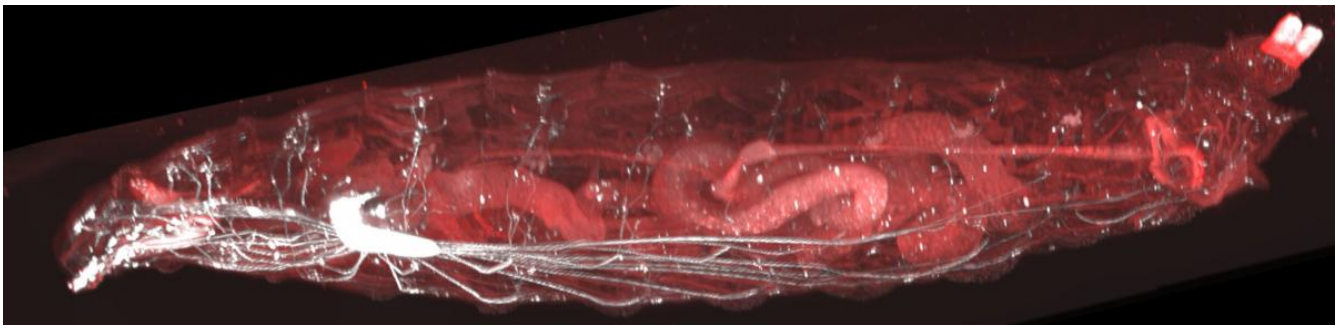
Light sheet microscopy of cleared *Drosophila* larvae

O.K. Kobler[1], T.S. Stöter[1], B.G. Gerber[2,3], U.T. Thomas[4]

[1] Leibniz Institute for Neurobiology, Department Combinatorial NeuroImaging Core Facility (CNI), Magdeburg; [2] Leibniz Institute for Neurobiology, Department Genetics of Learning and Memory, Magdeburg; [3] Center for Behavioral Brain Sciences (CBBS), Magdeburg; [4] Leibniz Institute for Neurobiology, Department Cellular Neurobiology, Magdeburg.

Abstract

Drosophila larvae are widely used to address questions in many biological research areas including molecular, cellular and systems neurobiology. Larval neuromuscular junctions, for instance, have proven an excellent model system to study principles underlying synaptic structure, function and plasticity in normal and disease-related conditions. Moreover, the implementation of EM volumes of the larval CNS and an ever more refined toolkit for transgenic and genomic manipulations in flies provide an excellent basis to decipher connectivity within the nervous system and thus to unravel how sensory inputs are translated into behavioural output. We have recently established a protocol for fast, robust, low-cost whole body multifluorescent imaging of *Drosophila* larva at cellular resolution (Kobler et al., 2021). The adapted procedure, based on a previously described organic solvent-based clearing method using Ethyl cinnamate (ECi; Klingberg et al. 2017), enables clearing of L3 *Drosophila* larvae with minimal handling time. The speed of our protocol, the low toxicity and the success rate allow for efficient analyses and low- to midscale screening approaches on a light sheet UltraMicroscope. We systematically tackled pitfalls associated with clearing of a small but cuticularized organism. Since the cuticle of *Drosophila* larvae constitutes an almost unbreakable barrier for antibodies, we assessed various fluorescent proteins (FPs) for suitability to monitor patterns of targeted or endogenous expression based on widely used transgenic approaches. Next to some conventional FPs, a recently introduced near-infrared FP proved to be particularly well suited. By using a white light laser source with appropriate filter combinations for excitation and emission, we are thus able to monitor, for instance, individual neurons in context with marker structures in the brain and with peripheral target areas. At present, we are aiming at automatizing the detection of 3D labelled structures for quantitative analysis. Such data will place the emerging knowledge of neural connectivity in the context of the whole larval body. This approach may be easily extended towards augmented reality to represent neuronal connectivity within whole body *Drosophila* larvae.



Whole *Drosophila melanogaster* L3 larvae, expressing UAS-CAAX-mCherry pan neural, were cleared and subsequently imaged.

References

1. Kobler, O., Weiglein, A., Hartung, K., Chen, Y. C., Gerber, B., & Thomas, U. (2021). A quick and versatile protocol for the 3D visualization of transgene expression across the whole body of larval Drosophila. Journal of neurogenetics, 35(3), 306–319.
2. Klingberg, A., Hasenberg, A., Ludwig-Portugall, I., Medyukhina, A., Männ, L., Brenzel, A., Engel, D. R., Figge, M. T., Kurts, C., & Gunzer, M. (2017). Fully Automated Evaluation of Total Glomerular Number and Capillary Tuft Size in Nephritic Kidneys Using Lightsheet Microscopy. Journal of the American Society of Nephrology : JASN, 28(2), 452–459.