In the past few years, improvements in imaging and automation techniques have made it easy for researchers to see hundreds of plates of cells partake in every activity from differentiation to apoptosis. But in living and breathing animals, we’re only just beginning to realize the potential of large-scale screens. “To take a whole animal and be able to perform high-throughput screening—that’s a new thing,” says Alan Mayer, assistant professor of pediatrics and cell biology at the Medical College of Wisconsin in Milwaukee. That’s worth the effort, he says, because you can do tests that you can’t do in cell culture, such as determining how changes in one gene can affect a living organism. What’s more, Mayer says, the techniques are a boon to animal physiology studies; a cell assay can tell you whether a drug is toxic, but only testing the drug in a living organism will show you how that drug is metabolized. Organisms such as Caenorhabditis elegans and zebrafish are a popular choice for such in vivo studies, because they are easy to raise and manipulate and they share molecular pathways with those of humans. Others, such as planarian flatworms, frog larvae, and Drosophila melanogaster, offer their own distinct advantages.

But working in vivo raises its own challenges. First, establishing these organisms as models for human disease isn’t always straightforward. Then, doing in vivo studies in hundreds of animals at once rather than one by one entails introducing automated components as well as making adjustments in everything from the volume and chemical make-up of the organisms’ surroundings to the brightness of the light in their living space. You’ve got to keep them alive, and non-stressed, during the entire process.

The Scientist talked to some researchers who have blazed new paths in whole-animal screens. Here’s what we found:

**Screening Whole**

**How to reel in high-throughput results using worms and fish.**

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The microfluidic worm sorter uses suction to immobilize worms against a flexible membrane, allowing researchers to automate their nerve regeneration screen.

The Yanik lab / MIT

**Immobilizing worms**

**User:** Mehmet Fatih Yanik, assistant professor of electrical engineering at the Massachusetts Institute of Technology, Cambridge

**Project:** Chemical and genetic screens of nerve regeneration in *C. elegans*

**Problem:** Several years ago, Yanik developed a way to sever nerves in *C. elegans* using ultrashort laser pulses. The surgery, done manually, took 10 minutes per worm. “I was sick and tired of doing it manually,” Yanik says. What’s more, such surgery, and high-resolution imaging to trace regenerating neurons, required using drugs to anesthetize the animals, a process that isn’t conducive to subsequent genetic and chemical screens since the anesthetic could alter gene expression or the chemical environment. He needed a way to eliminate the need for anesthesia and automate the surgery.

**Solution:** After months of brainstorming and experimenting, Yanik and his students came up with a way to use a microfluidic chip to sort, isolate, and immobilize individual worms. In 2007, they had a prototype of the chip, which consists of a series of channels and suction devices that direct the flow of worms, capturing individual worms and placing them into tiny chambers (*Proc Natl Acad Sci*, 104:13891-95, 2007).

The crucial component is the tiny pump, which is used to briefly suck living worms against a flexible membrane. This immobilizes the worm for a few seconds, eliminating the need for anesthesia while allowing Yanik’s team to take a high-resolution photo and cut select neuronal projections at a precise distance from the cell body, which acts as a reference point.

Finally, the pressure exerted by the pump is released, so the worm can flow back into its chamber. There, they can treat the animal with chemicals and

“*The first question was, ‘Are the zebrafish going to produce*
Tracking metabolites

User: Alan Mayer, assistant professor of pediatrics and cell biology, Medical College of Wisconsin, Milwaukee

Project: Assessing genetic programs of zebrafish metabolism during development

Problem: For decades, researchers have studied metabolism by placing small mammals into shoe box-sized chambers to measure oxygen consumption and carbon dioxide production. Mayer's group wanted to find a high-throughput way to assess metabolism in order to screen for genetic mutants that alter metabolic rate or drugs that can accelerate metabolism.

Zebrafish embryos are good candidates for such screens: They can be confined to fixed volumes, and ambient liquid can be analyzed for carbon dioxide. But the tests can only be conducted on a few embryos at a time, using single syringes to add and collect fluid.

Solution: About a year ago, Mayer's team began trying to scale up a zebrafish metabolic assay from one that uses 1 to 10 fish to one that uses a 96-well plate. They decided to try measuring the acidity of the solution, which would provide an indirect measure of carbon dioxide. "The first question was, 'Are the zebrafish going to produce enough acid for us to measure?'"

They had to settle on a volume that the embryo was confined in—small enough for the acid concentration to affect the ambient pH, but large enough in volume to physically accommodate the embryo. Then, they chose a simple indicator dye—phenol red—as a pH readout.

Considerations: You might not need special equipment for an assay like this, but you will need to think carefully about the experimental parameters. For example, Mayer had to tweak the buffer solution so that the production of acid could be measured over a reasonable amount of time. "Something less than a day but more than a couple of minutes, because it takes time to set the assay up," Mayer says. "You don't want the first embryos to be finished before the last embryos are in [the wells]," or you won't be able to monitor progression of the assay color change (i.e., CO₂ production).
Infection of *Candida albicans* in the *C. elegans* intestine progresses as the worm’s cells are replaced by metabolically active yeast cells (red).

Eleftherios Mylonakis and *PLoS Pathogens*

**Testing toxicity**

**User:** Eleftherios Mylonakis, assistant professor of medicine, Harvard Medical School, Boston

**Project:** Infecting *C. elegans* with fungal species and screening for potential antifungal agents

**Problem:** Many antifungal compounds can be toxic to mammalian cells. In vitro assays, however, measure potential efficacy of the antifungal agents, leaving toxicity for later stage trials. Mylonakis’s group needed to develop a whole-animal screen that would allow them to easily pinpoint safe antifungal agents.

**Solution:** The group can infect large numbers of worms, contained in 384-well plates, with a fungus and track the infections using microscope images. “The rest is easy from there,” Mylonakis says. “Just add screening compounds and monitor the survival of the worm and the expression of fungal virulence.”

Sounds simple, but coming up with the technique was a real challenge. When Mylonakis first started developing the technique a decade ago, he had minimal knowledge of how worms respond to fungal pathogens. He had to first demonstrate that the worms would ingest and become infected by the same species of fungus that serve as pathogens in humans. “When we started our work it was not even known if fungi can be a food source for *C. elegans,*” he recalls.

They were also able to make sure that the innate immune system molecules that detected such fungal pathogens were similar in humans and worms, in the case of a common, yeast-like fungus *Cryptococcus neoformans* (*J Exp Med*, 206:637-53, 2009).

**Considerations:** The most common mistake for beginners, says Mylonakis, is not adjusting the assay to fit the specific endpoints, whether it’s the effects of the antifungal compound or the worm’s immune response to the fungus. If it’s the latter, for example, then the assay should be adjusted to a fit a 4- to 6-hour window—the peak of a worm’s immune response. The former might take longer to see.
Genes involved in appetite, which researchers tagged with GFP, are activated in hungry worms during metabolism studies.

Eyleen O'Rourke

**Gene glow**

**User:** Eyleen O’Rourke, research fellow in the lab of Gary Ruvkun, Harvard Medical School, Boston

**Project:** Developing automated screens of *C. elegans* to identify genes controlling appetite

**Problem:** O’Rourke and her colleagues designed a worm that would glow green by activating certain hunger genes linked to a green fluorescent protein (GFP) when the animals were out of food. The worms could potentially provide great high-throughput data, but the researchers needed to develop a quantitative way to score brightness. Without that, says O’Rourke, “there was no statistical analysis to be done.”

**Solution:** The first challenge involved acquiring the images. Collaborating with two other labs, O’Rourke began by taking high-resolution photos of 20 worms at a time for analysis. At lower magnification, they could see more worms in the field, but they lost sensitivity. To help overcome that, they outfitted their microscopes with a more powerful ultraviolet light source, and scaled down GFP expression to reduce background noise in their samples.

The brighter UV had an unwanted effect, though: When they switched it on, the worms would instantly flee to the periphery of the wells. “We didn’t expect to lose all of them within a few milliseconds,” she says. So they redesigned the system to maintain the worms under normal bulb light, programming the microscope to find the correct focal plane before switching quickly to UV and snapping an image.

**Considerations:** For quantifying brightness, software advances have not yet caught up with the hardware advances made by O’Rourke and her colleagues. Currently, the group uses a freely available cell-imaging software, called CellProfiler, to analyze fluorescence in the worms. “It’s a very powerful and great tool, but worms have many specific issues, like they can overlap on top of each other” in the wells, she says. They are working with CellProfiler’s creator to develop software tailored specifically to worms.