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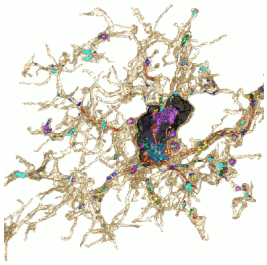
Explore the dataset from Three-dimensional nanostructure of an intact microglia cell



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We are still developing and optimizing this protocol

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Abstract

This protocol describe step by step how to download and explore the dataset provided by the article Three-dimensional nanostructure of an intact microglia cell of Bolasco et al 2018.

Guidelines

This protocol have been tested only with a Windows 10 configuration. If you encounter any kind problem following the step by step, we strongly encourage you to provide feedback to improve this protocol.

Troubleshooting

Safety warnings

The final size of the folder once all files will have been generated will be around 30GB. Also note that 3D modeling is demanding for the computer. A computer with suffisant amount of memory (8-16BG), a good CPU and a GPU is highly recommended.

Before start

In this protocol we describe how to navigate the dataset with Blender. Because it can be confusing at first, we recommend to follow a ten minute overview of Blender: https://www.youtube.com/watch?v=kes2qmijy7w&list=PLa1F2ddGya_8V90Kd5eC5PeBjySbXWGK1 and https://www.youtube.com/watch?v=qCkHNxOf9lE&list=PLa1F2ddGya_8V90Kd5eC5PeBjySbXWGK1&index=2

Other software can be used to navigate the 3D model, but this won't be covered by this protocol.

Do the downloads and installs

1 Download the dataset

Dataset	
Three-dimensional nanostructure of an intact microglia cell	NAME
https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/10201/	LINK

Download and install Fiji

Software	
Fiji	NAME

Download and install Blender

Software	
Blender	NAME

Download an addon for Blender

Software	
NeuroMorph_3D_Drawing.py	NAME

This last addon is part of the project NeuroMoprh. More tools and information can be find on the github repository. https://github.com/NeuroMorph-EPFL/NeuroMorph.

Link the addon to Blender

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1. Open Blender
2. Go to **Menu > File > User Preferences**
3. Go to the **Add-ons** tab
4. Click on "**Install from File**"
5. Locate and select the script NeuroMorph_3D_Drawing.py you just downloaded.
6. The file is now in the list of available plugins. Select it in the list and click on "**Save User Settings**".If you don't find the file, make sure that the search bar is empty.

Open the dataset in blender

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1. In Blender, go to Menu > File > Open and locate the Segmented.blend file (part of the dataset). Press open.
2. You can now navigate the dataset. Recommended navigation commands:

- Wheel to zoom in and out
- Wheel click and drag to move the dataset view
- Ctrl + wheel zoom in and out to move left/right
- Shift + wheel zoom in and out to move up/down
- Shift+F to enter "Fly mode". Then use WASD or Up,Down,Left,Right keys to fly. Adjust speed with mouse wheel. Left click to exit the fly mode (Right click to escape fly mode and reset view)

Generate the images of the stack in X and Y orientation Half resolution (recommended) (optional)

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1. Open Fiji
2. Open the two EM stacks Dataset1.tif and Dataset2.tif
3. For each Dataset, do: **Menu> Image > Scale** and set X, Y and Z Scale to 0.5 with all checkboxes checked.
4. Close the original datasets
5. Rename the downscaled datasets to Dataset1.tif and Dataset2.tif with
6. Go to **Menu > Plugins > Macro > Run** and select the script Generate_3D_image_stacks.ijm (included in the dataset).
7. The script ask for confirmation, press ok if you agree to get generate the 2.5 GB of data (The script mention 20 but it is only for the full resolution).

Wait approximately 5 minutes that the macro finishes.

Generate the images of the stack in X and Y orientation Full resolution (optional)

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1. Open Fiji
2. Open the two EM stacks Dataset1.tif and Dataset2.tif
3. Go to **Menu > Plugins > Macro > Run** and select the script Generate_3D_image_stacks.ijm (included in the dataset).
4. The script ask for confirmation, press ok if you agree to get generate the 20GB of images in each axis (required to vizualize slices in Blender).
5. Choose a folder where to save the 3 stacks

Wait approximately 15 minutes that the macro finishes.

Import the EM slices in Blender (optional)

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Safety information

You can do this step only if you have generated the EM stacks with steps 4 or 5.

1. Find and select the tab named **Neuromorph** on the left Panel
2. Go to the section **3D Drawing**
3. Set the values for x, y and z dimensions. **x=31.72 y=23.80 z=22.60**. These values does not depend on the resolution of your images. Set the **source X,Y and Z** to the matching folder you generated above.
4. Select the object **ImageStackLadder** from the object list, on the right panel.
5. Press **Tab key** when you mouse is in the object view panel.
6. Select a point from the **ImageStackLadder** object (with right or left click depending on your configuration)
7. In the section 3D drawing on the Left, press **"Show Image(s) at Vertex"**
8. You can now click and drag the EM images

Add/remove object from the view, color and transparency

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On the object list panel, click the eye icon to remove or add an object from the view

To add transparency to an object:

1. Select the object
2. Go to the **Neuromoprh** tab on the left panel
3. Go to the **3D drawing** section
4. Click on "**Add Transparency**"
5. You can now select the alpha (transparency intensity) and the color of the object.