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Bacteriorhodopsin oriented in polyvinyl alcohol films as an erasable optical storage medium

Zhongping Chen, Aaron Lewis, Hiroyuki Takei, and Isaiah Nebenzahl

Films of oriented bacteriorhodopsin have been formed in polyvinyl alcohol with excellent optical quality. Images with high contrast have been impressed and erased on these films. Second-harmonic microscopy has been used to read the image on a bacteriorhodopsin-polyvinyl alcohol film without erasure. The potential of these films for molecular information storage and computation is discussed.

Keywords: Erasable optical storage, bacteriorhodopsin, biomolecular electronic devices.

Introduction

Molecular information storage and processing have generated considerable interest in the past few years. This interest has focused on either organic or biological molecules as possible media for optical information storage and computation.¹ In this paper we present a unique biological material that exhibits interesting photochromic characteristics, has important optoelectric properties, and has extremely large optical nonlinearities that should be useful for both information storage and computation. This distinctive material is a relation to the visual pigment rhodopsin and is called bacteriorhodopsin (BR).² Many investigators have extolled the possible virtues of this protein pigment as a computational or recording medium. The proposed applications of BR have included the use of the pigment as an implementation of neural networks,³⁻⁵ the utility of the molecule as an ultrafast photosignal detector,⁴ and the use of this membrane as a medium for dynamic holograms and random access memory.⁶⁻¹⁰ However, in spite of all these suggestions, there is significant difficulty in forming uniform, thermally stable, and robust BR films. In this paper we demonstrate that films with all these qualities can be achieved when BR is embedded in a polyvinyl alcohol

(PVA) polymer matrix and, in addition, the BR included in such films is highly oriented. We report stable images on these films when the BR-PVA matrix is treated with the defined conditions detailed in this paper. The oriented BR molecules in this polymer environment not only allow image formation but also detection of photoelectric signals and nonlinear optical phenomena associated with the anisotropic nature of the film and its photochromic states.

Properties of Bacteriorhodopsin

Bacteriorhodopsin is a unique biological molecule since it is the only protein found in nature in a crystalline membrane. This crystalline membrane is called the purple membrane and BR is the sole protein component of this membrane produced by the bacterium *Halobacterium halobium*. The physiological role of BR is to convert light energy into a proton gradient across the bacterial cell membrane. Energy stored in the proton gradient is subsequently utilized in nature to synthesize energy rich adenosine triphosphate.² The light absorbing chromophore in BR is retinal.² In the process of light-induced proton pumping BR undergoes a series of rapid structural changes. Some of these structural changes are reflected in alterations in the absorption of the molecule. These changes are cyclic and in physiological conditions bring the molecule back to the initial state in 10 ms. Although there is still disagreement over the detailed photocycle, most of the intermediate states are well established.² Figure 1(a) shows the generally accepted scheme for the BR photocycle. The dashed lines in this diagram indicate photon-driven processes and the solid lines indicate thermal transformations. Figure 1(b) shows their corresponding absorption spectra.

In less than a picosecond after BR absorbs a photon it produces an intermediate with a red-shifted absorp-

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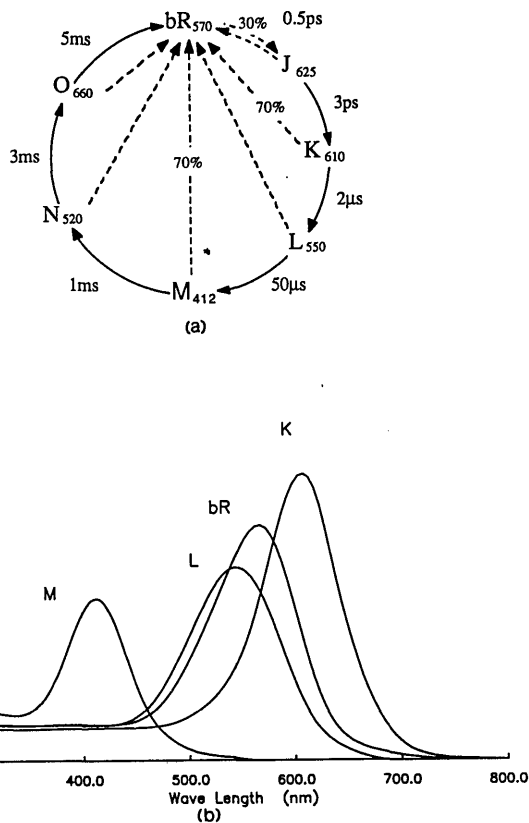


Fig. 1. (a) Photochemical cycle of the BR molecule. The dashed arrows indicate the photon-driven process. The solid arrows show the thermal decay path. Quantum yields for the forward photoreaction and back photoreaction of BR, K, and M are also indicated. (b) Absorption spectra of the corresponding intermediates of BR.

tion maximum that is called J.¹¹ This is the only light-driven event in the photocycle. The rest of the steps are thermally driven processes with K representing the first form of light-activated BR that can be stabilized by reducing the temperature to 90 K.¹² The decay times indicated in Fig. 1 are for room temperature. In addition to temperature, the decay rates of the intermediates can also be drastically altered by pH,¹² humidity,¹³ and the resuspension of the membranes in D₂O.¹⁴ The intermediates L and M are particularly susceptible to these chemical changes in the membrane. An important property of all the intermediates is their ability to be photochemically switched back to BR by shining light at a wavelength that corresponds to the absorption of the intermediate state in question. This property makes BR an ideal material for erasable optical storage.

For information storage and processing applications the K and M intermediate states are especially interesting. At 77 K, BR and K can be switched back and forth by the use of light with wavelengths corresponding to the absorption maxima of these intermediates. The quantum yields for the forward photoreaction and back photoreaction in this switching process are 0.3 and 0.7, respectively.¹⁵ The switching time is a few picoseconds and this makes it attractive for fast optical

information processing. Nonetheless, it requires low temperatures to stabilize K, and its absorption spectrum has a large overlap with that of the BR state, which reduces the contrast ratio. This overlap also causes a photostationary equilibrium to exist at low temperatures and thus prohibits complete switching of all the molecules between these two states.

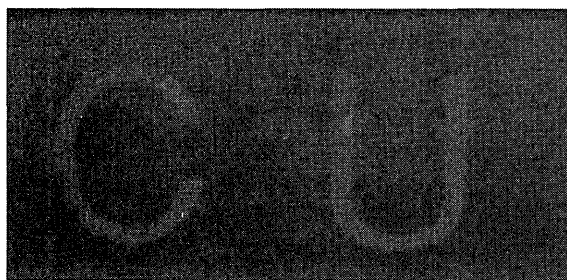
In view of these experimental complications, we concentrate on the M state that can be generated from BR. The quantum yields for the forward photoreaction and back photoreaction are identical to those for switching between BR and K.¹⁵ Although its switching time is not as fast as K, the absorption band of M is distinct from BR, which allows for the complete switching of BR to M and reduces, in comparison with other photochromics, the power needed to accomplish this optical switching. Furthermore, the temperature needed to stabilize this state is ~ 208 K, and this is much higher than the K state.² It should also be noted that the time required to initiate the transition between BR and M is much shorter than the actual transition time. This results because, once BR is photochemically switched to the primary photochemical product J in < 1 ps, all the molecules that have reached the J-state will thermally relax to the M state automatically.

In addition to the large quantum yields and distinct absorptions of BR and M, BR has several intrinsic properties of importance for optical engineering. First, this molecule exhibits a large absorption cross section. Second, the crystalline nature and adaptation to high salt environments makes the purple membrane robust to degeneration by light and by environmental perturbations and thus, unlike other biological materials, it does not require special storage. Dry films of purple membrane have been stored for several years without degradation^{16,17} and, in our laboratory, the film we made 2 years ago exhibits no noticeable changes in its characteristics. For example, this 2-yr old film has recently been switched once again between BR and M 10^6 times. Furthermore, of considerable significance is the fact that both the spectrum and kinetic aspects of the BR photocycle can readily be modified. This is accomplished by, in addition to the alterations described above of pH, humidity, and H₂O/D₂O exchange, replacing the light-absorbing component of the protein, which is a retinal (vitamin A like) chromophore. This chromophore can be replaced by natural and synthetic analogs that can shift the BR spectrum to virtually any color.¹⁷ It is also of significance that genetic mutants of BR can be readily produced by biotechnological procedures, and these mutants can affect both the kinetic and spectral properties of BR.^{18,19} Thus, with advances in both biotechnology and synthetic chemistry, it might be possible to optimize the properties of BR for optical information storage and processing. Finally, BR and M have large second-order nonlinear susceptibilities²⁰ that can be used to read without writing, and we demonstrate that it is possible to use second-harmonic microscopy to accomplish such nondestructive read operations.

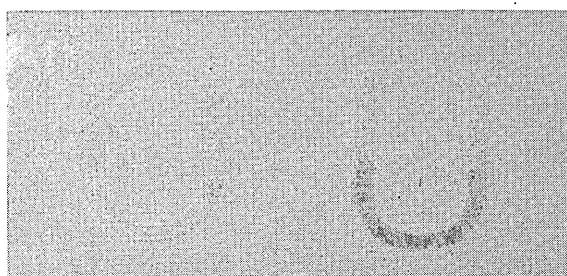
Film Preparation

The purple membrane was purified from *Halobacterium halobium* (S9) as described by Osterhelt and Stoerkenius.²¹ To make an oriented BR-PVA film, 15% (wt/vol) polyvinyl alcohol (PVA) with a molecular weight of 40,000 (purchased from Sigma) was dissolved in a 50-mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer by heating to 98°C. A volume of 1 mliter of a 0.15-mM BR solution dissolved in the same buffer was mixed with 9 mliter of the PVA solution after it had cooled down to room temperature. The BR-PVA solution was then degassed by spinning the solution at 5000 rpm in a centrifuge to remove any residual bubbles. The substrate, a 5-cm diam Pyrex window, was treated with chromerge at ~80°C. A volume of 4 mliter of the BR-PVA solution was then spread on a leveled glass substrate and filtered air was used to purge uniformly the film surface during the drying process. The film took ~24 h to dry. The dried BR-PVA film had a typical thickness of ~150–200 μm and an optical density at 570 nm of ~0.15. The absorption spectrum of such BR-PVA films showed no detectable difference from that of the purple membrane suspended in solution.

Although the M intermediate in these films is stable at approximately -65°C , we searched for a method to stabilize the M state in such films even at room temperature. Previous workers interested in the kinetics of the BR photocycle had reported that the half-life of the M state could be increased by 4 orders of magnitude when BR is suspended in saturated salt solutions of ether or high concentrations of guanidine hydrochloride.^{22,23} Other workers have shown that drying the purple membrane increases the M state half-life by ~3 orders of magnitude.¹³ We combined the procedures and found that the high concentration of salt used in the previous investigations made it impossible to generate uniform dry films of BR because the salt crystallized during drying. To overcome this problem we resorted to adjusting the pH of the BR solution to 10 without any salt, and we have observed a dramatic increase in the stability of the M state in a dry film treated with such a solution. This M stability was also observed in BR-PVA films treated with such a solution. The half-life of the M state in such a treated film was increased by 2 orders of magnitude compared with that for an untreated BR film. As a result, it was possible to impress images on the BR-PVA film even at room temperature. Figure 2 shows a room-temperature image impressed on such a film. In Fig. 2(a), the two letters C and U are formed by molecules that have been optically switched to the M state with background molecules in the purple BR state. The image is formed by illuminating a BR-PVA film through a mask with yellow light (100-W projection lamp filtered with a 550-nm long-pass filter). Figure 2(b) is the reverse image on the same film where the letters CU are formed by molecules in the BR state and the background by molecules in the M state. This image was formed by first erasing the letters in Fig. 2(a) with yellow light to switch all the BR to the M state and then



A



B

Fig. 2. Images on an optically switchable BR-PVA film. The image is formed at room temperature and can be erased either by blue light, which switches the yellow molecules to purple, or by yellow light, which switches the purple molecules to yellow. (a) Image formed by illuminating a BR-PVA film through a mask with yellow light. (b) Reverse image formed on the same film by first erasing the image in (a) with yellow light and then illuminating the film through the same mask with blue light.

by illuminating the film through the same mask with a blue light. The blue light was obtained with the same 100-W light source filtered with a Schott glass KG1 filter. The actual color contrast of the image on the BR-PVA film is better than what is shown in the picture because the strong light needed to photograph such images partially erased the letters.

There are other optical processes that can be used to read the image on this BR-PVA film. Holographic methods have already been suggested^{8,24} and, in addition, phase alterations have also been used as a means to write and read BR images.²⁵ With the appropriate choice of wavelengths, some of these processes may allow for nondestructive readout of the images. However, since the BR in PVA is oriented, such nonlinear processes as second-harmonic generation could also be applied to read these images. The orientation of BR in PVA is indicated by both photovoltage and second-harmonic measurements of BR-PVA films formed in neutral pH. Unfortunately, such PVA films with oriented BR do not form at the high-pH required to impress stable images. This was somewhat disappointing since neither photovoltage nor second-har-

monic generation could be excited without BR orientation. To overcome this problem we first formed the BR-PVA films in neutral pH and then diffused into the film the high-pH buffer. This procedure forms highly oriented, high-pH films with the M state visually stable at room temperature. In addition to this procedure we have also formed highly oriented films of BR without the polymer substrate by electrophoresis methods similar to the procedure that Varo has employed for his investigations of the electrical properties of dried BR films.²⁶ The M state in such films can also be stabilized by diffusing into the film a high-pH buffer. However, these films are inferior in their optical quality.

Orientation of BR in BR-PVA Films

Oriented BR films are important for a wide variety of fundamental studies on purple membrane. For example, these films have made important contributions to studies on the proton pump mechanism, the photocycle parameters, and energy transduction mechanisms.²⁷ In this section we compare the various methods of orientation of BR with particular emphasis on the use of such films for information storage and processing. In addition, the methods used to determine the orientation of BR in BR-PVA are described.

Oriented samples of purple membrane have been obtained in a number of ways. These include incorporation into positively charged bimolecular lipid membranes,²⁸ in polyacrylamide gels,²⁹ and adsorption to cationic surfaces.³⁰ In addition to these methods, dry films of oriented BR can also be made by electrophoresis of suspensions of purple membrane.²⁶ Such a method can be used because the cytoplasmic surface of the purple membrane carries a negative charge at neutral pH and, furthermore, there is a dipole moment associated with the purple membrane that is a result of the transmembrane α helices that constitute the BR molecules embedded in the membrane. However, the oriented films made by this technique are not uniform, especially at low BR concentration. Another technique that can be used to generate oriented BR films is based on Langmuir-Blodgett methods.^{31,32} However, since most applications require multilayer films of BR, Langmuir-Blodgett techniques have been difficult to apply since the BR protein sometimes denatures and unfolds at the air-water interface.³² Thus, in terms of films investigated to date, with good optical quality and a large degree of orientation, BR in a PVA film has the best qualities for nonlinear information processing applications. In addition, PVA has been widely applied^{33,34} and, therefore, the processing technology for PVA films is well developed. Two methods were used to demonstrate that the BR is oriented in the BR-PVA film produced at neutral pH. The first of these methods measured the photoelectric response of the films. This photoelectric response results from the well-known physiological role of BR as a light-driven proton pump. In essence, the electrical signal is associated with the photocycle (see Fig. 1) in which a proton is vectorially transported across the

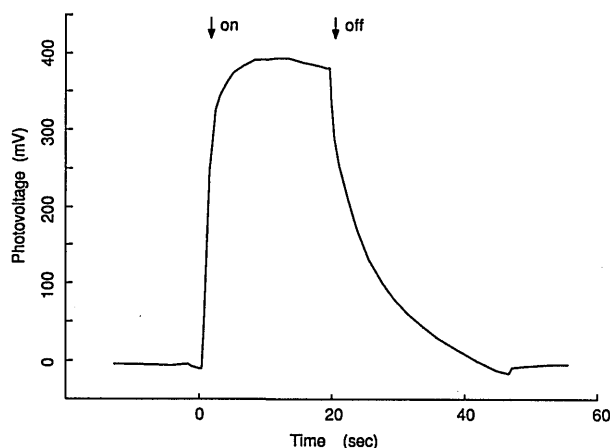


Fig. 3. Photovoltage from a BR-PVA film. The thickness of the film is $\sim 170 \mu\text{m}$ and the OD is 0.13.

membrane. Thus BR orientation is essential to detect this photoresponse.

A typical photoresponse of a BR-PVA film is shown in Fig. 3. To detect this signal, the BR-PVA film was peeled off from the substrate and cut into pieces of $0.5 \times 1 \text{ cm}$. The sample was then sandwiched between two transparent electrodes formed by glass that was coated with SnO_2 , and the signal was measured with an electrometer (Keithley 610C). The photovoltage was generated by illuminating the sample with a 100-W tungsten halogen projection lamp filtered with a long-pass 550-nm filter. The upper surface of the film (defined as the surface not in contact with the substrate on which the PVA film was dried) was connected to the positive electrode and the lower surface was connected to the ground. The photovoltage measured from these films with an optical density (OD) of 0.13 was $\sim 300 \text{ mV}$. This is quite large considering the relatively small OD of the film. The magnitude of the photovoltage is certainly comparable to samples that are oriented by electrical fields and Langmuir-Blodgett techniques.^{32,35} Since it is known that the photovoltage is also dependent on many other factors such as pH, humidity, etc., it is difficult to determine the quality of orientation in the films by simply considering the magnitude of photoresponse. However, the large photoresponse we observed clearly indicates that the BR is oriented in these BR-PVA films.

In addition to the photoelectric signal, second-harmonic (SH) signals have been detected from such BR-PVA films.^{20,36} To evaluate the extent of BR orientation in PVA films the magnitude of SH signals in these films was compared with the magnitude of SH signals generated from electric-field-oriented purple membrane on glass slides. A dry sample of oriented purple membrane made by electrophoresis has been shown by Varo²⁶ to give samples that consistently have a high degree of orientation. These samples have been shown by dichroism measurements to have the purple membrane lying flat on the glass substrate on which

they are deposited. Previous results have indicated the orientation of the purple membrane in BR-PVA²⁰ and thus, assuming perfect alignment by both methods of orientation, we can predict the ratio of the SH signal generated from samples produced by these two methods. In calculating this ratio it is assumed that the nonlinear susceptibility of the chromophore is dependent only on its immediate purple membrane surrounding and, thus, is the same in a dried film or in a polymer matrix. Any deviation from this calculated ratio will help us define the quality of the orientation in the BR-PVA films. We have measured SH signals generated from both the electric-field-oriented samples and BR-PVA films. The measured ratio of the signals from the PVA samples to the electric-field-oriented samples [$\eta = I_{\text{BR-PVA}}(2\omega)/I_{\text{EF}}(2\omega)$] is a factor of 2 larger than that predicted. Since the electric-field-orientation is thought to produce highly oriented samples, the fact that η is larger than 1 probably results from the signal losses caused by scattering caused by nonuniformity and the generally poor optical quality of the dry BR films oriented by using electric fields. These results indicate that BR is oriented in the BR-PVA films to at least the same degree as the dry BR films oriented by electric fields.

Nonlinear Optical Properties of the BR and M states in BR-PVA Films

The nonlinear optical properties of the BR and M states in BR-PVA films have been investigated by Huang *et al.*²⁰ These investigations showed that the nonlinear polarizability of the retinal chromophore in BR was ~ 10 times larger than that of the free chromophore. The free chromophore has a very large nonlinear polarizability ($\sim 10^{-28}$ esu) because of the conjugated double-bond structure giving rise to a strong π -electron delocalization. The difference in nonlinear polarizability in the free and bound chromophore indicates that the protein matrix enhances the dipole moment change after optical excitation. This may be crucial to the fast and efficient storage of photochemical energy in BR.³⁷

Of considerable importance for the next section of this paper is the observation that, when a Nd:YAG laser at $1.06 \mu\text{m}$ is used to generate the SH signal of the BR and M species in a BR-PVA film with an OD of 0.13 and a thickness of $170 \mu\text{m}$, a nonlinear susceptibility of 4.7×10^{-10} esu is measured for BR and a value of 5.0×10^{-11} esu is detected for M. Thus the nonlinear susceptibility is a factor of ~ 10 times larger for BR than M in a BR-PVA film. There are two explanations for the larger susceptibility of the BR species over the M state. First, the SH photons generated by illuminating a BR-PVA film with a $1.06\text{-}\mu\text{m}$ Nd:YAG laser are at 532 nm , which is resonant with the BR and not with the M absorption. Second, the retinal chromophore is complexed to the protein as a protonated Schiff base in BR and an unprotonated Schiff base in M, and this reduces the dipole moment change after optical excitation in the M intermediate. The differences in nonlinear optical properties of BR and M in

PVA films provide us with the possibility of using SH generation to read an image without erasure. It should also be pointed out that the susceptibility values given in this section are for a film with a dilute concentration of BR. Since the susceptibility is proportional to the molecular density and the film thickness used in the above measurement is considerably larger than the $18\text{-}\mu\text{m}$ coherence length of the PVA film,²⁰ it is not difficult to increase the susceptibility by an order of magnitude by reducing the film thickness appropriately while keeping the OD the same.

Nondestructive Read with SH Microscopy

A problem with all photochromic materials in optical memories is that erasure of the stored information is unavoidable when using a beam for readout that is in resonance with one of the states. However, the large and different nonlinear properties of BR and M allow for SH microscopy to be effectively applied to read an image without erasure. In essence, we can choose an infrared beam with a frequency out of the absorption of BR but with a SH frequency within the absorption of this species. With such a choice of laser a nondestructive read operation could be performed in which the infrared light has no one photon resonant absorption affect on BR. In addition, since the resonantly enhanced SH signal is relatively weak and a sensitive photomultiplier is used, there is no destructive effect on BR or M. Such a method requires a photochromic material with a large optical nonlinearity and this is certainly presented to us by BR.

To demonstrate that SH generation could in fact be used to perform such a nondestructive read operation, we detected the SH signal from an image formed by M states in a background of BR. The image on the BR-PVA film was formed by a focused, 514.5-nm line of an argon-ion laser that pumped the film in a localized region from the BR to the M state. Reading the image of this dot of M produced by the focused argon-ion laser was performed by the fundamental of a Q-switched Nd:YAG laser with a 10-Hz repetition rate and 10-ns pulse width. A power of 15 mW from the Nd:YAG laser probe beam was focused on the film with an incident angle of 30° . The film was then scanned through the probe beam with a $25\text{-}\mu\text{m}$ step size. SH photons at 532 nm were spectrally filtered and detected by a cooled RCA C31034 photomultiplier. At each step of the scan, fifty pulses of the Nd:YAG laser were averaged by a boxcar integrator. The resulting SH line scan through the M dot and the two-dimensional SH image are shown in Figs. 4(a) and 4(b), respectively. In Fig. 4(a) a line scan through the M spot is shown as a decrease in the SH intensity as the laser is scanned through the M dot. However, for clarity, the two-dimensional image in Fig. 4(b) is plotted with the z axis of the SH intensity reversed. The resulting image that was obtained clearly shows that SH generation is capable of detecting an image formed by BR and M states in a BR-PVA film. The noise in the image is principally caused by fluctuations of the Nd:YAG laser intensity. From the line scan that showed the largest contrast,

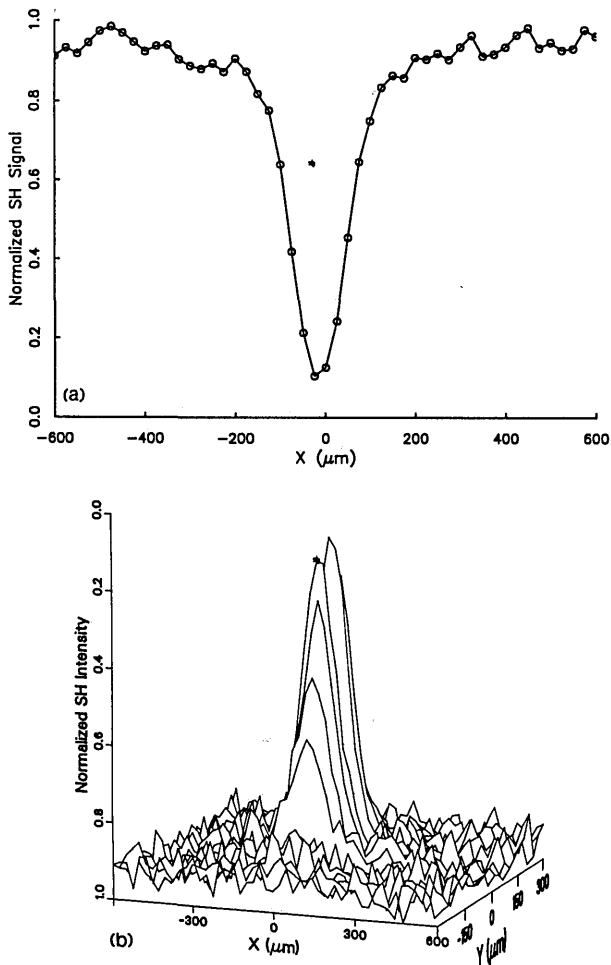


Fig. 4. Second-harmonic image of a dot of M-state molecules on BR-PVA film. The pattern is formed by a focused argon-ion laser beam that pumps the molecules in the illuminated region to the M state. The probe beam is a 1.06- μm Nd:YAG laser. (a) SH signal from a line scan across the pattern. The larger SH signal corresponds to molecules in the BR state and the smaller signal corresponds to M-state molecules. (b) Two-dimensional scan of the SH image of the pattern. Note that, for clarity, the two-dimensional image is plotted with the z-axis of the SH intensity reversed.

the ratio of the signal of BR to that of M was 10. Two main factors, in addition to the primitive nature of our experimental arrangement, contributed to this low contrast. First, the probe laser beam, at 1.06 μm and its SH photon at 532 nm, was not optimized for the absorption of BR and thus did not generate the maximum in resonant enhancement. Second, of great importance was the fact that it was impossible to pump 100% of BR to M at room temperature with 514.5-nm emission of the argon-ion laser because of weak residual absorption by M at this wavelength. The contrast ratio could be improved if a wavelength around 570 nm was chosen to switch the BR state to the M state.

In addition to the above, since the SH signal is proportional to the square of second-order nonlinear susceptibility of the medium, the theoretical limit on the contrast ratio can be predicted by calculating the

square of the susceptibility as a function of wavelength.^{38,39} The second-order susceptibility was calculated by the sum-over-states method.^{38,39} Because of the dominant contributions of the conjugated π electrons and of the resonant enhancement to the second-order susceptibility, only the lowest two excited states, $^1B_u^* +$ and $^1A_g^* -$, were considered for the BR chromophore for the exciting wavelengths concerned. The transition moment was estimated from the oscillator strength, and the dipole moment change was taken from previous SH measurements.²⁰ The damping constant used for BR was 1200 cm^{-1} , which is close to the reported results for femtosecond hole burning in BR.¹¹ The results of this calculation are shown in Fig. 5, where the solid curve is the normalized SH signal as a function of wavelength for the BR state and the dashed curve is for the M state. For a fundamental wavelength of 1.06 μm , the predicted ratio of SH signal from the BR and M states is 83, which is very close to the experimental value of 88.²⁰ It can also be seen from this graph that the wavelength for optimal contrast is 1.14 μm . At this wavelength, a contrast ratio of ~ 200 is predicted for the BR and M states found in native BR. Furthermore, increases in the signal-to-noise ratio for this contrast ratio could be obtained by using two nonerasing laser beams in which the SH wavelength of one is resonant with BR and the other with M. Such a measurement of the ratio of the BR/M response could enhance detectability of pixels even if complete switching has not occurred.

It should be noted that, although the SH signal is dependent on the square of the molecular density of the film, the contrast ratio of the SH signal from BR and M is independent of the film's molecular density. Thus reading with SH generation offers a significant advantage over readout methods based on absorption for which the maximum contrast ratio is determined by the optical density of the film. For a given optical density, A , which is proportional to the molecular density of the film, the contrast ratio is $R = 10^4$ for absorption-based readout methods. However, optical density is limited by constraints on the film thickness and the solubility of the BR in the polymer. For example, when the density of the stored information is maximized, the maximum sample thickness will be limited by the diffraction of the read or write beam. For a bit size of 1 μm^2 , the film thickness should be less than 3 μm for a Gaussian beam. Even for a BR film without any polymer matrix, the optical density of such a 3- μm film would be less than <0.8 , and the contrast ratio would be <6.3 if absorption readout were used. This is much smaller than the 200 predicted for SH readout, and, therefore, SH readout not only is a nonerasing method but also has a much higher contrast ratio than for absorption.

Read Power and Switch Power

Two additional questions need to be addressed to evaluate the applicability of BR-PVA films for optical memories. The first question concerns the power that would be needed to nondestructively read an image at

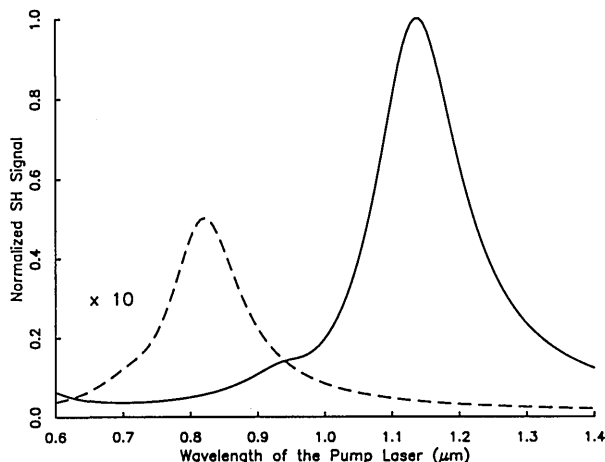


Fig. 5. Normalized SH signal as a function of the exciting fundamental wavelength. The SH signal from the BR state is shown by the solid curve and the dashed curve represents the SH signal from the M state. Note that the scale of the SH signal from the M state has been enlarged by ten.

the wavelength of maximum contrast calculated above. At this wavelength of $1.14 \mu\text{m}$, cw diode lasers readily exist. For a BR-PVA film with a thickness much less than the coherence length of the film, the number of SH photons generated, $N(2\omega)$, is approximately^{38,40}

$$N(2\omega) \approx \frac{16\pi^3\omega}{hc^3} |N_s\alpha^{(2)}|^2 \frac{P^2(\omega)}{S}, \quad (1)$$

where $P(\omega)$ is the fundamental pump laser power, S is the area of the pump beam on the sample surface, $\alpha^{(2)}$ is the second-order polarizability of the BR molecule, and $N_s = Nd = A/\sigma$ is the surface density of the BR molecule where N is the molecular density, d is the thickness of the sample, A is the OD of the BR-PVA, and σ is the absorption cross section of BR. Molecular polarizability $\alpha^{(2)}$ of the BR molecule has been previously determined for an exciting wavelength of $1.06 \mu\text{m}$. We use this near-resonance data to determine $\alpha^{(2)}$ for an exciting wavelength of $1.14 \mu\text{m}$, where its SH photon is in resonance. The value for $\alpha^{(2)}$ in resonance is 5.6×10^{-27} esu for a BR-PVA film with an OD of 0.2; 2.5×10^9 SH photon/s will be generated when 20 mW of a $1.14\text{-}\mu\text{m}$ cw laser is focused to $1 \mu\text{m}^2$. A microchannel photomultiplier, which is sensitive to as few as 100 photons, will allow a detectable signal to be accumulated in <50 ns. Thus a cw semiconductor laser could readily be used in reading the optical memory without destruction.

The second question of importance is the power and time required to switch between the BR and M states. Let us first consider the M—BR transition for which there is a switching time of 200 ns, an overlap in the absorption spectra of the species, and a 200-ns backphotoreaction. For monochromatic illumination and a BR-PVA sample with optical density A and thickness d , assuming that the Lambert-Beer law holds, the

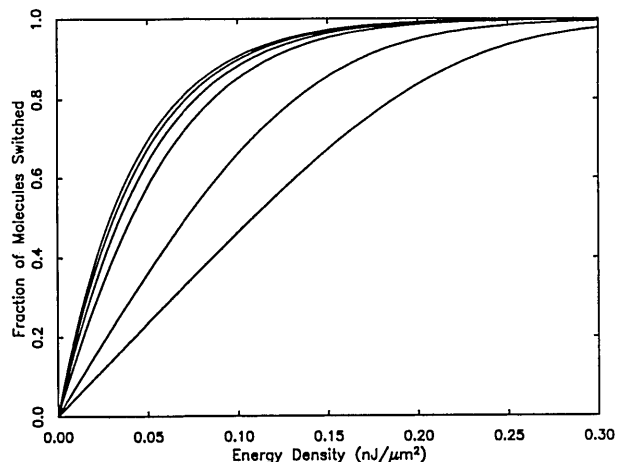


Fig. 6. Fraction of molecules switched from M to BR as a function of the energy density for BR-PVA films with different OD's. From left to right, each curve corresponds to a film with an OD of 0.01, 0.2, 0.5, 1, 3, and 5.

fraction of molecules switched from the BR to M state can be calculated analytically and is given by⁴¹

$$F = 1 - \frac{1}{A} \ln\{1 + [\exp(A) - 1]\exp(-\sigma\phi I_0 t)\}, \quad (2)$$

where $A = \sigma n_0 d$ is the OD of the film, σ is the absorption cross section of the M state, n_0 is the concentration of BR molecules, I_0 is the light intensity in units of photon flux, and $\phi = 0.7$ is the quantum yield for the backphotoreaction of the M state. In Fig. 6, the fraction of molecules switched from M to BR by a 410-nm beam ($\sigma = 1.6 \times 10^{-16} \text{ cm}^2$) is plotted as a function of energy density for films of BR-PVA with different OD's. For a bit size of $1 \mu\text{m}^2$ and an OD of 0.2, the required switching energy is ~ 0.2 nJ. Thus, for a 5-Mbit/s data rate, only a 2-mW cw laser at 410 nm is required. However, for BR to M switching the problem is complicated because, unlike the backphotoreaction from M to BR, the forward reaction occurs through a series of intermediates with absorptions that overlap with BR. This overlap limits the switching time of BR to M since quasi-photostationary equilibria will be generated that delay the rapid switching of BR to M. Using a BR photocycle model in which the backphotoreaction of the K state is included,²⁰ the predicted minimum time to initiate BR to M switching is $\sim 20 \mu\text{s}$. Thus for high bit-rate applications, the BR—K state transition is most promising. However, the high overlap in the absorption and low temperature (90 K) required to stabilize this intermediate is still a problem to be solved. Present efforts are aimed at generating new synthetic BR species that could alleviate this problem.

Conclusion

In summary, we have succeeded in producing oriented BR films with high optical quality in which images can be impressed and erased over $>10^6$ cycles. Using the

nonlinear optical characteristics of these films, we have shown that such images can be read nondestructively and with high contrast by using SH microscopy. Therefore, we have demonstrated the applicability of BR-PVA films as an erasable optical storage medium. The advances in genetic and synthetic techniques as applied to BR offer great promise to optimize this unique system for information processing and storage. Finally, it should be noted that BR also has unique photoelectric properties. The polarity of the photoelectric signal depends on the state of the protein and the wavelength of excitation, thus these photoelectric properties of BR can emulate both neural excitation and inhibition and this property of BR should open new applications for these films in schemes to implement neural processing.

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