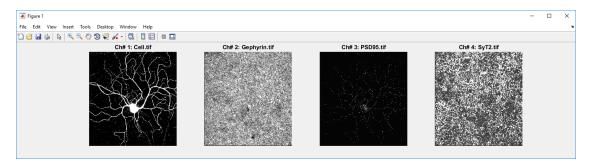
# **Object finder**

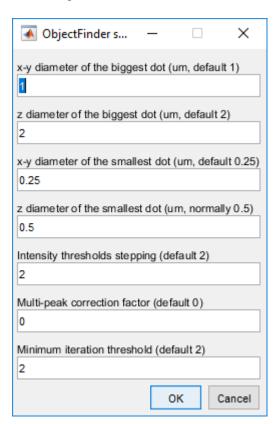
This program allows to analyze an image volume containing objects (i.e. labeling of synaptic structures) with the final goal of segmenting each individual object and computing its individual properties.

The basic steps implemented by this semi-automate approach involve:

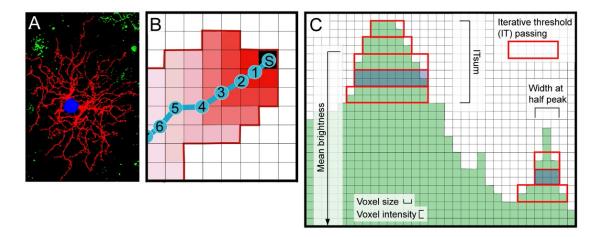
• Load image stacks of the volume to inspect and supplemental labelings (A mask is optionally provided to limit the search volume)



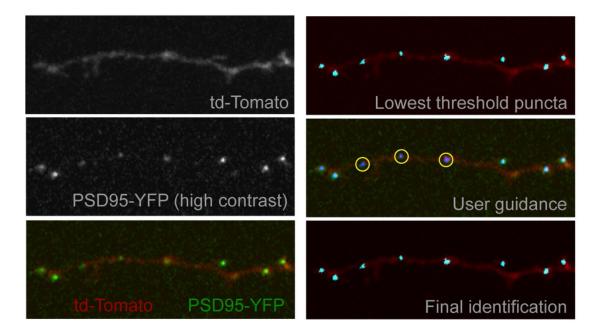
• Select options to restrict the search of candidate objects



 Candidate objects are detected automatically using an iterative thresholding method followed by watershed segmentation. This process is optimized for taking advantage of multi-core processors or processing remote clusters by multi-threading operations.



- The user is requested to filter candidate objects according to one or more parameters, by setting a threshold (usually using ITmax)
- The user refine the selection by 3D visual inspection using Imaris



- Object distribution and density is calculated in the volume or across the cell skeleton.
- If no skeleton is present, object density is plotted against Z depth

The main search loop follows the logic described in:

```
Developmental patterning of glutamatergic synapses onto retinal ganglion cells.

Morgan JL, Schubert T, Wong RO. Neural Dev. (2008) 26;3:8.
```

Originally written for the detection of postsynaptic PSD95 puncta on dendrites of gene-gun labelled retinal ganglion cells

# **Dependencies:**

- Imaris 7.2.3
- Image Processing Toolbox
- Parallel Computing Toolbox

# Change log

#### Version 3.0 created on 2017-11-03 by Luca Della Santina

- + Multi-threaded findObjects (times faster = number of cores available)
- + Complete multi-platform support (Windows / macOS / Linux)
- +  ${\tt Z}$  resolution is automatically detected from TIFF image description
- Removed median filtering of source images (unuseful to most people)
- Removed experiment detail description (unuseful to most people)
- % All settings are stored in Settings.mat (no more TPN and similar file)
- % Throw error if current working directory strucrue is invalid

#### Version 2.5 created on 2017-10-22 by Luca Della Santina

- + Display more than 4 images if present in the I folder
- + Automatically read x-y image resolution from tif files saved by ImageJ
- + Added text progress bars to follow progress during processing steps
- + Added debug=0/1 mode in subroutines to toggle text/graphic output
- Removed dependency from getVars() to get user input
- Removed redundances in user inputs (i.e. image resolution asked twice)
- Removed anaRa, anaRead, CAsampleCollect, StratNoCBmedian, Gradient
- Merged redundant scripts(anaCB/anaCBGrouped, anaRd/anaRdGrouped)
- % Unified Imaris XTensions under ObjectFinder\_ names
- % Imaris extensions provided visual confirmation dialog upon success
- % Restructured main look into 4 distinct operations for maintainability
- % Return Dots from objFinder and others instead of saving on disk
- $\ensuremath{\mathtt{\&}}$  Replaced GetMyDir dependency with pwd and work from current folder
- % filterObjects(TPN) skips questions and just reprocess Imaris dots
- % Stored ImageInfo (size,res) inside Grouped.InInfo for plotting

## Version 2.0 created on 2010-2011 by Hauhisa Okawa and Adam Bleckert

- % Improved speed of dot searching by resrtricting search within mask
- % Improved speed of dot searching by working on Uint8 values
- + Partial comments added to the original routines
- + Puncta linear density, distances now calculated along dendritic path

# Version 1.0 created on 2008 by Josh Morgan

#### **TODO**

Resolve minDotsize vs minFnalDotSize (current minDotSize fixed at 3px) Implement stepping=1 through gmode instead (current stepping of 2) Check LDSCAsampleCollect and LDSCAsampleUse calculate density differently When searching one signal (i.e. ctbp2 in IPL stack) prompt user several sample fields to choose an appropriate ITmax threshold findObjects() Post is broadcasted to every worker, slice Igm better grupFacingObjects, move this before imaris step to limit number of click

