Detection Limits for Chiral Amino Acids Using a Polarization Camera

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Abstract

The detection of biosignatures on a planetary surface is of high scientific interest, and enantiomeric excesses of organic molecules are one such signature. Enantiomeric excesses can be detected by their polarizing effects on transmitted light. As part of instrument development work for a microscopic imager, the Cold Lightweight Imager for Europa (C-LIFE), we assess the potential of polarization measurements to quantify enantiomeric excesses. We investigated the optical rotation of the amino acids serine and phenylalanine for a range of enantiomeric abundances. Measurements were made with mixtures of serine and phenylalanine as well as Europa-relevant salts to determine how well these combinations can be detected. We also conducted a small number of measurements on samples of bacteria taken from glacial environments. We found that concentrations greater than $10^{-3}$ M are needed to detect serine and concentrations greater than $10^{-4}$ M are needed to detect phenylalanine, with larger concentrations needed for smaller enantiomeric excesses. Salts do not have a significant effect on the optical rotation. Optical rotation of bacterial samples were detected at concentrations $>10^6$ cells ml$^{-1}$. Systematic errors in our polarization detector limited our sensitivity to optical activity changes of $\sim 0.008^\circ$, leading to an inability to distinguish enantiomeric abundances separated by 5%, but tests show that improvements to our laboratory technique can yield a factor of 20 improvement in sensitivity.

Unified Astronomy Thesaurus concepts: Astrobiology (74); Biosignatures (2018); Jovian satellites (872); Polarimetry (1278)

1. Introduction

The detection of biosignatures on a planetary surface is of significant scientific interest in the search for life beyond Earth. High enantiomeric excesses are one such biosignature that suggests the favoring or concentration of biogenic macromolecules. Enantiomers are each of the two non-superimposable mirror-image configurations of chiral molecules (Figure 1). In biological materials on Earth, the ratio of the L-enantiomer to the D-enantiomer of amino acids is high (Kvenvolden 1973), with a ~98% L-enantiomer abundance as a fraction of the whole for amino acids derived from bacteria (Aubrey 2008). In abiotically synthesized amino acids and the amino acids found in meteorites, the two enantiomers are generally found in approximately equal amounts (Kvenvolden 1973), although there are exceptions. L-enantiomer abundances of 50.5%–57.5% (excesses of 1%–15%; Pizzarello 2006), up to 60.5% (excesses up to 21%; Elsila et al. 2016), and even >70% (excesses of $>40$%; Glavin et al. 2012) have been reported for some amino acids in meteorites, and terrestrial contamination is unlikely to be the cause of the L-enantiomer excesses in all cases (Pizzarello 2006; Glavin et al. 2012; Elsila et al. 2016).

Icy moons of the outer solar system, such as Jupiter’s moon Europa and Saturn’s moon Enceladus, are targets in the search for life elsewhere in the solar system due to their global or regional subsurface oceans. Magnetometer and radio Doppler measurements by the Galileo spacecraft indicate that Europa hosts a liquid water ocean beneath a water ice shell (Anderson et al. 1998; Khurana et al. 1998; Kivelson et al. 2000). The composition of this subsurface ocean may include material critical for life as we know it, and the potential for biogeochemical cycling, which could provide a source of energy for life (Hand et al. 2009). Sulfates and chlorides of magnesium and sodium are likely solutes in the ocean (Zolotov & Kargel 2009). Magnesium and sodium salts are also indicated on the surface by spectra (Dalton 2007; Brown & Hand 2013; Fischer et al. 2015; Trumbo et al. 2019) and by color (Hand & Carlson 2015). The ocean’s conductivity implies that it is at least slightly, and potentially highly, saline; however, the upper limit on the conductivity is not well constrained (Hand & Chyba 2007; Schilling et al. 2007; Zolotov & Kargel 2009). Based on formation models, biologically essential elements such as carbon, nitrogen, phosphorous, and sulfur are likely present in Europa’s ocean (Hand et al. 2009) and carbon and sulfur compounds have been observed on Europa’s surface (Hand et al. 2009). Other species observed on Europa’s surface include radiolytically produced molecular oxygen and hydrogen peroxide, which are oxidants that could take part in important chemical cycles (Carlson et al. 2009).

The surface detection of biological material arising in the ocean is predicated on transport through the thick ice cover, leading to emplacement of ocean material on Europa’s surface. Water vapor plumes have been observed (Roth et al. 2014; Sparks et al. 2016, 2017, 2019; Arnold et al. 2019) potentially leading to material sourced from the ocean eventually being deposited on the surface. Various observations and formation hypotheses for European surface features also indicate that surface–ocean interactions may occur. Double ridges are
The abundance of L- and D-enantiomers impacts the observed optical rotation of light transmitted through a sample. The optical rotation imparted by a molecule is the angle by which plane-polarized light is rotated when it passes through a sample of the molecule. The measured optical rotation depends on the wavelength of light, temperature, path length, and concentration of the solution. The specific optical rotation, \( [\alpha]_\lambda \), at temperature \( T \) and wavelength \( \lambda \), in units of \( \text{deg ml}^{-1} \text{dm}^{-1} \), is given by

\[
[\alpha]_\lambda = \frac{\alpha}{l c},
\]

where \( \alpha \) is the observed optical rotation in degrees, \( l \) is the path length in decimeters, and \( c \) is the concentration in grams ml\(^{-1}\).

The two enantiomers of a chiral molecule will have optical rotations of equal magnitude and opposite sign. In racemic mixtures of two enantiomers (equal amounts of L and D), like those likely to be found in abiotic materials, no net optical rotation is expected. However, in nonracemic mixtures more likely to have a biogenic origin, a net optical rotation that can be used to constrain the abundance of that chiral molecule is expected. Because some enantiomeric excesses have been observed in meteorites, this optical rotation on its own would not be conclusive evidence of a biological origin for the sample. However, optical rotation could strengthen an argument for potential biology or quickly downselect what samples are most interesting for detailed analysis by other instruments. Variations on this approach have also been studied. For example, MacDermott et al. (1996) proposed using an array of multiple fixed polarizers at different angles to detect optical rotation. Kothari et al. (2008) tested a system for which the plane-polarized beam entering a sample was rotating, a technique that reduced the effect of depolarizing light scattering caused by biological samples. Thaler et al. (2006) utilized a polarimeter to measure the change in optical rotation over time during a racemization reaction.

2. Methods

2.1. Overview

The C-LIFE instrument tested here is a polarization camera with a micropolarizer array over the detector that measures the intensity of light at four different polarizations (Brook et al., 2011). These values can be used to find the angle by which the sample rotates linearly polarized light (angle of linear polarization, AoLP). We investigated the optical rotation of...
the amino acids serine and phenylalanine for a range of enantiomeric abundances. Both of these amino acids function in proteins and are found in organisms as well as fossils and sediments on Earth (Kvenvolden 1973). We also conducted a small number of measurements of bacterial samples taken from glacial environments to test whether the optical rotation of such samples could be measured using this instrument.

Serine has a specific optical rotation at $\lambda = 590$ nm of $-6.83$ deg ml g$^{-1}$ dm$^{-1}$ in water (O’Neill et al 2001, p. 1517). Phenylalanine has a specific optical rotation at $\lambda = 590$ nm and $20^\circ$C of $-35.1$ deg ml g$^{-1}$ dm$^{-1}$ in water (O’Neill et al 2001, p. 1305). Measuring the optical rotation of both of these amino acids allowed us to determine how the concentration detection limit for optical rotation differs for molecules with different magnitudes of optical rotation. These specific amino acids were also chosen for their stability over long periods of time.

For each amino acid, we tested L-enantiomer abundances of 100% L, 95% L, 90% L, 75% L, 60% L, and 55% L. Note that these correspond to L-enantiomer excesses ($%ee = %L - %D$) of 100%, 90%, 80%, 50%, 20%, and 10%. We also tested the corresponding majority D-enantiomer abundances (except for 95% D and 90% D). This range spans relative abundances of amino acids from that of a likely biological origin through abundances that are likely abiotically sourced and includes several abundances at intervals 5% apart, allowing us to test whether these intervals are distinguishable. For each amino acid and enantiomeric abundance, we measured the optical rotation at a range of concentrations in order to determine the concentration limit at which optical rotation could no longer be detected. The highest-concentration measured was based on the solubility of the amino acids. Phenylalanine is less soluble than serine (Yalkowsky et al. 2010) so a lower-concentration stock solution was used for the phenylalanine solutions than those of serine. Although L-serine and D-serine should have the same solubility, we observed that D-serine was slightly less soluble, which may be explained by differences in impurities in the samples. This limited the maximum concentration of solutions with a large proportion of D-serine.

Mixtures of materials were also investigated. Solutions containing different ratios of serine and phenylalanine were prepared, and their optical rotations were measured to determine how well these combinations can be detected. Salts have previously been found to affect the optical rotation of amino acids (Nostro et al. 2006; Rossi et al. 2007). Therefore, sodium chloride (NaCl) and magnesium sulfate heptahydrate (MgSO$_4$·7H$_2$O) were added to the amino acid solutions to determine the effect of potentially Europa-relevant salts (Dalton 2007; Brown & Hand 2013; Fischer et al. 2015; Hand & Carlson 2015) on the optical rotation detection limits. Table 1 provides a summary of all experiments performed.

### 2.2. Sample Preparation

Tested amino acids used were purchased from Acros Organics and had the following chemical purities: 98% (D-serine), 98.5% (L-phenylalalanine), and 99% (L-serine and D-phenylalanine). The enantiomeric purities of these amino acid samples are unknown but can be estimated by comparing specific optical rotation values measured by the manufacturer (at $\lambda = 590$ nm) to literature specific optical rotation values, assuming that the given compound is the only optically active substance in the sample. The measured L-serine optical rotation of $+14.8$ deg ml g$^{-1}$ dm$^{-1}$ in HCl is $\sim$99% of the literature optical rotation of $+14.95$ deg ml g$^{-1}$ dm$^{-1}$ (O’Neil et al. 2001, p. 1517), indicating an enantiomeric excess of $\sim$99% (corresponding to an L abundance of $\sim$99.5%). The measured D-serine specific optical rotation of $-15.5$ deg ml g$^{-1}$ dm$^{-1}$ is $\sim$104% of the expected value, making the enantiomeric excess unclear and indicating some of the chemical impurities may have been optically active. The L-phenylalanine optical rotation of $-33.6$ deg ml g$^{-1}$ dm$^{-1}$ is 96% of the expected value of $-35.1$ deg ml g$^{-1}$ dm$^{-1}$ in water (O’Neill et al. 2001, p. 1305), indicating that the enantiomeric excess is $\sim$96% (corresponding to $\sim$98% L abundance). The D-phenylalanine optical rotation of $+33.4$ deg ml g$^{-1}$ dm$^{-1}$ is $\sim$95% of the expected value. Note that L-abundances shown in figures do not take into account the enantiomeric purities discussed here.

Amino acids and distilled water (purified by steam distillation, filtration, and ozonation) were measured by mass, combined, and stirred (and in some cases heated slightly) until dissolved. Solutions with any visible insoluble particulates were filtered with an 11 μm filter. We did not filter out smaller particles and cells that might have been introduced by the water because impurities in the sample and control solutions should have the same effect on their optical rotation, and this effect should mostly cancel out as we utilize only the differences between these values. In addition, our measurements at 590 nm, e.g., for 100% L-serine are close to predicted values at concentrations $>10^{-2}$M, indicating that impurities do not provide significant optical rotation at those concentrations. At lower concentrations, the measured optical rotations deviate from expected values because instrument errors dominate the signal at those concentrations.

The masses of water and amino acid were used to calculate the molality of the solution, which was converted to a molarity. Serial dilutions were made in distilled water so that a range of concentrations could be measured, and the solutions were again filtered if any particulates were visible. The distilled water used as the solvent for these solutions served as a control for the polarization measurements. Measurements were typically made shortly after mixing solutions. If measurements were not made on the same day, the solutions were filtered again and stored in sealed containers at 4°C.

For the solutions to which salt was added, the amino acid stock solution and dilutions were made first, and the mass of salt to be added was determined by their volume. Europa’s ocean salinity is not well constrained, but terrestrial seawater salinity ($\sim$0.6 M) is among the range of possibilities. For Enceladus, the NaCl concentration of grains sourced from the plumes is 0.05–0.2 mol kg$^{-1}$ or $\sim$0.1–0.4 M (Postberg et al. 2009). Thus, we tested salt concentrations of 0.3, 0.6, and 1.2 M.

### 2.3. Optical Setup

Light from an LED was passed through a collimator and polarizer to produce plane-polarized light. Two apertures were used to control the amount of light entering a cuvette that held the sample. After passing through the cuvette, the light was detected by a polarization camera, which measures the angle and degree of linear polarization of the light. Figure 2 shows this experimental setup. The measurements were taken in a darkened room with light shields around the cuvette to decrease noise from ambient light.

Light sources used were 490 nm and 590 nm LEDs (Thorlabs M490L4, and M590L3, respectively) equipped with
a Glan–Thompson prism polarizer (Thorlabs, with extinction ratio >100:1). Specific optical rotation values for serine and phenylalanine have previously been measured only for 590 nm (O’Neil et al. 2001). However, shorter wavelengths tend to induce larger rotations (e.g., Polavarapu & Covington 2015), so in theory, lower concentrations could be detected using the 490 nm LED. Wavelengths shorter than 490 nm were not tested because radiation hard glasses doped with cerium, such as might be used in a realistic Europa Lander imager (e.g., Centurelli et al. 2018), are opaque at wavelengths shorter than ∼450 nm (Henson & Torrington 2001). In addition, in a realistic sample, optical scattering, which is worse at shorter wavelengths, could lead to depolarization. Also, optical rotation behavior for some molecules can change drastically at short wavelengths (Schreier et al. 1995), so we did not want to test at wavelengths far from 590 nm, where data were not available. All of the samples were measured using 490 nm light, and select samples were also measured at 590 nm for comparison to previously reported specific optical rotations (Figure 3).

| Compound | Enantiomeric Abundances | Concentration Range (M) | Wavelengths (nm) |
|----------|-------------------------|-------------------------|------------------|
| Serine   | 100% L                  | 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 | 490, 590 |
| Serine   | 95% L, 5% D             | 0.1, 0.05, 0.025, 0.01, 0.005, 0.001, 0.0005 | 490 |
| Serine   | 90% L, 10% D            | 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 | 490 |
| Serine   | 75% L, 25% D            | 0.1, 0.05, 0.025, 0.01, 0.005, 0.001 | 490 |
| Serine   | 60% L, 40% D            | 0.1, 0.05, 0.025, 0.01, 0.005, 0.001 | 490 |
| Serine   | 55% L, 45% D            | 0.05, 0.025, 0.01, 0.005, 0.001 | 490 |
| Serine   | 45% L, 55% D            | 0.1, 0.05, 0.025, 0.01, 0.005, 0.001 | 490 |
| Serine   | 40% L, 60% D            | 0.1, 0.05, 0.025, 0.01, 0.005, 0.001 | 490 |
| Serine   | 25% L, 75% D            | 0.1, 0.05, 0.025, 0.01, 0.005, 0.001 | 490 |
| Phenylalanine | 100% L              | 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490, 590 |
| Phenylalanine | 95% L, 5% D            | 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490 |
| Phenylalanine | 90% L, 10% D           | 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490 |
| Phenylalanine | 75% L, 25% D           | 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00001 | 490 |
| Phenylalanine | 60% L, 40% D           | 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00001 | 490, 590 |
| Phenylalanine | 55% L, 45% D           | 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00001 | 490, 590 |
| Phenylalanine | 45% L, 55% D           | 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00001 | 490, 590 |
| Phenylalanine | 40% L, 60% D           | 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00001 | 490, 590 |
| Phenylalanine | 25% L, 75% D           | 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00001 | 490, 590 |
| Equal parts Ser and Phe | 100% L              | 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490, 590 |
| 75% Ser, 25% Phe | 100% L             | 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490, 590 |
| 25% Ser, 75% Phe | 100% L            | 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490, 590 |
| Equal parts Ser and Phe | 75% L, 25% D         | 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490, 590 |
| Equal parts Ser and Phe | 25% L, 75% D         | 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490, 590 |
| Serine + 1.2M NaCl | 100% L             | 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 | 490, 590 |
| Serine + 0.6M NaCl | 100% L             | 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 | 490, 590 |
| Serine + 0.3M NaCl | 100% L             | 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 | 490, 590 |
| Phenylalanine + 0.6 NaCl | 100% L         | 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 | 490, 590 |
| Serine + 0.6 M MgSO4·7H2O | 100% L         | 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 | 490, 590 |
wavelengths, from 582 to 608 nm with a peak at 596 nm. No filter was used. Samples (56 ml) were measured in a 200 mm-long UV quartz cylindrical cell cuvette (FireflySci). The cuvette length is the path length used in Equation (1) for the prediction of optical rotation as a function of concentration that is shown in Figure 3.

Measurements were obtained with a PolarCam Snapshot Micropolarizer Camera made by 4D Technology. The PolarCam uses a wire grid polarizer array which contains a pattern of polarizers with 0°, 45°, 90°, and 135° polarizations that together form a superpixel which is repeated over the array (Figure 4; Brock et al. 2011).

The LED optics were focused at infinity, and the LED, polarizer, first aperture, and collimator were aligned. The first aperture was adjusted to prevent light hitting the edges of the polarizer. Then, the cuvette was placed in a stand at the correct height, and the second aperture and cuvette were aligned with each other and the other components. The second aperture was adjusted to prevent reflections off the sides of the cuvette. The PolarCam was then aligned with the cuvette, and the polarizer was rotated to approximately balance the intensity in two of the different polarization quadrants with polarizations of 0° and 45°, as we determined the angle of linear polarization is most accurately measured in this configuration. The exposure time

Figure 2. Picture and diagram of the optical setup. From left to right: PolarCam, cuvette, aperture 2, collimator, and polarizer with aperture 1 on the end, LED.

Figure 3. Absolute value of optical rotation of 100% L-serine at 490 and 590 nm. Curves shown are linear fits to the data points. The black dashed line shows the expected optical rotation at 590 nm using the specific optical rotation and Equation (1).
(typically a few milliseconds) was adjusted to receive as many photons as possible while avoiding saturation.

2.4. Data Collection

To find the optical rotation caused by the amino acid, the angle of linear polarization for a control measurement of distilled water must be subtracted from the angle of linear polarization measured for the amino acid solution. We reverse the polarity of the angle recorded by the system to be consistent with the definitions of positive and negative rotations for L- and D-enantiomers. Control measurements were taken before and after measurements of each amino acid solution. The cuvette was rinsed twice with distilled water and once with higher purity ASTM (American Society for Testing and Materials) standard type II deionized water (>1 MΩ cm) after each measurement of amino acid solutions.

2.5. Data and Error Analysis

PolarView software associated with the PolarCam outputs the intensity for each pixel of the micropolarizer array, i.e., the intensities of the 0°, 90°, 45°, and 135° linearly polarized components (I₀, I₉₀, I₄₅, I₁₃₅) for each superpixel on the detector. These can be used to find the Stokes vector describing the polarized light:

\[
S = \begin{bmatrix} S₀ \\ S₁ \\ S₂ \\ S₃ \end{bmatrix} = \begin{bmatrix} I₀ + I₉₀ \\ I₀ - I₉₀ \\ I₄₅ - I₁₃₅ \\ Iₗ𝐻𝐶 − Iᵣ𝐻𝐶 \end{bmatrix},
\]

where I₀, I₉₀, I₄₅, and I₁₃₅ are as described above, and Iₗ𝐻𝐶 and Iᵣ𝐻𝐶 are the left- and right-hand circularly polarized components (not measured by the PolarCam and not required for our

Figure 4. Diagram of the PolarCam charge-coupled device (CCD) sensor overlaid by the polarizer array, a portion of the polarizer array, and the arrangement of a single superpixel (based on Brock et al. 2011). The dimensions of the CCD sensor are 1700 x 1200 pixels, which are divided into four-pixel clusters by the polarizer array to form 850 x 600 superpixels.

Figure 5. AoLP of water every 30 s over an hour, with the camera warmed up for >1 hr.
calculations). The components of the Stokes vector can be used to find the degree of linear polarization (DoLP), which is the fraction of incident light that is linearly polarized:

$$\text{DoLP} = \frac{\sqrt{S_2^2 + S_3^2}}{S_0}. \quad (3)$$

They can also be used to find the AoLP, which is the polarization angle of the incident light relative to the detector axis:

$$\text{AoLP} = \frac{1}{2} \arctan \left( \frac{S_3}{S_2} \right). \quad (4)$$

Systematic errors were caused by the measured AoLP changing over time and this error depended significantly on the temperature of the camera. This error was partially mitigated, and results converged to their final values if the camera was allowed to warm up for at least 1 hr. Therefore, all of the measurements reported here were taken after the camera warmed up for at least 1 hr. After finding the AoLP and DoLP for each superpixel of the detector for each measurement, the average AoLP over all superpixels on the detector was determined. The large number of superpixels behave as many independent polarimeters to increase the resolution of the system and reduce the noise contributed by any single pixel in the micropolarizer array.

Error is dominated by the systematic drift in AoLP with time. Thus, measurements of the sample and control (at different times) cannot be simply subtracted. We mitigate the impact of drift of the control AoLP by linearly interpolating between the AoLP for the controls taken before and after the sample measurement to find what the control AoLP would be at the time the sample measurement was taken. We then subtract the AoLP of the sample from this control AoLP to get the optical rotation (this convention gives the appropriate sign for the optical rotation).

This approach depends on the AoLP measurements being taken close in time so that changes appear linear. To determine the accuracy of this approach, we took measurements of distilled water every 30 s for an hour (Figure 5). AoLP varied by up to 0.09° during this time at rates of up to $8 \times 10^{-5}$° deg s$^{-1}$. We used those measurements to find the difference between the measured AoLPs and AoLPs based on linear interpolation between two measurements at time $t_1$ and $t_1 + \Delta t_{cc}$, where $\Delta t_{cc}$ is the time between control measurements for a given amino acid solution. This linear interpolation was used to calculate a value for the AoLP at a time $t_1 + \Delta t_{cs}$, where $\Delta t_{cs}$ is the time between the first control measurement and the sample measurement for an amino acid solution. We calculated $|\text{AoLP}_{\text{measured}}(t_1 + \Delta t_{cs}) - \text{AoLP}_{\text{interpolated}}(t_1 + \Delta t_{cs})|$ and repeated this for every pair of measurements at a time $\Delta t_{cs}$ apart. The histogram of these differences based on $\Delta t_{cs}$ and $\Delta t_{cc}$ for one amino acid solution is shown in Figure 6. To be conservative in finding detection limits, we take the 90th percentile value of these differences as the error. This process was repeated for every amino acid solution because $\Delta t_{cs}$ and $\Delta t_{cc}$ are different for each. The average of the 90th percentile errors, for all amino acid solutions, is $\sim 0.008°$, but it ranges between 0.004° and 0.03°.

The error described above determines the consistency of results from multiple measurements of the same sample and is the error we base detection limits on. However, we also calculated the error for the AoLP from a single measurement. We propagated the standard deviation for each of the intensity measurements to get the error for the AoLP measurements. This gives an average AoLP error of $\sim 0.008°$.

### 2.6. Bacterial Sample Measurements

Bacteria contain a variety of metabolites and biomolecules including compounds such as L-amino acids and D-sugars, either of which can have positive or negative optical rotations. However, rotation in some direction is expected due to the low likelihood that the summed rotations of an abundance of chiral molecules would exactly cancel to zero. Previous polarimetric observations found that microorganisms have a negative rotation (Berthod et al. 2003) and that proteins exhibit optical activity because of both their asymmetric structure and their constituent amino acids (Jirgensons 1973). Here, we measured the optical rotations of three bacterial samples isolated from icy environments. These were (1) *Marinobacter gelidimuriae* (Chua et al. 2018), a moderate halophile originally isolated from a subglacial brine (salinity 8%) that discharges at a glacial feature known as Blood Falls in Antarctica; (2) a *Shewanella* strain designated Schw_1 from the surface ice around Blood Falls, where subglacial salts precipitate at the surface and freezing and thawing regularly occurs; and (3) an isolate denoted strain Easton_1, originally from snowpack on Easton Glacier, WA, USA. All cultures were initially grown in commercially available media. The *M. gelidimuriae* sample and Schw_1 were grown in a marine broth (DIFCO) and Easton_1 was grown in a lower nutrient, low conductivity broth (R2A; Bacto).

The bacteria were allowed to grow until they reached stationary phase at a concentration estimated to be $\sim 10^8$ cells ml$^{-1}$. Because they reached stationary phase, it is likely that some lysed cells were present in the measured samples. Before measurement, cells were separated from media by gentle centrifugation (1163 × g for 10 minutes) so that additional cells were not lysed by this process. Cell pellets were resuspended in pH 7 phosphate buffer (Fisher Chemical) and then serial-diluted.
at known concentrations. This work was done in a class II biosafety cabinet. Measurements were conducted in essentially the same way as the amino acid measurements, using the phosphate buffer as the control rather than water. We were not able to take optical density measurements of the samples; however, the highest-concentration samples were almost opaque, while the lower-concentration samples were more transparent, necessitating adjustment of exposure time and the size of the first aperture between each concentration.

3. Results

3.1. Serine and Phenylalanine

Measured optical rotation values for the serine experiments at 490 nm (Figure 7) confirm the expected result that more equal enantiomeric abundances result in a smaller optical rotation. The relation between optical rotation and concentration is linear down to ~0.005 M, below which optical rotations are not detectable. The apparent nonlinearity we observed at

![Figure 7.](image1.png)

**Figure 7.** Absolute value of optical rotation of serine for various L-enantiomer abundances and concentrations. Curves shown are linear fits to the data points with a prescribed intercept of (0, 0).

![Figure 8.](image2.png)

**Figure 8.** Absolute value of optical rotation of phenylalanine for various L-enantiomer abundances and concentrations. Curves shown are linear fits to the data points with a prescribed intercept of (0, 0).
the lowest concentrations is understood to be due to measurement limitations, rather than a physical difference in the dependence of rotation on concentration.

Measured optical rotation values for phenylalanine experiments at 490 nm (Figure 8) were higher at any given concentration than for serine. The relation between optical rotation and concentration is linear down to $\sim 0.0005$ M but at lower concentrations appears to be nonlinear, again likely due to measurement limitations.

In this study, we designated the concentration detection limit for optical rotation as the lowest concentration for which (1) the optical rotation plus or minus its error never crosses zero, (2) the optical rotation has the expected sign, and (3) every higher concentration measurement satisfies conditions (1) and (2). Detections could potentially occur at concentrations between this concentration and the next lowest investigated concentration that did not satisfy the conditions. Note that in the following, we present results using L or D abundances rather than enantiomeric excess.

We confirmed that the concentration detection limit for lower enantiomeric excesses is higher, e.g., compare abundances of 100% L- to 45% L-serine (Figure 9 and Table 2). In addition, it is observed that detection limits for solutions with some L-enantiomeric abundance and the equivalent D-enantiomeric abundance are generally the same. This is the case for 100% L- and 100% D-serine, 75% L- and 75%
D-serine, and 60% L- and 60% D-serine, but not for 55% L- and 55% D-serine. Although we found a detection limit for 55% L-serine, we did not find one for 55% L-serine, thus we cannot consistently detect such small enantiomeric excesses. Similarly, most detection limit trends are as expected for the phenylalanine measurements, except for 55% L- and 55% D-phenylalanine (Figure 10 and Table 2).

Table 3 shows the concentrations at which solutions with enantiomeric abundances 5% apart are distinguishable from each other, i.e., the concentrations at which their optical rotations including errors do not overlap. Only the concentrations that are above the detection limit for both solutions are included. We found that 100% L-serine and 95% L-serine were distinguishable at 0.1 M, but this result is not seen for other sets of serine measurements with enantiomeric abundances 5% apart. We also found that 100% L-phenylalanine and 95% L-phenylalanine were distinguishable at 0.005 M but not at the next higher concentration of 0.01 M, and no other sets of phenylalanine measurements with enantiomeric abundances 5% apart were distinguishable at any of the measured concentrations. Therefore, we conclude that no pairs of phenylalanine solutions with enantiomeric abundances 5% apart were consistently separable. Note that in Figures 7 and 8, these pairs are not clearly distinguishable because of the logarithmic scale.

Table 4 shows the concentrations at which the percent errors (error divided by absolute value of the optical rotation) were <5%. For serine, the percent error is <5% only at 0.1 M for L-enantiomeric abundances of 100% L, 95% L, and 90% L. For phenylalanine, the percent error is <5% only above 0.01 M for enantiomeric abundances of 100% L, 95% L, and 100% D.

### 3.2. Mixtures of Serine and Phenylalanine

The optical rotation of mixtures of amino acids is well approximated by averages of the optical rotations of the components, weighted by the proportion of the amino acid present in the mixture. Figures 11 and 12 compare measurements to predictions using a linear combination of the specific optical rotations (at 590 nm) of the two amino acids, weighted by the abundance of the amino acid as a fraction of the whole. Because phenylalanine has a higher specific optical rotation, solutions with a higher proportion of phenylalanine have a higher optical rotation. Higher L-enantiomer abundances, corresponding to higher enantiomeric excess, also lead to higher magnitudes of optical rotation. Because phenylalanine’s higher optical rotation, we expect mixtures of phenylalanine and serine to be detectable at lower concentrations than a 100% serine solution. This was the case for the 75% phenylalanine, 25% serine mixture (Table 5). However, the 50% phenylalanine, 50% serine mixture and the 25% phenylalanine, 75% serine mixture had the same detection limit as 100% L-serine because the increased optical rotation of the mixtures at smaller concentrations was not enough to surpass the error and decrease the detection limit.

The optical rotation of mixtures of amino acids is well approximated by averages of the optical rotations of the components, weighted by the proportion of the amino acid present in the mixture.
3.3. Effect of Adding Salts to Amino Acid Solutions

Adding NaCl and MgSO$_4$·7H$_2$O slightly decreased the optical rotation of serine (Figure 13) and phenylalanine. However, this difference is not enough to consistently affect the detection limits at the concentrations measured for the combination of salts and amino acids that we tested (Table A1).

3.4. Bacterial Measurements

The optical rotation of bacteria appears to be detectable at relatively high concentrations using this technique. Considering cells were harvested at stationary phase, some cells may have been lysed, thus measurements could include membranes of burst cells in addition to live cells and small molecules, such as glucose and amino acids which are a major source of optical rotation. However, the bacteria were pelleted and resuspended before measurement, removing any extracellular constituents that would have been present in the growth media, and the cells were deprived of nutrients for an extended period of time during the experiments, so levels of glucose and free amino acids produced by the bacteria may be low. Another source of optical rotation may be proteins within the cells or cell membranes, which are optically active due to both the constituent amino acids and the structure of the proteins themselves (Jirgensons 1973).

For concentrations >10$^6$ cells ml$^{-1}$, the optical rotation of Marinobacter gelidimuriae (Figure 14) is detectable and negative. Schw_1 and Easton_1 were detectable at the highest concentration at which they were measured, but unusual results were found at lower concentrations (Figures A2 and A3), including positive optical rotations and erratic jumps in the magnitude of optical rotation. The observed optical rotation for these measurements is generally below 10$^{-2}$ deg, where our amino acid optical rotation measurements were found to be unreliable. Therefore, these unusual results are most likely due

| Table 4 | Summary of Concentrations for All Serine and Phenylalanine Experiments at Which the Percent Error Was <5% |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
| Solution | Concentrations (M) for Which Error <5%                                                                                         |
| Serine 100% L | 0.1                                                                                                                              |
| Serine 95% L | 0.1                                                                                                                              |
| Serine 90% L | 0.1                                                                                                                              |
| Serine 75% L | None                                                                                                                             |
| Serine 60% L | None                                                                                                                             |
| Serine 55% L | None                                                                                                                             |
| Serine 45% L | None                                                                                                                             |
| Serine 40% L | None                                                                                                                             |
| Serine 25% L | None                                                                                                                             |
| Serine 100% D | None                                                                                                                             |
| Phenylalanine 100% L | 0.05, 0.01                                                               |
| Phenylalanine 95% L | 0.01                                                                 |
| Phenylalanine 90% L | 0.01                                                                 |
| Phenylalanine 75% L | None                                                                 |
| Phenylalanine 60% L | None                                                                 |
| Phenylalanine 55% L | None                                                                 |
| Phenylalanine 45% L | None                                                                 |
| Phenylalanine 40% L | None                                                                 |
| Phenylalanine 25% L | None                                                                 |
| Phenylalanine 100% D | 0.01                                                                 |

Figure 11. Absolute value of optical rotation of mixtures of 100% L-serine and 100% L-phenylalanine. Solid curves shown are linear fits to the data points, passing through (0, 0). Dashed curves are based on Equation (1) using a linear combination of the specific optical rotations for serine and phenylalanine.
Although in general we find that many of our measurements follow expected trends, there are some discrepancies, due primarily to variability in the magnitude of the systematic error caused by drift in measured AoLP over time. We mitigate this error by comparing our sample AoLP to a control AoLP value obtained by linearly interpolating between control AoLP measurements taken before and after the sample measurement, but this does not completely correct for the problem because the change in AoLP is not precisely linear. Measurements taken while the AoLP is changing nonlinearly have errors, and this may affect the detection limits found. In addition, slight variability in the amount of time taken between the control and sample measurements can influence the magnitude of errors (the longer the time between measurements, the larger the error if AoLP changes nonlinearly), and this may affect the detection limits found. Further improvements to the measurement protocol to decrease the time between the control measurement and the sample measurement or otherwise calibrate the system could decrease the errors.

4. Discussion

Although in general we find that many of our measurements follow expected trends, there are some discrepancies, due primarily to variability in the magnitude of the systematic error caused by drift in measured AoLP over time. We mitigate this error by comparing our sample AoLP to a control AoLP value obtained by linearly interpolating between control AoLP measurements taken before and after the sample measurement, but this does not completely correct for the problem because the change in AoLP is not precisely linear. Measurements taken while the AoLP is changing nonlinearly have errors, and this may affect the detection limits found. In addition, slight variability in the amount of time taken between the control and sample measurements can influence the magnitude of errors (the longer the time between measurements, the larger the error if AoLP changes nonlinearly), and this may affect the detection limits found. Further improvements to the measurement protocol to decrease the time between the control measurement and the sample measurement or otherwise calibrate the system could decrease the errors.

NASA’s Europa Lander report stipulated that enantiomeric excess should be quantified with an accuracy of 5% or better (Hand et al. 2017). Using the C-LIFE instrument, the percent error in the optical rotation is >5% at the detection limit, and solutions with enantiomeric abundances 5% different (corresponding to enantiomeric excesses 10% different) are rarely distinguishable through their polarization effects. Even when they are distinguishable, the concentrations at which they are distinguishable are more than an order of magnitude higher than the detection limits, meaning that even if an amino acid were detected at the C-LIFE instrument detection limits, the enantiomeric excess could not necessarily be determined to the desired accuracy.

The Europa Lander report also stipulates detecting amino acids (using an instrument like a separation mass spectrometer) to approximately the nM level (Hand et al. 2017). However, if amino acids were detected at this level, the polarization camera we tested could not determine their enantiomeric excess. A
much higher concentration, of \( >10^{-4} \text{ M} \), is needed to
determine the optical rotation of amino acids using this
approach as is.

To estimate the precision required to have a 5% error, we
consider the optical rotation of 0.05 M 60% L-serine, which
was \( \sim -0.02^\circ \), so an error of 5% would be 0.001\(^\circ\), which is
about four times lower than the lowest errors we found. One
way of decreasing the errors is decreasing the time between the
control and sample measurements. For example, we could
compare the control measurement and the sample measurement
to an intermediate measurement before comparing them to each
other. We tested measuring the LED polarization before and
after the control measurement and before and after the sample
measurement by removing the filled cuvette from the setup,
which took less than a minute, rather than the \( \sim 5 \) minutes
needed to switch the contents of the cuvette. An optical rotation
value for the control compared to the LED with no cuvette was
found by comparing the control AoLP measurement to the

Figure 13. Absolute value of optical rotation of 100% L-serine with various molarities of NaCl added. Curves shown are linear fits to the data points, passing through (0, 0).

Figure 14. Absolute values of optical rotation of *Marinobacter gelidimuriae*. 
LED-only AoLP measurements taken beforehand and afterwards. The error was estimated using interpolation of the LED-only measurements as described in Section 2.5. An optical rotation for the sample compared to the LED with no cuvette was found in the same way. The difference between the sample optical rotation relative to LED and control optical rotation relative to LED gives the optical rotation for the sample relative to control. This method yielded errors of ∼0.0004° (a 20-fold improvement), which would be less than a 5% error for a sample with an optical rotation of magnitude 0.01°.

To estimate the precision required to distinguish samples with L-abundances 5% different, we consider the optical rotation of 0.005 M 100% L- and 95% L-serine, which are different by 0.01°. Distinguishing them would require each of these measurements to have an error of less than half that, 0.005°. This could also be achieved using the modification described above. However, the ability to detect optical rotation at this precision depends on the concentration detection limit of the instrument, and this presents a more stringent requirement that is farther beyond the capabilities of this instrument. We can estimate the required concentration detection limit by considering the amino acid content in Earth’s polar oceans. The dissolved free amino acid concentration in winter circumpolar deep ocean water is ∼88 mM (Hand et al. 2017). To estimate the optical rotation required to detect these amino acids, we use the mass and optical rotation (at 590 nm) of the most abundant optically active amino acid in aquatic environments, serine (Moura et al. 2013). Assuming an Earth-like enantiomeric excess of 96% and using Equation (1), we find an optical rotation of ∼10−7 deg. In estimating this, we assume that the behavior of the measured optical rotation as a function of concentration is linear up to the detection limit and therefore Equation (1) is valid. Optical rotation measured with the PolarCam is approximately linear up to its detection limit, so with a similar but more sensitive instrument, we might also expect the results to be linear to smaller concentrations.

This more stringent requirement for the sensitivity of the instrument is beyond the capabilities of even current state-of-the-art laboratory instruments. For instance, the Anton Paar MCP-500 Sucromat saccharimeter is capable of measuring 0.0001° optical rotation. Other state-of-the-art instruments obtain polarization errors of 0.0007° (van Harten et al. 2014). However, these laboratory instruments are not easily adapted to a Europa-like temperature and radiation environment or the constraints of a small lander. This motivates our testing of an instrument with minimal active mechanical parts that could be integrated into another lander system like an imager.

There are other possible avenues for improving the sensitivity of the PolarCam that we have not explored. The limited sensitivity of the PolarCam is partly due to its low extinction ratio (∼20–100), as well as nonuniformity in the pixel-to-pixel polarizer characteristics. Improving these aspects of the sensor and polarizer array could be useful in increasing the sensitivity. The temperature was an important factor in the drift of AoLP over time, so further stabilizing the temperature with better heat sinking could be beneficial. Other possibilities for reducing noise are to further reduce the small amount of ambient light during the experiments, averaging more frames, and further reducing the gain while increasing the exposure (Brock et al. 2011, and personal communication).

A decrease in the magnitude of optical rotation of serine in the presence of NaCl has been observed in previous studies (Nostro et al. 2006; Rossi et al. 2007). We found that the addition of relevant salts, including NaCl, decreased optical rotation for serine and phenylalanine, but not significantly. While the salt concentrations tested in this study were relatively low, these results are encouraging as salts can challenge many analytic techniques.

Results from our microbial isolate measurements were equivocal, particularly at low concentrations. This is most likely due to instrument systematic errors or noise. However, it could be due in part to D-amino acid synthesis. While the role of D-amino acid synthesis in prokaryotic cells remains poorly understood, recent studies suggest a variety of cellular regulatory and ecological functions for D-amino acids (Cava et al. 2011; Hernández & Cava 2016). For example, evidence shows D-amino acid synthesis plays a role in regulating cell wall construction, sporulation, or biofilm disruption (Cava et al. 2011), with differences of D-amino acid concentrations potentially reflecting distinct growth conditions (Cava et al. 2011; Radkov & Moe 2014). Thus, the possibility exists to elucidate novel enantiomeric patterns that reflect various ecological conditions. To this end, future work could include measuring enantiomeric patterns at various stages of growth, mixtures of cultures, and environmental analog samples. Additionally, microorganisms respond to environmental conditions. Structures, such as spores, can provide microorganisms with protection under harsh conditions or in nutrient-limited environments, and similar cellular structures may be an important survival strategy on ocean worlds (Robinson & Mikucki 2018). Combining optical measurements of amino acid enantiomeric abundances using a polarization camera with the detection of other biosignatures for ecological status, such as dipicolinic acid, a biosignature for spore formation, could provide multiple lines of evidence for life detection.

5. Conclusions

We used polarized light to measure the optical rotation of serine and phenylalanine for various enantiomeric abundances and concentrations in order to determine the lowest concentration at which a nonzero optical rotation, indicative of a nonracemic mixture of enantiomers, could be detected. We found that concentrations greater than $10^{-8}$ M are needed to detect serine and concentrations greater than $10^{-4}$ M are needed to detect phenylalanine, with larger concentrations needed for smaller enantiomeric excesses. Mixtures of amino acids are well approximated by averages of the optical rotations of the components, weighted by the proportion of the amino acid present in the mixture. Europa-relevant salts do not have a significant effect on the optical rotation. Measurements of bacterial samples were made as proof of concept, showing that their optical rotation can be detected with this technique. Further studies using this approach with a wider range of microbial cells in a diversity of milieus may elucidate ecological patterns of microbial biosignatures. Continued effort to apply high technical readiness level instrumentation to life detection is critical for future exploration of ocean worlds such as Europa.

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Appendix

A.1. Effect of Adding Salts to Amino Acid Solutions

The addition of NaCl to phenylalanine did not significantly change the optical rotation (Figure A1). The addition of NaCl and MgSO₄·7H₂O to serine also did not significantly affect the detection limits for optical rotation (Table A1).

The detection limit for serine with 0.3 M NaCl and with 0.6 M MgSO₄·7H₂O is higher than that without salt, while all of the other detection limits are the same as with no salt added. The higher detection limit of 0.01 M serine with the addition of 0.3 M NaCl and 0.6 M MgSO₄·7H₂O is due to the optical rotations measured at 0.005 M serine being the wrong sign for both. The errors for these measurements are slightly larger than for the others (around 7 × 10⁻³ and 8 × 10⁻³ rather than 6 × 10⁻³) because of slightly more time being taken between measurements (∼100 s longer). However, taking errors into account, the optical rotations of all the measurements taken with salt added overlap.

Figure A1. Absolute value of the optical rotation of 100% L-phenylalanine with 0.6 M NaCl added. Curves shown are linear fits to the data points, passing through (0, 0). Salt does not significantly affect the optical rotation.

Table A1

| Amino Acid      | Salt       | Concentration Detection Limit (490 nm) (M, of amino acid) | Next Lowest Measured Concentration (M) |
|-----------------|------------|-----------------------------------------------------------|---------------------------------------|
| 100% L-serine   | none       | 0.005                                                     | 0.001                                 |
| 100% L-serine   | 0.3 M NaCl | 0.01                                                      | 0.005                                 |
| 100% L-serine   | 0.6 M NaCl | 0.005                                                     | 0.001                                 |
| 100% L-serine   | 1.2 M NaCl | 0.005                                                     | 0.001                                 |
| 100% L-serine   | 0.6 M MgSO₄·7H₂O | 0.01                              | 0.005                                 |
| 100% L-phenylalanine | none       | 0.0005                                                   | 0.0001                                |
| 100% L-phenylalanine | 0.6 M NaCl | 0.0005                                                   | 0.0001                                |
A.2. Bacteria Optical Rotation

The Schw_1 and Easton_1 samples (Figures A2 and A3, respectively) exhibit unusual results, including positive optical rotations and erratic jumps in the magnitude of optical rotation, which are most likely due to instrument noise.

**Figure A2.** Absolute values of the optical rotation of the Schw_1 sample.

**Figure A3.** Absolute values of the optical rotation of the Easton_1 sample.
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