High-contrast, synchronous volumetric imaging with selective volume illumination microscopy

Thai V. Truong, Daniel B. Holland, Sara Madaan, Andrey Andreev, Kevin Keomanee-Dizon, Josh V. Troll, Daniel E.S. Koo, Margaret J. McFall-Ngai & Scott E. Fraser

Light-field fluorescence microscopy uniquely provides fast, synchronous volumetric imaging by capturing an extended volume in one snapshot, but often suffers from low contrast due to the background signal generated by its wide-field illumination strategy. We implemented light-field-based selective volume illumination microscopy (SVIM), where illumination is confined to only the volume of interest, removing the background generated from the extraneous sample volume, and dramatically enhancing the image contrast. We demonstrate the capabilities of SVIM by capturing cellular-resolution 3D movies of flowing bacteria in seawater as they colonize their squid symbiotic partner, as well as of the beating heart and brain-wide neural activity in larval zebrafish. These applications demonstrate the breadth of imaging applications that we envision SVIM will enable, in capturing tissue-scale 3D dynamic biological systems at single-cell resolution, fast volumetric rates, and high contrast to reveal the underlying biology.
Understanding dynamic biological processes requires volumetric imaging tools that can faithfully image across hundreds of microns in three dimensions (3D) with cellular resolution within time scales as short as milliseconds. Conventional imaging approaches based on sequentially collecting signal from one point (confocal microscopy), one line (line-confocal), or one plane (light-sheet) at a time\textsuperscript{12} are often not fast enough to faithfully capture the relevant dynamics without distortion, as different parts of the 3D sample are observed at different times. Light-field microscopy (LFM; Fig. 1a) meets this challenge by capturing an extended sample volume in a single snapshot, enabling synchronous volumetric imaging\textsuperscript{3-5}. LFM records the extended light field coming from the sample space on a two-dimensional (2D) camera by positioning a micro-lens array at the image plane, and moving the camera to the focal plane of the micro-lens array. This permits the camera to capture information from the volume that extends above and below the native focal plane. Computational reconstruction is used to solve the inverse problem, reconstructing the image of the 3D sample from the recorded 2D image, sacrificing resolution for dramatically enhanced z-depth coverage\textsuperscript{3-5}. LFM conventionally employs wide-field illumination, exciting sample regions beyond the volume of interest (Fig. 1b), thus generating background signal that reduces the contrast of both the recorded 2D image and the final reconstruction. The limited contrast of conventional LFM has substantially limited its utility for imaging dynamic 3D biological tissues.

Taking inspiration from selective plane illumination microscopy (SPIM; also known as light-sheet microscopy)\textsuperscript{6}, which achieves low-background and high-contrast imaging by illuminating only the optical plane of interest (Fig. 1b), we reasoned that we could enhance the contrast of LFM by illuminating only the volume of interest. We thus created selective volume illumination microscopy (SVIM) by preferentially illuminating the volume of interest and then capturing the resulting fluorescence with light-field detection. SVIM reduces background, increases contrast, and produces an overall higher-quality reconstruction of the sample, while preserving the synchronous volumetric imaging capability of LFM.

**Results**

**Overview of SVIM instrument.** Our SVIM instrument combined selective volume illumination and LFM modules with an existing custom-built SPIM\textsuperscript{7}, permitting direct comparison between SVIM, conventional wide-field LFM, and SPIM imaging of the same specimen (Methods section, Supplementary Fig. 1, Supplementary Table 1). SPIM provided slower, but higher-resolution, “ground truth” images against which to judge the other imaging modalities. To achieve selective volume illumination, in either 1-photon (1p) or 2-photon (2p) excitation, we implemented galvanometer-based rapid scanning of the specified volume multiple times within a single camera exposure time\textsuperscript{8,9}, providing micron-level control over the spatial extent of the selected volume (Methods section, Supplementary Note 1). Our design of the light-field detection module drew upon previous efforts\textsuperscript{3,4}, and the light-field image reconstruction followed the 3D deconvolution approach\textsuperscript{4} using publicly available software\textsuperscript{5}. Supplementary Table 2 provides imaging and reconstruction parameters for all presented results. As previously described theoretically and experimentally\textsuperscript{3,4}, LFM image reconstructions are affected by non-uniform resolution and grid-like artifacts centered around the native focal plane, both of which were present in our results. SVIM performed as expected from the optical parameters used\textsuperscript{4}, achieving a nominal maximum resolution of ~3 μm laterally and ~6 μm axially, as approximated by the full-width half-maximum (FWHM) of sub-diffraction fluorescent beads, over a volume of 440 × 440 × 100 (x, y, z) μm\textsuperscript{3} (Supplementary Fig. 2). Our SVIM implementation provides a simple path for conventional SPIM instruments to be upgraded to SVIM.

![Fig. 1 Selective volume illumination microscopy enhances LFM for the synchronous imaging of 3D samples.](https://example.com/fig1.png)

**Fig. 1 Selective volume illumination microscopy enhances LFM for the synchronous imaging of 3D samples.** a LFM is a simple extension of a conventional microscope, which produces a magnified image of the sample (S) from the native focal plane (F) to the image plane (IP) using an objective lens (OL) and tube lens (TL). LFM places a micro-lens array (LA) at the IP, encoding 3D image information into a 2D light-field image (LF), which is captured by a planar detection camera. This permits LFM to synchronously capture information at z-positions above and below F; the 3D image of the sample is reconstructed from the LF image, based on knowledge of the optical transformation. b SVIM improves LFM by selectively illuminating the volume of interest within the sample. This decreases background and increases contrast when compared to wide-field illumination of the entire sample. SVIM was implemented through the use of light-sheet (SPIM) illumination that is scanned axially, so that the thin sheet of excitation is extended into a slab. In our work, the SVIM illumination axis was orthogonal to the detection axis (θ = 90°), but the benefits of reduced background can be obtained by using illumination from a different angle, and/or by employing non-linear optical effects to selectively excite the volume of interest. c SPIM and SVIM 3D images of the trunk vasculature of 5 dpf zebrafish larva reveal the compromises between resolution and volumetric imaging time. SPIM offers higher resolution but requires the collection of 100 sequential images to cover the 100-μm-depth z-stack; SVIM captures the same 3D volume in a single snapshot, two-orders-of-magnitude faster, but with lower resolution. Transgenic animal, Tg(kdrl:GFP), had its vasculature fluorescently labeled with green fluorescent protein (GFP). Inset shows the approximate location of the imaged volume along the trunk of the zebrafish larva. Scale bars, 50 μm.
SVIM imaging performance. The capabilities and compromises of SVIM are demonstrated by its single-snapshot capture of the entire depth of the trunk vasculature of a live larval zebrafish (Fig. 1c). Compared to the z-stack assembled from 100 higher-resolution SPIM snapshots, SVIM captured faithfully the 3D structure of the green fluorescent protein (GFP)-labeled vasculature. SVIM demonstrated modest reductions in resolution, but its single-snapshot acquisition offered two-orders-of-magnitude greater z-depth coverage and enhanced imaging speed, even after normalization for the number of resolvable voxels captured (Supplementary Table 2, Supplementary Note 2).

SVIM enhances image quality compared with wide-field LFM, as seen in the 3D images of the cranial vasculature of the same live zebrafish larva, with the illuminated volumes varying from 100-μm axial extent to wide-field illumination of the entire animal (Fig. 2a, Supplementary Fig. 3). Wide-field LFM produced the lowest-quality image with the highest background. SVIM produced progressively better-quality and higher-contrast images as the z-extent was reduced, approaching the ground truth images achieved by SPIM when the SVI was 100 μm (Supplementary Fig. 3g). Measurements of the quantitative image contrast (Methods section) show SVIM’s progressively increased performance as the illumination extent was confined to smaller volumes (Fig. 2b). The decreased contrast from background is consistent with our simulations (Supplementary Fig. 4), where increased levels of Poisson noise applied to the raw light-field images resulted in decreased contrast in the reconstructions. The higher contrast of SVIM mitigates against resolution-degrading effects of background noise, resulting in a better effective resolution even though SVIM and wide-field LFM utilize the same detection optics. This was demonstrated in imaging ~5-μm-diameter blood vessels where SVIM achieved up to 35% improved FWHM over wide-field LFM (Supplementary Fig. 5).

SVIM enhances imaging of biological components moving in 3D. The synchronous volumetric imaging capability and enhanced contrast of SVIM is ideal for imaging dynamic systems, where components undergo fast motion in 3D space. We employed SVIM to image the bacterial flows in seawater, surrounding the light organ of a Hawaiian bobtail squid, *Euprymna scolopes*, while it was selectively colonized by the bacteria *Vibrio fischeri*. The squid–bacteria symbiosis is an important model for understanding the effects of fluid flow during interactions between bacteria and epithelial surfaces. Previous 2D measurements of the bacterial flow field inadequately captured the 3D flows around the light organ. SVIM offered dramatically better image quality as compared to wide-field LFM (Fig. 3a, b). SVIM removed most of the background that severely compromised wide-field LFM, which came from the excitation of nearby auto-fluorescent tissues. In SVIM, the fluorescence of individual bacteria could be clearly imaged and tracked (Fig. 2b, Supplementary Movies 1, 2 and 3), yielding the position (Fig. 3c) and speed (Supplementary Fig. 3d) of the bacterial components. This demonstrates the possibility of monitoring the bacteria and epithelial surfaces simultaneously within the light organ with SVIM.
SVIM enhances brain-wide functional neuroimaging. Many recent developments of LFM have focused on functional neuroimaging\textsuperscript{5,19–24}, despite the challenges presented by the relatively slow image reconstruction algorithms, and the reduced image quality of LFM compared with state-of-the-art neuroimaging techniques\textsuperscript{2}. This is because the extraordinary imaging rate possible with LFM could be game changing for the simultaneous recording of large number of spatially distributed neurons. We tested if the enhanced contrast of SVIM would improve the recording of neural activity in larval zebrafish, as assayed through a genetically encoded calcium indicator expressed in all of its neurons (Fig. 4, Supplementary Figs. 8 and 9). The enhanced contrast from the reduced background of SVIM, in both 1p and 2p excitation modes (Supplementary Fig. 8), enabled better performance than wide-field LFM in recording the calcium transients that reflect the firing of single neurons across the zebrafish brain, capturing up to fourfold more neurons during spontaneous brain activity (Methods section; Fig. 4d–f). While both 1p and 2p excitation SVIM offered improved contrast, they present different compromises. Excitation with 1p offers simplicity and fast volumetric imaging rates, as demonstrated with LFM in general\textsuperscript{5,19–24}. However, 2p excitation can offer higher image quality, if the fluorophores are not photobleaching, as demonstrated with LFM in general\textsuperscript{5,19–24}.
The lower 2p excitation cross section limits the imaging rate of 2p-SVIM; however, we found that 2p excitation led to better contrast and a larger number of resolved active neurons (Fig. 4e, f, Supplementary Fig. 8). This detection of more active neurons, enabled by the improved contrast, results not only from the reduced tissue autofluorescence of 2p excitation, but also from additional mechanisms specific to our application. First, 2p laser light preserves its spatial profile better than 1p light as it penetrates deeper into the sample, due to the reduced scattering at longer wavelengths. Thus, the scanned selectively illuminated volume is more precisely defined spatially with 2p, leading to less extraneous background coming from outside of the illuminated region. Furthermore, the invisible near-infrared light used for 2p excitation avoids the visual responses triggered in the zebrafish by the visible 1p excitation25, eliminating the “always-on” visually activated neurons that would otherwise yield background fluorescence that suppresses the detection of neurons undergoing spontaneous activity. This last point suggests that 2p-SVIM is best suited for studies of visually sensitive behaviors, such as brain-wide responses to visual stimuli (Supplementary Fig. 9) or sleep26.

Discussion

The results presented here demonstrate that, by combining the strengths of SPIM and LFM, SVIM provides a powerful tool for high-contrast, synchronous volumetric imaging of dynamic systems. By optimizing the illumination pathway, SVIM offers single-cell resolution, with improved contrast over wide-field LFM. The SVI principle was implemented in a recent work27, where the sample was illuminated with a beam having a large cross-sectional area that filled up the volume of interest. This volume-filling strategy, compared with our volume-scanning strategy, is simpler to implement but gives up spatial precision in defining the volume of interest (Supplementary Note 1). SVIM is compatible and synergistic with recent innovations in LFM that optimize the detection pathway for more spatially uniform resolution and reduced grid-like artifacts in the reconstructions. These include implementation of multi-view light-field detection27, and methods that capture and process light-field information through phase masks28, diffusers29, or in the Fourier domain30. SVIM could also be further optimized by approaches that speed up the image reconstruction or information extraction pipeline. Together, these latest refinements of LFM and the high contrast of SVIM may enable LFM-based techniques to become the next-generation tools for imaging tissue-scale 3D dynamic biological systems. LFM-based methods belong to an emerging class of diverse computational imaging techniques31,32 that harness the power of physical modeling, signal processing, and computation to enable new performance spaces beyond conventional microscopy. The selective volume illumination strategy of limiting the illuminated sample volume, in improving the contrast of the acquired image data, is positioned to play a key role in optimizing a variety of computational imaging approaches for a wide range of biological applications.

Methods

Microscopy setup and implementation. The optical setup was based on an existing SPIM apparatus, with modifications to provide the selective volume illumination and light-field detection (Supplementary Fig. 1, Supplementary Table 2). Briefly, collimated beams from 1p excitation continuous wave and 2p excitation femtosecond-pulsed lasers were combined and directed at the sample through a pair of galvanometer scanners and scanning optics. The fluorescence signal was collected in the direction orthogonal to the illumination axis, through appropriate spectral optical filters, and directed to a detection module that allowed...
imaging in either SPIM or SVIM mode. For SVIM mode, a micro-lens array was placed at the conventional image plane to capture the light field coming from the sample. The data was subsequently recorded in 2D and reconstructed on a computer-controlled motorized stage to allow reproducible switching between SPIM and SVIM modes. To provide selective volume illumination, the galvos controlling the illumination light were adjusted to paint out the desired illuminated volume multiple times within a single camera exposure. Image acquisition was through Manager® and ImageJ (National Instruments). See Supplementary Fig. 1 and its caption for more detailed descriptions, and Supplementary Table 1 for a list of key components of the microscopy setup. Supplementary Note 1 provides further discussion on the advantages and disadvantages of our volume-scanning implementation of SVIM.

Sample handling and imaging procedure. Zebrafish experiments: Fish were raised and maintained as described in ref. [34], in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals by University of Southern California, where the protocol was approved by the Institutional Animal Care and Use Committee (IACUC). All zebrafish lines used are available from ZIRC (zebrafish.org). Zebrafish embryos were collected from mating of appropriate adult fish (AB/Tl strain) and raised in egg water (60 µg L⁻¹ of stock salts in distilled water) at 28.5 °C. At 20 hpf, 1-fluores-2-thioare (30 mL L⁻¹) was added to the egg water to reduce pigmentation in the animals. For imaging experiments, the samples were embedded in a 1-mm-diameter cylinder of 1.5% low-melting agarose (Sea Plaque) for imaging in the SPIM/SVIM setup, as described in ref. [3]. The imaging chamber was filled with 30% Danieau solution (1740 mM NaCl, 21 mM KCl, 12 mM MgSO₄·7H₂O, 18 mM Ca(NO₃)₂, and 150 mM HEPES, pH 7.5). Anesthetic was used (buffered Tricaine, 5 mM, Sigma) to control the immobilization of the animals during mounting and imaging. For the imaging procedures, except for the case of fluorescent calcium indicators, the samples were scanned by way (pitch = 150 µm, focal length = 3 mm) for two imaging conditions of (i) 32x magnification, 0.8 NA, and (ii) 20x magnification, 0.5 NA. With these parameters, and the reconstruction parameters listed in Supplementary Table 2, following we expect the 32x magnification reconstructions to have ~3 (and 6 µm) resolution laterally (and axially), and the 20x magnification reconstructions to have ~4 (and 12 µm) laterally (and axially).

Image analysis and presentation. Raw images were background subtracted to account for camera dark counts, for both SPIM (done manually) and SVIM (done automatically in the software LFDDisplay). All SPIM and SVIM images were scaled to fill the full 16-bit dynamic range. For visualization in the figures, unless otherwise noted, image pixel intensities were further scaled to minimum and maximum display contrast with 0.4% saturation. Unless otherwise noted, 3D images are presented as 2D averaged-intensity, instead of maximum-intensity, projections as this method provides a more accurate way to represent the background of 3D data in 2D format. Image processing and analysis in 2D were done in Fiji [35], while 3D rendering and analysis were done in Imaris (Bitplane). Zebrasfish vasculature: For zebrasfish vasculature images (Fig. 1d, Supplementary Figs. 3–5), the 3D datasets were displayed as an averaged projection in z, for the same volume section extending from z = 48 to −12 µm, where z = 0 µm is the native focal plane of the detection objective. This volume excludes the native focal plane of the imaged z-stack, where grid-like artifacts from the light-field reconstruction are most prominent. The native focal plane was experimentally set at ~200 µm into the zebrasfish head from its dorsal surface for the datasets shown in Fig. 1d and Supplementary Figs. 3–5. Bacteria–squid: For squids-bacterial results (Fig. 2a–c, Supplementary Fig. 6, Supplementary Movies 1–3), tracking and quantification of the bacterial flow field were carried out using the automatic spot segmentation and tracking functions in Imaris, followed by manual correction. Zebrasfish heart blood: For results describing the zebrasfish beating heart (Fig. 2d–g, Supplementary Fig. 7, Supplementary Movies 4–7), the sequentially acquired light-field time-series data from the heart blood were reconstructed separately. The reconstructed four-dimensional (4D) datasets, each spanning approximately four heart beats, were then synchronized in time by renumbering the endocardium frames such that the time point at which the atrium is most contracted in the endocardium movie matches the time point when, in the blood flow movie, the flow into the ventricle from the atrium momentarily stops. After synchronization, the two movies were overlaid to create a composite two-color movie. Tracking and quantification of 12 representative blood cells’ flow trajectories in the zebrasfish heart were carried out manually in Imaris. We analyzed and presented the flow trajectories as they were directly derived from the manual tracking, to mainly demonstrate the benefits of SVIM. Follow-up work that aims to draw biological and biophysical insights from the images beating heart and blood flow should take into account the non-uniform resolution and image artifacts inherent with LFM in general. Zebrasfish brain activity: For the zebrafish brain activity results (Fig. 2h–m, Supplementary Figs. 8 and 9), we used an analysis pipeline based on segmentation of the time-domain standard deviation of the 3D time-series data to find active neurons [36]. First, from the light-field reconstructed 3D time-series data, we calculated the standard-deviation-projection along the temporal axis. The resulting time-projected 3D dataset was a spatial map of where the signal intensity changed substantially during the time window, due to neuronal activity. Spot segmentation was then carried out on the time-projected 3D dataset using Imaris to find active neurons, with the constraint that neuronal nuclei appeared as ellipsoids with diameter of 5 and 10 µm in the lateral and axial direction, respectively, following the expected resolution of the light-field imaging and analysis parameters. Once found, spots were extracted from the analyses. Simultaneously, a Gaussian convolution was used functionally to extract the A/Fₐₙ activity traces from the original reconstructed 3D time-series data. Spontaneous activity time window was from t = 1 to 100 s (Fig. 2h–m, Supplementary Fig. 8), while the visually evoked activity time window was from t = 51 to 150 s with the LED evoking light turned on at t = 100 s (Supplementary Fig. 9). For the latter case, k-means clustering of the activity traces was used to group the active neurons, identifying a group that exhibited clear responses to the evoking light. Analysis was carried out using a combination of Fiji, Imaris, and MATLAB (MathWorks).

Image contrast, simulated noise, and effective resolution. Contrast: To compare the image contrast between SPIM, SVIM, and wide-field LFM, we measured the root mean square deviation of the pixel intensities from the respective images. The standard deviation σ of an image, which is the same quantity as root mean square contrast that appears in vision science [38,40], is given by

$$\sigma = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N}(x_i - \bar{x})^2},$$

in which $x_i$ is a normalized intensity-level value, so that 0 ≤ $x_i$ ≤ 65,535 and $\bar{x}$ is the
The average intensity of all pixel values $N$ in the image:

$$x = \frac{1}{N} \sum_{i=1}^{N} x_i.$$  

(2)

Putting the expressions above together, we have

$$Contrast = \frac{\sigma_x}{\bar{x}}.$$  

(3)

This measure of image contrast is independent of the total pixel count $N$, and thus provides a concise metric for direct comparison of contrast between SPIM, SVIM with various SVI extents, and wide-field LFM. For the vasculature results (Fig. 1e, Supplementary Fig. 4f), we calculated the contrast for individual $z$-slices of the static 3D reconstructions to provide a $z$-depth-dependent comparison between different imaging modalities. To match the intrinsically lower axial resolution of SVIM and LFM ($\sim 12 \mu m$), a moving average over 12 successive $z$-planes was applied to calculate the SPIM contrast curve. For the beating heart results (Supplementary Fig. 7c, f), the 4D reconstructions involved dynamic motion of the vasculature, thus we have

$$\frac{m_{SVIM} \text{ light-slices}}{m_{SPIM} \text{ light-slices}} = \frac{\text{spatially correlated with the original raw 100-\mu m light-microscopy}}{\text{raw light-microscopy}}.$$  

(4)

Effective resolution: To evaluate the effective resolution achieved with the various imaging modalities, we quantified the FWHM diameter of the same blood vessels captured by each modality (Supplementary Fig. 5, see its caption for full details). Briefly, starting in the SPIM 3D dataset, we selected a single $z$-slice that had four well-imaged blood vessels of approximately the same size. Matched $z$-slices in the SVIM and LFM datasets were found, and a MATLAB script generated line regions-of-interests across the selected blood vessels. Intensity line profiles were produced, normalized to a peak value of 1, from which the mean FWHM was calculated.

Statistics and reproducibility. No statistical tests were conducted.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Data underlying the plots in Figs. 2b and 4d-f are available as Excel files in Supplementary Data. All other relevant data are available from the authors upon request. Please send request to Thai Truong, tvtruong@usc.edu.

Code availability

All relevant custom-written LabVIEW and MATLAB scripts/codes are available from the authors upon request. Please send request to Thai Truong, tvtruong@usc.edu.

Received: 19 September 2019; Accepted: 15 January 2020; Published online: 14 February 2020

References

1. Pawley, J. B. Handbook Of Biological Confocal Microscopy. (Springer-Verlag, US, 2006).
2. Yang, W. & Yuste, R. In vivo imaging of neural activity. Nat. Methods 14, 349–359 (2017).
3. Levoy, M., Ng, R., Adams, A., Footer, M. & Horwitz, M. Light field microscopy. ACM Trans. Graph. TOG 25, 924–934 (2006).
4. Broxton, M. et al Wave optics theory and 3-D deconvolution for the light field microscope. Opt. Express 21, 25418 (2013).
5. Prevedel, R. et al Simultaneous whole-animal 3D imaging of neuronal activity using light-field microscopy. Nat. Methods 11, 727–730 (2014).
6. Huisken, J. Optical sectioning deep inside live embryos by selective plane illumination microscopy. Science 305, 1007–1009 (2004).
7. Trivedi, V. et al Dynamic structure and protein expression of the live embryonic heart captured by 2-photon light sheet microscopy and retrospective registration. Biomed. Opt. Express 6, 2056–2066 (2015).
8. Keller, P. J., Schmidt, A. D., Wittbrodt, J. & Stelzer, E. H. K. Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. Science 322, 1065–1069 (2008).
9. Truong, T. V., Supatto, W., Koos, D. S., Choi, J. M. & Fraser, S. E. Deep and fast live imaging with two-photon scanned light-sheet microscopy. Nat. Methods 8, 757–760 (2011).
10. McFall-Ngai, M. Divining the essence of symbiosis: insights from the squid–vibrio model. PLOS Biol. 12, e1001833 (2014).
11. Nyholm, S. V., Stabb, E. V., Ruby, E. G. & McFall-Ngai, M. J. Establishment of an animal–bacterial association: recruiting symbiotic vibrios from the environment. Proc. Natl Acad. Sci. USA 97, 10231–10235 (2000).
12. Nawroth, J. C. et al Motile cilia create fluid-mechanical microhabitats for the active recruitment of the host microbiome. Proc. Natl Acad. Sci. USA 114, 9516–9516 (2017).
13. Hove, J. R. et al Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. Nature 421, 172–177 (2003).
14. Vermot, J. et al Reversing blood flows act through flhA to ensure normal valvulogenesis in the developing heart. PLoS Biol. 7, e1000246 (2009).
15. Fahlbusch, E. O., Voigt, F. G., Schmid, B., Holmchen, F. & Fuchs, J. Rapid 3D light-sheet microscopy with a tunable lens. Opt. Express 21, 21010–21026 (2013).
16. Liebling, M., Forouhar, A. S., Gharb, M., Fraser, S. E. & Dickinson, M. E. Four-dimensional cardiac imaging in living embryos via postacquisition synchronization of nongated slice sequences. J. Biomed. Opt. 10, 054001 (2005).
17. Taylor, J. M., Girkin, J. M. & Love, G. D. High-resolution 3D optical microscopy inside the beating zebrafish heart using prospective optical gating. Biomed. Opt. Express 3, 3043–3053 (2012).
18. Mickleit, M. et al High-resolution reconstruction of the beating zebrafish heart. Nat. Methods 11, 919 (2014).
19. Cong, L. et al Rapid whole brain imaging of neural activity in freely behaving larval zebrafish (Danio rerio). Elife 6, e29158 (2017).
20. Pégard, N. C. et al Compressive light-sheet microscopy for 3D neural activity recording. Optica 3, 517–524 (2016).
21. Grosenick, L. M. et al Identification of cellular-activity dynamics across large tissue volumes in the mammalian brain. bioRxiv 132688, https://doi.org/10.1101/132688 (2017).
22. Nöbauer, T. et al Video rate volumetric Ca(2+)-imaging across cortex using seeded iterative demixing (SID) microscopy. Nat. Methods 14, 811–818 (2017).
23. Skocek, O. et al High-speed volumetric imaging of neuronal activity in freely moving rodents. Nat. Methods 15, 429–432 (2018).
24. Aimon, S. et al Fast near-whole-brain imaging in adult Drosophila during responses to stimuli and behavior. PLoS Biol. 17, e2006732 (2019).
25. Wolf, S. et al Whole-brain functional imaging with two-photon light-sheet microscopy. Nat. Methods 12, 379–380 (2015).
26. Lee, D. A. et al Genetic and neuronal regulation of sleep by neuropeptide VF. Elife 6, e25727 (2017).
27. Wagner, N. et al Instantaneous isotropic volumetric imaging of fast biological processes. Nat. Methods 16, 497–500 (2019).
28. Cohen, N. et al Enhancing the performance of the light field microscope using wavefront coding. Opt. Express 22, 24817–24839 (2014).
29. Antipa, N. et al DiffuserCam: lensless single-exposure 3D imaging. Optica 5, 1–8 (2018).
30. Guo, C. et al Fourier light-sheet microscopy. Opt. Express 27, 25573–25594 (2019).
31. Zheng, G., Horstmeyer, R. & Yang, C. Wide-field, high-resolution Fourier ptychographic microscopy. Nat. Photonics 7, 739–745 (2013).
32. Adams, J. K. et al. Single-frame 3D fluorescence microscopy with ultraminiature lensless FlatScope. Sci. Adv. 3, e1701548 (2017).
33. Edelman, A. D. et al. Advanced methods of microscope control using uManager software. J. Biol. Methods 1, e10 (2014).
34. Monte Westerfield. The Zebrafish Book (University of Oregon Press, 2000).
35. Troll, J. V. et al. Peptidoglycan induces loss of a cellular peptidoglycan recognition protein during host tissue development in a beneficial animal–bacterial symbiosis. Cell. Microbiol. 11, 1114–1127 (2009).
36. Dunn, A. K., Millikan, D. S., Adin, D. M., Rose, J. L. & Stabb, E. V. New rfp- and pES213-derived tools for analyzing symbiotic vibrio fischeri reveal patterns of infection and lux expression in situ. Appl. Environ. Microbiol. 72, 802–810 (2006).
37. Schindelin, J. et al Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682 (2012).
38. Muto, A., Ohkura, M., Abe, G., Nakai, J. & Kawakami, K. Real-time visualization of neuronal activity during perception. Curr. Biol. CB 23, 307–311 (2013).
39. Peli, E. Contrast in complex images. JOSA A 7, 2032–2040 (1990).
40. Bex, P. J. & Makous, W. Spatial frequency, phase, and the contrast of natural images. J. Opt. Sci. 19, 1096–111 (2002).
41. Taylor, M. A. & Bowen, W. P. Quantum metrology and its application in biology. Phys. Rep. 615, 1–59 (2016).
Acknowledgements
We thank E.G. Ruby (University of Hawaii-Manoa) for critical assistance and sharing of resources for the squid-bacteria experiments; Misha Ahrens (Janella) for sharing of zebrafish lines; and Le Trinh for advice and support on zebrafish husbandry. Funding was provided for by the Gordon and Betty Moore Foundation grant #3396 (Ruby, McFall-Ngai and Fraser); National Institute of Health, grant #1R01MH110728-01 (Arnold, Kesselman, Fraser), grant #R01AI150661 (McFall-Ngai and Ruby), grant #R01OD11024 (Ruby and McFall-Ngai); National Science Foundation, grant #1650406 (Dickman, Fraser, Truong), and grant #1608744 (Kanso, Fraser). S.M. was supported by the USC Provost Fellowship; and K.K.D. by the Alfred E. Mann Doctoral Fellowship.

Author contributions
T.V.T. conceived the idea, with further refinement from S.M., D.B.H., and S.E.F., T.V.T., D.B.H., S.M., A.A., J.V.T., M.J.M.N., and S.E.F. designed the experiments. T.V.T., D.B.H., and A.A. designed the microscopy setup. T.V.T. built the setup. T.V.T., D.B.H., S.M., A.A., and J.V.T. collected the data. S.M., D.B.H., D.E.S.K., A.A., and T.V.T. set up the light-field reconstruction pipeline. D.B.H., K.K.D., S.M., A.A., J.V.T., and T.V.T. analyzed the data and produced the figures. T.V.T., K.K.D., and S.E.F. wrote the manuscript, with inputs from all authors. T.V.T. and S.E.F. supervised the work. T.V.T., D.B.H., and S.M. contributed equally. A.A. and K.K.D. contributed equally.

Competing interests
A patent application has been filed by the University of Southern California, with inventors T.V.T., S.M., D.B.H., and S.E.F., for the methodology of SVIM. Application number: PCT/US2017/019512; pending. All other authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s42003-020-0787-6.

Correspondence and requests for materials should be addressed to T.V.T. or S.E.F.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020, corrected publication 2022