The Life Marker Chip (LMC) instrument - Antibody-based detection of organic molecules and biomarkers in Martian samples.
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Introduction & Overview: The Life Marker Chip (LMC) [1] is one of the instruments being developed for possible flight on the 2018 ExoMars mission. The instrument uses liquid solvents to extract organic compounds at low temperature from samples of martian regolith or crushed rock and transfers the extracts to detectors based on the use of antibodies in an immunoassay format. A key scientific aim of the instrument is to detect organic molecules in the form of biomarkers that might be associated with extinct and / or extant life as well as abiotic sources of organics. The instrument uses a novel solvent system comprising water / water miscible organic solvent / surfactant and bespoke, commercial and research-developed antibodies against a number of distinct biomarkers or other molecular types. The LMC comprises a number of subsystems designed to accept up to four discrete samples of martian regolith or crushed rock, implement solvent extraction, perform microfluidic-based multiplexed immunoassays for biomarkers and other targets, optically detect the fluorescent output of the assays, control the internal instrument pressure and temperature, and also includes associated instrument control electronics and software. The instrument principle can be extended to other configurations and missions as needed.

Scientific Objectives of the LMC: The primary scientific objective of the LMC on ExoMars is to detect a range of organics using a low temperature liquid extraction method from a crushed regolith or crushed rock sample acquired with the ExoMars drill or a rock corer and to characterise the amounts present or, provide upper concentration limits to, a number of key organic molecules including biomarkers. The aim is to detect and interpret any organic molecules within the categories of extinct and extant life, abiotic and Earth-derived contamination. The LMC (in its current ExoMars format) will simultaneously detect up to 25 different organic molecular targets in addition to conducting a pH measurement of the liquid extract prior to attempting organic detection.

Immunoassay Operation of the LMC: The LMC is based upon use of an immunoassay approach packaged in a lateral flow immunodiagnostic format. This is the same basic format that is used in pregnancy test kits for home use where antibodies are developed to recognise and bind specific molecular targets such as hormone molecules to indicate pregnancy or, in the LMC case, martian biomarkers. An inhibition immunoassay format is used as it is the most appropriate to detect small molecules such as the extinct biomarkers in an LMC context.

LMC Systems Design: The LMC configuration for ExoMars is designed to process and measure four samples. Other designs and configurations of the LMC are possible and several have been studied. In order to avoid potential problems with antibody binding under unusual conditions such as low pressure, temperature *etc*. the LMC is designed to allow the assays to occur under normal Earth-like pressures and temperatures. This results in a number of sub-systems to allow analysis to be performed on Mars. Figure 1 illustrates the basic systems architecture of the LMC in its current format and Figure 2 shows a CAD image of the LMC design.

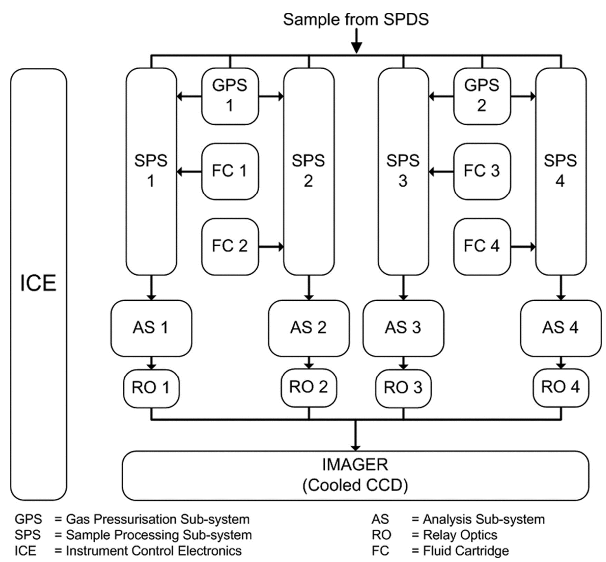

Figure 1: Scheme illustrating basic systems architecture of the LMC instrument.

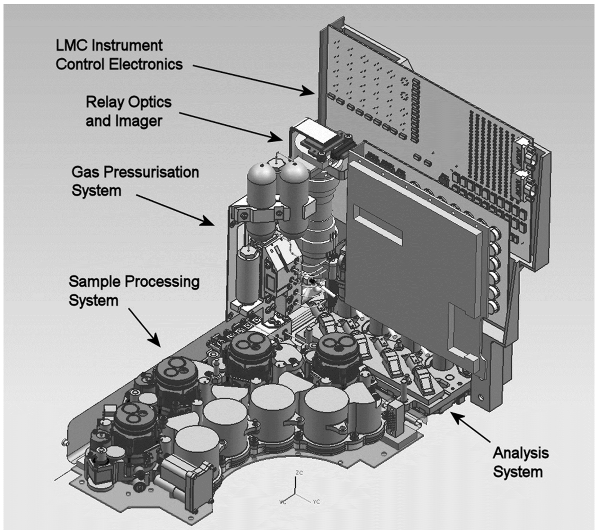

Figure 2: CAD image of LMC instrument design, correct as of December 2011 showing the various component subsystems of the instrument. The design mass is 4.7kg including contingency (for a four module / sample system).

The Sample Processing System (SPS) includes four, single-use modules. An inlet valve mechanism allows the martian sample to enter an experiment module and reseals the sample chamber. The SPS stores and mixes the solvents with the sample to extract biomarker molecules from the crushed sample.

The Gas Pressurisation System (GPS) provides argon gas to the instrument to generate Earth-like pressure for experimentation (0.2 to 1 bar), and gas pressure (up to ≈7bar) to actuate fluid cartridges and drive the sample around the fluidics system.

The Analysis System (AS) performs the multiplexed immunoassays and provides the interface for imaging and recording the fluorescent output of the assays (the immunoassays use fluorescent labels). Trade-off analysis determined that multiple, non-regenerative and therefore single use, microfluidic assay channels were the preferred option for the available mass and volume envelopes and timescales inherent in the ExoMars opportunity. The AS design has 4 single-use microfluidic channels with no cleaning or refurbishment of the immunoassays. Each individual channel has a dedicated laser diode to provide the fluorescent excitation light. For future implementation / future generations of the LMC, multiple-use, regenerative versions are being considered.

The Relay Optics and Imager collects from the AS, images of the multiplexed immunoassay (fluorescent images of the immunoassays formatted as microarrays) via imaging fibre optic cables and associated optics and passes onto an imager (CCD).

The Instrument Control Electronics (ICE) performs the majority of the control functions for LMC. A flex-rigid harness connects the ICE to a backplane board which sits under the four SPS modules, providing an interface to the subsystems and part of the Front-End Electronics (FEE). Utilisation of FEE and flexible harness minimises the number and mass of connections to the ICE.

Antibody Development: Antibodies to small molecules are well established in medical and environmental analytical situations however for many of the LMC small molecule targets, off-the-shelf antibodies do not exist and especially for small apolar target molecules which are significantly represented within extinct life biomarkers. Where possible commercially available antibodies or antibodies sourced from other research groups are used but for many targets *e.g.* steranes and hopanes, bespoke antibodies are needed and are under development including by the use of advanced recombinant antibody production techniques such as phage display library approaches. To maximise the ability to obtain such antibodies, multiple recombinant antibody production approaches are under investigation. Library types under investigation range from synthetic, through native naïve to immunised libraries. Aptamers are also under investigation as a future technology for LMC type instruments and molecular imprinted polymers remain a future possibility.

Summary Status: The LMC is under active development for use on the 2018 ExoMars mission and use on future *in situ* analysis missions. Solvent extraction of polar and apolar molecules and their detection with appropriate antibodies has been successfully demonstrated. Detailed engineering of the instrument for flight has occurred, the instrument design is currently being consolidated, and prototypes of key elements are under test. Further development of the instrument is underway (particularly in terms of the multiplexed immunoassay development) in order to meet the science objectives of the instrument. Other configurations of the instrument are possible and the LMC could be used in other mission scenarios. In addition a number of terrestrial applications of the technology are under active investigation and development.

References: [1] M. R. Sims *et al* (2012) *Planet. Space Sci.*, in press.