DETECTION LIMITS FOR CHIRAL AMINO ACIDS USING A POLARIZATION CAMERA. C. W. Cook[1], S. Byrne[1], D. Viola[2], C. Drouet d'Aubigny[1], J. Mikucki[3], [1]Lunar and Planetary Laboratory, University of Arizona, Tucson, AZ 85721 (clairec@lpl.arizona.edu), [2]NASA Ames Research Center, Mountain View, CA 94035, [3]Department of Microbiology, University of Tennessee, Knoxville, TN 37996

Introduction: The detection of biosignatures on a planetary surface is of significant scientific interest. Martian ice deposits (especially long-lived ice in the South Polar region) may preserve biosignatures by shielding them from UV radiation and slowing the rate of oxidative reactions common in the martian regolith [1]. High enantiomeric excesses are one such biosignature that a future mission that excavates subsurface ice could test for. Enantiomers are each of the two non-superimposable mirror image configurations of chiral molecules. In biological materials on Earth, the ratio of the L enantiomer to the D enantiomer of amino acids is high, while in abiotic materials, the two are found in approximately equal amounts [2].

High enantiomeric excesses in samples can be detected by their polarizing effects on transmitted light. The optical rotation of a molecule is the angle by which plane-polarized light is rotated when it passes through a sample of the molecule in solution. The two enantiomers of a chiral molecule will have optical rotations with equal magnitude and opposite sign. For abiotic mixtures of two enantiomers, the optical rotations will roughly cancel out. However, in biogenic samples, a net change in optical rotation may be imparted. Polarimetry has thus been proposed as a biosignature detection method [3-5] .

Here, we assess the potential of polarization measurements, specifically optical rotation, to quantify enantiomeric abundances. We determine the minimum concentration of amino acids that can be detected using this approach. Given that in-situ samples would likely include mixtures of several amino acids and other compounds such as salts, we also determine the effect of mixtures of amino acids and salts on the optical rotation.

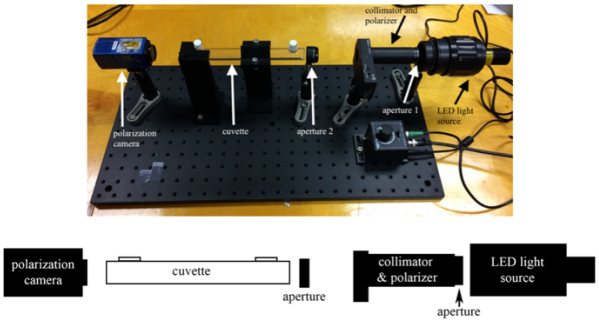

Figure 1: Picture and diagram of the optical set-up. From left to right: PolarCam, cuvette, aperture, collimator and polarizer with aperture on the end, LED.

Methods: The experimental set-up is shown in Figure 1. An LED light source is collimated and directed through a polarizer to create plane-polarized light, followed by a cuvette holding the sample, and the collimated beam is analyzed by a polarization camera (4D Technology's PolarCam Snapshot Micropolarizer Camera).

PolarCam uses a wiregrid polarizer array which contains a pattern of polarizers with 0, 45, 90, and 135 degree polarizations that together form a super pixel that is repeated over the array (Figure 2).

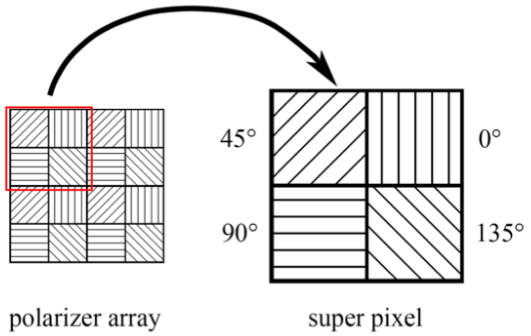

Figure 2: Diagram of a portion of the polarizer array and the arrangement of a single super pixel, based on [6].

We investigated two amino acids: serine (with a specific optical rotation at 590 nm of -6.83° [7]) and phenylalanine (with a specific optical rotation at 590 nm of -35.1° [8]). At shorter wavelengths, the specific optical rotation is higher [9] so we measured the optical rotation at 490 nm in addition to some measurements at 590 nm. Solutions of a single amino acid were measured for a range of enantiomeric abundances. In addition, some measurements were made with mixtures of serine and phenylalanine, with varying enantiomeric abundances. To determine the effect of salts on the optical rotation, measurements were also made with sodium chloride (NaCl) or magnesium sulfate heptahydrate (MgSO4 • 7H2O) added to the amino acids. In each case, the stock solution with the amino acid(s) dissolved in water was serially diluted to produce solutions for a range of concentrations. Control measurements of pure water were taken before and after sample measurements.

For each sample and control measurement, we found the Angle of Linear Polarization (AoLP) by combining elements of each super pixel, then the average AoLP over all super pixels on the detector. We mitigated systematic errors which caused the AoLP to drift over time, 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 subtracted the AoLP of the sample from this control AoLP to get the optical rotation of the sample. Figure 3 shows an example of the optical rotations obtained in this way for phenylalanine.

As concentration is reduced, the optical rotation shrinks to the detection limit and subsequently becomes noise with as many positive as negative results. Similarly as enantiomeric excess is reduced, larger concentrations are required for detection until the abundance of L-enantiomers is 50% (at which point these amino acids are undetectable with this method regardless of their concentration). We take the detection limit for optical rotation as the lowest measured concentration for which 1) the optical rotation plus or minus its error bars never crosses zero, 2) the optical rotation has the expected sign, and 3) every higher-concentration measurement satisfies conditions 1 and 2.

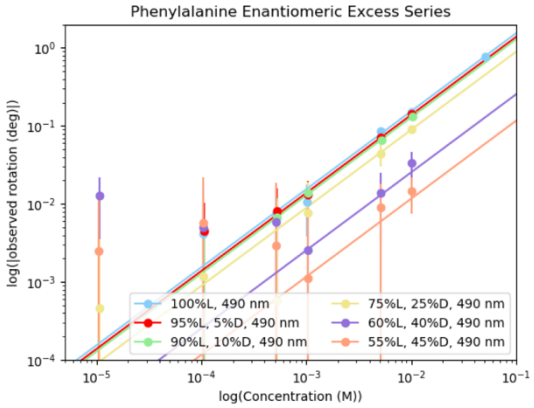

Figure 3: Absolute value of optical rotation of phenylalanine for various L-enantiomer abundances and concentrations. Curves shown are linear fits to the data points, passing through (0,0).

We also measured the optical rotations of three bacteria samples: *Marinobacter gelidimuriae* isolated from subglacial brine from Blood Falls, Antarctica [10], as well as a sample we denote Schw_1, from the glacial surface at Blood Falls, Antarctica, and a sample we denote Easton_1, isolated from snowpack on Easton Glacier, WA, USA.

Results: The detection limit for serine for 100% L is 0.005 M (moles/liter), decreasing to 0.05 M for 45% L. For phenylalanine, the detection limit for 100% L is 0.0005 M, decreasing to 0.01 M for 45% L.

As expected, the concentration detection limit for lower enantiomeric abundances is higher. In addition, detection limits for solutions with some L-enantiomer abundance and the equivalent D-enantiomer abundance are generally the same, as expected.

The optical rotations of the mixed amino acids are consistent with a linear combination of the optical rotations of the components. As expected, because phenylalanine has a higher specific optical rotation, the solutions with a higher proportion of phenylalanine have a higher optical rotation and lower detection limit. Adding salts does not significantly affect the optical rotation.

For concentrations $>10^6$ cells/mL, the optical rotation of *Marinobacter gelidimuriae* is detectable and negative, as one would expect for bacteria containing a majority of amino acids with negative optical rotations. Schw_1 and Easton_1 were detectable at ~10^8 cells/mL, but unusual results were found at lower concentrations, possibly indicating that the errors were large and the method was not effective at lower concentrations.

Discussion: Using this instrument, solutions with enantiomeric abundances 5% apart (corresponding to enantiomeric excesses 10% apart) are rarely distinguishable through their polarization effects. Even when they are, 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 detection limits that we found, the enantiomeric excess could not necessarily be determined to a high accuracy.

Biosignature detection via its polarizing effect on transmitted light offers a convenient and fast method to evaluate icy samples for more detailed analysis. However its detection thresholds are relatively high compared to other methods such as gas chromatography-mass spectrometry with chirality analysis.

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