Systems Biology through Mouse Imaging Centers: Experience and New Directions

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Abstract

The completed sequencing of genomes has forced upon us the challenge of understanding how the detailed information in the genome gives rise to the specific characteristics—phenotype—of the individual. This is crucial for understanding not only normal development but also, from a medical perspective, the genetic basis of disease. Much of the mammalian genome-to-phenotype relationship will be worked out in the mouse, for which powerful genetic-manipulation tools are available. Mouse imaging combined with powerful statistical methods has a unique and growing role to play in phenotyping genetically modified mice. This review outlines the challenges for image-based phenotyping, summarizes the current state of three-dimensional imaging technologies for the mouse, and highlights new opportunities in systems biology that are opened by imaging mice.
FROM GENOMES TO PHENOTYPES

There can be no dispute that one of the major accomplishments in biological research in the twentieth century was the sequencing of genomes. The sequencing of a human genome consisting of $3.3 \times 10^9$ base pairs was a phenomenal accomplishment—whether one considers either the planned governmental project (1) that was completed within budget and ahead of schedule or the competitive private-sector project with its higher-risk, more imaginative sequencing strategy and aggressive timetable (2, 3). Both of these projects ushered biology into the era of large-scale science. This style of big science, which was familiar to engineers through projects such as NASA and to physicists with particle accelerators and astronomical observatories, represented a cultural change for biological research.

Once the possibility of sequencing the genome of whole organisms had been demonstrated, researchers started mapping sequences for additional mammals, beginning with the mouse. There are now complete genome sequences for more than 1000 organisms (not including bacteria and archaea) (4), and the list grows by several per week (Figure 1). Specific genome sequences of several individual humans have now been reported, and it is expected that the cost of individual human sequences will fall to approximately $5000 per person next year (5). This incredible increase in sequencing capacity has been enabled by frequent new generations of sequencing machines that enhance both speed and parallelization.

Reaping the benefit of all this genome sequence information requires a detailed understanding of the relationship between genome and phenotype—the observable characteristics or traits of an organism such as its morphology, development, physiology, and behavior. Multiple questions need to be answered. How does the information contained as linear, digital data in the genome give rise to the specific phenotypic characteristics of the resultant individual? How do small differences in gene sequence produce differing phenotypes among individuals of the same species? How do environmental factors such as diet, learning, and socialization modulate the phenotype of
The number of completed genome sequences continues to grow exponentially. It is dominated by bacterial sequences, but the number of eukarya is becoming significant. Abbreviation: WGS, whole-genome shotgun. Figure courtesy of John Parkinson from the University of Toronto.

individuals with identical genomes? And from a healthcare perspective, what are the genetic and environmental determinants of human disease? Answering these questions constitutes the major goal of biomedical research in the twenty-first century.

When the detailed relationship between genome and phenotype is eventually worked out, it is not clear what the answer will look like. A scientifically pessimist view might be that the relationship will be so complex that it can only be represented as an algorithm within a supercomputer, which can receive a genome sequence as input and provide answers to any phenotypic queries as output. In this scenario, there will be no way to "understand" the relationship, and hence there will be no "stories" about genes and phenotype. It is more likely, given the remarkable conservation of many genes and molecular pathways over many diverse species, that there are general motifs that sketch out the broad-brush characteristics of genome-to-phenotype relationships across the evolutionary tree (6). These motifs can then be organized into hierarchical sets of explanatory "stories" that will provide general insight and understanding into how genetics control phenotypes.

Whatever the genome-to-phenotype relationship looks like, that it will not be simple is already clear. There are a few infrequent situations in which a single gene modification results in a single phenotypic change. Within medicine, most of these are rare genetic diseases that have already been identified by the observation of familial histories. More frequently, single gene changes give rise to multiple and often diverse phenotypes. This poignant cautionary tale is told by Nancy Jenkins from the National Cancer Institute at Fredrick in Maryland (7):

The “ashen” mouse has a gray coat color rather than shiny black because of a genetic abnormality in the vesicle transport motor that drags black melanin particles from the nucleus where they are made down to the distal parts of the hair cells. The “ashen” mouse also has neurological deficits. And that is because it is the very same vesicle transport motor that is used to deliver components of the synaptic vesicles along to the distal ends of neurons where they are essential for transmission of nerve impulses.

Thus a single gene change can change hair color to gray and surprisingly also diminish neuronal transmission. The genome-to-phenotype mapping will be complex and nonintuitive.
A map of the human protein-protein interactions obtained from the iRef Index database. The sphere at the right includes proteins that are highly connected to one another, whereas the sparse red dots represent proteins that have some neighbors that are connected only to themselves. The fine blue lines represent the interactions between these proteins. The image is generated by Cytoscape and provided courtesy of Xuejian Xiong and John Parkinson from The Hospital for Sick Children.

Also, most of the important and common diseases are already known to involve multiple genetic modifications.

Furthermore, the web of interactions of genetically coded proteins is highly complex. Gene products interact with other expressed proteins to form functional complexes. Gene products interact with DNA as regulatory molecules, and they interact with substrates exhibiting both positive and negative regulation. Because the number of connections and interactions (Figure 2) far exceeds the number of gene products (a mere ~25,000 in mammals), it is through these interactions that the complexity and stability of mammalian biology are maintained. Thus any attempt to understand genome-to-phenotype relationships must consider the biological organism as a whole.

SYSTEMS BIOLOGY

Embracing complex interaction networks in biology requires a new scientific paradigm. The straightforward cataloging of a myriad of specific details will never succeed in elucidating genome-to-phenotype relationships. Systems biology has been growing since 2000 as a new paradigm that focuses on how complex interactions between simple components can give rise to new “emergent” properties and behaviors:
Systems biology is about putting together rather than taking apart, integration rather than reductions. It requires that we develop ways of thinking about integration that are as rigorous as our reductionistic program, but different… It means changing our philosophy, in the full sense of the term. (8)

In anticipation of this change of paradigm, biologists (and to a lesser extent medical researchers) have extended an invitation to engineers and physicists to participate in this postgenome agenda. This invitation stems in part from a somewhat naive belief that physical scientists will know what to do with the large masses of data coming from genomics and phenotyping. At a deeper level, it represents a hope that these scientists will bring an expertise that is able to account for the discovery of sophisticated behaviors arising from multiple single interactions. This kind of invitation is attractive to physicists, some of whom are enamored with "complex adaptive systems" (9, 10) in which large numbers of simple components can give rise to remarkable and unexpectedly complex behavior, as that demonstrated in superconductivity and weather prediction. This also appeals to engineers, who appreciate the incredible potential and universality of digital computers that are composed of only 0s and 1s with simple interactions, albeit in very large numbers. These computers are capable of representing an astounding spectrum of human activities and interactions. A history of considering large numbers of rudimentary components with simple interactions as models of living things dates back to Conway’s game of life (11) and Dawkins’s evolutionary models (12).

This invitation from postgenomic biologists to physical scientists is embodied in a variety of new institutes such as the Institute for Systems Biology established by Leroy Hood in Seattle, Washington (http://www.systemsbiology.org); the Santa Fe Institute in Santa Fe, New Mexico (http://www.santafe.edu); the Systems Biology Institute in Tokyo, Japan (http://www.sbi.jp); and the Howard Hughes Medical Institute’s Janelia Farm Research Campus near Ashburn, Virginia (http://www.hhmi.org/janelia). To provide education in this new paradigm, the United States’ National Institutes of Health (NIH) and the Howard Hughes Medical Institute (HHMI) have jointly provided funding for the establishment of new graduate programs designed to bring engineers, physicists, and mathematicians into interactions with biologists. Whereas some of these programs that encourage basic science collaborations with postgenomic biology have a ring of naive hopefulness and enthusiasm, all of them reflect a strong conviction that understanding how genetic information gives rise to individuals requires a new approach to biology that embraces the whole individual as a complex system.

There are several different approaches to investigating the mapping between genomes and phenotype—all of which will be essential for an ultimate solution. The first is a bottom-up approach that begins with the identification of genes (and their control elements), determines which are transcribed (the transcriptome), finds the gene products and their structures (structural biology), identifies molecular interactions and pathways, and tries to build a reductionistic picture of the phenotype of a complex individual in terms of components. A second approach to genome-to-phenotype relationships—used particularly in medicine—is to conduct genome-wide association studies (GWAS). In GWAS, researchers begin with a group of individuals with a common phenotype (a disease, for example) and matched controls, and then look for shared signatures in the genetic sequences of the disease group that are not evident in the control group (13). This is more of a top-down approach that requires a mechanistic analysis once an implicated gene is identified. A third approach, which can be used only where deliberate genetic manipulation can be done, is to start with an inbred strain (an unlimited family of genetically identical individuals), make changes in the genome, and look for associated changes in the phenotype with respect to that of the inbred strain. This approach is more direct in that changes at the bottom (genetic modifications) are
robustly associated with phenotypic outcomes at the top. This kind of experiment cannot be done in humans, and therefore much of the mammalian genome-to-phenotype mapping is done in the mouse.

The mouse is an ideal model for mammalian genetics for a number of reasons:

1) The genes and pathways in mice are very similar to those in humans. In fact, there is a 99.5% probability that a gene from one species can be recognized in the other (14).

2) Mice exhibit clinical symptoms of the majority of human diseases.

3) Genome sequences are now complete for several strains of mice as well as for a growing number of humans.

4) Excellent inbred strains are available, providing genetically identical individuals with very similar phenotypes.

5) Mice breed rapidly (∼10 weeks per generation) and are relatively inexpensive to feed and house.

6) There are powerful techniques for genetic alternations in mice.

These techniques for genetic alternation include gene knockouts (KOs), in which a gene product is inactivated; knockins (KIs), in which a new gene is introduced from a species (such as green fluorescent protein from a jellyfish or luciferase from a firefly); conditional knockouts, in which a gene is inactivated in a spatially localized region or concurrently with an administered drug; and random point mutations that involve a chemical mutagen such as ethylnitrosourea (ENU) (15).

In addition, a worldwide collaborative consortium named the Knockout Mouse Project (KOMP) is working on knocking out each of the known 23,000 genes in the mouse, one at a time (16).

With this wealth of powerful techniques for genetic modification, comprehensive phenotyping is equally important if we are going to learn about genome-to-phenotype relationships. Phenotyping can focus on a wide variety of traits that can be generally grouped under anatomy, physiology, and behavior. Every lab in the world that works with mouse models is involved in phenotyping. In some labs that are focused on a specific disease model, the phenotyping may be attempting in great depth to obtain information about the molecular mechanisms of the disease. In other centers and companies that study a broad range of mutations, the phenotyping is more cursory and streamlined with a focus on throughput. An example involving structured phenotyping and a pipeline is the European Mouse Phenotyping Resource of Standardised Screens (EMPReSS) (17), which has been adopted as a standard in European labs and elsewhere.

IMAGING THE MOUSE

This brings us to the main subject of this review—imaging the mouse. Mouse imaging is a comparatively new field but one that is rapidly growing. Little work on mouse imaging was published before the early 1990s, but the field has grown rapidly since then: At the end of 2009, there were more than 15,000 papers. Remarkably, more than half of these were published in the past five years, demonstrating that the field is in a phase of exponential growth and that greater growth can be expected over the next decade. Much of this growth has been stimulated by a major NIH funding initiative named the Small Animal Imaging Resource Program, which has provided sizable equipment grants to establish mouse-imaging centers. Similar types of funding are also being provided in other countries.

There is a growing appreciation of imaging’s role in mouse phenotyping. The argument can (and has) been made that if we are interested in studying mouse mutants as models of human diseases, we should have the same diagnostic capabilities for mice that we have when we investigate human diseases in the human. In addition, it is hard to conceive of modern medical practice without medical imaging.
Some of the strengths that imaging brings to mouse phenotyping include the following:

1) Potential for live imaging: Many mouse mutations are difficult to breed and may even be represented by only a single mouse. Thus the ability to keep the mouse for subsequent studies is important. Also, many phenotypes are progressive, and longitudinal studies in the same individual are important.

2) Full animal coverage: Imaging in a mouse frequently covers the whole mouse. When single gene mutations result in a plurality of phenotypes, a whole-mouse survey is paramount. It is our experience that additional phenotypes beyond those originally anticipated often are found in the first imaging session.

3) Rich data sets: In contrast to physiological assays, which usually yield at most tens of measurements, imaging usually provides millions of independent voxel measurements. In fact, the data are so extensive that meaningful data-reduction techniques are necessary.

4) Quantitative analysis: Because modern imaging is digital, it allows for automated computer analysis and statistical measures of the significance of differences. This becomes increasingly essential given the richness of the imaging data.

5) Anatomical, physiological, and functional measure: Whereas imaging is targeted toward spatial differentiation and is primarily used for anatomical mapping, imaging techniques can increasingly also provide physiological measures such as cardiac function and blood flow and also functional mapping such as functional magnetic resonance imaging (fMRI) or molecular biodistributions.

6) High throughput: In the context of phenotyping, large numbers (thousands) of mice may be studied. New adaptations of imaging are enabling increased throughput. There is also potential for the greater use of robotic sample handling.

Not all of these imaging strengths have been fully realized for mouse phenotyping, and most of them still represent active areas of research.

### MOUSE IMAGING TECHNOLOGIES IN PHENOTYPING

The remainder of this review considers a variety of the major technologies that are used for mouse imaging (see Table 1). Mouse-imaging centers usually have several of these technologies; only a few centers have all of them. Illustrative examples are given regarding how these imaging methods are used to address genome-to-phenotype relationships in the mouse. Mouse imaging is also used for other studies that are not part of systems biology, such as toxicology and drug biodistributions. These are important studies and significant applications of mouse imaging. However, because they are not the focus of this review, the examples presented here are limited to genes and phenotypes.

#### Computed Tomography Imaging for Mouse Phenotyping

Computed tomography (CT) is readily adaptable from the clinic to the mouse (18). Smaller voxels can easily be obtained with finer detector elements. Cone-beam technologies easily provide full animal coverage. X-ray energies can be decreased because of the sample’s small size. However, good soft-tissue contrast is difficult to achieve because in CT, the grayscale discrimination is limited by the number of interacting X rays. If the voxel dimension is reduced from human imaging by 15-fold in the mouse, the voxel volume decreases by 3000 times, and the X-ray dose needs to be increased by 3000 times to attain equivalent soft-tissue contrast (18, 19). Nevertheless, CT is used for imaging high-density structures such as bone and vascular trees filled with X-ray-opaque contrast agents. Live imaging of soft-tissue organs at resolutions of coarser than 100 μm is also achievable at acceptable doses.
Table 1  Mouse-imaging technologies\(^a\) and their relative strengths\(^b\)

<table>
<thead>
<tr>
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<th>Resolution</th>
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<th>Molecular</th>
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<td>Luciferase transgenics</td>
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\(^a\)Abbreviations: BLI, bioluminescence imaging; CT, computed tomography; MR, magnetic resonance; OPT, optical projection tomography; PET, positron emission tomography; SPECT, single photon emission computed tomography; SPIM, single plane illumination microscopy; US, ultrasound.

\(^b\)Strengths are rated on a scale of – to ++++, with a minus sign indicating the lowest rating and four plus signs indicating the highest rating.

The first studies of CT in the mouse, which date from 1983, addressed lung density changes following radiation (20) and cysts in the brain (21). Presently, there is significant use of microCT (\(\mu\)CT) for imaging bone in the mouse. A group at Zurich has used \(\mu\)CT to image bone structure; from these images, the group has derived models that have been used to predict bone strength (22). As illustrated in Figure 3, we have used \(\mu\)CT to evaluate bone structures in a mouse mutant that shows cranial-facial abnormalities (23). Another common use of \(\mu\)CT is to evaluate vascular arborization structures and their aberrations with genetic mutations (24, 25). Figure 4 shows vascular patterning abnormalities in a mouse lung with a mutation in the Notch signaling pathway that is essential in early development. \(\mu\)CT can generate three-dimensional images of complex vascular patterning that are easier to interpret than histological sections. Recently, there has been growing interest in using \(\mu\)CT for evaluating mouse embryo development using contrast agents such as osmium tetroxide or iodine to achieve sufficient soft-tissue contrast or using the intrinsic contrast of bony structures (26, 27). The combination of \(\mu\)CT’s high spatial resolution on fixed samples and the potential of high throughput makes this method attractive.

**Ultrasound Biomicroscopy for Mouse Phenotyping**

Ultrasound scales readily from the clinic to the mouse. Increasing the spatial resolution is achieved when the frequency of the ultrasound is increased to the range of 30–50 MHz. This, in turn, limits the penetration of the ultrasound, but because the dimensions of the mouse are also smaller, the scaling is approximately proportional.

A long history of ultrasound measurements in the mouse dates back to the early 1950s. However, for the first 20 years, the research primarily addressed bioeffects and safety of ultrasonic radiation (28, 29). Serious mouse imaging began in the mid-1990s with the emergence of high-frequency instrumentation and its application to embryonic development (30–32). With the advent of commercially available equipment, ultrasound became the method of choice for cardiac imaging in the mouse (33) and is routinely used for cardiac physiological phenotyping of mutants (34–36). Because of its real-time capability, ultrasound is also the preferred method for studies of embryonic development (37) and functional embryonic assessment (38–40).
Figure 3
MicroCT images of mouse skull. An average of (a) five wildtype mice and (b) five mutant mice where the mutation is GJA1. (c) Vector deformations between the average wildtype and the mutant. (d) The magnitude of these deformations; the colored regions correspond to areas that demonstrate statistically significant differences. (e) Measures of the Jacobian or volume changes associated with these deformations. The color bar is a student’s t-test, and the highlighted regions on the image correspond to a false discovery rate of less than 5%. Images provided courtesy of Brian Nieman of the Mouse Imaging Centre in Toronto.

Ultrasound is also used for assessment of tumors in mice and their responses to treatment, particularly in the case of antiangiogenic treatments (41, 42). These studies are particularly facilitated by the availability of high-frequency bubble contrast agents (43).

The use of ultrasound for mouse phenotyping is likely to grow with the advent of array-based, high-frequency transducers that allow for color Doppler imaging (44) (see Figure 5). However, ultrasound will remain a labor-intensive modality with little opportunity to become a high-throughput modality.
Figure 4
MicroCT images of the vascular anatomy of the lung in an adult mouse. (a) A normal wildtype and (b) the vascular pattern of a mouse that has a mutation in the Notch signaling pathway. Images provided courtesy of Lisa Yu at the Mouse Imaging Centre in Toronto.

Magnetic Resonance Imaging for Mouse Phenotyping

Since the clinical introduction of magnetic resonance imaging (MRI) in the early 1980s, the magnetic resonance (MR) field has shown consistent expansion with the introduction of contrast agents, magnetic resonance angiography (MRA), spectroscopy, fMRI, cardiac imaging, rapid and parallel imaging, interventional MR, and molecular imaging. Thus clinical MR has remained an active research area. Most of this spectrum of clinical developments can be applied to MRI of the mouse. Although the MR signal from much smaller voxels is limited, the translation to the mouse is enabled by closer fitting coils and significantly higher magnetic field strengths (45). Nonetheless, the adaptation poses specific challenges—such as higher heart rates and the need

Figure 5
A Doppler ultrasound image of flow in an embryonic mouse heart at day 15.5. The image is courtesy of Yu-Qing Zhou at the Mouse Imaging Centre in Toronto. To view the corresponding video, follow the Supplemental Materials link from the Annual Reviews home page at http://www.annualreviews.org.

MRI: magnetic resonance imaging
MR: magnetic resonance
MRA: magnetic resonance angiography

Study name: Pregnant mice E15.5
Series name: Series 1
Frequency: 40 MHz
Wildtype Mutant

Figure 6

This figure illustrates the problem of finding phenotypic differences. These are high-resolution MR images of a wildtype (purple) mouse and a mutant (yellow) mouse. Computer analysis of significant anatomical differences has to cope with, among other things, postural differences between the mice being compared. The images are courtesy of Brian Nieman at the Mouse Imaging Centre in Toronto.

for anesthesia—and unique opportunities. Thus mouse MRI remains an actively evolving and incomplete field of research. As in the human, the high spatial resolution and soft-tissue contrast make MR particularly valuable for mouse phenotyping (see Figure 6).

The earliest examples of mouse MRI (46, 47) actually predate human imaging because it was easier to produce small-bore magnets that could accommodate only a mouse. Early clinical prototype imagers were also used for mouse imaging (48), with poor success by current standards. One of the earliest advocates and steadfast researchers of MR imaging in the mouse is G. Allan Johnson of Duke University (49), who showed how increasing fields, better coils, and novel applications to embryogenesis and phenotyping stimulated the growth of mouse MRI and magnetic resonance spectroscopy (MRS) (50).

Currently, the major research applications of mouse MRI are in neuroscience (51). Much of this work is oriented toward genetic models of neurodegenerative diseases such as Alzheimer’s disease (52–54) and Huntington’s disease (55) (see Figure 7) and mental health diseases such as schizophrenia (56). Other direct knockouts have been studied to identify the role of specific genes in neurodevelopment (57–59) and aging (60). It has been somewhat surprising to learn that 90% (29/32) of gene mutations in individuals that have learning or motor/neurological deficits also show abnormal anatomical phenotypes (61).

This work on MR evaluation of neuroanatomical phenotypes has been enabled by the development of average brain atlases (62–64) and quantitative computer analysis methods (65, 66). The anatomical reproducibility (a root mean square displacement error of only 100 µm) of brains from genetically identical mice makes the identification of phenotypic abnormalities much easier than it is in heterogeneous human populations. It also opens up the possibility of quantitative trait
loci (QTL) experiments through the use of neuroimaging in outbred lines such as those being generated by the Collaborative Cross (http://mouse.ornl.gov/projects/collabcross.html).

Beyond simple anatomical MR neuroimaging, there are a variety of functional measures such as blood oxygen level dependent (BOLD) MRI, fMRI (67), and active neuronal fiber tracking using manganese (68–70). Whereas fMRI is more easily related to clinical studies, it is more difficult in the mouse because of the limitations for stimulus presentations in the anesthetized animal. Manganese, which is too toxic to be used in humans, is easy to inject and track in the mouse. To date, none of these functional measurers has been used in primary phenotyping in mice. However, with the large and growing collection of mutant mice with neurofunctional deficits, there will be much more use of these methods for secondary phenotyping.

MR imaging of the mouse heart is also gaining momentum as a way to understand the role of genes in heart development and function. MR can provide an excellent characterization of cardiac function, flow dynamics, and valve function (Figure 8). The images have appropriate contrast for automated segmentation and computer analysis. Investigators are just beginning to look at knockouts to determine their roles in cardiac development and adult function (71, 72).

Mouse-embryo imaging benefits from the soft-tissue contrast of MRI. Mouse-embryo MR images from the mid-1990s are still regarded as a representative set showing embryo development over time (73). A more detailed set of data was published in 2008 (74). Figure 9 shows the kind of anatomical information that can be observed. The use of embryo MRI for heart development has been demonstrated through the use of a novel configuration of multiple embryos suspended in gel and imaged in a single coil (75). Over the past several years, this laboratory has imaged more than 5000 embryos.

One of the drawbacks of using MRI for phenotyping is throughput. High-quality MR images of mice usually take several hours to acquire the data for live mice and longer for fixed mice. This makes throughput a limitation. In addition to the multiple-embryo imaging mentioned above, we have developed fully parallel imaging for 16 live mice as shown in Figure 10 (76). In this system, the static magnet and the gradient are shared, but each mouse has its own shielded transmit-and-receive coil and independent acquisition electronics. At the end of an imaging session, 16 images are acquired with the same image quality that would be achieved for a single image (77). A similar 16-element coil array has been developed for fixed brain and fixed embryos. Other improvements that will continue to improve MRI for mouse phenotyping are significantly higher magnetic fields (78) and superconducting or supercooled receiver coils (79). All of these improvements will give MRI an enhanced role in mouse phenotyping.
Figure 8

MR images of cardiac function in the mouse. (a) A normal mouse in which the heart-wall displacements have been measured using Displacement Encoding with Stimulated Echoes (DENSE) as shown in panel b. (c) The degree of circumference shortening, which is uniform around the heart. (d) This mouse has a surgically induced coronary artery occlusion that shows up as (e) high asymmetric displacement imaging and (f) poor and nonuniform contraction. The image is provided courtesy of Fred Epstein and Moriel Vandsburger from the University of Virginia.

Positron Emission Tomography and Single Photon Emission Computed Tomography for Mouse Phenotyping

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are the two mouse-imaging technologies with the greatest molecular specificity; they have sensitivities down to the nanomole quantities. Both require the use of exogenous radioactive tracers, which can in turn be linked to any probe molecule, nanoparticle, vesicle, or cell that one wishes to use. The spatial resolution of PET is limited to 1 mm (80) by the recoil range of the positron. SPECT can improve on this resolution by using a pinhole camera with image magnification. A pinhole uses only an extremely small fraction of the emitted photons, seriously degrading the sensitivity of the method. To ameliorate this sensitivity loss, researchers use multiple pinholes (81) and more sophisticated image reconstruction. To maintain the sensitivity of PET and SPECT and to compensate for their limited resolution in mice, researchers combine nuclear imaging instruments with other high-resolution techniques such as CT and even MR (82).
Figure 9
MR microscopy images of a mouse embryo at day 18.5. These provide sufficient detail for anatomical phenotyping. Image courtesy of Michael Wong from the Mouse Imaging Centre in Toronto.

Figure 10
For parallel mouse MRI, an array of 16 receiver coils are assembled together in the upper left in a hexagonal array. In the center of the image, a mouse holder with 16 mice contained in centrifuge tubes is designed to be inserted into the multiple-receiver array. Individual mice are anesthetized and individually monitored during the MR imaging. The array was constructed by Jun Dazai from the Mouse Imaging Centre in Toronto.
Research using PET tracers for tumors (83) and neuroscience (84) in the mouse began in the early 1980s but was limited to ex vivo binding and biodistribution studies. Realistic instruments for PET imaging of live mice first appeared in 1991 (85, 86). Now PET and SPECT have a major role to play in mouse imaging with a focus on drug discovery (87), biodistributions, toxicology, and detailed mechanisms of diseases. More than 20% of all studies published about mouse imaging involved nuclear techniques. However, when it comes to phenotyping, particularly in the sense of phenotypic changes as a result of gene changes, there are few publications. The broad range of molecular markers available to nuclear imaging breeds uncertainty about how to use nuclear imaging for primary phenotyping as part of the systems-biology agenda. Over the next decade, there will be remarkable growth in the use of PET and SPECT for secondary phenotyping of specific molecular pathways and of disease models, but that will need to be the subject of a much larger review in the future.

Optical Imaging for Mouse Phenotyping

Of all the methods of imaging the mouse, the optical imaging methods are demonstrating the most rapid growth. Optical imaging of live mice includes bioluminescence (BLI) (88)—usually the luciferase gene from the firefly, but other BLI markers further toward the red end of the spectrum are anticipated. Although BLI had been used as a molecular marker in fixed tissues throughout the 1990s, its first use in live mice was in 1999 (89). Another form of in vivo optical imaging in mice is fluorescence imaging (also designated fluorescence molecular tomography, FMT), which may use autofluorescence of mouse tissues (90), in vivo knocked-in fluorophores such as green fluorescent proteins (91), or endogenous fluorophores including quantum dots that are selectively targeted with antibodies or other receptors. A third type of in vivo optical imaging in both the mouse and human is near infrared spectroscopy (NIRS), which is used primarily to detect blood and the oxygenation state of hemoglobin but also can be used for exogenous probes (92). Because optical scattering dominates the light transmitted, localization of the measurement is estimated from diffuse optical tomography (DOT) (93).

There is now a wide diversity of commercial and home-built devices for in vivo mouse optical imaging. Because photon scattering hinders spatial localization and thus results in poor resolution, there is an increasing interest in combining optical imaging devices with other imaging methods that provide good spatial resolution such as CT and MRI (94).

The major application of mouse optical imaging is in oncology: Tumor cells are labeled and then injected into the mouse (95, 96), and optical imaging is used to track these cells or to monitor growth in induced tumors. There are also applications of optical imaging for diffuse disease such as inflammation. However, when it comes to primary phenotyping in living mice, there are only a few general references (97). Optical imaging is similar to nuclear imaging in that it involves a wide spectrum of molecularly specific probes and only moderate spatial resolution. Thus the incredible variety of molecular imaging possibilities makes it difficult to decide what to pursue for phenotypic screening. Despite the similarities to nuclear imaging, optical imaging has one unique advantage over nuclear imaging—the capability of inserting the DNA for optical probes directly into the genome, allowing the optical probes to be expressed in specific tissues or to be used as reporters for any gene of interest. This capability will make optical imaging methods increasingly important for secondary phenotyping of molecular pathways and mechanisms in the mouse. However, the genetic manipulation of optical imaging reporters prevents the direct application of these methods in clinical studies.

Before I leave the subject of optical imaging in the mouse, I must point out the opportunities for fixed-sample optical imaging that will be used for phenotyping in studies of both normal and
Figure 11

An optical projection tomography (OPT) image of a fixed and cleared mouse embryo at day 12.5. (a) The image is volumetrically rendered in Amira with surface transparency. Because this is a 3D volumetric data set, sections can be viewed through it by computer, as in panels b and c. Images provided by Johnathon Walls from the Mouse Imaging Centre in Toronto.

mutant development (98). One such method is optical projection tomography (OPT) (99), which is a fluorescence analog of CT—or more accurately, SPECT (100)—that gives 3D, mesoscale microscopic (101) images of samples of up to 1–2 cm in size. Figure 11 shows an autofluorescent 3D isotropic OPT image of a mouse embryo from which sections can be viewed at arbitrary orientations. Because the method uses fluorescence, specific gene products can be specifically tagged to give patterns of gene expression, as shown in Figure 12 (102–104). Other emerging forms of 3D microscopy will also contribute to phenotyping (105).

NEW DIRECTIONS IN IMAGING FOR MOUSE PHENOTYPING

Mouse imaging is still in its early growth phase. This growth is driven by a mixture of demands and opportunities. These, in turn, will generate significant research opportunities for engineers, imaging scientists, and physicists.

Increasing Demand for Mouse Phenotyping

Thousands of current mouse models are of interest because they shed light on human diseases. Many of these models have been generated in academic environments, and that will continue to happen. However, there are also concerted efforts under way to generate large numbers of interesting mutant mice. The Jackson Laboratory in Bar Harbor, Maine, is the major supplier of research mice. It is also the repository of much of the data on genomes and phenotypes. It currently holds 6.7 million mouse sequence transcripts and has an index of 40,000 genes (including mutant genes). It has 145,000 gene ontologies, and it also has 500,000 mutant cell lines. All these numbers are growing daily.
Figure 12
An optical projection tomography (OPT) image of the embryonic vasculature at day 9, in which the vascular endothelium has been stained with platelet-endothelial cell adhesion molecule (PECAM). Image provided by Johnathon Walls from the Mouse Imaging Centre in Toronto. Click on figure to view a rotating movie of the data set.

The private sector is also involved. Lexicon Pharmaceuticals has almost completed determining the function of 5000 mammalian genes, and Regeneron is in the process of fulfilling an NIH contract to make 3500 different knockout mice involving some of the most difficult gene knockouts. In turn, the International Knockout Mouse Consortium (IKMC) (http://www.knockoutmouse.org) has generated stem cell KOs for 13,000 genes to date, which represents 56% of the total task.

All of these efforts are making important mutant mice that will eventually need to be phenotyped. Furthermore, imaging can have a big role to play in this phenotyping.

New Imaging Technologies
There is increasing demand for and interest in developing hybrid imaging systems, which would bridge the gap between molecular imaging and anatomical imaging. Even when development of the individual modality is mature, constructing hybrid systems still presents engineering challenges. Examples are PET-MRI (106), SPECT-CT, and optical systems, which can be combined with almost every other modality. Optical techniques themselves can still be improved in terms of resolution if the photon transport is handled in more detail, taking into account the optical properties and actual geometries of tissues in the mouse.

In MRI, the small size of the mouse means that we have not reached—and will never reach—the magnetic field limits imposed by radiofrequency penetration and B1 inhomogeneity. Thus there is plenty of opportunity to explore alternative and very high field systems with supercooled receiver coils for mouse phenotyping. Alternative parallel methods for high throughput can also easily be imagined, with decreased costs.

There are new and emerging imaging technologies that may become major contributors to mouse phenotyping, such as photoacoustic imaging (107) and flow-sensitive, optical coherence...
tomography (108). Furthermore, there are undoubtedly unknown techniques that have yet to be imagined or that have been bypassed because they are unlikely to be usable for clinical imaging.

Mouse imaging still needs to become an integrated part of phenotyping pipelines. Most of the mouse-imaging advances to date, including those summarized in this review, have comprised anecdotal evidence and proof-of-principle experiments. In the future, high-throughput pipelines will require more automation, including robot mouse handling and 24/7 imager operation without attending personnel.

Behavioral phenotyping is an increasingly important part of mammalian neuroscience (109). Much of this testing is manual with visual observation. There is an opportunity for more free-range behavioral testing in playgrounds with groups of animals, monitoring with computer vision tracking, and performing analyses in terms of comprehensive histories. This is not what is conventionally meant by mouse imaging, but it is imaging nonetheless.

Need for Better Molecular Contrast Agents

Because toxicity issues can be relaxed for in vivo mouse imaging and ignored when imaging fixed samples, there is much more scope for novel contrast-agent design than has been evidenced to date. With the added opportunity of incorporating the agents into the genetics of the animal, the possibilities become endless. To realize these possibilities, we need the following:

- Better contrast agents for vascular X-ray CT: a higher atomic number, increased attenuation, and more uniform delivery.
- Tagged ultrasound bubble contrast agents that can be selectively delivered anywhere in the animal, particularly beyond the vasculature.
- A “green fluorescent protein (GFP)” for MRI that can be built into the genome and read out via proton spins.
- Contrast agents (110) to use with multimodal imaging systems, in which one mode reports on agent concentration and the other reports on some interaction with the environment.
- Multiparameter Boolean contrast agents that we can use to request maps of A AND B but NOT C, to achieve better imaging discrimination and more sophisticated signatures of biomarkers.

Increased Challenges for Statistical Analysis of Image Data

Image registration has been well worked out in the mouse brain, where deformations are small and the tissue can be modeled as elastic. However, we also need registration methods that handle articulating joints and free-slip planes among organs in the abdomen. We also need 4D images to cope with changes in 3D anatomy over time for both embryonic development and disease progression.

Completed anatomical image-based mouse phenotyping will require automated segmentation of all organs to provide automatic, text-rich phenotypic reporting of individuals and their differences.

Rather than limit the analysis to only the points in a 3D image, we will need to develop methods to track connection—as in the proposed brain Connectome Project (111)—and more simply, methods for vascular tracking, arborization, and function (112).

As we move toward storing volumetric 3D image data as part of the mouse standard phenotype, we will need 3D data-mining tools that can respond to the query, “Show me other mice examples with a similar-looking structure.” As a general image-mining problem, this kind of similarity mining is probably intractable, but within the domain of mouse anatomy, it should be achievable.
Greater Need for Systems-Biology Modeling to Sort Out the Genome-to-Phenotype Relationship

With ever-growing databases such as the Mouse Genome Informatics, the Mouse Phenome Database, and the Gene Expression Atlas, there will be a high demand for engineers, mathematicians, physicists, and computer scientists to work with geneticists, developmental biologists, physiologists, and clinicians to understand the connection between genomes and phenotypes for the mouse and consequently to learn more about human development and the genetic basis of disease. There will be plenty of challenges for all of us!

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