**Instructions for generating the sample output from the sample data**

**Using ‘single’ mode**

In Matlab command line in the folder containing importData.m, enter:

>> [test\_data\_single, metadata, exp2] = importData('single', 'test\_data\_single');

When the dialog windows pop up, select the appropriate files. First, the configuration file “config\_2exp.csv” within configFiles. Then, within sampleData/voltageTimeSeries, the 5 voltage step files with “01-3” in the name. Then, from the same folder, the metadata file “2018-06-12\_sample\_metadata.csv.” Finally, from the same folder, the two IRF files (.sdt).

When the import is queued, review the information. If it is correct, type y and hit enter.

>> [test\_output\_single] = fitDataStruct(test\_data\_single,'test\_fits\_single',exp2);

This will generate (in matlab and in the folder with the data), a struct containing the results of the global fit for the summed photon histogram across each image.

**Using ‘global\_btm’ mode**

In Matlab command line in the folder containing importData.m, enter:

>> [test\_data\_gbtm, metadata, exp2] = importData('global\_btm', 'test\_data\_gbtm');

When the dialog windows pop up, select the appropriate files. First, the configuration file “config\_2exp.csv” within configFiles. Then, within sampleData/voltageTimeSeries, the 5 voltage step files with “01-3” in the name. Then, from the same folder, the metadata file “2018-06-12\_sample\_metadata.csv.” Finally, from the same folder, the two IRF files (.sdt).

When the import is queued, review the information. If it is correct, type y and hit enter.

The images will come up sequentially, with each region of interest shown with a random color. Follow the instructions on the screen to merge, delete, and save the regions of interest as suits you.

Once this process is finished, enter the following command to perform a global fit on decays extracted from each region of interest:

>> [test\_output\_gbtm] = fitDataStruct(test\_data\_gbtm,'test\_fits\_gbtm',exp2);

This will generate (in matlab and in the folder with the data), a struct containing the results of the global fit for the summed photon histogram for each region of interest you generated.

**Using ‘global\_fiji’ mode**

\*Note that for this to work, you have to already have exported your regions of interest (ROIs) to TIFF format in ImageJ. A macro ‘exportROItoTIFF.ijm’ is provided for this in the ImageJROIs folder. You must have an image open of the same dimensions as the target image to run the script, and you will have to edit the script such that the title of this image is listed in line 3.

In Matlab command line in the folder containing importData.m, enter:

>> [test\_data\_fiji, metadata, exp2] = importData('global\_fiji', 'test\_data\_fiji');

When the import is queued, review the information. If it is correct, type y and hit enter.

Type 2 to match ROIs to files using a metadata file. When the window comes up, provide the 5 TIFF masks in sampleData/voltageTimeSeries/imageJROIs, as well as the metadata file ‘2018-06-12\_imagej\_roi\_metadata.csv’

When the import is finished, enter the following in the command line:

>> [test\_output\_fiji] = fitDataStruct(test\_data\_fiji,'test\_fits\_fiji',exp2);

Note that you can also pair ROIs and the images based on the filenames of the ROIs if they are an exact match. However, the ‘parse’ function is not supported for time series, as it uses the end of the ROI name (as \_XX) to indicate multiple ROIs for a given field of view rather than time windows.

**Using ‘pxwise’ mode**

In Matlab command line in the folder containing importData.m, enter:

>> [test\_data\_px, metadata, exp2] = importData('pxwise', 'test\_data\_px');

When the dialog windows pop up, select the appropriate files. First, the configuration file “config\_2exp.csv” within configFiles. Then, within sampleData/voltageTimeSeries, the 5 voltage step files with “01-3” in the name. Then, from the same folder, the metadata file “2018-06-12\_sample\_metadata.csv.” Finally, from the same folder, the two IRF files (.sdt).

When the import is queued, review the information. If it is correct, type y and hit enter.

When the import is finished, enter the following in the command line:

>> test\_px\_2exp = pxwiseAnalysis(test\_data\_px, ["overlay\_tm"; "photons"], 'test\_output', exp2, [1.6 2.2]);

This will perform a 2 exponential fit to the five images. It will save out two kinds of TIFF images: the photon count (effectively intensity) and an overlay of the photon count with the weighted average lifetime, tm.

**Note:** Once you have imported the data once, you can use the same imported structure to evaluate various fit models and sets of parameters by repeatedly calling fitDataStruct or pxwiseAnalysis, as is appropriate.