

Video Article

In vivo Imaging of Intact *Drosophila* Larvae at Sub-cellular Resolution

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Abstract

Recent improvements in optical imaging, genetically encoded fluorophores and genetic tools allowing efficient establishment of desired transgenic animal lines have enabled biological processes to be studied in the context of a living, and in some instances even behaving, organism. In this protocol we will describe how to anesthetize intact *Drosophila* larvae, using the volatile anesthetic desflurane, to follow the development and plasticity of synaptic populations at sub-cellular resolution¹⁻³. While other useful methods to anesthetize *Drosophila melanogaster* larvae have been previously described^{4,5,6,7,8}, the protocol presented herein demonstrates significant improvements due to the following combined key features: (1) A very high degree of anesthetization; even the heart beat is arrested allowing for lateral resolution of up to 150 nm¹, (2) a high survival rate of > 90% per anesthetization cycle, permitting the recording of more than five time-points over a period of hours to days² and (3) a high sensitivity enabling us in 2 instances to study the dynamics of proteins expressed at physiological levels. In detail, we were able to visualize the postsynaptic glutamate receptor subunit GluR-IIA expressed via the endogenous promoter¹ in stable transgenic lines and the exon trap line FasII-GFP¹. (4) In contrast to other methods^{4,7} the larvae can be imaged not only alive, but also intact (i.e. non-dissected) allowing observation to occur over a number of days¹. The accompanying video details the function of individual parts of the *in vivo* imaging chamber^{2,3}, the correct mounting of the larvae, the anesthetization procedure, how to re-identify specific positions within a larva and the safe removal of the larvae from the imaging chamber.

Video Link

The video component of this article can be found at <https://www.jove.com/video/2249/>

Protocol

A) Assembly of the imaging chamber

1. Select a larva of chosen stage (e.g. early 3rd instar larvae leaving an observation interval of about 24 hours at 25°C until wandering stage).
2. Rinse the larva with water to clean it of the culture medium and dab it dry.
3. Coat the center of the bottom element of the chamber, the region to face the larva, with a thin film of halocarbon oil.
4. Put the larva with the ventral side facing the microscope objective in the chamber (allows neuromuscular junction (NMJ) 26 and 27 to be imaged) (Figure 1 A-E).
5. Place the plastic spacer onto the oil layer, with the air slots of the grid face upwards (Figure 1 A). Please verify at this step: The height of the spacer is roughly half the larval diameter, the width of the slit should be about twice the diameter of the larva.
6. Place the imaging chamber on the microscope

B) Anesthetization of the larva

7. Connect the two inlets of the chamber with an appropriate anesthetization device/vaporizer.
Please verify up-front:
The effective concentration of desflurane in the room-air should never exceed that specified by safety regulations! Only a very small total volume of desflurane is needed to anesthetize larvae. The application of 15% (v/v) of desflurane¹ has proven to be a good starting point for determining the ideal concentration to be used in an experiment. For safety reasons we suggest that the vaporizer should never contain more desflurane than needed for 2-4 hours of *in vivo* imaging.
8. Immerse the other end of the tube attached to the chamber outlet in a glass of water then open the valves controlling the flow of the anesthetic into the chamber for about five seconds.
Please verify at this step:
Do air bubbles ascend in the water? If not the chamber is leaky.

9. Close the valves for about three seconds.
10. Monitor residual larval movements in the microscope. Check both the heartbeat and muscle movements. If necessary open and close valves as described in step 8 and 9 until anesthetization of the larva is complete, then close all valves and start imaging.
Please verify at this step:
Residual muscle movement or heartbeat indicates that the larva is not properly anesthetized. Complete anesthetization is vital for high resolution images. The larva should normally not be anesthetized for longer than 15-20 minutes at a given time.

C) Imaging

11. Identify the correct position and image the structure of interest (Figure 2-4).

D) Recovery from anesthetization

12. After imaging is completed let air into the chamber.
Please verify at this step:
Check heartbeat and muscle contractions of the larva by transmitted halogen light. As muscles start contracting it is safe to remove the larva from the imaging chamber.
13. Detach the chamber from the anesthetization device and remove it from the microscope. Disassemble the chamber carefully and place the larva in mashed fly cultivation medium.
14. Store the dish in an incubator at the appropriate temperature.

E) Time series

15. Repeat the steps 2-14 until sufficient time points have been obtained.
Alternative protocol:
If the larva is to be imaged more than once within a 30 minute interval it might be practical to leave it in the imaging chamber until the next anesthetization time point. Repeat the steps 7-14 until sufficient time points have been obtained.
Please verify at this step:
The larva is not to be kept in the imaging chamber for more than two hours at a time, nor is it to be anesthetized for longer than 15-20 minutes at a time.

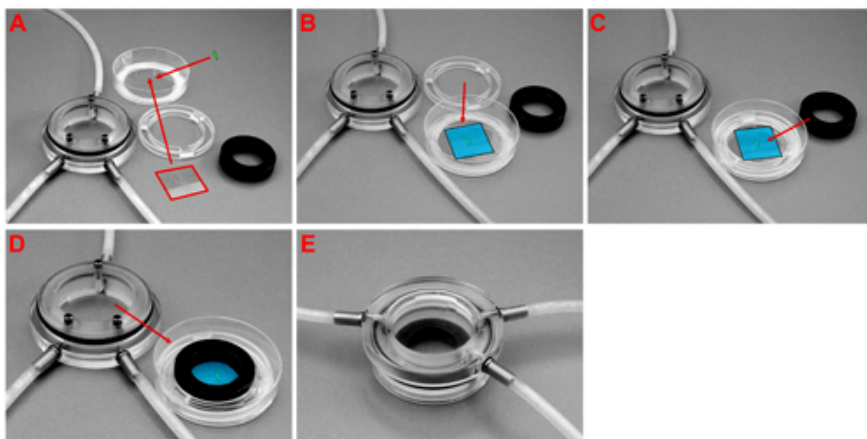


Figure 1 Assembly of the imaging chamber. (A) Place the larva and the plastic spacer onto the oil layer. (B) Place a 22 x 22 mm cover slip on the spacer and insert the plexiglas guide ring into the chamber, next (C) fix the position of the larva with the metal ring and (D) close the chamber. (E) Now the chamber is ready to be mounted on the microscope.

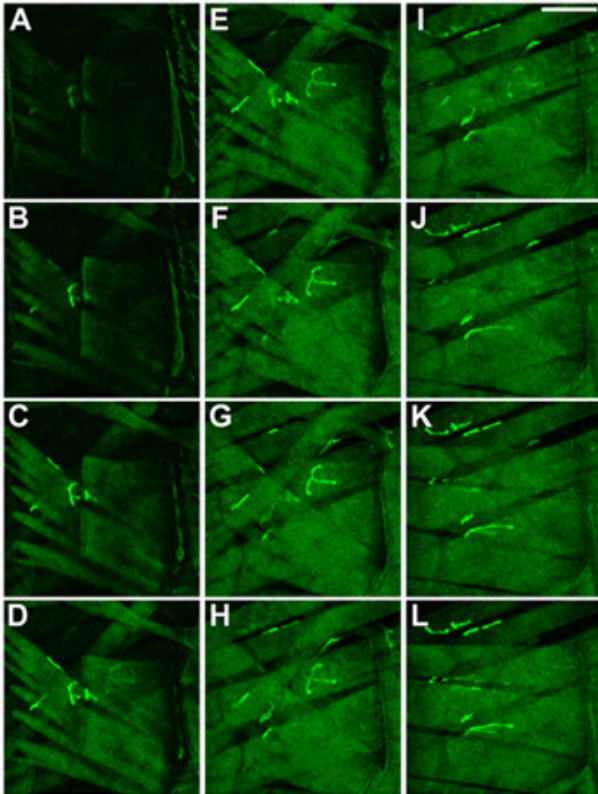


Figure 2 Body-wall muscles in *Drosophila* larvae. Muscles and NMJs were visualized by expression of a CD8-GFP-Sh fusion protein⁸. The muscles are shown as observed when focusing at the ventral side through the cuticle into the larva. In (A-C) the most superficial muscle, 27, is shown, in (K-L) the most interior muscles, 6 and 7, are shown. Scale Bar: 100 μ m, Δ Z between slices is 2 μ m.

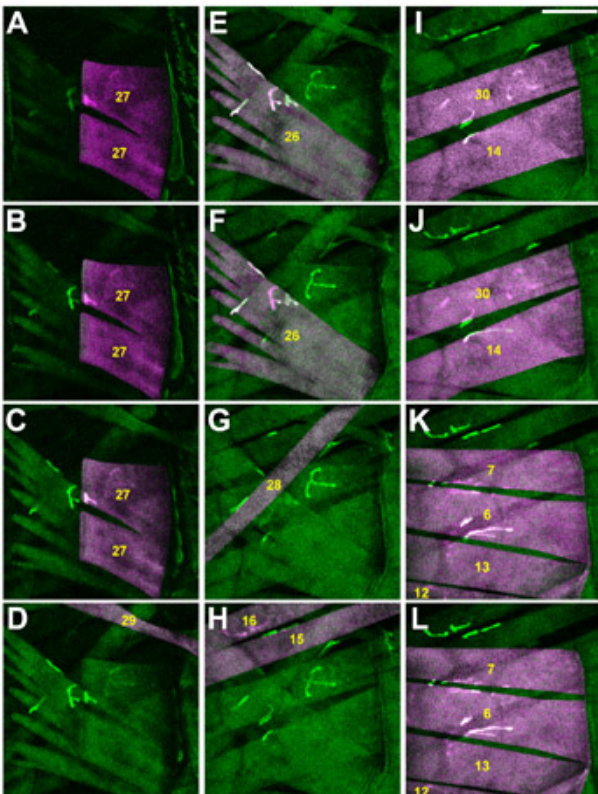


Figure 3 Identity of body-wall muscles in *Drosophila* larvae. The identity of muscles in L3 *Drosophila* larvae, segment A3, is shown. Muscles and NMJs were visualized by expression of a CD8-GFP-Sh fusion protein⁸. The muscles are displayed as observed when focusing at the ventral

side through the cuticle into the larvae. In A-C the most superficial muscle, 27, is shown, in K-L the most interior muscles, 6 and 7, are shown. Scale Bar: 100 μm , ΔZ between slices is 2 μm .

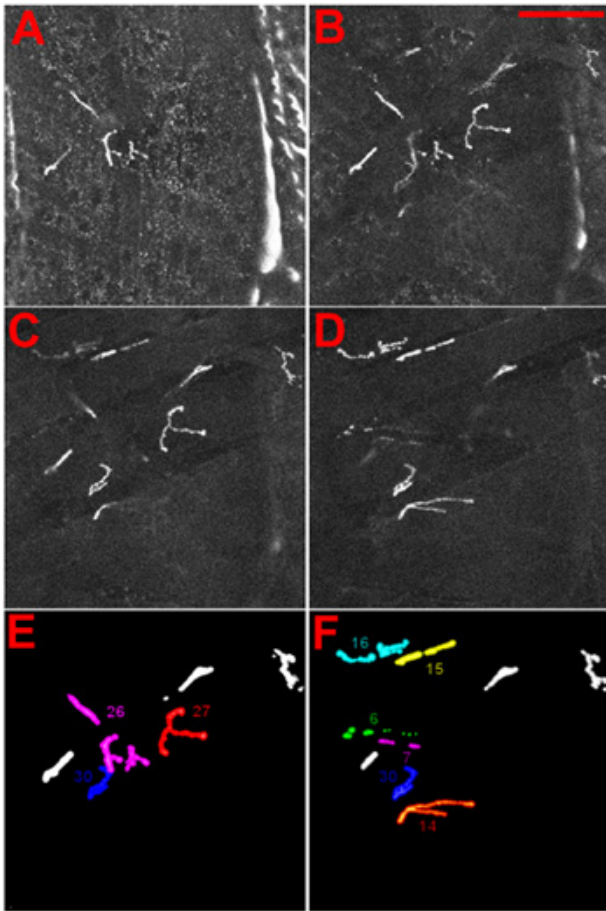


Figure 4 Identity of neuromuscular junctions of *Drosophila* larvae. (A-D) NMJs were visualized by expression of a DGluRIIA-mRFP fusion protein¹. The NMJs are shown as observed when focusing at the ventral side through the cuticle into the larva. In (A-B) the most superficial NMJ, 27, is shown, in C-D the most interior NMJs, 6 and 7, are shown. Scale Bar: 100 μm , ΔZ between slices is 5 μm . (E) and (F) the identity of the superficial (E) and more interior (F) NMJs is given for reference.

Discussion

The presented method was initially developed to study glutamatergic synapses on the body wall muscles of *Drosophila melanogaster* larvae. The *Drosophila* neuromuscular junction (NMJ) is characterized by a stereotypical cyto-architecture of muscles and neurons and is thus ideally suited for *in vivo* imaging. However, the described anesthetization protocol is not limited to imaging the NMJ; the transparency of *Drosophila* larvae facilitates the adaptation of the described protocol to study the development of organs, migration of cells, transport of axonal cargo and sub-cellular reorganization within cells.

Disclosures

No conflicts of interest declared.

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