Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Genetics & Development

Shedding light on the system: Studying embryonic development with light sheet microscopy

Raju Tomer, Khaled Khairy and Philipp J Keller

Light sheet-based fluorescence microscopy (LSFM) is emerging as a powerful imaging technique for the life sciences. LSFM provides an exceptionally high imaging speed, high signal-to-noise ratio, low level of photo-bleaching and good optical penetration depth. This unique combination of capabilities makes light sheet-based microscopes highly suitable for live imaging applications. There is an outstanding potential in applying this technology to the quantitative study of embryonic development. Here, we provide an overview of the different basic implementations of LSFM, review recent technical advances in the field and highlight applications in the context of embryonic development. We conclude with a discussion of promising future directions.

Address
Janelia Farm Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, VA 20147, USA

Corresponding authors: Tomer, Raju (tomerr@janelia.hhmi.org), Keller, Philipp J (kellerp@janelia.hhmi.org)

Current Opinion in Genetics & Development 2011, **21**:558–565

This review comes from a themed issue on
Developmental mechanisms, patterning and evolution
Edited by Sean Megason, Shankar Srinivas, Mary Dickinson and Anna-Katerina Hadjantonakis

Available online 19th August 2011

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2011.07.003

Introduction

Non-invasive three-dimensional imaging over time is indispensable for a quantitative understanding of biological processes at multiple scales, from molecular interactions to tissue morphogenesis. In particular, the *in vivo* study of early embryogenesis requires state-of-the-art imaging strategies that achieve high spatiotemporal resolution without compromising specimen integrity. Progress in sensor technologies, lasers and desktop computing in the past decades has led to a remarkable progress in imaging technologies and capabilities.

Specifically, the emergence of Confocal Laser-Scanning Fluorescence Microscopy (CLSM) provided means for non-invasive imaging of fixed as well as live samples for three-dimensional reconstructions and, therefore, confocal microscopes have become standard instruments in many laboratories. However, the ever-increasing demand to image for longer periods of time and at higher spatiotemporal resolution is rapidly exposing the limitations of CLSM. The point-scanning implementation of CLSM is inherently slow and causes high levels of photo-bleaching and photo-toxicity, owing to the iterative use of non-selective excitation with high-power beams. Additionally, tissue penetration depth is relatively low. These issues inspired the development of non-linear microscopy, specifically two-photon microscopy, which provided a substantial increase in the penetration depth and reduction in photo-bleaching, albeit, at the expense of spatial resolution and imaging speed.

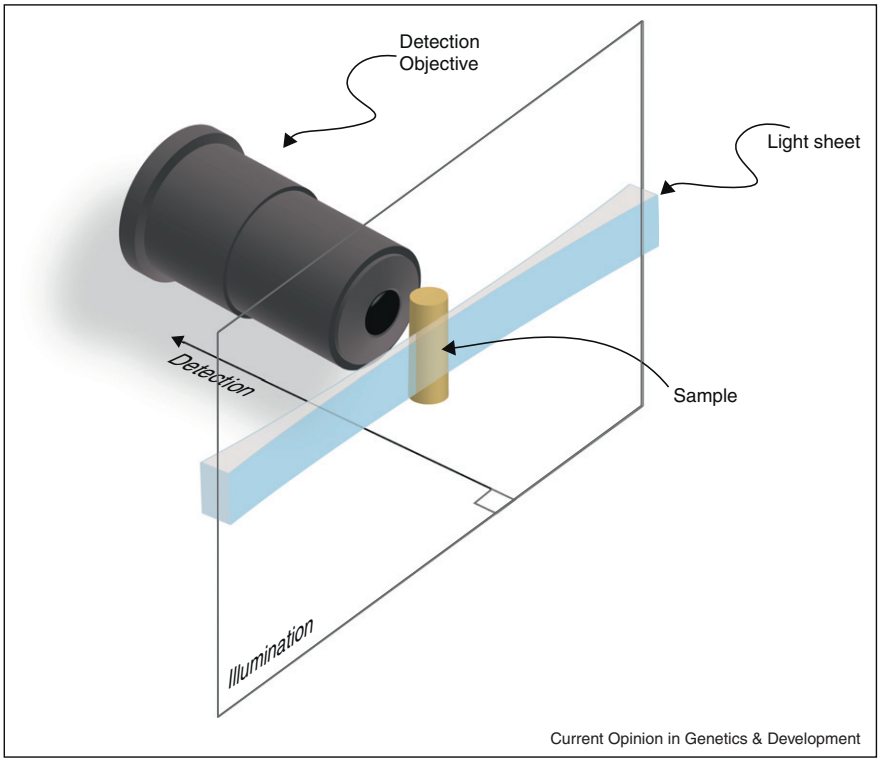
In the past decade, Light Sheet-Based Fluorescence Microscopy (LSFM) has emerged to fill the gap resulting from the inherent limitations of CLSM and point-scanning two-photon microscopy. The key concept behind LSFM is sample illumination in a thin volume section from the side, and fluorescence detection with an independent optical subsystem at a right angle to the illumination axis. This is in contrast to confocal microscopy and conventional wide-field microscopy, which typically use the same objective lens for illumination and detection. By illuminating only the in-focus plane, LSFM provides intrinsic optical sectioning and enables simultaneous detection of the fluorescence signal from an entire plane with highly efficient detectors. Thereby, LSFM combines several crucial properties, including high acquisition speed, high signal-to-noise ratio, minimal levels of photo-bleaching and good penetration depth. Moreover, sample preparation typically involves the use of low-concentration agarose cylinders for sample embedding, which represent a less stressful environment for live biological samples than the traditionally employed glass slide/coverslip. LSFM is thus naturally well-suited for studies of early embryogenesis, since it combines high-content dynamic imaging with a more physiological imaging environment.

Here, we discuss the various implementations of LSFM, review recent technical advances in the field, and highlight novel applications in the study of early embryogenesis. Other aspects of light sheet microscopy have been reviewed elsewhere [1–6].

Implementations of light sheet-based fluorescence microscopy

LSFM development has accelerated in the past decade. Many different variants with specialized features have emerged. However, the basic concept remains similar to the design of the first light sheet microscope by

Figure 1



Light sheet-based microscopy. The central concept behind light sheet-based microscopy is specimen illumination in a single plane with a thin sheet of laser light and recording of the fluorescence emitted by fluorophores in this thin section with a camera-based detection system oriented at a right angle to the light sheet. The optical subsystems for illumination and detection are decoupled, which allows using separate objectives optimized for low-NA specimen illumination and high-NA fluorescence detection. The light sheet is typically generated by scanning a pencil beam through the sample or by focusing a Gaussian beam along one direction into a sheet, using a suitable optical element such as a cylindrical lens.

Siedentopf and Zsigmondy [7]: the sample is illuminated with a sheet of light that overlaps with the focal plane of the detection optics (Figure 1).

Almost a century later, Voie *et al.* developed Orthogonal-Plane Fluorescence Optical Sectioning (OPFOS) [8]. The authors used a cylindrical lens to create a light sheet and imaged the internal architecture of the cochlea by scanning the sample through the sheet, achieving lateral and axial resolutions of 10 μm and 26 μm, respectively, for a field-of-view (FOV) of 1.5 mm. The shape of the light sheet can be well approximated by Gaussian beam optics and, thus, an intrinsic tradeoff exists between the central thickness of the light sheet, which directly influences axial resolution, and the size of the FOV within which the sheet's thickness remains sufficiently uniform. A high-resolution extension of OPFOS (HR-OPFOS) [9] was developed more recently, in which the authors used a large aperture lens to generate a thin light sheet. By moving the (fixed) sample stepwise along the illumination axis and recording the fluorescence signal from the thinnest part of the light sheet at each step, large specimens could be recorded with excellent axial resolution.

Thin Light Sheet Microscopy (TSLM) was developed to observe microbes in their natural setting with a millimeter-sized FOV [10]. Ultramicroscopy is a similar implementation optimized for imaging large fixed samples [11]. The sample is placed into a chamber containing the clearing solution with matched refractive index. Sample illumination is performed from two sides with aligned light sheets generated by cylindrical lenses. Both HR-OPFOS and Ultramicroscopy excel at imaging large fixed samples, but they are incompatible with the *in vivo* imaging of dynamic biological samples.

Selective Plane Illumination Microscopy (SPIM) was developed as an implementation of LSFM for non-invasive live imaging of early embryos [12]. Using fast CCD cameras and agarose cylinders for sample mounting, Huisken *et al.* reported long-term imaging of *Drosophila* embryonic development and high-speed imaging of the beating heart of a Medaka fish embryo. Since the one-sided illumination used in SPIM was often accompanied by shading effects resulting in stripe artifacts in the recorded images, the basic SPIM setup was further extended by multi-directional illumination (mSPIM) [13].

In addition, multi-view reconstructions and image deconvolution have been used to improve image quality [14,15]. In multi-view imaging, different views of the sample are obtained by rotating the sample. These data sets can then be combined computationally to achieve isotropic resolution in transparent samples [14] or to maximize coverage in partially opaque specimens [16,17].

Keller *et al.* reported the first beam-scanning implementation of light sheet-based microscopy (Digital Scanned Laser Light Sheet Fluorescence Microscopy, DSLM) [16] and used it to perform fast long-term imaging of entire developing zebrafish embryos. DSLM provides uniform intensity levels across the FOV, minimization of energy losses in the illumination process, a high-quality implementation of image contrast-enhancing structured illumination, and the capability to scan the light sheet through the sample for high-speed three-dimensional imaging. The beam-scanning approach introduced in DSLM has also been used as a basis for new LSFM techniques, such as Bessel plane illumination and efficient two-photon excitation [18••,19••].

Other LSFM implementations include Objective Coupled Plane Illumination (OCPI) [20], Highly Inclined Laminated Optical Microscopy (HILO) [21] and Oblique Plane Microscopy (OPM) [22]. In OCPI, the light sheet generating optics is coupled with the detection objective. The authors applied their technique to the fast imaging of neuronal activity in the mouse vomeronasal organ. HILO uses the same objective for illumination and detection. A highly inclined sheet of light is created and fluorescence observation is limited to a small FOV, where the light sheet and focal plane of the detection system overlap reasonably well. The basic HILO implementation has been extended in OPM, which enables an oblique plane in the specimen to be illuminated as well as imaged with the same objective lens. This approach allows recording a large FOV using conventional glass slide-based sample preparation.

Recent advances in light sheet-based fluorescence microscopy

Many aspects of LSFM have been improved over the past few years, including the development of better illumination and detection optics, instrument miniaturization, integration of other modalities, such as Fluorescence Correlation Spectroscopy (FCS), and efforts toward resolution enhancement and implementation of adaptive optics. Two particularly noteworthy improvements were recently reported: first, the use of self-reconstructing Bessel beams to create thinner light sheets and to reduce image quality degradation by light scattering, and second, the use of two-photon excitation in scanned light sheet microscopy.

Bessel beams are created by projecting an annular pattern at the rear pupil of an illumination objective. The central peak width is determined by the thickness of the annulus and can be decoupled from the Bessel beam's longitudinal extent. Fahrbach *et al.* conducted experiments with holographically shaped Bessel beams to demonstrate the self-reconstructing properties in three-dimensional scattering tissues, which reduce light scattering-induced image artifacts and increase penetration depth [18••,23]. The authors reported a prototype implementation (Microscope with Self-Reconstructing Beams; MISERB), which employs a Spatial Light Modulator (SLM) to generate scanned light sheets using Bessel beams.

Planchon *et al.* used an annular mask and an axicon to generate scanned light sheets from large numerical aperture Bessel beams [19••]. Bessel beams contain a substantial amount of energy in side lobes, which – if unaccounted for – lead to reduced axial resolution in light sheet-based microscopy in comparison to a Gaussian beam implementation. To address this problem, the authors used structured illumination and two-photon excitation, which eliminated the signal contribution of the side lobes to the recorded fluorescence images. The overall result is a marked improvement in axial resolution. Planchon *et al.* applied their technique to high-speed volumetric imaging of chromosomes in mitosis, three-dimensional imaging of protein pairs with isotropic resolution, and the observation of membrane dynamics in live cells. Photo-bleaching rates in scanned Bessel beam-based light sheet microscopes are significantly higher than those obtained with the scanned Gaussian beams used in DSLM. Thus, further experiments are required to determine if these principles can be extended to the live imaging of larger samples, such as entire developing embryos, over long periods of time.

Two-photon excitation has been implemented in scanning as well as non-scanning light sheet microscopy [19••,24•]. These implementations combine the intrinsic advantages of light sheet microscopy with the increased penetration depth resulting from the use of an infrared laser beam for fluorescence excitation.

Improvements in axial resolution can also be achieved by imaging the sample through the thinnest part of a light sheet created with Gaussian beams. Buytaert *et al.* used large aperture cylindrical lenses to generate highly focused light sheets and moved their sample step by step along the illumination axis. By stitching the resulting images, they obtained high axial resolution over a large FOV (HR-OPFOS) [9,25]. Similarly, Santi *et al.* reported TSLIM (Thin-Sheet Laser Imaging Microscope), where bi-directional light sheet illumination is used and the sample is moved through the thinnest part of the sheet to record a large FOV at high axial resolution [26]. Schacht *et al.* further improved TSLIM to provide a 70% reduction in scanning time and a 63% reduction in photo-bleaching [27]. These approaches are very useful for imaging large fixed samples at micron axial resolution. However, the

relatively slow acquisition speeds and high rates of photobleaching limit their applicability to live specimens.

Building upon the first implementation of scanned light sheet microscopy (DSLM) [16], Keller *et al.* implemented incoherent structured illumination in DSLM (DSLM-SI) to eliminate the contribution of scattered light to the fluorescence signal in partially opaque specimens [28••]. The authors demonstrated substantial improvements in signal-to-noise ratio, image contrast and lateral resolution, which allowed them to perform high-speed long-term imaging of zebrafish embryos and highly light-scattering *Drosophila* embryos. Similarly, Mertz *et al.* used scanned light sheet microscopy and a combination of uniform and incoherently modulated illumination to reject scattered signal light in their recordings of mouse brain tissue [29]. Lei *et al.* used a symmetric four-faceted pyramid to obtain a coherent three-dimensional structured illumination pattern. Combining this multi-layered light sheet geometry with the SI reconstruction algorithm introduced by Neil *et al.* [30] allowed the authors to discard out-of-focus light and improve axial resolution [31].

Some applications benefit greatly from miniaturized implementations of LSFM. Turaga *et al.* used an uniaxial gradient-index lens (GRIN), which replaces the cylindrical lens, to generate a light sheet in a miniaturized implementation of OCPI [32]. Similarly, Engelbrecht *et al.* used single-mode optical fibers, GRINs and a right-angle microprism to generate a light sheet in a miniSPIM setup [33•].

In addition to two-photon excitation, several other concepts have been successfully combined with LSFM. Friedrich *et al.* integrated stimulated emission depletion (STED) in SPIM (STED-SPIM) and achieved a 60% enhancement in axial resolution [34]. Turaga *et al.* introduced adaptive optics in light sheet microscopy. The authors implemented an image-based wavefront sensor using a variant of generalized phase-diverse imaging called multi-frame blind deconvolution to calibrate the deformable mirrors in an OCPI setup [35]. Wohland *et al.* combined SPIM with Fluorescence Correlation Spectroscopy (SPIM-FCS) and reported its *in vivo* application by imaging microspheres injected into the blood stream of a zebrafish embryo [36].

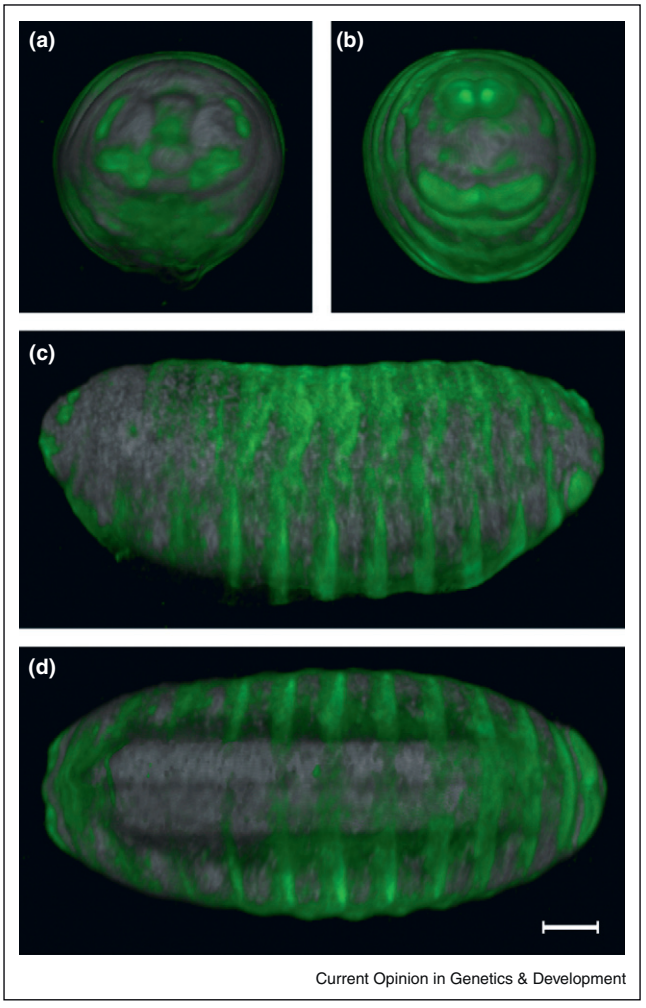
Applications in embryonic development

LSFM is particularly well suited for fast and long-term imaging of developmental processes at multiple spatial and temporal scales. Its applications range from imaging molecular interactions and gene expression patterns (Figure 2) to the reconstructions of early developmental dynamics (Figure 3) and *in toto* imaging of large fixed specimens (Figure 4).

Taking advantage of the high imaging speed and excellent signal-to-noise ratio of LSFM, Ritter *et al.* observed single molecules as deep as 200 μm within salivary gland tissue of *C. tentans* larvae [37,38]. The authors estimated the intra-nuclear viscosity parameters by imaging tracer molecules within the cell nuclei and were able to visualize the transport of single mRNA molecules tagged by fluorescently labeled RNA binding proteins. This study shows the potential of using LSFM to investigate gene activity in developing embryos at single molecule resolution.

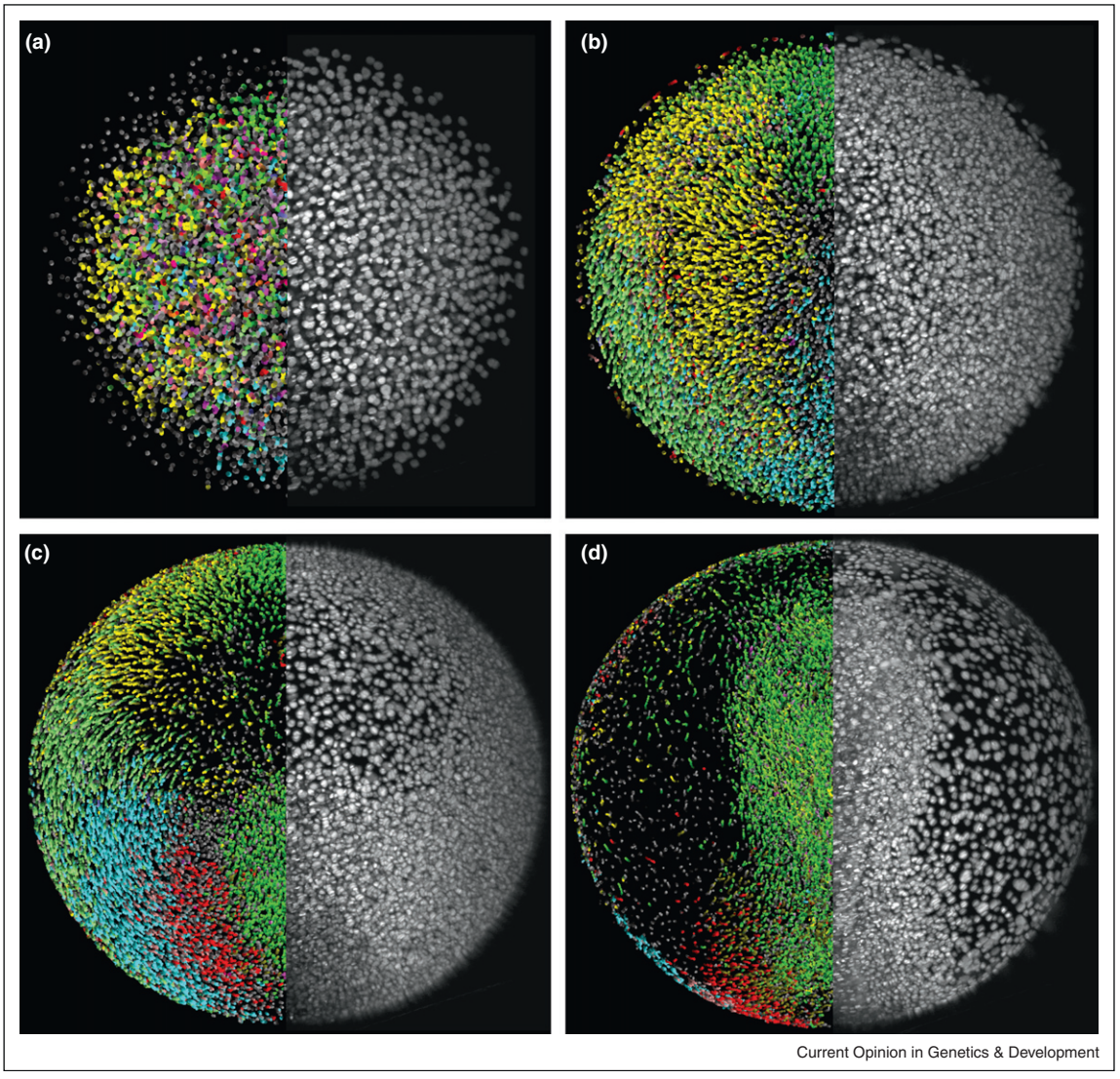
At the scale of entire embryos, Keller *et al.* used DSLM to image early development of wild-type and mutant

Figure 2



Light sheet-based imaging of gene expression patterns in *Drosophila* embryos. Three-dimensional rendering of a late-stage Drosophila pFlyFos-CG4702 embryo probed with anti-GFP antibody and DRAQ5 nuclear marker. The specimen was imaged with SPIM. Frontal **(a)**, caudal **(b)**, lateral **(c)** and ventral **(d)** views of the same embryo are shown. Scale bar = 50 μm. Credits: Reprinted from *Nature Methods*, vol. 6 no. 6, Ejsmont *et al.*, ''A toolkit for high-throughput, cross-species gene engineering in *Drosophila*'', 331–336, Copyright (2009), with permission from Macmillan Publishers Ltd.

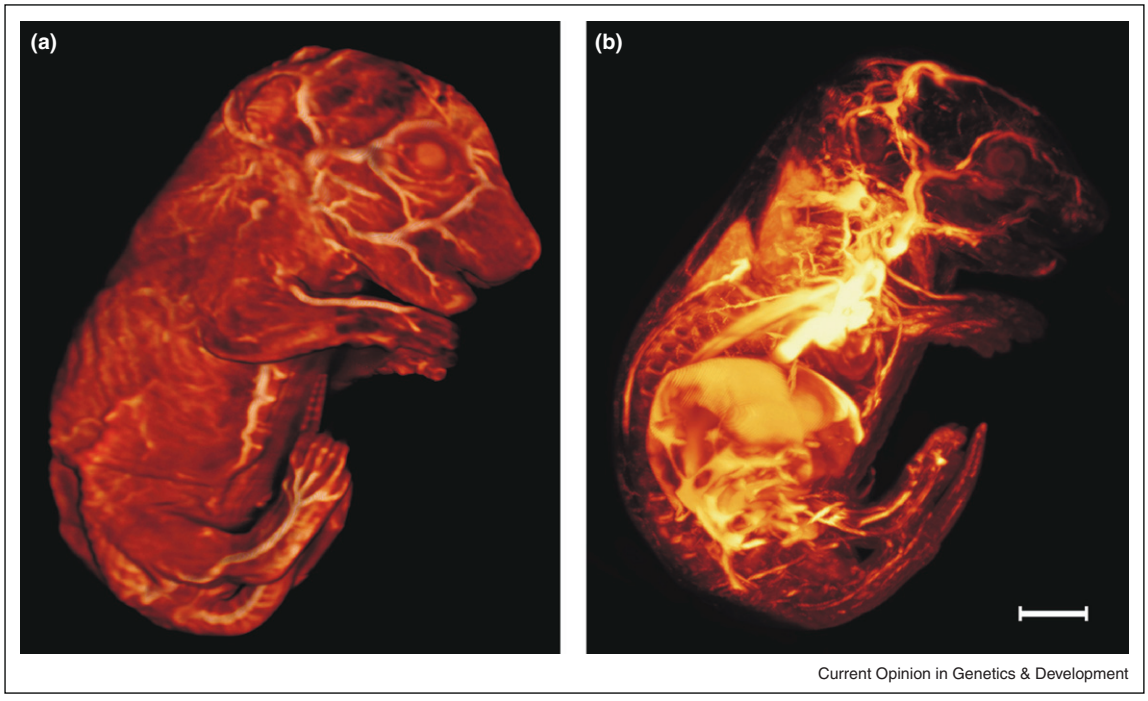
Figure 3



Global cell tracking in zebrafish embryos with scanned light sheet microscopy. DSLM microscopy data (right half of embryo: animal view, maximum-intensity projection) and the subsequently reconstructed 'digital embryo' (left half of embryo) with color-encoded cell migration directions. Time points: 289 min post fertilization (mpf) **(a)**, 368 mpf **(b)**, 599 mpf **(c)**, 841 mpf **(d)**. Color code: dorsal migration (cyan), ventral migration (green), toward or away from body axis (red or yellow), toward yolk (pink). Objective: Carl Zeiss C-Apochromat 10×. Databases and high-resolution movies of the digital zebrafish embryo are available at http://www.digital-embryo.org.
Credits: Reprinted from *Science*, vol. 322, Keller *et al.*, ''Reconstruction of Zebrafish Early Embryonic Development by Scanned Light Sheet Microscopy'', 1065–1069, Copyright (2008), with permission from AAAS.

zebrafish for 24 h [16]. The authors performed computational reconstructions of the microscopy data sets to generate 'digital embryos' (www.digital-embryo.org), which comprise quantitative information on cell coordinates, migratory tracks and cell division patterns for most of the cells in the developing embryo (Figure 3). This approach provided a detailed model of germ layer formation and revealed an initial morphodynamic symmetry break in the patterns of cell division, which identifies the orientation of the dorso-ventral body axis. Taking advantage of the capabilities of incoherent structured illumination, Keller *et al.* extended DSLM to DSLM-SI, to image and reconstruct early embryogenesis of partially opaque *Drosophila* embryos [28••]. Recently, Swoger *et al.* used SPIM to image and quantitatively analyze posterior lateral line (pLL) organogenesis in zebrafish [39•]. The authors used a triple-label fluorescence marker strategy for recording 4D data sets of the migration of the primordium and maturation of proneuromasts. The retrospective tracking analysis enabled the identification of hair cell progenitor lineages.

Figure 4



Imaging of chemically cleared mouse embryos with light sheet-based microscopy. Surface rendering of a mouse embryo **(a)** and view of the blood vessel system of the same embryo made visible by excitation of autofluorescence **(b)**. Objective: Carl Zeiss Plan-Apochromat 0.5×. Scale-bar = 2 mm. Credits: Reprinted from *Nature Methods*, vol. 4 no. 4, Dodt *et al.*, ''Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain'', 331–336, Copyright (2007), with permission from Macmillan Publishers Ltd.

Scherz *et al.* used high-speed mSPIM imaging to investigate the morphological and functional development of the zebrafish heart. Their analysis revealed that the atrioventricular valve (AVV) invaginates to directly form leaflets [40]. The authors furthermore disrupted ErbB receptors, TGF-β receptors and Cyclooxygenase 2 (Cox2)-dependent signaling to dissect the role of Cox2 in regulating myocardial cell shape. In a follow-up study, Arrenberg *et al.* combined optogenetics and light sheet microscopy to study the emerging function of the developing heart [41••]. The authors imaged zebrafish embryos with channelrhodopsin and halorhodopsin transgenes, which provided the capability to precisely control muscle cell activity. Using mSPIM in combination with well-defined photo-stimulation patterns for the systematic illumination of cell groups, they found that the pacemaker cells converge to the sinoatrial region in the developing heart. Systematic inhibition of these cells showed that only a few dozen of these cells are sufficient to maintain a functional system.

Future perspectives

Although the basic concept of light sheet illumination is over a century old [7], its modern form, laser light sheet-based fluorescence microscopy, has taken shape only recently. The spectrum of applications in the life sciences in general, and in early embryogenesis in particular, is extensive and further improvements can be envisioned in a multitude of ways.

Key parameters in any light microscopy technique are temporal resolution, spatial resolution, photo-bleaching/photo-toxicity and optical penetration depth. In the context of light sheet-based microscopy, further improvements are feasible in all of these aspects. Higher imaging speeds can be achieved by using faster detectors such as cameras based on sCMOS technology (http://www.scmos.com), integration of real-time electronics for microscope control and by parallelizing sample illumination and signal detection. Similarly, a further reduction of the energy load on the specimen – and thus a reduction of photo-bleaching and photo-toxic effects – is possible by using multiple detectors to collect more signal photons. Combining photoactivatable light microscopy (PALM, STORM) with the concept of light sheet illumination might provide enhanced super-resolution imaging capabilities in three dimensions. Further improvements in imaging depth could be achieved through the development of (infra-)red fluorescent proteins with good photostability and high quantum yield.

Qualitative advances and new experimental strategies are also conceivable and complement the quantitative improvements outlined above. For example, enhanced

capabilities in specimen interaction during imaging can be realized via optical subsystems for photo-activation, laser ablation and photo-stimulation. Automation of the experimental assay will enable the design of entirely new imaging-based studies, and could be achieved by using high-throughput approaches to sample loading via microfluidics or robotics and computational approaches to online data analysis. For example, using a reference database of embryonic development with single cell resolution ('digital embryo'), the control computer of a light sheet-based microscope could segment and structurally correlate a developing specimen online during image acquisition, thereby allowing statistical predictions of cellular or tissue-level identity for targeted laser ablation in functional studies. New approaches to *in vitro* tissue/organ culture may allow time-lapse imaging experiments in organisms that exhibit challenging optical properties, whereas new approaches to *in vivo* sample preparation may further enhance capabilities in physiological long-term imaging of entire developing animals.

Finally, it should be noted that large amounts of data result from high-speed and/or long-term *in vivo* light sheet-based microscopy experiments. For steady progress in the field, it will be imperative to develop advanced computational strategies to high-throughput data streaming, data management and storage, online processing and offline data analysis.

Acknowledgements

We thank the authors of Dodt *et al.* 2007 and Ejsmont *et al.* 2009 for kindly sharing their figure materials and permitting us to reprint their figures in this review article. This work was funded by the Howard Hughes Medical Institute.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Keller PJ, Pampaloni F, Stelzer EH: **Life sciences require the third dimension**. *Curr Opin Cell Biol* 2006, **18**:117-124.

2. Keller PJ, Stelzer EH: **Quantitative in vivo imaging of entire embryos with Digital Scanned Laser Light Sheet Fluorescence Microscopy**. *Curr Opin Neurobiol* 2008, **18**:624-632.

3. Huisken J, Stainier DY: **Selective plane illumination microscopy techniques in developmental biology**. *Development* 2009, **136**:1963-1975.

4. Khairy K, Keller PJ: **Reconstructing embryonic development**. *Genesis* 2010, **49**:488-513.

5. Reynaud EG, Tomancak P: **Meeting report: first light sheet based fluorescence microscopy workshop**. *Biotechnol J* 2010, **5**:798-804.

6. Santi PA: **Light sheet fluorescence microscopy: a review**. *J Histochem Cytochem* 2011, **59**:129-138.

7. Siedentopf H, Zsigmondy R: **Über Sichtbarmachung und Größenbestimmung ultramikroskopischer Teilchen, mit besonderer Anwendung auf Goldrubingläser**. *Ann Phys* 1903, **315**:1-39.

8. Voie AH, Burns DH, Spelman FA: **Orthogonal-plane fluorescence optical sectioning: three-dimensional imaging of macroscopic biological specimens**. *J Microsc* 1993, **170**:229-236.

9. Buytaert JA, Dirckx JJ: **Design and quantitative resolution measurements of an optical virtual sectioning three-dimensional imaging technique for biomedical specimens, featuring two-micrometer slicing resolution**. *J Biomed Opt* 2007, **12**:014039.

10. Fuchs E, Jaffe J, Long R, Azam F: **Thin laser light sheet microscope for microbial oceanography**. *Opt Express* 2002, **10**:145-154.

11. Dodt HU, Leischner U, Schierloh A, Jahrling N, Mauch CP, Deininger K, Deussing JM, Eder M, Zieglgansberger W, Becker K: **Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain**. *Nat Methods* 2007, **4**:331-336.

12. Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer EH: **Optical sectioning deep inside live embryos by selective plane illumination microscopy**. *Science* 2004, **305**:1007-1009.

13. Huisken J, Stainier DY: **Even fluorescence excitation by multidirectional selective plane illumination microscopy (mSPIM)**. *Opt Lett* 2007, **32**:2608-2610.

14. Swoger J, Verveer P, Greger K, Huisken J, Stelzer EH: **Multi-view image fusion improves resolution in three-dimensional microscopy**. *Opt Express* 2007, **15**:8029-8042.

15. Verveer PJ, Swoger J, Pampaloni F, Greger K, Marcello M, Stelzer EH: **High-resolution three-dimensional imaging of large specimens with light sheet-based microscopy**. *Nat Methods* 2007, **4**:311-313.

16. Keller PJ, Schmidt AD, Wittbrodt J, Stelzer EH: **Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy**. *Science* 2008, **322**:1065-1069.

17. Keller PJ, Stelzer EH: **Digital scanned laser light sheet fluorescence microscopy**. *Cold Spring Harb Protoc* 2010, **2010**: pdb top78.

18. •• Fahrbach FO, Simon P, Rohrbach A: **Microscopy with self-reconstructing beams**. *Nat Photonics* 2010, **4**:780-785.
The authors demonstrate self-reconstructing properties of holographically shaped Bessel beams in three dimensional scattering tissues, which lead to reduced scattering-induced image artifacts and increased optical penetration depth.

19. •• Planchon TA, Gao L, Milkie DE, Davidson MW, Galbraith JA, Galbraith CG, Betzig E: **Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination**. *Nat Methods* 2011, **8**:417-423.
The authors used an annular mask and an axicon to generate scanned light sheets from high aperture Bessel beams. Using structured illumination and two-photon excitation, the authors achieved a marked improvement in axial resolution, which allowed them to perform fast volumetric imaging of living cells with isotropic spatial resolution.

20. Holekamp TF, Turaga D, Holy TE: **Fast three-dimensional fluorescence imaging of activity in neural populations by objective-coupled planar illumination microscopy**. *Neuron* 2008, **57**:661-672.

21. Tokunaga M, Imamoto N, Sakata-Sogawa K: **Highly inclined thin illumination enables clear single-molecule imaging in cells**. *Nat Methods* 2008, **5**:159-161.

22. Dunsby C: **Optically sectioned imaging by oblique plane microscopy**. *Opt Express* 2008, **16**:20306-20316.

23. Fahrbach FO, Rohrbach A: **A line scanned light-sheet microscope with phase shaped self-reconstructing beams**. *Opt Express* 2010, **18**:24229-24244.

24. • Palero J, Santos SI, Artigas D, Loza-Alvarez P: **A simple scanless two-photon fluorescence microscope using selective plane illumination**. *Opt Express* 2010, **18**:8491-8498.
The authors used near-infrared lasers to achieve two-photon excitation with light sheets in a SPIM set-up (2p-SPIM). They performed optical sectioning of *C. elegans* as a proof-of-principle application of their implementation.

25. Buytaert JAN, Dirckx JJJ: **Tomographic imaging of macroscopic biomedical objects in high resolution and three dimensions using orthogonal-plane fluorescence optical sectioning**. *Appl Optics* 2009, **48**:941-948.

26. Santi PA, Johnson SB, Hillenbrand M, GrandPre PZ, Glass TJ, Leger JR: **Thin-sheet laser imaging microscopy for optical sectioning of thick tissues**. *Biotechniques* 2009, **46**:287-294.

27. Schacht P, Johnson SB, Santi PA: **Implementation of a continuous scanning procedure and a line scan camera for thin-sheet laser imaging microscopy**. *Biomed Opt Express* 2010, **1**:598-609.

28. •• Keller PJ, Schmidt AD, Santella A, Khairy K, Bao Z, Wittbrodt J, Stelzer EH: **Fast, high-contrast imaging of animal development with scanned light sheet-based structured-illumination microscopy**. *Nat Methods* 2010, **7**:637-642.
The authors introduced incoherent structured illumination to scanned light sheet microscopy (DSLM-SI) to enhance the image contrast in partially opaque specimens and to eliminate image artifacts arising from light scattering. They applied DSLM-SI to live imaging of zebrafish development for three days and fast imaging of early *Drosophila* embryogenesis, achieving subcellular resolution. The authors performed a computational reconstruction of the *Drosophila* recording, creating a digital fly embryo.

29. Mertz J, Kim J: **Scanning light-sheet microscopy in the whole mouse brain with HiLo background rejection**. *J Biomed Opt* 2010, **15**:016027.

30. Neil MA, Juskaitis R, Wilson T: **Method of obtaining optical sectioning by using structured light in a conventional microscope**. *Opt Lett* 1997, **22**:1905-1907.

31. Lei M, Zumbusch A: **Structured light sheet fluorescence microscopy based on four beam interference**. *Opt Express* 2010, **18**:19232-19241.

32. Turaga D, Holy TE: **Miniaturization and defocus correction for objective-coupled planar illumination microscopy**. *Opt Lett* 2008, **33**:2302-2304.

33. • Engelbrecht CJ, Voigt F, Helmchen F: **Miniaturized selective plane illumination microscopy for high-contrast in vivo fluorescence imaging**. *Opt Lett* 2010, **35**:1413-1415.
Combining graded index lenses (GRIN) and single mode optical fibers, the authors developed a miniaturized version of SPIM (miniSPIM).

34. Friedrich M, Gan Q, Ermolayev V, Harms GS: **STED-SPIM: stimulated emission depletion improves sheet illumination microscopy resolution**. *Biophys J* 2011, **100**:L43-L45.

35. Turaga D, Holy TE: **Image-based calibration of a deformable mirror in wide-field microscopy**. *Appl Optics* 2010, **49**:2030-2040.

36. Wohland T, Shi X, Sankaran J, Stelzer EH: **Single plane illumination fluorescence correlation spectroscopy (SPIM-FCS) probes inhomogeneous three-dimensional environments**. *Opt Express* 2010, **18**:10627-10641.

37. Ritter JG, Spille JH, Kaminski T, Kubitscheck U: **A cylindrical zoom lens unit for adjustable optical sectioning in light sheet microscopy**. *Biomed Opt Express* 2010, **2**:185-193.

38. Ritter JG, Veith R, Siebrasse JP, Kubitscheck U: **High-contrast single-particle tracking by selective focal plane illumination microscopy**. *Opt Express* 2008, **16**:7142-7152.

39. • Swoger J, Muzzopappa M, Lopez-Schier H, Sharpe J: **4D retrospective lineage tracing using SPIM for zebrafish organogenesis studies**. *J Biophotonics* 2011, **4**:122-134.
The authors applied SPIM to time-lapse imaging of posterior lateral line (pLL) organogenesis in zebrafish. A retrospective tracking analysis enabled the identification of the respective progenitor lineages.

40. Scherz PJ, Huisken J, Sahai-Hernandez P, Stainier DY: **High-speed imaging of developing heart valves reveals interplay of morphogenesis and function**. *Development* 2008, **135**:1179-1187.

41. •• Arrenberg AB, Stainier DY, Baier H, Huisken J: **Optogenetic control of cardiac function**. *Science* 2010, **330**:971-974.
The authors combined transgenic expression of channelrhodopsin/halorhodopsin and live imaging using mSPIM to precisely control and image the activity of muscle cells in the developing zebrafish heart. Using a systematic activation/inhibition approach, the authors showed that the pacemaker cells converge to the sinoatrial region, and that a few dozen of these cells are sufficient to maintain a functioning heart.