

Three-dimensional Surface Reconstruction from Serial Section

Three-dimensional (3-D) reconstruction is an important technique for clarifying the spatial configurations of biological objects including plant organelles. Surface reconstruction is particularly suitable for exploring the 3-D shape because reconstructed structures can be observed from any arbitrary angle by rendering 3-D models.

Installation of REANT-light

“REANT-light” is 3-D reconstruction software performing an interpolation between parallel planar contours based on the Euclidean distance transformation.

System requirements

- Debian GNU/Linux 4.0 i386: <http://www.debian.org/>
- GNU Fortran 95 compiler 4.1
- Python 2.4
- Python modules: numpy, numarray, psyco, pyvtk, PIL.
- MayaVi (scientific data visualization system)
- ImageJ (image processing software): <http://rsb.info.nih.gov/ij/index.html>

All required software except ImageJ is included in Debian GNU/Linux 4.0.

Installation of REANT-light

1. Download <http://www.biol.s.u-tokyo.ac.jp/users/hasezawa/kbi/reant-light/reant-light.tar.bz2> .
2. Extract files.

In the terminal ("% " represents a prompt of shell):

```
% tar xvfj reant-light.tar.bz2
```

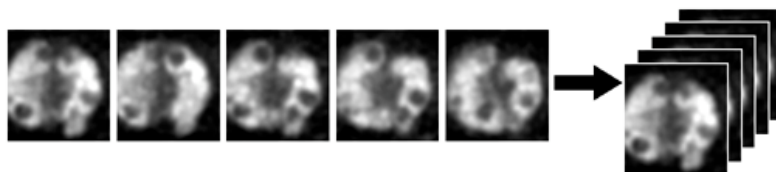
Preparation of TIFF stack file

1. Acquire a series of cross-sections as digital images.

Although we mainly utilize confocal microscopy for 3-D reconstruction, any approach can be used for the acquisition of sectional images, such as deconvolution algorithms and successive physical sections.

- * Pixel width (a.k.a. Δx) and pixel height (a.k.a. Δy) must be equal.
- * Distances between sections (a.k.a. Δz) must be constant in a single series.
- * Calculate and note the aspect ratio by $\Delta z / \Delta x$.

2. Convert the image series to a TIFF stack file.



If the image acquisition software does not handle TIFF stack format, the image-processing software “ImageJ” can be used to combine the multiple image files.

- 2-1. Load multiple image files by ImageJ menu **File – Import – Image sequence...** .
- 2-2. Save as a TIFF stack file by ImageJ menu **File – Save as – TIFF**.

Registration (optional)

If cross-sectional images are acquired by physical sectioning, the geometrical alignment of each section is required. This step is called “registration”. The ImageJ plugin “StackReg” (<http://bigwww.epfl.ch/thevenaz/stackreg/>) is a useful tool for automatic registration. Use the “Rigid Body” mode for transformation.

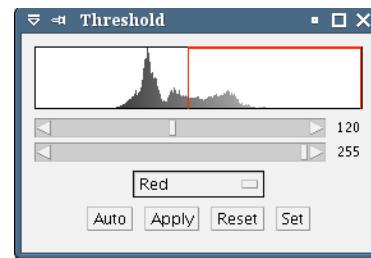
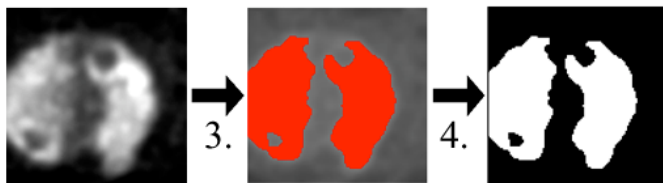
Segmentation

Prior to reconstruction, the original image must be converted to a bi-level image (black and white image). This step is usually called segmentation. The simplest method for the segmentation of fluorescence images is *intensity thresholding*.

Intensity thresholding using ImageJ

1. Open TIFF stack file by the menu **File – Open...**
2. Create “Threshold” window by ImageJ menu **Image – Adjust – Threshold...**
3. Adjust horizontal slide-bars in Threshold window.
4. Convert to bi-level image by **Apply** button.
5. Save as a TIFF stack file by ImageJ menu **File – Save as – TIFF**.

NOTE: Do NOT overwrite the original TIFF file !



Surface reconstruction using REANT-light

Assumptions:

Filename of original TIFF stack:	foo.tif
Filename of bi-level image:	foo-bi.tif
Aspect ratio ($\Delta z / \Delta x$):	1.618

In the terminal,

```
% python gen_vtk.py -r 2 -z 1.618 -V foo-bi.tif foo.tif
```

generates an OUT-pol.vtk file containing the 3-D model.

Rendering of 3-D model using MayaVi

With default parameters, REANT-light generates the 3-D model as a Visualization Tool Kit (VTK) file as described above. A scientific data visualization system called “MayaVi” performs the rendering (displaying the 3-D model) of VTK format files in Linux and Microsoft Windows.

1. Start MayaVi.
2. Load VTK file by MayaVi menu **File – Open – VTK file**.
3. Select “Z-coor-pt” as scalar (the depth of focus is used for the coloring of 3-D objects).
4. Display the 3-D model by MayaVi menu **Visualize – Modules – PolyData**.
5. Capture and save the rendered image to file by MayaVi menu **File – Save Scene to – TIFF image**.

The reconstructed 3-D model is interactively rendered in the right panel of MayaVi. The exploration of 3-D space is performed by a point-inputting device (usually a mouse or track-ball) as follows.

Operation of mouse:

Left-button: Rotate

Mid-button: Shift

Right-button: Zoom in/out

