FINAL SCRIPT: APPROVED FOR FILMING

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Title: Low-Cost Polyethylene Terephthalate Lamination Microfluidics Designs for Multiplexed Zebrafish Imaging

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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? **Yes**

   AmScope ZM-4TNZ3-80AM-3M3

   Videographer: Please capture the shots labelled SCOPE with a SCOPE KIT
   SCOPE: 4.3.1

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

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**, 500 m apart

Current Protocol Length

Number of Steps: 21
Number of Shots: 34

Introduction

Videographer: Obtain headshots for all authors.

REQUIRED:

1.1. **Qing Deng:** Our lab is interested in the mechanisms regulating neutrophil migration and activation in tissue injury, infection, and cancer, with a focus on characteristics and biological significance of intracellular calcium signaling in wound healing.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.5*

What are the current experimental challenges?

1.2. **Shelly Tan:** Conventional microfluidic design methods are costly and complex, slowing progress in fields like developmental biology that require diverse environments. We sought a rapid, efficient approach for developing and testing individual designs.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Shelly Tan:** This construction method provides greater design flexibility and spatial resolution than similarly simple protocols, while enabling faster, easier iteration than standard PDMS devices. Using it, we developed the RADISH to simultaneously image multiple zebrafish embryos within a single field of view.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.4*

What research questions will your laboratory focus on in the future?

1.4. **Qing Deng:** With this entrapment device that supports high-resolution live imaging, we are set to understand how diverse signals, including chemical and mechanical stimuli, are integrated to coordinate inflammation and wound healing.

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

Videographer: Obtain headshots for all authors.

Protocol

2. Assembly of the Polyethylene Terephthalate (PET) Microfluidics Device for Imaging Multiple Live Zebrafish Embryos

Demonstrator: Shelly Tan

2.1. To begin, design the pattern for the device using the software of choice **[1]**. Depending on the age and size of the embryo, adjust the width of the channel and the size of the central imaging chamber for the best fit **[2]**. Save each layer as an individual image file with identical dimensions **[3]**.

2.1.1. WIDE: Talent in front of the computer creating the device pattern.

2.1.2. SCREEN: 67313_2-1-2.mp4 00:00-00:35.

2.1.3. SCREEN: 67313_2-1-3.mp4 00:11-00:17 .

2.2. Now, initialize the cutting machine according to the manufacturer's instructions **[1]**.

2.2.1. Talent setting up the cutting machine.

2.3. In the design workspace, select the newly uploaded designs **[1]**. Group patterns intended for the same thickness of PET *(Pet)* sheet together in the same cut **[2-TXT]**.

2.3.1. SCREEN: 67313_2-3-1.mp4 00:00-00:16 .

2.3.2. SCREEN: 67212_2-3-2.mp4 00:00-00:19 . **TXT: If required, readjust the size of the patterns to the correct dimensions**

2.4. Confirm the number and type of patterns in the cut, then select the **cut material [1]**.

2.4.1. SCREEN: 67313_2-4-1.mp4 00:06-00:23.

2.5. Next, attach the PET sheet to the cutting mat at the desired thickness, pressing firmly to remove air bubbles **[1],** and load the mat into the cutting machine **[2]**. Follow onscreen instructions to prompt the cutting machine to begin the cut **[3]**. Then, remove the finished cuts from the cutting mat **[4]**.

2.5.1. Talent attaching the PET sheet to the cutting mat and presses it.

2.5.2. Talent loading the mat into the cutting machine.

2.5.3. SCREEN: 67313_2-5-3.mp4 00:00-00:13

2.5.4. Talent removing the finished cuts from the cutting mat.

2.6. Now, align the layers on a coverslip glass, either unassisted or with precut registration marks **[1-TXT]**.

2.6.1. Talent aligning the layers on coverslip glass. **TXT: Fasten multiple layers together before lamination for simultaneous alignment**

2.7. Secure the layers with office tape to avoid shifting during lamination **[1]**. Depending on the device size and laminator capacity, mount small designs to a larger backing with more tape to avoid jamming or clogging the laminator feed **[2]**.

2.7.1. Talent securing layers with office tape.

2.7.2. Talent mounting small designs to a larger backing using tape.

2.8. Afterward, turn on the laminator and laminate according to the manufacturer's instructions **[1]**. Remove the office tape before aligning the next layer **[2]**.

2.8.1. Talent turning on the laminator and initiating the lamination process.

2.8.2. Talent removing office tape. **TXT: Repeat the process until all layers are adhered**

2.9. Using cyanoacrylate glue, secure the device to a 35-millimeter dish drilled with a three-quarter-inch hole **[1]**.

2.9.1. Talent securing the device to the drilled dish with cyanoacrylate glue.

2.10. Then, waterproof the laminate by thoroughly coating the outer device edges with cyanoacrylate glue **[1]**. Use enough glue to fully cover the outer edges without gaps **[2-TXT]**.

2.10.1. Talent applying cyanoacrylate glue to the outer edges of the PET laminate.

2.10.2. Close-up of glue coverage, ensuring no gaps. **TXT: Allow the glue to cure for at least 2 h before use**

3. Positioning of the Zebra Fish Embryos within the RADISH and Preparation for Imaging

3.1. To begin, raise zebrafish embryos to at least 2 days post fertilization in E3 or another

suitable embryo-rearing medium **[1-TXT]**.

3.1.1. Talent at the working bench with zebrafish embryos being raised in a dish filled with E3 medium placed in front. **TXT: Stain/treat the embryos as per the experiment** *Videographer: Please take a still image of talent performing this action. Make sure that it is at least a half-body shot with the talent's face visible and zoom out so we have room for cropping.*

3.2. Then, anesthetized the embryos using 164 milligrams per liter of MS-222 *(M-S-Two-Twenty-Two)* **[1]**.

3.2.1. Talent adding MS-222 to a dish containing zebrafish embryos to anesthetize them.

3.3. Next, fill the holding dish containing the mounted device with 5 milliliters of E3 or another embryo medium containing 164 milligrams per liter of MS-222 **[1]**. Using a transfer pipette, deposit one anesthetized embryo into each holding chamber of the device **[2]**.

3.3.1. Talent filling the holding dish with 5 milliliters of medium containing MS-222.

3.3.2. Talent using a transfer pipette to place one anesthetized embryo into the holding chamber.

3.4. Then, using a hair loop, orient the embryos with the tail of each embryo protruding into the central imaging chamber, and the yolk immobilized in the wedge-shaped channel **[1]**. If desired, secure the head of the embryo by adding 1.5% low-melt agarose into the holding chamber using a micropipette **[2]**.

3.4.1. Talent using a hair loop to properly position the embryos in the device, ensuring correct orientation.

3.4.2. Talent using a micropipette to add 1.5% low-melt agarose into the holding chamber, securing the embryo's head.

3.5. Finally, move the device with embryos to the imaging system of choice to commence imaging **[1]**.

3.5.1. Talent carefully transfers the device with embryos to the imaging system.

4. Imaging of Calcium Transients after Zebra Fish Wounding Using Rotational Assistant for Danio Imaging of Subsequent Healing (RADISH)

4.1. Select the appropriate imaging system and objective for the experiment **[1]**.

4.1.1. Talent selecting the imaging system and objective lens suitable for the experiment.

4.2. Adjust the sample focus and imaging parameters, for optimal signal-to-noise ratio and acquisition speed **[1-TXT]**.

4.2.1. SCREEN: 67313_4-2-1.mp4 00:00-00:19. **TXT: Prepare the embryos for imaging in advance**

4.3. To wound the embryo via manual transection, apply pressure with a scalpel blade across the tail fin tissue at the tip of the notochord under a stereomicroscope **[1-TXT]**. Then, return the device to the stage **[2]**.

4.3.1. SCOPE: Talent using a scalpel to transect the tail fin tissue at the tip of the notochord under the stereomicroscope. **TXT: Embryo used: Tg(krt4:Gal4, UAS:GCaMP6f, cdh1-dTomato xt18)**
Videographer: Please capture using a SCOPE KIT

4.3.2. Talent returning the device with the wounded embryo to the imaging stage.

4.4. To wound the embryo via laser stimulation, define a region of interest near the edge of the tail fin fold and stimulate according to the available laser power **[1]**.

4.4.1. SCREEN: 67313_4-4-1.mp4 00:00-00:28 .

4.5. Image the embryo at room temperature using the GFP *(G-F-P)* and RFP *(R-F-P)* channel with a **frame interval** of **1 min** *(minute)* for a total of **1 h** *(hour)* **[1]**.

4.5.1. SCREEN: 67313_4-5-1.mp4 00:00-00:30

4.6. If necessary, release the embryos after imaging by gently suctioning near the head using a transfer pipette or by swirling the dish **[1]**.

4.6.1. Talent suctioning the embryos near the head with a transfer pipette or swirling the dish.

Pronunciation Guides:

1. **Embryo**
Pronunciation link: https://www.merriam-webster.com/dictionary/embryo
IPA: /ˈɛm.briˌoʊ/
Phonetic Spelling: em-bree-oh

2. **Coverslip**
   Pronunciation link: https://www.merriam-webster.com/dictionary/coverslip
   IPA: /ˈkʌv.ɚˌslɪp/
   Phonetic Spelling: kuh-ver-slip

3. **Cyanoacrylate**
   Pronunciation link: https://www.howtopronounce.com/cyanoacrylate
   IPA: /ˌsaɪə.noʊˈæk.rəˌleɪt/
   Phonetic Spelling: sy-uh-noh-ak-ruh-layt

4. **Laminator**
   Pronunciation link: https://www.merriam-webster.com/dictionary/laminator
   IPA: /ˈlæ.məˌneɪ.tɚ/
   Phonetic Spelling: la-muh-nay-ter

5. **Agarose**
   Pronunciation link: https://www.howtopronounce.com/agarose
   IPA: /ˈæg.əˌroʊs/
   Phonetic Spelling: ag-uh-rohs

6. **Micropipette**
   Pronunciation link: https://www.howtopronounce.com/micropipette
   IPA: /ˌmaɪ.kroʊ.paɪˈpɛt/
   Phonetic Spelling: my-kroh-py-pet

7. **Stereomicroscope**
   Pronunciation link: https://www.howtopronounce.com/stereomicroscope
   IPA: /ˌstɛr.i.oʊˈmaɪ.krəˌskoʊp/
   Phonetic Spelling: stair-ee-oh-my-kruh-skohp

8. **Notochord**
   Pronunciation link: https://www.merriam-webster.com/dictionary/notochord
   IPA: /ˈnoʊ.t̬oʊˌkɔrd/
   Phonetic Spelling: noh-toh-kord

9. **Zebrafish**
   Pronunciation link: https://www.merriam-webster.com/dictionary/zebrafish
   IPA: /ˈziː.brəˌfɪʃ/
   Phonetic Spelling: zee-bruh-fish