Chapter 8

Applications

In this chapter we will illustrate the relationship between the different algorithms and techniques we have described throughout this work by briefly sketching some biological applications.

8.1 Anatomical Insect Brain Models

Using our methods, together with the histology described in section 2.2, it became possible for the first time to create high-resolution accurate models of insect brains. This has been done in cooperation with the research groups of Prof. Heisenberg (Univ. Würzburg) and Prof. Menzel (FU Berlin). Some of the results are shown in Figure 8.1. Already the ability to interactively render and investigate the three dimensional data sets in almost real time has been a big step forward for the researchers. With the segmentation it became possible to accurately measure volumes for quantification of mutants or defects. In structures like the optic lobes, interpolation between successive parallel slices has proven to be an efficient and accurate tool. The polygonal models, that could be created from the segmentations allowed for studying the shape of anatomical structures even on smaller computers, e.g. when seeking structural mutants. Such models can easily be exchanged among colleagues and they serve as frame for integration of functional data as we detail in the following sections.

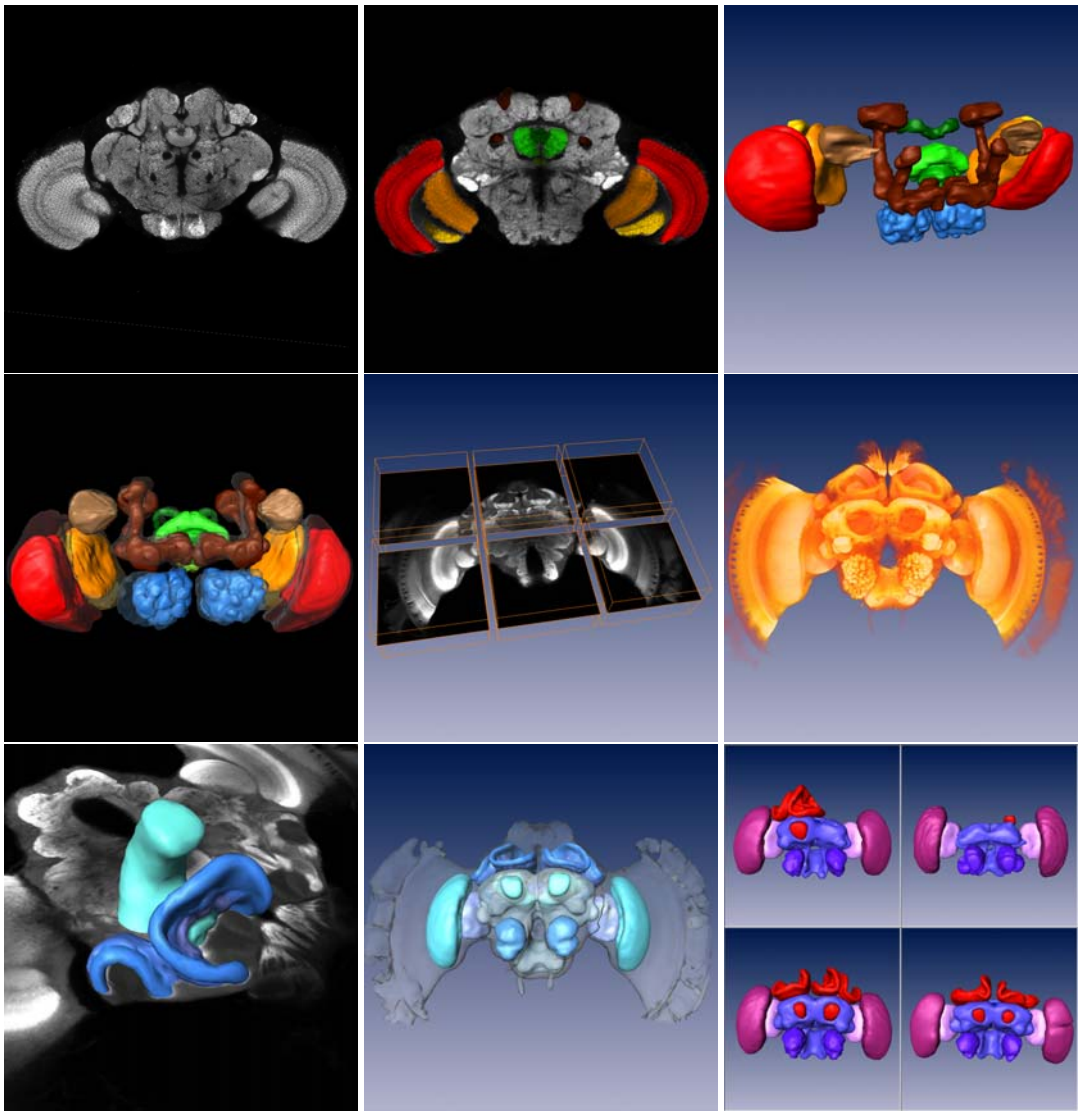

Figure 8.1: Digital insect brain models: The images show (from top left), (ii) Direct Volume Rendering of a confocal recording of the Drosophila brain, for a true three dimensional visualization of the image data. (iii) Labeling of some structures overlayed with the image data. Labeling is a prerequisite for quantification and geometry reconstruction. (iv) Surface models of a template brain model and a ROL-mutant (with Reduced Optic Lobes). (v) Confocal recording of bee brain; due to the limited field of view with high resolution objectives the specimen had to be recorded in 6 bricks which are then aligned and re-assembled in the computer. (vi) The resulting data set can be volume rendered. (vii) Surfaces of labeled sub-structures can be combined with the image data or rendered as a full atlas model (viii). Such surface models can be used to study structural defects. In images (ix) a toxin was applied in a specific phase of larval development, impacting the mushroom bodies. The models can not only be used to detect these quite significant differences, but also to verify that other structures are not pathologic.

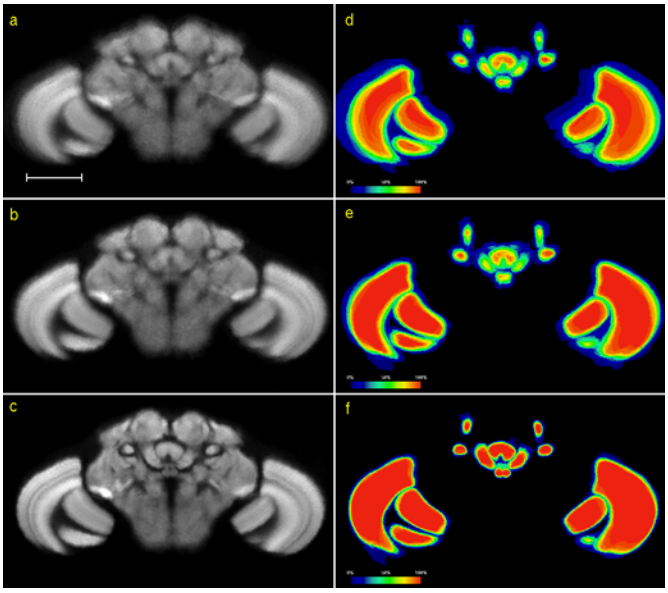

Figure 8.2: The image shows *average intensity maps* (left) and *probability maps* (right) for different alignment strategies. First row corresponds to rigid alignment, the second row is rigid alignment and global scaling, the third row is global scaling and per-structure rigid alignment combined with inter-structure interpolation. These maps can serve as a tool to detect regions of high variability or to estimate the accuracy of a specific interpolation scheme. For example one can assume the the variability (colored borders) in the second row could be due to relative positional deviation of the structures, while the variability in the third row is due to shape differences of corresponding structures.

8.2 Drosophila Standard Brain

In a joint work with K. Rein and M. Heisenberg from the Genetics department of University Würzburg we have used our methods for the generation of an anatomical atlas of the Drosophila brain. Unlike traditional anatomical atlases, the *Standard Brain* should be statistically secured. Here we summarize the important steps and results. For further details refer to [145, 150].

If data sets from only one individual are taken to create an atlas, one has no information about how representative the individual is. If deviations between the atlas and an individual are found it is not clear whether the atlas or the individual is pathologic. Or if only normal statistical variability is observed. This is a particular important aspect for Drosophila researchers, that deal a lot with structural mutants.

Therefore, about 30 male and 30 female flies, which have been raised under controlled and well defined conditions, were chosen. Their brains were dissected, stained, and recorded. From these representative templates were chosen using volumetric measures. After alignment to the template and labeling,*average intensity maps* and *probability maps* could be generated. Details are shown in Figure 8.2. The volumetric data was used to compare to different strains of Drosophila, namely CantonS, which has been kept in food vials for well over 1000 generations, and Lindelbach, which has been living in the lab since only 130 generations. Interesting findings were made, showing that brain structures believed to be important for in-flight navigation were reduced in size in the CantonS flies, while structures important for walking control were bigger. Volumetric results are

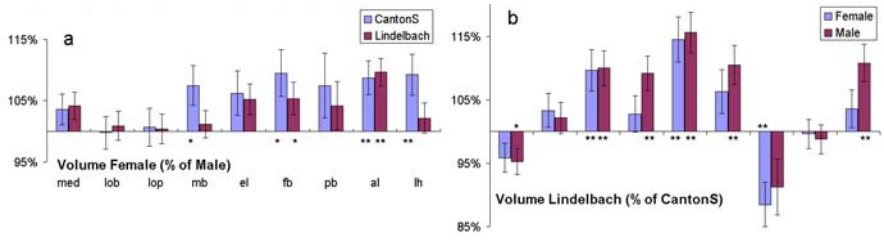

Figure 8.3: Volumetric comparison of four different groups of flies. An accurate volumetry requires an accurate segmentation. Significant (compare error bars) differences could be detected, which have interesting biological interpretations [145]

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depicted in Figure 8.3, more details are given in the paper [145].

8.3 Drosophila Gene Expression Atlas

Using specialized genetic techniques it is possible today, to visually detect the structures in which a specific gene is active for protein synthesis. Such gene expression lines are acquired from the Drosophila brain in many different laboratories around the world. A current problem is that these data sets are not directly comparable. A near future goal that we have pursued with the Drosophila Standard Brain was to establish a standardized reference frame for such functional data. In order to bring such data into the atlas, a registration is necessary. One way to achieve this is to record the neuropil simultaneously with gene expression using two different wave lengths. The neuropil can be labeled and used as an independent guide for registration. Figure 8.4 shows an example. Within the next year a first version of such a Drosophila gene expression atlas will be started with several hundred different lines under the lead of Prof. Heisenberg's group.

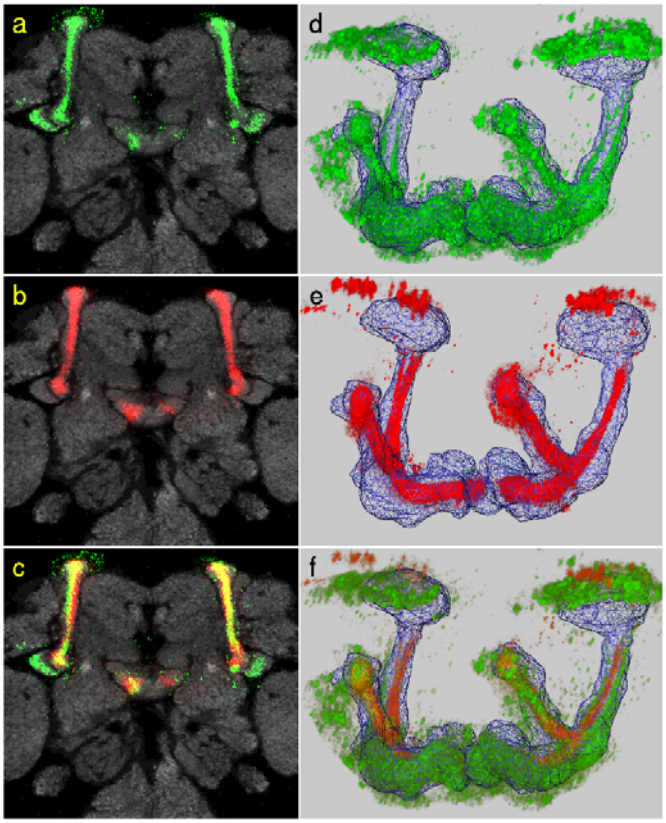

Figure 8.4: Two gene expression lines (green and red) in the mushroom body in two different animals overlayed on the simultaneously recorded neuropil. After per-structure rigid alignment of the mushroom bodies the expression patterns could be brought into a common reference frame, to study for example co-localization.

8.4 Honey Bee Antennal Lobe

The honey bee brain has two structures called antennal lobes, where the nerve signals from the antennas are processed. The bee smells with the antennas. Each of the antennal lobes consists of roughly 180 so called glomeruli. It is believed that each of the glomeruli is individually identifiable in every individual. The property that makes the glomeruli particularly interesting is the fact that a specific set of glomeruli shows neuronal activity when the bee perceives a specific odor [48]. Another odor will activate a different pattern of glomeruli. Using Ca^{2+}-imaging, it is possible to measure this activity with temporal and spatial resolution in the living animal. This way it is possible to see in the bee's brain how it smells, which is quite exciting. Two analyze and compare the excitation patterns, it is necessary to relate them to the individual glomeruli. In [35] all glomeruli of one individual were identified and given names. Producing the first labelings literally took months of colorizing the glomeruli in each slices in a painting program, and errors occurred.

With the new segmentation tools, especially the planar and non-planar interpolation methods, a segmentation can be achieved even by unexperienced users within a few hours. With the de-

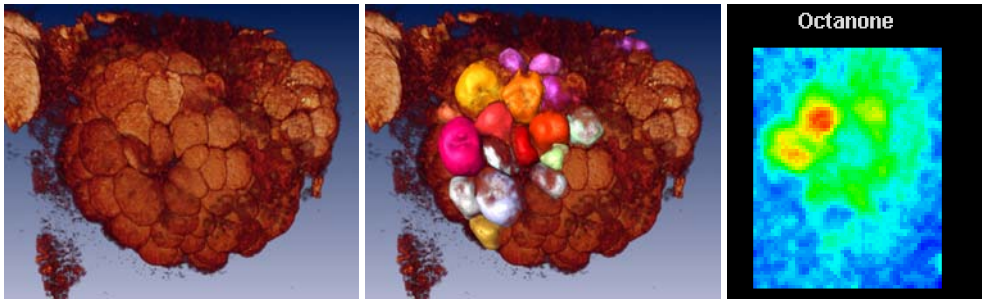

Figure 8.5: The left image shows a volume rendering representation of a high resolution confocal LSM scan of a honey bee antennal lobe. A labeling has been done and a surface model has been generated. Some surface patches enclosing selected glomeruli have been overlayed with the volume rendering in the middle image. The right image shows antennal lobe intensity patterns in living animals during odor exposure (patterns from Galizia *et. al*). Mapping of functional data onto anatomical structures is an important application of electronic anatomical atlases.

scribed geometry reconstruction methods, a polygonal model can be reconstructed that can be rotated interactively and looked at from arbitrary view points. When analyzing the experimental data the atlas can therefore be viewed from the same direction as the antennal lobe in the living animal in the specific experiment, allowing for an accurate identification of the glomeruli. Examples are given in Figure 8.5. Current research activities include detailed investigations of similarity of glomerular arrangement by registering (rigidly and and non-rigidly) a larger number of labeled antennal lobes and comparing overlaps of corresponding glomeruli. One of the goals is the development of an automated procedure for full identification of all glomeruli from only a few reference structures. These investigations are only possible due to the availability of the new tools, which allow a fast and reliable generation of numerous models.

8.5 Honey Bee Projection Neurons

From the antennal lobe the signals are distributed into other structures of the brain, where higher-level processing takes place. One such structure is the mushroom body with its two calyces. Specific neurons project from individual glomeruli into the calyces of the mushroom body. As described earlier, it is possible to measure the signals in single neurons and fill them with fluorescent dye and record them. By simultaneous staining and recording neuropil or using the auto-fluorescence, neuropil models can be generated. Figure 8.6 shows a model of a projection neuron in the context of the neuropil structures.

The procedure of filling individual neurons is difficult from the practical side, and it is in general not possible to target more than one neuron at a time. If the interaction of multiple neurons shall be investigated, it is necessary to bring them into a common reference frame. Again, we use the neuropil structures as reference for alignment. It is our next goal to create an atlas of the projection neurons, as well as selected inter-neurons, which connect individual glomeruli. This will be the basis for numerical simulation of these biological neuronal networks.

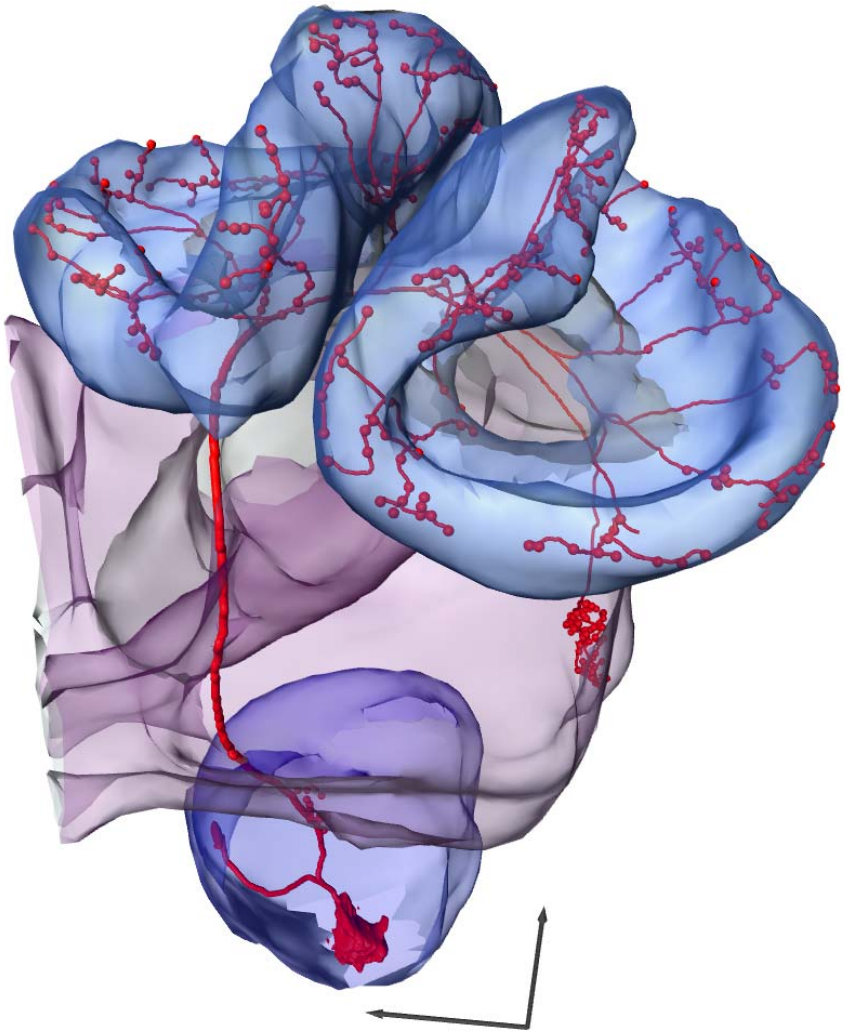

Figure 8.6: Geometrical model of a projection neuron in the bee brain, embedded in a surface model of its larger neuropil structures, compare [94]. The little spherical ’blebs’ are not artifacts, but extracted from the image data.