Brown's influential biomechanics X-ray technology grows ever more powerful

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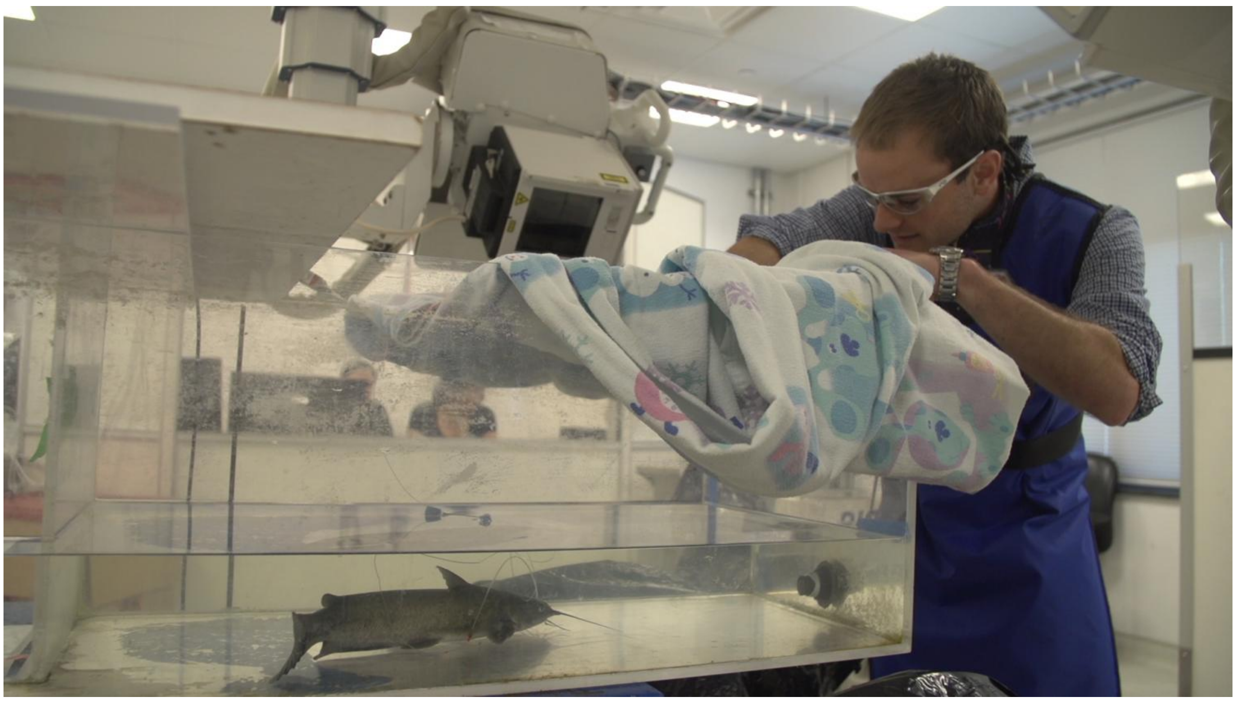


Postdoctoral scholar Aaron Olsen gets a catfish ready for its XROMM closeup. He's studying how they use their bones and muscles to generate suction for feeding. Stephen Crocker

It's right there in the word "discovery"—the idea of removing whatever cover may obscure a particular phenomenon from observation. And that's exactly what Brown University's X-ray Reconstruction of Moving Morphology technology does to reveal the inner workings of people and animals.

With two recent papers describing powerful new software and a way to track muscle contractions, XROMM can do even more than before. And as a result, so can the dozens of labs across the globe that have descended from XROMM's innovations over the last 10 years.

"The coolest things we've seen with XROMM are things you just absolutely can't see from the outside," said Beth Brainerd, director of the XROMM Technology Development Project and professor of ecology and evolutionary biology at Brown. "XROMM is exciting because we can see what's happening inside animals."

For one example, Brainerd cited turtles. Earlier this year, she and a team led by Clemson biologists Richard Blob and Christopher Mayerl published a study in the *Journal of Experimental Biology* showing that pond turtles swing their hips when they walk, much like people and other vertebrates do. No one had ever seen that before.

Brainerd and professor and co-director Stephen Gatesy first developed the system with seed funding from the University's Office of the Vice President for Research. Over the last decade, Brainerd, Gatesy and students and collaborators from around the world have published papers revealing the secrets under the fur, scales or skin of alligators, dogs, rats, frogs, pigs, ducks, geckos, fish, bats, iguanas and—using birds as proxies—dinosaurs.

"The range of animals is really part of the fun," Brainerd said.

It's not just that the system's X-rays can see through soft tissues. Housed in the W.M. Keck Foundation XROMM Facility, the system shoots high-frame-rate video from two different X-ray sources to allow its software to triangulate the three-dimensional positions (down to one-tenth of a millimeter) of bones as they move. XROMM, which over time has gained funding from the National Science Foundation and the W.M. Keck Foundation, combines the X-ray images with 3D computed tomography (CT) scans of bones to produce scientifically faithful animations of each animal or person's motions. The animations don't just show bones in motion but also show how each bone's shape and position make those motions possible.

"With XROMM, we can understand how joints work," Brainerd said. "Joints are all about the shape and motion of the bones."

In animals, the motions are tracked with tiny implanted metal markers. But the XROMM team at Brown also developed methods for tracking the motions of people using a markerless method.

In 2013, Brown orthopedics professor Dr. Braden Fleming published a study using the system to study differences in the knees of male and female athletes as they performed a sudden direction-change maneuver called a "jump cut." One of the research team's goals was to understand why women are injured more often than men in making these moves. They saw that female athletes landed the jump-cut maneuver with greater landing stiffness and less energy-absorbing knee bend, potentially putting them at greater risk for injury.

"For orthopedics research, XROMM provides information that is not available otherwise," said Brainerd, who noted that from its origins, the project has been a collaboration not only of biologists like her and Gatesy but also orthopedic bioengineers like Fleming.

Fluoromicrometry, fish and faster software

Recently, Brainerd and her team have published papers describing and validating two new capabilities for XROMM: new freely available software that radically speeds up the pace of research by making the system more automated and easier to use; and a method called "fluoromicrometry" that allows for measuring muscle motion alongside bone motion.

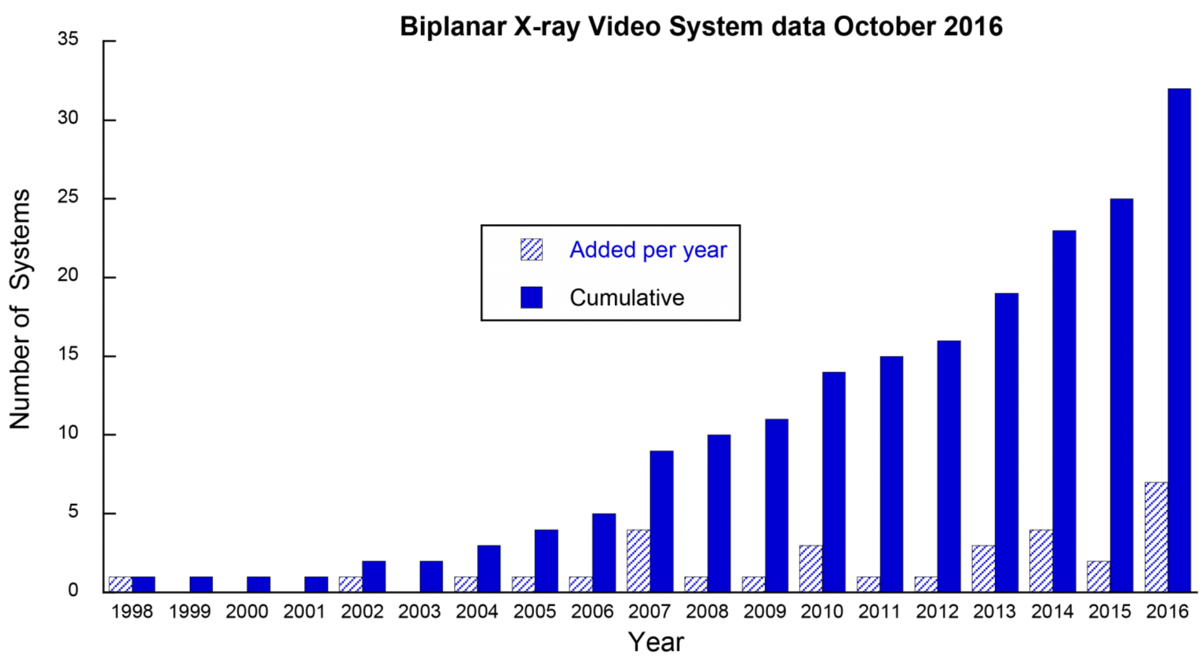
The fluoromicrometry method that Brainerd and her team describe in the Journal of Experimental Zoology involves implanting markers in muscles so that XROMM can track how much and how fast they shorten and bulge when they contract. Carefully documented and benchmarked demonstrations in leg muscles from frogs showed that fluoromicrometry can track muscle contractions precisely to within 0.09 millimeters.

Moreover, fluoromicrometry has a key advantage over "sonomicrometry," the 60-year-old method using sound waves that scientists currently use to measure muscle in moving animals, Brainerd said. Sonomicrometry requires that an animal be connected to instruments via wires, significantly inhibiting their motion, and there is a limit to how many measurements can be made at once. Fluoromicrometry is wireless and many markers can be implanted, allowing for more measurements per muscle.

The new paper formally validates the technology, but it has already led to discoveries. Last year Brainerd and Brown doctoral student Ariel Camp, lead author of the fluoromicrometry paper, led a study that combined standard XROMM and fluoromicrometry to show that bass use their body's swimming muscles to power their feeding, too. By imaging bones in the fish's head and muscles in the head and body, the researchers could see how the body muscles contributed to the rapid opening of the mouth, which created enough suction to slurp up prey.

Researchers continue to study suction feeding in other fishes. Brown postdoctoral scholar Aaron Olsen is working with catfish while George Washington University visiting scholar Patricia Hernandez is looking at carp using these methods.

Making all the work happen considerably more quickly is the new software described and validated for accuracy, precision and ease of use this fall in the *Journal of Experimental Biology*. Written by Benjamin Knörlein, an application scientist in Brown's Center for Computation and Visualization, the free, open-source XMALab program corrects for image distortions and streamlines online data storage—but most significantly, it automates much of the marker tracking previously done by hand, Brainerd said.



Especially in the last decade since XROMM came along, use of biplanar X-ray video has been growing fast. Credit: Beth Brainerd

"Previously, it took weeks or months to get to the point of even having one animation," she said. "Now, once we collect the data, we can have an animation within a day."

Indelible influence

Brainerd and Gatesy didn't invent X-ray video, though Gatesy is a pioneer of 3D X-ray video, Brainerd said. Overall, the contribution of XROMM has been to create a whole system—combining X-ray video with CT scans and software like XMALab for processing the data and making animations—that can enable biomechanics and orthopedics research, she said. Over the years, the XROMM lab has worked with two private companies, Radiological Imaging Services and Imaging Systems and Service Inc., to develop hardware configurations for animal or orthopedic studies that many other institutions have adopted.

Each year, the number of labs using XROMM-like technology has grown. Today, there are 34 facilities using "biplanar X-ray" ranging from North Carolina and California to Germany and Australia. The vast majority use at least some XROMM technology, but many also mix in other innovations, Brainerd said.

"The use of XROMM is really taking off," she said. "The slope of that curve is accelerating."

Meanwhile, for nearly a decade Brainerd and Gatesy have trained about a dozen visiting scientists every summer to use XROMM; and like Clemson's Blob and Mayerl, many researchers come to Brown to do experiments.

Altogether, the studies allow for a unique, moving-picture view of how life has evolved an incredible diversity of musculoskeletal structures to fit the survival needs of everything that moves.

Ultimately, Brainerd hopes that XROMM will not only continue to reveal these capabilities one by one, but also explain evolution across all animals.

"If we can understand some very basic relationships between the shape of bones and the way they move, we can begin to make sense of all that animal diversity," she said. "In the fossil record, all that we have are the shapes of bones—but if we can come up with these form-function relationships that help us interpret bone shape in terms of the way animals move, we can then use that fossil record much more powerfully to understand the evolution of animal diversity and the species we see on Earth today with all the amazing things that they do."

To gain that insight, you just have to be able to see inside.

More information: Benjamin J. Knörlein et al, Validation of XMALab software for marker-based XROMM, *The Journal of Experimental Biology* (2016). DOI: 10.1242/jeb.145383

Provided by Brown University

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