The Edinburgh Mouse Atlas: Using the CD

Richard Baldock, Jonathan Bard, Renske Brune, Bill Hill, Matt Kaufman, Kirstie Opstad, David Smith, Margaret Stark, Andrew Waterhouse, Yiya Yang and Duncan Davidson

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Abstract

This paper provides a simple introduction to the reconstructions and data-handling tools stored on the Edinburgh Mouse Atlas CD, together with some of the ways in which the viewers and software can be used to understand mouse development and analyse data. The key aspect of the Mouse Atlas is that the underlying models are a complete representation of the histology, which has not been constrained to a particular interpretation. This means, for example, that the current anatomy domains can be further subdivided as required to any resolution up to the resolution of the models (2–7 μm). In the CD of the early embryos described here, virtually all tissues that can be usefully distinguished either by the histology or morphologically have been delineated.

INTRODUCTION

Bioinformatics is a rapidly expanding field that has traditionally centred on sequences, structures and gene mapping. As the current challenge to genomics is to understand gene function in all its complexity, bioinformatics is having to take on a new role in facilitating query, comparison and analysis of the vast volume of information now being generated by biologists and being stored in databases. At the level of the genome, the underlying sequence provides a common framework onto which information can be mapped. For understanding gene function at the level of the organism, however, a new framework is required that can support the wide range of data now being produced that links gene-expression patterns, lineage information, cellular and tissue activity, mutations and morphology for a wide range of organisms. The Mouse Atlas, being produced at the MRC Human Genetics Unit and the Department of Biomedical Sciences, University of Edinburgh, can provide such a framework for mouse development. It also acts as a model for other organisms and is currently being used to provide a similar framework for human development. The key components of the Atlas are digital models for each stage of development. These are formed from 3D reconstructions generated from digital images of serial histological sections. The models are not just outlined organs or tissues, but 3D, full grey-level images of the embryo, which can be digitally resectioned at any orientation or visualised directly in 3D using rendering techniques to give whole-mount or surface style views. Most importantly, the models can be used to map any spatially organised data into a common spatial framework and thereby enable search comparison and analysis.

At present, the Mouse Atlas is composed of three main parts:

- One representative 3D grey-level model for each stage.
- A standardised nomenclature for anatomical components for each development stage; this includes a formalisation of the staging systems used in describing mouse development and some lineage links between the components.
- A set of 3D regions or domains that provide a mapping between the textual...
Each 3D image represents a substantial amount of data and, for efficient use of the Atlas, the whole image needs to be available at the user’s machine. For this reason the Atlas is provided as a series of CDs of which the first volume holds embryos from implantation (E5.5, Theiler stage 7) to E9 (Theiler stage 14). How this CD can be used to understand the underlying anatomy of the embryos and to analyse new data is described here. Because of the complexity of the data some techniques require the use of Unix machines for which data viewers and mappers have been developed. Most of the CD, however, is accessible with any computer that can run a Java-enabled browser. Displaying the anatomical nomenclature requires Internet access to the full anatomy database at the MRC. The CD can be obtained from the Mouse Atlas web site. An on-line version is also available from this site.

A spatial gene-expression database is also under development at the MRC. This uses this framework and is closely linked with the GXD mouse gene-expression database, which is part of MGI maintained at the Jackson laboratory. This database has been described elsewhere.\footnote{\textit{BRIEFINGS IN BIOINFORMATICS}, VOL. 2, NO. 2, 159–169, MAY 2001}

\textbf{BASIC FRAMEWORK}

The key requirement of a framework at the level of the whole organism is to represent space. Gene expression in the embryo represents a spatial organisation that, in many instances, does not follow the boundaries of other features in the embryo (eg anatomy). Furthermore if we want to represent more detailed structure such as gradients and complex patterning, only a framework that can capture the spatial features of the raw data will do. The simplest such representation is a digital reconstruction of the embryo itself, which is uninterpreted and therefore does not impose any constraints on the mapping process except for resolution. In order to ensure that specimens are representative of the developmental stage and to maintain consistency with existing reference material, where possible, we have reconstructed the embryos illustrated in the book ‘The Atlas of Mouse Development’\footnote{\textit{BRIEFINGS IN BIOINFORMATICS}, VOL. 2, NO. 2, 159–169, MAY 2001} for 8.5 dpc (Theiler stage 13) and older. These F2 CBA/C57Bl embryos had been embedded in wax and cut into 7 \(\mu\text{m}\)-thick sections. For younger stages, where higher resolution is essential, we have used new specimens staged and selected by Dr K. Lawson and MHK, embedded in plastic and sectioned at 2 \(\mu\text{m}\) thickness. The resolution (voxel size) of the older embryos is approximately 7 \(\times\) 4 \(\times\) 4 \(\mu\text{m}^3\) and for the younger 2 \(\times\) 2 \(\times\) 2 \(\mu\text{m}^3\). Tools for re-sectioning and viewing these models are described below.

The anatomy consists of two parts both of which are contained in an object-oriented database. The first is a component hierarchy of anatomical terms, which provides a controlled vocabulary as well as a natural description of scale. Each major anatomical component is subdivided into non-overlapping parts to form a ‘part-of’ tree. A separate tree is defined for each stage. There is provision for arbitrary re-grouping of these components; this is a necessary requirement for expressing the results of a gene-expression experiments and means the anatomy becomes a ‘directed-acyclic graph’ (DAG).

Additionally, the database allows lineage links between components so that progenitor and derivative tissues can be traced.

The second part of the anatomy is a set of 3D regions or \textit{domains} that correspond to selected anatomical components that can be identified in the grey-level image. These provide the mapping or link between the text and coordinate descriptions of embryo space. These three parts of the Mouse Atlas are illustrated in Figure 1.
ANALYSIS AND VISUALISATION TOOLS

In this section we describe what can be found on the CD and provide instructions for accessing and using the viewing software. Specific instructions that will make sense only if you are using the CD are in ‘how-to’ boxes. The CD has a very simple top-level page with thumbnail images providing links to the viewers for all architectures – PC/Windows, Macintosh and Unix workstations. This page has very little text and just enough instruction for producing views of the data on the screen. For more advanced use, access to the Unix-based viewers, detail on the data, user manuals, installation instructions and so on, follow the link at the bottom of the ‘picture index’ home page.

Setting up your computer for the CD

The CD is intended to be viewed using a Java-enabled web browser. This gives access to all of the viewers except SectionView and MAPaint which are Unix applications. Any machine that runs Internet Explorer 4.5 or later or Netscape Navigator/Communicator 4.5 or later can use this CD. This includes all Microsoft Windows 95, 98, 2000, NT, Macintosh

How to view the Stage criteria:

From the ‘Stage Definition’ column of the table on the CD home page click on the link for the embryonic stage of interest. This will bring up a page from the Genex web site giving the definition of that Theiler stage and the anatomical criteria. On this page click on ‘Stage Criteria’ to get the table of all stages.

How to view the anatomy hierarchy:

From the ‘Nomenclature’ column on the CD home page select the required stage either in a simple indented text form or with a Java browser. The Java viewer may request that you install a plug-in for your web browser. In the Java version the tree has ‘toggles’ to allow the tree to be expanded or collapsed as required. It is also possible to search, and by selecting a component with the right-hand button a new window with detail of the components is provided. This can be used to establish the unique ID of a term as well as lineage. Note this browser is from the Mouse Atlas web site and requires the Java 1.3 plugin available only for Windows, Solaris and Linux. For other machines use the text option.
and Unix machines (Sun, SGI and Linux). Sun workstations running Solaris, Silicon Graphics workstations running IRIX and PC compatibles running either Solaris or Linux can all use SectionBrowser and MAPaint. The recommended platform is a Sun microsystems Ultra 5/10 or a PC running Solaris 2.8 (free for academic use). The recommended browser is Netscape Navigator version 4.7 (Internet Explorer on Macintosh).

Once an appropriate browser has been installed, insert the CD, locate the start.html file and follow instructions from there. If you do not have a copy of the CD then you can use the on-line version, but you will not be able to access the full reconstructions for use within the SectionView and MAPaint applications. All other viewers are available, though by comparison with the CD, the download time will, of course, be longer for image data. Access to the full anatomy nomenclature trees and staging criteria requires connection to the Internet.

**Anatomy nomenclature and staging**

The traditional way to describe space in the embryo is to use anatomical terms. In this Atlas we have generated a formal anatomical hierarchy, or ontology, for each stage. Each hierarchy is defined for a particular developmental interval for which we have adopted the staging system defined by Theiler. In order that we can encode all of the types of data being generated, the anatomy ontology needs to be extended to include alternate groupings of components and more detailed lineage links. The anatomy ‘trees’ and the detailed mapping of the staging systems that have been adopted are available from the HGU Genex web site.

**Interactive 3D views**

For each stage there is a 3D grey-level image plus a set of anatomical domains. These can be displayed in 3D using rendering software. We have used the commercial package AVS/Express (Advanced Visual Systems) and the public domain Visualisation ToolKit (VTK). For most of these reconstructions, rendering the models takes some time and is not interactive. In order that the user can achieve a reasonably interactive response we have written a Java applet to allow the user to run through a preset sequence of images. This gives an interactive response on any machine, but cannot provide arbitrary views. Sets of interactive moves of this type are possible using the 3D visualization tools. Figure 2 shows the Java interface that enables browsing of the anatomy nomenclature database.

**Figure 2:** Java applet browser for viewing the anatomical nomenclature hierarchies. The tree is expanded and collapsed by clicking the ‘turn-buckles’ to the left of the component name. Details of any component can be displayed by clicking on the component with the right button.
How to rotate the embryo in an interactive 3D view:
From the EmbryoView columns in the table on the home page select a view by clicking on the image. This will take you to a page with the same image (a bit larger) with a caption giving the colours of the displayed anatomy. Click on the image to pop-up a new window with the view shown. It may take a little time to load to EmbryoView. Click and drag left–right to rotate the image. More than one window can be shown at the same time; simply select the required views. Use the close button on the top bar of the window to get rid of unwanted displays. It is useful to have an EmbryoView visible while using SectionBrowse. Bring up the EmbryoView first.

For example, this mapping is useful for analysing new gene expression patterns in terms of the known anatomy and is invaluable for navigation, indexing and teaching. On the CD we provide two whole-mount view

How to select a sagittal view of E9.0 (Theiler 14):
Find the ‘Section Browser’ column on the CD home page and click on the image in the Theiler 14 row (bottom); this will bring up a page from which the SectionBrowse applet can be selected. By default this applet displays the transverse view. In the navigator window select ‘sagittal’. In the section window pass the cursor over the image; the anatomical domain under the cursor will turn blue interactively. Click on the red line in the navigator window and drag left or right to change the displayed section. Click on the section image to select a component; this will remain filled-in and the anatomy hierarchy will be highlighted. In the tree any component with an asterisk after the name can be selected and the corresponding region in the section image will be filled in blue.

Figure 3: Interactive view of the Stage 12 embryo showing the neural tissue, gut, notochord and somites.

Section Browse
A key property of the Mouse Atlas is the link between the spatial description based on the coordinate frame and the corresponding anatomical name. This allows textual and spatial descriptions of patterns to be related in a consistent way.
3D navigation

Anatomy feedback

- The **navigator** window provides a cartoon of the embryo with a red line that can be adjusted interactively to reset the current displayed section. This window also allows the user to select transverse, frontal or sagittal sections.

- The **section** window displays the digital histology section through the embryo with an active display of the anatomy which can be displayed interactively by moving the cursor over the image. The spatial extent of the anatomical component under the cursor will be displayed in blue and the anatomical name will be shown at the top of the window. Clicking on the image will ‘fix’ the blue overlay and highlight the component in the anatomy window.

- The third window shows the full anatomy tree for the stage and is also active. The tree can be scrolled as required and all components that are visible in the current section can be selected with the same effect as selecting the anatomy from the choice menu.

Original digitised sections

The embryo models can be re-sectioned digitally at any orientation. This is important for data mapping and for analysing patterns from arbitrary sections and embryos. So that the new digital sections have roughly the same resolution in all directions we chose voxel sizes that are nearly isotropic. In practice this means that the resolution of the model is determined by the section thickness which is 7 μm for E8.5 and older and 2 μm for up to E8.0. The original sections are digitised at a much higher resolution and subsampled as required. This
reduction of resolution in the original section planes implies a loss of information. For this reason we provide software to view the original and unprocessed digital images of each histological section. This provides a much more detailed view of the tissues at the cellular level.

The viewer for the unprocessed 2D images is a Java applet which provides two windows (Figure 5). On the left is a navigator window, which displays a line, showing the approximate position of the displayed section and number of the plane in the 3D model. The right-hand image window displays the digital image of the histological section exactly as captured from the microscope. There has been no processing to remove background, debris and artefacts or to correct damaged areas of the sections. Because these are the original images, the \( x \pm y \) coordinates are not simply related to the \( x \pm y \) coordinates of the matching plane in the model. However, the plane numbers do correspond exactly so that it is possible, at any position in the 3D model, to view the histology at high resolution in the transverse plane. For certain research projects, it may be necessary to examine particular regions of the model at even higher magnification and, where time permits, the Mouse Atlas team will provide new images of the original sections upon request.

**Viewing at arbitrary orientations**

The primary purpose of the digital model embryos is to provide a context-free framework for spatial data in the embryo. The most detailed information is acquired by sectioning the embryo, which may be the only way to get the required resolution. The process of cutting sections will always yield variation in orientation and position particularly in the early embryos. Furthermore different studies may demand sectioning at non-standard orientations. Therefore, for more sophisticated access to the data we have implemented a user interface that allows arbitrary re-sectioning of the embryo. This interface works under Unix including Linux and Intel Solaris and is thus available for most PCs.

The program is called MAPaint and, when used for browsing only, it is referred to as SectionView. A screen-shot of MAPaint is shown in Figure 6. MAPaint reads in the grey-level voxel model, the anatomical nomenclature and the mapped anatomical domains and allows the user interactively to select arbitrary sections through the model. The user can control how the section is presented, review its location in 3D relative to named structures and show anatomy highlighted in colour. Each section view will also provide feedback of the 3D coordinates as well as the

**Figure 5:** Java browser for viewing the original digitised images. The left-hand window provides a navigator to select the required section to be displayed in the right-hand window.
3D navigator view

derive anatomy. Any number of views can be displayed and independently oriented and positioned in different windows.

The program is launched by selecting the requested stage on the Advanced Access page. Once SectionView is active, other Theiler stages can be selected as required using the Theiler stage menu. The first window to appear provides a simple 3D view of the reconstruction to give feedback for navigation through the volume. The coordinate bounds are displayed together with an outline of the embryo. The View menu provides a number of preset sections through the embryo. Selecting one such view will make a dialogue window pop up, showing the section and a number of controls. The controls allow the section to be moved interactively to any orientation and position, and, as the view moves, an outline of the view in the navigator window will move so the user gets feedback of the section position with respect to the overall volume.

As the cursor is moved over the image in the section window, the display will respond with the 3D coordinates, grey-value and the name of the anatomy component under the cursor. To highlight a given anatomical component in colour, use the domain menu to select a colour (see the ‘How-to’ box below) then use the anatomy menu to select the component. If the component is visible in any of the current views, then those pixels will acquire a see-through colour and the outline of the component will appear in the 3D navigator window. The 3D view can be adjusted by clicking and dragging the cursor within the navigator window; the embryo outline will rotate and can be shifted and expanded.

The basic controls for section view are two angles to define the orientation, and a distance and fixed point to determine the position of the section. Any position and orientation for the section is possible but the viewing model assumes that the user will be most interested in a series of parallel sections having determined the orientation, ie a digital microtome. For this reason the distance ‘slider’ is always visible and the orientation controls hidden until requested. Within the navigator window the intersecting lines of the section and the bounding box of the reconstruction indicate the plane of the section. These lines move interactively as...
the section is adjusted in angle and position. Within each section view a green line(s) shows the intersection of that view with other selected views.

The anatomy menu can be used to read in the domain of an anatomical component and the 3D extent of the domain will be visible in the navigator. This allows the user to manipulate the section in order to intersect specific morphological features and provides a 3D orientation of the 2D section within the volume.

**Painting and warping data onto the Atlas**

The grey-level models of each embryonic stage provide a spatial context onto which data can be mapped. This mapping can use the predefined anatomical domains but in general the anatomical components cannot provide a sufficient description of the spatial extent and distributions of gene-expression data. **MAPaint** allows the user to spatially transform the observed distribution so that it is in the framework used by the database. The simplest mechanism is to delineate the regions of expression manually by using the **painting** tools in the program. **MAPaint** allows the user to paint onto any section through the model and the domain is automatically stored in the model coordinates. The tools are straightforward and similar to other drawing programs with the exception that the delineation is onto any 3D section through the volume and that all views map data onto the same underlying 3D coordinate system.

Manual delineation relies on the user recognising all the areas of the gene-expression pattern and faithfully redrawing the pattern on the reconstruction. An alternative is to define a warp or morph transformation that determines the spatial mapping from the source image to the models and to use this to map the signal pattern. The data may come from a separate image that is coincident with the image used for mapping, for example, a dark-field image of the autoradiographic pattern coincident with a bright-field image, or the data may be obtained by segmentation (by colour or grey-scale) from the source. This process has the merit of capturing all the aspects of the data, but may still require editing to remove signal from artefacts and debris on the section. It is planned that the present prototype methods for warping will be extended to incorporate increasing levels of automation.

**How to display a section view through optic vesicles and otic pits of E9.0 (Theiler 14):**

- From the home page of the CD select Advanced Access home page then choose E9 TS14 from the SectionView menu. The navigator window will appear and show a 3D outline view of the embryo.

- Select **Anatomy** → **embryo** → **organs** → **sensory organ** → **ear** → **inner ear** → **otic pit** → **epithelium** → **epithelium domain** which will appear in red within the navigator window.

- Select **Domain** → **Select** → **domain_2.wlz** to change the colour of the next domain read in, then **Anatomy** → **embryo** → **organs** → **sensory organ** → **optic vesicle** → **optic vesicle domain**. This will now display in blue.

- Press and drag the left mouse button in the navigator window to rotate the 3D view so that the optic vesicle and the otic pit are viewed sagittally.

- From the view menu select ‘X–Y view’, a new dialogue window will appear showing a transverse section through the middle of the embryo.

- Use the distance slider to move the view so that it intersects the optic vesicles displayed in the navigator window.
The tool in MAPaint for mapping relies on the user finding the matching plane of section. This section through the model becomes the ‘destination’ image in the warp interface. The user then reads in the source image of the experimental embryo and marks a number of ‘tie-points’ that determine the transform either using a thin-plate spline or multi-quadric. The interface provides a review window, which shows an overlay of the two images after the transformation and more points can be added or points moved until the transform is sufficiently accurate. Then the signal image is read into a ‘mapping’ window which allows the user to apply some image-processing filters and thresholds to extract the signal or expression pattern. This part of the process is most accurate if the original section data are available for review under the microscope. Once the appropriate thresholds have been set the signal is automatically mapped on to the atlas and can be edited using the standard painting tools before being saved for submission to the database.

By mapping successive sections in this way a full 3D expression pattern can be established. Because the mapped data are in the context of the atlas the pattern can be analysed in terms of the anatomy, submitted to a gene-expression database or compared with other spatially mapped data.

### SUMMARY

This paper provides a simple introduction to the reconstructions and data-handling tools stored on the Edinburgh Mouse Atlas CD, together with some of the ways in which the viewers and software can be used to understand mouse development and analyse data. The key aspect of the Mouse Atlas is that the underlying models are a complete representation of the histology which has not been constrained to a particular interpretation. This means for example that the current anatomy domains can be further subdivided as required to any resolution up to the resolution of the models (2–7 μm). In the CD of the early embryos described here, virtually all tissues that can be usefully distinguished either by the histology or morphologically have been delineated, but a lower anatomical resolution will be assumed for the first version of the later stages. We would like to encourage community involvement in improving this resolution: groups might take on the detailed delineation of systems in which they are interested.

#### Acknowledgements

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#### References

1. The CD of Thelker stages embryos 7–14 is available by accessing the Mouse Atlas web site http://genex.hgu.mrc.ac.uk/. All data except those of the actual reconstruction are available on-line. Instructions for obtaining copies of the CD are available from the same address.


9. URL: http://genex.hgu.mrc.ac.uk/CDROM_online/


11. URL: http://www.avs.com/

12. URL: http://www.kitware.com/vtk.html

13. Using MAPaint and VTK, URL: http://genex.hgu.mrc.ac.uk/Software/UsingVTK.shtml