBT uncrosslinked removed from surface at t = 2hrs (main images). Scale bars denote 50 μm for (c.) and 10 mm for (b.)
Each sample was removed periodically for analysis by absorbance; emission spectroscopy (Fig. 24a) and optical microscopy (Fig. 24 b-c). Initially (0 hrs), all samples appeared uniform under an optical microscope and the absorbance and photoluminescence of the cross-linked PF8FBT-CL material showed a red-shifted peak (Fig 26d) with a peak of emission of 534 nm for the un-crosslinked-PF8FBT and a peak of emission of 547 nm for the cross-linked PF8FBT. While the absorption spectra of the cross-linked samples (PF8FBT-CL) were unaffected by immersion in the culture medium after 672 hours, the other PF8FBT-non-CL showed ~40 % drop in absorbance and photoluminescence due to oxygen quenching. Non-crosslinked PF8FBT is too fragile to sustain significant mechanical stress or to endure changing environments due to a weak bond with glass. Clear differences appeared in both the optical micrographs (figure 24 b-c), after 672 hours of immersion, the non-cross-linked sample PF8FBT-non-CL developed sizeable cracks and distorted folds, detaching from the glass. Over the same period of immersion, the cross-linked sample F8FBT-CL remained optically stable with no signs of film damage (apart from the ionization of its surface occurring for all conjugated polymers previously reported in chapter 2).

In chlorobenzene immersion, we observed partial dissolution over time (fig 24c cross-linked polymer partially removed from glass substrate at 336 hrs compared to non-cross-linked polymer totally dissolved at 2 hrs). Thus, we have demonstrated that the cross-linked polymer has an extreme morphological robustness and strong adhesion of PF8FBT to substrate glass and would be suitable for sustainable smart cell-culture glass or other bio-platform applications using glass cell-culture substrates [368-372].

3.4. Fabrication of freestanding light-emitting polymeric sheets

Here, we report the synthesis, by spin coating, of cross-linked free-standing film with relevant high mechanical, high homogeneity on large scale, (from very thin to very thick) adjustable thickness, high flexibility and photoluminescence properties.

Poly(styrene-sulfonate) (PSS) has been selected as a water-soluble sacrificial layer to obtain self-standing light-emitting sheets as it can survive the high annealing temperature needed for crosslinking to occur. P(F8-FBT)-modified is soluble in chloroform which does not dissolve the sacrificial layer of PSS when processed. The wettability of the two different films is shown Appendix A12 with the contact angle of DI-water and chlorobenzene with PF8FBT-CL. It is advisable to create a thick layer of PSS in order to ensure that there is no point of contact between the glass and the cross-linkable material.
The fabrication of the freely suspended light-emitting sheets consists of five steps, with steps three and four being repeatable in order to control the thickness of the film. First, plasma was applied to the substrate and followed by spin-coating about 200 nm thick of PSS. Next, in the step three, the cross-linkable modified polymer P(F8-FBT) is spin-coated onto the PSS-coated substrate in a nitrogen environment to ensure non-cross-linkability before forming the layer. Then the film is exposed to air and annealed at 120-degree Celsius temperature for 15 min in order to cross-link it. It is then possible to repeat steps three and four to control the thickness of the targeted freely standing light-emitting sheet, by returning the film in a nitrogen environment after the post-crosslinking step in air and to apply a second layer on top. Then it is possible to progress to the detachment of the cross-linked polymer from the glass by immersing the substrate/PSS/Polymer-CL structure into ethanol to activate the dissolution of PSS and to obtain the light-emitting sheet ultimately floating freely due to its hydrophobicity. The edges of the substrate have to be abraded to facilitate its detachment and immersion in ethanol for dissolution of the PSS sacrificial layer. A slow flow of ethanol around the substrate aids to create the separation of the biofilm from the glass/PSS. Finally, a fresh ethanol rinse is applied to remove PSS excess.
The resulting light-emitting film is flexible (see figure 25b image of the light-emitting sheet bent over a human hair), adjustable thickness from very thin 5 Layers figure 25c to very thick (50 Layers) figure 25d (Check Appendix A12 for optical characteristics of the light-emitting sheet at different thicknesses).

3.5. Cross-linkable polymers for efficient high operating voltage organic LEDs.

Conventional OLEDs composed of an anode (ITO; PEDOT) and a cathode (Al; Ca) sandwiching the active emissive layer of cross-linked F8FBT-CL in devices 1 and non-cross-linked F8FBT-non-CL in devices 2 were fabricated.

Figure 26| OLED Devices with cross-linked and un-crosslinked F8FBT. (a.) Device structure; (b.) IVL curves; (c.) OLED pixels of 75 mm² at 10V of PF8FBT and PF8BT-CL; (d.) Electroluminescence and photoluminescence of PF8FBT and PF8BT-CL; (e.) brightness efficiency and external quantum efficiency of PF8FBT and PF8BT-CL and (f.) variation of the luminescence and voltage for PF8FBT and PF8BT-CL.

The electroluminescence spectrum of device 2 (peak 535.6 nm) was very similar to the photoluminescence spectrum of the F8FBT-non-CL (peak 534 nm)), while a red-shift was measured for the cross-linked F8FBT-CL with the electroluminescence spectrum of device 1 (peak 533 nm) and photoluminescence spectrum of the F8FBT-CL (peak 546.2 nm). (See figure 26d). Figure 26b showing similar density of current at lower voltage 10 V leading to similar brightness of 100000 cd/m² for both type of devices but a strong decrease in performance occurs for non-crosslinked polymer at higher voltage while
for similar voltage the cross-linked polymer is showing high brightness that is remaining sustainable over long time. In Figure 26f, at high voltage the brightness lifetime of device 2 (non-crosslinked PF8FBT) is highly degrading when operating at 20V (LT50% of 40min) and approaching extinction at only 70 min. Device 2 is showing much longer stability for same voltage (LT50% of about 200 min) and achieving 95% of degradation of the brightness at 10 hours. Pictures of pixels in Fig 26c show degradation of pixel brightness at high voltage 20V only for short-time while pixels of device 1 show limited degradation of brightness at similar voltage and for longer periods of time. In addition, a higher efficiency (33 cd/A) and external quantum efficiency EQE (5.8%) were obtained at 7 V for the cross-linked device 1 compared to an (8.3 cd/A) and an EQE of (2 %) for the un-cross-linked device 2.

This leads to the conclusion that cross-linkable light emitting polymers using F8FBT-CL have electroluminescent stability and can sustain a higher voltage of operation, while having higher electroluminescent efficiency at low voltages. This would be beneficial for applying organic LEDs for the optical stimulation of neurons requiring high optical power.

3.6. Suitable interface of cross-linkable PF8FBT with cells upon irradiation.

This is of importance to understand the cellular response when an organic LED is breached leading to direct contact of the light emitting polymer with the bioenvironment. We further introduced the possible use of nanoparticles of polymer for photo-induced cell ablation applications. As previously observed, in chapter 2, different cell types grown on various conjugated polymers induced rapid and irreversible depolarization of the cell membrane potential due to the formation of radicals when irradiated at their peak of absorbance.

We investigated if PF8FBT non-crosslinkable and cross-linked polymers would react similarly. As expected from similarity with chapter 2, for all films and nanoparticles synthesized, the membrane potential of the cells was irreversibly depolarizing upon 473nm illumination directed to the cells via a microscope objective. We further checked if the thickness of films would influence cell responses. Figure 27 show that for 10 Layers of cross-linked PF8FBT, a marginally earlier mean depolarization occurs compared to thinner (5 Layers). When recording the membrane potential of cells pre-incubated with nanoparticles, we observed that the rate of cellular depolarization is greatly decreased relative to film.
Targeted Cell ablation. Photo-induced depolarization of cell (a.) by different thickness of cross-linked polymer and (c.) in nanoparticle forms. (b.) membrane potential of HEK293T cell grown on 5 layers (in red) and 10-layers (in black) of PFEFBT-CL. (d.) membrane potential of HEK293T cell grown on PFEFBT-CL at irradiation 0.8 mW/mm$^2$ in red and 10 mW/mm$^2$ in black.

The decreased rate is likely to have been contributed to by surface area considerations; while the polymer in film form has a large surface area in extreme vicinity with the cells membrane attached on top, the nanoparticles which are freely moving in the extracellular medium do not fully cover the membrane area at the tested concentration of 30 µg/L.

3.7. Discussion.

In this chapter, we found that the modified cross-linkable polymer is stable in various media, from corrosive cell-culture media to chlorobenzene, enabling multi-layer structure fabrication and more specifically enabling the fabrication of a freestanding and morphologically stable light-emitting sheet. The freestanding light emitting polymeric sheet can be manipulated, folded and unfolded in water without cracking, disaggregation or loss of optical properties. Its ease of fabrication, applying multi-layer solution processing from chlorinated solvents, makes it possible to design fully crosslinked organic LEDs. It is biocompatible when its biocompatibility is investigated without the use of light. Although it is found to photo-induce depolarization of HEK cells, its robust bonding properties to glass and stability in various
media might enable the sterilization of the surface of targeted material as well as for the synthesis of natural products and drugs \cite{345} by the photo-produced singlet oxygen.

Furthermore, the enhanced cross-linked active layer was able to achieve efficient Organic Light Emitting Diodes with various applications for indirect contact with the bio-environment due to the use of protective encapsulation materials. These applications may have potential in the fields of sensing and actuation, as well as in the biomedical field, e.g. as smart cell culture substrates with OLED pixels incorporated for cell culturing imaging and stimulation. Still to be investigated are crosslinkable adhesive materials with high charge transports and injection properties (e.g. hole transport/injection materials replacing PEDOT:PSS and electron transport/injection material replacing Ca/Al). These crosslinkable adhesive materials must have strongly sticking properties to bond to crosslinked light emitting polymers, such as F8FBT-CL, and to anode and cathode electrodes in order to obtain fully crosslinked and morphologically robust organic LEDs.

3.8. Methods

**Preparation of PF8FBT-CL-coated glass.** Glass substrates were rinsed with deionized (DI) water, acetone and 2-propanol, and then dried. Next, in the step one, the cross-linkable modified polymer P(F8-FBT) is spin-coated onto glass in a nitrogen environment to ensure non-cross-linkability before forming the layer. Then the film is exposed to air and annealed at 120-degree C temperature for 15 min in order to cross-link it. It is then possible to repeat to control the thickness of the films, by returning the film in a nitrogen environment after the post-crosslinking step in air and to apply a second layer on top.

**Preparation of freely suspended light-emitting sheet:** (Poly(styrene-sulfonate) PSS was obtained from Aldrich. The fabrication of the freely suspended light-emitting sheets consists of five steps, with steps three and four being repeatable in order to control the thickness of the film. First, plasma was applied to the substrate and followed by spin-coating about 200 nm thick of PSS. Next, in the step three, the crosslinkable modified polymer P(F8-FBT) is spin-coated onto the PSS-coated substrate in a nitrogen environment to ensure non-cross-linkability before forming the layer. Then the film is exposed to air and annealed at 120 degree Celsius temperature for 15 min in order to cross-link it. It is then possible to repeat steps three and four to control the thickness of the targeted freely standing light-emitting sheet, by returning the film in a nitrogen environment after the post-crosslinking step in air and to apply a second layer on top. Then it is possible to proceed to the detachment of the cross-linked polymer from the glass by
immersing the substrate/PSS/Polymer-CL structure into ethanol to activate the dissolution of PSS and to obtain the light-emitting sheet ultimately floating freely due to its hydrophobicity.

**Fabrication of OLEDs.** Conventional solution processing organic light emitting diodes were fabricated on glass by coating indium tin oxide (ITO of 10 ohm/sq.) with a 30 nm layer of poly(3,4-ethylendioxythiophene : poly(styrenesulfonate) (PEDOT:PSS). Cleaning of the ITO slides; film coating and electrode deposition were carried out all the same day to ensure optimum results. The glass substrates coated with ITO were supplied by Xin Yan Technology Lmtd. The glasses were sonicated in standard acetone and then in soap and DI water for 20 mins, in DI water 3 times for 5 min, in acetone twice for 5 mins and in isopropanol (IPA) for 5 mins, followed by blow-drying of the substrates. The ITO substrates were treated by oxygen plasma treatment. The PEDOT: PSS layers were deposited by spin coating at a spin speed of 3500 rpm for 40 sec and then annealed at 150°C for 20min. Polymeric devices followed conventional PLED structure using PEDOT:PSS for the injection of holes from the ITO anode and Calcium for the injection of electron from the Aluminum cathode. The precursor solutions were prepared the day before device fabrication and the solutions were stirred at <50 °C overnight. The precursor solutions of fluorescent polymers (F8FBT-CL and F8FBT) were prepared by dissolving 6 mg material in 1mL Chlorobenzene and were spin-coated in nitrogen for 30 s at 1200 RPM on top of PEDOT:PSS and followed by annealing at 120 degree Celsius in air. Following active layers deposition, Calcium Ca (25nm) and aluminum Al (100 nm) top-electrodes were deposited onto all the devices by thermal evaporation through a shadow mask at 10-6 mbar. Active areas measured 75 mm². The encapsulation of the devices was effected manually using another glass substrate of 1.1 mm adhered onto the device using flexible sealants from DELO-KATIOBOND and a low power UV light to set the glue.

**Device characterization.** The electrical characteristics of the devices encapsulated were measured in air at room temperature using a Keithley 2450 electrometer for the electrical current-voltage measurement. Emission spectra were measured using a fiber-coupled Ocean Optics 2000+ spectrometer. Absolute optical power intensity was determined using a large area calibrated Si PIN Photodiode (28 x 28mm active area, from Hamamatsu) coupled to a Keithley 6517A electrometer. Lifetime tests were carried out by constant current sources and the voltage was recorded by a Keithley 2450 electrometer. The luminance was recorded using an OPT101P Photodetector Amplifier from Texas Instruments connected to the National Instruments NI PCI-5112 100MHz Digital Oscilloscope Board.
**Electrophysiology.** Whole-cell patch-clamp recordings of cultured neurons were performed at room temperature using patch pipettes (4–8 M Ω), after attaining GΩ patch seals. Unless otherwise specified, traces were acquired in current-clamp mode using HEKA EPC10 amplifier and digitizer, and Patchmaster software (HEKA). The extracellular solution contained NaCl (135 mM), KCl (5.4 mM), MgCl₂ (1 mM), CaCl₂ (1.8 mM), HEPES (5 mM) and glucose (10 mM), and was adjusted to pH 7.4 with NaOH. The intracellular solution contained K-gluconate (126 mM), KCl (4 mM), MgSO₄ (1 mM), CaCl₂ (0.02 mM), BAPTA (0.1 mM), glucose (15 mM), HEPES (5 mM), ATP (3 mM) and GTP (0.1 mM), and was adjusted to pH 7.3 with KOH. Responses were amplified, low-pass-filtered at 10 kHz, digitized at 20-50 kHz, stored, and analyzed with Matlab (Mathworks).

**Cell Cultures.** Human Embryo Kidney (HEK293T) cells, obtained from ATCC, were cultured in Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 10 % fetal calf serum, 2 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (Life Technologies). The samples were sterilized in 70 % ethanol, prior to overnight incubation in 0.1% PLL solution for HEK293T experiments. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Primary cultures of hippocampal neurons were prepared from embryonic 18-day rat embryos (Charles River). Briefly, hippocampi were dissociated by a 15-min incubation with 0.25 % trypsin at 37 °C and cells were plated on poly-L-lysine-coated substrates (0.1% PLL in Borax solution overnight) in Neurobasal supplemented with 2 mM L-glutamine, 2% B27, 100 μg/ml penicillin and 100 μg/ml streptomycin, and with 10 % horse serum (Life Technologies) in the first 4 h of plating. Data reported for biocompatibility were obtained from two neuronal culture preparations.

**Nanoparticle synthesis.** Nanoparticle synthesis is based on the nanoprecipitation method. Polymer was dissolved in THF to a concentration of 0.1 mg/mL. The solution was filtered through a PTFE filter (0.2 μM). The resulting solution (1mL) was rapidly injected into ultrapure water (9 mL), under sonication and left for 3 min. The nanoparticle suspension was bubbled with nitrogen for 3 hrs to remove THF.

**Photo stimulation of cell protocols:** Illumination of HEK293T cells was carried out on a Nikon FN1 upright microscope (Nikon Instruments) by using Spectra X LED system (Lumencor) to target the respective absorption spectrum maxima of the targeted polymer and nanoparticles via dichroic mirror and 16x water immersion objective. LED employed had peak emission 473nm. Gate timing was controlled by digital outputs of HEKA patch clamp amplifier.
Absorption Spectra. Absorption spectra in the range 400 nm to 1200 nm were measured with a Shimadzu UV-3101 NIR-Vis-NUV spectrophotometer. The samples in cell culture medium were washed by rapid immersion in DI water prior to measurement.

Emission Spectra. Emission spectra in the range 400 nm to 1200 nm were measured with a FLS980 photoluminescence spectrophotometer. The samples in cell culture medium were washed by rapid immersion in DI water prior to measurement.

Imaging. Optical images in reflection and transmission mode were obtained using an optical microscope (Olympus BX51) fitted with a digital 9 MP camera (Conrad DP-M17. Unpolarised white light illumination was provided by a white light-emitting diode (Thorlabs LIU-PS).
Chapter 4: Use of SU8 as a stable adhesion layer for gold bioelectrodes

This chapter highlights the performance of an adhesive layer (SU8) for gold electrode. The investigation of the stability of the electro-optical properties of several commonly used electrode materials for organic LEDs is shown in Appendix A14. The aim was to identify their advantages and drawbacks for use in solid/liquid devices, as well as the benefits of an adhesion layer for applications requiring long term aqueous immersion. It presents transmission and reflection mode optical micrographs of the SU-8 based samples after the one-month immersion in Dulbecco’s medium, while the small insets show the same image taken on the sample before the immersion. The defects are evident and predominantly on the edges of the samples, but present, although in a lower number, also in the middle. We found that HEK293T cells were biocompatible when grown on ITO, gold and Aluminum in Appendix A15.

4.1. Introduction

The development of bioelectrodes that are transparent, biocompatible and capable of extended, stable operation in a broad range of biological media is a critical challenge for the field of bioelectronics. Optoelectronic devices such as light-emitting diodes,[297,298,355,379,380] photodiodes,[132,133,381,382] and phototransistors[383] have been deployed in multiple media, ranging from relatively mild interstitial fluids[384–389] to highly corrosive solutions.[390–392] The most important properties for bioelectrodes are high mechanical and chemical stability, high conductivity, high transparency, and excellent biocompatibility. For this reason, thin-film (<< 100 nm) gold is usually considered the electrode of choice for bioelectronic applications.[393–395] A further requirement of a bioelectrode, however, is strong adhesion to the surface on which it is deposited – often a transparent substrate material such as glass or quartz. Unfortunately, gold exhibits poor adhesion to both these materials, and typically requires the inclusion of a thin layer of an oxidative metal such as chromium to achieve adequate adhesion to the substrate.[396,397] Chromium, however, has significant disadvantages from a bioelectronic perspective, including cumbersome patterning procedures and potential cytotoxicity, leading to a strong demand for alternative adhesion layers.1

1 Note, while some other metals such as titanium have been successfully employed as adhesion layers for conventional electronic applications, the cytotoxicity of these metals and their oxides is also an open question, see e.g. Ref. 512.
The commercial negative-tone photoresist SU8 \(^{273}\) has previously been used as an alternative adhesion layer to chromium since it adheres well to multiple substrate materials and (once cross-linked) is chemically inert.\(^{274,275}\) SU8 has the further advantage of being photo-patternable, allowing patterned electrodes with feature sizes down to a few microns to be readily fabricated using a simple two-step expose/develop procedure.\(^{276}\) We have previously reported preliminary data,\(^{1,2}\) showing SU8-supported gold electrodes to have favorable and moderately stable electro-optical characteristics in cell culturing medium, making them a potentially attractive choice for bioelectronics. Unfortunately, we found that, after prolonged periods of immersion in culture medium, gold deposited on SU8 had a tendency to develop wrinkles and cracks on the surface, reducing electrode durability and performance.\(^{1,2}\) (See Appendix A14).

Here we show that the adhesion of Au to SU8 can be controlled by varying the relative amounts of thermal annealing before and after Au deposition, and report an optimized processing protocol that reduces the tendency for wrinkling and cracking. We further show that the viability of neurons grown on an exposed layer of SU8 exceeds that of neurons grown on chromium, implying that any localized loss of gold from an SU8/Au electrode should impair cell viability less than the equivalent loss from a Cr/Au electrode. Overall, the results suggest that SU8 can be used as an effective adhesion layer for gold bioelectrodes, offering levels of adhesion comparable to chromium, but with superior biocompatibility.

4.2. Biocompatibility of adhesion layers

Biocompatibility is a prerequisite for applying any material in a bioelectronic or biomedical device. Previous reports \(^{398-403}\) have shown that, owing to its chemical inertness, SU8 does not adversely affect cell lines in vitro, which has led to its extensive use as an encapsulant for biomedical devices. The effects of evaporated elemental chromium on cell lines, by contrast, are largely unknown, although there has been considerable speculation about the potential cytotoxicity of Cr(III) and Cr(VI) ions.\(^{404-409}\) While an adhesion layer should ideally remain physically isolated from the biological environment, there is always the risk that it may become partially exposed due to corrosion or physical cracking of the overlying metal layer. To evaluate the potential effects of such an exposure, we plated acutely prepared hippocampal neurons obtained from Sprague Dawley rats onto (i) bare glass, (ii) glass coated with 5 nm chromium metal, and (iii) glass coated with 1 \(\mu\)m fully cross-linked SU8-2 – a widely used SU8 formulation optimized for deposition of films < 5 \(\mu\)m. Samples for neuronal use were incubated for 24 hrs in culture medium at 37 °C, and again overnight in aqueous PLL [Poly-L-Lysine] coating solution at 37 °C. Samples were washed with deionized (DI)
water prior to cell plating. The same protocol was employed to prepare glass, chromium, and SU8 samples. Neurons grown directly on glass, as well as on glass coated with the two adhesion layers, were then investigated using a fluorescence-based assay of viability (7, 14, 21, 28 Days in vitro; DIV) and electrophysiological characterization (14-18 DIV) as described below.

Figure 28 – Viability of primary neurons on glass, cross-linked SU8-2 and chromium test-substrates (a) Representative images acquired at one-week intervals, with green regions signifying viable cells, red/purple regions signifying pyknotic cells, and blue regions signifying cells without intracellular esterase activity (Methods for details). (b) Stacked bar charts derived from fluorescence images, showing the distribution of viable, pyknotic and esterase-free cells versus time; error bars represent standard error of the mean. The plots show a significant influence of substrate material on cell viability (see main text), with the percentage of viable cells on chromium being significantly lower than on glass or SU8 at all-time points, and the percentage of pyknotic cells being significantly higher.

To compare the population vitality of the hippocampal neurons on bare glass, Cr-coated glass, and SU8-coated glass, time-course experiments were carried out using two fluorescent probes (see Methods): the first probe – a fluorescein-based intracellular esterase activity-dependent reporter – was used to monitor cellular metabolism; while the second probe – a propidium iodide (PI) based DNA-intercalating probe (that cannot permeate healthy cellular membranes) – was used to monitor membrane integrity. Cells that were stained only with fluorescein were designated viable (healthy); cells that were stained with PI were designated pyknotic (i.e. having damaged chromatin in the nucleus) \(^{410}\); and unstained cells were designated esterase-inactive (i.e. exhibiting dysfunctional cellular metabolism) \(^{410}\). For each substrate, data were obtained from 1280 ± 116 neurons at each time point, using images acquired from 3-4 replicate samples. Analysis was carried out by two-way ANOVA and Bonferoni post-test (p<0.05). Fig. 28 shows sample images at each time step, together with the quantification of viable, pyknotic and esterase-inactive populations versus time (see Methods). The time course measurements revealed no significant differences between glass and SU8 in terms of the percentage of viable (healthy) cells at each time point. By contrast,
the percentage of viable cells on Cr was found to be significantly lower at all-time points. Non-viable cells on glass and SU8 were predominantly esterase inactive while non-viable cells on Cr were predominantly pyknotic. The significant increase of pyknotic neurons measured at each point, indicates that the non-viable neurons on chromium were largely late apoptotic and necrotic.\cite{411,412}

![Figure 29](image)

**Figure 29** – Electrophysiological characterization of primary neurons on glass, cross-linked SU8-2 and chromium test substrates. (a) Plot of mean resting membrane potential (RMP, φ) versus substrate material, indicating a significantly higher mean RMP for neurons grown on chromium (φ = -58.79 ± 2.99 mV, N=14) than either glass (φ = -52.33 ± 5.3 mV, N=21) or SU82 (φ = -49.81 ± 7.81 mV, N=21). Statistical analysis was carried out using two-way ANOVA followed by Bonferroni’s multiple comparison test. The single asterisk (*) denotes P < 0.05. (b) Representative traces of elicited action potential versus time, recorded under current clamp conditions using a sequence of 500 ms current pulses, increasing from 0 to 375 pA with a step-size of 25 pA. (c) Bar charts showing for each substrate material the mean firing rate versus current pulse amplitude, indicating a higher action potential firing threshold for neurons grown on chromium but no significant differences in the firing rates between substrates.

Patch-clamp recordings\cite{373} were subsequently used to investigate the physiological state of hippocampal neurons on the three surfaces. The phenotype of a neuron is largely defined in terms of its ability to generate action potentials, the principal mechanism for rapid intra-neuronal data transmission and the initiator of inter-neuronal signaling at synapses.\cite{411,412} We first evaluated the functional state of the neurons by quantifying their resting trans-membrane potentials. (Fig. 29a) Neurons grown on chromium showed hyperpolarized resting membrane potentials compared to those grown on glass (meaning an increased stimulus was required to move the membrane potential to the action potential
threshold \(^{413-415}\)), while those grown on SU8 had similar resting membrane potentials to those grown on glass. We subsequently investigated the response of the neurons to electrical stimulation (Fig. 29b), using a current step protocol in which neurons were subjected to a train of 500 ms current pulses of progressively increasing amplitude (0 – 375 pA, step-size 25 pA, see Methods). At higher levels of current injection, neurons on bare glass, Cr-coated glass and SU8-coated glass all showed similar firing rate plateaus in the range of 8 to 13 Hz, with significance testing indicating no significant variations in firing rate at different current steps or between samples. However, neurons grown on Cr-coated glass showed significantly higher action potential firing thresholds (~150 pA) relative to those grown on bare glass and SU8 (~50 pA).

While it cannot be determined from these measurements if the alterations in neuronal behavior observed with chromium are directly caused by chromium or if they are a secondary effect of the increased cell death in the neuronal network, the findings indicate strong alterations in the functional properties of the neurons grown on the metallic adhesion layer. Similar findings of chromium toxicity were recently reported \(^{405}\) in orthopedic implant patients, where chromium-induced oxidative damage was posited to cause necrosis in the peri-implant tissue. *In vitro* studies of Cr\(_2\)O\(_3\) nanoparticles \(^{416}\) have further shown chromium particulate toxicity to derived cell lines. The striking reductions in cell viability and the altered electrophysiological character of neurons in contact with evaporated chromium films strongly motivate the use of alternative adhesion layers in bioelectronic and medical applications. Given the similar behavior of neurons on bare glass and SU8-coated glass, SU8 appears to be a promising candidate for such an adhesion layer, offering excellent biocompatibility even with sensitive brain tissue cultures.

4.3. Processing of SU8 adhesion layers

SU8 comprises a mixture of an epoxy-based monomer, a solvent, and a photoacid initiator.\(^{273}\) Under UV illumination the photoacid protonates epoxy moieties, enabling them to thermally react with any remaining neutral epoxy groups to yield a cross-linked polymer network. By varying the UV dose and curing time, the number of cross-linking sites can be controlled, and the mechanical stability of the film can therefore be modified.\(^{273,417-421}\) When using SU8 conventionally (i.e. as a photoresist), the SU8 film is normally subjected to an initial (soft) bake to drive off excess solvent and solidify the film, reducing the risk of subsequent swelling and adhesion failure.
**Figure 30** – Schematic showing conventional protocol for preparing Au-coated SU8 on glass plus several variants employed in this work. In the conventional procedure (A): SU8 is first deposited onto a glass substrate from solution, typically by spin-coating; the film is dried and solidified by heating to 65 °C, and then to 95 °C; it is then cooled to room temperature (RT) and exposed using 350-400 nm UV radiation to initiate photo-acid generation; it is then heated again to 65 °C, and then to 95 °C to induce complete cross-linking; and finally it is cooled to room temperature for metal deposition under vacuum. In the conventional approach (A), Au deposition occurs onto fully cross-linked SU8 at the final stage directly after the second 95 °C heating stage. In the variant methods, Au deposition occurs onto partially cross-linked SU8 at earlier stages in the process, either after the first 65 °C heating stage (D), the first 95 °C heating stage (C), or the second 65 °C heating stage (B). In cases (B) and (D), where gold deposition takes place after a 65 °C heating stage, an additional 65 °C heating stage is added prior to the 95 °C stage to avoid cracking the film. Uncoated SU8 films were prepared by carrying out the procedures up to (but not including) the point of gold deposition. The condition of the film at each stage of processing is indicated by the black lettering: “W” denotes a wet film containing significant residual solvent; “P-D” denotes a partially dried film containing some residual solvent; “F-D” denotes a fully dried film, containing minimal residual solvent; “X” denotes a UV-exposed film; “P-XL” denotes a partially cross-linked film; and “F-XL” denotes a full cross-linked film.
The soft-bake is typically carried out in a three-step process to reduce stress, with the film first being warmed to ~65 °C, then being held at this temperature for a few minutes to allow the polymer chains time to relax and realign, and finally being ramped to 95 °C to drive off the excess solvent. The film is then allowed to cool slowly to room temperature, ready for UV exposure through an inverse photo-mask. Following exposure, the SU-8 is subjected to a post-exposure bake (PEB) to complete the cross-linking, often using the same 65/95 °C heating procedure. The overall procedure is summarized in Fig. 30.

When using SU8 as an adhesion layer for gold bioelectrodes, it is not clear whether the standard processing protocol is the most appropriate for achieving optimal stability of the SU8/Au electrode in biological media. In particular, it is not obvious at which stage curing and UV exposure of the SU8 should best be carried out – before or after gold deposition? Fig. 30 shows that there are six possible stages at which the Au deposition can be carried out. Two of these stages (denoted by X’s in the figure), however, may be excluded on practical grounds: evaporation of Au onto as-deposited ‘wet’ SU8 gives poor quality films due to permeation of Au into the polymer resin, while evaporation of Au after UV exposure gives inconsistent results due to the difficulty of ensuring consistent diffusion of the photo-acid at the point of gold deposition. Four sets of SU8/Au films based on Au deposition at the remaining four stages (denoted by A, B, C and D in Fig. 30) were accordingly prepared: set A was prepared by carrying out the usual full protocol described above prior to Au deposition; set B was prepared by following the standard protocol up to and including the exposure step, followed by annealing at 65 °C to enable photoacid diffusion, Au deposition, and the remaining hard bake; set C was prepared by first carrying out a soft-bake, then depositing Au, and then carrying out the full standard procedure finally, set D was prepared by annealing at 65 °C prior to Au deposition, followed by the full standard procedure. All annealing times were set to five minutes. The UV dosage for the exposure step was set to 100 mJ/cm², a typical value for sub-micron film thicknesses. In passing from set A to set D, the degree of cross-linking at the time of metal deposition progressively decreased, meaning that Au was deposited onto a hard fully cross-linked film for set A, while it was deposited onto a tacky non-cross-linked film for set D. The effects of the different processing protocols on the physical properties of the resulting bio-electrodes were investigated in a series of comparative studies described below.
4.4. Stability of SU8 and SU8/Au films in culturing medium

*In vitro* bioelectronic investigations are typically carried out in cell culturing media \[^{303,374,375}\] that contain essential ions, peptides, lipids, enzymes, growth factors and other additives and products of metabolism of the cell lines under investigation.

Despite the typically corrosive nature of such media, \[^{422–424}\] it is often necessary for bioelectrodes to remain operational over many days or weeks, e.g. to monitor the cell state over physiologically relevant periods of time. To determine the suitability of electrodes A to D for use in culturing media, two sets of experiments were undertaken: the first one using bare SU8 films prepared according to the four protocols described above up to (but not including) the metal deposition, and the second one using Au-coated SU8 prepared according to the full protocols.

For the first set of measurements, SU8-coated glass substrates of types A to D (labeled SU8-A, SU8-B, SU8-C and SU8-D) were immersed in cell culture medium [Dulbecco’s Modified Eagles Medium (DMEM)] and removed intermittently for analysis by transmission spectroscopy (**Fig. 31a–d**) and optical microscopy (**Fig. 31 e–h)**. Initially (0 hrs), all samples appeared uniform under an optical microscope and exhibited

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**Figure 31** – Optical characterization of SU8 films before \((t = 0)\) and after \((t = 672 \text{ hrs})\) immersion in cell culture medium. Films were prepared by following protocols A to D in Fig. 30 up to (but not including) the point of Au deposition: (a–d) plots of transmittance versus wavelength at \(t = 0\) and \(t = 672 \text{ hrs}\), showing slight changes for SU8-A and SU8-B and much stronger changes for SU8-C and SU8-D after immersion; (e–h) micrograph images acquired at \(t = 0 \text{ hrs}\) (inset) and \(t = 672 \text{ hrs}\) (main images), showing cracks in SU8-A, wrinkles in SU8-C and SU8-D, and a largely intact film for SU8-B after immersion. Scale bars denote 50 μm.
broadly similar transmission spectra with transmittances of > 96 % in the wavelength range 400 – 1200 nm. However, clear differences in both the optical micrographs and the transmission spectra emerged with time. After 672 hours of immersion, the most cross-linked sample SU8-A developed sizeable cracks. Over the same period of immersion, the next most cross-linked sample SU8-B remained optically clear, while samples SU8-C and SU8-D both developed a cloudy, granular appearance with the grain size being largest in the case of the least cross-linked sample SU8-D. While the transmission spectra of the two most cross-linked samples (SU8-A and SU8-B) were broadly unaffected by immersion in the culture medium, the other two samples showed a substantial (~10 %) drop in transmission due to scattering by sub-micron sized features. From these measurements, it is evident that deposition of gold onto a fully cross-linked sample of SU8 may cause later damage to the gold layer due to fracturing of the underlying SU8. At the same time, insufficient cross-linking of the SU8 results in an unstable film that delaminates from the underlying substrate with a commensurate loss in transparency due to scattering. The above findings are consistent with previous investigations into the swelling, and subsequent deformation, of SU8 under exposure to solvents and solvent vapors, see e.g. Ref. 425. Deformation has been attributed to spatial variations in the cross-linking density, which in turn lead to spatial variations in the amount of swelling – and hence strain – within the film: strongly cross-linked regions undergo only slight swelling, leading to low strain, while weakly cross-linked regions undergo strong swelling, leading to high strain. The fracturing of the fully cross-linked sample SU8-A is consistent with that sample undergoing very non-uniform swelling under the described processing conditions.

Previous reports [1,2] involving the use of SU8 as an adhesion layer have typically entailed full processing of the SU8 film before the metal layer is deposited in accordance with protocol A. Fig. 32a shows a series of transmission spectra recorded at 168-hour intervals for a sample prepared in this way (SU8/Au-A). The initial transmission spectrum was broadly characteristic of the transmission spectrum of thin-film gold, with some additional oscillatory structure due to the weak cavity formed by the glass/SU8/Au structure. Over time, a slight increase in peak transmittance was observed, together with the emergence of stronger oscillatory features. The observed behavior is consistent with the localized removal of SU8/Au and/or Au from the three-layer stack, leading to a combination of the initial SU8/Au-A spectrum and strongly oscillatory features due to SU8-A and/or SU8/Au-A. This behavior can be seen in the corresponding reflectance (Fig. 32b) micrographs for sample SU8/Au-A: the formation of diagonal and parallel folds in the stack is evident at t = 168 hours, implying weak bonding of SU8 to the glass and/or weak bonding of Au to the fully cross-linked SU8 at this location; by t = 336 hours, stress due to immersion in the culture medium
had distorted these folds into a vortex-like pattern; while, by $t = 672$ hours, complete detachment had occurred. (Note, the beginning of a second detachment event is visible in the lower right portion of the 672 hour images).

Figure 32 – Evolution of optical characteristics of SU8/Au-A films with immersion time prepared by following Protocol A in Fig. 30. (a-d) Plots of transmittance versus wavelength at $t = 0$, 168, 326 and 672 hrs, showing good stability in the spectral characteristics up to 326 hrs, followed by significant changes thereafter. (e-h) Reflection micrographs at $t = 0$, 168, 326 and 672 hrs, showing wrinkling (168, 326 hrs) and eventual tearing of the SU8/gold bilayer (672 hrs). Scale bars denote 50 μm.

The SU8/Au samples prepared using protocols B, C and D (labeled SU8/Au-B, SU8/Au-C and SU8/Au-D) exhibited initial transmission spectra similar to SU8/Au-A (see Fig. 33a). However, they showed much weaker spectral changes with time, suggesting smaller changes in the morphology of the stack. However, comparing the reflectance (Fig. 33b) and transmittance (Fig. 33c) micrographs of the four samples obtained at $t = 672$ hours, it was evident that SU8/Au-B and SU8/Au-D had undergone qualitatively similar levels of degradation to SU8/Au-A, with both samples showing regions of wrinkling, cracking and delamination. Strikingly, sample SU8/Au-C remained optically uniform with no signs of film damage. Hence, taking the results from figures 2, 3 and 4 together, it is clear that substantial cross-linking of SU8 is required to achieve strong adhesion of SU8 to glass, while Au should be deposited before cross-linking of the SU8 is complete to achieve strong adhesion of SU8 to Au.
Figure 33 – Optical characterization of SU8/Au films prepared by following protocols A to D in Fig. 30: (a-d) plots of transmittance versus wavelength before (t = 0 hrs) and after (t = 672 hrs) immersion in cell culture medium, showing substantial changes for SU8/Au-A but comparatively small changes for the other films; (e-h) micrograph images of entire samples before and after immersion, showing significant cracking after immersion in all films except SU8/Au-C. Scale bars denote 10 mm. (i-l) micrograph images before and after immersion, showing significant cracking after immersion in all films except SU8/Au-C. Scale bars denote 50 μm.

Changes in the electrode performance as a result of prolonged immersion in the cell culture medium were assessed by carrying out four-point probe measurements of sheet resistance (see Methods) before immersion and after one day, two days, one week, two weeks and one month of immersion. Fig. 34 shows plots of the measured sheet resistance versus immersion time for the four SU8/Au samples. All four samples showed a progressive increase in sheet resistance with the immersion time from a similar starting point of ~4.8 Ω/sq. before immersion. However, the SU8/Au-C sample showed a significantly slower rate of degradation, increasing by a small amount to ~4.9 Ω/sq. after one month (compared to ~5.2 Ω/sq. for the other samples), consistent with the higher stability of the SU8/Au-C electrode evident from the micrographs of Fig. 33b.
The resilience of the SU8/Au films to mechanical disturbances was investigated using qualitative peel testing (see Fig. 35 and Methods). A strip of adhesive tape was applied to the gold surface and then peeled from the gold film at a constant speed of 50 mm/s at an angle of 90° to the surface. The process was repeated 50 times or until delamination of the gold film occurred. Samples SU8/Au-A and SU8/Au-B – i.e. the two films in which the SU8 was most heavily cross-linked at the time of gold deposition – suffered significant delamination within a single peeling step, indicating that Au does not adhere strongly to heavily cross-linked SU8. Samples SU8/Au-C and SU8/Au-D, by contrast, showed good resilience to repeated peeling steps and remained largely intact even after 50 repetitions. The improved adhesion of gold to SU8 in circumstances where cross-linking is initiated after gold deposition has occurred has recently been investigated by Merschrod and co-workers.[274] Their studies indicate that, during post-deposition cross-linking, the SU-8 chains cross-link around the bottom layer of large gold atoms. This cross-linking and the subsequent shrinkage of polymerized SU-8 (due to the reduction in free volume) trap the bottom layer of gold atoms, resulting in a gold-polymer composite structure with overall good adhesion of the gold layer to the cured SU-8.
Figure 35 – Images of SU8/Au films prepared according to protocols A to D in Fig. 30 after one peeling step (1st row) and fifty peeling steps (2nd row) using adhesive tape. In the case of SU8/Au-A and SU8/Au-B, images are shown only for the first peeling step due to immediate damage to the films.

On the basis of the peel tests, it is evident that the SU8 should be only weakly cross-linked prior to gold deposition to ensure good adhesion between the two layers. It is further evident from the images of Figure 33b that sample SU8/Au-C underwent the smallest amount of cracking and wrinkling when immersed in culture medium. Hence, on the basis of the data presented above, protocol C appears to be the most promising for the realization of stable bioelectrodes on glass substrates.

4.5. Biocompatibility of SU8/Au layers

To assess the effect of the various processing protocols on SU8 biocompatibility, we compared HEK293T cell adhesion and viability on bare glass, Cr-coated glass, glass coated with the differentially cross-linked SU8 samples (SU8-A-D) and glass coated with the optimized SU8/Au bilayer (SU8/Au-C). The samples were sterilized in 70% ethanol, prior to overnight incubation by 0.1% PLL solution. Adhesion was quantified using the Hoechst DNA stain, and cell vitality was assessed by FDA staining. All samples exhibited a similar level of cell adhesion, although the mean level of cell adhesion increased marginally from SU8-A to SU8-D. There were no significant differences in cell viability between bare glass, SU8-coated glass samples and SU8/Au-coated glass samples. However, for the Cr-coated glass sample, there was a significant (~20%) reduction in viable cell population relative to glass controls ($P < 0.005$, Mann-Whitney, one tailed),
providing further evidence of its cytotoxicity and the superiority of SU8 as an adhesion layer for bioelectrode applications (Fig. 36).

![Fluorescence micrographs](image)

**Figure 36** – HEK293-T cell viability on glass, SU8 films prepared according to protocols A to D in Fig. 3, Cr and Au coated SU8 prepared according to protocol C in Fig. 30 (a) Fluorescence micrographs of HEK293-T cells plated on samples of SU8-A-D, and SU8/Au-C, indicating no significant differences in cell adhesion or cell viability count with respect to glass. (b) Fluorescence micrographs of HEK293-T plated on chromium, showing a significant reduction in viable cell population with respect to glass.

**4.6 Discussion & Conclusion.**

In conclusion, we have described an effective protocol for fabricating mechanically and chemically stable gold bioelectrodes on glass, using a 1 μm layer of the negative-tone photoresist SU8 as an adhesion layer between the glass substrate and a thin layer of gold. Using a fluorescence-based assay of neuronal cell viability and electro-physiological characterization, SU8 was found to exhibit superior biocompatibility to chromium – the most widely used adhesion layer for gold on glass. Compared to neurons grown directly on glass, neurons grown on chromium-coated glass exhibited significant reductions in esterase activity and increased levels of cellular membrane permeability, consistent with elevated levels of apoptotic/necrotic processes; they also showed significantly higher action potential firing thresholds, suggesting an impairment of the neuronal phenotype. Neurons grown on SU8-coated glass, by contrast, showed a behavior similar to those grown directly on glass. Various treatment protocols for the SU-8 were investigated, with a view to attaining high transparency and good mechanical and biochemical stability. Thermal annealing to induce partial cross-linking of the SU-8 film prior to gold deposition, with further
annealing after deposition to complete cross-linking, was found to yield the optimum electrode properties. From a combination of optical microscopy, transmission spectroscopy and four-point probe sheet resistance measurements, we found that the optimized SU8/Au films (obtained using protocol C) exhibited good stability over twenty-eight days of immersion in cell culture medium. In addition, they showed excellent mechanical stability under repeated peel testing with adhesive tape, with the bioelectrode remaining intact over 50 repeat peeling steps. The adhesion and viability of HEK293T cells on the optimized SU8/Au electrode was found to be comparable to that of HEK293T cells on glass, confirming the biocompatibility of the electrode.

Finally we note that, although not specifically investigated here, SU8 has previously been used as a photopatterning adhesion layer for multiple substrate materials, including quartz and various plastics, together with a range of conductive materials such as carbon nanotubes, metallic nanoparticles, and conducting polymers such as PEDOT:PSS. Its excellent biocompatibility, combined with the ability to carry out patterning using a simple two-step expose/develop process via a technique known as interlayer lithography[140,276], suggest SU8-based electrodes will find diverse applications in bioelectronics devices.

4.7. Methods

**Cell Cultures.** Primary cultures of hippocampal neurons were prepared from embryonic 18-day rat embryos (Charles River). Briefly, hippocampi were dissociated by a 15-min incubation with 0.25 % trypsin at 37 °C and cells were plated on poly-L-lysine-coated substrates (0.1% PLL in Borax solution overnight), in Neurobasal supplemented with 2 mM L-glutamine, 2% B27, 100 μg/ml penicillin and 100 μg/ml streptomycin, and with 10 % horse serum (Life Technologies) in the first four hours of plating. Biocompatibility data were obtained using two neuronal culture preparations. Human Embryo Kidney (HEK293T) cells, obtained from ATCC, were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10 % fetal calf serum, 2 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (Life Technologies). Cells were plated at an equal density of 10000 cells/cm². Samples were sterilized in 70% ethanol, prior to overnight incubation in 0.1% PLL solution for HEK293T experiments. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

**Biocompatibility assays** For the fluorescence cytotoxicity assay, attached cells were rinsed twice with imaging solution (in mM: NaCl 140, KCl 2.5, MgCl₂ 1, CaCl₂ 1.8, HEPES 20) and were then incubated for 4 min in stain-supplemented imaging solution containing 15 μg/ml of Fluorescein diacetate (FDA; Sigma-
Aldrich), 5 μg/ml of Propidium iodine (PI; Sigma-Aldrich) and 3 μg/ml of Hoechst-33342 (Sigma-Aldrich). Cells were washed again twice with imaging solution. Each time point shown in Fig. 1 represents a mean of 1280 ± 116 neurons analyzed per culture substrate condition. For each time point, images were acquired from 3-4 samples per condition by a C91006 CCD Camera (Hamamatsu Photonics Italia) mounted on a Nikon Ti-E epifluorescence microscope (Nikon Instruments). Standard 4′,6-diamidino-2-phenylindole (ex: D350/50x, em: D460/50m, dic: 400dclp), fluorescein isothiocyanate (ex: D480/30x, em: D535/40m, dic: 505dclp) and tetramethylrhodamine isothiocyanate (ex: D540/25x, em: D605/55m, dic: 565dclp) filter sets were used to image Hoechst-33342, fluorescein diacetate and propidium iodine, respectively. Analysis was carried out manually, using the Hoechst signal as the region of interest for each cell. Cells were grouped as follows to generate population percentages: healthy cells were fluorescein positive and PI negative, pyknotic cells were PI positive, and Esterase Absent cells were those with neither a PI nor an FDA signal. HEK staining included FDA and Hoechst dyes, and was carried out on two duplicate samples per condition.

Neurons were patch-clamped using a HEKA EPC10 amplifier and digitizer coupled to a Nikon FN1 upright microscope (Nikon Instruments). Recordings were carried out in current-clamp mode and digitized with a 20 kHz sample rate. The current step protocol was administered after whole cell access, using 500 ms steps of 25 pA increasing from 0 to 375 pA. Standard intracellular (in mM: K-Glucuronate 126, NaCl 4, MgSO4 2, CaCl2 0.2, Bapta 0.08, Glucose 9.45, Hepes 5, ATP 3 and GTP 0.1) and extracellular (in mM: NaCl 135, KCl 5.4, MgCl2 1, CaCl2 1.8, HEPES 5, glucose 10 and pH adjusted to 7.4 with NaOH) solutions were used.

Preparation of SU8-coated glass. Glass substrates were rinsed with deionized (DI) water, acetone and 2-propanol, and then dried. The SU8 films were subjected to various treatment protocols as described in the main text. SU8-A was prepared by following the usual processing protocol, in which the following steps were carried out. The work reported here was carried out with the commercial formulation SU8-2 from MicroChem, which in its undiluted form is suitable for preparing thin films < 2 μm in thickness. As received SU8-2 was spin-coated onto the cleaned glass at 500 rpm for 6 s and then at 6000 rpm for 30 s. After annealing at 65 °C for 5 min then 95 °C for 5 min. Exposure of the sample was carried out through the glass substrate for a duration of 25 s, using near UV (350-400 nm) illumination at an energy density of 100 mJ/cm². Finally, the samples were subjected to post-exposure annealing at 65 °C for 5 min and then 95 °C for 5 min to complete the crosslinking process. SU8-B was prepared by following the standard protocol up to and including the exposure step, followed by annealing at 65 °C to enable photoacid diffusion. SU8-C was prepared by carrying out a soft-bake at 65 °C for 5 min and then at 95 °C for 5 min. SU8-D was prepared by annealing at 65 °C for 5 min.
Preparation of SU8/Au-coated glass. Glass substrates were cleaned as described above. As received SU8-2 was spin-coated onto the cleaned glass at 500 rpm for 6 s and then at 6000 rpm for 30 s. The SU8 films were coated with 30 nm of gold, using thermal evaporation at a pressure of $10^{-6}$ mBar. The samples were subjected to various treatment protocols before and after gold deposition as described in the main text. Exposure was carried out through the glass substrate for a duration of 25 s, using near UV (350-400 nm) illumination at an energy density of 100 mJ/cm$^2$. SU8/Au-A was prepared by following the full protocol described above for SU8-A coated on glass, followed by gold deposition. SU8/Au-B was prepared by following the standard protocol up to and including the exposure step, followed by annealing at 65 °C to enable photoacid diffusion, Au deposition, and the remaining hard bake after Au deposition. SU8/Au-C was prepared by first carrying out a soft-bake, then depositing Au, and then carrying out the full standard procedure. SU8/Au-D was prepared by annealing at 65 °C prior to Au deposition, followed by the full standard procedure.

Transmission Spectra. Transmission spectra in the range 400 nm to 1200 nm were measured with a Shimadzu UV-3101 NIR-Vis-NUV spectrophotometer. The samples in PBS and cell culture medium were washed by rapid immersion in DI water prior to measurement.

Imaging. Optical images in reflection and transmission mode were obtained using an optical microscope (Olympus BX51) fitted with a digital 9 MP camera (Conrad DP-M17. Unpolarised white light illumination was provided by a white light-emitting diode (Thorlabs LIU-PS).

Measurement of sheet resistance. Sheet resistances were measured at three locations per substrate, using a home-built four-point-probe set-up with equidistant probe spacing of $d = 1.5$ mm. Two Keithley 6514 source-measure units were used to supply a known current $I$ between the outer probes, while measuring the voltage $V$ across the inner probes. Sheet resistances $R_s$ were determined according to the thin-film equation: $R_s = \frac{\pi}{\ln 2} \left(\frac{V}{I}\right)$

Peel test procedure. The samples were secured to a wooden plate using double-sided adhesive tape on the underside of the substrate. Duct tape (Tesa®) was then placed on the sample and peeled away vertically, using a stepper motor-driven pulley system at a constant velocity of 50 mm/s. The procedure was repeated fifty times or until the film detached from the substrate, whichever occurred first.
Chapter 5: Biocompatible and freestanding organic LEDs using polymeric encapsulation for the interface with biology

5.1. Need for Encapsulation

The importance of encapsulation Organic Light Emitting Diodes (OLEDs) stems not only from the need to protect cells from the putative photochemical shock described in the previous chapters, but also to insulate the electrical current required for device operation, and to enhance device performance lifetime. Electrodes may delaminate due to intrinsic stress during deposition; catastrophic failure (high current density leading to higher temperature that generates leakage currents and resulting in a loss of luminance; or intrinsic degradation through moisture, oxygen or water having direct contact with the electrodes. Indeed, organic polymers and small molecules are sensitive to oxygen and moisture, and operation in such environments rapidly degrades the films.

A preliminary experiment was conducted to assess the viability of operating organic light-emitting diodes (OLEDs) for short periods of time in an aqueous environment. OLEDs based on F8BT described in the methodology section were immersed in an electrolyte (Phosphate Buffered Saline, PBS) and their degradation was measured over time (Figure 37 and 38). PBS [10 mM PO$_4^{3-}$, 137 mM NaCl, and 2.7 mM KCl] was chosen because the osmolarity and ion concentrations of this solution match those of the human body. Encapsulated devices using aluminum-capped calcium (Figure 37A) exhibited electroluminescent stability and were functional after 10 hours of immersion in PBS. Although the optical power reduced from 11-mW/m$^2$ at 1 hour to 7-mW/m$^2$ at 10 hours, the optical morphology remained fairly homogenous throughout the 10 hours (Figure 37A-c; d; e; f). Un-encapsulated devices using aluminum-capped calcium (Figure 37B) degraded in only 10 minutes when immersed in PBS. The optical power dramatically reduced from 7-mW/m$^2$ at 2 minutes to 1-mW/m$^2$ at 10 minutes, and the optical morphology deteriorated rapidly (Figure 37B-f). The current passing through the device (Figure 37B-a) dropped over time in a similar way to the optical power, suggesting a diminishing electron injection current from the cathode to the active layer. It is well known that calcium electrodes oxidize rapidly, especially in an aqueous environment, so the demonstration of this rapid electrical and optical degradation is in accordance with expectation (Figure 37A). Results to date suggest that a non-encapsulated device using calcium would not be sustainable in an aqueous solution, and therefore unsuitable for applications involving direct exposure of the device to biological media.
Figure 37 | Current (A-a.) and optical power density (A-b.) versus voltage curves for encapsulated aluminum-capped calcium devices at T=30 min in air (black square), T=1 hour in PBS (red circle), T=2 hours in PBS (green triangle) and T=10 hours in PBS (blue star). Current (B-a.) and optical power density (B-b.) versus voltage curves for non-encapsulated aluminum-capped calcium devices at T=5 min in air (black square), T=2 min in PBS (red circle), T=5 min in PBS (green triangle), T=10 min in PBS (blue star). Also shown are 2D intensity maps, obtained under 6 V operating conditions after each of the aforementioned treatments. For encapsulated aluminum-capped calcium devices at T=30 min in air (A-c), T=1 hour in PBS (A-d), T=2 hours in PBS (A-e) and T=10 hours in PBS (A-f). For non-encapsulated aluminum-capped calcium devices at T=5 min in air (B-c), T=2 min in PBS (B-d), T=5 min in PBS (B-e), T=10 min in PBS (B-f).
Figure 38 | Current (A-a.) and optical power density (A-b.) versus voltage curves for encapsulated aluminum devices at T=30 min in air (black square), T=1 hour in PBS (red circle), T= 2 hours in PBS (green triangle) and T= 10 hours in PBS (blue star). Current (B-a.) and optical power density (B-b.) versus voltage curves for non-encapsulated aluminum-capped calcium devices at T0=5 min in air (black square), T=2 min in PBS (red circle), T= 5 min in PBS (green triangle), T= 10 min in PBS (blue star). Also shown are 2D intensity maps, obtained under 6 V operating conditions after each of the aforementioned treatments. For encapsulated aluminum devices at T0=30 min in air (A-c), T1=1 hour in PBS (A-d), T= 2 hours in PBS (A-e) and T= 10 hours in PBS (A-f). For non-encapsulated aluminum devices at T=5 min in air (B-c), T=2 min in PBS (B-d), T= 5 min in PBS (B-e), T= 10 min in PBS (B-f).
Encapsulated devices using aluminum as an electrode (Figure 38A) were still functional after 10 hours immersion in PBS. The optical power reduced from 7 mW/m$^2$ at 1 hour to 6 mW/m$^2$ at 10 hours, which is less than the reduction observed in encapsulated calcium-based devices. The optical morphology remained fairly homogenous throughout the 10 hours (Figure 38A-c; d; e; f). Using non-encapsulated aluminum devices (Figure 38B) severe degradation was observed within just 10 minutes of immersion in PBS. The optical power reduced from 6 mW/m$^2$ at 2 minutes to 2.5 mW/m$^2$ at 10 minutes.

Fig. 39 shows photographs of the two un-encapsulated OLEDs after 10 mins immersion in PBS. Using Calcium/Aluminum cathode in Fig. 39a shows strong oxidation of calcium under water destroying the cathode electrode morphology. The improved resilience of the Al electrode remains clear, in Fig. 39b, but the device properties nonetheless degrade rapidly, rendering them unsuitable for operation in biological media. In the next phase of research, surface coatings that repel oxygen and water, whilst providing selective access to the electrode and/or active layer will be investigated.

![Figure 39](image)

Figure 39 | Image of a non-encapsulated OLED after 10 minutes immersed in PBS. a.) Using Calcium/Aluminum cathode; b.) Using only aluminum cathode. Scale bars denote 500 μm.

The final insulation of a device should present an oxygen and water barrier. Though oxide layers such as silicon oxide are well capable of limiting oxygen penetration, they are less well suited for creating effective water barriers. These latter task is well performed by polymeric insulators; whose chemical hydrophobicity repulses any water seeking to penetrate the device. The final exposed layer serves as contact layer with the biological medium or tissue in vivo, or as the culturing substrate for in vitro applications.
5.2. Suitable interface with cells

Accordingly, we have characterized ten commonly employed polymeric insulators (ABS, BCB, Ormostamp, Parylene-C, PDMS, PMMA, PS, PVA, PVC and SU8-2) for optoelectronic applications for their ability to adhere to cells, and hence their applicability as final barriers in OLED devices (Fig. 40).

5.2.1 Polymeric insulators for cellular patterning

![Figure 40](image)

**Figure 40 | Diverse Cell Adhesion Profiles of organic dielectric polymers**

A. Cell adhesion was evaluated 24 hrs after plating cells, before and after laminin treatment. While only ABS (black), PS (cyan), Ormostamp (dark brown), and SU8-2 (violet) showed adhesion before laminin treatment, all polymers could effectively be coated to gain cell adhesion character by laminin coating. B. Cells proliferated well on all investigated polymers following laminin treatment. C. RMS roughness of the investigated polymers’ surface, expressed as root mean square (nm). D. Scatterplot of cell density at 24hrs versus RMS roughness, before and after laminin treatment, blue and green dashed lines show best linear fit ($r^2 = 0.0727$ and 0.1277 for laminin and non-coated respectively). No correlation is found between roughness and cell adhesion following laminin treatment (Pearson R = 0.3574, p = 0.1553).
The protocol employed was similar to that presented in the previous section on characterization of the light emitting polymers, in which we employed HEK293T culture density after plating as an indicator of adhesion. The organic polymeric insulators used in the study are described in full in the materials and methods section. We find that before surface modifications of the insulators, ABS, ormostamp and PS present the strongest adhesion potential (Figure 40A, left column of pairs), with all other polymers exhibiting zero cells adhered to the surface following DAPI staining protocol. Interestingly, the use of laminin strongly dictates the location of cell adhesion of these latter polymers. This presents these polymers as ideal candidates for applications in which neurons could be patterned to the location corresponding to an underlying pixel of an OLED array. Conversely, the poor adhesion on these polymers when not coated indicates their strong potential applicability as final insulation layers for in vivo chronic implantation applications.

Given past reports citing relationships between cell adhesion potential and surface roughness of individual materials, we decided to evaluate the relationship across the materials tested here. Surface roughness was measured by atomic force microscopy for each of the polymeric dielectrics, which presented a range of values from 1.63 to 13.2 nm for the root mean square of scanned areas (Figure 40C). Before laminin treatment, the chemical potential of the polymer surfaces was dictated solely by their constituent parts, which vary between the groups. The effect of surface roughness is absent in this instance (Figure 40), as is well shown by PVA which with the highest surface roughness of 13.2 nm had zero cell adhesion.

Following surface modification by laminin coating, still no clear relation between the roughness of the polymer and the cell adhesion appears (Figure 40; $r^2 = 0.13$; Pearson $R = 0.34$, $p = 0.16$). Unlike comparisons within a single material, these results indicate that surface roughness is not a good indicator of cell adhesion when comparing materials. It seems instead that the individual surface properties of the insulators dictate cell adhesion for the range of surface roughness assessed here.

5.2.2 Suitable mode of operation of diodes immersed in cell culture media

Prior to implementation of these polymeric insulating layers for suitable encapsulation of organic LED, a thorough characterization of the electrochemical behavior at the interface with cell culture media is required. By using a three-electrode configuration, measurements were made using cell culture media, silver chloride Ag/AgCl as a reference electrode and platinum as a counter-electrode (see figure 41c). The working electrode is connected to the anode and/or cathode of the ohmic diode protected with SU8.
characterization analyzes the barrier performance while an ohmic diode (Al/ITO) is operating in saline solution.

**Figure 41 | Breaks from thin film polymeric SU8 insulating a ohmic diode.** a.) bubbles due to breaking of the polymer film protector. b.) The JV ohmic curve of the diode and the current at the interface with cells to record leakages. c.) the set-up. d.) schematic showing what may occurs if leakage. e.) & f.) Microscope image showing degradation of ohmic pixel after 10 V operation in media. Scale bars denote 50 μm.

Here we focus on SU8 as the polymeric encapsulation of an ohmic diode immersed in cell culture media. SU8 has been proved to enhance the adhesion of gold to glass (see chapter 4) and it has been proved to stop photo-induced cell responses caused by a direct contact of cell with organic semiconductors (see chapter 2). Different sets of ohmic diodes (ITO/Al) were protected with different thickness (T1=9 μm; T2=6 μm; T3=3 μm and T4=1 μm) of SU8 in order to optimize the operational device when immersed in media. In order to understand what might be the suitable thickness, the surface of the diode encapsulated was in contact with cell culture media while DC voltage was applied. At the same time, an electrochemical spectrometer was used in chrono-amperometer mode to sense any leakage occurring at the interface of the ohmic diode encapsulated with the media. **Figure 41** shows that the density of current at the interface is increased for all thicknesses of SU8.

Increasing DC diode voltage (from 10V) leads to a large increase of density of current at the interface and further increases are shown to produce bubbles, assumed to be gases issuing from a breakup of the
polymer. See figure 41e-f. Such deterioration is unwanted for targeted application in-vivo. Ultimate freestanding diodes will have to be insulated from the cathodic and anodic sides of the device in order that no electrodes are in direct contact with the media. It was observed that any leakage dramatically destroyed the operational pixel (see figure 41e-f).

To conclude this work, we decided to test a freestanding organic LED in cell culture media.

5.3. Freestanding OLEDs for bio applications

We fabricated a freestanding organic LED using SU8 and polystyrene as protective layers. In chapter 3, we described the fabrication of freestanding cross-linkable light-emitting sheet based on PF8FBT and the use of polystyrene sulfonate (PSS) as water based sacrificial layer provided an easy procedure to peel off the light-emitting sheet from glass by simply immersing them in ethanol. However, in this fabrication of freestanding organic LEDs with complex multi-layer structure of different materials and a usage of SU8 as an insulator, we found it impossible to peel off the device from glass in ethanol without destroying the structure.

5.3.1. Fabrication of free-standing organic LED

We use a freestanding polystyrene (PS) film already prepared to help lift off the device fabricated on top. (Polydimethylsiloxane) PDMS was used for sticking polystyrene during the solution processing of the organic diodes because it was found that the freestanding polystyrene deposited on glass were bending during processing top layers, leading to un-homogeneous films. On the other hand, polystyrene deposed on PDMS was found to not move during the deposition of top layers. See figure 42 step I and II.

PDMS is effectively double-sided scotch tape stuck onto glass to avoid bending issues during spinning and the freestanding PS film is positioned on its surface. The bio-electrode procedure of fabrication fully described in chapter 4 - SU8/AU-C first carrying out a soft bake, then depositing gold anode on PS, then carrying out the full crosslinking procedure. The procedure provided firstly insulation for the future freestanding device and, secondly, cross-linked the gold electrode to SU8 on the inner side of the device. Once the bio-gold was fully cross-linked, the next step consisted in spin-coating a hole injection/transport layer (PEDOT:PSS proved to not alter cell adhesion and health seen in Appendix A16.
On top of this, the emissive layer SY-PPV was deposited. It was proved to be biocompatible in darkness or under low intensity of irradiations in chapter 2).

**Fabrication of a self-standing biocompatible Organic Light Emitting Diode**

We then decided against using conventional calcium as the electron injection layer because we required a fully biocompatible freestanding device working in cell culture media. Aluminum work function of 4.2 eV is low enough to provide sufficient electron injection at the energy level required by the LUMO of SY-PPV. Aluminum electrodes do not alter cell adhesion and health and is often used as low work-function cathode in organic LEDs in chapter 4 and appendix 17. We then decided to fabricate a cathode electrode composed of 10 nm of Aluminum and 30 nm of gold. Aluminum helps gold electrode to bond well compared to a straight contact of gold to hydrophobic surfaces. (More study needs to be done in the subjected of improving the cross-linkability across materials). SU8 is required to protect the top layers.

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**Figure 42 | Step fabrication of freestanding OLED**

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order to do that, we protected the anode contacts with an epoxy/silver paste and thermally stable scotch tape around the edge, so that SU8 would easily be processed and fully cross-linked. To free the anode contacts, the Scotch tape should be removed after spin coating SU8 and before the first pre-baking steps. We then cleaned the edges of the device to avoid any possible contact of the electrodes with the media (see figure 43). Major issues can occur during each step, including thermal and motion stress that lead to cracks and wrinkles in the stacks.

Figure 43 | Degradations during process of fabrication. Scale bars denote 50 μm.

5.3.2. Characterization of a freestanding organic LED

Freestanding organic LED is foldable, bendable (figure 44 A,B sustaining operation at an humid egg ‘surface (figure 44) and full immersed in cell culture media (figure 44 E,F). We tested devices at 10V in order to not break the polymeric insulators. We can see video of the freestanding organic LED on an egg ‘surface at https://imperialcollegelondon.box.com/v/OLEDneuron2017. Drops of Di-water or immersion in cell-culture media do not alter the organic LED luminescence functions.
Figure 44 | Folded freestanding OLED a.) Switched off and b.) Switched on at 10V. Freestanding organic LED on the surface of a humid egg c.) Switched off and d.) Switched on at 10V. Freestanding organic LED immersed in cell culture media e.) Switched off and f.) Switched on at 10V. Video can be seen at https://imperialcollegelondon.box.com/v/OLEDneuron2017

Bending the device affected the freestanding diode becoming more resistive (Figure 45b) with lower electroluminescence (Figure 45a) while not much resistive degradation occurred due to the immersion of the device encapsulated by SU8/PS on both sides in media (Figure 45c-d). It seems that the polymeric insulator does protect effectively the device, but we notice a strong degradation of the device performance over 30 min probably explained by oxygen quenching and changes in energy alignments. Future work will need to improve oxygen barriers and material interfaces stability. Two critical challenges remain in order to achieve fully biocompatible OLEDs, namely (i) the identification of highly performant and water-stable
electron injection materials; and (ii) the identification of appropriate dielectrics, characterized by extremely low water and oxygen penetration, high electrical resistivity, high biocompatibility, and long lifetime.

Figure 45 | JVL curve before and after bending and immersion in cell culture media.

5.4. Discussion

The fabrication of a biocompatible freestanding organic LED prototype was described in this chapter. All materials were studied for their biocompatibility with cells in other chapters and these materials are used for the fabrication of freestanding organic LEDs as described here in chapter 5. The light emitting conjugated polymer SY-PPV as type-I material explored in chapter 2 was used here as a safe active emissive layer in the freestanding organic LED. Initial experiments studied the widely used PEDOT: PSS in OLEDs did not alter cell adhesion (see Appendix 16) and was used in this freestanding device as hole injection and transport layer. The stable and biocompatible bioelectrode investigated in chapter 4 with SU8/Au-C procedure was used as the anode and cathode in this freestanding organic device. In order to help the injection of electrons from the bioelectrode SU8/Au-C used as the cathode, a thin layer of aluminum was
placed between the gold and the light emitting polymer. Aluminum was investigated in previous work and reported in Appendix 15 as a biocompatible key electrode for opto-bioelectronics. This work is the first reported self-suspended organic LED using biocompatible materials and working in cell culture medium. However, it will be necessary to confirm the biocompatibility of the resulting freestanding organic LED by verifying long term safe operation of the device. It is extremely difficult to fabricate a device as stress occurring during fabrication may lead to movement that can lead to the breaking of contacts. Improvements in fabrication will hopefully lead to more stable devices. Furthermore, the temperature diffusion needs to be measured during aqueous operation in order to provide a safe voltage regime of operation, avoiding damage and providing minimal thermal diffusion to the cells. Further improvement with crosslinked materials as discussed in chapter 3 may provide prolonged stability.

The challenge of stretchable and ultra-flexible organic LEDs \[^{428}\] is to use inorganic/organic hybrid barrier and chemistry in packaging technologies \[^{429}\] for water and oxygen as encapsulation.\[^{430−436}\] Hybrid organic/inorganic or mixing thermostet plastics and thermoplastic\[^{437−439}\] encapsulation structures may protect the device against oxygenation and create an appropriate water vapor barrier.\[^{440}\] The design of a freestanding 3D device structure using hybrid hydrogel or hydrophilic components might be immersed in and surround solid insulated freestanding LEDs as a scaffold with many pores and pillars that could let pass large amounts of oxygen and water through a breathable emitting device. Holes and porosity in the solid device would help tissues to grow surrounding the holes and help good oxygen/water transmission while protecting functional pixels from the bio-environment using patternable thermostet materials such as SU8. A similar interface with neuronal network, muscle cells, contact lenses or cochlear implants\[^{155,441}\] may open new avenues to light-mediated controls for tissue engineering applications. Looking forward, embedding well encapsulated and high efficiency devices in hydrogel lenses would extend the range of application to treatment of retinopathies and virtual reality contact lenses.

5.5. Methods

**Materials.** Materials used were as follows: BCB: \{bis-benzocyclobutene\}(DOW Chemical Company); SU-8 2 (Microchem), PMMA, \{Poly\(\text{methyl 2-methylpropenoate}\)\} and Ormostamp (Microresist Technology). All others were acquired from Sigma-Aldrich. PDMS: \{poly(dimethylsiloxane)\}, BS: \{Poly\(\text{acrylonitrile-co-butadiene-co-styrene}\)\}, PVC: \{Poly \(\text{vinyl chloride}\)\}; PVA \{Poly \(\text{vinyl acetate}\)\}; Parylene-C \{2, 8-Dichlorotricyclo[8.2.2.24,7] hexadeca-1(12),4,6,10,13,15-hexaene\}; PS \{Polystyrene\}. All films were deposited on cleaned glass substrates. ABS films of 3 μm thickness were prepared by 2.5%wt of ABS in
Dichlorobenzene, sonicated in ultrasonic bath for 2 hrs; spin coated for 10 seconds at 300rpm and then 60 seconds at 600rpm. Films were dried for 2h at 130°C. Ormostamp films of 5μm were prepared from manufacturer solution, without further dilution, spin coating: 10 seconds at 300rpm and then 60 seconds at 1500rpm. Films were exposed to UV light (1000mJ at 385nm) and thermally cured at 130°C for 30min. PDMS films of 3μm were prepared with elastomer:curing agent in a weight ratio of 10:1. Further diluted with a weight ratio of 1:1 in Toluene. Spin coating was 10 seconds at 300rpm and then 60 seconds at 600rpm. Films were cured for 1h at 100°C. PMMA films of 1μm were spun as received from supplier. In order to achieve higher thickness 5 overlapping layers of PMMA were spun. Once one layer was spun, it was thermally cured, then the second one was spun. Spin settings were 10 seconds at 300rpm and then 60 seconds at 1500rpm, curing 10min at 130°C. PVC films of 3 μm were prepared from a 10%wt PVC in Tetrahydrofuran, and spin coated 10 seconds at 300rpm and then 60 seconds at 600rpm. PS films of 5 μm were made from 10%wt MEK solution, and were spincoated at 1000RPM for 30 seconds and dried for 2h at 130°C. BCB films of 5 μm was spun as provided from manufacturer for 30 seconds at 1500RPM. Next a soft baking for 20min at 100°C, preceded a post bake (1hr 150°C) and hard bake (4hrs 250°C). SU8-2 films of 5 μm were obtained by spin coating at 1500RPM; follow by soft baking (5min 60°C) and baking (5 min 100°C), brief low UV light exposure, and a repetition of baking steps. PVA films of 5 μm were prepared by 85% and PAA 15% for 0.1G in 1 mL ethanol, dissolved at 85 degrees for 3 h followed by addition of 0.2mL PEG400, 10% glyoxal, and 10 μl phosphoric acid. Solution was spin coated at 4000RPM, and films were dried 100°C overnight. Parylene C was deposited using an SCS Labcoater 2 to a thickness of 2 μm.

**Cell Cultures.** Human Embryo Kidney (HEK293T) cells, obtained from ATCC, were cultured in Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 10 % fetal calf serum, 2 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (Life Technologies). Cells were plated at an equal density. The samples were thermally sterilized at 120 degrees Celsius, prior to overnight incubation in 10 micromolar laminin solution for HEK293T experiments. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

**Ohmic LED fabrication.** Cleaning of the ITO slides; film coating and electrode deposition were carried out all the same day to ensure optimum results. The glass substrates coated with ITO were supplied by Xin Yan Technology Lmtd. The glasses were sonicated in standard acetone and then in soap and DI water for 20 mins, in DI water 3 times for 5 min, in acetone twice for 5 mins and in isopropanol (IPA) for 5 mins, followed
by blow-drying of the substrates. The ITO substrates were treated by oxygen plasma treatment. Evaporated Al on top of ITO

**OLED Fabrication.** Conventional solution processing organic light emitting diodes were fabricated on glass by coating indium tin oxide (ITO of 10 ohm/sq.) with a 30 nm layer of poly(3,4-ethylenedioxythiophene : poly(styrenesulfonate) (PEDOT:PSS). Cleaning of the ITO slides; film coating and electrode deposition were carried out all the same day to ensure optimum results. The glass substrates coated with ITO were supplied by Xin Yan Technology Ltd. The glasses were sonicated in standard acetone and then in soap and DI water for 20 mins, in DI water 3 times for 5 min, in acetone twice for 5 mins and in isopropanol (IPA) for 5 mins, followed by blow-drying of the substrates. The ITO substrates were treated by oxygen plasma treatment. The PEDOT: PSS layers were deposited by spin coating at a spin speed of 3500 rpm for 40 sec and then annealed at 150°C for 20 min. Polymeric devices followed conventional PLED structure using PEDOT:PSS for the injection of holes from the ITO anode and Calcium for the injection of electron from the Aluminum cathode. The precursor solutions were prepared the day before device fabrication and the solutions were stirred at <50 °C overnight. The precursor solutions of fluorescent polymers (F8BT) were prepared by dissolving 6 mg material in 1mL Chlorobenzene and were spin-coated for 30 s at 1200 RPM on top of PEDOT:PSS. Following active layers deposition, Calcium Ca (25nm) and aluminum Al (100 nm) top-electrodes were deposited onto all the devices by thermal evaporation through a shadow mask at 10-6 mbar. Active areas measured 75 mm². The encapsulation of the devices was effected manually using another glass substrate of 1.1 mm adhered onto the device using flexible sealants from DELO-KATIOBOND and a low power UV light to set the glue.

**Freestanding Organic LED fabrication.** PDMS substrates were cleaned with ethanol and double-sided scotch on glass. A freestanding plasticized polystyrene PS film were deposited on top of PDMS. As received SU8-2 was spin-coated onto the PS film at 500 rpm for 6 s and then at 6000 rpm for 30 s. The SU8 films were coated with 30 nm of gold, using thermal evaporation at a pressure of 10⁻⁶ mBar. The samples were subjected to treatment protocols before and after gold deposition as described in chapter 5. Exposure was carried out for 25 s, using near UV (350-400 nm) illumination at an energy density of 100 mJ/cm². SU8/Au-C first carrying out a soft-bake, then depositing Au, and then carrying out the full standard procedure described in Chapter 5. The PEDOT: PSS layers were deposited by spin coating at a spin speed of 3500 rpm for 40 sec and then annealed at 150°C for 20 min. Polymeric devices followed conventional PLED structure using PEDOT:PSS for the injection of holes from the ITO anode. The precursor solutions were prepared the day before device fabrication and the solutions were stirred at <50 °C overnight. The precursor solutions of
fluorescent polymers (SY-PPV) were prepared by dissolving 6 mg material in 1mL Chlorobenzene and were
spin-coated for 30 s at 1200 RPM on top of PEDOT:PSS. Following active layers deposition, aluminum Al (5
nm) and gold Au (60nm) top-electrodes were deposited onto all the devices by thermal evaporation
through a shadow mask at 10-6 mbar. The encapsulation of the devices was effected by spin coating SU8-2
onto the top electrodes at 500 rpm for 6 s and then at 6000 rpm for 30 s. The contacts were stabilized by
depositing silver epoxy at the cathode and anode contacts and protected by scotch during the deposition of
SU8. After removing the scotch and revealing the contacts, the exposure was carried out for 25 s, using
near UV (350-400 nm) illumination at an energy density of 100 mJ/cm^2. PS film on top was deposited before
fully crosslinking the top SU8 layer.

**OLED characterization.** The electrical characteristics of the devices encapsulated and non-encapsulated
were measured in air and PBS solution at room temperature using a Keithley 2450 electrometer for the
electrical current-voltage measurement. Emission spectra were measured using a fiber-coupled Ocean
Optics 2000+ spectrometer. Absolute optical power intensity was determined using a large area calibrated
Si PIN Photodiode (28 x 28mm active area, from Hamamatsu) coupled to a Keithley 6517A electrometer.
Chapter 6: Millisecond control of neural activity by an organic LED interface

Optogenetics combines optics and genetics to enable minimally invasive cell-type-specific stimulation in living tissue. For the purposes of bio-implantation – and hence to allow the technology to realize its full promise – there is a need to develop soft, flexible, transparent, and highly biocompatible light sources. Organic semiconducting materials have key advantages over inorganic counterparts, including low Young’s moduli, high strain resistances, and wide color tunability. However, it is unclear whether sufficient optical power can be generated for successful stimulation, while remaining within a biologically safe operating window. Here we investigate the use of blue and red Organic Light Emitting Diodes (OLEDs) under continuous and 10 kHz pulsed operation illumination. We show that under pulsed mode operation the operating temperatures are effectively reduced and allow for safe and controlled photo-stimulation of neurons expressing microbial opsins.

6.1. Introduction

The optical control of ion currents across the plasma membrane by light-gated ion channels or transporters has led to new realms of possibilities in a wide range of established and newly emerging scientific disciplines. The applications are diverse and include the ability to investigate neural circuits with high spatiotemporal control \cite{442}, treat a wide classes of degenerative \cite{443} and paroxysmal pathologies \cite{444}, regulate artificial organ function as photo-induced insulin release \cite{367}, and trigger bio-hybrid robot movements.\cite{445} The success of optogenetics derives from both the ability to genetically target specific cell types and the versatile nature of the protein tools which may be engineered to alter kinetics, ion selectivity, optical responsivity and wavelength sensitivity, resulting in a wide range of photosensitive actuators.\cite{446–449} With the technology already in clinical testing for the treatment of degenerative blindness \cite{450}, a renewed drive has emerged to develop thin, soft and flexible, efficient, multi-color and highly biocompatible light sources, for both wearable and implantable devices \cite{71,451}.

Currently, spectrally matched targeting of photosensitive proteins is carried out primarily by filtered halogen lamps, lasers or light emitting diodes, with the light beam commonly delivered via microscopes or by coupling to optical fibers.\cite{452,453} There have also been increasing numbers of reports on arrays of µLEDs to target individual neurons in a broader network with high spatial precision.\cite{313,454} While initial results
have been promising, the bulky and rigid nature of the light sources and associated optical components have prevented the development of fully-implantable light sources. OLEDs by contrast may be fabricated using layers of soft conformable materials that match well to the prerequisites of wearable and implantable devices. The thin layers used in the OLED device stack add minimal (sub-micron) thickness to the substrate thickness, making it feasible to incorporate the devices in many locations.\(^{73,74}\) OLEDs may be readily processed onto a wide range of substrate materials, allowing for the formation of flexible \(^{76}\) and stretchable devices.\(^{45,71}\) The flexibility and variability of the fabrication methods make it possible to achieve homogeneously emitting pixel surfaces covering microscopic (\(\mu m\)) to macroscopic (m) length scales \(^{455,456}\), with thicknesses as low as 2 \(\mu m\).\(^{72}\) From a biological perspective, this positions the technology for carrying out many useful tasks, ranging from highly localized sub-cellular targeting \(^{442}\) through to organ targeting.\(^{457}\) Rapid progress has been made in implementing organic electronics for optogenetic activation in systems including *Drosophila* larvae and *HEK293T* cells \(^{297,356,379}\), but until now optical stimuli lasted seconds to minutes, or employed bulky CMOS backplane architectures to be generated. Furthermore, the biocompatibility of the various polymeric and metalo-organic materials used in the manufacturing of OLEDs has not been addressed thus far. The application of pulsing mode operation also remained uninvestigated, despite its potential to increase device performance and efficiency, both critical issues in biomedical applications. Overall, the current challenge is to identify appropriate OLED materials to design suitable devices that meet the constraints in terms of biocompatibility and optical requirements to elicit biological effects.

Here, we report on the favorable biocompatibility of poly(p-phenylene vinylene)-based and fluorene-based light emitting polymers. We further identify key operating parameters for achieving effective stimulation of microbial opsin-expressing neurons, and demonstrate the ability to control the neuronal firing rate using long trains of microsecond pulses. Lastly, we describe the use of OLEDs for millisecond timescale control over neuron firing, successfully obtained in central nervous system neurons expressing the opsin ChrimsonR. The results reported here pave the way to fully solution processed, highly flexible, low temperature operating OLEDs capable of delivering the required optical energy for a control of neural activity with high temporal precision.

### 6.2. OLEDs for Optogenetic Activation

Two optogenetic actuators were targeted for neural control: the blue light-activated Sustained Step Function Opsin (SSFO) \(^{447}\) and the red-shifted ChrimsonR Opsin \(^{448}\). Both optogenetic effectors are light-
gated cation channels targeted to the cell membrane, whose conductance results in inward (depolarizing) currents when expressed in neuronal cells. To match the activation spectra of the opsins, a series of blue- and red-emitting OLEDs were fabricated using PEDOT:PSS-coated indium tin oxide as the anode and aluminum-capped calcium as the cathode. To find appropriate devices with high optical output and strong spectral matches for the targeted opsins, each of the blue and red OLED types were assessed by emission spectra and optical power output (Appendix A17). Among all solution processed devices that were fabricated, we selected the fluorescent type OLEDs F(1) for the blue OLED:SSFO activation system, and OLED F(2) for the red-shifted OLED:ChrimsonR system (Fig. 46a,b). The emissive layer of OLED F(1) is composed of the fluorene based Poly(9,9-di-octylfluorenyl-2,7-diyl) (PFO) for a deep blue emission, and Poly(9-vinylcarbazole) (PVK) employed for hole transport and electron blocking properties. OLED F(2) is centered on a phenyl alkoxyphenyl based PPV copolymer, Livilux™ PDO-124 (SO-PPV), with orange emission. While the opto-electronically active materials reside at the core of the device, chemical degradation of insulators, and/or mechanical wearing of devices imply an inherent risk of the underlying materials becoming exposed to the biological environment over time. We therefore assessed the acute toxicity of the polymers employed in OLED F(1) and F(2) by measuring the viability of primary hippocampal neurons on cast spin-coated films. The evaluation of primary membrane properties obtained by electrophysiological recordings, revealed no significant differences between neurons grown on polymers and glass controls (Appendix A18).

Two critical challenges remain in order to achieve fully biocompatible OLEDs, namely (i) the identification of highly performant and water-stable electron injection materials; and (ii) the identification of appropriate dielectrics, characterized by extremely low water and oxygen penetration, high electrical resistivity, high biocompatibility, and long lifetime. Several promising candidates have recently been reported and discussed elsewhere. [379] To effectively insulate and protect the diodes from the saline and redox environment, and in turn, protect cells from leak currents, we employed glass encapsulation in this proof of principle study on rapid photo stimulation (Fig. 46a,b). The glass also protects from the highly reactive Calcium, whose substitution is the subject of many ongoing research efforts.[461,462] The device architecture can however be subsequently adapted to flexible substrates.

To assess the device performance for cellular photo stimulation we utilize patch-clamp recordings. The technique requires the user to position a microelectrode on the surface of a cell membrane to gain a high electrical resistance contact. The membrane is subsequently ruptured by negative pressure to gain electrical continuity between the interior of the recording pipette and intracellular volume. The delicate
operation is most efficiently carried out with optical feedback, typically via a transmission microscope that is coupled to a camera to allow the user to adjust the position of the electrode. To obtain patch-clamp recordings on a standard electrophysiology setup, we used 100 nm aluminum electrodes, offering a full device transmission of 5% at 650 nm (peak 8% at 850 nm), and permitting the use near infrared imaging for cell observation and patch-electrode manipulation (Fig. 46 and Materials and Methods). This allowed us to evaluate the parameters of OLED operation that can be exploited for successful opsins activation.

Figure 46: Blue and Orange OLEDs based on SO-PPV and PFO for SSFO- and ChrimsonR-based photoactivation. (a) Devices were optimized for combining photo-stimulation with patch-clamp recordings, with high near-infrared transmission levels allowing for electrode manipulation on a standard patch-clamp set-up. (b) Device architectures based on the biocompatible polymers for blue F(1) and orange F(2) OLEDs. (c) Fluorescence images of neurons at the OLED F(1) surface expressing eGFP, alongside the near infrared transmission image, and a merge of the two. Scale bars denote 50 μm. (d) The blue F(1) electroluminescence emission targets the SSFO activation spectrum, while the Orange F(2) emission peak is well aligned to maximal activation of ChrimsonR activation. Inset representation of OLED F(1) and OLED F(2) operating at 10V
6.3. Organic LED performances at ultrafast pulses

Under typical DC operating biases < 7 V OLEDs commonly provide optical output powers ranging from hundreds to thousands of nW/mm². However, the typical excitation levels required for optogenetics range from hundreds to thousands of µW/mm². Under continuous bias operation, blue F(1) type OLEDs emitted a peak optical power of 15.14 µW/mm² at 8 V (0.3 A/cm²) (Fig. 47a) while the orange F(2) OLEDs emitted a peak optical power of 34.12 µW/mm² at 15 volts (0.52 A/cm²) (Fig. 47d). Increasing the DC bias further resulted in rapid degradation of the organic layer due to Joule heating. Pulsed mode operation has previously been shown to improve the efficiency of OLEDs, allowing lower drive voltages and current densities to be used for a given optical output power.[463]

![Graphs and diagrams showing device performance of OLEDs for targeted optical stimuli.](image)

**Figure 47 | Device performance of OLEDs for targeted optical stimuli.** (a,d) J-V-P characteristics. Current density (black traces) and optical power density (blue or orange traces) for continuous (dashed lines) or 10 kHz (solid lines) stimulation for (a) OLED F(1) and (d) OLED F(2). (b,e) Temporal train of optical power for 3000 ms pulse stimuli for (b) OLED F(1) and (e) OLED F(2). Continuous in blue/orange and 10 kHz in black. (c,f) The optical power characteristics (mean ± sem) at different sub-pulses for (c) OLED F(1) and (f) OLED F(2).

This is evident from Fig. 2a,d, which show for both tested device structures substantially lower current densities and higher optical output powers for pulsed operation (10 kHz, 50 % duty cycle) at drive voltages...
in the range 6 to 30 V. Pulsed mode operation has the further advantage of improving operating lifetimes as is clear from Fig 47c,d which show the optical output power versus time at a drive voltage of 30 V. Under DC operation, the optical output power of the devices dropped to 50 % of their starting value within ~250 ms of switching on the device. Under pulsed operation by contrast, the optical output power remained at > 98 % of its initial value over a 3s test period. The findings are re-iterated in Figure 47c which reports the average peak optical power of blue F (1) type OLEDs measured for pulse lengths of 50, 500 and 3000 ms performed either in continuous light or with sub-pulsing frequencies of 2 and 10 kHz (50% duty cycle). With 50ms pulse lengths, the devices performed in a stable state for any constant and sub-pulsed frequencies with maximal outputs ranging from ~90 to 100 \( \mu \text{W/mm}^2 \). Instead for the longer 500ms and 3000ms pulse lengths, pulsed operation confers stability to the optical output. Orange OLEDs (Fig. 47f) followed a similar instability: while constant pulse yielded an average of approximately 60 \( \mu \text{W/mm}^2 \) at 3000 ms, sub-pulsing rates of 2 and 10 kHz achieved a stable peak power of about 150 \( \mu \text{W/mm}^2 \). For shorter pulse lengths (50 ms), orange OLEDs performed in a stable state for any constant and sub-pulsed frequencies.

6.4. Surface temperature

Visible light absorption in tissue at the power densities described above is expected to generate negligible heating. However, cells must be protected from the heat that is conducted away from the semiconductor heterojunction of the device. Aside from enhancing performance stability, pulsing operation should also enhance the thermal safety of the organic device. To validate this assumption, we investigated the surface temperature taking readings at the OLED’s glass surface in contact with air or the cell culturing-medium, while administering stimuli in continuous and pulsed operation at different voltage settings. Figure 48 shows the pronounced joule heating effects in operating diodes as measured by temperature changes at the device surface. We first measured the temperature change in response to 3000ms stimuli (inset bars, Fig. 48a,b), operated under 7 and 30V bias (upper versus lower, black traces). The temperature deflections increased significantly as a function of the diode operating bias, from a few tenths of a degree under 7V bias, to increases of 2°C for F(1) and 3°C for F(2) under a 30V bias. Pulsing mode operation dramatically reduced the surface temperature rise measured in response to the 3000ms stimuli of OLED F(1) (colored traces, Fig. 48a) and OLED F(2) (colored traces, Fig. 48c). The mean temperature changes, measured 7000ms after the onset of the 30V bias stimuli are reported in Fig 48b,d. For all stimulus lengths tested a ~50% reduction in temperature rise was observed in pulsing mode for both OLED F(1) and OLED F(2). Previous studies have discussed the effect of the biological heat sink. While we observed reductions in the rate of surface temperature rise when adding a small (500 \( \mu \text{l} \)) volume of cell culture medium, no
significant difference were observed between the absolute temperature rise measured in air and medium (Fig. 48b,d). This trend was observed for the three pulse lengths tested. Although circulation in vivo may contribute to an increased cooling of device by rapidly carrying heat away from devices, increased device efficiency and/or pulsed-mode operation are critical in limiting device surface temperature during prolonged operation.

![Graphs showing temperature rise](image)

**Figure 48 | Temperature rise by device operated at sub-pulses and continuous mode.** (a,c) Voltage dependence of temperature rise for continuous (black) and 10 kHz operation (blue/orange) at 7V (lower traces) and 30V (upper traces) in response to 3000ms stimulus (inset colored bars); (a) OLED F(1) and (c) OLED F(2). (b,d) Pulse length dependence on temperature rise at continuous (black bars) and 10 kHz operation (blue/orange bars) in air, and at 10kHz operation in physiological medium (grey) for (b) OLED F(1) and (d) OLED F(2) under 30V bias.

### 6.5. Blue OLED and SFFO coupling for depolarization of cortical neurons at various sub-pulse rates

Having attained a high irradiance output from the organic diode within a safe thermal window, we set out to test the coupling to opsin-expressing neurons. We first employed a low density culture of cortical neurons prepared from E18 mouse embryos, transfected at 6-8 DIV with the bi-stable SSFO \[^{447}\] and recorded 24 hrs after transfection. SSFO has one of the lowest recorded Effective Power Densities for 50% activation (EPD50) \[^{446}\], lying between $10^{-2}$-$10^{-3}$ mW/mm$^2$, thereby bringing its use within reach of the stable blue OLED F(1) performances under pulsed operation. The low activation power is due to the bi-stable nature of the SSFO opsin, which stays in an open conductive state after illumination with blue light, and closes with a relaxation time of 29 min.\[^{447}\] This allows sequential pulses of light to have cumulative
effects on open channel populations, bypassing the current decay due to rapid channel inactivation that is experienced by other opsins. The channel closure can however be hastened by a secondary pulse of yellow light.\textsuperscript{447} To this aim, we employed an external yellow inorganic LED focused through the microscope objective to verify that depolarizations elicited during blue OLED emission were indeed driven by opsin-based currents.

Figure 49 | Blue OLEDs elicit depolarization of SSFO expressing cells at various pulse rates. (a) Neurons were transfected with SSFO constructs and recorded in current-clamp configuration. (b) Representative responses from individual neurons; at all tested sub-pulse rates, 500 ms blue light stimuli successfully depolarized neurons, leading to sustained activation that was fully reversed by yellow light stimulation (c) Individual neurons (symbols) and mean (colored bars) amplitudes of blue light-induced depolarization in response to 500 ms stimuli and yellow light-induced hyperpolarization in SSFO-expressing neurons (n=8-11). (d) Sample recording from a mature neuron transfected with SSFO (blue trace) and illuminated for 2 s that was successfully driven to firing threshold (black trace, control mock-transfected neuron). (e) Effects of the stimulus length on the mean depolarization, excluding action potentials, of SSFO-expressing neurons in response to 2 s stimuli; depolarizations increased significantly over the periods of 450-500 ms and 1950-2000 ms of illumination (n=6). Modulation in dark was quantified as the change in membrane potential between 0-50ms and 950-1000ms before OLED stimulus (f) Firing rate of SSFO-expressing neurons increased significantly during prolonged illumination; 1s bins before (dark) and during the blue light stimulus (n=4).
Firstly, we compared 500 ms stimuli driven either by a full duty cycle continuous DC bias, or a 50% duty cycle bias with sub-pulse frequencies of 10 kHz, 5 kHz, or 50 Hz. SSFO transfected neurons plated on glass coverslips were positioned at the surface of the OLED’s substrate surface (75 mm² area) and immersed in extracellular solution for patch-clamp recordings (Fig. 49a). We recorded the changes in membrane potential induced by the various light stimulation protocols in current-clamp mode, by repeating the selected stimulus 5 times per recorded cell. Sample recordings reported in Fig. 49b show that the OLED emitted blue light effectively activated SSFO channels and depolarized the neuron. At all tested stimulation frequencies, the depolarization elicited by the blue OLED extended well beyond the duration of the blue light illumination window, and was readily reversed when the neuron was exposed to yellow light, confirming that the voltage responses recorded in the neurons were driven by SSFO-generated currents.[447]

The mean (±sem) response amplitudes measured in the analyzed neurons (Fig. 49c) did not vary significantly across the tested sub-pulse rates (depolarization: 3.9±0.6 mV, n=11; 7.2±1.3 mV, n=8; 6.2± 1.1 mV, n=8; and 5.7±1.5mV, n=8; for 10 kHz, 5 kHz, 50 Hz and continuous operation, respectively one-way ANOVA; p>0.05). Importantly, the amplitude of the depolarization induced by blue light was equal to that of the hyperpolarization elicited by the secondary pulse of yellow light (Fig. 49b,c) testifying that the depolarizing effects of blue light irradiation were fully reversible across all of the tested conditions. For comparison, we repeated the experiment with the blue emitting phosphorescent OLED P(1) (Appendix A19).

As expected from the lower optical power output of the device under equal voltage bias, illumination led to significantly smaller depolarization at all sub-pulse frequencies and continuous bias operation (p < 0.05, Mann-Whitney). We next used 2 s pulses with the aim of driving mature neurons transfected with SSFO to their firing thresholds. To maximize the optical output of the device and minimize surface temperature changes, the OLED was driven with a high frequency sub-pulse rate of 10 kHz. Most of the recorded SSFO-expressing neurons (n=4 out of 6 neurons) were driven to firing thresholds, firing multiple action potentials during the period of light emission (Fig. 49d-f). When the firing rate of these cells was analyzed in bins of 1 s, a significant increase of firing rate during the illumination period was measured (Kruskal-Wallis, p<0.05; Dunn’s Multiple Comparison Test vs. baseline, p<0.05). When the first spike elicited per stimulus was considered, a mean delay of 893.7± 139.9 s relative to the light stimulus onset, and a mean 139.9±112.4 ms jitter (calculated as the standard deviation of latency per cell) were observed. The remaining cells (n=2 out of 6), although exhibiting a mean depolarization of 6.84±2.12 mV over the 2 s illumination periods, did not fire action potentials. Mock-transfected neurons under the very same light stimulation conditions showed
neither depolarization, nor changes in action potential firing rate (-0.57±1.08 depolarization and no firing during/after the light stimulus). Despite the large loss of the optical power by the refractive losses at material interphases, and the assumed lambertian pattern of emission, the broad electroluminescence of the blue PLED F(1), using the PVK interlayer between PEDOT:PSS and PFO active layer, efficiently generated the required level of optical power for activation of neural firing. However, the relatively slow SSFO current activation is a limitation for fast and synchronous neuronal activation.

6.6. Millisecond Scale Control of Spike Firing using kHz sub-pulse rates in Chrimson-R red-shifted OLED system

Although the long continuous stimulation described in the blue OLED-SSFO coupling is suitable for tasks like photo-mediated insulin release or photo-regulation of gene expression, where minute-scale control suffices, many optogenetic applications require a more precise temporal control of both the onset and offset of opsin currents. We therefore sought to test the coupling of red-shifted OLEDs based on the biocompatible OLED F(2) paired to the rapidly inactivating opsin ChrimsonR.

The opsin is currently under investigation for use in clinical applications [464], as the redshift of the full system implies less cell phototoxicity and enhanced tissue-penetration of the exciting light in vivo. To gain high and widespread opsin expression levels in our primary neural networks, we packaged the opsin in a lentivirus delivery vector that efficiently transfected large numbers of neurons in a mature primary network. In order to elicit action potential firing with a high temporal accuracy, we used a short stimulus, sub-pulsed at a 10 kHz rate. In response to the stimuli, ChrimsonR-expressing neurons displayed both subthreshold depolarizations and light-mediated action potentials, while neurons recorded from non-transfected control cultures were unresponsive to the light stimulus (Fig. 50).

ChrimsonR-expressing neurons with subthreshold activations showed a mean depolarization of 5.89±1.52 mV (n=10) during the short 50 ms stimulus (Fig. 50b). These subthreshold depolarizations were reliably elicited across multiple OLED light stimulations (see sample trace in Appendix A20). In contrast to SSFO-expressing neurons excited by the blue OLED, prolonged illumination (2 s, 10 kHz sub-pulse rate) of ChrimsonR-expressing neurons led to a mean (±sem) depolarization of 4.3±1.5 mV (n=3) during the period of 450-500 ms of illumination that did not increase over the remainder of the light pulse (mean depolarization ± sem at 1950-2000 ms = 4.01±1.6 mV; see sample trace in Fig. 50c).
Red-shifted OLEDs targeting ChrimsonR expressing neurons allow millisecond control of action potential firing. (a) Primary neurons were transduced with lentiviral vectors to express ChrimsonR and responses to OLED F(2)-mediated orange light illumination were recorded by patch-clamp. (b) Subthreshold responses to 50 ms illumination in ChrimsonR-expressing cells. Wild-type neurons without opsin expression (WT) show no responses to light (p < 0.05, Mann-Whitney). (c) Sustained subthreshold responses to prolonged (2 s) illumination in ChrimsonR-expressing cells (orange trace); mock-infected neurons without opsin expression show no response to prolonged illumination (black trace). (d) Overlay of membrane responses of a single ChrimsonR expressing neuron to 25 repetitions of 50 ms pulses, successfully triggering action potential firing. (e) Representative traces of neurons successfully firing action potentials during five 50 ms light stimuli, at various inter-stimulus repetition rates. (f) Firing probability vs. stimulus frequency (0.13-6.4 Hz), calculated over first 5 stimuli per cell (symbols); inset bar showing group mean. (g) Representative spiking activity in response to 5 trains of 5 1 Hz-stimuli administered at 0.1 Hz. (h) Firing probability over multiple repetitions (25-100) following the representative stimulation protocol shown in showing individual cells (symbols) and group mean (inset bar) (g). (i) Peristimulus time histogram for the time window 50 ms before/50 ms after the stimulus (1 ms bins). (j) Quantification of the delay and jitter of action potential firing based on the first spike per stimulus calculated across all recorded neurons.
Importantly, the largest subset (n=13 out of 26 neurons) of ChrimsonR-expressing neurons showed a repeatable firing pattern in response to light, successfully reaching firing threshold within the stimulus (sample traces, Fig. 50d). To check the temporal precision of neuronal activation by the OLED-Opsin system, we delivered consecutive light stimuli (50 ms, 10 kHz sub-pulse rate) at various frequencies (0.13-6.4 Hz). As highlighted by the representative traces of Fig. 50e, action potential firing followed the light cues with a high fidelity. When the firing probability was analyzed during the stimuli (Fig. 50f), the ChrimsonR:F(2) system achieved a 100% firing probability at 0.13 Hz (n=6), 94% at 0.5 Hz (n=10), 92.5% at 1 Hz (n=8), 80% at 3.3 Hz (n=7), and 70% at 6.4 Hz (n=4), showing that neuronal firing patterns were tightly locked to the OLED’s optical output.

To test the reliability of the system, we lastly undertook an experiment in which neurons under patch-clamp recording were exposed to a long series of repeated stimulations (25-100 stimuli). The experimental protocol (Fig. 50g) employed trains of stimuli composed of five bursts (0.1 Hz bursting rate) containing five 50 ms stimuli (10 kHz sub-pulse rate and 1 Hz pulse rate). The trains were administered up to for 4 times per cell. To assess the performance of the system, we quantified the mean firing probability per cell over each 25 stimuli train (Fig. 50h), revealing a reliable elicitation of action potentials across all quartiles of the protocol (mean\(_{Q1}\) = 90.5%, n=7; mean\(_{Q2}\) = 93% n=5; mean\(_{Q3}\) = 89%, n=4; mean\(_{Q4}\) = 93%, n=4). A similar performance was obtained using 2-s inter-stimulus intervals (mean\(_{Q1}\) = 94%, n=4; mean\(_{Q2}\) = 91% n=3; mean\(_{Q3}\) = 79%, n=3; mean\(_{Q4}\) = 76%, n=3). The peristimulus time histogram (PSTH), which was generated from the cumulative action potential distribution per cell (Fig. 50i), clearly reveals that the increased firing probability coincided with the onset of the light stimulus. Indeed, when the time between light onset and the peak of the first action potential per stimulus was calculated for all firing neurons tested with F(2) OLEDs, we found that the events were elicited with a mean (±sem) delay of 19.2±3.3 ms and a mean (±sem) jitter of 6.2±1.1 ms (Fig. 50l). In contrast to ChrimsonR-expressing neurons, non-transduced cells present in the same neural networks were largely unresponsive to light (mean depolarization in light= 0.09 ± 0.32 mV, n=6), though some delayed responses, likely mediated by synaptic network connectivity to opsin expressing neurons were observed (Appendix A20). In sum, the responses documented here highlight the ability of the F(2):ChrimsonR couple to photo-stimulate neurons, showing that light-emitting diodes based on organic conjugated polymers can be used to elicit neuronal activations over large areas.
Optogenetic activation relies on the interplay of several variables. Thus, we introduce a procedure for measuring the full OLED-Opsin coupling efficiency. For simplicity, we can consider the opsin photocurrent of the neuron versus the total radiative energy at the neuron as the essential parameters to approximate the experimental external quantum efficiency of the entire OLED:Neuron coupling system (see Equation 10 in the Appendix A22). The Quantum efficiency of OLED-OPSIN coupling for neuron activation ($\eta_{\text{oled-opsin}}$) is therefore the product of device external quantum efficiency, the loss during the transmission of the optical power to the neuron, and the subsequently generated ion current across the cell membrane. This simplification ignores the quantum efficiency of opsin activation, and the metabolic power supply of the cell which generates the requisites ionic gradient. To capture the necessity of spectral overlap between device emission and opsin activation spectrum, we introduce opsin specific OLED efficiency ($\eta_{\lambda}$). The term is analogous to the standard luminous efficiency measurements defined to the human eye’s light sensitive protein, but corresponds instead to the utilizable wavelength energy defined by the overlap of the targeted opsin’s wavelength responsivity and the OLED’s emission spectrum (see Equation 13 in Appendix A22). It reflects how much of the emitted light in the path of the cell can be used to activate the opsin channels. Finally, the specific activation efficiency ($\eta_{\lambda}$) corresponds to the total external quantum efficiency, including the spectral matching between the activation energy and the electroluminescence spectrum of the OLED (see Equation 15 in Appendix A22), and is a unitless measure of system efficiency.

Three conclusions can be drawn observing the efficiencies of the OLED F(1)-SSFO, OLED F(2)-ChrimsonR, and the OLED P(1)-SSFO systems. The first is that the current systems are inefficient, obtaining activation efficiencies on the order of $10^{-14}$ to $10^{-11}$%, OLED-Opsin couplings on the order of $10^{8}$ to $10^{7}$% and opsin-specific OLED efficiencies on the order of $10^{-1}$ to $10^{1}$ Wsr^{-1}A^{-1}. This is in part due to the use of a thick glass spacer between the emitting layers and the targeted cells, leading to extensive power loss by the geometrical spreading of the unfocused light. It is also due to overly broad electroluminescence spectrum, which means a significant fraction of the emitted light lies outside the activation window of the target opsin; the electroluminescent emission spectrum (EL) of the blue fluorescent OLED F(1) based on PVK/PFO, shown in Figure 1d, matches well with the activation energetic spectrum required for SFFO with a calculated 80.45% of overlap, compared to 75.21% of overlap using a blue phosphorescent OLED P(3). The EL of the Orange fluorescent OLED F(2) match with ChrimsonR activation energetic spectrum with a calculated 71.16% of overlap (see Fig. 46; Equation 12 in Appendix A22).
Figure 51 | Characterization of OLED-Opsin couple efficiencies. Influence of the pulse length on specific OLED efficiency (a), coupling OLED-OPSIN efficiency (b) and specific activation efficiency (c). Dark-blue squares: F(1):PVK/PFO using SFFO; orange squares: F(2):SO-PPV with CrimsonR. Influence of the sub-pulse rate on the specific OLED efficiency (d), coupling OLED-OPSIN efficiency (e) and the specific activation efficiency (f). Dark-blue squares: F(1):PVK/PFO using SFFO; light-blue circles: P(1):Firpic with SFFO.

Next, sub-pulsing the devices leads to large enhancements of the opsin activation efficiencies, owing to the increased device quantum efficiency under pulsed operation (Fig. 51). The excessive current density (>0.6 A/cm²) of the OLED at high voltages (30 V), which leads to degradation of devices in continuous mode, is reflected by the lower values of specific OLED efficiency, coupling OLED-OPSIN efficiency and specific activation efficiency. At higher sub-pulse rate, the current density decreases while optical power increases, playing a major role in the increase from \(~10^{-2}\) W·sr⁻¹·A⁻¹ (continuous) to \(~10^{-1}\) W·sr⁻¹·A⁻¹ (50 Hz) for specific OLED efficiency; \(~10^{-8}\)% (continuous) to \(~10^{-7}\)% at 50 Hz for coupling OLED:OPSIN efficiency; and \(~10^{-13}\)% (continuous) to \(~10^{-11}\)(50 Hz) for specific activation efficiency of device F(1). The phosphorescent OLED P(1) behaved similarly for the three efficiencies increase when comparing continuous to pulsing mode, but at lower values than for the fluorescent OLEDs F(1). Finally, activation efficiencies may change as a function of stimulus length. In the case of SSFO, which stays in an open conductive state following activation, the effective activation efficiency will continue to increase with time, until a full SSFO population activation is achieved. This explains the higher value of activation efficiency for long pulses for SSFO (Fig. 51), which is absent in the case of the more rapidly inactivating CrimsonR.
6.8. Discussion

We have described the utility of OLEDs for biological manipulations, using organic light-emitting polymers in devices for the optogenetic activation of primary neurons. Following a positive evaluation of PFO and SO-PPV biocompatibility, we fabricated and characterized devices based on those polymers, and compared continuous DC bias to pulsing mode operation. Using ultrashort length pulses, devices exhibited stable and high brightness output at elevated voltages (30 V), achieving 100 μW/mm² and 134 μW/mm² for the blue and orange OLED. Though causing delay in opsin responses, μs sub-pulses were successfully employed for ms scale control of neural activity. The operating mode proved particularly beneficial for long stimuli requiring maximal diode output, during which surface temperatures rapidly increase, and optical power decreases in DC continuous bias operation. The challenge for the next generation bio-OLEDs for optogenetics is to exploit the above-described OLED-Opsin coupling factors, in order to increase the low degree of external quantum efficiency. Synthesizing novel organic materials will give rise to further advances in mechanically and chemically stable OLEDs using cross-linked and biocompatible materials for interfacing with biology. Major gains will be obtained by minimizing the distance between emissive layers and the tissue with the use of thin and flexible and biocompatible polymeric insulators with matched refractive index and a high resistance to water and oxygen penetration. All of these factors will contribute to extending the operational lifetime of the organic diodes, which remain limited to a few 100s of stimulus cycles at the elevated voltages employed, even under the pulsed-operation mode. Until extremely high activation efficiency devices are developed, the decrease in the distance between the cell and diode will increase not only the optical power density, but also surface temperature. Thus, stimulus patterning with pulsed rate will remain a critical factor determining thermal safety and stability.

On this note, the increasing efficiency of other types of light sources, namely Light Electrochemical Cells (LECs) [317], along with improvements in their onset kinetics, may represent attractive alternatives for soft electronics applications requiring low operating temperatures. Along with Organic Light Emitting Transistors (OLETs), these alternative device architectures may yet prove their utility, although the maximal optical output described in the literature remains too low for successful optogenetic stimulation. Similarly, new types of light emitting materials based on Thermally Activated Delayed Fluorescence (TADF) [103], promise dramatic improvements of OLED IQE. In the fluorescent devices employed here, only singlet excitons generate photons, while triplet excitons remain unused, non-radiatively relaxing as heat. By introducing a heavy metal into the organic materials, phosphorescent OLEDs can enable the conversion of
triplet excitons into photons, which can improve the IQE\textsubscript{oled} to 100%. Although the phosphorescent devices fabricated here showed improved external quantum efficiency at low voltage compared to polymeric devices, this dropped at higher voltages, limiting their optical output power. To exploit the maximum external quantum efficiency of the current devices at low voltages, the distance between the cells and emitting layers must be minimized, or more sensitive optogenetic tools must be employed.

In the current architecture, the OLEDs fabricated on glass present the possibility of developing smart culture substrates for \textit{in vitro} experiments. To highlight the broad potential of OLEDs for optogenetics, we benchmarked performances of the solution processed OLEDs in comparison with the lowest reported optogenetic activation requirements, revealing a series of promising OLED-Opsin systems (Appendix A21). The listed optogenetic tools are primarily light-gated proteins that mediate ion currents across the membrane, but also include others tools as photo-gated enzymes, and photo-gated regulators of gene transcription. Similar to SSFO, the latter group of optogenetic tools often stay active for prolonged periods of times following activation, meaning that long pulses of low power light may be employed to achieve the intended biochemical manipulation. Accordingly, the lower end of the graph is mainly populated by such tools. In addition, ChR2-XXL \textsuperscript{[465]}, a blue light activated opsin with very large single channel cation conductance, has been repeatedly exploited to gain control of neural activity at extremely low powers. Though not a comprehensive list, it is evident that existing optogenetic tools and OLEDs present a wide array of compatible OLED-opsin couples, while the promising initial results of biocompatibility bode well for finding additional materials for an optimized matching of effector activation across the visible spectrum.

Looking forward, embedding well encapsulated and high efficiency devices in hydrogel lenses, would extend the range of application to treatment of retinopathies and virtual reality contact lenses. A hydrogel can also be used as a very flexible spacer for thermal dissipation in the device stack. Alternatively, a 3D device structure in which a core of solid insulated OLED device composed of scaffolds with many pores and pillars is immersed in hydrogel, may open new avenues to light-mediated controls for tissue engineering applications. Overall, pixel scale can be widely varied to make subcellular scale pixels or large pixels to cover entire organs. For the former, ultra-short pulses can be utilized to sequentially target distinct opsin population in the soma, axon, or individual synapses, to allow the stimulation speed necessary to mimic complex network inputs over large areas in parallel. The promise of OLED technology is a system that is easily implementable and devoid of external optics. The rapid pace of progress in both OLED and optogenetic technologies ensures that OLEDs will continue to find numerous applications in bioelectronics.
6.9. Methods

Materials. Materials employed were as follows; MEH-PPV: Poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] for F(4); CN-PPV: poly[2,5-di(hexyloxy) cyanoterephthalylidene] for F(5); PFO: Poly(9,9-di-n-octylfluorenyl-2,7-diyl); and PVK: Poly(9-vinylcarbazole) for F(1) devices; , were ordered from Sigma Aldrich. Super Orange (SO): Phenyl alkoxyphenyl PPV copolymer, conjugated polymers Livilux™ PDO-124 (SO-PPV) for F(2) devices were obtained from Merck PPF. Ir(MDQ)2acac : Iridium (III) bis(2-methyldibenzo[f,h]quinoxaline) (acetylacetonate) (ADS076RE) for P(5) and DFlr : Bis(2-(9,9-dibutylfluorenyl)-1-isoquinoline(acetylacetonate) for P(8) devices were purchased at American Dye Source, Inc. FlrPic : Bis[3,5-difluoro-2-(2-pyridyl)phenyl-(2-carboxypyridyl)iridium(III) (LT-E607) for P(1); Flr6 : (LT-N669): Bis(2,4-difluorophenylpyridinato)tetrakis(1-pyrazolyl)borate iridium(III) for P(2); FK306 : (LT-N620): Bis[4-tert -butyl-2,6'-difluoro-2,3'-bipyridine][acetylacetonate]iridium(III) for P(3); Hex-Ir(phq)3 : (LT-N741): Tris[2-(4-n -hexylphenyl)quinoline]iridium(III) for P(4); Hex-Ir(pic)3 : (LT-N754): Tris[2-(4-n -hexylphenyl)quinoline]iridium(III) for P(6); Ir(btp)2(acac) (LT-E709) : Bis(2-benzo[b ]thiophen-2-ylpyridine)(acetylacetonate)iridium(III) for P(7) devices and TPBi: 2,2',2"-(1,3,5-Benzinetriyl)-tris(1-phenyl-1H-benzimidazole), TAPC : Di-[4-(N,N -di-p -tolyl-amino)-phenyl]cyclohexane , PBD: (2-(4-Biphenyl)-5-(4-tert-butylphenyl)-1,3,4-oxadiazole), TBD: (N,N'-bis(3-methylphenyl)-N,N'-diphenylbenzidine) were obtained from Lumtec. For the biocompatibility assays, thin films of fluorescent polymers (PFO, PVK, SO-PPV) were prepared by dissolving 6 mg material in 1mL Chlorobenzene. Glass coverslips were spin-coated for 30 s at 1200 RPM, and annealed at 150°C for 20 min.

Electrophysiology. Whole-cell patch-clamp recordings of cultured neurons were performed at room temperature using patch pipettes (4–8 MΩ), after attaining GΩ patch seals. Traces were acquired in current-clamp mode using HEKA EPC10 amplifier and digitizer, and Patchmaster software (HEKA). The extracellular solution contained NaCl (135 mM), KCl (5.4 mM), MgCl2 (1 mM), CaCl2 (1.8 mM), HEPES (5 mM) and glucose (10 mM), and was adjusted to pH 7.4 with NaOH. The intracellular solution contained K-gluconate (126 mM), KCl (4 mM), MgSO4 (1 mM), CaCl2 (0.02 mM), BAPTA (0.1 mM), glucose (15 mM), HEPES (5 mM), ATP (3 mM) and GTP (0.1 mM), and was adjusted to pH 7.3 with KOH. Responses were amplified, low-pass-filtered at 3.9 kHz, digitized at 20-50 kHz, stored, and analyzed with Matlab (Mathworks).

Cell Cultures. Polymeric films and glass culture substrates were thermally sterilized at 120°C, prior to overnight incubation in 0.1% PLL solution. Primary cultures of hippocampal neurons were prepared from
embryonic 18-day rat and mouse embryos (Charles River). Briefly, hippocampi or cortex were dissociated by a 15-min incubation with 0.25 % trypsin at 37 °C and cells were plated on poly-L-lysine-coated substrates (0.1% PLL in Borax solution overnight) in Neurobasal supplemented with 2 mM L-glutamine, 2% B27, 100 μg/ml penicillin and 100 μg/ml streptomycin, and with 10 % horse serum (Life Technologies) in the first 4 h of plating. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

**Opsin Constructs, Transfection, Virus Production.** Neurons were transfected by Lipofectamine 2000 (Thermo Fischer Scientific) reagent using a 1 hrs incubation time and supplier protocol. pAAV-Ef1a-DIO hChR2(C128S/D156A)-EYFP was a gift from Karl Deisseroth (Addgene plasmid # 35503). FCK-ChrimsonR-GFP was a gift from Edward Boyden (Addgene plasmid # 59049). Third-generation Lenti-Virus were produced by transient four-plasmid co-transfection into HEK293T cells using the calcium phosphate transfection method. Supernatants were collected, passed through a 0.45 μm filter and purified by ultracentrifugation.

**Stimulation patterns.** For photoactivation of young neurons expressing SSFO, 500 ms pulses were applied with either full duty cycle continuous DC bias, or by 50% duty cycle stimulus with sub pulse frequencies of 10 kHz, 5 kHz, 50 Hz. For experiments on mature neurons expressing SSFO, 2000 ms pulses driven with 10 kHz sub-pulses at a 50% duty cycle were employed. Experiments on SO-PPV based device for excitation of ChrimsonR-expressing culture employed 50 ms and 2000 ms stimuli, composed of 10 kHz sub-pulses at 50% duty cycle. Unless otherwise stated, a 30 V bias was employed to drive the organic semiconductor based diodes.

**OLED Fabrication.** Conventional solution processing organic light emitting diodes were fabricated on glass by coating indium tin oxide (ITO of 10 ohm/sq.) with a 30 nm layer of poly(3,4-ethylenedioxythiophene : poly(styrenesulfonate) (PEDOT:PSS). Cleaning of the ITO slides; film coating and electrode deposition were carried out all the same day to ensure optimum results. The glass substrates coated with ITO were supplied by Xin Yan Technology Lmtd. The glasses were sonicated in standard acetone and then in soap and DI water for 20 mins, in DI water 3 times for 5 min, in acetone twice for 5 mins and in isopropanol (IPA) for 5 mins, followed by blow-drying of the substrates. The ITO substrates were treated by oxygen plasma treatment. The PEDOT: PSS layers were deposited by spin coating at a spin speed of 3500 rpm for 40 sec and then annealed at 150°C for 20min. Polymeric devices followed conventional PLED structure using PEDOT:PSS for the injection of holes from the ITO anode and Calcium for the injection of electron from the Aluminum cathode. The precursor solutions were prepared the day before device fabrication and the solutions were stirred at <50 °C overnight. The precursor solutions of fluorescent polymers (PFO, PVK, SO-PPV, MEH-PPV
and CN-PPV) were prepared by dissolving 6 mg material in 1mL Chlorobenzene and were spin-coated for 30 s at 1200 RPM on top of PEDOT:PSS. The phosphorescent devices used PEDOT: PSS for the injection of holes from the ITO anode, TAPC as hole transport and electron blocking layer and Calcium for the injection of electron from the Aluminum cathode, TPBi for electron transport and hole blocking layer. The precursor solutions of phosphorescent metallo organic dyes (P(1): Firpic; P(2): Fir6; P(3): FK306; P(3): FK306; P(4): Hex-Ir(phq)3; P(4): Hex-Ir(phq)3; P(5): Ir(MDQ)2acac; P(6): Hex-Ir(pic)3; P(7): Ir(btp)2(acac); P(8): DFIr) were prepared by dissolving 1 mg material blended with 4 mg PBD, 1.5 mg TBD and 10mg PVK in 1mL Chlorobenzene. Following active layers deposition, Calcium Ca (25nm) and aluminum Al (100 nm) top-electrodes were deposited onto all the devices by thermal evaporation through a shadow mask at 10^-6 mbar. Active areas measured 75 mm². The encapsulation of the devices was effected manually using another glass substrate of 1.1 mm adhered onto the device using flexible sealants from DELO-KATIOBOND and a low power UV light to set the glue.

**OLED pulsing characterization.** The electrical characteristics of the devices encapsulated were measured in air at room temperature using a Keithley 2450 electrometer for the electrical current-voltage measurement. Emission spectra were measured using a fiber-coupled Ocean Optics 2000+ spectrometer. Absolute optical power intensity was determined using a large area calibrated Si PIN Photodiode (28 x 28mm active area, from Hamamatsu) coupled to a Keithley 6517A electrometer. An OPT101P Photodetector Amplifier from Texas Instruments connected to the National Instruments NI PCI-5112 100MHz Digital Oscilloscope Board was used to detect fast pulsing mode imposed by the arduino uno microcontroller.

**OLED temperature characterization.** Surface temperature was measured and digitized by placing a thermistor sensor (TC-3444B, Warner Instruments), in firm contact with OLED surface. Output voltage trace responses were analyzed in Matlab (Mathworks). For recordings in culture medium, 500 µl of Hank’s Balanced Salt Solution (HBSS) were used to submerge sensor.

**Statistical analysis.** Statistical tests were selected based on the data distribution. Normal distribution of the data was verified using the D’Agostino and Pearson omnibus normality test (p value = 0.05). The sample size was indicated as number of recorded neurons (n). Analysis was carried out with Graphpad Prism 5.01 (Graphpad Software Inc.)

**Data availability.** The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary Information.
Chapter 7: Organic LEDs for *in vivo* optical imaging of neural activity

This chapter highlights the performance of an Organic Light Emitting Diode (OLED) for its use in intrinsic and extrinsic *in vivo* optical imaging of brain activities.

Experimental protocols have been approved by the Marseille Ethical Committee in Neuroscience (Approval A10/01/13, Official National Registration 71-French Ministry of Research).

7.1. Introduction

Optical imaging, in neuroscience, aims at visualizing the functioning brain surface *in vivo* to study either cortical development or its organization and functions. It provides us with a way to understand more about the mechanism of processing information. The benefit of using light provides high sensitivity of functional changes by either intrinsic contrast changes in absorption, fluorescence, scatter or the use of exogenous optical contrast agents.\(^{[466]}\) The light is used to map micro to meso-scale brain structure and function *in vivo* of an exposed animal brain. It leads to various applications – such as understanding the sensory and cognitive processing functions of the brain, the mechanisms of the neuronal response and the hemodynamic upon a stimulus or the effects of diseases and treatment on the brain.\(^{[467,468]}\)

A conventional excitation light source is provided by a halogen lamp, filtered at a specific wavelength according to use and both coupled or not to optic fibers.\(^{[469-472]}\) The light source is not easy to integrate into an implanted recording chamber. Moreover, their bulky nature and high Young’ modulus impose inherent restrictions on devices in terms of size, and flexibility to be compatible with the curvature of the brain. Due to this curvature in fact, the size of the recorded area is limited as just a small area is simultaneously in focus and homogeneously illuminated. Alternative light sources are needed to provide a large homogeneous emissive area with biocompatibility, sufficient irradiance, appropriate color tunability and mechanical flexibility properties that would integrate emitting devices in an optical chamber with the aim of providing larger homogenous illumination by the design of flexible light-emitting structure which might be integrated to the recording chamber.

Organic light-emitting diodes (OLEDs) are a potential alternative light source for intrinsic and extrinsic *in vivo* optical imaging for their unique properties such as homogeneity \(^{[73,74]}\), color tunability \(^{[13-18,473]}\), mechanical flexibility \(^{[70,155,156,314]}\) and transparency.\(^{[474-481]}\) As previously mentioned, organic semiconductors synthesized for OLEDs match well to the prerequisites of wearable and implantable devices.
Organic emissive layers have mechanical and optoelectronic properties that allow fabrication of ultra-thin layers (nanometer-scale) enabling incorporation of light emitters almost anywhere[73,74] leading to advances in flexible[70] and stretchable devices [45,71] that would perfectly follow the curvature of the cortex of the brain. The performances of transparent OLEDs are progressing rapidly and have now reached the market enabling new display and lighting application (e.g. mobile-phone display [482–485] and large-size OLED TVs [486–489], but their operating optical power is insufficient for use as a light source for optical imaging. Here we exploit a conventional OLED for intrinsic in vivo imagery and the use of a semi-transparent OLED for Voltage Sensitive Dye Imagery.

7.2. Organic LEDs for Optical imaging

Two techniques were targeted for optical imaging: two-dimensional intrinsic (or hyperspectral imaging of oxy- and deoxyhemoglobin dynamics) and two-dimensional voltage sensitive dye imaging VSDI (providing sensitivity to neuronal activity).

First, two-dimensional intrinsic of oxy- and deoxyhemoglobin dynamics can measure the hemodynamic response to functional activation in the brain. There is a special correlation between the hemodynamic response and the electrophysiological brain activity.[490] Hemoglobin in blood is the most important light absorber which is distinguishable under visible and NIR light (see spectrum figure 52b) and, as the brain functions, there are changes in the blood flow, volume and oxygenation. As oxygen is transported by blood to tissues leading to the conversion of HbO₂ to HbR, so alterations in the amount of oxygen in the blood indicate changes in the relative concentrations of HbO₂ and HbR. Changes in the absorption characteristics of the brain are modulated by the changes in these relative concentrations. The hemodynamic response to any stimulus can be monitored by mapping the changes of light intensity of an exposed cortex irradiated by an external light source (epi-illumination with optic fibers). Different types of map can be obtained from sensitive changes in the concentration of either HbO₂ or HbR by specific measurement at particular wavelengths related to HbR and HbO₂ absorption spectra (Fig 52b). Therefore, a light source emitting at different wavelengths is required. Here will focus on changes related to deoxyhemoglobin, which occur following activation of neuronal population after the presentation of a sensory stimulus.

Second, two-dimensional Voltage Sensitive Dye Imaging (VSDI) employs a fluorescent probe that is an electro-optical transducer which transcribes in real time the membrane potential changes to fluorescent intensity. It enables the detection of neuronal population activity (subthreshold modulation: spiking and
synaptic) \[^{[49]}\] of an exposed cortex *in vivo* with a much higher temporal resolution compare to intrinsic imaging. The cortex needs first to be stained with a dye that binds to the neuron’s membranes and fluoresces when a change in membrane potential occurs. A conventional optical imaging set-up halogen lamp, encounters filters and lenses, which are placed in the vertical illuminator before a dichroic beam splitter that results in coaxial illumination of the cortex. Such illumination is a priori more homogeneous compared to the intrinsic epi-illumination, but it is restricted to the apex of the imaged cortical area in focus, due to its curvature. In order to allow the separation of the excitation light directed to the cortex, the dichroic mirror is placed at 45 degrees to the propagation direction.

**Figure 52** | Orange OLED based on SO-PPV for optical intrinsic and extrinsic imaging. (a.) Device structure based on SO-PPV as emissive layer. (b.) Deoxy- and oxy- hemoglobin absorption spectrum and the electroluminescence of the orange OLED used for intrinsic imagery. (c.) The dye absorption and emission spectrum and the excitation spectrum from the orange OLED used for Voltage Sensitive Dye Imagery. (d.) Semi-transparent orange LED operating at 10 V. (e.) The side mode position of the opaque orange OLED tilted at 45° used for intrinsic imagery of rat’s cortex. (f.) The transparency mode position of the semi-transparent orange OLED used for extrinsic imagery of rat’s cortex.
In this way, without specific filtering, light returns to the CCD cells of the camera from the reflected and/or scattered light from the cortex and/or fluorescence from the dyes. Additional filtering can be applied to isolate the requisite wavelength related to functional signals. However, there is a need to increase the surface of the cortical area which receives homogenous illumination.

An orange emitting solution processing OLED was fabricated to match either the hemoglobin absorbance or the dye activation fluorescence spectra depending on what technique was used. Polymeric devices followed a conventional PLED structure using PEDOT: PSS for the injection of holes from the ITO anode and calcium for the injection of electrons from the aluminum cathode. A phenyl alkoxyphenyl PPV copolymer (SO-PPV) was used as emissive layer selected for spectrum electroluminescence to match with spectral absorption of hemoglobin and the chosen dye (fig 52).

![Graph](image)

**Figure 53 | Transmittance of the opaque and semi-transparent orange organic LEDs based on SO-PPV for optical intrinsic and extrinsic imaging.** The side mode position of the opaque orange organic LED (glass/ITO/Pedot:pss/SO-PPV/Ca/Al/glass with Al = 120nm) tilted at 45° used for intrinsic imagery of rat’s cortex. Transmittance of the opaque organic LED in red. However, the transparency mode position of the semi-transparent orange OLED (glass/ITO/Pedot:pss/SO-PPV/Ca/Al/glass with Al = 12nm) is used for extrinsic imagery of rat’s cortex. Transmittance of the semi-transparent organic LED in Al=20 nm in blue and Al = 12 nm in black.
Aluminum electrodes of 120 nm (for opaque devices) and aluminum electrodes of 12 nm (for semi-transparent devices) has been used to obtain organic LEDs integrated on a standard imaging setup. Here, opaque organic LEDs are used for intrinsic imaging and semi-transparent LEDs are used for Voltage Sensitive Dye imaging. The choice is due to the fact that the light scattered during intrinsic imaging is weak and cannot be detected through a semi-transparent device. However, the 30 % of transparency in the semi-transparent organic LEDs (fig 53) used for VSD dye imaging is sufficient to allow the luminescence of the dye activated to pass through the device and to be caught by the camera (on top). When the opaque device is positioned on the side outside the cortex view-point from the camera, it prevents interference with the image acquisition that will result in lower detectability and higher signal to noise ratio (See image in figure 52e). This type of device was found to be useful only for intrinsic optical imagery. On the other hand, when the semi-transparent OLED is positioned flat, perpendicular from the optic path to the camera, it allows illumination of the cortex and the functional emitted fluorescence to pass back through the device (See image in figure 52). This position allows evaluation of the parameters of OLED operation.

7.3. Organic LED performances

Device performance of OLEDs for targeted optical imaging tested on the cortex of a rat. In order to investigate that the device effectively provides uniform light, an opaque and a transparent organic LED was positioned on the side of (similar to fig 52e) and through (similar to fig 52f) the optical field of imaging and the electroluminescence irradiated the cortex of a monkey.

7.3.1. Semi-transparent and opaque organic LED performance at ultrafast pulses

Both opaque and semi-transparent devices were characterized at 5kHz. We have explained previously, in chapter 6, the benefits of applying the pulsing mode operation to decrease the Joule heating. Applying 5kHz, the orange opaque OLED emitted a peak optical power of 150 μW/mm² at 30 V (2 A/cm²), while the semi-transparent OLED emitted a peak optical power of 85 μW/mm² at 30 volts (0.5 A/cm²) (Fig 54). The semi-transparent OLED tested substantially increased the current density while decreasing the light intensity. The reduced thickness of (12 nm) of aluminum causes a reduction of charge injection from the cathode electrode to the SO-PPV active layer.
Figure 54 | Device performance of OLEDs for targeted optical imaging tested on the cortex of a rat. (a) J-V-P characteristics. Current density for opaque OLED (dash black) and semi-transparent OLED (solid black) and (b.) optical power density for opaque OLED (dash orange) and semi-transparent OLED (solid orange) at 5kHz. Green map of the cortex of a rat illuminated by (c.) Halogen; (d.) OLED at 10V; (e.) OLED at 20V and (f.) OLED at 30V.

We then experimented with semi-transparent organic LEDs positioned in transparency mode at the horizontal of the rat’s cortex. We aimed at investigating the appropriate voltage for sufficient optical intensity. As can be observed above, the map intensity increased as the voltage of the semi-transparent OLED was increased. We clearly observed vessels using halogen while the semi-transparent OLEDs placed on the way plays a role of an optical neutral density that reduces the recording, but we can already see vessels at 10V.

7.3.2. Organic LEDs to enhance light uniformity

The electroluminescence emitted from organic LEDs appears uniformly bright from all directions of view (approaching a lambertian emission). We exploited this property with an opaque organic LED positioned on side mode at 45 degrees from the horizontal plane to obtain a uniform working light (See figure 52 g.). The light uniformity on the cortex with no interferences with any loss of image contrast of vessels in a similar way than what conventional halogen is capable of providing. (See figure 55).
Figure 55 | OLED vs. Halogen uniformity of illumination tested on the cortex of a monkey. Comparison of cortical illumination using conventional halogen bulb with epi (optic fibers in (e.) & (f.) or coaxial illumination (dichroic mirror in (a.) & (b.) at two different wavelengths: 540nm for (a.) & (e.) and 605nm for (b.) & (f.) The map in (c.) correspond to image acquired through the transparent OLED positioned in VSD mode and the one in (g.) with the sided opaque device tilted at 45°. The gray scale histogram for (d.) OLED through (in blue) and conventional halogen bulb with epi illumination at 540 nm (in black) and 604 nm (in green) (h.) OLED side (in blue) and conventional halogen bulb with epi illumination at 540 nm (in black) and 604 nm (in green).

The gray scale histogram-(See figure 55 (d.) & (h.)) indicates the relative transmittance (or reflectance), and the standard deviation shows a dispersion measurement that can be used as an estimation of the spatial heterogeneity. Figure 55 d shows similar number of pixels emitting at different gray scale intensity (see spread black, green and blue histogram). We can see here that the positioned OLED at 45 degrees emitted a similar uniformity to the halogen coupled with fiber optics.

The large emissive area from organic LEDs provides unique uniform working light on a large scale. Large emissive pixels from a flat and semi-transparent organic LED are positioned horizontally near the upper top surface of the optical chamber. A high voltage was required to observe the blood vessels from the monkey cortex (See figure 55 c.). Although the image was blurred, we observed an enhancement of light uniformity. Figure 55 d shows a higher peak of the number of pixels regrouped at high intensity (in blue) compared to halogen light at 540 nm (in black) and at 605 nm (in green), having lower uniformity with the number of pixels emitting at different gray scale intensity.
7.4. Test of organic LEDs for functional brain signals

7.4.1. Organic LEDs for two-dimensional intrinsic imaging

We then tested OLEDs for functional brain signal. We found that when positioned in transparency mode it was not possible to use the semi-transparent OLED for an intrinsic experiment due to the low intensity of reflection and absorption from the cortex that is filtered by the semi-transparent OLED. We therefore focused on opaque devices positioned in side mode and with pixel-emission at 45 degrees from the cortex and outside from the camera view point. (See figure 52e)

In this experiment, a visual stimulus is presented to a rat (see method section) leading to a process of activation that can be monitored in the visual primary cortex area (V1). Indeed, the light excitation from the electroluminescence of organic LEDs are reflected and absorbed by the cortex and after stimulus, presentation changes of optical properties can be recorded via a camera. Each trial lasted 8 s and the beginning of each acquisition was triggered by the heartbeat of the animal. Two hundred milliseconds later (frame 0), the visual stimulus was displayed during 1 s. The trials were repeated 20 to 40 times per condition with an ISI of 3 s. To allow comparison across conditions, sessions, animals and stimuli (visual vs. electric) we performed temporal and spatial normalizations of optical imaging signals. The averaged time-course of the outer border (2 pixels width) of the image area (outside the region of interest) was subtracted from each pixel to remove non-functional temporal noise pattern. We then performed at each trial, an initial temporal normalization by subtracting the mean and dividing by the standard deviation, both estimated from the frame 0. Secondly, we averaged over trials and applied a spatial normalization.

Spatial z-score normalization was based on the averaged static map of the blank condition (non-visually stimulated condition: control condition). Pixel by pixel, we subtracted from each static map the mean value of the blank and divided the outcome by the blank standard deviation over space. Static maps were computed by averaging 0.5 to 1 s after stimulus onset (during the so called initial dip). The activation contours (black) were computed on a smoothed version of the maps (convolving the raw matrix with a 15x15 pixel flat matrix) and represents the 20th percentile of the amplitude modulation of the signal (max absorbance). The cortical center-of-mass (the red dot) was computed on the z-scored map. In order to quantify the extent and the shape of the activation contour, we computed the equivalent ellipse (Haralock and Shapiro, 1991). This provided the length and orientation of the equivalent ellipse, minor and major axis, as well as its equivalent diameter and geometric center (red cross).
**Figure 56 | Organic LEDs for two-dimensional intrinsic imaging.** (a.) the activation map averaged over the time period of interest (black line in b.) following the presentation of a visual stimulus (red line: period of stimulus presentation). Time course of the intrinsic signal (b.) averaged inside the black ROI in a.

The image of vessels is superposed to this functional map and is seen in transparency. The blue area on the map represents the consumption of oxygen (release of CO$_2$) from the blood that absorbs more light. The blue line in **Figure 56b** represents the time course of the absorbance inside the black contour in **Figure 56a**. The red line represents the period of stimulus presentation and the black one the period selected for frame averaging that leads to the map in **Figure 56a**. We observed that the visual stimulus induced a decrease of the z-score signal because of an increase in absorption led to a decrease of reflectance detected by the camera. The dynamics of such signal illustrated in b. corresponded well to the dynamics of intrinsic signal acquired with conventional illumination, an initial dip that follows a stimulus presentation with a delay for 500 to 1000ms, corresponding to local HbR concentration increase due neuronal population activity that uses oxygen, and a later rebond due to arrival of fresh HbO2 with an increases of local blood flow.

The opaque organic LED was successfully thus applied for intrinsic functional imaging.
7.4.2. Organic LED for two-dimensional voltage sensitive dye imaging

We focused on trying to apply semi-transparent OLEDs positioned through the pathway of the optical field, in order to excite uniformly the dye in the working area, and allow the emission of the dye fluorescence to pass through the semi-transparent device to be collected by the camera. (see Fig 52f)

By looking at the variation of fluorescence, we can observe a rapid increase of fluorescence after the presentation of visual stimulus with a shorter latency compared to the intrinsic signal (50ms vs. 500ms), which correspond well to the expected latency of such signal acquired using conventional light sources. The dye binds to the neuron membranes, under proper light excitation and when these neurons are depolarized (sub and supra-threshold), this dye emits fluorescence that is proportional to membrane potential modulations. This fluorescence, that pass through the transparent OLED, is then filtered and recorded by the camera. When a population of stained neuron is excited by light, we observe a gradual decrease of emitted fluorescence due to the dye bleaching (that is removed with normalization and detrending methods during data analysis). If the neurons are activated, a transient increase in fluorescence is observed on the top of this bleaching profile (the pic observed in Fig 57 b.).

In this experiment, a visual stimulus is also presented to a rat. Indeed, the light excitation from the electroluminescence of organic LEDs is directed to the cortex and after stimulus presentation changes of emitted fluorescence be recorded via a camera. Each trial lasted 8 s and the beginning of each acquisition was triggered by the heartbeat of the animal. Three hundred milliseconds later (frame 0), the visual stimulus was displayed during 600 ms. The trials were repeated 20 times per condition with an ISI of 3 s. To allow comparison across conditions, sessions, animals and stimuli (visual), we performed for each trial a first temporal normalization by dividing each time series by the mean estimated from the frame 0 (time interval before stimulus presentation). Second, we averaged over trials and applied a spatial normalization by subtracting the mean over time of the frame 0 and divided by the blank condition. Static maps were computed by averaging 400 to 700ms after stimulus onset (fluorescence peak).
Figure 57 | Organic LED for two-dimensional voltage sensitive dye imaging. (a.) the activation map averaged over the time period of interest (black line in b.) following the presentation of a visual stimulus (red line: period of stimulus presentation). Time course of the VSD signal (b.) averaged inside the black ROI in a.

Contour of cortical activation was achieved by applying a threshold at the 80th percentile of the amplitude modulation. The activation contour (black in fig 57a) was computed on a smoothed version of the maps (by convolving the raw matrix with a 15x15 pixels flat matrix). The cortical center-of-mass (the red dot) was computed on the z-scored map. In order to quantify the extent and the shape of the activation contour, we also computed the equivalent ellipse which provides length, orientation, minor and major axis, as well as the equivalent diameter and geometric center (red cross) of the activation equivalent ellipse. The image of vessels is superposed to this functional map and is seen in transparency. The reddish area on the map represents the increase in fluorescence linked to functional activation of neuronal population. The blue line in fig 57b represents the time course of the fluorescence inside the black contour in a. The red line represents the period of stimulus presentation and the black one the period selected for frame averaging that leads to the map in fig 57a.
We observed that the visual stimulus induces an increase of the signal because of the depolarization of the neuron membrane potential following the presentation of the visual stimulus. The dynamics of such signal illustrated in fig 57b corresponded well to the dynamics of VSD signal acquired with conventional illumination, an increase that followed a stimulus presentation with an averaged delay of 50 ms.

This increase indicates that the fluorescent signal did pass through the device and has been successfully recorded.

7.5. Discussion

We successfully demonstrated the use of organic LEDs for optical imaging of brain activities; a convincing proof of concept with a slight enouncement of light uniformity with high optical power was achieved with OLEDs in side and transparency mode. Using such technology could further improved these techniques by the packaging of the OLED in flexible substrates that can be either curved in cone shape and attach to the recording chamber for intrinsic imaging or embedded in curved glass to close the chamber for VSD which will enable proper illumination of a lager surface by compensating for the cortical curvature. We have confirmed here the successful application for intrinsic and voltage sensitive dye imagery techniques. It is clear that optical brain imaging is a rapidly expanding field, continually evolving to embrace new technologies, contrast mechanisms, and in-vivo applications. We hope organic optoelectronic devices will continue to answer the requirements of neuroscience (such as high temporal and special resolution, flexible and transparent LEDs). For instance, red-shifted OLEDs will allow non-invasive imaging directly through the intact human skull. New dyes have been developed that excite and emit at higher wavelengths, corresponding to regions where hemoglobin absorption is less significant. Acquisition of data is often triggered on the heartbeat and sometimes the breathing of the animal. Many repetitions are generally averaged, and “blank” acquisitions where no stimuli are presented are interleaved such that the final signal is given by the stimulated – blank data. The lifetime of OLEDs is still a constraint to be investigated.

A proposed alternative design using an open cone shape based on flexible substrate encapsulating an OLED could have advantages. The irradiating light towards the working area will result in a free space view for recording. (See Appendix A23). Another approach applying free standing organic LEDs as described in chapter 6 would provide emissive light source following the curvature of the cortex and may lead to higher uniformity.
7.6. Methods

**OLED Fabrication.** Conventional solution processing organic light emitting diodes were fabricated on glass by coating indium tin oxide (ITO of 10 ohm/sq.) with a 30 nm layer of poly(3,4-ethylenedioxythiophene : poly(styrenesulfonate) (PEDOT:PSS). Cleaning of the ITO slides; film coating and electrode deposition were carried out all the same day to ensure optimum results. The glass substrates coated with ITO were supplied by Xin Yan Technology Ltd. The glasses were sonicated in standard acetone and then in soap and DI water for 20 mins, in DI water 3 times for 5 min, in acetone twice for 5 mins and in isopropanol (IPA) for 5 mins, followed by blow-drying of the substrates. The ITO substrates were treated by oxygen plasma treatment. The PEDOT: PSS layers were deposited by spin coating at a spin speed of 3500 rpm for 40 sec and then annealed at 150°C for 20 min. Polymeric devices followed conventional PLED structure using PEDOT:PSS for the injection of holes from the ITO anode and Calcium for the injection of electron from the Aluminum cathode. The precursor solutions were prepared the day before device fabrication and the solutions were stirred at <50 °C overnight. The precursor solutions of fluorescent polymers (SO-PPV) were prepared by dissolving 6 mg material in 1mL Chlorobenzene and were spin-coated for 30 s at 1200 RPM on top of PEDOT:PSS. Following active layers deposition, Calcium Ca (25 nm) and aluminum Al (12 nm) top-electrodes were deposited onto all the semi-transparent devices and Calcium Ca (25 nm) and Al (120 nm) top-electrodes were deposited onto all the opaque devices by thermal evaporation through a shadow mask at 10-6 mbar. Active areas measured 75 mm². The encapsulation of the devices was effected manually using another glass substrate of 1.1 mm adhered onto the device using flexible sealants from DELO-KATIOBOND and a low power UV light to set the glue.

**OLED pulsing characterization.** The electrical characteristics of the devices encapsulated were measured in air at room temperature using a Keithley 2450 electrometer for the electrical current-voltage measurement. Emission spectra were measured using a fiber-coupled Ocean Optics 2000+ spectrometer. Absolute optical power intensity was determined using a large area calibrated Si PIN Photodiode (28 x 28mm active area, from Hamamatsu) coupled to a Keithley 6517A electrometer. An OPT101P Photodetector Amplifier from Texas Instruments connected to the National Instruments NI PCI-5112 100MHz Digital Oscilloscope Board was used to detect fast pulsing mode imposed by the arduino uno microcontroller.

**Surgical preparation.** Experiments were conducted on a male rhesus monkey (macaca mulatta, aged 8 years old). The monkeys were chronically implanted with a head-holder and a recording chamber located
above the cortical areas V1 and V2 of the right hemisphere. The dura was surgically removed over a surface corresponding to the recording aperture (18mm diameter) and a silicon made artificial dura was inserted under aseptic conditions. The experimental protocol was approved beforehand by the local Ethical Committee for Animal Research and all procedures complied with the French and European regulations on Animal Research (approval n°A12/01/13) as well as the guidelines from the Society for Neuroscience.

**VSD Animal preparation:** 1 Brown Norway male rat (3 months old, 250g) was anaesthetized using an intraperitoneal injection of urethane. The experimental protocol was approved beforehand by the local Ethical Committee for Animal Research and all procedures complied with the French and European regulations on Animal Research (approval n°A12/01/13) as well as the guidelines from the Society for Neuroscience. The bone and dura-mater over the primary visual cortex (V1) was removed surgically. Before recordings, the cortex was stained for 1.5 h with the VSD RH-1691 (Optical Imaging) prepared in artificial cerebrospinal fluid (aCSF) at a concentration of 0.2 mg/ml and filtered through a 0.2-μm filter. After this staining period, the cortex was rinsed thoroughly with filtered aCSF to wash off any supernatant dye and the craniotomy was closed with transparent agar and cover glass. Subsequently, optical signals were recorded from a focal plane ≈200 μm below the cortical surface. The semi-transparent OLED was then positioned at 0.5 cm above. Here the illumination was provided by semitransparent SO-PPV at 20v, 5kHz, at 0.5 cm from the cortex, for 1200 ms.

**For intrinsic imaging,** instead of a complete craniotomy, the bone was thinned with a drill until a clear optical access to V1 surface was obtained. Finally, transparent silicone was applied to the remaining bone and the preparation was covered with a glass slide. The cortex was illuminated with 2 optic fibers with a 100 W halogen bulb at 605 nm. Here it was with the opaque SO at 25v, 5KHz, tilted and sided at 0.5cm from the cortex, illuminated for 2000 ms.

**Visual stimulation:** Stimuli were displayed monocularly at 60 Hz on a gamma corrected LCD monitor (placed at 21.6 cm from the animal eye plane) covering 100° (W) x 80° (H) of visual angle using the Elphy software (Elphy®, Univ, Paris). In each trial, a gray screen with an averaged luminance of 0.5 Cd/m² was displayed during 300 ms (frame 0); the visual stimulus, a white square of 49 Cd/m², was then presented full field during 600ms for vsd imaging (followed again by a gray screen of 0.5 Cd/m2 during 7.1 s) and for 1 s for intrinsic imaging.

**Data acquisition:** We imaged 5*5 mm cortical windows using a Dalsa camera (Optical Imaging Inc, 504*504 pixels, 100 Hz for VSD and 30Hz for intrinsic imaging) controlled by the VDAQ data-acquisition
system (Optical Imaging®). Excitation light was provided by a 100-W halogen lamp filtered at 630 nm for vsd and 605 nm for intrinsic and fluorescent signals were high-pass filtered at 665 nm for vsd only.

Each trial lasted 8s and the beginning of each acquisition was triggered by the heartbeat. 300 milliseconds later (frame 0) the visual or electrical stimulus was displayed during either 600s or 1s. The blank condition consisted on a gray screen of 0.5 Cd/m2 of averaged luminance for visual stimulation. The trials were repeated 20 to 40 times per condition with an ISI of 3s.

Data analysis: Stacks of images were stored on hard-drives for off-line analysis. The analysis was carried out with MATLAB R2014a (Math-Works) using the Optimization, Statistics, and Signal Processing Toolboxes. Data were first preprocessed to allow comparison between animals and conditions.
Chapter 8: Conclusion

8.1. Summary

The attractive and innovative qualities of organic semiconductors for OLEDs render them a functional light source for organic neuro-optoelectronics. The specific topic was to apply Organic Light-Emitting Diodes (OLEDs) at the interface with biology.

In this research the design and engineering of stable and compatible OLEDs for incorporation into living tissues is optimized for their interface with functional environment by investigating the stability and biocompatibility of commonly used OLED materials.

Chapter one explains the background to organic Neuro-optoelectronics by introducing optical and electrical properties of organic semiconductor and their uses in device physics. The focus is mainly on the introduction of the suitability of OLED materials (light-emitting active layers, electrodes and encapsulation materials). Bio-applications using organic semiconductor and Organic Light Emitting Diodes (OLEDs) may be applied to this field. The background on cellular requirements, neuronal stimulation and bio-imagery is introduced.

In chapter two a protocol was established to investigate the suitability of organic semiconductor (OSC) materials at the interface with living cells, by investigating their functional stability in the bio-environment and their biocompatibility both in darkness as well as in light. A mechanism of cellular responses upon irradiation of cell interface with OSC was established and confirmed to occur in thin films and nanoparticles. Two groups of materials were investigated. For the first group of materials the possibility of utilizing organic semiconductor thin films to obtain inhibition of neural activity independent of genetic manipulation was demonstrated. For the second group of materials interacting with cells, a very specific irreversible depolarization at low power which was intensified at higher power was observed. This work led to understanding the requirements for insulation in devices to make them robust over long periods of time in bio-environmental conditions.

Chapter three highlighted the performance of a cross-linkable Light Emitting Polymer (LEP) demonstrating its diverse uses in various biological applications. A synthesized cross-linkable polymer was shown to be biocompatible in the dark and suitable for multi-layer structure leading to a robust and freely standing light-emitting sheet. The cross-linked polymer was shown to be stable in prolonged immersions in
cell culture and chlorobenzene. The material was found to be functional in a working organic LED and shown to be robust and with a longer electroluminescence lifetime at high operational voltages. To further investigate the suitability of this crosslinked material, the biocompatibility was assessed under irradiation. We found similar cellular responses as described in chapter three. When irradiated by light, the light-emitting sheet might not be exploitable in direct contact with the bio-environment but would find other appropriate applications when encapsulated in organic LEDs. The robust bonding properties of crosslinked F8FBT to glass and its stability in various media might enable the sterilization of the surface of targeted material as well as for the synthesis of natural products and drugs \cite{345} by the photo-produced singlet oxygen.

**Chapter four** showed the investigation of the stability of the electro-optical properties of several commonly used electrode materials for organic optoelectronics. The aim was to identify their advantages and drawbacks for use in solid/liquid devices, as well as the benefits of an adhesion layer for applications requiring long term aqueous immersion. Key electrodes (Ag, Al, Au and ITO) were exposed to culture media and the stability of their electrical, optical and mechanical properties was characterized over time. The prolonged immersion in cell culture media led to surface modifications involving oxygenation and delamination of thin film metal. An effective protocol for fabricating mechanically and chemically stable gold bioelectrodes on glass, using a 1 \( \mu \)m layer of the negative-tone photoresist SU8 as an adhesion layer between the glass substrate and a metallic layer of gold was proposed with modifying the cross-linking procedure. The optimized glass/SU8-Au electrodes displayed improved stability in biological culturing medium with respect to conventional glass/Cr-Au electrodes, and offered the further advantage of enabling facile electrode patterning via selective UV-illumination. The adhesion and viability of HEK293T cells on the optimized SU8/Au electrode was found to be comparable to that of HEK293T cells on glass, confirming the biocompatibility of the electrode.

In **Chapter five**, polymeric insulators were shown to be efficient as protection of organic devices and compatible with cell for safe interaction against putative photochemical stress. Thicknesses of the film were optimized for operational diodes while the cells were protected against charge leakages from devices. Using the techniques of bioelectrode fabrication described in **Chapter four** enabled the fabrication of freestanding OLEDs with simple structure and with morphologically robust protection against direct contact of the organic semiconductor with cell applications. The critical challenges to achieve fully biocompatible OLEDs with higher efficiency and stability remains by the identification of high performing and water-stable electron injection and transport materials that the framework of this thesis does not investigate. The
presence of hole and electron transport layers reduces the energy barriers for the ease of injection of charge carriers from electrodes. Hole transport material as PEDOT: PSS has proven electrical stability in cell culture media. (See Appendix A16) However, it is subject to current delamination. The investigation of hole and electron transport materials with electrochemical compatibility with cells and high mobility of charges with adequate energy levels for good injection of charges will improve the efficiency and stability of organic LEDs suitable for opto-bioelectronics.

The focus of chapters six and seven was to investigate the limits of OLEDs for their optical power and their optimal use in optogenetics (in Chapter six) and bioimagery (in chapter seven).

In chapter six, conventional solution processed OLEDs were pushed to operating limits, assessing how light delivered at pulse rates from 10 kHz to continuous illumination affected the optical and thermal character of the devices. The pulsing mode of operation effectively enhanced performance stability at high voltage operation and lowered operating temperature, allowing for safe and controlled photo-stimulation of neurons expressing microbial opsins. We developed a light emitting diode for activation of optogenetic tools and we successfully achieved opsin activation. Though causing delay in opsin responses, μs sub-pulses were successfully employed for ms scale control of neural activity. The operating mode proved particularly beneficial for long stimuli requiring maximal diode output, during which surface temperatures rapidly increased, and optical power decreased in DC continuous bias operation.

Chapter seven examined OLEDs as a light source for optical bio-imagery and they were employed for intrinsic and extrinsic in vivo optical imaging of functional cortical architecture and dynamics. Both opaque and semi-transparent organic LEDs were found to provide sufficient optical power to function in two position—both to the side of (with opaque OLEDs) and through (with semi-transparent OLEDs) the optical field. Semi-transparent devices provided the highest uniformity and intensity while the opaque devices were found to be similar to conventional lamps. The work will lead to alternative uses of light sources, e.g. optical imaging of an active animal.

8.2 Future work

The cell adhesion and function compatibility of representative materials used in plastic electronics such as polymeric encapsulator in chapter 5, light emitting polymers in chapter 2, host polymers with metallo-organic phosphorescent emitters (see Appendix A3 related to chapter 2), charge injection and transport interlayers (see Appendix A16 related to chapter 2) and metal electrodes in chapter 4 were investigated.
This led to an initial prototype of a simple structure of a freestanding organic LED, using polymeric encapsulation material as substrate with high cell adhesion properties, to be successfully fabricated.

The work has led to a good understanding of the interface of solid state devices with biology requirements. Organic LEDs have successfully been used for stimulation and imagery of HEK293T cells and neurons. The devices may be used as a source of excitation for green fluorescent protein GFPs, for secondary anti-body and fluorescent immune assays for biological marker, fluorescence imaging and for therapeutic drug monitoring.

Synthesizing novel organic semiconductors will give rise to further advances in mechanically and chemically stable OLEDs using cross-linked and biocompatible materials for interfacing with biology. However, direct contact of the organic semiconductor may be exploited for targeted cancer treatment with nanoparticles that can be functionalized with anti-bodies.

Overall, pixel size can be widely varied to make subcellular scale pixels or large pixels to cover entire organs. For the former, ultra-short pulses can be utilized to sequentially target distinct opsin population in the soma, axon, or individual synapses, to allow the stimulation speed necessary to mimic complex network inputs over large areas in parallel. Epicortical implant with Matrix electrode arrays (MEA) combined with transparent OLEDs would lead to a large area of optical stimulation and recording of cells simultaneously.

The OLEDs fabricated on glass present the possibility of developing smart culture substrates for in vitro experiments. I have recently started a project using a 3D printed cell culture incubator with OLEDs or MEA electrodes incorporated inside and a homemade pump fabricated in John de Mello’s Laboratory, with the aim of providing fresh cell culture media in flow for cell stimulation, imagery and monitoring while cells are gradually growing. The realization of a fully-printed perfusion chamber, where cells can be grown independently outside a conventional incubator and directly analyzed with means of fluorescence imaging performed with an OLED/OPD combination. The perfusion chamber might integrate pH and temperature sensing capabilities, realized with means of solution processable electronics, along with energy harvesting components and printed antennas.

The challenge of stretchable and ultra-flexible organic LEDs [428] is to use inorganic/organic hybrid barrier and chemistry in packaging technologies [429] for water and oxygen as encapsulation. [430–436] Hybrid organic/inorganic or mixing thermoset plastics and thermoplastic [437–439] encapsulation structures may protect the device against oxygenation and create appropriate water vapor barrier.[440] The design of a
freestanding 3D device structure using a hybrid hydrogel or hydrophilic components might be immersed in and surround solid insulated freestanding LEDs as a scaffold with many pores and pillars that could let pass large amount of oxygen and water throughout a breathable emitting device. Holes and porosity in the solid device would help tissues to grow surrounding the holes and help good oxygen/water transmission while protecting functional pixels from the bio-environment using patternable thermoset materials such as SU8. A similar interface with neuronal network, muscle cells, contact lenses or cochlear implants \cite{155,441} may open new avenues to light-mediated controls for tissue engineering applications. Looking forward, embedding well encapsulated and high efficiency devices in hydrogel lenses, would extend the range of application to treatment of retinopathies and virtual reality contact lenses.

The promise of OLED technology is a system that is easily implementable and devoid of external optics. The rapid pace of progress in both OLED and biotechnologies ensures that OLEDs will continue to find numerous applications in bioelectronics.
APPENDIX:

A1. Reversible and irreversible cellular photo stimulation at the interface with organic emitting semiconductors

A1.1. Morphological and chemical degradation of organic emitting semiconductors

Appendix A1 | Tracking photo-induced and darkness chemical degradation of (a.) PFO and F8BT, (b.) SO-PPV and SY-PPV, (c) MEH-PPV and CN-PPV and (d.) P3HT and PVK by FTIR. The arrows show the backbone degradation with representative bands decreasing. The stars represent the C-H aliphatic region that is increasing by irradiation of light. The triangles represent the carbonylic region that is increasing over time and accentuated by irradiation of samples in media.

The Appendix A1 shows the carbonylic region (at 1600 – 1800 cm\(^{-1}\)) and C-H aliphatic region (at 3000 – 2750 cm\(^{-1}\)) absorbance bands appearing for all aged conjugated polymers soaked for two hours and for 672 hours in cell-culture media; a result that is accentuated when the samples are irradiated for two hours. The phototoxicity in certain buffer is well known.\(^{341,342}\) Here we used a media free from phenol-red to avoid the phototoxicity effects, and in order to only focus on understanding the phototoxic coming from light
emitting polymer investigated. Double bond saturation via hydrogen abstraction may form alcohol (shown with star symbols in Appendix A1 at 3460 cm\(^{-1}\)); and cage reaction forming aromatic ketone (shown with triangles in Appendix A1 at 1680 cm\(^{-1}\)).

Appendix A2 | Morphological changes by bio-functionalization and under prolonged soakage in bio-environments. Micrograph images of morphological changes after 24 hrs immersion in media of (a.) PFO (b.) MEH-PPV (c.) P3HT (d.) PVK:Firpic and (g.) SY-PPV. Micrograph images of morphological changes after (e.) laminin biofunctionalization BF-1 and (f.) horse serum biofunctionalization BF-2. Micrograph images of morphological changes of SY-PPV after over a month with neuron grown on top. Scale bars denote 50 μm for micrograph image.

It was found that the irradiation of type-II: F8BT, CN-PPV and PFO with cells grown on top evokes a specific irreversible depolarization of cells at low optical power of exposure. However, similar irreversible depolarization was observed only for type-I: P3HT, SY-PPV, SO-PPV and MEH-PPV at longer exposure while having reversible depolarization at short pulse of high optical power of irradiation. Those type-I materials investigated here are safe to be used with cells at low and short optical power. Type-I and type-II of materials represent similar morphological instability and is due to a problem of adhesion to glass. Appropriate adhesive layer should be investigated in order to go further in progress of multi-stack layers for the fabrication of morphologically stable organic LEDs for opto-neuro-electronics.

For the purpose of understanding the application of solution processing phosphorescent film, the PVK as host blended with the metallo-organic phosphorescent dopant Firpic were shown in Appendix A2d not
to be destroyed at 24 hours. However, the insoluble particles, are assumed to be released from the host PVK and diffused toward the aqueous solution to accumulate at the surface. For the purpose of understanding the application of solution processing phosphorescent organic LEDs, hole transport/electron blocking layer TPBi; the electron transport/hole blocking layer TAPC and the active layer based with PVK as the host polymer blended with metallo-organic phosphorescent dopant were shown in Appendix A3, to respond with different interface interaction material/cells during irradiation.

Appendix A3 | Phosphorescent OLEDs represented in (a.) with their active layer number number P(x) and following Glass/ITO/Pedot:Pss/TAPC /P(x) /TPBi/Ca/Al structure with FlrPic for P(1); Flr6 for P(2); FK306 for P(3); Hex-Ir(phq)3 for P(4); Ir(MDQ)2acac for F(5); Hex-Ir(pic)3 for P(6); Ir(btp)2(acac) for P(7); DFir for F(8) devices. The energy required for 50% of the maximum of cell depolarization represented in (b.) for phosphorescent active layer following Glass/P(x) structure with PVK blended with PBD and doped with FlrPic; Flr6; FK306; Hex-Ir(phq)3; Ir(MDQ)2acac; Hex-Ir(pic)3; Ir(btp)2(acac) and DFir. (usually used for solution processed phosphorescence organic LEDs).

The energy required for 50% from the maximum of depolarization of cells is used (see Appendix A3b) to quantify the photo-induced cell responses of various phosphorescent thin films. The higher the EP50% the safer the film is for its application in organic phosphorescent LEDs. Ir(MDQ2(acac) blended with PVK and PBD shows higher than 10% of ED50%. TPBi and TAPC are in similar range of values and are represented as relatively safe for in-vitro experiments. Base on this initial result, a phosphorescent organic LED can be made using Ir(MDQ2(acac) blended with PVK and PBD and TPBi as electron blocking layer and TAPC as hole blocking layer. See Appendix A3a for the representation of the device.
A1.2. Applications with nanoparticles: targeting cell ablation

Nanoparticles are used in many applications in direct contact with living cells which operate based upon their photo-excitation and their relaxation process. The understanding of photo-induced toxicity is critical in order to carefully choose suitable nanoparticles to carry out subsequent clinical, diagnostic, or fundamental research work.

The encapsulation of hydrophobic conjugated polymers into a biodegradable matrix of Poly(ethylene glycol) methyl ether-block-poly(lactic-co-glycolide) (PEG-PLGA) by solvent displacement technique has shown to be a facile production method with high product yield of conjugated polymer nanoparticles (CPNs) designed for biomedical applications.[353] The darkness biocompatibility of CNPs encapsulated was reported as healthy at low concentration. We aim at understanding under which condition the NPs becoming dangerous for their uses in bio applications and if the use of PEG-PLGA encapsulation would protect the cells and the NPs.

A1.2.1. Synthesis of green to red emitting PEG-PLGA encapsulated CPNs toolkit

Generation of CPNs is made through a modified bio-silification method involving nano-precipitation. After CPNs concentration (Figure 22), nanoparticles containing SY-PPV and SO-PPV presented the highest product yield (≥95%), followed by CN-PPV (66%), F8BT (56%) and 100% CN-PPV (46%). Except for F8BT, all CPNs present a redshift in emission in comparison to the conjugated polymer in THF: ~30 nm (SY-PPV),~40 nm (SO-PPV),~80 nm (CN-PPV) and ~90 nm (100% CN-PPV) (Appendix A4). Such shift is compatible with the data reported in literature for CN-PPV and other PVV derivatives, and has been related to interactions between segments of the polymer chain and intramolecular energy transfer within the particle.

Following manufacture, all the nanoparticle system embedding the polymers within the core of PEG<sub>5K</sub> - PLGA<sub>55K</sub> significantly increased nanoparticle hydrodynamic diameter in comparison to PEG-PLGA alone, in a polymer dependent fashion. In particular, SO-PPV and SY-PPV showed the bigger hydrodynamic diameters – possibly due to their higher molecular weight leading to a higher viscosity of the organic solution. PEG-PLGA also reduced the zeta potential of the systems nanoparticles in comparison to bare CN-PPV CPNs and it is consistent to what reported by Abelha[492] et al on similar systems. More specifically, bare CN-PPV presented a highly negative zeta potential (-40.8±1.83) due to the exposure of the nucleophilic CN groups, while empty PEG-PLGA nanoparticle are much closer to neutral values (-10.97±0.85). PEG-PLGA CPNs
presented a range of intermediate values, where the intrinsic conjugated polymer hydrophobicity is mitigated by the more neutral PEG-PLGA.

In sum, we were able to generate a multicolor toolset of PEG-PLGA encapsulated nanoparticles presenting high manufacturing yields, with nanometer scale diameters between 85 and 145 nm, ideal for a whole range of biomedical applications including but not limited to subcellular scale imaging applications, drug delivery and diagnostics.

Appendix A4 | Nanoparticles characterization. a.) Solvent displacement steps: I. PEG-PLGA and CP are injected in distilled water, under stirring; II. Nuclei grow until saturation; III. Upon solvent evaporation nanoparticles are formed b). Nanoparticles zeta potential and hydrodynamic diameters c). Nanoparticles after preparation (i) and after concentration (ii), from left to right: 100% PEG5K-PLGA_{55K}, SY-PPV, F8BT, CN-PPV, SO-PPV and 100% CN-PPV.
Appendix A5 | NPs optical characterization. Absorption and emission spectra of conjugated polymers in THF and emission spectra of nanoparticles in water

A1.2.2 Photo-inducing cell ablation using OSC nanoparticles

Appendix A6 | Patch Clamp set-up with cells grown on glass and with organic semiconductors nanoparticles. Green circles representing the nanoparticles of OSCs

Using similar detection techniques as on films, radical oxidative stress production was measured upon irradiation of the NPs at 1 mW/mm² for 10 minutes. (See appendix A7). The detection of superoxides for F8BT nanoparticles were evident, while a weaker detection on SY-PPV at the edge of the tail of its spectrum was difficult to identify. SO-PPV and CN-PPV nanoparticles were both able to form singlet oxygen and oxidative stress with CN-PPV producing a larger amount of detectable singlet oxygen. The encapsulation of CN-PPV NPs and films with PEG-PLGA was not protecting the interface from the formation of radicals upon light while the encapsulation of films with polystyrene seems to protect well against radical formation.
Appendix A7 | Singlet oxygen, oxidative and superoxide’s detection for nanoparticles NPs in Di-water.

For the set on the left and middle, a fast-growing mouse neuroblastoma cell lines, N2a cell, were plated 24hrs before adding nanoparticles. For the set on the right, the nanoparticles were added while plating the cells. Green fluorescence represents the fluorescein signal (reflecting intracellular esterase activity) and the purple-red fluorescence represents the propidium iodide (reflecting compromised membrane permeability - apoptosis/necrosis). The images are tiles of 35 individual images, covering the 12mm diameter coverslips (dashed white line). The illumination covers the area indicated by the dashed white line.

While the excitation light does appear to be toxic by itself (Note at 11.2 J/cm² full cell death by blue light alone, when giving illumination one hour after plating with NP), toxicity is accelerated by the presence of the NP. It is evident that NP induce cell death at light dose of 59J/cm², at a rate far exceeding that induced by NP in the dark (see cells outside illuminated area), or N2a cell illuminated in absence of nanoparticles. Therefore, nanoparticles may be used for targeted cell ablation. However, the high power of light over time requirements to obtain such results may limit clinical applicability, which require high rates targeted ablation, at a safe irradiation on non-cancerous tissue. The tool is likely more appropriate for in vitro use, where single or multi cell ablation studies can be employed to understand the roles of individual cells on network-wide neural activity.
Appendix A8 | N2A imaging. After 24 hours incubation while irradiated with 0; 11.2; 22.5 and 59 J/cm² light dose. The images are tiles of 35 individual images, covering the 12mm diameter coverslips (dashed white line). The illumination covers the area indicated by the dashed white line.

Photovoltaic retinal prosthetics applications using type-I of conjugated polymer P3HT, SY-PPV, MEH-PPV and SO-PPV may have little effect on cells at low powers, but it does not mean that radicals are not being generated at lower volumes and that those radical species are not likely to act as secondary messengers in cell pathway communications. In fact, it is important to avoid high optical input to avoid further damage to patients’ eyes. While it is not the focus of this thesis it seems from initial experiments that the nanoparticles of type-II OSCs (CN pure, CN-PEG-PLGA, F8BT-PEG-PLGA) are reactive at the cellular membrane, leading to depolarization and swelling of the cell under illumination. The depolarization is evident in HEK293T (see Appendix A9), N2A (see Appendix A8), and neurons (see Appendix A9).
Appendix A9 | Electrophysiology of HEK293T and N2a cells with presence of light emitting nanoparticles. 

A.) Examples image of HEK293T with CN-PEG-PLGA under illumination while recording by patch clamp. B.) Membrane potential of HEK293T in response to 1 minute of illumination. C.) Examples image of hippocampal neurons with F8BT-PEG-PLGA. D.) Membrane potential of Neurons in response to 1 minute of illumination, blue bar inset shows illumination, dashed line is mean potential before illumination. Nanoparticles @ [30ug/ml]. E.) Membrane potential of N2A in response to 1 minute illumination, blue trace is control without NP, cyan trace with F8BT-PEG-PLGA Nanoparticles @ [30ug/ml]. F.) Membrane capacitance of N2A in response to 10 seconds illumination, dashed line showing mean capacitance (pF) before illumination.

In all cases the stimulation does not reverse in the recorded time window. From the HEK293T we can see that the amplitude of the depolarizations is dependent on both light intensity and on nanoparticle concentrations. In neurons, stimulation leads to intense action potential firing. The amplitude of the action potentials decreases over the illumination period, before firing of the cell is silenced. This is also reflected...
over entire neural networks. While cellular swelling was observed by the patch clamp operator during recordings, a more direct evidence of this is presented in subpanel F of electrophysiology Appendix A9.

Appendix A10 shows Multi Electrode Arrays (MEAs) recording before and after the illumination of neuron culture incubated with CN-PPV nanoparticles. A large amount of silencing of neural activity after illumination (shown by absence of spiking activity in each respective recorded channel) can be observed.

![Graph showing neural activity before and after illumination](image)

*Appendix A10* | Matric electrode Array MEA recording neuron activity (12hrs incubation of nanoparticles) before and after light irradiation.
A2. Novel application of cross-linkable light emitting polymer in bioelectronics

A2.1 Synthesis of cross-linkable modified polymer (by Adam Creamer)

Synthesis of 4,7-Dibromo-5-fluoro-2,1,3-benzothiadiazole was based on literature procedure\textsuperscript{493}. We show here report the novel incorporation of trimethoxysilane terminated side-chains onto 4,7-Dibromo-5-fluoro-2,1,3-benzothiadiazole by nucleophilic aromatic substitution of the fluorine on the fluorobenzothiadiazole (FBT) unit with (3-mercaptopropyl)trimethoxysilane, (Appendix 11 scheme 1-a).

\begin{center}
\begin{figure}
\centering
\includegraphics[width=\textwidth]{synthesis.png}
\end{figure}
\end{center}

Appendix A11 | Synthesis of P(F8FBT) modified

The modification occurs in very simply one-step process, post-polymerisation. The only requirement for the modification is the presence of the FBT unit, a popular monomer used in OLEDs, no modification of monomers prior to polymerisation is required. The trimethoxysilane group hydrolyses and self-condenses forming an insoluble cross-linked polymer (Appendix A11 scheme 1-b) without the need for additives, external stimuli or exposure to air. (See Appendix A11 for further details).

The process is greatly accelerated by the presence of heat (24hrs at 120°C) and/or aqueous environment and making it suitable for cell-culture environment.
Synthesis of PF8FBT:

Synthesis of 4,7-Dibromo-5-fluoro-2,1,3-benzothiadiazole was based on literature procedure\(^{(93)}\)

**PF8FBT polymer synthesis.**

![Chemical structure of PF8FBT](image)

9,9-Dioctyl-9H-fluorene-2,7-diboronic acid bis(pinacol) ester (251.3 mg, 0.391 mmol), 4,7-dibromo-5-fluoro-2,1,3-benzothiadiazole (122.0 mg, 0.391 mmol) and Pd(PPh\(_3\))\(_4\) (9 mg, 0.008 mmol) and a stirrer bar were added to a 20 mL high pressure microwave reactor vial. The vial was then sealed with a septum and flushed with argon, before degassed toluene (10 mL), degassed aqueous 2M K\(_2\)CO\(_3\) (2 mL) and 2 drops of aliquat 336 were added. The whole solution was then degassed again for 30 min before the reaction was heated to 120 °C for 3 days. The mixture was then cooled to room temperature, precipitated in methanol (200 mL), stirred for 30 min and filtered through a Soxhlet thimble. The polymer was then extracted (Soxhlet) using methanol, acetone, hexane and CHCl\(_3\) in that order under argon. The CHCl\(_3\) fraction was collected and concentrated to ~10 mL before being precipitated into methanol (200 mL), stirred for 30 min and filtered to yield a green solid (194 mg, 92%); \(M_n = 92\) kDa, \(M_w = 212\) kDa, \(M_w/M_n (\mathcal{D}) = 2.3\). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.15 – 7.78 (m, 7H), 2.37 – 1.90 (m, 4H), 1.26 – 0.88 (m, 26H), 0.84 – 0.78 (m, 6H).

**Silane-F8BT**

![Chemical structure of Silane-F8BT](image)

\(\text{PF8FBT.}\) (30 mg, 0.055 mmol), and K\(_2\)CO\(_3\) (500 mg, 3.62 mmol) were added to a 20 mL high pressure microwave reactor vial. The vial was sealed with a septum and degassed with argon, before anhydrous
chlorobenzene (4.5 mL) and DMF (1.5 mL) were added. (3-Mercaptopropyl)trimethoxysilane (0.1 ml, 0.54 mmol) was then added and the solution was degassed for 30 min. The solution was heated at 120 °C for 30 min in the microwave. After cooling the solution was precipitated into MeOH 100 mL and filtered. The solid was washed with MeOH and hexane to remove excess thiol and K$_2$CO$_3$. The yellow solid was then dried under vacuum to leave a yellow solid (30 mg, yield 76%); $^1$H NMR (400 MHz, CDCl$_3$) δ 8.09 – 7.89 (m, 5H), 7.67 – 7.59 (m, 2H), 3.53 – 3.49 (m, 9H), 3.02 – 2.92 (m, 2H), 2.26 – 1.93 (m, 4H), 1.82 – 1.72 (m, 2H), 1.38 – 0.65 (m, 47H).

**C$_8$-F8BT**

![Image of C$_8$-F8BT structure]

**PF8FBT.** (5 mg, 0.009 mmol), and K$_2$CO$_3$ (10 mg, 0.72 mmol) were added to a 2 mL high pressure microwave reactor vial. The vial was sealed with a septum and degassed with argon, before anhydrous chlorobenzene (1.5 mL) and DMF (0.5 mL) were added. 1-Octanethiol (0.1 ml, 0.58 mmol) was then added and the solution was degassed for 30 min. The solution was heated at 120 °C for 30 min in the microwave. The mixture was then cooled to room temperature, precipitated in methanol (20 mL), stirred for 30 min and filtered through a Soxhlet thimble. The polymer was then extracted (Soxhlet) using acetone and CHCl$_3$ in that order under argon. The CHCl$_3$ fraction was collected and concentrated to ~10 mL before being precipitated into methanol (20 mL), stirred for 30 min and filtered to yield as a yellow solid (4.4 mg, 73%); $^1$H NMR (400 MHz, CDCl$_3$) δ 8.09 – 7.91 (m, 5H), 7.70 – 7.60 (m, 2H), 3.02 – 2.93 (m, 2H), 2.24 – 1.93 (m, 4H), 1.32 – 1.07 (m, 36H), 0.88 – 0.77 (m, 9H).
A2.2 characterization of freestanding light-emitting sheet

Appendix A12 | Characterization of PF8FBT sheet (a.) Cross-linked PF8FBT of 1; 5 and 50 Layers and (b.) contact angle of Di-water and Chlorobenzene on the cross-linked and non-cross-linked polymer.

A2.3. Nanoparticle synthesis

Appendix A13 | Scheme nanoparticles of PF8FBT

Nanoparticle synthesis in Appendix A13 is based on the nano precipitation method.\(^{[494]}\) Polymer was dissolved in THF to a concentration of 0.1 mg/mL. The solution was filtered through a PTFE filter (0.2 μM). The resulting solution (1mL) was rapidly injected into ultrapure water (9 mL), under sonication and left for 3 min. The nanoparticle suspension was bubbled with nitrogen for 3 hrs to remove THF.
A3. Use of SU8 as a stable adhesion layer for gold bioelectrodes

This Appendix section of the chapter 4 highlights the performance of an adhesive layer (SU8) for key electrodes. The aim was to identify their advantages and drawbacks for use in solid/liquid devices, as well as the benefits of an adhesion layer for applications requiring long term aqueous immersion.

A3.1 Use of SU8 as a stable adhesion layer for key electrodes

Appendix A14 | Micrograph stability of gold, silver and aluminum immersed in cell culture media. Reflection and transmission optical microscope image of the electrode materials after 672h in medium, with a magnification of 10x. The images clearly show the formation of wrinkles and cracks in the SU-8 layer, which determine a deterioration of all the considered electro-optical parameters. The insets show the images taken with the same parameters at the baseline.

The investigation of the stability of the electro-optical properties of several commonly used electrode materials for organic LEDs is shown in Appendix A14. It presents transmission and reflection mode optical micrographs of the SU-8 based samples after the one-month immersion in Dulbecco’s medium, while the small insets show the same image taken on the sample before the immersion.
A3.2 Biocompatibility of key electrodes

Appendix A15 | Biocompatibility of key electrodes used in OLEDs. Top: time-course of FDA, PI, Hoescht viability assay on the various electrode substrates. Middle: average number of attached cells per acquired image. Bottom: basic cell membrane parameters (left: resting potential and right: input resistance) assessed by patch clamp. Statistical analysis was performed using one-way ANOVA followed by the Dunnett’s multiple comparison test versus glass controls.

The defects are evident and predominantly on the edges of the samples, but present, although in a lower number, also in the middle. We found that HEK293T cells were biocompatible when grown on ITO, gold and Aluminum in Appendix A15. In organic LEDs, the presence of hole and electron transport layers reduces the energy barriers for the ease of injection of charge carriers from electrodes. Hole transport material as PEDOT: PSS has proven electrical stability in cell culture media. (See Appendix A16). However, it is subject to current delamination. The investigation of hole and electron transport materials with electrochemical compatibility with cells and high mobility of charges with adequate energy levels for good injection of charges will improve the efficiency and stability of organic LEDs suitable for opto-bioelectronics.
A4 Biocompatible and freestanding organic LEDs using polymeric encapsulation for the interface with biology

The fabrication of a biocompatible freestanding organic LED prototype was described in this chapter. All materials were studied for their biocompatibility with cells in other chapters and these materials are used for the fabrication of freestanding organic LEDs as described here in chapter 5. The light emitting conjugated polymer SY-PPV as type-I material explored in chapter 2 was used here as a safe active emissive layer in the freestanding organic LED. This Appendix section of the chapter 5 support with initial experiments studied the widely used PEDOT: PSS in OLEDs did not alter cell adhesion (see Appendix 16) and was used in this freestanding device as hole injection and transport layer. The stable and biocompatible bioelectrode investigated in chapter 4 with SU8/Au-C procedure was used as the anode and cathode in this freestanding organic device. In order to help the injection of electrons from the bioelectrode SU8/Au-C used as the cathode, a thin layer of aluminum was placed between the gold and the light emitting polymer. Aluminum was investigated in previous work and reported in Appendix 15 as a biocompatible key electrode for opto-bioelectronics.

A4.1 Biocompatibility of PEDOT/GAGs and PEDOT:PSS

Appendix A16 | Biocompatibility of PEDOT samples. (a) Nº of cells per sample on PEDOT (control), PEDOT:PSS, PEDOT:Dextrose (D), PEDOT:Guar (G), PEDOT: Xantham Gum (Xg) and PEDOT:Agar (A) on experiments performed at 1 and 5 DIV. Nº of cells per sample on PEDOT (b), PEDOT:PSS (c) and PEDOT:D (d) with different concentrations of PSS at 1 and 5 DIV. A statistical difference was observed. (*p<0.1, ** p<0.01, *** p<0.005 ANOVA one-way). It seems that commercially available PEDOT:PSS used for conventional OLEDs is biocompatible with HEK293T cells.

Different biomolecules have been used as a substitute for PSS in order to improve biocompatibility. In this context studies with dopants such as hyaluronic acid, fibrinogen, and nerve growth factor have been
conducted. Nevertheless, doping PEDOT with large biomolecules can have a negative side effect by altering some of the desirable properties of PEDOT. Here we present the biocompatibility of crosslinked PEDOT doped with four different polysaccharides, Dextran, Guar, Agarose and Xanthum Gum. In order to assess the biocompatibility of the materials, we plated Human embryonic kidney cells 293 (HEK 293) in the PEDOT with Dextran (D), Guar (G), Agarose (A) and Xanthum Gum (XG) using PEDOT:PSS as control. Presto blue assay was used and fluorescent measurement was carried out at 1 and 5 DIV. Appendix A16 shows that at 1 DIV the number of cells that adhered in the different substrates was very similar to the number of cells that adhered to PEDOT:PSS with the exception of PEDOT:A where the number of cells was significant lower. Meanwhile, at 5 DIV the number of cells on PEDOT:D was higher than on PEDOT:PSS while in the rest of conditions the number was comparable to the number of cells in the control sample. Results suggest an improved biocompatibility of PEDOT:D with respect to PEDOT:PSS as the number of cells that adhere is comparable at 1 DIV and significant higher at 5 DIV suggesting an improved proliferation on this material.

**Synthesis of Bio-PEDOT dispersions.** All bio-PEDOT dispersions containing 4% solids content were synthetized following the indicated procedure. 0.44 g of each polysaccharide (Dextran, Guar, Agarose, and Xanthan Gum) were dissolved in a 50mL round bottom flask filled with 21mL of MILLI Q water and vigorously agitated during 1-3h with a magnetic stirrer at 0°C under N₂ until complete solubility. 0.44g of EDOT were added followed by 1.5 eq of the oxidant, (NH₄)₂S₂O₈, and 5mg of the catalyst, Fe₂(SO₄)₃. After 96 h at 0°C under N₂, the dispersions were dialyzed at 5°C for 72 h to remove any unreacted monomer as well as excess oxidant and catalyst. All the dispersions were kept at 5°C until utilization. EDOT monomer, Agarose, and Dextran were purchased from ACROS while Ammonium sulfate, Iron persulfate, Xanthan Gum, and Guar were purchased from Sigma Aldrich. All the chemicals were used as such without further purification. The dialysis membrane 12-14kDa was supplied by Spectrunlabs.

**Cell Cultures and biocompatibility.** Human Embryo Kidney (HEK293) cells, were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM), supplemented with 10 % fetal bovine serum, 100 ȝg/ml penicillin, and 100 ȝg/ml streptomycin, directly on the surface of the investigated substrates. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Presto blue (PB, Life Technologies) viability assay was used according to the manufacturer’s protocol to study the viability of HEK 293 cells on PEDOT substrates at 1 and 5 DIV (days in vitro) using glass + PDL (Poly-D-Lysine) as control. Cells plated on the substrates were incubated at 37°C for 2h with PB that was diluted 1:10 in the medium used for cell culture. After that 100 ¦L were taken and then placed into a 96 well plate for fluorescence measurement in a Thermo
Scientific™ Varioskan™ Flash Multimode Reader at an emission and excitation wavelength of 585 nm and 570 nm respectively.

A5. Millisecond control of neural activity by an organic LED interface

A5.1 Characterization of fluorescent and phosphorescent organic LEDs

### Fluorescent OLEDs

- F1: PVK/PFO
- F2: SO-PPV
- F3: PFO
- F4: MEH-PPV
- F5: CN-PPV

### Phosphorescent OLEDs

- P1: F1rpic
- P2: F1r6
- P3: FK306
- P4: Hex-Ir(phq)3
- P5: Ir(MDQ)2(acac)
- P6: Hex-Ir(piq)3
- P7: Ir(btp)2(acac)
- P8: DF1r

---

**Appendix A17** | Characterization of a series of blue and red solution processed OLEDs for **Optogenetics**. Top showing device structure and naming legends representing the device number to their active layer with at the right the fluorescent device structure and on the left the phosphorescent structure. The middle showing electroluminescent spectra of devices. The bottom row show maximal stable output in continuous bias operation (black bars), or under 10kHz 50% duty cycle pulsed mode operation (colored bars).
**A5.2 Biocompatibility of the active layer**

![Graphs showing biocompatibility](image)

**Appendix A18** | Neuronal cultures are unaffected by conjugated polymers. Primary hippocampal neurons prepared from embryonic brain tissue were plated on semiconducting polymers and recorded by patch-clamp between 14-21 DIV. No differences were found in (a.) resting membrane potential, (b.) capacitance or (c.) membrane resistance for the neurons grown over glass versus those grown on the tested polymers (p > 0.05, Mann-Whitney), inset bars represent group mean.

**A5.3 Metallo-organic LEDs for SSFO activation**

![Graphs showing metallo-organic LEDs](image)

**Appendix A19** | Neuronal depolarization by SSFO during illumination of metallo-organic based device. (a) Control neurons and neurons transfected with SSFO were placed at the surface of the OLED substrate and recorded in current clamp mode. (b,c) Bar graph displaying mean maximal responses at 20, 30 and 40 V for continuous 500 ms stimuli, or 500 ms 50% duty cycle trains at 30 kHz, 10 kHz, 5 kHz (control n=2, SSFO n=3). (d) Example response of a neuron to 500ms blue light stimulus (40V continuous bias).
A5.4 Fluorescent organic LEDs for ChrimsonR activation

Appendix A20 | Neuronal Response Types of high density cortical cultures expressing ChrimsonR to illumination by SO-PPV. Responses of both opsin expressing and control neurons in cultures infected by viral vector carrying ChrimsonR under the CaMKIIa promoter. (a) Example trace of a wild-type neuron exposed to 50ms light stimuli. In contrast, ChrimsonR-positive cells displayed action potential firing (main text) and subthreshold depolarization (b). Wild-type neuron with consistent delayed action potential firing in response to 50ms stimulus (c). Several neurons, including both control and ChrimsonR-expressing cells displayed putative inhibitory postsynaptic potentials in response to light, representative trace (d).
A5.5 List of organic LEDs for various opsin activation

Appendix A21 | Maximal optical output of solution processed blue OLEDs (in blue) and red OLEDs (in red), plotted alongside benchmarks of optogenetic activation requirements (in black) reported in literature. Fluorescent OLEDs characterized and represented with their active layer number F(x) and following Glass/ITO/Pedot:Pss/ F(x) /Ca/Al structure with PFO and PVK for F(1) devices; Super Orange (SO-PPV) for F(2) devices ; PFO for F(3); MEH-PPV for F(4); CN-PPV for F(5). Phosphorescent OLEDs characterized and represented with their active layer number number P(x) and following Glass/ITO/Pedot:Pss/TAPC/P(x) / TBPl/Ca/Al structure with FlrPic for P(1); Flr6 for P(2); FK306 for P(3); Hex-Ir(phq)3 for P(4); Ir(MDQ)2acac for F(5); Hex-Ir(pic)3 for P(6); Ir(btp)2(acac) for P(7); DFlr for F(8) devices. References for OPSIN tools are ChR2-XXL [465]; Rhodopsin [433]; Jaws [405]; ChR2(H134R) [496]; Killer Red [497]; GtACR1 [498]; CsChrimson [499]; GtACR1* [499]; Melanopsin [500]; bPAC [501]; REST-LOV [502]; ChR2(H134R)* [503]; C1V1(E162T) [503]; mPAC [504]; Opto-mFGFR1 [505]; ChR2(H134R)** [506]; ReaChR [465]; eNpHR3.0 [446]; eArch3.0 [446]; Chronos [448]; iChloC [507].
A5.6 Equation for the OLED-Opsin couple efficiency (Appendix A22):

**OLED external efficiency.** External quantum efficiency $\eta_{\text{oled}}$ of the OLED, with its electrical current $I_{c,\text{oled}}$, electric charge $q$, Plank’s constant $\hbar$ and speed of light $c$ and wavelength $\lambda$ with a range of $[\lambda_1, \lambda_2]$ according to the minimum $\lambda_1$ and maximum $\lambda_2$ of the electroluminescent spectral distribution of the optical power flux $\Phi'_{E_{\text{oled}}} (\lambda)$ and is normalized spectrum $N(\lambda)$.

$$\eta_{\text{oled}} = \frac{q}{\hbar c I_{c,\text{oled}}} \int_{\lambda_2}^{\lambda_1} \Phi'_{E_{\text{oled}}} (r_0, h_0, t_0, \lambda) \lambda \, d\lambda \quad (1)$$

$$\Phi'_{E_{\text{oled}}} (r_0, h_0, t_0, \lambda) = \frac{I_{c,\text{oled}}(\lambda)}{\sigma(\lambda) f(r_0, h_0, t_0, \lambda)} \quad (2)$$

While, $f(r_0, h_0, t_0, \lambda) < 1$ the fraction of light emitted that is coupled to the photodetector is negligible ($f(r_0, h_0, t_0, \lambda) = 1$) when using a larger photodetector compared to the OLED pixels with a known responsivity $\sigma(\lambda)$ and a measured photocurrent $I_{c, PD}$ at the OLED glass surface. The total amount of photons emitted by an OLED $\Phi_{E_{\text{oled}}} (r_0, h_0, t_0)$ in [W], with initial spatial coordinate $r_0$, initial radiation direction $h_0$ and initial time $t_0$.

$$\Phi_{E_{\text{oled}}} (r_0, h_0, t_0) = I_{c, PD} \frac{\int_{\lambda_1}^{\lambda_2} N(\lambda) \, d\lambda}{\int_{\lambda_1}^{\lambda_2} \sigma(\lambda) f(r_0, h_0, t_0, \lambda) \cdot N(\lambda) \, d\lambda} \quad (3)$$

**Spatial loss factor.** Evaluating the spatial loss factor $S(\vec{r}, \vec{h})$ at the interface between the OLED and the neuron enable to estimate the total amount of power, $\Phi_{E_{\text{neuron}}} (\vec{r}, \vec{h}, t)$ in [W], observed by a neuron of a surface $A_{\text{neuron}}$ and with a spatial coordinate $\vec{r}$, radiation direction $\vec{h}$ and time $t$. It corresponds to the transmittance of the path and it’s allowing to estimate the fraction of light emitted by the OLED that is coupled to the neuron. It is represented by the shape of the light distribution $l(\vec{r}, \vec{h})$, the geometrical
spreading of the unfocused light \( g(\hat{h}) \) that correspond to the law of conversion of energy and the scattering associated with the mass attenuation coefficient and density of different components of the biological environment properties \( m(\vec{r}, \hat{h}) \) explained with general theory of light propagation \cite{508-511}.

\[
S(\vec{r}, \hat{h}) = l(\vec{r}, \hat{h}).g(\hat{h}).m(\vec{r}, \hat{h}) \tag{4}
\]

\[
\frac{\Phi_{E\text{ neuron}}(\vec{r}, \hat{h}, t)}{A_{\text{neuron}}} = S(\vec{r}, \hat{h}). \frac{\Phi_{E\text{ oled}}(r_0, h_0, t_0)}{A_{\text{oled}}} \tag{5}
\]

When considering the geometry independently, following Lambert’s cosine law, the energetic luminance (Radiance) \( R(\vec{r}, \hat{h}, t, \Omega) \) in \([\text{W.sr}^{-1}.\text{m}^{-2}]\) with a projected surface of the OLED pixel \( s_E = \cos(\theta) \cdot A_{\text{oled}} \) and a projected surface at the neuron viewpoint \( s_V = \cos(\theta) \cdot A_{\text{neuron}} \)

\[
R(\vec{r}, \hat{h}, t, \Omega) = \frac{\Phi_{E\text{ oled}}(r_0, h_0, t_0)}{\Omega_E \cdot s_E} = \frac{\Phi_{E\text{ neuron}}(\vec{r}, \hat{h}, t)}{\Omega_V \cdot s_V} \tag{6}
\]

A simplification for \textit{in-vitro} studies, with a negligible media transmittance of \( m = 1 \), and for the assumption of a lambertian light pattern of emission giving a solid angle \( \Omega_E = \pi. \) \cite{511} Therefore,

\[
S = \frac{\Omega_V}{\Omega_E} = \frac{A_{\text{neuron}}}{\pi \cdot D_{\text{neuron}}^2} \quad \text{with } D_{\text{neuron}} \text{ being the estimation of the distance of the neuron to the glass.}
\]

\[
\frac{\Phi_{E\text{ neuron}}(\vec{r}, \hat{h}, t)}{A_{\text{neuron}}} = \frac{\Phi_{E\text{ oled}}(r_0, h_0, t_0)}{\pi \cdot A_{\text{oled}}} \cdot \frac{A_{\text{neuron}}}{D_{\text{neuron}}^2} \tag{7}
\]
**Neuron external efficiency.** External quantum efficiency \( \eta_{ \text{neuron} } \) of the neuron, with incremental photocurrent generated in the neuron \( I_{c, \text{neuron}} \) by the electroluminescent spectral distribution dependence of the OLED optical power \( \Phi_{E_0, \text{neuron}}(r_0, h_0, t_0, \lambda) \), electric charge \( q \), Plank’s constant \( h \) and speed of light \( c \). Membrane currents \( (I_c) \) were obtained applying Ohm’s Law to the experimentally measured membrane voltages changes and neurons’ membrane resistance.

\[
\eta_{ \text{neuron} } = \frac{h \cdot c \cdot I_{c, \text{neuron}}}{q} \int_{\lambda_1}^{\lambda_2} \frac{1}{\Phi_{E_0, \text{neuron}}(r_0, h_0, t_0, \lambda)} \lambda \, d\lambda \quad (8)
\]

Here, we exclude the cell’s metabolic power supply, which generates the requisite ionic balances across the membrane, for the passive ionic flux following opsin activation. Alternate methods may choose to define \( \eta_{ \text{neuron} } \) using the fraction of maximum attainable current.

**Coupling activation efficiency.** An accurate External quantum efficiency of OLED-to-Opsin coupling \( \eta_{\text{OLED-to-opsin}} \) for neuron activation correspond to the product of the quantum efficiency of the OLED, the loss during the transmission of the optical power to the neuron and the quantum efficiency of the neuron.

\[
\eta_{\text{OLED-to-opsin}} = \eta_{\text{oled}} \cdot \eta_{\text{neuron}} \quad (9)
\]

\[
\eta_{\text{OLED-to-opsin}} = \frac{1}{S(r, h)} \cdot \frac{I_{c, \text{neuron}}}{I_{c, \text{oled}}} \quad (10)
\]
Spectral matching. The activation spectrum normalized of the opsin $V_{L, opsin}(\lambda)$ under study correspond to the conversion of energetic optical power $\Phi_{E, oled}(r_0, h_0, t_0)$ to the specific visual optical power for activation of the opsin $\Phi_{L, oled}(r_0, h_0, t_0)$. The spectral matching SM in % is including the loss of energy at the opsin convergence.

$$\Phi_{L, oled}(r_0, h_0, t_0) = \int_{\lambda_1}^{\lambda_2} V_{L, opsin}(\lambda) \Phi'_{E, oled}(r_0, h_0, t_0, \lambda) \, d\lambda \quad (11)$$

$$SM = \frac{\Phi_{L, oled}}{\Phi_{E, oled}} \times 100 = \frac{\Phi_{L, neuron}}{\Phi_{E, neuron}} \times 100 \quad (12)$$

Specific OLED efficiency. By considering the spectral matching, the specific OLED efficiency $\eta_L$ in $[W.sr^{-1}.A^{-1}]$ and the specific OLED efficacy $\eta_P$ in $[W.sr^{-1}.W^{-1}]$ of the OLED can be calculated.

$$\eta_L = \frac{\Phi_{L, oled}}{\Omega_E \cdot I_{c, oled}} \quad (13)$$

$$\eta_P = \frac{\Phi_{Loled}}{\Omega \cdot I_{c, oled} \cdot V} \quad (14)$$

Therefore, a specific activation efficiency $\eta_A$ of the OLED-to-Opsin coupling can be estimated by experimental and prediction. $\eta_A = \frac{\eta_L \cdot \eta_P \cdot I_{c, neuron}}{\Phi_{limit, required}} \quad (15)$
A6 Organic LEDs for in vivo optical imaging of neural activity

A6.1 Organic LEDs made in a cone shape for optical chamber integration

A proposed alternative design using an open cone shape based on flexible substrate encapsulating an OLED could have advantages. The irradiating light towards the working area will result in a free space view for recording (See Appendix A23). Another approach applying free standing organic LEDs as described in chapter 6 would provide emissive light source following the curvature of the cortex and may lead to higher uniformity.

If you join 1. and 2. you will obtain a cone emissive OLED with the contacts (+ / -) outside.

Appendix A23 | Proposed alternative design of an open cone of an OLED emissive pixel that emits light uniformly on the observed area and allow free space for no interferences with the view point leading to no loss of contrast. Arrow in red represents the fluorescent or intrinsic signal of brain activity. Arrows in orange represent the emission of light from the open OLED cone.
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