The Entrapment and Rotation of Cells using Dielectrophoresis

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Abstract:

Dielectrophoresis in microfluidics offers an inexpensive, yet an efficient, method in separating particles. This method induces motion of dielectric particles selectively in a non-uniform electric field. We designed a simple device using printed circuit board that allowed electric current to flow through the electrodes of the device. We applied an electromagnetic field by using voltages from 5V to 40V and frequencies of 10Hz, 30Hz, and 300Hz according to our calculations using MATLAB and theoretical simulations using COMSOL Multiphysics. Micro-beads, similar to spherical cells, were used for initial testing in this research, and the goal was to gather the particles to the center of the electromagnetic field. Once this process can be replicated reliably and accurately, experiments on living cells will be carried out.

Introduction

A patient's chances for recovery are closely related to how soon the diseases can be detected; therefore, an efficient method in detecting diseases is needed in the medical field nowadays[1-5]. The application of electromagnetic fields for entrapments and electro-rotation of cells is one method being explored. The idea is that cells of different sizes resonate at different electrical frequencies. In other words, the selected cells would separate from other cells and move toward the center of the electromagnetic field using dielectrophoresis. With time and refinement, this research could be used for detecting illnesses by separating the diseased cells from the healthy ones, which allows the medical professionals respond to diseases quicker and increases the patient's chances for recovery.

Many different types of methods had been tried for the entrapment and electro-rotation of cells in other research since finding the most reliable and efficient method and design is essential. Dielectrophoresis, used by many research, offers an inexpensive method in separating particles selectively and effectively by inducing motion of dielectric particles in a non-uniform electric field[4]. When exposed to an electric field, electrical charges are induced in the cells. The generation of dipole allows for movement of the cells along a non-uniform electric field based on their polarizability. With two electrodes at 180 degree angles to one another, the cell will move either toward the electrodes or toward the center depending on the conductivity and permittivity (Figure 1a). While in uniform e-field, the cells would receive a net force of zero. In other words, the net force would not be zero when the e-field is not uniform. Since the cells are at a distance away from the surface of the electrodes, the e-field that they receive would be non-uniform. The force received by the particles at different position would vary in a non-uniform e-field. When cells are captured by the e-field, a reliable means of stimulating rotation must be employed. This can be accomplished by employing four electrical signal phases at 90 degree angles to one another to create an electromagnetic field that will push the entrapped cells into the center and stimulate rotation as shown in Figure 1b. In some experiments, researchers introduced nano- and micro-particles such as iron oxide, gold and silicon to enhance effectiveness of the electromagnetic field[5,6]. The main point in these experiments was to observe and analyze the sample particles by entrapment.

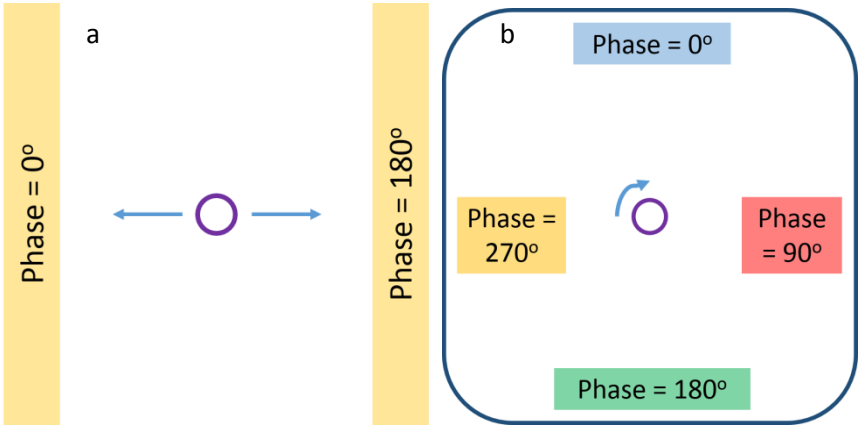


Figure 1: A conceptual image of the suggested process for the cells viewed from the top; a) a negative force was generated from the two electrodes to maneuver cells toward the electrodes, b) with four phases active to create the e-field to entrap and rotate a cell[3, 4].

In order to proceed to the actual entrapment of cells, this research focused on directing the movement micro-beads by moving the particles toward the electrodes as shown in Figure 1a. Once this process can be done reliably, living specimens would then be introduced, and finally, the entrapment and rotation of non-spherical cells similar to Figure 1b. The design in Figure 2 was used in this research to direct particles in a certain direction by introducing AC current to both electrodes. This design would be discussed further later.

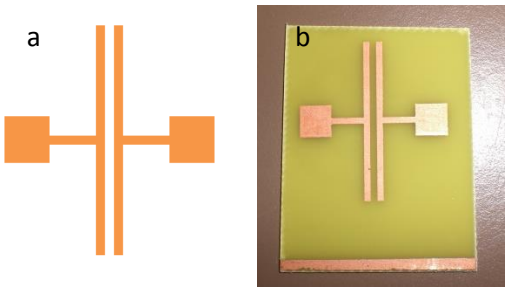


Figure 2: a) The basic design using PowerPoint, and b) the actual device used in this research, where the two vertical lines are the two electrodes. AC current would be applied to both electrodes to generate an electromagnetic field.

When the non-charged particles are between and above the two electrodes, they would sense dielectrophoretic (DEP) force from the alternating current. This non-uniform electric field helps to induce polarization and motion of the particles. The DEP force acting on a spherical cell can be found from the following relation:

$$F_{DEP} = \frac{3}{2} v_{cell} \varepsilon_{med} \, \mathrm{Re}[K_{CM}^{*}(f)] \nabla E^{2} \quad . \tag{1}$$

with v_{cell} being the volume of the cell and E the "root-mean-square" value of the external electrical field, and K_{CM}^{*} the complex Clausius-Mossotti (C-M) factor[2].

C-M factor depends on the complex dielectric constants of medium (ε_m^*) and particle (ε_p^*)[7],

$$[K_{CM}^{*}(f)] = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{2}$$

and the expression of complex dielectric constants is given by:

$$\varepsilon^* = \varepsilon - \frac{i\sigma}{2\pi f} \tag{3}$$

where ε is the permittivity, σ is the conductivity, f is the frequency of the applied electric field, and i is the imaginary number[2].

Therefore, the C-M factor relies on both the permittivity and conductivity of the medium and the particle as well as the frequency applied. These variables have the potential to change the sign and the magnitude of the C-M factor, which would inverse the direction and reduce the magnitude of DEP force. Ideally, the electric field gradient would be similar to Figure 3, where the DEP force is positive and points toward the middle of device, when $\sigma_m < \sigma_p$ and $\varepsilon_m > \varepsilon_p$. However, the DEP force would result as a negative force when $\sigma_m > \sigma_p$ and $\varepsilon_m < \varepsilon_p$, and the electric field gradient would be pointing away from the center.

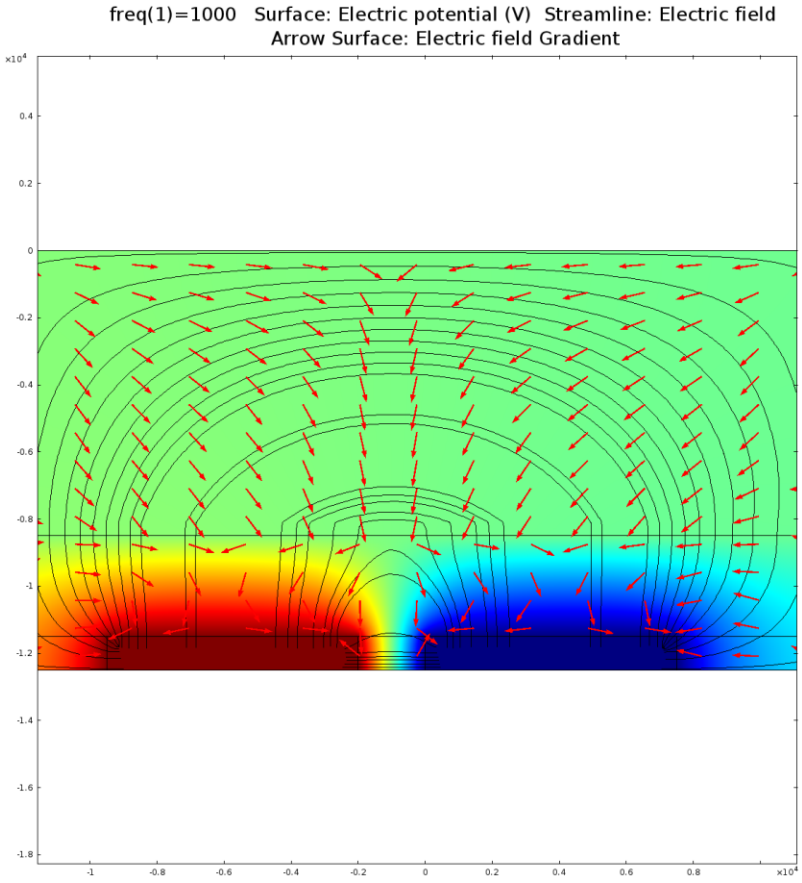


Figure 3: A simulation of a positive DEP force generated by COMSOL that shows the direction of the electric field of a device with a voltage of 15 V and a frequency of 1000 Hz using a low conductive medium.

The strength and direction of DEP force depends on the electric field gradient ∇E. The figure above shows the directions of the DEP force pointing toward the center of the two electrodes. As a result, the particles move towards the center of the design, where the largest magnitude of electrical field is located.

Device design

Previously, electrodes were made using a copper-coated glass slide by the deposition of copper onto a standard glass microscope slide at a thickness of 91 nm. The deposition process was carried out in the physics department on Central Michigan University's campus.

With this method, however, the copper was not evenly distributed throughout the slide. Light was able to penetrate spots on the coating when viewed with a light microscope. Since the thickness of the copper layer was thin, it was very easy to remove the copper layer, even by scratching. Some copper was also removed during photolithography. With the copper layer distributed unevenly and leading to the problems listed above, the electrical field cannot be generated properly; thus, it is not suitable for our research.

Printed circuit boards (PCBs) was used as an alternative for electrode devices because a thicker layer of copper (about 30 μm thick) was available. Although there were still some tiny spots without copper, the copper layer was much more uniformed. However, the width of the electrodes on a PCB was not as thin as the copper-coated slide. The width of electrodes of the copper-coated slides was 508 μm, and this width was impossible to attain on a PCB.

The original device was designed with 5 to 4 electrodes "fingers" as shown in Figure 4a. In order to simplify the device and increase the width of electrodes, we changed the device with only two electrode "fingers" with a width of 1 mm shown in Figure 2. In addition to the design, the PCBs cannot be viewed with a light microscope, where the light source of the microscope is located at the bottom of the device. The light source could not penetrate the plastic layer of the PCBs, and it was difficult to focus on the device. Therefore, a top-lighting compound microscope was used to observe and capture pictures on the PCB device. However, since the top-lighting compound microscope do not have much depth of focus, it is important to keep the total thickness of device as small as possible. The device consisted of: the PCB layer at the bottom, a polydimethylsiloxane (PDMS) polymer channel that was 1 mm thick, where the micro-beads were introduced, and a coverslip on top with thickness of about 130 μm that would seal off the channel as shown in Figure 5.

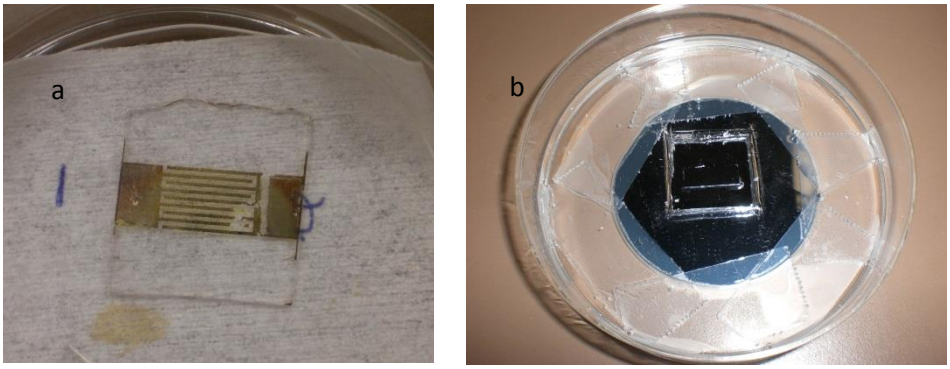


Figure 4: a) The original design of the electrodes device on a glass slide, where copper layer was destroyed during etching. b) The mold used to create channels for this research.

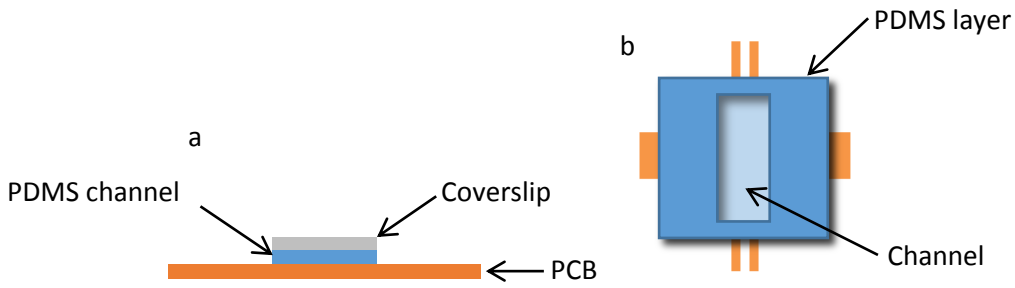


Figure 5: The device used in this research with; a) cross-sectional view, and b) top view without coverslip.

Fabrication of the electrodes

The electrodes were fabricated through photolithography and wet etching of PCBs, which were originally coated with copper layer and photoresist layer by CamCon Chemical. The photoresist layer for the PCBs is a negative photoresist. The exposed regions of the negative photoresists would harden after exposing in UV light and become much more resistant to developer. The unexposed regions would be removed during photolithography, and the mold shape would be left intact. The mold of the device was created using Microsoft PowerPoint and was printed on transparency film. The mold was then placed on top of the Pressmatch DTP Ultra Violet (UV) exposure frame, and the exposure would happen in a dark room. The untreated PCBs were placed on top of the transparency flim with the photoresist layer facing towards the UV bulbs. The samples were exposed in UV light for 45 seconds and were left in exposer for 15 minutes before removing the samples.

Sodium carbonate (Na_2CO_3) was used to develop the photoresist. 1 g of Na_2CO_3 was dissolved in 100 mL of deionized water to make up the developer solution. The solution was heated up to about 50°C on a metal hot plate. Each sample was submerged in the developer solution until the photoresist layer was removed, except the photoresist of the UV-exposed region. Each sample was rinsed thoroughly with deionized water and dried before the etching take place.

Sodium persulfate ($Na_2S_2O_8$) was used as the etchant, where 25 g of $Na_2S_2O_8$ was dissolved in 100 mL of deionized water. The etchant was heated up to around 60°C, and the samples were placed in the etchant until the copper that was not covered with photoresist was completely removed. Then, each sample was rinsed with deionized water and dried. Since the photoresist of the exposed regions remained on the PCBs, the PCBs were placed in the developer solution (heated to 50°C) again to remove the remaining photoresist to expose the copper circuit as shown in Figure 2b. The PCBs were then cleaned with DI water and put in a sonicator for approximately three minutes to remove any trace of photoresist.

Fabrication of PDMS channel

Before the PDMS channels could be fabricated, the mold of the channels had to be made first. The mold in this research was created using a negative photoresist, SU-8. SU-8 has a high

viscosity and would function as a suitable mold. Photolithography and wet etching were again used to create the PDMS channels.

A 2-inch diameter silicon wafer was cleaned with DI water, isopropyl alcohol, and acetone, and it was then centered onto the spin-coater. A pipette was used to apply a sufficient amount of SU-8 to the wafer. Due to its thick nature, the SU-8 needed to be spread out with a spatula to ensure an even coating. Once ready, the spin coater was set into two cycles. The first cycle was 500 rpms for 5 seconds, and the second was 3000 rpms for 30 seconds. After both spin cycles, the wafer underwent two hotplate treatments to prepare it for exposure in the mask aligner. The sample was first set on a hotplate set to 65°C for five minutes and then allowed to cool; it was then placed on a hotplate set to 95°C for 20 minutes and then allowed to cool to room temperature. The wafer would be ready for the exposure process after the two treatments.

The wafer was exposed in the mask aligner for a time interval of 10 seconds. After the exposure had completed, the sample wafer would receive a post-bake on the hot plates with the same temperatures—5 minutes at 65°C and then 20 minutes at 95°C. Once the molds had been cooled down to room temperature, wafer was immersed in the SU-8 developer for 30 minutes and rinsed with DI water and isopropyl alcohol. After the development process had completed, the mold was hard baked on a hot plate set to about 200°C for 10 minutes. This was done to remove any lingering moisture that may have remained within the mold. This technique was also useful for correcting minor structural defects in the SU-8. Figure 4b shows the fabricated mold used in this research.

The PDMS channels was made with PDMS curing agent and PDMS in a 1/10 ratio. The curing agent and PDMS were mixed thoroughly until bubbles were formed in the solution. The solution was left in fume hood to remove the bubbles. When all bubbles were removed, the PDMS solution was poured onto the mold made with SU-8. The mold would be left in the fume hood overnight. At this point, the PDMS was still a thick solution and uncured. The mold and PDMS were then heated at 65°C for one hour to cure the PDMS. The PDMS would be hardened up and turned into a rubber like structure after heating and would be able to remove by cutting.

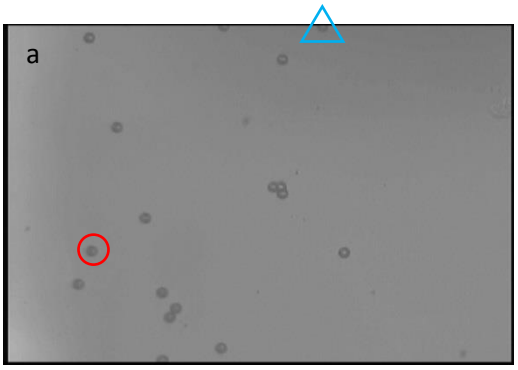
Methods

With the device set under the top-lighting microscope in the clean room showed in Figure 6, AC current of different voltage and frequencies were applied. For early trials, 5 V was applied with frequencies of 10 Hz, 30 Hz, and 300 Hz. These frequencies were determined using calculations from MATLAB and COMSOL Multiphysics. The particles were moving towards the two electrodes at a slow speed because the conductivity of the solution was higher than the conductivity of the micro-beads. Calculations showed that the DEP force of a high conductive medium between a high frequency (~1 MHz) and low frequency (~10 Hz) would be about the same magnitude. Instead of trying to reduce the conductivity of the solution, the conductivity was increased by adding one drop of sodium chloride solution to the channel. This caused the magnitude of the DEP force increase but remain as a negative force that push the particles away from the center and toward the electrodes. After a drop of sodium chloride was added to the solution, 5 V to 40 V with frequencies of 10 Hz, 30 Hz, and 300 Hz were tested. Two wires

where added and applied the same current and voltage on top of the coverslip to increase the DEP force and speed of particles.

Results

After the conductivity of the solution was increased, the DEP force had increased. Movements of certain micro-beads were more observable. Figure 7a through 7e were taken from one of the trials that offered a clear view of the moving particles. From Figure 7a through 7e, two particles moved toward the left electrode and stopped when they reached the edge of the channel. Red circles and blue triangles were added after the pictures were taken to help visualize the movement of the particles. Note that the particles move diagonally toward the white spot on the left (the copper electrode), and the particle circled with a red circle remain stationary once it reached the edge at the 6-minute mark. The particle circled with a triangle started from the top and ended at the bottom left hand corner when it was about 13 minutes after the observation began. While the two particles, shown in Figure 7, moved, the rest of the particles remained stationary. The same situation occurs to trials using frequencies of 30 Hz and 300 H, where some particles remained stationary. The speed of particles with different frequencies showed no significant change. There was no difference in speed by applying different voltage of the same frequency as well.



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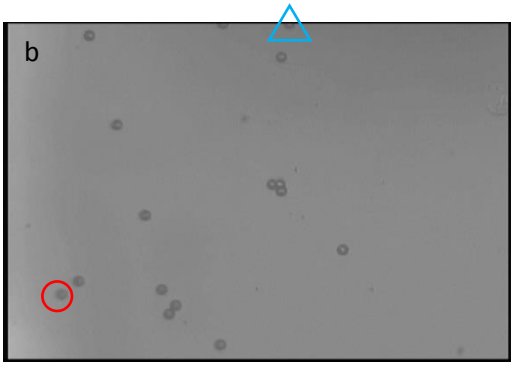

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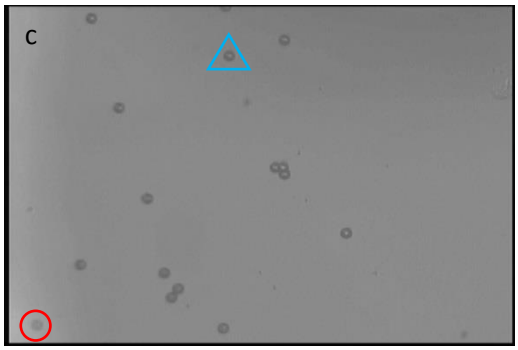

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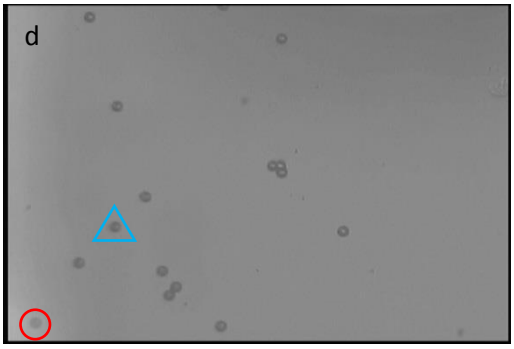

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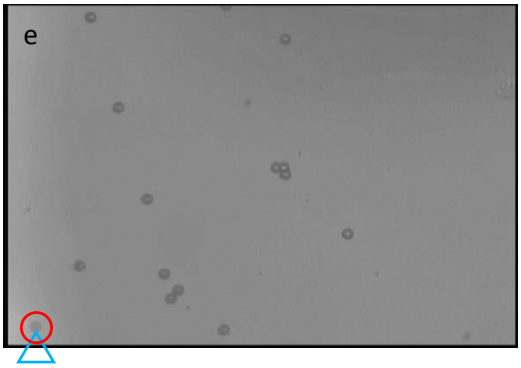

Time = 13 min

Figure 7: One of the trials used a frequency of 10 Hz and was observed over 15 minutes. Pictures were taken a) before voltage was applied, b) 3 minutes after voltage was applied, c) 6 minutes after voltage was applied, d) 9 minutes after voltage was applied, and e) 13 minutes after voltage was applied.

It could be due to the fact that the particle and solution had been sitting in the channel for a while, which caused some particles to sink and attach to the channel. The attachment to the PDMS channel caused an increase of drag force of the particles; therefore, the DEP force was not strong enough to move the particles, which remained stationary. The DEP force was able to

push the particles that were still floating in the solution toward the electrodes since the drag force was not as strong. The particles did not have much space to move around due to the limited width and depth provided by the channel; therefore, it was difficult to observe the movement of some particles. As mentioned above, Figure 7 offered a better view of the moving particles than other pictures taken from other trials, and therefore, was used in this paper.

Conclusion

The need of a reliable device for detecting illness with efficiency is in demand and is being explored by many other researchers. Although this research focused on directing the movement of non-living cells instead of the entrapment and rotation of cells, the results were promising. The device designed in this research was functioning as the calculated results predicted; granting, some minor changes, such as increase the volume of the PDMS channel, would allow better results. The next step for this research would be testing the improved device with living cells and directing the cells toward the electrodes. Once this movement of cells can be done consistently, the final stage would be the entrapment and rotation of non-spherical cells. The testing of entrapment and rotation of cells requires a new device, and it would be tested with cells such as *Escherichia coli* and *Bacillus cereus*.

Acknowledgements

Special thanks to Thomas White for his assistance of fabrication of PDMS mold and PDMS channel. We want to thank Dr. Qin Hu for allowing us to use her lab for COMSOL simulation. We would also like to thank chemistry department and biology department of Central Michigan University for aiding us while tracking the problem of the dissolved electrodes on the glass slide.

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