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Protocol for dissection of *Drosophila* abdomens for fluorescent imaging

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Abstract

Fluorescent imaging in *Drosophila* species is indispensable for investigating dynamic biological processes and visualizing gene expression patterns with cellular precision. This technique leverages the transparency of *Drosophila* larvae and pupae, combined with advanced microscopy, to enable real-time observation of developmental events such as morphogenesis and organogenesis. Genetically encoded fluorescent proteins and dyes allow specific labeling of cells and proteins, facilitating detailed studies of spatial and temporal dynamics within intact tissues. Techniques like confocal and two-photon microscopy provide high resolution and depth penetration, essential for 3D reconstruction and quantitative analysis of complex biological structures. Fluorescent imaging in *Drosophila* supports disease modeling, drug screening, and therapeutic exploration, bridging insights from basic biology to potential clinical applications. It is therefore necessary to develop imaging techniques and protocols that accurately capture and profile gene expression patterns in a wide range of *Drosophila* tissues. In this study, we present a detailed protocol for preparing and imaging transgenic *Drosophila* abdomen, which will enable researchers investigate gene expression patterns underlying fundamental biological processes in the abdomen.

Keywords: *Drosophila*; Imaging; Fluorescent reporters; Abdomen

1. Introduction

The fruit fly species of genus *Drosophila* have served as excellent experimental models to study various aspects of development such as differentiation, pattern formation, organogenesis, morphogenesis, pigmentation and phylogenetics [1-6]. Elucidating the localization of genes in *Drosophila* tissues is the basis of understanding development and often requires imaging tissue specimen. Fluorescent imaging in *Drosophila* plays a crucial role in elucidating dynamic biological processes, visualizing gene expression patterns, and studying cellular and subcellular structures within a living organism. The transparent cuticle of *Drosophila* larvae and pupae, coupled with advancements in microscopy techniques, allows researchers to perform high-resolution imaging of fluorescently labeled tissues and organs in real-time. This capability is instrumental in studying developmental processes such as morphogenesis, organogenesis, and tissue remodeling, providing insights into how genes regulate these processes.

Moreover, single-cell RNA sequencing (scRNA-seq) has revolutionized the study of *Drosophila* biology by enabling transcriptomic profiling of different tissues at the single-cell level [7-12]. By analyzing gene expression in individual cells, researchers can uncover cellular heterogeneity, identify rare cell types, and explore gene regulatory networks with unprecedented detail [13-15]. This approach complements fluorescent imaging techniques, offering a deeper understanding of spatial and temporal dynamics in gene expression and cellular interactions within intact tissues. Additionally, fluorescent proteins and dyes can be genetically encoded or introduced into *Drosophila*, enabling specific labeling of cells, organelles, or proteins of interest. This dual approach facilitates comprehensive studies by combining

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spatial information from imaging with transcriptomic data from scRNA-seq, enhancing our understanding of how genes and proteins orchestrate biological processes at a cellular and molecular level.

Furthermore, fluorescence microscopy techniques like confocal microscopy and two-photon microscopy provide the depth and resolution necessary to capture intricate details within *Drosophila* tissues, supporting quantitative analysis and 3D reconstruction of complex biological structures.

In biomedical research, the integration of scRNA-seq with fluorescent imaging in *Drosophila* contributes to modeling human diseases, evaluating drug efficacy, and assessing therapeutic interventions. By combining transcriptomic analysis with visualizing disease-related phenotypes and molecular mechanisms *in vivo*, researchers can validate disease models, screen for potential treatments, and explore the underlying genetic and cellular basis of human disorders[13-15]. Therefore, the synergistic use of scRNA-seq and fluorescent imaging techniques enhances our ability to investigate fundamental biological questions and translate findings into applications that benefit human health and medicine.

Fluorescent and non-fluorescent imaging of *Drosophila* tissues has become an essential technique for studying developmental biology, genetics, and cell biology. The high resolution and specificity of fluorescent markers, combined with traditional light microscopy, allow researchers to visualize and analyze the expression patterns and localization of proteins, cellular structures, and genetic elements within developing tissues[16]. The *Drosophila* pupal and adult bodies, in particular, provide a valuable model for investigating processes such as morphogenesis, pattern formation, and tissue differentiation[3, 5, 6, 17].

It is crucial to carefully prepare and dissect the pupal abdomens to achieve a high resolution understanding of gene expression patterns. In this study, we present a protocol that outlines a detailed method for the dissection and preparation of *Drosophila* abdomens for fluorescent imaging, ensuring the preservation of tissue integrity and the acquisition of high-quality data essential for detailed quantitative and qualitative analyses.

2. Material

- 70% ethanol (for disinfecting and tissue preparation)
- Transgenic *Drosophila* pupae (expressing fluorescent markers)

These items are essential for the various steps involved in the dissection, preparation, and imaging of *Drosophila* pupal abdomens. They enable researchers to carefully handle and mount specimens, ensuring optimal conditions for high-resolution imaging and detailed analysis of developmental processes and gene expression patterns.

2.1. Equipment

- Wet brush
- Petri dish
- 9-well glass dish
- Forceps (sharp, preferably two pairs)
- Dissection scissors
- Fluorescent microscope
- Light microscope (for non-fluorescent imaging)
- Microscope slides
- Double-sided tape
- Halocarbon oil 27 (or mounting medium)
- Kim wipes
- Paper towels
- Clear nail polish (for sealing coverslips, if needed)

2.2. Procedure

2.2.1. Pre-screening Pupae for Fluorescence

- Using a wet brush, gently collect a few pupae of the desired developmental stage.
- Place a Kim wipe in a Petri dish with a few droplets of water. Gently place the pupa in the wet Kim wipe. This prevents the pupa from drying out, which may cause tissue damage.

- Pre-screen the pupae for fluorescence under a fluorescent scope to ensure the presence of the desired signal.

2.2.2. Preparation of Ethanol Solution

- Fill one well of a 9-well glass dish with 1 mL of 25 to 50% ethanol.

2.2.3. Ethanol Treatment

- Using forceps, pick a transgenic pupa and immerse it in the ethanol for 30 seconds. This helps to disinfect and slightly dehydrate the specimen, making dissections easier. Process one or two pupae at a time.
- While ethanol is effective in disinfecting and slightly dehydrating the specimen, prolonged exposure or improper handling during the ethanol treatment step can lead to over-dehydration or tissue shrinkage, potentially affecting the morphology and fluorescence of the sample

2.2.4. Drying the Pupa

Remove the pupa from the ethanol and briefly dry it on a paper towel by gently tapping the pupa to the paper towel. Care must be taken to prevent tissue damage.

2.2.5. Mounting the Pupa

- Attach a double-sided tape to a microscope slide and then place the pupa sideways on the slide. Make sure the pupal case sticks to the tape on the slide.
- Ensure that the pupa is positioned with its head region facing to the right if you are right-handed (reverse this orientation if dissecting with left hand).

2.2.6. Dissection of the Pupal Case

- Using forceps in your right hand, carefully open the pupal case above the head. Be gently to avoid ripping the entire pupal case.
- Gently pull the body out while stabilizing the bottom of the puparium with the forceps in your left hand.

2.2.7. Removal of the Clear Membrane

- Transfer the extracted body into the 9-well glass dish containing 25 to 50% ethanol.
- Remove any remaining clear membrane that may still be attached to the body.

2.2.8. Trimming Appendages

- Use dissection scissors and forceps to cut off the wings and legs, leaving only the abdomen. The wings can be dissected in this step and used for imaging if required. Add few microliters of mounting medium on a glass slide and transfer to wings to the glass slide. Place a cover slip on the glass slide and seal the cover slip to the slide using clear nail polish. The wings are ready for imaging.
- The dissection process, especially the removal of the pupal case and clear membrane, can be delicate and may result in damage to the tissue if not performed carefully. This can compromise the integrity of the sample and affect the quality of both fluorescent and non-fluorescent imaging.

2.2.9. Drying the Body

- Once the appendages are dissected out, place the body on a dry paper towel to remove excess ethanol. Be gentle to avoid damaging the specimen. Failure to remove excess ethanol may interfere with fluorescent imaging.

2.2.10. Preparation of Mounting Slide

- Prepare a clean microscope slide and add a small drop of Halocarbon oil 27 on the slide. Mounting medium can also be used if Halocarbon oil is not available.

2.2.11. Mounting in Oil

- Immediately place the body into the drop of Halocarbon oil 27 on the slide.

2.2.12. Orientation of the Specimen

- Align the body so that either the dorsal side, the dorsolateral side, or the lateral side faces up. Note that all three orientations need to be imaged, and multiple pupae may be required to capture all necessary views due to photo bleaching.
- Make sure that the pupa does not move once placed in the oil. Using a Kim wipe gently remove any excess oil on the slide. Having excess oil on the slide may lead to detachment of the pupa from the slide and can leave the pupa unsettled interfering with imaging.
- Proper alignment of the specimen in Halocarbon oil 27 is crucial for obtaining clear and accurate images. Misalignment can result in suboptimal imaging of the desired anatomical regions, necessitating repeated dissections and imaging attempts.

2.2.13. Imaging

- Using a fluorescent or light microscope, take image stacks of approximately 25 images. Start from the top focal plane and end at the bottom focal plane.
- Ensure all images of the same construct are saved in the same folder for consistent organization and analysis.
- Fluorescent signals can diminish over time due to photo bleaching, especially during prolonged imaging sessions. This limits the duration for which high-quality fluorescent images can be obtained and may require multiple specimens to capture all desired orientations and views.

Notes

- Handle pupae with care throughout the process to avoid damage.
- Ensure the imaging environment is clean to prevent contamination of the samples.
- Adjust the imaging parameters as needed to obtain the best possible resolution and signal-to-noise ratio.
- Document each step carefully to ensure reproducibility and accuracy in subsequent experiments.

By following this detailed protocol, researchers can efficiently dissect and image *Drosophila* abdomens, facilitating high-resolution analysis of both fluorescent and non-fluorescent signals within the specimens.

3. Results

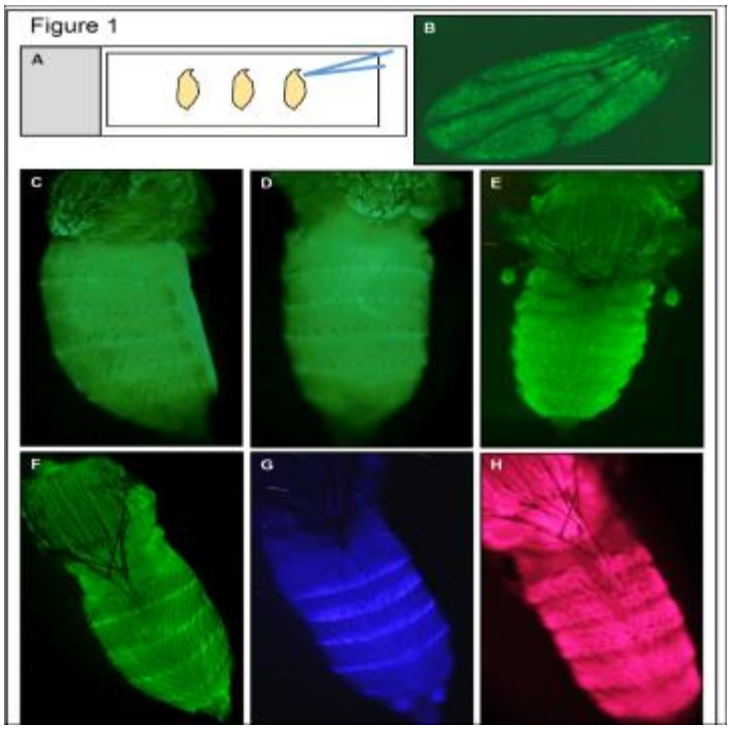

Figure 1 Fluorescent imaging of *Drosophila* abdomens A. schematic illustration of pupal arrangement and dissection on a side B. *Drosophila* wing displaying fluorescent reporter expression C-D control abdomens showing no reporter activity E-H abdominal image demonstrating reporter gene expression

Figure 1B-H demonstrates an application of this protocol in imaging transgenic *D. melanogaster* abdomens. Transgenic pupae carrying enhancers driving reporters specifically in the abdomen were imaged using this method. The images clearly depict reporter expression patterns, thereby validating the protocol's efficacy. Furthermore, this protocol can also be used for abdomen immune-stained with different antibodies as well.

4. Discussion

Fluorescent imaging in *Drosophila* abdomens represents a pivotal approach in developmental and cell biology research. This protocol outlines a systematic method for preparing and dissecting pupal abdomens to maximize imaging quality and preserve tissue integrity. The use of transgenic lines expressing fluorescent markers ensures precise visualization of developmental processes and molecular interactions within live organisms[3, 5, 6, 17]. Ethanol treatment aids in specimen preparation by disinfecting and facilitating dissection, although careful handling is crucial to avoid tissue damage and fluorescence quenching.

Successful imaging relies on meticulous handling during dissection and mounting in Halocarbon oil 27 to minimize artifact and ensure stable specimen orientation. Imaging parameters are optimized to capture clear, high-resolution image stacks necessary for comprehensive analysis. Challenges include potential tissue damage during dissection, variability in fluorescence intensity, and photo bleaching over extended imaging periods, necessitating careful experimental design and replication. Moreover, the protocol is widely applicable to other organisms and also for non-transgenic *Drosophila* or other organisms.

Integrating single-cell RNA sequencing (scRNA-seq) with fluorescent imaging can significantly enhance the depth of developmental biology studies in *Drosophila*. scRNA-seq allows for the resolution of transcriptomic profiles at the single-cell level, providing insights into the heterogeneity and specific gene expression patterns within individual cells of the pupal abdomen. This approach complements fluorescent imaging by linking visual phenotypes with underlying gene expression dynamics, offering a holistic view of developmental processes [7-12] .

5. Conclusion

Overall, fluorescent imaging in *Drosophila* offers unparalleled insights into developmental biology and disease mechanisms, enhancing our understanding of genetic regulation and cellular dynamics. By integrating advanced microscopy with genetic manipulation, researchers can elucidate fundamental biological processes and explore therapeutic avenues with potential clinical relevance. Future advancements in imaging technology and genetic tools will further expand the utility of *Drosophila* as a model organism for biomedical research

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflicting interests.

Authors' Contributions

B.P.G. and K.K.B.R. prepared and imaged the specimen. The manuscript was prepared by B.P.G and K.K.B.R. and reviewed by K.K.B.R.

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