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Microscopic marvels

Microscopes are changing the face of biology. Researchers should innovate and collaborate if they want to be part of the new vision.

Watching molecular-scale events unfold in a living cell can be an inspiring experience. The inner workings of the nucleus, the shuttling of cellular cargo, the passage of messages through a membrane — seeing this tumultuous activity up close can fire the scientific imagination in a way that abstract data from genetic sequences or chemical analyses can never quite equal.

This helps to explain why microscopes are such essential tools in science, and why scientists' desire to see more is driving rapid innovation in the field. Five microscopes representative of these innovations are featured in this issue, starting on page 629. They range from 'super-resolution' devices that use light to reveal details once visible only with electron microscopes, to an electron microscope that can peer into thick samples once accessible only to optical ones.

Few of these new imaging technologies come cheaply, however. One new-generation light microscope can easily cost US$500,000 or more, not counting the staff and training required to use it. There is no room for error on an instrument so sensitive that the slightest vibration or misstep in the experimental set-up creates artefacts.

As a result, biologists may have to get used to sharing their instruments. Research groups often prefer to buy their own microscopes so that lab members can use them whenever they want. But to stay at the forefront of image-led science, they will increasingly have to work with their departments, universities and funding agencies to create shared microscopy facilities staffed by specialists.

Getting engineers into the mix will be a good idea, and computational biologists will be a must. Automation, computer processing and image reconstruction are now central to microscopy and imaging. This means that there is increasing separation between the sample placed in the microscope and what scientists actually see, and that there are numerous points at which inaccuracies can inadvertently be introduced. The only way to interpret the images the system spits out is to understand (and explain alongside the final results) precisely what went into their creation. Not that this problem is new: scientists have struggled with how to interpret microscope images since the instrument's earliest days (see page 642).

As well as sharing microscopes, cell and molecular biologists will need to share the instruments' output. A good way to do that would be through a central repository or framework for biological images modelled on existing data repositories such as GenBank. Such a resource would not only help to make the images universally available — using widely accepted data standards — but could be a driver for the development of analysis techniques that extract deeper meaning from existing images. A database launched last year by *The Journal of Cell Biology* (http://jcb-dataviewer.rupress.org) is already a start towards this goal. Such experiments deserve the community's support and participation.

"The era of systems microscopy could soon be here."

Meanwhile, some labs are exploring an alternative to the fat-price-tag rule: instead of buying one machine for $500,000, buy 50,000 microscopes for $10 apiece. By creating microscopes that are small, cheap and even disposable, these researchers hope to accelerate the development of microscopy into a high-throughput, automated procedure that can quickly collect data on living cells as systematic changes are made to one gene, protein or receptor at time. The era of systems microscopy could soon be here.

The wonderful thing about thinking visually is that it is so easy to think big. And that's exactly what researchers should do as they move ahead. By thinking about what they really want to see, they will help to devise microscopes — as well as computation, labelling and sample-preparation techniques — that make that vision possible. Thinking big might lead to microscopy that allows individual molecules to be tracked across thousands of cells in real time in living tissue; that can watch a single cell over the months or years that lapse from birth to death; or that can map the intricate form of every neuron in the brain across multiple species. The deeper biologists look, the more they will find there is to see. ■

MAGNIFYING POWER

New microscopes are revealing sights that have never been seen before. *Nature* profiles five machines that are changing how biologists view the world.

Close-ups of cork, lice and fly's eyes do not inspire the rhapsodies that they did more than 300 years ago when Robert Hooke first observed them under a microscope. But other pictures do — the boughs and twigs of a branching neuron in its forest; the scuttle of vesicles delivering molecular loads; the endless thrill of a cell carving itself in two again — and again, and again — as an embryo buds into being. Now, as then, microscopy is central to the understanding of living systems. In this special section, *Nature* reporters look at five microscopes that are resolving aspects of life in stunning new ways.

Microscopes today still do the job that Hooke asked of his: gathering information on details that the human eye cannot resolve and magnifying them to a size that it can.

P. KELLER, A. SCHMIDT; G. MARSHALL; K. YOSHIDA/UHVEM, OSAKA UNIV.; B. G. SAAR, Y. ZENG; K. WILLIG, V. NÄGERL, N. URBAN, T. BONHOEFFER, S. W. HELL/MAX PLANCK INST. BIOPHYS. CHEM.

The microscopes that greet undergraduate biologists in university teaching labs are remarkably similar to those that Carl Zeiss developed in the nineteenth century. On that basis of continuity, one might think that the technology was mature. But although the laws of optics have not changed, the ways they are applied are in constant flux.

Part of the flux is due to the ingenuity of engineers and scientists. Applying the laws of optics to electrons, rather than to light, was possibly the greatest development in microscopy of the twentieth century — and it is still yielding dividends in the twenty first, as the ultrahigh-voltage electron microscope at Osaka demonstrates (page 634). Recently some of that ingenuity has explored the possibility of doing with electronics what used to be done with carefully crafted glass, producing technologies that do away with lenses altogether. The microscope-on-a-chip featured here could turn microscopes into a disposable commodity (page 632).

But perhaps the greatest potential for progress is not so much in the engineering of microscopes, as in the engineering of what can be seen with them. Hooke and his successors used the microscope to see the world as it was, revealing seemingly miraculous detail at scales far too fine for human craft — powerful evidence, so it seemed, for the infinite superiority of divine craftsmanship.

Today's microscopes are used more and more to look at systems that are carefully prepared to make their workings visible. The dramatic fruits of this transformation can be seen in the green-fluorescent-protein revolution of the past decades, which has made it possible to engineer appropriate illumination into organisms under study. Its full impact will only be realized when microscopes can capture everything that these organisms can reveal.

This is why there is so much excitement around the arrival of the stimulated emission depletion (STED) microscope (page 638) and other 'super-resolution' techniques that are allowing light microscopy to resolve details on the nanometre scale, something once thought impossible. And the unassuming single plane illumination microscope (SPIM) microscope, with its way of imaging life without killing it, could herald an era of 'systems microscopy' (page 630). New ways of manipulating life will supply — and indeed demand — new ways of seeing what is going on. At the same time, there will also be those who prefer to make observations without the interference of labels. For those researchers, there are improved ways to identify molecules by their intrinsic chemical properties, such as the stimulated Raman scattering microscope sitting in a Harvard basement (page 636).

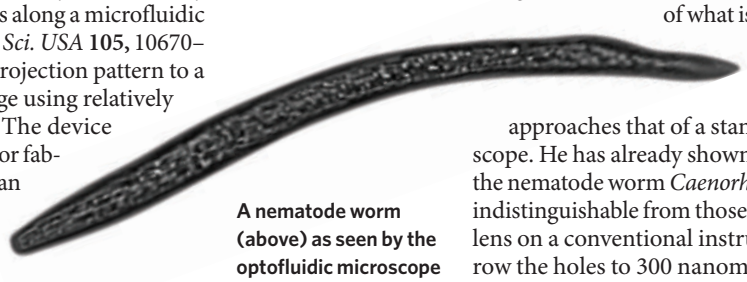
All these developments share one thing in common: computers. As tools for the construction, manipulation and distribution of images, whether moving or still, in two-dimensions or three, computers are almost as central to the microscope now as the lens. The startled eye at the eyepiece, as rendered on our cover, may be increasingly a thing of the past, as all that microscopes show comes to be seen on screen. The shock of new discovery, though, will remain — and perhaps, even, intensify — for as long as the workings of life become ever more variously and acutely examined. ■

See Editorial, page 615, Essay, page 642 and online at http://tinyurl.com/microspecial.

MICROSCOPE FOR THE MASSES

Blurry specks in the eye seem an unlikely source of inspiration for a revolutionary microscope. But 'floaters' — tiny debris that floats inside the eyeball — led Changhuei Yang at the California Institute of Technology in Pasadena to devise a microscope so small, cheap and mass-producible that it could, according to its inventor, transform the way that microscopy is done.

The human eye registers floaters when bright light casts the shadow of debris directly onto the retina. On the tiny 'optofluidic' microscope that Yang and his colleagues invented, the sample casts a shadow directly on to an array of commercial light sensors as it floats along a microfluidic channel (X. Cui *et al. Proc. Natl Acad. Sci. USA* **105,** 10670–10675; 2008). The sensors feed the projection pattern to a computer, which constructs an image using relatively simple image-processing software. The device itself is assembled using semiconductor fabrication techniques and is smaller than an American dime. When mounted into a device with a USB port so that it can transfer information to a computer, it is still just 3 centimetres square.



X. CUI *ET AL.*

A nematode worm (above) as seen by the optofluidic microscope (shown en masse and slightly larger than life, opposite, and in schematic below).

Yang says that his microscopes, which could cost as little as US$10 apiece, could have the same revolutionary impact on science that the integrated circuit has had on the electronics industry. "When people were building transistors individually, it was still a pretty expensive proposition to build circuits out of them," he says. "But the move to build integrated circuits moved the semiconductor industry forwards because you could build things comparably cheaply with high functionality. If we can start to put 10–100 microscopes on a single chip and link a bunch of them up to operate in parallel to do high-throughput processing of a large number of samples, this opens up the opportunity to do experiments you might not otherwise do."

With cheap, high-throughput imaging, researchers could perform drug assays, genomic or proteomic screens and rapidly observe the outcome of hundreds or thousands of manipulations on the shape or behaviour of living cells. "It's very clever work," says Charles DiMarzio, director of the Optical Science Laboratory at Northeastern University in Boston, Massachusetts. "This is a way of making a [high-power] microscope that is very low cost, maybe even disposable, and that's something that we haven't had before."

Yang recognized that it would be difficult to shrink the lens and other delicate optics in a high-end instrument — so his 'direct projection' technique did away with lenses altogether. Other scientists have worked out similar techniques before, but they couldn't resolve anything smaller than 5 micrometres, because that's as small as the pixels on most digital light-sensing chips get. Yang coated the sensing chips with a thin layer of metal, than punched 500-nanometre holes into the metal to create apertures that are smaller than a pixel and that are patterned along the path of the microfluidic channel (see graphic). As the sample floats along, the chip captures repeated but staggered snapshots of what is passing overhead.

With 500-nanometre holes, Yang's optofluidic microscope has a resolution that approaches that of a standard laboratory light microscope. He has already shown that it can capture images of the nematode worm *Caenorhabditis elegans* that are almost indistinguishable from those collected with a 20× objective lens on a conventional instrument. He is working to narrow the holes to 300 nanometres, a resolution capable of distinguishing the finer details of cells.

Besides transforming research microscopy, Yang's microscope could boost low-cost science and medicine in developing nations. The scope is rugged, works with sunlight, needs only the amount of computational power found in an iPod and, Yang wrote in his paper last July, might be "a boon for a health worker who needs to travel from village to village". That statement struck home for Ricardo Leitão, a postdoctoral fellow at New York University School of Medicine, who is founding a non-profit group called Tek4Dev — Science & Technology for Sustainable Development — which is putting together a tool kit to enable 'telemedicine' (using networks such as the Internet to facilitate clinical care) in poor countries. Leitão wrote to Yang on the day the paper was published to propose a collaboration. Yang, Leitão and Ana Rodriguez, a malaria researcher also at New York University, are now testing the microscope's ability to diagnose malaria-infected red blood cells based on their shape and those of the parasites inside them. Microscopy is still the standard method for diagnosing malaria, but microscopes can be few and far between in malaria-endemic areas. "Having a diagnostic tool as powerful as Yang's integrated with our hardware and 'tele' ability would be of tremendous clinical value," says Leitão.

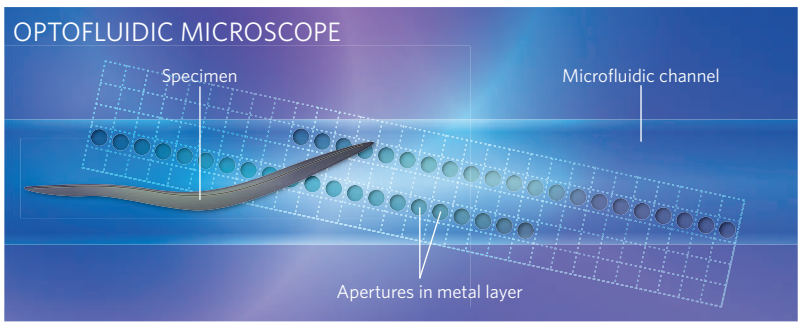
Yang admits that by trying to do more with less, he is thinking differently from many of his peers. "In the field of biomicroscopy, there is a very strong drive towards building more sophisticated microscopes, giving you ever better resolution," he says. "But I think there's another axis to pursue, which is if you're actually building this in a comparably low-cost fashion, it can create experimental formats that are currently not doable using a traditional microscope or any other high-end microscope that other research groups are pursuing."

What is certain is that by making microscopes exceedingly small, Yang has actually been thinking very, very big. ■

Erika Check Hayden

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

X. CUI *ET AL.*



G. MARSHALL