SBFSEM-tools tutorial

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Goal: basic analysis support requiring minimal setup and background in computers, math, etc.

Current capabilities:

- ► Import relevant Tulip data (single neurons and networks)
- Count up synapses and condense synapses spanning multiple slices
- ► Summarize synapses with statistics and plots
- Sync with Blender images and cell fills
- Basic network analysis

Next:

- Compare neurons
- Additional network analysis

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Tulip Import

15Sept2017:

Matlab's webread has replaced the Tulip import system as the default. Support for Tulip import remains, however, as it can be useful for certain situations.

Some changes since the first release:

- ► All display units are now in microns
- Stratification histogram (use the Add Cell Skeleton checkbox while the Z-axis histogram is active)
- Export current figure to a new window
 [Menu -> Export ->]
- ▶ Plot soma mosaic with vissoma.m

Install

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First download or clone SBESEM-tools. Make sure SBSFEM-tools is added to your MATLAB path like so:

addpath(genpath('C:\...\sbfsem-tools'));

If you already have JSONLab installed, make sure

1 which loadison

returns the version in sbfsem-tools. Otherwise, you might get some errors.

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The Neuron class is the basic representation of a cell exported from Viking. To create a Neuron:

```
1 % cellName = Neuron(cellID, 'source');
2 c207 = Neuron(207, 'temporal');
```

Note: Source can be 'inferior', 'temporal' or 'rc1'. Also, abbreviating to 'i', 't' and 'r' works as well. Open the Neuron class in the user interface

1 NeuronApp(c207);

To update a neuron in the workspace:

1 c207.update();

Neuron

Properties

Neuron has the following publicly accessible properties:

- data
- viking Structure of the neuron's info from viking
- nodes Table of all annotations
- edges Table of links between annotations
- volumeScale Units are nm/pix for X,Y and nm/section for Z
- synapses Table of each child structure.
- geometries Table of closed curve geometries
- analysis Containers.map of analyses
- ▶ lastModified Last update of neuron from OData

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Create a single data object to hold related Neurons:

 $_1$ h1hc = sbfsem.NeuronGroup([28, 447, 619]);

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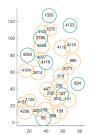
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To plot the somas of all Neurons in the NeuronGroup:

```
1 h1hc.somaPlot();
2 h1hc.somaPlot('addLabel',true); % Label with ID
3 h1hc.somaPlot('ax',gca); % Add to existing axis
4 % Two methods for controlling plot color:
5 h1hc.somaPlot('Color', [0 0.8 0.3]);
6 h1hc.setPlotColor([0 0.8 0.3]); h1hc.somaPlot;
```



Mosaic of H1 and H2 somas

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The NeuronAnalysis class helps keep population data organized by managing input parameters and results of common analyses. To create a new analysis, subclass NeuronAnalysis and edit the doAnalysis and visualize methods.

See Tutorial.m for information on these existing classes:

- ▶ DendriticFieldHull uses convex hull to estimate dendritic field area, includes methods for removing axons prior to analysis.
- ► PrimaryDendriteDiameter returns the median dendrite diameter at a given distance from the soma.

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This entire panel is designed with a future directory class in mind. As of now, you don't need to save each Neuron, so only set these if you have a specific reason for doing so.

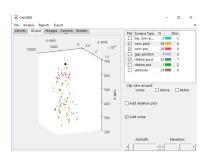
- If known, the cell type and subtype will be helpful for connectivity analysis.
- ► The other properties will eventually be used for cell queries but don't have much use yet.

After changing any of the attributes on the Cell Info Panel, make sure to press the [Add to cell data] button. This will make sure your changes are reflected next time you open the UI.

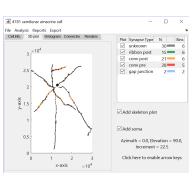
3d plot

Components

You can add and remove each synapse type, the soma node and the skeleton independently using the checkboxes. Rotate the plot with the elevation and azimuth sliders.



All amacrine cell synapses



Semilunar synapses & skeleton

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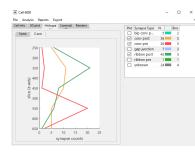
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There are two histograms. These plot synapse count as a function of:

- Distance from soma
- Section number (z-axis)

Use the Synapse Table to edit the number of bins. Add the cell skeleton to see dendrite stratification.



Z-axis synapse distribution for a putative All AC. This synapse asymmetry isn't news but is still good to see.

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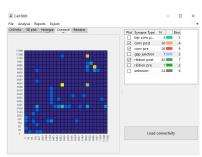
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Tulip Import

To add connectivity data, save a network map in Tulip as a JSON file, as described above. Then in Matlab:

c207.addConnectivity('c207hops.json');



The connectivity matrix is weighted by the number of unique synapses between two cells (dark blue for 0 synapses). Directed synapses will only register a contact from the pre \rightarrow post-synaptic neuron.

Connectivity for the All AC.

Connectivity

The network data is split into edges and nodes, each with their own table. The tables can be exported to Excel as .csv or as a text file from the UI menu bar.

To print a easily readable table to the command line use the networkTable function

networkTable(c207);

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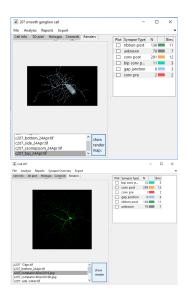
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Blender Renders and Cell Fills



For now, set the renderDir in getFilepaths.m to the file your images are saved into. The UI find the images if their filename includes the letter 'c' followed by cell number (like 'c207'). I hope to improve this at some point.

This isn't limited to renders and could include whatever images and diagrams are helpful. For example, I can compare my ON-smooth cell reconstructions and cell fills.

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This is pretty limited... Right now you can generate two reports:

- ► Location IDs of unknown synapses
- An overview of all synapse types

The report name is auto-generated and will overwrite any existing reports of the same type for that neuron. Let me know any other reports that would be of use.

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Tulip Import

Neurons do not need to be saved, as the underlying data is replaced with each update. However, if you would like to save a neuron for offline work, here's some guidelines:

Save Cell Info

After adding information to the Cell Info Panel, make sure to press the button [Add to cell info].

This saves cell attributes to the Neuron object.

Save Neuron

To save the Neuron object itself, go to File->Save Cell or save from the command line to the current directory.

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These are found in the defaults folder.

- 1. Directories to save time navigating to the correct directory each time, edit getFilepaths.m
- Cell types and subtypes go to getCellTypes.m and getCellSubtypes.m. Let me know if any are missing.
- 3. **Synapse colors** go to getStructureColors.m to change the default colors for each synapse.

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The NeuronAnalysis class helps keep population data organized by managing input parameters and results of common analyses. To create a new analysis, subclass NeuronAnalysis and edit the doAnalysis and visualize methods.

See Tutorial.m for information on these existing classes:

- ▶ DendriticFieldHull uses convex hull to estimate dendritic field area, includes methods for removing axons prior to analysis.
- ► PrimaryDendriteDiameter returns the median dendrite diameter at a given distance from the soma.

Mosaic Class

The Mosaic class is a first attempt at support for groups of neurons. This allows the most important properties of each neuron to be accessed and analyzed as a group.

Mosaic is the parent class that should not be used directly. Instead use Generic or one of the cell type specific subclasses (BipolarCells, Photoreceptors, HorizontalCells).

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These core methods are common to all Mosaic subclasses. not just Generic.

```
1 % Create a Mosaic by passing a Neuron
_2 bip = Generic (c142);
3 % Add a description of the mosaic
4 bip.describe('s-off bipolar cells');
5 % Add Neuron to the mosaic
6 bip.add(c1411);
7 % Remove a Neuron
8 bip.rmNeuron(1411); % by cell number
9 bip.rmRow(2); % by row number
10 % To view the neurons in the cmd line:
11 bip
12 % Mosaic is essentially matlab's table. To use the
       full range of table methods, cast to table:
T = mosaic2table(bip);
```

Mosaic Visualization

The Mosaic class grew from functions designed to view the photoreceptor mosaic. Accordingly, the visualization methods are most developed.

```
1 % Basic plot of cell 'somas'
2 % Plot to existing axis
3 bip.somaPlot('ax', axesHandle);
4 % Include cell ID labels
5 bip.somaPlot('lbl', true);
6 % Set the color and linewidth
7 bip.somaPlot('co', [1 0 0], 'lw', 1);
```

Additional cell-type specific parameters can be found in each Mosaic class' description.



Cone mosaic with cellID labels

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Here's some information and methods that are less essential:

- ► Links
- Tulip import method
- ▶ Will add more with next update...

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The SBFSEM-tools repository can be found on Github. I included two open source matlab toolboxes: JSONLab and the GUI Layout Toolbox.

The software used for annotations is Viking, developed by Jamie Anderson and the Marc Lab at University of Utah. Useful free programs involved in these analyses:

- ► Tulip supports graph visualization. The documentation for Tulip's Python package can be found here.
- Blender for 3D renders of neurons.

SBFSEM-tools was developed in the Neitz lab at University of Washington.

Set the file name* and file path:

```
outputFile = "C: \... \c207.json"
```

Then run these two lines:

```
1 params = tlp.getDefaultPluginParameters('JSON
     Export', graph)
```

```
2 success = tlp.exportGraph('JSON Export', graph,
     outputFile, params)
```

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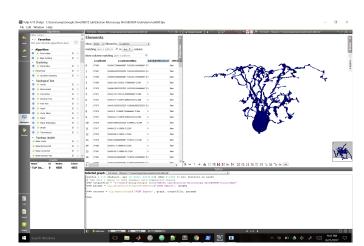
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Step One: Tulip



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Step One - Tulip

If you're comfortable with Python, you can skip opening Tulip's UI entirely.

First get the Tulip modules:

1 from tulip import tlp

1 \$ pip install tulip—python

This will allow you to export .tlp (or compressed .tlp.gz) files to JSON from a command line without Tulip's UI.

```
2 graph = tlp.loadGraph("C:\...\morph-207.tlp")
3 outputFile = "C:\...\c207.json"
4 params = tlp.getDefaultPluginParameters('JSON Export', graph)
5 success = tlp.exportGraph('JSON Export', graph, outputFile, params)
```

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Load in the JSON file and create a Neuron object:

```
1 c207 = Neuron('c207.json');
```

A dialog box will ask for the cell number and source (temporal, inferior, rc1). To avoid that, include them while creating the Neuron object:

```
1 % output = Neuron(filename, cellNumber, source);
2 c207 = Neuron('c207.json', 207, 'temporal');
```

To update the underlying data for an existing Neuron:

```
1 c207.updateData('c207.json');
```

Open up the user interface:

c207.openUl;