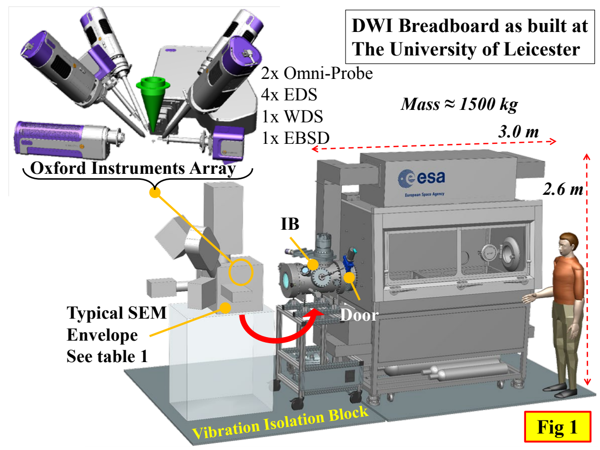
A DOUBLE WALLED ISOLATOR SCANNING ELECTRON MICROSCOPE FOR INVESTIGATING MARS SAMPLES IN FULL BIOSAFETY CONTAINMENT. J. M. C. Holt[1], A. Martindale[1], A. O. Norbury[1], R. E. Jones[2], J. Bryon[2]; F. Gaubert[3] and J. Vrublevskis[4]; [1]The University of Leicester, email: jmch1@le.ac.uk, [2]Oxford Instruments NanoAnalysis, email: Rosie.jones@oxinst.com, [3]European Space Agency, [4]Thales Alenia Space, UK

Introduction: At the time of writing, the anticipated landing of NASA's Perseverance rover at Jezero crater will signal the first milestone of a major global effort to return the first samples from the surface of Mars. Mars Sample Return (MSR) requires material from the near surface to be acquired, documented and cached by Perseverance with subsequent retrieval by an ESA fetch rover and ultimate return to Earth. If successful, these mission components will deliver up to 38 tubes (+ 5 witness tubes) of approx. 15 g/tube of Cat V, restricted material to Earth, c. 2031/33. Pristine rock, regolith and atmosphere must be isolated to prevent both contamination of the samples and any possible transfer of harmful materials into the terrestrial biosphere while enabling curation and preliminary scientific investigations for which they were retrieved.

The engineering challenges of maintaining the highest bio-safety containment possible (BSL-4) and enabling the samples to be analyzed, after the sample tubes are opened inside a Sample Receiving Facility (SRF), are significant. By comparison, the engineering complexities to build a rover within the confines of planetary protection are understood (and the costs known), but a SRF must meet the same standards (as unprecedentedly clean, sample intimate components of the rover) and address additional requirements to mitigate any risk of backward contamination, with returned material. To this end, a Double Walled Isolator (DWI) [1] offers, i) controlled containment/isolation, ii) contamination control, iii) scientific instrument interfaces, and iv) sample articulation/manipulation. Moreover, operating analytical techniques in a way that maintains cleanliness and high containment, will demand technical innovation to enable the SRF to serve the community by exploiting leading commercial instruments such that "preliminary sample characterization" [2] is of the highest scientific standard possible. This abstract describes one method for the customized integration of a scanning electron microscope (SEM) onto a DWI and further describes the benefits of coupling a coordinated suite of analytical instruments within a DWI/SEM envelope to investigate Martian samples during that initial characterization.

SEM Envelope Accommodation: SEM's may be 'tuned' for a wide range of user applications, but the aim of this development is to consider physical adaptations that provide a scientific advantage to MSR. Fig 1: shows the current DWI Breadboard and potential accommodation of a multi-instrument SEM envelope.



By combining expertise in DWI technologies and SEM instrumentation (Oxford Instruments), we are developing an "Instrument Box" (IB) prototype [1, 3] to test preferred geometries for an SEM/IB platform that will enable optimized analysis of samples in full containment while potentially minimizing sample preparation. Separated by a door on the side of DWI, the IB is as much a methodology as it is a physically engineered chamber that facilitates movement of a solid sample into an adjacent, controlled environment (e.g. magnetic, acoustic, gas, temp, pressure, oxygen/water content vacuum, biological/organic, particulate & VoC's) where it can be safely analyzed without breaking containment. In this sense, the IB may be any size, geometry, or material and is proposed as a facilitating feature to maximize end user flexibility**.** The exact analytical methods for samples will be proposed by the MSPG and wider scientific community, but, early DWI design/testing ensures the engineering is uniquely flexible by including necessary interface technologies that can be tailored to current and future, commercially available analytical techniques. Here we discuss this in the context of a bespoke SEM package.

An SEM Workhorse: The DWI enables many instrument accommodation modalities that must serve the multidisciplinary science envisaged for MSR. SEM imaging and electron-beam based analysis is a fundamental tool used for the characterization of both terrestrial and extra-terrestrial geological specimens, which must be considered within the context of an SRF. For example, using the same instrumentation to identify potential contamination and thereby helping to protect the scientific integrity of the samples. Imaging modes (e.g. SE, BSE, CL) reveal information about surface topography and composition while additional instruments may be added to the SEM chamber to collect signals generated by the interaction of electron beams with an analyte (or ion/laser beam in the case of a FIB-SEM). For instance, Energy Dispersive X-ray Spectrometry (EDS) and Wavelength Dispersive X-ray Spectrometry (WDS) are typically utilized to give elemental abundances, and Electron Backscatter Diffraction (EBSD), to obtain crystallographic and microstructural information. However, the SEM is rapidly evolving to meet the advancing needs of industry sectors like novel materials for batteries, electronics and life sciences. To this end an EC funded project, Universal SEM (grant ID: 280566), developed a multimodal prototype SEM that also included optical imaging, Raman and TOF-SIMS capability**.** Evidently, high-throughput and complementary, multi-instrument technologies are particularly relevant to cross-disciplinary research and, when combined with isolator technology, address many problems related to planetary protection [4] and science in containment.

Minimizing Sample Handling and Preparation: A key requirement for the SRF is to limit sample damage and contamination. It is proposed that SEM adaptations serve to reduce preparation in some modes of operation, minimize sample handling and ultimately expedite the transition of material through the curatorial and cataloguing process before comprehensive analysis in PI labs. As a case in point, electron imaging, quantitative compositional data (e.g. EDS, WDS), and high-quality crystallographic information (e.g. EBSD), typically requires geological samples are cut/polished flat, and a conductive coating (e.g. 10-20 nm of carbon/noble metal) applied to prevent sample charging. Obviously, these steps alter the sample, and therefore other and potentially novel solutions must be sought for MSR (see Oxford Instruments concept array in Fig 1). In particular : (1) using the SEM in 'variable pressure' or 'environmental' mode to reduce sample charging; (2) using micro and nano-manipulators (e.g. Oxford Instruments OmniProbe) to manipulate samples into suitable orientations, for optimum analysis, and to remove local charge build up (by localized contact); (3) using multiple EDS detectors to reduce the effect of shadowing caused by surface topography, and to increase speed and sensitivity; and (4) co-location of complementary instrument techniques in the same SEM/IB housing to minimize sample handling (unnecessarily moving a sample between instruments).

Conclusions: Inevitably, there is a 'sample cost' to an intended scientific outcome particularly where a proportion of the sample is altered. While damage may be negligible, it cannot be ignored if it occurs during routine handling of pristine samples. Supporting technology (DWI, instruments/detectors and manipulators) should be optimized such as to minimize the impact of a specific technique on the sample and indeed the quantity of material that is expended during any processes. This abstract presents a methodology for instrument accommodation via the DWI IB and proposes future studies to ensure that efficiency/sensitivity/resolution are maximized for any given instrument such that 'sample cost' is acceptable. It is suggested that the use of multiple instrument probes (of the same type) may alleviate some challenges of analyzing unprepared samples. Table 1 shows a non-exhaustive list of commercially available instruments that could be co-located inside a customized SEM/IB housing and connected to an SRF DWI via a door mechanism, so as to enable preliminary sample analysis within an acceptably clean, high containment environment.

Indicative DWI/SEM Instrument Configuration Matrix

Key: ☑ = Analysis is possible; ■ = Analysis is possible, but sub-optimal; X = Analysis is not possible

			Sample Coating		Surface Preparation (incl. coating)			
	Technique	**Primary datasets/information**	*No coating (variable pressure)*	*Coating (high vacuum)*	*None (i.e. rough)*	*Cut flat*	*Polished*	*Fib/laser cut*
Single DWI/SEM Envelope	Optical	Macro/micro imaging, correlative mapping	☑	☑	☑	☑	☑	n/a
	SE	Imagining - topography and morphology	☑	☑	☑	☑	☑	☑
	BSE	Imaging - composition (atomic contrast with dual detector)	[1]■	☑	☑	☑	☑	☑
	CL	Imaging - composition/crystal defects, cementation, μ-fracturing	■	☑	■	☑	☑	n/a
	[5]EDS	Major-minor element chemistry	[2, 3]■	☑	[2, 3]■	[3]■	☑	☑
	[5]EBSD	Cystallographic/microstructural (low energy/beam current)	☑	☑	X	■	☑	☑
	[5]WDS	Minor-trace element chemistry	X	☑	■	■	☑	☑
	[5]OmniProbe	Nano-manipulation and reduces charging	☑	☑	☑	☑	☑	☑
	Raman	Molecular and organic identification	☑	☑	☑	☑	n/a	n/a
	Micro XRF	Min sample preparation, heavy element	[4]■	☑	☑	☑	n/a	n/a
	TOF-SIMS	Chemical/isotopic information	X	☑	n/a	n/a	n/a	☑
	[5]AFM	Topographic imaging, force measurement, mechanical properties	X	☑	☑	n/a	n/a	n/a
	X-Ray CT	Internal structure and density, sub-micron voxel	☑	☑	☑	n/a	n/a	n/a

Notes: [1]*High resolution is impacted* [2]*Semi-quantitative* [3] *Possible with multiple detectors* [4]*Possible loss of low z signal* [5]***Oxford Instruments***

Table 1

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References: **[1]** Holt, J. M. C., J. C. Bridges, J. Vrublevskis and F. Gaubert (2019). Double Walled Isolator Technology For Mars Sample Return Facilities. 50th Lunar and Planetary Science Conference, The Woodlands, TX. **[2]** MSPG (MSR Science Planning Group: D. W. Beaty *et al* (2019) The Relationship of MSR Science and Containment*.
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*https://mepag.jpl.nasa.gov/reports.cfm
[4] COSPAR/IAU, COSPAR "Planetary Protection Policy (As amended)". 2011