

# Design and Fabrication of Optical Filters for Fluorescence-Based Monitoring of Organ Viability

Thin-film optical filter fabrication, fluorescence sensing of pH and oxygen, and experimental validation using ex vivo organs

Master Thesis  
Filippos Bersis

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Thin-film optical filter fabrication, fluorescence  
sensing of pH and oxygen, and experimental  
validation using ex vivo organs

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# Preface

This thesis, titled Design and Fabrication of Optical Filters for Fluorescence-Based Monitoring of Organ Viability, marks the conclusion of my Master's journey in Biomedical Engineering at TU Delft.

My involvement in this field began in April 2025 when my supervisor, Prof. Paddy French, introduced me to a remarkable project being developed under his guidance by postdoctoral researcher Dr. Avik Sett. The research centred on creating a single, bi-functional, thin-film, biocompatible optical sensor for simultaneous pH and oxygen monitoring during pre-transplant normothermic perfusion on organ grafts. Coming from an electrical and electronics engineering background, several aspects of this project were new to me. Yet, the enthusiasm it sparked was immediate. Following our discussions, we decided to focus my thesis on designing and fabricating the optical filters to further refine the data collected by this monitoring system.

As I delved into the research, I fell in love with the nobility of creating an impactful, patient-centric medical device. My involvement deepened, leading to opportunities to represent the project at scientific conferences and research showcases. When Dr. Sett later relocated to India, I took on the responsibility of conducting ex vivo experiments on human grafts. This was made possible through a profound collaboration with the Erasmus MC Organ Perfusion team, who graciously provided discarded human livers for our experimentation.

This project holds immense personal significance. It not only validated our engineering designs but also awakened a deep passion for academic research. Witnessing the direct impact that real-world solutions can have on advanced preservation protocols and transplant logistics has solidified my desire to pursue a PhD in this field. It took the dedication of many hard-working people to push this project forward, proudly resulting in multiple publications in globally recognised journals, most notably in Nature's, Microsystems & Nanoengineering.

None of this would have been possible alone. I wholeheartedly thank Prof. French and Dr. Sett for this life-changing opportunity, for entrusting me to be part of their team, and for their unwavering guidance, support, and personal time.

I extend my deep gratitude to Dr. Jeroen de Jonge and PhD candidates Puck Groen and Annelot Muntinga from Erasmus MC for their clinical expertise, interest, and fantastic collaboration. Thank you to Dr. Ger de Graaf and MSc Robbert Friendwijk for their crucial foundational contributions to the project, and to Dr. Massimo Mastrangeli, whose time, support, and resources were indispensable. I am also incredibly grateful to PhD candidates Ceren Kutucu and Zhengwei Liao, whose guidance helped me navigate multiple hurdles.

Working in the labs was vastly improved by the technical expertise of EKL's equipment engineer Dylan Loozen, who generously shared his knowledge and ideas in the cleanrooms, and TU Delft's bioelectronics technician Brian Nanhekhan, whose help in the optics lab was highly impactful.

Lastly, I want to thank my significant other, Victoria, my parents, and my entire family. Your daily support has been paramount; you shared every feeling and difficulty with me, every step of the way. To my friends, thank you for your continuous encouragement and for graciously sharing your belief in me.

*Filippos Bersis  
Delft, September 2026*

# Abstract

This thesis details the development, optimisation, and clinical evaluation of a comprehensive biomedical optical monitoring device designed for the real-time, non-invasive assessment of metabolic parameters in transplantable organs. Addressing the critical need for localised, cellular-level data during ex vivo normothermic machine perfusion (NMP), the research bridges the gap between foundational optical engineering and real-world clinical application. A core focus of this work was the computational modelling and cleanroom fabrication of selective multi-cavity Fabry-Perot (MCFP) optical interference filters. Constructed from alternating dielectric layers, these thin-film stacks were meticulously tuned to achieve the precise spectral separation of excitation and emission wavelengths required for accurate fluorescence detection. Concurrently, bi-functional luminescent hydrogel sensors were synthesised, encapsulated with optimised protective overcoats, and integrated into the device for the continuous monitoring of tissue pH and oxygen ( $O_2$ ) via dual-wavelength excitation (405 nm and 450 nm).

Clinical validation was conducted during the NMP of human donor livers at the Erasmus Medical Center (EMC) across two distinct experimental models. Initial trials on a non-viable, defrosted liver confirmed the sensor's capability to instantaneously track mechanically driven transient shifts in local oxygenation and pH. Subsequently, the platform was deployed on a viable, freshly declined human liver for over six hours, with localised optical readings continuously cross-referenced against gold-standard clinical perfusate analysis. While local  $O_2$  measurements demonstrated high reliability and reflected active biological recovery, pH monitoring faced analytical challenges from hydrogel swelling, blood diffusion barriers, and photobleaching. Notably, UV excitation (405 nm) induced an exponential signal drift, whereas blue light excitation (450 nm) proved significantly more robust for continuous tracking. Ultimately, these trials successfully validated the device's fundamental architecture, demonstrating strong proof-of-concept capabilities while identifying targeted modifications to enhance long-term clinical transplant monitoring.

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# Nomenclature

## Abbreviations

| Abbreviation                            | Definition                                                                                      |
|-----------------------------------------|-------------------------------------------------------------------------------------------------|
| ATP                                     | Adenosine triphosphate                                                                          |
| CCD                                     | Charge-coupled device                                                                           |
| CMOS                                    | Complementary metal-oxide-semiconductor                                                         |
| COF                                     | Covalent organic framework                                                                      |
| COP                                     | Covalent organic polymer                                                                        |
| CVD                                     | Chemical vapour deposition                                                                      |
| DBR                                     | Distributed Bragg reflector                                                                     |
| DCD                                     | Donation after circulatory death                                                                |
| DM                                      | Demineralised                                                                                   |
| DSP                                     | Double-side polished                                                                            |
| ECD                                     | Extended criteria donors                                                                        |
| EMC                                     | Erasmus Medical Center                                                                          |
| ETS                                     | Electron transfer system                                                                        |
| FP                                      | Fabry-Perot                                                                                     |
| FWHM                                    | Full width at half maximum                                                                      |
| HBOC                                    | Haemoglobin-based oxygen carriers                                                               |
| HBW                                     | Half bandwidth                                                                                  |
| HMP                                     | Hypothermic machine perfusion                                                                   |
| HPV                                     | Hypoxic pulmonary vasoconstriction                                                              |
| HPTS                                    | 8-hydroxypyrene-1,3,6-trisulfonic acid (Pyranine)                                               |
| IC                                      | Internal conversion                                                                             |
| ICP-CVD                                 | Inductively coupled plasma chemical vapour deposition                                           |
| IRI                                     | Ischaemia-reperfusion injury                                                                    |
| ISFET                                   | Ion-sensitive field-effect transistor                                                           |
| ISC                                     | Intersystem crossing                                                                            |
| LED                                     | Light-emitting diode                                                                            |
| LPCVD                                   | Low-pressure chemical vapour deposition                                                         |
| MBE                                     | Molecular beam epitaxy                                                                          |
| MCFP                                    | Multi-cavity Fabry-Perot                                                                        |
| MEMS                                    | Microelectromechanical systems                                                                  |
| MIM                                     | Metal-insulator-metal                                                                           |
| MOSFET                                  | Metal-oxide-semiconductor field-effect transistor                                               |
| Na <sup>+</sup> /K <sup>+</sup> -ATPase | Sodium-potassium adenosine triphosphatase (sodium-potassium pump)                               |
| ND                                      | Neutral density                                                                                 |
| NMP                                     | Normothermic machine perfusion                                                                  |
| OD                                      | Optical density                                                                                 |
| PdOEP                                   | Palladium octaethylporphyrin                                                                    |
| PDMS                                    | Polydimethylsiloxane                                                                            |
| PECVD                                   | Plasma-enhanced chemical vapour deposition                                                      |
| PEG                                     | Polyethylene glycol                                                                             |
| PEGDMA-NIPAAm-AA                        | Poly(ethylene glycol) dimethacrylate, <i>N</i> -isopropylacrylamide, and acrylic acid copolymer |
| PET                                     | Photoinduced electron transfer                                                                  |
| PHEMA-co-PAM                            | Poly(2-hydroxyethyl methacrylate-co-acrylamide)                                                 |

| Abbreviation | Definition                                          |
|--------------|-----------------------------------------------------|
| PRBC         | Packed red blood cell(s)                            |
| PS           | Polystyrene                                         |
| PtOEP        | Platinum octaethylporphyrin                         |
| PtTFPP       | Platinum(II) meso-tetra(pentafluorophenyl)porphyrin |
| RF           | Radio frequency                                     |
| ROS          | Reactive oxygen species                             |
| SCS          | Static cold storage                                 |
| USB          | Universal Serial Bus                                |
| UV           | Ultraviolet                                         |

## Symbols

| Symbol                       | Definition                                               | Unit                 |
|------------------------------|----------------------------------------------------------|----------------------|
| Ag/AgCl                      | Silver/silver chloride                                   | [-]                  |
| AlGaAs                       | Aluminium gallium arsenide                               | [-]                  |
| cBase(B)c                    | Base excess in blood                                     | [mmol/L]             |
| $c\text{Ca}^{2+}$            | Concentration of ionized calcium                         | [mmol/L]             |
| $c\text{Cl}^-$               | Concentration of chloride                                | [mmol/L]             |
| $c\text{Glu}$                | Concentration of glucose                                 | [mmol/L]             |
| $c\text{HCO}_3^-(\text{P})c$ | Concentration of bicarbonate in plasma                   | [mmol/L]             |
| $c\text{K}^+$                | Concentration of potassium                               | [mmol/L]             |
| $c\text{Lac}$                | Concentration of lactate                                 | [mmol/L]             |
| $c\text{Na}^+$               | Concentration of sodium                                  | [mmol/L]             |
| $\text{CO}_2$                | Carbon dioxide                                           | [-]                  |
| ctHb                         | Concentration of total hemoglobin                        | [g/dL]               |
| $D$                          | Diffusion coefficient                                    | [cm <sup>2</sup> /s] |
| $d_m$                        | Physical thickness of an individual mirror layer         | [m]                  |
| $d_p$                        | Optical penetration depth                                | [m]                  |
| $d_r$                        | Physical thickness of the resonant cavity                | [m]                  |
| $E$                          | Fluorescent signal intensity                             | [a.u.]               |
| $F$                          | Coefficient of Finesse                                   | [-]                  |
| $F_i\text{O}_2$              | Fraction of inspired oxygen                              | [%]                  |
| $\text{H}^+$                 | Hydrogen ion (proton)                                    | [-]                  |
| Hct <sub>c</sub>             | Hematocrit                                               | [%]                  |
| $I$                          | Luminescence or transmitted light intensity              | [a.u.]               |
| $I_0$                        | Unquenched or initial light intensity                    | [a.u.]               |
| $\text{IP}_3$                | Inositol trisphosphate                                   | [-]                  |
| $k_{SV}$                     | Stern-Volmer quenching constant                          | [L/mol]              |
| KCl                          | Potassium chloride                                       | [-]                  |
| $L$                          | Physical thickness of the sample                         | [m]                  |
| $\text{N}_2$                 | Nitrogen                                                 | [-]                  |
| $n$                          | Refractive index (general)                               | [-]                  |
| $n_m$                        | Refractive index of a specific thin-film mirror material | [-]                  |
| $n_r$                        | Refractive index of the resonant cavity material         | [-]                  |
| $\text{O}_2$                 | Oxygen                                                   | [-]                  |
| $p\text{CO}_2$               | Partial pressure of carbon dioxide                       | [kPa]                |
| $p_a\text{O}_2$              | Arterial partial pressure of oxygen                      | [kPa]                |
| pH                           | Potential of hydrogen                                    | [-]                  |
| $p\text{O}_2$                | Partial pressure of oxygen                               | [kPa]                |
| Pt                           | Platinum                                                 | [-]                  |
| $Q$                          | Quality factor                                           | [-]                  |
| $q$                          | Interference order                                       | [-]                  |

| Symbol                            | Definition                                                   | Unit                          |
|-----------------------------------|--------------------------------------------------------------|-------------------------------|
| $R$                               | Reflectance                                                  | [-]                           |
| $r$                               | Amplitude of reflected light                                 | [-]                           |
| $[\text{Ru}(\text{dpp})_3]^{2+}$  | Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II)          | [-]                           |
| $[\text{Ru}(\text{phen})_3]^{2+}$ | Tris(1,10-phenanthroline)ruthenium(II)                       | [-]                           |
| $S_n$                             | Singlet state ( $n$ -th excited state)                       | [-]                           |
| $\text{Si}_3\text{N}_4$           | Silicon nitride                                              | [-]                           |
| $\text{SiH}_4$                    | Silane                                                       | [-]                           |
| $\text{SiO}_2$                    | Silicon dioxide                                              | [-]                           |
| $s\text{O}_2$                     | Oxygen saturation                                            | [%]                           |
| $T$                               | Transmittance (general)                                      | [-]                           |
| $T_{\text{half}}$                 | Transmittance at half of the peak's maximum height           | [-]                           |
| $\text{TiO}_2$                    | Titanium dioxide                                             | [-]                           |
| $T_{\text{max}}$                  | Maximum transmittance (peak height)                          | [-]                           |
| $T_n$                             | Triplet state ( $n$ -th excited state)                       | [-]                           |
| $t_{\text{res}}$                  | Response time                                                | [s]                           |
| $VR$                              | Vascular resistance                                          | [mmHg·min/L]                  |
| $x$                               | Total thickness of the filtering layer                       | [m]                           |
| $\alpha$                          | Fluorescent signal ratio; Absorbance; Absorption coefficient | [-]; [-]; [ $\text{m}^{-1}$ ] |
| $\theta$                          | Angle of incidence                                           | [°]                           |
| $\lambda$                         | Wavelength                                                   | [nm]                          |
| $\rho$                            | Ratio of optical admittances                                 | [-]                           |
| $\tau$                            | Excited-state decay time (lifetime)                          | [s]                           |
| $\tau_0$                          | Unquenched excited-state decay time (lifetime)               | [s]                           |
| $\phi$                            | Total phase change due to propagation                        | [rad]                         |

# Introduction

## 1.1. The Limitations of Transplantable Organ Monitoring

The transplantation of solid organs remains the only curative treatment option for end-stage liver failure [1, 2]. However, the number of patients on the waiting list continues to grow and far exceeds the number of livers available for transplantation [3]. To bridge this discrepancy, clinicians increasingly rely on extended criteria donors (ECD) and donation after circulatory death (DCD) donors; yet, these organs carry a higher risk of early allograft dysfunction and primary non-function [3].

Historically, Static Cold Storage (SCS) has been the standard for preservation due to its simplicity. However, SCS is limited by its inability to reverse sustained organ injury or allow for the assessment of organ viability prior to transplantation [2, 4]. Furthermore, the lack of oxygen during cold storage contributes to ischaemia-reperfusion injury (IRI), which can severely compromise graft outcomes [2].

Normothermic Machine Perfusion (NMP) has emerged as a revolutionary alternative, designed to mimic physiological conditions by perfusing the organs at 37°C with oxygenated blood or blood substitutes [1, 3]. This technique allows for the resuscitation of the organ and, crucially, the assessment of its viability before implantation [3]. The experimental work in this study utilises the Liver Assist (Organ Assist, Groningen, The Netherlands), a dedicated device that provides pressure-controlled pulsatile flow to the hepatic artery and continuous flow to the portal vein [1, 5].

Despite these advancements, current clinical assessment protocols on devices like the Liver Assist heavily rely on the analysis of the "global" perfusate, the fluid circulating through the entire organ [2]. This standard approach presents two significant limitations:

- **Global Averaging:** Viability criteria currently include measures such as perfusate lactate clearance, pH regulation, partial pressure of oxygen ( $pO_2$ ), and glucose metabolism [3, 1]. However, measurements taken from the organ's venous outflow or bulk perfusate represent an average of the entire organ's metabolism. This global view may mask regional heterogeneity or localised ischaemia, as the perfusate reflects the aggregate function rather than specific tissue zones.
- **Discrete Sampling:** Standard blood gas analysis requires the manual extraction of perfusate samples via syringe at set intervals [1]. This discrete sampling method provides only "snapshots" of the organ's status, failing to capture rapid metabolic fluctuations or immediate responses to haemodynamic changes in real-time.

## 1.2. The Shift Towards Optical, Localised Sensing

To overcome the limitations of global averaging and discrete sampling, there is a critical need for continuous, localised monitoring directly at the tissue surface. Optical sensing, particularly fluorescence-based techniques, offers a powerful non-invasive solution [6, 7]. By utilising hydrogel-coated sensors that operate on fluorescence quenching principles, it is possible to continuously measure specific local biomarkers, such as pH and oxygen, at the cellular level without physical fluid extraction. This approach

eliminates the need for discrete sampling and provides a continuous stream of data, offering a highly granular view of the organ's metabolic microenvironment.

### 1.3. Engineering Challenges in Optical Monitoring

While fluorescence sensing provides a high-resolution image of the organ's health [7], deploying these sensors in a compact, real-world biomedical device presents significant optical engineering challenges. Reliable fluorescence detection requires the precise separation of the relatively weak emitted fluorescent signal from the high-intensity excitation light.

In traditional bench-top setups, this separation is achieved using bulky, expensive optical components. For a miniaturised, integrated biomedical device suitable for clinical perfusion settings, highly specific, thin-film optical filters must be custom-designed and fabricated. These filters must possess high transmission in the emission bands and deep blocking in the excitation bands, all while maintaining a form factor compatible with the sensing apparatus.

### 1.4. Research Objectives and Proposed Solution

This thesis aims to bridge the gap between bench-top optical sensor development and real-world clinical application. To achieve this, a comprehensive biomedical device was developed, moving from foundational optical engineering to ex vivo validation. The specific objectives of this research are three-fold:

1. **Filter Design and Fabrication:** To design and fabricate highly selective thin-film optical filters capable of accurately separating excitation and emission wavelengths for precise fluorescence detection.
2. **Sensor Synthesis and Integration:** To recreate and optimise chemical-based fluorescent optical sensors tailored for the real-time monitoring of pH and oxygen.
3. **Clinical Validation:** To deploy and validate the fabricated sensing apparatus in a clinical research setting.

The validation phase was conducted as part of an internship in collaboration with the Erasmus Medical Center (EMC) in Rotterdam, within the research group dedicated to organ perfusion and transplantation. By applying the developed hydrogel-coated sensors directly to the tissue surface of ex vivo organs during NMP, this study evaluates the device's capability to provide a continuous stream of localised metabolic data.

### 1.5. Clinical Significance and Future Impact

NMP has already demonstrated the ability to rescue grafts that would otherwise be discarded, improving utilisation rates [3, 4]. By providing a higher-resolution image of organ health through local sensing, this technology aims to further reduce the rate of false negatives, scenarios where potentially viable organs are discarded [3]. Furthermore, establishing a reliable, non-invasive monitoring platform is a prerequisite for the development of advanced preservation protocols, paving the way for technologies that can extend organ storage times and improve transplant logistics.

The following chapter will cover the pathological dangers associated with transplantable organs to underscore the critical need for continuous monitoring. This context leads into a discussion of NMP, the current clinical standard for preserving and assessing graft health. Finally, the theoretical framework details the core technologies underlying the developed monitoring device, specifically focusing on optical oxygen sensing, pH sensing, and multiple-layer optical filtering.

# Theoretical Framework

For patients suffering from end-stage organ failure, such as end-stage liver disease, transplantation frequently remains the sole curative therapy; however, a severe global shortage of viable human donor organs has led to substantial wait-list mortality [1]. Recent data highlights this stark imbalance: thousands more patients are added to waiting lists annually than there are available organs, leaving a massive national and global deficit [3]. To expand the donor pool and bridge this gap, clinicians increasingly consider older donors and organs sourced from donation after circulatory death (DCD). Unfortunately, these extended-criteria organs are inherently more susceptible to complications such as early allograft dysfunction and primary non-function. Due to these elevated risks and the uncertainty surrounding organ quality, nearly one in ten procured livers is ultimately discarded rather than transplanted [3].

A primary limiting factor that exacerbates this shortage across all solid organ transplantations is the physiological damage sustained during the preservation and transfer phases. Specifically, traditional static cold storage (SCS) and unavoidable periods of warm ischaemia inevitably trigger ischaemia-reperfusion injury (IRI) [2]. This cascade of cellular damage restricts how long an organ can be safely preserved and makes it exceptionally difficult to assess its true viability prior to implantation, hindering both clinical outcomes and ex vivo research advancements [2].

Throughout the transplantation process, organs are subjected to critical windows where their oxygen supply is severely hindered. Whether during the initial procurement, standard SCS, or the subsequent reperfusion phase, the development of hypoxic conditions leaves the tissue highly vulnerable to sustained cellular injury. Consequently, precise monitoring of local biomarkers is essential. Advanced monitoring not only ensures that only truly viable organs are selected for transplantation, but it also provides the real-time feedback necessary to intervene, resuscitate, and preserve borderline organs that might otherwise succumb to these lethal stressors.

To fully grasp the importance of this monitoring and the mechanisms behind organ degradation, it is first necessary to understand the precise physiological stressors the tissue undergoes.

## 2.1. Hypoxia, Hypoxaemia, and Ischaemia

### 2.1.1. Defining Oxygen Deficiency

Hypoxia is fundamentally defined as a state of oxygen deprivation in which the oxygen levels reaching the tissue are insufficient to maintain adequate homeostasis and meet the metabolic demands of the cells [8, 9, 10]. It must be distinguished from hypoxaemia, which specifically refers to low oxygen content within the blood itself (a reduction in arterial oxygen tension,  $p_aO_2$ , typically due to ineffective pulmonary oxygenation). While hypoxaemia is a primary aetiology of tissue-level oxygen deprivation, hypoxia refers to the localised tissue state [8, 9]. Furthermore, hypoxia differs from anoxia, the complete absence of oxygen (0.0 to 0.5%  $O_2$ ), which is tolerated by oxygen-dependent organisms for only brief periods before irreversible damage occurs [11, 10].

Crucially, hypoxia cannot be defined by a single, universal oxygen tension threshold; it is highly relative

to the "normal" baseline oxygenation of a specific tissue [12]. Different organs operate at vastly different physiological baselines. For instance, alveolar epithelial cells in the adult lung reside at a high partial pressure of oxygen ( $pO_2$ ) of approximately 100 mm Hg, whereas normal arterial levels in the brain are around 37 mm Hg [12, 13]. Therefore, an oxygen tension that triggers a severe hypoxic response in pulmonary cells may be entirely adequate for cellular function elsewhere [12].

### 2.1.2. Cellular Consequences

When a donor organ is subjected to static cold storage (SCS) or periods of interrupted perfusion, it enters a state of ischaemia, a severe restriction in blood supply that causes profound local hypoxia. At the cellular and bioenergetic level, tissues can sustain basal respiration until extracellular  $pO_2$  drops below a critical bioenergetic threshold (often 5 to 7 mm Hg, or less than 1%  $O_2$ ) [12, 9]. Once this threshold is breached, a destructive molecular cascade begins:

**Mitochondrial Dysfunction and Oxidative Stress** Contrary to the assumption that low oxygen simply halts oxidation, hypoxia actively increases the generation of highly toxic Reactive Oxygen Species (ROS) [12]. As physiological  $pO_2$  falls (e.g., below  $\sim 17$  mmHg in neural tissue), mitochondrial respiration is inhibited, and the electron transfer system (ETS) reverses, particularly at Complexes I, II, and III [9, 13, 12]. This leads to a surge in ROS (such as superoxide) released into the intermembrane space. This ROS surge causes lipid peroxidation, the oxidative degradation of cell membranes, which in turn activates  $IP_3$  receptors, triggering a massive, toxic release of intracellular calcium ( $Ca^{2+}$ ) [13]. Ultimately, excessive ROS surpasses cellular antioxidant defences, leading to structural damage, metabolic toxicity, and cell death [9, 11].

**Metabolic Failure, Acidosis, and pH Fluctuations** Oxygen is the essential terminal electron acceptor required for the production of adenosine triphosphate (ATP) [9, 11]. In hypoxic conditions, cells undergo "hypoxic conformance", preserving ATP for obligatory survival functions, but eventually must switch to anaerobic respiration [12]. This anaerobic shift produces significantly less energy and results in the rapid accumulation of lactate [9]. In an ischaemic organ, the danger is compounded by a lack of "washout". The enzymes responsible for cellular oxygen sensing catalyse reactions that release carbon dioxide ( $CO_2$ ) and succinate. Without active blood flow to clear these metabolic byproducts, they accumulate alongside lactate, severely disrupting homeostasis and manifesting as localised tissue acidosis (a sharp drop in pH) [11]. This acidic environment creates a vicious cycle: it negatively affects haemoglobin's oxygen-carrying capacity, hinders the growth of new blood vessels (angiogenesis), and promotes the overgrowth of hypoxia-favourable anaerobic bacteria, which produce further acidic byproducts [9].

**Organ-Specific Structural Impairment** Hypoxia also drives macroscopic structural pathologies that severely compromise graft viability. For example, in donor lungs, hypoxia triggers the internalisation of  $Na^+/K^+$ -ATPase pumps from the cell membrane. While this conserves ATP, it destroys the alveoli's ability to clear fluid, leading to pulmonary oedema [12]. Furthermore, sustained hypoxia causes Hypoxic Pulmonary Vasoconstriction (HPV), leading to increased arterial pressure and chronic vascular remodelling [12]. At the cellular level, prolonged hypoxia can also manifest as dysoxia (histotoxic hypoxia), where structural damage to enzymes like cytochrome C oxidase renders the cells completely unable to utilise whatever oxygen is provided [8].

**Reperfusion Injury and Oxygen Toxicity** The physiological response to oxygen deprivation dictates that simply resupplying an ischaemic organ with oxygen is not a guaranteed cure. In fact, prolonged delivery of high oxygen concentrations (increasing the fraction of inspired oxygen,  $FiO_2$ ) can cause severe oxygen toxicity [8]. Furthermore, the rapid re-introduction of oxygen to ischaemic tissues often triggers Ischaemia-Reperfusion Injury (IRI). Upon re-oxygenation, specific homeostatic resets are required to inactivate hypoxic survival factors; if the balance between oxygen supply and consumption fails, the sudden influx of oxygen reacting with compromised mitochondria exacerbates ROS generation, leading to extensive cellular damage and death [11, 2].

Because the margins between viable tissue and irreversible ischaemic or reperfusion damage are narrow, precise environmental control and monitoring are paramount. The continuous monitoring of organ viability, specifically of factors like localised oxygenation and pH, is a critical imperative to prevent tissue death. Having established the physiological necessity of tracking these parameters to save vulnerable organs, it is essential to examine how this monitoring is currently implemented in clinical practice.

## 2.2. Normothermic Machine Perfusion (NMP)

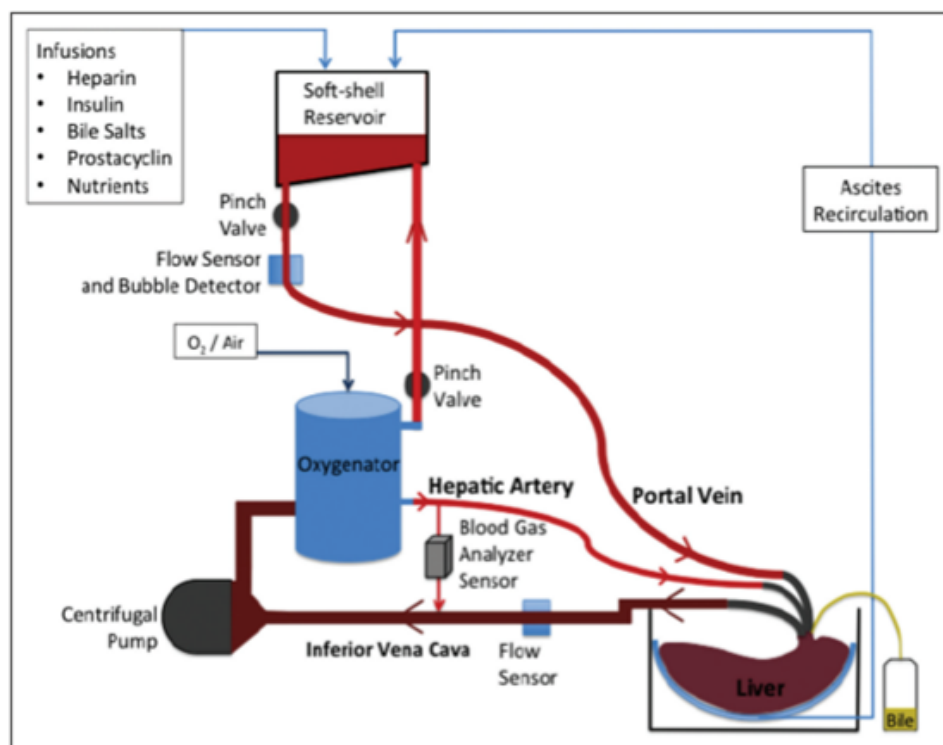
To combat the profound limitations of traditional Static Cold Storage (SCS), the field of organ transplantation has increasingly turned to dynamic preservation techniques. The most prominent and revolutionary of these is Normothermic Machine Perfusion (NMP).

### 2.2.1. The Modern Standard of Ex Vivo Preservation

NMP systems are advanced medical devices designed to preserve donor organs *ex vivo* by closely mimicking physiological conditions [2, 3]. While traditional SCS and Hypothermic Machine Perfusion (HMP) aim to minimise ischaemic damage by slowing cellular metabolism using cold temperatures (typically 4°C to 12°C), NMP takes the opposite approach. It maintains the organ at a normothermic body temperature (34°C to 38°C) while continuously perfusing it with a calibrated mixture of oxygen and nutrients [4, 3]. This active state of physiological simulation keeps the organ metabolically functional rather than in a state of suspended animation, fundamentally altering how clinicians handle and evaluate donor organs [2, 1].

### 2.2.2. System Operation

Establishing a successful NMP circuit requires highly regulated hardware to replicate the complex haemodynamics of the human body. A standard NMP system consists of a sterile organ chamber, fluid pumps (often centrifugal or rotary), an oxygenator for gas exchange, a heat exchanger to maintain normothermia, and an array of integrated sensors to monitor flow, pressure, and temperature (Figure 2.1) [2, 3].



**Figure 2.1:** Normothermic machine perfusion pump system schematic. Reproduced from [3].

The experimental validation in this thesis relies on the **Liver Assist** (Organ Assist, Groningen, The Netherlands) (Figure 2.2), a dedicated Class IIa medical device explicitly engineered for ex vivo liver perfusion [5, 4]. *It should be noted that the Liver Assist machine is the specific NMP system with which the experiments associated with this thesis project were conducted.* To accurately replicate the liver's natural dual blood supply, the Liver Assist utilises a dual-perfusion mechanism [5, 4]:

- **Hepatic Artery Circuit:** A pump unit delivers pressure-controlled, pulsatile flow (maintaining a mean pressure of approximately 70 mmHg) [1, 4, 5].
- **Portal Vein Circuit:** A separate pump unit provides continuous, low-pressure flow (approximately 11 mmHg) [1, 4, 5].



**Figure 2.2:** Liver Assist (Organ Assist, Groningen, The Netherlands) NMP System. Reproduced from [5].

Circulating through this hardware is the perfusate. Because oxygen demands are exceptionally high at 37°C, the base nutrient solution must be supplemented with a highly efficient oxygen carrier. While packed red blood cells (PRBCs) are the traditional standard, recent advancements have introduced Haemoglobin-Based Oxygen Carriers (HBOCs), such as HBOC-201 [1, 3]. These polymerised, bovine-derived haemoglobin molecules are significantly smaller than human erythrocytes, allowing for deeper tissue penetration, lower fluid viscosity, and an improved release of oxygen to remote tissue regions, all while eliminating the logistical hurdles of human blood matching [1].

### 2.2.3. Clinical Impact

The adoption of NMP has transformed organ transplantation logistics and patient outcomes in several critical ways:

1. **Expanding the Donor Pool:** By keeping the organ metabolically active, NMP allows surgeons to safely utilise "marginal" organs, such as those from Donation After Circulatory Death (DCD) or elderly donors, which carry higher risks of early allograft dysfunction [3]. NMP has demonstrated "rescue rates" of over 70% for livers that would have otherwise been discarded [3].
2. **Mitigating Ischaemia-Reperfusion Injury (IRI):** NMP substantially reduces the ischaemic period. Systems like the Liver Assist also permit controlled oxygenated rewarming, attenuating the shock and massive ROS generation that typically occurs when an ice-cold organ is suddenly reperfused with warm blood in the patient [2, 4].
3. **Platform for Therapeutics:** Maintaining a functional organ ex vivo creates a unique window for direct medical intervention prior to implantation. Emerging techniques include "defatting" steatotic livers, administering thrombolytic drugs to dissolve microthrombi, and delivering targeted gene therapies [2, 3].

### 2.2.4. Viability Monitoring

The most profound advantage of NMP over cold storage is that it transforms organ assessment from a subjective visual judgement into an objective, data-driven analysis [3]. Because the organ is metabolically active, clinicians can conduct real-time functional testing to determine if the graft is truly viable.

- **Oxygenation and Lactate Clearance:** The liver's ability to perform aerobic respiration is the ultimate test of viability. Clinicians use lactate clearance as a primary proxy for this oxygenation [2, 3]. A rapid decline in perfusate lactate levels (falling to  $\leq 2.5$  mmol/L) indicates that the hepatocytes are well-oxygenated, metabolically competent, and successfully producing ATP [3, 1]. Conversely, a failure to clear lactate strongly indicates severe hypoxic injury or metabolic failure.
- **pH Homeostasis:** The liver is central to acid-base homeostasis. A viable liver must be able to maintain the perfusate pH within a healthy physiological range ( $> 7.2$ , ideally  $\geq 7.3$ ) without requiring massive external supplementation of sodium bicarbonate [3, 1]. Furthermore, monitoring the pH of the bile produced during NMP acts as the "canary in the coal mine" for biliary health. A bile pH above 7.6 is a robust criterion indicating healthy biliary function, whereas a pH below 7.4 signals severe injury to the biliary epithelium and a high risk of post-transplant strictures [3].

While these metrics provide unprecedented insight into the organ's overall status, they are ultimately derived from the bulk perfusate exiting the organ. This "global" fluid analysis represents an average of the entire liver's metabolism, presenting a significant limitation when attempting to identify localised zones of ischaemia or tissue damage, a limitation that drives the need for localised sensing technologies.

## 2.3. Oxygen Sensing

Having analysed the biological dangers of oxygen deprivation and the current status quo of graft monitoring, the critical importance of reliable oxygen measurements is clear. Accurate quantification of localised oxygen tension is paramount, especially for assessing the metabolic viability of donor organs during dynamic perfusion [14].

### 2.3.1. Electrochemical Approach

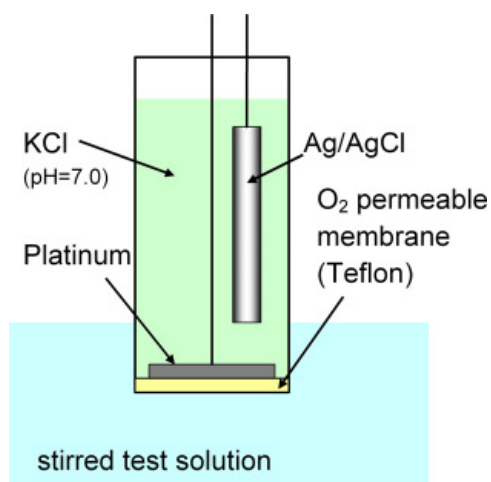
Historically, the quantification of oxygen tension ( $pO_2$ ) has been dominated by electrochemical methods, with the Clark-type electrode serving as the undisputed gold standard for decades [15, 16].

The Clark electrode operates on the principle of amperometric electrochemical reduction. In its standard configuration, the sensor consists of a noble metal working electrode (e.g. platinum or gold) that acts as a cathode, paired with a reference/counter electrode (e.g. Silver/Silver Chloride, Ag/AgCl) acting as an anode [16, 17, 18]. These electrodes are immersed in an electrolyte solution and separated from the biological sample by a gas-permeable, polymer membrane, such as Teflon or polydimethylsiloxane (PDMS) [18, 16] (Figure 2.3).

The fundamental sensing mechanism relies on a diffusion-limited reduction reaction. A polarising voltage is applied to the cathode [17]. As dissolved oxygen from the surrounding tissue or perfusate diffuses across the permeable membrane and reaches the cathode, it is electrochemically reduced [16]. This continuous reduction generates a measurable electrical current that is directly proportional to the local partial pressure of oxygen [17, 18].

#### Advantages in Clinical Monitoring

When scaled down using modern microfabrication techniques, miniaturised Clark cells offer rapid and highly localised detection. As demonstrated by Gray, Marland, Dunare, et al. [17], micro-electrodes with an active diameter of just  $50\ \mu\text{m}$  can be placed directly on the serosal surface of an organ, detecting localised ischaemic events within 1 to 2 minutes without inducing parenchymal damage [17]. Furthermore, advanced protective coatings, such as Nafion (a sulfonated ionomer), can be applied to exclude macromolecules and interfering anions, thereby reducing biofouling and stabilising the sensor for hours during in vivo monitoring [17].



**Figure 2.3:** Clark electrode schematic, consisting of Pt and Ag/AgCl electrodes, with a Teflon membrane. Reproduced from [19].

### Limitations of Electrochemical Sensing

Despite its historical dominance, the traditional Clark electrode possesses several inherent practical limitations that restrict its utility in continuous, long-term organ perfusion and microfluidic applications:

1. **Oxygen Consumption:** The most critical flaw of the amperometric method is that the electrochemical reduction physically consumes the oxygen molecules it is attempting to measure [16, 15]. In micro-environments or under hypoxic conditions where oxygen diffusion is mass-transfer limited, the electrode can consume oxygen faster than it is replenished, leading to severe underestimations of true tissue oxygenation [20]. Consequently, accurate measurements often require continuous sample stirring or high flow rates [15].
2. **Signal Drift and Biofouling:** The generated electrical current is highly dependent on the diffusion characteristics of the permeable membrane. When deployed in protein-rich biological media (such as organ perfusate), proteins and cells rapidly adhere to the membrane (biofouling). This alters the oxygen diffusion rate over time, causing severe long-term signal drift [16].
3. **Material Degradation and Invasiveness:** The Ag/AgCl anode slowly degrades during continuous operation, significantly limiting the working life of miniature cells [20]. Furthermore, these permanent, non-biodegradable materials carry a risk of inducing a chronic inflammatory response if left in situ, and their required electrical connections present a physical pathway for bacterial contamination in sterile bioreactors or perfusion loops [21, 16].

### Biodegradable Galvanic Cells

To circumvent the issues of permanence and external power requirements, a novel electrochemical approach has been developed by She and Allen [21], the biodegradable galvanic corrosion cells [21]. Instead of relying on an external polarising voltage, these self-powered sensors operate via a spontaneously corroding electrochemical couple (e.g., a Magnesium anode and a Molybdaenum cathode). In this system, the local oxygen concentration directly influences the reduction reaction at the cathode, generating a measurable shift in the sensor's output voltage [21]. Crucially, because the materials are entirely biodegradable and utilise the biological fluid itself as an electrolyte, they are highly suited for transient biomedical applications like organ transport, eliminating the need for subsequent surgical removal and reducing the risk of chronic inflammation [21].

### 2.3.2. Optical Approach

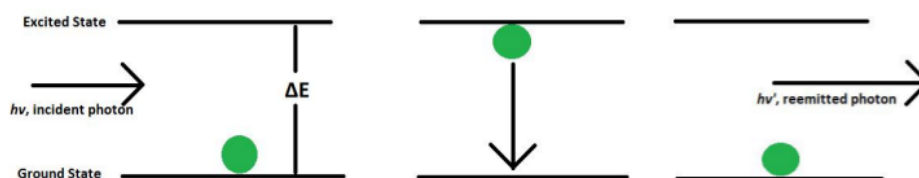
Given the significant limitations of conventional electrochemical sensors, optical sensing techniques have emerged as a robust and adaptable alternative for monitoring oxygen in biological systems [15, 16, 22]. Optical sensors do not consume the target analyte, require no reference electrode, and remain immune to electromagnetic interference and flow rate variations [22].

### Photoluminescence and Fluorescence Quenching

The scientific principle driving optical oxygen sensors is the dynamic (or collisional) quenching of photoluminescence, a contactless optical phenomenon where matter emits light following the absorption of a photon [23, 24, 14]. Photoluminescence encompasses both fluorescence and phosphorescence, which are distinguished by their specific electron spin states and emission timescales [25, 24].

To fully understand the sensing mechanism, one must examine the energy state transitions within the luminescent indicator dye (the luminophore) [15]. The process begins with photo-excitation. When the luminophore is illuminated by an external light source (such as an LED), its electrons absorb the electromagnetic energy and are promoted from a stable, ground electronic state ( $S_0$ ) to a higher, unstable excited electronic and vibrational state (e.g.,  $S_1$  or  $S_n$ ) [26, 25, 24].

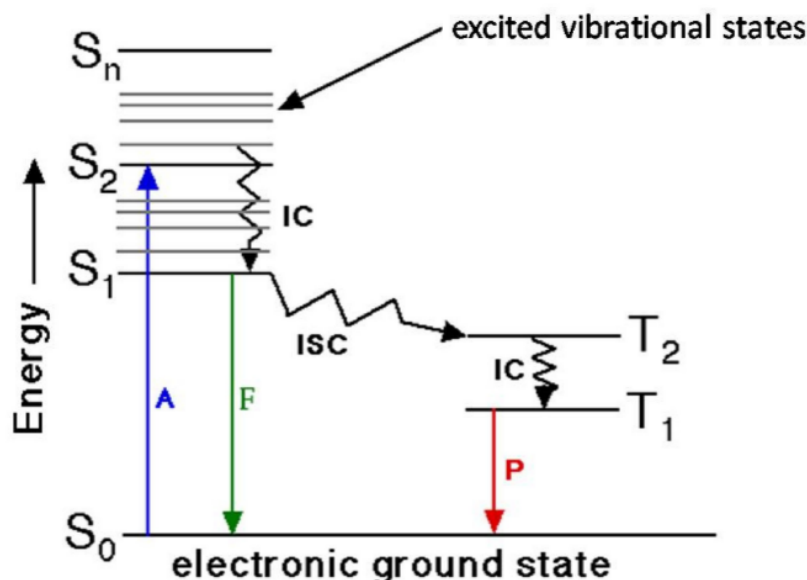
Because this highly energetic state is unstable, the molecule rapidly undergoes internal conversion [24]. During this phase, the electrons lose a fraction of their vibrational energy to the surrounding environment as they relax to the lowest vibrational level of  $S_1$  [26, 24]. From this metastable state, the electrons return to their equilibrium ground state ( $S_0$ ), releasing their remaining excess energy as a photon of light (Figure 2.4) [25, 24, 15]. Because a portion of the initial energy is inherently lost during internal conversion prior to emission, the emitted light always possesses a lower energy and correspondingly, a longer wavelength than the excitation light [26, 25, 24]. This predictable optical displacement is known as the Stokes shift [26, 24].



**Figure 2.4:** Upon excitation by incident light, an electron can return to a lower energy state by emitting a photon. Reproduced from [24].

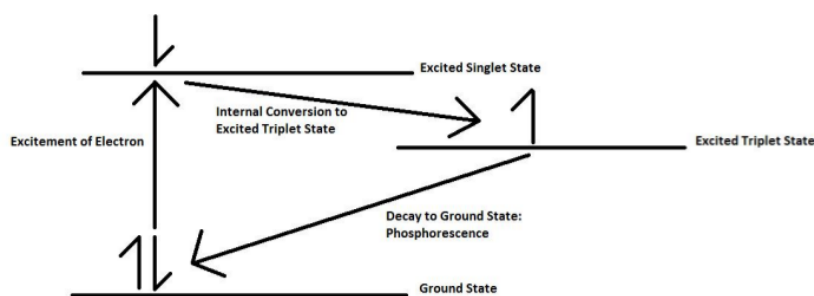
The nature, efficiency, and duration of this photon emission are dictated by the electron's spin state, dividing the phenomenon into either fluorescence or phosphorescence.

**Fluorescence** Fluorescence occurs when the excited electron returns directly from the singlet excited state ( $S_1$ ) to the singlet ground state ( $S_0$ ) without altering its spin (Figure 2.5) [26, 24]. Because this transition is "spin-allowed" under quantum mechanics, fluorescence is a highly rapid and efficient radiative process, typically lasting only  $10^{-9}$  to  $10^{-7}$  seconds [25]. Consequently, the emission ceases almost immediately after the excitation light source is removed [25]. For optical oxygen sensing, certain fluorescent complexes are favoured due to their high quantum yields, bright signal intensities, and robust photostability [16]. Fluorescent probes, such as ruthenium-based complexes, provide fast sensor response times (often within one second) and reliable signal transduction, making them the dominant choice for real-time, continuous monitoring in standard physiological and microfluidic environments [16, 27].



**Figure 2.5:** Jablonski diagram illustrating the main photophysical processes: absorption (A), fluorescence (F), phosphorescence (P), singlet state (S), triplet state (T), internal conversion (IC), and intersystem crossing (ISC). Reproduced from [24].

**Phosphorescence** Alternatively, the excited molecule may undergo a process called intersystem crossing [24]. In this scenario, the electron transitions from the singlet state ( $S_1$ ) into a metastable triplet state ( $T_1$ ), where its spin becomes parallel to the ground state electron (Figure 2.5, 2.6) [26, 24]. Because returning to the ground state from this triplet configuration requires a reversal of electron spin, a "spin-forbidden" conversion, the resulting emission is significantly delayed [24]. This delayed emission, termed phosphorescence, produces a characteristic "afterglow" with much longer lifetimes ranging from microseconds ( $10^{-4}$  seconds) to hours ( $10^4$  seconds) [25, 24]. While phosphorescent emissions are inherently weaker than fluorescence and require strict encapsulation to protect the fragile triplet state from background noise, this extended lifetime provides a much larger temporal window for oxygen collisions [15, 14, 24]. Therefore, phosphorescent dyes (like metalloporphyrins) are exceptionally sensitive, serving a highly specific role in monitoring deeply hypoxic or low-oxygen environments [15, 14].



**Figure 2.6:** Phosphorescence occurs when an electron relaxes from an excited triplet state to the singlet ground state, releasing energy in the form of an emitted photon. Reproduced from [24].

**Dynamic Quenching by Oxygen** Whether the specific emission pathway relies on fluorescence or phosphorescence, the introduction of a target analyte like molecular oxygen ( $O_2$ ) disrupts the radiative decay through dynamic quenching [26, 14, 16]. As oxygen molecules diffuse through the sensor matrix,

they collide with the luminophore while it is still trapped in its excited state ( $S_1$  or  $T_1$ ) [14, 16]. During this collision, the excited state energy of the indicator molecule is non-radiatively transferred to the oxygen molecule [15]. This collisional interaction immediately deactivates the luminophore, forcing it to return to its ground state without the emission of a photon (radiationless deactivation) [14, 15]. Consequently, an increase in local oxygen concentration leads to a predictable, quantifiable reduction in both the intensity of the emitted light and the overall lifetime of the excited state [26, 15, 14].

This photoluminescent quenching behaviour is mathematically modelled by the Stern-Volmer equations, which describe the non-linear dependence of the quenching process on the target analyte concentration [28, 15]:

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + k_{SV}[O_2] \quad (2.1)$$

In this equation,  $I_0$  and  $\tau_0$  represent the unquenched luminescence intensity and excited-state decay time in the absolute absence of oxygen, while  $I$  and  $\tau$  represent these respective values when oxygen is present [28, 15, 16]. The Stern-Volmer quenching constant is denoted by  $k_{SV}$ , and  $[O_2]$  represents the concentration of dissolved oxygen permeating the sensor (often interchangeable with  $pO_2$  for partial pressure) [28, 15, 16].

### Sensor Architecture

The basic physical architecture of an optical oxygen sensor typically involves three primary components: an excitation light source, the sensing dye, and a photodetector [18, 29] (Figure 2.7). Light Emitting Diodes (LEDs) or optical fibres are commonly used to deliver the excitation light, while photodetectors such as CCD cameras, microscopes, photodiodes or optical fibres are used to capture the emitted luminescent signal [15, 18].

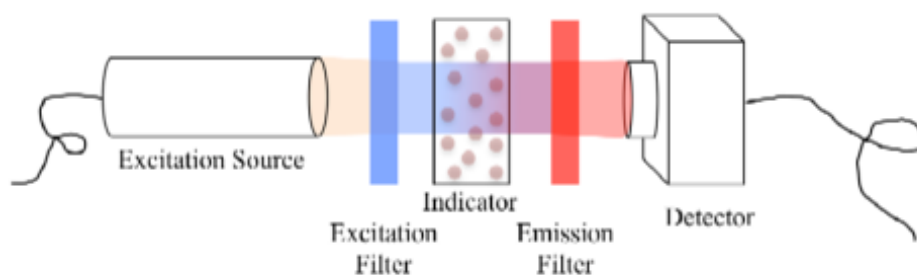
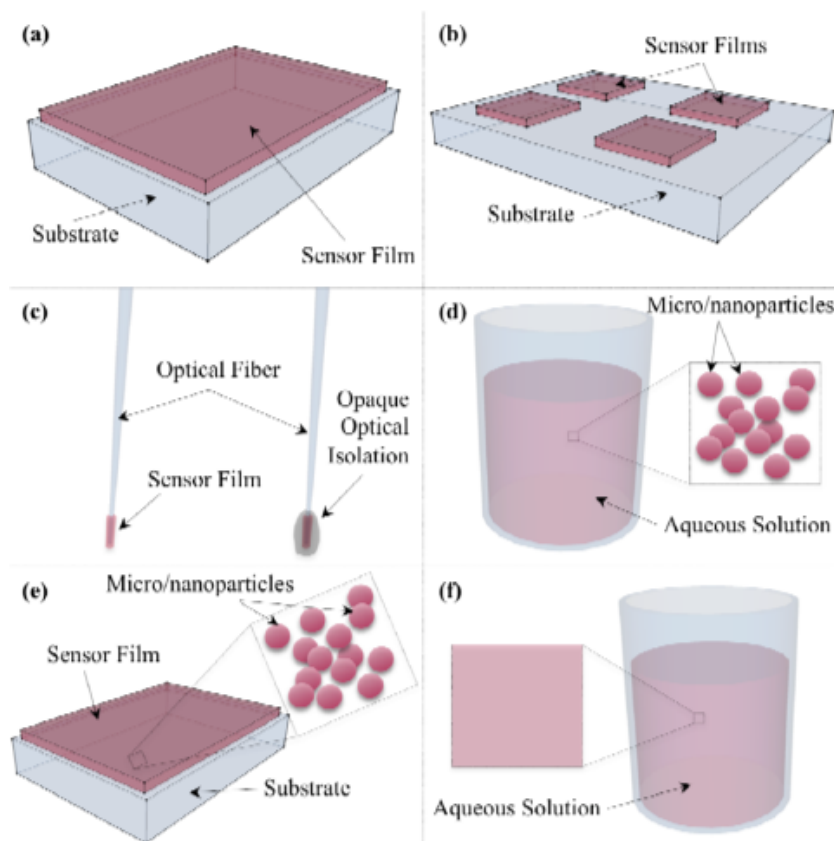


Figure 2.7: Optical sensor setup example. Reproduced from [15].

Because they rely entirely on light transmission, optical sensors can be miniaturised into a variety of form factors (Figure 2.8) suitable for different biological environments:

- **Thin-Film Sensors:** The sensing matrix is pipetted or spin-coated directly onto rigid substrates like glass or flexible polymers, creating planar sensing patches [15].
- **Fibre-Optic Probes:** The sensor film is attached to or photopolymerised directly onto the distal tip of an optical fibre, allowing for targeted probe insertion [15, 27].
- **Nanoparticles and Microparticles:** Indicator dyes are encapsulated within polymer or silica microbeads [18, 14]. These can be dispersed into culture media or even taken up intracellularly for highly localised 3D sensing [18].



**Figure 2.8:** Representative oxygen sensing formats: (a) planar thin-film sensor; (b) patterned thin-film configuration; (c) tapered optical fibre sensor with and without opaque polymer isolation (rendered partially transparent for clarity); (d) micro/nanoparticle sensors dispersed in aqueous media; (e) micro/nanoparticles incorporated into a thin-film layer; (f) water-soluble sensor molecules dissolved in solution. Reproduced from [15].

### Detection Modalities

Optical sensors utilise three primary readout strategies to translate the quenched light into actionable oxygen data. Each method offers its own advantages and limitations.

- **Intensity-Based Sensing:** This method directly measures the brightness or intensity of the emitted light by capturing the raw amplitude of the fluorescent emission using a camera or photodiode [18]. While it is the easiest method to implement and is highly compatible with standard fluorescence microscopes, it is highly susceptible to errors caused by dye photobleaching, toxic dye leaching, uneven dye distribution and thickness, and intensity variations from the excitation light source [15].
- **Lifetime-Based Sensing:** This method measures the decay time ( $\tau$ ) of the luminescence, determining how long the fluorophore remains in its excited state before decaying [15]. This can be done either in the time domain (using pulse-and-gate methods) or the frequency domain (measuring phase shifts) [15]. Lifetime measurements are widely considered superior and more robust than intensity measurements because they are fundamentally independent of probe concentration, initial light source intensity, and detector efficiency [30]. The primary drawback is that this method requires a significantly more complicated measurement setup featuring fast, time-resolved imaging technology [14].
- **Ratiometric Sensing:** This approach is designed to stabilise intensity-based readings without the need for complex lifetime imaging hardware [30, 15]. The sensor incorporates two distinct dyes into its matrix: an oxygen-sensitive indicator dye and an oxygen-insensitive reference dye, allowing the system to measure the ratio of the two emission intensities [15, 30]. Because both dyes are subjected to the same optical path variations and light source fluctuations, the calculated ratio remains stable even if the absolute intensities drift [30]. This offers enhanced robustness

while only requiring a standard fluorescence microscope for readout [30].

### Materials

The overall performance, sensitivity, and biological safety of an optical sensor depend on the specific luminophore (indicator dye) selected and the matrix used to encapsulate it [15].

### Luminophores

- **Ruthenium Complexes:** Dyes such as  $[Ru(dpp)_3]^{2+}$  and  $[Ru(phen)_3]^{2+}$  are widely utilised because they exhibit proven photostability, high quantum yields, and large Stokes shifts [15, 16]. However, they possess relatively short excited-state lifetimes (100 ns to 5  $\mu$ s), which results in lower sensitivity [15, 14]. This makes them less ideal for monitoring extremely low-oxygen environments [15]. Furthermore, free ruthenium compounds exhibit documented cytotoxicity and carry potential carcinogenic and mutagenic risks. However, these physiological hazards can be fully mitigated through proper encapsulation; securely immobilising the luminophores within a biocompatible matrix, such as a hydrogel, completely isolates the compounds to prevent toxic leaching, thereby ensuring safe application within delicate biological environments.
- **Metalloporphyrins:** These include Platinum and Palladium-based dyes such as PtOEP, PdOEP, and PtTFPP [15, 18]. They feature long excited-state lifetimes (10  $\mu$ s to 1 ms), which significantly increases the probability of an oxygen collision [15]. This grants them high sensitivity, making them the ideal choice for detecting low-oxygen conditions [15]. However, a notable disadvantage of certain variants, such as PtTFPP and older PtOEP dyes, is their susceptibility to photobleaching over time, which can cause significant signal drift during long-term monitoring [15, 30].

**Immobilisation Matrices** If a luminophore is placed directly into a biological sample, it risks toxic leaching into the tissue and rapid degradation due to nonspecific binding [15, 16, 27]. Therefore, dyes must be immobilised within oxygen-permeable matrices [15].

- **PDMS and Polystyrene (PS):** Polydimethylsiloxane (PDMS) and polystyrene are highly common matrices [15]. PDMS is particularly favoured for its high oxygen diffusivity (with a diffusion coefficient of  $D = 4.1 \times 10^{-5} \text{ cm}^2/\text{s}$ ), allowing ambient oxygen to rapidly permeate and reach the embedded dye [15, 18].
- **PEG Hydrogels:** To prevent the severe long-term signal drift associated with biological fouling, dyes can be immobilised in biocompatible poly(ethylene glycol) (PEG) hydrogels [16]. PEG is scientifically renowned for its ability to inhibit protein and cellular adhesion [16]. Utilising a low molecular weight PEG creates a tightly cross-linked network that physically traps the dye to prevent toxic leaching, while pre-hydrating the gel ensures optimal conditions for gaseous oxygen diffusion [16].
- **Covalent Co-polymerisation:** To solve the problem of physical dye leakage entirely, some advanced sensors utilise chemically modified luminophores (such as attaching acrylamide monomers to a ruthenium complex) [27]. This chemical modification allows the dye to covalently bond directly to the polymer network, providing absolute stability and preventing leaching even in long-term aqueous environments [27].

For a comprehensive overview of the properties associated with various indicator dyes and various immobilisation matrices, please refer to Table A.1 in Appendix A.

## 2.4. pH Sensing

Just as monitoring dissolved oxygen is vital for detecting tissue hypoxia, tracking pH is an indispensable marker for understanding tissue homeostasis and metabolic viability [31]. During periods of ischaemia or cardioplegia, oxygen-deprived cells are forced to switch to anaerobic glycolysis to produce ATP, resulting in the rapid accumulation of lactic acid and hydrogen ions ( $H^+$ ) [32]. This precipitous drop in pH serves as a critical, real-time early warning for cellular damage, ischaemia-induced acidosis, or bacterial infection within a perfusion loop [31, 32].

### 2.4.1. Electrochemical Approach

Historically, well-calibrated pH electrodes provided accurate baseline measurements, but their rigid and bulky nature renders them unsuitable for continuous, minimally invasive monitoring inside surgical cavities or microfluidic chips [26].

Modern electrochemical pH sensing is frequently achieved using the potentiometric ISFET (Ion-Sensitive Field-Effect Transistor) principle [33, 34]. These miniaturised sensors utilise thin, robust dielectric layers, such as 60-nm thick silicon nitride ( $Si_3N_4$ ), deposited over bare platinum working electrodes [33, 34]. According to the site-binding model, the  $Si_3N_4$  surface contains specific binding sites (salinol and amine) that dynamically associate with or dissociate from  $H^+$  ions in the surrounding cell-culture medium [33, 35]. As the pH fluctuates due to cellular metabolism, the surface charge of the  $Si_3N_4$  layer changes, creating a measurable electrical potential difference against a standard reference electrode (e.g., Ag/AgCl) [33, 34]. This voltage shift is directly and linearly proportional to the pH step, allowing for continuous tracking of the acidification rate [34].

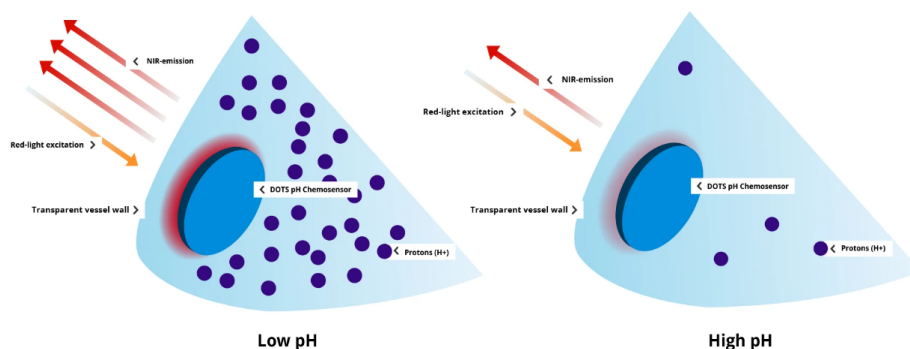
### 2.4.2. Optical Approach

To circumvent the electrical interference inherent to traditional electrodes and enable affordable, non-invasive monitoring, optical pH sensors have seen rapid development [6, 36].

#### Fluorescence and Luminescence

Unlike oxygen sensors that rely on collisional quenching, fluorescent pH sensing predominantly operates on the principle of protonation-dependent ionic equilibria (Figure 2.9) [26]. Fluorophores such as fluorescein derivatives or pyranine (HPTS) alter their molecular structure and optical properties based on the ambient  $H^+$  concentration [6, 26].

For example, HPTS shifts dynamically between a protonated (phenol) state in highly acidic environments and a deprotonated (phenolate) state in basic environments [32]. Under neutral or basic conditions, the phenolate form dominates; it absorbs excitation light and emits a strong green fluorescence [32]. As the pH drops, the equilibrium shifts toward the phenol form, which does not emit fluorescence under the same excitation, causing a measurable decrease in signal intensity [32].



**Figure 2.9:** Schematic of an optochemical pH sensor showing protonation-dependent fluorescence intensity with an inverse optical response to pH changes. Reproduced from [37].

Conversely, certain fluorophores exhibit a reverse optical response to protonation (Figure 2.9) [26]. For instance, coumarin-based dyes integrated into Covalent Organic Polymers (COPs) frequently incorporate an imidazole ring that remains protonated and highly fluorescent in neutral to near-neutral environments [26]. As the pH increases into highly basic ranges (e.g., pH 10 to 13.6), the ring gradually loses its proton to form an imidazole anion [26]. Unlike HPTS, this deprotonated state actively quenches the coumarin fluorescence through Photoinduced Electron Transfer (PET), transferring an electron to the coumarin core and resulting in a signal decrease as the environment becomes more alkaline [26]. In other specific dye molecules, quenching can also be driven by the formation of non-fluorescent intra-ester isomers [6].

Because fluorescence intensity is highly vulnerable to optical noise, photobleaching, and changes in the physical light path, dual-wavelength ratiometric readout methods are highly preferred for pH sensing [32]. By utilising dyes with multiple distinct absorption bands (such as HPTS or Pyranine-integrated Covalent Organic Polymers), the sensor can be excited by a pH-sensitive wavelength (e.g., 475 nm) and a pH-insensitive reference wavelength (e.g., 405 nm) sequentially [32, 26]. Specifically, the ratio  $\alpha(pH)$  can be mathematically calculated by comparing these two readings [32]:

$$\alpha(pH) = \frac{E(pH)_{\lambda_{high}}}{E(pH)_{\lambda_{low}}} \quad (2.2)$$

where  $E(pH)_{\lambda}$  represents the intensity of the fluorescent signal associated with the respective excitation wavelength  $\lambda$  [32]. The calculated ratio of the resulting emissions cancels out the absolute dye concentration and background stray light, ensuring robust, self-calibrating accuracy [32].

#### Absorbance and Waveguide Mechanics

Beyond fluorescence, optical pH sensing can be achieved through absorbance spectroscopy and physical waveguide transduction.

- **Absorbance Sensing:** This modality utilises entrapped dyes (such as bromophenol blue) that fluctuate their specific optical absorption spectrum as the pH changes [28]. When light is guided through an optical fibre coated with this dye, the evanescent field extends into the matrix. [28]. The amount of light absorbed from this evanescent wave acts as a direct indicator of the local pH level [28]. The absorbance  $\alpha(\lambda)$  can also be mathematically calculated, based on Beer's law, by measuring the transmittance  $T(\lambda)[\%]$  [32]:

$$\alpha(\lambda) = -\log(T(\lambda)) = -\log\left(\frac{T(\lambda)[\%]}{100}\right) \quad (2.3)$$

Moreover, the optical penetration depth ( $d_p$ ) which describes the physical distance light must travel through the sample before its transmitted power drops to  $1/e$  (or 36.8%) can be formulated as [32]:

$$d_p(pH, \lambda) = \frac{-L}{\log(T(pH, \lambda))} \quad (2.4)$$

where  $L$  represents the physical thickness of the sample [32].

- **Hydrogel Swelling Mechanics:** Novel, 3D waveguide sensors measure pH via osmotic pressure [38]. As carboxylic groups within specific hydrogels (like PEGDMA-NIPAAm-AA) become charged due to pH changes, counterions rapidly flood the nanoporous structure [38]. This creates an osmotic pressure differential that forces the hydrogel to take up water and swell [38]. This mechanical expansion closes the physical gaps of integrated diffraction gratings, effectively converting a chemical pH shift into a sudden, macroscopic loss of optical light scattering [38].

#### Sensor Materials and Matrices

As established with oxygen sensors, unprotected indicator dyes are highly susceptible to toxic leaching and rapid signal degradation when exposed directly to biological environments [6, 26]. Consequently, pH-sensitive luminophores must also be securely immobilised within specialised encapsulation matrices.

- **Sol-Gel Glasses:** One foundational approach to immobilisation is the use of sol-gel processing to create microporous silica or titania glass matrices [28]. Analyte-sensitive species are permanently entrapped within a nanometre-scale, mesh structure that allows smaller target analytes (like  $H^+$  ions) to permeate the interconnected pores [28].
- **Hydrogels and Microbeads:** Biocompatible hydrogels are extensively utilised for pH sensing in microfluidics and multi-well plates [6, 32]. To eliminate dye leaching, modern formulations (such as PHEMA-co-PAM) utilise chemical conjugation [6]. Alternatively, dyes like HPTS can be primarily immobilised inside microscopic anion exchange resins (such as AmberChrom microbeads) before being uniformly suspended in a softer hydrogel matrix like HydroMed D4 [32].

- **Covalent Organic Polymers and Frameworks (COPs/COFs):** As an advanced alternative to hydrogels, COPs and COFs are crystalline, porous materials linked by strong covalent bonds [26]. Unlike traditional small-molecule chemosensors, they are insoluble in water and organic solvents, inherently preventing any leaching and allowing for repeated use [26].

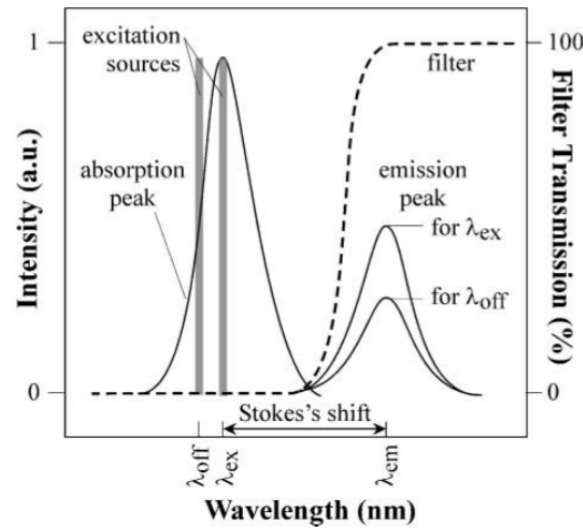
#### Design Challenges and Interferences

Translating theoretical optical pH sensing into complex, dynamic biological environments presents several distinct engineering challenges.

- **Optical Interference and Turbidity:** Biological mediums are inherently complex; they are often deeply coloured, turbid, and contain high concentrations of cells and opaque suspended particles [36]. These fluids intensely absorb and scatter excitation light, creating a high background emission that severely interferes with accurate optical readings [36]. To overcome this, sensing matrices can be capped with a black, microporous filter membrane (e.g., 0.45  $\mu m$  pore size) stained with water-insoluble ink [36]. This acts as a selective optical shield, blocking scattered light and cellular matter from reaching the detector while allowing hydrogen ions to diffuse freely into the sensing film [36].
- **Signal Drift and Photobleaching:** The intensity of a fluorescent emission will naturally degrade over time due to reagent photobleaching, ageing of the light source, or physical shifts in the optical path [28, 32]. To isolate the pH signal from these hardware issues, sensors tend to rely on dual-wavelength ratiometric measurements or lifetime-decay analysis rather than simple intensity measurements [28, 32].
- **Oxygen Cross-Talk:** Certain luminescent complexes (particularly ruthenium-based dyes) used for pH sensing exhibit a cross-sensitivity to molecular oxygen via dynamic quenching [36]. If unaccounted for, fluctuating dissolved oxygen levels in the perfusate might skew the pH readings. To resolve this interference, sensing platforms should utilise distinct probes with separated, non-overlapping excitation and emission spectra to prevent optical cross-talk or operate under strictly controlled environmental conditions [6, 36].

## 2.5. Optical Filtering

The ability to isolate specific wavelengths of light is a fundamental requirement for any optical sensor, particularly when monitoring transplantable organs in a perfusion environment [39]. In fluorescence spectroscopy, the excitation light is often orders of magnitude brighter than the resulting fluorescent emission [39]. Without an optical filter to block these intense excitation photons while allowing the weaker emission to pass, the critical biological signal would be completely drowned out by optical noise [39]. Consequently, optical filters serve as essential tuning mechanisms, reshaping the light spectrum to capture the exact data required by the detector (Figure 2.10) [40].



**Figure 2.10:** Illustration of the principle of optical filtering in fluorescence sensing. The excitation spectrum (left) and emission spectrum (right) highlight how a wavelength filter (dashed line) suppresses excitation light while transmitting fluorescence emission, ensuring accurate signal detection. Excitation at off-peak wavelengths results in lower emission intensity. Reproduced from [39].

### 2.5.1. Physics and Principles

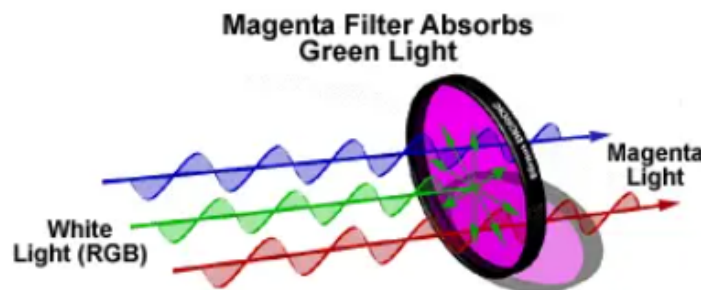
Optical filtering relies on manipulating the interaction between electromagnetic radiation and physical matter [40]. The two most prominent scientific principles governing this manipulation are selective absorption and wave interference.

#### Absorptive Filtering

Absorptive filtering relies on the inherent chemical or material properties of a substrate to absorb specific, unwanted wavelengths while allowing others to pass through (Figure 2.11) [41, 39]. This attenuation is strictly governed by the Beer-Lambert law, where the reduction in light intensity is a function of the material's absorption coefficient and the optical path length [39, 42]. Mathematically, this relationship is expressed as:

$$I = I_0 e^{-\alpha x} \quad (2.5)$$

In this equation,  $I_0$  denotes the initial intensity of the incoming light,  $I$  represents the final intensity of the transmitted beam,  $\alpha$  defines the material's wavelength-specific absorption coefficient, and  $x$  corresponds to the total thickness of the filtering layer [39]. In semiconductor-based absorptive filters, this principle is dictated by the material's bandgap: photons with energies lower than the bandgap pass through unimpeded, while high-energy photons are absorbed [39]. By operating through a single functional layer, absorptive filtering bypasses the nanometre scale thickness tolerances and multi-step deposition process required for multilayer interference stacks [39].



**Figure 2.11:** Schematic representation of an absorption filter showing selective wavelength attenuation, where green light is absorbed by the filter material and red and blue wavelengths are transmitted. Reproduced from [43].

Beyond inorganic semiconductors, organic absorption filters incorporate non-fluorescent chromophores into a polymer matrix via backbone integration or host-guest blending [39]. These filters enable low-cost, room-temperature processing (e.g., spin-coating), with out-of-band rejection scaling directly with chromophore concentration up to matrix saturation [39].

Absorptive filters offer low angle sensitivity, maintaining spectral stability across wide incident angles without passband blue-shifts, alongside simple fabrication, low stress, and high mechanical robustness [41, 39]. Conversely, their response is constrained by intrinsic material properties rather than stack geometry, preventing ultra-steep edge transitions [41, 39]. Additionally, non-zero out-of-band absorption can attenuate weak fluorescence signals, while absorbed light may induce thermal loading under high-power illumination [41, 39].

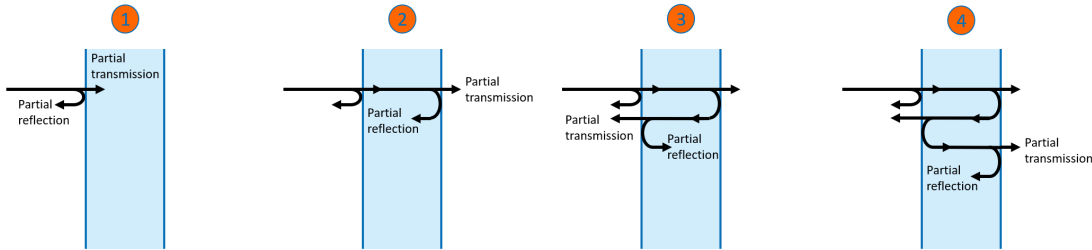
### Multiple-Layer Interference Filtering

To achieve high spectral precision required for modern fluorescence sensing, optical systems rely on multiple-layer interference filters [39]. Unlike absorptive filters that convert unwanted light into heat, interference filters manipulate the fundamental wave nature of light.

The core principle involves depositing alternating thin-film layers of materials with differing refractive indices ( $n$ ) onto a transparent substrate [39, 44]. As broadband incident light passes through this multilayer stack, a portion of the light is reflected at every discrete interface between a high-index and a low-index material [39]. The magnitude of this reflected amplitude at any single boundary is quantitatively defined by the relationship:

$$r = \frac{1 - \rho}{1 + \rho} \quad (2.6)$$

where  $r$  denotes the amplitude of the reflected light and  $\rho$  represents the ratio of the optical admittances between the two adjoining media [39]. When light reflects off a boundary of a medium with a lower refractive index, it undergoes a specific  $180^\circ$  phase shift (Figure 2.12) [39]. By precisely engineering the physical thickness of each layer, the filter forces specific target wavelengths to undergo constructive interference (reinforcing their amplitude and allowing transmission) while forcing unwanted wavelengths to undergo destructive interference (cancelling them out and reflecting them away) [39, 44].

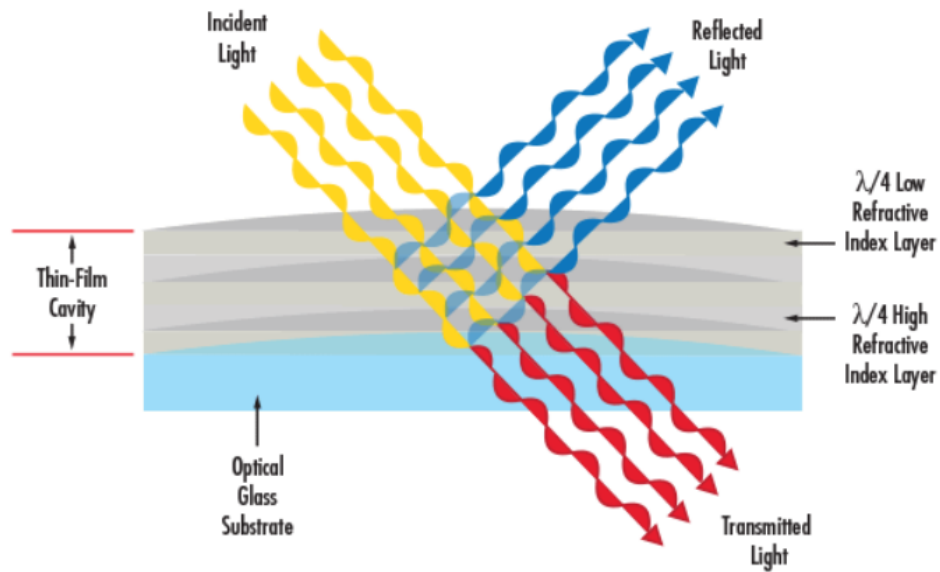


**Figure 2.12:** Illustration of the fundamental principle of interference filtering, where light experiences multiple partial reflections and transmissions at dielectric layer interfaces. Reproduced from [45].

### 2.5.2. Distributed Bragg Reflectors (DBRs)

The foundational architecture for these interference filters is the quarter-wave structure, commonly known as a Distributed Bragg Reflector (DBR) or Bragg mirror [39].

In a standard DBR, the physical thickness of each alternating dielectric or semiconductor layer is rigorously designed to be exactly  $\lambda/4n$ , where  $\lambda$  is the target centre wavelength and  $n$  is the refractive index of that specific material layer (Figure 2.13) [39]. This quarter-wave geometry ensures that all reflections from the multiple interfaces recombine in phase. By stacking many of these alternating pairs (e.g., using dielectrics like  $SiO_2$  and  $TiO_2$ , or compound semiconductors like AlGaAs), the DBR achieves near-perfect reflectivity for a specific band of light, acting as a highly efficient, wavelength-selective mirror [39].

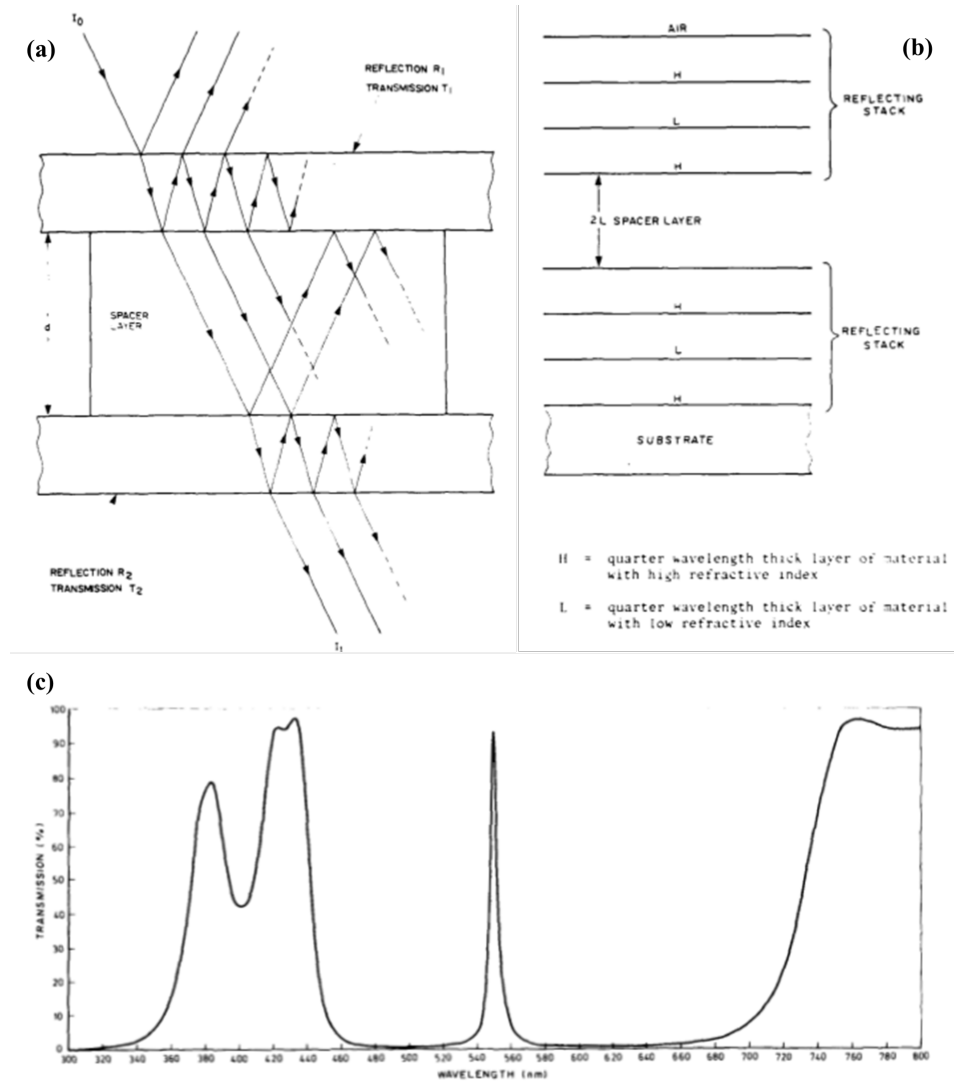


**Figure 2.13:** Illustration of a multilayer interference filter composed of alternating high- and low-refractive-index dielectric layers forming a quarter-wave stack structure, a fundamental architecture used in distributed Bragg reflectors and thin-film optical filters. Reproduced from [46].

Ultimately, the precise manipulation of quarter-wave DBR stacks serves as the foundational building block for more complex, highly selective optical geometries, most notably Fabry-Pérot interferometers and Notch filters.

### 2.5.3. Fabry-Perot Filter

The most prominent application of multiple-layer interference in miniaturised optical sensing is the Fabry-Pérot (FP) interferometer, frequently implemented as a solid-state optical interference filter [47, 48]. An FP filter is fundamentally composed of a central transparent resonant cavity (or spacer layer) positioned between two strictly parallel, highly reflective mirrors (Figure 2.14) [49, 47, 48, 50]. Due to its compact size, high spectral selectivity, and compatibility with standard semiconductor manufacturing, the FP architecture is heavily utilised in integrated lab-on-a-chip devices and CMOS-compatible biosensors [51, 52].



**Figure 2.14:** Fabry–Perot interference filter principle and response: (a) schematic representation of a Fabry–Perot interferometer, illustrating incident light undergoing multiple reflections and transmissions within a spacer layer confined between two thin-film mirror stacks; (b) example of a Fabry–Perot optical filter, showing dielectric mirror stacks composed of alternating high- and low-refractive-index layers and a central spacer layer with an optical thickness of approximately half a wavelength; (c) typical transmission spectrum of a Fabry–Perot filter, highlighting the main transmission peak and associated sidebands. Adapted from [48].

**Operating Principles and Physics** The filter operates on the principles of multiple-beam amplitude-division interferometry [50]. When incident light enters the structure, it undergoes multiple internal reflections between the two boundary mirrors (Figure 2.14a) [51]. The transmission of light through the filter is dictated by the phase difference of these reflected wavefronts. When the optical path difference aligns as an exact integer multiple of the wavelength, the emerging wavefronts undergo constructive interference, thereby maximising transmitted intensity and minimising reflection [48, 51].

Assuming normal incidence (where the angle of incidence  $\theta = 0$ ), the specific resonant wavelength ( $\lambda$ ) that successfully transmits through the cavity is governed by the resonance condition:

$$q\lambda = 2n_r d_r \quad (2.7)$$

where  $q$  is an integer representing the interference order,  $n_r$  is the refractive index of the resonant cavity material, and  $d_r$  is the physical thickness of the cavity [49, 51]. Furthermore, in the case of employing dielectric mirrors consisting of thin-film multilayers, the physical thickness ( $d_m$ ) of each individual mirror

layer is strictly dictated by the condition:

$$4n_m d_m = q\lambda \quad (2.8)$$

where  $n_m$  is the refractive index of that specific thin-film material [49, 51]. At all other wavelengths, destructive interference drives the transmitted intensity toward zero, effectively reflecting those out-of-band wavelengths away [51]. In the absence of optical absorption within the cavity, this transmitted intensity distribution ( $I$ ) is mathematically described by the Airy function:

$$I = \frac{T_1^2}{(1 - R_1)^2} \cdot \frac{I_0}{1 + F \sin^2 \phi} \quad (2.9)$$

where  $I_0$  is the incident intensity,  $T_1$  and  $R_1$  are the transmittance and reflectance of the mirrors,  $F$  represents the Coefficient of Finesse, and  $\phi$  is the total phase change due to propagation through the spacer layer [48]. This mathematical relationship visually manifests as the characteristic sharp transmission peaks surrounded by broad reflection zones (Figure 2.14c).

**Mirror Design and Materials** The performance of an FP filter, specifically the sharpness of its transmission band, relies heavily on the reflectivity of its boundary mirrors. While some designs utilise Metal-Insulator-Metal (MIM) configurations (employing thin films of silver or aluminium to achieve high reflectivity), metallic layers inherently absorb a fraction of the incident optical energy [53, 52]. Unlike transparent dielectrics, metals rely on free electrons for reflection; during the multiple internal bounces required for Fabry-Pérot resonance, this results in compounded absorption losses that reduce the filter's overall peak transmittance. Furthermore, introducing certain metals into pristine cleanroom environments can cause severe cross-contamination in standard CMOS or Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD) equipment.

Consequently, modern highly-selective biomedical filters predominantly utilise all-dielectric mirrors, specifically the Distributed Bragg Reflectors (DBRs) discussed previously [47, 49, 51]. By using alternating quarter-wave layers of low refractive index materials (such as Silicon Dioxide,  $SiO_2$ ) and high refractive index materials (such as Titanium Dioxide,  $TiO_2$  or Silicon Nitride,  $Si_3N_4$ ), the filter achieves near-perfect reflectivity with exceptionally low optical absorption [47, 49, 51]. The central resonant cavity is typically formed by depositing a precisely controlled half-wave layer of the low-index material (e.g.,  $SiO_2$ ) between the two DBR stacks [49, 51].

**Key Characteristics:** The spectral profile of a Fabry-Pérot filter is defined by several critical metrics:

- **Half Bandwidth (HBW) / FWHM:** The Full Width at Half Maximum (FWHM) defines the spectral width of the transmission peak [48]. Narrow FWHM values indicate high selectivity, which is vital for isolating specific fluorophore emissions from background noise.
- **Finesse and Q-Factor:** Finesse is the ratio of the distance between adjacent transmission peaks (the Free Spectral Range) to the FWHM [48]. The Quality factor (Q-factor) explicitly quantifies the sharpness of the resonance, defined mathematically as the ratio of the peak-transmission wavelength ( $\lambda_0$ ) to the FWHM ( $Q = \lambda_0/\text{FWHM}$ ) [53]. Higher mirror reflectivity directly increases both finesse and Q-factor, yielding a sharper filter [48, 53].
- **Wavelength Tuning:** The exact spectral position of the transmission line is directly proportional to the physical height of the cavity [47]. While some advanced MEMS filters feature tunable air-gap cavities actuated by electrostatic forces, solid-state sensors rely on fixed-width cavities tailored during the deposition process [47, 52].

**Fabrication Challenges and Limitations** While thin-film FP filters offer exceptional integration potential, they are bound by stringent fabrication constraints [49, 52]. Filters are typically fabricated using Radio Frequency (RF) sputtering or PECVD [49, 52]. Because the target wavelength is dependent on the cavity thickness, a deposition thickness error of merely 0.1% can significantly displace the passband [48, 49]. Additionally, the theoretical limits of a narrow FWHM assume perfectly flat, parallel mirrors; any surface roughness or lack of parallelism across the substrate will severely degrade the filter's optical resolution [48]. Finally, like all geometric interference structures, FP filters are highly sensitive to the angle of incidence [48]. Illuminating the filter with non-normal light alters the effective optical path length, shifting the passband toward shorter wavelengths and broadening the FWHM [48, 53].

### 2.5.4. Filter Characterisation Parameters

To engineer or select the appropriate optical filter, several defining parameters must be carefully evaluated [41]:

- **Transmittance and Stopband:** Transmittance defines the precise fraction of incident light that successfully passes through the filter, whereas the stopband dictates the spectral range where light is strictly attenuated or blocked [41].
- **Bandwidth and Edge Steepness:** The bandwidth represents the specific width of the transmitted wavelength range [41]. Steep spectral transitions at the edges of this band are crucial for ensuring sharp cutoffs and high-resolution wavelength control [41].
- **Optical Density (OD):** This logarithmic metric quantifies a filter's efficiency at blocking unwanted light [41]. High OD values represent high light suppression, which is critical for preventing excitation light bleed-through in highly sensitive fluorescence detectors [41].
- **Angle Sensitivity:** This parameter measures how a filter's spectral performance shifts when light strikes it at non-perpendicular angles, a common vulnerability in interference-based filters due to their thin-film geometry [41].

### 2.5.5. Categorisation of Optical Filters

Based on how they manipulate the spectral profile, filters are generally categorised into several functional types [54, 41]:

- **Bandpass Filters:** These transmit a strictly defined, isolated range of wavelengths while blocking all light above and below that band [54, 41]. They are the standard choice for isolating specific fluorophore emissions.
- **Edge Filters (Longpass and Shortpass):** These operate based on a specific wavelength cutoff point. A longpass filter allows wavelengths longer than the cutoff to pass (ideal for capturing red-shifted fluorescence), while a shortpass filter transmits shorter wavelengths while blocking longer ones [54, 41].
- **Notch Filters (Minus Filters):** Functioning as the inverse of a bandpass filter, notch filters are designed to completely reject a highly specific, narrow band of wavelengths (the stopband) while efficiently transmitting the surrounding spectrum [41, 55, 56]. This targeted attenuation makes these filters well-suited for blocking specific laser excitation lines while preserving closely adjacent biological emission signals [41].
- **Neutral Density (ND) Filters:** These uniformly reduce the overall intensity of light across all wavelengths without altering the colour balance or spectral profile, operating purely on intensity-based attenuation [54, 41].

### 2.5.6. Fabrication Materials, Techniques, and Limitations

The evolution of optical filters has shifted from bulk absorptive materials to precision-engineered thin films [54, 24].

Historically, coloured filter glasses and gelatine films were the standard [44, 24]. While cheap and durable, glass filters possessed fixed spectral ranges that could not be easily tailored [54]. More critically, many traditional coloured glasses contained luminescent materials themselves; when struck by UV or high-intensity excitation light, the filter itself would inadvertently fluoresce, ruining the baseline reading of the sensor [24]. Furthermore, their spectral transmission was highly susceptible to degradation from temperature and humidity shifts [44].

Consequently, modern integrated sensing relies on thin-film interference coatings. Integrating these multilayer structures into miniaturised lab-on-a-chip biosensors requires sophisticated low-temperature deposition techniques, such as Plasma-Enhanced Chemical Vapour Deposition (PECVD), Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD), sputtering and Molecular Beam Epitaxy (MBE) [39].

Despite their exceptional optical performance, the primary limitation of interference filters lies in the manufacturing precision they demand [39]. Because the optical phase shifts dictate the entire spectral

response, the deposition equipment must maintain stringent, nanometre control over the thickness of every single layer [39]. A physical thickness variation of as little as 5% across the substrate will drastically shift the cutoff wavelength, entirely ruining the filter's optical resolution and rendering it useless for precise fluorescence isolation [39]. Furthermore, because their function relies strictly on geometric optical path lengths through these thin films, interference filters are highly sensitive to the angle of incoming light [39, 41]. If incident light strikes the filter at non-anticipated angles, the effective path length through the layers changes, causing an undesired shift in the transmitted spectral band [39, 41].

## Methodology

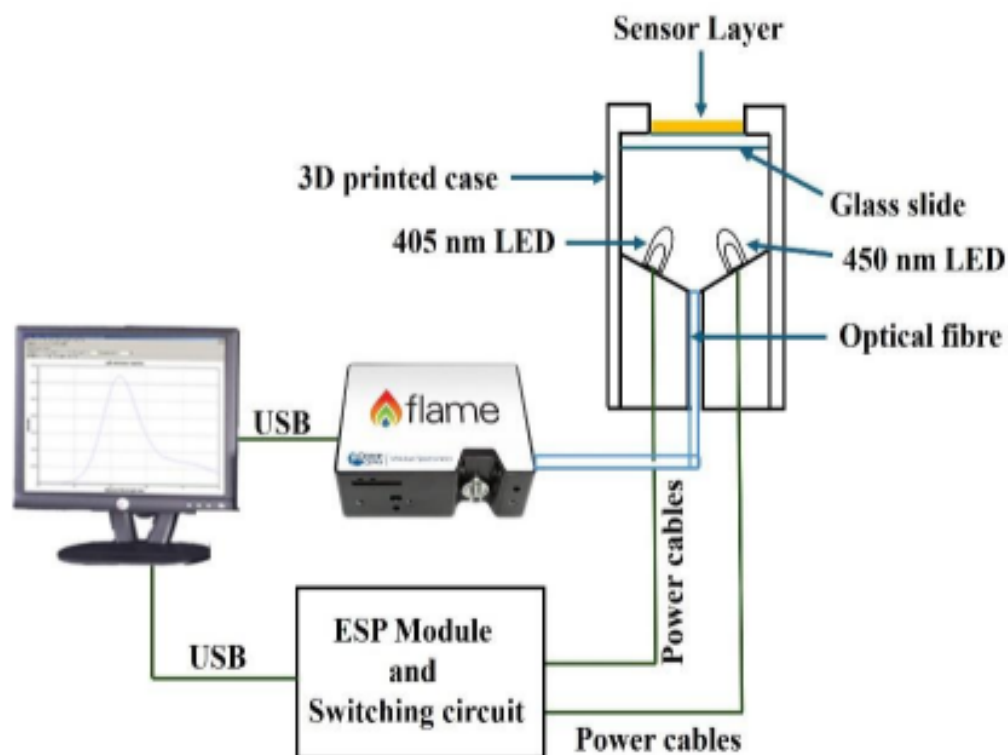
### 3.1. System Overview

This section details the overall architecture of the fluorescence-based sensing system designed for real-time organ viability monitoring. The sensing platform employs a unified, bi-functional optical paradigm specifically engineered to simultaneously monitor pH and dissolved oxygen levels during normothermic machine perfusion. This architecture consolidates two distinct fluorescent probes within a single, biodegradable hydrogel matrix. By utilising two single-wavelength excitation sources (405 nm and 450 nm), the system triggers dual-wavelength sensitive emissions. This configuration enables the independent, real-time tracking of both biomarkers without necessitating complex, multi-sensor hardware arrays. Furthermore, the integration of dual single-wavelength sources facilitates continuous ratiometric measurements, which minimises signal artefacts, enhances data accuracy, and strengthens the overall validity of the physiological readings.

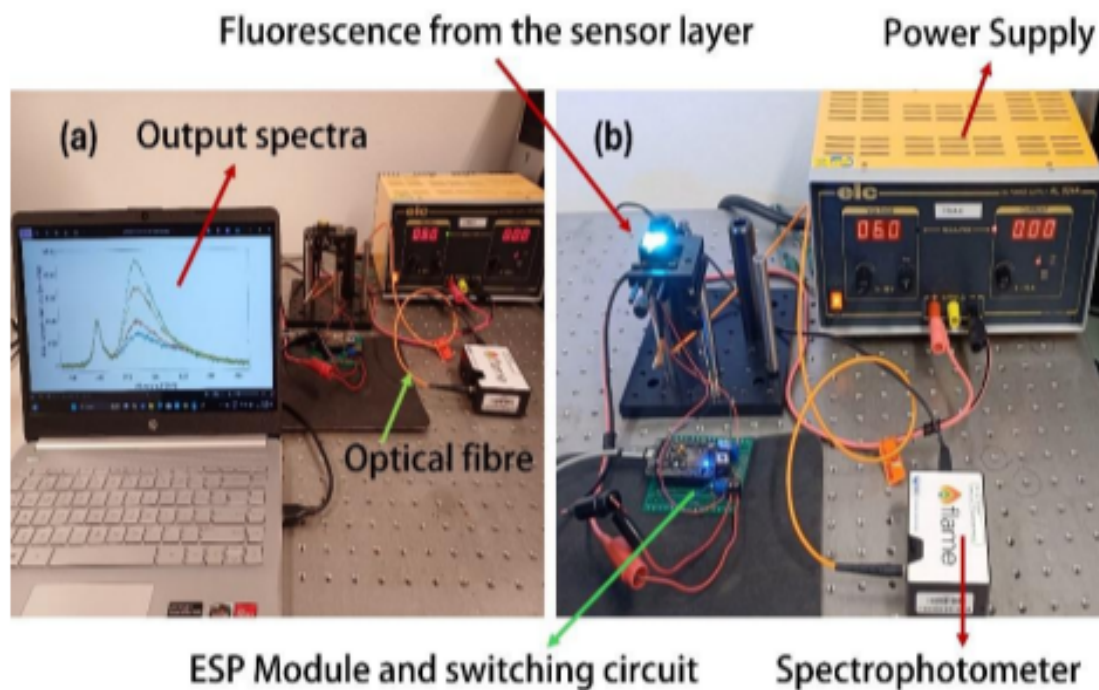
The simultaneous monitoring of these two biomarkers is driven by distinct fluorescence mechanisms. The pH detection operates on the principle of protonation-dependent fluorescence, where the local pH correlates directly with the measured fluorescence intensity; specifically, a more alkaline (higher) pH results in a proportionately higher emission intensity. Conversely, the oxygen detection mechanism relies on the dynamic quenching of fluorescence by molecular oxygen. In this process, the presence of dissolved oxygen proportionally suppresses the emission signal of the oxygen-sensitive probe.

The overall functional flow and hardware architecture of the sensing platform are illustrated in Figures 3.1 and 3.2. System operation is driven by custom MATLAB® scripts that interface with an ESP microprocessor, which in turn controls the switching and illumination of the two LED excitation sources. In the designated operational geometry, the dual-probe hydrogel sensor is placed in direct contact with the organ tissue, positioned directly in front of the LED array. Upon illumination by the LEDs, the fluorophores within the hydrogel become excited. Through the principles of fluorescence, the sensor transmits the emission light backward. This backscattered light bypasses the LED plane and travels toward an optical fibre linked to a spectrophotometer. While the thin-film optical filters were fully fabricated and characterised, they were not directly deployed during the ex vivo experimental validation on human grafts. Nevertheless, the overall optical system and spectral specifications were designed for their eventual integration, where they would be positioned immediately in front of the optical fibre entrance.

To successfully implement this bi-functional optical paradigm and isolate the critical emission signals, the custom thin-film optical filters, positioned immediately in front of the optical fibre entrance, were engineered to meet strict spectral and physical parameters. Primarily, the filters must exhibit high transmittance at the critical emission peaks of 520 nm (the pH-sensitive peak) and 600 nm (the oxygen-sensitive peak). Concurrently, it is imperative to achieve high optical density for excitation rejection at 405 nm and 450 nm. This ensures that the high-intensity excitation light originating from the LED sources is strictly filtered out before reaching the optical fibre, thereby preventing spectrophotometer saturation and signal masking. Finally, the maximum filter thickness was capped at 1.5  $\mu\text{m}$ . This



**Figure 3.1:** Schematic overview of the integrated dual-parameter optical sensing system. The diagram illustrates the complete hardware architecture, including the bi-functional sensor matrix, the customised optical probe housing the dual-excitation light sources (405 nm and 450 nm), the spectrometer for emission acquisition, the ESP microcontroller for hardware synchronisation, and the central laptop interface for real-time data processing. Reproduced from [57]



**Figure 3.2:** Photographs of the experimental measurement setup during operation. (a) The complete system layout, featuring a laptop displaying real-time output spectra. (b) A detailed view of the hardware components, highlighting the active sensor layer, power supply, ESP microcontroller and switching circuit, optical fibre, and spectrophotometer. Reproduced from [57]

precise physical threshold was established to minimise fabrication complexity and mitigate the risk of machine-related deposition errors during the Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD) process.

## 3.2. Optical Filter Design and Simulation

### 3.2.1. Design Requirements

The integration of optical filters was established as a critical requirement following preliminary filterless system testing. These initial tests revealed substantial signal interference from the high-intensity excitation sources, as well as ambient noise from external lighting environments, such as operating rooms. To mitigate this and optimise the signal-to-noise ratio, the optical filters were engineered to selectively transmit the target fluorescence emission wavelengths while decisively rejecting the excitation light.

The primary spectral criteria for the filters included high transmission bands at 520 nm and 600 nm, alongside robust blocking bands at the excitation wavelengths (405 nm and 450 nm). Initially, the theoretical filter architecture was designed utilising titanium dioxide ( $\text{TiO}_2$ ) as the high refractive index material, paired with silicon dioxide ( $\text{SiO}_2$ ) as the low refractive index layer. However, as the project progressed toward physical fabrication, practical constraints necessitated a material substitution. To optimise cleanroom compatibility, leverage pre-existing Chemical Vapour Deposition (CVD) machine infrastructure, and utilise highly validated deposition recipes, silicon nitride ( $\text{Si}_3\text{N}_4$ ) was selected to replace  $\text{TiO}_2$ . Both  $\text{Si}_3\text{N}_4$  and  $\text{SiO}_2$  exhibit excellent biocompatibility, are readily accessible within the university's cleanroom facilities, and have been successfully deployed in previous institutional research, providing a high degree of confidence in achieving the target optical properties. Based on the spectral requirements, both Fabry-Perot resonance and distributed bandpass filter architectures were selected as the ideal configurations for the sensing setup.

### 3.2.2. Simulation Method

The theoretical modelling of the thin-film stacks was initiated through analytical calculations of optical thickness. The alternating dielectric mirrors, which act as Bragg reflectors, were calculated using the quarter-wave optical thickness equation:

$$d = \frac{\lambda}{4n} \quad (3.1)$$

where  $d$  is the physical thickness,  $\lambda$  is the target centre wavelength, and  $n$  is the refractive index of the respective material. For the Fabry-Perot resonance cavities, the half-wave optical thickness equation was utilised to establish the transmission peaks:

$$d = \frac{\lambda}{2n} \quad (3.2)$$

Preliminary computational simulations were initially conducted based on the theoretical  $\text{TiO}_2$  and  $\text{SiO}_2$  pairing. Following the strategic material substitution to  $\text{Si}_3\text{N}_4$ , a new series of iterative simulations was performed to optimise the final optical configuration and adapt the thickness calculations to the new refractive index. While OpenFilters and TFCalc were initially explored, the final optimisation was conducted using the Filmetrics Spectral Reflectance Calculator (KLA Corporation) due to software licensing constraints. The simulation environment was defined with air as the incident medium and a silica glass substrate ( $n \approx 1.457$  for  $\text{SiO}_2$  [58]). During this phase, standard library values for the refractive indices of  $\text{Si}_3\text{N}_4$  ( $n \approx 2.023$  [59]) and  $\text{SiO}_2$  ( $n \approx 1.457$  [58]) were utilised, pending empirical characterisation of the specific cleanroom-deposited materials. Filter performance was evaluated by analysing the resulting transmittance versus wavelength spectra, focusing specifically on transmission efficiency at the emission peaks, the Full Width at Half Maximum (FWHM), and the depth of the out-of-band rejection.

**Table 3.1:** Layer configuration profiles for all simulated Fabry-Perot filter iterations targeting the 520 nm peak. Models are grouped by their primary high-index material ( $\text{TiO}_2$  vs.  $\text{Si}_3\text{N}_4$ ), with total physical thickness denoted at the bottom of each stack.

| L#           | TiO <sub>2</sub> Models |             |             |    |            | Si <sub>3</sub> N <sub>4</sub> Models |             |             |             |             |             |
|--------------|-------------------------|-------------|-------------|----|------------|---------------------------------------|-------------|-------------|-------------|-------------|-------------|
|              | M                       | F1          | F2          | M  | F3         | M                                     | F4          | F5          | F6          | F7          | F8          |
| 1            | Si                      | 60          | 80          | Ti | 45         | Si                                    | 45          | 60          | 90          | 60          | 70          |
| 2            | Ti                      | 30          | 50          | Si | 80         | Ni                                    | 45          | 50          | 60          | 60          | 70          |
| 3            | Si                      | 80          | 90          | Ti | 45         | Si                                    | 45          | 60          | 90          | 60          | 70          |
| 4            | Ti                      | 40          | 30          | Si | 180        | Ni                                    | 140         | 165         | 120         | 170         | 70          |
| 5            | Si                      | 80          | 80          | Ti | 45         | Si                                    | 45          | 60          | 90          | 60          | 70          |
| 6            | Ti                      | 45          | 40          | Si | 80         | Ni                                    | 45          | 50          | 60          | 60          | 175         |
| 7            | Si                      | 40          | 80          | Ti | 45         | Si                                    | 45          | 60          | 90          | 60          | 70          |
| 8            | Ti                      | 60          | 45          | –  | –          | Ni                                    | 180         | 170         | 120         | 60          | 70          |
| 9            | Si                      | 180         | 40          | –  | –          | Si                                    | 60          | 60          | 90          | 60          | 70          |
| 10           | Ti                      | 45          | 60          | –  | –          | Ni                                    | 60          | 50          | 60          | 170         | 70          |
| 11           | Si                      | 80          | 180         | –  | –          | Si                                    | 60          | 60          | 90          | 60          | 70          |
| 12           | Ti                      | 40          | 45          | –  | –          | Ni                                    | 180         | 165         | 120         | 60          | 175         |
| 13           | Si                      | 80          | 80          | –  | –          | Si                                    | 50          | 60          | 90          | 60          | 70          |
| 14           | Ti                      | 30          | 40          | –  | –          | Ni                                    | 50          | 50          | 60          | 60          | 70          |
| 15           | Si                      | 90          | 80          | –  | –          | Si                                    | 50          | 60          | 90          | 60          | 70          |
| 16           | Ti                      | 50          | 30          | –  | –          | Ni                                    | –           | –           | –           | 170         | 70          |
| 17           | Si                      | –           | 90          | –  | –          | Si                                    | –           | –           | –           | 60          | 70          |
| 18           | Ti                      | –           | 50          | –  | –          | Ni                                    | –           | –           | –           | 60          | 175         |
| 19           | –                       | –           | –           | –  | –          | Si                                    | –           | –           | –           | 60          | 70          |
| 20           | –                       | –           | –           | –  | –          | Ni                                    | –           | –           | –           | –           | 70          |
| 21           | –                       | –           | –           | –  | –          | Si                                    | –           | –           | –           | –           | 70          |
| 22           | –                       | –           | –           | –  | –          | Ni                                    | –           | –           | –           | –           | 70          |
| 23           | –                       | –           | –           | –  | –          | Si                                    | –           | –           | –           | –           | 70          |
| <b>Total</b> |                         | <b>1030</b> | <b>1190</b> |    | <b>520</b> |                                       | <b>1100</b> | <b>1180</b> | <b>1320</b> | <b>1470</b> | <b>1925</b> |

**Legend:** L# = Layer Number, M = Material (shared per column group). All thickness values are in nm.

**Materials:** Ti =  $\text{TiO}_2$ , Si =  $\text{SiO}_2$ , Ni =  $\text{Si}_3\text{N}_4$ . All models utilise an Air medium and  $\text{SiO}_2$  substrate.

**Filters:** F1: 16-L, F2: 18-L, F3: 7-L base, F4–F6: Drafts 1–3 (15-L), F7: Measured index (19-L), F8: Extended (23-L).

### 3.2.3. Final Filter Design

The iterative simulation process resulted in two distinct filter architectures, both carefully constrained to remain under the maximum thickness limit of  $1.5\ \mu\text{m}$  to ensure reliable fabrication via Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD).

The first design (3.1) is a 19-layer multi-cavity Fabry-Perot filter tailored for the 520 nm emission peak. The stack sequence alternates between  $\text{SiO}_2$  and  $\text{Si}_3\text{N}_4$ , primarily utilising 60 nm layers for the Bragg reflectors. To induce the necessary transmission resonances, three thicker  $\text{Si}_3\text{N}_4$  cavity layers of 170 nm are strategically placed at layers 4, 10, and 16. The total physical thickness of this design is  $1.47\ \mu\text{m}$ .

The second design (3.2) is a 21-layer bandpass filter optimised for higher wavelength transmission, particularly addressing the 600 nm oxygen-sensitive emission. This stack features an alternating sequence of  $\text{Si}_3\text{N}_4$  and  $\text{SiO}_2$  with varying layer thicknesses to broaden the transmission band. The first nine layers alternate between 50 nm ( $\text{Si}_3\text{N}_4$ ) and 70 nm ( $\text{SiO}_2$ ), while the subsequent layers shift to an 80 nm / 60 nm sequence to manipulate the long-wave pass characteristics. The total physical thickness of this design is  $1.36\ \mu\text{m}$ .

Both designs represent a calculated trade-off between spectral selectivity and fabrication complexity. While increasing the layer count would theoretically improve the narrowness of the FWHM and the depth of the out-of-band rejection, doing so would exceed the  $1.5\ \mu\text{m}$  threshold, thereby significantly

increasing the risk of thin-film delamination, residual stress, and machine-related deposition errors during the CVD process.

**Table 3.2:** Layer configuration profiles for all simulated filter iterations targeting the 610 nm oxygen peak. Models are grouped by their primary high-index material and starting layer architecture, with total physical thickness denoted at the bottom.

| L#    | TiO <sub>2</sub> Models |     | Si <sub>3</sub> N <sub>4</sub> Models |     |      |      |    |      |
|-------|-------------------------|-----|---------------------------------------|-----|------|------|----|------|
|       | M                       | F1  | M                                     | F2  | F3   | M    | F4 | F5   |
| 1     | Ti                      | 50  | Si                                    | 90  | 90   | Ni   | 50 | 50   |
| 2     | Si                      | 90  | Ni                                    | 60  | 60   | Si   | 70 | 70   |
| 3     | Ti                      | 30  | Si                                    | 90  | 90   | Ni   | 50 | 50   |
| 4     | Si                      | 80  | Ni                                    | 155 | 165  | Si   | 70 | 70   |
| 5     | Ti                      | 40  | Si                                    | 90  | 90   | Ni   | 50 | 50   |
| 6     | Si                      | 80  | Ni                                    | 60  | 60   | Si   | 70 | 70   |
| 7     | Ti                      | 45  | Si                                    | 90  | 90   | Ni   | 50 | 50   |
| 8     | Si                      | 280 | Ni                                    | 155 | 165  | Si   | 70 | 70   |
| 9     | Ti                      | 45  | Si                                    | 90  | 90   | Ni   | 50 | 50   |
| 10    | Si                      | 80  | Ni                                    | 60  | 60   | Si   | 80 | 80   |
| 11    | Ti                      | 40  | Si                                    | 90  | 90   | Ni   | 60 | 60   |
| 12    | Si                      | 80  | Ni                                    | 155 | 165  | Si   | 80 | 80   |
| 13    | Ti                      | 30  | Si                                    | 90  | 90   | Ni   | 60 | 60   |
| 14    | Si                      | 90  | Ni                                    | 60  | 60   | Si   | 80 | 80   |
| 15    | Ti                      | 50  | Si                                    | 90  | 90   | Ni   | 60 | 60   |
| 16    | —                       | —   | —                                     | —   | —    | Si   | 80 | 80   |
| 17    | —                       | —   | —                                     | —   | —    | Ni   | 60 | 60   |
| 18    | —                       | —   | —                                     | —   | —    | Si   | 80 | 80   |
| 19    | —                       | —   | —                                     | —   | —    | Ni   | 60 | 60   |
| 20    | —                       | —   | —                                     | —   | —    | Si   | 80 | 80   |
| 21    | —                       | —   | —                                     | —   | —    | Ni   | 60 | 60   |
| Total | 1110                    |     | 1425                                  |     | 1455 | 1370 |    | 1370 |

**Legend:** L# = Layer Number, M = Material (shared per column group). All thickness values are in nm.

**Materials:** Ti = TiO<sub>2</sub>, Si = SiO<sub>2</sub>, Ni = Si<sub>3</sub>N<sub>4</sub>. All models utilise an Air medium and SiO<sub>2</sub> substrate.

**Filters:** F1: TiO<sub>2</sub> base (15-L), F2: Draft 1 (15-L), F3: Draft 2 (15-L), F4: Notch Draft 1 (21-L), F5: Measured index (21-L).

### 3.3. Thin-Film Fabrication

#### 3.3.1. Materials and Substrate Preparation

The optical filters were fabricated using alternating layers of silicon nitride (Si<sub>3</sub>N<sub>4</sub>) and silicon dioxide (SiO<sub>2</sub>). The substrate selected for deposition was a 1-inch, 500 µm thick, double-side polished (DSP) fused silica JGS2 glass wafer (MicroChemicals GmbH). Fused silica was chosen primarily for its exceptional transparency and minimal optical interference. Furthermore, the 500 µm thickness provided sufficient mechanical rigidity to prevent fracture during handling and integration, while the 1-inch diameter ensured compatibility with both the available optical testing setup and the spatial constraints of the organ-monitoring hardware without requiring substantial physical alteration.

To preserve the pristine surface quality of the optical glass, the substrates were utilised strictly as received from the supplier. Because the wafers arrived pre-packaged in a highly controlled, clean state, they intentionally bypassed any additional chemical cleaning or ultrasonic wet bench procedures within the cleanroom prior to deposition. This methodology was implemented to prevent the inadvertent introduction of micro-particulates, organic contaminants, or solvent residues that can occasionally occur during manual handling and standard solvent-based cleaning protocols.

#### 3.3.2. Deposition Principles and Equipment

The thin-film fabrication was conducted in the EKL cleanroom 100 utilising a PlasmaPro 100 Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD) system (Oxford Instruments). Unlike

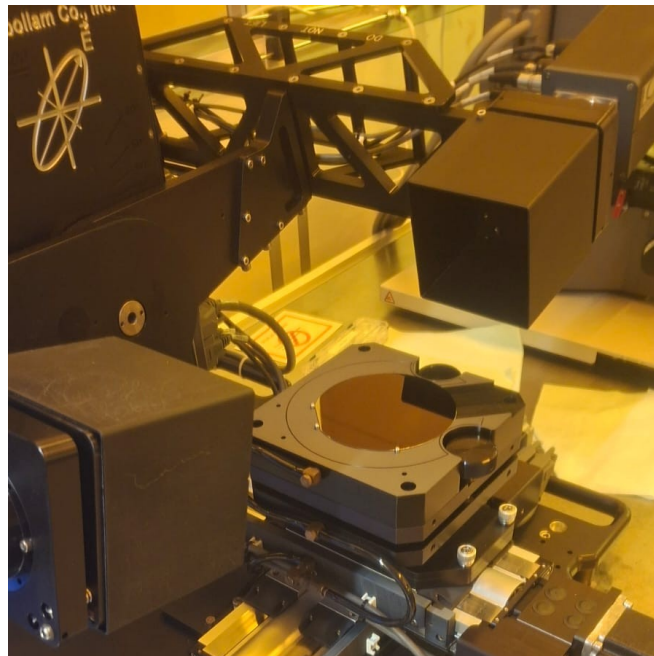
standard Plasma-Enhanced Chemical Vapour Deposition (PECVD), ICP-CVD separates the plasma generation from the substrate biasing. By utilising an inductive coil to generate a high-density plasma at relatively low chamber pressures, ICP-CVD allows for the deposition of high-quality, dense, and low-stress dielectric films at lower substrate temperatures. For this study, the deposition temperature (controlled via the table heater) was maintained at 300°C, a parameter optimised in consultation with the cleanroom process engineers to balance film density with thermal stress.

### 3.3.3. Process Characterisation and Recipe Optimisation

Given the precise nanometer-scale tolerances required for optical filters, accurate control over the deposition rate was paramount. Because there were no pre-existing standard recipes for this specific continuous multi-layer architecture, two custom recipes were developed: "SiO<sub>2</sub>-SiN<sub>x</sub> Filter 300c MC FP Filter" for the multi-cavity Fabry-Perot filter and "Notch Filter SiO<sub>2</sub>-SiN<sub>x</sub> 300C" for the bandpass filter.

To ensure accuracy, a rigorous daily calibration protocol was executed before any fabrication. First, the ICP-CVD chamber was cleared of residual contaminants using a standard "short plasma clean" recipe. Next, a silicon dummy wafer was introduced for a 3-minute preconditioning phase. Crucially, the material deposited during this preconditioning was specifically chosen to be the opposite of the first layer of the intended filter (e.g., if the filter's first layer was Si<sub>3</sub>N<sub>4</sub>, the preconditioning was done with SiO<sub>2</sub>). This step was designed to coat the chamber walls and mimic the internal plasma chemistry that occurs during the actual fabrication, where each subsequent layer is deposited immediately following a layer of the alternating material.

Following chamber conditioning, single-layer depositions were performed on fresh silicon dummy wafers for exactly 1 minute for both SiO<sub>2</sub> and Si<sub>3</sub>N<sub>4</sub>. These calibration wafers were immediately analysed using an M-2000 Ellipsometer (J.A. Woollam) (Figure 3.3). This analysis provided the precise deposition rate and the exact refractive index of the materials for that specific session, yielding values of  $n \approx 1.55$  for SiO<sub>2</sub> and  $n \approx 1.92$  for Si<sub>3</sub>N<sub>4</sub>. These empirical deposition rates were then used to translate the simulated physical thicknesses of the filter designs into exact deposition times in the custom ICP-CVD recipes.

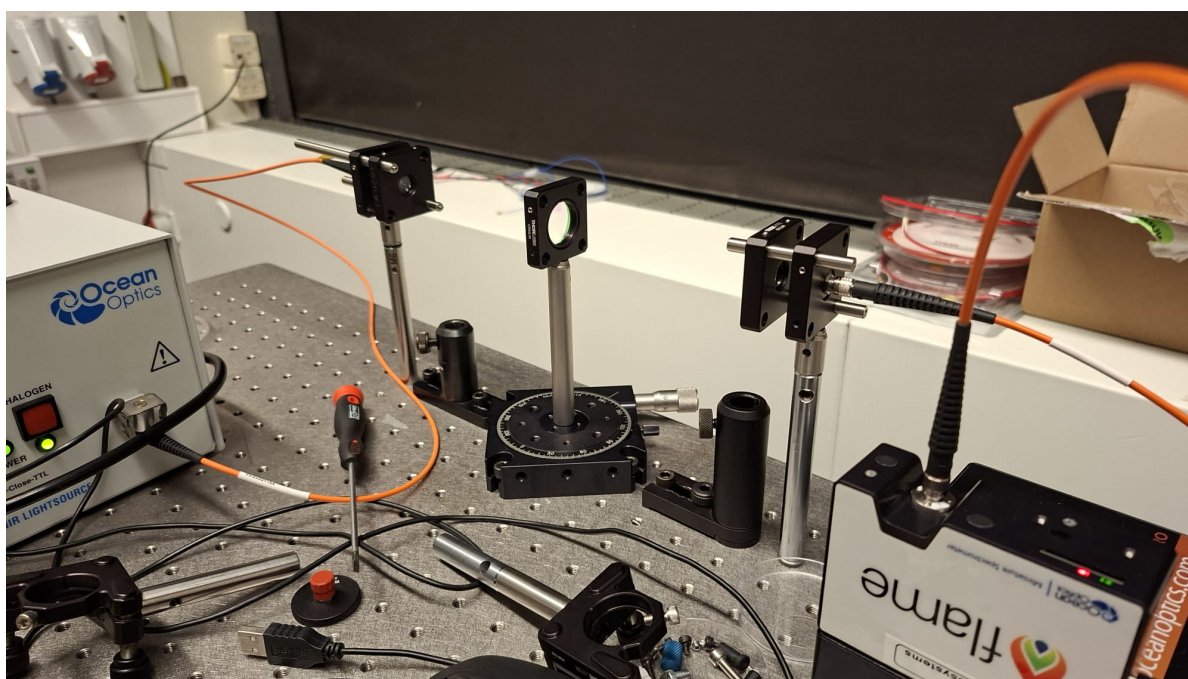


**Figure 3.3:** Photographic documentation of the optical characterisation setup within the cleanroom. Calibration wafers were analysed using an M-2000 Spectroscopic Ellipsometer (J.A. Woollam) to precisely determine the deposition rates and refractive indices of the individual thin-film layers prior to the complete fabrication of the optical filters.

### 3.3.4. Fabrication Process

During the actual filter fabrication, all gas flows, pressures, and plasma powers were kept constant, with layer thickness controlled strictly by adjusting the respective deposition phase durations. For the  $\text{SiO}_2$  layers, the deposition utilised 26.0 sccm of silane ( $\text{SiH}_4$ ) and 32.0 sccm of oxygen ( $\text{O}_2$ ), operating at a stabilised pressure of 2.5 mTorr with an ICP RF power demand of 1500 W. Conversely, the  $\text{Si}_3\text{N}_4$  layers were deposited using 26.0 sccm of  $\text{SiH}_4$  and 27.0 sccm of nitrogen ( $\text{N}_2$ ), operating at 2.0 mTorr with an ICP RF power demand of 750 W.

To conduct the deposition on the highly transparent 1-inch fused silica wafers, they were mounted on top of a standard silicon carrier wafer. This arrangement served a dual purpose: it mechanically secured the smaller glass substrate via the machine's robotic handling system, and it provided an opaque backing, which is necessary for the system's optical sensors to recognise the presence of the wafer in the load lock. Once loaded, the automated recipe executed the entire multi-layer stack sequentially. The system transitioned between the  $\text{SiO}_2$  and  $\text{Si}_3\text{N}_4$  deposition steps seamlessly without removing the wafer or breaking the chamber vacuum, minimising interfacial defects and contamination. Upon completion, the fabricated filters were transferred to sealed cleanroom containers for transport to the optics laboratory at the EWI building (TU Delft) for spectral characterisation (Figure 3.4). For the detailed thin-film filter fabrication process, refer to Appendix B.



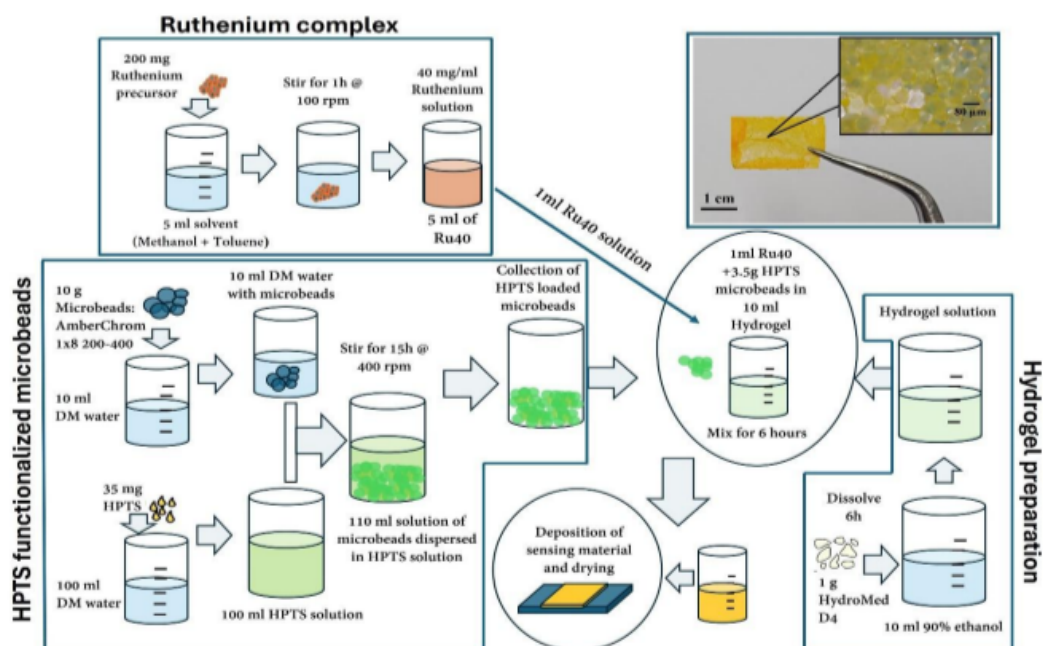
**Figure 3.4:** Benchtop optical measurement configuration utilised for the spectral characterisation of the fabricated thin-film filters. The setup features a broadband light source (left) coupled via optical fibre to a collimator. The light is transmitted through the filter sample, which is securely mounted on a precisely graduated rotational stage. The transmitted optical signal is subsequently collected by a receiving optical fibre (right) and routed to an Ocean Optics Flame miniature spectrometer for real-time spectral analysis.

## 3.4. Sensor Fabrication

The fundamental chemical architecture and base fabrication protocol of the bi-functional sensor were developed by the project's leading scientist, Assistant Professor Avik Sett [60]. During the *ex vivo* experimental phase, these sensors were reproduced following the established methodology (Figure 3.5), with an additional structural enhancement introduced to improve sensor robustness during tissue contact.

### 3.4.1. Fluorescent Materials

The bi-functional sensing paradigm requires two distinct fluorophores optimised to minimise cross-sensitivity [60].



**Figure 3.5:** Step-by-step fabrication workflow of the bi-functional pH and oxygen-sensitive sensor matrix. The sequential deposition and encapsulation steps are outlined, with the finalised, integrated sensor device shown in the upper right panel. Reproduced from [57]

For the pH-sensitive element, pyranine was utilised [60]. Specifically, 35 mg of 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) was dissolved in 100 ml of demineralised (DM) water [60]. Simultaneously, 10 g of AmberChrom microbeads were soaked in 10 ml of DM water [60]. The microbead solution was then combined with the HPTS solution and stirred for 15 hours at 400 rpm to allow functionalisation, after which the HPTS-loaded microbeads were collected via filtration [60].

For the oxygen-sensitive element, a ruthenium bathophenanthroline complex was employed [60]. The precursor solution was prepared by dissolving 200 mg of the ruthenium complex into 5 ml of a methanol and toluene solvent mixture (4:1 ratio) [60]. This was stirred for 1 hour at 100 rpm to yield the final "Ru40" solution (40 mg/ml ruthenium concentration) [60]. This specific concentration was rigorously chosen because deviations from it resulted in severe cross-sensitivity between the pH and oxygen emission peaks [60].

### 3.4.2. Sensor Matrix and Immobilisation

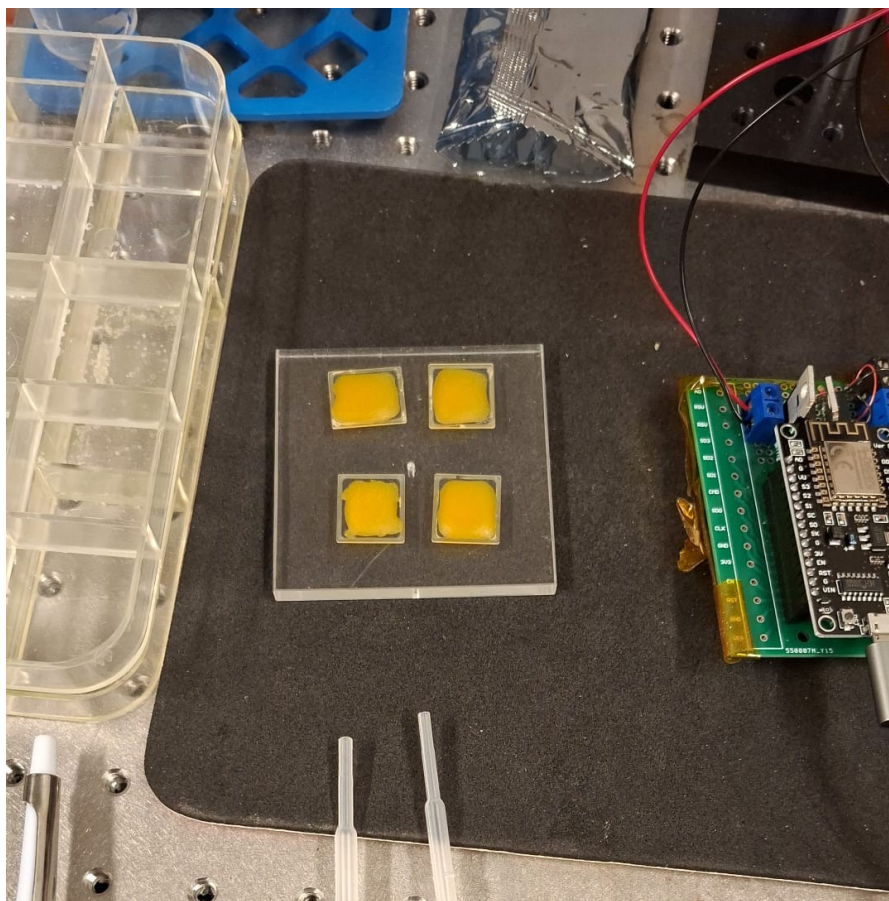
Both sensitive probes were encapsulated within a biocompatible hydrogel matrix [60]. The foundational hydrogel solution was formulated by dissolving 1 g of HydroMed D4 precursor into 10 ml of 90% ethanol [60]. This mixture was continuously stirred for approximately 6 hours at room temperature to ensure a uniform consistency [60].

To form the bi-functional sensing material, 3.5 g of the prepared HPTS-functionalised microbeads and 1 ml of the Ru40 solution were introduced into the 10 ml hydrogel solution [60]. This composite mixture was stirred for an additional 6 hours at room temperature to achieve a well-dispersed suspension of the probes within the matrix [60]. To greatly diminish cross-sensitivity during measurements, the final optimised ratio of ruthenium to HPTS within the matrix was carefully controlled at 1:88 by weight ( $HPTS_{88}Ru$ ) [60].

### 3.4.3. Sensor Geometry and Substrate

The primary immobilisation technique utilised for sensor fabrication was drop-casting. Specifically, 0.2 ml of the well-dispersed hydrogel composite was drop-casted directly onto a standard glass slide substrate (Figure 3.6) [60].

Following the initial deposition, the base sensing layer was allowed to dry completely at room temperature [60]. Once dried, a novel enhancement was applied to increase the physical durability of the sensor for the *ex vivo* tissue experiments. An additional protective overcoat of approximately 0.1 ml of pure, probe-free hydrogel mixture was drop-casted over the active sensor area. This top layer was subsequently allowed to dry, effectively encapsulating the active fluorescent matrix. This overcoat served a dual purpose, mitigating mechanical degradation and preventing the potentially harmful leaching of sensor materials onto the organ graft during prolonged direct contact. For the detailed fabrication process of the bi-functional sensor, refer to Appendix C.



**Figure 3.6:** Photographic documentation of the sensor fabrication process. The bi-functional luminescent sensing solution is shown manually drop-casted into predefined square containment wells on a transparent substrate. The sensors are pictured during their mandatory drying phase prior to the application of the secondary protective hydrogel layer.

## 3.5. System Hardware and Integration

The structural foundation, electronic circuitry, and mechanical housing of the data acquisition platform were adapted from prior system development conducted by Robbert Friendwijk, MSc [61]. For this current study, the established architecture was utilised to accommodate the integration of the custom-fabricated thin-film optical filters.

### 3.5.1. Electronic Design and Illumination

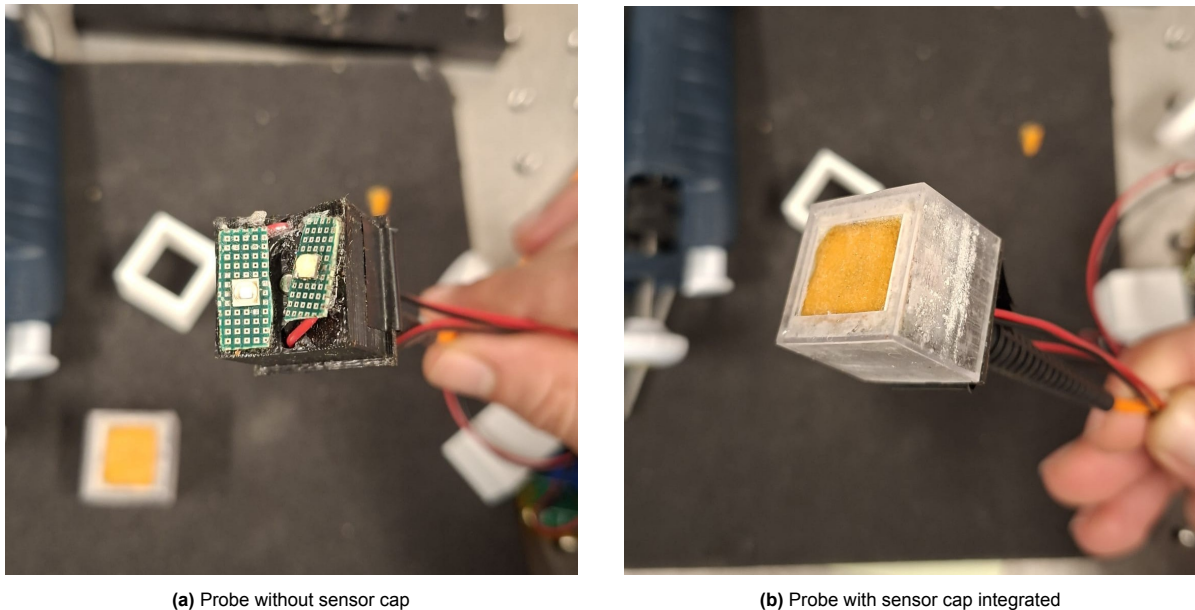
The system's excitation circuitry is governed by an ESP microcontroller module, which dictates the synchronised illumination sequence of the two single-wavelength light sources: the 405 nm and 450 nm LEDs [61]. These LEDs are integrated into the circuit via MOSFETs, with the ESP module connected directly to their respective gates [61]. This configuration allows the microcontroller to precisely modulate the on/off states of the LEDs to facilitate the required excitation cycles. The assembled circuitry is housed on an ESP prototype board, powered by an external power supply, and interfaced with a central computer via a standard USB connection (Figure 3.1 and 3.2) [61].

### 3.5.2. Optical Detection Pathway

For signal acquisition, the system utilises a Flame-S Miniature Spectrometer (Ocean Optics) coupled to an optical fibre as the primary photodetector [61]. During operation, the induced fluorescent emission from the hydrogel sensor matrix is captured by the optical fibre and transmitted to the spectrometer. To optimise this established setup and isolate the critical emission peaks, the custom-fabricated thin-film optical filters (as detailed in Sections 3.2 and 3.3) are designated to be placed directly in front of the optical fibre entrance. This critical integration step ensures that the high-intensity excitation light is physically blocked before entering the optical pathway, preventing spectrometer saturation. Once the filtered light is analysed by the spectrometer, the digitised spectral data is relayed back to the computer for real-time processing [61].

### 3.5.3. Mechanical Housing and Optical Alignment

To guarantee precise optical alignment and shield the sensitive components from ambient environmental noise, custom 3D-printed enclosures designed in SolidEdge and SolidWorks were utilised [61]. This mechanical housing employs a two-piece sliding architecture to create an isolated optical environment [61]. The base unit mounts the LEDs and features a fitted aperture to secure the optical fibre, while the secondary sliding cover firmly holds the glass wafer hosting the bi-functional sensor layer (Figure 3.7) [61]. Integrating these two pieces forms a completely enclosed, light-tight modular box, ensuring that the LEDs efficiently illuminate the active sensor area and that the resulting fluorescence is optimally directed into the optical fibre without external interference [61].



**Figure 3.7:** Two-piece sliding architecture of the mechanical probe housing. **(a)** The main body of the optical probe prior to the attachment of the cap. **(b)** The fully assembled probe with the sensor cap securely integrated into the sliding mechanism.

### 3.5.4. Software Integration

Complete system automation and data synchronisation are achieved through a centralised computer interface. Because both the ESP module and the Flame-S Miniature Spectrometer are connected to the same computer, the entire data acquisition process is seamlessly controlled and synchronised utilising custom MATLAB<sup>®</sup> scripts [61]. The complete MATLAB code is provided in Appendix D.

## 3.6. Calibration Procedures

To establish a robust correlation between the recorded fluorescence intensities and the target biomarker concentrations, rigorous calibration protocols were executed prior to experimental deployment.

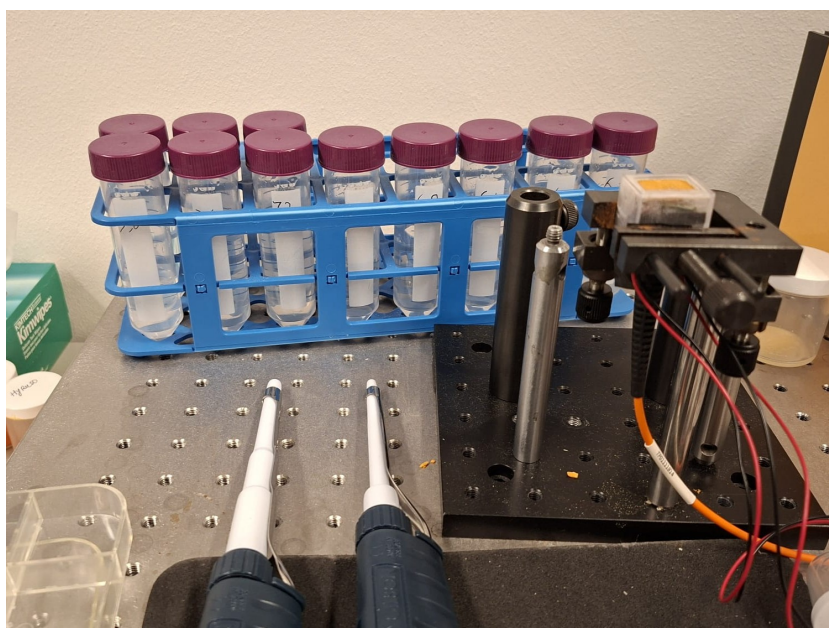
### 3.6.1. Oxygen Calibration

The oxygen-sensitive quenching response was calibrated within a controlled aqueous environment. The optical probe, housing the bi-functional sensor, was submerged in a beaker containing a solution of known, constant pH, alongside a Compact Dissolved Oxygen Meter (Extech Instruments), a gold-standard electrode-based oxygen meter. To establish a baseline of complete oxygen absence (0 mg/ml) and to manipulate subsequent dissolved oxygen concentrations, nitrogen gas was introduced directly into the solution via a submerged tube. By carefully adjusting the release valve on the nitrogen gas tank to control the output flow, the dissolved oxygen levels in the solution were precisely titrated.

Measurements were taken at stabilised dissolved oxygen concentrations of 0 mg/ml, 5.0 mg/ml, 8.5 mg/ml, and 10.0 mg/ml, as continuously verified by the reference oxygen meter. At each targeted concentration level, the resulting decrease in fluorescence intensity at the 600 nm peak was recorded by the probe. These optical measurements were subsequently correlated with the gold-standard meter readings to accurately map the dynamic quenching behaviour of the ruthenium complex and generate the standard calibration curve (Figures 4.16, 4.17, and 4.18).

### 3.6.2. pH Calibration

The relationship between local pH levels and the corresponding fluorescence emission intensity at the 520 nm peak was mapped using a series of standardised buffer solutions (Figure 3.8), adapting a protocol previously established by Friendwijk [61]. The calibration range spanned from pH 6.2 to 8.2, utilising discrete increments of 0.4 pH units. To ensure highly precise baseline values, the accurate pH of each solution was independently verified using a Lab 850 pH meter (Schott Instruments) equipped with an N64 pH combination electrode (SI Analytics).



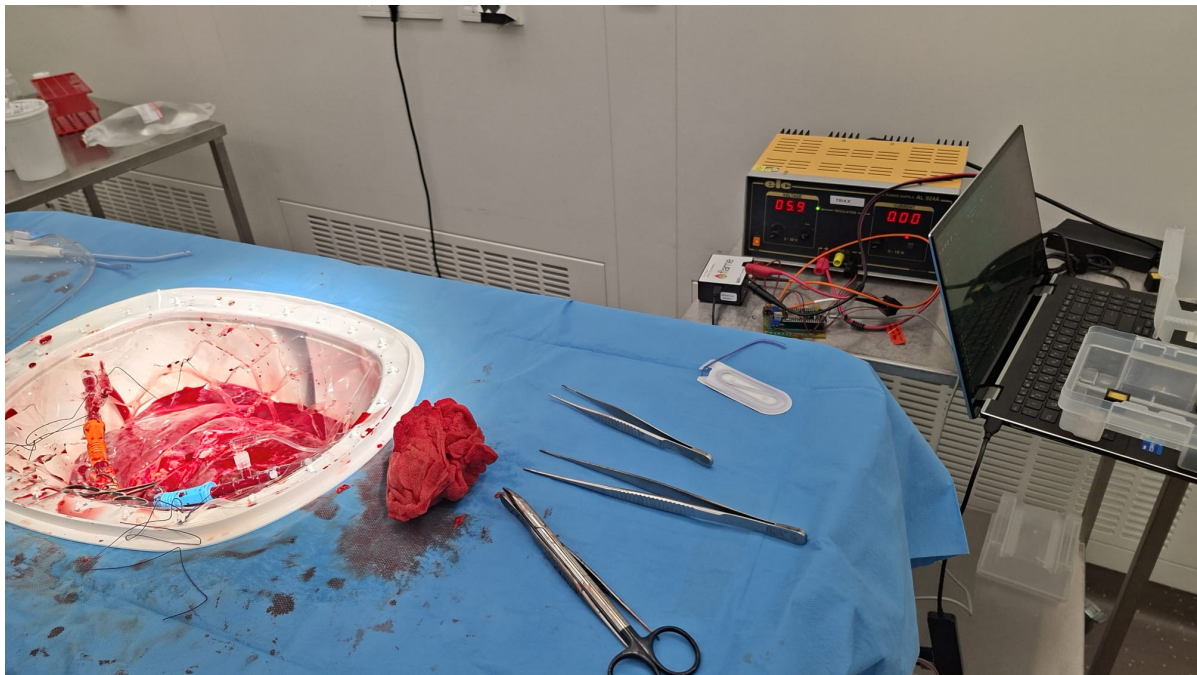
**Figure 3.8:** Benchtop experimental setup for pH calibration. The arrangement features the standardised buffer solutions (ranging from pH 6.2 to 8.2) in the background, precision micropipettes in the foreground, and the optical probe mounted in an upward-facing orientation on the right to facilitate the direct deposition of the buffer solutions onto the sensor matrix.

For each measurement, exactly 50  $\mu\text{L}$  of the respective buffer solution was deposited directly onto the active sensor layer using a micropipette. The system was then left undisturbed for 15 minutes to ensure the hydrogel matrix and the buffer reached complete chemical equilibrium. Following the discrete intensity measurement, the sensor was thoroughly rinsed with deionised water and allowed to dry for approximately 30 minutes to prevent cross-contamination between successive tests. This entire deposition and measurement cycle was repeated for all buffer solutions in both strictly incremental and randomised sequences to verify repeatability and rule out temporal bias or hysteresis [61]. These calibrations were conducted on the benchtop within the optics laboratory, where the ambient atmospheric oxygen concentration was considered constant, ensuring that the isolated intensity variations were

solely attributable to the pH buffer changes.

### 3.7. Experimental Setup

The *ex vivo* experimental validation phase was conducted within a dedicated operating room at the Erasmus Medical Center (EMC, Rotterdam, Netherlands) (Figure 3.9). The experiments were executed in direct collaboration with, and under the supervision of, clinical PhD candidates Puck Groen and Annelot Muntinga. Strict clinical hygiene and safety protocols were maintained throughout the procedures, including the mandatory use of standard operating room apparel, sterile gloves, and the rigorous disinfection of the optical probe using an alcohol-based solution prior to any tissue contact.



**Figure 3.9:** Clinical experimental setup during the *ex vivo* normothermic machine perfusion (NMP) of a human donor liver at the Erasmus Medical Center (EMC). The photograph illustrates the custom bi-functional optical sensing platform (right) deployed alongside the NMP Liver Assist circuit containing the viable organ graft (left).

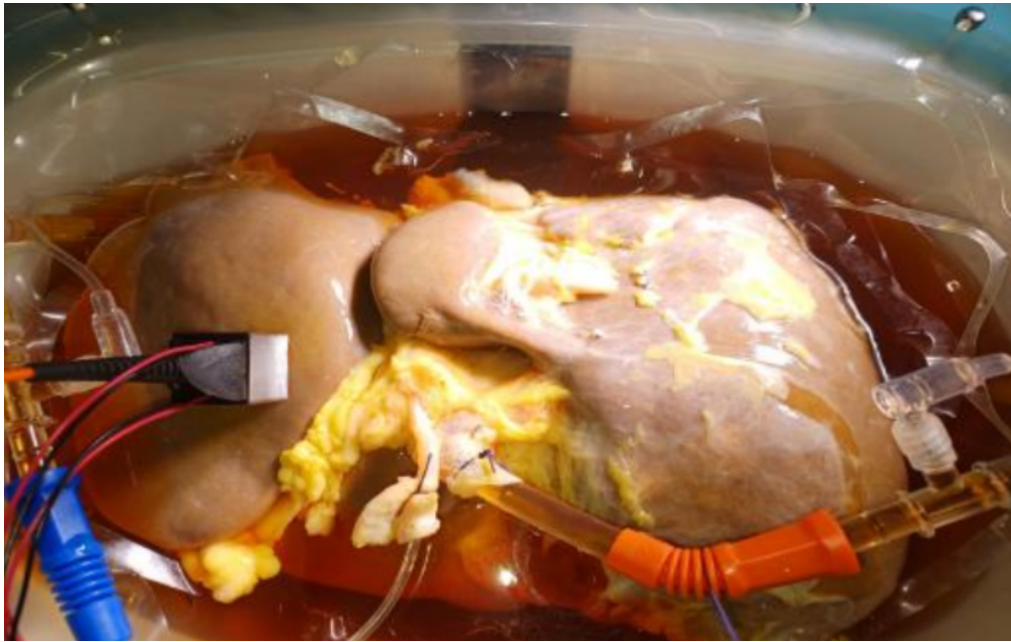
#### 3.7.1. Organ Preparation and Handling

The experimental subjects were human livers that had been formally declined for transplantation, provided by the EMC. The validation was divided into two distinct experimental paradigms utilising different organ states. The first experiment utilised a previously frozen (defrosted), non-viable liver to serve as a baseline model for testing the system's response to extreme, artificially induced parameter shifts (Figure 3.10). The second experiment utilised a freshly declined liver, approximately two hours post-declination, which was deemed to be in a substantially healthy, viable state, providing a highly realistic physiological model for real-time viability monitoring (Figure 3.11).

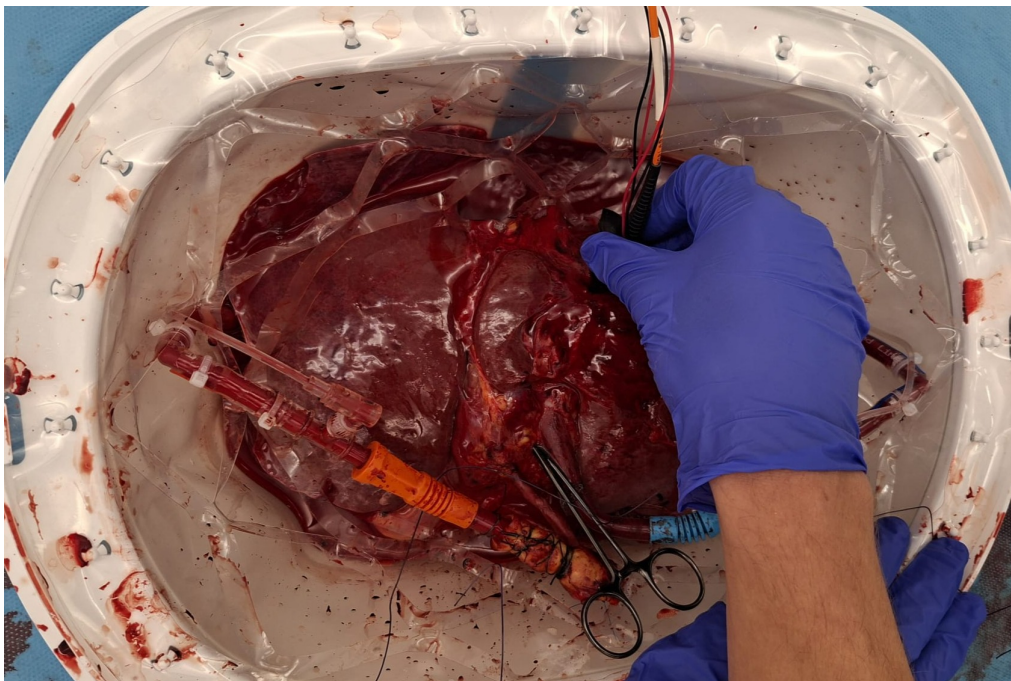
#### 3.7.2. Measurement and Perfusion Conditions

During testing, the livers were sustained using a clinical-grade normothermic machine perfusion (NMP) system (Liver Assist, Organ Assist). The NMP system maintained a constant physiological temperature of 37°C and continuously circulated an acellular perfusion solution.

Based on the system's pump unit telemetry during the viable organ experiment, the perfusion hemodynamics were carefully regulated. The hepatic artery was maintained at a flow rate of 241 mL/min with a perfusion pressure of 41 mmHg and a vascular resistance (VR) of 0.16 mmHg/mL/min. Concurrently, the portal vein was maintained at a flow rate of 1.42 L/min with a pressure of 3 mmHg and a VR of 1.92 mmHg/L/min. For the optical readings, the sterilised sensor probe was placed directly onto the organ tissue, with measurements specifically localised at multiple discrete positions across the posterior



**Figure 3.10:** Photographic documentation of the preliminary *ex vivo* trial utilising a defrosted human liver model within the normothermic machine perfusion (NMP) circuit. The custom dual-parameter optical probe is shown positioned directly on the left lobe of the organ. Reproduced from [60]



**Figure 3.11:** Photograph of the healthy human donor liver maintained within the normothermic machine perfusion (NMP) system during the second experimental trial. The custom optical sensing probe is shown being manually positioned against the right lobe of the organ to acquire localised measurements.

surface (back) of the liver.

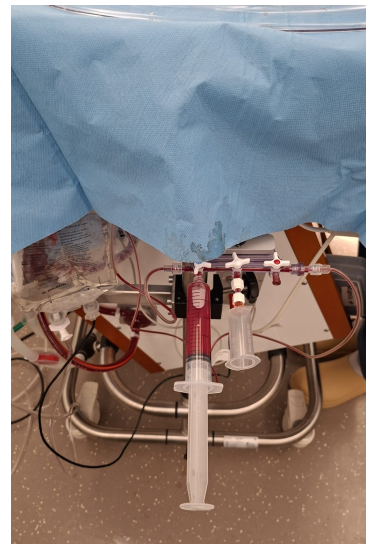
### 3.7.3. Data Acquisition and Validation

The data acquisition strategy varied between the two experimental models to comprehensively evaluate sensor performance. During the first experiment (defrosted liver), the oxygen supply to the NMP system was manually modulated, alternating between 1 L/min and 6 L/min. During these dynamic shifts, 10-minute continuous transient response measurements were recorded at various tissue locations alongside discrete spot measurements. In contrast, the second experiment (fresh liver) was conducted under a static oxygen supply, focusing on the sensor's ability to monitor the organ's natural physiological baseline over time. Across both experimental setups, multiple identical sensor patches were deployed to compare localised readings, ensure measurement consistency, and validate the reproducibility of the fabricated sensing platform.

To validate the localised optical sensor readings against the global physiological state of the organ, reference samples were taken sequentially after every optical measurement cycle. The supervising clinicians extracted samples of the circulating perfusate via syringe (Figure 3.12). These samples were immediately analysed using the hospital's clinical gold-standard ABL90 FLEX analyser (Radiometer, Brønshøj, Denmark). This provided a comprehensive global biomarker panel, including pH,  $p\text{CO}_2$ ,  $p\text{O}_2$ , cBase(B)c,  $\text{cHCO}_3^-$ (P)c, ctHb, Hctc,  $s\text{O}_2$ ,  $\text{cK}^+$ ,  $\text{cNa}^+$ ,  $\text{cCa}^{2+}$ ,  $\text{cCl}^-$ , cGlu, and cLac. The global pH and  $p\text{O}_2$  values obtained from the perfusate analysis were subsequently cross-referenced with the localised optical fluorescence data to evaluate the accuracy and physiological relevance of the sensing platform.



(a) Sampling port on NMP machine with an connected, empty syringe.



(b) Connected syringe fully filled with extracted blood perfusate.

**Figure 3.12:** Experimental setup for perfusate sampling. The clinician uses the connected syringe system on the NMP device to extract samples for clinical perfusate analysis. (a) Depicts the setup with an empty syringe. (b) Depicts the syringe filled with extracted blood perfusate.

## 3.8. Data Processing and Analysis

### 3.8.1. Optical Filter Characterisation and Iterative Optimisation

Prior to integration, the fabricated thin-film filters underwent rigorous spectral characterisation to ensure their transmission profiles matched the computational simulations. This was conducted using a dedicated benchtop optical setup comprising an Ocean Optics DH2000 Deuterium Tungsten Halogen light source and the Flame-S Miniature Spectrometer. Two TH-M151L01 optical fibres (Thorlabs) were utilised, one to guide the broadband light from the DH2000, and the other to transmit the filtered light to the spectrometer. The fibres were secured on a specialised alignment stand, positioned opposite each other at equal heights.

The sample filters were mounted in a 1-inch sample holder secured on a rotating stage precisely midway between the two optical fibres. During all measurements, the filters were oriented at a 0° angle of incidence, ensuring the surface was strictly perpendicular to the incoming light beam. Data acquisition was managed via OceanView 2.0 software (Ocean Optics) using a fixed integration time of 10 ms. The resulting transmittance versus wavelength spectra were systematically compared against the ideal computational models generated during the design phase. Based on these empirical comparisons, iterative modifications ranging from 5% to 10% were applied to the theoretical deposition thicknesses of the individual layers in subsequent fabrication runs. This optimisation feedback loop was repeated until the fabricated filters' optical properties successfully converged with the targeted simulation profiles.

### 3.8.2. Sensor Calibration Data Processing

Calibration data processing was critical for quantifying the fluorescence signals obtained during the *ex vivo* experiments. To translate the raw optical intensities into precise biological units, empirical regression models derived from the initial calibration mappings were applied. For oxygen sensing, regression curves were established by correlating the dynamic quenching of the ruthenium complex at the 600 nm emission peak with the specific dissolved oxygen concentrations (mg/ml) validated by the reference meter. Similarly, for pH sensing, regression curves derived from the controlled buffer experiments were applied to the 520 nm emission peak to accurately calculate the local tissue pH.

Furthermore, the calibration data was comparatively analysed to validate sensor functionality and verify the absence of optical crosstalk between the two luminophores. Specifically, this analysis was performed to ensure that a relative increase in local pH corresponded to a measurable increase in intensity at the 520 nm peak and that rising oxygen concentrations yielded a distinct quenching of intensity strictly at the 600 nm peak. Finally, dynamic transient response measurements were evaluated to calculate the sensor response time under changing analyte conditions.

### 3.8.3. Ex Vivo Experimental Data Analysis

Raw spectral data acquired during the *ex vivo* perfusion experiments were digitised and systematically tabulated for rigorous statistical analysis. For every discrete measurement cycle, the excitation light source intensities were recorded alongside the corresponding emission intensities. To ensure robustness, data extraction focused not only on the absolute peak values at 520 nm and 600 nm but also incorporated metrics within defined spectral windows (520–530 nm for pH, and 593–604 nm for oxygen). Within these specific bands, the maximum, minimum, min-max average, mean, and median intensity values were calculated for both excitation sources.

This comprehensive data extraction protocol was repeated for every measurement point, across various physical locations on the liver, and across multiple sensor replicas to validate reproducibility. The processed data was subsequently visualised through a series of comparative plots. These included graphs correlating emission intensity per measurement and emission intensity relative to the specific excitation source (405 nm vs. 450 nm), which critically enabled the system's ratiometric analysis. Furthermore, the temporal shift of signal intensity was tracked and mapped not only throughout the entirety of the perfusion experiment but also over the specific duration of use for each individual sensor. Variables such as sensor placement location and sensor identification number were continuously used to classify these graphical analyses.

### 3.8.4. Clinical Validation and Dynamic Response Evaluation

To validate the diagnostic accuracy of the optical platform, the optically derived biomarker trends were rigorously cross-referenced against the discrete, gold-standard clinical measurements obtained from the hospital's ABL90 FLEX analyser.

During the dynamic phases of the experiment, where the NMP oxygen supply was intentionally modulated, the transient optical response measurements were tracked against the known supply shifts. This allowed for the evaluation of the sensor's temporal tracking capabilities and its signal stability during periods of static oxygen supply. Finally, overlapping measurements from multiple sensors under identical physiological conditions were statistically compared against one another to confirm high sensor reproducibility in a true clinical environment.

# 4

## Results

### 4.1. Optical Filter Fabrication and Characterisation

#### 4.1.1. Preliminary Simulations and Material Evolution

The design phase of the optical filters began with a rigorous literature review of biocompatible dielectric materials suitable for Fabry-Perot (FP) cavity architectures. Initially, a material pairing of silicon dioxide ( $\text{SiO}_2$ ) and titanium dioxide ( $\text{TiO}_2$ ) was selected.  $\text{TiO}_2$  was chosen primarily for its exceptionally high refractive index, which provides strong light scattering capabilities and highly efficient attenuation of ultraviolet (UV) and near-UV radiation.

The preliminary computational simulations focused on designing two distinct single-cavity FP filters using the  $\text{TiO}_2/\text{SiO}_2$  material stack. These were specifically targeted to isolate the anticipated 520 nm (pH) and 610 nm (oxygen) emission peaks while decisively rejecting the 405 nm and 450 nm excitation wavelengths. At first evaluation, the simulations yielded highly promising theoretical optical density. The models demonstrated robust attenuation of the unwanted excitation spectrum, reducing transmittance well below 10% at the 405 nm and 450 nm marks, and approaching 0% across other non-target bands, while successfully maintaining over 90% transmittance at the target emission wavelengths.

However, detailed analysis of the transmission spectra revealed a critical vulnerability regarding the Full Width at Half Maximum (FWHM). To quantify this parameter, the threshold transmittance ( $T_{\text{half}}$ ) was first determined at half of the peak's maximum height ( $T_{\text{max}}$ ):

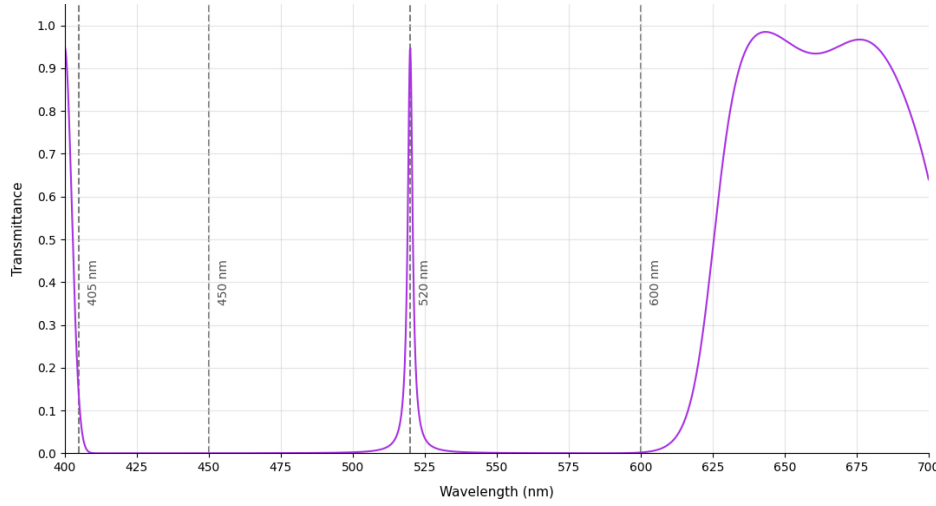
$$T_{\text{half}} = \frac{T_{\text{max}}}{2} \quad (4.1)$$

The spectral width was then calculated by finding the two wavelength points ( $\lambda_1$  and  $\lambda_2$ ) where the curve crossed this half-maximum value:

$$\text{FWHM} = \lambda_2 - \lambda_1 \quad \text{where} \quad T(\lambda_1) = T(\lambda_2) = T_{\text{half}} \quad (4.2)$$

For the initial simulation targeting the pH emission peak (Figure 4.1), the visual profile indicated an estimated peak height of  $T_{\text{max}} \approx 0.95$ , yielding  $T_{\text{half}} = 0.475$ . Evaluating the intercept wavelengths at this intensity ( $\lambda_1 \approx 518.2$  nm,  $\lambda_2 \approx 521.7$  nm) resulted in a critically narrow width:

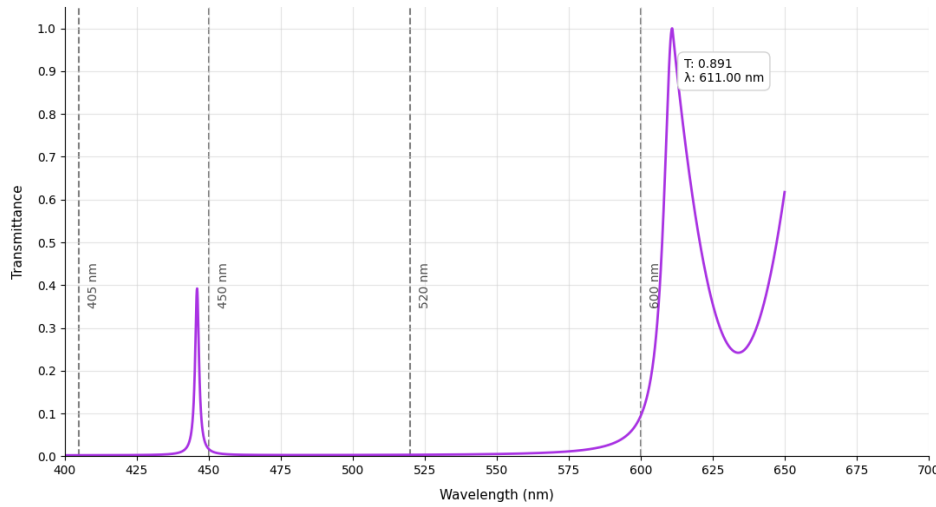
$$\text{FWHM} = 521.7 \text{ nm} - 518.2 \text{ nm} = 3.5 \text{ nm}$$



**Figure 4.1:** Simulated transmittance spectrum for the  $\text{TiO}_2/\text{SiO}_2$  single-cavity Fabry-Perot filter targeting the 520 nm pH emission peak, demonstrating an ultra-narrow FWHM.

Similarly, for the 611 nm oxygen target simulation featuring an asymmetric peak (Figure 4.2), the exact peak height was  $T_{\text{max}} = 0.891$ , giving a half-maximum of  $T_{\text{half}} = 0.4455$ . The intercept wavelengths ( $\lambda_1 \approx 606.0$  nm,  $\lambda_2 \approx 616.0$  nm) yielded a width of:

$$\text{FWHM} = 616.0 \text{ nm} - 606.0 \text{ nm} = 10.0 \text{ nm}$$



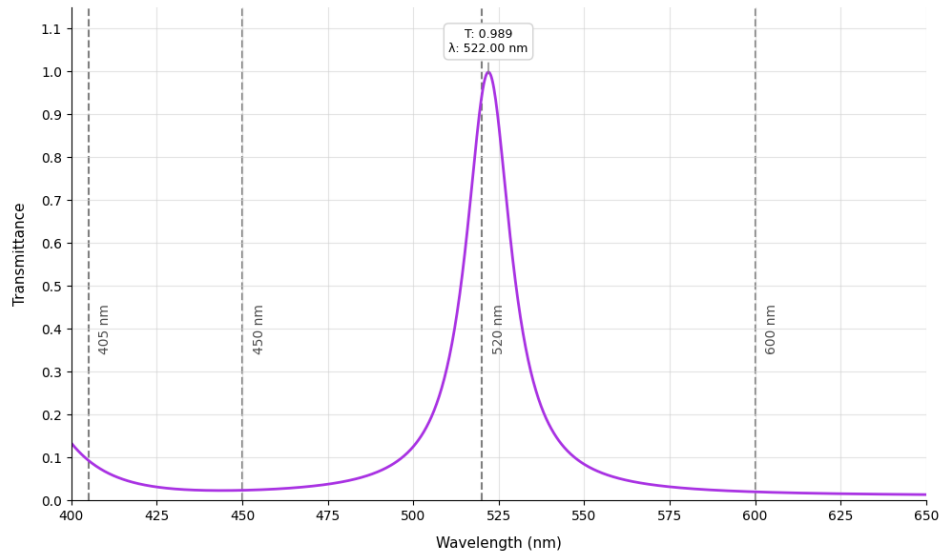
**Figure 4.2:** Simulated transmittance spectrum for the  $\text{TiO}_2/\text{SiO}_2$  single-cavity filter targeting the 611 nm oxygen emission peak.

From a practical fabrication standpoint, these ultra-narrow tolerances introduced an unacceptable level of risk. Any minor manufacturing inconsistency, such as deposition rate fluctuations, minute thickness mismatches, machine errors, or ambient cleanroom contaminants, would easily shift the physical pass-band away from the target wavelength, effectively blocking the very fluorescence signals the system was designed to detect.

Subsequent simulation iterations were conducted in an attempt to widen the FWHM, resulting in a standard bandpass peak design for the 522 nm target (Figure 4.3). With an exact peak height of  $T_{\text{max}} = 0.989$  and  $T_{\text{half}} = 0.4945$ , the intercept wavelengths ( $\lambda_1 \approx 514.0$  nm,  $\lambda_2 \approx 530.0$  nm) successfully broadened the width to:

$$\text{FWHM} = 530.0 \text{ nm} - 514.0 \text{ nm} = 16.0 \text{ nm}$$

The theoretical thin-film layer stack configuration required to achieve this widened profile is detailed in Table 4.1.



**Figure 4.3:** Subsequent simulation attempt aiming to widen the FWHM for the pH target peak (522 nm) while maintaining out-of-band rejection.

**Table 4.1:** Thin-Film Layer Stack Configuration

| Layer / Position | Material Type             | Thickness (nm) |
|------------------|---------------------------|----------------|
| <b>Medium</b>    | Air                       | —              |
| 1                | TiO <sub>2</sub> - Rutile | 45             |
| 2                | SiO <sub>2</sub>          | 80             |
| 3                | TiO <sub>2</sub> - Rutile | 45             |
| 4                | SiO <sub>2</sub>          | 180            |
| 5                | TiO <sub>2</sub> - Rutile | 45             |
| 6                | SiO <sub>2</sub>          | 80             |
| 7                | TiO <sub>2</sub> - Rutile | 45             |
| <b>Substrate</b> | SiO <sub>2</sub>          | —              |

Nevertheless, successfully applying a similar broadening technique to the 610 nm target while simultaneously constraining the total physical thickness of the filter to below the 1.5  $\mu\text{m}$  threshold proved unfeasible without heavily compromising the requisite out-of-band rejection characteristics.

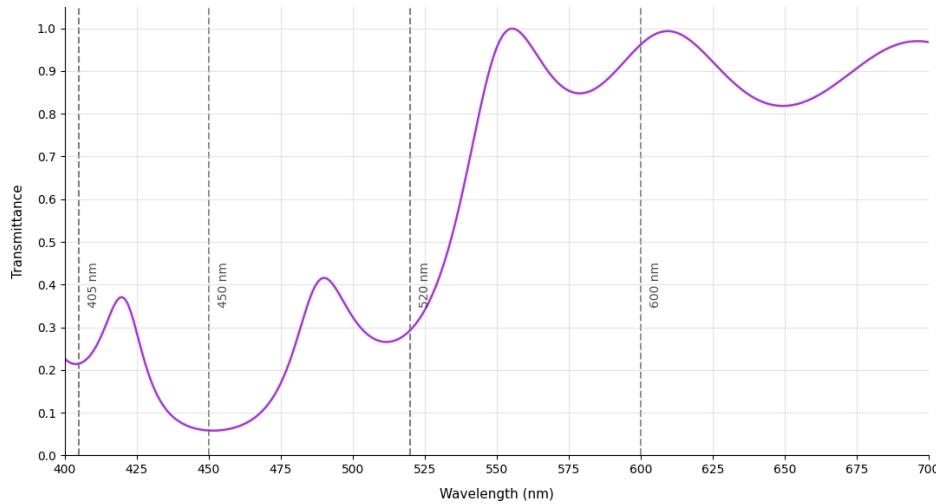
Consequently, a strategic material pivot was made, substituting TiO<sub>2</sub> with silicon nitride (Si<sub>3</sub>N<sub>4</sub>). Beyond theoretically yielding a more accommodating, wider FWHM for the target passbands, Si<sub>3</sub>N<sub>4</sub> presented overwhelming practical advantages for fabrication within the EKL cleanroom environment. The existing Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD) infrastructure was already seamlessly connected to Si<sub>3</sub>N<sub>4</sub> precursor gases. Furthermore, the cleanroom engineering staff possessed extensive familiarity with Si<sub>3</sub>N<sub>4</sub> deposition rates and behaviours, and the material had a proven, highly reliable track record in previous successful FP filter fabrications by prior researchers within the institution. This pivot ensured a balance between optimal optical performance and high-fidelity manufacturability.

#### 4.1.2. Fabricated Spectral Performance vs. Simulation

Following the theoretical validation and the strategic material shift to silicon nitride (Si<sub>3</sub>N<sub>4</sub>) and silicon dioxide (SiO<sub>2</sub>), the fabrication of the optical filters was initiated using the Inductively Coupled Plasma

Chemical Vapour Deposition (ICP-CVD) system. The computational simulations were updated to reflect the empirically measured refractive indices of the specific cleanroom precursor materials:  $n \approx 1.55$  for  $\text{SiO}_2$  and  $n \approx 1.92$  for  $\text{Si}_3\text{N}_4$ .

**Bandpass Filter Fabrication (600 nm Target)** Initial efforts to design a Fabry-Perot cavity that could simultaneously meet the strict sub- $1.5\ \mu\text{m}$  thickness constraint while effectively isolating the 600 nm peak and rejecting the 405, 450, and 520 nm wavelengths proved computationally unfeasible. To satisfy all design prerequisites, the architecture for the oxygen-sensitive emission filter was adapted into a 21-layer bandpass configuration (Figure 4.5). The final simulated stack sequence, optimised for the true refractive indices, is detailed in Table 4.2, and the corresponding simulated spectral profile is shown in Figure 4.4.



**Figure 4.4:** Simulated transmittance spectrum of the 21-layer  $\text{Si}_3\text{N}_4/\text{SiO}_2$  bandpass filter optimised for the 600 nm oxygen-sensitive emission peak.

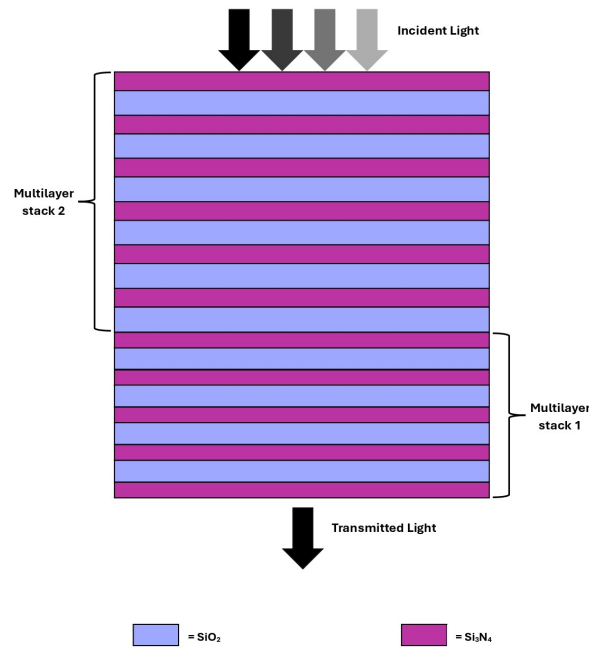
**Table 4.2:** Layer Stack Configuration for the 600 nm Bandpass Filter

| Layer                  | Material                | Refractive Index ( $n$ ) | Thickness (nm) |
|------------------------|-------------------------|--------------------------|----------------|
| <b>Medium</b>          | Air                     | 1.00                     | -              |
| 1, 3, 5, 7, 9          | $\text{Si}_3\text{N}_4$ | 1.92                     | 50 (each)      |
| 2, 4, 6, 8             | $\text{SiO}_2$          | 1.55                     | 70 (each)      |
| 11, 13, 15, 17, 19, 21 | $\text{Si}_3\text{N}_4$ | 1.92                     | 60 (each)      |
| 10, 12, 14, 16, 18, 20 | $\text{SiO}_2$          | 1.55                     | 80 (each)      |
| <b>Substrate</b>       | $\text{SiO}_2$          | 1.55                     | -              |
| <b>Total Sum</b>       |                         |                          | <b>1370 nm</b> |

On the day of the primary fabrication, empirical deposition rates were calibrated at 55.23 nm/min (0.9205 nm/s) for  $\text{Si}_3\text{N}_4$  and 75.70 nm/min (1.2617 nm/s) for  $\text{SiO}_2$ . The fabricated filter (Figure 4.6) was subsequently evaluated in the optics laboratory using the Ocean Optics Flame-S spectrometer setup.

The empirical spectral performance (Figure 4.7) demonstrated an exceptional correlation with the simulated model. The physical transmittance graph exhibited almost identical morphological characteristics; spikes and crevasses aligned with the expected theoretical intensities. Crucially, the fabricated bandpass filter successfully suppressed the 405 nm excitation wavelength to nearly 25% transmittance and the 450 nm wavelength to less than 5%. Additionally, the 520 nm emission interference was restricted to below 35%, while the target 600 nm wavelength maintained a robust 90% transmission efficiency.

To validate reproducibility, the fabrication protocol was repeated during a subsequent session after recalibrating the deposition rates ( $\text{Si}_3\text{N}_4$ : 55.01 nm/min;  $\text{SiO}_2$ : 76.95 nm/min). Incorporating these



**Figure 4.5:** Schematic cross-section of the 21-layer  $\text{Si}_3\text{N}_4/\text{SiO}_2$  bandpass filter optimised for the 600 nm oxygen-sensitive emission peak. Multilayer stack 1 consists of alternating layers of 50 nm  $\text{Si}_3\text{N}_4$  and 70 nm  $\text{SiO}_2$ , while stack 2 features alternating layers of 60 nm  $\text{Si}_3\text{N}_4$  and 80 nm  $\text{SiO}_2$ . Note: Vertical layer thickness is exaggerated  $100,000\times$  ( $\sim 10^5\times$ ) relative to the lateral scale for visual clarity.

adjusted rates into the recipe yielded an identical spectral profile, confirming the high stability and reliability of this bandpass design.

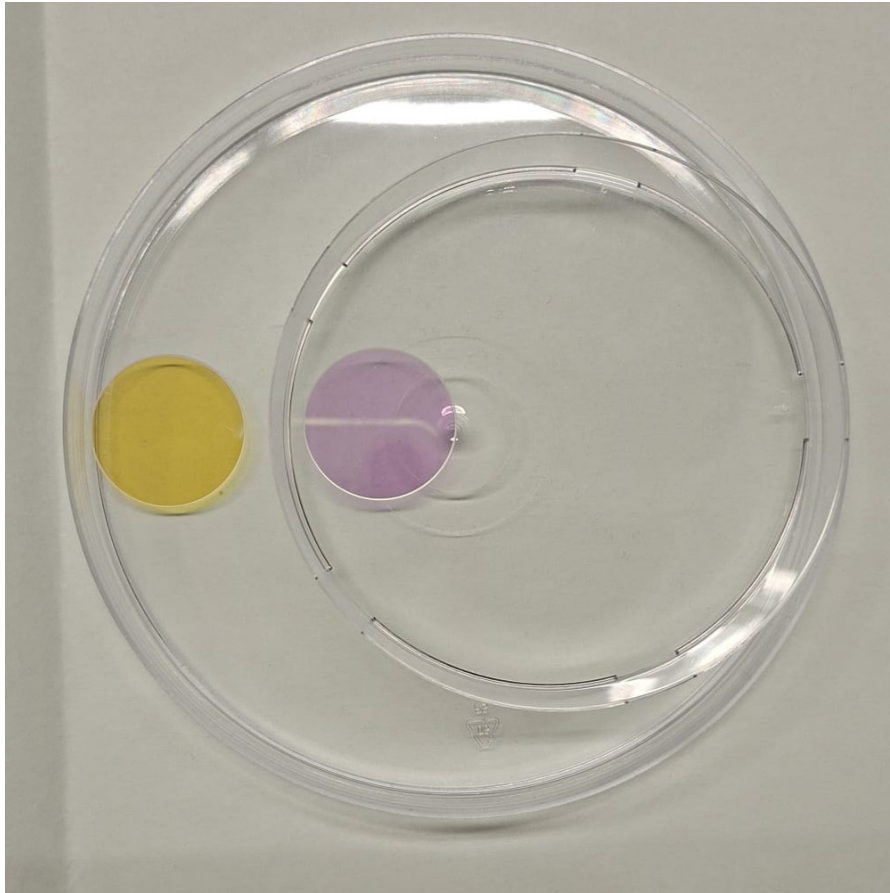
**Multi-Cavity Fabry-Perot Filter Fabrication (520 nm Target)** For the isolation of the 520 nm pH-sensitive peak, the fundamental Fabry-Perot design was retained but upgraded from a single-cavity to a 19-layer multi-cavity architecture (Figure 4.10). This structural complexity was necessary to achieve the desired cut-offs for 405, 450, and 600 nm wavelengths while strictly remaining under the  $1.5\ \mu\text{m}$  thickness threshold.

The updated simulation, illustrated in Figure 4.8, achieved the necessary sub-20% transmittance for the excitation light sources. While the cut-off for the 600 nm target was theoretically limited to 60% transmittance, this was deemed a highly acceptable trade-off due to the inherently low-intensity nature of the oxygen-associated fluorescence peak. The layer sequence is detailed in Table 4.3.

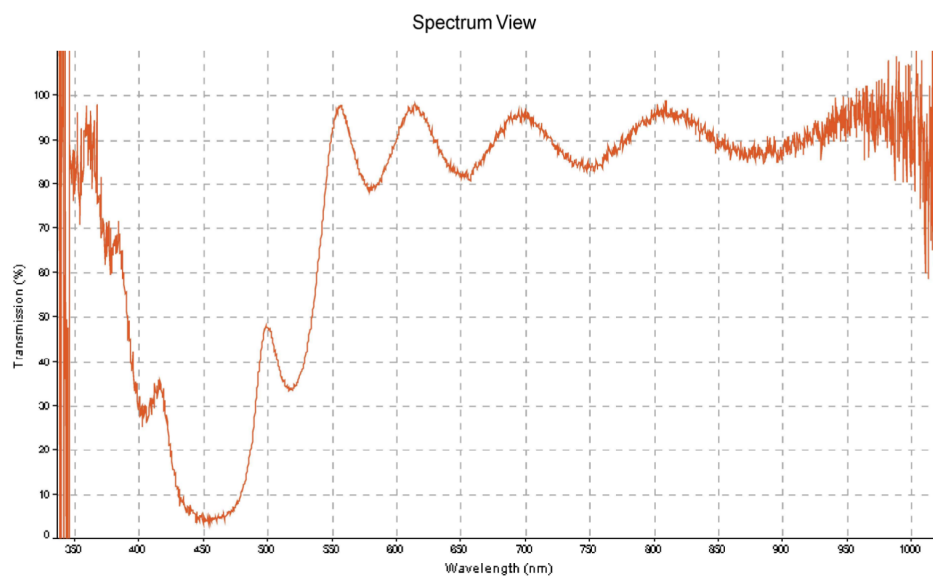
**Table 4.3:** Layer Stack Configuration for the 520 nm Multi-Cavity Fabry-Perot Filter

| Layer                             | Material                | Refractive Index ( $n$ ) | Thickness (nm) |
|-----------------------------------|-------------------------|--------------------------|----------------|
| <b>Medium</b>                     | Air                     | 1.00                     | -              |
| 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 | $\text{SiO}_2$          | 1.55                     | 60 (each)      |
| 2, 6, 8, 12, 14, 18               | $\text{Si}_3\text{N}_4$ | 1.92                     | 60 (each)      |
| 4, 10, 16 (Cavities)              | $\text{Si}_3\text{N}_4$ | 1.92                     | 170 (each)     |
| <b>Substrate</b>                  | $\text{SiO}_2$          | 1.55                     | -              |
| <b>Total Sum</b>                  |                         |                          | <b>1470 nm</b> |

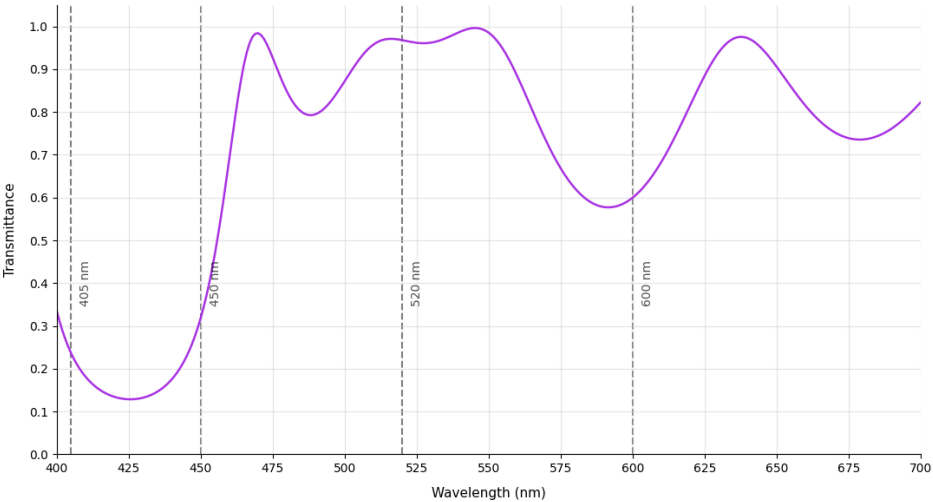
Fabrication was executed following daily rate calibrations of 70.71 nm/min (1.1785 nm/s) for  $\text{SiO}_2$  and 54.88 nm/min (0.9147 nm/s) for  $\text{Si}_3\text{N}_4$ . Upon empirical testing, the fabricated filter (Figure 4.6) yielded a spectral profile (Figure 4.9) that strongly mirrored the morphological nature of the simulation's curves, spikes, and crevasses. Notably, the physical iteration demonstrated superior out-of-band rejection at the 600 nm mark, achieving a deeper cut-off at approximately 35% transmittance compared to the 60% theoretical prediction.



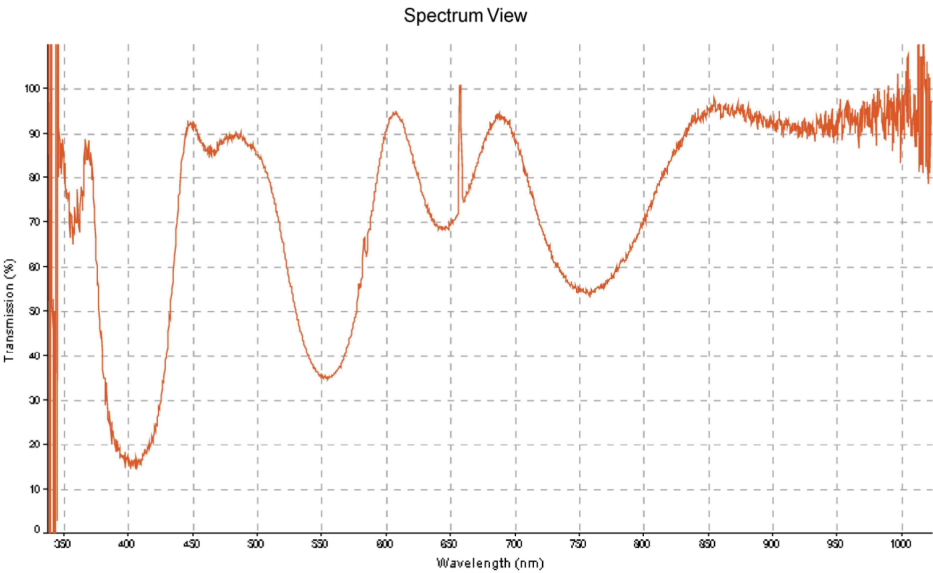
**Figure 4.6:** Physical realisation of the fabricated optical interference filters. The bandpass filter, engineered for the 600 nm oxygen emission target, is shown on the left. The multi-cavity Fabry-Perot (MCFP) filter, targeted for the 520 nm pH emission peak, is shown on the right.



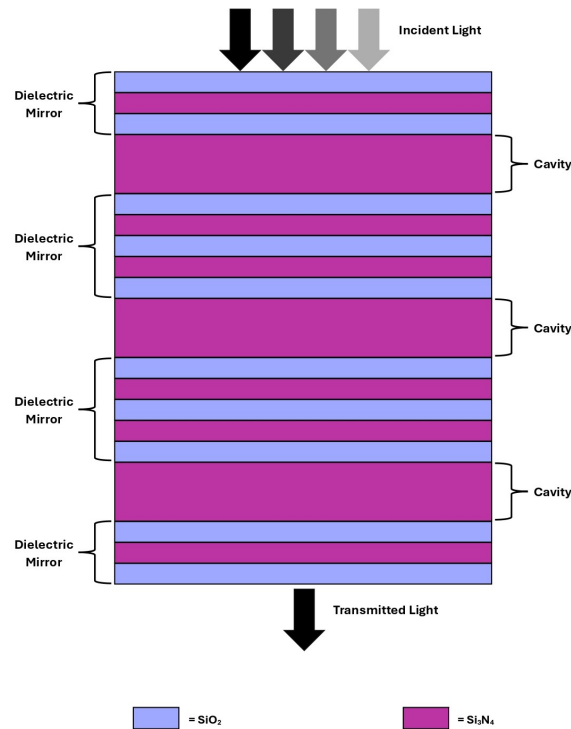
**Figure 4.7:** Empirical transmittance spectrum of the fabricated 21-layer bandpass filter measured via the Ocean Optics spectrometer.



**Figure 4.8:** Simulated transmittance spectrum of the 19-layer multi-cavity Fabry-Perot filter optimised for the 520 nm pH-sensitive emission peak.



**Figure 4.9:** Empirical transmittance spectrum of the initially fabricated 19-layer multi-cavity Fabry-Perot filter, demonstrating the 25–30 nm blue-shift.



**Figure 4.10:** Schematic cross-section of the 19-layer Multi-Cavity Fabry-Pérot (MCFP) filter with a 10% global thickness enlargement. The structure incorporates dielectric mirrors composed of alternating 66 nm  $\text{Si}_3\text{N}_4$  and  $\text{SiO}_2$  layers, separated by 186 nm thick resonant cavities. Note: Vertical layer thickness is exaggerated  $100,000\times$  ( $\sim 10^5\times$ ) relative to the lateral scale for visual clarity.

However, a critical discrepancy emerged: the entire spectral graph experienced a blue-shift of approximately 25 to 30 nm relative to the simulation. This uniform shift caused the filter to misalign with both the target passbands and the intended cut-off wavelengths, rendering this specific iteration inappropriate for immediate clinical deployment. Despite this alignment error, the preservation of the highly complex multi-cavity curve shape proved that the underlying architecture was fundamentally sound. The predictable nature of the blue-shift provided a reliable baseline, indicating that targeted modifications to the individual layer thicknesses would successfully shift the spectrum into alignment.

#### 4.1.3. Iterative Fabrication Adjustments

As observed in the initial fabrication of the 19-layer Multi-Cavity Fabry-Perot (MCFP) filter, the structural morphology of the spectrum was successfully achieved, but it suffered from a systematic blue-shift of approximately 21 nm relative to the simulation. To correct this misalignment and shift the transmittance graph toward the desired longer wavelengths (red-shift), a strategy of uniform percentage-based thickness enlargement was implemented across all layers.

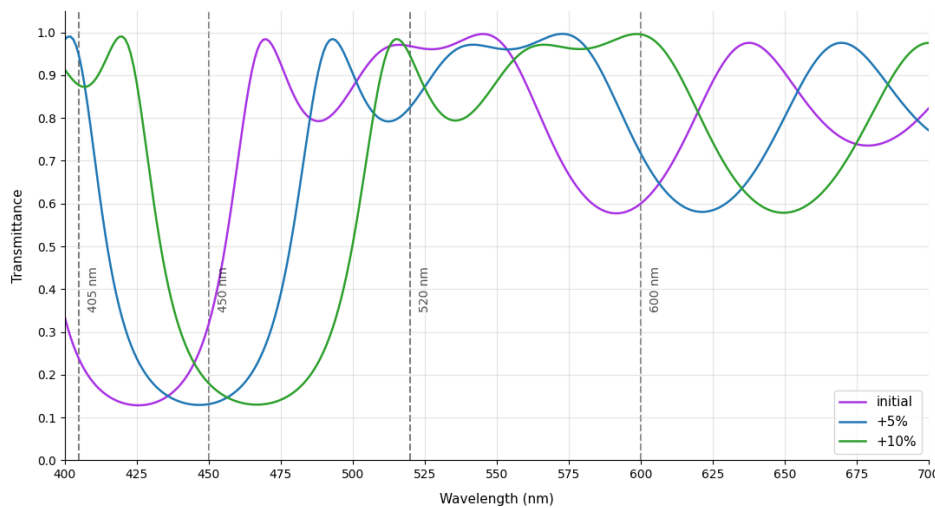
**Proportional Thickness Enlargement (+5% and +10%)** The first iteration involved a 5% global increase in the targeted layer thicknesses. Fabrication was conducted with measured deposition rates of 76.57 nm/min (1.2762 nm/s) for  $\text{SiO}_2$  and 52.98 nm/min (0.8830 nm/s) for  $\text{Si}_3\text{N}_4$ . While this adjustment yielded a positive shift of approximately 7.5 nm toward the target spectrum, it remained insufficient for clinical application. Furthermore, comparing the +5% simulation to its empirical measurement revealed that the predictive error margin had widened to approximately 30 nm. This led to the critical assumption that the deposition discrepancy (error) scaled proportionally with the total thickness of the deposited layers.

To test this, a second iteration featuring a 10% global thickness enlargement was fabricated (layer specifications detailed in Table E.1). Deposition rates for this run were calibrated at 80.41 nm/min (1.3402 nm/s) for  $\text{SiO}_2$  and 55.71 nm/min (0.9285 nm/s) for  $\text{Si}_3\text{N}_4$ .

**Table 4.4:** Layer Stack Configuration for the +10% 19-Layer MCFP Filter

| Layer                             | Material                       | Refractive Index ( $n$ ) | Thickness (nm) |
|-----------------------------------|--------------------------------|--------------------------|----------------|
| <b>Medium</b>                     | Air                            | 1.00                     | -              |
| 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 | SiO <sub>2</sub>               | 1.55                     | 66 (each)      |
| 2, 6, 8, 12, 14, 18               | Si <sub>3</sub> N <sub>4</sub> | 1.92                     | 66 (each)      |
| 4, 10, 16 (Cavities)              | Si <sub>3</sub> N <sub>4</sub> | 1.92                     | 187 (each)     |
| <b>Substrate</b>                  | SiO <sub>2</sub>               | 1.55                     | -              |
| <b>Total Sum</b>                  |                                |                          | <b>1617 nm</b> |

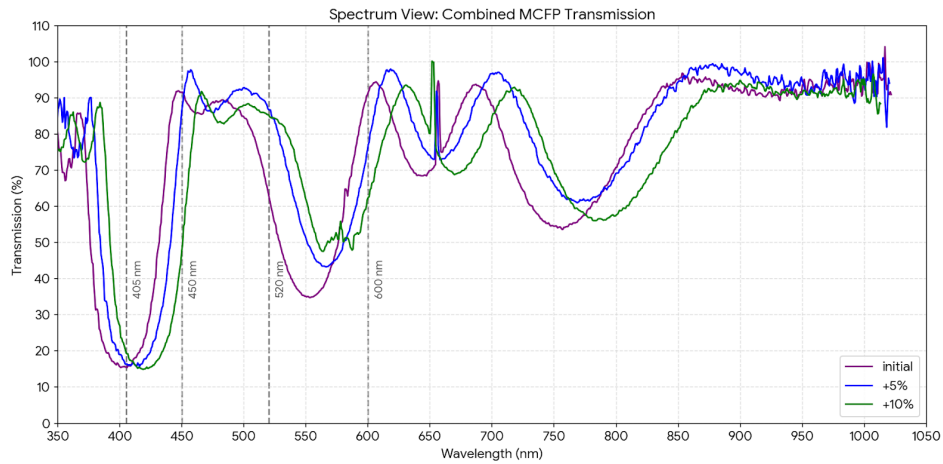
This +10% iteration produced an additional 7.5 nm red-shift. At this stage, the empirical properties of the filter were deemed satisfactory: the 405 nm excitation peak was suppressed below 20% transmittance, the 450 nm peak was kept below 45%, and the 600 nm oxygen-associated peak matched the initial prediction at approximately 60% transmittance. Crucially, the target 520 nm pH emission peak was successfully maintained at over 85% transmission. As predicted by the scaling assumption, the measurement-to-simulation error in this thicker iteration grew to approximately 40 nm. The comparative evolution of the simulated and empirical spectra across the initial, +5%, and +10% iterations are illustrated in Figure 4.11 and Figure 4.12, respectively.

**Figure 4.11:** Simulated transmittance spectra comparing the initial, +5%, and +10% global thickness modifications.

Alongside the global thickness adjustments, an alternative refinement approach was also tested wherein only the cavity thicknesses were increased by 10% while maintaining the original mirror dimensions. However, empirical testing revealed that the resulting spectrum closely mirrored the initial, unadjusted filter. Because this localised modification failed to induce the necessary spectral shift, this specific configuration was disregarded from further optimisation. The associated data and transmittance graphs for this cavity-only adjustment are available in Appendix E.

**Extended 23-Layer Pre-Compensated Architecture** Having quantified the fabrication error scale, a final experimental iteration was conducted that deliberately bypassed the strict 1.5  $\mu\text{m}$  physical thickness constraint. This was done to investigate if incorporating additional alternating layers could sharpen the out-of-band rejection crevasses. The model was upgraded to a 23-layer architecture (Table 4.5). Crucially, the simulation for this iteration (Figure 4.13) was designed with an intentional pre-compensated shift of 40 nm to proactively counteract the scaling error observed in the previous fabrication runs.

The theoretical properties of this 23-layer model showed significant improvement: the primary rejection crevasse expanded to a width of 48 nm at 20% transmittance (theoretically compressing both the 405 nm and 450 nm peaks below 20%), while the 600 nm crevasse deepened to 27%, all while preserving

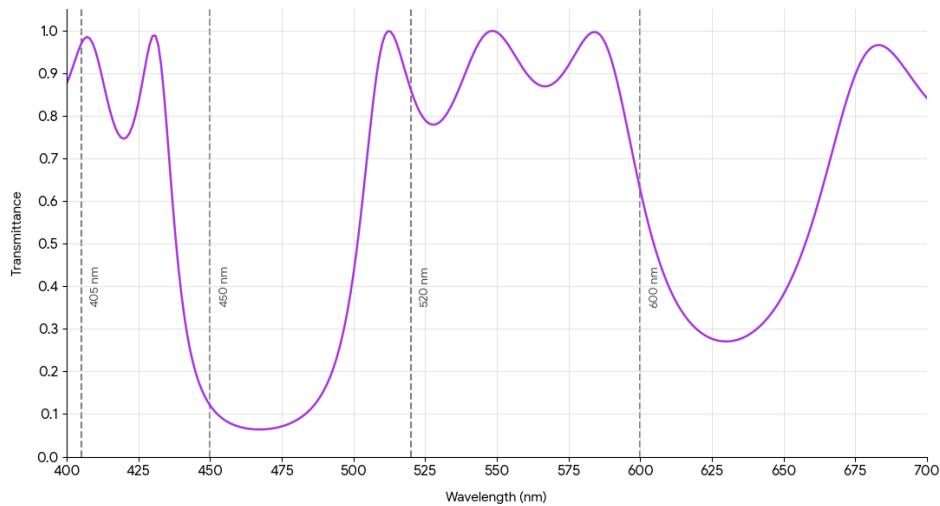


**Figure 4.12:** Empirical transmittance spectra measured via spectrometer, illustrating the progressive red-shift achieved by the +5% and +10% thickness adjustments.

**Table 4.5:** Layer Stack Configuration for the Extended 23-Layer MCFP Filter

| Layer                                     | Material                       | Refractive Index ( $n$ ) | Thickness (nm) |
|-------------------------------------------|--------------------------------|--------------------------|----------------|
| <b>Medium</b>                             | Air                            | 1.00                     | -              |
| 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 | SiO <sub>2</sub>               | 1.55                     | 70 (each)      |
| 2, 4, 8, 10, 14, 16, 20, 22               | Si <sub>3</sub> N <sub>4</sub> | 1.92                     | 70 (each)      |
| 6, 12, 18 (Cavities)                      | Si <sub>3</sub> N <sub>4</sub> | 1.92                     | 175 (each)     |
| <b>Substrate</b>                          | SiO <sub>2</sub>               | 1.55                     | -              |
| <b>Total Sum</b>                          |                                |                          | <b>1925 nm</b> |

a 520 nm target peak above 90%.

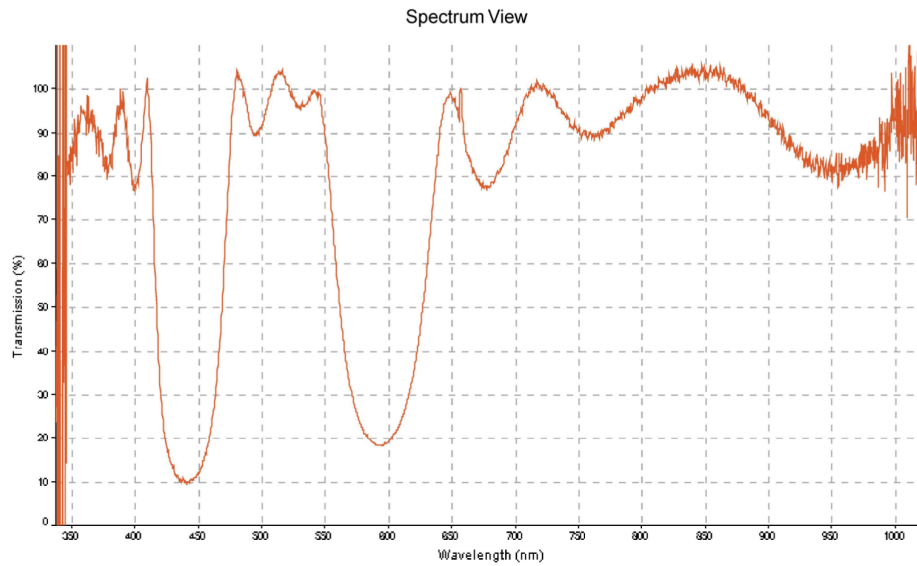


**Figure 4.13:** Simulated spectrum of the 23-layer MCFP architecture, deliberately pre-compensated with a 40 nm shift to offset empirical deposition errors.

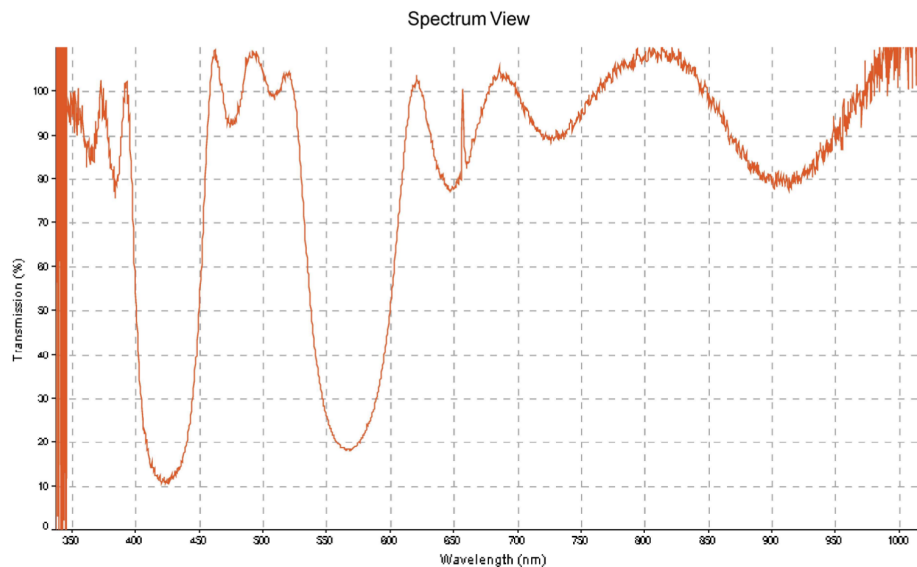
Fabrication was executed with deposition rates of 72.10 nm/min (1.2017 nm/s) for SiO<sub>2</sub> and 51.61 nm/min (0.8602 nm/s) for Si<sub>3</sub>N<sub>4</sub>. The empirical measurement taken at a standard 0° angle of incidence (Figure 4.14) yielded a highly promising morphological match to the simulation, though it remained slightly misaligned, leaving the 405 nm peak insufficiently compressed.

To explore angular tuning, the filter was subsequently measured at a 30° angle of incidence relative

to the incoming light beam (Figure 4.15). This angular rotation induced the desired spectral blue-shift, resulting in a highly effective operational profile: both the 405 nm and 450 nm excitation peaks were attenuated to approximately 50% transmittance, the 600 nm peak was suppressed to 50%, and the target 520 nm emission peak achieved near 100% transmittance.



**Figure 4.14:** Empirical transmittance of the 23-layer filter measured at a 0° angle of incidence.



**Figure 4.15:** Empirical transmittance of the 23-layer filter measured at a 30° angle of incidence, demonstrating the corrective blue-shift induced by angular tuning.

## 4.2. Sensor Calibration and Baseline Performance

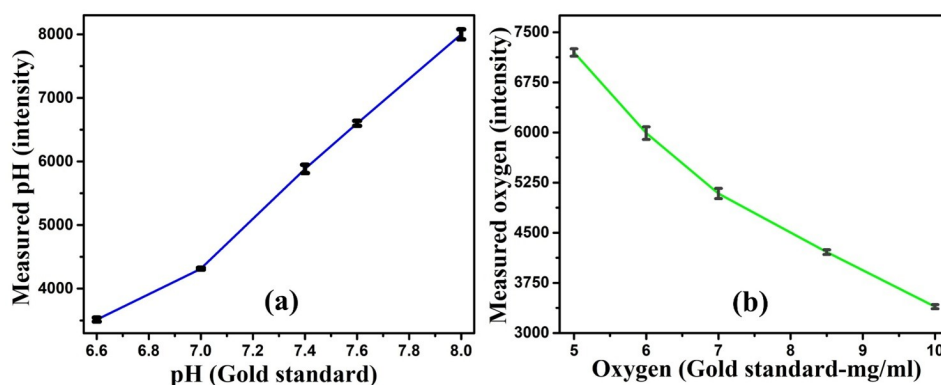
Before deploying the dual-parameter sensor in dynamic *ex vivo* tissue environments, a comprehensive benchtop characterisation was conducted to establish baseline calibration curves, verify the absence of luminophore cross-talk, and determine the transient response times.

### 4.2.1. Calibration Curves

The isolated responses of the bi-functional sensor to varying analyte concentrations are presented in Figure 4.16.

As depicted in Figure 4.16(a), the pH-sensitive luminophore demonstrated a proportional, protonation-dependent fluorescence response. As the environmental pH was incrementally increased from 6.6 to 8.0, the emission intensity at the 520 nm peak exhibited a corresponding rise from approximately 3500 to 8000 counts.

Conversely, Figure 4.16(b) illustrates the expected quenching behaviour of the oxygen-sensitive ruthenium complex. As dissolved oxygen concentrations increased from 5.0 mg/ml to 10.0 mg/ml, the resulting emission intensity at the 600 nm peak underwent significant attenuation, dropping from approximately 7200 to 3400 counts. This inverse relationship confirms that the oxygen-related luminophore interacts correctly with the local oxygen molecules, successfully validating the dynamic quenching principles required for quantification.



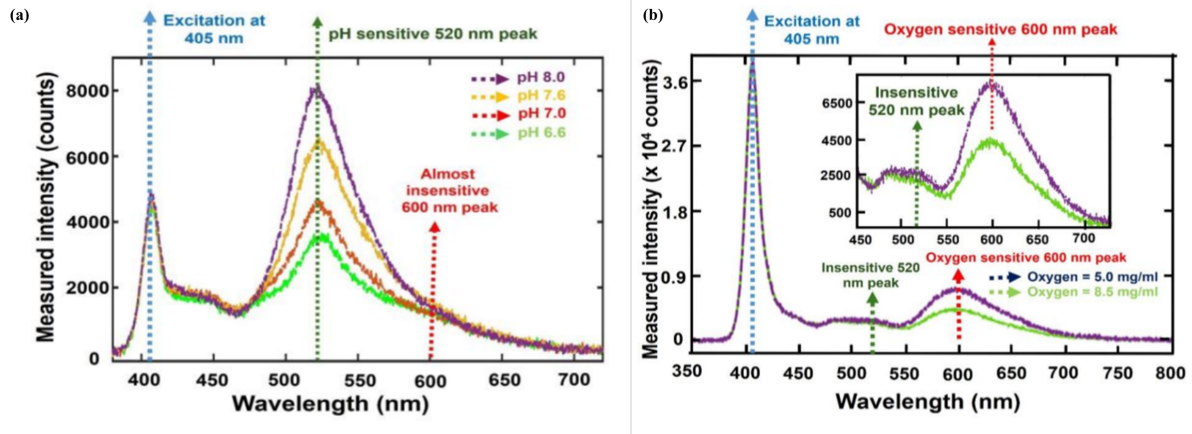
**Figure 4.16:** Baseline calibration curves demonstrating (a) the proportional increase in 520 nm emission intensity across a pH range of 6.6 to 8.0, and (b) the fluorescence quenching at the 600 nm peak across an oxygen concentration range of 5.0 to 10.0 mg/ml. Reproduced from [57].

#### 4.2.2. Cross-Sensitivity Analysis

A critical requirement for the bi-functional sensor is the independent operation of its two embedded luminophores. To validate this, the sensor was subjected to environments where one analyte was modulated while the other was held strictly constant (Figure 4.17).

Figure 4.17(a) demonstrates the spectral response when the sensor was exposed to a varying pH environment (6.6 to 8.0) while maintaining a static dissolved oxygen concentration of 5.0 mg/ml. Under 405 nm excitation, the 520 nm pH peak scaled dramatically, whereas the 600 nm oxygen peak remained almost entirely insensitive to the pH fluctuations.

Similarly, Figure 4.17(b) depicts the sensor's response in an environment with a fixed pH of 7.2 and alternating oxygen levels (5.0 to 8.5 mg/ml). The 600 nm peak quenched exactly as expected, while the 520 nm peak remained remarkably stable (highlighted in the figure's inset). These results provide strong empirical reassurance that there is no substantial optical cross-talk or interference between the two luminophores, allowing for the reliable, simultaneous extraction of both parameters from a single optical reading.

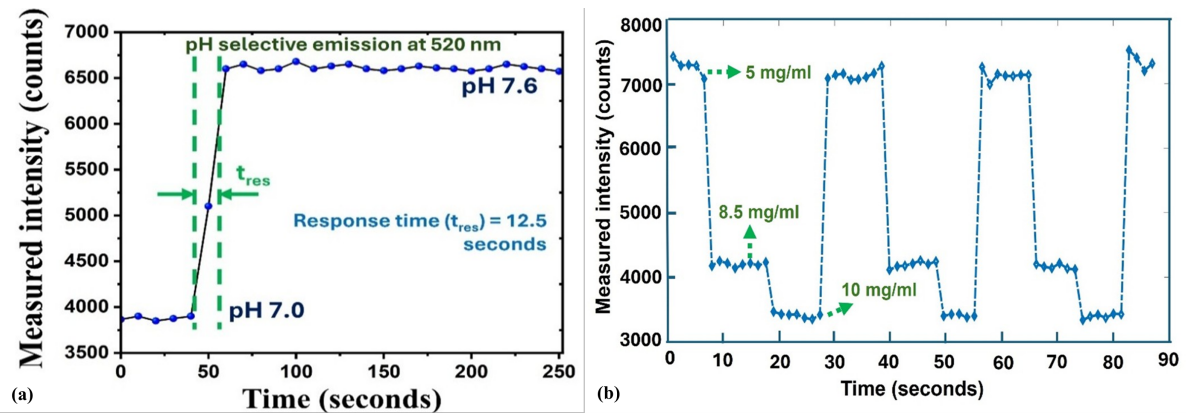


**Figure 4.17:** Cross-sensitivity analysis under 405 nm excitation. (a) Modulating pH (6.6–8.0) at a constant oxygen level (5.0 mg/ml) yields fluctuations strictly at the 520 nm peak. (b) Modulating oxygen (5.0–8.5 mg/ml) at a constant pH (7.2) yields quenching strictly at the 600 nm peak. Reproduced from [60]

#### 4.2.3. Dynamic Response Time

To ensure the sensing platform is capable of capturing rapid physiological shifts during organ perfusion, the temporal dynamics of the hydrogel matrix were evaluated. The transient response curves for both parameters are presented in Figure 4.18.

The transient response for pH detection (Figure 4.18(a)) illustrates a rapid signal shift from approximately 3750 counts to 6750 counts following a step change in pH from 7.0 to 7.6. The sensor reached equilibrium with a response time ( $t_{res}$ ) of 12.5 s. The transient response for oxygen detection (Figure 4.18(b)) demonstrated even faster dynamics; as oxygen levels were shifted sequentially from 5.0 to 8.5 to 10.0 mg/ml, the sensor reacted with a response time of  $t_{res} < 2$  s. These rapid response characteristics confirm that the diffusion kinetics through the protective 0.1 ml hydrogel overcoat are highly efficient and suitable for real-time monitoring of rapid metabolic changes.



**Figure 4.18:** Dynamic response analysis of the bi-functional sensor matrix. (a) Transient fluorescence response to a discrete pH step change from 7.0 to 7.6, demonstrating a response time of 12.5 s. (b) Transient fluorescence quenching response to sequential step changes in oxygen concentration (5.0, 8.5, and 10.0 mg/ml), demonstrating a rapid sub-2-second response time. Reproduced from [57].

### 4.3. Ex Vivo Validation: Dynamic Perfusion Model (Defrosted Liver)

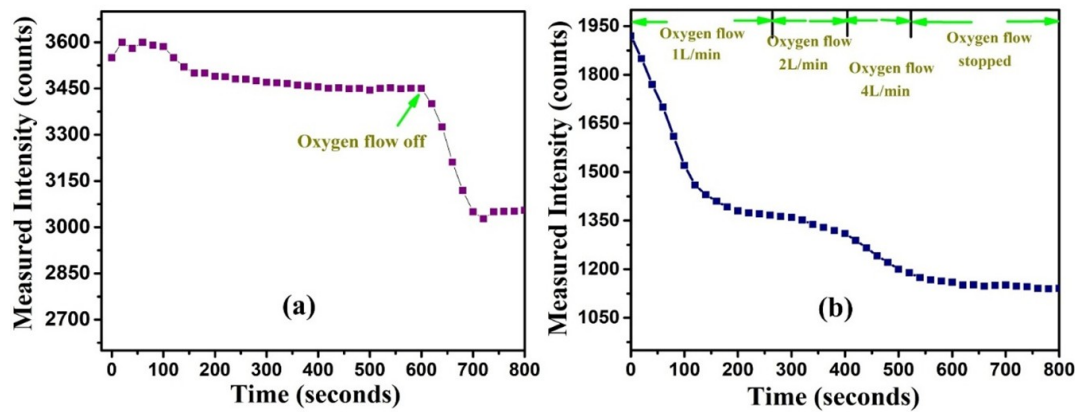
The initial phase of human tissue testing was conducted in collaboration with the Erasmus Medical Center (EMC). This foundational experiment utilised a discarded, defrosted human liver that was biologically non-viable. The primary objective of this trial was to evaluate the transient response capabilities of the integrated optical sensors, specifically monitoring real-time shifts in local tissue pH and oxygenation as the perfusion parameters were actively manipulated.

### 4.3.1. Optical Tracking of Oxygen Shifts

During this experiment, two continuous 10-minute measurements were recorded at distinct spatial locations on the surface of the organ. To induce a transient metabolic response, the oxygen supplied to the normothermic machine perfusion (NMP) system was manually altered using the primary tank release valve.

It should be noted that these initial measurements were conducted utilising solely the 450 nm (blue) excitation light source. At this stage of testing, blue light excitation was deemed the most reliable and sufficient for capturing the quenching dynamics. Consequently, dual-wavelength ratiometric data was not collected for this specific experiment. However, an alternative ratiometric analysis comparing the isolated oxygen and pH transient responses, alongside the unrefined graphs and extended raw intensity data, is provided in Appendix F.

Figure 4.19 (b) details the continuous tracking of the oxygen sensor's fluorescence intensity in response to manual, stepwise increases in the perfusate's oxygen flow rate. As systemic oxygen delivery escalated, the recorded optical signal exhibited a proportional decrease in fluorescence, indicating standard quenching behaviour at the tissue surface. Concurrently, Figure 4.19 (a) presents the localised tissue pH readings over the identical 10-minute window. Throughout the initial fluctuations in oxygen provision, the pH profile maintained a steady baseline. However, following the complete discontinuation of the oxygen supply, a progressive downward shift in pH was recorded, temporally aligning with the cessation of flow.



**Figure 4.19:** Refined transient metabolic response of the defrosted liver model over a 10-minute period. **(a)** Local tissue pH fluctuations, with an indicator marking the exact moment oxygen flow was completely cut off. **(b)** Local oxygen ( $O_2$ ) response, with annotations indicating the manual adjustments made to the oxygen flow rate (ranging from 1 L/min to 6 L/min).  
Reproduced from [57].

### 4.3.2. Clinical Gold-Standard Comparison

Discrete clinical perfusate analysis, such as intermittent blood gas sampling, was not conducted during this preliminary trial. Instead, the optical sensor readings were presented alongside the known mechanical inputs of the NMP system. The machine's oxygen flow was mechanically regulated at specific intervals of 1 L/min, 2 L/min, and 4 L/min, concluding with a complete flow cut-off at 0 L/min. The optical sensing data corresponded with these step-wise systemic adjustments. As the macroscopic oxygen supply was increased, the local tissue  $O_2$  readings showed corresponding decreases in fluorescence intensity. Upon the complete cessation of flow (0 L/min), a distinct shift was recorded in both profiles, characterized by an increase in oxygen sensor fluorescence and a subsequent drop in the pH reading.

## 4.4. Ex Vivo Validation: Viable Organ Monitoring (Fresh Liver)

Following the initial functional validation on the defrosted liver model, the sensor platform was subsequently evaluated in a fully viable clinical scenario utilising a freshly declined human liver undergoing normothermic machine perfusion.

#### 4.4.1. Clinical Baseline and Measurement Protocol

To capture a comprehensive spatial profile of the graft's viability, discrete optical measurements were recorded across multiple distinct anatomical locations on the posterior surface of the liver. To simultaneously evaluate the reproducibility of the fabricated sensor patches and rule out localised sensor degradation, three separate sensors were utilised sequentially throughout the experiment. Furthermore, Measurement 4 was conducted twice at the exact same anatomical location (labeled 4.1 and 4.2) but with different sensors (Sensor 2 and Sensor 3, respectively) to allow for a direct comparative analysis.

The spatial mapping and sensor distribution were executed as follows:

- **Measurement 1:** Posterior Right Medial region (New Sensor 1)
- **Measurement 2:** Posterior Left Medial region (New Sensor 2)
- **Measurement 3:** Posterior Right Lateral region (Sensor 2)
- **Measurement 4.1:** Posterior Left Lateral region (Sensor 2)
- **Measurement 4.2:** Posterior Left Lateral region (New Sensor 3)
- **Measurement 5:** Superior Posterior Medial region (Sensor 3)

Simultaneous to the optical readings, clinical reference samples of the perfusate were extracted and analysed by the hospital's ABL90 FLEX analyser. As detailed in Table 4.6, the clinical data confirmed that the liver maintained a highly stable and normal physiological baseline throughout the duration of the monitoring protocol, with pH values ranging from 7.446 to 7.575 and  $pO_2$  levels ranging from 20.7 to 22.5 kPa.

**Table 4.6:** Clinical reference parameters (pH and  $pO_2$ ) recorded by the hospital analyser concurrently with the optical measurement index.

| Measurement Index | Sensor Index | Clinical pH | Clinical $pO_2$ (kPa) |
|-------------------|--------------|-------------|-----------------------|
| 1                 | 1            | 7.560       | 22.5                  |
| 2                 | 2            | 7.575       | 22.4                  |
| 3                 | 2            | 7.456       | 21.6                  |
| 4.1               | 2            | 7.497       | 21.0                  |
| 4.2               | 3            | 7.497       | 21.0                  |
| 5                 | 3            | 7.446       | 20.7                  |

#### 4.4.2. Optical Data Acquisition and Aggregation

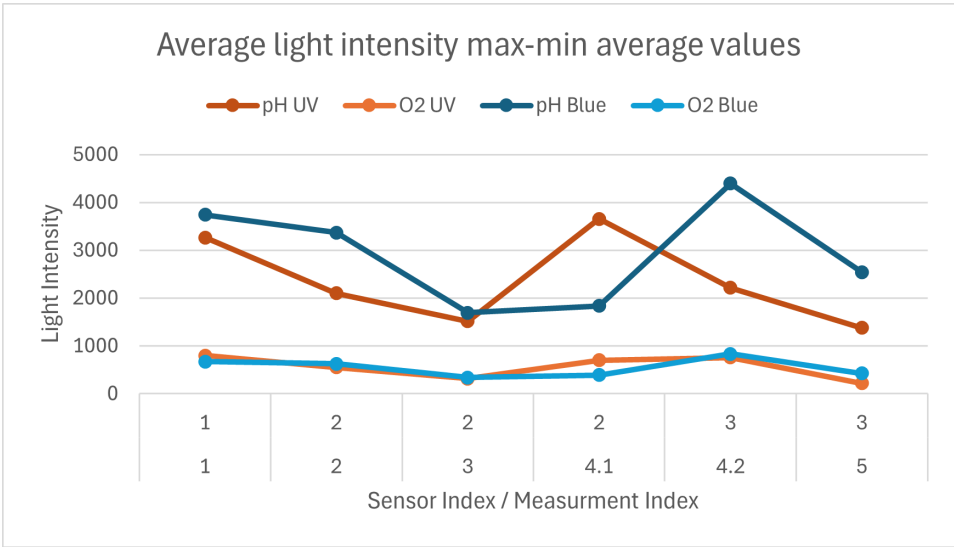
Due to the inherent micro-fluctuations in real-time continuous spectral acquisition, a standardised data aggregation methodology was applied to translate the raw spectral data into stable discrete data points.

For every designated measurement location, ten sequential intensity readings were recorded. For each individual reading, the maximum and minimum intensity values were extracted within strict spectral windows corresponding to the target analytes and excitation sources: 520–530 nm (pH emission), 593–604 nm (oxygen emission), 405–410 nm (UV excitation), and 445–455 nm (Blue excitation). The arithmetic average of these max-min boundaries was calculated for each of the ten readings. Finally, a single, overarching average was computed from these ten values to represent the definitive intensity for that specific measurement index.

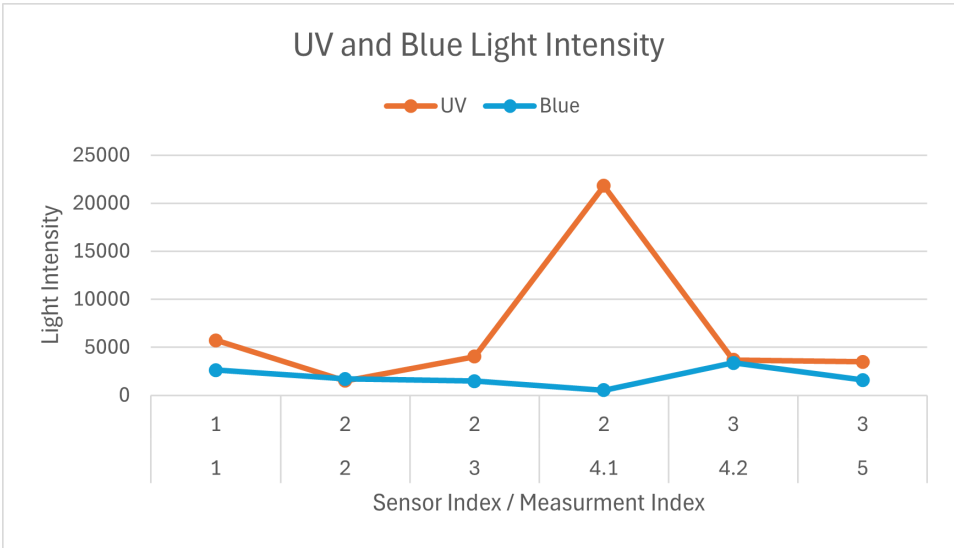
#### 4.4.3. Ratiometric Fluorescence Tracking

The aggregated max-min average values for the target emission peaks across the entire experiment are visually summarised in Figure 4.20. The graph tracks four distinct emission responses relative to the measurement index: the pH (520 nm) and  $O_2$  (600 nm) emission intensities under both UV and Blue light excitation. Mapping the dual-wavelength responses concurrently facilitates the ratiometric normalisation required for accurate signal processing in dynamic tissue environments.

Simultaneously, the intensities of the excitation light sources returning to the spectrometer were tracked and plotted in Figure 4.21 to account for any positional or geometric variations during probe placement on the uneven liver surface.



**Figure 4.20:** Average max-min light intensity values for the pH and O<sub>2</sub> target peaks, tracked across the sequence of measurement indices and under dual-excitation modes (UV and Blue).



**Figure 4.21:** Recorded intensity variations of the UV and Blue excitation light sources corresponding to each measurement location on the liver.

The complete dataset, detailing the absolute, maximum, minimum, and aggregated max-min average values for every measured parameter and excitation modality, is catalogued comprehensively in Table 4.7. Notably, the transition between Measurement 4.1 and 4.2 (sensor swap at the same location) highlights the system’s ability to maintain relative signal tracking despite the introduction of a new sensor matrix and a documented physical shift in the UV source intensity at that specific timepoint. For exhaustive documentation, additional graphs and data tables detailing every individual reading from every measurement and for each sensor utilised during this experiment are provided in Appendix G.

**Table 4.7:** Comprehensive breakdown of the raw and aggregated optical intensity values recorded across all measurement indices for both excitation sources.

| Meas. | pH (UV Excitation) |      |      |      | O <sub>2</sub> (UV Excitation) |     |     |     | pH (Blue Excitation) |      |      |      | O <sub>2</sub> (Blue Excitation) |      |     |     | Source |      |
|-------|--------------------|------|------|------|--------------------------------|-----|-----|-----|----------------------|------|------|------|----------------------------------|------|-----|-----|--------|------|
|       | 520 nm             | Max  | Min  | Avg  | 600 nm                         | Max | Min | Avg | 520 nm               | Max  | Min  | Avg  | 600 nm                           | Max  | Min | Avg | UV     | Blue |
| 1     | 3175               | 3459 | 3074 | 3267 | 768                            | 947 | 652 | 800 | 3737                 | 3931 | 3552 | 3741 | 645                              | 803  | 544 | 673 | 5707   | 2613 |
| 2     | 2087               | 2267 | 1934 | 2101 | 572                            | 676 | 431 | 554 | 3418                 | 3585 | 3149 | 3367 | 639                              | 749  | 499 | 624 | 1500   | 1709 |
| 3     | 1455               | 1668 | 1359 | 1513 | 296                            | 452 | 175 | 314 | 1663                 | 1852 | 1530 | 1691 | 350                              | 474  | 202 | 338 | 4021   | 1468 |
| 4.1   | 3439               | 3919 | 3394 | 3657 | 712                            | 845 | 561 | 703 | 1679                 | 2049 | 1623 | 1836 | 411                              | 518  | 266 | 392 | 21825  | 521  |
| 4.2   | 2125               | 2378 | 2051 | 2214 | 723                            | 894 | 621 | 758 | 4416                 | 4583 | 4201 | 4392 | 778                              | 1009 | 654 | 831 | 3675   | 3368 |
| 5     | 1381               | 1524 | 1233 | 1379 | 208                            | 347 | 86  | 217 | 2560                 | 2716 | 2361 | 2539 | 405                              | 557  | 296 | 426 | 3495   | 1581 |

# 5

## Discussion

### 5.1. Summary of System Efficacy

This study successfully demonstrated the design, fabrication, and clinical validation of a novel dual-parameter optical sensing platform capable of real-time, ratiometric monitoring of pH and dissolved oxygen in viable tissue. To overcome the inherent optical interference associated with high-intensity excitation sources and ambient clinical lighting, bespoke multi-layer dielectric optical filters were engineered. While initial theoretical models explored titanium dioxide, the strategic transition to silicon nitride ( $\text{Si}_3\text{N}_4$ ) and silicon dioxide ( $\text{SiO}_2$ ) enabled the successful cleanroom fabrication of robust band-pass and multi-cavity Fabry-Perot architectures.

Benchmark characterisation confirmed that the integrated luminophores exhibited highly independent, cross-talk-free responses, alongside rapid transient kinetics essential for capturing acute metabolic fluctuations. Furthermore, the secure encapsulation of these optical probes within a biocompatible hydrogel matrix ensured structural integrity and successfully mitigated the inherent cytotoxicity of the ruthenium complexes, ensuring safe direct contact with biological tissues.

Ultimately, the deployment of this integrated system during the *ex vivo* normothermic machine perfusion of a human liver validated its clinical viability. By leveraging a dual-excitation (405 nm and 450 nm) ratiometric methodology, the sensor matrix maintained robust signal stability across varied anatomical locations. The platform successfully translated raw, localised optical intensity readings into reliable physiological data, tracking the organ's metabolic baseline without disrupting the established clinical workflow.

### 5.2. Evaluation of Optical Filter Fabrication and the "Blue-Shift"

#### 5.2.1. Fabrication Strategy and Calibration Challenges

A fundamental objective of the filter design was to utilise a streamlined, rapid, and highly secure fabrication process. To achieve this, the project adapted a continuous deposition methodology previously demonstrated in thin-film optical filter fabrication by Ceren Kutucu, PhD [62]. By depositing the entire filter stack within the Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD) machine in a single, uninterrupted recipe, the vacuum seal remained unbroken. This "one-go" approach significantly reduced fabrication time compared to Low-Pressure CVD (LPCVD) processes and successfully shielded the ultra-thin layers from external environmental contaminants.

However, this method introduced a critical trade-off: because the deposition chamber was not purged and cleaned between alternating material layers, cross-contamination from residual precursor gases was highly probable. To mitigate the accumulation of these remnants and maximise optical accuracy, strict structural constraints were imposed, limiting the architectures to approximately 20 layers and a maximum total thickness of 1.5  $\mu\text{m}$ .

The preparation and calibration of the ICP-CVD machine also presented substantial practical chal-

allenges. Initial attempts to map the deposition rates involved running one-minute test depositions, breaking the vacuum to measure the wafer, and repeating the process throughout the day to track rate drift. This proved highly inconsistent due to daily machine variations and environmental exposure during measurement. Consequently, the protocol was revised to the final methodology detailed in Chapter 3, relying on immediate, back-to-back calibration runs right before the final filter fabrication to capture the most accurate possible snapshot of the deposition rates.

### 5.2.2. Material and Architectural Evolution

As established, the initial simulations utilised a  $\text{TiO}_2/\text{SiO}_2$  pairing. While theoretically sound, the resulting Full Width at Half Maximum (FWHM) was critically narrow (e.g., 3.5 nm for the 520 nm peak). Given the inherent cross-contamination risks of the continuous ICP-CVD process, it was determined that the fabrication precision required to hit such a narrow passband was practically unattainable. The strategic pivot to  $\text{Si}_3\text{N}_4$  provided the necessary "wiggle room" by inherently widening the FWHM, thus drastically increasing the probability of a successful fabrication despite minor deposition inaccuracies.

This material shift necessitated architectural adaptations. While the oxygen-targeted filter was simplified into a highly reproducible 21-layer bandpass design, the pH-targeted filter was upgraded from a single-cavity to a Multi-Cavity Fabry-Perot (MCFP) configuration. Influenced by existing thin-film literature, expanding to three cavities proved to be the optimal configuration to maintain the strict layer constraints while achieving the necessary spectral isolation.

During the MCFP simulations, achieving a deep rejection band for the 600 nm peak proved difficult without exceeding the layer limit. The theoretical model yielded a 60% transmittance cut-off (which empirically improved to approximately 35–50% in the fabricated filters). This partial attenuation was deemed clinically acceptable because the inherent fluorescence intensity of the oxygen-sensitive peak is more than five times lower than the 520 nm pH peak, and drastically lower than the excitation source intensities, rendering its residual interference negligible.

### 5.2.3. Spectral Anomalies and the "Blue-Shift" Analysis

A prominent feature across all empirical and simulated FP transmittance spectra is the presence of a "ripple effect" in the transmission bands, particularly at wavelengths above 600 nm. This is a well-documented and expected shortcoming inherent to Fabry-Perot architectures [39]. For future refinements, this ripple effect can be minimised by ensuring the optical admittance of the filter stack is perfectly matched to that of the underlying substrate [39].

The most significant fabrication anomaly was the 25–40 nm spectral blue-shift observed in the MCFP filters. This systematic error is attributed to a combination of factors inherent to the cleanroom environment: unrecorded micro-fluctuations in the ICP-CVD deposition rates, the accumulation of residual material contamination during the continuous deposition process, and slight calculation errors in the baseline refractive index measurements extracted via spectroscopic ellipsometry.

Interestingly, while these factors caused the spectrum to shift, they also resulted in an empirically sharper 600 nm rejection crevasse than initially simulated. As the layer thicknesses were globally increased by 5% and 10% to correct the shift, the sharpness of this crevasse was observed to smooth out, converging back toward the original simulation morphology. Extrapolating this trend, an additional 5% thickness increase (for a total +15% adjustment) would likely align the spectrum even closer to the theoretical ideal and represents a highly recommended parameter for future fabrication attempts.

### 5.2.4. Architectural Complexity and Engineering Workarounds

To test the boundaries of the system, a final 23-layer pre-compensated filter was fabricated, intentionally bypassing the strict layer minimisation rule. The addition of just four extra layers instantly yielded significantly sharper rejection crevasses and superior out-of-band blocking. This confirms that, given access to highly precise deposition machinery that mitigates cross-contamination (e.g., automated cleaning cycles between layers), future iterations should confidently deploy more complex, higher-layer-count mirror stacks to approximate ideal optical properties.

Ultimately, the challenges encountered during fabrication highlighted the value of practical engineering workarounds. When standard deposition resulted in the persistent 40 nm scaling error, deliberate the-

oretical pre-compensation and physical angular tuning (such as the 30° incident rotation) proved to be highly effective, viable strategies to successfully force the optical passbands into functional alignment.

## 5.3. Sensor Dynamics, Stability, and Safety

### 5.3.1. Calibration and Signal Independence

The foundational laboratory testing confirmed that both embedded luminophores exhibited their anticipated optical behaviours relative to environmental analyte fluctuations. As established in Figure 4.16, the pH-sensitive matrix demonstrated proportional fluorescence scaling, while the ruthenium-based oxygen matrix underwent expected dynamic quenching.

However, the most significant achievement of the sensor's chemical architecture is the confirmed absence of optical cross-sensitivity, as decisively demonstrated in Figure 4.17. In multiplexed optical sensing, achieving two completely independent signal outputs from a single, co-localised matrix is a notoriously difficult task well-documented in the literature. Without strict signal independence, variations in one analyte can easily cause false or misinterpreted readings in the other. The lack of cross-talk in this bespoke platform ensures that the data collected during highly variable clinical procedures remains untainted by inter-luminophore interference. This clean signal segregation elevates the sensor to a state-of-the-art diagnostic tool, validating its novel bi-functional architecture.

### 5.3.2. Clinical Relevance of Transient Response

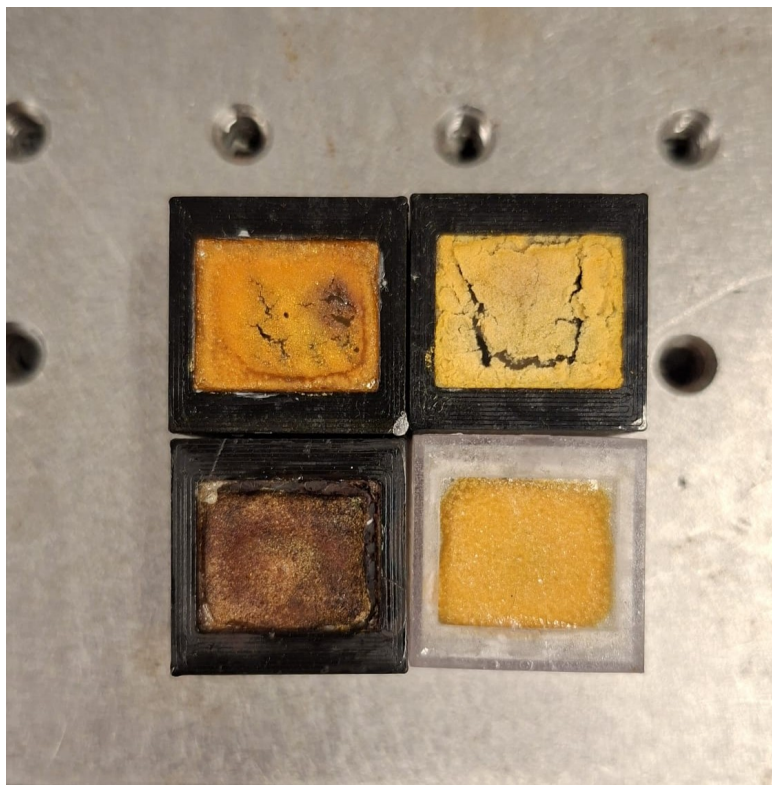
For an optical sensor to be viable within a dynamic clinical environment, such as an operating room or an organ perfusion circuit, it must possess diffusion kinetics capable of matching the speed of physiological changes. The transient response times recorded in Figure 4.18, less than 2 seconds for oxygen and 12.5 seconds for pH, prove that the sensor is highly capable of real-time monitoring. These rapid response characteristics are clinically critical; they guarantee that medical professionals are provided with instantaneous measurements, allowing for immediate alarms and rapid interventions in the event of any acute or negative metabolic shifts within the graft.

### 5.3.3. Biocompatibility and Hydrogel Encapsulation Optimisation

While ruthenium complexes provide excellent photostability and quantum yields, their inherent cytotoxicity poses a severe barrier to direct tissue contact. Initially, to minimise potential optical interferences and maximise signal intensity, only a minimal volume of hydrogel was utilised for sensor encapsulation. However, preliminary trials utilising a frozen liver model revealed that this ultra-thin encapsulation was insufficient to fully enclose the chemistry; upon prolonged exposure to the moist tissue, the sensor showed signs of structural degradation, leaching toxic ruthenium compounds and leaving distinct orange stains on the organ's surface.

To enhance durability and ensure absolute biocompatibility, a much more robust encapsulation protocol was engineered. A thicker hydrogel coating layer, calibrated to approximately 0.1 ml, was introduced (Figure 5.1). The application process was divided into two distinct phases to ensure stability: first, the sensor solution was dispensed onto the glass substrate and subjected to an overnight drying period. Subsequently, the hydrogel solution was dispensed and mechanically brushed to guarantee a uniform, hermetic seal across the entire matrix, followed by a second overnight drying phase.

Subsequent experimental observations on the viable liver model confirmed that this optimised coating successfully locked the sensor chemistry in place, preventing the matrix from dissolving over time and completely shielding the delicate organ tissue from toxic leaching. Crucially, as evidenced by the rapid transient response times, this thicker protective layer did not impede the diffusion of target analytes. However, comparative analysis between the two experiments revealed a distinct optical trade-off: the added structural encapsulation significantly attenuated the signal, lowering the baseline fluorescent intensities for both the pH and oxygen peaks by approximately 3000 counts. While this attenuation required recalibration of the baseline values, the relative dynamic quenching behaviour and the overall sensitivity of the sensor remained functionally intact.



**Figure 5.1:** Comparison of sensor probe caps illustrating degradation patterns based on hydrogel encapsulation and deployment. **Top left (TL):** No supplementary hydrogel, in-laboratory use only. **Top right (TR):** No supplementary hydrogel, post-Experiment 1. **Bottom left (BL):** Optimised supplementary hydrogel, post-Experiment 2. **Bottom right (BR):** Optimised supplementary hydrogel, in-laboratory use only.

## 5.4. Clinical Translation during Ex Vivo Perfusion

The translation of the bi-functional sensing platform from controlled laboratory conditions to a functional clinical workflow was evaluated across two distinct normothermic machine perfusion (NMP) models: a non-viable defrosted liver (Experiment 1) and a viable, freshly declined human liver graft (Experiment 2). The combined experimental observations provided critical insights into the biological, mechanical, and optical variables that govern real-time tissue monitoring in blood-rich, dynamic environments.

### 5.4.1. Biological Context and Metabolic Dynamics

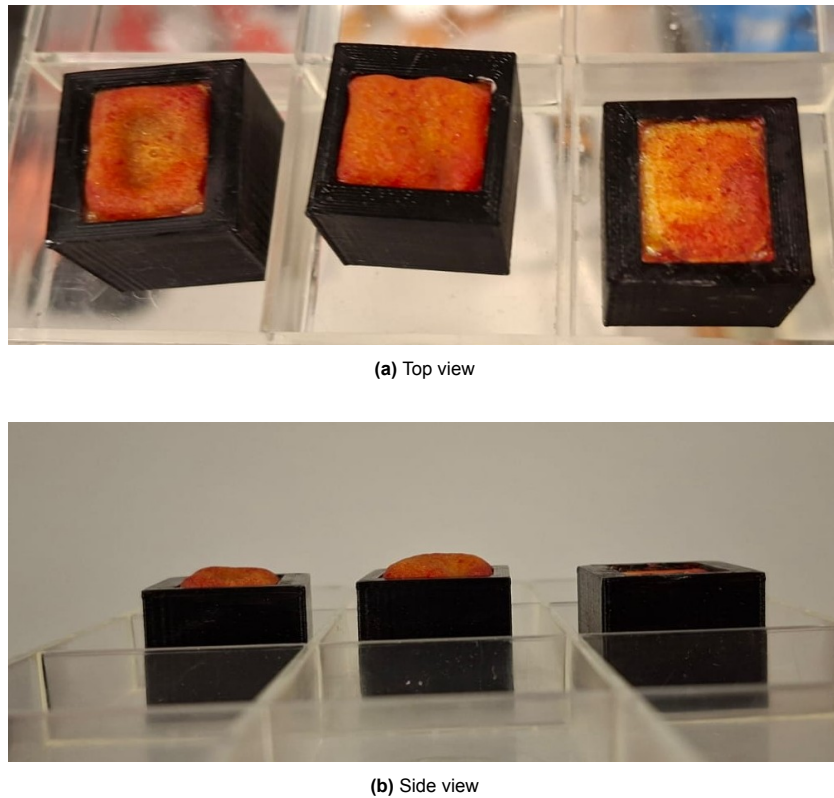
The initial trials on the non-viable, defrosted liver successfully validated the foundational functionality and practical deployment capabilities of the optical sensing platform. Because the frozen graft was biologically inactive, it did not consume oxygen at the cellular level. Therefore, the integrated device acted as a direct monitor of the mechanical perfusate flow. As the oxygen flow rate in the perfusate was manually escalated, the sensor exhibited a short response time and yielded a clear, proportional decrease in fluorescence intensity, accurately reflecting the expected dynamic quenching behaviour. Furthermore, the localised tissue pH remained highly stable throughout the fluctuations in oxygen provision; a significant drop in pH was only recorded following the complete discontinuation of the oxygen supply, indicating an expected acidic shift in response to the induced ischaemic environment.

In stark contrast to the mechanically driven shifts of the first experiment, data extracted from the viable human liver graft established a pristine baseline haemodynamic profile, confirming that the organ was biochemically active. Maintained under normothermic conditions for over six hours, the graft's metabolic signature was characterised by a progressive downtrend in perfusate oxygenation, with partial pressure ( $pO_2$ ) dropping from 22.5 kPa to 20.7 kPa. Rather than indicating an unstable oxygen supply from the perfusion circuit, this steady decline demonstrates active cellular respiration and biological consumption as the living tissue recovered from preservation.

However, local optical measurements were profoundly influenced by the anatomical landscape of the liver. The organ exhibited a significantly thinner physical profile at its peripheral boundaries, particularly along the medial and lateral segments. These thin regions proved highly susceptible to localised compression and pressure artifacts during probe application. Conversely, the thicker medial segments provided the most structurally consistent morphology for stable contact. Localised pathological anomalies, specifically visible parenchymal fold marks and a minor haematoma, introduced reading variances, highlighting the necessity of multi-site spatial tracking to differentiate localised tissue trauma from global organ status.

#### 5.4.2. Sensor Micro-Structure, Leaching, and Temporal Stability

Beyond the initial signal attenuation caused by the optimised encapsulation layer, the physical and structural evolution of the sensor matrix emerged as a critical operational variable across both experiments. Upon deployment, the highly porous hydrogel overcoat underwent visible swelling and reversible deformation immediately upon contact with the aqueous perfusate (Figure 5.2). This fluid-driven swelling behaviour, where the polymer matrix fully returns to its original physical state upon subsequent drying, matches the baseline characteristics recorded during prior laboratory buffer calibrations and does not negatively influence signal propagation.



**Figure 5.2:** Post-Experiment 2 condition of the sensor probe caps (ordered left to right: Sensor 3, Sensor 2, Sensor 1). **(a)** Top and **(b)** side views demonstrate the significant swelling and physical deformation of the porous hydrogel overcoat. The visible yellow-to-red discolouration confirms the absorption of blood and perfusate during the deployment.

In contrast, the temporal stability of the sensor chemistry emerged as a significant operating limitation during the second experiment. Notable measurement inconsistencies were tracked across the experimental timeline and traced directly to advanced sensor age. The sensor patches utilised for the viable liver had been fabricated more than two weeks prior to the perfusion experiment, using a luminophore precursor solution prepared several months in advance. While established protocols dictate optimal sensor reliability within a strict 10-day window post-fabrication, these older matrices suffered from premature degradation, amplifying the system's susceptibility to operational drift under continuous excitation.

### 5.4.3. Apparatus Optimisation and Mechanical Setup

The clinical trials highlighted several operational constraints in the physical apparatus, from which specific structural recommendations can be derived to optimise future clinical iterations. To improve intra-operative manoeuvrability and ease of use around the crowded perfusion circuit, extending the physical length of the fibre-optic cabling is essential.

Furthermore, integrating a robust, easy-to-clean, and completely waterproof protective sheath over the optoelectronic components is mandatory to safeguard the hardware from biological fluids, especially when the sensor head must be positioned underneath the posterior surface of the organ.

Finally, to eliminate the risk of parenchymal tissue damage resulting from direct contact pressure, the rigid geometry of the device head must be modified. Rounding the sharp edges of the probe tip or overcoating it with a soft medical-grade silicone casing would protect the liver capsule from mechanical trauma. Critically, this mechanical refinement would also promote seamless sensor-to-tissue conforming contact, effectively sealing out ambient operating room light and reducing boundary-layer air interference.

### 5.4.4. Optical and Ratiometric Signal Analysis

During the viable liver experiment, a clear divergence was recorded between the global parameters tracked in the bulk perfusate and the localised readings captured by the optical probes. While the machine perfusion system reported a highly stable, tightly bound global pH range (7.560 to 7.446), the local sensors captured distinct, cellular-level microenvironment variations across the liver capsule.

The dual-excitation methodology (405 nm and 450 nm) revealed completely distinct photophysical behaviours between the two interrogation light sources:

- **Excitation Source Behaviour and Photobleaching:** A critical finding was the exponential increase in the returning 405 nm ultraviolet (UV) intensity over repeated measurement cycles. Notably, during Measurement 4.1 (marking the third consecutive interrogation block using Sensor 2), the recorded UV intensity spiked by a factor of 14.5 relative to its baseline value. This massive exponential rise indicates that the aging chemical matrix reached an absolute saturation or photobleaching threshold, causing it to cease absorbing the UV light and instead begin completely reflecting/scattering the excitation beam back into the spectrometer. In stark contrast, the 450 nm blue excitation source exhibited an exceptionally stable, highly predictable linear decline across all measurements, confirming its superior resilience against photobleaching.
- **Oxygen ( $pO_2$ ) Dynamics:** The oxygen measurements derived from the sensor matrix displayed strong morphological consistency across both excitation paths, aligning with the stable metabolic baseline of the graft. However, an initial stabilisation period was found to be mandatory: the first two data points within any given measurement bundle consistently yielded artificially high intensities, indicating that the hydrogel matrix requires a brief equilibration window upon tissue contact before achieving true chemical equilibrium. Beyond this stabilisation phase, a highly stable or slightly downward trend was maintained across all bundles, with the sole exception of Measurement 4.1 under UV illumination, where severe photobleaching induced an artificial value spike. Under blue light excitation, the max-min average intensities remained exceptionally stable within a tight envelope of 673.8 to 426.8 counts. The isolated anomaly recorded during Measurement 4.2 ( $\sim 831$  counts) is attributed to localised parenchymal tissue fatigue resulting from repetitive pressure at that specific anatomical site.
- **pH Inconsistency and Blood Diffusion Barriers:** The pH data exhibited substantial baseline instability compared to the highly resilient oxygen metrics. This instability is a direct consequence of the prolonged fabrication timeline compounded by complex fluid dynamics. The UV-excited pH signals drifted dramatically during Measurement 4.1, showing an artificial intensity incline from  $\sim 2319$  to  $\sim 5368$  counts due to the aforementioned UV matrix bleaching, an anomaly that was entirely eliminated in subsequent tests (Measurement 4.2) when a fresh sensor was introduced.

Conversely, the blue-light-excited pH channels remained stable, mirroring the reliable trends established during benchtop calibration. A pronounced analytical latency (hysteresis) was recorded upon initial tissue contact, where the blue-light intensities peaked sharply before slowly decaying to a stable baseline. This latency is partially driven by the rapid accumulation of blood on the

hydrogel surface. Although the blood perfusate functions as a continuous solution that theoretically reflects local tissue pH, its complex biomacromolecular composition presents severe optical and physical barriers. Haemoglobin and plasma proteins act as highly effective optical absorbers across both the UV and blue spectral regions. Furthermore, the high viscosity of whole blood, combined with the presence of multiple interfering cellular solutes, creates a dense physical diffusion barrier. This barrier establishes a temporal lag, delaying the diffusion of hydronium ions to the sensing matrix and lagging the optical detection of local pH shifts relative to the instantaneous fluctuations occurring within the free fluid.

#### 5.4.5. Strategic Paradigm Shifts and Future Horizons

To circumvent the analytical limitations imposed by blood-contact interference and sensor aging, future iterations of this platform should explore a fundamental paradigm shift in analyte target selection. While real-time pH monitoring remains an invaluable metabolic metric, transitioning the secondary sensing channel from pH to carbon dioxide ( $O_2 \rightarrow CO_2$ ) could yield vastly superior signal stability.

Physiologically, pH and  $CO_2$  are intrinsically coupled via the bicarbonate buffering system. However, from an analytical engineering perspective, a  $CO_2$  sensor relies on gas diffusion principles identical to those employed by the highly successful oxygen quenching channels.

Unlike ionic pH measurements, which are inherently vulnerable to the fluctuating ionic strength, protein fouling, and viscosity of whole blood, a gas-permeable sensing modality can be overcoated with an ion-impermeable, lipophilic membrane. This membrane completely seals out non-volatile blood components, haemoglobin absorbers, and viscous proteins while permitting the rapid, unhindered diffusion of gaseous molecules. Transitioning to a gas-permeable dual-parameter ( $O_2/CO_2$ ) ratiometric platform would fundamentally minimise the chemical signal interference and optical drift observed in this study, providing a highly robust framework for tracking organ viability in complex, haemo-rich clinical environments.

#### 5.4.6. Conclusions

The clinical translation data demonstrates that while the 450 nm blue excitation path provides consistent readings and clearly captures dynamic transient responses, the 405 nm UV path is highly prone to severe operational drift driven by sensor aging and photobleaching. The exponential rise in returning UV intensity marks a definitive transition from light absorption to active backscattering, fundamentally compromising the validity of UV-derived pH measurements over extended monitoring windows. Furthermore, while additional protective hydrogel layers successfully prevent luminophore leaching, their signal-attenuating properties must be strictly calibrated. Future designs must incorporate pre-compensated calibration curves that account for both degradation timelines and structural attenuation or prioritise blue-light-derived optical metrics to ensure robust, long-term analytical accuracy during extended organ perfusion.

### 5.5. Study Limitations

The findings and technical outcomes of this study must be contextualised within the logistical, temporal, and financial boundaries inherent to a Master's thesis research framework. These practical constraints significantly influenced both the engineering methodologies and the scope of the clinical validations.

#### 5.5.1. Logistical Constraints and Chemical Aging

The fabrication of the bi-functional sensor matrices was impacted by prolonged procurement and delivery lead times for the specialised luminescent reagents and precursor chemicals. This temporal bottleneck severely restricted the capacity to perform multiple, iterative fabrications of fresh sensor batches immediately prior to clinical testing. Consequently, logistical necessity dictated the reuse of aging precursor solutions during the *ex vivo* perfusion experiments. As analysed previously, this directly contributed to the accelerated photobleaching, signal saturation, and significant UV signal drift observed during prolonged tissue contact, limiting the ability to evaluate the sensor's true theoretical lifespan under optimal chemical conditions.

### 5.5.2. Cleanroom Fabrication and Shared Infrastructure

The physical development and refinement of the dielectric optical filters were fundamentally limited by the shared nature of the university's cleanroom infrastructure. Given unrestricted, exclusive access to the Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD) machinery, the ideal fabrication protocol would have incorporated a rigorous chamber-cleaning plasma cycle between every alternating material layer. This would have effectively eliminated the residual precursor cross-contamination that caused the spectral anomalies. However, a standard cleaning recipe requires approximately 1 hour and 15 minutes to execute. Applying this step between every layer of a 21- or 23-layer architecture would monopolise the shared equipment for multiple days, which is unfeasible in a multi-user institutional facility. Thus, the continuous "one-go" deposition method was a mandatory compromise, directly resulting in the observed 40 nm spectral blue-shifts and necessitating post-fabrication engineering workarounds like angular tuning.

### 5.5.3. Biological Specimen Scarcity and Statistical Sample Size

Finally, the *ex vivo* clinical validation was constrained by the availability of viable biological models. Because the primary institution does not house an internal biological laboratory equipped for maintaining living whole-organ tissues, the *ex vivo* trials relied entirely on external collaboration and scheduling with the Erasmus Medical Center (EMC).

Furthermore, the experimental protocol was dependent on human donor grafts that had been explicitly rejected for clinical transplantation yet remained physiologically viable for research. Donor organs are inherently scarce, making the specific subset of research-eligible rejected organs exceptionally rare. This severe supply limitation inherently restricted the statistical sample size of the perfused organs. Consequently, it limited the ability to conduct repeated, large-scale longitudinal trials that would be necessary to fully map the sensor's reproducibility and robustness across a diverse array of physiological and pathological organ profiles.

## 5.6. Future Directions

While this study successfully established the foundational viability of the dual-parameter optical sensing platform, the experimental limitations and observations pave the way for several critical avenues of future research and optimisation.

### 5.6.1. Optical Fabrication and Hardware Refinements

- **Eliminating the Spectral Blue-Shift:** Future optical filter fabrication must prioritise eliminating the 25–40 nm scaling error at a  $0^\circ$  angle of incidence. If dedicated cleanroom machine time permits, this can be achieved by integrating rigorous plasma cleaning regimes into the ICP-CVD recipe between every alternating material deposition to eliminate residual cross-contamination.
- **Architectural Complexity and Ripple Mitigation:** As demonstrated by the 23-layer prototype, increasing the layer count yields significantly sharper rejection bands. Future designs should confidently pursue higher layer stacks (incorporating the +15% thickness compensations) while ensuring the optical admittance of the filter stack matches the substrate to minimise the characteristic Fabry-Perot transmittance ripple effect.
- **Probe Miniaturisation and Flexibility:** To transition from open-surgery or *ex vivo* settings to minimally invasive clinical applications, the optoelectronic footprint must be miniaturised for endoscopic compatibility. Furthermore, transitioning the rigid sensor substrates to highly flexible, biocompatible polymers would allow the matrix to seamlessly conform to dynamically expanding tissues, such as ventilating lungs or beating hearts, without risking localised tissue trauma or sensor delamination.

### 5.6.2. Sensor Chemistry and Biomaterial Optimization

- **Non-Toxic Luminophore Alternatives:** Although the optimised 0.1 ml hydrogel overcoat successfully mitigated chemical leaching, the inherent cytotoxicity of ruthenium remains a regulatory and physiological hurdle. Future iterations should explore transitioning away from ruthenium toward inherently non-toxic, biocompatible oxygen-sensitive luminophores that exhibit equivalent photostability and quantum yields.

- **Shelf-Life and Reusability Quantification:** The severe photobleaching and UV signal drift observed during the clinical trials underscore the necessity of using freshly fabricated matrices. Comprehensive longitudinal studies must be conducted to establish a strict, quantifiable shelf-life for the prepared sensors. Additionally, the maximum number of times a sensor can be cleaned and reused before signal degradation compromises data integrity must be statistically defined.
- **Multiplexing Additional Metabolic Parameters:** Expanding the diagnostic capability of the platform is a primary objective. As discussed, transitioning from pH to  $p\text{CO}_2$  sensing via gas-permeable membranes would drastically reduce optical interference from whole blood. Future matrices should aim to multiplex  $\text{O}_2$ ,  $\text{CO}_2$ , and critical ischaemic biomarkers such as lactic acid into a single, cohesive sensing patch.

### 5.6.3. Clinical Translation and System Integration

- **Spatial Mapping via Multi-Probe Arrays:** Given the localised morphological variances observed across the liver capsule, future setups should utilise multiple synchronised probes simultaneously. Creating a distributed spatial mapping network across the organ would provide clinicians with a comprehensive, 3D physiological profile of the entire graft rather than an isolated reading.
- **Expansion to Diverse Organ Models:** The successful liver validation provides a strong rationale for adapting the platform to other highly metabolic donor organs, specifically focusing on *ex vivo* kidney, lung, and heart perfusion models where continuous real-time monitoring is equally critical.
- **Automated Closed-Loop Perfusion Systems:** The ultimate clinical trajectory for this technology is its integration into an automated, control-loop-based Normothermic Machine Perfusion (NMP) system. By feeding the real-time, multiplexed optical data directly into the NMP control unit, the perfusion machine could autonomously adjust flow rates, dynamically titrate oxygen supply, and inject buffering agents in real-time, ensuring optimal, continuously corrected graft preservation prior to transplantation.

# 6

## Conclusions

The primary objective of this research was to bridge the critical gap between foundational optical engineering and real-world clinical application, specifically addressing the limitations of global perfusate averaging during Normothermic Machine Perfusion (NMP). By designing, fabricating, and validating a miniaturised, non-invasive optical sensing platform, this study successfully demonstrated the viability of continuous, localised metabolic monitoring of transplantable organs directly at the cellular level. To achieve this, custom multi-cavity Fabry–Pérot (MCFP, 19-layer) and bandpass (21-layer) optical interference filters were deposited via Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD) using alternating dielectric layers of  $\text{Si}_3\text{N}_4$  and  $\text{SiO}_2$ . By exploiting the refractive index contrast between  $\text{Si}_3\text{N}_4$  ( $n \approx 1.92$ ) and  $\text{SiO}_2$  ( $n \approx 1.55$ ), these thin-film architectures incorporated resonant cavity spacers and quarter-wave reflectors engineered to yield defined spectral edge transitions and effective out-of-band rejection. This allowed the filters to effectively isolate weak fluorescent emission signals at the target 520 nm (pH) and 600 nm ( $\text{O}_2$ ) emission peaks from high-intensity excitation light without relying on bulky benchtop optics. Concurrently, a novel bi-functional hydrogel sensor was synthesised to track oxygen and pH with rapid transient kinetics and strict signal independence. The application of a rigorously calibrated 0.1 ml hydrogel overcoat successfully locked the ruthenium complexes in place, preventing cytotoxic leaching and ensuring safe, direct tissue contact. The platform's efficacy was decisively validated across two ex vivo NMP models, proving its capability to track mechanically driven transient shifts on a defrosted organ and dynamically map the metabolic baseline of a viable, haemo-rich human liver.

While the foundational device demonstrates strong proof-of-concept capabilities, transitioning from a controlled laboratory environment to an ex vivo clinical setting highlighted specific operational boundaries. Shared cleanroom logistics necessitated a continuous deposition process that introduced a 25 to 40 nm spectral blue-shift in the optical filters, and the structural necessity of the robust hydrogel overcoat attenuated the baseline fluorescent intensities by approximately 3000 counts. Furthermore, prolonged tissue contact revealed that the 405 nm UV excitation path is highly susceptible to severe photobleaching. The complex biomacromolecular nature of whole blood also acted as a dense physical diffusion barrier, establishing a temporal lag in localised pH detection.

These practical realities provide a definitive roadmap for the next generation of optical transplant monitors. To circumvent the analytical limitations imposed by blood-contact interference, future iterations should explore transitioning the secondary sensing channel from pH to carbon dioxide ( $\text{CO}_2$ ). Utilising a gas-permeable, ion-impermeable lipophilic membrane would seal out viscous blood proteins and haemoglobin absorbers, drastically minimising optical drift. Furthermore, future hardware must prioritise the highly stable 450 nm blue excitation path to ensure longitudinal accuracy. As fabrication processes are refined, the mechanical footprint of the device can be further miniaturised onto flexible, biocompatible polymers to form distributed spatial multi-probe arrays. Integrating this real-time, high-resolution data directly into automated, closed-loop perfusion circuits will pave the way for dynamic, autonomous organ resuscitation, ultimately reducing discard rates and improving clinical outcomes in solid organ transplantation.

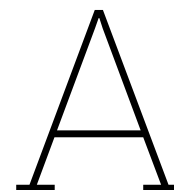
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# Characteristics of Common Luminophores and Encapsulation Matrices

**Table A.1:** Overview of indicator material properties in different encapsulation matrices. Adapted from [15, 14]

| Indicator                                    | Encapsulation Matrix | Unquenched Lifetime ( $\mu\text{s}$ )                   | Quantum Yield                                                            | Reported Sensitivity                                                                                          | Excitation Peaks (nm) | Emission Peaks (nm) |
|----------------------------------------------|----------------------|---------------------------------------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-----------------------|---------------------|
| $([\text{Ru}(\text{dpp})_3])^{2+}$           | Polystyrene          | 5                                                       | Not Reported                                                             | 22% signal decrease from $\text{N}_2$ to air                                                                  | 450                   | 600                 |
| $([\text{Ru}(\text{dpp})_3])^{2+}$           | Plasticized PVC      | 5                                                       | Not Reported                                                             | 50% signal decrease from $\text{N}_2$ to air                                                                  | 450                   | 600                 |
| $([\text{Ru}(\text{dpp})_3])^{2+}$           | None                 | 6.3 at 23 °C (silicone-soluble ion pair in 2-butanone)  | 0.3 (in water/ethanol)<br>0.35 (silicone-soluble ion pair in 2-butanone) | $k_Q(\text{dissolved O}_2) = 2.5$<br>( $10^9 \text{ dm}^{-3} \text{ mol}^{-1} \text{ s}^{-1}$ ) (in methanol) | 460                   | 613, 627            |
| $([\text{Ru}(\text{phen})_3])^{2+}$          | None                 | 0.74 at 23 °C (silicone-soluble ion pair in 2-butanone) | 0.08 (silicone-soluble ion pair in 2-butanone)                           | $k_Q(\text{dissolved O}_2) = 4.2$<br>( $10^9 \text{ dm}^{-3} \text{ mol}^{-1} \text{ s}^{-1}$ )               | 447, 421              | 605, 625            |
| $[\text{Ru}(\text{Ph}_2\text{phen})_3]^{2+}$ | Sol-gel silica       | 5.8                                                     | Not Reported                                                             | $\tau_{\text{N}_2}/\tau_{\text{O}_2} = 5$                                                                     | Not Reported          | Not Reported        |
| $([\text{Ru}(\text{bpy})_3])^{2+}$           | None                 | 0.6                                                     | 0.042                                                                    | $k_Q(\text{dissolved O}_2) = 3.3$<br>( $10^9 \text{ dm}^{-3} \text{ mol}^{-1} \text{ s}^{-1}$ )               | 423, 452              | 613, 627            |
| $([\text{Ru}(\text{bpy})_3])^{2+}$           | Sol-gel silica       | 1.26                                                    | Not Reported                                                             | $\tau_{\text{N}_2}/\tau_{\text{O}_2} = 2$                                                                     | Not Reported          | Not Reported        |

Table A.1: Continued

| Indicator                                                                   | Encapsulation Matrix                     | Unquenched Lifetime ( $\mu$ s) | Quantum Yield | Reported Sensitivity                         | Excitation Peaks (nm) | Emission Peaks (nm) |
|-----------------------------------------------------------------------------|------------------------------------------|--------------------------------|---------------|----------------------------------------------|-----------------------|---------------------|
| PtOEPK                                                                      | Polystyrene                              | 61.4 at 22 °C                  | 0.12          | High                                         | 398, 592              | 759                 |
| PtOEPK                                                                      | PDMA                                     | Not Reported                   | Not Reported  | $Q_{DO} = 97.5\%$                            | Not Reported          | 754                 |
| PdOEPK                                                                      | Polystyrene                              | 480 at 22 °C                   | 0.01          | Very high                                    | 410, 602              | 790                 |
| PtOEP                                                                       | Polystyrene                              | 94.7 at 20 °C                  | Not Reported  | $\tau_0/\tau_{air} = 3.60$                   | 383, 535              | 647                 |
| Pd-coproporphyrin                                                           | None (aqueous solution)                  | 530 (no BSA), 1200 (BSA)       | 0.2           | $k_Q = 195 \text{ mmHg}^{-1} \text{ s}^{-1}$ | 393, 545              | 667                 |
| Pt-coproporphyrin                                                           | None (aqueous solution)                  | 100                            | 0.4           | Not Reported                                 | 380, 535              | 650                 |
| Pd-meso-tetra-(4-carboxy-phenyl) tetrabenzoporphyrin dendrimer (Oxyphor G2) | None (BSA solution at pH 6.8, 23.5 °C)   | 276                            | 0.12          | $k_Q = 195 \text{ mmHg}^{-1} \text{ s}^{-1}$ | 442, 632              | 800                 |
| Pd-meso-tetra-(4-carboxyphenyl) porphyrin dendrimer (Oxyphor R2)            | None (BSA solution at pH 6.8, 23.5 °C)   | 738                            | 0.1           | $k_Q = 270 \text{ mmHg}^{-1} \text{ s}^{-1}$ | 415, 524              | 700                 |
| Pd-meso-tetra-(4-Carboxyphenyl) Porphine (Oxyphor R0)                       | None (albumin solution at pH 6.8, 23 °C) | 705                            | 0.06          | $k_Q = 246 \text{ mmHg}^{-1} \text{ s}^{-1}$ | 416, 523              | 687                 |
| PdTPCPP                                                                     | None (water)                             | ~800                           | ~0.1          | Not Reported                                 | 415, 524              | 690                 |
| PdTCPTBP                                                                    | None (water)                             | 240                            | 0.12          | Not Reported                                 | 442, 632              | 790                 |
| PtCP                                                                        | None (water)                             | ~60                            | 0.28          | Not Reported                                 | 380, 535              | 650                 |
| PtTBP                                                                       | None (DMF)                               | 50                             | 0.50          | Not Reported                                 | 416, 609              | 745                 |
| PtCPK                                                                       | None (water)                             | 24                             | 0.01          | Not Reported                                 | 400, 596              | 767                 |
| Ir(III)(C <sub>x</sub> ) <sub>2</sub> (acac)                                | None (CHCl <sub>3</sub> )                | 11.3                           | 0.54          | Not Reported                                 | 472, 444              | 563                 |
| ([Ru(bpy) <sub>2</sub> (picH <sub>2</sub> )] <sup>2+</sup>                  | None (water)                             | 0.7–0.9                        | 0.07          | Not Reported                                 | 460                   | 607                 |

# Experimental Protocols: Thin-Film Filter Fabrication

This appendix presents a step-by-step protocol for the calibration, setup, and automated execution of thin-film optical interference filters using Inductively Coupled Plasma Chemical Vapour Deposition (ICP-CVD).

## Equipment, Substrates, and Processing Parameters

**Table B.1:** Substrate specifications, cleanroom equipment, and ICP-CVD processing parameters.

| Parameter / Facility         | Specification / Operating Condition                                                                             |
|------------------------------|-----------------------------------------------------------------------------------------------------------------|
| <b>Cleanroom Facility</b>    | Else Kooi Laboratory (EKL) Cleanroom 100                                                                        |
| <b>Deposition Tool</b>       | PlasmaPro 100 ICP-CVD System (Oxford Instruments)                                                               |
| <b>Characterisation Tool</b> | M-2000 Spectroscopic Ellipsometer (J.A. Woollam)                                                                |
| <b>Primary Substrate</b>     | 1-inch, 500 $\mu\text{m}$ thick, Double-Side Polished (DSP) Fused Silica JGS2 Glass Wafer (MicroChemicals GmbH) |
| <b>Carrier Substrate</b>     | Standard Silicon (Si) carrier wafer (for load-lock optical sensing)                                             |
| <b>Substrate Temperature</b> | 300 °C (controlled via table heater)                                                                            |

**Table B.2:** ICP-CVD material deposition recipes and baseline optical properties.

| Process Parameter                                | Silicon Dioxide ( $\text{SiO}_2$ ) | Silicon Nitride ( $\text{Si}_3\text{N}_4$ ) |
|--------------------------------------------------|------------------------------------|---------------------------------------------|
| <b>Silane (<math>\text{SiH}_4</math>) Flow</b>   | 26.0 sccm                          | 26.0 sccm                                   |
| <b>Oxygen (<math>\text{O}_2</math>) Flow</b>     | 32.0 sccm                          | —                                           |
| <b>Nitrogen (<math>\text{N}_2</math>) Flow</b>   | —                                  | 27.0 sccm                                   |
| <b>Chamber Pressure</b>                          | 2.5 mTorr                          | 2.0 mTorr                                   |
| <b>ICP RF Power Demand</b>                       | 1500 W                             | 750 W                                       |
| <b>Typical Refractive Index (<math>n</math>)</b> | $\approx 1.55$                     | $\approx 1.92$                              |

## Step-by-Step Fabrication Workflow

### Step 1: Chamber Decontamination and Preconditioning

1. Load a dedicated cleaning dummy wafer into the ICP-CVD chamber.
2. Run the standard "**short plasma clean**" recipe on the empty ICP-CVD chamber to eliminate residual film contaminants.
3. Load a dedicated Silicon dummy wafer into the chamber.

4. Execute a **3-minute preconditioning deposition** using the dielectric material *opposite* to the first layer of the intended filter (e.g., precondition with  $\text{SiO}_2$  if the filter begins with  $\text{Si}_3\text{N}_4$ ). This coats the chamber walls to replicate steady-state plasma conditions.

## Step 2: Deposition Rate Calibration

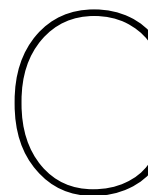
1. Load a fresh, clean Silicon calibration wafer.
2. Deposit a single test layer of  $\text{SiO}_2$  for exactly **60 seconds** using the parameters in Table B.2.
3. Repeat the process on a second fresh Silicon wafer for  $\text{Si}_3\text{N}_4$  for **60 seconds**.
4. Immediately measure both test wafers on the J.A. Woollam M-2000 Spectroscopic Ellipsometer to extract the exact empirical deposition rate (nm/min) and refractive index ( $n$ ).
5. Update the phase durations in the custom automated machine recipes based on the day's measured deposition rates to achieve the target layer thicknesses:
  - **Bandpass Filter (600 nm  $\text{O}_2$  Target):** Custom Recipe "*Notch Filter SiO<sub>2</sub>-SiNx 300C*" (21-layer stack).
  - **Multi-Cavity Fabry–Pérot Filter (520 nm pH Target):** Custom Recipe "*SiO<sub>2</sub>-SiNx Filter 300c MC FP Filter*" (19-layer stack).

## Step 3: Substrate Mounting and Carrier Preparation

1. Retrieve the 1-inch DSP fused silica JGS2 substrate directly from the supplier packaging inside Cleanroom 100. *Note: Intentionally bypass wet bench chemical cleaning to avoid solvent residue or particulate contamination.*
2. Mount the 1-inch glass substrate centrally onto a standard 4-inch Silicon carrier wafer.
3. Confirm that the silicon backing provides complete opacity beneath the glass wafer to ensure proper detection by the ICP-CVD load-lock optical sensors.

## Step 4: Continuous Multilayer Stack Deposition

1. Load the prepared carrier wafer containing the 1-inch fused silica substrate into the ICP-CVD load-lock chamber.
2. Transfer the wafer assembly into the main reaction chamber (300 °C).
3. Initiate the corresponding custom automated recipe. The ICP-CVD system will alternate sequentially between  $\text{SiO}_2$  and  $\text{Si}_3\text{N}_4$  steps without breaking vacuum or performing inter-layer cleans.
4. Upon sequence completion, unload the completed thin-film filter.
5. Transfer the finished filter directly into a sealed cleanroom container for storage prior to optical transmission characterisation on the spectrometer setup in the optics lab LB 02.430.



# Experimental Protocols: Sensor Fabrication

This appendix provides a step-by-step protocol for synthesising and depositing the bi-functional pH and oxygen ( $O_2$ ) hydrogel sensor matrix, including exact chemical specifications and catalogue references.

## Reagents and Specifications

**Table C.1:** Chemical reagents and polymer specifications used in sensor fabrication.

| Component            | Chemical Name / Description                                                                           | Supplier                                       | Catalogue No.   |
|----------------------|-------------------------------------------------------------------------------------------------------|------------------------------------------------|-----------------|
| pH Indicator         | 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS / Pyranine, $\geq 97\%$ , CAS: 6358-69-6) | Sigma-Aldrich                                  | H1529           |
| $O_2$ Indicator      | Dichlorotris(1,10-phenanthroline)ruthenium(II) hydrate (98%, CAS: 207802-45-7)                        | Sigma-Aldrich                                  | 343714          |
| Anion Exchange Beads | AmberChrom 1 $\times$ 8 resin, 200–400 mesh, $Cl^-$ form                                              | Thermo Scientific / Fisher Scientific          | AC472971000     |
| Hydrogel Matrix      | HydroMed D4 (Ether-based aliphatic polyether polyurethane hydrogel resin)                             | AdvanSource Biomaterials / Mitsubishi Chemical | ASB P/N: 100028 |
| Solvents             | Ethanol (90% v/v), Methanol, Toluene, Demineralised (DM) Water                                        | Standard Grade                                 | Cleanroom —     |

## Step-by-Step Fabrication Workflow

### Phase 1: Preparation of Fluorophore Precursors

#### Step 1: Synthesis of HPTS-Functionalised Microbeads (pH Element)

1. Dissolve **35 mg** of 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (*Sigma-Aldrich, Cat. H1529*) into **100 mL** of DM water.
2. Separately suspend **10 g** of AmberChrom 1 $\times$ 8 resin beads (*Fisher Scientific, Cat. AC472971000*) in **10 mL** of DM water.
3. Add the microbead suspension to the HPTS solution.

4. Stir the mixture for **15 hours at 400 rpm** at room temperature (20–25°C) to allow full functionalisation.
5. Isolate the HPTS-loaded microbeads via filtration and retain for matrix compounding.

### Step 2: Synthesis of "Ru40" Solution ( $O_2$ Element)

1. Prepare a **4:1 (v/v)** solvent mixture of methanol and toluene.
2. Dissolve **200 mg** of dichlorotris(1,10-phenanthroline)ruthenium(II) hydrate (*Sigma-Aldrich, Cat. 343714*) into **5 mL** of the methanol/toluene mixture.
3. Stir for **1 hour at 100 rpm** at room temperature until fully dissolved, yielding a **40 mg/mL** ("Ru40") precursor solution.

## Phase 2: Formulation of the Hydrogel Matrix

### Step 3: Base Hydrogel Precursor Solution

1. Dissolve **1.0 g** of HydroMed D4 resin (*AdvanSource Biomaterials, ASB P/N: 100028*) into **10 mL** of 90% ethanol (in DM water).
2. Stir continuously for **6 hours** at room temperature until a uniform, transparent solution is obtained.

### Step 4: Bifunctional Sensing Composite ( $HPTS_{88}Ru$ Matrix)

1. Introduce **3.5 g** of the HPTS-functionalised AmberChrom microbeads (from Step 1) into the **10 mL** base HydroMed D4 solution (from Step 3).
2. Add **1.0 mL** of the Ru40 solution (from Step 2) into the composite mixture, maintaining a strict **1:88** ruthenium-to-HPTS weight ratio.
3. Stir the mixture for **6 hours** at room temperature to achieve a homogeneous dispersion of both indicator species.

## Phase 3: Substrate Deposition and Protective Overcoating

### Step 5: Drop-Casting and Encapsulation

1. Clean glass slide substrates thoroughly using ethanol and allow to dry completely.
2. Drop-cast **0.2 mL** of the fully dispersed sensing hydrogel composite (from Step 4) onto the designated target area of the glass slide.
3. Allow the active sensing layer to dry completely at room temperature.
4. Apply a protective overcoat by drop-casting **0.1 mL** of pure, dye-free HydroMed D4 solution (from Step 3) directly over the dried sensing patch.
5. Air-dry at room temperature until fully cured. This top layer seals the active luminophores within the hydrogel network, preventing chemical leaching and enhancing physical durability during direct organ contact.

# D

## System Control MATLAB Code

### Code Attribution and Provenance:

The MATLAB control software presented in this appendix was developed by **Robbert Friendwijk, MSc.** Further details regarding the design, architecture, and original implementation of this codebase can be found in the TU Delft Repository [61] at <http://repository.tudelft.nl/>.

### D.1. Direct Spectrometer Control (`directSpectrometer.m`)

Listing D.1: Direct spectrometer interfacing and control routine (`directSpectrometer.m`).

```
1 % close all;
2 sensor = '_6';
3 ph_value = '6_2';
4 %%Connect to spectrometer and LED controller
5 port = 'COM6';
6 s = serialport(port,115200);
7 flame = icdevice('OceanOptics_OmniDriver.mdd');
8 connect(flame);
9
10
11 %% initialize spectrum parameters
12 int_time = 3000; %integration time in us normal 250
13 scans = 30; %scans to average
14 enable = 1; % enable signal
15 spectrometerIndex = 0; % Spectrometer index to use (first spectrometer by
    default).
16 channelIndex = 0; % Channel index to use (first channel by default).
17
18 %% Set the parameters
19 invoke(flame, 'setIntegrationTime', spectrometerIndex, channelIndex,
    int_time);
20 invoke(flame, 'setCorrectForDetectorNonlinearity', spectrometerIndex,
    channelIndex, enable);
21 invoke(flame, 'setCorrectForElectricalDark', spectrometerIndex,
    channelIndex, enable);
22 invoke(flame, 'setScansToAverage', spectrometerIndex, channelIndex, scans)
    ;
23 writeline(s,'o');
24
```

```

25
26 %%
27 [wavelength, backgroundIntensity] = getSpectrumBlue(s,flame,'o');
28 [wavelengthblue, intensityblue] = getSpectrumBlue(s,flame,'b');
29 [wavelengthUV, intensityUV] = getSpectrumBlue(s,flame,'u');
30 writeline(s,'o');
31
32 outputArrayBlue = [wavelengthblue (intensityblue-backgroundIntensity)];
33 outputArrayUv = [wavelengthUV (intensityUV-backgroundIntensity)];
34
35 writematrix(outputArrayBlue, strcat(ph_value,sensor,'_blue'));
36 writematrix(outputArrayUv, strcat(ph_value,sensor,'_uv'));
37 % writematrix(outputArrayBlue, strcat(ph_value,'_blue'));
38 % writematrix(outputArrayUv, strcat(ph_value,'_uv'));
39
40 %% Plot Data
41 plot(outputArrayBlue(:,1),outputArrayBlue(:,2));
42 hold on;
43 plot(outputArrayUv(:,1),outputArrayUv(:,2));
44
45 %% Disconnect from spectrometer and LED controller
46
47 clear s;
48 disconnect(flame);
49 delete (flame);
50 delete(instrfindall);

```

## D.2. Wavelength Identification (findWavelength.m)

Listing D.2: Wavelength identification algorithm (findWavelength.m).

```

1 function mag = findWavelength(wavelength,spectrum)
2     index = find(round(spectrum(:,1)) == wavelength);
3     final_index= round(mean(index));
4     mag = spectrum(final_index,2);
5 end

```

## D.3. Maximum Wavelength Peak Detection (findWavelength\_max.m)

Listing D.3: Maximum wavelength peak detection algorithm (findWavelength\_max.m).

```

1 function mag = findWavelength_max(wavelength,spectrum)
2 if(wavelength == 520)
3     index1 = find(round(spectrum(:,1)) == 510);
4     final_index1= round(mean(index1));
5     index1 = find(round(spectrum(:,1)) == 530);
6     final_index2= round(mean(index1));
7     mag = max(spectrum(final_index1:final_index2,2));
8
9 elseif(wavelength == 445)
10     index1 = find(round(spectrum(:,1)) == 420);
11     final_index1= round(mean(index1));
12     index1 = find(round(spectrum(:,1)) == 455);
13     final_index2= round(mean(index1));
14     mag = max(spectrum(final_index1:final_index2,2));
15 end
16 end

```

## D.4. Blue Spectrum Acquisition (getSpectrumBlue.m)

**Listing D.4:** Blue excitation spectrum acquisition routine (getSpectrumBlue.m).

```

1 function [wavelengths,spectralData] = getSpectrumBlue(esp,specObj,led)
2 fprintf(esp,led);
3 while esp.BytesAvailable == 0
4     % Wait for response
5 end
6 response = fscanf(esp);
7 display(response);
8
9 pause(0.1);
10 wavelengths = invoke(specObj, 'getWavelengths', 0, 0);
11 spectralData = invoke(specObj, 'getSpectrum', 0);
12 end

```

## D.5. Reference Spectrum Visualisation (refPlot.m)

**Listing D.5:** Reference spectrum visualisation script for plotting intensity over wavelength (refPlot.m).

```

1
2 % folder = "422_2";
3 % folder = "417_wholeSensUP";
4 folder = "516_1";
5 % folder = "422";
6
7
8 fileNameblue = '*blue*';
9 fileNameUv = '*uv*';
10 wavelength = 520;
11 wavelength_ref = 405;
12 wavelength_blue = 445;
13 isobestic_blue = 502;
14 isobestic_uv = 499;
15 blue = dir(fullfile(folder,fileNameblue));
16 uv = dir(fullfile(folder,fileNameUv));
17 ratioblue = zeros(1,length(uv));
18 peaksblue = zeros(1,length(uv));
19 peaksblue_450 = zeros(1,length(uv));
20 peaksUV = zeros(1,length(uv));
21 peaksUV_450 = zeros(1,length(uv));
22 peaksblue_iso = zeros(1,length(blue));
23 peaksUV_iso = zeros(1,length(uv));
24 pH_values = [6.2 6.6 7 7.4 7.8 8.2];
25
26 for i = 1:length(ratioblue)
27     peaksblue(i) = findWavelength_max(wavelength,table2array(readtable(
28         fullfile(folder,blue(i).name)))); %important
29     peaksUV(i) = findWavelength_max(wavelength,table2array(readtable(
30         fullfile(folder,uv(i).name))));
31     peaksUV_450(i) = findWavelength_max(wavelength_blue,table2array(
32         readtable(fullfile(folder,uv(i).name)))); %important
33     peaksblue_450(i) = findWavelength(wavelength_blue,table2array(
34         readtable(fullfile(folder,blue(i).name))));
35     peaksblue_iso(i) = findWavelength(isobestic_blue,table2array(readtable(
36         fullfile(folder,blue(i).name))));

```

```

32     peaksUV_iso(i) = findWavelength(isobestic_uv,table2array(readtable(
        fullfile(folder,blue(i).name))));
33 end
34 ratio= peaksblue./peaksUV_450;
35
36 % ratioblue = peaksblue./peaksblue_iso;
37 % ratioUV = peaksblue./peaksUV_iso;
38 % [f, gof] = fit(pH_values(:), ratioblue(:), 'gompertz');
39 % plot(f, pH_values, ratioblue);
40 plot(pH_values,ratio, 'LineWidth',1 );
41 hold on;
42 % plot(pH_values,ratioblue, 'LineWidth',1 );
43 xlabel("pH");
44 ylabel("Measured intensity ratio");

```

## D.6. Response Time Measurement (Response\_time\_measurement\_script.m)

Listing D.6: Sensor response time analysis script (Response\_time\_measurement\_script.m).

```

1 %% Close all figures
2 close all;
3
4 %% Sensor and measurement parameters
5 sensor      = '_1';
6 ph_value    = '8_2';
7
8 %% Connect to spectrometer and LED controller
9 port = 'COM21';
10 s = serialport(port, 115200);
11
12 flame = icdevice('OceanOptics_OmniDriver.mdd');
13 connect(flame);
14
15 %% Initialize spectrum parameters
16 int_time      = 1000; % Integration time in µs (default: 250 µs)
17 scans         = 200; % Number of scans to average
18 enable        = 1;   % Enable signal
19 spectrometerIndex = 0; % Spectrometer index (0 = first spectrometer)
20 channelIndex   = 0; % Channel index (0 = first channel)
21
22 %% Set spectrometer parameters
23 invoke(flame, 'setIntegrationTime', spectrometerIndex,
    channelIndex, int_time);
24 invoke(flame, 'setCorrectForDetectorNonlinearity', spectrometerIndex,
    channelIndex, enable);
25 invoke(flame, 'setCorrectForElectricalDark', spectrometerIndex,
    channelIndex, enable);
26 invoke(flame, 'setScansToAverage', spectrometerIndex,
    channelIndex, scans);
27
28 %% Initialize communication with LED controller
29 writeline(s, 'o');
30
31 %% Experiment parameters
32 N = 400; % Number of iterations

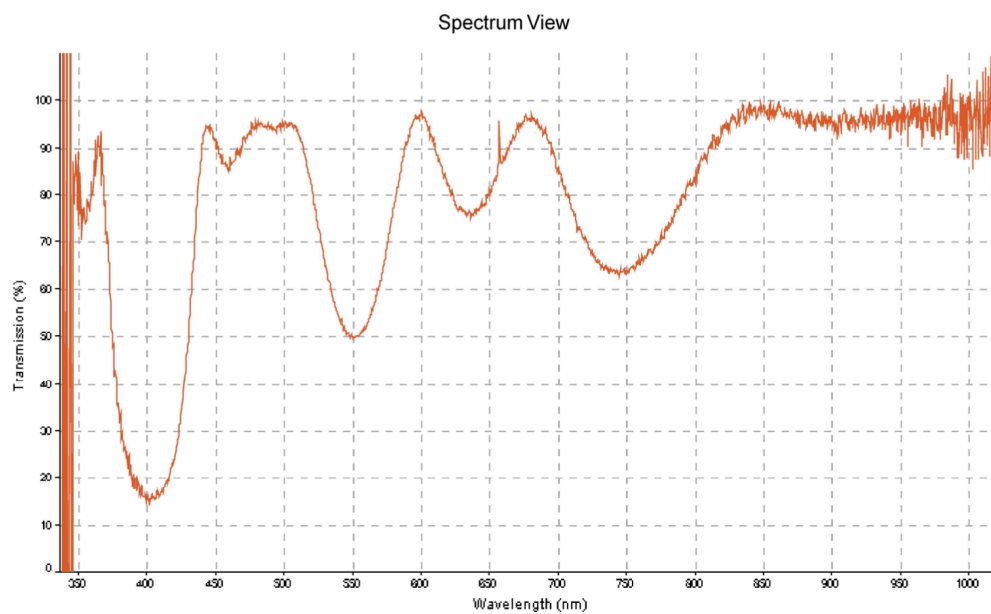
```

```
33 wavelength_blue = 520;    % Reference wavelength (blue)
34 wavelength_UV   = 435;    % Reference wavelength (UV)
35
36 % Preallocate arrays
37 peaks_blue      = zeros(1, N);
38 peaks_UV_450    = zeros(1, N);
39 ratio           = zeros(1, N);
40
41 %% Main loop
42 Tstart = tic;
43 for i = 1:N
44
45     % Acquire background, blue, and UV spectra
46     [wavelength, backgroundIntensity] = getSpectrumBlue(s, flame, 'o');
47     [wavelengthBlue, intensityBlue]   = getSpectrumBlue(s, flame, 'b');
48     [wavelengthUV, intensityUV]       = getSpectrumBlue(s, flame, 'u');
49
50     % Reset LED controller
51     writeline(s, 'o');
52
53     % Subtract background and build output arrays
54     outputArrayBlue = [wavelengthBlue, (intensityBlue -
55         backgroundIntensity)];
56     outputArrayUV   = [wavelengthUV, (intensityUV - backgroundIntensity)
57         ];
58
59     % Find peaks
60     peaks_blue(i) = findWavelength(wavelength_blue, outputArrayBlue);
61     peaks_UV_450(i) = findWavelength(wavelength_UV, outputArrayUV);
62
63     % Compute ratio
64     ratio(i) = peaks_blue(i) ./ peaks_UV_450(i);
65
66     % Optional: save output (uncomment as needed)
67     % writematrix(outputArrayBlue, strcat(ph_value, sensor, '_blue'));
68     % writematrix(outputArrayUV,   strcat(ph_value, sensor, '_uv'));
69
70     % Pause briefly between acquisitions
71     pause(1);
72 end
73 Tstop = toc(Tstart);
74
75 %% Disconnect from spectrometer and LED controller
76 clear s;
77 disconnect(flame);
78 delete(flame);
79 delete(instrfindall);
```

# Characterization of the +10% Cavity Modified MCFP Filter

**Table E.1:** Layer Stack Configuration for the 19-Layer MCFP Filter with +10% Cavity Thickness

| Layer                             | Material                       | Refractive Index ( $n$ ) | Thickness (nm) |
|-----------------------------------|--------------------------------|--------------------------|----------------|
| <b>Medium</b>                     | Air                            | 1.00                     | -              |
| 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 | SiO <sub>2</sub>               | 1.55                     | 60 (each)      |
| 2, 6, 8, 12, 14, 18               | Si <sub>3</sub> N <sub>4</sub> | 1.92                     | 60 (each)      |
| 4, 10, 16 (Cavities)              | Si <sub>3</sub> N <sub>4</sub> | 1.92                     | 187 (each)     |
| <b>Substrate</b>                  | SiO <sub>2</sub>               | 1.55                     | -              |
| <b>Total Sum</b>                  |                                |                          | <b>1617 nm</b> |

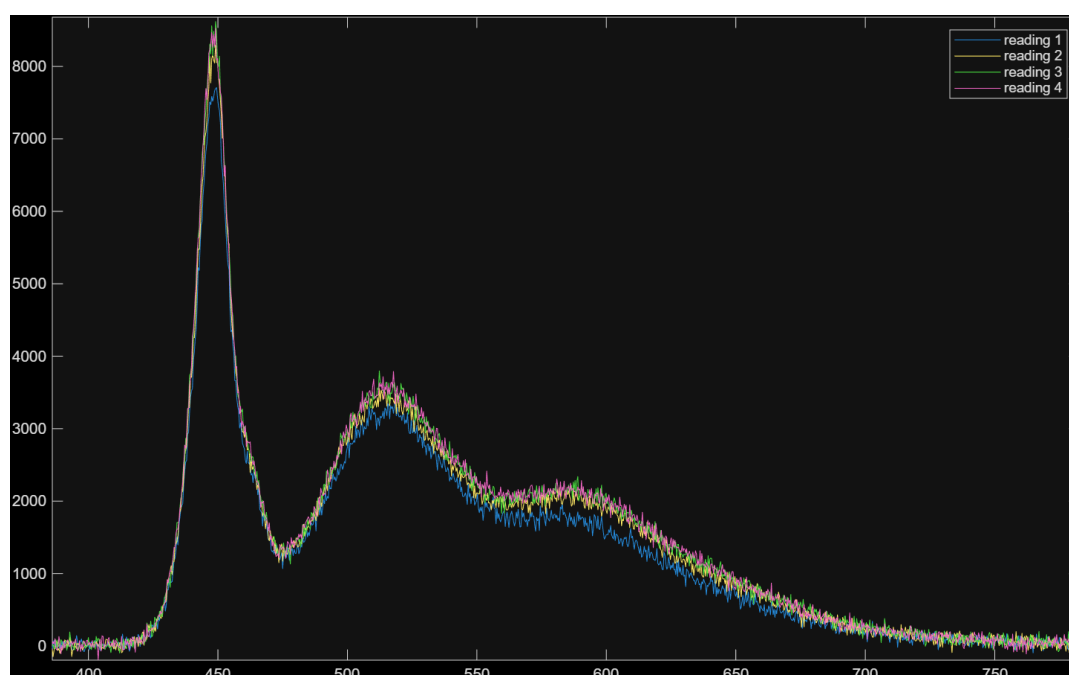


**Figure E.1:** Empirical transmittance spectrum of the fabricated 19-layer multi-cavity Fabry-Perot filter featuring a +10% increase in resonance cavity thickness.

# F

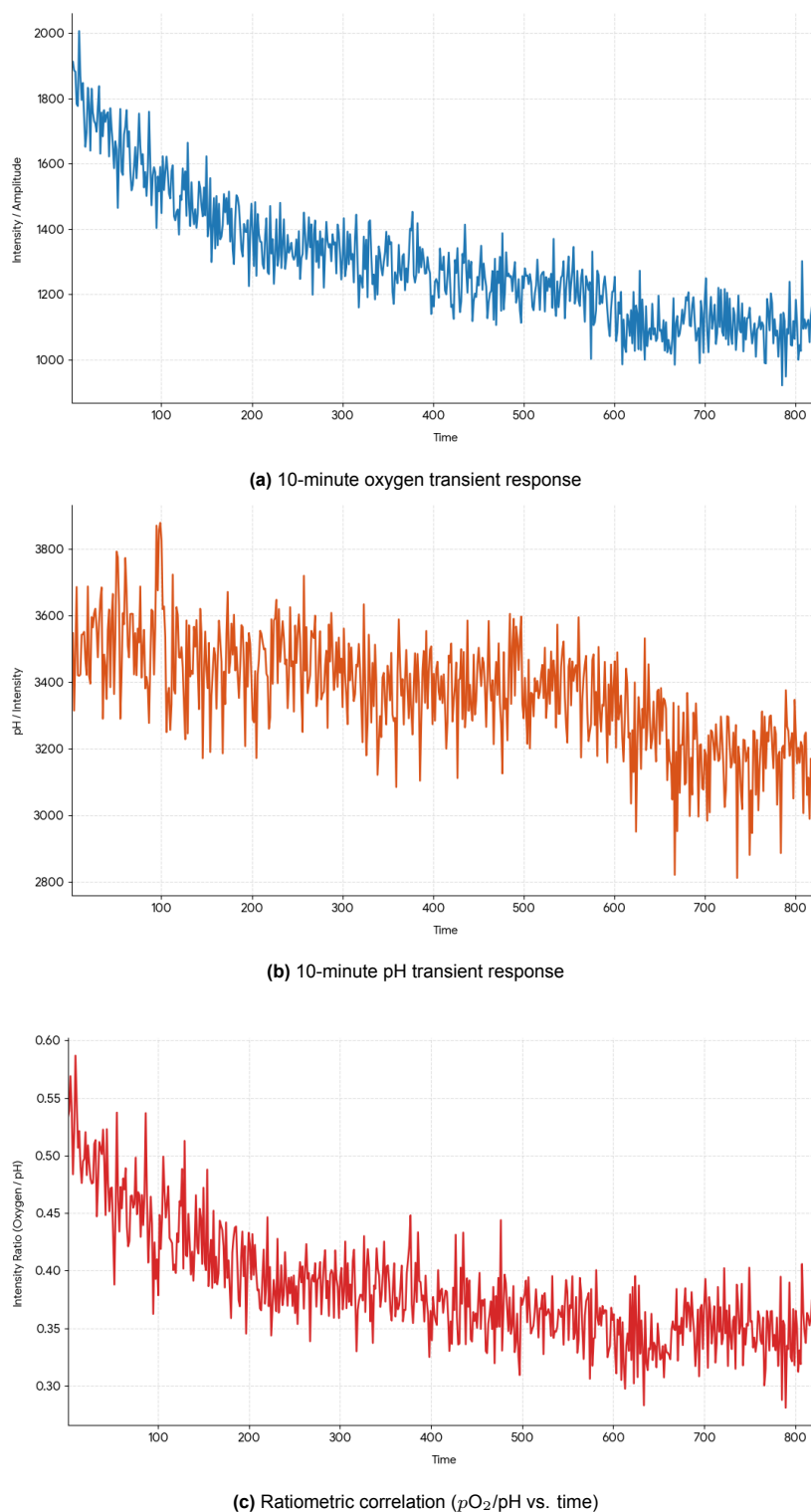
## Supplementary Clinical Data – Experiment 1

### F.1. Spectral Intensity Measurements



**Figure F.1:** Spectral distribution of light intensity across the visible wavelength range. This graph displays four distinct readings acquired from a single, static spatial location on the defrosted liver model. The 405 nm emission peak is distinctly absent, as the ultraviolet excitation source was not utilised during this specific experimental phase, resulting in no associated baseline fluorescence for that spectrum.

## F.2. Unrefined Transient Responses and Ratiometric Analysis



**Figure F.2:** Unrefined longitudinal tracking data acquired during the 10-minute transient response test on the defrosted liver model. **(a)** The isolated transient response reflecting local oxygen variations. **(b)** The corresponding transient response mapping local tissue pH fluctuations. **(c)** The calculated ratiometric trend, plotted as the ratio of the oxygen response to the pH response over the identical 10-minute testing window.

# G

## Supplementary Clinical Data – Experiment 2

### G.1. Measurement 1: Sensor 1 Raw Data and Clinical Reference

#### G.1.1. Clinical Perfusate Analysis (EMC)

| EMC Measurements                    |                                    |        |        |
|-------------------------------------|------------------------------------|--------|--------|
| Blood gas values                    |                                    |        |        |
| pH                                  | pH                                 | 7.560  | —      |
| pCO <sub>2</sub>                    | Partial pressure of carbon dioxide | 3.39   | kPa    |
| pO <sub>2</sub>                     | Partial pressure of oxygen         | 22.5   | kPa    |
| cBase(B)c                           | Base excess                        | 0.7    | mmol/L |
| cHCO <sub>3</sub> <sup>-</sup> (P)c | Bicarbonate concentration          | 22.8   | mmol/L |
| Oximetry values                     |                                    |        |        |
| ctHb                                | Total hemoglobin concentration     | 4.3    | mmol/L |
| Hctc                                | Hematocrit                         | 0.210  | —      |
| sO <sub>2</sub>                     | Oxygen saturation                  | 100.3% | %      |
| Electrolyte values                  |                                    |        |        |
| cK <sup>+</sup>                     | Potassium                          | 4      | mmol/L |
| cNa <sup>+</sup>                    | Sodium                             | 156    | mmol/L |
| cCa <sup>2+</sup>                   | Ionized calcium                    | 0.72   | mmol/L |
| cCl <sup>-</sup>                    | Chloride                           | 97     | mmol/L |
| Metabolite values                   |                                    |        |        |
| cGlu                                | Glucose                            | 10.6   | mmol/L |
| cLac                                | Lactate                            | 1.9    | mmol/L |

**Figure G.1:** Gold-standard clinical perfusate measurements extracted via the ABL90 FLEX analyser corresponding to Measurement 1.

## G.1.2. Optical Sensor Data Logs

| Values at 520 nm and 600 nm |                   |                |                     |                |
|-----------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #   | UV light (405 nm) |                | Blue light (450 nm) |                |
|                             | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                           | 3343.570          | 1078.110       | 4273.840            | 945.082        |
| 2                           | 3487.540          | 879.565        | 3729.820            | 680.756        |
| 3                           | 2956.090          | 701.544        | 3752.830            | 619.518        |
| 4                           | 3043.630          | 656.513        | 3497.330            | 580.302        |
| 5                           | 2949.750          | 675.850        | 4209.390            | 675.369        |
| 6                           | 3310.800          | 674.826        | 3593.940            | 471.258        |
| 7                           | 3399.760          | 790.499        | 3621.720            | 648.550        |
| 8                           | 3217.430          | 811.334        | 3955.500            | 774.405        |
| 9                           | 3102.710          | 783.333        | 3445.190            | 529.146        |
| 10                          | 2944.300          | 630.077        | 3297.260            | 535.523        |
| Mean                        | 3175.558          | 768.165        | 3737.682            | 645.991        |
| Median                      | 3160.070          | 742.439        | 3675.770            | 634.034        |

(a) Standard intensity values

| Maximum values (520-530 nm) (593-604 nm) |                   |                |                     |                |                       |              |
|------------------------------------------|-------------------|----------------|---------------------|----------------|-----------------------|--------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                | Light from the source |              |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> | 405 - 410 nm          | 445 - 455 nm |
| 1                                        | 3489.770          | 1218.060       | 4290.190            | 1039.720       | 1176.860              | 1891.190     |
| 2                                        | 3632.780          | 1015.570       | 3972.430            | 806.188        | 2936.940              | 2320.610     |
| 3                                        | 3239.080          | 934.054        | 3891.620            | 873.892        | 3695.300              | 2882.940     |
| 4                                        | 3371.640          | 956.232        | 3710.050            | 725.346        | 4634.590              | 2870.420     |
| 5                                        | 3173.360          | 859.202        | 4376.170            | 866.576        | 4506.010              | 4017.950     |
| 6                                        | 3638.180          | 966.431        | 3937.470            | 758.418        | 8097.600              | 2320.450     |
| 7                                        | 3803.920          | 939.261        | 3775.720            | 724.361        | 8394.100              | 2407.080     |
| 8                                        | 3521.280          | 892.748        | 4226.020            | 825.879        | 8094.590              | 2730.580     |
| 9                                        | 3438.650          | 881.646        | 3707.830            | 738.719        | 7965.180              | 2448.400     |
| 10                                       | 3286.110          | 815.492        | 3428.520            | 672.147        | 7573.860              | 2249.540     |
| Mean                                     | 3459.477          | 947.870        | 3931.602            | 803.125        | 5707.503              | 2613.916     |
| Median                                   | 3464.210          | 936.658        | 3914.545            | 782.303        | 6104.225              | 2427.740     |

(b) Maximum intensity values

| Minimum values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                        | 3129.570          | 905.105        | 3999.600            | 776.936        |
| 2                                        | 3330.330          | 689.156        | 3535.950            | 514.162        |
| 3                                        | 2919.540          | 631.286        | 3581.150            | 538.090        |
| 4                                        | 2963.780          | 607.556        | 3394.630            | 501.447        |
| 5                                        | 2728.610          | 581.062        | 3987.910            | 623.965        |
| 6                                        | 3291.200          | 674.824        | 3396.110            | 471.258        |
| 7                                        | 3359.940          | 661.301        | 3473.200            | 492.409        |
| 8                                        | 3069.100          | 636.581        | 3792.100            | 593.934        |
| 9                                        | 3102.710          | 587.139        | 3269.560            | 487.729        |
| 10                                       | 2851.280          | 547.327        | 3090.690            | 445.192        |
| Mean                                     | 3074.606          | 652.134        | 3552.090            | 544.512        |
| Median                                   | 3085.905          | 633.934        | 3504.575            | 507.805        |

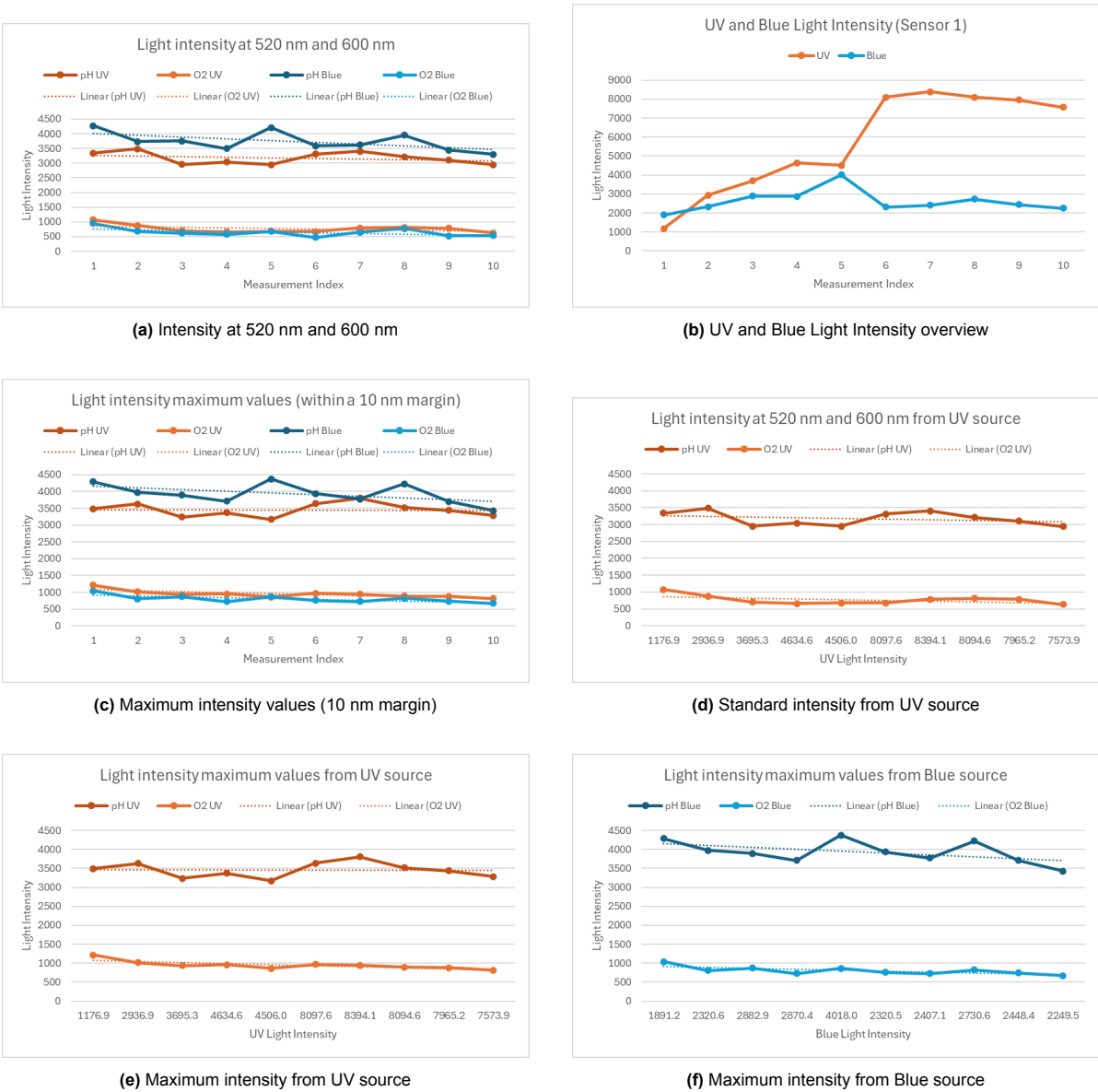
(c) Minimum intensity values

| Min Max Average values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|--------------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                        | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                                  | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                                | 3309.670          | 1061.583       | 4144.895            | 908.328        |
| 2                                                | 3481.555          | 852.363        | 3754.190            | 660.175        |
| 3                                                | 3079.310          | 782.670        | 3736.385            | 705.991        |
| 4                                                | 3167.710          | 781.894        | 3552.340            | 613.397        |
| 5                                                | 2950.985          | 720.132        | 4182.040            | 745.271        |
| 6                                                | 3464.690          | 820.628        | 3666.790            | 614.838        |
| 7                                                | 3581.930          | 800.281        | 3624.460            | 608.385        |
| 8                                                | 3295.190          | 764.665        | 4009.060            | 709.907        |
| 9                                                | 3270.680          | 734.393        | 3488.695            | 613.224        |
| 10                                               | 3068.695          | 681.410        | 3259.605            | 558.670        |
| Mean                                             | 3267.042          | 800.002        | 3741.846            | 673.818        |
| Median                                           | 3282.935          | 782.282        | 3701.588            | 637.507        |

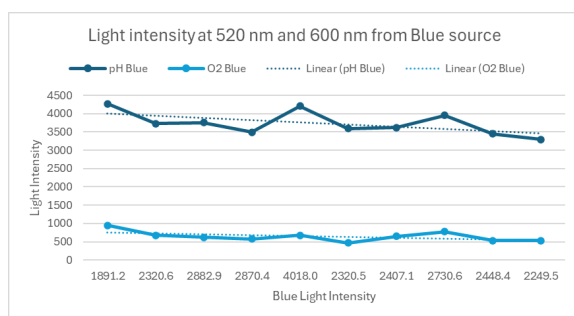
(d) Averaged intensity values

**Figure G.2:** Detailed numerical data logs extracted from Sensor 1 during Measurement 1. The tables break down the raw intensity readings into (a) standard values, (b) maximums, (c) minimums, and (d) averages, categorized by excitation wavelength.

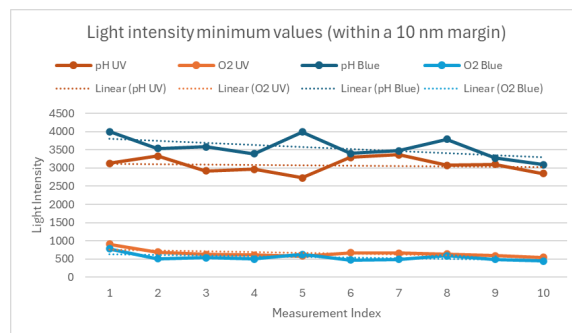
G.1.3. Comprehensive Intensity Response Graphs



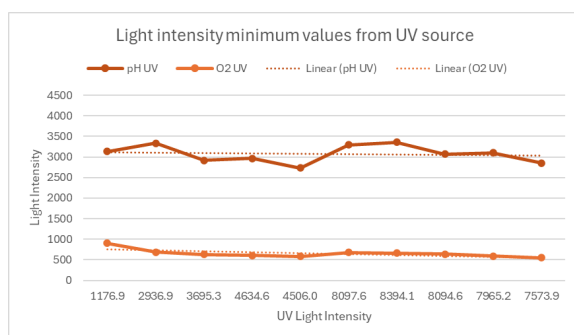
**Figure G.3:** Comprehensive graphical representations of the recorded light intensities for Measurement 1, detailing general trends, excitation source baselines, and maximum peak behaviors (Part 1).



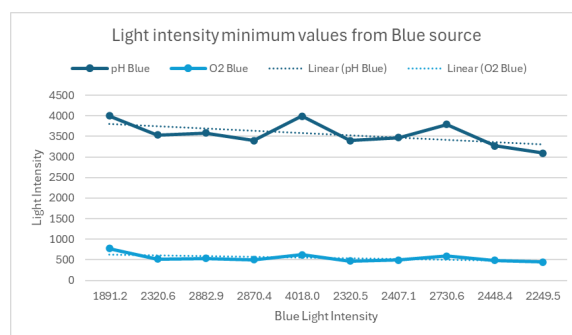
(g) Standard intensity from Blue source



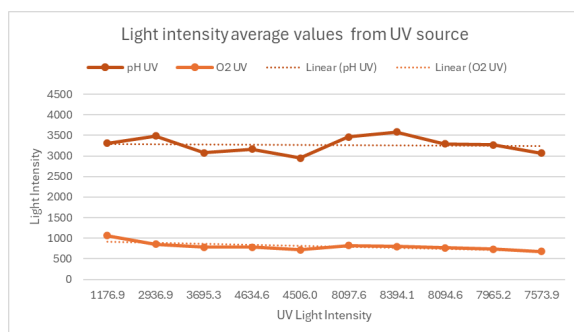
(h) Minimum intensity values (10 nm margin)



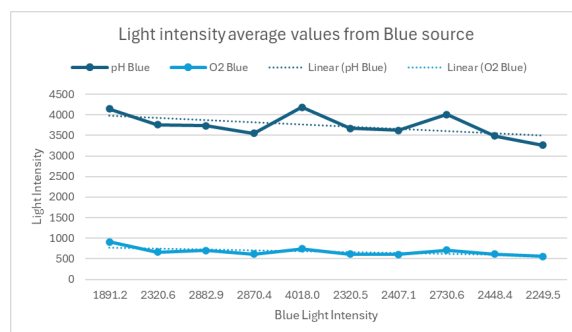
(i) Minimum intensity from UV source



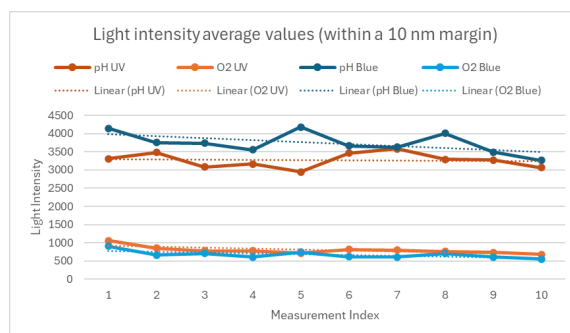
(j) Minimum intensity from Blue source



(k) Average intensity from UV source



(l) Average intensity from Blue source



(m) Combined average intensity (10 nm margin)

**Figure G.3:** Comprehensive graphical representations of the recorded light intensities for Measurement 1 (Part 2). The complete dataset maps out standard, maximum, minimum, and average recorded intensities sequentially across both UV and Blue excitation modes.

## G.2. Measurement 2: Raw Data and Clinical Reference

### G.2.1. Clinical Perfusate Analysis (EMC)

| <b>EMC Measurements</b>                 |                                    |        |        |
|-----------------------------------------|------------------------------------|--------|--------|
| <b>Blood gas values</b>                 |                                    |        |        |
| <b>pH</b>                               | pH                                 | 7.575  | —      |
| <b>pCO<sub>2</sub></b>                  | Partial pressure of carbon dioxide | 3.23   | kPa    |
| <b>pO<sub>2</sub></b>                   | Partial pressure of oxygen         | 22.4   | kPa    |
| <b>cBase(B)c</b>                        | Base excess                        | 0.7    | mmol/L |
| <b>cHCO<sub>3</sub><sup>-</sup>(P)c</b> | Bicarbonate concentration          | 22.4   | mmol/L |
| <b>Oximetry values</b>                  |                                    |        |        |
| <b>ctHb</b>                             | Total hemoglobin concentration     | 4.5    | mmol/L |
| <b>Hctc</b>                             | Hematocrit                         | 0.222  | —      |
| <b>sO<sub>2</sub></b>                   | Oxygen saturation                  | 100.3% | %      |
| <b>Electrolyte values</b>               |                                    |        |        |
| <b>cK<sup>+</sup></b>                   | Potassium                          | 3.9    | mmol/L |
| <b>cNa<sup>+</sup></b>                  | Sodium                             | 158    | mmol/L |
| <b>cCa<sup>2+</sup></b>                 | Ionized calcium                    | 0.72   | mmol/L |
| <b>cCl<sup>-</sup></b>                  | Chloride                           | 97     | mmol/L |
| <b>Metabolite values</b>                |                                    |        |        |
| <b>cGlu</b>                             | Glucose                            | 10.6   | mmol/L |
| <b>cLac</b>                             | Lactate                            | 1.7    | mmol/L |

**Figure G.4:** Gold-standard clinical perfusate measurements extracted via the ABL90 FLEX analyser corresponding to Measurement 2.

G.2.2. Optical Sensor Data Logs

| Values at 520 nm and 600 nm |                   |                |                     |                |
|-----------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #   | UV light (405 nm) |                | Blue light (450 nm) |                |
|                             | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                           | 2416.990          | 874.879        | 3535.080            | 780.005        |
| 2                           | 2641.510          | 748.494        | 2792.940            | 598.302        |
| 3                           | 2557.530          | 677.317        | 3656.840            | 767.284        |
| 4                           | 2623.980          | 645.112        | 3733.500            | 689.057        |
| 5                           | 2008.620          | 499.373        | 3400.390            | 633.416        |
| 6                           | 1799.980          | 484.383        | 3639.230            | 664.069        |
| 7                           | 1681.330          | 395.663        | 3455.160            | 569.606        |
| 8                           | 1810.080          | 456.431        | 3272.690            | 544.246        |
| 9                           | 1715.990          | 462.476        | 3348.630            | 622.001        |
| 10                          | 1614.430          | 485.391        | 3345.570            | 522.455        |
| Mean                        | 2087.044          | 572.952        | 3418.003            | 639.044        |
| Median                      | 1909.350          | 492.382        | 3427.775            | 627.709        |

(a) Standard intensity values

| Maximum values (520-530 nm) (593-604 nm) |                   |                |                     |                |                           |
|------------------------------------------|-------------------|----------------|---------------------|----------------|---------------------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                | Light from the source     |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> | 405 - 410 nm 445 - 455 nm |
| 1                                        | 2544.880          | 977.834        | 3674.220            | 859.750        | 1016.780 1421.560         |
| 2                                        | 2716.340          | 840.368        | 2914.270            | 685.312        | 827.418 893.565           |
| 3                                        | 2732.470          | 726.467        | 3865.450            | 857.141        | 1323.240 1328.630         |
| 4                                        | 2753.660          | 737.717        | 3970.740            | 787.634        | 1405.750 1477.530         |
| 5                                        | 2167.210          | 674.349        | 3569.770            | 758.641        | 2145.190 1535.230         |
| 6                                        | 1911.090          | 511.394        | 3763.880            | 708.009        | 1516.230 2001.610         |
| 7                                        | 1990.970          | 559.932        | 3578.620            | 711.948        | 1658.150 2138.370         |
| 8                                        | 1972.500          | 596.083        | 3506.090            | 683.275        | 1650.260 2090.410         |
| 9                                        | 1973.560          | 593.925        | 3481.030            | 757.627        | 1705.600 2046.040         |
| 10                                       | 1911.960          | 551.360        | 3531.510            | 686.331        | 1759.610 2166.700         |
| Mean                                     | 2267.464          | 676.943        | 3585.558            | 749.567        | 1500.823 1709.965         |
| Median                                   | 2079.090          | 635.216        | 3574.195            | 734.788        | 1583.245 1768.420         |

(b) Maximum intensity values

| Minimum values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                        | 2256.750          | 746.931        | 3334.790            | 621.591        |
| 2                                        | 2353.150          | 597.489        | 2526.500            | 425.007        |
| 3                                        | 2283.910          | 502.208        | 3371.070            | 575.870        |
| 4                                        | 2483.050          | 532.955        | 3540.510            | 563.247        |
| 5                                        | 1898.870          | 367.753        | 3263.700            | 499.251        |
| 6                                        | 1591.650          | 273.157        | 3311.420            | 461.177        |
| 7                                        | 1577.250          | 327.330        | 3120.890            | 479.387        |
| 8                                        | 1617.990          | 380.000        | 3092.900            | 454.280        |
| 9                                        | 1687.560          | 254.089        | 2960.690            | 479.897        |
| 10                                       | 1595.960          | 329.414        | 2976.820            | 437.237        |
| Mean                                     | 1934.614          | 431.133        | 3149.929            | 499.694        |
| Median                                   | 1793.215          | 373.877        | 3192.295            | 479.642        |

(c) Minimum intensity values

| Min Max Average values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|--------------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                        | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                                  | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                                | 2400.815          | 862.383        | 3504.505            | 740.671        |
| 2                                                | 2534.745          | 718.929        | 2720.385            | 555.160        |
| 3                                                | 2508.190          | 614.338        | 3618.260            | 716.506        |
| 4                                                | 2618.355          | 635.336        | 3755.625            | 675.441        |
| 5                                                | 2033.040          | 521.051        | 3416.735            | 628.946        |
| 6                                                | 1751.370          | 392.276        | 3537.650            | 584.593        |
| 7                                                | 1784.110          | 443.631        | 3349.755            | 595.668        |
| 8                                                | 1795.245          | 488.042        | 3299.495            | 568.778        |
| 9                                                | 1830.560          | 424.007        | 3220.860            | 618.762        |
| 10                                               | 1753.960          | 440.387        | 3254.165            | 561.784        |
| Mean                                             | 2101.039          | 554.038        | 3367.744            | 624.631        |
| Median                                           | 1931.800          | 504.546        | 3383.245            | 607.215        |

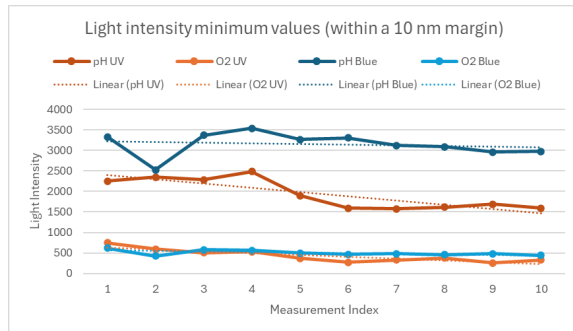
(d) Averaged intensity values

Figure G.5: Detailed numerical data logs extracted during Measurement 2. The tables break down the raw intensity readings into (a) standard values, (b) maximums, (c) minimums, and (d) averages, categorized by excitation wavelength.

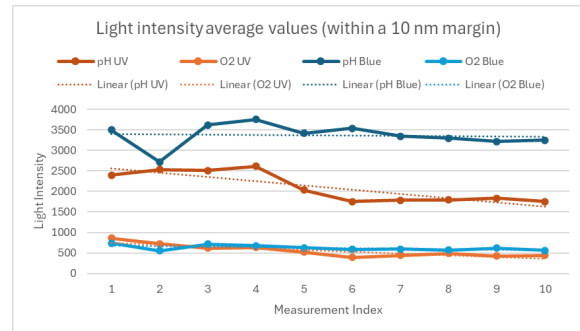
G.2.3. Comprehensive Intensity Response Graphs



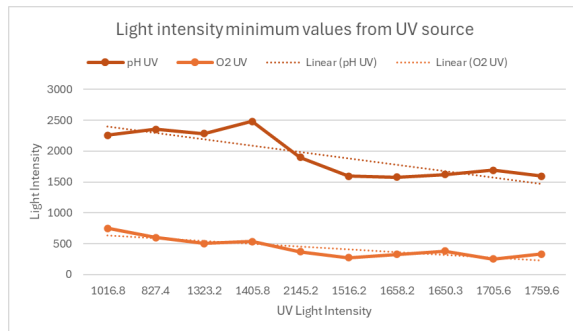
Figure G.6: Comprehensive graphical representations of the recorded light intensities for Measurement 2 (Part 1).



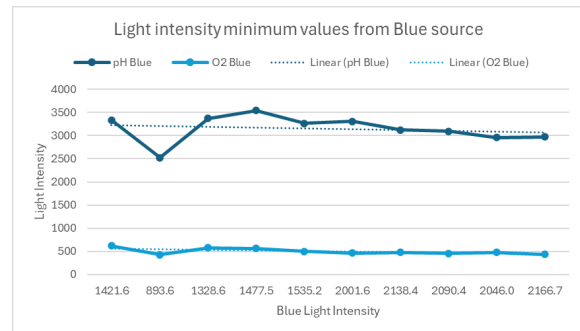
(g) Minimum intensity (10nm margin)



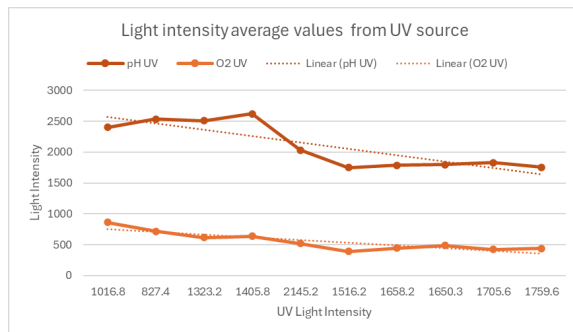
(h) Average intensity (10nm margin)



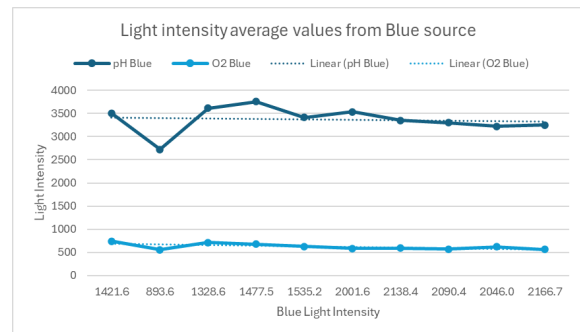
(i) Minimum intensity from UV source



(j) Minimum intensity from Blue source



(k) Average intensity from UV source



(l) Average intensity from Blue source

**Figure G.6:** Comprehensive graphical representations of the recorded light intensities for Measurement 2 (Part 2). The complete dataset details standard, maximum, minimum, and average recorded intensities, systematically separated by wavelength and excitation source.

## G.3. Measurement 3: Raw Data and Clinical Reference

### G.3.1. Clinical Perfusate Analysis (EMC)

| <b>EMC Measurements</b>                 |                                    |        |        |
|-----------------------------------------|------------------------------------|--------|--------|
| <b>Blood gas values</b>                 |                                    |        |        |
| <b>pH</b>                               | pH                                 | 7.456  | —      |
| <b>pCO<sub>2</sub></b>                  | Partial pressure of carbon dioxide | 4.54   | kPa    |
| <b>pO<sub>2</sub></b>                   | Partial pressure of oxygen         | 21.6   | kPa    |
| <b>cBase(B)c</b>                        | Base excess                        | 0.2    | mmol/L |
| <b>cHCO<sub>3</sub><sup>-</sup>(P)c</b> | Bicarbonate concentration          | 24     | mmol/L |
| <b>Oximetry values</b>                  |                                    |        |        |
| <b>ctHb</b>                             | Total hemoglobin concentration     | 4.5    | mmol/L |
| <b>Hctc</b>                             | Hematocrit                         | 0.221  | —      |
| <b>sO<sub>2</sub></b>                   | Oxygen saturation                  | 100.3% | %      |
| <b>Electrolyte values</b>               |                                    |        |        |
| <b>cK<sup>+</sup></b>                   | Potassium                          | 3.7    | mmol/L |
| <b>cNa<sup>+</sup></b>                  | Sodium                             | 159    | mmol/L |
| <b>cCa<sup>2+</sup></b>                 | Ionized calcium                    | 0.74   | mmol/L |
| <b>cCl<sup>-</sup></b>                  | Chloride                           | 96     | mmol/L |
| <b>Metabolite values</b>                |                                    |        |        |
| <b>cGlu</b>                             | Glucose                            | 10.5   | mmol/L |
| <b>cLac</b>                             | Lactate                            | 1.7    | mmol/L |

**Figure G.7:** Gold-standard clinical perfusate measurements extracted via the ABL90 FLEX analyser corresponding to Measurement 3.

## G.3.2. Optical Sensor Data Logs

| Values at 520 nm and 600 nm |                   |                |                     |                |
|-----------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #   | UV light (405 nm) |                | Blue light (450 nm) |                |
|                             | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                           | 1026.270          | 300.846        | 1852.340            | 450.652        |
| 2                           | 1440.660          | 206.040        | 1707.570            | 324.956        |
| 3                           | 1687.190          | 401.838        | 1724.810            | 385.502        |
| 4                           | 1316.550          | 250.070        | 1650.270            | 305.454        |
| 5                           | 1353.630          | 332.911        | 1872.760            | 423.225        |
| 6                           | 1625.050          | 269.220        | 1610.670            | 273.727        |
| 7                           | 1584.470          | 287.346        | 1577.980            | 321.784        |
| 8                           | 1524.430          | 298.818        | 1504.750            | 338.978        |
| 9                           | 1486.610          | 256.894        | 1586.520            | 361.305        |
| 10                          | 1510.270          | 360.598        | 1542.380            | 322.150        |
| Mean                        | 1455.513          | 296.458        | 1663.005            | 350.773        |
| Median                      | 1498.440          | 293.082        | 1630.470            | 331.967        |

(a) Standard intensity values

| Maximum values (520-530 nm) (593-604 nm) |                   |                |                     |                |                           |
|------------------------------------------|-------------------|----------------|---------------------|----------------|---------------------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                | Light from the source     |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> | 405 - 410 nm 445 - 455 nm |
| 1                                        | 1141.480          | 600.614        | 2060.630            | 551.547        | 202.185 333.292           |
| 2                                        | 1685.380          | 447.782        | 1951.290            | 513.905        | 1467.090 1090.510         |
| 3                                        | 1867.770          | 445.436        | 1864.410            | 450.872        | 3131.360 1841.840         |
| 4                                        | 1533.510          | 414.939        | 1916.370            | 445.164        | 2690.120 1942.780         |
| 5                                        | 1598.200          | 428.220        | 2007.630            | 540.826        | 3063.920 2012.340         |
| 6                                        | 1787.460          | 423.028        | 1719.190            | 504.087        | 5996.570 1445.510         |
| 7                                        | 1820.980          | 451.718        | 1712.500            | 434.126        | 5970.260 1443.660         |
| 8                                        | 1806.880          | 466.309        | 1772.230            | 448.804        | 5980.050 1506.500         |
| 9                                        | 1697.980          | 410.484        | 1793.290            | 427.241        | 5923.280 1544.750         |
| 10                                       | 1743.920          | 440.196        | 1725.800            | 426.291        | 5793.330 1521.760         |
| Mean                                     | 1668.356          | 452.873        | 1852.334            | 474.286        | 4021.817 1468.294         |
| Median                                   | 1720.950          | 442.816        | 1828.850            | 449.838        | 4462.345 1514.130         |

(b) Maximum intensity values

| Minimum values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                        | 849.970           | 83.881         | 1529.930            | 294.131        |
| 2                                        | 1359.220          | 178.776        | 1632.940            | 289.528        |
| 3                                        | 1609.950          | 140.955        | 1537.550            | 165.780        |
| 4                                        | 1221.950          | 156.882        | 1650.270            | 215.146        |
| 5                                        | 1312.200          | 142.726        | 1669.720            | 239.259        |
| 6                                        | 1467.060          | 233.550        | 1434.620            | 185.890        |
| 7                                        | 1501.580          | 194.043        | 1435.950            | 176.463        |
| 8                                        | 1464.870          | 213.827        | 1425.590            | 125.146        |
| 9                                        | 1409.630          | 237.649        | 1520.220            | 142.416        |
| 10                                       | 1399.370          | 174.159        | 1463.810            | 190.533        |
| Mean                                     | 1359.580          | 175.645        | 1530.060            | 202.429        |
| Median                                   | 1404.500          | 176.468        | 1525.075            | 188.212        |

(c) Minimum intensity values

| Min Max Average values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|--------------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                        | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                                  | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                                | 995.725           | 342.248        | 1795.280            | 422.839        |
| 2                                                | 1522.300          | 313.279        | 1792.115            | 401.717        |
| 3                                                | 1738.860          | 293.196        | 1700.980            | 308.326        |
| 4                                                | 1377.730          | 285.911        | 1783.320            | 330.155        |
| 5                                                | 1455.200          | 285.473        | 1838.675            | 390.043        |
| 6                                                | 1627.260          | 328.289        | 1576.905            | 344.989        |
| 7                                                | 1661.280          | 322.881        | 1574.225            | 305.295        |
| 8                                                | 1635.875          | 340.068        | 1598.910            | 286.975        |
| 9                                                | 1553.805          | 324.067        | 1656.755            | 284.829        |
| 10                                               | 1571.645          | 307.178        | 1594.805            | 308.412        |
| Mean                                             | 1513.968          | 314.259        | 1691.197            | 338.358        |
| Median                                           | 1562.725          | 318.080        | 1678.868            | 319.284        |

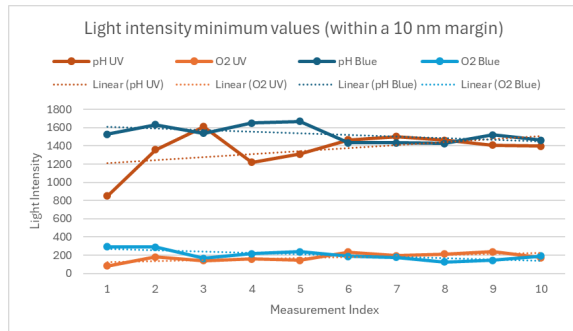
(d) Averaged intensity values

**Figure G.8:** Detailed numerical data logs extracted during Measurement 3. The tables break down the raw intensity readings into (a) standard values, (b) maximums, (c) minimums, and (d) averages, categorized by excitation wavelength.

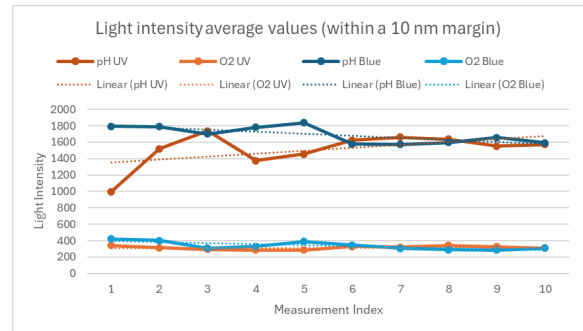
G.3.3. Comprehensive Intensity Response Graphs



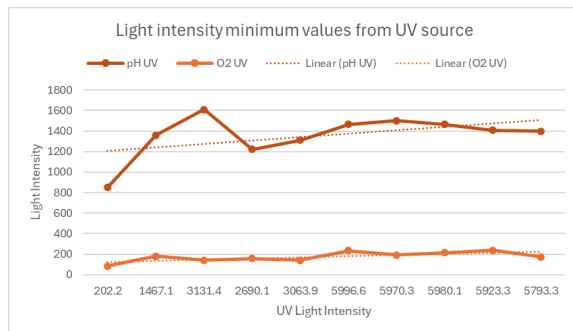
Figure G.9: Comprehensive graphical representations of the recorded light intensities for Measurement 3 (Part 1).



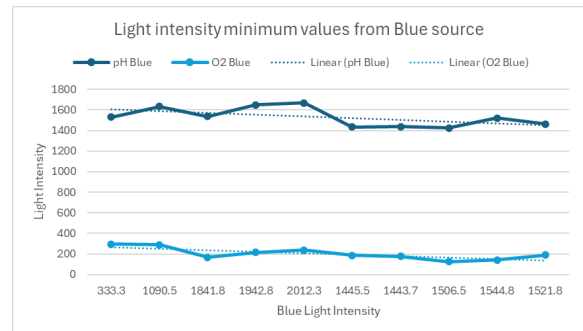
(g) Minimum intensity (10nm margin)



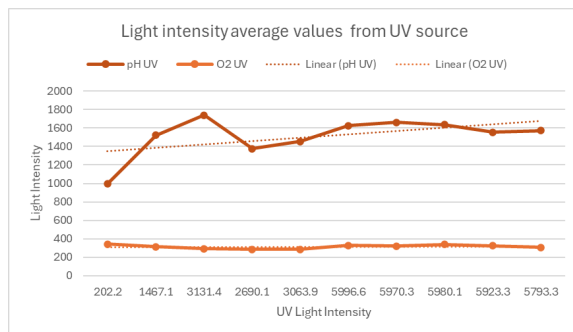
(h) Average intensity (10nm margin)



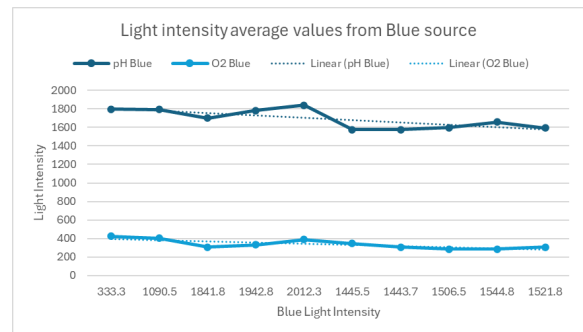
(i) Minimum intensity from UV source



(j) Minimum intensity from Blue source



(k) Average intensity from UV source



(l) Average intensity from Blue source

**Figure G.9:** Comprehensive graphical representations of the recorded light intensities for Measurement 3 (Part 2). The complete dataset details standard, maximum, minimum, and average recorded intensities, systematically separated by wavelength and excitation source.

## G.4. Measurement 4.1: Raw Data and Clinical Reference

### G.4.1. Clinical Perfusate Analysis (EMC)

| <b>EMC Measurements</b>                 |                                    |        |        |
|-----------------------------------------|------------------------------------|--------|--------|
| <b>Blood gas values</b>                 |                                    |        |        |
| <b>pH</b>                               | pH                                 | 7.497  | —      |
| <b>pCO<sub>2</sub></b>                  | Partial pressure of carbon dioxide | 4.07   | kPa    |
| <b>pO<sub>2</sub></b>                   | Partial pressure of oxygen         | 21     | kPa    |
| <b>cBase(B)c</b>                        | Base excess                        | 0.6    | mmol/L |
| <b>cHCO<sub>3</sub><sup>-</sup>(P)c</b> | Bicarbonate concentration          | 23.7   | mmol/L |
| <b>Oximetry values</b>                  |                                    |        |        |
| <b>ctHb</b>                             | Total hemoglobin concentration     | 4.5    | mmol/L |
| <b>Hctc</b>                             | Hematocrit                         | 0.224  | —      |
| <b>sO<sub>2</sub></b>                   | Oxygen saturation                  | 100.1% | %      |
| <b>Electrolyte values</b>               |                                    |        |        |
| <b>cK<sup>+</sup></b>                   | Potassium                          | 3.5    | mmol/L |
| <b>cNa<sup>+</sup></b>                  | Sodium                             | 162    | mmol/L |
| <b>cCa<sup>2+</sup></b>                 | Ionized calcium                    | 0.72   | mmol/L |
| <b>cCl<sup>-</sup></b>                  | Chloride                           | 98     | mmol/L |
| <b>Metabolite values</b>                |                                    |        |        |
| <b>cGlu</b>                             | Glucose                            | 9.9    | mmol/L |
| <b>cLac</b>                             | Lactate                            | 1.4    | mmol/L |

**Figure G.10:** Gold-standard clinical perfusate measurements extracted via the ABL90 FLEX analyser corresponding to Measurement 4.1.

## G.4.2. Optical Sensor Data Logs

| Values at 520 nm and 600 nm |                   |                |                     |                |
|-----------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #   | UV light (405 nm) |                | Blue light (450 nm) |                |
|                             | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                           | 2319.430          | 585.821        | 1717.300            | 518.313        |
| 2                           | 2818.030          | 628.694        | 1438.840            | 404.670        |
| 3                           | 3056.660          | 639.452        | 1708.830            | 419.038        |
| 4                           | 3323.740          | 651.690        | 1850.860            | 384.927        |
| 5                           | 3342.770          | 695.282        | 1850.300            | 474.172        |
| 6                           | 3195.940          | 571.622        | 1637.570            | 347.771        |
| 7                           | 3165.480          | 610.415        | 1724.200            | 322.540        |
| 8                           | 3873.370          | 930.549        | 1693.460            | 410.343        |
| 9                           | 3933.930          | 770.247        | 1459.690            | 341.580        |
| 10                          | 5368.740          | 1041.970       | 1715.080            | 492.757        |
| Mean                        | 3439.809          | 712.574        | 1679.613            | 411.611        |
| Median                      | 3259.840          | 645.571        | 1711.955            | 407.507        |

(a) Standard intensity values

| Maximum values (520-530 nm) (593-604 nm) |                   |                |                     |                |                       |              |
|------------------------------------------|-------------------|----------------|---------------------|----------------|-----------------------|--------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                | Light from the source |              |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> | 405 - 410 nm          | 445 - 455 nm |
| 1                                        | 2765.210          | 623.154        | 2086.470            | 556.683        | 11132.700             | 498.976      |
| 2                                        | 3340.120          | 724.367        | 2007.960            | 560.792        | 14597.900             | 473.845      |
| 3                                        | 3709.270          | 811.732        | 2067.090            | 520.471        | 17420.300             | 522.887      |
| 4                                        | 3820.440          | 796.996        | 2218.810            | 546.641        | 20774.500             | 566.189      |
| 5                                        | 3892.760          | 781.598        | 2282.250            | 545.450        | 22174.900             | 517.901      |
| 6                                        | 3574.100          | 798.241        | 1982.340            | 465.397        | 21643.000             | 509.066      |
| 7                                        | 3509.480          | 815.416        | 1982.950            | 484.358        | 21750.900             | 522.632      |
| 8                                        | 4370.100          | 950.587        | 2059.920            | 585.789        | 27124.300             | 558.287      |
| 9                                        | 4317.830          | 933.815        | 1720.020            | 407.166        | 25972.000             | 503.017      |
| 10                                       | 5894.000          | 1216.150       | 2091.020            | 514.298        | 35667.000             | 546.923      |
| Mean                                     | 3919.331          | 845.206        | 2049.883            | 518.705        | 21825.750             | 521.972      |
| Median                                   | 3764.855          | 804.987        | 2063.505            | 532.961        | 21696.950             | 520.267      |

(b) Maximum intensity values

| Minimum values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                        | 2319.430          | 426.494        | 1717.300            | 276.860        |
| 2                                        | 2818.030          | 499.918        | 1438.840            | 292.349        |
| 3                                        | 3056.660          | 524.454        | 1708.830            | 287.813        |
| 4                                        | 3327.550          | 496.464        | 1850.860            | 313.889        |
| 5                                        | 3342.770          | 549.870        | 1756.700            | 265.917        |
| 6                                        | 3057.570          | 461.925        | 1621.760            | 251.663        |
| 7                                        | 3082.690          | 506.558        | 1612.420            | 237.613        |
| 8                                        | 3873.370          | 652.699        | 1660.170            | 305.794        |
| 9                                        | 3835.800          | 579.193        | 1342.540            | 185.253        |
| 10                                       | 5233.350          | 916.079        | 1522.040            | 246.266        |
| Mean                                     | 3394.722          | 561.365        | 1623.146            | 266.342        |
| Median                                   | 3205.120          | 515.506        | 1640.965            | 271.389        |

(c) Minimum intensity values

| Min Max Average values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|--------------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                        | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                                  | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                                | 2542.320          | 524.824        | 1901.885            | 416.772        |
| 2                                                | 3079.075          | 612.143        | 1723.400            | 426.571        |
| 3                                                | 3382.965          | 668.093        | 1887.960            | 404.142        |
| 4                                                | 3573.995          | 646.730        | 2034.835            | 430.265        |
| 5                                                | 3617.765          | 665.734        | 2019.475            | 405.684        |
| 6                                                | 3315.835          | 630.083        | 1802.050            | 358.530        |
| 7                                                | 3296.085          | 660.987        | 1797.685            | 360.986        |
| 8                                                | 4121.735          | 801.643        | 1860.045            | 445.792        |
| 9                                                | 4076.815          | 756.504        | 1531.280            | 296.210        |
| 10                                               | 5563.675          | 1066.115       | 1806.530            | 380.282        |
| Mean                                             | 3657.027          | 703.286        | 1836.515            | 392.523        |
| Median                                           | 3478.480          | 663.361        | 1833.288            | 404.913        |

(d) Averaged intensity values

**Figure G.11:** Detailed numerical data logs extracted during Measurement 4.1. The tables break down the raw intensity readings into (a) standard values, (b) maximums, (c) minimums, and (d) averages, categorized by excitation wavelength.

G.4.3. Comprehensive Intensity Response Graphs

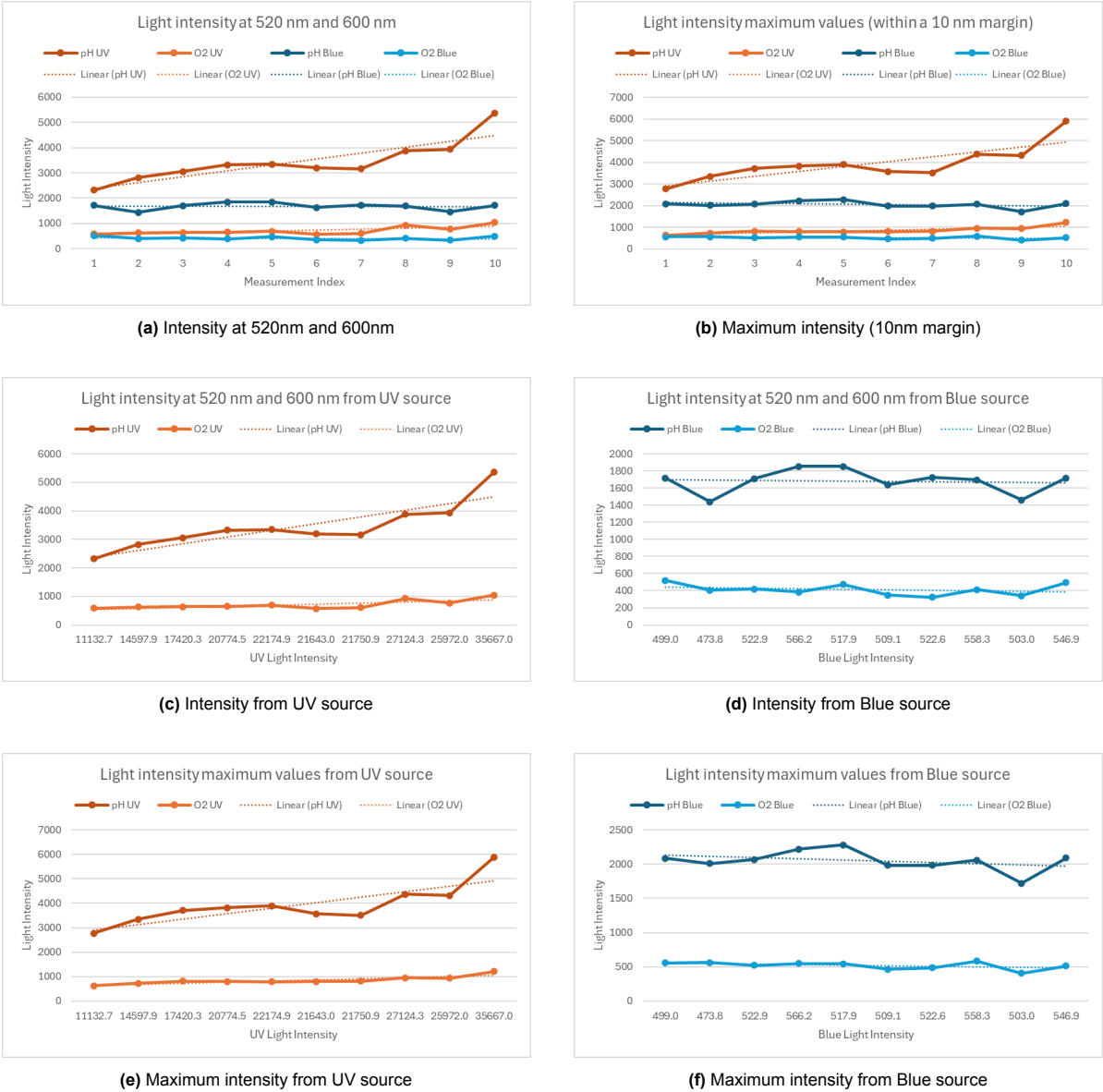
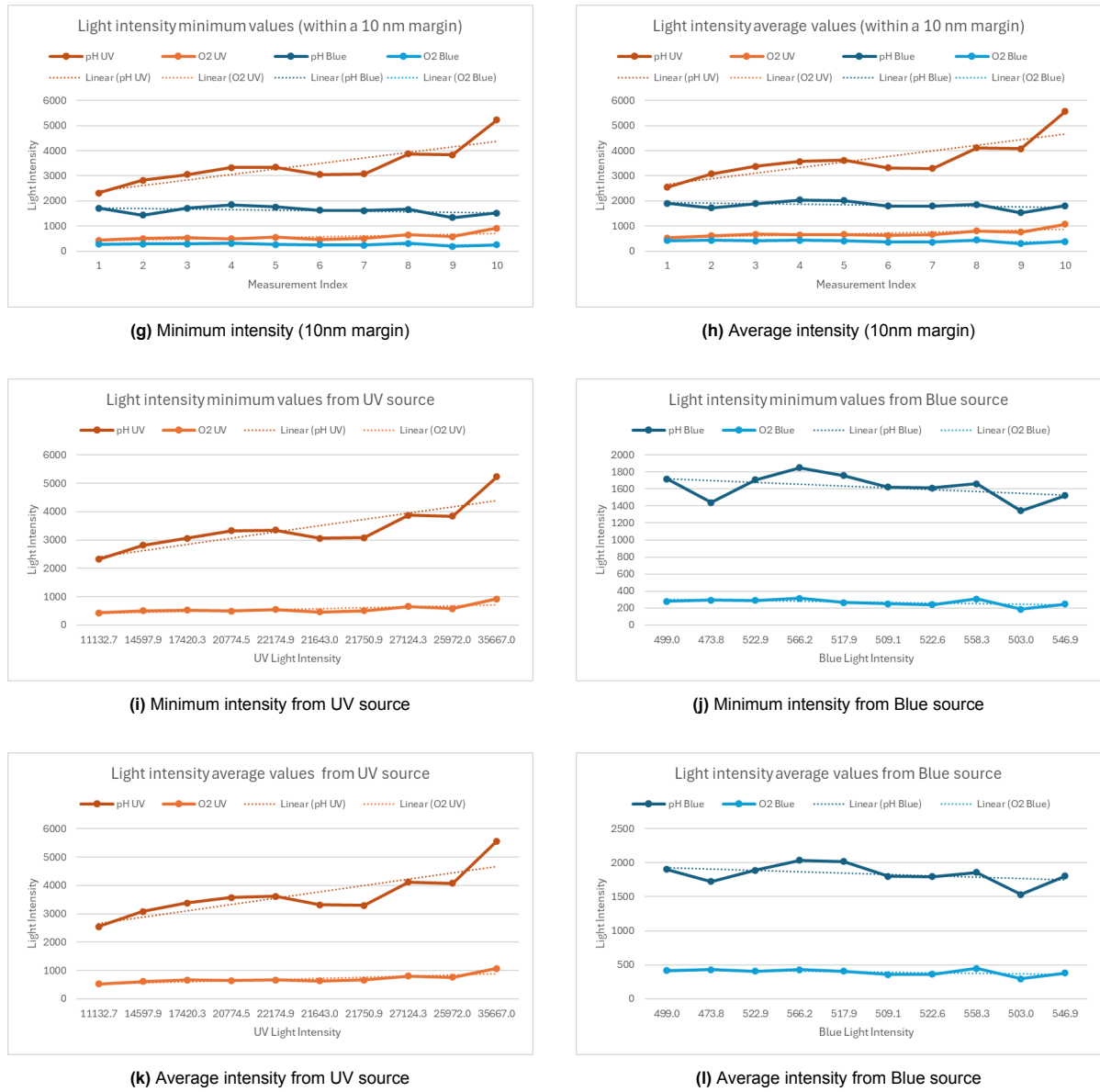


Figure G.12: Comprehensive graphical representations of the recorded light intensities for Measurement 4.1 (Part 1).



**Figure G.12:** Comprehensive graphical representations of the recorded light intensities for Measurement 4.1 (Part 2). The complete dataset details standard, maximum, minimum, and average recorded intensities, systematically separated by wavelength and excitation source.

## G.5. Measurement 4.2: Raw Data and Clinical Reference

### G.5.1. Clinical Perfusate Analysis (EMC)

| <b>EMC Measurements</b>                 |                                    |        |        |
|-----------------------------------------|------------------------------------|--------|--------|
| <b>Blood gas values</b>                 |                                    |        |        |
| <b>pH</b>                               | pH                                 | 7.497  | —      |
| <b>pCO<sub>2</sub></b>                  | Partial pressure of carbon dioxide | 4.07   | kPa    |
| <b>pO<sub>2</sub></b>                   | Partial pressure of oxygen         | 21     | kPa    |
| <b>cBase(B)c</b>                        | Base excess                        | 0.6    | mmol/L |
| <b>cHCO<sub>3</sub><sup>-</sup>(P)c</b> | Bicarbonate concentration          | 23.7   | mmol/L |
| <b>Oximetry values</b>                  |                                    |        |        |
| <b>ctHb</b>                             | Total hemoglobin concentration     | 4.5    | mmol/L |
| <b>Hctc</b>                             | Hematocrit                         | 0.224  | —      |
| <b>sO<sub>2</sub></b>                   | Oxygen saturation                  | 100.1% | %      |
| <b>Electrolyte values</b>               |                                    |        |        |
| <b>cK<sup>+</sup></b>                   | Potassium                          | 3.5    | mmol/L |
| <b>cNa<sup>+</sup></b>                  | Sodium                             | 162    | mmol/L |
| <b>cCa<sup>2+</sup></b>                 | Ionized calcium                    | 0.72   | mmol/L |
| <b>cCl<sup>-</sup></b>                  | Chloride                           | 98     | mmol/L |
| <b>Metabolite values</b>                |                                    |        |        |
| <b>cGlu</b>                             | Glucose                            | 9.9    | mmol/L |
| <b>cLac</b>                             | Lactate                            | 1.4    | mmol/L |

**Figure G.13:** Gold-standard clinical perfusate measurements extracted via the ABL90 FLEX analyser corresponding to Measurement 4.2.

## G.5.2. Optical Sensor Data Logs

| Values at 520 nm and 600 nm |                   |                |                     |                |
|-----------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #   | UV light (405 nm) |                | Blue light (450 nm) |                |
|                             | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                           | 1742.820          | 997.979        | 4693.280            | 1122.300       |
| 2                           | 1885.860          | 758.992        | 5188.300            | 999.201        |
| 3                           | 2147.140          | 727.068        | 4492.710            | 601.077        |
| 4                           | 1944.470          | 638.000        | 4677.020            | 809.831        |
| 5                           | 2331.340          | 801.083        | 4714.850            | 912.780        |
| 6                           | 2464.030          | 733.320        | 4291.460            | 676.981        |
| 7                           | 2356.440          | 762.481        | 3785.640            | 660.136        |
| 8                           | 2347.360          | 659.158        | 4183.480            | 655.588        |
| 9                           | 2061.380          | 543.775        | 4042.130            | 692.804        |
| 10                          | 1969.840          | 611.428        | 4092.810            | 656.982        |
| Mean                        | 2125.068          | 723.328        | 4416.168            | 778.768        |
| Median                      | 2104.260          | 730.194        | 4392.085            | 684.893        |

(a) Standard intensity values

| Maximum values (520-530 nm) (593-604 nm) |                   |                |                     |                |                           |
|------------------------------------------|-------------------|----------------|---------------------|----------------|---------------------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                | Light from the source     |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> | 405 - 410 nm 445 - 455 nm |
| 1                                        | 2035.360          | 1229.530       | 4927.240            | 1391.360       | 2336.440 2902.420         |
| 2                                        | 2165.870          | 813.493        | 5238.930            | 1113.620       | 2750.420 3256.080         |
| 3                                        | 2565.730          | 899.207        | 4582.710            | 993.785        | 3951.080 3243.170         |
| 4                                        | 2246.130          | 831.721        | 4945.090            | 1032.740       | 3909.490 3887.910         |
| 5                                        | 2420.740          | 880.296        | 4737.740            | 996.874        | 3824.050 3839.180         |
| 6                                        | 2570.280          | 826.846        | 4296.900            | 903.493        | 3455.820 3328.320         |
| 7                                        | 2693.600          | 939.690        | 4146.620            | 848.420        | 3859.440 3221.890         |
| 8                                        | 2528.090          | 847.706        | 4487.330            | 892.895        | 4117.260 3479.440         |
| 9                                        | 2324.520          | 831.104        | 4223.760            | 1001.500       | 4149.960 3262.230         |
| 10                                       | 2233.240          | 845.215        | 4249.350            | 918.982        | 4397.960 3259.380         |
| Mean                                     | 2378.356          | 894.481        | 4583.567            | 1009.367       | 3675.192 3368.002         |
| Median                                   | 2372.630          | 846.461        | 4535.020            | 995.330        | 3884.465 3260.805         |

(b) Maximum intensity values

| Minimum values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                        | 1682.330          | 937.912        | 4415.010            | 978.664        |
| 2                                        | 1827.910          | 562.318        | 4921.490            | 735.066        |
| 3                                        | 2147.140          | 686.684        | 4170.43             | 601.077        |
| 4                                        | 1944.470          | 564.849        | 4566.390            | 689.972        |
| 5                                        | 2177.130          | 563.306        | 4263.400            | 688.966        |
| 6                                        | 2340.370          | 621.895        | 4041.150            | 638.769        |
| 7                                        | 2356.440          | 631.625        | 3778.150            | 556.384        |
| 8                                        | 2213.310          | 601.787        | 4105.460            | 571.093        |
| 9                                        | 1941.930          | 524.828        | 3948.280            | 474.605        |
| 10                                       | 1879.650          | 524.368        | 3802.830            | 605.842        |
| Mean                                     | 2051.068          | 621.957        | 4201.259            | 654.044        |
| Median                                   | 2045.805          | 583.318        | 4137.945            | 622.306        |

(c) Minimum intensity values

| Min Max Average values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|--------------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                        | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                                  | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                                | 1858.845          | 1083.721       | 4671.125            | 1185.012       |
| 2                                                | 1996.890          | 687.906        | 5080.210            | 924.343        |
| 3                                                | 2356.435          | 792.946        | 4376.570            | 797.431        |
| 4                                                | 2095.300          | 698.285        | 4755.740            | 861.356        |
| 5                                                | 2298.935          | 721.801        | 4500.570            | 842.920        |
| 6                                                | 2455.325          | 724.371        | 4169.025            | 771.131        |
| 7                                                | 2525.020          | 785.658        | 3962.385            | 702.402        |
| 8                                                | 2370.700          | 724.747        | 4296.395            | 731.994        |
| 9                                                | 2133.225          | 677.966        | 4086.020            | 738.053        |
| 10                                               | 2056.445          | 684.792        | 4026.090            | 762.412        |
| Mean                                             | 2214.712          | 758.219        | 4392.413            | 831.705        |
| Median                                           | 2216.080          | 723.086        | 4336.483            | 784.281        |

(d) Averaged intensity values

**Figure G.14:** Detailed numerical data logs extracted during Measurement 4.2. The tables break down the raw intensity readings into (a) standard values, (b) maximums, (c) minimums, and (d) averages, categorized by excitation wavelength.

G.5.3. Comprehensive Intensity Response Graphs

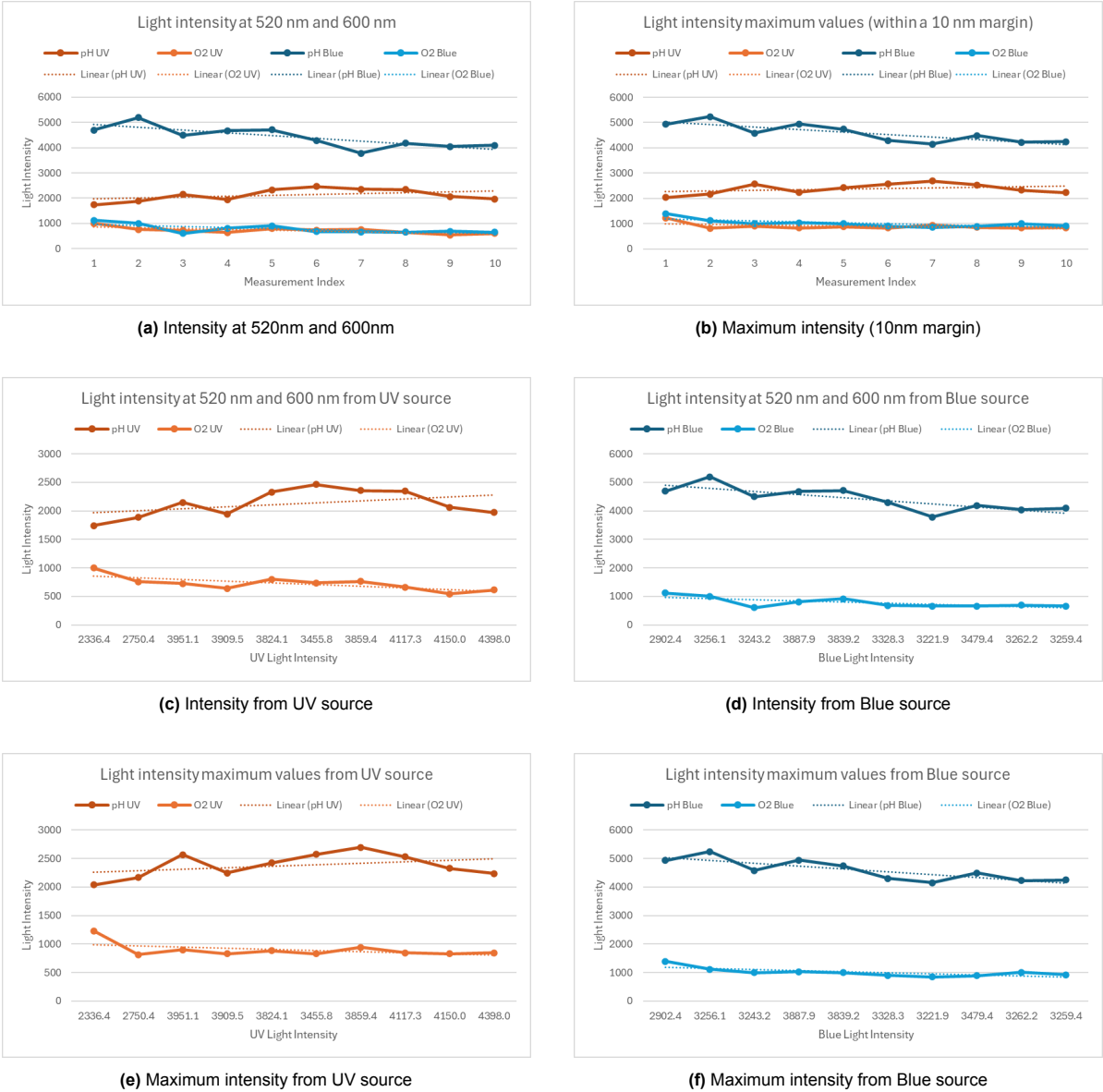
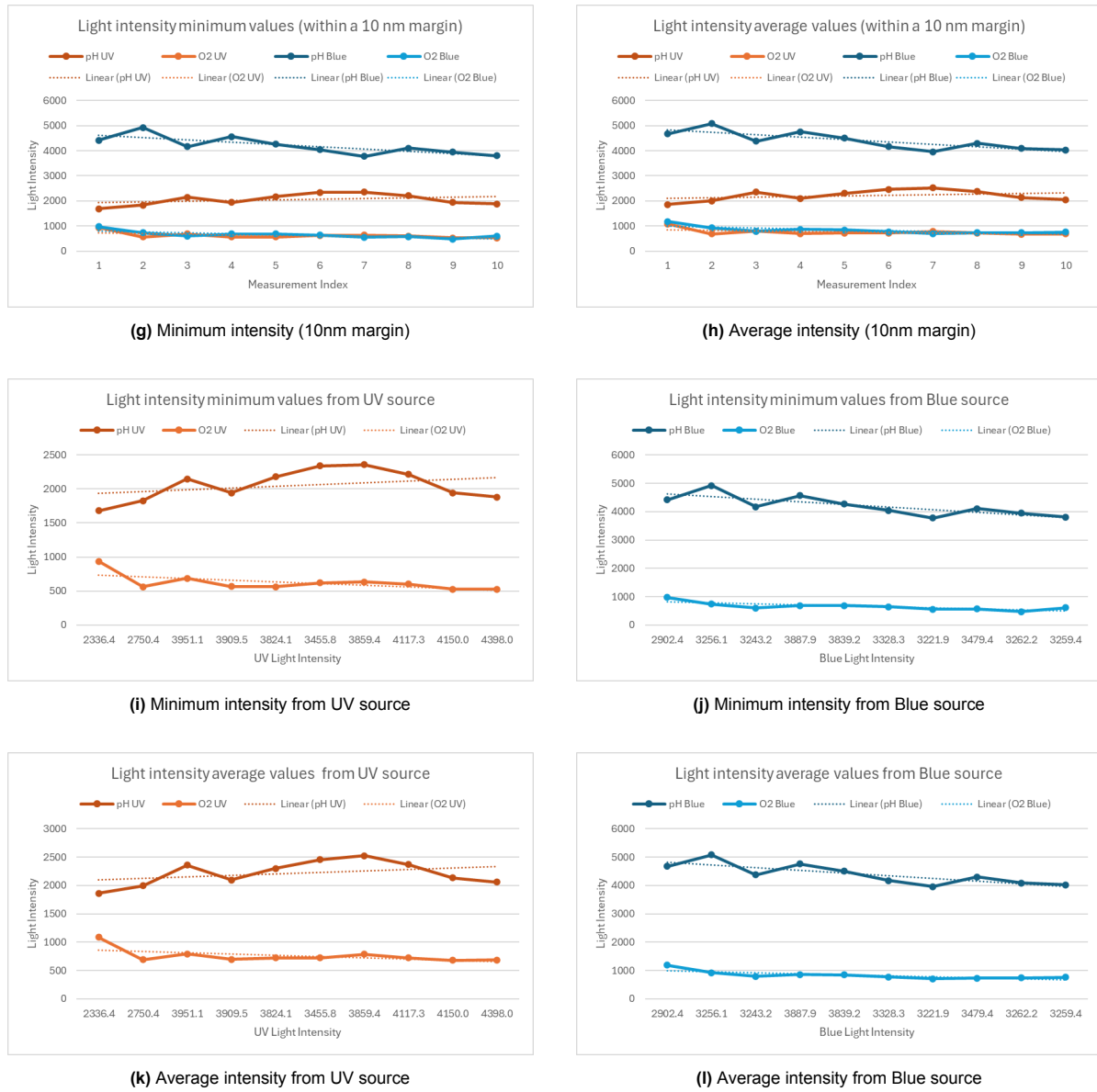


Figure G.15: Comprehensive graphical representations of the recorded light intensities for Measurement 4.2 (Part 1).



**Figure G.15:** Comprehensive graphical representations of the recorded light intensities for Measurement 4.2 (Part 2). The complete dataset details standard, maximum, minimum, and average recorded intensities, systematically separated by wavelength and excitation source.

## G.6. Measurement 5: Raw Data and Clinical Reference

### G.6.1. Clinical Perfusate Analysis (EMC)

| <b>EMC Measurements</b>                 |                                    |        |        |
|-----------------------------------------|------------------------------------|--------|--------|
| <b>Blood gas values</b>                 |                                    |        |        |
| <b>pH</b>                               | pH                                 | 7.446  | —      |
| <b>pCO<sub>2</sub></b>                  | Partial pressure of carbon dioxide | 4.59   | kPa    |
| <b>pO<sub>2</sub></b>                   | Partial pressure of oxygen         | 20.7   | kPa    |
| <b>cBase(B)c</b>                        | Base excess                        | -0.3   | mmol/L |
| <b>cHCO<sub>3</sub><sup>-</sup>(P)c</b> | Bicarbonate concentration          | 23.7   | mmol/L |
| <b>Oximetry values</b>                  |                                    |        |        |
| <b>ctHb</b>                             | Total hemoglobin concentration     | 4.5    | mmol/L |
| <b>Hctc</b>                             | Hematocrit                         | 0.224  | —      |
| <b>sO<sub>2</sub></b>                   | Oxygen saturation                  | 100.1% | %      |
| <b>Electrolyte values</b>               |                                    |        |        |
| <b>cK<sup>+</sup></b>                   | Potassium                          | 3.6    | mmol/L |
| <b>cNa<sup>+</sup></b>                  | Sodium                             | 163    | mmol/L |
| <b>cCa<sup>2+</sup></b>                 | Ionized calcium                    | 0.72   | mmol/L |
| <b>cCl<sup>-</sup></b>                  | Chloride                           | 98     | mmol/L |
| <b>Metabolite values</b>                |                                    |        |        |
| <b>cGlu</b>                             | Glucose                            | 9.9    | mmol/L |
| <b>cLac</b>                             | Lactate                            | 1.6    | mmol/L |

**Figure G.16:** Gold-standard clinical perfusate measurements extracted via the ABL90 FLEX analyser corresponding to Measurement 5.

## G.6.2. Optical Sensor Data Logs

| Values at 520 nm and 600 nm |                   |                |                     |                |
|-----------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #   | UV light (405 nm) |                | Blue light (450 nm) |                |
|                             | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                           | 1382.310          | 312.940        | 2737.970            | 455.953        |
| 2                           | 1261.930          | 129.053        | 2304.480            | 342.906        |
| 3                           | 1416.010          | 179.050        | 2771.510            | 447.597        |
| 4                           | 1504.210          | 209.250        | 2719.390            | 452.322        |
| 5                           | 1259.310          | 232.392        | 2455.160            | 419.336        |
| 6                           | 1503.590          | 287.789        | 2707.740            | 382.569        |
| 7                           | 1331.720          | 128.846        | 2403.220            | 397.538        |
| 8                           | 1503.070          | 279.662        | 2544.650            | 458.312        |
| 9                           | 1294.520          | 193.901        | 2581.560            | 368.826        |
| 10                          | 1359.660          | 127.926        | 2375.890            | 330.559        |
| Mean                        | 1381.633          | 208.081        | 2560.157            | 405.592        |
| Median                      | 1370.985          | 201.576        | 2563.105            | 408.437        |

(a) Standard intensity values

| Maximum values (520-530 nm) (593-604 nm) |                   |                |                     |                |                       |              |
|------------------------------------------|-------------------|----------------|---------------------|----------------|-----------------------|--------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                | Light from the source |              |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> | 405 - 410 nm          | 445 - 455 nm |
| 1                                        | 1450.620          | 337.494        | 2737.970            | 622.185        | 3134.230              | 1334.170     |
| 2                                        | 1353.430          | 354.302        | 2384.890            | 509.871        | 3730.820              | 1539.680     |
| 3                                        | 1466.980          | 329.802        | 2893.200            | 562.429        | 3491.300              | 1700.600     |
| 4                                        | 1629.520          | 343.320        | 2875.500            | 554.114        | 3636.210              | 1670.000     |
| 5                                        | 1397.230          | 314.414        | 2615.170            | 583.035        | 3138.110              | 1644.700     |
| 6                                        | 1653.380          | 397.996        | 2855.560            | 555.500        | 3660.450              | 1615.820     |
| 7                                        | 1635.500          | 331.524        | 2688.360            | 532.006        | 3777.180              | 1563.810     |
| 8                                        | 1672.640          | 372.708        | 2765.900            | 556.548        | 3712.690              | 1591.590     |
| 9                                        | 1507.430          | 358.383        | 2753.960            | 558.039        | 3320.910              | 1673.560     |
| 10                                       | 1481.880          | 336.389        | 2595.770            | 538.594        | 3351.890              | 1484.020     |
| Mean                                     | 1524.861          | 347.633        | 2716.628            | 557.232        | 3495.379              | 1581.795     |
| Median                                   | 1494.655          | 340.407        | 2745.965            | 556.024        | 3563.755              | 1603.705     |

(b) Maximum intensity values

| Minimum values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                          | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                        | 1146.590          | 71.572         | 2303.900            | 411.768        |
| 2                                        | 989.880           | 24.676         | 2114.900            | 243.856        |
| 3                                        | 1150.460          | 71.977         | 2555.720            | 261.410        |
| 4                                        | 1316.750          | 140.603        | 2515.120            | 364.585        |
| 5                                        | 1153.810          | 70.190         | 2293.610            | 298.182        |
| 6                                        | 1315.520          | 126.251        | 2566.050            | 286.881        |
| 7                                        | 1331.720          | 128.846        | 2315.500            | 290.986        |
| 8                                        | 1385.600          | 109.481        | 2298.930            | 277.587        |
| 9                                        | 1278.080          | 40.190         | 2402.940            | 286.807        |
| 10                                       | 1263.890          | 83.944         | 2251.200            | 242.779        |
| Mean                                     | 1233.230          | 86.773         | 2361.787            | 296.484        |
| Median                                   | 1270.985          | 77.961         | 2309.700            | 286.844        |

(c) Minimum intensity values

| Min Max Average values (520-530 nm) (593-604 nm) |                   |                |                     |                |
|--------------------------------------------------|-------------------|----------------|---------------------|----------------|
| Intensity<br>Measurment #                        | UV light (405 nm) |                | Blue light (450 nm) |                |
|                                                  | pH                | O <sub>2</sub> | pH                  | O <sub>2</sub> |
| 1                                                | 1298.605          | 204.533        | 2520.935            | 516.977        |
| 2                                                | 1171.655          | 189.489        | 2249.895            | 376.864        |
| 3                                                | 1308.720          | 200.890        | 2724.460            | 411.920        |
| 4                                                | 1473.135          | 241.962        | 2695.310            | 459.350        |
| 5                                                | 1275.520          | 192.302        | 2454.390            | 440.609        |
| 6                                                | 1484.450          | 262.124        | 2710.805            | 421.191        |
| 7                                                | 1483.610          | 230.185        | 2501.930            | 411.496        |
| 8                                                | 1529.120          | 241.095        | 2532.415            | 417.068        |
| 9                                                | 1392.755          | 199.287        | 2578.450            | 422.423        |
| 10                                               | 1372.885          | 210.167        | 2423.485            | 390.687        |
| Mean                                             | 1379.046          | 217.203        | 2539.208            | 426.858        |
| Median                                           | 1382.820          | 207.350        | 2526.675            | 419.129        |

(d) Averaged intensity values

**Figure G.17:** Detailed numerical data logs extracted during Measurement 5. The tables break down the raw intensity readings into (a) standard values, (b) maximums, (c) minimums, and (d) averages, categorized by excitation wavelength.

G.6.3. Comprehensive Intensity Response Graphs

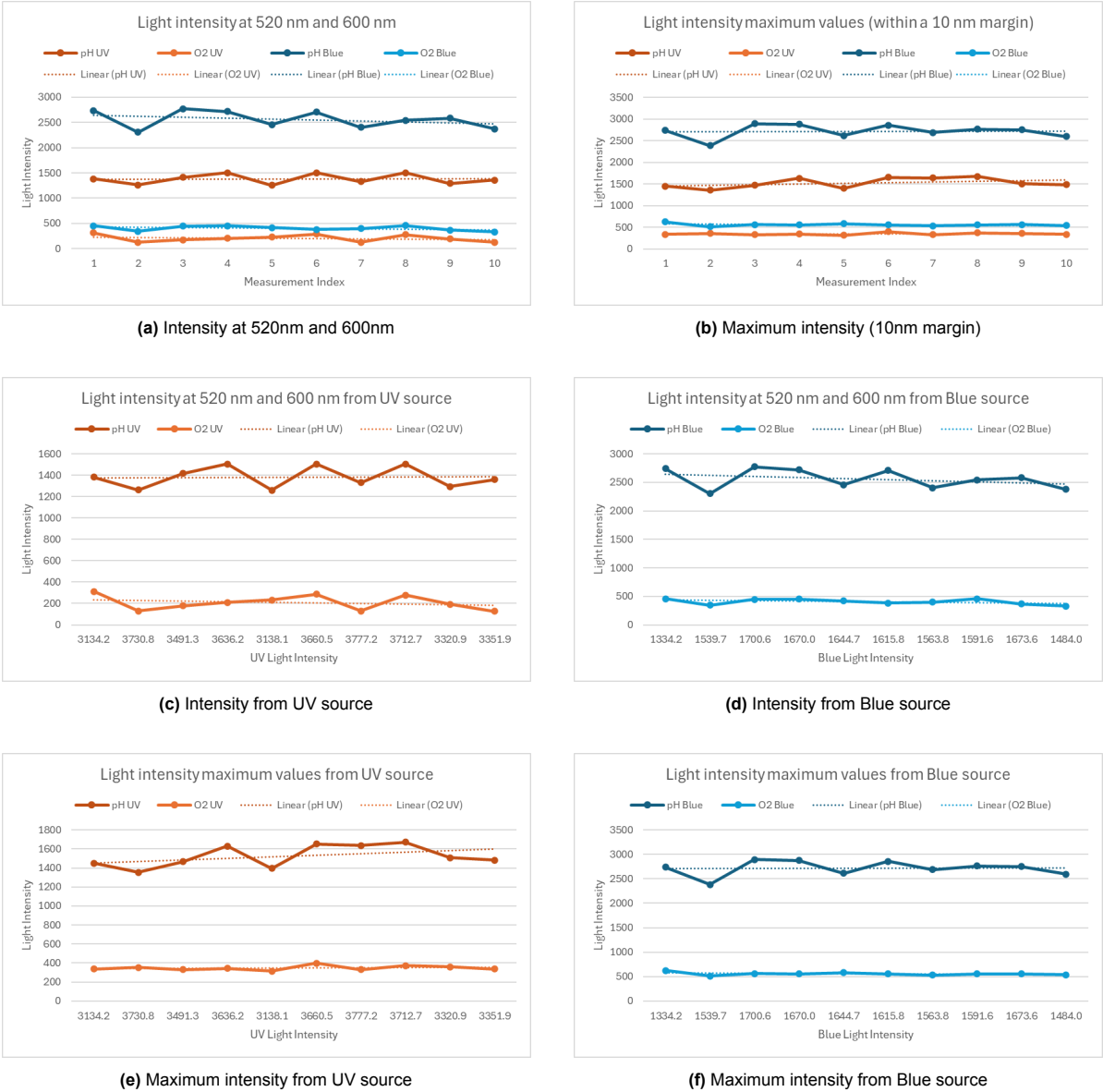
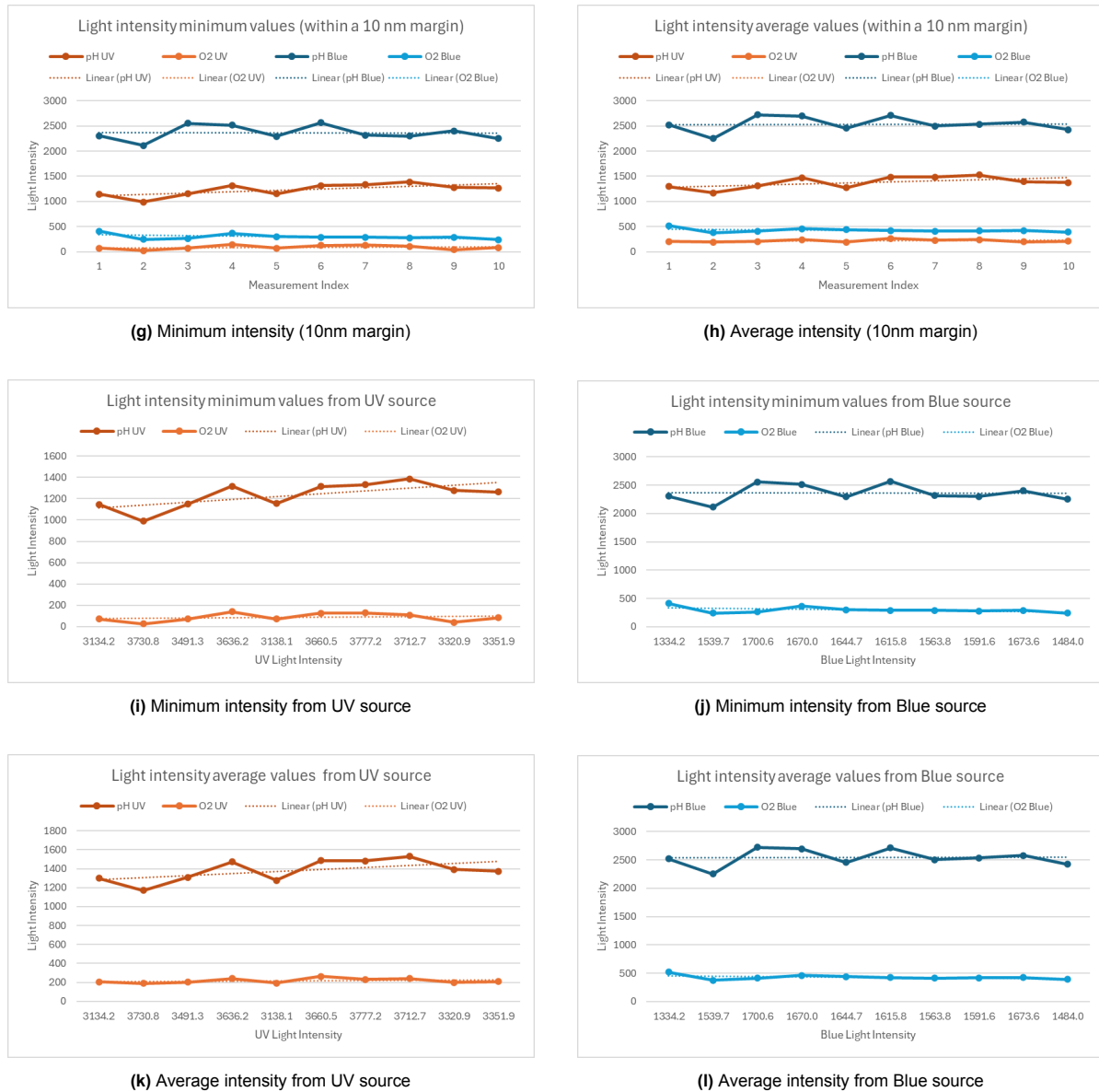
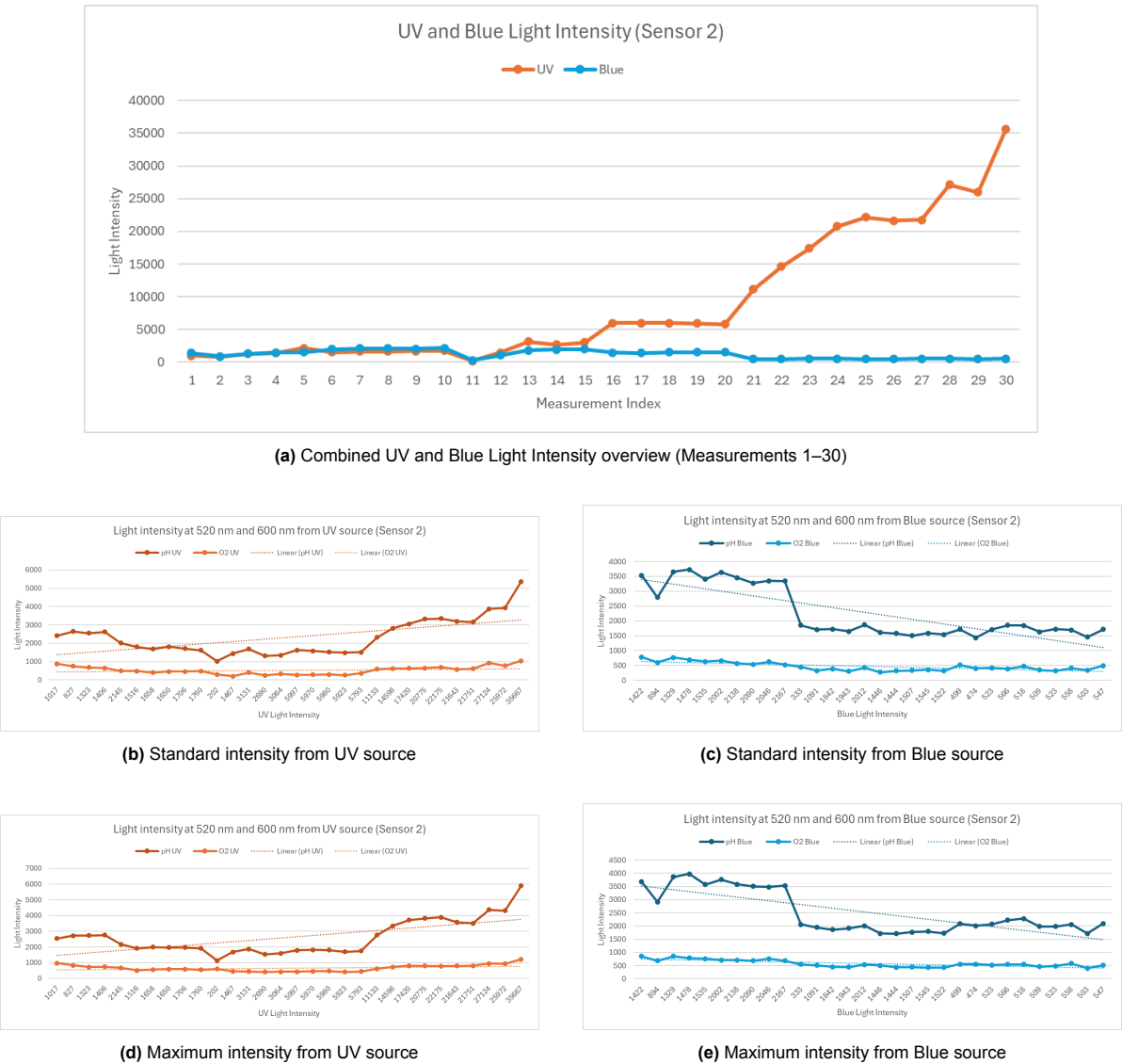


Figure G.18: Comprehensive graphical representations of the recorded light intensities for Measurement 5 (Part 1).

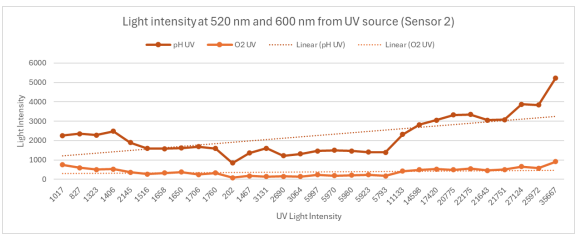


**Figure G.18:** Comprehensive graphical representations of the recorded light intensities for Measurement 5 (Part 2). The complete dataset details standard, maximum, minimum, and average recorded intensities, systematically separated by wavelength and excitation source.

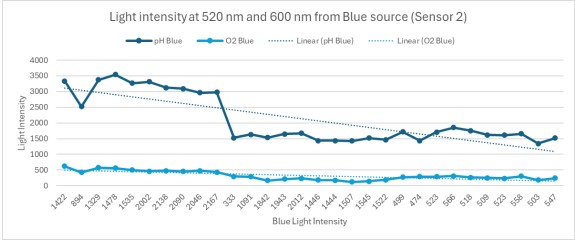
G.7. Combined Measurements: Sensor 2  
G.7.1. Longitudinal Intensity Response Graphs



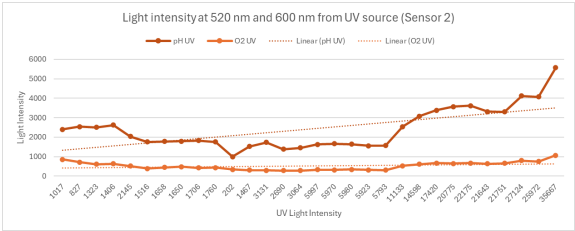
**Figure G.19:** Comprehensive longitudinal graphical representations of the recorded light intensities for Sensor 2 across all experimental measurements (Part 1).



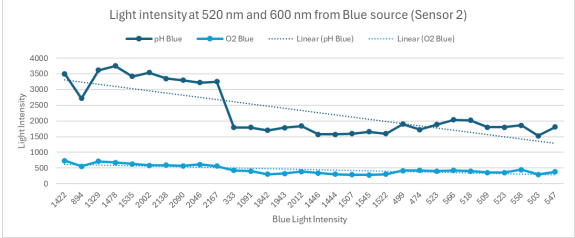
(f) Minimum intensity from UV source



(g) Minimum intensity from Blue source



(h) Average intensity from UV source

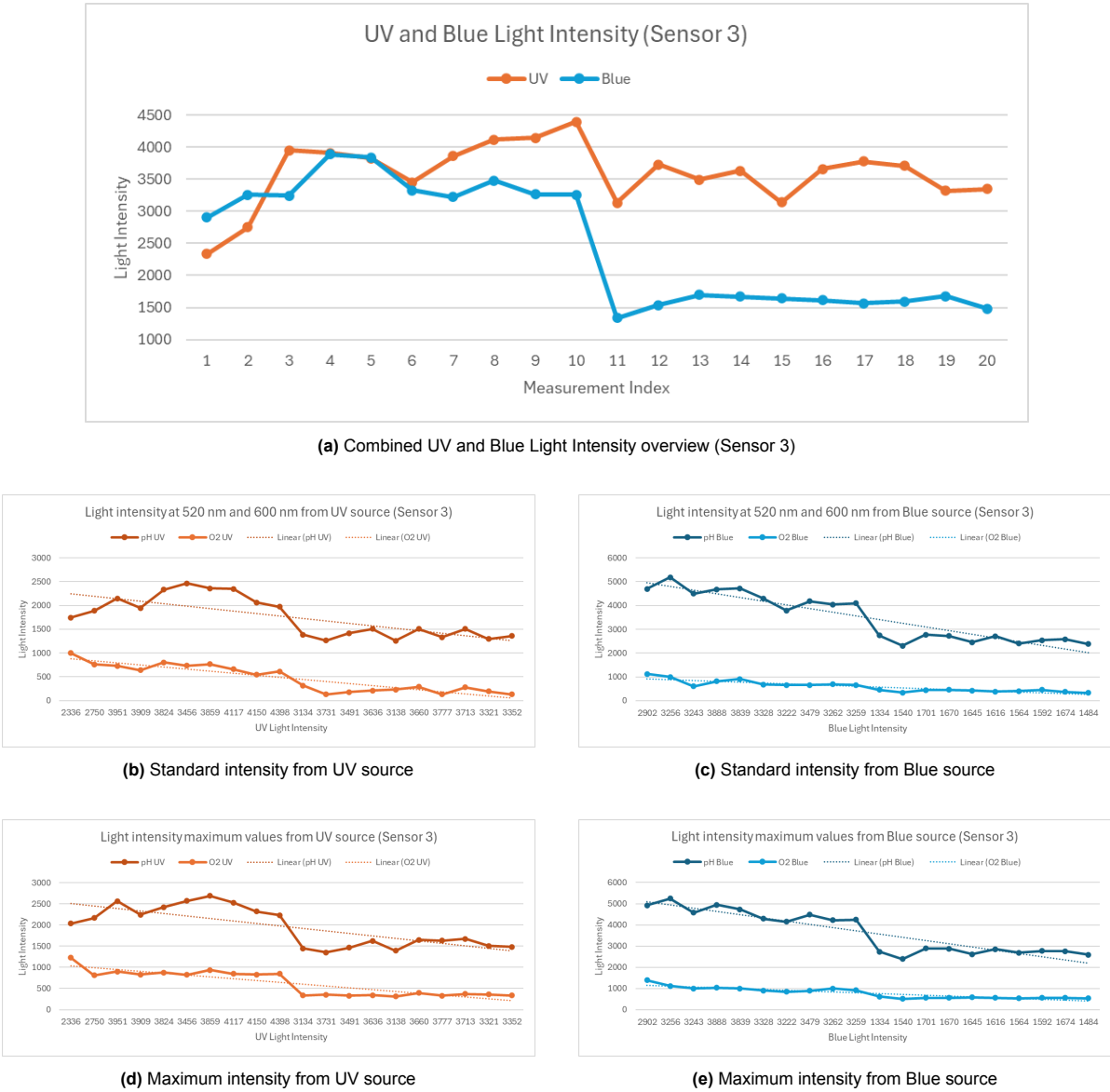


(i) Average intensity from Blue source

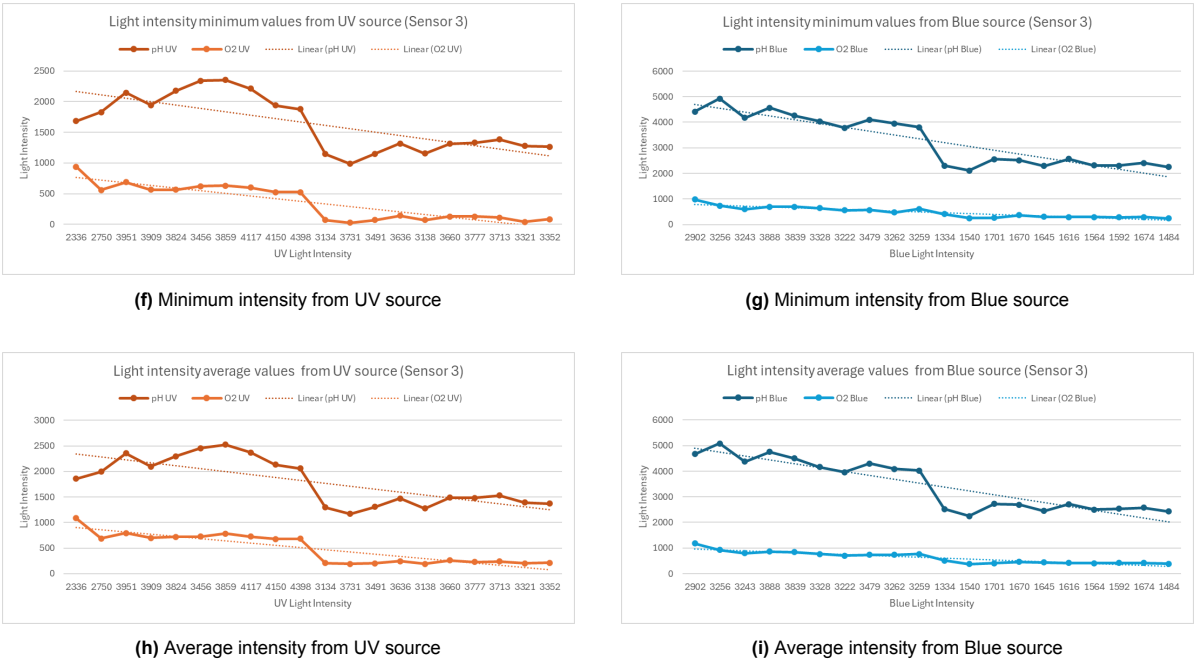
**Figure G.19:** Comprehensive longitudinal graphical representations of the recorded light intensities for Sensor 2 across all experimental measurements (Part 2). The dataset details standard, maximum, minimum, and average recorded intensities to map the sensor's full deployment lifecycle.

G.8. Combined Measurements: Sensor 3

G.8.1. Longitudinal Intensity Response Graphs



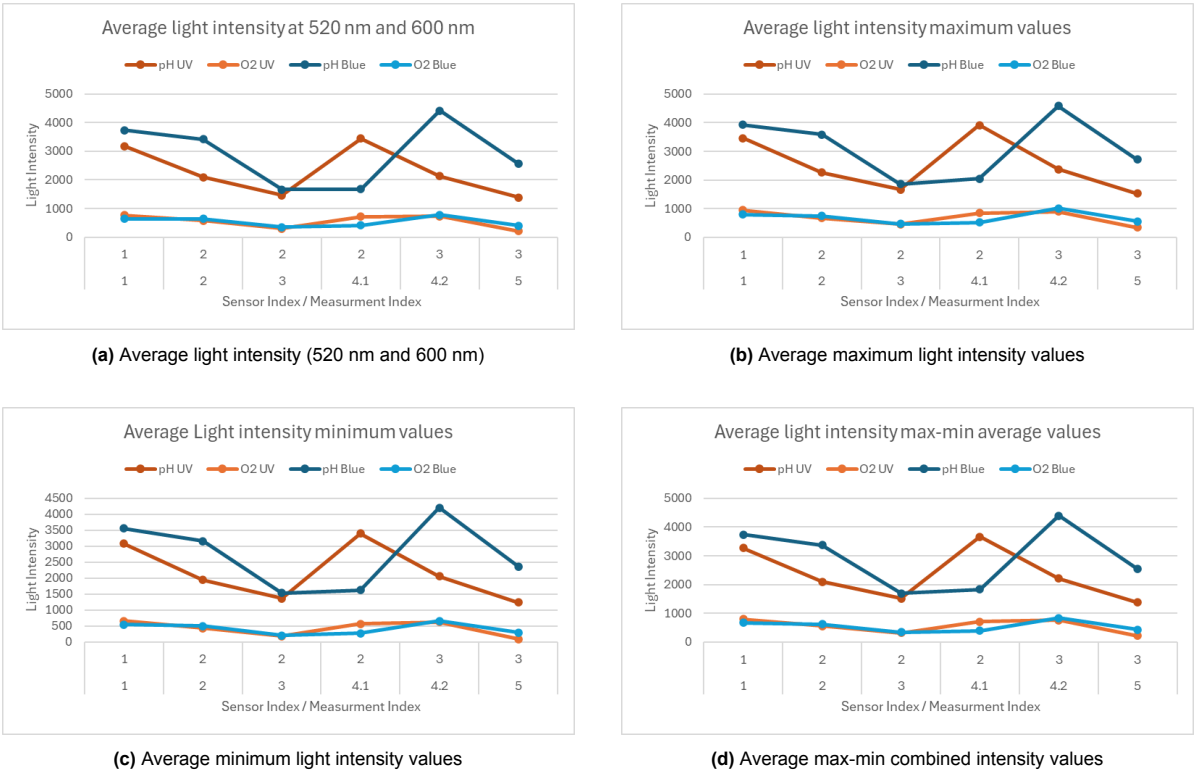
**Figure G.20:** Comprehensive longitudinal graphical representations of the recorded light intensities for Sensor 3 across all experimental measurements (Part 1).



**Figure G.20:** Comprehensive longitudinal graphical representations of the recorded light intensities for Sensor 3 across all experimental measurements (Part 2). The dataset details standard, maximum, minimum, and average recorded intensities to map the sensor's full deployment lifecycle.

## G.9. Combined Measurements: Whole Experiment Summary

### G.9.1. Global Intensity Response Graphs



**Figure G.21:** Global summary of the light intensity trends across the entire experiment. The graphs map the (a) standard averages, (b) maximums, (c) minimums, and (d) combined max-min averages across all respective sensor and measurement indices.