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Photostimulator allowing independent control of rods and the three cone types

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Abstract

This report describes a second-generation photostimulator with four primary lights that allows independent control of the stimulation of the four receptor types in the human eye. The new design uses LEDs (with light levels controlled by eight drivers that include voltage-to-frequency converters that provide 1-μs pulses at frequencies up to 250 kHz), with four center channels being combined by use of a fiber optic assembly, and likewise for four surround channels. Four fiber optic bundles are merged into a single bundle whose output is fed into a spatial homogenizer terminated by a diffuser. An interference filter is sandwiched between each LED and the fiber optic bundle. Two camera lenses collimate light from the diffusers, one for center and one for surround. The center-surround field configuration is formed by a photometric cube with a mirrored ellipse on the hypotenuse. A field lens places images of the diffusers in the plane of an artificial pupil. The fields are highly uniform. Following alignment and calibration, the center and surround fields are indistinguishable. An observer calibration procedure, designed to compensate for prereceptoral filtering, is shown by calculation to correct also for normal observer receptoral spectral sensitivity variation. With the instrument calibrated for the individual observer, a peripherally fixated 200-ms 40% contrast rod center field pulse, highly conspicuous under dark adaptation, is invisible following light adaptation.

Keywords: Optics, Instrumentation, Colorimeter, Rods, Cones, Prereceptoral filtering

Introduction

Shapiro et al. (1996) gave a theoretical introduction to the use of a four-primary colorimetric system to achieve independent control of the stimulation of the four receptor types in the human eye. They developed a general method for adjusting the radiances of four lights to silence up to three photoreceptor classes and change the excitation of the nonsilenced classes in a specified manner (see also Knoblauch, 1995). Sun et al. (2001*a–c*) described the implementation of an eight-channel photostimulator. The device had eight channels, four for a central field and four for a surround, and thus allowed generation of center-surround differences visible to any combination of the four photoreceptors.

The channels originated from light-emitting diodes (LEDs) and interference filters (10-nm half-bandwidth). The eight channels were optically combined in a Maxwellian view system. To obtain a homogenous field, eight channels had to be aligned precisely relative to each other. This required a rigid mounting of over 40 optical components; alignment was a laborious procedure. The design goal for the Generation II photostimulator was to create an optically simpler device that would be relatively easy to construct and align.

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Materials and methods

Design

Fig. 1 shows the optical layout; Fig. 2 is an overhead view photograph of the instrument. The dimensions are length, 760 mm; width, 180 mm; and height, 120 mm.

The new design continues to use LEDs and interference filters to produce narrow-bandwidth primaries. The primary wavelengths are near those of the Generation I photostimulator: 460, 516, 558, and 660 nm. These primaries were chosen to allow high modulation of each of the four photoreceptor types within the constraints of the spectra and efficiencies of commercially available LEDs. When viewing a light metameric to the equal energy spectrum, the maximum modulation depths for the four photoreceptor types are in the range of 20–40%.

The four center channels are combined by use of a fiber optic arrangement that has four separate fiber optic bundles merging into a single bundle, with random spatial locations for the individual fibers composing the bundle (custom made by Fiberoptics Tech, Inc., Pomfret, CT. Technical description: four branch randomized light guides, 1 foot long, 3/16 inch at branch bundle, 3/8 inch at collective and PVC sheathing). An interference filter and a slot to allow insertion of a neutral density filter are sandwiched between each LED and the fiber optic bundle. The output of the bundle is coupled to an integrating bar that serves as a spatial homogenizer.

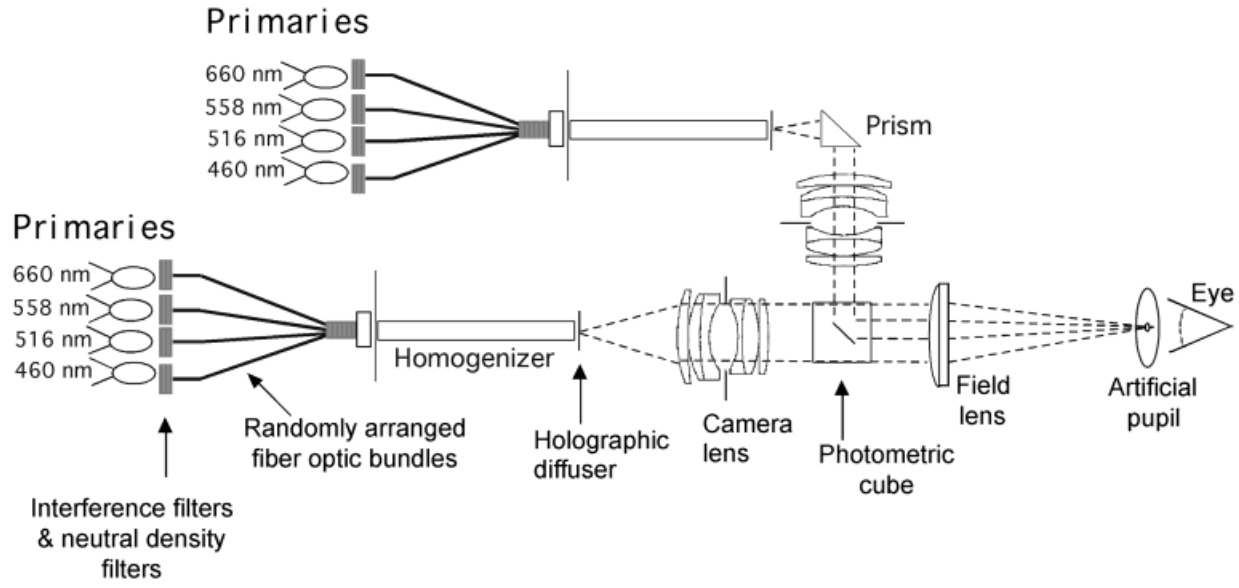

Fig. 1. Optical layout of the photostimulator.

The integrating bar is a 100-mm length of clear acrylic 9.525 × 9.525 mm square rod with 1.27 mm raised lathe cut 9.525 mm circles at each end that are then polished. The integrating bar is mounted in a stainless-steel housing that couples to the 3/8-inch termination of the fiber optic bundle. Holographic light-shaping diffusers (30 deg, Physical Optics Corp, Torrance, CA) at the ends of the center and surround integrating bars serve as sources for the Maxwellian view. For compactness, the center and surround channels are parallel to each other; a right angle prism deflects the center beam 90 deg for combination with the surround channel. Two 35-mm camera lenses (Minolta Maxxum AF50/1.7) collimate light from the diffusers.

A photometric cube creates a central circular field within a surround that is delimited by a circular aperture. The cube is formed by cementing together two right angle prisms, one with a mirrored ellipse (3 × 4.24 mm) on its hypotenuse (we are not aware of a commercial source for photometric cubes; from past experience, custom fabrication can be expensive). Stimulus configurations other than center-surround can be produced inexpensively (e.g. a bipartite field created by the edge of a front surfaced mirror). A field lens (105 mm coated achromatic doublet, Rolyn Optics, Covina, CA) places images of the diffusers in the plane of an artificial pupil.

The LED light levels are controlled by drivers that include voltage-to-frequency converters (Texas Instruments VFC320CP) that provide 1-μs pulses at frequencies up to 250 kHz (Swanson et al., 1987). In the output circuit, the LED cathode is connected to ground and the anode is connected to +15 volts through a current-limiting resistor. The output of the voltage-to-frequency converter is interfaced to the LED by a Signetics SN75451 driver. The NPN output transistor of the SN75451 shunts the LED, and is set to conduct to provide the LED off-state. This low-impedance output circuit makes the length and positioning of the driver to LED cables noncritical. The light levels are controlled by 12-bit digital to analog converters on a National Instruments (Austin, TX) interface board (NI PCI-6713) in an Apple Macintosh G4 computer.

Narrowband primaries are created by filtering the LED outputs with circular 1-inch, INTOR (Socorro, NM) 10-nm half-bandwidth interference filters (IF). The LED specifications and the interference filter peak wavelengths are in Table 1. Each LED is mounted on a small heatsink to minimize thermal effects (Watanabe et al., 1992). The thermal stability of each LED was assessed by monitoring the light at the exit of the optical device over time following a step from the off-state to the maximum light output level. The Nichia LEDs all showed drifts of $<$1% over 1 min with the current set at the maximum recommended by the manufacturer (150 Ohm current-limiting resistor). Corresponding thermal stability for the Marktech LED could be achieved at a lower current (180 Ohm current-limiting resistor). Light levels are stable over time, remaining unchanged with routine use of the instrument for a period of more than 1 year.

The device can produce retinal illuminances $\geq$300 trolands throughout the chromaticity gamut. The LED-interference combination that produced the 560-nm primary limited the maximum light output. We did not locate a highly efficient LED with maximal output near 560 nm. The Nichia NSG500S-HT with a peak near 535 nm and a broad spectral distribution proved more efficient than LEDs with longer wavelength peaks when combined with the interference filter. If higher light levels are required with reasonably narrowband primaries, the maximal output of the system can be increased by a factor of 2.5 by substituting a Toshiba TLPGE23TP LED (distributed by Marktech, Menands, NY) for the Nichia LED-interference filter combination. This produces a 558-nm dominant

Table 1. *Primary LED and interference filter combinations*

Primary	LED	Interference filter
460 nm	Nichia[a] NSB500S	460.0 nm
516 nm	Nichia[a] NSG500S-GT	514.5 nm
558 nm	Nichia[a] NSG500S-HT	560.0 nm
660 nm	Marktech[b] MT5000A-UR	660.0 nm

[a]Mountville, PA.
[b]Menands, NY.

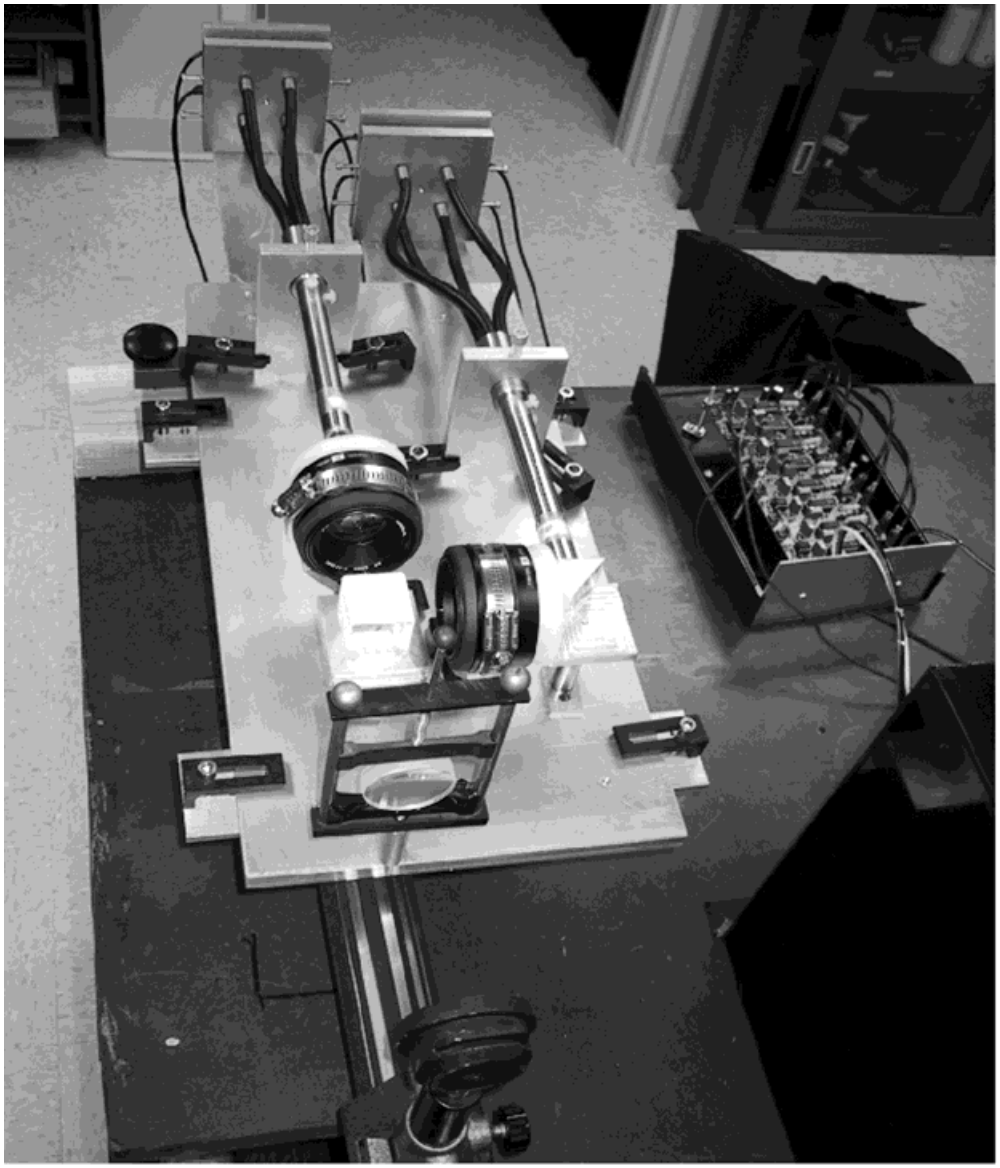

Fig. 2. Overhead photograph of the photostimulator. The LEDs are mounted on the far side of the metal housings at the top, and the artificial pupil at the bottom. At the right is the 8-LED driver printed circuit board.

wavelength, 14-nm hbw primary with a thermal stability of ~2% (150 Ohm current-limiting resistor). For applications where narrow-band primaries are not a requirement, the LEDs without interference filters are capable of producing >10,000 trolands of light metameric to the equal energy spectrum. For this, the device in essence becomes a three-primary system since the primary wavelengths (and half-bandwidths) are 660(20), 520(44), 516(40), and 462(28) nm.

To realize the light level resolution afforded by the D/As, it is necessary to set the light levels of the individual LEDs so the maximum to be employed is achieved near the maximal D/A output. Thus, in this four-LED system, the maximal light level is determined by the dimmest LED with the light levels of the other three of the LEDs being attenuated. There are three ways this can be accomplished. First, the current-limiting resistor can be changed. This has the disadvantage that an LED change would necessitate disassembling the driver and soldering in the correct resistor. The second method is to attenuate the D/A output voltage with a potentiometer serving as a voltage divider. This works for small adjustments (our drivers allow for attenuation by a factor of 2). However, making large changes in maximal light level by greatly reducing the operating range of the voltage to frequency converter results in a loss of precision. The third method is to insert neutral density filters in the LED light paths. Our procedure for setting light levels is as follows: After establishing the attenuation required for a given channel, we make a coarse adjustment with a polyester neutral density filter (Lee Filters, Burbank, CA) that can be placed adjacent to the interference filter, and then we make a fine adjustment of the input voltage to the driver.

Calibration

There are two stages of calibration, one dealing with the light output of the instrument and the other with the observer. The first stage of calibration is a physical measurement of the spectral and luminance output of each LED-interference combination, and a characterization of the light output of each LED as a function of input voltage. To implement a four-primary colorimetric system, it is also necessary to have accurate estimates of an individual observer's receptoral spectral sensitivities, in our case, expressed at the cornea (i.e. the product of the receptoral spectral sensitivity and prereceptoral filtering). The sensitivities should correspond to the retinal eccentricity at which the experiment will be carried out. The purpose of the second stage of calibration is two-fold. It corrects for individual differences in prereceptoral filtering, and it obviates the need for precise calibration of the relative energies of the primaries. The second stage of the calibration is therefore to ask the observer to set a photopic color match that allows us to correct for differences in (corneal) spectral sensitivity between our observer and the standard.

Physical calibration

The spectral output of each of the primary lights was measured at 2-nm intervals with an Optronics (Orlando, FL) OL754 spectroradiometer positioned at the eyepiece. The pulse-frequency modulation technique offers high LED output linearity over a 3-log-unit range for modulation around a mean level (Swanson et al., 1987). However, as described by Sun et al. (2001*a*,*b*), sustained changes in mean level, which presumably result in thermal effects due to the level of current in the diode junction (Watanabe et al., 1992), did produce small but systematic deviations from linearity. Linearization of each LED output was achieved by fitting low-order polynomials to measurements of light output that were obtained with a PIN silicon photodiode, a current amplifier, and a Fluke (Everett, WA) precision voltmeter. Second-order polynomials were sufficient for the 460-, 516-, and 558-nm primaries; a third-order polynomial was required for the 660-nm primary.

The maximum photopic illuminance output of the center 558-nm LED was measured with an EG&G (Gaithersburg, MD) 550 radiometer/photometer and was used as a reference to calculate the illuminances of the other LEDs.

Observer calibration

Sun et al. (2001*a*) developed a method to assess whether, at the wavelengths of their primaries, an observer's photopigment spectral sensitivities (expressed at the cornea) could be characterized by linear transforms of the CIE (1964) 10-deg Standard Observer sensitivities. The strategy exploited the fact that there are no known rhodopsin polymorphisms in human observers with normal visual function (Sung et al., 1991). This means that differences in scotopic luminance sensitivity for an individual observer and the Standard Observer can be solely attributed to differences in prereceptoral filtering. Scotopic matches can be made for wavelengths where the scotopic sensitivity is higher than the photopic sensitivity, in Sun et al.'s case for the 459-, 516-, and 561-nm primaries. At long wavelengths, however, the cones and the rods have similar sensitivities, preventing the use of a scotopic luminance match to calibrate individual prereceptoral filtering of the 664-nm primary. For this primary, Sun et al. (2001*a*) used a photopic color match between a mixture of the 459-nm and the 561-nm primaries ("blue" + "greenish-yellow"), and a mixture of the 516-nm and 664-nm primaries ("green" + "red"). A chromaticity diagram with the locations of the primaries is shown in Fig. 2 of Sun et al. (2001*a*). For an individual observer, the values of the 459-, 516-, and 561-nm primaries were first corrected for the individual prereceptoral filtering differences measured by the scotopic luminance matches, and then fixed at the matching values of the Standard Observer for the color match. With a button press the observer toggled between the two mixtures. If the observer could make a color match by adjusting only the radiance of the 664-nm primary, his corneal spectral sensitivities at the four primary wavelengths could be approximated by linear transforms of the Standard Observer data. The difference between the 664-nm setting for the individual observer and the Standard Observer will be largely due to differences in prereceptoral transmittance at 664 nm, but may also include differences in receptoral sensitivity.

The above procedure is arduous since the scotopic matches need be made with the very high precision of color matches. Given that the seven observers who went through this procedure were all able to make a satisfactory color match with the three primaries set for the Standard Observer, we decided to simplify the procedure. The appropriateness of this simplification is confirmed by calculations presented below.

We asked observers to make photopic color matches for the central field viewed at a retinal eccentricity of 4 deg. The procedure was similar to the color match described above. Observers toggled between two displays, one containing a mixture of 460- and 558-nm lights, the other a mixture of 516- and 660-nm lights. The 558-nm primary served as the reference, and the observer made a match by varying three parameters: the luminance of the 460-nm light, the luminance ratio of the 516- and 660-nm lights, and the combined luminance of the 516- and 660-nm lights. By comparing the relative radiances of the four lights required by the individual, with the values theoretically required by the CIE (1964) 10-deg Standard Observer, we estimated the differences in sensitivity between the individual and the Standard Observer at the wavelengths of our primaries. As above, this method assumes that an individual observer's spectral sensitivities at the primary wavelengths do not differ significantly from linear transforms of the Standard Observer color-matching functions.

Our observer-calibration method can be shown to be effective by calculation. In practice, the standard deviation on our empirically determined match settings results in a residual uncertainty in receptor class isolation of around $\pm 2\%$. How about the now well-documented variation in the L- and M-cone spectral of color-normal individuals? Consider, for example, the common L (A180) and L (S180) polymorphism, which causes a shift in the corneally measured L-cone λ_{max} of about 2.6-nm (Sharpe et al., 1998). Without correction, a light that offers a 25% modulation to the M-cone of a CIE (1964) 10-deg Standard Observer (while silencing the other receptors) would present a 3.2% L-cone modulation to a 10-deg L (A180) observer (modified 10-deg fundamental calculated as described by Stockman & Sharpe, 2000). This artifact is reduced to 1.7% by scaling the LED outputs by the differences in radiances required by the two observers for the color match.

With eccentric fixation, the surround field may include areas with substantial differences in prereceptoral filtering. If an observer was to perform the same observer-calibration procedure as was described for the center field, the match for the surround could differ from the match for the center. When presented together, the center and surround might not match. For this reason, we com-

pleted our calibration by requiring observers to make pair-wise matches between center and surround fields for each of the LEDs.

The observer-calibration procedure should be tailored to suit the planned experiments. The method described here works well for detecting 2-deg pulses at 4-deg eccentricity in a steady surround. Other possibilities include making the color match in the surround (if this is to be used as the test field), and then fixing the center settings with the paired-matching technique. Additionally it may sometimes be more appropriate to use a 2-deg standard observer, rather than a 10-deg observer.

The fields are highly uniform and, following alignment and calibration, the center and surround fields are indistinguishable. With the instrument calibrated for the individual observer, a peripherally fixated 200-ms 40% contrast rod center field pulse, highly conspicuous under dark adaptation, is invisible following light adaptation (at 300 photopic trolands or 620 scotopic trolands). This is consistent with Aguilar and Stiles' (1954) measurements on rod saturation. At a field intensity of 2.8 log scotopic trolands, they measured increment thresholds of around 2.5 log scotopic trolands. Importantly, this indicates that our observer calibration, performed under photopic conditions, did indeed afford accurate isolation of the rods.

In principle, the instrument can be used with nonhuman primates or other animals. What is required are accurate estimates of the spectral sensitivities of the photoreceptors, either at a retinal or corneal level, and the identification of a behavioral or physiological response that is determined only by a single receptor system. By finding the settings of the primary lights that yield equivalent response in this single receptor system, the experimenter can determine the correction factors required to calibrate the output of the primary lights. The amount of light from each of the primaries (specified in relative energy units) that is required to yield equivalent response, should be set in inverse proportion to the receptoral sensitivity (specified at the level of the retina or cornea) at the wavelength of the primary. For nonhuman primates, as for humans, this condition can be satisfied by the rod spectral sensitivity at three of the primary wavelengths. A photopic color match analogous to that described for humans can provide the correct normalization for the long-wavelength primary. The number of primaries needed to silence all but one of the receptors is equal to the number of receptor spectral sensitivities. Thus for a dichromatic species, three primaries would suffice, allowing independent modulation of rods and two cone types.

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