

## Microscope renaissance

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### **With genetic engineering and advances in imaging, scientists have entered a colorful new world where they can watch neurons connect and viruses enter cells**

By Gareth Cook, Globe Staff | November 6, 2006

The field of splotches across the middle of the image evoke Claude Monet's impressionistic brushwork. The rich colors -- from fiery reds to grass greens and sky blues -- would be at home in his famous painting of a sunny poppy field near Argenteuil, France.

But the image, created in a dimly lit Harvard University laboratory on an exquisitely sensitive microscope equipped with lasers, shows nerve cells in the brain of a mouse. Each of the brain's cells, or neurons, has been tinted a different hue, allowing researchers to distinguish individual neurons in the tangle and better understand how brains are wired. In the lab, they call this the "Brainbow."

The Brainbow is part of a renaissance in microscopy that could have far-reaching implications for biology and, perhaps, for the diseases that some biologists study.

In just the last few years there have been dramatic improvements in biologists' ability to highlight features in a cell they want to see by using fluorescent colors. This, combined with genetic engineering and advances in the microscopes themselves, is illuminating living worlds never seen before.

Now scientists can watch as a solitary virus enters a cell or a single neuron reaches out to make a new connection inside a living brain.

"It is just jaw-dropping," said Jeff W. Lichtman, a Harvard biologist who directed the Brainbow work and is an investigator of the Howard Hughes Medical Institute. "There has never been a time like right now."

Imaging advances drive progress in many areas of science. MRIs, which provide detailed pictures of the body, have changed the way medicine is practiced. Atomic force microscopes have brought a new understanding of materials down to the scale of individual molecules. Telescopes like the space-based Hubble have provided stunning views of distant galaxies and stars being born from veils of gas.

In biology, the imaging renaissance was helped, in part, by the jellyfish. In the 1960s, scientists found that a particular jellyfish makes a protein that glows, known as green fluorescent protein. In the 1990s, using genetic engineering, researchers found that they could take the gene that makes GFP and insert it into the DNA of other cells. This makes different parts of the cell glow -- and thus easier to see in a microscope -- depending on where the gene is placed.

Since then, scientists have moved beyond the basic green and made the colors brighter by finding fluorescent proteins in other species and genetically modifying them. Their creators seem to delight in naming them: A new yellow fluorescent protein is "Venus"; blue is "Cerulean"; red is "Cherry" or "Tomato."

The creation of the Brainbow mouse, which was led by Harvard postdoctoral fellow Jean Livet in Lichtman's lab as part of a collaboration with the Harvard laboratory of Joshua R. Sanes, uses some of these new colors, but with a twist. The mouse is genetically engineered so its neurons produce fluorescent proteins, but each cell produces a random combination of the colors. These colors mix, giving each cell a different color, the way the basic colors of a television screen mix to produce a range of hues. The images can be taken in a living mouse, and the genetic engineering does not harm the mouse.

Now the team can follow the path of a single neuron.

The researchers said they were stunned at how well it worked -- and the sheer beauty of the images. When the first image appeared on the screen of Tammy Weissman , a postdoctoral fellow in Lichtman's lab, she ran to get Lichtman.

"It was amazing," she said.

Fluorescent proteins have also made it possible for biologists to more deeply explore viruses, from the ones that cause the common cold to more-dangerous threats.

In 2004, Tom Kirchhausen of Harvard Medical School and the CBR Institute for Biomedical Research created the first movie of a virus entering a living cell. In the movie, the virus appears as a red dot floating toward the surface of the cell. It pauses at the surface, tricks the cell into letting it in and then moves inside, where it can begin its mischief. Now that scientists can see this process, they expect to be able to develop new strategies for stopping viruses.

Earlier this year in the journal *Science*, Harvard scientist X. Sunney Xie published a movie of dividing *E. coli* cells. His equipment was able to pick up a flash of fluorescent Venus yellow every time the cell made a single molecule of a protein, allowing biologists to more precisely study how cells create proteins -- some of which are involved in diseases.

The one weakness of both the *E. coli* movie and the brainbow image: The resolution is limited, so fine details within the cell are too small to be seen.

But scientists are working on new, so-called "super-resolution" microscopes that go beyond that barrier. In September, a team led by Eric Betzig of the Howard Hughes Medical Institute published a paper describing a microscope that can achieve a resolution high enough to see tiny structures inside a cell. Other microscopes are being developed with exotic names like "structured illumination" and STED . None of the super-resolution microscopes can work on living cells because the cells move in the time it takes for them to record a complete image.

At the same time, other scientists are working on new cellular imaging techniques that can work on living tissue but don't depend on any of the fluorescent proteins. This is an important advantage because it means the scientist can see a completely natural system and potentially look at people.

Harvard's Xie, for example, has been developing a technique called CARS microscopy that illuminates tissue using laser light without causing any harm.

Researchers in Xie's team, led by Harvard graduate student Conor Evans , have produced images below the skin of a living mouse. In one, a single strand of hair can be seen jutting toward the surface, ringed at its base by a gland. Xie said that he is developing the technology for use in medical imaging.

All of the new high-tech imaging technology could encourage a return to an older style of science, according to Lichtman. Much of biology today is carried out in a familiar mode: A scientist has a theory about how something works and sets out to test it.

But Lichtman says he approaches his work more like a 19th-century naturalist. He watches the quarry -- in his case a neuron -- in its natural habitat. When a neuron does something surprising, then he thinks of ways to understand what he has seen. And he says the surprises come often.

"You see a lot of things," Lichtman said, "that make you wonder."

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(Correction: Because of a reporting error, Dr. Jeff W. Lichtman was misidentified in a story about new microscopic techniques in yesterday's Health/Science section. He is a professor of molecular and cellular biology and member of the Center for Brain Science at Harvard University.) ■

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