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# Fabrication of Thin Film Silver/Silver Chloride Electrodes with Finely Controlled Single Layer Silver Chloride

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## TITLE:

# Fabrication of Thin Film Silver/Silver Chloride Electrodes with Finely Controlled Single Layer Silver Chloride

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## KEYWORDS:

Ag/AgCl electrodes, electrode impedance, galvanostatic fabrication, micro electrodes, single layer coating, cathodic cleaning

## SUMMARY:

This paper aims to present a method to form smooth and well-controlled films of silver chloride (AgCl) with designated coverage on top of thin film silver electrodes.

## ABSTRACT:

This paper aims to present a protocol to form smooth and well-controlled films of silver/silver chloride (Ag/AgCl) with designated coverage on top of thin film silver electrodes. Thin film silver electrodes sized 80 µm x 80 µm and 160 µm x 160 µm were sputtered on quartz wafers with a chromium/gold (Cr/Au) layer for adhesion. After passivation, polishing and cathodic cleaning processes, the electrodes underwent galvanostatic oxidation with consideration of Faraday's Law of Electrolysis to form smooth layers of AgCl with a designated degree of coverage on top of the silver electrode. This protocol is validated by inspection of scanning electron microscope (SEM) images of the surface of the fabricated Ag/AgCl thin film electrodes, which highlights the functionality and performance of the protocol. Sub-optimally fabricated electrodes are fabricated as well for comparison. This protocol can be widely used to fabricate Ag/AgCl electrodes with specific impedance requirements (e.g., probing electrodes for impedance sensing applications like impedance flow cytometry and interdigitated electrode arrays).

## INTRODUCTION:

The Ag/AgCl electrode is one of the most used electrodes in the field of electrochemistry. It is most commonly used as the reference electrode in electrochemical systems due to its ease of fabrication, non-toxic property and stable electrode potential[1-6].

Researchers have attempted to understand the mechanism of Ag/AgCl electrodes. The layer of chloride salt on the electrode has been found to be a fundamental material in the characteristic redox reaction of the Ag/AgCl electrode in a chloride containing electrolyte. For the oxidation path, the silver at the imperfection sites on the surface of the electrode combines with the chloride ions in the solution to form soluble AgCl complexes, in which they diffuse to the edges of the AgCl deposited on the surface of the electrode for precipitation in the form of AgCl. The reduction path involves the formation of soluble AgCl complexes using the AgCl on the electrode. The complexes diffuse to the silver surface and reduces back to elemental silver[7,8].

The morphology of the AgCl layer is a pivotal influence in the physical property of Ag/AgCl electrodes. Various works showed that the large surface area is key to form reference Ag/AgCl electrodes with highly reproducible and stable electrode potentials[9-12]. Therefore, researchers have investigated methods to create Ag/AgCl electrodes with a large surface area. Brewer et al. discovered that using constant voltage instead of constant current to fabricate Ag/AgCl electrodes would result in a highly porous AgCl structure, increasing the surface area of the AgCl layer[11]. Safari et al. took advantage of the mass transport limitation effect during AgCl formation on the surface of silver electrodes to form AgCl nanosheets on top of them, increasing the surface area of the AgCl layer significantly[12].

There is a rising trend to design AgCl electrode for sensing applications. A low contact impedance is crucial for sensing electrodes. Thus, it is important to understand how the surface coating of AgCl would affect its impedance property. Our previous research showed that the degree of AgCl coverage on the silver electrode has a pivotal influence on the impedance characteristic of the electrode/electrolyte interface[13]. However, to correctly estimate the contact impedance of thin film Ag/AgCl electrodes, the AgCl layer formed must be smooth and have well-controlled coverage. Therefore, a method to form smooth AgCl layers with designated degrees of AgCl coverage is needed. Works have been done to address this need partially. Brewer et al. and Pargar et al. discussed that a smooth AgCl can be achieved using a gentle constant current, fabricating the AgCl layer on top of the silver electrode[11,14]. Katan et al. formed a single layer of AgCl on their silver samples and observed the size of individual AgCl particles[8]. Their research found that the thickness of a single layer of AgCl is around 350 nm. The aim of this work is to develop a protocol to form fine and well-controlled films of AgCl with predicted impedance properties on top of silver electrodes.

## PROTOCOL:

### 1. Fabrication of a Cr/Au adhesion layer using liftoff

1.1. Spincoat HPR504 positive photoresist of 1.2 µm thickness onto a quartz wafer using a spread speed of 1,000 rpm for 5 s and a spin speed of 4,000 rpm for 30 s.

1.2. Softbake the photoresist on the quartz wafer at 110 °C for 5 min on a hot plate.

1.3. Using a mask aligner, expose the wafer such that locations for Cr/Au deposition are exposed with ultraviolet (UV) light. The exposure power density and time is 16 mW/cm$^2$ and 7.5 s respectively (exposure energy density = 120 mJ/cm$^2$).

1.4. Develop the wafer by submersing it in positive resist developer FHD-5 for 1 min. Rinse the wafer with deionized (DI) water after the development process.

1.5. Dry the wafer using a nitrogen ($N_2$) gun. Put the wafer in an oven for 5 min at 120 °C.

1.6. Using electron beam (e-beam) evaporation, deposit a 5 nm Cr layer, followed by a 50 nm Au layer on the wafer. The deposition rates are 1 Å/s and 2 Å/s, respectively.

1.7. Place the e-beam evaporated wafer into a container. Pour copious amount of acetone inside.

1.8. Close the container using a lid. Place the lidded container in an ultrasonic cleaner for 10 min or until the liftoff process has completed.

1.9. Flush the wafer using isopropanol (IPA) followed by DI water. Dry it using $N_2$ gun and oven afterwards.

NOTE: The protocol can be paused here.

## 2. Fabrication of thin film Ag electrodes on the adhesion layer using liftoff

2.1. Spincoat AZ P4620 positive photoresist of 7 µm thickness onto the wafer using a spread speed of 1,000 rpm for 5 s and a spin speed of 4,000 rpm for 30 s.

2.2. Softbake the photoresist on the wafer at 90 °C for 450 s on a hot plate.

2.3. Using a mask aligner, expose the wafer such that locations for Ag deposition are exposed with UV. The exposure power density and time is 16 mW/cm$^2$ and 45 s respectively (exposure energy density = 720 mJ/cm$^2$).

2.4. Develop the wafer by submersing it in FHD-5 for 2 min. Rinse the wafer with DI water after the development process.

2.5. Dry the wafer using a $N_2$ gun. Put the wafer in an oven for 5 min at 120 °C.

2.6. Sputter a 1 µm Ag layer on the wafer. The sputtering rate is ~86 nm/min.

2.7. Place the sputtered wafer into a container. Pour copious amount of acetone inside.

2.8. Close the container using a lid. Place the lidded container in an ultrasonic cleaner for 10 min or until the liftoff process has completed.

2.9. Flush the wafer using IPA followed by DI water. Dry it using $N_2$ gun and oven afterwards.

**3. Passivation of the wafer to expose only the electrodes and contact pads**

3.1. Passivate the whole wafer surface with a 2 µm silicon dioxide ($SiO_2$) layer using plasma enhanced chemical vapor deposition (PECVD).

3.1.1. Passivate a small silicon dummy sample (a silicon wafer fragment) together the wafer simultaneously.

3.1.2. Measure the thickness of the oxide layer of the dummy sample.

NOTE: The protocol can be paused here.

3.2. Spincoat AZ 5214E dual tone photoresist of 1.4 µm thickness onto the wafer using a spread speed of 1000 rpm for 5 s and a spin speed of 3000 rpm for 30 s.

3.3. Softbake the photoresist on the wafer at 90 °C for 150 s on a hot plate.

3.4. Using a mask aligner, expose the wafer such that the locations for pad opening are exposed with UV. The exposure power density and time is 16 $mW/cm^2$ and 2.25 s respectively (exposure energy density = 36 $mJ/cm^2$).

3.5. Develop the wafer by submersing it in FHD-5 for 75 s. Rinse the wafer with DI water after the development process.

3.6. After briefly drying the wafer using $N_2$ gun, further dry and hard bake the wafer in an oven for 15 min at 120 °C.

3.7. Perform descum of photoresist on the wafer for 1 min using a plasma asher to ensure complete removal of unwanted photoresist.

3.8. Perform reactive ion etching on the wafer and the dummy sample to expose the thin film electrodes and contact pads.

3.8.1. After performing the etching process for a short period of time (e.g., 5-10 min), stop the operation and take out the dummy sample.

3.8.2. Measure the thickness of the oxide layer on top of the dummy sample. Compare it with the result obtained in step 3.1.2.

3.8.3. Calculate the rate of $SiO_2$ etching of the machine to fine tune the etching duration in order to achieve a 10% overetch.

3.8.4. Continue the etching process without the dummy sample.

3.9. Resist strip the etched wafer by plasma ashing for 30 min, followed by a positive photoresist stripper MS2001 bath at 70 °C for 5 min.

3.10. Flush the wafer using DI water. Dry the wafer using $N_2$ gun and oven.

NOTE: The protocol can be paused here.

## 4. Preparation for the fabrication of thin film Ag/AgCl electrodes (chip)

4.1. Dice cut the wafer to obtain different testing chips.

4.2. Polish the electrode surfaces on the chips using fine sandpaper.

4.3. Bond the contact pads on the chip to an external printed circuit board for interfacing purposes in further steps.

4.4. 3D-print an acrylic hollow rectangular container to hold the electrolyte on the thin film electrodes. The dimensions of the rectangular container should allow placement of a wire and a pipette inside the void comfortably.

4.5. Mix a small amount of polydimethylsiloxane (PDMS) prepolymer and its curing agent thoroughly. The ratio should be 10:1.

NOTE: It is very common to degas the PDMS mixture to obtain high quality PDMS devices; however, it is not needed in this case as the mixture is only used as an adhesive.

4.6. Place the acrylic container on the diced chip in a fashion such that all the silver electrodes are inside the cavity of the container.

4.6.1. Using a toothpick or a fine rod, smear the uncured PDMS mixture on the outer edge where the container and the chip touch each other.

4.6.2. Carefully place the chip on a flat hot plate and cure the PDMS for 2 h at 80 °C or until the container is securely affixed onto the chip.

## 5. Preparation for the fabrication of thin film Ag/AgCl electrodes (reagents)

5.1. Using DI water and concentrated hydrochloric acid (HCl), obtain 0.01 M HCl solution.

5.2. Using DI water and potassium chloride (KCl) powder, obtain 3.5 M KCl solution and 0.1 M KCl solution.

NOTE: The protocol can be paused here.

**6. Preparation for the fabrication of thin film Ag/AgCl electrodes (macro electrodes)**

6.1. Cut some silver wires.

6.2. Polish the surface of the silver wires with fine sandpaper.

6.3. Submerse 80% of the silver wires into household bleach for 1 h.

NOTE: The color of the wire will change from silvery to dark purple. This shows the formation of AgCl on the surface of the silver wire.

6.4. Flush the Ag/AgCl wire with DI water.

6.5. Make an Ag/AgCl reference electrode using one of the Ag/AgCl wires referencing to Hassel et al. with modifications[15].

NOTE: The modifications are using a pipette instead of a glass capillary, using 3.5 M KCl as the electrolyte, ditching the polymer block and the gold-plated connector and replace it with parafilm.

6.6. Store the Ag/AgCl electrodes by submersing them in 3.5 M KCl solution. Make sure the silver part does not come in contact with the solution.

6.6.1. Cut several pieces of Ag/AgCl wires and put them into the KCl solutions mentioned in step 5.2.

NOTE: The protocol can be paused here.

**7. Cathodic cleaning of the micro Ag electrodes**

NOTE: All of the following processes use the CHI660D electrochemical analyzer/workstation and its accompanying software.

7.1. Flush the chip using IPA followed by DI water.

7.2. Pour 0.01 M HCl solution into the acrylic container.

7.3. Wipe dry the macro Ag/AgCl reference electrode’s pipette exterior (fabricated in step 6.5) and a macro Ag/AgCl electrode (fabricated in step 6.3) using laboratory clean wipes.

7.4. Connect the chip and the macro electrodes to the analyzer such that a thin film Ag electrode on the chip is defined as the working electrode, the macro Ag/AgCl reference electrode is defined as the reference electrode, and the bare macro Ag/AgCl electrode is defined as the counter electrode.

7.5. Place the macro electrodes into the container. Use blu-tack as the lid of the container to anchor the macro electrodes.

7.6. Place the setup into a Faraday cage.

7.7. In the CHI660D software, click on the **Setup** tab at the top left corner of the window. Then click **Technique | Amperometric i-t Curve | OK** to perform cathodic cleaning of the electrodes.

7.8. In the pop-up menu, modify the parameters for cathodic cleaning.

7.8.1. Set the **Init E** (**V**) as **-1.5**.

7.8.2. Set the **Sample Interval** (**sec**) as **0.1** (**Default**).

7.8.3. Set the **Run Time** (**sec**) as **900**.

7.8.4. Set the **Quiet Time** (**sec**) to be **0** (**Default**).

7.8.5. Set the **Scales during Run** as **1** (**Default**).

7.8.6. Set the **Sensitivity** (**A/V**) appropriately. For an 80 µm x 80 µm electrode, set it as **1e-006**.

7.9. Press **OK**. Start the process by pressing the **Start** icon under the menu bar.

7.10. Let the experiment run and finish.

7.11. Open the Faraday cage.

7.12. Remove the macro reference and counter electrode. Wipe dry their surfaces.

7.13. Pour the used electrolyte in a waste container. Flush the acrylic container using DI water.

## 8. Fabrication of single layer AgCl on top of the thin film Ag electrodes

8.1. Pour 0.1 M KCl solution into the acrylic container.

8.2. Connect the chip and the macro electrodes to the analyzer such that the cleaned thin film Ag electrode on the chip is defined as the working electrode, the macro Ag/AgCl reference electrode is defined as the reference electrode, and the bare macro Ag/AgCl electrode is defined as the counter electrode.

8.3. Place the macro electrodes into the container. Use blu-tack as the lid of the container to anchor the macro electrodes.

8.4. Place the setup into a Faraday cage.

8.5. In the CHI660D software, click on the **Setup** tab at the top left corner of the window, then click **Technique | Chronopotentiometry | OK** to perform galvanostatic fabrication of single layer AgCl on silver electrodes.

8.6. In the pop-up menu, modify the parameters for such process.

8.6.1. Set the **Cathodic Current** (**A**) as **0** (**Default**).

8.6.2. Set the **Anodic Current** (**A**) such that the current density applied to the thin film electrode is 0.5 mA/cm$^2$.

8.6.3. Keep the High and Low **E limit** and **Hold Time** as default.

8.6.4. Set the **Cathodic Time** (**sec**) as **10** (**Default**).

8.6.5. Set the **Anodic Time** (**sec**) correspondingly to achieve the degree of AgCl coverage needed.

NOTE: With reference to Faradays Law of Electrolysis, the time needed for 100% coverage is 262 s. The time needed varies linearly with the coverage percentage.

8.6.6. Set the **Initial Polarity** as **Anodic**.

8.6.7. Set the **Data Storage Intvl** (**sec**) as **0.1** (**Default**).

8.6.8. Set the **Number of Segments** as **1** (**Default**).

8.6.9. Set the **Current Switching Priority** as **Time.**

8.6.10. Uncheck the **Auxiliary Signal Recording When Sample Interval >= 0.0005s** (**Default**)**.**

8.7. Press **OK**. Start the process by pressing the **Start** icon under the menu bar.

8.8. Let the experiment run and finish.

8.9. Open the Faraday cage.

8.10. Remove the macro reference and counter electrode. Wipe dry their surfaces.

8.11. Submerse the macro electrodes in 3.5 M KCl solution for storage.

8.12. Pour the used electrolyte in a waste container. Flush the container using DI water.

8.13. Cover the opening of the acrylic container using parafilm for further processing.

## REPRESENTATIVE RESULTS:

**Figure 1** shows an 80 µm x 80 µm Ag/AgCl electrode with a designed AgCl coverage of 50% fabricated following this protocol. By observation, the area of the AgCl patch is around 68 µm x 52 µm, which corresponds to around 55% of AgCl coverage. This shows that the protocol can finely control the amount of AgCl coverage on the thin film Ag electrodes. The AgCl layer fabricated is also very smooth, as evident by the clumping of adjacent AgCl particles. Furthermore, the layer of AgCl is only a single layer, which is proved by the absence of stacked AgCl particles and a distinctive Ag/AgCl intersection. **Figure 2** shows more successful examples of thin film Ag/AgCl electrodes fabricated using this protocol, which are 80 µm x 80 µm electrodes with a designated AgCl coverage of 70% and 30%, together with 160 µm x 160 µm electrodes with a designated AgCl coverage of 75% and 90%, confirming the robustness of this protocol.

[Place **Figure 1** here]

[Place **Figure 2** here]

**Figure 3** illustrates a negative result where the polishing step is omitted (i.e., step 4.2). **Figure 3a** shows a polished electrode surface whereas figure 3b shows an unpolished electrode surface. For the unpolished electrode, finger-like structures can be observed on the surface, which is illustrated in **Figure 4**, where the polished electrode surface is smooth with minor scratch marks caused by the polishing process. Figure 5 shows an unpolished 80 µm x 80 µm Ag/AgCl electrode with a designed AgCl coverage of 50%. By observation, the area of the sparsely covered AgCl is only around 40 µm x 40 µm, which is 25% of the apparent electrode surface area. Furthermore, compared to **Figure 1** where the protocol is properly observed, for the unpolished electrode, the AgCl formed appears to be recessed inwards instead of protruding outwards.

[Place **Figure 3** here]

[Place **Figure 4** here]

[Place **Figure 5** here]

## FIGURE AND TABLE LEGENDS:

**Figure 1: Exemplar SEM image of the thin film Ag/AgCl electrode with a dimension of 80 µm x 80 µm and designated AgCl coverage of 50%.** The observed AgCl coverage is 55%, demonstrating the effectiveness of the protocol. This figure has been modified from Tjon et al.[13].

**Figure 2: Exemplar SEM images of thin film Ag/AgCl electrodes with various electrode areas**

**and AgCl coverages.** (**A**) 80 µm x 80 µm with 70% AgCl coverage. (**B**) 80 µm x 80 µm with 30% AgCl coverage. (**C**) 160 µm x 160 µm with 75% AgCl coverage. (**D**) 160 µm x 160 µm with 90% AgCl coverage. These figures have been modified from Tjon et al.[13].

**Figure 3: SEM images for bare silver electrodes.** (**A**) Polished 160 µm x 160 µm electrode (**B**) Unpolished 40 µm x 40 µm electrode.

**Figure 4: Zoomed SEM image for unpolished silver electrodes.** Finger-like structures can be observed.

**Figure 5: Suboptimal fabrication of thin film Ag/AgCl electrodes.** Without polishing, the degree of coverage of the formed AgCl on the surface of the electrode is smaller than the predicted value. The designed AgCl coverage for this 80 µm x 80 µm thin film silver electrode is 50% but the actual coverage is only 25%.

## DISCUSSION:

The physical properties of an Ag/AgCl electrode is controlled by the morphology and the structure of the AgCl deposited on the electrode. In this paper, we presented a protocol to precisely control the coverage of a single layer of AgCl on the surface of the silver electrode. An integral part of the protocol is a modified form of the Faraday's Law of Electrolysis, which is used to control the degree of AgCl on the thin film silver electrodes. It can be written as:

$$X \cdot \ P\% = \frac{j \cdot M \cdot t}{F \cdot D}$$

Where X is the thickness of a single AgCl layer in cm (350 nm = $3.5 \times 10^{-5}$ cm); P% is the percentage of AgCl coverage on the surface of the Ag electrode (100% = full coverage); j is the applied current density in A/cm$^2$ (0.5 mA/cm$^2$), M is the molar weight of AgCl (143.5 g/mol), t is the duration of anodization in s (262 s for 100% coverage); F is the Faraday's constant (~96485 C/mol); D is the density of AgCl (5.56 g/cm$^3$). To ensure the success of the protocol, several critical steps in the protocol must be observed. Step 4.2, which is about the polishing of the surface of the thin film silver electrode, is crucial to define the surface area of the electrode prior to the galvanostatic formation of AgCl on the electrode surface. From **Figure 3** and **Figure 4**, the difference in the surface structure and roughness of the thin film silver electrodes fabricated by sputtering can be seen clearly; the unpolished silver surface has finger-like structures, whereas the polished silver surface is mostly smooth with minor scratch marks caused by sandpaper rubbing. This creates a major problem as the finger-like structures effectively increases the surface area of the electrode. This makes the determination of the surface area of the electrode and subsequently the degree of AgCl coverage on the electrode impossible. The effect of this is well-illustrated in **Figure 1** and **Figure 5**. The protocol compliant Ag/AgCl electrode has a smooth, single layer of AgCl with a well-controlled AgCl coverage, whereas the electrode without observing the polishing step has an overestimated coverage of AgCl on the electrode. Step 8.6.2, which is about the use of a constant current density of 0.5 mA/cm$^2$ to form the AgCl layer on the thin film Ag electrode, is crucial to create a smooth AgCl layer with single layer thickness. Newly formed AgCl deposits at the edges of existing AgCl on the surface due to its low energy[7,8]. This allows the AgCl particles to form a single layer first before growing thicker. However, if a high current density is applied during the galvanostatic formation of the AgCl layer, the newly formed AgCl could have enough energy to form directly on the electrode other than along the existing AgCl edges, creating a rougher AgCl surface[14]. This makes the degree of AgCl coverage on the electrode unable to be controlled as the AgCl formation sites cannot be predicted under such condition. Also, this makes the determination of the AgCl surface area impossible as its roughness affects the surface area, which was shown to affect the electrode's impedance characteristics in our previous work[13].

There are several ways to troubleshoot whether a single layer of AgCl is formed properly. Firstly, to check whether the polishing step is carried out successfully. The sample should be observed under an SEM microscope without gold coating after the polishing step to see whether the finger structure is replaced by a smooth surface. Furthermore, when the surface of the electrode is fully covered with AgCl, further galvanostatic oxidation will cause a sudden increase in the applied potential to the system as the thickening of the AgCl increases the ohmic resistance of the AgCl layer. This can be used to determine whether the surface of the electrode is fully covered with AgCl already.

There is a major limitation concerning the use of this method to fabricate thin film Ag/AgCl electrodes with good control of the AgCl coverage. Electrodes fabricated using this method are not reworkable. During the process of galvanostatic oxidation of the silver electrode to form the AgCl deposit layer, the sites of imperfection on the surface of the electrode will grow in size in an unpredictable way. If the electrode is reduced to revert AgCl back to Ag, it is unable to guarantee that these sites at the electrode surface will be filled back the way it was. Instead, the surface will become rougher. If the surface is re-polished using sandpaper after an attempted rework, some silver will be removed from the surface during polishing. Hence, one can only do this for a few times before the underlying gold layer becomes exposed.

This method, when compared to typical Ag/AgCl electrode fabrication methods, focuses on a fine control of the coverage of AgCl on the surface of the thin film Ag electrode, whereas other methods focus on creating a porous layer of AgCl. From the best of the author's knowledge, this is the first time a protocol is developed to fabricate a single layer of finely controlled AgCl on top of a silver electrode. This is due to different design aims. Most previous works aimed to achieve a reference Ag/AgCl electrode with high electrode potential stability, while our protocol aims to design sensing Ag/AgCl electrode with low contact impedance for impedance sensing systems, such as impedance flow cytometers and interdigitated electrode arrays.

Future experiments can include a more sophisticated polishing step, for example using a polishing system to achieve an even smoother surface. Further investigations can also be carried out to evaluate the quantitative relationship between the thickness of the AgCl layer and the faraday's equation of electrolysis.

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## DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

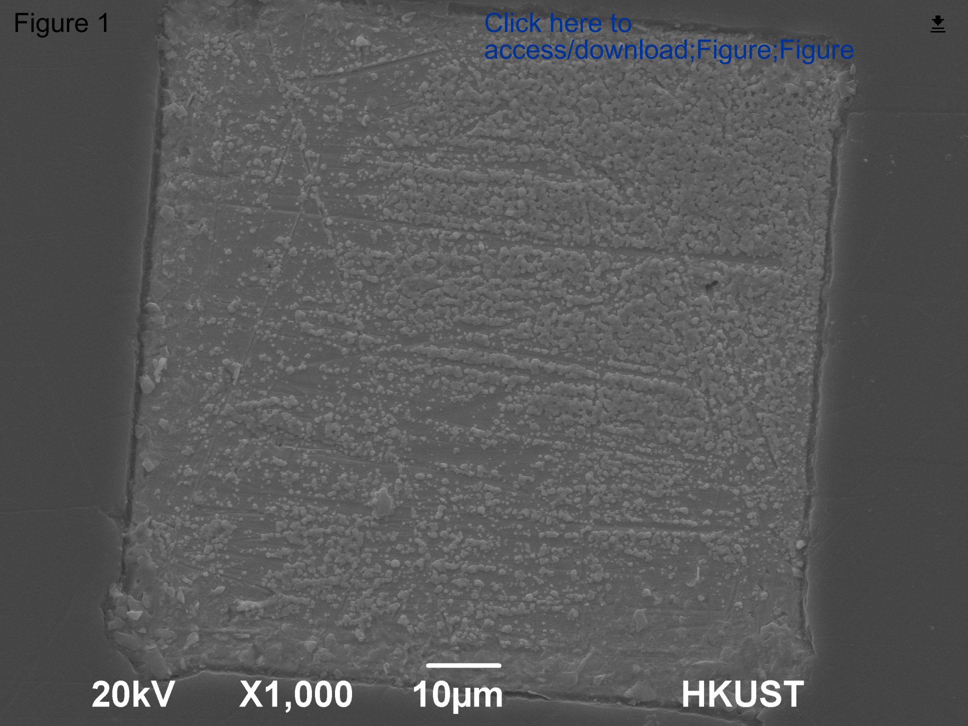
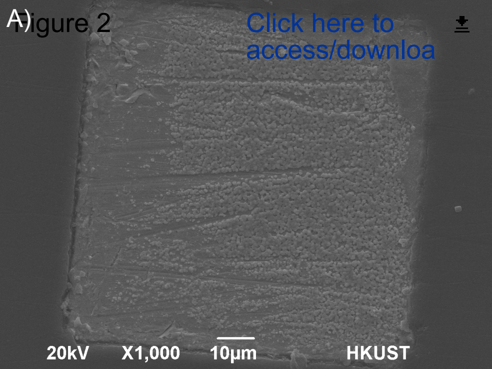
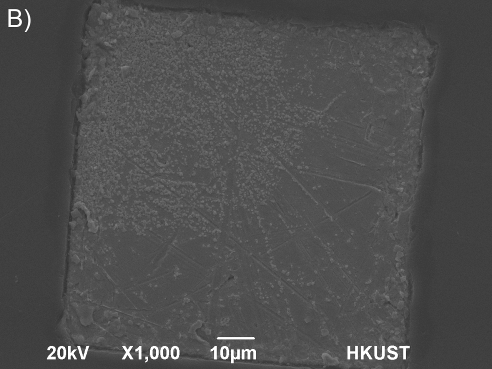


Figure 2




B)
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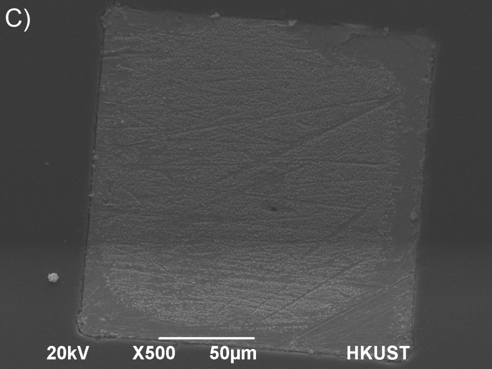
C)



D)

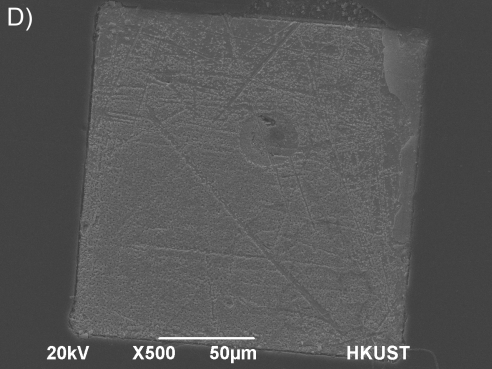
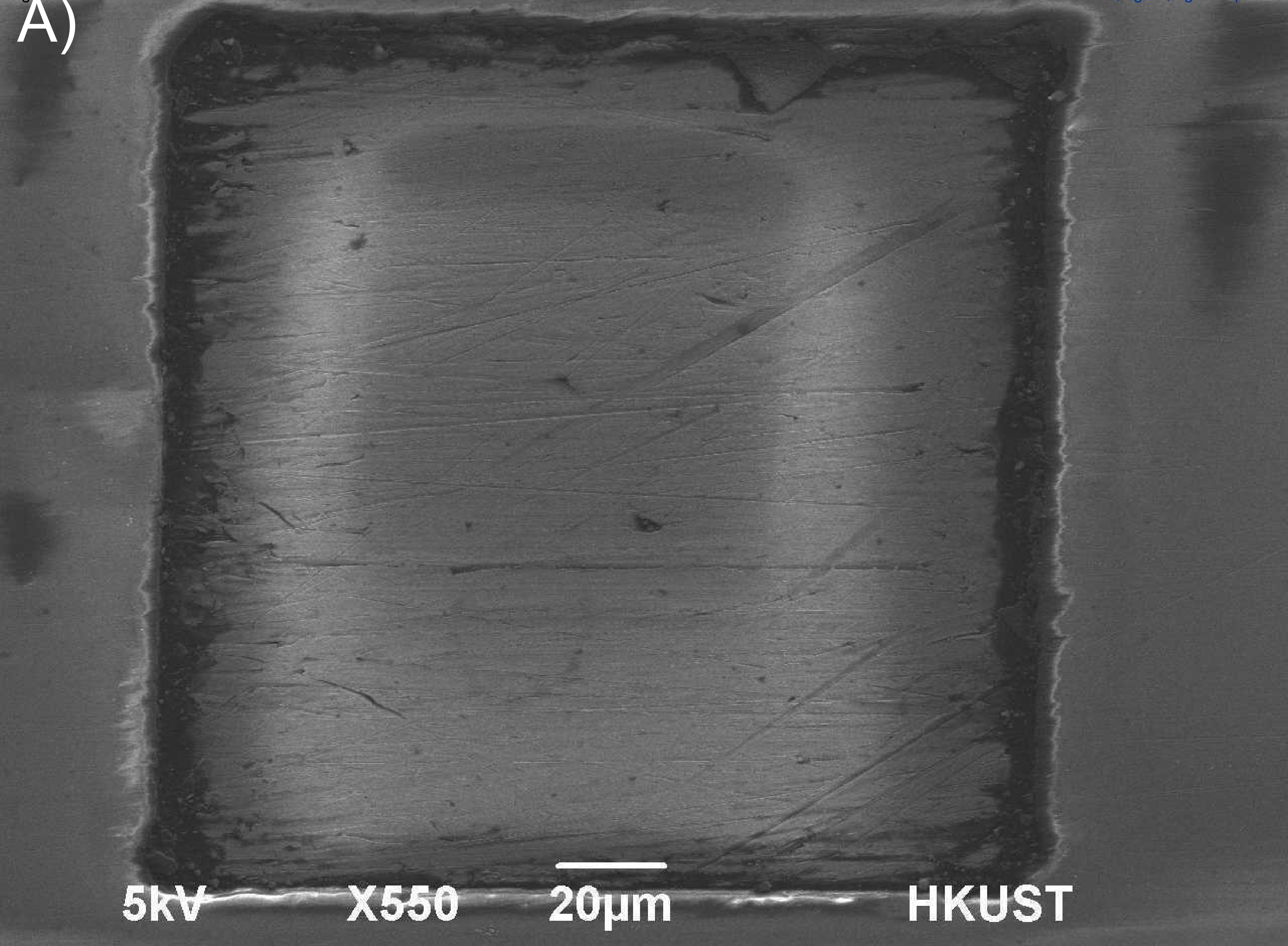


Figure 3



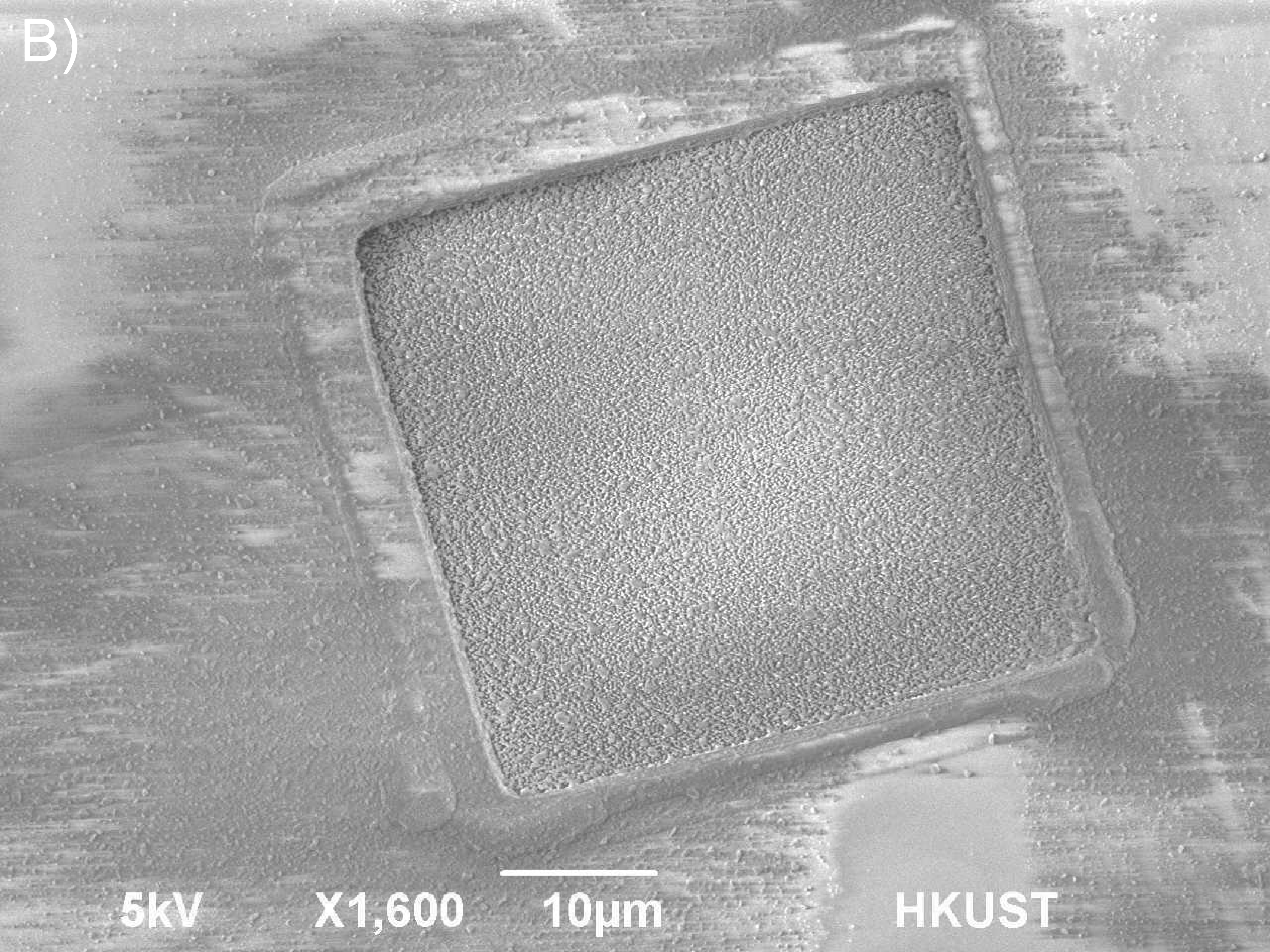

B)
5kV
X1,600
10µm
HKUST

Figure 4

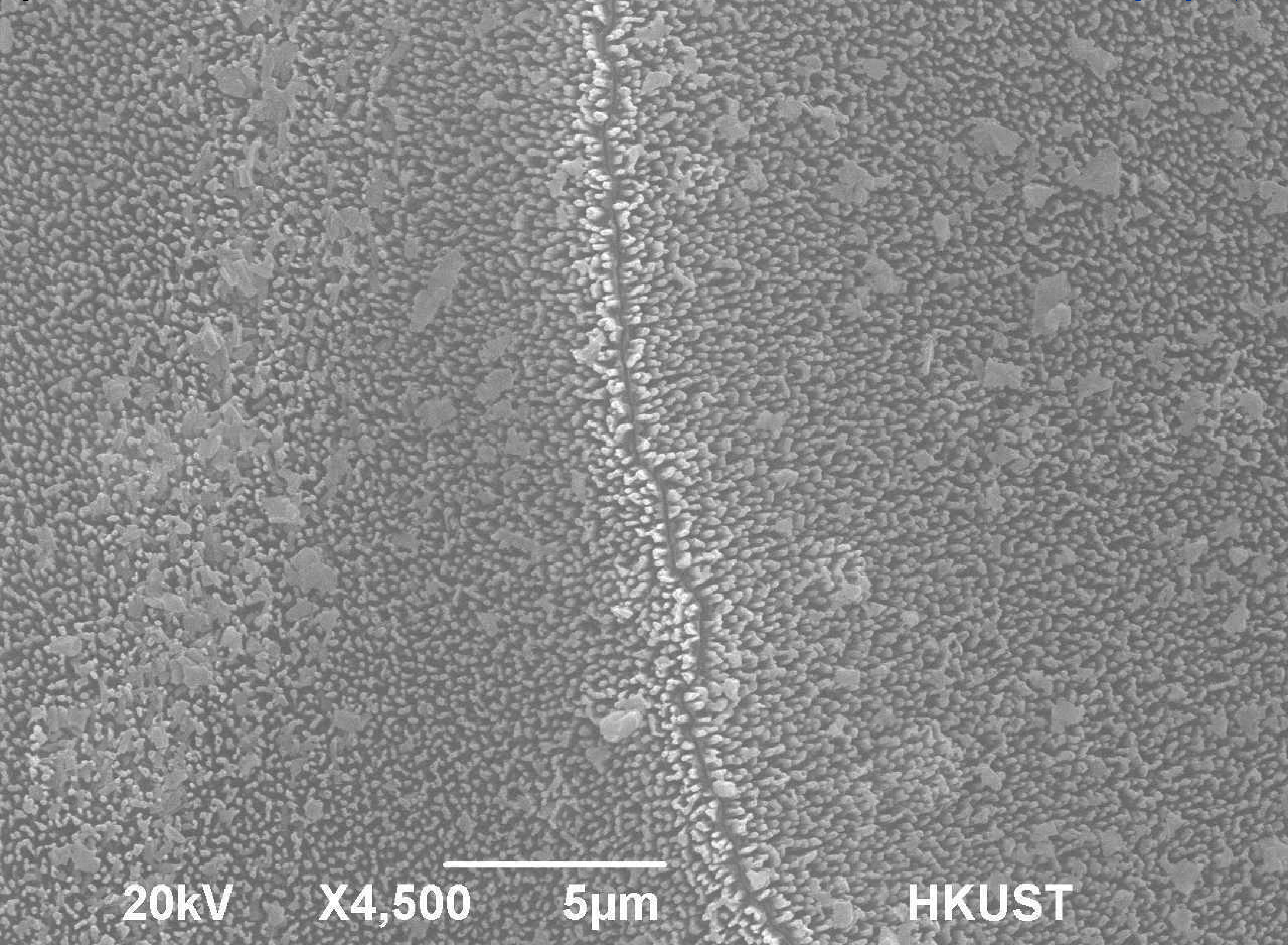
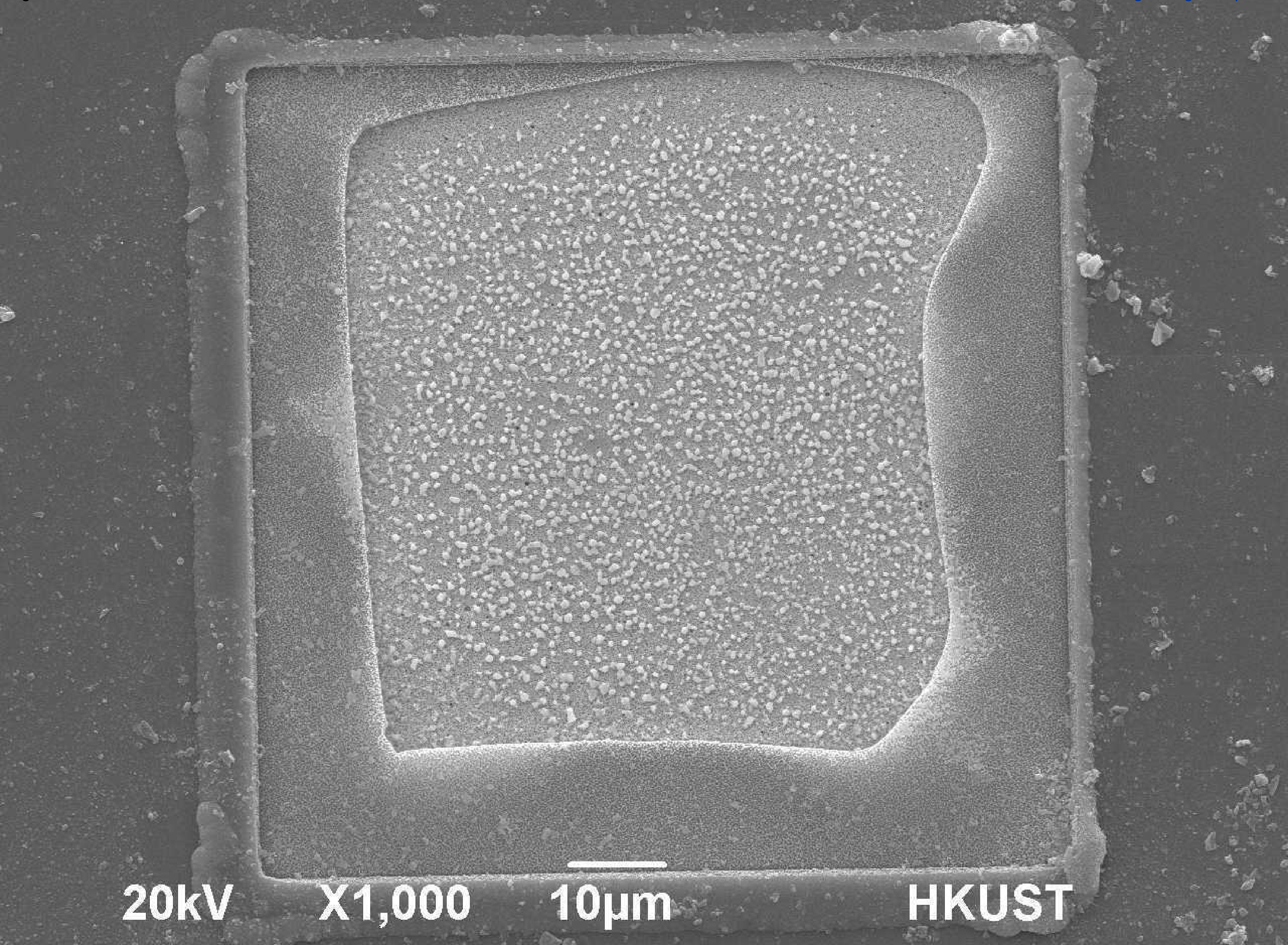


Figure 5



| Name of Material/ Equipment | Company | Catalog Number |
|---|---|---|
| AST Peva-600EI E-Beam Evaporation System | Advanced System Technology | |
| AZ 5214 E Photoresist | MicroChemicals | |
| AZ P4620 Photoresist | AZ Electronic Materials | |
| Branson/IPC 3000 Plasma Asher | Branson/IPC | |
| Branson 5510R-MT Ultrasonic Cleaner | Branson Ultrasonics | |
| CHI660D | CH Instruments, Inc | |
| Denton Explorer 14 RF/DC Sputter | Denton Vacuum | |
| FHD-5 | Fujifilm | 800768 |
| HPR 504 Photoresist | NV | |
| Hydrochloric acid fuming 37% | VMR | 20252.420 |
| J.A. Woollam M-2000VI Spectroscopic Elipsometer | J.A. Woollam | |
| Multiplex CVD | Surface Technology Systems | |
| Oxford RIE Etcher | Oxford Instruments | |
| Potassium Chloride | Sigma-Aldrich | 7447-40-7 |
| SOLITEC 5110-C/PD Manual Single-Head Coater | Solitec Wafer Processing, Inc. | |
| SUSS MA6 | SUSS MicroTec | |
| Sylgard 184 Silicone Elastomer Kit | Dow Corning | |

| Comments/Description |
|---|
| For Cr/Au Deposition |
| Photoresist for pad opening |
| Photoresist for Ag liftoff |
| Ashing |
| Liftoff |
| Electrochemical Analyser |
| For Ag Sputtering |
| Photoresist Development |
| Photoresist for Cr/Au liftoff |
| Making diluted HCl for cathodic cleaning |
| Measurement of silicon dioxide passivation layer thickness on dummy |
| Silicon dioxide passivation |
| For Pad opening |
| Making KCl solutions |
| For spincoating of photoresist |
| Mask Aligner |
| Adhesive for container on chip |

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3. Please use h, min, s for time units.

Answer: The manuscript is modified accordingly.

4. Please define all abbreviations before use.

Answer: The manuscript is modified accordingly.

Reviewers' comments:

Reviewer #1: The manuscript entitled "Fabrication of Thin Film Silver/Silver Chloride Electrodes with Finely Controlled Single Layer Silver Chloride" submitted by Prof. Jie Yuan described a protocol to obtain electrodes modified with a thin film of Ag/AgCl combining sputtering and electrochemical deposition.

The protocol is well stablished, but some minor aspects should be revised.

Q1.1 Please the acronyms used should be defined the first time used (for example FHD-5 or DI, but there are others along the manuscript).

Answer: Thank you for your suggestion. The manuscript is modified accordingly. However, for FHD-5 and MS2001, they are not acronyms. Nonetheless, I stated the function of the chemicals before their names for clarification (positive resist developer FHD-5, positive photoresist stripper MS2001).

Q1.2 The reactants used should be on capital letter only at the beginning of a sentece.

Answer: Thank you for your suggestion. The manuscript is modified accordingly.

Q1.3 There are some technical and specific words that which would be of great use to the reader if its meaning were indicated, albeit in a very brief form (for example, "descum").

Answer: Thank you for your suggestion. The manuscript is modified accordingly. The term ‘descum’ is elaborated.

Q1.4 In the manuscript the section 7 is yellow highlighted. I do not understand why. Is something wrong or that the authors want to change?

Answer: Thank you for your question. The yellow highlighted sections are requested by the journal for video capturing.

Q1.5 A list of the instruments used should be included (for example the SEM)

Answer: Thank you for your suggestion. The list of instruments used is included in the 'Table of Materials' spreadsheet. Concerning the SEM, as it is NOT involved in the fabrication of the thin film silver/silver chloride electrodes, we find it prudent to not include it in the 'Table of Materials'.

Q1.6 Revise the references. There are some reference where not all the authors are cited.

Answer: Thank you for your suggestion. For reference 2, not all the authors are cited on purpose to fulfil the journal's requirements on references.

Reviewer #2: Dear authors,

The authors want to develope a new method to fabricate the Ag/Agcl reference electrode. But the results seem too simple to publish. So the major revision is needed before consideration of publication.

Major Concerns:

The authors need to make some major revision and answer some questions like:

Q.2.1 Why can the bare macro Ag/AgCl electrode be defined as the counter electrode in step 7.4 and 8.2?

Answer: Thank you for your question. The reason why the bare macro Ag/AgCl electrode can be defined as the counter electrode is because it has a large surface area. It can act as an electron sink to complete the current flow between the CHI660D machine, the working electrode and the electrolyte while not interfering with the working electrode's measurement as its interfacial impedance with the aqueous 0.1M KCl solution is quite small compared to the working electrode due to its high surface area.

Q2.2 How is the AgCl coverage calculated?

Answer: Thank you for your question. The AgCl coverage can be calculated using the Faraday's Law of Electrolysis detailed in the beginning of the discussion section. For clarity, the equation and the description is slightly modified to better explain the calculation for AgCl coverage.

Q2.3 What is the corresponding AgCl coverage when the Ag/AgCl electrode has lowest impedance? What is the lowest impedance?

Answer: Thank you for your question. This is answered by our previous publication (Tjon, K. C. E., Yuan, J., Impedance characterization of silver/silver chloride micro-electrodes for bio-sensing applications. *Electrochimica Acta*. **320**, 134638 (2019).). From our findings, the $160\mu m \times 160\mu m$ Ag electrode with 90% AgCl fabrication using this protocol has the lowest impedance. Its impedance varies with changing frequency; at 100Hz the impedance is around 20kΩ: at 100kHz the impedance is around 4kΩ.

Q2.4 How are its reproducibility and stability when the Ag/AgCl electrode is used as reference electrode?

Answer: Thank you for your question. The focus of this line of work is to design Ag/AgCl electrode for sensing, not for reference. This is a new development of the Ag/AgCl electrode application, which needs discussion. We feel the discussion of its usage for reference electrode will diverge the topic.

Reviewer #3:

Q3.1 The samples after fabrication should be characterized more, for example, the AFM, XRD, EDS, Raman spectra... to confirm that the AgCl layer was really formed.

Answer: Thank you for your suggestion. AFM is a scanning probe microscopy method which we deemed that is not needed to confirm the existence of AgCl on the surface of the electrode as from the SEM images a layer of crystals can already be observed on the surface of the electrodes. XRD and Raman spectroscopy were attempted. However, as the AgCl layer is too thin, XRD is unable to detect the characteristic crystalline structure of AgCl. For Raman spectroscopy, the thin layer of the AgCl causes focusing issues for the optical microscope and the incomplete AgCl coverage of the electrodes leads to a strong background signal as the metal layers underneath the AgCl layer are being detected by the Raman spectroscope, thus for both of the methods the characteristic peaks cannot be observed. With this thin layer of AgCl, SEM seems to be the best characterization method.

Q3.2 The SEM image of the cross-section is expected to include to see each layer.

Answer: Thank you for your suggestion, however this SEM image cannot be obtained from our Ag/AgCl electrode. To obtain the SEM image of the cross-section, the AgCl is needed to be formed on the surface of the electrode first, then the chip is needed to be diced by a chip dicer which involves constant flushing of the sample using deionized water before SEM observation of the chip. As the AgCl layer is thin and fragile, during the flushing step the layer of AgCl is easily damaged and flushed away and thus the AgCl layer would not be included in the cross-section image, which defeats the purpose of this SEM scan and thus not included.

Q3.3 The literature review: The author should refer to the recent involved publication (Tien Minh Huynh et al 2019 Adv. Nat. Sci: Nanosci. Nanotechnol. 10 015006: Fabrication of thin - film Ag/AgCl reference electrode by Electron Beam Evaporation method for potential measurements).

Answer: Thank you for your suggestion, this publication is included in the references as reference no. 6.

**K.C.E. Tjon** received the B.Eng degree in electronic and computer engineering from The Hong Kong University of Science and Technology (HKUST), Hong Kong, in 2017. He is currently pursuing the Ph.D. degree in electronic and computer engineering from HKUST. His research interest is on developing microfluidic lab-on-chips for biomedical applications, including microfluidic design, impedance biosensing frontends and data acquisition circuits.

Biography:

Prof. Jie Yuan (M06-SM12) received his Ph.D in Electrical Engineering from University of Pennsylvania, Philadelphia, in 2006. He received his B.S in Electrical Engineering from Tsinghua University in 2000. Since 2006, Prof. Yuan has been a faculty member of the Electronic & Computer Engineering Department in Hong Kong University of Science & Technology. Currently, he is an Associate Professor at HKUST.

Prof. Yuan's research expertise is on sensory IC design. His research group has made contributions to the fields of ADC, CMOS imaging sensor, bio-cellular recording systems, human body communication, energy harvesting etc.

Prof. Yuan is currently the chair of the Analog Signal Processing Technical Committee of IEEE CAS society. He served as the chair of the Biomedical Circuits and Systems Technical Committee of IEEE CAS 2012-2014. He served as the Technical Program Committee Co-Chair of IEEE Biomedical Circuits and Systems Conference in 2009. He is an Associate Editor of the IEEE Transactions on Biomedical Circuits and Systems. Prof. Yuan was an Associate Editor of the IEEE Transactions on Circuits and Systems II: Express Briefs, and a guest editor of the IEEE Transactions on Circuits and Systems I: Regular Papers.

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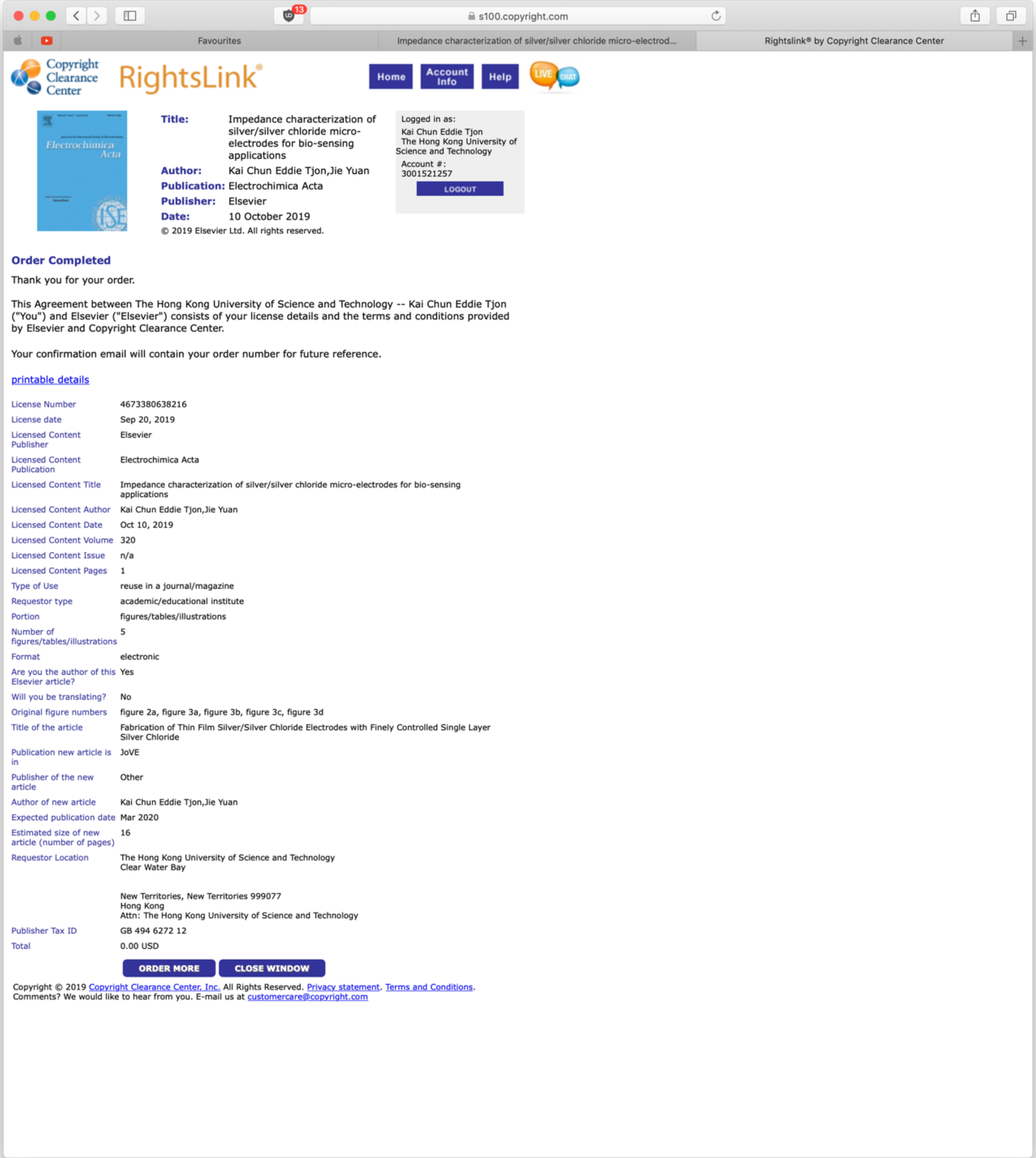

**Title:** Impedance characterization of silver/silver chloride micro-electrodes for bio-sensing applications
**Author:** Kai Chun Eddie Tjon,Jie Yuan
**Publication:** Electrochimica Acta
**Publisher:** Elsevier
**Date:** 10 October 2019

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| Publication new article is in | JoVE |
| Publisher of the new article | Other |
| Author of new article | Kai Chun Eddie Tjon,Jie Yuan |
| Expected publication date | Mar 2020 |
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