**Redox Cycling at an Array of Interdigitated Bipolar Electrodes for
Enhanced Sensitivity in Biosensing**

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Abstract

An array of many bipolar electrodes (BPEs) can be controlled by a single pair of driving electrodes yet allows for multiplexed analysis of many individual biomarkers or single cells at once. A wide range of bipolar electrochemical sensors have been devised, many of which operate under battery power and produce visible signals (e.g., luminescent, electrochromic) appropriate for smartphone or naked eye readout. These features of BPEs are advantageous in the context of clinical and environmental sensing applications at the point of need. However, the sensitivity of BPEs is poor in comparison to direct measurement of current at an individual electrode, and therefore, the enhancement of signals obtained at BPEs is an active area of research. Here, we describe signal amplification by redox cycling accomplished by interdigitation of each BPE in an array with a shared driving electrode. We evaluate amplification obtained for interelectrode spacing in the range of 35 μm to 15 μm . Each interdigitated BPE (IDBPE) in the array has an independent, reproducible, and linear response to a reversible electroactive analyte. Therefore, this universal amplification strategy allows for multiplexed or spatially resolved sensing in point-of-need applications.

1 Introduction

In this work, bipolar electrodes (BPEs) are integrated with redox cycling facilitated by interdigitated electrodes (IDEs) for signal amplification in sensing applications (**Figure 1**). These interdigitated BPEs (IDBPEs) retain the primary advantage of BPEs in that tens to thousands of electrodes can be operated in parallel with a single pair of electrical leads. To achieve amplification at each BPE, the cathodic pole is divided into microband 'digits' that are interdigitated with microbands extending from the anodic driving electrode, and the narrow gap between bands allows reversible redox species to cycle between them undergoing repeated electron transfer. This advancement is important because it provides a straightforward approach to achieve enhanced sensitivity at an array of many electrochemical sensors. Our results demonstrate that an array of IDBPEs having uniform geometry, behave identically, while a decrease in the gap size

Figure 1. (a) Schematic illustration of redox cycling that occurs within the gaps between microbands of the IDBPEs, the gap size indicates the distance between microbands, (b) Brightfield micrograph of the microfluidic device with IDBPEs. A 'sensing channel' is positioned over the sensing end of the IDBPEs, a 'reporting channel' over the reporting end of the IDBPEs, and an insulating wall of PDMS is located between the channels, (c) Micrograph of the array of IDBPEs under dark conditions. The sensing channel contains 3.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer (pH 6.9) with no flow. The reporting channel contains ECL solution (5.0 mM $\text{Ru}(\text{bpy})_3^{2+}$ and 25.0 mM TPrA in 0.5 M phosphate buffer) with no flow. Under a driving voltage of 2.0 V, a luminescent signal is showing at the BPE anodes within the reporting channel. This reporting reaction at each BPE anode is electrically and stoichiometrically coupled to ferriyanide reduction occurring at each BPE cathode, within the sensing channel.

leads to signal enhancement. The dimensions employed here (15 μm to 35 μm) are readily accessible by conventional photolithography. Gap dimensions of 15 and 25 μm , respectively, yield 2.6-, and 1.2-fold higher current than a 35 μm gap. Extension to narrower gaps is anticipated to yield still greater amplification. Finally, we show that the IDBPEs respond independently from one another. This feature is key to their application in array-based sensing, in which the desired outcome is to quantify many distinct analytes or to map the spatial distribution of a single analyte.

BPEs have found application in many areas including electrochemical imaging,¹ electrokinetic separations,^{2–5} materials synthesis,^{6,7} electrocatalyst screening,⁸ micromotors,^{9,10} single-cell analysis,^{11–13} visual voltammetry,^{14,15} point-of-care sensing¹⁶ and biosensing.¹⁷ Recent advancements in bipolar electrochemical methods of analysis are discussed in a recent review by Rahn et al.¹⁸ A BPE comprises a conductor immersed in an electrolyte solution. When a potential is applied to the electrolyte by an external pair of driving electrodes, the interfacial potential difference that develops between the solution and the BPE drives faradaic reactions at its opposing ends (**Scheme 1**).^{5,6,19–21} Because the potential is applied to the electrolyte instead of directly to the conductor, the BPE is considered wireless, allowing for multiple BPEs to be arranged in an array format and to be operated with a simple DC power source.^{1,20,21} Importantly, oxidation at the

Scheme 1. Schematic representation of the IDBPE system. (a) A plot of the potential of the driving potential of the cathodic and anodic driving electrodes of the IDBPE (E_{drive}) and of the BPE (E_{BPE}). An interfacial potential difference creates an anodic and cathodic overpotential available to drive faradaic reactions. The insulating wall indicates this is a closed BPE system. (b) Schematic illustration of the faradaic reactions at an individual IDBPE. A driving potential is applied to the anodic driving electrode allowing for $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ to be cycled through oxidation and reduction between the microbands of the IDBPE, electrons are transferred to the opposite end of the BPE (round sensor) where oxidation of $TPrA + Ru(bpy)_3^{2+}$ occurs, followed by reduction of H_2O at the cathodic driving electrode.

BPE anode is electrically coupled to a reduction reaction occurring at the BPE cathode such that the currents are opposite in sign and equal in magnitude. This feature has allowed these electrodes to be utilized as sensors because one of the reactions can be leveraged to generate a visible signal (**Figure 1b,c**). Electrogenenerated chemiluminescence (ECL) is broadly used as a reporting reaction because it provides a visible signal that Crooks and coworkers have shown is linear over a wide range of current densities – a feature that facilitates accurate quantitation.²² The BPE configuration utilized in this work is a closed BPE configuration. In a closed BPE system, the two ends of the BPE are fluidically separated by an electrical insulator. This arrangement prevents intermixing of the reaction volumes and further requires the total current passed between the driving electrodes to flow through the BPE. The latter feature provides an indirect measure of the current through the BPE (or BPE array).

Despite their many advantages, BPEs have characteristically suffered from poor limits of detection when employed for biosensing. In the context of ECL as the reporting reaction, there are two main categories of strategies employed to amplify the

signal generated by BPEs: modification of the reaction or the geometry at either the 'sensing pole' or the 'reporting pole'. For example, increases in surface roughness or the geometric area of the sensing pole or the incorporation of catalytic labels augment the current generated. At the reporting pole, strategies to enhance ECL include substitution of the ECL co-reactant and adjustment of the ECL chemical environment by addition of surfactants or a change in pH.^{23–26} As an example of the magnitude of enhancement, Li and Zu were able to show a 50-fold increase in maximum ECL intensity by adding a nonionic fluorosurfactant to their system.²⁴ In some cases, sensing and reporting occur on the same pole. In such cases, the signal generated by ECL reactions has been amplified through the addition of metal nanoparticles, dendrimers, or liposomes.^{23,27–31} In particular, Kim et al. demonstrated that modification of indium tin oxide (ITO) with dendrimer-encapsulated Pt nanoparticles, enhanced the catalytic activity of the electrochemical oxidation of ECL producing a 213-fold increase in ECL emission compared to bare ITO.²⁹

These strategies result in amplification of the ECL signal on a BPE; however, many of these methods are not universal, add cost to the system, or complicate manufacture of the electrodes. Conversely, redox cycling is an amplification strategy that is universal, tunable, and produces consistent results, and further, it can be integrated with some of these existing strategies to achieve multiplicative gains. Redox cycling is the repeated reduction and oxidization of redox active molecules at two closely spaced electrodes and occurs when the diffusion layers of the electrodes overlap.^{32,33} In the context of 2- and 3-electrode cells, redox cycling has been utilized to amplify the signal arising from reversible faradaic reactions. The degree of amplification is determined primarily by the distance between the electrodes.^{32–35} Continuous redox cycling allows for hundreds of electrons to be

exchanged with the electrodes by one reversible redox-active molecule.^{36,37} In addition to amplifying the signal through redox cycling, selection of reversible over irreversible faradaic reactions is realized.

To achieve signal amplification, redox cycling has been utilized in three main geometries: scanning electrochemical microscopy (SECM),^{37,38} nanogap devices,^{39,40} and interdigitated electrodes (IDEs).^{32–36} Fan and Bard correlated the current obtained from faradaic reactions of single molecules to the distance between the SECM tip and the substrate.³⁷ Further, Neugebauer and coworkers demonstrated a 3.6-fold current amplification of the signal obtained during a DNA assay using SECM in feedback mode.³⁸ Nanogap devices are comprised of two electrodes spaced at a distance of 100 nm or less. Nanogap devices encompass various designs such as: nanocavities,^{39–43} ring-disk electrodes,^{44,45} and nanopore electrodes.^{46,47} Using ring-disk electrodes defined by a metallized nanoporous membrane, Bohn and coworkers were able to show a 30-fold current amplification.⁴⁵ In IDEs, employed in this work, redox cycling takes place in the gap between closely spaced microbands (**Figure 1a**). Heineman and coworkers utilized IDEs for the detection of electrochemical enzyme-linked immunoassays and were able to attain a detection limit of 10 ng/mL.³⁵ Dam et al. reported 4-fold and 9-fold signal enhancement with a 7 μm -thick IDE having 2 μm gaps between 4 μm - and 2 μm - wide microbands, respectively.⁴⁸ Lemay and coworkers demonstrated that with confinement of redox species higher amplification could be achieved. They reported 110-fold signal amplification at 250 nm-spaced IDEs confined by a 75 nm-deep nanochannel.³⁶ Most recently, interdigitation with 120 nm gaps was utilized to detect tumor-derived extracellular vesicles (tdEVs) with a limit of detection of 5 tdEVs/ μL .⁴⁹

Interdigitation has been used in conjunction with BPEs in one previous application by the Bohn group.⁴⁷ Bohn and coworkers'

designed a microfluidic device with an embedded working electrode (WE), a quasi-reference electrode (QRE), and an array of BPEs. The WE and QRE each comprised 8 microbands and were positioned such that each intersected one of two parallel microchannels. Independent microband BPEs were positioned between the 8 WE/QRE microbands such that they interconnected the two microchannels. Each BPE was spaced 10 μm from each the WE and QRE to facilitate redox cycling. Bohn's group utilized this configuration to map chemical gradients. This work is related to the current work in that it implements interdigitation in the context of BPEs.⁴⁷

In contrast to the work by Bohn and coworkers, the work described herein demonstrates the combination of interdigitation and BPEs for the purpose of signal amplification, redox cycling occurs solely at the sensing (cathodic) pole of the BPE, and ECL (not fluorescence) reports the signal. This device combines the inherent advantages of BPEs (wireless arrays of electrodes, a simple power source, and uncomplicated device design) with the added benefit of redox cycling to amplify the ECL signal. To this end, we have designed an IDBPE array that yields an equivalent response for each IDBPE across the array, which allows for multiple sensing opportunities with a single readout at once. In addition, we demonstrate signal amplification by comparing the luminescence intensity and current obtained under matched conditions at IDBPES having distinct spacing between the microbands (**Figure 1b,c**). Such amplification lowers the limits of detection and increases sensitivity to increase applicability of BPEs to bioanalysis. Finally, we demonstrate that each of the IDBPES within the array respond independently (i.e., there is no crosstalk), which allows for multiplexed or spatially resolved biosensing within one array. We anticipate that the IDBPE concept is scalable and that gaps between the microbands can be decreased to achieve greater amplification.

2 Experimental

Chemicals. The following reagent grade chemicals were used as received. Potassium ferricyanide (Acros Organics), potassium ferrocyanide trihydrate (Alfa Aesar), 1-methyl-2-pyrrolidinone (Acros Organics), and tripropylamine (TPA) (TCI America) were purchased from Fisher Scientific (Waltham, MA). Tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate ($\text{Ru}(\text{bpy})_3^{2+}$) was purchased from Strem Chemical (Newburyport, MA). ECL solution contained 5.0 mM $\text{Ru}(\text{bpy})_3^{2+}$ and 25.0 mM TPA in 0.5 M phosphate buffer (pH 6.9). All solutions were made with double deionized water (18.2 M Ω ·cm, Sartorius Arium Pro, Göttingen, Germany).

Device Fabrication. The sensing and reporting microchannels of the device (indicated in **Figure 1**) were each 1.34 cm long and 50 μm tall and had widths of 125 μm and 250 μm , respectively. The thin metal film IDBPE array consisted of 20 distinct BPEs arranged in parallel (**Figure S1**). From each BPE, 3 microbands extended across the sensing channel and were interdigitated with an identical set of 3 microbands extending from a single rectangular driving electrode, which ran parallel to the full length of the array. Each microband was 25 μm wide and 340 μm long (of which 125 μm was exposed to the sensing microchannel). Three such IDBPE architectures, distinguished by the gap size (15, 25, 35 μm) between the microbands, were used in this work. Each IDBPE was spaced from the neighboring IDBPE as follows: 270 μm (15 μm gap device), 215 μm (25 μm -gap device), and 162 μm (35 μm -gap device). The middle segment of each BPE was 50 μm wide and 500 μm long, extending underneath the PDMS wall and into the reporting channel. The opposing (reporting) end of each BPE was terminated with a 75

μm -diameter circle spaced 100 μm from a 25 μm -wide rectangular driving electrode located within the reporting channel and extending the full length of the array. A complete drawing of the device is included as **Figure S1** of the Supporting Information (SI).

Au electrodes were made on glass slides using standard photolithographic and etching methods.¹¹ In brief, positive photoresist AZ P4620 (Integrated Micro Materials (IMM), Argyle, TX) was spin-coated onto Au-coated glass (5 nm-thick Cr adhesion layer and 100 nm-thick Au top layer, EMF Corp., Ithaca, NY) and exposed to UV light using a photomask printed with the electrode design. The Au-coated slide was developed with AZ 400K developer (IMM), wet etched with 4% KI/ 1% I₂ followed by chrome etchant (Sigma Aldrich, St. Louis, MO), and rinsed with DDI water. The slide was next agitated in 1-methyl-2-pyrrolidinone at 65°C for 20 min to remove the AZ photoresist and then dried with a stream of N₂ gas. For removal of any remaining residue, the slide was exposed to UV ozone cleaning (HELIOS-500, UVFab, Walnut Creek, CA) for approximately 1 h followed by a 30 s rinse with isopropanol and drying with a stream of N₂.

A poly(dimethylsiloxane) (PDMS) monolith with embedded microfluidic channels was made by casting Sylgard 184 (Dow Corning, Midland, MI) to a master mold made of negative photoresist (SU-8 2050 Kayakli Advanced Materials, Westborough, MA) that was photolithographically patterned on a Si wafer. Specifically, PDMS precursor (10:1 base to curing agent) was poured onto the SU-8 mold and cured at 65°C for 12 h. The PDMS device was then cut from the mold, and 4 mm-diameter reservoirs were punched at the ends of each of the two microchannels. The PDMS was then thoroughly rinsed with ethanol and dried with N₂. The PDMS channels and glass substrate with Au electrode were exposed to air plasma (PDC-001, Harrick Scientific, Ithaca, NY) at medium power for 60 s to activate the surfaces for permanent bonding. Second, a few drops

of ethanol were applied on the glass slide to delay bonding to the PDMS and to facilitate alignment of the electrodes to the microchannels. The device was aligned and baked at 65°C to drive off the ethanol and to encourage bonding. After 3 h of baking, the device was removed from the oven to apply silver paint (Cl 1001, Engineered Materials Systems, Delaware, OH) to the gold flag of the electrode design and then returned to the oven for at least 12 h. Prior to performing experiments, the Au electrodes were cleaned by filling the device with 50 mM H₂SO₄ and cycling the potential between the two leads over the potential range +1.00 V to -1.00 V with the initial and final potential as 0.00 V with a scan rate of 50 mV s⁻¹. The channel was subsequently flushed three times with double deionized water followed by a 10 min wait time which allowed the system to return to quiescent conditions. The device was then used for electrochemical measurements.

Electrochemical Measurements. Prior to electrochemical measurements, the sensing channel was filled with a sample solution composed of 0.5 M phosphate buffer and a 1:1 mixture of ferricyanide and ferrocyanide ($\text{Fe}(\text{CN})_6^{3-}:\text{Fe}(\text{CN})_6^{4-}$) at a concentration indicated for each experiment of the *Results and Discussion* section. The reporting channel was filled with ECL solution. Linear sweep voltammetry and chronoamperometry were performed on a Pine Research WaveDriver 10 Potentiostat (Durham, NC). Electrical contact to the two leads of the driving electrodes of the IDBPE device was made by applying copper tape coated with silver paint. The lead for the working electrode of the potentiostat was attached to the anodic driving electrode nearest the cathodic (interdigitated) pole of the BPE. A combined auxiliary and reference lead were connected to the cathodic driving electrode.

Linear sweep voltammograms (LSVs) were obtained over a potential range of +1.00 V to +3.50 V at a scan rate of 5 mV s⁻¹. In chronoamperograms (CAs), the potential was stepped from open circuit to a potential between +2.00 V and +2.50 V, as indicated in the experiments described in the *Results and Discussion* section, and then maintained constant for 10 s. A set of luminescence micrographs of the ECL response at the IDBPEs were recorded at 1 frame/s with an exposure time of 100 ms for the length of the experiment. In several chronoamperometric experiments as indicated in the *Results*, a voltage bias (ΔE_{tot}) was applied to the driving electrodes using a BK Precision 1687B power supply (Melrose, MA) instead of a potentiostat. Unless otherwise indicated in the text, all experiments were performed under quiescent (no flow) conditions in both the sensing and reporting channels. The ECL solution, in the reporting channel, was refreshed between individual experiments. This refresh involved removal of 20 μ L of ECL solution from one reservoir of the reporting channel to drive fluid flow during a 30 s wait time. This step was followed by replacement of 20 μ L of fresh ECL solution into the same reservoir. An additional 30 s wait time allowed the system to return to quiescent conditions.

To demonstrate that each IDBPE in the array responded independently, we performed a set of experiments, in which electroactive species were gradually introduced into the sensing channel. To achieve this goal the sensing channel of the device with 35 μ m gaps between microbands of the IDBPE was filled, initially, with 0.5 M phosphate buffer (and the reporting channel was filled with ECL solution). Then, a chronoamperometric experiment was begun. At $t = 10$ s following application of a potential step, 40 μ L of 0.5 M phosphate buffer in the inlet of the sensing channel was replaced with 40 μ L of 5.0 mM Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ in 0.5 M phosphate buffer. At $t = 20$ s, 20 μ L of solution

was withdrawn from the outlet reservoir. Also starting at $t = 20$ s, a series of luminescence images was recorded at 1 frame per sec (fps) over 1 min.

Luminescence Micrographs. A microscope (Nikon AZ100, Nikon Co., Tokyo, Japan) and a sCMOS camera (Andor Zyla 4.2, Oxford Instruments, Abingdon, Oxfordshire, England) were used to obtain luminescence micrographs in dark room conditions with 100 ms or 1 s exposure time. Nikon Imaging Software was used to process this imaging data. The signal obtained during CAs was analyzed as follows. First, the current measured at the driving electrodes during each CA at $t = 7$ s (following application of the potential step) was averaged across the trials. The ECL intensity was analyzed at the frame corresponding to $t = 7$ s to match. The ECL intensity was summed within a circular ROI over the reporting pole of each electrode (BPE anode) and then divided by the area of that pole.

3 Results and Discussion

Redox Cycling. Redox cycling occurs when two oppositely polarized electrodes are placed in close proximity and a species that undergoes a reversible electron transfer reaction is present. When the diffusion layer thickness exceeds the interelectrode distance, redox molecules reduced at the cathode diffuse along this concentration gradient to the anode, where they are subsequently re-oxidized. The result is an amplified current, and in the context of bipolar electrochemistry, the visible signal generated by ECL at the BPE anode will be increased proportionally. As has been amply demonstrated, as the spacing between the microbands decreases the degree of amplification increases.^{32–35} The following

IDE equation (Eq. 1) predicts the limiting current (i_{lim}) to be generated by an IDE.⁴⁸

$$i_{lim} = nmFDCb [0.637 \ln 2.55(1 + W/G) - 0.19(G/(W + G))^2] \quad \text{eq 1}$$

Where n and m are the number of electrons transferred per redox molecule and number of microbands of the IDE, respectively. F is Faraday's constant, D is the diffusion coefficient of the redox species, and C is its bulk concentration. The length of the microbands is b , while W and G refer to the width of the microbands and the edge-to-edge gap between them, respectively. This equation was derived under the assumption of semi-infinite diffusion of a single redox species to the electrode from the bulk solution. In the present study, the IDE is embedded in a microchannel having a depth of 50 μm , which equals the thickness of the diffusion layer at $t = 0.9$ s following a potential step, and therefore, our results deviate from the current predicted by eq. 1. However, the general trend is that i_{lim} increases exponentially with decreased G at fixed W . In this work, W is constant at 25 μm , however, G is varied (15, 25, and 35 μm). The amplification factor is defined as the ratio between the current (or the luminescent signal) obtained for smaller G to that obtained in a device with larger G .

In the current study, the amplification by redox cycling was achieved at an array of BPEs by interdigitating the cathodic end of each BPE with the anodic driving electrode. **Figure 1** illustrates the design of the resulting array which is composed of 20 individual IDBPEs. An individual IDBPE is illustrated in **Figure 1a** and includes 6 microbands, 3 of which extend from the driving electrode (anode) and 3 of which extend from the BPE cathode. The microbands are positioned in close proximity to facilitate redox cycling. ECL at the BPE anode reports the BPE current, and a cathodic driving electrode.

A brightfield micrograph of the device (**Figure 1b**) shows the alignment of the IDBPEs to overlying microchannels. One microfluidic channel (the 'sensing channel') overlies the interdigitated microbands and is filled with a solution containing the analyte to be sensed while a 'reporting channel' containing the ECL reagent solution encloses the circular reporting end of the BPE. **Figure 1c** is a micrograph of the reporting channel showing the luminescence that results at each BPE anode following application of a potential bias (2.0 V) between the driving electrodes when the sensing channel is filled with 3.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer.

Equivalency of IDBPEs within an array. A key assumption of this arrayed configuration is that all of the IDBPEs respond identically when exposed to a uniform analyte concentration. **Figure 2a** is a plot of the spatially averaged ECL intensity ($n \geq 6$) observed at the circular anode of each IDBPE obtained at four distinct concentrations (2.0, 3.0, 4.0, and 5.0 mM) of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer in a 25 μm -gap device. Prior to the first experiment on each device, 40.0 μL of ECL solution was pipetted into each end of the reporting channel and the lowest concentration of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ (2.0 mM) was pipetted into the sensing channel (40.0 μL per end of the channel). Then, a wait time of at least 10

min was allowed to ensure quiescent conditions in both channels. Finally, a 2.0 V potential bias was applied between the driving electrodes for 10 s and the current monitored while a video of the ECL response at seven of the IDBPEs was recorded at 1 frame/s and an exposure time of 100 ms. This chronoamperometric measurement was repeated six to eight times, after which the $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}$ solution was exchanged for the next concentration. An ECL solution refresh (as described in the *Experimental* section) was performed between each exchange of the $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}$ solution. The resulting ECL intensity, obtained at 10 s following each potential step, plotted in **Figure 2a**, shows uniformity across the array of IDBPEs. Within the device the standard deviation in ECL intensity from electrode to electrode (for each of the several $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}$ concentrations measured) ranged from 8–10%. For individual electrodes, from run-to-run, the standard deviation of the signal intensity ranged from 6–12%. The observed variation in results for each subsequent concentration is attributed to changes in the electrode surface occurring over multiple trials performed all in the same device. Most notably, byproducts of the ECL reaction led to the appearance of solid deposits on the BPE anodes.

Figure 2. (a) Plot of the average sum ECL intensity of each IDBPE of a 25 μm gap size device at four concentrations of $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}$ (2.0 mM red, 3.0 mM black, 4.0 mM blue, 5.0 mM green) in 0.5 M phosphate buffer, at 2.0 V applied potential ($n \geq 6$). (b) Bar graph of average sum ECL intensity of three representative 15 μm gap size devices in the presence of 2.0 mM $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}$ in 0.5 M phosphate buffer, showing an equivalent response in three separate IDBPE devices ($n \geq 3$).

An evaluation of the entire array of IDBPEs was achieved by performing three trials obtaining multiple images between which the microscope stage was repositioned. The order in which the IDBPEs were imaged was alternated in each successive trial. These results, plotted in **Figure S2** of the *Supporting Information*, show similar uniformity with the exception that the ECL intensity increased at the IDBPEs imaged last. These results demonstrate that the IDBPEs within an array are equivalent. This feature of the device is important because it allows for multiple sensing opportunities with a simultaneous single readout.

Next, to evaluate reproducibility we conducted experiments in three identical devices. **Figure 2b** is a plot of ECL intensity obtained in three separate 15 μm -gap IDBPE devices in the presence of 2.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer (averaged over 3 trials per device). The luminescent response was 0.728 counts μm^{-2} with a standard deviation of 0.011 counts μm^{-2} (1.5%), demonstrating low variability between the devices. This result is important because it allows for comparison of results from device to device. In combination, the results presented in **Figure 2** elucidate the reproducibility of these measurements and therefore lay a foundation for subsequent evaluation of the dependence of ECL signal on concentration, voltage, and interelectrode spacing (G).

Linearity of the response of the IDBPE array. Evaluation of the linearity of the response of the IDBPE array, in both ECL intensity and current, to analyte concentration, motivated the next set of experiments. First, linear sweep voltammograms (LSVs) were obtained to determine the potential bias that would provide the most stable response. We employed the same method as was described in the preceding subsection to infuse the sensing channel sequentially with increasing concentrations of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ and to refresh the ECL solution between trials. **Figure 3a** shows LSVs acquired at four concentrations of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ (2.0, 3.0, 4.0, and 5.0 mM) in 0.5 M phosphate buffer in the 25 μm -gap IDBPE device. The results demonstrate that current increases linearly with concentration and that a mass transfer limited current on the order of 100 nA per IDBPE (2.0 μA for 20 IDBPEs at 3.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$) is achieved for the redox reaction at approximately 2.0 V. In the absence of redox cycling, the quasisteady state current expected at the three microband ultramicroelectrodes of the BPE cathode 1.5 mM $\text{Fe}(\text{CN})_6^{3-}$ (oxidized form only) is approximately 50 nA. The inset of **Figure 3a** is the current sampled at 2.0 V during the LSVs, which exhibits a linear correlation ($R^2 = 0.99$) with $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ concentration. Based

Figure 3. (a) LSVs obtained in a 25 μm -gap device at four concentrations of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ (2.0 mM solid, 3.0 mM fine dash, 4.0 long dash, 5.0 mM dot dash) in 0.5 M phosphate buffer, in the range of 1.0 V to 3.0 V. Inset: Plot of current obtained at 2.0 V versus $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ concentration. (b) Bar graph of average ECL intensity obtained in a 25 μm -gap device at the same four concentrations (as in (a)) at $t = 7$ s following a step in potential to 2.0 V ($n=6$). Inset: Linear fit to this ECL response. (c) Plot of average ECL intensity when voltage is applied from a standard DC voltage power source, in a 25 μm -gap device in the presence of 3.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer. Applied DC voltage ranged from 2.0 V to 2.5 V and the ECL response was recorded for 30 s with a 1 s exposure time and average at $t = 7$ s ($n = 3$).

on these results, a bias of 2.0 V was selected for subsequent chronoamperometric experiments.

Next, CAs were obtained at the same four concentrations of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in a 25 μm -gap device. A potential bias of 2.0 V was applied for 10 s at the driving electrodes, and both the current and ECL intensity were monitored. A video of the ECL response at eight of the IDBPEs was recorded at 1 frame/s and an exposure time of 100 ms. The ECL intensity was analyzed as previously described in the *Experimental* section. This chronoamperometric measurement was repeated six times, after which the $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ solution was exchanged for the next concentration and the ECL solution was refreshed (as described in the *Experimental* section). A linear response in current was obtained (**Figure S3**). **Figure 3b** is a plot of the ECL intensity averaged over 6 trials. The inset of **Figure 3b** shows that a linear fit of these data exhibits $R^2 = 0.97$ (inset, **Figure 3b**). This direct proportionality of ECL intensity to concentration (and to current) is important because it indicates that these experimental conditions are appropriate for characterization of redox cycling and for application of this device to sensing.

Finally, we evaluated the reproducibility of the ECL signal as a function of applied voltage bias. **Figure 3c** is a plot of ECL intensity (averaged over 3 trials and over 8 IDBPEs in each trial) obtained in response to a potential step from open circuit to each of a series of voltages between 2.0 V and 2.5 V. This range of applied voltages was selected to fall within the mass transfer limited regime observed in the LSV (**Figure 3a**). These trials were carried out in a 25 μm -gap device with a sensing channel containing 3.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer. The experimental procedure for infusion of the $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ and ECL solutions was performed as described in preceding paragraphs; however, a standard DC power supply was used instead of a

Figure 4. (a) Brightfield micrograph of 3 gap size (left to right, 15 μm , 25 μm , 35 μm) IDBPE devices. (b) Plot of the current produced during CAs of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer, at $t = 7$ s following a step in potential to 2.0 V ($n=3$), in 15 μm (black) gap size device (0.5 mM, 1.0 mM, 2.0 mM, 3.0 mM), 25 μm (red) gap size device (1.0 mM, 2.0 mM, 3.0 mM, 4.0 mM, 5.0 mM), 35 μm (blue) gap size device (3.0 mM, 4.0 mM, 5.0 mM). (c) Plot of the average sum ECL intensity of 3 gap size (15 μm , 25 μm , 35 μm) IDBPE devices of various concentrations of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer, at $t = 7$ s following a step in potential to 2.0 V ($n=3$). Concentrations by gap size device included, 15 μm (black) gap size device (0.5 mM, 1.0 mM, 2.0 mM, 3.0 mM), 25 μm (red) gap size device (1.0 mM, 2.0 mM, 3.0 mM, 4.0 mM, 5.0 mM), 35 μm (blue) gap size device (3.0 mM, 4.0 mM, 5.0 mM).

potentiostat. The data of **Figure 3c** show that ECL signal is constant over this range of voltages (cumulative standard deviation, 0.007 counts μm^{-2} , (0.2%)) but is more variable at 2.5 V (0.5%) than at 2.0 V (0.2%). These results indicate that a simple power supply or even a coin cell battery can be employed to operate this sensor array and that deviations of several hundred millivolts in applied voltage can be tolerated. This versatility allows for the IDBPE device to be adapted to point-of-need applications where a multiplex signal, simple setup, and ease-of-use are required.

Signal amplification at an array of IDBPEs through redox cycling.

Signal amplification was a primary goal of this work. As previously

discussed, it was anticipated that decreasing the gap size between the microbands of an individual IDBPE would amplify the signal. **Figure 4a** is made up of three brightfield micrographs showing, from left to right, one 15 μm -, 25 μm -, and 35 μm -gap IDBPE (from separate devices). In all three cases, the width of the microbands is 25 μm . **Figure 4b and 4c** show the dependence of current and ECL intensity, respectively, on the concentration of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in IDBPE devices at each of these three gap sizes ($n = 3$). CAS were obtained as previously described using a potentiostat, and in each trial, the current and luminescence intensity were averaged at $t = 7$ s following a potential step to 2.0 V. **Figure 4b and 4c** show that current and ECL intensity increase as the gap size decreases. As predicted by eq. 1, at 3.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ where all three gap sizes are represented, ECL intensity increases logarithmically with decreasing gap size.

The greatest amplification in ECL signal is observed between the 35 μm - and 15 μm - gap IDBPEs at 224% (2.24-fold relative amplification). These results demonstrate amplification by redox cycling at an array of BPEs through their interdigitation with a shared driving electrode. This outcome is significant because this is a universal strategy to lower the limits of detection of BPEs for reversible redox species. This enhanced sensitivity opens a pathway to more diverse applications of BPEs. Interdigitation can broaden the type of biomarkers that can be sensed by BPEs at the single-cell level – for example, electrochemical enzyme linked immunosorbent assay of surface antigens on tumor cells produced a signal equivalent to 0.1 mM redox active product per cell within a ~ 1 μL microfluidic chamber.⁵⁰ Further, in instances where IDEs are used currently for amplification, such as in the detection of extracellular vesicles,⁴⁹ BPEs would afford facile multiplexing and spatial resolution.

Independence of IDBPEs. The final goal of this work was to demonstrate that each IDBPE of the array responds independently. **Figure 5a** shows a series of micrographs of the ECL response at the anodes of the eight IDBPEs nearest the two inlets of the device (left-hand side, **Figure 5a**). These images were obtained in a 35 μm -gap device under dark room conditions at four separate time points following introduction of an electroactive analyte to the sensing channel. To obtain these conditions, first, the reporting channel was filled with ECL solution and the sensing channel with

Figure 5. (a) Micrograph of the IDBPE array under dark conditions, where 5.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer was gradually added to the sensing channel of a 35 μm gap size IDBPE device filled with 0.5 M phosphate buffer, micrographs at time periods of t_1 (9 s), t_2 (10 s), t_3 (11 s), t_4 (12 s). (b) Plot of an IDBPE array line trace responses where 5.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer was added to the sensing channel of a 35 μm gap size IDBPE device filled with 0.5 M phosphate buffer, micrographs at time periods of 9 s (black), 10 s (red), 11 s (blue), 12 s (green) recorded at 1 s exposure time.

0.5 M phosphate buffer. After quiescent conditions were obtained by a 10 min wait time, a potential bias of 2.0 V was applied at the driving electrodes. After 10 s, 40.0 μL of 0.5 M phosphate buffer was removed from the inlet of the sensing channel and replaced by 40.0 μL of 5.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ in 0.5 M phosphate buffer. After an additional 10 s, 20.0 μL of solution was withdrawn from the outlet of the sensing channel to induce fluid flow towards the outlet (left to right, **Figure 5a**). Simultaneously, video capture at 1 fps was initiated (defined as $t = 0$). A key point is that the ECL solution in the reporting channel remained quiescent.

The frames shown in **Figure 5a** were obtained 9 s (t_1), 10 s (t_2), 11 s (t_3) and 12 s (t_4) after initiating flow in the sensing channel. At t_1 , ECL was observed at the anodes of the two IDBPEs nearest the device inlets while the rest of the array remained dark. As time progressed (t_2 to t_4) each subsequent IDBPE produced a luminescent response. These observations can be explained as follows. Prior to introduction of $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$, no redox active species were present in the sensing channel, and therefore, no luminescence was observed in the reporting channel. As the leading edge of the $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ solution reached each IDBPE cathode, faradaic reactions were initiated and an ECL response became visible at each IDBPE anode in turn (**Movie S1**). **Figure 5b** shows line traces taken across the midpoint of the IDBPE anodes at t_1 to t_4 . When plotted, the gradual increase in luminescence becomes apparent and underscores the spatiotemporal resolution of the array. This result is important because the independence of the response of the individual IDBPEs allows for time-resolved multiplexed biosensing or spatial mapping.

4 Conclusions

In this study, an array of BPEs was interdigitated with a shared driving electrode to achieve redox cycling. The resulting IDBPE array allowed for signal amplification while retaining the advantage that the entire array of BPEs can be operated with a single power supply. Importantly, with the IDBPE device we demonstrated (i) an equivalent luminescence signal under uniform conditions across the IDBPE array, (ii) a linear response over a wide range (500 mV) of applied voltages, (iii) increased sensitivity with a relative amplification of 224% when decreasing the gap size from 35 μm to 15 μm , and (iv) an independent response from each individual IDBPE of the array. These are each critical

features of multiplexed or spatially resolved biosensors. Ongoing research in our group is focused on developing this IDBPE device further for array-based analysis of individual tumor cells. We are also further reducing the gap size of the IDBPEs to enhance sensitivity. It is anticipated that with a gap size of 100 nm, we will bring the LOD into the micromolar range, which is relevant for a wide range of direct and indirect biosensing approaches.⁴⁹

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