The 200-year effort to see the embryo

Innovations in technology and thought help to visualize the embryo as it really is

By John B. Wallingford

The year 2018 was a watershed moment for the science of embryos. Building on the recent development of single-cell transcriptomic approaches, time-resolved, single-cell atlases of gene expression in an array of developing embryos were reported (1). Unleashing the full power of these datasets will require precise and dynamic registration of gene expression changes with the constant changes in each cell’s position in the embryo. It is therefore important that emerging methods are also allowing the visualization of embryonic morphogenesis with unprecedented accuracy. 2018 saw the report of continuous imaging of the entire mouse embryo at single-cell resolution over multiple days of early development (2). Given these advances, it is compelling to consider the more abstract question of what it means to really “see” the developing embryo.

Modern biologists take for granted that they can collect four-dimensional (4D) movies of an embryo (i.e., 3D imaging over time), but they continue to struggle with distilling and presenting the immense complexity of embryos’ changing forms over time. As the historian of science Janina Wellmann noted, challenges posed by the “intricate entanglement of seeing movement and understanding form” are not limited to time-lapse data or even to biology, but rather they affect visual representation of any dynamic process (3). In this context, the recent advances in single-cell sequencing and in toto imaging also highlight the transformational power of “descriptive” embryology and of the key role that technology plays at moments of breakthrough.

Another breakthrough occurred 200 years earlier, after two recent graduates of an Estonian university sought out a copper engraver in Germany. Christian Pander and Karl Ernst von Baer were pursuing studies together when their adviser Ignaz Döllinger insisted that understanding embryonic development would require what is now called an interdisciplinary team. At Döllinger’s recommendation, Pander recruited Eduard d’Alton, a prodigiously skilled artist with an abiding passion for the life sciences. d’Alton was also a gifted engraver, which was essential for mass production of images at the time. In an unsettling reflection of today’s funding climate, von Baer repeatedly emphasized d’Alton’s independent wealth as a key contribution to the team. Pander and d’Alton worked together on embryo development for years (4).

Pander first published his treatise on chick embryogenesis without pictures, and von Baer found it “incomprehensible” (4). The follow-up reports, however, contained d’Alton’s extensive and beautifully rendered illustrations (see the figure) and sparked von Baer to his own investigations. Pander and von Baer’s work is widely considered to represent the dawn of modern embryology, and the 19th century was marked by the increasingly sophisticated deployment of images to explain embryonic development and to systematize our understanding of it (5, 6).

Nearly a century later, in December 1895, the first motion pictures were shown in a basement in Paris. It was a period of wide-ranging technical innovation with the use of film, and a largely forgotten innovator warrants some discussion: Just a few months before those movies were shown in Paris, Friedrich Kopsch presented what may have been the very first recorded images of moving cells. These cells were in a developing embryo.

Kopsch collected a series of photographs of amphibian gastrulation, the period of development when massive cell movements set up the initial form of the animal body plan. Rather than simply photographing the embryos, he kept the camera shutter open for long periods. In the resulting images, the nuclei of moving cells appeared blurred, indicating the direction and speed of cell movement; stationary cells remained in sharp focus. Kopsch’s images seem not to have survived, but he compiled the data into diagrams of cell trajectories during gastrulation that are impressively accurate (3). The counter-directional flows precisely reflect the results of now-classic fate mapping studies published decades later. Unfortunately, Kopsch’s work was never embraced and has largely been forgotten by developmental biologists (3).

Despite Kopsch’s efforts, and even though movies depicting embryogenesis in sea urchins are among the earliest scientific uses of video (7), for more than half a century, the medium was rarely used by developmental biologists (3). This is surprising because cell biologists such as Ronald Canti and Michael Abercrombie benefited tremendously from time-lapse imaging during the same period (8). Indeed, cinematography brought with it not only the power to resolve movements in space but also the power to manipulate time in the eyes of the observer (7). Here was a tool with which to understand movement and, moreover, to perceive a nascent form as it arises.

The use of time-lapse movies by embryologists finally exploded in the 1960s, and the work of Antone Jacobson and his colleagues prompted the modern paradigm of putting hard numbers to videos of developing embryos. By projecting films of amphibian neural tube closure onto butcher paper and hand-tracing trajectories, they generated high-resolution data on the speed and direction of individual cell movements. From the same movies, they mapped the position of markers and, using D’Arcy Thompson’s method for monitoring the transformation of grids overlain on the images over time, they extracted quantitative data on the deformation of the tissue as a whole. The result was the first systematic attempt to quantify embryonic morphogenesis (9).

A decade later, Jacobson promulgated the concept of morphodynamics, in which experimental developmental biology merged with formal mathematics and predictive computer modeling to meet the complex 4D challenge posed by morphogenesis (10) (see the figure). The foresight of this early work cannot be overstated. Although developed when computational modeling required feeding paper cards into computers the size of cars, this blueprint for morphogenesis research remains the standard of the field today (11).

In the following decades, advances in imaging and improvements in data storage and software greatly enhanced embryologists’ ability to systematically track large numbers of cells over long developmental periods. The development of confocal and two-photon microscopy and the discovery of fluorescent proteins [such as green fluorescent protein (GFP)] allowed embryologists to envision the possibility of in toto imaging, in which every cell in the embryo is discretized and tracked across the full course of development. Although simple to describe, this was a radical notion in a time when
Visualizing the embryo: 1818–2018

Key milestones in imaging embryo development over the past ~200 years are depicted.

most imaging studies took a necessarily piecemeal approach, imaging only one part of the embryo or another. The simplicity and optical clarity of invertebrate embryos such as Caenorhabditis elegans and Drosophila melanogaster made them the first candidates for such studies, which in more recent years have been extended to vertebrates such as zebrafish and mice (11-13).

Technical advances in both microscopy and computation have consequently spurred progress in embryo imaging (11, 12). For example, light sheet microscopy provided a faster and far less phototoxic imaging modality (14), although the datasets were almost invariably large. The first in toto movies of zebrafish development (15) were finished in a timely manner only because the Large Hadron Collider was under maintenance owing to a vacuum leak, leaving the CERN supercomputers unoccupied.

The recent effort to image the mouse embryo employed light sheet microscopy, and it also required a wide array of technical advances (2). For example, precise culture conditions are essential to keep the embryos healthy outside the uterus. In addition, the embryos’ relentless growth makes immobilization on the microscope stage difficult, and growth also brings with it constantly changing optical properties. Improvements in embryo culture, better mounting methods, and a newly configured multiview light sheet microscope with adaptive optics met these challenges. To provide better resolution in the thicker tissues of the larger, late-stage mouse embryos, transgenic mouse strains expressing near-infrared reporters were developed. To make data processing more manageable, an improved block-based image file type for loss-less compression was also developed. Using mosaic expression of tissue-specific reporters, McDole et al. precisely correlated cell lineage, cell movement, and gene expression (2).

Perhaps the most remarkable achievement of their study is the statistical fate map of the mouse embryo. Since the 19th century, embryologists have struggled with the selection of specific embryos (and images of those embryos) for presentation in publications. The use of so-called “normal stages” helped embryologists tackle the challenge posed by the variability inherent in embryos (and in all of biology). That is, investigators must use images to depict specific embryos, but they must also provide images that represent our collective conception of what a normal embryo of a given species looks like at a particular stage of its development (5). McDole et al. achieved a major advance, computationally registering all the cells in multiple mouse embryos in space and time. Thus, the dataset provides not only specific information spanning single-cell trajectories to whole-embryo shapes for individual embryos, but it also provides a rendering of the “average” embryo (2). That is, it provides normal stages of mouse development that are quantitative and derived directly from data.

By integrating these newly developed tools for visualizing the embryo (2) with those for analyzing gene expression (1), it is possible to peer deeper into what has been called the “cytoskeletal universe” (13). This refers to what may be the ideal of modern developmental biology: To describe the state of the entire genome (g) across time (t) in all three cardinal axes of space (x, y, z). In addition, the recent advances in imaging now allow researchers to contemplate even higher dimensions of understanding. For example, future generations of genetically encoded biosensors will allow the integration of space, time, and gene expression but also physiology, as calcium dynamics, metabolite intermediates, and even mechanical strain are tracked. Coupled with the recent explosion of optogenetic tools (light-mediated activation of gene expression), the advances in embryo imaging will also provide an exceptional opportunity for a finer-grained mechanistic dissection of gene and protein function in living embryos.

Pander, von Baer, Kopsch, and even Jacobson likely would not grasp the power of optogenetics or the impact of single-cell genomics, but they would understand the images and how they advance the effort beyond crude diagrams and even beautiful engravings. They would also grasp the power of descriptive triumphs to drive understanding. In such moments, no hypothesis is tested and no new model emerges. Rather, new technologies and thinking lead to quantum advances in our ability to describe, to visualize, and thus to understand the self-organized transformation of a single fertilized egg into a complex animal form.

REFERENCES AND NOTES
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ACKNOWLEDGMENTS
I thank J. Wellman and J. Wittbrodt for thoughtful conversations and E. Roberson and D. Dickinson for critical reading. This work is supported by the NICHD and by the NHLBI.

10.1126/science.aaw7565
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Science 365 (6455), 758-759.
DOI: 10.1126/science.aaw7565