Pipeline for the Creation of Surface-based Averaged Brain Atlases

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ABSTRACT

Digital atlases of the brain serve as a spatial reference frame which can be used to relate data from different image modalities and experiments. In this paper we describe a standardized pipeline for the creation of extendable surface-based anatomical insect brain atlases from 3D image data of a population of individuals. The pipeline consists of the major steps imaging and preprocessing, segmentation, averaging, surface reconstruction, and surface simplification. At first, 3D image data sets from confocal microscopy are resized, stitched, and initially displayed using standard image processing and visualization tools. Then brain structures, such as neuropils and neurons, are labeled by means of manual segmentation and line extraction algorithms. The averaging step comprises affine and elastic registration and a mean shape selection strategy. Finally non-manifold surfaces of the labeled and aligned structures are reconstructed using a generalized surface reconstruction algorithm. These surfaces are simplified and adapted to further needs by decimation and retriangulation. The chosen methods of each step are adequate for a variety of data. We propose an iterative application of the pipeline in order to build the atlas in a hierarchical fashion, integrating successively more levels of detail. The approach is applied in several different neurobiological research fields.

Keywords: Digital neuroanatomy, brain atlas, surface reconstruction.

1. INTRODUCTION

One specific aim of neuroscience is to analyze learning and behavior. Understanding such neural functions is based on knowledge about the wiring of the brain and functional properties of individual neurons as well as parts of the nervous system. Therefore anatomical and functional properties of neural networks are investigated.

Particularly suited for such analysis are insect brains, since they are easy to access and brain substructures as well as important single neurons are already known and identified. Furthermore, due to the small size of the structures one is able to examine them as a whole down to a level of single neurons. Much knowledge is extracted by comparing anatomical structures and their functions of individuals at several development stages or from individuals that equal or differ genetically.

In the last decade 3D imaging methods, like confocal microscopy or transmission electron tomography, have become standard together with sophisticated staining methods. Nowadays they provide large amounts of data that has to be organized, analyzed and explored. Almost a revolution from the biological point of view are population-averaged standard atlases that for the first time in biology provide spatial references. These allow the researchers to relate data from different experiments and different data modalities as well as to accumulate information with spatial reference. Furthermore they facilitate visualization of neurobiological data and form a basis for investigating the relation between brain morphology, brain function, and genetic factors. In the long term this will help to turn neuroanatomy into a more quantitative science.

We describe a generalized pipeline for the generation of insect brain atlases from 3D image data of a population of individual brains. We consider surface-based atlases, since these are easier to deal with than volumetric atlases. The pipeline roughly comprises imaging, segmentation, averaging, and surface reconstruction. All methods required within

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the pipeline were integrated in the open visualization platform Amira [SWH04].

This paper is organized as follows: In section 2 we give an overview about existing brain atlases. Section 3 describes the concept and the steps of the atlas generation pipeline. Applications of the procedure and the resulting atlas are presented in Section 4. We discuss the limits of our approach and conclude in Section 5.

2. RELATED WORK

For the human brain a variety of digital population-based atlases have been introduced and continuously enhanced. Techniques for the generation of such brain atlases, for instance, are described in [GMT00] and [TT01]. They focus on the development of registration and averaging strategies using cerebral CT and MRT scans. A processing environment that enables researchers to combine and execute established methods is presented in [RMT03]. It has been used successfully to build average intensity atlases with fully automated processing. The general steps for atlas generation of human brains are similar to the ones for the generation of insect brain atlases. Nevertheless imaging modalities and application dependent objectives require the development of more specific and appropriate techniques.

Meanwhile invertebrate brain atlases also do emerge. In [RZM*02] and [RBMM01] the generation of atlases for the fruitfly *Drosophila melanogaster* and the honeybee *Apis mellifera* are described. Besides generation methods supplemental information on neurobiological backgrounds and application fields of insect brain atlases can be found in [BRR*05] and [PH04]. Web interfaces (http://www.neurofly.de; http://www.neurobiologie.fu-berlin.de/BeeBrain) allow downloading and interactive viewing of currently existing models. The analysis of insect brains is still an explorative research field. So far little attention has been paid to the integration and sequencing of all steps needed for a complete atlas generation into one single visualization platform. The insertion of a taxonomic description of brain structures and the usage of advanced visualization and interaction techniques also remain challenging tasks.

3. ATLAS GENERATION PIPELINE

The proposed pipeline for the generation of brain atlases is composed as follows: First the image data is preprocessed and initially visualized. In a second step the structures of interest are segmented and a label is assigned to each of them. The averaging takes place in step three. Therefor all labeled data sets have to be registered into a common reference and a mean or median has to be derived. Finally three-dimensional surface models of the averaged brain structures are reconstructed. Figure 1 shows a schematic overview of the proposed pipeline.

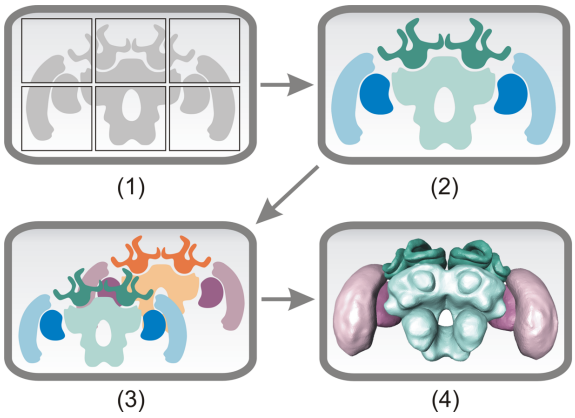

Figure 1. Schematic overview of the pipeline for the generation of surface-based insect brain atlases. After imaging and preprocessing (1), structures are segmented (2). The averaging of individuals (3) is followed by a surface reconstruction(4).

The underlying methods of each step have to fulfill several requirements. They must be applicable to the present imaging data and provide robust results. The techniques shall allow for modifications of final and intermediate data. For example the insertion and deletion of structures shall be supported. In addition the methods have to consider the user's skills and wishes. Finally the received results shall enable further processing.

We propose an iterative application of the pipeline. First coarse structures like neuropils are reconstructed and an initial atlas is generated. Using the same work steps, more detailed information such as substructures of neuropils or neurons is integrated. Thereby the initial standard serves as a reference frame for the registration procedure and the final visualization. This approach allows for a hierarchical organization of data, which in turn establishes a basis for the representation of the natural hierarchical organization of brain structures.

In the following we will describe the steps of the pipeline by means of brain data of the honeybee. Section 4 shows that the pipeline and its results are much more generally applicable.

Imaging and Preprocessing

Presently insect brains such as the fly brain or the honeybee brain are imaged with confocal laser scanning microscopy. After preparation including dissection and staining, the size of a honeybee brain (~$2.5 \times 1.6 \times 0.8\,mm$) extends the maximum field of view of the microscope with a common configuration. Therefore multiple stacks are used to acquire entire brains. Typically 2×3 partially overlapping tiles, each using

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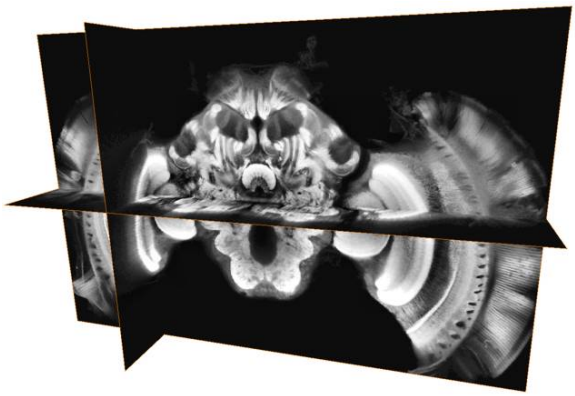

Figure 2. Slice view of a honeybee brain confocal microscopy image in xy-, yz-, and xz-plane. The data set is composed of six separately acquired image stacks.

between 80 and 120 slices of 512×512 pixels in plane and a thickness of $8\,\mu m$ are imaged.

For the combination of the image stacks landmarks that define corresponding points are placed manually. Afterward the stacks are merged using a conventional pyramidal blending. The landmarks are used to define the regions that will be blended. The scanning technique usually causes a shortening of distance in the z-direction. This is compensated by applying a linear scaling to the data [BRR*05].

By means of slicing or direct volume rendering or the combination of the two a first insight into the edited data is gained and the image and preprocessing quality can be checked. A slice view of a complete bee brain data set can be found in Figure 2.

Segmentation

In the next step anatomically distinct structures of the brain are segmented and assigned unique labels. The results are stored in so-called label fields in which each voxel represents a coding for a particular brain structure. These label fields will be used in the following averaging step and form the basis of the surface reconstruction. For several insects a coarse division and nomenclature of the brain into neuropils already has been defined and can be used as a guideline for the segmentation. In the honeybee brain we currently distinguish 22 major compartments.

The development of fully automatic segmentation methods is impeded by several difficulties. For instance, the borders of neuropils are often fuzzy, thus lacking strong gray value gradients. Gray values of structures highly depend on the chosen staining. Additionally they vary within the regions and between different individuals. Furthermore research in neurobiology still remains explorative aiming at discovering new structures and correlations. So frequently there is little a priori information and results are strongly influenced by the user's knowledge and experience.

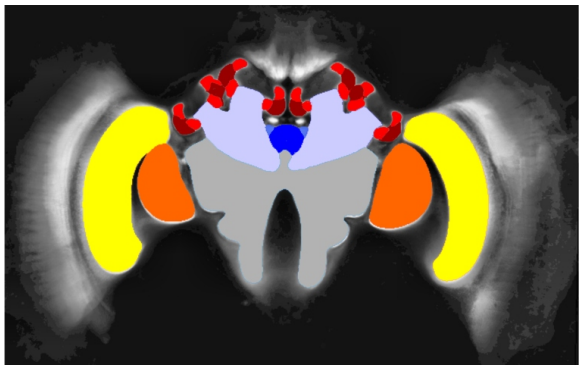

Figure 3. Segmentation of neuropils in the honeybee brain. Anatomical structures of interest were segmented and assigned individual labels. Here they are overlaid their corresponding gray value data set.

For the segmentation of neuropils we provide simple but robust, and user-friendly tools, which support manual segmentation. The user can choose from freehand boundary drawing, interval thresholding, and propagating contours based on a level set method [WnZ03]. For the latter a small circle is placed on mouse click and then the contour of the circle is extended as the user moves the mouse. All tools require the adjustment of only few parameters and allow for an interactive correction of the segmentation results. Their efficiency is improved by the integration of interpolation and extrapolation of label segmentation between slices. At any time structures can be added, removed or refined easily within the provided editor.

Figure 3 shows an example segmentation of honeybee brain neuropils.

Averaging

From the preprocessed and labeled data sets of individuals an atlas representing the brain characteristics of the analyzed species has to be derived. For this purpose an average of the sample is determined. This is usually done by computing the mean or median. In general the procedure comprises a registration of the data sets to a common reference and a subsequent averaging step.

We use an iterative averaging scheme which is based on the work presented in [RBMM01]. First global differences in position, orientation, and size of all data sets are compensated and an initial average image is generated. Using a nonrigid registration the data sets are then registered to the initial reference. This leads to a new less blurred average image to which the transformed images are registered again, and so forth.

Affine registration. The procedure starts with an affine registration of the individual images to an arbitrarily chosen template. Usually a subjective choice of a data set such as the original individual brain which appears most average to the user works as an adequate

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first reference. In order to allow for standardization we select the individual brain which has the smallest deviation from the mean volume over all components in the reconstruction as it has been used in [RZM*02]. Our affine registration implementation is based on the multiresolution search method presented in [SHH97].

Label and intensity averaging. Having registered all original individual images, a mean or median can be determined from them. Here the computation of the voxelwise arithmetic average of data values forms the most intuitive and general approach. For label fields, which are non-numerical images, a non-arithmetic average is needed. We do use a method that has been proposed in [BRR*05]. It selects the label that occurs most frequently and represents at least t percent of the valid voxels. A result voxel is termed 'undecided' if there is no unique most frequent label.

Elastic registration. Due to the remaining local shape varieties an average image solely based on a global affine registration might be inaccurate resulting in blurred average intensities and average labels with a large quantity of undecided voxels. Application of elastic registration methods helps to reduce this inaccuracy.

Our pipeline contains a non rigid registration method described in [RZM*02]. The algorithm computes an individual rigid transformation for each labeled brain structure. These transformations are relatively small due to the prior global alignment. For each voxel within a segmented substructure the transformation vector is calculated based on the per-structure alignment. Afterward the piece-wise defined vector field between the substructures is component-wise interpolated. This is done by using a heat transfer equation: at locations where the vectors are known the 'temperature', which means the component of the transformation vector, is kept fixed and in-between the resulting equilibrium state is computed.

We also offer a multiresolution and multigrid cubic B-spline registration based on the developments published in [RBMM01] and [RSH*99]. This approach pays more attention to fine details than the above outlined method.

In general, elastic registration is a delicate step, since the transformation might eliminate not only preparation artifacts like mechanical deformations, but also anatomical variability. If one is interested in this, elastic registration should be employed particularly conservatively.

Convergence. For some applications an average resulting from the affine registration step may be sufficient. It preserves possibly relevant shape differences and requires a comparatively lower computation time. However, to minimize the number of undecided voxels and to avoid disappearance of small structures the application of subsequent non-rigid registration often is necessary. Here the fraction of undecided voxels in the average label images and a visual observation coupled with an entropy analysis define the choice of the algorithm and the termination of the averaging procedure. In [RBMM01] a maximum iteration number of four has been proposed. No significant decrease in entropy and the number of undecided voxels could be observed in subsequent registration steps.

Surface generation

In this step surfaces are created that separate regions with different labels. If more than two differently labelled regions meet in space, complex topological situations may arise and the resulting separating surface in general is not a manifold. In order to generate such non-manifold surfaces from label fields, we utilize a generalized surface reconstruction algorithm [HSSZ97]. It produces interfaces between all neighboring voxels of different type, nicely stitched together. Like standard marching-cubes, it works by processing each hexahedral cell (dual to the voxel grid) individually and utilizing tables that contain triangulations for different equivalence classes. An equivalence class is a set of cells with labeled corners, where the cells can be transformed into each other by discrete rotations, reflections, and/or label permutations. While in the marching-cubes case $1 + 13$ such topologically distinct classes exist, for more than 2 labels on a cell more classes may exist. The number of equivalence classes can be computed by group theoretical means [BLS05, Heg98]: employing e.g. all before mentioned symmetry groups simultaneously, for 1 to 8 different labels on the eight corners of a cell there are 1, 13, 44, 66, 43, 15, 3, 1 different classes. Employing less symmetry groups, these numbers are significantly higher. Practically we use pre-computed tables only for configurations with up to three different labels. More complex cases occur rarely and the corresponding triangulations are computed on-the-fly. Figure 4(a) depicts the outer average surfaces of the major substructures of the honeybee brain. For alternative algorithms see [BDS*03] and [BRvL*05].

The number of triangles resulting from the surface generation algorithm typically has to be reduced. Since down-sampling the labeled input data may result in loss of tiny but relevant structures, we generate the surface in high resolution and simplify it afterwards, using the method presented in [GH97]. Figures 4(b) and 4(c) show detailed views after reducing the number of triangles to $20\,\%$ and $5\,\%$, respectively, of the original number of triangles. The simplified surface finally is remeshed in Figure 4(d), using

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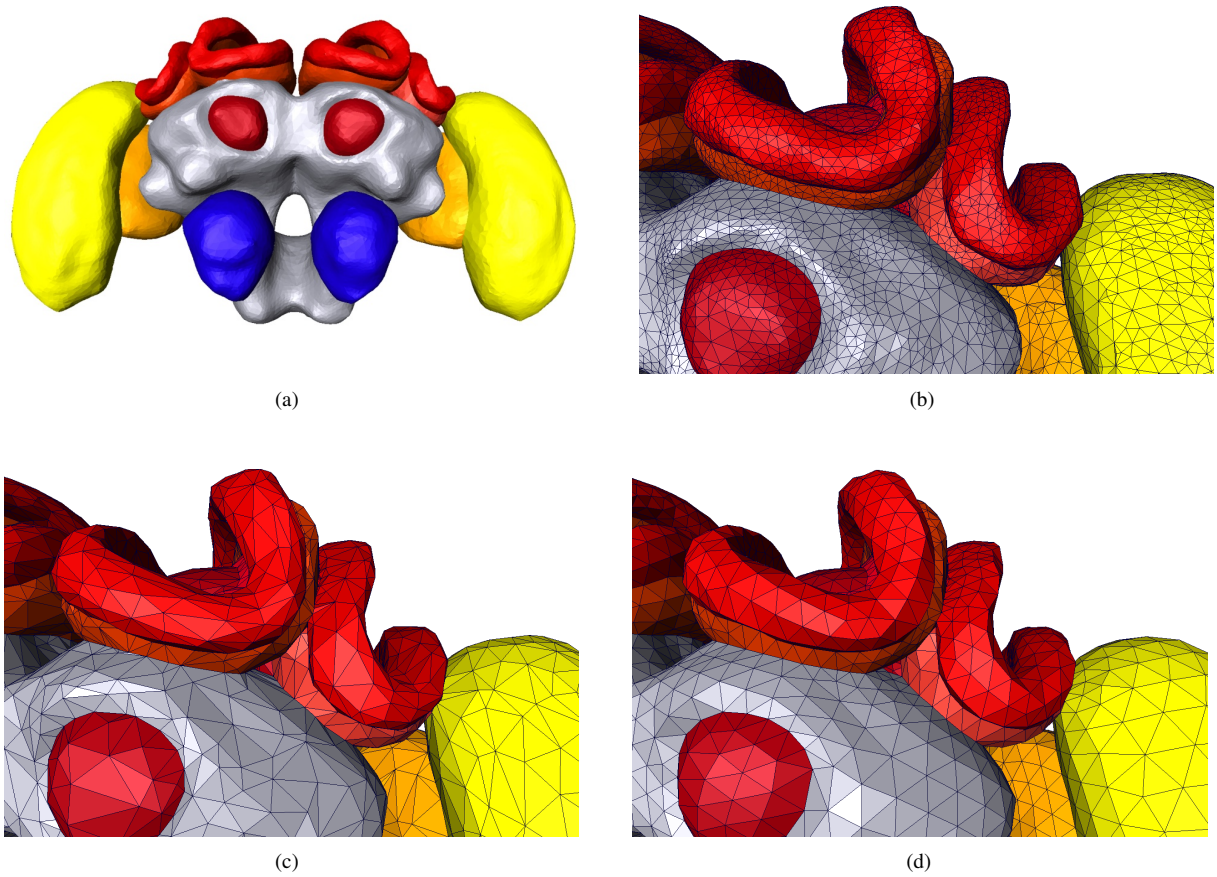

Figure 4. Generation of surfaces separating different biological structures. (a) outer average surfaces of the major substructures of the honeybee brain, (b) after reducing the number of triangles to $20\,\%$, (c) after reducing the number of triangles to $5\,\%$, and (d) after remeshing the simplified surface.

an implementation based on algorithms proposed in [SG03].

A surface editor enables the modification of the resulting surface. It provides the subdivision, collapse, removement or translation of selected triangles. Additional patches can be defined. Structures can be selected and their associated triangles can be saved as individual surfaces. These tools are mainly used for final visualization purpose. They also support further processing such as the insertion of new structures and the subdivision of existing ones.

Data integration

The resulting atlas represents a group of structures which can be regarded as a certain level of a hierarchy. On the next level substructures as for example single neurons or the glomeruli are added. The integration of additional data follows the above process. Again preprocessing, segmentation, averaging and surface reconstruction are needed in order to insert more detail into the existing atlas. We exemplary describe the approach for the integration of a projection neuron. See Figure 5 for the visualization of some work steps.

Image acquisition and preprocessing. Projection neurons (PN) are stained and imaged using confocal microscopy. Typically 2×2 tiled stacks of 350 sections each with 1024×1024 voxels are scanned. The integration of the PN also requires an acquisition of its surrounding neuropil regions which are needed for the registration. Therefore a generic counterstaining is applied and imaging is done according to the above protocol.

To avoid loss of detail usually neither resampling nor merging of the PN image stacks takes place. In contrast aiming at lower computation times in the registration step the size of the neuropil data is reduced and the stacks are composed into one data set. Neuron images usually contain only parts of the brain and subsequent steps of the pipeline have to be restricted to these parts. For this purpose several regions in the label image of the atlas have to be removed and the volume has to be cropped.

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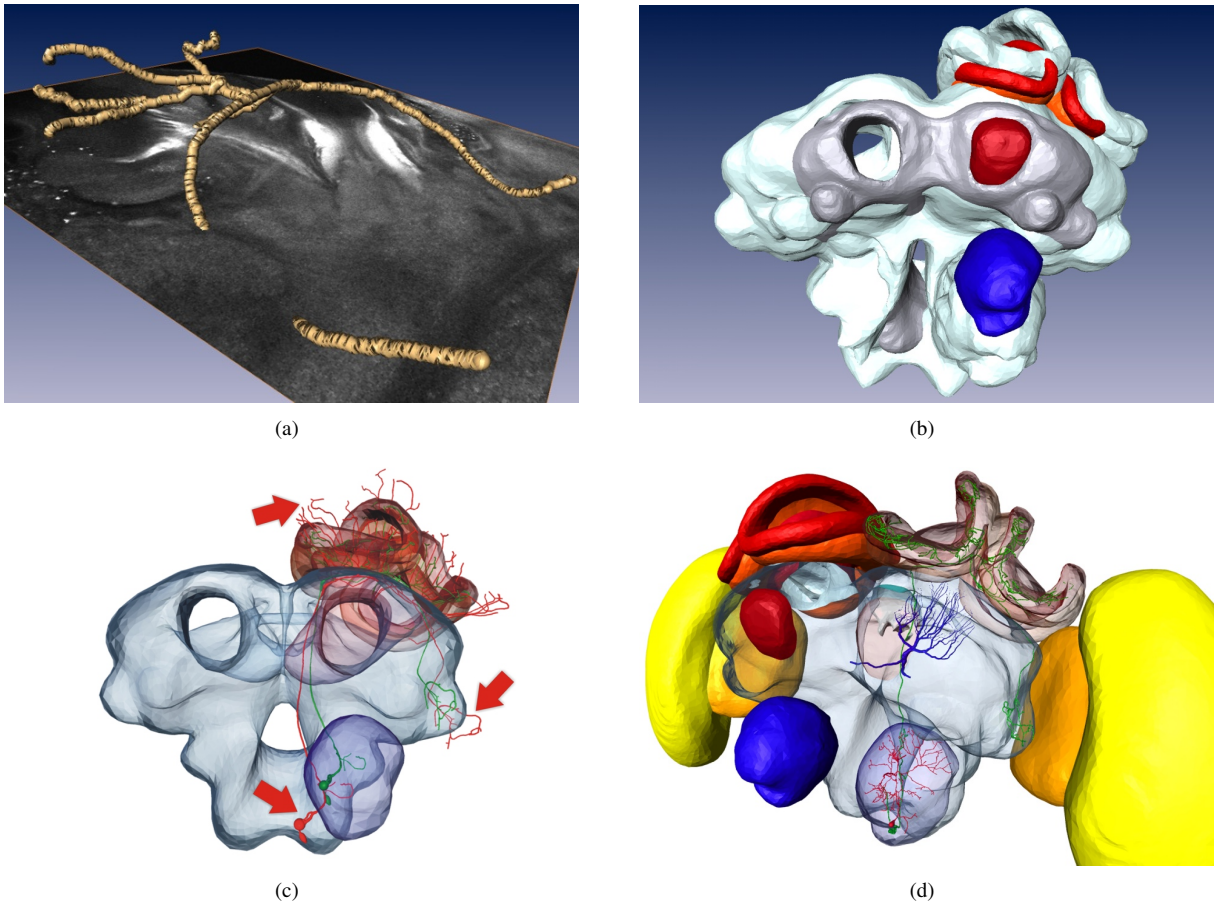

Figure 5. Neuron integration. (a) Segmentation of a neuronal part in one of the four commonly acquired image stacks. (b) Combined display of surface reconstructions of neuropils surrounding the considered neuron. The individual (light) and the standard neuropils (dark) differ in size and orientation. (c) After affine registration of the individual to the standard neuropils, the neuron (arrows) sticks out the atlas. Nonrigid registration can compensate for this inaccuracy. (d) View of a complete bee brain with a selection of integrated neurons

Neuron segmentation. The neuropils belonging to the considered neuron are segmented as described in Section Segmentation. For the segmentation of the neuron itself more specific methods are needed. Due to staining intensity variations, the existence of fine structures close to the resolution limit, and a partly insufficient resolution in z-direction fully automatic segmentation has not been achieved yet. In our pipeline promising results can be obtained by means of line extraction algorithms [SED*04, WnZ03]. Thereby the user manually sets branching or end points thus roughly tracing the graph like structures of the neuron. The centerline and thickness of the neuronal segment between these points are then detected automatically for example by minimizing an energy functional.

Registration of surrounding neuropils. For the integration of the neuron into the atlas the individual neuropils and the corresponding neuropils of the standard are registered using the same methods as for computing the standard. Afterward the resulting transformation is applied to the neuron data.

Surface reconstruction. Using a thickness-annotated graph, which represents the neuron, a surface reconstruction can be easily obtained. Our pipeline contains an implementation of the method described in [WnZ03]. Here the neuronal surface is initially described as a union of a set of spheres and cylinders. Applying an implicit surface algorithm, consistent and closed surfaces are generated that can be used for volume or surface rendering.

Computational performance

The computation time for the generation of an atlas highly depends on the number of individual data sets and the chosen registration strategy. An averaged atlas composed of ten individual brains can be created within one week. The following measurements apply to a single data set of size $650 \times 400 \times 120$. Alignment and merging of the stacks took about two min-

utes. Manual segmentation of the neuropils required between two and six hours depending on the number of neuropils and the user's knowledge. Affine registration needed one hour. Non-rigid registration according to [RZM*02] required one hour whereas the method presented in [RBMM01] used up to 12 hours. Label voting and intensity averaging required about two minutes. The surface was generated within 38 seconds and produced $1.2 \cdot 10^6$. triangles. The simplification down to $2 \cdot 10^4$ triangles required 168 seconds and the final remeshing took about 3.5 minutes. The computation times for the steps of the integration of neuron data are similar. Speed-up can be observed during registrations. This results from the reduction of the data to only concerning neuropils. All techniques were tested on a current PC (3.0GHz Intel Pentium 4 processor).

4. APPLICATIONS

Using the proposed pipeline, a standard atlas of the honeybee brain has been generated [BRR*05]. This standard initially composed of 22 neuropils, is currently extended and refined. In [KMK*04] progress has been made in the investigation of the temporal dynamics of olfactory coding, and the neuronal wiring within different integration areas of the olfactory path. Here experimental data were acquired by stimulating the antennae with a range of odors and recording the response from single projection neurons. Individual neurons and accordant neuropils were reconstructed and collected into the framework of the standard atlas of the honeybee brain. Thus morphological as well as physiological properties of brain structures have been integrated. This work directly supports the analysis of neuronal circuits and explores the role of single neurons within the network.

The standardized atlas of the fly brain (see [RZM*02] for details) is upgraded by an average model of the *thoracic-abdominal nervous system*, which contains deeply analyzed insect motor pattern generators and most of the insect individually identified neurons. Multiple stained whole-mount preparations of the thoracic ganglia are averaged (see Figure 6 for first results). Afterward morphological details such as projections of legs, wings and halteres are added to the standard. On the next level neuropil structures are subdivided based on functional issues and finally groups of identified neurons are integrated into the atlas.

Parts of the pipeline have also been used for the generation of a standard atlas out of ten individual locust (*Schistocerca gregaria*) brains. Here neuropils belonging to the first hierarchy of the brain structure ontology have been averaged. The atlas is intended to form a spatial reference for the analysis of the central complex, its substructures, and its nervous system down to single neurons [KSH05].

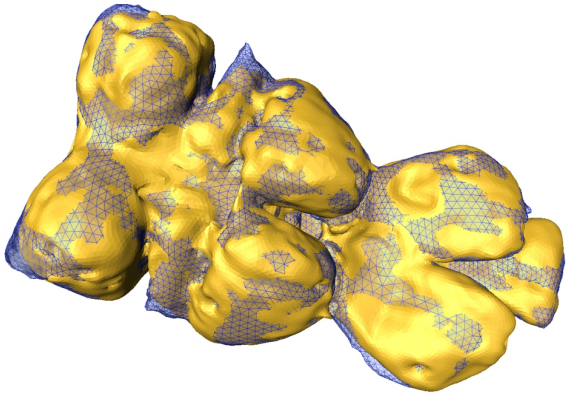

Figure 6. Atlas of fly thoracic ganglion. Shown are the average surface reconstruction of three individual fly thoracic ganglions and an overlaid individual surface reconstruction (grid)

The most time-consuming step in geometry reconstruction from image data is image segmentation. Using atlases, fully automatic segmentation of image data from an unknown subject can be performed. In [RBMMJ04] confocal microscopy images of 20 individual bee brains have been segmented by nonrigid registration to an atlas. The results were compared to a manually generated gold standard and showed a very high accuracy for most of the considered structures.

5. CONCLUSION AND FUTURE WORK

We presented a standardized pipeline for the creation of population-averaged atlases from 3D image data. The atlases are surface-based and extendable. Our pipeline consists of a sequence of steps, of which some require fine-tuned and sophisticated image and geometry processing algorithms. The proposed pipeline has been used to create a honeybee 'standard brain' and it is applied in a variety of other biological fields now.

Atlases resulting from the proposed procedure serve as spatial references, into which data from other experiments can be integrated – while maintaining their spatial context. Thus a natural organization of information is achieved that is well suited for visually supported analysis. Utilization of such reference models, augmented with empirical data of diverse provenance, greatly facilitates many scientific investigations. Examples in neurobiology are investigations of relations between brain morphology, brain function, and genetic factors.

In the future we will also associate ontological information with the spatially referenced substructures. This will provide not only a visual representation of the natural ontology of biological structures, but also will enable new navigation techniques.

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