Review

A coming of age: advanced imaging technologies for characterising the developing mouse

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The immense challenge of annotating the entire mouse genome has stimulated the development of cutting-edge imaging technologies in a drive for novel information. These techniques promise to improve understanding of the genes involved in embryo development, at least one third of which have been shown to be essential. Aligning advanced imaging technologies with biological needs will be fundamental to maximising the number of phenotypes discovered in the coming years. International efforts are underway to meet this challenge through an integrated and sophisticated approach to embryo phenotyping. We review rapid advances made in the imaging field over the past decade and provide a comprehensive examination of the relative merits of current and emerging techniques. The aim of this review is to provide a guide to state-of-the-art embryo imaging that will enable informed decisions as to which technology to use and fuel conversations between expert imaging laboratories, researchers, and core mouse production facilities.

Why image

Imaging has revolutionised biomedical research over the past four decades, and innovations are continuing at an increasing pace. The immense challenge of annotating the entire mouse genome [1] has led to the development of cutting-edge imaging tools in a drive to discover novel structural and functional information with a particular relevance to human pathobiology. The emphasis is now increasingly on application of these techniques, data extraction, multi-scale screening, and dissemination of technologies out to the community for user annotation and detailed follow-up studies. Furthermore, sophisticated computational techniques are being combined with novel data acquisition and a deep understanding of biological processes to characterise mutant mice.

Over the coming decade, the scientific community has a unique opportunity to discover a wealth of information about embryonic development and gene function through large-scale international efforts to systematically knock out every gene in the mouse genome [2] and interrogate the phenotypic consequences. The International Mouse Phenotyping Consortium (IMPC) aims to create 20 000 knockout strains by 2021, and at least one third of these are expected to be embryonic lethal in homozygous form [3,4], precluding further investigations in adult mice. Characterising these mutants is vital for a comprehensive understanding of gene function in embryonic development [5], and there is now a major push by the IMPC to develop a dedicated embryonic lethal phenotyping pipeline to facilitate this endeavour [3].

The ability to observe the embryo, either at specific stages or 'live' as morphogenesis proceeds, is a fundamental goal of developmental biology. For decades this has relied on optical light microscopy of intact embryos or histological sections, which remains a key approach in most laboratories, providing high-resolution 2D data for visual assessment of morphological phenotypes and tissue sections that may be stained for gene and protein expression. Additional imaging opportunities have been provided by the advent of confocal microscopy, enabling collection of

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optical sections through thicker samples. Episcopic imaging is a further development, in which the autofluorescence of each tissue slice is photographed and combined to generate high-resolution 3D datasets. Nevertheless, despite vast improvements in technology and the corresponding information that can be acquired, these approaches are time-consuming, labour-intensive, and prone to distortions, presenting challenges for high-throughput phenotyping and restricting the amount information that may be obtained. Furthermore, conventional methods do not lend themselves to longitudinal studies (particularly in mammalian systems) or computational analyses, which are becoming increasingly important. Therefore, there is a clear need for complementary imaging techniques that can fill these gaps.

A major driver for the adoption of novel imaging techniques has emerged from international efforts to systematically characterise the rapidly increasing number of mutant lines being generated, for which there is frequently little or no embryonic phenotype information. State-of-the-art 3D imaging has the potential to expedite this ambitious task, ideally providing high-throughput phenotyping approaches that are applicable at a range of stages of mouse development, coupled with advanced computational methods of analyses and user-friendly remote access to data for widespread analysis.

Another key driver is the specific and varied requirements of individual research groups. For example, abnormal morphogenesis of complex developing structures can be difficult to visualise by external examination of the embryo or 2D sections. Thus, the ability to generate 3D images is highly advantageous. In addition, emerging technologies for *in utero* imaging will create exciting opportunities for longitudinal studies of normal and abnormal development.

This review aims to enable informed choices and encourage dialogue between expert imaging laboratories, core mouse production facilities, and individual researchers, and prime the community with new information about emerging state-of-the-art imaging tools. By aligning imaging techniques with biological needs, the maximal number of novel phenotypes may be discovered to pique the interests of the life science and preclinical research community, and provide primary evidence to justify detailed follow-up studies.

Imaging technologies: established and emerging

Magnetic resonance imaging (MRI)

MRI has become a versatile powerhouse of the preclinical imaging world over the past few decades. Already well-established for high-throughput mouse embryo imaging [6–8], MRI users can leverage a variety of methodologies to address different needs. From a phenotyping perspective, it is ideally suited for detailed morphological screening of mid- to late-gestation embryos *ex vivo*, as demonstrated in studies that have characterised development [9,10] (Figure 1A), identified subtle cardiac and brain defects in mutants [6,11–14] (Figure 1D,E), and visualised the cardiovascular system [15–17].

The general method developed by Smith and Johnson *et al.* in the 1990s [18] is still widely used today. Essentially, embryos are dissected from the uterus and imaged *ex vivo* at microscopic resolutions (as fine as 18 μm). Approximately 40 late-gestation embryos can be imaged simultaneously in a single overnight scan [6–8], reducing imaging costs and enabling high-throughput screening, which are important factors for both widespread use and large-scale pipelines.

Because MRI is not limited by the optical properties of tissue, 3D images are produced with excellent soft-tissue contrast that can be readily manipulated. A common approach is to incorporate an MRI contrast agent in the tissue fixation process, which boosts the image contrast [11]. Although this process generally extends the imaging protocol by several weeks, contrast agents provide markedly improved image quality for a fraction of the scan-time and enable visualisation of many additional tissue structures. Embryos may also be perfusion-fixed with a contrast agent mixed in gelatin to provide enhanced visualisation of the vasculature, which is difficult to capture using conventional histological methods. For example, this approach has enabled identification of structural defects in *Gli2*$^{-/-}$ mutant embryos [15].

Despite the wide array of available contrast agents, MRI is currently limited by a lack of tissue staining capabilities that provide the flexibility and target specificity offered by conventional histological methods. A recent study demonstrated that different agents can produce spatially-distinct MRI staining patterns in embryos [19], promising a host of MRI stains that provide tissue-specific contrast enhancement in a fashion synonymous to conventional histological stains, thus expanding the range of pathologies and defects that may be detected. A wide-scale assessment is necessary to identify potential MRI histological stains, as well as further investigation of the underlying mechanisms to fully exploit their capabilities and facilitate the engineering of novel agents.

Additional contrast may be obtained in the embryo using diffusion-based MRI techniques, which non-invasively probe tissue microstructure and provide unique neuroanatomical information about the pre-myelinated embryo central nervous system, such as discrete delineation of multiple cortical layers, grey-matter structures, and various white-matter tracts [20–22]. Diffusion MRI has enabled volumetric analysis of whole brain and sub-structures [23], facilitated 3D mapping of gene expression data [20], contributed to the characterisation of normal brain development at multiple stages [21,23–25], and enhanced phenotypic assessment of mutant embryos [26–28]. Practical implementation of this technique in an embryonic screening pipeline and widespread routine use for embryo phenotyping are currently restricted by an extended scanning time (an order of magnitude higher than conventional structural imaging). Methodological investigations are currently underway to enable wider adoption of this technique.

Although *ex vivo* imaging remains the method of choice for phenotyping in view of higher resolution and throughput (e.g., not having to anesthetise an animal during imaging), it was recently demonstrated that MRI can produce stunning high-resolution 3D images of embryos *in utero* as early as embryonic day (E)10.5 [29]. This method has been used in combination with a contrast agent to identify brain defects in mutants [30], demonstrating its