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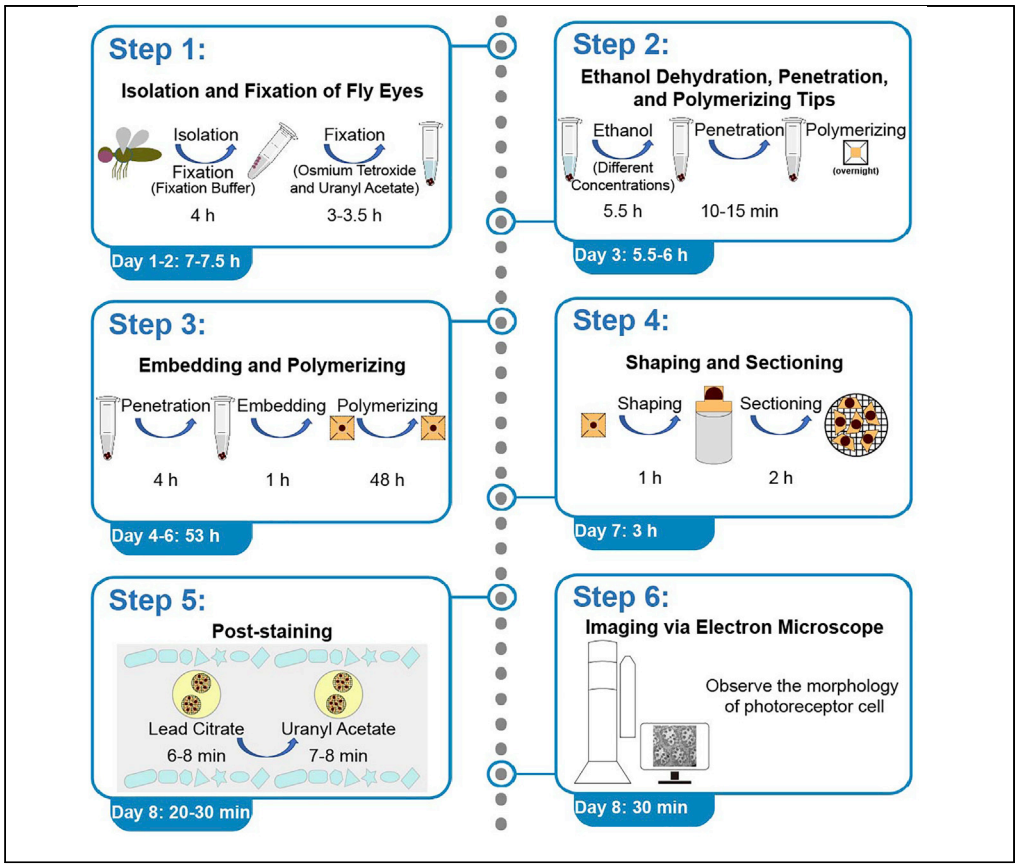
Protocol for electron microscopy of *Drosophila* photoreceptor cells



In *Drosophila*, mutations in genes that prevent normal Ca^{2+} influx after light stimulation usually cause light-dependent retinal degeneration or neurodegeneration, detectable by defects in eye morphology. Here, we present a protocol based on electron microscopy (EM) to observe the morphological structure of photoreceptor cells in *Drosophila*. We detail how to fix, dehydrate, embed, and polymerize compound eye samples, followed by sectioning, post-staining, and image acquisition, to assess the eye morphology at the ultrastructural level.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

A protocol for revealing the ultrastructure of *Drosophila* photoreceptor cells

Preparing compound eye samples for electron microscope observation

Dissecting molecular mechanisms underlying retinal degeneration

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Protocol for electron microscopy of *Drosophila* photoreceptor cells

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SUMMARY

In *Drosophila*, mutations in genes that prevent normal Ca^{2+} influx after light stimulation usually cause light-dependent retinal degeneration or neurodegeneration, detectable by defects in eye morphology. Here, we present a protocol based on electron microscopy (EM) to observe the morphological structure of photoreceptor cells in *Drosophila*. We detail how to fix, dehydrate, embed, and polymerize compound eye samples, followed by sectioning, post-staining, and image acquisition, to assess the eye morphology at the ultrastructural level. For complete details on the use and execution of this protocol, please refer to Gu et al. (2020).

BEFORE YOU BEGIN

The electron microscopy protocol enables the observation of a sample's morphological structure at the ultrastructural level (Harris, 2015). However, we'll describe below how we applied this procedure in *Drosophila* photoreceptor cells further down.

All the stock solutions in the tables (materials and equipment section) need to be prepared in advance and stored at the reported temperature.

Fly husbandry and harvest

1. The flies are raised in standard medium at 25°C, with 60%–80% relative humidity, in 12 h light/dark cycles unless special treatment.
2. The flies are collected after emerging from the pupas.

Note: Collecting flies according to the purpose of the experiment. For example, when exploring the relevance between age and retinal degeneration in *Drosophila*, it is required to collect flies of various ages in groups. To avoid light-dependent retinal degeneration, all flies should be reared in the dark and examined at different ages.

Prepare buffers

Timing: 10 min

3. Prepare 10% PFA in ddH_2O for further use.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Paraformaldehyde (PFA)	Aladdin	Cat#C104188
Glutaraldehyde solution	Sigma	Cat#G7651
Sodium cacodylate trihydrate	Sigma	Cat#C0250
NaOH (flaky)	BBI	Cat#A100583
Osmium tetroxide	Ted Pella	Cat#18456
Uranyl acetate	SPI	Cat#6159-44-0
Ethanol absolute	Sangon Biotech	Cat#A500737
Propylene oxide	Aladdin	Cat#P118604
Spurr's resin	SPI	Cat#02680-AB
Toluidine blue staining kit	BBI	Cat#E670105
Lead citrate	SPI	Cat#10099-74-8
Experimental models: Organisms/strains		
Drosophila: Canton-S	Bloomington Drosophila Stock Center	RRID: BDSC_64349
Software and algorithms		
Adobe Illustrator	Adobe	https://www.adobe.com/products/illustrator.html
Other		
Tweezers	Electron Microscopy China (EMCN)	Cat#EP5622
Brush	N/A	N/A
Safety razor blade	Flying eagle brand	Cat#98758915
1.5 mL Eppendorf tube	Eppendorf	Cat#0030125150
Electronic balance	Sartorius	Cat#BS124S
Stereomicroscope	Nikon	Cat#SMZ645
Tin foil	N/A	N/A
Disposable beakers	BBI	Cat#F606144
Pasteur pipette	Sangon Biotech	Cat#F621006
Embedding mold	PELCO	Cat#10585
Vacuum drying oven	Shanghai Yiheng Technology	Cat#DZF-6021
Sandpaper	Electron Microscopy China (EMCN)	Cat#HB240
Glue	N/A	N/A
Cylindrical plastic base	N/A	N/A
EM Ultramicrotome	Leica	Cat#UC7
Slide	Sangon Biotech	Cat#F518205
Eyelash with handle	Ted Pella	Cat#113
Gilder grids	Electron Microscopy China (EMCN)	Cat#BZ10023a
Diamond knife 45	Diatome	N/A
Transmission electron microscope sample box	Electron Microscopy China (EMCN)	Cat#DP160
Parafilm	Parafilm	Cat#F104002
Petri dishes	N/A	N/A
Qualitative filter paper	Sangon Biotech	Cat#F503316
Transmission electron microscope	Hitachi	Cat#7650

MATERIALS AND EQUIPMENT

Stock solutions

0.5 M sodium cacodylate (pH 7.2)		
Reagent	Final concentration	Amount
sodium cacodylate trihydrate	0.5 M	53.5 g
ddH_2O	n/a	up to 500 mL
Total	**n/a**	**500 mL**

Store at 4°C for up to a year.

Note: Add ddH_2O to ~450 mL, adjust the pH to 7.2 with 0.1 N HCl, add the remaining ddH_2O to 500 mL.

⚠ CRITICAL: Sodium cacodylate is toxic. Personal protective equipment such as face shields and latex gloves must be worn and the preparation process must be done in a fume hood.

0.1 M sodium cacodylate (pH 7.2)		
Reagent	Final concentration	Amount
0.5 M sodium cacodylate	0.1 M	100 mL
ddH_2O	n/a	up to 500 mL
Total	**n/a**	**500 mL**
Store at 4°C for up to a year.		

2% uranyl acetate		
Reagent	Final concentration	Amount
uranyl acetate	2%	0.2 g
ddH_2O	n/a	up to 10 mL
Total	**n/a**	**10 mL**
Store at 25°C for up to a year and protect the solution from light.		

Note: 2% uranyl acetate should be wrapped in tin foil and stored away from light.

⚠ CRITICAL: Uranyl acetate is toxic. Personal protective equipment such as face shields and latex gloves must be worn and the preparation process must be done in a fume hood.

1% lead citrate		
Reagent	Final concentration	Amount
lead citrate	1%	0.1 g
ddH_2O	n/a	up to 10 mL
Total	**n/a**	**10 mL**
Store at −80°C for up to a year.		

Note: Milky white lead citrate is a suspension, 1 mol/L NaOH is added dropwise, and the solution is shaken until the solution is clear. Dilute with distilled water to the final volume.

⚠ CRITICAL: Lead citrate is toxic. Personal protective equipment such as face shields and latex gloves must be worn and the preparation process must be done in a fume hood.

Fresh solutions

10% PFA		
Reagent	Final concentration	Amount
PFA	10%	0.1 g
ddH_2O	n/a	up to 1 mL
Total	**n/a**	**1 mL**

Note: Add 20 μL of NaOH (1 mol/L) and put the capped Eppendorf tube in a 70°C water or metal bath to accelerate dissolution.

⚠ CRITICAL: PFA is toxic. Personal protective equipment such as face shields and latex gloves must be worn and the preparation process must be done in a fume hood.

Fixation buffer		
Reagent	Final concentration	Amount
10% PFA	4%	800 μL
glutaraldehyde (50%)	1%	40 μL
0.5 M sodium cacodylate	0.1 M	400 μL
ddH_2O	n/a	up to 2 mL
Total	**n/a**	**2 mL**
Prepare fresh at 4°C.		

Note: Glutaraldehyde is stored at −20°C. It should be dissolved completely on ice before use. Glutaraldehyde can stabilize glycogen and preserve some protein structures that are poorly preserved with osmium fixation alone, which has little damage to enzyme activity. With glutaraldehyde fixation alone, the microtubules and smooth endoplasmic reticulum are fixed well, while the fat protection is poor, and the image contrast is bad. So osmium tetroxide fixation is essential (Prentø, 1995).

Note: The table shows the amount of fixation buffer used for one sample.

⚠ CRITICAL: The regents are toxic. Personal protective equipment such as face shields and latex gloves must be worn and the preparation process must be done in a fume hood.

1% osmium tetroxide		
Reagent	Final concentration	Amount
osmium tetroxide	1%	0.01 g
0.1 M sodium cacodylate	n/a	up to 1 mL
Total	**n/a**	**1 mL**
Prepare fresh at 25°C.		

Note: The table shows the amount of 1% osmium tetroxide used for one sample. A 2% stock solution is advised for simpler weighing, or you can purchase a commercial stock solution directly.

⚠ CRITICAL: Osmium tetroxide is toxic, evaporable, and a strong oxidizer. It should be weighed quickly in a fume hood. Personal protective equipment such as face shields and latex gloves must be worn. If a store solution is prepared, keep the store solution in a bottle of dark glass and wrapped in foil. Tightly wrap two to three layers of parafilm around the cap and bottle neck. It is advised to store the stock solution in the dark and preferably at 4°C. The solution can be stored for months as long as the color remains yellow.

1% uranyl acetate		
Reagent	Final concentration	Amount
2% uranyl acetate	1%	0.5 mL
ddH_2O	n/a	up to 1 mL
Total	**n/a**	**1 mL**
Prepare fresh at 25°C.		

Note: The table shows the amount of 1% uranyl acetate used for one sample.

10% ethanol		
Reagent	Final concentration	Amount
ethanol absolute	10%	0.1 mL
ddH_2O	n/a	up to 1 mL
Total	**n/a**	**1 mL**
Prepare fresh at 4°C.		

30% ethanol		
Reagent	Final concentration	Amount
ethanol absolute	30%	0.3 mL
ddH_2O	n/a	up to 1 mL
Total	**n/a**	**1 mL**
Prepare fresh at 4°C.		

50% ethanol		
Reagent	Final concentration	Amount
ethanol absolute	50%	0.5 mL
ddH_2O	n/a	up to 1 mL
Total	**n/a**	**1 mL**
Prepare fresh at 4°C.		

70% ethanol		
Reagent	Final concentration	Amount
ethanol absolute	70%	0.7 mL
ddH_2O	n/a	up to 1 mL
Total	**n/a**	**1 mL**
Prepare fresh at 4°C.		

85% ethanol		
Reagent	Final concentration	Amount
ethanol absolute	85%	0.85 mL
ddH_2O	n/a	up to 1 mL
Total	**n/a**	**1 mL**
Prepare fresh at 4°C.		

95% ethanol		
Reagent	Final concentration	Amount
ethanol absolute	95%	1.9 mL
ddH_2O	n/a	up to 2 mL
Total	**n/a**	**2 mL**
Prepare fresh at 4°C.		

Note: The solutions for ethanol dehydrations show the amounts of different concentrations of ethanol for one sample.

Embedding reagents (Spurr's resin)		
Reagent	Final concentration	Amount
ERL 4206	24.75%	5 g
DER 736	9.90%	2 g
NSA	64.36%	13 g
DMAE	0.99%	0.2 g
Total	**n/a**	**20.2 g**

Prepare fresh at 25°C.

Note: The table shows the amount of Spurr's resin used for one sample. The Spurr's resin should be prepared before use. Add the above components sequentially into a disposable polythene beaker and mix with a variable-speed electric stirrer. To facilitate weighing, Pasteur pipettes can be used.

⚠ CRITICAL: The Spurr's resin should be mixed very thoroughly for 1 h.

⚠ CRITICAL: The Spurr's resin is toxic. Face shields and latex gloves must be worn.

Propylene oxide/Spurr's resin mixture		
Reagent	Final concentration	Amount
propylene oxide	50%	0.5 mL
Spurr's resin	n/a	up to 1 mL
Total	**n/a**	**1 mL**

Prepare fresh at 25°C.

Note: The table shows the amount of propylene oxide/Spurr's resin mixture used for one sample. Ethanol and Spurr's resin are not mutually soluble, so when using ethanol dehydration, propylene oxide is used as an intermediate solvent. Make sure the samples are immersed in the propylene oxide.

⚠ CRITICAL: Propylene oxide is a toxic, colorless, ether-smelling, low boiling point and flammable liquid. Vapors irritate eyes, skin, and respiratory system. It should be stored in a cool, ventilated and dry place below 25°C, and should not be directly exposed to sunlight and isolated from fire sources. Face shields and latex gloves must be worn.

STEP-BY-STEP METHOD DETAILS

Isolation and primary fixation of fly eyes (day 1)

Timing: 4 h for those who choose fixation for 2 h

The purpose of this step is to ensure that the cell ultrastructure of the isolated eye remains intact.

Note: The purpose of primary fixation is to preserve the ultrastructural details of the cells as completely as possible in the living state, so as to avoid autolysis caused by the decomposition of their own enzymes or corruption caused by the invasion and reproduction of external microorganisms, resulting in the destruction of the ultrastructure of the cells. At the same time, the various components in the cell can be fixed to avoid dissolving and loss during subsequent washing and dehydration.

1. Prepare the fixation buffer and keep it on ice.

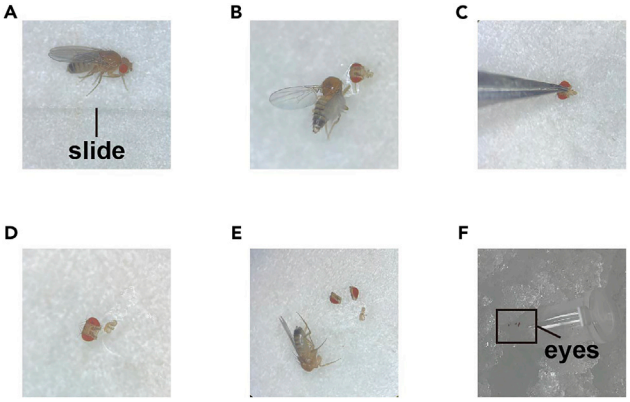

Figure 1. Isolation and primary fixation of fly eyes

(A) Anesthetize flies with carbon dioxide.

(B) Use the blade to cut heads off on a slide.

(C) Use tweezers to expose the mouthparts.

(D) Remove the mouthparts of the heads.

(E) Cut the heads into two halves from the middle.

(F) Place the tube diagonally on ice to ensure the eyes are fully immersed in fixation buffer.

2. Isolation of samples.
   a. Take the required strains of flies, anesthetize them with carbon dioxide, and quickly cut off their heads with a blade on a slide (Figures 1A and 1B).
   b. Quickly remove the mouthparts and cut the heads into two halves from the middle (Figures 1C–1E).

   Note: The whole process of sample isolation should be done quickly and the eyes should be put into fixation buffer immediately after isolation, so that the tissues and cells can keep their original living conditions as much as possible.

   Note: Choose a sharp cutting instrument such as a fresh blade to reduce pulling or squeezing tissues.

3. Primary fixation.
   a. Add 1 mL of fixation buffer to the Eppendorf tube.
   b. Use tweezers to clamp the samples gently and insert them into the fixation buffer.
   c. Slowly place the Eppendorf tube on ice, keeping the eyes immersed in the fixation buffer (Figure 1F).

   Note: The eyes are light and float easily on the surface of the fixation buffer. Make sure all the eyes are immersed in the fixation buffer.

   d. Fix the samples for 20 min on ice.
   e. Change the samples to 1 mL of fresh fixation buffer in the Eppendorf tube, maintaining all the eyes' immersion in the buffer (Figure 1F).
   f. Fix the samples for 2 h on ice or 12 h at 4°C.

   Note: The red-eyes of flies have pigments. It will damage the structure of the eyes if they are fixed for 12 h at 4°C. So, if the flies' eyes are red, fix the samples for 2 h on ice. If the flies' eyes are white, you can choose to fix them for 2 h on ice or 12 h at 4°C.

   ⚠ CRITICAL: During the fixation process, bubbles will appear in the Eppendorf tube. Pay attention and make sure all the eyes are immersed in the fixation buffer.

4. Wash samples: Change the samples into 1 mL of fresh 0.1 M sodium cacodylate in the Eppendorf tube on ice to wash for 5 times, 15 min/time.

   Note: Make sure all the eyes are immersed in the 0.1 M sodium cacodylate.

5. Storage: Change the samples to 1 mL of fresh 0.1 M sodium cacodylate in the Eppendorf tube and store them at 4°C for 12 h.

Post-fixation with osmium tetroxide and uranyl acetate (day 2)

Timing: 3–3.5 h

The purpose of this step is to use osmium tetroxide and uranyl acetate to fix and ensure that fixation works well.

Note: Osmium tetroxide can react with most of the components in cells and protect fat, but the protection of carbohydrates, sugars and nucleic acid is poor (Palay et al., 1962). Osmium tetroxide has poor permeability and a high molecular density. The tissue fixed by osmium tetroxide can obtain good contrast under transmission electron microscope.

Note: Uranyl acetate is both a fixative and a stain. It can react with phosphate groups to fix DNA and RNA, as well as phospholipids containing phosphate groups, thus helping to preserve membrane structures. In addition, it can react with acidic groups (e.g., aspartic acid residues, glutamic acid residues) and basic groups (e.g., lysine residues) in the protein, thus acting as a fixed protein. But uranyl acetate does not preserve glycogen very well (Ackermann, 2009). After the fixation with aldehydes and osmium tetroxide, a third fixation with uranyl acetate is often performed.

6. Wash samples: Change the samples to 1 mL of fresh 0.1 M sodium cacodylate in the Eppendorf tube on ice to wash for 3 times, 15 min/time.

   Note: The samples should be at the bottom of the Eppendorf tube at this time.

7. Fixation with 1% osmium tetroxide: Change the samples to 1 mL of 1% osmium tetroxide solution for 1 h at 25°C in a fume hood.

   Note: For the best contrasting color, proper osmium tetroxide fixing time is very important. For compound eyes, 1 h always works well.

   ⚠ CRITICAL: Osmium tetroxide is highly toxic and volatile reagent that causes strong irritation of the respiratory tract and must be operated in a fume hood. The waste must be collected in a closed container. Be careful during the experiment!

8. Wash samples: Change the samples to 1 mL of fresh distilled water in the Eppendorf tube at 25°C to wash for 5 times, 15 min/time.

   Note: Once the fixation with 1% osmium tetroxide is finished, suck out the osmium tetroxide quickly. Add 1 mL of fresh distilled water to rinse and quickly suck out the water before the washing process to ensure the osmium tetroxide is diluted.

   ⚠ CRITICAL: The waste should be recycled into the corresponding waste liquid bottle and be treated by the department.

9. Fixation with 1% uranyl acetate.
   a. Prepare 1 mL of 1% uranyl acetate in a fume hood carefully.
   b. Change the samples to 1 mL of 1% uranyl acetate for 12 h at 25°C, protected from light in a fume hood.

Note: The Eppendorf tube could be wrapped in tin foil to protect samples from light.

Ethanol dehydration, penetration, and polymerizing tips (day 3)

Timing: 5.5–6 h

The purpose of this step is to dehydrate and gradually permeate the tissue with the embedding agent (Spurr's resin).

Note: Dehydration is the step leading to the complete removal of free water from tissues (Mollenhauer, 1993). Since most commonly used embedding agents are water-immiscible resins, they can only be immersed into tissues if the free water in biological tissues is removed. Ethanol extracts less cell matter and shrinks less tissue, but it is poorly soluble with resin, so propylene oxide is used as an intermediate solvent when ethanol is used for dehydration.

10. Wash samples: Change the samples to 1 mL of fresh distilled water in the Eppendorf tube at 25°C to wash for 5 times, 15 min/time.

Note: Once the fixation with 1% uranyl acetate is finished, suck out the uranyl acetate quickly. Add 1 mL of fresh distilled water to rinse and quickly suck out the water before the washing process to ensure the uranyl acetate is diluted.

⚠ CRITICAL: The waste should be recycled into the corresponding waste liquid bottle and be treated by the department.

11. Dehydration with different concentration gradients of ethanol.
   a. Prepare 1 mL 10%, 30%, 50%, 70%, 85% ethanol and 2 mL 95% ethanol on ice.
   b. Change the samples to 1 mL of 10% ethanol on ice for 15 min.
   c. Change the samples to 1 mL of 30% ethanol on ice for 15 min.
   d. Change the samples to 1 mL of 50% ethanol on ice for 15 min.
   e. Change the samples to 1 mL of 70% ethanol on ice for 15 min.
   f. Change the samples to 1 mL of 85% ethanol on ice for 15 min.
   g. Change the samples to 1 mL of 95% ethanol on ice for twice, 30 min/time.
   h. Change the samples to 1 mL of ethanol absolute on ice for 3 times, 30 min/time.

⚠ CRITICAL: Dehydration should strictly follow the gradients of ethanol concentrations mentioned above. Rapid dehydration can cause cell contractions. This step should be phased rather than rapid dehydration.

12. Prepare Spurr's resin mixture at 25°C in a disposable measuring beaker.

Note: Mix the Spurr's resin very thoroughly for 1 h when dehydrating with ethanol absolute the second time.

13. Penetration: Replace dehydrating regents in tissue with embedding regents gradually.
   a. Change the samples to 500–800 μL of 100% propylene oxide for twice, 5 min/time.

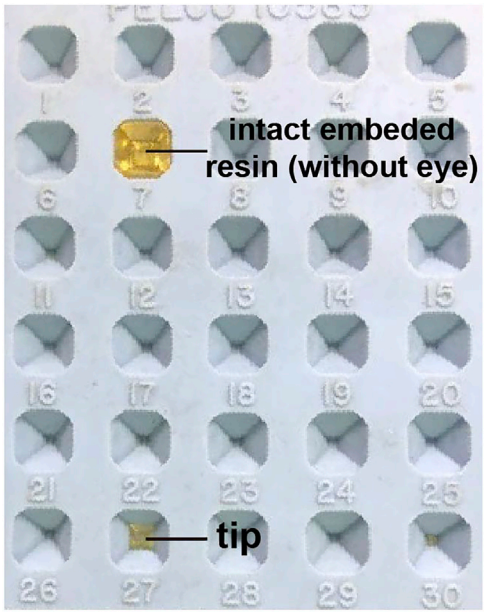

Figure 2. Tip and intact embedded resin samples in the Pyramid-Tip Mold

Each groove of the embedding mold is in the shape of an inverted pyramid. Polymerize tips first, and then place the eyes on the tip's surface so that the embedded eyes are in a favorable location, not too deep for shaping, and the resin surrounding the eyes per slice is reasonable (see step 19 for details).

Note: Ethanol and Spurr's resin are not mutually soluble, so when using ethanol dehydration, propylene oxide is used as an intermediate solvent. Make sure the samples are immersed in the propylene oxide.

⚠ CRITICAL: Propylene oxide is a toxic, colorless, ether-smelling, low boiling point and flammable liquid. Vapors irritate eyes, skin, and respiratory system. It should be stored in a cool, ventilated and dry place below 25°C, and should not be directly exposed to sunlight and isolated from fire sources.

b. Prepare 100% propylene oxide/Spurr's resin mixture in an Eppendorf tube and invert the tube till they mix well.
c. Change the samples to 1 mL of propylene oxide/Spurr's resin mixture and leave them cupped at 25°C in a fume hood for 12 h. Using a shaker to improve resin infiltration is recommended. Shake gently to prevent the sample from flinging out of the resin.

Note: The Spurr's resin is in the form of oil droplets, and it can be sucked with a Pasteur pipette.

14. Polymerizing tips.
    a. Use the vacuum drying oven by heating it to 65°C.
    b. Add one drop (about 10 μL) of Spurr's resin (Figure 2) to each well of the embedding mold and polymerize in the vacuum drying oven at 65°C for 12 h.
15. Use detergent to clean the disposable measuring beaker containing the Spurr's resin and dry it in the vacuum drying oven at 65°C for 12 h.

Embedding and polymerizing (day 4–6)

Timing: 53 h

The purpose of this step is to penetrate the tissue with the Spurr's resin and finally embed the tissue.

16. Take out the embedding mold containing the tip from the vacuum drying oven.

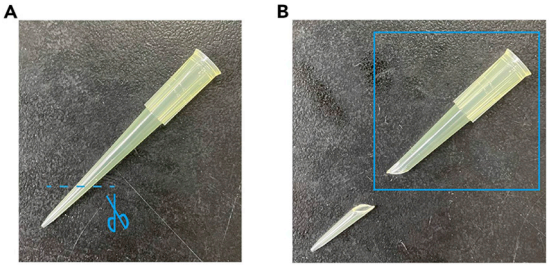

Figure 3. Preparation of the pipette tip for absorbing the Spurr's resin

(A) Use the scissors to cut off the pipette tip at the position indicated by the blue dotted line.

(B) Use the remaining pipette tip in the square to absorb the Spurr's resin and gently blow the eyes.

17. Prepare Spurr's resin mixture.

Note: Take out the disposable measuring beaker from the vacuum drying oven and mix the Spurr's resin in it very thoroughly for 1 h.

18. Penetration: Change the samples to 1 mL of Spurr's resin mixture three times, 1 h/time, at 25°C, in a fume hood.

Note: The Spurr's resin is in the form of oil droplets. Use the scissors to cut off the pipette tip at the appropriate position for the convenience of the experiment (Figure 3A). Use the remaining pipette tip to absorb the Spurr's resin and gently blow the samples (Figure 3B), so that the samples are suspended in the Spurr's resin.

19. Embedding and polymerizing.
    a. Prepare the vacuum drying oven by heating it to 65°C.
    b. Fill with Spurr's resin and place a compound eye in each hole of the embedding mold containing the tip.
    c. Under a stereoscopic microscope, move the compound eye with tweezers to adjust its direction so that the most prominent part of the compound eye is as far down as possible (Figure 4).

Note: When embedding the compound eyes, try to make the sample in the field of vision appear round to ensure that the sample is aligned (Figure 4).

    d. After placing the sample in each groove of the embedding mold, slowly place the embedding mold into the oven at 65°C for 1 h.

⚠ CRITICAL: Avoid violent shaking to make the adjusted sample orientation change.

    e. Take out the embedding mold, fine-tuning the compound eye's direction with tweezers under a stereoscopic microscope to ensure the sample is aligned.
    f. Return the embedding mold to the vacuum drying oven at 65°C for 48 h.

⚠ CRITICAL: The polymerizing time depends on the specific situation. Excessive solidification will make the sample too brittle and affect the later sectioning.

Note: After embedding, the blocks containing the samples are pyramid-shaped (Figure 4). They can be kept at 25°C for up to a year.

Shaping and sectioning (day 7)

Timing: 3 h for one sample

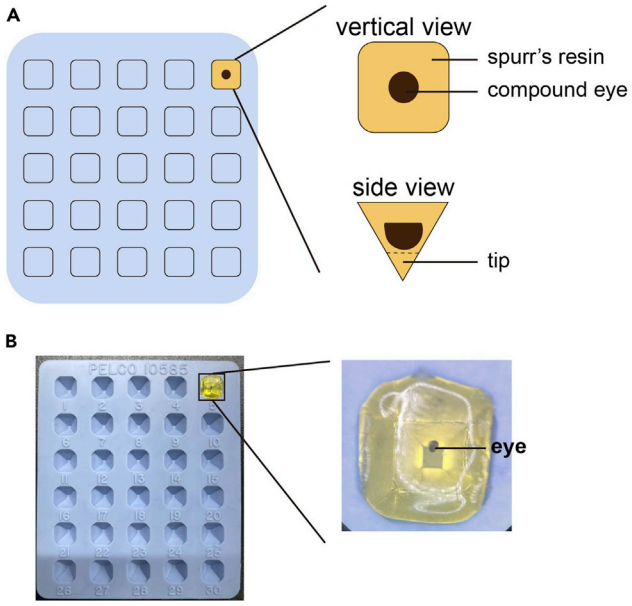

Figure 4. Embedding the compound eyes
(A) Schematic diagram of the sample orientation in the resin.
(B) One intact embedded sample in the embedding mold.

The purpose of this step is to shape and section to facilitate subsequent staining and sample observation.

20. Shaping.
    a. Use a fresh blade to trim away excess resin from the block underneath the embedded compound eye under the stereomicroscope (Figure 5A). Shape the upper part, which contains the eye, into an equilateral trapezoid (Figure 5B), and below that is a square base.

Note: When sectioning, feeding from the short base of the shaped equilateral trapezoid is helpful to protect the diamond sectioning knife.

    b. Adjust the initial section angle of the top surface according to the direction of the compound eye after embedding.

Note: Take care to avoid cutting too close to the compound eye to prevent damage to the sample. The initial rough trimming will reduce the time spent on achieving the trapezoid face shape when sectioning.

⚠ CRITICAL: Take note of the distance between the sample and the top surface; too far will result in more early empty slices, while too close will make it easy to cut and expose the sample accidentally. In addition, the top surface should be smooth as far as possible to avoid diamond sectioning knife marks.

    c. Check whether the embedded sample is free of any imperfections or debris after reaching the area of interest and achieving the trapezoid surface shape. Otherwise, start shaping a new one.
    d. Use the sandpaper to rough the bottom surface of the shaped block flat.
    e. Apply a small drop of super glue on a cylindrical plastic base and put the shaped resin-encased compound eye on. Glue the bottom surface of the shaped block to the cylindrical plastic base (Figures 5A and 5C).

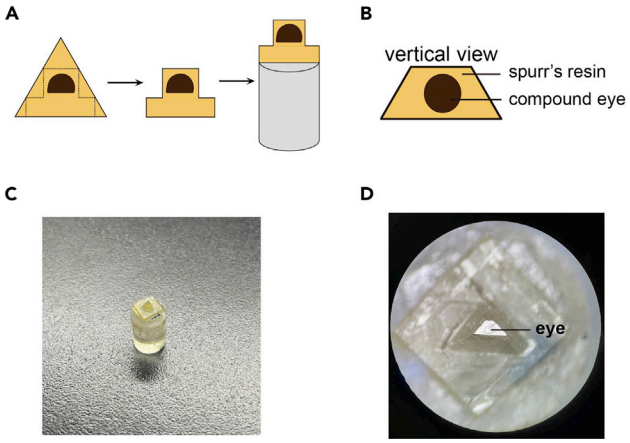

Figure 5. Shaping the sample block

(A) Diagram of the shaping of an embedded sample prior to sectioning. Glue the bottom surface of the shaped block to the cylindrical plastic base after shaping.

(B) A vertical view of the upper part of the shaped block.

(C) The shaped block is glued to the cylindrical plastic base.

(D) A vertical view of the shaped block that is glued to the cylindrical plastic base.

f. Allow the glue to dry before sectioning.

21. Alignment and sectioning.
    a. Place the cylindrical plastic base glued with the shaped block into the chuck on the ultramicrotome and tighten it firmly (Figure 6).
    b. Place the diamond sectioning knife on the sectioning knife holder stage on the ultramicrotome (Figure 6).
    c. Add a little excess water to the groove of the diamond sectioning knife to make it convex and ensure that the blade is soaked.
    d. Allow the diamond sectioning knife to soak up distilled water for about 15 min.
    e. A portion of the water is then slowly drawn under the microscope with a syringe or Pasteur pipette. Adjust the lighting system so that when the liquid surface becomes concave, a curved surface reflecting light can be seen, which is the correct liquid surface and appears white under the microscope. As the liquid evaporates during sectioning, distilled water should be added to ensure the correct liquid level.

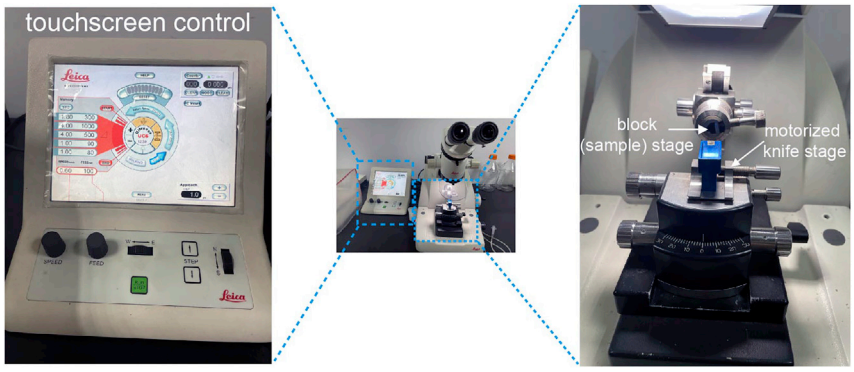

Figure 6. Use the ultramicrotome to section

Set parameters on the touchscreen control panel after adjusting the direction of the block and the angle of the diamond sectioning knife, start the automated movement of the ultramicrotome specimen arm.

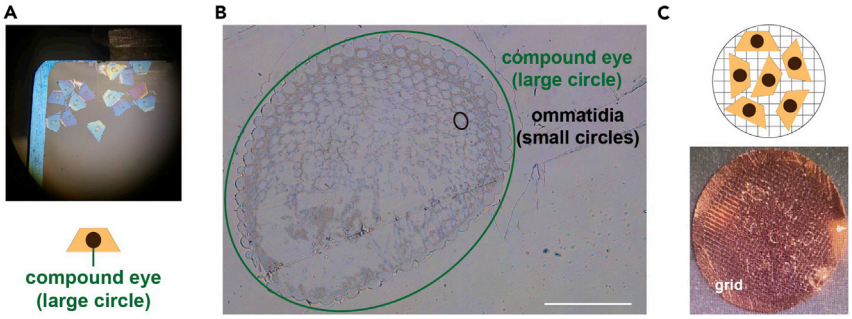

Figure 7. The light microscope image of the section

(A) A large circle (compound eye) is visible on each section. Top, actual picture. Bottom, schematic diagram of one section.

(B) The image of a toluidine blue stained section. Under a light microscope, within the large circle (compound eye), many small circles (ommatidia) can be seen. Scale bar: 100 μm.

(C) A large circle (compound eye) is visible on each section on the grid. Top, schematic diagram. Bottom, actual picture.

f. Adjust the sample under the microscope such that the short side of the trapezoid is facing down, so that sectioning from the short edge will cause less damage to the diamond knife blade.
g. Adjust the knife angle so that the two parallel sides of the trapezoid are parallel to the blade. At this point, the compound eye is perpendicular to the cross section of the knife.
h. Move the knife to align the available part with the block surface.
i. Raise the block until it stops slightly above the knife blade.
j. Set parameters in the control panel: feed of 400 nm, and a rate of 2 mm/s. Start the automated movement of the ultramicrotome specimen arm.
k. Pause sectioning when a large circle (compound eye) appears in the slice (Figure 7A). Place the sections on the slide, stain with 0.1% toluidine blue and use a light microscope to check if the ommatidia (small circles) have been sliced (Figure 7B).
l. When ommatidia begin to appear on the section (the big circle is relatively small at this time), continue to cut 200 sections using the parameter of 200 nm thickness. During this process, the size of the big circle increases gradually, and the number of ommatidia appearing in one section approaches its maximum.
m. Pause sectioning, clean the groove of the diamond sectioning knife and replace it with fresh distilled water. Adjust the liquid level and sectioning parameters. At a speed of 1 mm/s, cut sections of 90–100 nm thickness.

Note: The section thickness can be determined according to the interference color. The section thickness of 90–100 nm is golden, with good resolution and contrast, and is easy to observe under a transmission electron microscope.

n. Stop the automated movement after cutting 40 sections. Use an eyelash brush to gently move the ribbon sections away from the blade.
o. Carefully collect the sections on a grid. Make sure that there are 5–6 sections on one grid (Figure 7C).
p. Place the grids with sections into the transmission electron microscope sample box.

Note: Keep the room quiet without vibration during the whole process of sectioning, so as not to affect the sectioning.

Post-staining with lead citrate and uranyl acetate (day 8)

Timing: 20–30 min

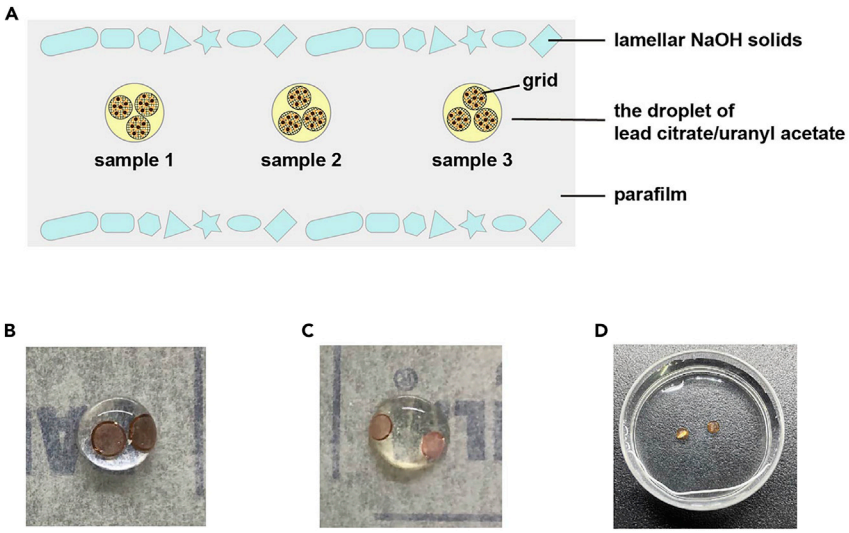

Figure 8. Post-staining with lead citrate and uranyl acetate

(A) Post-staining diagram with lead citrate and uranyl acetate.

(B) A lead citrate droplet with grids.

(C) A uranyl acetate droplet with grids.

(D) The Petri dish used for washing grids.

The purpose of this step is to enhance contrast by staining with lead citrate and uranyl acetate to facilitate the observation of ultrastructure.

Note: The following steps describe how post-staining is done (Figure 8). Lead citrate has high electron density and wide affinity for cell ultrastructure, and can improve the cell membrane system and lipid contrast. Lead citrate is easily contacted by CO_2 in the air to form lead carbonate precipitate and pollute sections (Cattini and Davies, 1983). Staining for a long time with lead citrate can increase all of the contrast, which is not conducive to observation. At present, uranyl acetate is the most widely used dye (Ackermann, 2009). It can be combined with most components and can stain nucleic acid, nuclear protein, connective tissue fiber, glycogen, secreted particles and lysosome, but it has a poor staining effect on membrane structure. Prolonged staining with uranyl acetate can cause glycogen extraction and tissue deformation (Daddow, 1983).

22. Prepare a parafilm of about 5 cm in width (the length depends on the number of samples, e.g., about 10 cm), and place lamellar NaOH solids in rows on both sides (Figure 8A).
23. Add a drop of 1% lead citrate (about 100 μL) between two rows of NaOH, one drop for each sample (Figure 8A).
24. Insert the grid face up into the droplet and let it stand for 6–8 min (the specific time can be adjusted according to the final staining degree) (Figures 8A and 8B).

Note: During the staining, it is necessary to minimize the contact with CO_2 in the air, so some solid NaOH or filter paper soaked with 0.1 mol/L sodium hydroxide is added to the staining environment to adsorb CO_2 and reduce the pollution of CO_2 in the environment.

25. Prepare three 2 cm Petri dishes, fill them with distilled water, and put the leaded grids into Dish 1 for a rinse for 10 s. Move the grids to Dish 2 for washing for 10 s, then move them to Dish 3 for washing for 10 s, and finally place them on filter paper to dry (Figures 8A and 8D).
26. Prepare a fresh parafilm of about 5 cm in width with lamellar NaOH solids in rows on both sides (Figure 8A).

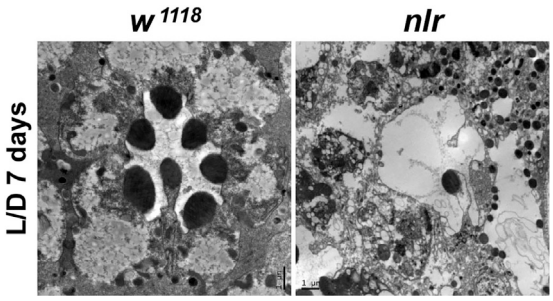

Figure 9. Depletion of all *Gαq* splice variants (*nlr*) results in severe retinal degeneration

EM analysis reveals that *nlr* mutants underwent rapid retinal degeneration. Flies were raised in 12 h light/dark conditions for the indicated time. Each picture shows a single ommatidium. Scale bar: 1 μm.

27. Add a drop of 2% uranyl acetate (about 100 μL) between two rows of NaOH, one drop for each sample (Figure 8A).
28. Invert the grids to the surface of the uranyl acetate droplet by using the surface tension of the droplet (the side where the sections are placed is attached to the surface of the droplet), and the grids are left for 7–8 min (Figures 8A and 8C).
29. Replace the three dishes in step 25 with fresh distilled water and wash in the same way for three times, 10 s/time (Figures 8A and 8D).
30. After drying the grids on the filter paper, place them in the transmission electron microscope sample box.

Imaging (day 8)

Timing: 30 min for one sample

The purpose of this step is to observe the ultrastructure of the sample.

31. Acquire images using a Hitachi 7650 transmission electron microscope operated at 80 kV.
32. Collect images of photoreceptor cells from the compound eye.

EXPECTED OUTCOMES

This protocol describes a method based on EM to observe the morphological structure of photoreceptor cells in *Drosophila*. Our results show that depletion of all *Gαq* splice variants (*nlr*) results in severe retinal degeneration (Gu et al., 2020). The photoreceptor cells of the fly are obviously absent and morphologically abnormal (Figure 9). This protocol can be applied to detect the changes in the morphological structure of different fly strains according to the specific experimental conditions.

LIMITATIONS

This protocol was used to detect the morphological structure of photoreceptor cells in *Drosophila*. The quality of electron micrographs depends on the quality of the fixation and subsequent processes. The sample is required to be very light and can be soaked in Spurr's resin. When using other samples, such as mouse muscle tissue for EM, the resin may need to be changed to ensure tissue infiltration. By the way, the embedded mold also needs to be replaced.

TROUBLESHOOTING

Problem 1

Bubbles appear in the tube during fixation, which makes fly eyes float on the fixation solution, resulting in poor fixation effect (step isolation and primary fixation of fly eyes (day 1), 3).

Potential solution

Place the sample gently on the tube wall with tweezers and gently place the tube diagonally on ice. Keep an eye on whether the eyes are fully immersed in the fixative and adjust them at any time (Figure 1F).

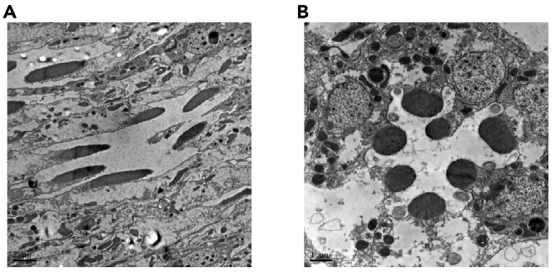

Figure 10. EM images of contrasting photoreceptor morphology

(A and B) The bad embedded sample shows that photoreceptor cells are sharply elliptic (A), while the good one shows round or elliptical (B). Scale bar: 2 μm (A) and 1 μm (B).

Problem 2

Osmium solid is difficult to weigh because of the small amount of use, and a strong oxidizer (step post-fixation with osmium tetroxide and uranyl acetate (day 2), 7).

Potential solution

Buy liquid osmium tetroxide and store it in the -80 refrigerator. Dilute it with 0.1 M sodium cacodylate before use.

Problem 3

After polymerizing for 48 h, the Spurr's resin is too brittle, making the shaping difficult (step shaping and sectioning (day 7), 20).

Potential solution

Adjust the amount of DMAE, shorten the polymerization time and check the hardness of the tips every hour when approaching 48 h.

Problem 4

It is difficult or takes a long time to section the sample (step shaping and sectioning (day 7), 21).

Potential solution

Cut off the Spurr's resin at the top of the eye as far as possible when shaping. Ensure that the eye is not cut, exposed to the air, or damaged.

Problem 5

Damage to the sections or failure to retrieve the sections when using the grids (step shaping and sectioning (day 7), 21).

Potential solution

The grids used should be carbon-supporting film and have some hydrophilic properties.

Problem 6

The photoreceptor cells in the embedded samples may be found to be sharply elliptic when imaging (step imaging (day 8), 31) (Figure 10).

Potential solution

Ensure that the most protruding part of the eye is at the bottom of the block, and that the eye appears round or oval when looking down.

Problem 7

Scratches may be found in the picture when imaging (step imaging (day 8), 31) (Figure 11).

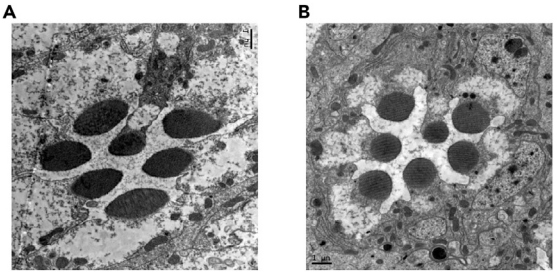

Figure 11. EM images of the contrasting slice smoothness

(A) Scratches have been found in (A), due to the damaged diamond sectioning knife.

(B) A good one has no scratches. Scale bar: 1 μm (A and B).

Potential solution

Sectioning with an undamaged diamond sectioning knife and keep the room quiet and do not vibrate while sectioning.

Problem 8

The background of the imaged picture is too deep or too shallow (step imaging (day 8), 31) (Figure 12).

Potential solution

Make sure the fixation time with osmium tetroxide does not exceed 1 h. The subsequent staining time of lead citrate and uranyl acetate should not be too long or too short.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Junhai Han (junhaihan@seu.edu.cn).

Materials availability

We did not generate any new materials.

Data and code availability

We did not generate any dataset or code.

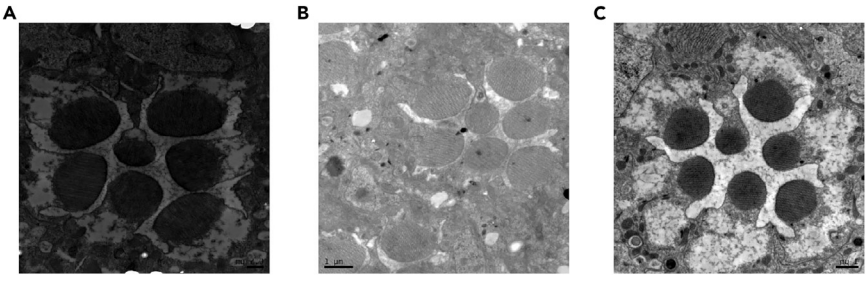

Figure 12. EM images of a contrasting slice background

EM images with various slice backgrounds. (A) is too deep. (B) is too shallow. (C) is successful while complying with the time of fixation with osmium tetroxide and post-staining. Scale bar: 0.5 μm (A) and 1 μm (B and C).

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AUTHOR CONTRIBUTIONS

Q.G. and J.H. designed the protocol, Q.G. and Y.T. acquired data and wrote the manuscript, and J.H. analyzed the data and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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