

# **Topologizer for Analysis and Visualization of Microscopic Slide Stack by VTK**

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microscope (see fig. X) are more difficult to use for the segmentation analysis.

## Abstract

In TclTk/Perl a tool (Topologizer v0.25) has been designed (for Unix and Windows) to visualize and analyze a stack of serial slides from a microscope sections. From the brain of a beetle, three examples of the neuropils olfactory glomeruli are reported. Segmentation by objects and segmentation by measured concentration are demonstrated. Slide stacks from confocal laser-scanning microscopy and non-confocal light-microscopy are used. The goal is to analyze the visualization results in context of the systems topology.

## 1. Background

Microscopes and NMR-Tomographs are common devices for presenting a 3D object as a slide stack. We used a confocal laser scanning microscope (Laica TCS NT) and a simple fluorescence non-confocal microscope (Olympus IMT-2 with an epifluorescence device and phase contrast, with a high resolution CCD camera attached) to analyze brain slices. In the later case, an image slide is produced by focusing one z-layer of the object slice and digitizing the image with the CCD-camera. When the z altitude is changed the next slide is entered, and each slide is saved as a BMP file.

In contrast, the confocal laser scanning microscope produces slides of extreme precision without any blurr. The simplified optical path is shown in fig. X. Here a laser of a wavelength adjusted to the fluorescence spectrum of a secondary antibody, is used as light source. The beam is focused by a dichroic mirror and directed into the target. The antibodies contained in the focus point inside the object slice are emitting light, which is proportional in intensity to the concentration of antibodies in this xyz-voxel. This light is passing through the beam-splitter and the confocal aperture into the photomultiplier where the light is transformed into a signal. Since the laser beam can be focused with extreme precision into a voxel, only light from that focused voxel can be emitted. Therefore scattered light from voxels around the focused one cannot reach the detector. The situation is opposite in the focused layer of a light microscope, where at least all voxels in the focused z-layer contribute to the image. The beam-splitter is now oriented in such a way that the focused voxel can move in lines through the object slice, whereby all lines assemble one z-layer. The signals of all voxels in the z-layer are digitized to produce a single image (slide). All image slides are saved in a multi-image TIFF file (for simplicity called "TIFF-stack-file"), ready for analysis.

Confocal image slide stacks are ideal for segmentation purposes. Blurred image slides made with a light

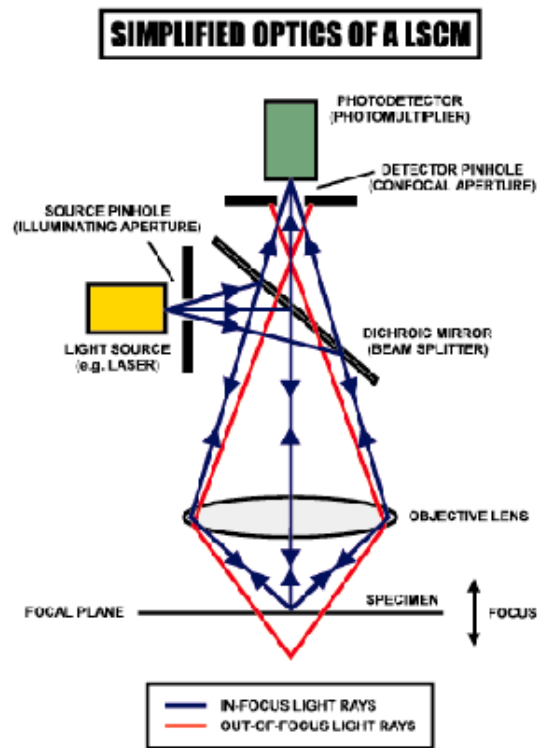


Figure 1: Simplified optics of a LSCM.

## 2. Software Design

The program "Topologizer v0.25" is created with the intention of analyzing the dynamic and topological characteristics of abstract complex data, i.e. to build Complex-System-Interfaces. As one of the simplest possible application, the visualization of microscopic slide stacks was chosen to understand and demonstrate the fundamental features of visualization techniques. The name "Topologizer" is a word creation deduced from topology. It studies properties that an object retains under deformation and characterizes its inherent relations in space. The name anticipates the final goal which is to analyze dynamic invariant properties of complex data.

### 2.1 Components and Functions

The program is using the free packages VTK and netpbm+. It is written in of C++, TclTk82+ and Perl5+. C++ is used in VTK and in added tool kits. Tcl is used to apply the available functions of libraries and to manage the information flow during processing. Perl manages the interface to the application of graphic conversion and

analysis tools. Finally Tk provides the GUI on the top of all.

## 2.2 Information Process Flow

The first step is to read the data as a series of BMP files, a TIFF stack or a set of AVI files (AVI-stack).

BMP files are produced with a light microscope or as edited raw images for processing. Fig X shows a raw 2D file and fig X the edited file, in regions of the same interest (tissues) are assigned each with one specific gray value. The BMP format is used since it is possible to control each pixel value (opposed e.g. to JPEG) and it is independent of the platform used.

TIFF (Tagged Image File Format) stacks are produced by the Laica laser scanning microscope. Many stack specific information are stored in the file header and provide a flexible control to pixel level.

AVI stacks are produced with a microscope that is used to examine the time dependence of a developing 3D object (like a growing C.elegans). The growth of the organism C.elegans is just one example. A dump program converts an AVI file stack into a 3D frame sequence in which one frame is stored in a TIFF stack. This sequence may be analyzed in a later version and processed in the High Density File format (HDF) or visualize an evolving phenomena.

## 2.3 The GUI

The shell displays all relevant information in every stage of analysis processing. The processing steps can be controlled and are discussed in the following.

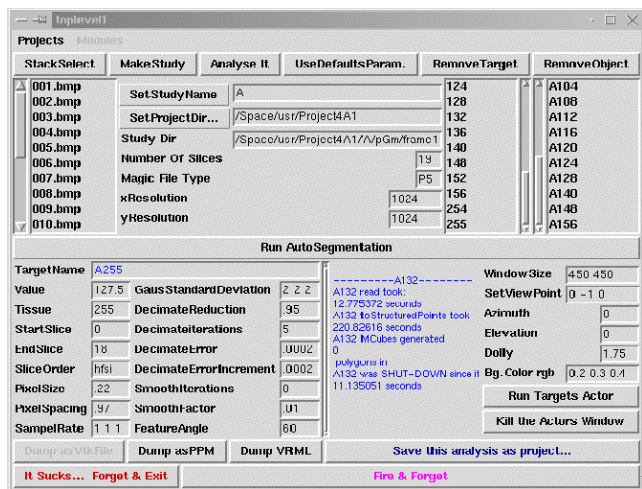


Figure 2: The Topolozizer v0.25 GUI.

**Selection:** Firstly the data to be analyzed are selected. Depending on the type of input (BMP- TIFF- or AVI-

stack) the appropriate conversion routines are chosen for the next step. In case of AVI-stacks, this may take considerable time and disk space.

**Make a Study:** Before building the studies, the studies name and path can be chosen or set by default. The conversion process is so launched that the study (or study sequence) can be found under the specified location as PGM files.

**Analyze the Study:** Now consistency checks are run on all slides analyzing the resolution, on the file types and on the file contents. Additional header information (like xyz voxels) can be extracted and are delivered to the shell for subsequent segmentation. To each stack, a parameter array is attached, that surfaces the relevant information later in the appropriate name-spaces of the processing situation.

**Segmentation:** The necessary parameters and possible segmentation targets are now available. After choosing one target, a set of targets, or simply by default all targets, the segmentation starts using the default parameters. These can be individually adjusted before processing the segmentation and are valid for the specified targets list. As the segmentation progress is shown, the rendering objects are created.

**Rendering:** After adjusting the parameters for rendering (or using the defaults), the render window is opened and the specified object list is shown.

**Project:** Any stage of analysis can be saved as a project file. Thus it is possible to re-enter the same analysis in an earlier stage and proceed with other segmentation parameters, a new target list or other chosen objects. It is advised to save the project status before and after running the segmentation since some processes are very time consuming.

**Dumping the results:** The rendering results can be important for further analysis with plug-in modules. Therefore these results can be saved as simple PGM files, as VRML or native VTK files.

## 2.4 Other Systems

Since the software bus is maintained to be very common, it should be easy to create interfaces to other system (f.e. MATLAB or a database). Thus modules of any purpose can be attached.

## 3. The Visualization Results

Three sets of the olfactory glomeruli (processor like entities of the brain) slices are analyzed by the algorithm.

Firstly, a confocal slide stack of edited brain slices is segmented for tissue assigned objects. Fig. 3 shows an unedited slide of the stack. The colors scales correspond to the fluorescence of secondary antibody concentration. In fig. 4, the 2D regions that are believed to belong to a specific 3D object are assigned with specific gray valued pixels. The edited slide stack is analyzed with default parameters.

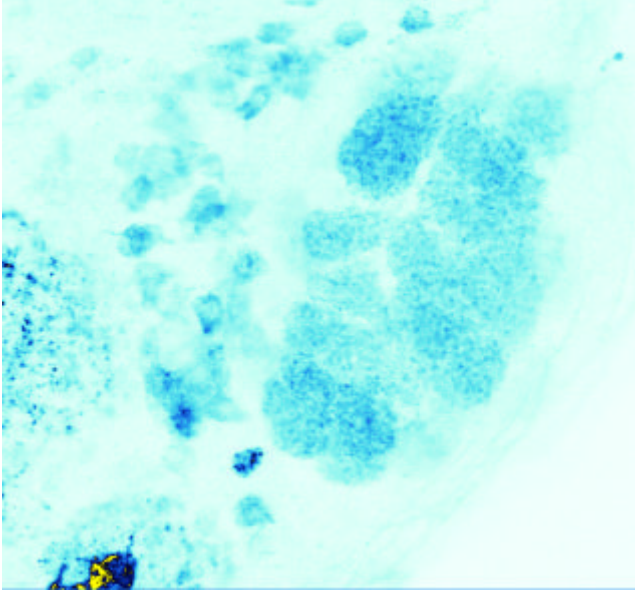


Figure 3: The raw data LSCM slide



Figure 4: The edited raw data LSCM slide

The run took 2.5 hours and 66 mb additional disk space. Fig 5 renders the scene of 3D objects which can be recognized as compact glomeruli. Actually, this result satisfies our imagination about these objects. But in fact, it

is not clear whether this image the reality fits best, since the content of the information (in fig. 3) acquired by measurements is a varying distribution of antibodies in space and this is changed (in fig. 4) by assigning tissue values to pixel groups in the 2D raw data.

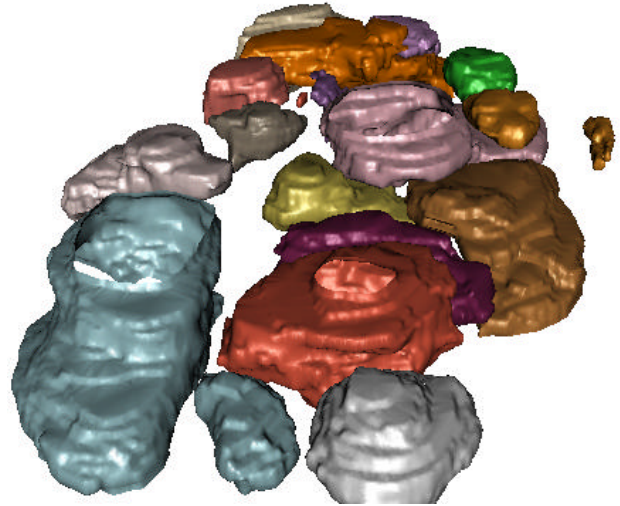
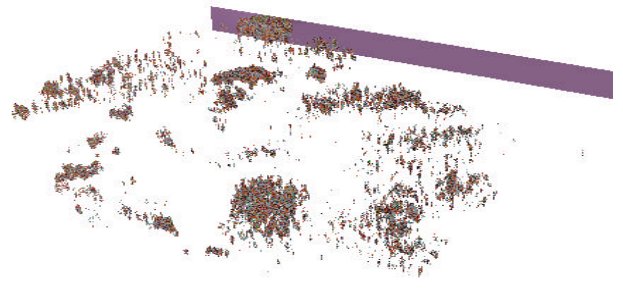


Figure 5: The rendered scene of edited slides. All glomeroli of all concentrations are shown.

Secondly, the same unedited slide stack is segmented for the concentration of secondary antibodies. The parameter setting for the Gaussian Standard Deviation (GSD) was set to (0.5 0.5 0.5). Fig. 6 shows a 3D scene assembled of a set of different distributed antibody concentrations, i.e. only specific antibody concentration are shown. Compared with fig. 5, the shape of the selected imaginary 3D objects is fairly good approximated and the content of the measurements information is not changed so





far.

Figure 6: The rendered scene of raw slides. Only glomeroli of specific concentrations distribution are approximating some objects in fig 5.

Thirdly, a TIFF stack (phase-contrast sliced organism *C.elegans*) is processed. A non-confocal slide stack is sub-optimal for segmentation purpose, since the images are blurred. Fig 7 shows one representative slide of an analyzed stack. A non-confocal slide stack of the organism *C.elegans* is extracted from an AVI stack and analyzed. The goal is analyze this AVI stack in 4D.

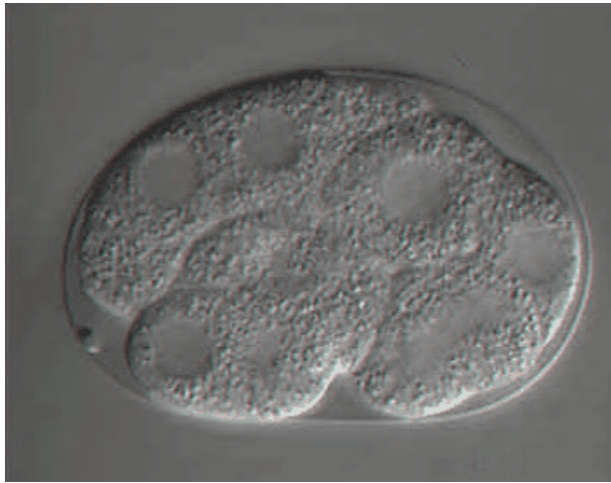


Figure 7: One raw data slide of an AVI stack showing the organism *C.elegans* in its 8 cell status of growth.

Fig 6 renders a scene equivalent to the one shown in fig. 8. This time the parameters for Pixel Spacing were set to 1.27 and for GSD was kept. A comparison of fig. 6 and fig. 8 show that the results obtained with a confocal laser scanning microscope are good data source for segmentation. But it is not necessary to use these expensive devices to produce acceptable results. Therefore confocal laser scanning microscopes make sense if the content of single pixel information is relevant.



Fig. 8 shows a 3D scene of assembled concentrations distribution approximating the cell compartments of *C.elegans* in the 3 cell status.

#### 4. Lessons Learned

The segmentation and volume rendering methods do have their limits. For complex data structures it becomes increasingly difficult to construct a useful description for an observed phenomena. It appears to be necessary to develop complexity reducing methods, thus segmentation of information is done on an algebraic basis.

#### 5. Conclusions

A) Simple segmentation and volume rendering methods are useful for 3D microscopy. Laser Scanning Confocal Microscope (LSCM) are good, but not a necessary condition to produce acceptable results.

B) It is necessary to develop a generalized method for segmentation and rendering in 4D. Thus measured information can finally be described as an assemble of space-time compartments.