Principles and Practice of Clinical Electrophysiology of Vision

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Monitoring Equipment and Calibration

J. Vernon Odom

A critical aspect of running any clinical electrophysiology laboratory is standardization of procedures. Only standardized stimulation and recording procedures can elicit normal values with minimal variability. Without consistent normal values, abnormal responses become more difficult to detect. Standardization requires calibration since stimuli must be presented at the same luminance, contrast, frequency, and same distance subtending the same visual angles. For the normalcy of responses to be determined, the gain of the system, the artifact rejection levels, and delays associated with filtering must be determined. Calibrations should be repeated on a regular basis. If changes are found, the equipment should be adjusted. To perform the calibrations indicated below several instruments are required: (1) a volt-ohm or impedance meter, (2) a light meter (photometer), and (3) an oscilloscope or averager. Several other items such as photodiodes, waveform generators, and frequency counters may be useful.

MEASUREMENT OF SOURCE IMPEDANCE

As discussed in the chapter on amplifiers and special-purpose data acquisition systems (Chapter 24), an important requirement of modern amplifiers is low impedance of the electrodes; otherwise, large line interference is likely. Depending on the availability, one may employ either an impedance meter to measure impedance or a volt-ohm meter to measure resistance. Resistance measured in kilo-ohms quantifies the resistance to a minute direct current passed through the electrodes and intervening skin. Because the current is directional, the electrodes may polarize with repeated or prolonged measurement. Therefore, one should reverse the positive and negative electrodes after a few seconds or on alternate measurements. Impedance quantifies resistance and capacitance as alternating current (ac) is passed through the electrodes. Electrode polarization is not a problem in impedance measurement. Because current must be passed through the electrode to measure resistance or impedance, it is not customary to measure the impedance of corneal electrodes even with the small current levels used. Following subject preparation, the impedance of the subject's electrodes should be less than 5 to 10 kΩ.

While the measurement of impedance is a necessary first step in the recording process, continual monitoring of the recorded signal on an oscilloscope or averager also is advisable, especially when recording the electroretinograms (ERGs) or visual evoked potentials (VEPs) of children or active adults. Their activity may loosen the electrodes, which can alter the impedance and introduce line interference. A flowchart used at West Virginia University is presented in Figure 25–1 as an example of the role of measuring impedance and monitoring the signal as part of the entire recording procedure.

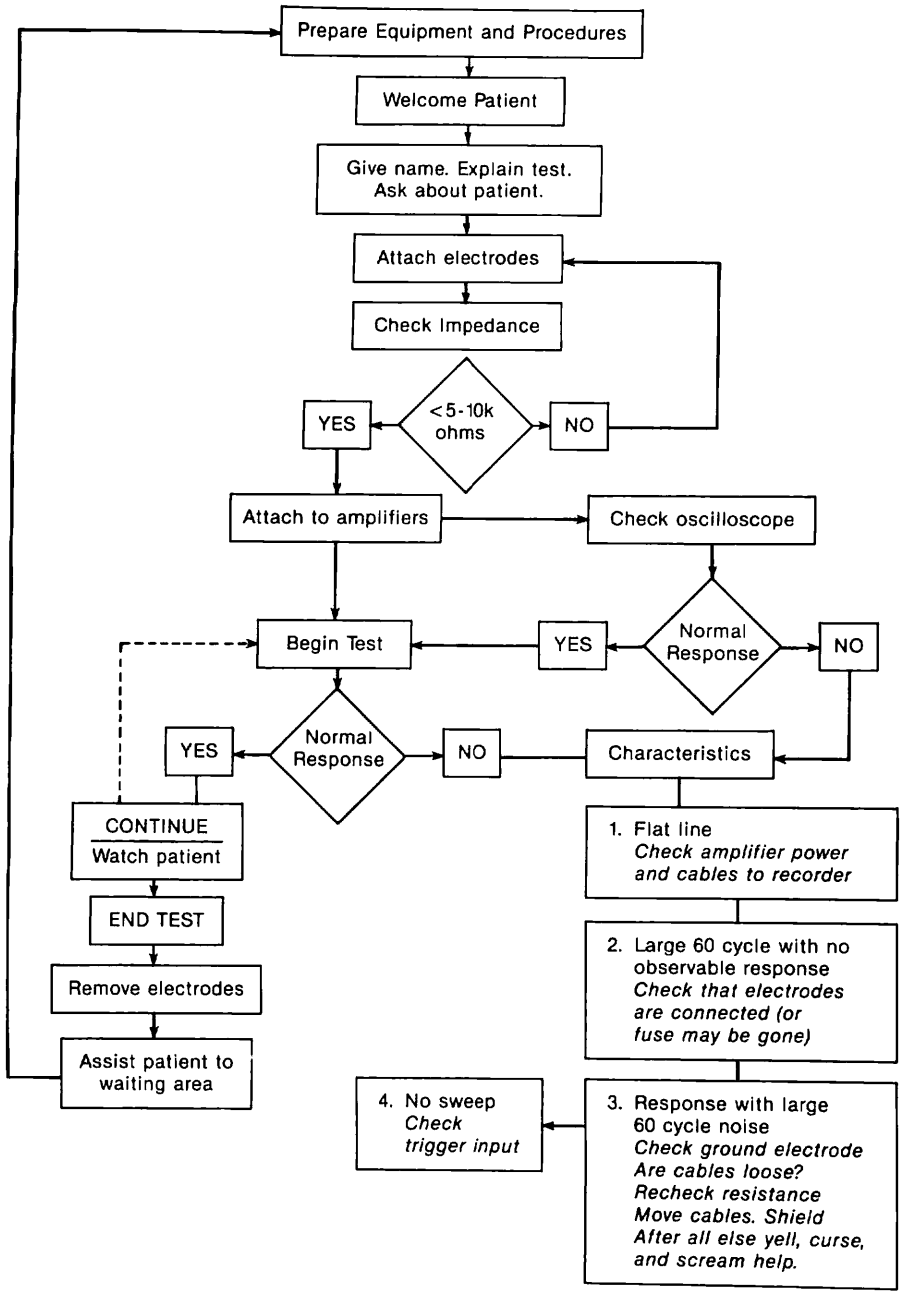


FIG 25–1.
Flowchart illustrating how measurement of electrode impedance fits into electrophysiological tests. (Courtesy of West Virginia University Retinal Function Laboratory.)

CALIBRATION OF AMPLIFIERS AND GAIN

The general characteristics of amplifiers in terms of their frequency-response characteristics and a general description of the means of calibrating them has been given in Chapter 24. Unless one has reason to doubt the manufacturer's specifications, the frequency-response function may be accepted. Some contemporary data acquisition systems have built-in amplifiers so that the signal amplification is known and the real voltages can be provided by the system.

To determine the original signal voltage it is necessary to know the system gain, or amplification. If the system has separate amplifiers, several amplifier stages are cascaded, or if the system is older, it will

be necessary to calculate and provide the amplified voltages. To verify system gain, one must have some calibrated source, ideally 100 μV or less. Many preamplifiers and oscilloscopes include a calibrated signal. If not, one may be constructed by taking a higher-voltage signal and passing it through voltage divider. The calibrated voltage passes through the entire system and the output is measured by using an oscilloscope, averager, or plotter (oscillograph). Knowing the input (e.g., 20 μV) and the output as read from the oscilloscope (e.g., 0.2 V) one can determine the amplification of the system (e.g., $0.2/0.000002 = 1 \times 10^5$). If one employs the oscillograph or plotter output, one determines the relationship between the deflection amplitude in millimeters (or inches) and the input amplitude in volts of the signal.

ARTIFACT REJECTION

Although there are a number of sophisticated artifact rejection routines including adaptive filtering, only amplitude rejection is commonly employed in commercially available clinical electrophysiology equipment. The use of amplitude rejection assumes that if a signal is too large it cannot be a signal of interest and must be an artifact; therefore, the sweep in which it occurs is rejected. In many systems, the amplitude at which artifact rejection occurs is selected by the examiner. Therefore, one needs some knowledge of the expected size of the signal of interest. The chapter on amplifiers and special-purpose data acquisition systems (Chapter 24) includes a table of the typical signals encountered in clinical electrophysiology and their upper amplitude limits. Consideration of this information may be of assistance in determining the appropriate artifact rejection level.

CALIBRATION OF DELAYS DUE TO FILTERING

In general, it is best to have fixed-frequency bandwidths, with the upper frequency limit at 100 Hz or higher. On some occasions however, it may be necessary to adjust the upper frequency limit to reduce artifacts. As discussed in Chapter 24, this will introduce phase delays and result in a delayed peak response (and in some cases an altered waveform). Several strategies exist to determine the latency of the original response. One may (1) compare the latency differences of normal subjects under the two conditions to determine a mean correction factor or (2) perform inverse filtering on the digitized signals.[10, 18] Method 1 has the advantage of simplicity in the clinical situation. Method 2 is more precise but unavailable on most commercial systems.

MEASUREMENT OF LIGHT (PHOTOMETRY)

Radiometry is the measurement of quantities of electromagnetic energy and power generated by radiant energy sources. Photometry began as an experimental science in the 19th century in an effort to compare and scale the subjective brightness of different sources of radiant energy. In the 20th century it has developed into a set of rules, definitions, and standards, the purpose of which is to enable the experimenter, clinician, or engineer to abstract a subjectively meaningful single number with which to describe light sources.[1, 4, 5, 12, 13, 17, 19, 22]

Light is the portion of the electromagnetic energy spectrum that stimulates the photoreceptors of the eye and elicits visual sensation. The frequency of light is the fundamental unit in describing light because it does not vary with the speed of light as it passes through various optical media. However, light is usually measured in terms of its wavelength. Wavelength is most often expressed in nanometers but is occasionally expressed in millimicrons or angstroms, where $1\ \mu m = 1\ m \times 10^{-6}$, $1\ nm = 1\ m \times 10^{-9}$, and $1\ \text{Å} = 1\ m \times 10^{-10}$. The wavelength of light ranges from about 400 to about 700 nm.

The basic operation in photometry consists of weighting measurements of electromagnetic energy or power (radiometric functions) by the sensitivity of the individual or "standard" human observer. Because human sensitivity varies dramatically under light-adapted and dark-adapted conditions, two weighting functions are used, a light-adapted (photopic) function and a dark-adapted (scotopic) function. The weighting functions for the standard or average observer are adopted and occasionally modified by the Commission International de l'Eclairage (CIE) and the national and international agencies that regulate standards. It should be remembered that the standard observer represents a mean value and does not faithfully represent the value of any one human observer. If determining the weighting function for a single observer is important, one should employ one of the standard psychophysical procedures described by Walsh[19] or Wyzcecki and Stiles.[22]

Table 25–1 lists at 10-nm intervals the relative

TABLE 25–1.
Important Values for Vision*

Wavelength λ, nm (in Air)	Frequency $\times 10^{14}$ $\nu = c/\lambda$ sec^{-1} (Tetrahertz)	Relative Photopic Luminosity Factor V_λ	Relative Energy (Ergs) for Equal Brightness $1/V_\lambda$	Energy ϵ $\times 10^{-12}$ per Quantum, $\epsilon = h\nu$	No. Quanta $\times 10^{15}$/ sec/Lumen, Q_λ	Relative Scotopic Luminosity Factor V'_λ
390	7.687	0.0001	10,000	5.092	28,870	0.00221
400	7.495	0.0004	2,500	4.964	7.406	0.00929
410	7.312	0.0012	833	4.843	2.529	0.03484
420	7.138	0.0040	250	4.728	777.6	0.0966
430	6.917	0.0116	86.20	4.618	274.5	0.1998
440	6.813	0.023	43.48	4.513	141.6	0.3281
450	6.662	0.038	26.32	4.413	87.70	0.455
460	6.517	0.060	16.67	4.317	56.75	0.567
470	6.378	0.091	10.99	4.225	38.24	0.676
480	6.246	0.139	7.194	4.137	25.57	0.793
490	6.118	0.208	4.808	4.052	17.43	0.904
500	5.964	0.323	3.096	3.972	11.41	0.982
510	5.878	0.503	1.988	3.893	7.508	0.997
520	5.765	0.710	1.408	3.819	5.421	0.935
530	5.656	0.862	1.160	3.746	4.553	0.811
540	5.552	0.954	1.048	3.678	4.188	0.650
550	5.451	0.995	1.005	3.611	4.092	0.481
555	5.402	1.000	1.000	3.578	4.110	0.402
560	5.353	0.995	1.005	3.546	4.167	0.3288
570	5.259	0.952	1.050	3.483	4.434	0.2076
580	5.169	0.870	1.149	3.424	4.936	0.1212
590	5.081	0.757	1.321	3.366	5.770	0.0655
600	4.996	0.631	1.585	3.309	7.044	0.0315
610	4.914	0.503	1.938	3.255	8.980	0.01593
620	4.835	0.381	2.624	3.202	12.05	0.00737
630	4.758	0.265	3.774	3.152	17.70	0.003335
640	4.684	0.175	5.714	3.102	27.08	0.001497
650	4.612	0.107	9.346	3.055	44.96	0.000677
660	4.542	0.061	16.39	3.008	80.14	0.0003129
670	4.474	0.032	31.2	2.963	154.8	0.0001480
680	4.409	0.017	58.8	2.920	296.1	0.0000715
690	4.345	0.0082	122	2.878	623.4	0.00003533
700	4.283	0.041	244	2.837	1,264	0.00001780

*Adapted from Boynton RM: Vision in Sidowski JB (ed): *Experimental Methods and Instrumentation in Psychology*, New York, McGraw-Hill International Book Co, 1966.

photopic and scotopic weighting functions for the standard observer. These two functions are known as the photopic relative luminous efficiency function (V_λ) and the scotopic relative luminous efficiency function (V'_λ). λ indicates that wavelength is varied. The prime indicates scotopic conditions. Each function has a maximum value of 1, is defined between 380 and 780 nm, and is zero at all other wavelengths. Table 25–1 indicates several other variables of interest in vision research, including the frequency (in tetrahertz, 10^{14} Hz) corresponding to wavelengths between 380 and 780 nm and the quantal energy at each frequency.

There are four photoreceptor types in humans: the rods, the short-wavelength sensitive cones (blue), the middle-wavelength sensitive cones (green), and the long-wavelength sensitive cones (red). Therefore, the scotopic relative luminance efficiency function represents the relative sensitivity of the rods and rod pathways as a function of wavelength. At photopic adaptation levels, cones function and rod signals are suppressed. The photopic weighting function represents the relative sensitivity of the set of three cone types and the cone pathways as a function of wavelength, specifically the sensitivity of the fovea.

Each cone type has its own weighting function (see Table 25–3).[3] Before these were known, it was recognized that almost all phenomena of color vision could be explained on the basis of three 'fundamental' mechanisms. Any natural light stimulates more

than one, but it is possible to define imaginary stimula that excite only one (or more class of cone). This is the basis of the CIE color coordinate system which defines hues in terms of imaginary primaries x, y, and z. The quantities are scaled so that x + y + z = 1. Thus the third dimension is implicit.

Light is transmitted in packets of energy called quanta or photons. The amount of energy contained in an individual quantum of light increases with its frequency. The initial event in vision is the activation of photopigments by individual quanta of light. Individual photoreceptors act much like quantum counters.

One important principle in photometry is univariance. The principle of univariance means that the response of any one photoreceptor varies from 0 to some maximum value based on two variables, the wavelength of the stimulus and the number of quanta of that wavelength that activates the photoreceptor. Any quantum that activates a photopigment has exactly the same effect, irrespective of its wavelength. The differential effects that are observed are due to the differential probabilities of quanta of different wavelengths activating a photopigment. Consequently, for a single photoreceptor type it is possible to make any two wavelengths elicit the same response by adjusting them so that the number of quanta activating the photopigment are the same. This is the basis for the use of silent substitution as a stimulation method (see below).

Additivity is a general assumption in the rules of photometry. It is assumed that if one wavelength has a relative brightness, A, and a second wavelength has a relative brightness, B, then if those two wavelengths were added, the stimulus would give a sensation of brightness equal to A + B, or C. Because only one photoreceptor type (rods) is involved, the assumption of additivity is generally valid for scotopic photometry. Because three photoreceptor types (red, green, and blue cones) are involved under photopic conditions, there may be large deviations from additivity but color matching and brightness matching obey additive laws if the intensities are not high. However, if a bright red light is added to an equally bright green light, the resulting yellow light may not appear twice as bright as either light alone, although it will appear brighter than either light alone.

Common Photometry Units

The candela is the basic unit of photometry and is one of the basic units of the Système International (SI) system. It is defined such that the luminance of a full radiator at a temperature of 2024 K (the temperature of freezing platinum) is equal to 6×10^5 cd m^{-2}. There are two related difficulties with the candela as a measurement unit. First, it is a basic unit only because of a definition and, second, the definition is made without reference to photopic or scotopic sensitivity. The weighting functions in Table 25–1 (and Table 25–2) are relative and dimensionless. Consequently, one customarily assigns a dimension *photopic* to V_λ and *scotopic* to V'_λ. To convert from radiometric to photometric units requires a conversion factor, K. The photopic function is maximal at 555 nm, and K_{max} is 673. The scotopic function is maximal at 510 nm, and K'_{max} is 1,725.

Table 25–3 illustrates the principle types of radiant and photometric measures. Understanding these units of measurement is dependent on understand-

TABLE 25–2.

Relative Spectral Absorbances of Human Cones*†

Wavelength λ (nm)	SWS‡ Cones	MWS‡ Cones	LWS‡ Cones
370	0.59		
80	0.67		
90	0.76		
400	0.88	0.35	0.36
10	0.96	0.35	0.36
20	1.00	0.34	0.33
30	0.96	0.34	0.30
40	0.86	0.35	0.29
450	0.68	0.38	0.28
60	0.50	0.42	0.30
70	0.36	0.49	0.34
80	0.25	0.56	0.39
90	0.18	0.67	0.47
500	0.12	0.78	0.55
10	0.08	0.88	0.63
20	0.05	0.95	0.73
30	0.03	0.99	0.83
40		0.99	0.91
550		0.93	0.96
60		0.82	0.99
70		0.67	0.98
80		0.53	0.93
90		0.40	0.85
600		0.29	0.74
10		0.20	0.61
20		0.14	0.47
30		0.09	0.34
40		0.06	0.24
650		0.04	0.16
60			0.105
70			0.068

*Data from Bowmaker JK, Dartnall HJA: *J Physiol* 1980; 298:501–511.
†These values do not allow for intraocular filtering and cannot be used as human "primaries."
‡SWS = short wavelength sensitive; MWS = medium wavelength sensitive; LWS = large wavelength sensitive.

TABLE 25–3.

Types of Radiant and Photometric Measures and Their Relationships*

	Radiant Measure		Photometric Measure	
Geometry of Rays	Type	Units	Type	Units
	Radiant energy	Joules (J)	Luminous energy	Lumen-second (lm-sec) (Talbot)
	Radiant flux	Watts (W) joules/second (J/sec)	Luminous flux	Lumens (lm) (1 candle emits 4π lm)
	Radiant intensity	Watts/steradian (W/sr)(1 sr = 1 unit of solid angle)	Luminous intensity (candle power)	Lumens/steradian (lm/sr) (1 lm/sr = 1 candela = 1 cd)
	Irradiance	Watts/square meter (W/m^2)	Illuminance	Lumens/square meter (1 lm/m^2 = 1 lux = 1 lx)
	Radiance	Watts/steradian/square meter ($W/sr/m^2$)	Luminance	Lumens/steradian/square meter ($lm/sr/m^2$) (1 $lm/sr/m^2$ = 1 cd/m^2 = 1 nit)
	—	—	Retinal illuminance	Trolands (1 cd/m^2 viewed through 1 mm^2 pupil) (T)

*Adapted from Armington JC: The *Electroretinogram.* New York, Academic Press, Inc, 1974.

ing their geometrical relationships. Figure 25–2 illustrates two surfaces that subtend the same solid angle. An ideal luminous source (a point source) will radiate in all directions. Therefore, at any distance r from the source one may create a sphere of radius r. The surface area of a sphere is $4\pi r^2$. A steradian (sr) is a solid angle subtended by a portion of the spherical area equal to r^2. Therefore, there are 4π steradians in a sphere.

As light radiates from the source, the amount of light (*luminous flux* or power, ϕ) passing through one steradian is always the same, as can be seen for the two surfaces in Figure 25–2. If the point source has a luminance of 1 candela (cd), the luminous flux

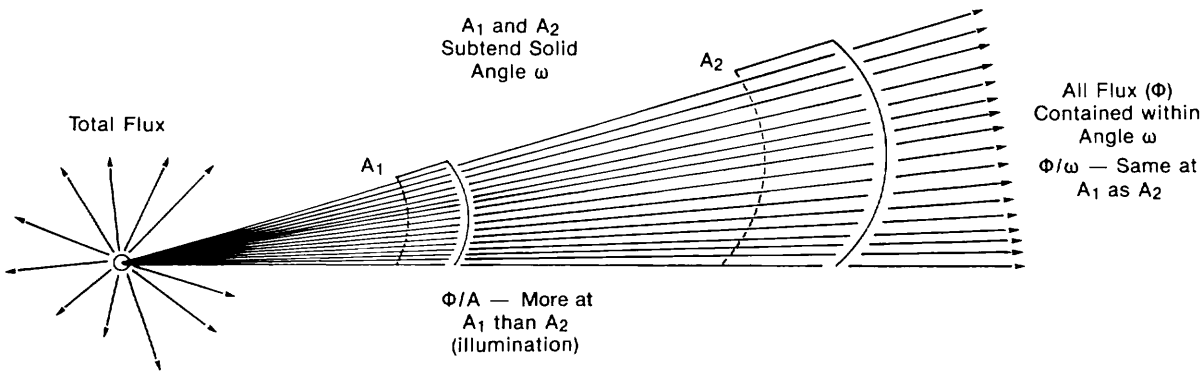


FIG 25–2.

Geometrical relationships associated with a point source. A point source emits luminous flux in all directions. A_1 and A_2 represent two surfaces at different distances from the source that subtend the same solid angle. The number of lumens is related to the flux per solid angle. Illumination is related to the flux per area subtended by the solid angle.

TABLE 25–4.
Conversion Factors for Illuminance Units*

	Lux	Phot	Milliphot	Foot-Candle
Lux (meter-candle)	1	0.0001	0.1	0.092903
Phot	10,000	1	1000	929.03
Milliphot	10	0.001	1	0.92903
Foot-candle	10.764	0.0010764	1.0764	1

*The value in units in the left-hand column times the conversion factor equals the value in units shown at the top of the column.

of 1 steradian is 1 lumen (lm); i.e., a point source of 1 cd emits 4π lm. A source may be considered a point source if it is measured at a distance no less than ten times the maximal dimension of the source. The *luminous intensity* or candlepower of a light source measured in candelas is provided by the number of lumens per steradian.

A primary light source is said to *illuminate* a surface. As can be seen in Figure 25–2, if the solid angle subtended by two surfaces remains the same as their distance from the light source increases, their area must increase with the square of the distance. The *illuminance* of each surface is equal to the total luminous flux within the solid angle subtended by the surface (lumens) divided by the surface area. If the area is measured in square meters, the measurement unit is a lux (lx). If the area is measured in feet, the measure is the foot-candle (fc or ft-c). Table 25–4 provides the conversion factors for various units of illuminance.

One measures the *luminance* of surfaces which reflect illuminated light (secondary light sources) or of extended primary light sources. Figure 25–3 illustrates the geometrical principles involved in measuring luminance. The measuring instrument (or eye) will occupy some solid angle relative to the projected surface. The total luminous flux reaching the instrument divided by the area of the projected surface is equal to the luminance of the surface. If the surface area is measured in meters, the unit of measurement is the candela per square meter or, in older units, the nit (nt).

If the surface represented in Figure 25–3 is a perfect diffuser, it is said to be a lambertian surface. The light exiting from its surface will radiate equally in all directions, and its luminance can be shown to be

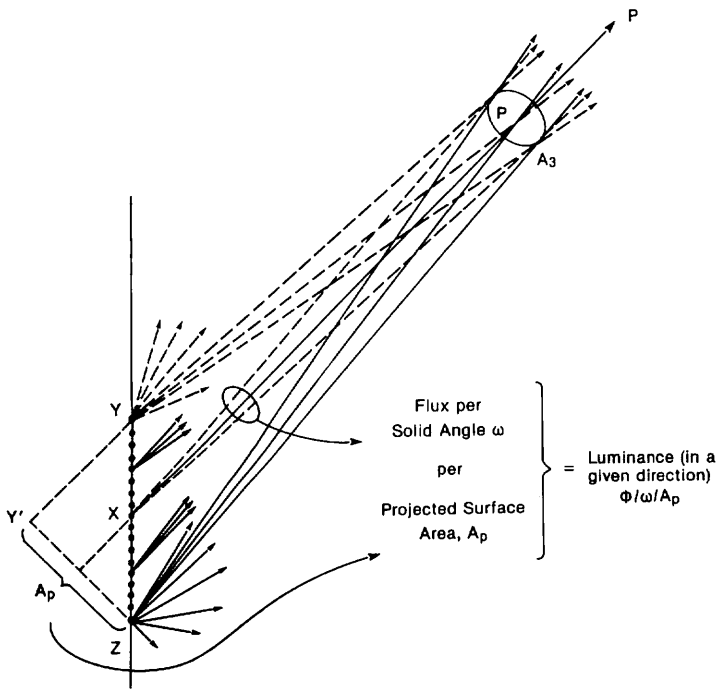


FIG 25–3.
Geometrical relationships associated with extended primary and secondary sources. Luminous flux is determined by the luminous emittance (primary) or exitance (secondary) of an extended source. The detector (or pupil) is the area $A_3(P)$ centered on line P at some angle from the source. Luminance in a given direction is the flux per solid angle per projected area.

TABLE 25–5.
Conversion Table—Units of Luminance*

	Nit	Stilb	Apostilb	Lambert	Millilambert	Foot-Lambert	Candle/ft^2
1 Candela per square meter (cd/m^2)	1	10^{-4}	3.14	3.14×10^{-4}	0.314	0.2919	9.29×10^{-2}
1 nit (nt) $\frac{1 \text{ Candela}}{m^2}$	1	10^{-4}	3.14	3.14×10^{-4}	0.314	0.2919	9.29×10^{-2}
1 stilb (sb) $\frac{1 \text{ Candela}}{cm^2}$	10^4	1	3.14×10^4	3.14	3.14×10^3	2.919×10^3	229
1 apostilb (asb) $\frac{1 \text{ Candela}}{\pi cm^2}$	0.3183	3.183×10^{-5}	1	10^{-4}	10^{-1}	9.29×10^{-2}	2.957×10^{-2}
1 lambert (L) $\frac{1 \text{ Candela}}{\pi cm^2}$	3.183×10^3	0.3183	10^4	1	10^3	9.29×10^2	295.7×10^{-2}
1 millilambert (mL) $\frac{1 \text{ Candela}}{\pi cm^2}$	3.183	3.183×10^{-4}	10	10^{-3}	1	0.929	0.2957
1 foot-lambert (fL) $\frac{1 \text{ Candela}}{\pi ft^2}$	3.426	3.426×10^{-4}	10.764	1.0764×10^{-3}	1.0764	1	0.3183
1 candle per sq. ft. $\frac{1 \text{ Candela}}{ft^2}$	10.764	1.0764×10^{-3}	3.382×10	3.382×10^{-3}	3.382	3.14	1

*Examples: x apostilb = x(0.1 mL) = x blondel; x nit = x(cd/m^2) = x(0.2919 fL)

equivalent to the luminance defined above divided by π. In the SI system, 1 apostilb is 1 cd divided by π times the area in squared meters. If the extended surface is an integrating sphere such as a cupola, the number of apostilbs would equal the number of candelas per square meter, i.e. there is no need for the factor π. Table 25–5 provides conversion factors for the various units of luminance.

The retina is illuminated by the external world. If in Figure 25–3 the region *P* represents the pupil, the total luminous flux entering the eye will increase as the pupillary diameter increases. The basic relationship is that *retinal illuminance* is luminance multiplied by pupillary area. Retinal illuminance is usually measured in trolands, 1 troland being defined as 1 cd/m^2 viewed through a 1-mm^2 pupil. The effectiveness of light in stimulating photoreceptors varies with pupillary eccentricity (the Stiles-Crawford effect). Consequently, the effective pupillary area is smaller than the measured area. Le Grand[12] suggested that retinal illuminance should be corrected for the Stiles-Crawford effect under mesopic and photopic conditions to determine the effective pupil area (see Table 25–6). The effective pupil size in square millimeters multiplied by the luminance is cd/m^2, yields the effective retinal illuminance as a function of luminance.

PROBLEMS IN MEASUREMENT

Illuminance and luminance are measured by using either a subjective or electronic photometer. Several models of each exist. Subjective photometers have a calibrated light source, and the luminance of the calibrated source is adjusted, usually with a neutral-density wedge, until it appears to have the same brightness as the light source of interest. If the source of interest is brighter than the brightest setting of the photometer, a neutral-density filter may be placed between the photometer and the source to reduce its brightness.

Digital electronic radiometer/photometers operate by measuring the output of a photosensitive device such as a photodiode. A chromatic filter is usually provided that compensates for the spectral sensitivity of the photosensor so that its output approximates the human eye's photopic relative luminous efficiency function. The measurements of digital photometers are more objective and reliable than those made by human observers. They also have some limitations. First, the filter provided only approximates the human luminosity function. The approximation is adequate as long as broad-spectrum light sources are measured. If the light is in a narrow band, the adequacy of the approximation may depend on the spectral region of the light. Second, few photometers have a scotopic filter. Therefore, at lower intensities the electronic photometer extrapolates the photopic function and does not provide a good approximation to the scotopic function, V'_λ. Therefore, direct measures of luminance and illuminance at mesopic and scotopic levels are not accurate when using electronic photometers unless the photometer comes with a scotopic filter or setting. Otherwise, the photopic values obtained must be converted to their scotopic equivalents.

In clinical electrophysiology, one must calibrate

TABLE 25–6.

Retinal Illuminance, Pupil Diameters, and Areas at Various Luminances*

L† (Candelas/m^2)	d (mm)	S (mm^2)	S_e (mm^2)	$L \times S$ (Trolands, T)	$L \times S_e$ (Effective Trolands, T_e)
1×10^{-6}	7.95	49.5	24.5	5.0×10^{-5}	
2×10^{-6}	7.94	49.4	24.5	9.9×10^{-5}	
5×10^{-6}	7.91	49.2	24.5	2.5×10^{-4}	
1×10^{-5}	7.89	48.9	24.4	4.9×10^{-4}	
2×10^{-5}	7.86	48.5	24.4	9.7×10^{-4}	
5×10^{-5}	7.81	47.9	24.3	2.4×10^{-3}	
1×10^{-4}	7.77	47.4	24.2	4.7×10^{-3}	
2×10^{-4}	7.70	46.6	24.1	9.3×10^{-3}	
5×10^{-4}	7.60	45.4	23.8	2.3×10^{-2}	
1×10^{-3}	7.50	44.2	23.6	4.4×10^{-2}	
2×10^{-3}	7.38	42.8	23.3	8.6×10^{-2}	
5×10^{-3}	7.18	40.5	22.8	0.20	
1×10^{-2}	6.99	38.4	22.3	0.38	
2×10^{-2}	6.77	36.0	21.6	0.72	
5×10^{-2}	6.43	32.4	20.5	1.6	
0.1	6.14	29.6	19.5	3.0	1.95
0.2	5.82	26.6	18.3	5.3	3.7
0.5	5.36	22.5	16.4	11.3	8.2
1	5.00	19.7	15.0	19.7	15
2	4.64	16.9	13.4	34	27
5	4.18	13.7	11.3	69	57
10	3.86	11.7	9.96	117	100
20	3.57	10.0	8.72	200	174
50	3.23	8.17	7.30	408.5	365
1×10^2	3.01	7.12	6.46	712	646
2×10^2	2.82	6.24	5.73	1.248×10^2	1.15×10^3
5×10^2	2.62	5.39	5.01	2.695×10^3	2.5×10^3
1×10^3	2.50	4.91	4.59	4.91×10^3	4.6×10^3
2×10^3	2.40	4.52	4.25	9.04×10^3	8.5×10^3
5×10^3	2.30	4.15	3.92	2.075×10^4	1.96×10^4
1×10^4	2.23	3.90	3.70	3.90×10^4	3.7×10^4
2×10^4	2.19	3.77	3.58	7.54×10^4	7.2×10^4
5×10^4	2.14	3.60	3.43	1.80×10^5	1.72×10^5
1×10^5	2.11	3.50	3.34	3.34×10^5	3.3×10^5
2×10^5	2.09	3.43	3.27	6.86×10^5	6.5×10^5
5×10^5	2.06	3.33	3.18	1.665×10^6	1.59×10^6

*Adapted from LeGrand Y: *Light, Color and Vision*, ed 2. Bloomington, Ind, Indiana University Press, 1967.
†L = luminance; d = pupil diameter; S = pupil area; S_e = effective pupil area; T = retinal luminance; T_e = effective retinal luminance.

the illuminance or luminance of brief, bright flashes in addition to constant background lights.[6, 14] Both subjective and electronic photometers have difficulties in quantifying these light sources. A light flash delivered by a xenon arc lamp rapidly increases in brightness, reaches its maximum brightness, and gradually diminishes in brightness. The total time period of the flash is brief, 10 to 20 μs. When stimulated by brief stimuli, the eye integrates over time (Bloch's law) so that the perceived brightness is less than the maximum stimulus brightness.[8] Because the eye integrates brightness over time, the appropriate photometric measures involve integration of illuminance or luminance measurements over time. Therefore, the appropriate measures are lux-seconds or cd/m^2-seconds. Most photometers are not calibrated in these units, but the units may be approximated by following several steps. First, determine the duration in seconds of the flash at one third of its peak amplitude (e.g., 10 μs). Second, determine the peak intensity (I) of the flash (e.g., 36,000 cd). Third, multiply these values (0.36 cd-sec). Fourth, convert to other units if necessary. To convert candela-seconds to lumen-seconds, multiply the candela-seconds by the surface area of measurement and divide by the squared distance from

TABLE 25–7.
Calibration of Flash Luminance When Using a Subjective Photometer

I. Determine the flash duration in seconds (i.e., the time period between the ascending and descending limbs at one-third amplitude) by using the manufacturers specifications or direct measurement by recording a photodiode output on an oscilloscope
II. Determine the maximum brightness
  A. Flash the strobe unit repetitively so that it appears as a constant, steady light
    1. The flash rate at which the light appears constant will be lower the dimmer the light; therefore, attenuate the strobe intensity by using neutral-density filters
    2. Strobe candle power may vary with the intensity setting and the repetition rate
  B. Match the luminance of the standard and the steady light
  C. Multiply the flash duration by the number of flashes per second to obtain the total flash duration during 1 sec.
  D. Divide 1 sec by C to obtain the weighting factor
  E. Multiply the luminance determined in I by D
  F. Adjust E by any attenuation of the strobe, e.g., neutral-density filters, etc
III. Multiply I and IIF to obtain the integrated light value
IV. Convert to the desired units. If the flash is presented in an integrating sphere and luminance was calculated in lamberts (fL, mL, etc.), the factor π is not required in converting to other values

the flash (D^2). Several authors propose strategies that involve nonintegrated light measures.[7, 9] The use of digital and subjective photometers in performing these steps are detailed in Tables 25–7 and 25–8.

SCOTOPIC/PHOTOPIC EQUIVALENCE AND SILENT SUBSTITUTION

One may wish to compare the ERG or VEP elicited by stimuli of two wavelengths whose luminances have been either scotopically or photopically matched. The two most common objectives are to isolate specific photoreceptor mechanisms by using silent substitution and to separate rod and cone mechanisms by using chromatic stimulation, i.e., one selects two wavelengths, usually a short and long wavelength, that give very similar responses in the dark-adapted eye and then observes their responses as the adaptation level is increased.

Matching the photopic wavelengths of two sources is relatively easy. One may use any of several psychophysical procedures, subjective photometers, or an electronic photometer. One should be aware that while any of these methods is acceptable, the matches obtained will differ depending on the method. If one has an electronic photometer, photopic matches may be performed more simply and more reliably. One (1) measures the luminance or illuminance of the two sources and (2) adjusts their output, usually by using neutral-density filters, until the photometric readings are equal.

Determining the equivalent scotopic luminances

TABLE 25–8.
Calibration of Flash by Using a Digital Photometer

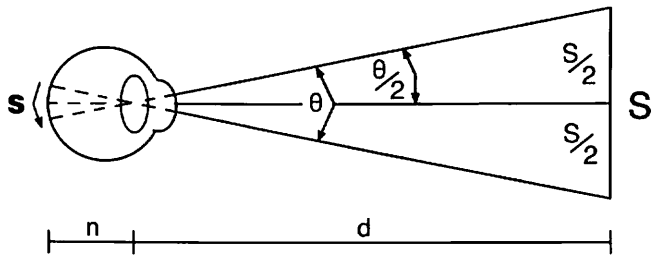
I. Determine the period in seconds between the rising and descending limbs of the flash at one-third amplitude
II. Determination of peak intensity
  A. Flash the strobe repetitively
  B. Measure the luminance (illuminance)
  C. Calculate the total flash period (flash period × frequency in Hz)
  D. Divide 1 sec by total duration (c)
  E. Multiply the calculated value in B by the conversion factor in D
III. Multiply the duration (I) by the peak intensity (IIE) to obtain the integrated light value
IV. Convert to desired units. If the flash is presented in a full-field integrating sphere, the conversion from lambertian luminances to other values do not require the factor π; Therefore, values obtained by using Table 25–7 should be divided by 3.1416

of two wavelengths is relatively straightforward if one employs either a psychophysical approach[22] or a subjective photometer. In either case, one (1) selects the wavelengths of the two sources; (2) attenuates them, probably by using neutral-density filters; (3) dark-adapts the observer (the light sources should be dim enough that they appear approximately gray to the dark-adapted observer); and (4) further adjusts the light sources, probably by using neutral-density filters, until they appear of equal brightness to the dark-adapted observer (i.e., the observer adjusts the subjective photometer to the same value for the two light sources).

Scotopic matching by using an electronic photometer is less straightforward than in the photopic case unless the photometer has a scotopic filter or setting. An approximation of the scotopic luminance of a *narrow-band source* may be calculated by using the formula Sx = Px (V'_λ/V_λ) where Px indicates the measured photopic luminance and Sx is the calculated scotopic luminance.

Silent substitution is a strategy for eliciting ERG or VEP responses from single-photoreceptor mechanisms. It is based on the principle of univariance (see above). The basic strategy is to alternate between two wavelengths of light that effectively isolate specific photoreceptor mechanisms. For the sake of simplicity, assume two receptor types. If the two wavelengths are selected such that they are equally effective in stimulating receptor type 1 but one wavelength is much more effective in stimulating receptor type 2, substitution of the two wavelengths will be silent for receptor type 1 and elicit a response in receptor type 2 that is proportional to the chromatic contrast between the two wavelengths for receptor type 2.[11, 16] To employ silent substitution one must estimate the relative response of the four photoreceptor types and especially for the three cone types. Such information is provided in Tables 25–1 and 25–2. Further estimates are available in the work of Wyszecki and Stiles.[22]

To isolate red cones from the green cones, one might (1) choose two wavelengths above 550 nm, e.g., 550 and 650, so that the blue cones would not be stimulated; (2) adjust the brightness of the two lights so that they would be equally bright for the green mechanism; and (3) alternate the two lights. The isolation of blue cones would be similar, but one would employ wavelengths of about 450 and 520 nm. The brightness would be matched for the red and green cones. Adaptation of rods might be ensured by the addition of a middle-wavelength adapting light of about 570 nm.



$$S = 2[\arctan \frac{(S/2)}{d}] \text{ or } \arctan \frac{S}{d} \text{ in degrees}$$

$$s = \frac{nS}{d} \text{ or } \frac{17S}{d} \text{ in millimeters}$$

FIG 25–4.
Calculation of visual angles and retinal image size. Visual angles are determined relative to the nodal point(s) of the eye, although this is often approximated by measuring the distance from the object to the cornea (or less commonly the pupillary plane). Retinal image size is calculated depending on the distance from the nodal point to the retina, about 17 mm.

SPECIAL TOPICS

Maxwellian view and line or point targets are seldom used in clinical electrophysiology. However, if the reader chooses to employ these stimulation procedures, he is referred to the indicated references on the topic.[2, 15, 20, 21]

Contrast is a ratio of the brightness of two light sources or of two regions of the same source. If the stimulus is a symmetrical, repeating pattern, the Michelson formula is usually employed: $(L_{max} - L_{min})/[(L_{max} + L_{min})]$. If the stimulus is asymmetrical or nonrepeating, several different formulas may be employed, depending on the aim. The most common is L^l/L^b, i.e., the brightness of the target region (usually the dimmer area) is divided by the brightness of the background (usually the brighter area).

Visual angle may be calculated in several different ways as well. One difficulty is that the eye is spherical while most measured objects are approximately flat. Consequently, there is a slight error in the calculations. Technically, visual angle is measured from the nodal point. Figure 25–4 illustrates the geometry of the calculation of visual angles and retinal image size.

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