Spatio-temporal framework for integrative analysis of zebrafish development studies

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Citation

Belmamoune, M. (2009, November 17). *Spatio-temporal framework for integrative analysis of zebrafish development studies*. Retrieved from https://hdl.handle.net/1887/14433

CHAPTER 3

THE 3D DIGITAL ATLAS OF ZEBRAFISH: AN INTEGRATIVE TOOL FOR ZEBRAFISH ANATOMY

Based on:

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The 3D digital atlas of zebrafish: 3D models visualization through the Internet.
(Submitted, 2009)

Partially published in:

S.A. Brittijn, S.J. Duivesteijn, M. Belmamoune, L. F.M. Bertens, W.B., J.D. de Bruijn,
D.L. Champagne, E. Cuppen, G. Flik, C.M. Vandenbroucke-Grauls, R.A.J. Janssen,
I.M.L. de Jong, E.R. de Kloet, A. Kros, A.H. Meijer, J.R. Metz, A.M. van der Sar, M.J.M.
Schaaf, S. Schulte-Merker, H.P. Spaink, P.P. Tak, F. J. Verbeek, M.J. Vervoordeldonk,
F.J. Vonk, F. Witte, H. Yuan and M.K. Richardson.
Zebrafish development and regeneration: new tools for biomedical research.
Int. J. Dev. Biol. (2009) 53: 835-850.

Abstract

We have designed and implemented a 3D digital Atlas of zebrafish development. 3D digital Atlas models have an explicit formal-ontological representation of their anatomical entities at multiple levels of granularity. This data representation is an important requirement to facilitate 3D models processing and data understanding. The Atlas is representing standard histology of the zebrafish developing embryo. Zebrafish has been established as a genetically flexible model system for investigating many different aspects of vertebrate developmental biology. It has become the focus of a major research effort into understanding the molecular and cellular events throughout the development of vertebrate embryos. The increasing use of zebrafish as a model system for developmental studies has necessarily generated interest in the anatomy of this species at different developmental stages to map the many key aspects of organ morphogenesis that take place. 3D standard anatomical resources and references that encompass the zebrafish development at early developmental stages are absent and there is therefore an urgent need for such resource to understand how different organ systems and anatomical structures develop throughout the early lifespan of this species. We have built a 3D digital Atlas of zebrafish containing a range of zebrafish 3D models. 3D models at different stages of early embryonic development are annotated with standard and formal ontological anatomical nomenclature and are made available through the internet. We have created a web-application to search, inspect and browse 3D atlas models at different levels of granularity.

3.1 Introduction

The study of anatomy during embryonic developmental is an intrinsically three-dimensional (3D) endeavour. Anatomists wish to grasp the full 3D complexity of the anatomical structures they study. A 3D shape offers an intrinsic beauty and moreover, it helps to understand how they are related to their adjacent organs and more importantly how their complex shapes are created during embryonic development. Here we present the 3D digital Atlas of zebrafish. This 3D Atlas provides the standard histology of zebrafish model organism. A 3D model consists of a variety of anatomical structures that exist at different levels of complexity or levels of biological organization (also called levels of granularity). Levels of granularity can be observed also on the level of anatomical structures functions, for instance brain (finer level of granularity) and the central nervous system (coarse level of granularity). We applied the principle of granularity to organize anatomical structures in the Developmental Anatomy Ontology of Zebrafish (DAOZ; Belmamoune and Verbeek, 2007). Furthermore, we used DAOZ ontological terms to annotate anatomical structures in the 3D Atlas models. This annotation enables Atlas data to be organized at multiple levels of granularity too. We believe that such data organization facilitates anatomical structures access; from coarse entities we get into structures at finer levels. Moreover, when anatomical entities have different levels of representation, their identity is not hampered over time than when they are treated as pure entities with one level of abstraction; for instance functions are continuous entities that preserve their identity over time. Therefore data integration becomes also stronger when entities are tracked using their attributes that navigate through different levels and over time. The work presented here is an extension of the already published 3D digital Atlas of zebrafish (Verbeek el al, 2000, 2002). 3D models of zebrafish at early developmental stages were produced and published to allow users to learn more about anatomy of the developing zebrafish embryo. In its new version, 3D models of the Atlas were evaluated and annotated with standard nomenclature from the

DAOZ and reorganized in an object oriented database. Through internet tools users are able to interact with the Atlas database and perform queries against the 3D models. A 3D Atlas model is an entity that through these internet tools could be accessed and browsed as a whole as well as through its instances. For example users could view a 3D model of 36 hours post fertilization (hpf) as a whole or in sub-structures by composing a search query such as: “select all anatomical structures from a 36 hpf embryo that belong to the central nervous system and located in head”.

Zebrafish (Danio rerio) has emerged as a useful model system to study vertebrate development. The zebrafish model system has the considerable advantage of holding complex developmental systems not present in other model invertebrates such as C. elegans and Drosophila (Lieschke, 2001). Prior to our 3D digital Atlas, no detailed 3D anatomical reference for the early zebrafish embryo was available. The stages of zebrafish development have been intensively studied over the last decades, and an embryological staging series (Kimmel et al, 1995) has been provided including key events in embryo development. However, a detailed 3D documentation of zebrafish development describing the whole anatomy of the embryo was not available. Previous detailed descriptive studies of zebrafish development were presented in 2D or limited to a particular functional system (Isogai et al, 2001). A holistic understanding of the anatomy of an organism is critical to dissecting the development and function of different organs and tissues in space and time.

Imaging methodologies such Optical Projection Tomography (OPT) and Magnetic Resonance Imaging (MRI; Kabli et al, 2006) were developed to rapidly and easily generate digital data on the internal structure of tissues without the necessity for cutting the sample, although even today the results are most commonly viewed in two dimensions, as virtual sections. This is the approach used by FishNet project that uses OPT to generate 3D models of larval zebrafish (Bryson-Richardson et al, 2007). With the 3D Atlas of zebrafish we attempt to build a 3D reference framework with real standard anatomical structures and at high resolution. Moreover, this 3D framework should be ready for interoperability with other resources for an improved developmental study.

The 3D models of our digital Atlas are the result of 3D reconstruction from serial physical sections (Verbeek et al, 1998; Brune et al, 1999; Weninger et al, 2002). Each anatomical domain in a 2D section is outlined by a closed contour and annotated by a standard nomenclature. With this imaging method we get high quality models providing detailed view of the anatomical structures in the plan of section images. This technique is preferred over other non-destructive methods such as OPT and MRI. The most obvious advantage of using histology is the high level of detail which can be achieved. Furthermore it enables the use of staining methods, thereby enhancing the contrast between tissue types, which makes images annotation (next section) easier. This process of 3D models generation might be qualified as time-consuming however each embryo, i.e. 3D model could be considered as a standard model for developmental biology and therefore justifies the amount of effort involved in its production.

The effectiveness of any model organism is restricted by the availability of accurate anatomical information for that model organism. With the 3D Atlas we strive to broaden the understanding of the biological development of the zebrafish and provide stakeholders with information on the scope of the size and shape of anatomical domains so as to enable an anatomical structure to be compared with its well described presumed "standard". For zebrafish developmental studies gene expression patterns are produced at different stages of development. To understand this wealth of molecular data the 3D Atlas serves as a spatial and temporal mapping system for gene expression data submission and retrieval.

The 3D digital Atlas of zebrafish consists of a number of canonical stages of the zebrafish embryo and we have chosen to report on the early stages that have been completed; these are 24, 36, 48 and 72 hpf. These stages correspond to those of the staging series of the early work of kimmel et al. For each of these developmental stages an acquisition database was produced. 3D models, i.e. 3D images in this database were graphically and semantically annotated. The graphical annotation is realized by means of closed contours around anatomical domains while the semantic annotation is given by conveying to each anatomical domain its presumed anatomical name.

The 3D Atlas is intended to be used as a standard framework to understand development of the zebrafish embryo. Development is a spatial and temporal event; therefore the 3D models of the Atlas need a spatio-temporal description. The spatial, temporal as well as function information in the 3D models is provided from the DAOZ (cf. chapter 2).

At present, biological research information is preferred in a digital computer readable form so that this information can be shared and linked to other digital resources. This is in particular important for anatomical Atlases of model systems. We have, therefore made our 3D digital Atlas available through internet. Atlas images and annotations are organized in a database system. Furthermore, we developed a web-application, i.e. ZFAtlasServer (http://bio-maging.liacs.nl/ZFAtlasServer) that enables users to render 3D models on the fly. More importantly it allows users to search in a 3D model for specific instances or anatomical entities that are subsequently displayed on the fly, in 2D and 3D formats.

The remaining part of this work is structured as follows: section 2 contains a detailed description of the adapted methods to acquire and construct 3D models in the Atlas. In section 3 we describe how the 3D model are annotated and in section 4 we presents the way 3D models are managed in an object oriented database. Furthermore, in section 5 we give a detailed description of our internet tools for 3D models retrieval and visualization. Section 6 is dedicated to present our results and discussions. While our future works are discussed in section 7.

3.2 3D Models Acquisition

Here we present the model's acquisition. So far we produced a number of embryos along the time axis at the following developmental stages 24, 36, 48 and 72 hpf (staging was based on Kimmel et al., 1995). The models presented here are acquired on normal and high resolutions.

3.2.1 Imaging methodology

Our starting point is a series of serial sections (Verbeek et al, 1995, Verbeek, 2000 and Verbeek el al, 2000). From these sections, section images were acquired with our dedicated acquisition station (Verbeek & Boon, 2002); Zeiss microscope equipped with CCD camera (JAI-Vision) and a xy-stage (Marzhauser) controlled by a MAC 4000 (Marzhauser). The stage controller was connected to the computer through the serial interface (RS 232) while the CCD camera was connected to the computer via a PC Vision frame grabber. Our acquisition software, 3Dacq, vs. 2.0 (Verbeek et al., 1998; Verbeek and Boon, 2002) controlled both the XY stage and the camera/frame grabber and thereby also the image capturing as well as the positioning of the region of interest in the field of view of the microscope. The PCVision frame grabber facilitated video overlay and this was utilized in the sequential acquisition of the section images. While the previous image section was kept in overlay, the next section was acquired and aligned with the overlay. In this manner an accurate registration of the consecutive initial section images was obtained. The logistics of the acquisition process were assembled in an acquisition database that was transposed to a data set suitable for annotation.

3.2.2 Normal Resolution

The images of normal resolution were acquired using standard camera output. The whole process of producing an initial set of section images at standard resolution for the 3D modelling was very well documented in a database. This documentation is required for high resolution acquisition (as described in the next section). After processing, all section images were stored in Portable Network Graphics format (PNG; http://www.w3.org/Graphics/PNG/).

3.2.3 High Resolution

At high resolution models histology could be seen in more details than at normal resolution. The XY position and angle φ of the camera during standard resolution acquisition has been stored in the acquisition database for each section image and were used to obtain a second set of images at a higher magnification. High resolution series were produced for the developmental stages of 36 and 72 hpf embryos. In the high resolution series each image was constructed from multiple tiles. An overlap of 32 pixels between the tiles was used to be able to assemble the tiles into a complete image section after acquisition. The obtained tiles were combined into image sections and preprocessed using the methods described in Verbeek and Boon, 2002. The images acquisition was accomplished using the normal resolution image stacks. The image stack was annotated and visualized in the same way as at normal resolution.

3.3 3D Models Annotation

Image stacks for several developmental stages have been produced and annotated. Each image has been segmented into multiple anatomical areas (or structures). Each anatomical structure is outlined by a closed contour; this makes it an anatomical domain.

Furthermore, we assigned an anatomical name to each domain. This anatomical nomenclature is extracted from the DAOZ database.

The annotation and subsequent visualization of the resulting 3D models was realized with a software suite that we developed for generating 3D models out of plan parallel sections, i.e. the TDR-3Dbase (Verbeek et al, 1995 and 2000) (cf. Figure 1). This phase of the 3D reconstruction starts with transfer of the acquisition database with the section images and other relevant information to TDR-3Dbase; together with the annotated structures this transposed to a (XML) database dedicated to the 3D reconstruction.

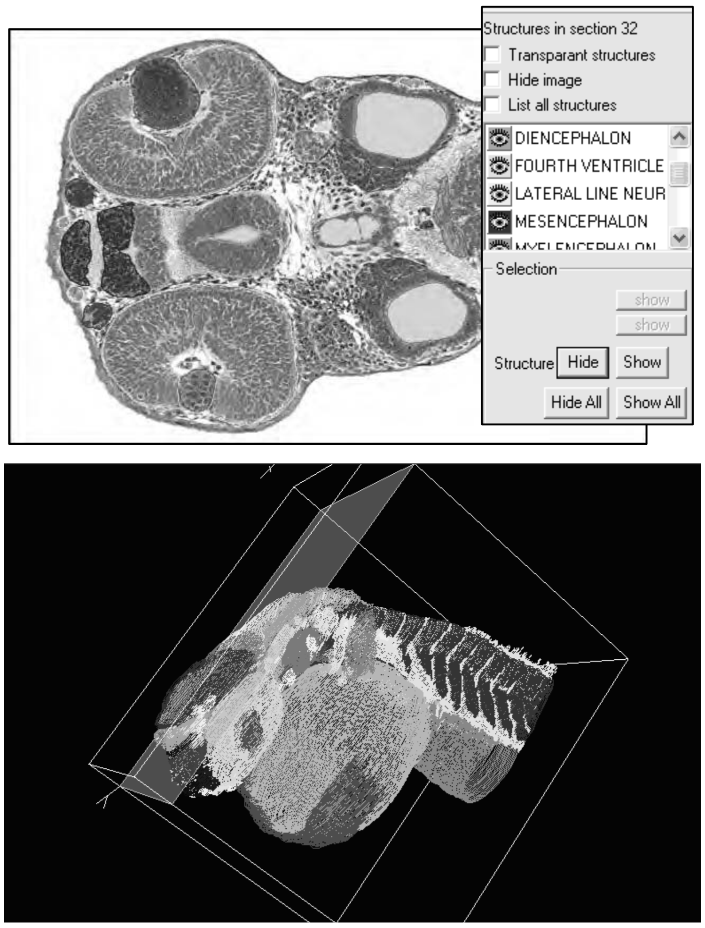


Figure 1: (Above) shows a section image that has been segmented in multiple anatomical domains (closed contours) and to each anatomical domain a color and an anatomical name are assigned, these are given in the form of an anatomical concepts list. (Below) illustrates the 3D contour model; it is the result of 3D reconstruction using contour elements.

The 3D Atlas consists, therefore, of two parts; one is a textual description of the anatomical structures and the other is a graphical annotation realized by segmenting each section image into multiple domains. The first part is stored in an object oriented database while the second part is stored on the server file system. Both parts are accessible through the internet for 3D models visualization.

3.3.1 Graphical annotation

To enable a wide range of users to understand what anatomical information is present in a section image a graphical annotation is required. This form of annotation indicates a domain in the image (area or volume) in which an anatomical concept is observed. In each of the section images anatomical structures were traced using a WACOM LCD tablet (PL series, WACOM, Europe). To that end, each anatomical structure was specifically named and attributed a color label.

3.3.2 Textual annotation

A textual annotation is realized by attaching a name to each anatomical domain in every section image. Anatomical domains are annotated with anatomical names from the DAOZ. The annotation of the Atlas 3D models with a domain-specific ontology implies its data integration with a broad range of resources. Additionally, anatomical structures in the DAOZ are organized at different levels of abstraction. To each anatomical term a number of path to that term have been conceptualized such as its spatial location, functional system and stages of development. This organization allows structures to be grouped in units at different levels of granularity. This data organization is inherited by the annotated anatomical domains. It provides therefore a novel mechanism to retrieve and group anatomical domains at different levels of details (from gross to finer levels of granularity) for example from functional system (central nervous system) to structure (brain) to sub-structure (midbrain). Moreover, a wide range of users can readily search the Atlas. They are able to search in a 3D model for detailed information (instances) using coarse anatomical entities. Anatomical entities in an Atlas 3D model are described at multiple levels of granularity which enables their identity to remain maintained through time. This makes of the 3D digital Atlas a stronger and more persistent data integration framework where anatomical structures and attributes traverse multiple levels.

3.4 3D models Pre-processing and Management

For reasons of flexibility, we choose XML to be the native format of our data. As XML is involved in the whole processing pipe, it is relatively easy to add entries and/or attributes to the XML files without having to rewrite all software. This scalability feature is indispensable for a project which is subject to adaptation and update so as new insights are added to constantly improve the quality of the data. The Atlas data comes from two resources; one being the image acquisition software and the other being the TDR-3Dbase software package (Verbeek et al, 2002). Using the TDR-3Dbase 3D reconstruction from serial sections is realized and for each 3D model an XML file is generated.

An XML file includes entries for images and anatomical terms. Each anatomical structure is attached to an encoded file that stores contour information of its corresponding anatomical domain. These entries are extracted from the original XML files and stored in the Atlas database; PostgreSQL is the Database Management System (DBMS) that we are using. This process is realized using a parser software that takes an XML file as input and organizes its content into the Atlas database. Organizing data in an object oriented database facilitate data access and retrieval. In other words, search queries could be performed against the database to choose models or sub-models of interest.

3.5 Data Delivery: An interface for the Atlas database

Having created models covering the important early stages of embryonic development we designed a web-application, ZFAtlasServer, to allow easy online access to the full Atlas dataset. From an earlier application we have elaborated and produced an extension for the web-aplication: AtlasBrowser (Verbeek et al, 2002). The AtlasBrowser is a java applet. It allows data display and browsing in 2D and 3D formats. The ZFAtlasServer extended the AtlasBrowser by enabling 3D models to be queried and generates query results on the fly.

ZFAtlasServer is a three-tier application with three layers (cf. Figure 2). (1) The presentation layer or the Graphical User Interface (GUI). Through the GUI users send search requests and get the results prompted in an applet. (2) The application logic layer. This layer processes that services data requests between the user and the databases. (3) The databases or the data layer is where data is managed.

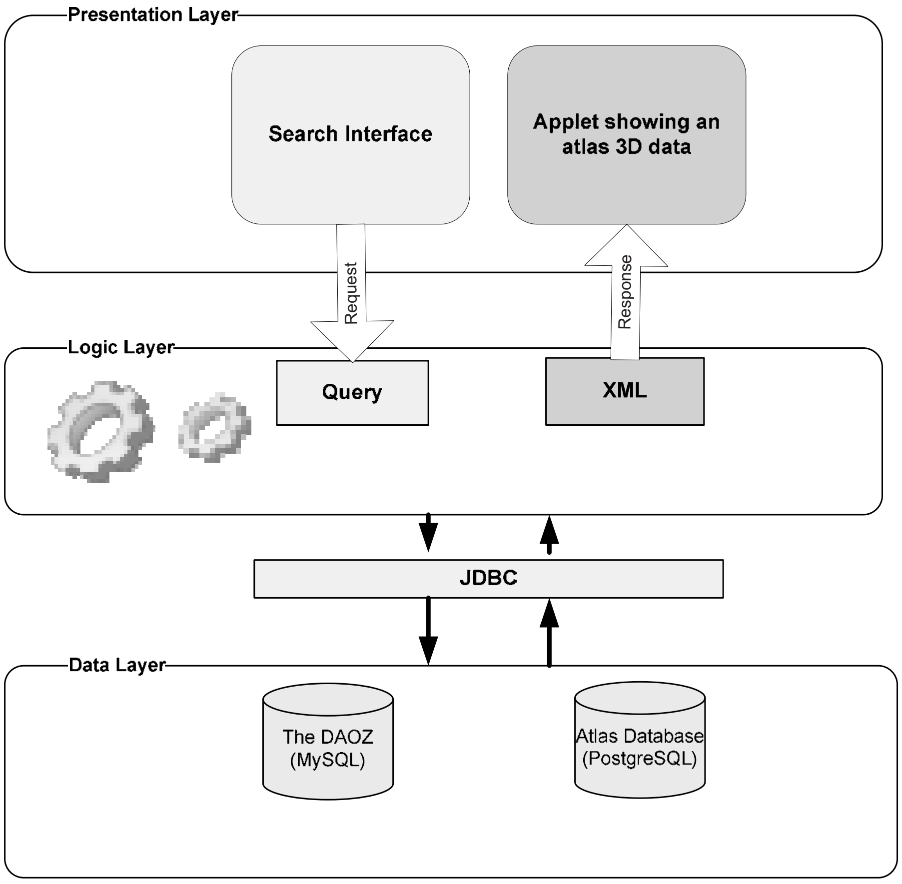
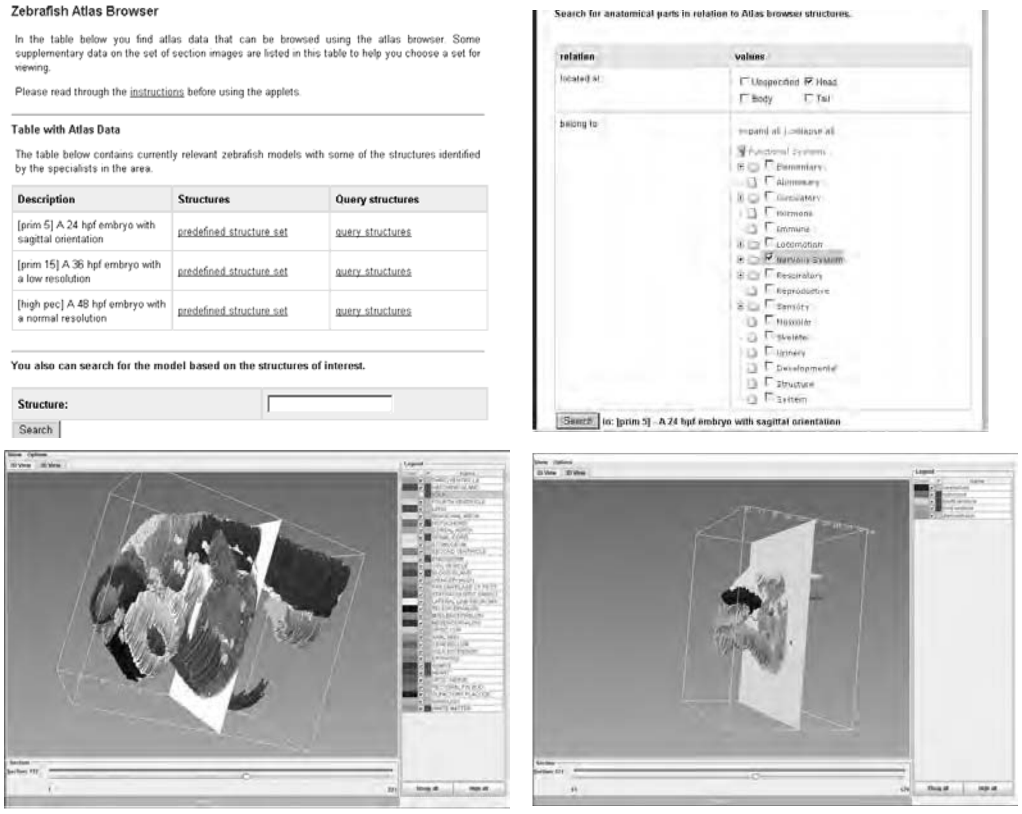


Figure 2: The user-server architecture of the web application: ZFAtlasBrowser.

Through a search interface, a search query could be performed. A query is composed using conceptual paths to the annotated anatomical domains, e.g. "select in the 36 hpf embryo all anatomical domains that belong to the nervous system and located in head". This query is executed against both the DAOZ and the Atlas databases. A list of anatomical structures is returned. This list is further used by the services layer to generate an XML file with all required elements as entries to the images set and their textual and graphical annotations. This XML file is passed to the java applet application. Through the applet, a volume rendering of the retrieved anatomical domains is prompted to the user (cf. Figure 3). The user is able to navigate through the data in 2D or 3D.

We have generated two versions of 3D models: at normal and high resolutions. Users can choose to query and browse the normal resolution models before selecting a higher resolution copy to examine in more detail. In this way, the volume data can be easily browsed in an intuitive manner on the wide range of operating systems and internet connections used by researchers. The ZFAtlasServer includes the following features:

1. Display a whole 3D model
2. Search in a 3D model for specific anatomical structures
3. Display the search results
4. Display 2D section images
5. Display contours present in each section image
6. List of anatomical structures nomenclature
7. Graphical legend of the anatomical structures
8. Tabs for 2D and 3D visualizations
9. Select/deselect anatomical structures options
10. Features to navigate, zoom in/out and to manipulate the images opacities.



Zebrafish Atlas Browser

In the table below you find atlas data that can be browsed using the atlas browser. Some supplementary data on the set of section images are listed in this table to help you choose a set for viewing.

Please read through the instructions before using the applets.

Table with Atlas Data

The table below contains currently relevant zebrafish models with some of the structures identified by the specialists in the area.

Description	Structures	Query structures
[prim 5] A 24 hpf embryo with sagittal orientation	predefined structure set	query structures
[prim 15] A 36 hpf embryo with a low resolution	predefined structure set	query structures
[high pec] A 48 hpf embryo with a normal resolution	predefined structure set	query structures

You also can search for the model based on the structures of interest.

Structure:	

Search

Search for anatomical parts in relation to Atlas browser structures.

relation	values
located at:	☐ Unspecified ☑ Head ☐ Body ☐ Tail
belong to:	expand all | collapse all Functional Systems ☐ Elementary ☐ Alimentary ☐ Circulatory ☐ Hormone ☐ Immune ☐ Locomotion ☑ Nervous System ☐ Respiratory ☐ Reproductive ☐ Sensory ☐ Muscular ☐ Skeletal ☐ Urinary ☐ Developmental ☐ Structure ☐ System

Search **in: [prim 5] - A 24 hpf embryo with sagittal orientation**

Figure 3: (Left, above) the first screen where users could directly start the java applet to view a complete 3D model. (Right, above) The query interface is where users are able to compose a search query using entities from the DAOZ ontology. (Left, below) A whole 3D model of a 36 hpf embryo. (Right, below) A 3D sub-model (the central nervous system in the head region) of the 36 hpf embryo; it is the result of the following query: "Select all structures present in the 36 hpf embryo, belong to the central nervous system and located at in head".

The ZFAtlasServer supports other geometrical representations. We developed another application to display surface models. The TDR-3Dbase uses contour information to generate 3D object models. For the object models, a triangulation algorithm is used, i.e. Boissonnat algorithm (Boissonnat, 1984; Verbeek et al, 1993) that enables acquisition of triangular surface tiles between consecutive contours in section images. The triangulation operation is used to create visually attractive 3D surface models (Verbeek and Huijsmans, 1998). Triangulated objects are created for each anatomical domain. Entities are added into the database to describe each anatomical domain with its surface model information (cf. Figure 4). To enable surface models visualization, the Atlas web-application has been extended with the object viewer tool. This extension is needed to offer an advanced form of data visualization through the internet.

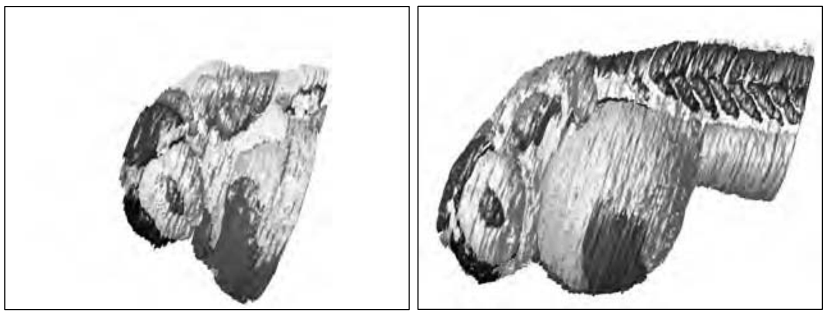


Figure 4: (Left) 3D surface model of the hear region of a 36 hpf embryo. (Right) The 3D surface model of a whole 48 hpf embryo.

3.6 Results and Discussion

We collected numerous samples and we made the best ones available online: at 24, 36, 48 and 72 hpf. These samples (or 3D models) represent important embryonic development stages of the zebrafish. We presented here the technology that we adopted to generate the 3D Atlas models. The models are the result of 3D reconstruction from physical serial sections. This method allows detailed cells observation and is therefore still preferred over other non-destructive methods such as OPT and MRI. The overall advantage of physical sections is that one can observe cells at different resolutions and study the cells in the context of the histological tissues and textures in the object. Each embryo, i.e. 3D model generated with this powerful method could be used as a standard by the scientific community.

Section images of a 3D model are annotated both textually and graphically. Textual annotation helps to understand what is in the image while it does not help to indicate where in the image a certain concept is observed. Words help to trace images, not domains in the images. Sometimes the annotation is very obvious; in other cases a graphic clue is added to help to locate a concept in an image. The simplest form of a graphic aid is to add an arrow or a line pointing to an area in which a concept is observed. This is not unambiguous in all cases. It can be easily implemented by using Scalable Vector Graphics (SVG; http://www.w3.org/Graphics/SVG/) which provides XML structures to do this. A more precise way of using a graphic aid is to apply a true graphical annotation by indicating in an image a domain, i.e. a group of pixels with similar characteristic. In our case, we segmented each section image by drawing closed contours around anatomical structures sharing similar anatomical functionality. Coordinates of each contour element (or graphical annotation) are saved as part of the annotation in a contour file.

The graphical annotation is completed by assigning to each anatomical domain an anatomical nomenclature. We used anatomical concepts from our formal ontology, i.e. DAOZ. These concepts are organized at different levels of representation. Therefore, a gross level granularity could be used to access anatomical information at a finer level of granularity. Annotated anatomical domains could also be accessed at different levels of granularity and grouped in units according to users' queries. Therefore, from one sample embryo in the database, multiple instances could be generated on the fly according to users search desires. Additionally, viewing an instance instead of the whole embryo reduces enormously the load time since we have less data transfer.

The DAOZ anatomical structures nomenclature is the same as this known and used inside the zebrafish research community. DAOZ concepts enable the 3D Atlas data to be integrated with other resources such as ZFIN; objects in the Atlas could be integrated with others resources which enable our data to be presented into a large integrated research network. Anatomical domains are annotated with continuous entities such as functional systems and stages of development which means that they preserve their identity over time even when lower level anatomical nomenclature are changed. This feature facilitates Atlas data access and integration to other model systems.

Annotated models are organized in an object oriented database, i.e. the Atlas database. This database includes information of each 3D model. This information is related to a model anatomical structures nomenclatures, graphical annotation as well as pointers to their set of section images. A query interface has been setup to access objects in this database. From this interface, straightforward queries could be performed using ontological characteristics of the anatomical structures. Users are not required to have a deep knowledge of the anatomy to interact with the Atlas. Therefore, we trust that a wide range of users can made use of our 3D Atlas.

When a search query is sent to the database an XML file of the query model is generated containing query results. The XML file is used as an input for the applet application. This application enables models visualization and manipulation on the fly. In addition, a model could be freely manipulated, such as structures could be isolated to be studied individually or in the context of their neighbors.

The zebrafish 3D Atlas provides a novel and valuable tool for researchers studying zebrafish embryonic development and can be applied to a range of research areas, including the identification of abnormal anatomical patterning in transgenic lines. In our case, the 3D Atlas of zebrafish already serves as a model for 3D gene expression information submission and retrieval (Belmamoune et al, 2006, 2008). The techniques we have developed and employed to acquire, manage and present the data have been successfully applicable to many other model systems and anatomical structures (De Jong et al, 2005; Welten et al, 2005 and Bertens et al, in preparation).

3.7 Future work

The Atlas presents a novel, accessible, intuitive approach for studying zebrafish anatomy that will facilitate analysis of embryo morphology. We also expect that it will be an excellent reference tool for a broad range of the scientific community and be more useful as an educational tool. By propagating the Atlas use in the community, we will get feedback from users about any inconsistencies in data annotation and how features of the datasets and software can be improved. Work has been realized to improve mapping the Atlas models to our 3D spatio-temporal patterns of gene expression (cf. chapter 5). Moreover, mapping the zebrafish Atlas to Atlases such as the Edinburgh Mouse Atlas Project (http://genex.hgu.mrc.ac.uk) and the Edinburgh Mouse Gene Expression Atlas (http://genex.hgu.mrc.ac.uk) is under consideration. As more data are added to the Atlas database we will continue to improve our tools to access this data set to disseminate the use of this valuable 3D resource.