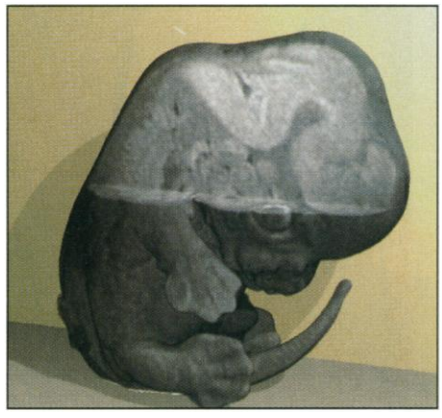
A Womb With a View

Noninvasive imaging techniques move from the clinic to the laboratory, doing for developmental biologists what Technicolor and CinemaScope did for the movies

For researchers studying how embryos develop, model organisms such as sea urchins, nematodes, and zebrafish have a clear advantage: lucidly transparent embryos that develop outside their mothers' bodies, making it easy to observe their development under a microscope. By contrast, scientists' picture of mammalian embryos, which grow deep in a darkened womb, has long been relatively opaque. Not until the invention of ultrasound imaging in the 1980s did they get their first live—albeit grainy and cryptic—pictures of fetuses romping in the womb. Now, however, mammalian embryology's dark ages may finally be coming to an end.

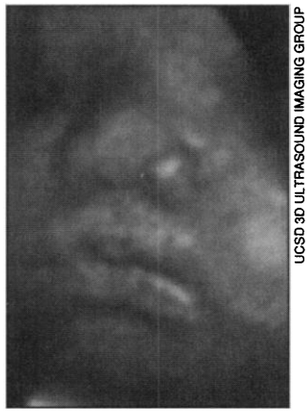
By adapting established technologies such as ultrasound imaging, confocal microscopy, and magnetic resonance imaging (MRI), researchers are developing new ways to make images of once-hidden embryos. They are also harnessing computers to represent existing molecular data on development in its anatomical context. As a result, they are getting noninvasive views of mouse embryos and preserved human embryos that are sharper, deeper, more dynamic, and—most important—more informative than ever before.

The new technologies are adding a third and a fourth dimension—depth and time—to the two-dimensional (2D) still images found in most scientific reports in embryology. "When we studied [embryogenesis] in school, all we had to look at was a series of tissue sections, and it would take weeks to figure out how they were connected," says developmental biologist Steven Klein of the National Institute of Child Health and Human Development (NICHD) in Bethesda, Maryland. "Here's the chance to take all those sections, reconstruct them into a computer model, and do this for different stages. The result is an interactive movie: You watch it from the front, from the top, and from the side, and you understand development completely."



RUSSELL E. JACOBS

Pristine perspective. MRI gives a noninvasive view of a mouse embryo's innards.



UCSD 3D ULTRASOUND IMAGING GROUP

Flawless fetus. 3D ultrasound image reveals no facial defects in this fetus.

These new imaging capabilities couldn't have come at better time, developmental biologists say. Over the past decade, advances in molecular biology have allowed researchers to identify many of the genes that drive mammalian embryogenesis. But as Sally Moody, a developmental neurobiologist at the George Washington University Medical Center in Washington, D.C., points out, "Gene function is extremely difficult to ascertain if you don't know the morphology of what's actually going on in the embryo." Now, she says, the high-resolution, three-dimensional (3D) movies of embryogenesis that can be created with the new tools are bringing about "a real melding of genetics and morphology. It's necessary, and it's wonderful."

Moody's comment captures the enthusiasm evident at a developmental imaging workshop held this September at NICHD.* Participants said the new techniques can help answer questions they couldn't even ask in the past. How do neural precursor cells fare, for example, when they are transplanted from one spot of the embryonic mouse brain to another? How do subtle malformations in an embryo's cardiac muscles disrupt blood flow through the heart? And how do the membranes and subcellular components of individual cells throb and undulate as the cells migrate to their destined locations?

Beyond such basic-science questions, the meeting even gave a few hints of the eventual practical benefits of the techniques. Physicians at the University of California, San Diego, for example, showed 3D ultrasonograms of fetal faces so clear and lifelike that some pregnant mothers have claimed to see a family resemblance—and have stopped smoking or drinking as a consequence.

New dimensions

The approaches to embryo imaging laid out at the NICHD workshop ranged from the dizzyingly high-tech—for example, miniaturized MRI machines reminiscent of Isaac Asimov's *Fantastic Voyage*—to methods not much more sophisticated than those used to make topographical maps. On the high-tech end, neurobiologist Russell Jacobs and colleagues at the California Institute of Technology in Pasadena are using MRI to add new dimensions to the atlases of development often consulted by researchers and students.

Existing photographic atlases offer 2D images of selected slices of mouse, chick, frog, and other embryos at specific times in development. Jacobs's goal is to produce 3D versions of these 2D atlases, then bring in the missing time dimension, stringing together 3D snapshots like an animated cartoon. Magnetic resonance is well-suited to these tasks. Because it uses electromagnetic pulses, rather than harmful x-rays or radioactive dyes, it can be repeatedly applied without damaging an embryo. With the aid of a computer, MR images can then be rendered either as solid, 3D volumes or as 2D cross sections sliced in any direction.

In his first effort, Jacobs has produced MR images of mouse embryos removed from the uterus that are so detailed it's possible to distinguish tissue layers only 50 micrometers thick. But ultimately, Jacobs would like to create such images without removing the embryo from its sanctuary. That's a challenge, he explains, as clinical MRI machines typically produce images with "voxels," or 3D pixels, of about 1 cubic millimeter, but high-resolution images of tiny mouse embryos require voxels 10 million times smaller. When the object is so small relative to an MRI machine's receiver coils, its signal tends to get swamped by background noise, especially if it's surrounded by a lot of other tissue—the mother.

But by experimenting with higher magnetic field strengths, different pulse frequencies, and other adjustments to conventional MR imaging machines, Jacobs and his colleagues are gradually increasing signal-to-noise ratios to acceptable levels. "Once you can do [imaging]

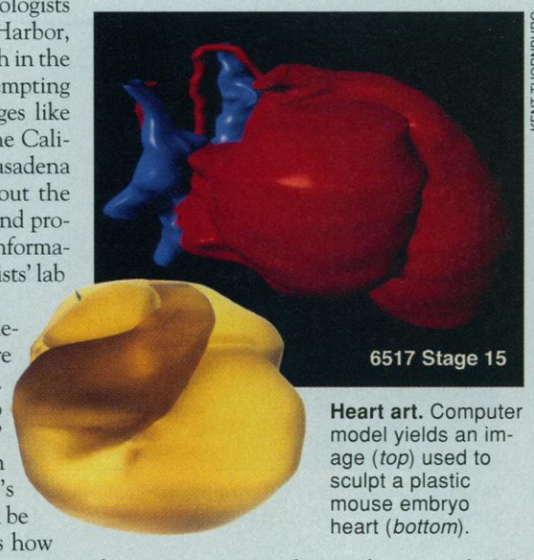
* See abstracts, images, and links at http://sdb.bio.purdue.edu/sdbnews/i_wrkshp/i_wrkshp.htm

Information Displays Go 3D

Some of the most intriguing technologies shedding new light on mammalian embryos don't produce images, but better ways of displaying and studying existing data. Biologists at the Jackson Laboratory in Bar Harbor, Maine, and the University of Edinburgh in the United Kingdom, for example, are attempting to meld three-dimensional (3D) images like those produced by Russell Jacobs of the California Institute of Technology in Pasadena (see main text) with information about the shifting networks of gene expression and protein activity that mold the embryo—information piling up in developmental biologists' lab notebooks and hard drives.

"Ninety-five percent of the data [developmental biologists] generate they are not able to store in appropriate ways. ... We have to develop an infrastructure to store that data and make it accessible," explains molecular biologist Martin Ringwald, leader of the Jackson Lab's team. The project's eventual result will be a kind of "virtual embryo" that shows how gene activity and the production of the corresponding proteins vary with time and location in the embryo. The database will be accessible to all researchers via the World Wide Web, and it could go a long way toward helping biologists understand how the signals that pulse through developing tissues, turning genes on and off or incrementally adjusting their expression, guide development.

At Oregon Health Sciences University in Portland, developmental physiologist Kent Thornburg is pioneering a more tangible way to display information about embryonic development: producing actual 3D models of human fetal hearts. In collaboration with Adrianne Noe, director of the National Museum of Health and Medicine at the Armed Forces Institute of Pathology in Washington, D.C., Thornburg takes a relatively low-tech approach, first tracing the outlines of tissue layers in hearts in AFIP's embryo collection onto a computerized drawing tablet. The computer stacks these outlines into wire-frame simulations the researchers can examine from any angle, including from inside the heart's inner cavities. Using "morphing" technology, the researchers can also examine how different tissue layers expand and move as the heart develops.



KENT THORNBURG

Heart art. Computer model yields an image (*top*) used to sculpt a plastic mouse embryo heart (*bottom*).

The same data can be fed into a stereolithography machine that sculpts a solid model from plastic. In this way, Thornburg has used computer maps of hearts that are only 0.8 millimeters across in the embryo to build 5-centimeter-wide models of hearts that he can hold in his hand. By forcing water through the lumen, or inner cavities, of normal and malformed model hearts, Thornburg can see how specific developmental flaws alter the flow of blood through the heart. "This wouldn't be possible in any other model," says Thornburg. "It's a beautiful thing." –W.R.

in vivo, a lot of things open up that are hard to do in vitro," he says. "For one thing, you can follow events over time in the same specimen."

At Duke University Medical Center's Center for In Vivo Microscopy in Durham, North Carolina, researcher Bradley Smith and colleagues are taking a different approach to the noise problem: shrinking the MR receivers down to embryo size, to reduce the size differential between receiver and subject. Smith, who started out as a medical illustrator and then earned a Ph.D. in anatomy so he could "get a peek inside these objects I was drawing," uses the devices to flesh out collaborators' studies of mice or rats with developmental mutations. "They'll perform some manipulation, then turn a pregnant mouse specimen over to me and ask me to investigate the embryos to determine when and where changes are occurring," Smith explains.

Using MR microscopy, for example, Smith has helped researchers compare the vasculature of 12-day-old mouse embryos treated with retinoic acid, a compound that induces birth defects, with normal embryos. The treated embryos lacked blood vessels in their tails and lower limbs, indicating that retinoic acid interferes with developmental signals in the rear half of the embryo.

Right now, getting a high-resolution image requires sacrificing the embryos in order to fix them inside the miniature MR coils. But like Jacobs, Smith says he and other Duke researchers will soon be using the devices to examine live embryos in utero. And in the meantime, other, more serendipitous, uses for the technology are emerging.

One came from a team of physicists, who asked Smith's help in working out how much radiation human embryos are likely to absorb when, for example, female nuclear workers or scientists using radioactive chemicals are exposed before realizing they are pregnant. The physicists needed data about the size and volume of embryonic organs at different times in development in order to estimate how much of the radiation reaching the womb an embryo would actually absorb. Smith provided this data by doing MR studies of the human embryos in the historical Carnegie Collection at the Armed Forces Institute of Pathology in Washington, D.C., and then deriving 3D images of the embryos.

A feeling for the organism

At the Skirball Institute of Biomolecular Medicine at New York University School of Medicine in Manhattan, developmental neurobiologist Dan Turnbull is adapting another established imaging technique to carry out delicate microsurgical procedures on mouse embryos still in the womb. Turnbull studies the transformation of ectodermal tissue into brain cells in the mouse embryo. To learn exactly when neural progenitor cells in different regions of the primordial mouse brain become committed to their ultimate fates, he and coworkers Martin Olsson and Kenneth Campbell are using high-frequency, high-resolution ultrasound imaging to guide the needles used in cell transplantation experiments.

When the researchers grafted marked cells from the forebrains of 13-day-old mouse embryos to specific spots in mid-hindbrain and vice versa, they reported in the October issue of *Neuron*, they found that the dislocated forebrain cells metamorphosed into hindbrain cells, while the grafted hindbrain cells failed to adapt to their new location—indicating that the fates of hindbrain cells are set before those of forebrain cells.

Just as important as this result was the precedent set by the experiment. Such cell transplants had never before been attempted on such young mouse embryos. But the technique allowed the researchers to distinguish the tiny structures—the entire embryonic

mouse brain at that stage is only a few millimeters thick—sufficiently well to perform the transplants. "A lot of people who have come to our institute and seen us doing these procedures have been getting excited," says Turnbull. "Everybody sees the future applications where we can start introducing labeled cells into mutant mice, to look at how the differentiation process is altered."

Perhaps the greatest excitement at the NICHD workshop, however, was sparked by another effort to image individual cells. Researchers at the University of Iowa's W. M. Keck Dynamic Image Analysis Facility in Iowa City have melded a confocal microscope with a 3D movie camera and a computer to create the world's only instrument for monitoring the full range of movements and shape changes that cells undergo during development. The microscope changes its focal plane 30 times per second, and the computer records the resulting "optical sections" for reconstruction into a 3D computer model that highlights cell membranes and internal surfaces such as those of the nucleus, mitochondria, and vesicles. The process is repeated every 2 seconds, and a QuickTime movie is the result.

So far, the technique has been used only on cells that can crawl in a lab dish. David Soll, director of the Keck facility, handed out red-and-blue glasses at the workshop and treated viewers to a 3D movie of the sluglike colonies that the normally unicellular slime mold *Dictyostelium* forms when it needs to reproduce. Like all amoeboid creatures, the *Dictyostelium* colony moves by continuously assembling and disassembling its internal skeleton, made of the protein actin. Soll's movie showed how the colonies pulsate as their actin-filled pseudopods appear and disappear, dragging along the entire mass.

Using the 3D motion analysis system, Soll and his collaborators have demonstrated that *Dictyostelium* strains engineered to lack certain of the proteins known to regulate actin display specific flaws in the way they create or absorb pseudopods. These flaws are a sign that the numerous cytoskeletal regulators aren't redundant, but specialized. According to George Washington's Moody, that result would probably elude a researcher viewing mutant *Dictyostelium* colonies under a conventional 2D microscope.

And that, in the end, may be the strongest rationale behind the new surge in developmental imaging. Researchers using 2D still images of their subjects have to spend years acquiring an ethereal, intuitive "feeling for the organism" before they can understand its behavior in three dimensions over time, Moody argues. "But having these new technologies out there means that people will quickly be able to form a visual understanding they can rely on. ... It's going to be terrific."

–Wade Roush

MEETING BRIEFS

How Does HIV Overcome the Body's T Cell Bodyguards?

MARNES-LA-COQUETTE, FRANCE—In 1854, Emperor Napoleon III created an elite squadron called the "Cent Gardes" for his own protection. Thirty years later, Louis Pasteur turned one of the squadron's barracks in this small town just outside Paris into laboratories. Pasteur died here in 1895, but his disease-fighting tradition lives on: Today, the Cent Gardes building hosts one of the world's most prestigious AIDS meetings. At this year's gathering,* an elite squadron of researchers grappled with still-unsolved questions about how HIV destroys the immune system and how they can fend off its attacks.

The Life and Times of T Cells

It might be said that AIDS researchers have come to know the virus that causes the disease, HIV, inside and out. They have isolated its proteins, sequenced its genome, and identified the receptors it uses to dock onto the CD4 T lymphocytes that are the virus's primary target. Yet the central mystery of AIDS remains unresolved: How does the virus cause the severe loss of CD4 cells, which wrecks the immune system, that is the hallmark of the disease? This question has stimulated heated discussion in recent years, and new findings presented at the meeting by David Ho, director of the Aaron Diamond AIDS Research Center in New York City, and immunologist Paul Johnson of Harvard Medical School in Boston are fanning the flames of the debate.

Different calls. Frank Miedema (*left*) and David Ho disagree about how T cells are lost.

For many researchers, a major clue to the riddle was revealed in January 1995 with the publication of two papers in *Nature* indicating staggeringly high rates of HIV replication and CD4 cell turnover in a typical HIV-positive patient. The findings—by Ho and his collaborator Alan Perelson of the Los Alamos National Laboratory in New Mexico, and by George Shaw at The University of Alabama, Birmingham, and his co-workers—which have since been refined in more recent papers, suggest that about 100 billion new viral particles are produced every day and 1 billion to 2 billion CD4 cells are dying and being regenerated each day as well.

These extraordinarily high numbers led Ho to propose what has come to be known as the "sink model" for CD4 cell loss. In Ho's view, the high levels of HIV production keep both the sink's tap (the immune system's production of new CD4 cells) and its drain (their destruction by the virus) wide open. Because the body's ability to generate new cells can only be stretched so far, the sink slowly empties, until the CD4 cells are lost and the immune system is exhausted.

Last year, however, the sink model was challenged by a team of researchers led by immunologist Frank Miedema of the Netherlands' Red Cross Blood Transfusion Service in Amsterdam (*Science*, 29 November 1996, p. 1543). Miedema and his co-workers made their own estimate of CD4 cell turnover by measuring changes in the length of the T cells' telomeres, the extreme ends of chromosomes, which shorten slightly each time a cell divides. The telomere length can provide an estimate of how many times a cell has divided during its lifetime, and thus an indication of overall turnover rate in a cell population. The Amsterdam team found that the telomeres in CD4 cells from HIV-infected people were not appreciably shorter than those of uninfected controls, and the team concluded that turnover rates in these two groups were essentially the same—a result that directly contradicted Ho's sink model. Miedema's team proposed that the loss of CD4 cells was not due to a major increase in their rate of destruction—an open drain—but rather that HIV was interfering with production of new cells, thus turning down the tap.

At the meeting, Ho delivered a riposte to

* 11th Colloquium of the Cent Gardes, Marnes-la-Coquette, France, 27 to 29 October 1997.