Astronomy Meets Biology: EFOSC2 and the Chirality of Life

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Homochirality, i.e., the exclusive use of L-amino acids and D-sugar in biological material, induces circular polarisation in the diffuse reflectance spectra of biotic material. Polarimetry may therefore become an interesting remote sensing technique in the future search for extraterrestrial life. We have explored this technique and performed a laboratory experiment making an exotic use of an astronomical instrument. During a period when EFOSC2 was detached from the Nasmyth focus to host a visitor instrument at the NTT, we have observed various samples of biotic and abiotic material and measured their linear and circular polarisation spectra. Among the various targets, we have included samples of the hypolithic cyanobacteria species *Chroococcidiopsis* isolated from the Coastal Range of the Atacama Desert. To our knowledge, these are the first and highest precision measurements of circular polarisation using living material and obtained with an astronomical instrument.

Motivation

The building blocks of life are *chiral*. Their molecular structure lacks an internal plane of symmetry, and their mirror image cannot be superimposed on their original image. The term chirality is specifically used when a molecule (or an object) exists in both mirror-symmetric configurations. The human hands are the classic example that illustrates the concept of chirality, and the term chiral itself comes from the Greek word for hand, *χειρ*.
In chemistry, the two images of a chiral molecule are called enantiomers, and the two forms are generally referred to as right-handed and left-handed, or dextrorotatory and levorotatory.

The term *homochirality* is used when a molecule (or a crystal) may potentially exist in both forms, but only one is actually present. Homochirality characterises life as we know it: all living material on Earth contains and synthesises sugars and nucleic acids exclusively in their right-handed form, while amino acids and proteins occur only in their left-handed representation. However, in all these cases, both enantiomers are chemically possible and energetically equal. The reasons for homochirality in living material are unknown, but they must be related to the origin of life. It is still disputed whether bioactive molecules (and with them small enantiomeric excesses) were delivered to Earth (e.g., by meteorites) or whether (pre-)biotic chemistry started on Earth (Bailey et al., 1998). Undoubtedly, however, chemical and biological processes on Earth must have favoured the selection of one-handed biomolecules leading towards homochirality. If similar evolutionary scenarios naturally occur elsewhere in the Universe, homochirality may be a universal hallmark of all forms of life.

Chirality induces optical activity: each enantiomer rotates the reflected (or transmitted) light in opposite directions, and homochirality guarantees that there will be an excess of circularly polarised light in one direction. This opens up the interesting possibility that biosignatures could be sensed remotely by means of polarimetric techniques.

This chain of arguments, which had been raised in various articles in the scientific literature (e.g., Wolstencroft et al., 2004), was also discussed during the workshop Astrobio 2010[1] held in Santiago de Chile last January. For the first time, an international and interdisciplinary conference that aimed to cover major topics in astrobiology was organised and hosted in Chile. The topics covered included the origins of life, the chemistry of the Universe, extrasolar planetary systems, and the search for life in the Solar System.
A prominent topic of discussion was the Atacama Desert as an example of an extreme environment on Earth, and the microbial colonisation of subsurface layers in halites (rock salt) and quartz rocks by specific cyanobacteria. In the most hostile environments (exceptional aridity, salinity, and extreme temperatures), a primitive type of cyanobateria, *Chroococcidiopsis*, can be the sole surviving organism. This has interesting implications for the potential habitability, and eventual terraforming, of certain areas on Mars (Friedmann & Ocampo-Friedmann, 1995).

The idea of using the ESO Faint Object Spectrograph and Camera (EFOSC2) to investigate samples of *Chroococcidiopsis* extracted from the underside of Atacama Desert quartzes and to measure their circular polarisation in a laboratory experiment arose at this conference.
The idea looked appealing, because only limited, and sometimes contradictory, reports about circular polarisation measurements of biotic material as a signature of homochirality are available in the literature. Moreover, the successful use of an astronomical instrument for the first time for that purpose could serve as a benchmark for further applications of this method in astrophysics.

A detailed feasibility study as well as the production of significant quantities of *Chroococcidiopsis* were prepared and initiated in the following weeks. Since there is no formal process in place to obtain "observing time" for laboratory experiments, the ESO Director General was asked for authorisation. He approved the experiment under the condition that it did not pose any risk to the instrument. This could be achieved by using EFOSC2 when it was not attached at its nominal Nasmyth focal station (i.e. during an extended visitor instrument run), but keeping it in a horizontal position (which avoids the possibility of any material falling on the entrance window). Our "laboratory" is shown in Figure 1, left. EFOSC2 is detached from the New Technology Telescope (NTT). A microphotograph of our main target, *Chroococcidiopsis*, is displayed on the right.

Experiment

During one week in June 2010, three of us (Pontificia Universidad Católica

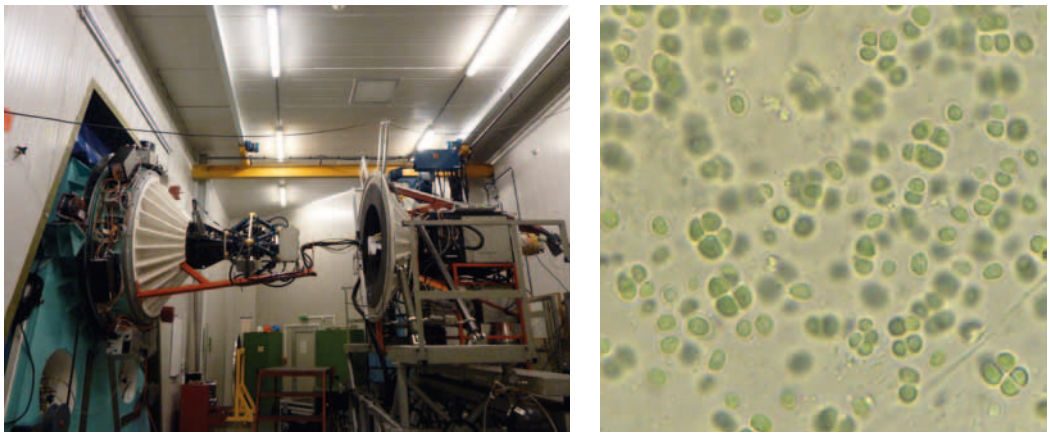

Figure 1. Left: The EFOSC2 instrument is shown detached from the NTT. The instrument attached to the Nasmyth focus (on the left) is ULTRACAM. Right: A microphotograph of the cyanobacteria *Chroococcidiopsis*, enlarged 100 times.

student Fabiola Salinas, Stefano Bagnulo and Michael Sterzik) were busy using EFOSC2 to observe samples of minerals and paints (quartz, salt, sugar, white flat-field screen), leaves (*Philodendron*, *Ficus*, *Schefflera*) and cyanobacteria films deposited on filter paper. All samples were prepared as thin sheets inserted in the flat-field screen position. An integrating sphere (the usual calibration lamp mechanism for EFOSC2 when attached to the 3.6-metre telescope) was used for diffuse illumination. EFOSC2 covers large spectral regions in the spectropolarimetry mode: we used mostly grism 13 to cover the range 370–930 nm, with a spectral resolution of ~ 2.3 nm (we adopted a 1-arcsecond slit width). Both linear and circular polarisation measurements of the samples were obtained, using the λ/2 and λ/4 retarder waveplates, respectively (we note that the λ/4 retarder waveplate is a recent addition to the instrument, see Saviane et al. [2007]).

Polarimetric measurements were taken by combining several pairs of exposures obtained with different position angles of the retarder waveplates (–45°, 45°, 135°, 225° for circular polarisation, and 0°, 22.5°, 45°, ... 335.5° for linear polarisation measurements, measured from the principal plane of the Wollaston prism). This "beam swapping" technique minimises the instrumental effects — for a detailed description of the measuring strategies, see, e.g., Bagnulo et al. (2009). For inorganic samples and for leaves, the typical observation cycles lasted 20 minutes, dominated in practice by overheads (readout time and retarder waveplate setting), and allowed us to measure the polarisation level with an error bar of the order of 10^{-4} per spectral bin. Measurements of the cyanobacteria lasted several hours, but for a total integration time of just about 10 minutes. This exposure allowed us to reach an error bar of 10^{-6} per spectral bin. These figures refer only to the statistical errors due to Poisson noise. With our ultra-high signal-to-noise ratio measurements, we have certainly hit the limits imposed by the polarimetric optics and the experimental conditions — we will come back later to this important point. Here we note that the reliability of the error bars (in terms of statistical error) has been validated with the use of so-called null-profiles (i.e. the difference between Stokes profiles obtained from different pairs of exposures). Null profiles were found scattered around zero within the error bars.

Results and outlook

Figures 2 and 3 show the results obtained from our circular polarisation measurements (Stokes *V* normalised to the intensity) of a *Philodendron* leaf, and a film of *Chroococcidiopsis* deposited on filter paper, respectively. We also obtained calibration measurements of a white screen flat-field, produced by a barium sulphate based white reflectance coating. The circular polarisation measured for the screen flat-field was found as a negative continuum of about –0.05 %, with the absolute value slowly increasing towards shorter wavelengths. No narrow features or signals are present in the polarisation spectra of this reference source.

In the *Philodendron* leaf we detected both broad polarised features (with an amplitude of ~ 0.5 %), and, superposed on them, a narrow feature at about 680 nm (with an amplitude of ~ 0.05 % over the continuum), both well above the statistical noise (the green line shows the null profile). The behaviour of Stokes *V* in the continuum closely follows the reflectivity, shown with a solid black line (this is known as the Cotton effect). This behaviour closely resembles the diffuse reflectance circular dichroism spectra of leaves as seen by Wolstencroft et al. (2004). The narrow feature around 680 nm is very similar to the results shown by Gregory & Raps (1974) in their transmission spectroscopy of chloroplasts and is related to the chlorophyll-a pigment response.

The interpretation of the results for *Chroococcidiopsis* is the most interesting. In the continuum, Stokes *V* shows a behaviour similar to that observed for the white screen field, and hence is likely to be of instrumental origin. A number of narrow, low amplitude features appear superposed on the continuum. In order to prove that these features are real (and not spurious signals, e.g., caused by the CCD readout noise) we also obtained ultra-high signal-to-noise ratio measurements with the retarder waveplate at 0°, 90°, 180° and 270°. With these retarder waveplate settings, we would theoretically expect a null signal. The resulting profile, shown with the red line in Figure 3, and arbitrarily rectified to zero for display purposes, shows no high frequency signatures. This curve, together with a consistently flat null profile (shown

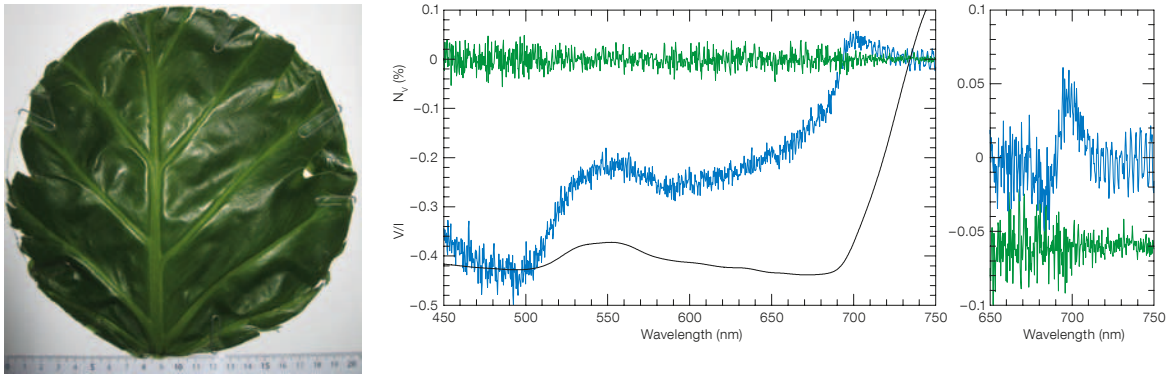

Figure 2. The *Philodendron* leaf is shown at left and its polarised spectrum measured with EFOSC2 at middle and right. The blue lines show the circular polarisation (V/I). In the rightmost panel, V/I is rectified to zero. The green lines show the null profile (offset by −0.06 % in the rightmost panel, for display purposes) demonstrating the statistical errors. The black line is the reflectivity (in arbtirary units).

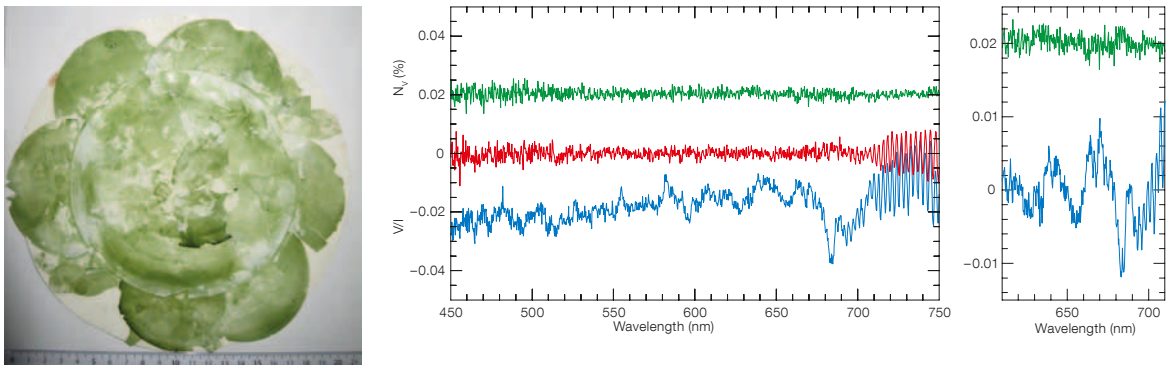

Figure 3. The sample of *Chroococcidiopsis* is shown at left and its polarised spectrum in the middle and right panels. The blue line shows circular polarisation and the green line is the null profile (offset by + 0.02 %). The red line shows the spectrum obtained with the retarder waveplate at position angles offset by 45º, rectified to zero. In the rightmost panel, V/I is rectified to zero.

with the green line offset to + 0.02 % in the right panel) suggests that the narrow features are indeed real. We notice again the prominent chlorophyll-a feature around 680 nm, but may also infer signatures of other pigments like carotinoids and phycocyanins. Our measurements also appear consistent with polarisation spectra obtained from a different type of cyanobacteria by Sparks et al. (2009).

While we kept the noise confidently at a very low level, the polarimetric mode of EFOSC2 is lacking the full characterisation needed to reach ultra-high and absolute precision polarimetric measurements under the conditions of our experiment. Using special calibration techniques, in some astronomical observations it is already possible to achieve a precision of 10^{-6} (e.g., Bailey et al., 2010), but in our experiments we are limited by an instrument setup in which most of the signal comes from off-axis rays. Patat & Romaniello (2006) and Bagnulo et al. (2009) have discussed the spurious polarimetric effects observed off-axis with the FORS instrument of the VLT. Some of these effects may also play a role in EFOSC2. We must also remark that the prepared samples are not fully reproducible in their configuration, and thus likely introduce some effects due to their different backscattering properties.

In conclusion, the polarised spectra of Figures 2 and 3 show that the polarimetric techniques employed by us are sensitive to the presence of biotic material. We detect chiral signatures of pigments involved in biotic photosynthetic reaction chains. Signatures of chlorophyll pigments can be captured both in samples of leaves and cyanobacteria of species *Chroococcidiopsis*. More accurate measurements of the polarised signal amplitudes require experiments to be carried out with samples prepared under better controlled laboratory conditions. It will also be interesting to observe the polarimetric signatures of *Chroococcidiopsis* in their natural habitat beneath translucent rock surfaces. This will allow the feasibility of detecting this type of bacteria on other Solar System bodies in the future with extremely large telescopes to be assessed.

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Links

[1] http://www.astro.puc.cl/astrobio2010