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Methodologies and Techniques for Detecting Extraterrestrial (Microbial) Life

Why Raman Spectroscopy on Mars?—A Case of the Right Tool for the Right Job

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ABSTRACT

We provide a scientific rationale for the astrobiological investigation of Mars. We suggest that, given practical constraints, the most promising locations for the search for former life on Mars are palaeolake craters and the evaporite deposits that may reside within them. We suggest that Raman spectroscopy offers a promising tool for the detection of evidence of former (or extant) biota on Mars. In particular, we highlight the detection of hopanoids as long-lived bacterial cell wall products and photosynthetic pigments as the most promising targets. We further suggest that Raman spectroscopy as a fibre optic-based instrument lends itself to flexible planetary deployment. Key Words: Mars exploration—Raman spectroscopy—Astrobiology—Robotic exploration. Astrobiology 3, xxx–xxx.

INTRODUCTION

ALL ROBOTIC ASTROBIOLOGY MISSIONS to Mars, both past and planned, have focussed on exploring the surface or the near surface with little consideration of the issue of targeting sampling sites with the highest astrobiological potential. We present an argument that any such future astrobiology mission should employ Raman spectroscopy as the primary instrument. Raman spectroscopy is particularly suited to the detection of biosignatures—these may be defined as macroscopic molecules directly linked to biogenic metabolism or other cellular functions (Schulze-Makuch *et al.*, 2002).

Raman spectroscopy is based on the inelastic scattering of incident monochromatic laser light from a target, with the scattering being dependent on the molecular vibrational and rotational in molecules of the sample. Raman spectroscopy detects the vibrational states of molecules, with these states being determined by the molecule's structure (i.e., the bond types and other structural factors of organic molecules). The scattered Raman signal makes up $\sim 10^{-6}$–10^{-8} of the incident energy. The transmitted laser light is shifted in frequency—the incident photons gain or lose energy. The Stokes lines are shifted to lower frequencies than the illumination source generated by molecular ground states of the sample, while anti-Stokes lines are shifted to higher frequencies generated by excited vibrational states. Within

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the Raman spectrum, the dominant Rayleigh scattering line is normalised to a wavenumber of 0 so all Raman lines are relative. The detected light is passed through a grating and separated into different wavelengths, which impinge on different pixels of a charge-coupled device (CCD) detector. It is the distribution of intensity as a function of wavelength that gives a compound's so-called "Raman spectrum." This vibration spectrum is centred on the dominant elastic Rayleigh scattering frequency. The Raman spectrum of a given compound consists of a unique fingerprint of all its atoms, groups, and bonds and their interactive effect (stretching, deformation, rotation) on each other. Raman spectroscopy does not require sample preparation and offers fibre-optic delivery, cleaner spectra than that obtainable from infrared spectrometry, and a range below 400 cm^{-1}, which provides for identification and differentiation of organic and inorganic species.

Raman spectroscopy offers the prospect for compact and robust instrumentation and lends itself to a distributed instrument comprising two parts, which may be physically separated—a small sensor head probe and a separate CCD-based spectral analyser connected by a tether to carry electrical power and optical fibre data.

THE MARTIAN ENVIRONMENT

The average atmospheric pressure on Mars lies between 6 and 10 mbar, close to the triple point of water (at 273 K and 6.11 mbar) (Pollack and Yung, 1980). At latitudes between ±30°, the temperatures range from 170 to 290 K, rising above 0°C at mid-day during the summer. At martian atmospheric pressure, ice is unstable and sublimes to vapour below latitudes of 40°. It is expected, on the basis of geophysical models (Carr, 1987), that such regions are depleted of water ice above a 500 m depth. At higher latitudes of 40–80°, however, water ice is stable at depths exceeding 1 m. The cryosphere (subsurface region impregnated with water ice) has a thickness of 2.5 km at the equator and 6 km at the poles, below which geothermal energy is sufficient to melt the permafrost into liquid (Clifford, 1993). It is conceivable that such depths could support chemolithotrophic organisms (Boston *et al*., 1992). Most chemosynthetic energy mechanisms are aerobic in nature and will be reliant on the co-evolution of photosynthetic organisms—however, as pointed out by Boston *et al.* (1992), methanogens use carbon dioxide as an oxidant and could prosper beneath the surface. The Beagle 2 Lander of the European Space Agency's Mars mission will be searching for indirect evidence of such extant biota through the detection of emissions of reactive gases such as H_2, H_2S, CH_4, NH_3, HCN, NO_x, and SO_x. The depths at which liquid water would exist are inaccessible to current technology for direct analysis.

The observation of water-cut fluvial valleys and outflow channels suggests that liquid water once flowed on the martian surface in earlier epochs (Carr, 1986, 1987). An oft-postulated martian hydrological model suggests four epochs of hydration on Mars with equivalent habitats in Antarctica (Clifford, 1993; Wynn-Williams, 1999):

(i) The first (Noachian) hydrological cycle ~4.2–3.8 Ga ago with abundant surface and subsurface water and characterised by life originating and evolving to photosynthetic cyanobacterial colonies in surface waters and riverbeds
(ii) The second hydrological cycle ~3.8–3.1 Ga ago with surface water restricted to ice-covered, hypersaline lakes with benthic cyanobacterial stromatolites (calcified deposits from microbial mats) beneath the ice, similar to those in Lake Hoare, Antarctica
(iii) The third hydrological cycle ~3.1–1.5 Ga ago with water restricted to moisture in porous rocks as in the translucent Beacon sandstone of Victoria Land, Antarctica, which force desiccation-tolerant cyanobacterial communities to near-starvation conditions
(iv) The fourth (Amazonian) hydrological cycle <1.5 Ga ago to the present time, characterised by the desertified of the martian surface underlain by permafrost, possibly with anhydrobiotic microbes and deep chemolithotrophic life at depths of 2–6 km beneath the surface

The McMurdo Dry Valleys of Antarctica represent the closest terrestrial analogues of the martian surface with an mean annual temperature of −20°C and an average annual precipitation of <10 mm. There are two major biological communities in the deserts of Antarctica—cryptoendolithic communities, which survive in the highland regions, and algal mats, which survive within ice-covered lakes (Friedmann, 1986). Such

communities may have direct analogues on Mars. Of particular interest is the possibility that ice-covered lakes may have resided on Mars for ~500 Ma after the ambient temperature dropped below freezing—such environments would resemble perennially ice-covered lakes in the Antarctic Dry Valleys, which contain dissolved atmospheric gases at elevated concentrations above that for equilibrium with the atmosphere (McKay, 1991). On Mars, the loss of the martian atmosphere and gradual cooling would have caused drying up of the early surface water—extreme dehydration of any cyanobacteria would require increasing amounts of water-replacement molecules such as trehalose to maintain protein structures. Trehalose is often manufactured as a water-replacement molecule to maintain structural and functional integrity of proteins and membranes in cyanobacteria under desiccation conditions (Potts, 1999). However, such water-replacement molecules are not long-lived in sediment. Salts would have accumulated as water was gradually lost. As temperatures dropped below freezing, martian ice-covered lakes would resemble the perennially ice-covered lakes of the McMurdo Dry Valleys of Antarctica such as the glacially dammed 4 km × 1 km Lake Hoare, which has a temperature close to 0°C but which supports microbial communities including benthic cyanobacterial mats (Anderson *et al.*, 1995). These lakes would eventually become hypersaline like the lakes of the Antarctic Dry Valleys, including the Don Juan Pond, which consists of saturated calcium chloride solution that does not freeze until −53°C. Clifford (1993) described how groundwater that resides in the Earth's crust for approximately Ma generally evolves into highly mineralized brine (a saturated mixture including chlorides, carbonates, and sulfates) and suggested that this process would probably be accelerated on Mars by the influx of minerals leached from the crust by low-temperature hydrothermal circulation. The hypersalinity of such lakes prevents water convection so the bottom waters can maintain temperatures of up to 25°C relative to the freezing surface. The ice-covered lakes accumulate hypersaline bottom water leached from the soil minerals. Viable but dormant bacteria can survive within environments such as Don Juan Pond. Lake Vanda has bacterial mats living within water with ~113 g L^{-1} of dissolved salts. The increased concentration of dissolved salts would require the manufacture of the antifreeze glycine betaine by cyanobacteria to reduce osmotic stresses, though it is costly to manufacture. Such communities are often the source of stromatolitic deposits. Ice-covered lakes may have survived on Mars until ~3 Ga ago (Wharton *et al.*, 1989). Such lakes would have survived until the ambient temperature dropped below −35°C, when the lakes would have frozen solid and subsequently ablated. Martian palaeolakes with lacustrine and evaporite sediments forming smooth, level, featureless plains within basins offer possible reserves of biotic fossils while affording high trafficability and few obstacles to a mission landing (De Hon, 1992). Those palaeolakes that are in proximity to drainage systems such as outflow channels are the most favoured targets, especially within crater basins—palaeolake craters. Those that lie at the terminal portion of an outflow channel are the most likely to possess evaporite deposits (e.g., the Gusev crater, which lies at the terminus of 700 km long, 15 km wide Ma'adim Vallis). Palaeolake beds are typically large in extent and readily targeted by modern landers, which tend to have large landing error ellipsoids.

SITE SELECTION

Landing sites for Mars missions must satisfy certain engineering constraints to ensure a safe landing yet offer scientifically interesting targets:

(i) There must be sufficient atmosphere for parachute descent.
(ii) There must be sufficient sunlight for solar power generation.
(iii) There must be few rocks greater than 0.25 m in diameter distributed around the landing region.
(iv) Regions of high radar reflectivity indicative of significant dust / regolith depths should be avoided lest the lander should be buried too deeply.

There are several possible locations for former or even extant life on Mars, not necessarily exclusively (Westall *et al.*, 2000):

(i) Regions where water existed for significant periods of time, such as palaeolakes and water-cut channels including ancient dry lakebeds with evidence of sedimentary layering (Doran *et al.*, 1998)

(ii) Hypersaline brines, as found in Antarctic Dry Valley lakes, or evaporite deposits indicative of salt mineral deposition in water (Rothschild, 1990)
(iii) The permafrost/water interface, which may potentially harbour life associated with glacial deposits such as the Argyre Basin at the South Pole
(iv) Localised hydrothermal regions at shallower depths, perhaps as shallow as 500 m (Walter and Des Marais, 1993), possibly created by localised volcanic activity—such localised water deposits could persist as liquid for as long as 10–100 Ma to support a limited ecology similar to the earliest terrestrial life based on hyperthermophilic organisms (McKay, 1991)
(v) Impact craters, which are another possible source of hydrothermal heating, particularly as sites of lakes from catastrophic outflows that could have persisted for 10,000–100,000 years

Terrestrial polar ice and permafrost can yield viable ancient freeze-dried bacteria of approximately Ma old and, of course, gases such as methane, ammonium, hydrogen sulphide, sulphur oxides, and nitrogen oxides, perhaps in clathrate form. Some populations of microbes may still be preserved freeze-dried in a dormant state in subsurface permafrost of Mars. Viable but hypometabolic prokaryotic organisms have been recovered from Siberian permafrost up to 3–5 Ma old (Vorobyova *et al*., 1997). Permafrost is a constant and stable habitat for bacterial communities, especially methanogenic bacteria, which have been the primary biota recovered and cultured from such sites. Regions of terrestrial permafrost can be ~0.5–1.0 km in thickness with an approximately constant temperature gradient of 1.5°C temperature drop per 100 m depth, reaching −10 to −12°C in Arctic permafrosts and −25 to −30°C in Antarctic permafrosts. Typical recovered permafrost cell counts have been $\sim 10^6$–10^9 cells/g dry weight with a viable cell count of $\sim$10–10^{-7} cells/g dry weight. Viable cell densities vary significantly, with higher viable cell fractions associated with Arctic permafrosts (0.1–10%) than with Antarctic permafrosts (0.001–0.01%). Nonetheless, this indicates the possibility of long-term survival over $\sim 10^6$ years of viable, culturable biota from permafrost.

The most promising sites for the recovery of biota on Mars are evaporite deposits. Evaporites are mineral salt deposits that precipitate from aqueous solution and incorporate inclusions of fluid during precipitation, which often capture ambient microorganisms and biomolecules. Indeed, evaporite deposits offer the most likely sites for the recovery of viable and dead bacteria (Rothschild, 1990). Evaporites are characterised by salt deposits such as halites (NaCl), gypsum ($CaSO_4$), and carbonates (Farmer, 1998). Soda lakes on Earth are an example of phosphor-rich environments in which the precipitation of phosphate salts is partly aided by biogenic processes—plankton take in phosphorus for skeleton building, which is eventually deposited as sediment onto the seafloor. However, phosphate deposits on Mars are not known to exist. Evaporation of lakes causes the deposition of soluble salts that are often inhabited by halophilic bacteria, which can be preserved in silica or barite minerals. Evaporites formed from seawater exhibit a characteristic order based on the least soluble fraction precipitating first: calcium and iron carbonates, calcium sulphate, halite, magnesium and potassium chlorides, and finally sulphates. They are characterised by carbonates at the periphery of the deposit with an increase in deposited chlorides and sulphates towards the centre. Catling (1999) suggested that Martian evaporites will exhibit a characteristic deposition sequence of $FeCO_3$ (siderite), $Si(OH)_4$ (silica), $CaCO_3$ (calcite with some $MgCO_3$), $Mg_4(CO)_3(OH)_2$ (hydromagnesite), $CaSO_4 \cdot 2H_2O$ (gypsum), and highly soluble salts such as NaCl and Na/Mg sulphates. The deposition of siderite would be diagnostic of a high atmospheric pressure ~0.1 bar during deposition—lower pressures would induce the deposition of Fe silicates such as greenalite [$Fe_3Si_2O_5(OH)_4$] due to the equilibrium reaction: $3FeCO_3 + 2SiO_2 + 2H_2O \Leftrightarrow Fe_3Si_2O_5(OH)_4 + 3CO_2$. On Earth, halobacteria are restricted to halite inclusions and cannot survive in bittern brines (chloride salts) (Grant *et al*., 1998). Evaporites could similarly have formed on Mars—the ubiquitous duricrusts are believed to be dominated by salts such as NaCl, $MgSO_4$, and $CaCO_3$ mixed with Fe and Al oxides (Clark and van Hart, 1981). Hypersaline environments—evaporite deposits in particular—provide an extreme habitat for halophilic bacteria. Halophiles can survive high salt concentrations because of their proteins having high proportions of charged amino acids for tight binding to water. Halophiles such as *Halococcus salifodinae* can survive up to 25–30%

NaCl solution and exhibit high longevity, metabolising at a low rate (Stan-Lotter *et al.*, 2001). Halophiles are Archaea with ether-linked isoprenoid lipids in their cell membranes, a distinguishing characteristic of Archaea. Halobacteria are the dominant population in hypersaline waters and often impart a red colouration to such deposits due to their possession of red pigments of bacteriorhodopsin, which they employ to capture solar radiation for photosynthesis. The red pigment is based on C_{50} carotenoid, which would be detectable through Raman spectroscopy. Deeply buried evaporite deposits could be brought to the Martian surface by water flows. Halophilic bacteria can survive within brine inclusions in salt crystals for almost a year as demonstrated experimentally though their viability over geological time scales is suspected. There is evidence that viable bacteria (*Bacillus permians*) can survive within fluid inclusions of buried halite crystals up to 250 Ma (Vreeland *et al.*, 2000). However, it is currently not clear whether such bacteria were direct descendents of an earlier trapped population, or whether they are in fact later contaminants, but evidence is accumulating in favour of the former (McGenity *et al.*, 2000). Indeed, entrapment of living halobacteria within fluid inclusions may affect the physical morphology of the inclusion, making it larger and thus more amenable to probing by techniques such as Raman spectroscopy (Norton and Grant, 1988). At the least, examination of the fluid within fluid inclusions offers insights into the climatic conditions during their deposition. "White Rock," which lies within an 80–km-diameter crater in the Sinus Sabaeus region at 8°S 335°W on Mars, is believed to represent a possible evaporite deposit. Generally, however, evaporite deposits are associated with lacustrine environments with salt deposits of sulphates and halides deposited during lake evaporation.

Although studies have shown significant survival rates of some bacteria from the *Bacillus* and *Clostridium* genera, under simulated martian conditions for long periods (Koike *et al.*, 1995), we suggest that the presence of viable biota on the present-day martian surface is unlikely to be due to the lack of liquid water availability currently on Mars (McKay and Stoker, 1989; Clark, 1998). Alternatively, their fossils buried in stromatolitic sediments may have been flushed onto or near the surface by relatively recent transient hypersaline flows. The first priority is to investigate sedimentary deposits of chert, carbonates, silicified, or evaporite (e.g., phosphates) deposits to maximise the possibility of detecting biotic fossils.

Fossilisation and the preservation of organic matter are enhanced under anoxic conditions, rapid burial rates, rapid desiccation, hypersaline conditions, and subzero temperatures. Much organic material on Earth, especially lipid-rich polymers, decays into kerogens, stable macromolecules of aliphatic and aromatic structure. Most of the organic material is broken down into "kerogen," an amorphous mixture of straight- and branched-chain hydrocarbons, lipid-based steroids, and hopanoids. Hopanoids are lipid hydrocarbons of the form C_{3x} with a primary pentacyclic hopane skeleton of four cyclohexane rings and one cyclopentane ring—2α-methyl hopanes of the C_{31}–C_{35} form are indicative of cyanobacteria. Hopanoids are prokaryotic membrane lipids with similar functions to sterols like cholesterol (with C_{27} form, which require aerobic conditions for their synthesis) in eukaryotic cells—both determine the permeability and rigidity of the cell wall. Hopanoids are often produced in high-stress environments, and they are not known to form abiotically. Hopanoids are readily detected by Raman spectroscopy (Fig. 1).

F1

There are a number of potential signatures all within the terpenoid hydrocarbon class (defined by the basic C_5 isoprene structural unit) that can provide distinctions between major kingdoms—hopanoids indicate eubacterial (including cyanobacteria but excluding purple and green sulphur bacteria) origin, acyclic steroids indicate eukaryotic origin (but also in mycoplasmas), and isoprenoids indicate archaebacterial origin. Green and purple sulphur bacteria lack hopanoids but possess distinctive aromatic carotenoid pigments. Archaebacteria possess ether lipids that incorporate a number of distinctive isoprenoid chains such as C_{21}–C_{25} isomers. Higher plant and green algae steroids can be characterised by steroids with 24-ethyl groups, while cholesterol is specific to metazoa and red algae. Terpenoid hydrocarbons are resistant to thermal degradation and have been found in abundance in Proterozoic deposits on Earth as early as 2.5 Ga ago (Summons and Walter, 1990). The detection of macromolecules such as kerogen or oligonucleotides on Mars would be evidence for the presence of biogenic processes.

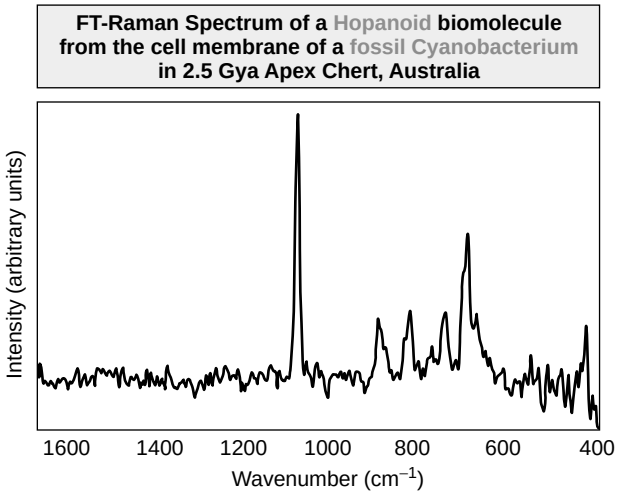

FIG. 1. Fourier transform-Raman spectrum of a hopanoid biomolecule from the cell membrane of a fossil cyanobacterium in 2.5-Ga-old apex chert from Australia.

NATURE OF MARTIAN BIOTA

Early terrestrial life used chemosynthetic sources of energy from redox gradients due to Fe sulphides under anoxic conditions (Russell and Hall, 1999). They were most likely sulphur-metabolising hyperthermophiles like *Pyrolobus*, which inhabits deep-sea hydrothermal vents at tectonic margins on the seafloor and can grow in temperatures of up to ~113°C (Nealson, 1997). However, the migration of life to the Earth's surface and the evolution of photosynthesising cyanobacteria were rapid. One estimate for the evolution of cyanobacteria from the prebiotic condition is $\sim 7 \times 10^6$ years (Lazcano and Miller, 1994). This included bottlenecks imposed by the origin of self-replication and the emergence of protein biosynthesis before and after the RNA world, respectively, and the constraint of complete recycling of the ocean through prebiotically destructive deep-sea vents every 10^7 years. If this is accurate, or even a vast underestimate, there was sufficient time for the evolution of photosynthetic cyanobacteria on Mars during the period 4.0–3.5 Ga ago. We hypothesise that life on Mars expanded into illuminated surface habitats, and that the selective pressure of solar radiation on evolution would have driven the original microbiota to develop photosynthetic and UV-protective pigments to harness the ubiquitous and energy-efficient photosynthetically active radiation (PAR), which lies in the spectral range 400–750 nm. The current irradiance of PAR on the martian surface is 6.5 μmol m^{-2} s^{-1} compared with 1,500 μmol m^{-2} s^{-1} for the Antarctic Dry Valleys—this would have been depressed by 30% to 45 μmol m^{-2} s^{-1} 3.5 Ga ago because of the Sun's reduced luminosity (Boothroyd *et al.*, 1991). This would still be sufficient for the survival of cyanobacteria through photosynthesis—on Earth, microbial mat communities are exposed to only 1.5 μmol m^{-2} s^{-1} of PAR. The Dry Valley lakes of Antarctica are overlain by 3–4.5-m-thick ice, which permits solar radiation penetration to 70 m depth [e.g., Lake Hoare permits PAR penetration to cyanobacterial communities of 15 μmol m^{-2} s^{-1} (Wynn-Williams and Edwards, 2000a,b)]. Photosynthesis is the most efficient energy extraction process used by life, and solar flux on Mars would represent the most bountiful source of energy. Photosynthetic bacteria utilise inorganic chemicals for electron transport to extract energy. Any primitive photosynthesis requires the ability to transfer electric charge across a membrane—this implies the need for a porphyrin pigment, a quinone electron donor, and a carotene electron acceptor. Fe^{3+} may be an important energy source for early forms of photosynthesis—it can act as an UV screen as exhibited by *Thiobacillus ferrooxidans*, which secretes waste Fe^{3+} as a sheath around its cell. Early terrestrial photosynthesis was based on anaerobic metabolism—green photosynthetic bacteria (e.g., *Chloroflexus*), purple sulphur bacteria (e.g., *Chromatium*), and non–purple sulphur bacteria (e.g., *Rhodopseudomonas*)—but these forms of photosynthesis are much less efficient than oxidative photosynthesis characterised by cyanobacteria (e.g., *Nostoc*). Cyanobacteria are the dominant photosynthetic organisms in the Antarctic Dry Desert Valleys, which are the harshest environments on Earth.

Cyanobacterial mat communities use a number of techniques for survival in extreme environments such as the use of mycosporine-like amino acids and the sunscreen pigment scytonemin (Wynn-Williams and Edwards, 2000b). Communities under extreme stress, as in hot and cold deserts, adopt a stratified community structure and avoidance strategies for survival with the upper layers composed of dead cells. To protect their DNA from damaging solar UV radiation, cyanobacteria also utilise pigment molecules. Photosynthetic cyanobacteria in polar environments harness PAR at 400–750 nm and protect

themselves against UVB radiation at 280–320 nm. DNA absorbs UVB near its absorption maximum at 254 nm, which is particularly harmful. UVB radiation is also damaging to membrane lipoproteins, and photosynthetic pigments. On Earth, the ozone layer absorbs the most damaging UV radiation below 260 nm, but on Mars, carbon dioxide absorbs only wavelengths under 190 nm, which permits the surface flux of damaging UV to the surface as its ozone layer column density is only 2% that of Earth. Cyanobacteria would have been restricted to surface and near-surface zones penetrated by PAR and UV radiation. Cyanobacteria produce organic pigments, which are used for functions of both photosynthesis and protection by trapping visible wavelengths while screening against UVB radiation (Wynn-Williams *et al.*, 2001). Such pigments have characteristic aromatic ring structures, which provide the basis for photon absorption [e.g., the quinone ring, which has a distinctive Raman feature at 1,659 cm^{-1} (Wynn-Williams *et al.*, 2002a)]. Pigments are vital to the survival of any surface microbiota on a terrestrial-type planet of the inner Solar System.

Sunscreen pigments allow cyanobacteria to trap light and direct it to chlorophyll, giving them the capability to survive with very faint illumination levels beneath the ice-covered lakes and translucent rock in Antarctica (Wynn-Williams *et al.*, 2002b). Scytonemin is a sunscreen pigment that is secreted as part of the outer protective sheath of many ancient cyanobacterial species, which resists degradation and would be an excellent biosignature for cyanobacteria. Such colonies would result in a stratified biofilm. Such biofilms typically contain high concentrations of photosynthetic and UV-protective pigments with distinctive functional molecular structures produced as a response to environmental stress. The occurrence and spatial distribution of preserved pigments or their derivatives in the near-subsurface profile beneath the oxidised zone of Mars should be detectable *in situ* by nondestructive laser Raman spectroscopy, as on Earth. Both photosynthetic and UV-protective pigments generate distinctive Raman spectra, independent of their hydration state. Microbial communities in extreme Antarctic desert habitats analogous to those of early Mars indicate that these key biomolecules can be detected *in situ* within the biochemical pool of mixed populations (Edwards *et al.*, 1997, 1999). Photosynthetic cyanobacteria that are dominant in extreme Antarctic desert habitats near the limits of life on Earth are good sources of pigments and other biomolecules that could act as analogues of martian biosignatures for former life on Mars. Other biosignatures are possible. The cyanobacteria *Nostoc*, and other cyanobacteria, produce the UV-pigment scytonemin within an external mucilage sheath, which absorbs both UVB (280–320 nm) and UVC (190–280 nm) radiation (with a characteristic Raman band at 1,590 cm^{-1}) (Fig. 2). Scytonemin is particularly long-lived in sediment and would act as a specific cyanobacterial marker. UV avoidance by living in stratified habitats requires accessory pigments to chlorophyll (with absorption maximum at 640 nm) such as phycocyanin (with absorption maximum at 560 nm) to harvest low levels of photons.

F2

Microorganisms under stressed conditions of low temperature and dryness have retreated into porous rock as cryptoendoliths, especially translucent rock such as sandstone—all endolithic communities (which usually include lichens, fungi, and algae) require photosynthetic cyanobacteria to fix inorganic carbon. The rocks are warmed by solar insolation to temperatures above that of the ambient atmosphere. Such cryptoendolithic organisms within Antarctic rocks illustrate the capacity of life to survive under cold, dry conditions utilising the scarce capillary wa-

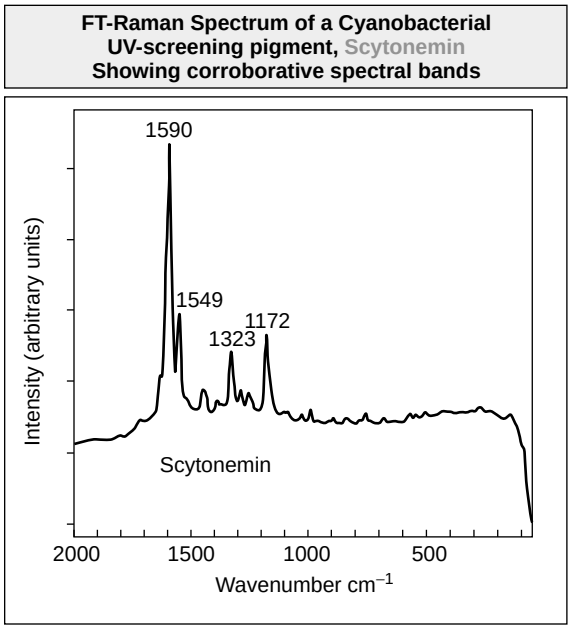

FIG. 2. Raman spectrum of the cyanobacterial UV-screeening pigment scytonemin showing corroborative spectral bands.

ter trapped within the rock while living at the limits of their physiological adaptability (Friedmann and Weed, 1987; Freidmann and Koriem, 1989). These communities would be difficult to detect using metabolic tests such as those adopted on the Viking Mars lander of 1976. These communities leave a characteristic leaching pattern of Fe oxyhydroxide coatings on the sandstone grains even once all fossil organic material has become silicified (Friedmann, 1986). Such biota may be representative of life on Mars—frost forms on the rocky surface of Mars for half of the year, indicating that liquid water may be available within the pore spaces near rock surfaces. As the climate degraded and water receded on Mars, any indigenous surface life that may have existed on early Mars would have retreated into porous rock as endoliths (Edwards and Newton, 1999; Wynn-Williams and Edwards, 2000a). Cyanobacteria inhabit the Earth's harshest environments such as the Dry Desert Valleys of Antarctica within sandstone rocks and soil. Sandstone is sufficiently translucent for light to penetrate for their photosynthesis—the cyanobacterium *Chroococcidiopsis* lives ~8 mm beneath the surface of sandstones in the interstitial spaces between quartz minerals. *Chroococcidiopsis*, which is an endolithic cyanobacterium, utilises accessory pigments such as phycocyanin (with a characteristic Raman spectrum) to trap sparse photons to augment chlorophyll. Endolithic cyanobacteria metabolise at a reduced rate from their normal unstressed condition—photosynthetic and respiratory activity may be separated by as much as 10^4–10^5 years. Metabolism is essentially limited by the small amount of liquid water available in such environments, but they can be readily detected by Raman spectroscopy (Fig. 3). The anti-oxidant pigment β-carotene acts to screen UV radiation by absorbing UVA (320–380 nm) and UVB (280–320 nm) radiation (with a characteristic Raman band at 1,524 cm^{-1}). Raman spectroscopy can detect fossilised residues of such pigment molecules as biosignatures of ancient cyanobacteria. The overall Raman spectrum of such deposits not only reveals organic matter and the composition of its mineral substratum, but also reveals the nature of biomolecules present and their potential function (such as UV absorption). For this reason, we favour shallow searches for the remains of photosynthetic bacterial and cyanobacterial communities rather than deep drilling for chemolithotrophic bacteria.

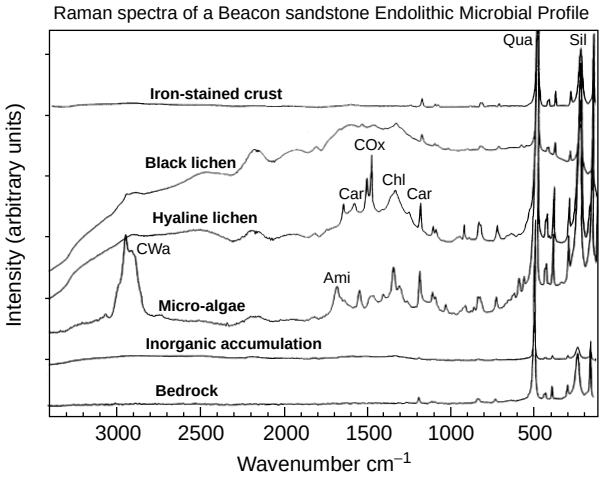

FIG. 3. Raman spectrum of a Beacon sandstone endolithic microbial community.

We suggest that the search for evidence of former martian life should be for biomolecular species with molecular structures that define their functionality, with such functionality being fundamental to all terrestrial microorganisms. The derivatives of photosynthetic and UV-protective pigments are well defined. Chlorophyll degrades to porphyrins, carotenoids (such as bacteriorhodopsins or β-carotene) degrade into isoprenoids (derived from ether lipids), and cell membranes degrade into hopanoids (derived from bacterio-hopanetretol), all of which can act as long-lived fossil biosignatures stable to degradation that are also recognisable by the Raman spectra of their molecular characteristics. Hopanoid derivatives from cyanobacterial cell walls have been identified from relict stromatolite deposits of photosynthetic cyanobacteria in 2.5-Ga-old Precambrian chert (Summons *et al.*, 1999) when there would still have been liquid water on Mars (Table 1).

Hopanoid biosignatures of photosynthetic cyanobacteria have been detected by Raman spectroscopy (Wynn-Williams and Edwards, 2000b). The distinctive Raman spectra of hopanoids and pigments add to their suitability as biosignatures. By definition, their near-surface location also makes analysing them by a nonintrusive system such as Raman spectroscopy technologically feasible. Such evidence of surface biota may remain preserved in stratified sedimentary layers beneath the oxidised zone of the near-subsurface martian regolith. Hence, the detection of hopanoids and other related biomolecules would

TABLE 1. TIME SCALES FOR MICROBE AND BIOMOLECULAR INTEGRITY

Microbe/biomolecule	*Location*	*Period*	*Time scale (Ma)*
Fossil cyanobacteria?	Apex chert, Pilbara, Australia	Precambrian	3,500
Cyanobacterial hopanoids	Apex chert, Pilbara, Australia	Precambrian	2,500
Porphyrins	Siberian shales	Cambrian	~550
Chlorobiaceae carotenoids	Vena-del-Gesso, Italy	Ordovician	~450
Halobacteria	Cleveland potash, UK	Permian	270
Isoprenoids	Lower Albial shale, France	Cretaceous	100
Viable bacteria	Mt. Feather permafrost, Antarctica	Holocene	~3

provide unequivocal evidence of former biotic activity—furthermore, each biomolecular type yields unique Raman spectra. The evolutionarily ancient endolithic cyanobacterium *Chroococcidiopsis* exhibits a Raman spectrum including the signatures of carotenoids, hopanoids, and isoprenoids. These pigments are also induced in the cyanobacterium *Nostoc* when exposed to UV radiation. For the search for fossilised biota, the Raman spectrometer is currently one of the instruments of choice in palaeobiology (Schopf, 2002). Raman spectroscopy has been used extensively in the study of Antarctic samples of cryptoendolithic microorganisms (Russell *et al.*, 1998; Newton *et al.*, 2000).

RAMAN SPECTROSCOPY

There is a need for a scientific instrument to characterise *in situ* the microstructure and inorganic composition of potential microbial habitats on Mars with concurrent analysis of molecular components of organic material and biomolecules in particular, without any preparation or prior identification of compounds. Specific requirements are shown in the following list:

(i) Nonintrusive *in situ* analysis of the microstructure and inorganic composition of potential microhabitats for microbial life
(ii) Nonselective analysis of organic compounds in near-surface substrata below the oxidised regolith zone without any extraction or preparation
(iii) Diagnosis of biomolecules from their functional components (e.g., aromatic compounds, porphyrins, isoprenoids, carotenoids, oxalates, trehalose, etc.), without prior knowledge of the identity of compounds
(iv) Diagnosis of focused strata of biomolecules (e.g., light-constrained pigments in the profile of palaeolake sediments) in spatially dispersed cores/samples to minimise the chance factor of heterogeneity
(v) Spectrum from 200 to 4,000 cm^{-1} covering most vibration modes including organics [e.g., ν(CH) band near 3,000 cm^{-1}] and metal oxides
(vi) Emphasis on ecological function of component parts (e.g., UV-absorbing rings for former surface microbial survival) in environmentally challenging conditions
(vii) Establishment of a database of terrestrial microbial biomolecules (especially photosynthetic and photoprotective pigments from potentially analogous primitive microbes on Earth) (e.g., photosynthetic bacteria and cyanobacteria)
(viii) Simultaneous microscopic imagery of the habitat structure and any fossil or preserved microbes
(ix) Proven suitability for detection of similar analogues in Antarctic samples
(x) Capacity for miniaturisation of the instrument with a low power requirement for a Mars lander or rover mission

Raman microspectroscopy fulfils these requirements. Raman spectroscopy can be used to definitively identify rock-forming minerals and organic species on the surface or in the subsurface of planetary bodies. It is an active technique, and the laser power is set as low as possible to minimise sample degradation whilst maintaining signal quality, with ~10–20 mW being typical. However, the use of high powers allows the incident laser beam to "burn off" overlying material such as rock coatings, which can be ~15–20 μm in thickness. Raman spectroscopy is considered to be almost as powerful as x-ray diffraction spectroscopy for mineralogy but without the requirement for pow-

TABLE 2. RAMAN SPECTRAL BANDS AND ABSORPTION MAXIMA FOR MICROBIAL PHOTOPIGMENTS

Pigment	*Pigment type*	*Raman vibrational bands (wavenumber cm^{-1})*					*Absorption peaks (nm)*				
							UVC (<280)	*UVB (280–320)*	*UVA (320–400)*	*Visible (>400)*	
Usnic acid	Cortical acid	2,930		1,607	1,322	1,289	>220	290	325	>400	
Pulvinic dilactone	Pulvinic derivative		1,672		1,405		>246	290	367	>400	
Parietin	Anthraquinone		1,675		1,099	551	>257	288		431	422
Calycin	Pulvinic derivative			1,611		1,379	>269			>400	
Antranorin	*para*-Depside	2,942	1,666	1,303	1,294	1,266	>274			>400	
Gyrophoric acid	Tridepside		1,661		1,290		275	304		>400	
Fumarprotocetraric acid	Depsidone		1,642	1,630	1,290	1,280	273	315		>400	
Emodin	Quinone		1,659					291		440	
MAA (*Nostoc*) 7437	Mycosporine amino acid							(>310)	330	>400	
Scytonemin	8-ring dimer		1,590	1,549	1,323	1,172	252	300	370	>400	
β-Carotene	Carotenoid			1,524	1,155		>246	283	384	429	451
Rhizocarpic acid	Isoprenoid		1,665	1,620	1,596		NA	NA	NA	>400	
Porphyrin	Tetrapyrrole ring			1,453						>650	
$Chlorophyll_a$ (Cyano)	Tetrapyrrole ring			1,360	1,320					680	700
Bchia (*R. spheroides*)	Tetrapyrrole									850	870
Chlorobium Chi	Tetrapyrrole									650	660
Phycocyanin	Phycobilin		1,630	1,351						560	620
Phycoerythrin	Phycobilin	NA								544	

NA, not applicable.

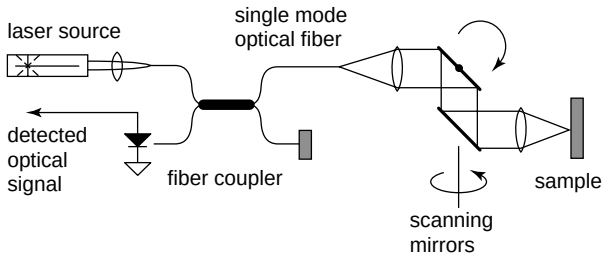

FIG. 4. Raman spectrometer/confocal imager architecture (from Dickensheets and Kino, 1998).

dering the sample—to date, no Mars mission has provided direct mineralogical measurements. Each group of oxyanionic minerals—carbonates, sulphates, nitrates, phosphates, and silicates—and most oxides, sulphides, and hydroxides have characteristic Raman spectra [i.e., most rock-forming minerals (Haskin *et al.*, 1997; Wang *et al.*, 1998; Popp *et al.*, 2001)]. Instruments using Raman spectroscopy have been developed for the mineralogical analysis of carbonates, silicates, and sulphates. However, their short wavelengths (514.5, 670, 885, and 785 nm) preclude them from organic measurement of pigments because of autofluorescence excitation, which tends to mask the Raman spectra of organic materials. Photosynthetic pigments (chlorophyll and accessory pigments such as phycocyanin) and certain photoprotective UV screening and quenching pigments (such as carotenoids) autofluoresce under blue / green illumination. Their response typically lies within 200–4,000 cm^{-1} wavenumbers—phycocyanin fluoresces at 530–560 nm. Although autofluorescence of pigments is useful for epifluorescence microscopy, for Raman spectroscopy, it swamps the Raman signal. To reduce organic autofluorescence, incident wavelengths longer than 852 nm are required. Thus, visible Raman spectroscopy is not ideal for organics (pigments in particular) analysis because of pigment autofluorescence, which favours near-infrared Raman spectroscopy. Although suited to biomolecule detection, near-infrared Raman spectroscopy is inferior to visible wavelength Raman spectroscopy for mineralogy because of the reduced resolution. However, at near-infrared wavenumbers between 500 and 100 cm^{-1}, there exist a number of inorganic bands that are valuable for describing the mineral composition of the habitat (e.g., quartz at 464 cm^{-1} and haematite at 400 cm^{-1}, 350 cm^{-1}, and 260 cm^{-1}). Biomolecules, especially pigment derivatives, may be detectable beneath the oxidised zone in the near-subsurface profile of Mars. Pigments such as carotenoids and chlorophyll give Raman bands in the 1,000–1,700 cm^{-1} range, while structural organics give bands in the 2,7503,000 cm^{-1} range (Table 2). Doran *et al.* (1998) have shown recognisable stromatolitic layers in the beds of Antarctic palaeolakes, and it is these types of profiles that would make good targets for the detection of biomolecules on Mars.

T2

Raman spectroscopy offers the prospect for compact and robust instrumentation and lends itself to a distributed instrument comprising two parts, which may be physically separated—a small sensor head probe and a separate CCD-based spectral analyser connected by a tether to carry electrical power and optical fibre data (Fig. 4).

F4

The first part comprises a light-weight spectrometer, a distributed Bragg reflector diode laser source (at 852 nm), and control electronics, which are all to be housed in the electronics bay of a surface rover. These are fibre-coupled to a second part, a compact probe comprising the MEMS-based confocal microscope and Raman filters. An optical fibre core of ~125–200 μm in diameter will provide maximum mechanical flexibility—opti-

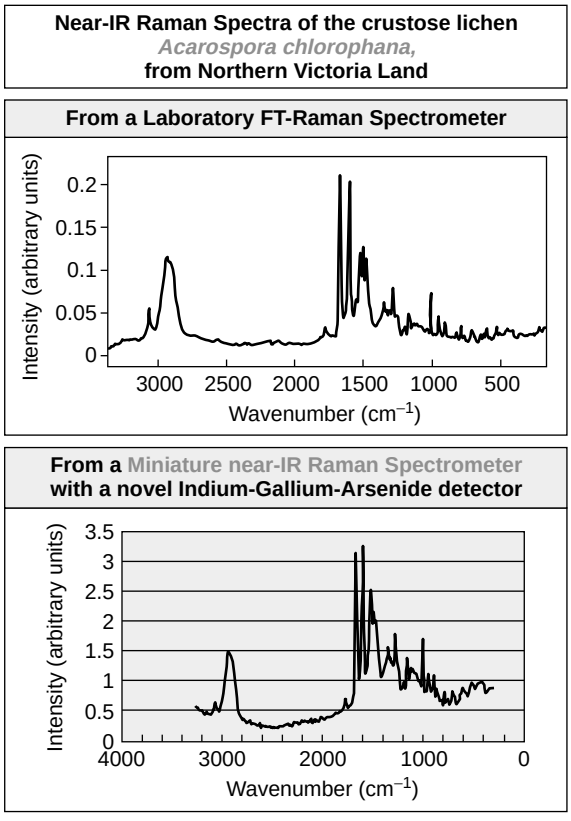

FIG. 5. Laboratory and micro-Raman IR spectra.

cal fibre integrity is highly sensitive to mechanical curvature induced by the spooling of such fibres. The micro-objective can be used to magnify the target so that the laser beam illuminates a selected spot that can be as small as 1 μm in diameter. This is within the resolution required to detect prokaryotes, which are typically <2 μm in size, though larger cells up to 10 μm do occur, but smaller cells down to 0.1 μm would be undetectable. Using the same fibre-optic path as the Raman laser, confocal microscopy can be used to image the target before Raman analysis. In contrast to many spectrometers, the Raman spectrometer can perform its analysis within minutes to a small number of hours per sample.

The CMaRS (Confocal Microscope and Raman Spectrometer) comprises an Si MEMS-based scanning mirror and lenses for the microscope with confocal imaging at 30 frames/s (Dickensheets and Kino, 1998; Dickensheets *et al.*, 2000). The Rayleigh-scattered light is detected and focussed to form the confocal image. The Raman scattered light is detected via a set of Raman filters by a miniaturised dispersive spectrometer based on infrared-sensitive InGaAs detectors in a linear CCD-based array with a spectral resolution of 8 cm^{-1} through the range 400–1,800 cm^{-1} with a 20$\times$ microscope objective. The laser transmitter is a Bragg reflector diode-pumped Nd:YAG laser with a spot size of 1 μm with a field of view of 250×250 μm at the optimal 1,064 nm laser wavelength. A narrow laser beam is preferred as a broad laser beam $\sim$20 μm induces a high fluorescence background (Israel *et al.*, 1997). It has a fixed grating (i.e., no moving parts), incorporating a linear axis. This linear axis provides insensitivity to temperature fluctuations, and a rugged and compact design. It can perform spot chemical analysis at any selected point within the borehole, while the scanning confocal microscope can provide images of soil/rock morphology, crystal structure, and microscopic images of suspected life forms. The CMaRS prototype is expected to undergo field trials in the near future (D.W.-W., unpublished data; D. Dickensheets, personal communication). Such trials will attempt to replicate realistic conditions for the robotic deployment of a Raman spectrometer—indeed, it is conceived that such an instrument may be incorporated into a ground-penetrating mole (Richter *et al.*, 2001a,b). The CMaRS instrument offers good resolution in comparison with a laboratory Raman (Fig. 5).

F5

The high potential of Raman spectroscopy has led to a mission concept called Vanguard, which exploits the separation of the instrument head from the instrument electronics (Ellery and Wynn-Williams, 2002; Ellery *et al.*, 2002a–c, 2003). The electronics reside within a micro-rover, while the sensor heads reside within three ground-penetrating moles that are designed to be deployed independently to analyse the subsurface of Mars to a depth of 5 m using Raman spectroscopy.

CONCLUSION

We have outlined the scientific case for former and extant biota on Mars, and the likelihood that evidence of such biological systems would be available for analysis near to the surface. A palaeolake crater environment offers the optimal landing site location. The centrepiece scientific instrument for any Mars astrobiology mission should be the near-infrared Raman spectrometer to detect evidence of long-lived biomolecules in the sedimentary deposits. Such an instrument may be augmented by a confocal imager. Fibre-optic-based instruments (Culshaw, 1995; Gratton and Sun, 2000) such as the Raman spectrometer provide the means to employ remote sensing of difficult to access environments such as subsurface boreholes, eliminating the need for physical recovery of soil samples. This approach can minimise the robotic complexity of instrument deployment on Mars by:

(i) The use of moles for modest depth capability over drills, which require autonomous assembly of the drill string for depths >1 m (Ellery *et al.*, 2002a–c)
(ii) The descent-only trajectory of each mole eliminates the requirement for mole recovery, for tether tension strengthening, respooling the tether, and mole-launch tube mating on recovery
(iii) No need to ensure hole integrity through the reinforcement of the borehole by casing
(iv) Elimination with the lack of physical sampling of the need for direct sample analysis instruments such as the gas chromatograph–mass spectrometer

AU1

By minimising the complexity of the robotic infrastructure, we enhance reliability, and thereby maximise the probability of mission success.

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ABBREVIATIONS

CCD, charge-coupled device; CMaRS, Confocal Microscope and Raman Spectrometer; PAR, photosynthetically active radiation.

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As meant?