Implementing receptive fields with excitatory and inhibitory optoelectrical responses of bacteriorhodopsin films

Hiroyuki Takei, Aaron Lewis, Zhongping Chen, and I. Nebenzahl

The sign of the optoelectrical response of bacteriorhodopsin is highlighted as a means to emulate excitation and inhibition in neural computation. A classic example of a neural computation that is based on such excitation and inhibition is chosen to highlight the unique applicability of bacteriorhodopsin in highly parallel computational schemes. The classic example chosen is that of the ganglion receptive field, which is a fundamental element in retinal edge detection. Dried bacteriorhodopsin films are constructed that effectively act as receptive fields because of the sign of their photoresponse. The results on these simple bacteriorhodopsin receptive fields are extended to schemes that incorporate with greater elegance this unique ability of bacteriorhodopsin to exhibit excitation and inhibition. Experiments are presented that test some of these advanced ideas in bacteriorhodopsin parallel computation. *Key words:* Bacteriorhodopsin, molecular electronics, biomolecular device, edge detection, optoelectrical device, excitatory and inhibitory transduction.

I. Introduction

Parallel computation based on neurobiological principles is presently an area of great interest in terms of both advancing our knowledge on the fundamental basis of how the brain works and developing devices that can emulate neural networks. Although a number of schemes for such computational device applications have been suggested [for examples, see Ref. 1 for detailed descriptions of analog very large scale integrated circuit (VLSI) technology], none of the materials that are presently being considered has the inherent ability at the material level to mimic a crucial component which is present in all neural networks. Specifically all neural networks have as a fundamental component an ability to elicit excitatory and inhibitory responses which are essential for neural processing. Emulating such excitation and inhibition with semiconductor materials requires differential amplifiers with a large number of active elements that need a large area of chip implementation. In this paper we present a molecule that has, in addition to its photochromic character, intrinsic optoelectrical characteristics that can simulate both excitation and inhibition. The unique molecule that encompasses these characteristics is a relative of the visual pigment rhodopsin and is called bacteriorhodopsin (bR). The object of this investigation is to demonstrate the bR emulation of excitation and inhibition by simulating some elementary properties of the ganglion receptive field found in the retina.

Receptive fields are extensively used in all stages of visual processing. By performing real-time spatial filtering and differentiation operations on an image projected on the retina, ganglion receptive fields provide preliminary information needed for the detection of a sharp intensity variation in the image space, so-called edge detection. In addition to defining spatial variations, retinal receptive fields also have the ability to detect the motion of objects by having different response times for the excitatory and inhibitory regions of the receptive field. Furthermore, these intensity and temporal variations are accomplished with high spatial resolution.

The bR films have the ability to mimic all these properties of retinal tissue. The resolutions of the films are better than 0.17 μm and probably can be considered to have molecular resolution.[2] The excitatory and inhibitory response times can be made to be different, and eventually the technology discussed in this paper can be extended to 2-D computations. However, in this paper we focus on the excitatory and inhibitory nature of the transitions and consider two

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different approaches for obtaining receptive fields with bR.

A. Bacteriorhodopsin Characteristics

Bacteriorhodopsin is the sole protein found in a specialized bacterial membrane called the purple membrane (PM). This membrane grows in the bacterium *Halobacterium halobium* and is the only crystalline membrane found in nature.[3] The molar extinction coefficient of bR at 570 nm, 63,000 M^{-1} cm^{-1}, is one of the highest among biological pigments.[4] Oesterhelt and Stoeckenius[4] discovered that bR acts as a light driven proton pump that generates a proton gradient across the purple membrane. Energy stored in the proton gradient is subsequently utilized in nature to synthesize energy rich ATP.

Bacteriorhodopsin is composed of a retinal light absorbing chromophore embedded in a protein. When a photon is absorbed by the retinal chromophore, a photocycle consisting of a number of photointermediates is initiated with a quantum efficiency of 0.3.[5] Within a picosecond after light absorption, the retinal undergoes a molecular transformation, which can be detected as an electrical signal due to vectorial charge movements induced by light.[6] The ensuing change in the electrical charge environment induces a shift in the absorption spectrum of the retinal, which can be seen in the *K* intermediate of the photocycle in Fig. 1. Although the exact number of photointermediates and pathways connecting them have not been established unequivocally, Fig. 1 shows the generally accepted scheme for the photocycle.[7] Each photointermediate is characterized by a distinct absorption spectrum as shown in Fig. 2, and in physiological conditions, the cycle is complete in 10 ms.[8] Most of the transitions are thermal in nature, and by varying the temperature, pH, relative humidity, etc., the lifetimes of these photointermediates can be altered significantly.

A number of technical applications have been proposed to exploit some of the above unique characteristics of bR. In the area of computation these include the use of bR films as a neural network[9] and an optical memory.[10,11] In this paper we describe the first workable computational device that we know of that is based on bR. We demonstrate the fabrication of edge detection hardware based on long lasting, thin, and dried bR films with controlled heterogeneity. The results of our studies are encouraging for the eventual exploitation in a number of ways of the capabilities of the bR molecules in molecular electronics applications.

B. Edge Detection

Edge detection is one of the most important tasks in the early stages of pattern recognition in biological as well as artificial visual systems. The visual system in higher vertebrates is characterized in part by its massive parallelism, spatial filtering, and differentiation capabilities within the retina. There have been numerous attempts to emulate similar preprocessing capabilities in artificial implementations. The goal of

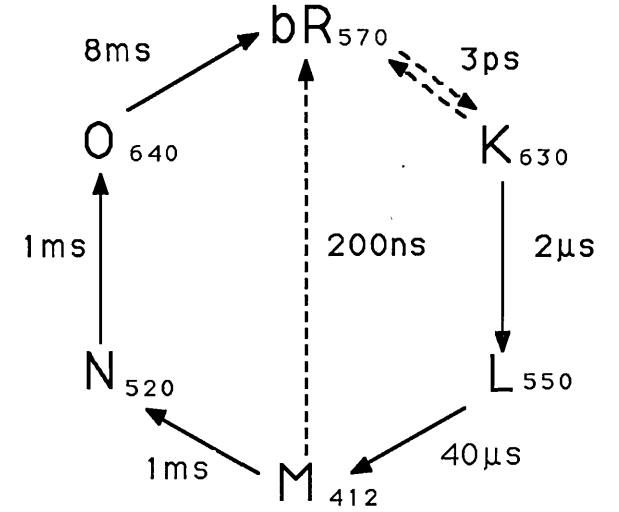

Fig. 1. Generally accepted photocycle of light adapted bR. Photochemical reactions are indicated by broken arrows and thermal reactions are indicated by solid arrows. Subscripts accompanying each photointermediate are the wavelengths in nanometers at the absorption maximum.

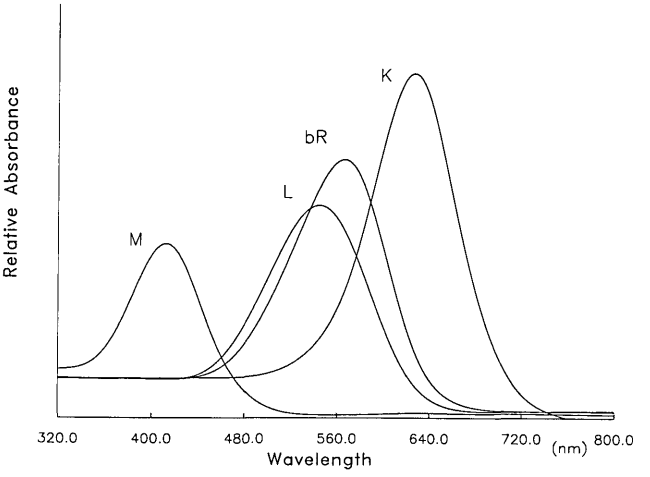

Fig. 2. Absorption spectra of bR and some of its photointermediates.

achieving real-time edge detection has been pursued with electrical, optical, or hybrid hardware. One recent attempt by Mead and Mahowald[12] resulted in an artificial retina based on the VLSI technology. Another approach by Armitage and Thackara[13] takes the form of an optical implementation that is based on a combination of a photoconductive material and a liquid crystal.

Edge detection involves the detection and location of spatial variations in image intensity. Detection of spatial variations is generally achieved by a combination of a spatial smoothing process of the original image followed by a spatial differentiation operation. Preliminary smoothing is necessary because a differentiation operation is highly susceptible to high frequency noises, which can be particularly difficult to eliminate in real-time applications where time averag-

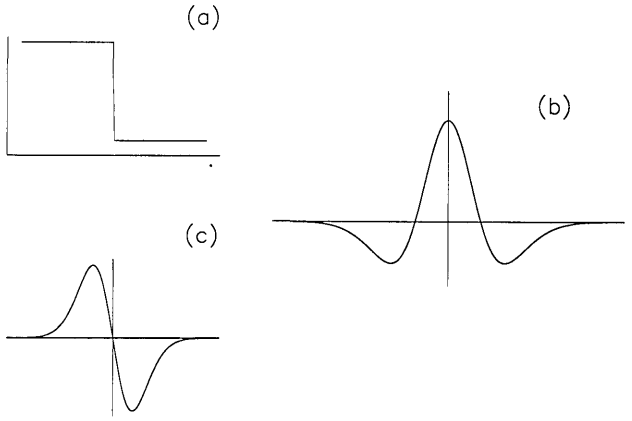

Fig. 3. Schematic demonstration of how a zero crossing (ZC) of the second derivative can be used to locate an edge: (a) sharp edge in intensity; (b) DOG filter; (c) result of processing (a) with (b). The location of the sign change in (c) coincides with the position of the edge in (a).

ing is not possible. Rodieck and Stone[14] were among the first to give a mathematical description of an edge detection scheme in visual perception. Using the concept of the receptive field as the basic unit of edge detection, where an excitatory region is surrounded by an inhibitory region, they proposed that the profile of the response of this receptive field to light could be described as a difference of two Gaussian distributions (DOG) with different sigma values. One significant aspect of the DOG is that it is a good approximation to the spatial second derivative of a Gaussian distribution.[15] If the receptive field can be so described, projecting an image on the receptive field amounts to the following: first, features in the projected image are smoothed by a Gaussian filter, and then the second derivative of the smoothed image is taken.

Such a detector that is capable of obtaining the spatial second derivative of the image intensity can be used to locate a sharp spatial variation or in other words an edge.[15] Figure 3(a) portrays such an edge. The result of processing this edge with the DOG filter shown with Fig. 3(b) is Fig. 3(c). The presence of an edge in (a) leads to a change in the sign of the second derivative in (c). Such a point is called a zero crossing (ZC), and its detection leads to the location of an edge in the image. Note that in Fig. 3 the sign of the graph in (c) is opposite the actual second derivative, because the filter in (b) has an excitatory rather than inhibitory region in the center.

The present paper employs bR to form receptive fields. Such bR receptive fields can subtract and differentiate with molecular resolution. Both these operations cannot be implemented in other devices without an additional external connection. Due to the ability of bR to imitate both excitation and inhibition, the bR receptive fields can effectively compute functions even with negative values. In addition to the above we conceptually extend the simple bR receptive fields studied in this investigation to more complex bR edge detection devices. Experiments are presented that support the eventual implementation of such highly parallel devices with bR.

II. Materials and Methods

The S7 strain of *Halobacterium halobium* is cultivated in standard conditions.[16] At the end of the cultivation period, PM is isolated by generally accepted procedures except for the sucrose gradient step which is skipped since the presence of minute amounts of red membrane would not adversely interfere with our application. For best film deposition PM is rinsed with ultrapure water (~8 MΩ cm) a number of times. This procedure starts with incubation of a PM solution (0.6 mg/mliter) with an equal volume of 1-M NaCl solution for 30 min. Each of the rinsing cycles consists of spinning at 20K rpm in a Beckman Centrifuge with a Type 50 rotor. Subsequently, the supernatant is decanted, and the pellet is redissolved in ultrapure water. This is repeated between 4 and 7 times. It should be noted that excessive rinsing turns PM blue.[17] This can be avoided by simply setting aside a small portion of the PM solution and always subjecting it to one extra rinse cycle than the remaining portion. When this sample turns blue, it signals an end to the rinsing process for the remaining PM solution. The final concentration of the PM solution is adjusted to ~1.5 mg/mliter.

To fabricate electronic devices based on the bR molecule, it is necessary to form a thin film in which all molecules are oriented in the same direction. Fortunately there are a number of methods that allow orientation of molecules so that the direction of the induced photocurrent in such a film is unidirectional as well as perpendicular to the surface of the film.[18] One such method is by electrophoresis in which the net charge and intrinsic dipole moment of PM are employed.[19] At neutral pH, PM has a net negative charge, and the direction of the dipole moment is such that one side of the membrane is more positively charged. When a drop of PM solution is placed between two electrodes and a weak voltage is applied, PM is oriented and becomes deposited on the positive electrode. An ~0.5-mliter volume of the concentrated PM solution is placed between two parallel 3.8- × 3.8-cm SnO_2 coated Pyrex glass plates (obtained from F.J. Gray and Co., New York) separated by a 1.4-mm gap. A voltage of 4.6 V is applied between the two electrodes with the lower electrode being the anode. The duration of the voltage application is crucial. If the voltage is applied too briefly, the adhesion of the PM to the anode is poor, whereas if it is applied for too long, the PM turns blue.[19] Although the exact duration depends on voltage, electrode gap, degree of rinsing, etc., 45 s seems to be an appropriate value; insufficient rinsing also leads to poor adhesion and bubble formation on the electrodes due to electrolysis of the water. After deposition of the PM on the anode, the remaining solution is carefully removed with a pipette, and the deposited film is allowed to dry slowly in a high humidity environment (~80% relative humidity) to prevent crack-

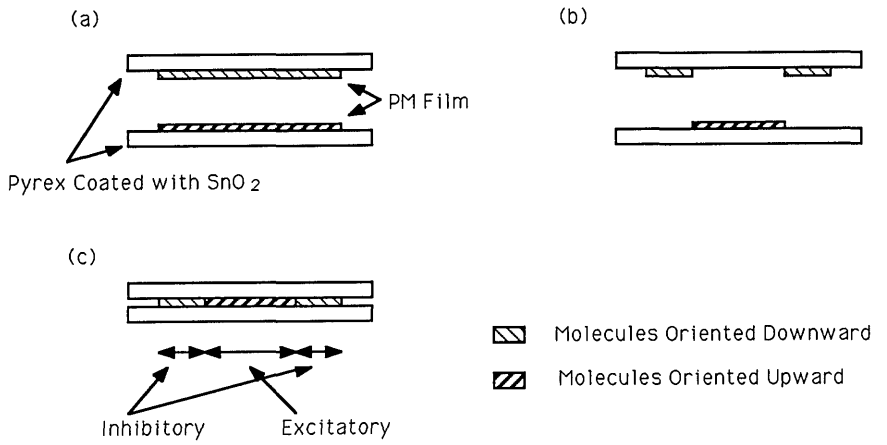

Fig. 4. Implementation of a receptive field consisting of excitatory and inhibitory regions: (a) Two equivalent PM films are oriented face to face. (b) The appropriate section is removed from each film. (c) Two PM films are brought into contact with each other. Electrically conductive SnO_2 films act as electrodes.

ing. The resulting thin and dried film generally has a surface area of 1 cm^2, a thickness of 25 μm, and an optical density of ~2.0 at 570 nm. Resistance is in the 10^9–10^{10}-Ω range, and such a film generates photovoltage when illuminated by white light, with the extracellular side being positive. Dried PM films are structurally robust, and a number of laboratories have reported undiminished photoelectrical responses in thin bR films for as long as two years[20,21] after preparation.

To measure photovoltage, an identical SnO_2 coated Pyrex glass plate is placed on top of a PM film, and, although not absolutely necessary, a thin Mylar film is placed between the two electrodes as this tends to prevent decay of the photovoltage. No special precaution is taken to maintain the PM film at a particular relative humidity, but the film is usually kept at the ambient humidity of ~15–40%. A 100-W tungsten halogen lamp in combination with a KG-3 IR absorbing filter is used as a light source, and the electrometer used is a model 610C by Keithly Instruments. With this setup, photovoltage of a few volts is routinely observed when a PM film is continuously illuminated with an intensity of 50 mW/cm^2.

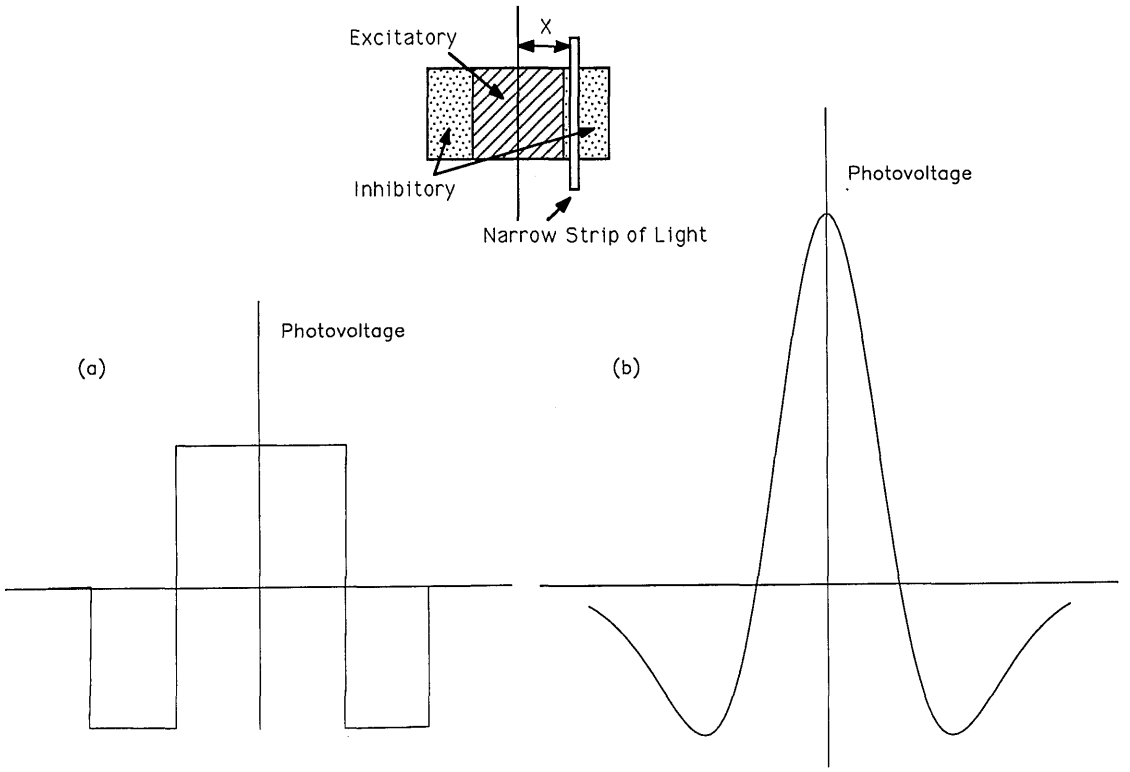

Fig. 5. Two types of photoresponse profiles investigated. Response profiles were measured by detecting the photovoltage generated by illuminating a thin light strip on the detector as shown in the inset and scanning it across the detector surface. The smooth variations in (b) were obtained by locally adjusting the width of the edge detector.

We use two such films with equivalent photovoltage to fabricate an edge detector. After the two films are brought into contact with each other in a face-to-face orientation [see Fig. 4(a)], photovoltages generated in the two films on illumination tend to cancel each other. If the PM is locally removed from one of the films, the net photovoltage can be made positive or negative depending on which PM film has been physically etched away [see Fig. 4(b)]. Thus in this fashion it is possible to implement excitatory and inhibitory regions of a desired receptive field [Fig. 4(c)]. In an excitatory region, there are bR molecules oriented in one direction, whereas in the flanking inhibitory regions the bR molecules are oriented in the opposite direction.

In our investigations we have considered only 1-D ZC detectors in which the edge to be located is aligned only along one axis. For optimal differentiation, the edge has to be aligned parallel to the symmetry axis of the detector. To implement different response profiles with such a 1-D ZC detector, the width of the excitatory or inhibitory regions, that the ZC detector is composed of, can be adjusted. Figure 5 shows two different photoresponse profiles that have been measured by illuminating the ZC detector with a moving slit of light parallel to the regions of the detector. In Fig. 5(a) the central excitatory region is twice the width of the flanking inhibitory regions with all regions of equal length. This produces a rectangular photoresponse profile. In Fig. 5(b) the excitatory and inhibitory regions are of varying length but similar width. This produces a DOG profile.

III. Results and Discussion

A. Demonstration of Simple ZC Detection

First, we investigated the dependence of photovoltage on illumination intensity and duration by exposing a PM film to pulses of white light. A mechanical shutter is used to generate light pulses whose intensity is adjusted by the light source voltage and the use of a neutral density filter. Figure 6 shows that the relationship between photovoltage and illumination intensity is linear over almost 4 orders of magnitude for various pulse durations.

Second, we tested the response of each ZC detector by projecting on it a sharp edge which was parallel to the symmetry axis of the detector, and the net photovoltage was measured as the edge was incrementally scanned across the detector. Thus initially the entire detector was dark, and, as the edge was scanned, increasing widths of the PM film were exposed to light. Therefore, at the end of the scan the entire PM film was exposed to light. Figure 7 shows how detectors of different photoresponse profiles, rectangular and DOG (see Fig. 5), respond to such a scanning sharp edge. In both cases, a ZC is observed when the position of the sharp edge coincides with the center of the receptive field. Errors in locating an edge can arise from a number of sources such as nonuniformity in the quality of the bR film, unbalanced etching of excitato-

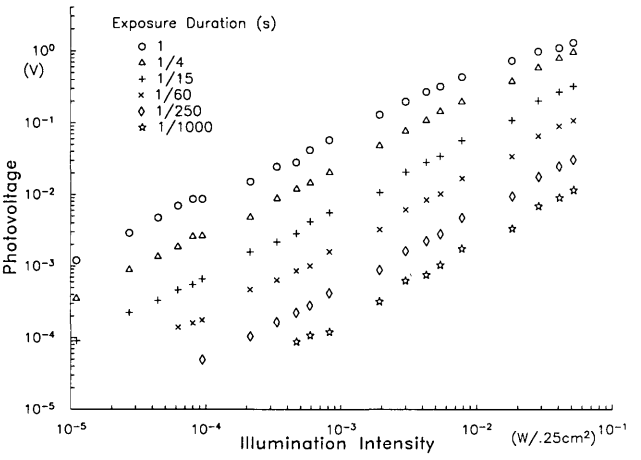

Fig. 6. Relationship between photovoltage and illumination intensity. The photovoltage exhibits a dependence on illumination duration because the photovoltage reflects charges accumulating across the PM film over the period of illumination.

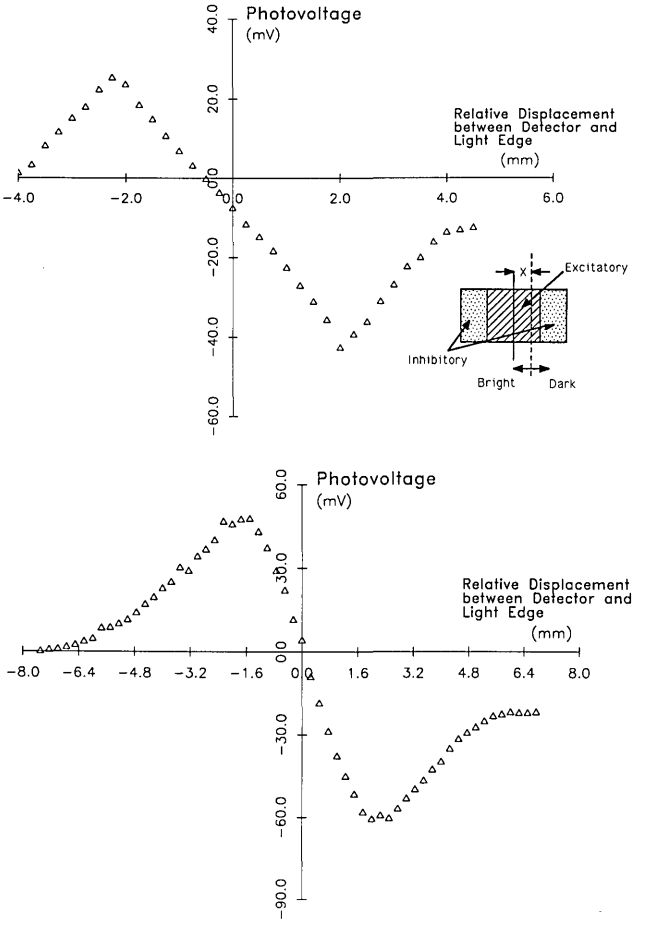

Fig. 7. Responses of actual edge detectors patterned after types (a) and (b) in Fig. 5. A sharp edge whose position is indicated by the broken line is cast on the detector. The shadow is to the right of the broken line. × indicates the relative displacement between the detector center and edge. The photovoltage is measured as × is varied incrementally.

ry and inhibitory regions, and variations in the contact between the electrode and bR films. On the other hand, the upper bound of errors is determined by the dimension of the receptive field so that smaller receptive fields will have smaller errors. Note that a nonzero response is observed when the entire receptive field is uniformly illuminated at the point in the scan when the edge is at +4.5 m [Fig. 7(a)] and +6.5 mm [Fig. 7(b)]. This is due to an unintentional net imbalance between the excitatory and inhibitory regions. More careful processing should eliminate this.

Even with this simple method of producing bR ZC detecting films it is possible to extend this methodology to produce a 2-D receptive field. In a 2-D scheme, it would not be possible to arbitrarily change geometries of excitatory and inhibitory regions to accomplish a particular photoresponse profile. It is possible, however, to obtain any desired photoresponse profile in such 2-D implementations by exploiting the molecular nature of the bR light detector and deactivating molecules in a particular region using intense light to either permanently photobleach molecules or temporarily produce another intermediate of the photocycle by regulating the intensity of the bleaching light. Furthermore, in some applications, it may be of interest if the time-course of the detector can be adjusted. Two methods can be envisioned. The first method would involve locally modifying the LC constant of the bR light detector by increasing resistance and/or capacitance which would change the time course of the electrical signal. This could be accomplished in a variety of ways, such as having an array of electrodes with variable RC characteristics. The second method would involve selecting different phototransitions with different transition rates, although this approach does not permit continuous variability in the time-course modification of the electrical signal.

B. Toward the Goal of a PM Device with Some Retinal-Like Capabilities

With this demonstration of simple PM zero crossing detecting films, advanced schemes for the use of these films in ZC detection can now be considered. Thus this section is divided into three parts. First, the unique properties of bR are described in terms of their relevance to computational schemes that are fundamental to the development of a PM artificial retina. Second, these unique bR properties are integrated into a method of implementing a versatile device with some retinal-like capabilities. Third, experiments are described to test the validity of some of these ideas that form the basis of these advanced implementations.

1. Purple Membrane Film Characteristics Important for Computation

Speed, compactness, and flexibility are all characteristics of PM films important to the implementation of simple computational devices.

The speed of the response of the bR films is based on the observation that within 5 ps of photon absorption an electrical response is observed.[22] This electrical response is thought to be associated with the first light-induced molecular transformation which takes place in 450 fs, and thus, it is generally believed that with improved detection schemes the onset of the electrical response is considerably faster.

The compactness of a PM film device arises from several factors. In such films that are needed to cover a single excitatory or inhibitory region there is no need (as is the case of semiconductor edge detector implementations) to wire together electrically and intricately modify the strengths of the interconnections of a large number of discrete photodetectors. Instead the PM film is a single continuous photodetector with the possibility (see below) of local variations in the photoresponse. These local photoresponse variations are in place of the modified electrical interconnections among discrete photodetectors that exist in conventional solid state implementations to endow such solid state devices with edge detection capabilities. In addition, because photovoltage is generated by a charge displacement confined within each molecule, the crosstalk between two adjacent molecules is unlikely. Thus the unique light-to-electrical transduction mechanism of bR allows fine local variations in photoresponse and promises great reductions in size.

Locally variable photoresponses can endow the PM film with computational flexibility. Such a variation is accomplished by changing the population density of the bR molecules continuously. Thus, relative to conventional detectors, PM films could be readily fine-tuned to implement a variety of response profiles. Implementing such a response profile by manipulating with light the molecular population densities requires neither excessively complicated electrical connections among a large number of discrete photodetection elements, as is the case in VLSI implementations, nor extra computational power, which is required for digital image processing.

Finally, further computational flexibility and edge detection with ultimate elegance can be achieved with bR by fully implementing the subtleties of the bR photovoltage characteristics. To understand these subtleties the reader is referred to Fig. 1, in which a generally accepted photocycle scheme is shown. One important pathway not considered thus far is the so-called backphotoreaction induced by illumination of the *M* intermediate with blue light. This is accompanied by a reverse proton translocation across the bR molecule and a reversed polarity of the photovoltage normally observed.[20]

To successfully incorporate this photovoltage with reversed polarity into edge detection computation a thin PM film is treated with a high pH buffer (BuffAR pH 10 from Mallinckrodt). With this treatment the lifetime of the *M* photointermediate is extended significantly at room temperature.[23,24] The exposure of such a film to yellow light converts molecules into the *M* photointermediate. In this process one observes a change in color in the film from purple to yellow because the *M* photointermediate has an absorption spectrum peaked at 412 nm with a molar extinction

coefficient of 33,000 M^{-1} cm^{-1}.[4] Due to the extended lifetime of M in a high pH film, such a film remains yellow for almost a minute. If the M intermediate in this film is then exposed to blue light, the initial bR state is recovered via a backphotoreaction with a half-time of 200 ns.[25] Thus high pH PM films are capable of producing photovoltages with polarities that depend on the ratio of bR and M at a particular site in the film.

2. *Integrating the Unique Characteristics of PM Films into an Advanced Edge Detector*

Our proposed advanced edge detection scheme takes advantage of the bR to M and M to bR photoactivated transitions in PM films treated with a high pH buffer. To form an edge detector in a PM film, it is necessary to implement excitatory and inhibitory regions; a photon landing on an excitatory region should contribute positively to the overall response of the receptive field, while another photon on an inhibitory region should contribute negatively to the overall response. In our advanced scheme for bR edge detection, the overall response is measured as the photovoltage across the entire area of a receptive field, which is sandwiched between two transparent electrodes. If a region within the receptive field consists of molecules in the bR state, subsequent exposure of the region to light that is absorbed by bR results in a photovoltage in this bR region. On the other hand, if a region consists of molecules in the M state, its exposure to light in the M absorption results in a photovoltage of the opposite polarity to the one measured in the bR region. Thus, if one type of region is arbitrarily defined to be excitatory, the other type can be considered to be inhibitory. Figure 8 shows the procedure for implementing a bR/M edge detector. First, the entire area is exposed to blue light to reset molecules to the bR state (a). Then a mask is placed over the film, and only a selected region is exposed to yellow light, converting molecules there into the M state (b). Now the edge detector consists of excitatory and inhibitory regions ready to process an image (c).

To process an image the edge detector, generated by blue and yellow serial illuminations described above, must be illuminated with light consisting of both blue and yellow wavelengths. As a first example of image processing consider the presence of an edge projected with white light (containing blue and yellow wavelengths) onto a bR/M edge detector with an M zone flanked on either side by equivalent bR zones. If the edge is placed exactly in the middle of the M zone, no photoresponse would be detected. On the other hand, if an edge between light and dark is placed at some point other than the middle of the M zone, a response will be detected indicating the position of the edge at some point away from the M zone center. Thus, if such a bR/M combination is considered as one receptive field, an artificial retina composed of many such receptive fields, whose individual electrical responses can be integrated, could define the nature of an image projected on this PM retina. Therefore, such a mosaic of receptive fields refreshed between detection events will allow for a rapid, compact, and flexible PM device with some capabilities of neural processing in the retina.

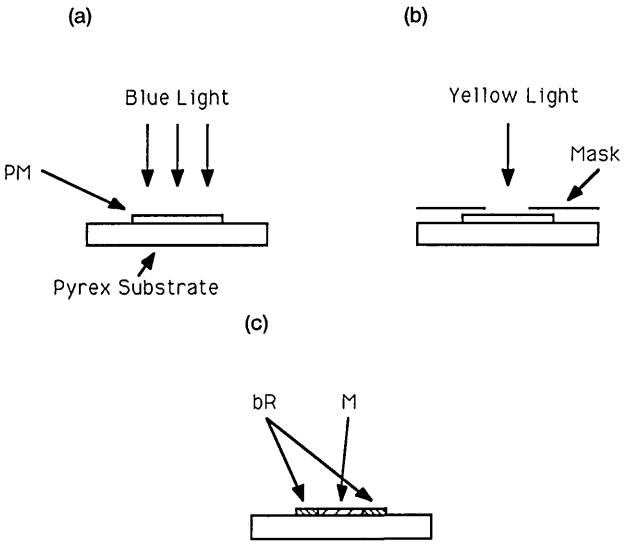

Fig. 8. Formation of excitatory and inhibitory regions in a uniform PM film treated with a high pH buffer. (a) The entire film is exposed to blue light to set bR molecules to the bR state. (b) By using an optical mask, molecules in a selected region are exposed to yellow light. (c) PM film consisting of excitatory and inhibitory regions is ready to locate an edge.

3. *Feasibility of Key Features in Advanced BR Edge Detection*

To check the feasibility of implementing an advanced bR/M edge detector, we investigated some characteristics of bR films treated with a high pH buffer that are relevant to this implementation. The treatment with the buffer involves simple spraying of an oriented dried PM film with the buffer and subsequently drying the films. The results from such a film are now described and discussed.

1. BR to M Conversion Time. Since the advanced schemes of bR edge detection proposed above is based on bR to M and M to bR transitions, the speed of these transitions is an important parameter in developing a device that promises to be practical in the future. In spite of the speed of the photochemically induced conversions of bR the time to convert bR to the M intermediate is limited by the presence of other intermediates that are produced between bR and M. These intermediates unlike the M state do not have distinct absorption spectra and also have photoreactions that drive them back to bR. Therefore, photochemical cycling between bR and the intermediates that precede M occurs and lengthens the time needed for complete bR to M conversion. The same problem does not occur in the M to bR transition since the time-course of the intermediate in this back photoreaction is 3 orders of magnitude faster. Therefore, if a bottleneck exists in the required cycling between bR and M, it would be in the bR to M conversion. Thus we have measured the

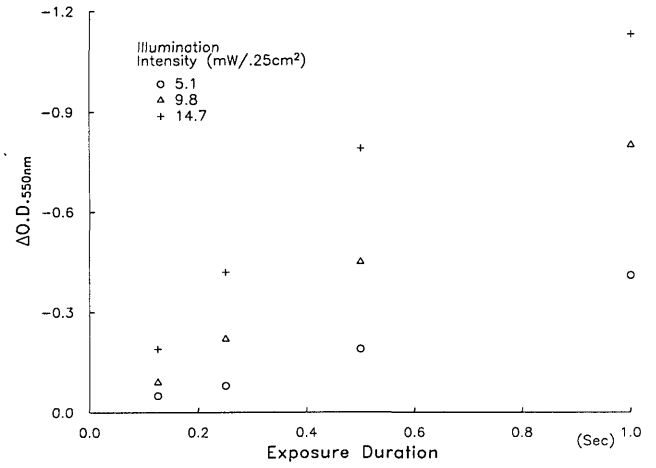

Fig. 9. Bacteriorhodopsin to M conversion. Although not shown here, saturation is achieved after a few seconds. The total $\Delta O.D._{550nm}$ after a 10-s exposure to yellow light is ~1.45.

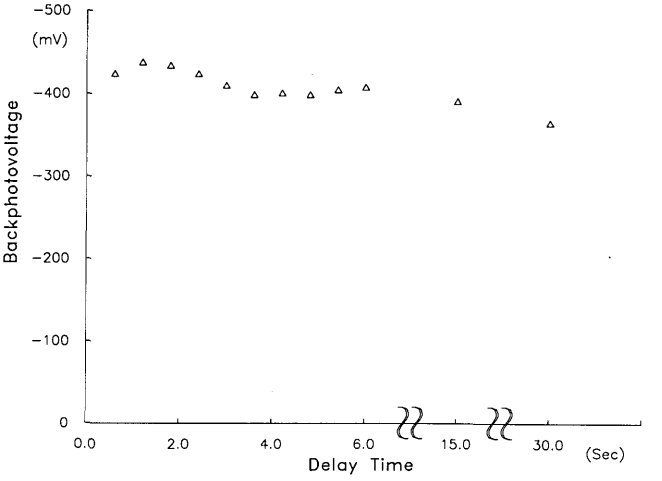

Fig. 10. Effect of time delay on backphotovoltage.

rate of this conversion in various intensities of illumination for the high pH films that are used.

For this measurement a PM film (~2.0 optical density) is placed in the sample chamber of a spectrophotometer (Cary model 14) with the monitoring light wavelength set at 550 nm. With the shutter to the detector of the spectrophotometer closed, the PM film is exposed for 10 s to blue light from a 100-W tungsten halogen lamp with a blue filter (cutoff above 490 nm). This procedure insures that most bR molecules are in the bR state. After the blue light is turned off, the spectrophotometer shutter is momentarily opened to record the optical density (O.D.) at 550 nm. Then the bR film is exposed to a pulse of yellow light (a 100-W tungsten halogen lamp with a yellow filter that cuts off below 530 nm) followed by recording once again the O.D. at 550 nm. The intensity of the yellow light as well as the pulse duration is varied, and Fig. 9 shows the dependence of $\Delta O.D._{550nm}$ on the above two variables. Because of the modest light intensity of 4 mW/cm^2, it takes fully 1 s for 80% conversion from bR to M, but judging from the extrapolation of the $\Delta O.D._{550nm}$ vs intensity graph, we believe that the conversion can be speeded up considerably with the availability of intense light sources. For example, using optimal laser pulses in the yellow and blue region of the spectrum complete switching between bR and M.[10] However, such intense and complicated light sources would not be needed because even small differences in bR and M can produce effective edge detectors, and these differences can be rapidly impressed with halogen light sources that are 2 or 3 times the intensity of the simple halogen lamp used in our measurements. In addition it should be noted that even the bR to K transition could conceivably be used in such edge detectors in spite of incomplete switching between these states. The speed of edge detection based on this pair of states could potentially be in the picosecond regime.

2. *Stability of the M Intermediate.* Another parameter of interest in this implementation of edge detection is the stability of the M intermediate in a PM film treated with the high pH buffer. The stability of an intermediate, such as M, used for the backphotovoltage is important because a population of the intermediate that is constant in time is needed to ensure the reproducibility of the backphotovoltage. Thus this was checked by considering the effect of the time delay between yellow and blue pulses on the amplitude of the backphotovoltage. First, the PM film is exposed to yellow light so that the bR to M conversion is saturated. Subsequently, the yellow light is blocked, and the PM film is exposed to a 0.25-s pulse of blue light following a certain time delay. The dependence of the backphotovoltage amplitude on the time delay between pulses is then observed. Figure 10 shows this relationship. We confirmed that the M intermediate is sufficiently stable so that the decay of M should cause no difficulty in the edge detection of a projected image.

3. *Effect of White Light on the bR to M and M to bR Transitions.* The effect of white light on the bR to M and M to bR transitions was investigated for two reasons. First, in the advanced implementation scheme it is required for multiple wavelengths to be used that are within the absorption of both bR and M. However, since the presence of blue light with yellow illumination or yellow light with blue illumination has an inhibitory effect on each respective transition,[26] it is important to evaluate the two photovoltages induced by one color of light in the presence of the other. To test such combinations of excitatory and inhibitory effects, an experimental arrangement was used that allowed exposure of a PM film to white light in which the relative intensity of the blue and yellow components could be adjusted. The intensity of the blue component is kept constant, whereas that of the yellow component is varied by insertion of a neutral density filter. Both bR to M and M to bR transitions are observed by exposing a PM film to a 0.25-s pulse of this white light after the PM film is converted to either the bR or the M state by blue or yellow light, respectively. Figure 11 shows the results from such a measurement where the intensity of the yellow light is varied and the

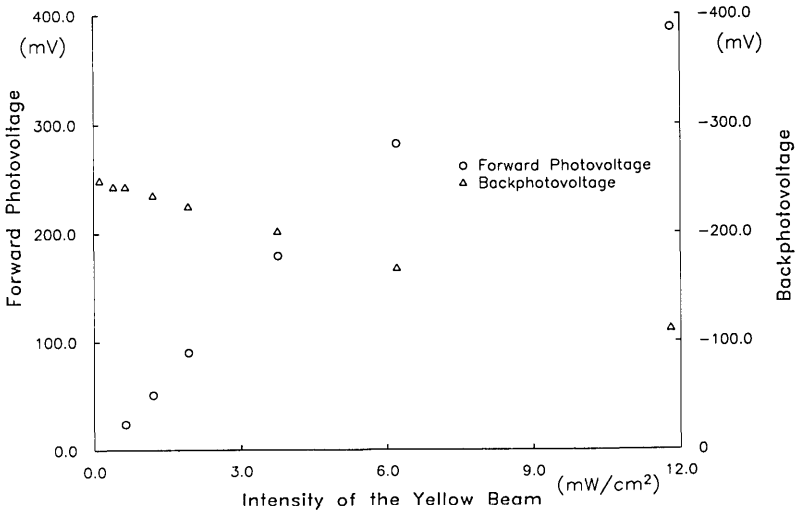

Fig. 11. Effect of the spectral content of the illumination on the forward and backphotovoltages. The intensity of the blue beam is held constant at 4.6 mW/cm².

blue light is kept at 4.6 mW/cm². Thus, when the yellow component's intensity is 0 mW/cm², the back photovoltage indicated by the triangles in the figure is at a maximum. As the yellow component of the light is increased in intensity, there is an increase in the forward photovoltage as would be expected. However, this also has an inhibitory effect on the back photovoltage due to the photoinduced reversal of some bR molecules that were created by the blue light.

Eventually a yellow light intensity is reached where the forward and back photovoltages are nearly equivalent. It is important to note that this point is reached when both photovoltages still have a comparable magnitude. Thus this allows for a readily observed inhibitory effect.

IV. Conclusion

The intrinsic ability of bR to exhibit both excitation and inhibition has been applied to construct bR receptive fields which mimic some of the functions of this computational approach that is used by the brain. Bacteriorhodopsin is a robust biomolecule which is characterized by various interesting electrical and optical properties. Its initial photoinduced electrical response is observed within 5 ps after photon absorption with the polarity of the electrical response of the sample depending on both the wavelength of the light and prior conditioning. We have exploited these optoelectrical characteristics to fabricate receptive fields consisting of two oppositely oriented PM films with specific heterogeneity introduced at certain points in the film. We have demonstrated that such receptive fields are capable of locating edges by detecting zero crossings. We have also proposed another edge detection scheme based on the *M* to bR backphotoreaction and have presented some relevant characteristics of a PM film treated with a high pH buffer to confirm the feasibility of the idea. Our studies have indicated that bR is indeed a most interesting system for implementing schemes of parallel computation with high speed, high density, and simplicity.

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