Efficient and cost-effective 3D cellular imaging by sub-voxel-resolving light-sheet add-on microscopy

Xinlin Xie¹⁺, Yicong Yang¹⁺, Hao Jiang¹⁺, Yi Li²⁺, Xuechun Wang¹, Hao Zhang¹, Haibo Jia³, Sheng Liu⁴, Mei Zhen⁵, Shangbang Gao³* and Peng Fei¹,²*

¹ School of Optical and Electronic Information, Huazhong University of Science and Technology, Wuhan, 430074, China.
² Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan, 430074, China.
³ College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, 430074, China.
⁴ School of Power and Mechanical Engineering, Wuhan University, Wuhan, 430072, China.
⁵ Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, M5G 1X5, Canada.
* These authors contribute equally to this work
* Correspondence: feipeng@hust.edu.cn

Abstract: Light-sheet fluorescence microscopy (LSFM) is an emerging technique for 3D imaging of biological samples at high-speed and low photo-toxicity. Unlike various large-format LSFM modalities based off widely-used conventional microscopes, we report here a sub-voxel-resolving light-sheet add-on microscopy (SLAM) method that enables fast, voxel super-resolved light-sheet fluorescence imaging on a common wide-field microscope. A miniature add-on device is developed to comprise: a horizontal laser-sheet illumination path that can confine fluorescence excitation to the vicinity of the focal plane for imposing sharp optical sectioning on the samples, and a unique off-axis scanning strategy that can encode raw LSFM images with sub-voxel spatial shifts for reconstructing a two-fold higher-resolution volume through a sub-voxel-resolving (SVR) computation. It thus empowers a 2D wide-field microscope for 3D LSFM imaging of various biological samples, such as behaving C. elegans, live zebrafish embryos, and cleared mouse brains, at significantly higher spatiotemporal resolution. Heartbeat of developing zebrafish larva and changing calcium activities of aging C. elegans muscle can be reliably extracted using this method. SLAM represents an efficient and cost-effective solution to convert the vast number of in-service microscopes for advanced light-sheet imaging with super-resolution capability.

1. Introduction

Light-sheet Fluorescence Microscopy (LSFM) has emerged as an imaging technique of choice for developmental biology, histology, and neuroscience. With sharp plane illumination and orthogonal wide-field detection, LSFM allows rapid imaging of various live samples with excellent axial resolution and low photo-toxicity. A number of variants of LSFM, such as selective plane illumination microscopy (SPIM), multidirectional SPIM (mSPIM), ultramicroscopy, digitally scanned light sheet microscopy (DSLM), and Bessel plane illumination microscopy, have been continuously developed in the past decade to further increase the resolution and scale of imaging from single living cells to an entire cleared mouse. Despite the diversity of reported modalities, LSFM is fundamentally characterized by the separate laser-sheet...
illumination path, which provides fast optical sectioning of the samples. Otherwise, LSFM uses the same wide-field fluorescence detection as those used in conventional microscopes. Therefore, development of LSFM based on conventional wide-field microscope is believed to greatly simplify the system construct. However, most of advanced LSFM modalities are currently independent from either conventional microscopy or confocal scanning microscopy, thus containing relatively complicated setting with large form factors and high maintenance. Though a few of LSFM methods, such as OCPI and iSPIM, that can be established on existing inverted microscopes, they also contain full set of illumination and detection optics. The host microscopes simply provide bright field or epifluorescence observation without being involved in the LSFM imaging. Several LSFM methods, e.g. open-SPIM, with smaller format factor and simplified illumination/detection structures, have been developed to disseminate advanced biological imaging for less-funded environments. Given the number of epifluorescence microscopes in service, an even simpler way to access LSFM imaging is creating a laser-sheet illumination and making full use of existing conventional microscope for fluorescence readout. Fluid-based LSFM methods use capillary or specially designed chip to drive the sample flow through a Gaussian laser-sheet, enabling conventional microscope to record the images from the consecutively illuminated planes of the flowing samples. On a portable optofluidic platform, this class of methods can achieve basic LSFM imaging at high throughput, but they can only handle certain types of samples with highly specialized sample loading/scanning strategy by motorized pumps. Our previous development of plane illumination plugin device, which contains a compact laser-sheet illumination and sample mounting parts, renders a simple method to implement LSFM imaging on an inverted microscope. We demonstrated by proof-of-concept that a conventional microscope can obtain rapid, 3D imaging in an extremely cost-effective way, though its performance and reliability are limited due to the use of improvised elements.

Herein, we report a sub-voxel-resolving light-sheet add-on microscopy (SLAM) that readily enables multidimensional, high spatiotemporal resolution imaging of multicellular organisms on a conventional wide-field microscope. A light-sheet add-on device is designed to be compatible with conventional wide-field microscopes, the working horse by most biomedical researchers. It contains a compact plane illumination path that replaces the epi-illumination system of host microscope. The low profile, horizontal laser-sheet SLAM outputs selectively illuminate a thin plane of the samples, thus confining the axial excitation in the vicinity of the focal plane to generate high-contrast signals. Meanwhile, the camera-based wide-field detection from the host microscope rapidly records fluorescence images from the consecutively illuminated planes, enabling dynamics volumetric imaging of live specimens. In addition to converting a conventional wide-field microscope into a light-sheet microscope, SLAM is integrated with a unique off-detection-axis scanning scheme plus a sub-voxel resolving (SVR) computation strategy, which together can obtain a volumetric image with three-dimensionally enhanced resolution. Therefore, SLAM can be free from either high magnification optics or mechanical stitching when imaging large specimens, such as whole embryos and thick organs. Using fast GPU-based computation, SLAM is able to further increase the space-bandwidth-product and throughput, which are originally coupled to the physical limitation of system optics.

We demonstrate the success of SLAM method by volumetrically imaging a broad range of samples, including live zebrafish embryo, behaving C. elegans, and clarified mouse brain tissue. In comparison to the original wide-field fluorescent imaging, SLAM provides improved contrast, significantly enhanced resolution (over 10 folds) and 3D imaging capability to its host microscope, with achieving high speeds up
to 3 volumes per second. Various quantitative analyses, such as the cardiovascular dynamics and distinct physiological and pathological motor activity outputs, are also enabled based on SLAM imaging results.

2. Results

2.1. The design and operation procedures of SLAM.

SLAM is based on an add-on device that consists of several compact components: a collimated fiber laser (illumination source), a cylindrical lens with a slit (for the generation of tunable laser-sheet), a magnetic holder (for mounting the sample close to the objective), a glass chamber (for immersing the sample into index-matched liquid), and a tilt stage (for scanning the sample through the laser-sheet along off-z-axis direction) (Fig. 1a and Supplementary Table S1). All these components are integrated and self-aligned into a portable aluminum base. This assembled device is currently designed to fit the host microscope (Olympus IX73), and thus can be easily attached for sustained imaging (Fig. 1b and Supplementary Fig. S2). In working status, SLAM first generates a Gaussian-type laser-sheet (~3 - 15 microns) at the horizontal plane, which is orthogonal to the detection axis of the host microscope. This laser-sheet replaces the wide-field illumination of the inverted microscope and therefore imposes a sharp plane illumination on the sample at the vicinity of detection objective’s focal plane (Fig. 1c). The fluorescent signals, emitted from the illuminated planes of the samples, are collected by the detection objective and imaged onto the camera via the intrinsic optical path of the host microscope (Supplementary Fig. S1). A conventional microscope is at this moment switched from wide-field, epi-fluorescence imaging (Fig. 1e) to in-focused, light-sheet fluorescence imaging (Fig. 1f). The vignettes in Fig. 1e and 1f indicate that, due to reduced out-of-focus excitation, the raw image acquired from SLAM already exhibit improved intensity and contrast.

The sample stage contains a motorized actuator for rapid 3D imaging (Z812B, Thorlabs, USA, Supplementary Table S1). The actuator scans the sample through the stationary light-sheet, either continuously with certain velocity or discretely with fine step-size. The sample stage includes a customized tilting plate (Fig. 1a), which makes the actuator’s scanning axis oblique to the horizontal laser-sheet. Thus, unlike standard LSFM imaging, SLAM scans samples along a vector with typically 70-80 degree angles with respect to the x and y directions of the camera pixel grid (Fig. 1c, d). The microscope simultaneously detects the fluorescence plane by plane with an ultra-small step size (Fig. 1d). This unconventional off-z-axis scanning mode in conjunction with an ultra-small step size encrypts lateral and axial shifts into the raw SLAM image sequence. These 3D spatial shifts are then modeled in the subsequent SVR computation to generate output image with enhanced resolution.
Figure 1. Schematic of the sub-voxel-resolving light-sheet add-on microscopy (SLAM) and its working principle. (a) The structure of the add-on device, consisting of several compact mechanical parts and small optical elements on a device base (D.B.). The major components include: a collimated fiber laser (F.L.) as illumination source; a cylindrical lens (C.L.) combined with an adjustable slit (A.S.) for generation of tunable laser-sheet; a magnetic holder (M.H.) for mounting the sample close to the detection objective (D.O.); a glass chamber (G.C.) for housing the sample into an index-matched liquid environment; and a sloped stage (S.S.) driven by a motorized actuator (M.A.) for scanning the sample through the laser-sheet along a direction off z axis. (b) SLAM in working status. The device can be easily fixed on an inverted microscope (Olympus IX73) for sustained LSFM imaging. (c) For 3D sub-voxel scanning LSFM imaging, the sample is first mounted on a transparent FEP plate using 0.5% hydrogel. Then, via a user-friendly magnet adapter, the plate is firmly immersed into a liquid-filled chamber, with an angle of 30 degrees to the horizontal plane. (d) Sub-voxel 3D scanning off the z-axis. During image acquisition, the sample is scanned through the laser-sheet by a motorized actuator, along a direction with typically 10-20 degree angles off the z axis. As sample being consecutively illuminated layer by layer, the fluorescence detection system of the microscope simultaneously collects the plane-excited fluorescent signals, record them using a high-speed camera and finally obtain a stack of raw images encoded with spatial shifts beyond the native solution. (e)-(f) Drastic imaging improvement brought by SLAM. After SLAM being activated, a conventional microscope can be immediately switched from wide-field, epi-illumination (e) to in-focused, plane illumination (f). (g) Sub-voxel-resolving (SVR) reconstruction based on sub-voxel scanning SLAM data. First, the raw image sequence is separated into several LR image stacks according to Nyquist Sampling theorem along z-axis. Through the sub-voxel scanning with ultra-fine step size, each subdivided LR image stack presents native system resolution and further carries 3D, sub-voxel information that cannot be resolved in raw data (step 1). Following the reversion of image degradation process in spatial domain, these LR images are inputted into the VSR computation procedure to iteratively solve a voxel-super-resolved estimate containing details...
beyond the native resolution (step 2, 3). At last step, a voxel re-alignment is applied to the VSR estimate to recover the accurate shape of the sample from the slight deformation induced by sub-voxel scan (step 4).

2.2. SVR reconstruction based on SLAM images.

The raw, over-sampled SLAM image sequence is first subdivided into a number of low-resolution (LR) image stacks by appropriate resampling rate (Fig. 1g, φ). Each segmented SLAM image stack presents standard resolutions simply accepted from the optics combination of SLAM (axial resolution) and inverted microscope (lateral resolution). Thus, each LR stack, considered as a regular SPIM result, contains sub-voxel-resolution, 3Dimensional shifts relative to all the other LR stacks. Then based on these LR measurements, a SVR computation procedure is designed to model their spatial correlation and iteratively estimate a final output with encompassing high resolution across a large volume^32 (Supplementary Fig. S4). In practice, the high-resolution (HR) solved via minimizing the following cost function,

\[
\hat{I} = \text{ArgMin}_{I} \left[ \sum_{k=1}^{N} \rho(I_k, D_k, O_k, S_k) + \lambda Y(I) \right]
\]

Here \(I_k\) is the Kth LR measurement, and \(D_k, O_k, S_k\) represent the down-sampling, blurring and sub-voxel shifting operations, which simulate the camera digitalization, optical imaging and off-z-axis scanning in the real light-sheet imaging process, respectively. The equation iteratively seeks a final HR solution, which has the maximum likelihood (evaluated by \(\rho\) and regularized by \(Y\)) to the LR measurements after the aforementioned degradation operations being successively applied to it (Fig. 1g, ⊙®). A voxel re-alignment is applied at the final step to recover an accurate reconstruction of the sample from the slight deformation induced by the off-z-axis scanning procedure (Fig. 1g, 4). Compared to the SLAM-raw image, which already shows significant quality improvement in contrast and axial resolution because of the introduction of plane illumination, SLAM-SVR procedure further increases the space-bandwidth-product (SBP) fundamentally limited by the system optics, rendering itself a superior tool for high-resolution mapping of large organisms.

2.3. SLAM-based LSFM imaging under different modes.

SLAM is developed to be adaptive to various samples when it works on a host microscope. Through tuning the width of an adjustable slit and truncating the incident Gaussian beam, the confocal range of the laser-sheet can vary from hundreds of microns to millimeters, for adopting samples from small embryos to mesoscale thick organs. The axial extent of the laser-sheet correspondingly varies from ~3 to 15 microns, indicating different axial resolution of the optical sectioning (Supplementary Fig. S3).

Using fluorescent beads as point source (~400 nm in diameter), we characterized the performance of four typical imaging modes, which were 4x-wide-field mode (4X/0.16 illumination and detection), 4x-SLAM mode (0.03 NA illumination + 4X/0.16 detection), 4x-SLAM-SVR mode, and 10x-SLAM mode (0.07 NA illumination + 10X/0.4 detection), on a SLAM-enabled conventional microscope. Fig. 2a-d show the x-y and x-z planes of resolved micro-bead using 4x-wide-field excitation, 4x-SLAM, 4x-SLAM-SVR, and 10x-SLAM, respectively. We also plotted the line intensity profiles of the resolved beads along transverse (x) and axial (z) directions (Fig. 2c, f). The full width at half-maximum values (FWHMs) were labeled (dash lines) to indicate the lateral and axial resolving powers of these methods. Compared to the wide-field mode, here
the addition of plane illumination from SLAM greatly enhances the axial resolution at ~5 times, shown as over 60 μm for 4x wide-field versus ~12 μm for 4x-SLAM. With the SVR computation applied, resolutions of 4x-SLAM-SVR are further improved from ~5 μm and 12 μm (4x-SLAM, raw) to ~2 μm and 5.5 μm, which are similar to the 10x-SLAM (~2 μm and 5 μm), whereas the imaging field-of-view (FOV) of 4x-SLAM-SVR is over 6 times larger than that of 10x-SLAM (Fig. 2g-i). Compared to regular SLAM imaging that has enhanced the conventional microscope with better axial resolution and higher contrast, SLAM-SVR goes further with the capability to image large organisms at cellular level, such as whole zebrafish embryo (Fig. 4), due to its increased space bandwidth product (FOV * resolution).

Figure 2. Characterization of SLAM imaging. A sub-resolution fluorescent bead (~500 nm) is resolved by: basic wide-field imaging (a, 4X/0.16 N.A. illumination/detection), low N.A. SLAM imaging (b, 0.03 N.A. illumination + 4X/0.16 detection), high N.A. SLAM imaging (c, 0.066 N.A. illumination + 10X/0.4 N.A. detection), and low N.A. SLAM imaging with VSR computation (d). The lateral and axial extents of the resolved bead, are shown in x-y (left) and x-z (right) plane images, respectively. (e, f) For each method, the Intensities of linecuts across the lateral (e) and axial extents (f) of the bead are plotted. 50% intensity levels (dashed lines) are shown to estimate the FWHM values, which indicate the achieved system resolutions. After the VSR reconstruction, the highest lateral and axial resolutions of low N.A. SLAM are improved to ~1.6 μm (compared to ~4.5 μm) and ~4 μm (compared to 10 μm), respectively, which are not only far beyond the original resolutions of wide-field microscope, but superior to SLAM with higher N.A. configuration too. (g-i) The comparisons of resolving power as well as uniform illumination area between low N.A. SLAM (raw), high N.A. SLAM (raw), and low N.A. SLAM (VSR). SLAM (VSR) shows the largest illumination field while achieves highest resolution at the same time.
2.4. High-speed, dual-color SLAM imaging of beating zebrafish embryo heart.

Imaging fast dynamics in fluorescent samples, such as cardiac muscle contraction and blood flow, often poses technical challenges to light microscopy. Wide-field method exhibits the ability of fast acquisition. The image quality, however, is limited by the excessive out-of-focus excitation. Confocal or two-photon excitation method requires laser scanning on each sample plane, hence the compromised speed being inadequate for freezing the fast motions. Through the combination of plane illumination and wide-field detection, LSFM is capable of capturing quick bio-dynamics with high frame rate as well as contrast\textsuperscript{26,28-31,33}. Here we demonstrate that, using dual-color SLAM imaging, a conventional microscope can be modified to investigate the fast cardiac hemodynamics inside a beating heart of zebrafish embryo.

Transgenic zebrafish embryos (\textit{cmlc2: GFP-Gata1: DsRed}) with myocardium and red blood cells specifically labelled were imaged on an inverted microscope (Olympus IX73) using x10 detection (Olympus UPLNAPO 10x/0.4 objective) and sCMOS camera (Hamamatsu ORCA Flash 4.0, V2). The consecutive frames of beating myocardium (\textit{cmlc: GFP}) and flowing red blood cells (RBCs, \textit{gata1: DsRed}) were simultaneously recorded using a beam splitter (Hamamatsu W-VIEW GEMINI) and merged afterward, to visualize the fast cardiac hemodynamics. The beating embryonic heart in one cardiac cycle was first imaged using built-in epi-illumination mode (10 ms exposure). The wild-field frames were consecutively recorded with 90 ms time interval (Fig. 3c). For the reasons discussed above, the images were obviously blurred to demarcate cardiac structures from excessive hazes. Then the same sample was observed under SLAM mode (laser-sheet 6 μm in thickness). The plane illumination significantly improved the contrast by confining the axial excitation and reducing the image contamination from the out-of-focus fluorescing. Two selectively illuminated planes of the beating heart were imaged at high spatial resolution as a function of time, revealing the dynamic changes in the endo, epicardial boundaries and blood pumping (Fig. 3a, b and Supplementary Fig. S6). SLAM model thus helps a conventional microscope to freeze the fast cardiac motions, enabling the clear identification of myocardium as well as the localization of flowing RBCs.

Follow-up studies on cardiac hemodynamics were also enabled as a result of the improved visualization. We first segmented the dynamic boundaries of the beating heart and use CFD analysis to compute its time-variant strain rate (Supplementary Fig. S6, Fig. S7). Fig. 3d shows the strain rate variation of beating ventricle, which is consistent with the periodic diastole and systole stages of the heat. Also, at a certain state, we estimated the inflow/outflow velocities of the heart based on the amount of pumped RSCs, and further calculated the transient pressures of each point at the inner surface (Fig. 3e, f). In addition, the continuous pressure change in one complete cardiac cycle was analyzed (Supplementary Fig. S7 and Video S1). It is noteworthy that, with the improved axial resolution, further reconstruction of the complete cardiac cycle from the 3D beating heart could be realized using either a high-speed camera or retrospective synchronization algorithm\textsuperscript{35}. 
Figure 3. Dual-color SLAM imaging of the beating zebrafish embryo heart unravels the fast cardiac hemodynamics. In (a) and (b), through the sharp optical sectioning by SLAM, high-contrast images from selective planes (z=70 μm and 100 μm) can be rapidly obtained at a high speed of 200 fps. The consecutive frames of beating myocardium (cmlc: GFP) and flowing red blood cells (RBCs, gata1: DsRed) are simultaneously recorded and merged afterward, to capture the fast cardiac hemodynamics. (c) Heart images taken by the same conventional microscope without SLAM combined. Compared to SLAM-LSFM imaging, epi-illumination mode suffers from excessive out-of-focus excitation which causes obvious photon contamination in the images. With clearly identifying the dynamic inner and outer boundaries of beating heart, the strain rate of myocardium throughout one cardiac cycle is plotted and corresponded to the shapes/stages of the heart (d). (e) The segmentation of beating heart based on SLAM images. (f) The pressure changes of different critical points at the heart boundary are plotted.

2.5. Voxel-super-resolved SLAM imaging of entire zebrafish embryo.

We demonstrate that, after SLAM combined, the development of a whole zebrafish embryo can be 3D imaged and quantitatively analyzed on a conventional microscope. A 72 h.p.f. zebrafish embryo (Tg Fli1: GFP) with endothelium labelled was imaged on Olympus IX73 inverted microscope, using 4x-SLAM-SVR mode described above. The reconstructed 3D blood vessels across the entire embryo encompassed 12 giga-voxels after SVR computation (Fig. 4a). The magnified views of the super-resolved sub-intestinal veins (SIVs) are three-dimensionally shown in Fig. 4b. Fig. 4c-e further compares the same structures imaged by 4x-SLAM-raw, 10x-SLAM and wide-field illumination, respectively. Compared to the wide-field result (Fig. 4e), 4x-SLAM imaging has shown much improved qualities (Fig. 4c), and 4x-SLAM-SVR (Fig. 4b) further breaks the SPB limit of raw 4x-SLAM, reconstructing a digital embryo with finer details visualized at global scale.
Different vessel structures spreading across the embryo, such as mesencephalic vein (Msv), central artery (CtA) and Intersegmental vein (IsV) etc., can be clearly identified, accurately segmented, and readily quantified (Fig. 4f).

Figure 4. 3D visualization and quantitative analysis of whole zebrafish embryo using SLAM. (a) A 72 h.p.f. zebrafish embryo (Tg Fltl: GFP) with labelled endothelium is imaged under SLAM (VSR) mode. The visualized blood vessels system of the whole embryo encompasses 12 giga-voxels after VSR computation. The resolved endothelium features of a sub-intestinal vein at the anterior part of the fish, are magnified and three-dimensionally shown in (b). (c-e) further compares the same structure imaged by low N.A. SLAM (raw), high N.A. SLAM and wide-field illumination, respectively. The voxel-super-resolved SLAM imaging renders a high-resolution digital embryo, which is unachievable by conventional microscope. Following the clear image reconstruction, different vessel structures across the embryo, such as mesencephalic vein (Msv), central artery (CtA) and Intersegmental vein (IsV), are all segmented, as shown in (f).

2.6. 3D rapid functional volumetric imaging of behaving C. elegans using SLAM.

Using SLAM-enabled conventional microscope, we further imaged the muscular Ca\textsuperscript{2+} activities of behaving C. elegans in five dimensions (3D space + time + spectrum). A transgenic C. elegans hps600 (L2 larva stage), in which muscles expressed calcium indicator GCaMP6 and wCherry as background fluorescence reference
(see Methods), was used for functional imaging. Employing an x20 wide-field detection (Olympus 20X/0.45 objective) plus 4 μm light-sheet sectioning configuration, continuous volumetric imaging of the entire larva was performed at a high speed of ~1.7 dual-color volumes per second (C. elegans larva were imaged in 1% agarose). The dynamic muscle activities from body bend to relaxation were readout by the fluctuation of GCaMP6 fluorescence signals. The imaging process lasted for at least 12 seconds with totally over 20 image volumes recorded (Fig. 5a). As shown in six consecutive volume renderings (Fig. 5b) and the magnified view at 8.4 seconds (Fig. 5c), the high spatiotemporal profile of SLAM imaging is capable of intoto visualizing the dynamic worm, with indicating the time-varying Ca\(^{2+}\) signals in three dimensions (Supplementary Fig. S8). Based on the complete 5-D visualization, the muscle bend correlated with the local Ca\(^{2+}\) signal variations, consistent with that muscle Ca\(^{2+}\) activities drive muscle contraction (Fig. 5d). Thus, with this compact and cost-effective SLAM device, the conventional microscope substantively gains the capability of rapid functional imaging of dynamic samples in three dimensions and thereby brings a broad range of applications to the next level.

![Figure 5. Dual-channel 3D imaging of muscular activities of behaving C. elegans larva.](image)

(a) An L2 stage transgenic C. elegans is five-dimensionally (3D space + time + spectrum) imaged using 3 μm laser-sheet illumination (achromatic cylindrical lens, f=20 mm) plus 20X/0.45 NA wide-field detection (Olympus PLANAPA 20X/0.45 objective). GFP/wCherry two-channel signals are obtained simultaneously by a single sCMOS camera (Hamamatsu, Flash 4.0 V3) in conjunction with an image splitter (Hamamatsu, W-view). The plane images are recorded under the sequential mode with a high acquisition rate of 200 frames per second. Under the aforementioned conditions, each worm is continuously recorded for 12 seconds, with finally 20 consecutive LSFM volumes being generated. (b) Time-lapsed volume rendering sequence (6 of 20) that intoto visualizes the behaving worm in real time. (c) Vignette high-resolution views of 6-second time point with x-y, x-z projections and volume rendering shown respectively. The imaging volume is 665.6×166.4×128 μm\(^3\) with voxel size being 0.325 × 0.325 × 1.5 μm\(^3\). (d) The correlation plots of the muscle bending curvatures (ventral...
and dorsal) versus the Ca²⁺ signal variations. Muscle contractions induced the body curvature changes have relatively high correlation coefficients with the fluctuation of the local Ca²⁺ signals.

2.7. Cellular activities in Amyotrophic Lateral Sclerosis syndrome model animals

To verify SLAM method is capable of assisting distinct investigations at physiological and pathological function levels, we compared the cellular activities in wildtype worms and Amyotrophic lateral sclerosis (ALS) diseased model animals in vivo. ALS is a fatal neurodegenerative disorder that primarily induced by progressively loss of motor neurons. Mutation of FUS501 protein (lacking the C-terminal 25 amino acids of FUS) was constructed based on its high similarity to several human C-terminal splicing/frame-shifting truncation mutations that associated with severe ALS. When FUS501 protein is ectopic expressed in C. elegans motor neuron, the animals exhibit strong ALS-like phenotypes, including obvious FUS cytoplasmic accumulation, motor neuron degeneration as well as eventual mobility reduction. Indeed, we observed that the thrashing frequency of FUS501-expressed C. elegans is significantly lower than that of wildtype animals in both young and aged animals (Supplementary Fig. S9), especially more obviously in aged worms. We then used SLAM to record ALS and wildtype C. elegans at 1st and 6th days post L4 larva, respectively, for ~14 seconds with total 37 LSFM volumes obtained at a high rate of 0.38 second per volume (Supplementary Video S2). Compared to wildtype animals, ALS animals exhibited obvious muscle activity defects along with the development from day 1 to day 6 (Fig. 6a). Quantitatively, for each group, the averaged volumetric Ca²⁺ intensity of all the 10 worms at 37 time points were drawn into a 370 pixel color map, to indicate the overall muscular activities (Fig. 6b). Ca²⁺ intensity histogram of each group was plotted with Gaussian fitting (Fig. 6c). It is apparent that the muscular activities curves from ALS animals were left shift compared to those of the wildtype animals. Finally, averaged Ca²⁺ intensity showed significant reduction in ALS groups compare to wildtype groups in both day 1 and day 6 adult animals (Fig. 6d). These quantitative analyses were consistent with the thrashing frequency defect in ALS (Supplementary Fig. S9), and confirmed the observation on ALS-induced progressive loss of neuronal activities during aging. Therefore, this experiment demonstrated that SLAM-enabled microscope could completely follow the in-vivo cellular activities under diverse conditions, thereby showing its great potentials for a wide range of applications.
Figure 6. Quantitative study of the Amyotrophic lateral sclerosis (ALS) syndrome at different life stages of C. elegans. (a) Serial SLAM volumetric reconstructions of wildtype and ALS C. elegans adults at day 1 and day 6, respectively. The blinking of GCaMP6 fluorescence signals are acquired at ~3 volumes per second, indicating the dynamics of muscle contractions in the acting worms. The total 37 volumes continuously captured in 14 seconds with ~0.38 second interval are shown in Supplementary Fig. S8. (b) The fluorescence intensity color maps completely illustrate the dynamic changes of each wildtype and ALS animals at different days (n = 10 for each group). (c) Histograms of all volume fluorescence intensity in wildtype and ALS. The Gaussian fitting curves are applied to show the approximate intensity distributions. At day 6, the shift of the Gaussian-fitting curve is larger than that of at day 1. (d) The averaged volume fluorescence intensity of wildtype and ALS animals in day 1 and day 6, respectively. Scale bars, 300 μm.
3. Methods

3.1. Sample manipulation for SLAM experiment.

Live samples, such as trans-genetic zebrafish embryo and *C. elegans*, were mounted on a thin, transparent FEP slide using agarose solution (low melt-point, 0.5% to 1%). This step can be as easy as standard sample operation on glass coverSLAM. Then the prepared sample slide was clamped on the wedge-shaped adapter, thereafter being sucked to the holder by magnetism. Since FEP material has a refractive index very similar to water (~1.34), it minimizes the light deflection at solid-liquid interface as the sample slide being immersed into the cuvette filled with phosphate buffered solution (PBS) \(^1\). For clarified brain tissue, the sample was embedded into transparent resin for better preserving fluorescence of the sample and reducing the photobleaching. Then the brain was packed onto a FEP board and stabilized into a glass chamber, which was filled with refractive index-matched solvent (benzyl alcohol + benzyl benzoate + diphenyl ether, BABB-D).

3.2. Off-z-axis scanning for SVR reconstruction.

Unlike conventional stepwise z-scan applied in 3D microscopy, in SLAM-SVR mode, the samples were continuously moved along a vector with 78-degree angles with respect to the x and y directions of the camera pixel grid. Also based off the Nyquist sampling that is often used, SLAM scanned the samples through the laser-sheet with a sampling step-size significantly smaller than the light-sheet thickness, and hence created nanoscale shift components simultaneously in both lateral and axial directions through a simple 1-D motion. The unconventional off-z-axis scanning mode in conjunction with ultra-small step size encrypted subtle lateral and axial shifts into the raw SLAM image stack. As a reference point, for SLAM-SVR imaging of the zebrafish whole embryo, the sample was scanned through the laser-sheet with a 133 nm step-size, resulting an incremental lateral and axial shift of ~17 nm and 79 nm. A low magnification detection objective (4X/0.16, Olympus) was used to cover the large FOV of the large samples, such as zebrafish embryo and brain block, and a sCMOS camera was used to record the images at high speed up to 100 fps. The data acquisition time of sub-voxel scanning was only 85 seconds for zebrafish embryo (4250 frames in total). Then the recorded plane images were transferred from the camera to a RAID 0 array of two solid state drives (SSDs). Finally, 48 groups of low-resolution, 3D images were extracted from the raw image stack to compute the SVR image.

Taking the incomplete camera sampling into account, the effective lateral and axial resolution of each LR fish image are limited, being ~4 μm and 12 μm, respectively, with yielding ~300 megavoxels SBP over a ~3 mm\(^3\) volume. After modeling the LR image sets with known shifts, optical aberration and digital decimation, the SVR image encompassing 12 gigavoxels could be obtained by iteratively seeking a high-resolution solution which has maximum likelihood to the sum of the LR measurements after serial degradations being applied\(^34\). It is noteworthy that our GPU-based SVR computation procedure is fast, with achieving over 2-orders acceleration when compared to traditional CPU-based computation. With excavating the capacity of two NVidia GTX 1080ti graphical cards, the processing time was dramatically reduced to around 1 minutes. The speed can be easily improved by using more GPUs.

3.3. Dual-color SLAM imaging of live dynamics.

By using a high-speed actuator, we scanned the behaving *C. elegans* through a dual-color laser-sheet (488
and 590 nm) back and forth rapidly (0.3 mm/s). The sample was optically sectioned with a step-size of 1.5 μm and three-dimensionally imaged at both its forward and backward states with a short exposure of 5 ms and maximum acquisition rate of ~200 frames per second. We used an image splitter (Hamamatsu W-VIEW GEMINI, A82101-01) to simultaneously image dual-channel fluorescent signals onto a single sCMOS camera (Hamamatsu Flash 4.0 V3). After the mixed fluorescence going through the splitter, the GFP signals were projected onto the upper half of the sensor while the wCherry signals were directed to the lower half (Supplementary Fig. S2). Therefore, we were able to obtain dual-channel images with one capture. The consecutively acquired time-lapsed, dual-color image volumes (“665.6 μm × 166.4 μm × 128 μm” in size) can be furthermore translated into five-dimensional visualization via reversing the images captured at backward state and merging the two channels.

3.4. Zebrafish embryo culture.

Adult Tg (fli1a: EGFP) and Tg (cmlc2: GFP-Gata1: DsRed) transgenic zebrafish are raised in the zebrafish core facility of Huazhong University of Science and Technology (HUST). All the experiments were performed in compliance with the approval of GLA Institutional Animal Care and Use Committee (IACUC) protocol (Zebrafish IACUC Protocol number: 101004-14). The zebrafish maintained with filtered fresh water under 14 hours of incandescent light and 10 hours of dark conditions. In Tg(fli1a:EGFP) fish, the fli1a promoter-driven enhanced green fluorescent protein (EGFP) was expressed predominantly in vascular endothelial and endocardial cells. In Tg (cmlc2:GFP) fish, the cmlc2 promoter-driven GFP was expressed exclusively in myocardia cells. The embryos incubated in 31°C with petri dish. To maintain transparency of zebrafish embryos, embryos were incubated with egg water containing 0.2 mM 1-phenyl-2-thio-urea (Sigma) to suppress pigmentation at 24 hpf. The live fish embryos are anaesthetized with 0.04mg/ml of tricaine (MS-222, Sigma) before they are mounted to the slide for sustained imaging.

3.5. C. elegans preparation.

All strains were maintained at 22°C on NGM plates with OP50 E. coli as food. The strains used in this study were: ZM5848: hpIs237 [psu006::GFp-fus(del501)], ZM9140: hpIs600 [Pmyo-3::GCaMP6::wCherry], SGA45: hpIs237; hpIs600. In thrashing experiments, animals cultured 16h post L4 larva were scored as Day 1 adults. Individual animals were transferred into 5 μl of M9 buffer on a glass slide. Thirty seconds after the transfer, the frequency of the body bending was counted for 1 min. A single thrashing was defined as a complete sinusoidal movement through the head and tail. The same protocol was used to assess the motor function in Day 6 adults.

4. Conclusion

While conventional epi-fluorescence microscopy is currently still the backbone of many research purposes despite its inadequate axial performance and inefficiencies in 3D imaging, the emerging light-sheet fluorescent microscopy is apparently an attractive tool that provides advanced visualization and greatly reduced photo-toxicity to samples. Though constructing a benchtop LSFM system may not be feasible for resource-limited environments, an extra light-sheet illumination part in conjunction with conventional
microscopes could be a good alternative, given the fact that LSFM is also employing wide-field detection. The development of SLAM is motivated by these similarities between epifluorescence microscopes and LSFM. Some of the pioneering research, such as OpenSPIM had developed step-by-step guides on how to build LSFM systems\textsuperscript{25,35,42}. These user-friendly instructions aim for the spread of advanced light sheet imaging in a way of standardized design, moderate cost, and easy operation. SLAM shares the similar spirit of these methods, while it goes further on simplifying the design and reducing a significant amount of cost via fully utilizing the conventional microscopes (Supplementary Fig. S2). A simple LSFM add-on will still have the fundamental resolution limit after it brings the host microscope to a level close to a benchtop SPIM system (Fig. 1b, comparison see Supplementary Fig. S3). SLAM furthermore contains sub-voxel-scanning mode that can accomplish three-dimensional resolution enhancement after a probability-based SVR computation applied to the raw shift-modulated images. With handling the generated sub-voxel spatial shifts, the SVR procedure estimates the super-resolved 3D output that best suits a certain conditional probability in spatial domain, and computationally increases the SBP which is originally clamped by the physical limitations of system optics. It is thereby capable of further providing resolution and throughput increase for raw SLAM image, without the need of mechanical stitching or patterned excitation. In addition to the contrast and axial resolution enhancement immediately brought by sharp plane illumination, the SVR function based on sub-voxel-scanning mode further isotropically improves the numerical aperture of raw light-sheet image over 2 folds, resulting a multiplicative resolution enhancement over 10 times to original epifluorescence images.

It is noteworthy that without the incomplete sampling or shifting, the SVR method would have the same limits as deconvolution. Therefore, the algorithm is better suited to fluorescent imaging of large specimens, for which a large field-of-view is required and the recorded digital images are generally under-sampled. Furthermore, the continuous sub-voxel scanning process in our SLAM system is very simple and efficient. It works under a high acquisition rate up to hundreds of frames per second, and can be easily achieved through a small retrofit of actuator’s orientation. In hybrid, SLAM-SVR together offers a simple and cost-effective strategy, which remarkably extend the imaging capability of the existing wide-field microscopes. The dramatically improved spatial-temporal performances of SLAM-enabled conventional microscope are further verified by imaging beating zebrafish heart, behaving \textit{C. elegans}, and whole zebrafish larva. These results are compared to those of epifluorescence and benchtop light-sheet fluorescence microscopes, and can be further quantitatively studied as a result of superior visualization. As a reference point, in the SLAM imaging of live \textit{C. elegans}, we have carefully explored the potential of our device for \textit{in-vivo} animals study, including the measurements of transient Ca$^{2+}$ signals and spatiotemporal analyses on the behaviors of different worm models. Rapid 5-D functional imaging of acting wildtype and ALS model \textit{C. elegans} were successfully achieved, showing their muscular activity changes versus the disease progression being quantified.

In summary, the ability to achieve high-speed, multi-dimensional imaging on various specimens with scalable space-bandwidth product on a wide-field microscopy renders SLAM a valuable tool for many biomedical applications such as embryo development, tissue pathology and neuroscience. Furthermore, its compact add-on format allows the full use of pre-owned equipment and renovation at an affordable expense, which could substantially benefit smaller and less-funded departments / laboratories for not limiting the access to greater biological research in the absence of commercial light-sheet systems. Furthermore, we
believe SLAM could be more transformative in future, as its open design provides a general platform that can be potentially added with other useful modules, e.g. dual-side illumination and multi-view functions, per the evolving biological demands.

**Funding**

National Key R&D program of China (2017YFA0700500), Research Program of Shenzhen (JCYJ20160429182424047), the National Natural Science Foundation of China (21874052, 31671052), the National Science Foundation of Hubei (2018CFA039), Wuhan Morning Light Plan of Youth Science and Technology (2017050304010295) and the Junior Thousand Talents Program of China (Peng Fei and Shangbang Gao).

**Acknowledgements**

We thank Haiwen Li for technical assistance. We thank Yanyi Huang for discussions and comments on the manuscript, Dan Zhu and Tingting Yu for the discussion on the imaging.

**Disclosures**

The authors declare that there are no conflicts of interest related to this article.

**See Supplement 1 for supporting content**

**References**


