ELECTROHYDRO_DYNAMIC JET PRINTED MULTI-MATERIAL, MULTI-LAYER ELECTRONICS AND INTERFACIAL LAYERS FOR USE IN BIOELECTRONIC INTERFACES

A Thesis Presented

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To My Parents, Sisters, and Christine
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# TABLE OF CONTENTS

**ABSTRACT** .................................................................................................................................................. viii

**Chapter 1 Introduction** ............................................................................................................................... 1

  1.1 Rationale – Electrohydrodynamic Printing ......................................................................................... 3
  1.2 Points of Focus ...................................................................................................................................... 5
  1.3 Organization of This Thesis .................................................................................................................. 7

**Chapter 2 Electrohydrodynamic Jet Printer** .............................................................................................. 8

  2.1 Background .......................................................................................................................................... 8
    2.1.1 Printing ......................................................................................................................................... 8
    2.1.2 Inkjet Printing .............................................................................................................................. 8
    2.1.3 Electrohydrodynamic Printing .................................................................................................... 10
  2.2 Adopted Design .................................................................................................................................. 12
    2.2.1 Overall Design ............................................................................................................................ 13
    2.2.2 Stage ........................................................................................................................................... 13
    2.2.3 Amplifier ...................................................................................................................................... 13
    2.2.4 Camera ......................................................................................................................................... 14
    2.2.5 Leveler ......................................................................................................................................... 14
    2.2.6 Nozzles ....................................................................................................................................... 14
    2.2.7 Code ........................................................................................................................................... 14
  2.3 Modifications of the UIUC Design ......................................................................................................... 15
    2.3.1 DAQ boards ................................................................................................................................. 15
    2.3.2 Hood ........................................................................................................................................... 15
    2.3.3 Ejection Modalities ....................................................................................................................... 16
    2.3.4 Heated Vacuum Chuck ................................................................................................................. 22
    2.3.5 Three Nozzle Setup ..................................................................................................................... 37
    2.3.6 Back Pressure System ................................................................................................................ 41
    2.3.7 Nozzle Fabrication ....................................................................................................................... 42
    2.3.8 LabVIEW Code ........................................................................................................................... 48
  2.4 Ink Formulation ..................................................................................................................................... 55
  2.5 Printer Setup and Function .................................................................................................................... 56
  2.6 Conclusion ........................................................................................................................................... 57

**Chapter 3 Electrochemical Sensor – Nitric Oxide** ...................................................................................... 59

  3.1 Background .......................................................................................................................................... 59
5.4.2 Biohybrid Robotics ................................................................. 141
5.4.3 Rationale for Bioelectronic Fabrication and Use ...................... 142
5.4.4 Bioelectronic LED Interface ................................................... 143
5.4.5 Bioelectronic Electrochemical interface ................................. 143
5.4.6 Bioelectronic Photodiode Interface ....................................... 147

5.2 Methods .................................................................................. 148
5.2.1 S. oneidensis Electrochemical Sensor Interface ....................... 148
5.2.2 V. fischeri Photosensor Interface .......................................... 152

5.3 Results .................................................................................... 156
5.3.1 Interfacial Layers: Collagen and Gelatin ................................ 156
5.3.2 S. oneidensis Electrochemical Sensor Interface ....................... 157
5.3.3 V. fischeri Photosensor Interface .......................................... 158

5.4 Discussion .............................................................................. 159
5.4.1 Electrochemical Sensor Based Interfaces ............................... 159
5.4.2 Photosensor Based Interfaces .............................................. 160

5.5 Conclusion ............................................................................. 161

Chapter 6 Conclusions and Future Work ....................................... 162
6.1 Thesis Summary ...................................................................... 162
6.2 Contributions and Conclusions .............................................. 163
6.3 Future Work ........................................................................... 164

REFERENCES ........................................................................... 171
ABSTRACT

Bioelectronic interfaces and biohybrid technologies have emerged as powerful solutions for sensing and manipulation applications. A unique method of underwater chemical sensing via a combination of engineered microbes and bioelectronic interfaces is discussed here with the goal of reporting to autonomous robotics through an electronic nervous system. The fabrication of these interfaces warrants a deposition method capable of precisely implementing the electronic, interfacial, and biological layers necessary for reliable devices. Electrohydrodynamic jet (e-jet) printing has been shown capable of doing so at a biologically relevant scale of 240 ± 50 nm. An e-jet printing system was fabricated and modified to use additive manufacturing to fabricate heterogeneous interfaces for the biological reporters Nitric Oxide and luminescence. These tasks included the fabrication of electronics that typically have multiple materials and thus multiple layers in their architecture; a potential point of failure in this liquid phase deposition method. The implementation of code based ejection modalities and a heated vacuum chuck allow for controlled volume deposition and rapid solvent evaporation, alleviating dissolution events in the sublayer. By eliminating these events, controlling film thickness to deter pinhole formation, and generating inks with solvents that were incompatible with sublayers, multi-layer electrochemical nitric oxide sensors and photosensors were produced. The fabricated multi-layer nitric oxide sensor has a lower limit of detection range of 188.5 nM - 314.5 nM; levels at which RAW 264.7 macrophage depolarization can be monitored in real time. An alternative bioelectronic interface is being studied wherein a modified version of this sensor is interfaced with *Shewanella oneidensis* to detect aqueous toxins. The photosensor architecture is comprised of a transparent PET substrate, an ITO cathode (pre-deposited), a bulk heterojunction P3HT:PC_{71}BM active layer, and a silver anode, with a final maximum spectral response of 7 mA/W and 90 mA/W/mm² at 514 nm, with a broad visible light spectrum from 400 nm to 620 nm. In interfacing these sensors with *Vibrio fischeri*, emitting light at 490 nm, the concentration of auto inducers in solution has been
monitored in real time. These studies have created a clear path toward the fruition of olfactory
guided, underwater autonomous robotics.
Chapter 1 Introduction

In the Baltic and North Seas alone, there are an estimated 1.6 million metric tons of conventional munitions and over 500,000 metric tons of chemical munitions that have yet to be found from WWII [1]. Since then, underwater mines have sunk or severely damaged four times as many U.S. Navy ships as any other weapon, costing billions of dollars; the impact of the loss of life immeasurable [2]. Though sites are being uncovered intentionally and otherwise, there seems to be a need for a safer method of discovery and deactivation, both in time of peace and in time of war. With the development of autonomous biomimetic robotics [3-7], the platform is in place for broad sweeping searches though a reliable method of munitions recognition is found lacking; specialized bioelectronic interfaces are uniquely suited to fill this void.

The robotics produced in the Ayers lab are unique in their use of an electronic sensory and motor nervous system. The biomimetic descriptor is used in both visualization and in function in that, as in simple animals, the central neural network comprised of command neurons, coordinating neurons, and central pattern generators are modulated by sensory feedback. Currently installed in the RoboLobster are sensory inputs for compass mediated heading control, optical flow mediated stabilization and compensation, bump mediated obstacle avoidance, and antennae bending mediated rheotaxis and surge compensation, the neural networks for which are shown below in Figure 1.1 [8]. The introduction of bioelectronic interfaces aims to provide the autonomous robots with a sense of smell for locating underwater munitions via the specificity of an engineered biological response, reported through a robust electronic network.
Figure 1.1: RoboLobster neural sensory network and subsequent motor dependencies. The subsystems, A: compass impacting rotation and yaw directly and forward and backward motion indirectly, B: optical flow impacting forward and backward motion directly, C: collision detection, halting forward motion, and D: antennal bend impacting rotation and yaw directly and forward and backward motion indirectly, are shown together with their neural dependencies in E. Image adapted from [8].

The long term goal of the current research being conducted in Dr. Ayers’ lab is to create integrated bioelectronic circuitry between a single cell and multiple printed electronics, creating an olfactory taxis for the biomimetic autonomous robotics produced therein; RoboLobster, RoboLamprey, and RoboBee are shown in Figure 1.2 below. The fruition of this long term goal will yield multiple modes of sensory input and sensory confirmation, which can be harnessed for navigation, danger avoidance, and target locating (namely explosives). The achievement of this hinges on the ability to fabricate individual sensors and integrate them with biologicals.

Figure 1.2: A: RoboLobster B: RoboLamprey C: RoboBee
There are a number of methods, both in research and in industry, by which electronics may be fabricated. Traditionally, the bulk of research based electronics have been produced on silicon or other substrates, with a number of clean room lithographic processes, including, but not limited to, vapor phase deposition or doping, metal depositions, photoresist steps, and etching steps. While the clean room environment is key to repeatable production, the number of steps that go into manufacturing something like a photosensor become expensive to set up and wasteful to run using traditional lithography. Thus, other processes have been investigated, many of which fall into the printed electronics category, including screen [9-11], inkjet [12-19], and e-jet printing [20-27]. Though many of these solution based methods are noncontact (to the substrate) and have the benefit of reducing chemical waste, by which saving money, inkjet and e-jet have the additional benefit of being variable. These systems are not only variable in what types of ink they print, but how many inks they can print at one time or sequentially. This advantage allows for both speed and the ability to set up the system once and let the machine do all of the work, reducing man hours and the potential for contamination [25]. Until recently, ink jet processing methods have not come close to the resolution needed to be reproducible and compete with current manufacturing practices. Inkjet printing currently boasts a resolution (narrowest width and spacing of lines) of about 20-30 μm and have become reliable with ink tuning [12, 28, 29]. However, since the formation of a Taylor Cone at the tip of the nozzle produces a droplet that is proportionally smaller than that of inkjet printing upon ejection, e-jet printing can manage reported line widths of 240±50 nm [23]. Additionally, at sufficiently high electric fields, these droplets become a steady stream, creating a uniform line instead of a series of dots forming a scalloped line.

1.1 Rationale – Electrohydrodynamic Printing

Since the inception of electrohydrodynamic jet (e-jet) printing, the fluid dynamics, print quality, and variability of the system have been well studied. With careful preparation of solutions, known as ‘inks’, various crystalline, organic, polymeric, and nanoparticle suspensions can be deposited through noncontact printing methods. Though the e-jet printing platform has been shown to lend itself well to the printing of conductive lines and geometries, electrical components, and various steps of sensor fabrication, there is a noticeable void in the construction of multi-layered electronics and fully printed sensors [22, 23, 26, 27]. However, with additions and alterations to published setup variants, the electrohydrodynamic printing system assembled
herein is uniquely suited to print multi-layered, multi-material electronics. Furthermore, it is well understood that printing technologies are capable of depositing various biologicals, including DNA and protein arrays, cell adhesives such as collagen and gelatin, and even cells themselves [24]. Consequently, in conjunction with the aforementioned sensor printing capabilities, the e-jet printing system is uniquely suited to integrate micro-scaled electronics with biologicals [23]. Examples of e-jet printed features and scale references from Park et al are shown below in Figure 1.3.

![Figure 1.3 E-jet printed features. A: Ring oscillator circuit B, C: 2 μm wide gold lines D: Printed gold source and drain E: AFM image of the source and drain. Reprinted by permission from Macmillan Publishers Ltd: Nature, 2007 [23].](image-url)

The specific objective of the research conducted was to investigate whether electrohydrodynamic jet printing is a viable deposition methodology for multi-layered systems with biological integration. A number of studies have shown the capabilities of printing techniques using a single ink to produce conductive interconnects as well as the deposition of one layer while using other methodologies to deposit the more complex portions of the resultant electronic device [21, 22, 27, 30]. Subsequently, there has been little to no study into the deposition of consecutive layers in these complex electronics exclusively via printing. The deposition of multi-layered bioelectronic interfaces could lead directly to an industrial deposition method and academic research platform, and is thus, the most important immediate contribution of the herein research.
The communication between electronics and living organisms is an ongoing focus of research in both higher education and industry. By harnessing the light generation of bacteria such as *Vibrio fischeri* and *Vibrio harveyi* in conjunction with the printed photosensor [31-37], as well as a nitric oxide detection limit that would allow the detection of nitric oxide produced by RAW 264.7 macrophages during rapid depolarization [38], the work herein demonstrates a method for full bioelectronic deposition, in addition to showing the viability of a ‘bomb sniffing’ robotic nose. Furthermore, the expansion of the nitric oxide sensor into an electron detection method is currently being tested and highlights the long term viability of the produced electrochemical sensor in cellular culture. In terms of the robotics research done in the Ayers laboratory, the ability to sense light and specific chemicals, namely nitric oxide (NO), is the first step in parallel research to create bomb ‘sniffing’ robotics. Yeast and bacterial cells that detect dinitrotoluene (DNT), a product of TNT degradation, and report NO through NO synthase and/or light through luciferase expression can then be harnessed to selectively detect underwater mines when integrated into the preexisting electronic nervous system of the autonomous underwater robotics currently under research in the Ayers lab [3-7]. These proposed bioelectronic sensors can assist in the autonomous search, identification, and destruction of underwater explosives. Furthermore, the ability to e-jet print onto a number of substrates and in various geometries allows for the customization of sensors and the maximization of surface area for a robotics field that continues to see a reduction in platform volume. This contribution is significant because it has the potential to provide the current robotics landscape with a variable deposition method capable of producing custom, singularly selective, sensing platforms.

### 1.2 Points of Focus

In order to electrohydrodynamic jet print multi-layer, multi-material functional electronics that are capable of detecting biological expressions, there are a number of challenges and parameters that need to be considered. These challenges and parameters are briefly outlined in this section.

**Inks:** Ink selection and formulation is paramount to reproducibly printing using micro and nanometer inner diameter nozzles. There are three main parameters to consider; viscosity, surface tension, and concentration. These parameters need to be optimized to achieve appropriate droplet ejection.
**Substrate Selection:** The choices made in the setup of the e-jet printer limit the thickness of the potential substrates. Too thick (approximately >1.0 cm, but depends on conductivity of substrate) and the electric field will be too altered to control printing. Additionally, the electronic being printed impacts the substrate choice in that LEDs and photosensors need a transparent substrate. Substrate longevity is of concern as the end goal for these sensors implies long term exposure to salt water or cellular media.

**Adhesion to Substrate:** The degree to which the deposited inks attach to the chosen substrate is important, again due to exposure to aqueous solutions. Any delamination event would impact signal or destroy the sensor completely.

**Dissolution of Underlying Layers:** Of major focus in the studies herein is the dissolution of the underlying layer when printing multiple layers. In order to print functional electronics, the deposition of multiple layers is necessary, as is when printing advanced electronic structures. Solvent selection, low volume deposition, and rapid curing are points of emphasis to combat this issue.

**Curing Methodologies:** As inks are cured, their internal characteristics and interaction with adjacent layers depends on the curing practices employed. Silver resistivity, glass transition points, heterojunction dispersion, and substrate longevity all rely on the curing methodology to create functional, long lasting electronics.

**Encapsulation:** The process of encapsulating the final electronics is necessary not only due to planned aqueous solution exposure, but also because a number of inks and cured films react readily with the air and its moisture content. A long lasting encapsulation method should not dissolve the underlying electronics, or alter their performance to a significant degree. Furthermore, it should still allow interaction between the electronics and the biologicals, thus creating a bioelectronic interface.

**Interfacial Layers:** Whether the sensors are used in cellular solution, under a biofilm, in agar, or otherwise, some sort of cellular attachment, preferably controllable attachment, is necessary to achieve the most direct interaction between the biologicals and the printed electronics.

**Biological Integration:** Cellular growth, concentration, and attachment are all necessary to demonstrate the feasibility of these electronics and their potential use in munitions detecting
bioelectronic interfaces. The goal of this research is to provide the groundwork needed to reach this final functional form.

1.3 Organization of This Thesis

This thesis is organized as follows. The thesis is opened up above with a brief description of the overall objective of the funded research and the need for the work presented here, with a brief explanation into why e-jet printing was chosen as the deposition methodology. Chapter 2 further investigates the e-jet printer and discusses the challenges, solutions, and improvements made in order to be able to create, print, and cure the various inks required as layers in the discussed sensors. Chapter 3 describes the multi-layer deposition methodology of a photosensor and its subsequent sensor characteristics. Chapter 4 touches on the various configurations of the multi-layer nitric oxide electrochemical sensor, as well as its possible use as a general chemical sensor in detecting other molecules. Chapter 5 shows the use of the photosensor as a bioelectronic interface and continued research with the electrochemical sensor with a potential electron detection interface, demonstrating the feasibility of using the printed sensors as bioelectronic interfaces and their flexibility in doing so. A conclusion provides an overview for the discussed research herein as well as future directions for these promising results.
Chapter 2 Electrohydrodynamic Jet Printer

2.1 Background

The goal of this research is to join multiple electrohydrodynamic printed organic, polymer, metallic, insulating, and biological layers resulting in interfaces between electronics and microbes to enable a sense of olfaction to mediate odor guided rheotaxis in biomimetic underwater robots [39, 40]. It integrates contributions from additive manufacturing, organic electronics, robotics, and biohybrid technology based on principles of synthetic biology. There are distinct advantages to a liquid based deposition process with noncontact ejection modalities; commonly, this deposition methodology is known as ‘printing’.

2.1.1 Printing

Additive printing technologies came into popularity in the mid-1970s, improving upon the deposition methods of the time through an improved reliability, precision, and scale [41]. Though printing methods were primarily studied for their ability to efficiently print memoranda, the decade saw an expansion in applications, a deeper understanding of the deposition characteristics, and an improved quality of print.

2.1.2 Inkjet Printing

In the most basic terms, inkjet printing technology is the spraying of ‘ink’ onto a substrate. This can be done many different ways and onto many different types of substrates [12, 13, 15, 17-19]. The rise of this technology occurred in the mid-1970s, when printing companies realized the potential for inkjet printing to replace the current matrix dot printing methods [41]. Thus, in its infancy, the main monetary and scientific contributors came from printing companies like IBM, Canon, and Epson. As research progressed, into the early 1980s, the methodology of ejecting droplets onto the substrates began to fork. IBM began to use continuous inkjet printing by ejecting charged particles onto a surface [42], Canon focused on using thermodynamics to push
droplets out on demand, while Epson utilized piezoelectric effects to achieve the same [43]. Along with government entities such as NASA [44], various other scientific communities began to see the benefit of inkjet printing as a graphical and analytical platform [45, 46]. It wasn’t until the late 1990s that inkjet printing began to be thought of as a feasible platform that could be employed, not only to further scientific research, but may also be useful in industries such as electronics fabrication and drug discovery [47].

Consequently, the end of the 20th century saw an expansion in research that utilized the inkjet platform, printing viable mammalian cells (Figure 2.1 A1-A4) [48], electronics incorporating various conductive polymer and metal inks (Figure 2.1 B) [49, 51, 52], protein arrays, DNA arrays, transistors (Figure 2.1 C1-C2) [18], light emitting devices (Figure 2.1 D1-D3) [50, 53, 54], and
biosensors [12, 55]. Following this line of progression, more recently, research has trended toward making the inkjet printing system industrially viable, including printing disposable and flexible electronics [28, 29, 56-58], printing graphene (a strong, electrically efficient conductor) [59], printing polymers that may replace ITO (a clear conductor used in LEDs and photoelectronics) [60], and in tissue engineering and regenerative medicine [61]. In these cases, the smallest droplet sizes created are approximately 10-20 μm in diameter, with a standoff height > 1mm [18, 58, 62], resulting in the narrowest continuous lines or reliably created gaps being 20-30 μm in width when not utilizing pre or post processing steps [12, 28, 29]. Inkjet printing has also shown the capability to print fully developed microelectromechanical systems (MEMS) [63] as well as biohybrid systems [64], further demonstrating the capabilities and potential for solvent based micro/nano deposition methodologies in laboratory and manufacturing roles. Though these studies show the flexibility and potential value to using liquid phase deposition, the studies presented have two distinct disadvantages over traditional lithography; the size scale does not approach that of lithography and there is a deficiency in the demonstration of producing multiple layers exclusively using inkjet printing. However, electrohydrodynamic jet printing significantly reduces the theoretical and demonstrated scale, and the studies herein have produced functional layered electronics.

2.1.3 Electrohydrodynamic Printing

Electrohydrodynamic jet (e-jet) printing, in name and function, relies on electrohydrodynamics (EHD) via the study of the ionized particles and their interaction with electric fields and surrounding fluids [65]. In essence, it is the study of the direct conversion of electrical potential into kinetic energy; the first observed examples of which go back to Niccolo Cabeo in 1629 when he noticed that sawdust was attracted towards an electrified body, and once in contact with said body, would proceed to be repelled by it [66]. This was subsequently coined by Francis Hauksbee in 1709 as electric wind [67], observed by Tiberius Cavallo whilst watching a fly in 1777 [68]. Later in 1834 [69], Michael Faraday correctly hypothesized that the wind was due to the collisions of charged and uncharged gas particles, which is now commonly referred to as ionic wind. A basic representation of the EHD effect and its resultant particle stream between two electrodes is shown in Figure 2.2 and provides a visualization for how the deposition theory works [70, 71].
Figure 2.2 Ionic Wind: Positively charged ions stream from a source to a collector, running into neutrally charged particles, creating a ‘wind’. Adapted with permission from 2009 IEEE [71]

Recently, this phenomenon has been exploited to deposit complex geometries onto various substrates; the first major quantitative demonstration of which was by Jang-Ung Park via printing high resolution images on a micrometer scale as well as printed electronics [23]. This study not only showcased the feasibility of printing with the same resolution (10μm) as inkjet printing, but took it further, managing line widths as small as 240±50 nm. The reason that the e-jet system can produce smaller line widths is because as the voltage is increased on the anode (nozzle), a Taylor cone is formed within the inner diameter from which the droplets are ejected. Since this Taylor cone apex is inherently smaller than the inner diameter of the nozzle, a comparatively smaller droplet is ejected. In 2008, Hong Kyoong Choi developed a mathematical model to explain the jet pulsations and the scaling laws involved in the same e-jet printing system, highlighting the importance of the viscosity and surface tension of the inks, along with the length of the nozzle itself [72]. As systems shrink in size to the micro and nano-scale, as in the fluid dynamics governing droplet ejection, the parameter influence changes; Choi et al mathematically evaluated these scaling laws. Figure 2.3 shows a schematic visualization of the e-jet printing system, including a AuPd sputter coated pulled glass nozzle, Taylor cone formation as voltage to the nozzle is increased, and material ejection [23, 25].
The e-jet system holds additional advantage over other printing systems due to the innate charge contained within ejected droplets, the polarity of which is controlled via the applied polarity to the nozzle [73]. It has been shown that these charges, of controlled magnitude, can be retained over time when in an amicable environment (low humidity), and show potential uses via printed security codes, controlled assembly of charged particles, and modulation of biological system activity [73] Park utilized these charges to deposit oligonucleotide arrays [24]. As these initial studies demonstrated the functionality of e-jet printing, research into the deposition of various inks has displayed its versatility; including the printing of nanoparticle solutions such as gold, copper, and silver [21, 22, 74, 75], the printing of biopolymer scaffolds, nano-hydroxyapatite, and other biomaterials [26, 76, 77], and even 3D printing a rectangular walled container from zirconia [78]. Furthermore, though the one nozzle system is attractive for research purposes, a multi-nozzle system allows for the printing of multiple lines to save time during the printing process, and lends itself to becoming a multi-nozzle, multi-ink system for rapid printing of multiple layer electronics and multiple sensor types using similar inks [21].

2.2 Adopted Design

E-jet printing requires a number of separate systems to make up the whole of the printer. These systems provide three dimensional planar and motion control, high voltage supply and wave form generation, back pressure, various curing methodologies using both heat and UV light, and microscopic optics for stage visualization. This section discusses the original design, construction, and implementation of these subsystems as well as the various solutions to design constraints and their advantages and disadvantages.
2.2.1 Overall Design

The general layout of the e-jet printing system, as well as the major components necessary for basic printer function were supplied by Sutanto et al [25, 79]. Though modifications were later made, motion in the x-y planes, amplified connection to nozzles, printing optics, substrate leveling system, and conductive nozzle design were initially replicated via Sutanto et al. All parts discussed herein were attached to a standard optics breadboard to keep everything in place and to control the distances between various pieces of equipment such that the nozzle is centered over the substrate.

2.2.2 Stage

The design of the e-jet printer has an x-y plane static nozzle, thus requiring a dynamic substrate. The motion of the substrate needs to be fluid, precise, and capable of stepping at the nano and micro scale, based on the planned features. Commercially available stages have the desired characteristics. The optimal stage is available from Aerotech and included an A3200 controller as well as X and Y drivers that can be controlled via software (Motion Composer) provided by the company. This system was later integrated into a LabVIEW GUI used to control the printer. The stage travel is 110 mm in both the x and y with 1 nm resolution, while having a wide range of precise velocity options from μm/sec to hundreds of mm/sec. The vacuum chuck described later is designed to attach to the stage via standard threaded holes on the top of the stage.

2.2.3 Amplifier

To generate the high voltages necessary for ink ejection an amplifier of some sort is necessary. As specified by Sutanto et al, an amplifier from TREK (Model 667N-L-CE) with a gain of 200 that can be controlled via LabVIEW was integrated into two SCC-68 I/O DAQ boards from National Instruments. The voltage is controlled via an analog output of -10 to 10 volts from the DAQ boards to the amplifier translating to a -2000 to 2000 V output to the nozzle. The various ejection modalities, discussed below, are controlled using the -10 to 10 volt range and result in an amplified modality.
2.2.4 Camera

Since printing is currently not automated as in production inkjet printers, a vision system is necessary to see where and how well the printer is depositing material. We adapted a Lumenera Infinity 2-2 microscope color camera as well as a 2x fixed focal length lens, a microscope zoom lens, lens holders, and x, y, and z stages for full range of motion, all purchased from Edmund Optics [25]. The camera was interfaced to the LabVIEW GUI produced herein via USB connection. An MI-150 fiber optic Quartz Halogen illumination system Edmund Optics to illuminate the substrate.

2.2.5 Leveler

The offset height of the e-jet nozzle is a major factor in the controlling of the electric field and thus the ejection rate. During stage motion, if the substrate is not level this offset height will change and runs the risk of substrate/nozzle contact. A small leveling stage was purchased from Edmund Optics (E-970569) to level the substrate in reference to the nozzle, independent of the rest of the e-jet setup. Once set, this leveling does not need to be altered, assuming precise substrate width.

2.2.6 Nozzles

The initial nozzle setup was adapted from Sutanto et al, wherein a pre-pulled glass pipette purchased from World Precision Instruments was sputter coated, using an AuPd target on a Denton Vacuum Desk II. This allows for a precise nozzle inner diameter and less variability during initial prints. These nozzles would be replaced by ones produced in house, as discussed below.

2.2.7 Code

The initial basic LabVIEW code suite was provided by Sutanto and Alleyne and supported the control of the DC electrical connection and back pressure for one nozzle. This initial code helped greatly to test the mechanical and electrical setup as opposed to troubleshooting code, but would later be vastly modified as discussed herein.
2.3 Modifications of the UIUC Design

To adapt the initial UIUC (Sutanto and Alleyne) design [25, 80] to fabricated the bioelectronic interfaces needed for biohybrid sensing, we explored a variety of adaptations and configurations to optimize the sensitivity and reproducibility of printed interfaces.

2.3.1 DAQ boards

The data acquisition (DAQ) boards used to send and receive information with the e-jet printer and its various parts is handled through two SCC-68 I/O connectors from National Instruments (NI). Both communicate to the computer through one internal NI card in an Apple tower. Initially, the LabVIEW GUI and other software were handled in Windows XP, though the final LabVIEW GUI is planned for a Mac OS release to utilize the original tower design.

2.3.2 Hood

The addition of a hood was necessary for two separate reasons; general purpose laminar flow to keep dust out, and ductless fume expulsion during ink production and nozzle filling. A general purpose Purair Basic ductless fume hood was purchased to serve the latter purpose. The breadboard was placed on a large piece of plywood painted black, and the hood was placed over it as seen in Figure 2.4 below, sealing the edges for one point of airflow through the window in front. The ductless version was purchased in order to limit the need for outside duct work. This system was modified to produce a laminar flow from the top of the installation down through the working area, such that it could easily be changed back if a large volume of any volatile chemical was used. It was also believed that a modification from ductless to laminar was easier than the reverse orientation. The laminar flow through various filters provides a dustless environment during printing and a bio-friendly environment to ensure monoculture retention when cellular solutions are being handled before, during, and after printing.
2.3.3 Ejection Modalities

The frequency of the ejection of droplets from the Taylor cone not only relies on the amplitude of the voltage applied to the nozzle [72], but also the electric field created by the ejection modality. This form of the electric field can change the ink deposition from a constant line ejection, to a high frequency droplet ejection even though the same voltage is used. Furthermore, it can lead to more consistent printing practices by creating a neutral film potential, as well as modulating the amount of material deposited. Provided in each of the modalities below is a figure showing the corresponding block diagram on the top and the output taken via oscilloscope on the bottom of the figure.
DC Mode

The DC ejection modality is a constant voltage set by the user, shown as a constant voltage in the bottom of Figure 2.5. It is the modality brought over from the Sutanto and the Alleyne group and can deliver droplet ejections at a frequency prescribed by the set voltage, as well as constant droplet ejection (line) at a higher voltage. The frequency of these droplets is capped by this conversion to constant ejection, thus high frequency droplet ejection is not possible with this modality, which would be advantageous to use, for example, in rapid motion deposition circumstances.

![Figure 2.5 DC ejection modality block diagram with the user input of ± 2000 volts being translated to ± 10 volts that is output to the 200 gain amplifier. The user is supplied a display of the output amplitude and modality that they have selected. The voltage is output based on the modality radio button and the ‘Send Signal’ Boolean, which, when ‘False’, outputs an amplitude of 0 volts regardless of the modality selected.](image)

(top) Resultant waveform at 100 volt amplitude (bottom).
Pulse Mode

The Pulse ejection modality prescribes a spike amplitude and a frequency at which this amplitude is pulsed at via the block diagram seen in Figure 2.6. The pulses always originate from zero and the duty cycle is set to 10% internally but can be modified within the Simulated DC Signal 2 block. The spike height needs to be greater than the ejection voltage ($V_E$) necessary for deposition, with voltages higher than $V_E$ creating larger drops as the spike height is increased. This modality cannot create a constant ejection, though a deposited line can be created by tuning the frequency with the stage movement. This line can be thinner in both height and width than the DC voltage and cure rapidly thanks to the smaller volume ejected in each pulse compared to the DC modality. The pulsed square wave is shown on the bottom of Figure 2.6.

Figure 2.6 Pulsed ejection modality block diagram with the user input of ± 2000 volts being translated to ± 10 volts that is output to the 200 gain amplifier. The user is supplied a display of the output amplitude and modality that they have selected. The voltage is output based on the modality radio button and the ‘Send Signal’ Boolean, which, when ‘False’, outputs an amplitude of 0 volts regardless of the modality selected. (top) Resultant wave form at 100 volt amplitude at 10 Hz (bottom).
Pulse Around Mode

The Pulse Around ejection modality, governed by the block diagram seen in Figure 2.7, allows the user to set a voltage from which to pulse around, as well as the spike amplitude above and below this voltage and the frequency of these pulses. Similar to the Pulse modality, the duty cycle is set internally at 50% and can be altered in the Simulate DC Signal 3 block. Theoretically this modality would have quicker switching than that of the Pulse modality, though this was not used during production of any sensors because of the additional tuning in the set voltage and the spike height required for full turn off at the lower voltage and ejection at the higher. The resultant voltage is shown in the bottom of Figure 2.7.

![Block diagram of Pulse Around mode](image)

Figure 2.7 Pulsed Around ejection modality block diagram with the user input of ± 2000 volts being translated to ± 10 volts that is output to the 200 gain amplifier. The user is supplied a display of the output amplitude and modality that they have selected. The voltage is output based on the modality radio button and the ‘Send Signal’ Boolean, which, when ‘False’, outputs an amplitude of 0 volts regardless of the modality selected. (top) Resultant waveform pulsing around a 100 volt amplitude with a spike height of 25 volts at 10 Hz (bottom).
Hover Pulse Mode

The Hover Pulse ejection modality, governed by the block diagram in Figure 2.8, is similar to the Pulse Around in that it allows you to set a higher starting voltage, but this time set point is where you pulse from, not around. The spike amplitude governs the amplitude of this pulse and the frequency is again set by the user. The duty cycle is set to 50% and can be altered in Simulate Signal 5. This modality is easier to tune than the Pulse Around, though it was not tested in produced electronics. Similar to the Pulse Around modality, the wave form is shown in the bottom of Figure 2.8.

![Hover Pulse ejection modality block diagram](image)

Figure 2.8 Hover Pulse ejection modality block diagram with the user input of ± 2000 volts being translated to ± 10 volts that is output to the 200 gain amplifier. The user is supplied a display of the output amplitude and modality that they have selected. The voltage is output based on the modality radio button and the ‘Send Signal’ Boolean, which, when ‘False’, outputs an amplitude of 0 volts regardless of the modality selected. (top) Resultant wave form pulsing from a 100 volt amplitude with a spike height of 25 volts at 10 Hz (bottom).

Switch Mode

The Switch ejection modality pulses from the set voltage, to the negative of the set voltage, to the positive of the set voltage, and back to the set voltage in a square wave at a duty cycle of 50% set in the Simulate DC Signal 4 block; a visualization of this is shown in the bottom of Figure 2.9. This modality, mainly used when printing silver, ejects markedly higher volumes of ink
in the form of droplets or in a ‘broken constant flow’ type ejection state, depending on the set frequency. The switch from positive to negative (or vice versa) collapses the Taylor cone and ejects a larger droplet than otherwise ejected. If the voltage is set to a state where droplet ejection occurs, positively charged droplets will eject when on the positive side of the square wave and negative when on the negative side. If set to a point where the ejection state is continuous, then a positively charged line will eject on the positive side of the square wave and negative on the negative side. The advantage this delivers is the possibility of repeatedly depositing a comparatively large area, key in producing and layering silver contacts for sensor connection and for creating large uniform sensor bases that may otherwise cure before the next line of material is deposited next to it, creating curing overlaps. Additionally, this creates a net neutral deposition state by depositing equal volumes of positive and negatively charged droplets before the ink is cured on stage.

Figure 2.9 Switch ejection modality block diagram with the user input of ± 2000 volts being translated to ± 10 volts that is output to the 200 gain amplifier. The user is supplied a display of the output amplitude and modality that they have selected. The voltage is output based on the modality radio button and the ‘Send Signal’ Boolean, which, when ‘False’, outputs an amplitude of 0 volts regardless of the modality selected. (top) Resultant wave form switching around zero with an amplitude of 100 volts and a frequency of 10 Hz. (bottom).
2.3.4 Heated Vacuum Chuck

Once the e-jet printer was assembled with the base components and the GUI was established to control them, the first major physical addition to the printer was a vacuum chuck to hold various substrates level during printing. The original vacuum chuck did not fit the necessary design parameters [80]. These parameters included the ability to hold large and small substrates, hold firm and flexible substrates, the material under the substrate had to be electrically conductive to function as a ground to the nozzle’s high voltage, and the base needed to be an insulating material to isolate the conductive ground from the rest of the printer.

![Figure 2.10 3D rendering of polyoxymethylene base (left) and aluminum chuck (right) for the fabricated vacuum chuck](image)

As seen in Figure 2.10 a two-piece design was employed wherein an aluminum plate, with 1mm through holes, was used as a ground that would hold the substrates, connected to amplifier ground via the screw connection that attached it to the insulating polyoxymethylene base. This two part vacuum chuck was attached to the top of the Aerotech stage. The screws that connect the aluminum to the amplifier ground also attach it to the insulating base, creating a seal outside of the square of holes with an o-ring. A hole from within the base is attached to a Rocker 300 oil-less vacuum pump, thus creating a point of vacuum when a substrate is placed on this 20 mm by 20 mm square. This design can facilitate substrates that are smaller than 400 mm² by using Kapton tape to cover unneeded vacuum area, as well as substrates up to 40 mm by 40 mm, which is the geometry of the aluminum plate.
Chuck Thermal Management

Solvent selection is a key ink preparation decision. Not only does it have implications on ink ejection performance, but also impacts resolution and deposition characteristics once on the substrate. The more unwelcome ink/substrate interactions can be mitigated by ink preparation, substrate manipulation, and as discussed below, through rapid primary curing, called ‘soft curing’ herein. When the first two techniques fall short due to stubborn particles or solutes, and/or purchased stable inks are made with solvents that remain in liquid phase post ejection, rapid soft curing can be used to improve resolution, reduce ink spreading, and stabilize the drying process. In order to employ this soft curing process, a heated vacuum chuck was designed and installed, utilizing a PID controller, a solid state relay (SSR), and resistance wire heating element (P2128N80DGS, Pelican Wire).

There were a number of goals and constraints that were observed during the design of this component of the printer. First and foremost, the system needed to deliver a temperature capable of curing the high boiling point silver ink (approximately 45°C). Furthermore, the rise time must be under 10 minutes and must maintain temperature under vacuum. The final control of the system must be able to be integrated into the LabVIEW GUI. Since the aluminum chuck sits on a plastic holder, the temperature applied to the chuck cannot exceed the melting temperature of polyoxymethylene (175°C). Finally, the current applied to the heating element must not interfere with the electric field used for droplet ejection from the nozzle.

Various configurations have been studied to understand how to optimize performance, though the SSR (switch to regulate current flow through resistance wire), high current resistors (to optimize current amplitude), cooling fans (to cool high current resistors), glass insulated K-type thermocouple (5TC-GG-K-36-72) from Omega Engineering (real time monitoring of plate temperature), and mechanical on/off switch (to mechanically ensure power is off when not in use) remained constant. Figure 2.11 below shows the initial electrical diagram, which used an off the shelf PID controller to handle the set temperature, PID settings, thermocouple, SSR, and incoming power.
The configurations, as discussed above, revolved mainly around the application of the resistance wire to the chuck itself, chiefly to examine how heat transfer functioned with the materials being used. The first configuration was to tape the resistive heater to the bottom of the vacuum chuck and apply heat while not on the stage. (Blue dots in Figure 2.14)

The second configuration involved milling grooves along the bottom of the aluminum chuck wide and deep enough to insert the heating element. This approach reduces the thickness of aluminum that the heat from the element has to traverse in order to reach the surface and substrate. A CNC mill was used to generate a pattern 0.9 mm deep and 0.75 mm wide that was large enough to fit the 0.55 mm thick heating element without abrading its insulation during installation. For this configuration, this insulation is the only layer between the resistance wire and shorting the aluminum ground. The pattern was designed to maximize the amount of area covered by the element while being constrained by the o-ring for the vacuum chuck. The designed and fabricated aluminum chucks are shown in Figure 2.12.
Figure 2.12 Fabrication of the heated vacuum chuck. A 3D rendering was used to determine an appropriate milling pattern (left). The aluminum chuck was milled to allow enough room to avoid insulation abrasion (center). A representation of the heating element and thermocouple in their final orientation (right); the tape was replaced with thermally conductive, electrically insulating epoxy.

Once again, the heating element was taped into these grooves using Kapton tape, and the surface temperatures were recorded for incremental set temperatures on the PID controller. As shown in Figure 2.14 (red dots), the slope of the linear relationship increased, giving higher surface temperature per set temperature as expected.

Finally, a portion of the grooves were used to permanently install the heating element using a thermally conductive, electrically insulating one part epoxy (50-3122 One Component Epoxy) from Epoxies Inc. (cured at 175°C for 20 minutes). At the same point, the epoxy was used to permanently install a K-type thermocouple on the underside of the chuck, seen in Figure 2.13. The wires used to access the heating element and thermocouple were bundled together, exiting the chuck holder through a sealed access hole.
Figure 2.13 Alignment of thermocouple wire and exposed region (red) in reference to heating element. The thermocouple wire (black) was not fixed to the chuck, but was bundled with the heating element connection wires and access through a sealed hole in the insulated chuck holder.

In order to deliver soft curing (solvent evaporation) and hard curing (sintering) temperatures that can be compared to other heat cured systems, the temperature at the center of the hot plate surface was recorded with a thermocouple for each configuration. The actual surface temperature was plotted as a function of the set temperature for the different configurations such that the temperature at the actual deposition location is known. Although each configuration has a linear relationship between set and surface temperatures, the set temperature does not come close to the surface temperature since the thermocouple is placed on the bottom (or inside) of the hotplate so that it does not interfere with the printing process. To verify how well the final configuration was working, temperature verifications were performed under ambient conditions while attached to the vacuum chuck, as well as under vacuum, using a PET substrate to block the holes (grey dots and yellow dots respectively in Figure 2.14). We notice that, though the temperatures are lower with the vacuum on, the slope of the line remains consistent with other configurations.
Figure 2.14 Surface temperature vs set temperature calibration. The surface temperature (y-axis) was measured either directly on the heated aluminum (blue, red, grey) or on a PET substrate (yellow) in reference to the set temperature (x-axis). The iterations of the aluminum chuck were measured and plotted in direct comparison to each other with the iteration signifiers in the legend.

Once installed, the system was cycled from room temperature to a set temperature of 85°C 50 times, and the temperature analysis was run again. Surface temperatures ($T_s$) were obtained through measuring the temperature of a PET substrate held on by vacuum. Surface temperatures at six random points on the PET substrate were logged manually via an HH11A thermometer with a K-type thermocouple from Omega Engineering after allowing the temperature to settle for 20 minutes at each set temperature. The resultant calibration curve, seen in Figure 2.15, was used for surface temperature calculations after this point. There is a decline in the surface temperature response compared to the previous temperature measurements that may be due to either the resistance wire and/or insulating epoxy ‘breaking in’, or possibly due to a deterioration of the sealant around the wire access point in the chuck base.
Figure 2.15 Heated vacuum chuck set temperature calibration curve. The surface temperature (y-axis) of PET substrates was taken in six different locations at nine different set temperature points (x-axis). The error is shown as standard deviation, though the bars do not show up in the graphic.

**Silver Film Sheet Resistance Curing Temperature Dependency**

The impact that these curing techniques have on the sheet resistance and conductivity of the cured metallic inks was examined, varying surface temperatures to soft cure silver films and recording the sheet resistance via 4-point probe. Two 4 mm by 8 mm layers of silver ink were deposited on cleaned PET substrates using a 30 μm inner diameter tip on three substrates for each of 70, 80, 90, and 100°C (corrected for in the data set for surface temperature) set temperatures. As the set temperature increases, the sheet resistance (standard units of Ohms/square) decreases exponentially, as seen as circles in Figure 2.16. Error is shown as standard deviation. Of note is the wider sheet resistance distribution at lower temperatures. The lowest calculated sheet resistance in these experiments was 11.058 ± 2.508 μΩ-cm, when the silver was soft cured with a set temperature of 100°C (around 58°C) and hard cured at 250°C, which is similar to the advertised 11 μΩ-cm on the Sigma website.

Additionally, for each temperature (with an additional room temperature substrate), a hard cure at 230°C in air was performed for 30 minutes and the sheet resistance was taken as well. Shown as diamonds in Figure 2.16, a similar exponential pattern is evident, though all sheet resistances are lower than before due to the hard curing. This exponential trend was unexpected
and notes the importance of hard curing as a whole, but also to soft cure while printing as high a temperature as possible.

![Figure 2.16 Sheet resistance (y-axis) of silver films after soft (circles) and hard curing (diamonds) (x-axis). Each film was printed and fully soft-cured on the stage at the corresponding temperatures. Films were then hard-cured at 230°C. The two experiments are plotted together to show the dependency of both the soft and hard curing stages. Error is shown as standard deviation.](image)

To further ascertain appropriate hard curing practices for the silver ink and its interaction with the PET substrates, a 4 mm by 8 mm silver pad was printed with a stage set temperature of 85°C, corresponding to ~51°C surface temperature. Though the silver purchased was said to have a low curing temperature (100°C – 150°C) on plastics, initial acceptable resistances were not observed at such a low curing temperature. Thus, each substrate was soft cured completely and then hard cured at 160, 185, 210, 230, 240, and 250°C. After curing for 20 minutes, the films were examined for sheet resistance to evaluate the effectiveness of sintering under each temperature, shown in Figure 2.17 below. As the hard curing temperature increases, the sheet resistance decreases and delivers an acceptable sheet resistance above 230°C, with a minimum sheet resistance of 5.238 ± 1.650 μΩ-cm at 250°C, which is less than half the sheet resistance advertised by Sigma. At temperatures higher than 250°C, however, the PET breaks down dramatically, becoming brittle, curling on itself, and impacting PET light transmittance. A regular hard curing
temperature of 230°C – 240°C is considered acceptable. Any temperatures reported higher than this herein were used before these experiments were conducted.

![Figure 2.17 Impact of hard-curing temperature on sheet resistance. Silver film sheet resistance (y-axis) at the same soft curing temperature (51°C) using different hard curing temperatures (x-axis). Error is shown in standard deviation.](image)

The results from the hard curing temperatures of silver, alone, are misleading. PET at these temperatures, however, shows results that have a reduced sheet resistance at 160°C and an unexpected sheet resistance increase and then decrease at higher temperatures. This change in resistance may be due to PET’s crystal transition temperature range. PET was observed to ‘soften’ when hard cured at 200°C and break down above 250°C (around PET’s melting temperature $T_m$), becoming opaque and brittle. What is thought to be happening is that temperatures below 185°C are seeing a reduction in sheet resistance due to the improved sintering at elevated temperatures, though at around 185°C, it is believed that the deposited silver film is beginning to fuse into the PET substrate, creating a stronger bond, but also initially increasing the resistance due to PET being dispersed into the Ag lattice and effectively reducing the pristine Ag film thickness. As temperatures increase past this fusion point, the continued impact of the reduction in sheet resistance due to increased temperature is observed again. This is supported by the provided adhesion data that shows no dissolution from the PET surface at hard cure temperatures of 210°C and above, with decreasing retention rates as the hard curing temperature is reduced.
Silver Curing Temperature Dependent Dissolution

This data was collected through attachment experiments conducted on the films from the same processes as the sheet resistance experiments, being deposited in the same manner. These films were submerged in non-agitated water for 12 hours followed by a low force attachment test by applying 3M clear tape to the silver surface and removing it via parallel motion. These tests are meant to remove any silver that has delaminated from the PET substrate, but not apply extra removal force beyond dissolution. Images were taken via microscope camera and analyzed in ImageJ. The originally deposited silver area was calculated via pixel count and then the image was converted to a binary format to determine the remaining silver coverage in pixel count, which is shown in Figure 2.18 as a percentage of the original.

Figure 2.18 Retention percentage (y-axis) of silver when submerged in non-stirred water for 12 hours as a function of the hard curing temperature used (x-axis). Delaminated silver was removed via low force adhesion (3M clear tape). The retention percentage was found using ImageJ through binary analysis and the remaining amount of silver in pixels was divided by the original number of pixels. Error is shown in standard deviation.

Silver Durability Under Mechanical Stress

To briefly evaluate the durability of the printed silver films, a bend test was conducted, wherein the PET substrate would be bent until the ends of the substrate touched, then bent in opposite direction in the same manner, constituting one bend cycle. The results from this test done on the lowest sheet resistance film in the soft curing study, soft cured at 58°C and hard cured
at 230°C, is shown in Figure 2.19 as a model for this test to evaluate the degradation of the best film possible. The sheet resistance was taken at 0, 1, 20, 60, and finally 140 bend cycles. As seen, there is an evident breakdown in sheet resistance due to visible micro-fractures in the film lattice. Shown is a nonlinear breakdown trend, which was consistent among all (five total tests at varying hard cure temperatures) though no trend in the amount of breakdown was evident with regards to hard cure temperature. Though this test is not exhaustive, it provides sufficient preliminary data warranting further investigation.

![Figure 2.19 Degradation of silver resistivity per bend cycle (left) and SEM micro-fractures (right). Bend cycles are counted by flexing the film in one direction until the ends of the substrate meet and again in the opposite direction, constituting as one bend cycle. Error is shown in standard deviation.](image)

**Line Thickness Advantage**

In addition to the heated vacuum chuck providing reliable curing practices, it also provides the ability to layer films in quick succession and increase resolution. In Figure 2.19, the impact of additional layers on resistance is evaluated, 1 being a single deposited layer, 10 being 10 total deposited layers. Sheet resistance was not recorded here, though thin (~200 μm intended width) lines were produced with the respective assigned number of layers, all fully cured at 51.4°C (set temperature was 85°C), and hard cured at 230°C. The test data, labeled as a ‘Stacked’ orientation and shown as squares, was printed, allowed to fully soft cure, and then printed again as another layer. As a control, the same number of passes were deposited sequentially, without allowing the initial layer to soft cure, ultimately depositing the same amount of material. Resistance was taken via multimeter and film height was taken on four different lines using a Dek-tak 3030/3ST film thickness analyzer. Additional layers for both data sets generate a downward resistance trend as additional material is deposited. As seen in Figure 2.20, the control did have a slightly higher
resistance, though the most interesting difference between the two test groups is the ability for the heat to significantly change the line morphology, increasing the height of the printed geometry through stacking.

Figure 2.20 Vertical deposition of silver via non-stacked (triangles) and stacked (squares) method controlled using the number of times a feature was printed (x-axis Number of Repeats). Lines of the same intended width (100 μm) were printed using both methodologies and resistances (y-axis left) of the two were compared. The resultant line height (y-axis right) of each was plotted using data collected via a Dek-tak 3030/3ST film height measurement system. Error is shown in standard error.

Though the upper height limit of this was not tested, it is of note that the height became over twice the width towards the higher end of the stacked test group with no indication of an impending height plateau. Furthermore, the stacking method had markedly higher printing resolution, as seen in the resultant average line width of 192.9 ± 22.0 μm compared to an average non-stacked line width of 326.7 ± 42.5 μm. Both data sets did not have significant changes in line width with increased silver deposition.
Figure 2.21 The cross sectional profile has the classic ‘coffee-ring’ shape and demonstrates that the e-jet printed lines can attain at least a height four times the width via printed lines. The figure shown is of 10 stacked layers (Dek-tak 3030/3ST).

Room for improvement, however, lies in the peak-and-valley like cross sectional area shown in Figure 2.21, which was prevalent at 200 μm widths but may be less prevalent in thinner lines. This curing tography is not uncommon for heat cured printed materials and likely stems from a classic issue with liquid based deposition of droplets known as the ‘coffee ring’ effect [81-83]. There has been work to reduce these effects by altering the shape of the colloids in solution [83], optimizing the curing temperature (ink dependent, though the reference study shows lower temperatures are better) [82], and optimizing surface tension [81], though these methodologies would likely have a negative impact on the resultant film resistivity reported here.

PID LabVIEW Integration and Graphical User Interface

Integration of the PID controller into the LabVIEW GUI, allows for real-time control of the temperature, more effective monitoring, remote use, and further separation between user control and the printing chamber. The majority of the hardware was retained, however the PID controller was scrapped and transferred into LabVIEW using an NI USB temperature logger and a digital output via the NI DAQ boards to control the SSR. As seen in the block diagram (Figure 2.22), the PID variables are constant, but can be manipulated prior to running the program. The main variables that should be controlled by the PID are rise time and overshoot to allow for flash curing without burning.
Figure 2.22 Heated vacuum chuck integration into LabVIEW GUI. Electrical diagram of heated vacuum chuck with the addition of the DAQ board and thermocouple I/O’s. All remaining components are run as before.

The three main portions of a PID controller are Proportional, Integral, and Derivative control, adjusting the output for current error, past error, and future error respectively. Since the suction from the vacuum, air exposure, and size of the aluminum compared to the heating element naturally control for temperature overshoot to a certain degree due to thermal inertia, the Derivative parameter was left out of the controller, since this parameter mainly reduces settling time and overshoot at the expense of rise time. The initial proportional gain was set to 80 and the integral gain to 0.015 in the example that the diagram was based from and was used after testing showed acceptable overshoot. Since the current was the limiting factor in the rise time, any overshoot seen took a significant amount of sampling time to reach, at which point the proportional and integral gains shut off the supply current.

Figure 2.23 Block diagram while loop used in e-jet system GUI with input from the thermocouple using USB-TC01 (DAQ Assistant2), PID gains, and digital SSR control (DAQ Assistant6)
These parameters were input into the PID controller in LabVIEW, shown in Figure 2.23, to control the SSR while completing the feedback loop with a K-type thermocouple, purchased from Omega Engineering, and a USB National Instruments thermocouple connection with built in cold junction (USB-TC01). The block diagram (left) and the physical manifestation of the electronics used (right) are shown below in Figure 2.24.

![Figure 2.24](image)

**Figure 2.24** Physical wiring (right) of heated vacuum chuck contains a labeled SSR, high power resistors, cooling fans, power switch, fuse, and wall outlet with the LabVIEW connection to the SSR.

The controller was tested with two different set temperatures to evaluate functionality. To test visually inspect the rise time and overshoot, the two set temperatures were used sequentially of 75°C and 110°C without the use of vacuum. The set temperatures and thermocouple temperature were recorded and are displayed in Figure 2.25 below. Upon completion of the experiment, the ‘send temperature’ function was turned to ‘off’ and the vacuum was turned on to evaluate how quickly a reduction in temperature could be had in the current system. Visual observations were made that the overshoot (less than 1°C) and rise time (less than 10 minutes) were acceptable for current heated vacuum chuck use as a soft curing mechanism. This LabVIEW integration allowed for testing with rapid stage movement which requires higher flow rates and higher stage temperatures.
Figure 2.25 The set temperature of the chuck was set to 75°C and then 110°C (square steps) to evaluate a step-wise rise time. The thermocouple recorded the temperature (y-axis) seen as the recorded data. The overshoot of the temperature and the rise time (x-axis) were recorded separately. At the end of the experiment, the vacuum pump was turned on to visualize the time needed to cool the chuck under ideal conditions.

2.3.5 Three Nozzle Setup

Looking ahead at the proposed electronic devices discussed later, the one piece that they have in common is that they all have multiple materials and multiple layers that are either currently deposited via e-jet printing or will be deposited via e-jet printing when looking at a manufacturing or mass fabrication standpoint. Thus, an early plan for the e-jet printer was to use multiple nozzles to either print the same ink at once to expedite the printing process or, more useful herein, to print different inks sequentially to eliminate the chance for deposited inks to react if printed in air. For example, silver, NOA 74, and interfacial layers are used in both the chemical sensor and the photosensor bioelectronic interfaces produced herein. When both devices are printed on the same substrate, which is a design goal for the olfactory taxis used on autonomous robotics, it would behoove the user to load these three inks into individual cartridges and print an array without the need to stop and reload.

Mechanical Design

Though the Alleyne group created a multi-nozzle system, the system was used to quickly switch out nozzles using a rotating multi-cartridge system. It could ejected from multiple nozzles simultaneously and the switch time relied on the rotation and the offset correction via a vision...
A simpler setup was opted for herein, where a main nozzle could be used as a one nozzle system or two additional syringes can be installed for a three nozzle system. Based on previous research, the Taylor cone formed at these tips is not impacted by the tilt shown in Figure 2.26 [79, 84].

Figure 2.26 3D rendering of the three nozzle system. The adapter to the z-stage and the syringe holders were modeled in SolidWorks and fabricated in-house or through Proto Labs. The manipulators are shown a trivial geometric shapes with similar knobs.

The adapter that holds the central nozzle and the manipulators for the flanking nozzles, as well as the syringe holders with voltage connection were designed in SolidWorks and fabricated in-house or through Proto Labs (Maple Plain, MN).

Figure 2.27 Substrate and nozzle offset orientation diagram, showing the standard nozzle offset in the Y-direction and the Z-direction, both of which are handled through micromanipulation.
The offset height from the substrate is controlled via LabVIEW using a precision manipulator and a stepper motor driven by communication to an Arduino Leonardo that controls a stepper driver. The code from the LabVIEW GUI delivers digital signals to the Leonardo in the form of a binary input that governs the speed of the stepper motor. The offset of the two additional nozzles from the main nozzle (Figure 2.27) is done via mechanical precision micromanipulator to bring each tip parallel to the center nozzle in the Z direction. The Y direction, the dimension that brings the tips closer to each other in Figure 2.28 below, is controlled by the same manipulator and controls the separation of the printed features. Any X offset due to imperfect nozzle creation is currently controlled by manually rotating the nozzle until the tip is parallel with the center nozzle. Since the holder is designed to have all of the nozzles in parallel with the assumption that the nozzles are perfectly straight, only the center nozzle needs to be relatively straight to bring these nozzles to parallel using this rotation based technique.

![Figure 2.28 Fabricated three nozzle print head system attached to e-jet printer and its orientation to the substrate holding heated vacuum chuck.](image)

**Three Nozzle Electronics**

The main challenge with the three nozzle setup, since the voltage and modality required for ejection is governed not only by the viscosity and surface tension of the ink but also of any
variance in the nozzle length, taper, and inner diameter, is the wide voltage range that is being handled from -2000 V to 2000 V. The initial setup, where the amplified voltage is sent directly from the amplifier to the nozzle worked well at both ends of the range with constant voltage and also allowed for high frequency monopolar or bipolar switching. To retain these features, on which the single nozzle deposition method had come to rely, a high voltage (7500VAC, 7500VDC) Reed relay was used (5501-05-1 from Coto Technology). Since these relays, shown in Figure 2.29, use an induction to close the circuit, they tend to have a slow (3 ms) switch time that would hinder the aforementioned voltage switching. To combat this, the relays are left closed for the ‘on’ nozzles and open for the ‘off’ nozzles, chosen via LabVIEW.

![Electrical diagram of three nozzle system](image)

Figure 2.29 Electrical diagram of three nozzle system. LabVIEW is used to send digital 5V signals to three MOSFETs that can be triggered independently or simultaneously. These trigger 5 volts with the current supply needed to trigger the Reed relay that allows the amplified voltage to reach the nozzle.

These relays are turned on and off via a 5 V digital signal from the NI DAQ board. Since the Reed relays need 125 mA to close the relay, in actuality the digital signal triggers a MOSFET (IRL3103PBF - Infineon Technologies) that allows the 5 V 125 mA to flow to the relay switch, thus
allowing the chosen ejection modality to flow to the selected e-jet nozzle as shown in the block diagram below (Figure 2.30). Currently, there is only one amplifier, thus whichever ejection modality and voltage level are chosen would be sent to all nozzles set to the ‘on’ position. If required, particularly if creating sequential grids of sensors or drawing multiple silver electrical connections, all three nozzles could be turned on at once using this setup, though the only way the ejection rate can be controlled would be to move each nozzle away from or towards the stage while keeping the other two stationary. The radio button icon in the black diagram is changed in this type of setup to allow multiple nozzle selection, and the center nozzle voltage modality and amplitude are governed to all selected nozzles. This however is not the best method of rapidly changing ejection rates or troubleshooting in real time. The best practical way to accomplish this real time control would be with three different amplifiers due to the level of voltage being dealt with and the accuracy and precision required in the voltage delivered. This, however, is only necessary if printing using multiple nozzles simultaneously is of interest; something more attractive to a manufacturing setting than a lab setting and thus was not implemented herein.

Figure 2.30 Three nozzle system block diagram for switching voltage between nozzles. The user selects one of the three nozzles, or can switch all nozzles to the ‘Off’ position (default). This selection sends the 5V signal to the MOSFET.

2.3.6 Back Pressure System

As prescribed by the equations in Choi et al [72], the pressure differential from behind the ink and the entrance of the nozzle impacts ejected volumetric flow, as well as the mode of ejection (i.e. droplet, line, spray). Thus, a back pressure system was designed and installed to use either compressed air or compressed inert gasses in one to three nozzles from one gas source. A quick connect attached to a compressor (Porter Cable 150 PSI, 6 Gal Oil-Free Pancake), limited via the onboard regulator, supplies compressed air while nitrogen or argon compressed air cylinders (Airgas), limited in pressure via a gas regulator supply, inert gas.
The ability to control this incoming gas flow and direct it to individual supply lines is the final addition that completes the three nozzle system. The ability to control three different nozzles at different prescribed pressures based on the ink properties in each nozzle is important. A 0 psi to above 15 psi control range was also important, as many inks print using no back pressure whatsoever, while a high pressure is needed to clear clogs.

As shown in Figure 2.31, a ControlAir Inc 438-544-005 manifold with Minuteman three 550-CIT 0-30 psi transducers were chosen to control the gas flow for the back pressure, as it can control from 0-30 psi via linear analog input. A constant supply over 40 psi is needed, which can be supplied via both compressed gas options. Furthermore, each nozzle is controlled independently of its neighbor, completing the independently controlled three nozzle system. The control for these regulators is done in LabVIEW, all three nozzles being included in the final GUI.

2.3.7 Nozzle Fabrication

There are a number of published configurations that generate the electric field needed to produce the Taylor cone ejection characteristics that define e-jet printing, though the most functionally relevant method for purposes herein is the conductive nozzle [23, 25]. There are also a number of methods to fabricate this nozzle. The original method [25] where pulled glass pipettes are attached to a Luer lock syringe and coated in a conductive material via sputter coating, was initially adopted for a number of reasons. The ability to order or produce various diameter nozzles,
at micrometer to nanometer scale allows for variability in what and how materials are deposited. Additionally, since the technique uses a glass capillary tube, any solvent can be used without damaging the ink, or the nozzle, making the nozzles solvent agnostic. Finally, this method allows for the most control in deposition characteristics and reduces the voltage required for ejection, as the nozzle is positioned within a millimeter of the ground.

**Custom nozzles**

Though ordering nozzles has its advantages in work time and regularity in tip diameter, the lead time, cost, and planning were distinct disadvantages that lead to the practice of producing tips in house using a Sutter Instruments Brown-Flaming P-87 micropipette puller. A capillary tube is placed close to a filament coil that heats the glass to a user specified temperature, until the two ends of the capillary tube start to separate as the glass is melted. A computer controlled solenoid then applies a pattern of tension as the glass softens. Once a velocity, specified by the user, is reached by the ends of the pipette as they are pulled apart, the filament turns off and the glass is cooled. This process is repeated until the capillary is pulled into two tips. By storing this heat/tension profile into a library of pulling profiles, a number of different tip diameters and geometries can be reproducibly generated based on demand.

In testing the instrument and developing a methodology for the appropriate tip geometry, various tips were generated and scanned using SEM as seen in Figure 2.32, with scale bars in the bottom right to reflect the measurements taken. These initial pipettes contained filaments, though purchased pipettes and the borosilicate glass OD 1.0 mm, ID 0.50 mm, 10 cm length fire polished capillary tubes used to make the final nozzles did not use this for fear it would alter the Taylor cone shape, and thus the ejection mechanics.
Since the micropipette puller is designed to produce inner diameter 3 μm and smaller, any nozzle diameter larger than that, sizes typically used herein to test inks and devices, has to be truncated from a smaller diameter tip. Upon testing various tip geometries for ejection consistency, production percentage, and ease of use, it was clear that a short taper, thick walled variety allowed for consistent truncations up to 30 μm in diameter with a quality starting diameter from 1-3 μm. Thus, a patch clamp type design, shown in Figure 2.33 (2 μm ID tip, 3-4 mm taper under 400x magnification), was adopted using a heat of 655, a pull velocity of 6, and a pressure of 500 (all other parameters set to 0) [85].

Once the pipettes are pulled, they are immediately sputter coated using a AuPd target for 90 seconds, then rotated and sputtered for another 90 seconds for full coverage. At this point, the nozzles are used in one of two ways; either they are permanently attached to a Luer lock adapter, used for the majority of the inks, or they are temporarily attached to a Luer lock adapter using a compression fitting, used primarily for the silver ink.

During the initial stages of nozzle production experiments, the produced micropipettes were initially affixed to the Luer adapters prior to sputter coating. This was changed when the
connection from the amplifier produced better results when directly touching the coated glass pipette as opposed to the adapter; the opposite of what was originally hypothesized was determined to be the better of the two practices. Thus, as opposed to sputtering 4-6 nozzles at a time, bulk (20+) sputtering of just the pipettes was adopted, followed by their affixation to the Luer connectors. This also limits the exposure time of the adapter to potential nozzle clogging dust particles.

**Sputter Coater**

All nozzles discussed herein were coated using a Denton Vacuum Desk II desktop sputter coating system. Sputter deposition uses heavy gas molecules, argon herein, to bombard a target (AuPd), ejecting parts of this target with high energy, that then deposit themselves throughout the low pressure chamber that this technique is conducted in. Inside of this chamber is the substrate in question, the nozzles in this instance. By using this technique, materials with high melting points, like many metals, can be easily deposited on nearly anything in the chamber. This metal coat creates the conductive film that carries the application voltage to the tip of the nozzles for the e-jet printer.

**UV Box Design and Use**

In order to attach the pulled pipettes, an adhesive was necessary, preferably one that was cured quickly without the use of heat, since the adapters are plastic. NOA 74, an optically transparent adhesive made by Norland Products cures under direct UV light in under 10 minutes with the amount of adhesive required to affix the pipettes. In order to be used as an adhesive for the micropipettes in conjunction with a clear plastic Luer lock adapter (CFLL-332V-PC Industrial Specialties Mfg.), a UV light needed to be sourced and a way to safely use it needed to be determined.

The design parameters were to generate enough UV light to cure a small amount of adhesive within minutes, provide potential ozone additives for use in substrate preparation, have a workable area that could include multiple nozzles or multiple substrates at a time, and cost under $600. Though desktop UV Ozone treatment enclosures are available and meet a majority of the design parameters, they often cost thousands of dollars.
Figure 2.34 UV curing system parts. The purchased BA3500 Ozone Air Purifier (left) is placed inside the fabricated tinted acrylic box on the right. There are two doors, one of which can access the ozone adjustment and one that opens to the curing chamber. Three air inlet holes supply air to the system via three 12V fans, exiting the enclosure via exhaust line at the top. Power is supplied to the fans, and to the UV lamp via a sealed AC Inlet and an internal surge protector.

A BA3500 Ozone Air Purifier was purchased as both the UV and potential ozone source. The ozone is generated through electrical contacts with aluminum grating inside of the enclosure, while the UV light is located through a rear door panel. The ozone system is normally turned off while being used herein, but can be turned on through a switch on the front. Since UV light is harmful to the eyes and skin and ozone is harmful to the respiratory tract, a tinted acrylic enclosure was constructed with fans that blow air in through the side, and exhaust is lifted through the top via a duct system. The box itself is a standalone product with power adapter and on/off switch used for NOA 74 curing and UV Ozone treatment experiments, as seen in Figure 2.34.

The second way to connect the coated pipettes is via a compression fitting. Permanently affixing the coated pipettes to the plastic Luer locks is the longest part of the nozzle production process, as only two nozzles can fit in the UV system at any point in time without overcrowding. To eliminate this process, nickel plated brass k Systems TSD931-31 RN to Luer adapters were purchased, along with Hamilton 55750-01RN Compression Fittings as shown in Figure 2.35.
The combination of these two adapters, though not designed for this application, lead to the ability to switch out coated pipettes as opposed to the pipette and Luer adapter as a whole. This allowed for the easy termination of compromised nozzles, as well as variable diameter printing using the same ink cartridge. The TSD931-31 had to be bored out to accommodate the compression fittings that close around the coated pipette as the threaded metal piece is tightened.

This new setup was tested with the rapidly evaporating P3HT:PC$_{71}$BM photosensor active layer, as well as the silver ink. Though both of these inks could be printed with similar ejection characteristics as with the original glued setup, this new adapter setup became appropriate only for the silver ink due to the slow evaporation of the solvents. This ‘ink cartridge’ can be left between prints without clogging and the nozzle can be readily changed between prints to accommodate larger or smaller nozzle diameters when needed.

**Tip Diameter Truncation**

As previously discussed, the Brown & Flaming micropipette puller used to forge the nozzles has high tip diameter resolution at ≤ 3 μm. Pipettes larger than this need to be truncated, by either scoring them with glass pipettes or with a ceramic tile and then broken to size. Though
these methods can produced the diameters desired, the process produced inconsistent inner diameters, and failed over 50% of the time to produce a usable flat nozzle. This success rate was confirmed by colleagues, as well as by WPI customer service. Thus, the tips are left as-is at 1-3 μm until needed, and then are broken using a vertical collision technique if a larger diameter is required. A PET substrate is held on the chuck via vacuum and the tip is slowly brought to the surface, the proximity to which is determined by half the distance between the observed tip and the shadow created on the substrate, as seen in Figure 2.36. The nozzle is then slowly brought into contact with the substrate and raised again. This process is repeated with a progressive negative distance into the substrate until the desired nozzle size is produced due to the breaking of the nozzle in response to the collision. This technique not only produces usable tips over 90% of the time, but also allows for variability in tip diameter instead of being locked into one diameter upon break or upon purchase of pipettes.

Figure 2.36 Nozzle vertical truncation technique with an image prior to truncation (left) and after a suitable diameter is achieved (right) ~10 μm ID.

2.3.8 LabVIEW Code

As discussed earlier, the original code was given to us by Dr. Alleyne at UIUC, which employed a one nozzle system with back pressure and voltage control from -800 V to 800 V, some of which was used in the final configuration of the GUI.

Configurations

Improvements were made in progressive configurations reflecting the development of the printer itself, adding code mirroring physical implementation. All configurations discussed below contain the upgrades stated as well as the upgrades of previous configurations. The block
diagrams for the heated vacuum chuck and the three electrode switching system were provided earlier, however the rest of the subsystems are presented here, prior to a time table of their implementation.

**Backpressure System**

The backpressure control system block diagram is shown in Figure 2.37 below. The upper portion takes a user input from 0-30 psi and converts it to a 0-5 volt DAQ output to the 550-CIT pressure transducer. Once the ‘Center’ button is pressed, the analog output opens the transducer to the prescribed psi if the pressure input to the transducer is at least 40 psi, using a DC signal generator and the DAQ assistant. The user input can either be typed in, toggled with ‘+’ and ‘-’ buttons, or via the input wheel, the programming for which is shown in the bottom portion of the block diagram.

![Figure 2.37 Pressure system block diagram. The user input in PSI is converted to an analog 5V output to the backpressure transducer. The buttons and display that make this user friendly is shown in the loop on the bottom.](image)

**Light Source**

The light source block diagram is simple and uses an analog output because the digital output did not deliver the voltage necessary to open the SSR used to switch the light source on. The block diagram uses a button to deliver 10 volts via a simulated DC signal and the DAQ assistant block (Figure 2.38).
Z-Stage Control

Though LabVIEW does have the capability of controlling a stepper motor, the z-stage movement was effectively outsourced to an Arduino Leonardo and stepper motor controller to avoid the use of another frequency dependent output, which caused DAQ issues for rapid travel as discussed below in Configuration 2.3.1. As briefly discussed earlier, digital signals are sent to the Arduino corresponding to the user selected speed from an arbitrary 1-7 decoded to a binary based recognition system on the Leonardo. From here a coded rotation speed is prescribed to each of the binary states, and the direction is governed by the top two digital signals into the DAQ assistant. There are two movement options, both of which are coded in the top box (Figure 2.39); a single step mode, and a continuous jog mode. The movement is done via a stepper motor linked to a 100 threads per inch manipulator (AJS100-2 Newport) attached to the linear stage (443-4 Newport) that holds the nozzle arm, effectively moving the nozzle in the vertical z-direction.
Substrate Viewing – Microscope Camera

The substrate is continuously viewed through the USB based Infinity 2-2 microscope camera, introduced into the GUI via the block diagram below (Figure 2.40). The camera is open and initiated upon GUI start up and closed when the stop button is pressed. The IMAQdx module continuously grabs the image provided by the camera and displays on the front panel with preset resolution.

Stage Control – From Aerotech

The vast majority of the stage control was delivered by Aerotech in their install disk and was modified to fit the e-jet GUI (not shown due to Aerotech IP considerations by installing their block diagrams into the central while loop of the final GUI. Ultimately, the stage is controlled via g-code written in the native Aerotech software A3200 Motion Composer, saved to a file, and loaded in the file lookup, seen in the final GUI front panel configuration.

Configuration 2.0

Two specific changes were made in the original upgrade of the received e-jet code; first was the implementation of the back pressure system which had three different pressure regulators and second was the addition of a pulsed ejection mode to give the first modality advantage over the retained ‘DC Mode’ in certain situations.

Configuration 2.1

This configuration expanded on the ejection modalities, adding ‘Hover Pulse’ and ‘Pulse Around’ modes for planned future use and providing ejection troubleshooting options.
Additionally, a visualization of the intended voltage supplied to the nozzle was introduced for use as positive confirmation of user selected ejection options.

**Configuration 2.2**

The z-stage was added in configuration at 2.2. Digital communication with an Arduino was used to drive a stepper motor attached to a precision manipulator to move the nozzle in the vertical direction at a high rate to clear the nozzle from the stage as well as under fine motion when adjusting the nozzle offset.

**Configuration 2.2.1**

The ‘Pulse’ ejection modality was introduced here thanks to observation during deposition and out of necessity as discussed later in the printing of top layer of the photosensor.

**Configuration 2.3**

This was the point at which the heated vacuum chuck was taken from the purchased PID controller and transferred to LabVIEW. The PID parameters are set via constants, while the set temperature, the ‘send’ button, and actual temp, recorded through an NI purchased thermocouple attachment (TC-01) and thermistor wire, are on the front panel.

**Configuration 2.3.1**

This configuration altered the sampling rate and tied all input and output into the same DAQ assistant controller, allowing a stage velocity of over 1.0 mm/sec. There were two issues that caused this motion impairment, both revolving around handling DAQ I/O commands.

After troubleshooting the original layout designed by UIUC, it was noticed that the back pressure system was operating at a different analog sampling rate when compared to the Aerotech stage and the voltage amplifier. Combining the back pressure and voltage amplifier analog output DAQ block resolved this sampling rate problem, allowing for normal travel at any speed.

The second issue was executing an analog input from the USB-TC01 thermocouple in the same while loop as the digital and analog outputs for the other subsystems. The bundling of these blocks was necessary initially because the DAQ output that trips the SSR to allow current to heat the stage requires 10 volts from an analog output (5 volt digital output created ‘dimming’ and did
not deliver enough power). All of the analog output blocks need to be in the same while loop because they need to be executed from the same DAQ assistant block or an error message will be triggered saying the utility is already in use. With the initial bundled configuration, the system ran well at 1 mm/sec or slower but had glaring problems handling high frequency stepper motor control on the stage. To work around this, a MOSFET (IRL3103PBF) was used as a switch, controlled by a 5V digital output, to trip the SSR handling the heated stage current flow. At this point, the heated vacuum chuck block diagram was bundled in its own while loop such that the sampling rate did not govern the rest of the block diagram. The original block diagram shown in the previous section discussing the heated chuck is the final configuration used.

The resolution of these two I/O inefficiencies allowed for rapid stage movement and rapid printing. This rapid printing process led to rapid cured silver as well as a set g-code file that prints a silver nitric oxide base skeleton.

Configuration 2.4

Thought to be a point of deterioration for the photosensor during deposition, the stage light was integrated into the LabVIEW GUI instead of a standalone mechanical switch inside of the hood. This allows the user to turn the light on to check deposition and off to allow deposition without direct light. After testing, this did not have a significant impact on final photosensor performance, but was kept because it enhanced the remote deposition capabilities by allowing the user to shut off the light during long term deposition and on when the user logs in to check the deposition state from a VPN outside of the lab.

Configuration 2.5

The three nozzle system was completed at this stage, adding voltage control and output visualization for each nozzle, as well as the ability to select a specific nozzle or none at all. The layout of the GUI was changed drastically to focus on the three nozzle setup. The stage visualization was also implemented into this GUI configuration, as was the stage control for the Aerotech stage.

Final Integrated Design

Two final GUls were generated, one with a single nozzle and one with three nozzle control. The thought is that during testing, one nozzle is necessary and cleaner to the eye, though
final structure printing would be done in a closed environment, so three ink cartridges or more would be necessary.

Figure 2.41 Final single nozzle GUI with all discussed integrated system

Figure 2.42 below shows an obvious rearranging of the control buttons deemed minor to active printer operation to allow the user to actively control the three nozzle system. Though the appearance is changed, the majority of the controls that were considered less necessary to alter during deposition were moved to the left column. The only addition here was the individual selection the desired nozzle and a control and display for each applied voltage.
2.4 Ink Formulation

One similarity between all printing methodologies is the need to optimize specific ink characteristics to achieve quality ejection; namely viscosity and surface tension. Drop on demand mode inkjet printing generally requires viscosities less than 40 cP and surface tension greater than 20 dyne/cm. Though lower viscosities lead to higher ejection control, they also cause a reduction in damping in post-drop oscillation within the Taylor cone, leading to a bounded upper frequency of droplets and/or multiple droplets where one is desired; thus optimization, not simple reduction is critical to successful deposition. Though typically unnecessary, in the case of high density inks, viscosity and surface tension should be converted to their kinematic forms to determine if their properties fall within the specified ranges. Particle suspensions, including metals, ceramics, and oxides, retain these property guidelines as well, provided the particle size is less than 10% of the orifice inner diameter. The numbers provided are quite ambiguous because of the property range narrowing as the nozzle diameter decreases, though exhaustive studies into ink formulation and ejection characteristics has yet to be done [86].

Printing methods are quite similar in their use of conical nozzle based ejection orifices, thus these viscosity and surface tension ranges remain relatively true in e-jet printing as well; the main difference being that there is a voltage dependent transition from frequency droplet
deposition to continuous line deposition that relies on these properties, as opposed to just droplet frequency in the case of inkjet as seen in Choi et al [72].

Figure 2.43 Formulated inks from left to right with labeled additives for repeatable printing: PEDOT:PSS, Graphene, P3HT:PC71BM, ZNO. The purchased silver ink (right) was used as-received.

The inks produced herein are solvent based inks meant to keep the solute in solution for at least 24 hours. Since there were no colloidal inks produced herein, the stability of the inks did not come into play, though all were shaken, sonicated, and/or filtered prior to each use. In general, 1.5 mL batches of the inks were produced and stored at 4°C in septum jars, seen in Figure 2.43. Viscosity and surface tension were controlled via solvent dilution and surfactant (Capstone FS-31 DuPont) addition. The only ink purchased and used as-received was the silver dispersion from Aldrich (736465). Though the actual parameters were not measured, the addition of further diluted inks approach parameters similar to the solvents selected. Additionally, surfactant additional was done via 0.03% additions to inks until desired ejection characteristics were observed.

2.5 Printer Setup and Function

Putting the information and data provided above together, the printing method takes approximately 30 minutes to set up initially and has printed continuously for over 24 hours. To start the setup process, the ink is removed from its storage at 4°C and shaken for approximately 20 minutes, probe sonicated for 1 minute, and shaken again until the nozzle and cartridge are ready to be filled. The LabVIEW GUI is initialized, the stage is ‘homed’, the stage illumination is
turned on, and in the case of the silver ink, the heated chuck is brought to 51°C (85°C set temperature in the GUI). During this second shaking, the nozzle is prepared by attaching whichever Luer lock system (based on the ink) will be used, to a 3cc sterile syringe. This formed cartridge is then put into the holder, affixed to the amplifier connection, and the collision break process is undergone to form the desired nozzle diameter. Once broken, the nozzle is loaded with ink and the experiment substrate is placed on the chuck. Before printing, the substrate is cleaned based on the device being printed and is simply wiped with ethanol once on the vacuum chuck. The program to be printed is loaded into the GUI and the nozzle is prepared for deposition by priming it with a 1000V charge and a rapid 30 psi burst of pressure to bring the ink to the tip of the nozzle. Once primed, the ejection voltage is determined by ramping the applied voltage modality until the desired ejection characteristic is observed. The stage is centered to the starting position of the prescribed printing geometry and the print is started. Upon completion, the nozzle is brought up and the substrate brought towards the user to examine the print and complete any post processing steps. With the exception of the silver ink cartridge, the nozzle and the cartridge are disposed of once printing is completed for the day and the system is shut down completely, unless printing overnight or remotely. If another ink is needed, the same process is followed in creating a new ink cartridge and the stage is registered with the new nozzle at the point where the previous layer was primed.

2.6 Conclusion

The results herein are improvements focused on particular deposition constraints and hurdles that required attention to print functional versions of the sensors discussed in the coming chapters. Ink preparation allowed for reproducible film construction resulting in individual material deposition as well as multi-layer electronics. Though similar in properties, these inks require different nozzle diameters, lengths, and fabrication processes. One ink in particular required further attention once deposited, in examining the method by which the silver ink would be cured. Accomplished by the addition of a custom heated vacuum chuck, the investigation into soft and hard curing practices allowed for reproducible silver films used as electronic connections, reference electrodes, and top photosensor electrode. Finally, the integration of these improvements and the idea that less mechanical manipulation of the e-jet printing process itself would lead to higher quality films that were deposited with reproducible metrics, led to a LabVIEW GUI that allowed the user to control nearly every aspect of the process once appropriately set up.
Though future work would include a user focused version of this GUI, it can currently be controlled remotely without a user present in the lab. Future work would also focus on more than three nozzles (since there are more than three layers in current and potential sensors), atmospheric control (reactive materials benefit from an inert surrounding during deposition), on stage hard curing, user geometric layout control and automatic g-code conversion, wavelength controlled LED light source for stage camera (many films are sensitive to certain wavelengths of light), electronic camera position, magnification, and focus control, direct UV curing on stage, and other similar enhancements that are geared towards improving the performance of individual films that in return, improve the overall function and reproducibility of total sensors. Though these improvements would ultimately benefit the e-jet printer and the electronics produced by it, the printer, as delivered herein, can produce functional multi-layer and multi-material electronics as previously hypothesized.
Nitric oxide is a common byproduct of routine microorganism processes in both prokaryotes [87] as well as eukaryotes [88] and is thus broadly available as a reporter. Targeting the nitric oxide synthase eNOS that produces extracellular nitric oxide capable of detection, there are various detection/reporting pairs that can be made in these microorganisms, which will be discussed in a later section. The logical progression was to develop an e-jet deposited general electrochemical sensor sensitive to nitric oxide in pursuit of a bioelectronic interface capable of single entity detection (i.e. analytes, wavelength of light, etc.).

3.1 Background

An electrochemical sensor is a device that quantifies chemical information, such as chemical concentration in solution, through electrical energy. One way to do this is through amperometry, wherein the sensor detects ions in solution and reports their concentration, or the change in concentration, via an electrical current. By applying a voltage across two electrodes (reference and working electrodes), amperometric sensors can detect the concentration by observing the change in current as the electroactive analyte is oxidized or reduced at an analyte dependent redox potential. This detection methodology possesses the advantages of not only being sensitive and specific, but also detects these changes in real time [89]. The reference electrode can be further split into two electrodes, the reference and the counter (or auxiliary), wherein the reference is typically positioned close to the working electrode to apply the potential at a theoretical single point. This three electrode system has the distinct advantage of measuring only one half of the cell, meaning that the changes occurring at the working electrode are measured independently of the counter electrode, increasing the accuracy of the cell. Reading the current through this counter electrode also eliminates any reactive change at the reference
electrode which requires stability to maintain an accurate redox potential with the working electrode. Using this three electrode setup, the produced e-jet printer functionality was investigated through printing multiple heterogeneous layers, resulting in a Au functionalized graphene working electrode sensitive enough to detect ≤ 1.0 μM nitric oxide in solution.

A powerful vasodilator and important free radical in mammalian cellular signaling and organ function, nitric oxide (NO) is used in pharmaceuticals to help treat anything from pulmonary hypertension in neonatal patients [90], to salvage therapy in patients with acute right ventricle failure, though it has a short (~455 seconds) half-life in aqueous solution [91]. Furthermore, the breakdown of nitric oxide to N₂O and NO₂ post fossil fuel (namely diesel and biodiesel) combustion in machines and automobiles has major implications on greenhouse gas concentration [92, 93]. These molecules also arise from the decomposition of certain explosives and may be harnessed as a detection mechanism [94-96]. In order to selectively detect various molecules, electrodes are coated with a selective membrane to eliminate the possibility of competitive reactions and amplified, inaccurate results. In the case of nitric oxide, it is important to prevent the unintended measurement of similar anions, namely nitrite and nitrate. Various coatings have been studied, including nickel complexes [97], o-phenylenediamine [98, 99], and polymerized eugenol (4-allyl-2-methoxyphenol) [100], though the most widely studied coating is Nafion; a sulfonated tetrafluoroethylene based fluoropolymer-copolymer discovered by Walther Grot at DuPont in the late 1960s. Though Nafion has shown selectivity for glucose [101] and nitric oxide [102] in biosensor applications, perhaps its most important feature is that it is biocompatible and stable in cell culture and in vivo, [103] making it an ideal selectivity filter for bioelectronic interface design.

Improving sensitivity is also important, as nitric oxide is typically in solution at pM - μM range. Many electrochemical sensors use nickel complex coatings to increase sensitivity, Ni(II) Tetrasulfonated Phthalocyanine (NiTsPc) a popular choice among them for its ease of application and well-studied conductivity improvements, and thus sensitivity benefits. The versatility of NiTsPc has led to use in detecting Methyl-parathion [104, 105], p-Nitrophenol [105, 106], hydrazine [107], dopamine [108], and more relevantly, nitric oxide [97, 98], as well as being used in conjunction with Nafion®[96, 104]. Recent studies have used gold nanoparticle deposition to further amplify the signal to concentration ratio of nitric oxide and other molecules, even compared to the widely studied Ni compounds. [109-115]
We have developed a methodology for electrohydrodynamic printing a nitric oxide sensor. The main aspects of this methodology include the printing of carbon and Ag based inks, an encapsulation to define the working area, the conversion of Ag to AgCl as a reference electrode, and the deposition of a nitric oxide selective layer on the working electrode. Both dissolution of printed material and efficacy of multiple layers have been studied herein. In terms of robotics, and the general research done in the Ayers laboratory, the ability to sense chemicals, namely nitric oxide, is the first step in parallel research to create bomb ‘sniffing’ robotics via a bioelectronic olfactory sensor suite as microbes that integrate dinitrotoluene (DNT) detection [117] (the breakdown from TNT can be seen in Figure 3.1) and reporting through NO production [88] are a goal of our collaborators. In conjunction with the autonomous underwater robotics currently under research in the Ayers lab, the proposed nitric oxide sensor can assist in the autonomous search, identification, and neutralization of underwater explosives. The demonstration of this device gives precedent and direction to further chemical sensor fabrication via e-jet printing and creates a new category of biohybrid sensors based on an NO reporter.

3.2 Materials

3.2.1 Nitric Oxide Solutions

In pursuit of a nitric oxide test solution that could be calibrated and easily reproduced, two methodologies in particular were examined. The first involved bubbling nitric oxide into
solution but was abandoned before testing due to its comparatively high cost, low stability, short working time, and toxicity. The second was to use a well-studied nitric oxide donor (S-Nitroso-N-acetylpenicillamine: SNAP) seen in Figure 3.2 below. This molecule has a high solubility in water, has a steady and previously quantified/easily quantifiable release rate, and is readily available (Cayman Chemical, Ann Arbor, MI) [118].

![Molecular SNAP](image)

**Figure 3.2 Molecular SNAP**

SNAP was purchased in 10 mg vials with 98% purity. Stock solutions were made by dissolving 10 mg of SNAP into 10 mL 3.4 mM EDTA. This molarity of EDTA was recommended by World Precision Instruments during the calibration of the nitric oxide sensor purchased from them [119]. EDTA regulates the SNAP release of NO over time by chelating free metal ions. A control solution was made of 3.4 mM EDTA and will be referenced as the control in nitric oxide experiments from here on unless otherwise stated.

### 3.2.2 Nitric Oxide Production and Calibration

Based on previous publications, it was assumed that 1.0 mM SNAP maintained a concentration of 0.5 μM nitric oxide over the course of at least two hours when in the presence of EDTA [118]. To reproduce this data in the current laboratory environment, a nitric oxide sensor ISO-NOP from World Precision Instruments (WPI, Sarasota, FL) was purchased and calibrated. To calibrate it, the purchased sensor was polarized for 12 hours in 0.1 M cupric (II) chloride at 0.853 V, which is the voltage setting that came with the free radical analyzer from WPI. Cupric (II) chloride rapidly breaks down SNAP into nitric oxide at an approximately 60% conversion rate. The
calibration curve was set by injecting aliquots of known SNAP concentrations into the stirred solution and recording the current output from the sensor, the results of which are shown below in Figure 3.3. Based on this calibration curve, SNAP was tested by placing the probe in EDTA while injecting aliquots of SNAP/EDTA and once again recording the current output. By doing so, it was seen that 1.0 mM SNAP produces 490 ± 20 nM nitric oxide in EDTA. All NO concentrations are based on this calibration curve of the NO donor SNAP in EDTA.

Figure 3.3 WPI ISO-NOP calibration curve. The probe was placed in CuCl₂ and aliquots of SNAP were introduced into stirred solution, plotted as controlled concentration of NO (x-axis). The resultant current change was plotted (y-axis).
3.3 Methods

3.3.1 How a three electrode electrochemical cell works

Electrochemistry, in name and function, uses an electrical input and output to detect chemical species in solution. A basic setup employs two electrodes, wherein a potential (voltage) or current is set between the two, a reference electrode and a working electrode and the current or voltage is read out respectively; in amperometry, the case herein, a potential is input and current is then output. High currents, however, can alter the state of an electrode and thus a third (counter) electrode is added to supply the electrons to the system so that the reference electrode does not have to, thus preserving its stability.

![Diagram of three electrode setup](image)

**Figure 3.4 General three electrode setup**

Thus, in the three electrode setup used and shown in Figure 3.4, the reference electrode (RE) is used as a baseline to set a potential (voltage offset) between it and the working electrode (WE) where a redox reaction takes place. To balance this redox reaction, the aforementioned counter electrode (CE) was added, which is also where the output current is read. In order to have a functional and repeatable fabricated sensor, all three of the working electrodes needed attention, having their own individual sets of challenges and resolutions. The following sections (3.3.2-3.3.5) focus on the each of the electrodes individually.
3.3.2 Reference Electrode fabrication and optimization

In electrochemical cells, the reference electrode is a stable electrode from which a potential or current is passed to the working electrode to induce a redox reaction at that potential or current. Ag:AgCl is a common material to use as a reference electrode in a three electrode cell, especially when cost and the e-jet deposition method are taken into account. The potential between a stable reference electrode and a working electrode allows the user to determine how the working electrode is reacting to the solution in question; reacting to nitric oxide in this case.

**Printing Ag:AgCl**

Initially, an e-jet compatible AgCl ink was synthesized by adding various concentrations of AgCl powder to a commercial silver ink, both purchased through Sigma (204382 and 736465 respectively). For commercial screen printing inks, a concentration of 70:30 or 60:40 Ag:AgCl is used for disposable reference electrodes [30]. Given that the Ag ink is 20 wt%, 42.86 mg and 66.67 mg of AgCl were added to 0.5 mL Ag ink, and diluted with ethanol to produce the aforementioned ratios. This fabricated ink has been pipetted onto glass cover slips and sintered at 220°C, yielding a conductive thin film layer. The next step was to adapt the ink to e-jet printing requirements.

One major challenge throughout the development of the nitric oxide architecture, was the notion of depositing AgCl in bulk form. In previous work, including the paper some of the architecture is based on, Ag/AgCl ink was screen printed in bulk [99]. Screen printing uses highly viscous inks, cut screens, and back pressure to deposit material. Based on assumptions that dilution would reduce viscosity and surface tension to values similar to those of the diluent, an AgCl ink was developed and printing was attempted with a silver ink and AgCl in powder form, at ratios around 60:40. Though in some instances deposition was possible, it was unclear whether the deposited material was 60:40 AgCl, a different a AgCl ratio, or simply Ag. The advantage of screen printing is that these highly viscous inks tend to be less reactive, as the higher viscosity often arises due to the amount of bulk material in solution, thus reducing the amount of diffused oxygen to the majority of the material. Per conversations with manufacturers of AgCl screen printing paste at Creative Materials Inc., AgCl in a low viscosity liquid state tends to be reactive, and thus has not been successfully deposited via any low viscosity liquid deposition method to the best of their knowledge and my research. Thus, a more reasonable process was adopted,
wherein silver was deposited as the reference electrode and converted into AgCl via electrodeposition.

**Printing Ag for Reference Electrode**

Silver ink was the next logical route to a stable reference electrode as silver wire can be readily converted to AgCl using electrodeposition, and it was anticipated that the same conversion can be made on a silver film. Thus, the silver ink was printed using 30 μm nozzles at 0.8 mm wide and varying lengths.

**Ag conversion to Ag:AgCl**

The basic method to convert Ag to AgCl is to submerge Ag into 1.0 M HCl and electrodeposit the Cl\(^-\) onto the Ag anode and the H\(^+\) onto a platinum cathode. Typical methodologies using Ag wire used a constant current source with 1-10 mA/cm\(^2\) and allowed the voltage to settle over a period of time. The methodology herein uses a constant voltage source (0.3–0.5 V) to set an initial current of 15–30 μA (5-10 mA/cm\(^2\)) and attend as the current settles to zero over time (minutes). In order to test for adequate Cl\(^-\) addition to the Ag, a purchased AgCl electrode is placed in 3.0 M KCl with the printed AgCl, and the voltage (mV scale) difference between the two was recorded. Ideally this voltage difference is 0.0 mV, though any difference between the two can be recorded and compensated for later.

Initial electrodes were not cured on the heated stage and had some film inconsistencies that arose from their rapid sintering at 250\(^\circ\)C in the oven that led to inconsistencies in the AgCl conversion process, namely leaving unconverted areas that created noisy and inconsistent potentials. Additionally, initial electrodes were bare, and the size of the electrode was determined simply by the amount of the electrode that was submerged in HCl. To minimize these two reference potential inconsistencies, the heated chuck was introduced to help evaporate the solvents in the silver ink, creating a consistent Ag film, and NOA 74 was applied to the electrode before AgCl conversion to define the active area (discussed later). By doing this, a consistent potential (voltage comparison between two electrodes) between fabricated and commercial (MF-2078 RE-6 BASi, West Lafayette, IN) Ag/AgCl electrodes of ±10mV has been achieved. This small difference is recorded and can later be compensated for when comparing to another reference electrode standard.
Importance of Reference Electrode Layer Thickness

Through testing, the importance of the initial Ag thickness for the reference electrode became apparent. Regardless of the manner in which the reference electrode was converted to AgCl, the results were imprecise. This was due to the fact that a monolayer (printed monolayer, not atomic monolayer) was being deposited. Often times, this layer would be thick enough where the conversion process would penetrate the silver until the current executing the conversion approached zero. However, if the monolayer was too thin, due to user error in the perceived deposition, the conversion penetrated to the substrate, causing an erosion of silver layer meant to be the reference electrode. As seen in the Figure 3.5, the reference electrode that is over converted leads to a noisy response during working electrode coating (pictured) and during voltammetric detection. Pictured is a reference electrode that was over converted, the noisy response, and a response using the same printed working and counter electrodes with a commercial reference electrode. The process shown is the Au coating of the working electrode (discussed later) and shows that the response is the same, though the noise in the broken electrode is unacceptable. The additional response shown is that of a fully printed three electrode setup with a reference electrode that was not over converted, displaying the same low-noise response as the commercial sensor.

![Graph](image)

**Figure 3.5** Comparison between a under-deposited (broken-red), commercial (runs beneath broken-blue), and a properly deposited (Working-grey) reference electrodes while coating a working electrode with Au.
Impact of the location of Reference Electrode on the stability of the WE and CE

Generally speaking, the reference electrode needs to be as close to the working electrode as possible. Simply put, in comparing two electrodes in a concentration, one wants to compare them in exactly the same location as the difference between the two would reflect the actual concentration of the analyte at that precise location. Since we are dealing with planar surfaces, the next best thing is to deposit the reference as close as possible to the site of detection. In the pursuit of having an overall sensor with a square geometric shape that is easy to print and implement, the reference electrode was placed on the outside of the square (working electrode being the interior) closest to the working electrode (450 μm separation) and further away from the counter electrode. A representation of the initial base layer of this setup is shown below in Figure 3.6, with an AgCl reference electrode and a working and counter electrode waiting for graphene deposition.

![Diagram showing the location of electrodes](image)

**Figure 3.6 Printed silver base before graphene addition to WE and CE. The RE was converted to Ag:AgCl for visualization of color change.**

Due to the reactive nature of HCl with Ag, something that is necessary with the reference electrode but not with the other two electrodes, the location of the reference electrode had to be tested to see if the silver for the working and counter electrodes would react with HCl in solution. The final configuration of the architecture was initially printed with all silver electrodes (as seen in Figure 3.6 above) that are then altered during post processing; reference to be converted to AgCl, working to be capped with graphene, and counter to remain silver or also
If either the working or counter electrode converted slightly to AgCl (obvious color change) during the electrodeposition of the reference, then either the printing process or the reference electrode location would have to be altered, in order to preserve the integrity of the underlying silver pads. Occasionally during the AgCl conversion process the silver would be delaminated by the HCl process, which was helpful in identifying the inappropriate soft/hard curing process discussed earlier. Outside of these delamination events, there was no visual chemical alteration due to this process because of spontaneous reaction between the Ag pads and the HCl, and functioning sensors were produced using this reference electrode location.

**Size of Reference Electrodes**

The size of the reference electrode, was a major factor in the overall stability of the reference electrode. Due to the way in which the printing process was executed in the early stages of testing, it was rather difficult to obtain a ‘large’ (greater than ~0.3 mm²) silver surface that was uniform throughout. The primary factor was that the pooling of the ink would cause defects in the cured electrode when put into the 230°C oven, because the cohesion of the ink would pull material towards the center as the more exposed edges cured. This would leave a comparatively thicker circle in the center, which also occurred with the photosensor silver curing process when using ‘large’ volumes of silver. Once the heated chuck was developed, it was possible to soft cure on the chuck, but due to the way in which data was sent out to various electronics from LabVIEW, the stepper motors of the stage could move beyond 1 mm/sec, and curing would occur before another pass could be executed, creating line defects in the silver due to overlapping. The defects arose from the practice of printing a long line, stepping half of the width of this line, and printing the next line with half of the material overlapping the old section and half printing on new substrate. This printing process, used to eliminate portions of the deposited film where hole defects expose the substrate or underlying layer, commonly known as ‘pinholes’, left a ‘scale’ like texture at low speeds since the printed silver would cure prior to the overlap print. Once the LabVIEW GUI was retooled (Configuration 2.3.1) as discussed earlier, it was possible to generate full films without any defects at various sizes since the deposition would occur prior to curing, creating a low volume pool which cured as a single film.

The reason these defects impact the final reference electrode is attributed to the non-uniform conversion from Ag to AgCl due to the thickness of the film and sometimes because of a reduced connection to various parts of the film due to inadequate silver coverage. Once the
heated chuck and rapid printing was introduced on the e-jet system, these defects were eliminated, while simultaneously, it became apparent that the size of the reference electrode is independent of its ability to serve as a reference. This means that if the reference electrode was 1/10th the size of the working or counter electrode, it would work the same as if it was 10 times the size. This became valuable because the larger the printed surface, the higher chance of defects that would not convert to AgCl, making the electrode useless. Thus, the reference electrode was limited to ~0.3 mm², where the thickness of the silver was observed to be uniform at the tip of the printed electrode. This uniformity was accomplished by printing the connection to the reference electrode, which required multiple passes, and printing 1-2 layers of silver 0.8 mm x 1.6 mm that soft cured into a defect free thin film. This 1.6 mm was then reduced to approximately 0.4 mm using NOA 74.

**NOA 74/Aquaseal/super glue**

The limiting of the reference electrode functional area was done during post processing as opposed to during the printing stage. NOA 74, Aquaseal, and super glue were all tested as electrically insulating materials as all were readily available and transparent, useful in determining how they visually impacted the silver film. Aquaseal, though water proof, did not adhere to the PET and was difficult to work with. The super glue delaminated during water testing and was brittle once cured. The NOA 74 was easy to work with and retained its effectiveness in solution, as well as under high heat at 250°C. This was used to limit the size of the reference electrode to 0.3 mm² before conversion in to AgCl. The coating also was resistant to HCl, even under applied potential, as only the location that was converted was that which was left exposed to the HCl solution, as seen in Figure 3.7.
3.3.3 Electrical Contacts

An electrical connection to the working, reference, and counter electrode is needed in order to have any distance between the functional area, exposed to an aqueous environment, and the robots electronics. To achieve this, a conductive and resilient material is needed, that can be easily printed, and encapsulated to protect the measurements from unwanted reaction between the test solution and the printed connections.

As alluded to above, silver ink will be the main component in the contact fabrication process due to its conductivity, ease of printing, and familiarity from the previous chapter. Once all of the electrodes and connections are implemented, this also allowed for the rapid printing of a silver base structure, which will be discussed below. With the implementation of the heated chuck, the fast curing process allows for the layering of silver to reduce the resistance of the connections. Finally, silver did not have any observed adverse reactions with NOA 74 and did not delaminate or dissolve when encapsulated, which allowed for active geometry selection as well as electrode insulation.
10 mm per second change allows for rapid based printing and layering

Further improvement on the LabVIEW code used to control the e-jet system was realized when diagnosing an issue with the Aerotech stage integration, as discussed in configuration 2.3.1. Though it ran well at 1mm/s in the X direction, there was a noise occurring when traveling in the Y direction, but only when the LabVIEW suite was running. Above that speed, there was significant noise when proceeding in any direction.

The resolution discussed in configuration 2.3.1 presented the possibility of comparing silver pads printed at 1mm/s to pads printed at 10mm/s, which cut the print time of the larger nitric oxide sensor silver base layout from 120 minutes to 12 minutes. There is a noted color change between the slow and fast deposition methods, the latter of the two creating a reflective uniform film.

In order to investigate the difference in functionality, electrochemical sensors were created. There was no difference in Ag:AgCl deposition and resulting potential. The resistance of the lines however were at least two fold lower in the rapidly printed electrodes compared to the slower versions. The initial thought is that due to the rapid curing of the silver, and the tenfold decrease in printing time, there is less time to react with the air around the silver and thus is printed with less resistance.

Insulation

To test the nitric oxide sensor, an insulating layer was applied to isolate the active geometries from the connection pads and conductive lines. The insulating layer, shown below in Figure 3.8, restricts reactions to the defined geometric areas of each electrode and not their conductive connecting tracks. Though the insulation herein is not printed, the UV light cured Norland Optical Adhesive 74 (NOA 74) serves well in that it is easily deposited and rapidly cured using the aforementioned UV curing chamber. The low surface tension and appropriate viscosity of NOA 74 lend well to e-jet printing and cure well, without bubbles with a standard UV bulb.
To test the process of encapsulating the silver electrodes, NOA 74 was applied cold (~4°C) and spread over the electrodes utilizing a drop cast technique, using micro tweezers to spread it, setting the active geometry. The electrodes were placed into the UV curing station previously discussed in the first chapter, and cured for 20 minutes. The electrodes were tested in various liquids, including PBS, 3.4 mM EDTA, and nitric oxide solutions and no shorting or reaction occurred at any point along the encapsulated connections in any tested solution. As discussed previously, silver reacts when current is applied in chlorinated solutions. Though NOA 74 has not been printed for this purpose to date, it has been readily printed as a test solution, shows no delamination of silver, and would likely function the same way despite potentially long film exposure times to the NOA 74.

### 3.3.4 Counter Electrode fabrication and repeatability

We considered three materials for the counter electrode, PEDOT:PSS, Graphene, and Silver based. PEDOT:PSS readily dissolves in water, though the option to coat the electrode with Nafion was explored and the deposited material was retained over time but the less than optimal
conductivity rendered PEDOT:PSS unsuitable as a counter electrode. Graphene ink was selected for the working electrode and was tested as a counter electrode as well. In printing the counter electrode using the graphene ink, inconsistencies were noticed with the printing process and low conductivity was observed. Due to these irregularities in the printing process, the graphene ink was abandoned as the counter electrode.

Silver ink was determined to be the most consistent to print, exhibiting the highest conductivity and, after the heated chuck and rapid stage movement were introduced, the most consistent film deposited. Thus the entire counter electrode has been printed using the silver ink and was used in early studies. The final configuration of the counter electrode, however, used silver as a sublayer and applied graphene as the active coated counter electrode as seen in Figure 3.8 above. These graphene counter electrodes were used in the final tests using amperometric response discussed herein.

**Counter Electrode Size**

The size of the counter electrode is critical to stable function of the three-electrode setup. The role of the counter electrode is to supply the current response required from the redox reaction occurring at the working electrode, maintaining the potential set by the user. When the reaction occurs at the working electrode, the current rises to maintain the potential set by the user, forcing a redox reaction. To supply this current the counter electrode must be much larger than the working electrode. Though there is no prescribed size difference, the higher the counter to working electrode ratio the better, with an infinite ratio being ideal. Herein a ratio of at least 3:1 has been set for the final electrode configuration, where the counter electrode is 22.75 mm² and the working electrode is 7.5 mm² for the large working electrode discussed in the coming sections and 7.25 mm² and 1.28 mm² respectively for the small working electrode setup.

**Counter Electrode Curing**

When printed at room temperature, surface tension tended to cause pooling of the ink and uneven film consistency. By using the heated vacuum chuck, solvent pooling was controlled and the electrodes became more consistent. The slower curing process allows for a more uniform evaporation of solvents, though the center does still tend to be final point of curing. It was noticed however that this process tends to leave some regions with less silver due to the rapid movement of the stage and change in electric field when there is no previously deposited material. The
majority of these defects are located along the edges of the counter electrode. Though these points are still functional in the sense that they do not break electrical connection with the silver contacts, they are still less than ideal for the entire functional area when an even current distribution is key to the success of the counter electrode and device as a whole. Thus, immediately after printing the counter electrode, a second layer is printed. Since the counter electrode is relatively large, it partially cures as the print progresses. By the time the regions are is reprinted, the silver is hot and is more thermally conductive than the PET, thus the printed silver cures rapidly on top of it, delivering a more uniform film. Following the hard cure, the electrode is exposed to HCl during reference electrode AgCl conversion. The time interval between the soft cure and the subsequent hard cure in the oven was critical. As little as 15 minutes between soft curing and hard curing leads to a delamination event in all solutions tested, not simply an acidic HCl solution. However, delamination does not occur if the hard cure takes place directly after the soft cure.

3.3.5 Working Electrode fabrication

As discussed earlier, the working electrode in an electrochemical cell is the point of specification for the analyte in question because this is where the forced reaction occurs. There are a number of ways to make the working electrode specific, including using a material that reacts specifically with the analyte making it more selective and also using a film to rebuff possible points of noise due to reaction with other molecules. The most effective electrode setup here was found to be a silver base to conduct the signal, a stable graphene cap that is altered with gold electrodeposition to amplify response, and a Nafion coating to filter common molecules that are known to cause noise during electrochemical nitric oxide detection, including NO$_2^-$ and NO$_3^-$. There have, however, been many iterations of the working electrode to get to this point, involving a number of inks, geometries, sizes, and additives.

As with the counter electrode, we evaluated several different ink candidates including PEDOT:PSS, carbon, graphite flake, flocculated graphene, and reduced graphene oxide, with less than optimal results. Sigma recently released an ink formulated for inkjet printing, which consisted of nanoparticle graphene sheets that would easily fit through micron sized nozzles and higher. We found that clogging events with the nanoparticle graphene could be minimized by the following reformulation. A dilution of 4:1 toluene to graphene ink dilution was made in 2.0 mL
septum bars (an air tight glass vial with a rubber gasket that can be accessed via needle and syringe) that was probe sonicated for 10-15 seconds to break up any large particles. Once shaken, 1 μl of Dupont™ Capstone® FS-31 was added per 0.5 mL of ink to reduce surface tension. This specific surfactant has been previously used in inks to improve PEDOT:PSS functionality [120]. Once shaken, this ink is stored at 4°C indefinitely, though sonication and shaking is done again before each print. This ink was used in all subsequent working electrodes, as well as the aforementioned counter electrode construction.

3.3.6 Voltammetry

There are a number of electroanalytical methods used herein to test the printed electrochemical devices. Voltammetry, a category of these methods and a subcategory of amperometry, inputs a voltage difference between the reference and working electrodes as before, and reads out currents as these voltages are altered in various ways.

Square Wave Voltammetry

Of these electroanalytical methods is square wave voltammetry, which is used herein to evaluate the working electrode architectures with a test analyte known as 1,1, ferrocene dimethanol. A form of linear potential sweep voltammetry, the potential between the reference and working electrode is swept using a staircase type voltage increase with a square wave superimposed on top of it as seen in the Figure 3.9 below.

Figure 3.9 Square wave voltammetry applies a square wave to a user defined linearly increasing voltage between the RE and WE, shielding the recorded data from non-faradaic currents [121].
Use of this method produces minimal contribution from non-faradaic currents to the resultant current allowing for high sensitivity analyte investigations. In theory this process creates peaks at anodic (oxidation) and cathodic (reduction) points, however, herein scans were done using a positive potential and thus an anodic peak is seen in the graphs provided. All square wave scans herein, regardless of the potential range used, used a frequency of 15 Hz, an $E_{\text{step}}$ of 0.004 V, and an $E_{\text{amp}}$ of 0.05 V.

**Cyclic Voltammetry**

Cyclic voltammetry has great sensitivity and good selectivity, making it a popular choice of voltammetric analysis. As the potential is increased, shown in Figure 3.10, a peak is formed based on the analytic properties in solution wherein the analyte loses an electron during an oxidation phase. Once this peak is seen, the potential direction is reversed and a reduction peak is seen wherein an electron is added. The currents seen in the resultant cyclic voltammogram are proportional to the concentration in solution. There are a number of additional parameters and kinetic information one can discern from the voltammogram, though herein concentration will be the major focus. All cyclic scans herein, regardless of the potential range used, had a scan rate of 0.1 V/s and a potential step ($E_{\text{step}}$) of 0.01 V.

![Cyclic voltammetry diagram](image)

*Figure 3.10 Cyclic voltammetry applies a user defined linearly increasing and then decreasing voltage between the RE and WE, triggering a redox reaction of analytes in solution.*
3.4 RESULTS

3.4.1 Reference Electrode Consistency and Repeatability

During the course of printing, testing configurations, and multiple methodologies, the electrodeposition process was logged for each sensor developed after the NOA 74 addition was introduced. During this process, 130 reference electrodes were recorded, 89.3% of which were functional. Electrode failure was due to a number of reasons, including 3.05% that were due to leaving the cured silver on the surface for too long before ‘hard curing’ as discussed earlier. Additionally, these electrodes were printed, often using silver from nozzles used in previous prints, as the majority of the silver ink was still functional. Many times however, the first electrode that was printed would delaminate as well due the silver at the tip of the nozzle being corrupt for an unidentified reason, potentially due to oxidation during stagnancy. This occurred with 5.34% of the total electrodes, meaning 2.29% of the failing devices cannot be explained as of yet. This means that of the explainable and avoidable problems in the future, around 97.7% of the reference electrodes were functional, which is fair to expect in the future as of now.

![Graph showing the distribution of potential offsets](image)

**Figure 3.11** Reference electrode fabrication variance from a commercial Ag:AgCl electrode. The distribution (y-axis) of the offset (x-axis) is shown. The 100+ electrodes had a mean of 0.2 ± 0.5 mV.

Repeatability is also a metric of importance for the sensors printed and those to be printed in the future. The 117 functional devices were categorized as functional if the potential vs a commercial (MF-2078 RE-6 BASi) reference electrode was ±20 mV. The average potential was -
0.2 ± 0.5 mV with a standard deviation of 4.9, meaning 95\% of the printed and future electrodes fall within ~10 mV of 0 as shown in variance Figure 3.11 above.

3.4.2 Counter Electrodes

**Silver vs Platinum**

Platinum is one of the prominently used counter electrodes in laboratory electrochemical setups due to its inert nature and availability in high purity wire form. Silver was chosen for its ease of deposition in small and large geometries and its resilience in solution. Thus, a comparison between the two metals is in order to determine how effective the silver counter electrode is. As seen in the Figure 3.12 below cyclic scans were taken at 0.1 V/sec in 2.156 \( \mu \)M NO for the printed silver counter electrode and platinum wire using the same working and reference electrodes that were printed on a PET substrate with the silver counter electrode tested here. The hysteresis seen in Figure 3.12 is material dependent, and is one of the reasons Pt is desirable as a CE, though there may be additional hysteresis due to silver CE oxidation.

![Cyclic voltammogram of a WE and RE pair using a Silver (blue) and Platinum (red) CE. The hysteresis is material dependent and may be due to partial Ag oxidation.](image)

*Figure 3.12 Cyclic voltammogram of a WE and RE pair using a Silver (blue) and Platinum (red) CE. The hysteresis is material dependent and may be due to partial Ag oxidation.*
3.4.3 Working Electrodes

Working Electrode Adhesion Evaluation

Since the intended application for the proposed nitric oxide sensor is in solution, it is important that the proposed materials can survive this type of environment. Silver and its adhesion to various substrates has been examined as it is part of the Ag:AgCl electrode as well as the counter electrode and the base of the working electrode. Three substrates per experiment were used; one unclean, one cleaned with ethanol, and one cleaned in an isopropanol bath sonication. Three 0.5 μl drops of silver ink (Sigma) were deposited on each substrate and then baked at 250°C for 20 minutes in air to form a test dot. Each substrate was let to cool for 10 minutes prior to a tape test on one of the dots (I) on each substrate. This tape test involved simply putting fresh Scotch™ tape over the dot and pulling the tape off directly parallel to the substrate, away from the dot in question. Each substrate was placed in water for 1 hour and left without agitation. Substrates were dried using argon and another tape test was done on a second dot (II). The substrates were then placed in 2-propanol and sonicated for 15 minutes, followed by a tape test on the final dot (III). Graphene ink was also tested in the same manner using 300°C for 15 minutes and a composition of 0.1 mL graphene ink, 0.4 mL toluene, and 0.1 μl of F-31 surfactant.

Two substrates were examined; glass and PET. Though both substrates saw no loss of silver after their first tape test (post baking), there was a glaring deficiency in attachment to glass following the water bath. While PET saw very little reduction in silver (cohesion failure, no pinholes formed), glass saw severe wrinkling in the silver dots after water treatment and the majority of dots were blown off during drying. The remaining dots were subjected to the tape test and lost at least 30-40% of the remaining silver due to this wrinkling in the water.

In order to simulate a long-term harsh environment exposure, both substrates were subjected to a bath sonication in isopropanol and while there were slight cohesion problems with the PET, no pinholes were evident and nearly all of the deposited silver remained on the surface of the substrate. There was no lift off on either substrate containing graphene until the final propanol sonication step.

Though more graphene was removed from the PET substrate after 2-propanol sonication, harsh solvents aren’t expected in testing or in practice. Thus, in light of the stark difference in silver adhesion to the substrates, PET was selected as the substrate for all electrodes. Further
studies using the silver/graphene multi-layer working electrode on PET showed that delamination was not an issue with the chosen substrate. Kapton tape was also used to mask the counter electrode during Nafion application and did not delaminate the silver or the silver/graphene multi-layer counter electrode.

**Graphene Alone**

The first functional working electrodes produced were graphene electrodes printed with the fabricated ink discussed earlier on PET substrates. These electrodes were printed through a 30 μm nozzle with DC voltage resulting in a working area of 3.0 mm². The portion of the graphene that touched the silver connection and the connections themselves were covered with NOA 74 and cured under UV light. These first devices also had an AgCl electrode within 1 mm distance from the working electrode and a silver counter electrode that was more than twice the size of the working electrode. AgCl conversion was done prior to graphene printing to avoid graphene contact with HCl and KCl. Graphene was cured at 250°C for 30 minutes, followed by the NOA 74 capping of the small amount of graphene that overlapped the silver, as discussed above.

**Graphene Doped with NiTsPc**

Graphene and carbon electrodes are fairly nonreactive, though doping or additives can be used to make them more reactive with an analyte in question. This necessity is actually a benefit in that various doping agents can be used to turn a normalized electrochemical substrate into a reactive agent for a number of different analytes, meaning this graphene electrode can now be post processed with different materials to detect different things. Improving sensitivity is important, as nitric oxide is typically in solution at pM-μM range. Many electrochemical sensors use nickel complex coatings to increase sensitivity, Ni(II) Tetrasulfonated Phthalocyanine (NiTsPc) being one of these complexes used to detect a number of biologically relevant molecules as discussed earlier; i.e. nitric oxide.
Figure 3.13 NiTsPc application using cyclic voltammetry from 0.0 – 1.2 volts. The increase in concentration is clearly seen by focusing on the redox peaks just above 0.4 volts and below 0.3 volts. As material is added, the response increases at these peaks.

The electrodeposition of NiTsPc can be done via cyclic voltammetry. The scan is typically run from 0.0 V to 1.2 V vs an AgCl electrode, showing telltale peaks as layers of NiTsPc are added to the electrode as shown by Miserere et al [99]. There is a shift in the peak location shown in the cyclic voltammogram of the e-jet printed working electrode, though the spacing between the anodic and cathodic peaks are consistent. To clearly visualize the application of the NiTsPc a zoomed in version of this is shown in Figure 3.13 with increased concentration of deposited NiTsPc along the provided arrows.

As the Nickel compound NiTsPc is applied, the peaks increase in amplitude. Once the NiTsPc was applied, cyclic voltammetry and square wave analysis were done to determine device functionality, both of which are shown in Figure 3.14.
Figure 3.14 Graphene/NiTsPc based WE detecting a 1.0 mM concentration of 1,1 ferrocene dimethanol in PBS using cyclic (left) and square wave (right) voltammetry.

These tests were done using a 1.0 mM concentration of 1,1 ferrocene dimethanol in PBS, a molecule that has a reversible redox reaction and known anodic and cathodic peaks at ~0.3 V and ~0.15 V respectively. As seen once again, there is a similar shift in the peak location compared to previous studies, though the spacing in the cyclic voltammogram remains true. Additionally, the oxidation peak shows a sharp response in the square wave unlike the shallow peaks in the cyclic voltammogram. This highlights the ability to detect lower concentrations via square wave analysis, but also that the oxidation peaks in both analyses are consistent.

**Silver Alone**

Though these experiments do not necessarily reflect the response of these architectures to nitric oxide, there is some translation in architecture repeatability as well as comparing newer architectures to older models. This analyte also helps in that it is an inexpensive and stable alternative to the reactive, short lived nitric oxide donor used otherwise. The detection response is rather low for the molarity tested, thus a pursuit for an architecture that delivers a higher response per concentration was in order.

Since silver is readily printable, it was printed as a working electrode, using the same geometry as in the carbon based version; 3.0 mm$^2$. Though a square wave response was registered, the declining response over voltammograms of the same concentration was drastic. This was likely due to the reactive nature of the silver electrode which turned a black color similar to the reaction that takes place in coating the AgCl electrode. The silver working electrode was an unsustainable and unquantifiable option. Furthermore, the response was shifted almost 0.2 V towards zero compared to the carbon electrode above which didn’t correlate well with the
expected response. Similar to the PEDOT:PSS electrode, this response does show some promise and may warrant another experiment with a Nafion cap, though this was not pursued herein.

**Silver with Secondary Graphene Layer**

The final working electrode base architecture that was explored was a silver base layer and graphene top layer. The theory here was that the graphene, in the form of a film, was forced to carry signal too far along its length, thus reducing the effective working area and response of the electrode itself. If instead signal simply had to flow directly from a material up through the thickness of the graphene, evenly distributing the potential throughout the intended functional area, then response would improve. Thus, silver was deposited as a base material, which was printed with the discussed soft and hard curing stages, upon which graphene was deposited as a secondary layer. The AgCl conversion was also done prior to the graphene deposition so that the graphene would not come into contact with the HCl or KCl solutions. It was observed that the thickness of this graphene mattered for two different reasons; first because it capped the silver and protected it from delivering potential to the solution itself (discussed later), and secondly it provided more complete doping of the active area. Thus the graphene was applied past the point at which no silver could be seen through the deposited graphene layers under a dissection microscope, after which it was baked at 230°C for 30 minutes.

Initial configurations were tested with cyclic and square waves as before with 1.0 mM 1,1, ferrocene dimethanol in PBS to gauge how the new base configuration compared to a Ni doped graphene configuration. This is not a direct comparison of the two bases since graphene alone did not generate a reportable response without Ni doping in previous experiments.
Figure 3.15 Silver base WE amplification (red) over its non-silver aided predecessor (blue) using square wave voltammetry to detect 1,1 ferrocene dimethanol. The anomaly seen around 0 V is likely due to incomplete coverage of the Ag by the deposited Graphene.

As seen in the Figure 3.15 provided above, the silver base increases the response 4 fold, even without any additives. Of note here is a response close to zero. This is likely due to less than full coverage of the silver base that was rectified in subsequent fabricated sensors.

Fully rapid printing

At this point, and for the remainder of the studies herein, it may be beneficial to state that the ‘base’ of each of the nitric oxide sensor is silver. The contacts are silver, layered 5-10 times on themselves to increase the height, thus reducing the resistance, and are 0.8 mm wide. The reference electrode is silver that is then converted to AgCl via 1.0 M HCl. Additionally, the base of the working and counter electrodes are silver (in some instances are simply silver themselves) to increase electron flow to less conductive materials. Thus, a system for producing this base was designed to print sensor bases as consistent and as quickly as possible.

The printing of this silver base layer fully utilizes the modifications to the LabVIEW GUI, as well as the heated vacuum chuck described previously, to print and rapidly cure the printed silver. Throughout testing, observations and alterations were made until a final product was created, wherein the stage movement rate is 10 mm/sec and the heated chuck set temperature is 85°C. Since delamination occurs, as discussed earlier, when large areas are printed early and
not hard cured within minutes, the contacts are printed first, 5 or 10 layers thick via printing each contact and then repeating the print. The small amount of time it takes to print the entire base is enough to soft cure the previously printed silver and add another layer without simply adding to the pool of uncured silver. On the last cycle, the electrodes are printed, starting with the reference electrode. One or two layers are used for both the reference and working electrodes because these are small enough where the film cures quickly and uniform, thus one may be employed in certain circumstances. The counter electrode on the large electrode setup is too large to have a uniform print with one layer, thus two are employed, leaving a base on the first pass with cured edges and a liquid center, finished by adding to that pool and allowing for a uniform curing of the counter electrode via soft curing. Once the totality of the base has soft cured, it is hard cured at 230°C for 30 minutes. Finally, the NOA 74 insulation is applied, the reference electrode is converted to AgCl, and the base is ready for any post processing steps, including the application of graphene and any additives.

**Effect of size of working electrodes**

As the introduction of the silver base improved the response, the doubling of the surface area of the working electrode was expected to do the same. The initial working area went from 3.75 mm² to 7.5 mm², thus to facilitate the extra current, the counter electrode went from 17.25 mm² to 22.75 mm² to preserve the 3:1 ratio. The AgCl geometry remained the same in these studies as size doesn’t matter for the reference electrode. All other parameters, including printing, curing, and post processing steps remained constant. To directly compare the results, 1.0 mM 1,1 ferrocene dimethanol in PBS was used as an analyte in a square wave analysis, shown in Figure 3.16.
Figure 3.16 Square wave voltammogram showing an increased sensitivity toward 1,1 ferrocene dimethanol when the working electrode active area is increased in size (grey). Due to complete silver coverage, the peak around 0 volts is no longer present.

As seen, the response more than doubles in amplitude for the same concentration. The reason it more than doubles, despite a doubling in actual active area is likely due to the added deposition of graphene along the edges of the silver base to ensure full encapsulation, thus avoiding the aforementioned response due to silver/solution interaction.

Effect of Doping with Ni

As previously discussed, a NiTsPc addition improves the response of a graphene electrode, though it was unclear if this would be the same with the silver base. At this point, there was the possibility that the graphene could simply be shielding the silver, limiting the working area, thus regulating its response, instead of the graphene doing the bulk of the sensing. Thus, it was uncertain if doping would quantitatively improve existing detection methods or systematically alter the analysis output by making the graphene ‘screen’ conductive. At this point, either a Ni addition would improve upon the existing response, or prove that the graphene was acting as a selective film. The original size of the working electrode, 3.75 mm$^2$, was printed as before, with the original counter electrode size as well.
The NiTsPc was applied using the same cyclic voltammetry method discussed previously. Though there are more cycles during Ni addition for the device shown in Figure 3.17, there is a stark difference in the current produced, even at the beginning of the nickel application, compared to the original non-silver based device discussed previously. This is supported by the 13 fold improved response of the nickel doped silver/graphene based working electrode compared to a nickel doped graphene based working electrode of the same size as seen in Figure 3.18. Of interest as well is that, despite being the original, smaller working area, the nickel doped electrode out performs the non-doped working electrode that is twice as large.

Figure 3.17 The addition of the silver base layer amplifies the response during NiTsPc application.

Figure 3.18 Square wave voltammogram depicting the increase in sensitivity by applying NiTsPc to the silver based graphene WE (yellow). The amplification is seen by a non-additive WE twice its size (grey) and a similarly doped WE without the silver base (blue).
Effect of Doping with Gold

At this point, tests using the previously printed sensors were not yielding results detecting sufficiently low nitric oxide concentrations, thus alternatives were explored for working electrode configuration. There have been a few studies suggesting gold electrodes and gold nanoparticles can detect nitric oxide [110-114, 122]. Since reports suggest that gold ink has a curing temperature that is too high for PET, a nanoparticle addition to a carbon base was explored. HAuCl₄ has been shown to donate its Au component to a number of different substrates in a number of different methodologies, though the most relevant use was to coat RGO with Au nanoparticles via electrodeposition [112]. Since the graphene base herein has been shown to stand up to electrodeposition in the past, this methodology was adopted, though slightly altered from the publication.

The protocol used herein is to apply a -50 mV potential between the AgCl reference electrode and the silver graphene base working electrode via the three electrode setup in a 1.0 mM HAuCl₄ solution in 1.0 M HCl, thereby coating the working electrode with Au nanoparticles. Experiments were done with a Pt electrode instead of the printed silver electrode as a coating counter electrode with no observed coating differences.

![Figure 3.19](Image)

Figure 3.19 Square wave voltammogram showing the final doping iteration. The gold doped graphene WE with silver base (blue) shows the highest sensitivity to 1,1 ferrocene dimethanol at a standard size and holds trend as the working electrode size is increased (green).
As seen in the Figure 3.19 above, the Au nanoparticle doped silver/graphene base electrode was approximately 40% better than with the Ni addition with a lower background current. Of note is the same spike towards zero potential that is likely due to a silver base that was not fully encapsulated by graphene. Additionally, a large Au coated silver/graphene base working electrode more than doubled the response of its smaller counterpart, showing similar results in a previous comparison between non-doped versions of the base electrode. Of note again is the amplified response close to zero volts of the Au doped working electrode, highlighting the need to fully cover the underlying silver layer with graphene.

3.4.4 Nitric Oxide Detection

Detecting Nitric Oxide Using Au Doping

Though this ferrocene analyte testing setup allows for direct comparison of functionality of the working electrode, the main goal of the research is to detect nitric oxide. Thus aliquots of nitric oxide were established with varying concentrations of nitric oxide. For most experiments 1 mM of SNAP (0.49 μM NO via internal calibration) and a stock solution (2.156 μM NO) were the main concentrations investigated for ease of testing and consistently comparable results. A 4.41 mM stock concentration of SNAP was made in 0.34 mM EDTA. The concentration of EDTA was taken from the WPI look-up table for calibration of commercial nitric oxide sensors and is used to regulate the degradation of SNAP and thus the production of nitric oxide in solution [119]. This EDTA concentration was maintained through using a stock 0.34 mM EDTA solution to dilute the stock SNAP solution down to various concentrations; i.e. 1 mM SNAP.
Figure 3.20 Nitric oxide detection via cyclic (left) and square wave (right) voltammetry using a Ag/Graphene/Au WE, using a Ag CE and Graphene CE respectively. Concentrations of NO were derived through calibrated SNAP concentrations.

Figure 3.20 above depicts the comparison of the two methods of analysis that were discussed earlier; cyclic and square wave voltammetry, utilizing a silver and silver/graphene counter electrode respectively. Seen on the left is the comparison of the stock SNAP concentration (2.156 μM NO) to the EDTA control, thus controlling for the background current due to EDTA in solution. Of note are the identifiable oxidation peak at ~0.9 V and the pronounced reduction peak at ~0.4 V, which are consistent with previously published peak locations [123]. The right portion of Figure 3.20 depicts nitric oxide detection via square wave voltammetry. There is a distinct difference between the three concentrations with identifiable oxidations peaks. Of note is the shift in the oxidation peak to the left compared to the cyclic voltamogramm which may have been due to a compromised reference electrode, since this was not consistent with all silver/graphene counter electrode setups.

**Cyclic Staircase Test**

To elaborate on the tests conducted above, a cyclic voltammogram was produced in various concentrations of nitric oxide to construct a concentration curve. This concentration curve could then be used to investigate the concentration of test solutions by measuring the peak oxidation response. A 7.5 mm² Ag/Graphene/Au working electrode with a AgCl reference electrode and Ag counter electrode was used in this experiment.
Figure 3.21 Cyclic voltammogram of increasing nitric oxide concentration using a Ag/Graphene/Au working electrode (left). EDTA background currents were subtracted from the recorded data. The response (y-axis) for each concentration (x-axis) was plotted (right).

As seen in Figure 3.21, 1.5 mL samples were produced at increasing SNAP concentrations, correlating to the nitric oxide concentrations listed. EDTA concentrations were correlated to the ratio described above for each sample at 12.97:1 SNAP:EDTA. Each data set had the background EDTA concentration subtracted out afterwards, revealing the presented graphic. Though peak drift occurred, the majority of the oxidation peaks centered on 0.91 V, which is typical for nitric oxide detection and AgCl reference, and was used to compile the concentration gradient above, wherein the current response for each concentration was taken at the 0.91 V potential and displayed together. The concentration gradient presented is characteristic of this individual sensor and did not translate to other sensors tested due to graphene deposition variations between sensors. The trend line is presented as linear since this type of relationship is expected from cyclic concentration gradients.

**Time-series Monitoring**

As opposed to testing the sensor via cyclic voltammetry, amperometric detection is used for real time-series detection. The peaks found in cyclic voltammetry are key to detection using amperometry in that the peak potentials are used as a consistent potential set between the reference and the working electrode to induce a constant redox reaction. When the analyte in question becomes present, the current response changes directly in real time. In this way the presence and quantity of the analyte can be consistently interrogated without a complex scanning protocol as seen in cyclic and square wave voltammetry detection methods.
Though two electrode setups were not printed, a low current potentiostat was purchased from World Precision Instruments (TBR1025) for use with the commercial nitric oxide two electrode sensor used in the calibration protocol discussed earlier. This instrument was used to initially test the printed sensors as well to determine functionality in an amperometric setup due in part because of this low current level detection but also because of its increased sample rate compared to the three electrode potentiostat used for cyclic voltammetry.

The Figure 3.22 below depicts the real-time two electrode setup with a 7.5 mm² working electrode, electroplated with Au at -0.05 V potential vs printed AgCl electrode with the same AgCl electrode as a reference, though it was printed as a three electrode architecture with a silver counter electrode. This electrode was first tested in 1.0 mM SNAP using cyclic voltammetry to determine its functionality. The working electrode was then coated with Nafion via spin coating at 5500 rpm for 60 seconds and then baked at 150°C for 20 minutes. To determine continued functionality after Nafion coating, it was tested in 1.0 mM SNAP via cyclic voltammetry.

The real-time device was acclimated at 0.853 V (set by instrument) in 10 mL 3.42 mM EDTA for over 5 minutes with agitation via stir bar and being recorded in LabScribe. Once the output current was stabilized, a 4.42 mM SNAP 0.34 mM EDTA solution was added via pipette in 1 ml intervals corresponding to the increases in the current response in Figure 3.22. At 140 seconds, 2 mL of solution was removed from the chamber and 1 mL increments of EDTA solution were added, diluting the nitric oxide concentration. A final 1 mL of SNAP solution was added at the end. Responses are shown in nM of nitric oxide, calibrated through the commercial sensor discussed previously.
Figure 3.22 Two electrode amperometric NO concentration detection at 0.853 V. SNAP aliquots were added, corresponding to the first two increases in current (y-axis), followed by two dilution steps, and one final addition step.

To visualize the concentration response, Figure 3.23 above shows the concentration compared to the response directly. Though the ~200 nM was detected, the use of a concentration gradient is that a response can be registered via the sensor and referenced to calculate the molarity in solution. This response resembles the beginning of a typical ‘S’ curve for a dose response, becoming linear at 300 nM with a 0.4 pA/nM response and can be used to responsibly estimate the concentration based on future low molarity amperometric responses, though the full ‘S’ curve was not completed. This simple two electrode configuration has a base detection limit of between 200 nM - 300 nM.
Figure 3.23 Two-probe dose response plotting the current change (y-axis) based on the concentration of nitric oxide added to solution.

This experiment demonstrates the ability for the sensor to respond to concentration changes both positively and negatively. It also demonstrates the use of the Nafion layer (discussed later with three electrode architectures) and the ability to detect changes in concentration in nitric oxide solution.

Real-Time Measurement with a Large WE, Three Electrode Configuration

Though the two electrode setup is a good basis for examining functional printed devices, the three electrode setup was the proposed long term electrochemical configuration. Thus, a Model 842C (CH Instruments) three electrode potentiostat was employed to derive amperometric calibration sequences for various devices. This potentiostat was setup in a Faraday cage and was grounded at multiple points to combat electronic harmonic noise. The printed device shown in Figure 3.24 employed a 7.5 mm$^2$ working electrode, electroplated with Au at -0.05V potential vs printed AgCl electrode and wider (0.8 mm vs 0.4 mm) and higher (10 stacked) silver contacts to improve the conductivity and connection. This electrode was first tested in 1.0 mM SNAP using cyclic voltammetry to determine functionality. The device was acclimated at 0.918 V in 10 mL 3.42 mM EDTA for over 5 minutes. This potential was taken from the cyclic voltammogram done prior to amperometric detection. Once the output current was stabilized, a 4.42 mM SNAP 0.34 mM EDTA solution was added via pipette in 0.5 ml increments. In Figure 3.24 is the dose response of this sensor with a lower limit of detection (LOD) of 188.5 nM, showing the ‘S’ type dose response discussed in the previous section with the two electrode setup.
Figure 3.24 Three electrode amperometric NO detection dose response curve showing the increase in current (y-axis) as the nitric oxide concentration (x-axis) increases. A Ag/Graphene/Au working electrode was used. The potential, determined through cyclic voltammetry, was set to 0.918 V.

Three Electrode Configuration with Nafion

The selectivity of Nafion stems from its ability to shield the working electrode from negatively charged molecules, namely NO$_2^-$ and NO$_3^-$, two of the major byproducts of the NO oxidation. To test and calibrate a proposed Nafion deposition methodology, commercially screen printed sensors, supplied with the commercial potentiostat (DropSens μStat200 Asturias, Spain) were used. These sensors utilize carbon working and counter electrodes but have a silver reference electrode which causes a slight shift in the voltammograms compared to the AgCl reference electrodes used in the E-Jet printed sensors. A spin coating methodology was studied, as previous tested drop cast films were washing out the detection abilities of the sensors, signifying that the film was too thick. A 5% dispersion of Nafion in alcohol from Sigma was cast for 1 minute at 500 – 6500 rpm in 1000 rpm increments with a drop cast control (too much Nafion), a propanol spin coat control (to control for the addition of the Nafion solvent), and a non-additive control (to control for any modification to the sensor due to the Nafion curing process). All samples were cast onto the working electrode using Kapton tape as a mask for the rest of the functional area and cured, with the tape on, at 150° C for 1 hour immediately after casting. The
films were tested in 1.0 mM 1,1-ferrocene dimethanol prior to coating and after curing and tape removal using cyclic voltammetry from -0.1 V to 0.4 V with scan rate of 0.1 V/s. The non-additive control matched the cyclic voltammograms from before the coating process as did the propanol control. Figure 3.25 below depicts the non-additive control (blue) with the drop cast control (red) for comparison.

Figure 3.25 Cyclic voltammogram in 1,1 ferrocene dimethanol with no Nafion (blue) and a thick layer of Nafion (red) (left) and a progressive thickness range (right). Nafion thickness was governed through the spin coating speed used during Nafion application.

The drop cast control shows a major washout of the oxidation peak as well as a major shift and washout of the reduction peak. The test group is also shown in Figure 3.25, showing this process at work with a slow washout of the oxidation peak and a shift and amplification of the reduction peak.

For clarity, the oxidation (top right) and reduction (bottom left) peaks were mapped with their potential and their amplitude in Figure 3.26, with arrows signifying an increase in film thickness (reduction in spin speed). As discussed earlier, the oxidation peak becomes washed out with little shift in potential, while the reduction peak amplifies and shifts to more negative potentials. Of note however, is that all of the spin speeds tend to group into two major peak locations and intensities (circled), allowing for a broader range of spin speeds to study when determining the ability to shield against noise creating analytes.
Figure 3.26 Oxidation and reduction peak location (x-axis) and amplitude (y-axis) of Nafion coated WE’s. Arrows shown the direction of increasing Nafion thickness (reduced spin speed). Circled regions correspond to regions of further research for analyte selection. Non-coated and propanol wash controls were used but not plotted.

**Nafion with detection and shielding**

The main use of Nafion herein is the ability to block negatively charged molecules that may create interference while attempting to detect nitric oxide. Two of the main byproducts of nitric oxide oxidation are NO$_2^-$ and NO$_3^-$. Thus, sensors comprised of a gold coated working electrode, a Ag/Graphene counter electrode, and an AgCl reference electrode. The goal was to coat the working electrode to the point where high concentrations (1 mM in 0.34 mM EDTA) compared to the analyte at hand (~1μM NO in EDTA) are eliminated. That point was reached at 5500 rpm as seen in Figure 3.27 and successfully washed out NO$_2^-$ and NO$_3^-$ while retaining nitric oxide detection, though the reduction peak did shift as expected.

Figure 3.27 Nafion coating selectivity using 5500 rpm spin speed, detecting NO (left) but not NO$_2^-$ (center) or NO$_3^-$ (right) analyzed using cyclic voltammetry. The concentration of NO is 0.49 μM, and 1 mM for both NO$_2^-$ and NO$_3^-$. Blue signifies the response prior to Nafion and application and grey signifies after.
A more realistic test was done with 1.0 μM concentrations of NO₂⁻ and NO₃⁻ without Nafion and the same setup. This architecture could detect nitric oxide, as shown earlier, but did not detect the interfering molecules in solution at these concentrations.

**Real-time measurement of nitric oxide w/ Nafion**

In order to evaluate the final setup, four fully printed three electrode nitric oxide sensors were produced with a AgCl reference electrode, a 1.28 mm² Ag/Graphene/Au/Nafion working electrode, and a 7.25mm² Ag/Graphene counter electrode. Each of the four was tested for functionality using cyclic voltammetry in 1.0 mM SNAP/EDTA solution. The respective oxidation potentials were used during amperometric detection and a dose response was created as before. These responses were averaged together and the resultant dose response is shown in Figure 3.28 below with a lower limit of detection (LOD) of 314.5 nM.

![Figure 3.28](image)

**Figure 3.28** Three electrode amperometric NO detection dose response curve showing the increase in current (y-axis) as the nitric oxide concentration (x-axis) increases. The potential, determined through cyclic voltammetry, and an average of four devices is shown. A Ag/Graphene/Au/Nafion working electrode was used with a graphene CE.

Though Nafion decreased the lower LOD of the nitric oxide sensor, the added selectivity broadens the environments in which the sensor would be deemed appropriate for use, including in cellular solution. A visualization of this data is shown in Table 3.1, along with the maximum tested selectivity concentration, and the R² fit of the dose response.
Table 3.1 Nitric Oxide detection limits and sensitivity with and without Nafion

<table>
<thead>
<tr>
<th>Nafion</th>
<th>Lower LOD (nM)</th>
<th>Maximum Selectivity (μM Competition)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>188.5</td>
<td>1.0</td>
<td>0.99936</td>
</tr>
<tr>
<td>Yes</td>
<td>314.5</td>
<td>1000.0</td>
<td>0.99258</td>
</tr>
</tbody>
</table>

3.5 Discussion

Within the results provided, there are four major subsections; the reference electrode, the counter electrode, the working electrode, and the overall sensor function. The functionality of each electrode was evaluated independently of each other since their independent function is paramount to the sensor as a whole.

With regards to the reference electrode, 97.7% functionality within ±10 mV of a commercial AgCl electrode with respect to avoidable failures, outpacing the 90% functionality within this range set out at the beginning of the experiment. To achieve these results, however, it is paramount that unoxidized silver (i.e. clearing 10 μl from the tip of stagnant cartridges) is deposited with two printed layers of thickness using the heated vacuum chuck set to 85°C (~51°C surface temperature). This ensures enough of a pristine silver thickness to allow for full AgCl conversion without penetrating to the substrate.

The counter electrode requires identical printing conditions with two layers of Ag base thickness. As seen in Figure 3.12, the anodic peak is relatively similar in potential, though there is a noticeable shift in the cathodic peak. Typically, this hysteresis is CE material dependent, with Pt having low hysteresis and Ag showing slightly higher impacts in resolution. There also may be oxidation on the silver films produced due to the heat curing process that can amplify hysteresis, which should be a focus in future work. This would explain both shifts and the disparity of a taller voltammogram for the platinum. Never the less, the disparity is acceptable, especially when looking at the location of the anodic peak. The addition of graphene on top of the Ag was also tested and was used in the final three electrode setup, displaying adequate amperometric response despite its relatively lower conductivity.

Though the results showed a consistent progression in working electrode response, the only functional working electrode chemistry in nitric oxide detection was the Ag/Graphene/Au
configuration. The Nafion tests showed a range of possible spin rates that were then tested on this working electrode configuration, with 5500 rpm being the first speed in that progression, where significant concentrations of competing analytes were repelled and nitric oxide detection was retained.

The inconsistencies in detection characteristics stem from potential deposition issues with the graphene ink. Though the ink is readily printed without issue, the ink itself has a high surface tension when printed on the silver sublayer. This leads to inconsistencies in the deposition of graphene, a material that is polished in traditional electrochemical cells to achieve a featureless working area. This is not possible at this time as any polishing may either create shorts between the closely printed electrodes or create pinholes in the working electrode graphene; consequently this has not been attempted to this point. Since the toluene has a lower boiling point, the heated vacuum chuck has only been tested at lower set temperature in attempts to quicken the evaporation process. The impacts of this heating has not been examined to this point and the subsequent sensors were not included in the results section.

The fabricated Ag:AgCl reference electrode, Ag/Graphene counter electrode, and Ag/Graphene/Au/Nafion working electrode setup was able to consistently detect nitric oxide below the 1.0 μM detection limit set out at the beginning of these studies. Though there is some disparity between the lower LOD of this configuration, a range of 188.5 – 314.5 nM was established with size of the working electrode possibly contributing to this inconsistency. These detection levels are lower than those of previously produced nitric oxide sensors using screen printing [99]. Regardless, this LOD range and the fact that the final configuration can block competing reaction with NO₂⁻ and NO₃⁻ at high (1 mM) concentrations bode well for use as a general chemical sensor and a bioelectronic interface, as discussed later herein.

3.6 Conclusion

The work herein has demonstrated the ability to deposit various materials resulting in the fruition of a sensor capable of selectively sensing nitric oxide on the nanomolar scale and above via various voltammetric detection methodologies, including cyclic voltammetry, square wave voltammetry, and amperometric detection. The key enablers for the fruition of the design goals were the heated vacuum chuck, development of a graphene ink, and post processing methodologies. Though sensitivity routinely remains sub-micromolar, the concentration gradient
is relatively imprecise from device to device, likely due to the inability to create consistently uniform graphene films at the moment. Future work would include development of a methodology to resolve these and other precision and repeatability issues, as well as reducing the overall functional area of the sensor itself for use in micrometer level detection. Overall, the design goal of Nafion induced selectivity (<1% detection of NO$_2^-$ and NO$_3^-$ compared to NO) in detecting nitric oxide concentrations $\leq$ 1.0 $\mu$M was met and exceeded, though the <5% metric variability was not achieved though may be in the near future.
Chapter 4 Photosensor

4.1 Introduction

One possible pathway to target in microorganisms is that of bioluminescence. As discussed later, there are naturally occurring bacteria that generate enough light in response to quorum sensing to elicit a current change in traditional and printed photosensors. The goal of the proposed research in Dr. Ayers’ lab aims to harness this luminescence to specifically detect harmful agents (munitions, chemical weapons, etc.) in the water column and on the sea floor using biomimetic autonomous robotics. The next stage in the evolution of these robotics is to use the photosensor discussed in this section as a munitions detecting bioelectronic interface. In the methods and results delivered below, the electrohydrodynamic printing system functionality is investigated through the fabrication of functional, reproducible (>90% intended devices, <5% metric variability), multi-layer photosensors that are capable of detecting ≤ 0.3mW/cm².

A photodiode is a photon detector that is able to convert light energy into electrical energy, via either current or voltage, based on the mode of operation of the sensor. Similar to regular semiconductor diodes in design, the sensitive part of the diode is left exposed, or packaged with a window, such that light may reach it. When a photon of light of sufficient energy impacts this sensitive zone, an electron-hole pair is created and, if struck in the depletion zone (conductive region where the mobile charge carriers have diffused away), these carriers are swept from the junction; holes towards the anode and electrons toward the cathode. The resultant current is the summation of the current due to the photon impact, and the current produced in the absence of light (dark current). This dark current must be minimized in order to create a sensitive photodiode [124]. Traditionally, semiconductors have been used in photodiodes, including silicon, germanium, indium gallium arsenide, lead(II) sulfide, and mercury cadmium telluride; each of which has a unique photon electromagnetic wavelength range [125].
Photodiodes are simply phototransistors without the emitter connected; thus it is appropriate to assign the bulk of the credit of invention of the photodiode to the inventor of the phototransistor, John N. Shive, who used germanium and bronze electrodes to prove that holes could diffuse through bulk germanium, and not just along the surface as previously thought [126-128]. Though commercial photodiodes are fabricated mainly via clean room methodologies, there has been some recent research into the fabrication capabilities of spin coating and ink jet printing using polymer based photosensitive chemicals, and clear conductive electrodes [129, 130].

The photosensor is one of the most studied sensor classes because of their application in the energy conversion industry, namely solar cells [131-134]. Though the photosensor produced herein does not aim to meet industry standards in photon detection sensitivity and efficiency, the methodology of electrohydrodynamic printing the sensor is of major significance. The ability to precisely print predefined areas using a variable system not only cuts down on fabrication time and chemical costs, it can ultimately cut down on cost to return ratios. The ability to print onto a number of substrates and in various geometries allows for the customization of sensors and maximum use of surface area for a robotics field that continues to see a reduction in platform volume. The devices produced in this chapter will have an immediate testing scenario as a sensor on the robotic lobster [7] currently under research in the Ayers lab.

Previous research have demonstrated the ability to fabricate photodiodes and solar cells via printing methods [20, 135-140]. Often times in these studies, the transparent electrode (chiefly indium tin oxide ITO) is pre-deposited via sputter coating, the active layer is deposited via inkjet printing or spin coating, and the final electrode (aluminum in most cases, silver herein) is deposited via thermal evaporation or sputter coating. Though these experiments can show how printing methods may be harnessed to create photodetectors, the fabricated detectors tend to be large in size, and utilize other deposition methods in conjunction with printing without showing how these layers will react to layering of printed materials.
The basic design of the photodetector emulated herein comes from the research conducted on organic photosensors by Gang Yu et al, who utilized poly(3-octyl thiophene) P3OT and [6,6]-PCBM as an active layer [129], though these materials were replaced with P3HT:PC_{71}BM upgrades, pictured in Figure 4.1. These materials create an electron-hole pair when impacted with a photon of sufficient energy (specific wavelengths of light). Though various derivatives of these structures have been studied since the original publication, the majority of the chemicals used are commercially available. The four main components of the proposed design herein, which were modified from its original form to improve upon function, printability, and stability, are an ITO clear cathode (pre-deposited and commercially available), P3HT:PC_{71}BM active layer, PEDOT:PSS hole transport layer (HTL), and silver anode [141-143]. With the exception of the ITO cathode, the rest of the layers reported below have been deposited via e-jet printing in a number of geometries.

4.1.1 Basic Printed Structure and Theory of Operation

The basic printed structure is quite similar to the structure adopted in initial trials, where P3OT:PCBM was used as an active layer. The final structure, however, uses a P3HT:PC_{71}BM heterojunction format instead of the original P3OT:PCBM due to a number of publications showing the advantages of adopting this upgrade [141-143]. In Figure 4.2 below are two bandgap
diagrams from two publications; one with an aluminum electrode and one with a silver electrode [144, 145]. The structure shown on the right in Figure 4.2 is different than typically produced photosensor (left in Figure 4.2) in that by changing the anode, it flips the direction of the current flow. As seen in the graphic, the original configuration (left) is setup to allow holes to move along the Highest Occupied Molecular Orbital (HOMO) of each layer, acting as steps, to reach the anode (ITO). Conversely the Least Unoccupied Molecular Orbitals (LUMOs) are setup, acting as steps for electrons when the electron-hole pair is created. Though the path of the holes and electrons along the HOMOs and LUMOs remains the same respectively, the ‘staircase’ is essentially reversed in direction due to the Al to Ag conversion.

Figure 4.2 Traditional (left) and flipped (right) photosensor configuration [144]. Both configurations have a clear ITO electrode, though the traditional form benefits from clean room processes that increase the work function. The substitution of Ag for Al allows the use of the lower ITO work function, altering the direction of the electron flow, depicted with black arrows. Copyright 2008 National Academy of Sciences.

ITO treatments have been developed to increase the bandgap energy of ITO to make the difference between ITO and Al greater these actually would reduce the current flow by creating a higher ‘step’ for the electrons to overcome in the flipped orientation. Instead, the ITO substrates used in this study are cleaned with bath sonication in acetone, followed by 2-propanol, and then spin coated with a DI water bath [145]. This leads to a comparatively lower ITO bandgap, potentially even lower than that of Ag (4.26 eV to 4.74 eV), around 4.09 eV to 4.20 eV, though these numbers are difficult to obtain experimentally and vary from film to film [146-148]. A simple way of determining which way the electrons flow is to study the response of the device to light, which confirmed a flipped architecture in our case, as the current flowed in the opposite direction.
Furthermore, Figure 4.2 highlights the use of PEDOT:PSS as a means to gradually step to a final work function or band gap energy at the hole accepting electrode. PEDOT:PSS is used as a hole transport layer in polymer and organic devices for this specific purpose [136, 149, 150], and as an electron blocking layer to ensure that electrons do not enter zones in which they would cause noisy or inefficient response; thus the opposite orientation.

This orientation, though it seems to be out of the ordinary in terms of recent research trends, is actually advantageous. ITO is difficult to deposit via solution deposition methods and anneals at high temperatures, with varying degrees of optical transmittance, which is why chemical vapor or sputter coating deposition is preferred. Additionally, and more importantly, X-ray photoelectron spectroscopy has been used to examine the vertical orientation of the two materials that make up the bulk heterojunction. As the heterojunction is annealed after deposition, it is heated to a temperature that allows the motion of these materials and alters their final vertical orientation. The temperature and length of time during annealing impacts how these materials are orientated, which alters overall device performance. Despite the method of annealing and deposition in this X-ray photoelectron spectroscopy study, all heterojunctions examined were found to have the PCBM mainly located closer to the bottom electrode (ITO herein), with the P3HT located closer to the air, and eventually the top electrode (Ag herein). As seen in Figure 4.2, this is advantageous for the inverted architecture used herein over the traditionally deposited polymer and organic photosensors, as it increases the efficiency of the direction of electron and hole flow [151].

4.2 Methods/Materials

4.2.1 Substrate choice

In pursuit of constructing a functioning photodiode, the selection of a substrate and a transparent electrode are the first logical steps to take. Based on a number of publications [129, 130, 136, 139, 144] and commercial availability, it was determined that the active layer would be printed onto an ITO coated substrate, known for its stability and high light transmittance. Currently, there are two substrates that are commercially available with a pre-deposited ITO film at various sheet resistances; glass and polyethylene terephthalate (PET). In order to choose between the two substrates, a number of parameters were considered, including transparency,
potential and actual electrode adhesion, dissolution in potential media, and longevity of printed films.

**PET/ITO/Glass Transmittance**

The proposed devices have a clear ITO electrode so that the excitatory wavelengths of light can reach the active layer. This also means that the light has to traverse the substrate that the ITO is deposited on. The difference in substrate can impact how much and which wavelengths of light make it to the active layer due to their transmittance spectrums; thus these spectrums were examined. Light from a tungsten halogen lamp (LS-1, Ocean Optics) was passed via fiber optics through an open space into a collector (USB4000, Ocean Optics) that examines the light beam received. A light and dark control were gathered and then samples were placed in the path of the light. To examine the substrates, cleaned (acetone and 2-propanol sonicated) samples were placed in the path of the beam, substrate side facing the light as would occur in printed devices. As shown in Figure 4.3 ITO itself (spectrum from PET with ITO minus spectrum from PET) has an absorption spectrum, meaning a reduction in light level is already occurring due to ITO, regardless of the substrate. Furthermore, there is a significant difference between the glass and PET substrates with the former having the advantage in regards to overall transmittance. To examine this difference, the light control was instead run through the ITO coated glass substrate as a light control to get a direct comparison to ITO coated PET, which became the sample. This is shown in Figure 4.3, showing that PET transmits 5-15% less light in the spectrum of concern.

![Figure 4.3 ITO transmittance (left) and PET vs Glass transmittance comparison (right) taken over the visible spectrum.](image)
Potential adhesion evaluation through wettability testing

Ink adhesion to ITO as well as to the substrate itself is important for final device structures since devices may be in cell media and/or ocean water, and any dissolution due to liquid contact, or delamination due to mechanical stress during testing will impact final device performance. One method of increasing the adhesion of inks to the ITO surface is to process it by chemical, mechanical, and photo methodologies [152-156]. A literature search had a number of pretreatment options for the ITO film that have contributed to an improvement in final device function and overall adhesion. The treatments are meant to either clean the surface of the ITO [145], or alter it to improve the work function of the ITO [152-156], thus improving the bandgap of the device. The study herein adopted a widely used cleaning process as well [145]. The cleaning process utilizes bath sonication to agitate the surface of the ITO in acetone for 1 minute and in 2-propanol for 10 minutes, followed by spin coating the substrate with a DI water wash to rid the surface of any oils or organic substances. One surface altering method that was considered is UV Ozone treatment, wherein short wavelength light is used to excite and dissociate the remaining impurities (hydrocarbons absorb 253.7 nm) on a pre-cleaned surface while simultaneously degrading molecular oxygen (184.9 nm) and ozone (253.7 nm) into atomic oxygen. The continually excited impurities on the surface of the ITO react with the continually produced atomic oxygen, changing these impurities into simpler, volatile entities that desorb from the surface of the substrate. This was attempted by utilizing a handheld UV light (Versalume PP-FLS) without oxygen amplification. Additionally, there is evidence [157, 158] that hexamethylene disilazane (HMDS) improves the adhesion of various organic films to ITO and ZNO surfaces through binding with the methyl groups in HMDS. This has helped to improve the efficiency of photosensors in the past and would be a simple spin coated addition to the architecture if it indeed improves adhesion.

The contact angle of the ink on any given substrate is one parameter to consider when investigating substrate adhesion. Each substrate contains its own surface energy and the contact angle of a known solution (DI water) can be used to compare these energies. If the contact angle of a droplet of DI water is 0 degrees, the DI water is said to have completely wetted the substrate. Likewise, between 0 and 90 degrees (Figure 4.4), high wetting occurs, 90 and 180, low wetting occurs, and at 180 degrees, no wetting occurs; glass has a small contact angle and is thus highly wetted by water (~27 degrees after isopropanol cleaning) [159].
In order to determine the effects that cleaning, HMDS, and UV treatment has on the surface of proposed substrates, an experiment was run in a particle free room. The contact angle of a droplet (~17 μl) of DI water on a polyethylene terephthalate (PET) substrate was measured using a Phoenix 150 contact angle measurement system. The angle was measured following a rinse in DI water (dried with nitrogen), sonication in isopropanol for 10 minutes (dried with nitrogen), direct UV light (Versalume PP-FLS) for 15, 30, and 60 minutes, HMDS spin coating (deposit then spin and deposit while spinning), and HMDS spin coating followed by 60 minutes of direct UV light. The contact angle was recorded in the center, on the edge, and in the corner of the substrate after each treatment and was analyzed three times per droplet.

As shown in Figure 4.5, the contact angle and wetting on PET significantly change once cleaned, showing higher wetting, however remain fairly constant during the attempted
treatments (HMDS previously shown to increase contact angle). Similar results were shown for glass. This is not to say that the treatments have no impact on the substrate, however there is no change in the wettability of the substrate. UV treatment was tested here because of its uses in further cleaning the substrate after isopropanol, and though there is some sign of an increase in contact angle and coincident decrease in wetting energy over time, this may be a product of time itself and not the UV treatment, which deserves further study. Previous studies have shown that UV application, specifically under high O\textsubscript{2} concentrations, has shown a decrease in contact angle, which is not shown here, possibly due to this lack of oxygen supply. This implies that the current UV treatment does not have as much an impact on the substrate herein was thus eliminated from the cleaning process after the study. HMDS has been shown to increase the contact angle in past studies, but does not seem to have more of an impact than isopropanol, thus was not used in subsequent studies.

![Figure 4.6 Contact angle measurement setup showing a water droplet before (left) and after (right) deposition with the latter showing the automated contact angle measurement.](image)

These studies also allow a declaration of higher potential printing resolution on PET (58.32 ± 0.82\(^\circ\)) than on glass (27.20 ± 0.06\(^\circ\)), as a higher contact angle indicates less spreading of the ejected droplet upon impact. With an ideal contact angle being 90\(^\circ\) as far as resolution is concerned, PET is still not perfect (Figure 4.6), though it is significantly better than glass, as far as water is concerned. Unfortunately, with the solvents used herein having drastically lower surface tension than that of water, spreading will occur and printing resolution will be impacted. One major route of additional research would be a processing methodology that increases the contact angle of the substrate without impacting film adhesion and device function.
PET/SILVER Durability

Glass substrates coated with ITO have been the overwhelming choice of substrate for testing photosensors of this variety in the literature. Although adhesion to ITO was sufficient, observations and tests regarding the nitric oxide sensor have shown that the adhesion of the silver electrode was poor on glass but excellent on PET. Initial variability with functioning photosensors was largely due to electrode failure during testing, delamination being principle among these failures. In attempts to stabilize failing electrodes, copper tape was employed, as seen in Figure 4.7. This tape did not adhere over a long period of time and moved slightly on the glass, eventually failing as well. Examining the adhesion of silver to substrates under various environments was important for just this reason.

![Figure 4.7 Copper tape applied to photosensor silver electrodes](image)

Silver Dissolution

Since the adhesion of silver to the bare glass substrate was the initial problem, two substrates (bare glass and PET) and three cleaning practices per experiment were used; one unclean, one cleaned with ethanol, and one cleaned in an isopropanol bath sonication. As discussed in the previous section, substrates saw no loss of silver after their first tape test (post hard-curing). There was, however, poor attachment to glass following a 60 minutes water bath showing wrinkles, while PET saw very little reduction in silver (no pinholes formed). The remaining dots on the glass substrate were subjected to the tape test and lost at least 30-40% of the remaining silver.
Figure 4.8 PET (left) and glass (right) delamination of silver. On each substrate the dots were evaluated for adhesion after I: curing at 230°C II: 60 minutes in non-agitated DI water III: sonication in isopropanol. Three substrates were used to interrogate the cleaning process with one uncleaned, one cleaned with ethanol, and one cleaned via isopropanol sonication.

Both substrates were subjected to a bath sonication in isopropanol and while there were slight cohesion problems with the PET, no pinholes were evident and nearly all of the deposited silver remained on the surface of the substrate. The remaining two dots of the original nine on the glass substrates were interrogated with the tape test, one of which was removed all together, the other removing all but about 20% of the original dot (only about 60% left after water) as shown in Figure 4.8 above.

PET/P3HT Durability

An additional experiment was run with the cleaning steps used in photodiode production to examine if the cleaning steps had an impact on active layer adhesion, as the active layer would come into contact with the substrate itself when used as an insulator to prevent ITO/Silver shorting, as discussed later. The same adhesion tests were run by depositing P3HT:PC71BM via pipette onto an uncleaned and cleaned glass and PET substrate in triplicate, which were left to dry in air fully, and then baked at 160°C. On both cleaned and uncleaned substrates, there were multiple rings created around a solid active layer center. The same observations were made for the cleaned and uncleaned glass substrates, wherein the solid center of the active layer was removed via the tape test after curing, though the rings remained for each. After curing the PET substrate, the entirety of the active layer was removed via tape test, though the rings remained on the cleaned version, suggesting some advantage over the uncleaned substrate tests though neither substrate retained a substantial amount of active layer and cleaning did not significantly improve upon this.
Interestingly, the outside of these depositions were the points retained. This may suggest that when there is higher crystallization of the active layer, due to greater film thicknesses, as in the center of the deposition in these experiments, there is an increase in internal film strength greater than the adhesion to either substrate, which was less than the adhesion of the tape; hence complete delamination and not partial separation or top layer lift-off.

![Microscope camera image showing a large delamination event due to small active layer pinhole](image)

This could be due to thickness of the active material as suggested, though an alternative theory is that it could be due to the fact that the outer rings are either P3HT or PC_{71}BM, but not both, as both are drawn to the center in a slow, 20 minute, evaporation process, creating a heterojunction with high internal crystallization and low substrate adhesion. This doesn’t necessarily lead to reduced production in devices, but it has been observed that if a pinhole is accessed by liquid silver ink for an extended period of time, the silver tends to delaminate a comparatively large portion of the active layer as it cures by relieving any adhesion that may have been present between the active layer and the substrate as seen in the resultant Figure 4.9, taken from the bottom of the substrate.

**Conclusion**

Though there is a decrease in transmittance, the glaring reduction in adhesion of the silver electrode, an electrode that will be partially exposed in a robotics environment and potentially manipulated during function and during testing, forces the selection of the PET substrate over the glass. The switch was made to an ITO coated PET substrate and the improvement in production
percentage was readily apparent and thus quantitative testing could occur. Furthermore, the flexibility of the substrate, and the ease of manipulation in overall size were distinct advantages, however the curing temperature of silver is just below that of the PET failure temperature, (becomes extremely brittle, rolls on itself cracking the ITO, and becomes hazy, decreasing transmittance) thus careful temperature and timing control must be observed.

4.2.2 Etching ITO

Initial ink testing and device fabrication were done on substrates without alteration to the layout of the ITO, contacting the electrodes via 3D printed electrical probes. This was done to ensure any alteration to the ITO was not the cause of the failure or parameter shift in early devices. For a more practical approach, the ITO was etched to allow silver electrode deposition, and remote connection to functioning devices. Both glass and PET substrates were examined by applying Kapton tape over the geometry to be retained, Kapton tape being a material known to be stable in various chemicals and under relevant temperature ranges. The substrates were then submerged in 1.0 M HCl for a set period of time. Initial studies were done on glass substrates, observed every 20 minutes for the first hour and then every hour after that. A working time zone from 12-16 hours was found to etch unwanted ITO from the glass surface, leaving a straight ITO/glass interface to use as a deposition guide. Attempts to replicate this experiment with PET were initially unsuccessful due to the relatively rapid etching of the ITO on the PET substrate. A significantly shorter working time of between 9.5 and 10.5 minutes was found to leave a straight interface, as seen in Figure 4.10 below, similar to that on the glass substrate. Two unwanted effects occur if this time period is overshot for both substrates; HCl etches the edges of the interface at the weakest point of tape adhesion (left Figure 4.10), leaving the edges unusable for straight printing and, if left long enough, HCl will begin to penetrate the line itself, destroying the interface initially (center Figure 4.10), and fully etching the ITO if left indefinitely. The images provided paint a nice picture of these possibilities compared to the desired result.
Figure 4.10 PET slightly over etched (left), over etched (center), appropriate etching (right) after varying times in 1.0 M HCl

4.2.3 Active Layer Deposition

Ink Formulation

The main goal in this portion of the project was to develop a printed photosensor to interface with engineered cells. Part of this aim is to print the sensor, and thus ink formulation is a major focal point. The governing mathematics behind the electrohydrodynamic ejection of the droplets has been previously published by Choi et al [72].

Equation 4.1 Volumetric ejection from nozzle

\[
Q \approx \frac{\pi d_N^4}{128 \mu L} \left( \Delta P + \frac{1}{2} \varepsilon_0 E^2 - \frac{4 \gamma}{d_N} \right)
\]  

Looking at the volumetric flow (Q) from this publication (Equation 4.1), two variables in particular become glaringly important in regards to ink optimization; ink viscosity (\(\mu\)) and surface tension (\(\gamma\)). The remaining parameters can be tuned in nozzle preparation and during printing if necessary, though an ink that balances viscosity and surface tension within the provided equation allows for flexibility during printing [72]. These include solvent evaporation rate, solvent/solute compatibility, sintering/curing methods, film absorbance/transparency, layering edge effects, and adhesion to surfaces. If the evaporation rate (boiling point) of the solvent is too low, the solvent can evaporate during points in the printing process when ejection is paused, leaving a buildup of solute as a clog. However if it’s too high, the resolution of the printed material falls upon deposition. The solvents used must not only be compatible with the solute, but also be incompatible with any layers underneath it so that dissolving does not occur. Curing methods have to be appropriate in terms of temperature (too high and substrate or other layers deteriorate) and method (chemical sintering can reduce resolution, laser sintering is impractical
in early stages of printer development). Some of the films need to allow light through on the photosensor, and thus must be functional and printable at high transparency, or absorb light at specific wavelengths, while transmitting those we wish to detect. Layering can also cause problems around the edge of a pre-deposited film when curing or due to evaporation of a film on top, shrinking the top layer and pulling the bottom layer, creating shorts in the device. Finally, the films need to adhere to each other, as well as to any substrate used to create a working and long lasting device.

The original formula for the ink used in the P3OT trials was taken from a previous publication [129] that used spin coating as their method of deposition. Adopting this protocol, the same formula was used, with chloroform as the solvent. After a number of trials, it was noticed that this solution was not appropriate for the e-jet setup due to constant and irreversible clogging of the nozzle. Five points of improvement were investigated at this time. A back pressure system containing inert gas was installed in order to readily relieve any small clogs, though this was not intended to reduce the number of clogging events. A probe sonication step was used in the ink blending process that was meant to agitate the molecules in solution to reduce the number of initial clumps that may be causing clogging. In conjunction with this, a filtration step was introduced after sonication and immediately before printing in order to eliminate any large particles that still remained in solution. Though these steps did reduce the clogs that occurred at the start of printing, there were still significant clogging events before any devices could be fully printed. The final two steps taken were to switch from chloroform to chlorobenzene and to dilute the mixture. Chlorobenzene has a higher boiling point than chloroform and still dissolves organic active materials. This higher boiling point reduced clogs due to the rapid evaporation of chloroform causing solute aggregation at the tip of the nozzle, though the boiling point advantage did not lead to satisfactory printing processes on its own. A master solution was produced, wherein the original concentration was kept constant and underwent all processes as before, which was later stored at -80°C to eliminate solvent evaporation. A portion of this solution was then diluted 1:9 master to chlorobenzene and underwent the same ink preparation processes as the master. This dilution step led to only minor clogs that could be remedied via the new backpressure system and other declogging methods.
Upon adoption of the final P3HT:PC_{71}BM heterojunction chemistry, the same processes were followed to determine the best ink preparation steps, though no differences in ejection characteristics were observed after the active layer alterations.

**Pinholes and Gridding**

As discussed above, P3OT:PCBM was the active layer in the original photodiode structure. P3OT and PCBM were both purchased from Sigma in powder form. Per Yu *et al*, a master solution of 40 mg P3OT and 8 mg PCBM in 1.5 mL of an organic solvent was prepared. As discussed above, though both chloroform and chlorobenzene were tested, the latter was chosen for its higher boiling point (131°C) to reduce tip clogging, and appropriate surface tension (32.99 dyne/cm at 25°C) and viscosity (0.8 cP at 20°C) to reduce the turn on voltage (voltage needed to initiate ejection). The master solution was shaken initially and when thawed during ink preparation for 5 hours to deter coagulation at the tip of the nozzle and ensure uniform dispersion. This dilution step, however, forces the deposition process to repeat a number of times before all pinholes are eliminated and appropriate layer thickness is achieved; an example of why this is necessary is shown in the center in Figure 4.11, one deposition pass, on the right, two deposition passes.

![Figure 4.11 Pinholes in center of an active layer film (left) and on the edge of the film (center). Pinhole occurrences was reduced via multiple layer gridded deposition. (right)](image)

It has been well documented [50, 160, 161] that due to the low volume during single droplet deposition via printing methods and the reduction in volume as the nozzle is scaled down, the presence of small areas of the underlying substrate remain uncovered by the deposited material. These ‘pinholes’, as they are commonly known, are shown on the left in Figure 4.11 and can be reduced in number with the implementation of layering; that is printing over the same pattern more than once. It has been shown not only to improve the conductivity of deposited metals [162], but is also relevant in preventing shorts once the top electrode is deposited atop
the active layer. These pinholes, though shown with P3OT:PCBM remained an issue with P3HT:PC$_{71}$BM and adopted the same resolution discussed below.

**Gridding vs lines**

As stated, one of the major difficulties with liquid phase deposition technology is the presence of pin holes, tiny vacant or weak points in the deposited film that allow afore and post deposited layers to come into contact with each other. In the case herein, the active layer is prone to pinholes causing subsequent shorting of the devices from ITO to Ag. There are a number of reasons these pin holes arise, including low active concentration, dissolution of a layer due to harsh or competitive solvents of a post deposited layer, and inappropriate or insufficient post processing of the deposited layers. Ironically, another reason, which was discovered in the work herein, is due to the uniformity of the printing process. What is meant by this is that as solvents evaporate, printed lines tend to cure in the same geometry as previously printed material instead of flowing into vacant portions (pinholes). Various methods to combat this have been attempted, including increased ejection rate (more deposited material), reduced line spacing causing deposition overlap, and slower stage movement to instantaneously repeat deposition areas. The most effective method however, has been to introduce a grid pattern with negative line spacing (instant overlap) during the printing process. Most of the geometries attempted herein are square or rectangular, geometries that lend themselves well to this approach. The printer generally starts in the top right corner of the aforementioned rectangle, working its way across the geometry horizontally, stepping vertically to the bottom of the geometry where it ends on the bottom right. At this point, the geometry is switched vertically, wherein the print moves vertically bottom to top, and steps left horizontally until it ends in the bottom left. This continues two more times, horizontally ending in the top left, and then vertically ending in the top right again for one ‘grid.’ In doing so, the tendency for the print to follow a pre-laid pattern is broken by intersecting it perpendicularly. Over 98% area production percentage was achieved after the introduction of this method during photodiode deposition, a metric which will be discussed later.

To evaluate the grid printing process with P3HT:PC$_{71}$BM, active layers were printed with increasing number of repeated grids, and analyzed for pinholes and defects under magnification using the e-jet printer camera (Infinity 2-2 CCD). The grids were 1.2 mm x 1.2 mm with a 10 μm step between printed lines using a 30 μm ID nozzle. As shown in Figure 4.12 below, pictures were taken starting with 4 repeated grids (top right) and increased 1 grid per iteration, ending with 13
Pinholes can be seen quite clearly at 4 grids and reduce each time until pinholes are seen only on the ITO/PET barrier at 7-8 repeated grids. This is still not ideal for printing, as it is not evident from simple observation that these pinholes are exclusively located atop the PET and won’t create shorts with the ITO. No pinholes are observed when 9-10 grids are deposited and this trend persists through 13 grids, as does a deepening of color, signifying an increase in film thickness. This leads to two important conclusions; 10 grids will be used for final device fabrication and the testing of thin films (sub 10 grids) deposited via e-jet printing is not feasible at this time. In the center of the Figure 4.12 is a final active layer with 10 repeated grids just after being deposited, thus the slight angle in comparison to the rest of the images, which were taken vertically. This also shows ITO/PET barrier well with the active layer overlapping it with less than 100 μm on the PET to ensure no shorts will occur when printing the silver electrode due to any anomaly in the straight barrier edge.

Figure 4.12 Pinhole reduction with increasing number of grids shown from the top right, counter clockwise to bottom right; images taken from top of film. The center image shows an active layer after 10 grids taken by the stage camera (center).

4.2.4 PEDOT:PSS Deposition

As previously discussed, electron and hole flow from the active layer is facilitate by gradual stepping of the bandgap in the materials installed in the architecture, meaning the LUMO and HOMO of each material used provides a gradual step towards their respective electrodes. Often times, layers are added around the active material to provide this gradual state. One such
layer is used to facilitate hole flow while blocking electron flow. This material can be classified as an Electron Blocking Layer (EBL) or a Hole Transport Layer (HTL), with the latter being the more popular of the two descriptors. A proposed HTL herein is PEDOT:PSS which, as seen earlier, provides a final step before the silver electrode for hole transport.

Four different PEDOT:PSS formulas were purchased and tested for conductivity via a simple deposition and resistance measurement. A cleaned glass slide was used as a substrate where 0.1 ml of each chemistry was deposited and cured at 160°C for 30 minutes. After the substrates were allowed to cool, film observation was done as well as a quick resistance test on the film. Of the films, only one was fully intact after curing and registered a resistance within the kΩ range and was thus chosen. Though this ‘ink’ was purchased through Sigma, the hydrodynamic properties are meant for inkjet printing and tuning was necessary to achieve consistent printing. Per Sigma documentation, the purchased ink solvents are mainly primary alcohols. Successful viability tests were done by printing 200 proof ethanol without additives, thus, in pursuit of bringing the surface tension and viscosity of the PEDOT:PSS ink closer to that of its solvent, a 9:1 dilution of ethanol to PEDOT:PSS was made. During testing of this ink, it was noticed that once printing, the ink flowed close to the way the ethanol did, though the ejection voltage was higher than expected. A surfactant was found that had been used in previous studies to increase the hole transport characteristics of the PEDOT:PSS and was used herein to reduce the surface tension as well by adding 1.0 μl FS-31 surfactant to 0.5 mL prepared dilution [120]. This became the final ink formula used throughout the rest of the studies herein.

4.2.5 Silver Deposition

Though aluminum and other metals are often used as electrodes for traditionally fabricated photosensors, the reactivity of aluminum in air is not ideal for the current e-jet printer setup. Silver is more stable in air and is one of the first nanoparticle solutions to have been produced for inkjet printing and deposited via e-jet printing, with previous research conducted on deposition methods, substrate choice, and ejection characteristics [21, 22, 27]. Silver nanoparticle solution (<150nm particle size at 20 wt. % dispersion) was purchased from Sigma and used as received. As shown in Figure 4.13, silver has been printed in lines 10 μm in width via a continuous e-jet printing process with a 5 μm diameter nozzle, showing the level of resolution and uniformity that can be achieved with this type of printing.
During initial layering testing, it was observed that a silver layer deposited on top P3OT:PCBM will dissolve the underlying layer along the circumference of the silver if left in liquid phase for an extended period of time (minutes). Though still functional, this is not ideal as it may cause shorts around the edges of the structure. This is eliminated if the dwell time, that is the time the silver layer is allowed to stand on the active layer, is shortened; this will be discussed in more detail below. Additionally, limiting the amount of silver atop the printed active layer reduces this rate of dissolution.

These layering effects have yet to be studied in a published format. This unwanted layering dissolution, as seen in the dissolving of the active layer of the photodiode around the edges of the silver layer atop it, can create silver to ITO contact, resulting in short circuits. This dissolution is shown in Error! Reference source not found., where silver was pipetted on top of a 3OT:PCBM active layer, seen both under 4x and 20x magnification (Infinity 2-2 CCD with 2x Fixed Focal Length Extender and 2.5x – 10x VZM 1000i 35mm standoff lens). This particular layer issue was caused by a combination of printing factors; volume, sublayer solvent affinity, temperature,
and solvent boiling point. These issues were combatted through the use of smaller (5 μm – 30 μm) nozzles, highly controlled ejection modalities, and the heated vacuum chuck.

4.2.6 Photodiode Construction

In order to demonstrate the layering process of the photodiode fabrication, both active and silver electrode layers must be printed. For testing and proof of concept purposes, photodiodes were constructed using a 30 μm nozzle for the active layer, and a 5-10 μm nozzle for the silver layer. These nozzles were chosen specifically to reduce the time to print the larger surface area of a multi-device active layer, whereas the silver was meant to be precise and cover only the amount of area specified for final device geometry. All three layers (anode, active layer, cathode) must be geometrically aligned in order for the device to function, thus the silver electrode can be the limiting factor for the functional area of the final device.

![Figure 4.15 Lack of edge dissolution under 4x (left) and 20x (right) thanks to lower deposition volumes and heated vacuum chuck.](image)

As seen in Figure 4.15, there is evidence that at least some of the edge evaporation is alleviated when the silver electrode is printed rather than pipette deposited due to the considerable reduction in volume of the silver droplets causing rapid evaporation, as previously discussed.

One final observation with this printing process is the high surface tension of the liquid phase silver while printing this final layer, likely due to the high surface energy of the deposited active layer. Surface energy is a disruption of intermolecular bonds at the surface of a material, decreasing the contact angle of dropped fluids. This invokes a unique set of challenges including pooling of silver nanoparticles, loss of full coverage and the necessity of multiple printing passes upon initial silver drying. To combat the issues with layering and silver deposition, a heated
vacuum chuck was designed and fabricated, the implementation of which has solved the majority of these issues.

4.2.7 Utilization of Heated Vacuum Chuck

The resolution that had the highest impact on repeatable layering practices was using the heated vacuum chuck to rapidly soft cure the silver on top of the active or PEDOT:PSS layers. During fabrication, the vacuum chuck was set to 75°C – 85°C (46°C – 51°C surface temperature) while using a 10 μm nozzle on pulse mode between 10 – 16 Hz. Depending on the voltage applied, the combination of this pulse mode and the elevated chuck temperature create a 10 μm wide ‘mist’ or a 5-10 μm stream that cures within 2 seconds and allows silver electrode layering for better connection without dissolution or melting of the layer being printed on. After the top silver electrode was printed, a silver contact was deposited for each sensor to use during testing and for later use in a 3D printed card bus. This was done by layering the active heterojunction over the etched ITO, using this as a bridge for the silver to reach the substrate without shorting the photosensor. The difference between the deposition of the electrode and their connection is that the voltage used while printing the connections is higher and is ejected using a switching ejection modality wherein the voltage is pulsed using a square wave around zero using a chosen amplitude. This ejection modality allows for rapid deposition of a non-charged silver electrode. The rapid printing creates a large connection of silver in a short period of time to ensure that the soft cured silver electrodes do not become compromised before their hard curing stage at 230°C. Attaching an extra connection to the ITO surface before hard curing also allows for total sensor encapsulation via NOA 74.

4.2.8 Encapsulation

Through a literature search and observation with printed sensors, it was noted that over time, deposited materials react with atmosphere, especially in a high humidity environment. During testing of the devices produced in these studies, a number of the devices aged and decayed over time. All devices were left without encapsulation and open to the atmosphere though they were protected from light as they aged. These devices were observed to have a dark current that began to radically drift and amplify. Though not significantly observed in a weeklong experiment to evaluate device decay, this change was observed within weeks and months after fabrication and is not favorable for long term experiments nor for implantation into the planned robotic
scheme. Thus encapsulation methods were considered; namely using super glue, silicon sealant, NOA 74 with glass cap, and NOA 74 without a cap. The super glue sealed well but pealed easily when introduced to water, destroying the devices while doing so. The NOA 74 with the glass cap had a similar failure, though it only caused a few device failures after delamination. The silicon sealant stood up well to the water, though the device function fell off drastically, perhaps due to a more difficult sensor/light centering portion of device testing. Finally, the NOA 74, cured under UV light without a cap on it, sealed well and lasted indefinitely when exposed to water and culture media and was selected for all subsequent devices.

4.2.9 Spectral Analysis

As photons impact the active area of the device, electrons are excited and an electron-hole pair is formed. This response is dependent on the wavelength (energy) of the incoming photon. When the response is compared to the incoming photon, the ‘spectral response’ is the resultant graphic. The spectral response allows us not only to select an appropriate emission wavelength for planned cellular interfaces, but also allows for a back calculation downstream to determine how much light the cells are giving off in response to a stimulus, and thus quantify the stimulus itself. To obtain these recordings, a calibrated monochromator (37-597, Edmund Optics) was used and the devices were excited via a HL-2000-HP-FHSA 20W light source on a 300-800 nm range. A high torque motor was applied to the monochromator to control the direction and pace of the scan range. Preliminary scans were done over 21 seconds and recorded using LabScribe. These initial scans were run forward and backward at the same rate. Though the resultant shape of the response was similar, the forward and backward scans were offset on the wavelength axis shown in Figure 4.16, implying that the scan was faster than the rise time of the fabricated devices. Shown in Figure 4.16 is one scan from four different sensors running in one direction; the blue and grey began at 800 nm and the orange and red began from 300 nm.
Figure 4.16 Spectral offset due to rapid wavelength scan showing one scan from four different sensors running in one direction; blue and grey began at 800 nm and orange and red began from 300 nm. The offset is due to the slow rise time of the sensors.

The scan rate was decreased 10 fold and the wavelength shift was rectified. The spectral response was taken for P3HT:PC$_{71}$BM bulk heterojunction devices on ITO coated PET with printed silver electrodes on top. The composition of the active layer remained constant, as did the silver composition and the nozzle sizes of each. Though different device efficiency parameters arose due to various inconsistencies during the current production process, a direct comparison of device spectral response shape can be made if normalized. Thus, each device’s spectral response, both forward and backward, was normalized and compared for difference in shape.

**Generating Amps/Watts**

In order to generate a spectral response in amps output per watts input for the printed devices, the wattage input to the sensors had to be analyzed, as white light have spectral variability over the visible color spectrum. A commercial photodetector was purchased from DigiKey (PDB-C107) and run through the same testing methodology as the printed sensor to produce a current response to the light input. This was then combined with the published spectral response of the sensor to get a wattage output of the light in the same setup used for device evaluation. The results are shown in the Figure 4.17 below and are interpolated to the same wavelength interval as the printed devices in order to divide their response by the light input to generate a functional spectral response.
Figure 4.17 Light input spectral analysis. The response of a PDB-C107-ND photodiode was taken from 300-800 nm (left) and the published spectral response of the sensor (center) was used to calculate the resultant light intensity per wavelength (right) delivered to the tested devices.

Each wavelength run has a slightly different scan time and has to be processed using the coding software R, which interpolates each data set to a prescribed interval from 300 nm to 800 nm for comparison, normalizes each data set, generates a spectral response (amps output per watts of light input), and combines that into one data file for each device iteration.

4.3 Results

4.3.1 P3OT:PCBM Photodiode

The layering process is new and unpublished. Thus, it is important to take steps during the fabrication process in order to fully understand the implications of adding liquid phase layers on top of crystalline ones. The initial photodiodes fabricated consisted of a high flow rate nozzle with undefined area of coverage of a P3OT:PCBM active layer onto a cleaned ITO (Sigma 30-60 Ω) coated glass slide, followed by a curing step for 20 minutes at 160°C. The silver electrode was then deposited onto this active layer via pipette and hard cured at 220°C for 1 hour to sinter the nanoparticles together into one electrode that was tested via a probe station. This initial architecture was used as a starting point for all further improvements, a baseline from which all changes can be compared.

The output of these resultant photodiodes was then examined. In order to characterize these initial photodiodes, calibrated LEDs of various wavelengths were illuminated at increasing applied voltages through a cleaned ITO coated glass slide into a calibrated photodiode; all were purchased through DigiKey. The LEDs input levels and photodiode output levels were processed through LabVIEW with timestamps to line up the LED steps with the photodiode response. From there, the same process was done with each printed photodiode. By comparing the output current from the calibrated photocurrent at the prescribed wavelengths, the photon output of
the LEDs at various voltages can be used to find the spectral response at those wavelengths for the printed photodiodes. In Figure 4.18 below, the normalized spectral response shows the amps output per photon input. This was normalized to have a base for comparison with P3HT devices and their spectral shapes. The error is shown in standard error.

![Normalized P3OT:PCBM spectral response using six different internally calibrated LEDs. Error is shown in standard deviation.](image)

**Figure 4.18** Normalized P3OT:PCBM spectral response using six different internally calibrated LEDs. Error is shown in standard deviation.

### 4.3.2 Film Transmittance

As previously discussed, there is the potential to add layers between the heterojunction active layer and the two electrodes. These layers are used to do a number of things, including reducing the energy needed for an electron or hole to jump from the active layer to an electrode, limiting the bandwidth of the incoming light to make a wavelength specific device, reducing or increasing the work function of the electrodes, and other useful purposes. Since many of these layers would impede or alter light coming into the active layer, transmittance experiments were set up to determine how much and what wavelength of light would be let through based on the number of layers of the additive in question.

The sensitivity of the photodetector is paramount in sensing biological reporting. One major addition to the architecture of the photodiode that has been shown to improve the sensitivity is that of a hole transport layer or an electron transport layer. Of these, PEDOT:PSS [136, 149, 150], an HTL, and Zinc Oxide (ZNO) [133, 163-165], an ETL, have been shown to be
strong candidates for solution based deposition and are readily available from Sigma. Thus, the transparency of an increasing number of layers deposited on a glass substrate was analyzed using an LS-1 light source and a USB4000 from Ocean Optics as discussed earlier.

**PEDOT:PSS**

As discussed earlier, the original architecture of the proposed photosensor uses the HTL between the transparent electrode and the active layer, thus transparency is paramount. Though in the flipped orientation, this layer will be on the opposite side, there is the possibility to use other electrode materials that would force PEDOT:PSS to the transparent side of the photosensor. To examine the transparency of the printed films, PEDOT:PSS was printed in increasing layers, no gridding, using a 30 μm nozzle, and the transmittance from 400 nm – 900 nm was examined. The results of the transparency tests are shown in Figure 4.19, with an initial transparency at 490 nm close to 100%. As the number of layers increases, the transmittance goes down as expected as well. Discussed later, the supplied wavelength specific data was evaluated at 490 nm because a potential use with *Vibrio fischeri*. This microorganism has a natural emission wavelength of 490 nm, though there is an engineered version (Y1) that emits around 570 nm.

![Figure 4.19 PEDOT:PSS layer transmittance (y-axis) through glass with increasing number of deposited layers (left). The transmittance (y-axis) trend in reference to the number of printed layers (x-axis) at the emission wavelength (490 nm) of *V. fischeri*.](image)

After transmittance was recorded, the sheet resistance of each film was analyzed and referenced to transmittance, as well as the number of layers. This is used to determine the appropriate number of layers to be deposited and would also be a way to back calculate the thickness of a deposited layer and its conductance by measuring the transparency. As seen on the right of Figure 4.20, there transmittance remains above 90% while the sheet resistance drops
significantly with only a few deposited layers. This zone gives room for error during the printing process. Whether there is a little more or less material deposited, it does not drastically impact the resistance or the transmittance of the PEDOT:PSS film.

![Graph showing PEDOT resistance vs transmittance and sheet resistance vs number of layers](image)

*Figure 4.20* PEDOT:PSS sheet resistance (left y-axis) compared to the measured transmittance (right x-axis). An alternative format is shown compared to the number of printed layers (right x-axis).

This PEDOT:PSS layer was switched in orientation when the reversed photosensor configuration was selected in order to use silver as an electrode. Though this data does not directly impact the sensor at hand, it is valuable for future uses of this popular material in future printing studies and as an HTL between the active layer and the silver electrode.

**ZNO**

ZNO may be an appropriate addition to the architecture discussed herein, delivering more efficient transport of electrons to the transparent ITO electrode. Working as an ETL, this ZNO will have to be placed between the active material and the transparent electrode, thus transparency is vital and required interrogation.

**ZNO Regular and D49**

To test the transmittance of deposited ZNO films, two inks were used, both deposited via a 30 μm nozzle in increasing layers, not grids, each being cured at 160°C for 20 minutes. The first ink, seen below, was unchanged in its delivered state from Sigma, the other was diluted 49:1 in ethanol. As seen in the Figure 4.21 below, the transmittance was taken for each with similar layer distribution. The original solution restricts around 45% of incoming light at 490 nm with just one layer, something that is not usable as a transparent layer in a photosensitive device.
Further diluting the ZNO solution improves this transmittance by around 50% where only 20% of the incoming 490 nm light is restricted. Looking at these numbers, 80% is still quite low, especially when considering that this is with one printed layer, where previous studies in film deposition have suggested multiple layers are necessary for full film coverage deposited thus far.

4.3.3 ZNO addition to P3HT architecture

In the case of an inverted photosensor, whichever material chosen as an ETL would need to be transparent at a functional film thickness. ZNO [133, 163, 166, 167] and doped versions of ZNO, namely aluminum doped zinc oxide (AZO) [168, 169] are prescribed as such and have been shown to improve efficiency. Thus, in hopes of improving the response of the photosensor, ZNO was integrated between P3HT and the ITO electrode to see if the advantage gained by adding an ETL outweighed the 20% decrease in photons impacting the active zone.

The ZNO layers were deposited on cleaned ITO coated glass using 4.44% ZNO nanoparticles in ethanol and a 10 μm nozzle, increasing ¼ grid of thickness per device, and curing at 160°C for 20 minutes. P3HT:PC71BM was printed with 8 grids of thickness on top of these ZNO layers, including a control without a ZNO addition. The results (Figure 4.22) show that at this concentration, the addition of ZNO, even at a minimum, is a detriment to the response and efficiency, most likely due to the lack of transparency, leading to a conclusion that ZNO without doping is inappropriate for the architecture at this concentration and via this deposition method.
Figure 4.22 Degradation of response as the number of layers increases, shown with rise time.

Instead of the previously used 490 nm, each device was exposed to identical intensity light at 570.3 nm (possible emission wavelength of engineered *Vibrio fischeri* [170]), with an active area of 1.96 mm².

4.3.4 P3HT:PC₇₁BM Spectral Response Shape

Early P3OT:PCBM devices were evaluated using calibrated LEDs and photodiodes to get a rough estimate of the spectral response. As seen in Figure 4.18 Normalized P3OT:PCBM spectral response, the normalized spectral response has a limited number of data points, though does form a consistent and distinctive curve that may be used for future comparison.

Regardless of amplitude, there should be little shape deviation between sensors if normalized. The spectral response is the evaluation of the response (current) at individual wavelengths of incoming light and is intrinsic to the materials used in the device. Thus, if the materials remain constant in their composition and orientation, there should be no deviation in the shape of this spectral response.
After the final photosensor architecture was determined (PET/ITO/P3HT:PC$_{71}$BM/Silver/NOA74), the spectral response of the 23 devices from 5 different printing sessions were analyzed. Shown with standard error in red around the blue data, Figure 4.23 above depicts the normalized spectral response for the final architecture.

### 4.3.5 NOA74 Encapsulation

Once a reproducible testing method for the response was in place, the evaluation of how to encapsulating printed devices was examined, the need for which was discussed earlier. In order to evaluate how the chosen NOA 74 encapsulation method may impact the sensor function and if any dissolution occurred, spectral responses were taken before and after the NOA 74 was applied to the surface. Any dissolution would impact that spectral response in shape, and likely render the device nonfunctional, which did not occur in any devices. Furthermore, since a UV light is used to cure this encapsulating liquid into a solid, this test is also needed to evaluate if direct UV exposure impacts the shape as well. As seen in the Figure 4.24 the pre and post NOA 74 application normalized responses are depicted, with all tested sensors mirroring these results. Furthermore, there was no drop in any spectral amplitude due to this encapsulation method, which was monitored during two different print sessions.
Figure 4.24 Impact of NOA 74 encapsulation method. The normalized response of a device before (blue) and after (red) encapsulation. Intensity (not shown) of the response did not change and no device tested malfunctioned due to this encapsulation method.

4.3.6 P3HT:PC$_{71}$BM Spectral Response Amplitude

Based on observation and later experimental design, it was noticed that sonication plays a larger role in the amplitude of the response than just printability of the active layer. Since iteration 20 of the device, 3 iterations (7 total devices) were printed without first sonicating the P3HT:PC$_{71}$BM active layer ink, while 2 iterations (16 total devices) were sonicated. The comparison of the average spectral response amplitude for these two different scenarios is shown in Figure 4.25 below. The red regions in the figure are standard error for the respective data sets.
Figure 4.25 Sonication of the active layer ink is imperative to amplitude of spectral response. The blue line indicates the devices fabricated using a sonicated and filtered ink and the green using just filtration. The error (red) is shown as standard error.

Of the 17 sonicated devices produced, 16 were functional. Of those 16, there were 13 that were 10 fold higher in magnitude than their non-sonicated counterparts. In order to provide a geometric component to the data provided above, these 13 devices were averaged and divided by their active area, shown in Figure 4.26 with standard error in gray.

Figure 4.26 Spectral response for appropriately fabricated devices, shown as A/W/mm². The error (gray) is shown as standard error.
4.3.6 Reproducibility

To validate the printing process and determine how useful this deposition method is in a laboratory setting, the reproducibility of the process is an important metric to consider. Early stages in the development of this process have seen many device failures, for a number of different reasons, as discussed earlier. The examination of this parameter is only appropriate once these obstacles have been resolved, occurring at device iteration 20, when a final architecture was achieved for the basic photosensor (ITO/P3HT:PC_{71}BM/Ag/NOA 74). Though at this point a final architecture was achieved, testing was still done on this architecture in attempts to implement additional layers, so the evaluation of reproducibility did not include experimental forms of the photosensor. Three variables in reproducibility are important to evaluate for our purposes; production percentage (a percentage to compare functional devices to fully printed devices), spectral response shape, and spectral response amplitude, the latter two of which were previously discussed.

Production percentage is needed to evaluate how well the selected printing parameters are doing at a base level; does the sensor work or not. As testing commenced, various sizes and numbers of devices were printed on each substrate. Each substrate was logged in Table 4.1 with device number, with 20 being the first fully encapsulated, functional, long term product. Each device number shows the number of the intended devices (fully printed P3HT, regardless of if silver was deposited or not), the device size (size of printed silver electrode), and the number of functional devices (regardless of functional amplitude). A device was deemed nonfunctional if there was no response given spectral stimulus, or if the signal to noise ratio was too low to get a reliable spectral response. Table 4.1 shows the results via production percentage for each intended device number as well as intended area (larger sensors require longer prints).
Table 4.1 Photosensor production percentage

<table>
<thead>
<tr>
<th>Device</th>
<th>Intended</th>
<th>Functional</th>
<th>Area Per Device (mm²)</th>
<th>Production Percentage Device</th>
<th>Production Percentage Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3</td>
<td>3</td>
<td>0.16</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>21</td>
<td>6</td>
<td>5</td>
<td>0.09</td>
<td>83.33</td>
<td>83.33</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>23</td>
<td>12</td>
<td>12</td>
<td>0.09</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>3</td>
<td>0.09</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>26</td>
<td>5</td>
<td>4</td>
<td>0.09</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>28</td>
<td>16.52</td>
<td>87.5</td>
<td>98.1052</td>
</tr>
</tbody>
</table>

Table 4.2 below is compiled of the devices that employed a sonication step in the ink preparation. Before the active layer was passed through a filter, it was sonicated using a probe sonicator, typically used to break up biological samples, but used here to break apart and remaining clusters that may clog the nozzle.

Table 4.2 Photosensor production percentage with sonication step

<table>
<thead>
<tr>
<th>Device</th>
<th>Intended</th>
<th>Functional</th>
<th>Area Per Device (mm²)</th>
<th>Production Percentage Device</th>
<th>Production Percentage Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>12</td>
<td>12</td>
<td>0.09</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>26</td>
<td>5</td>
<td>4</td>
<td>0.09</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>16</td>
<td>0.09</td>
<td>94.11764706</td>
<td>94.11764706</td>
</tr>
</tbody>
</table>

4.4 Discussion

The results provided above show a progression in the printing and testing process, resulting in photosensor parameters within the design constraints set out at the beginning of these studies. The original P3OT spectral response analysis was limited in its wavelength data points, though it is of note that the peaks, valleys, general shape, and average emission maximum wavelength of P3OT and P3HT are comparable to each other and previously published results.
Of the three repeatability metrics that are being considered herein, the amplitude of the spectral response is the most difficult to demonstrate precision with. This could be due to a number of reasons, including the film thickness being too large and difficult to measure while printing, the current inability to create thin (nanometer scale thickness) films, as well as the length of time that each sensor is exposed to light and air due to the pace of printing, among other possible points of contamination. Of note, however, is the maximum response around 514 nm, which sits close to both the natural wavelength of emission of *Vibrio fischeri* (490 nm) and the engineered Y1 wavelength (570 nm). The spectral response also shows a wide band above 70% maximum response from approximately 480 nm to 610 nm. Furthermore, the standard error from these devices shows minimal deviation in shape. The resultant mA/W ranges have a minimum detection limit in the 10’s to 100’s of nW range, which is lower (device sizes did not approach 1.0 cm²) than the 0.3 mW/cm² design constraint set at the beginning of these experiments, though the response for these levels requires pA detection methods.

There is a stark difference in device performance when sonication is used. This may be due to the fact that sonication breaks up clumps of P3HT or PC$_{71}$BM that would be removed from the ink by the filter without sonication, thus retaining the original solution ratio and delivering a more consistent ink. Only 1 of the 17 sonicated devices did not function after printing, 13 of the remaining 16 having increased spectral response amplitude over 10 fold higher than their non-sonicated counterparts. The 3 that did not have this 10 fold amplification still had higher spectral responses than non-sonicated devices. Of note in Figure 4.25 is the seven fold amplification sonication has over non-sonication and the noticeable error in amplitude for the sonicated devices, having a broad amplitude range of sensitivity. The selected sonicated devices also show that the response is dependent on the size of the sensor, with a maximum response around 90 mA/W/mm².

Though basic, this structure was intended to contain more layers that did not enhance the efficiency to a point where there was a justification to complicate the printing process in order to retain these layers. As seen in the ZNO trials, there is an exponential trend in the decrease in current response with each additional layer, though there is not a significant change in rise time (~200 ms), suggesting there is simply less light impacting the active zone. This result is expected as the transmittance was substantially hindered by additional layers. Though not displayed in the results, PEDOT:PSS was added as a HTL between the active layer and the silver electrode. There
was no change in the spectral shape and there was no significant change in the spectral response amplitude in either direction, and was thus eliminated from the architecture as well. Both applications (ETL and HTL) warrant further examination.

The production percentage of the devices is just under 90%, though the production percentage in area approached 100%. These production numbers are a direct reflection of the substrate selection, ink preparation processes, curing processes, and ejection modalities that were chosen after the various experiments and results provided herein.

4.5 Conclusion

The work herein demonstrates a number of new liquid phase deposition techniques, as well as a fabrication methodology for repeatable multi-layer e-jet printed photosensors. Though photoactive layers have been deposited in the past, including via liquid phase deposition by using spin coating and inkjet printing, the layering process of stacked liquid phase printed layers has not been published. Thanks to the implementation of the heated vacuum chuck herein, the layering process has been repeatedly demonstrated in the form of a multi-layer photosensor. Additionally, the sensitivity of the photosensor surpasses the 0.3 mW/cm\(^2\) sensitivity set out in the proposal (capable of detecting nW range with less than 1.0 mm\(^2\)), as well as the demonstrating close to the 90% intended devices, exceeding this number in intended area. The variability of the intended devices is still an issue for future consideration. The demonstration of the printing process was described herein but the essential material science behind device efficiency optimization and precision has not been investigated to this point. While optimizing these metrics should be the first step in additional work, other points of investigation would be focusing on developing thin films, perhaps using post processing wash steps to eliminate pinholes, implementing additional layers which have been shown to enhance efficiency, and a look into the impact of flexible substrate deformation has on immediate and long term photosensor performance. In summary, the hypothesis that an electrohydrodynamic printing system can produce a functional, reproducible (>90% intended devices, <5% metric variability), multi-layer photosensor that is capable of detecting ≤ 0.3mW/cm\(^2\) has been confirmed, less the metric variability which may prove possible in the near future.
Chapter 5 Bioelectronic Interfaces

5.1 Background

Bioelectronic interfaces are unique in that they take something that is reliable, precise, and able to be integrated into existing electronic platforms, and couples it with something as powerful and specific as biology. The methodology discussed below utilizes e-jet printed electronics, and couples them with biologics using previously tested and/or biologically appropriate cellular adhesives and biocompatible substrates and encapsulating materials. Since, the fabrication of both electronics and biologics has yet to be accomplished utilizing a deposition method capable of depositing both sides of the interface, any communication between the two entities will provide ground work for future studies in the field. In this aim, the constructed electrohydrodynamic jet printing system was used to fabricate bioelectronic interfaces between engineered cells and printed electronics.

5.4.1 Biorobotics

The pursuit of biologically inspired robotics has been an objective of scientists for well over 2000 years. The general pursuit to mimic biologics via robotics gained structure in Ancient Greece in the form of automata. Examples of early automata include the construction of a water serving waitress and performing mechanical musicians during the 13th century by scholar Al-Jazari [171]. It wasn’t until the mid-20th century however, that a more directly relevant form of biorobotics was produced, paralleling the rise in cybernetic approaches. An example of the improvement in biomimicry was the robotic tortoises created by W. Gray Walter, complete with locomotion (via wheels), headlight and light sensor, and a hard ‘shell’, complete with pressure sensors underneath it. More importantly, the behavior of these early biorobots were controlled via electronic neural networks, and included exploration, positive and negative phototropisms, and obstacle avoidance [172].
The study of biorobotics exploded from the 1980s to the 1990s, namely with the work done by Rodney Brooks, in which he argued that machinery can interact with the environment around it to generate nontrivial robotic behavior, and showcased these interactions via insect-like walking demonstrations [173]. Equally important during this time period was the work done by Marc Raibert on hopping and legged robots, highlighting the role of energetics in general stabilization and locomotion of robotics [174]. Later scientific contributions during the 1990s sought to expand the types of locomotion, as well as the quality of sensors based on biological taxes. These include work done by Hirose on serpentine motion, Beer, Quinn, Chiel, and Ritzmann on hexapod locomotion, Triantafyllou and Triantafyllou on swimming locomotion, Franceschini on insect like eyes, Webb on cricket phototaxis, Grasso on lobster chemical orientation, and Lambrinos on ant homing via polarized light [175-182]. Within Dr. Joseph Ayers’ lab, various autonomous biomimetic platforms are currently under research, including a undulatory lamprey [3, 4], a walking lobster [7], and a flying bee [5, 6]; though mainly the completed research herein will revolve around testing on the RoboLobster platform.

5.4.2 Biohybrid Robotics

In recent years, there has been an increase in focus in biohybrid robotics. These systems are formed by at least one biological and one artificial component that pass information either in one or both directions, and aim at solving engineering issues through integrating an evolutionarily optimized component with a versatile one. As opposed to emulating a biological system using only mechanical and electrical parts, biohybrid research seeks to harness biology itself. The applications can range from propelling beads using attached bacteria and chemical gradients, [183] to a biohybrid olfactory sensing system [184]. Medical research has worked towards more commercially relevant biohybrids; namely a biohybrid visual prosthetic [185] and a retinal prosthetic [186]. More robotics based biohybrid interactions can be seen in Parker et al and their phototactic guided tissue-engineered robotic ray [187]. Bioelectronic interfaces aim to further advance biohybrids by directly integrating engineered cells and with electronics creating a specificity that is difficult to manage in current electronic detection setups. The following is recent work in unidirectional communication, though that direction can be from the electronics to the biologicals or vice versa.
5.4.3 Rationale for Bioelectronic Fabrication and Use

Though photodetectors and electrochemical sensors are useful tools in their own right, they also lend themselves to specialized applications. Bacteria cells may be engineered to detect various chemicals, kinetics, or wavelengths of light, and report a different wavelength of light. Coupled with a photodiode, engineered to detect this peak wavelength, the resultant bioelectronic interface is a uniquely specific and electrically efficient device. Through their unique specificity, bioelectronic interfaces have the potential to be used for biosensing, interface biology, cell expression monitoring, and biomedical purposes. The communication between electronics and living organisms is an ongoing focus of research in both higher education and industry. The ability to more closely monitor and support the human body, both during everyday life and medical emergencies is of major interest to the medical community. With interesting advancements like wearable electronics [188-190] and personalized medicine, the interaction between electronics and biology is expanding and proving beneficial in many different ways. One such example are the scaffolds printed via ink-jet printing for patterned dissociated neurons, though these scaffolds aimed to produce many cells per feature whereas e-jet printing can print many features per cell [64]. Furthermore, the utilization of biology that has evolved in order to solve a unique problem, can have an endless number of applications, as does the realm of engineering robust microorganisms to develop specificity in bioelectronic interfaces, including creating explosive seeking robotics.

The major innovation and most significant contribution of the bioelectronic interface studies herein is in the fabrication of reliable bioelectronic interfaces, that are produced via a deposition methodology capable of depositing all of the different parts of the interface. The fruition of these studies demonstrates the ability to print various sensors, pattern a specific ‘cell glue’ geometry, and deposit specialized living cells in defined regions or submerge the sensors in cellular solutions. Furthermore, it demonstrates the variability of the electrohydrodynamic printing system, and thus the specialization of the electronics side of the interface as well as the biological side.

Discussed below are means of cell attachment, regardless of the sensor used, bioelectronic interface possibilities for the nitric oxide sensors and demonstration of a bioelectronic interface for the chemical sensor, concluding with a demonstration of another bioelectronic interface for the photosensor.
5.4.4 Bioelectronic LED Interface

Some of the most promising recent research has been using biohybrid [191-194] interfaces for locomotion [184, 195] applications, including past grant based research in Dr. Ayers’ lab. This research aimed at utilizing engineered muscle tissue that responds to intense blue light by flexing when illuminated, and relaxing in its absence [196-198]. By depositing a blue electroluminescent device on a flexible substrate, and seeding these engineered sells atop of it, an undulatory ‘tail’ can be produced. A similar approach was used by Sakar et al, though the light was not directly interfaced with the muscle [196]. Figure 5.1 shows a basic schematic for the design of a ‘tadpole’ type biohybrid robot, complete with chemical for navigation and detection. Through e-jet printing, the printing of this type of LED device coupled with muscle cells may yet be possible.

![Figure 5.1 Miniature biohybrid robot diagram that would employ a bioelectronic chemotaxic guidance system, an onboard thin film battery, and bioelectronic muscle/LED locomotion system.](image)

5.4.5 Bioelectronic Electrochemical interface

Nitric oxide is a common byproduct of routine microorganism processes and the research into its production is extensive, including studies into its function in immune response of various organisms [199-201], its suppression [202, 203], and its uses in the human body [204-206]. This reactive, short lived molecule can be produced by microorganisms in standard culture at millimolar concentrations each hour. Unfortunately, the best methodology for detection is to allow nitric oxide to react with oxygen, forming nitrates, nitrites, or other easily detectable stable molecules and back calculate the nitric oxide produced. Based on the reactivity of nitric oxide in cellular solution, the produced nitric oxide sensor does not make sense for use in these long exposure type experiments, as the nitrates and nitrites are not detectable by design.
A possible biological to interface with the completed nitric oxide sensor are those currently being studied at Newcastle University. Targeting the nitric oxide synthase protein eNOS Orr Yarkoni et al were able to insert light-oxygen-voltage (LOD) domains that effectively cause the cells to create NO in the presence of direct light. Plasmids containing gene constructs to achieve this effect are transfected into a Chinese Hamster Ovary (CHO-K1) cells line, creating viable mammalian cells that see light and release nitric oxide [207]. Coupled with the produced nitric oxide sensor, this bioelectronic interface may be able to sense more accurate and precise levels of light. Furthermore, this nitric oxide reporting characteristic may be able to be coupled with the DNT sensing feature discussed above.

Justification for NO Sensor

Nitric Oxide synthase is present in prokaryotes [87] as well as eukaryotes [88] and is thus broadly available as a reporter and quite unambiguous in the environment when compared to ambient light. Macrophages produce nitric oxide using nitric oxide synthase (iNOS) in order to neutralize and decompose threats within the human body [204]. Typically, oxides are released in small areas around the cell in zepto and femtomole amounts, resulting in micro and millimolar concentrations in close proximity. Recently, the investigation into how these macrophages use nitric oxide, as well as other chemicals, to fight viral and microbial infection has recruited the use of microelectrodes and electrochemical techniques to quantify some of the processes at work [38].

Diagram of setup

In order to quantify the emission flux of nitric oxide and other chemical species during macrophage immune response, Amatore et al used a carbon fiber based microelectrode placed 5 ± 1 μm from a RAW 264.7 macrophage in solution using a microscope and micromanipulator. Set to a constant potential at 650 mV versus a sodium-saturated calomel reference electrode, the working microelectrode was used to monitor the rapid depolarization of the macrophage using a fine tipped microelectrode to puncture the macrophage cellular membrane, as represented in Figure 5.2 below.
Figure 5.2 Schematic of the nitric oxide electrochemical sensor setup from Amatore et al [38] (left) used to analyze the rapid depolarization of macrophages. A schematic of a potential replication of these experiments using printed sensor (right) may perform better thanks to the lack of spatial separation.

In a similar, and more user friendly setup, the working electrode of the e-jet printed nitric oxide sensor would act as substrate for the macrophage, producing a contact reading of the depolarization event, delivering more reliable readings than a microelectrode positioned with a level of dimensional uncertainty. As seen in Figure 5.2, the three electrode setup could be used on these scales with the reference electrode being positioned closer than the microelectrode setup in Amatore et al. Some level of overhang is used on the working electrode such that the depolarization can be monitored under the cell as well as around the cell. RAW 264.7 macrophages are approximately 11 μm in diameter, [208] thus a 30 μm diameter working electrode could be used to monitor a distance of 0-10 μm from the edge of the cell.

**Sensitivity**

Using these dimensions, it is possible to mathematically determine if the printed sensor is sensitive enough to detect the nitric oxide flux produced by these macrophages. Upon depolarization of the cell membrane, Amatore et al reports a 14 fmole release of nitric oxide over approximately 30 seconds, with a peak flux of approximately 2.45 fmole/sec. Given that the half-life of nitric oxide in 37°C aqueous solution is 445 seconds [209], approximately 1 fmole would degrade over this 30 second time period, leaving 13 fmole. Given the maximum radius of the working electrode beneath the macrophage is 15 μm, a hemispherical volume of 7069 μm$^3$ (7.069 pl) is used to calculate a total molarity of 1.839 mM produced over 30 seconds. Since diffusion (using $D_A = 3.0 \times 10^{-5}$ cm$^2$/sec @ 37°C [210]) would dissipate these 13 fmoles into a volume of
approximately 0.01 mm³ (10 nl) over the 30 seconds, the final concentration would likely be around 1.3 μM. The maximum nitric oxide flux from the cell is approximately 2.45 fmole/second, which translates to 346.6 μM/sec in the sensor working electrode volume and 245 nM/sec over the final volume of 10 nl. A schematic of the macrophage depolarization and the resultant nitric oxide diffusion is provided below in Figure 5.3.

![Diffusion schematic](image)

**Figure 5.3** Visualization of macrophage depolarization and nitric oxide diffusion over time based on published metrics in Amatore et al [38].

Given that these numbers fit into the dose response data (via cyclic and amperometric detection) discussed earlier, it is reasonable to state that the nitric oxide sensors produced via the e-jet deposition method are suitable candidates for use as mechanic disruption detection bioelectronic interfaces with the RAW 264.7 macrophages and warrant further study as research tools therein.

**Obligate Symbiotic Bacteria**

Outside of nitric oxide detection, there are a number of biologically important chemicals that are detectable via electrochemistry in biologically relevant quantities. Of current interest, however, is the detection of extracellular electrons, given off by various microorganisms during normal respiration.
*Shewanella oneidensis* MR-1 is a unique microorganism in that through its normal metabolic state, it is able to transfer electrons to extracellular solid minerals and electrodes, creating current flow through respiration [211]. It does so through the Mtr pathway, allowing the detection of any material that is toxic towards its normal metabolic rate once a baseline is set [212]. Thus, a *Shewanella* electrochemical bioelectronic interface that utilizes a similar setup as the nitric oxide sensor is capable of monitoring presence and concentration of chemicals such as pyruvate. Published work has attempted to use these microorganisms in electrosynthesis, power generation, and biosensing, including the possibility of using them to detect toxic molecules like formaldehyde, benzene, and heavy metals [213-215]. The most intriguing application, however, is that this pathway may be harnessed and assigned to the detection of a specific molecule, creating a specific bioelectronic interface; i.e. DNT.

### 5.4.6 Bioelectronic Photodiode Interface

The utilization of the photosensor produced in the Chapter 4 as a component in a bioelectronic interface relies on the use of bioluminescent microorganisms. There have been studies that utilize the rat olfactory receptor Olfr226 engineered within *Saccharomyces cerevisiae* that allows these yeast cells to ‘smell’ 2,4-dinitrotoluene (DNT), an explosive residue mimic, which then cause a report of green light [216]. Thus, coupled with a photodiode sensor, a bioelectronic interface may be designed to sense explosives, though an obtainable replication or derivative of these yeast cells has yet to be located.

**Vibrio Luminescence Detection**

The main goal of the bulk of the cellular interface experiments herein is proof of concept. More pointedly, the goal of the experiments with the photosensor is to detect light given off by living biologicals, with similar proof of concept experiments for the nitric oxide sensor.

In looking at light production, the *Vibrio* genus was a standout in the literature in that it was biosafety level 1, it has a number of high school level experiments that are easy to follow, the growth and maintenance of the cell line is well documented and simple as far as cell culture is concerned, and the expression system is well documented for a number of species. *Vibrio fischeri* and *Vibrio harveyi* were two species that fit the design specifications particularly well. The light expression of these species is achieved thanks to auto inducers (AIs) produced by the cells themselves, causing light production at high cell density, and thus high AI concentration [37, 217].
In addition, these AIs are available for commercial purchase due to the rather significant amount of research that has been conducted on *V. fischeri*. This species is a symbiont with the squid *Euprymna scolopes* in the Pacific. A light organ is used by the squid to actively culture this cell line. They use this organ to carefully maintain a counter illumination to down-welling moonlight thus camouflaging themselves via silhouette reduction [218-220].

While conducting a literature search, it became evident that the main design specifications for the cells used to interface with the printed electronics were overall safety and ease of use due to limited lab space for culture, high expression rates, whether that be light or nitric oxide production, and finally, an on/off switch for this expression. *Vibrio fischeri*, *Vibrio harveyi*, and *Shewanella oneidensis* fit these design specifications well.

### 5.2 Methods

#### 5.2.1 *S. oneidensis* Electrochemical Sensor Interface

In conjunction with Dr. Caroline Ajo-Franklin at Berkeley Lab and her students, Jose Cornejo and Lin Su, we have been integrating the electrochemical technology discussed herein with their previous work in detection of *Shewanella* electron production. *Shewanella* can be induced to form a biofilm [221] and thus, in principle, can be directly integrated onto the electrode. Their current setup utilizes a comparatively large 2400 mm² carbon felt working electrode, an Ag/Cl reference electrode, and a platinum counter electrode. Due to the potential toxic redox agents produced at the counter electrode it is currently necessary to isolate it from the cellular solution, only allowing current flow through a membrane. Thus, in early testing phases, a two electrode setup was agreed upon, to evaluate the both the working electrode, reference electrode, and the setup as a whole [211]. The pyruvate induced electron production and signaling pathways are shown in Figure 5.4 below.
Figure 5.4 *Shewanella oneidensis* pyruvate response over time (left) and signaling pathways (right) with potential of using two electrode printed electrochemical sensor [211] Adapted with permission 2015 IEEE.

Reference Electrodes Cellular Solution Viability

A major concern, brought up by Ajo-Franklin, was that silver, an abundant material in the produced chemical sensor, is inherently toxic to many microorganisms. Though silver was not planned as an exposed material for this bioelectronic interface and has been converted to AgCl or masked by graphene in the final chemical sensor design, there was concern of silver leeching from unconverted AgCl, through the NOA 74 protective layer, and/or through the graphene layer.

Three reference electrodes were printed on three PET substrates (9 total electrodes) at a 51°C surface temperature, fully soft cured on the stage, and hard cured at 230°C. NOA 74 was applied to the electrodes, leaving both ends of each electrode uncovered, and cured under direct UV light. The silver was then fully converted to AgCl and affixed to a glass slide using silicon caulk.

These electrodes were sent to Jose Cornejo and Lin Su who tested them for 36 hours by submerging them in 30 ml M9 minimal salts medium and 80 mM sodium lactate inoculated with *Shewanella oneidensis*. Optical density of the solution was taken at the start (0 hours), at 18 hours, and at 36 hours, at the conclusion of the experiment. These tests sought to interrogate the AgCl conversion and the NOA 74 encapsulation layer for possible points of silver toxicity.
**Large WE Two Probe Sensors**

A typically sized 0.32 mm$^2$ reference electrode and a rather large working electrode (0.45 cm$^2$), soft cured at 85°C and hard cured at 230°C for 30 minutes were used as the two probe setup requested. The reference electrode was converted to AgCl as before, using NOA 74 as a mask, and the working electrode was coated with graphene via e-jet deposition, cured again at 230°C without a soft cure. If an Au coating was applied to an electrode, the working electrode and reference electrode were used with a platinum wire counter electrode (since these two electrode setups do not have a standalone counter electrode) to electrodeposit Au onto graphene in an HAuCl$_4$:HCl solution as before. No selective coatings were applied, as the Nafion used for the NO sensor restricts negatively charged ion flow, allowing neutral and positively charged ions through, which is the opposite of the reporting modalities in *Shewanella oneidensis*.

**Biocompatibility Testing**

In order to test if the printed graphene was a substantially thick enough barrier to protect microorganisms from the silver beneath, Berkley Labs submerged two electrodes, one Ag/Graphene/Au and one Ag/Graphene, into *Shewanella oneidensis* cell culture to grow on the electrodes while submerged as in the reference electrode experiment. Pictures were taken to examine cellular growth on each electrode and viability was determined.

**Functionality Testing**

Functionality tests were carried out in Ajo-Franklin’s laboratory in California. The size used for this working electrode was almost six times larger than any tested in the three electrode setup described earlier. Thus, functionality was tested in a well-known solution to assess this new two electrode setup, as well as determine if the packaging method was appropriate. The two electrode setup and a platinum wire counter electrode was submerged in potassium ferrocyanide at 1.0 mM, 2.0 mM, and 4.0 mM concentrations and the potential was brought from -1 Volt to 1 Volt using cyclic voltammetry.

**Sealing the Bioreactor**

As previously stated, the flexible PET was affixed to a glass slide. This was necessary to seal the bottom of the bioreactor, a schematic of which is shown in Figure 5.5. The top has a rubber septum screw top that can be used to injection solution, air supply, broth supply, and/or
electrodes (counter in this case). The bottom utilizes an o-ring and a clamp to create a liquid tight seal around the working and reference electrodes.

The initial substrates used were too small to properly utilize the o-ring, thus attaching these substrates to larger glass substrates was necessary. When attaching the two electrode setup to the bioreactor however, the slight lip created by attaching the PET to the glass obstructed the o-ring from creating a seal. To work around this, the same silicone caulk was applied on the glass, over the lip, encircling the sensor, creating a slightly flexible material that was dimensionally higher than the sensor and thus becomes flat. This was accomplished by applying the caulk and using a sheet of Parafilm and another glass slide to evenly flatten the caulk. The top leveling glass was removed and the caulk/Parafilm was left to dry slightly. After approximately 10 hours, the caulk was dry enough where the Parafilm could be pulled off without sticking, and a solid film was left behind; the resultant setup is shown in Figure 5.5

Figure 5.5 Bioreactor/electrochemical setup (left) and potential sealing method (right) using silicon caulk to interface the o-ring on the bottom of the bioreactor.

Though this method is functional in that it eliminates leaks around the o-ring, the long term attachment of the caulk is inconsistent. Current tests into replacement sealants, as well as larger substrates are underway.
5.2.2 *V. fischeri* Photosensor Interface

**Agar Culture**

In pursuit of developing a methodology to culture *Vibrio fischeri* and *Vibrio harveyi*, agar and broth tests were run simultaneously with previously published media chemistries (Table 5.1) [32, 222]. Agar plates were prepared in triplicate for each media inoculated using sterilized wire loops on two of each media recipe, leaving one as a control. The plates were covered and incubated for 24 hours at 25°C. Glowing colonies were observed after 24-48 hours of incubation, and were re-plated as necessary.

**Table 5.1 Media chemistries**

<table>
<thead>
<tr>
<th>Media A:</th>
<th>Media B: (adjust pH to 7.5)</th>
<th>Media C: Autoinducer Biosassay (AB) Media (adjust pH to 7.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 2.5 g NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 10 g Oxoid nutrient broth #2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 13.5 g NaHPO4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2 g Na2HPO4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1 L DI water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 15 g Agar*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1.5 g CaCl2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 5.5 g MgCl2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 6.9 g MgSO4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 5 g Peptone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 0.7 g KCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 28.2 g NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 3 g Yeast extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1 L DI water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 15 g Agar*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 17.5 g NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 12.3 g MgSO4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2 g Casein hydrolysate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1 L DI water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 15 g Agar*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 3N NaOH for adjusting pH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Only add agar if making plates – DO NOT USE IF MAKING MEDIA BROTH*

**Broth Culture**

It was quickly noticed that the light production from the agar plates was limited. Broth media was prepared in a similar fashion, though agar was not added. During initial experiments, one broth in particular, Broth A (Figure 5.6), showed the highest visual light production and biomass yield and was thus adopted as the only culture media used. There are a number of cell culturing recommendations in the literature, thus in order to optimize cell production in the facilities at hand, multiple culture setups were studied. Initially, cells were cultured in the broth without agitation of any kind.
The progression in setup for the culturing system was a logical one, in that cells were cultured without agitation initially, followed by the addition using a magnetic stir stick, and finally, with the addition of an air bubbling setup to saturate the media with nutrients (via broth), air (via filtered airline), and provide agitation (without the use of a magnetic stir stick). This final setup bubbled filtered air into culture media with a blow-off tube through DI water, fully enclosed within an incubator for 24 hours set to median water temperature near Hawaii (25°C) and was used throughout the experiments revolved around the photodiode bioelectronic interface [32].

**Freezing Cell Cultures**

To extend the length of time for which the Vibrio cell lines could be studied, frozen stocks were prepared, from which inoculations could be lifted when needed. A broth culture was prepared as previously outlined, and centrifuged at 4000 rpm for 10 minutes to concentrate high cellular density. From this concentrated cellular solution, 500 μl was removed and combine in a 2.0 ml screw-top cryovial with 500 μl of 60% glycerol solution that was previously autoclaved. The tube was inverted repeatedly to thoroughly combine the solution and was frozen at -80°C.

When needed, a previously autoclaved inoculation loop was used to scrape a portion of the frozen cellular/glycerol solution, which was placed into 1.5 ml of autoclaved broth and inverted repeatedly to mix thoroughly. This was added to 500 ml of autoclaved broth and cultured for 24 hours. Tests were not run during this time period and would rely on additional broth culture sessions.
Long Term Cell Growth

Though the rise time of the electronic portion of the bioelectronic interfaces produced herein are important for rapid detection, the longevity of the sensor and constant/consistent detection is perhaps more important for the current application of underwater chemical detection with the available cell detection methods. The reasoning here is twofold. Firstly, the locomotion of the current robots produced has a top speed of less than 1 knot, meaning that munitions that are detected will be located via detection of a gradual increase in chemical stimulus as the robot hones in on the source. A rapid sensor is needed for accurate readings, however a consistent and long lasting sensor is needed to accurately locate the chemical source. Secondly, the current available biologicals and pathways used for response are not rapid quick to do so like the electronic portion of the interfaces. Many of the available mechanisms used respond to a stimulus over hours as opposed to milliseconds, and thus, for current testing purposes, long term detection is a valuable and necessary parameter to examine.

In light of these design constraints, an experiment was formulated to determine if the printed sensors were viable for long term detection. 800 ml of Broth A was inoculated with *V. fischeri* that were not illuminated at the time. Filtered air was bubbled into the borosilicate glass volumetric flask containing these 800 ml and kept in a 25°C incubator with the lights off. A PDB-C107 photosensor was set up on the outside of the glass, as was a printed sensor with a functional area of 16 mm², encapsulated in NOA 74 and previously tested for spectral response characteristics. The commercial sensor had its voltage recorded in LabVIEW as the current from the printed sensor was recorded in LabScribe, both at 1 Hz over the course of 30 hours. The hypothesis was that both would show a quantitative increase in response as the cells gave off light over time. The setup (left) and subsequent light production (right, using normal exposure time) are shown in Figure 5.7 below.
As the *V. fischeri* divide and consume the media, they begin to produce auto-inducers (AIs), used for communication and causing bioluminescence; a function that serves them well in a symbiotic relationship with *Euprymna scolopes*. For the purposes here, this gradual illumination, and subsequent decline as the media is consumed and oxidized, is useful to simulate a response to a chemical stimulus and the gradual location of the source.

**Cell Attachment – *V. fischeri***

To evaluate the innate cell attachment of the PET substrate, three conditions were examined; one substrate was submerged in broth with active *Vibrio fischeri* culture using stainless steel monofilament wire and stainless steel nuts for weight for 15 minutes, one had a droplet of this culture pipetted onto it and dried at 50°C, and one did not come into contact with any cellular solution as a control. The submerged film was not heat fixed after being pulled from the broth but was air dried. All three were stained using a classis Gram negative stain, as *V. fischeri* are gram negative bacteria. To execute this stain, crystal violet was placed on the substrate, and then rinsed thoroughly with water to stain any present bacteria. Next, Gram’s iodine was used to trap the stain in the cell wall of any present Gram positive bacteria. The substrate is then washed with ethanol and, since these cells are Gram negative, the crystal violet is washed away due to a thin peptidoglycan layer that protects crystal violet from binding the cell wall. Finally, safranin is
pipetted onto the substrate, staining the gram negative cells red and showing contrast between positive and negative cell lines. For the experiments herein, it is a method of visualization and a semi-confirmation that the cells are at least gram negative. Since a monoculture was used, the assumption is that the culture remains only *V. fischeri*. Furthermore, the washing steps should remove any bacteria that did not fully adhere to the PET substrate itself.

### 5.3 Results

#### 5.3.1 Interfacial Layers: Collagen and Gelatin

Cell attachment and/or biofilm formation is of major focus when discussing the fabrication of bioelectronic interfaces. Though there is variation in how and what different cell types will attach to, collagen and gelatin have a wide range of cell attachment applications, with collagen being one of the most biologically relevant materials studied today. The e-jet printer was used to deposit 5 mg/ml of collagen type I (Sigma; C-7661), as well as gelatin onto cell attachment resistant agarose covered glass slides and compared against each other.

![Collagen and Gelatin](image)

**Figure 5.8 Mouse myoblast C2C12 cell attachment to printed collagen and gelatin lines**

By printing on top of agarose, cells may attach only where the collagen/gelatin binding ‘glue’ is printed, creating lines in prescribed geometries and resolution. As shown in Figure 5.8, lines of C2C12 cells (mouse myoblast cell line) were created, approximately 100 μm (4 cells) wide and 1 mm long, while gelatin generated lines that were significantly thinner (30 μm, 1 cell wide) lines almost twice as long in parallel lines. This variation in line width is thanks to the intentional printing of thicker collagen lines, not necessarily that collagen cannot produce thinner lines. Though sufficient studies have not been performed on cell attachment and e-jet printing, and this is not a direct comparison between collagen and gelatin, the studies performed show a proof of concept for cell attachment as well as promise and direction for future studies.
5.3.2 *S. oneidensis* Electrochemical Sensor Interface

**Reference Electrodes Cellular Solution Viability**

As seen in the Figure 5.9 below, there is little difference between the reference electrodes tested and the control. Since the optical density trended the same, the reference electrode chemistry was deemed to be safe for the microorganisms in which they were tested. Similarly, the working electrodes tested, both with and without the addition of Au (seen in Figure 5.9), did not impact cellular growth in broth. The working electrode tests further suggested the safety of the reference electrode AgCl chemistry as these were present on the two electrode setups tested.

<table>
<thead>
<tr>
<th>Time hr</th>
<th>Control</th>
<th>Electrodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>18</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>36</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Figure 5.9 Sensor exposure viability; RE setup (bottom left), and optical density (top left) over culture time. A visualization of the WE (right) under *Shewanella oneidensis* culture and subsequent biofilm formation.

**Ag/Graphene WE Functionality**

Figure 5.10 below provides a visualization for the setup used in the Ajo-Franklin Lab to test the functionality of the two electrode setup that was sent to them. Pictured is the setup with the basic Ag/Graphene working electrode with a platinum counter electrode. The results show a graded response to increased concentrations of a known analyte, as would be expected. The cyclic scan was run repeatedly and little drift is observed, suggesting stable working and reference electrodes.
5.3.3 *V. fischeri* Photosensor Interface

A normalized response is shown (left of Figure 5.11) over a period of 20 hours, showing the rise and decline in light production from both the commercial and printed sensor, simulating the graded response of a potential stimulus over time. An identical test was done where the control was blocked using electrical tape, registering no response while the printed sensor showed a response to the *Vibrio fischeri* light production similar to what is shown in the figure.

The printed sensor used herein averaged approximately 8.8 pA at max *V. fischeri* luminescence (right Figure 5.11). Having created a spectral response for each produced sensor at that stage of production, this sensor had response of 1.178 mA/W at 490 nm, translating to 7.47 nW produced in culture. Given that the sensor had a 16 mm² active area, this sensor was able to read 46.7 nW/cm²; well under the photosensor design specifications set herein.
Cell Attachment

Seen in the Figure 5.12 below, a black and white photograph was taken with a 10 μm scale bar of the PET substrate with cellular solution pipetted onto it. The density of the cells is significant due to the droplet added to the surface. Though there was high cell density, there was little to no observed biofilm formation, again due to the droplet deposition. As seen in the top right portion, there were points of observed delamination from the rinsing process. This same bare patch is seen in the color photograph next to it (center) for reference. As seen, the color confirms the gram negative morphology. The final picture is that of the submerged PET substrate, showing attachment on both sides of the substrate (out of focus portions) as well as a high cell density and slight biofilm formation after 15 minutes.

![Image of cell attachment](image)

Figure 5.12 *Vibrio fischeri* attachment to PET via pipetted (left, center) culture and after 15 minutes of submersion in live culture (right). The affixed *V. fischeri* was stained using a Gram negative stain.

5.4 Discussion

5.4.1 Electrochemical Sensor Based Interfaces

By far the most widely used reporting mechanism by microorganisms, chemical signaling should be of major focus in the pursuit of bioelectronic interfaces. Discussed in the methods and results above is a variable chemical sensor printed on the e-jet platform that can be used to detect biologically dependent nitric oxide, electron production, or any number of different biologically produced chemicals in real time using a number of different electrochemical techniques.

Discussed in the methods section, the nitric oxide sensor is capable of selectively detecting nitric oxide at biologically relevant levels during a rapid depolarization event of RAW
264.7 macrophages. Though this shows theoretical success as a chemical sensor based bioelectronic interface, it potentially has a wider impact in macrophage biology as a possible disposable platform to investigate individual macrophage cells.

Investigating the viability of the chemical sensors in long term cellular solutions, the Berkeley Lab experiments showed the durability of the chemical sensor parts and as a whole in bioelectronic interface conditions. Their ability to remain functional without causing cellular toxicity is paramount to long lasting bioelectronic interfaces in adverse environments.

The demonstration of functionality of the larger working electrode and altered chemical sensor setup in known analytes highlights the versatility of the chemical sensor backbone formulated herein as well as the adaptability of the e-jet printer to overcome various design constraints. The graded cyclic voltammetry response in the presence of increasing concentrations of the analyte reflects the different electrochemical detection methods that this sensor setup provides.

The variability of these printed sensors allows the user to tune the chemical sensor base structure to detect any number of potential response chemicals from stimulated microorganisms; in this case through mechanical stress and pyruvate stimulation. Though neither of these bioelectronic interfaces detects underwater munitions, these interfaces are important in their respective fields and it is a reasonable next step in the progression of the research herein to integrate munition detecting engineered cells.

### 5.4.2 Photosensor Based Interfaces

As a bioelectronic interface for long term detection, the printed photosensor and the *Vibrio fischeri* were setup to detect auto-inducers (AIs) as an example of the potential these e-jet printed interfaces hold. Shown in Figure 5.11, there is significant response in the pA range, detecting in the nW range at peak luminescence. The normalized responses show lower sensitivity at low light levels compared to a commercial sensor, however these sensors are of a basic design and have room for improvement in low light level detection and efficiency through hole transport layer and electron transport layer implementation, among other possible additions and modifications. This does however show a concentration graded response to light production in direct response to AI cellular interaction yielding an AI concentration detecting bioelectronic interface.
The cell attachment results show that there is innate cell attachment to the PET substrate, without applied adhesion agents. This natural adhesion, over a longer period of exposure, could lead to easier biofilm formation on the PET substrate with biofilm promutors in broth that should profoundly improve sensitivity. There is need to test if this is the case and if an adhesion agent is necessary at all. It is evident however that an anti-adhesion agent is necessary to repel cell attachment to the PET in unwanted zones. Furthermore, three substrates were submerged in the same way for 48 hours to determine cellular toxicity. These were placed in newly inoculated broth and luminescence was observed. There was no change in the rate at which the Vibrio fischeri began luminescing and the procedure was thus deemed not significantly toxic to the cell culture. This is significant in that the PET substrates need to interact with cellular solution for extended periods of time and rely on a nontoxic relationships to produce functional, long term bioelectronic interfaces.

5.5 Conclusion

Bioelectronic interfaces open an interesting field; one with more possibilities than examples at this point in time. The provided bioelectronic interfaces provide three examples, in detecting mechanical manipulation, pyruvate, and AIs. Apparent is the lack of munition detection that these three examples provide, though the framework of the testing methodologies, electronics construction via e-jet printing, and viability in long term cellular solutions provides a clear path to testing engineered cells in the same manner. Since there are more possibilities than examples, the potential future work is broad in scope and diverse in functionality. A few major points of emphasis that translate to nearly every application is the demonstration of robust biofilm formation and communication on printed sensors, rapid time based communication with instantaneously responsive microorganisms, and the opposite of what is demonstrated here in a reporter based electronic and a receiver based microorganism as discussed earlier with a blue LED and a flexing muscle cell. Regardless of the next step, with the addition of the fabricated bioelectronic interfaces herein, the future of the field is open and promising. The results and discussion in this chapter provide a confirmation of the tested hypothesis that the e-jet printer is a viable deposition methodology for quantifiable bioelectronic interface fabrication.
Chapter 6 Conclusions and Future Work

This chapter concludes the thesis with a brief discussion of the whole. The following sections deliver a chapter summarization, the contributions and conclusions of the results herein, and proposed future work respectively.

6.1 Thesis Summary

In order to clearly summarize the thesis, a summary of each chapter is provided for clarity and reference.

Chapter 1: Introduction/Background

This first chapter opened the thesis, highlighting the exciting emerging field of synthetic biology and the high impact it is expected to have with a broad array of industrial and research based application. A discussion is provided regarding the potential that liquid based printer deposition, namely e-jet printing, has to deposit electronics, interfacial layers, and cellular solutions to fabricate synthetic bioelectronic interfaces with applications in autonomous underwater munitions detection via biomimetic robotics.

Chapter 2: E-jet Construction and Modification

The design, fabrication, and testing of a multi-nozzle, heated vacuum chuck aided, electrohydrodynamic printing system is discussed in this chapter. Focus is directed towards subsystem design, code, and implementation, as well as the impact that the heated vacuum chuck had on silver deposition and the deposition of multiple layers as 3D structures.

Chapter 3: Nitric Oxide Electrochemical Sensor

The first electrical component produced by the assembled e-jet printer is the focus of the third chapter. The sections discuss the fabrication of the subcomponents of the device, with major focus on functionalizing the working electrode. The chapter concludes with results and discussion
on its use as a nitric oxide sensor, highlighting the ability to print functional multi-material, multi-layer electronic components via an e-jet printing system. The design goal of Nafion induced selectivity (<1% detection of NO$_2^-$ and NO$_3^-$ compared to NO) in detecting nitric oxide concentrations $\leq 1.0$ μM was met and exceeded, though the <5% metric variability was not achieved though may be in the near future.

**Chapter 4: Photosensor**

The second sensor produced via e-jet printing is discussed in the fourth chapter; the photosensor. A major early issue with the photosensor was the layering process on a fragile material, and the resolutions to this problem are highlighted. Furthermore, the path to a stable spectral response is discussed, with the results of this process and its repeatability finishing the chapter. The hypothesis that an electrohydrodynamic printing system can produce a functional, reproducible (>90% intended devices, <5% metric variability), multi-layer photosensor that is capable of detecting $\leq 0.3$mW/cm$^2$ has been confirmed, less the metric variability which may prove possible in the near future.

**Chapter 5: Bioelectronic Interfaces**

Three different bioelectronic interfaces are discussed in the fifth chapter, bringing the topics set out in the introduction to fruition. A functional photosensor based bioelectronic interface is an example of the potential the produced devices have in detecting molecules singularly. The nitric oxide sensor is also shown capable of detecting mechanical manipulation via macrophage depolarization, with a modified version of the sensor being used to detect electron production in a separate bioelectronic interface. This chapter showed a confirmation of the hypothesis that the e-jet printer is a viable deposition methodology for quantifiable bioelectronic interface fabrication.

**6.2 Contributions and Conclusions**

The use of the heated vacuum chuck aided e-jet printer to deposit fully functional electronics and interfacial layers opens new potential research and industrial applications for this deposition technology. As synthetic biology expands in its stability and scope, the need for a reliable deposition method, which can be used in both laboratory and industrial settings, will continue to increase.
The impact of the demonstration of multi-layer liquid phase deposition and its use in multi-material electronics encompasses nearly every known electronic device. The general electrochemical sensor provides a framework for various research opportunities and has already shown use in detecting pH in ongoing research in the Ayers lab. Nitric oxide was detected at printed sensor relevant levels though demonstrates wider adaptability in the printing process. Consequently, the produced nitric oxide sensor and its base form may have applications in the study of macrophages, disposable biological sampling, aqueous pollutant detection, and various other high impact research areas.

The photosensor devices produced are fully functional, yet still have room for improvement with additional layers that would enhance their efficiency. One major challenge of producing these layers was achieving adhesion without dissolution of the sublayer or delamination during use. Through the use of the heated vacuum chuck, as well as tunable ejection modalities, low volume deposition is rapidly cured, eliminating dissolution effects on both the photosensor and nitric oxide sensor.

The integration of these multi-layer devices with microorganisms demonstrates the use of the e-jet printer to produce functioning bioelectronic interfaces, which, with the e-jet printers ability to deposit interfacial layers like collagen and gelatin for cell attachment, have the potential of becoming ever more sensitive with the e-jet aided formation of biofilms on the sensors themselves. Multiple demonstrations of these interfaces have shown the potential to detect molecules, physical manipulation, and biologically produced current. Though these are the first demonstrations using these printed sensors, their use in underwater munitions location, macrophage research, and water pollutant evaluation have been discussed. Demonstrations of these bioelectronic interfaces open the door for their use with these or countless other applications.

6.3 Future Work

The experiments and results discussed within this thesis provide several different possible research paths. Looking at chapters 2-4, there are various improvements that can be done to the e-jet printer as a whole, the electrochemical sensor, and the photosensor as well as developing other multi-layer sensors like LEDs and thin film batteries.
Future work on the e-jet printer would include a user focused version of the provided GUI. Since there are more than three layers in current and potential sensors, adding more than three nozzles would open opportunities to rapidly print complex sensors. The printer could also stand to add subsystems like atmospheric control (reactive materials benefit from an inert surrounding during deposition), on stage hard curing, user geometric layout control and automatic g-code conversion, wavelength controlled LED light source for stage camera (many films are sensitive to certain wavelengths of light), electronic camera position, magnification, and focus control, direct UV curing on stage, and other similar enhancements that are geared towards improving the performance of individual films that in return, improve the overall function and reproducibility of total sensors, as discussed earlier.

One of the most prominent deficiencies in current printing processes and discussed briefly in the work presented here, is inconsistent deposited film morphology, which severely impacted the device performance of the fabricated electronics discussed earlier. This is seen in the ‘coffee ring’ effect in Figure 2.21, as well as in the metric variability in the nitric oxide sensors and photosensors between devices. These inconsistencies are due in large part to the inconsistencies in the morphology of the deposited films, namely due to inconsistent film thickness across the entire film and across fabricated devices, and due to film roughness. Major focus in future studies should be spent in reducing this metric variability by addressing substrate surface energy, ink internal force reduction (surface tension), and film post processing steps.

Wettability, as previously discussed, is the interaction of a liquid with a solid surface, specifically, its tendency to spread on this surface, creating a low contact angle and high adherence. Though this interaction can be addressed in ink formulation, it is a two parameter problem (liquid and solid) that must be investigated from two directions. Of particular future focus should be the ability to reduce the surface roughness of the material upon which the active material is being deposited. This can done by addressing the substrates themselves, creating low roughness films with high wettability, containing the other parameters that are needed for device fabrication (transparency, flexibility, etc.). The experiments necessary to do this, however, would take a specialized laboratory. Another method to achieve a pristine surface is through surface additives that may produce high wettability and low surface roughness without addressing the chemistry or production of the substrate itself.
An extensive experiment into increasing the wettability via additives is a logical progression on the sensor fabrication presented here. It has been shown that ferrocene polymers [223], ZircSOx [224], crosslinked PVP and poly(melamine-co-formaldehyde) in PGMEA [225], HMDS [157, 158], polystyrene, PMMA, and polyimide improve surface roughness on various substrates [226]. Additionally, since solvents may be hydrophobic or hydrophilic and wettability relies on the interaction of both the solid and liquid, ferrocene polymers can be reversibly transitioned to hydrophobic or hydrophilic using a redox reaction applied to the surface, and ZrO₂:B treatment combined with UV-Ozone treatment has shown the ability to create super-hydrophilic films out of typically hydrophobic ones, increasing wettability of the resultant film [226]. Finally, UV-ozone has been widely used to improve wettability, though does not typically alter surface roughness [227].

The solvent interaction with the substrate or substrate additive depends on both the solvent and the surface. Thus in order to plan for various solvents (chlorobenzene is hydrophobic and triethylene glycol monomethyl ether is hydrophilic) all of these surface treatments should be examined. PET is an attractive substrate, shown capable of handling current device fabrication. Since the provided studies to not examine the function of the additives on PET, a full experiment should be run to fully understand their adhesion and morphological relationships upon deposition. All of the treatments listed above can be applied via spin coating, thus spin coating speed should be altered from 500 rpm to 6500 rpm, with controls for thick applications (drop cast) and no application (cured at the same temperature as additive). Each produced film and control should be first analyzed for surface roughness (typically in the 10-100s nm range), using a standard AFM technique, and then analyzed for hydrophobicity and wettability via contact angle measurements with DI water (<90° = hydrophilic, >90°=hydrophobic). Each additive and produced additive thickness should also be examined using UV-Ozone from 0-5 minutes tested at 1 minute increments for contact angle, as well as running the same test using the ZrO₂:B additive with controls being carried throughout. In this way, low surface roughness may be achieved with both a hydrophobic and super-hydrophilic coating, completing half of the effort to regular e-jet printed films.

As layers are added to devices, these same steps should be investigated, with additives based on the film in question, to improve surface morphology and wettability for subsequent layers. Though dielectric materials and insulation materials may be inappropriate between
functional layers, UV Ozone treatment and other ultra-thin films may improve wettability and function of previously printed films.

Though substrate additives and alterations are important for the initial printed layer, ink formulation is imperative to depositing consistent films as they are layered within the device. Once the droplets ejected during e-jet printing impact the surface, they begin interacting with previously deposited droplets and the surface beneath, whether it is a deposited film or the substrate itself. Future work has been set out to control the wettability of these surfaces (above), though the ‘coffee ring’ effect and film roughness depends highly on the ink itself. As solution based materials begin to evaporate, re-circulatory flow due to the surface tension of the liquid and the gradients involved in this evaporation result in a coffee ring type effect in droplets and more complex drying morphologies in films. These gradients can be reduced by reducing the surface tension of the liquid via surfactant or dilution, as well as by altering the particle geometry if using a nanoparticle ink [81-83]. FS-31 [120], used here, other fluorosurfactants [228], EGBE [136], and Surynol 2502 [229] have all been used in inks as surfactants to reduce their surface tension. Furthermore, methanol [230], DMSO [229], and glycerol [136] have been used to create low surface roughness solution based films. The use of these molecules to control film deposition relies on the primary solvent used and its polarity.

Ink formulations are dependent on the active molecule solubility in a primary solvent and the miscibility of ink additions into that solvent. Thus, any future experiment described here would be a methodological framework and not a workable experiment for every device layer, though this sort of experiment is necessary in optimizing film morphology. Each ink, however, should be printed on the optimized substrate or film from the experiment described above. In the case of the P3HT:PC71BM active layer, the solvent is hydrophobic chlorobenzene. In order to reduce the surface roughness of the produced film, increasing added concentrations of DMSO and methanol should be printed with active material concentrations remaining constant, depositing 10 dots, and 10 lines, tested for surface roughness using an AFM and topography using the Dek-tak 3030 system or a similar film thickness analyzer. The same process should be done for each surfactant, with an expected reduction in the coffee ring effect as the surfactant concentration increases. The surface tension and viscosity for each additive concentration should be analyzed, which would result in an expected relationship between surface tension and
topography uniformity and may yield further relationships between ink properties and film morphology.

The impact of both of these additives, a surface roughness reducer and a surface tension reducer, requires evaluation within devices as well. Each of the formulations should be used to create 10 devices, analyzing the primary parameters of the device; the shape and amplitude of resultant spectral response with controls of the original formulation for example when optimizing the P3HT:PC71BM active layer.

The same process should be followed in combing the surface roughness and surface tension reduction additives, using a concentration matrix to scale each substance concentration, again testing each ink for surface tension and viscosity, each deposited dot and line on its optimized substrate/film for surface roughness and topography, and each ink for its impact on device performance.

In addition to optimizing the deposition surface and the deposition solution, there is evidence that post processing these films can have an impact on film function and morphology. Both methanol [231] and chlorobenzene [232] washes have been shown to improve PEDOT:PSS film function in optoelectronic devices. Though these treatments were not evaluated for subsequent film deposition morphology, they did improve the overall device function. An appropriate future examination would be to pretreat films with the solvents used in the subsequent layers, potentially creating an interface for deposition similar to that of which is being deposited next.

Various film curing and sintering methods have also been shown to improve the morphology and active molecule density of deposited solution based films. Heat based silver sintering, as discussed earlier, has a place in producing silver films, though other sintering methods should be examined in the future, including UV radiation [233], plasma treatment [234], laser-aided sintering [235], and chemical based sintering [236], though these treatments are largely active material dependent, as would be the experiments conducted.

The nitric oxide sensor has suffered from detection precision and repeatability issues which should be a major focus in continued research on the chemical sensor base. Reducing the overall functional area of the sensor itself for use in micrometer level detection would provide a micro framework for single cell interfaces.
The essential material science behind photosensor device efficiency optimization and precision has not been investigated to this point, and would include further investigating the active layer deposition characteristics and how they impact sensor construction. While optimizing these metrics should be the first step in additional work for the sensor, other points worth investigating would be the development of thin films, perhaps with a post processing wash step to eliminate pinholes, in order to implement additional materials that have been shown to enhance efficiency.

Long term exposure experiments should be another focal point of future research. Though sensors were kept in culture broth during short term (15 minutes) and long term (96 hours) experiments, the goals of these experiments was to investigate bioelectronic communications techniques. Experiments on the mechanical stability of the substrates and encapsulation layers should be done in salt water, possibly through evaluating reduction in adhesion using Instron manipulation, in addition to documenting any decline in sensor performance. Additionally, biofouling, an issue not discussed in the results above, is a routine and complex problem impacting long term ocean-based sensing platforms [237-239]. Though, as discussed, cell attachment is encouraged for increased sensing capabilities due to proximity and molecule diffusion rates, there is the potential that these films become too thick and/or begin to have a reduction in signal quality. Long term cell culture exposure experiments should be carried out wherein biofilm thickness is monitored and signal quality is recorded over culture times. For full bioelectronic interfaces, this would entail introducing the signal which the biological is engineered to sense in controlled doses, and monitoring the resultant electrical signal. Some optimized biofilm thickness is expected, though this will likely be species, if not cell line dependent.

Robust biofilm formation and subsequent communication with printed sensors, rapid time based communication with instantaneously responsive microorganisms, and the opposite of what is demonstrated here, in a reporter based electronic and a receiver based microorganism as discussed earlier with a blue LED and a flexing muscle cell should be some of the next points of study from what has been established here as bioelectronic interfaces. Furthermore, the general goal of the research herein is to provide a framework for munitions detection with multiple modes of confirmation using a low cell count.
The author believes that, with the presented results herein, the pursuit of multiple electronic devices per cell would ultimately create a robust olfactory taxis for underwater autonomous robotics and would be a next logical step in the progression of this research.
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