Bacteriorhodopsin as a Photochromic Retinal Protein for Optical Memories

Norbert Hampp

Institute for Physical Chemistry, University of Marburg, D-35032 Marburg, Germany, and Materials Science Center, D-35032 Marburg, Germany

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I. Introduction

Photochromic proteins are very rare in nature as far as the number of different models is regarded. However, those few proteins play a key role in photosynthesis and visual perception, e.g. photo reaction centers and visual pigments. Due to their enormous importance, numerous studies have been done to elucidate the function of these molecules, their interaction with light, and the mechanisms of how the light energy is transformed into chemical energy or into physiological signals. The molecular mechanisms have been found to be rather complicated as well as very effective—often close to the physical limits. In an extreme ecological niche, a rather simple representative of the class of photochromic proteins was discovered about 3 decades ago, which was named bacteriorhodopsin (BR).[1] Bacteriorhodopsin (BR) is produced by halobacteria and is the key protein of their photosynthetic capabilities. Rather soon after its discovery, the first proposals for technical applications of this protein were brought up. Various technical and in particular optical applications of BR have been explored since that time in several research groups. In this paper the area of photochromic applications of BR is reviewed. Is it possible to consider a technical use of BR to be realistic? What makes BR so attractive over other proteins and conventional inorganic or organic photochromic materials? First, BR wild-type—the form which is found in nature—already has quite attractive properties. One of these is that it occurs as a two-dimensional crystal, which causes its astonishing stability toward chemical and thermal degradation. But even more important is that the photosensitivity and cyclicity to illumination of this biological photochrome is far beyond that of synthetic materials. Second, BR can be modified to a large extent and serves as a platform for a whole new class of materials. The key to this is that the physical mechanisms of BR are understood on a molecular level today and the genetic tools to redesign the protein have been developed. These reasons make BR not only an attractive candidate to be the first photochromic biomolecule in a technical application but also allow exploration of strategies how biomaterials with technically interesting physical functions can be used as components in technical devices. Gene technology

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Norbert Hampp was born in Neuburg in 1957. He studied pharmacy and physics at the University of Munich, where he received a Ph.D. in pharmaceutical biology in 1986. During his post-doc time he came in touch with bacteriorhodopsin. He finished his habilitation thesis in physical chemistry in 1992. After a time in industry he moved to the University of Marburg in 1995, where he holds a chair for physical chemistry. There he built up an interdisciplinary research group for applied research on the physical properties of biomaterials and their use in technical applications. Optical applications of bacteriorhodopsin are one of the focuses of this research direction. Others are electrochemical processing of biomaterials and near field microscopy of biological macromolecules. The general goal of this research is the use of evolutionary optimized biological materials in technical applications. He has published more than 80 articles and holds about 10 patents in this field. In 1993 he received the Philip-Morris research award. Currently he is Dean of the Chemical Department of his university.

may play a key role in the exploitation of evolutionary optimized functions found in nature for technical applications. This approach was unique when it was used for the first time with BR.

II. Biological Function and Structure of Bacteriorhodopsin

BR is the key protein of the halobacterial photosynthetic system.[2,3] Only a single protein—the BR molecule—is needed to convert light energy into chemical energy. Compared to the chlorophyll-based photosynthetic systems, BR is extremely simple. The harsh environment where *Halobacterium salinarum* (formerly *Halobacterium halobium*) lives may be an explanation why this presumably older evolutionary development to manage the conversion of sunlight into chemical energy survived—it is simple and robust. These are the best features a molecule can have if an implementation into a technical system should have a chance of success, making BR a model compound for functional biological molecules in physical applications.

A. Halobacteria and Their Ecological Niche

H. salinarum lives under extreme conditions. Warm concentrated salt solutions with low oxygen tension, these are the conditions found in salterns (Figure 1) and salt lakes and this is the ecological niche this species adapted to. When you fly over coastal areas where seawater is used in natural or artificial salines to produce salt, quite often you may see an intense purple color in the basins. This is caused by the halobacteria growing there. The physiological conditions can be called "autosterile" because hardly any

Figure 1. Salinas del Rio on Lanzarote Island is a beautifully located, typical, old-fashioned saline. The purple color in the different basins is caused by halobacteria. The intensity of the coloring depends on the time passed since fresh seawater was pumped in.

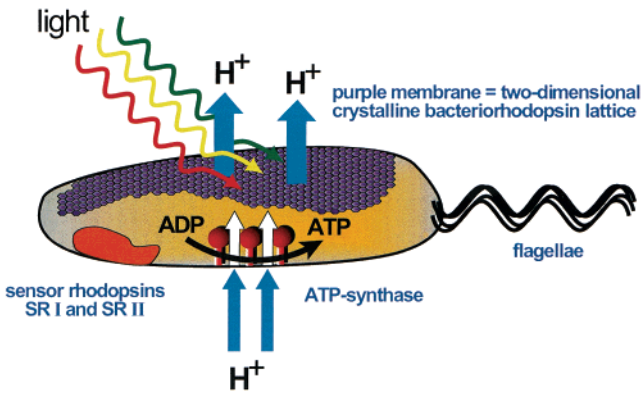

Figure 2. Schematic representation of a halobacterial cell and its main functional systems for phototaxis and photosynthesis. The halobacterial cell contains in its cell membrane a two-dimensional crystalline array consisting of unidirectionally oriented bacteriorhodopsin (BR) molecules and lipids which is called the purple membrane (PM) due to its intense color. Other components contributing to the photosynthetic capabilities of BR are a membrane-bound ATPase, sensory rhodopsins I and II, halorhodopsin, and the flagellar motor.

other organism can survive the enormous salt concentration there. From time to time fresh seawater of comparably low salt but loaded with organic molecules splashes into the natural saline basins. The organic material can be used as a source for living under oxidative conditions and for reproduction. After a while when oxygen is consumed the main energy source remaining is sunlight. During evolution *H. salinarum* adapted to this by developing phototrophic capabilities. The key molecule of its photosynthetic system is BR. The efficiency for the conversion of sunlight energy into chemical energy is about 15% with the BR system and about 35% with the chlorophyll system.[4]

BR is found in halobacteria in the form of a two-dimensional crystal integrated into their cell membrane (Figure 2). These patches are called purple membranes (PM). Up to 80% of the surface may be covered by one or more PM patches, which consist of BR and lipids only. All the BR molecules are uniformly oriented with their carboxy termini located in the inner portion of the cell. Each BR molecule

Table 1. Structural and Physicochemical Properties of Purple Membranes (PM)

PMs consist of lipids and BR at a molar ratio of about 10:1
PMs are two-dimensional, hexagonal crystalline arrays of BR trimers which are uniformly (outward) oriented
PMs have a buoyant density of 1.18 g/cm^3
PMs have a refractive index of $n_{PM} = 1.45-1.55$
PM patches are of irregular shape with lateral dimensions up to 5 μm and a constant thickness of 5 nm.
PMs are stable toward
exposure to sunlight in the presence of air/oxygen for years
temperatures over 80 °C (in water) and up to 140 °C (dry)
pH values from 0 to 12
high ionic strength (3 M NaCl)
digestion by most proteases
PMs preserve their color and photochemical activity when water is removed
PMs are sensitive to polar organic solvents such as EtOH and acetone, especially when they are mixed with water, but they are stable in unpolar solvents such as hexane

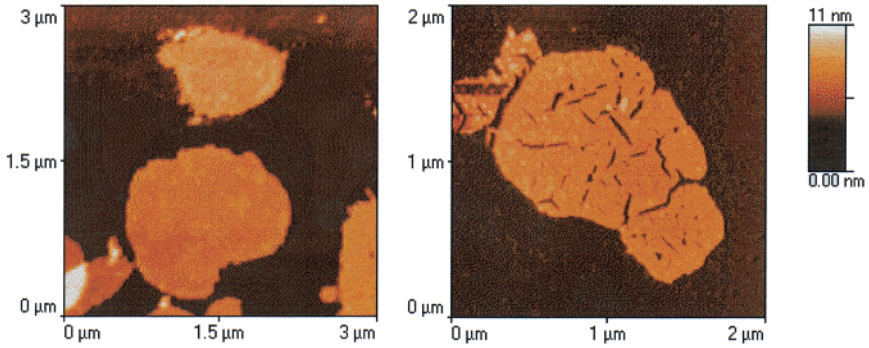

Figure 3. Isolated purple membranes. PM patches adsorbed onto mica were imaged by atomic force microscopy in air at room temperature. (left) Contact-mode imaging with silicon nitride standard tips at about 1.5 nN force. (right) Non-contact-mode AFM with high resonance frequency tips (HRF, Topometrix). Upon drying of the PM cracks appear with a typical 120° symmetry, which corresponds to the hexagonal lattice of BR inside the lipid bilayer.

acts as a light-driven, outwardly directed, proton pump. After absorption of a photon by the molecule, the transport of a proton from the cytoplasm of the cell to the outer medium is observed. By this process light energy is converted into chemical energy and a proton gradient across the cell membrane is generated which serves for energy storage. A membrane-bound ATPase is driven by proton influx from the gradient and regenerates ATP from ADP, which is used by halobacteria, as well as all other living cells, for energizing the cellular molecular processes. In addition, there are two so-called sensor rhodopsins (SR I and SR II) that enable the halobacteria to detect whether light contains the required wavelengths to drive the photocycle of BR, accomplishing light energy conversion. Green-yellow light drives the photocycle, but additional blue light hinders it. The different wavelengths are sensed by SR I and SR II as detector molecules that trigger a signal cascade that controls the flagellar motor. By this rudimentary process of visual perception, halobacteria move themselves into light of suitable spectral composition. In addition, there is halorhodopsin (HR), a light-driven chloride pump that creates the effective level of chloride anions in the cell. All those molecules, SR I, SR II, and HR, and the flagellar motor are very interesting and may also be considered for technical uses.[5−16]

B. Purple Membrane—the Biological Solar Cell of Halobacteria

Proteins occur in soluble or membrane-bound form. It is very rare that they are arranged in crystalline arrays. Apart from BR and the purple membrane (PM), the so-called S-layers[17,18] are important representatives of this class. The physicochemical properties of PM are summarized in Table 1. Isolated PM patches are of irregular shape and have diameters of several hundred nanometers (see Figure 3). The thickness of the PM sheets is about 5 nm. The crystalline array formed by trimeric BR and the lipids has hexagonal symmetry, but also an orthorhombic structure is described.[19] A phase transition of PM at about 70 °C is observed which is slowly reversible.[20−22]

The crystalline structure causes a substantial increase of the BRs stability toward chemical and thermal degradation.[23,24] The thermal stability of dry layers up to 140 °C was reported.[25] Upon solubilization of the PM into monomeric or trimeric BR this property is lost. Astonishing is the mechanism of how the crystallization of the BR inside the lipid membrane is mediated. Mainly only a few amino acid side chains act like an anchor between protein and lipid.[26] Deletion of these amino acid functions probably will lead to a loss of the self-organization capabilities of BR. PM is particularly sensitive to mixtures of polar organic solvents[27] with water but stable toward unpolar solvents such as hexane.[28−30]

PM shows several analogous functions for protons such as a silicon solar cell for electrons. The BR molecules individually convert sunlight into chemical energy by transporting protons from the cytoplasm to the outer medium. The PM does not allow passive diffusion of the protons from the generated gradient back into the cell. The tightest packing of proteins and lipids is in a crystalline state.

C. Bacteriorhodopsin—Structure and Photocycle

All halobacterial retinal proteins belong to the seven-transmembrane helix family of membrane proteins. The structure of BR is analyzed with a resolution of 2.5 Å, i.e. at near atomic resolution,[31,32] whereas from structural data of BR from cryoelectron microscopic analysis of PM patches, a resolution of 3.5 Å parallel and 7.8 Å perpendicular of the membrane plane was obtained.[33−35] The resolution was enhanced to 4.7 Å perpendicular to the membrane by employing additional computational methods and structural information. The higher resolution of 2.5 Å was obtained from three-dimensional crystals of BR.[36] The photochemical conversions of BR have been investigated by several groups, and a detailed understanding of the molecular mechanisms resulted. Presently, BR is the best investigated membrane protein.

1. Structure of BR

Bacteriorhodopsin consists of 248 amino acids which are arranged in seven α-helical bundles inside the lipid membrane and form a cage where a retinylidene residue attached to lysine 216 is located. The retinylidene residue is tilted about 20° toward the outer side. In Figure 4, a molecular model of BR is shown and the proton path through the molecule is indicated. Only the loops connecting the α-helical domains are outside of the lipid bilayer and accessible for proteases and other degrading or modifying enzymes.

2. Molecular Function and Photocycle of BR

The chromophoric group of BR consists of the retinylidene residue and an inner shell of interacting amino acids that influences the absorption properties and controls the photochemical pathways of the retinylidene residue. It revealed that a covalent linkage of the retinylidene residue to the protein moiety is not essential for its function.[37,38] This inner group of amino acids may be considered as an enzyme with a substrate specifity for *all-trans*/13-*cis*-retinal and catalyzes this isomerization at room temperature. Furthermore, the amino acid part of the chromophore is responsible for the suppression of isomerizations different from all-trans ↔ 13-cis which would occur in free retinal, e.g. isomerizations leading to 7-*cis*-, 9-*cis*-, and 11-*cis*-retinal. The rest of the protein moiety carries the proton conduction pathway and shields the photochromic inner group from influences from the outer environment.

The molecular function of BR is that of a light-driven proton pump. The wild-type molecule needs about 10 ms for a complete catalytic cycle. As BR does not have any refractory period[39] after completing a photocycle under light saturating conditions, 100 protons per second could be transported by a single molecule from the inner area of the cell to the outer medium. However, even in bright sun the molecule is far from light saturation. Since the photocycle of BR contains several thermal steps, the overall photocycle shows a dependence on the temperature but only little dependence on the salt concentration as well as the external pH value. The proton conduction pathway[40] (see Figure 4) contains at least

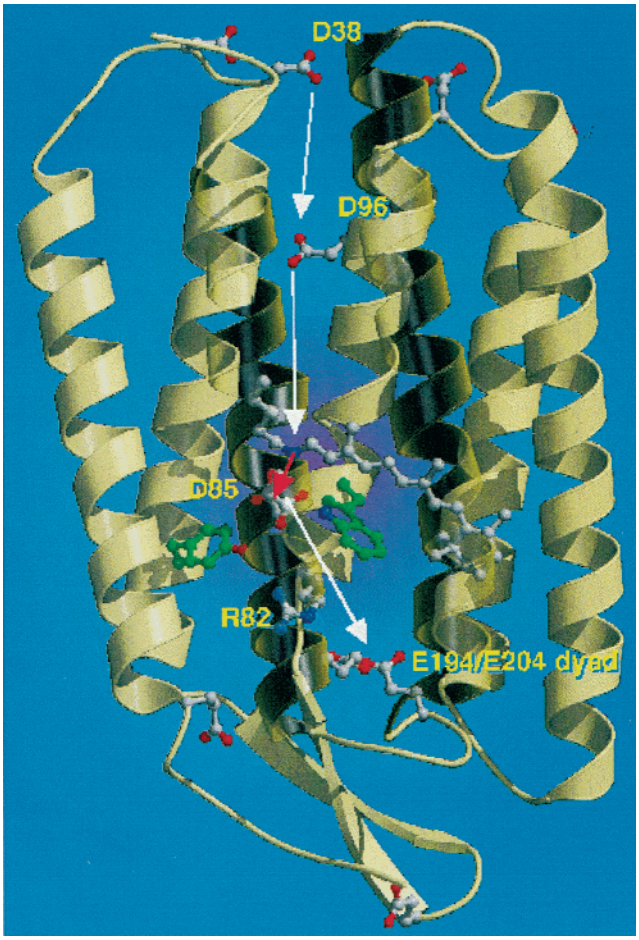

Figure 4. Schematic representation of the three-dimensional structure of BR. The seven α-helical domains form a transmembrane pore. The retinylidene residue is linked to the protein moiety via a Schiff base linkage to lysine-216 (K216). The proton pathway is shown and, in particular, the positions of the aspartic acids 85 and 96 (D85; D96) are given, which act as proton acceptor and donor for the reversible protonation and deprotonation of the Schiff base linkage during the photocycle.

E194/E204 ← R82 ← D85 ← Schiff base ← D96 ← D38

and several water molecules in the core of BR[41−43] The proton transport starts with the release of a proton on the extracellular side and ends with a proton uptake from the cytoplasmatic side. The proton transport is accompanied by a cyclic series of spectral changes of the protein which is called the photocycle of BR (see Figure 5). Twenty years of research have resulted in a more and more detailed understanding of the photocycle; however, still today details of the proton transport are under investigation.[44−46]

The photochemical cycle of BR consists of several intermediates which can be identified by their spectroscopic properties, mainly their absorption maxima. Figure 5 shows only a simplified model of the BR photocycle. The intermediates are given by the common single letter code with their approximate absorption maxima as subscripts. Photochemical and

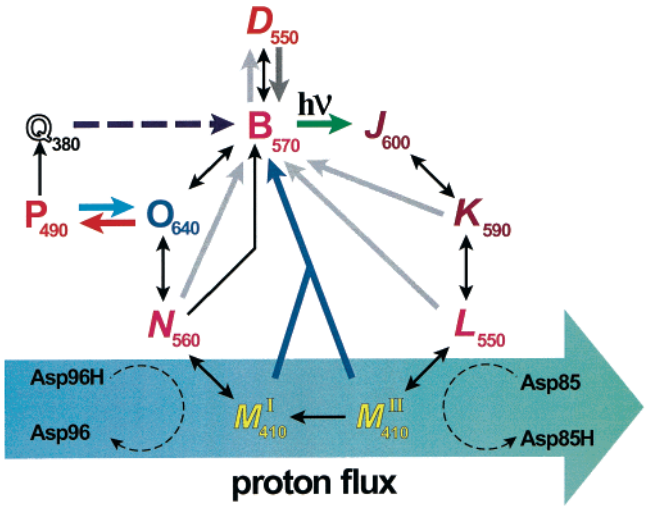

Figure 5. Photocycle of BR. The different intermediates of the photocycle of BR are represented by their common single letter abbreviations with their absorption maxima as subscripts. Upright letters represent *all-trans*-retinal and slanted letters 13-*cis*-retinal containing states. During the L → M transition, the Schiff base is deprotonated and Asp85 becomes protonated. From there the proton moves toward the outer side of the extracellular part of the proton channel. During the M → N transition the Schiff base is reprotonated. Aspartic acid 96 serves as a proton donor for this step. Asp96 is reprotonated through the cytoplasmatic proton half-channel.

thermal conversions are shown as thick and thin arrows, respectively. Upright letters indicate an all-trans conformation of the retinal, i.e. B and O, and slanted letters represent 13-*cis*-retinal. In the dark BR relaxes into a 6:4 mixture of D and B state.[47] This mixture is called dark-adapted BR. Upon exposure BR becomes light-adapted, i.e. all the BR material returns to the B state. From there it starts into the photocycle which results in the transport of a proton. This part may be addressed as the core cycle. Whereas all the conversions between the intermediates are reversible, the transition from M^I to M^{II} is considered to be the main irreversible step in the photocycle.[48,49] During this transition the nitrogen in the Schiff base group no longer becomes accessible from the extracellular part of the proton channel but only to the cytoplasmatic half-channel.[50] This may be accomplished through a conformational change from 13-cis/14-cis to 13-cis/14-trans.[51,52] Larger conformational changes of the protein molecule are not excluded.[53] This reorientation of the Schiff base accessibility for protons, i.e., M^I and M^{II}, is the molecular origin for the vectorial proton transport through the protein.[54−56]

The physicochemical properties of the intermediates are summed in Table 2. They are spectroscopically characterized by the wavelength of their absorption maxima λ_{max} and the specific molar extinction coefficient ϵ.[57] The protonation of the Schiff base group and the configuration of the retinylidene residue affect the position of the absorption bands. The photocycle of BR requires several temperature-dependent conformational changes in the protein. The formation of most of the intermediates can be suppressed by cooling the molecule. The value T_{freeze} gives the temperature where an intermediate can be thermally stabilized, i.e. the transition to the next intermediate is inhibited. The quantum efficiency Φ of photoconversions is given in the last column.

Apart from the core photocycle there are some states that are not populated at physiological conditions. The P and Q states contain 9-*cis*-retinal in their photochromic groups.[58,59] This is reached after photochemical excitation of an *all-trans*-retinal when at the same time Asp85 is protonated, i.e., is in the Asp85H form. This can be achieved in wild-type BR either by low pH values[60−62] or deionization,[63−65] where so-called blue membranes are formed.[66] Another approach is to replace Asp85[67] for an amino acid having a different pK_a value that stays in its uncharged form at the pH values of interest.[68−71] A third possibility is to remove the carboxylate group by mutation. Asparagin (BR-D85N) is a typical example of such a mutation. Blue membrane from wild-type BR is difficult to prepare and to keep in a stable state. It tends to aggregation and irreversible bleaching. Mutated BRs such as BR-D85N make the 9-cis photochemistry available at ambient conditions—this is their main advantage. But in one aspect the blue membrane and the mutated BRs are unfortunately identical: they share the low quantum efficiency for the all-trans to 9-cis conversion, which is in the range of 0.02%.[72] The photochemical back reaction from 9-cis to all-trans is about 50-times more efficient, but it is still in the 1% regime. Monomeric BR has its isoelectric point at about pH 4−5.[73] In Table 3 the most important molecular properties of BR are summarized.

Table 2. Characterization of the Intermediates of the Bacteriorhodopsin Photocycle

name	λ_{max} (nm)	ϵ ($M\ cm^{-1}$)	retinal configuration	SB[a] conformation	SB[a] protonation	T_{freeze} (°C)	Φ (%)
B	570	63000	all-trans	anti	yes	- - -	$64_{B\rightarrow K}$
K	586	52100	13-cis	anti	yes	−190	
L	544	48900	13-cis	anti	yes	−100	
M^I	409	48800	13-cis	anti	no	−30	
M^{II}	409	48800	13-cis	anti	no	−10	
N	562	46100	13-cis	anti	yes	−60	
O	629	61900	all-trans	anti	yes		$0{,}02_{O\rightarrow P}$
D	550	51000	13-cis	syn			
P	485	39000	9-cis				$1_{P\rightarrow B}$
Q	390		9-cis				

[a] SB = Schiff base.

Table 3. Molecular Properties of Bacteriorhodopsin (BR)

BR consists of a single polypeptide chain of 248 amino acids and a retinylidene residue attached to the protein via lysine-216
BR has a molecular weight of MW = 26 784 Da
BR has its isoelectric point at about pH 4−5
the retinylidene residue in BR is
tilted 20° from the membrane plane toward the extracellular side and
its cyclohexene ring is oriented perpendicular to the membrane plane
after photochemical excitation the photochromic group in BR undergoes
a reversible change of the all-trans ⇄ 13-cis configuration
a reversible de-/reprotonation of the Schiff base nitrogen
a reversible conformational change which switches the nitrogen accessibility for protons
the quantum efficiency of the primary photoreaction of BR is $\Phi_{B\rightarrow J} \geq 0.64$ (=64%)
the photochemical cycle of BR has no refractory period

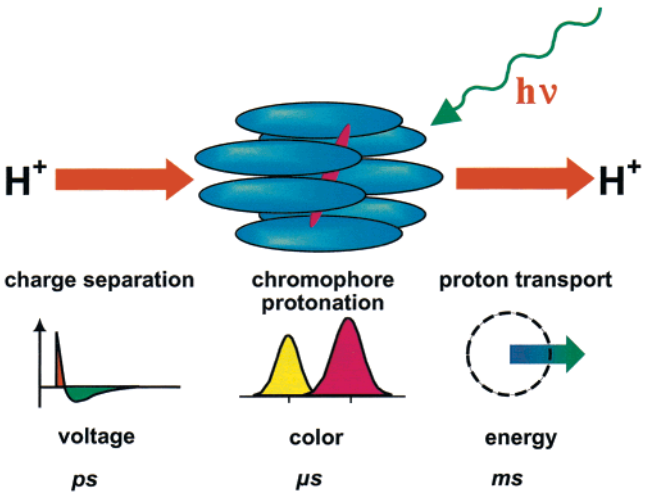

Figure 6. Basic molecular functions of BR. The proton transport is initialized by photon absorption and a charge separation step on the picosecond time scale. After about 50 μs the deprotonation of the Schiff base leads to the main photochromic shift during the photocycle. After about 10 ms the proton transport is completed.

III. Overview on Technical Applications Suggested for Bacteriorhodopsin

In Figure 6 the basic molecular functions of BR and their corresponding physical effects are schematically shown. The light-driven proton transport process is initialized by absorption of a photon and an immediately following charge separation step. This initial part of the proton transport accounts for the photoelectric properties of BR. The charge transport through the molecule requires a deprotonation and reprotonation of the Schiff base linkage in the photochromic group. This causes a significant shift in the absorption maximum of more than 150 nm. The optical properties of the B and M states allow the use of BR as a photochromic optical recording material. The photoconversion of BR results in the transport of a proton, which may be used on a macroscopic scale, e.g. in artificial photosynthesis or in seawater desalination.[74−76]

In Table 4 applications of BR that are described in the literature are summarized and grouped in view of the basic molecular functions of BR. By far the most applications reported utilize the photochromic properties of BR.

A. Photoelectric, Photochromic, and Charge Transport Applications

Each class of BR applications (see Table 5) differs in the complexity of the related devices and the demands toward the biological components. For the integration of BR into technical systems two hurdles have to be overcome: first, the structuring of the biomaterial itself and, second, the interface of the biocomponent with the rest of the conventional system.

The technical level for both criteria is the lowest for photochromic applications. No long-range order between PM patches, i.e. macroscopic orientation, is required. A homogeneous distribution of PMs in an optically inert matrix material fulfills the needs. Because the interface is an optical one, it is easy to protect the BR media. They may be sealed completely between protecting glass layers. For these reasons most of the applications discussed for BR are based on its photochromic properties and range from long-term data storage to optical and holographic processor (see the next section).[77−81]

Table 4. Technical Applications Suggested for Bacteriorhodopsin

charge transport
ATP generation in reactors
desalination of seawater
conversion of sunlight into electricity
photoelectric
ultrafast light detection
artificial retinas
motion detection
miscellaneous
2nd harmonic generation
radiation detection
biosensor applications
etc. .....
photochromic
information storage
2-D storage
3-D storage
holographic storage
associative memories
information processing
optical bistability/light switching
optical filtering
signal conditioning
neural networks
spatial light modulators
phaseconjugation
pattern recognition
interferometry

Table 5. Main Classes of Technical Applications of BR, Their Differing Microstructural Demands and the Type of Interface to the Technical System

microstructural demands	photochromic	photoelectric	charge transport
orientation of PMs	none	high	very high
porosity of the PM layer	not relevant	low	very low
thickness of the PM layer	not relevant	few layers	monolayer
interface	optical	electrical	chemical

More challenging in this respect are photoelectric devices. To generate a maximal voltage per photon absorbed, a macroscopic orientation of the PM patches is a key issue. Langmuir−Blodgett[82−85] as well as immunochemical methods[86] for the preparation of highly orientied monolayers and thin layers of PM have been developed. Somewhat thicker PM layers are prepared by electrosedimentation of PM from an aqueous dispersion.[87,88] Interfacing between the light-dependent proton motive force generated by BR and the other part of the system requires that at least one of the electrodes is transparent. The transformation of BR's proton motive force into an electromotive force of a photoelectric cell is often supported by an embedded electrolyte containing layer.[89,90] Whereas in the beginning super-fast photodetection[91] was investigated, now much more sophisticated uses of BR in systems for motion detection[92−94] and artificial retinas[95−97] are considered.

The use of the biological function of BR as a light-driven proton pump was one of the first suggestions for a technical application of BR.[98−100] Unfortunately, it was revealed to be very difficult to bring it to a technically useful scale. The figure-of-merit for this type of application is easy to define and may be described as a huge artificial purple membrane, several square meters large, with exactly the same properties as natural PM, i.e., unidirectional embedded BR and no passive proton flux through pores or holes. To date the preparation of macroscopic PM-like separator membranes was not successful. But even if this could be realized, another difficult problem still remains with the interface. The separator membrane needs to contain a monomolecular layer of oriented BR without any passive pores. A chemical interface to the liquid which shall be deprotonated, as well as a surface exposed to the sun, is required. If the active monolayer is destroyed in any place, a short circuit for the generated proton gradient results. The only way to overcome this would be a self-curing construction of the PM modules.

B. Miscellaneous Applications

Apart from the major areas of BR applications there appeared several proposals for unconventional routes. In several cases the proposals were really astonishing, but often a detailed investigation of the effects described cannot be found in the literature. Some of these proposals shall be mentioned here briefly. The largest group in this section describes the effects of anesthetics on the BR photocycle and its potential uses,[101−103] Also biosensors for monitoring, for example, pesticides[104,105] and enzymic reactions where BR acts as an indicator[106] are described. Even for the detection of high-energy radiation[107] BR seems to be applicable. Second harmonic generation[108] with BR is an option, but it does not seem to be competitive with conventional materials. Those applications cannot be discussed further here, but they illustrate that in the future potentially more unexpected applications of BR might appear.

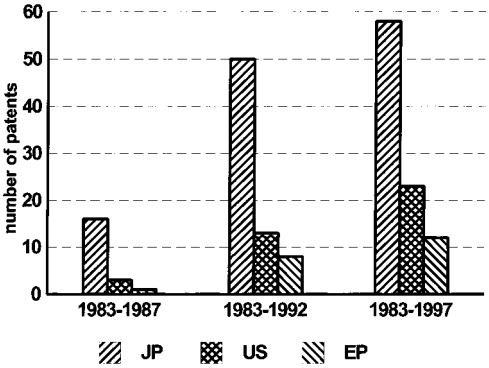

Figure 7. Patents related to BR. Shown is the number of patents filed in Japan, the United States, and Europe during the last 15 years. All these patents claim the use of BR in different applications or describe the preparation of materials employing BR. *(Not counted are patents where BR is mentioned in the general context but is not in the main focus.)*

C. Patents on Bacteriorhodopsin Materials and Applications

Patents are an indicator anticipating the commercial exploitation of a new material or a new technique. Currently about 60 Japanese, 20 United States, and 10 European patents exist which claim materials, devices, and applications based on BR. The earliest one dates back to 1980 and claims the conversion of solar energy to chemical and electrical energy.[109] The number of patent applications has increased continuously since then. In Figure 7 the number of patents in Japan, the United States, and Europe are plotted for a time range of 15 years in reference to their priority dates. The situation to date may be interpreted in such a way that it is still not clear which type of application of BR will be commercialized first but also that a growing community is convinced that this unique biological material will be of technological and commercial importance soon.

IV. Bacteriorhodopsin as a Photochromic Molecular Material

During the photocycle of the BR several molecular switches operate sequentially: first the photoinduced isomerization around the C13−C14 double bond ("isomere-switch"), then the deprotonation of Asp85 to a more outward-located proton release group ("amino acid protonation switch"), immediately followed by the deprotonation of the Schiff base linkage

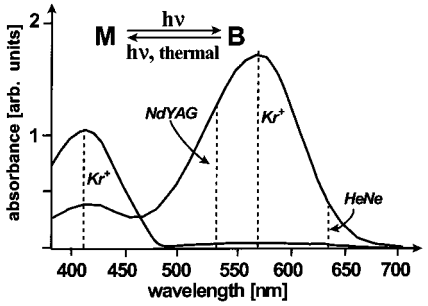

Figure 8. Absorption spectra of the B and M states of bacteriorhodopsin. The purple B state is the initial state of BR after light adaptation. The M state is the longest living intermediate and the most blue-shifted from B. For photochromic applications the photochemical interconversion of B and M is preferrably used because of the large photochromic shift of about 160 nm.

("Schiff base protonation switch"), and a conformation change at C15 ("conformation switch"). Isomerization, Schiff base protonation, and conformation switch are reset and the molecule is ready to start into a new cycle. The reprotonation of the Schiff base linkage occurs from Asp96 instead of Asp85, reflecting the vectorial proton transport. All of the listed "switches" have some technical importance, even if it is not possible to separate them completely. For photochromic applications the most important one is the "Schiff base protonation switch" because it causes the large spectral shift of the chromophore with almost completely separated absorption bands.

In Figure 8 the absorption bands of the B and M states are given, and the position of some important laser wavelengths is indicated. Whereas for lab applications krypton gas lasers are the best choice for the work with BR, they have emission lines at 568 and 412 nm, which coincide with the maximal absorption wavelengths of the B and M state almost perfectly, technical applications better use solid-state laser, e.g., a diode pumped frequency-doubled Nd:YAG or laser diodes which are available down to 633 nm from the shelf.

In the following sections how this "deprotonation switch" can be modified in its properties is discussed. In principle, photochromic applications with earlier states of the BR photocycle could be also done, e.g., at lower temperatures, but most of the photochromic applications described in the literature use the interconversion of the B and M states at room temperature.

A. Modeling the Photoresponse of Bacteriorhodopsin

Due to the complexity of the photocycle of BR, mathematical models are rather complicated. The reaction rates of the thermal conversions depend on the sample temperature. Furthermore, it is important whether continuous wave (cw) or pulsed excitation is used. Under cw excitation the photochemical conversion of intermediates needs to be considered. A multiexponential fit with up to seven components is used to approximate the photoelectric,[110] absorptive,[111] and refractive changes[112] in BR.

Often an extremely simplified photocycle is used, regarding the B and M states only, for a qualitative

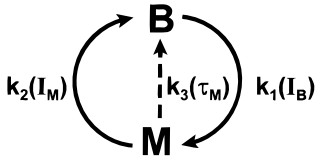

Figure 9. Simplified photocycle model of BR considering the initial B and the longest living intermediate M only. The photochemical transitions k_1 and k_2 depend on the intensities of the writing and erasing wavelength. For simplification it is assumed that each wavelength drives only one transition. The thermal pathway from M to B is given by k_3, which depends on the lifetime of the M state. A material with unlimited thermal M lifetime would be the best photochrome on the basis of BR.

or even semiquantitative treatment of the population of the intermediates (see Figure 9).

Astonishingly, this model is good enough for many discussions as long as certain restrictions are considered. Looking at the time scale of the different transitions, the population of the K and L states is negligible compared to the M states as long as the time of observation is long enough to allow the formation of the M states. In particular, when the system is mainly under photon-control, e.g. blue and green-yellow wavelengths are used in combination, the contributions of later intermediates do not play an important role and may be neglected. However, at low and high light levels of green-yellow light alone, significant deviations from this simple model must be taken into account. At very low light levels the BR material tends to relax into the so-called dark-adapted state. This means that a significant contribution of the D state is present in the sample that needs extra light to be converted back into the B state. With low levels of signal light, periodic flashing of the material with actinic light to keep it in the light-adapted state is required. At high light levels samples with low proton mobility have a problem restoring the protonation state in the proton pathway before a second photon is absorbed. In this case changes in the kinetics of the different states are observed.

As long as the restrictive preconditions for the simple model shown in Figure 9 are taken into account, it is a comfortable tool for semiquantitative estimations. The rate equation for this model is

$$\frac{dB}{dt} = -k_1(I_B)B + [k_2(I_M) + k_3(\tau_M)]M \qquad (1)$$

with the intensity-dependent reaction rates k_1 and k_2 and the thermal decay rate k_3. Due to the cyclic conversions, $B_0 = B + M$ holds for all times, where B_0 is the initial concentration of BR, assuming that no dark adaptation occurs. Solving this equation leads to the time-dependent population density of M

$$M(t) = \frac{B_0}{k_{123}}(k_1 + k_{23}e^{-k_{123}t}) + Ce^{-k_{123}t} \qquad (2)$$

where terms such as k_{123} represent the sum $k_1 + k_2 + k_3$. C is an integration constant that has to be determined from a known population for M. At time $t = 0$, all the material is in the B state; thus, $M(0) =$

0. The resulting function describes the rise of M when BR is bleached, e.g. in a BR film.

$$M(0) = 0 \Rightarrow M(t) = B_0 \frac{k_1}{k_{123}}(1 - e^{-k_{123}t}) \qquad (3)$$

Assuming that no blue light is employed ($k_2 = 0$) the typical bleaching kinetics is described. Analyzing eq 4 shows that the material is completely converted into the M state only when the denominator is close to 1.

$$k_2 = 0 \Rightarrow M(\infty) = B_0 \frac{1}{1 + \frac{1}{k_1(I_B)\tau_M}} \qquad (4)$$

This is the case for $k_1(I_B)\tau_M \rightarrow \infty$. Assuming $\lambda_B = 532$ nm, $\epsilon_B = 63.000$ L mol^{-1} cm^{-1}, $\Phi_{B\rightarrow J} = 0.64$, eq 5 results

$$M(\infty) = B_0 \frac{1}{1 + \frac{1}{0.4 I_B \tau_M}} \qquad (5)$$

with I_B to be given in mW/cm^2 and τ_M in seconds. This equation shows that an increase in the M lifetime, e.g. by a factor of 2, causes that the light intensity required to reach the same maximal bleaching rate decreases by the same factor of 2. For this reason BR materials with extended M lifetime are of great interest for optical recording materials. The perfect BR material would be a bistable one where the thermal decay of M is completely suppressed.

B. Modification of the Photochromic Properties of Bacteriorhodopsin

The modification of the photochromic properties can be accomplished by changing or modifying one or more of the essential components in BR. In Figure 10 the different functional modules of BR are schematically shown.

Table 6 summarizes the means by which the different components can be modified. All the listed approaches have been implemented already. Amino acid exchanges are accomplished by genetic engineering of the gene coding for BR and expression of the resulting BR variant in halobacteria. Genetic methods are of great importance for the future development of new BR variants for technical uses.

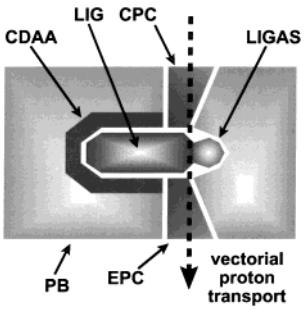

Figure 10. The different functional components of BR are sketched, i.e. LIG = light interacting group, LIGAS = light interacting group attachment site, CPC = cytoplasmatic proton channel, EPC = extracellular proton channel, CDAA = chromophore determining amino acids, PB = protein backbone. The protein moiety in total mediates also the long-range effects (pK_a changes).

Besides the changes in the molecule itself, alterations of the physicochemical environment of BR and its mutated versions allow its photochemical properties to be influenced to a great extent. Decreasing the temperature leads to the stabilization of photocycle intermediates. The lower the temperature, the earlier the photointermediate is, which is not further converted. Since the protein motions are required for the long-distance effects in the photocycle, e.g. to change the pK_a value of an amino acid which may be about a nanometer away from the chromophoric group,[113] these couplings are hindered and lead to the "freezing" of intermediates. In Table 2 the values for the different intermediates are summarized. Whereas it takes liquid nitrogen to stabilize the K state, the M state can be "frozen" at −10 to −30 °C. Decreasing the temperature does not influence the photochemistry itself but the time constants for the inner molecular processes. External electric fields are another approach to alter the photochromism of BR.

Chemicals added[114−118] to the BR can have rather complex interaction with the molecule and its photocycle. In most cases these effects have been discovered accidentally. For example, it is well-known that

Table 6. Functional Modules in BR and Their Possible Modifications and the Technologies Used for Their Realization

functional modules in BR	alterations	technology[a]
proton pathways (EPC & CPC)	chemical additives	chem
	amino acid exchanges	gene
retinylidene residue (AG)	retinal analogues	chem
amino acids in chromophore (CDAA)	amino acid exchanges	gene
Schiff base linkage (AGAS)	attached or free chromophore	gene
	different attachment sites	gene
protein backbone (PB)	chemical modifications	chem
	amino acid exchanges	gene
external fields	electric fields	phys
	magnetic fields	phys
substrate (protons)	pH-value	phys
	proton availability (drying)	phys
	proton mobility (additives)	phys
	deuterium	phys

[a] chem = chemical modifications, phys = physicochemical means, gene = genetic engineering.

Table 7. Strategies To Modify the Photochemical Properties of BR

	temperature decrease	chemical additives	retinal analogues	genetic engineering
time constants	high	medium	minor	very high
color	minor	minor	high	high
reversibility	?	(decrease)	?	?

some primary amines lead to an increased lifetime of the M intermediate. Such samples are often called chemically modified bacteriorhodopsins. Sodium azide shows the opposite effect.[119] BR-D96N reverts its M lifetime to that of the wild-type when azide is mixed into the sample.

Retinal analogues[120−125] replace the retinylidene residue in BR. First a BR without a retinylidene residue is needed. This can be achieved either by extraction of BR with hydroxylamine[126] or by production of white membrane which is obtained from Ret$^-$ strains that are deficient in retinal synthesis. Quite a number of molecules have successfully been tested and are able to form a chromophoric group in BR.

The addition of chemicals and the use of retinal analogues share one problem. Today it is not possible to predict how the reversibility of the molecule is affected. It is difficult to predict the photochemistry of retinal analogues, but it is impossible to predict the side reactions of the photoconversions and thermal transitions. Those—after maybe hundreds and thousands of cycles—lead to the loss of the photochromic properties of the material.

Genetic engineering has become a valuable tool to produce mutated BRs at will. Expression in halobacteria allows in most cases production of the purple membrane form of the mutants. This is much desired because of the stabilization effect of the PM form mentioned earlier. In Table 7 the different approaches to modify the photochemical properties of BR are summarized and a qualitative estimation of their effect on the time constants, in particular the M lifetime, the initial color of the material, and its reversibility are given.

C. Relation between Changes of Absorption and Refractive Index

For many optical and, in particular, holographic applications, the refractive index of the material and the spectral dependence of the light-induced refractive index changes are important. The molar refraction and the molar absorption coefficient are the real and imaginary parts of a single complex value. The spectral dependence between both values is described by the Kramers−Kronig relation for the case of an undisturbed idealized chromophore.

In solid-state materials, due to matrix effects, significant deviations from this relation are observed. The refractive index of BR was measured by several groups and values of 1.47−1.55 were found.[127,128] The spectral dependence of the refractive index change and the absorption change, astonishingly, is in quite good agreement with the Kramers−Kronig relation (see Figure 11). This finding is of great value for the characterization of new BR variants. The spectral dependence of the light-induced refractive index change of such a material has to be measured at only one wavelength. Assuming the Kramers−Kronig relation also holds for the new material, then this immediately supplies the whole spectral dependence, because the computed curve has only to be scaled by the one experimentally analyzed value.

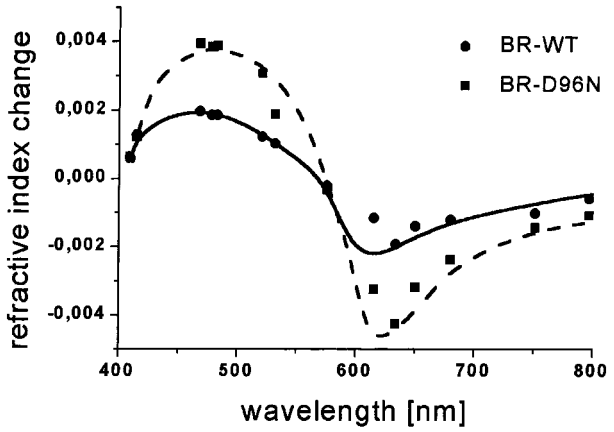

Figure 11. Spectral dependence of the light-induced refractive index changes in BR films. Experimental values (●, ■) and values computed on the basis of the Kramers−Kronig model (solid and broken lines) are compared. The good correspondence between experimental and theoretical values holds for both wild-type bacteriorhodopsin (BR-WT, solid line) as well as the bacteriorhodopsin variant BR-D96N (broken line) (modified from ref 127).

The finding that the Kramers−Kronig relation is an appropriate approximation for $n(\lambda,I)$ was unexpected, because in the dense packing of BR in the BR films charge motion induced long-range effects would be expected. It shows that the amino acid moiety shields the chromophore region very well and decouples it from outer physicochemical conditions.

D. One-Photon and Two-Photon Excitation of Bacteriorhodopsin

The primary photochemical events of BR have been analyzed in detail by many groups.[129,130] The single photon excitation from the B ground state which results in a 64% forward reaction to the K ground state is not a matter of further discussions. The assignment of intermediates before K as ground states or excited states is a bit more complicated but less important in this context, because these intermediates cannot be used for technical applications. With a molar extinction of 63 000 L mol^{-1} cm^{-1} and a quantum efficiency of 64%, BR is a very effective chromophore. Less than two photons are needed per molecule for switching.

Apart from one-photon excitation, two-photon excitation of BR was investigated in detail because two-

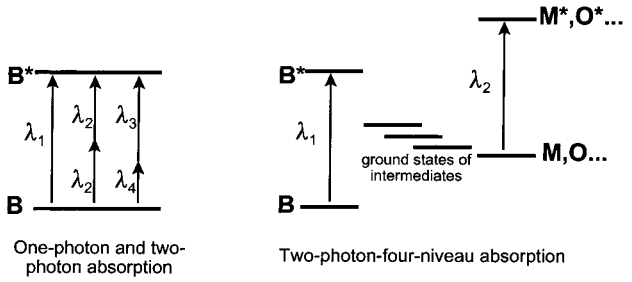

Figure 12. One-photon and two-photon excitation of BR. (Left) Both mechanisms lead to the formation of a K intermediate. For symmetry reasons the processes of one-photon and two-photon absorption are different in their mechanism. This simple diagram takes into account energy only. (Right) The combination of two one-photon steps is used when the M state is photochemically converted to the B state as well as when the O state is converted into the so-called P state. Both absorptions are decoupled, but the second one comes only into effect when triggered by the first one.

photon excitation is an attractive method for volume storage of data. Two-photon excitation of chromophore molecules (see Figure 12) is not surprising, but the cross section for two-photon absorption of BR is astonishingly high compared to other materials.[131] Since the two photons have to coincide at the molecule under very strict conditions, the absorption probability is rather low compared to one-photon absorption. In the literature such processes are often named "biphotonic absorption", because there tends to be confusion with two consecutive one-photon absorption processes.

For photochromic excitation the combination of two one-photon excitations is a key element in applications. Whereas one wavelength is used for writing information into the BR material, the other one is used to erase it. The absorption bands of B and M have only little overlap. For this reason this combination is preferred for photowriting/photoerasing applications. The photochemical generation of the state which is used for recording was first demonstrated with BR for holographic recording and was named M-type holography.[132] The main advantage is that the read-out light is not disturbing the contrast of the recorded information, instead it is required. The more the information is read-out, the better the contrast is. Last but not least, long-term storage with BR is obtained by the same approach. It was known that the so-called blue membrane, which is obtained from purple membranes by acidification or removal of divalent cations, converts under irradiation to the so-called pink membrane. The pink membrane is stable and its color does not revert to the initial blue color at room temperature. Erasure with light, however, allows the return to the initial blue state. It was found that the pink membrane contains a 9-*cis*-retinal group. This is obtained from *all-trans*-retinal by photochemical excitation with a low quantum yield of less than 1%.[133] Since the protein moiety is not able to catalyze the 9-cis → all-trans isomerization at room temperature, a second photon is needed to overcome the activation barrier.

E. Optical Recording Media Made from Bacteriorhodopsin

Whether the theoretical limits of an optical system based on BR can be reached depends mainly on the quality of the recording media, in particular their (1) homogeneity, (2) light sensitivity, (3) maximal contrast, (4) size (aperture), (5) storage time, and (6) long-term stability.

For a long time the availability of BR and its high price was a limitation which hindered the development of optical recording media, but this limitation has been overcome in the meantime. Other restrictions still remain, for example, that no organic solvents can be used in the preparation of BR-based recording media. This fact reduces the chemical methods applicable to a great extent. When PM patches with dimensions up to 1 μm have to be processed into a homogeneous mixture with matrix polymers, care has to be taken because this mixture tends to separate during water removal, which is often done by simply drying in air. Usually glass is used as substrate for the recording media, but other substrates such as plastics are suitable too. In most cases the use of gelatin or poly(vinyl alcohol) is used for matrix embedding of BR. Attempts have been made to embed BR into sol−gel glasses.[134,135] In some cases attempts were made to use BR suspensions directly.[136,137] The importance of the media preparation for the different research groups may be the reason for an interesting observation: In most papers the preparation procedure of the optical media is not sufficiently described nor are the ingredients listed completely. Furthermore, the quantitative portions of the ingredients are not given. This situation is not satisfying from a scientific point of view but becomes reasonable when the commercial aspects are also taken into account. The preparation of the recording media is a key technology in the application of BR. The reason that authors do not describe in full detail how this is done presumably is, that as soon as a commercial use of BR begins, these technologies will determine who will win the race.

For most optical applications, films made from BR are employed, but for 3-D storage, cubes filled with BR have been used. Due to the size of PM, rotational and lateral diffusion is not a severe problem. Only for long-term storage applications both parameters have to be considered carefully, because any type of diffusion would cause fatigue of the stored information and reduce the useable storage density.

Quite often optical BR films with small diameters are used. This requires that lenses have to be used for imaging, which usually limit the optical resolution of the systems. For lensless recording, e.g. interferometry, films with large, 4 in. wide apertures have been developed[138] (see Figure 13). The optical and holographic properties of BR films are summarized in Table 8.

Figure 13. Bacteriorhodopsin film with large aperture. These films have free aperatures of 90 mm × 90 mm, their outer dimensions are 120 mm × 120 mm. They are tuned to maximal light sensitivity because their primary use is in holographic interferometry.

Table 8. Optical and Holographic Properties of BR Films

spectral range	400−700 nm possible
transient recording	
B → M	520 nm−640 nm
M → B	400 nm−430 nm
long-term recording	
O → P	630 nm−700 nm
P → B	430 nm−530 nm
resolution (optical)	≥5000 lines/mm
optical density (570 nm)	1−5 OD_{570}
maximal bleaching ratio	95%
index of refraction	1.47
refraction index change	0.001−0.01 (depends on OD)
diffraction efficiency	1−3%, max 7%
light sensitivity	0.1−20 mJ/cm^2
polarization recording	possible
reversibility	≥10^6 cycles
shelf life	years
film thickness	10−500 μm, typ 20−40 μm
rise and decay times	ms−s
aperture	unlimited

V. Data Storage Applications

High-capacity long-term data storage is one of the areas where optical techniques already have been established. Data safety, storage density, data transfer rate, and access time are the critical parameters. The system should need a minimal number of moving parts, because this significantly increases its reliability. Stored data must be safe even if the power supply goes down.

These demands are hard for BR to match. Not only BR but all photochromic materials have an intrinsic problem because writing as well as erasing is accomplished by light. Undesired exposure to light destroys the stored data. For rewriteable long-term optical data storage, so-called "gating" is a must. Reading of data shall be possible with light at any time, but writing requires an additional physical input. For example, in magnetooptical recording writing is possible only as long as an electromagnetic field—the laser beam—and a comparably strong magnetic field coincide. The lack of a magnetic field still allows reading of the information, but no change or erasure occurs. Such a disk can be handled without too much care.

In long-term storage not that many write−read−erase (WRE) cycles are required. A few hundred thousand WRE cycles are enough, but storage density is a major issue. Limitations arise more from the wavelength of the light used than from the physical capabilities of the recording materials. Theoretically every 5 nm a binary information could be recorded in a BR layer by switching a single BR molecule or a BR trimer from B to M. But today there is no convenient technique which allows one to address single molecules optically. State-of-the-art scanning nearfield microscopes (SNOM) have a resolution in that range, but they are analytical instruments and cannot reach the data transfer rates required. A 10 nm spacing would mean about 100 000 lines/mm resolution. With conventional optics a resolution of 500 nm is obtained with an upper limit of 2000 lines/mm.

Photochromic materials without gating are not of interest for long-term data storage. This holds also for biological materials such as BR in such applications. The impetus for exploring BR in long-term storage applications is much beyond the existing 2-D storage techniques. Volume data storage expands the capacities of a system dramatically. Also holographic storage systems have their attractiveness because they allow realization of content-addressable and associative memories.

In Table 9 the different data storage techniques are listed, and the advantages and limitations which BR has in these uses are indicated.

A. 2-D Data Storage

For 2-D data storage the same principle setup may be used like in CD-ROMs. The storage medium moves under a head which is positioned and used for reading and writing. The resolution of such a system is limited by the mechanical precision for positioning the read−write head. The next limitation would be the wavelength of light which is used for storage. The standard today is light in the range of 670 nm, which can be supplied easily and cheap from laser diodes. The physical resolution of most recording materials is far beyond the technical limits. Photochromic materials in principle would allow resolutions in the subnanometer regime, but even with near-field optical microscopy only resolutions in the range of 10 nm are reached currently. The main efforts concentrate today on the development of reliable light sources with shorter wavelengths, in particular blue laser diodes. Decreasing the wavelength by a factor of 2 increases the capacity in two-dimensional storage by a factor of 4. About 10^4−10^5 WRE cycles are sufficient for such rewritable media. Even in the case that a photochromic long-term storage would be desired, there are synthetic photochromic materials which can reach this number of WRE cycles (see the papers authored by M. Irie and Y. Yokoyama in this issue).

For quite a while the effects of external electric fields on the BR photocycle were known.[139] In combination with mutated BR-D85N, an interesting new effect was reported, the first "gating mechanism" in

Table 9. Comparison of Long-Term Storage Techniques with Bacteriorhodopsin

	2-D storage	3-D storage	holographic storage
advantages	fast writing speed (<0.5 ps)	no long range effects in the material, high two-photon cross section	high resolution, i.e. many coexisting page holograms possible
limitations		no gating mechanism ⇒ nondestructive reading difficult	
	wavelength of light (not a material limit)	read-out procedure difficult	low diffraction efficiency
state-of-the-art	magneto-optic disk		photorefractive crystals

BR.[140] This may point into a new direction of research on BR in long-term storage applications, but today it is not possible to estimate whether this will become technically competitive.

Another form of optical gating can be done by using a two-photon−four-niveau scheme, as described in Figure 12. It is well-known that BR in blue membranes can be photochemically converted into 9-*cis*-retinal and forms the so-called pink membrane. It has been shown that the O intermediate and the blue forms of BR are functionally identical and excitation of BR with a first photon to form O and excitation of O with a second photon to form P can be achieved even in wild-type BR.[141] This is the functional basis of a so-called "branched photocycle" scheme for storage of information in BR,[142,143] which also may be applied for 3-D storage.

B. 3-D Data Storage

The situation in 3-D storage looks somewhat better for BR. Three different types of volume storage techniques are investigated. The first is page-oriented holographic storage, the second is based on a so-called branched photocycle scheme, and the third one uses two-photon excitation of individual data points in the volume of a material.

The latter is accomplished by the intersection of two laser beams.[144] Each beam carries photons with just half the energy to switch BR from B to M or vice versa. Two-photon absorption depends on the product of the contributing intensities ($\propto I^2$). In the volume of intersection the probability for two-photon absorption is high enough that a measurable shift in the population distribution between B and M can be induced. The wavelengths employed must fulfill the condition $1/\lambda_{excite} = 1/\lambda_1 + 1/\lambda_2$. This may be achieved by choosing $2\lambda_{excite}$ for both beams, e.g. 820 and 1140 nm, correponding to one-photon excitations with 410 and 570 nm, respectively.[145] In the pass of the light beams, undesired two-photon excitations must be taken into account. For this reason it might be advantageous to use two different wavelengths which fulfill the given equation. It is of great advantage that wavelengths can be chosen where the material is almost transparent and one-photon absorption is not a competing reaction. It revealed that BR has an astonishingly high cross section for two-photon absorption, and for this reason BR should be considered as a material for volume data storage via two-photon excitation.[146] The longevity of the stored information is currently possible only if the BR-based storage medium is cooled. A similar two-photon storage operation can be performed with photorefractive crystals without such precautions necessary.[147] A problem, not only for BR, is the read-out procedure.

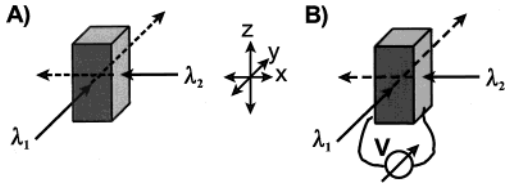

Figure 14. Principle of 3-D data storage in BR using two-photon absorption. (A) Writing: with $\lambda_1 = \lambda_2 = 1140$ nm, B → M and with $\lambda_1 = \lambda_2 = 820$ nm, M → B is induced at the intersection of the beams. The storage medium is positioned in three axis *x*, *y*, and *z* by microactuators. (B) Reading: first the address is chosen by mechanical positioning. Then a write pulse is employed. If the BR present is in the B state, it will be switched to M. This transition is accompanied by a transient voltage that may be detected by external electrodes. Care must be taken to restore the initial state of the storage cell after reading. Cell dimensions are 3−6 μm.

Addressing of an individual data point in the volume must create a signal which allows the return of information as to whether the photochromic state is a "1" or a "0". This is a fundamental problem which was discussed in detail by several authors.[148] For BR its photoelectric properties may be employed for read-out purposes.

As shown schematically in Figure 14, writing is done by two beams with wavelength λ_1 and λ_2 that intersect in the volume. Using $\lambda_1 = \lambda_2 = 1140$ nm the photochemical transition from B to M can be initiated. B and M state, or certain ratios of B and M, are assigned to "0" and "1". Switching from M to B is obtained by overlapping two waves of 820 nm. Spot sizes of 3−6 μm have been proposed.

During read-out the same procedure is applied. First, two 1140 nm beams are used and if BR was in the B state, it switches now to M. The initial charge separation leads to a photovoltaic effect which can be detected by two electrodes on the outer surface of the volume storage medium. Care has to be taken to restore the original information state after reading. To enhance the photovoltaic effect, an orientation of the PM patches during embedding into the matrix material for the volume storage medium is desired. The same electrodes can be used to induce the preorientation of the PM patches.

The other approach mentioned for long-term data storage with BR uses two one-photon transitions in BR. The scheme for this process is given in Figure 15. First a green laser pulse initiates the photochemical B to J transition.[149] From there BR, through a sequence of thermal steps, reaches the O state. After a characteristic delay time $\tau_{J\rightarrow O}$ all of the BR material exited by the first green laser pulse should arrive in the O state. Second, a red laser pulse converts it from there into the P state which has a 9-*cis*-retinal

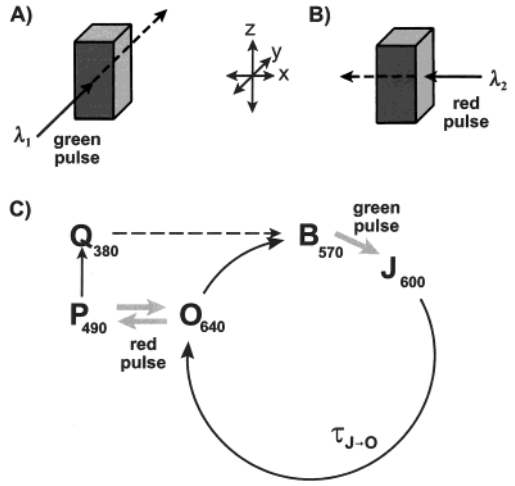

Figure 15. Principle of 3-D storage in BR using two one-photon photochemical transitions. (A) A first laser pulse drives the BR from the initial B state to the J state from where it relaxes thermally to the O state. The time needed for this thermal pathway is $\tau_{J\rightarrow O}$. (B) Upon arrival in the O state a second red laser pulse can convert the material to P, which contains 9-*cis*-retinal and thus does not thermally decay to the B state. The P state relaxes slowly to the Q state. Both states can photochemically be shuttled back into the conventional photocycle. If no second red laser pulse is applied, the BR returns to the initial state. (C) A simplified photocycle for the description of this process is shown. Because BR exited in B with the first laser pulse reaches the O state with a typical delay, a second laser pulse can selectively address those molecules and switch them out of the conventional photocycle photochemically. This scheme was named "branched photocycle".

configuration. A thermal relaxation of the P state to the Q state is observed and may be assigned to a deliberation of the retinal molecule from the Schiff base linkage. Only photochemical excitation of the BR material in the P and Q states returns it to the initial B state. This procedure potentially allows a mass storage application of BR, but the conversion efficiency from O to P must be improved to reach competitive data transfer rates compared to conventional hard disks.

C. Holographic Storage and Associative Memories

In holographic storage, complete data pages are stored during each recording. Today about 10^6 binary data points can be processed in parallel. After recording one page either the recording material is shifted or rotated or, alternatively, the angle between reference beam and data beam is changed. These procedures are named angular multiplexing and shift multiplexing (see Figure 16). Then the next data page is stored in the same location. The more data pages that are stored in a single location, the lower their signal-to-noise ratio becomes, because a data page written later diminishes the contrast in earlier recorded pages.

The information is restored by exposing the recording medium to the reference beam in exactly the same geometry as it was used during recording. Then from the addressed hologram the complete data page is read-out in parallel.

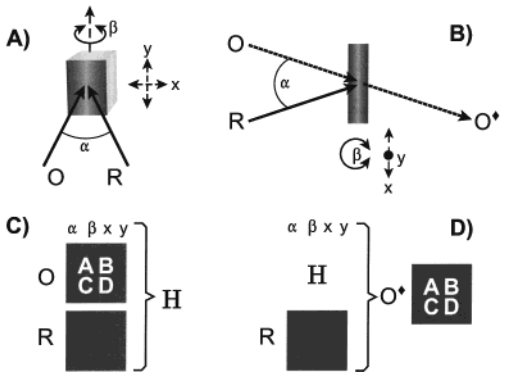

Figure 16. Principle of holographic storage. (A) An object wave O and a reference wave R interfere in the holographic medium. The angle α between the two beams determines the carrier frequency of the recorded hologram. In addition, the position of the holographic medium in two axes, *x* and *y*, and its rotation around the *z*-axis (angle *β*) are further parameters of recording. (B) The same setup as in panel A viewed from the top. During reconstruction of holograms by exposing the recording medium to the reference beam only, the original object wave O♦ is reconstructed. (C) The recording process: An information carrying wave O is overlapped with the reference wave R. A hologram H is recorded. (D) The hologram can be restored only if the geometric parameters are matched (α, *β*, *x*, *y*) and the hologram H is irradiated by the reference wave R.

One unique feature of holographic storage is that so-called associative memories or content-addressable memories are easy to realize. When not a plane reference wave is used for reconstruction but an object wave, which carries part of the information stored in one page, the location of this page can be derived from the signal returned. Content-addressable memories are a domain of holographic storage today and cannot be realized by conventional electronics.

D. Conclusions

Using BR instead of conventional chemicals is justified only if there is at least one property which is an exceptional feature for a group of applications, e.g. long-term rewritable optical storage. In principle, BR might be used for this purpose; however, it is difficult to argue why BR is needed for such applications and which advantage BR offers over conventional materials here. Maybe P and Q states and the electrochromic effects of BR variants are ways to raise the power of BR for long-term storage.

VI. Information Processing

The main area for technical applications of BR presumably will be in optical data processing. Here the number of WRE cycles required is much higher. For example, in real-time image processing at video frame rate, about 10^6 WRE cycles for a single day of operation are required. This blocks out most of the synthetic organic photochromic materials. The reversibility of BR is astonishingly high. Because it is extremely difficult, if not impossible, to design or to produce synthetic chemicals with similar properties, it is reasonable to employ the development which is found in nature. Biotechnology allows production of

Table 10. Applications of BR in Information Processing

application	absorption modulation	refractive index change	light sensitivity	nonlinearity	thermal decay	two photoactive states	polarization recording	reversibility
nonlinear filtering	+	−	±	++	++	++	−	++
spatial light modulators	+	±	±	−−	+	++	−	++
phase conjugation	−	++	−	±	+	±	±	++
pattern recognition	−	+	−−	++	±	++	++	++
interferometry	−	++	++	±	−−	++	++	++

large quantities of such a material, so availability is not a roadblock.

BR is a first choice material in all optical applications where the number of WRE cycles is a critical issue. This is the reason real-time applications such as pattern recognition and interferometry are estimated to be areas where a technical use of BR seems feasible.

Apart from the reversibility, there are other properties that are attractive, but they alone would not be sufficient to make BR a technically relevant material. As long as only a single parameter is considered there are other substitutes for BR, but in the combination of attractive features BR is unique. In Table 10 several optical applications of BR are listed and the properties of BR which are of particular relevance for these are weighted.

Obviously reversibility is important for all of them. Two photoactive states allow light-controlled erasure but also "controlling light by light". The polarization recording feature is of highest importance where an excellent signal-to-noise-ratio (SNR) is required. The advanced information processing techniques such as pattern recognition and interferometry benefit more from this possibility than more simple applications. The thermal decay of state M to B signifies that under irradiation a steady state is reached. The nonlinearity of the steady-state bleaching of BR is advantageous for some applications and disadvantageous for others. Whereas it is the basis for nonlinear filtering for spatial light modulators, a linear intensity response would be much more desired. In holographic techniques the refractive index changes are more important than the changes in the absorption, but absorption is required for the interaction with light.

A. Nonlinear Filtering

Two types of nonlinear filtering with BR already have been described. The first is based on the nonlinear photoresponse of the BR material and the other on Fourier filtering. In both cases optional use of light of a second wavelength allows active control of the local transmission of the BR.

1. Contrast Enhancement

The nonlinear absorption properties of BR arise from the cyclic photochemistry. If the simple two-state model (see Figure 9) is used, a typical bleaching curve for BR with a lower threshold, a nonlinear rise, and a saturation regime results. In Figure 17 a theoretically calculated and an experimentally measured curve are compared. The maximal bleaching ratio depends on the M lifetime of the material.

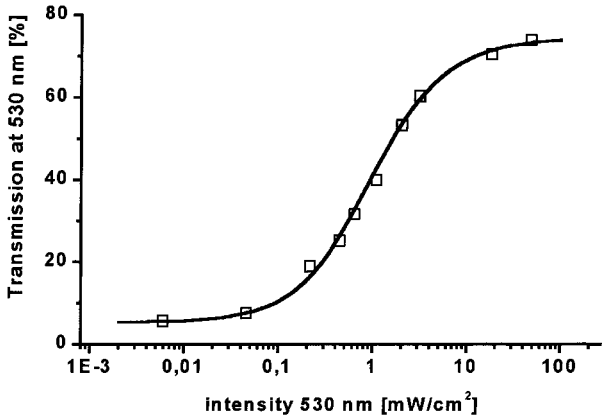

Figure 17. Steady-state bleaching curve of a BR-D96N film. The initial absorbance at 570 nm of the film was OD(570) = 1.92, which corresponds to a value of OD(532) = 1.39. Experimental values (□) and values computed using the simple two-state model of the BR photocycle (solid line) are compared.

Applying such a BR-based nonlinear filter causes the percentage of light absorption for low intensities to be higher than for high intensities.

To use BR for the contrast enhancement of microfiches is an older proposal.[150] More effective is the use of BR for nonlinear filtering in the Fourier plane.[151,152]

2. Fourier Filtering

The intensity distribution in the Fourier plane of a lens is very wide. The zeroth order is the strongest and represents the average brightness of the input image. Often an exponential intensity relation between the different Fourier orders occurs. This allows—as in far field—suppression of noise just by transmitting the Fourier image through a BR film and thereby removing the weaker Fourier orders. Active photochemical transmission control of the BR film with a second light source enables a simple form of optical processing (see Figure 18).

With the described optical setup the suppression of Fourier orders of Obj 1 with corresponding Fourier orders of Obj 2 can be realized. If Obj 2 is just a blank, only a zeroth Fourier order is obtained. For $\lambda_1 = 568$ nm, and $\lambda_2 = 412$ nm, the zeroth order of the yellow light path will be suppressed, since the blue light prevents bleaching of the BR film in the corresponding position. After retransformation, an edge-enhanced image is obtained (see Figure 18B). For a mathematical modeling of how the two light waves interact in the BR layer, the two-state model (Figure 9) and the equations given are sufficient.

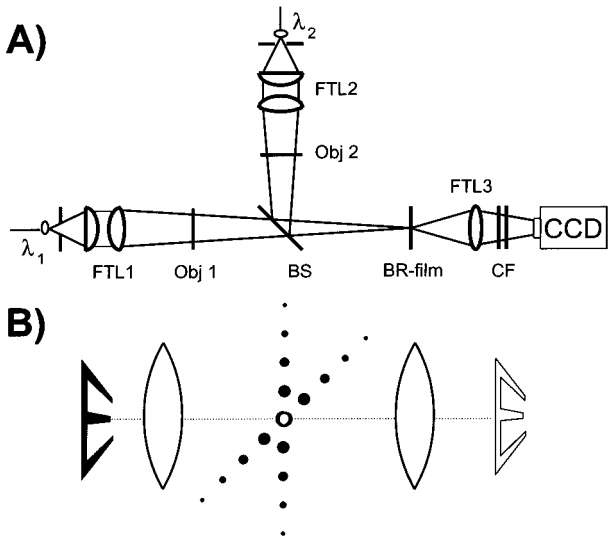

Figure 18. Experimental setup for Fourier filtering. The first input pattern (Obj1) is optically transformed using a first wavelength λ_1 and the 2-D Fourier transform is obtained in the plane of the BR film. A second, e.g. blue, wavelength is used to transform a second input pattern (Obj2) and to obtain the corresponding Fourier pattern overlapping the first one in the BR film. Coupling of the second lightpath onto the first one is done by means of a beam splitter (BS). A third Fourier transform lens accomplishes the retransformation and color filters (CF) separate the first and second wavelength. The filtered image is recorded by a CCD camera.

B. Neural Networks

The photochromic properties of BR, in particular its ability to be switched "forward" with light in the green to red wavelength regime and "backward" with blue light, make it a potential candidate as a medium for the implementation of optical neural networks. Several groups explored different approaches to use BR in optical networks, using either only the photochromic properties of BR or, in addition, its photoelectric capabilities.[153−156] The optical implementations take advantage of the photoinduced M to B transition, but even more, the reversibility of BR is required, because in optical neural networks a continuous writing and reading process is needed.

C. Spatial Light Modulators

Optically addressed spatial light modulators (OA-SLMs) are multipurpose devices for "controlling light by light". For all OA-SLMs, where high optical resolution and high contrast in the visible wavelength regime is required, BR might form the photoactive layer that is required to allow the interaction of the different light waves. The basic principle is shown in Figure 19. A BR film is locally irradiated and thereby its absorption and its refractive index are changed. Either the absorption change is used to control the transmission of a second light wave in an absorptive way[157] or the refractive index change is used in a Fabry−Perot cavity[158] for tuning its transmission.

One of the main functions of BR in such an OA-SLM is to act as a short-term memory material for serial-to-parallel conversion. Information sequentially written from a laser scanner to the BR layer is read-out in parallel by illuminating it with light from the rear. High contrast ratios are easy to obtain. Assuming a BR layer with an initial optical density of OD = 5 is 90% bleached, the contrast ratio between the dark and bright regions is more than 1:1000. The optical resolution of such a system is not limited by the BR material which is capable of recording several thousand lines/millimeter.

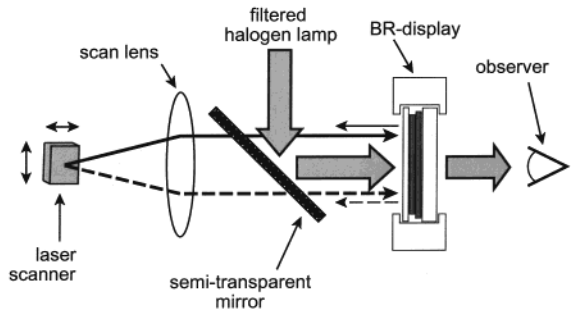

Figure 19. Schematic setup of a spatial light modulator with a photochromic BR layer. A laser scanner is used to write information onto the BR display. Due to internal reflective layers, no laser light is transmitted to the side of the observer. Filtered light, e.g. from a tungsten lamp, is used for the illumination of the image on the BR display.

OA-SLMs based on BR may be used as incoherent−coherent converters.[159] An incoherent image projected onto a BR−SLM is used here to modulate the transmittance of a coherent wave. Because the information carried on the incoherent beam is then transferred to the coherent beam, such devices are called incoherent−coherent converters. Another use of BR−SLMs is in projection displays.[160] Also, displays for direct-view can be made from BR. A set of dielectric layers is used in contact with the photoactive BR layer which has a high reflection for the light used for addressing the OA-SLM. In this way the transmission of light for writing information to the BR−SLM can be almost completely omitted. This allows one to view such displays directly with the naked eye.[161]

Another interesting embodiment for an BR−SLM is the combination of the photoelectric behavior of an oriented PM layer and a liquid crystal layer. The photovoltage generated by BR directly controls a liquid crystal layer and affects its orientation.[162,163]

The number of groups working on BR-based OA-SLMs may be an indicator that this application has a potential for a technical use in the very near future.

D. Phase Conjugation

A phase-conjugating mirror does not reflect light in the conventional way. In Figure 20 a few beams approaching the phase-conjugating mirror are schematically shown. A phase-conjugated wave travels back exactly the light path of incidence. This is often called a "time-reversed" wave.[164] In a more practical view this means that a wave that passed through an inhomogeneous medium, after "reflection" at a phase conjugating mirror, generated a back-propagating wave such that all distortions are exactly compensated for after passing a second time through the distorting medium. There are several ways to gener-

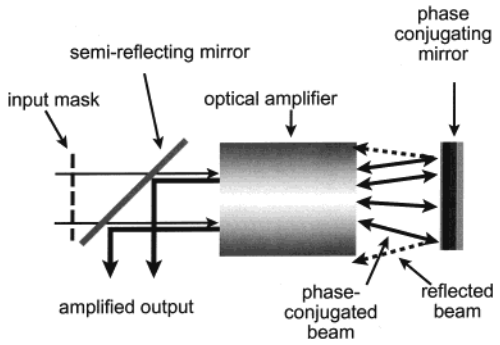

Figure 20. Application example for a phase-conjugating mirror. In photolithography a partially transparent mask is illuminated by light to expose a light sensitive layer on the wafer. The higher the light intensity is, the shorter the exposure time required. When laser light is used it could be amplified in an optical amplifier after passage through the mask. This causes distortions in the wave front. A phase-conjugated mirror reflects light back in a way that these distortions are exactly compensated. After a second passage the amplified output could be decoupled by a semitransparent mirror.

ate a phase-conjugated wave, but with BR only so-called four-wave mixing is important, which is a holographic process. A dynamic volume hologram is formed in a BR film in an arrangement so that the incoming signal wave will generate the phase-conjugated wave.[165] In Figure 20 the laser beams which are necessary to generate the phase-conjugating mirror in BR are not shown. Optical phase conjugation with BR has been explored by several groups.[166−168] Because a holographic setup for phase conjugation, which is a four-wave mixing process, is very difficult to stabilize, a dynamic medium like BR has some particular advantages. It is capable of compensating slow changes and drifts in the setup which is the attractiveness of BR in this application. The main drawback is the low diffraction efficiency of BR films, which is in the range of a few percent.

One application where phase-conjugating mirrors might be useful is in optical lithography for the semiconductor industry. To maximize the throughput, a short exposure time per chip is requested. For this reason the light intensity should be as high as possible. Weak light passed through a mask could be passed through an optical amplifier to increase the light intensity. This causes distortions in the wave front. If a phase-conjugating mirror is used, which reflects back the distorted wave through the optical amplifier, all distortions would be compensated. Then the amplified image can be decoupled by a semitransparent mirror and irradiate the substrate with the desired intensity.

E. Pattern Recognition

Holographic pattern recognition is the comparison of two simultaneous input images. Maybe you are looking for a specific word in a text. In this case the whole text is displayed in one channel of the correlator and the word you are seeking for in the other channel. The correlation signal will be a peak pointing to the location of occurrence of the searched word. In addition there will be some weaker signals. These correspond to words which are similar but not identical with the original word. A holographic correlator does not only indicate the position but also gives a measure for the degree of similarity. In case of identity, the correlation signal reaches its maximal value, and in case of similarity, e.g. if only part of the word occurs, a lower intensity of the correlation signal results.

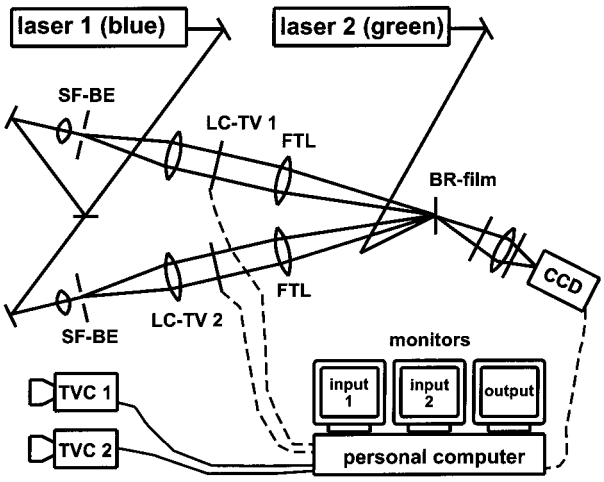

Figure 21. Schematic setup of a holographic dual-axis-joint-Fourier transform-correlator for pattern recognition. As inputs, the signals of two video cameras can be used directly. Also, artificial patterns may be used alternatively. The correlation signals (output) are viewed on the third monitor. The information which shall be correlated is modulated onto the laser beams by means of LC-TV spatial light modulators. Output signals are recorded by a CCD camera. A high signal-to-noise-ratio is obtained because polarization recording in the BR film is employed which allows separation of scattered light and signal light.

The demands of holographic pattern recognition toward the recording material almost perfectly match the strong properties of BR and none of the weaker ones.

In Figure 21 the optical setup of a BR-based holographic real-time correlator is shown. Light from laser 1 emitting in the blue, e.g. 412 nm from a krypton ion laser, is split by a polarizing beam splitter into two perpendicular polarized components. Both beams pass through a spatial filter (SF) for homogenesation, then they are expanded (BE) and pass through electrically addressed spatial light modulators (EA-SLMs). In the described experiment, liquid crystal television (LC-TV) displays were used. In the SLMs an external video signal controls spatially the phase shift applied to the transmitting light wave. The modulated wave passes through a lens and in its focal plane the optical Fourier transform is obtained. At the BR layer the Fourier transform of the first arm of the correlator is overlapped with the Fourier transform from the light wave in the other arm of the correlator. Only in positions where both Fourier patterns share common Fourier components are holograms formed locally. As long as the readout beam is not turned on, nothing happens in the BR film. Only when a beam in the green−yellow−red regime irradiates the BR film does BR start into its photocycle and the M state is generated. Now the overlapped blue Fourier patterns can interact with the material, and holograms are formed. In this

application the pump beam acts at the same time as a read-out beam which is diffracted at the Fourier components in common. After retransformation by another Fourier lens, the resulting correlation signal is monitored by a CCD camera.

Several years ago a lab system of a holographic correlator with BR was built.[169,170] Its speed and the signal-to-noise ratio of the correlation signals were excellent. The experimental values reached almost the theoretical limits.[171] Even an adaptive correlator, which can compensate for the tracked objects changing their size or rotating, was made.[172] There is hardly any other material which can compete with BR in this application today, but there are only very few technical applications where a holographic correlator is needed. And, in addition, the EA-SLMs which are used for data input limit the total data throughput, because their resolution is too low. As soon as the data input capabilities are increased by 1 or 2 orders of magnitude, this BR application should be revisited.

F. Holographic Interferometry

Holographic interferometry is a powerful technique for the analysis of the deformation of objects on the scale of $^1/_{10}$ to $^1/_{100}$ of a wavelength. Nondestructive testing and vibration analysis are technical areas where holographic interferometry is employed.

The first papers where BR was used in this context appeared on signal-conditioning in a fiber-optical interferometer.[173,174] Heterodyne interferometers with BR films as recording media with one or two wavelengths were used for real-time vibration analysis[175,176] and for monitoring thermodynamic processes such as crystal growth.[177,178] The use of two different wavelength makes the monitoring process very easy, but the holograms and the interferogram are not properly scaled. In proportion to the difference in the wavelengths they appear to be of different size. But even more problematic is that the Bragg condition will limit severely the object sizes which can be analyzed.

In holographic interferometry a complex optical signal has to be processed. Illuminating an object with coherent light generates a scattered light wave. Part of this is captured by the recording medium, in this case by the BR film. Together with a reference wave from the opposite direction, a reflection-type hologram of the object is recorded in the BR film. A schematic setup for homodyne interferometry with BR films is given in Figure 22. A laser beam is divided by a beam splitter (BS) into an object beam and reference beam. The intensity of the object beam is adjusted by a Glan−Taylor (GT) polarizer and a lambda half-plate ($\lambda/2$). The beam expander (BE) finally magnifies the beam and illuminates the object. From there the scattered light is reflected and part of it illuminates the BR film. The reference beam is expanded and overlaps with the object wave in the plane of the BR film to form a reflection-type hologram. Then the object is deformed and the procedure is repeated. After that at least two holograms coexist in the BR film. For reconstruction of both together,

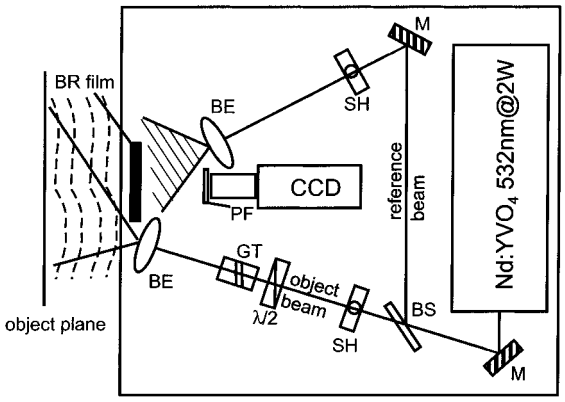

Figure 22. Sketch of the holographic camera for nondestructive testing. A frequency-doubled Nd:YVO_4 laser is used for the illumination of the object. The holograms are recorded in the BR film in a reflection type geometry, i.e. reference beam and light reflected from the object coincide from opposite sides in the BR film. The holograms are recorded as reflection-type polarization holograms. For reconstruction of the holograms, the object beam is switched off and the reference beam irradiates the hologram. A polarization filter in front of the CCD camera is used to increase the signal-to-noise ratio.

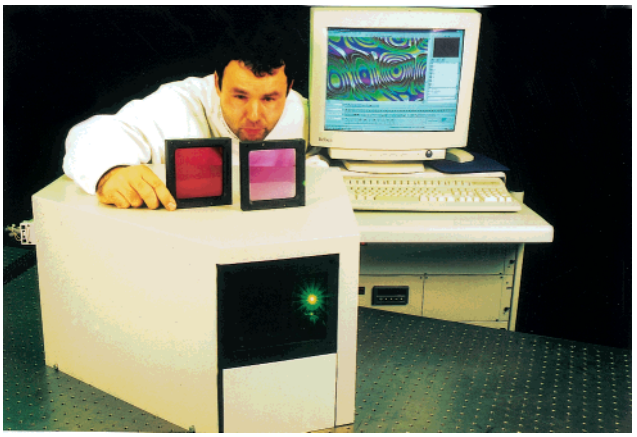

Figure 23. Prototype of a holographic camera for interferometric applications. All optical components are inside the camera head in the front. Bacteriorhodopsin films as they are used in the system are shown on top of the housing. In the rear the control unit can be seen. The operation of the system is completely under software control.

the object beam is switched off and only the reference beam is on. The timing of the beams is controlled by means of mechanical shutters (SH). The reconstructed holograms interfere and the resulting intensity distribution is monitored by a CCD camera. The polarizer in front of the camera (PF) is needed since the polarization recording employed allows separation of the signal light from the scattered light. The polarizer is used to suppress the scattered light.

The light waves from the object are recorded directly without any lenses between the object and the BR film. Because the optical resolution of lenses is in the range of only several hundred lines/millimeter, they usually are the components which limit the resolution of the system. For this type of setup, comparably large recording media are required. The BR films employed here have a free aperture of 90 mm × 90 mm (see Figure 13). In Figure 23 a

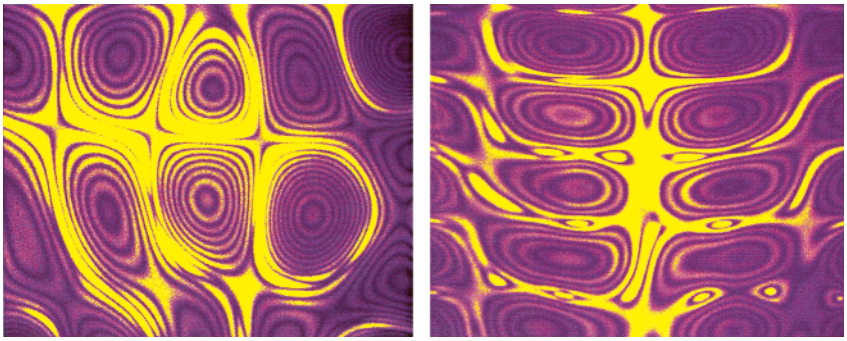

Figure 24. Vibrational modes of a ceramic substrate for electronic circuitries. The vibrational modes are induced by coupling a piezoelectric actuator to the investigated substrate. The frequency is scanned and the vibrational mode is recorded where a resonance is found. The pattern is very sensitive to the mechanical properties of the investigated part. Cracks, changes in thickness, etc. cause the vibrational modes obtained to differ significantly from those of a reference part.

Table 11. Bacteriorhodopsin—A Model for a New Class of Materials

Model system for the "re-engineering" of biomaterials
Technical utilization of evolutionary optimized molecular modules
Adaptation to the technical needs by genetic methods
Model system for thermodynamic stabilization of biomaterials
Solubilized, 2-D crystalline and 3-D crystalline materials are available
Transition from solution/dispersion to solid state
Model system for studying the interface problem with biomaterials
Optical, electronic, and chemical interface with the same material
Optical interface causes minimal impact to the biomaterial
Model system for the integration of a biomaterial into technical systems
Biomaterial → Biocomponent → Biosystem
Biomaterials are not one-to-one replacements for conventional materials

prototype of a system for holographic interferometry is shown.

Figure 24 shows results from the nondestructive analysis of ceramic substrates for electronic circuits obtained with the BR system shown. A whole analysis cycle is finished in several tens of seconds, and no consumables are required.

G. Conclusions

The domain of BR is dynamic applications where hundreds or thousands of cycles between the B and the M state are required. The excellent reversibility of BR distinguishes this material from conventional ones. Because of the problems with optical recording materials within the last 10 years, not many of the promising optical techniques received broad attention. The power of computers increased dramatically during the same period. With a new and reliable recording material, the use of optics not only for storage purposes but also for processing should be reconsidered. Among the optical applications of BR, holographic interferometry obviously is the first field of a commercial use of BR.

VII. Summary

The use of bacteriorhodopsin as a molecular switch in optoelectronic and other applications seems to be technically feasible (Table 11). But this is not enough to leave the scientific level and reach the commercial one. For technical systems the only reason to use a biomaterial instead of a conventional organic material or electronics is if the system benefits from this choice and its performance increases. BR combines photochromism and color fastness. Since the photochromism is part of the natural function, evolution has optimized this function over millions of years. Genetic engineering is now used for a "second evolution", which aims to amplify the photochromic properties without losing the excellent reversibility of the molecule. Such materials cannot be obtained by statistic mutagenesis and any type of selection procedure because the BR variants which are most interesting for the applications almost lost their native biological function to be an efficient energy converter. Gene technology is a powerful tool for this type of nanoengineering. Last, but not least, biotechnology is capable of producing technically interesting amounts of such redesigned BRs at a competitive price.

Bacteriorhodopsin is a test example for a new approach in nanotechnology where gene technology is used as a tool for material design and biotechnology for the production process. Bacteriorhodopsin meets the requirements to be the first "test site" for this idea: it has a technically interesting function, its thermal and chemical stability is high enough for the prospected applications, the structure function relation is available, techniques to modify its function exist, and methods to produce modified material in large quantities have been developed.

The potential for technical applications of BR was discovered more than 20 years ago. Quite a lot of reviews have been published in this field, and even a book is dedicated to this topic.[179] The current research focuses on the integration of BR variants into technical systems. BR is not a one-to-one replacement product. There are no technical systems where you can take out a component and simply replace it with a better new one made from BR. It is

necessary to redesign the technical systems in order to match with this new product.

BR is not an isolated exceptional molecule; it has become the technological platform where a variety of tools are used to derive many new and useful materials. Also BR is not the only molecule where a technical exploitation of its physical properties seems attractive. Even in the same organism there are several more, as described shortly in the Introduction. It is hard to imagine how many of them we could find in nature, but most nanotechnologically desired functions are already developed, i.e., sensors, actuators, motors, and valves. It is really worthwhile to learn how this natural treasure can be employed for technical systems. Furthermore, the commercial impact will be significant. If only few of such functional biomolecules are technically useful, a whole new industry may be born.

VIII. Abbreviations

BR	Bacteriorhodopsin
BR-WT	Wild-type BR
BR-D96N	BR variant with the aspartic acid (=D) in position 96 replaced by asparagine (=N)
BR-D85N	BR variant with the aspartic acid (=D) in position 85 replaced by asparagine (=N)
PM	Purple membrane
WRE cylces	write−read−erase cycles
SR I	Sensor rhodopsin I
SR II	Sensor rhodopsin II
HR	Halorhodopsin
AFM	Atomic force microscopy
SLM	Spatial light modulator

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