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Expert Panel Recommendations for Enabling Cell Science Requirements

June 2013

International Space Station National Laboratory – Cell Biology Research

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Table of Contents

Executive Summary ii
1.0 Introduction 1
1.1 External expert panelists 1
1.2 Additional participants 2
1.3 Cell biology in microgravity 3
1.4 Fields of research 3
2.0 Recommendations 5
2.1 Overarching strategies 5
2.2 Cryopreservation 9
2.3 Culture 12
2.4 Sample analysis and storage 18
2.5 Quality 21
3.0 Appendix A – Additional Considerations 24

Executive Summary

In March 2013, an external panel of experts from various cell biology, microbiology, and tissue engineering fields was convened to develop and recommend a set of scientific requirements that could be used to steer strategic planning and tactical execution of these fields of research on the International Space Station (ISS) National Laboratory. Panel discussions focused on feasibility assessments, culture and specimen types, the in-flight culture environment, in-flight experiment handling and processing, and sample return.

The expert panel made a total of 41 recommendations for the future of cell biology experiments on the ISS National Laboratory. These recommendations fall within five major categories, as listed below:

Overarching strategies – 11
Cryopreservation – 4
Culture – 13
Sample analysis and storage – 7
Quality – 6

Key findings include these:

- Ground-based studies remain critical for identifying important areas of cell biology research on the ISS (feasibility testing) and ensuring proper flight definition.
- On-orbit capabilities should approximate those in ground-based laboratories to the greatest extent possible.
- A strategy of experiment iteration allows immediate continuity of experiments with promising findings, and maximizes scientific return on investment.
- An integrated approach to cell biology and tissue engineering experiments that uses standardized cell models to coordinate investigators from different disciplines will maximize scientific output and return on investment.
- The use of physiologically (or pathologically) relevant cell or tissue models will improve the likelihood that results can be extrapolated to homeostasis or disease in the whole animal.

1.0 Introduction

As noted in NASA's fundamental Space Biology Plan, "Cell based science in microgravity serves many areas of basic and applied research for space exploration and for Earth applications. Use of cells for investigations in microgravity carries the advantages of low mass and power, many replicates, adaptability to mission scenarios, minimum crew requirements, and amenability to real time analysis. Additionally, there are a number of analog settings that can be used to define and refine flight experiments thereby increasing the probability for a successful experiment in space."

In March 2013, Johnson Space Center and Ames Research Center cell biology and tissue engineering teams convened an expert panel to discuss cell biology research that would utilize the International Space Station (ISS) National Laboratory. The goal of the panel discussion was to put forward a set of scientific requirements that could be used to steer strategic planning and tactical execution of this sector of research.

The panel convened in person and by teleconference on March 4 and 5, 2013, at the Universities Space Research Association meeting facility in Houston, TX. In attendance were experts representing various basic science fields and industry concerns, who had direct links to cell science research. The invited external expert panelists and additional participants are listed here.

1.1 External expert panelists

- Tom Cannon, Co-Founder & Executive Vice President, Tissue Genesis
- Jack Dean, Ph.D., Sc.D. (Hon.), DABT, President, Drug Development Advisors, LLC, & Professor, Pharmacology & Toxicology Department, University of Arizona
- Cyrus Ghajar, Ph.D., Bioengineering Fellow, Bissell Laboratory, Lawrence Berkeley National Laboratory
- Lynne Haber, Ph.D., DABT, Associate Director, Toxicology Excellence in Risk Assessment
- Rosemarie Hunziker, Ph.D., Director, Tissue Engineering and Regenerative Medicine, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health
- Kurt Kasper, Ph.D., Faculty Fellow, Department of Bioengineering, Rice University
- Stanley Kleis, Ph.D., Associate Professor of Mechanical Engineering, University of Houston
- Cheryl Nickerson, Ph.D., Professor of Life Sciences, The Biodesign Institute, Center for Infectious Diseases and Vaccinology, Arizona State University
- Graham Scott, Ph.D., Assistant Professor, Department of Molecular and Cellular Biology & Center for Space Medicine, Baylor College of Medicine, & Vice President & Institute Associate Director, National Space Biomedical Research Institute
- Arun Sreekumar, Ph.D., Associate Professor, Department of Molecular and Cellular Biology, Baylor College of Medicine
- David Wolf, M.D., Consultant, Universities Space Research Association

- Brian York, Ph.D., Instructor, Department of Molecular and Cellular Biology, Baylor College of Medicine

1.2 Additional participants

- Eduardo Almeida, Ph.D., Chief, Space Biosciences Research Branch, Ames Research Center (ARC)
- Patricia A. Bahr, Chief, Biomedical Projects Branch, Johnson Space Center (JSC)
- Tacey Baker, Project Scientist, Wyle Science, Technology & Engineering Group, Space Cell Biology Laboratory, JSC
- Kirt Costello, Ph.D., Assistant International Space Station Program Scientist, JSC
- Brian Crucian, Ph.D., Immunology Laboratory, JSC
- Todd Elliott, Wyle Science, Technology & Engineering Group, Space Cell Biology & Microbiology Laboratories, JSC
- Clifford Folmes, Ph.D., Mayo Clinic Center for Regenerative Medicine & Center for the Advancement of Science in Space (CASIS)
- Charlene Gilbert, Project Manager, Space Cell Biology, JSC
- Judith Hayes, Chief, Biomedical Research and Environmental Sciences Division, JSC
- Antony Jeevarajan, Ph.D., Deputy Chief, Biomedical Research and Environmental Sciences Division, JSC
- Shannon Langford, Ph.D., Wyle Science, Technology & Engineering Group, Toxicology Laboratory, JSC
- Torin McCoy, Chief, Environmental Science Branch, JSC
- Valerie Meyers, Ph.D., DABT, Toxicology Laboratory, JSC
- Mark Ott, Ph.D., Microbiology Laboratory, JSC
- Duane Pierson, Ph.D., Microbiology Laboratory, JSC
- Michael Roberts, Ph.D., Chief Scientist, CSS-Dynamac, & Research Scientist, CASIS
- Julie Robinson, Ph.D., International Space Station Program Scientist, JSC
- Kevin Sato, Ph.D., Project Scientist, Lockheed Martin, ARC
- David Tomko, Ph.D., Program Executive, Human Research Program and Space Biology, NASA Headquarters
- Honglu Wu, Ph.D., Radiation Laboratory, JSC

The expert panel members were charged with identifying core enabling requirements, including key ground-based and in-flight culturing, processing, and analysis capabilities, that are needed to fully utilize the ISS National Laboratory for animal cell science and tissue engineering. Panel members discussed the need to establish a world-class research facility befitting a US National Laboratory for cell science and tissue engineering aboard the ISS, which can address the 2011 National Research Council (NRC) Decadal Survey mandate to provide research that both "*enables space exploration*" and is "*enabled by access to space*." Given the limited lifespan of the ISS, panel members were made aware that these requirements should be implemented immediately and with urgency. Furthermore, the panel was asked to elucidate new sample processing and analysis capabilities that are needed to maximize scientific return for the benefit

of human health and quality of life.

1.3 Cell biology in microgravity

Many unique scientific discoveries have come from extreme environments. Experiments with extreme heat led to breakthroughs in molecular biology with the discovery of the heat-shock response and the microorganism *Thermus aquaticus,* from which the DNA polymerase required for development of the polymerase chain reaction was derived. The panel agreed that microgravity is another example of an extreme environment with great potential. All life on Earth has evolved in a 1*g* environment, and reduced gravity provides a novel opportunity to reveal important aspects of cell biology that had previously been masked, such as weak forces in cell adhesion, signaling and developmental pathways, biomechanics, mechanosensing, and mechanotransduction. Although no one can guarantee that cell biology in microgravity will produce the next-generation breakthrough, high-risk science has the potential for a high-impact payoff.

In general, the panel agreed that the unique capabilities of the ISS outlined in the NRC Decadal Survey in 2011 – "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era" (hereinafter referred to as the Decadal Survey) should be utilized to the fullest extent possible, specifically with respect to the abilities to **1)** perform experiments of extended duration, **2)** continually revise experiment parameters on the basis of previous results, and **3)** have flexibility in experimental design provided by both human operators and automation.

1.4 Fields of research

To determine the scope of the critical enabling requirements needed for successful ISS National Laboratory research, the panel first delineated the specific cell science fields in which space-based cell science research is most likely to produce high-impact discoveries. Cell science research experiments that have been, are being, or will most likely be conducted utilizing the ISS National Laboratory include but are not limited to these:

- Scientists in the field of developmental biology would investigate cell development, organogenesis, tissue engineering, and regenerative medicine using stem cells (embryonic and adult), cell lines, and primary cells. Key questions to be answered include how cells sense and respond to culture in microgravity and microgravity analogs, and how those responses drive cell replication, plasticity, differentiation, regeneration and repair, morphogenesis, and tissue-specific function. These studies would include characterization of cell-cell and cell-extracellular matrix interactions, co-culture of multiple cell types, and studies of other microenvironments, such as those subject to mechanotransduction. The goal of these experiments would be to determine the role of the microenvironment in regulating tissue-specific three-dimensional structure and the function of such a structure as it maintains homeostasis or allows disease progression. The sustained and unique gravity field aboard the orbiting ISS National Laboratory cannot be matched on Earth and therefore represents a distinctly singular facility where

this research can be accomplished. NRC study committees and other advisory groups, as noted in the Decadal Survey, have consistently highlighted this field of research.

- Scientists in the field of cancer biology would study whether morphogenic programs are altered and result in disrupted tissue architecture, a phenotypic hallmark of cancer, under microgravity conditions. In addition, cancer biologists could design experiments aimed at testing whether microgravity alters drug sensitivity of specific cancer cell lines.
- Scientists in the field of radiation biology would conduct basic space cell biology studies to investigate chromosome damage and repair.
- Scientists in the field of toxicology would use space cell biology to elucidate unique factors affecting the toxicity of chemicals found in spacecraft environments. Identifying these factors would help toxicologists better protect space crews and hardware on missions in the near future as well as on exploration-class missions.
- Scientists in the field of pharmacology would use space cell biology research to characterize altered efficacy of drugs used by space flight crews and to unmask new drug targets. Marked space flight-related human physiological changes as well as long-term stability of drugs in the altered radiation environment aboard space vehicles are key factors that can affect current space missions as well as future exploration missions.
- Scientists in the field of chronobiology, the study of biological rhythms, would use space cell biology to evaluate changes in circadian rhythm genes, which play a very important role in normal body homeostasis and are thought to greatly influence a myriad of cellular functions both in the normal state and in disease processes such as cancer. The altered cycles of day and night, as well as schedule-shifting, that are experienced by space crews could offer a valuable investigatory tool to help cancer researchers better understand the role of circadian rhythm in causation and progression of neoplasms and metastatic disease. This line of investigation is also in line with the research area proposed in the Decadal Survey to study the interaction between circadian rhythm and cardiovascular and immune function in simulated and true microgravity environments.
- The field of microbiology would use space cell biology to further advance our understanding of host-microbe interactions, including those with pathogens, commensals, and beneficial microorganisms. These studies could provide valuable insight into pathogen infectivity, host immunity, and microbiomes (with translational implications for the general public and also insight into what might be expected by space crews in the future to mitigate disease risks). Evidence already gleaned from space-based microbiological studies demonstrates that microorganisms are profoundly affected by exposure to the unique environment found inside the habitable volumes of spacecraft. These changes include global alterations in gene expression and, in human pathogenic organisms, changes that have consequences for virulence and pathogenesis, as noted in the Decadal Survey.

2.0 Recommendations

Panel recommendations for key enabling requirements for space-based cell science research are summarized below.

2.1 Overarching strategies

The program should provide a suite of hardware and similar workflow on the ISS National Laboratory that is analogous to basic hardware and workflow in state-of-the-art cell science laboratories on the ground.

> *Rationale: A suite of hardware similar in function to hardware in current ground-based laboratories will facilitate a similar workflow from which to conduct experiments.*
>
> *This suite should include basic hardware analogous to a cell culture incubator, sample centrifuge, microscope, fluid-transfer capability (e.g., ground-based pipettes), cold stowage, and other hardware. The components of this suite should ideally be modular and compatible with cell handling and analysis subcomponents (so that, for example, a sample can be removed from the incubator and inserted in the microscope or centrifuge as needed). Accuracy and reproducibility of sample transfer and pipetting are essential.*

The program should provide near-real-time flexibility in experiment execution on orbit, similar to ground-based research.

> *Rationale: The ability to alter an experiment based on macro- and/or microscopic observation and chemical analysis in near real time is required to maximize scientific value and utilization of ISS National Laboratory resources.*
>
> *The panel agreed that it was very important to determine the status and health of a cell culture before continuing with the next phase of an experiment, such as introducing a toxicant or an infectious pathogen. This determination could be made visually by a crewmember or by some automated means available to the investigator on the ground. If the flight cell culture is not at that predetermined phase, the forward schedule must be flexible enough to delay the continuation of the experiment. The panel acknowledged that this practice could affect the crew schedule. These impacts could be mitigated by automation of the experiment or by training multiple crewmembers to interact with the experiment.*

The program should implement a strategy of experimental iteration.

> *Rationale: On-orbit experiment flexibility will facilitate meaningful data capture for cell science experiments, allow immediate continuation of experiments with promising findings, and maximize science return on investment.*
>
> *In a normal ground laboratory, an iterative cycle of experimentation exists that involves data gathering, analyses, and determination of the next planned step—all based on the outcome of the previous step. A strategy of iteration would greatly benefit the pace of microgravity research, which currently requires years to repeat or follow up an*

experiment. The Decadal Survey similarly noted that "Spaceflight science should also maximize repeated, multi-sample experiment designs." It also stated that "…there should be a continued emphasis on keeping scientists engaged during the conduct of the experiment, to allow the experiment to be facile and responsive to the flight profile or experiment progress. That emphasis could be accomplished by designing autonomous hardware to be communicative and responsive to remote input."

The panel agreed that, although some success has been achieved in the past, the program should move away from "an experiment in a can" approach to space cell science. This consensus opinion was balanced with the understanding that cell culture is labor intensive, and limitations on crew time may necessitate maximization of automated functions in cell science hardware. The goal should be to strive for a balance between hardware automation and crew hands-on interaction. This goal is consistent with the idea that "Biological experiments in space will benefit from a considered intermingling of automated and scientist-in-the-loop implementation," as stated in the Decadal Survey.

When trade studies are performed on hardware in the cell biology hardware suite, the program should prioritize technologies that minimize variability in the experiment.

Rationale: Minimizing or eliminating variability in equipment function and processes is critical for overall experiment quality.

Mitigating background variability, such as hardware temperature and other environmental parameters, and optimizing reproducibility and accuracy in experimental protocols, will help to remove uncertainty in data that is unrelated to actual experiment findings. The panel foresees the use of trade studies and prioritization of hardware capabilities. The minimization of variability should be the highest priority during this phase of development. Biological systems are inherently variable, and the more variability that is taken out of the hardware, the better. Other factors that the panel understands will need to be compared in trade studies are mass, volume, power, and safety.

The program should have a balanced approach to the cell biology hardware requirements for automation and crew interaction.

Rationale: Certain tasks are more suited to automation than others.

There is a limit to the scope and success of onboard cell science experiments conducted solely by means of automated functions and processes (i.e., the concept of "experiment in a can"). For this reason, the panel agreed that the program should move away from this strategy.

In general, the panel agreed that automation should be emphasized for time-consuming, day-to-day phases of experiments, whereas hands-on crew interaction should be utilized during high-value, strategic points in experiments; to facilitate science-related iterative steps; or to access cell culture hardware for troubleshooting tasks.

Some panelists cautioned that "if the hardware needs crew intervention, it will not fly as many times as hardware that does not need crew intervention." The concepts of iteration of experimentation and real-time decision-making and troubleshooting will require late-notice changes to the crew schedule and require a lot of real-time work.

Therefore, a balanced approach is needed that utilizes automation where possible, but allows for crew access and intervention. As mentioned previously, this is consistent with the Decadal Survey, which states, "Biological experiments in space will benefit from a considered intermingling of automated and scientist-in-the-loop implementation."

The program should enable an integrated approach to cell biology experiments that coordinates investigators from different disciplines by using a standardized cell model.

Rationale: Because of the unique logistical constraints and extremely valuable resources particular to the ISS National Laboratory, involving multiple investigators with each study to be conducted on orbit will maximize potential scientific return.

The panel agreed that the program could get more science for its dollar by bringing in researchers from different disciplines to study the same model and gather data from the same flight experiment. The Decadal Survey supports this approach as well: "Larger-scale multiple investigator experiments, with related science objectives, methods, and data products, would result in the production of large datasets and would emphasize analysis over implementation." The capability for collecting "matched samples," another recommendation of the panel, would help enable this strategy.

The program should support the use of ground-based studies using microgravity analogs to identify important areas of cell biology research on the ISS.

Rationale: Ground-based microgravity analog studies provide an important mechanism for identifying potential cellular and molecular responses to the microgravity environment and determining compelling reasons to utilize space for cell biology research.

This recommendation agrees with the Decadal Survey, which stated, "The space biology research programs will advance rapidly when supported by a robust ground research program. The ground research program will produce and refine the questions to be addressed in space." This recommendation is also corroborated by recent successes in ISS research, including Salmonella *virulence research, which were predicted in ground-based studies.*

The program should ensure the proper flight definition of the experiment on the ground.

Rationale: Ground-based pilot studies are demanded by the unique logistical constraints, highly specialized and sophisticated space cell culture hardware, and extremely valuable and limited resources encountered when cell science research is conducted on board the ISS. Conducting pilot studies will ensure that experiments can be developed with a rationale for needing the microgravity environment and will improve the probability of success on orbit.

Pilot studies help define the onboard experiment and mitigate differences between the Earth-based and space-based environments. Many of the indeterminate parameters of the experiment can be resolved during proper definition of the flight experiment. These include parameters such as the temperature, culture length, cell types, cell numbers, cell health, behavior of the model in the culture hardware, scaffolding, and cryopreservation specifications.

The program should provide capabilities for on-orbit sample analysis and sample return.

Rationale: A combination of capabilities for onboard analysis and return of samples to the ground will maximize achievable science by facilitating iterative onboard experimentation and enabling exploration-capable analysis technologies.

The panel preferred to move space cell science beyond the limited "experiment in a can" by facilitating as much sample analysis as possible on the ISS National Laboratory. The Decadal Survey noted, "Telemetric science without sample return will greatly facilitate increased sophistication in the design of space biology experimentation." However, the survey authors recognized that not all analyses could be performed on board and that there would still be a desire to return samples from the ISS National Laboratory. The panel agreed that "nice to have" technologies, such as mass spectrometry or reverse-phase protein microarray, can be performed more easily and cost-effectively on the ground. A good criterion for justifying on-orbit sample analysis technology is whether that technology would be used in deep space on exploration missions where samples cannot be returned. The flow cytometer is a good example of a technology useful for analyzing immune cells midflight, which will be required for evaluating crew health on deep-space missions.

The program should utilize the ISS as an exploration platform to test new, cutting-edge sample analysis technologies.

Rationale: The prolonged microgravity, intense radiation, and extreme isolation of the ISS environment make it the only venue where new space exploration-enabling technologies can be developed and proof-tested under actual operational conditions.

An example suggested by the panel is deep sequencing (or RNA-Seq), a next-generation high-throughput sequencing technology that provides unparalleled base-level resolution and quantitation of RNA sequence content in a given sample.

The Decadal Survey also notes that "Modern analytical techniques such as those employed in genomics, transcriptomics, proteomics, and metabolomics offer an immense opportunity to understand the effects of spaceflight on biological systems."

The program should make data accessible to other researchers, not only in the form of publications, but also in the form of the underlying data collected. The data should be accessible at multiple levels, and intellectual property issues should be addressed so that others can access and mine the data.

Rationale: Because of the unique logistical constraints and extremely valuable resources particular to the ISS National Laboratory, potential scientific output can be maximized through open data sharing and access.

In the past, more data have been gathered than are available in the published literature. These unpublished data may be useful to other researchers. To realize the greatest return on investment, data should be released to other investigators so that it can be mined for any significant scientific purpose not addressed by the original investigator. This data mining could also reveal other compelling scientific questions to pursue in further studies. The panel raised a concern about proprietary data and publication rights. These are issues that would need to be addressed, perhaps by a "wait period" before public release of the data.

The Decadal Survey similarly addressed data sharing. "The creation of a formalized program to promote the sharing and analysis of such data would greatly enhance the science derived from flight opportunities. Elements of such a program would include guidelines on data sharing and community access, with a focus on rapid release of these datasets while respecting the rights of the investigators conducting the experiments."

2.2 Cryopreservation

The program should develop cryogenic capability for the ISS National Laboratory and vehicles for launch and return.

Rationale: Cryopreservation and storage of cells would allow researchers to initiate cell cultures on orbit, allowing investigators to isolate the effects of microgravity, reduce the impact of launch delays and dependence on crew schedule, and enable iterative experimentation.

The current practice of transporting metabolically active cell cultures makes it difficult to isolate the effects of microgravity on the cells from the effects of launch acceleration and reentry on gene expression and cell signaling. It also makes the cells especially sensitive to launch delays because of concerns about overgrowth, viability, and feeding. Finally, it has had an impact on crew schedule by requiring crewmembers to shift their sleep period to receive the cells upon arrival of the transport vehicle.

In the Decadal Survey, cryopreservation systems were recommended as an enabling technology. The panel agreed that development of a cryogenically stored stock of cell cultures would enable launch of metabolically static cells, which would eliminate launch acceleration effects. Cryopreservation also enables iterative experimentation by allowing multiple cell stocks to be stored on orbit. Iterative experimentation would enable "cell science experimental flow" and allow prepositioning of cell stocks for multiple

investigators, which not only reduces variability, but enables a wider breadth of scientific investigation. A cryogenic stock of cells would also enable a "job jar" concept for cell biology research experiments—short-term experiments to be performed during slack time in the crew schedule.

The panel agreed that using stocks of cryogenically preserved cells was the nominal way to transport and store animal or human cell cultures, and that full cryogenic capability was a current major gap in technology for the ISS National Laboratory.

Generally, the vapor phase of a liquid nitrogen cryogenic freezer is used for the cryogenic storage of animal cell cultures. These freezers can reach cryogenic temperatures between -135°C and -196°C. It is known that storage of mammalian cells at -70°C can lead to cell degradation and loss of viability.

The panel understood that the freezer currently on the ISS National Laboratory, General Laboratory Active Cryogenic ISS Experiment Refrigerator (GLACIER), has a minimum temperature of -160°C, which is below the glass transition point of water at -135°C, and may be sufficient; however, short-term studies to elucidate any differences between storage in GLACIER at -160°C and storage in vapor phase liquid nitrogen may be needed to verify its utility.

Maintenance of a stock of cryogenically preserved cells would require the use of GLACIER full time during that period. GLACIER units on board transport vehicles, such as SpaceX Dragon, are incapable of maintaining a minimum temperature of -160°C, so other means of maintaining cryogenic temperature for transport should be used, such as liquid nitrogen dewars, which have been used in the past.

The program should provide hardware that can perform a standardized thaw of frozen and cryogenic samples.

Rationale: Uniform, rapid thawing is required to initiate viable cultures on orbit.

The thawing process is as critical to cryopreserved cells as the freezing rate and storage temperature. Generally, cryopreserved cells are removed from cryogenic temperatures and incubated in a 37°C environment (water bath) for 1 to 2 minutes until the frozen solution is just melted, or around 1°C. The solution is then dispensed into a medium or buffer at 37°C for further processing.

In past on-orbit experiments, cryopreserved cells were thawed by hand. This process not only introduces an uncontrolled variable into the experiment, it requires protective gloves and can consume a notable amount of crew time when multiple vials are required to initiate an experiment.

Developing a simple, standard method of thawing, such as a warming block, would reduce variability and save crew time. Automation of this task might further reduce variability and crew time.

The program should provide hardware that can remove the cryopreservative from the cells after thawing.

Rationale: Most cryopreservatives become toxic at culture temperatures (typically 37°C).

Mammalian cells are cryogenically stored using a cryopreservative, usually dimethyl sulfoxide (DMSO). DMSO is known to have negative effects on cells at the dilution used for cryopreservation (~10% by volume) at temperatures above 4°C. Removing or sufficiently diluting the cryopreservative can avoid these negative effects. In ground-based labs, the freshly thawed cells in cryopreservatives are typically added slowly (to account for osmotic pressure of DMSO) to culture medium in a centrifuge tube. The cell suspension is centrifuged so that the cells form a pellet at the bottom of a centrifuge tube. The liquid is removed, and fresh culture medium is used to resuspend the cell pellet from the tube. In past cell biology space flight experiments, the DMSO was diluted into culture medium in the culture vessel, then the medium was removed from the cells using a filter, and fresh medium was added.

The program should provide hardware that can freeze a sample at the rate of -1°C per minute down to -80°C, followed by cryostorage.

Rationale: This capability is required for returning viable cells from the ISS to the ground laboratory for further study.

The panel indicated a desire to return viable cells to the ground laboratory. To evaluate temporary or permanent phenotypic changes due to microgravity alone (and not due to stresses incurred during landing), mammalian cells must be cryopreserved.

In ground-based cell biology laboratories, a single cell suspension of mammalian cells is added to a medium or buffer with a cryopreservative, usually DMSO, and the suspension is frozen at a known rate, usually -1°C per minute. This can be attained using either a programmable freezer or a low-tech isopropanol bath-based Nalgene Freezing Container, also known as a "Mr. Frosty." The cells are added to the Mr. Frosty, then put into a -80°C mechanical freezer overnight. Then the samples are transferred to a liquid nitrogen vapor-phase cryogenic freezer.

A low-tech method using existing freezers on the ISS National Laboratory that are similar to Mr. Frosty would be acceptable, but the hardware would have to be modular to accept different configurations of transport containers.

2.3 Culture

The program should provide cell biology culture hardware that can support physiologically or pathologically relevant models, including three-dimensional culture of cells.

Rationale: The recapitulation of cell behavior in a cell model so that it is physiologically relevant and behaves like tissue in the body is critical for extrapolating experimental results to the whole animal. This recapitulation requires a three-dimensional growth environment, co-culture of several codependent cell types, scaffolding to enable cell attachment, extracellular matrix, and other components of the tissue-specific microenvironment.

Evidence exists that cell morphology and tissue architecture influence the pathway to cell replication, plasticity, differentiation, regeneration and repair, programmed cell death (apoptosis), morphogenesis, gene expression, signal transduction, and tissue-specific function. Because two-dimensional cell culture in flasks or plates does not generally recapitulate normal cell morphology and tissue architecture , cell-cell interactions, differentiation, or extracellular matrix formation, many experts in the field have argued that two-dimensional culture is not physiologically or pathologically relevant to tissues in the body, which function in three dimensions. Thousands of scientific papers have demonstrated that appropriate cytoarchitecture is one of the major requirements for recapitulating tissue structure and function found in the body. To extrapolate a response from cultured cells to the whole animal, one must create a three-dimensional tissue culture model with cells that behave like they do in the body. For example, mammary epithelial cells cultured in two-dimensional monolayers in plastic T-flasks will not express milk proteins even when stimulated with prolactin. However, when they are provided with the proper extracellular matrix, basement membrane, and three-dimensional architecture, they organize properly and start to express proteins. In addition, three-dimensional organotypic cell culture models of intestinal, respiratory, and reproductive tissues mimic the differentiated form and function of parental tissues in the body. The responses of these models to infection with pathogens, their toxins, and antimicrobials mimic the responses of the human body in key ways and cannot be observed in flat, two-dimensional monolayer cultures.

The program should provide hardware that can monitor, control, and/or alter as needed, and maintain essential cell culture parameters, including pH of the medium, carbon dioxide (CO_2) and oxygen (O_2) tensions, glucose levels, and temperature.

Rationale: Monitoring and controlling these parameters is vital to cell and tissue growth and survival.

The panel agreed that environmental monitoring was essential for determining the health of cultures; however, monitoring alone is not sufficient. Control of these parameters is required for maintaining optimal cell and tissue health. The set points for control of these parameters must have flexibility so that they can be modified to meet the needs of the specific tissue model.

CO_2 needs to be managed and maintained at the proper physiological level, usually at 35-45 mmHg (5%). The system must be capable of adding CO_2 when required and removing CO_2 as a culture progresses and metabolism increases. The pH of the culture can be controlled through the addition of buffer or fresh medium once the desired CO_2 tension is obtained.

Control of O_2 concentration is especially important for maintaining the correct physiological environment for the specific cell model. For example, some cell models, such as bone, cartilage, and stem cells, require hypoxic conditions for normal differentiation and development, whereas other cell models require normoxic conditions.

Control of glucose is important for maintaining a physiological and stable environment. Maintenance of the glucose level through delivery of a stock glucose solution or fresh medium enables the culture environment to remain stable and avoid large swings in glucose levels.

The panel agreed that the culture may need to be maintained at temperatures above and below the standard temperature of 37°C. One panel member related a story of an experiment involving cow embryos that was negatively affected because it required a temperature lower than 37°C, but the hardware was not capable of adjusting. Conversely, temperature is also important to heat-shock response studies, which typically involve temperatures higher than 37°C.

The program should provide cell biology hardware that can allow the culture of multiple cell types in the same culture vessel.

Rationale: Co-culture—or the culture of multiple cell types—is critical in creating physiologically relevant tissue models.

Tissues in the body are not composed of a single cell type. Physiologically or pathologically relevant models to be used as human surrogates must be able to mimic the actual three-dimensional tissue structure/architecture, function, and multicellular complexity found in the body. Important considerations are cell-cell interactions and whether cell types secrete or are activated by soluble cytokines. In these microenvironmental processes, multiple cell types clearly have different needs and make different contributions. Examples include stem cells, which require feed layers to provide essential growth factors; fibroblasts, which provide structural integrity to connective tissues by secreting extracellular matrix proteins; and immune cells that mimic the full complement of innate, adaptive, and mucosal immunity. The inclusion of multiple cell types, including but not limited to those mentioned above, is important for development of organotypic tissue models that mimic parental tissues in vivo. These studies may also include host and bacterial cells for studies of pathogenic, commensal, and beneficial bacteria.

The program should provide cell biology culture hardware with culture compartments that have been designed to mitigate or eliminate unique fluid dynamics phenomena, including nutrient starvation, reduced cellular- or tissue-level oxygen tension, and toxic waste accumulation, that may be present in a reduced-gravity environment.

Rationale: Fluid dynamics in microgravity will have a significant impact on the behavior of the cell culture hardware, and it will be critical to understand the effects of mass transport, mixing, and fluid shear, as well as bubble formation and removal.

An evaluation of fluid dynamics should include an understanding of not only the behavior of the fluid itself, but also how the cell aggregates interact with the nutrients and waste products in the fluid in microgravity. For example, 200 µm is the limit of diffusion in a gravity-dependent system with cells in scaffolding, meaning that nutrient and waste molecules diffuse across only 200 µm in an unmixed system. Beyond that, cells are not oxygenated well without a vascular network to permeate the structure. This traditional diffusion limitation is encountered in static cultures. Bioreactor technologies exist that could be translated to space flight to mitigate those issues.

The program should provide cell biology culture hardware that allows variable sample volumes to be withdrawn from culture chambers.

Rationale: Different investigations require different sample volumes, depending on the analyses that are being conducted.

The panel discussed the many different volumes they would expect to need to sample, and it was clear that they covered a broad range, from microliters to milliliters. This recommendation ties into the recommendation for accuracy, precision, and reproducibility as well as the need for definition of the flight experiment.

The program should provide cell biology culture hardware that allows sequential, independent sampling to facilitate collection of multiple samples over a predefined time period (kinetic analyses).

Rationale: Time-course data are needed to understand the processes that contribute to the final outcome.

Many studies in cell biology consist of a kinetic analysis over a period of time, meaning that several samples are taken at different time points; e.g., time zero, 1 hour, 2 hours, 4 hours, 8 hours, and so on. Time-course experiments may be used to determine the sequence of events and/or the timing of events leading up to the final outcome (e.g., a protein must be cleaved before the effect is seen or a compensatory mechanism must be overwhelmed before the effect is seen). There should be enough flexibility so that the time scale could occur on the order of minutes, days, weeks, or months.

This capability would be very useful and important to the cell biology community. A small volume could be drawn out of the culture vessel at each time point, or the entire culture vessel (one of a large array of culture vessels) could be harvested at each time.

The program should provide cell biology culture hardware that can use different types and sizes of scaffolding.

Rationale: Anchorage-dependent cells, which are used frequently in cell biology, require a structure, or scaffolding, on which to grow.

Many types of scaffolding, including beads, sponge-like meshes, hollow fibers, semi-solid gels, or even decellularized tissue, can be used to provide structure in three-dimensional cell culture and are critical for physiological cell and tissue structure and function. The type of scaffolding will depend on the investigation, and the hardware should be flexible enough to accommodate different types of scaffolds used in the cell biology discipline.

Scaffold-based approaches to tissue engineering are invaluable for constructing in situ functional tissues that recapitulate key aspects of the differentiated form and function of their parental tissues and facilitate greater mechanistic understanding of cell-cell and cell-matrix interactions, tissue organogenesis, and three-dimensional structure and function. In scaffold-based approaches, one can go from micrometer scale to macro scale. Having the capability of accommodating different types and sizes of scaffolding would appeal to a broad range of investigators.

The program should provide cell biology culture hardware that can make it possible to view cell cultures in situ without removing the cells from their respective culture chambers.

Rationale: Visualization of the cells is a basic capability required for routine monitoring of culture health and is critical to the iteration paradigm of experimentation.

The panel discussed the criticality of viewing the cell cultures during the course of the experiment. They agreed that the cell culture hardware should not be "entirely closed in a black box," but should be flexible enough to allow visualization to determine culture health and status (i.e., whether the culture is ready to proceed to the next experiment phase).

Two different kinds of visualization that are critical to cell and tissue culture are represented in normal laboratory operations. One is visualization by in vitro *microscopy, which is microscopic examination of the cells in the culture vessel, usually to inspect general cell behavior, contamination, and health of the cells. The other distinct type is analytical microscopy, which is microscopy of a sample that has been taken out of the vessel and processed, and its morphology has been analyzed with the microscope by a user for a specific measurement. The lack of visualization hardware is a gap that was identified in the NASA Space Cell Biology Project Proposal in 2010, and filling it is essential for successful experimental outcomes. The Decadal Survey similarly identified "in situ imaging systems to visualize changes in cell shape, configuration, and molecular tags" as an enabling technology. A microscope of this type would most likely require phase contrast capability with a magnification range of 40X to 200X, to visualize mammalian cells in cell biology experiments.*

The panel understood that this process would require crew time and that there will be a need to build in some process or schedule availability to accommodate the process.

The program should provide cell biology culture hardware that can accommodate a broad range of cell numbers and/or tissue construct sizes.

Rationale: Culture size depends on the end goal of the experiment and may vary widely.

The panel agreed that the capability should exist to have a broad range of culture volumes based on total cell number and size, because of the broad range of different cell types in co-culture and different subdisciplines in cell biology.

The initial number of total cells may range from 1 million to more than 100 million, depending on the desired experimental outcome. For example, analysis of the cell culture by deep sequencing, proteomics, or microarrays may require few cells or multicellular aggregates 200-300 µm in size, whereas a tissue-engineered construct may consist of hundreds of millions of cells and be several centimeters in size.

The program should provide cell biology culture hardware that can be used for short-term and long-term experiments.

Rationale: Experiment duration depends on the outcome being measured.

Some cellular changes, such as phosphorylation of important signaling molecules, may occur rapidly (within minutes or hours), whereas changes in protein expression may require hours or days. Weeks may be required for development of cell aggregates for exposure to a study stimulant such as a drug, toxicant, or pathogen. Studies of the functional effects of long-term microgravity may require several months to a year or more. Given this wide range of potential study durations, the panel agreed that a broad duration capability should exist for the culture phase of the experiment, from weeks to at least 1 year, depending on the investigation. Some long-term cultures may require subculture, as described below.

The program should provide cell biology culture hardware that enables serial passaging or splitting of cells.

Rationale: In cultures maintained for any substantial period of time (more than several hours to a few days), subculture may be necessary to avoid cell death due to overgrowth.

Cells cannot be held in culture indefinitely because a gradual rise in toxic metabolites, use of nutrients, and number of cells is associated with growth. Subculture, the process of removing a small population of cells from an active culture vessel and starting a new culture in a new culture vessel with fresh nutrients, is therefore used to reduce cell density and prolong the experiment. Subculture is especially important for proliferating cells, but is also important for terminally differentiated cells.

In combination with cryopreservation, subculture can also be a tool to support iterative experimentation and reduce the need for reflight of cells.

The program should provide cell biology culture hardware that may be accessed on orbit by crewmembers to facilitate assessment of and situational response to culture parameters, sampling of cells and media, and maintenance of culture conditions as needed.

Rationale: Conducting cell biology research in ground laboratories involves daily access to the experiment for these types of routine assessments.

Access to the culture hardware is a basic capability required for the maintenance and health of cell cultures. The panel agreed that cell biology in microgravity should move away from the concept of "experiment in a can" or in a "black box" and incorporate crew interaction where possible. However, the panel understood that this requirement must be evaluated in the context of crew time availability.

The program should provide hardware that can maintain cell culture chambers in an induced gravity field that is selectable from microgravity up to and including 1*g* (9.81 m/s^2) continuously for the duration of the culture.

*Rationale: This capability would support the addition of 1*g *on-orbit controls and fractional gravity studies.*

*Flight and lunar-surface centrifuges were identified as an enabling technology in the Decadal Survey. A 1*g *on-orbit cell culture environment provides additional experimental controls that will include other environmental factors, such as experiment operations, space radiation, and other factors that cannot be replicated in ground laboratories.*

The additional capability of fractional gravity would provide insight into the kinetic effects of gravity on cell biology (e.g., differences at ¼, ½, ¾ g*). This capability would also provide a platform for evaluating cellular-level effects of fractional gravity during exploration of near-Earth objects, the Moon (~$^1/_6$* g*), and Mars (~$^1/_3$* g*).*

For each of the uses mentioned here, this capability would be required for the duration of the culture phase of the experiment, which may range from hours to months. It would also require that different types of culture hardware be modular and able to interact with and fit within the centrifuge or other instrumentation that is reintroducing gravity.

2.4 Sample analysis and storage

The program should provide cell biology culture hardware that supports an analytical microscopic view of cell, tissue, or media samples obtained from culture chambers.

Rationale: An analytical microscope would provide critical on-orbit capability for live cell imaging, fluorescent staining, and evaluation of DNA double-strand breaks.

The panel agreed that a need existed for real-time microscopy of the cells after sample processing. This need was similarly identified in the 2010 NASA Space Cell Biology Project Proposal.

This microscope would need an automated stage, light microscopy, phase-contrast microscopy, and multi-wavelength fluorescence microscopy with magnifications from 40X to 1000X, analogous to a high-fidelity microscope found in ground-based state-of-the-art cell biology laboratories. It should be capable of interacting with several different types of culture hardware. There may also be a need to integrate with a glove box for containment of toxic chemicals.

The program should provide centrifugation hardware that can be used in culture manipulations and sample processing.

Rationale: Centrifugation is a fundamental capability, universal to cell biology laboratories, that would enable fractionation.

Centrifugation is frequently used in ground laboratories to separate suspensions of cells or cell fractions (cell membranes and nuclei) from liquids by using acceleration to force cells to the bottom of a test tube into a pellet. Centrifugation would also enable the use of column purification of DNA, RNA, and protein, which is critical to achieving the recommendation of collecting matched samples. Centrifugation may also help with removal of bubbles from the culture suspension.

This sample centrifugation hardware should be modular in that it can be used by a broad range of cell biology hardware.

The program should provide a means of on-orbit pre-analysis processing of biological samples.

Rationale: Biological material is fragile and may be damaged or lost if not properly processed before it is returned to Earth.

For example, RNA samples are important for gene expression analysis but are very fragile and rapidly degrade if they are not stabilized quickly. Multiple stabilization techniques exist for RNA, including flash freezing, immersion in an aqueous sulfate solution such as the commercially available RNALater, and reverse transcription of RNA into the more stable complementary, or cDNA, strand. Another example is the use of TRIzol, a reagent that separates proteins from genetic material such as DNA and RNA. TRIzol has the additional advantage of being able to simultaneously isolate RNA, DNA,

and protein into separate fractions, which would facilitate the recommendation to collect matched samples.

Proteins and other molecules may also be stabilized by "snap-freezing," which usually consists of freezing a sample very quickly in liquid nitrogen. This is not to be confused with cryopreservation, which requires a slow freeze in mammalian cells. Quick freezing will soon be performed on orbit for the MICRO-5 experiment using a pre-chilled block, which will then be inserted into a freezer. In this case, a 2-ml sample will be frozen within a few minutes, which was acceptable to the investigator. However, more rapid freezing may be required for other applications, and freezing time will depend on sample volume.

The program should investigate expanding the ISS catalog of available and allowed chemicals for biological sample processing.

Rationale: The use of better biological sample-processing chemicals, such as phenol, TRIzol, and trichloroacetic acid (TCA), would improve sample stability, resulting in improved scientific quality and return on investment.

TRIzol is used in a method of RNA-DNA-protein preparation from cell samples called guanidinium thiocyanate-phenol-chloroform extraction. The panel agreed that having the ability to use TRIzol would significantly improve the quality of sample return by enhancing DNA, RNA, and protein sample stability and improving molecular genetics assays. As mentioned previously, the use of TRIzol would also enable the collection of data from matched samples, which the panel agreed was critical to ensuring that the investigator gets the most value out of the material.

The panel acknowledged that these chemicals are toxic and would require handling precautions. One panelist affirmed that TCA has been used in the past. Another panelist explained how TRIzol had been approved and its containment in hardware developed before his program was canceled.

Alcohols are another group of common biological sample-processing chemicals. The panel acknowledged that the use of alcohols is restricted on the ISS because of their impacts on the Environmental Control and Life Support System (ECLSS) that result from their volatility and water solubility. The panel agreed that the current gap in the use of alcohol-based fixatives should be evaluated to determine whether an acceptable amount or mitigation method exists to allow the use of such fixatives on the ISS.

The program should provide the capability to collect matched samples.

Rationale: Collection of matched samples reduces variability and ensures that the greatest scientific value is realized from a single experiment.

Analysis of matched samples (identical samples split for different analyses) is standard practice in ground-based research laboratories. The objective of using matched samples is to obtain better estimates of true differences by "removing" the possible side effects of experimental variables–e.g., by analyzing RNA, DNA, and protein from the same sample.

Matched samples would also enable data or sample sharing, which maximizes the scientific value of a single space flight experiment. For example, one investigator may be interested in examining gene expression or epigenetic changes, and another may be interested in differential phosphorylation of a particular protein. The use of TRIzol as a sample processing agent would enable extraction of the sample into three separate macromolecular fractions.

The program should provide additional cell biology analysis hardware, including a microscope with live cell imaging and fluorescence capability, a centrifuge for sample processing, and a flow cytometer for cell counting, sorting, and biomarker detection.

Rationale: On-orbit analysis would reduce requirements for down-mass, access to transport vehicles, transfer logistics (both on orbit and on the ground), and docked operations.

The panel discussed on-orbit analysis at length and agreed that an urgent and immediate need exists for real-time analysis. The reasons for on-orbit analysis include these:

- ***Analysis of extremely sensitive endpoints:*** *Cold storage and/or chemical fixation of samples acquired on orbit for later ground-based processing, highlighted earlier in this report, is sufficient to facilitate a wide range of data analyses. However, for some biological components, which are highly labile, transient, or incompatible with cold storage and/or chemical fixation, expedited on-orbit analysis is required to obtain data before the samples degrade. Returning samples to the ground laboratory can add an extra confounder to interpretation of the data. Achieving the goal of analyzing samples on orbit would also require the minimization of perturbations, hardware glitches, and external environments. The only reason to freeze such samples would be to analyze them as a batch to reduce variation or to process excess samples at a later time.*
- ***Experiment modulation:*** *Having the capability to analyze samples on orbit would enable investigators to modulate their experiments according to outcomes. In other words, a result obtained immediately after the experiment is performed can be tested in a follow-up experiment, rather than waiting months for return of samples.*
- ***Utility:*** *The capability to analyze samples on orbit will increase the utility of the ISS National Laboratory.*
- ***Variability:*** *The capability to analyze samples on orbit using the same crew technician under the same conditions, with replication as needed, will greatly reduce variability.*
- ***Failure:*** *The capability to analyze samples on orbit could detect failure in a flight experiment long before the samples are returned to the ground and allow troubleshooting and reiteration of the experiment to maximize scientific value.*

The panel acknowledged that the ISS cannot accommodate all of the analytical capabilities available on the ground and that it will be important to retain return capability. However, the group determined that a microscope with live cell imaging and

multiple-wavelength fluorescence capability, a centrifuge to support sample processing and fractionation, and a flow cytometer for sample analysis and to support on-orbit crew health evaluations are three critical pieces of hardware that the program should develop to support high-quality cell science on the ISS National Laboratory. The panel also discussed repurposing existing hardware, such as using the bone densitometer as an x-ray source for radiation biology experiments.

The program should provide cold-storage capabilities suitable for storage of experiment supplies and experiment samples, for all phases of space-based cell science research.

Rationale: Noncryogenic storage capabilities at 4°C, -20°C, and -80°C are required for culture supplies and samples because of specific limitations of various biological and chemical components of these research-related items.

2.5 Quality

The program should support the use of numbers of replicates that will produce statistically significant results according to experimental design.

Rationale: The minimum sample size required for adequate statistical power is determined by the experiment, but will require multiple biological and technical replicates.

Sample size will vary according to the magnitude of the outcome being measured and is experiment-dependent. The important thing is to have a sufficient number of samples to be confident in the outcome (i.e., have sufficient statistical power to detect a true difference between groups). Generally, a minimum biological replicate number of n *= 5 is used in animal studies, and* n *= 3 is commonly used for most cell and molecular studies. A larger* n *increases confidence in reproducibility to build on existing data and produce a meaningful result. However, the panel agreed that a single experiment (*n *= 1) can provide valuable data, especially when those data are reproduced in multiple technical replicates and/or numerous ground-based analog experiments. For example, very drastic changes were seen in cell cultures in a single rotating-wall perfused system on STS-107. Some circumstances, such as accidental toxic exposure, cannot be repeated for ethical reasons but provide valuable human exposure data.*

The panel discussed the distinction between biological replicates and technical replicates in cell biology. Biological replicates are samples taken from three different people, animals, or cell cultures. They measure the variability between those different sources of biological material and are often referred to as n*. Technical replicates are samples taken from the same source but measured multiple times in the same assay as a means to measure variability in the assay. Replicates are important to help mitigate the risk of contamination and technical failure of hardware.*

The number of biological replicates is usually limited by the hardware. Hardware containing multiple vessels or chambers is preferred; however, the panel agreed that one could use existing hardware with a single vessel or chamber if that is the only available option. Investigators have to ask questions that can accommodate the

limitations of the hardware, but it is equally important that hardware design keep up with current cell culture needs. A trade-off between statistical utility and hardware practicalities may be necessary.

The program should continue to support the use of ground controls for cell biology experiments on the ISS National Laboratory.

*Rationale: It is critical that ground-based controls be included in all in-flight studies (regardless of the presence of onboard 1*g *controls) to distinguish the reactions of cell models in a 1*g *environment and a simulated microgravity environment from reactions of the models in the reduced-gravity field experienced aboard the ISS.*

Access to ground-based hardware and experiments maximizes the interactions possible between co-investigators and technical staff, which greatly enhances interpretive power for collected data, rapid and efficient iterative experiment planning, and hands-on diagnostics of anomalies that may arise in experiments and/or experiment hardware.

The program should require robust characterization of the cells used in space cell biology experiments and should standardize cell models being used for space cell biology studies.

Rationale: Components of an experiment model (e.g., cell type in this requirement) must be well characterized and documented to delineate inherent variability in responses to normal environmental stimuli before effects of an experimental stimulus can be teased out of study data.

The basic characteristics of the cells should be known and standardized to control variability in the experiments that is unrelated to variability that may be caused by an experimental stimulus (e.g., exposure to microgravity). Knowledge of these characteristics includes knowing how the cells are cryopreserved, knowing their passage number or how many times they have been subcultured from the original population of cells from the cell repository, and knowing their growth phase characteristics. These different parameters should be standardized. Standardization will help control variability in the experiment as iterations are performed, and allow other investigators to more easily replicate and build on previous experimentation.

The program should support the standardization of cell models being used in cell biology experiments.

Rationale: Cell and tissue models must be standardized to reduce or eliminate variability that may occur each time the model is used in an experiment.

As noted in the Decadal Survey, "Model systems offer increasingly valuable insights into basic biology." To maximize the usefulness of these systems, the panel agreed that the program will need to make a concerted effort to standardize cell models across the program so they can be replicated in other ground laboratories and with the flight hardware as faithfully as possible. Standardization does not preclude development of

new models, but rather ensures that all flight models are well characterized and can be replicated by others.

The program should provide a fluid sampling and dispensing system that is analogous to a pipette or micropipettor and has reproducibility, accuracy, and precision comparable to accepted research standards.

Rationale: Consistency, accuracy, and reproducibility in fluid sample handling operations such as injections and withdrawals are critical to cell and molecular biology experimentation and to reducing and/or eliminating intra- and interexperiment variability.

Definitive knowledge of critical parametric data concerning the quantities of delivered constituents (e.g., cell culture media, nutrient supplements, experimental treatments) is important in understanding how well background variability is controlled in the experiment.

The program should support the use of internal controls for cell biology experiments.

Rationale: The inclusion of internal standards (e.g., molecular weight markers) provides quality control markers to assess the consistency, accuracy, and reproducibility of experiment manipulation processes and provides known standards by which to interpret experimental findings.

The reaction of the internal standard to experimental conditions and sample-handling processes allows these global effects to be separated from any potential effects due to the variable being studied. An example of using an internal standard is injecting a known amount of a compound into the culture being studied, then analyzing for that compound and measuring the variance between samples so that it can be corrected. The panel agreed that it would be important to include sample-handling standards and controls to analyze sample handling. This is a standard approach in metabolomics research, and it should be an ISS National Laboratory strategy.

3.0 Appendix A – Additional Considerations

This appendix represents a number of topics that the authors and/or panel members believed to be useful considerations for the future of cell biology on the ISS National Laboratory but that were not specifically discussed during the face-to-face meeting in March. This list was reviewed by the panel during the development of this report.

Enumerating single-cell suspensions of viable cells. The enumeration of single-cell suspensions of viable cells is a critical daily operation to estimate a cell density (cells/mL) based on physical counts of cells in a representative sample. Enumeration is used to prepare a culture vessel with a known number of cells for experimentation. Enumeration will be critical if cell cultures are subcultured from stock cultures of cells before the experimental phase—a routine technique used in ground laboratories. Subculture is also a strategy used to negate the effects of cryopreservation on the cells by culturing the cells for days or weeks after thawing. Some flow cytometry-based technologies that could enumerate cell densities, such as the Guava Personal Cell Analyzer, have been verified in parabolic flight to operate successfully. Enumeration also will be important to iteration of cell experiments.

Subculturing cells from three-dimensional cell aggregates. No standard method exists to subculture a culture of three-dimensional cell aggregates into a single-cell suspension of measurable cell density (cells/mL) to seed a new culture vessel. Routine ground laboratory operations for subculture involve the enzymatic dissociation of two-dimensional monolayers of cells from plastic flasks, enumeration of viable cells, and dispensation of a known quantity of viable cells into either a plastic flask or a three-dimensional culture vessel. Subculture of cell aggregates grown in three-dimensional culture vessels should be investigated and developed for future cell biology experiments on the ISS National Laboratory.

Making sample-processing holding temperatures selectable from 0°C – 100°C. Sample processing will require a method of holding samples during processing steps at different specific temperatures for specific reasons related to the chemistry of the processing step. For example, it will be necessary to hold samples at 1°C, analogous to keeping them on ice. It will also be necessary to hold samples during a processing step at 37°C. Sample-processing technologies should have enough flexibility to hold an elevated temperature at different points up to 100°C. The technologies used will be analogous to ice baths, water baths, and heating blocks routinely used in cell biology laboratories on the ground. These technologies will need to be modular with the culture and sample-processing hardware so that they can be used by different experiments and culture hardware.

Using industry standards for liquid volume manipulations. Industry standards exist, including American Society for Testing and Materials E542 – 01, “Standard Practice for Calibration of Laboratory Volumetric Apparatus,” and International Organization for Standardization 8655, “Piston-Operated Volumetric Apparatus,” that may be useful and applicable for developing a fluid sampling and dispensing system.

Monitoring and recording the experiment-level gravity environment. The program should provide the means to monitor and record the acceleration due to gravity during the conduct of

each cell science experiment. Steady-state and transient “spike” gravity loads should be measured and recorded for future reference in interpretation of experiment findings. Records of steady-state acceleration may be obtained from ISS station-wide monitoring devices, and recordings of transient gravity loads should be measured at a location in or very near the experiment location. Transient load events exceeding a magnitude of $1 \times 10^{-3} g$ will be sufficient to meet this requirement.

Monitoring and recording the experiment-level radiation environment. The program should provide the means to monitor and record the total received radiation dose as well as the time-dependent dose at the location of each cell science experiment aboard the ISS. The ISS, by the nature of its location in space, is exposed to a radiation environment different from that found at the surface of the Earth. Furthermore, different locations on board the ISS may be shielded from environmental radiation, so that the overall exposure profile will be modified even further. For these reasons, principal investigators must have a record of the radiation exposure profile and total radiation dose to meaningfully interpret the data they receive from onboard experiments.

Creating a set of standardized cell and tissue models that can be used by investigators. The existence of a baseline set of physiologically relevant cell and tissue models would be useful. The models should be standardized for many parameters such as scaffolding type, culture medium formulation, cell types, number of cells, and order of cell seeding. A collaboration or consortium of experts should work to define the models and perform analyses to characterize their cytoarchitecture and functionality. The models and data should be published or made available for scientists to use or modify to meet their experiment requirements.

Performing benchmarking with other national laboratories. Many national laboratories utilize shared core facilities for culture, environmental exposure, and analyses to support biological research. The ISS National Laboratory should consider benchmarking other national laboratories that support similar research. An example of such a lab is the Brookhaven National Laboratory, where established relationships exist to study radiation environments. This benchmarking should be done to 1) better outfit and equip the ISS National Laboratory with analytical tools and instrumentation, and 2) develop the most effective method to ensure that sufficient access is readily available to investigators’ experiments and that the equipment is always in working order. Not all data obtained from the benchmarking of other national laboratores will be applicable to the ISS because of the unique environment and operations on board. A panel of experienced investigators and payload developers should guide the ISS National Laboratory in determining the needs that should have highest priority and in using the resources most efficiently.

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In March 2013, an external panel of experts from various cell biology, microbiology, and tissue engineering fields was convened to develop and recommend a set of scientific requirements that could be used to steer strategic planning and tactical execution of these fields of research on the International Space Station (ISS) National Laboratory. Panel discussions focused on feasibility assessments, culture and specimen types, the in-flight culture environment, in-flight experiment handling and processing, and sample return. The panel made 41 recommendations for the future of cell biology experiments on ISS National Laboratory. Recommendations fall within these categories: overarching strategies; cryopreservation ; culture ; sample analysis and storage ; and quality. Panel members discussed the need to establish a world-class research facility that can address the 2011 National Research Council Decadal Survey mandate to provide research that both “enables space exploration” and is “enabled by access to space.” Panel members were made aware that requirements should be implemented immediately and with urgency. Furthermore, the panel was asked to elucidate new sample processing and analysis capabilities needed to maximize scientific return for the benefit of human health and quality of life..

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