**MICROBIAL LIFE IN ICE: HABITATS, METABOLISM, AND SURVIVAL ON MARS.** P. B. Price[1] (bprice@berkeley.edu), R. A. Rohde[1], and R. C. Bay[1], Physics Dept., Univ. of California, Berkeley, CA 94720, and N. E. Bramall[2] (nebramall@gmail.com), SETI Institute, NASA Ames Research Center, Mountain View, CA 94035

**Introduction:** Microbial life may have originated in glacial ice on Earth as well as elsewhere [1]. In the last decade, experiments have shown that microbes, including methanogens, can extract energy and essential elements while living in liquid veins at triple junctions of ice crystals [2], on clay grains in ice [3], or even within an ice crystal lattice [4]. Microbes immobilized in terrestrial ice have been shown to metabolize at a rate proportional to exp(-Q/RT) that is just sufficient to repair spontaneous damage due to amino acid racemization and DNA depurination [5,6]. Their lifetimes are constrained mainly by the rate of double chain breaks of DNA in cellular nuclei penetrated by alpha particles from Th and U impurities within a range ~20 μm in soil and rock [7]. Putative Martian microbes, if encased in relatively pure low-radioactivity ice, may have survived to the present day. In order for metabolism to account for the rapidly time-varying methane flux on Mars, methanogens probably live within a few tens of meters below the present surface. A mechanism suggested by Michael Mumma is via a crack that seals up with ice in winter and becomes accessible to the surface by sublimation of that ice in summer. As a result of gardening by micrometeorites, microbes now at depths less than several meters from the surface are unlikely to have survived earlier exposure to solar UV and highly oxidizing chemical compounds. If the methane bursts are mainly of microbial origin, their present concentration is estimated from their temperature-dependent metabolic rate to be as high as 1 cell per $cm^3$. Gaseous participants in microbial metabolism such as CO, $N_2O$, and $NH_3$ should be sought in spatial and temporal coincidence with $CH_4$ bursts [6].

**Scanning Laser Fluorimetry:** In October 2009 we used our laser fluorimeter to map tryptophan (Trp) and chlorophyll (Chl) in ice cores from the GISP2 core (Summit, Greenland), three sites in West Antarctica (Siple Dome, RIDS, and WAIS Divide), the South Pole, and Vostok Station in East Antarctica. Using an excitation wavelength of 224 nm, the fluorimeter records fluorescence in wavelength bands centered at 300, 320, 340, 360, 380, 670, and 710 nm. Trp has its peak emission at 320 to 340 nm and Chl has its peak emission at 670 nm. Micrometer-size mineral dust particles fluoresce very weakly and with non-interfering spectral distribution. Volcanic ash, with strong emission at both 670 and 710 nm, is recorded at certain depths but does not interfere with Trp and Chl signals. Residues of organic fluids (e.g., kerosene or butyl acetate) used to drill holes deeper than ~300 m may be trapped in microcracks at the surfaces of deep cores and may produce a background fluorescence that interferes with the Trp signal. Figure 1 shows our Trp and Chl data for cores down to 300 m that were drilled without any fluid, as well as some Chl data for fluid-drilled cores planed just before fluorimetry to remove ice that may contain residual fluid. The laser beam records fluorescence in a cylindrical volume of ice with diameter ~1 mm and depth ~1 cm. Data are collected once every 700 μm along a core in a time of ~2 minutes, not including an additional ~3 minutes to change and align a new core. Each data point in Figure 1 is the average of readings along a core of typical length ~1 m. The scatter is due to counting statistics and to the stochastic rate of arrival of wind-blown microbes onto a snow surface and subsequent compression into the growing ice pack.

The most striking feature of the data is that at every site the intensity falls off rapidly with depth in the upper ~100 m and then levels off to an intensity that shows no systematic depth-dependence. Our tentative interpretation of the steep decrease is that most microbes transported onto a growing ice cap die and decompose with loss of much of their fluorescence as a consequence of failing to adapt to the hostile conditions inside the ice. Those that do adapt will metabolize at a rate sufficient to repair spontaneous molecular damage [5,6]. As future data at depths below a few hundred meters are acquired, we will be looking for correlations with climate.

Figure 2 shows concentrations of microbial cells that others have measured by direct counting. We excluded data obtained by culturation because they may greatly underestimate cell concentrations, and we excluded data from flow cytometry because of inaccuracies associated with clumping onto mineral grains.

To gain a quantitative understanding of the fluorescence data, we have begun to study concentrations, size distributions, and quantitative PCR of cells at selected depths. Figure 3 shows an image from WAIS Divide ice at 84 m. Most of the ~5 x $10^4$ cells $cm^{-3}$ in that sample are in tetradic clusters similar to those exhibited by the psychrophile *Kocuria polaris* found in cyanobacterial mat found in a frozen Antarctic pond.

**Discussion:** The data in Figs. 1 and 2 are complementary: Fig. 1 gives the big picture but no details on taxonomy, cell sizes, genomics, or fraction that are still alive in the ice.

When drilling is complete in 2011 the WAIS Divide core will extend to bedrock at a depth of ~3600 m at a site in West Antarctica chosen in the expectation of providing resolution of annual layers and a record of climate, chemistry, gases, and volcanism that will extend over at least 150,000 years. When operated in absolute darkness, our scanning fluorimeter can be integrated into the Core Processing Line for the WAIS Divide ice core.

**References:** [1] Price P. B. (2007) *FEMS Microbiol. Ecol. 59*, 217-231. [2] Price P. B. (2000) *PNAS, 97*, 1247-1251. [3] Tung H. C. et al. (2006) *Astrobiol., 6*, 69-86. [4] Rohde R. A. and Price P. B. (2007) *PNAS, 104*, 16592-16597. [5] Price P. B. and Sowers T. A. (2004) *PNAS, 101*, 4631-4636. [6] Rohde R. A et al. (2008) *PNAS, 105*, 8667-8672. [7] Price P. B. (2009) *Can. J. Microbiol. 55*, 1-11. [8] Abyzov S. S. et al. (1998) *Adv. Space Res. 22*, 363-368. [9] Abyzov S. S. et al. (2007) *in Climate Change and Polar Research*, 11-39. [10] Abyzov S. S. et al. (2004a) *Adv. Space Res. 33*, 1222-1230. [11] Christner B. C. et al. (2006) *Limnol. Oceanog. 51*, 2485-2501. [12] Abyzov S. et al. (2004b) *Polar Biosci. 17*, 106-116. [13] Miteva V. et al. (2009) *Environ. Microbiol. 11*, 640-656. [14] Yung P. T. et al. (2007) *FEMS Microbiol. Ecol. 59*, 300-306. [15] Tung H. C. et al. (2005) *PNAS 102*, 18292-18296.

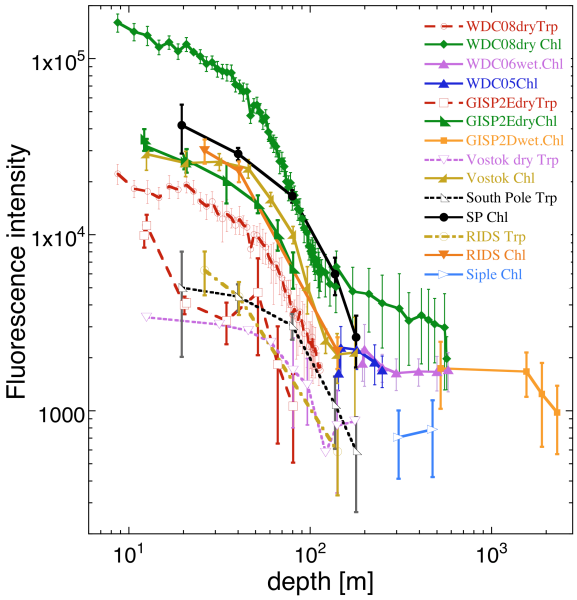


Figure 1. Scanning fluorimetry of Trp and Chl from one Greenland site (GISP2) and five Antarctic sites. Each data point is the average of up to $10^6$ measurements along an ice core up to 1 m in length.

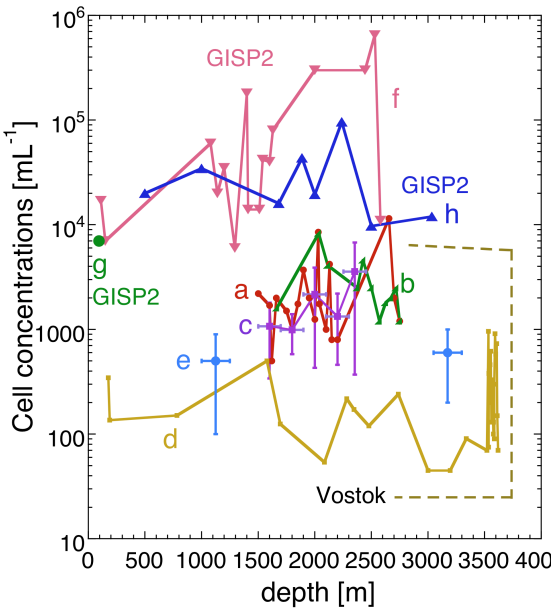


Figure 2. Cell concentrations measured by direct counts. The large scatter and poor statistics are due both to the labor involved in direct cell counts and to the preference of most biologists for studies of microbial taxonomy and genomics. Note that the data do not extend to shallow enough depth to reveal the dramatic decrease with depth in the top 100 m found by Trp and Chl fluorescence. Labels to references are given by: a = ref. [8]; b = ref. [9]; c = ref. [10]; d = ref. [11]; e = ref. [12] f = ref. [13]; g = ref. [14]; h = ref. [15].


Figure 3. Cells in WAIS Divide ice at a depth of 84 m. Essentially all of the cells in that sample had the form of tetrads.