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The Phoenix Ganzfeld ERG

Designed for rodents using the Maxwellian view illumination technique and LED light sources

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The Company invites comments to this document

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1. Introduction

The Phoenix Ganzfeld ERG is optimized for testing the visual function of mice and rats and is not an adaptation of clinical devices. The unique Phoenix design features a stable and reliable means for achieving corneal electrodes, provides UV and Green light sourced from LEDs to access the S-cones, M-cones, and rods, and, through software, delivers pulse control in intensity, pulse length, delay, background, and flicker.

Key to the design is the use of Maxwellian view illumination, which, while selected for its convenience and small size, also has the advantages of being insensitive to the dilation diameter and assures that the animals gaze is appropriate. While the Maxwellian view approach is described in the literature perhaps its advantages have not been widely appreciated.

In this paper the Phoenix Ganzfeld ERG is described in detail and a comparison of delivered areal energy density at the retina is made between systems using the historic back illuminated bowl and the Maxwellian view illumination.

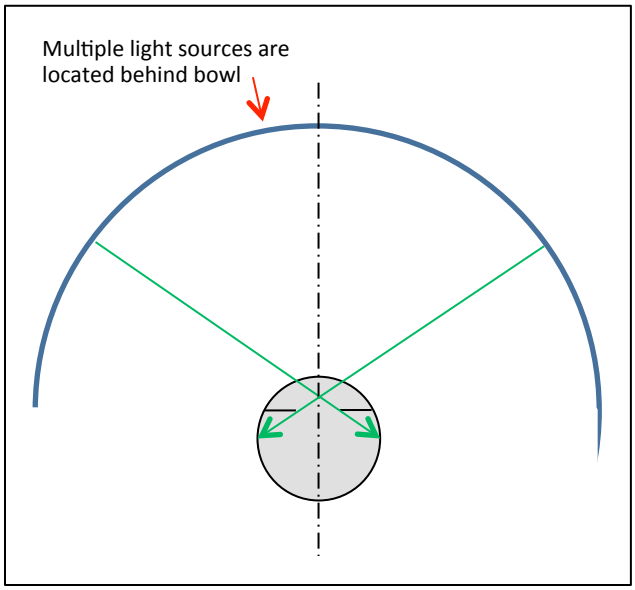
And, analysis and experiments are presented connecting the ISCEV 2008 standard for clinical application, which is based on white light illumination measured in Lumens, to the Phoenix Maxwellian view illumination, which uses narrow band green or UV LEDs or flash lamps. A schematic comparison of the historic Ganzfeld dome and Maxwellian view approaches is shown in Figure 1.

2. Requirements for studying the mouse and rat eye

The rodent eye is a useful analogue of the human eye in many respects but different in significant ways. In particular the rodent retina has a unique distribution of rods and cones and, quite importantly, the rodent eye has two not three classes of cones as does the human eye. The rodent photoreceptors include the S-cones, responsive in the ultraviolet, and the M-cones and rods, responsive in the green.

To separately study the S-cones, M-cones, and rods light at the peak of the S-cone response is provided at 360 nm and at 504 nm at the peak of the M-cones and rods. There are LEDs available at each of these wavelengths and the spectral output of both of these LEDs are displayed along with the photoreceptor spectral response in Figure 2.

LEDs make excellent sources for the Maxwellian view illumination due to their ease of use, low electrical noise, low heat dissipation, and ease of modulation making them an ideal light source. However, there may be applications where

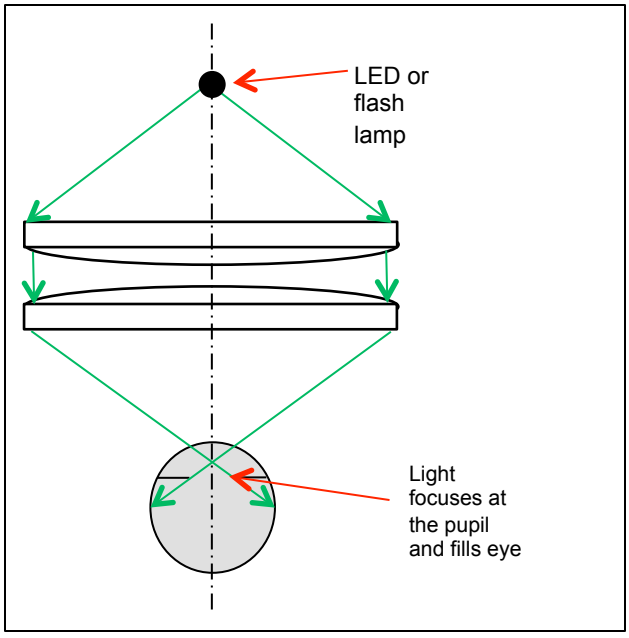


Ganzfeld ERG shown using the traditional back-lit bowl illumination

Historic back lit illuminated bowl for Ganzfeld testing (not drawn to size, bowls are typically 1 meter in diameter.)

Lumens sec/mm^2 delivered to the retina depends on animal dilation, surface brightness of bowl, and pulse length

It is difficult to determine the gaze angle of the rodent eye in a “dark lab”



Maxwellian view Ganzfeld illumination

Maxwellian view Ganzfeld illumination uses a single light source and illuminates the retina by projecting this onto the eye pupil with high divergence

Joules/m^2 at the retina depends on the power of the light source and pulse length and not dilation. Direction of gaze of animal under test is easily verified

Figure 1. Comparison of historic back illuminated bowl vs. Maxwellian view projected illumination

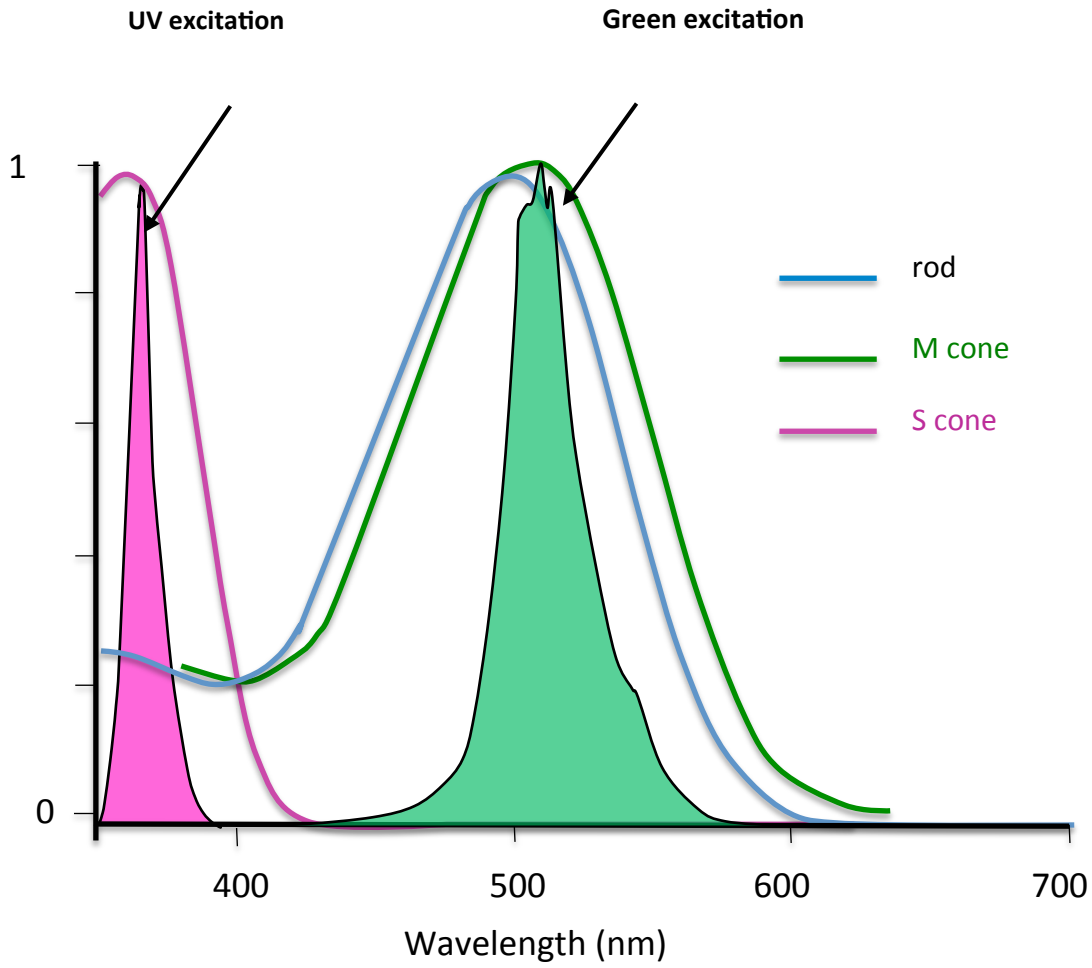


Figure 2. Spectral response of photoreceptors in the rodent eye shown with the spectral output of the LEDs used in the Phoenix Ganzfeld Maxwellian view ERG*

*Chart adapted from J. Neurosci., May 25 2011 – 31(21):7670-7681 7673

the short micro second pulse of a Xenon flash would be preferred and this is an option for the Phoenix Ganzfeld.

The second requirement is temporal and intensity control of the illumination. The Phoenix stimulation/reception control is provided by a highly integrated and low-noise microprocessor based technology and is controlled entirely by software and there are means to drive three different LEDs and control pulse length from 0.2 to 500 milliseconds, set LED power over a range of 10^6, set continuous backgrounds, set delays for any pulse, and to provide flicker over a wide range of conditions.

The third requirement is to deliver the upper and lower limits of aerial energy density delivery as specified by the ISCEV* standard and that is up to 100 Cd sec/m^2 and down to 0.01 Cd sec/m^2 as measured at the surface of the classical Ganzfeld bowl. The Phoenix Ganzfeld can set values at many levels and with a range far exceeding the ISCEV standard at both high and low values.

It is noted that the ISCEV standard is established for white light and therefore the use of photometric units. The photometric units are natural when white light is used. With these, the spectral response of the human eye is multiplied by the spectral distribution of the illumination source to determine the response of the eye irrespective of the detailed nature of the light source. And, the standard refers to the Brightness (lumens/steradian/m^2) (at the surface of the bowl) times the pulse length; this in units of Cd sec/m^2.

As noted the spectral response of the mouse eye is different from the human, and again, the objective is to separately test the M and S cones and rods using monochromatic light of particular wavelengths. In this instance it is important to look at the situation when the light is either narrow band green or UV.

The geometry of the illumination systems as related to this calculation is shown in Figure 3. The bowl is back illuminated and the brightness of the surface measured in Luminance (Lumens/steradian/m^2) is defined as **B**.

*Doc Ophthalmol (2009) 118:69–77
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ISCEV Standard for full-field clinical electroretinography
(2008 update)
M. F. Marmor, A. B. Fulton, G. E. Holder, Y. Miyake, M. Brigell,
M. Bach (for the International Society for Clinical Electrophysiology of Vision)

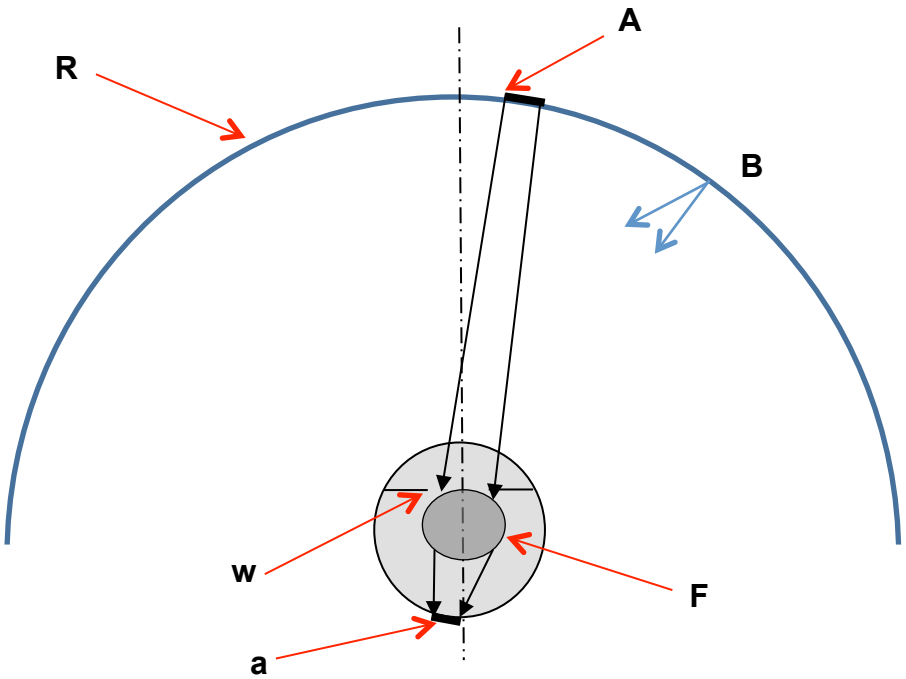


Figure 3. Calculating the delivered aerial energy density for the Ganzfeld bowl

If the pupil diameter is defined as **w**, the focal length of the eye as **F**, the bowl radius **R,** then the total light energy **E** collected by the pupil for a small area **A** at the bowl surface is

$$E = B\ A\ t\ (w / R)^2 \qquad (1)$$

This energy from this small spot **A** is projected onto an area on the retina **a** given by

$$a = A\ (F/R)^2 \qquad (2)$$

Combining (1) and (2) leads to the energy density on the retina per flash defined as **e** of

$$e = B\ t / f^2 \qquad (3)$$

Where **f** is the **f** number of the eye defined by

$$f = (F/ w) \qquad (4)$$

The final equation

$$e = B\ t / f^2 \qquad (5)$$

highlights the role that the eye **f** number plays in determining the Lumens sec/m^2 at the retina. Ultimately, the aerial energy density is the quantity of interest, not bowl brightness. Typical dilation for humans is 6 mm and the effective f number is 3 leading to an f^2 equal to 9. However, for the mouse the typical dilation is 1.2 mm for an effective f number of f = 1.5 giving an f^2 of 2.25. Accordingly, to deliver the same Lumens sec/m^2 on the mouse eye *retina* as the human eye *retina by the bowl method* at full dilation it is necessary to reduce the classical high number of 100 Cd-sec/m^2 for humans by (2.25/9) giving 13 Cd-sec/m^2 as the appropriate flash energy for rodents.

For the comparison it is assumed that both systems are designed to test only the M-Cones/rods and that both only provide green light at 504 nm. Since it is desired to work with narrow band light the use of watts and Joules is a more natural set of units. Using the CIE Standard Luminous Efficiency Functions for photopic vision the conversion at 504 nm is 4.6 x 10^-3 watts/Lumen. For the most intense pulse in the ISCEV 2008 standard equivalent to 100 Cd sec/m^2, and converting from m^2 to mm^2, this would deliver an energy density at the retina of the rodent of

$$e = 5.1 \times 10^{-8} \text{ joules/mm}^2$$

If both approaches deliver the same aerial energy density to the retina then they will serve to precisely perform the identical ERG Ganzfeld test. For the Maxwellian view the aerial energy density is the optical power times the pulse length divided by the retinal area **K.**

$$e = P\ t / K \qquad (6)$$

As an example use **t** of 1 millisecond and power of 1 milliwatt gives for the mouse eye and energy density at the retina of

$$e = 5.6 \times 10^{-8} \text{ joules/mm}^2$$

This is above the ISCEV high standard and for a short pulse. For the rat eye, to achieve the same aerial energy density, the power times the pulse length would have to be increased by four times.

Looked at from another view the necessary LED power to equate to nominated level of effective 100 Cd sec/m^2 bowl brightness is 0.91 milliwatt for mice and 3.6 milliwatt for rats for a 1 millisecond pulse.

> **For mice a Maxwellian view Ganzfeld LED at 0.91 mw for 1 millisecond will deliver to the retina the same aerial energy density at 504 nm as the Ganzfeld bowl at 100 Cd sec/m^2 also operating at 504 nm. And, for the rat the same results will occur at a power setting of 3.6 milliwatt.**

For UV radiation at 360 nm there is no meaningful value for the CIE luminosity function. Phoenix proposes that the ultimate basic element of interest would be better identified as the number of photons/mm^2 as opposed to watts/mm^2. To this end for the same number of photons/mm^2, since the UV photons are (510/360) more energetic, the equivalent joules for the UV vs. the green be increased by 1.4 times.

That would increase the UV equivalent aerial energy density specification to

e = 7.1 x 10^-8 joules/mm^2 .

to deliver the same aerial density of photons. For a one millisecond pulse the diode would need deliver 1.3 milliwatt for mice and 5.1 milliwatt for rats.

3. Operational necessities and innovations

A complete instrument must include all means necessary to implement the study. Phoenix has in particular focused on providing innovative techniques necessary for working in a “dark laboratory”; meaning a room in which there is no significant illumination that will prevent the animal from being dark adapted. For example there must be means to align the animal and to detect the state of alignment, a technique for providing a reliable and stable corneal electrode is a major need as the contact lens or wire electrodes are hard to place, fall off in many instances, and, of course all of this has to be accomplished in a dark laboratory

The Phoenix “dark lab” technology

By “dark lab technology” it is met that the animal will remain very well dark adapted

by even the most stringent standards while performing the ERG tests. This is not well defined in the literature and opinions vary. Accordingly, in this section Phoenix will precisely specify what it means by the term “dark lab technology.”

Besides maintaining dark adaption of the rodent eye there are two operational functions that must be accomplished in a “dark” environment. One is the general operation of the equipment, placing the mice in the holder, placing the electrodes, operating the alignment controls, and so forth. The second function is to carefully align the animal to the corneal contact electrode, aiming the gaze in the best direction, and obtaining some clues as to locating the corneal electrode.

In Figure 4 the sensitivity of the mouse retina from its peak in the green and into the NIR is presented on a semi log scale. As will be described below, the Phoenix Maxwellian view Ganzfeld will illuminate the rodent eye with NIR at 850 nm during alignment. This radiation is not visible to humans or rodents and the mouse eye response is down 10^-8 down from its the peak in the green.

It is the Company’s belief that the 850 nm radiation can be used to illuminate the rodent eye without light adaptation.

However since the 850 nm radiation is *not* visible to the human eye the rodent eye is imaged by an infrared camera to give the user clues for alignment. The alternative of using night vision goggles was tested and it was concluded that these were extremely difficult to use and were not a useful approach.

Radiation at 750 nm has a response that is down nearly 10^-7 from the peak in the green for the rodent eye is750 nm is visible to the human eye. It was decided that general laboratory lighting would be provided at 750 nm but not as a laboratory flood light and not projected towards the animal eye.

It is the Company’s belief that this radiation can be used for general laboratory lighting without light adapting the rodent eye.

Keyboards would be illuminated at 750 nm and the display would be covered with a red filter and text and graphs would be presented as white on black to minimize the visible radiation in the laboratory.

Animal temperature control

It is known that the animal temperature will drop with sedation and it is necessary to keep the animal warmed artificially. The Phoenix ERG system places a small heater under the animal on the animal stand with a conformal shape to the animal body. Test results are shown in Figure 5 and the animal temperature was measured

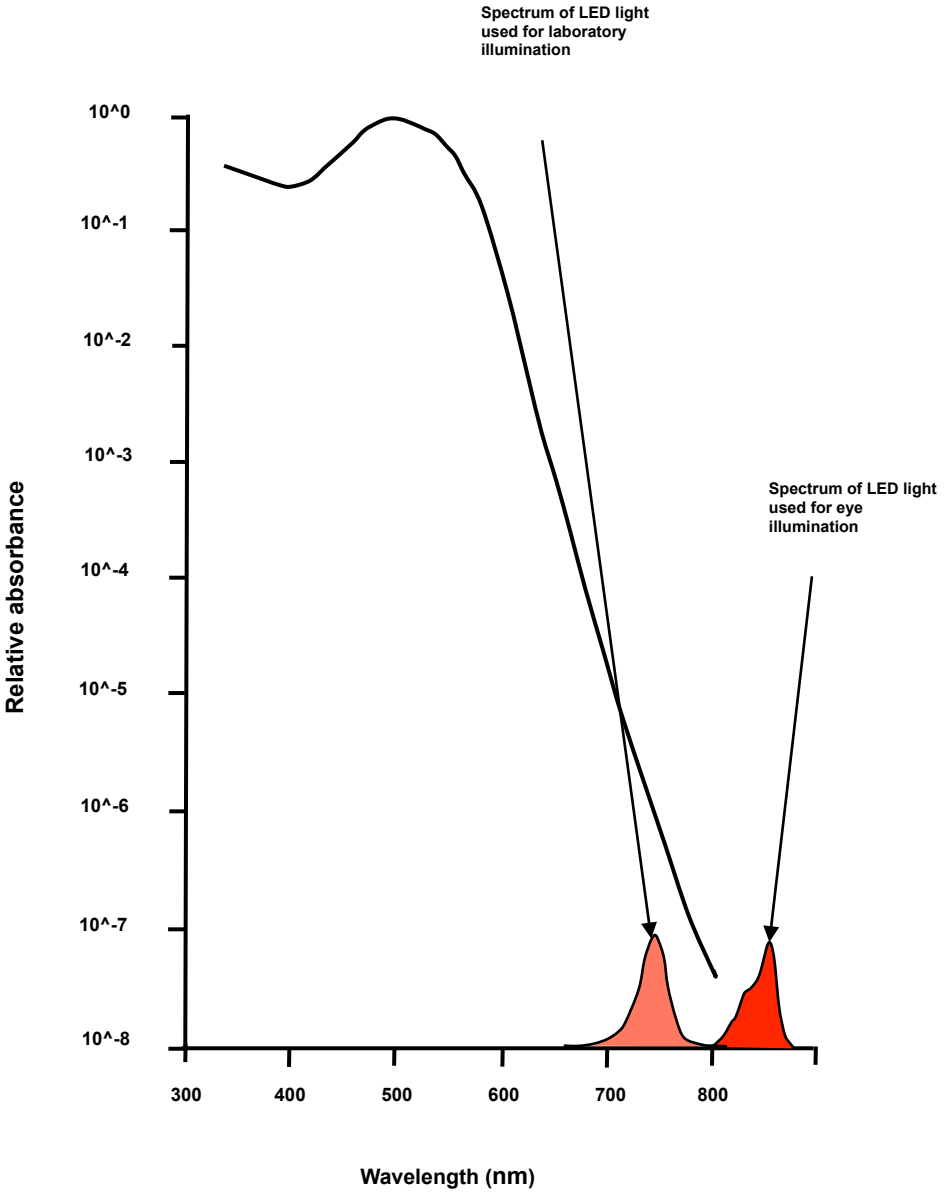


Figure 4. Sensitivity of the mouse eye from visible to near infrared plotted on a logarithmic scale*

*Private communication
Edward N. Pugh, Jr.
Professor of Physiology & Molecular Biology,
Cell Biology & Human Anatomy

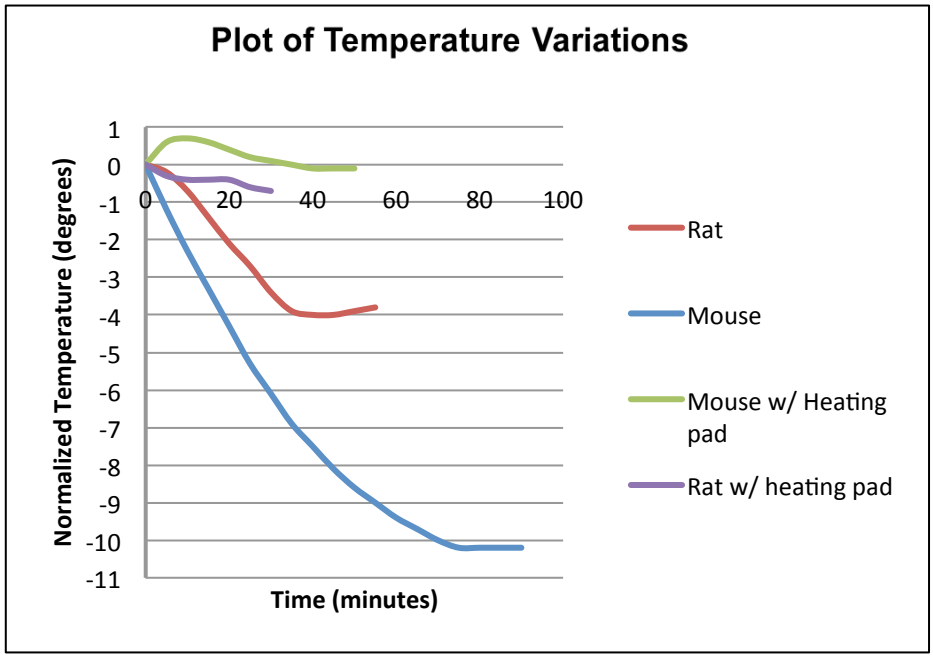


Figure 5. Animal temperature measured rectally with and without the Phoenix animal warmer.

with a rectal thermometer and plots of body temperature vs. time are shown with and without the heaters.

Physical design and electrodes

The Ganzfeld optical head is comprised of three LEDs with center wavelengths of 360, 504 and 850 nm. These sources are combined using dichroic beam splitters and the 850 nm light also returns to the infrared camera seen in the top right in Figure 6. The front objective assembly is removable and has separate lens selections for mouse, rat, and alignment. During alignment the lens closest to the eye has a long focal length and the eye is illuminated with 850 nm. The apparatus including the animal goniostage, animal heater, and some of the adjustment mechanisms, is shown in Figure 7. For alignment the optical head is backed away from the eye using a rack and pinion adjustment that moves the optical head back and forth along its optical axis. Using the NIR image the eye pupil is centered in the field of view. Then the animal is rotated in the supporting goniostage such that the cornea appears round and centered. The eye is now aligned to the optical head.

The rodent Ganzfeld objective lens with a vey small f number lens that will

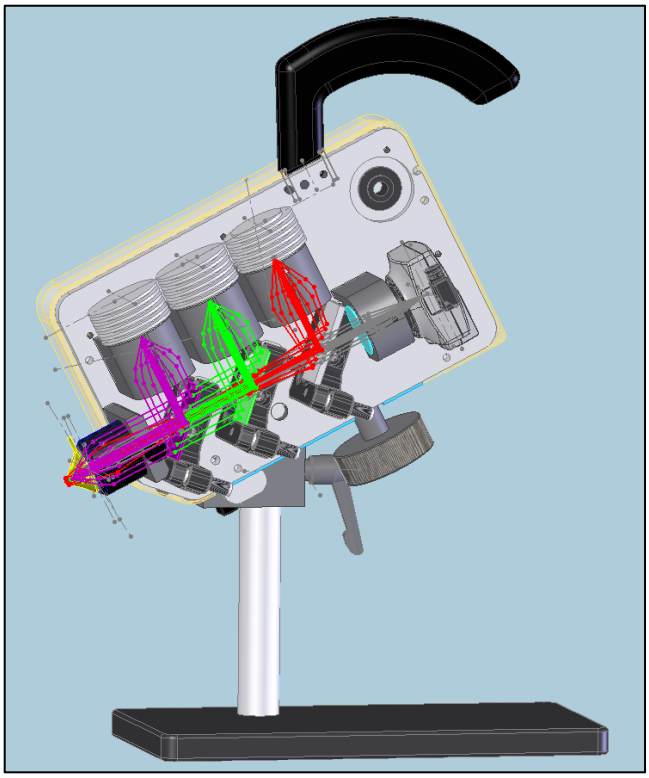


Figure 6. The Phoenix Ganzfeld ERG showing interior optical system

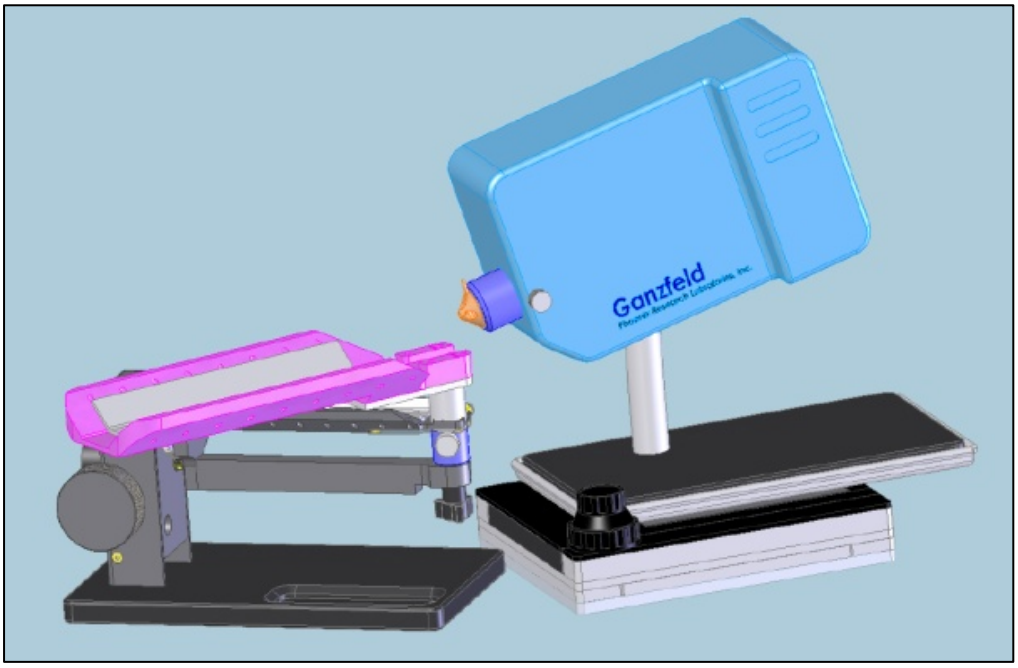


Figure 7. Animal goniostage and camera with x, y stage and rack and pinion and angle adjustment

flood the entire retina is then inserted. The light cone leaving this lens is surrounded by a Gold coated metal frame that is electrically isolated from the camera body with a hole in the tip the diameter of the mouse or rat iris. A cross section of the actual device is shown in Figure 8 This is then moved forward until it touches the cornea and this forms the corneal electrode. The ground electrode is set in the tail and the reference is provided by a bit bar.

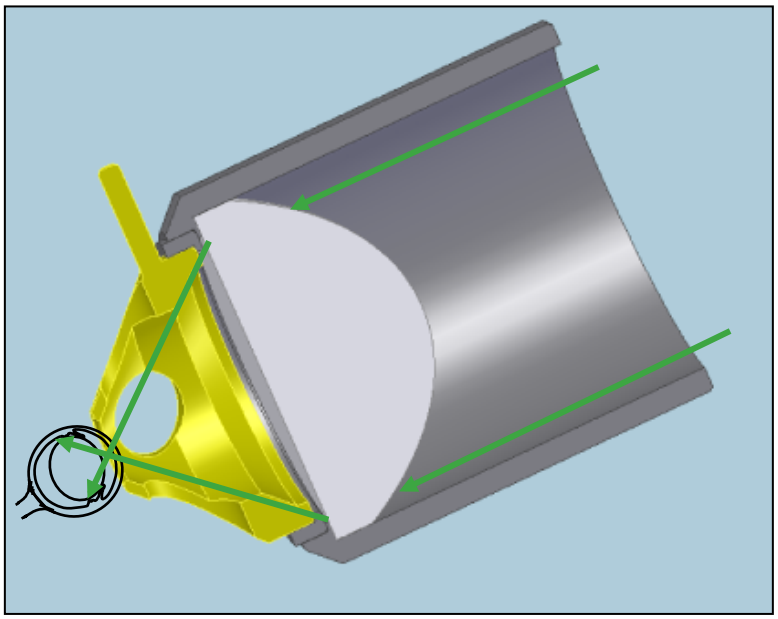
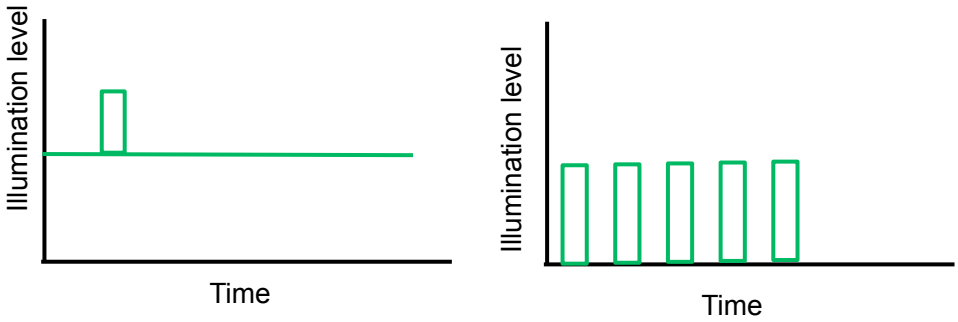


Figure 8 Phoenix Ganzfeld ERG shown in cross section for the actual physical design

Light and acquisition controls and data processing and display

Each LED is separately controllable from the software that runs the data acquisition. The reference power level is set such that is equivalent to 30 Cd sec/m^2 at 1 millisecond. From there the power can be adjusted up or down by factors of two and the pulse length is continuously adjustable over a range of 0.2 ms to 500 msec. The total power range is 20 bits setting the ratio of highest to lowest power at 10^6.

There is in addition to ability to set time delays between pulses over a similar range. Finally, any LED can be set to emit continuously or to operate in “flicker” mode as seen in Figure 9. In Table and Figure 10 is shown the enormous range of aerial energy densities delivered by the Phoenix Ganzfeld and a comparison to various natural light sources.



By providing a continuous background the rod response can be saturated and only the cone response measured

By providing a "flickering" signal of sufficient rate the rod response is suppressed due to its lower response time as compared to the cones

Figure 9 Phoenix Ganzfeld ERG is enabled for a variety of temporal modulation formats

Source	Energy density at retina Joules/mm^2
Aerial energy density at maximum guaranteed power of 3 mW and at 8 ms	1.3 x 10^-6
Sunlight reflected of a Lambertian surface of reflectance of 0.5 for 1 ms	7.4 x 10^-8
100 Cd sec/mm^2	5.1 x 10^-8
0.01 Cd sec/mm^2	5.1 x10^-12
Lowest system illumination 0.2 ms / 3 x 10^-6 mW	3.4x 10^-14
Starlight reflected of a Lambertian surface of reflectance of 0.5 for 1 ms	7.4 x 10^-17

Table 1. Comparison of aerial energy density at retina for the Phoenix Ganzfeld as compared to the ISCEV standard and common light sources

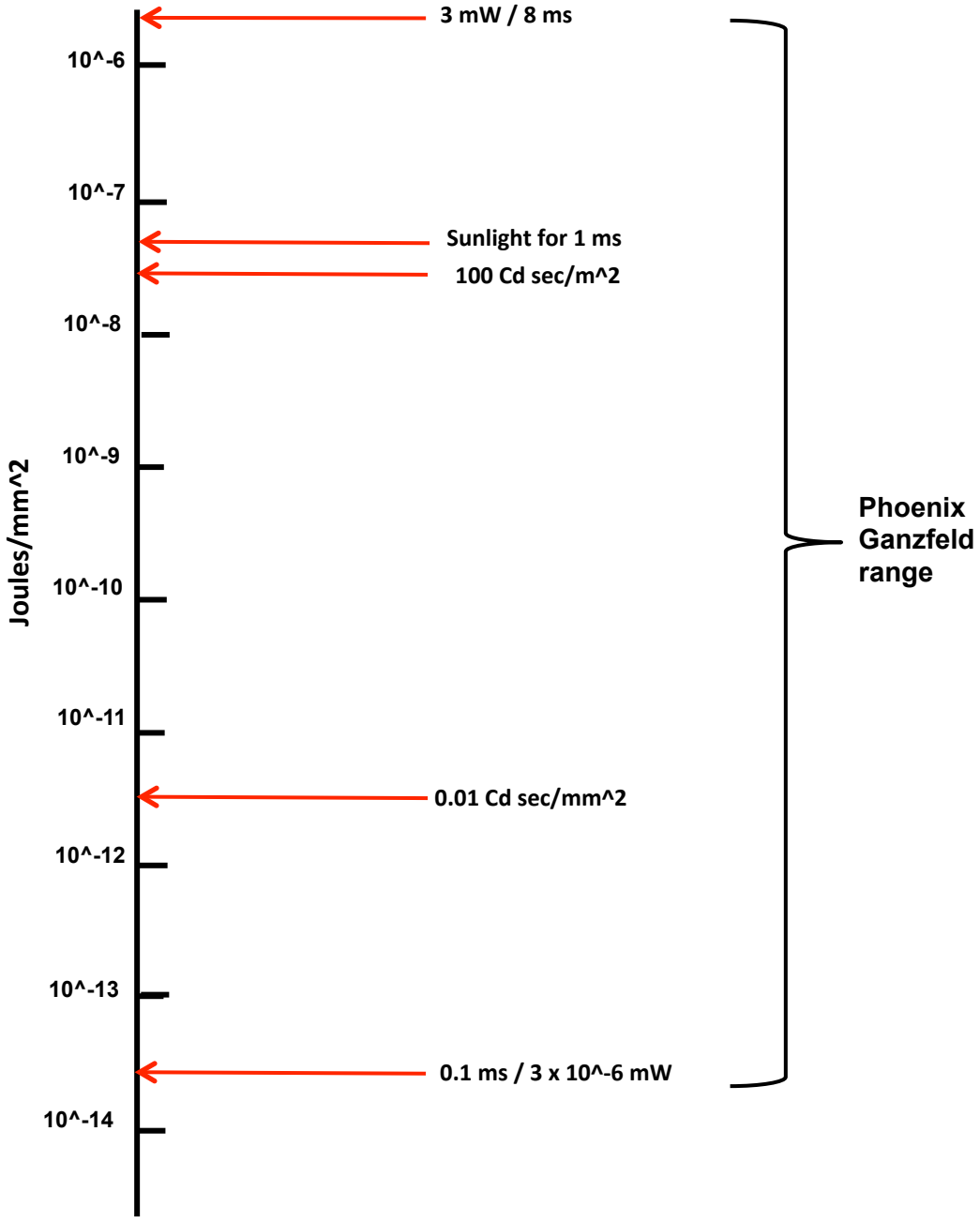


Figure 10. Comparison of aerial energy density at retina for the Phoenix Ganzfeld as compared to the ISCEV standard and common nature light sources

Data acquisition controls, processing, analysis, display, and export

Under software control the length of scan, the sample rate, and the bandwidth is selected. If the signal is low level or the environment especially noisy, the system can be set to acquire a number of traces for averaging. In the display in Figure11 is show a plot of a number of repeated responses and the average. The software conveniently allows for the identification and deletion of traces with especially noisy responses.

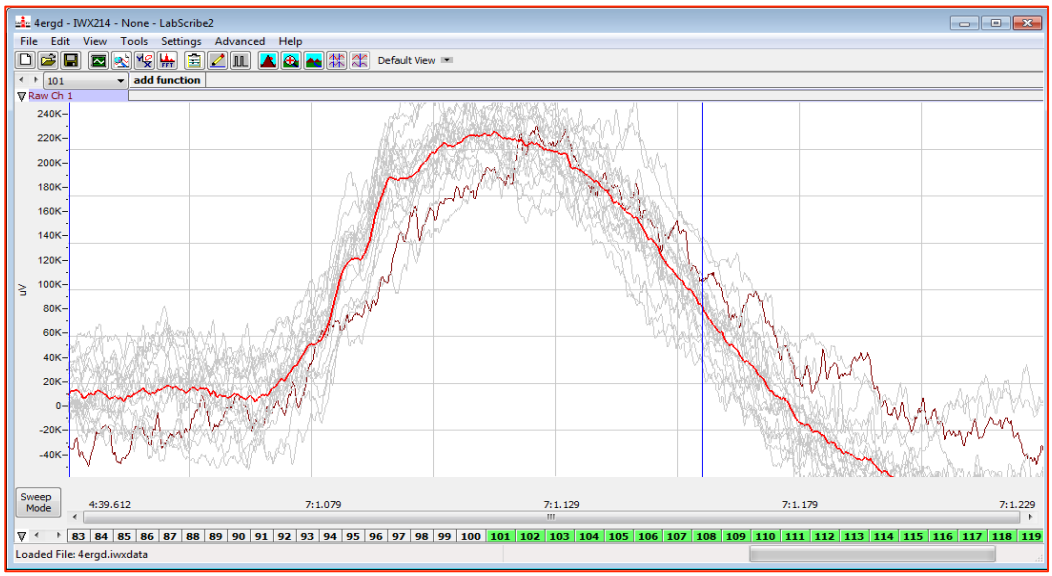


Figure 11. Display of a number of traces and the average

Also under software control the system can automatically generate a “water fall” display. In this display each trace vertically is represented the response of a factor of two increase in light level; this shown in Figure 12.

In Figure 13 is shown a simple extraction of the OP.

Data can be exported as images or Excel files.

4. Example Ganzfeld traces

To provide a few useful examples of the output of the prototype Ganzfeld ERG the system was run at several stages of dark adaption and light levels. These experiments were not designed to be a comprehensive, methodical evaluation of the system under a well considered protocol but serve the useful purpose of

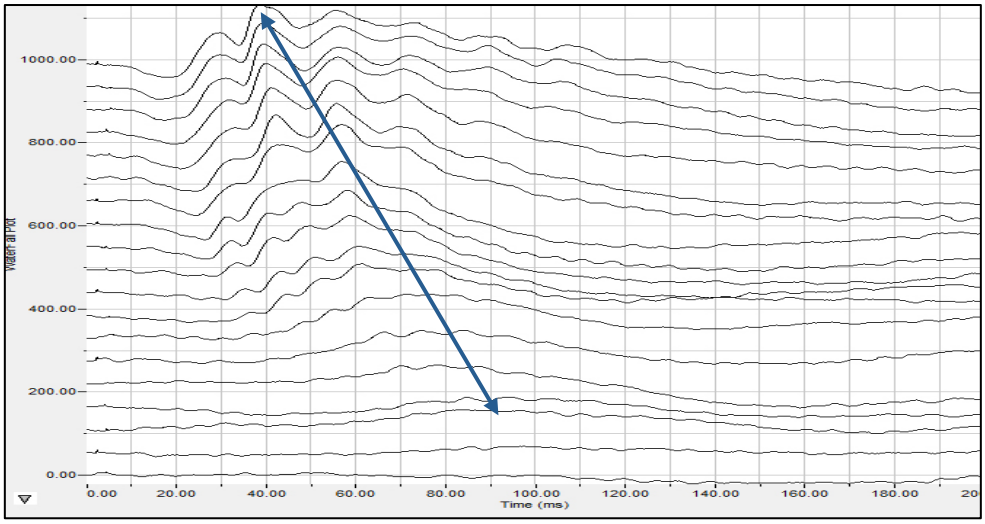


Figure 12. Display of the Phoenix automatic "water fall" display with the shorting of the implicit time with increasing signal noted by the arrow

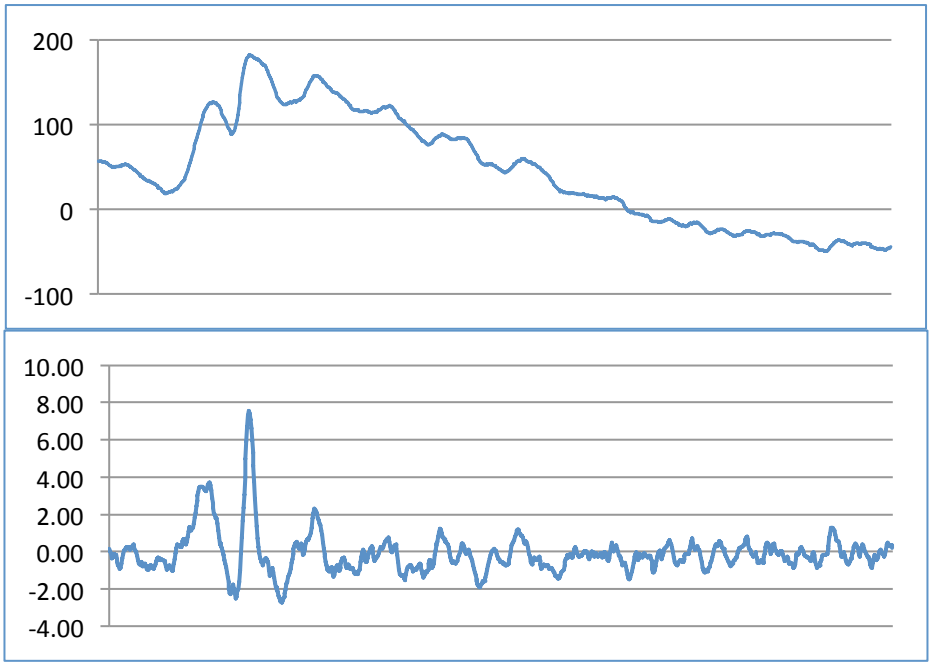
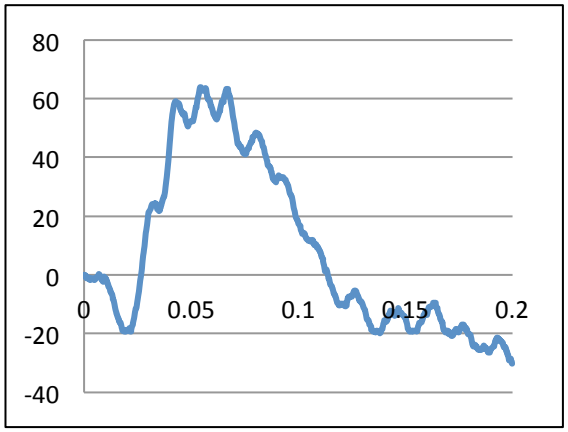


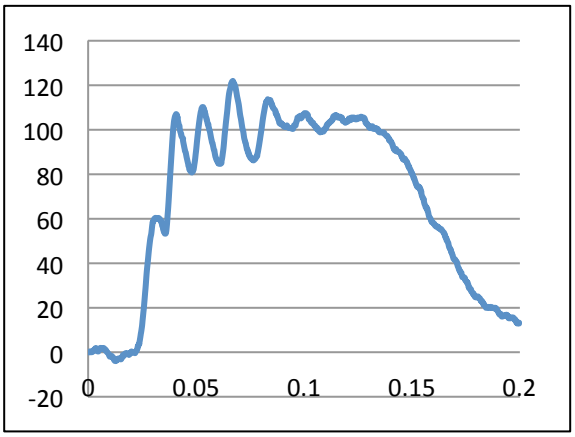
Figure 13. The oscillatory potential can be extracted with simple processing

demonstrating functionality. All traces come from a 10 msec pulse and were obtained on Brown Norway rats; vertical scale in microvolts and horizontal is 200 milliseconds.

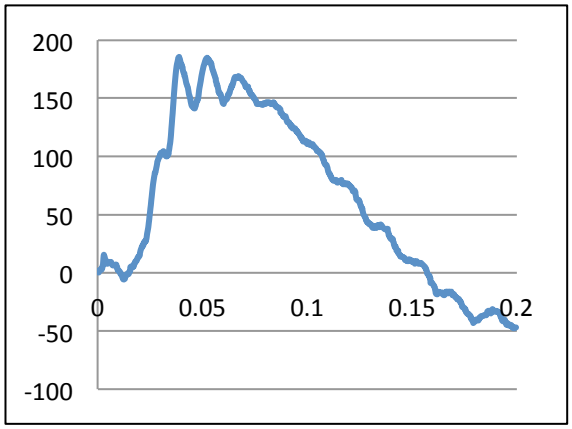


This trace shows a clear but limited amplitude A wave and a distinct OP that occur at the peak of the B wave.

There is some rod response in the UV which may play a role in this curve.



With the light level up and perhaps less dark adaption the B wave saturates and the C wave is delayed.



A higher amplitude B was is seen under different illumination and dark adaptation.

Figure 14 UV Ganzfeld for Brown Norway rat

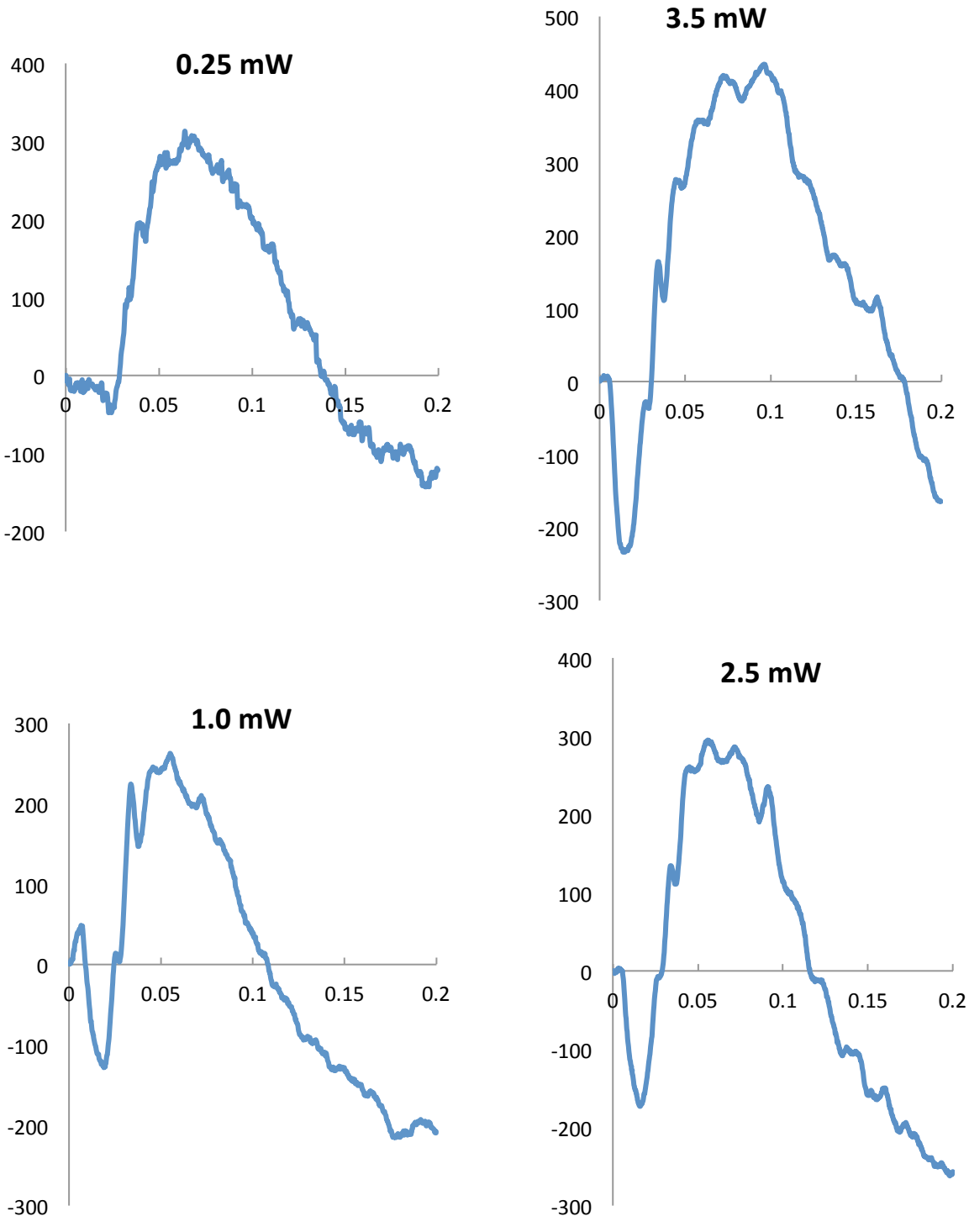


Figure 15 Green Ganzfeld for Brown Norway rat