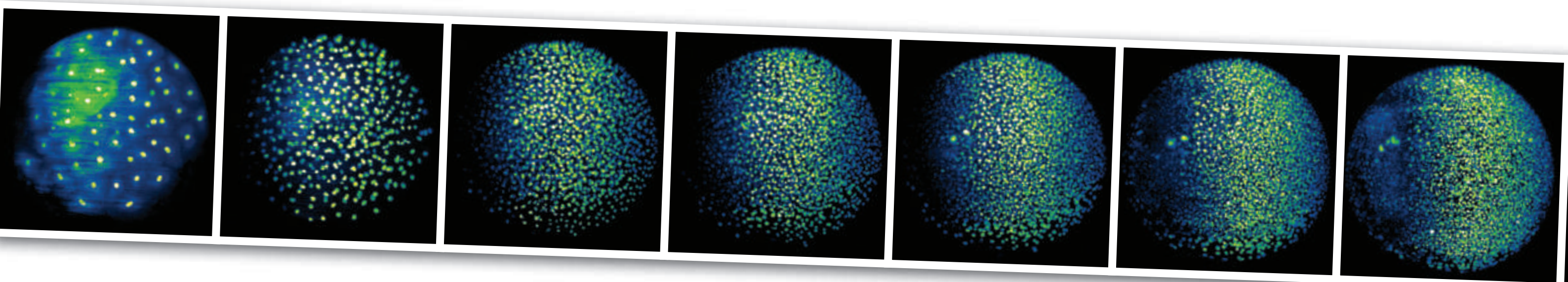
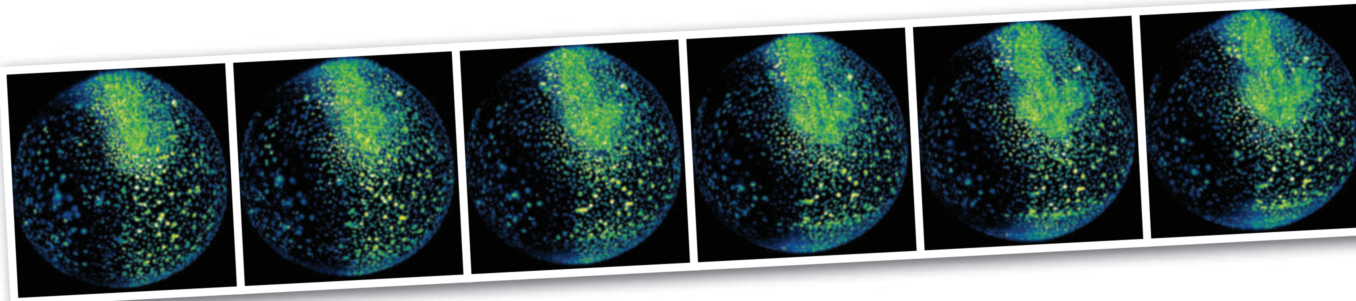


SEEING THE SYSTEM



P. KELLER, A. SCHMIDT

"We don't pretend to have invented new physics," says Ernst Stelzer modestly, standing next to an equally modest layout of lasers, mirrors and lenses. "It has all just been plain common sense."

Stelzer has been applying his common sense to microscopes ever since he arrived at the European Molecular Biology Laboratory in Heidelberg, Germany, in 1983 as a fresh-faced physics PhD student, and stayed on to head a team developing three-dimensional light microscopy. A quarter of a century in the field has now led to single plane illumination microscopy (SPIM), the technique with a modest face but an extravagant view: beautiful and unprecedented moving images of whole organisms as they grow one cell division at a time.

When Stelzer started out in the 1980s, a method called confocal fluorescence microscopy was beginning to show huge potential. The technique exploited the labelling of molecules in cells with a fluorescent tag to build up a three-dimensional image. But it still relied on traditional light microscopy procedures by which the biological sample is attached to a two-dimensional slide and a beam of light passes through the lens and through the entire sample, stimulating fluorescent emission that is detected by a camera.

Two problems with this approach bothered Stelzer. First, the squashed, two-dimensions of the slide were an unnatural environment for cells that live in three-dimensional tissue. And second, fluorescent microscopy slowly destroys the very thing that researchers are trying to make visible. The undiscriminating beam illuminates and triggers fluorescence from the entire sample, not just the focal plane. Yet photons are damaging to cells, which — apart from those in skin, eyes or other body surfaces — receive little daylight. Light also 'bleaches' the fluorescent tags, which limits the number of times that the sample can be observed.

> "This idea had been around for a century, but biologists didn't realise its potential."
> — Ernst Stelzer

Stelzer determined to develop a microscope that could visualize living biological samples, for long time periods, in conditions that approximate normal physiology. To optimize the use of light, he turned a laser-light source at 90° to the camera and lens. In 2002, he added the element that is central to SPIM — mechanics that create a micrometre-thin sheet of light in a single plane that sweeps gradually through the specimen (see graphic). "This idea had been around for a century," he says, "but biologists didn't realize its potential for high-resolution." Only the fluorescent tags in this plane of light are excited, and the camera efficiently captures the photons emitted. The specimen is then rotated and the procedure repeated along half a dozen or so additional axes. Eventually all the planar images are merged together computationally into a three-dimensional whole. By eliminating excess illumination and dye photobleaching, the whole process can be repeated every two minutes or less for more than 24 hours. The specimen to be viewed — intact, in three dimensions — passes the time in a tiny, transparent cylinder filled with agarose gel to dampen any movement and perfused with physiological levels of gases such as oxygen and carbon dioxide.

Stelzer, working with colleague Joachim Wittbrodt, has shot spectacular movies of the first day in the life of a zebrafish embryo, a species loved by microscopists for its transparent skin (P. J. Keller *et al. Science* **322,** 1065–1069; 2009). As development is triggered, a single oval cell undulates, seeming to squeeze out additional complexity. More

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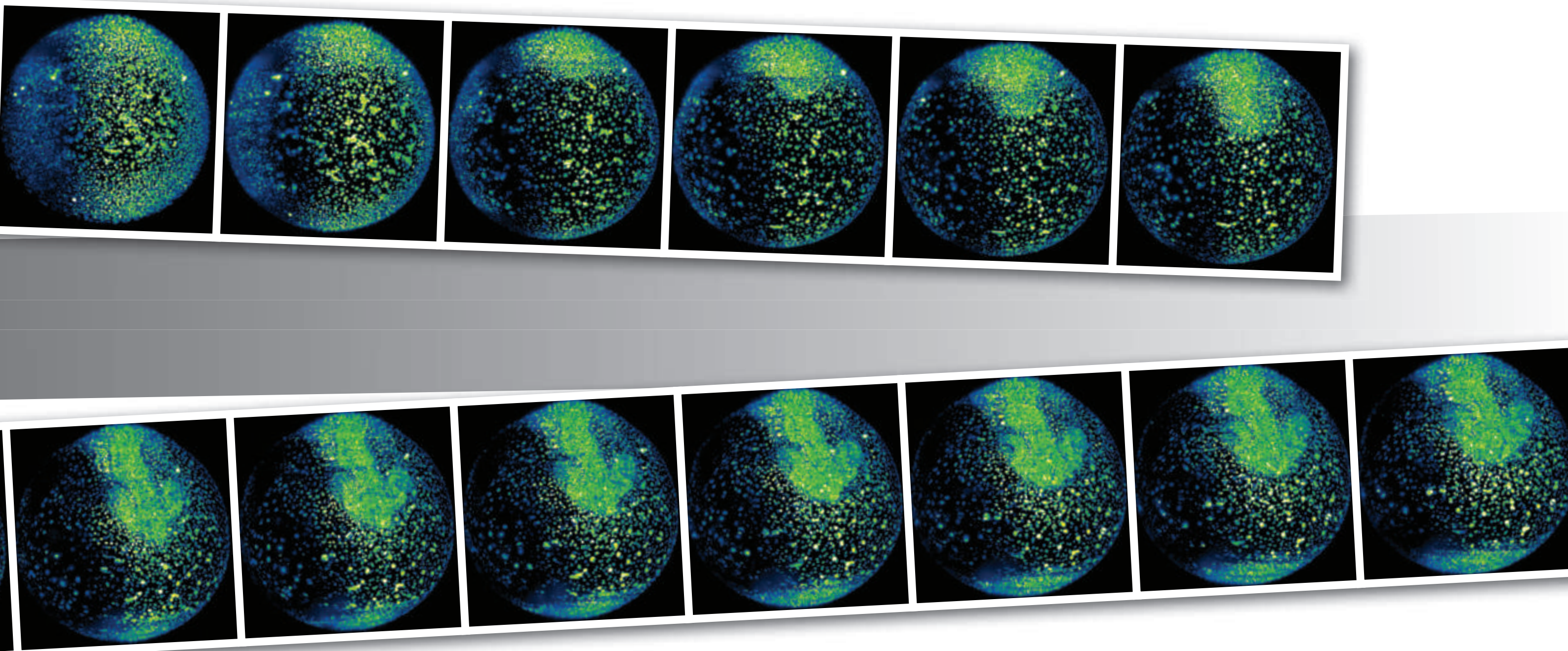
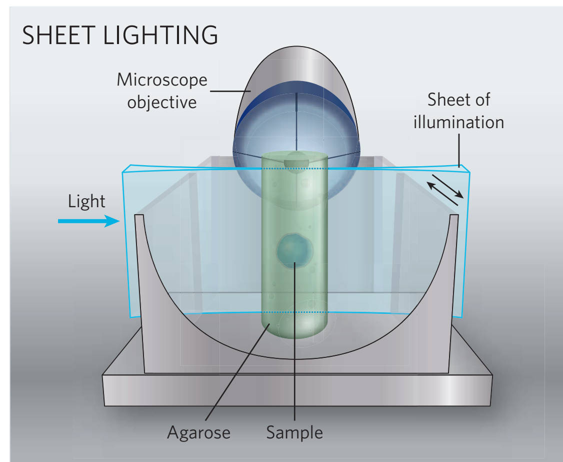


Hourly snapshots taken during during zebrafish embryo development, starting 100 minutes after fertilization (top left).

and more new cells shoot out along purposeful paths in a coordinated and symmetrical way. Emerging shadowy shapes soon become recognizable as organs. It all happens at a dazzlingly swift and confident pace until the one cell has multiplied to some 20,000 cells.

"It gives you a good feeling to see what is happening — you understand things more intuitively," says Carl-Philipp Heisenberg, from the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany. But Heisenberg says that the real wonder is in the data rather than in the movies. "That is where the real information is hidden — it is immensely helpful that the technique allows you to track all of the cells all of the time." For developmental biologists interested in tracking the destiny of each and every cell, these global, dynamic data are a boon, he says.



Stelzer's dream would take this further — into the realm of systems biology. He sees a future in which movies of thousands of developing embryos, each with one gene mutated, are recorded and compared. "Now we can think about systematizing the process to see the consequences of every possible gene mutation on development of the embryo, or of organs — or the effect of small changes in conditions such as temperature or acidity," says Stelzer. "It's a systems-biology microscope," agrees Wittbrodt, who talks about mapping the expression of genes on to the system too.

The process is computationally very demanding. Twenty-four hours of data for the zebrafish embryo took around 1,000 hours to process using their system. Stelzer is working on robust hardware and software so the microscope can be used by non-specialists. He and others are also refining the ability of SPIM to record movies of biological processes in real time. "Already you can see how an organ such as the beating heart develops, or how blood vessels develop, in the embryo", says developmental geneticist Didier Stainier, who is working with Stelzer's former PhD student Jan Huisken at the University of California, San Francisco.

Back in Heidelberg, Keller puts an adult fly into the container and flicks a switch. A fuzzy image of its eye, from a single-plane shot, appears on the computer screen. A few seconds later he flicks another switch to line up the images from hundreds of planes, and an intensely detailed view of the eye materializes like a rabbit from a magician's hat. But "it is not magic", says Stelzer. "It's simple technology." ■

Alison Abbott

See also page 629 and online at http://tinyurl.com/microspecial.

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