# Digital Scanning Lightsheet Microscopy (DSLM) for Drosophila Whole Brain Functional Imaging

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## Abstract

Intravital imaging has presented a powerful tool for tracking cellular function and structural dynamics in the real environment of a complex multicellular organism. However, most commercial microscopy systems are limited by optical design, allowing only long-term live imaging of transparent samples, such as Drosophila embryo, zebrafish embryo, and suffering from slow image acquisition speeds. Consequently, they can only generate two-dimensional time-lapse images at selected optical planes. Here, we introduce a high-resolution Bessel beam based vertical digital scanning light sheet microscopy (V-SPIM) for real-time imaging of the adult fruit fly brain. Compared to traditional multiphoton imaging or multi-point confocal microscopy, Bessel beam based light sheet microscope exhibits lower photodamage and faster acquisition volume rates (V.R.) higher than 1Hz (with proper exposure time (E.T.) and signal to background ratio (SNR)) for each volume($330\times330\times162\mu m$), allowing us to perform long-term volumetric time-lapse functional imaging in adult animals. This novel approach allows us to visualize the olfactory coding of various odors in a multitude of cells within a single fly brain.

**Keywords**: Drosophila Melanogaster, Neuron calcium imaging, Olfactory system, Odor preference and behavior, Bessel beam, Lightsheet microscopy

## I Introduction

Live imaging has revolutionized biomedical sciences by providing real-time insights into cellular and neurological dynamics in live animals[1]. Advances in fluorescence markers and microscopy have expanded applications to tissues and organs, demanding high spatiotemporal resolution. Traditional methods like confocal and multiphoton microscopy are limited by their scanning systems, impacting imaging speed[2]. Although technologies like TAG lenses in multiphoton microscopy enhance speed, they struggle with large-scale high-speed imaging with high contrast[3].

Spinning disk microscopy significantly boosts acquisition speed but faces photobleaching issues[4]. Light field microscopy offers rapid volumetric imaging but suffers from resolution and field of view trade-offs, limiting its effectiveness in non-transparent tissues[5]. Light sheet microscopy reduces light damage and achieves subcellular resolution but struggles with tissue scattering in dense or non-transparent tissues.

Bessel beams, with their non-diffracting properties, offer better penetration in dense tissues and maintain high intensity over long distances. Bessel scanning light sheet microscopy has shown promise in capturing cellular activities in transparent organisms and super-resolution imaging in expanded samples[6-8]. However, its effectiveness in mature non-transparent animals, especially for functional imaging in dense cellular structures crucial for regenerative and neuroscience studies, remains to be fully validated.

To address the unmet need for imaging dense structures in vivo, we present an up-right digital scanning Bessel light sheet microscopy (vSPIM). This technique utilizes the properties of the Bessel beam to perform high-speed scanning (100 fps) over the full

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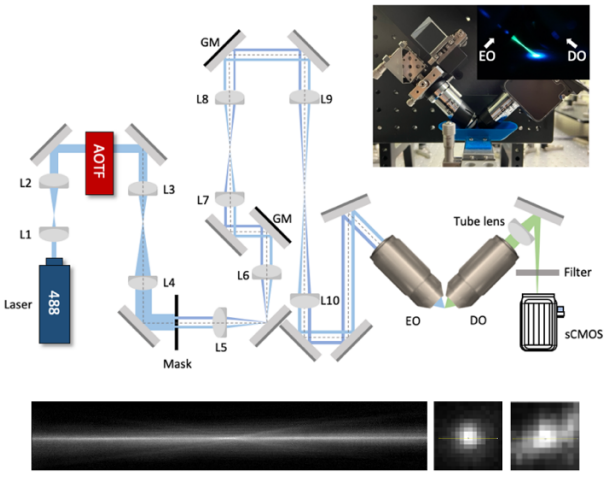
field of view of the objective while maintaining subcellular resolution in both lateral and axial dimensions. We demonstrate the significance of vSPIM in neuroscience research by capturing high-resolution calcium images of odor responses in the densely packed synapses of olfactory neurons in the near-complete brain of mature fruit flies at a speed of 2-3 volumes per second. We used GH146-Gal4 Drosophila to study PNs' responses to various odors using V-SPIM. Odors were alternately delivered, and volumetric samples of boutons in the Calyx region were collected. Our alignment program minimized movement artifacts, allowing us to classify response types from fluorescence data. We found significant differences in response types between odors, particularly higher prolong responses to aversive odors and higher off responses to food odors. This study demonstrates vSPIM's capability in capturing detailed neural activity, providing valuable insights for neural network research.

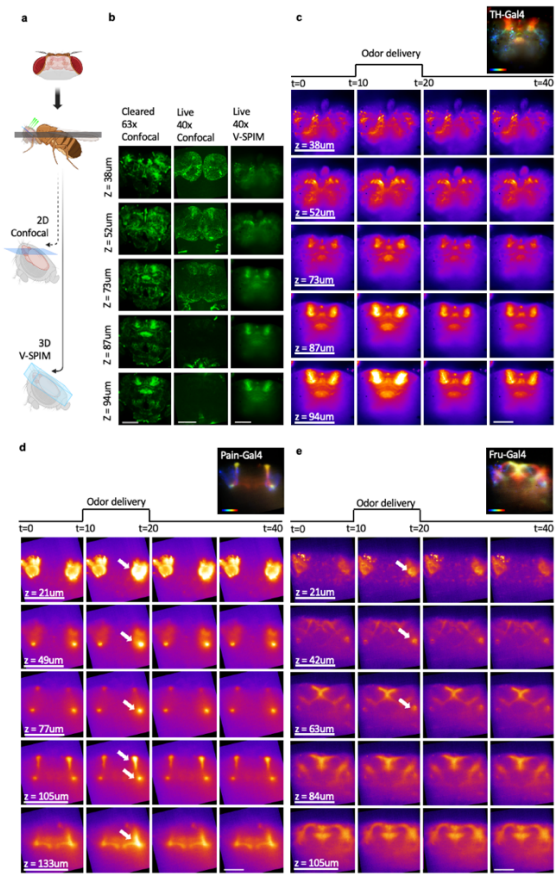
## II Result

**Fig.1** vSPIM system description



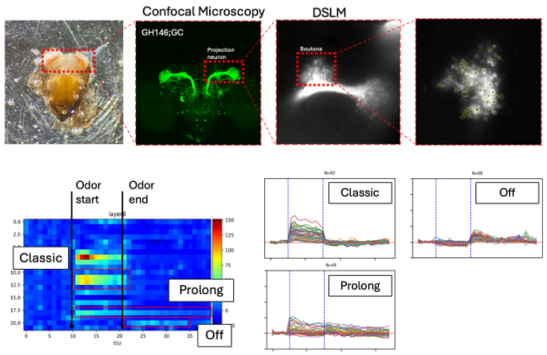

Bessel beam based digital scanning up-right lightsheet microscopy system (vSPIM) schematic diagram, with sub-cellular resolution description.

**Fig.2** Drosophila whole brain imaging

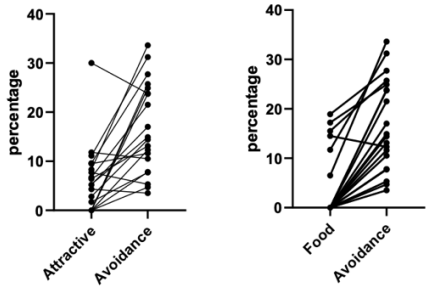



We tested V-SPIM for functional imaging in Drosophila brains, addressing challenges in confocal microscopy with higher z-axis penetration. vSPIM maintained consistent contrast deep in the brain, unlike confocal microscopy. We imaged neural activity in response to odors in various Drosophila lines, confirming V-SPIM's effectiveness for large-scale brain activity observation and neural network studies.

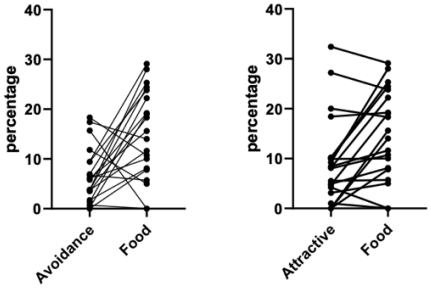
**Fig.3** Quantitative odor respond and analyzation for dense neuron synaptic structure in Drosophila



Prolong respond type on PN's boutons




Off respond type on PN's boutons




We used the Drosophila line GH146-Gal4 with UAS-GC6m to label most PNs and tested five different odors (two aversive: OCT, MCH; two attractive: MSC, GA; one food-related: ACV) on flies (n=20) fixed under vSPIM with volume rate 1Hz, covering all boutons in the Calyx region (330 x 330 x 52um). We manually identified odor-responsive boutons from the high-resolution data and categorized them into 3 response types (classic, prolong, off). Analysis revealed significant differences in response type proportions between odors. Aversive odors had a higher proportion of prolong responses, while the food odor had more off responses.

## III Acknowledgements

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