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Circular polarization in scattered light as a possible biomarker

W.B. Sparks [a,*], J.H. Hough [b], L. Kolokolova [c], T.A. Germer [d], F. Chen [e], S. DasSarma [e], P. DasSarma [e], F.T. Robb [e], N. Manset [f], I.N. Reid [a], F.D. Macchetto [a], W. Martin [b]

[a] Space Telescope Science Institute, Baltimore, USA
[b] Center for Astrophysics Research, University of Hertfordshire, UK
[c] Department of Astronomy, University of Maryland, USA
[d] National Institute of Standards and Technology, USA
[e] Center of Marine Biotechnology, University of Maryland Biotechnology Institute, USA
[f] Canada-France-Hawaii Telescope, USA

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ABSTRACT

Biological molecules exhibit homochirality and are optically active. Therefore, it is possible that the scattering of light by biological molecules might result in a macroscopic signature in the form of circular polarization. If this is the case, then circular polarization spectroscopy, which may be utilized in remote sensing, can offer a powerful indicator of the presence of a universal biosignature, namely homochirality. Here, we describe laboratory experiments designed to investigate this idea. We focus on photosynthetic microorganisms, and also show results from macroscopic vegetation and control minerals. In the microorganisms, we find unambiguous circular polarization associated with electronic absorption bands of the photosynthetic apparatus. Macroscopic vegetation yields a stronger and more complex signature while the control minerals produce low-levels of circular polarization unrelated to their spectra. We propose a heuristic explanation of our results, which is that the polarization is produced by circular dichroism in the material after the light has undergone its last scattering event. The results are encouraging for the use of circular polarization spectroscopy in remote sensing of a generic biomarker from space or the ground.

1. Introduction

All known living material exhibits a remarkable property which is homochirality—the complex chiral molecules of biology occur almost exclusively with only a single handedness (chiral: from Greek, χείρ, *cheir*, hand).

For example, the vast majority of organisms use only left-handed L-amino acids in proteins and right-handed D-sugars in nucleic acids. More generally, a chiral compound contains one or more asymmetric centers and thus can occur in non-superimposable mirror-image forms. This chiral property is believed to be a necessity for self-replication, and as a consequence, it is likely to be a generic property of all biochemical life whether similar to terrestrial or not.

Biological material displays optical activity, arising from either circular dichroism (different absorption coefficients for left and right circularly polarized light) or differential scattering of left and right circularly polarized light by its component chiral molecules and macrostructure; cells, membranes, nuclei etc. [1–3]. Circular dichroism spectroscopy is a standard analysis technique for studying protein structure [4,5]. For example, chlorophyll has significantly circularly dichroic

* Corresponding author. Tel.: +1 410 338 4843.
E-mail address: sparks@stsci.edu (W.B. Sparks).

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absorbance bands which lead to a distinctive dimer circular polarization signature [6]. Circular intensity differential scattering (CIDS) considers the circular polarization induced by scattering from chiral samples [7]. Circular polarization can also be caused by optical interaction associated with the birefringence of macroscopic biopolymer membranes of a biological structure, while light emitted through fluorescent processes, commonly associated with molecules of biological significance, may also exhibit circular polarization. A variety of microbes were found to produce fractional polarizations $\sim 10^{-2}$–10^{-3} [8]. In photosynthesis (discussed below) circular dichroism and related phenomena are induced by the intrinsic chirality of the molecules, from excitonic coupling between chromophores in molecular complexes and from macroscopic organization of the system [9]. Here, we are interested in the fact that interaction between light and living organisms can produce a detectable circular polarization signature by means of several mechanisms, and hence that circular polarization spectroscopy may be useful as a remote sensing indicator of the presence of biological material.

Abiotic contributions to circular polarization arise from atmospheric aerosols and from mineralogical scattering processes. Some minerals and crystals are optically active, however, integrating over a large naturally produced sample (many individual crystals) is expected to give equal fractions of enantiomorphs so that the optical activity of the macroscopic sample averages to zero. Reflection from absorbing materials and total internal reflection from non-absorbing materials can introduce circular polarization, but only when the optical geometry has chirality. Observationally, geometric chirality typically produces a smooth distribution recognizably related to the geometry of the scattering [10,11] while mineralogical optical activity typically has a very broad, smooth spectral dependence. This is in contrast to electronic transitions associated with biological material which present relatively narrow absorption bands with which the strongest circular polarization signal is associated (the Cotton Effect). Even if local enantiomeric excesses of optically active minerals were to exist, it is expected that they would be distinguishable from biological matter by their spectral properties [1,5]. Empirically, measurements of bodies within the Solar System reveal low integrated fractional circular polarization levels $\sim 10^{-4}$–10^{-5} [10,12,13], some two or three orders of magnitude smaller than possible biological effects.

An especially promising type of interaction between light and living organisms is photosynthesis and phototrophy (“light harvesting”; hereafter we loosely use photosynthesis to include phototrophy). In photosynthesis (and phototrophy), the organism extracts energy by triggering an electronic excitation of the active pigment in diffuse absorbance bands.

From an astronomical perspective, photosynthetic activity is extremely attractive, since it is largely a *surface phenomenon*, which can therefore be detected through external optical observation. Photosynthesis generally requires interaction with light of the host star, hence is tuned to the *wavelength of maximum flux* and is further tuned to the *wavelength of maximum transmission* of the planetary atmosphere [14]. These conditions optimize the possibility of observing photosynthesis.

Biophysically, the interaction between organisms and light is particularly strong in photosynthesis, and the strongest signatures of chirality are manifested in the circular dichroism of the electronic absorption bands that characterize photosynthetic activity [9]. Hence, by focussing on photosynthetic processes we optimize our chances of finding a circular polarization signature.

Photosynthesis offers enormous evolutionary advantages and arose early in the history of the Earth. Once present, over 3 Gyr in the past, photosynthesis by oceanic microbial life dramatically changed the atmosphere of the Earth, giving rise to oxygen in large quantity, and has played a fundamental role in life’s progression ever since. Hence photosynthesis, oxygenic or otherwise, is likely to be a common and successful life strategy that extraterrestrial organisms could certainly utilize. Therefore, a microbial dominated photosynthetic biosphere is one of the most likely possibilities for a randomly chosen extrasolar planet.

For these reasons, Sparks et al. [15] studied the circular polarization spectra of scattered and transmitted light from photosynthetic cyanobacteria, which were found to produce a significant circular polarization spectral signature associated with the photosynthetic pigments. They also presented results from the bacteriochlorophyll-based purple non-sulfur bacteria *R. rubrum*, and an example of a leaf and mineral for context. Here, we present new experimental results for the cyanobacteria, specifically comparing low and high optical depths, we describe initial attempts to use halobacteria, and present new macroscopic vegetation results and additional mineral control samples.

2. Experimental concept and choice of samples

Our aim is to understand whether astrobiologically important microbes produce a macroscopic circular polarization signature in scattered light. To address this we established a dedicated laboratory configuration, described below, at the National Institute of Standards and Technology (NIST) and obtained samples of microbes in liquid suspension from the Center of Marine Biotechnology (COMB), part of the University of Maryland Biotechnology Institute.

Our primary microbial targets are marine cyanobacteria, which are photosynthetic prokaryotes possessing chlorophyll *a* and their antenna pigments phycocyanin and phycoerythrin. They have an extensive fossil record that may date back 3.5 Gyr [16]. With the early development of a photosynthetic capability, they are widely thought to have been the primary agent responsible for the rise of oxygen in the primitive atmosphere on Earth. Cyanobacteria are abundant and ubiquitous in marine and freshwater systems; are found in cold and hot environments; tolerate conditions of high desiccation; and can produce biochemical products that provide protection from ultraviolet (UV) damage. High diversity and great adaptability have made cyanobacteria extremely competitive on the planet Earth. These species have been studied extensively as part of

exobiological research on the limits of life in the Solar system [17] and are now being studied as an analog for past (or perhaps even present) life on Mars. Endolithic (living embedded in the surface of rocks) communities of photosynthetic cyanobacteria have been found in subsurface layers in the Mars analog arctic Svalbard rocks and elsewhere, including the driest regions of the Atacama desert, suggesting additional strategies for life that may find extraterrestrial application [18,19]. By residing just below the surface of the rock, light useful for photosynthesis reaches the organisms, while potentially harmful UV radiation does not.

We also initiated a study of phototrophic haloarchaea, which are salt-loving extremophiles, robust against UV radiation and tolerant of extreme desiccation. Haloarchaea are commonly found in salt-rich lake environments, and imbedded in evaporative products such as halite associated with those environments. In recent experiments they have been shown to possess exceptional hardiness to Solar UV and electron beam radiation [20]. This hardiness and the tolerance of haloarchaea to harsh cold, dry, salty conditions are qualities that a microbial species alive on Mars either now or during a moist past would need to share [21,22]. They use a light harvesting system that contains the retinal-based pigment bacteriorhodopsin in their metabolism [23].

For context, we present measurements of examples of macroscopic vegetation chosen largely by availability near the laboratory, and control minerals sulfur (which has a strong spectral "edge") and a Mars regolith analog, JSC-1.

3. Experimental methods

We purchased a sophisticated dedicated polarimeter optimized for measurement of circular polarization in the presence of significant linear polarization. The polarimeter is a Hinds Instruments, Series II/FS42–47 dual photoelastic modulator (PEM) precision optical polarimeter. Our requirements were that a circular polarization degree of 10^{-4} be measurable in the presence of linear polarization of degree 0.03 and that the polarimeter be tunable from 400 to 800 nm. The long wavelength cut-off was designed to extend beyond chlorophyll's red edge at 700 nm while covering the important absorption bands in the optical spectrum. The equipment is hosted at NIST in the optical metrology department. It has been well-characterized and found to meet its design requirements.

Details of the experiment may be found in [15], including a schematic of the experimental configuration. The polarimeter is oriented vertically with a direct unobscured view of the sample. Our reflection experiments utilize an integrating sphere which depolarizes and diffuses the illuminating light and provides a distribution of incidence angles onto the sample. The source of illumination can also be targeted onto a white plaque below the sample, and light passes from there, through the sample into the polarimeter, yielding a transmission spectropolarization measurement to

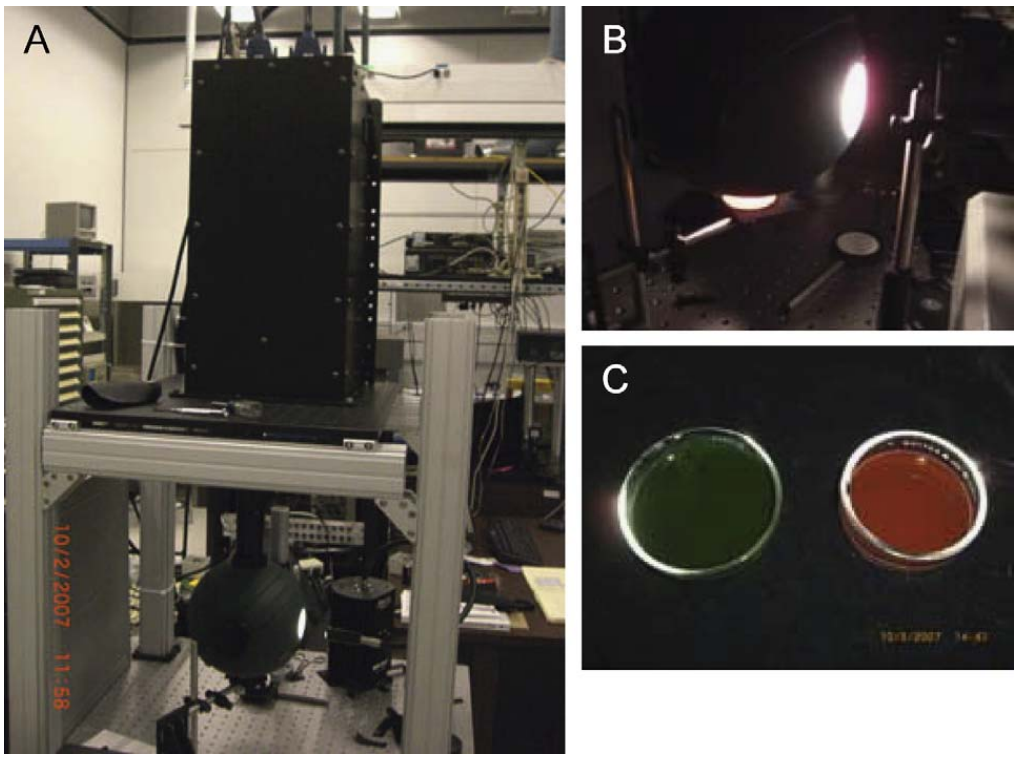

Fig. 1. Hinds dual PEM polarimeter installed in the laboratory at NIST. The polarimeter views vertically down through an open port at the top of an integrating sphere (A). The sample is directly viewed at an open port below the sphere, and for reflection experiments (B) a source of illumination enters a third port onto the interior of the sphere without direct illumination of the sample. The net result is illumination of the sample by diffuse unpolarized light. Two cyanobacteria samples are shown in (C).

complement the reflectance experiments. The transmission configuration measurement is analogous to a classical circular dichroism measurement. The polarimeter and laboratory configuration are shown in Fig. 1.

4. Experimental results

4.1. Cyanobacteria

Detail description of the sample preparation techniques may be found in [15]. One of the results presented therein was a comparison of the transmission and absorption polarization spectra for specimen *Synechococcus* WH8101 which appears green due to the presence of the light harvesting pigment phycocyanin. It was shown that the strongest circular polarization features in both transmission and absorption are related to the electronic absorption bands of the photosynthetic pigments. In order to obtain an initial insight into the influence of microbe concentration on polarization levels, we carried out an additional experiment in which a very much more concentrated sample of the same organisms was measured in the reflection configuration. To the eye, the appearance of the suspension was very dark green, almost black. Numerically, the reflected light intensity of the concentrated sample is 5–30 $\times$ fainter than the "shallow" sample for wavelengths blueward of 700 nm. Fig. 2(A) summarizes the polarization results for the shallow, less concentrated reflection and transmission experiments for WH8101, as described in the caption. Fig. 2(B) shows the data for the "deep", more concentrated, dark sample. Overlaid for comparison are the polarization curves for both the shallow reflected experiment and the transmission experiment. It can be seen that the shallow reflection and transmission measurements result in essentially the same degree of polarization. There are prominent features at the location of chlorophyll *a* ($\approx$680 nm), phycocyanin ($\approx$620 nm) and a mix of carotenoids in the blue. Both a relatively well-defined absorption band, and a significant circular polarization within the band, characterize each feature. We interpret this as the Cotton Effect, where the relatively strong circular dichroism of the electronic transitions is manifested in the transmission and reflection spectra as circular polarization. For the chlorophyll-*a* band, the polarization switches sign at the location of the absorption maximum, evidence of the orbital excitonic dimer transition where chlorophyll molecules function in pairs.

The concentrated sample has a similar but not identical spectrum. The phycocyanin absorption band is no longer evident, and the blue wing of the chlorophyll absorption is reduced, though the red carotenoid absorption is slightly higher. This can be understood as adaptation to the strong illuminating light needed to acquire a measurable polarization signal. (These data were smoothed more heavily in the spectral dimension to increase the signal-to-noise ratio per point.) The phycocyanin pigment either saturated or the organism suppressed its production. Apart from these changes, the character of the polarization spectrum is similar, with features at the absorption features and an overall quantitative polarization

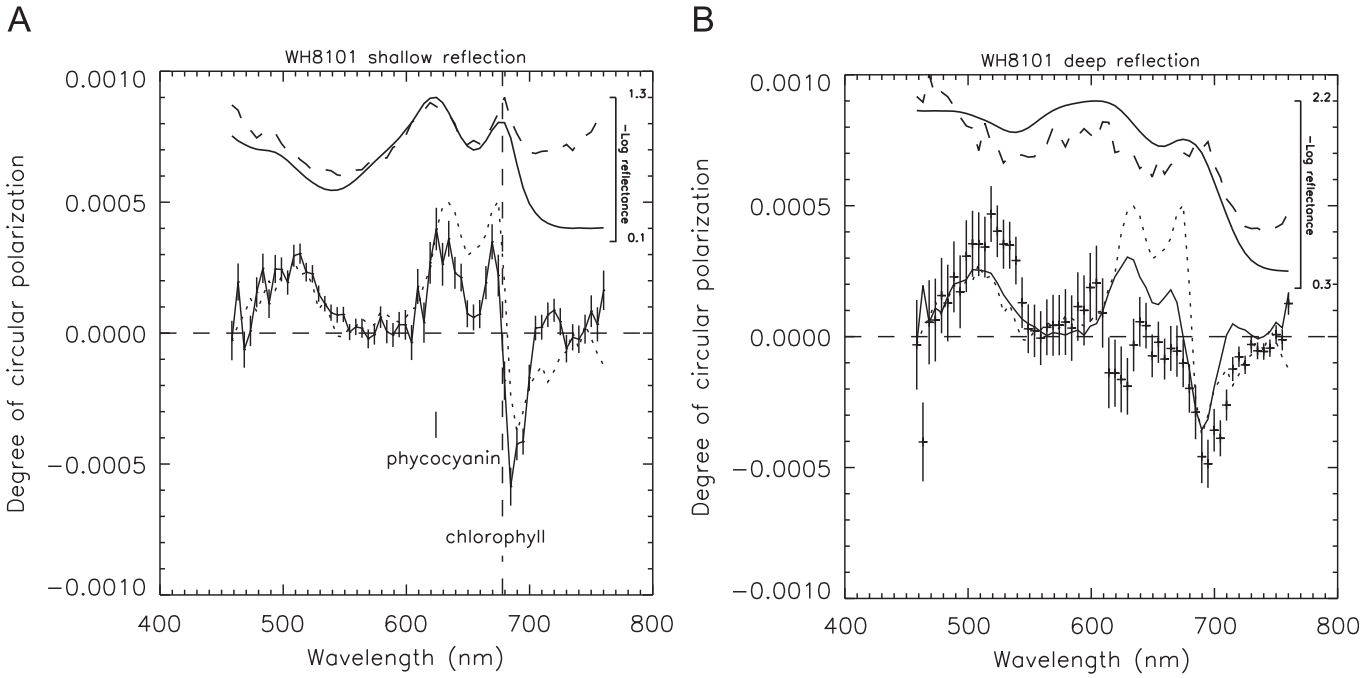

Fig. 2. Polarization analysis for cyanobacteria WH8101: (A) shows the shallow reflection circular polarization spectrum with ± 1–σ error bars. Overlaid for comparison, dotted line, is the data from the transmission experiment. Above, are the absorbance (solid line with scale bar) and arbitrarily scaled degree of linear polarization (dashed line) to illustrate spectral relationships between absorption features and circular polarization features. In general the degree of linear polarization is much smoother than the circular polarization and (B) shows the concentrated microbial samples, which have lower S/N given they are darker. Overlaid are the shallow reflection (solid line) and transmission (dotted line) polarization spectra. Above, the absorbance (solid line with scale bar) and arbitrarily scaled linear polarization (dashed line) spectra.

level that is very close to the polarization degree returned by the shallow sample. We propose, below, that this is due to an optical depth effect, whereby circular polarization is produced in the circularly dichroic layer at low optical depths.

4.2. Halobacteria

In similar fashion, we obtained spectropolarization measurements of halobacteria in suspension, *Halobacterium* species strains S9 and NRC-1. These experiments revealed absorption spectra that did not contain prominent relatively narrow bands characteristic of the cyanobacteria photosynthesis. Instead, the spectra were smooth with only a small amplitude, very broad feature at the location of the anticipated light harvesting pigment bacteriorhodopsin, which has a 550 nm absorption maximum. The presence of gas vesicles within the microorganisms and possible additional scattering substances in the suspension resulted in a smooth spectrum characteristic of a broad band scattering phenomenon, rather than the light harvesting absorption spectra. Overall circular polarization levels were either low (less than 10^{-4}) or were offset globally from zero as might be expected from a scattering scenario. In some cases, there were hints of structure in the polarization spectrum, however, these were at a very low level. More work is needed to understand the situations in which these organisms produce a light-harvesting dominated spectrum, and hence we may then predict the expected polarization levels from the circular dichroism properties of bacteriorhodopsin.

4.3. Vegetation

For context, we also acquired polarization spectra of a variety of easily obtained macroscopic vegetation from the NIST grounds. An example of a maple leaf circular polarization spectrum was presented in [15]. The average circular polarization reflection spectrum shown in Fig. 3 is an average of scans of three maple leaves, three oak leaves, two clover leaves and one leaf from a flowering fruit tree. The reflectance intensity spectra are all very similar, the flowering tree leaf shown as an example. The circular polarization reflection spectra, however, show substantial diversity. The average spectrum brings out the common features, which are a strong polarization signal associated with chlorophyll absorption between 650 and 700 nm, and non-zero polarization with a broader spectral dependence to the blue. Polarization is low at wavelengths longer than the chlorophyll red edge at 700 nm. In a planetary context, it will be of interest to establish the polarization characteristics of large scale, sometimes heterogeneous, vegetation-dominated scenery such as forests or grasslands. In

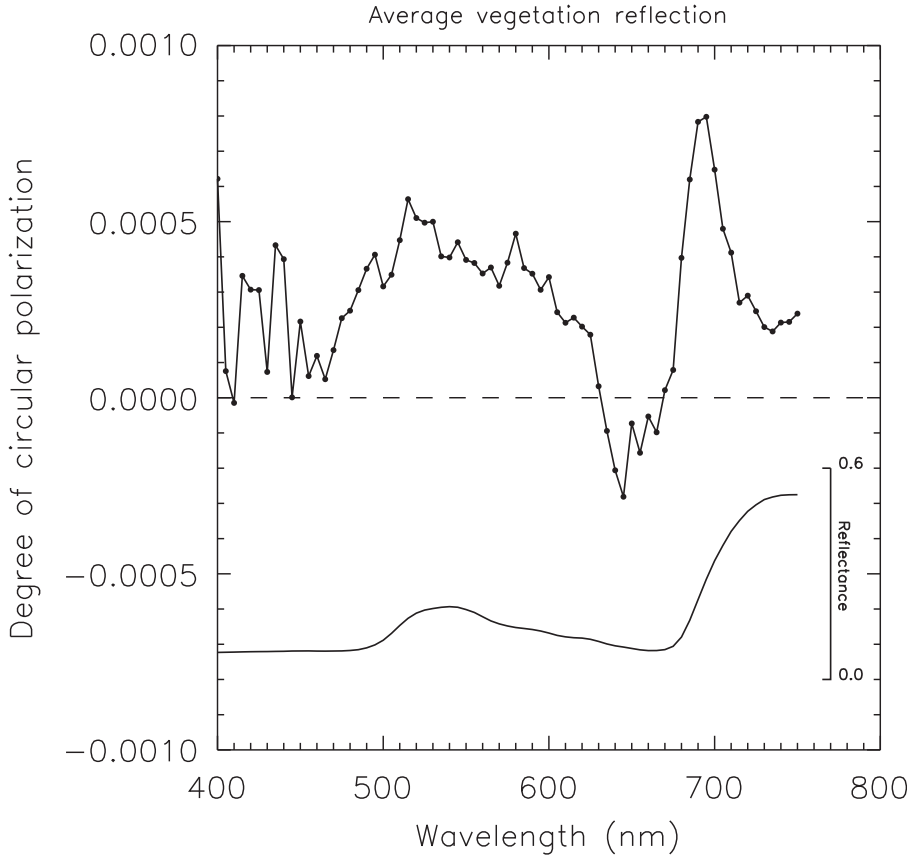

Fig. 3. The average circular polarization reflection spectrum of a miscellaneous sample of macroscopic vegetation showing significant levels of polarization throughout, but especially in the chlorophyll-*a* absorption band between 650 and 700 nm. An example reflectance spectrum is shown below with scale bar.

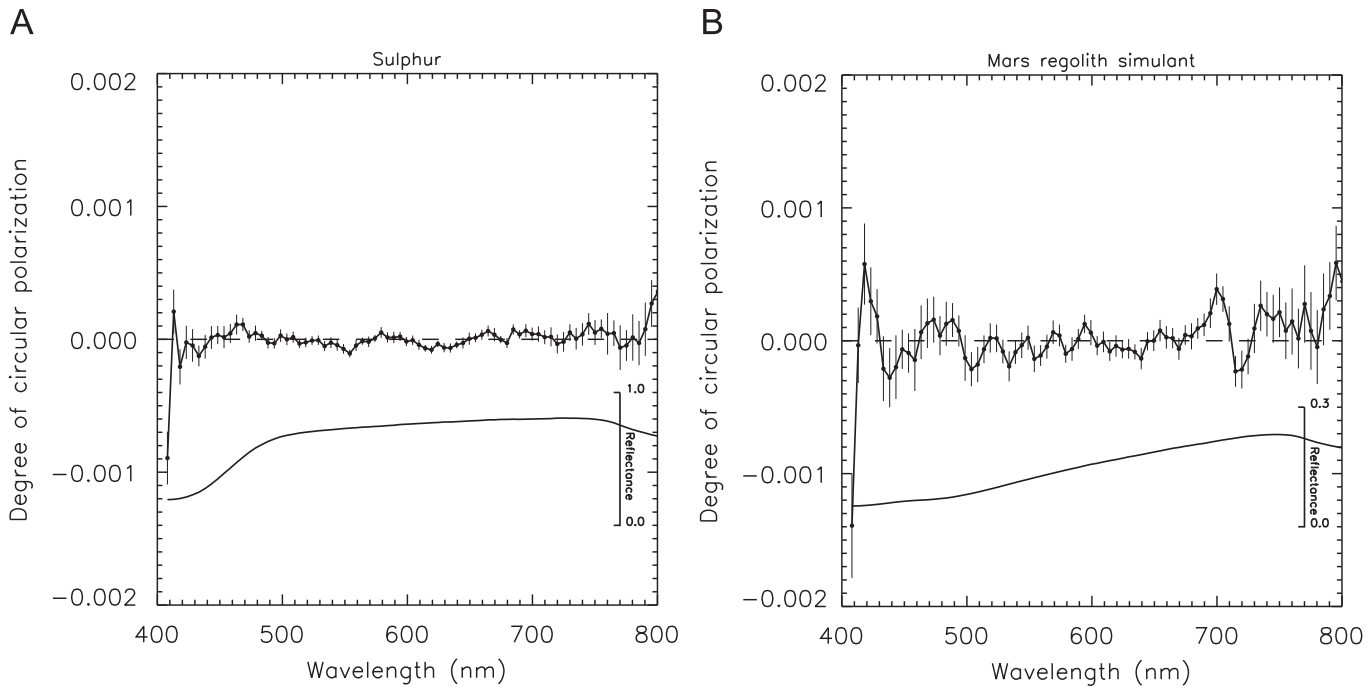

Fig. 4. Circular polarization reflection spectra of control minerals (filled circles with error bars) and reflection spectra (solid line with scale bar below): (A) shows the spectrum of a sulfur rock with a prominent absorption edge in the blue and (B) shows the Mars regolith simulant JSC-1 which has a smooth spectrum. While non-zero polarization levels are measured, they do not show any correlation with features in the intensity spectra.

addition to the mix of scattering vegetation, there is a mix of scattering angles and the potential for multiple scattering within the forest canopy, or between closely packed grasses. Empirical measurements are needed to determine whether a circular polarization signal is present, the physical processes by which it is produced, and whether in turn it may provide a useful diagnostic observable.

4.4. Mineral controls

To guard against possible instrumental polarization artifacts arising from strong spectral features, we made measurements of two minerals exhibiting significant strength "edges" in their spectra. The results from the analysis of one of these, iron oxide, were presented in [15]. The other was from a naturally occurring sulfur rock from Hawaii. The rock appears to be essentially pure sulfur, with some coarse sulfur crystalline structures imbedded in the matrix. Fig. 4 presents the polarization spectrum derived from a series of measurements of this rock. While there are small non-zero polarization levels evident, they do not share the distinctive character of relationship to the intensity spectra that the photosynthetic cyanobacteria or leaves do. The character of the polarization spectrum of this mineral is quite different to that of the biological specimens. Additional polarization spectra of a variety of other minerals were presented by Pospergelis [1] and this was true in all cases.

We also obtained spectropolarimetry of a popular Mars regolith analog. Martian regolith simulant JSC Mars-1 is made of weathered volcanic ash collected from a cinder cone of the Island of Hawaii [24,25]. The collected tephra was dried and passed through 1 mm sieves to keep only the finer material. This simulant has spectral similarities to the Martian bright regions—the visual spectrum contains a relatively featureless ferric absorption edge and an indication of a ferric absorption band in the 800–1000 nm region. To the eye, the material appears to be a fine grained, smooth, gray powder. The spectrum obtained with the Hinds polarimeter is also smooth with essentially no structure across the wavelength region to which we have access. The circular polarization level is very close to zero throughout. There are small non-zero ripples in the polarization spectrum which may be due to multiple reflections and interference within the powder, however, as for the sulfur, there are no correlations with spectral features.

5. Discussion

Our series of experiments was initiated to test the notion that circular polarization spectroscopy could provide a powerful biomarker, being directly sensitive to the chirality of the scattering medium. Homochirality presents an essentially unique biosignature, while circular polarization spectroscopy is amenable to standard astronomical or remote

sensing applications. Hence it may be envisaged that major surveys of the surfaces of astrobiological targets including Mars, Europa and Titan could be undertaken in advance of landing.

We found that the polarization signal in reflection was almost the same for a very dark suspension as it was for a less-concentrated lighter one. Our conjecture is that this approximate constancy of polarization signal in reflected light is due to an optical depth effect. The optical depth is $\tau = (\sigma_k + \sigma_s)nd$, where d is depth, n the number density, and σ_k, and σ_s are scattering and absorption cross-sections, respectively. If τ is dominated by bulk scattering (or absorption) which is relatively insensitive to circular polarization, such as the macrostructure of the cells, then interior multiple scattering destroys any polarization information. Viewed from the outside, the surface of last scattering may be considered to be an unpolarized surface from which light emerges and passes through the optically thin layer between it and the surface of the medium. In the vicinity of electronic absorption bands, the circular dichroism of the medium is strongest, and passage of unpolarized light through a circularly dichroic layer imprints a circular polarization signature. In the case of shallow reflection the unpolarized surface is provided by the reflection from the white spectralon plaque beneath the sample; in the concentrated case, by the internal scattering and absorption of the medium. Thus, in both cases we have light that was depolarized by multiple-scattering which then passes through the optically thin circularly dichroic layer. This results in a similar circular polarization for both cases. This heuristic scenario requires detailed modeling to properly understand the interplay between scattering, absorption and resonant electronic absorption spectral features. Concerning application as a biomarker, if valid, the relevant parameter is optical depth rather than physical depth. So, for example, in deep clear water, a low concentration of microbes could yield approximately the same polarization signal as a more concentrated suspension provided that the optical depth remains dominated by the microbes.

Of course, in the Solar System, there are no other terrestrial-like oceans visible on the surface. Europa and other moons of Jupiter may have subsurface oceans, and Titan may have surface lakes of hydrocarbons that could potentially be accessible. Europa is one of the most important astrobiological targets in the Solar System. While Mars may have hosted liquid water oceans in the geological past, it is likely that Europa has a saline liquid water ocean at the present time [26]. Europa may therefore be able to sustain life. It is also thought that transport occurs between the interior ocean and the surface, from morphological studies of terrain features most reasonably explained by sub-glacial activity and geological youth of the surface [27,28]. There are complex areas of brownish-red material that have apparently seeped onto the surface from the ocean below whenever the ice shell is fractured, punctured by impact or melted by internal heating. This reddish material appears to be a complex mixture of sulfate hydrates and other materials [29]. The possibility of a microbial origin for features seen by the Galileo Near-Infrared Mapping Spectrometer (NIMS) has even been considered [30]. A detailed surface spectroscopic and polarimetric analysis of this previously interior material could reveal important information on its origin and constituents and represents an approach that should precede potential landers and ice penetrators.

Cyanobacteria and other photosynthetic microbes also exist in solid surface communities, and even in endolithic communities (within rock). Endolithic communities of hardy, photosynthetic cyanobacteria have been found just millimeters below the surface in halite evaporite rocks from the most hostile, hyperarid parts of the Atacama desert [18]. This extremely dry, saline, evaporite environment is a plausible terrestrial Mars analog site [19]. The thin rock layer above the microbes admits light useful for photosynthesis while preventing damaging UV radiation from penetrating, which could prove to be a useful survival strategy on Mars. It would be extremely interesting to know whether a distinctive polarization signature survives for both solid exposed surface and endolithic communities of cyanobacteria. There is likely to be additional scattering from surface roughness, and considerations of optical depth are replaced by the surface coverage of microbes, or equivalently their dilution by unobscured rock. This is work for the future.

Life at the surface of Mars has long been considered unlikely because of the lack of water, the high levels of UV radiation, and the lack of organics as determined from Viking soil analyses [31,32]. On Mars, however, there are surface features that indicate the presence of considerable flows of water in the past, and recent results from the Mars Express and other missions show that substantial quantities of water and perhaps even methane are present [33–35]. These considerations, when taken together with the tolerance of halophilic archaea (salt-loving and often found in evaporative conditions) to extreme environments and the possibility of organisms being preserved in the Martian permafrosts [21,36], enhance the probability that life may be present at some surface locations, or may have been present in the past. If so, the timescale for racemization has been estimated to exceed the lifetime of the planet and a fossil record of chirality may exist at the present time [37]. The notion that the chemical changes seen by the Viking lander experiments may have a biological origin was also recently revived [38].

It is appropriate therefore to consider conducting (passive) remote sensing surveys of astrobiological targets. For Mars, polarization imaging from the ground can achieve a resolution of $\approx$200 km at the surface [39], while smaller more distant Solar System targets are only minimally resolved. By contrast *in situ* orbiters such as High Resolution Imaging Science Experiment (HiRISE) camera on NASA's Mars Reconnaissance Orbiter can achieve spatial resolutions of order 1 m on the Mars surface. The detected polarization signal from a hypothetical (chiral) target sample depends on the dilution of its scattered light by light from unrelated material within the field of view. The higher the spatial resolution, the better the ability to discriminate the target, minimize dilution and maximize the polarization signature. Unless chiral signatures are widespread, it is evident that an orbiting remote sensing spacecraft would offer the best prospects for success within the Solar System, though initial exploration can certainly be undertaken using the much larger telescopes available on the ground.

Circular polarization imaging of a portion of the Mars surface was carried out at two wavelengths [39], but with marginally insufficient sensitivity to detect biological circular polarization at the degrees reported in this paper. However, in principle there are sufficient photons to reach these levels, and it would seem prudent to obtain more extensive and precise polarization spectroscopy of the Mars surface to determine whether or not there are any locations that display significant circular polarization, which in turn could be an indication of past or present chirality in the surface material.

6. Conclusions

We have presented new evidence that microbial photosynthetic organisms can produce a measurable circular polarization signature in their spectra. The primary polarization signatures arise in concert with electronic spectral absorption bands, as in the Cotton Effect. Hence, for utility as a biomarker, it is essential that the circular polarization spectrum be viewed together with a critical examination of the intensity spectrum. We showed that the strength of the polarization signal did not change substantially even with an increase of over a factor ten in the microbial concentration. We interpreted this as an optical depth effect and predict that in liquid suspension, the polarization signal in reflected light will reach saturation at approximately unit optical depth. Control minerals exhibiting spectral edges in their intensity distribution did not yield a spectropolarization signature, and the Mars regolith simulant studied did not yield a significant spectropolarization signature. Macroscopic vegetation did provide strong circular polarization, with features linked to characteristic absorption edges. Not only may circular polarization spectroscopy measurements be made remotely, but also they depend for the presence of a significant signature upon a characteristic of life which is thought to be exceedingly general and a likely property of all biochemical life, namely its homochirality. In a variety of situations therefore, it appears the circular polarization spectroscopy could provide a powerful biomarker.

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