Light-sheet imaging for systems neuroscience

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Developments in electrical and optical recording technology are scaling up the size of neuronal populations that can be monitored simultaneously. Light-sheet imaging is rapidly gaining traction as a method for optically interrogating activity in large networks and presents both opportunities and challenges for understanding circuit function.

Early light-sheet microscopy

Although light-sheet microscopy has become popular in the life sciences only recently, the key concepts underlying this method were developed over a hundred years ago by Richard Zsigmondy and Henry Siedentopf. Working for Carl Zeiss, a manufacturer of optical systems, Zsigmondy and Siedentopf invented the ultramicroscope in 1902, a dark-field illuminator that allowed them to determine the size of colloidal nanoparticles in 'gold ruby' glass[1]. Their idea was to illuminate samples using a thin sheet of light, which was generated by an illumination arm oriented at a right angle to the microscope's detection system (**Fig. 1**). Zsigmondy improved this microscope further and, in 1925, was awarded the Nobel Prize in Chemistry for his research on colloid solutions and the development of the ultramicroscope[2].

Light-sheet microscopy continued to be used primarily in chemistry and materials science through the 20th century. Then, in pioneering work in the early 1990s, Voie and colleagues developed modern laser light-sheet fluorescence microscopy[3]. Their orthogonal-plane fluorescence optical sectioning technique focuses a laser beam into a thin sheet of laser light and images the thin section of the sample illuminated by this laser light sheet with a camera. By rotating the specimen relative to the light sheet, Voie *et al.* could reconstruct the three-dimensional architecture of the guinea pig cochlea. About a decade later, this modern form of laser light-sheet fluorescence microscopy was beautifully applied to live imaging of biological specimens, focusing on applications in microbial oceanography[4] and developmental biology[5]. Since then, the field of light-sheet microscopy has truly taken off. Throughout the past decade, there have been numerous advances in both methods development and the exploration of novel applications.

Light sheets in systems neuroscience

One such application is in large-scale neural recordings. The strength of light-sheet microscopy for systems neuroscience is that it gives researchers the ability to record from many cells at the same time. The importance of population recordings has long been recognized in neuroscience: most neurons are embedded in circuits and are unlikely to act independently; instead, they may best be described by their role within, say, a sensorimotor transformation or a memory retrieval system. These systems often span multiple brain areas, such as pathways from retina to higher visual cortices. Understanding certain aspects of such global systems may hinge on our ability to observe them in their entirety. Indeed, measurements of neural activity across the whole brain, or at least a very large fraction of it, have been something of a holy grail for neuroscientists. Large-scale optical recording methods such as light-sheet imaging are starting to provide these types of data.

Light-sheet microscopy was first applied in systems neuroscience to map olfactory responses in the mouse vomeronasal organ. In single experiments, the responses to a bank of chemical stimuli across a large population of cells could be mapped[6]. Subsequently, the technique we and others applied to study circuit activity in larval zebrafish[7,8], whose small size and transparency makes it a natural target for optical physiology (**Fig. 2**). Imaging from about 100,000 neurons—most of its central nervous system—generated neural activity data with

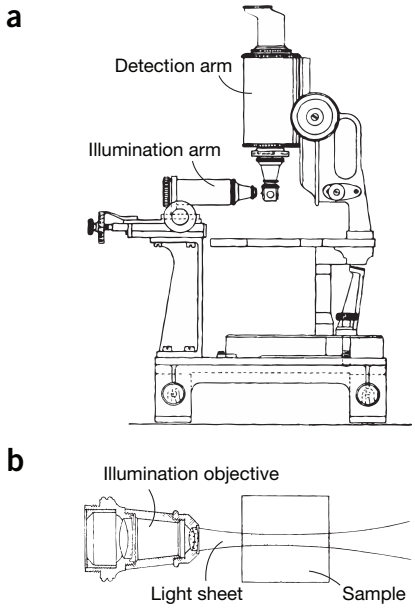

Figure 1 | The ultramicroscope, the first light-sheet microscope. (**a**) The original design of the ultramicroscope by Zsigmondy and Siedentopf[1]. (**b**) Light-sheet illumination of a glass sample in the ultramicroscope. Images reproduced from Siedentopf, H. and Zsigmondy, R. (1902), Uber Sichtbarmachung und Größenbestimmung ultramikoskopischer Teilchen, mit besonderer Anwendung auf Goldrubingläser. *Ann. Phys.*, **315:** 1–39. doi:10.1002/andp.19023150102.

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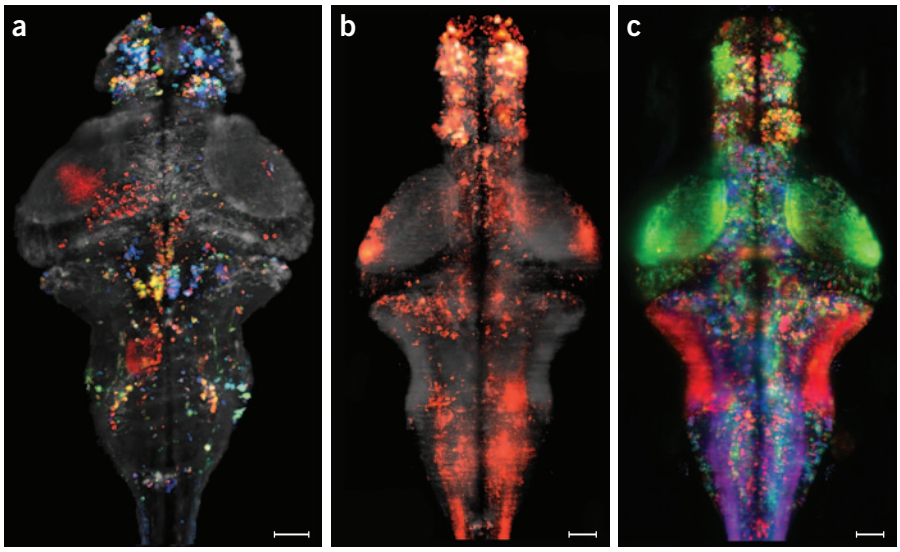

Figure 2 | Modern light-sheet microscopy in systems neuroscience. (**a**) Activity in the brain of a larval zebrafish expressing a genetically encoded calcium indicator, imaged with a fluorescence laser light-sheet microscope[7]. Each color represents neural activity at a different time point. Reprinted from *Methods*, **62**/3, cover, Copyright (2013), with permission from Elsevier. (**b**) Activity in the brain of a larval zebrafish at a single time point, measured with a genetically encoded calcium indicator. In **a** and **b**, overall brain anatomy is shown in gray. (**c**) Computational map of correlated activity across the same brain as in **b**. Shared colors indicate common dynamic patterns of activity. Scale bars, 50 μm.

rich structure in both space and time; when computational techniques were applied to these data, repeating patterns of coordinated population activity could be recovered. Whereas some of these patterns involved thousands of neurons, others involved 100 neurons or fewer. The latter result is important because it shows that small neural populations with specialized functions can be extracted from a sea of thousands. In further work, we combined light-sheet microscopy with visual stimulation and behavior in zebrafish by introducing a fictive readout of intended locomotion[9]. This represents an optically accessible nervous system behaving in virtual reality under a microscope so that neural function can be directly related to swimming behavior.

In the future, we hope to see large-scale microscopy—light sheet and other techniques—used to record neural activity in more model organisms, including adaptations for use in larger brains such as those of mice. In addition, neuroscientists are developing new genetic model organisms, an effort made easier in part by transgenic tools such as the CRISPR-Cas9 system. Thus, more optically accessible organisms may become genetically accessible; or genetically accessible organisms may be made more transparent by genetic manipulation and thereby more amenable to optical interrogation and perturbation.

Challenges and prospects

Calcium imaging of large neuronal populations with state-of-the-art light-sheet instruments has already led to new insights into nervous system function[6–8,10]. Despite this progress, functional imaging techniques still have many limitations, and further advances are imperative: temporal resolution, spatial resolution and physical coverage of nontransparent specimens still need to be improved.

Fairly transparent specimens, such as the brain of larval zebrafish, can be effectively imaged with current light sheet–based functional imaging. To obtain similar coverage in larger or less transparent nervous systems, however, better methods will be needed: for example, more advanced strategies for simultaneous multiview imaging. So far, volumetric imaging speeds in the range of a few tens of seconds per volume have been reached for medium-sized samples such as *Drosophila* embryos[11,12]. Pushing these speeds into the range of at least a few full-animal volumes per second would enable calcium imaging with good coverage in less optically accessible specimens.

Even for more transparent samples, the current volumetric acquisition speeds, although sufficient for basic calcium imaging experiments, are generally far below the temporal resolution required to detect the timing of single neuronal spikes that represent true information flow across the brain. In addition, although spikes can in principle be decoded from calcium transients, genetically encoded calcium indicators are indirect reporters. As such, increases in speed and the further development of genetically encoded voltage indicators are exciting and necessary. Current imaging approaches are also not optimal for investigating certain types of neuronal computations, such as processing of visual information in the retina, owing to their use of one-photon excitation and the possibility of photoreceptor stimulation. By improving two-photon light-sheet imaging assays[13] to deliver speeds compatible with calcium imaging, one could reduce unwanted side effects of the imaging process.

Finally, light-sheet functional imaging at the scale of the entire brain is subject to the fundamental tradeoff between temporal and spatial sampling. Although lateral resolution (i.e., resolution within the image plane) is usually fairly high, axial resolution (i.e., resolution perpendicular to the image plane) is often at least partially constrained by axial sampling as a result of the need to minimize the number of image planes covering the sample volume. Further improvements in spatial resolution are generally desirable but must be realized without affecting imaging speed.

Data interpretation

Given the large size and complexity of whole-brain light sheet–based measurements of neural activity, significant computational and theoretical challenges lie ahead. At the most basic level are image processing steps such as registering volumes, aligning to a common reference and filtering time series. Many key techniques already exist—those, for example, from the medical imaging and human neuroimaging communities—but when every experiment can be several terabytes in size, as is typical for light-sheet measurements, merely storing and interfacing with the data, and performing such routine operations, becomes a challenge.

Very quickly, another issue ensues: how do we extract signals of biological interest? There are many options to explore and continually re-evaluate. For example, some analyses first segment and extract signals from individual cell bodies, whereas others are performed at the level of the individual

voxels[7] that make up both cell bodies and other neural structures such as axons and dendrites. The former offer large reductions in data size and the capability for more complex analyses downstream, but the latter can offer a more unbiased view of the data and in principle capture a wider range of signals.

The most interesting problems, however, are at a level higher; we must make sense of neural activity by relating it to properties of the outside world, to the animal's observable behavior or to internal states that we cannot observe directly—and, in most cases, to some combination of all three. The only thing agreed upon so far is that there is no agreed-upon way to do this. Many techniques, drawn from existing work in computational neuroscience and neuroimaging, are useful. These range from regression, dimensionality reduction[14,15] and clustering to network and graph theoretic models of coupling and nonlinear time series analysis[16]. But given the complexity of any single experiment, no single technique is 'most informative'; we need to try many—or invent new ones. Light-sheet imaging will increasingly yield data with a combination of spatial scale and temporal precision that is unique among other measurement techniques, and there are surely analysis methods—if not entire branches of computational theory—that will need to be developed for such data.

We will need to answer questions such as: How do networks in one brain area control or modulate one another? Are there theoretical principles underlying the organization and dynamics of neural networks, and should we look for their signatures in data or attempt to extract them from data directly? How can we relate large-scale recordings with other complementary data—for example, structural connectivity, morphology and gene expression—obtainable in the same systems? Across all of these levels, a key theme is that there are many ways to look at any given data set. We need computational approaches and interfaces not only for standardized processing of large data sets but for flexible, exploratory data analysis[17,18].

In the end, large-scale imaging and computation must be part of a bigger endeavor that includes neural perturbation techniques, electrical interrogation, anatomical characterization, connectivity analysis and the study of neural development, to help build a comprehensive understanding of what nervous systems do and how they do it. Continuing developments both within and across these fields will be enormously exciting for neuroscience.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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