SCAPE Microscopy for High Speed, 3D Whole-Brain Imaging in *Drosophila Melanogaster*

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Abstract:
SCAPE microscopy permits high-speed 3D imaging of neuronal activity throughout the brain of behaving, adult *Drosophila*, as well as activity in the entire nervous system of freely crawling *Drosophila* larvae. Latest results will be presented.

1. Introduction

Understanding complex, distributed neural circuits depends heavily on our ability to observe them in action, but the spatial and temporal resolution needed for large-scale neural imaging is a major challenge. Although the size and the scale of the *Drosophila melanogaster* brain, and the availability of fluorescent indicators with fast kinetics, makes it well-suited for whole brain microscopy, standard confocal and two-photon approaches cannot achieve sufficiently high volumetric imaging rates over large enough fields of view to capture this whole-brain activity. While conventional multi-objective light sheet configurations are improving in speed, they are not suitable for imaging the intact, adult Drosophila brain, nor the dynamics of freely crawling larvae [2, 3]. Swept confocally aligned planar excitation (SCAPE) microscopy is a recently developed technique that combines light-sheet optical sectioning with confocal de-scanning to permit very fast 3D imaging through a single objective lens [1]. These features make SCAPE well suited for in-vivo brain imaging in awake, behaving animals. Here, we demonstrate the use of our latest SCAPE microscopy system design to image brain activity of both larval and adult behaving *Drosophila,* recording multi-region brain activity at cellular resolution at 10 volumes per second for both head-fixed walking adult fly and freely crawling larva.

2. Methods

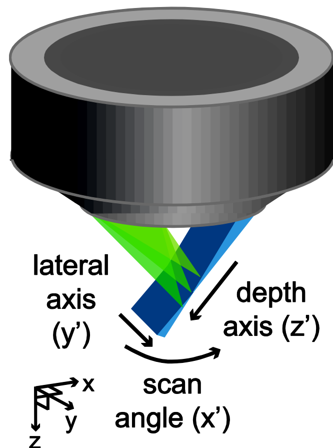

Fig. 1. The imaging geometry of SCAPE microscopy. An oblique light sheet emerges from the edge of the objective lens, and light excited in the sample is detected through the same objective. The 3D image is formed by sweeping the sheet back and forth within the sample (in the x-direction) (see [1]). Scanning is achieved by moving a singe galvanometer mirror, which changes the angle of the light entering objective, sweeping the light sheet back and forth. The galvanometer also serves to descan the returning light, causing the plane incident on the camera to always stay aligned with the light sheet as it moves through the sample. Volume rates of 20 VPS can be achieved by scanning the galvanometer mirror at only 20 lines per second. Volumetric imaging is achieved without needing to translate the objective lens or the sample, making SCAPE well-suited for imaging small, sensitive samples such as Drosophila and zebrafish.

SCAPE microscopy is a single-objective, light-sheet illumination technique capable of imaging fields of view larger than 550x900x300 µm at speeds up to and exceeding 20 volumes per second. By passing a light sheet off-axis through the back aperture of an objective lens optical sectioning is achieved over an oblique plane of laser excitation (currently 488 nm). This plane is scanned through the sample using a galvanometric mirror, descanned onto a stationary intermediate image plane and mapped onto a high-speed sCMOS camera. The basic schematic of the system is shown in Fig. 1. Also, taking advantage of the sCMOS camera's large sensor size, we can optionally incorporate a dual-channel splitter in front of the camera to perform simultaneous red-green imaging, which is important for cell tracking of the moving sample such as crawling larva. Since the objective les does not need to translate in order to generate a 3D image, it is straightforward to add light sources for wide-field or spatially patterned optogenetics, such as with

ReaChR, during high-speed volumetric imaging of neural activity. The fact that SCAPE uses light-sheet illumination minimizes photodamage and bleaching compared to confocal and epi-fluorescence techniques since only the tissue section being imaged is illuminated at any point in time.

Imaging adult *Drosophila melanogaster*

The adult *Drosophila* imaging setup was designed to include comprehensive stimulus control, whole brain GCaMP imaging and recording of fly walking behavior. Our experiments to date have focused on the olfactory circuit of the adult fly, as it is currently well understood from electrophysiology measurements and two-photon recordings of discrete regions [4, 5]. To acquire data, the fly was fixed on plastic tape with a small cut window. The brain is then exposed in saline by removing the cuticle on the head and fat around the brain. Muscle 16 was cut to minimize the brain motion. Under this experiment setup, the fly can behave normally and stay in healthy imaging condition for several hours. This configuration is shown in Figure 2 along with an example SCAPE data set acquired in a fly expressing pan neuronal GCaMP6f and dsRed/mCherry. The whole brain was imaged at 10 volumes per second over a field of view of is 228 x 619 x 240µm. The light sheet is scanning with a step size of 2µm. In ongoing experiments, we are able to visualize the patterns of neural activity throughout the brain, with cellular resolution, in response to a range of different odors, while relating brain activity to the walking behavior of the fly in real-time.

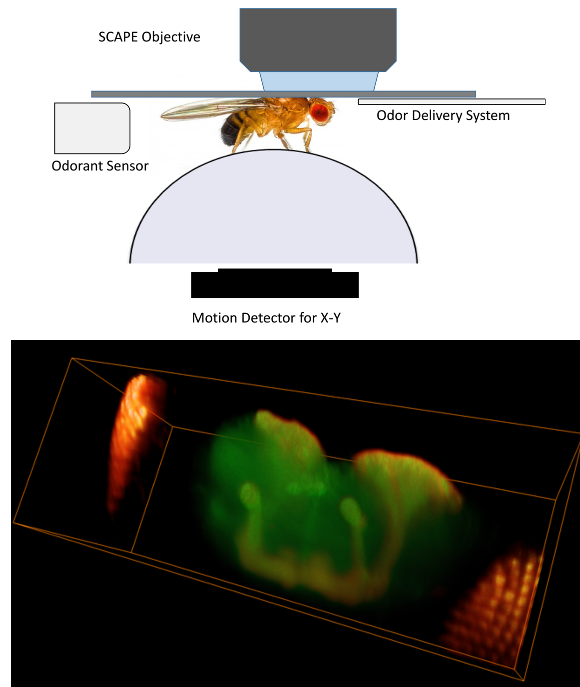

Fig. 2. Top: The schematic of the experiment setup for adult fly brain imaging using an upright layout with odor delivery and fly walking tracking system. **Bottom:** Example of SCAPE data acquired with dual-color emission in 0.1 seconds, capturing the entire adult Drosophila brain**.** The yw;UAS-GCaMP6f,MB-dsRed;Nsyb-Gal4 model produces dense pan-neuronal GCaMP6f to indicate neuronal activity and expression and dsRed expression in all the kenyon cells in the mushroom body. The orange structures at the sides are the fly's eyes.

Imaging crawling *Drosophila* larvae

The question that we are addressing with crawling larva imaging is to understand the role that specific neuronal sub-types play in sensorimotor integration and proprioception [6]. In the data shown below, we recorded the function of neurons in the ventral nerve cord, and ventral proprioceptive neurons. To image the ventral surface of the larva, SCAPE can be easily reconfigured to image in an inverted configuration, by flipping the objective lens and raising the sample stage. Larval *Drosophila* were positioned on a cover glass bottomed petri dish and embedded in a 0.5% low gelling point agarose to restrict motion (similar to their native environment) but still allow free crawling on the cover glass. This inverted layout positions the ventral nerve cord close to the objective which minimizes the scattering effect of the thick fat around the larva. Also, taking advantage of the live preview and high volumetric speed of the SCAPE system, we can manually or automatically move the stage during the acquisition to keep the larva within the field of view while crawling normally. Unlike in the adult *Drosophila* experiment setup, there is no surgical preparation of the larva, making it possible to explore learning/developmental activity on the same animal using SCAPE system.

For the freely behaving larva experiment, we used both pan neuronal and subset labeled GCaMP6f and tdtomato fly stock. The larvae were imaged at 2nd instar, which have a limited amount of fat and a full behaving pattern. At 10 volumes per second, the field of view is 405x748x121µm (Fig.3), which is several times larger than the ventral nerve cord and the stage can be adjusted to keep the larva in the acquired volume for long term imaging. Cellular resolution is achieved and some of the large sensory dendritic branches are visible. Higher volumetric speeds can be achieved with smaller fields of view if we want to capture fast burst activity while keeping the ability to still image the whole ventral nerve cord.

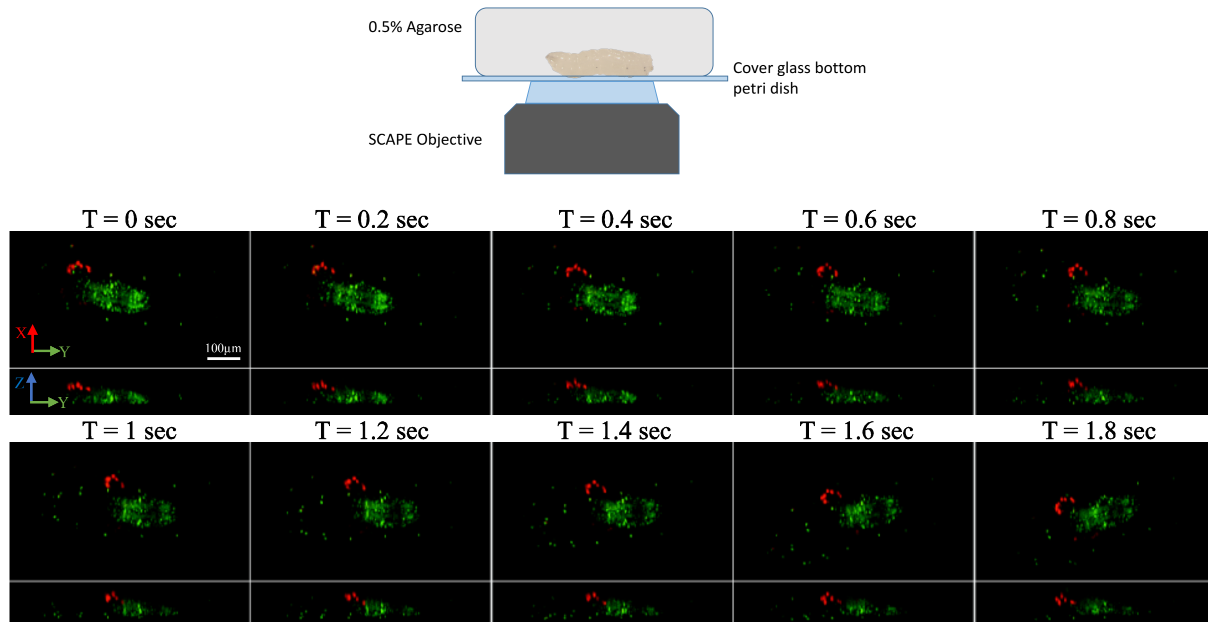

Fig. 3. Top. SCAPE imaging configuration to capture neural activity in freely crawling *Drosophila* larvae. **Bottom:** Dual color imaging of a 2nd instar 410-Gal4;UAS-GCaMO6f/UAS-tdtomato larva, which has a subset of neurons labeled in ventral nerve cord together with some sensory input neuron around the cuticle. Images show time lapse of maximum intensity projections through z (top frame) and through x (bottom frame) of the acquisition. Every other acquired frame is shown (10 VPS acquisition).

4. Conclusion and Discussion

SCAPE provides a powerful new tool for imaging both larval and whole adult *Drosophila* brain. SCAPE is a dramatic improvement over traditional methods that can only probe small regions of the brain's circuits during complex behaviors. The capability to image freely moving animals offers a new way to study sensorimotor integration and understand circuit to cellular level neuronal function in networks. The ability of SCAPE to perform continuous, long-term observations of learning and development is also very useful compared with other invasive imaging/electrophysiology approaches.

In addition to fruit fly, SCAPE microscopy is also effective for imaging a wide range of other in-vivo samples including zebrafish brains and heart, the awake, behaving mouse brain, and even intact fresh tissues for histopathology-like imaging. A current challenge is to manage data throughput, storage, analysis and visualization. Cell tracking algorithms for dual color imaging, registration and cell segmentation for cellular level GCaMP activity are being developed. We will present our latest results applying SCAPE to imaging the in-vivo *Drosophila* system.

5. Acknowledgements

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6. References

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