ISCEV Guidelines for calibration in clinical electrophysiology of vision: 2022 update


Key words: electroretinography, luminance, photometry, visual evoked potentials, ISCEV guidelines, calibration
1. Introduction

Clinical electrophysiology of vision comprises non-invasive tests which assess visual function including different types of electroretinogram (ERG), visual evoked potential (VEP) and electro-oculogram (EOG) [1]. The International Society for Clinical Electrophysiology of Vision (ISCEV) establishes and regularly updates clinical Standards and approves extended protocols for clinical electrophysiology testing of vision, to promote reliable diagnosis and monitoring with comparable results across testing centres [2]. ISCEV also publishes Guidelines that provide context for the ISCEV Standards and extended protocols. The ISCEV guide to visual electrodiagnostic procedures [1] introduces the principles and common clinical applications of ISCEV Standard tests. These Guidelines to calibration aim to provide concise information for practitioners and instrument manufacturers, and updates the 2003 Guidelines [3].

ISCEV standards for the full-field ERG [4], EOG [5], VEP [6], pattern ERG (PERG) [7], multifocal ERG (mfERG) [8], and eight ISCEV extended protocols [9–16] specify stimulus and recording parameters. For all tests, the electrical potentials of the retina or cortex evoked by visual stimuli are detected from surface electrodes, amplified, filtered and digitized then measured, typically with averaging to extract the signal from background noise. As both stimulus and acquisition system characteristics can have substantial effects on the waveform, peak time and amplitude of responses, meaningful interpretation requires stimulators and acquisition systems to be accurate. Regular calibration of stimulus and recording parameters is essential to ensure the reliability and stability of equipment and stimuli, to allow comparison with control data or for monitoring.

This document provides guidelines for calibration\(^1\) of stimulus and recording systems specific to clinical electrophysiology of vision; Appendix A provides additional reading. As for all ISCEV Standards and Guidelines, these Guidelines do not contain safety standards or standards for clinical care and management; users must choose instruments approved for clinical use in their jurisdiction and follow local requirements for clinical safety and care.

\(^1\) Calibration: the process of measuring and, if necessary, adjusting a parameter to establish its value and to ensure that it falls within a pre-specified range.
These Guidelines were adopted by the ISCEV Board of Directors as an official publication on [date].

2. Stimulus calibration

2.1 Background

Three types of stimuli are used in ISCEV Standard tests and extended protocols. Luminance flash stimuli provide uniform stimulation across the extent of the retina with full-field uniform steady backgrounds for light-adapted tests. Pattern stimuli are high contrast black-and-white checkerboards presented as either contrast reversal or onset/offset from a uniform grey field². Stimuli for multifocal testing comprise an array of hexagonal elements presented as on or off according to a pre-determined pseudorandom m-sequence.

ISCEV stimuli are measured using photometric quantities, a system that weights electromagnetic radiation by the nominal spectral sensitivity of a typical human observer, i.e. describes visible light. Luminance is the photometric quantity pertinent to extended sources such as a visual display screen or ganzfeld stimulator and hence is of most relevance to clinical electrophysiology. For brief flashes, the pertinent quantity is time-integrated luminance. ISCEV Standards stipulate achromatic stimuli. Pattern stimuli are specified as black-and-white and light flashes and backgrounds are specified as visibly white with a scotopic to photopic ratio between 2.25 and 2.75 [4, note 1]. Stimulus strength is specified in photopic units based on the relative sensitivity of the light-adapted cone system, except for dark-adapted recordings of full-field ERGs when both photopic and scotopic units are specified. Chromatic (non-white) flashes and backgrounds are used in several ISCEV extended ERG protocols [9–11, 14, 15]. It is necessary to comply with scotopic specifications for chromatic stimuli used under dark-adapted, i.e. rod dominated, conditions because the scotopic to photopic ratios of chromatic stimuli vary markedly.

2.2 Visual stimulus generators

Flash and pattern stimulus generators should be capable of matching the specifications of the Standards and preferably allow generation of a broader range of stimuli for ISCEV extended protocols and additional customised testing. Stimuli should be calibrated and remain stable

² Grating stimuli are used for the ISCEV extended protocol for VEP methods of estimation of visual acuity [16].
over time, with regular verification. Adjustment is required when a measurement of a visual stimulus falls outside the limits specified by a Standard.

Most manufacturers offer stimulus calibration as part of ongoing maintenance or service contracts. If practitioners do not undertake their own photometric calibrations, they should purchase manufacturer calibrations. The previous version of these Guidelines recommended calibration at a maximum interval of 6 months [3]. However, stability of stimuli is highly dependent on the technology used. In these current Guidelines, maximum intervals for calibration based on technology are tabulated in Appendix B. More frequent checks may be required to check stability if values change substantially between successive measurements. The onus is on practitioners to ensure adequate calibration of stimuli and to interpret recordings with appropriate consideration of stimulus accuracy and stability.

Calibration requires photometer(s) capable of measuring the luminance of both extended sources, including small areas, pattern elements, and time-integrated luminance of brief flashes. The photometer should be capable of accurate measurement of low luminance levels, e.g. the pattern VEP dark check of approximately 0.1 cd/m². It should be equipped to measure in photopic and scotopic units. The photometer field of view should be several degrees and preferably tens of degrees to measure ganzfeld stimuli. It requires a time-integrating function capable of measuring a single brief flash as well as multiple brief flashes over several seconds. It should be sufficiently sensitive to measure weak flashes (0.01 phot cd.s.m⁻², 0.03 scot cd s m⁻²). Photometers themselves require calibration, typically annually, which should use a source traceable to an international standard.

2.3 Measurement of luminance stimuli

For continuously lit or reflecting sources such as display screens and ganzfeld light-adapted backgrounds, the appropriate measure is luminance (units cd/m²). This is a measure of the steady-state luminance level of the surface. For brief flashes, the appropriate measure is time-integrated luminance (units cd·s/m²). This is a measure of the total luminance delivered by the flash. For clinical electrophysiology of vision, brief flashes range from less than 100 microseconds for discharge lamps up to 5 ms for LEDs and other sources. Appendix C describes methods for measuring brief flashes, flicker stimuli and ganzfeld backgrounds used in light-adapted full-field ERG and EOG tests.

2.4 Measurement of pattern stimuli
Visual display units (VDUs) or other optical imaging systems that generate pattern stimuli are used in clinical electrophysiological tests, including VEPs, PERGs and mfERGs. Appendix B lists the main types of VDUs with information about their application to clinical electrophysiology of vision and calibration frequency. Pattern stimuli require calibration of their mean luminance, contrast, pattern element size and the field size.

**Mean luminance:** The mean luminance is of the utmost importance for pattern stimuli as it affects amplitudes and peak times, particularly for PERGs and mfERGs. Mean luminance is expressed as:

\[
\frac{L_{\text{max}} + L_{\text{min}}}{2}
\]  

(1)

where \(L_{\text{max}}\) and \(L_{\text{min}}\) are luminances of light and dark pattern elements respectively, when these are equal in size and duty cycle. The ISCEV Standards require mean luminances of 40–60 cd/m\(^2\) for the VEP [6], > 40 cd/m\(^2\) for the PERG [7] and > 50 cd/m\(^2\) for the mfERG [8].

**Luminance transients:** Pattern reversal or pattern onset/offset stimuli must not have transient changes in mean space-averaged luminance of the whole display. Pattern onset/offset stimuli are particularly prone to unwanted luminance artifacts, where the uniform grey field does not match the patterned field in mean space-averaged luminance. Any transient or step change in luminance with each pattern change creates a luminance artifact that may contaminate the pattern response with a luminance response. A qualitative method of checking for luminance transients can be achieved by observation. With room lights off to enhance sensitivity, a sheet of white paper is held in front of, and parallel to, the plane of the stimulus at a distance of \(\approx 30\) cm and viewed by an observer standing behind the display while the stimulus is delivered. The diffused reflection of the display on the paper should appear to have constant brightness. If any brightness change is perceived, the stimulus must be adjusted to remove it.

**Contrast:** The Michelson contrast of pattern stimuli is defined as:

\[
\text{Contrast} \, (\%) = \frac{L_{\text{max}} - L_{\text{min}}}{L_{\text{max}} + L_{\text{min}}} \times 100
\]  

(2)

where \(L_{\text{max}}\) and \(L_{\text{min}}\) are the luminances of the light and dark elements, respectively. Thus, contrast ranges from 0% for a homogeneous field to 100% when the dark checks have a luminance of zero. Although ISCEV Standard VEPs require a minimum contrast of 80% [6], VEP waveforms change very little for contrast levels above approximately 50%. However, mfERG and PERG amplitudes increase linearly with contrast so that high contrast levels and
stable calibration of contrast are critical; minimum contrast levels are 90% for mfERGs and 80% for PERGs. It is advisable to protect manual controls for ‘brightness’ and ‘contrast’ to prevent accidental changes. Methods for verification and calibration of pattern mean luminance and contrast are presented in Appendix D.

_Element and field size:_ Pattern evoked potentials show spatial tuning and thus are affected by the size (angular subtense) of the pattern elements. For guidance, a pattern element of 1 cm subtends ≈1° at a viewing distance of 57 cm. The elements in checkerboard stimuli are specified by check width in degrees or minutes of arc. The hexagons in mfERG stimuli are scaled as specified in the mfERG Standard with a smaller central hexagonal element surrounded by elements that increase in size with eccentricity [8]. The field sizes for pattern stimuli are specified by the angular subtense of both height and width. Procedures for calculating visual angles for fixed viewing distances and for choosing a viewing distance to obtain a desired visual angle are given in Appendix D.

3. Acquisition systems

3.1 Background

Electrophysiological signals produced by the retina and visual cortex typically have small amplitudes in comparison to noise sources such as line noise (50 or 60 Hz), electrical noise from electronic equipment (often 100 or 120 Hz) and physiological ‘noise’ generated by muscle (electromyogram, EMG) and brain (electroencephalogram, EEG) activity or eye movements. Differential amplifiers amplify the difference between the two inputs, rejecting signals common to both inputs. Electrodes placed near the relevant signal source, distant from it, and at an indifferent point are connected to the positive, negative and common inputs of the acquisition system, respectively. High amplitude artifacts, such as those generated by eye movements or blinks, are typically excluded from the signal on-line or post hoc using rejection criteria based on amplitude; for example, the VEP and PERG Standards [6, 7] suggest a ±100 µV rejection threshold. Stimulus-locked signal averaging increases the signal to noise ratio; the choice of the number of averages should be governed by signal to noise conditions and meet the requirements of the specific Standards and extended protocols.

The amplified signal is digitised into arrays of time and voltage values using an analogue-to-digital convertor, and processing such as filtering and averaging reduces the noise further.
Sampling frequency and some filter characteristics are specified in ISCEV Standards and extended protocols. Analogue filters and digital emulations introduce frequency-dependent phase changes and can alter waveforms and peak times which should be borne in mind if filter settings differ, for example between serial recordings or between a recording and reference data. Amplifier gain should be adjustable and should ensure high amplitude resolution and avoid any non-linearities at the extremes of the range which could distort signals (e.g. clipping of the peaks).

3.2 Measurement of electrode impedance

Electrode impedance: Impedance is measured by passing a low amplitude (≤1 µA) alternating current (AC, 10–100 Hz) through the tissue between a pair of electrodes. Impedance depends on frequency but typically varies little between 10 and 100 Hz. DC ohmmeters must not be used as these typically apply relatively high currents and will polarize electrodes, resulting in unreliable measures. Each ISCEV Standard and extended protocol specifies the upper limits of inter-electrode impedance differences. Impedance is typically low for electrodes in contact with the tear film so impedance measurement may not be required, particularly for thin fibre electrodes which may be damaged by even the small currents used to measure impedance. Electrode leads may add noise to the signal via capacitive coupling, and such interference can be minimised by having low and matched input impedances. These specifications are pertinent to ‘passive’ electrodes: no specifications are made for ‘active’ electrodes which incorporate pre-amplification, as they have less stringent impedance requirements.

3.3 Measurement of amplification systems

Calibration and verification of amplifier gain is assessed by passing a known signal, with amplitude and timing in the expected range of the physiological signals, through the amplification system. This known signal should pass through the entire system, including any pre-amplifiers incorporated into the electrode connection box or the electrodes themselves. The amplitude of the output should closely resemble that of the input multiplied by the gain

---

3 Impedance: denotes resistance at frequencies other than direct current (DC). At the relatively low frequencies used in clinical electrophysiology there is little quantitative difference between the impedance and resistance.
and take into account the effect of any applied filters. A method to verify amplification systems using a signal generator is given in Appendix D.

Most manufacturers offer calibration of acquisition system as part of ongoing maintenance or service contracts. If practitioners do not undertake their own calibrations, they should purchase manufacturer calibrations. ISCEV encourages manufacturers to provide methods for practitioners to perform interim verification of the amplification systems, for example to investigate suspected malfunction. Calibration of amplification and filtering should be performed as specified by the manufacturer based on the known stability of the system. If there are no specified intervals, we recommend a maximum interval of one year. The onus is on practitioners to perform or commission adequate calibration of the system.

Acknowledgements
References


2. https://iscev.wildapricot.org/standards


Appendix A: Further reading

*Headed by topic and arranged in chronological order by publication date.*

**Bioelectric potentials recording:**


**Photometry and Colorimetry**


Non-invasive visual bioelectric potentials and signal detection

Appendix B: Visual displays

The major types of visual display units (VDUs) for pattern stimulation are compared in the table below.

<table>
<thead>
<tr>
<th>Display type</th>
<th>Brief technical description</th>
<th>Comments pertinent to visual electrophysiology</th>
<th>Frequency of calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathode ray tube (CRT)</td>
<td>Vacuum tube containing electron guns whose beams repeatedly scan the front of the tube to display images on a phosphorescent screen</td>
<td>No longer produced. Continuous pixel size is useful for some applications, e.g., for visual acuity estimation. Small patterns lose contrast.</td>
<td>At least every 6 months. More frequently for older models e.g., every month.</td>
</tr>
<tr>
<td>Liquid crystal display (LCD) with thin film transistor (TFT)</td>
<td>Image elements (pixels) are capacitors with an insulating liquid crystal layer between transparent conductive layers, driven by column and row transistor switches</td>
<td>Widely available and relatively inexpensive. Unacceptable luminance artifact with pattern change unless specially adapted e.g., by inserting flicker at every frame, modulating a banded backlight, using feedback or feed-forward adjustments.</td>
<td>Annual. Or more frequently based on hours of use</td>
</tr>
<tr>
<td>Plasma display</td>
<td>Pixels are phosphor lined cells that form plasma (gas of ions) when voltage is applied causing excitation of visible photons in the plasma.</td>
<td>Capable of high luminances. Pixels ≈3× larger than LCD.</td>
<td>Annual</td>
</tr>
<tr>
<td>Organic light emitting diode (OLED) display</td>
<td>Panel of light-emitting diodes (LEDs) in which the electroluminescent layer is a film of organic compound that emits light in response to an electric current.</td>
<td>Technology evolving. Has potential to optimise stimulus presentation. Some have ‘picture processing’ detrimental to precise stimulation</td>
<td>Annual or less frequently as directed by manufacturer</td>
</tr>
<tr>
<td>Digital light processing (DLP) display</td>
<td>Projection technique - matrix of microscopic mirrors on a semiconductor chip oriented rapidly to reflect light either through the lens or onto a heat sink.</td>
<td>Array of tiny mirrors, used for projection. Timing of the trigger is not straightforward.</td>
<td></td>
</tr>
</tbody>
</table>
i. CRTs perform optimally with mean luminance between 25 and 100 cd/m²; nonlinearities can occur with \( L_{\text{max}} > 200 \) cd/m². Luminance is typically greater in the centre than at the periphery. The current ISCEV mfERG Standard \([8]\), require less than a 15% difference between the centre and periphery of the stimulus array. For Standard pattern VEPs, which are less dependent on eccentric stimulus elements, the maximum acceptable difference is 30% \([6]\)

ii. For example, Eizo monitors should be calibrated after 200–300 hours of use [Color Management Resources Eizo] \(https://www.eizo.com/library/management/calibration/\)
Appendix C: Measurement of luminance stimuli

Flashes

A method for measurement of the time-integrated luminance of brief, single-flash stimuli is summarized below.

I. Preparation
   a. Turn on the photometer and allow sufficient time to stabilise.
   b. Add appropriate photopic or scotopic filters if indicated.
   c. Set the photometer to time-integration mode, measuring time-integrated luminance in units of cd·s/m².
   d. Place the detector at the position occupied by the eye during a test.

II. Measurement
   a. Darken the room and turn off fixation lights and any background lights or infra-red monitoring devices e.g. lamps for cameras in the ganzfeld.
   b. Zero the photometer.
   c. Measure a single stimulus flash, noting the reading once stable. Repeat at least five times to ensure stability of measurements.
   d. Select the median value as the flash luminance. If individual values differ by >10%, consult the manufacturer.
   e. Alternatively, deliver a series of a known number of flashes separated by at least 1 s, and divide the measurement by the number of flashes. This method does not provide a measure of inter-flash variability.

Flickers

A method for measurement of rapidly presented flashes, such as the 30 Hz flicker stimulus is given below. To calibrate flicker stimuli, it is typically necessary to measure in integration mode over a known period of time. If the exact flicker frequency is known, the number of flashes delivered can also be known. If measuring flicker stimuli delivered by flash units based on discharge lamps, it is important to delay the start of measurement until after the
output has stabilized: discharge lamp flashes typically are not full strength when operating at 30 Hz.

I. Preparation
   a. Turn on the photometer to stabilise, as above.
   b. Use an appropriate photopic filter.
   c. Set the lamp to flicker and allow it to stabilise (typically >1 s for discharge lamps / not necessary for LEDs with more consistent output).
   d. Operate the photometer in time-integration mode.

II. Measurement
   a. Measure the output for a known, fixed time.
   b. Calculate the number of flashes delivered in the time interval and divide by the number of flashes to obtain the strength of individual flashes in the flicker stimulus.

Backgrounds

Luminance of extended backgrounds such as the full-field light-adapting background for light-adapted ERG testing are measured in photopic cd.m$^{-2}$.

I. Preparation
   a. Turn on the photometer to stabilise, as above.
   b. Use an appropriate photopic filter.
   c. Use an extended field if available (e.g. 10 degrees).
   d. Set the photometer to steady-state mode, measuring luminance in units of cd·m$^{-2}$.
   e. Place the photometer at the location of the patient’s eye during testing.

II. Measurement
   a. Darken the room, or match the room lighting to the level used during testing. Room lighting must not alter the luminance of the surface to be measured. Turn off any fixation light or infra-red source.
   b. Zero the photometer, typically using a light-excluding cap.
   c. Note the measurement once stable, and repeat at least twice more to ensure stability.
d. Take 5 measurements discard the high and low values and average the 3 remaining values. If measurements differ by >10% consult the manufacturer. For discharge lamps replace the bulb if indicated and re-calibrate.
Appendix D: Measurement of pattern stimuli

Luminance of light and dark pattern elements

This method requires a spot photometer, also known as a luminance meter, equipped with optics for measurement over a small area of surface and usually with a means of monitoring the region that is being measured. Some devices work by directly contacting the emissive surface or screen, others are focussed from a short distance away. Direct contact measurements will not incorporate any additional luminance from room illumination.

I. Prepare the equipment
   a. Turn on the photometer and allow sufficient time to stabilise.
   b. Add appropriate photopic filter if indicated.
   c. Set the photometer to steady-state mode, measuring luminance in cd/m²
   d. Display a stimulus with large pattern elements, e.g., 2 deg checks on the display
   e. Slow, or if possible, stop, the pattern changes

II. Measurement
   a. Adjust room lighting conditions to those used during testing
   b. Zero the photometer with the detector covered
   c. Where appropriate, focus the photometer optics
   d. Position the detector so that it is perpendicular to the screen and so that the measurement field is no more than half the size of the bright or dark element being measured
   e. Obtain stable measurement of light and dark elements at the centre of the screen, usually the four elements surrounding the centre.
   f. Repeat each measurement at least twice more to ensure stability.
   g. Repeat e) and f) for the four elements at the outer corners of the checkboard near the screen periphery.

III. Calculations
   a. Calculate mean luminance using Equation (1) with median values of light elements (L_{max}) and dark elements (L_{min}) at the centre of the screen.
b. Calculate mean luminance for elements at the periphery of the screen.

c. Calculate stimulus contrast using Equation (2) with luminance values obtained from the centre of the stimulus.

If values differ from requirements of the standards, consult the manufacturer, or perform the required adjustments as appropriate.

**Measurement of visual angles.**

I. Measurement of pattern element size at a fixed viewing distance

   a. Measure the size of 10 elements (e.g. 10 check widths, whether black or white) across the centre of the screen and divide by 10 to obtain the mean element size. Do this horizontally and vertically to verify symmetry of square checks.

   b. Measure the distance from the patient’s eye to the centre of the screen.

   c. Divide the mean element size by this viewing distance.

   d. Determine the angle whose tangent is equal to this value by using the inverse tangent (\(\tan^{-1}\) on a calculator), converting from degrees to minutes of arc (min) if necessary by multiplying by 60 (e.g., 0.25° equals 15 min.) Check sizes given in the ISCEV VEP Standard (1° and 0.25°, i.e. 60 min and 15 min) refer to the width (equal to the height) of a check.

II. Calculation of viewing distance for desired visual angle

   a. Measure the size of 10 elements (e.g. 10 check widths, whether black or white) across the centre of the screen and divide by 10 to obtain the mean element size.

   b. Look up the tangent of the desired visual angle of one element (i.e. tangent of the desired visual angle).

   c. Divide the mean element size measured in step a by the tangent of the desired visual angle obtained in step b to obtain the viewing distance.

III. Field size calculation

   a. Measure the diameter of the stimulus area (width and height)

   b. Measure the viewing distance from the patient’s eye to the centre of the screen.

   c. Divide the diameter by this viewing distance.
d. Determine the angle whose tangent is equal to this value by using the inverse tangent (e.g. $\tan^{-1}$ on a calculator). Consult the relevant ISCEV Standard to verify that your field size and the ratio of width to height meets the requirements.

_____________________

i. Element sizes are small angles so that the tangent, check width divided by distance, is approximately equal to the angle in radians. Angle in degrees = angle in radians $\times \frac{180}{\pi}$

ii. Note that checkerboard stimuli are sometimes described using the diagonal dimension of the check, which is the size of 1 cycle of the fundamental spatial frequency of the pattern. See Fahle M, Bach M (2006) (Appendix A) for an explanation.

iii. Systems that allow input of the viewing distance to automatically calculate check width and field size will have a size calibration protocol for the monitor. This monitor calibration can be verified using the method above.
Appendix E: Verification of amplifiers for clinical recording

Verification requires a signal generator capable of producing low amplitude output in the physiological amplitude range, i.e. 1 µV to 1 mV. Sinusoidal signal inputs allow both amplification and filter settings to be assessed, and any harmonic distortion to be detected. Simulated electrophysiologic signals can be used to determine the effects of the amplification system on measurable characteristics of the signal of interest. A square-wave pulse signal allows detection of any ‘ringing’ in response to an abrupt voltage change. Signal generators themselves will require regular calibration. If amplifiers are not performing as expected, or if distortions of signals are observed, the equipment needs to be repaired. A useful check that all amplifier channels are working similarly is to pass an identical signal through each channel with identical settings.

A suggested method for amplifier verification is given below. Note that verification of amplification systems must not be done with a person connected to the system.

Amplifier measurement method

I. Preparation
   a. Switch on the amplifiers and signal generator and allow to stabilise (typically a minimum of 15 minutes)
   b. Connect the signal generator to the electrode inputs of the amplifier
   c. Generate signals (1 µV to 1 mV).

II. Measurements
   a. Acquire and measure simulated electrophysiologic calibration signals as for a patient recording. If signal averaging is used, the signal generator must trigger the data acquisition system.
   b. Verify the nominal filter band pass characteristics by measuring multiple sinusoidal signal frequencies that begin below and extend above low-pass and high-pass filter settings
   c. Optionally to verify the accuracy of the amplitude calibration and waveform, acquire and measure simulated electrophysiologic calibration signals without the filters used for clinical testing (i.e. maximum band-pass available, ‘open’ or no filter settings)
   d. If values differ from nominal values to a degree that would make a clinically significant difference, consult the manufacturer.