DETECTION OF DNA AND DNA ANALOGUES USING TIME CORRELATED SINGLE-PHOTON COUNTING. T. J. Whitaker[1] and F. S. Anderson[1], [1]Department of Space Operations, Southwest Research Institute, 1050 Walnut Road, Boulder, CO 80302. Email: whitaker@boulder.swri.edu, anderson@boulder.swri.edu.

Introduction: We describe a straightforward technique enabling flight instrumentation for identifying deoxyribonucleic acid (DNA) or DNA-like molecules on extraterrestrial bodies such as Europa or Enceladus. The technique does not give sequence information about the unknown DNA but it does determine the presence or absence of DNA or DNA analogue with high fidelity and *without DNA amplification*, thus eliminating issues with false positives inherent to amplification methods.

The approach uses "molecular beacons" (MBs), which are synthetic single-stranded oligonucleotides consisting of a "loop" sequence sandwiched between two short, complementary "stem" sequences having a fluorophore and quencher attached, respectively, to the 5' and 3' termini [1]. MB fluorescence is greatly enhanced when the loop sequence hybridizes to complementary DNA, separating the fluorophore and quencher, and disrupting resonance energy transfer to the quencher (Fig. 1). In addidion to sensitive detection of DNA, MBs can hybridize to sequences of some alternative polymers that may serve as a substitute for DNA in extraterrestrial life, such as those recently described by Pinheiro et al. [2]. We follow Pinheiro's convention in calling these DNA analogues XNAs.

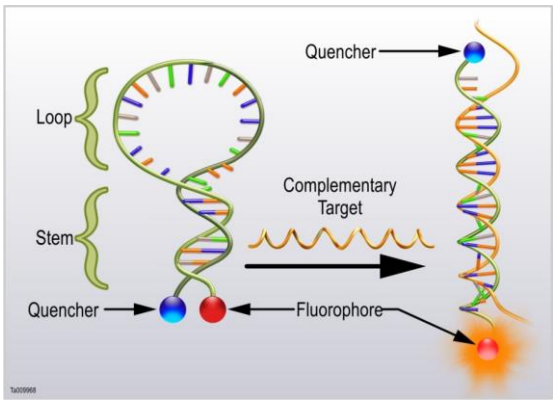

Figure 1. Conventional Molecular Beacon. The fluorophore is quenched in the self-hybridized conformation on the left but fluoresces when hybridized to complementary DNA or XNA.

The ability to detect an unknown DNA/XNA is made possible by using one or more universal bases in a shorter than normal (9- to 11-base) MB loop region to increase the probability of complementarity. If needed, stability of the hybrid is increased by using locked nucleic acids. Careful design of multiple MBs allows a high probability of detection of a random DNA/XNA having length comparable to bacterial DNA on earth (~6 Mbase pairs). Longer lengths, such as found in plants on earth (~100 Mbase pairs to multi-Gbase pairs) have an even higher detection probability. Some MBs can be designed to target highly conserved sequences in Earth organisms while still using the short loop design to detect random sequences.

Instrumentation:

Once a liquid sample is delivered by the spacecraft, it will be lysed and concentrated in a device similar to the SimplePrep® system (Clarmont Biosystems) presently onboard the International Space Station. Molecular beacons are then introduced and heat-cycled, followed by fluorescence analysis using time correlated single-photon counting (TCSPC). A combination of photon burst size, burst duration, and fluorescence decay time from the TCSPC analysis sensitively detects down to a single molecule of hybridized MB, even in the presence of an excess of unhybridized or damaged MBs [3].

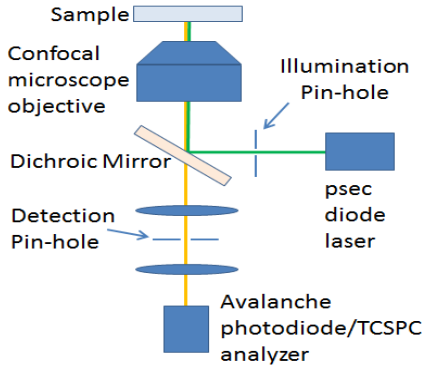

Figure 2. Sketch of the TCSPC Instrument.

Radiation Issues: On some extraterrestrial bodies, particularly Europa, high radiation levels may cleave the MBs. We have developed a mitigation using short oligonucleotide scavengers with 3'-quenchers to reduce background from this damage. Tests are needed to determine the level of radiation damage that can be tolerated by a combination of the scavengers and TCSPC analysis before false positives become an issue.

References: [1] Goel, G., Kumar, A. Puniya A. K., Chen W., and Singh K., (2005) J of Appl. Microbiol. 99, 435. [2] Pinheiro V. B., et al., (2012), Science 336(6079), 341. [3] Knemeyer, J.-P., Marmé N., and Sauer M.. (2000), Anal. Chem. 72, 3717.