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Digital three-dimensional models of *Drosophila* development

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Digital models of organs, cells and subcellular structures have become important tools in biological and medical research. Reaching far beyond their traditional widespread use as didactic tools, computer-generated models serve as electronic atlases to identify specific elements in complex patterns, and as analytical tools that reveal relationships between such pattern elements that would remain obscure in two-dimensional sections. Digital models also offer the unique opportunity to store and display gene-expression patterns, and pilot studies have been made in several genetic model organisms, including mouse, *Drosophila* and *Caenorhabditis elegans*, to construct digital graphic databases intended as repositories for gene-expression data.

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Abbreviations

3D	three-dimensional
DA1	dorsoanterior 1 glomerulus of antenna lobe
DAL	dorsoanterior lateral group of secondary lineages
DALv3	dorsoanterior lateral group, ventral 3 secondary lineage
GFP	green fluorescent protein
SPI	SuPerImposing

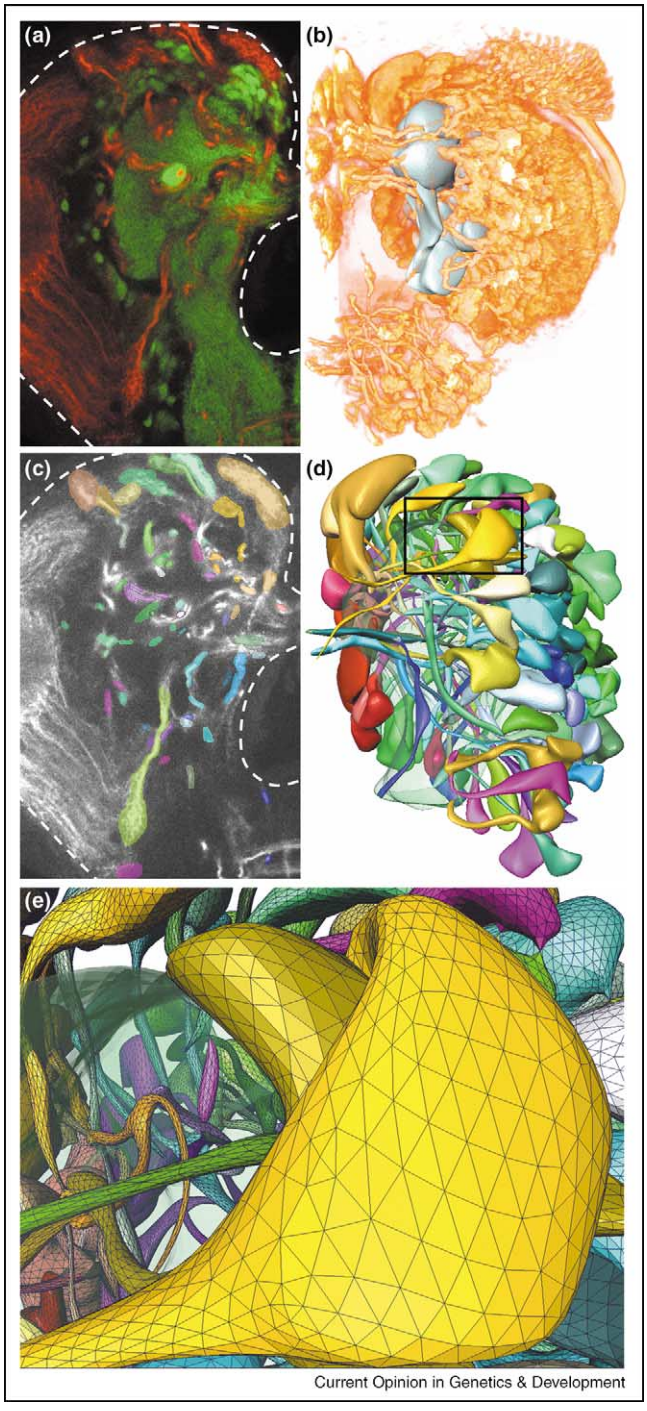
Introduction

Anatomical science gains most of its data from sectioned material, be it sections that are physically cut with a knife, or (more recently) optical sections obtained by sophisticated microscopic techniques. Since it is often difficult for the human mind to accurately grasp the spatial relationship between elements of a complex structure that appear on different sections along a z-axis, attempts have always been made to reassemble sections, in graphical or physical form, into three-dimensional models that can be viewed from different angles. Such models of a large variety of biological specimens have been used as didactic tools for many generations. Impressive examples are the models of embryos that were based on slices of wax representing sections [1]. Other materials that were used included plastic slices shaped according to the profiles of sectioned specimens, or acetate sheets on which such profiles were drawn and which were then stacked in a special holding device.

Computers entered the field of anatomical modeling in the sixties and seventies. The use of computers coincided with the introduction of new anatomical tools, such as electron microscopy or the injection of individual cells with dyes. It was predominantly in the field of neurobiology where computer-generated three-dimensional (3D) models found their first widespread application. The aim was to ''study the detailed structure of simple nerve nets so that the anatomy of individual nerve cells and their fibers can be recorded in three dimensions and the morphologically visible synapses connecting them noted'' (from [2]; see also [3,4]). The first published models represented 'wire frame models' in which profiles of a biological structure (e.g. a neuron) that appeared in serial sections were digitized and transformed into paths that could be stacked electronically on top of each other, sheared, and rotated.

During the past few decades, computer technology has come a long way, and with it has developed the applications of 3D digital modeling in anatomical work. In particular, the enormous increase in computing speed and data-storage capacity has provided the opportunity to generate virtual surfaces on digitized objects, to produce virtual sections at arbitrary angles, and to integrate multiple models of related structures into one by 'warping'. These capabilities have vastly extended the usefulness of digital anatomical models. In regard to their didactic use, the fact that virtual surfaces can be rendered in such a way as to simulate different textures, lighting and transparency makes it possible to present complex nested structures in a comprehensible way. Versatile surface-rendering capabilities, along with the ability to generate virtual sections at any desired plane, allow for the use of computer models as digital atlases. Thus, by comparing any new set of histological or optical sections of a given structure to a similarly oriented virtual section prepared from the digital model, a researcher can identify elements of that structure included and annotated in the digital model. Finally, standard models of embryos or embryo parts may serve as matrices on which the patterns of expression of particular genes or proteins can be overlaid by warping (see below). Used in this way, digital models may serve as 3D archives of expression patterns. As expression data for multiple genes can be displayed simultaneously, digital models will become analytical

Figure 1



Creation and visualization of three-dimensional models. **(a)** A sample confocal section of the *Drosophila* third-instar larval brain is shown in which the secondary lineages (red) and neuropile compartments (green) are labeled. **(b)** The volume-rendering technique is used to visualize the secondary lineages (orange) and surface reconstruction is used to visualize neuropile compartments (gray). **(c)** The stack of confocal images was manually segmented, so that each secondary lineage and each neuropile compartment is a separate structure. Using the data (c) created during segmentation of the secondary lineages, surfaces were reconstructed **(d)** by triangulation for each of the secondary lineages. Also shown **(e)** is an enlarged view corresponding to the boxed area in (d) in which the triangles that constitute the surfaces are visible.

tools that allow one to form hypotheses about gene function and gene interactions. In this review, we discuss the use of digital models in *Drosophila*, surveying the types of models that exist and the way in which they are adapted to their special purpose.

Making and using digital models

Data collection

Generating three-dimensional reconstructions of biological specimens begins with acquiring digitized images of consecutive physical or optical sections through the subject [5–8]. The digitized images are initially stored as separate files (section files; Figure 1a), which are then combined into a single file (stack file). The stack file is an ordered collection of all the sections through a specimen including the distance in between adjacent sections. The use of confocal microscopy results in automatically aligned section files as virtual sections are taken from an intact specimen so no alignment is needed. If the sample has been physically cut, section files are created for each cross-section separately which leads to misalignment relative to each other. Before a three-dimensional reconstruction can be made, sections within the stack file must be aligned using both rigid (translation and rotation) and non-rigid transformations (deformation, if the physical sections were warped during their preparation). Several computer programs have been developed to assist in the alignment process and these range from allowing manual, semi-automatic to fully automatic alignment [9–13] (see below for a list of software).

The final aligned dataset consists of an array of three-dimensional pixels, called voxels, which have an x and y component composed of the pixel from the section they originated from, as well as a z component made up of the section thickness. Several academic and commercial programs have been created especially to assist with data acquisition, section alignment, as well as creating both volume and surface-rendered models. Some of the software packages used are Amira (http://www.amiravis.com/), AVS (http://www.avs.com/), Imaris (http://www.bitplane.com/), IRIS-Explorer (http://www.nag.co.uk/Welcome_IEC.html), ParVis (http://cap.anu.edu.au/cap/projects/parvis/), SurfDriver (http://www.surfdriver.com/), VayTek Image (http://www.vaytek.com/index.html), Vitrea2 (http://www.vitalimages.com/products/vitrea2.php), VolVis (http://www.cs.sunysb.edu/~vislab/volvis_home.html), and VoxBlast (http://www.vaytek.com/VoxBlast.html) among others.

Digital modeling through volume rendering

Once aligned, the next step for the dataset within the stack file is to be visualized as a 3D physical object. The

illusion of three-dimensionality on a two-dimensional surface is created by displaying the image with several depth cues (optical information that can be used to help understand the spatial layout) such as occlusion of far structures by near structures, false shadows, size gradients (far structures are displayed smaller than near structures) as well as taking advantage of the kinetic depth effect in which a moving object displayed using depth cues appears to be more three-dimensional than a static view. There are two techniques that can be used to visualize the aligned stack file in 3D: volume rendering and surface reconstruction.

Volume rendering is an automatic computer-intensive technique that uses the mathematical process known as ray-tracing to visualize the 3D structure within the dataset. The mechanism behind ray tracing is similar to projecting hand puppets on a screen using a light source such as a projector. Ray-tracing software traces virtual rays of light that emanate from a point source (the point of view), through the volume of the dataset and onto a final screen (the display), each ray of light corresponds to one pixel in the final display. Although this process has a high computational cost, even modern low-end computers are capable of generating real-time volume renderings so that the user can freely rotate the object by changing the point of view and investigate the 3D structure in this way (Figure 1b).

Surface reconstruction

Whereas volume rendering excels in providing the investigator with an interactive image of all structures in the dataset, the technique of surface reconstruction adds new functionality to the generated models. Surface-reconstructed models can be used for quantitative analysis, virtual dissections (in which user-selected structures can be removed from view allowing examination of obscured structures), generation of atlas models (for example, for use with descriptive anatomy) as well as a framework for gene-expression data (described below). Creating surface reconstructions is a two-step process involving, first, image segmentation, in which the boundaries of all relevant structures within the dataset are demarcated, and second, surface generation, that uses the defined boundaries to create surfaces of distinct color and transparency.

Image segmentation can be performed in one of three ways: user-defined thresholding, atlas-based segmentation or manual demarcation. Thresholding, the simplest and quickest procedure, simply creates a surface around clusters of voxels that have an intensity higher than a user-defined threshold. Atlas-based segmentation is an automatic method in which *a priori* information (within the atlas image) about a sample is used by software to demarcate the boundaries on a new dataset. This technique has successfully been used in the biomedical field for automatically segmenting both MR and CT images [14–18] as well as more recently for several biological specimens, including the honey bee brain [19••]. Although the potential benefits of this technique, such as being fully automated as well as providing reproducible segmentation from one dataset to the next, are quite attractive, this technique works well only for structures that have clear boundaries between them or are well contrasted. In addition, the procedure is computationally expensive, requires the orientation of the sections in the new dataset to be nearly identical to the orientation of the atlas, and significant deviations in topology, even changes introduced by variability, are not handled consistently by current algorithms. In contrast to thresholding and atlas-based segmentation, manual segmentation requires that the investigator draw in boundaries of all structures directly into the dataset. This technique is the slowest of the three but is required in several cases, for example when segmenting a previously undescribed structure, and in cases where the boundaries between structures are not visible in every section (Figure 1c).

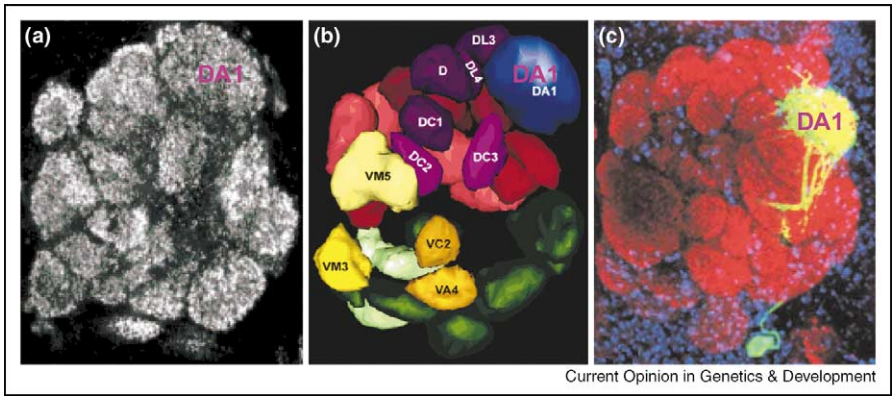
Following segmentation, the image-processing software uses the contours to create surfaces that can be displayed in different colors, textures and degrees of transparency (Figure 1d). The generation of surfaces is performed by a mathematical process known as triangulation, which involves connecting consecutive contours of a structure by a band of triangles resulting in a faceted surface representing each structure [18,19••] (Figure 1e).

Models of the *Drosophila* CNS: neurons and neuropile compartments

The nervous system of the hatching *Drosophila* larva comprises $\sim 10^4$ neurons and glial cells. This number increases by more than an order of magnitude during the larval and pupal stage when adult specific (secondary) neurons and glial cells are added to the primary cells born during embryogenesis. Axons and dendrites, accompanied by glial sheaths, assemble into a central neuropile, whereas neuronal cell bodies form an external cortex at the periphery of the brain. The neuropile is subdivided into numerous compartments, based on the structure of axons and dendrites and the expression of molecules (neurotransmitters, channels, and adhesion molecules), and surrounding glial septa. Compartments are highly conserved among different insect groups, and at least some compartments can be viewed as functional modules that deal with defined aspects of insect sensory processing and behavioral control (e.g. the antennal and optic lobe, the mushroom body and the central complex).

Digital models have been generated both for compartments and for individual neurons, or small clusters of neurons. Links to models and photographic information on the *Drosophila* brain can be found on the FlyBrain database (http://flybrain.neurobio.arizona.edu/). Compartment models illustrate the size, shape and position

Figure 2



Atlas model of the *Drosophila* adult antennal lobe. **(a)** Confocal section of antennal lobe labeled with the global neuropile marker nc82 (from [20], with permission). **(b)** Frontal view of a surface rendered model of antennal lobe; rostral layer of glomeruli removed (from [20], with permission). **(c)** Confocal section of antennal lobe (red) containing GFP-labeled interneuron (yellow; shown as Z-projection) whose dendrites arborize in the DA1 glomerulus (from [28•], with permission).

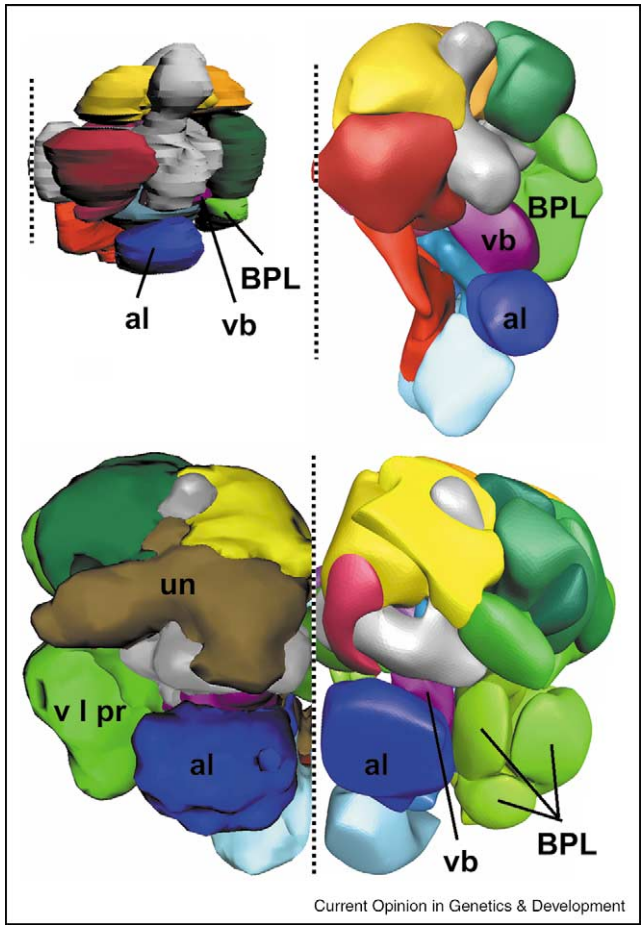
of compartments and have proven invaluable as a 'digital atlases', as well as providing tools to investigate gene function. Laissue *et al.* [20] present a digital atlas of the *Drosophila* adult antennal lobe (Figure 2a,b); similar models have been published for several other insect species [21,22•,23,24]. The antennal lobe represents the primary sensory neuropile for antennal olfactory neurons. Similar to the vertebrate olfactory bulb, the antennal lobe is subdivided into a large number (~40 in *Drosophila*) of glomeruli that are separated from each other by glial septa. Olfactory neurons sharing common receptors converge upon one glomerulus, and second-order olfactory neurons whose dendrites fill out that glomerulus project to a distinct domain in the secondary olfactory neuropile of the calyx and lateral horn. The detailed digital atlas of the antennal lobe has served as the basis to recognize and label sensory afferents and second-order neurons in a suite of recent studies addressing connectivity in the olfactory system, and visualizing neural function in the live brain [25,26•–28•,29] (Figure 2c).

Heisenberg and collaborators have created a digital model of the adult *Drosophila* brain that encompasses most of the currently identified compartments [30,31••] (left hemisphere of Figure 3c). This model, unlike most other models surveyed here, is based on averaging anatomical data from 20 adult brains (therefore 'standard brain'). A similar standard model exists for the bee brain [17] and the adult cockroach brain [32]. Given the high level of precision at which neuropile compartments are displayed in the standard brain, as well as the capabilities of measuring sizes and volume, it was possible to determine variability among individuals, male and female, and, most importantly, to establish structural aberrations in brain morphology of several mutants that would have been difficult to determine without a digital model. The standard brain is easily accessible and interactive and forms the cornerstone of future modeling efforts addressing the expression and function of specific molecules in the *Drosophila* nervous system.

Digital models of neuropile compartments have been generated for the larval brain [33••] (Figure 3b) and are under preparation for embryonic, pupal and adult stages (right hemisphere of Figure 3c). It came as a gratifying realization that most of the compartments that constitute the central brain of the adult are already recognizable in the early larva; other compartments, including the layers of the optic lobe and the central complex, are formed *de novo* during larval and pupal development, respectively. Digital models greatly enhance the ease with which progressive changes of three-dimensional structures such as compartments can be appreciated (Figure 3).

Models of individual neurons or clusters of contiguous neurons differ in character and purpose from the compartment models discussed above. In the simplest case, neuron models are generated in an automated way as Z-projections of confocal images, in which it becomes possible to visualize all structural elements of a neuron in one plane. All software packages that accompany a confocal microscope include the capability of generating Z-projections, and typically also go one step further to build volume rendered models that can be rotated and artificially sectioned. These procedures can generate highly informative images, in particular if Z-projections or volume rendered models of neurons are presented against a backdrop of a confocal image or model that shows compartment boundaries. Following this approach, models of antennal lobe interneurons were related to glomeruli, as well as to the calyx and lateral horn where their axons terminate [26•–28•,29] (Figure 2c).

Figure 3



First instar **(a)**, third instar **(b)** and adult **(c)** neuropile compartment models of the *Drosophila* brain created by manual segmentation of compartment boundaries visualized by a reporter driven by the choline acetyltransferase enhancer ([a,b], right hemisphere of [c]) and from the FlyBrain (http://flybrain.neurobio.arizona.edu/) database (left hemisphere of [c]) which was visualized using a different neuropile marker. Compartments such as the antenna lobe (al) and ventral body (vb) undergo an increase in volume as development progresses from an early larvum to the adult. Compartments, such as the basoposterior lateral (BPL), are subdivided into several new compartments in addition to growing in overall volume through this developmental time. Note that the ventrolateral protocerebrum (vlpr; the name given to the adult compartment corresponding to the larval BPL compartment) has been modeled as one structure whereas this structure is subdivided into six sub-compartments in the right hemisphere. The use of the choline acetyltransferase reporter allowed the visualization of these subdivisions that was not seen with the neuropile marker used for making the FlyBrain model. This difference is also seen with the unnamed neuropile (un; left hemisphere) compartment that encompasses several compartments from the left hemisphere. Models are shown in anterior view, with each compartment consistently rendered in the same color. Only the right hemispheres are shown in panels (a) and (b). Dotted line indicates midline.

Superb models were also generated for honey bee mushroom bodies to visualize the spatial ordering of subsets of Kenyon cells [34], optic lobe commissures [35•], and cockroach accessory medulla where different classes of interneurons intermingle with retinal axon terminals [36••].

Surface rendered models of compartments, neuron clusters (in this case, neurons related by cell lineage) and axon tracts were combined in a recent series of studies of the *Drosophila* larval brain [37–39,40•]. During embryogenesis, a stereotyped set of neuropile founder tracts lay down a pattern of axon bundles that interconnect brain and ventral nerve cord, as well as different parts of the brain. These tracts remain visible throughout development; starting in the late larva, they are supplemented by a much more complex pattern of secondary axon tracts. A model relating the primary axon tracts to compartment boundaries has been generated [33••,38]; further models of secondary tracts, as well as of the pattern of trachea that travel with the glial septa in between compartments, are under construction.

The larval compartment model was also used in a first attempt to insert subsets of neurons by 'warping' (for more detail, see below). Thus, neural lineages derived from a specific domain of the head called the eye field were visualized by their expression of a reporter gene driven by the *sine oculis* promoter. These lineages were modeled and inserted into the larval compartment model [41].

Models of non-neuronal cells, external surfaces and subcellular structures

Digital anatomical modeling outside the nervous system has been relatively modest to date in *Drosophila* (for attempts to construct models in the context of gene expression documentation, see below). In many instances, Z-projections or volume rendering of confocal section, or time lapse videomicroscopy, has proven to be sufficient in analyzing and documenting complex morphogenetic processes, such as nuclear movement in the blastoderm, epithelial folding during gastrulation, germ band elongation, dorsal closure, tracheal branching, and gut development, to name but a few examples. 'True' surface rendered digital models of the developing hindgut (DD Iwaki, JA Lengyel, personal communication) proved highly valuable to visualize cell movement driving the convergent extension of this structure [42]. Digital models have also been prepared for sperm cells following fertilization [43] and retina cells [44]. Schematic animated models of a variety of organ systems are collected as didactic tools in the FlyMove website (http://flymove.uni-muenster.de/), which serves as a great resource for students and researchers in *Drosophila* development.

Taking advantage of the autofluorescence of the cuticular surface, digital models of the exoskeleton have recently been generated that approach scanning electron microscopy in resolution and detail [45,46,47•]. Such models

lend themselves to functional studies addressing the distribution of strain within the cuticula in relationship to mechanoreceptors and muscle insertion sites.

Digital modeling based on transmission electron microscopy or electron cryo-microscopy sections has a long-standing tradition as a technique to visualize organelles and macromolecular assemblies. Using *Drosophila*, this approach is enhanced by the availability of structural mutants that, among other advantages, help overcome technical difficulties. For example, preparations of thin filaments from myosin-less *Drosophila* flight muscles were used to generate a digital model of the troponin-actin complex which can be used for the analysis of steric regulation [48•]. Models of the complex between microtubules and tubulin-based motors were able to visualize structural difference between minus-end and plus-end directed motor proteins [49]. Similarly, models were prepared for the *Drosophila* proteasome complex [50] and synaptonemal complex [51], among others.

Digital models as repositories and analytical tools to study gene expression

Digital databases aimed at incorporating gene expression are under way for several model organisms, including mouse, *Drosophila* and *Caenorhabditis elegans*. Their primary goal is to illustrate the spatial expression of many gene products within the framework of a surface reconstructed organism or tissue. A spatial database has several beneficial applications, including: first, the position of a gene product may give insight about this gene's function; second, 'virtual' experiments can be conducted to determine if two genes are co-expressed without performing the corresponding 'wet' experiment; and third, investigators will be able to communicate the expression pattern of a new gene within an existing framework at a resolution much better than a representative whole-mount or section image.

To construct a gene expression database, it is necessary to incorporate the data from many gene expression experiments into one framework: the atlas model. Due to inter-individual variability, misalignment, staining variation, and different orientations of sections, the sample data will need to be matched to the atlas model. Matching can be done in one of two approaches: the gene expression data can be manually annotated or the gene expression needs to be warped to match the atlas model. Manual annotation is a demanding task in which the annotating investigator examines the gene expression data and assigns this gene expression to one or several of the structures within the master model. This approach has the benefit of using the complex pattern recognition ability of the human mind but is also subject to variation depending on the capability of the annotating investigator and becomes less practical with the large scale gene expression experiments that are often desired.

Warping, the other approach, seeks to have software calculate what distortions are needed to match the spatial conformation of each gene expression experiment to the atlas model (this process is also known as morphing or registration). Various algorithms have been developed which can detect edges, regions or other aspects within a dataset and use this information to try to match the gene expression dataset to that of the atlas. However, the efficiency of these endeavors is still quite low, and these efforts have become semi-automatic, requiring an investigator to manually fix inaccuracies that occur and, in some cases, to provide landmarks that are used to help the software match points between the experiment and the atlas.

The prevailing difficulty of warping gene expression data onto a digital atlas using automatic procedures is illustrated vividly by a recent study in *C. elegans*. Although this organism with few cells and fixed lineages is ideal for identifying specific cells, software created for warping 2 to 28 cell embryos encountered a lower than expected efficiency rate. The ability of the software, called SPI (SuPerImposing), to determine the developmental stage of an embryo decreased rapidly with increasing age; for example, at the 6- and 8- cell stage, with a well-contrasted dataset, automatic prediction was 65.3% accurate when assayed by visual examination by a human researcher [52••]. The position of nuclei of embryos later than the 8-cell stage needs to be entered by the researcher using an interactive semi-automatic procedure. Approximately 30% of the gene expression data that were warped into the appropriate staged atlas model required manual adjustments to ensure proper registration. Although the efficiency level of SPI is sufficient and practical for the study of maternal genes in early *C. elegans* embryos, it provides insight into the difficulties of applying this automated process to an organism with more cells of varied position.

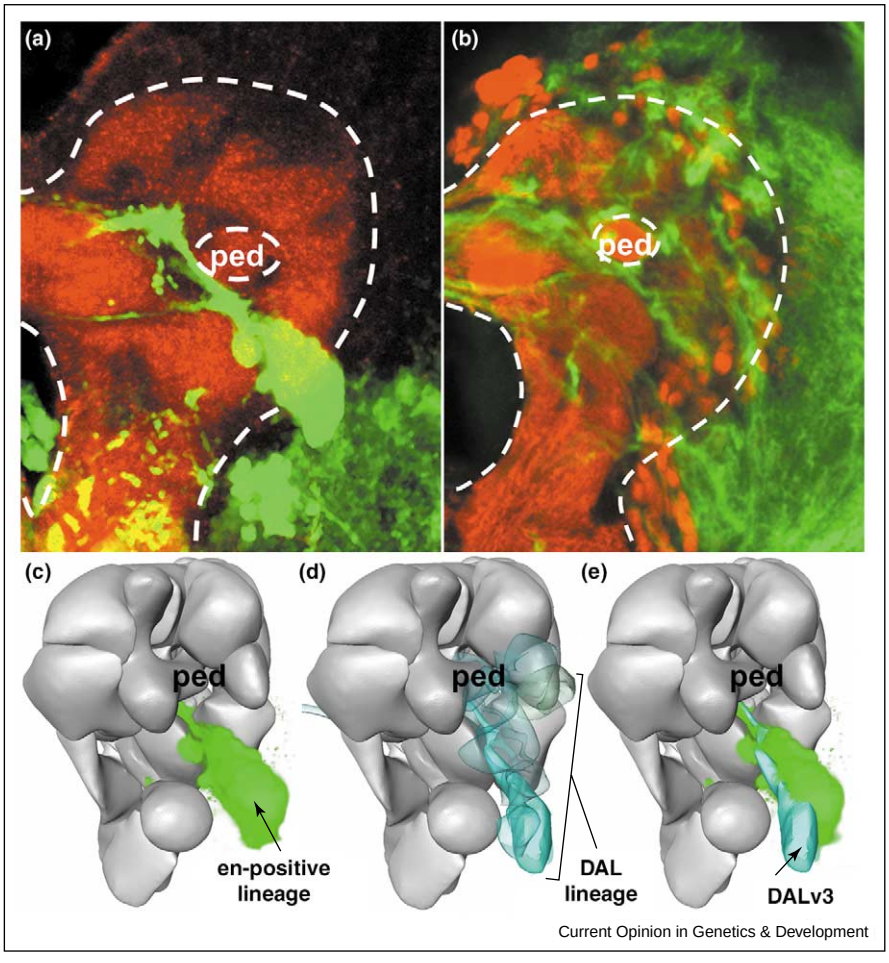
As a first step towards a digital database of gene expression in mouse, the Edinburgh Mouse Atlas Project (EMAP, http://genex.hgu.mrc.ac.uk/) has created a framework of computer models representing successive stages of mouse embryogenesis onto which gene expression data are inserted (Edinburgh Mouse Atlas Gene-Expression Database [EMAGE]) [53–55]. Both surface reconstructed and volume rendered models can be visualized simultaneously with gene expression data to provide a proper spatial context. The resolution available allows spatial comparison of gene expression data relative to organ primordia [55]. A similar approach was recently published by another group [56]. Given the large cell number and considerable variability in shape of mouse embryos, the resolution achieved by warping gene expression domains on the digital master model remains at the level of tissues, not individual cells. Gene expression patterns within the database are available to the

public and can be searched online either through textual or spatial queries. The establishment and maintenance of this database has helped to define the elements that are needed to successfully set up a functional gene expression database.

Several databases of gene expression exist in *Drosophila* [57,58,59•,60] (among others), but with the exception of several recent papers pertaining to early expressed segmentation genes that form regular stripe-like domains within the blastoderm [61,62•,63•], no digital three-dimensional atlas into which gene expression patterns are deposited by an automated warping procedure has been generated yet. Models of specific organs, such as the standard fly brain [31••], the antennal lobe [20], or the larval brain [33••,40•] present a significant first step towards generating the atlas model that will ultimately serve as the repository for expression patterns.

Given the high number of cells in the brain (and other organs), as well as the typically complex expression patterns of genes, the level of resolution at which expression patterns can be managed meaningfully will initially almost certainly not be single cells. A level of resolution

Figure 4



Transfer of gene-expression patterns into an atlas model. For this figure, the *engrailed* (en) gene expression pattern **(a)**, visualized by a lacZ reporter construct (neuropile labeled by anti-Syntaxin in red), was transferred **(b)** into an atlas model of the late third instar brain that contained neuropile compartments and secondary lineages. Warping was performed manually using the Amira software package using only rigid transformations such as translation, rotation and uniform scaling. The first step was to register the neuropile within the *engrailed* dataset (red in [a]) with the neuropile of the atlas model (red in [b]) to ensure that the orientation and dimensions of both matched closely. The registration step was refined by matching landmarks within each of the neuropiles (dashed outlines in [a,b]); for example, the contours of the neuropile surface had to match each other, as well as the location of the peduncle (ped) of the mushroom body. Arrows indicate the branch point of the *engrailed*-positive lineage in both (a) the *engrailed*–LacZ dataset and (b) the atlas model dataset in which all secondary lineages are labeled. Once warping is completed, the *engrailed* expression pattern is visualized **(c–e)** using volume rendering within the framework of the surface-rendered atlas model. In (c), the *engrailed*-positive lineage (green) is shown against the backdrop of the neuropile compartments (gray). In (d) the lineages of the atlas model that belong to the dorsoanterior lateral (DAL) group are visualized. By comparing the position of the volume-rendered *engrailed* lineage with each of the DAL lineages consecutively, it was determined (e) that the DALv3 lineage expresses *engrailed*.

that appears to be appropriate in current attempts of our group to document and model gene expression patterns in the larval brain is that of neural lineages. Thus, during the third larval instar, the brain cortex of each hemisphere is studded with approximately 90 lineages, each one located at more or less invariant position, and represented by an axon bundle that forms an invariant pattern in the neuropile [40•].

Shown in Figure 4 is the expression pattern of the *engrailed* gene in the *Drosophila* third-instar larva warped into an atlas model of the same stage. Warping was performed manually using the Amira software package using only rigid transformations such as translation, rotation and uniform scaling. By comparing the position of the volume-rendered *engrailed* lineage with each of the lineages consecutively, it was determined that the DALv3 lineage expresses this gene (Figure 4e). We consider it to be realistic to expect that other expression patterns that coincide with discrete lineages (or groups of lineages) can be deposited in the same manner into the larval brain model.

Conclusions

In conclusion, digital 3D models of anatomical structures, in particular elements of the nervous system, have made a significant impact on the visualization and interpretation of data in the field of *Drosophila* development. Improvement of modeling software has come a long way and will continue further, so that the effort of generating models from serial sections, confocal or histological, diminishes. Attempts at producing atlas models that can serve as repositories for gene expression data are ongoing in the *Drosophila* field. It is to be expected that these models will soon provide comprehensive gene-expression archives in which expression data of multiple genes can be displayed at a high level of resolution, thereby enabling the user to infer hypotheses about gene function and gene interactions.

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- •• of outstanding interest

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Segmentation (identifying different elements on digitized sections) is a crucial step in the model-making process. Atlas-based segmentation attempts to use *a priori* information within an atlas dataset to automatically segment an experimental dataset. Atlas models can be generated in one of several ways and this paper evaluates the predictive ability and efficiency rate of each method of creating an atlas model. Several different approaches for atlas image selection — registration to an individual atlas image, registration to an average-shape atlas image, registration to the most similar image from a database of individual atlas images, and registration to all images from a database of individual atlas images with subsequent multi-classifier decision fusion — are assessed. The results of this study are highly relevant for ongoing efforts of segmentation.

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21. Galizia CG, McIlwrath SL, Menzel R: **A digital three-dimensional atlas of the honeybee antennal lobe based on optical sections acquired by confocal microscopy**. *Cell Tissue Res* 1999, **295**:383-394.

22. • Berg BG, Galizia CG, Brandt R, Mustaparta H: **Digital atlases of the antennal lobe in two species of tobacco budworm moths, the Oriental Helicoverpa assulta (male) and the American Heliothis virescens (male and female)**. *J Comp Neurol* 2002, **446**:123-134.

Papers [20,21,22•] present comprehensive three-dimensional atlas models of the antennal lobe of *Drosophila*, honey bee, and tobacco budworm (Helicoverpa). The models are based on confocal microscopic analysis of glomeruli stained with a neuropile-specific monoclonal antibody. Individual glomeruli are recognized and annotated on the basis of their shape, size, position, and intensity of antibody labeling. The authors experiment with attempts to further increase the utility of their model by subdividing the relatively large number of glomeruli into arrays, each of which is defined by a prominent landmark glomerulus. The models were used to establish interindividual glomerular variability, and variability between males and females. The *Drosophila* model has been used as a digital reference atlas of the antennal lobe in recent functional and developmental studies of the fly olfactory system (see [26•–28•]).

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26. • Marin EC, Jefferis GS, Komiyama T, Zhu H, Luo L: **Representation of the glomerular olfactory map in the *Drosophila* brain**. *Cell* 2002, **109**:243-255.

See annotation [28•].

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See annotation [28•].

28. • Wong AM, Wang JW, Axel R: **Spatial representation of the glomerular map in the *Drosophila* protocerebrum**. *Cell* 2002, **109**:229-241.

Papers [26•–28•] investigate the ordered projection pattern of antennal lobe interneurons in the calyx and lateral horn, two second-order olfactory neuropiles of the central brain. Individual antennal interneurons were labeled by GFP expression. It is concluded that a 'glomerulotopic' projection pattern exists, such that the association of the dendrite with a certain glomerulus is correlated with the central arborization of the corresponding axon. In identifying glomeruli, the authors took advantage of the existing atlas model of the antennal lobe (see [20]) and employed the nomenclature introduced in this model.

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30. Rein K, Zockler M, Heisenberg M: **A quantitative three-dimensional model of the *Drosophila* optic lobes**. *Curr Biol* 1999, **9**:93-96.

31. •• Rein K, Zockler M, Mader MT, Grubel C, Heisenberg M: **The *Drosophila* standard brain**. *Curr Biol* 2002, **12**:227-231.

This and the previous paper [30] outline the method of generating an atlas model of a *Drosophila* standard brain that is based on warping and averaging confocal stack files from 20 individuals. Brains were labeled with a global neuropile marker. The three-dimensional datasets were automatically aligned into a common coordinate system and intensity averages calculated, resulting in a three-dimensional probabilistic map. Means and variances for several aspects of size and shape of the brain were established. Most significantly, the applicability of the model to analyze mutants and gene expression patterns was demonstrated.

32. Chiang AS, Liu YC, Chiu SL, Hu SH, Huang CY, Hsieh CH: **Three-dimensional mapping of brain neuropils in the cockroach, *Diploptera punctata***. *J Comp Neurol* 2001, **440**:1-11.

33. •• Younossi-Hartenstein A, Salvaterra P, Hartenstein V: **Early development of the *Drosophila* brain IV. Larval neuropile compartments defined by glial septa**. *J Comp Neurol* 2003, **455**:435-450.

In this paper, we present a digital model of the larval neuropile compartments and their relationship to the pattern of long-axon tracts. On the basis of their location, larval compartments could be identified with compartments defined by structural and functional criteria for the adult fly brain. Changes in brain compartmental organization occurring during larval growth are described. The model of compartments is currently being used as an atlas model into which specific brain structures, labeled by their expression of genes or proteins, are entered.

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35. • Reischig T, Stengl M: **Optic lobe commissures in a three-dimensional brain model of the cockroach *Leucophaea maderae*: a search for the circadian coupling pathways**. *J Comp Neurol* 2002, **443**:388-400.

The authors have reconstructed the pattern of optic lobe commissures in the brain of the cockroach *Leucophaea*. The paper illustrates the way in which a volume rendered projection of neuronal subsets — the optic commissures, here reproduced by camera lucida — can be combined with a digital model of the major neuropile compartments in such a way that spatial relationships become exceedingly clear.

36. •• Reischig T, Stengl M: **Ultrastructure of pigment-dispersing hormone-immunoreactive neurons in a three-dimensional model of the accessory medulla of the cockroach *Leucophaea maderae***. *Cell Tissue Res* 2003, **314**:421-435.

Here the authors have taken the first step in constructing a volume-filling digital model of a small neuropile compartment, the accessory medulla which integrates visual input with several sets of interneurons, thereby generating a circuit that is pivotal in circadian rhythmicity. Digital models of volume-rendered PDH neurons (which express the Period protein) were combined with compartment models reconstructed from serial sections. The paper provides an excellent example for the potency of digital models as didactic and analytical tools in neuroanatomy.

37. Nassif C, Noveen A, Hartenstein V: **Embryonic development of the *Drosophila* brain I. The pattern of pioneer tracts**. *J Comp Neurol* 1998, **402**:10-31.

38. Hartenstein V, Nassif C, Lekven A: **Embryonic development of the *Drosophila* brain II. The glia cells of the brain**. *J Comp Neurol* 1998, **402**:32-48.

39. Nassif C, Noveen A, Hartenstein V: **Early development of the *Drosophila* brain III. The pattern of neuropile founder tracts during the larval period**. *J Comp Neurol* 2003, **455**:417-434.

40. • Dumstrei K, Wang F, Nassif C, Hartenstein V: **Early development of the *Drosophila* brain. V. Pattern of postembryonic neuronal lineages expressing Shg/DE- cadherin**. *J Comp Neurol* 2003, **455**:451-462.

We used expression of *Drosophila* E-cadherin in secondary lineages to model the position of these cell clusters and their axon tracts in relationship to the neuropile. Axons of neurons belonging to one lineage form tight bundles. The model shows that the trajectories of these bundles are correlated with the location of the neuronal lineages to which they belong. Thus, axon bundles of lineages that are neighbors in the cortex travel parallel to each other and reach the neuropile at similar positions. On the basis of topology and axonal trajectory, the lineages of the central brain can be subdivided into eleven groups that can be followed throughout successive larval stages. This model of secondary lineages is instrumental in current efforts to construct a comprehensive atlas model of the late larval brain (W Pereanu, V Hartenstein, unpublished).

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rdgA, at early stages of degeneration. *Cell Tissue Res* 1988, **252**:293-300.

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The authors use stack files of the autofluorescent insect exoskeleton (*Drosophila* male and female genitalia) to generate high-resolution models of parts of the outer surface of the fly. The study indicates that confocal laser scanning microscopy and 3D reconstruction are excellent techniques for visualizing small, complex, autofluorescent structures in *Drosophila*.

48. • Cammarato A, Hatch V, Saide J, Craig R, Sparrow JC, Tobacman LS, Lehman W: ***Drosophila* muscle regulation characterized by electron microscopy and three-dimensional reconstruction of thin filament mutants**. *Biophys J* 2004, **86**:1618-1624.
Wild-type and mutant thin filaments from 'myosinless' *Drosophila* flight muscles were serially sectioned. Digital models of filaments were able to resolve the exact spatial relationship between tropomyosin and actin in both the presence and absence of calcium, demonstrating that the complex formed by these structural molecules is indistinguishable from that in vertebrate striated muscle. A model of thin filaments from the *Drosophila* troponin mutant *heldup(2)* confirmed the previous hypothesis that this allele of troponin no longer reacts sterically in the absence of calcium.

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The authors introduce an automated procedure to morph gene-expression patterns into atlas models of early *C. elegans* embryos. This is one of the few studies where the ability of a program to 'recognize' cells in extremely simple embryos with fixed lineage (2–28 cells) is put to a test. Before expression can be mapped, the number and position of each cell must be determined. The accuracy of this software to stage these early embryos declined from 90.0% (2-cell), to 89.8% (4-cell) and down to 65.3% at the 8-cell stage. Embryos later than the 8-cell stage required the investigator to enter the exact stage and to identify the location of nuclei using a built-in interactive classification procedure. Both the technology used here as well as the outcome of the test are relevant for ongoing efforts to generate similar models in *Drosophila*.

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58. Montalta-He H, Reichert H: **Impressive expressions: developing a systematic database of gene-expression patterns in *Drosophila* embryogenesis**. *Genome Biol* 2003, **4**:205.

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This paper introduces a web-based searchable database of *Drosophila* gene expression patterns. 2179 genes were analyzed by *in situ* hybridization to fixed *Drosophila* embryos. Of the genes assayed, 63.7% displayed dynamic expression patterns that were documented with 25,690 digital photomicrographs of individual embryos. The photomicrographs were annotated using controlled vocabularies for anatomical structures that are organized into a developmental hierarchy. The database provides a tool that allows for a systematic analysis of annotated patterns of gene expression.

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61. Myasnikova E, Samsonova A, Kozlov K, Samsonova M, Reinitz R: **Registration of the expression patterns of *Drosophila* segmentation genes by two independent methods**. *Bioinformatics* 2001, **17**:3-12.

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See annotation [63•].

63. • Poustelnikova E, Pisarev A, Blagov M, Samsonova M, Reinitz J: **A Database for Management of Gene Expression Data in Situ**. *Bioinformatics* 2004, in press.
Papers [61,62•,63•] describe a gene-expression database (FlyEx) that aims at integrating the expression pattern of segmentation genes in the *Drosophila* blastoderm. Two methods of warping are used to incorporate data from multiple blastoderm-stage models, in which the position and number of nuclei is variable. The first method, one-dimensional sampling, seeks to extract data from a longitudinal stripe of the embryo corresponding to ~10% of the dorso-ventral width. This is meaningful in this context because the segmentation genes are responsible for creating the anterior–posterior axis and so the resulting expression database consists of one-dimensional expression profiles for each of the segmentation genes. The second morphing technique attempts to integrate different images of gene-expression data by creating the concept of a unified discrete scale. In this, an average embryo is calculated, using information from each dataset, and gene-expression profiles are morphed into this framework for visualization.