Optical Sectioning Deep Inside Live Embryos by Selective Plane Illumination Microscopy

Jan Huisken,* Jim Swoger, Filippo Del Bene, Joachim Wittbrodt, Ernst H. K. Stelzer*

Large, living biological specimens present challenges to existing optical imaging techniques because of their absorptive and scattering properties. Modern life science research often requires multidimensional imaging of samples up to a few millimeters in size. Techniques that provide noninvasive (optical) sectioning, as opposed to those that destroy the sample, are indispensable for live studies. Optical projection tomography can image fixed embryos at high resolution. Magnetic resonance imaging (MRI) and optical coherence tomography (OCT) feature noninvasive imaging, but do not provide specific contrasts easily.

In optical microscopy, green fluorescent protein (GFP) and its spectral variants are used for high-resolution visualization of protein localization patterns in living organisms. When GFP-labeled samples are viewed, optical sectioning (which is essential for its elimination of out-of-focus light) is obtainable by laser scanning microscopy (LSM), either by detection through a pinhole (confocal LSM) or by exploitation of the nonlinear properties of a fluorophore (multiphoton microscopy). Despite the improved resolution, LSM suffers from two major limitations: a limited penetration depth in heterogeneous samples and a marked difference between the lateral and axial resolution.

We developed selective plane illumination microscopy (SPIM), in which optical sectioning is achieved by illuminating the sample along a separate optical path orthogonal to the detection axis (Fig. 1 and fig. S1). A similar approach in confocal theta microscopy has been demonstrated to improve axial resolution (10–12). In SPIM, the excitation light is focused by a cylindrical lens to a sheet of light that illuminates only the focal plane of the detection optics, so that no out-of-focus fluorescence is generated (optical sectioning). The net effect is similar to that achieved by confocal LSM. However, in SPIM, only the plane currently observed is illuminated and therefore affected by bleaching. Therefore, the total number of fluorophore excitations required to image a 3D sample is greatly reduced compared to the number in confocal LSM (supporting online text).

GFP-labeled transgenic embryos of the teleost fish Medaka (Oryzias latipes) were imaged with SPIM. In order to visu-
analyze the internal structure, we imaged the transgenic line Arnie, which expresses GFP in somatic and smooth muscles as well as in the heart (14). A 4-day-old fixed Arnie embryo (stage 32 (15)) is shown in Fig. 1. SPIM was capable of resolving the internal structures of the entire organism with high resolution (better than 6 μm) as deep as 500 μm inside the fish, a penetration depth that cannot be reached using confocal LSM (fig. S6). The axial resolution in SPIM is determined by the lateral width of the light sheet; for the configuration shown in Fig. 1, the axial extent of the point spread function (PSF) was about 6 μm, whereas without the light sheet it was more than 20 μm (supporting online text).

Any fluorescence imaging system suffers from scattering and absorption in the tissue; in large and highly scattering samples, the image quality decreases as the optical path length in the sample increases. This problem can be reduced by a multiview reconstruction, in which multiple 3D data sets of the same object are collected from different directions and combined in a postprocessing step (16–18). The high-quality information is extracted from each data set and merged into a single, superior 3D image (supporting online text). One way to do this is by parallel image acquisition, using more than one lens for the detection of fluorescence (18).

We collected SPIM data for a multiview reconstruction sequentially by generating multiple image stacks between which the sample was rotated. Sample deformations were avoided with a rotation axis parallel to gravity (Fig. 1). In contrast to tomographic reconstruction techniques (such as those in (4)), which require extensive processing of the data to yield any meaningful 3D information, rotation and subsequent data processing are optional in SPIM. They allow a further increase in image quality and axial resolution compared to a single stack, but in many cases a single, unprocessed 3D SPIM stack alone provides sufficient information.

We performed a multiview reconstruction with four stacks taken with four orientations of the same sample (figs. S2 and S3). Combination of these stacks (supporting online text) yielded a complete view of the sample, ~1.5 mm long and ~0.9 mm wide. In Fig. 2, the complete fused data set is shown and the most pronounced tissues are labeled. The decrease in image quality with penetration depth is corrected by the fusion process. It yielded an increased information content in regions that were obscured (by absorption or scattering in the sample) in some of the unprocessed single views.

The method of embedding the sample in a low-concentration agarose cylinder is nondisruptive and easily applied to live embryos. We routinely image live Medaka and Drosophila embryos over periods of up to 3 days without detrimental effects on embryogenesis and development. To demonstrate the potential of SPIM technology, we investigated the Medaka heart, a structure barely accessible by conventional confocal LSM because of its ventral position in the yolk cell. We imaged transgenic Medaka Arnie embryos and show a reconstruction of the inner heart surface (Fig. 3A) derived from the data set shown in Fig. 2. This reveals the internal structure of the heart ventricle and atrium. In a slightly earlier stage, internal organs such as the heart and other mesodermal derivations

![Image](https://www.sciencemag.org/content/305/5687/1008/F1.large.jpg)
tives are deeply buried in the yolk sphere, under the body of the embryo (Fig. 3B). In Fig. 3, C to E, three optical sections at different depths illustrate GFP expression in the muscles of the living heart. Fast frame recording (10 frames per s) allows imaging of the heartbeat (movies S3 and S4); similar imaging has previously only been demonstrated at stages when the heart is exposed and by cooling the embryo to reduce the heart rate (19).

To demonstrate that SPIM can also be used to image the internal structures of relatively opaque embryos, we recorded a time series (movie S5) of the embryogenesis of the fruit fly Drosophila melanogaster (Fig. 4). GFP-moesin labeled the plasma membrane throughout the embryo (20). Even without multiview reconstruction, structures inside the embryo are clearly identifiable and traceable. Stacks (56 planes each) were taken automatically every 5 min over a period of 17 hours, without refocusing or realignment. Even after being irradiated for 11,480 images in total, the embryo was unaffected and completed embryogenesis normally.

In summary, we present an optical wide-field microscope capable of imaging protein expression patterns deep inside both fixed and live embryos. By selective illumination of a single plane, the excitation light is used efficiently to achieve optical sectioning and reduced photodamage in large samples, key features in the study of embryonic development. The method of sample mounting allows positioning and rotation to orient the sample for optimal imaging conditions. The optional multiview reconstruction combines independently acquired data sets into an optimal representation of the sample. The implementation of other contrasts such as scattered light will be straightforward. The system is compact, fast, optically stable, and easy to use.

SPIM is well suited for the visualization of high-resolution gene and protein expression patterns in three dimensions in the context of morphogenesis. Heart function and development can be precisely followed in vivo using SPIM in Arnie transgenic embryos. Because of its speed and its automatable operation, SPIM can serve as a tool for large-scale studies of developing organisms and the systematic and comprehensive acquisition and collection of expression data. Even screens for molecules that interfere with development and regeneration on a medium-throughput scale seem feasible. SPIM technology can be readily applied to a wide range of organisms, from whole embryos to single cells. Subcellular resolution can be obtained in live samples kept in a biologically relevant environment within the organism or in culture. Therefore, SPIM also has the potential of being used in the promising fields of 3D cultured cells (21) and 3D cell migration (22).

References and Notes
14. Materials and methods are available as supporting material on Science Online.
23. We thank S. Enders and K. Greger for contributions to the instrumentation and F. Jankovics and D. Brunner for providing the Drosophila samples. The beating-heart data was recorded by K. Greger.

Supporting Online Material
www.sciencemag.org/cgi/content/full/305/5686/1007/DC1
Materials and Methods
SOM Text
Figs. S1 to S6
References and Notes
Supporting Online Material
Movies S1 to S5
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1 Material and methods

1.1 Setup

Figures 1 and S1 show the main components of the Selective Plane Illumination Microscope (SPIM). A series of lasers (several HeNe, one multi-line Ar-ion) provide lines for fluorescence excitation (e.g. 488 nm, 543 nm). An optical system that includes a cylindrical lens focuses the laser light to a thin light sheet. The sample is mounted in a transparent, low concentration (0.5 %) agarose gel. This agarose is prepared from an aqueous solution adequate for the sample, in our case phosphate buffered saline (PBS), providing a suitable environment for a live sample. The cylinder of agarose containing the sample is immersed in PBS, which virtually eliminates refractive imaging artifacts at the agarose surface. The cylinder containing the sample is supported from above by a micropositioning device. By using the four available degrees of freedom (3 translational, 1 rotational), the sample can be positioned such that the excitation light illuminates the plane of interest. An objective lens, detection filter and tube lens are used to image the distribution of fluorophores in the illumination plane onto a CCD camera (Hamamatsu Orca-ER, 12 bit, 1344×1024 pixels), with the detection axis arranged perpendicular to the axis of illumination. A variety of lenses (preferably designed for imaging in water without a cover slip) can be used, with magnifications ranging from 2.5× to 100×. The light sheet thickness is adapted to the detection lens, i.e. the light sheet is made as thin as possible while keeping it uniform across the complete field of view of the objective lens. Its thickness is typically between 3 and 10 µm: e.g., for a 10×, 0.30 objective lens, the light sheet beam waist can be reduced to 6 µm, and the resulting width will vary less than 42% across the field of view of 660 µm. Translations of the sample along the detection axis and successive image acquisitions deliver a three-dimensional stack of the sample’s fluorophore distribution. We generally achieve recording speeds of 1–4 planes per second at image sizes of 1344×1024 pixels and a dynamic range of 10–12 bits.
1.2 Medaka transgenic line

The transgenic line *Arnie* was generated by injecting Medaka embryos at the one cell stage with a construct containing 5 Kb of Fugu genomic region upstream of the fugu Ath5 gene and the Green Fluorescent Protein (GFP) coding region as reporter, flanked by I-Sce meganucleases recognition sites (1). The *Arnie* line shows GFP expression in the ganglion cells, driven by the Fugu Ath5 promoter, as well as in the developing muscle tissue, by an enhancer trap effect. For the experiments shown in Figs. 1, 2, and S2, four day old embryos were fixed for one hour in 4% PFA/PBS and then dechorionated. The yolk was then removed, and the embryo was mounted in low melting temperature agarose and imaged in the SPIM as described above. For the experiment shown in Fig. 3 live embryos were only dechorionated and mounted for in-vivo imaging.

1.3 Image processing outline

A single 2D slice acquired with the SPIM has a maximum size of 1344 by 1024 pixels (pixel pitch in the camera is 6.45 µm) and a nominal dynamic range of 12 bits. An axial stack is usually acquired with a step size of 0.5 µm to 5 µm between slices. For the multi-view reconstruction, multiple stacks are recorded, rotating the sample between stacks. Most multi-view data sets consist of 4-8 views with 200-300 planes per stack. The time lapse function allows consecutive recordings over time.

The data processing stages required for the fusion of our multi-view SPIM images are:

I. **Pre-processing**

   This includes cropping of the region of interest in all three dimensions (to reduce computation times), rescaling along the detection axis (to make the lateral and axial voxel dimensions equal), and rotation of the data sets.

II. **Registration**

   This is the process of aligning the different views of the sample so that features visible in more than one view overlap spatially. For the purpose of the registration, the stacks are high-pass filtered (to reduce background-induced artefacts) and cross-correlated. The position of the resulting correlation peak determines the translation that is applied to register the pre-processed images obtained in step I.

III. **Fusion**

   The final stage is to fuse the pre-processed and registered views into a single, optimal image, i.e. to extract the high resolution features from each view and combine them into a single data set. The data sets were fused by:

   a) Fourier transforming the individual views, yielding complex value data sets.
b) For each spatial frequency (i.e. each voxel in Fourier space), we select the (complex) value from the view with the largest magnitude, and insert it into the new, fused data set.

c) Inverse Fourier transforming to obtain the final, fused image.

Because the data stacks were quite large (there are $1201 \times 659 \times 688$ voxels in the data sets shown in Figs. 1, 2, and S2), the fusion was done sequentially on smaller sub-regions from which the final data set was assembled as a 3D mosaic. This not only simplifies the processing of large data sets, but also permits different views to determine the weighting of the same spatial frequency in the different sub-regions of the sample. Thus only the high-information-content portions of each view contribute to the final fusion.

If the data sets overlap sufficiently in the multi-view reconstruction, the lateral resolution determines the axial resolution, i.e. ideally the multi-view reconstruction compensates the poor axial resolution from any single view with information from others, and provides a nearly isotropic resolution (2).

Figure S2 shows four pre-processed data sets projected along two axes. The combination of these stacks yielded a complete view of the sample, ca. 1.5 mm long and ca. 0.9 mm wide (Fig. 2). Regions in the single views that contributed most to the final result were those requiring minimal optical path lengths inside the sample. The drop in image quality with penetration depth is compensated by fusing the multiple views. Figure S3 shows volume renderings of the individual views and the fused data.

The above algorithm is non-iterative, which makes it possible to implement it in a reasonably computationally efficient form. For the processing of the images presented in this work, the algorithms were implemented in MATLAB 6.1, running on a 1.8 GHz Windows 2000 based personal computer. The total processing time required to produce the fusion shown in Figs. 2 and S2 was $\approx 24$ hours; however, the processing time can be reduced considerably by implementing the algorithms in an optimized, compiled computer language.

The above processing algorithm is in principle applicable to other optical microscopies. However, the traditional method of mounting the sample between a glass slide and a cover slip mean that recording stacks from multiple directions is not generally practical.
2 Supplemental results and discussion

2.1 Lateral resolution

The SPIM provides high resolution throughout thick samples which cannot be imaged with high NA lenses because of their intrinsic short working distances. It is not intended to replace traditional confocal or deconvolution microscopes for applications involving, e.g., thin (<10µm), flat cultured cells.

The lateral resolution of the SPIM is limited either by the NA of the detection lens or the pixel size of the camera. For the data set presented in Figs. 1, 2, and S2 an area of $1.5 \times 0.85$ mm$^2$ is imaged with a 1.4 megapixel camera. In this case, the lateral resolution of the detection lens (1.1µm for the 5×, 0.25 lens) is not fully exploited and the images are undersampled (3). However, SPIM technology can be applied to any magnification and NA. Water dipping lenses with working distances of 1–3 mm (NA $\approx$ 0.3–1.0) are particularly well suited for the current implementation of the SPIM.

The primary niche of the SPIM is in imaging thick, intact samples such as whole embryos of 100s of µm to mm in size, which are generally imaged with relatively low magnification and NA (e.g. 5×, NA = 0.25 in Fig. 2). However, it is also possible to use the technique for higher magnification and resolution imaging, as demonstrated in Fig. S4, which shows slices from a single-view SPIM stack. Here the pole cells of a Drosophila embryo in the cellular blastoderm stage are imaged with a NA = 0.8 water dipping lens at a resolution well below 1µm. The individual cell membranes, and the distribution of the spherical pole cells on top of the hexagonal somatic cells and the cortex are clearly visible. Although there is some degradation of image quality with depth (the side of the sample on which the illumination is incident, i.e. the bottom in Fig. S4A, is sharper than the opposite side), even without multi-view image fusion the optical sectioning provided by the SPIM allows imaging with high resolution of the interior of this optically diffuse embryo.

2.2 Light sheet thickness

There are two distinct yet related aspects of the light sheet dimensions that are relevant to the SPIM. First of all, the light sheet provides the optical sectioning in the SPIM, and the extent of this sectioning capability is dependant on the thickness of the light sheet. Secondly, the light sheet significantly improves the axial resolution if it is thinner than the axial extent of the detection PSF. The axial resolution is then dominated by the light sheet thickness and not by the detection lens NA.

It is important to note, however, that even if the light sheet is thicker than the axial extent of the objective PSF, it can still significantly improve the resolution in a thick fluorescent sample. This is because in practice the resolution is affected by the image contrast (3). In a 500µm thick sample imaged in the SPIM with a 10µm wide light sheet, the contrast will be improved by a factor of up to 50× compared to imaging with uniform illumination. In addition to this, in the SPIM a further increase in resolution can be obtained by multi-view reconstruction.
For optimal performance the light sheet thickness is adapted to the detection optics. Ideally, the NA of the illumination system is such that the light sheet has a uniform thickness across the full field of view of the camera. For example, with the 10× detection lens the SPIM has a field of view of 660 µm. A light sheet can be formed that has a thickness of between 5.8 µm and 8.2 µm across the field of view in such a system. This significantly reduces the axial extent of the system PSF from 14 µm to about 7 µm. By multi-view reconstruction this can theoretically be further reduced to ≈ 1 µm. A high-NA lens such as the 100×, NA = 1.0 has an axial PSF width of 1.08 µm. The optimal light sheet (thickness variation < 42% over the field of view) for this lens has a thickness of 0.95 µm. In this case, while the light sheet does not significantly decrease the size of the PSF, it can still contribute to the image quality by providing optical sectioning. The profile of the light sheet that is used for the 5× lens is shown in Fig. S5, in which the optical sectioning of the SPIM is readily apparent.

Variants of light sheet illumination have been utilized in oceanography to image bacteria (4) following an idea introduced by Siedentopf et al. (5) and in 3D light scanning macrography to scan the surface of small specimens (6).

2.3 Comparison with confocal microscopy

Optical sectioning in fluorescence microscopy has been obtained in the past mainly by confocal laser scanning microscopy (CLSM) (7). The limited working distance of high numerical aperture (NA) objective lenses, which are required for high-resolution imaging, and the severe drop in signal intensity with increasing depth in heterogeneous specimens are responsible for the limited accessible depth when imaging with a CLSM. For example Hecksher-Sørensen et al. (8) had to generate as many as 24 physical sections 70 µm thick to obtain a full expression pattern in mouse using a CLSM. A multi-photon microscope can image at greater depths, but at the expense of lower resolution and higher focal plane bleaching. A spinning-disk microscope (7) provides images at a much higher speed than the beam-scanning LSM but still inherits its other drawbacks. Confocal theta microscopy, which is similar to the SPIM in its orthogonal illumination and detection arrangement, has been demonstrated to improve the axial resolution (9).

In a CLSM, fluorescence light is collected during the time the focal spot rests at each pixel (1–10 µs). In contrast, a sensitive CCD camera is used in the SPIM to detect fluorescence. Integration times of 0.1 s to 1 s mean that the laser intensity can be decreased, reducing the effects of fluorophore saturation (7). As an example situation of interest, we compare the power densities used in the imaging for Fig. S6. For the confocal images shown in (A) and (C) we estimate a confocal spot size in the sample of ≈ 1.3 µm and a total power of 250 µW, which means a power density of ≈ 20 kW/cm². For the SPIM images in (B) and (D) the light sheet was ≈ 10 µm × 5 mm and the power ≈ 5 mW, making the power density ≈ 10 W/cm². It is clear that although the confocal microscope may suffer from saturation, the SPIM power density is still more than 3 orders of magnitude lower.

Moreover, in a CLSM the process of imaging a single plane illuminates the entire volume
of the sample. When a stack of images is required to determine the full 3D fluorophore distribution in a thick sample, excessive photo-bleaching can occur because the entire sample is illuminated many times. In contrast, in the SPIM only the plane currently being observed is illuminated, and is therefore affected by bleaching. The total number of fluorophore excitations required to image a 3D sample is therefore greatly reduced. Even though a total of 1000 images were taken to generate the data shown in Fig. 2, photo bleaching was not noticeable due to the economical use of excitation light and the efficient collection of fluorescence light.

Selective plane illumination intrinsically provides optical sectioning, since no out-of-focus light is generated. The net effect is similar to that achieved with a CLSM. However, in the CLSM out-of-focus light is generated and rejected by the pinhole. Moreover, in the CLSM the overall signal decreases as the focal plane is moved deep into scattering tissue, because aberrations cause the confocality to fail. Scattering of the illumination in any direction will degrade the confocal image quality. In contrast, in the SPIM only scattering of the illumination in one dimension (along the detection axis) causes the broadening of the light sheet that can deteriorate the image quality. Moreover, the low NA used in the illumination ensures that aberrations in the illumination process are minimal (7).

In Fig. S6 we illustrate the differences between confocal and SPIM imaging of Medaka fish embryos. Although the confocal gives excellent resolution near the surface of the sample, the penetration depth is minimal, and very little can be determined regarding the interior structure of this sample (note that contrast enhancement by using $\Gamma = 0.5$ was required to make any internal structure visible at all). If one were solely interested in surface features, the confocal system would be ideal, and the resolution could be further improved by using a higher NA objective lens. However, this would absolutely preclude imaging the entire sample because currently available high NA lenses do not have sufficient working distance. In contrast, in the SPIM images one can see details of the structure throughout the sample, although there is naturally some degradation of the resolution towards the center of the embryo.

2.4 Penetration depth and aberrations with SPIM

Depending on the optical properties of the sample there will be aberrations both in the illumination and detection processes. The image quality is degraded the deeper one penetrates into the sample. As for any other microscope, this is true for single data stacks taken with the SPIM. However, in the SPIM we can compensate for these effects by multi-view image fusion. For a given region of the sample, if high resolution information is available in at least one view, the reconstruction algorithm will favor this over the low resolution information in other views. The outcome is a high resolution over a much larger volume than in a single unprocessed stack. If the penetration depth is at least half the thickness of the sample, high resolution throughout the whole sample can be obtained by multi-view combination.

If the illumination beam is scattered or absorbed by features in the sample, shadowing along the illumination direction can appear. These effects can be present in all optical microscopies; however, in the SPIM these effects can be more pronounced because the illumination is colli-
mated, rather than being incident on the sample from many directions. Multi-view combination can compensate for these artifacts, at least in part.
Fig. S1. Basic components of the SPIM. Laser light emanating from a fibre is collimated. A cylindrical lens focuses the light in one dimension and forms a light sheet that penetrates the sample. This plane of illumination is then imaged onto a camera by a microscope objective lens and a tube lens. The fluorescence emission filter rejects scattered excitation light and selects the spectral detection band. See also Fig. 1.
**Fig. S2.** Medaka embryo (same as in Figs. 1 and 2) imaged in the SPIM with different orientations. The sample was rotated mechanically and for each orientation (0°, 90°, 180°, 270°) a stack was recorded. The stacks were then re-oriented in the computer to align them with the stack recorded at 0°. Lateral (A-E) and dorsal-ventral (F-J) maximum projections are shown. Particularly well resolved are parts that were close to the detection lens and facing the illumination plane (arrow heads). E.g. the left eye is best resolved in orientation 0° (F) whereas the right eye is best seen in view H (180°). The fusion of these four data stacks yields a superior representation featuring similar clarity and resolution throughout the entire specimen (E,J). The image combination procedure inherently favors well resolved and bright over poorly resolved and less well visible features. Images were taken with a Zeiss Fluar 5×, 0.25 objective lens.
Fig. S3. Volume rendering of the data sets shown in Fig. S2 in an anterior orientation. The four pre-processed data sets are shown on the outside, and the fused image stack is in the center. The fused data set represents a complete image of the fish and all details from the individual data sets are preserved.
Fig. S4. Pole cells of a Drosophila embryo in the cellular blastoderm stage imaged in the SPIM. Two individual slices of unprocessed data (single view, no multi-view combination) are shown: x-y-slice (A) and x-z-slice (B) with z being parallel to the detection axis. Pixel size is 0.16µm, plane spacing is 1µm. (B) has been scaled by a factor of 6.2 along z to give an aspect ratio of 1:1. Objective lens: Zeiss Achromat 40×, 0.8. The organism and the labelling are the same as the one shown in Fig. 4.
Fig. S5. Sectioning performance of the SPIM in reflection mode. The image of a mirror surface is shown, taken with the Fluar 5 ×, 0.25 lens with (A) plain illumination (lamp) and (B) SPIM illumination. The configuration is shown in the inset (C). In (A) the large depth of focus and the lack of sectioning is obvious. In contrast the SPIM provides sectioning and reduces the depth of focus (B). (D) shows the profile of the light sheet: the FWHM is 6.5 µm.
Fig. S6. Projections (A,B) and slices (C,D) from 3D reconstructions of the head region of a Medaka *Arnie* embryo, taken with a confocal microscope (A,C) and with the SPIM (B,D).

(A,C) The sample was imaged in an inverted Zeiss LSM 510 with a C-Apochromat 10×, 0.45W. Excitation wavelength 488 nm, detection filter LP510 nm. The direction from which the sample was imaged is indicated by the arrow. (A) Maximum value projection, (C) single slice, $\Gamma = 0.5$.

(B,D) Fusion of four SPIM views. For all of the individual views, both the illumination and detection axes lie in the plane of the image shown, and the rotation axis was perpendicular to the image. Objective lens: Fluar 5×, 0.25; excitation at 488 nm, detection filter: BP500-550 nm. (B) Maximum value projection, (D) single slice.
4 Movies

Movie S1. Movie comparing the wide-field (left), SPIM (center), and multi-view SPIM maximum projections (right) of the Medaka embryo shown in Fig. 1, 2, and S2.

Movie S2. 3D rendered movie of the Medaka fusion shown in Fig. 2.

Movie S3. Focussing through a Medaka embryo from dorsal to ventral as shown in Fig. 3. The recoding frame rate was 6.6 fps. It is shown at 10 fps.

Movie S4. Beating heart of a Medaka embryo. Same as in Fig. 3 and Movie S3. The recoding frame rate was 10.7 fps. It is shown at 10 fps. The sum of each row is shown on the right as it changes periodically over time.

Movie S5. Time-lapse movie of the Drosophila embryogenesis of which selected frames are shown in Fig. 4
References and Notes


10. The data set of the beating heart has been recorded by K. Greger. We wish to thank J. Beaudouin and J. Ellenberg for help on the confocal microscope. We gratefully acknowledge contributions to the instrumentation by S. Enders and K. Greger. We wish to thank F. Jankovics and D. Brunner for providing the Drosophila samples.