PuPl Manual

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1 Getting started

PuPl (**pu**pillometry **pi**pe**l**iner) is an Octave-compatible library of Matlab functions for processing pupillometry data and a user interface that lets you run these functions without having to write any code.

1.1 Initializing

Change Matlab's working directory to the PuPl folder (which should contain `pupl.m`, `LICENSE.md`, etc.). You can do this either using Matlab's "Current Folder" panel or from the Command Window using the `cd` function. Run `pupl init` in the Command Window to add the source code to Matlab's path, check the web for a new version, initialize the global data variable (by default named `eye_data`), initialize the user interface, and load whichever add-ons are in the `add-ons/` folder. Any of these last 4 steps can be skipped by adding `noWeb`, `noGlobals`, `noUI`, and/or `noAddOns`, respectively, as command-line arguments to `pupl init`.

1.2 Configuring PuPl

Most users can probably safely skip this step. If you want to change any of the global options (e.g. how many timeline steps to keep track of for undo/redo operations, what the global data variable should be called, what extension PuPl should use to save data in its native format, whether to use single or double precision), these are set early in the file `pupl_init.m`.

1.3 Getting the example dataset

At `github.com/kinleyid/PuPl-worked-example`, you can find a worked example of how to use PuPl to reproduce published results from an experiment on sustained attention [12].

1.4 Importing raw data

Under `File > Import`, you will see options for importing raw data. If the format you use is not yet supported, please send an email to kinleyid@mcmaster.ca and I can look into adding support. Note: if you import raw data from a BIDS-formatted folder [1], event logs will not automatically also be imported. See 1.6 to find out how to import event logs from a BIDS-formatted folder. If you want a relatively quick way to organize your data according to the BIDS specification, I have another project that might help: `pypi.org/project/dastr/`.

1.5 Inspecting and manipulating data

Loaded data will appear on the user interface. Hovering your cursor over the name of recording will cause a quick summary to appear (sample rate, number of events, etc.). To inspect a recording in more detail, double click it in the workspace or run `openvar eye_data`. This will open Matlab's built-in tool for viewing and editing variables. See 2 to find out how to visualize your data.

Unselected recordings are ignored by the functions run by the user interface. To delete recordings from Matlab's workspace, go to `Edit > Remove recordings`. Data is also accessible in the Command Window through the global variable `eye_data`. See 7.1 for details.

1.6 Importing event logs

After you have loaded some data, you will be able to attach event logs to it. Go to `Trials > Event logs > [Import/Import from BIDS]` to see your options for importing raw event logs. Not that this step only establishes a correspondence between recordings and event logs—to actually load events from the event logs, go to `Trials > Event logs > Synchronize with eye data`. See 1.7 for details.

1.7 Loading events from imported event logs

There are in general two situations when loading events from event logs. In the simpler and more common situation, the same computer records both eye tracker data and event log events, meaning all timestamps are recorded according the same system clock. If this is your situation, see 1.7.1. Alternatively, it could be that different computers are recording the eye tracker data and event log events, and that sync triggers are sent to both computers at the same time. If this is your situation, see 1.7.2.

1.7.1 By timestamps

Go to `Trials > Event logs > Synchronize with eye data > By timestamps` to select the events from the event logs you wish to load/attach to the eye data for further analysis.

1.7.2 By shared events

The different clocks are used to measure timestamps in the event log and the eye data may drift and/or be offset relative to one another. To solve this problem, PuPl uses linear regression to find a mapping between the two sets of sync markers. To do this, go to `Trials > Event logs > Synchronize with eye data > By shared events`. You will be able to specify which events are sync markers. After this, you will be able to select the events from the event logs that should be copied to the eye data and specify whether the pre-existing events in the eye data should be deleted.

2 Visualizing data

2.1 Plotting continuous

It is a good idea to visualize your data throughout processing to see how it is being altered. Under `Plot > Plot continuous` there are tools for plotting continuous datastreams.

2.2 Plotting gaze coordinates

To see how gaze positions are distributed for each recording, go to `Plot > Gaze scatterplot`.

2.3 Plotting pupil size

To see how pupil size measurements are distributed for each recording, go to `Plot > Pupil diameter histogram`

2.4 Visualizing pupil foreshortening error

The pupil foreshortens as it turns away from the eye tracker, being measured as smaller than it really is [2]. E.g. if the eye tracker is placed below a computer screen, the pupil will tend to be measured as smaller when looking at the top of the screen and either side. This will appear as a (roughly) linear trend in a plot of pupil size vs gaze y coordinate and a (roughly) quadratic trend in a plot of pupil size vs gaze x coordinate.

Under `Plot > Pupil foreshortening error`, there are tools to examine whether there is a systematic relationship between gaze location and measured pupil size. The tools explained in 3.11 can correct for such a relationship.

2.4.1 Plotting pupil size as a function of single gaze dimension

To plot pupil size as a function of either gaze x or gaze y coordinate, go to `Plot > Pupil foreshortening error > Pupil size vs gaze [x/y]`.

2.4.2 Plotting pupil foreshortening error surface

To visualize pupil size across the whole gaze field, go to `Plot > Pupil foreshortening error > Error surface`. This divides the gaze field into a grid and computes the mean pupil size for points falling within a square centered on each node.

2.5 Plotting epochs

Once epochs have been defined (see 5.3), they can be plotted using `Plot > Plot epochs`.

2.6 Plotting epoch sets

Once epoch sets have been defined (see 5.6), they can be plotted using `Plot > Plot epoch sets`.

2.6.1 Line plots with error bars

Under `Plot > Plot epoch sets > Line plot with error bars`, you will see options for generating these types of plots. If you select more than one recording at a time (e.g. if you plot the data for one experimental condition), you will be able to weight the individual epochs either equally/by trial (each individual epoch has an equal influence over the appearance of the final plot) or by participant (each participant's epochs are averaged together before being averaged for the final plot, i.e., a participant with 50 valid epochs would have the same influence over the appearance of the final plot as a participant with 10 valid epochs.

2.6.2 Data matrix images

This type of plot is conceptually the same as EEGLAB's erpimage (see `sccn.ucsd.edu/wiki/Chapter_08:_Plotting_ERP_images`). Essentially, it's a grid/raster image where each row is a different trial, each column is a different timepoint relative to the timelocking event, and the color of each grid cell/pixel indicates to the pupil size at that trial and timepoint. To change the colours used, see `www.mathworks.com/help/matlab/ref/colormap.html`.

3 Processing raw data

All functions for preprocessing raw data can be found under `Preprocess`.

3.1 Monocularizing recordings

If you have a lot of data from a high-quality eye tracker, you may not need to include both eyes in your analysis. To remove the pupil size data from one or the other, go to `Edit > Make recordings monocular`. If you are concerned about memory usage, you may also want to use low numerical precision and a limited undo/redo timeline—see 1.2 for more info.

3.2 Cropping recordings

There may be sections of data before, after, or between trials that are not of interest. To reduce memory usage, you can crop these sections using the tool under `Edit > Crop recordings`. If you are concerned about memory usage, you may also want to use low numerical precision and a limited undo/redo timeline—see 1.2 for more info.

3.3 Concatenating recordings

It is not unusual to record data from the same participant in several blocks, resulting in 2 or more separate data files for the same participant. To concatenate recordings together, go to `Edit > Concatenate recordings`. If the block number is of interest, you can add a suffix to every event from a given block (e.g. `_block1`, such that an event recorded as `trial1` would be renamed to `trial1_block1`).

3.4 Excluding recordings

If a participant moved around a lot during recording or if the eye tracker did not calibrate properly, the entire recording may be unusable. To exclude such recordings, go to `Preprocess > Exclude recordings`. Recordings can be excluded manually or on the following bases:

- High percent missing data
- High standard deviation of pupil size
- Low correlation between left and right pupil size measurements
- High number of rejected epochs (see 5.3)

3.5 Converting between pupil area and diameter measurements

Some eye trackers record pupil area, while others record pupil diameter. To convert between these, go to `Process > Convert pupil size`. To see which size measurement your tracker uses, read the y-axis on the plot of the continuous data (2.1)

3.6 Z-scoring data

To z-score the pupil size data, go to `Preprocess > Z-score pupil size data`.

3.7 Removing bad data samples

3.7.1 Trimming extreme pupil size measurements

Go to `Process > Trim data > Trim extreme dilation values` to remove pupil size measurements that are too high or low. In the window that opens, you can specify cutoff points as explained in B.2. Note: the gaze measurements corresponding to rejected pupil size measurements will also be removed. If you plan to run the same pipeline on multiple participants, it is best to specify relative cutoffs since different participants will likely have different average pupil size measurements (unless, of course, you z-score the data as in 3.6).

3.7.2 Trimming extreme gaze measurements

Go to `Process > Trim data > Trim extreme gaze values` to remove gaze measurements that are too extreme (e.g. off-screen or too far from a fixation cross). You can specify cutoff points as explained in B.2. Note: the pupil size measurements corresponding to rejected gaze measurements will also be removed. If you plan to run the same pipeline on multiple participants, it is best to create absolute gaze cutoffs since screen x and y values are likely to have the same meaning across participants.

3.7.3 Trimming extreme dilation speeds

Go to `Process > Trim data > Trim by extreme dilation speed` to trim datapoints that represent an abrupt change in pupil size [5].

3.7.4 Trimming isolated samples

Go to `Process > Trim data > Trim isolated samples` to remove temporally isolated islands of data that are unlikely to be meaningful or accurate measurements [5] [6]. See B.1 to understand how to specify the max length of these islands of isolated data and the maximum allowable distance from the nearest other datapoint.

3.8 Blinks

Blinks are problematic for 2 reasons: first, obviously, they represent lost data. Second, the pupil is partially obstructed shortly before and after the eye fully closes during blinks, making samples recorded near blinks unreliable. To deal with these issues, PuPl first identifies which data samples were recorded during blinks and then removes both these samples and, optionally, adjacent ones.

3.8.1 Identifying blinks

There are several methods for identifying blink samples, including the pupillometry noise [3] and velocity profile [7] methods. These are excellent methods if you're using a high-end eye tracker that records a relatively smooth drop-off and then increase in measured pupil size around blinks (see 1). However, lower-end eye trackers may not be able to do this (see 2). You should inspect your data (2.1) to see which case best matches yours. If your data does not contain the characteristic slopes used by these sophisticated algorithms, you may be best off using the simpler method of identifying blinks as sections of consecutive missing data.

Note that eye trackers are inconsistent in the way that they treat blink samples. Some set the pupil size to 0 during blinks (as in figure 1) while others treat these samples as missing (as in figure 2). The blink identification algorithms all require blink samples to be missing, so it is necessary to remove pupil size measurements below some threshold (a plausible value for the dataset in figure 1 might be 100; see 3.7.1).

3.8.2 Removing blink and possibly blink-adjacent samples

To remove blink samples and possibly blink-adjacent samples, go to `Blinks > Remove blink samples`. It is best to start by removing only the blink samples themselves (i.e. providing `0s` as input to the dialog box that asks how much data to remove immediately before and after blinks) and then inspecting the continuous data to decide whether blink-adjacent data also needs to be removed (see 2.1). If it does, click the same menu and provide a non-zero input to the dialog box this time (see B.1 for an explanation of valid inputs).

3.9 Filtering data

To apply a moving mean or median filter to pupil size or gaze data, go to `Process > Moving average filter`. If your data contains large jumps (as in figure 2), you may be best off using a moving median filter.

3.10 Downsampling

It is not uncommon to downsample data to save memory and accelerate subsequent processing. To do this, go to `Preprocess > Downsample`. If your data contains high-frequency artifacts (as in figure 2), make sure to first filter it (see 3.9) to avoid aliasing. If you are concerned about memory usage, you may also want to use low numerical precision and a limited undo/redo timeline—see 1.2 for more info.

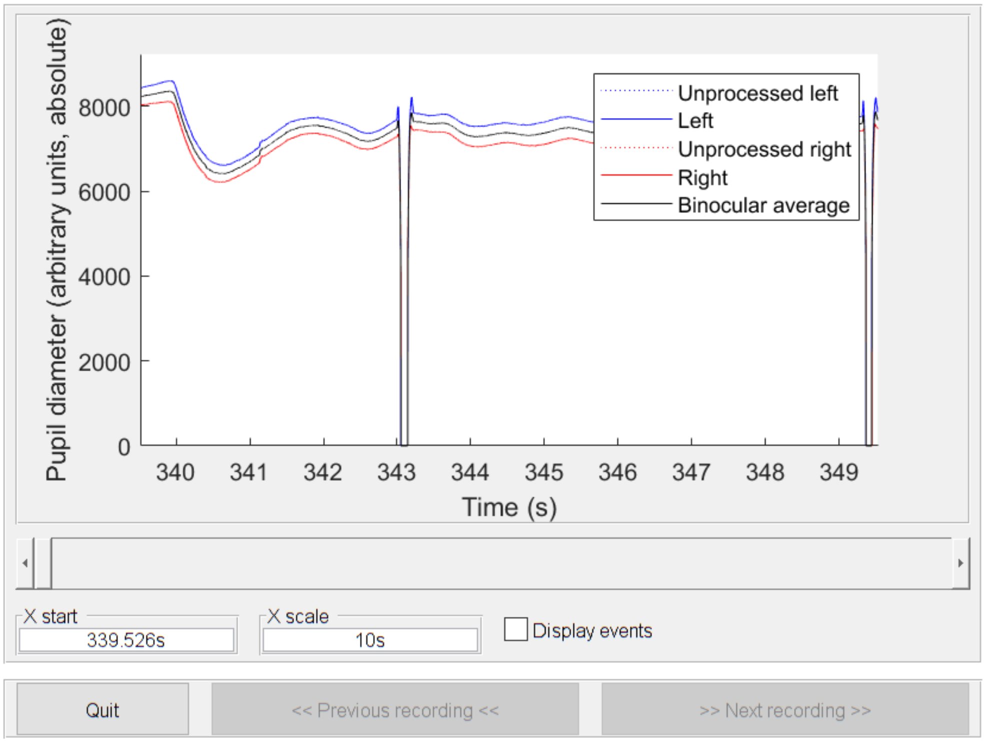


Figure 1: Data from a higher-end eye tracker

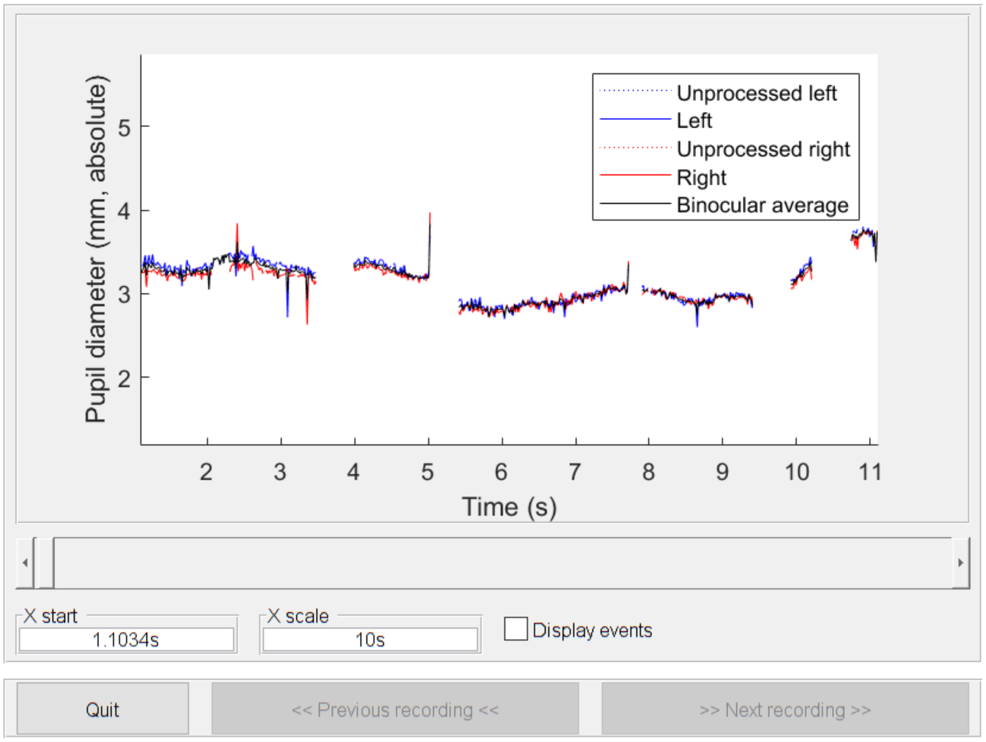


Figure 2: Data from a lower-end eye tracker

3.11 Pupil foreshortening error correction

For an explanation of pupil foreshortening error (PFE), see 2.4 [2]. The PFE can be corrected using the options under `Process > Pupil foreshortening error correction`. However, since PFE correction makes use of gaze measurements, it is important to first ensure that these are accurate.

3.11.1 Identifying saccades and fixations

Algorithms available for identifying saccades and fixations can be found under `Process > Identify saccades and fixations` [9]. Note: data points themselves are not labelled as saccades or fixations, only the periods of time between them. The justification for this is as follows: if gaze samples 1, 2, and 3 are all at the same coordinates and gaze samples 4, 5, and 6 are at a far-away coordinate, it doesn't make sense to label any of those points themselves as a saccade. Clearly the participant was fixating at points 1, 2, and 3 and then again at points 4, 5, and 6, and the saccade took place between points 3 and 4.

3.11.2 Mapping to fixations

After identifying fixation periods, the data during these periods can be reassigned to their centroids (spatial means; [9]) using `Process > Map gaze data to fixation centroids`.

3.11.3 Geometric PFE correction

The pupil foreshortening error can be corrected using straightforward geometry if the coordinates of the experimental setup are provided [2]. First, gaze coordinates need to be converted from units of pixels to units of millimeters; go to `Process > Pupil foreshortening error correction > Convert gaze units from pixels to millimeters` to do this. Then, you can add the coordinates of the experimental setup under `Process > Pupil foreshortening error correction > Add coordinates of experimental setup`. A dialog box will appear explaining the coordinate system to use when providing these coordinates. Once you have done so, go to `Process > Pupil foreshortening error correction > Geometric PFE correction` to apply the geometric correction.

3.11.4 Detrending PFE correction

Options to correct for a linear relationship between pupil size and gaze y position and a quadratic relationship between pupil size and gaze x position can be found under `Process > Pupil foreshortening error correction`. Note that at the time of writing these tools have not been thoroughly tested and use only a first approximation of the PFE.

3.12 Interpolating missing data

To interpolate missing pupil size and gaze data using either linear interpolation or cubic spline interpolation, go to `Process > Interpolate missing data`. Dialog boxes will open asking for the maximum length of time and the maximum distance to interpolate over (see B.1 and B.2 for explanations of how to provide input for these, respectively). Note: it is a good idea to be conservative when specifying the maximum gaze distance to interpolate over since, if saccades take place during long periods of missing data, linear interpolation will make these look like periods of smooth pursuit.

4 Saving data

To save data, go to `File > Save`. A separate filesave dialog will open for each active recording. The `.pupl` extension on saved recordings is merely an alias for version 6 `.mat` files, which are readable using Octave.

4.1 Batch saving data

To save all active data to the same folder using filenames corresponding to recording names, go to `File > Batch save`. This will open a single folder selection dialog.

4.2 Saving data to BIDS format

If you loaded your data from a BIDS-formatted folder, you can likewise save it to one under `File > BIDS > Save` (e.g. you may want to save your formatted data to a `sourcedata/` folder for quick loading later using `File > BIDS > Load`).

5 Working with trials

5.1 Event variables

Event variables refer to variables such as numbers or strings that are associated with events. By default, events only have two attributes of interest: their name and their time of occurrence. However, you may want to add other attributes, such as whether an event was the onset of a congruent or incongruent Stroop trial. The following sections explain how to do this.

5.1.1 Reading event variables from event names

Often, event variables are encoded in event names. E.g. the onset of a congruent trial Stroop trial may be called `trial_cong1` and the onset of an incongruent trial may be called `trial_cong0`. To read these variables, we use regular expression captures, which search for for a given pattern in a piece of text and return anything that matches.

In the example dataset (see 1.3), events have names like `Response:2 Hit=2/3,FA=1`. The numbers after `Hit=` indicate the number of detected gaps and the number of gaps total and the number after `FA=` indicates the number of false alarms, all of which are of interest.

Regular expressions capture information using round brackets. For example, the regular expression `Hit=(.)/(.)` would look for the string `Hit=`, then get the character immediately following that, then skip over the following backslash and get the following character. I.e. running that regular expression on `Hit=1/2` would return `1` and `2`.

You can read event variables from event names in this way using the tool under `Trials > Event variables > Read event variables from event names`. You will be able to:

1. select a subset of events to read event variables from (e.g., all those containing the string `Hit=`; see B.4)
2. provide a regular expression that will capture your variables of interest (e.g., `Hit=(.)/(.)`)
3. name the resulting event variable(s) (e.g., `#nhits; #maxhits`)
4. specify whether these should be converted to numbers or left as strings (we would want to convert `#nhits` and `#maxhits` to numbers)

5.1.2 Manually entering event variables

It is possible (though not efficient) to manually enter event variables using Matlab's variable viewer. This can be done by running `openvar eye_data`and then clicking on the structures under `event`. By populating a new column in the table that appears, you will be adding a new event variable.

5.1.3 Computing event variables

If you want to compute higher-order event variables (e.g., hit rate = n. hits / max. possible hits), go to `Trials > Event variables > Compute event variables`. You will be able to:

1. select a subset of events on which to compute your higher-level event (e.g., all those where `#nhits` is not empty; see B.4)
2. specify an expression that will return a new event variable (e.g., `#nhits / #maxhits`)
3. specify the name of the resulting event variable (e.g. `#HR`)

4. specify whether the resulting variable should be converted to a string or converted to a number (we would select "Numeric" for `#HR`

5.1.4 Homogenizing event variables within trials

Usually, there will be information about a participant's response encoded in the name of the event that marks that response (e.g. key pressed, hit or miss). However, often we want to use trial onsets, not responses, as timelocking events for event-related pupillometry (see 5.3). To deal with this scenario, it makes sense to read the response-related event variables from the names of the events that mark responses and then copy them to all the other events occurring within their trials (including the events that mark a trial's onset).

In the example dataset, we want `#HR` to be an attribute not only of the events that summarize the preceding trials, but also of the events that indicate the onset of a trial (which we will later use as timelocking events; see 5.3).

To accomplish this, go to `Trials > Event variables > Homogenize event variables within trials`. You only need to specify which events mark the onset of a trial and which events mark the end of a trial (in the example dataset, these are events whose names contain the string `Scene` and events whose names contain the string `Response`, respectively).

5.1.5 Mapping string event variables to numeric event variables

Matlab does not treat strings as atomic/scalar data types, meaning that, for example, the expression `'wxyz' == 'abc'` does not just return `false` but an error. To compare strings, you have to use the `strcmp` function. If you want to select events on the basis of a event variable which has been stored as a string (see B.4), it can be cumbersome to repeatedly type `strcmp`.

One solution to this is to map each possible value of a string event variable to a different number. For example, you might have a event variable called `#congruency` that takes on the values `'con'` or `'inc'` to specify whether the corresponding Stroop trial is congruent or incongruent. You could map these to the values 1 and 0 and name the resulting variable `#iscongruent`.

To do this, go to `Trials > Event variables > Make string event variables numeric`.

5.1.6 Computing reaction times

To compute reaction times, go to `Trials > Event variables > Compute reaction times` to specify which events mark trial onsets and which trials mark responses. Reaction times are stored in the event variable `#rt` and are associated with both the trial onset events and the response events.

5.2 Defining compound events

Often, we want to analyze responses to specific serial combinations of events (e.g. a trial of a certain type followed by a response of a certain type). To define these "compound events", go to `Trials > Find compound events`.

The steps involved in this are the following:

1. specify the name of the compound event you wish to define
2. specify the "primary" events (these are the events whose onset times will be used as the onset times of your compound event)
3. specify the "secondary events" that, if they occur in some serial position and/or time window relative to the time-locking events, will indicate the presence of your compound event
4. optionally, specify a time window centered on the primary events in which the secondary events must occur (e.g. Start: `-2s`, End: `0s`; see B.1 for an explanation of how to specify this window; if none is specified, it defaults to the entire duration of the recording)

5. optionally, specify the relative serial positions, at one of which the secondary events must occur (these can be specified using Matlab's colon operator, e.g. `-3:-1`; if left empty, any serial position is allowed)
6. optionally, specify a criterion, based on event variables, that will determine whether a secondary event has been found. You can use event variables from both primary and secondary events to define this criterion. Event variables prefixed with `#1` (e.g. `#1rt`) will be read from the primary event and event variables prefixed with `#2` (e.g. `#2rt`) will be read from the candidate secondary event (e.g. `#2rt >= #1rt` sets the criterion that secondary events must have reaction times at least as long as their primary events; if left empty, no such event variable-based criterion will be used)
7. specify whether compound events should be marked based on the presence or the absence of the secondary events within the specified window and/or serial position list
8. specify what should be done when a compound event is found (options are to rename the primary event or add a event variable to the primary event)

If you choose to add a event variable to the primary event, the procedure for doing this is the same as in 5.1.3, with one difference: event variables prefixed with `#1` (e.g. `#1rt`) will be read from the primary event and event variables prefixed with `#2` will be read from the first found secondary event.

5.2.1 Example: computing reaction times the long way

To compute reaction times using compound events, you would:

1. set the primary events to be trial onsets
2. set the secondary events to be responses
3. leave the relative time window empty, since there is not reason not to compute reaction times that are greater than, e.g., 3 seconds
4. set the relative serial positions to `1` (assuming there are no other events that occur between trial onset and responses)
5. specify that compound events should be marked by the presence rather than the absence of the secondary events
6. specify that you want to add a event variable to the primary events
7. specify the expression to compute said event variable as `#2time - #1time` (the time of the response minus the time of the trial onset)
8. give the resulting event variable the name `#rt`
9. specify that `#rt` is a numeric variable

5.3 Defining epochs

Epochs are windows of data defined relative to timelocking events. Epochs can be defined using the tool under `Trials > Define epochs`. You can specify the timelocking events (as explained in B.4) and the relative starts and ends of the time windows centred on the timelocking events (as explained in B.1).

In the example dataset, trials should be extracted from `0s` to `29.5s` relative to each event whose name contains the string `Scene`.

5.4 Baseline-correcting epochs

Due to variability in pre-trial pupil size measurements, you may wish to baseline correct your epochs (however, be aware that baseline pupil size can contain valuable information—for example, pre-decision pupil size could reflect bias toward exploration or exploitation [4]).

Pupil size can be baseline corrected by subtracting the baseline mean, computing the percent change from baseline mean, or z-scoring based or baseline statistics (i.e. subtracting baseline mean and normalizing by baseline standard deviation).

To do this, go to `Trials > Baseline correction`. The mapping from baseline periods to trials can be one-to-one (e.g. baselines periods are the 200 ms before each event onset), one-to-all (e.g. one single baseline period occurs at the beginning of the experiment), or one-to-some (e.g. one baseline period occurs before a block of 5 trials). If the one-to-some mapping is selected, trials are baseline-corrected using the most recent baseline period. The durations of baseline periods can be specified as explained in B.1.

5.5 Rejecting epochs

Epochs can be marked for rejection using the tools under `Trials > Epoch rejection` on the following bases:

- proportion missing data
- presence of extreme pupil size measurements
- presence of blinks (see 3.8)
- presence of saccades (see 3.11.1)
- event variables (see B.4)

It is also possible to reject trials on the basis of the amount of data missing within a specific window (e.g. the baseline period) using `Trials > Epoch rejection > Reject by proportion missing data within window`.

5.6 Defining epoch sets

It is useful to average together trials of multiple types to get reliable estimates of pupillary response. E.g. you may want to examine responses to both a particular stimulus and a category of stimuli to which it belongs. To define sets of epochs for later analysis, go to `Trials > Define epoch sets`. Epochs can be selected as in B.4.

Note: even when you do this the first time, you will be asked if you want to overwrite the pre-existing trial sets. This is because initially extracting trials initially trial sets with a one-to-one mapping to trial types.

6 Exporting for statistical analysis

By design, PuPl does not contain its own built-in statistical analysis tools. Instead, it contains a flexible set of data export options that allow for subsequent analysis by dedicated statistical software such as the R programming language [8], which are far more comprehensive that PuPl could ever hope to be.

6.1 Exporting statistics

To export statistics (e.g. mean pupil diameter) as a spreadsheet, go to `Experiment > Write statistics to spreadsheet`. You have the option to compute statistics on individual epochs or on averages of epochs within epochs sets.

You can compute multiple statistics within different time windows. For example, you may want to compute the mean in a baseline period (e.g., from `-1s` to `0s` relative to timelocking events) and the max pupil size during the actual trial (e.g., from `0s` to `5s`).

Computing statistics on individual trials enables the use of linear mixed effects models, which are powerful tools for detecting effects of interest while ignoring noise (e.g. between-subjects differences). These models enable you to, for example, measure effects on baseline pupil size while still being able to perform baseline correction (by simply adding baseline mean as a predictor term in a mixed effects model of pupil dilation response).

6.2 Exporting data

You can export epochs to a spreadsheet either as raw data or as downsampled/binned data by going to, respectively, `Experiment > Export undownsampled data` or `Experiment > Export downsampled/binned data`.

7 Using PuPl programmatically

7.1 Processing data in the command window

The central data structure used by PuPl is by default called `eye_data`. This is the variable that the user interface operates on. After initialization, this variable will appear in Matlab's global workspace. You can manipulate it the way you would any other variable using the Command Window.

If you are about to alter the data variable and want the option to return it to its present state, run `pupl cache`. This saves a copy of the data that can be reinstated using `Edit > Undo`.

If you process data in the Command Window and want to update the appearance of the user interface (e.g. if you have defined epochs in the Command Window and want the `Plot epochs` menu to be enabled), run `pupl redraw`.

7.2 Automating processing pipelines

When a processing function is run, it saves the equivalent command as a string to the `history` field of the data. All of these commands together constitute a processing pipeline. To export the processing history to a Matlab script, go to `Tools > Save processing history as script`. To view the processing history within the command window, run `pupl history`. To run a processing pipeline on the currently active data, go to `Tools > Run processing script`.

A Worked example

To demonstrate how PuPl works, I have prepared a worked example based on the data from [12]. I am extremely grateful to Drs Zhao and Chait for their help in understanding the dataset. The following explains how to reproduce the results in their paper.

A.1 Getting the data

First, download the repository `github.com/kinleyid/PuPl-worked-example` and unzip it in some folder on your computer. Then unzip the file `data-raw.zip`. This folder contains the raw EDF files. The names of these files indicate the subject number and block number.

If you want to convert the data to BIDS format, you can run the script code/toBIDS.py, which relies on dastr (`pypi.org/project/dastr/`).

There are 198 individual recordings, each of which is roughly 23-24 MB, so it takes a long time to load all the data. The best approach is to first load in the first 6 recordings (cS1_b1.edf, cS1_b2edf, ... , cS1_b6edf), which are blocks 1–6 for participant 1. The import tool to use for this is `File > Import > From EyeLink EDF`.

A.2 Preparing the data

First, remove the data from the right eye (see 3.1). Then, concatenate the recordings together (see 3.3). You can manually select first cS1_b1, then cS1_b2, then cS1_b3, etc., but this will mean that the processing history will only be applicable to these first 6 recordings (see 7.2). Since we want to eventually use the processing history as a macro for processing the entire dataset, it's best to select the first recording using

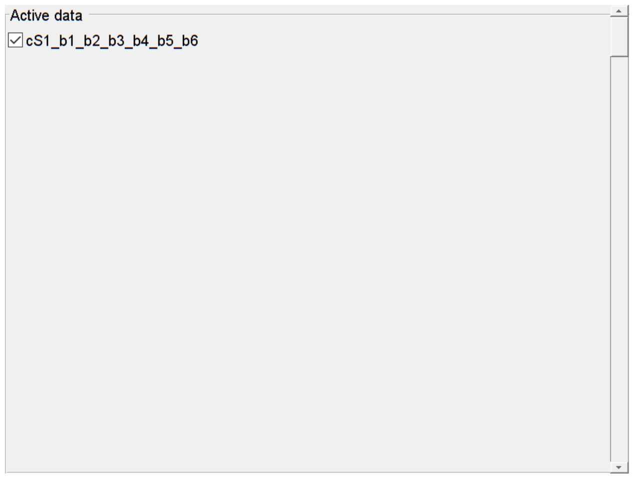


Figure 3: The user interface, after concatenating the first 6 recordings

the regular expression “b1”, the second using the regular expression “b2”, the third using “b3”, etc. (see B.3).

If you add the suffix “_b2_b3_b4_b5_b6” to the first recording, the output in the Command Window should appear as follows:

```
Concatenating as follows:
    cS1_b1_b2_b3_b4_b5_b6 = cS1_b1 + cS1_b2 + cS1_b3 + cS1_b4 + cS1_b5 + cS1_b6
```

And the user interface should now show only one recording (see figure 3.

A.3 Processing the continuous data

A.3.1 Trimming pupil size 0 samples

By inspecting the continuous data (see 2.1), you will see that blink samples are measured as 0 rather than missing. Blink correction requires these samples to be marked as missing (i.e., set to NaN), so first trim all pupil size measurements below 200 (see 3.7.1). The upper pupil size cutoff can be set to **inf** to avoid cutting any data from the higher end of the pupil size distribution (see figure 4).

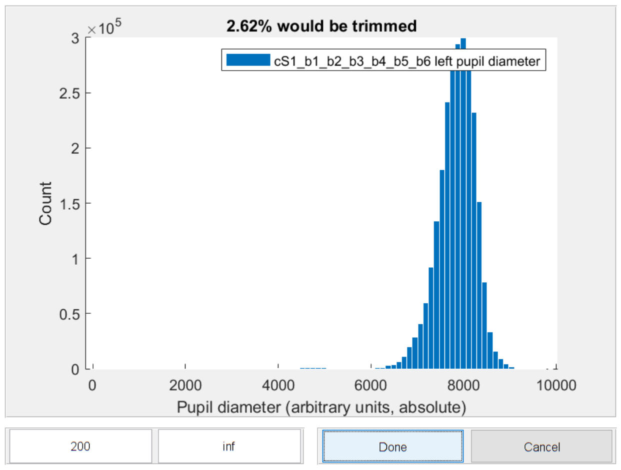
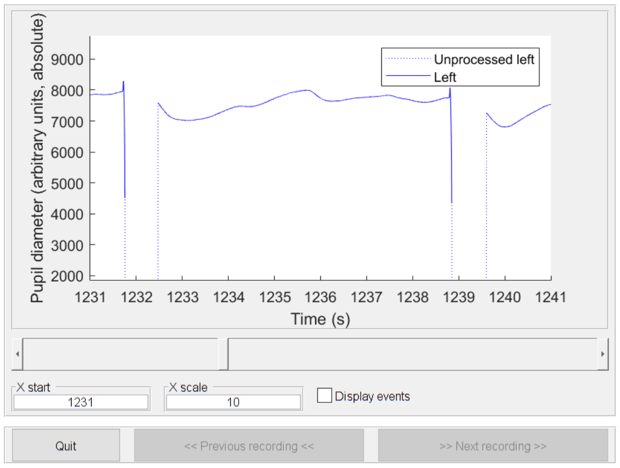


Figure 4: The user dialog for trimming extreme pupil size measurements

A.3.2 Correcting blinks

You can use any of the blink identification algorithms (see 3.8), but for the purpose of this example I am using the simple consecutive missing data method with minimum blink length 50ms and maximum length 1s. This may seem like an implausibly large range, but there are some very long blinks in this recording (see figure 5).



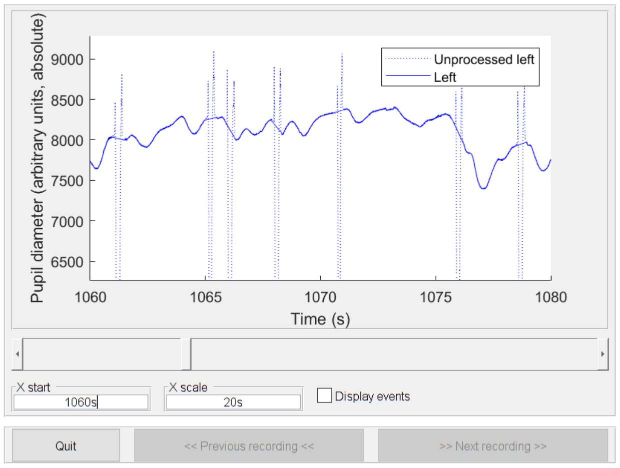
X start: 1231
X scale: 10

Figure 5: The big blinks

After identifying blinks, trim 200ms of blink-adjacent data on either side. (Note that, if you have access to Matlab's curve fitting toolbox, you should use the pupillometry noise method since it allows you to remove less data [3]).

After this, linearly interpolate (see 3.12) over a max. of 1.2s and across a maximum gap of 3 standard deviations (i.e. `3‘sd`; see B.2). If you inspect the data (see 2.1), you will see that this does a reasonable job of correcting for blinks (see figure 6).

Note that trials are approximately 30 seconds long in this experiment. For with shorter trials, it is probably unwise to interpolate across so much data (1.2 seconds could contain most of the pupil dilation response being analyzed in a short trial).



X start: 1060s
X scale: 20s

Figure 6: Bink-corected data

A.3.3 Filtering

To deal with any high-frequency artifacts in the data, use a Hann window moving average filter with a width of 150ms (see 3.9). This step smooths the data (see figure 7).

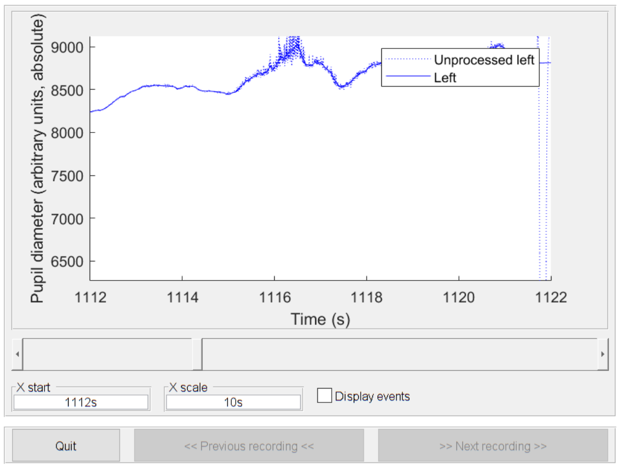


Figure 7: A high-frequency artifact has been smoothed

A.3.4 Downsampling

To speed up subsequent analysis, you can downsample by a factor of 50 to obtain data with a new sample rate of 20 Hz (see 3.10).

A.4 Working with trials

A.4.1 Reading event variables

Trials have the following structure in this experiment:

1. `Trial:[n] [stimulus information]`
2. `Trial:[n] EndStim`
3. `Response:[n] Hit=[n. hits]/[max. hits],FA=[n. false alarms]`

We want to compute the hit rate and the number of false alarms in order to exclude trials where participants may not have been on task.

To compute the number of false alarms, read event variables from events whose names contain the word `Response`. The regexp to use for reading from the event names is `FA=(\d+)` (see B.3) and the resulting event variable can be named `#FA` and will be numeric (see 5.1).

To compute the hit rate, we need to first read the number of hits and the max. possible hits. To do this, read event variables from events whose names contain the word `Response` again. This time, the regexp to use for reading from event names is `Hit=(\d)/(\d)` and the event variables can be named `#n_hits` and `#max_hits` (both numeric).

Next, to actually compute hit rate, we divide the number of hits by the max possible hits. To do this, we compute an event variable (see 5.1.3) for events whose names again contain the word `Response`. The expression to compute the new event variable will be `#n_hits / #max_hits` and the resulting event variable can be called `#HR` (it will be numeric).

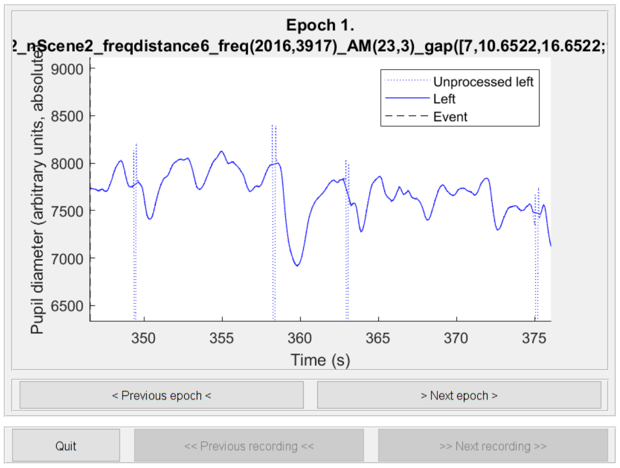


Figure 8: Plot of a single epoch

A.4.2 Homogenizing event variables within trials

So far, the event variables that have been derived are attached to the `Response` events. However, we want to attach these to the corresponding `Trial` events, which will be used as timelocking events for epoching.

Therefore we will homogenize event variables within trials (see 5.1.4). Trial onsets will be marked by events whose names contain the word `Scene` and trial ends will be marked by events whose names contain the word `Response`.

A.4.3 Epoching

The next step is to define epochs (see 5.3). Epochs will be defined relative to events containing the word `Scene`, from the onset of these events (`0s`) til `29.5s` afterward. After epoching, you will be able to visualize individual epochs (see figure 8).

A.4.4 Baseline correction

Epochs are baseline corrected by subtracting the average pupil diameter between `4s` and `4.5` after the event signalling the onset of a trial (there will be a one-to-one mapping between baseline periods and epochs; see 5.4).

A.4.5 Rejecting epochs

Epochs will be rejected if they contain more than 1 false alarm or if the hit rate is any less than 100%. We will therefore be rejecting epochs on the basis of event variables (see 5.5).

Epochs will be rejected according to the expression `#HR < 1 | #FA > 1`. In total, 7 epochs will be rejected from this first set of recordings.

A.4.6 Defining epoch sets

The next step is to group epochs by difficulty level (easy, medium, or hard). The difficulty level is encoded in the names of the trial onsets: `Trial:[n]...Scene[x]...` where `x` is 1 for easy, 2 for medium, and 3 for hard. Create the epoch set `Easy` out of epochs containing the characters `Scene1`; the set `Medium` out of the epochs containing the characters `Scene2`; and the set `Hard` out of the epochs containing the characters `Scene3` (see 5.6). Each epoch set should contain 24 trials. Once you've done this, you can plot these epoch sets to obtain figure 9 (see 2.6.1).

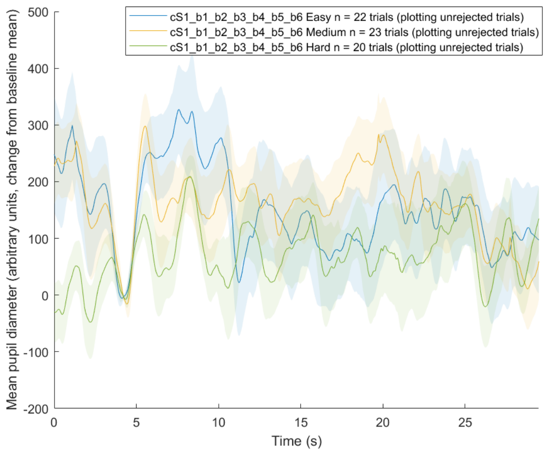


Figure 9: The epoch sets for participant 1

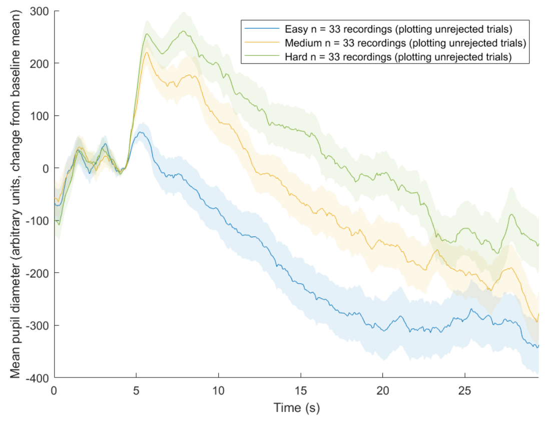


Figure 10: The epoch sets for the entire dataset

A.5 Processing the entire dataset

The processing steps outlined above define a general-purpose pipeline that can be re-used on the entire dataset. To do this, first export the processing history as a script (see 7.2). It should match the annotated script `code/preprocess.m`.

Next, remove the processed data from PuPl's workspace (see 1.5) and import all of the EDF files in `data-raw/` (this step takes around a half hour on my laptop, since there are 198 in total).

Once the data has been imported, you can process it all at once using the script you exported (see 7.2). This step is also fairly time-consuming for me, but hopefully you have a more powerful computer.

Once the analysis script has finished running, you can plot the epoch sets (grand average by condition, using the default unlabeled condition that contains all recordings; see 2.6.1) to obtain figure 10.

A.6 Exporting and analyzing the data

To actually statistically analyze the data, you have to export it to a csv table (see 6).

We want to know whether the pupil diameter over the course of the 25-second trial differs depending on the difficulty level (just imagine it's not blatantly obvious from figure 10).

The sample R code in the file `code/analysis.R` will run the statistical analyses and generate the plots in this section.

A.6.1 Exporting for standard analysis

For standard statistical analysis, we use a participant's average pupil size within trials of a certain type as the unit of analysis. To do this, go to `Experiment > Write statistics to spreadsheet` and specify that epoch set averages (rather than individual epochs) should be analyzed.

Specify that the data of interest occurs between `4.5s` and `29.5` into the trial. Name this statistics window "trial" and specify that the mean pupil size should be computed in it (as well as whatever other statistics you may be interested in).

Save the table to `export/stats-basic.csv` and then run the first section of `analysis.R` to generate the following ANOVA table:

```
Analysis of Variance Table

Response: trial_mean
           Df  Sum Sq Mean Sq F value    Pr(>F)
difficulty  2  909080  454540  10.283 8.986e-05 ***
Residuals  96 4243475   44203
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

You can also run a linear model to estimate the magnitude of the effect of difficulty:

```
Call:
lm(formula = trial_mean ~ difficulty, data = data)

Residuals:
    Min      1Q  Median      3Q     Max
-397.94 -166.32   -2.27  136.68  648.19

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)       -200.11      36.60  -5.468 3.61e-07 ***
difficultyMedium   139.11      51.76   2.688  0.00848 **
difficultyHard     233.29      51.76   4.507 1.85e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 210.2 on 96 degrees of freedom
Multiple R-squared:  0.1764,Adjusted R-squared:  0.1593
F-statistic: 10.28 on 2 and 96 DF,  p-value: 8.986e-05
```

Finally, you can generate figure 11.

A.6.2 Exporting for mixed effects analysis

For mixed effects models, the unit of analysis is the mean pupil size during a single trial. To export this type of data, specify that each epoch should be analyzed individually.

Again, define the window of interest as `4.5s` into the trial until `29.5s` into the trial, name this window "trial", and specify that the mean pupil size should be computed in this window. Save the resulting table to `export/stats-long.csv`.

The script `analysis.R` contains code to generate the following summary of a linear mixed effects model fit using the Analysis of Factorial Experiments (afex) package [10]:

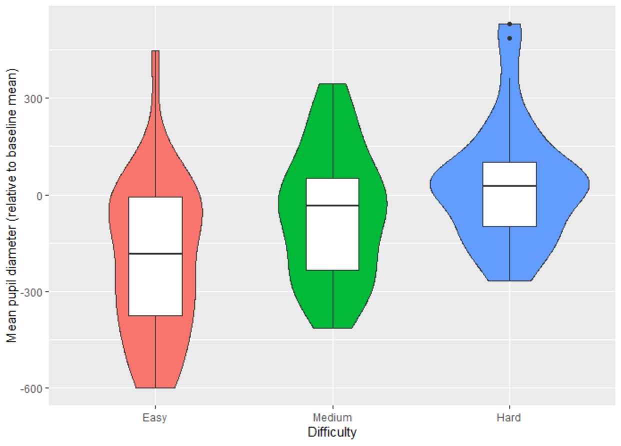


Figure 11: Mean pupil diameter by difficulty level

```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: trial_mean ~ difficulty + (1 | recording)
   Data: subset(data, rejected != 1)

REML criterion at convergence: 26364.3

Scaled residuals:
    Min      1Q  Median      3Q     Max
-4.1244 -0.5964 -0.0412  0.5418  6.1894

Random effects:
 Groups    Name        Variance Std.Dev.
 recording (Intercept)  28423   168.6
 Residual              192619   438.9
Number of obs: 1754, groups:  recording, 33

Fixed effects:
                 Estimate Std. Error      df t value Pr(>|t|)
(Intercept)       -202.85      33.65   43.88  -6.029 3.09e-07 ***
difficultyMedium   149.36      24.29 1723.07   6.149 9.67e-10 ***
difficultyHard     245.02      27.04 1728.04   9.061  < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) dffclM
diffcltyMdm -0.328
diffcltyHrd -0.294  0.418
```

A.6.3 Exporting downsampled data

If you want to generate your own plots from scratch, you can export the data itself (rather than summary statistics such as mean pupil size) to a table, downsampled so that the size is not unmanageable, in wide or long format.

To do this, go to `Experiment > Export downsampled/binned data` and specify that you want to export epoch averages (exporting individual epochs generates a table that's approx. 140,000 lines long) and specify the following downsampling parameters:

Start: `0s`
Width: `500ms`
Step size: (leave empty)
End: `29.5s`

This will compute pupil size in averages of 500-millisecond chunks, which will result in a smaller output file.

Output the data in long format, which is well-suited for plotting with R, as a file called `export/ds-long.csv`.

The script `analysis.R` contains code to generate figures 12 and 13. Users who are familiar with ggplot [11] can will probably be able to generate even more sophisticated visualizations.

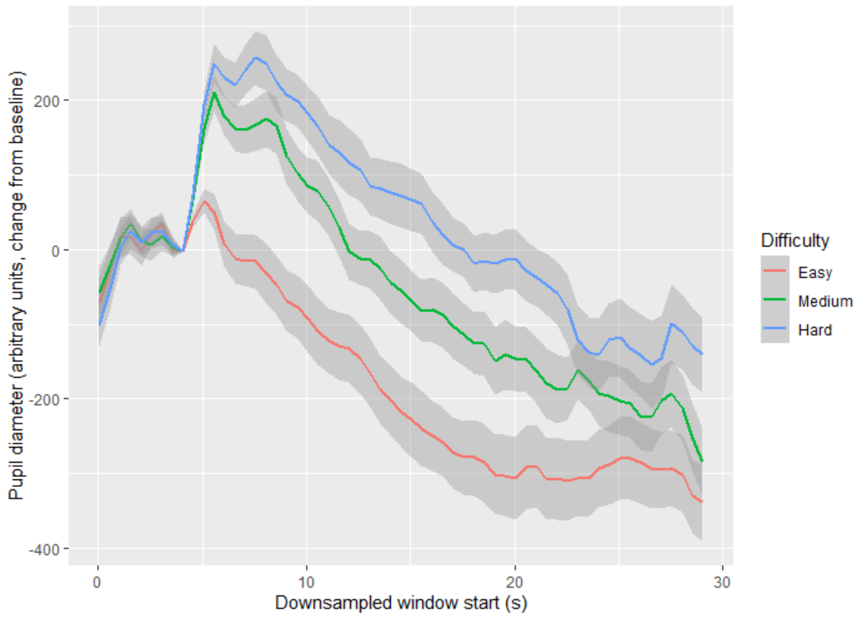


Figure 12: Pupil size over the course of a trial, by difficulty. Error bars reflect the standard error of the mean.

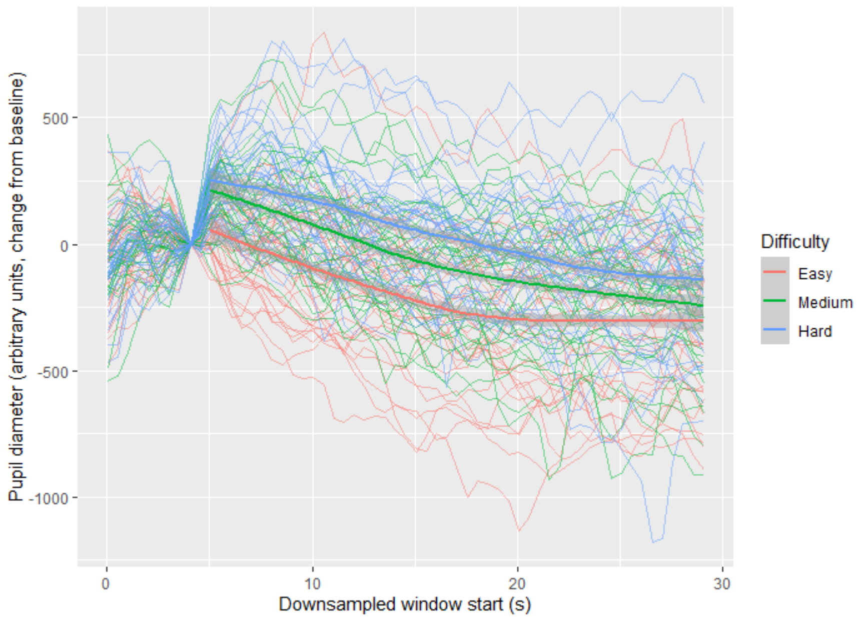


Figure 13: Pupil size over the course of a trial, by difficulty, showing each participant's average plus a LOESS estimate of the average by difficulty level.

B Recurring user dialogs

B.1 Specifying durations

You may use units and arithmetic to specify lengths of time. Valid units are as follows:

- `ms`: milliseconds
- `s`: seconds
- `m`: minutes
- `dp/d`: datapoints

For example, `1s + 2 dp - 100 ms - 2d` translates to one second plus two datapoints minus 100 milliseconds minus two datapoints.

B.2 Specifying relative quantities

You can specify relative quantities by writing any valid Matlab expression using `$` to refer to the data in question. E.g. `max($)` would return the max. You can also use the following shortcuts to compute common statistics (note that the backticks are to avoid conflicts with other Matlab expressions):

- `` `mu ``: mean
- `` `md ``: median
- `` `mn ``: min
- `` `mx ``: max
- `` `sd ``: standard deviation
- `` `vr ``: variance
- `` `iq ``: interquartile range
- `` `madv ``: median absolute deviation

- `%`: percentile (e.g. `15%` computes the 15th percentile)

E.g. `‘mn - 2‘sd` would compute the mean minus two standard deviations. Note that this would be much faster than `mean($) - 2*std($)`, because the latter requires converting the data to a string, substituting that string for every instance of `$`, and evaluating the resulting expression.

B.3 Regular expressions (regexp)

Many of PuPl's user dialogs contain input fields that are intended for regular expressions. A regular expression is a string of characters that specifies a search pattern. For example, the regular expression "abc" would look for any instance of "abc" ("abcdef" would be a match, "acb" would not).

Regular expressions can have special characters to make searches very specific (or very general). A few useful examples:

- a period matches any character. So the regular expression "a." looks for an "a" followed by any character ("abcdef" and "acb" would both match, "cba" would not because the "a" is not followed by another character).
- a plus means "one or more instances of the immediately preceding pattern." For example, "ab+" would look for "a" followed by one or more instances of "b" ("abb" and "abc" would match, "acb" would not).
- an asterisk means "zero or more instances of the immediately preceding pattern." For example, "ab*" would match "abb", "abc", and "acb".
- "\d" matches any digit from (0-9).
- square brackets match any character within them. For example, "a[bc]" looks for an "a" followed by either a "b" or a "c" ("abc" and "acb" would both match, "aa" would not).

A full list of Matlab's regular expression rules can be found here: `mathworks.com/help/matlab/ref/regexp.html#btn_p45_sep_shared-expression`.

Regular expressions can be frustrating at times, but they're extraordinarily useful once you get the hang of them. When I'm struggling to pick the right regular expression, I find it helps to remember that the computer is doing exactly what I'm telling it to.

B.4 Selecting events

Many of PuPl's tools require you to select a subset of events. (e.g. timelocking events for epoching; see 5.3). When this occurs, a window called "Event filter" will appear (figure 14). This window allows you to select subsets of events either manually, by regular expression, or by event variables (see 5.1).

B.4.1 Manual event selection

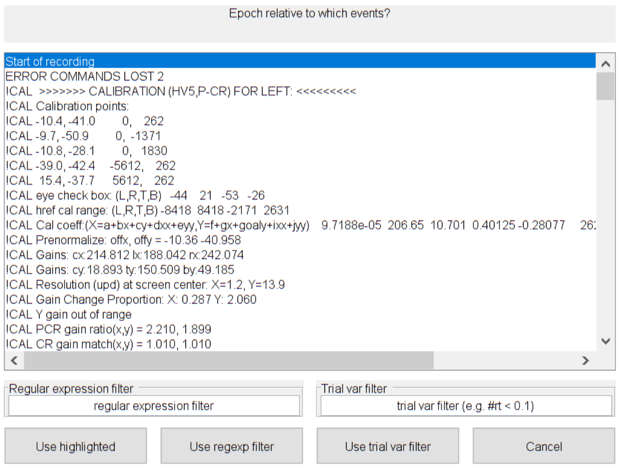
To select events manually, simply highlight them by hand.

B.4.2 Regular expression filter

To select events whose names match a certain pattern, put that pattern in the box labeled "Regular expression filter" (see B.3). E.g. if you wanted to select all events whose names include the word `Response`, you would write `Response` in that box (then press enter to highlight the events that include that word).

B.4.3 Event variable filter

To select events that meet some criterion defined by their event variables (see 5.1), put an expression that returns `true` if that criterion is met in the box labeled "Trial var filter". To refer to a event variable, prefix its name with a hash symbol. For example, if you wanted to select events with reaction times (see 5.1.6) greater than 500ms, you would write `#rt > 0.5` in that box (then press enter to highlight the events that meet that criterion). Entering `regexp(#name, 'x'` would be the same as writing `x` in the regexp filter box.


Epoch relative to which events?
Regular expression filter
regular expression filter
Trial var filter
trial var filter (e.g. #rt < 0.1)
Use highlighted
Use regexp filter
Use trial var filter
Cancel

```
Start of recording
ERROR COMMANDS LOST 2
!CAL  >>>>>>> CALIBRATION (HV5,P-CR) FOR LEFT: <<<<<<<<<
!CAL Calibration points:
!CAL -10.4, -41.0        0,    262
!CAL -9.7, -50.9        0,  -1371
!CAL -10.8, -28.1        0,   1830
!CAL -39.0, -42.4    -5612,    262
!CAL  15.4, -37.7     5612,    262
!CAL eye check box: (L,R,T,B)  -44    21   -53   -26
!CAL href cal range: (L,R,T,B) -8418  8418 -2171  2631
!CAL Cal coeff:(X=a+bx+cy+dxx+eyy,Y=f+gx+goaly+ixx+jyy)   9.7188e-05  206.65  10.701  0.40125 -0.28077    26
!CAL Prenormalize: offx, offy = -10.36 -40.958
!CAL Gains: cx:214.812 lx:188.042 rx:242.074
!CAL Gains: cy:18.893 ty:150.509 by:49.185
!CAL Resolution (upd) at screen center: X=1.2, Y=13.9
!CAL Gain Change Proportion: X: 0.287 Y: 2.060
!CAL Y gain out of range
!CAL PCR gain ratio(x,y) = 2.210, 1.899
!CAL CR gain match(x,y) = 1.010, 1.010
```

Figure 14: The "Event filter" window.

B.4.4 Testing filters

To see which events would be selected by a given filter, go to `Trials > Event variables > Test filter`. This lets you ensure a filter is working as expected before using it in data processing.

B.4.5 Using filters

Which events actually get selected depends on which button you press. If you click "Use highlighted", all of the highlighted events will be selected, regardless of what's written in the text boxes. If you click "Use regexp filter", the regular expression filter will be used, regardless of which events are highlighted. Similarly, if you click "Use trial var filter", the event variable filter will be used, again regardless of what's highlighted.

For example, if you use the regular expression filter to select all events containing the word `Start`, and then you manually deselect the event `Start of recording`, and then you click "Use regexp filter", `Start of recording` will still end up being selected.

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