

1. Introduction. Earth's upper atmosphere is an extreme environment: dry, cold, and irradiated. It is unknown whether our aerobiosphere is limited to the transport of life, or there exist organisms that grow and reproduce while airborne (aerophiles); the microenvironments of suspended particles may harbor life at otherwise uninhabited altitudes[2]. The existence of aerophiles would significantly expand the range of planets considered candidates for life by, for example, including the cooler clouds of a hot Venus-like planet.

The X project is an effort to engineer a robotic exploration and biosampling payload for a comprehensive survey of Earth's aerobiology. While many one-shot samples have been retrieved from above 15 km, their results are primarily qualitative; variations in method confound comparisons, leaving such major gaps in our knowledge of aerobiology as quantification of populations at different strata and relative species counts[1]. These challenges and X's preliminary solutions are explicated below.

2. Current Status. X's primary balloon payload is undergoing a series of calibrations before beginning flights in Spring 2012. A suborbital launch is currently planned for Summer 2012. A series of ground samples taken in Winter 2011 is being used to establish baseline counts and identify likely background contaminants.

3.1 Physical and Environmental Factors.

Physical: No single craft can provide extended sampling times from 0 to >100 km; however, a single sampler improves repeatability. Our payload is adaptable for suborbital rockets (<140km), balloons (<40 km) and ground locations.

Environmental: High-altitude measurement ranges are -70°F-120°F, .0001-1 atm, full UV spectrum 400-250 nm, 10-100 ft/s airspeed, and <0.1% RH. Ground measurements include location, altitude, and wind.

Kinetic: Particulate biases (size, nonisokinetic, particle bounce, etc.) dependent on sampler airflow are expected to be the single largest source of uncertainty; characterization of as many of these as is possible is a top priority.

Sterility: The design of the payload is the first defense against sample contamination. Pre-launch sterilization should meet forward-contamination planetary protection standards. A positive control of sterile 1 µm fluorescent beads will also be used.

3.2 Sampling and Analysis Factors.

Detection Range: Our expected sample size ranges from ~100-~1x10⁶ organisms (incl. spores) of unknown and varied type. We anticipate analyzing such sparse samples with a combination of electron microscopy and whole-genome analysis.

Capture Efficiency: A review of previous work suggests that, for 1-15 µm particles of interest, 1/L may be an appropriate target up to altitudes of 30 km. Our first results will inform later design revisions.

Recovery Efficiency: The extraction of particles of interest from the raw sample introduces many biases (differential survival, filter recovery, etc.) which together are expected to be the second-largest source of uncertainty. We plan empirical identification of these effects.

Species Identification: The sparse sample size and expected species variety strongly indicate *in situ* culture-independent analysis. We are in the process of developing a

microfluidics backend to conduct [whole genome amplification? 16s RNA sequencing? DNA microassays?] aboard the ballooning payloads. For our preliminaries, tests will be run on returned samples in a laboratory environment.

Particle Effects: In returned samples, after areas of interest have been identified by optical microscopy and DAPI staining, SEM+EDS will be used to look for correlations between non-living particulate matter and the presence and type of microbes.

4. Proposed Sampling Program. Our intended first balloon launch sites are Wyoming, Nevada, California, Maine and Brazil, with potential follow-ons in the mid-Atlantic and the west coast of Africa.

5. References.

- [1] Burrows et al. (2009). "Bacteria in the global atmosphere - Part 1." *Atmos. Chem. Phys.* 9, 9263-9280.
- [2] Womack, Bohannan, & Green. (2010). "Biodiversity and biogeography of the atmosphere." *Phil. Trans. R. Soc. B.* 365, 3645-3653.