Seeing is understanding

Improvements in computer software and hardware are revolutionizing three-dimensional imaging in biology

A picture is worth a thousand words. For scientists in particular, it is often easier to draw a signal transduction pathway or a large organic molecule in order to understand it than it is to describe it using words or numbers. As human sensory equipment relies heavily on visual input, our brains find it much easier to process information from an image—the question is how to “represent data in a way that humans can analyse and understand it,” according to Forbes Dewey, Professor of Mechanical Engineering and Bioengineering at the Massachusetts Institute of Technology (Boston, MA, USA) and co-director of the International Consortium for Medical Imaging Technology.

Of course, drawing a sketch or a rough illustration of a cell membrane, a ribosome or a nuclear pore is not a problem for an experienced illustrator. But the challenge increases with the amount of information available, the level of detail required and the need to represent reality exactly—drawing a ribosome in which every single atom is shown in its correct position for a particular state would overwhelm even the best graphics department.

It is here that the computer—or, more specifically, rendering software—steps in to create such realistic images. Although architects, engineers, the movie industry and computer-game developers have used these programmes for more than two decades, biologists are only beginning to discover three-dimensional (3D) graphics software as a powerful tool to visualize molecular and cellular structures. And it does not stop there: new methods and graphics-supported modelling software that make use of the highly developed human visual system could greatly enhance the understanding of any research data. “Graphics is very important in helping to visualize [...] predictions,” said Dave Ritchie, a lecturer in the University of Aberdeen’s Department of Computing Science in the UK. Although biologists, like

their colleagues in chemistry, have long used computers to draw complex molecules or proteins at the atomic level, the increased power of today’s computers, combined with improved software to render realistic 3D images, now enables completely new applications without the need for expensive graphics workstations. “All modern PC graphics capabilities are certainly sufficient for molecular biology visualizations,” Ritchie said. “We’re exploiting the hardware.”

The theoretical foundations for creating realistic 3D images were laid as early as the 1960s, when the first ray-tracing algorithms to render virtual scenes were developed (Appel, 1968; Goldstein & Nagel, 1971). Continually improving over the years, ray-tracing software now produces highly realistic images by a process that could be described as ‘reverse seeing’. Instead of collecting the light from light sources and objects as an eye or a camera does, the computer shoots rays from a virtual camera representing the viewer’s position into a scene to calculate the colour of every pixel. If the ray does not hit an object, the pixel adopts the background colour. If the ray hits an object, the computer assigns the colour of the object to the pixel, and then creates new rays bouncing away from it, or if the object is transparent, passing through it. The process is repeated until the rays hit the background or a light source, or the number of rays exceeds a pre-determined

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limit. The cumulative colour information from all the rays is then used to calculate the final colour of each pixel.

Another method—more often used in architecture and movies—is radiosity, which creates more realistic light distribution and shadows. It first decomposes a scene into polygons and then computes how much of the light reflected from each polygon would reach other polygons. All polygons are set to black except those that represent primary light sources, then the algorithm determines how the light from each polygon causes others to be lit. The process iterates to calculate how these second-class light sources light up other polygons, and so on until things finally come to a rest when the light intensity drops below a certain level.

Although the idea behind these algorithms is relatively simple, a computer must still solve highly complex integrations to render a realistic image, which takes an enormous amount of processor time. Personal computers from the 1980s and 1990s easily took hours or even days to produce just one still image. However, improved software, more powerful computers and specialized graphic chips, which perform most of the raw number-crunching in rendering and leave the main processor free to handle other tasks, have greatly accelerated this process. According to Ritchie, it was primarily the development of sophisticated computer games with increasingly realistic 3D graphics that spurred the improvement of graphics-handling hardware.

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The Max Planck Institute for Biochemistry in Munich, Germany, is one of the beneficiaries, using commercial rendering software to create 3D images of cellular structures on the basis of cryo-electron tomography. The 'artists', led by the institute's director, Wolfgang Baumeister, shock-freeze cells and take a series of electron microscopic pictures from various angles. By aligning the individual images and removing background noise digitally, the computer first creates a density map of the cellular structures that the researchers are interested in (Nickell *et al*, 2005). The institute's image processing group, led by Reiner Hegerl, has developed its own software to perform this task almost automatically. The individual structures and proteins are then separated from each other and from the background, a step that requires considerable human expertise and can take days even if supported by several computers working in parallel. "The extraction of information can become very intricate," Hegerl said. "This still largely depends on the experience of the operator." In the last step, the scenery is rendered based on the shape and distribution of individual proteins and complexes. Their work has led to numerous images from inside the cell, such as graphic representations of protein complexes, herpes virus, nuclear pores, flagella and the actin network in an amoeba (Nickell *et al*, 2006; Nicastro *et al*, 2005; Grünwald *et al*, 2003; Medalia *et al*, 2002).

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For Hegerl, the real challenge is not the visualization itself but the extraction of meaningful information from the raw data. "Alignment, noise reduction and segmentations are the main problems we are still coping with," he said, and despite all the computer support, "[the separation] still needs to be corrected by the human eye and hand." With the ultimate aim to reconstruct the shape of all proteins in smaller cells, Hegerl's group also plans to link their software to protein databases, to search for related structures with template-matching algorithms, a task that is computationally very intensive.

Ritchie has also created his own software, called HEX, to model proteins docking with each other or with macromolecules, such as DNA, by using spherical polar Fourier correlations (Ritchie, 2003; Ritchie & Kemp, 2000). As he pointed out, "a major problem in protein docking are false positive predicted complexes," and to weed those out, HEX makes use of the human visual processing system. The software includes a rendering module, based on the OpenGL graphics standard, which creates a 3D image of the model to check the quality of the fit. "3D computer graphics are almost essential to help users assess the quality of predicted protein complexes," Ritchie said.

These applications mainly create static images and do not make much use of the ability of the software to produce animated simulations—indeed, animations of complex molecular events are still rare. Among them is a five-minute movie showing protein synthesis at the ribosome (Fig 1), created by Said Sannuga, an independent animation consultant in York, UK. "The elucidation of the ribosome and other associated molecules is a remarkable achievement in structural biology and it inspired me to produce a movie that shows the workings of all these components together to synthesize proteins," explained Sannuga. The movie, produced on a standard dual-processor PC using the Maya® animation software (Autodesk; Toronto, Ontario, Canada), is a similarly remarkable achievement for its level of detail, and took about seven months to produce, four of which Sannuga spent working with Venki Ramakrishnan at the MRC Laboratory of Molecular Biology in Cambridge, UK.

Sannuga, who holds a PhD in molecular biology from Imperial College, London (UK), trained himself in computer graphics and worked as a special effects animator before becoming an independent animator. "My interest in molecular visualization was a natural progression for any researcher who requires a deeper understanding of the theoretical side of molecular interactions," he commented. He has just finished an animation of the ATPase catalytic mechanism for Sir John Walker, Director of the Medical Research Council Dunn Human Nutrition Unit in Cambridge, UK. Indeed, a growing demand, not only from academia but also from the pharmaceutical industry, is supporting freelance animators and commercial studios who specialize in scientific and medical

animations, such as Hybrid (Minneapolis, MN, USA), Nucleus (Kennesaw, GA, USA) and Animated Biomedical Productions (Westmead, NSW, Australia).

Although such animations are mainly used for presentations, they could also become a scientific tool. "Animation will easily become a complementary approach in visualization projects as the number of resolved, large molecular complexes grows," Sannuga believes, adding that "animation technology could become a powerful tool to construct, integrate and manage complex visual scientific information." Dewey, whose work involves analysing the mechanics of the actin cytoskeleton—including visualizations (Cheng *et al*, 2000)—agrees. For example, he would like to see a movie showing the movement of myosin along an actin fibre: "The kinetics of some of these processes are immensely easier to understand with movies." Similarly, animated representations of a stochastic model of "proteins wandering around and bumping into each other" could help to better understand protein interactions and docking and to test hypotheses. Hegerl also pointed out the Max Planck Institute's similar work on understanding how macromolecules are transported through a nuclear pore into the cytoplasm, "where we try to visualize the substrate's path".

However, calculating and rendering complex events in real time requires significantly more computing power than is available in the average molecular biology department. As Dewey pointed out, basic protein-docking representations could be achieved with a single computer, but more advanced uses—such as modelling the physical dynamics of a cell membrane in real time and three dimensions—would require much more number-crunching capacity: "We're talking about tens of Silicon Graphics rendering engines working in parallel."

Visualizing molecular structures and processes may be just the beginning. Combined with new information-handling systems, computer graphics could be used "to create an environment that a person can use to extract information from very large datasets", according to Dewey. An example he gave is an information system combined with a graphical interface to track and analyse relationships between various proteins based on microarray data.

Fig 1 | Still shots from Said Sannuga's ribosome animation. Courtesy of Said Sannuga and Venki Ramakrishnan, MRC Laboratory of Molecular Biology in Cambridge, UK.

Such applications depend on smart information systems to handle and prepare different research data from many experiments—one of the projects Dewey is working on. Such systems already exist for medical applications in which data from different sources, such as X-ray images or CAT scans, are processed and displayed in various formats,

for example, to help surgeons plan an operation. As Dewey pointed out, biology could easily benefit from this work. “Right now, biology is receiving a bonus from what was done in the medical imaging area,” he said. “And it is only now that people in biology are realizing that data has to be shared and analysed.” Consequently, he expects an

explosion of new programmes in data analysis and imaging in the years to come: “It’s coming along very rapidly now. Biology, by and large, now has the funds to support that kind of effort.”

As computers become more powerful, the use of visualization software will definitely spread, Hegerl and Ritchie agree.

"For quite a long time, it was very expensive to do image creation and processing, but PCs and graphic cards are today so cheap that even small institutes can afford them," Hegerl said. In addition, improvements in the software make the technology more accessible for non-specialists. "In the future, [scientists] may become more able to do this themselves, so they no longer need a computer expert... which removes the need for people like myself," Ritchie said. However, Dewey warned that scientists should not leave the field to commercial developers but should stake their own claim. "It really is very important that all of these processes use open standards and that all software is open," he commented, to prevent monopolization by companies or the problem of competing software based on proprietary data.

Buying the software and hardware does not automatically guarantee exciting insights or fascinating animations; it still requires considerable expertise. "First you need a good idea," and experience in knowing which applications and processes are optimal for analysing and visualizing the object in question, Hegerl said. "I generally spend more time thinking about the problems than doing the calculations," Ritchie commented. Similarly, animations such as Sannuga's ribosome or the ATPase machinery are not completed within a few days. "To master an advanced software such as Maya, one would require considerable training, maybe a year or more," Sannuga commented. "Scientists with limited resources and animation skills would be able to produce less complex animations, but they would still require experts in molecular animation when producing more challenging animations."

Beyond their scientific use, these images and movies can fascinate laypeople too. "It is really impressive to be able to see into a cell," said Hegerl, describing the attraction of such images. Furthermore, the fascination and sometimes beauty of computer-generated scientific images and animations would make powerful tools for presentations and in education. "This is coming and it makes sense. I think we should use it in schools in the long term," Hegerl said, adding that the institute already regularly receives requests from universities to use their images for teaching.

It is not only educators who are interested, but also people specialized in the arts. The latest attraction at the Denver Museum of Nature and Science (CO, USA) is a 23-minute scientific animation of a flight through a black hole—to "the other side of infinity", as their website explains. The simulation, using animations created by Andrew Hamilton, Professor of Astrophysics at the University of Colorado, Boulder, is based on hundreds of hours of computing time at the University of Illinois National Center for Supercomputing Applications, to calculate how light behaves at the event horizon and inside the black hole (Johnson, 2006). If children can be thrilled by flying through a black hole, they might be equally exhilarated by flying through a cell. To this end, computer graphics are not only a great new tool for the researcher but also a way to fascinate the general public with science.

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doi:10.1038/sj.embor.7400695

The infection connection

Helicobacter pylori is more than just the cause of gastric ulcers—it offers an unprecedented opportunity to study changes in human microecology and the nature of chronic disease

When Barry Marshall drank a broth of *Helicobacter pylori* in the mid-1980s, he hoped to prove that the bacteria cause gastric ulcers, a common complaint worldwide. Sure enough, Marshall, then at the Royal Perth Hospital in Western Australia, soon developed gastritis, the precursor to stomach ulcers and gastric cancer. In addition to earning him the 2005 Nobel Prize in Physiology or Medicine with his colleague Robin Warren, this unorthodox—and now infamous—experiment proved that a bacteria caused a chronic disease that had previously been attributed to

genetic and lifestyle factors. During the same period, researchers have proposed bacterial and viral triggers for many other chronic diseases, such as cancers and coronary heart disease. But Marshall and Warren's discovery has far wider implications than curing a single disease. "Human microecology is changing, and these changes have manifestations in terms of disease," said Martin Blaser, Chair of the Department of Medicine at New York University (NY, USA). To understand the impact of these changes, researchers need to take a closer look at host-pathogen interactions—both good and bad.