DETECTION, MANIPULATION, ISOLATION, AND AMPLIFICATION OF SINGLE MICROBIAL CELLS IN OPTOFLUIDIC PLATFORM FOR WHOLE GENOME SEQUENCING OF VERY LOW BIOMASS SAMPLES Y. Liu[1], P. Jeraldo[1], S. McDonough[2], J. Wadsworth[3], J. Jen[2], D. Schulze-Makuch[4], J.-P. de Vera[5], C.Cockell[3] and M. Walther-Antonio[1], [1]Department of Surgery, [2]Genomic Analysis Core, Mayo Clinic, USA, [3]School of Physics and Astronomy, University of Edinburgh, UK, [4]Center of Astronomy and Astrophysics, Technical University Berlin, Germany, [5]German Aerospace Center (DLR), Institute of Planetary Research, Management and Infrastructure, Astrobiological Laboratories, Germany

Introduction: Genome sequencing of single microbial cells in low biomass settings such as in extreme conditions could add crucial detection capabilities to astrobiological pursuits. Typical microbial sequencing requires high biomass and averages over genomically heterogeneous populations, concealing the valuable information hidden within very few cells [1]. Single cell whole genome sequencing (SC-WGS) allows for identifying unculturable microbes with low representation often neglected or undetectable in traditional studies [2, 3]. Using SC-WGS, detection of rare mutation events in single cells can also allow for mutagenic and detailed evolutionary work. In this work, we use an optofluidic platform to obtain single cells of *Gleocapsa sp*., *Sphaerocystis sp*. Arctic strain CCCryo 101-99 and *Nostoc sp.* Antarctic strain CCCryo 231-06 for SC-WGS to identify the genomic variation among the cells exposed to space and simulated Mars conditions on the International Space Station (ISS) in addition to the simulated space and Mars conditions on ground.

Optofluidic platform: This platform integrates advanced microscopy (Nikon), optical tweezers (Thorlabs) and microfluidic technology (Fig. 1) [4]. In this platform, single cells can be selected, moved into an isolated chamber and transported for SC-WGS.

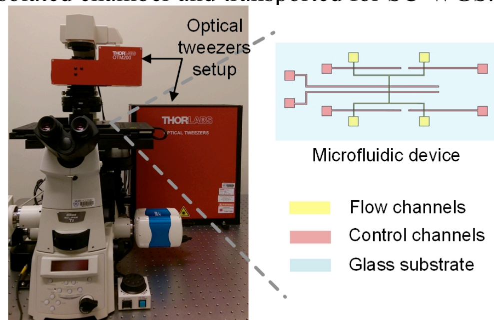

Fig. 1. The optofluidic platform. Optical tweezers are integrated into a microscope system, with a microfluidic chip.

Microfluidic device: The device consists of two channel layers separated by a flexible membrane, enabling gating functions for chamber creation (Fig. 2(a)). When a flow channel and a control channel cross, a gate is formed. By pressurizing the control channel, the membrane pinches off the flow channel, closing the gates and forming the chamber (Fig. 2(b), (c)).

Optical tweezers: Optical tweezers rely on the refractive index difference between an object and the suspension to form optical traps. Single cells can be selected and trapped based on visually discernable traits. Trapped cells can be moved passing an open gate into a chamber, and the gate can be closed to isolate the cells. (Fig. 3)

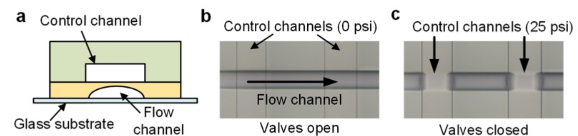

Fig. 2. (a) Cross-section of a microfluidic chip, with a membrance separates the top and bottom layer. (b),(c) Gates open and closed.

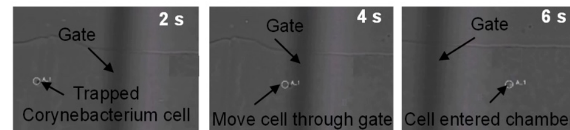

Fig. 3. A time-elapsed image of the trapping and moving of a single cell to pass through an open valve to be isolated in a chamber.

Experimental: Suspension of *Gleocapsa sp*., *Sphaerocystis sp*. and *Nostoc sp*. exposed to the aforementioned conditions will be introduced into a microfluidic device respectively. Single cells in each sample will be trapped and isolated in a chamber. The isolated single cells will then be transported and collected from the device outlet for amplification and sequencing. This platform will allow for unparalleled study of single cell mutagenic events within a population and observation of the raw rate of mutation events that a single cell experiences under the various conditions.

Future work: We envision that the optofluidic platform can be integrated with various microfluidic devices for different single cell applications such as single cell interactions, transcriptomics, proteomics and metabolomics (Fig. 4)

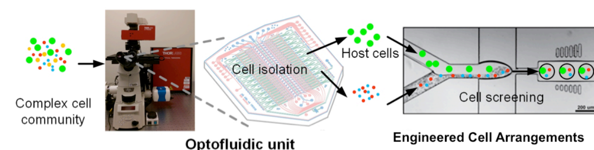

Fig. 4. Integration of microfluidic devices into optofluidic platform for single cell analyses.

References: [1] T. Ishoey et al. (2008), *Current opinion in microbiology,* vol. 11, no. 3, pp. 198-204. [2] N. Navin et al. (2011), *Nature,* vol. 472, no. 7341, pp. 90-94. [3] J. Eberwine, et al. (2014), *Nature methods,* vol. 11, no. 1, pp. 25-27. [4] Z. C. Landry et al. (2013), *Methods in enzymology,* vol. 531.