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Creating Virtual Insect Brains with Amira

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Abstract

By combining techniques of preparation, histology, confocal microscopy, data visualization and data processing, we have created and recently published a Standard Brain model for drosophila and honey bee brains. This report describes the algorithms and implementation of the corresponding software modules. At the same time it serves as a users guide for scientists who want to reproduce the results for different species or mutants as well as those who want to incorporate new data into the drosophila Standard Brain model.

1 Introduction and Overview

All algorithms and tools described in the following have been implemented as custom modules within the commercially available Amira software system [2]. We make these modules available free of charge for scientific and non-commercial use. In order to use these modules a recent license for the Amira system is required. Details are given at [3].

This document is not self-contained in the sense that a basic knowledge of the Amira visualization system is assumed. We recommend to become acquainted with at least the *Getting Started* tutorial, the *Visualizing 3D Images* tutorial and the segmentation part of the *Grid Generation* tutorial of the Amira online documentation.

Also it is assumed that the concepts described in [1] are known.

1.1 Overview

This document is organized as follows:

Section 1 introduces the concepts and the terminology used.

Section 2 describes the data sets that have been published as the Drosophila Standard Brain model and how these can be loaded in the Amira system.

Section 3 gives hints on how to incorporate new data like gene expression patterns into the Standard Brain model.

Section 4 describes the methods as well as the modules and scripts that were used to create the Standard Brain. This section is especially interesting for

scientists who wish to reproduce the results published in [1] or who want to create a similar model for different species.

Finally section 5 gives answers to questions regarding technical details, questions that are raised and referenced throughout the document.

1.2 Concepts and Terminology

The anatomical approach discussed in this paper is based on high-resolution three dimensional images of *whole-mount brain preparations*. Such images can be obtained by using a *confocal microscope* after an appropriate staining and histology procedure (see [1] for details).

The resulting data sets are three dimensional arrays of intensity data. Typical dimensions are 512 x 512 x 200 *voxels* with a voxel size of e.g. $2\mu m$ x $2\mu m$ x $2\mu m$.

Each voxel of a data set was labeled to indicate, in addition to its intensity value, the anatomical structure to which it belongs. The resulting array of labels is referred to as *LabelField*.

2 The Standard Brain Datasets

We have released the important datasets described in [1] to the scientific community. They come along with online documentation and a number of self-running demo scripts for the Amira system at [3].

After installation, the demo section in the Amira online User's Guide (available from Help/Demos) contains a new entry 'Drosophila Standard Brain'. From that page you can launch Amira scripts by clicking on the respective icons.

All data sets are stored in a subdirectory `data/vib` in the Amira installation directory and can also be loaded individually via the *File/Load* menu. Included are the following files:

`template.grey`: The image data set that was chosen as template in [1]. The data set has been resampled to a grid with a suitable orientation. Resolution has been downsampled to reduce the file size for internet download.

`template.labels`: The label field corresponding to template.grey with 16 structures labeled.

`template.surf`: A three dimensional surface model which has been generated from template.labels.

`CantonM43c.grey`: The image data set of another individual. This can serve as an example/tutorial data set for your own studies. The data set has been aligned with the template and resampled to its grid.

`CantonM43c.labels`: The label field corresponding to CantonM43c.grey.

`CantonM43c.surf`: The surface generated from CantonM43c.labels.

`AverageIntensity-rigid.am`: Average intensity map created by averaging the rigidly aligned intensity data sets.

`AverageIntensity-scale.am`: Average intensity map created by averaging the scaled and then rigidly aligned intensity data sets.

`AverageIntensity-interp.am`: Average intensity map. The important structures have been aligned independently after a global scaling was applied. The transformation field has been interpolated in the space between the structures.

3 Importing into the Standard Brain

3.1 Preparations and Alignment

Bringing data from new studies into the Standard Brain is easy. Simply load one of the data sets described in section 2 into Amira. You may refer to FAQ 5.1 for details. Make sure that you specify the voxel size in μm. Then you should register the dataset with the Standard Brain template. There are multiple ways to do that: First you can interactively rotate and translate your data set as described in FAQ 5.2. To refine that transformation you can use the Align module for an automated grey-value-correlation based alignment. If you have done a segmentation of your data set you can also use the LabelFields as input for the Align module.

Once you have found a transformation for alignment, you can retrieve this information by typing

`<dataset> getTransform`

into the Amira console (where `<dataset>` is the name of your data set). You can copy and paste the resulting 16 numbers to a text editor and save them to disk.

Next you can actually *apply* that transformation to the data. For image data this means that it is re-sampled onto a new grid: Choose `Compute/Apply-Transform` from your data set's right-mouse menu. Connect the ApplyTransform's `Reference` input port to the template.grey data set and click 'DoIt'. For highest quality you may choose Lanczos instead of standard interpolation.

If you have multiple data sets to which you want to apply the same transformation, like e.g. a second channel from your recording or an Amira surface object that was created prior to alignment, you can do so by typing

`<new dataset> setTransform p1 p2 ... p16`

where p1,...,p16 are the numbers you have previously saved.

3.2 Comparison

In order to visually compare an individual with the template or different individuals with each-other, the whole range of visualization methods can be employed. One particularly useful tool is the MultiChannel field.

This tool requires that the data sets are defined on the same grid (same voxel size, same dimension, no transformation). This is the case after a transformation has been applied as described above.

Choose `Create/MultiChannelField` from the Amira menu. Connect the MultiChannelField's channel1 input with your first data set and the MultiChannelField's channel2 with the second one. Then you can attach e.g. an OrthoSlice to the MultiChannelField.

4 Creating the Standard Brain

This section describes the methods, tools, and scripts used to create the drosophila Standard Brain. It mainly serves as a reference and user's guide for scientists who wish to reproduce the creation of an average brain model for a different species.

We here assume that all preparations have been imaged with a confocal microscope and are available as 3D-image stacks.

The next step is to label all structures of interest for each data set. Image data sets and LabelFields should be named consistently and all should be stored in a particular directory.

Finally the configuration file for the VIB scripts has to be adjusted accordingly (the path names need to be specified) and the scripts have to be executed.

The details for each step are given below:

4.1 Recording and Image Data

For the work described in [1] four different groups of about 30 animals each where prepared and recorded. In order to get meaningful results, it is recommended to standardize the whole procedure as much as possible. In particular it is helpful to record all animals with the same resolution. Also further processing may be more convenient if the preparations have roughly the same orientation already at recording time.

After recording, the image data sets can be imported into the Amira program. After setting the correct voxel size it is recommended to save the data sets again in Amira's native AmiraMesh format. Depending on acquisition parameters, such as the type of lens (dry, water or oil immersion) and embedding medium (aqueous, glycerol etc...), it may be necessary to scale voxels in z-direction. E.g. a specimen embedded in methyl salicylate and imaged with a dry lens must be scaled by a factor of about 1.6, while a specimen imaged in aqueous solution with a water immersion objective needs no scaling. For details refer to [4].

For the following steps and for the specific scripts to work properly it is assumed that all the image data files are stored in one directory and have canonical names ending with `.grey`. An example could be: F01.grey, F02.grey, ..., F29.grey, M01.grey, M02.grey, ..., assuming you have one group of female and one group of male species. If you do not want to distinguish different groups in the subsequent analysis only the ending `.grey` is important.

4.2 Labeling

Clearly the most time consuming step in our approach is the manual labeling of the individual anatomical structures. This step has to be performed for each data set individually.

It is important that you have labeled exact the same structures in all data sets. Also the spelling (including upper/lower case) as well as the order of the structures in LabelField must be identical across all LabelFields. In order to achieve this we recommend to create a LabelField for one of the data sets with the complete list of structures in a suitable order and with the colors nicely chosen. Then use this as a template for all the other data sets (see 5.3 in the FAQ part for details).

All LabelFields should be stored in one directory and should have the same name as the corresponding image data sets with the file extension `.labels` . E.g. F01.labels, F01.labels, ...

4.3 Installation and Script Configuration

After the installation of the Amira VIB extension from [3] your Amira installation directory (typically `c:/Program Files/Amira-2.3/`) will contain a directory directory `VIB-Root`. `VIB-Root` contains a directory tree which is suitable for storing all input and result data sets. We therefore recommend to move this directory with all its sub-directories to an appropriate location in your file system and move the previously created image and label field data sets into the subdirectories `images` and `labels` thereof. Please be aware that you will need enough disk space in that file system and that a local file system on the computer where the computations will take place will be much faster than a network drive.

Next you should take a look at the file `VIB-Root/scripts/config.txt`. Load this file into a text editor like wordpad and adjust the path and group settings if necessary following the instructions in that file. If you have followed the above recommendations, in many cases you will need to adjust the group definitions in that file.

4.4 Downsampling

In general the images files are rather large. Therefore, often it is convenient to have a downsampled version of the image data files for quick browsing. Some of the scripts like the grey-value based alignment will operate on a downsampled version of the data sets by default.

To automatically create a version which is downsampled by a factor of two in X and Y direction, load the file `VIB-Root/scripts/VIBresample.scro`, choose '*.grey' as group and click Compute. The downsampled versions are automatically stored in the directory specified in `config.txt`, by default this is `images-small`. Computation may take a couple of minutes.

4.5 Volumetry

The first script to be executed is the VIBtissuestat.scro. This script will create and store a statistics file for each individual data set. The statistics files contain volumetric information for each anatomical structure; later they are also used to store the per-individual transformations in the registration steps. Therefore, be aware that re-calling the VIBtissuestat script at a later time will overwrite any previously computed transformations.

Load the file `VIB-Root/scripts/VIBtissuestat.scro`. As group choose '*.grey' and click `Compute`. This will create an Amira spread sheet for each individual containing volumes per structure. The spread sheets are stored in the subdirectory specified in the config.txt file (`statistics` by default).

Then you can compute average volumes and deviations for all animals or individual sub-groups: load the script `VIB-Root/scripts/VIBvolumetrie.scro` and click `Compute`. The script will create two spread sheets: One containing the average and standard deviation of each structure's volume for the selected group and a second (big) one containing all volumes for all data sets individually. The latter can be used for quick manual checking to reveal significant segmentation errors. For handling the spreadsheets please see FAQ 5.4.

4.6 Alignment

An important step that enables you to average or further compare individual data sets is the registration. This means that the data sets which have been recorded in a rather arbitrary orientation have to be brought into a common coordinate system. In [1] we have proposed three different methods for alignment. The first takes into account the centers of gravity of the labelled structures in the two data sets and finds the rigid transformation (rotation+translation) which minimizes the sum of these distances. A uniform scaling factor is computed such that the sum of the volumes of all structures match the template.

The script `VIBcentertrans.scro` computes transfomations in this way. After loading the script, a template and a group of data sets to work on can be specified. After clicking on `Compute` the transformation is computed for each data set and stored as a parameter (see FAQ 5.5) in the corresponding statistics file. The parameter name is `${template}SCenterTransformation`, e.g. if the template is F01 it would be `F01SCenterTransformation`. This computation takes only fractions of a second.

The transformation obtained with `VIBcentertrans.scro` can be refined by iteratively modifying it such that the correlation between the grey images (template vs. individual) or label fields is maximized. This can be done using the scripts `VIBlbltransform.scro` and `VIBgreytransform.scro`. These scripts expect a parameter `${template}SCenterTransformation` to be present in the statistics file which they use as starting position. They will store the result in a parameter `${template}SLabelTransformation` and `${template}SGreyTransformation`, respectively. Note that the `VIBgreytransform.scro` will by default operate on downsampled versions of the grey images to save run-time. Therefore it is necessary to run the `VIBresample` script first.

4.7 Averaging

Once the alignment transformation has been computed for each data set of a specific group, average intensity maps can be computed.

Load the file `VIB-Root/scripts/VIBaveragegrey.scro` and choose the group of data sets you want to average and the template you want to use. The template is only used to define the grid (bounding box and resolution) on which the average is computed.

Type the name of the transformation you want to use into the field `Transformation`. The expression ${template} will automatically be replaced by the name of the template you have chosen. See above for an explanation of the different transformation names.

After setting all parameters, click 'Compute'. This will setup some modules and ask you to hit the 'DoIt' button in the newly created AverageBrain module. Do so and go to fetch a cup of coffee since the following computation will take a while.

As a result an average intensity map named 'Average' will be created. You should manually save this to an apropriate place.

In a similar way the module `VIB-Root/scripts/VIBaveragelbl.scro` works which 'averages' labels. As explained in [1] averaging labels requires a completely different definition of an average. Therefore this module will create for each material individually a probability map encoding the probability that within this group at one particular location the specific label will occur.

To start the computation load `VIB-Root/scripts/VIBaveragelbl.scro`, adjust all settings, click on `setup`, then click `compute`. Note: Since a full run across all data sets is required for *each* material, this computation will take significantly longer than the average intensity map creation. The resulting probability maps (one for each label) will automatically be saved to the output directory specified in the `config.txt` file.

5 FAQ

5.1 How do I import my own image files into Amira ?

Amira supports a number of standard image file formats. Therefore in many cases it is sufficient to choose *File/Load* from the menu and select the 3D image volume file. If your microscope software stores the images in separate files for each slice we recommend to use a standard image file format like TIFF for the slices. Then you can select all files forming your image volume by holding down the shift key in the Amira file browser. After clicking OK a new dialog which allows you to specify the voxel size will pop up. We recommend to use μm here. More details on file import are given in the Amira User's Guide.

5.2 How do I use the transform editor for alignment ?

The transform editor allows you to interactively shift, rotate, and/or scale a data set relative to another data set or a reference coordinate system. If a data set is to be aligned with another data set, the first step is to visualize both data sets simultaneously. This can be achieved e.g. with multiple OrthoSlice modules or with iso-surfaces (choose different colors for the two-iso surfaces).

The transform editor is invoked by selecting the data set's green icon in Amira. After switching the Amira viewer to interaction mode (arrow button), you can pick the transform editor's box on its faces or edges to translate or rotate the data set.

More details are described in the editors section in the Amira users guide.

5.3 How do I reuse the names/colors of materials in a particular LabelField for a new data set ?

Load the existing LabelField (the template) into Amira. Load the new data set into Amira. Create a new LabelField by selecting Labeling/LabelField from the data set's right mouse menu. Then attach a Compute/Relabel module to the new LabelField. Connect the template input port of the Relabel module to the template LabelField. Activate the *Modify Input* option in the Relabel module and click 'DoIt'.

5.4 What can I do with spreadsheets ?

The Amira spread sheet object is intended to store and provide a quick preview to statistical data. For further processing it should be exported to a dedicated spread sheet or statistical application like Excel or Statistica. In order to do so, save the Amira spread sheet in CSV (comma separated values) or in ASCII format. Make sure that your system uses '.' as decimal point.

In order to be able to read the spread sheets back into Amira they should be stored in AmiraMesh format.

5.5 What are Amira parameters ?

Each Amira data object (green icons) can be annotated with arbitrary name/ value pairs, called parameters. Depending on the file format the parameters are stored to disc and recreated when loading. The AmiraMesh file format supports this.

Parameters can be edited using the "Parameter Editor": Select the data set's icon in the Amira object pool and activate the Parameter Editor using the respective green editor button. Refer to the Editor section in the Amira User's Guide.

The VIB scripts use parameters to store the transformation matrices from the different alignment steps. They are stored along with the statistics files created in the volumetry step, not with the grey images or the label fields. The reason

for that is that the statistics files are very small compared to the image data files and can therefore be loaded and saved very quickly.

References

[1] Karlheinz Rein, Malte Zöckler, Michael Mader, Cornelia Grübel, and Martin Heisenberg. *The Drosophila Standard Brain* , Current Biology 12 (2002)

[2] *Amira: An Advanced 3D Visualization and Volume Modeling System.* `http://www.amiravis.com` (2001)

[3] *The Virtual Insect Brain Project.* `http://www.amiravis.com/vib` (2001)

[4] Bucher D, Scholz M, Stetter M, Obermayer K, Pfluger HJ *Correction methods for three-dimensional reconstructions from confocal images*, I. Tissue shrinking and axial scaling, J Neurosci Methods 2000 Jul 31 ;100(1-2):135-143