nature protocols

https://doi.org/10.1038/s41596-024-00957-5

Protocol

Check for updates

Modular segmentation, spatial analysis and visualization of volume electron microscopy datasets

In the format provided by the authors and unedited

Supplementary Information

Modular segmentation, spatial analysis, and visualization of volume electron microscopy datasets

Andreas Müller, Deborah Schmidt, Jan Philipp Albrecht, Lucas Rieckert, Maximilian Otto, Leticia Elizabeth Galicia Garcia, Gunar Fabig, Michele Solimena, and Martin Weigert

1. Supplementary workflows

Binning of subvolumes with IMOD - TIMING: 5 min

As an alternative to FIJI, binning of the raw vEM volumes is also possible in IMOD.

1) Install IMOD by downloading the version corresponding to your system specifications at https://bio3d.colorado.edu/imod/.
2) For binning use the binvol program which is documented here: https://bio3d.colorado.edu/imod/doc/man/binvol.html.
3) Open the console and type in “binvol”.
4) Choose options (e.g. binning factor) and define your input_file and output_file.
5) Press “Enter”
6) Examine the result after binning has finished.

Overlay visualization with 3Dscript - TIMING: 15-30 min

We used 3Dscript mainly for visualizing the two dimensional overlays of raw and segmentation data (cf. Demo Data 3)1. To make the overlays follow these steps:

1) Open the raw files and the segmentation files you want to visualize in FIJI.
2) Go to Image . Color . Merge Channels.
3) Define the colors for each channel (these do not have to be your final colors, you can set them later in 3Dscript) and click OK.
4) Go to Plugins . 3D script . Interactive Animation.
5) FIJI will now open the 3Dscript window and the "Interactive raycaster" and you will see a 3D rendering of your file.
6) Change the Rendering algorithm to "Combined transparency".
7) Now change the color of each organelle to your pre-defined one by double-click on the color block next to the channel and double-click on the colored rectangle that appears. You can now set the HTML color code for the "Foreground color".
8) To make 2D overlays of raw and segmentation data go to Cropping . Show and set the z-range to only a small value of 1-2 slices. Press Enter and a 2D view will appear. Zoom in if necessary.
9) Export an image of the area in the window by clicking Animation . Show . Start text-based animation editor.
10) Go to Record . Record transformation and set the frame to 1.
11) Click "Run" and save the result as TIFF or PNG file.
12) You can also make complex 3D animations with the text-based editor and by changing the values of the axes of the organelle channels. We recommend to watch the 3Dscript tutorial video (https://bene51.github.io/3Dscript/gallery.html).

Visualization with ORS Dragonfly - TIMING: 60-90 min

1) Install ORS Dragonfly according to installation instructions at https://www.theobjects.com/dragonfly/index.html. We recommend requesting a non-commercial license for academic use.
2) Open ORS Dragonfly.

3) Load your individual raw and segmentation masks by going to File ▷ Import Image Files... ▷ Add... and choose the file you want to open. Click "Next" and define the voxel dimensions of your dataset. Click Finish. Repeat this step for all the individual masks.
4) Right-click on the image and select 3D for rendering.
5) On the left panel you can change the "Background color" in the "Scene's View Properties" tab. You may change it to white or black.
6) Now set the colors for the organelles according to your color palette (Fig. 12a). You do so by clicking on your file in the "Data Properties and Settings" tab. Go to the "Window Leveling" tab on the left side. Set one of the preset colored LUTs (*e.g.* green). Now right-click on the colored "Value Box" and change the HTML value as it is defined in your palette. Click OK. Set the levers below the histogram in a way to achieve a uniform color. Now click "More..." next to LUT and define a name (ideally the organelle name) for the new histogram. Repeat this step for all organelles.
7) In order to make animations or export single frames (Fig. 12b) right-click on the main window and click "Show Movie Maker". Now additional options below the main window will appear that allows the setting of key frames. For creating animations it makes sense to activate the "Clip box" by pressing the "Clip" button in the "Clip" tab. You can then rotate, move or clip the data and add new key frames by pressing "Add Key".
8) You can run the animation by pressing "Play" and once you are satisfied with exporting it by clicking on the "Export Animation" button. Choose the desired speed, pixel dimensions and file format and press "Save as Video File".

2. Supplementary Tables:

Tool	Version	Purpose	Link
ORS Dragonfly	2022.2	Software for 3D rendering.	https://www.theobjects.com/dragonfly/index.html
3Dscript		Fiji plugin for 3D rendering. We use it for creating 2D overlays.	https://imagej.net/plugins/3dscript
IMOD	4.11	Software for tomogram reconstruction and manipulation.	https://bio3d.colorado.edu/imod/

Supp. Table 1: Additional Software

3. Supplementary Videos:

Supplementary Video 1: *Album/CSBDeep training*
We show how to use the Album GUI to install and execute the *CSBDeep* Album solution that trains a U-Net for semantic segmentation of Golgi from FIB-SEM volumes. This includes vizualisation of the loss and intermediate results via tensorboard.

Supplementary Video 2: *Album/StarDist training*
We show how to use the Album GUI to install and execute the *StarDist* Album solution that trains a StarDist network for instance segmentation of secretory granules from FIB-SEM volumes. It additionally shows how to set and change training parameters and how to visualize the loss and intermediate results via tensorboard.

Supplementary Video 3: *Album/CellSketch project creation*
We show how to install the protocol solutions catalog and how to create a *CellSketch* project via the Album GUI. In the video, several cell component annotations are added to the project.

Supplementary Video 4: *Album/CellSketch spatial analysis*
We show how to run the automated spatial analysis routine on an existing *CellSketch* project via the Album GUI. We also demonstrate how to visualize the analysis results in BigDataViewer and as Jupyter Notebook plots via Album solutions.

Supplementary Video 5: *Album/CellSketch 3D rendering with Blender*
We show how to convert pixel based datasets from an existing *CellSketch* project into meshes via the Album GUI. We demonstrate how to visualize these meshes in VTK and how to automatically create a Blender scene including these meshes. Finally, the scene is rendered in Blender.