Light-sheet imaging of mammalian development

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Tackling modern cell and developmental biology questions requires fast 3D imaging with sub-cellular resolution over extended periods of time. Fluorescence microscopy has emerged as a powerful tool to image biological samples with high spatial and temporal resolution with molecular specificity. In particular, the highly efficient illumination and detection scheme of light-sheet fluorescence microscopy is starting to revolutionize the way we can monitor cellular and developmental processes in vivo. Here we summarize the state-of-the-art of light-sheet imaging with a focus on mammalian development – from instrumentation, mounting and sample handling to data processing.

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1. Introduction

Embryonic development is a highly dynamic process spanning several spatial and temporal scales: from subcellular molecular processes which can happen in a fraction of a millisecond to large-scale tissue morphological transformations on time scales of minutes to several hours [1]. Techniques that allow monitoring these interdependent developmental processes by bridging both spatial and temporal scales are vital for a deeper and quantitative understanding of morphogenesis.

Fluorescence live imaging now enables not only to image morphological processes in vivo, but also to quantify the abundance, turnover, and concentrations of key biological players with molecular specificity. Fluorescence microscopy is currently the only method that allows spatial and temporal resolved data acquisition [2]. This is one of the reasons why confocal microscopes have been the workhorse imaging devices so far, and many important discoveries on mammalian development have been possible with their usage, especially on cultures [3–10]. This imaging technique, however, comes with a price: in addition to genetic modification to express fluorescent marker molecules, which can be difficult to implement depending on the organism of choice, extensive illumination of biological samples can cause phototoxic effects that impair the normal development of the specimen. Especially in the case of long-term live imaging of large three-dimensional samples, it is essential to minimize the total light dosage used for imaging. In particular for confocal microscopy, the illumination and detection directions are equal, meaning that sample is illuminated below and
above the region of interest with the same light density. As a result, total light exposure scales with the number of imaging planes comprising the 3D stack. Light-sheet fluorescence microscopy (LSFM), on the other hand, is a microscopy technique that offers a particularly efficient illumination and detection scheme, which yields comparable resolution to confocal microscopy while dramatically reducing the amount of light exposure [11, 12]. As a comparative example, imaging a single 3D dataset of a pre-implantation embryo (spherical shape with a diameter of about 100 μm) on a confocal microscope using 1 μm interplane distance yields an equivalent light exposure as imaging a single plane 100 times. In classical light-sheet microscopy, two lenses are used to image the sample: one to illuminate the sample and one to collect the emitted photons. The perpendicular arrangement of the illumination objective relative to the detection allows excitation of fluorescent molecules only in the close vicinity of the focal plane of the detection objective. In the pre-implantation embryo case, for example, a typical light-sheet thickness of 2 μm would yield a reduction of a factor 50 compared to confocal imaging. This, together with the ability to perform faster imaging over entire volumes, makes light-sheet microscopy the technique of choice for long-term recordings of organisms in toto and in vivo.

Here we review light-sheet imaging of mammalian development. For mammals, the viviparous nature of the embryonic development complicates fluorescent live imaging approaches, as embryos are not directly accessible in the uterine environment [1, 13]. Moreover, for live imaging of mammalian embryos careful atmospheric control and specific medium concentrations are indispensable, while the high photosensitivity demands very low light doses [14]. All these properties complicate in vivo imaging, posing a strong challenge which motivates specific microscopy solutions. These aspects can be tackled by light-sheet microscopy in different ways, and we review some of the many microscope setups that are designed to best match the sample requirements. In contrast to classical petri-dish sample mounting approaches that were developed for confocal and widefield setups, light-sheet microscopy also enables more flexible mounting approaches. As sample mounting is an essential step in the whole imaging process we also review current techniques with a focus on mouse imaging.

The paper is organized as follows: first, the fundamental concepts of light-sheet microscopy are reviewed, and particular implementations of the light-sheet concepts are presented and compared. An overview of light-sheet microscope subsystems and mammalian imaging is presented. The next three paragraphs review the light-sheet imaging of pre-implantation, post-implantation and adult mouse development.

2. Light-sheet microscopy

The basic idea behind light-sheet microscopy is to generate a thin optical sectioning by illuminating the sample from the side. In modern setups a laser beam is focused through an illumination objective lens in order to create a thin sheet of light orthogonal to the detection axis. This overlaps with the focal plane of the detection objective lens which then collects the emitted signal (Fig. 1A). Unlike a conventional confocal microscope, where the desired region of interest needs to be point scanned, a process which can easily take seconds, light-sheet microscopy can illuminate the entire field of view at once. Furthermore, light-sheet microscopy allows the sample to be imaged from multiple directions, providing a full 3D image as a result.

The first development of light-sheet microscopy dates back to 1902 with the work from Zsigmondy and Seidentopf. In their microscope a high intensity light-sheet was created in order to visualize dispersed sub-micrometer colloidal particles immersed in solution and was called the ultramicroscope [15]. It took several years until light-sheet imaging technique was rediscovered under the name of orthogonal-plane fluorescence optical sectioning (OPFOS) [16]. In this work, entire three-dimensional structures of stained and cleared guinea pig cochlea could be reconstructed [17]. Soon after that, a more versatile version of light-sheet microscopy was developed at EMBL and named single plane illumination microscopy (SPIM) [11]. This work allowed light-sheet microscopy to become visible to a broader part of the scientific community, largely contributing to the acceleration of the development of new techniques since then. Below we describe some of the most important imaging approaches and developments which followed.

SPIM was the first technique which allowed the sample not only to translate in all directions, but also to rotate around a vertical axis. This is an important asset, especially for highly scattering samples, which rapidly introduce noise into the quality of light-sheet illumination and fluorescence emission. By rotating the sample, different complementary sides of the specimen can be illuminated and all the different views brought together in order to obtain an entire 3D dataset made only from high quality illumination data. Though effective, sample rotation usually needs to be performed in a controlled fashion so to avoid sample drift, which makes it an inherently time consuming process. As a consequence, after many rotations, the final dataset may not provide an accurate snapshot of the living sample. One way to improve acquisition speeds and avoid too many rotations can be done by introducing another illumination path to the opposite side of the first one. This way it is possible to sequentially illuminate the sample from each direction without the need to rotate it 180° [18]. This approach is now commercialized under the name of Zeiss Light-Sheet Z1.

The possibility to obtain 3D datasets without sample rotation becomes possible only after introduction of an opposite detection path. This way a full 3D dataset can in principle be acquired in two steps due to the sequential illumination and dual simultaneous detection. The increase in imaging speed allows the full embryonic development to be acquired at rates fast enough to enable cell tracking even in the case of fast developmental processes, such as ventral furrow in Drosophila melanogaster embryos (MuVi-SPIM, SimView, four-lens SPIM) [19–21].

In all these SPIM microscopes, however, the objectives are mounted on the horizontal plane, and the specimen needs to be placed in a vertical mount, usually embedded in a gel. There are applications, however, where mounting the sample on horizontal direction is preferred. One example is mounting in upright position where the objectives face down toward the sample. Although in this configuration normally only two objectives are used, the utilization of dichroic mirrors allow both objectives to perform illumination and detection in a sequential manner. This is the way that the dual-view iSPIM (diSPIM) was first used to image embryonic development of C. elegans with close to isotropic resolution [22]. This kind of approach has also been recently published to design more symmetrical MuVi-SPIM setups, allowing all four objectives to be used for illumination and detection purposes [23]. Another possibility is to use the larger illuminated field of view to perform massively parallel fluorescence correlation spectroscopy, using each camera pixel to record fluorescence fluctuations over time [24]. A third possible geometry is to mount both objectives in an inverted fashion. It allows multiple samples to be immersed in a medium different from that of the objectives, and its development allows for the first time the acquisition of entire 3D datasets of multiple preimplantation mouse embryos up to blastocyst stage [25].

Due to the decoupled illumination and detection paths, light-sheet setups allow higher flexibility on the optics design. On the one hand, light-sheets can be generated in different ways. Probably the easiest one is the utilization of a cylindrical lens in order to shape the illumination beam by focusing it in only one direction [11]. This creates a light-sheet at once, since the entire field of
Fig. 1. (A) Sketch of the main important properties of light-sheet microscopy. A plane of light is created on the corresponding field of view of detection, where the specimen is located. This way most of the illumination light can be utilized to gently illuminate only the plane to be recorded by the camera. (B) Top row: Typical workflow for imaging with light-sheet microscope setups. An idea for relative comparison of amount of storage necessary for the relevant steps is depicted by black-and-white cylinders. Concrete examples for each step given in second row (i–iii.) From left to right: detection cameras spool all images acquired for each recorded stack with high transfer rates which can be up to 2 GB/s (i) raw images. Stacks need to be usually pre-processed right after acquisition, this way 3D datasets from different views can be obtained (ii) (background subtraction, multiple views registration, fusion). After that, through fast gigabit connections (e.g. 10GB/s), data can be passed onto another computer, where more time demanding post-processing steps can take place. Ultimately, aiding in segmentation and further analysis (iii) deconvolution, segmentation, tracking… Finally the recorded datasets and analyzed data can be copied to long-term storage systems. (C) Light-sheet setups for best imaging different developmental stages of mouse embryonic development. Arrows depict the average sizes embryo sizes for each developmental time window. (i) From first stages of development up to blastocyst (E0–E4), multiple embryos can be imaged per experiment, utilizing simple illumination and single detection inverted upright light-sheet setups. (ii) Typically after E4.5 up to E8.5, multifield light-sheet microscopes with sample rotation and dedicated mounting can be used for live in vivo imaging of development. (iii) Due to increased size and scattering effects on late post-implantation stages (>E8.5), tissue clearing techniques and light-sheet microscopes with dedicated optics allow for multiview stitching of multiple stacks across entire organs. Resources from EMAP eMouse Atlas Project (http://www.emouseatlas.org) [70].

view is instantaneously illuminated, and is therefore the fastest way to illuminate a sample. However, this kind of illumination also introduces long shadows which can so far only be overcome by using a galvanometric mirror to tilt the light-sheet dynamically while imaging, thus illuminating the sample through different angles [18]. Another approach is to let the camera exposing while a focused Gaussian beam scans throughout the field of view, creating a virtual light-sheet. The illumination beam properties can be tuned to match the needs of the illumination sheet (length, thickness), and scanning is performed with a galvanometric mirror. This technique is the principle behind the digitally scanned light-sheet microscope (DSLM) [26]. Less illumination scattering can be achieved by using a pulsed infrared laser for two photon excitation [27]. Better optical sectioning requires very thin Gaussian beams which need to be tightly focused, at the expense of a shortening of their focused length (Rayleigh range) due to the diffractive nature of light. This is the main proposition for the use of Bessel beams in order to produce thin and long illumination lines [28]. The profile of Bessel beams is nonetheless characterized by fairly strong side lobes, which contribute to background noise, and to higher levels of phototoxicity [29]. One way to minimize this is to make use of the quadratic dependence of the illumination intensity for two photon excitation processes, or to block the acquisition of the side lobes on the camera sensor [29,30]. Yet another approach is to use multiple Bessel beams to interfere with each other in order to create a Bessel beam plane, which minimize the effects of side lobes [31].

Finally, detection in SPIM is in its simplest form based on the widefield microscope and consists of a detection objective, filters, tube-lens and camera. Scattered photon detection can be minimized by implementation of a confocal detection which can follow the image of the scanning beam as it moves across the field of view in the specimen. Though mechanical slits have been proposed [32], the dynamic control of pixel lines on the camera sensor allow for the creation of an electronic slit which provides confocality without any hardware addition to the detection path [33–35].

To make the most out of light-sheet microscopy, however, specialized optics is not enough: image processing (Fig. 1B) is an integral part of imaging, and it has been coupled to SPIM since its first realization [11]. For example, in the case of larger and/or less transparent samples only a single view cannot provide an image of the whole specimen because of scattering effects. Thus, imaging from multiple views is necessary for completeness. Later, however, these recordings have to be fused together to provide a complete 3D representation. The fusion process consists of finding the same structures within all different views, and determining the transformation parameters that will move them onto a common reference frame. This process is called image registration. It can be carried out by several different methods; it may be image or bead based, and rely on different types of algorithms [36].

After registration, the fusion step can be performed. This is usually highly specimen dependent, and also here, many different algorithms can be implemented. The most straightforward method is a simple (weighted) average between views. Although this does
not improve resolution, it can still be effective to provide a complete reconstruction of the sample [19].

For highly transparent samples, however, imaging with multiple views has the additional benefit of providing extra information for other views, which can be used to ultimately improve resolution [37]. This is due to the fact that the axial resolution is inherently lower than the lateral resolution of any objective, resulting in anisotropic 3D point-spread-function (PSF). By imaging the same sample from different directions, and utilizing multiview deconvolution-fusion methods, the high resolution information from one view can be used to complement the low resolution information from another and vice versa [38]. In this case a multiview-deconvolution fusion of all datasets has to be carried out. This step is usually based on the Richardson-Lucy deconvolution algorithm [22,39,40], or on a derivation of it [41].

Although these image processing steps can be very involved, different open source packages are being developed to help streamline these steps. Especially useful can be the Multiview Reconstruction plugin for Fiji [42], which contains both the bead based registration [36] and Bayesian-based multiview deconvolution [41].

After fusing the data, evaluation and data analysis can finally begin. Since light-sheet based experiments can easily take up from hundreds of gigabytes to terabytes of storage space, just viewing the data can already pose a significant challenge, making its handling rather difficult. Ideally one would be able to visualize any point in space from arbitrary views while also freely moving through the time course of the experiment. Two independent tools which are available to use are ClearVolume [43], and BigDataViewer [44].

Overall, light-sheet microscopy is a technology which allows thin optical sectioning with high contrast and image quality, being able to image large samples in their entirety. It offers itself as a new imaging technique which further expands the field of microscopy. Since it has the ability to be gentle in the photon budget, light-sheet microscopy is very suitable in live embryonic imaging for long periods of time. Furthermore, this technology has the ability to be utilized in many different forms, with microscope setups being built around the sample. This fact can be exemplified by looking at the different light-sheet setups designed for imaging mouse embryos in different developmental stages (Fig. 1C). Different objective orientations allow easier sample mounting and aids in optimal imaging conditions. Even for commercialized light-sheet microscopes, where the optics are kept fixed, new developments in sample mounting or clearing techniques allow careful control of conditions in order to maintain embryos stable throughout the imaging experiment. This makes light-sheet microscopy a versatile tool through large temporal and spatial scales.

### 3. Mouse pre-implantation development

Throughout the first three days of development, the fertilized zygote becomes a fully grown blastocyst with 64 cells, ready to attach to the uterine wall. Embryo sizes change from around 100 μm to ca. 120 μm, while maintaining a spheroidal shape. At blastocyst stage the embryo has already undergone the first important cell fate assignments, with trophoderm (TE) and the inner cell mass (ICM) cells being readily recognizable. TE cells, which are in contact with the exterior environment, will form then the extraembryonic tissues, whereas ICM cells lead to the generation of the embryo proper.

In total real-time imaging of early mouse development has been a challenge due to embryonic high light sensitivity and demanding culture conditions. Phototoxicity effects occurring during confocal time-lapse imaging of early mouse embryos at 15 minute intervals can inhibit normal development already after 8 h [25]. Traditionally, confocal microscopy techniques have been utilized to image specific aspects of development in a particular developmental time window; for an example on the cell-fate patterning see Dietrich and Hiragi [45] and references therein. Novel approaches on how to minimize phototoxicity effects may include utilization of infrared lasers for two-photon excitation light [46–48], or longer waiting times between stacks [49]. These solutions require either expensive optics with ultrashort laser pulses, or a reduction in time resolution. Light-sheet imaging of pre-implantation embryos allows visualization, with single photon excitation, of the early divisions and morphological changes for whole live embryos, in real time. A particular example is the work of Strnad et al. [25], which focused on the first systematic in toto imaging of mouse pre-implantation development ranging from zygote to blastocyst with a dedicated inverted light-sheet setup (Fig. 2A). Embryos were mounted onto a thin transparent plastic foil attached to a multi-sample chamber (Fig. 2A, i), where droplets of medium could be added for live imaging conditions. The illumination and detection objectives, mounted in an inverted configuration (Fig. 2A, ii) stay immersed in water, focusing on the embryos through an optically transparent foil (Fig. 2A, iii). With this setup, Strnad and colleagues performed a complete lineage tracking by visualizing (Fig. 2B), segmenting (Fig. 2C) and following all cell divisions up to blastocyst stage (64 cells). These datasets corresponded to up to 39h of image acquisition, and rely on the high degree of mechanical, atmospheric and thermal stability of the whole microscope setup, allowing for normal in vitro development.

### 4. Post-implantation development

During normal development, after implantation into the maternal uterus, the mouse embryo grows and reshapes, with cell-fate assignment of the different tissues. These ultimately give rise to the embryo’s rudimentary body plan. Special attention is devoted to the developmental time window corresponding to the late blastocyst (~E4.5), through gastrulation (~E6.5) up to the somite formation (~E8.5). Although many of the main molecular pathways which drive these morphogenetic processes have been defined, a validation through imaging techniques has become necessary. Until recently, in situ visualization techniques have been the method of choice, providing still images of the several developmental processes [50]. These, however, do not allow for an in toto image of the developmental process of a single embryo, and rely on the high degree of reproducibility. Here, just as in the case of pre-implantation embryos, light-sheet microscopy is capable of acquiring 3D datasets of the entire embryo over long periods of time, allowing the observation of internal nuclear migration, gastrulation, yolk sac expansion and other important dynamical events with sub-cellular resolution. Such datasets can give a live, in toto perspective of the way that each single mouse embryo develops. They also provide the unprecedented ability to move toward quantification of such important developmental events.

Since early post-implantation embryos are delicate and small to handle, gentle care is required for the dissection step. With this in mind, it is also important to evaluate mounting procedures in order to perform the imaging whilst maintaining conditions which do not hinder embryo growth. Recently there have been two different ways of mounting post-implantation mouse embryos in dedicated holders for light-sheet microscopy. In the work of Ichikawa et al. [51] mounting requires the fabrication of a special acrylic rod which contains carefully drilled holes with different sizes. This way the embryo can be inserted via the Reichert’s membrane into the best fitting hole, leaving the embryo body free to develop (Fig. 3A, i, ii). With this it is possible not only to observe the formation of the different cell types during different stages of development (Fig. 3A,
iii at E6.5), but also to observe and characterize cell movement during interkinetic nuclear migration (during E5.5–6.5). Imaging time-lapses can typically be of 3 h without any phenotypical developmental failure. Another way of mounting, which encompasses embryos at stages ranging from E6.5 to E8.5, comprises of placing the embryo inside a hollow agarose cylinder filled with the necessary medium for optimal embryo survival (Fig. 3B–F). By fabricating different cylinder sizes it is possible to accommodate embryos of various stages, allowing them to grow freely inside. With this method Udan et al. [52] have been able to record 24 h time-lapses.

**Fig. 2.** (A) Inverted light-sheet microscope for multiple early mouse embryo imaging. (i) A sample holder (SH), containing a transparent FEP membrane (M) allows multiple embryo samples (S) to be placed in line for multisample imaging. (ii) Inverted objective orientation with side view of the sample holder. One possible configuration is to use a 10 × 0.3 NA illumination objective (IL) and another 100 × 1.1 NA detection objective placed at a right angle to the illumination. (iii) Close up on side view of sample on FEP membrane with both objectives. Since the FEP membrane is transparent on water, it provides no hindrance to the illumination beam in penetrating the sample or for the emitted fluorescence on reaching the detection objective. (B) Still images of one particular timelapse experiment, and (C) corresponding segmented nuclei. The star depicts the polar body. Adapted from Strnad et al. [25].

**Fig. 3.** (A, i, ii) Mounting technique for E5.5 to E6.5 embryos. A tip-truncated 1 mL syringe holds an acrylic rod, cut and drilled with holes of different size in order to best fit the mouse embryo by its Reichert’s membrane, leaving the embryo free inside the medium. (iii) Maximum intensity projection of a 13 μm slice at 78 μm from distal end of an E6.5 mouse embryo. The different tissues corresponding to the rudimentary body plan are annotated. Scale bar: 20 μm. (B) For stages ranging between E6.5 and E8.5, mounting using a hollow agarose cylinder has also successfully been proposed. Optimal sizes for the corresponding embryonic stage to be imaged can be produced, so that the embryo can grow with least hindrance. (C–F) Steps for mounting the mouse embryo inside the agarose cylinder. The inner volume of the cylinder can be filled with optimal medium, allowing the much larger chamber volume to have less expensive medium. (G–H) Example images of a 9.8 h timelapse with the mounting shown in (B) where the expansion of the yolk sac can be observed in different directions. (i) In order to aid multiview light-sheet setups in overcoming the higher scattering properties of embryos at this stage, and to allow faster and easier data recording, electronic confocal slit detection allows better quality images to be taken at shorter acquisition times. Scale bar: 20 μm. Adapted from Ichikawa et al. [51], Udan et al. [52] and de Medeiros and Norlin et al. [35].
of living mouse embryos, focusing particularly at gastrulation and yolk sac formation, and expansion events (Fig. 3G–H).

For these datasets to be acquired, it is necessary to be able to image the whole embryo over time. Since scattering increases as the embryo develops and becomes more densely compact, optimal imaging conditions must be able to keep up with high imaging quality. As previously discussed, one way to minimize scattering effects in large specimens is to perform multiview imaging. Another possibility is to use electronic confocal slit detection in combination with multiview imaging techniques [35]. Here the camera’s sensor dynamically activates only a certain number of pixel lines, which follow the illumination beam while it scans over the field of view. This has been shown to strongly minimize the detection of scattered light (Fig. 3I) [33,34]. The combination of confocal slit detection and multiview light-sheet microscopy allows a 4-view dataset to be acquired with simultaneous illumination and detection at once. Furthermore, opposing camera stacks can be readily added after registration in order to obtain a 3D dataset without the need for sample specific fusion.

5. Adult mouse imaging

Imaging adult mice poses several challenges, such as maintaining cellular resolution while significantly increasing the field of view and overcoming tissue scattering to enable imaging of deeper regions of the specimen. Light-sheet microscopy is already very well suited for fast imaging of large field of views, however in this case, to encompass the entire specimen, usually multiple recordings are necessary followed by computational stitching to achieve the necessary field of view. Although this increases imaging time, this is still 2 orders of magnitude faster than conventional 3D imaging methods (confocal microscopy, 2-photon microscopy).

The other, more significant challenge is light scattering and absorption in the adult tissue, especially in the brain. This depends partly on the size of the specimen, but also on the composition. Proteins inside the cells locally change the refractive index which induces scattering effects, while lipids absorb the light. Both of these effects reduce the effective light penetration in the tissue, severely limiting the imaging depth. Thus, one needs to overcome these two problems in order to achieve a higher light penetration in the tissue by optical clearing.

Because of these limitations, light-sheet microscopy is usually applied only to specific organs, instead of the whole mouse, most notably the brain. Since imaging depth is limited to a few mm-s even for cleared specimens, usually multiple directional illumination is necessary, when the sample is imaged from multiple orientations, which are later computationally fused. A similar method can be used to increase the field of view by imaging multiple stacks and stitching them computationally after the acquisition.

The first demonstration of light-sheet microscopy on cleared whole mouse brains was performed by Dodd et al. [53], who built a custom ultramicroscope which consists of two illumination arms that generate double-sided horizontal light-sheets; and one detection objective to image the fluorescence (Fig. 4A). After clearing the sample, it is placed in a glass chamber filled with the clearing solution (Fig. 4B) and imaged from top and bottom after a 180° rotation. Depending on the application needed, it is possible to image the whole brain at once with a smaller magnification objective (Fig. 4C), or smaller dissected parts such as the hippocampus at higher magnifications (Fig. 4D). Using higher magnification and higher NA objectives, individual cell-cell connections can also be visualized (Fig. 4E), and even dendritic spines can be observed (Fig. 4F).

Since then, numerous other clearing methods were developed such as ScaleA2 [54], 3DISCO [55,56], ClearT2 [57], SeeDB [58], CLARITY [59,60], CUBIC [61], and iDISCO [62]. All of these try to address both problems of scattering and absorption by somehow removing/substituting the lipids while preserving structure, and matching the higher refractive index with special imaging mediums. CLARITY by Chung et al. [59] for example uses
formaldehyde together with a hydrogel monomer to fix the samples, followed by lipid extraction in an electrophoretic chamber. This clears the sample while simultaneously preserving its structure and the fluorescent proteins, to enable deep tissue imaging for the whole brain. Its application with light-sheet microscopy was demonstrated by Tomer et al. [60] who imaged full mouse brains with subcellular resolution. Other applications include assessing axon regeneration after spinal cord injury [56], analyzing immunolabeled axonal tracts in 3D [63], studying neuronal development and degeneration [62], lymphangiogenesis [64], mapping the nervous system in the whole brain [32,60,61], or imaging the whole body [65,66].

As demonstrated, light-sheet imaging of cleared samples are becoming more and more accessible to a wide range of researchers because of the availability of the different clearing methods, and commercially available microscope solutions (UltraMicroscope, LaVision BioTec). However, because of the necessary optical requirements, i.e. having at least two objectives perpendicular to each other and in the close vicinity of the sample, imaging the brain of live adult mice with standard light-sheet methods is extremely challenging. Two recently developed methods however have promising potential in this field, since both of them require only one objective while still allowing for fast 3D imaging with optical sectioning. Axial plane optical microscopy (APOM) [67] and swept confocally-aligned planar excitation (SCAPE) [68] microscopy both only use only one objective to create the light-sheet and collect the fluorescence by optically rotating either the imaging plane (APOM), or both the imaging and illumination plane (SCAPE). This way, it becomes possible to image neuronal activity in live mouse brain through a cranial window using light-sheet microscopy [68].

6. Discussion

Light-sheet microscopy is currently transforming the way we can image biological processes in living organism. It allows for imaging samples of vastly different sizes with subcellular and unprecedented temporal resolution for extended periods of development. The fundamental difference between light-sheet microscopy and other fluorescent imaging techniques – confocal and widefield – is the uncoupling the sample illumination from the emitted photon detection. In contrast to confocal and widefield imaging, where for every optical section the entire sample is illuminated, the perpendicular arrangement of illumination and detection directs the excitation light to the close vicinity of the imaging plane. This reduces the total light exposure of the specimen and enables to monitor unperturbed specimen development. In particular for delicate mammalian samples, this gentle optical sectioning will enable new live imaging approaches to address cellular and developmental biology questions, in particular in such delicate mammalian samples.

The gentle optical sectioning of light-sheet concept allows acquiring images with the maximum frame rate of modern sCMOS cameras which yields high temporal resolution, but also demands efficient and sophisticated image processing and analysis techniques, as single experiments can easily yield a few terabytes of data. Another bottleneck at the moment is the visualization of raw and processed data in real-time as the data arrives from the microscope. An interdisciplinary community effort is needed – and in parts already happening - in order to develop these computational methods [42,69].

It is still the early days in light-sheet imaging and future technological improvements are to be expected. The areas of current light-sheet technology development can be broadly categorized in methods that optimize the illumination light distribution to achieve better optical sectioning, or to improve the light detection branch to, for example, improve image quality of opaque samples. The flexibility of design, together with the very active community provide a fertile creative ground for novel microscope setups to be developed. This enables further steps toward the observation of biological events with an even closer spatial-temporal look.

Competing interests statement

The authors declare no competing financial interests.

Contributions

All authors wrote the manuscript.

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Methods

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