This file contains instructions on the installation of software and how to use custom-written scripts for preprocessing and data analysis as used in the manuscript "Neural circuits in the mouse retina support color vision in the upper visual field" by Szatko and colleagues.

SYSTEM REQUIREMENTS

The software and custom-written scripts have only been tested on the Windows system.

In our study, we have used the following software:

- IGOR Pro 6.3/7.08/8.0 for Windows (Wavemetrics)
- Custom-made imaging software (ScanM v2.04 by M. Müller and T. Euler) in IGOR Pro
- Custom-written analysis code, partially based on the image analysis toolbox SARFIA in IGOR Pro (Dorostkar et al. 2010)
- Morphological reconstructions of cells: Simple Neurite Tracer plugin implemented in Fiji (https://imagej.net/Simple_Neurite_Tracer).
- For statistical analysis: R (https://www.r-project.org/) and packages lme4 and mgcv

INSTALLATION GUIDE

In order to install the above mentioned software, please follow the detailed instructions provided on the official websites (see links below). The whole procedure should not exceed 30 minutes.

In order to install IGOR Pro, visit the website:
https://www.wavemetrics.com/downloads/current.

Next, install the image analysis toolbox SARFIA (www.igorexchange.com/project/SARFIA) and custom-written software ScanM v2.04
https://github.com/eulerlab/ret_preproc/tree/development/ScanM).

DEMO AND INSTRUCTIONS FOR PREPROCESSING

In the following, we include instructions on preprocessing and analyzing an exemplary GCL scan field. Scripts used for OPL and IPL recordings use the same principle. All analysis scripts can be viewed using e.g. Notepad.

You will find the exemplary recording field (Exemplary_GCL_ScanField.pxp) in the subfolder Preprocessing.

In order to load the data, please open IGOR Pro 32-bit, go to File -> Open Experiments and choose the IGOR experiment saved as .pxp file. Loading the experiment should not exceed 30 seconds.

After loading the experiment, open the preprocessing GUI (Official Scripts, OS) that uses custom-written algorithms (ScanM -> Open OS GUI). The instructions presented in the window will guide you through the preprocessing steps. Next, open the Image Analysis window (SARFIA -> Image Analysis) that was designed for basic data analysis and displaying stacks, graphs and images.

In the Data Browser window, you will find all the recordings made for this particular field. In the name of each recording you will find the stimulus used (e.g. Chirp or MovingBars). Drag the red

arrow to the recording that you want to work on. The files within the recording already contain the regions of interest (ROIs) mask created using a semi-automatic algorithm (Autom. CellLab function in the OS GUI). To extract the traces (single trials and averages) of chirp and moving bar responses, please use the provided script AverageLoops.ipf. To estimate stimulus and event kernels, use StimulusKernels.ipf and EventKernels.ipf, respectively. Drag the selected .ipf file into the IGOR Pro workspace. In order to execute the script, type the name of the function into the command line. In the subfolder you will find the following scripts:

AverageLoops.ipf – the script detrends the traces of single ROIs, cuts the raw traces into different trials (Responses), computes the average responses (ResponsesAverage) and estimates the quality index (Quality). Adjust parameters to moving bar or chirp stimulus as described in the file.

StimulusKernels.ipf – the script detrends the traces of single ROIs and uses the matrix SC_Stimulus (stimulus sequence of UV and green center and surround) to estimate stimulus kernels from the calcium traces (Kernels). Adjust the parameter Color as described in the file.

EventKernels.ipf – the script uses the matrix Stimulus (generated by StimulusKernels.ipf) to find timepoints where both center and surround stimulus were on (onset of full-field illumination) or off (offset of full-field illumination) and estimates the mean calcium event of each ROI (Events_On, Events_Off).

ANALYSIS SCRIPTS FOR POSTPROCESSING

The following scripts were used for data analysis subsequent to preprocessing and can be found in the folder Postprocessing. Scripts are sorted in subfolders (Cones and IPL_GCL). Please download the data from the online repository (https://zenodo.org/record/3760607#.XsPLFWgzaUk). The following scripts were used for IPL and GCL data:

KernelQuality.ipf – the script calculates the area under the curve for temporal kernels and events and computes quality indices.

SpectralContrast.ipf – the script calculates the spectral contrast of center and surround using the matrix AreaKernels estimated in KernelQuality.ipf.

OpponencyEvents.ipf – the script calculates the opponency index as a correlation coefficient between the events for onset and offset full-field UV and green stimulation (Events_Correlation).

The following scripts were used for OPL recordings:

SpectralContrast.ipf – the script calculates the spectral contrast based on the area under the curve of full-field, center and surround flashes.

SortVentralDorsal.ipf – the script assigns OPL scan fields to dorsal or ventral retina depending on mean spectral contrast of center responses (estimated in SpectralContrast.ipf). This was necessary as we did not systematically record retinal orientation for OPL scans.