Scott Fraser: live action embryology

Using cutting-edge approaches to live imaging, Scott Fraser captures the dynamics of development.

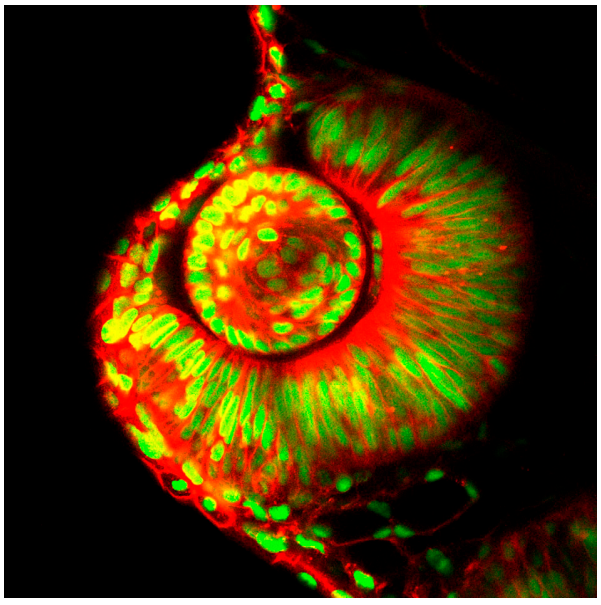
Scott Fraser has likened trying to understand embryonic development to trying to understand the rules of football—you have a much better chance of grasping them by watching a game in real-time than by looking at a bunch of static photographs (1).

Fraser's dedication to achieving a dynamic document of embryo development (2) has led him to push the boundaries of live imaging, in terms of both microscopy and experimental techniques. Over the course of this pursuit, Fraser and his colleagues have captured some remarkable footage of embryonic development, including the remodeling of neuronal arbors during tadpole eye development (3), cell movement and lineage development in the frog embryo (4), the migration of cells during somite and neural crest development in the chick embryo (5, 6), and polarized cell divisions in the patterning of the zebrafish gastrula (7), to name just a few.

> "I made the mistake of looking at embryos, and it was a slippery slope."

The results are visually stunning and have greatly enriched our understanding of the rules of the developmental game. Now, as director of the Biological Imaging Center at Caltech's Beckman Institute (Pasadena, CA), Fraser and his team continue to develop innovative approaches for imaging the live embryo. They are now also using these techniques to view human disease systems.

When I recently convinced Fraser to peel his eyes away from the microscope for a few moments to discuss his work, he explained that a major source of innovation is the departmental coffee pot.



FRASER

Confocal microscopy captures every cell mitosis, migration and death in the eye of a living zebrafish embryo (green: nuclei, red: cell membrane).

BEGINNINGS

I understand you started out studying physics. What made you switch to embryology?

While studying physics, I became more and more fascinated by the tight interchange between theory, technology development, and experiment. But one of the things that I found frustrating was the scale of many of the experiments. They were so huge that they didn't have the sort of personal feel that experiments in cell or developmental biology have. So I was looking for ways to apply the same sort of approaches to things that are much more personal, and where the time between thinking of an experiment and getting a result was much shorter. So I did a biophysics Ph.D.

Scott Fraser

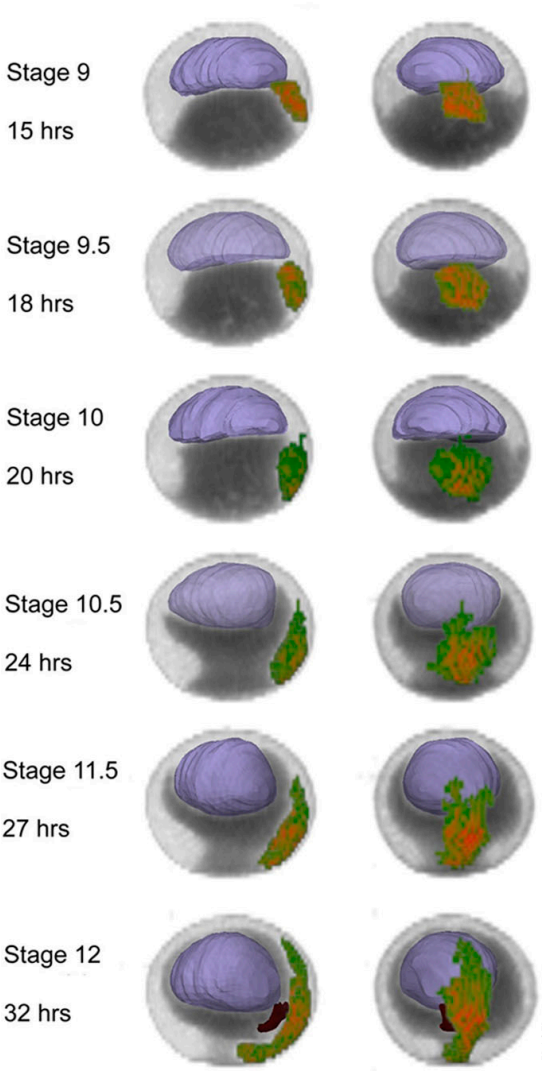
I was attracted to biophysics because I saw it as a place where microscopy and instrumentation could have an impact. And then I made the mistake of looking at embryos, and it was a slippery slope, I was just pulled down into working on the embryo. Well, "down" is probably not the right word [laughs].

Did microscopy get you into embryology or was it the other way around?

It was a little of both. To me, the cell is the quantum, and microscopy's the way to get to that. So it's very much the microscopy driving my interest in embryology and the embryology driving my interest in microscopy. It's a whole loop.

And that slippery slope of embryology led you to the University of California at Irvine?

Being at Irvine with a really strong community in developmental biology, and also a very strong community in biophysics, was an ideal incubator space for me as an assistant professor. There were people like Hans Bode, who was working on hydra, and Peter Bryant working on the *Drosophila* imaginal wing disk. They had been defining formal rules and making predictions about how cells should interact, but it was hard to go from how they should interact to how they actually do interact. That's where



The descendents of a single labeled cell of a frog embryo can be followed by MRI. Right column shows a 90° rotation (purple: blastocoel).

the imaging tied in nicely; it allowed us to ask questions about cell interactions in the intact embryo.

How did you go about asking these questions?

People like Gunther Stent and his colleagues at Berkeley had been using vital dyes to trace lineages in the leech. And similar work was going on in David Bentley's lab and Corey Goodman's lab on the patterning of the insect nervous system. In these systems, the cells are large and somewhat convenient for applying vital dyes. What we decided to do is to take those technologies and apply them to cells that are much more challenging. We chose inconvenient systems like the vertebrate nervous system or imaginal disk cells, or things of that sort. The cells of the imaginal disk, for example, are orders of magnitude smaller than the cells that had been studied in the leech.

Speaking of inconvenient systems, you also image chick embryos. How do you physically get the egg under the microscope?

Well, it's really funny, in fact it was a riot trying to convince the microscope manufacturers to sell us the equipment. We needed a microscope with more room between the objective and the stage. The first microscope we used for this was one that had actually been built for the electronics industry, for inspecting big things like integrated circuits. The manufacturers said, "Oh, no, no, no, that's not for a biologist, that's for the electronics people," and we had to go back and forth, and finally they agreed to sell it to me. It was sort of odd to have to convince them to let me spend my money.

Once you had your equipment, how did you look inside the egg?

The way we do it is really painfully simple. There's a couple different ways you can window the egg. One of them is to just use a pair of fine scissors to actually cut a porthole right in the top. People had operated on chicken eggs before, like Nicole LeDouarin and my wife, Marianne Bronner-Fraser, who was working on chick neural crest. So we appropriated the technology, and what we added to it was going in and labeling cells, or only one cell, in some cases, so that we'd know that we could follow one lineage.

I also collaborated with Andrew Lumsden, Roger Keynes, and Claudio Stern on their systems: on somites, the hindbrain, and the spinal cord. In each case, they provided this huge background knowledge of the snapshots of what might be going on. And we animated it. So we could then follow the individual cells, ask questions about what they do and how those snapshots relate to the real cell behaviors that are going on.

"All of this MRI started because of a very, very good colleague at UC Irvine, Russell Jacobs, and because we shared a coffee pot."

GOING DEEPER

For these sorts of studies you use light microscopy such as confocal and two-photon. But you also use magnetic resonance techniques. How did you get into that?

I wanted to be able to look inside of a frog embryo—all the important interactions are happening deep down inside. The problem was that, with something the size of an embryo, you're completely blind to everything below the outer few tens of microns, if you're looking at it with a light microscope.

If you want to see deep into an embryo, that's just like a neurologist wanting to look down into somebody's head, or like an orthopedist wanting to look into somebody's knee, so MRI jumps out at you. The problem is that, if you look at a frog embryo with normal MRI, you'd get a picture with one bright dot, because the resolution of the clinical MRI is almost exactly the same as the size of a frog embryo. That's when we started thinking, "Would it be possible? Could you push the resolution to the microscopic scale?"

Text and Interview by Ruth Williams
ruth.williams@rockefeller.edu

What made it possible?

I should say that all of this MRI started because of a very, very good colleague at UC Irvine, Russell Jacobs, and because we shared a coffee pot. Once a day we would bump into each other at the coffee pot, and I would ask a question, and he would tell me it was impossible. And the next time I'd see him, he'd say, "I thought about it. It's actually not impossible," and I'd say, "Oh, but that method won't work."

"We can literally watch lineages as they divide and watch cells as they interact."

After weeks of drinking at the same coffee pot, we eventually got to a point where it was clear we could apply the technology to embryos and do a microscopic MRI instead of a macroscopic MRI.

The conventional wisdom at the time was that as you made the resolution smaller and smaller, the time it takes to get the image goes up astronomically. The back of the envelope calculation tells you that—to go from clinical instrument down to something that would let you see single cells, it would require about 10^{36} times as long for the image. So, say if I loaded you into the MRI, it would take a few minutes to take a nice image of your brain. Well, 10^{36} minutes is a really long time. It would be blurred by plate tectonics, that sort of time scale!

What Russ came up with in this whole dialogue was a way to go to higher resolution by using a much stronger magnet. Looking back on them, the first images we got were horrible, but in other ways they were spectacular because we were seeing inside of the embryo.

We've been pushing hard over the years to get down from the millimeter scale to the micron scale, and we just now have a couple of papers coming out with some of these recent images of the frog embryo. We can literally watch lineages as they divide and watch cells as they interact—it's letting us address all the things we were blind to before.

You owe a lot to that coffee pot.

Yes, Russ and I moved together to Caltech, and the first thing we did when we set up our labs here was to buy a very good espresso machine, restaurant-quality, and put it in the conference room. I would say at least three dozen patents have come out of that coffee pot!

A wise investment then.

Yeah, a very, very good investment!

So what recent ideas have bubbled up from the Caltech pot?

Recently we've been using labeling agents that we can see in both the MRI as well as the light microscope to play those technologies off against one another.

You literally use the same embryo in both techniques?

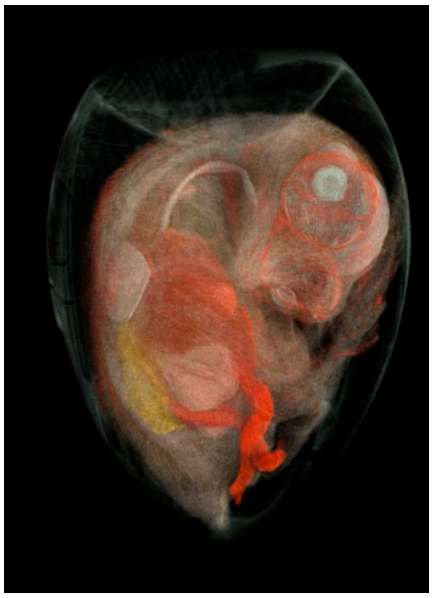
Yes, so then we can validate the results between the two and jump orders of magnitude between the two. In fact, one of the people in the lab right now, Mike Tyszka, is trying to make a stage microscope to look at embryos by MRI. Normally, in MRI, you mount the embryos inside something that's not convenient for light microscopy. What Mike's trying to do is make something that, from above, we'll be able to look at embryos in a Petri dish with a light microscope, and from below we'll be able to look at them with an MRI attachment.

GOING FURTHER

Any other future projects?

We're also now trying to apply our imaging tools to disease models and to clinical medicine. For example, we'd love to look in and ask things about a tumor, about the way that the cells respond to chemotherapy, or other therapies, not by waiting until the cells die or the lump grows or the lump gets smaller, but to really ask, are you hitting that cell? Are you inflicting damage on that cell? Are you hitting the cancer stem cells as well as the non–stem cells?

Similarly, we're now trying to now make microscopes that can look into somebody's eye and image the earliest



FRASER

MRI reveals a quail embryo as it develops in its eggshell.

events in macular degeneration. You want to be able to catch the process before somebody's lost their vision. So, we think that these imaging tools could allow us to understand the disease progression and to identify the earliest antecedents that would provide an alarm sign. Then, we could do proactive therapy instead of trying to save somebody's eyes once they've already lost a good bit of their vision.

There's a variety of other people in the lab trying to make similar leaps to clinical applications, as translation is something we really believe in. JCB

1. Lichtman, J.W., and S.E. Fraser. 2001. *Nat. Neurosci.* 4:1215–1220.
2. Ruffins, S.W., R.E. Jacobs, and S.E. Fraser. 2002. *Curr. Opin. Neurobiol.* 12:580–586.
3. O'Rourke, N.A., and S.E. Fraser. 1990. *Neuron.* 5:159–171.
4. Jacobs, R.E., and S.E. Fraser. 1994. *Science.* 263:681–684.
5. Kulesa, P.M., and S.E. Fraser. 2000. *Development.* 127:1161–1172.
6. Kulesa, P.M., and S.E. Fraser. 2002. *Science.* 298:991–995.
7. Gong, Y., C. Mo, and S.E. Fraser. 2004. *Nature.* 430:689–693.