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Joshua Miller MEMS Independent Study Report – Fall 2019

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Abstract

μ-BEC:

The study of microbes performing extracellular electron uptake (EEU) has multiple uses for biotechnological applications. To that end, a high-throughput platform comprising an array of bioelectrochemical cells (essentially small chambers for electrochemical analysis of biological cells) is necessary to provide the opportunity for proper study of microbes performing EEU. Utilizing traditional cleanroom microfabrication techniques (photolithography, metallization, and lift-off), a 16-chamber micro bio-electrochemical cell (μ-BEC) array was fabricated. Each cell utilized a 3-electrode system, achieving successful electrochemical measurements for 8 of 16 chambers. The 8 chambers that failed exhibited peeling of electrodes due to processing errors. More robust fabrication processes are being explored to improve yield.

IDTs:

The field of microscale acoustofluidics is extremely useful for numerous bioanalytical applications involved with studying swimming cells including bacteria. To that end, an experiment was performed using acoustic focusing devices that produce standing surface acoustic waves (SSAWs) excited in a piezoelectric substrate by micropatterned interdigital transducers (IDTs). These waves were used to trap bacterial cells at nodes and antinodes of the SSAWs, creating visible

patterns. Experiments proved that 2 μm average diameter swimming cells can be successfully patterned with a 24.1-MHz actuation frequency.

Introduction & Background

μ-BEC:

Extracellular electron uptake (EEU) is the ability of some microbes to directly take up electrons from solid-phase conductive substances, or SPCSs, for use in electron transfer, electron transduction, and CO_2 fixation. During this process, the cells perform microbial oxidation-reduction reactions while attached to the surface of the SPCS. EEU has been linked to photosynthetic electron transfer, and understanding this natural phenomenon has numerous uses for biotechnological applications. In the proposed project, EEU was to be investigated using a novel high-throughput platform comprising arrays of micro bio-electrochemical cells (μ-BEC) that could allow for sequential and/or simultaneous electrochemical, imaging, and spectrometric analyses of the microbes and EEU processes. Current methods of studying EEU by these microbes suffer from: i) low throughput that precludes acquisition of sufficient data to achieve statistical relevance in study outcomes and ii) an inability to image EEU processes at the microscopic scale due to the use of bulk (macroscale) reactors that output only aggregate electrochemical data. Microfluidics enable researchers to address both shortcomings, while also providing the unique capability to flush out all non-surface-attached microbes, isolating only those microbes actively performing EEU on SPCSs and reducing noise.

Photolithography and metallization were used to create the desired 3-electrode pattern on the μ-BEC device. Photolithography is a three-step process involving an ultraviolet light sensitive polymer called photoresist: (1) the photoresist is spread over the surface of a substrate via spin coating, (2) the photoresist is preferentially exposed to UV light to create the desired pattern, and

(3) the photoresist is placed in a developing solution that selectively washes away the exposed photoresist (assuming use of a positive photoresist). A 2-step LOR10B and S1805 photolithography process is shown in Fig. 1. Photolithography is followed by metallization, where metal is deposited onto the substrate surface and subsequently removed with dissolved unexposed photoresist from unwanted areas in a process called "lift-off". Again, the lift-off process is detailed in Fig. 1.

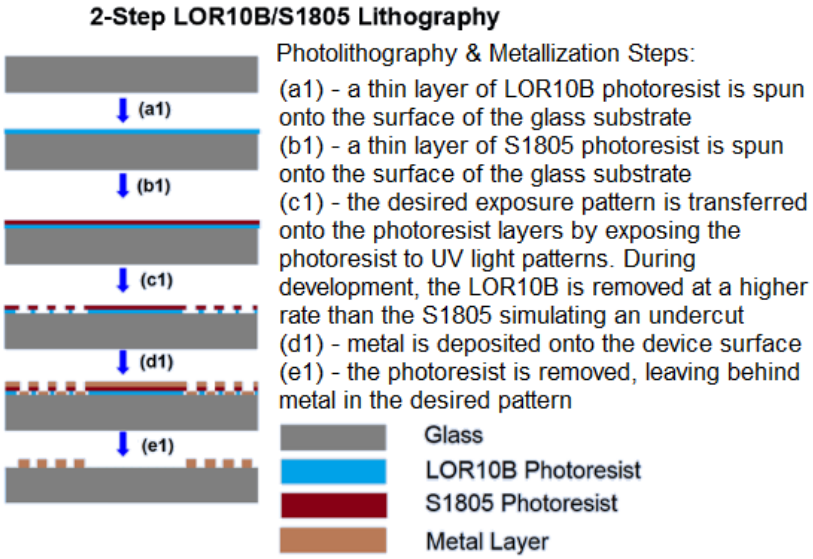

Figure 1: 2-Step lithography and metallization/lift-off processes.

IDTs:

Microscale acoustofluidics is an effective method for trapping and analysis of how motile cells swim. A primary method to perform this manipulation is via standing surface acoustic waves (SSAWs), which arise due to constructive/destructive interference of acoustic waves traveling along a surface. In the field of microscale acoustofluidics, this actuation relies on generating acoustic waves in piezoelectric materials. SAW devices incorporate interdigital transducers (IDTs) that diffract SAW through fingers to create a field of parallel wave fronts called a Fresnel region,

and further, a broadly diffracted region called a Fraunhofer region. A sample IDT design is shown in Fig. 2.

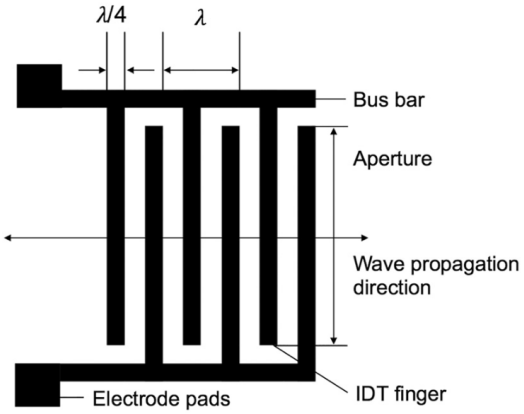

Figure 2: Typical IDT design.[1]

To minimize losses in the Fresnel and Fraunhofer regions, it is desirable to contain the aperture within the Fresnel region. To ensure that the aperture is contained in the Fresnel region, it is necessary that $F<1$ in the following equation:[1]

$$F = \frac{4\lambda D_f}{a^2} \tag{1}$$

Where D_f is the distance to the IDT edge, a is the aperture width, and λ is the SAW device wavelength. In the field of microscale acoustofluidics, SAW propagate along a substrate until they encounter a fluid droplet or enclosed fluidic channel, into which the SAW leak, diffracting and forming standing waves or driving fluid recirculation. Alternatively, the wave sheds into a microchannel forming a standing wave pattern if a pair of IDTs are made to produce counter-propagating SAW that meet within the channel. My goal was to run an experiment examining acoustic wave patterning of cells using SSAWs that are induced by IDTs fabricated on a lithium niobite substrate. Here, a radio frequency (RF) signal is applied to two IDTs to produce individual SAW that constructively/destructively interfere with each other, resulting in SSAW and a periodic distribution of pressure nodes and anti-nodes within a straight microchannel. SSAW encounter a

liquid medium and leak into the liquid creating pressure fluctuations throughout. The resulting pressure field scatters off of particles (or cells) generating acoustic radiation forces that push the suspended microparticles toward either the nodes or anti-nodes.[2]

Procedure

μ-BEC:

To create a higher-throughput μ-BEC device, standard microfabrication techniques were first utilized to manufacture a 16-chamber (3-electrodes per chamber) μ-BEC platform on a 500-μm thick, 150-mm diameter glass wafer. This wafer was then bonded to another wafer with 100-μm deep wet-etched microfluidic channels. To create the 3-electrode system, multiple lithography and deposition steps had to occur in series, with extra care taken to ensure proper electrode alignment. A two-step LOR10B / S1805 lithography process was used to create features with undercuts that assist in removal of unwanted material (or "lift-off") following a "bottom up" approach (lithography, metallization, lift-off). This procedure was repeated four times, first to create chromium (Cr) alignment marks and then for each 50-nm thick electrode layer: i) the working electrode in indium tin oxide (ITO), ii) the counter electrode in platinum (Pt), and iii) the reference electrode in silver (Ag). Working and counter electrodes were deposited over 10-nm thick Cr adhesion layers. Microchannels were wet-etched into the other glass piece using a mixture of hydrofluoric acid, nitric acid, and deionized water. Inlet/outlet holes were drilled into the channel wafer. The channel and electrode wafers were bonded together using a stamp-and-stick method with SU-8 photoresist. The bonded wafers were then sectioned into individual multi-chamber devices and fitted with a 3D printed cover for holding the devices on the microscope stage. Initial electrochemical demonstration experiments were performed by biological collaborators in the Bose laboratory.

IDTs:

For the microscale acoustofluidics experiment, a function generator and amplifier were attached to an IDT-patterned lithium niobite chip bonded to a 545-μm wide glass channel. These channels were introduces to the IDT chip via pipette (cells were provided by biological collaborators in the Bose laboratory). Before the cell solution was loaded into the microfluidic flow device, they were centrifuged, concentrating the solution to about 5x its original concentration, to improve the clarity of and ability to see the cells testing. Various frequencies and voltages were applied to the IDTs, and cell patterning was observed via brightfield microscope imaging.

Analysis

μ-BEC:

In fabricating the devices, numerous equipment failures were encountered. Because of this, the quality of the ITO deposition did not yield a transparent electrode as desired (note this has since been addressed by PhD mentor Stoica). Furthermore, due to issues with the physical vapor deposition machine, the counter electrode exhibited poor adhesion. The lithography process used had a resolution of around 2–5 μm, which was sufficient but could be improved via further process refinement. Additionally, the height of the electrodes was not optimized as no minimum height requirement for a functional electrode was established. Otherwise, quality of the fabrication processes was adequate, although it is anticipated that additional process development time will allow for better process control in all deposition steps, leading to better quality electrodes.

IDTs:

For this experiment, numerous things could have been improved for data collection. First, the microscope lens was blurry, limiting the image resolution possible. Additionally, the

swimming cells were not particularly easy to see, so some sort of fluorescent tracers would have been helpful. The data collected was otherwise good, and despite some sticking at wall, the cells were able to be focused.

Results

µ-BEC:

Of the 16 electrode units fabricated for the µ-BEC device, 8 produced a proper electrical signal. The remaining 8 electrodes failed, as the platinum counter electrodes peeled from the surface of the device due to poor adhesion. The device, prior to being placed in its holder, is shown in Fig. 3 below.

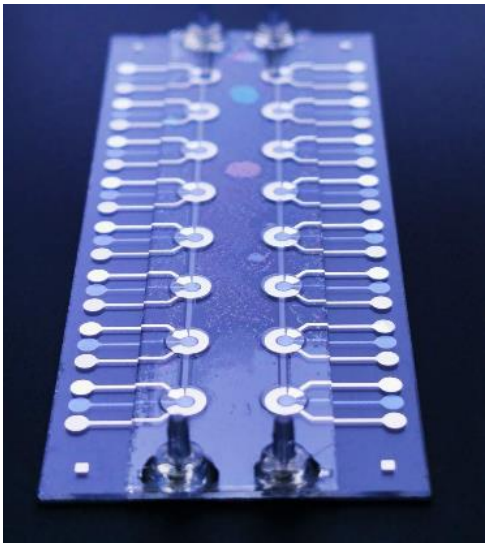

Figure 3: 16-chamber µ-BEC device.

For the working electrodes, the ITO was not fully transparent, and thus, while it was functional as an electrode, it may not be used for transmitted light microscopic imaging of microbes.

IDTs:

In the SSAW patterning experiment (see Fig. 4 below), swimming cells were focused into relatively straight lines when actuated. The gradient in focused population density is likely due to channel geometry and (potentially swimming-induced) flow characteristics.

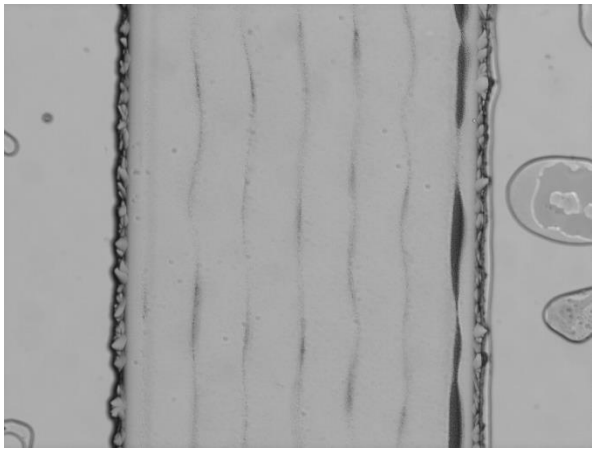

Figure 4: Focused swimming cells in an acoustic field driven at 24.1 MHz.

Discussion

µ-BEC:

The outcome of the initial 16-chamber µ-BEC testing demonstrates that with a bit of parameter adjustment a workable device can be manufactured with the protocols used. As a proof-of-concept, the µ-BEC is largely successful and demonstrates the potential scalability of the manufactured design. Ultimately, given more time, reliable equipment, and better-characterized processes, the imperfections in the electrodes can be corrected, and optical and electrochemical properties will be of the quality necessary to facilitate testing on microbes performing EEU.

IDTs:

As seen in Fig. 4, the focusing experiment showed that swimming cells of an average diameter of 2 µm can be effectively pushed into patterns by SSAWs. The cell size made imaging difficult until strong focusing was achieved. Further, the cells were seen to continue to flow even

when trapped at nodes and antinodes of the SSAWs. This flow could be due to a pressure gradient in the channel or possibly to an induced flow occurring from the cells themselves, as the swimming of densely packed cells can induce a flow. An additional way to improve the experiment would be to use tracers to better track cells (if bound to cells) or the bulk flow, but ultimately the experiment successfully demonstrated SSAW driven patterning of swimming cells at around 24.1MHz.

Conclusion

μ-BEC:

Ultimately, fabrication of the μ-BEC platform served as a useful proving ground for the viability of traditional microfabrication techniques in creating a high-throughput and versatile bio-electrochemical testing device. By improving processing equipment and characterization of materials, the device can be easily improved both in performance and cost, as well as to enable imaging through optically transparent ITO working electrodes.

IDTs:

The IDT focusing experiment demonstrated that properly actuated IDTs will produce a strong enough SSAW to trap swimming cells in the 2-μm diameter size range. These traps worked well enough to produce noticeable patterns that corresponded to nodes and antinodes of the SSAWs. While the imaging setup was not ideal for observing such small cells, the resulting images was clear enough for proof-of-concept. Again, this experiment could be improved via the addition of tracers, allowing us to better image the flow and to see individual cell behaviors.

References:

1. Connacher, William, et al. "Micro/nano acoustofluidics: materials, phenomena, design, devices, and applications." *Lab on a Chip* 18.14 (2018): 1952-1996.***Lab Chip***, 2020.
2. Shi, Jinjie, et al. "Focusing microparticles in a microfluidic channel with standing surface acoustic waves (SSAW)." *Lab on a Chip* 8.2 (2008): 221-223.