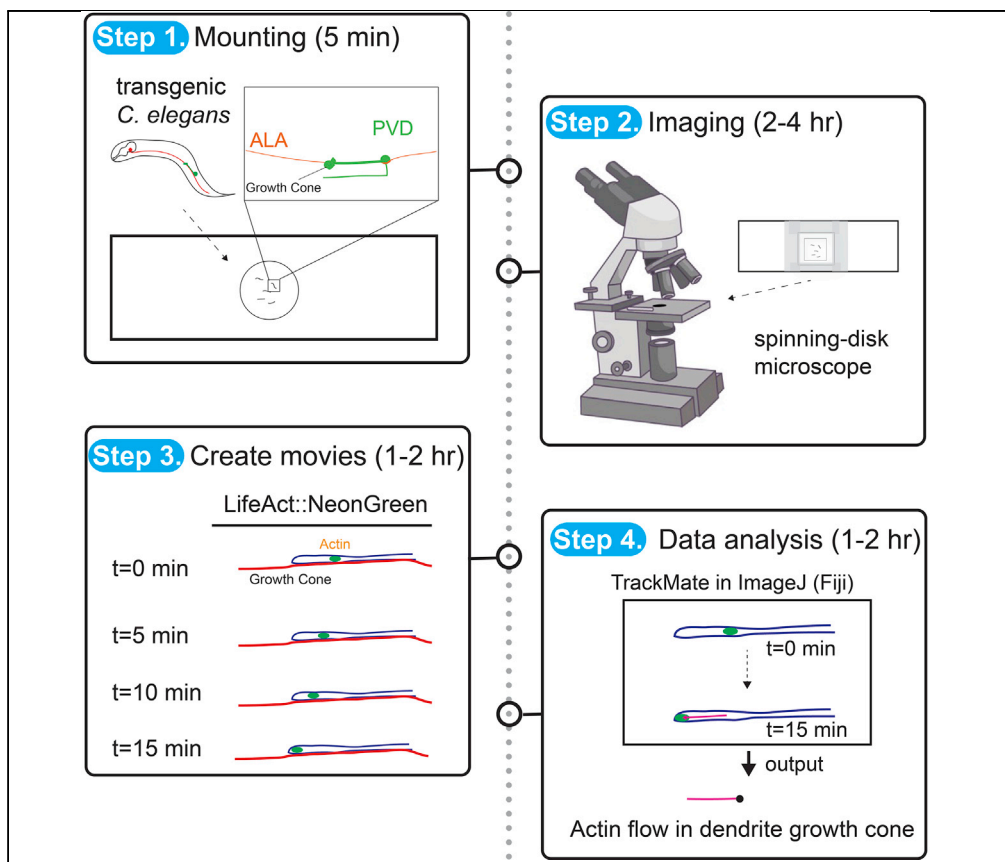


Protocol

Live-cell imaging of PVD dendritic growth cone in post-embryonic *C. elegans*



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HIGHLIGHTS

Conceptual framework to study real-time dendrite and axon development in *C. elegans*

Live-cell imaging of PVD dendrite growth cones by spinning-disk confocal microscopy

Analysis of F-actin dynamics in growth cones by TrackMate of Fiji (ImageJ)

Live-cell imaging analysis provides tremendous information for the study of cellular events such as growth cone migration in neuronal development. Here, we describe a protocol for live-cell imaging of migrating PVD dendritic growth cones in the nematode *C. elegans* by spinning-disk confocal microscopy. Fluorescently labeled growth cones and cytoskeletal proteins could be continuously observed for 4–6 h in mid-stage larvae. This protocol is suitable for revealing the dynamic molecular and cellular events in dendrite and axon development of *C. elegans*.

Chen & Pan, STAR Protocols 2,
100402
June 18, 2021 © 2021 The
Author(s).
[https://doi.org/10.1016/
j.xpro.2021.100402](https://doi.org/10.1016/j.xpro.2021.100402)



Protocol

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<https://doi.org/10.1016/j.xpro.2021.100402>

SUMMARY

Live-cell imaging analysis provides tremendous information for the study of cellular events such as growth cone migration in neuronal development. Here, we describe a protocol for live-cell imaging of migrating PVD dendritic growth cones in the nematode *C. elegans* by spinning-disk confocal microscopy. Fluorescently labeled growth cones and cytoskeletal proteins could be continuously observed for 4–6 h in mid-stage larvae. This protocol is suitable for revealing the dynamic molecular and cellular events in dendrite and axon development of *C. elegans*.

For complete details on the use and execution of this protocol, please refer to Chen et al. (2019).

BEFORE YOU BEGIN

This protocol provides a detailed description for performing live-cell imaging analysis of developing dendritic growth cones in *C. elegans* larvae. The essential materials are transgenic *C. elegans* strains, spinning-disk confocal microscope, commercialized Zen software (Carl Zeiss), and the open source software Fiji (ImageJ). Transgenic *C. elegans* strains are generated by standard germline transformation method. Expression of fluorescent proteins in free form or in fusion with other proteins or peptides, such as Lifeact, enables the visualization of growth cone morphology and cytoskeleton dynamics. Here we describe procedures to generate transgenic *C. elegans* strains that express GFP or Lifeact::NeonGreen in PVD neurons.

Generating transgenic *C. elegans*

⌚ Timing: 1 week

1. Place wild-type L4 larvae hermaphrodites on Nematode Growth Medium (NGM) plates with OP50 bacteria as the food source and cultivate the animals 12–16 h at 20°C (Brenner, 1974).
2. Prepare the injection mixture with *Pser2prom3::GFP* (50 ng/μl) or *Pser2prom3::LifeAct::NeonGreen* (20 ng/μl), coinjection marker *Pflp-7::mCherry* (50 ng/μl), and empty vectors pBlue-script (Mello et al., 1991) (Table 1). *Pflp-7::mCherry* (50 ng/μl) is added in the mixture as the cotransformation marker. *Pflp-7::mCherry* labels several head and tail neurons including ALA interneuron that is fasciculated with PVD primary dendrite. The use of *Pflp-7::mCherry* facilitates locating PVD dendritic growth cones in live-cell imaging.



Table 1. Constructs used in microinjection

Construct name	Injection amount (final concentration in the injection mixture)	Function
<i>Pser2prom3::GFP</i> or <i>Pser2prom3::LifeAct::NeonGreen</i>	50 ng/μl or 20 ng/μl	label PVD, OLL and one tail neurons
<i>Pflp-7::mCherry</i>	50 ng/μl	label head and tail neurons, including ALA interneurons. ALA axons are fasciculated with PVD primary dendrites
pBluescript	100 ng/μl	empty vector; used for reaching the optimal DNA concentration of around 200 ng/μl in the DNA mixture for microinjection

Note: In addition to the *ser2prom3* promoter, promoters of *F49H12.4* and *mec-3* could be used for labeling PVD neurons as alternatives.

- Load the needle with 1 μL of DNA mixture on the end of injection needle and wait for 5 min until the needle is filled by capillary action.
- Install Injection needle onto microinjector, and push against a glass tube to create an opening under 40× objective.
- Immerse animals in an oil drop, and gently push them down on the 2% (w/v) dried gel pad.
- Insert the injection needle to the distal core cytoplasm of one gonadal arm, and inject the DNA mixture.
- Recover the worms by adding a drop of M9 buffer, and pick the worms back to OP50-seeded NGM plates. Incubate at 20°C for three days.
- Search F1 animals with expression of the cotransformation marker (mCherry in the head) by fluorescent stereomicroscope and isolate each F1 animal into a single OP50-seeded NGM plate.
- If the F2 progeny inherit expression of the cotransformation marker, keep the plate as one individual transgenic line. Keep all transgenic lines for further analysis.
- Use the compound microscope to verify the expression pattern of the transgenes at L3-L4 stages. *Pser2prom3::GFP* labels the entire PVD neuron, including the axon and dendritic arborization. *Pser2prom3::LifeAct::NeonGreen* is enriched in dendritic growth cones at early L2 stage, and is enriched in the high-order branches of PVD neurons at later developmental stages (Figure 1).

△ **CRITICAL:** High expression of *LifeAct::NeoGreen* may disrupt neuronal development (Figure 1B). Be sure to keep the concentration of injected DNA low (<25 ng/μl) and screen multiple transgenic lines to select an optimal one for live-cell imaging. The optimal transgenic lines are selected by two criteria. First, growth cones are clearly visible at late L2 stage without aggregation of GFP or NeonGreen signal. Second, over 80% of transmission rate and stable expression level among individuals. In some cases, abnormal branches at primary dendrite and abnormal protrusions at dendritic growth cone are observed, suggesting that the transgene interferes PVD branch development.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
<i>E. coli</i> : strain OP50	<i>Caenorhabditis Genetics Center</i> (CGC)	WormBase:OP50
Chemicals, peptides, and recombinant proteins		
Polybead (0.1 μm)	Polysciences Inc	Catalog number: 00876
Levamisole	Sigma-Aldrich	Catalog number: FL-31742

(Continued on next page)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Agarose	PanReac AppliChem	Catalog number: A22114
KH ₂ PO ₄	PanReac AppliChem	Catalog number: 121512
Na ₂ HPO ₄	PanReac AppliChem	Catalog number: 131678
NaCl	PanReac AppliChem	Catalog number: A2942
MgSO ₄	PanReac AppliChem	Catalog number: A4101
NaOH	PanReac AppliChem	Catalog number: 211687
Recombinant DNA		
<i>Pser2prom3::LifeAct::NeonGreen</i>	Chen et al., 2019	N/A
<i>Pser2prom3::GFP</i>	Chen et al., 2019	N/A
<i>Pflp-7::mCherry</i>	Chen et al., 2019	N/A
pBluescript II KS(-)	Addgene (Catalog number: 212206)	N/A
Experimental models: Organisms/strains		
<i>C. elegans</i> : strain CLP937: <i>twnEx398(Pser2prom3::GFP, Pflp-7::mCherry)</i>	Chen et al., 2019	N/A
<i>C. elegans</i> : strain CLP1078: <i>sax-7(nj48); twnEx398(Pser2 prom3::GFP, Pflp-7::mCherry)</i>	Chen et al., 2019	N/A
<i>C. elegans</i> : strain CLP928: <i>twnEx400(Pser2 prom3::LifeAct::NeonGreen, Pflp-7::mCherry)</i>	Chen et al., 2019	N/A
Software and algorithms		
Fiji (ImageJ) (Schindelin et al., 2012)	NIH	https://imagej.net/Fiji.html
Other		
ZEISS Axio Examiner	Carl Zeiss	N/A
Yokogawa CSU-XI spinning disk	Yokogawa	N/A
Rolera EM-C2 EMCCD	QImaging, Surrey, Canada	N/A

MATERIALS AND EQUIPMENT

M9 buffer		
Reagent	Final concentration	Amount
KH ₂ PO ₄	22 mM	3 g
Na ₂ HPO ₄	42 mM	6 g
NaCl	85 mM	5 g
ddH ₂ O	N/A	up to 1 L

After sterilization by autoclaving, cool it down to room temperature (20°C–25°C), and then add 1 mL 1M MgSO₄. Mix well and store at room temperature (20°C–25°C) for up to 1 month.

Alkaline-bleach solution		
Reagent	Final concentration	Amount
NaOH (10N)	0.8 N	0.4 mL
Commercial bleach	5%	0.25 mL
ddH ₂ O	N/A	up to 5 mL

Store at room temperature (20°C–25°C) for up to 1 week.

STEP-BY-STEP METHOD DETAILS

Synchronizing transgenic *C. elegans*

⌚ Timing: 4 days

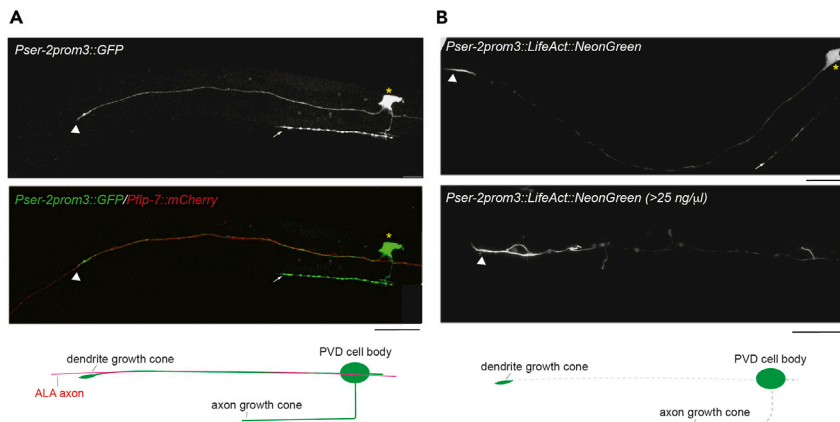


Figure 1. Representative figures of the PVD neurons at early L2 stages

(A) PVD neurons labeled by *Pser2prom3::GFP* (top) and merge image (middle) of PVD and the ALA axon labeled by *Pflp-7::mCherry*. (Bottom) Schematic diagram of the PVD neurons and ALA axon at early L2 stage. (B) PVD neurons labeled by *Pser2prom3::LifeAct::NeonGreen* at low (top) and high (middle) expression level. (Bottom) Schematic diagram of PVD neurons labeled by *LifeAct::NeonGreen* at early L2 larva stage. Arrowhead, dendritic growth cone. Arrow, axon growth cone. Asterisk, PVD cell soma. Scale bar, 20 μm .

The primary PVD dendrites begin to develop at the L2 stage (~ 20 h post-hatching) and complete their extension within 3–4 h. To image PVD dendritic growth cones at the right developmental stage, start with synchronized L1 larvae obtained by bleaching gravid adult transgenic *C. elegans* and hatching the embryos in M9 buffer one day before the experiment to accurately time the developmental stage.

1. Pick 20 transgenic *C. elegans* at L4 stage with expression of GFP or *LifeAct::NeonGreen* in PVD neurons on each feeding NGM plate (5.5 cm). Distribute 20 worms onto four plates (5 per plate) for each experiment.
 2. After cultivation for 3 days at 20°C, over 100 gravid hermaphrodites are obtained in each plate.
 3. Wash down *C. elegans* with 1 mL M9 buffer per plate, and collect hermaphrodites into a single 15 mL glass tube.
 4. Centrifuge at 1500 \times g for 5 min, and remove the supernatant.
 5. Add 10 mL of M9 Buffer to wash the sample. Repeat steps 4 and 5 twice until the supernatant becomes clear.
 6. Remove the supernatant, resuspend the worm pellet with alkaline-bleach solution, and seal the opening with parafilm.
 7. Place the 15 mL glass tube on the rocking plate and gently rock for no longer than 5 min.
 8. Visually inspect the worm-containing solution against the room light and make sure that worm carcasses are gone, leaving the solution in a fine-grain appearance.
- △ CRITICAL: Time in the bleach is critical. Soaking in bleach solution over 7 min will kill the embryos.**
9. Stop the reaction by adding 10 mL M9 buffer.
 10. Centrifuge at 4000 \times g for 5 min, and remove the supernatant.
 11. Wash the embryos with M9 buffer as step 5. Repeat this step for three times.
 12. Resuspend the embryos in 1 mL M9 buffer and seal the opening with parafilm.
 13. Incubate the embryos without bacterial food at 20°C for 12–16 h on the rocker.
 14. On the next morning, centrifuge the arrested L1 larvae at 1500 \times g for 5 min, and remove the supernatant.

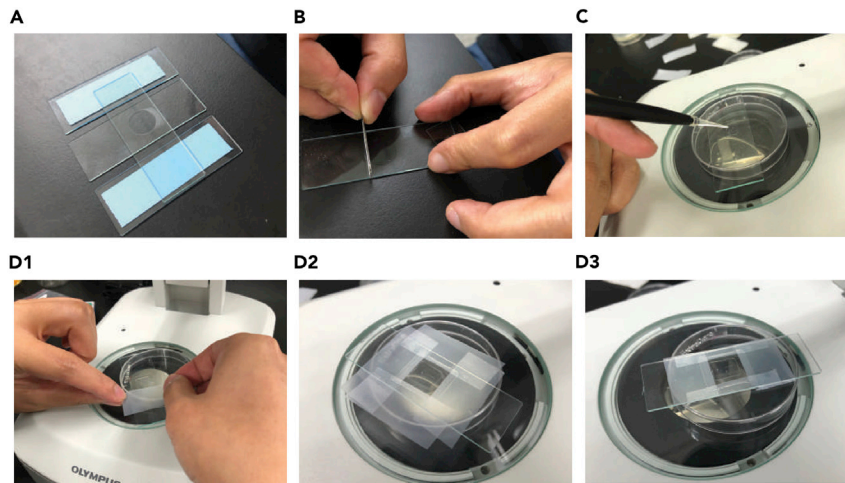


Figure 2. Steps for making a 10% agarose gel pad for live-cell imaging

- (A) Leave a drop of 10% melted agarose on a slide flanked by two taped slides, place another slide perpendicular to the center slide, and gently press against the top slide to make a round pad.
 (B) Use razor to trim excessive parts of the gel pad.
 (C) Drop the levamisole/Microbeads mixture on the gel pad and pick animals into the solution.
 (D1) Seal the edge of the coverslip with 3M tape.
 (D2-3) Trim the 3M tape to fit the slide.

15. Resuspend with 50 μ L M9 buffer and drop 2–5 μ L worm solution on NGM plates seeded with OP50 bacteria.
16. Cultivate the L1 larvae for 20 h at 20°C.

Mounting *C. elegans* onto an agarose pad

⌚ Timing: 10 min

Adequate immobilization of worms is the key step for a successful live-cell imaging experiment. This part will provide a detailed description for mounting a live L2 larva with the anesthetic mixture onto an agarose gel pad for microscopic observation.

17. Make an agarose pad (Figure 2):
 - a. Tape two glass slides with lab tape. The thickness of the tape will be the thickness of the agarose pad made later.
 - b. Place a third slide between the two taped slides.
 - c. Use a Pasteur pipette to put a drop of freshly melted 10% (w/v) agarose (dissolved in distilled water) onto the center of the untapped slide.
 - d. Immediately lay another untaped slide on the melted gel perpendicular to the third slide, and press this slide gently to make a circular gel pad. Wait for 10 s until the gel pad is cool down.
 - e. Once the agarose pad is set, carefully remove the fourth slide by sliding it, and use a razor blade to trim the pad to fit the size slightly smaller to coverslip.
18. Prepare the anesthetic mixture by mixing 1 μ L 5% (w/v) levamisole, 0.25 μ L liquid OP50 culture at 0.4–0.6 optical density (600 nm) and 0.75 μ L microbeads.

Note: Levamisole causes the animal to contract, which results in shorter body length.

19. Place around 10 synchronized L2 *C. elegans* larvae in the anesthetic mixture and wait for 2 min until the movements of larvae are significantly reduced.
20. Put on the coverslip and then gently seal the edge of coverslip by vaseline or 3M tape (Figure 2).

Note: We tried several methods for sealing including regular wax, vaseline, and commercial mounting reagents. The 3M tape is chosen because it is faster and easier compared with other reagents.

△ **CRITICAL:** 10% agarose gel instead of regular 5% agarose gel is used in live-cell imaging because it is stiffer, resulting in better immobilization.

Imaging dendritic growth cones by spinning-disk confocal microscopy

⌚ **Timing:** 3–4 h

Because PVD dendritic growth cones are relatively thick and highly dynamic in *C. elegans*, we use spinning-disk confocal microscopy to acquire z-stack images at high speed. PVD neurons have anterior and posterior primary dendrites. The dendrite outgrowth of posterior primary branches is not controlled by axon-dendrite fasciculation through L1CAM-Robo signaling. This protocol primarily focuses on anterior primary dendrites to study the influence of axon-dendrite fasciculation on dynamics of dendritic growth cone. Nevertheless, it is also suitable to image growing growth cone of posterior primary dendrite. It is notable that the outgrowth of posterior primary dendrites is abolished in some mutant strains, such as the *sax-7* mutant. For the imaging setting, the interval between each z-stack series is 30 s or longer, and worms are able to survive for 4–6 h on the gel pad.

21. Place the slide on the microscope stage and search for transgenic *C. elegans* under the 10× objective.
22. Image growth cone morphology under the 63× objective (immersed in oil) and mark the location of PVD growth cones for each animal.
23. Set the laser intensity at 20% and exposure time at 200 ms as the default. Adjust the exposure time and laser intensity until the dendritic growth cone is clearly observed. If the signal is dim and unclear and cannot be improved by adjusting the setting, go to the [problem 3](#) for [troubleshooting](#). If the signal is quickly diminished, it is likely due to the photobleaching of images. Go to the [problem 4](#) for [troubleshooting](#).

Note: GFP and LifeAct::NeonGreen are weakly expressed by the *ser2prom3* promoter at L2–L3 stage. Locate the PVD cell body first and move horizontally to locate the growth cone of the horizontal primary dendrite. Alternatively, the ALA axon can be used as another landmark to locate the PVD dendritic growth cone. Increasing the intensity of laser is not recommended because of rapid bleaching of fluorescence.

24. Acquire z-stack images of the dendritic growth cone under the 63× objective by spinning-disk confocal microscope at 2-min (growth cone morphology) or 30-s (cytoskeletal proteins) interval for 2–4 h. The average acquisition time is around 10–15 s with optimal thickness (around 0.25 μm) recommended by Zen software (Carl Zeiss).

Note: Sometimes the development of the PVD neuron stops after imaging a few frames. If the growth cone arrests for more than 30 min, search another animal to begin a new imaging series. Discard the slide when imaging time is over 5 h. Usually the growing dendritic growth cone has relatively low GFP or LifeAct::NeonGreen expression ([Figure 1](#)) with continuous changes in morphology and F-actin signals. The average pixel intensity of dendritic growth cones ranges from 40 to 100 in the 8-bit grayscale with minimal background noise lower than 10 pixel intensity measured by Fiji (ImageJ). By contrast, dendritic growth cones with strong expression of GFP or LifeAct::NeonGreen (average pixel intensity > 200) are mostly stalled and stationary.

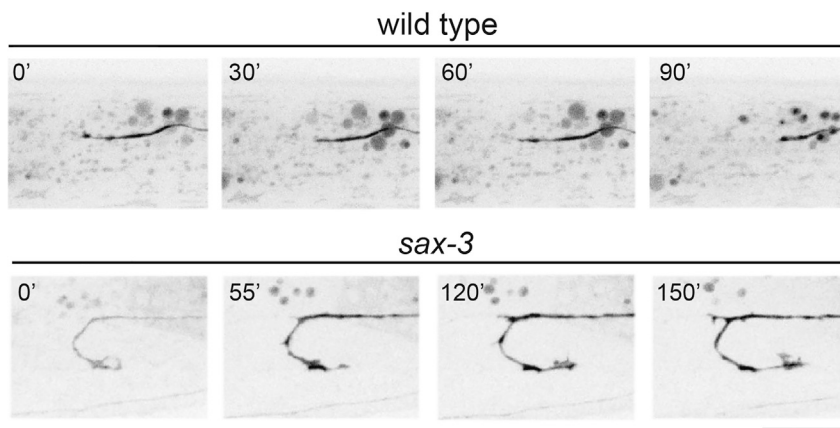


Figure 3. The dynamic morphology of PVD dendritic growth cone in the wild-type (top) and the *sax-3* mutant (bottom)
Scale bar, 10 μm .

- Obtain maximal projection images of the z-series by Zen software (Carl Zeiss). Sequential images are imported to Fiji (ImageJ) to produce videos.

EXPECTED OUTCOMES

Following this protocol, the morphology of PVD dendritic growth cones could be observed in live *C. elegans* by spinning-disk fluorescent confocal microscopy (Figure 3). In addition, cytoskeletal dynamics can be monitored by expressing actin or microtubule binding peptides such as LifeAct::NeonGreen or EB1/EBP-2::GFP (Figure 4). The migration of the PVD dendritic growth cone usually completes within 3–5 h. In wild-type animals, PVD dendritic growth cones are highly dynamic and alternate between palm- and torpedo shapes with unidirectional F-actin flow towards the leading edge. In contrast, mutants with axon-dendrite fasciculation defects have enlarged but relatively stationary dendritic growth cone. The F-actin flow moves in multiple directions, showing defective cytoskeletal dynamics in the absence of axon-dendrite fasciculation (Chen et al., 2019) (Figure 5). The actin trajectories measured by TrackMate analysis is strongly correlated with extending directions of PVD dendritic growth cones, suggesting that the assembly of F-actin cytoskeleton predicts the direction of growth cone migration and dendrite pathfinding.

QUANTIFICATION AND STATISTICAL ANALYSIS

Lifeact::NeonGreen shows punctate pattern and traffic along in growing dendritic growth cone. To understand the dynamics of F-actin assembly in dendritic growth cone, the puncta movements of Lifeact::NeonGreen are subsequently analyzed by TrackMate Plugin in Fiji (ImageJ) (Figure 6).

Data analysis of F-actin dynamics by TrackMate in Fiji (ImageJ)

⌚ Timing: 1–2 h

- For F-actin dynamics analysis, open the sequential images of LifeAct::NeonGreen by Fiji (ImageJ) (Figure 6A).
- Examine the sequential images. If the images are drifting, consider aligning images by using Plugin linear stack alignment with SIFT (see problem 5).
- Open Plugin>TrackMate (Tinevez et al., 2017). A new window will show up for setting conditions (Figure 6B).
- Use default of calibration and crop settings, and then click Next.
- Select LoG detector to detect Gaussian-like particles in the presence of noise and click Next.

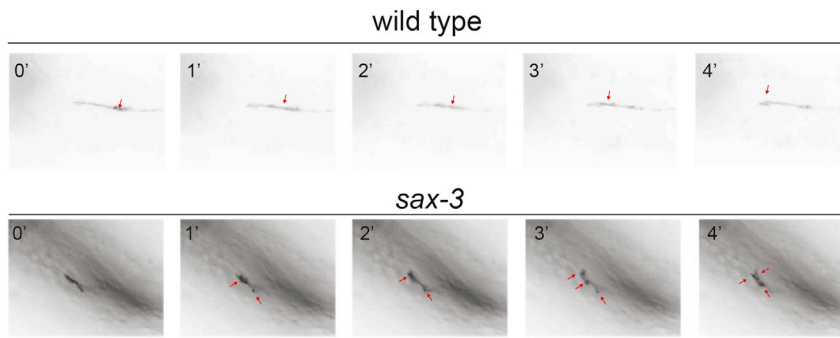


Figure 4. F-actin dynamics of the PVD dendritic growth cone in the wild-type (top) and the *sax-3* mutant (bottom)
Scale bar, 10 μm . Arrows, F-actin signals.

6. Defined puncta with estimated size of 1 μm and preview the labeling. Manually increase the threshold until it only detects puncta along dendritic growth cone and then click Next (Figure 6C).
7. For the initial thresholding, click Auto to set quality control automatically and click Next (Figure 6D).
8. Set the color by manual color to distinguish different tracks and click Next.
9. Select LAP tracker and click Next.
10. For the setting of LAP tracker, keep the maximum distance at 15 μm and the maximum gap at 1 frame, and click Next (Figure 6E).
11. Set the color by Track ID and click Next (Figure 6F).
12. Tracks of puncta will be seen after the analysis, and modify the display options to label spots and tracks by Track ID (Figure 6G).
13. The raw data of puncta tracing could be retrieved by clicking analysis. Tracks over 10 frames are preserved for further analysis.
14. Save the images as TIFF files.
15. Draw a horizontal line and a circle in Adobe Illustrator. Import images and adjust the orientation to align the PVD primary dendrite with the horizontal line (Figure 6H).
16. Move the images to match the starting point of puncta with the circle (Figure 6H).
17. Manually plot the trajectory of F-actin puncta for F-actin assembly trace. Assemble all the tracks to present the actin flow in PVD dendritic growth cone (Figures 5A and 6H).

Note: The automatic output of moving tracks generated by TrackMate could be available to plot figures when the newer version of TrackMate is released.

18. Quantify the directions of individual trajectories by measuring the angle of vectors with the anteroposterior axis align with PVD primary dendrite (from 0° to 360°). Statistical analysis could be performed by directly comparing the absolute values of the angle in the wild type and the mutant animals. Alternatively, fractions of growth cones showing angles in binned angle sections could be compared as part of the analysis of actin dynamics in dendritic growth cones.

Note: A polar figure summarizing actin trajectories in each 30° section is present as an example to compare actin dynamics in the dendritic growth cones of the wild type and the *sax-3* mutant (Figure 5B).

LIMITATIONS

While this live-cell imaging protocol provides detailed information about the dynamic subcellular events during neuronal development in *C. elegans*, one major limitation is that the developmental timing of observed cells is usually delayed compared with unanesthetized animals. In some cases,

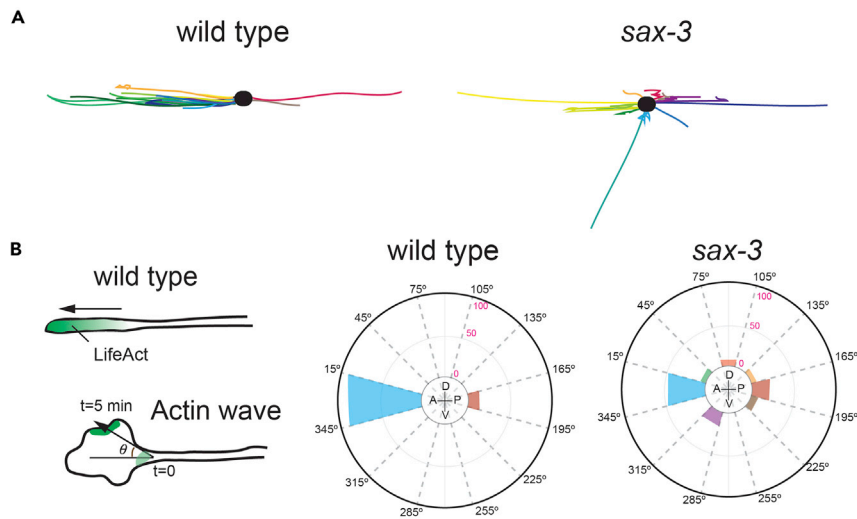


Figure 5. Trajectory of F-actin puncta in PVD dendritic growth cones

(A) Individual trajectories are labeled in different colors. Numbers are: 17 puncta from 12 animals in the wild type and 14 puncta from 10 animals in the *sax-3* mutant.

(B) Quantification of the portion of actin trajectories in PVD dendritic growth cones in the defined angle sections (30°).

neuronal cells are arrested during development, which have also been reported somewhere else (Berger et al., 2018; Chai et al., 2012).

TROUBLESHOOTING

All the steps mentioned here are referred to the steps in “step-by-step method details” section.

Problem 1: Inadequate animals for imaging

Sometimes only a few L2 larvae survive after synchronization following the protocol. It is likely due to the prolonged incubation time in bleach solution (step 7).

Potential solution

Reduce the time soaking in bleach solution from 5 min to 3 min with vortex. Vortex will accelerate the bleaching process without damaging eggs.

Problem 2: Dehydration of animals

C. elegans larvae die quickly in dehydrated condition. If dying larvae are constantly observed, consider the possibility that animals are dehydrated during mounting procedures (Step 17).

Potential solution

Remake the 10% agarose gel solution. Melted 10% agarose gel kept in heated dry bath over 3–4 h are likely dehydrated. It is recommended to use the freshly melted 10% agarose for all experiments. Incomplete sealing of 3M tape is also a major cause for dehydration of animals during live-cell imaging. Make sure the edge of the coverslip is properly sealed without gaps.

Problem 3: Low quality of images

Two main reasons for obtaining images of low quality:

- In adequate immobilization of the animals (step 19)
- Low fluorescent signal intensity (step 23)

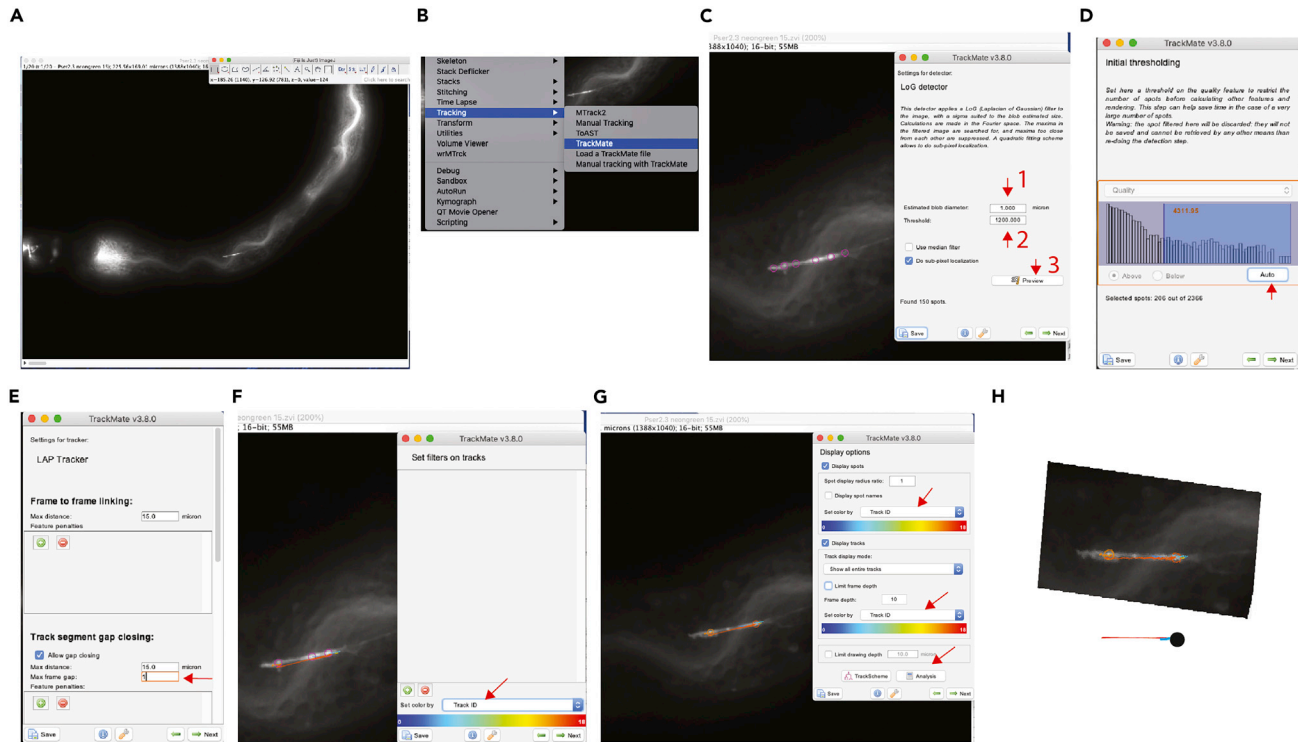


Figure 6. Steps for analyzing image series of LifeAct::NeonGreen puncta in the dendritic growth cone

- (A) Import the sequential images into Fiji (ImageJ).
 (B) Open plugin and choose TrackMate in the tracking section.
 (C) Set up 1 μm as the diameter of puncta, and manually adjust the threshold of pixel density, and preview the labeling. Manually adjust threshold until it only detects puncta in the growth cone.
 (D) Set automatic thresholding for quality control.
 (E) Choose the LAP tracker and set the maximum frame gap as 1 frame.
 (F and G) The trackers of puncta will appear on the images. Label the tracks by track ID to differentiate independent tracks.
 (H) Export the images and manually depicts the tracks using Adobe Illustrator. Assemble all the tracks to show the actin flow in dendritic growth cone.

Potential solution

Inadequate immobilization of the animals:

The commercial microbeads are usually soaked in solvent, so the final concentration of the microbeads in the anesthetic mix is often overestimated. If animals are constantly moving during imaging, remove excess solvent of microbeads by centrifuging at low speed (1000 \times g).

Low fluorescent signal intensity:

Increase the power of light source or replace GFP with brighter fluorescent proteins such as NeonGreen (Shaner et al., 2013). Strong blue light is noxious to *C. elegans*, and may induce avoidance behaviors. Therefore, replacing with brighter fluorescent proteins is preferable if the signal is hard to observe in live-cell imaging. Or consider to add a *lite-1* mutation to the strain to reduce avoidance response induced by blue light.

Problem 4: Photobleaching of fluorescent imaging

Because the promoter activity of *ser2prom3* is weak at early L2 larva stage (\sim 20 h post-hatching), the fluorescent signal might rapidly decay at the beginning of the live-cell imaging. A guideline and a

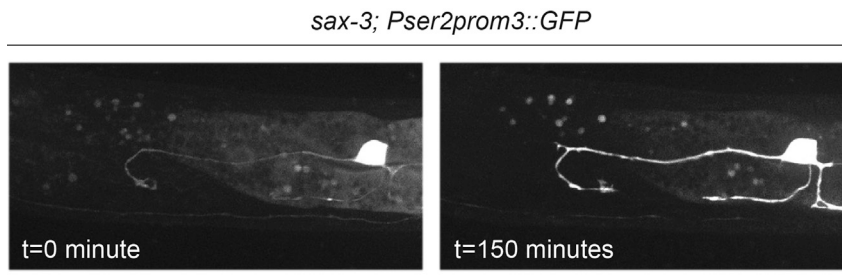


Figure 7. Representative images of PVD neurons in the *sax-3* mutant at time = 0 min or 150 min
Scale bar, 20 μ m.

solution are listed below. After 21 h post-hatching, the *ser2prom3* promoter activity is significantly increased (Figure 7), which is thus unlikely to suffer from photobleaching (step 23).

Potential solution

In live-cell imaging, rapid decay of fluorescent signal is usually due to the strong laser intensity. Thus, longer exposure time is recommended over higher laser intensity if the fluorescent signal is below detectable level. Here we recommend choosing optimal exposure time and laser intensity with the guideline below. Set the laser intensity to 20%–40%, and increase the exposure time until the outline of dendritic growth cone is clearly observed. If exposure time is below 300 ms, use this condition for live-cell imaging. The average pixel intensity of growing dendritic growth cones is around 40 to 100 in the 8-bit grayscale. If the exposure time is higher (>300 ms), increase laser intensity for 5%–10%. Repeat the process to search for optimal conditions for live-cell imaging.

Problem 5: Drifting of images

Although *C. elegans* is anesthetized by levamisole, it sometimes trembles with spontaneous muscle contractions. This will cause drifts of images in live-cell imaging that impedes the analysis of imaging data (step 19).

Potential solution

Two potential solutions for the drift of images:

Longer time of anesthetizing can significantly prevent the trembling of animals along the imaging process.

Correct the drifting of images by algorithm in Fiji (ImageJ). Open the plugin, choose the registration, and open rigid registration for correcting the shifts in sequential images. Click Plugin>Registration>Linear Stack Alignment with SIFT>expected transformation: Rigid. Corrected images are available for TrackMate analysis.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Chun-Hao Chen (chunhaochen@ntu.edu.tw)

Materials availability

For transgenic *C. elegans* strains and plasmids used in this protocol, please refer to [Chen et al., 2019](#). All strains and constructs are available upon request. All other materials are available commercially.

Data and code availability

This paper does not generate any dataset or code.

ACKNOWLEDGMENTS

This protocol was funded by the Ministry of Science and Technology (MOST), Taiwan, to C.-L.P. (MOST-107-3017-F002-002, MOST-106-2320-B-002-051-MY3, and MOST-109-2320-B-002-019-MY3) and C.-H.C. (MOST-109-2636-B-002-016). C.-L.P. is also supported by the Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE 109L901402A). The authors are grateful to Hua-Man Hsu for her assistance in spinning-disk confocal microscopy and the First and Third Core Labs, National Taiwan University College of Medicine, for imaging. The authors are also grateful to Jen-Wei Weng for her assistance in preparation of materials.

AUTHOR CONTRIBUTIONS

Conceptualization, C.-H.C. and C.-L.P.; methodology, C.-H.C.; writing – original draft, C.-H.C.; writing – review and editing, C.-H.C. and C.-L.P.; funding acquisition, C.-H.C. and C.-L.P.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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