MouseEyeTracker is Python software for acquisition and analysis of video data of the mouse eye.

Dependencies

- Python 2 or 3 (image acquisition from camera tested with PC running Python 2.7; analysis of saved data tested with PC running Python 2.7 and Mac running Python 3.4)
- Numpy
- PyQt4
- PyQtGraph
- OpenCV3
- h5py (if saving acquired video data or opening saved data files)
- Pymba (if acquiring data with AVT camera)
- PyDAQmx and nidaq.py (if using NIDAQ board for digital input/output)

Hardware

Currently we use an Allied Vision GigE GC660 IR camera (659W x 494H pixels, max 119 frames/s at full resolution, grayscale) with a Navitar Zoom-6000 lens (adapter mount 1-6010 and body tube 1-6265). Four IR LEDs (Thorlabs, LED851L, 8060-2) spaced equally around the lens (parallel to the video image) are used to determine the center of the corneal surface closest to the camera. Additional IR LEDs (Mightex SLS-0208-A) are used for illumination. A single-axis manipulator (Thorlabs, MT1/M) moves the camera parallel to the image axis for calibrating mm/pixel. A National Instruments board (USB-6009) is used to trigger data acquisition and/or broadcast timing of saved frames.

Starting MouseEyeTracker

From the command line:

```
python MouseEyeTracker.py
```

From a Python or IPython console:

```
import MouseEyeTracker
MouseEyeTracker.start()
```

Acquiring data

To acquire images from a camera, select 'Use Camera' from the camera menu and press the Start Video button. To save images, check the Save Video Data checkbox. Alternatively, saving can be triggered by a digital input to a NIDAQ board after selecting Camera menu > NIDAQ IO > Use Save Trigger (NIDAQ Input P0.0). To broadcast the timing of saved frames to other devices, select Camera menu > NIDAQ IO > Signal Saved Frames (NIDAQ Output P1.0).

Video data is saved to a hdf5 file. Each frame is a dataset named by the frame number. The file has attributes 'frameRate', 'numFrames', and 'mmPerPixel'. Frames have the attribute 'acquisitionTime', which is a timestamp in seconds from the camera's internal clock.

Analyzing data

To analyze data from an hdf5 file saved by MouseEyeTracker or a video file, select 'Open' from the file menu. Press the Start Video button to play the video. Press the '>' and '<' keys to navigate the video one frame at a time.

If the pupil is detected (see 'Tracking the pupil'), the pupil area, horizontal position, and vertical position plots will continuously update. Press the 'n' key to set these values to NaN for the current frame. To move the video to a particular frame, drag the vertical red line in one of the data plots or use the frame number box below these plots (type in it or press the arrows). Double-clicking on one of the plots resets the x range to the full video duration.

Frames can be analyzed more quickly by selecting Tools menu > Analyze All Frames to analyze all frames from the current frame to the end of the video without updating the display (until the final frame).

Use File menu > Save to save the pupil area/position data (and frame times, if available) to a hdf5 file. This data can be reloaded with Tools menu > Load Analyzed Data. With File menu > Save it is also possible to save a video or annotated video showing pupil/reflection markers.

Setting a region of interest (ROI)

Using an ROI increases performance and reduces file size. Pressing the Set ROI button resets the ROI to the full image size. Drag the bottom-right corner of the ROI box to resize the ROI. Click within the ROI box and drag the mouse to re-position the ROI. Alternatively, use the + and – keys the resize the ROI and the arrow keys to change its position. Pressing the Set ROI button again (or any other button) causes the ROI to fill the image window.

Tracking the pupil

Press the Find Pupil button and double click on the image near the center of the pupil. The clicked point serves as the pupil center 'seed' that MouseEyeTracker uses to find the pupil edges, which are fit to an ellipse. The center of the ellipse is the pupil center and serves as the seed for the next frame. The pupil center seed can be reset at any time (including while the Start Video button is depressed) by double clicking on the image.

The pixel intensity along lines at different angles from the calculated pupil center ('radial profiles') are shown in the top plot. Drag the horizontal red line in this plot up or down to change the pupil edge threshold.

The middle plot shows the running sum of the radial profiles up to the 'minimum number of pixels threshold'. This threshold determines the minimum consecutive number of pixels that must be above the pupil edge threshold. Drag the horizontal red line in this plot to change the minimum number of pixels threshold.

The bottom plot shows the distance of the pupil edge points from the center of the ellipse. Points above or below the horizontal red lines in this plot are excluded for fitting the ellipse. Dragging these lines adjusts their offset and separation relative to the edge distance mean and standard deviation.

Image masking for pupil detection

For pupil detection, specific regions of the image (LED reflections, for example) can be masked. Press the Set Mask button and double-click on the image near the center of a region to mask. A small ROI box should appear; adjust the size and position of this box as described under 'Setting a region of interest (ROI)'. Double-click on the image again to set additional mask regions. Press the 'delete' key to delete a mask region. Pixel intensities within masked regions are set to 0 for the purposes of pupil detection when the Use Masks checkbox is checked.

Tracking corneal reflections

Pupil position is typically calculated relative to the location of a corneal reflection to better isolate movements caused by eye rotation. When the location of the corneal reflection is the center of the corneal curvature closest to the camera, this point can be used to estimate angular rotation (see 'Estimating rotational angle of the mouse eye').

Press the Find Reflection button and double click the image near the center of a reflection to cause a small ROI box to appear. Adjust the size and position of this box as described under 'Setting a region of interest (ROI)'. Press the Find Reflection button again and the reflection center is shown at the centroid of pixels within the reflection ROI that are above the reflection threshold. The reflection threshold is set by selecting Tools menu > Reflection > Set Threshold.

The reflection can be a single spot or a ring of four small spots resulting from LEDs placed around the camera. In the latter case, select Tools menu > Reflection > Set Type > Ring and double click of the four spots. The reflection center is then calculated as the centroid of the centers of the four spots.

Calculating mm per pixel

Calculating the mm per pixel conversion of the video image is required to estimate the rotational angle of the eye and/or display pupil area in units of mm^2 rather than $pixels^2$. To determine this value while in acquiring camera images, find track a corneal reflection as described above and select Tools menu > mm/pixel > Measure. A window will appear to provide directions. Move the camera along the image plane 0.5 mm, then press 'Ok' on the pop-up window. The camera can then be moved back to any location. The mm/pixel value can also be set manualy using Tools menu > mm/pixel > Set.

Estimating rotational angle of the mouse eye

Video-based eye tracking involves locating the center of the pupil on an image and converting this position to angular rotation of the eye. One approach to the image-to-angle calibration problem is to have the subject fixate to known visual angles on a screen. A second approach, more practical in mice, is to move the camera instead (i.e. rotate the camera around the corneal surface while the pupil is still; Stahl et al. 2000, Zoccolan et al. 2010). There are several disadvantages to this method: (1) it requires an apparatus for complicated movements of the camera; for some rigs this might necessitate suboptimal placement of the camera and use of mirrors; (2) the calibration process it time consuming and must be performed at the beginning of every experiment; this reduces the time available for the actual experiment; (3) the eye must be still during the calibration process; (4) it assumes that the center of rotation of the corneal surface and the eyeball are equivalent; this is known to be erroneous.

Another approach to eye tracking in mice is described by Sakatani et al. 2004. The intersection of the corneal reflections of 4 IR LEDs placed around the camera lens indicates the center of the corneal curvature closest to the camera. On the video image, the position of the pupil center relative to the center of corneal curvature is related to the center of rotation of the eyeball as follows (Fig. 5 in Sakatani et al. 2004):

$$\frac{\mathbf{x}_0 - \mathbf{x}_p}{\mathbf{x}_c - \mathbf{x}_p} = \frac{\mathbf{R}_{pupil}}{\mathbf{R}_{pupil} - \mathbf{Offset}_{eye\text{-}cornea}}$$

or

$$\mathbf{x}_0 = (\mathbf{x}_c - \mathbf{x}_p)\frac{\mathbf{R}_{pupil}}{\mathbf{R}_{pupil} - \mathbf{Offset}_{eye\text{-}cornea}} + \mathbf{x}_p$$

where,

x_p = image coordinate of pupil center
x_c = image coordinate of corneal reflection intersect
x_0 = image coordinate of center of eye
R_{pupil} = rotational radius of pupil
$Offset_{eye\text{-}cornea}$ = offset between the rotational centers of the eye and cornea

The rotation angle of the pupil is:

$$\text{pupil rotation} = \arcsin\left(\frac{x_p - x_0}{R_{pupil}}\right)$$

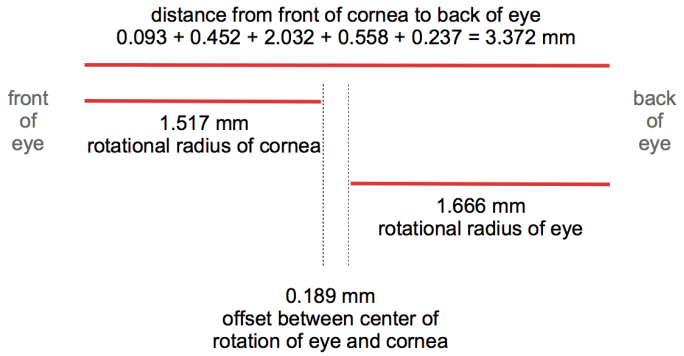
thus,

$$\text{pupil rotation} = \arcsin\left(\frac{(x_c - x_p)\dfrac{R_{pupil}}{R_{pupil} - Offset_{eye\text{-}cornea}}}{R_{pupil}}\right)$$

Using this equation and the values of R_{pupil} and $Offset_{eye\text{-}cornea}$ discussed below, one can determine the vertical and horizontal rotational angles of the eye from the image coordinates of the pupil center and corneal reflection intersection. Note that by tracking and comparing the movement of both the corneal reflection intersection and the pupil each frame, rotations of the pupil are isolated from translational movement of the eyes (with movement of the head, for instance; DiScenna et al. 1995, Stahl et al. 2000).

One potential disadvantage of this method is that is uses values of R_{pupil} and $Offset_{eye\text{-}cornea}$ derived from the literature (Remtulla and Hallett 1985) rather than measured for each mouse. However the variation in these parameters across mice of different age and sex is small (less than 10% after the mice are 6 weeks old; Puk et al. 2006, Tkatchenko et al. 2010).

From Table 1 in Remtulla and Hallett 1985 (note this table is erroneously labeled 'rat' rather than 'mouse') we can derive the following:



Similarly, the offset between the center of rotation of the eye and pupil is:

$Offset_{eye\text{-}lens}$ = |2.032+0.558+0.237-1.248-1.666| = 0.087 mm
$Offset_{eye\text{-}lens}$ is negative because the center of rotation of the lens is offset towards the back of the eye

relative to the rotational center of the eye.

Sakatani et al. 2000 round $Offset_{eye\text{-}cornea}$ to 0.2 mm and $Offset_{eye\text{-}lens}$ to 0.1 mm.

Correcting for pupil size (Sakatani et al. 2000),

$$R_{pupil} = \sqrt{R_{lens}{}^2 - P_{radius}{}^2} - Offset_{eye\text{-}lens}$$

where,

R_{lens} = rotational radius of the lens = 1.25 mm (Remtulla and Hallett 1985)
P_{radius} = pupil radius (dilation; as opposed to the rotation radius, R_{pupil})

References

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Tkatchenko et al. (2010). Analysis of postnatal eye development in the mouse with high-resolution small animal magnetic resonance imaging. Invest Ophthalmol Vis Sci

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