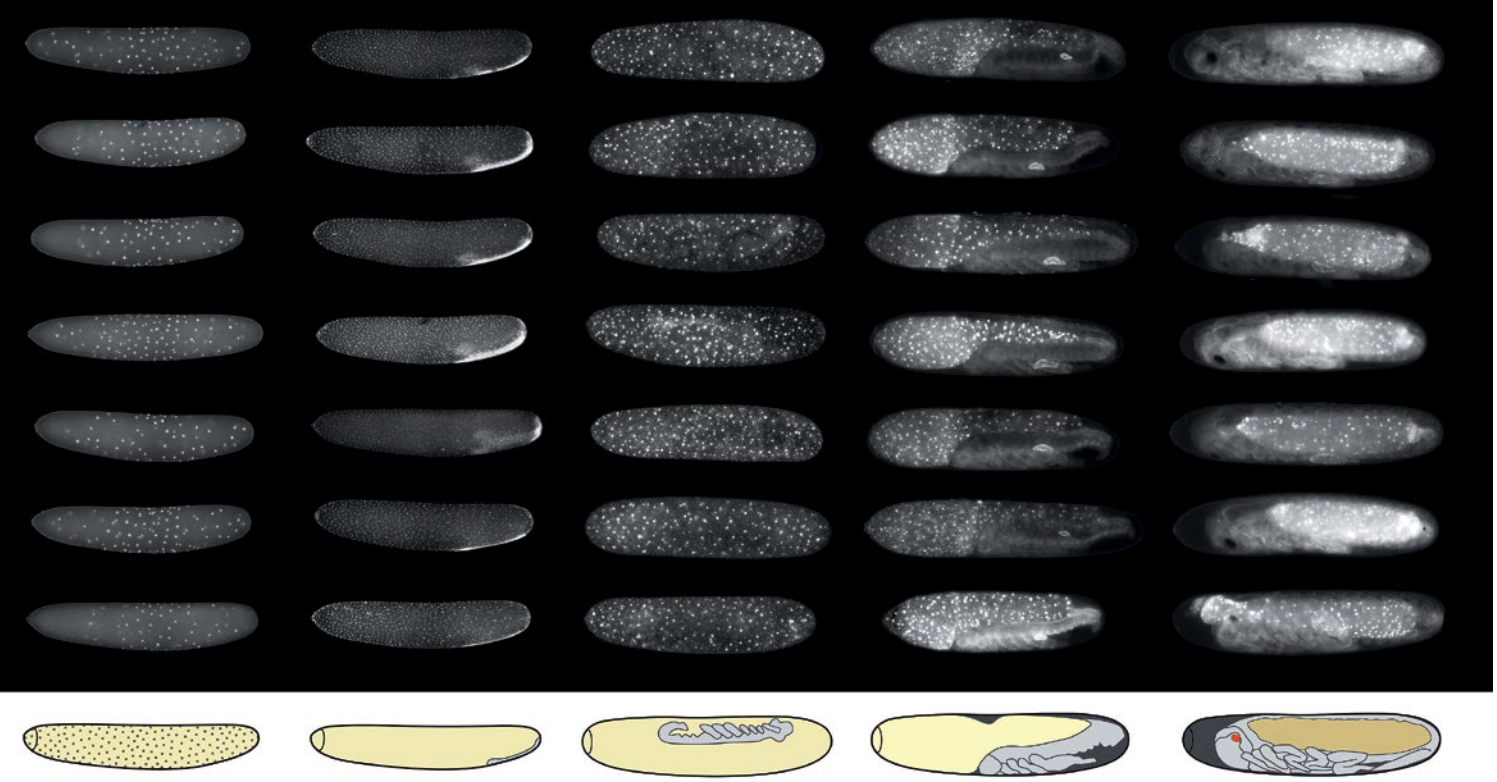

High-Throughput, Long-Term Live Imaging

Automated Microscopy of Insect Development

Seth Donoughe[1] and Sebastian Gliem[2]

The study of arthropod embryogenesis can provide insight into the evolution of development mechanisms. Many aspects of development are most effectively studied by live-imaging the development of many embryos. Historically, this has been difficult, but new tools are making the collection of such datasets easier than ever.

Fig. 1: *ZEISS Celldiscoverer 7. The right module of the system contains the main imaging unit; the left module hosts units for environmental control and an additional camera port.*

Introduction

With regard to the well-studied animal model systems such as zebrafish, nematode, and fruit fly, the last few decades have been enormously fruitful for our understanding of the process of animal development. For those animals, much is known about how cells divide and move in the process of forming an organized body. However, comparatively little is known about how developmental mechanisms evolve. The research group led by Cassandra Extavour at Harvard University aims to uncover aspects of development evolution by characterizing arthropod embryogenesis in a number of emerging model species [1, 2, 3, 4]. By comparing among these species, it is possible to infer the course of evolutionary events that generated some of the great diversity seen in nature.

An ongoing project of the Extavour Lab and its collaborators is to describe embryogenesis of the two-spotted field cricket *Gryllus bimaculatus* (fig. 2A, B). Recent technological advances, such as light sheet and confocal microscopy, have made it possible to explore the dynamics of cellular-level phenomena in emerging model organisms – like the cricket – by sensitive time-lapse imaging. However, although the resulting data have relatively high spatial resolution in three dimensions, these imaging techniques are well-suited for recording at most a few embryos at a time. With such small sample sizes, it is difficult to assess developmental variation and timing [5]. Moreover, pharmacological treatments and functional genetics approaches often produce a broad range of phenotypes (e.g. [6]), which are difficult to characterize without imaging dozens of treated embryos.

This raises the need for a complementary approach in which many samples can be mounted at once, and then recorded during a long-term live imaging experiment with high sensitivity and precise environmental control. A new microscope system, Celldiscoverer 7 (Carl Zeiss Microscopy, Jena, Germany; fig. 1) has provided the opportunity to address these demands. It has been very useful for imaging dozens or even hundreds of samples in parallel, in several different sample carrier types. Therefore, microscopy of this type will potentially provide better insight into many aspects of insect development.

Mounting Embryos

An acrylic mold is used to create low-melt agarose troughs in a glass-bottom dish or in each well of a glass-bottom 6-well plate. All imaging is done on embryos from a trans-

VITA

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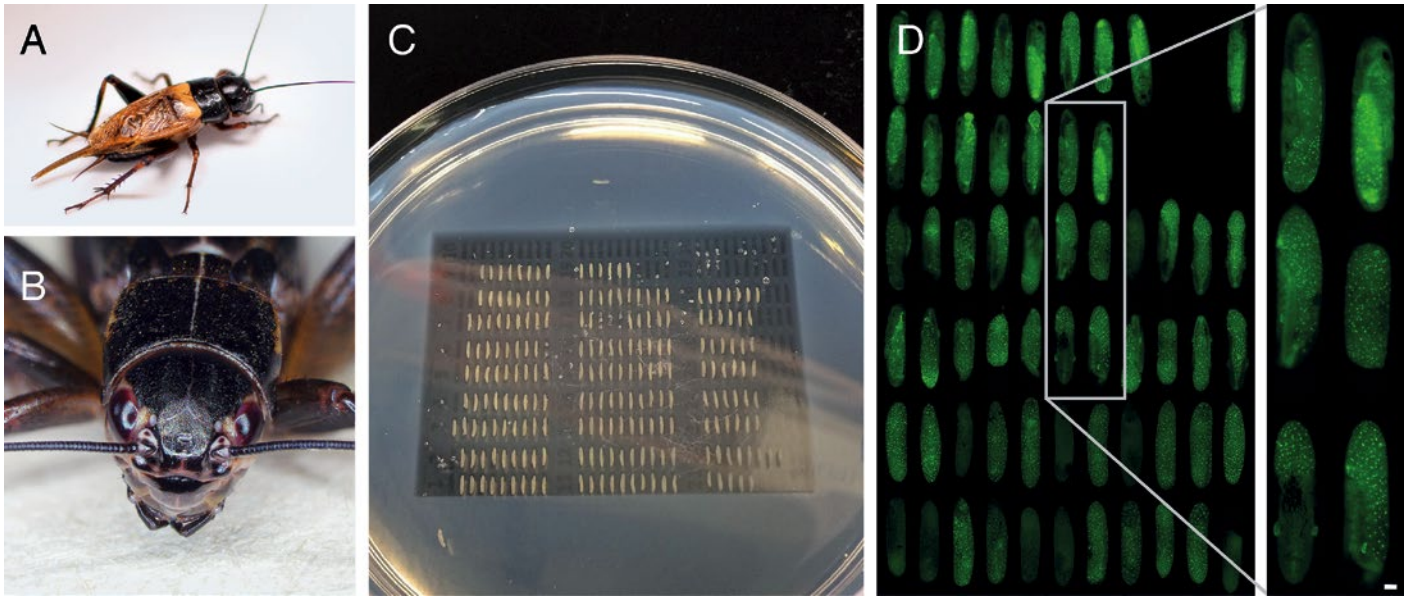
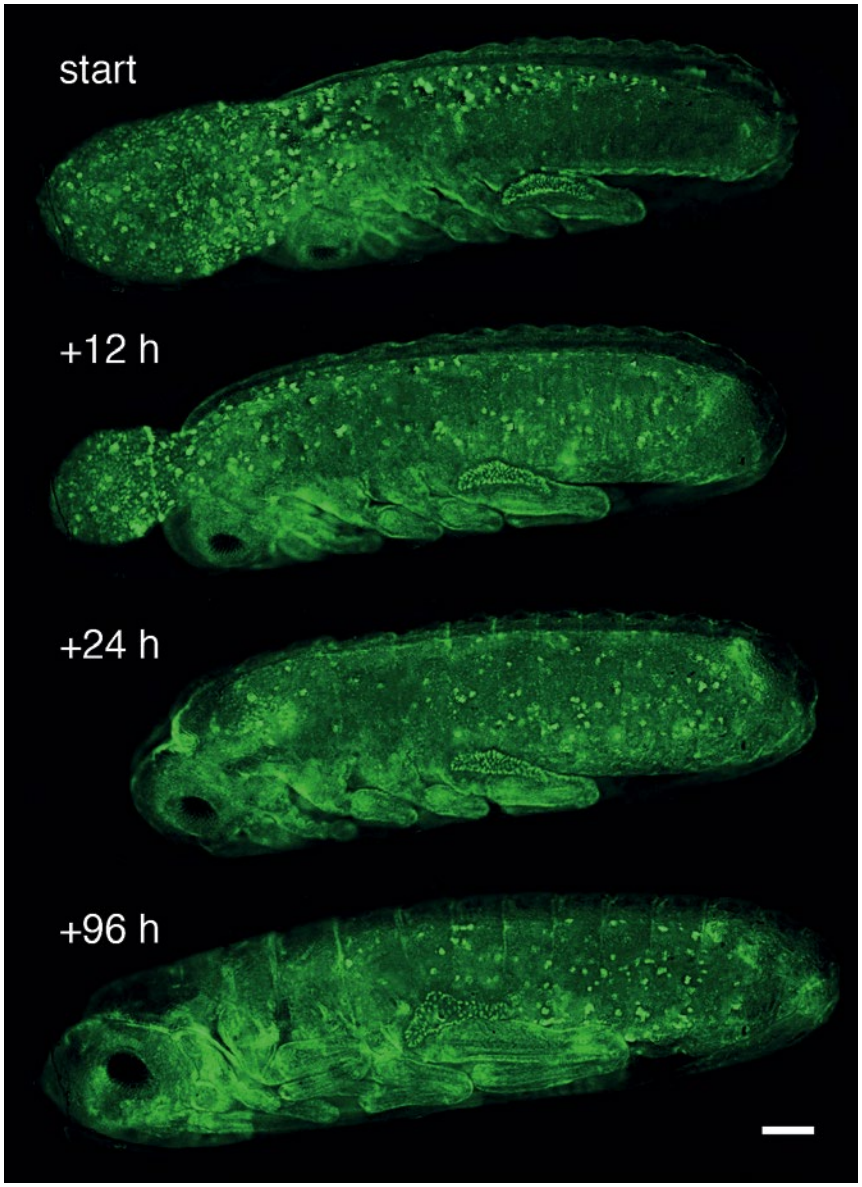


Fig. 2: *Mounting an array of cricket eggs for imaging. A, B: Adult two-spotted field crickets. C: A customized mold insert was used to make a plate of low-melt agarose microwells. After eggs were placed into the microwells, additional low-melt agarose was used to hold eggs in place. D: This technique was used to mount transgenic eggs containing embryos from several different developmental stages. This image was generated from 15 individual tiles that were acquired at 2.5x magnification on Celldiscoverer 7. Scale bar: 200 µm.*



◀ ***Fig. 3:*** *A developing cricket embryo. During the development from egg stage 15 to egg stage 21, dorsal closure as well as limb and body growth can be observed. All images were acquired at 2.5x magnification. They were extracted from a multi-position time series (5-minute sampling interval) and deconvolved from a 130 µm thick z-stack. Scale bar: 200 µm.*

genic cricket line that ubiquitously expresses the cricket Histone2B protein fused to Enhanced Green Fluorescent Protein [7]. Freshly laid cricket eggs are placed into the troughs, and then a few drops of low-melt agarose are added to the troughs to hold the eggs in place (fig. 2C). Water or Robb's Saline is then poured into the dish/well to keep the eggs hydrated. The sample carrier is then loaded into the microscope. The microscope automatically detects the carrier type, measures the cover glass thickness, and adjusts the correction collars of the objectives to minimize optical aberrations. Using a 2.5x magnification, many embryos can be easily imaged at each timepoint by tiling an array over the dish (fig. 2D). At each (x,y) position a z-stack is captured. This approach results in less z-resolution than a confocal or light sheet microscope can provide, but in exchange it is possible to image a much larger sample size while still retaining cellular-level resolution in the x-y plane (fig. 2D).

Long-Term, High-Quality Imaging

The microscope provides precise environmental control for the imaging chamber. Cricket embryos at several different developmental stages could be live-imaged for more than five days with no loss of fluorescence intensity.

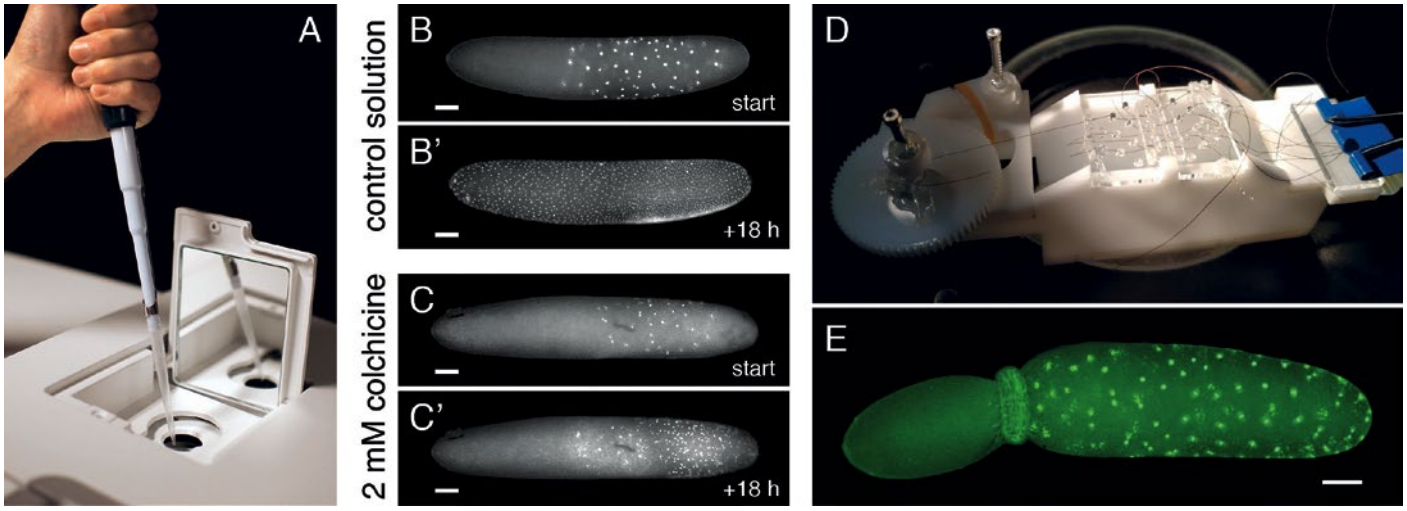


Fig. 4: *Investigating the effect of chemical treatment and physical manipulation on cricket development. A: Celldiscoverer 7 has a fluid handling chamber that can be used to add chemical compounds to the embryos without interrupting incubation. B: Robb's Saline with DMSO was added to embryos as a control condition. This resulted in normal embryonic development up through 18h. C: Robb's Saline containing 2 mM colchicine (a disruptor of microtubule polymerization) inhibited nuclear movements within the egg. D: Device for embryo constriction. A ratchet mechanism applied incrementally higher tension to a human hair wrapped around a cricket egg. The tension was applied while watching the embryo under a dissection microscope. The hair was then fixed in place for imaging. E: By imaging the fluorescently labeled nuclei, it is clear that the constriction impaired movement of nuclei into the tip of the egg. Scale bars: 200 µm.*

Moreover, the survivorship of the embryos was >90% (similar to non-imaged controls). Even with low magnification, the optics are sufficiently sensitive to detect and track nuclei from egg fertilization through the formation of the germ band (see micrographs at top of of the article) and in the extraembryonic tissues throughout development. It is also possible to observe appendage elongation and dorsal closure, an important process during embryonic development in all insects (fig. 3). The large field of view reduces the number of tiles that is required to cover the full array of embryos, thereby decreasing the sampling interval of the time-lapse. For instance, an array of 30+ embryos could be imaged at 2-minute intervals.

Investigating Chemical and Physical Treatments on Embryo Development

Experimental manipulations are an especially valuable application of high-throughput embryo time-lapse recording. Colchicine is a small molecule that inhibits microtubule polymerization. It was dissolved in dimethyl sulfoxide (DMSO) and then diluted in Robb's Saline to five different concentrations, ranging from 2 µM to 20 mM. An array of 20 cricket embryos were mounted in each well of a 6-well glass-bottom plate. Each well was filled with a different concentration, plus a control treatment of DMSO only. By recording time-lapses over 18 hours, a presumptive arrest of nucleus movement within the embryo could be observed. This indicated that cricket cell movements during the formation of the blastoderm are sensitive to microtubule disruption (fig. 4B, C).

Mechanical manipulation is another way to probe the physical mechanisms at work during embryogenesis. A custom ratchet device was developed to wrap a human hair around an individual egg, and then slowly increase tension on the hair, thereby constricting the egg (fig. 4D). This device was used to constrict many embryos, and then live-image the effect on their development. Such manipulations produce effects with a high variance. Some embryos fail to develop, while others may eventually burst from the strain. Only by imaging in a high-throughput manner it is possible to capture numerous instances of embryos that continue development within their artificially truncated eggs (fig. 4E). This technique will be used to assess whether a change in the density of cells/nuclei causes them to change their divisions and movements.

Conclusion

An epifluorescence microscope such as Celldiscoverer 7 with its large field of view but high quality optics, precise environmental control, and sophisticated stage control system serves as a useful complement to confocal and light sheet microscope systems. In the example case of cricket development, it was straightforward and advantageous to conduct the fast, high-throughput time-lapse recordings that facilitate the characterization of developmental variation and high-variance experimental manipulations of embryogenesis.

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