

# A-2000 Small Animal Pupillometer System

## Instructions for Use



## Notices & Safety Information

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### Intended Use Notice

The NeurOptics® A-2000 Pupillometer is an optical scanner which captures and analyzes a digital video of an animal pupil responding to light stimulation. **It should only be operated by properly trained personnel and should not be used on human eyes.**

**Trade names:** NeurOptics™ A-2000 Animal Pupillometer

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If you have a question regarding this product, please contact NeurOptics at one of the numbers given in Appendix B.


## Safety Information

### Please review the following safety information prior to operating the system.

Read the Operating Instructions fully before attempting to use the A-2000 Pupillometer. Attempting to operate the system without fully understanding its features and functions may result in unsafe operating conditions and/or inaccurate results.

If you have a question regarding the installation, set up, operation, or maintenance of the system, contact NeurOptics as shown in Appendix B.

**Warnings:** Warnings and Cautions appear throughout this manual where they are relevant. The Warnings and Cautions listed here apply generally any time you operate the system.

**Animal use only. Not for use on Humans, serious eye damage may  occur.**

**Use of the Pupillometer** - The Pupillometer is intended for use by trained personnel. If a problem is recognized while operating the system, the system must be removed from use and referred to qualified personnel for servicing. Using an inoperative system may result in inaccurate readings.

**Electric shock hazard** - Do not open any parts of the system. There are no user serviceable parts. Refer all servicing to a NeurOptics authorized service technician. Opening up the system will void the warranty.

Use only power adapters which are shipped with the NeurOptics® A-2000 Pupillometer system. Using unauthorized parts will void the warranty.

**Trusted Media** - The A-2000 System is shipped without antivirus protection. Attach only to trusted media.

## Cautions

DO NOT submerge the system or pour liquids over or into any components of the system.

DO NOT use acetone to clean any surface of the Pupillometer.

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## Section 1 - Introduction

The A-2000 Animal Pupillometer is a multi-chromatic binocular/dual camera system that tracks both pupils and stimulates both eyes. It is specifically designed for measuring the pupils of small animals (e.g., mice) but it can also be applied to larger species (i.e., canines, primates). The video stream is captured at 30Hz. The video frame is digitized into 640X480 pixels with 8-bit gray level resolution. The profile of the light stimulation can be customized using the graphic user interface (GUI). Pupillary variables are automatically analyzed, reported graphically in a special window and saved into a results file. Light stimulation has four different chromatic options: Red (622 nm), Green (528 nm), Blue (463 nm) and White. Light is emitted through a diffusing screen (approx. 50°x 35° of visual angle).

## Section 2 – Set-up

The NeurOptics® A-2000 system is packed into a shipping box. Included with your system are:

1. The A-2000 Pupillometer System
2. Small Animal Table
3. Serial, Firewire, HDMI and Power Cables
4. Slim Desktop Computer with Monitor
5. Keyboard & Mouse
6. Instructions for Use

Please check the contents of your box when delivered. Figure 1 & 2 shows packed Animal System and Computer components. **Please handle the cameras carefully. Cameras should not be carried by cables.**

**If anything is damaged or missing please contact NeurOptics at once at 1-949-250-9792. DO NOT ATTEMPT TO USE A DAMAGED SYSTEM!**

**The system includes a desktop computer that has specific technical characteristics and cannot be replaced with a different model.**

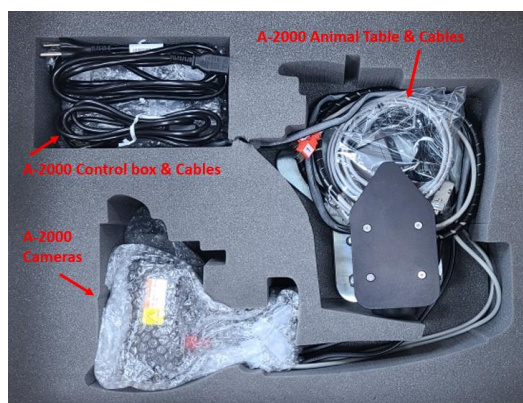


Figure 1

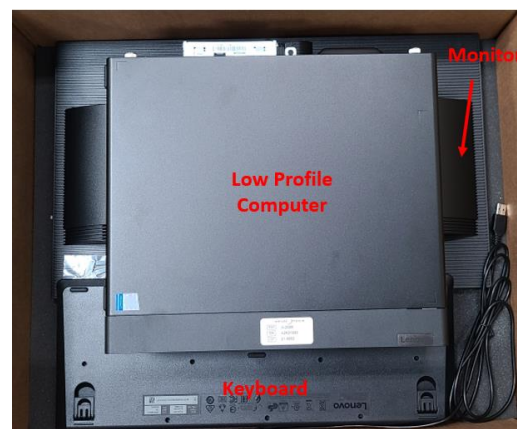


Figure 2

Place the Computer, Monitor, Keyboard, and mouse on a stable surface. Connect Monitor, Keyboard and Mouse and power cables to the back of the computer and monitor. Place Small Animal Table next to the computer. See Figure 3 & 4.



Figure 3



Figure 4

Place the Camera Units (Unit B) on the Small Animal Table and lock into place by twisting the lever on the Magnetic Base to ON, which activates the magnet. See Figure 5.



Figure 5

Connect the grey Serial Cable (Labeled 4) and 9V power supply (Labeled NEUR-PWR4) to the open ports of the Control Box (Unit A) as shown below. See Figure 6. The other end of the Serial Cable (Labeled 3) is connected to the back of the computer as shown on the right. See Figure 7.

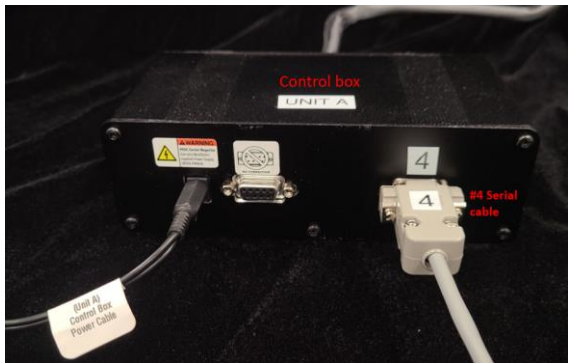


Figure 6

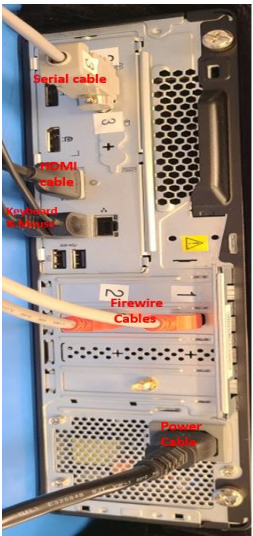


Figure 7

Connect both Firewire cables labeled 1 and 2 to the back of the computer in the Firewire port labeled 1 and 2. See Figure 8.



Figure 8



Plug in the power adapter for the Control Box and power cables for the Monitor and Computer into surge protector that is plugged into the wall. We highly recommend using a power surge protector. Example shown in Figure 9.



Figure 9

Press the power button on the computer. Click on the A-2000 icon on the screen to get started. See Figure 10.



Figure 10

You can adjust the camera position by loosening the levers under the camera. Raise or lower the table as needed by turning the knob on the Small Animal Table. See Figure 11.

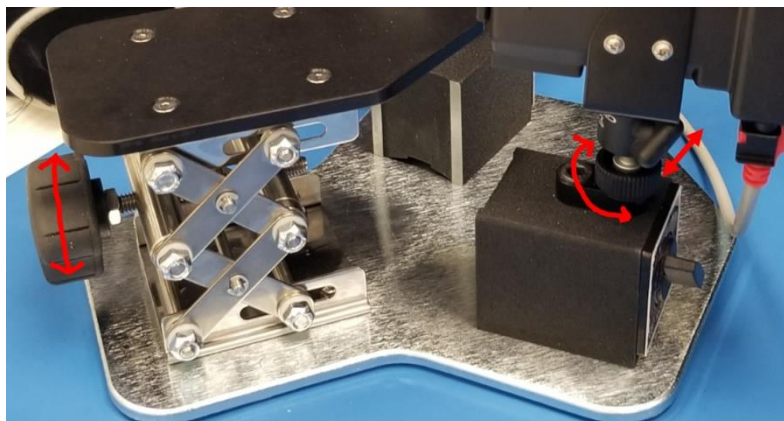


Figure 11

## Section 3 – Taking a Measurement

All possible options to operate the program are listed in the left most side of the GUI (see Figure 12). The live video from the two cameras is displayed in the same GUI. Pupil tracking is represented by a circle fitting the contour of the pupil– the subject’s left eye is displayed on the right and the subject’s right eye is displayed on the left. This is to maintain the natural perspective of the examiner facing the subject during the examination. The left pupil (OS) is tracked with a yellow circle and the right pupil (OD) with a green circle. This association (left-yellow and right-green) is also used for display purposes (see below). Light stimulus waveform and the corresponding pupil response dynamics are shown together in the graph located at the bottom of the GUI. Note: the software version can be checked by pressing the Version button.

**Subject ID**

The first two windows on the top of the list (Figure 12) serve to enter information regarding the current measurement (Subject ID and Note). This information is attached to the master data result file produced at the end of the measurement and used for defining the name of the result file.

### Edit Stimulus Profile

The Edit Stimulus Profile button allows the user to define and to save a new light stimulus profile or to load a predefined profile. A profile is defined by one or more light steps is flashed to the eye and then turned off (i.e. a pulse). Light pulses can be repeated a number of times, thereby forming a section. Pulses and/or sections can be concatenated in order to define the final light stimulus profile. The Edit Stimulus Profile GUI (Figure 13) is used to define all the elements of the light stimulus profile.

Light intensities are defined in Lux or pure radiometric units ( $\text{W}/\text{m}^2$ ). Two different definitions of Lux are available. They correspond to the scotopic or photopic CIE human luminosity sensitivity function and they can be selected using the drop-down menu on the top-left side of the GUI.

Lux or radiometric levels can be set using the horizontal slide button (see arrows) and they can be incremented by 0.1 log units by clicking on the bar. When a Lux or W/m<sup>2</sup> value cannot be achieved by the system, the number (see the dashed arrow, Figure 13) is displayed in red and a lower intensity must be selected instead.

A pulse defines single pupil stimulation and a section is a repetition of pulses having the same intensity, timing and wavelength characteristics. One or more pulses define a section.

**For example, Section 1** in Figure 13 is defined by two pulses of red light of 2.20 logLux flashed for 1000 msec and then turned OFF for another 2000 msec (total duration 3 seconds). The eyes are stimulated alternatively with the right eye (OD) stimulated first.

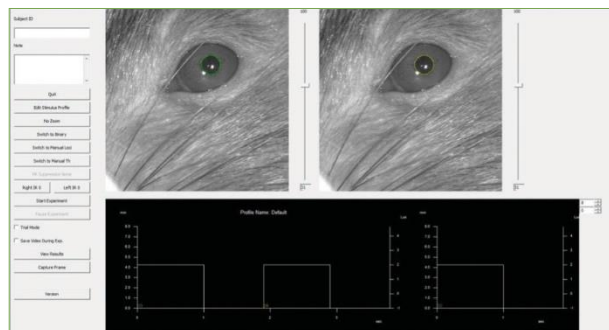


Figure 12 – The main program graphic user interface (GUI).

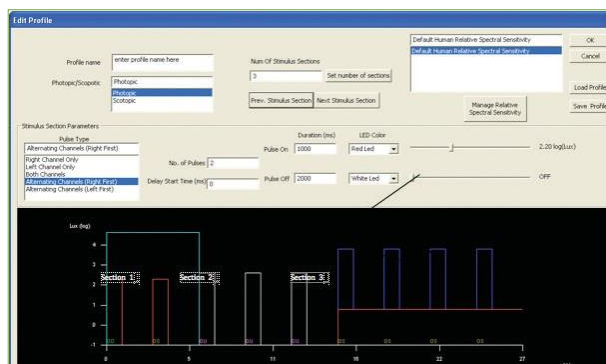


Figure 13 – The Edit Profile GUI, Section 1.

**Section 2** (Figure 14) is defined by three pulses of white light– 2.60 logLux, 1000msec ON and 2000msec OFF, both eyes stimulated simultaneously.

**Section 3** (Figure 15) is defined by four pulses of blue light over a red background – intensity of the blue light is 3.80 logLux and intensity of the red background is 0.80 logLux. Only the left eye (OS) is stimulated.

All the elements of the pulse are definable using the corresponding input window in the Edit Stimulus Profile GUI (Figures 13, 14 and 15). A new Section can be added or deleted by just entering the proper number in the Num. Of Stimulus Section box. Sections can be browsed and edited using the Prev. Stimulus Section and Next Stimulus Section button. The final profile can be named, saved (it will have a .SPF extension) and retrieved later for execution using the buttons Load Profile and Save Profile. Once the profile is defined (or loaded if previously defined) press OK – that light stimulus profile is now ready to be executed. Note that the Stimulus Profile can be changed and defined also by editing the profile file using Microsoft Excel.

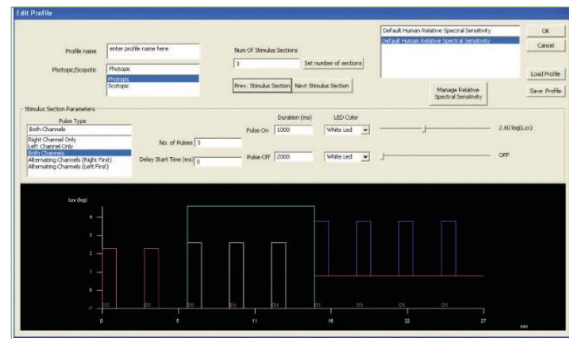


Figure 14 – The Edit Profile GUI, Section 2

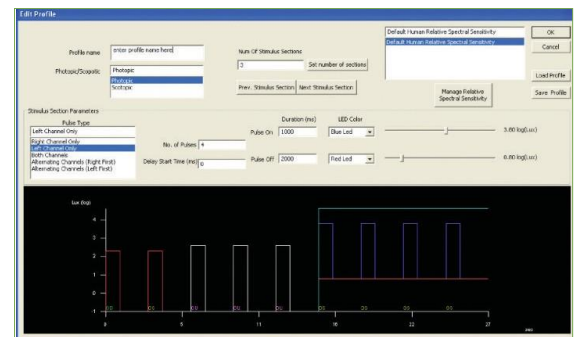


Figure 15 – The Edit Profile GUI, Section 3

The file contains all the instructions necessary for the editing and it has all the variables available in the GUI. Open the file with Excel, and once the new profile is defined in the spreadsheet and ready to be used in an experiment, then the file should be closed and saved as Text (Tab delimited) keeping the same extension SPF.

With the Manage Relative Spectral Sensitivity feature it is possible to upload and use a different Relative Spectral Sensitivity function than the one used by default (for human) but this needs to be provided by the user. The new Relative Spectral Sensitivity must be provided as an ASCII file of two columns for photopic and scotopic defining the function within the range 400nm and 700nm and increment of 1nm. Each row corresponding to a 1nm increment. Please contact NeurOptics for further instructions.

## Preparing the subject for the experiment and checking the tracking.

The subject should be positioned close to the cameras. The cameras can then be positioned in front of each eye and adjusted so the eye is visible and well in focus in the center of the video display in the main GUI (Figure 12). A green circle should automatically track the right eye (displayed on the left) and a yellow circle tracks the left eye (displays on the right). It is very important to carefully check the accuracy of the tracking before initiating the experiment. If the circles are not precisely around the pupil or there is no circle or there is only a red circle moving erratically around the video frames and tracking other portions of the image or the green (or yellow) circle turns occasionally into red – all of these are signs of bad tracking and a few corrective steps are necessary before initiating the measurement. Below is the list of all the available options.

## Digital Zoom.

The zoom button toggles between the No Zoom and the 2x Zoom video option. When the zoom is enabled, the video of both eyes is shown magnified. This feature is included in the GUI solely for assisting the user in focusing and/or adjusting the camera position or the pupil threshold. It has no effect on the tracking algorithm or the optical configuration.



## Binary/Grayscale Switch.

This switch changes the video display mode between grayscale and black and white (binary) display. It does not affect the tracking algorithm and it may be used simply for visualizing the tracking algorithm's performance and helping with the setting of the threshold. When the binary view is enabled (Figure 16) the pupil must look like a black solid circle surrounded by white.

## Manual Loci/Automatic Loci Switch.

When the Manual Loci feature is enabled, the user can manually locate the region of interest for searching for the pupil. This region of interest is a sub-window of the entire video frame containing the pupil. It is where the tracking algorithm will be searching for the pupil. This region is usually the entire field of view. However, in some cases, for example when strong dark patches such as hair/whiskers or eyelashes are also included in the field of view or in the case of some types of iris pigmentation, the detection of the pupil can be problematic. In these cases, it is advisable to manually restrict this region to a smaller and more specific portion of the field of view. Left mouse click on the desired top-left corner of the restricted region of interest and then drag the region down to the proper size. A red rectangle overlapped on the live video indicates the position and size of the pupil searching region of interest (Figure 17).

## The Manual/Automatic Threshold (Th) Switch.

Manual pupil threshold is set using the Manual/Automatic Threshold (Th) Switch button. This option must be used in conjunction with the binary view introduced above. It serves to refine the binary threshold defining the pupil. The pupil must appear as a uniform black patch. A good threshold is when the pupil is clearly isolated from the rest of the searching region of interest appearing in white (as displayed

in Figure 17 or 18). The threshold can be changed using the vertical scroll bar on the right side of each video. If the threshold is too high (Figure 19, video on the left) the pupil looks fuzzy, blackness is sparse and not contiguous and the tracking circle is red which means poor tracking. If the threshold is too low (Figure 20, video on the left) the image looks too dark and the pupil is not clearly isolated from the background.

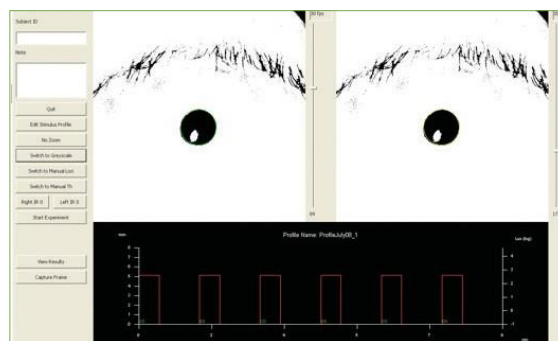


Figure 16 – Binary view enabled

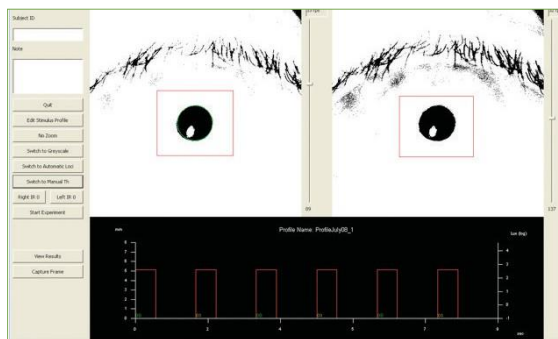


Figure 17 – The Manual Loci feature can improve pupil detection and tracking by restricting the region of the interest for searching the pupil (see red rectangular)

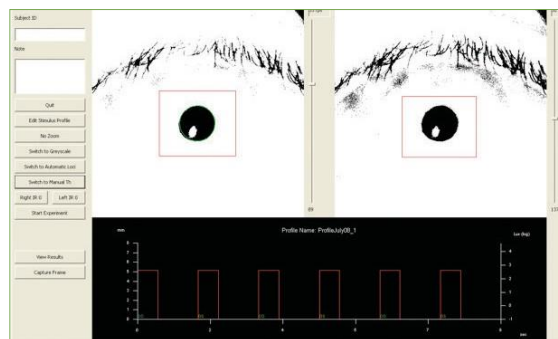


Figure 18 – The Manual Loci feature can improve pupil detection and tracking by restricting the region of the interest for searching the pupil (see red rectangular)

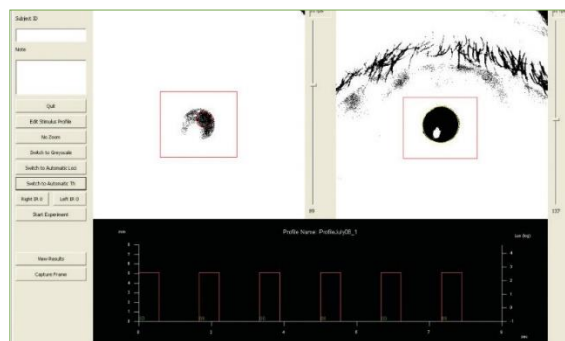


Figure 19 – Threshold too high on the left video (right pupil) - looks fuzzy, blackness is sparse and not contiguous. Note the tracking circle in red which indicates poor tracking.

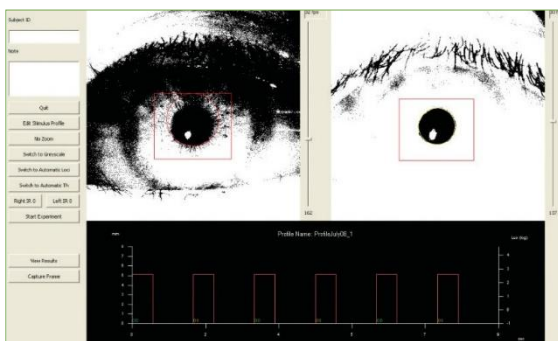
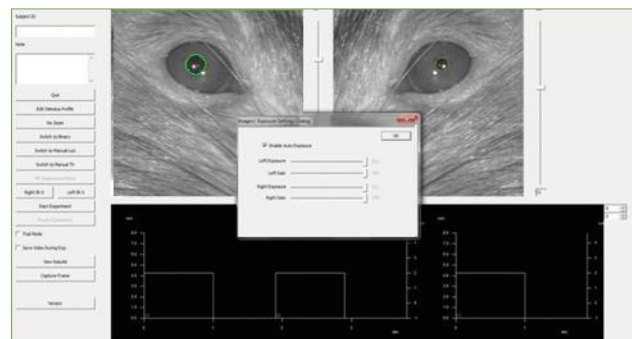


Figure 20 – The Threshold too low on the left video - the image looks too dark and the pupil is not clearly isolated from the background.

## Auto-Exposure Setting

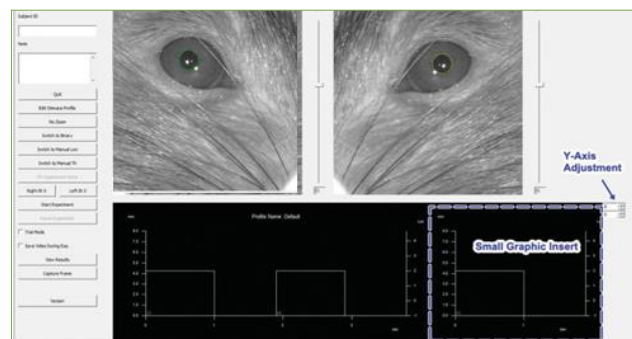
The auto-exposure or the value of the exposure and the gain of the two video cameras can now be set manually. This is to optimize the quality of the video when necessary.

To change the exposure and the gain of the two cameras, right click on one of the two live videos and open the “Imager’s Exposure Setting Dialog” window.



## Small Graphic Inset

A small graphic inset is added in the main window, next to the original graphic. The new graphic displays in real time the current pulse and the two pupil waveforms at larger magnification and it is updated at each pulse transition. The range of the y axis range of the new graphic can be set using two small scroll boxes located on the top-right side of the graphic. The new graphic allows the user to magnify the display of each pulse in real time during the measurement.



## Start Experiment.

If both pupils are tracked correctly, a press of the Start Experiment button will initiate the execution of the light stimulus protocol. The subject should be kept firmly in front of the cameras – blinking or similar artifact should be minimized. A live progressing trace of the two pupils' dynamics (yellow for the left pupil and green for the right pupil) are shown on the bottom inset of the main GUI (Figure 21, note a blink at second 21). At the end of the measurement – the results file is saved in folder C:\NeurOptics\A2000\DATA and results are displayed in the View Results GUI. Note that the Start Experiment switches to Abort Experiment as soon as the measurement is initiated and it may be used to abort the experiment. Another switch, Pause/ResumeExperiment

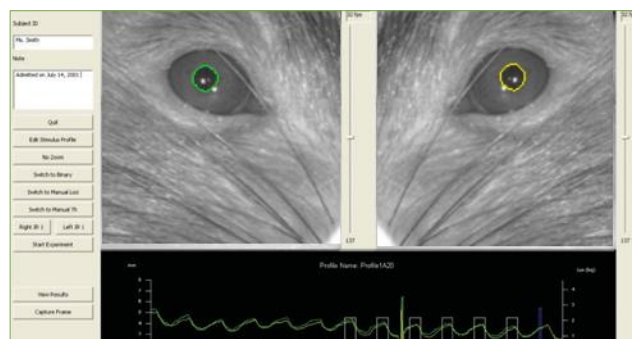


Figure 21 – During the experiment, the responses of the two pupils are displayed in real time in the graph at the bottom of the main GUI - yellow is for the left pupil and green is for the right pupil.

(added in the new version of the software and not shown in Figure 20) serves to interrupt momentarily (and then resume) the ongoing measurement. The Pause/Resume switching operations can be performed also by simply pressing the space bar on the keyboard.

## Right IR – Left IR.

Each camera has two pairs of IR LEDs (0 and 1) that can be toggled using the IR buttons for the left and right channel. This can be useful for small pupils, if one of the IR corneal reflections is overlapping on the pupil, disrupting the tracking. Switching to the other pair of IRs can, often, resolves the problem and improves the visibility of small pupils.

## Capture Frame.

Two snapshots of the two videos being displayed in the GUI can be saved as bitmaps with a press of this button prior the start of a measurement. A new pair of bitmaps are generated and saved in memory at each press.

## Section 4 – Viewing the Results

Pupil variables are reported graphically and numerically in the View Results GUI (Figure 22). The small inset on the right (A) shows each single pulse and different pulses can be viewed by pressing the Previous Pulse and the Next Pulse button. The horizontal gray lines overlapped on the plot indicate the Maximum and Minimum pupil size (before the constriction and at the peak of the constriction) – the vertical line indicates the latency of the constriction. The user can select which pulse to show: both pulses, left pupil only or right pupil only. The entire pupil waveform and the light stimulus protocol are shown together on the long graph at the top of the GUI (B) – similarly to the Edit Stimulus Profile GUI; a light-blue rectangle indicates a section. In the example (Figure 22), the 5th pulse in the list is shown in the small inset A and that is part of the first section (pulses are enumerated starting from zero, so the 5th pulse is pulse no. 4). Pupil variables and pulse information are reported in C. The information includes the ID of the subject and Notes about the subject and the measurement, duration, type and intensity of the light stimulation. Below, still in the inset C, the pupil variables for the right and left pupil light reflex are listed – the variables measured are:

**Max** = pupil diameter baseline before the constriction

**Min** = pupil diameter at the peak of the constriction

**Delta** = Max – Min

**CH** = (Max – Min)/Max, given in percentage

**CLAT** = latency of the constriction (delay of the onset of the pupil constriction)

**MCV** = maximum constriction velocity

**DV** = mean dilation velocity (recovery after the constriction)

If the pulse belongs to a section composed of many pulses (repeated in time) then the analysis in C returns the average of all the above variables for all the pulses composing the section. RPupil Ave is the average of all the right pupil responses (for both right and left eye stimulation), LPupil Ave is the average of all left pupil responses (for both right and left eye stimulation), RStim Ave is the average of all the responses (left and right pupils together) corresponding to all the right eye stimulations and LStim Ave is average of all pupil responses to all the left eye stimulation.

In case of a blink (or similar artifact) as, for example, for pulse no. 7 (belonging to the second section, see Figure 23), pupillary variables are reported in red and some of them might not have been even calculated (and reported with "--", see Figure 23). A new feature has been added to the View Results GUI that allows the recovery of the affected pulse (Figure 24). The blink, or gap in the pupil profile, can be linearly interpolated by selecting the option L. Pupil Fill Data Gap for the left pupil or R. Pupil Fill Data Gap for the right pupil – in this case, a line is drawn automatically over the gap (see example in Figure 14, right inset) to indicate the fitting. If the linear fitting is not properly performed (or the gap is too large to be interpolated efficiently), the MAX, MIN and LAT of the pupil reflex can be set manually using the three input cursors at the bottom of the graph (Figure 24). Changes in the MAX, MIN or LAT values are reflected by the position of the three gray lines in the plot to give visual feedback of the values being changed (two horizontal lines for MAX and MIN, and one vertical line for LAT). The pupil variables reported in table C (Figure 22) are automatically updated after the linear fit option has been selected or a value during manual setting has been changed. Finally, the options R. Pupil Valid or L. Pupil Valid (Figure 24) serve to flag as abnormal (and to report in red) a reflex that is otherwise considered normal. This GUI feature is provided to manually correct those pulses where the tracking error was missed by the algorithm and that cannot be included in the analysis.

Data are automatically saved after the measurement in the C:\NeurOptics\A2000\DATA folder and they can be loaded and viewed later using the Load Data button in the same GUI. Remember to use a different name for the data file if a fitting or a manual override has been performed on the data – this is to preserve the original file. Data files can also be viewed with Excel.

Different segments corresponding to different information are defined in a data file:

The segment <STIMULUS-DEFINED> reports the stimulus settings as defined in the SPF stimulus profile file.

The segment <STIMULUS-GENERATED> reports the exact sequence and values of the lights emitted during the experiment. The first column indicates the section that particular light intensity belongs to; the second column is which eye was stimulated (0=right, 1=left, 2=both, 3 or 4 if background light); then Stimulus start time; (seconds elapsed from start of measurement); the Color of the stimulation for the Right eye (0=white, 1=red, 2= green, 3=blue); Intensity of the stimulation for the Right eye (given in radiometric units); Intensity of the stimulation for the Right eye (given in lux); the Color of the stimulation for the Left eye (0=white, 1=red, 2= green, 3=blue); Intensity of the stimulation for the Left eye (given in radiometric units); Intensity of the stimulation for the Left eye (given in lux). The right and left stimulus intensities could slightly differ due to the differences in the LEDs light emittance.

Raw pupil tracking data are included in the data file; they are reported under the label <RIGHT-PUPIL-DATA> or <LEFT-PUPIL-DATA>; for each time sample (line) the second column is time, the third column is pupil diameter, the third and fourth column is the x and y position of the pupil. The first column indicates whether that sample was successfully tracked (1) or it was affected by a blink or similar artifact (0) Example.

total number of lines for right pupil good (1) or bad (0) sample  
sample time (negative is prior the start)  
pupil diameter  
pupil position (x and y)

```
<RIGHT-PUPIL-DATA>
232 1
1 -0.033000 2.731023 241.943726 347.149994
1 -0.002000 2.704758 241.599976 346.993744
1 0.029000 2.731023 241.943726 347.149994
1 0.061000 2.704758 241.599976 346.993744
1 0.092000 2.731023 241.943726 347.149994
1 0.123000 2.704758 241.599976 346.993744
1 0.154000 2.731023 241.943726 347.149994
...
```

The results of the analysis are reported in different parts of the file and different formats Tables <RIGHT-PUPIL-ANALYSIS> and <LEFT-PUPIL-ANALYSIS> are obsolete and can be ignored: in this table there is one line for each pulse in the stimulus waveform and a total of 25 values. From left to right those values correspond to: Pulse index; Section index; which eye was stimulated (0=right, 1=left, 2=both); Stimulus start time; (seconds elapsed from start of measurement) ; Stimulus duration (sec) ; Pulse duration (sec) ; Intensity of the stimulation for the Right eye (lux) ; Intensity of background illumination for the Right eye (lux) ; Intensity of the stimulation for the Left eye (lux) ; Intensity of background illumination for the Left eye (lux) ; the 10th value is obsolete and should be ignored ; the Color of the stimulation of the Right eye (0=white, 1=red, 2= green, 3=blue) ; the Color of the background illumination for the Right eye ; the Color of the stimulation of the Left eye; the Color of the background illumination for the Left eye ; the 16th value is binary indicating the presence of a blink or artifact (1=tracking ok, 0=tracking not ok, pulse should be discarded) ; Max pupil diameter (mm) ; Min pupil diameter (mm) ; Max pupil area (mm2) ; Min pupil area (mm2) ; Latency of the constriction (sec) ; Average constriction velocity (mm/sec) ; Maximum constriction velocity (mm/sec) ; Average dilation velocity (mm/sec). Each line represents a single pulse in the list of the stimulus waveform; the two sections <RIGHT-PUPIL-ANALYSIS> and <LEFT-PUPIL-ANALYSIS>, have the initial fields describing the stimulation populated with identical parameter settings.



Figure 22– The View Result GUI. The entire pupil profile (and stimulus waveform) is displayed in B. Pulse can be individually inspected in A. Pupil variables (belonging to that specific pulse in A) are reported in C. In this example, the 5th pulse as the first section has been selected in the displayed

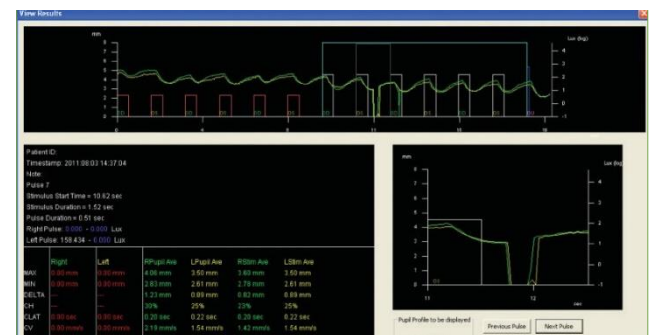


Figure 23 – In the case of a blink (or a similar artifact) as in the example for pulse 7, (belonging to the second section) pupillary variable are reported in red or not reported.



What follows in the same file is a list of tables in the same format as they were displayed in the View Results GUI of the program, one for each of pulse and for all the pupil and measurement variables. The right and left pupil profiles are reported again below this list of tables. Finally, a complete table is provided at the very bottom which includes again all stimulus and variables values. This table can be selected and pasted onto a clean new Excel spreadsheet for further analysis and statistics. The names of the columns should be self-explanatory. Note that the third and fourth columns named Stimulus Sections Start Index and Stimulus Sections End Index indicate the index of the pupil profile in sections <RIGHT-PUPIL-DATA> or <LEFT-PUPIL-DATA> corresponding to that specific row (pupil light reflex) in the table. This is to allow further analysis of the raw pupil profile corresponding to that analysis.

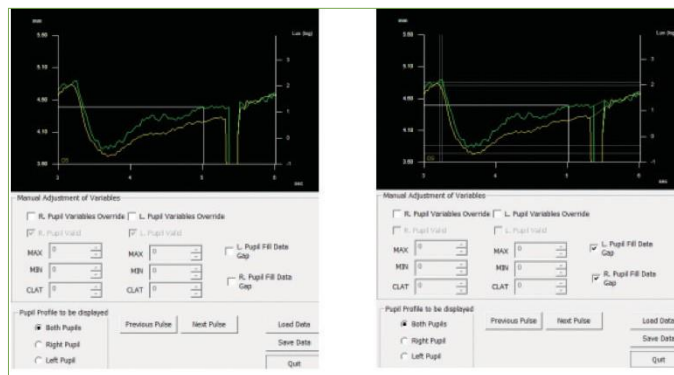


Figure 24 – The new feature added to the View Results GUI allows the recovery of those pulses affected by pupil tracking artifacts (such as eye blinks). If the Fill Data Gap option (see right panel) is selected, a linear fit is applied to the artifact and pupil variables are re-analyzed accordingly (and reported again in C). If the linear fit cannot be properly conducted, the user can also set manually the MAX, MIN and LAT of the pupil light reflex.

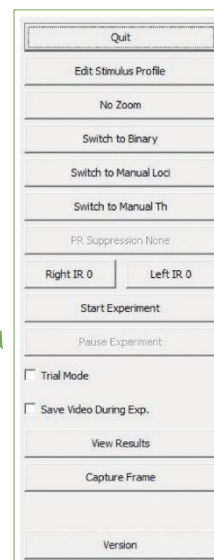
NeurOptics provides a Matlab script that automatically parse and upload the result data file into the Matlab workspace. Contact NeurOptics for information.

### For the check box “Trial Mode”

The trial mode allows to make a measurement without saving the results at the end of the measurement. It is recommended only when the experimental logistic is being tested, to avoid many insignificant “test” result files in the DATA folder.

### For the check box “Save Video During Exp.”

If the box is checked, video frames are saved in the hard drive during the experiment and they can be accessed and viewed later. Only one parameter needs to be specified which is the length of the video, in terms of number pulses to be recorded and saved. The two videos from the left and right eye are saved in a binary format, they can read using, for example, a Matlab script provided by NeurOptics. Contact NeurOptics for information.



## Section 5 – Cleaning & Maintenance

The A-2000 Animal Pupillometer is a non-sterile product

To clean the surfaces of the A-2000 pupillometer, use a soft, lint-free wipe with a quaternary disinfectant.

DO NOT attempt to sterilize any part of the A-2000 system, as sterilization may damage the system.

DO NOT immerse or drip liquids on any component of the system & DO NOT use acetone to clean any surface of the pupillometer.

The Desktop LCD and keyboard should be cleaned using a soft, lint-free wipe with IPA. Clean the lens with lens cleaning solution and a lint free cloth.



## Appendix A — Troubleshooting

Issue	Possible Reason	Solution
<b>Error Window: “Stimulus Hardware Not Detected”</b>	Control Box not powered or connected	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Check power connection to the control box and serial cable connections.</li> <li>• Restart the A-2000 program.</li> </ul>
	Firewire cable to Hub not connected	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Check all power and video connections.</li> <li>• Restart the A-2000 program.</li> </ul>
	Connection error or malfunctioning hardware due to missing serial adaptor driver	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Check power connection to the control box and serial cable connections.</li> <li>• Restart the A-2000 program.</li> <li>• Reboot computer, contact Manufacturer if error persists.</li> </ul>
<b>Error Window: “FiInitialize failed with error: ubCore stack initialization error.”</b>	ubCore stack is not active	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Reboot computer, contact NeuroOptics if error persists.</li> </ul>
<b>Error Window: “Initializing Please Wait”</b>	System unable to read settings	<ul style="list-style-type: none"> <li>• Contact NeuroOptics.</li> </ul>
<b>Error Window: “Settings file A2000Settings.dat can’t be read.”</b>	Missing or corrupted system data file	<ul style="list-style-type: none"> <li>• Contact NeuroOptics.</li> </ul>
<b>Program becomes unresponsive.</b>	Serial Cable unplugged	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Check USB-Serial adaptor and cable connections.</li> <li>• Restart the A-2000 program.</li> </ul>
<b>Program fails to start or crashes</b>	Program corrupted	<ul style="list-style-type: none"> <li>• Contact NeuroOptics if error persists.</li> </ul>

## Appendix A — Troubleshooting Continued

Issue	Possible Reason	Solution
<b>Error Window:</b> <b>FiCreateIsochReceiveEngine failed with error: Error in video streaming initialization.</b>	System failed to process video	<ul style="list-style-type: none"> <li>• Quit the A-2000 program and restart if error persists, reboot computer.</li> </ul>
<b>Error Window:</b> <b>FiLocateCamerasEx failed with error: error in video streaming initialization</b>	System failed to process video	<ul style="list-style-type: none"> <li>• Quit the A-2000 program and restart if error persists, reboot computer.</li> </ul>
<b>Error Window:</b> <b>"FiOpenCameraHandleEx failed with error: FIREi STATUS CAMERA READ FAIL"</b>	Control system does not detect the camera(s).	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Check all power and video connections.</li> <li>• Restart the A-2000 program.</li> </ul>
<b>Error Window:</b> <b>"FiOpenCameraHandleEx failed with error: FIREi STATUS INVALID PARAMETER"</b>	Camera(s) not powered or connected.	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Check all power and video connections.</li> <li>• Restart the A-2000 program.</li> </ul>
<b>Error Window:</b> <b>FiQueryCameraRegister failed with error: Error in video streaming initialization</b>	System failed to process video	<ul style="list-style-type: none"> <li>• Quit the A-2000 program and restart if error persists, reboot computer.</li> </ul>
<b>Error Window:</b> <b>FiStartCamera failed with error: Error in video streaming initialization</b>	System failed to process video	<ul style="list-style-type: none"> <li>• Quit the A-2000 program and restart.</li> </ul>

## Appendix A — Troubleshooting Continued

Issue	Possible Reason	Solution
<b>Error Window:</b> <b>FiStartCamera failed with error:</b> <b>FIREi STATUS CAMERA ALREADY RUNNING</b>	System failed to process camera image	<ul style="list-style-type: none"> <li>• Quit the A-2000 program and restart.</li> </ul>
<b>Number of cameras detected by application is less than two</b>	Camera(s) not powered or connected	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Check all power and video connections.</li> <li>• Restart the A-2000 program.</li> </ul>
<b>"Initialization Video" error on one or both cameras</b>	System failed to process camera image	<ul style="list-style-type: none"> <li>• Quit the A-2000 program and restart.</li> </ul>
<b>Error Window:</b> <b>Can't save data</b>	The DATA subfolder is not write-enabled or missing	<ul style="list-style-type: none"> <li>• Verify the DATA subfolder is located under the C:/NeuroOptics/A-2000 directory and is not write protected.</li> <li>• Create a DATA folder if necessary.</li> </ul>
<b>Error Window:</b> <b>Loading Profile...</b> <b>Can't open selected file -</b> <b>Can't open profile file</b>	Camera(s) not powered or connected.	<ul style="list-style-type: none"> <li>• Quit the A-2000 program.</li> <li>• Check all power and video connections.</li> <li>• Restart the A-2000 program.</li> </ul>
<b>Error Window:</b> <b>FiQueryCameraRegister failed with error: Error in video streaming initialization</b>	Possible missing file or the file has no read permission	<ul style="list-style-type: none"> <li>• Verify file properties are not write protected.</li> <li>• Verify parent folder properties are not write protected.</li> </ul>
<b>Error Window:</b> <b>Can't read from selected file -</b> <b>can't load the profile file data</b>	Possible corrupted file	<ul style="list-style-type: none"> <li>• Delete corrupted file and re-edit.</li> </ul>

# Appendix B — Contact & Ordering Information

The following **options and accessories** can be ordered from NeurOptics:

<b>A-2000</b>	NeurOptics Small Animal Pupillometer – US
<b>NEUR-PWR4</b>	Control Box Power Supply – US
<b>NEUR-COMP-04</b>	Lenovo Desktop Computer

For more information, please contact NeurOptics or visit [www.NeurOptics.com](http://www.NeurOptics.com)

**Distributed by:**



NeurOptics, Inc.

**US Customer Service:**

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Irvine, CA 92618 | US

Tel: 1 949.250.9792 or North America Toll-Free 866.99 PUPIL

Fax: 1 949.250.9796

[info@NeurOptics.com](mailto:info@NeurOptics.com)

**A. Standard Limited Warranty.** NeurOptics (the Manufacturer) warrants to the original end user purchaser (the Purchaser) that the enclosed product (Product) purchased by the Purchaser, at the time of delivery to the Purchaser, shall be substantially free from defects in material and workmanship. The Manufacturer makes no warranty (express, implied, or statutory) for Products that are modified (except as expressly contemplated herein) or subjected to unusual physical stress, misuse, improper operation, neglect, improper testing, use in combination with other products or components other than those for which the Products were designed, or use in any manner or medical procedure for which the Products are not indicated. If the Product is opened up by anyone other than an authorized service technician of NeurOptics, the warranty shall be void.

**B. Remedy.** Purchaser's exclusive remedy and the Manufacturer's sole liability for breach of the foregoing warranty shall be, at the Manufacturer's sole option and election, to replace the Product or credit Purchaser for the net amount actually paid for any such Product; provided that (i) the Manufacturer is notified in writing within one (1) year after Purchaser's receipt of the Product that such Product failed to conform, including a detailed explanation in English of any alleged nonconformity; (ii) such Product is returned to the Manufacturer within one (1) year after Purchaser's receipt of the Product as designated by the Manufacturer using a Returned Good Authorization number issued by the Manufacturer and (iii) the Manufacturer is reasonably satisfied that the claimed nonconformities actually exist. Except as expressly provided in this paragraph, Purchaser shall not have the right to return Products to the Manufacturer without the Manufacturer's prior written consent.

**C. Exclusion of Other Warranties.** EXCEPT FOR THE LIMITED WARRANTY PROVIDED IN (A) ABOVE, NEUROPTICS GRANTS NO OTHER WARRANTIES OR CONDITIONS, EXPRESS OR IMPLIED, AND MANUFACTURER SPECIFICALLY DISCLAIMS THE IMPLIED WARRANTIES AND CONDITIONS OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. NEUROPTICS NEITHER ASSUMES NOR AUTHORIZES ANY OTHER PERSON TO ASSUME ANY OTHER LIABILITIES ARISING OUT OF OR IN CONNECTION WITH THE SALE OR USE OF ANY **PRODUCT**.

### Returned Goods Policy

The A-2000 Pupillometer is a custom-made product and no returns will be allowed unless the Product is defective. Products must be returned using a Returned Goods Authorization number issued by the Manufacturer and sent well wrapped and protected via Fedex or UPS with insurance for replacement value, to be accepted for replacement or repair. Determination of a product defect will be made by NeurOptics, which determination will be final.



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A2K-IFU-01 Rev K (HOMA-8LJQG2)