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SPOTLIGHT

Advances in live imaging early mouse development: exploring the researcher's interdisciplinary toolkit

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ABSTRACT

Live imaging is an important part of the developmental biologist's armoury of methods. In the case of the mouse embryo, recent advances in several disciplines including embryo culture, microscopy hardware and computational analysis have all contributed to our ability to probe dynamic events during early development. Together, these advances have provided us with a versatile and powerful 'toolkit', enabling us not only to image events during mouse embryogenesis, but also to intervene with them. In this short Spotlight article, we summarise advances and challenges in using live imaging specifically for understanding early mouse embryogenesis.

KEY WORDS: Image analysis, Imaging developmental processes, Microscopy, Mouse embryology

Introduction

The first live imaging 'cinematographs' of mouse development, using film to record through a microscope, were of pre-implantation embryos undergoing cleavage as far as the blastocyst stage (Kuhl and Friedrich-Freska, 1936). However, because of the difficulty in developing consistent *in vitro* culture conditions that supported mouse development, it was not until the 1960s that detailed time-lapse films of early mouse embryonic development were achieved by multiple labs (Borghese and Cassini, 1963; Cole, 1967; Mulnard, 1967). Since these pioneering imaging experiments, huge strides in a wide range of technologies have revolutionised our ability to live image developmental processes in the mouse embryo. Our ability to gain insights into mammalian development has also been facilitated by parallel advances in culture technology, microscopy hardware, cell and tissue labelling approaches combined with the ability to intervene optically in a controlled manner, and computational approaches to analyse image data. Here, we explore these key aspects of the researcher's 'toolkit' with regard to imaging early mouse embryogenesis (Fig. 1). We highlight how live imaging is an intrinsically interdisciplinary endeavour, and we discuss the importance of advancing each of these complimentary technologies, any one of which can become limiting. This is not a comprehensive review of live imaging technologies; we point the reader to reviews in this special issue (Boka et al., 2021; Vieites-Prado and Renier, 2021; Wolf et al., 2021) and the existing literature (de Medeiros et al., 2016; Nowotschin and Hadjantonakis, 2014). We apologise in advance to colleagues whose contributions we are unable to reference in this brief article due to space limitations.

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Mouse embryo culture for live imaging

Owing to the intrauterine development of mammalian embryos, a fundamental part of the live imaging toolkit for mice relates to approaches for culturing embryos *ex utero*. The isolation and *in vitro* culture of mouse pre-implantation embryos is relatively easy and has become widely used, with embryos being cultured from the one-cell stage through to the blastocyst stage using static culture conditions and defined media, enabling live imaging on a range of microscopic set-ups (Hiiragi and Solter, 2004; McDole and Zheng, 2012; Strnad et al., 2016; Watanabe et al., 2014). For post-implantation-stage embryos, well-established approaches exist for maintaining embryos *ex utero* using roller culture, in which the continuous rolling is understood to promote gas exchange (Beddington, 1987; Lawson et al., 1986; Tam, 1998). However, these methods preclude contemporaneous imaging. Static culture approaches have therefore been developed to image post-implantation embryos, for example to study anterior visceral endoderm cell migration (Srinivas et al., 2004) and to follow cell movements over several days continuously during gastrulation (McDole et al., 2018). Recently, such approaches have been extended to the culture of mouse embryos from pre-gastrulation stages onwards for 5-6 days using a combination of static and roller culture (Aguilera-Castrejon et al., 2021). Although this set-up does not currently incorporate continuous long-term live imaging capability, it takes us one step closer to developing other approaches, for example those using microfluidic chip-based devices, that have been used in cell culture experiments (Coluccio et al., 2019) to give precise control over culture conditions, thereby potentially enabling long-term culture along with live imaging.

Advances in microscopy hardware

Microscopy hardware is naturally a key component of the live imaging toolkit. Live imaging mouse embryos during development is a delicate balancing-act; it requires a limited photon budget to achieve sufficient signal-to-noise and to maximise spatial/temporal resolution while minimising photo-toxicity so as to ensure normal development. The application of fluorescence microscopy to developmental biology, using electromagnetic radiation to excite fluorophores that label a specific structure or cell type, has enabled dynamic cellular and sub-cellular processes to be visualised, thereby revolutionising our understanding of early embryo development. However, exposure of embryos to the illumination required to excite fluorescence can lead to photothermal and photochemical damage, arising from the heating of the sample and the generation of free radicals, respectively. For example, although single-photon confocal microscopy has the advantage of optically sectioning a sample, it still exposes the entire depth of the sample to illumination. This limits its use for live imaging early mouse development. Multi-photon microscopy reduces phototoxicity by using near-infrared wavelength pulsed laser excitation (Benninger

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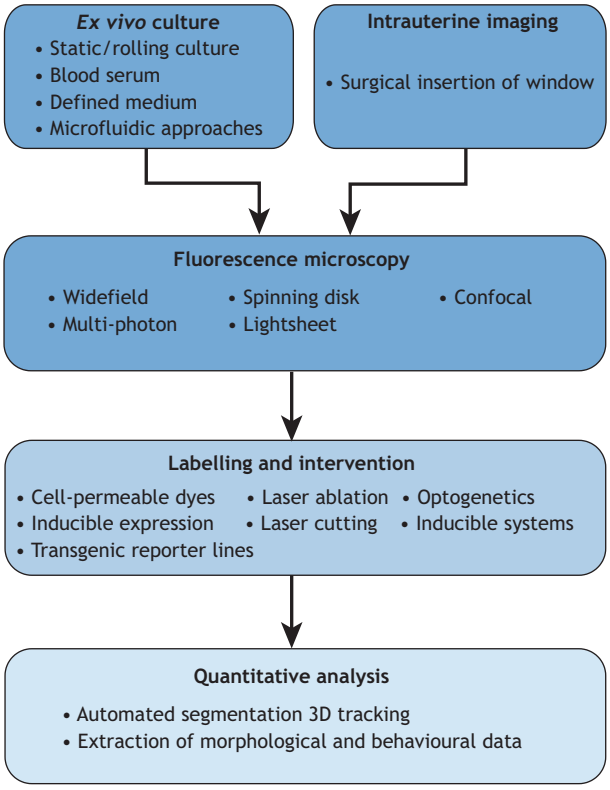

Fig. 1. A toolkit for live imaging mouse embryogenesis. The researcher's 'toolkit' with regard to live imaging mouse embryos involves various approaches and technologies. Advances on all these fronts will greatly facilitate the use of live imaging for investigating mouse embryogenesis.

and Piston, 2013). Here, a fluorophore is only excited when two low energy photons are absorbed. This non-linear effect only occurs at the focal point, thereby providing optical sectioning while restricting photochemical damage. Such approaches have been used successfully for studying pre-implantation-stage mouse embryos, which are particularly sensitive to perturbations, at high resolution (McDole and Zheng, 2012).

An increasingly popular way of minimising photodamage involves using light-sheet microscopy. This technique was first described using a bright-field illumination with a slit-aperture in the early 1900s (Siedentopf and Zsigmondy, 1902) but was later adapted to fluorescence microscopy by using a cylindrical lens or scanning laser beam to create a thin 'lightsheet' for illuminating the sample (Huisken et al., 2004; Keller and Stelzer, 2008). By positioning the excitation lightsheet orthogonal to the imaging objective and placing the sample in this sheet, the embryo can be imaged, while illuminating only the plane being imaged. Optically sectioning through the entire sample requires moving the sample through the fixed sheet of light that is positioned at the focus of the detection objective. This set-up enables full image volumes to be rapidly acquired while minimising photodamage, allowing higher temporal resolution and longer duration than generally possible with confocal or multi-photon microscopy. In addition, multiple views of the embryo can be captured by rotating the sample or alternately, in instruments with two imaging objectives, by imaging from two angles acquired simultaneously (Huisken and Stainier, 2007; Krzic et al., 2012). Such multi-view imaging therefore enables one to image further into the sample, as the highest quality images from each view angle can subsequently be computationally combined into a single volume.

Light-sheet imaging has been used to generate high-resolution 3D whole-volume images of mouse embryos and has been used to image pre-implantation development (Strnad et al., 2016), early gastrulation (Ichikawa et al., 2013; Mathiah et al., 2020) and post-gastrulation stages (McDole et al., 2018; Udan et al., 2014). Light-sheet microscopy has been a little slow to be adopted for studying mouse development owing to the challenging aspects of suspending the sample between the objectives. However, several commercial systems with an inverted set-up are now available and allow one to image the sample in a dish. These include the InVi SPIM (Bruker-Luxendo) and the Lattice Lightsheet 7 (Zeiss). Furthermore, modifications incorporating multi-photon (Truong et al., 2011), beam-shaping (Chen et al., 2014) and airy scanning technology (Vettenburg et al., 2014) have also been developed. These next-generation light-sheet microscopes, integrated with advanced culture methodologies, have the potential to become an established part of the mouse embryologist's toolkit in the future.

Genetic labelling and optical modulation of cell function

In combination with advances in microscopy hardware, the ability to label cells with genetically encoded fluorescent proteins has revolutionised the field of live imaging. The use of genetically modified mouse fluorescent reporter lines can provide temporal and spatial information about gene expression, cell lineage, and the position and behaviour of labelled proteins (reviewed by Xenopoulos et al., 2012). Furthermore, mouse lines exist that encode sensors of specific behaviours or cellular processes (e.g. cell cycle progression; Abe et al., 2013).

To achieve a mechanistic understanding of mouse development, some sort of perturbation or intervention is required. In the context of live imaging, this has generally been achieved by imaging genetic knockout mutant embryos or via the exposure of embryos to pharmacological inhibitors. However, there can be drawbacks to these approaches; inhibitors may affect all cells in the embryo making it difficult to disentangle their effect on particular cells or tissues, and knock-outs of genes in mouse embryos, even if generated in a tissue-specific manner, may have such a large effect that it becomes difficult to interpret the phenotype. One approach that can provide more precise spatiotemporal control, enabling

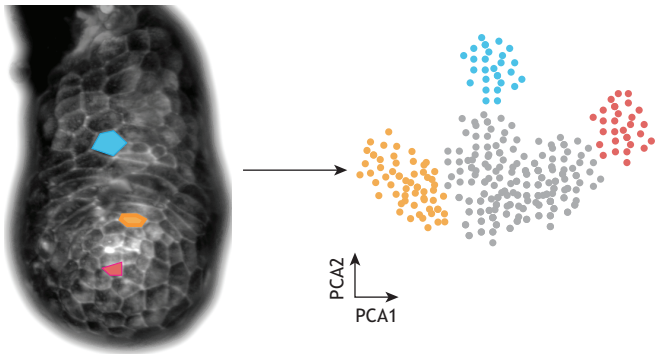

Fig. 2. An analytical framework for understanding complex multi-dimensional image data. Quantitative analysis of cell behaviour from multiple embryos requires new ways of considering and displaying complex multi-dimensional image data. For example, principal component analysis (PCA) of cells from multiple embryos clustered according to behaviour and morphology might enable distinct sub-populations of cells to be identified. In this illustration, three cells with different geometric and behavioural properties have been coloured in the light-sheet image of a pre-gastrulation embryo on the left. Cells from multiple embryos with quantitatively similar characteristics to these highlighted cells cluster together, as depicted in the plot on the right.

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localised acute intervention, is laser ablation. This allows one to not only ablate entire cells but also break sub-cellular structures, such as actin-myosin cables, to provide insight into mechanical forces within tissues. This approach has been widely used in *Drosophila* embryos (Kiehart et al., 2000; Shivakumar and Lenne, 2016) and has also been adapted for mouse embryos (Angelo and Tremblay, 2013; Fierro-Gonzalez et al., 2013). Going forward, optogenetic approaches that enable genes to be switched on and off in groups of cells (Konermann et al., 2013), or that control the localisation of proteins within cells (Buckley et al., 2016), combined with live imaging technology, have the potential to transform the precision with which we can intervene with cellular processes during mouse development.

Quantitative analyses of image data

Advances in embryo culture, microscopy hardware and labelling technologies allow us to record developmental processes in great detail. However, one increasingly frequent limiting factor in fully exploiting these technologies lies in our ability to analyse computationally the large and complex multi-dimensional datasets that can be generated. For example, multi-view light-sheet imaging experiments of mouse embryos developing over several days can generate 10 terabytes per embryo (McDole et al., 2018). Such datasets require dedicated computational approaches for both image processing (e.g. fusing images from multiple view angles, applying spatiotemporal registration, allowing image augmentation) and data analysis (e.g. cell tracking in 3D for thousands of cells across hundreds of time points). Machine learning-based approaches to automate analysis are proving effective (see Hallou et al., 2021, in this issue), and several software packages have been developed for such image processing and 3D cell-tracking requirements, including MaMut (Wolff et al., 2018), BigDataViewer (Pietzsch et al., 2015) and RACE (Stegmaier et al., 2016).

It is clear that a major challenge for future live imaging-based studies will involve the development of bespoke computational analysis pipelines to extract and analyse specific behaviours. For example, a quantitative understanding of tissue remodelling at the single-cell level will require approaches for integrating high-resolution image data from multiple embryos within an analytical framework that can accommodate natural variations in mouse embryo size, morphology and developmental timing. It will also require a way to consider and display such complex data in a unified space, for which we can take inspiration from the approaches used to analyse multi-dimensional data in single cell '-omics' studies (Fig. 2).

Conclusions

Live imaging mouse development is on the cusp of a 'golden-age' due to a convergence of several factors: new microscopy technologies, mature genetic reporter technologies and novel experimental set-ups that provide researchers with exquisite spatial and temporal control over experimental interventions. These are being 'turbo-charged' by the development of novel analytical approaches to extract quantitative insights into cell behaviour during development. Ultimately, the coordinated development of these complimentary technologies will start to the push the field into the realms of 'Big Data' science. Exciting times indeed!

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Competing interests

The authors declare no competing or financial interests.

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