UTAS

EMWin Software

User's Manual

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CE 2797

Rx only

Part No. 96-022-EN

EN - http://www.lkc.com/IFUsPrintable instructions for use (IFU) in multiple languages are stored on the UTAS computer as PDF files in the IFU folder on the computer desktop screen, or go to www.lkc.com/IFUs

DE - Druckbare Nutzungsanweisungen (IFU) in mehreren Sprachen werden auf dem UTAS-Computer als PDF-Dateien im IFU Ordner auf Ihrem Desktop gespeichert. Alternativ können Sie www.lkc.com/IFUs besuchen.

ES - En el ordenador UTAS hay almacenadas como archivos PDF instrucciones imprimibles de uso en varios idiomas, en la carpeta IFU del escritorio del ordenador, o acceda a www.lkc.com/IFUs

FR - Des instructions d'utilisation à imprimer (IFU) dans plusieurs langues sont stockées sur l'ordinateur UTAS sous forme de fichiers PDF dans le dossier IFU présent sur le bureau. Vous pouvez également les obtenir sur www.lkc.com/IFUs

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European regulatory Data

Instructions for USE (IFUs) in other languages may be found at www.lkc.com/IFUs
To request a printed copy of this manual please send an email to support@lkc.com and include the following information:

1) Company name
2) Your Name
3) Mailing address
4) The Serial Number of your device
5) The part number of the manual you need
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Your manual will be shipped to you within 7 days.

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LKC PRODUCT LIFETIME POLICY

UTAS is the Trade Name for this device and all associated software. The lifetime of a UTAS system is 7 years from the original shipment date of the UTAS system. LKC will service any UTAS system that is within its lifetime.

SOFTWARE LICENSE

The UTAS software is a copyrighted product of LKC Technologies, Inc. and is included with the UTAS system under the following license agreement:

The software may be used in conjunction with the UTAS system only. The purchaser of the UTAS system may make copies of the software for convenience of use, provided the LKC copyright notice is preserved with each copy. This license specifically prohibits the use of this software in a system that does not include an LKC Technologies, Inc. UTAS Interface Unit. Additional copies of the software may be purchased to produce reports of UTAS data using a stand-alone computer system.

	**Cautions**: • This software is for use ONLY with a LKC UTAS system. • To assure operator and patient safety consult the UTAS Visual Electrodiagnostic System Hardware User's Manual which was shipped with your UTAS system. • To assure other regulatory compliance requirements, consult the UTAS Visual Electrodiagnostic System Hardware User's Manual.
	Read the software use directions, before use, to ensure safety.

INTRODUCTION

LKC's UTAS visual electrodiagnostic test system is designed for electroretinogram (ERG), visual evoked response (VER) (also called visual evoked potentials (VEP)), and electro-oculogram (EOG) testing. It can be upgraded with additional software allowing for multifocal ERG and multifocal VEP. The additional testing software are covered in different manuals. The UTAS is a fully automated system providing features needed for both clinical and research applications. The UTAS meets all the specifications and requirements of the International Society for the Clinical Electrophysiology of Vision (ISCEV).

This manual is divided into three components: The **UTAS EMWIN** Software portion, the **Testing the Patient** portion, and the **GLP/GCP Compliance** portion. The Software component covers all aspects of the software. Explicit instruction on testing patients is covered in the Testing component of the manual. It is important to be familiar with the Software Manual and the separate Hardware Manual before you go through the Testing Manual. Reference 96-020 UTAS System Hardware User Manual for details on UTAS hardware and regulatory information. The GLP/GCP compliance portion is applicable for those who have purchased the additional GLP/GCP compliance software package.

This software is offered for sale only to qualified Health Professionals. The improper use of this software with the associated hardware may cause injury to the patient.

Content

1 Introduction

1.1 Overview

This EMWin User's Manual is divided into two sections; the Software, and Testing manuals. A separate UTAS Hardware Manual accompanies the UTAS system when it is purchased. The Hardware Manual will explain how the system is connected, the specifications for the system, how to use the hardware features, and how to assist LKC in servicing the system should trouble arise. The Software Manual and the Testing manual, will explain how to use the software and the details of performing a patient test.

1.2 Warnings and Symbols

	**Cautions**: • This software is for use ONLY with a LKC UTAS system. • To assure operator and patient safety consult the UTAS Visual Electrodiagnostic System Hardware User's Manual which was shipped with your UTAS system. • To assure other regulatory compliance requirements, consult the UTAS Visual Electrodiagnostic System Hardware User's Manual.
	**WARNING: The installation of any software on the UTAS Windows based computer that is not provided directly by LKC can cause the system to stop functioning, crash unexpectedly, or disrupt the timing of the stimulus presentation and data collection.**

The LKC UTAS Visual Electrophysiology System is a precision standalone medical device. The computer provided with the system has been manufactured and configured for this specific purpose. It is absolutely essential that the timing of the stimulus presentation and data collection not be impeded by any non-LKC provided software products.

The warranty on the UTAS system does not cover problems caused by installation of non-approved software on the computer. The UTAS system is a medical device that uses a Windows-based computer. Installation of additional software on the UTAS computer may result in improper operation of the UTAS system. It is the customer's responsibility to ensure that any additional software installed on the UTAS computer does not affect the performance of their UTAS system. LKC is not liable or responsible for improper operation of the UTAS system caused by customer-installed software.

Therefore, LKC strongly recommends that the system be used as a standalone medical device. LKC also strongly recommends that:

1. **The user does not change any user privileges or software settings.**
2. **No non-LKC approved software products be installed on the system**

2 Introduction

The UTAS software is called EMWIN; it handles all of the details of performing a test – presenting a stimulus, collecting and analyzing the data, storing results, and printing a report. This section of the manual details the use of the EMWIN software. This manual assumes familiarity with the basic operations of the Windows operating system.

	**Note: The computer must be powered by one of the isolated outlets on the Interface Unit. This means that the computer will lose power when the Interface Unit is turned off. Make sure to save all data BEFORE and properly shut down the computer turning off the Interface unit.**

3 Using the System

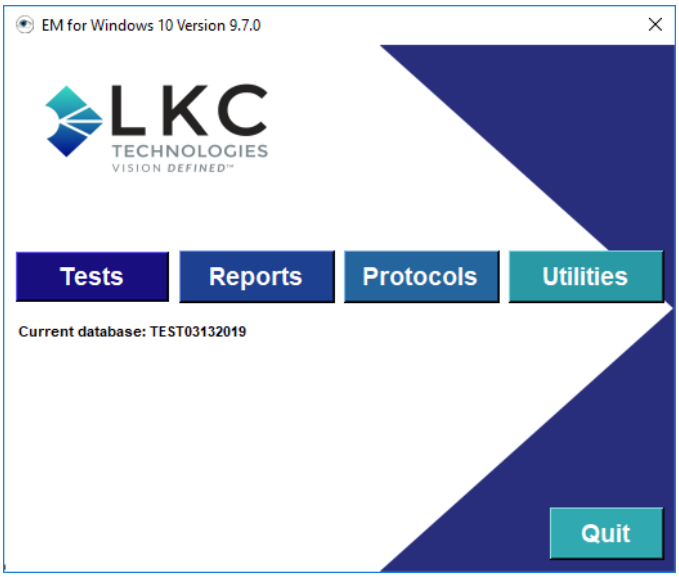
3.1 Starting and Quitting the System

To start using the system, first turn on the power of the Interface unit. Then make sure all peripherals are on (pattern monitor, printer, user monitor, and turn on the computer.

Once the computer boots the EMWIN software will automatically open up to the main menu.

To turn off the system click on **QUIT** in EMWIN main menu, turn off the computer by going to Start -> Turn OFF Computer -> Turn OFF.

Once the computer has powered down, power off the Interface this will turn all the peripherals off as well.



3.2 The Main Menu

The **Main Menu** is used to access all of EMWin's features. The four sections are Perform Tests, Create Reports, Design and Manage Protocols and Utilities.

While the Protocols or Utilities functions may not be used on a daily basis, it is still necessary to understand their functions.

TESTS: Clicking on this button will allow the user to perform all visual electrodiagnostic tests that the instrument is configured for. This segment of the program allows for the collection and storage of data and will be the most frequently used function of EMWin.

REPORTS: This module allows the user to not only print reports, but also analyze the data. Stored data can be recalled and displayed before being analyzed and the reports printed out.

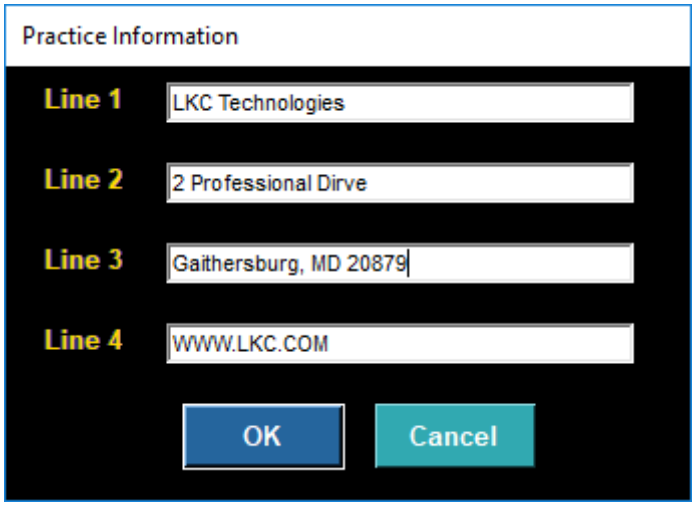
PROTOCOLS: EMWin allows the user to design their own testing protocols. This part of the program allows for the specification of the individual parameters to create specific protocols.

UTILITIES: This section of the program allows the user to change the practice information, edit data and databases, and change the system setup.

3.3 Setting Preferences in Utilities

Although the software and hardware for the LKC Visual Electrodiagnostic Test System are, for the most part, set up at the factory before shipping, there are a few tasks that must be performed to complete the setup procedure.

3.3.1 Entering the Report Header



EMWin will print out up to four lines of text at the top of each printed report. This text is normally the name and address of the practice, but it may be any other four lines of text wanted. In order for EMWin to print out this information, the information must be provided in this section.

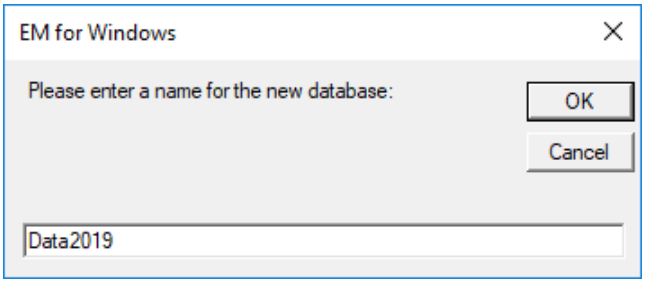
From the Main Menu, click on **Utilities** and choose **Change Practice Information.** A Text Fill-In menu will appear that will allow the user to specify the name and address to appear at the top of the report. (If this information is left blank, no information will be printed.) Each line may have no more than 32 characters in it, so plan the header carefully. Because this information will appear at the top of every report that is printed, make sure that there are no errors.

3.3.2 Creating the Storage Database

EMWin allows for the creation of Access **databases** in which waveforms can be stored. All of the waveforms in one database are hidden from the waveforms in other databases. One method for organizing the files is to create different databases to store them. EMWin will allow the user to specify which database is to be used to store and retrieve waveforms. All of the waveforms in this database are unique and different from those in other databases. For example, it is possible to have several subdirectories called data2007, data2008, data2009, etc. Waveform number 1 in data2008 is totally unrelated to waveform number 1 in data2009.

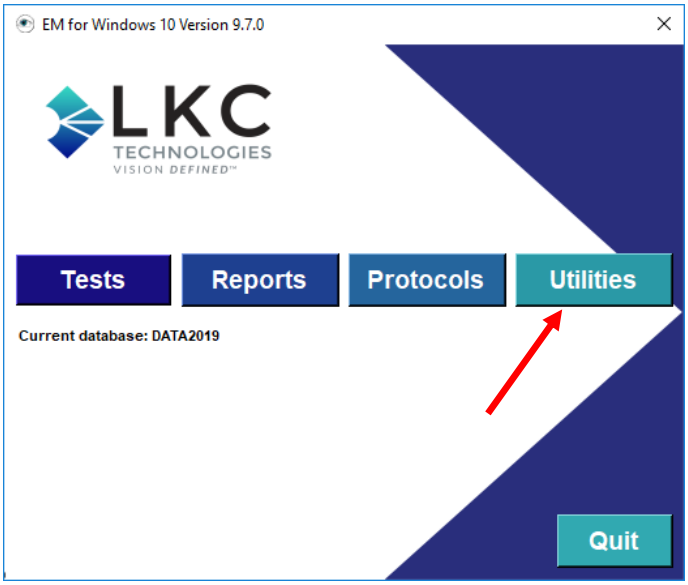
Although each database can theoretically hold an unlimited number of waveforms, the search performance of the computer slows when there are more than a few thousand waveforms in a single database. Therefore, it is recommended to take some time to decide how to organize the storage of waveforms. Depending on how many waveforms will be recorded per month, there can be different database for each month or each year, or per different studies…

Select **Utilities** from the **Main Menu**, and then choose **Create New Database** and **Standard Database** from the popup menu. A screen will then allow for the naming of the new database.



NOTE: The database should only contain letters A-Z or a-z and/or numbers 0-9 (no spaces allowed)

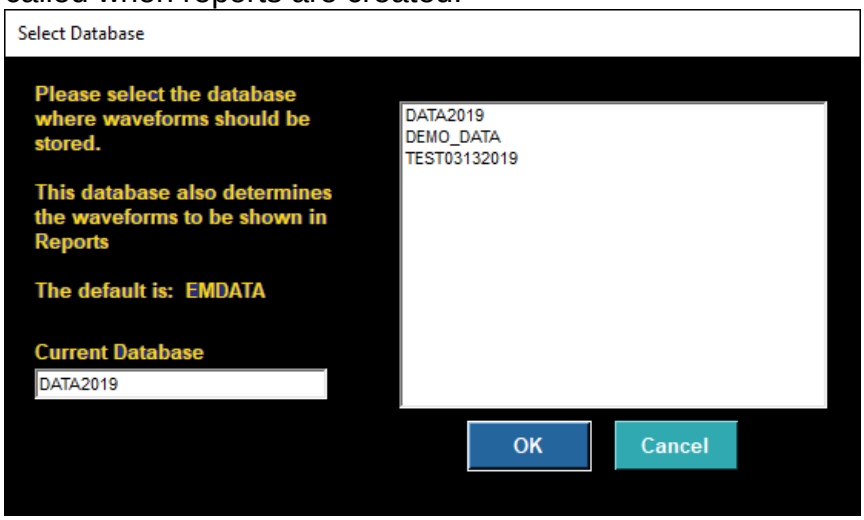
Once the database has been created, don't forget to select it to start storing waveforms in the created database. See the next section for selecting a database.



3.3.3 Selecting the Storage Database

EMWin allows the user to store waveforms in different databases. To select which database to work with, select **Utilities** from the **Main Menu**. Choose **Select Database and Standard Database** from the popup menu. A screen appears that lets the user choose the database desired and also informs them of the current database. This selection

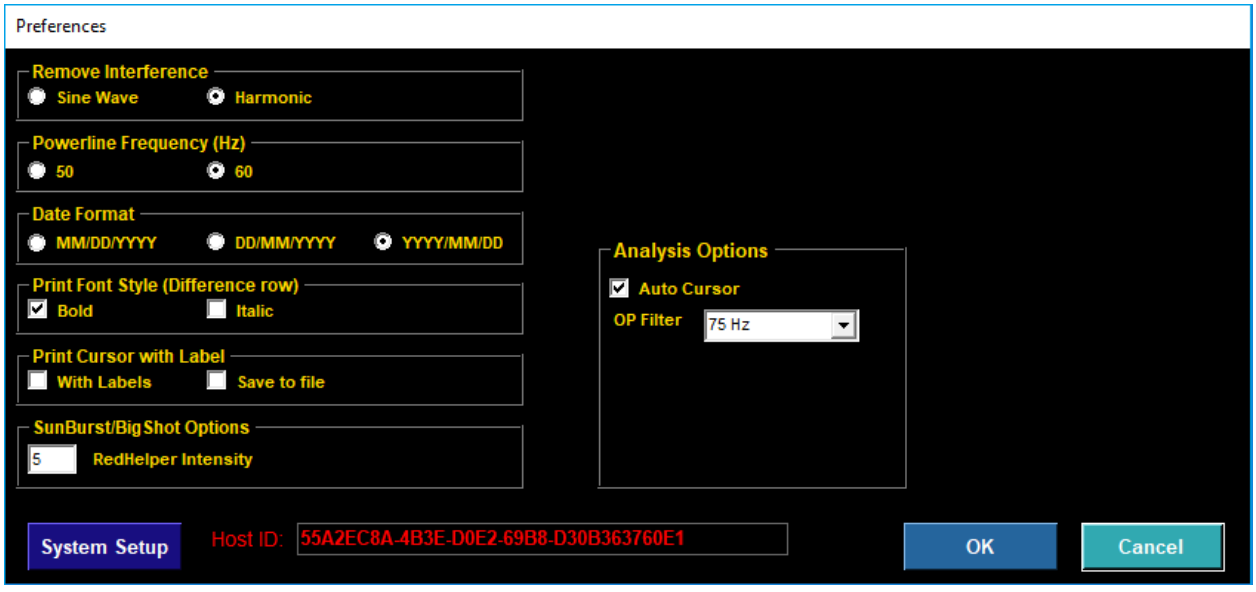
determines the location where test data will be stored, as well as which database will be called when reports are created.



EMWin's Main Menu automatically displays the current database in use.

3.3.4 Selecting the Date Format

The date format is defaulted to YYYY-MM-DD; however, the date format may be changed to DD/MM/YYYY or MM/DD/YYYY by going into Utilities and System Preferences.



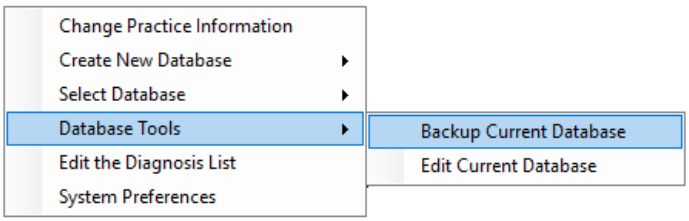
3.3.5 Selecting the OP Filter

The software can be used with custom OP filters. The software is preloaded with the following OP filter frequencies: 45 Hz (Mouse), 75 Hz (Human), 100 Hz, 125 Hz, and 150 Hz. To use any of the filters, select them from the dropdown list (see preferences window in section 10.3.4). If user defined filters would like to be used (up to 500 Hz), simply type in the drop-down box the filter frequency desired.

3.4 More on Utilities

Initial set up of preferences for Practice Information, Database and Date format are covered in section 10.3.

The next section of the manual will be devoted to covering the remainder of the Utilities menu.



3.4.1 Backing Up Databases

Utilities -> Database Tools -> Backup Current Database. It is good practice to frequently backup the data. How often depends on how much data is willing to be lost. To backup a whole database, go to Utilities -> Backup Database. Select the database to backup (the currently used database is displayed on the bottom part of the main menu).

Highlight the database to be backed up and then select which location it should be saved in. It is recommended that databases be backed up to a different filesystem (such as an external USB drive) than the original database.

3.4.2 Editing a Database / Patient Information

Utilities -> Database Tools -> Edit Current Database. It's possible to change the information in the database; to delete waveforms and edit patient information. For example, if a patient was tested and the patient's last name was accidentally misspelled, the problem can be fixed by going to edit current database, finding the waveform of the patient (by using the waveform number) and clicking on edit patient information. There, it is possible to edit: first name, last name, date of birth, label of the eye (R, L, OD, and OS), diagnosis and comments. Then proceed to the next waveform that needs editing.

3.4.3 Editing the Diagnosis List

When entering the patient information, it is possible to select a diagnosis to associate with the patient data. A new diagnosis can be selected from the drop-down list or typed directly in the patient information window (the program will automatically add to the diagnosis list). However, if you need to edit or delete some of the diagnoses you will need to go to **Utilities -> Edit the Diagnosis List**.

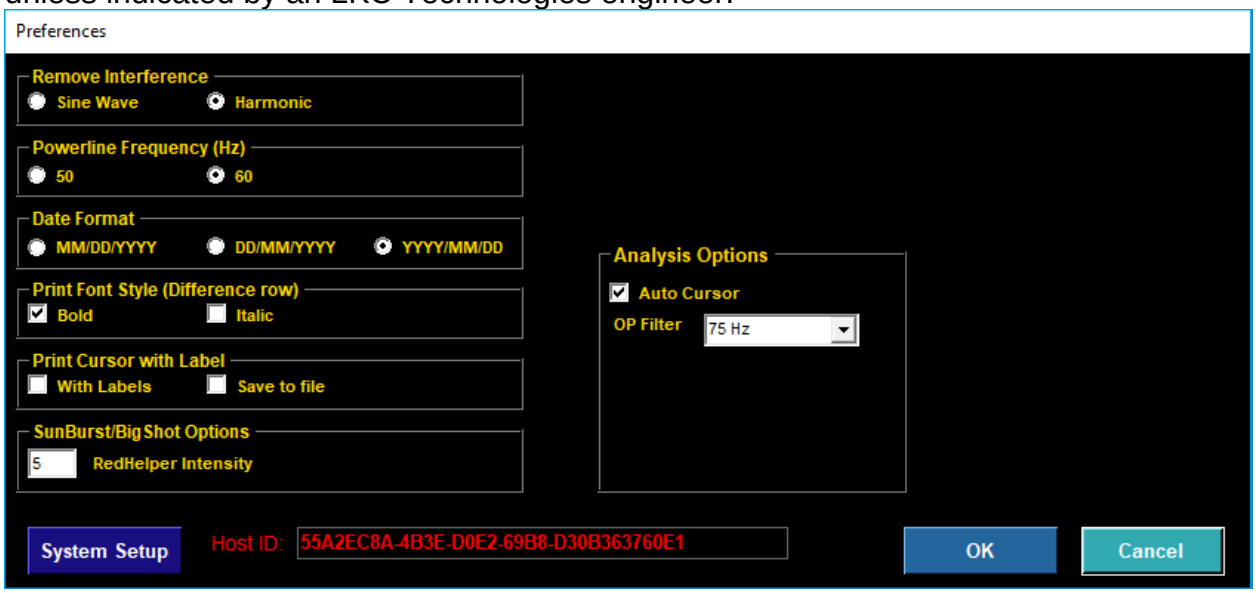
3.4.4 System Preferences

Powerline Frequency (Hz) – Set this to whatever power line frequency is used in your location. This setting helps EMWin reduce powerline interference in test results.

Print Font Style (Difference Row) - This specifies the difference row of the cursor printout in bold or italic. Note that the choice is possible only when Print Cursor with Label is not checked

Screen Size
Used to optimize viewing when using a secondary computer for data analysis.

System Setup contains all information specific to the system and should not be changed unless indicated by an LKC Technologies engineer.



3.5 Performing a Test

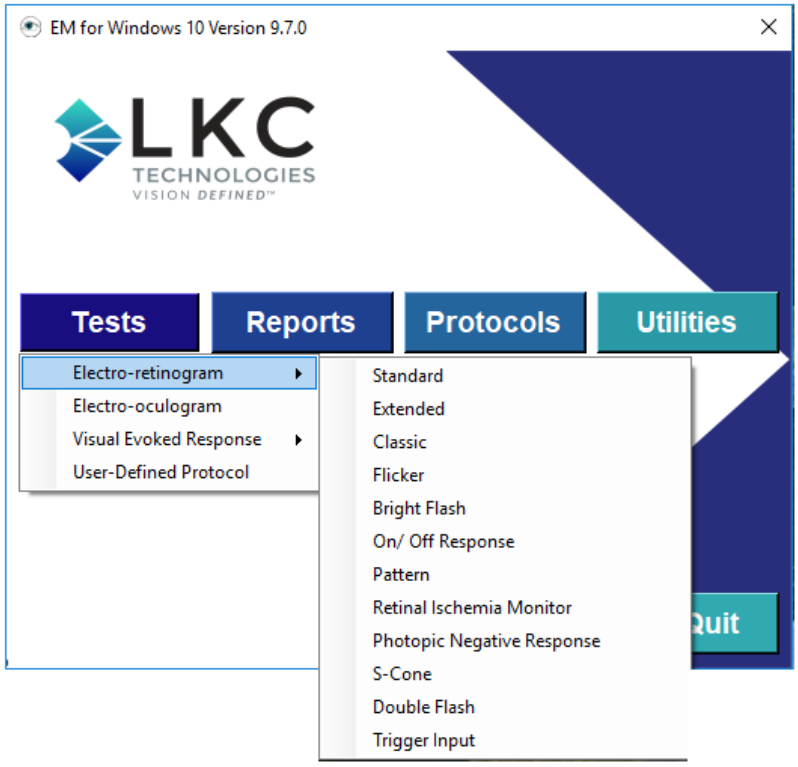
This section covers procedures that are common to all tests run using the UTAS.

3.5.1 Selecting a Test

To perform a test with the EMWin system, click on **Tests** from the Main Menu, and choose the test to be performed. The options are:

- Electroretinogram
- Electro-oculogram
- Visual Evoked Response
- User Defined Protocol

After the type of test that you wish to perform has been selected, another menu will appear asking for the specific test to perform. Click on the appropriate test from that menu.



3.5.2 Patient Information

After the test has been chosen, EMWin will provide a screen to enter patient information.
It is important to stay consistent in the format of entered information, otherwise, data analysis and retrieval could be more difficult.

If more than one test is run in the same EMWin session, the demographic information from the last patient tested will appear as the default values for the next test. To clear this information, simply click on the **Clear** button at the bottom of the menu. This will delete all information and allow new patient information to be stored. Fill in all of the items deemed appropriate (it is not *required* that all fields be filled).

Depending on whether the test subjects are animals or humans, there can be two different patient information windows. EMWin is defaulted to the human information window. If the animal version is required, go to **My computer -> C:\EMWIN**, open the **temp** folder, and rename the file **Animal_Demographics.txt** to **Demographics.txt**.

When the data is retrieved after testing, the patient information entered can be used to make data retrieval easier. The searchable items are marked with an asterisk. (*)

Last Name, First Name*, Middle Initial** - The first letter of names does not have to be capitalized, since EMWin will automatically capitalize them when data is stored. There is space for 16 characters in both **Last Name** and **First Name** and 2 characters in **Middle Initial**.

Sex and Birthdate** - The **Sex** field should be entered with either M or F.

*Identification** - There is space for 16 characters in the **Identification** field. Any alphanumeric combination can be used for this information. It could be the patient's Social Security number, or their medical ID code.

Pupils Dilated - Record in this field whether the patient's pupils were dilated with mydriatic eye drops for this test.

Diagnosis - A diagnosis can be typed into this field or choose one from the pull-down menu. The typed diagnosis will be automatically remembered and added to the list. The list can be edited to add or remove or correct spelling by going into **Utilities** and **Edit the Diagnosis List** (see section 10.4.3).

Electrode Type - For ERG testing, choose the type of the electrode used in the test from the drop-down list.

Dark Adapt Time - Record the amount of time in minutes that the patient was dark adapted before ERG testing.

*Other** - This field allows any additional information to be recorded. When the records are retrieved, this field is searchable. Thus, it may be useful for recording information such as participation in studies.

Database – This window shows the current database where the data will be stored. Another database can be selected from the pull-down menu. However, to permanently store the data in a database other than the one defaulted; it must be changed in the **Utilities** Tab. Refer to section 10.3.3 for instruction on how to "Select the Storage Database".

Comment - Enter comments here about the patient that will be stored with the data. There is no restriction in length. Typical comments might include symptoms the patient presented with, referring physician, or classification in a study group.

The "Continue" button -When you have completed all of entries, click on this button to continue.

The "Cancel" button - This will return the screen back to the main menu.

The "Clear" Button - This will clear out all of the information in the fields.

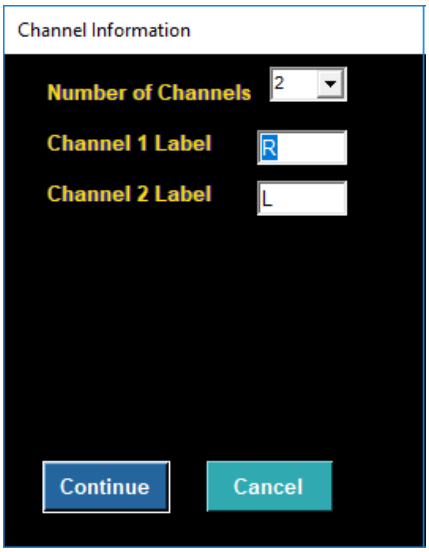
The "Search + Fill" button – This will search the current waveform database for matches. Fill in the patient's Last Name and click on this button. If the system finds a match, it will fill in the remaining items based on the information stored in the database.

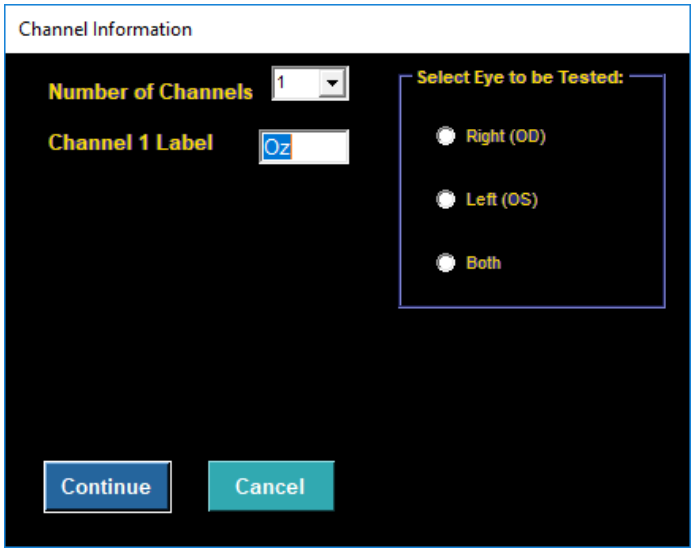
If no matches are found, a notice will appear, and the information may be entered manually.

3.5.3 Channel Information

Once the patient information has been entered, click the continue button, and the channel information menu will appear. Select the number of channels to be recorded from and place the Label for each channel.

The software defaults R in channel 1 and L in channel 2 for ERG and Oz in channel 1 for VEP. Note that for VEP testing you will also need to select the eye to be tested.





ERG *VEP*

If only one eye is tested, channel 1 should be used regardless of which eye is being tested.

3.6 Icons and Menus

3.6.1 Parameters

By clicking on parameters in the upper menu bar or the Amplifier (gear), or the light bulb icon, the parameter window will open.

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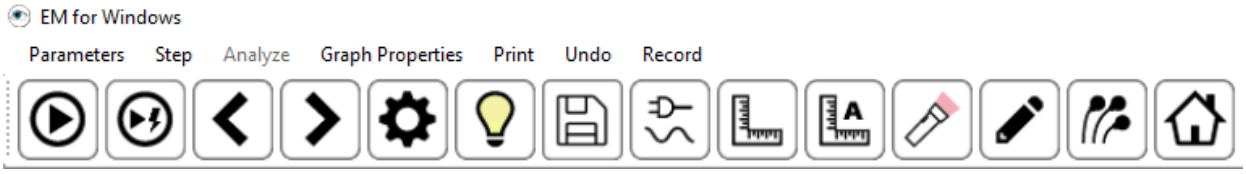
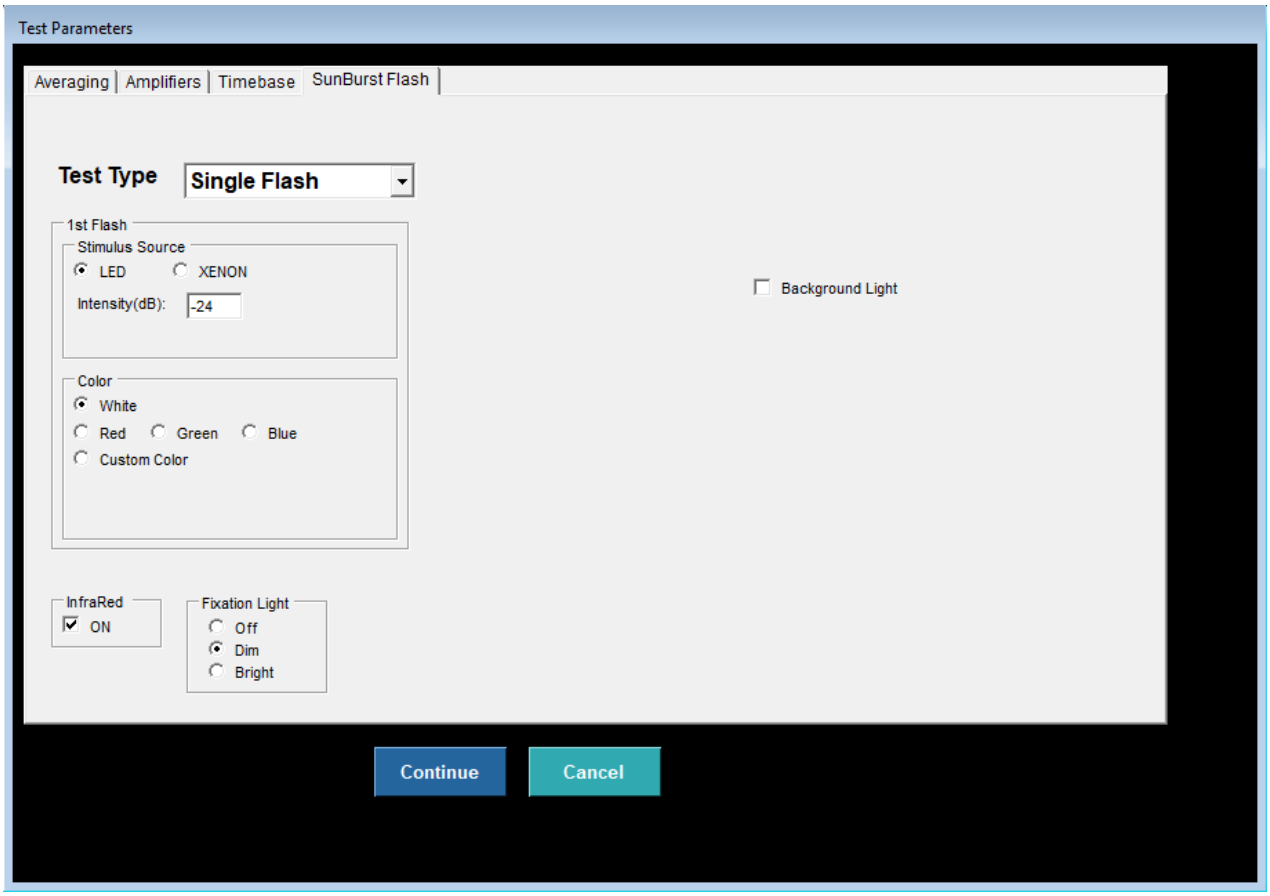


The parameters window contains four tabs: SunBurst Flash, Averaging, Amplifiers and Timebase.

SunBurst/BigShot Flash Parameters

This window allows the user to change the flash color, intensity, flicker rate as well as the background light intensity and fixation light.

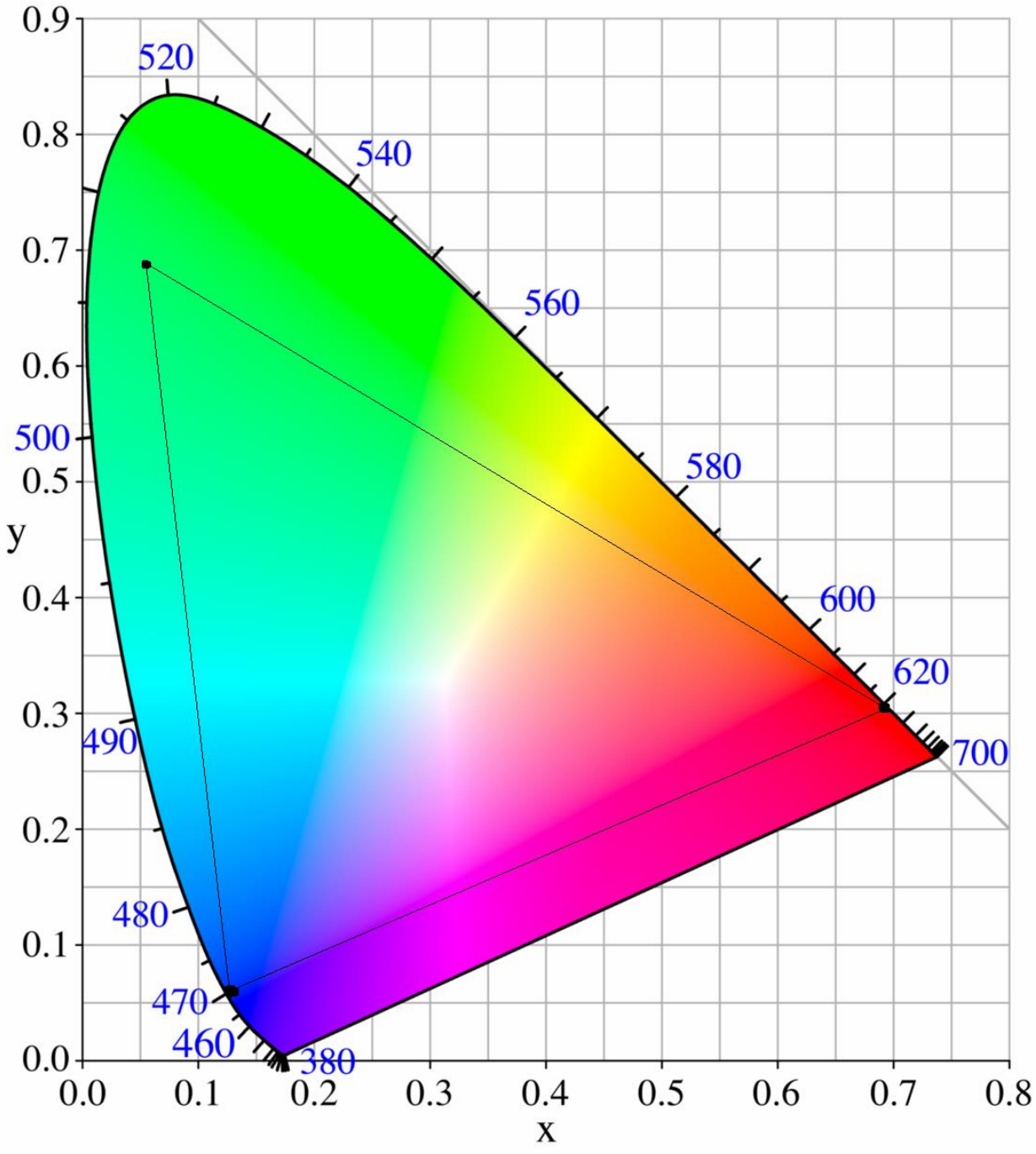


There are 4 different options for the Ganzfeld flashes:

1. Single Flash
2. Double Flash
3. Flicker

4. On/Off

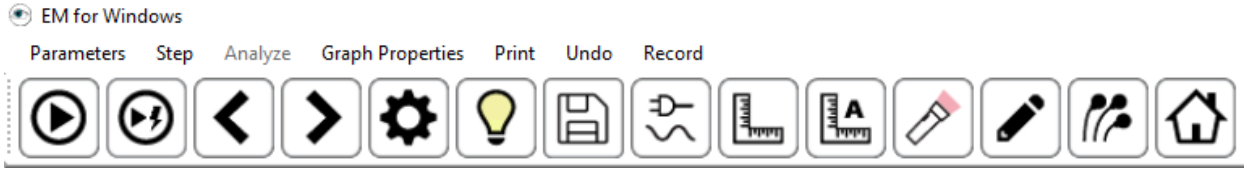
The range of the intensities depends on the stimulus source (LED, Xenon or UV if applicable)
For LED flashes, the x, y coordinate of color can be entered according to the CIE color diagram below. Note that only colors within the inner triangle can be achieved.



CIE Color Diagram

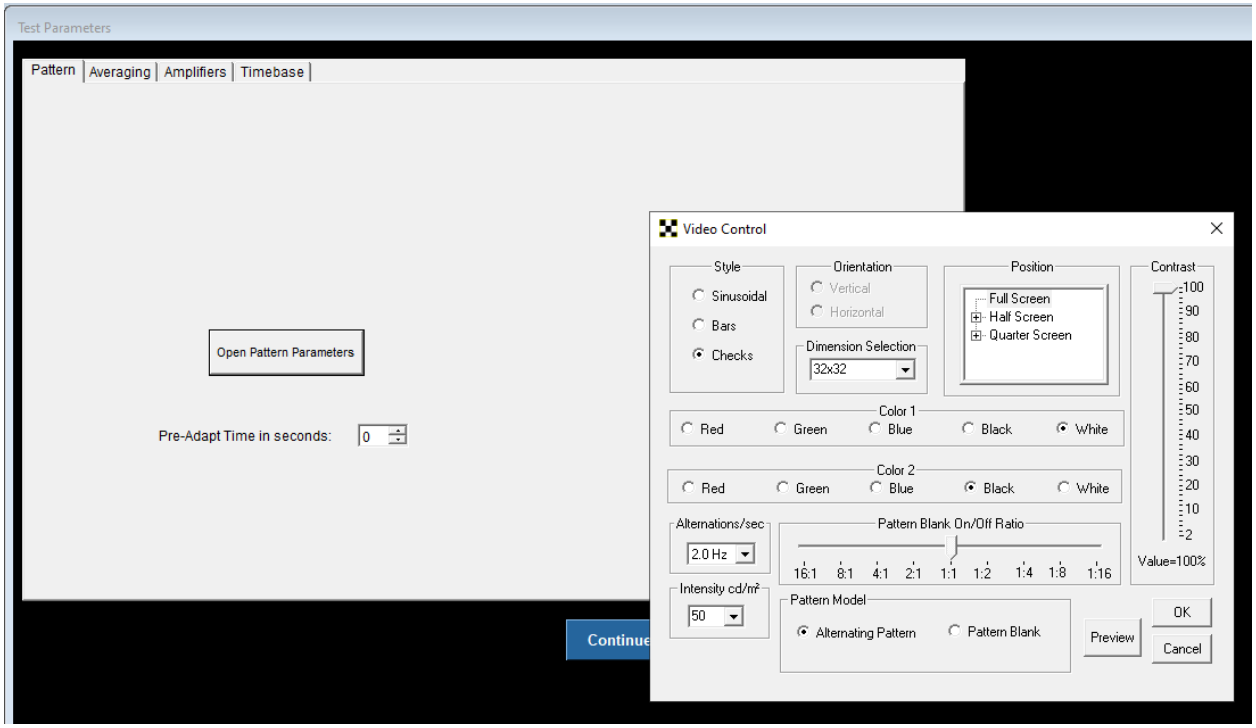
In order to produce:
- Pure red LED stimulus enter x = 1, y = 0
- Pure blue LED stimulus enter x = 0, y = 0
- Pure green LED stimulus entre x = 0, y = 1

Pattern Parameters

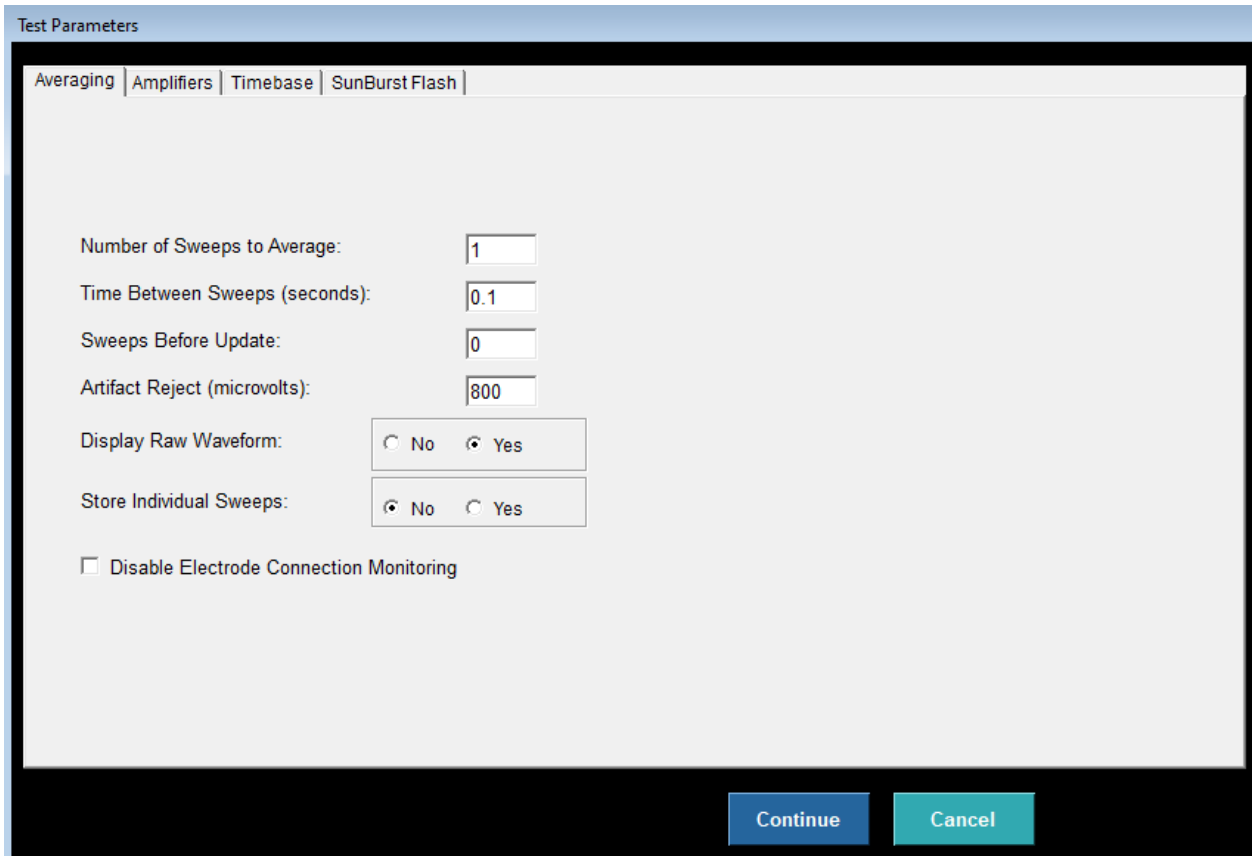


In the case that a pattern stimulator is being used, the icons above will appear. The Checkerboard icon will open the pattern parameters.

Pre-Adapt Time specifies how long the pattern will alternate before the actual recording starts.



Averaging Parameters



The Averaging Menu will appear, allowing the modification of the following options:

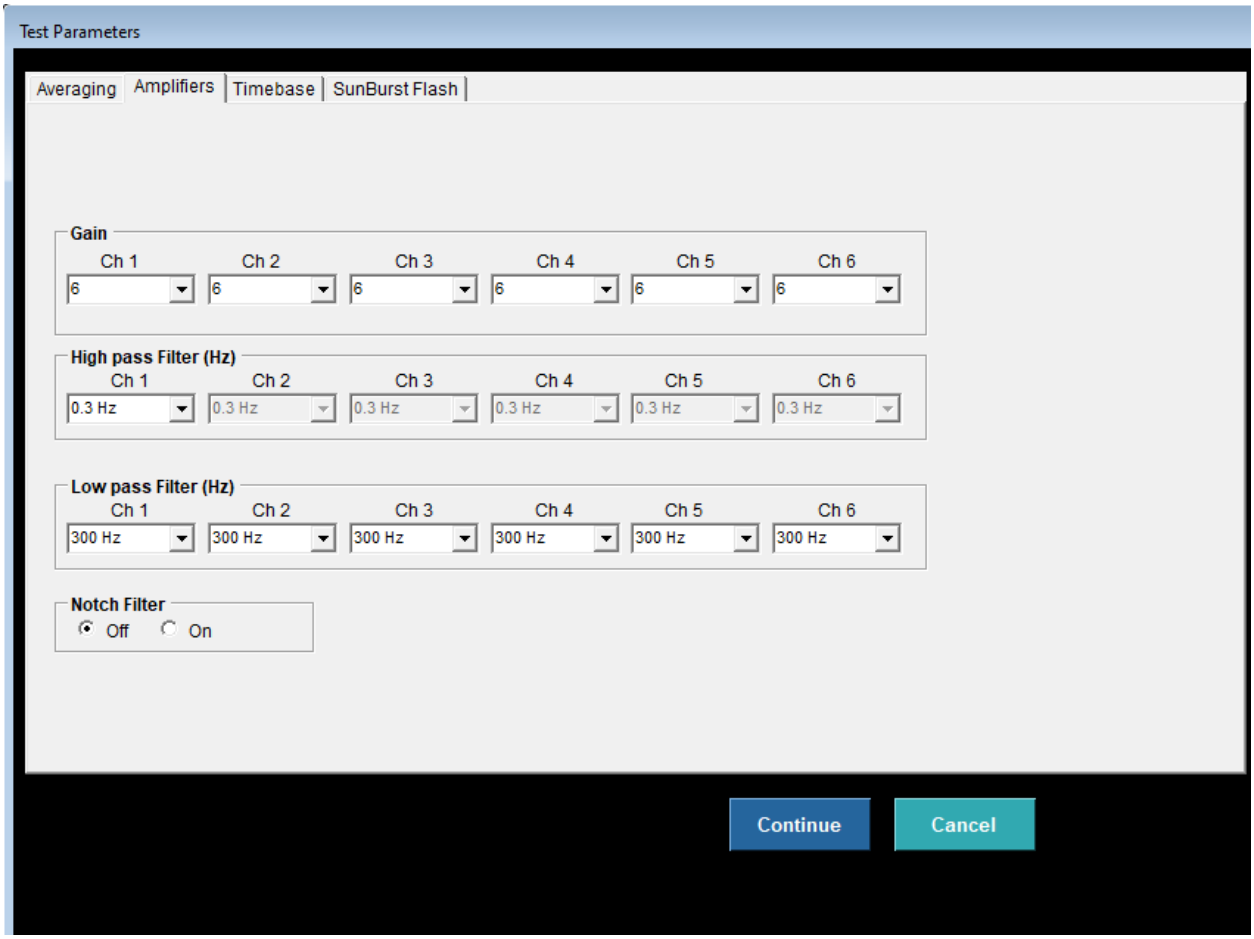
- Number of sweeps to average
- Time between sweeps (seconds): At least 5 - 10 seconds between flashes is necessary for scotopic testing to avoid light-adapting the subject during averaging. To manually reject waveforms, it is recommended that this be set to at least 2 seconds.
- Sweeps before update: Number of sweeps to acquire between screen displays of averaged waveform data.
- Artifact reject (microvolts): Threshold for rejecting artifacts. This option allows automatic rejection of waveforms during averaging that exceed an amplitude criterion. If zero is entered, the artifact reject option is disabled. If the artifact reject threshold is set to a value that is higher than the input range of your chosen amplifier gain setting, EMWin will instruct the user to lower the artifact reject threshold.
- Display raw waveform: Whether or not to display each waveform as it is acquired. For single flash ERGs, each response is usually viewed as it is generated. However, for VEP tests, each individual response is typically meaningless since it is usually embedded in noise. To manually inspect waveforms and reject them individually, each waveform must be displayed as it is acquired.
- Store individual sweeps. Typically, EMWin only stores the final average of all sweeps from one test, and the raw responses that make up the average are

discarded. If **Store Individual Sweeps** is selected, all raw responses will be stored in the database as well (up to 30 responses). In Report mode the raw responses are included or rejected from the averaging process. Using this feature adds an additional step of manually choosing each response when preparing reports.

- Disable electrode connection monitoring: Allows users to select whether the software should detect if an electrode becomes disconnected.

Setting any of these parameters to “0” disables that option.

Amplifier Parameters



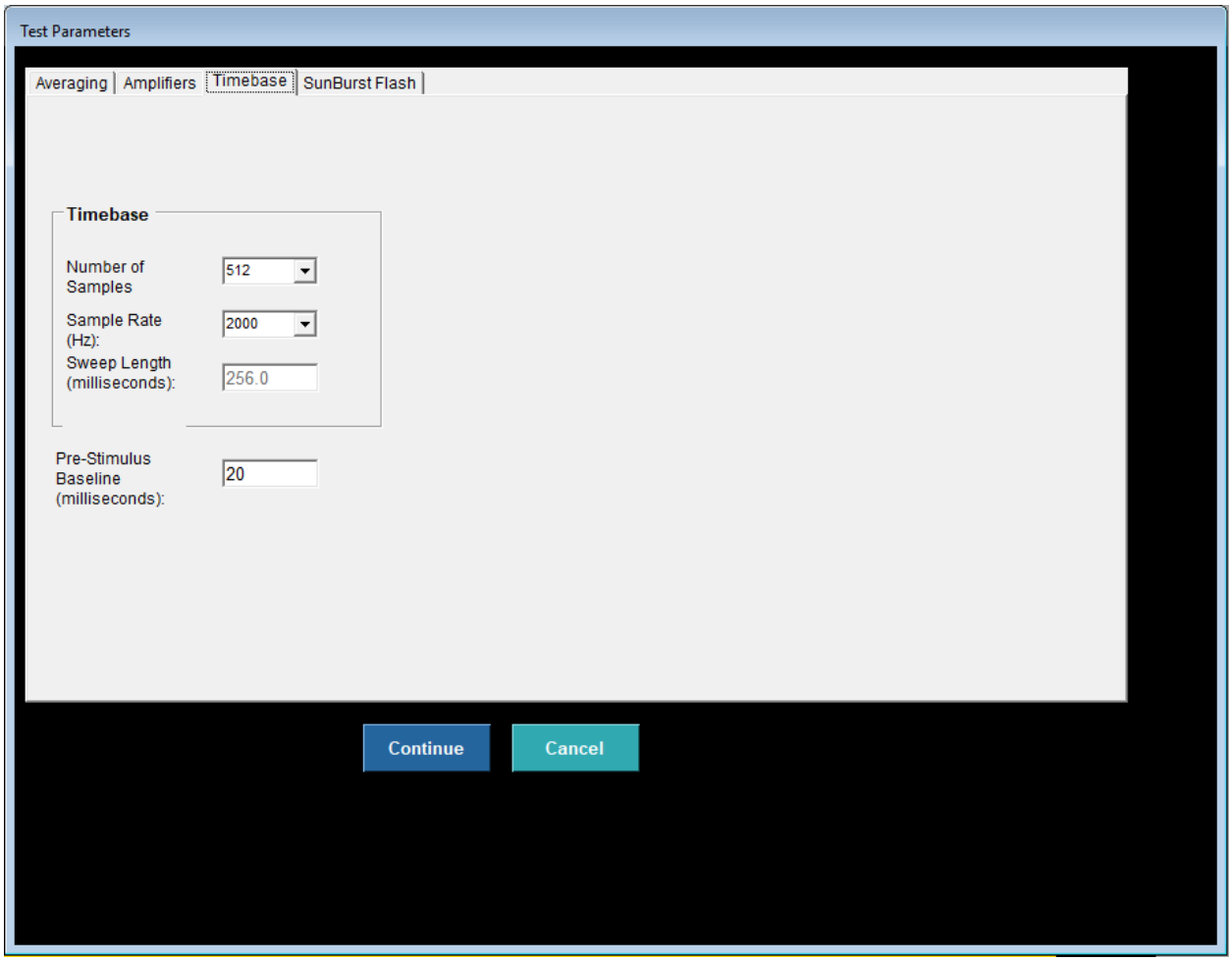
This selection is used to change the amplifier gain or filter settings for the protocol.

If the default values of the High Cut and Low-Cut filters are changed, the waveform may significantly be altered.

The “Notch Filter” will selectively reduce 60 or 50 Hz powerline interference. Typically, excessive powerline interference can be reduced by better electrode placement or by

moving the patient away from powerline sources. However, the notch filter can be turned ON if all other noise reduction techniques fail.

Timebase Parameters

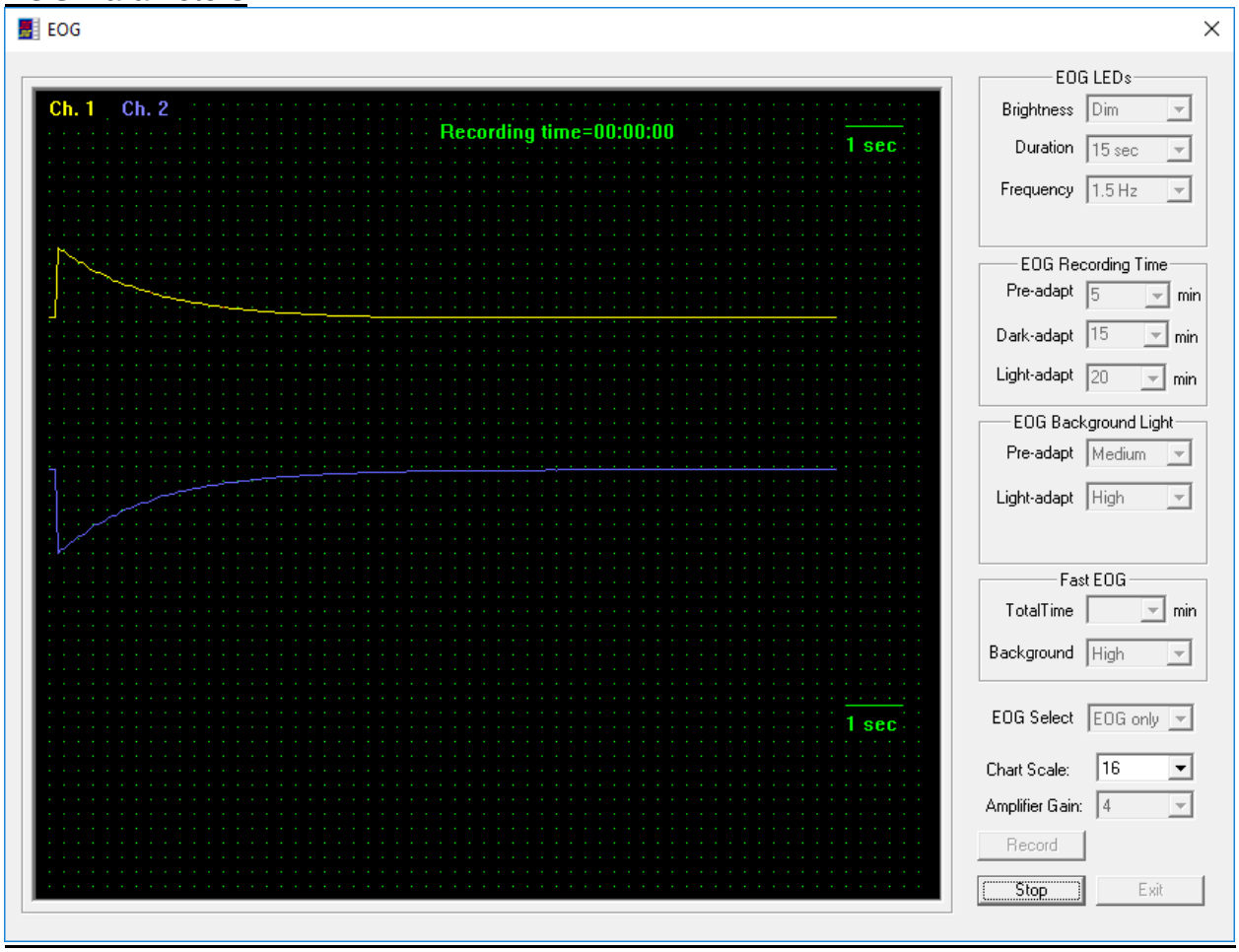


- *Sample Rate and Sweep Frequency* - This screen allows the user to control the sampling frequency (default is 2 kHz). A single recording sweep always includes 512 samples. If the Sample Rate is changed, the Sweep Length will automatically be updated.

- *Pre-Stimulus Baseline* - A Pre-Stimulus Baseline allows the user to adjust the amount of data to be collected before the stimulus is presented. Typically, this is used to indicate the amount of baseline noise present just prior to a flash stimulus.

A "Pre-Stimulus Baseline" cannot be entered when performing a flicker test and the software will automatically set this parameter to 0 if attempted.

EOG Parameters



Brightness - If the patient is having trouble seeing the Red LEDs, the brightness of the LEDs can be set to a higher setting.

Duration - The duration of the recording time is 15 sec, meaning that the LEDs will alternate from right to left for every first 15 sec of each minute.
Frequency - If the patient has problems following the LEDs, the alternation frequency can be slowed.

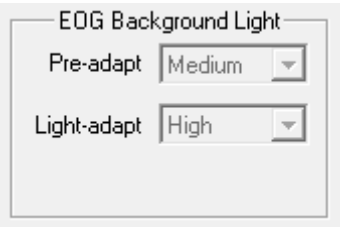
Auto Save Sweeps – The raw data of every sweep can be saved to file. The files will be saved under C:\EMWIN\EOGData. The sweeps will be saved as sweep_001.txt and so on up to sweep_999.txt

The settings in EOG recording time are preset to the ISCEV standard and usually do not need to be changed. However, if the patient has reached the lower value in the dark adapt time, the time may be shortened while the test is being run. If the patient has reached the peak in the light-adapt phase, the test can be stopped; all information needed has been recorded.

The EOG backgrounds are set to low for Pre-adapt and Medium for Light-adapt. According to ISCEV standard the eyes of the patient must be dilated for EOG, so it is not recommended to use the high intensity background on dilated eyes.

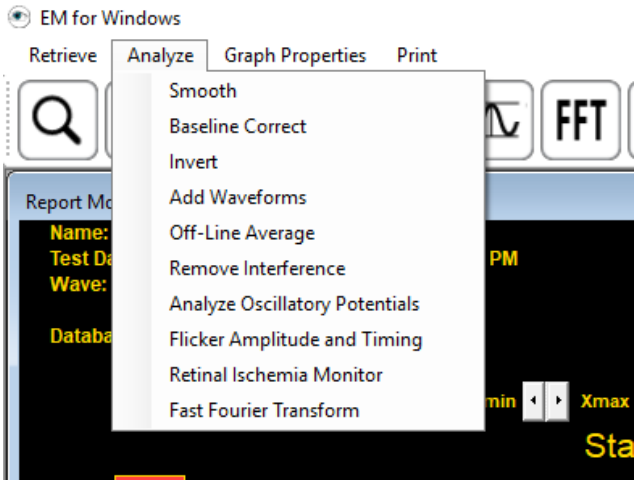
Display Scale - If the data seems too large for the window, change the display scale to a lower setting.

Custom Intensity Background Light - for UTAS SunBurst and BigShot user, the light adapt phase can be set to a custom intensity; just select Custom in the Light Adapt drop down list and type in the desired value.



3.6.2 Analyze

Once waveforms are displayed, many analysis features can be implemented. For each function, the system will ask which waveforms are to be acted on.



<u>*Smooth*</u> –

Smoothing can be achieved by going to *Analyze -> Smooth* or by clicking on the icon.

The smoothing algorithm is useful for removing excess high frequency noise from a waveform. The smoothing procedure consists of replacing each point in the waveform by the average of the surrounding 11 points (five on each side plus the point itself). This process acts as a high-cut filter that preserves the latency of all of the features of the waveform. If smoothing the waveform once does not provide sufficient noise reduction, the smoothing operation can be performed multiple times.

Warning: Smoothing a single flash ERG waveform will remove any oscillatory potentials and may change the initial slope of the a-wave.

<u>*Baseline Correct*</u>

Baseline Correct can be achieved by going to *Analyze -> Baseline Correct* or by clicking on the icon.

The purpose of baseline correction is to remove any slope or ultra-low frequency noise from the data that might interfere with estimating amplitudes. Baseline correction is accomplished by performing a linear regression on the data and subtracting the resulting line from the waveform. If the stimulus is flickering, the slope of the line is estimated from the entire waveform; otherwise it is estimated from the pre-stimulus baseline. Thus, if a waveform has an overall upward slope, baseline correction will remove it. If the waveform goes first up and then down (or vice-versa), the baseline correction function may not be useful.

Invert

Invert can be achieved by going to *Analyze -> Invert* or by clicking on the icon.

A waveform may be inverted (make positive-going potentials appear below baseline and negative-going potentials appear above baseline). This feature is useful if you wish to make the waveform appear as if you had interchanged the + and - connections to the patient amplifiers. It is also useful for subtracting waveforms. (See Add Waveforms)

Add Waveforms

Add Waveforms can be achieved by going to *Analyze -> Add Waveforms* or by clicking on the icon.

Two or more waveforms may be added together by selecting Add Waveforms. All of the waveforms chosen will be added together and will replace waveform 1 on the screen. (The original waveforms will be removed, leaving only the summed waveform.)

Two waveforms can be subtracted by first inverting one of them (see Inverting Waveforms above) and then adding the two together. For example, it is possible to subtract a Scotopic Red ERG from a matched Scotopic Blue ERG to remove the cone contribution and obtain a good estimate of the isolated rod ERG.

Off-Line Average

Off-Line Average can be achieved by going to *Analyze -> Off-Line Average* or by clicking on the icon.

It is possible to retrieve and average waveforms that have previously been stored to disk. This process is called off-line averaging. First, open all of the waveforms to be averaged, placing all of them on the screen at one time. Then select Off-Line Average. All of the waveforms chosen will be averaged together and will replace waveform 1 on the screen. (The original waveforms will be removed, leaving only the averaged waveform.)

Remove Interference

Sometimes, despite all precautions, a recording will contain some power line interference. If desired, EMWin will estimate the amount of power line

interference in the waveform and remove it. Of course, after the waveform is stored, there's no way to know how much of the signal at the power line frequency (60 or 50 Hz) is noise and how much is part of the original signal, so EMWin subtracts all of it out. Except for 30 Hz flicker (where there may be a substantial real component at 60 Hz), this subtraction should cause no problems.

Removing power line interference after the test has been performed can clean up a waveform that has some noise in it, but it will not rescue an otherwise uninterpretable waveform. If the power line interference is larger than the original signal, the waveform should be discarded.

Analyze Oscillatory Potentials

Analyze Oscillatory Potentials can be performed from *Analyze -> Oscillatory potentials* or by clicking on the icon.

Oscillatory potentials (OPs) are fast wavelets on the ascending edge of the b-wave of the flash ERG. They have been shown to be a good predictor of progression of neovascularization in patients with diabetic retinopathy or with central retinal vein occlusion (CRVO). There are two methods to obtain OPs from the EMWin system.

The first method that can be used to extract OPs (and the one that LKC recommends) is to record the flash ERG with the low-cut filter set normally, and use the software filtering system to extract the OPs. The software filtering method results in a more accurate representation of both the amplitude and latency of the individual OP wavelets.

To use the EMWin software to determine oscillatory potentials, record the OPs using the either the Standard or the Bright Flash ERG protocol (The Standard protocol follows the ISCEV guidelines for recording oscillatory potentials, while the Bright Flash protocol more closely approximates the technique of Bresnick et al.). Store the raw waveforms. Click on *Analyze* and select *Analyze Oscillatory Potentials*. After the waveforms from which to extract Ops have been chosen, EMWin will filter the waveforms and display them on the screen. The waveforms may then be stored again.

After the waveforms have been filtered to extract OPs, EMWin will ask if to automatically place cursors on the OP wavelets. If this is desired, click *Yes*. The program will then place cursors on the oscillatory potentials (at its best guess for the maximum and minimum for each one) and will determine the summed OP amplitude. EMWin will place up to 10 cursors on up to 5 OP wavelets. To access the cursors of these wavelets, use the *Cursors* option from the Reports Menu. The summed OP amplitude will be reported at the top of each analyzed waveform.

The second method is to record the flash ERG with the low cut filter of the patient amplifiers set to 75 Hz. The 75 Hz filter will remove the low-frequency components of the ERG, leaving only the higher frequency components, including the oscillatory potentials. (Step 3 of the Standard ERG Protocol implements this filtering.)

The cursors may also be manually placed on the Ops by selected Cursors from the Reports tab or the button bar.

Flicker Amplitude and Timing

Flicker Amplitude and Timing can be performed from *Analyze -> Flicker Amplitude and Timing* or by clicking on the following icon.

EMWin provides a means of determining the implicit time and amplitude of the flicker electroretinogram. This technique only works for flicker rates of 20 Hz or faster. The implicit time estimates are derived somewhat differently from the normal technique of placing the cursor at the peak of the response. The technique used by EMWin estimates implicit time based on finding the center of the response; this technique has been proven effective in prediction of neovascularization of the iris in central retinal vein occlusion. (CRVO)

To automatically determine implicit time and amplitude of a flicker ERG, click on *Analyze* and select *Flicker Amplitude and Timing*. Select each waveform to analyze. The analysis will then be performed.

After the analysis is complete, the estimates of Amplitude and Implicit Time (abbreviated *Ampl.* on the screen) will be displayed. This technique does not affect the placement of cursors on the waveform, nor does it place any cursors of its own.

Retinal Ischemia Monitor

This can be found under *Analyze -> Retina Ischemia Monitor*

The *Retina Ischemia Monitor* (RIM) algorithm developed under a grant from the National Institutes of Health, analyzes 30 Hz flicker ERG waveforms to provide information about the extent of ischemia (inadequate blood supply) in the retina. Implicit time provides more information than amplitude. The implicit time reported by Retinal Ischemia Monitor is not the same as the implicit time of the peak of the waveform.

Fast Fourier Transform

Fast Fourier Transform can be performed from *Analyze -> Fast Fourier Transform* or by clicking on the following icon.

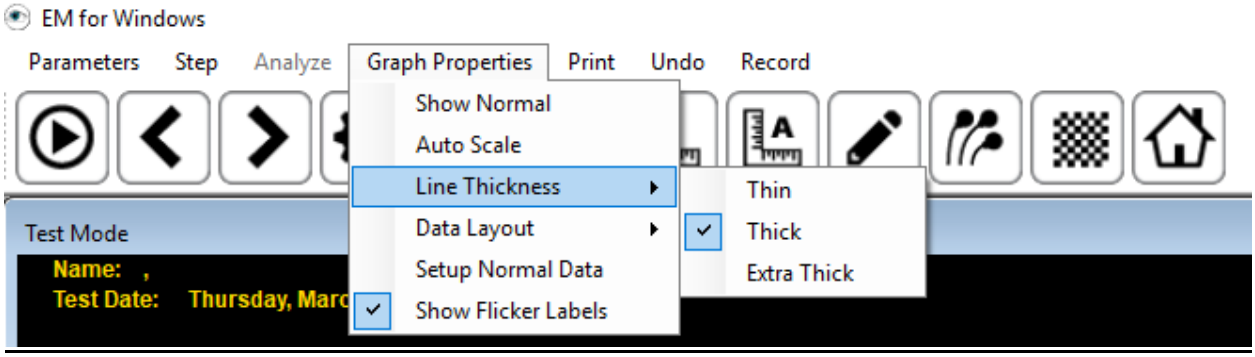
The Fast Fourier Transform is computed for selected waveforms, giving information on specific frequency components of waveform data. It is possible to determine the amplitude and phase of a specific component using the mouse.

3.6.3 Undo

This will remove all the analysis that was performed on the waveforms prior to saving (smooth, analyze OP, auto scale….)

3.6.4 Graph Properties

These options control how the data is displayed on the graph.



Show Normal - This function will write the upper and lower normal limits of the standard ERG adjusted to the age of the current patient. This function is toggled on/off to add/remove the normal data from the waveforms. LKC only provides normal values for the standard 5 step ERG protocol.

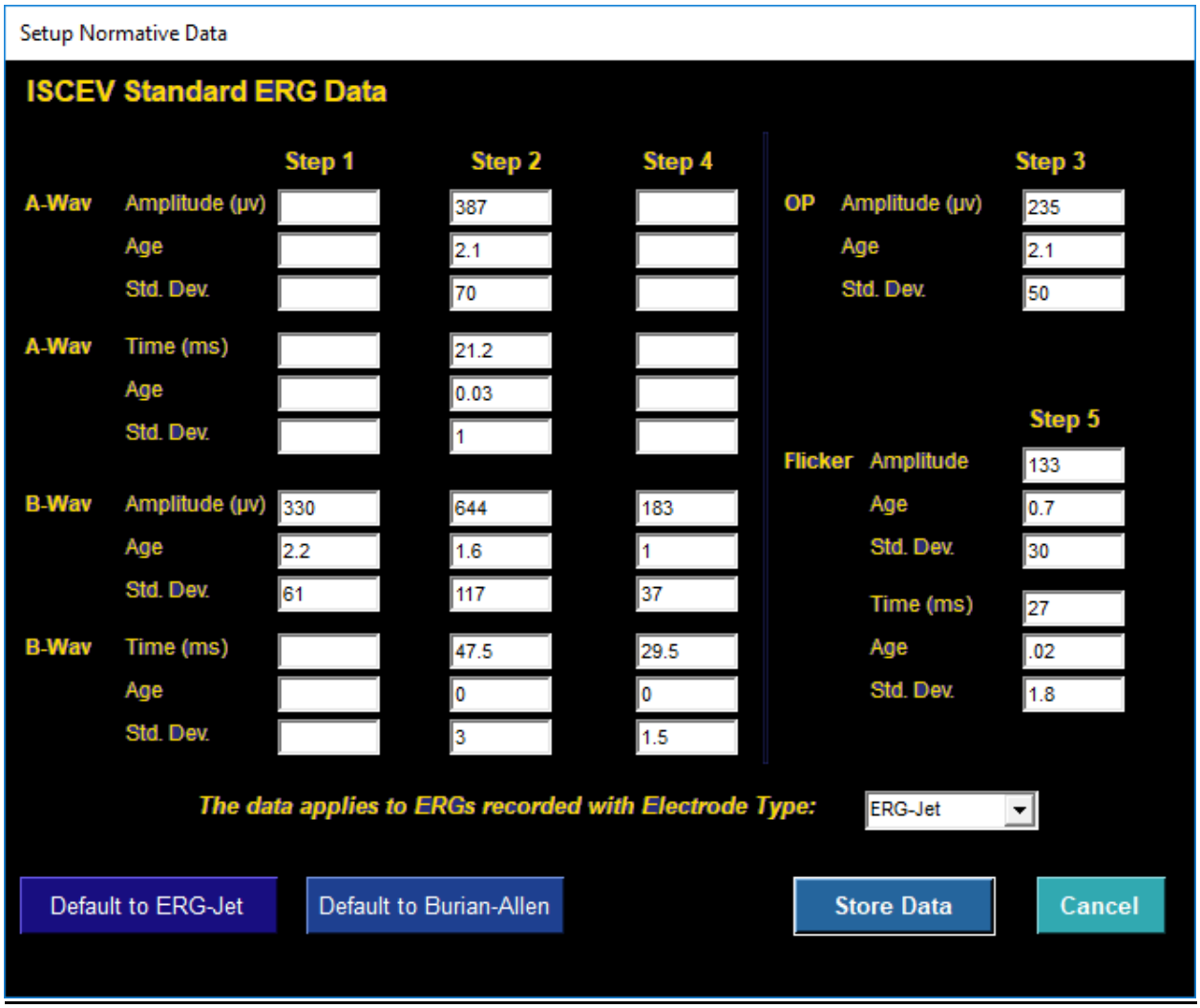
Auto Scale - This will expand all waveforms to fit closely within the waveform's axes. Although the Auto Scale feature make waveforms look larger in amplitude, these large amplitudes can be deceiving, since even small-amplitude signals will be expanded to fit the entire graph. Please use this feature with caution when analyzing waveforms.

Line Thickness - This option allows the user to choose from among Thin, Thick, and Extra Thick for the thickness of the line. The default is Thin. Thicker lines may be useful for easier visualization, especially on printouts and faxes.

Data Layout - This allows the data to be displayed as separate data sets or overlapping data sets.

3.6.4.1 Setup Normal Data –

This allows practice-specific normal data to be entered for the standard 5 step ERG protocol so that it displays when "show normal" is selected. It is recommended that each institute develop their own normal data. Normal data differs for each electrodes type (ERG-Jet, Burian Allen…) particularly in amplitudes. Only use this feature for 5 step ERG protocols.



Show Flicker Labels - Choose whether each flash will be represented by an upside-down triangle on flicker waveforms.

3.6.5 Retrieve

Search -This option returns the screen back to the Patient Information Screen and allows a new search to be performed.

Next - Selecting Next will display the next waveform(s) stored in the current database.

Previous - Selecting Previous will display the previous waveform(s) stored in the current database.

Re-Read - Clicking on Re-Read will redisplay the current waveform. This is especially useful when analyzing data and the original, unedited data is needed.

Add More - Clicking on Add More will open the search waveform window and allow adding more waveforms to the report.

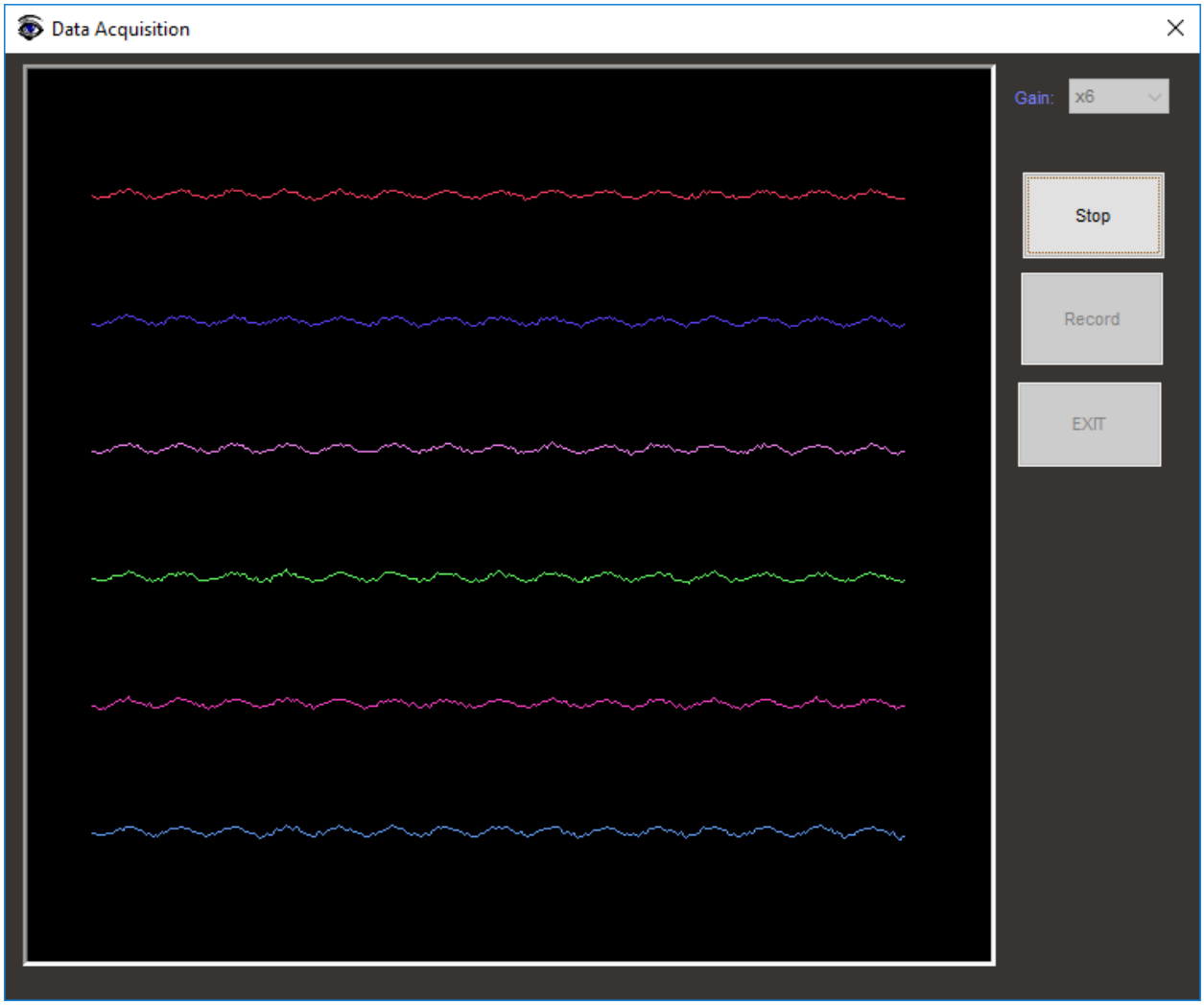
3.6.6 Record

This will open a window streaming the real-time data: baseline.

Running the baseline is the first action to do when starting a test. Once this button is clicked, real time acquisition of data is observed. A good baseline be reasonably flat and should have minimal 50/60 Hz cycle noise.

Baseline – starts streaming real time data, click stop to stop the baseline recording.

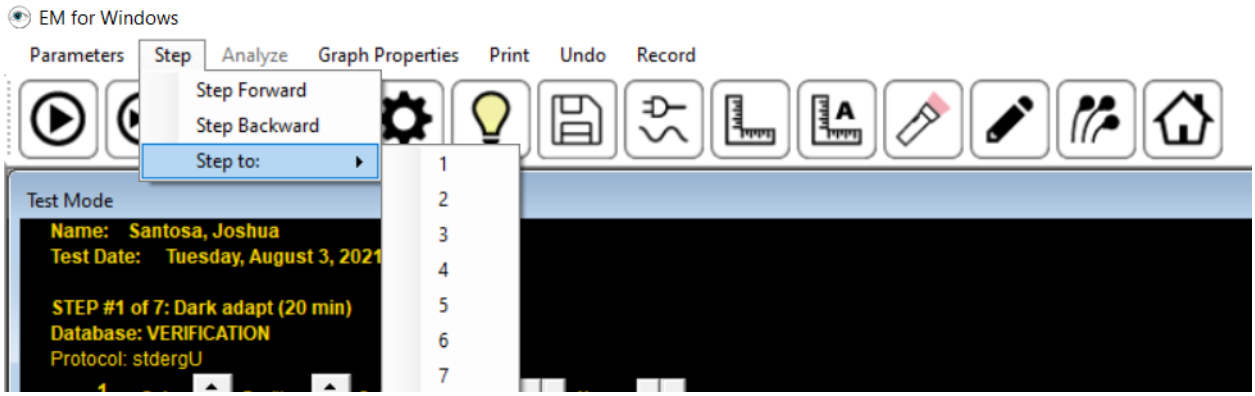
Record – From the baseline menu it is possible to start recording immediately by clicking on Record Data



3.6.7 Store

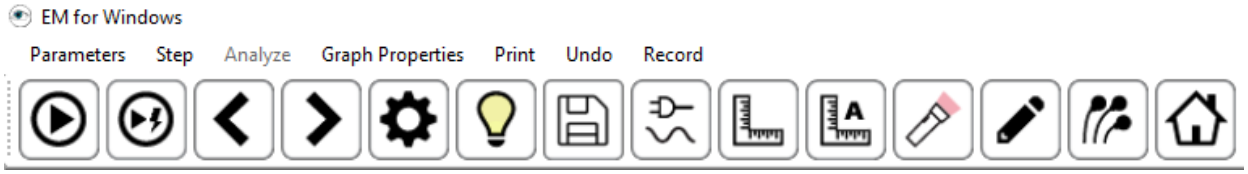
This stores the waveforms in the currently selected database. Choose to save all displayed waveforms or select the one to be saved. The waveforms are saved back to back and given a number. The latest saved waveforms will be the waveform with the highest waveforms number in the database.

3.6.8 Step



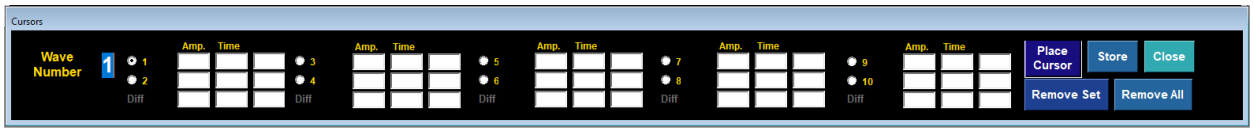
To proceed to another step in the protocol, click on step. Select **Step Forward** to proceed to the next step in the protocol, or select **Step Backward** to proceed to the previous step. The appropriate stimulus conditions and step number for the step will appear at the bottom of the screen.

There are several ways to move forward and backward. Use either the arrow icons, pointing left to move backward pointing right to move forward or by clicking on Step, then Forward or Backward or Step To, to jump to any of the steps in the protocol. Finally, it is possible to use the keyboard F3 to go backward and F4 to go forward.



3.6.9 Place Cursors

In order to measure the amplitude and time of specific locations of waveforms, use the **Place Cursors** feature. It is possible to place up to 10 cursors on each waveform, although only two or four cursors for a single response are typical.



- A new box (picture above) will be shown at the bottom of the screen, which indicates that the cursors will be placed on **Waveform 1**. Also, notice that a set of cross hairs is placed on the first (top) waveform on the screen.

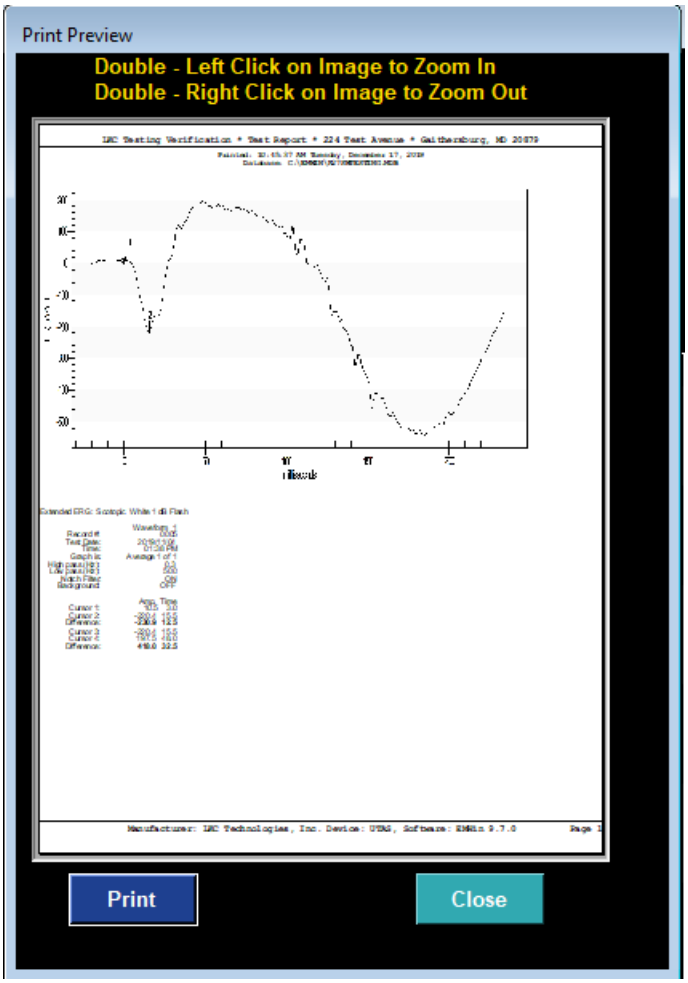
- Left-Click on the waveform on which you want to place cursors on.
- EMWIN will automatically select **Cursor 1** to start with, which indicates that cursor 1 is the cursor to be placed on the waveform.
- Use the **Left Arrow (←)** and **Right Arrow (→)** keys to slowly scroll through a waveform. As the cross-hair moves, the amplitude and time data will automatically update. Hover the mouse arrow over the point to place the cursor and left click on the mouse, this will also place the cursors.
- To move through a waveform faster, use the **Page Up** and **Page Down** keys.
- When the cross-hair is located at the data point of your choice, press the **Enter** key. This will place a marker on that waveform. Use the **Place Cursor** button at the bottom of the screen.
- After **Enter** is pressed, notice that the selected option is now **2**, since EMWin assumes the cursors will be placed in sequential order.

3.6.10 Print

The currently displayed waveforms can be printed to any standard Windows printer. After clicking Print (from either the menu option or the toolbar button), a Print Preview of your data will appear, showing how the data will appear when printed. Words that may appear jumbled on the preview screen will be expanded to appear normal on the printout. Double-click the left button to zoom in or double-click the right mouse button to zoom out. To print the data, click on the **Print** button.

The first page will have the patient name, their birthdate, the waveform pictures, and the cursors for each waveform. It also shows protocol information, number of average waveforms and test date. The second and third pages contain all the patient information, comments entered and all analysis routines that have been performed on each waveform.

The user also has the option of printing the report to a pdf format. After clicking Print (from the menu option or toolbar button), the Print Preview appears. Click on the Print button after the pages desired have been selected. Another window will appear, where the printer to be used can be selected. Select *Microsoft Print to PDF* as the printer in the scroll down menu, and press print. Instead of printing, a save as box will appear where the destination and name of the report can be entered.



If printing with the purpose of faxing the waveforms, the lines will need to be thickened (see section 10.4.4). Graph Properties -> Line Thickness -> Thick

3.6.11 Update Patient Information

This allows the user to change the patient information or enter comments during recording. The changes need to be made before the waveform is saved to the database.

3.6.12 Change Channel Information

This icon allows the user to view the number of channels that are being used at that time. From this window, the labels for the channels (automatically filled in as R in channel one and L in channel two) can be changed.

3.6.13 Measure Interference

The measure interference button collects one sweep of data from each channel and determines the amount of main interference (50 Hz or 60 Hz, depending on configuration); measured in µV. (Higher numbers are worse.) The information will dictate whether a good recording from the patient can be obtained. Excessive interference is often caused by poor electrode contact, so if the interference value is high, check the electrode contact and re-measure.

3.6.14 Red Background Light

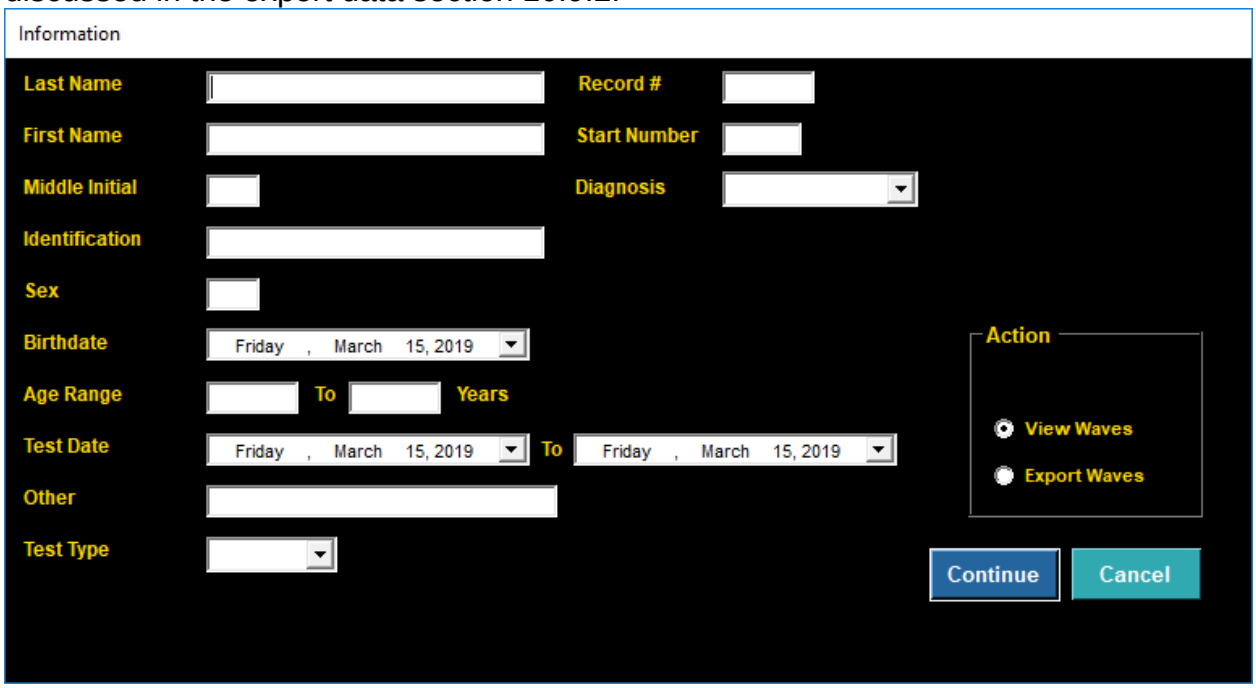
This will turn the background light of the Ganzfeld red. This is meant to help assist the technician when placing the electrodes without ruining dark adaptation of the patient.

3.7 Creating Reports

3.7.1 Selecting Patient's Data

The first step in generating a report is to select the waveforms that will be displayed. From the main menu, go to *Create Report*. EMWin will put up a Patient Information Screen to fill in and search through the waveforms with. The more filled in, the fewer waveforms that must be searched through to find the desired ones.

In the Action window, View Waves is selected as default, allowing the waveforms to be displayed in order to create the reports. Export Waves and Export Cursors will be discussed in the export data section 10.9.2.



Patient Information Screen

In many cases, filling in the patient's last name will be sufficient to search through the large database.

Filling in the Age Range as **45 - 55** would select all patients aged 45 to 55. Note that this computes the age of the patient at testing, not the present age of the patient.

Filling in Test Date as **11/1/1990 - 11/30/1990** would select all tests performed in November, 1990.

Any menu item which is left blank is assumed to match all items. As an example, to find the ERGs of all patients named Smith who were tested in July of 1990, fill out the menu as follows:

Last Name	:	Smith
First Name	:	
Middle Initial	:	
Identification	:	
Sex	:	
Birthdate	:	
Age Range	:	
Test Date	:	7/1/1990 - 7/31/1990
Other	:	
Test Type	:	ERG
Record Number:		
Start Number	:	
Diagnosis	:	

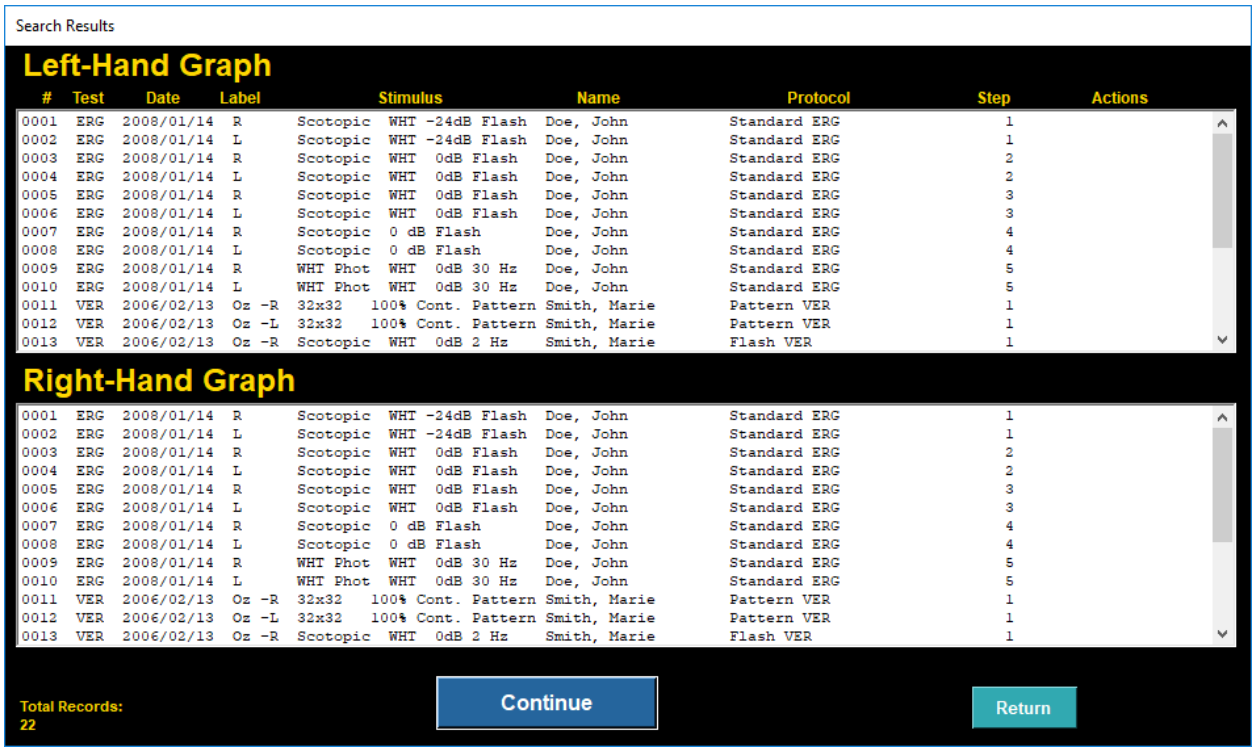
If EMWin reports *No Matches Were Found*, either the name was either misspelled in the search or the patient name may have been mistyped when the test was performed. It is also possible that the waveform is stored in a different database or has been deleted. Use wildcards (*) to help reduce errors. For example, the search term: Sm* will find "Smith", "Small", "Smythe," etc.

EMWin will search through all of the waveforms and display on the screen all of those that match the items filled in on the menu. Select the waveforms to display by clicking anywhere on the line of the appropriate waveform. If a mistake is made, click on the box again to deselect it.

Notice the list of waveforms in the “Search Results” box. The waveforms that are selected will be displayed on the next screen.

3.7.2 Selecting the Display View

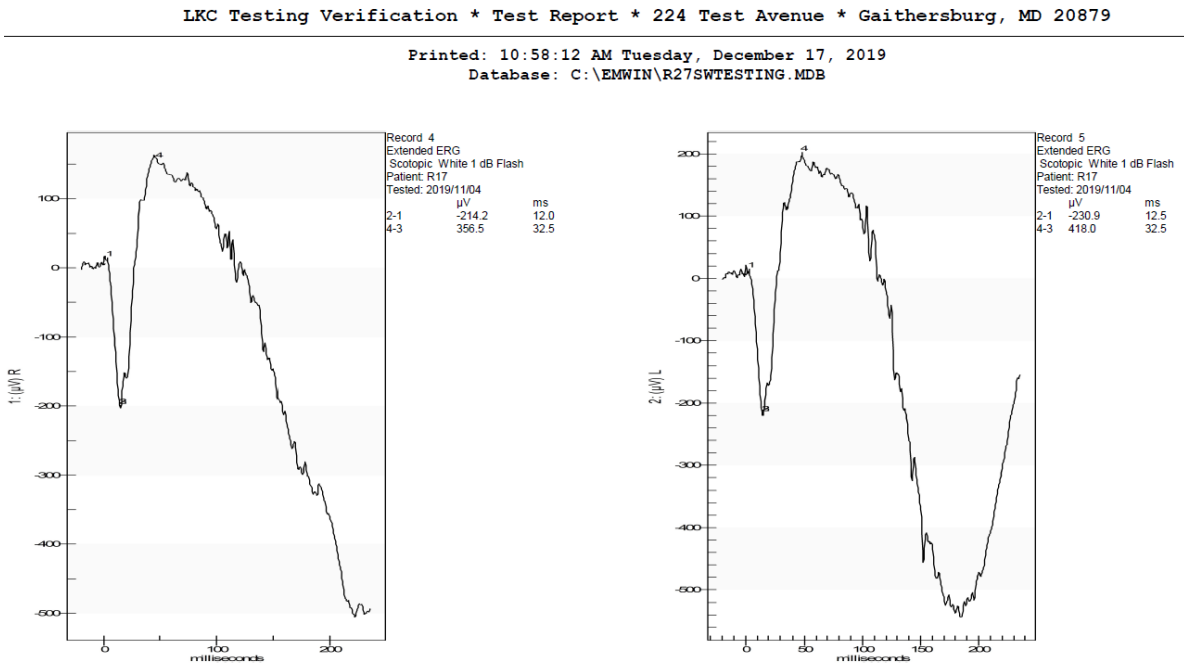
Once the search parameters have been entered, the search result box will appear as follows:



It has two main windows that contained the data waveforms. Left-hand Graph refers to the left half page of the report; Right-Hand Graph refers to the right half page of the report.

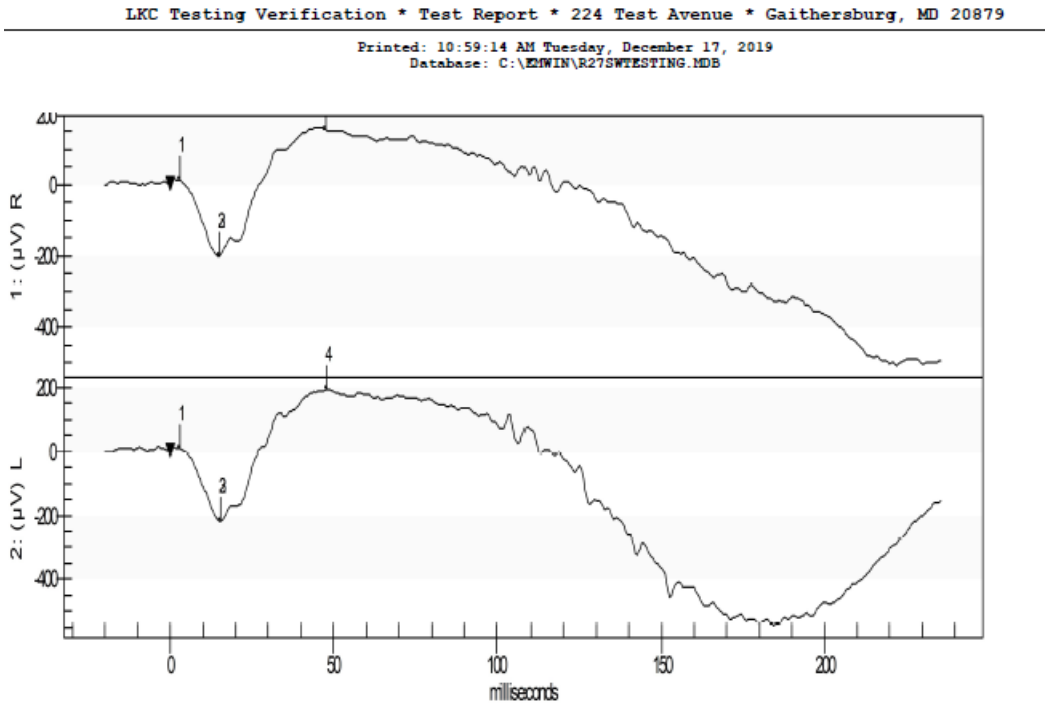
There are two different ways to display the data:

Option 1: Select waveform 2 (in this case L eye ERG) in Left-hand Graph and waveform 1 (in this case R eye ERG) in Right-hand Graph then the data will display as below.



Option 1

Option 2: If the two waves are selected in the Left-hand Graph window then they will appear on top of each other in the report.



Waveform 1: Extended ERG: Scotopic White 1 dB Flash
Waveform 2: Extended ERG: Scotopic White 1 dB Flash

	Waveform 1		Waveform 2	
Record #:	0004		0005	
Test Date:	2019/11/04		2019/11/04	
Time:	01:38 PM		01:38 PM	
Graph is:	Average 1 of 1		Average 1 of 1	
High pass (Hz):	0.3		0.3	
Low pass (Hz):	500		500	
Notch Filter:	ON		ON	
Background:	OFF		OFF	
	Amp.	Time	Amp.	Time
Cursor 1:	10.9	3.0	10.5	3.0
Cursor 2:	-203.3	15.0	-220.4	15.5
Difference:	-214.2	12.0	-230.9	12.5
Cursor 3:	-203.3	15.0	-220.4	15.5
Cursor 4:	153.2	47.5	197.5	48.0
Difference:	356.5	32.5	418.0	32.5

Option 2

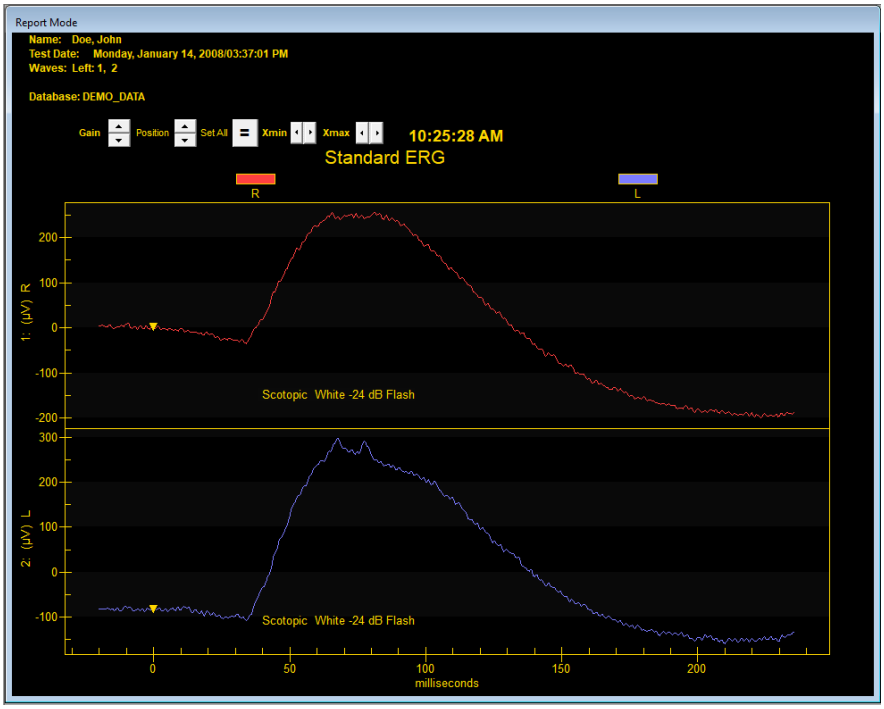
Both options have advantages and inconveniences.

In option 1, up to 5 waveforms can be selected on each side, but the waveforms will appear a lot smaller since it is only using half of the page.

In option 2, the waveforms are more visible because they are using the whole width; however, there is a maximum of 5 waveforms that can be displayed with this option.

3.7.3 Adjusting the Data Display

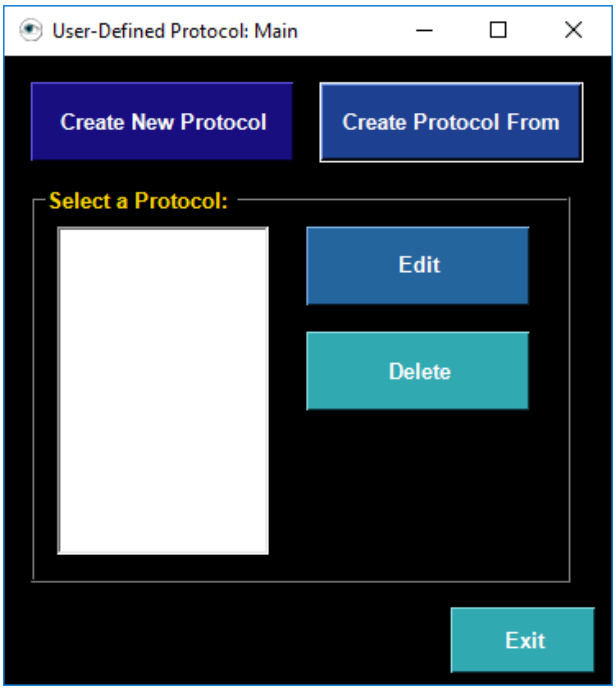
There are several things that can be done to adjust the display of the data on the reports. Along the right and left time axis, the waves can be zoomed in on. (see example below)



To adjust the time scales (for display purposes only) first click on the waveforms and then move the Xmin and Xmax tabs to the desired location.

The gain buttons allow the user to zoom in and out in amplitude. To change the amplitude of one curve and have the second one match:
Select one of the waveforms, adjust the gain and click on the = icon. This will set all waveforms currently on display to the same amplitude scale.

The position buttons allow the user to move the waveforms up and down on the displays.



3.7.4 Retrieving data

Once the first report is done, use the retrieve menu to select the next waveform to avoid going back to the main menu. See section 10.6.5 about the retrieve menu.

3.8 User-Defined Protocols (UDP)

3.8.1 Creating user-defined protocols

To write a User-Defined Protocol, choose **Design Protocols** and choose **Design** from the **Main Menu**. The option of writing a new protocol, editing an existing User-Defined Protocol, deleting a protocol, or printing out a summary of a protocol will appear.

To create a new protocol, click on **CREATE NEW PROTOCOL**. To create a new protocol that closely resembles one already existing, click the protocol you want to clone and choose **CREATE PROTOCOL FROM.** This will copy all the information from the selected protocol and with the opportunity to make changes to it.

If creating a new protocol, then the screen below will appear. This screen is used to enter the **Protocol Name**, **Test Type**, and **Stimulator Type**.

The **Protocol Name** must be unique or the system will ask to replace the existing file. At this time, ERG and VEP are the only available options for **Test Type**. Only the stimulators that the equipment is configured for will be offered as choices under **Stimulator Type**. The other stimulators will be grayed out. Depending on which stimulator is used, the appropriate parameter window will open. See section 10.6.1 on parameters. Be sure to check all 4 tabs and select the values desired (stimulator parameter, amplifiers, timeframe and averaging).

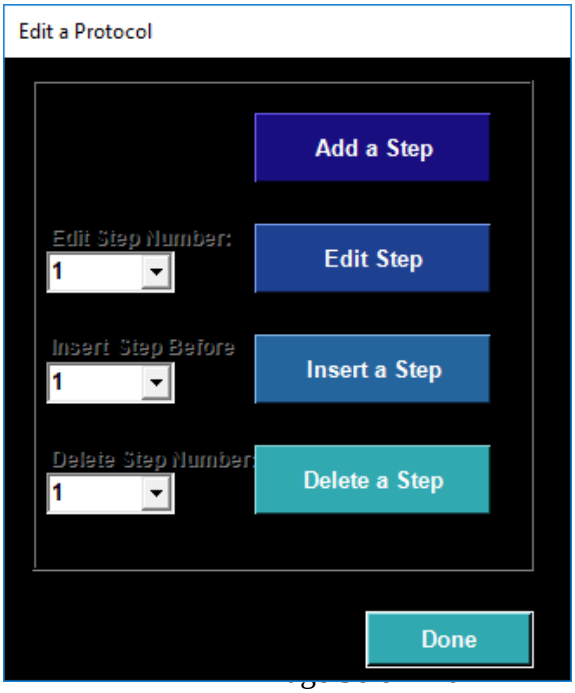
> **Note: All flicker stimuli should have a pre-stimulus baseline of 0 in the timeframe tab**

Once all the tabs for the first step of your protocol are checked, click continue, and the choice to add another step or finish the protocol (which would create a one-step protocol) will come up. If add another step is selected, the software will automatically use the values selected in the previous as default.

3.8.2 Editing user-defined protocols

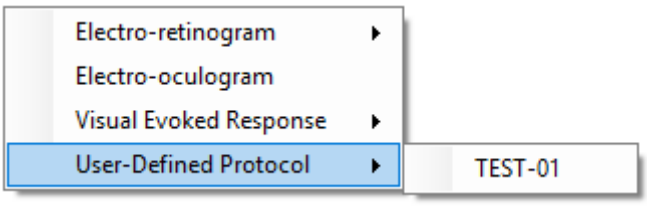
UDPs can edit any user defined protocols by going to *Design Protocol -> Design* selecting the protocol to be edited and clicking on *Edit*.

The choices given are to add, edit, insert or delete steps.



3.8.3 Using user-defined protocols

To use the UDP that has been created, go to *Perform Test -> User Defined Protocols* and select that protocol from the list.



3.8.4 Changing Standard Protocols

EMWIN comes with a preset number of ISCEV standard protocols such as On/Off, standard, double flash, etc. It is possible to change those protocols to fit the specific clinic's needs using the methods described above.

The standard protocols are stored in C:\EMWIN and have .PRO extensions. See Appendix 3 for the list of all standard protocols, their names and parameters. If the settings of a standard protocol are changed, the extension of the protocol file must also be changed from, UDP to.PRO. The protocols will then be able to be edited as described in section 10.8.2. Once done editing the protocol, don't forget to change the extension name back to .PRO

3.8.5 Printing Standard and User Defined protocols

To print protocols, go to Design Protocol -> Print. A list of protocols will appear. The files with UDP extensions are the user defined protocol, the .PRO are the standard protocols. All the UTAS protocols have a capital U as the last letter of their name such as stdergU.pro for the standard ERG.

SunBurst / BigShot Error Sounds

SunBurst and BigShot have a built-in speaker. It will play music when it is first turned on. There are some occasions where the Ganzfeld might emit sounds.

Ganzfeld Sounds and Meanings:

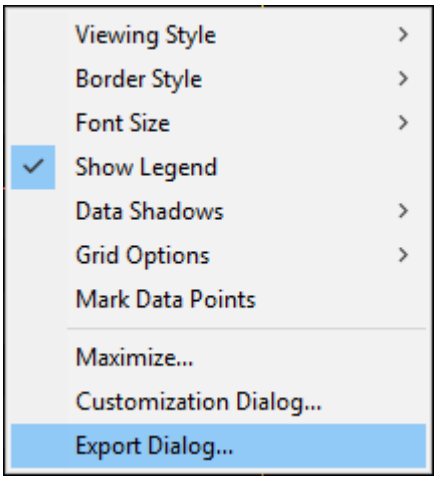
1. Ganzfeld is playing the Jeopardy theme song “Think”. This means the Ganzfeld is overheating. SunBurst will keep playing the music and not respond to any other commands until the temperature goes down enough.
2. Single Beep. This indicates a wrong command was sent to the Ganzfeld.
3. Two Beeps. The two-beep scheme is used to signal that the flash or background light that is commanded is either out of the possible color or luminance range.

—— —— Short Beep of Medium Frequency followed by Short Beep of Higher Frequency: Color out of bound

———— —— Long Beep of Medium Frequency followed by Short Beep of Lower Frequency: Luminance out of allowed range

3.9 Other Features

3.9.1 Exporting Images



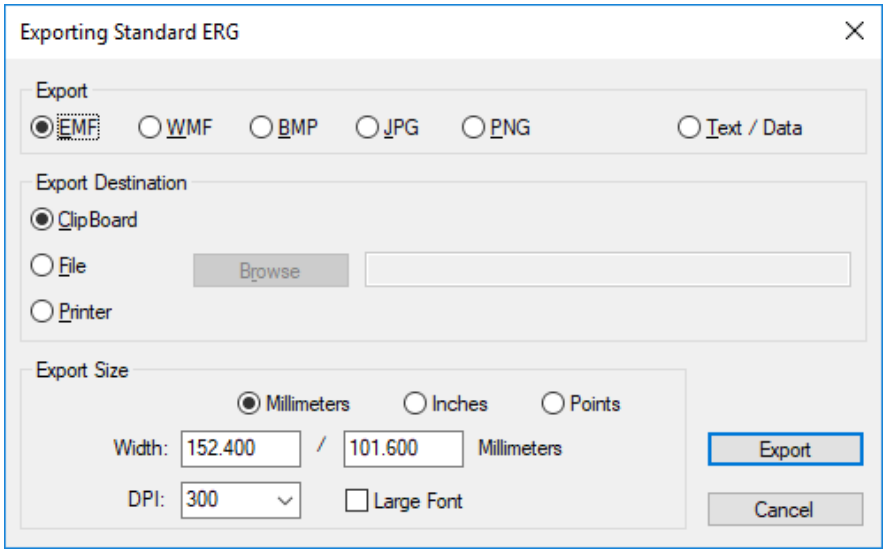
Exporting the patient data for analysis or visualization in other programs is very simple. When a graph of data is on the screen (either in Test Mode or Report Mode), click anywhere on the background of the graph (left side or right side) with the right-mouse button. A pop-up menu will appear with a list of features. Select the item labeled “Export Dialog” and the Exporting Electroretinogram window will appear.

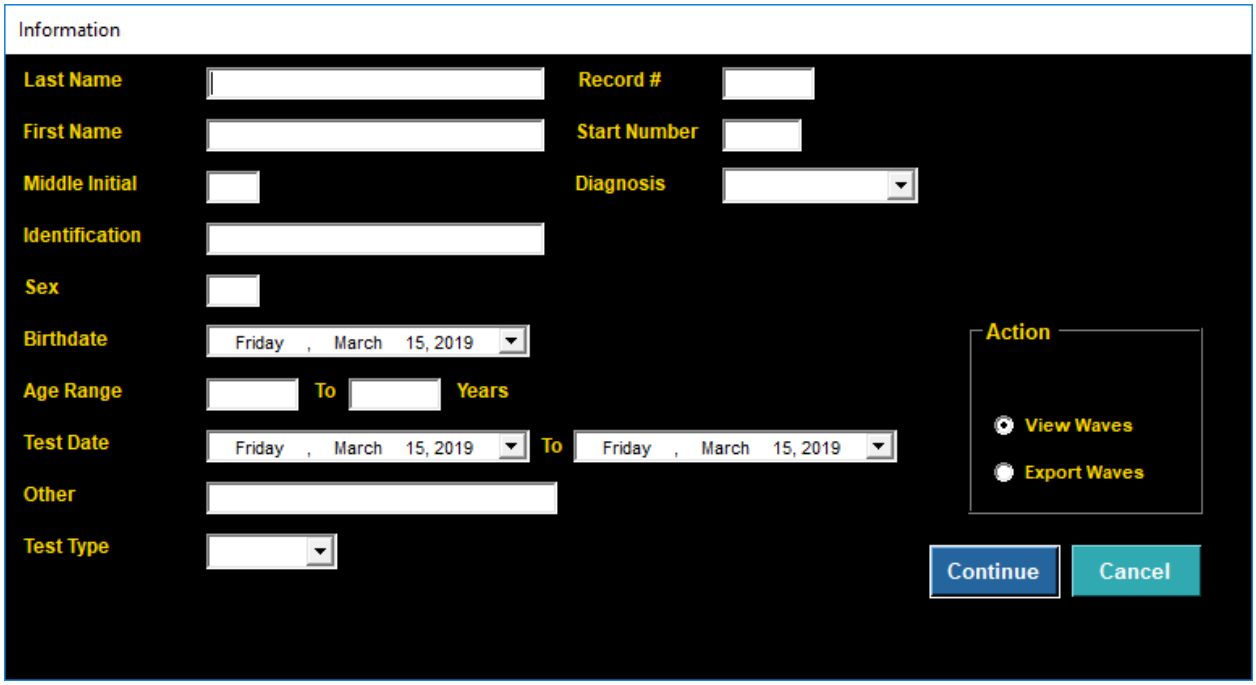
The picture can be exported in Metafile or Bitmap format to the Clipboard, a specific file of the user’s discretion, or the printer.

3.9.2 Batch Exporting Waveform Data and Cursors

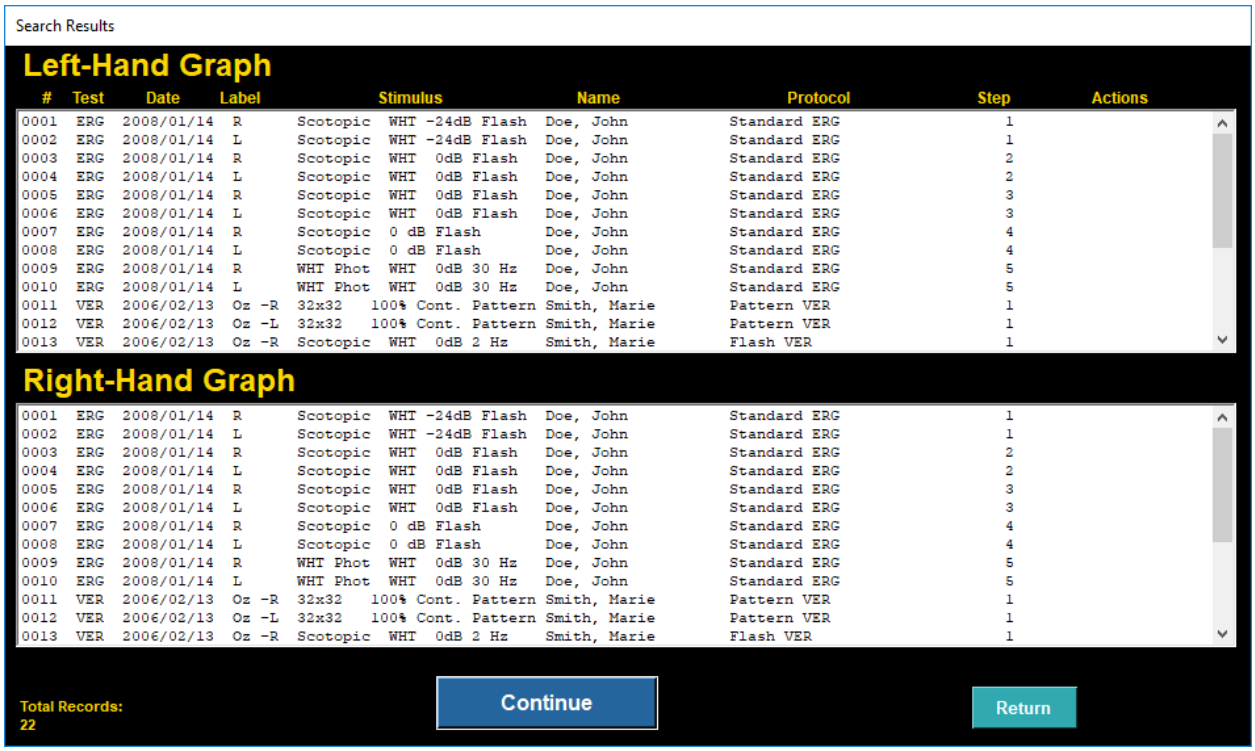
EMWIN has the capability to export up to 1000 waveform and cursors of 1000 waveforms into a .CSV file format.

In order to export the waveforms, Cursor, and patient information into this type of file, go to *Report* and select ***Export Waves*** in the *Action* window.





Then click on *Continue*, this will bring up the list of all waveforms.



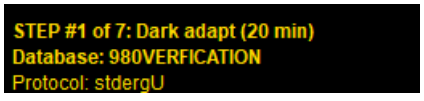
Use the Ctrl or Shift key to select multiple waveforms to export. The default name of the export file is Export.CVS. If needed, the name can be changed by typing the desired new name in the *Export File Name* window.
Once Continue is clicked, the export file will be created and saved under *C:\EMWIN\Export.*

Note: if multi-data (created a protocol where in one step the software will automatically collect more than 1 waveform and save all individual waveforms) are recorded, all the waveforms will be exported back to back; however, only the cursors on the first waveform will be exported.

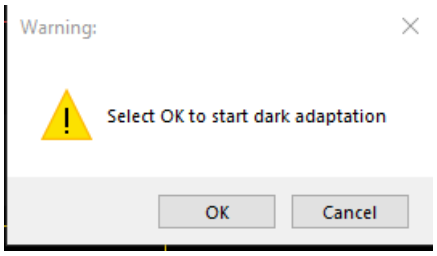
3.10 Adaptation Steps

EMWin has introduced the creation of adaptation steps in built-in and custom protocols. These adaptation steps enable users to track the length of dark adaptation and light adaptation of subjects using separate software enabled steps.

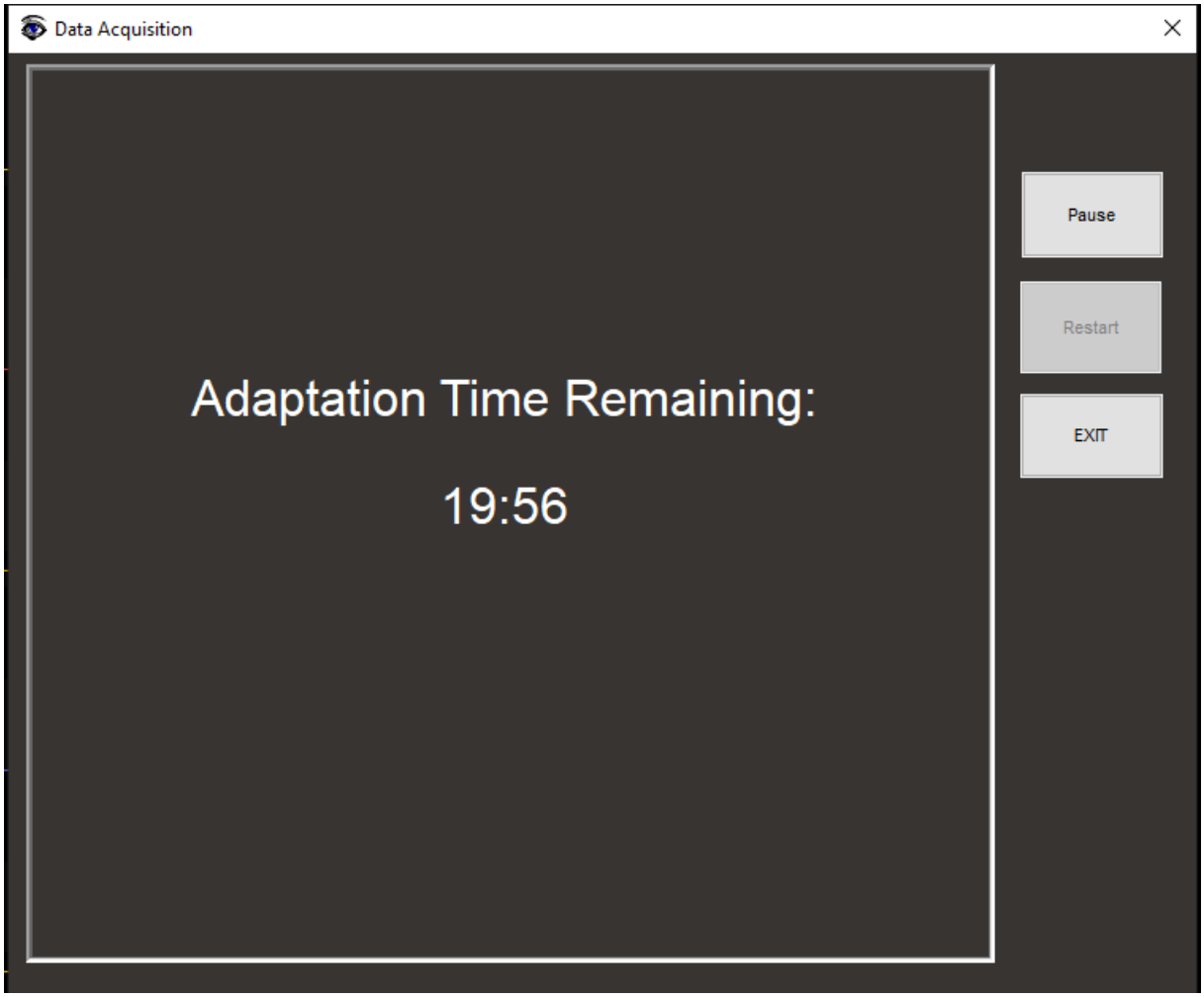
The adaptation steps will be labeled as such in the test step info:



When the user selects to start the test, the software will prompt the user to begin the adaptation step:



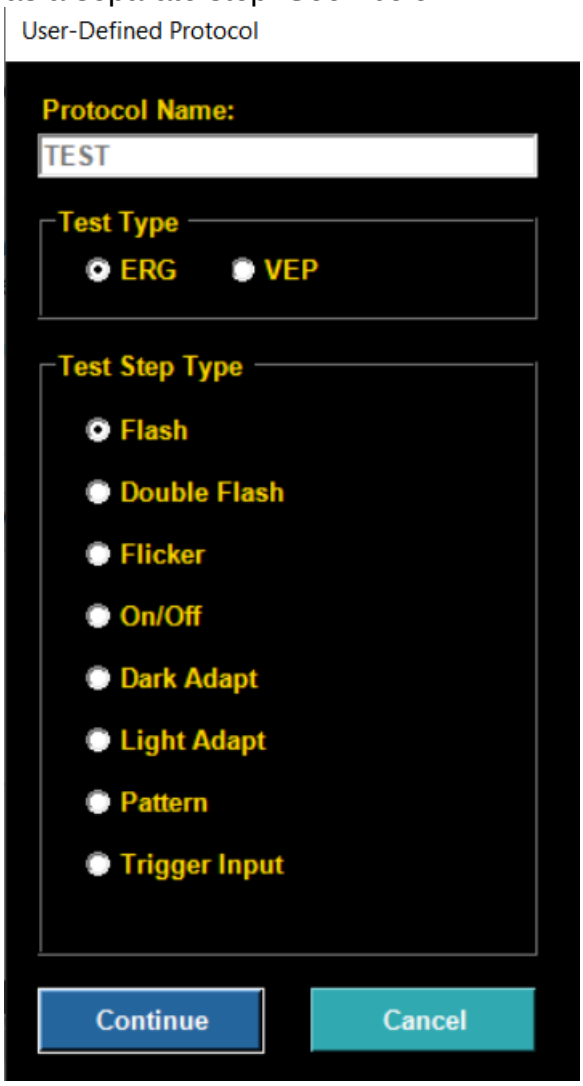
Once the adaptation step begins, a timer will appear allowing the user to track the adaptation step.



The adaptation timer will allow the user to select whether to pause the timer, restart the timer, or exit out of the adaptation step. If the user is performing an auto-run protocol, the timer will also allow the user to skip over the adaptation step.

Built-in ISCEV protocols will contain the adaptation steps for Dark Adaptation, and Light Adaptation. These will be automatically included for users. These adaptation steps are

also available through custom protocol design. The adaptation control panel is shown as a separate step. Seen below:



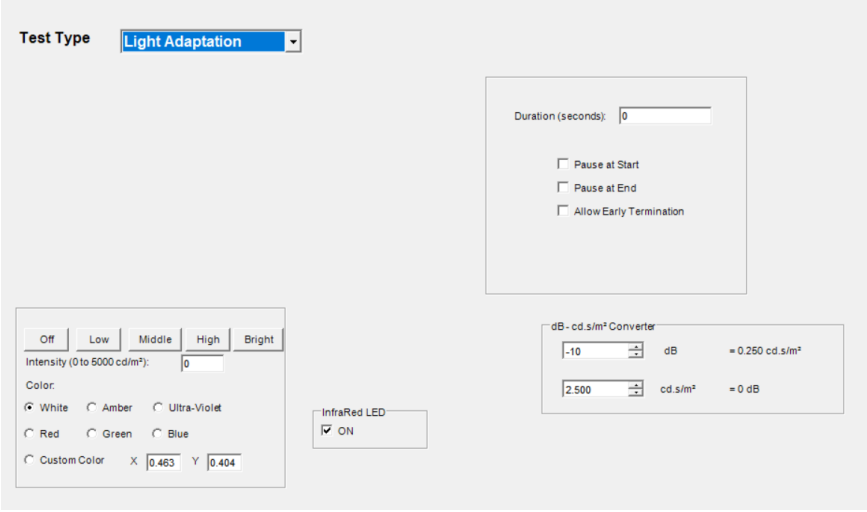
Users are able to select dark adapt or light adapt.

Dark adaptation design:

- Users are able to choose how long a duration the dark adaptation timer records
- Users are able to prompt users prior to the start, or at the end of start
- Users are able to enable whether skipping or early stopping of adaptation is available during recording:

Light adaptation design:

- Users are able to choose how long a duration the light adaptation timer records
- Users are able to select the color of the background adaptation light
- Users are able to select the luminance of the background adaptation light
- Users are able to prompt users prior to the start, or at the end of start
- Users are able to enable whether skipping or early stopping of adaptation is available during recording:

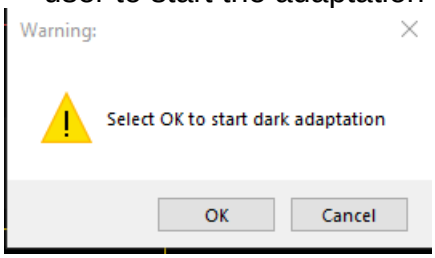


3.11 Automated Testing

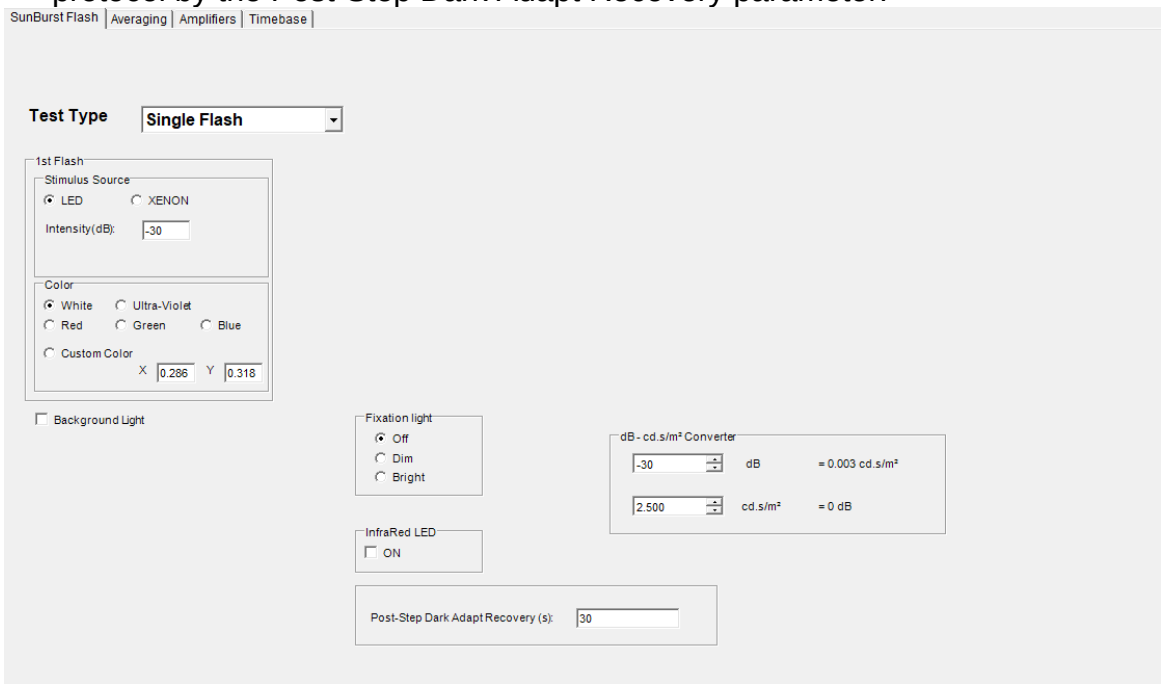
EMWin has introduced automated protocol testing. This feature enables the end user to perform an entire protocol with the push of a button. All built-in protocols or custom protocols have this feature. The automated testing button will be found in the toolbar at the top during the start of each test. This is shown with the following icon:

This icon allows the user to start the autorun feature in EMWin. Once selected, the following actions will occur:

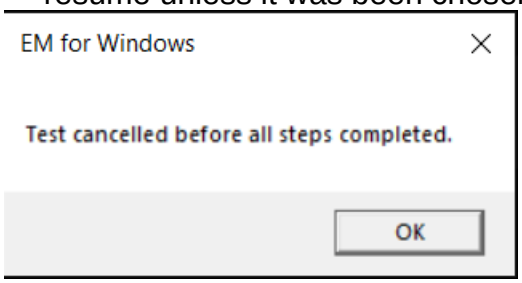
- If the protocol has an adaptation step, the automated protocol will first prompt the user to start the adaptation timer. As shown below:



- If the protocol does not have an adaptation step, the protocol will go through each step in the protocol automatically. The automated test protocol will automatically save files as dictated by the protocol.
- If performing scotopic tests, the automated test feature will include a time delay in between each step to prevent unintended light adaptation, set in a custom protocol by the Post-Step Dark Adapt Recovery parameter.



- Users may interrupt the autorun feature which will cancel the current step and require the user to repeat that specific step. Autorun will not automatically resume unless it was been chosen again.



- The autorun feature is designed for nonstop continuous testing. It is best implemented for research purposes in subjects under anesthesia. It may also be appropriate for highly cooperative patients.

4 Introduction

This portion of the User's Manual contains instructions for testing patients with LKC's UTAS system. The manuals for hardware and software components of the system should already be understood. This section will focus only on how to use the instrument to test patients

5 The Electroretinogram (ERG)

5.1 Overview

The electroretinogram (ERG) exposes the patient to some visual stimuli and measures the electrical response of the retina. The most commonly used stimuli are a flash of light (either bright or dim) or an alternating pattern of light flashes.

In clinical testing, the electrical response of the retina is measured by placing one electrode on the cornea and a second electrode on a reference location, usually the forehead, and measuring the electrical difference between the two. This difference is measured by a sensitive amplification system which can detect millionths of a Volt (called microvolt, and abbreviated as μV). In contrast, a typical wall outlet produces more than 100 Volts, more than a million times greater than the typical ERG signal.

Typically, ERGs are performed with a flash of light that covers the entire retina. To ensure that this happens, a device called a *Ganzfeld* is used. A Ganzfeld is a device that resembles a bowl covering the patient's vision field and is coated with highly reflective paint. The flash of light stimulus evenly illuminates the interior of the Ganzfeld and allows the most penetration of light into the patient's eye.

The flash ERG can be used to separately measure the response of the retinal rods and cones. The response of the rods is measured by first dark adapting the patient and then stimulating the eye with dim flashes of light. Using a bright flash of light tests both the rod and cone response. The rod function is suppressed when the patient's eyes are exposed to an adapting light so that only the response of the cones is measured. Rapidly flickering lights is also used to measure cone function. The most commonly used flash ERG test is the "standard ERG" which encompasses all of the above tests.

Other types of ERGs include the multifocal ERG (measures the function of the macula) and the pattern ERG (measures the function of the inner retinal layers, including the ganglion cells). The Standard ERG is the most commonly used, so we will focus on it for most of the section while also mentioning some of the other types.

5.2 ERG protocols

If the system contains a mini Ganzfeld with the SunBurst or BigShot, some of the protocols will be the same; such as the Standard and Standard with Mini-Ganzfeld

protocols. The only difference is the instrument used to deliver the stimuli and the fact that with the mini-Ganzfeld unit, it is possible to only test one eye at a time.

5.2.1 Standard ERG Protocol

This protocol currently recommended by the International Society for the Clinical Electrophysiology of Vision[1] (ISCEV); they explain:

> In 1989 a basic protocol was standardized so that ERGs could be recorded comparably throughout the world. This standard was updated most recently in 2008. Standards for five commonly obtained ERGs were presented: (1) ERG to a weak flash (arising from the rods) in the dark-adapted eye (2) ERG to a strong flash in the dark-adapted eye (3) Oscillatory potentials (4) ERG to a strong flash (arising from the cones) in the light-adapted eye (5) ERGs to a rapidly repeated stimulus (flicker)

The rod response is particularly useful to determine hereditary night-blinding disorders as well as conditions that involve peripheral retinal function, such as diffuse inflammatory disease. The cone response is used to diagnose cone dysfunction in hereditary or acquired disease. Flicker responses document ERG timing that can help diagnose vascular disease or discriminate between dystrophies and acquired degenerations. The maximal response provides an overall indication of the retina function, and can be used to diagnose conditions such as traumatic retinal damage or evaluate visual impairment in infants. The oscillatory potentials are used to determine disorders that cause retinal ischemia, such as diabetic retinopathy.

ERG Conditions - Standard Protocol:

1	Scotopic	Single Flash	-24 dB	0.01 cd/m^2	Rod Response
2	Scotopic	Single Flash	0 dB	2.5 cd/m^2	Maximal Response
3	Scotopic	Single Flash	0 dB	2.5 cd/m^2	Oscillatory Potentials
4	Photopic	Single Flash	0 dB	2.5 cd/m^2	Cone Response
5	Photopic	30 Hz Flicker	0 dB	2.5 cd/m^2	Flicker Response

Unless specified otherwise in the protocol, 10 sweeps will be averaged for 30 Hz flicker (stimulus condition 5).

20 minutes of dark adaptation is required in the Standard Protocol prior to any stimulation

1 International Standardization Committee. Standard for clinical electroretinography (2008 update). *Documenta Ophthalmologica* **118:** 69-77, 2009.

5.2.2 Extended ERG Protocol

In the 2008 update of the ISCEV Standard for full-field clinical electroretinography, it is recommended that an additional flash is performed while the patient is dark adapted. This flash stimulus is called the Dark-adapted 10.0 ERG and occurs after the standard step 3 in the protocol at an intensity of 6dB. The purpose of the additional gives is to produce a response with a larger, more defined a-wave and identifiable oscillatory potentials. The more intense stimulus can also be useful for eliciting a maximal response from patients with denser opacities who would normally not be able to produce the response to step 2.

5.2.3 ERG Conditions – Extended Protocol

1	Scotopic	Single Flash	-24 dB	0.01 cd/m^2	Rod Response
2	Scotopic	Single Flash	1 dB	3.0 cd/m^2	Maximal Response
3	Scotopic	Single Flash	1 dB	3.0 cd/m^2	Oscillatory Potentials
4	Scotopic	Single Flash	6 dB	10.0 cd/m^2	Larger a-wave Response
5	Photopic	Single Flash	1 dB	3.0 cd/m^2	Cone Response
6	Photopic	30 Hz Flicker	1 dB	3.0 cd/m^2	Flicker Response

5.2.4 Classic ERG Protocol

Until the adoption of the Standard Protocol by ISCEV, the Classic Protocol was the most commonly utilized ERG diagnostic protocol. It also consists of 5 steps, as follows. (1) The *Scotopic Blue* stimulus. Since rods are more sensitive to short wavelength light (blue), this step isolates the rod response. (2) The *Scotopic Red* stimulus, displays both the rod and cone response on a single waveform. Interpretation of this waveform may be difficult. (3) The *Scotopic White* stimulus provides a maximal response of both the rod and cone systems. This is the same stimulus as step 2 of the Standard Protocol. (4) The *Photopic White* stimulus is designed to suppress rod participation. This is the same stimulus as condition 4 of the Standard Protocol. (5) The *30 Hz Flicker* stimulus isolates the cone response because the flicker is too fast for the rods to follow. This stimulus is done without background light.

ERG Conditions - Classic Protocol

1	Scotopic	Single Flash	Blue - 38 dB	0.0004 cd/m^2	Rod Response
2	Scotopic	Single Flash	Red +8 dB	16.0 cd/m^2	Rod+Cone Resp.
3	Scotopic	Single Flash	0 dB	2.5 cd/m^2	Maximal Response
4	Photopic	Single Flash	0 dB	2.5 cd/m^2	Cone Response
5	Photopic	30 Hz Flicker	0 dB	2.5 cd/m^2	Flicker Response

Unless specified otherwise in the protocol, 10 sweeps will be averaged for 30 Hz flicker (stimulus condition 5).

30 minutes of dark adaptation are required in the Classic Protocol.

5.2.5 Bright Flash ERG Protocol

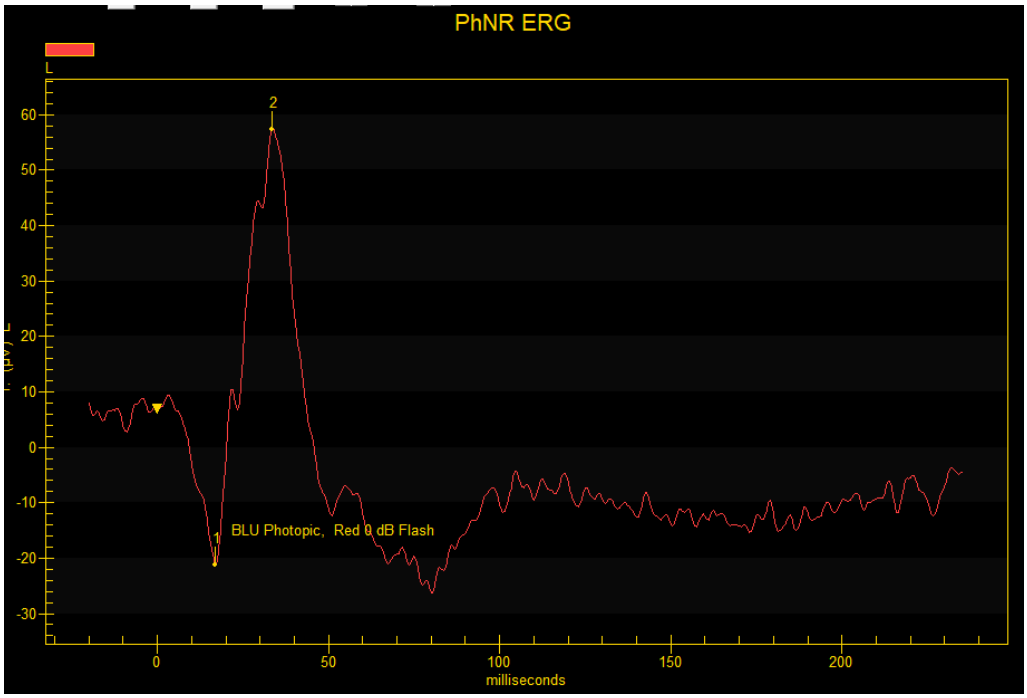
The Bright Flash protocol presents a stimulus that is much brighter than the intensity of the standard flash. It is normally used for subjects with sufficiently dense media over the eye that the standard flash cannot elicit a response from. The Bright Flash protocol should *not* be used to attempt to elicit a larger response from subjects with relatively normal media (unless attempting to determine the asymptotic PI, amplitude, or measure the photoreceptor kinetics). The Bright Flash Protocol may also be used to elicit oscillatory potentials. If the Bright Flash receives maximum amplitude Ops, use a conditioning flash followed by the recording flash 30 seconds later.

When testing a subject with one relatively normal eye and one with opaque media, the normal, dilated, eye should be patched to prevent severe discomfort from the bright flash stimulus.

5.2.6 Photopic Negative ERG Protocol

The photopic negative response (PhNR), originating from the inner retina, appears after the b-wave (and if the flash duration is long enough, it appears again after the d-wave). The PhNR arises as a consequence of the spiking activity of the retinal ganglion cells. PhNR may be a sensitive measure of retinal dysfunction in patients with diseases that affect the inner retina such as glaucomatous damage.

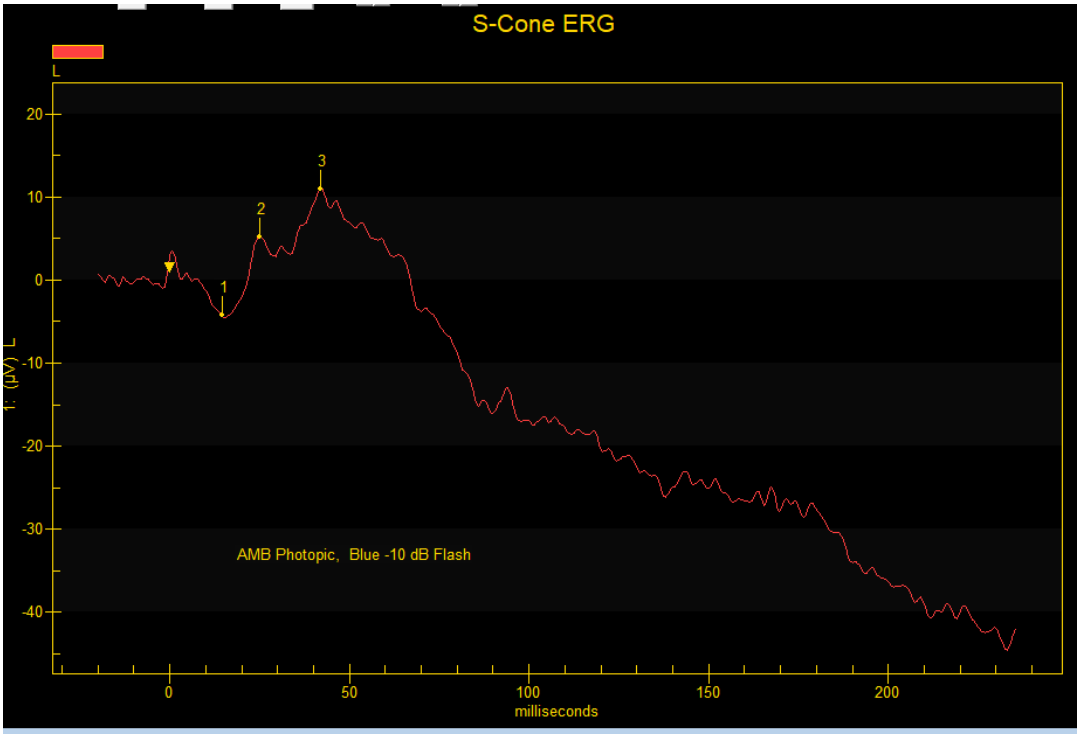
The photopic negative response is elicited using a 200 ms red (630 nm) stimulus with a blue background (470 nm).



5.2.7 S-Cone ERG Protocol

S-Cone ERGs facilitate the determination of enhanced S-cone syndrome, a rare disorder related to mutation in NR2E3. By enabling a more detailed assessment of the relative involvement of S-cones in other genetically determined disorders, they contribute to a more accurate description of the phenotype.

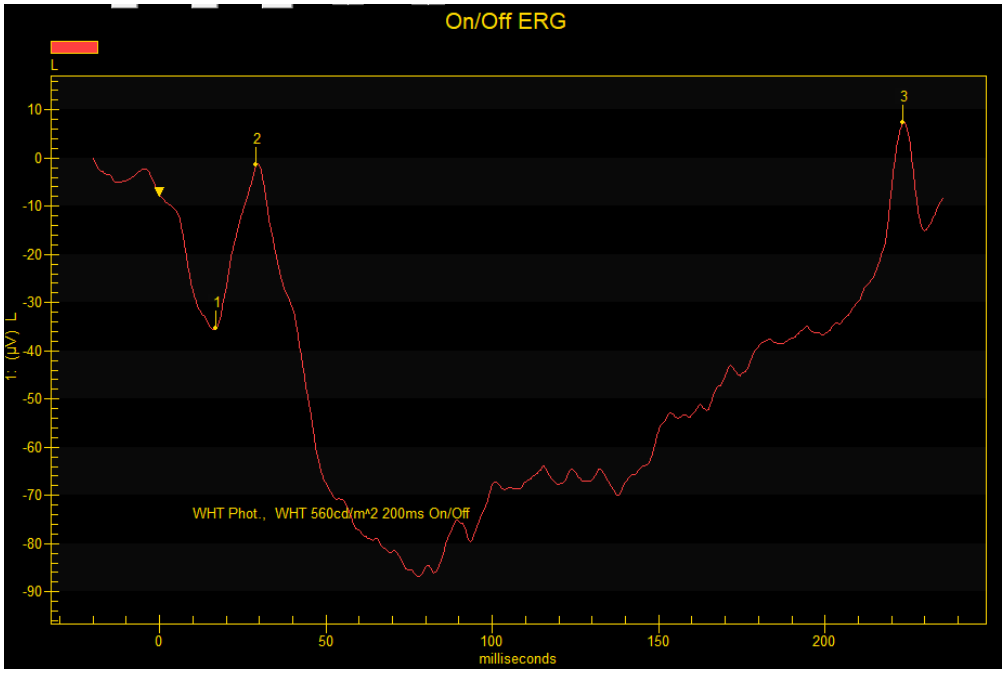
S-Cone recording requires a bright photopic background to suppress the rod and L-cone and M-cone function. The typical protocol uses a blue flash (470 nm) on a bright amber (590 nm) background.



5.2.8 On/Off Response

The L- and M- cones cells signal via both ON- (depolarizing) and OFF- (hyperpolarizing) bipolar cell pathways. Rods do not communicate with OFF- bipolar cells, and no S-Cones OFF bipolar cell have been identified. Long duration stimulation enables the functional separation of the cone ON- and OFF- responses.

ON- and OFF- response recording enables an assessment of the post-phototransduction cone pathways. This may reveal abnormalities not usually indicated by a more conventional cone ERG protocol and may therefore contribute to an accurate determination of the site and nature of the cone system dysfunction. This knowledge may help in the management of both inherited and acquired retinal disease.



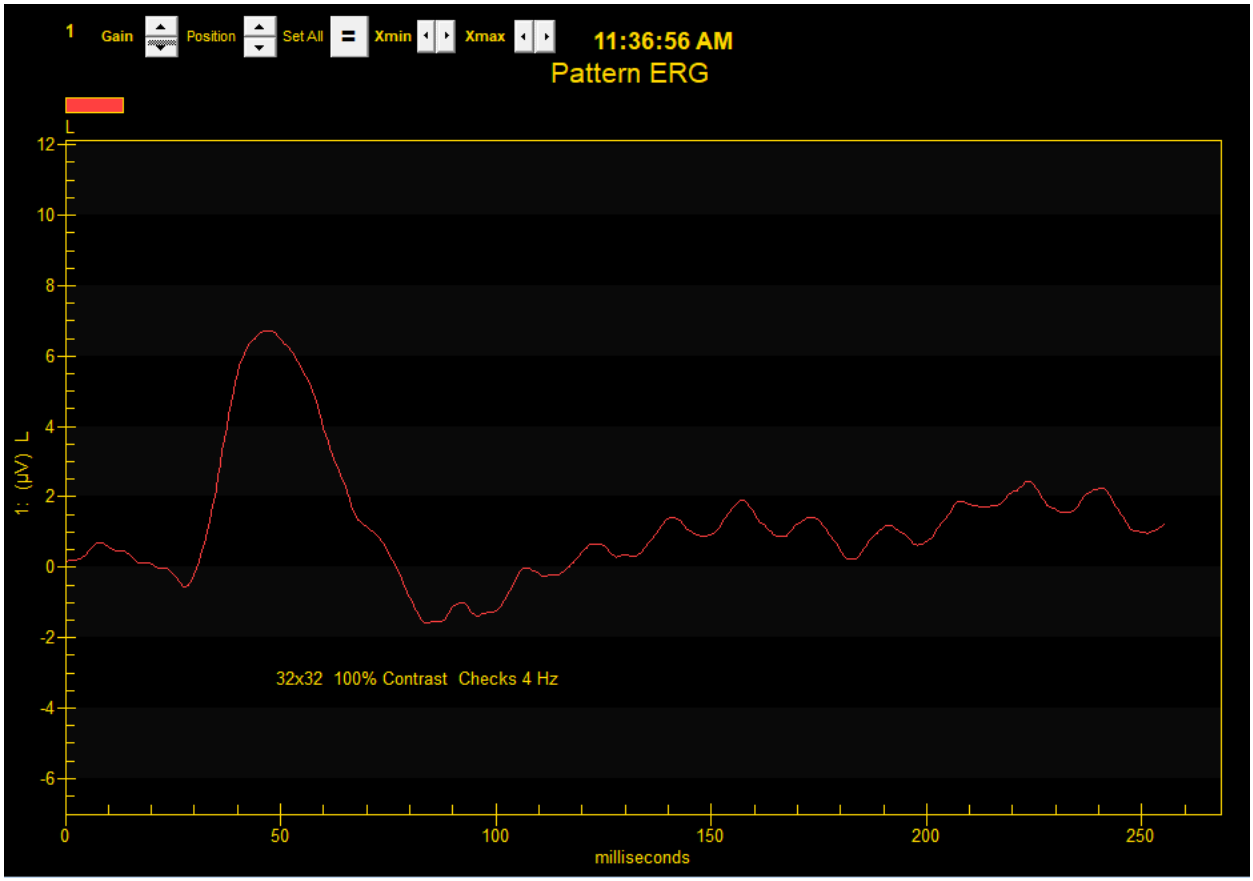
5.2.9 Flicker ERG Protocol

The flicker ERG protocol is one that measures cone response by presenting a flickering stimulus that is too fast for rods to respond to. The protocol presents flickering stimuli at 5, 10, 15, 20, 25, 30, 35, and 40 Hz. It is used mostly in research investigations.

5.2.10 Pattern ERG Protocol

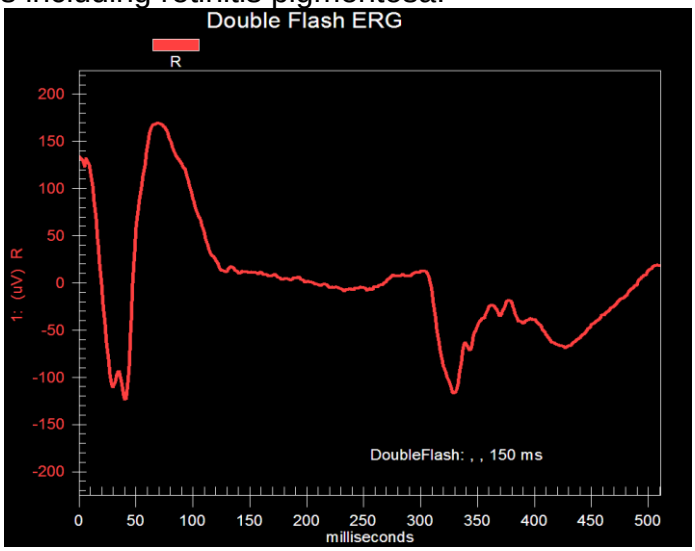
This protocol uses an alternating checkerboard pattern to elicit a retinal stimulus, unlike the flashing light stimuli in other protocols. Because equal amounts of the retina are stimulated as the checkerboard alternates, the response is a measure of neuron and ganglion cell activity of the inner retina rather than the rod and cone activity. As such, the pattern ERG is useful in diagnosing inner retina disorders.

The pattern ERG is a photopic test, so dilation and dark adaptation are not necessary. Because the edges of the checkerboard are important components of the stimulus, any refractive error should be corrected. If cycloplegic drugs are used, a one diopter plus added should be used to compensate for the typical screen distance of one meter.



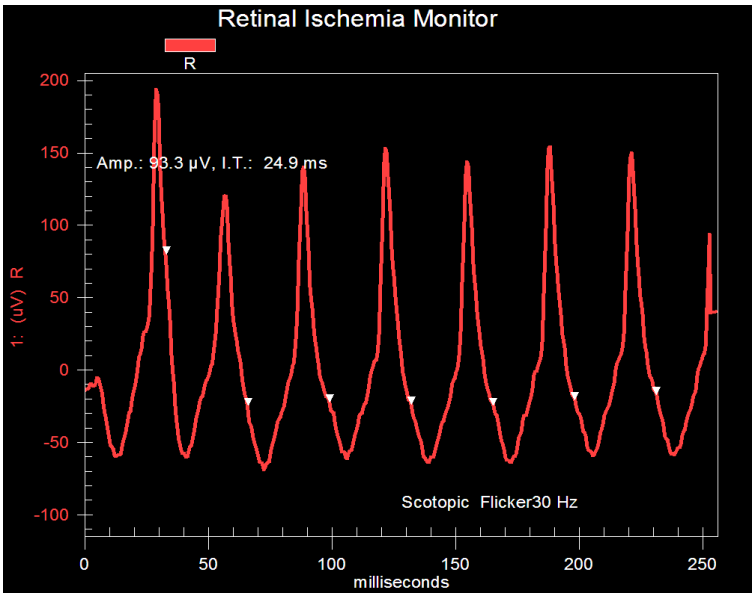
5.2.11 Double Flash ERG Protocol

The double flash protocol is used to study the recovery of photoreceptors. It consists of a bright conditioning flash followed by a measurement flash. The amplitude of the measurement flash as a function of time will provide the information needed. The recovery time of the photoreceptors, such as between the two flashes, is affected in several retinal disorders including retinitis pigmentosa.



5.2.12 Retinal Ischemia Monitor (RIM) Protocol

The RIM protocol can be used to assess ischemic retinopathies such as Diabetic Retinopathy, Ocular Ischemic Syndrome, Central Retinal Vein Occlusion (CRVO), Branch Vein Occlusion (BVO), Central Retinal Artery Occlusion, and Sickle-Cell Retinopathy. RIM has been shown to be 92% accurate in predicting the outcome in CRVO and has a strong correlation to the severity of Diabetic Retinopathy.



5.3 Patient Preparation

5.3.1 Standard ERG

The first step in the standard ERG is to dilate the patient's eyes with a mydriatic (any medium-duration cycloplegic, such as Tropicamide, will be sufficient). If the patient is already dilated from ophthalmoscopy, no further dilation is required. It is more comfortable for the patient if a few drops of local anesthetic are administered to the eye before the mydriatic dilating drops.

The next step is to dark adapt the patient. This step is **critical** to obtaining good results. The patient should be dark adapted for at least 20 minutes before the test is administered (dark adapting for longer than the 20 minutes will not alter the results, but dark adapting for less than 20 minutes will create problematic responses). To dark adapt the patient; simply place them in a completely darkened room (such as the room in which you will perform the ERG). Another method to dark adapt the patient is to

securely patch the patient's eyes so that no light can get through and return them to the waiting room.

After the patient has been dark adapted for at least 20 minutes, they should be brought into the testing room. The testing room must be completely dark (*a dim red* light is acceptable which may be provided by the Ganzfeld's background red LED if needed)

5.3.2 Pattern ERG

For the pattern ERG protocol, do NOT dilate the patient's eyes, as the patient must be able to focus on the pattern stimulus (dilation paralyzes the patient's ability to focus). The patient should not be exposed to bright lights, such as sunlight or a slit lamp, for at least 10 minutes before testing. Use the patient's best lens correction while running the test (with either the patient's glasses or trial lenses).

5.3.3 MultiFocal ERG

Dark adaptation is not required for the multifocal ERG protocol because it tests only cone function. However, the patient should not be exposed to bright lights, such as sunlight or a slit lamp, for at least 10 minutes before testing.

It is best to perform this test with the eyes dilated; although it doesn't largely affect the test results, it will make the technician's job much easier. To dilate the eyes, apply a cycloplegic agent and allow the patient to sit for about 15 minutes for the drug to take effect. If the patient is already dilated from a previous procedure, no further dilation is needed.

This test is only available if you have the multifocal ERG upgrade.

5.3.4 Other ERGs

There are several other types of ERGs, such as the intensity-response ERG, the flicker ERG, and the bright flash ERG. All of these tests require dilation of the eye and dark adaptation (the exception to this is if the eye to be tested is damaged by trauma in which a cycloplegic may be contraindicated). After applying dilating drops in the eye, dark adapt the patient for the amount of time shown in the table below.

Dark Adaptation Period for Various ERG Tests

ERG Test	Dark Adapt Time
Standard	20 minutes
Intensity-Response	45 minutes
Flicker	10 minutes

Bright Flash	20 minutes

5.4 Electrodes (Type BF Applied Parts)

Note: The instructions below related to anesthetization, insertion and removal of corneal electrodes, and post testing eye cleaning are provided as a general aid. Follow your clinic's procedures and guidelines for these procedures as well as adhering to the manufacturer's use instructions.

After the patient has been dilated and dark adapted (if needed), the next step is to attach the electrodes to the patient. All ERG tests require three different electrode connections; the corneal electrode, the reference/indifferent electrode, and the ground electrode. Use only electrodes enumerated in Appendix 1 of the UTAS System Hardware manual.

> **Note: If the protocol requires that the patient be dark adapted, the electrodes must be applied in the dark room, lit with only a dim red bulb (the Ganzfeld's dim red background light may also be used).**

To apply the electrode, anesthetize the cornea with several drops of a medium duration anesthetic, such as proparacaine hydrochloride (do not use a short-duration anesthetic - it will wear off before the test is over). While waiting for the anesthetic to take effect, apply the electrodes to the locations other than the eye.

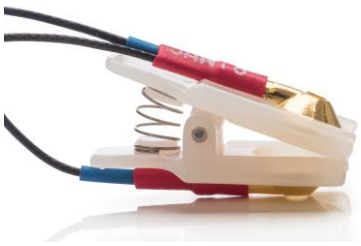
5.4.1 The Indifferent/Reference Electrode

The indifferent/reference electrode should be applied first. It is a separate electrode, often an ECG (EKG) electrode, such as the Silvon® electrode. The Burian-Allen electrode has the indifferent electrode built in (making it a *bipolar* electrode and does not require a separate indifferent electrode).

The indifferent electrode is applied to the center of the forehead and used as a reference for both eyes. With an **electrode prep pad,** scrub the center of the forehead to remove all traces of skin oils and makeup. Let the alcohol dry off for a few seconds. Before the indifferent electrode is applied, check it to make sure the center gel is still wet (if it has dried out, discard the electrode and get a new one as it won't be able to obtain a sufficient signal from the patient).

If you are testing two eyes, connect a splitter to the - inputs of recording channels 1 and 2 of the UBA. (A splitter is a Y-shaped cable with plugs on two sockets and an arm on the third.) Connect the indifferent electrode to the splitter.

5.4.2 The Ground Electrode



The ground electrode, typically a gold cup ear clip electrode, should be applied after the indifferent electrode.

The electrode is applied to the patient's ear lobe. With an **electrode prep pad,** scrub the ear lobe to remove all traces of skin oils. Let the alcohol dry off for a few seconds. Fill both cups of the ear clip generously with electrode gel and clip it onto the ear lobe. Plug the ground electrode into the appropriate ground channel of the amplifier.

5.4.3 The Corneal Electrode

Finally, the corneal electrodes should be applied. The most common corneal electrodes are the ERG-Jet, Burian-Allen, and DTL electrodes (shown below). Other types of electrodes may be used.

ERG-Jet Electrode

Burian Allen

DTL Electrode

Contact Lens Electrode

Before inserting the contact lens electrodes, place several drops of a lubricating solution that contains methylcellulose (such as Goniosol or Liquid Tears) in each of the contact lens electrodes. If you are using the ERG-Jet electrodes or the Burian-Allen electrodes, Liquid Tears may minimize damage to the cornea.

Gently insert a contact lens into the eye. Leave a small loop of excess electrode wire (about 1" - 2" in diameter), and tape it to the patient's cheek. Repeat the procedure for the other eye, if appropriate.

DTL Electrode

DTL Electrodes are used with patients that cannot tolerate the ERG-Jet contact lens electrode. They are single use, silver nylon thread electrodes. They should be placed on the eye so that the fiber is running gently across the cornea (not too tight to avoid corneal abrasion).

Gold Foil Electrode

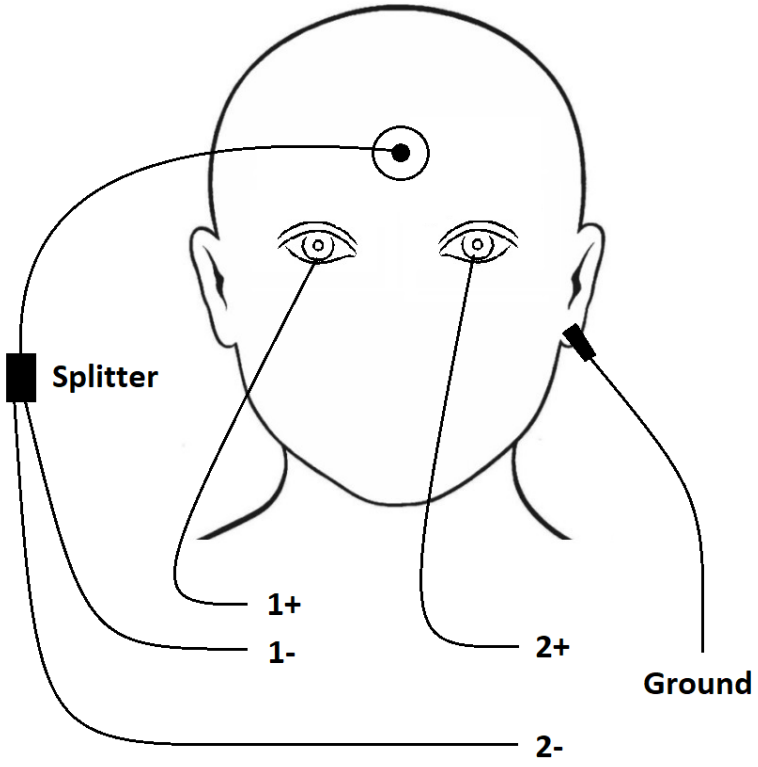
The gold foil electrode is used to avoid blurring the patient's vision, where contact lens use is not possible (such as in eyes with keratoconus), or where topical anesthetic is

not desired. The gold foil electrode has a thin coating of gold deposited onto a Mylar substrate.

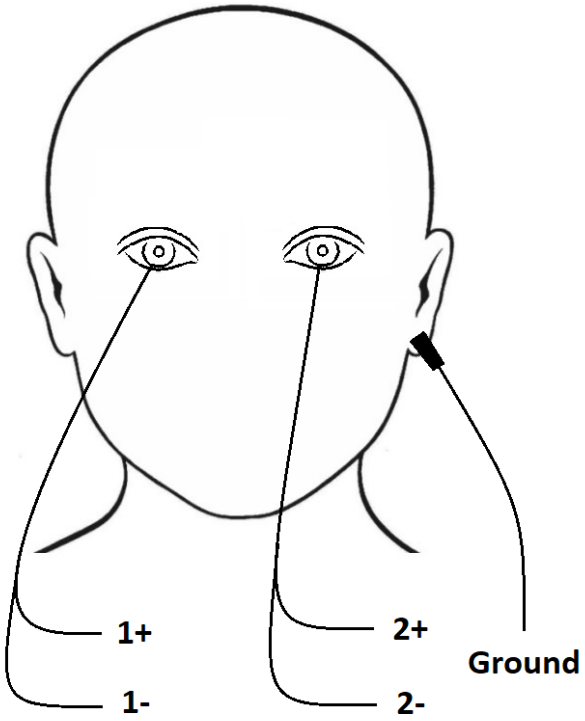
To insert the electrode into the patient's eye, fold the electrode in half lengthwise with the foil side out, so that it forms an inverted "V". Gently retract the patient's lower lid and insert the end of the electrode into the space between the lower lid and the sclera. Tape the electrode wire to the patient's cheek to hold the electrode in place.

Note that blinking or rapid eye movements easily dislodges the gold foil electrode. It is important that the patient looks straight ahead and does not blink excessively once the electrode has been inserted.

Always connect the corneal electrodes (active electrode) to the + input of each channel and references to the – input of each channel of the UBA. Connect the ground electrode into the ground channel.



Monopolar Electrode Placement (ERG-Jet, DTL...)



Bipolar Contact Lens Electrode Placement

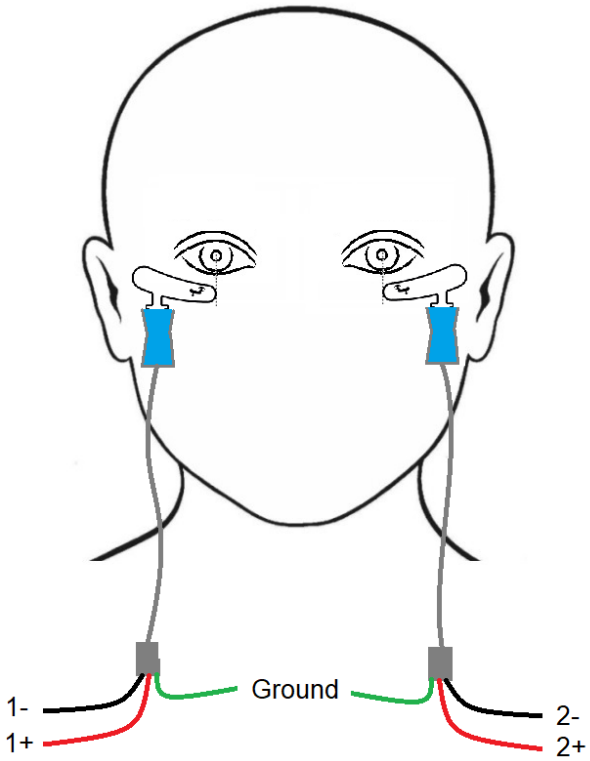
5.4.4 An alternative to Corneal Electrodes

In 2014 LKC introduced Sensor Strip electrodes for visual electrophysiology measurements. Sensor Strips are adhesive, skin contact, electrodes which include the three necessary electrode connections and so they can take the place of all three of the electrode types mentioned above. Sensor Strips are single-use so there is no cleanup of messy electrode gels or pastes. For optimal use the skin below the eye should be prepared with a pre-electrode attachment cleaning method. LKC recommends use of Nuprep or a similar product. Signals collected using Sensor Strips will be of smaller magnitude than corneal electrodes, this should be considered before use.

LKC Sensor Strips are intended for human use only.

One Sensor Strip to DIN connector cable (LKC # 91-201) is provided with all SunBurst systems. Two cables are required for binocular testing.

Bipolar Contact Lens Electrode Placement



Sensor Strip Electrode Placement

5.5 Recording Data

After the electrodes have been inserted, the test may be started. The patient information may be inserted into the program before the electrodes are placed on the patient to minimize the amount of time the patient must have the electrodes inserted and to speed up the total testing time.

In this section, the Standard ERG protocol will be used. Other protocols are very similar. In order to understand this section, familiarity with the UTAS Software manual is necessary.

5.5.1 Setting up the test

- From the main menu select *Tests -> Electroretinogram -> Standard*
- Fill in the patient information (see section 10.5.2 for more info on the patient info window) and click on *Continue*
- Select the number of channels to be used and label them. Label the channels with the eye to be tested. If testing both eyes, select two channels (the convention is to have Channel 1 labeled as Right Eye and Channel 2 as Left).

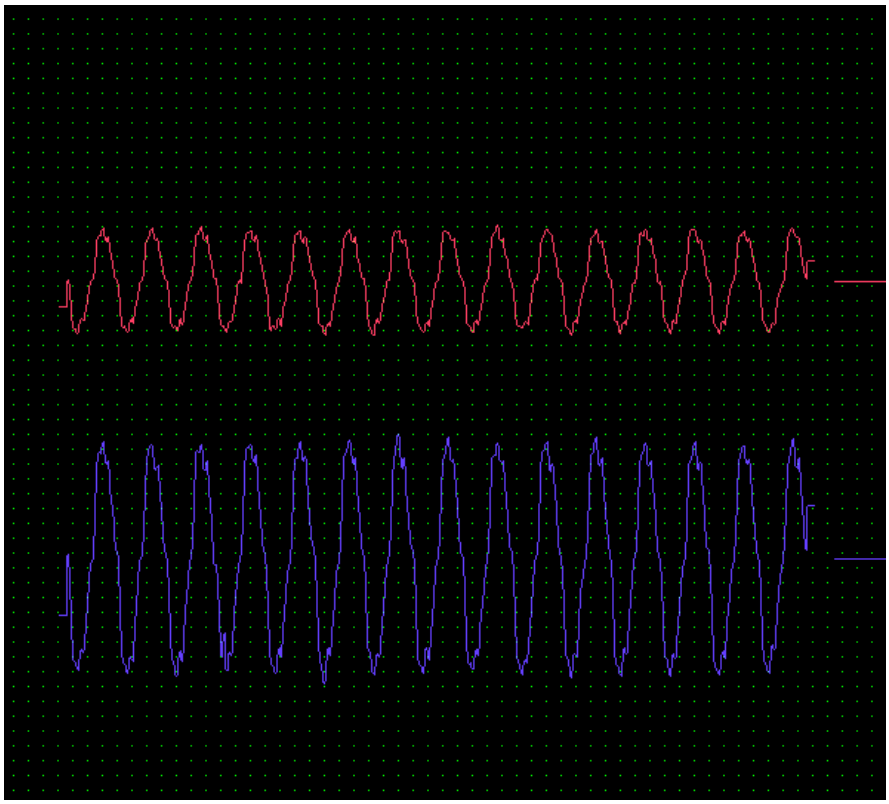
You are now ready to start the test. Be sure the patient is seated comfortably with their face in the Ganzfeld. Instruct the patient to look straight ahead at the red light.

5.5.2 Record – Checking the baseline

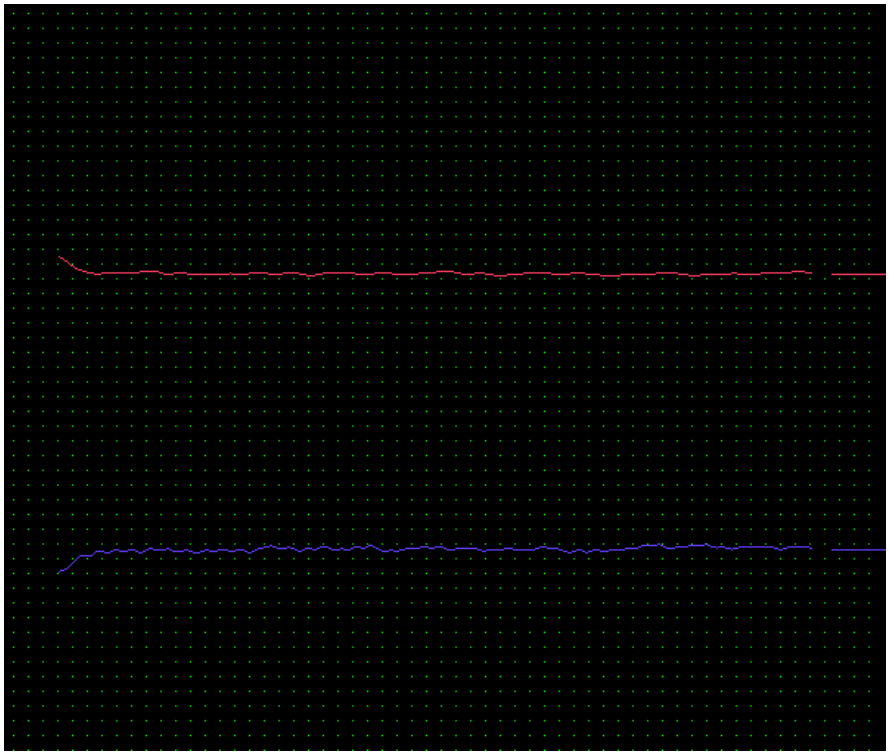
Click on Record (which is the first icon of the toolbar). The baseline is a check to make sure that everything is working correctly; that the electrodes are connected properly and making good contact with the patient, that the patient isn't clenching their face muscles, that no power line interference (maybe from the laser next door) is being detected, etc.

An example of a poor baseline is shown below. This baseline has a large amount of power line interference in it. If the baseline looks similar to this one, suspect a bad connection in the system. The indifferent electrode may not be making good contact with the forehead or there may be a problem with the contact lens electrode. Of course, there are other potential sources of interference as well. For more information about interference see **Appendix 2: Artifacts in Electrophysiology** in the UTAS system hardware manual.

Also shown below is an example of a good baseline. Attempt to make the baseline as close to this horizontal line as possible. If the baseline does not look good, if it has a lot of vertical lines or other noise and occupies most of the screen – it needs to be fix the problem before continuing with the test.



Bad ERG Baseline



Good ERG Baseline

5.5.3 Recording Data

Step 1: Rod Response

At the bottom of the screen there should be a line reading "Scotopic White -24 dB Flash".

Click on **Record** (this can be found on the top menu or the record Icon)**.** Once the baseline is stable, click stop and then click on record. If the response looks good (examples of good waveforms are shown in the *Analysis* section below), click on **Store** (using the diskette icon)**.** If the response does not look good (at this point, usually because of a blink or other reflex motion), wait at least two seconds before repeating the flash.

Note: Always wait at least 2 seconds between dim flashes (if required) of step 1 to avoid light adapting the subject.

After storing a good waveform, move to the next step. Click on the **Step Forward** icon**.**

Step 2: Maximal Response

The bottom of the screen should now read "Scotopic White 0 dB Flash".

Again, click on **Record** to measure a baseline and make sure noise hasn't developed. If the baseline looks OK, click **Stop** and then **Record.** The response will look different this time - it will be larger and less rounded. An example of a good response for the second step in the protocol is shown in the *Analysis* section below. If the response is good, click on **Store** to save the waveforms. If they're not good, wait at least **15** seconds before trying again to avoid light adapting the patient.

After storing a good waveform from step 2, click the **Step- Forward** icon to proceed to Step 3.

Step 3: Oscillatory Potentials

The stimulus description at the bottom of the screen won't change for this step since the stimulus isn't different for oscillatory potentials, only the recording technique. Again, click on **Record** to make sure no noise has developed.

To record oscillatory potentials properly:

- Click on **Record.** Do not store this waveform.
- Wait 15 seconds
- Click on **Record.** If this waveform looks good, store it. If not, wait another few seconds and repeat the steps.

The first flash is called *a conditioning flash.* It is used to sensitize the retina to maximize the oscillatory potentials recorded with the second flash, which is measured to give the waveform to be stored.

Step 4: Photopic response

When you click on **Step Forward** to move to step 4 of the protocol, the background light inside the Ganzfeld will turn on. For this step, the patient needs to be light adapted using this background light so be sure the patient doesn't close their eyes for extended periods. The bottom of the screen should now read "WHT Photopic 0 dB Flash".

Wait 10 minutes for light adaptation before recording.

Repeat the **Record,** and **Store** sequence performed above in step 3.

Step 5: Flicker response

This is the last step of the protocol. The bottom of the screen should read "WHT Photopic 0 db 30 Hz Flicker". Click on the **Record** button**.** The remainder of this step is automated, so the program will automatically start the flash flickering at 30 flashes per second, waiting 5 seconds, then averaging the 10 sweeps. The result will be displayed on the screen. To save the waveforms, click **Store.**

The test is now finished. Click on **Return** until to reach the Main Menu.

5.5.4 Cleanup

First, gently remove the corneal electrodes from the patient's eyes. Next, remove the indifferent/reference and ground electrodes from the patient's forehead and ear. Use an alcohol pad or electrode prep pad to remove the sticky material that might have been left by the electrodes on the patient's skin. The patient is now completed with the test.

5.6 Reports and Analysis

To prepare a report for a specific patient:

- Retrieve the patient's waveforms (see section 10.6.5 on how to retrieve waveforms).
- Place cursors on the waveforms or use another method to analyze them (see section 10.6.9 on how to place cursors using EMWIN).
- Print out the reports (see section 10.6.10).

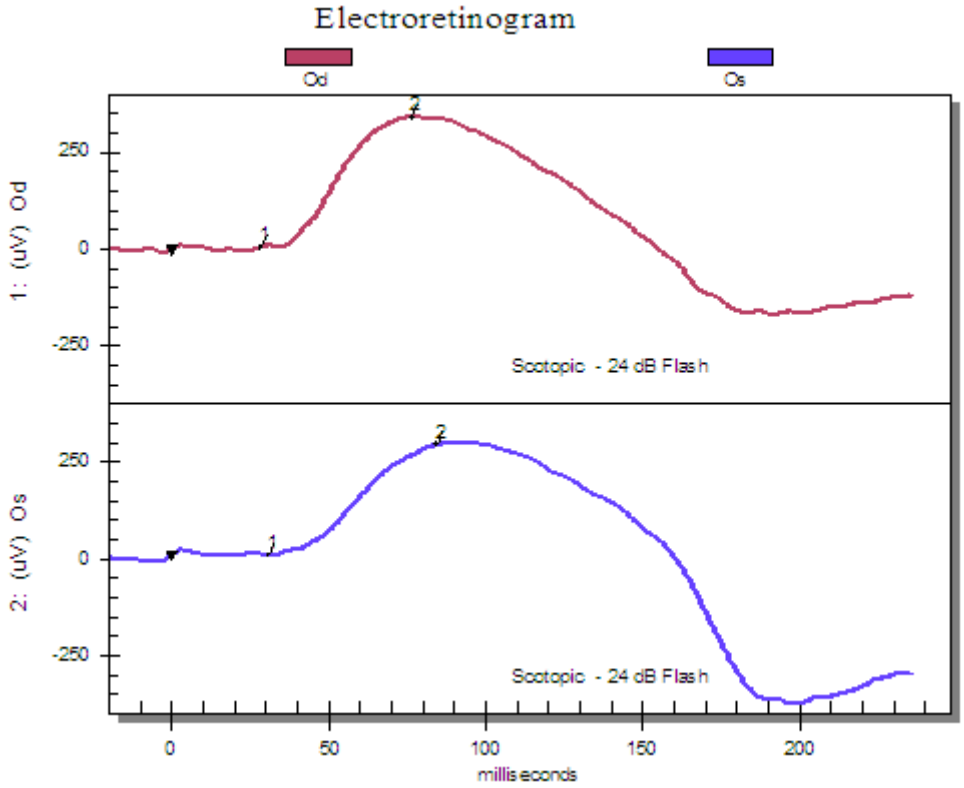
5.6.1 Retrieving the waveforms

In order to retrieve the waveform, go to the main menu and select Create Reports. Fill in the necessary information to search for the waveforms. For more detailed information about searching for waveforms, see section 10.6.5

5.6.2 Step 1: Rod Response Analysis

Retrieve the first two waveforms, labeled "Scotopic -24 dB". Next, click on the **Place Cursors** icon**.** Place two cursors on the waveform as shown in the figure below. Place Cursor 1 on a flat spot in front of the waveform and place Cursor 2 at the peak of the waveform (see section 10.7 on how to create report and place cursors). The calculated difference between cursors 1 and 2 gives the amplitude of the b-wave. B-wave latency is represented by the timing of cursor 2.

After the cursors are placed properly on both waveforms, click on **Store** to save the cursor positions with each waveform.



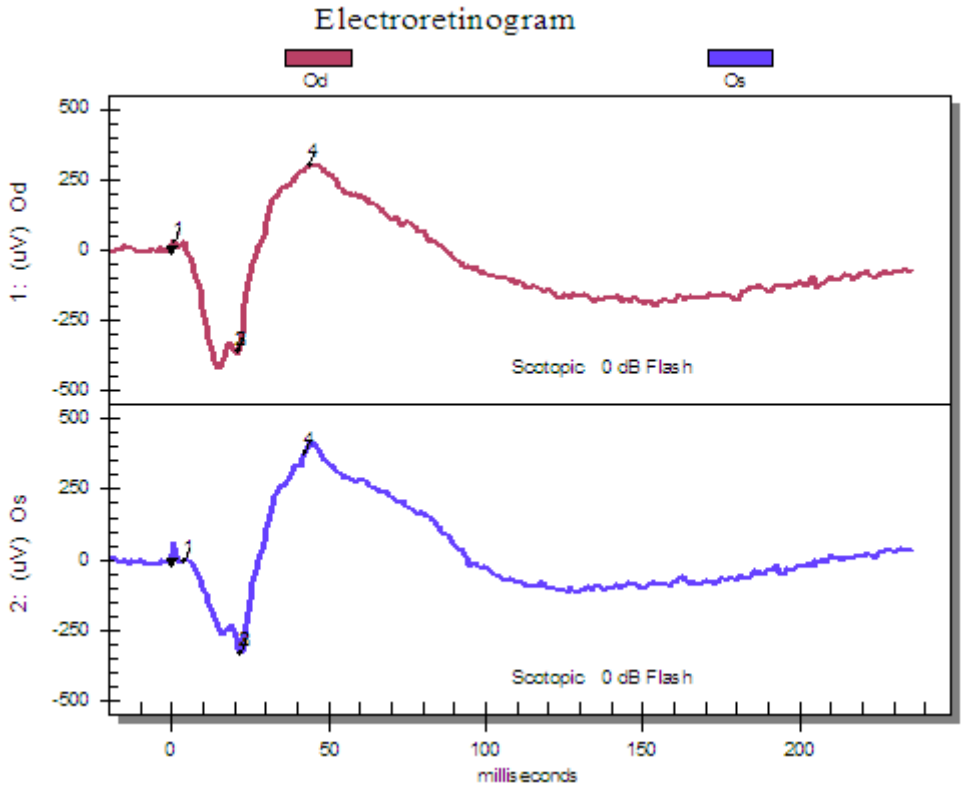
5.6.3 Step 2: Maximal Response Analysis

After storing the cursors with the waveforms, click **Return** to return to the Reports Menu. Next, retrieve the following pair of waveforms (either by clicking on **Retrieve -> Next** by clicking on the **Search For Waveforms** icon or by clicking

on Next). These waveforms should say "Scot W 0 dB SF." Place the cursors as described below:

Cursor 1 should be placed on a flat spot of the waveform before the amplitude decreases in the a-wave. Cursor 2 and 3 should be placed on top of each other at the trough of the a-wave. Cursor 4 should be placed at the peak of the b-wave. If there is a small bump at the top of the b-wave, place the cursor to one side of the bump, not directly on the top.

The calculated difference between cursors 1 and 2 represents the amplitude of the a-wave, while the calculated difference between cursors 3 and 4 represents the amplitude of the b-wave. After the cursors are placed properly on both waveforms, click **Store** to save the cursor positions with the corresponding waveforms.



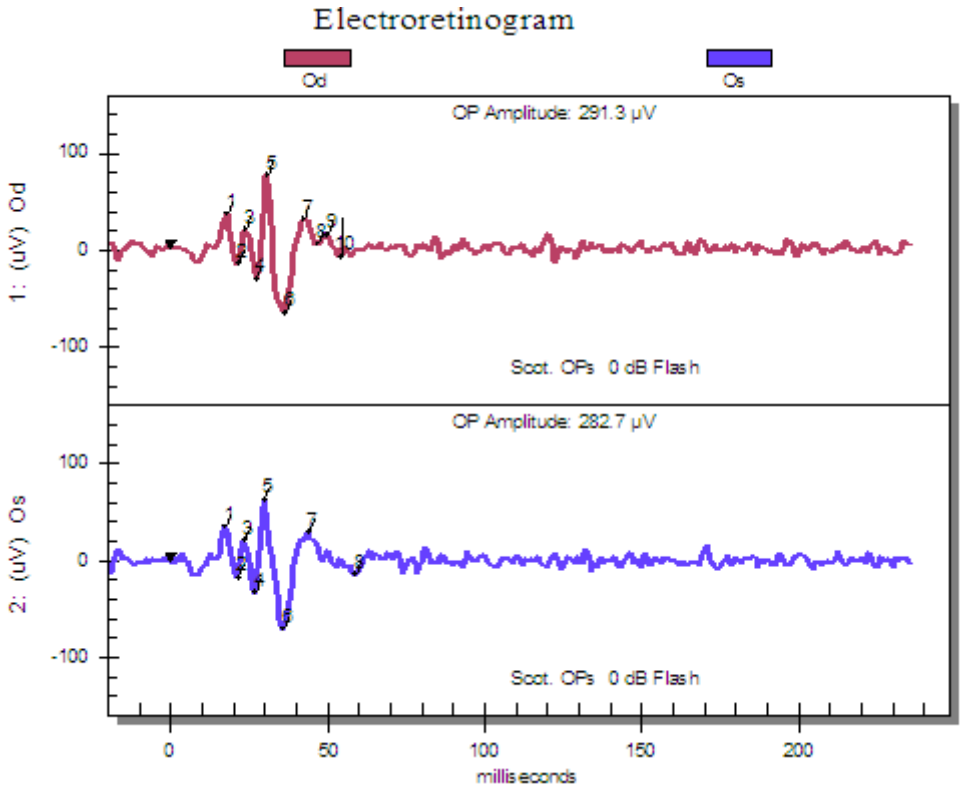
5.6.4 Step 3: Oscillatory Potential Analysis

Retrieve the next set of waveforms that correspond to step 3. These waveforms should read "Scot OPs 0 dB." They will be the recorded measurements of the oscillatory potentials and will resemble the one shown below.

With this particular waveform, the program will automatically place cursors. Click

on the **Analyze Oscillatory Potentials** icon or use the menu tool bar and select **Analyze** -> **Oscillatory Potentials.** When the program asks, tell it to place cursors on the Ops. The results will be similar to the ones on the waveform below.

Note that the cursors placed by the program will not be saved on the original waveforms; instead, a new waveform with the oscillatory potential cursors will be created in the database.



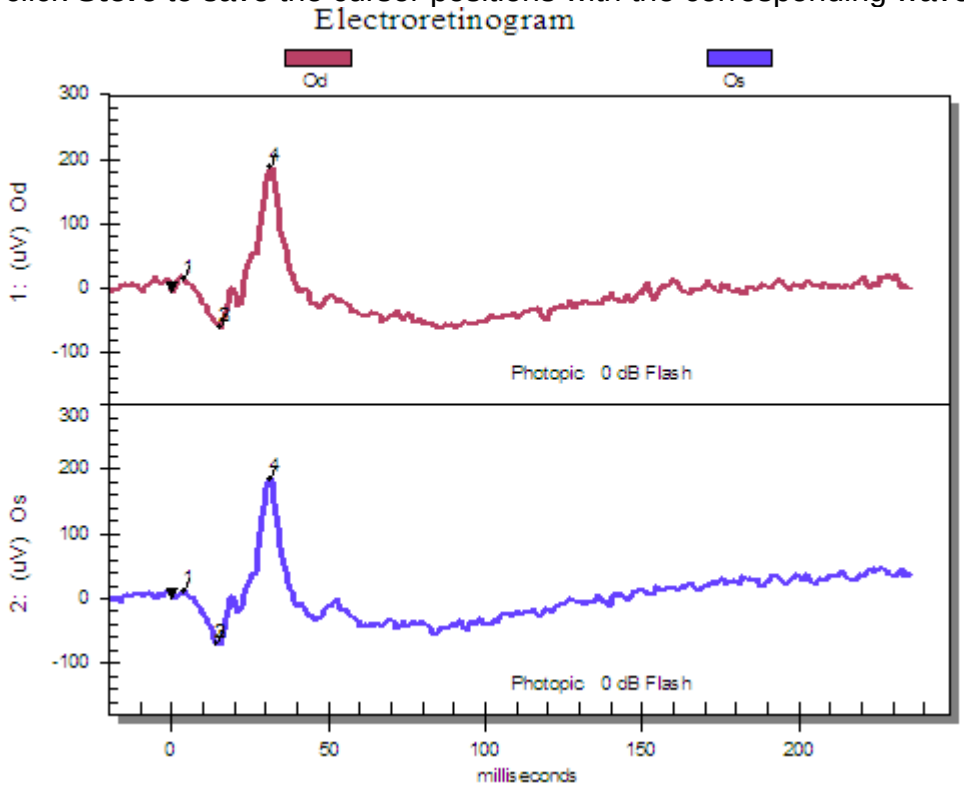
5.6.5 Step 4 Photopic Response Analysis

Retrieve the next set of waveforms, from step 4, labeled "Phot W 0 dB SF." Place cursors (and store them) as dictated below. The Photopic waveform is generally faster and smaller than the Scotopic waveforms because only cone function is being tested.

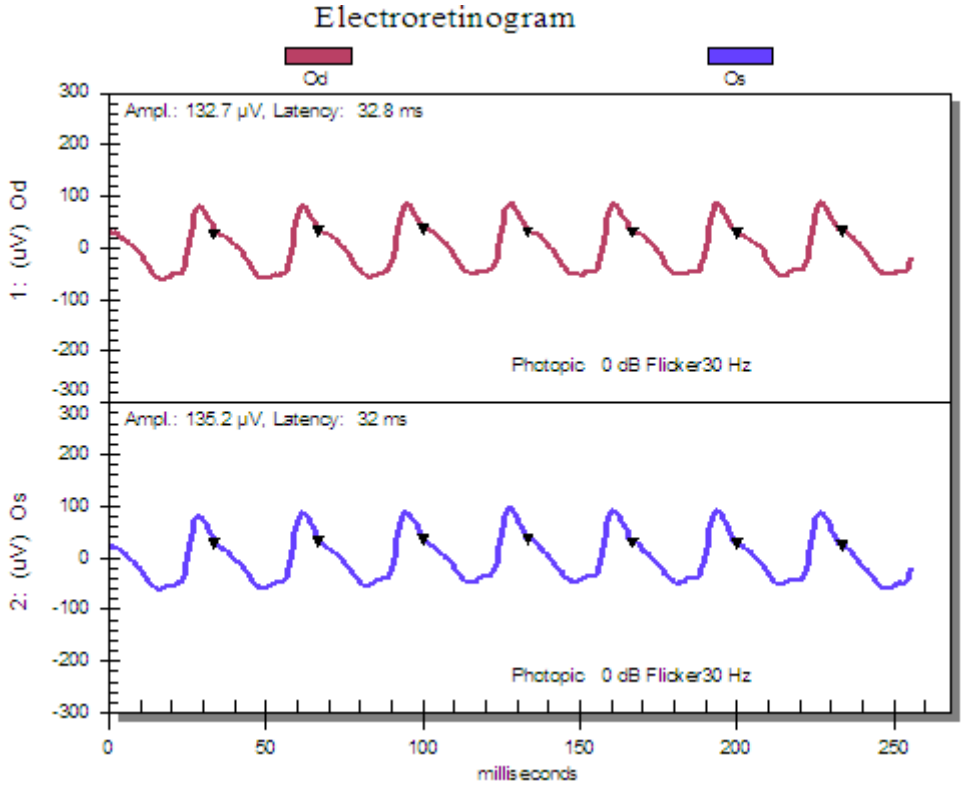
Cursor 1 should be placed on a flat spot of the waveform before the amplitude decreases in the a-wave. Cursor 2 and 3 should be placed on top of each other at the trough of the a-wave. Cursor 4 should be placed at the peak of the b-wave. If there is a small bump at the top of the b-wave, place the cursor to one side of the bump, not directly on the top.

The calculated difference between cursors 1 and 2 represents the amplitude of the a-wave, while the calculated difference between cursors 3 and 4 represents the

amplitude of the b-wave. After the cursors are placed properly on both waveforms, click **Store** to save the cursor positions with the corresponding waveforms.



5.6.6 Step 5: Flicker Analysis



Finally, call up the last set of waveforms from step 5. These waveforms will be labeled "Photopic 0 dB 30 Hz" And they correspond to the flicker tests.

To analyze these waveforms, go to the menu and select **Analyze** ->**Flicker Amplitude and Timing,** or use the **Analyze flicker icon.** This analysis will not put cursors on the waveform like the oscillatory potential analysis. Instead, it will produce a comment display that will contain the amplitude and timing information as determined by the analysis program.

THE FOLLOWING ARE STEP BY STEP QUICK GUIDES FOR ERG
May be copied and kept with your system for reference

STEP-BY-STEP INSTRUCTIONS FOR PERFORMING THE STANDARD ERG

Patient Preparation

- Anesthetize the patient eye(s) to be tested.
- Put dilating drops into the eye(s).
- Completely patch the eye(s) to be tested or seat the patient in a dark room for dark adaptation. During dark adaptation no light may get into the eye (a red light, like the background red light from the Ganzfeld, can be used to see and does not affect dark adaptation). *The patient must be dark adapted* for at least *20 minutes.*

Computer Setup Before the Patient is Brought for Testing

- Turn the system on and wait for the Main Menu to appear.
- On the Menu, select *Test -> Electroretinogram. -> Standard.*
- Enter the patient information. Do not use spaces or punctuation in the data fields, as this may interfere with the search and retrieval of waveforms.
- Add channel information. The right eye is hooked up to channel 1 and should be labeled OD or R. The left eye gets hooked up to channel 2 should be labeled OS or L. Click continue

Patient Hook-Up

- Seat the patient in front of the Ganzfeld globe, clean the forehead with an electrode prep pad and allow the alcohol to dry.
- Turn the red background light of the Ganzfeld on to provide light to place the electrodes.
- Place an EKG electrode on the forehead. Connect the pinch connector to the nipple of the EKG electrode. Plug the pin end of the pinch connector lead into the receptor of the one-to-two splitter.
- Place the two leads from the splitter into the 1- and 2- positions of UBA.
- Connect the two Monopolar (ERG-Jet, DTL) electrodes into the 1+ and 2+ positions of UBA.
- Before the ERG electrodes are actually placed on the corneas, put another drop or two of the anesthetic in the eye. The anesthetic wears off after approximately fifteen minutes.
- If using a lens electrode, fill the lens of the electrodes with Goniosol or any Methyl cellulose solution and place the 1+ electrode on the right cornea and the 2+ electrode on the left cornea, and tape wires to the cheeks. (Follow the directions on the ERG-Jet electrode box.)

Performing the Test

- Click on the *Record* icon.
- If the Baseline appears to be good, click on *Record.*
- If the waveforms are good, click *Store* to save them. Otherwise, wait a period of time before repeating the step (wait 2 seconds for step 1, 10 seconds for step 2, and 15 seconds for step 3). Select *Step,* then *Forward.* The display will indicate that you are on the next step.
- Repeat these steps (from the Performing the Test section) until the waveforms from steps 1-5 have been recorded and stored.

Testing the Patient

- The patient is now completed with the testing. All electrodes can be removed properly. The affected skin should be cleaned and the corneas washed with saline solution.
- The waveforms can now be retrieved and analyzed, and the reports printed.

Afterward the appointment

- The forehead rest should be cleaned and disinfected using a mild disinfectant such as a Benzalkonium Chloride wipe or an isopropyl alcohol wipe.

6 The Visual Evoked Response (VER)

6.1 Overview

The Visually Evoked Response (VER) - also called the Visually Evoked Potential (VEP) or Visually Evoked Cortical Potential (VECP) – is a test used to measure the electrical response of the primary visual cortex when visually stimulated. The response is measured from Brodman's Area 17 of the cortex, an area concerned mostly with foveal vision. The most commonly used visual stimulus is an alternating checkerboard pattern, although a flash of light can also be used.

In clinical testing, the electrical response of the visual cortex is measured by placing one electrode on the scalp directly over the visual cortex, a second on a reference location (such as the ear) and measuring the difference between these two responses. The sensitive amplification system is able to measure the difference (typically millionths of a Volt, or microvolts, µV). Note that a typical wall outlet produces over 100 Volts which is about ten million times greater than the typical VER signal.

A normal VER is indicative of a properly working visual pathway - from the foveal retina through the optic nerve to the visual cortex. The VER can provide useful information for the diagnosis and treatment of many conditions; including, optic neuropathies, differential diagnosis of unexplained acuity loss (with the focal ERG), and malingering blindness.

Typically, the VER is recorded in response to an alternating checkerboard stimulus. The electrical response to this pattern stimulus consists of an initial negative wavelet followed by a positive one (the VER may contain a few additional wavelets, but only the first two are of primary clinical significance). There are many factors that can influence the amplitude of the VER (including skull thickness and electrode location), so the timing of the waveform is the most useful diagnostic measure in this test. The negative wave, which generally occurs at 75 milliseconds (ms), is called N75 while the positive wavelet, which occurs at almost exactly 100 ms, is known as P100. Disease states that affect the VER prolong the waveform, leading to an increased timing in the P100 wavelet.

The flash VER is most useful in evaluating ocular trauma. It provides evidence of some foveal vision, indicating that a reconstruction of the eye is likely to be successful. The flash VER is a complicated waveform that is quite varied between subjects. Usually, the useful information gathered from the flash VER waveform is whether or not it is present.

6.2 Patient Preparation

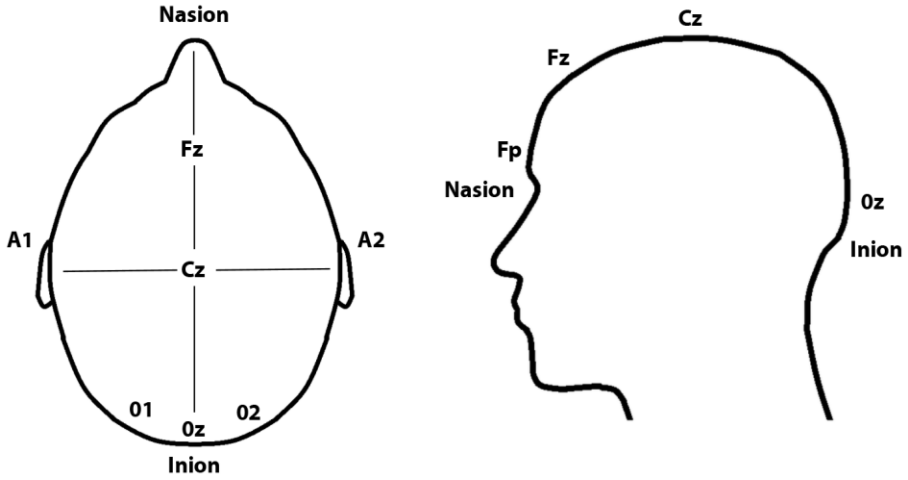
Because electrodes will be attached to the scalp, the patient should be advised to wash their hair within 24 hours of the test and not to use any hair products the day of testing.

Testing the Patient

Before starting the test, make sure that the patient is properly refracted.

Errors in refractive correction will give inaccurate test results. The patient can simply wear their normal prescription eyeglasses or contacts during the test. If the patient's glasses prescription is not be correct, be sure to check and correct before proceeding with the test.

The first step is to decide the locations from which to place the electrodes and record. Usually, a single electrode at OZ and one reference electrode will be sufficient. If the purpose of the test is to diagnose pre- versus post-chiasmal optic nerve defects, electrodes must be placed at both 01 and 02.



Typical recording sites and landmarks for the VER

To identify the recording sites to place the electrodes on the patient's scalp, first identify the **inion,** the bony protrusion at the back of the skull.

> **If you are recording on an adult with a normal-sized head, the Oz is located about 2.5 cm (1 inch) above the inion on the midline. 01 and 02 are located 2.5 cm (1 inch) to the left and right of Oz.**

If the subject has an abnormal-sized head, is an infant, –or if it is important that the electrodes are placed in the exact locations, making a few measurements will determine the locations for the recording sites. First, identify the **nasion,** the bony ridge along the brow line just above the nose on the front of the head. Measure the distance from the nasion, over the head, to the inion. Next, locate the two **pre-auricular points,** the bony protrusions of the mastoid bone just in front of the ear and measure the distance around the back of the scalp between the two pre-auricular points.

- The Oz point is located on the midline, 10% of the distance from the inion to the nasion above the inion.
- O1 is located at the same elevation as Oz, 10% of the distance between the

pre-auricular points to the left of midline

- O2 is located at the same elevation as Oz, 10% of the distance between the pre-auricular points to the right of midline

6.3 VEP Electrodes

The VEP is measured using three types of electrodes: the recording (positive) electrodes, the reference (negative) electrode, and the common (ground) electrode. The positive electrodes are gold cup electrodes, as shown on the right. This electrode is typically placed in the Oz location. The negative electrode is typically either an ECG electrode or another gold cup electrode. This is typically placed on the forehead, or along the Fp site. The common electrode is typically an ear clip.

Clean the electrode site thoroughly to remove all skin oils and other debris that might inhibit the electrode to making a good electrical contact.

Fill the cups of the ear clip electrode with electrode **gel** (not cream) and clip it to the patient's ear lobe. Then connect this to the ground/common site on the amplifier.

Locate the positive electrode site(s). Part any hair to expose the skin at the recording site, and *vigorously* scrub the skin with an electrode prep pad. (If the patient's hair is long, bobby pins or other clips should be used to hold the hair out of the way during this process.)

Note: It is important to clean the scalp thoroughly to obtain a good electrode contact.

Using a generous dollop of electrode **cream** (not gel); paste the hair on each side of the part to the scalp or use bobby pins to securely hold down the hair. The important thing is to keep the scalp exposed. Next, put a generous portion of electrode cream in the cup of the electrode and press the electrode *firmly* into place on the scalp. Cover the electrode with a 2 to 3 cm (1 to 1½ inch) square of tissue paper, and press firmly again.

Repeat this procedure for each electrode being used. Plug the other end of the electrode into the positive (+) side of the Amplifier unit, taking note of which electrode is plugged into which channel if you are using more than one.

The negative (reference) electrode is typically an ECG electrode that is placed on the forehead, attached to a wire and place in the negative side of the amplifier unit. If you have more than one positive electrodes, attach a splitter to the end of the negative electrode and place them in the corresponding negative channels on the amplifier.

6.4 Recording data

6.4.1 Setting Up the Test

After the electrodes have been attached to the patient, the test can begin. This section of the manual contains information that was explained in the *Software Manual*.

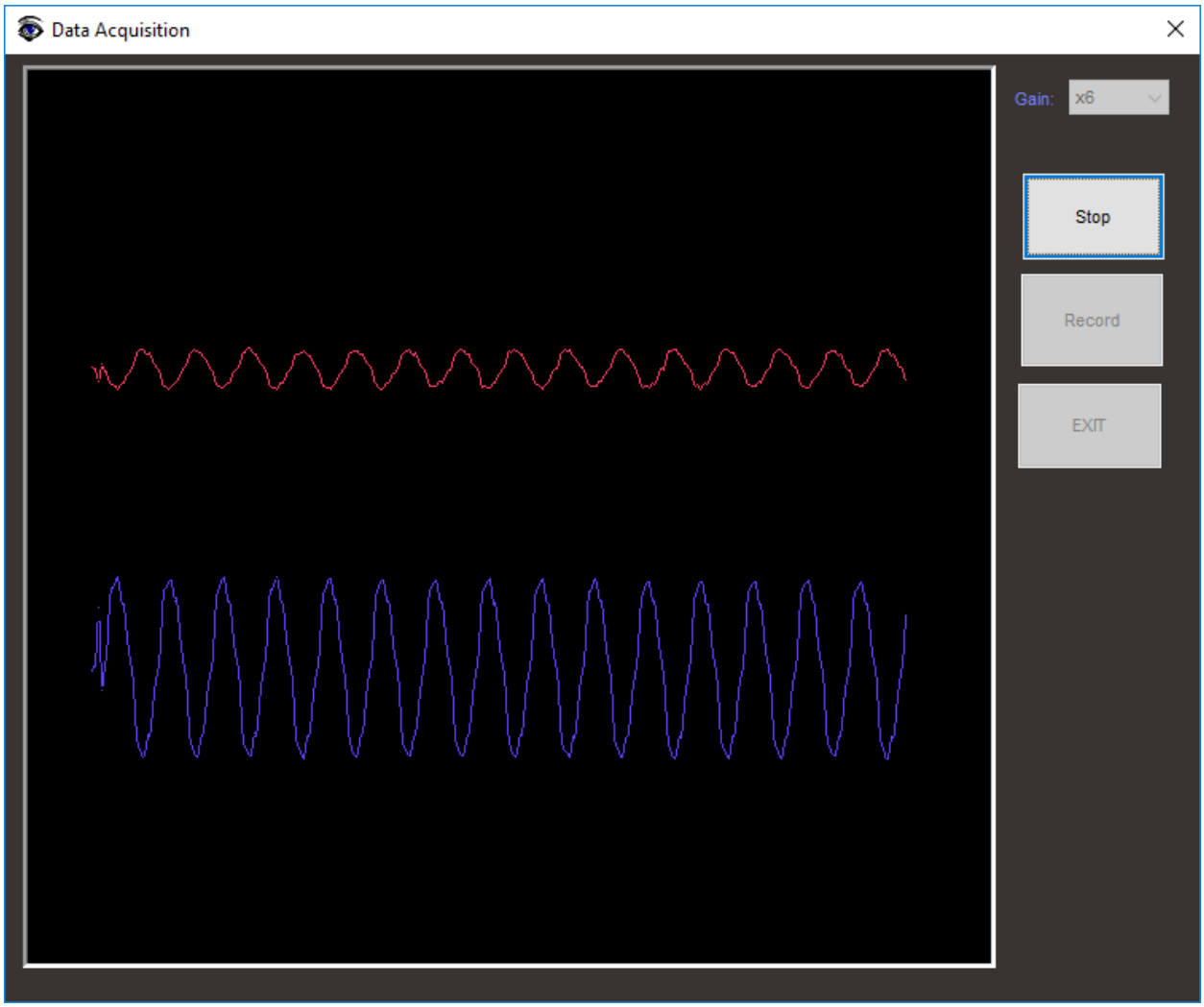
- From the Main Menu, click on **Perform Tests -> Visual Evoked Response -> Pattern.**
- Fill in the patient information (as much as possible, but at least the First and Last Names).
- Enter the number of channels used/electrodes to be recorded from. Label the channels with the name of the site (O_Z, O1, etc.) Select which eye is being recorded and click continue.

The test is now ready to begin. Make sure the patient is seated comfortably and located the correct distance away from the screen. This distance is specified on a label on the pattern monitor.

Note: Any muscle contraction may be picked up as noise on the recording, so it is important that the patient is as relaxed as possible in order to get a decent recording.

6.4.2 Record

Now click on the **Record Icon.** The baseline should look somewhat like a good baseline of other tests. There should be about 20 to 50 µV of signal visible on the screen (most of this is EEG activity generated by the brain).



Bad VEP Baseline

The shown baseline is an example of a bad baseline. The patient may be tensing his neck/shoulder muscles so that a large amount of EMG (muscle electrical activity) is measured. If the patient's baseline looks like this one, encourage the patient to relax as much as possible.

If the baseline appears to have a large number of vertical lines on the screen spaced about 15 milliseconds apart (20 milliseconds apart in Europe and Asia), then there is power line interference. The most likely cause of this baseline is poor electrode contact. Be sure to press firmly on each of the scalp electrodes and try another baseline.

Note: Poor electrode contact is the most common reason for poor VER recordings!

6.4.3 Recording Data

When the baseline is acceptable, it's time to record. Just before clicking on **Record,** instruct the patient to carefully watch the screen and to think about something else (about

20% of patients are able to reduce their VER amplitude by concentrating on other topics).

The default VER protocol will average 80 responses to obtain a waveform. The number of responses averaged appears in the lower right portion of the screen. If, while watching the waveform (which displays every 10 sweeps), it does not significantly change, the test may be safely stopped. In most cases, as few as 30 responses will give you a satisfactory recording.

When the recording has finished, instruct the patient to relax. Patient fatigue can affect the test results, so encourage them to close their eyes and relax. It is a good idea to wait a few seconds between the tests to allow the patient to recover.

The first stimulus presented in the pattern VER protocol is a checkerboard composed of 32 x 32 alternating squares. For most purposes, this stimulus will be the only one needed. If other responses are desired, the check sizes in the default pattern VER protocol will be:

32 x 32, 8 x 8, 16 x 16, 64 x 64, 128 x 128

When testing multiple responses to different check sizes, click on **Step Forward Icon** to go to the next check size. 

Remember to store the waveforms (by clicking **Store**). If the waveforms are not stored, the software will automatically prompt you if you want to save them or not. 

When the test is finished, return to the Main Menu by clicking on the **Return Icon.** 

6.4.4 Cleanup

Gently remove the electrodes from the patient's scalp. The electrode cream should be cleaned out of the patient's hair using warm, damp paper towels. Electrode cream is not soluble in alcohol, so electrode prep pads will not be able to remove it.

6.5 Reports and Analysis

To prepare the reports on a particular patient,

- Retrieve the waveforms for that patient (see section 10.6.5 for more info).
- Place the appropriate cursors on those waveforms (section 10.6.9 for more info).
- Print out the reports (section 10.6.10 for more info).

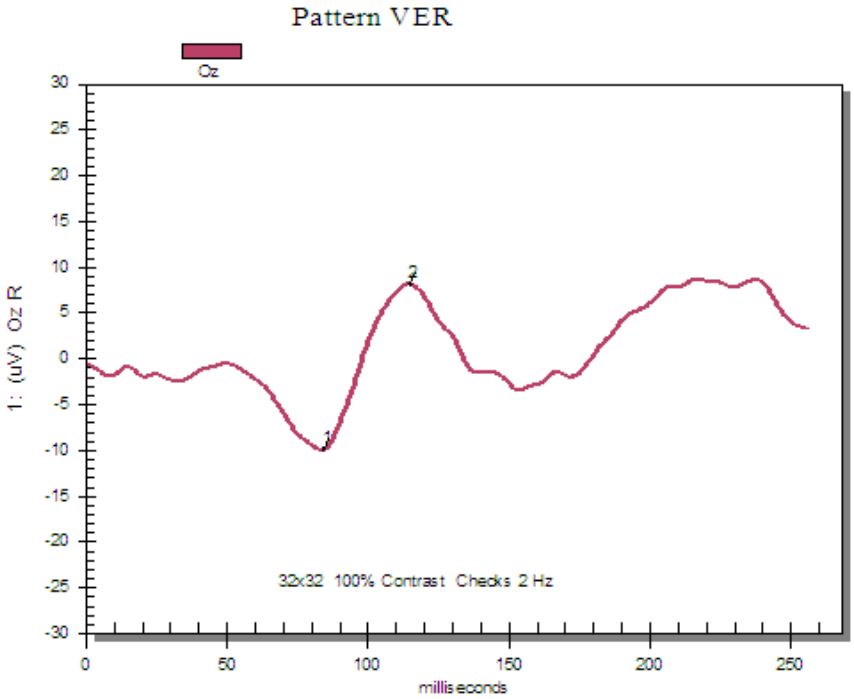
6.5.1 Retrieving waveforms

To retrieve the waveforms, start from the main menu and select Create Reports. Fill in the necessary information to search for and retrieve the waveforms (for more detailed information see section 10.6.5).

6.5.2 Analysis

To analyze the waveforms retrieved, click on the **Cursors** icon**.** Place cursors on the waveform as shown in the figure below. Place Cursor 1 at the bottom of the major trough, which usually occurs at 70 milliseconds. Place Cursor 2 at the top of the peak, which usually occurs at 100 milliseconds.

Note: Patients with a disease may have significantly altered peak/trough timing.



After the cursors are placed properly on the waveform, click on the **Store** icon to save the cursor positions with the waveform.

6.5.3 Smoothing

Before printing the waveform, it may be a good idea to smooth it. Smoothing the waveform will remove the small bumps. To smooth, click on

Analyze ->**Smooth** or use the smooth icon**.** If you smooth the waveform and then click on the save icon it will save as a new waveform. See section 10.6.2 for more info on smoothing.

THE FOLLOWING ARE STEP BY STEP QUICK GUIDES FOR VEP
May be copied and kept with your system for reference

STEP-BY-STEP INSTRUCTIONS FOR PERFORMING THE VEP

Computer Setup before Patient Preparation

- Turn the system on.
- Select *Perform Test -> Visual Evoked Response.*
- Select the type of VER to be done (*Pattern or Flash).*
- Add the patient information. If recording from only one channel, the electrode at the midline is labeled O z . If recording from two channels, the left channel is labeled O1, and the right channel is labeled 02.
- Select the eye to be tested (Right, Left, Both).

Patient Preparation

- Seat the patient at the specified distance on the label at the bottom of the screen from the pattern stimulator.
- Clean the ear lobe and the OZ location in the back of the head with alcohol prep pads, allow the alcohol to dry.
- Fill both cups of the ear clip electrode with electrode gel and attach it to the ear lobe. This electrode should be connected to common ground (green) channel on the UBA.
- Part the hair at the midline.
- Hold the hair down on the scalp by using secure bobby pins or electrode cream on both sides of the part,
- Fill the gold cup electrode with electrode cream and place it in the middle of the part, directly on the scalp, pressing down firmly on the electrode.
- Use a one square inch of Kleenex tissue and place it over the electrode to insure the electrode is attached tightly to the scalp.
- Connect the electrode to the 1+ location of the UBA.
- A reference electrode is placed using an additional gold cup electrode, or alternatively an EKG forehead patch.

Performing the Test

The Standard *Pattern VER* Test contains a five-step protocol. In each step the pattern size is changed. For systems with manual Pattern Stimulators, it is essential to change the pattern size according to the stimulus conditions found at the bottom of the computer screen.

- Select *Record.*
- If the baseline appears normal, click *Record.* During the test, the number of sweeps averaged is displayed at the bottom of the screen.
- When the waveform is sufficient, select *Stop* and then *Store.* It is possible to finish the averaging and not record a perfectly smooth waveform. It is up to the technician to decide whether to repeat the test or to reposition the electrodes and then repeat the test.
- Once a satisfactory waveform has been stored for a step, select *Step* and *Forward.* This will take the test to the next step.
- Repeat the last four steps until step 5 has been successfully recorded and stored.
- At this point, if a *Normal Intensity Flash* VER or *Bright Flash* VER is not needed, the patient can be disconnected, cleaned or electrode gel and released.
- The waveforms can then be retrieved, cursors placed and analyzed, and reports printed.

7 The Electro-oculogram (EOG)

7.1 Overview

The electro-oculogram (EOG) measures changes in the standing potential of the eye in dark and light conditions. The standing potential of the eye is generated across the retinal pigment epithelial (RPE) layer, thus the EOG primarily measures RPE function.

The EOG is a somewhat more difficult test than the ERG or VER, taking more than 30 minutes to complete. Consequently, it is less common than other visual electrodiagnostic tests. Nevertheless, there are some conditions where the EOG is quite useful; it is necessary in the diagnosis of Best's disease.

The standing potential of the eye causes it to act like a weak battery. The anterior (corneal) pole of the eye is more positive than the posterior pole. It is not possible to measure the standing potential of the eye directly, as this would involve inserting an electrode behind the globe, so the EOG is measures the potential indirectly.

To perform the EOG, two electrodes are placed on the skin next to the eye - one is placed near the temporal canthus and the other is placed near the nasal canthus. The patient then looks left and right. As the eye swings toward the nasal electrode, it becomes more positive than the temporal one. As the eye swings toward the temporal electrode, it becomes more positive than the nasal one. The difference between the temporal and nasal values is related to the potential across the eye.
Because the potential measured by this technique is related to many factors including the placement of the electrodes and the geometry of the eye, the value is of little use by itself. The clinically useful value is the ratio of the peak value in the light to the minimum value in the dark. This is called the Arden Ratio:

$$Arden\ Ratio = \frac{V_{light\ peak}}{V_{dark\ trough}}$$

Patient Preparation

According to ISCEV standards, the patient should be maximally dilated using a medium-acting mydriatic, such as Tropicamide. The patient should not be dark adapted before the test.

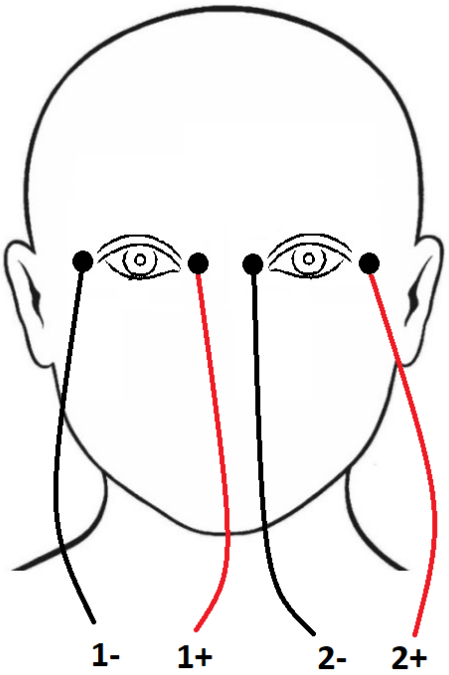
> **Note: The patient should not be exposed to bright lights, such as a slit lamp or ophthalmoscope, for at least 20 minutes before the test.**

7.2 Electrodes

The EOG uses four EOG recording electrodes (two for each eye).

Using an electrode prep pad, **carefully** clean the skin near the nasal and temporal canthi as well as one ear lobe. Take care to remove all skin oils, but don't get alcohol in the patient's eye. The EOG electrodes are attached to the patient using electrode washers, thin disks with adhesive on both sides. Remove the protective covering from one side of the electrode washer and affix it carefully to the EOG electrode. Align the washer so that its tab is near the electrode wire. Next, fill the cup of the EOG electrode with electrode **gel** (not cream). The gel should protrude slightly from the surface of the electrode washer. Remove the protective covering from the second side of the electrode washer and affix the electrode as close to the canthus of the eye as possible. A gold cup ear clip electrode is used for ground electrode and should be plugged into the ground connection of the amplifier.

Attach the EOG electrodes as follows:



7.3 Obtaining Data

After you have attached the electrodes, the test may begin. This section requires an understanding of the system software. For more information on the software, read the *UTAS Software* Section first.

7.3.1 Setting up the Test

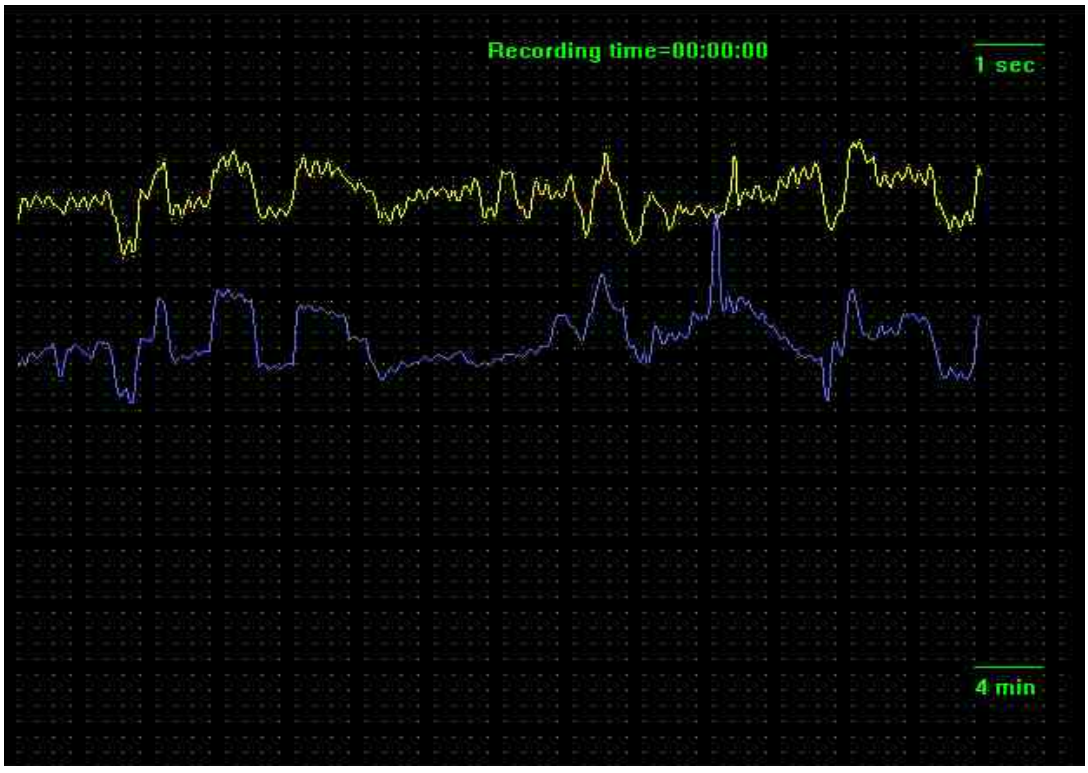
- From the Main Menu, click on **Tests -> Electro-oculogram.**
- Fill in the patient information (at least the First and Last Names).
- Enter the number of channels (electrodes) to be recorded. Usually with both eyes, channel 1 is labeled R and channel 2 is labeled L.

Seat the patient in front of the Ganzfeld. Make sure that the patient is comfortable, as they will be sitting there for more than half an hour without a break. Instruct the patient to look at the red light in the center of the Ganzfeld bowl. Tell the patient that when the lights begin to move, they should follow the lights with their eyes without moving their head.

7.3.2 Baseline

The software automatically starts in baseline mode. The LEDs will move from right to left immediately and the patient should follow these lights. See below for examples of a good and bad EOG baseline. To check for good electrode contact, perform an impedance check (which should be less than 40KΩ). If the wave is too large, interrupt the baseline, adjust the scale display to a lower setting, and run the baseline again.

Good EOG Baseline



Bad EOG Baseline

7.3.3 Recording Data

Once a good baseline has been established, the recording can begin. Click on **Interrupt** to stop the baseline and then click on **Record** to start the test.

The EOG collects data for the first 15 seconds of every minute. During these 15 seconds, the patient must consistently follow the alternating EOG fixation lights.

> **Note: An important part of the technician's job during the test is to help the patient remain alert (and stay awake) and to inform them of their progress.**

The clock at the top right of the screen will measure the time of the test. At 55 seconds into **each** minute, warn the patient that the lights will begin flashing in five seconds. When each minute starts, the EOG lights will begin flashing. The patient's eye movements can be monitored on the screen. If they are not moving their eyes with the lights, encourage the patient to follow the lights. At 15 seconds into each minute, the EOG lights will stop flashing and the center fixation light will turn on. Let the patient know that they can relax while still looking at the fixation light.

The EOG test consists of three phases:

- A pre-adapt phase (light on), lasting 6 minutes.
- A dark adapt phase (light off), lasting 16 minutes.
- A light adapt phase (light on), lasting 14 minutes.

Under normal circumstances, these times will not need to be changed.

> **Note: Make sure the patient does not close his or her eyes during the pre-adapt or light adapt portions of the test.**

When the test is finished, the software will automatically display the result. Be sure to store the data. After, the electrodes can be removed from the patient and they can be released.

7.3.4 Saving EOG raw data

After each 15 seconds segment of alternating LED, the Save button will be available. Clicking this icon will save the raw EOG data for that segment into C:\EMWIN\EOG. To save all of the raw data, click on Save after each 15 second segment is recorded.

7.4 Report and Analysis

To prepare the patient reports:

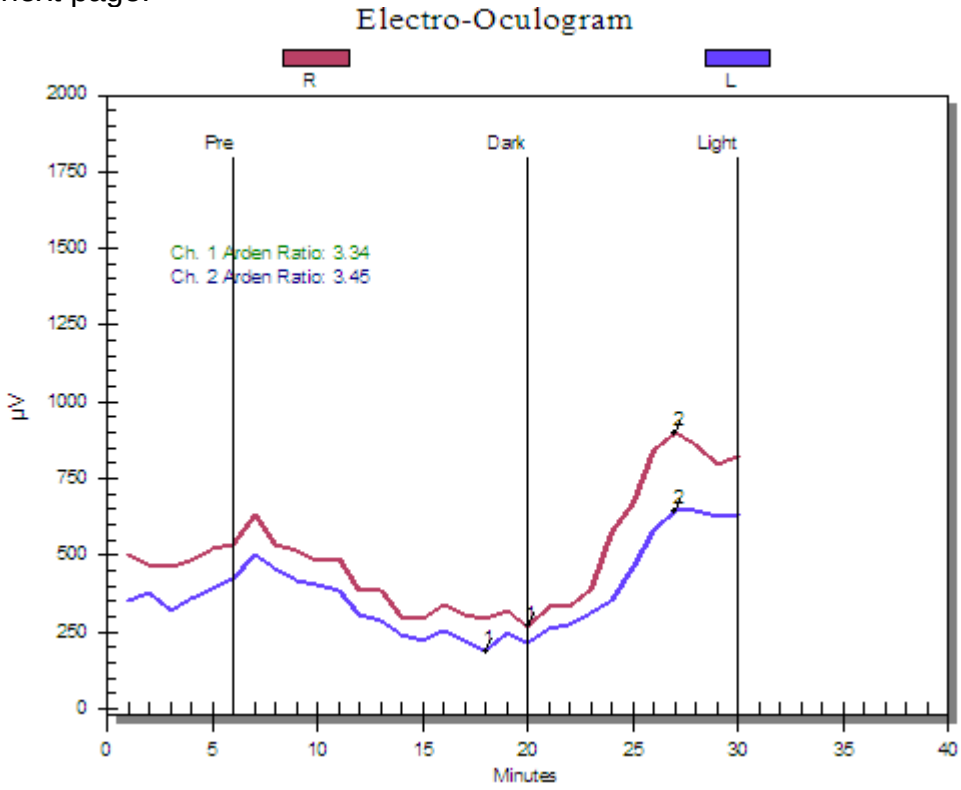
- Retrieve the specific waveforms (see section 10.6.5).
- Place cursors on the waveforms (see section 10.6.9).
- Print out the reports (see section 10.6.10).

From the Main Menu, click on the **Create Reports**. Fill in the necessary patient information and retrieve the waveforms (they will be labeled "Electro-oculogram").

To place the cursors automatically to calculate the Arden Ratio, click **Analyze -> Computer Arden Ratio**. The program will place cursors on the lowest point in the dark trough and the highest point in the light peak. Occasionally, the cursor will not be placed in the best locations. ISCEV guidelines state that cursors should be placed based on an appropriate imagined smooth curve through the data.

For example, if the patient fell asleep during the dark adapt portion of the test, there may be a few minutes where the computed value was close to zero. The program may select one of those values and give you an artificially high Arden ratio (in which case, cursors should be manually changed).

To change a cursor value, click on the **Cursors** icon. Move the cursor to the correct location. An example of an EOG with proper cursors is shown on the next page.



7.5 Fast Oscillation EOG

"The fast osciollation EOG is an optional additional test that has a different mechanism to the clinical EOG owing to the shorter dark and light intervals used. At light onset,

there is a fall in potassium in the sub-retinal space that causes a strong outward hyperpolarizing potassium current across the RPE's apical membrane and is reflected in the c-wave of the electroretinogram (ERG). The fall in sub-retinal potassium also reduces the transport of chloride ions into the RPE. The reduction in chloride ions causes the basolateral membrane to hyperpolarize and lowers the TEP generating the trough of the FO 35–45 s after light onset. The TEP returns to normal, as ionic homeostasis is restored and a peak is recorded during the subsequent dark period after a further 35–45 s. The alternation between dark and light at 1-min intervals establishes a continuous oscillation that is dependent on changes in ionic permeability at the apical and basal membranes and the electrical coupling between these membranes by tight junctions.

The FO has the opposite polarity to the EOG. Light causes a decrease in the standing potential, while in darkness there is an increase in the standing potential.

The FO is recorded using the same technical specifications as the EOG (amplifier, electrode placement, fixation targets, background luminance and 1/s saccades). However, the saccades and the recording should be continuous for the duration of the test. Light and dark intervals are alternated at 60 or 75-s intervals to induce the FO, which has a near sinusoidal appearance. The total number of light–dark intervals should be at least 4. Pre-adaptation does not affect the FO, so this test can be performed either independently or before the EOG."[2]

2 Constable PA, Bach M, Frishman LJ, Jeffrey BG, Robson AG; International Society for Clinical Electrophysiology of Vision. ISCEV Standard for clinical electro-oculography (2017 update) [published correction appears in Doc Ophthalmol. 2017 Apr;134(2):155]. *Doc Ophthalmol*. 2017;134(1):1–9. doi:10.1007/s10633-017-9573-2

THE FOLLOWING ARE STEP BY STEP QUICK GUIDES FOR EOG
May be copied and kept with your system for reference

STEP-BY-STEP INSTRUCTIONS FOR PERFORMING THE EOG

Computer Set up Before the Patient Arrives

- Turn the system on.
- Select *Test -> Electro-oculogram.*
- Add the patient information line by line. Add comments as needed, up to three lines.
- The EOG test is always a two-channel test and the channels are labeled automatically. The patient can now be prepped for the test.

Patient Hook-Up

- Clean the forehead, ear lobe, and the temporal and the nasal canthi of both eyes with alcohol pads. Pat the areas dry. Take care to avoid getting alcohol in the patient's eyes.
- Stick the electrode washers to the flat inner side of four EOG electrodes, leaving the paper cover on the exposed side. Position each "washer" so that its tab aligns with the electrode wire.
- Fill the electrodes with gel.
- Remove the washer tape covers and place the electrodes on the patient.
- Each eye should have one + electrode and one – electrode (one of each color).
- Connect the electrodes to the corresponding channels of the UBA.

Performing the Test

- The software will automatically start in Baseline mode. Check the eye movements (the size of the peaks) to see if they seem uniform and of good amplitude, if so, then click *Interrupt.*
- Select *Record* to start the test.
- The patient's eye movements should be constantly monitored to ensure the patient remains alert and does not close their eyes during any phase of the test.
- The machine will automatically place cursors on the waveforms and display the Arden Ratio at the end of the test.
- If there are anomalous high points in the light adapting phase or anomalous low points in the dark adapt phase, the Arden Ratio will not be correct. The cursor positions will need to be changed by selecting *Cursors* from the menu.

Appendix 1: LKC Normal Data
Normative Data for the Clinical Electroretinogram

Means and Standard Deviations as a Function of Age for the Most Commonly Measured Parameters of the International Standard (ISCEV) ERG Protocol

Monopolar Electrodes (e.g. ERG-Jet)

Stimulus	Parameter	Change With Age	S.D.
-24 dB Scotopic Flash	**b-wave amplitude**	330 µV - 2.2 µV/year	61 µV
0 dB Scotopic Flash	**b-wave amplitude**	644 µV - 1.6 µV/year	117 µV
	b-wave implicit time	47.5 ms	3 ms
Oscillatory Potentials	**summed amplitude**	235 µV - 2.1 µV/year	50 µV
0 dB Photopic Flash	**b-wave amplitude**	183 µV - 1.0 µV/year	37 µV
	b-wave implicit time	29.5 ms	1.5 ms
30 Hz Photopic Flicker	**Amplitude**	133 µV - 0.7 µV/year	30 µV
	implicit time	26.8 ms + 0.02 ms/year	1.8 ms

Bipolar Electrodes[3] (e.g. Burian-Allen)

Stimulus	Parameter	Change With Age	S.D.
-24 dB Scotopic Flash	**b-wave amplitude**	260 µV - 1.7 µV/year	48 µV
0 dB Scotopic Flash	**b-wave amplitude**	507 µV - 1.3 µV/year	92 µV
	b-wave implicit time	47.5 ms	3 ms
Oscillatory Potentials	**summed amplitude**	185 µV - 1.7 µV/year	39 µV
0 dB Photopic Flash	**b-wave amplitude**	144 µV - 0.8 µV/year	29 µV
	b-wave implicit time	29.5 ms	1.5 ms
30 Hz Photopic Flicker	**Amplitude**	105 µV - 0.6 µV/year	24 µV
	implicit time	26.8 ms + 0.02 ms/year	1.8 ms

How To Use the ERG Normative Data

[3] ERG amplitudes for bipolar electrodes, such as the Burian-Allen bipolar electrode are 0.79 (±0.03) times those of a monopolar electrode, such as the ERG-Jet

Appendix 1

ERG amplitudes decrease with age, while some implicit times increase with age. Consequently, some of the normative data provided are expressed as a value plus or minus a change per year of age. ERG amplitudes also depend on the type of electrode (monopolar or bipolar) that is used. Make sure to use values from the table that is appropriate for the electrodes used. For example, to calculate the mean value of b-wave amplitude for the 0 dB scotopic flash response of a 67 year old patient using an ERG-Jet electrode:

$$Mean = 644\ \mu V - \left(\frac{1.6\ \mu V}{year} \times 67\ years \right)$$

ERG amplitudes and implicit times will vary among individuals and all are approximately normally distributed. Accordingly, 95% of all normal values will fall below 1.65 standard deviations from the mean. For the 67-year-old patient above, the 0 dB scotopic flash response would have a 5% chance of being normal if it were below 537 - (1.65 x 117) or 344 µV. To determine cutoff probabilities other than 5%, use the table below to select the appropriate multiplier.

Probability	Multiplier
2.5 %	1.96
5 %	1.65
10 %	1.28

Notes:

- These normative data are valid for subjects between the ages of 20 and 80. Extrapolations to younger or older ages should be made with care.

- Oscillatory Potential amplitudes were determined using the *Analyze Oscillatory Potentials* function of LKC's software package. They were *not* determined using the analog filtered waveform.

- The 30 Hz flicker amplitude and implicit time were determined using the *Flicker Amplitude and Timing* function of LKC's software package. They were *not* determined by placing cursors on the waveform.

The data reported here were collected under a grant to LKC from the National Eye Institute. ERG data was collected from one eye of 122 subjects with ophthalmoscopically normal fundi and no history of diabetes. Their ages ranged from 22 to 79, with approximately equal representation in each decade. Cursors were placed

on waveforms by experienced technicians. Linear regression was used to determine the age-dependence of each parameter.
Normative data for other ERG parameters of interest was measured with a monopolar electrode.

To determine the normal value for any age, add the constant value to the product of the patient's age and the change per year (if any). For bipolar electrodes, divide amplitude values by 1.26. The 95% limits of normal are the mean ± 2 S.D.

Stimulus and Parameter	Normal Value (Linear Regression)	S.D.	Distribution Shape
0 dB Scotopic Flash a-wave amplitude	387 µV - 2.1 µV/year	70 µV	Normal
0 dB Scotopic Flash a-wave implicit time	21.2 ms + 0.03 ms/year	1.0 ms	Unknown
30 Hz Scotopic Flicker amplitude	126 µv - 0.3 µv/year	30 µV	Normal
30 Hz Scotopic Flicker implicit time	27.5 ms +0.06 ms/year	2.2 ms	Unknown
Naka-Rushton *log* **K**	-2.68 + 0.006/year	0.20	Lognormal*
Naka-Rushton $\mathbf{R}_{max}$	558 µV - 0.83 µV/year	113 µV	Normal

* Data are lognormal after addition of a constant.

Appendix 1

Limits of Normal for the Clinical Electroretinogram

95% Limits of normal as a function of age for the most commonly measured parameters of the International Standard (ISCEV) protocol using **monopolar** electrodes

-24 dB Scotopic Flash b-wave Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	185	163	141	119	97	75

0 dB Scotopic Flash b-wave Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	419	403	387	371	355	339

0 dB Scotopic Flash b-wave Implicit Time should be **below**:

Age	20	30	40	50	60	70
Time (ms)	52	52	52	52	52	52

Oscillatory Potential Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	110	89	75	61	50	50

0 dB Photopic Flash b-wave Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	102	92	82	72	62	52

0 dB Photopic Flash b-wave Implicit Time should be **below**:

Age	20	30	40	50	60	70
Time (ms)	32	32	32	32	32	32

30 Hz Photopic Flicker Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	70	63	56	49	42	35

30 Hz Photopic Flicker Implicit Time should be **below**:

Age	20	30	40	50	60	70
Time (ms)	30	30	30.5	30.5	31	31

Appendix 1

Limits of Normal for the Clinical Electroretinogram

95% Limits of normal as a function of age for the most commonly measured parameters of the International Standard (ISCEV) protocol using **bipolar** electrodes

-24 dB Scotopic Flash b-wave Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	146	129	111	94	77	59

0 dB Scotopic Flash b-wave Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	331	318	306	293	280	268

0 dB Scotopic Flash b-wave Implicit Time should be **below**:

Age	20	30	40	50	60	70
Time (ms)	52	52	52	52	52	52

Oscillatory Potential Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	87	70	59	48	40	40

0 dB Photopic Flash b-wave Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	81	73	65	57	49	41

0 dB Photopic Flash b-wave Implicit Time should be **below**:

Age	20	30	40	50	60	70
Time (ms)	32	32	32	32	32	32

30 Hz Photopic Flicker Amplitude should be **above**:

Age	20	30	40	50	60	70
Amplitude (µV)	56	50	44	39	33	28

30 Hz Photopic Flicker Implicit Time should be **below**:

Age	20	30	40	50	60	70
Time (ms)	30	30	30.5	30.5	31	31

Appendix 1

Normative data for ERG b/a amplitude ratio

Methods: Data was collected from the right eye of 110 normal subjects aged 22 to 79. There was approximately the same number of subjects in each decade. ERG responses were measured from the "maximal response" step (0 dB scotopic flash) of the ISCEV standard ERG protocol. Cursors were placed on the waveform by experienced technicians. A-wave amplitude was measured from a quiet spot on the baseline to the trough of the a-wave. B-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave.

B-wave/A-wave amplitude ratios were computed and the change with age was determined by linear regression.

Results: B-wave/A-wave amplitude ratio changed significantly with the age of the patient ($p = 0.0011$, t-test); however, there is a large standard deviation which results in a low correlation coefficient ($R^2 = 0.09$). Linear regression of B-wave/A-wave amplitude ratio results in the following relationship:

$$Mean\frac{b}{a} = 1.64 + \left[.0095 \times Age(years)\right]$$

Limits of Normal for Visually Evoked Potentials

95% Limits of Normal for P_{100} Latency of the Pattern Visually Evoked Potential

Checks on Screen	Check Size (minutes of arc)	Upper Limit of Normal (ms)
4 x 4	199'	119
8 x 8	100'	110
16 x 16	50'	109
32 x 32	25'	118
64 x 64	12'	123
128 x 128	6'	141

Check sizes are given for the recommended viewing distance of 1.0 meter

Limits of Normal for Features of the Flash Visually Evoked Potential

Feature	Lower Limit	Upper Limit
N_{40}	36	60
P_{71}	50	90
N_{91}	70	120

P_{114}	95	155
N_{154}	115	200

Note: Amplitudes of the pattern and flash Visually Evoked Potential are highly variable and are seldom of clinical interest.

Appendix 2: Recommended Literature and Website

Website:

ISCEV (International Society for Clinical Electrophysiology of Vision)
Website: https://iscev.wildapricot.org/

Books:

Fishman, GA, Birch, DG, Holder, GE and Brigell, MG (2001) Ophthalmology Monograph 02: Electrophysiologic testing in disorders of the retina, optic nerve, and visual pathway. American Academy of Ophthalmology, 2nd Edition 2001.

Principles and Practice of Clinical Electrophysiology of Vision, Second Edition
Edited by John R. Heckenlively and Geoffrey B. Arden
Steven Nusinowitz, Graham E. Holder and Michael Bach, Associate Editors

Appendix 3: Standard Protocols

The following pages contain the software settings for each of the LKC standard protocols that come with the UTAS system. Some of the protocols are repeated for different stimulators (e.g., the Standard ERG protocol appears for the Grass Flash and Kurbisfeld stimulators).

The protocols are organized by test: ERG protocols, VER protocols, and EOG protocols.

2008 STANDARD ERG PROTOCOL

Page 1

Protocol in C:\EMWin\Standard Protocols\stdergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1	2	3	4	5	6	7
High Pass Channel 1 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 2 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 3 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 4 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 5 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 6 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Pass Channel 1 (Hz):	300	300	300	300	300	300	300
Low Pass Channel 2 (Hz):	300	300	300	300	300	300	300
Low Pass Channel 3 (Hz):	300	300	300	300	300	300	300
Low Pass Channel 4 (Hz):	300	300	300	300	300	300	300
Low Pass Channel 5 (Hz):	300	300	300	300	300	300	300
Low Pass Channel 6 (Hz):	300	300	300	300	300	300	300
Notch Filters:	OFF	OFF	OFF	OFF	OFF	OFF	OFF
Stimulator:	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld
Flash Intensity:	-24 dB	-24 dB	0 dB	0 dB	0 dB	0 dB	0 dB
LED/ XENON:	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED
Color X:							
Y:							
Single Flash / Flicker:	Flicker	Flash	Flash	Flash	Flicker	Flash	Flicker
Flicker Rate (Hz):	10.0				10.0		30.3
Pre-Adapt (sec):	10				10		3
Background Light:	OFF	OFF	OFF	OFF	30cd/mm	30cd/mm	30cd/mm
White/Color/ Amber:					UV LED	UV LED	UV LED
Color X:							
Y:							
Fixation Light:	OFF	Dim	Dim	Dim	OFF	Dim	Dim
Number to Average:	1	1	1	1	1	1	10
Time Between Sweeps(sec):	0.100	0.100	0.100	0.100	0.100	0.100	0.033
Sweeps Before Update:	0	0	0	0	0	0	0
Artifact Reject (uv):	800	800	2000	2000	800	800	500
Display Raw Waveform:	YES	YES	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512	512	512
Pre-Stim Baseline (msec):	20	20	20	20	20	20	0

Appendix 3

ERG EXTENDED PROTOCOL

Protocol in C:\EMWin\Standard Protocols\ISCEVextended2011.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1	2	3	4	5	6	7	8
High Pass Channel 1 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 2 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 3 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 4 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 5 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 6 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Pass Channel 1 (Hz):	500	500	500	500	500	500	500	500
Low Pass Channel 2 (Hz):	500	500	500	500	500	500	500	500
Low Pass Channel 3 (Hz):	500	500	500	500	500	500	500	500
Low Pass Channel 4 (Hz):	500	500	500	500	500	500	500	500
Low Pass Channel 5 (Hz):	500	500	500	500	500	500	500	500
Low Pass Channel 6 (Hz):	500	500	500	500	500	500	500	500
Notch Filters:	ON	ON	ON	ON	ON	ON	ON	ON
Stimulator:	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld
Flash Intensity:	-24 dB	-24 dB	1 dB	1 dB	6 dB	1 dB	1 dB	1 dB
LED/ XENON:	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED
Color X:								
Y:								
Single Flash / Flicker:	Flicker	Flash	Flash	Flash	Flash	Flicker	Flash	Flicker
Flicker Rate (Hz):	10.0					10.0		30.3
Pre-Adapt (sec):	10					10		3
Background Light:	OFF	OFF	OFF	OFF	OFF	30cd/mm	30cd/mm	30cd/mm
White/Color/ Amber:						UV LED	UV LED	UV LED
Color X:								
Y:								
Fixation Light:	OFF	Dim	Dim	Dim	Dim	OFF	Dim	Dim
Number to Average:	1	1	1	1	1	1	1	10
Time Between Sweeps(sec):	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.033
Sweeps Before Update:	0	0	0	0	0	0	0	0
Artifact Reject (uv):	800	800	2000	2000	2000	800	800	500
Display Raw Waveform:	YES	YES	YES	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512	512	512	512
Pre-Stim Baseline (msec):	20	20	20	20	20	20	20	0

Appendix 3

CLASSIC ERG PROTOCOL

Protocol in C:\EMWin\Standard Protocols\clasergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1	2	3	4	5	6	7
High Pass Channel 1 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 2 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 3 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 4 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 5 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 6 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Pass Channel 1 (Hz):	500	500	500	500	500	500	500
Low Pass Channel 2 (Hz):	500	500	500	500	500	500	500
Low Pass Channel 3 (Hz):	500	500	500	500	500	500	500
Low Pass Channel 4 (Hz):	500	500	500	500	500	500	500
Low Pass Channel 5 (Hz):	500	500	500	500	500	500	500
Low Pass Channel 6 (Hz):	500	500	500	500	500	500	500
Notch Filters:	ON	ON	ON	ON	ON	ON	ON
Stimulator:	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld
Flash Intensity:	-34 dB	-34 dB	-8 dB	0 dB	0 dB	0 dB	0 dB
LED/ XENON:	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED
Color X:							
Y:							
Single Flash / Flicker:	Flicker	Flash	Flash	Flash	Flicker	Flash	Flicker
Flicker Rate (Hz):	10.0				10.0		30.3
Pre-Adapt (sec):	10				10		3
Background Light:	OFF	OFF	OFF	OFF	30cd/mm	30cd/mm	30cd/mm
White/Color/ Amber:					UV LED	UV LED	UV LED
Color X:							
Y:							
Fixation Light:	OFF	Dim	Dim	Dim	OFF	Dim	Dim
Number to Average:	1	1	1	1	1	1	10
Time Between Sweeps(sec):	0.100	0.100	0.100	0.100	0.100	0.100	0.033
Sweeps Before Update:	0	0	0	0	0	0	0
Artifact Reject (uv):	800	800	1000	2000	800	800	500
Display Raw Waveform:	YES	YES	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512	512	512
Pre-Stim Baseline (msec):	20	20	20	20	20	20	0

Appendix 3

FLICKER ERG PROTOCOL

Protocol in C:\EMWin\Standard Protocols\flickergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1	2	3	4	5	6	7	8
High Pass Channel 1 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 2 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 3 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 4 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 5 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Pass Channel 6 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Pass Channel 1 (Hz):	300	300	300	300	300	300	300	300
Low Pass Channel 2 (Hz):	300	300	300	300	300	300	300	300
Low Pass Channel 3 (Hz):	300	300	300	300	300	300	300	300
Low Pass Channel 4 (Hz):	300	300	300	300	300	300	300	300
Low Pass Channel 5 (Hz):	300	300	300	300	300	300	300	300
Low Pass Channel 6 (Hz):	300	300	300	300	300	300	300	300
Notch Filters:	OFF	OFF	OFF	OFF	OFF	OFF	OFF	OFF
Stimulator:	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld
Flash Intensity:	0 dB	0 dB	0 dB	0 dB	0 dB	0 dB	0 dB	0 dB
LED/ XENON:	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED
Color X:								
Y:								
Single Flash / Flicker:	Flicker	Flicker	Flicker	Flicker	Flicker	Flicker	Flicker	Flicker
Flicker Rate (Hz):	5.0	10.0	14.9	20.0	25.0	30.3	34.5	40.0
Pre-Adapt (sec):	3	3	3	3	3	3	3	3
Background Light:	30cd/mm	30cd/mm	30cd/mm	30cd/mm	30cd/mm	30cd/mm	30cd/mm	30cd/mm
White/Color/ Amber:	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED	UV LED
Color X:								
Y:								
Fixation Light:	Dim	Dim	Dim	Dim	Dim	Dim	Dim	Dim
Number to Average:	10	10	10	10	10	10	10	10
Time Between Sweeps(sec):	0.200	0.100	0.067	0.050	0.040	0.033	0.029	0.025
Sweeps Before Update:	0	0	0	0	0	0	0	0
Artifact Reject (uv):	500	500	500	500	500	500	500	500
Display Raw Waveform:	YES	YES	YES	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512	512	512	512
Pre-Stim Baseline (msec):	0	0	0	0	0	0	0	0

Appendix 3

PATTERN ERG PROTOCOL

Protocol in C:\EMWin\Standard Protocols\patergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1
High Pass Channel 1 (Hz):	0.3
High Pass Channel 2 (Hz):	0.3
High Pass Channel 3 (Hz):	0.3
High Pass Channel 4 (Hz):	0.3
High Pass Channel 5 (Hz):	0.3
High Pass Channel 6 (Hz):	0.3
Low Pass Channel 1 (Hz):	300
Low Pass Channel 2 (Hz):	300
Low Pass Channel 3 (Hz):	300
Low Pass Channel 4 (Hz):	300
Low Pass Channel 5 (Hz):	300
Low Pass Channel 6 (Hz):	300
Notch Filters:	OFF
Stimulator:	Pattern
Style:	Checks
Horizontal:	32
Vertical:	32
Color #1:	Black
Color #2:	White
Position:	Full
Contrast(%):	100
Alternation Type:	Alternating
Alternation Rate(Hz):	4
On/Off Time:	1:1
Number to Average:	100
Time Between Sweeps(sec):	0.266
Sweeps Before Update:	5
Artifact Reject (uv):	250
Display Raw Waveform:	NO
Store Individual Sweeps:	NO
Rejection of Clipped Data:	Unchecked
Sample Rate (Hz):	2000
Number of Sample:	512
Pre-Stim Baseline (msec):	0

Appendix 3

BRIGHT FLASH ERG PROTOCOL

Protocol in C:\EMWin\Standard Protocols\bergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1
High Pass Channel 1 (Hz):	0.3
High Pass Channel 2 (Hz):	0.3
High Pass Channel 3 (Hz):	0.3
High Pass Channel 4 (Hz):	0.3
High Pass Channel 5 (Hz):	0.3
High Pass Channel 6 (Hz):	0.3
Low Pass Channel 1 (Hz):	300
Low Pass Channel 2 (Hz):	300
Low Pass Channel 3 (Hz):	300
Low Pass Channel 4 (Hz):	300
Low Pass Channel 5 (Hz):	300
Low Pass Channel 6 (Hz):	300
Notch Filters:	OFF
Stimulator:	Ganzfeld
Flash Intensity:	25 dB
LED/ XENON:	XENON
Color X:	
Y:	
Single Flash / Flicker:	Flash
Flicker Rate (Hz):	
Pre-Adapt (sec):	
Background Light:	30cd/mm
White/Color/ Amber:	UV LED
Color X:	
Y:	
Fixation Light:	Dim
Number to Average:	10
Time Between Sweeps(sec):	0.356
Sweeps Before Update:	0
Artifact Reject (uv):	2000
Display Raw Waveform:	YES
Store Individual Sweeps:	NO
Rejection of Clipped Data:	Unchecked
Sample Rate (Hz):	2000
Number of Sample:	512
Pre-Stim Baseline (msec):	20

Appendix 3

DOUBLE FLASH ERG PROTOCOL

Protocol in C:\EMWin\Standard Protocols\dbergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1
High Pass Channel 1 (Hz):	0.3
High Pass Channel 2 (Hz):	0.3
High Pass Channel 3 (Hz):	0.3
High Pass Channel 4 (Hz):	0.3
High Pass Channel 5 (Hz):	0.3
High Pass Channel 6 (Hz):	0.3
Low Pass Channel 1 (Hz):	300
Low Pass Channel 2 (Hz):	300
Low Pass Channel 3 (Hz):	300
Low Pass Channel 4 (Hz):	300
Low Pass Channel 5 (Hz):	300
Low Pass Channel 6 (Hz):	300
Notch Filters:	OFF
Stimulator:	DoubleFlash
Flash Intensity #1:	25 dB
LED/ XENON:	XENON
Color X:	0.289
Y:	0.320
Flash Intensity #2:	0 dB
LED Color:	White
Color X:	
Y:	
Delay (msec):	200
Background Light:	OFF
Background Color:	
Color X:	
Y:	
Fixation Light:	Dim
Number to Average:	10
Time Between Sweeps(sec):	0.556
Sweeps Before Update:	0
Artifact Reject (uv):	1500
Display Raw Waveform:	YES
Store Individual Sweeps:	NO
Rejection of Clipped Data:	Unchecked
Sample Rate (Hz):	2000
Number of Sample:	512
Pre-Stim Baseline (msec):	20

Appendix 3

ON/OFF ERG PROTOCOL

Protocol in C:\EMWin\Standard Protocols\onoffergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1
High Pass Channel 1 (Hz):	0.3
High Pass Channel 2 (Hz):	0.3
High Pass Channel 3 (Hz):	0.3
High Pass Channel 4 (Hz):	0.3
High Pass Channel 5 (Hz):	0.3
High Pass Channel 6 (Hz):	0.3
Low Pass Channel 1 (Hz):	300
Low Pass Channel 2 (Hz):	300
Low Pass Channel 3 (Hz):	300
Low Pass Channel 4 (Hz):	300
Low Pass Channel 5 (Hz):	300
Low Pass Channel 6 (Hz):	300
Notch Filters:	OFF
Stimulator:	Ganzfeld
Single Flash/Flicker/Onoff:	On/Off
ResponseIntensity(cd/mm):	560
Background LED Color:	UV LED
Color X:	
Y:	
OnTime(ms):	200
Background Light:	160cd/mm
White/Color/ Amber:	UV LED
Color X:	
Y:	
Fixation Light:	Dim
Number to Average:	60
Time Between Sweeps(sec):	0.556
Sweeps Before Update:	0
Artifact Reject (uv):	300
Display Raw Waveform:	YES
Store Individual Sweeps:	NO
Rejection of Clipped Data:	Unchecked
Sample Rate (Hz):	2000
Number of Sample:	512
Pre-Stim Baseline (msec):	20

Appendix 3

PHOTOPIC NEGATIVE RESPONSE ERG PROTOCOL

Protocol in C:\EMWin\Standard Protocols\pnrergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1
High Pass Channel 1 (Hz):	0.3
High Pass Channel 2 (Hz):	0.3
High Pass Channel 3 (Hz):	0.3
High Pass Channel 4 (Hz):	0.3
High Pass Channel 5 (Hz):	0.3
High Pass Channel 6 (Hz):	0.3
Low Pass Channel 1 (Hz):	300
Low Pass Channel 2 (Hz):	300
Low Pass Channel 3 (Hz):	300
Low Pass Channel 4 (Hz):	300
Low Pass Channel 5 (Hz):	300
Low Pass Channel 6 (Hz):	300
Notch Filters:	OFF
Stimulator:	Ganzfeld
Flash Intensity:	0 dB
LED/ XENON:	UV LED
Color X:	
Y:	
Single Flash / Flicker:	Flash
Flicker Rate (Hz):	
Pre-Adapt (sec):	
Background Light:	10cd/mm
White/Color/ Amber:	Custom Color
Color X:	0
Y:	0
Fixation Light:	Dim
Number to Average:	10
Time Between Sweeps(sec):	0.356
Sweeps Before Update:	0
Artifact Reject (uv):	250
Display Raw Waveform:	YES
Store Individual Sweeps:	NO
Rejection of Clipped Data:	Unchecked
Sample Rate (Hz):	2000
Number of Sample:	512
Pre-Stim Baseline (msec):	20

Appendix 3

S-CONE ERG PROTOCOL

Protocol in C:\EMWin\Standard Protocols\sconergU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1
High Pass Channel 1 (Hz):	0.3
High Pass Channel 2 (Hz):	0.3
High Pass Channel 3 (Hz):	0.3
High Pass Channel 4 (Hz):	0.3
High Pass Channel 5 (Hz):	0.3
High Pass Channel 6 (Hz):	0.3
Low Pass Channel 1 (Hz):	300
Low Pass Channel 2 (Hz):	300
Low Pass Channel 3 (Hz):	300
Low Pass Channel 4 (Hz):	300
Low Pass Channel 5 (Hz):	300
Low Pass Channel 6 (Hz):	300
Notch Filters:	OFF
Stimulator:	Ganzfeld
Flash Intensity:	-10 dB
LED/ XENON:	UV LED
Color X:	
Y:	
Single Flash / Flicker:	Flash
Flicker Rate (Hz):	
Pre-Adapt (sec):	
Background Light:	200cd/mm
White/Color/ Amber:	UV LED
Color X:	
Y:	
Fixation Light:	Dim
Number to Average:	50
Time Between Sweeps(sec):	0.356
Sweeps Before Update:	0
Artifact Reject (uv):	250
Display Raw Waveform:	YES
Store Individual Sweeps:	NO
Rejection of Clipped Data:	Unchecked
Sample Rate (Hz):	2000
Number of Sample:	512
Pre-Stim Baseline (msec):	20

Appendix 3

PATTERN VEP PROTOCOL

Protocol in C:\EMWin\Standard Protocols\pvepU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1	2	3	4	5
High Pass Channel 1 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 2 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 3 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 4 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 5 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 6 (Hz):	0.3	0.3	0.3	0.3	0.3
Low Pass Channel 1 (Hz):	300	300	300	300	300
Low Pass Channel 2 (Hz):	300	300	300	300	300
Low Pass Channel 3 (Hz):	300	300	300	300	300
Low Pass Channel 4 (Hz):	300	300	300	300	300
Low Pass Channel 5 (Hz):	300	300	300	300	300
Low Pass Channel 6 (Hz):	300	300	300	300	300
Notch Filters:	OFF	OFF	OFF	OFF	OFF
Stimulator:	Pattern	Pattern	Pattern	Pattern	Pattern
Style:	Checks	Checks	Checks	Checks	Checks
Horizontal:	32	8	16	64	128
Vertical:	32	8	16	64	128
Color #1:	Black	Black	Black	Black	Black
Color #2:	White	White	White	White	White
Position:	Full	Full	Full	Full	Full
Contrast(%):	100	100	100	100	100
Alternation Type:	Alternating	Alternating	Alternating	Alternating	Alternating
Alternation Rate(Hz):	2	2	2	2	2
On/Off Time:	1:1	1:1	1:1	1:1	1:1
Number to Average:	80	80	80	80	80
Time Between Sweeps(sec):	0.500	0.500	0.500	0.500	0.500
Sweeps Before Update:	5	5	5	5	5
Artifact Reject (uv):	250	250	250	250	250
Display Raw Waveform:	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512
Pre-Stim Baseline (msec):	0	0	0	0	0

Appendix 3

PATTERN VEP ISCEV 2011

Protocol in C:\EMWin\Standard Protocols\ISCEV2011VEP.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1	2	3	4	5
High Pass Channel 1 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 2 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 3 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 4 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 5 (Hz):	0.3	0.3	0.3	0.3	0.3
High Pass Channel 6 (Hz):	0.3	0.3	0.3	0.3	0.3
Low Pass Channel 1 (Hz):	500	500	500	500	500
Low Pass Channel 2 (Hz):	500	500	500	500	500
Low Pass Channel 3 (Hz):	500	500	500	500	500
Low Pass Channel 4 (Hz):	500	500	500	500	500
Low Pass Channel 5 (Hz):	500	500	500	500	500
Low Pass Channel 6 (Hz):	500	500	500	500	500
Notch Filters:	ON	ON	ON	ON	ON
Stimulator:	Pattern	Pattern	Pattern	Pattern	Pattern
Style:	Checks	Checks	Checks	Checks	Checks
Horizontal:	32	128	8	16	64
Vertical:	32	128	8	16	64
Color #1:	Black	Black	Black	Black	Black
Color #2:	White	White	White	White	White
Position:	Full	Full	Full	Full	Full
Contrast(%):	100	100	100	100	100
Alternation Type:	Alternating	Alternating	Alternating	Alternating	Alternating
Alternation Rate(Hz):	2	2	2	2	2
On/Off Time:	1:1	1:1	1:1	1:1	1:1
Number to Average:	80	80	80	80	80
Time Between Sweeps(sec):	0.500	0.500	0.500	0.500	0.500
Sweeps Before Update:	5	5	5	5	5
Artifact Reject (uv):	250	250	250	250	250
Display Raw Waveform:	NO	NO	NO	NO	NO
Store Individual Sweeps:	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512
Pre-Stim Baseline (msec):	0	0	0	0	0

Appendix 3

ISCEV PATTERN ONSET PROTOCOL

Protocol in C:\EMWin\Standard Protocols\patternBlankVERU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1	2
High Pass Channel 1 (Hz):	0.3	0.3
High Pass Channel 2 (Hz):	0.3	0.3
High Pass Channel 3 (Hz):	0.3	0.3
High Pass Channel 4 (Hz):	0.3	0.3
High Pass Channel 5 (Hz):	0.3	0.3
High Pass Channel 6 (Hz):	0.3	0.3
Low Pass Channel 1 (Hz):	300	300
Low Pass Channel 2 (Hz):	300	300
Low Pass Channel 3 (Hz):	300	300
Low Pass Channel 4 (Hz):	300	300
Low Pass Channel 5 (Hz):	300	300
Low Pass Channel 6 (Hz):	300	300
Notch Filters:	OFF	OFF
Stimulator:	Pattern	Pattern
Style:	Checks	Checks
Horizontal:	32	128
Vertical:	32	128
Color #1:	Black	Black
Color #2:	White	White
Position:	Full	Full
Contrast(%):	100	100
Alternation Type:	Pattern Blank	Pattern Blank
Alternation Rate(Hz):	2	2
On/Off Time:	2:1	2:1
Number to Average:	80	80
Time Between Sweeps(sec):	0.602	0.602
Sweeps Before Update:	5	5
Artifact Reject (uv):	246	246
Display Raw Waveform:	YES	YES
Store Individual Sweeps:	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000
Number of Sample:	512	512
Pre-Stim Baseline (msec):	0	0

Appendix 3

FLASH VEP PROTOCOL

Protocol in C:\EMWin\Standard Protocols\fvepU.pro

LKC Technologies
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1
High Pass Channel 1 (Hz):	0.3
High Pass Channel 2 (Hz):	0.3
High Pass Channel 3 (Hz):	0.3
High Pass Channel 4 (Hz):	0.3
High Pass Channel 5 (Hz):	0.3
High Pass Channel 6 (Hz):	0.3
Low Pass Channel 1 (Hz):	500
Low Pass Channel 2 (Hz):	500
Low Pass Channel 3 (Hz):	500
Low Pass Channel 4 (Hz):	500
Low Pass Channel 5 (Hz):	500
Low Pass Channel 6 (Hz):	500
Notch Filters:	OFF
Stimulator:	Ganzfeld
Flash Intensity:	0 dB
LED/ XENON:	UV LED
Color X:	
Y:	
Single Flash / Flicker:	Flicker
Flicker Rate (Hz):	2.0
Pre-Adapt (sec):	3
Background Light:	OFF
White/Color/ Amber:	
Color X:	
Y:	
Fixation Light:	Dim
Number to Average:	80
Time Between Sweeps(sec):	0.500
Sweeps Before Update:	0
Artifact Reject (uv):	150
Display Raw Waveform:	YES
Store Individual Sweeps:	NO
Rejection of Clipped Data:	Unchecked
Sample Rate (Hz):	2000
Number of Sample:	512
Pre-Stim Baseline (msec):	0

Appendix 3

INTENSITY RESPONSE PROTOCOL

Protocol in C:\EMwin\IRergU.pro

LKC Technologies, Inc.
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1	2	3	4	5	6	7	8
Amplifier Display Scale (uv):	250	250	250	250	250	500	500	500
Amplifier Gain Settings:	X64	X64	X64	X64	X64	X64	X64	X64
Low Cut Channels 1 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 2 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 3 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 4 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Cut Channels 1 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 2 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 3 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 4 (Hz):	500	500	500	500	500	500	500	500
Notch Filters:	OFF	OFF	OFF	OFF	OFF	OFF	OFF	OFF
Stimulator:	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld
Flash Intensity:	-40 dB	-38 dB	-36 dB	-34 dB	-32 dB	-30 dB	-28 dB	-26 dB
LED/ XENON:	White LED	White LED	White LED	White LED	White LED	White LED	White LED	White LED
Color X:								
Y:								
Single Flash / Flicker:	Flash	Flash	Flash	Flash	Flash	Flash	Flash	Flash
Flicker Rate (Hz):								
Pre-Adapt (sec):								
Background Light:	OFF	OFF	OFF	OFF	OFF	OFF	OFF	OFF
Background LED:								
Color X:								
Y:								
Fixation Light:	Dim	Dim	Dim	Dim	Dim	Dim	Dim	Dim
Number to Average:	1	1	1	1	1	1	1	1
Time Between Sweeps(sec):	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Sweeps Before Update:	0	0	0	0	0	0	0	0
Artifact Reject (uv):	0	0	0	0	0	0	0	0
Display Raw Waveform:	YES	YES	YES	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512	512	512	512
Pre-Stim Baseline (msec):	20	20	20	20	20	20	20	20

Appendix 3

Protocol in C:\EMwin\IRergU.pro

LKC Technologies, Inc.
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	9	10	11	12	13	14	15	16
Amplifier Display Scale (uv):	500	500	500	500	500	500	500	500
Amplifier Gain Settings:	X64	X64	X64	X64	X64	X64	X64	X64
Low Cut Channels 1 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 2 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 3 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 4 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Cut Channels 1 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 2 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 3 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 4 (Hz):	500	500	500	500	500	500	500	500
Notch Filters:	OFF	OFF	OFF	OFF	OFF	OFF	OFF	OFF
Stimulator:	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld
Flash Intensity:	-24 dB	-22 dB	-20 dB	-18 dB	-16 dB	-14 dB	-12 dB	-10 dB
LED/ XENON:	White LED	White LED	White LED	White LED	White LED	White LED	White LED	White LED
Color X:								
Y:								
Single Flash / Flicker:	Flash	Flash	Flash	Flash	Flash	Flash	Flash	Flash
Flicker Rate (Hz):								
Pre-Adapt (sec):								
Background Light:	OFF	OFF	OFF	OFF	OFF	OFF	OFF	OFF
Background LED:								
Color X:								
Y:								
Fixation Light:	Dim	Dim	Dim	Dim	Dim	Dim	Dim	Dim
Number to Average:	1	1	1	1	1	1	1	1
Time Between Sweeps(sec):	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Sweeps Before Update:	0	0	0	0	0	0	0	0
Artifact Reject (uv):	0	0	0	0	0	0	0	0
Display Raw Waveform:	YES	YES	YES	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512	512	512	512
Pre-Stim Baseline (msec):	20	20	20	20	20	20	20	20

Appendix 3

Protocol in C:\EMwin\IRergU.pro

LKC Technologies, Inc.
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	17	18	19	20	21	22	23	24
Amplifier Display Scale (uv):	500	500	1250	1250	1250	1250	1250	1250
Amplifier Gain Settings:	X64	X64	X64	X64	X64	X64	X64	X64
Low Cut Channels 1 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 2 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 3 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 4 (Hz):	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
High Cut Channels 1 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 2 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 3 (Hz):	500	500	500	500	500	500	500	500
High Cut Channels 4 (Hz):	500	500	500	500	500	500	500	500
Notch Filters:	OFF	OFF	OFF	OFF	OFF	OFF	OFF	OFF
Stimulator:	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld
Flash Intensity:	-8 dB	-6 dB	-4 dB	-2 dB	0 dB	2 dB	4 dB	6 dB
LED/ XENON:	White LED	White LED	White LED	White LED	White LED	White LED	White LED	White LED
Color X:								
Y:								
Single Flash / Flicker:	Flash	Flash	Flash	Flash	Flash	Flash	Flash	Flash
Flicker Rate (Hz):								
Pre-Adapt (sec):								
Background Light:	OFF	OFF	OFF	OFF	OFF	OFF	OFF	OFF
Background LED:								
Color X:								
Y:								
Fixation Light:	Dim	Dim	Dim	Dim	Dim	Dim	Dim	Dim
Number to Average:	1	1	1	1	1	1	1	1
Time Between Sweeps(sec):	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Sweeps Before Update:	0	0	0	0	0	0	0	0
Artifact Reject (uv):	0	0	0	0	0	0	0	0
Display Raw Waveform:	YES	YES	YES	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512	512	512	512
Pre-Stim Baseline (msec):	20	20	20	20	20	20	20	20

Appendix 3

Protocol in C:\EMwin\IRergU.pro

LKC Technologies, Inc.
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	25	26	27	28	29
Amplifier Display Scale (uv):	1250	1250	1250	1250	1250
Amplifier Gain Settings:	X64	X64	X64	X64	X64
Low Cut Channels 1 (Hz):	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 2 (Hz):	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 3 (Hz):	0.3	0.3	0.3	0.3	0.3
Low Cut Channels 4 (Hz):	0.3	0.3	0.3	0.3	0.3
High Cut Channels 1 (Hz):	500	500	500	500	500
High Cut Channels 2 (Hz):	500	500	500	500	500
High Cut Channels 3 (Hz):	500	500	500	500	500
High Cut Channels 4 (Hz):	500	500	500	500	500
Notch Filters:	OFF	OFF	OFF	OFF	OFF
Stimulator:	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld	Ganzfeld
Flash Intensity:	8 dB	10 dB	12 dB	14 dB	16 dB
LED/ XENON:	XENON	XENON	XENON	XENON	XENON
Color X:					
Y:					
Single Flash / Flicker:	Flash	Flash	Flash	Flash	Flash
Flicker Rate (Hz):					
Pre-Adapt (sec):					
Background Light:	OFF	OFF	OFF	OFF	OFF
Background LED:					
Color X:					
Y:					
Fixation Light:	Dim	Dim	Dim	Dim	Dim
Number to Average:	1	1	1	1	1
Time Between Sweeps(sec):	0.100	0.100	0.100	0.100	0.100
Sweeps Before Update:	0	0	0	0	0
Artifact Reject (uv):	0	0	0	0	0
Display Raw Waveform:	YES	YES	YES	YES	YES
Store Individual Sweeps:	NO	NO	NO	NO	NO
Rejection of Clipped Data:	Unchecked	Unchecked	Unchecked	Unchecked	Unchecked
Sample Rate (Hz):	2000	2000	2000	2000	2000
Number of Sample:	512	512	512	512	512
Pre-Stim Baseline (msec):	20	20	20	20	20

Appendix 3

ULTRAVIOLET ERG PROTOCOL (non humans only)

Protocol in C:\EMwin\UVergU.pro

LKC Technologies, Inc.
2 Professional Drive
Gaithersburg, MD 20879
WWW.LKC.COM

Step:	1
Amplifier Display Scale (uv):	500
Amplifier Gain Settings:	x64
Low Cut Channels 1 (Hz):	0.3
Low Cut Channels 2 (Hz):	0.3
Low Cut Channels 3 (Hz):	0.3
Low Cut Channels 4 (Hz):	0.3
High Cut Channels 1 (Hz):	500
High Cut Channels 2 (Hz):	500
High Cut Channels 3 (Hz):	500
High Cut Channels 4 (Hz):	500
Notch Filters:	OFF
Stimulator:	Ganzfeld
Flash Intensity:	0 dB
LED/ XENON:	UV LED
Color X:	
Y:	
Single Flash / Flicker:	Flash
Flicker Rate (Hz):	
Pre-Adapt (sec):	
Background Light:	OFF
Background LED:	
Color X:	
Y:	
Fixation Light:	Dim
Number to Average:	1
Time Between Sweeps(sec):	0.100
Sweeps Before Update:	0
Artifact Reject (uv):	0
Display Raw Waveform:	YES
Store Individual Sweeps:	NO
Rejection of Clipped Data:	Unchecked
Sample Rate (Hz):	2000
Number of Sample:	512
Pre-Stim Baseline (msec):	20

Appendix 3

GLP/GCP Compliance Package Software

1 General Information

1.1 Symbols

The following symbols are used in this portion of the manual:

Caution! Read this section carefully.

1.2 Software License

The GLP/GCP Compliance Pack software is a copyrighted product of LKC Technologies, Inc. and may only be used under the following license agreement:

The software may be used in conjunction with an LKC UTAS system only. The purchaser of the UTAS system may make one copy of the software for convenience of use, provided the LKC copyright notice is preserved with each copy. This license specifically prohibits the use of this software on any computer that is not directly connected to an LKC Technologies, Inc. UTAS Interface Unit. The software may be used by the purchaser of the UTAS system, however, to produce reports of UTAS data using one, and only one, stand-alone computer system.

LKC warrants only that the software will conform to the specifications described in this manual. If the software is determined not to conform to any specification (a bug) LKC will take measures necessary to cause the software to conform to specification(s) (bug fix) as quickly as is possible in LKC's sole judgment. LKC does not warrant this software to be suitable for any specific business purpose nor will LKC be responsible for any incidental or consequential damages associated with its use including loss of revenue, delay of revenue opportunity or any business impact of associated testing.

2 General Overview

*2.1 What are GLP/GCP and 21 CFR 11?**

The US Food and Drug Administration (FDA) regulated industries, such as Bio-Pharmaceutical, Personal Care Products, Medical Devices and Food and Beverage, are required to document and acknowledge conditions and events during development, verification, validation, and manufacturing of products.

---

* ***LKC Technologies' provides this overview and associated Internet links for your convenience. Standards change and evolve on a regular basis so you should verify the latest requirements and not rely upon this brief overview as being complete or comprehensive.***

Most governments now require compliance with GLP and GCP (Good Laboratory and Clinical Practice, respectively) regulations for studies demonstrating the safety or efficacy of regulated medical products. These regulations require users to prevent electronic data from being improperly manipulated during the study. Internationally, the standards for Good Laboratory Practices are maintained by the Organization for Economic Cooperation and Development (OECD). An index of the relevant guidelines can be found at
http://www.oecd.org/document/63/0,2340,en_2649_34381_2346175_1_1_1_1,00.html

If you are retaining electronic records in compliance with GLP or GCP, "*system design should always provide for the retention of full audit trails to show all changes to the data without obscuring the original data. It should be possible to associate all changes to data with the persons making those changes by use of timed and dated (electronic) signatures. Reasons for change should be given.*" (***Application of the Principles of GLP to Computerized Systems, OECD, 1995)***.

If you are retaining electronic records for submission of results to FDA, you are required to comply with 21 CFR Part 11 (***Electronic Records, Electronic Signatures*****,** US Code of Federal Regulations, 1997). These regulations are very similar to the GLP/GCP requirements and define the conditions under which the FDA can assure "*electronic records, electronic signatures, and handwritten signatures executed to electronic records to be trustworthy, reliable, and generally equivalent to paper records and handwritten signatures executed on paper*."
GLP compliance is required for all non-clinical pharmacological and pharmacokinetic studies designed to test drug safety and efficacy and GCP compliance is required for similar clinical studies. Additionally, FDA mandates that all FDA-required records stored in electronic format *in place of or in addition to a paper record* must be compliant with 21CFR Part 11.
FDA's guideline for 21 CFR Part 11 stipulates requirements for creating, maintaining, approving, archiving, retrieving, printing, and transmitting electronic documentation. It provides the first definitive set of guidelines for records security. To comply with 21 CFR Part 11, pharmaceutical companies and medical device manufacturers must limit system access to authorized individuals (§11.10 (d)). In addition, they must be able to maintain audit trails for all significant activity and system events (§11.10 (e)). It is the foundation for good laboratory/clinic practice (GLP / GCP).

The provisions of 21 CFR Part 11 can be summarized as follows:

- Accurate Record Generation (§*11.10(b)*)
- Program Timeouts (§*11.10(d), §11.200(a)*)
- Audit Trails (§*11.10(e)*)
- Event Logging (§*11.10(e), §11.300(d)*)
- Input Checks (§*11.10(h)*)
- Controls for Electronic Signatures (§*11.200*)
- Password Expirations (§*11.300(b))*

LKC Technologies' EMWin GLP/GCP Compliance Pack is designed to be compliant with the provisions of 21 CFR Part 11. It features restricted user access

to the computer, verification of users' identities, device operation, and data. All the records in the GLP/GCP studies are secured so that only the assigned users can access them using LKC's software. All the operations for the study are stored to provide an audit trail, including the name of the user, access date and time, action (recording, analyzing, creating reports, printing, etc.).

2.2 Will LKC's GLP/GCP Compliance Pack Guarantee My Study's Compliance?

LKC's GLP/GCP Compliance Pack provides a series of safeguards which are necessary for LKC's proprietary EMWin software to be GLP, GCP, and 21 CFR Part 11 compliant. However, compliance with these principles requires more than just a software package. The most recent FDA guidance on application and enforcement of 21 CFR 11 (***Guidance for Industry Part 11, Electronic Records; Electronic Signatures — Scope and Application***,
See https://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm125125.pdf for a copy) states that the FDA intends to enforce provisions related to
"

- *limiting system access to authorized individuals*
- *use of operational system checks*
- *use of authority checks*
- *use of device checks*
- *determination that persons who develop, maintain, or use electronic systems have the education, training, and experience to perform their assigned tasks*
- *establishment of and adherence to written policies that hold individuals accountable for actions initiated under their electronic signatures*
- *appropriate controls over systems documentation*
- *controls for open systems corresponding to controls for closed systems bulleted above (§11.30)*
- *requirements related to electronic signatures (e.g., §§ 11.50, 11.70, 11.100, 11.200, and* 11.300)"

Clearly, many of these provisions require the establishment and enforcement of Standard Operating Procedures and are beyond the ability of any software package to provide compliance.

The GLP/GCP Compliance Pack software provides functionality that when properly implemented as part of a compliant process, will ensure that the correct testing protocols will be followed throughout the course of the study, that raw data will be preserved, that manipulated data or procedures will be appropriately documented, that all records will be properly archived, and that access to data can be audited.

LKC has developed this GLP/GCP Compliance Pack software in conjunction with a major pharmaceutical company to be used in their GLP/GCP environment. LKC believes that this system provides the functionality necessary to achieve GLP/GCP compliance when implemented as a component of a compliant process including all of the necessary business processes and controls necessary to achieve GLP/GCP compliance. LKC

cannot and does not warrant that your process will be GLP/GCP compliant and strongly recommends that you evaluate the underlying functionality of the GLP/GCP Compliance Pack software offering to ensure that the functionality provided will allow for compliance in your environment. Any claim that your process is GLP/GCP compliant is yours and yours alone based upon your analysis and resulting process implementation.

This manual often refers to the User's Manual of your UTAS system and the EMWin software package User's Manual. We recommend that you keep all of the manuals together.

3 Installing and Enabling the GLP/GCP Compliance Pack

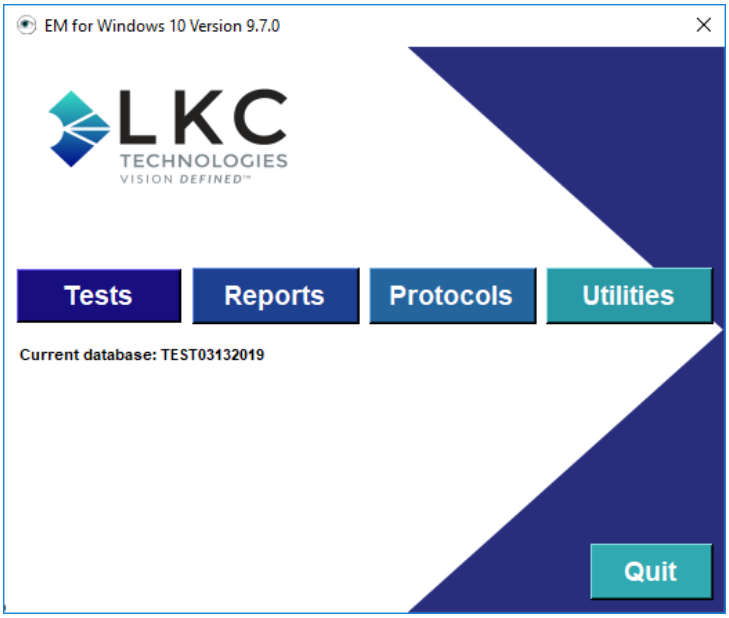
- **If you purchased the GLP/GCP Compliance Pack at the same time as your UTAS system, the software has been pre-installed and configured on your computer. You can skip this section.**
- If you purchased additional licenses of the GLP/GCP Compliance Pack for data analysis on additional computers follow the instructions to get a software key to enable GLP/GCP.

3.1 Installing additional licenses & software

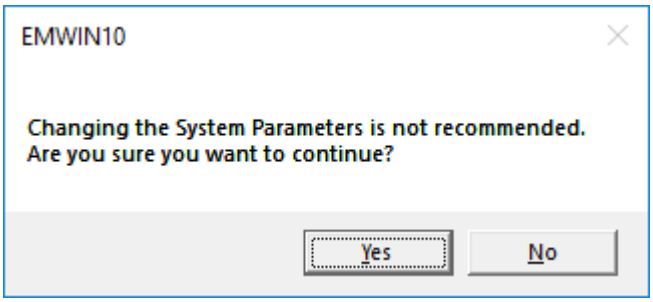
Install EMWIN on the new computer using the media provided by LKC when you purchased additional licenses for analysis, following the onscreen instructions.

3.2 Enabling GLP/GCP and acquiring the Software Key file

Open EMWin by double clicking on the EMWin software icon. The main menu of EMWin will appear.

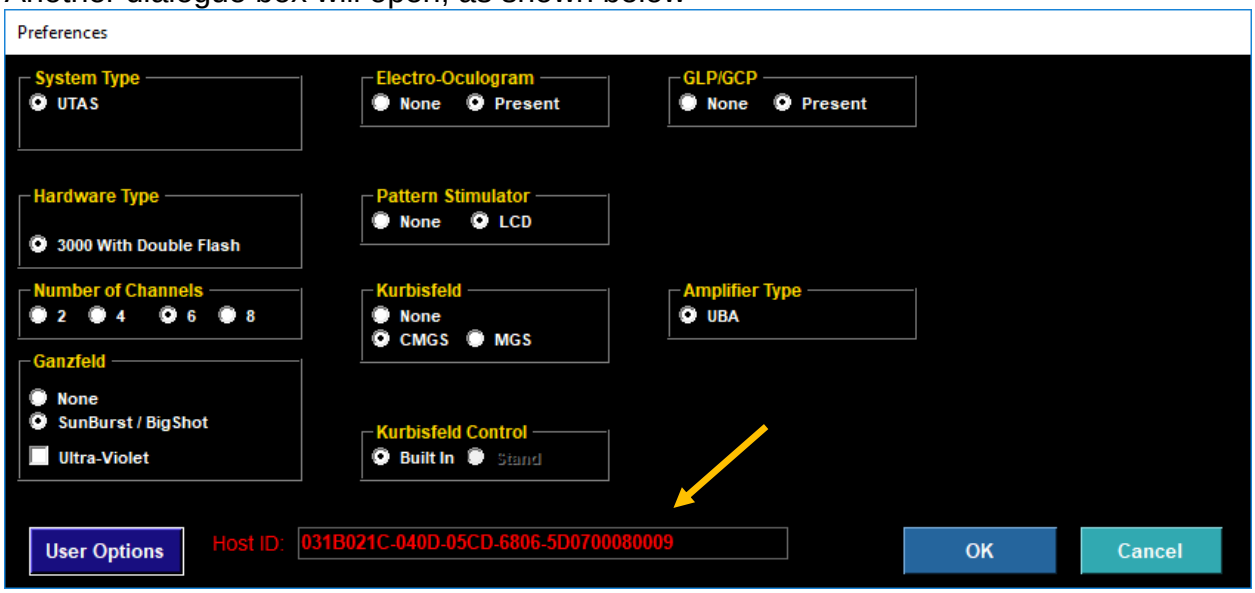


From the main menu, select *Utilities* → *System Setup*. A warning message will pop up.



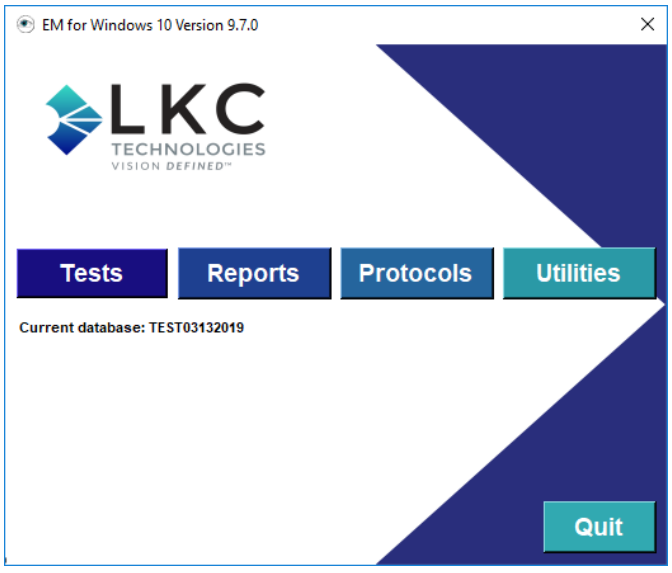
Click on *Yes*.

Another dialogue box will open, as shown below

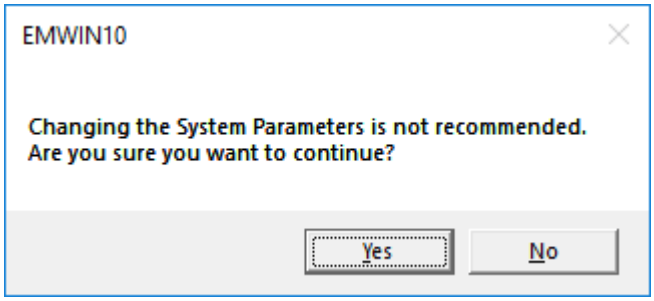


Copy the long red Host ID into an email and send to support@lkc.com along with your company name. LKC will email you back a file named GLP.KEY, copy this file to the C:\EMEin directory on the computer. Close EMWin if it is open.

Open EMWin by double clicking on the EMWin software icon. The main menu of EMWin will appear.

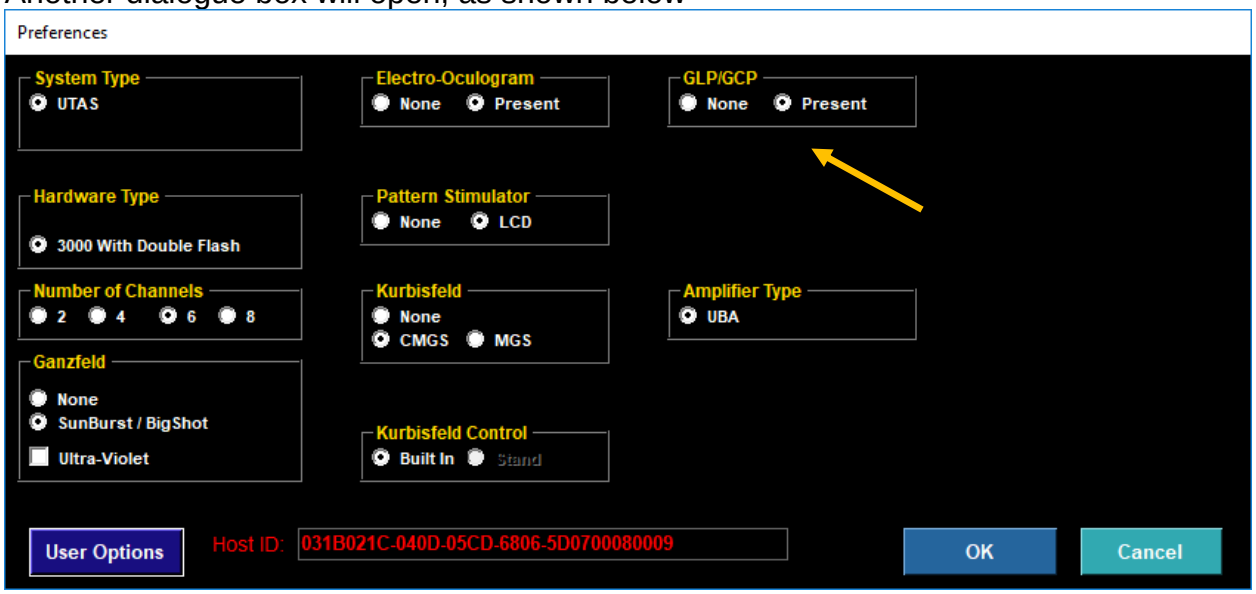


From the main menu, select *Utilities* → *System Setup*. A warning message will pop up.



Click on *Yes*.

Another dialogue box will open, as shown below



In the GLP/GCP section, change the selection from *None* to *Present*, and click the *OK* box.

EMWin software will return to the Main Menu.

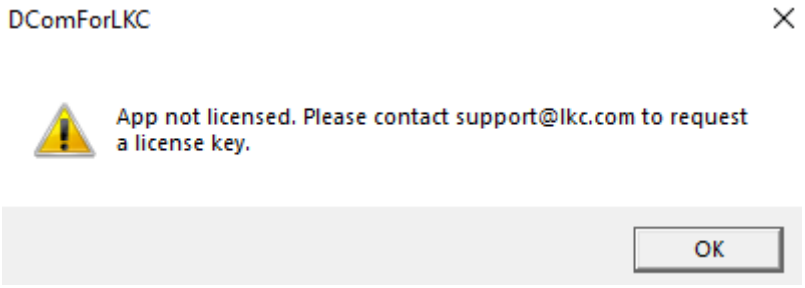
3.3 Operating System and Networking.

The database containing all of the test results and other information will reside on the computer of your UTAS system. However, it is possible for other computers to access the data over a network.4 A separate license for EMWin and the GLP/GCP Compliance Pack software must be purchased for each computer that will access the data. Contact

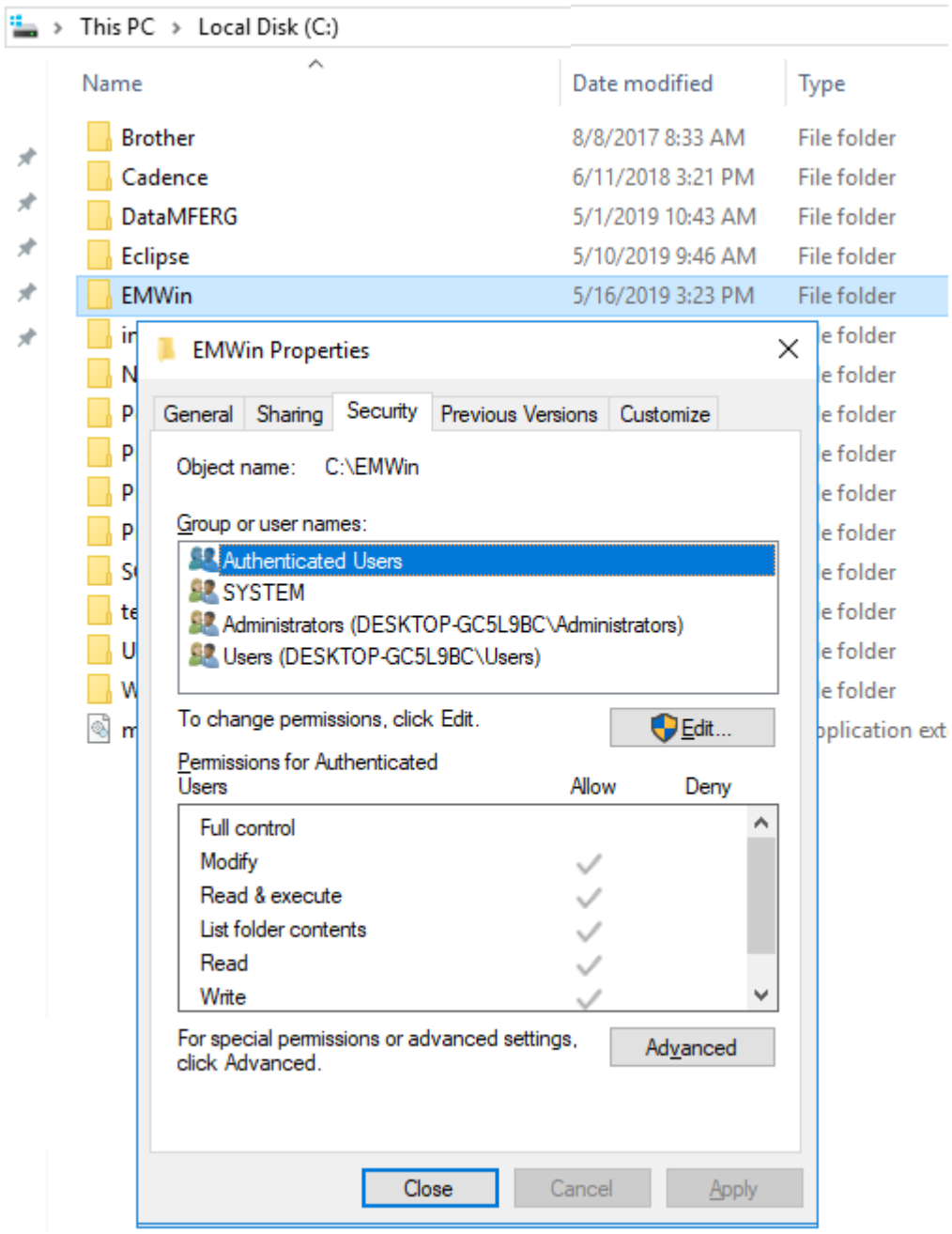
4 To maintain patient electrical safety, your UTAS system must not be connected to a wired local area network while human patients are being tested.

LKC Technologies for more information about installing and using the software on networked computers.

Software Key Prompt



To allow other users to use the EmWin and GLP/GCP compliance pack you must make the C:/EMWIN a share folder. Go to C:/EMWIN folder and right click, select Properties. An EMWIN property window will pop up (see figure 7) select *Security* and choose the users you wish to have access and give them full control.



EMWIN Folder Sharing Properties

Then create new Windows account for all the users. Log into each newly created account and reinstall EMWIN from their account.

4 Creating a GLP/GCP Database

There are 3 steps in creating a new GLP/GCP database:

1. **Define the testing protocol**
2. **Add users and define their roles and privileges**

3. Fill in information to be stored and printed (Institution, Address, report title, etc.)

The first step in the creation of a study is to create the protocol that will be used. EMWin has a number of predefined protocols and will also let you create your own User Defined Protocols (UDP). Please refer to your UTAS User's Manual for instructions on how to create a User Defined Protocol.

	Make sure that you have defined and stored the protocol that will be used for testing (or are using a standard protocol supplied with your system) before creating the GLP/GCP database
	All Users of the CLP/GCP Compliance Pack software must have a Windows account on the UTAS system's computer before they can access a GLP/GCP database. The User Name in the GLP/GCP database must match their Windows User Name.

All the GLP/GCP recorded data and related operation for each study will be stored in its own encrypted database. To create a new database, start from EMWin main menu and select: *Utilities* → *Create New Database* → *GLP/GCP Database*. Type in a name for the database and click on *Enter*. The GLP/GCP User's Role window will pop up (Figure 7).

The person creating the database is defined as the SuperUser, also known as the Administrator. The SuperUser's information (UserName, Password and Role) are automatically entered, and are those of the SuperUser's Windows account on the computer. The SuperUser can change his/her EMWin GLP/GCP Compliance Pack password by typing a new password in the *Password* field and clicking the *Modify* button.

	The UserName entered for each individual in the GLP/GCP Compliance Pack software must be the same as their Windows user name on the computer. Any user names that are not Windows users can not login to the GLP/GCP study, even their names are assigned in the study.
	Only the Administrator can create a new GLP/GCP database and assign users and their initial passwords. It is recommended that each user should change their password immediately after logging in for the first time

The SuperUser must enter the User Name for all other users and set their role. For each user, the SuperUser enters the User Name and password, selects a Role (SuperUser, Analyzer, Viewer or Disabled) and clicks on *Add.* While the User Name entered in the GLP/GCP database must match their Windows User Name, the password can be different from the user's Windows password.

The SuperUser should also determine the privileges associated with each role by clicking on the *Set GLP Options* button; the GLP/GCP option window will open (Figure 8) and the SuperUser can select the different privileges that he/she wants to assign to different roles. Privileges are enabled by checking the associated box for that user type. Once finished, click *OK.*

The first 5 GLP options are specific to GLP/GCP Compliance Pack protocols:

Channels: Allows the user to change the number of recording channels. When disabled, protocols default to two recording channels.

Step Backwards: Allows a user to go to previous step in the protocol.

Step to…: Allows the user to jump to any step in the protocol.

Save Selection: Allows the user to choose which waveforms to save. If disabled the user can only save ALL waveforms.

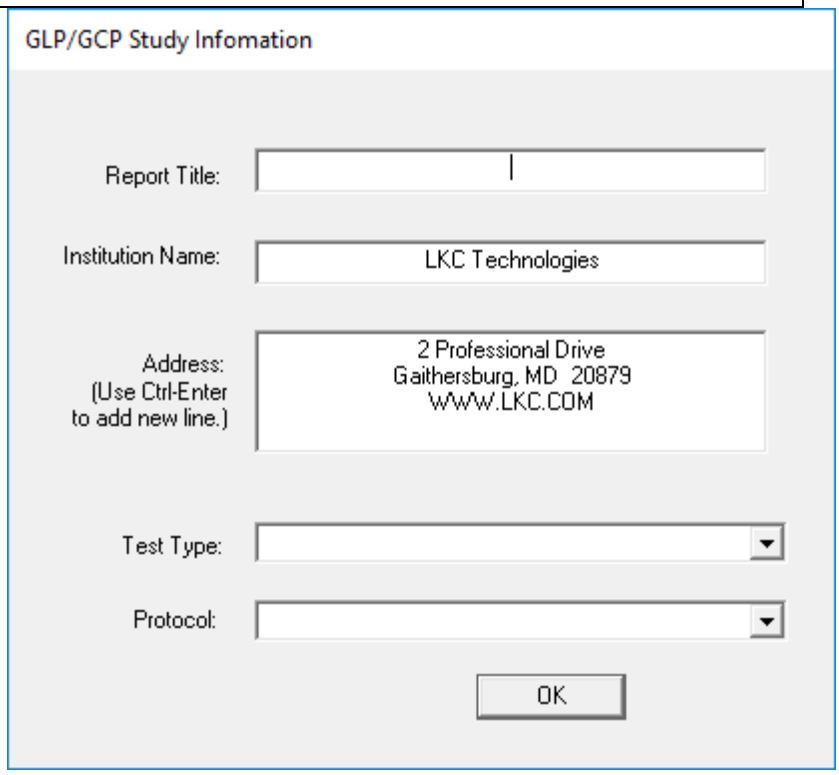
Auto Save: When enabled, data will automatically be saved after each recording and the study will move forward to the next step in the protocol.

	***The Auto Save can cause difficulties if you are using an ERG protocol with both dark adapted and light adapted steps. Once the last dark-adapted step is performed, the software will immediately***

	turn on the background light. If you need to repeat the dark-adapted step, the eye will have already become partially light adapted, making it impossible to repeat last the dark-adapted step. It is strongly recommended that you NOT enable the Auto Save function if you are using an ERG protocol with both scotopic and photopic steps.

All of the other options on the list of User Privileges are covered in the UTAS User Manual; please refer to it if needed.

Once all users have been added and their roles defined, click on *Quit.* The study information window will appear (figure to the right). From the protocol drop down menu, select the protocol that will be used during the study. All data collected in the database being created will use the selected protocol.



Study Information Screen

If you select a standard protocol provided by LKC, the report title and test type fields will be filled out automatically. The SuperUser can change these entries by typing in the corresponding field.

If you select a User Defined Protocol, the SuperUser must enter both the report title and the test type.

Once all information has been entered select the *OK* button. The entered information will be printed at the top of the page of each report. The software will then return you to the Main Menu.

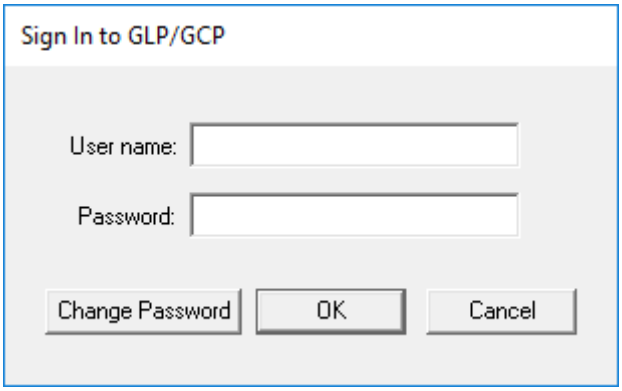
5 Selecting a Database

To use the database you just created, it must first be selected.

From Main Menu, go to *Utilities* → *Select Database* → *GLP/GCP Database,* choose the correct database from the list and click *Open*.

A sign-in window will open. Enter your User Name and your GLP/GCP Compliance Pack password and click *OK.*

The software will return you to the Main Menu.



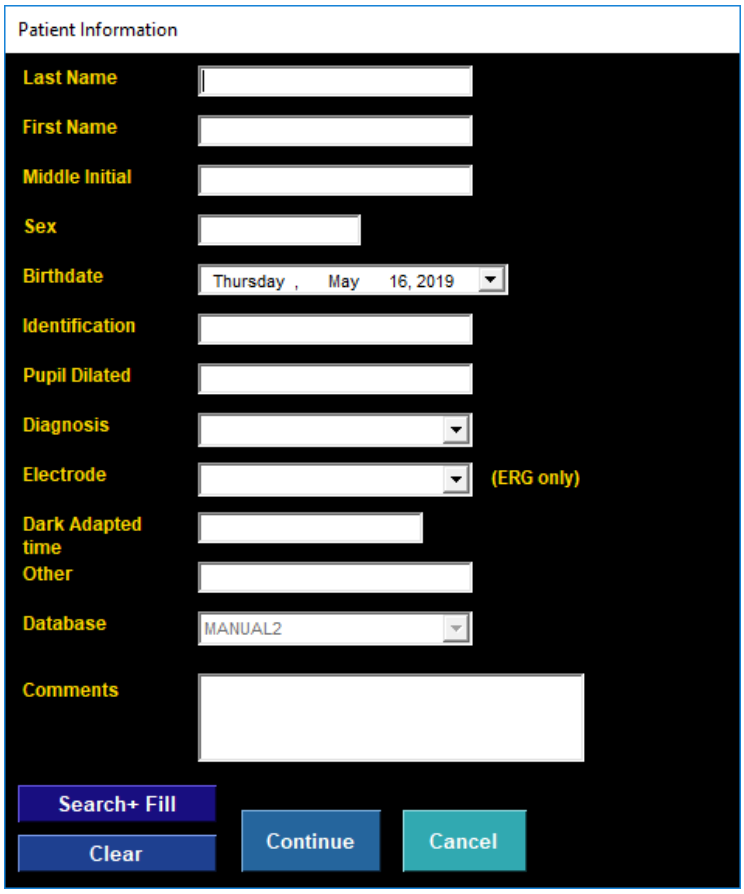
Log on Window

6 Recording data in GLP/GCP mode

From the EMWin Main Menu, you can start testing by selecting *Perform Test → GLP/GCP Study*

The patient information window will appear (Figure 11). The minimum information that must be entered in order to proceed is the information in the first 4 fields of the form. The default categories are:

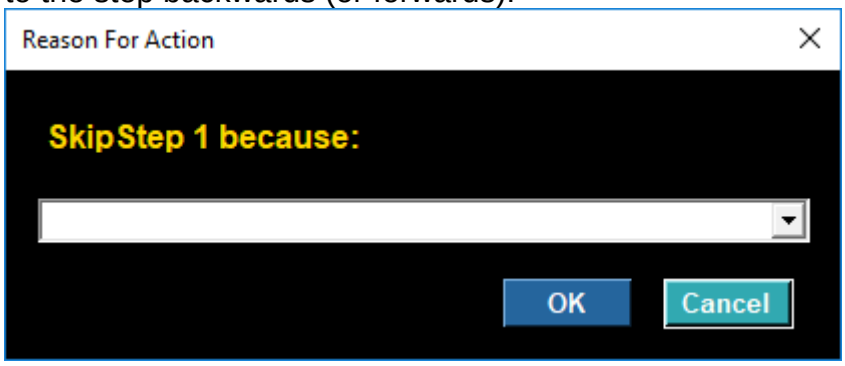
- Animal ID
- Session
- Sex
- Age



Each field has limited length accepting the characters. The first field (such as LastName or AnimalID), the second field (such as FirstName or Session), and the third field (such as MI or Species) are 30 ASCII characters (15 Unicode characters) long, meaning that user can enter the longer names up to 30 characters. For the fields of Sex and Age, the allowable length is 15 characters. Most fields accept up to 30 characters, whereas the field Other accepts up to 100 characters and Comments accepts up to 300 characters.

	***There are certain reserved characters that should not be used to fill in ANY information in the database. The reserved characters are:*** *** % ? _ # ! - [ ]**

There is one main difference between the regular EMWin software and the GLP/GCP mode. In the GLP/GCP mode each time the user saves the waveform the software will automatically go forward to the next step of the protocol. If for any reason the user needs to go back a step and record again, he/she will be asked to enter the reason for his/her action (Figure 12). The justification reasons can be preset by a text file c:\EMWin\jlist.txt. Click "OK" button to accept the typed reason or click "Cancel" button to the step backwards (or forwards).



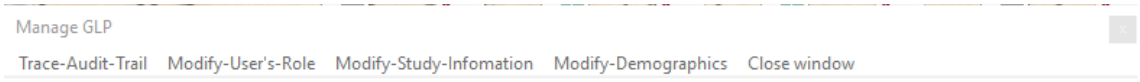
Prompt for moving back one step in the protocol

The recording procedure is similar to recording in a non-GLP/GCP database; refer to your UTAS User's Manual for details on how to collect data.

7 Managing a GLP/GCP database

Only the defined SuperUser can manage the database. In order to manage a GLP/GCP database you must first select the database (refer to Section 5). Then from the Main Menu go to
Utilities → Manage GLP/GCP information.

The managing option window will appear



Managing GLP/GCP database menu

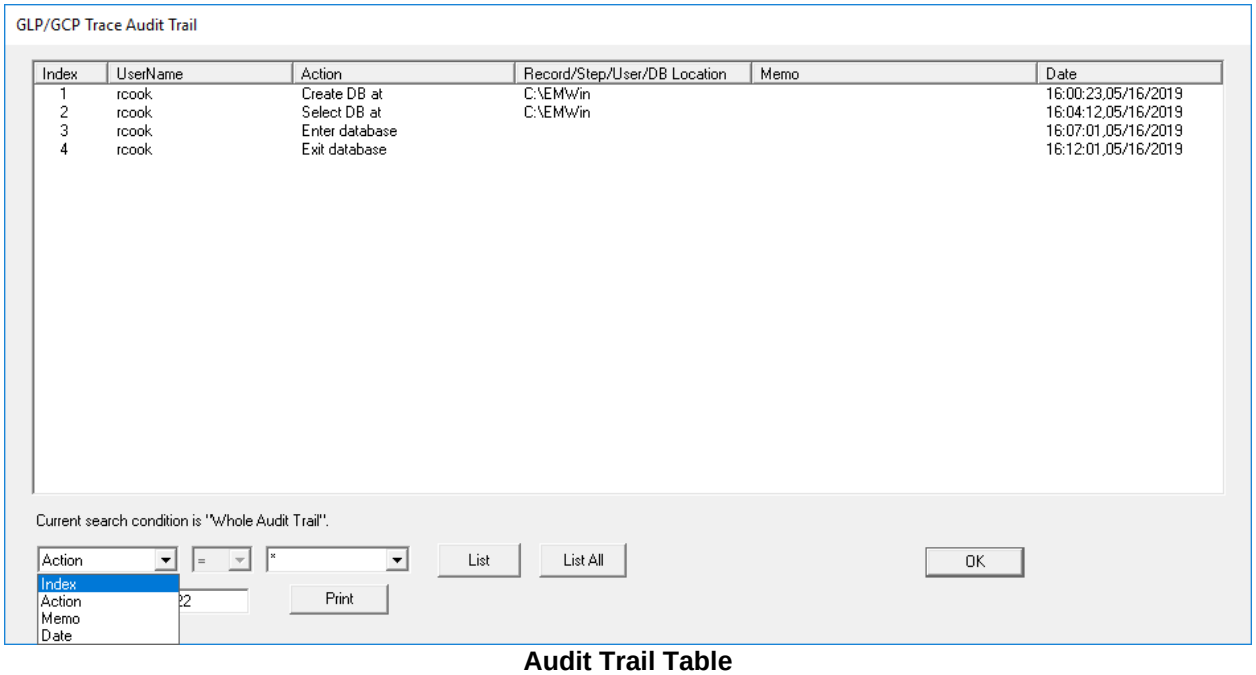
The Administrator has four possible activities: look at the audit trail, modify user roles and privileges, modify the study information and modify the demographics.

7.1 Trace Audit Trail

The GLP/GCP Audit Trail is presented as a table. Each entry in the table has an Index number, a User Name, the Action that the user performed (create, select, save, report...), information about the action and any reason that was given, and the date and time of the action.

This table can be searched by User Name, Index, Record / Step Number, Reason or Date.

The audit trail list can also be printed. The printing action itself will be stored in the audit trail.

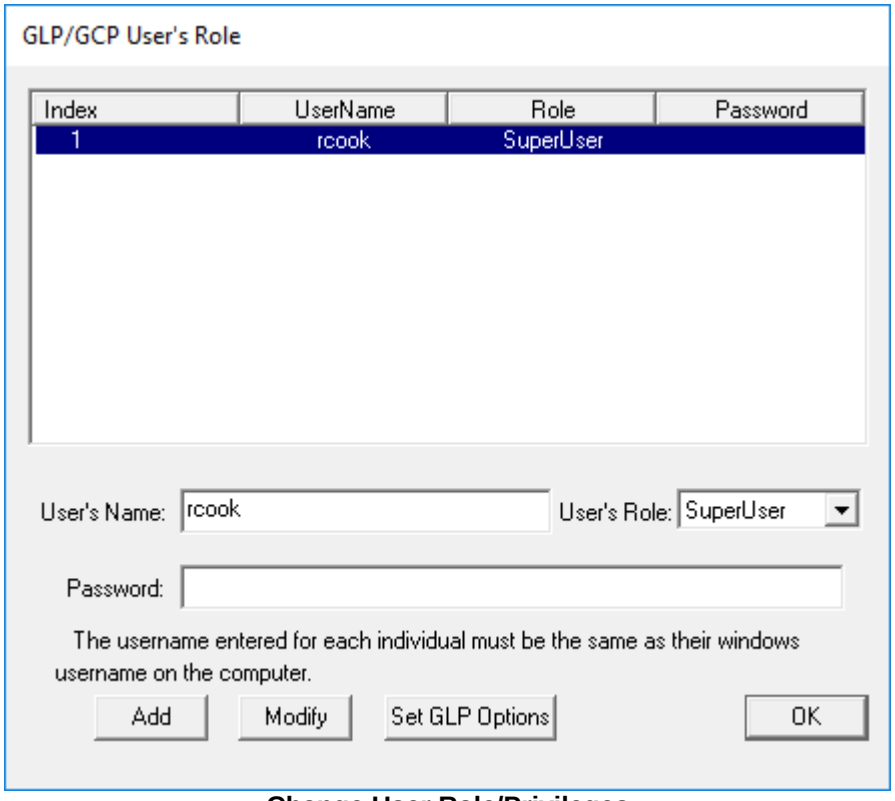


Audit Trail Table

7.2 Modify User's Role

A SuperUser can change any user's role and password from the GLP/GCP User's Role window; the privileges associated with each role can also be changed anytime during the study (refer to Section 4).

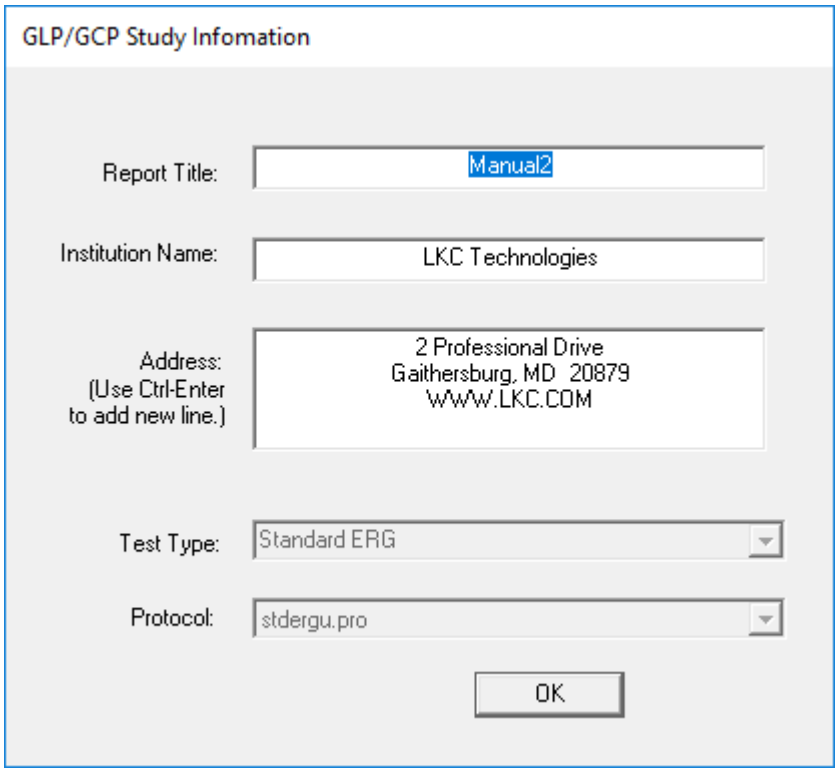
	Users cannot be deleted from the user list. If a user is to be denied access to the data, then his/her role should be set to Disabled.



Change User Role/Privileges

7.3 Modify Study Information

The SuperUser can modify the study information such as Report Title, Institution Name, and Address. The Test Type and Protocol values will be shown but cannot be changed.



Modify Study Information

7.4 Modify Demographics

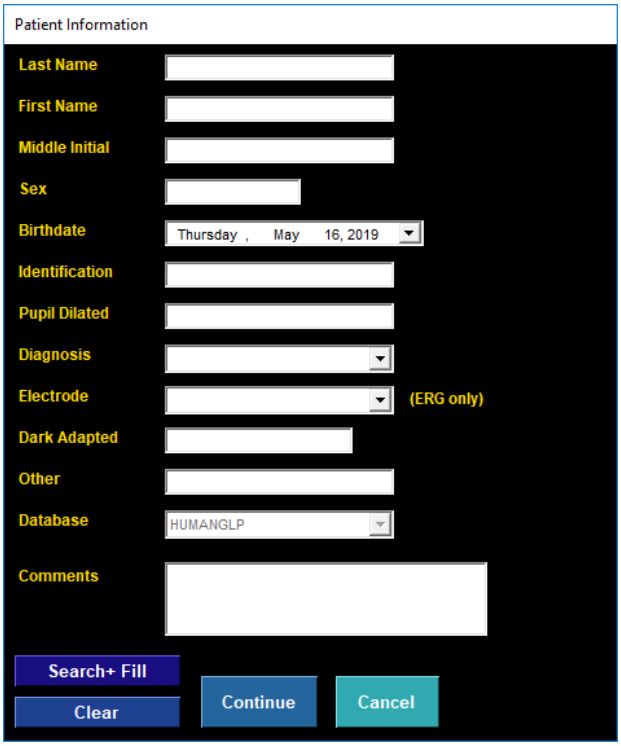
The EMWin software provides for the collection of demographic data. The default demographic data are:

- Patient Name (Last, First, MI)
- Identification
- Sex
- Birthdate
- Diagnosis
- Electrode Type
- Pupils Dilated
- Dark Adapt Time

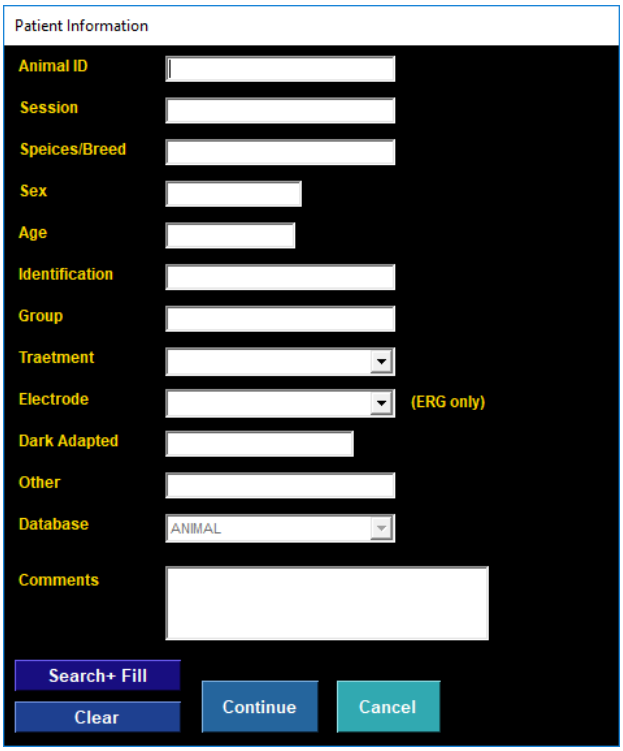
These prompts may not be appropriate for your study. You can change the names of the demographic data fields using the Modify Demographics option. The definitions are stored in a text file **C:\EMWin\temp\ Demographics.txt**, which can also be modified using a text editor.

Figure 17 shows the Demographics form that is used to rename each of the demographic data fields. In the example below, the first field in the form, which would be labeled “Last Name” in normal usage, will be used to hold Animal ID in this study.

Figure 18 shows the resulting Patient Information screen as it will appear during testing after the Demographics form has been filled out as shown below.



Modify Demographics



Patient Information Window as it appears after Demographics are filled out as in Figure 16

Changes made to the demographic information are first made in the Demographics.txt file as noted above. When the GLP/GCP database is created, this information is copied into the database, but also remains in the Demographics.txt file. Thus:

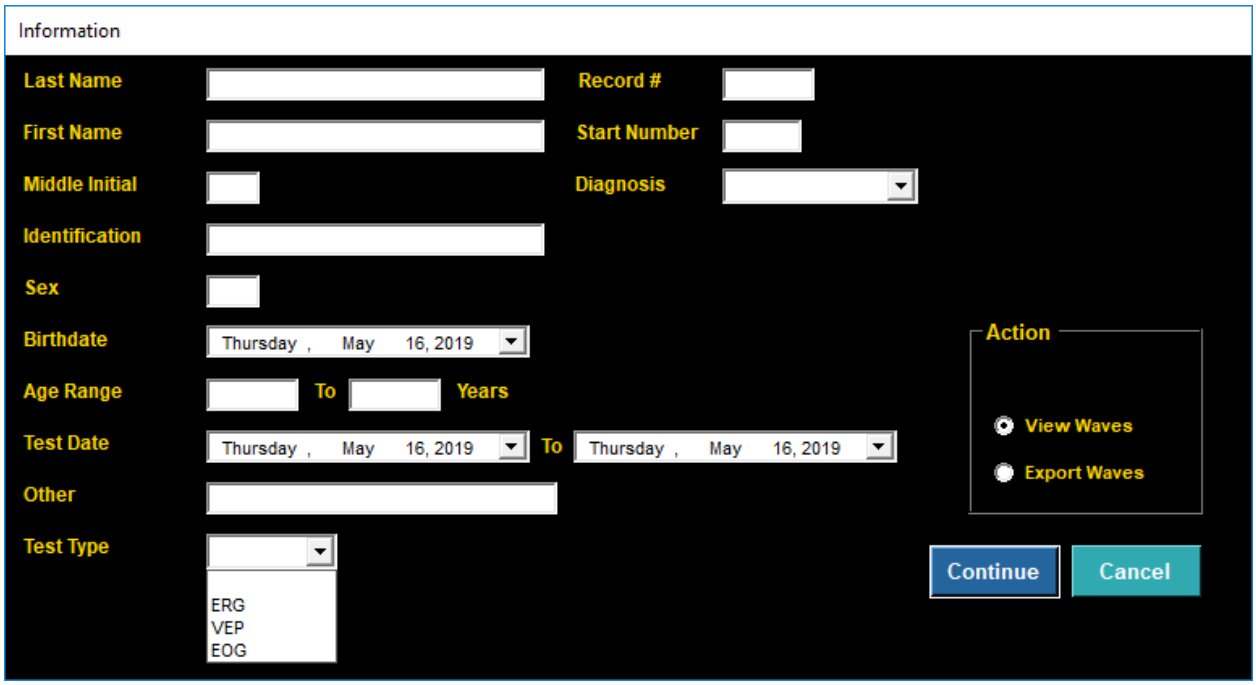
- If you want to change the demographic information in your GLP/GCP studies, you must make these changes before you create the GLP/GCP database. Items related to GLP/GCP data collection cannot be changed once they are stored in the database.
- After you create the database, the demographic information will be changed for non-GLP/GCP operation of the software until you change the demographic information back again.

The Birthdate field includes checks to make sure that a valid age or date of birth is entered. This is true even if you change the name of this field to something else. The format for date of birth may be

MM/DD/YYYY or DD/MM/YYYY (defined in System Setup). Age format is either a number, or a number followed by a letter (d, m, or y). For example, 5d indicates 5 days old, 2m indicates 2 months old, and 10y indicates 10 years old.

8 Analyzing and Reviewing Data

In order to analyze the data, the user will have to select the GLP/GCP database of interest, and log in. Then, from the Main Menu select *Reports*, You can narrow your search by entering data in the various search fields. If you leave all fields empty, all of the data stored in that database can be accessed.



Retrieving Data

The array of possible activities of the user depends on its role and associated privileges. Refer to the UTAS User Manual for more information on the different available functions (smooth, print, export data...)

9 Creating reports

Creating report in the GLP/GCP mode is exactly the same as non-GLP/GCP mode. Please refer to your UTAS User's Manual for more information on how to create reports.

	***All actions in creating reports will be recorded and stored in the audit trail.***