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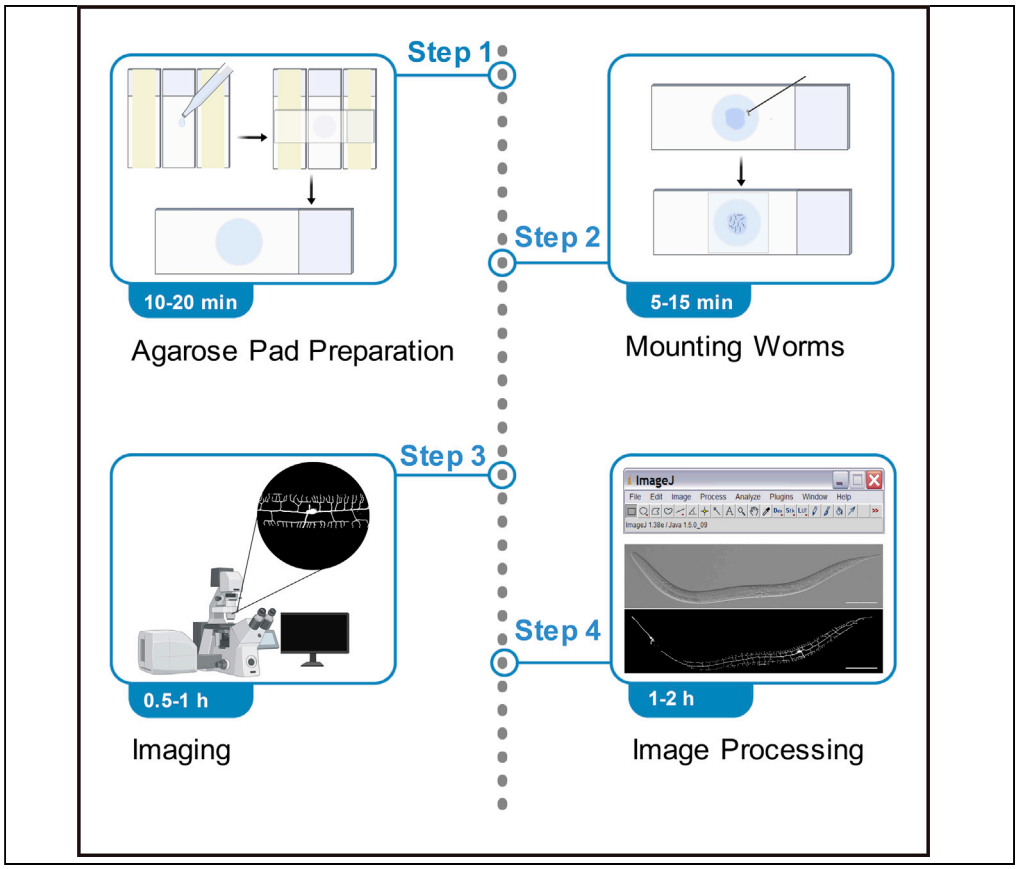
In vivo imaging of a PVD neuron in *Caenorhabditis elegans*



The nematode *Caenorhabditis elegans* nociceptive PVD neurons have highly ordered dendritic branches, making this an ideal model to study the development and organization of dendrites. A *ser-2*-promoter-driven GFP reporter line *wyIs592*[*ser-2prom-3p::myr*-GFP] provides a comprehensive visualization of PVD anatomy. Here, we describe the detailed procedures for imaging a PVD neuron using *wyIs592* at late L4 larval stage *in vivo* by confocal microscopy. This protocol can also be applied to imaging other cells in *C. elegans*.

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HIGHLIGHTS

Protocol for fluorescence imaging in *C. elegans*

Worm strain cultivation for stress-sensitive neurons

Acquiring and assembling pictures for a large neuron with highly elaborate dendrites

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In vivo imaging of a PVD neuron in *Caenorhabditis elegans*

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SUMMARY

The nematode *Caenorhabditis elegans* nociceptive PVD neurons have highly ordered dendritic branches, making this an ideal model to study the development and organization of dendrites. A *ser-2*-promoter-driven GFP reporter line *wyIs592*[*ser-2prom-3p::myr*-GFP] provides a comprehensive visualization of PVD anatomy. Here, we describe the detailed procedures for imaging a PVD neuron using *wyIs592* at late L4 larval stage *in vivo* by confocal microscopy. This protocol can also be applied to imaging other cells in *C. elegans*.
For complete details on the use and execution of this protocol, please refer to Feng et al. (2020).

BEFORE YOU BEGIN

The nematode *Caenorhabditis elegans* is commonly maintained in the laboratory on Nematode Growth Medium (NGM) agar plate using *Escherichia coli* strain OP50 as a food source (Brenner, 1974). Additional resources about the techniques described here also can be found in WormBook, "Maintenance of *C. elegans*" by Stiernagle, T., (http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html) (Stiernagle, 2006).

NGM Petri plates preparation

Timing: 2–3 days

1. Weigh 3 g NaCl, 17 g agar, and 2.5 g peptone. Mix them in a 2 liter Erlenmeyer flask and add 975 mL double distilled water (ddH_2O).
2. Put a magnetic stir bar into the flask and cover the flask with aluminum foil. Autoclave for 20 min.
3. Set a hotplate magnetic stirrer at 55°C. Air-cool the autoclaved flask to 55°C on the hotplate with gentle stirring.

   Alternatives: Water cooling the flask with gentle stirring at 55°C for 15 min.

4. Add 1 mL $MgSO_4$ (1 M stock solution), 1 mL cholesterol in ethanol (5 mg/mL stock solution), 1 mL $CaCl_2$ (1 M stock solution) and 25 mL KPO_4 buffer (1 M stock solution, PH 6.0, see table in Materials) into NGM solution respectively and swirl well.

⚠ CRITICAL: Pay attention to aseptic operation in the process of adding reagents. All these reagents are already sterilized except 5 mg/mL cholesterol (cholesterol is dissolved in ethanol and should not be autoclaved).

Optional: Add an antibiotic to the NGM media (for example, streptomycin), and cultivate a modified OP50 strain with corresponding resistance in step 8 (Büchter et al., 2015). This helps prevent contamination from other bacteria and extends the storage time of NGM plates (Note after step 10).

5. Dispense the NGM solution into petri plates using a peristaltic pump. For 60 mm dish, add about 10 mL, and for 35 mm dish add about 4 mL of NGM solution.

   Alternatives: The peristaltic pump is not a necessary equipment. Liquid pipette is also an option.

   Note: Several sizes of petri plates are available. Medium size plates (60 mm diameter) and smaller plates (35 mm diameter) are commonly used for general strain maintenance.

6. Place NGM plates at room temperature (15°C–25°C) for 2–3 days before use to allow excess moisture to evaporate.
7. Pack NGM plates upside-down in an air-tight container to prevent water loss as well as steam condensation on the lid. NGM plates stored at room temperature (15°C–25°C) can be usable for about one week. For longer usage, store them for up to 2–3 weeks at 4°C.

Bacterial food source

Timing: 2–3 days

8. Pick a single clone of OP50 *E. coli* from an LB agar plate (see table in Materials). The clone is aseptically inoculated into LB liquid medium (see table in Materials) at 37°C for 12–14 h.

   Optional: If you add an antibiotic to the NGM media (Optional after step 4), pick a modified OP50 strain with corresponding resistance into LB liquid medium (Büchter et al., 2015).

   Note: OP50 *E. coli* clone should be prepared at least one day before worm culture. OP50 *E. coli* is streaked on an LB agar plate and cultured for 14–16 h at 37°C.

9. Under sterile conditions, add approximately 100 μL of OP50 *E. coli* culture liquid on an NGM plate. Spread to create a larger lawn.

   Note: The volume of OP50 *E. coli* culture liquid is depended on the worm culture dishes, such as 100 μL for a 60 mm dish, and 50 μL for a 35 mm dish.

   Note: Avoid spreading the lawn to the edges of the plate. Keep the lawn in the center of the dish.

10. Allow the OP50 *E. coli* lawn to grow for 12–16 h at room temperature (15°C–25°C). If not used immediately, the seeded plates could be packed upside-down in an air-tight container and stored for up to 1 week at room temperature or 2–3 weeks at 4°C.

    Note: NGM plates with streptomycin can be stored for up to 2–3 months at 4°C.

    Alternatives: The OP50 *E. coli* lawn also can grow for 8 h at 37°C.

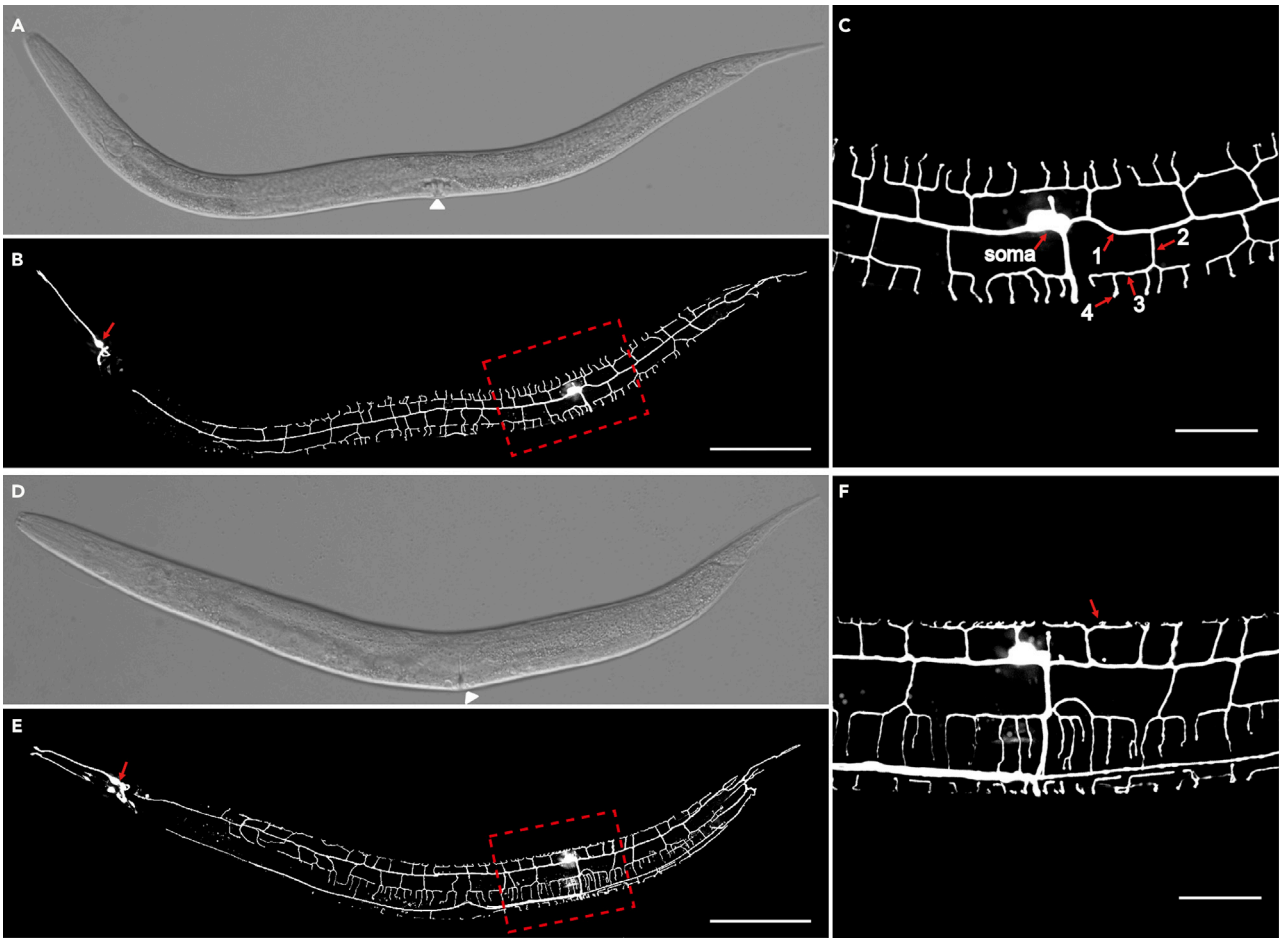

Figure 1. PVD neuron displays an elaborate dendritic arbor that envelops the animal in a net-like array

(A) DIC image showing that the mounted worm adopts a lateral position. The vulva pointed by the triangle indicates that the worm is at late L4 stage.

(B) Fluorescent image of a PVD neuron with whole web-like array dendrites visualized by the *wyIs592* reporter in the same worm as (A). The red arrow points to the OLL neuron in the head which is also labeled by the *ser-2* promoter.

(C) Inset shows a highly magnified image of the region surrounding PVD soma in (B). The red arrows note 1°, 2°, 3°, and 4° dendritic branches and soma.

(D) DIC image showing that the mounted worm at late L4 stage adopts the dorsal-lateral position. The triangle indicates the position of vulva.

(E) Fluorescent image of a PVD neuron with partial web-like array dendrites visualized by the *wyIs592* reporter in the same worm as (D). The red arrow points to the OLL neuron in the head.

(F) Inset shows a highly magnified image of the region surrounding PVD soma. The red arrow indicates partially visualized ventral side of PVD dendrites. Scale bars in (A and B) and (D and E), 100 μm. Scale bars in (C) and (F), 20 μm.

Worm strain preparation

Timing: 3 days

A pair of PVD neurons PVDL and PVDR extend their dendrites to cover the left and the right side of worm body respectively (Figure 1B and Methods Video S1). For clearly imaging PVD neurons, here we choose *wyIs592*[*ser-2prom3p::myr-GFP*] with a PVD-specific GFP reporter to present its elaborate and highly ordered dendritic branches morphology (Wei et al., 2015; Zou et al., 2015). *wyIs592* also labels OLL neurons in the head, whose signal would not interfere with PVD fluorescence when imaging (Figure 1B). In addition, wyIs592 has a co-injection marker *odr-1p::DsRed* which may assist tracking when making crosses.

Alternatives: In addition to the *wyIs592* strain, you can also inject the modified plasmid (from the backbone plasmid: Addgene 164223) with *ser-2* promotor driving any fluorescent reporters to label PVD neurons according to your specific aims.

11. Pick 4–6 L4 stage *wyIs592* worms onto each NGM plate, and maintain them at 20°C in thermostat incubator.
12. Three days later, the hermaphrodite animals at late L4 stage are anesthetized for imaging.

Note: PVD neurons mature with 4° dendritic branches at late L4 larval stage (Cody et al., 2010), which is visually determined by the vulva morphology (Schindler and Sherwood, 2013). Briefly, at late L4 larval stage, vulval lumen is about to close so that its "Christmas tree" shape becomes narrower (Figure 2).

⚠ CRITICAL: Worms must be cultivated under optimal physiological conditions without starvation or other stresses.

Pharmacological agent preparation

Timing: 10–30 min

Using a pharmacological agent to paralyze the worms allows us to mount multiple worms on one slide easily (Byrne et al., 2011; Kim et al., 2013). Levamisole, a cholinergic agonist that causes muscle relaxation, is widely used in *C. elegans* (Chung et al., 2013). Here, 5 mM levamisole is recommended.

13. Dissolve 2.407 g levamisole in M9 buffer (see table in Materials) to obtain 10 mL 1 M stock solution.
14. Aliquot 100 μL of 1 M levamisole stock solution into a 1.5 mL centrifuge tube. Store it at −20°C for up to 12 months.
15. Just before use, dilute to 5 mM levamisole in M9 buffer.

Note: Diluted 5 mM levamisole in M9 buffer can be kept at room temperature (15°C–25°C) for 1–2 weeks.

Alternatives: According to your own laboratory resources, you can also use 10 mM muscimol (a $GABA_A$ agonist) (Norris et al., 2009), 0.1% Tricaine plus 0.01% tetramisole (McCarter et al., 1999) or 10 mM (0.7%) sodium azide (NaN3) (Massie et al., 2003) as a pharmacological agent. However, GFP fluorescence is photobleached rapidly when choose sodium azide as an anesthetic (http://wbg.wormbook.org/wli/wbg13.2p13/).

⚠ CRITICAL: Sodium azide is hazardous. Sodium azide is a mutagen and easily explosive. Avoid inhalation and direct contact.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
OP50 *E. coli*	Caenorhabditis Genetics Center	OP50
Recombinant DNA		
Pser2prom3::myr-mcherry	Addgene	164223
Chemicals, peptides, and recombinant proteins		
Sodium chloride	SCR	Cat#10019318
Agar	MDBio	Cat#101-9002-18-0
Peptone	Sangon	Cat#PN5247
Magnesium sulfate	SCR	Cat#20025117

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Cholesterol	Ourchem	Cat#690082004
Ethanol	SCR	Cat#10009259
Calcium chloride	Sigma-Aldrich	Cat#V900269
Monopotassium phosphate	SCR	Cat#10017608
Dipotassium phosphate	SCR	Cat#10017518
Yeast extract	Sangon	Cat#BN5245Y
Tryptone	SCR	Cat#69024138
Agarose	Biowest	Cat#BY-R0100
Levamisole hydrochloride	Sangon	Cat#A506644-0100
Sodium azide	Sigma-Aldrich	Cat#S2002
Latex beads, polystyrene, 0.1 μm mean particle size	Sigma-Aldrich	Cat#LB1-1ML
Experimental models: organisms/strains		
C. elegans: wyIs592[ser-2prom-3p::myr-GFP; odr-1p::Dsred]	Zou et al., 2015	N/A
Software and algorithms		
ImageJ	Schneider et al., 2012	https://imagej.nih.gov/ij/
Other		
Nikon Spinning Disk confocal microscope	Nikon	TI2-E+CSU+W1
Microscope camera	Teledyne Photometrics	Prime 95B
Zeiss dissecting stereomicroscope	Zeiss	Stemi 2000
Petri plates, 35 mm	Huilun	Cat#HL-E01
Petri plates, 60 mm	Huilun	Cat#HL-E03
Petri plates, 90 mm	Huilun	Cat#HL-E06

MATERIALS AND EQUIPMENT

KPO_4 buffer		
Reagent	Final concentration	Amount
KH_2PO_4	N/A	108.3 g
K_2HPO_4	N/A	35.5 g
ddH_2O		up to 1 L
Adjust PH to 6.0 and use after high pressure sterilization. Store at room temperature (15°C–25°C) for up to 1 month.		

LB agar		
Reagent	Final concentration	Amount
NaCl	N/A	5 g
Yeast extract	N/A	5 g
Tryptone	N/A	10 g
Agar	N/A	15 g
ddH_2O		up to 1 L
Use after high pressure sterilization. Cool to 55°C–60°C. Pack into 60 or 90 mm petri dishes. Store at 4°C for up to 2 weeks.		

LB liquid medium		
Reagent	Final concentration	Amount
NaCl	N/A	5 g
Yeast extract	N/A	5 g
Tryptone	N/A	10 g
ddH_2O		up to 1 L
Use after high pressure sterilization. Store at 4°C for up to 2 weeks.		

M9 buffer		
Reagent	Final concentration	Amount
KH_2PO_4	N/A	3 g
Na_2HPO_4	N/A	6 g
NaCl	N/A	5 g
ddH_2O		up to 1 L

After high pressure sterilization, cool to under 55°C, add 1 mL sterilized 1 M $MgSO_4$ and mix well. Store at room temperature (15°C–25°C) for up to 1 month.

Equipment

- A Nikon Spinning Disk confocal microscope (TI2+CSU+W1) is used in this study with Photometrics Prime 95B camera, W1 spinning disk head, the 488 nm excitation laser, emission filters (B525/50), pinhole size (50 μm) and 40×/1.30 N.A oil immersion objective (0.28 μm/pixel).
- A dissecting stereomicroscope Zeiss Stemi 2000 is used in this study with standard 10× eyepieces and objectives which range from 0.6× to 5×.

STEP-BY-STEP METHOD DETAILS

Agarose pad preparation

Timing: 10–20 min

This step describes how to make agarose pads, which are used to mount worms. Additional resources about the techniques described here also can be found in WORMATLAS, "Mounting Animals for Observation with Nomarski DIC Optics" by Monica Driscoll (https://www.wormatlas.org/agarpad.htm)

1. Dissolve 2% (w/v) agarose in M9 buffer in a microwave oven. Heat until it is fully dissolved. Put on a hot plate or a metal bath (65°C) to keep it melted during agarose pad preparation.

   Optional: It is recommended to aliquot 5 mL of agarose solution into a 15 mL Corning centrifuge tube, which can be stored at 4°C for up to 2 months. When needed, take 1 tube into a metal bath (80°C) and heat it to dissolve completely.

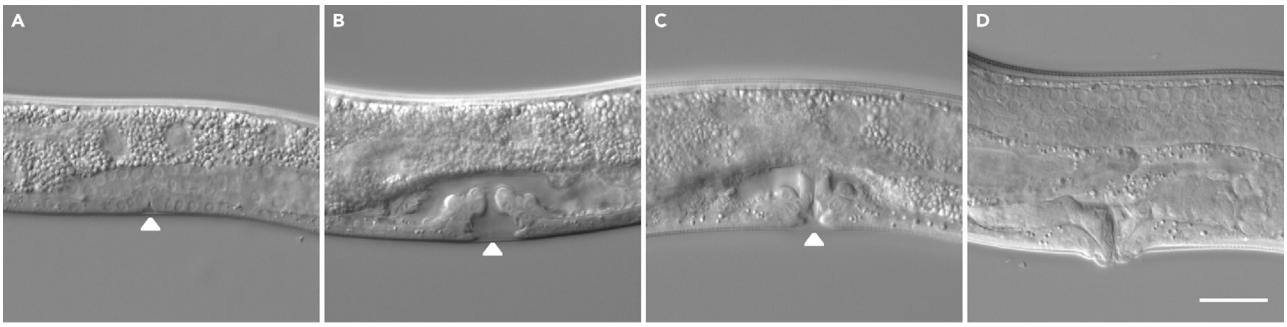

Figure 2. Vulva morphology from early L4 to adult stages (related to Figures 1 and 6)
(A) DIC image of the developing vulva of an early-L4 animal in the lateral view. The vulval sheet and vulval cells start to bend inward. This invagination step establishes the beginnings of the vulval lumen.
(B) DIC image of the developing vulva of a mid-L4 animal in the lateral view. The vulval lumen continues to expand and give rise to a characteristic shape of "Christmas tree."
(C) DIC image of the developing vulva of a late-L4 animal in the lateral view. As morphogenesis continues, the vulval lumen starts to get smaller.
(D) DIC image of the vulva of a young adult animal in the lateral view. The closed lumen generates the adult vulva protruding from the surface of the body.
Scale bar, 20 μm. The triangles in (A)–(C) point to the vulval lumen.

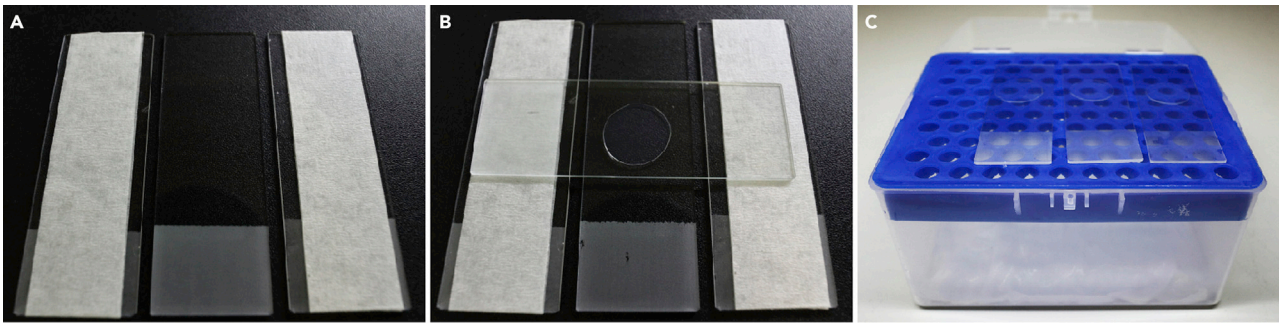

Figure 3. Agarose pad preparation

(A) Three microscope slides are placed in parallel, with laboratory tape on the two side slides.

(B) An Agarose pad is made by sandwiching a small drop of molten agarose between two glass slides spaced by the laboratory tape.

(C) The agarose pads are stored in a humidity chamber (a pipette tip box with wet paper inside).

2. Place three clear microscope slides in parallel. Adhesive tapes are pasted on the two side slides, which provides approximately 200 μm thicker space than the middle slide (Figure 3A).
3. Add one drop (approximately 100 μL) of 2% molten agarose onto the center of the middle slide and quickly, but gently, lay down another slide orthogonally on the drop. Make sure the top slide across the three parallel bottom slides to create a flat agarose surface (Figure 3B). The thickness of the pad is determined by the tape on the two adjacent slides.

   Note: Avoid bubbles when putting the glass slide onto the agarose drop. The bubbles will turn into sunken holes for worms.

   Optional: To strictly prevent the animals from turning and moving, levamisole can be added into the 2% molted agarose (5 mM work solution) before making the pads.

4. After the agarose pad is solidified (about 1 min), separate the slides with the agarose pad so that the pad remains attached to one slide (Figure 4A). Then, store the slide in a humidity chamber at room temperature (15°C–25°C; a pipette tip box with wet paper towels on its bottom) (Figure 3C).

   Optional: We also recommend 10% agarose pads with 0.1 micron polystyrene beads, especially for long-term or time-lapse imaging (Fang-Yen et al., 2012). The preparation of 10% agarose pads is the same as described above in this section steps 1–4.

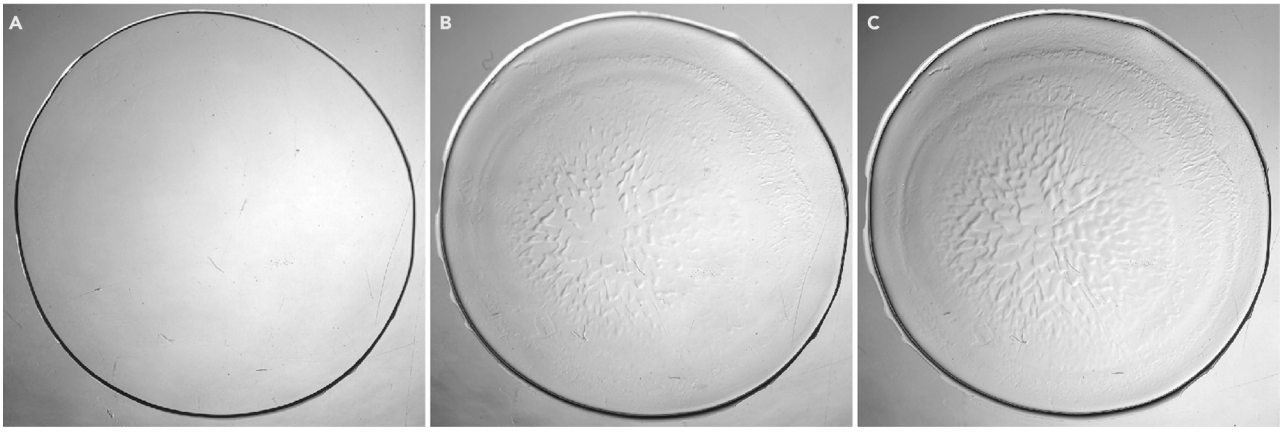

Figure 4. Shrink and dehydrated agarose pads after long time storage

A freshly made agarose pad with smooth surface (A), a shrinking agarose pad after a short period (B), and a severe dehydrated agarose pad for a long period (C).

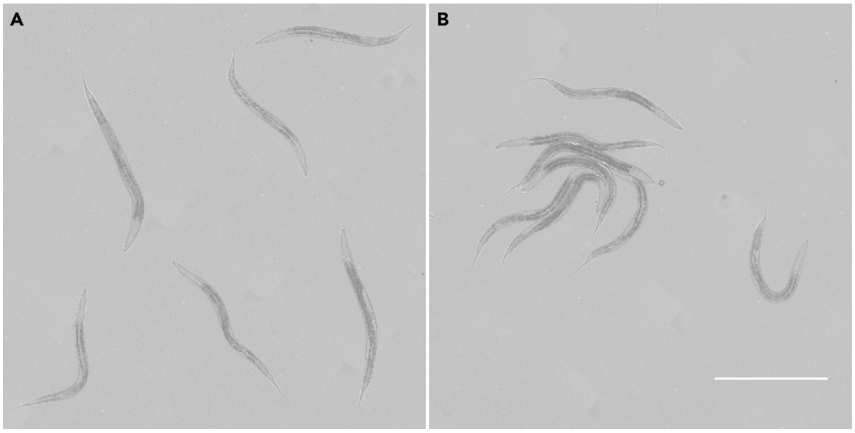

Figure 5. Worms on the agarose pad

The mounted worms are spread out sparely (A) versus stacked together (B). Scale bar, 500 μm.

Note: If not used immediately, agarose pads should be transferred to a humidity chamber before mounting the worms.

Pause point: The agarose pads can be kept in a humidity chamber for 1 h although the fresh agarose pad is always recommended. If some agarose pads shrink or appear dehydrated (Figures 4B and 4C), they cannot be further used.

Mounting worms

Timing: 5–15 min

This step describes how to mount worms on agarose pads, which are used for imaging.

5. Add 5 μL of 5 mM levamisole onto an agarose pad.
6. Pick 10–20 worms at late L4 stage into the levamisole solution on the pad and gently swing the pick to spread the worms sparsely (Figure 5A). The worms are allowed to be anesthetized for 1–2 min in 5 mM Levamisole.

Note: Too much levamisole solution can cause worms to float off the agarose pad. You should not exceed to add more than 5 μL of liquid to the agarose pad. This is especially important for long-term imaging. If necessary, you can limit the volume of levamisole as low as 1 μL to restrict further movement of worms. In this case, place the coverslip as fast as possible to avoid worms from dehydrating.

Note: If not imaging PVD neurons at different developmental stages on purpose, late L4 stage worms are recommended. The high order dendrites of PVD neurons are just fully developed at late L4 stage (Figure 6A), yet without exuberant branches in aged worms (Figure 6B). The typical vulva morphology at late L4 stage is shown in Figure 2.

⚠ CRITICAL: When picking worms, the residual OP50 *E. coli* on the pad should be reduced to the least. The presence of OP50 will enhance fluorescence background, and may induce worms to move during imaging.

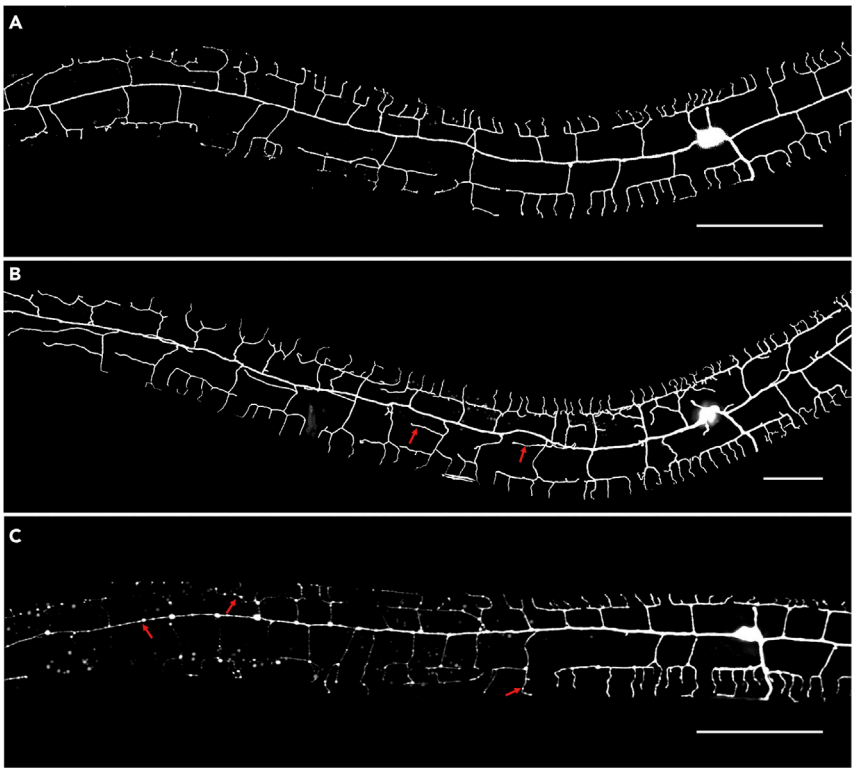

Figure 6. The high order dendrites of PVD neurons are fully developed at late L4 larval stage under optimal physiological conditions

(A) Fluorescent image of a PVD neuron at late L4 larval stage visualized by the *wyIs592* reporter under optimal physiological conditions.

(B) Fluorescent image of a PVD neuron at day 2 adult stage visualized by the *wyIs592* reporter. The PVD dendrites grow excessively with exuberant branches as indicated by the red arrows.

(C) Fluorescent image of a PVD neuron at late L4 larval stage visualized by the *wyIs592* reporter after being mounted for about 3 h. The degenerating dendrites are indicated by the red arrows. Scale bars in (A)–(C), 50 μm.

⚠ CRITICAL: The mounted worms should be spread out sparsely and not stacked together (Figure 5). In this way, a single worm can be found in a field of microscope when taking pictures.

⚠ CRITICAL: Worms must be picked gently. PVD neurons are very vulnerable to stresses. PVD dendrites in wounded worms usually appear weaker fluorescence or degenerating (Figure 6C).

7. Cover the worms with a coverslip. Store the slide in a humidity chamber and wait for another 5–10 min before imaging.

⚠ CRITICAL: Cover the worms immediately after 1–2 min anesthesia. The complete morphology of highly ordered dendrites of a PVD neuron can be well visualized only in the lateral view (Figures 1A–1C). However, longer waiting time before putting on the coverslip will cause high percentage of the mounted worms to lie in the dorsal or ventral view (Figures 1D–1F).

⚠ CRITICAL: It is critical that the worms remain strictly still throughout the imaging period. The total anesthetized procedure needs at least 5–10 min after mounting.

Note: When covering with a coverslip, avoid bubbles in the process, which will interfere with the refractive projection of fluorescence. If the slide has bubbles, avoid collecting images near the bubbles for analysis.

Optional: If you choose 10% agarose pads, after step 5 and before step 6, add 1 μL of 0.1 micron polystyrene beads in water (total liquid volume less than 5 μL) onto the pad. Vaseline can be used to seal the coverslip to prevent evaporation of the sample for long-term or time-lapse imaging.

Imaging

Timing: 0.5–1 h

8. Place the well-prepared slide on the microscope stage and properly focus to find the PVD neuron under the microscope.
   a. First, find the worms using bright field illumination under low magnification (5 or 10× objective). Choose separated worms with a perfect lateral view.
   b. Then, switch to 20× magnification and turn on the 488 nm laser to select worms with whole branches (1°, 2°, 3°, and 4°) of a PVD neuron for imaging.
   c. Finally, switch to high magnification (40×/1.3 N.A oil immersion objective) and focus on the PVD neuron.

Note: When previewed in the fluorescence field, the worm should be observed by low fluorescence intensity and low exposure to avoid fluorescence quenching.

⚠ CRITICAL: It is best to choose a field without worms sticking to each other. Otherwise, the fluorescence intensity of the imaging worm may be disturbed by the fluorescence of the neighboring worms, or this may cause problems in later image processing.

9. Acquire microscopy images.
   a. Adjust the major parameters (Channel, laser intensity, exposure times, pinhole and bit depth) for imaging appropriately. The goal is to see the PVD soma and all its branches (1°, 2°, 3°, and 4°) clearly in the image.

Note: When imaging, enhance the excitation intensity to clearly show the 4° branches, while the cell body may be a little over-exposed.

Note: For *wyIs592*, the exposure value is generally 100–200 ms and the laser intensity is 5%–10% using Nikon Spinning Disk confocal microscope. Of course detail imaging parameter settings can be modified according to your microscope and sample preparation. Exposure time and excitation light level will depend on the baseline signal and expression level of the fluorescence.

   b. We typically image 15–30 Z steps (0.5 μm/step) until all branches can be photographed clearly. The whole image of a PVD neuron is performed using 3–4 visual fields under 40× magnification.

⚠ CRITICAL: Based on our experience, the imaging process is better to be accomplished within 1 h. Longer time sometimes leads to weaker fluorescence intensity or degenerations of PVD dendrites.

Image processing

Timing: 1–2 h

10. The *.nd format images are opened with ImageJ (NIH) software (Schneider et al., 2012). Convert the image type to 8 bit and the color to gray. Then Maximum-intensity Z projection is generated.
11. Adjust "Brightness/Contrast" for optimal fluorescent intensity and weak background signal. Capture screen and calculate scale bar. Then save pictures. Assemble images from 3–4 visual fields to get a complete dendrite structure of a single PVD neuron. The final representative worm images are showed with the anterior to the left, the posterior to the right, and vulva facing downward (Figure 1A).

Note: In some cases, it is possible to use lower magnification (20× or 40× objective) depending on what feature of PVD you are interested in. 20× objective can only show the overall dendrite organization, while 40× objective can clearly show the structure of the neuron at all levels. If you want to see more details on dendrites, such as organelles and subcellular localization of certain proteins, you can go for a higher magnification (60× objective).

EXPECTED OUTCOMES

Image of *wyIs592* or other similar PVD reporters reveals the PVD neuron terminated near the base of the pharynx in the head region and posteriorly in the tail, and its highly branched arbor of dendritic processes enveloped with a web-like array directly beneath the skin. The PVD soma is located in posterior-lateral (Figure 1B). A 1° dendritic branch defined as the branch extends from the PVD cell soma along the anterior-posterior axis. The branch protruding from 1° dendrite is recorded as 2° dendrite, by parity of reasoning. A single 2° branch can be seen as the "stem" for a "menorah-like" collection of 3° ("base") and 4° ("candles") branches (Figure 1C). Orthogonal arrays of 2°, 3°, and 4° dendritic branches envelop the animal along the dorsal-ventral and anterior-posterior axes to produce a network of sensory processes (Oren-Suissa et al., 2010). A mature wild-type PVD neuron usually exhibits about 38 menorah-like dendritic architecture structures (Cody et al., 2010). The complete morphology of highly ordered dendrites of PVD neuron on both dorsal and ventral sides can be two- and three-dimensionally visualized only in a lateral view (Figures 1A–1C and Methods Video S1). If the mounted worms lie in a dorsal or ventral view, only the dorsal or ventral side of dendrites of PVD neuron can be fully visualized (Figures 1D–1F).

LIMITATIONS

This protocol just describes the imaging of the mature PVD neuron morphology at one time point, which is not perfect for dynamically detecting the changes of PVD neuron development or degeneration. Alternatively, if you want to observe the dynamic changes of PVD neurons development or degenerative morphology, you can recover the worm from the pads and then repeat the protocol at different time points or use long-term/time-lapse live imaging.

TROUBLESHOOTING

Problem 1

Too much bubbles are generated in the process of covering the agarose pad with a coverslip (Note after step 7). The bubbles will affect the refractive projection of fluorescence.

Potential solution

Slowly place the coverslip on the pad with lowering at an oblique angle, and the droplet containing your specimen but not the agarose pad should touch the coverslip first.

Problem 2

Only part of the dendrites of a PVD neuron can be seen under the microscope (step 8; Figures 1E and 1F).

Potential solution

When mounting worms, in the process of anesthetization, the time should not exceed 2 min (Critical after step 7), then the cover slide will be covered to prevent the worms from changing their position to lying in a dorsal or ventral view.

Problem 3

The fluorescence intensity of PVD dendrites is low or PVD dendrites are degenerating (step 9; Figure 6C).

Potential solution

Worms must be cultivated under optimal physiological conditions without starvation or other stresses (Worm Strain Preparation, steps 11–13).

Pick worms gently when mounting the slide (Critical after step 6). Wounding may lead to PVD dendrite degeneration or weak fluorescence intensity.

The mounting and total imaging procedure should be completed within 1 h (Critical after step 9).

Problem 4

Worms move intermittently during imaging (step 9).

Potential solutions

Reduce the total volume of anesthetic (Note after step 6).

The presence of *E. coli* food on the slide may induce worms to move. Reduce the *E. coli* residue on the slide when mounting worms (Critical after step 6).

Anesthetize the worms completely with freshly diluted pharmacological agent and enough anesthesia time (Critical after step 7).

Strong fluorescent light is a stimulus to worms (Note after step 8). When turn on fluorescent light, worms sometimes move a little bit. To avoid this, turn down the intensity of fluorescent light or wait for a few seconds to start imaging.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Yan Zou (zouyan@shanghaitech.edu.cn).

Materials availability

Materials used in this study are either available from the Lead Contact with a completed Materials Transfer Agreement or can be purchased (details listed in the Key resources table).

Data and code availability

This study does not generate any unique datasets or code.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.xpro.2021.100309.

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AUTHOR CONTRIBUTIONS

Conceptualization, X.W. and Y.Z.; investigation, X.W., T.L., J.H., Z.F., R.Z, W.N., X.Y., and Y.Z.; writing – original draft, X.W.; writing – review & editing, Y.Z.; funding acquisition, Y.Z.; supervision, Y.Z.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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