FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68755
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Title: Fabrication of a Solution-gated Indium–Tin–Oxide-based One-piece Transistor Enabling Sensitive Biosensing

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

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

Authors: Please create screen capture videos of the shots labeled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible: **https://review.jove.com/account/file-uploader?src=20970323**

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

Current Protocol Length
Number of Steps: 30
Number of Shots: 55

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Toshiya Sakata:** The scope of this research is to demonstrate a simple and rapid method for fabricating a solution-gated one-piece transistor for pH sensing and highly sensitive biosensing.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 1*

What technologies are currently used to advance research in your field?

1.2. **Ritsu Katayama Naganuma:** To increase the detection sensitivity of solution-gate FET biosensors, the technologies of miniaturization and lower dimensioning of materials have been mainly used.

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

What significant findings have you established in your field?

1.3. **Ritsu Katayama Naganuma:** We have found that a solution-gated FET can be easily fabricated by simply etching a portion of a conductive ITO thin film to a thickness that exhibits semiconductivity, providing an "all-by-ITO" technology.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.8*

What advantage does your protocol offer compared to other techniques?

1.4. **Ritsu Katayama Naganuma:** Using this method, a solution-gated FET can be fabricated more easily than with other protocols, and the fabricated device shows a steep subthreshold slope which is related to sensitivity.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 3*

What new scientific questions have your results paved the way for?

1.5. **Toshiya Sakata:** The proposed method provides an "all-by-ITO" technology, leading a pH sensing and highly sensitive biosensing because the source, drain electrodes, and channel are fully integrated without any interfaces.

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

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Ritsu Katayama Naganuma:** We expect that JoVE will effectively explain the fabrication of the device by etching with current measurement in this study by visualizing the process.

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Ritsu Katayama Naganuma:** The protocol for this study is simple and we would be grateful if someone would use it and I hope it will start a collaborative study based on it. We also hope it will save us time in teaching new students.

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

Protocol

2. Fabrication of One-Piece ITO-ISFET

Demonstrator: Ritsu Katayama Naganuma

2.1. To begin, wash the glass substrate by sonicating it in acetone, methanol, and water for 5 minutes each **[1]**. Using a nitrogen gas blower, dry the substrate thoroughly **[2]**, and bake it on a hot plate at 110 degrees Celsius for more than 5 minutes to ensure it is completely dry **[3]**.

2.1.1. WIDE: Talent placing the glass substrate in a sonication bath filled with acetone.

2.1.2. Talent drying the substrate using a nitrogen gas blower.

2.1.3. Talent placing the dried substrate on a hot plate set to 110 degrees Celsius.

2.2. Spin-coat the dried glass substrate with an OFPR-800 *(O-P-F-R-Eight-Hundred)* layer at 500 rpm for 5 seconds, followed by 3000 rpm for 30 seconds **[1-TXT]**.

2.2.1. Talent placing the substrate onto the spin coater and setting it to 500 revolutions per minute for 5 seconds. **TXT: Examine the surface for uniform coating**

2.3. Prebake the coated substrate on a hot plate at 110 degrees Celsius for 5 minutes **[1]**. Allow the substrate to cool down to room temperature before proceeding **[2]**.

2.3.1. Talent placing the coated substrate on a hot plate set to 110 degrees Celsius.

2.3.2. Talent removing the substrate from the hot plate and setting it aside to cool to room temperature.

2.4. Position the photomask film on the photoresist-coated side of the substrate and use tape to fix it securely in place **[1]**. Then, expose the substrate to ultraviolet light using a photolithography machine for 40 seconds **[2]**.

2.4.1. Talent placing the photomask film on top of the coated substrate and fixing it with tape.

2.4.2. Talent placing the prepared substrate into the photolithography machine.

2.5. Now, take the substrate out of the photolithography machine and carefully remove the photomask from the surface **[1]**.

2.5.1. Talent removes the exposed substrate and removes the photomask.

2.6. To develop the photoresist, immerse the substrate in NMD-3 *(N-M-D-Three)* developer for 1 minute **[1]** and verify that a sharp pattern is formed **[2]**. Then, dip the substrate in water to wash away the developer **[3]**.

2.6.1. Talent submerging the substrate in a container filled with NMD-3 developer.

2.6.2. Talent inspecting the photoresist pattern for clarity and sharpness.

2.6.3. Talent dipping the developed substrate into a water bath for rinsing.

2.7. After drying the washed substrate by blowing nitrogen gas, bake it on a hot plate at 110 degrees Celsius for 5 minutes **[1]**.

2.7.1. Talent placing the dried substrate on a hot plate set to 110 degrees Celsius for post-baking.

2.8. To begin deposition, fix the substrate onto a substrate holder using tape **[1]** and introduce it into the vacuum chamber of a sputtering machine **[2]**. Pump down the sputtering chamber pressure to below 10^{-3} pascals **[3]**.

2.8.1. Talent placing the substrate onto a holder and taping it down securely.

2.8.2. Talent inserting the taped holder into the vacuum chamber of the sputtering machine.

2.8.3. Show the sputtering machine interface as the vacuum pump is engaged and the chamber pressure drops below 10^{-3} pascals.

2.9. Then, deposit a 100-nanometer thick layer of indium tin oxide via radio frequency sputtering at 4 nanometers per minute under argon, without substrate heating **[1].**

2.9.1. Show the sputtering parameters on instrument display, including target composition, 4 nanometers per minute deposition rate.

2.10. To lift off the photoresist, sonicate the substrate in acetone, methanol, and then water for 5 minutes **[1-TXT]**.

2.10.1. Talent placing the substrate in an acetone bath and starting sonication. **TXT: Ensure no ITO fragments remain on substrates**

2.11. Then, blow the substrate dry using a nitrogen gas blower **[1]** and bake it on a hot plate at 110 degrees Celsius until fully dry **[2]**.

2.11.1. Talent blowing the substrate surface dry with nitrogen gas.

2.11.2. Talent placing the substrate on a hot plate set to 110 degrees Celsius for drying.

2.12. To spin-coat the clean substrate with SU-8 *(S-U-Eight)* 3005 *(Three-Zero-Zero-Five),* spin at 500 rpm for 5 seconds, followed by 6000 rpm for 30 seconds **[1-TXT]**. Prebake the SU-8-coated substrate on a hot plate set to 95 degrees Celsius for 5 minutes **[2-TXT]**.

2.12.1. Talent placing the substrate onto the spin coater and setting the initial spin to 500 revolutions per minute for 5 seconds. **TXT: Check the coated surface for uniformity**

2.12.2. Talent placing the coated substrate on a hot plate at 95 degrees Celsius. **TXT: Cool the substrate to RT**

2.13. Then, place the photomask film on the photoresist-coated substrate and secure it with tape to prevent any movement during exposure **[2]**.

2.13.1. Talent aligning the photomask film over the SU-8 coated substrate and taping it onto the substrate.

2.14. Expose the SU-8-coated substrate to ultraviolet light for 7 seconds using a photolithography machine **[1]**. Then, remove the substrate from the machine and carefully detach the photomask **[2]**.

2.14.1. Talent placing the substrate in the photolithography machine and initiating the 7-second ultraviolet exposure.

2.14.2. Talent removing the substrate and peeling off the photomask.

2.15. Postbake the exposed substrate first at 65 degrees Celsius for 2 minutes, then at 95 degrees Celsius, and continue baking for 5 minutes **[1]**.

2.15.1. Talent transferring the substrate to a hot plate set to 95 degrees Celsius.

2.16. Immerse the substrate in SU-8 developer for 3 minutes under strong agitation to develop the photoresist **[1]**. Rinse the substrate in 2-propanol for 1 minute **[2-TXT]** and dry the developed substrate using a nitrogen gas blower **[3]**.

2.16.1. Talent places the substrate in a SU-8 developer container and shaking vigorously.

2.16.2. Talent dipping the developed substrate in 2-propanol for rinsing. **TXT: Ensure that the photoresist is fully developed**

2.16.3. Talent blowing nitrogen gas across the surface of the cleaned and developed substrate.

2.17. To begin forming the semiconductive indium tin oxide channel, prepare 0.1 molar hydrochloric acid solution **[1]**. Then, connect the source and drain electrodes to a semiconductor parameter analyzer **[2]**.

2.17.1. WIDE: Talent measuring and preparing 0.1 molar hydrochloric acid in a labeled beaker.

2.17.2. Talent attaching source and drain pads on the substrate to the semiconductor parameter analyzer.

2.18. Turn on the B1500A *(B-Fifteen-Hundred)* device and start the EasyEXPART *(Easy-Expart)* software. Then, open the **Workspace** interface to begin setup **[1]**.

2.18.1. SCREEN: 68755_screenshot_1ver2.avi: 00:00-00:02, 00:09-00:12, 00:21-00:22, 00:55-00:57

2.19. On the analyzer interface, select the **Classic Test** tab, then choose the **I/V-t Sampling** *(I-V-T-Sampling)* option. Set the sampling interval to 0.5 seconds **[1]**. Apply a voltage of 1 volt between the source and drain electrodes and record the initial current **[2]**.

2.19.1. SCREEN: 68755_screenshot_2ver2.avi: 00:00-00:06; 68755_screenshot_3ver2.avi: 00:00-00:06

2.19.2. SCREEN: 68755_screenshot_4ver2.avi: 00:00-00:19

2.20. Using a micropipette, place a 30-microliter drop of the prepared hydrochloric acid solution directly onto the exposed indium tin oxide channel area **[1-TXT]**.

2.20.1. Talent using a micropipette to carefully dispense a 30 microliter drop of hydrochloric acid onto the channel. **TXT: Ensure the droplet fully covers the entire channel region**

2.21. Monitor the current between the source and drain electrodes continuously and observe the conductivity change during etching. Continue the process until the current drops to 10 percent of the initial value **[1]**.

2.21.1. SCREEN: 68755_screenshot_5ver2.avi: 00:04-00:10; 68755_screenshot_6ver2.avi: 00:35-00:45

2.22. Then, immediately rinse the etched indium tin oxide channel with deionized water to stop the etching once the current reaches 10% of the initial current **[1]**. Dry the one-piece transistor by gently blowing nitrogen gas over the rinsed substrate using a nitrogen gas blower **[2-TXT]**.

2.22.1. Talent using a pipette to rinse the channel area thoroughly with deionized water.

2.22.2. Talent drying the device with gentle nitrogen gas flow focused on the channel area. **TXT: Store the dried devices in a vacuum desiccator until further use**

3. Electrical Measurements of One-piece ITO-ISFET

3.1. Using a test fixture, connect the source and drain electrodes of the one-piece transistor to the semiconductor parameter analyzer **[1]**. Place a silicone O-ring around the surface of the indium tin oxide channel to form a liquid well **[2]**.

3.1.1. Talent connecting probe wires from the test fixture to the source and drain terminals on the one-piece transistor.

3.1.2. Talent positioning and gently pressing a silicone O-ring onto the substrate to surround the ITO channel.

3.2. Add 30 microliters of phosphate buffer or another standard pH buffer solution into the silicone O-ring well while making full contact with the indium tin oxide channel surface **[1]**.

3.2.1. Talent pipetting 30 microliters of buffer solution into the silicone well.

3.3. Then, insert a silver or silver chloride reference electrode into a saturated potassium chloride solution, which is connected to the gate electrolyte by a salt bridge **[1]**. Connect the silver or silver chloride reference electrode to the semiconductor parameter analyzer so that it functions as the gate electrode **[2]**.

3.3.1. Talent placing the Ag/AgCl reference electrode into a pippet chip containing a salt bridge and saturated potassium chloride.

3.3.2. Talent attaching the wire from the reference electrode to the gate input port of the semiconductor parameter analyzer.

3.4. On the semiconductor parameter analyzer, select the **Classic Test** tab, then choose the **I/V Sweep** mode **[1]**. Connect the source electrode to ground to establish a reference potential **[2]**.

3.4.1. SCREEN: 68755_screenshot_7ver2.avi: 00:00-00:05

3.4.2. SCREEN: 68755_screenshot_8ver2.avi: 00:00-00:05

3.5. Set the applied drain voltage to a constant value of 1 volt **[1]**. Set the sweep range of gate voltages, from minus 0.8 volts to plus 0.8 volts, and adjust delay, integration time, and measurement interval **[2]**.

3.5.1. SCREEN: 68755_screenshot_9ver2.avi: 00:00-00:06

3.5.2. SCREEN: 68755_screenshot_10 ver2.avi: 00:05-00:09, 00:12-00:15, 00:19-00:22, 00:25-00:29

3.6. Then, set the number of iterations to 10 cycles and use the data from the final cycle for analysis **[1]**. Simultaneously, ensure that the leak current between the source and gate electrodes is recorded and start the measurement **[2]**.

3.6.1. SCREEN: 68755_screenshot_11ver2.avi: 00:00-00:07

3.6.2. SCREEN: 68755_screenshot_12ver2.avi: 00:03-00:05; 68755_screenshot_13ver2.avi: 00:02-00:08

3.7. On the semiconductor parameter analyzer, select the **CMOS** category in the **Application Test** tab, then choose the **Id-Vd** mode **[1]**. Set the gate voltage to increase sequentially from 0 volts to 0.8 volts in 0.1 volt intervals **[2]**. For each gate voltage value, set the drain voltage to sweep from 0 volts to 1 volt in 0.01-volt intervals and start the measurement **[3]**.

3.7.1. SCREEN: 68755_screenshot_14ver2.avi: 00:00- 00:15

3.7.2. SCREEN: 68755_screenshot_15ver2.avi: 00:00- 00:13

3.7.3. SCREEN:68755_screenshot_16ver2.avi: 00:00- 00:25

3.8. Now, remove the silicone O-ring from around the channel surface **[1]**, then rinse the channel area thoroughly with deionized water **[2]**. Dry the one-piece indium tin oxide device using a nitrogen gas blower **[3-TXT]**.

3.8.1. Talent gently peeling off the silicone O-ring from the substrate.

3.8.2. Talent rinsing the ITO channel area using a pipette filled with deionized water.

3.8.3. Talent drying the cleaned device with a nitrogen gas blower. **TXT: Store the fully dried device in a vacuum desiccator**

Results

4. Results

4.1. The indium tin oxide ion-sensitive field-effect transistor demonstrated a steep subthreshold slope of approximately 81 millivolts per decade**,** indicating near-ideal switching behavior at 25 degrees Celsius **[1].**

4.1.1. LAB MEDIA: Figure *3A* *Video editor: Highlight the text "SS = 81 mV/dec." on the inset graph*.

4.2. The gate leakage current remained four orders of magnitude lower than the drain current, even when the channel was directly exposed to the electrolyte solution **[1].** The drain current increased with the drain voltage and showed saturation behavior at each gate voltage, confirming good field-effect transistor characteristics **[2].**

4.2.1. LAB MEDIA: Figure 3B.

4.2.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the set of blue curves, especially focusing on the highest curve labelled "VGS = 0.8 V*".

4.3. The on-off current ratio exceeded 10,000 for channel thicknesses below 20 nanometers **[1]** but dropped drastically at greater thicknesses **[2].**

4.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight the cluster of red dots above 10^4 on the left side of the vertical dashed line.*

4.3.2. LAB MEDIA: Figure 4. *Video editor: Highlight the group of red dots near 10^0 to 10^1 on the right side of the dashed line.*

4.4. The one-piece indium tin oxide ion-sensitive field-effect transistor responded linearly to pH changes, exhibiting a voltage shift of approximately 51 millivolts per pH unit **[1].** The pH sensitivity increased slightly after 5 days of wet storage, approaching the ideal Nernstian limit **[2]**, and was retained even after 16 days **[3].**

4.4.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the dashed trendline labelled "51 mV/pH" in the inset plot.*

4.4.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the second bar (5 days)*

4.4.3. LAB MEDIA: Figure 5B. *Video editor: Highlight the third bar (16 days)*

1. substrate
Pronunciation link:
https://www.merriam-webster.com/dictionary/substrate
IPA: /ˈsʌbˌstreɪt/
Phonetic Spelling: sub-strate

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2. sonicating / sonication
Pronunciation link:
https://www.merriam-webster.com/dictionary/sonication
IPA: /ˌsɒnɪˈkeɪʃən/ (American)
Phonetic Spelling: son-i-KAY-shun

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3. acetone
Pronunciation link:
https://www.merriam-webster.com/dictionary/acetone
IPA: /ˈæsɪˌtoʊn/
Phonetic Spelling: AS-i-tone

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4. methanol
Pronunciation link:
https://www.merriam-webster.com/dictionary/methanol
IPA: /ˈmɛθəˌnɔl/
Phonetic Spelling: METH-uh-nol

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5. photomask
Pronunciation link:
https://www.merriam-webster.com/dictionary/photomask
IPA: /ˈfoʊ-toʊ-ˌmæsk/
Phonetic Spelling: FO-to-mask

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6. photolithography
Pronunciation link:
https://www.merriam-webster.com/dictionary/photolithography
IPA: /ˌfoʊtoʊlɪˈθɑgrəfi/
Phonetic Spelling: FO-to-li-THOG-ra-fee

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7. developer (in context of photoresist)
Pronunciation link:
https://www.merriam-webster.com/dictionary/developer
IPA: /dɪˈvɛləpər/
Phonetic Spelling: di-VEL-uh-per

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8. NMD-3

No confirmed link found (proper name of a specific developer formulation).
IPA: /ˌɛn-ɛm-ˈdi-θri/
Phonetic Spelling: en-em-DEE-three

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9. photoresist
Pronunciation link:
https://www.merriam-webster.com/dictionary/photoresist
IPA: /ˈfoʊˌtoʊrɪˈzɪst/
Phonetic Spelling: FO-to-re-zist

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10. indium tin oxide

- **indium**:
  Pronunciation link: https://www.merriam-webster.com/dictionary/indium
  IPA: /ˈɪndiəm/
  Phonetic Spelling: IN-dee-əm
- **tin**:
  Pronunciation link: https://www.merriam-webster.com/dictionary/tin
  IPA: /tɪn/
  Phonetic Spelling: tin
- **oxide**:
  Pronunciation link: https://www.merriam-webster.com/dictionary/oxide
  IPA: /ˈɑksaɪd/ or /ˈɑkˌsaɪd/
  Phonetic Spelling: OX-ide

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11. sputtering
Pronunciation link:
https://www.merriam-webster.com/dictionary/sputtering
IPA: /ˈspʌtərɪŋ/
Phonetic Spelling: SPUT-er-ing

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12. microliter (µL)
Pronunciation link:
https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪkrəˌli·tər/
Phonetic Spelling: MY-kro-lee-ter

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13. SU-8
No confirmed link found (specific resin name).
IPA: /ˌɛs-ju-ˈeɪt/
Phonetic Spelling: S-U-eight

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14. hydrochloric acid

- **hydrochloric**:
  Pronunciation link: https://www.merriam-webster.com/dictionary/hydrochloric
  IPA: /ˌhaɪdrəˈklɔrɪk/
  Phonetic Spelling: hy-dro-KLO-rik
- **acid**:
  Pronunciation link: https://www.merriam-webster.com/dictionary/acid
  IPA: /ˈæsɪd/
  Phonetic Spelling: AS-id

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15. phosphate
Pronunciation link:
https://www.merriam-webster.com/dictionary/phosphate
IPA: /ˈfɑsˌfeɪt/
Phonetic Spelling: FOS-fate

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16. analyzer (as in parameter analyzer)
Pronunciation link:
https://www.merriam-webster.com/dictionary/analyzer
IPA: /əˈnæl̩əˌzɚ/
Phonetic Spelling: uh-NAL-uh-zer

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17. subthreshold
No confirmed link found (compound technical term).
IPA: /ˌsʌbˈθreʃhoʊld/
Phonetic Spelling: sub-THRESH-hold