Color sensitive retina based on bacteriorhodopsin

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Abstract

Bacteriorhodopsin (BR), a membrane protein of a microorganism Halobacterium salinarium has been studied since the 80’s as a potential material for information technology. The information processing applications of BR employ either photochromic or photoelectric properties of the protein. In this study we discuss about design principles and describe our study of the use of bacteriorhodopsin as a sensor material for a color sensitive artificial retina. This retina includes low-level processing of input information. The design of a color sensitive matrix element, the self-organizing color adaptation algorithm and a system model for the retina are presented. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Although all parts of the eye are important for perceiving a good image, the most vital one is the retina. The retina is a part of a brain tissue, which directly interacts with the light and images of the outside. It is the only part of the brain that is visible from outside the skull. The retina contains photoreceptors and five layered neural structure, which is claimed to have some capability of simple processing of visual information (Dowling, 1987). Even a simple two-layer neural network is capable of low level image processing (Mantere et al., 1992).

The whole human visual system includes also the lateral geniculate nucleus, ‘a relay station’ in the thalamus and the visual cortex. Actually, the image is processed not only on one cortical region, but on a network of over 20 cortical areas (Kosslyn, 1996). The exact meaning of each component for visual processing is not known, but certain low level processing is done in the retina and a high level conceptual processing in the cortical areas (Zeki, 1993). Like other neural or-
ganism, the visual system is modified through learning, mainly during early years of infancy (Teller, 1997).

An important attribute of human visual system is color. The sense of color originates from responses of three types of color sensitive photoreceptors in the retina, cones, to a spectrum of electromagnetic radiation originated from an object observed (Wandell, 1995). Several distinct codings of color exist in the visual system. The triplets of cone responses can be regarded as the initial one. The receptor responses are transformed into achromatic and color-opponent signals (Buchsbaum and Gottschalk, 1983). This transformation occurs in the retina and is retained until the early cortical areas. These broadband signals are afterwards transformed to narrowband responses of cells at the higher cortical areas, namely area V4 (De Valois and De Valois, 1993; Kosslyn, 1996).

The human visual system has been a common source of inspiration in machine vision research (Marr, 1982; Jain, 1989). For more than the last 10 years, many sensors, which include both the photosensors and parallel processing elements have been reported. Mainly these are silicon based special circuits, often called vision chips, smart sensors or artificial retinas (Moini, 1997). There are also some constructions of bacteriorhodopsin-based detectors (Miyasaka et al., 1992; Chen and Birge, 1993; Martin et al., 1997). These detectors use a photoelectric property of bacteriorhodopsin (BR). Miyasaka et al. (1992) constructed BR-based detector in a matrix form. They have immobilized BR on a silicon circuit by the Langmuir–Blodgett method, forming a matrix of size $8 \times 8$ pixels. They used S-9 strain of *Halobacterium salinarium* with the maximum photocurrent at the wavelength 560 nm, with the width at half maximum being about 100 nm. Therefore, it can be used as a monochromatic detector.

We have developed a model for a color sensitive artificial retina, in which photosensitive elements are based on BR. This retina includes low-level processing of input information. The processing consist in color space transformation. In order for the retina to operate in similar fashion as the natural system does, we employ an artificial neural network learning algorithm, to tune color space transformation parameters for the environment in which the retina operates. In this report, we present the design of a color sensitive matrix element, the learning algorithm and the system model for the color sensitive, protein based, artificial retina.

2. Bacteriorhodopsin

BR is produced by a microorganism *H. salinarium* as a membrane protein. BR is composed of the protein part bound to the chromophore (retinal) with Schiff base linkage. Natural function of BR is to act as a light-driven proton pump, converting sunlight into chemical or electric energy (Brauchle et al., 1991; Hampp et al., 1992). The proton pumping is coupled with photochemical conversions, occurring during a photocycle of BR (Lozier et al., 1975) (Fig. 1).

![Fig. 1. Scheme of the photocycle of bacteriorhodopsin](image-url)
The photocycle has intermediates with different lifetimes and absorption spectra. The transition times vary from ps to ms. In the dark, an equimolar mixture of the D and B-state is found which is called dark-adapted BR. When illuminated, a pure population of the B-state occurs, called light-adapted BR. The B-state has a broad absorption band with maximum near 570 nm. Absorption of a photon, causes protein to move from the B-state, through a number of intermediates into the M-state. The M-state has the absorption maximum at 412 nm and it has the longest lifetime of all the excited states. The transition from the B to the M-state takes \( \approx 50 \mu s \), during which a proton is released from the Schiff base. From the M-state protein it thermally decays in \( \approx 10 \) ms back to the initial B-state. During the transition the Schiff base is reprotonated. The transition from the M to the B-state can be also triggered directly by illumination with blue light (Hampp et al., 1992).

The proton release and acceptance, during switching to and from the M-state, causes charge dislocation within bacteria membrane, or an oriented protein film (Birge, 1990; Oesterhelt et al., 1991). This photoelectric property is a base for construction of optoelectric devices.

If we use only wild type BR, the sensor has certain wavelength dependency and only different levels of luminosity can be sensed, but not color. The wavelength response can be changed by modifying the BR biotechnically. In our earlier work (Silfsten et al., 1996) we have studied two variants: 4-keto and 3,4-dehydro. As it has been shown also by the others (Drachev et al., 1989; Beischel et al., 1991), both variants retained optoelectric activity. The measurements have also shown that the optoelectric response with respect to wavelength was different among the protein variants. The differences allow color recognition with BR. Absorption spectra and optoelectric responses of natural form of BR and the two variants are drawn in Fig. 2.

3. The model of the system

The model of the color sensitive artificial retina is outlined in Fig. 3. The retina consists of two layers, a photosensitive layer and a preprocessing layer. The input to the retina is an image. The image first interacts with the photosensitive layer, which converts the image into electrical signals. The signals are passed for further transformations to the preprocessing layer. The output of the retina is communicated to the processor.

The photosensitive layer consists of one or more arrays of photosensitive elements. The elements can be ordinary CCD elements, cones in the retina, patches of BR film, or any other suitable material. There is no restriction on a geometrical arrangement of the elements either. Fig. 4 shows some examples of photosensitive layers the model accommodates. An output of the preprocessing layer is an array of \( n \)-tuples. The value of \( n \) depends both on physical arrangement of elements within the layer and on number of those layers (RGB camera is a sensor with \( n = 3 \)).

The preprocessing may include, e.g. simple feature extraction, as edge detection, simple image processing or color coordinate transformations. The preprocessing layer can also do just identity mapping. That in practice, corresponds to the case, when the preprocessing layer is missing.

The preprocessing layer can operate in two modes: off and on-line mode. The purpose of off-line or learning mode is to adjust internal parameters of the preprocessing layer, according to the information about the environment. In on-line mode, the preprocessing layer recodes the output of photosensors according to the parameters adjusted during the learning phase.

4. The constructed system

We have constructed a simple artificial retina, based on the previously described model and we have studied its performance in color recognition. In our retina, the photosensitive layer is a matrix of BR elements. The electric signal generated by the elements is amplified and connected to a computer. The computer simulates the preprocessing layer.
4.1. Photosensitive layer

BR in the photosensitive layer was used in a form of purple membrane isolated from *H. salinarium* wild type (S9). The membrane was isolated as described by Oesterhelt and Stoeckenius (1974). Two variants of wild type BR were prepared by reconstituting bleached BR with synthetic retinal analogues: 4-keto and 3,4-dehydro retinals. Optoelectric elements were produced from the three proteins as follows:
Polyvinylalcohol (PVA) films were prepared by mixing 750 μl of 15% PVA with 200 μl of BR solution and spread onto a conductive glass substrate. After drying for 24 h, a gold layer of about 40 nm thickness was sputtered on the PVA film to form counter electrode for the conductive glass. Thin wire was connected to the corner of the gold layer by silver paint, to form an electric connection from the gold layer (Fig. 5). The element system containing altogether six such elements was made (Fig. 6). There are three pairs of elements in the system, each pair containing one of the three proteins. All elements have the same size, about 18 × 17 mm.

Color is represented and communicated using color space. Most of color research and standard color representation is based on some three dimensional color space (Wyszecki and Stiles, 1982), as CIEXYZ and its linear transformations. This is due to the fact that the human eye have three different classes of color sensitive cells in the retina.

Color representations based on CIEXYZ color space are closely related to the trichromatic human visual system and their use is limited to applications related to human vision. Other living species may have much different color vision. For example, vision of most mammals is dichromatic. Old world primates (Catarrhini), including man, has evolved trichromatic color vision, but the spectral distribution of the cone-pigment sensitivity peaks differs among subspecies (Osorio and Bossomaier, 1992). Visual pigments can also cover a part of spectrum, which is not visible to humans, as for example in the honey bee (Backhaus, 1993). Another disadvantage of standard CIE color spaces is that they define a fixed representation of color.

The preprocessing layer in the constructed retina, has been designed to learn the color space which makes the retina adaptable to an environment. The learning is done by training the self-organizing map (SOM) with the output of photosensitive layer. Hence, we have a model of an independent color vision, which has protein sensors and which forms its ‘visible’ color space by a self-organization process from shown color samples.

The SOM introduced by Kohonen (1982, 1995) is a form of unsupervised learning in which a set of data (feature) vectors in an N-dimensional data space, is summarized in terms of a set of reference vectors which are connected into a regular equal or lower (usually two) dimensional grid. The reference vectors are commonly called neurons. The training of SOM starts with reference vectors initialized at random. After that, the reference vectors are iteratively updated using the
Fig. 4. Examples of photosensitive layers. (a) CCD is used in many image detection devices, for example in ordinary CCD camera. In color camera there are three CCD arrays, each of them is presented with input image filtered through red, green, or blue filter. The input image is so transformed into the array of triplets. (b) The retina — the photosensitive layer of the retina, consists of an array of tightly packed cells — rods and cones. For each spatial position, the layer outputs one value. (c) BR based sensor — the sensor has one array of patches of BR film. Therefore, as in the retina, for every spatial position, there is only a single output value. One possibility to get a tuple of values for each spatial position, is to form groups of patches, so that every group will contain all kinds of proteins positioned next to each other. Each group is then assumed to represent single spatial position. The same principle is used for example in color CRT.
5. Results

The constructed system has been tested by training it using a collection of 84 plastic filters having different colors. These filters were 'shown' to the constructed retina by setting them between the retina and a flash light. A triplet of responses, one for each type of BR, was recorded for each filter (Frydrych et al., 1998). This set of triplets was used for training the SOM. Two SOM were trained, one with rectangular grid of reference vectors and the other one with hexagonal grid, respectively. Both grids consisted of 5 × 5 vectors. The maps were trained using 10,000 iterations.

Figs. 7 and 8 show the color spaces formed by training the SOM with responses of photosensitive layer. Fig. 7a and Fig. 8a show the learned color spaces. Each cluster of color patches are the colors assigned to one reference vector in the map after the training has finished. Fig. 7b and Fig. 8b show in 3-D data space the training data vectors (responses of photosensitive layer) and the self-organizing MAP embedded into the data space. The data vectors are denoted by stars. The reference vectors are the vertices of the grid, lines connecting them symbolize the grid connections. It can be seen from Fig. 7a and Fig. 8a, that the colors have been sensibly organized. The similar colors have been clustered to occupy separate regions of the color space.

6. Conclusions

We have built a simple artificial retina using BR protein and its two artificial variants. The particular set of color lights may be regarded as the representation of the environment in which the retina operates. We have given an example of color spaces learned for one such an environment, the artificial environment created using a collection of plastic filters. The color spaces learned with SOM will differ for other environments (Frydrych, 1998).

The number of different proteins in the system is, of course, not limited to three. One can build a retina containing four or more proteins, their actual number will depend on an application and availability of particular proteins.
Fig. 7. Visualization of the color space for BR responses. 5 × 5 rectangular SOM.
Fig. 8. Visualization of the color space for BR responses. 5 × 5 hexagonal SOM.
The spatial resolution of our system shall be improved, but in this study we have showed that it is possible to produce an artificial color sensitive retina based on protein sensors. Compared to the previous study (Miyasaka et al., 1992) our retina is much easier to produce and it is sensitive to color.

The artificial retina based on BR combined with a color space defined using an artificial neural network demonstrates the system that learns colors and adapts to an environment. Because of an ability to learn and use of biological material the system can be thought as an intelligent color sensitive artificial retina.

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