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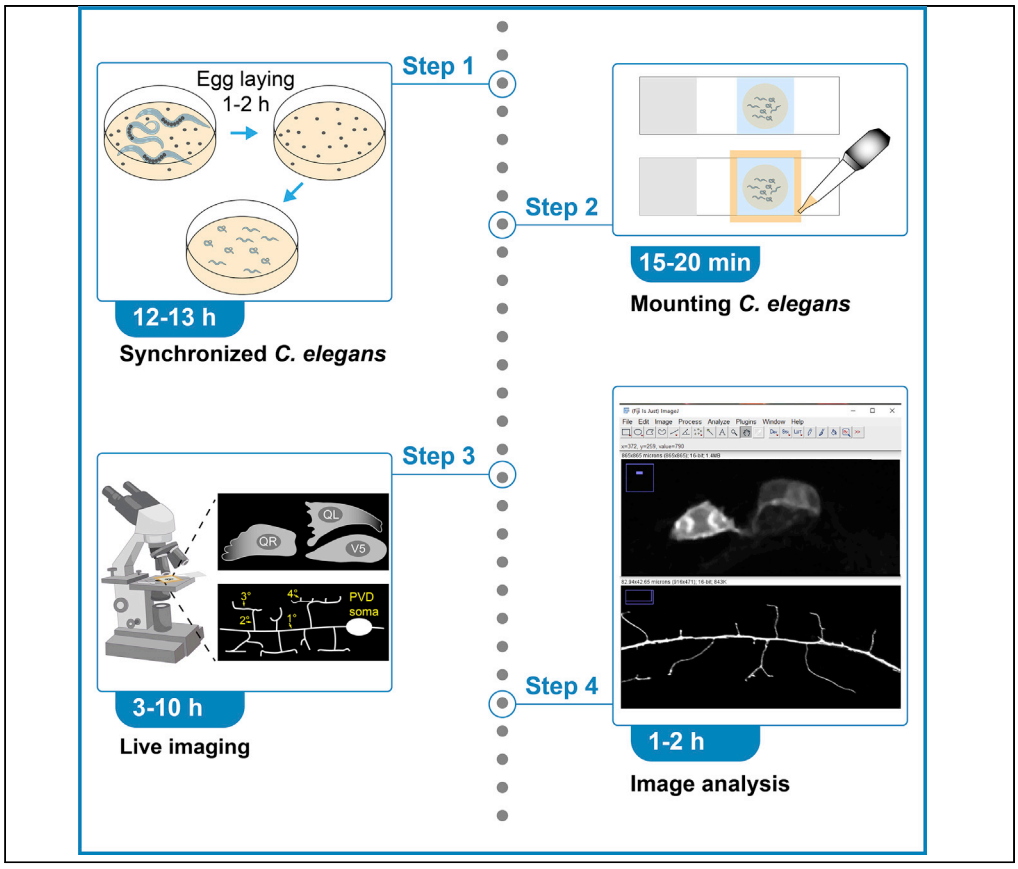
Live imaging of postembryonic developmental processes in *C. elegans*



Live imaging is an important tool to track dynamic processes such as neuronal patterning events. Here, we describe a protocol for time-lapse microscopy analysis using neuronal migration and dendritic growth as examples. This protocol can provide detailed information for understanding cellular dynamics during postembryonic development in *Caenorhabditis elegans* (*C. elegans*).

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Highlights

Protocol for synchronization and immobilization of *C. elegans*

Time-lapse imaging of postembryonic developmental processes

Details for imaging analysis and quantification

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Live imaging of postembryonic developmental processes in *C. elegans*

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SUMMARY

Live imaging is an important tool to track dynamic processes such as neuronal patterning events. Here, we describe a protocol for time-lapse microscopy analysis using neuronal migration and dendritic growth as examples. This protocol can provide detailed information for understanding cellular dynamics during postembryonic development in *Caenorhabditis elegans (C. elegans)*.
For complete details on the use and execution of this protocol, please refer to Feng et al. (2020), Li et al. (2021), and Wang et al. (2021).

BEFORE YOU BEGIN

The protocol described here is used to record dynamic events during *C. elegans* development. Live imaging may provide detailed information for various developmental processes in mutant analysis. For example, absence of a certain cell may be attributed to defective cell division or apoptosis, which is controlled by different signaling pathways. Decreased neurite length can be the outcome of defective neurite growth, pruning or anchoring. Therefore, monitoring the developmental processes by live imaging can differentiate between these alternative scenarios, and thus pointing out the directions for further genetic analysis.

The maintenance of *C. elegans* in the laboratory is performed as described (Wang et al., 2021). *C. elegans* is maintained on Nematode Growth Medium (NGM) agar plate and using *Escherichia coli* strain OP50 as a food source in the laboratory. Detailed information about preparation of growth media can be found in WormBook chapter "Maintenance of C. elegans" written by Stiernagle T. (Stiernagle, 2006) (http://www.wormbook.org/).

Preparation of synchronized *C. elegans*

Timing: 4–5 days

C. elegans is cultured on NGM plates and can be visualized using a dissecting stereoscopic microscope equipped with a transmitted light source. Worms used for imaging should be healthy, so the worm needs to be transferred to a fresh plate regularly.

There are several methods to transfer animals from old plates to fresh plates. Chunking NGM agar is a quick and convenient method for culturing animals. The agar chunk usually contains hundreds of worms which will crawl out of the agar and spread to the bacterial lawn. However, the disadvantage

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of chunking worms is that a lot of worms are transferred, causing the bacteria to be quickly consumed. Another method is to use a worm picker to pick animals into a fresh NGM plate. A worm picker can be made by installing a 1.5 cm platinum wire on the tip of a Pasteur pipet. The advantage of the platinum wire is that it can be heated and cooled quickly and can be flamed frequently to avoid contaminants.

Some cell fate markers may not be early enough for the developing processes since they usually come from terminal differentiation factors. Therefore, it is important to select specific and early-expressing promoters for tracking neuronal development. In addition, the choice of fluorescent proteins with high brightness, most photostable, least phototoxic, and fast chromophore maturation are important to obtain good results of live imaging in *C. elegans* (Hutter, 2012).

We choose *wyIs592* to label the entire PVD neuron/ including axon and dendritic branches morphology (Wang et al., 2021; Wei et al., 2015) and the integrated marker *rdvIs1* for Q neuroblast (Ou et al., 2010).

It is crucial to obtain enough healthy synchronized larvae to maximize the efficiency of live imaging. There are two ways to obtain synchronous *C. elegans*: time limited egg-laying or bleaching gravid adults to isolate eggs. The egg-laying produces synchronized population of much closer age range within only a few hours, but it cannot be applied to animals with egg-laying defects. Synchronized animals obtained by bleaching can release many eggs and are suitable for strains with a slow rate of egg-laying. Moreover, the contaminants are easily removed by bleaching.

Synchronization by timed egg-laying.

1. Transfer about 50 reproductively active adults to a fresh NGM plate seeded with bacteria OP50. Allow the adults to lay eggs at 20°C for 1–2 h.
2. If the worms have produced enough eggs, remove adult worms from the plate. Allow the eggs to hatch and develop to the right time to image. The developmental timings of *C. elegans* at different temperatures can refer to (Byerly et al., 1976).

⚠ CRITICAL: The degree of synchronization is lesser for longer time. Recording exactly how many adults are on each plate can ensure that all adults are removed.

Note: Temperature affects nematode synchronization efficiency. Higher temperatures slightly speed up development and make developmental differences greater, resulting in poor synchronization. The lower temperature slowed development and egg-laying rates, making it take longer to obtain enough eggs, ultimately resulting in poorer synchronization.

Synchronization by bleaching adult worms.

3. Wash worms off 3–4 plates (each plate should have many gravid adults) with sterile M9 buffer and pipette them into a 15 mL conical tube.
4. Centrifuge the tube for 2 min at 1,100 × *g*, remove the supernatant and leave the pellet in 1 mL liquid, then add sterile M9 to a total volume of 3.5 mL.
5. Make the bleach solution freshly just before use by mixing 0.5 mL 5 N NaOH and 1 mL household bleach (5% solution of sodium hypochlorite).
6. Add the bleach solution to the 15 mL conical tube with washed worms.
7. Shake well or vortex the tube every 2 min, and monitor worms under the dissecting stereoscope until adult materials are lysed (within 10 min). Add sterile M9 to a total 10 mL volume.
8. Spin the eggs down for 2 min at 1,100 × *g*. Aspirate the liquid to 1 mL. Add sterile M9 to a total 10 mL volume and vortex for a few seconds.
9. Repeat step 6 twice.

10. After the last centrifugation, aspirate the liquid to 1.5 mL, resuspend washed eggs in M9 solution. Use a Pasteur pipe to transfer the eggs to NGM plates seeded with OP50. Allow the eggs to hatch.

⚠ CRITICAL: Longer bleaching periods reduce the hatching rate.

Prepare reagents for coverslip seal

Timing: 10–30 min

The humidity is essential for *C. elegans* development, but the glass slides with agarose pad usually dry out within 2 h. We seal the gap between the glass slide and the coverslip with 1:1 Vaseline/Paraffin to maintain the humidity (Smith et al., 2010). Paraffin wax and Vaseline are solid at 20°C–25°C, and melt into liquid when the temperature rises above 60°C.

11. The paraffin wax in the plastic bottle is usually lumpy and bulky. Cut the wax into small pieces and put them into a 15 mL centrifuge tube. Put the tube in a Dry Bath (80°C) to heat and melt the paraffin wax.
12. Vaseline in the bottle at room temperature (20°C–25°C) is paste-like and viscous. It is troublesome to pick up and put into a 15 mL tube with a medicine spoon. Use a thin stick to roll the Vaseline into a 15 mL centrifuge tube and place it on the Dry Bath.
13. After the paraffin and Vaseline are melted, add 1:1 volume to a new 15 mL tube, mix them in the tube and place the tube in a Dry Bath for later use.

Note: The mixture of paraffin and Vaseline can be stored at 20°C–25°C and melted on the Dry Bath (80°C) when needed.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
OP50 *E. coli*	Caenorhabditis Genetics Center (CGC)	OP50
Chemicals, peptides, and recombinant proteins		
NaCl	SCR	Cat#10019318
NaOH	SCR	Cat#10019718
$MgSO_4$	SCR	Cat#C1707042
KH_2PO_4	SCR	Cat#10017608
Na_2HPO_4	SCR	Cat#20040618
Paraffin Wax	General-Reagent	Cat#8002-74-2
Vaseline commercial grade	Sangon Biotech	Cat#A510146-0500
Agarose	Coolaber	Cat#CA1341B
Levamisole hydrochloride	Sangon	Cat#A506644-0100
Polybead Microspheres 0.10 μm	Polysciences	Cat#00876-15
Experimental models: Organisms/strains		
C. elegans: *wyIs592[ser-2prom-3p::myr-GFP; odr-1p::Dsred]*	(Zou et al., 2015)	N/A
C. elegans: *rdvIs1 [egl-17p::Myri-mCherry::pie-1 3′UTR + egl-17p::mig-10::YFP::unc-54 3′UTR + egl-17p::mCherry-TEV-S::his-24 + rol-6 (su1006)]*	Caenorhabditis Genetics Center (CGC)	N/A
Software and algorithms		
ImageJ	(Schneider et al., 2012)	https://imagej.nih.gov/ij/
NIS-Elements AR 4.30.02	Nikon	N/A
NIS-Elements AR 5.20.00	Nikon	N/A

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Other		
Zeiss dissecting stereomicroscope	Zeiss	Stemi 2000
Nikon Spinning Disk confocal microscope	Nikon	TI2-E+CSU W1
Nikon A1R laser scan confocal	Nikon	Ti-E+A1R SI
Immersion Oil Type F For Microscope 30cc (MXA22168)	Nikon	N/A
Mini Dry Bath	+UXI	HX-10
Glass slide	FANYIGX	Cat#ZBP-7105
35 mm plate	JET BIOFIL	Cat#MCD000035
60 mm plate	JET BIOFIL	Cat#MCD000060
1.5 mL microcentrifuge tube	PULLEN	Cat#PL01001
15 mL microcentrifuge tube	PULLEN	Cat#PL02001

MATERIALS AND EQUIPMENT

Levamisole

Reagent	Final concentration	Amount
Levamisole	1 M	2.407 g
M9 buffer	N/A	10 mL

Aliquot 100 μL of 1 M levamisole stock solution into a 1.5 mL centrifuge tube. Store it at −20°C for a year. Just before use, dilute the stock to 2.5 mM working solution in M9 buffer. Levamisole solution can be used for 1–2 weeks at 20°C–25°C and up to 1 year when stored at −20°C.

M9 buffer

Reagent	Final concentration	Amount
KH_2PO_4	22 mM	3 g
Na_2HPO_4	42 mM	6 g
NaCl	86 mM	5 g
ddH_2O	N/A	up to 1 L

The M9 buffer after autoclaving can be stored at 4°C for up to 1 year. Add sterile $MgSO_4$ to a final concentration of 1 mM before use.

M9 with $MgSO_4$ can be stored at 20°C–25°C for one month.

Equipment

- For imaging Q Cell migration, we use Nikon Spinning Disk confocal microscope (TI2+CSU+W1) with Photometrics Prime 95B camera, W1 spinning disk head, the 561 nm excitation laser, pinhole size (50 μm) and 60×/1.4 N.A oil immersion objective.
- For the live imaging of PVD dendritic growth, we use Nikon A1R laser scan confocal equipped with 488 nm excitation laser, emission filters (B525/50), pinhole size (20.4 μm) and 60×/1.49 N.A oil (working distance 0.13 mm).
- A dissecting stereoscopic microscope Zeiss Stemi 2000 equipped with a transmitted light source, standard 10× eyepieces and objectives (0.6×-5×) for culturing and picking *C. elegans*.

Note: The disadvantage of the Nikon Spinning Disk confocal microscope for imaging is that the boundary signal is weak when the excitation light focuses on the plane, which affects the image quality after PVD stitching. Therefore, we use a laser scan confocal for imaging PVD dendritic growth since PVD neurons have elaborate architecture.

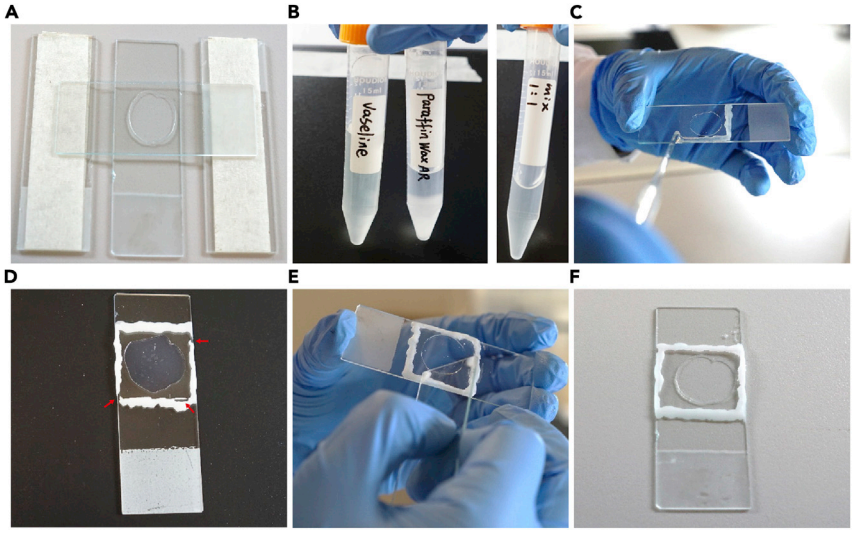

Figure 1. Prepare 10% (w/v) agarose pad for live-cell imaging

(A) Glass slide flanked by two slides with a layer of paper tape. Drop the 10% melted agarose on the middle slide and gently place another glass slide across the three slides to make an agarose pad.

(B) Vaseline and Paraffin are placed in a thermostatic Dry Bath (80°C) to be melted and mixed at a 1:1 volume ratio.

(C) The gap between the glass slide and the coverslip is sealed with the mixture of Vaseline and Paraffin.

(D) If the gap is not sealed completely as shown by the red arrow, add more mixture to the unclosed area.

(E and F) Use another glass slide to slowly scrape the protruding white wax until the thickness becomes uniform.

STEP-BY-STEP METHOD DETAILS

Preparation of agarose pad and mounting *C. elegans*

Timing: 15 min

10% (w/v) agarose pad is used to immobilize worms for live-cell imaging. High quality agarose pad with stable humidity is the key step for live-cell imaging of *C. elegans*.

1. Prepare 10% (w/v) agarose solution: Boil 2 g agarose in 20 mL M9 buffer in a 50 mL Erlenmeyer flask. Aliquot 5 mL into a 15 mL centrifuge tube, and place the tube on a thermostatic Dry Bath (80°C) to keep the solution as liquid.

   Note: The aliquoted agarose solution in 15 mL centrifuge tube can be stored at 4°C for up to 2 months. When needed, place the tube in a beaker containing water, unscrew the cap of the tube and place it in a microwave oven to heat. Be careful to minimize boiling over to reduce evaporation. Put the tube in the Dry Bath to keep the agarose in the molten state.

2. Prepare two glass slides, and paste a layer of paper tape on both sides. Put the two glass slides on the table in parallel, and place a clean glass slide in the middle (Figure 1A).
3. Add a drop (50–100 μL) of 10% molten agarose onto the center of the middle slide and immediately place another slide across the three slides, forming an agarose sandwich (Figure 1A).
4. Wait about 1 min for the solidification of the agarose, then remove the top slide by gently sliding. Place the middle slide with the agarose pad in a humidity chamber.

   Note: Although the agarose pads can be kept in a humidity chamber for 1 h, we recommend a freshly made agarose pad. The agarose pads that have been stored for a long time become wrinkled (Wang et al., 2021), which affects the image quality.

5. Place 10–20 synchronized larval *C. elegans* into the 2.5 mM levamisole solution on the pad with a worm picker. Wait a while until the movement of the larvae is not obvious. Put the coverslip on the pad.

   Alternatives: We recommend washing the worms with M9 buffer in 1.5 mL microcentrifuge tube, spinning the tube in a tabletop centrifuge at 1,000 × *g* for 15 s. Aspirate to 100 μL and add 100 μL 5 mM levamisole. Use a Pasteur pipet to transfer worms (25–50 μL) to the agarose pad and absorb the excess liquid with filter paper. Wait for 30 s and put the coverslip on the pad.

   Note: The stages of worms depend on the developmental time window of the dynamic processes you are interested in. For example, we use late L2 larval worms (about 30 h after egg laying) for the live imaging of PVD dendritic growth and early L1 larvae (12–14 h after egg laying) for imaging Q cell migration.

   Note: Transferring worms with a worm picker is simple and quick, but it is easy to cause damage to the larvae. The method of M9 buffer washing down the worms has little damage to the larvae, but the operation is cumbersome and takes a longer time.

6. Make a 1:1 mixture of Paraffin: Vaseline. Put the mixture on the edge of coverslip to seal the gap between the coverslip and the slide (Figures 1B and 1C).

   ⚠ CRITICAL: After adding the mixture of Paraffin and Vaseline, check the gap between the cover glass. If there is a hole (Figure 1D), make sure to fill it. If the solidified Paraffin: Vaseline mixture is too thick, use another glass slide to slowly flatten it until it is slightly higher than the coverslip (Figures 1E and 1F).

Imaging Q neuroblast migration using spinning-disk confocal microscopy

Timing: 8–10 h

Q cells are born on the left (QL) and right (QR) lateral sides during late embryogenesis. After hatching, both QL and QR neuroblasts need to undergo 3 rounds of asymmetric cell divisions to yield 2 apoptotic cells and 3 neurons. The QL cell produces PQR, PVM and SDQL, whereas the QR cell produces AQR, AVM and SDQR (Middelkoop and Korswagen, 2014).

About 4 h after hatching, Q cells complete the initial migration, in which QL migrates a short distance towards the posterior to a position above the V5 seam cell, and QR migrates forward to a position above the V4 seam cell, then Q cells divide into equal-sized daughter cells Q.a and Q.p (Figure 2A) and migrate a long distance along the anteroposterior axis. The daughter cells Q.a divide once and Q.p twice to produce 3 descendants. The descendants of QL migrate toward the tail of the worm, whereas QR descendants migrate anteriorly to the head (Figure 3A).

7. Preparation of Microscopy and photography equipment.
   a. Use Nikon Spinning Disk confocal microscope with an air lens 10×/ 0.5 N.A objective for finding worms and an oil lens 60×/1.4 N.A objective for high-resolution imaging.
   b. Image with a spinning disk confocal scanner unit CSU-W1 with NIS-Elements AR 5.20.00 and a Photometrics Prime 95B camera. The parameters of CSU-W1 are as follows: 4,000 rpm disk-spin speed, pinhole 50 μm and 561 nm excitation laser wavelengths. We usually use 10% of the full power of the 561 nm laser with 200 ms exposure time.

   Note: Using minimal laser power and exposure time to minimize phototoxicity. Set the room temperature at 20°C–25°C for imaging.

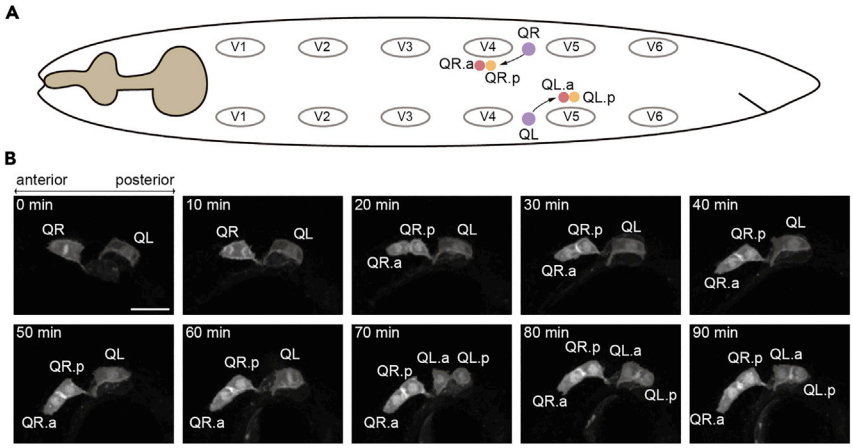

Figure 2. Q neuroblast undergoes migration and division

(A) The diagram shows the birthplace and migration of Q neuroblast (dorsal/ventral view). Q neuroblasts are born between V4 and V5 within the two later rows of seam cells (V1-V6). The QR cell is located on the right lateral side and migrates anteriorly, while QL cell on the left side migrates posteriorly. About 4 h after hatching, QR initially migrated to position above V4 seam cell and QL migrated to position above V5 seam cell, and then began to the first cell division.

(B) Images show Q cells (QR and QL) migration and cell division. The marker *rdvIs1* labels the plasma membrane and chromosomes by mCherry fused with myristoylation signal and histone (HIS-24) (Ou et al., 2010). The time is shown on the top left. Scale bar, 10 μm. See also Methods video S1.

8. Live imaging.
   a. Place the slide on the inverted fluorescence microscope stage and find worms under air lens 10×/ 0.5 N.A objective, using the automated stage of acquisition software to mark the location of worms.
   b. Use a higher-magnification 60× objective to select healthy L1 larvae and examine the development stage of Q cells. Q cells are born between V4 and V5 seam cells and start to migrate. We choose the stage before the first division and mark its position.
   c. To follow Q cell migration, acquire a stack of mCherry images with 18 planes at 1 μm interval every 10 min and automated stages for multiple position acquisition.

Note: Try to avoid following worms for imaging. The worms appear straight, shrunken or curled after immobilization with levamisole solution. The straight or shrunken worms die quickly, whereas the curled worms tend to live longer, but too much curling up may affect Q cell division and migration.

Note: Do not select worms that are close to each other to image, as this will affect the imaging quality.

⚠ CRITICAL: Choose the worm with a little curl and only a single worm in the field to image.

   d. Finding enough worms to image is important for the efficiency of long-term live imaging. We normally choose no more than 5 worms on one slide to image.

Note: The more worms to image and the longer exposure time result in more phototoxicity. Too many (>5) marked positions may result in out-of-focus problems during imaging.

   e. For imaging acquisition: set up acquisition parameters, including the excitation laser wavelengths at 561 nm, 10% power, 200 ms exposure time, Z stack at 1 μm interval and a frame rate of 10 min. Create a new data folder and set parameters for automatically saving the new live-imaging data.

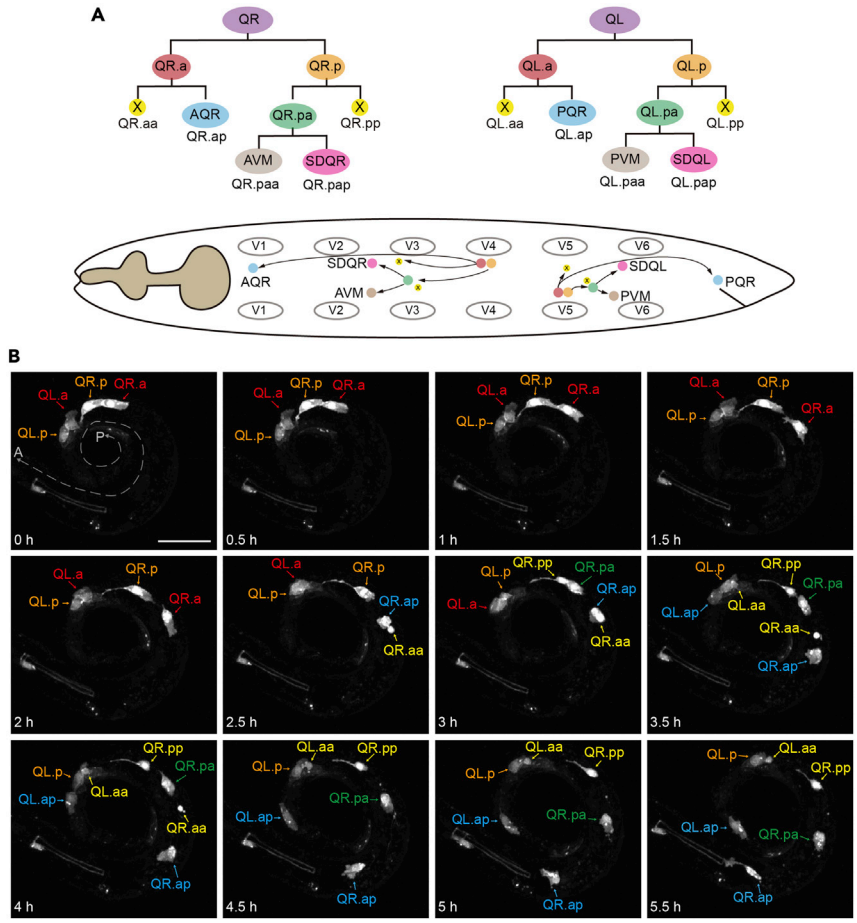

Figure 3. Q neuroblast lineage and their descendant's migration

(A) The schematic overview of Q neuroblast lineage and their descendant's migration at L1 larval stage. QR and QL neuroblasts undergo symmetric and asymmetric cell division to yield two apoptotic cells (QR/L. aa and QR/L. pp) and three neurons (A/PQR, A/PVM and SDQR/L). QR descendants migrate anteriorly to the head, whereas QL descendants migrate posteriorly to the tail. Color code in the migration of Q neuroblast descendants is the same as in the Q neuroblast lineage.

(B) Images of Q descendants' migration visualized by a fluorescent marker *rdvIs1* at indicated time points after the cell first division. The dashed line indicates the position of the worm with arrowheads pointing to A (anterior) and P (posterior). The colors of the text and the arrows corresponds those in (A). Scale bar, 20 μm. See also Methods video S1.

Note: A longer frame interval and less Z stacks can reduce photodamage, but they may cause a lot of details to be lost.

Note: To studying asymmetric cell division within 1 h duration, we recommend setting the time interval at 30–60 s.

Note: To examine the cell migration and apoptosis for more than 3 h duration, we set the time interval at 5–10 min. Tracking the development completion of Q cell lineage takes about 10 h. For physiological activity and high-quality images of worms, we recommend a maximum time of no more than 4 h for live imaging.

Imaging PVD dendritic growth using laser scan confocal microscopy

Timing: 3–4 h

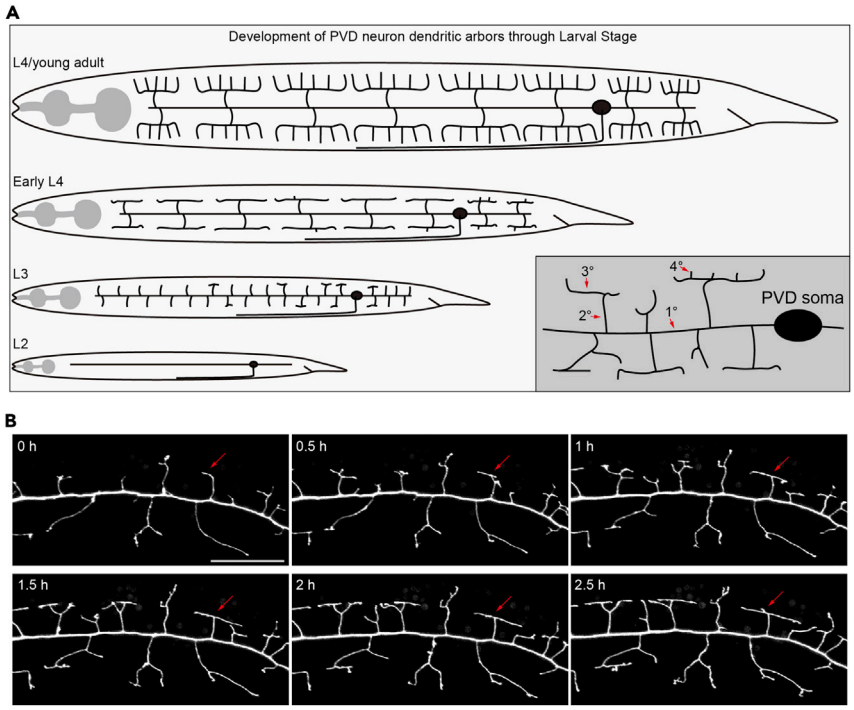

Figure 4. The development of PVD neurons dendrites

(A) The cartoon shows that PVD neurons gradually develop a complete dendritic morphology as the process of development (modified from wormatlas.org). The pattern diagram of the dendritic branch structure of PVD neurons is shown in the bottom right corner. The numbers and red arrows indicate the 1°, 2°, 3°, and 4° dendrites.

(B) The representative images show the dynamic process of PVD neuron dendritic development. The red arrow indicates the process of dendritic growth. Scale bar, 20 μm. See also Methods video S2.

PVD is derived from V5 cell lineage and is born post embryonically, located on the posterolateral sides of the body with PVDL on the left and PVDR on the right. In the larval stages, the 1° dendrites grow along the anterior and posterior axis, then 2° dendrites grow perpendicular to the 1° dendrites, reaching the axial boundary of the medial edge of body muscle cells. In this position, each 2^0 dendrite turns horizontally to project 3° dendrite, and each 3° branch eventually adopts a vertical angle, leaving a gap between adjacent menorahs and giving rise to a 4° dendrite. Finally, PVD dendrites forms well-ordered, repeating candlestick-like units at L4 stage (Figure 4A). We use laser scan confocal microscopy to collect a Z-stack image of PVD neuron dendritic branches, especially for the growth process of 2°–4° dendrites.

9. Open Nikon Microscope Acquisition Software NIS-Elements AR 4.30.02. Place the prepared slide on the stage of inverted fluorescence microscope and find worms under air lens 10×/ 0.5 N.A objective, marking the location of worms.

   Note: For imaging PVD dendritic growth, we mount 10–15 synchronized L2 *C. elegans* on the agarose pad. Select healthy L2 larvae with proper straight body and without other worms nearby to interfere with.

10. Examine the development stage of the PVD dendritic at 20× objective, then switch to a higher-magnification 60× objective.
11. Set up image parameters: laser scan confocal PMT (HV 86), 20.4 μm pinhole size, one way scan direction, 2.288× zoom, laser scan speed at 0.25 fps, laser wavelength at 488 nm, 5% laser Power, Z-stack at 0.5 μm, 10 min time interval for 3.5 h duration.

Note: To follow dendrite branching and self-avoidance in detail, we recommend setting the time interval to 30 s–60 s within 1 h.

⚠ CRITICAL: To minimize phototoxicity, we use minimal laser power and exposure time. If the PVD dendrites stop growing for more than 30 min, find other worms or make a new slide to start a new imaging.

EXPECTED OUTCOMES

Using live imaging of dynamic cellular events in postembryonic *C. elegans*, we can study the asymmetric cell division, cell migration and apoptosis of the Q cell development (Figures 2 and 3). After hatching, the QL migrates a short distance towards the posterior whereas QR migrates towards the anterior. After the Initial short-distance migration, QL and QR cells first divide symmetrically to generate daughter cells Q.a and Q.p, which migrate to the same direction as the progenitor cells. The second division is asymmetric to produce Q.aa/Q.pp (small) and Q.ap/Q.pa (larger), then Q.aa and Q.pp undergo apoptosis, while the larger daughter cells Q.ap and Q.pa survive. Q.ap differentiates into the sensory neuron AQR (QR.ap)/PQR (QL.ap), Q.pa undergoes the third round of symmetrical cell division to generate Q.pap and Q. paa, and finally differentiates into SDQL/R and P/AVM neurons (Methods video S1).

Following this protocol, we can also study the development and organization of dendrites. During the L2 larvae stage, PVD neurons are born and the soma is located in the posterior-lateral of the body. 1° dendrites of PVD grow along the anterior-posterior axis, then 2° dendrites grow perpendicular to the 1° dendrites. 2° dendrites protrude 3° dendrites along the horizontal direction, and each 3° dendrite grows 4° dendrites vertically, leaving gaps between each adjacent 3°. We can monitor a dynamic process of dendrites development (Figure 4 and Methods video S2) to study neurite growth, guidance, anchoring, self-avoidance or regeneration.

QUANTIFICATION AND STATISTICAL ANALYSIS

The live-imaging raw data (nd2 suffix format) are opened with Fiji (ImageJ) software, analyzed and processed. The raw data of live imaging analyses, at least ten high-quality images are required.

Raw data preparation.

1. For live-cell imaging analysis with ImageJ, Open Image> Adjust> Brightness/Contrast> Set Display Range.
2. Pick high-quality frames for Maximum-intensity Z projection.
3. To ensure high quality analysis, make sure that the fluorescence signal is not overexposed, and the kinetic parameters remain the same in the sequential images. Set each processed image to gray and saved as a tiff file in a new folder.
4. Adjust the position of the *C. elegans*: head left and tail right, dorsal up and ventral down. Process: Image> Transform> Rotate.
5. Set scale bar, Analyze> Tools> Scale bar.
6. Movie production: arrange each picture in time sequence, merge it into the same series, and save as an avi format file. Process: Image> Stacks> Tools> Concatenate> File> Save As> AVI. For next statistical analysis, the images need to be saves as an 8-bit image. Go to Image > Type > 8-bit.

   Note: The *C. elegans* appear twisted or even slightly rotated, causing image drift. Manually calibrate the position of the worm in each image, keep it in a relative position and crop it to the same size.

Q cell migration speed and direction.

7. Select a relatively stationary point within the animal as a reference point. Auto-fluorescent particles in the gut are good reference points.
8. Measure the distance from the cell to the reference point along the midline of the worm at the time of data collection, calculate the relative movement in every 10 min interval, and obtain the average velocity.
9. For each frame, draw a line through the cell along the anterior-posterior axis of the animal. The line pointing to the head is set as 0° (0 degrees). Draw a line from the nucleus to the middle of the lamellipodia morphology, and measure the angle between this line and 0°, which is the cell migration direction defined at this time (Chai et al., 2018).

PVD dendritic growth statistics.

NeuronJ is a generally used plugin for semi-automatic tracking and measurement of neurites in ImageJ. NeuronJ plugin can be downloaded from the ImageScience website (https://imagescience.org/meijering/software/neuronj/).

10. Open the ImageJ and go to Plugins > Install Plugin. The installation Plugin update needs to close the ImageJ. Open the ImageJ again and go to plugins > NeuronJ. This will change the toolbars in ImageJ and the toolbar is described in the NeuronJ manual (https://imagescience.org/meijering/software/neuronj/manual/).
11. Open the 8-bit image in NeuronJ using "Load image/tracings" (fold style). Select "Set parameters" (white squares with lines style) to set Neurite appearance at Bright (image appears as bright structures on a dark background). However, tracing Dark neurites on a bright background to set Neurite appearance at Dark.
12. When tracing the neurite, the image needs to be zoomed in as much as possible to improve accuracy. Use "Add tracing" button to trace neurite and "Delete trace" button to remove the mistake traces.
13. Select "Label tracing" (four color squares) to label the tracing. By assigning different tracing ID, cluster, and color, you can mark multiple dendrites simultaneously. Select "Measure tracings" to measure the neurites.
14. Select "Save tracings", save the statistical data as a CSV file with the same name as the image. Repeat the above process for all images.

Note: The length should be read in μm instead of pixels. To calibrate distances, an image with a known scale is needed. Open the image in ImageJ and select the straight line from the toolbar. Make a straight line between two points with a known distance at the proper magnification. Then go to Analyze -> Set Scale.

LIMITATIONS

This protocol for live imaging provides information about the cellular dynamic events during *C. elegans* postembryonic development. The main limitations of this protocol are: (1) The protocol cannot be applied to the imaging of later embryonic development since worms begin to writhe within the eggshell in the later stages of embryonic development and could not be immobilized with anesthetic. The resources about live imaging of embryogenesis can be found in Wormbook or reported studies (Bao and Murray, 2011; Laband et al., 2018; Zhao et al., 2021). (2) The physiological functions of *C. elegans* are affected after the imaging time longer than 4 h, leading to slowing down in the speed of cell division and migration, which is also reported elsewhere (Chai et al., 2012).

TROUBLESHOOTING

Problem 1

Mounting *C. elegans* on the agarose pad leaves too much liquid (step 5).

Potential solution

Add a small drop (~2 μL) of levamisole solution on the pad and quickly transfer animals using a worm picker (step 5). Alternative, elute the worms into a 1.5 mL tube, removing excess liquid to ensure the density of the worms. We recommend that the L1-L2 larvae contain about 50 worms in 10 μL of liquid. Absorb excess liquid on the pad with filter paper and wait for 30 s before placing the coverslip on the pad.

Problem 2

Too many worms on the agarose pad results in crowding, which affects the imaging quality (step 5).

Potential solution

Don't pick too many worms. Alternatively, control the density of washed worms in M9 buffer and drop a little on the slide.

Problem 3

The *C. elegans* larvae easily die or developmentally arrest during imaging (steps 8 and 11).

Potential solution

Pick up as gently as possible to avoid damage when transferring the *C. elegans* and minimize bacteria as much as possible to avoid nematodes anoxia, which may result in developmental arrest or death (step 5). Besides, normal physiological processes are slowed down when imaging exposure exceeds 3 h (Chai et al., 2012). For long-term fluorescence time-lapse imaging, we recommend reducing the amount of levamisole anesthesia and add microbeads for physically immobilization (Middelkoop et al., 2012). It is also helpful to use lower laser power, imaging frequency and time to reduce phototoxicity. In addition, segmented imaging is performed for imaging with a development time of more than 3 h (steps 7 and 11).

Problem 4

The *C. elegans* movement is frequent, causing image drift (steps 8 and 11).

Potential solution

To fully immobilize the worms for imaging, check the concentration of levamisole. And the levamisole solution should be freshly prepared. A good method for immobilizing the worms is: soaking the worms in a solution of 0.1% tricaine and 0.01% levamisole in M9 for 20 min prior to imaging (Dong et al., 2013). Microbeads also can be used to physically immobilize *C. elegans* (Polysciences-00876 2.5% w/v aqueous suspension) (Middelkoop et al., 2012) (step 5).

Problem 5

The fluorescence signal is weak and background signal is strong (steps 8 and 11).

Potential solution

Increasing the exposure time is better than higher laser intensity (steps 8 and 11), and leaving OP50 bacteria as few as possible on the agarose pad (step 5).

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.2022.101336.

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AUTHOR CONTRIBUTIONS

Conceptualization, T.L. and Y.Z.; Investigation, T.L., X.W., Z.F., and Y.Z.; Writing – Original Draft, T.L.; 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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