FINAL SCRIPT: APPROVED FOR FILMING

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Title: Probing Surface Electrochemical Activity of Nanomaterials using a Hybrid Atomic Force Microscope-Scanning Electrochemical Microscope (AFM-SECM)

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Yes, all images will be recorded using software so scope kit is not needed.**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Vid NOTE: A lot of it was shot in 4K because needed to get closer up on the protocol, but the interviews were also shot in 4K so need to scale to fit in 1080 timeline. All of the people who were interviewed had thick accents so they were a little difficult to understand.

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Wen Zhang:** This protocol employs AFM-SECM to demonstrate the electrochemical mapping and measurement on faceted crystalline nanomaterials and nanobubbles in water.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Wen Zhang:** AFM-SECM is capable of mapping the electrochemically active sites based on the tip current images and enables simultaneous acquisition of nanoscale surface structural and electrochemical information.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Xiaonan Shi:** The sample preparation for this method requires that the solid particles are immobilized on the substrate completely and the binding between samples and substrates ensures electrical conductivity. The choice of the redox mediator is critical as well.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Vid NOTE: File 6H0A0811 - is shot 1.3.1, it was not slated

1.4. **Qingquan Ma:** Visual demonstration is necessary to clearly show the detailed and delicate operations in this protocol such as sample preparation, system set-up and the imaging process.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Sample Preparation for AFM/SECM

2.1. Deposit 10 microliters of epoxy on a cleaned silicon wafer using a pipette tip and tile it with a clean glass slide **[1]**. After about 5 minutes, drop 10 microliters of the copper oxide nanoparticle suspension on the epoxy-coated silicon wafer substrates **[2]**. Then, vacuum dry the substrate at 40 degrees Celsius for 6 hours **[3]**. *Videographer: This step is important!*

2.1.1. Talent putting epoxy on the silicon wafer and tiling it.

2.1.2. Talent dropping nanoparticle suspension on the wafer. *Video Editor: Show figure 1 B here as an inset.*

2.1.3. Talent putting the substrate to dry. Vid NOTE: 2.1.3 was shot out of order and was the very last shot we did in this protocol (file 6H0A0887)

2.2. To prepare oxygen nanobubbles, directly inject compressed oxygen through a tubular ceramic membrane into deionized water. Deposit 1.8 milliliters of the nanobubble suspension on a gold substrate in the electrochemical sample cell and stabilize it for 10 minutes **[1]**.

2.2.1. Talent depositing the nanobubble suspension onto a gold substrate.

3. Setup and Operation of AFM-SECM

3.1. Replace the existing sample chuck with the SECM chuck **[1]**, then screw it in place using two M3 6-millimeter socket head cap screws and a 2.5-millimeter hex wrench **[2]**. Install the strain-release module onto the AFM scanner **[3]** and connect it to the working electrode connector on the spring connector block with an extension cable **[4]**.

3.1.1. Talent replacing the existing sample chuck with SECM chuck. NOTE: Use 2nd take

3.1.2. Talent screwing the chuck in place.

3.1.3. Talent installing the stain-release module.

3.1.4. Talent connecting the module to the working electrode connector.

3.2. Double-click the two software icons to initialize the AFM system and the bipotentiostat control interface **[1]**.

3.2.1. Talent at the computer double-clicking the software icons. Vid NOTE: there is only 1 take and it's labeled shot 3.2.1 take 2

3.3. Prepare the electrostatic discharge field service package including an antistatic pad, electrostatic discharge protective probe stand, wearable anti-static gloves, and wrist strap **[1]**.

3.3.1. Contents of an electrostatic field service package (antistatic pad, electrostatic discharge protective probe stand, wearable anti-static gloves, and wrist strap).

3.4. To prevent the AFM scanner from exposure to liquid, use a protective boot during AFM-SECM testing **[1]**. Put the probe holder onto the electrostatic discharge protective probe stand **[2]** and use a pair of plastic tweezers to attach the protective boot to the tip holder **[3]**.

3.4.1. Talent demonstrating the protective boot.

3.4.2. Talent putting the probe holder onto the electrostatic discharge protective probe stand. Vid NOTE: use take 2 as talent was able to do the step more smoothly than 1st take

3.4.3. Talent attaching the protective boot to the tip holder.

3.5. Then, align the small cut in the protective boot to the notch in the probe holder **[1]**. Open the box of AFM-SECM probes with a tip tweezer and grab the probe from both sides of the grooves **[2]**.

3.5.1. Talent aligning the small cut of the boot to the notch in the holder. Vid NOTE: take 3 (file 6H0A0863) - I had the talent indicate where the small cut was since it was very small and not visible unless you manipulated the protective boot. This is probably the best take to use to demonstrate this as you couldn't see the small cut in the previous two takes

3.5.2. Talent opening the probe box and grabbing the probe.

3.6. Use the disk gripper to hold the probe holder on the stand and put the probe wire into the hole of the stand **[1]**, then slide the probe into the slot of the probe holder **[2]**. After the probe is inside the slot, use the flat end of the tweezer to push it in **[3]**. *Videographer: This step is important!*

3.6.1. Talent putting the probe wire into the hole of the stand while using the disk gripper to hold the probe holder. NOTE: 3.6.1 – 3.6.3 in one take

3.6.2. Talent sliding the probe into the probe holder.

3.6.3. Talent pushing the probe in with the tweezer.

3.7. Attach the whole probe holder to the scanner **[1]** and use the PTFE tip tweezer to grab the wire right below the copper ring and connect it to the module **[2]**. Then, put the scanner back to the dovetail **[3]**.

3.7.1. Talent attaching the probe holder to the scanner.

3.7.2. Talent grabbing the wire right below the copper ring and connecting it to the module.

3.7.3. Talent putting the scanner back to the dovetail.

3.8. Put the previously assembled electrochemical sample cell with the test sample on the central point of the SECM chuck **[1]**. Then, connect the pseudo-reference electrode and the counter-electrode to the spring connector block **[2]**.

3.8.1. Talent putting the EC sample cell on the central point of the SECM chuck.

3.8.2. Talent connecting the counter-electrode and the pseudo-reference electrode to the spring connector block. Vid NOTE: 4 takes, the talent had a little trouble attaching the wires so we did multiple takes

3.9. In the AFM-SECM software, select SECM-Peak Force QNM to load the workspace. In **Setup**, load the SECM probe **[1]**, and then align a laser on the tip using an alignment station **[2]**.

3.9.1. SCREEN: 3.9.1.mov. 0:56 – end. *Video Editor: Speed this up as necessary but make sure to show setup selections.*

3.9.2. SCREEN: 3.9.2.mov. *Video Editor: Speed this up as necessary.*

3.10. Go to **Navigation** and move the scanner downwards slowly to focus on the sample surface. Adjust the position of the electrochemical sample cell slightly to make sure the scanner does not touch the glass cover of the sample cell while moving. After focusing on the sample, click **Update Blind Engage Position [1]**.

3.10.1. SCREEN: 3.10.1.mov.

3.11. Click **Move to Add Fluid Position** and add approximately 1.8 milliliters **[1]** of the buffer solution into the sample cell, making sure that the level of the solution is lower than the glass cover and that the wires are immersed in the solution **[2-TXT]**. Use a pipette to agitate the solution to remove any bubbles and wait for 5 minutes **[3]**.
Videographer: This step is difficult and important!

3.11.1. SCREEN: 3.11.1.mov. 0:00 – 0:15.

3.11.2. Talent adding buffer to the sample cell. **TEXT: Filter solution before use ; Store at 4°C after preparation**

3.11.3. Talent agitating the solution with a pipette. Vid NOTE: we did two takes, the first take he agitated the solution with a pipette to get rid of the air bubbles. In the second take, which is a continuation of the first take, he used a hypodermic needle to get rid of an air bubble because it was not possible to reach it with the pipette

3.12. Click **Move to Blind Engage Position**, which will make the tip move back into the buffer solution. Adjust the laser slightly to make sure the laser is aligned on the tip **[1]**.

3.12.1. SCREEN: 3.12.1.mov. 0:00 – 0:47.

3.13. Open the electrochemical workstation software and click on the **Technique** command on the toolbar to open the tech selector. Select **Open Circuit Potential – Time** and use the default setting to run the OCP measurement, which should be almost zero and stable **[1]**.

3.13.1. SCREEN: 3.13.1.mov.

3.14. Click the **Technique** command again and select **Cyclic Voltammetry**, then enter the Cyclic Voltammetry parameters and proceed with SECM imaging **[1]**. Go back to the AFM-SECM software and click **Engage [2]**.

3.14.1. SCREEN: 3.14.1.mov. *Video Editor: Speed this up as necessary.*

3.14.2. SCREEN: 3.14.2.mov.

3.15. Next, select **Chronoamperometry** and set the chronoamperometry parameters with the initial E as -0.4 Volts, the pulse width as 1000 seconds, and the same sensitivity as the CV scan **[1]**.

3.15.1. SCREEN: 3.15.1.mov. 0:00 – 0:40.

3.16. With the program is running, go back to the AFM-SECM software, check the real-time reading on the strip chart, and click on **Start**. Save the images in the AFM-SECM software **[1]**.

3.16.1. SCREEN: 3.15.2.mov.

3.17. Using the electrochemical sample cell as a clean water container, move the tip in and out of the liquid with the blind engage functions in the navigation panel, changing the water three times **[1]**. Then, use clean wipes to carefully remove residual water from the probe holder and put the probe back in the probe box **[2]**.

3.17.1. Tip moving in and out of the sample cell.

3.17.2. Talent wiping the water from the probe holder with wipes.

Results

4. Results: Topography and current imaging of ONBs and Cu_2O NPs by AFM-SECM

4.1. This protocol was used to characterize individual oxygen nanobubbles, revealing both morphological and electrochemical information **[1]**. A comparison of the topography and the current image demonstrates the correlation between the locations of the nanobubbles and the current spots **[2]**.

4.1.1. LAB MEDIA: Figure 9 A and B.

4.1.2. LAB MEDIA: Figure 9 A and B. *Video Editor: Emphasize A when VO says "topography" and B when VO says "current image".*

4.2. The topography and current images of copper-oxide nanoparticles are shown here. The tip current image indicates that the nanoparticle visible in the topography image is associated with an evident electric current spot, whereas the background current corresponds to the flat silicon substrate **[1]**.

4.2.1. LAB MEDIA: Figure 10 A and B.

4.3. Here are five representative cyclic voltammetry curves of the AFM-SECM tip at approximately 1 millimeter away from the substrate. The diffusion-limited tip current did not decrease with time **[1]**.

4.3.1. LAB MEDIA: Figure 11 A.

4.4. The changes of the tip current as the tip moves towards the sample surface are plotted here. The AFM-SECM tip approached the substrate surface in the Z direction until it reached a setpoint indicating the physical tip-substrate contact or bending **[1]**.

4.4.1. LAB MEDIA: Figure 11 B.

Conclusion

5. Conclusion Interview Statements

5.1. **Qingquan Ma:** When attempting this protocol, make sure that the solid particles are immobilized on the substrate completely with electrical conductivity and that there are no bubbles in the solution in the EC sample cell.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.11.3.*

5.2. **Xiaonan Shi:** These sample preparation methods are relevant to a wide range of applications that involve nano-objects, especially for nano-object characterization.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

5.3. **Xiaonan Shi:** The AFM-SECM technique can be used to acquire simultaneous topography and electrochemistry images at nanoscale, which is important in the development and application of nanomaterials in research fields such as material science, chemistry, and life science.

5.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Vid NOTE: File 6H0A0814 is interview shot 5.3.1 we had to stop about the 1:30 mark because somebody was speaking in the background and the talent did not speak again in this file, we did additional takes of this shot in file 6H0A0815.