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Title: Imaging Worm Early Development: A Practical Manual for New Researchers

Authors and Affiliations:

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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, done**

If **Yes**, can you record movies/images using your own microscope camera?

Yes.

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Scope shots:

2.2.2., 4.1.1., 5.3.1., 5.3.2., 5.4.1., 5.4.2., 6.2.1., 6.2.2., 6.2.3., 6.3.1., 6.4.1., 6.4.2.

Authors: Please use your microscope camera to film the SCOPE shots and upload the clips to your project page. Please refer to the guidelines at the end of this script

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? **Yes.**

If **Yes**, how far apart are the locations? **Just different rooms in same building.**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No.**

Current Protocol Length

Number of Steps: 26

Number of Shots: 53 (11 Scope, 14 Screen)

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Authors: The answers are slightly edited in accordance with the journal guidelines.

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Guoye Guan:** Our research focuses on the development of organisms from germ cells, aiming to answer how freshmen can obtain high-quality imaging data.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Pei Zhang:** Current methods provide limited visual guidance for germline and embryo preparation. This protocol offers detailed instructions and practical tips to improve reproducibility and image quality.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Tina Thuy Nguyen Hoang:** This protocol enables researchers to use time-lapse imaging to study cellular events during germline development and embryogenesis, including meiosis and fertilization.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Tina Thuy Nguyen Hoang:** This procedure can be combined with quantitative imaging, cell tracking, and computational analysis to further study developmental processes and cellular mechanisms.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Pei Zhang:** Future studies can use this protocol to investigate molecular mechanisms of development, cell division, and organ morphogenesis by integrating advanced imaging and computational approaches.

- 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.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Culturing *Caenorhabditis elegans*

Demonstrator: Pei Zhang

2.1. To begin, culture *Caenorhabditis elegans* on NGM agar seeded with the food bacterium *Escherichia coli* OP50 [1] at 20 degrees Celsius for the wild-type and at 16 degrees Celsius or 25 degrees Celsius for the temperature-sensitive mutants [2-TXT].

2.1.1. WIDE: Talent placing the worms on the NGM agar plates.

2.1.2. Talent placing the plate in incubator. **TXT: NGM: Nematode Growth Medium**

2.2. Transfer the worms to new plates before starvation, when significantly fewer *Escherichia coli* OP50 bacteria are observed under the microscope [1 and 2]. Maintain the animals on the new plates for approximately 2 to 3 days to keep them healthy for subsequent experiments [3].

2.2.1. Talent transferring the animals to a new plate before starvation.

2.2.2. SCOPE: C004. *Video editor: Show 2.2.2 as an inset in 2.2.1*

2.2.3. Talent placing the plate in incubator.

3. Preparing Agarose Pads

3.1. To prepare the agarose buffer, add 3 grams of agarose to a 250-milliliter conical flask [1]. Then, add 100 milliliters of M9 buffer, mix well [2], and heat it in a microwave oven for 2 to 3 minutes until the agarose is fully dissolved [3]. **Authors: I have changed this step as I think using fresh is good for video demonstrations**

3.1.1. WIDE: Talent adding agarose to 250 ml conical flask.

3.1.2. Talent adding M9 buffer and mixing.

3.1.3. Talent placing the flask in an oven.

3.2. Using scissors, cut off the end of a 200-microliter pipette tip to widen the opening [1]. Place a drop of the agarose buffer onto a soda-lime glass slide measuring 75 by 25

millimeters [2].

3.2.1. Talent cutting off the tip of a 200 microliter pipette tip.

3.2.2. Talent placing a drop of the melted agarose buffer onto the glass slide.

3.3. Immediately cover the agarose drop with another glass slide [1]. Gently press the agar into a thin, round circle, ensuring no air bubbles are trapped [2].

3.3.1. Talent placing another glass slide on the agarose drop.

3.3.2. Talent gently pressing the agar into a thin, round circle surrounding each coverslip without trapping any air bubbles.

3.4. Allow the agar to cool for at least 3 minutes until it is fully solidified before use [1].

3.4.1. Talent placing the slide-agar aside.

3.5. Next, gently remove the upper slide, leaving the agarose pad on the bottom slide [1].

3.5.1. Talent gently removing the upper slide while leaving the agarose pad on the bottom slide. **Authors:** This step is moved here for better flow

4. Mounting Live Worms on Agarose Pads for Imaging

4.1. Select NMY-2::GFP-labeled (*N-M-Y-2-G-F-P*) worms [1]. Add 5 to 10 microliters of 0.5% tetramisole hydrochloride, diluted in M9 buffer, onto a coverslip [2], and place 10 to 20 worms in the buffer [3]. **Author's NOTE:** We did not use L4 worms and hence modified the script as "place 10 to 20 worms"

4.1.1. SCOPE: C006 01:42-01:49

4.1.2. Talent adding tetramisole hydrochloride diluted in the M9 buffer on a coverslip.

4.1.3. Talent transferring 10 to 20 NMY-2::GFP-labeled animals into the solution. **Author's NOTE:** We did not use L4 worms and hence modified the script as "place 10 to 20 worms"

4.2. Incubate the worms for 1 minute until they are fully anesthetized [1].

4.2.1. SCOPE: C007 00:06-00:08 and 00:59 *Video editor: Show 00:59 still frame at the*

end to show that worms are not moving.

- 4.3. Invert the slide with the agarose pad [1]. Gently touch the agarose pad to the worms in the buffer to avoid forming air bubbles [2] and slowly flip the slide with the coverslip facing upward [3].
 - 4.3.1. Talent inverting the slide with the agarose pad.
 - 4.3.2. Talent gently lowering the agarose pad onto the worms in the buffer without trapping any air bubbles.
 - 4.3.3. Talent flipping the slide so that the coverslip faces upward.
- 4.4. Apply additional agarose buffer around the edges of the coverslip [1]. Next, using a 200-microliter pipette tip, apply pre-heated liquid petroleum jelly along the boundary of the coverslip [2] and let it cool to secure the coverslip to the slide [3].
 - 4.4.1. Talent applying additional agarose buffer around the four corners of the coverslips.
 - 4.4.2. Talent applying the heated liquid petroleum jelly along the boundary of the coverslip with a 200 microliter pipette tip.
 - 4.4.3. Talent placing the slide aside.
- 4.5. Place the slide on the microscope stage for imaging [1]. Observe an oocyte entering the spermatheca from the gonad, where it is fertilized by the stored sperm [2], before entering the uterus as a one-cell embryo [3].
 - 4.5.1. Talent placing the slide on the microscope stage for imaging the germ cell development.
 - 4.5.2. SCOPE: 4.5.1-SCOPE 00:01-00:08
 - 4.5.3. SCOPE: 4.5.1-SCOPE 00:09-00:12

5. Preparing an Embryo Chamber and Imaging

Demonstrators: Pei Zhang and Tina Thuy Nguyen Hoang

- 5.1. Create a 10-millimeter central hole in a 35-millimeter Petri dish using a drill to eliminate the influence of the pressure from the coverslip and the agarose pad [1]. Seal the underside with a 22-millimeter microscope cover glass to create a reusable chamber for the inverted confocal microscopy [2].

- 5.1.1. Medium Shot of Talent machining a 10 millimeter central hole in a 35 millimeter Petri dish using a lathe drill.
- 5.1.2. Talent sealing the underside of the Petri dish with a 22 millimeter microscope cover glass.
- 5.2. Add approximately 30 microliters of the M9 buffer into the chamber [1].
 - 5.2.1. Talent adding approximately 30 microliters of the M9 buffer into the imaging chamber.
- 5.3. Select the hermaphrodites [1] and transfer approximately 20 young adults into the M9 buffer in the imaging chamber [2].
 - 5.3.1. SCOPE: 5.3.1.-SCOPE.
 - 5.3.2. SCOPE: C010 00:17-00:25.
- 5.4. Using a syringe needle, make a transverse cut at the spermatheca twice to release more early-stage embryos [1]. Then, with an eyelash picker, remove the body [2].
 - 5.4.1. SCOPE: C010 03:00-03:10 and 04:05-04:06.
 - 5.4.2. SCOPE: : C010 04:40-04:45.
- 5.5. Cover the Petri dish with its lid to prevent liquid evaporation [1].
 - 5.5.1. Talent placing the lid on the Petri dish.
- 5.6. For the non-fluorescent worms, determine the developmental stage of the early embryos based on the location of the cell nucleus [1]. Assess the fusion status [2] and examine the morphology of the cleavage furrow [3].
 - 5.6.1. SCOPE: 5.3.1-5.6.3-SCOPE 00:00-00:03 *Video editor: Show only grey scale footage and Play in loop.*
 - 5.6.2. SCOPE: 5.3.1-5.6.3-SCOPE 00:07-00:10 .
 - 5.6.3. SCOPE: 5.3.1-5.6.3-SCOPE 00:14-00:20 . *Video editor: Zoom in*

6. Fluorescence Microscopy

Demonstrator: Tina Thuy Nguyen Hoang

- 6.1. Place the sample on the confocal microscope under the lowest magnification of 10x to search for the newly fertilized embryos at the desired stage [1].
 - 6.1.1. WIDE: Talent placing the slide onto the microscope.
- 6.2. For the fluorescent worms, identify the developmental stages based on the protein distribution. Assess the cell morphology to confirm the stage [1].
 - 6.2.1. SCOPE: 6.2.1.-6.2.2.-SCOPE.mp4.
- 6.3. After locating the embryos, switch to a higher magnification of 60x or 100x [1].
 - 6.3.1. SCREEN: 6.3.1-SCREEN 00:18-00:24.
- 6.4. For the fluorescent strains susceptible to phototoxicity or photobleaching, reduce the laser power and shorten the exposure time during the long-term imaging [1]. Start the Z-stack video recording or capture a picture snapshot with a Z-stack interval of approximately 1 micrometer and a scanning interval as low as 1 second [2]. Author's NOTE: Swap 6.4. and 6.4.2
 - 6.4.1. SCREEN: 6.4.2-SCREEN 00:06-00:12 and 00:17-00:19.
 - 6.4.2. SCREEN: 6.4.1-SCREEN-20260824.mp4 00:01-00:12.
- 6.5. Save all the images in the TIFF (*tiff*) format while preserving the original imaging information [1].
 - 6.5.1. SCREEN: 6.5.1-SCREEN 00:03-00:14.

7. Processing Confocal Images in Fiji

Demonstrator: Xiangchuan Wang

NOTE: For this section: Use the video uploaded on 22-08-2026

- 7.1. Open the acquired images in Fiji. Adjust the brightness and contrast [1]. Select **Image** followed by **Hyperstacks** and **Stacks to Hyperstacks** to separate the channels, the Z (*zee*) layers, and the time points in a single TIFF (*tiff*) image [2]. Then, select **Image**, followed by **Color**, and **Channels Tool** to optimize the color balance for exhibition [3].
 - 7.1.1. SCREEN: 7.1-stack-and-hyperstack. 00:00-00:04

NOTE: For this section, Use the video uploaded on 22-08-2026. Do not use the old ones

7.1.2. SCREEN: 7.1-stack-and-hyperstack. 00:05-00:13.

7.1.3. SCREEN: 7.1-stack-and-hyperstack. 00:14-00:22.

7.2. Go to **Image, Transform**, and choose **Rotate [1]**. Choose the **angle and** check the **Enlarge image** box to rotate each image so that the worm is oriented horizontally with the head facing left **[2]**. If needed, remove unnecessary blank regions by selecting **Image** followed by **Crop [3]**.

7.2.1. SCREEN: 7.2-rotate-and-crop 00:00-00:04.

7.2.2. SCREEN: 7.2-rotate-and-crop 00:06-00:09 and 00:12-00:14

7.2.3. SCREEN: 7.2-rotate-and-crop 00:17-00:21

7.3. Select **Image** followed by **Stacks** and **Z Project** to generate a Z-projection from the representative frames with sufficient signal **[1]**. Then, select **File** followed by **Save As** and **Tiff** to save the projected images for the subsequent analysis **[2]**.

7.3.1. SCREEN: 7.3-z-projection 00:00-00:07.

7.3.2. SCREEN: 7.3-z-projection 00:08-00:13.

7.4. Finally, select **Analyze** followed by **Set scale** to calibrate the image scale according to the confocal microscope used so that the pixels are converted to micrometers **[1]**. After the calibration, add a scale bar by selecting **Analyze, Tools** and **Scale Bar [2]**.

7.4.1. SCREEN: 7.4-scale-bar 00:00-00:11.

7.4.2. SCREEN: 7.4-scale-bar 00:12-00:14 and 00:18-00:20.

Results

8. Results

8.1. NMY-2::GFP marked the overall structure of the U-shaped gonad, the rachis, and the cortical region of primary spermatocytes [1]. mCherry::Histone (*m-cherry-histone*) showed the nuclear morphology and the developmental stage [2].

8.1.1. LAB MEDIA: Figure 1B. *Video editor: Show upper image, Highlight the green fluorescence signals.*

8.1.2. LAB MEDIA: Figure 1B. *Video editor: Show upper image, Highlight the magenta fluorescence signals.*

8.2. The most proximal primary spermatocytes were at the karyosome stage [1], indicating that these cells were beginning their first division.

8.2.1. LAB MEDIA: 8.2-Script-movie.avi

8.3. Mitotic divisions of the primordial germ cells could also be captured in the distal region of the gonad [1].

8.3.1. LAB MEDIA: Figure 1

8.4. Continuous imaging allowed more than 500 minutes of time-lapse recording that encompassed the entire process from fertilization through muscle twitching [1]. The protocol was also used to capture furrow dynamics in the hum-2 ok-596 mutant for visual comparison with the control [2].

8.4.1. LAB MEDIA: Figure 2. *Video editor: Sequentially highlight panels A through T.*

8.4.2. LAB MEDIA: Supplemental Figure S3

1. *Caenorhabditis elegans*

Pronunciation link: <https://www.howtopronounce.com/caenorhabditis-elegans>

IPA: /kiː.noʊ.ɪ.hæbˈdaɪrɪs ˈelɪɡənz/

Phonetic Spelling: kee·noh·hab·DYE·tuhs EL·ih·guh·nz

2. *Escherichia coli*

Pronunciation link: <https://www.howtopronounce.com/escherichia-coli>

- IPA: / ɛʃəˈnkiə ˈkoulai/
Phonetic Spelling: esh·uh·RIK·ee·uh KOH·lye
3. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: / ˈæɡəˌroʊs/, / ˈɑːɡəˌroʊs/, / ˈæɡəˌroʊz/, / ˈɑːɡəˌroʊz/
Phonetic Spelling: AG·uh·rohs / AH·guh·rohs / AG·uh·rohzh / AH·guh·rohzh
 4. Tetramisole
Pronunciation link: No confirmed link found
IPA: / ˌtɛtrəˈmɪzoʊl/
Phonetic Spelling: tet·ruh·MIZ·ohl
 5. Hydrochloride
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrochloride>
IPA: / ˌhaɪdrəˈklɔːraɪd/
Phonetic Spelling: hy·druh·KLOR·yde
 6. Oocyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/oocyte>
IPA: / ˈoʊəˌsaɪt/
Phonetic Spelling: OH·uh·syte
 7. Spermatheca
Pronunciation link: No confirmed link found
IPA: / ˌspɜːrməˈθiːkə/
Phonetic Spelling: spur·muh·THEE·kuh
 8. Gonad
Pronunciation link: <https://www.merriam-webster.com/dictionary/gonad>
IPA: / ˈɡoʊˌnæd/
Phonetic Spelling: GOH·nad
 9. Fertilized
Pronunciation link: <https://www.merriam-webster.com/dictionary/fertilize>
IPA: / ˈfɜːtəˌlaɪzd/
Phonetic Spelling: FUR·tuh·lyzd
 10. Uterus
Pronunciation link: <https://www.merriam-webster.com/dictionary/uterus>
IPA: / ˈjuːtərəs/
Phonetic Spelling: YOO·tuh·ruhs
 11. Confocal
Pronunciation link: <https://www.howtopronounce.com/confocal>
IPA: / kənˈfoʊkəl/
Phonetic Spelling: kuhn·FOH·kuhl
 12. Hermaphrodites
Pronunciation link: <https://www.merriam-webster.com/dictionary/hermaphrodite>
IPA: / hɜːˈmæfrəˌdarts/
Phonetic Spelling: hur·MAF·ruh·dytes
 13. Transverse
Pronunciation link: <https://www.merriam-webster.com/dictionary/transverse>

- IPA: /'trænzˌvɜːs/, /trænz'vɜːs/
Phonetic Spelling: TRANZ·vurs / tranz·VURS
14. Embryos
Pronunciation link: <https://www.merriam-webster.com/dictionary/embryo>
IPA: /'embriouz/
Phonetic Spelling: EM·bree·ohz
15. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'resəns/
Phonetic Spelling: floo·RES·uhns
16. Phototoxicity
Pronunciation link: No confirmed link found
IPA: /ˌfotootɔːk'sisəti/
Phonetic Spelling: foh·toh·tok·SIS·uh·tee
17. Photobleaching
Pronunciation link: No confirmed link found
IPA: /ˌfotou'bliːtʃɪŋ/
Phonetic Spelling: foh·toh·BLEE·ching
18. Hyperstacks
Pronunciation link: No confirmed link found
IPA: /'haɪpərˌstæks/
Phonetic Spelling: HY·per·staks
19. Z-projection
Pronunciation link: No confirmed link found
IPA: /'ziː prə'dʒɛkʃən/
Phonetic Spelling: ZEE·pruh·JEK·shuhn
20. Rachis
Pronunciation link: No confirmed link found
IPA: /'reɪkɪs/
Phonetic Spelling: RAY·kiss
21. Cortical
Pronunciation link: <https://www.merriam-webster.com/dictionary/cortical>
IPA: /'kɔːrtɪkəl/
Phonetic Spelling: KOR·tih·kuhl
22. Spermatocytes
Pronunciation link: <https://www.howtopronounce.com/spermatocytes>
IPA: /spɜː'mætəˌsaɪts/
Phonetic Spelling: spur·MAT·uh·sytes
23. Karyosome
Pronunciation link: No confirmed link found
IPA: /'kæriəˌsoʊm/
Phonetic Spelling: KAIR·ee·uh·sohm
24. Mitotic
Pronunciation link: <https://www.merriam-webster.com/dictionary/mitotic>

- IPA: /maɪˈtɑːtɪk/
Phonetic Spelling: my·TOT·ik
25. Primordial
Pronunciation link: <https://www.merriam-webster.com/dictionary/primordial>
IPA: /praɪˈmɔːrdiəl/
Phonetic Spelling: pry·MOR·dee·uhl
26. Fertilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/fertilization>
IPA: /ˈfɜːtələˈzeɪʃən/
Phonetic Spelling: fur·tuh·luh·ZAY·shuhn
27. mCherry
Pronunciation link: No confirmed link found
IPA: /ˌɛmˈtʃəri/
Phonetic Spelling: em·CHAIR·ee
28. Histone
Pronunciation link: <https://www.merriam-webster.com/dictionary/histone>
IPA: /ˈhɪsˌtoʊn/
Phonetic Spelling: HIS·tohn
29. Morphology
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>
IPA: /mɔːrˈfɑːlədʒi/
Phonetic Spelling: mor·FOL·uh·jee
30. Micrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪˈkraːmɪtər/, /ˈmaɪkrəˌmiːtər/
Phonetic Spelling: my·KROM·uh·ter / MY·kroh·mee·ter