New technology for high-speed study of zebrafish larvae works in seconds

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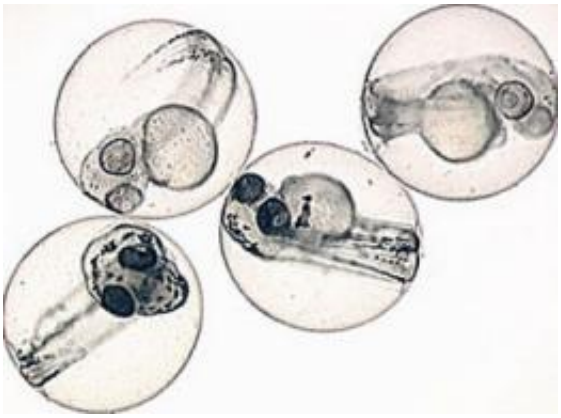

Zebrafish embryos, seen here, are transparent and have internal organs that can be easily seen as they develop.

(PhysOrg.com) -- One of the most commonly studied laboratory animals is the zebrafish — a tiny fish with transparent embryos, or larvae, whose internal organs can be easily seen as they develop.

Because they are genetically similar to humans and have complex organs, biologists often use zebrafish as a model for human diseases such as cancer, liver disease and heart disease. However, one limitation of zebrafish studies is that it takes several minutes to visually examine each larva. This has kept researchers from using the fish in experiments that require a large number of animals, such as testing the effects of many different drugs.

With the aim of speeding up the process and enabling large-scale studies, engineers at MIT have developed a new technique that can analyze larvae in seconds. The researchers, led by Mehmet Fatih Yanik, associate professor of electrical engineering and computer science, describe the new technology in the July 18 issue of the journal *Nature Methods*. First authors of the Nature Methods paper are graduate students Carlos Pardo-Martin and Tsung-Yao Chang.

"There is significant need for high-throughput [automated]studies on whole animals, at high resolution," says Yanik. "People are currently doing this manually, which is too slow. Ours is the only system that can take a large library of chemicals and screen it on thousands of vertebrates."

Although humans and zebrafish may not appear to be closely related, many zebrafish organs and much of its biochemistry are similar to those of humans. For example, zebrafish and humans share the same liver enzymes, so the fish are useful for testing drugs that might cause liver damage. They also make good subjects for studies of cancer, Parkinson's disease, Alzheimer's, diabetes, amyotrophic lateral sclerosis (ALS) and other diseases, says Yanik.

Zebrafish take only seven days to fully develop, and most of their organs are formed by the third day of development, which makes zebrafish studies faster than those with mice or other slow-growing mammals. Best of all, the transparency of the larvae lets researchers directly see the effects of drugs or genetic mutations.

However, inspecting the animals is tedious and time-consuming. "We have to manually look at each embryo in a dish, which involves a lot of positioning and repositioning," says Leonard Zon, professor of hematology and oncology at Harvard Medical School, who was not involved in the research. "Having the ability to flow the embryos through

a machine and image them on the fly is going to be very helpful."

With the new MIT system, larvae are pumped from a holding area to an imaging platform, where they are automatically rotated so the area of greatest interest can be seen. This is important because if the larvae are in the wrong position, the yolk or pigmentation on the skin may block the organs that the researcher wants to observe. The animals remain unharmed throughout the process.

The microscope's resolution is high enough to image individual cells, and the entire process takes about 19 seconds per animal, compared to about 10 minutes for manual inspection. To demonstrate the system's effectiveness, the MIT team imaged the neurons that project from the zebrafish retina to the brain. The system could also be used to observe tumor growth, organ regeneration or stem-cell migration, says Yanik.

The researchers have also devised a way to rapidly perform laser surgery on the larvae. This would allow them to damage organs and then study how they regenerate.

Yanik's team has applied for a patent on the device and is now looking into commercial applications to use the technology to screen large numbers of drugs on various zebrafish disease models. The researchers are also working on further speeding up the system and developing ways to process the huge amounts of data generated by the imaging machine.

"The development of these powerful technologies for the monitoring and manipulation of model organisms, such as individual zebra fish in a rapid fashion, is representative of one type of high-impact research that the NIH Director's New Innovators Award program was designed to promote. Professor Yanik demonstrated tremendous creativity and accomplishment early in his independent career, and the New Innovator Award helped provide him and his team the freedom to vigorously

pursue their ideas," says Jeremy M. Berg, director of the National Institute of General Medical Sciences, who helps lead the New Innovator Award program at the NIH.

More information: "High-throughput subcellular-resolution in vivo vertebrate screening platform," by Carlos Pardo-Martin, Tsung-Yao Chang, Bryan Kyo Koo, Cody L. Gilleland, Steven C. Wasserman, Mehmet Fatih Yanik. Nature Materials, 18 July 2010.

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