Biotech Highlight

# Meeting report: First light sheet based fluorescence microscopy workshop

*Emmanuel G. Reynaud*[1] *and Pavel Tomancak*[2]

[1] School of Biology & Environmental Science, University College Dublin (UCD), Ireland
[2] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Selective Plane Illumination Microscopy (SPIM) and its derivative techniques have captured the imagination of scientists and the general public in recent years by providing unprecedented recordings of large, dynamic biological systems with resolution sufficient to distinguish and follow the behavior of individual cells throughout a living organism. Yet relatively few researchers are aware that SPIM represents one of the useful variations of an old idea in microscopy to use light sheet for illumination and optical sectioning. In September 2009 the First Light Sheet based Fluorescence Microscopy workshop in Dresden gathered, for the very first time, all researchers developing, using or simply interested in the various flavors of light sheet microscopy. In two exciting days full of talks and moderated discussion sessions, participants covered all critical aspects of this technology starting from building the light sheet microscopes, through processing of the data and finally identifying the optimal biological applications. In this report we inform the scientific community about the latest developments in this nascent field of microscopy and convey the general enthusiasm for the technology displayed abundantly by the workshop participants.

In Jena, at the turn of the century, Richard Zsigmondy (1865–1929), working together with the physicist Heinrich Siedentopf, had the brilliant idea to use a light sheet to illuminate from the side the colloid solution they were investigating [1]. From there the idea of light sheet illumination vanished from the scientific spotlight until it was revived it in the 90's as Orthogonal-Plane Fluorescence Optical Sectioning (ORFOS) [2] and theta confocal microscopy [3]. At the beginning of the new millennium, Ernst Stelzer and colleagues developed Selective Plane Illumination Microscopy (SPIM) and applied it to live imaging of model organism embryos [4, 5]. Since then the light-sheet illumination approach proved instrumental for a wide variety of biological disciplines ranging from marine biology [6], to fundamental developmental [7, 8] and cell biology research questions [9, 10]. Yet the technology remains largely confined to the laboratories that orchestrated its revival and the benefits of light sheet illumination are inaccessible to the broad scientific community. It was the purpose of the First Light Sheet based Fluorescence Microscopy workshop to bring together the key players in the light sheet technology and to establish effective communication among them that will hopefully lead to better integration of light sheet microscopy into scientific mainstream. It is symbolic that the workshop took place at the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden only some 100 miles away from Jena where it all started.

**Correspondance:** Dr. Pavel Tomancak, Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, D-01307 Dresden, Germany
**E-mail:** tomancak@mpi-cbg.de

**Abbreviations: SPIM**, Selective Plane Illumination Microscopy; **LSFM**, Light Sheet Fluorescence Microscopy

The workshop aimed at founding a community and to encourage communication and sharing between the different groups developing, using or simply interested in this technology for their research. In preparation for the workshop we established a Wiki where every invited participant could describe his research and sign up for a presentation in one of the sessions (https://spim.mpi-cbg.de). This self-organizing approach worked very well, as most of the participants were in fact presenting in the session of their choice and all speakers received equal amount of time to present their data.

The presentations were divided in three main sections:

1. Light Sheet Fluorescence Microscope (LFSM) building and engineering (chairmen: Jan Huisken and Olaf Selchow),
2. Image processing for LSFM (chairmen: Uroš Kržič and Stefan Preibisch) and
3. Sample preparation and biological applications (chairmen: Emmanuel G. Reynaud, Pavel Tomancak and Ernst H.K. Stelzer).

Each session was followed by a moderated discussion where many useful ideas surfaced. This report highlights the main issues and trends addressed during the meeting that covered all aspects of LSFM from instrument design to biological applications; from single particle tracking to whole embryo imaging.

## Building an LSFM block by block

The first session was dealing with the core of the technology: the machines themselves. Since the last decade or so, many applications have been presented that use a light sheet for side illumination of the sample. The most common approach is to use a single light sheet to scan the sample from multiple angles (views) and Ernst H. K. Stelzer introduced the benefits and caveats of this technology usually referred to as Selective Plane Illumination Microscopy (SPIM). He also enlightened us about the origin of light sheet technology in his laboratory going back to the Theta microscope [11] and the futuristic tetrahedral set-up [12, 13]. The very first SPIM was built by Jan Huisken when he was a student in Ernst Stelzer laboratory [4]. Jan went on to further develop the technology and presented at the workshop the mSPIM that uses two opposite and alternating light sheets formed using two water dipping lenses for the illumination [14]. The two light-sheet arrangement reduces the amount of views required to cover the sample in multi-view acquisition and increases the overall information obtained from a single view. Additionally Jan Huisken introduced the light-sheet pivoting that reduces the notorious SPIM stripes and should broaden the appeal of the technology for the more aesthetically oriented researchers. In those two approaches, the sample is moved through a fixed light sheet, while in the Objective Coupled Planar Illumination microscopy (OCPI) presented by Diwakar Turaga the sample is fixed on a slide and the light sheet is moved to scan it [15]. He described how the OCPI arrangement can be used for fast *in vivo* imaging of neuronal activity in the brain of anesthetized mice [16].

The light sheet represents the core of this technology and its formation and properties are crucial for obtaining the best results. Florian Fahrbach proposed phase-modulated Bessel beams as an alternative to the standard Gaussian beams [17]. As a prominent member of the class of non-diffracting beams, Bessel beams promise a number of advantages, such as a more isotropic resolution over a larger field of view or the ability of self-reconstruction behind obstacles [18], leading to a more homogeneous sample illumination.

Not only did this session address the technology itself but also how it relates to other techniques such as Optical Projection Tomography (OPT) presented by Jim Swoger [19], which, analogously to SPIM, relies on multi-view reconstruction and is geared towards imaging large samples such as

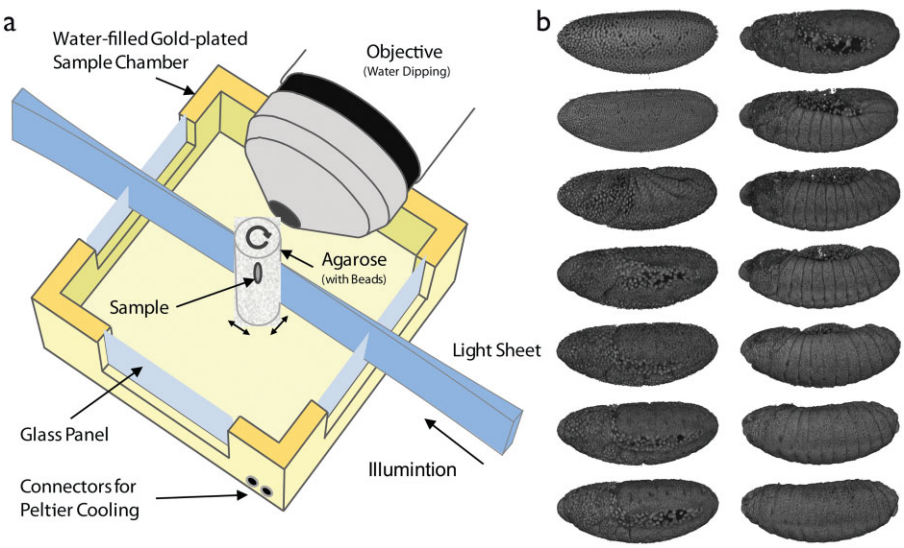


**Figure 1. SPIM microscope and long-term time lapse data.** (a) Schematic drawing of SPIM. The specimen embedded in agarose is illuminated by a laser light sheet that achieves optical sectioning. The sections are imaged with a CCD camera behind the perpendicularly positioned objective lens focused on the center of the light sheet. The sample can be moved through the light sheet and multiple 3d acquisitions of the sample can be taken by rotating the agarose column. (b) 3d renderings of long-term time-lapse SPIM recording of *Drosophila* embryogenesis (ubiquitous His-YFP, imaged from 6 views every 5 minutes, time-points at the end of every hour in the first 15 hours of development are shown).

mouse embryos [20, 21]. Günter Giese was one of the several participants who built their own light sheet microscope of the SPIM flavor for their biological applications. He showed multicolor recordings of fixed, optically cleared mouse brain tissue and pointed out the lack of low magnification objectives suited for the aggressive tissue clearing fluids (*e.g.* a benzyl alcohol/benzyl benzoate mixture). The session was appropriately closed by a joint presentation from Olaf Selchow and Chris Power from Carl Zeiss Microimaging GmbH, Germany, who presented the problems they are facing in bringing LSFM technology to the market and discussed the early prototypes that will hopefully give birth to a final product soon.

The debate that followed this session focused in particular on the cost of building LSFM instruments with estimates ranging from 50 000 to 150 000 Euros. Clearly the main part of the cost lies in the laser units and the cameras followed closely by the precise motorized stages. The lack of optimal lenses for such a microscopy was illustrated by Gunter Giesse's talk and further discussed among the participants. Other points mentioned were solutions for software control of the instrument, time required to build a LSFM and the recommended camera types.

## Handling the images

When we talk about three dimensional imaging we often think about great images that catch the eyes and allow a better understanding of biological phenomena we want to investigate, but we forget the difficulties in handling those datasets arising from their sheer size where Megabytes turn quickly into Gigabytes. In LSFM these problems are multiplied by the fact that the most popular flavors use multi-view acquisitions to fully cover large specimens. Therefore image processing is a great challenge in LSFM and the second session focused on this aspect.

LSFM is using a side illumination that results in a specific type of artifacts across the field of view. Two talks given by Maja Temerinac-Ott and Hwee Kuan Lee, presented various ways of restoring LSFM images by means of image processing. The most obvious LSFM artifact are the so-called "stripes" caused by absorption of the illuminating light sheet and the consequent shadow casting behind large fluorescent objects. Lee Hwee Kuan introduced a physics inspired approach attempting to simulate the behavior of individual photons in the sample that allows *in silico* signal intensity compensation and stripe suppressions. Hwee Kuan's approach represents an alternative to hardware solutions that achieve stripe suppression by modifying the illumination set-up as shown in the previous session by Jan Huisken.

The second image processing challenge in LSFM is in taking full advantage of the rotation of the sample to acquire image stacks of the same specimen from multiple angles (in SPIM jargon views). These views must be combined, that is registered and fused, to generate a final output image. This is not a brand new problem in microscopy but the limited amount of microscopes allowing multi-view did not favor the development of algorithms for such a purpose [22, 23]. Jim Swoger presented the early concepts of multi-view reconstruction that require stable set-up enabling registration simply by view rotation followed by translation-only adjustment and fusion in Fourier space [12, 24]. The use of reference points or fiduciary markers is a well-known approach used commonly in electron tomography and medical imaging. Stephan Preibisch has developed a bead-based registration technique that is sample independent, fast and completely unguided allowing efficient registration without prior knowledge of the angles (Fig. 2a) [25, 26]. Its use is thus not restricted to LSFM but can also be applied to other imaging platforms where sample rotation can be improvised such as on upright single photon confocal. The bead-based approach clearly outperforms intensity-based approaches in terms of speed [27] and therefore is particularly suitable for processing of long-term time-lapse acquisitions. Multi-view imaging offers key advantage for image restoration by deconvolution approaches [28] as it provides multiple observations of the same point in the samples. Uroš Kržič exploited this property with an elegant deconvolution-mediated image fusion achieved by iterative expectation maximization, which will lead to improved isotropic resolution in multi-view acquisitions (Fig. 2b,c, data provided by Stephan Preibisch). As his approach is also very fast its combination with Preibisch's bead-based registration presented itself as an obvious direction to follow up after the meeting. Lutz Schaefer, a collaborator of Zeiss, presented an extensive comparison of the various fusion techniques that have been published and applied them to model LSFM datasets critically evaluating their advantages and disadvantages.

Finally, Dan White made a passionate plea for using open source development concepts to generate image processing solutions for LSFM and make them as widely available as possible. He proposed Fiji (Fiji Is Just ImageJ, http://pacific.mpi-cbg.de) as a common software platform, which the LSFM

 

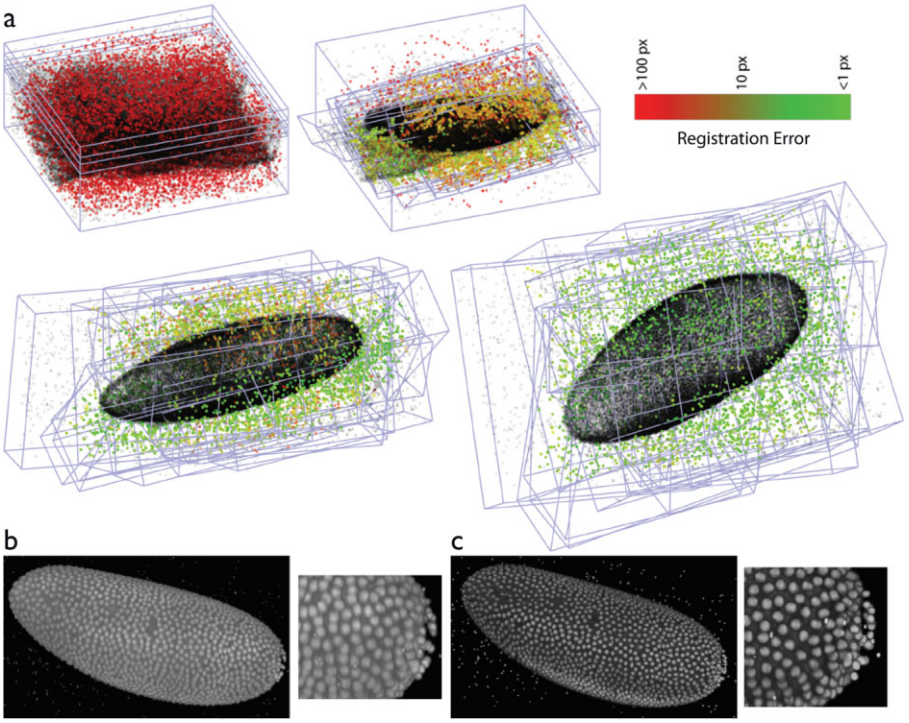


**Figure 2. Processing of multi-view SPIM acquisitions.** (a) 3d visualization of the registration progress on multi-view acquisition of fixed *Drosophila* embryo mounted in agarose in a custom sample rotation chamber and imaged on a single photon confocal microscope in tiling mode from 15 angles. Displacement of corresponding bead descriptors is color coded from red (maximum displacement) to green (minimal displacement). The global optimization is initialized with all views on top of each other and four iterations are shown. The registration process takes a few seconds (see link to Fiji YouTube www.youtube.com/user/fijichannel#p/u/11/yOAzf6hehFs). 3d rendering of pre-cellular blastoderm stage *Drosophila* embryo expressing His-YFP in all cells imaged with SPIM from 7 angles before (b) and after (c) multi-view deconvolution (posterior end magnified).

community could use, but his main point was clearly to foster the spirit of collaboration on common platforms in the open source world.

The debate was very lively as this session addressed a key element in LSFM. The questions discussed were mainly focused on the time it takes to register and fuse the LSFM data that was reduced from days to hours by using smarter algorithms, but that ultimately needs to be in the range of minutes to enable interactivity for average users. The community also recognized the need for sharing standard datasets of various kinds for the purpose of benchmarking and comparisons of the algorithmic solutions. The Dresden group committed to open the gates of their bead-based registration approach by making it fully open source and providing a usable solution under Fiji (http://pacific.mpi-cbg.de/wiki/index.php/SPIM_Registration) and we hope that others will follow.

## Do we really need an LSFM in our l(ife)aboratory?

The third session moved the attention from the electronics, the laser and the computers towards the samples itself. Often in LSFM, the sample is not on a glass slide but must be held in front of the lens in an aqueous medium. Emmanuel G. Reynaud introduced the problem and described the four main possibilities of achieving this: hooking, embedding, flattening or enclosing. This is clearly an area where innovation and experience are key and sharing of clever solutions is a must (Fig. 3a-d).

The meeting was attended by several leaders of microscopy facilities at large academic institutions. They brought a unique perspective to the meeting, highlighting the requirements for an emerging microscopy technology in multi-user environment. Corinne Lorenzo presented her experience with their home-made SPIM used to image cancer spheroids and *Xenopus laevis* development. Jan Peychl described the collective experience of his users on the Zeiss SPIM prototype placed at MPI-CBG Dresden for over two years. He showed us the limits of the technology, the requests from users that cover many scales of biological inquiry and the whole zoo of model organism and pointed out what is needed to make this technology mainstream. We all hope that the Zeiss people present were listening.

The debate that followed this session focused on users and their needs not only in terms of sample mounting but also in the ease of use of the technology. It was clear that the majority of people image large objects and that developmental biologist were amongst the most avid users of this technology so far. But the technology has numerous applications in cell biology, in particular in 3d cell culture imaging as was highlighted by Francesco Pampaloni

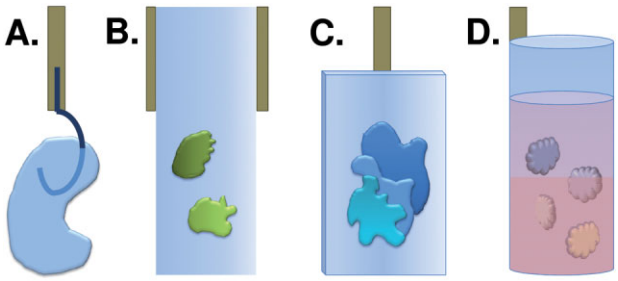


**Figure 3. Sample preparation strategies.** SPIM samples are typically suspended by gravity in water filled sample chamber. The strategies for attaching the sample range from simple hooking (a), through the most widely used embedding in agarose inside a glass capillary or plastic syringe (various diameters can be used for specimen of different size, the agarose column is pushed out by a plunger) (b), to more sophisticated, sculpted sample holders usually also made from agarose such as blocks (c) and baskets (d) filled with medium for tissue culture cells.

[10]. Dedicated set-ups geared towards specific applications are currently the norm and it is a great challenge for the Zeiss commercial solution to cover all the scales and sample types to the satisfaction of the demanding biologists.

## Yes we do and here is why!

The publications on LSFM technology have increased over the last five years as did the number of light sheets used for illumination (from one to two). However, what's coming next was the point of the last session. Ernst Stelzer illustrated the simplicity of LSFM set-up by presenting a monolithic design that should allow the production of a suitcase SPIM that we will probably carry along with us to the next LSFM conference. The monolithic LSFM uses the Digital Scanning Light Sheet (DSLM) principle that combines the advantages of side illumination and line scanning confocal allowing introduction of additional tricks such as structured illumination. It takes advantages of the diode laser now available in many colors and of the limited number of moving parts needed for an LSFM set-up. Ernst presented the work of his student Phillip Keller that pushes the limits of the LSFM technology in developmental biology allowing *in toto* recording of large embryos such as zebrafish [5].

Ulrich Kubitscheck showed that LSFM technology is certainly not limited to multi-view imaging of large specimens by describing real-time observations of single RNA molecules in living tissues [29]. Gregory Harms took advantages of a free STED left by a former colleague to combine LSFM and STED further strengthening the notion that LSFM has a lot to offer even on the smallest of scales. Ulrich Dodt presented applications covering broad range of scales from neural network to full mouse embryo imaged using an Ultramicroscope which represents a pure LSFM approach without sample rotation applicable particularly to imaging of cleared, fixed specimen [30, 31].

Jan Huisken, in his second contribution, proposed to us to buy a projector on E-bay to create a highly flexible FRAP system where you can literally draw the pattern of illumination yourself. He used it to study and manipulate heart function in zebrafish and enlightened us with a musical experience: an unlikely combination of FRAP, sound effects and LSFM [32]. Nadine Peyrieras then showed us that the power of LSFM in developmental biology is not limited to zebra-fish but is applicable to many other systems such as amphioxus and sea-urchin. The computational challenges in analyzing the data are enormous [33], but her presentation left us with a sense of wonder of what it will mean in the future to describe a developing system moving from one snapshot to several terabyte large multi-view, multi-channel, time-lapse SPIM monster datasets.

Finally, Pavel Tomancak and Emmanuel Reynaud closed the session and the meeting with two talks on what's on the horizon in terms of large-scale application of LSFM. Pavel wants to image large collections of transgenic fly embryos as part of an ongoing effort to describe patterns of gene expression in *Drosophila*, genome-wide with the maximum available resolution in both space and time [34, 35]. Emmanuel will use LSFM to scan for the future generations of scientists massive numbers of planktonic species collected as part of Tara Oceans expedition (http://oceans.taraexpeditions.org), a three year journey around the globe to analyze oceanic biodiversity in our troubled times of climate change. They will not need one LSFM, but a whole army of these machines to jumpstart the revolution in biology similar in scale to efforts of Celera on human genome sequencing.

The LSFM workshop clearly showed to all the participants that the revival of light sheet technology is not episodic and it is here to stay. Now we only need to convince the rest of the scientific community to use it to do exciting biology. It will certainly require a commercial solution available to everyone, but there is also the need for better access to the solutions developed by the experts in the LSFM community especially in areas like instrumentation, image processing and sample mounting. We truly hope that the Wiki pages that helped us organize the workshop will mature into a repository of ideas, tricks and solutions concern-

 


**Figure 4. The First Light Sheet based Fluorescence Microscopy Workshop.** (a) The front page of the conference booklet. (b) Farewell picture showing all participants in front of the MPI-CBG in Dresden. The names of participants in alphabetical order: Buytaert Jan, Dodt Hans-Ulrich, Ejsmont Radoslaw, Ewald Andrew, Fahrbach Florian, Gay Guillaume, Giese Guenter, Harms Greg, Hufnagel Lars, Huisken Jan, Kržič Uroš, Kubitscheck Ulrich, Lee Hwee Kuan, Lorenzo Corinne, Nitschke Roland, Nowell Cameron, Pampaloni Francesco, Peychl Jan, Peyriéras Nadine, Preibisch Stephan, Reynaud Emmanuel G., Ronneberger Olaf, Schaefer Lutz, Selchow Olaf, Stelzer Ernst H.K., Swoger Jim, Temerinac Maja, Theer Patrick, Tomancak Pavel, Turaga Diwakar, Wachsmuth Malte, White Dan and Yakimovich Artur.

ing LSFM. It is the responsibility of the LSFM community that was established at the meeting, to make it happen (Fig. 4).

The last debate of the meeting ended up with a vote to organize a second LSFM workshop in autumn 2010 in Dublin where we shall meet again and reflect on the promises made in Dresden. In particular the French participants rejoiced on the prospects of moving on from the great Saxonian wine testing held at Schloss Wackerbarth in Dresden to the undoubtedly equally spectacular Irish Whiskey tasting in Dublin. We hope to meet you there.

The next LSFM Workshop will be held at the School of Biology and Environmental Sciences, University College Dublin, Dublin, Ireland in autumn 2010 and is organized by Corinne Lorenzo and Emmanuel G. Reynaud.

*We would like to thank MPI-CBG for hosting the event and Carl Zeiss Microimaging GmbH for sponsoring the social event. Special thanks goes to Sandy Schneider for administrative assistance, Costas Margitudis for seamless operation of the audio/visual equipment and to Veronika Marcalikova for the design of the conference booklet (Fig. 4a).*

*The authors have declared no conflict of interest.*

## Useful links

- ↗ https://spim.mpi-cbg.de (LSFM conferences Wiki)
- ↗ spim-lsfm-info@lists.sourceforge.net
- ↗ http://pacific.mpi-cbg.de (Fiji Wiki)
- ↗ http://oceans.taraexpeditions.org

## References

[1] Zsigmondy, R., *Colloids and the Ultramicroscope: A Manual of Colloid Chemistry and Ultramicroscopy*, Kessinger Publishing 1914.
[2] Voie, A. H., Burns, D. H., Spelman, F. A., Orthogonal-plane fluorescence optical sectioning: three-dimensional imaging of macroscopic biological specimens. *J. Microsc.* 1993, *170*, 229–236.
[3] Stelzer, E. H., Lindek, S., Fundamental reduction of the observation volume in far-field light microscopy by detection orthogonal to the illumination axis: confocal theta microscopy. *Opt. Comm.* 1994, *111*, 536–547.
[4] Huisken, J., Swoger, J., Del Bene, F., Wittbrodt, J., Stelzer, E. H., Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science* 2004, *305*, 1007–1009.
[5] Keller, P. J., Schmidt, A. D., Wittbrodt, J., Stelzer, E. H., Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. *Science* 2008, *322*, 1065–1069.
[6] Fuchs, E., Jaffe, J., Long, R., Azam, F., Thin laser light sheet microscope for microbial oceanography. *Opt. Express* 2002, *10*, 145–154.
[7] Keller, P. J., Stelzer, E. H., Quantitative in vivo imaging of entire embryos with Digital Scanned Laser Light Sheet Fluorescence Microscopy. *Curr. Opin. Neurobiol.* 2008, *18*, 624–632.
[8] Huisken, J., Stainier, D. Y., Selective plane illumination microscopy techniques in developmental biology. *Development* 2009, *136*, 1963–1975.
[9] Keller, P. J., Pampaloni, F., Stelzer, E. H., Three-dimensional preparation and imaging reveal intrinsic microtubule properties. *Nat. Methods* 2007, *4*, 843–846.
[10] Pampaloni, F., Reynaud, E. G., Stelzer, E. H., The third dimension bridges the gap between cell culture and live tissue. *Nat. Rev. Mol. Cell Biol.* 2007, *8*, 839–845.
[11] Lindek, S., Stelzer, E. H., Resolution improvement by nonconfocal theta microscopy. *Opt. Lett.* 1999, *24*, 1505–1507.
[12] Swoger, J., Huisken, J., Stelzer, E. H., Multiple imaging axis microscopy improves resolution for thick-sample applications. *Opt. Lett.* 2003, *28*, 1654–1656.

[13] Huisken, J., Swoger, J., Stelzer, E. H., Three-dimensional optical manipulation using four collimated intersecting laser beams. *Opt. Express* 2007, *15*, 4921–4928.
[14] Huisken, J., Stainier, D. Y., Even fluorescence excitation by multidirectional selective plane illumination microscopy (mSPIM). *Opt. Lett.* 2007, *32*, 2608–2610.
[15] Turaga, D., Holy, T. E., Miniaturization and defocus correction for objective-coupled planar illumination microscopy. *Opt. Lett.* 2008, *33*, 2302–2304.
[16] Holekamp, T. F., Turaga, D., Holy, T. E., Fast three-dimensional fluorescence imaging of activity in neural populations by objective-coupled planar illumination microscopy. *Neuron* 2008, *57*, 661–672.
[17] Durnin, J., Miceli, J., Jr., Eberly, J. H., Diffraction-free beams. *Phys. Rev. Lett.* 1987, *58*, 1499–1501.
[18] Bouchal, Z., Wagner, J., Chlup, M., Self-reconstruction of a distorted nondiffracting beam *Optics Commun.* 1997, *151*.
[19] Sharpe, J., Ahlgren, U., Perry, P., Hill, B., *et al.*, Optical projection tomography as a tool for 3D microscopy and gene expression studies. *Science* 2002, *296*, 541–545.
[20] Boot, M. J., Westerberg, C. H., Sanz-Ezquerro, J., Cotterell, J., *et al.*, In vitro whole-organ imaging: 4D quantification of growing mouse limb buds. *Nat Methods* 2008, *5*, 609–612.
[21] Alanentalo, T., Asayesh, A., Morrison, H., Loren, C. E., *et al.*, Tomographic molecular imaging and 3D quantification within adult mouse organs. *Nat. Methods* 2007, *4*, 31–33.
[22] Bradl, J., Hausmann, M., Ehemann, V., Komitowski, D., Cremer, C., A tilting device for three-dimensional microscopy: application to in situ imaging of interphase cell nuclei. *J. Microsc.* 1992, *168*, 47–57.
[23] Matula, P., Kozubek, M., Staier, F., Hausmann, M., Precise 3D image alignment in micro-axial tomography. *J. Microsc.* 2003, *209*, 126–142.
[24] Swoger, J., Verveer, P., Greger, K., Huisken, J., Stelzer, E. H., Multi-view image fusion improves resolution in three-dimensional microscopy. *Opt. Express* 2007, *15*, 8029–8042.
[25] Preibisch, S., Saalfeld, S., Schindelin, J., Tomancak, P., Software for bead-based registration of selective plane illumination microscopy data. *Nat. Methods* 2010, *7*, 418–419.
[26] Preibisch, S., Saalfeld, S., Rohlfing, T., Tomancak, P., *SPIE Medical Imaging 2009: Image Processing* 2009.
[27] Preibisch, S., Rohlfing, T., Hasak, M. P., Tomancak, P., in: Reinhardt, J. M., Pluim, J. P. W. (Eds.), *SPIE Medical Imaging 2008: Image Processing.*, pp. 69140E-69140E-8 2008.
[28] Verveer, P. J., Swoger, J., Pampaloni, F., Greger, K., *et al.*, High-resolution three-dimensional imaging of large specimens with light sheet-based microscopy. *Nat. Methods* 2007, *4*, 311–313.
[29] Ritter, J. G., Veith, R., Siebrasse, J. P., Kubitscheck, U., High-contrast single-particle tracking by selective focal plane illumination microscopy. *Opt. Express* 2008, *16*, 7142–7152.
[30] Dodt, H. U., Leischner, U., Schierloh, A., Jahrling, N., *et al.*, Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. *Nat. Methods* 2007, *4*, 331–336.
[31] Becker, K., Jahrling, N., Kramer, E. R., Schnorrer, F., Dodt, H. U., Ultramicroscopy: 3D reconstruction of large microscopical specimens. *J. Biophoton.* 2008, *1*, 36–42.
[32] Scherz, P. J., Huisken, J., Sahai-Hernandez, P., Stainier, D. Y., High-speed imaging of developing heart valves reveals interplay of morphogenesis and function. *Development* 2008, *135*, 1179–1187.
[33] Zanella, C., Campana, M., Rizzi, B., Melani, C., *et al.*, Cells Segmentation from 3-D Confocal Images of Early Zebrafish Embryogenesis. *IEEE Trans Image Process* 2009.
[34] Ejsmont, R. K., Sarov, M., Winkler, S., Lipinski, K. A., Tomancak, P., A toolkit for high-throughput, cross-species gene engineering in Drosophila. *Nat Methods* 2009, *6*, 435–437.
[35] Preibisch, S., Ejsmont, R., Tomancak, P., *5th IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, Paris, France 2008, pp. 324–327.

 