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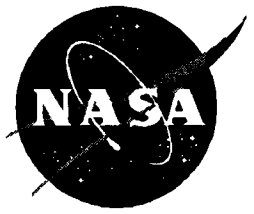

Biotechnology System Facility: Risk Mitigation on *Mir*

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ACRONYMS AND ABBREVIATIONS

A, amp	Ampere
BCM	biotechnology system computer module
BCSP	biotechnology cell science program
BEM	biotechnology experiment module
BSTC	biotechnology specimen temperature controller
BTF	Biotechnology Facility
BTR	biotechnology refrigerator
BTS	biotechnology system
DACS	data acquisition and control system
ECC	experiment control computer
EDU-M	engineering development unit – Mir
EE	engineering evaluation
EPROM	erasable programmable read only memory
GSM	gas supply module
GSS	gas supply system
HDD	hard disk drive
I/O	input/output
IBMP	Institute for Biomedical Problems
IFM	in-flight maintenance
ISS	International Space Station
JSC	Johnson Space Center
KSC	Kennedy Space Center
LCD	liquid crystal display
LED	light-emitting diode
Mini-PIC	Miniature Payload Integration Center
ML	milliliter
MOST	*Mir* Operations Support Team
MPOSA	microgravity payload operations support area
MSFC	Marshall Space Flight Center
PCMCIA	Personal Computer Memory Card International Association
PM	power module
psi	pounds per square inch
psia	pounds per square inch at atmospheric pressure
psig	pounds per square inch gauge
PTFE	polytetrafluoroethylene
PUP	payload utility panel
ROM	read only memory
RRS	radiation recovery software
SBC	single-board computer
SCSI	small computer standard interface
SRAM	station rundown access memory
STES	single locker thermal enclosure system
SVGA	super video graphics adapter
TCM	tissue culture module
TEC	thermoelectric cooler
UCB	urine containment bags
USL	United States Laboratory
Vdc	volts direct current
W	watts

1 INTRODUCTION

The National Aeronautics and Space Administration (NASA) is working with its international partners to develop space vehicles and facilities that will give researchers the opportunity to conduct scientific investigations in the low-gravity environment of space. As part of this activity, NASA's Biotechnology Cell Science Program (BCSP) at the Johnson Space Center (JSC) is developing a world-class biotechnology laboratory facility for the International Space Station (ISS). Figure 1-1 shows the Biotechnology Facility (BTF) design concept. Operating the BTF on the ISS will allow investigators to use NASA cell culture technology and the microgravity of space to advance groundbreaking research in biomedical science. Currently, the BTF is designed to support investigations in the promising areas of microgravity-based cell culture and the engineering of tissues for research, transplantation, and production of biopharmaceutical agents.

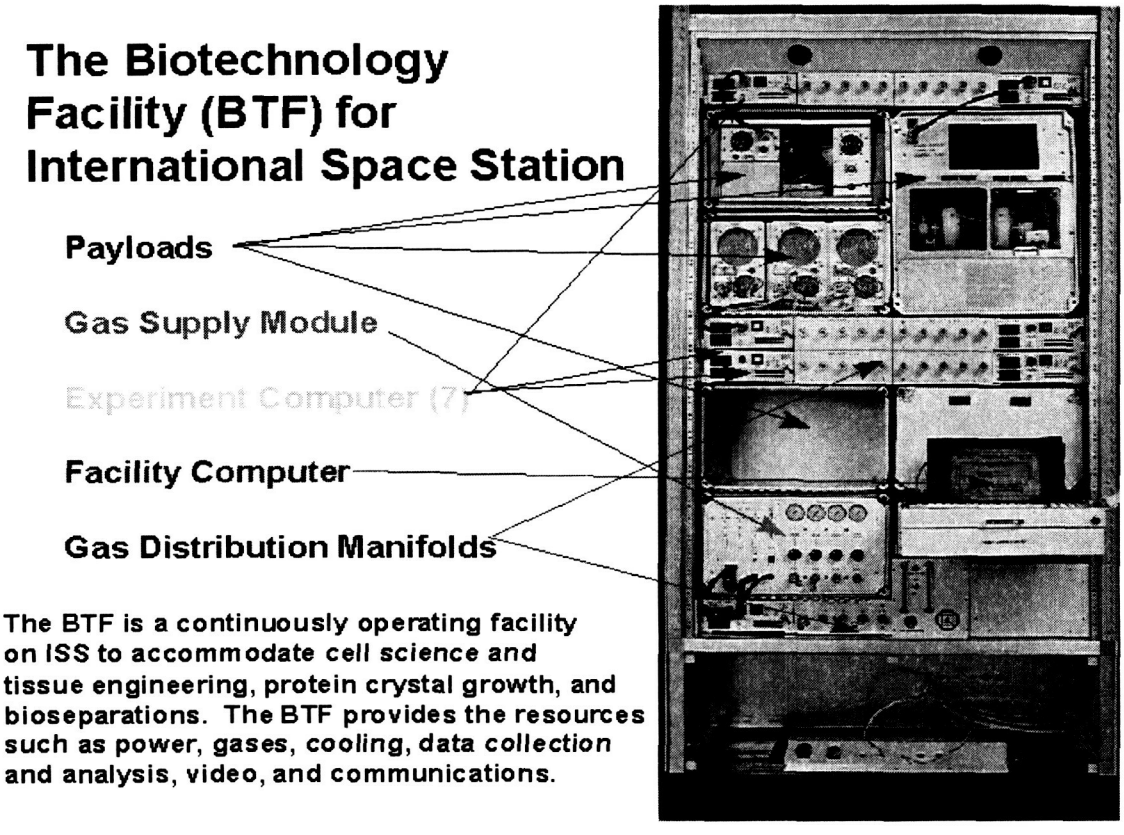

Figure 1-1. Biotechnology Facility.

The Cellular Biotechnology Program at JSC has used a phased approach in developing bioreactors and support systems for on-orbit biotechnology investigations. In the recent past, the primary vehicle for microgravity experiments has been the Space Shuttle. Biotechnology cell culture flight experiments began in 1992 on short-duration missions in the Space Shuttle's middeck and were extended to 16 day-missions during the Extended-Duration Orbiter Medical Project (EDOMP) (1). In parallel with the phased ISS Program, the Cellular Biotechnology

Program conducted substantial risk mitigation of the BTF design concept during Phase I of the ISS Program. The Russian *Mir* space station was used as the space platform during Phase I for nearly 2.5 years for both fundamental cellular biotechnology science experiments and on-orbit evaluation of BTF hardware and system components. Phase I provided the Cellular Biotechnology Program with an opportunity to conduct long-duration experiments in the microgravity environment in a rack-type facility, a forerunner of the BTF. Another important goal of Phase 1 was to reduce the end-to-end risk of designing, developing, and operating multiple cellular biotechnology payloads in the ISS. Phase 2 of the ISS Program is the ongoing development, assembly, and operation of the ISS. This report will focus on the JSC BCSP's design of the BioTechnology System Facility, its integration and launch in the Priroda module, and its 2.5-year operation on *Mir*. In addition, this report summarizes the lessons learned from the BTS experiments performed on *Mir* that are documented in this report, which can and will be used to "mitigate the risk" of designing, developing, integrating, and operating the BTF on the ISS.

The NASA cellular biotechnology cell culture and tissue engineering equipment and technology used on *Mir* was a forerunner to the elements of the BTF, which is planned for the ISS. The Biotechnology System (BTS) facility was launched in the *Mir* Priroda module in April 1996. The BTS contained the hardware and critical resources required to support the long-duration fundamental science investigations planned for its tenure on *Mir*. The BTS design was based on the BTF concept of a single-rack facility. The design included all the hardware and capabilities planned for the BTF but, unlike the BTF, the BTS was designed to support one experiment module at a time. This report describes the BTS design and operations on the *Mir*. The *Mir* space station has been the cornerstone of the Russian space program for over a decade. *Mir*, shown in Figure 1-2, has been in low Earth orbit from 1986 to 2001 (2). *Mir* had accommodations for both pressurized (internal) and unpressurized (external) payloads. Most U.S. payloads flown as part of the ISS Phase 1 NASA-*Mir* Science Program were operated in the pressurized volume of *Mir*. The *Mir* pressurized volume has been expanded over time by adding modules. The Priroda and Spektr modules were added to *Mir* as a result of U.S.-Russian cooperation on the NASA-*Mir* Science Program to provide living quarters for U.S. crewmembers and to serve as a location for performing U.S. science experiments in a variety of disciplines. The NASA cell science experiments on *Mir* were performed in the Priroda module, shown in Figure 1-3. A series of eleven Shuttle missions were performed as part of the Shuttle-*Mir* Program from February 1994 to June 1998. Nine of these missions involved docking the Shuttle with *Mir*. One U.S. crewmember remained on *Mir* between dockings to operate the U.S. experiments and help the Russian cosmonauts maintain the space station. Another primary objective of the ISS Phase 1 Program was to cultivate a good working relationship between the U.S. and Russian space programs at various levels. The BTS played a prominent role in working toward all goals and objectives of the Phase 1 Program as evidenced by the large number of long-duration experiments performed using the BTS.

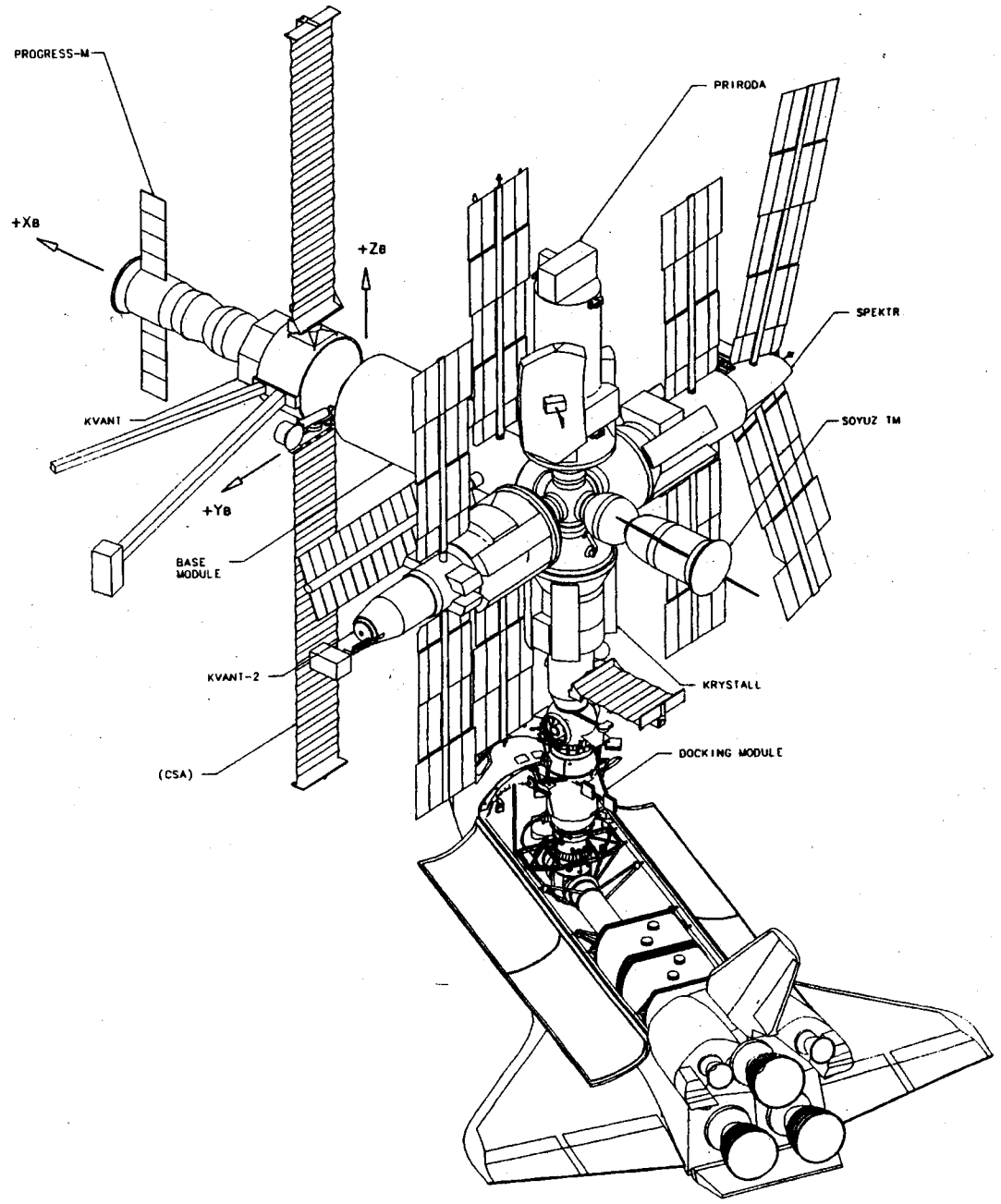

Figure 1-2. Mir space station.

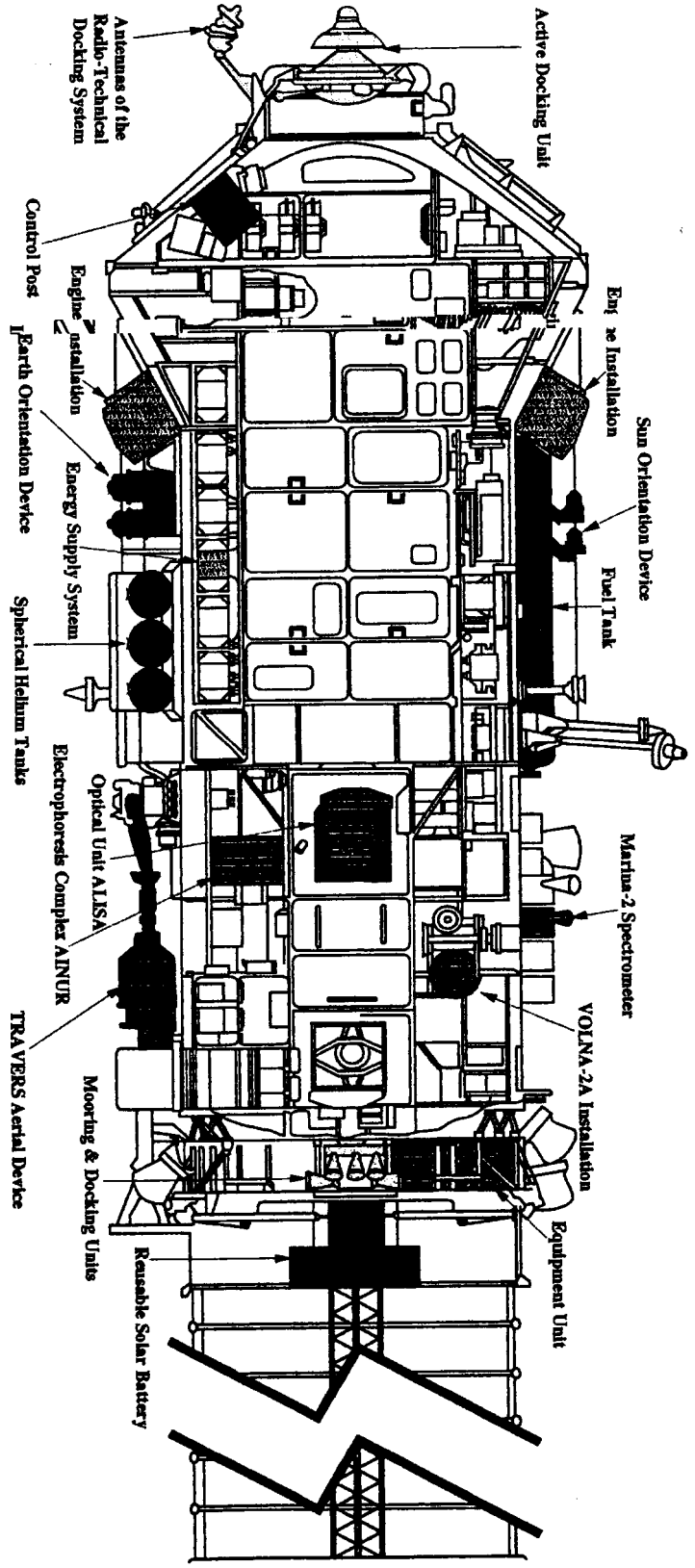

Figure 1-3. The Priroda module.

This report describes the overall NASA BCSP, including the role of the BTS in this comprehensive flight program of peer-reviewed science. We identify the purpose and objectives of the BTS. We also include a detailed description of BTS facility design and operational concept, BTS facility and experiment-specific hardware, and scientific investigations conducted in the facility. This report also identifies the objectives, methods, and results of risk mitigation investigations of the effects of microgravity and cosmic radiation on the BTS data acquisition and control system (DACS). The results of these investigations may apply to many other space experiments that use commercial, terrestrial-based data acquisition technology. Another focal point of this report is a description of the end-to-end process of integrating and operating biotechnology experiments on a variety of space vehicles, including the launch, on-orbit, and landing phases of this process. The identification of lessons learned that can be applied to future biotechnology experiments is an overall theme of the report.

Astronauts performed five major experiments in the BTS facility during the NASA-*Mir* Science Program. The NASA BCSP was one of the most active science and engineering teams during the program because of the amount of hardware flown and the complexity of crew operations required to perform the experiments. These investigations involved multiple flights to *Mir*. Both the Shuttle and Russian space vehicles ferried NASA Cellular Biotechnology Facility and experiment hardware and biological samples to and from *Mir*. The risk mitigation and fundamental biology experiments were performed during increments 2-7 of the Phase I program. An increment is a period, between Shuttle dockings, during which long-duration U.S.-Russian experiments were performed on *Mir*. The increments typically lasted 120 days, but some were longer because of unplanned Shuttle launch delays. A different U.S. astronaut was assigned to each increment. This dictated a requirement for essentially continuous training activities for both the BTS facility and the experiment-specific hardware. The NASA cell science program on *Mir* was successful in several respects, not the least of which is the scientific results achieved from the cell science and risk mitigation experiments.

The report includes a brief summary of the science results, but this is not the focus of the report. Detailed science results are documented in the scientific journals dedicated to the disciplines of cell science, tissue engineering, space biotechnology, and other related sciences. The report provides some discussion on the successful 130-day tissue engineering experiment performed in BTS on *Mir*, the longest continuously operating bioreactor experiment in space. The report also describes a seminal gene array investigation that identified a set of unique genes that are activated in space. These landmark biotechnology experiments on *Mir* also occurred in conjunction with some historic moments in the annals of human spaceflight. Astronauts were performing the NASA biotechnology cell science experiments on *Mir* during the only on-orbit fire ever recorded in a piloted spacecraft. Also, a collision between the *Mir* and the Russian Progress resupply vehicle caused a partial decompression and almost caused the joint Russian and U.S. crew to abandon the station. Both of these events were highly publicized and captured the attention of the world community. The heroic efforts of the Russian and American crewmembers saved *Mir* and allowed the Phase 1 Program to be completed in its entirety, albeit with some limitations and replanning of the science program.

1.1 Program Description

The Cellular Biotechnology Program (CBP) is responsible for developing both the BTS facility and BTF at JSC. The Cellular Biotechnology Program is in the Microgravity Division of the Office of Biological and Physical Research (OBPR). The interrelationships of these programs and the roles played by the BTS and BTF within them are described in this section. The descriptions are provided as background information to illustrate the overall goals and objectives of the BCSP and explain the logical progression of technology development needed to accomplish the science objectives.

1.1.1 OBPR Biotechnology Program

OBPR has identified cell culture and tissue engineering, protein crystal growth, and molecular separation as areas with opportunities for significant advancements through low-gravity experiments. The focus of the coordinated ground- and space-based BCSP is the use of the low-gravity environment of space to conduct fundamental investigations leading to major advances in basic and applied biotechnology. Results from planned investigations have potential to advance our understanding of cell biology in space and provide new insights and strategies in understanding and using cell processes on Earth, offering the prospect of engineering three-dimensional tissues for research, transplantation, and production of biopharmaceuticals.

1.1.2 Cellular Biotechnology Program

The CBP uses NASA cell culture technology and the microgravity of space to advance groundbreaking research in biomedical science. The program emphasizes research in: 1) the engineering of tissue for research, transplantation, and biopharmaceutical production; 2) production of tissues for disease modeling such as cancer; 3) vaccine production through propagation of microorganisms; and 4) space cell biology as it relates to the transition of terrestrial life to low-gravity environments and to the exploration of space. Our investigator community extends throughout the country and includes august research organizations such as the National Institutes of Health. The Program has produced technologies that introduce a new age to cell culture. The developments are patented, licensed, and commercially produced. The research and technological achievements are the basis for space act agreements, commercial ventures, and industrial partnerships. The Program is rife with opportunity for applied science in ground-based applications and will extend into the use of living cells as reporter systems in sensors, and basic knowledge for human exploration and the search for life beyond our planet.

The CBP evolved from the space bioprocessing concepts of the 1970s. The purpose of the Program is to use NASA technology and the microgravity of space to provide a unique cell culture setting that addresses applied and fundamental cell science challenges. In this context, the Program addresses several goals:

1. Develop ground-based and space bioreactors that serve the needs of the scientific community in the investigation of cell biology and tissue engineering.

2. Develop ground-based basic and applied science programs that use microgravity technology to investigate cellular processes.

3. Use the expertise and technology of NASA and its academic and commercial partners to advance tissue engineering to provide three-dimensional, functional tissues for research, transplantation, and commercial applications.

4. Establish space cell biology as an academic discipline.

5. Contribute to space exploration by providing technological advances in life support, health care, living exploratory probes, and space research. In the past decade, the BCSP has developed state-of-the-art space bioreactors and successfully used them on the Space Shuttle and in the BTS facility on Mir for seminal discoveries. These discoveries are creating a new venue for investigating cell biology and offer the prospect for new vistas in understanding cell function as it relates to disease etiology, tissue modeling, and drug development. Through NASA Research Announcement grants, a cadre of 70 scientists from the nation's universities, medical centers, and other institutions—including the National Institutes of Health—are using NASA-designed bioreactors to improve the quality of life on Earth and to advance NASA's goals of the Human Exploration and Development of Space spaceflight research endeavor.

1.1.3 Biotechnology Facility

The deployment of a long-duration, dedicated ISS facility introduces a new era for space cellular biotechnology research. The BTF operational concept will support a continuously operating facility, thereby maximizing the use of microgravity exposure time for biotechnology experiments. Continuous, long-term on-orbit operations will enable sufficient sample replicates and repeat experiments within 90 days (typical length of an ISS crew increment) to allow for robust statistical analysis of data and facilitate rapid publications of results. This profile contrasts with the Shuttle-based research, in which repeat and confirmatory experiments can easily span several years.

We have adopted a multilevel approach for the design of the BTF. Core elements of the BTF will include basic science and engineering support hardware comprising the base rack structures, robust versatile incubator systems, modularized support hardware for on-orbit tissue engineering, metabolic gas supply systems, research-grade water supply systems, and robust computer workstations. Supplementary elements of the BTF will include sub-ambient sample storage, quick sample freezing apparatus, cryogenic transport containers, physicochemical parameter monitoring devices, and other analytical tools.

1.1.4 Biotechnology System Facility

The BTS facility's four operational modules and three stowage modules provided all the resources needed for long-duration biotechnology investigations: a data acquisition and control system (DACS), refrigeration, gases to support cell culture, and a module in which to house experiment-specific hardware. Developed to meet the science and hardware resource

requirements for the facility's scheduled 2-year operation aboard *Mir*, all modules were designed to accommodate the evolution of science requirements and to facilitate the easy change-out of experiment-specific hardware. Figure 1-4 shows four of the seven BTS modules that were launched to *Mir* in the Priroda module in their contiguous operational configuration. The BTS facility configuration remained unchanged during its 2-year operation while accommodating changes in experiment specific hardware (bioreactors) or facility hardware upgrades (refrigerator, experiment control computers). Experiment-specific hardware and software, biological samples and specimens, and experiment consumables were transported to and from the facility aboard the Space Shuttle and the Russian Progress vehicle.

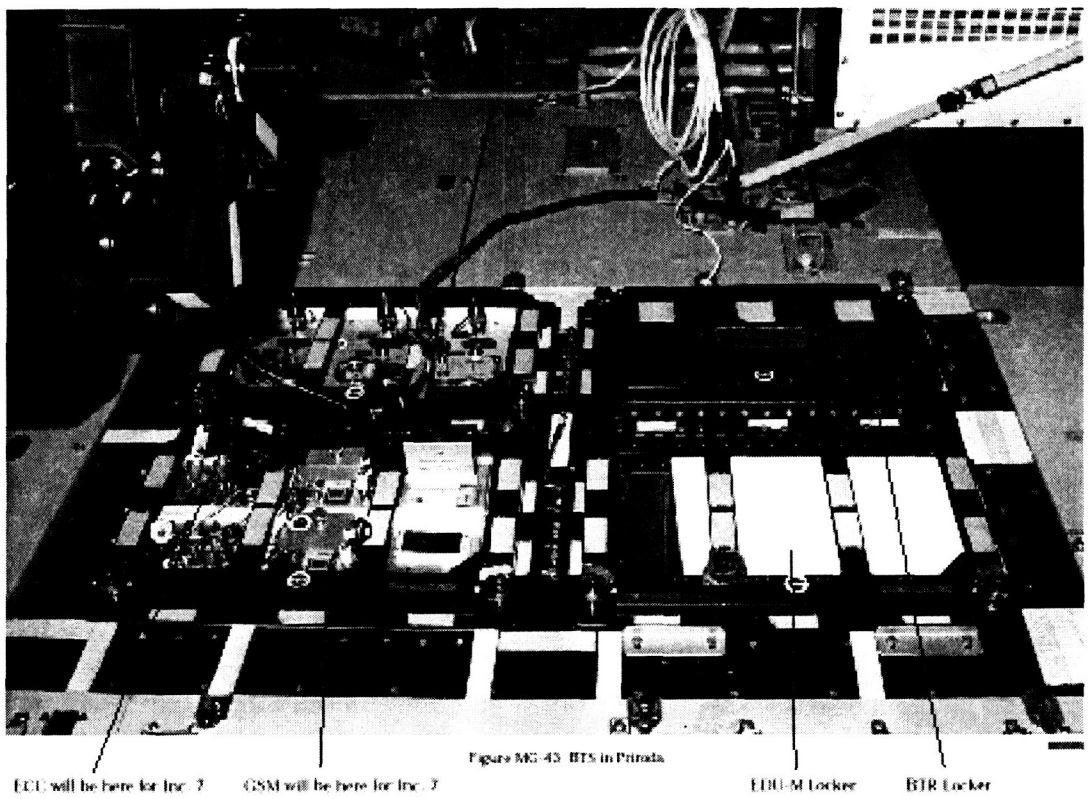

Figure 1-4. BTS-Priroda launch configuration.

1.2 Purpose: BTS Risk Mitigation

The long-duration operation of the BTS facility on *Mir* enabled the BCSP team to validate BTF concepts and systems, and mitigate risk by on-orbit use of BTF components and verification of BTF procedures. In addition, we are using the results obtained from conducting fundamental science investigations in the BTS to clarify the science requirements for the BTF on the ISS and to optimize the BTF design.

1.3 Approach

The BTS facility was designed around the BTF concept of a modular rack facility that was under design for launch on the Space Shuttle and for long-duration operation on an orbiting space station. A ground-based emulation of the BTF, the Mini-Payload Integration Center (Mini-PIC) was developed to support the integration and testing of BTS components during development and during on-orbit operations (see Section 3.5). It was designed to accommodate middeck lockers, the primary payload physical accommodation on both the Space Shuttle and the U.S. portion of the pressurized volume in the *Mir* Priroda module.

1.3.1 Mini-Payload Integration Center

JSC's Cellular Biotechnology Program developed the Mini-PIC as a ground-based emulation of the BTF. This system provides flight-approved scientists with a duplicate of the BTF's interfaces early in their experiment development process. Each Mini-PIC can accommodate two middeck-sized payloads and provides the same DACS, power and data connections, and gas, water, and vacuum resources used in the BTF. The Mini-PIC is a working model of the BTF interfaces with which researchers can build, conduct, and evaluate their experiments in their own laboratories at their home institutions. The ability to integrate and test experiments on the ground in the same facilities they will use in flight both reduces risks and increases scientific returns from the ISS. The CBP team successfully used the Mini-PIC, as described in detail in a later section (Section 5.3), during Increment 3 to duplicate, in near real time, the incorrect integration of the NASA space bioreactor into the BTS Facility and to identify a corrective action that enabled a successful and complete 130-day continuous tissue engineering investigation.

1.3.2 BTS Hardware Description

The BTS facility consists of 7 modules (Table 1-1): 4 contiguous modules for active, powered operation, and an additional 3 modules for passive stowage.

Table 1-1. Components of the BTS

Module Name	Quantity	Function
BTS Experiment Module (BEM)	1	A location that houses and supports experiment-specific cell biotechnology hardware (bioreactors)
BTS Computer Module (BCM)	1	A location that houses 2 experiment control computers, which are used for data acquisition, control, and power supply for the BEM
BTS Refrigerator Module (BTR)	1	A location for cold storage of experiment consumables and samples
BTS Gas Supply Module (GSM)	1	A location housing the apparatus that supplies mixed research-grade gas to the BEM
BTS Stowage Module (BSM)	3	A location that houses passive stowage equipment and supplies required for experiment operations, diagnostics, and maintenance

The BTS required a volume of 7 lockers on *Mir*. During experiment operations the first four modules were contiguous. The 3 BTS stowage modules (BSMs) were distributed at other locations within the Priroda module.

1.3.3 Objectives

The Program's objectives for developing, launching, and operating the on-orbit BTS facility were to:

- demonstrate technology and systems to support biotechnology investigations.
- validate BTF concepts and systems through long-duration operations.
- verify BTF operational and training procedures.
- verify procedures for the launch, landing, and transfer of operating experiments between orbiting spacecraft.
- perform fundamental science investigations.

All of the objectives were realized during the ISS Phase 1 Program.

2 DESCRIPTION OF TECHNOLOGY AND SYSTEMS

The BTS facility was designed around the BTF concept of a modular rack facility that was under design for launch on the Space Shuttle and for long-duration operation on an orbiting space station. The cell science experiments performed on *Mir* required unique technology and systems that were designed to meet both the specific scientific requirements and vehicle interface requirements. The BTS facility was designed to provide the resources and hardware that, in combination, provide an environment suitable for sustained cell growth. In addition, the BTS was designed to mount and operate in different space vehicles that were required during various phases of the experiment. The BTS was designed to remain operational during the pre-launch, launch, ascent, on-orbit, and descent phases of the mission in order to meet the science requirements during all phases of the mission. The BTS components were designed to operate in the Shuttle middeck, *Mir*'s Priroda module, and the Spacehab module, which is flown in the Shuttle cargo bay. All of these vehicles have similar mechanical and electrical power interfaces, but are managed by completely different organizations within the U.S. and Russian Space Programs. This section describes the technology and systems composing the BTS that were flown on the vehicles identified above.

Many factors influence the design of space payloads and systems. Both the scientific and vehicle interface requirements must be satisfied for successful operations and science results. Safety requirements must be met before an experiment can be approved for flight and operation on the host carriers. Because of high launch costs, both the volume available for experiment hardware and electrical power are at a premium in space vehicles. This dictates that all space science hardware be compact and make maximal use of the limited electrical power available for experiment operations. Also, human resources, such as crew time, are extremely limited, making automation an essential design requirement. All of these factors shape the design and

operational planning for space-based biotechnology experiments. Section 2-1 describes in detail the individual BTS modules identified in Table 1-1. The section identifies the function and design characteristics of the BTS modules, and discusses the role of each module within the overall system, including the interaction and interfaces between the modules. Figure 2-1 shows the BTS facility in the Priroda module as configured during Increment 3.

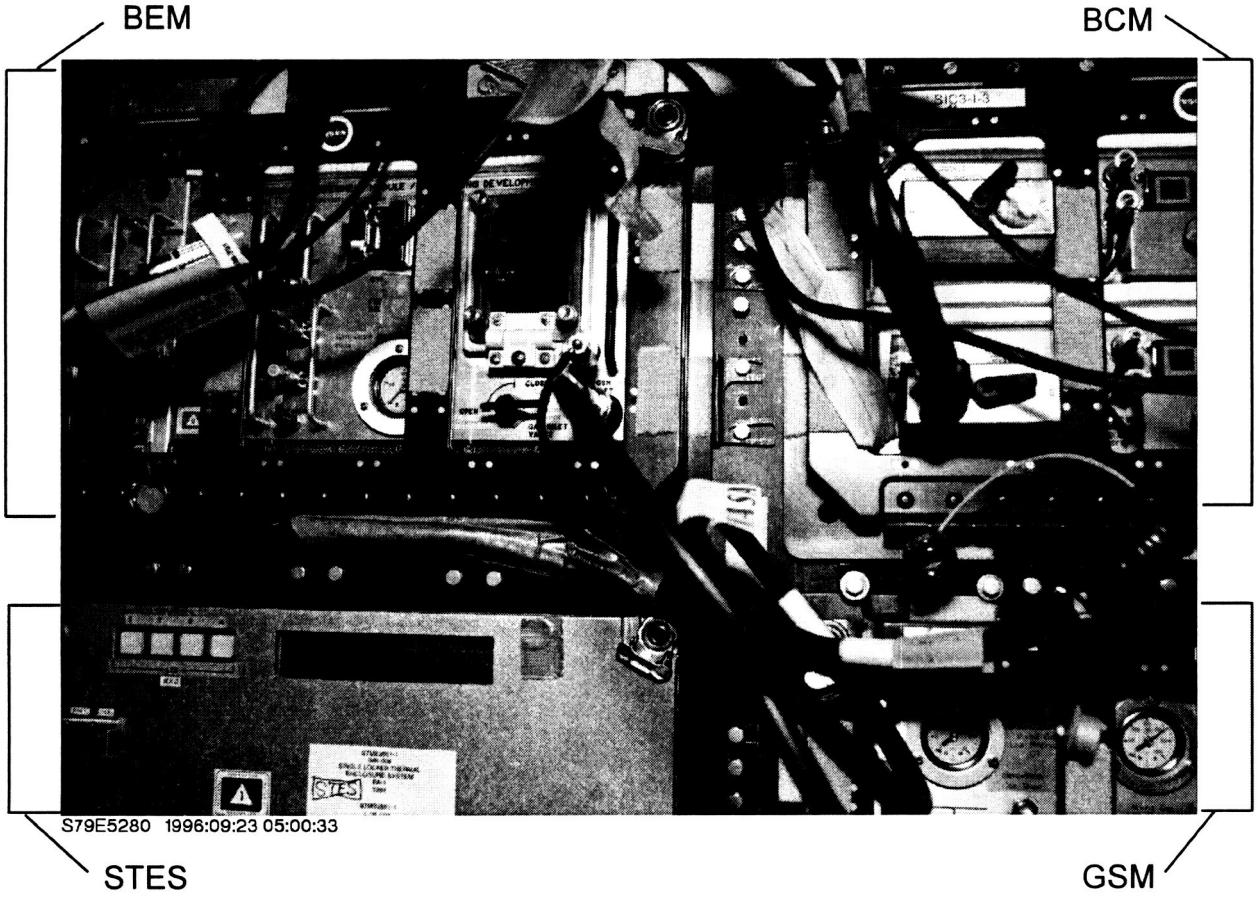

Figure 2-1. BTS Increment 3 configuration.

Note: STES = Single locker thermal enclosure system, BCM = biotechnology system computer module, GSM = gas supply module, BEM = biotechnology system experiment module.

2.1 FACILITY HARDWARE

2.1.1 BTS Computer Module (BCM)

The BCM contains 2 experiment control computers (ECCs). The ECCs are used for data acquisition, commanding, and control, and as an electrical power supply for cell culture experiments supported by the BTS. The 2 ECCs are housed in a single Priroda storage locker as shown in Figure 2-2. A foam cushion surrounds each ECC to dampen launch and landing vibrations. Personal Computer Memory Card Industry Association (PCMCIA) cards implement the experiment-unique software. Each ECC has 2 PCMCIA drives. All electrical and data

interfaces are through cables attached directly to the front panel. In the air-cooled ECC, air is pulled in through slots cut in the foam on the front and side of the ECC and exhausted to the rear of the locker via internally mounted cooling fans. The ECC consists of a control module (CM), a power module (PM), and an interface cable connecting the two units in the rear.

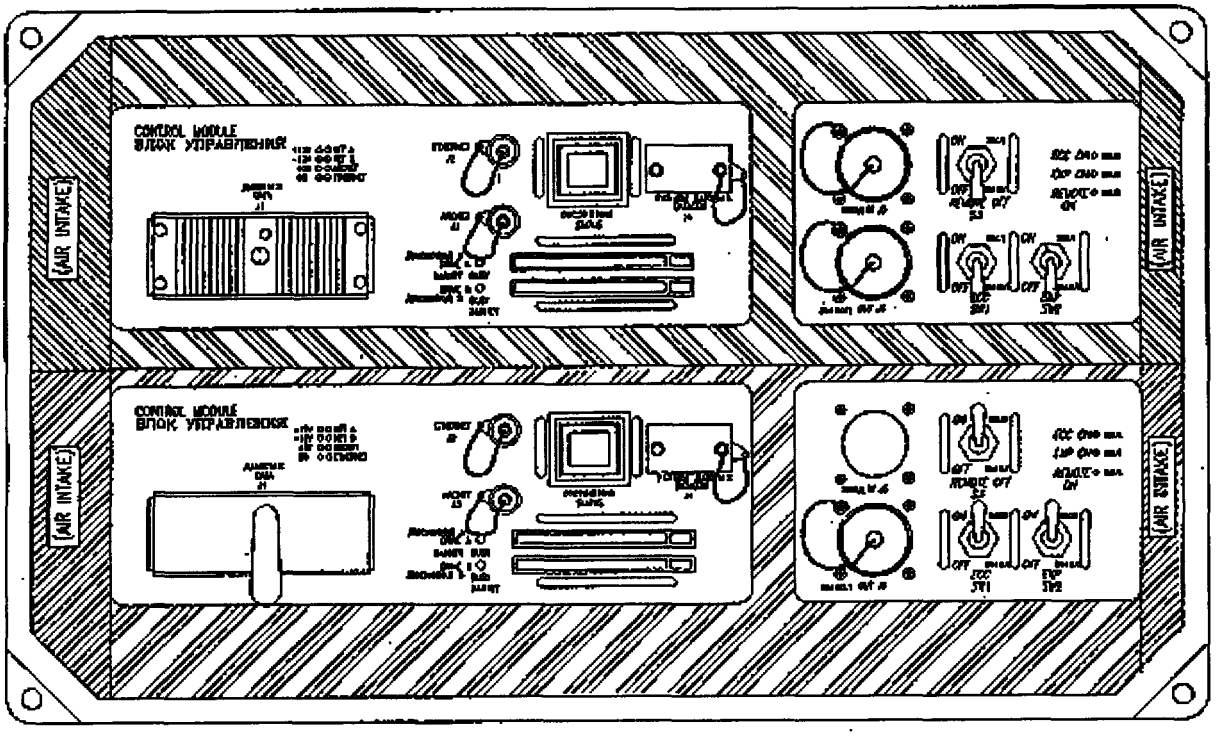

Figure 2-2. BCM front panel.

The ECC's primary function is to execute the principal investigator's (PI) experiment protocols, provide autonomous control of the experiment, monitor the health of the experiment-unique hardware, and archive experiment and hardware data. During the *Mir* experiments, the BCM interfaced directly only with the BTS experiment module (BEM), or experiment-unique equipment. Future versions of the ECC built for the ISS may interface with other BTS subsystem components in addition to the BEM.

2.1.1.1 Control Module

The CM is capable of autonomous real-time multitasking of experiment operations, analyzing and managing data, remotely controlling through common network interfaces, monitoring experiment health and status, reconfiguring experiments via a PCMCIA card, collecting data and storing it on PCMCIA cards, and communicating with the crew using a liquid crystal display (LCD). A single-board computer (SBC) within the ECC controls these features. Table 2-1 identifies the specific features of the CM.

Table 2-1. CM Indicators and Controls

Panel nomenclature	Type of feature	Description	Function/significance
DATA J1	Experiment interface connector	220-pin connector	Experiment data cable interface
DATACOM J4	RS-232 data connector	Pin-type data connector	For use with external computer
STATUS	LCD switch	Momentary push-button display switch	Displays ECC and experiment diagnostic codes; push to acknowledge
DRIVE A DRIVE B	PCMCIA Drives (2) PCMCIA drive A (B) power on (2) PCMCIA drive A (B) in use (2)	PCMCIA card slots (2) Left white LED (1 per drive) Rich white LED (1 per drive)	Store facility and experiment programs and data. Indicates power for drive A (B) Indicates access to PCMCIA card in drive A (B)
	PC-card eject buttons (one each Drive)	Push-to-eject buttons (one per drive)	Ejects PC card installed in Drive A (B)
ETHERNET J2	Ethernet connector	Network interface connector	Interface for Ethernet communications
ARCNET J3	ARCNET connector	Data interface connectors	Interface for ARCNET communications
	ECC status LEDs (8)		
+12V (-12V, +5V)	+12V (-12V, +5V) Power (3)	Green LEDs (1 each)	ON indicates +12 (-12, +5) Vdc power available to ECC
WD	Watchdog Activity Timer Indicator	Green LED	ON indicates nominal, OFF indicates automatic shut-off of experiment power if REMOTE OFF S3 switch is in ON position and ECC resetting
NET A NET B	Internal ARCNET status (2)	Green LEDs (1 each)	ON indicates internal communications in progress
ARCNET (ETHERNET)	ARCNET (Ethernet) status (2)	Green LEDs (1 each)	ON indicates ARCNET (Ethernet) communications in progress

The SBC components include a 486 DX2 66M microprocessor, a real-time clock, a watchdog timer, a power-failure detection current, and 32 MB of dynamic random access memory (DRAM). The SBC analyzes and manages experiment data and controls the systems within the BEM hardware. The CM provides the following interfaces through a single 220-pin connector on the front panel: Ethernet, Arcnet, RS-232 Serial Communications, RS-422/485 Serial Communications, small computer interface (SCSI) for 2 hard disks, 48 digital input/output lines, 16 analog input lines, and 2 analog outlet lines. The large variety of interfaces permits the ECC to be used for many types of BEMs.

The LCD switch provides display and mode monitoring for the ECC. The LCD communicates to the crew by displaying status codes. It will also flash red or amber to provide a more highly visible indication of off-nominal conditions associated with the ECC or experiment. After the

off-nominal conditions have been connected, the LCD can be reset to green. The CM also has green light-emitting diodes (LEDs) that serve as status indicators for operating voltages.

2.1.1.2 Power Module

The PM houses the power connectors and performs the ECC's power control, distribution, and protective functions. It receives 27 +5/-4 Vdc power from the *Mir* payload utility panel (PUP) and provides and controls power to the CM and experiment-unique hardware. It provides +5 Vdc and 12 Vdc power to the CM and provides circuit protection for the CM via a 5-amp magnetic breaker. The PM monitors power levels provided to the BEM and can shut off power if it detects abnormal power usage. The PM provides 27 ± 4 Vdc power to the BEM and provides circuit protection via a 10-amp magnetic breaker. Table 2-2 indicates the PM front panel indicators and controls.

Table 2-2. PM Indicators and Controls

Panel nomenclature	Type of feature	Description	Function/significance
IN J5	PUP input power interface	Pin-type power connector	27-Vdc power feed from PUP
OUT J6	Experiment power interface	Pin-type power connector	27-Vdc power feed to experiment equipment (not used until experiment installed)
REMOTE OFF S3	ECC control of experiment power	Three-position toggle	ON indicates experiment power remote control enabled (ECC can turn experiment power OFF), OFF indicates disabled, center position is not used (center position is functionally same as the OFF position)
ECC SW1	ECC ON/OFF toggle switch	Two-position toggle	ON indicates control module power enabled, OFF indicates power disabled to both experiment and ECC
EXP SW2	Experiment power switch	Two-position toggle	ON indicates 27 Vdc to experiment, OFF indicates no power to experiment
ECC ON	ECC power ON/OFF status light	Green LED	ON indicates 27 Vdc power available to ECC
EXP ON	Experiment status indicator light	Green LED	ON indicates 27 Vdc power available at J6 connector (power to experiment)
REMOTE ON	ECC remote power status indicator	Green LED	ON indicates that REMOTE OFF S3 switch is in ON position and ECC can shut off experiment power

The ECC (PM and CM) requires approximately 65 W to operate. The ECC was used successfully for several cell science and risk mitigation experiments on *Mir*. These experiments are discussed in Section 6.0.

2.1.2 Gas Supply Module (GSM)

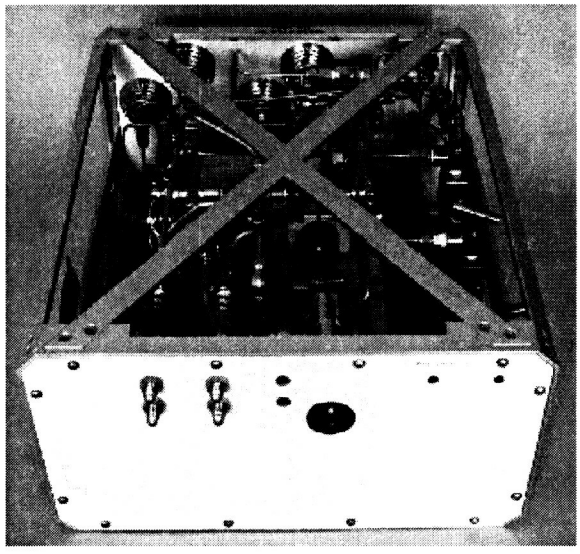

Figure 2-3. GSM rear and top.

The GSM (Fig. 2-3), a major and essential element of the BTS, is a high-pressure system that provides high-purity gases to bioreactors housed in the BEM. The gases are required for cell metabolism and pH control. The GSM is a single middeck locker-sized hardware item that contains 3 high-pressure gas cylinders, valves, gauges, pressure regulators, and other components for delivering the required gas mixture at 40 pounds per square inch (psi) pressure to the space bioreactor. The GSM was launched to *Mir* in the Priroda module. It was pressurized with a mixture of 90% medical grade air and 10% medical grade CO_2 in all three cylinders. Two of the pressure cylinders contained 367 liters and the other cylinder contained 121 liters of mixed gases based on standard temperature and pressure (STP) conditions. The GSM can automatically mix precise amounts of pure gases via front panel valve settings. However, this capability was not used during the Phase 1 Program on *Mir*. The gas mixture is routed to the BEM via a flexible gas hose (flexline) and front panel connections.

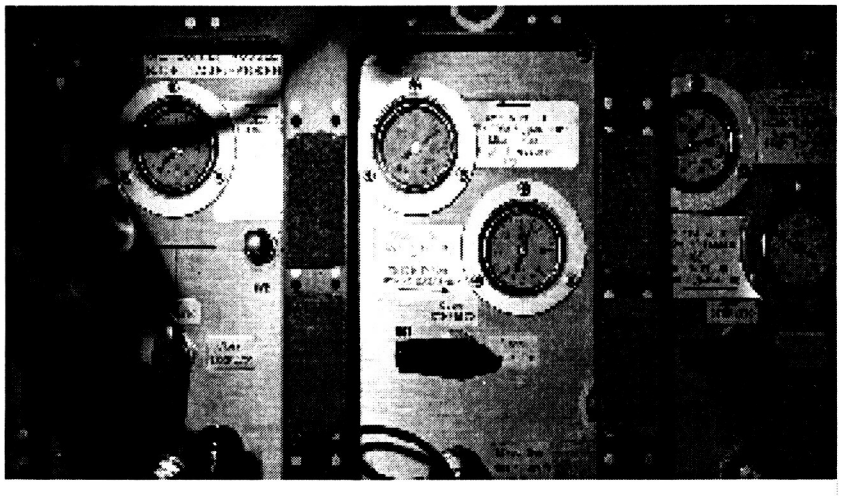

Figure 2-4. GSM front panel.

The GSM was used during 2 increments on *Mir*, but GSM status checks were performed periodically from the time the GSM arrived on *Mir* until the end of the Phase 1 Program. A brief definition of the primary components of the GSM is provided in Table 2-3.

The GSM does not require electrical power, nor does it have an interface to the BTS command and data handling systems, specifically the ECC. The BTS project management decided to make the GSM completely passive. The primary reason for this was the short period of time between NASA's decision to launch the BTS to *Mir* and the Russian Priroda module launch date. There simply was not enough time to design, build, and test the GSM and incorporate automated data system interfaces and still deliver the hardware in time to be launched on Priroda. Since U.S. payloads had no data downlink from the *Mir*, the U.S. crewmember on board *Mir* had to visually check pressure gauges on the GSM front panel, record the readings in BTS log books,

and periodically send the GSM status information to the ground via either voice communication or e-mail.

Table 2-3. GSM Components

Component	Function
Gas cylinder	Stores high-pressure gas; fiberglass filament-wound composite cylinder rated at 3000 psig maximum working pressure.
Pressure switch	Sends pressure-included electrical signal to the audible alarm that indicates the pressure relief valve is near opening condition
Check valve	Maintains one-way gas flow
Pressure gauge	Analog pressure indicator
Flow restrictor	Maintains gas flow at a specified maximal value
Alarm test switch	Tests the Sonalert (audible alarm) circuit and battery and turns the over-pressure alarm circuit on and off
Metering valve	Adjustable valve that permits various gas-flow rate settings. Preset before launch based on specific experiment requirements
Shutoff valve	Manually operated valve
Relief valves	Automatic valves that are set to open at a specified maximal pressure

Future versions of the GSM on the BTF will incorporate a data systems interface for logging equipment status and downlink of performance data to ground controllers. The GSM performed flawlessly on *Mir* during two separate increments. The system did not lose pressure over time, despite a relatively wide range of environmental conditions on *Mir*. Obviously, future designs of the GSM for BTF on the ISS should consider similar pressure system design features and components.

2.1.3 Single-Locker Thermal Enclosure System (STES)

The STES (Fig. 2-5) is a refrigeration and incubation module for conducting microgravity and biotechnology research in the Shuttle middeck, in the Spacehab module, or on *Mir*. The STES's internal volume temperature can be controlled to any desired temperature between 4° and 40°C. The STES is designed to control temperature to 0.5°C. During Increment 3, the STES was set to control temperatures at 7°C, based on BTS experiment refrigeration requirements.

The STES maintains thermal control by conducting heat in or out of its internal enclosure through the left wall. This heat is transferred to a heat exchanger mounted on the left wall of the STES, which is heated or cooled by Shuttle Orbiter cabin air circulated by a fan in the unit. STES operations require a maximum 128 W of nominal 28-Vdc electrical power.

The STES, which replaces a single middeck locker, is approximately 20.3 in. long, 18.1 in. wide, and 10.87 in. high, and weighs approximately 69 lb (3). The STES does not have a data interface with the ECC. However, temperature history data are logged internally and can be retrieved after the STES has been returned to the ground.

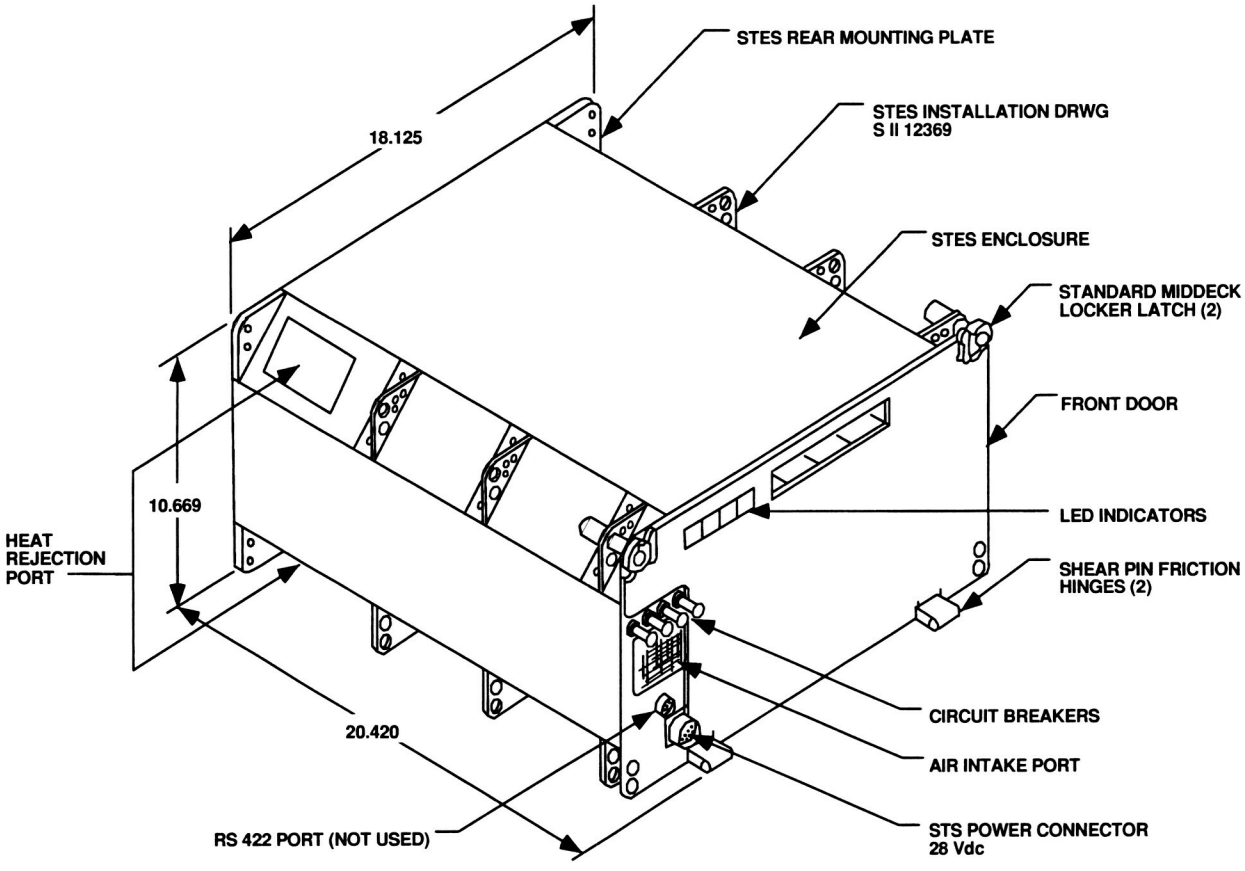

Figure 2-5. Single-locker thermal enclosure system.

On *Mir*, the STES was used to store temperature-labile items and medium samples that were required during the Increment 3 experiment. The items included powdered media, portable chemical blood analyzer (PCBA) cartridges, and other temperature-sensitive items.

2.1.4 Biotechnology Refrigerator (BTR)

The BTR (Fig. 2-6 and 2-7) is a single middeck locker equivalent that provides approximately 0.58 ft^3 of temperature-controlled storage volume to support biotechnology experiments. It is a general-purpose refrigerator for BTS supplies and samples. The BTR provides a controlled thermal environment with a set point of 4.0°C and a control accuracy of ±1.0°C. It uses thermoelectric cooler (TEC) technology to control temperatures in the storage volume.

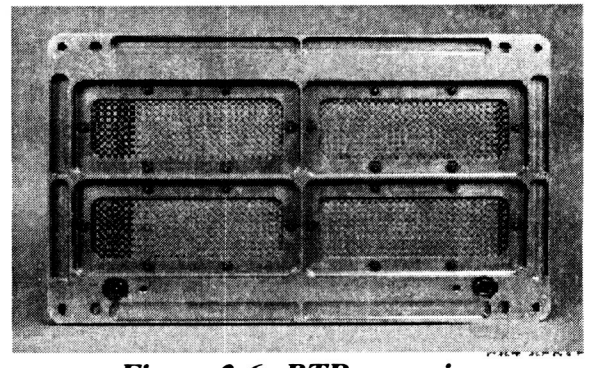

Figure 2-6. BTR rear view.

The BTR consists of the following major components: 1) locker housing, 2) temperature-controlled storage volume, and 3) front panel controls and displays. The front panel controls and displays include a connector for 28 Vdc input power, a data interface connector, a magnetic circuit breaker, a power status LED, a toggle switch to adjust the set-point temperature, and an LED bar graph display to show actual temperature versus set-point temperature. We built the BTR to replace the STES for BTS experiments on *Mir*.

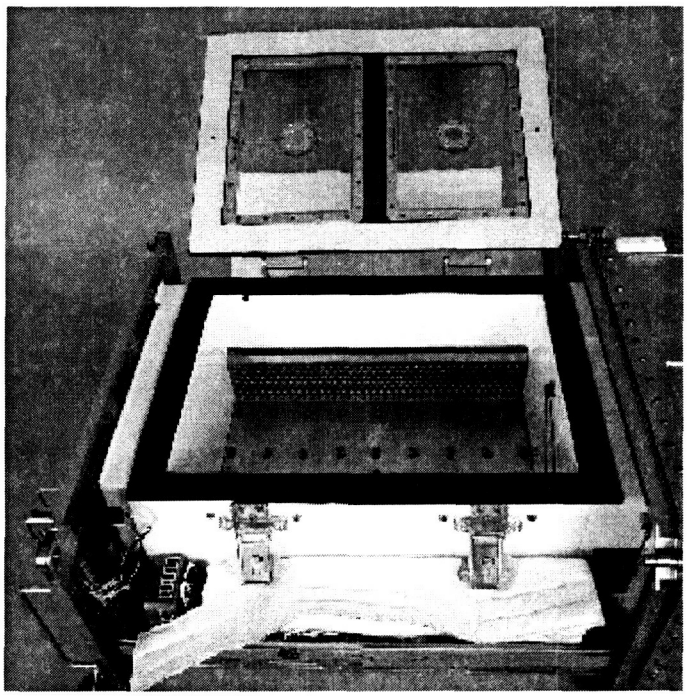

Figure 2-7. BTR cooling chamber with access door open.

The BTR slides in and out of its housing on guide rails, enabling the crew to access the temperature-controlled volume, or tub, for nominal operations and maintenance. The guide rails lock in the extended position. The crew must actuate latches on both guide rails to slide the BTR back into the locker housing.

The BTR uses cabin air for cooling. Passive cabin air cools external BTR surfaces and two internal fans cool internal components by forcing cabin air through the unit. Air is drawn into front panel ports under the BTR tub, and then exhausted from side panels of the BTR.

The BTR does not have a data interface with the ECC, but it has internal data logging capabilities. The recorded data can be retrieved using the data connector on the BTR front panel. The BTR weighs approximately 64 lb (29 kg) and has a peak power requirement of 171 W.

2.1.5 BTS Stowage Module

The BSM stores experiment consumables, diagnostic equipment, and other supplies required for experiment operations under ambient conditions. In the BTS Priroda configuration, the BSM was a single locker location in the 4-locker BTS facility configuration. Other BSMs were placed at other locations on *Mir*. Each BSM typically contained stowage items packed in two half-locker single stowage trays. Two of the BSMs launched on Priroda contained 48 one-liter bags of sterile, ultrapure water that was used with the BTS cartilage in space (BTS–CART) experiment during Increment 3. The BSM is a general stowage location, using standard locker accommodations, which accommodates a wide variety of equipment required for biotechnology experiments.

2.2 EXPERIMENT-SPECIFIC HARDWARE

The experiment-unique hardware flown as part of the BTS was the engineering development unit–*Mir* (EDU–M) and the biotechnology specimen temperature controller (BSTC). Each of these space bioreactors was integrated in the BEM. The EDU–M was used during two increments and the BSTC during one increment on *Mir*. This section describes the design characteristics and capabilities of both of these units.

2.2.1 Space Bioreactor

The EDU-M (Figs. 2-8 and 2-9) is an automated bioreactor designed for supporting long-duration cellular biotechnology investigations in low-gravity environments.

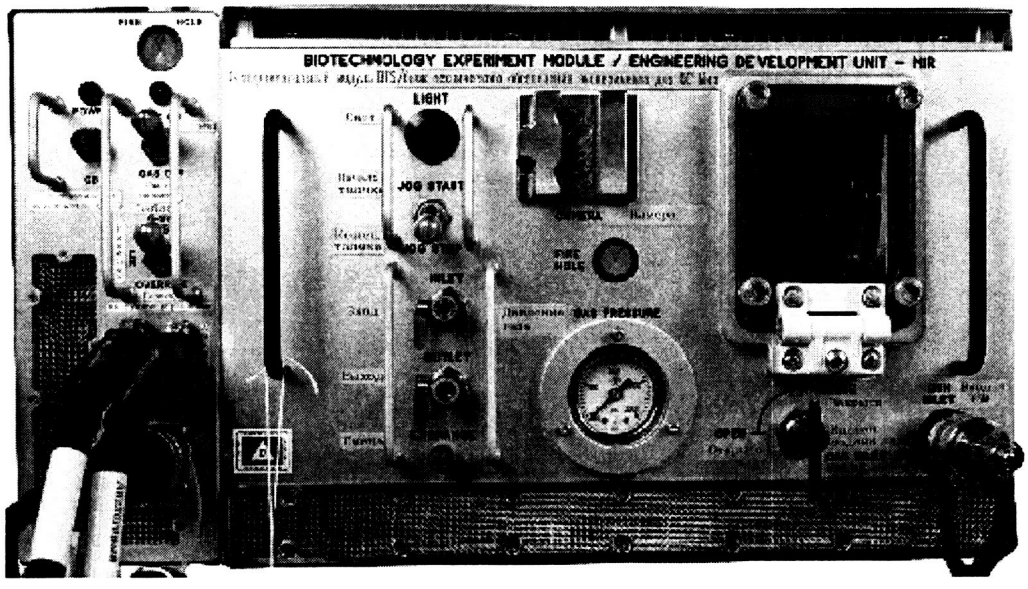

Figure 2-8. Space bioreactor/engineering development unit/Mir.

The EDU–M, 226 mm high, 414 mm wide, and 516 mm deep, weighs approximately 25 kg and fits in a standard middeck locker. The EDU-M is surrounded by 1/2 in. (12.7 mm) of foam to cushion the unit during launch and landing. Its housing is fabricated primarily of 6061-T6 aluminum alloy.

The EDU–M was connected to the ECC by a power and data cable. It remained powered through ascent, orbit, and docking with *Mir*. Power was removed for no more than 45 minutes for transporting the unit to *Mir* and installing it. The EDU–M nominal power is 110 W, and it has a maximal power of 155 W. Use of the ECC with the EDU–M requires frequent use of the *Mir* interface to payload system (MIPS-2L) laptop computer and other stowed items.

The EDU–M houses a rotating wall perfused vessel (RWPV) in a controlled atmosphere held at 36 ± 1°C, and is maintained by a cell support system with accompanying electronics. The EDU–M incubator volume contains a vessel with a 125-mL volume. After cells have been added to a

sterile EDU–M before launch, the vessel begins rotating at a predetermined rotation rate. Upon reaching orbit, the rotation speed automatically changes to microgravity levels.

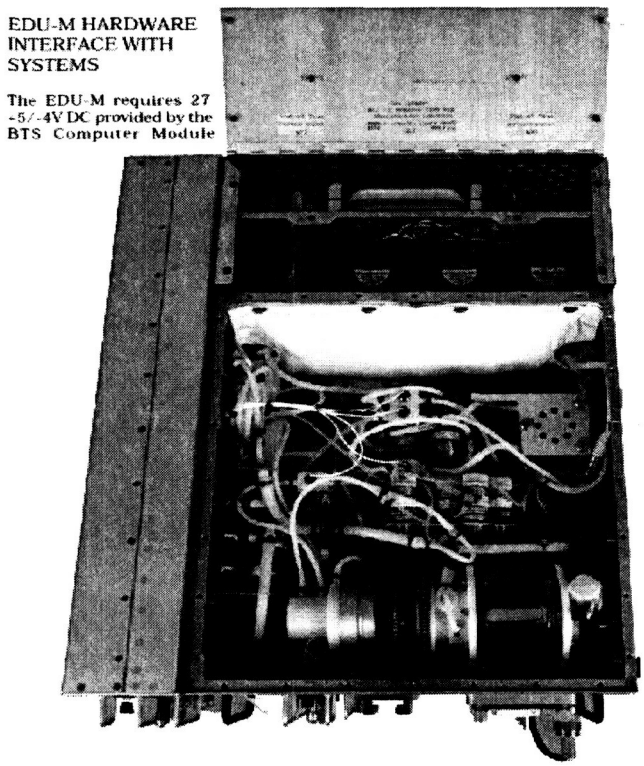

Figure 2-9. Space bioreactor, internal view.

Vessel fluid support hardware supports mammalian cell growth for an experiment period of 90 or more days on orbit. During this period, the system maintains proper temperature, monitors and oxygenates the nutrient medium, and facilitates the removal of medium samples and waste products.

The EDU–M has five major components:

- Fluid system
- Reactor vessel
- Temperature control system
- Electronics
- Gas supply system

Each of these subsystems is described below.

2.2.1.1 EDU–M Fluid System

The fluid system is located in the incubator module of the EDU–M. Tubing in the system is a combination of C-Flex and silicon. A two-roller peristaltic pump driven by a stepper motor

circulates fluids in the system. Although power usage varies with the perfusion rate, the stepper motor uses a maximum of 10 W of power.

The fluid system is part of the first level of containment for the biological materials in the EDU–M. This first level of containment comprises the reactor vessel, oxygenator, tubing, fittings, and fluid storage vessels of the fluid system. The second level of containment is the aluminum incubator housing surrounding the fluid system. Multiple levels of containment for all fluids is a safety requirement for the Shuttle and *Mir*. A 0.2-μm polytetrafluoroethylene (PTFE) hydrophobic membrane on the inside of the housing covers the fire hole in the front panel of the incubator housing to ensure the level of containment. This was done because the *Mir* contains no suitable fire extinguisher (Shuttle-type) for the EDU–M.

During flight, the crewmembers took medium samples from the outlet port to monitor key parameters, including pH, pCO_2 (blood carbon dioxide tension), pO_2 (blood oxygen tension), HCO_3^- (bicarbonate ion), Na^+, K^+, Cl^-, and glucose. During the sampling process, they took great care to avoid contaminating the medium and tissue culture inside the EDU–M. Indeed, during this phase of the process, the greatest risk is not contamination of *Mir*—since the medium and culture are sterile, as described below—but, rather, the risk of contaminating the medium and culture. The crewmembers used sterile gloves with benzalkonium chloride wipes, a sterile syringe and cannula, and cleansed the septum with methanol wipes both before and after sampling. To collect a sample, the crewmember attaches the sterile syringe with cannula to the Luer lock on the outlet port (the cannula punctures the septum during attachment), opens the outlet port valve, draws approximately 5 mL of medium into the syringe, and closes the outlet port valve. The crewmember then removes the syringe with cannula from the Luer lock on the outlet port, removes and discards the cannula, and carefully injects a small amount of medium (approximately 1 mL) from the syringe into each of 2 PCBA cartridges and closes the fill ports on the cartridges. Any overflow is immediately wiped up with a benzalkonium chloride towelette. The cartridges are in turn loaded into the PCBA and the medium solution parameters are read. The PCBA cartridges and syringe are then discarded using a triple containment technique. This sampling procedure and the use of the PCBA for analysis is nearly identical to procedures used for collecting and analyzing crewmembers' blood on orbit. In fact, less risk is involved since the medium and cell cultures are sterile and pathogen-free, as described elsewhere in this document. At no time during this procedure is there a risk of a spill of more than 5 mL of medium.

Another port in the front panel is the exchange port. It was used during flight to remove the internal expended medium and to refill the internal medium storage area with fresh medium. The first level of containment at this port is an internal, computer-operated, three-way solenoid valve, and the second level is a Luer lock cap that covers the port when not in use. Any time a Luer lock cap is removed from the port (for a fluid transfer operation, for example), it is discarded and replaced with a sterile cap to maintain sterility of the port. The solenoid valve is normally closed. To empty or fill medium, a crewmember removes the port cap and discards it, then installs a medium bag by mating the Luer lock connector. The crewmember then opens the three-way valve by entering a command on the MIPS-2L laptop computer. Expended medium is removed when the ECC determines that enough fresh medium has been infused to warrant use of the expended medium bag. As with the medium sampling procedure, the crewmember empties and refills the medium bag using sterile techniques, including wearing surgical mask and gloves, cleansing the

gloved hands with benzalkonium chloride wipes, and cleansing the sample port and all Luer connectors with methanol wipes before and after transfers. To remove waste medium, an empty medium bag is attached to the port, and when the valve is opened the waste medium flows from the waste storage area in the incubator through valves and out the exchange port into the empty bag. Once the waste medium has been removed, fresh medium can be loaded. When the fluid system is replenished with fresh medium, sterile water from the BSM is first added to one of the powdered medium bags from the STES to rehydrate the medium. After the powdered medium has been mixed and dissolved in the water, its bag is connected to the exchange port via the Luer lock connector. A valve is then commanded open and the medium is filtered through a 0.22-micron hollow-fiber membrane filter. It is routed through valves to the reversible peristaltic pump, which pumps the medium to the incubator medium storage area. The expended medium (waste) bag inside the fluid system has a capacity greater than the total volume of medium in the fluid system. In the event that the expended medium bag becomes full before a crewmember empties it, the pressure transducer provides to the ECC a change representative of this condition to notify the crewmembers that a medium exchange is necessary. At a perfusion rate predetermined by the ECC or by crew input, the ECC initiates the perfusion procedure to oxygenate the medium in the reactor vessel. Based on the results of the PCBA analysis of glucose levels in medium samples from the bioreactor, a determination is made whether to request the computer to initiate infusion of fresh medium. For infusion, fresh medium is pumped from the medium storage area into the flow loop.

Crewmembers sealed medium samples and bags with expended medium in zip-lock bags and then stored them in one of the urine containment bags (UCBs) that were on board to support crew metabolic experiments. The zip-lock and UCB maintained three levels of containment for the expended medium. The UCB, with samples and waste medium, were discarded on a Russian Progress vehicle.

JSC microbiologists and toxicologists analyzed the powdered nutrient and antibiotic mixtures, the reconstituted (liquid) growth medium and antibiotic solutions, the cell culture, the waste medium (including the waste products of cell metabolism and growth), and solution mixtures. The toxicologists characterized the powdered mixtures as Level 1 (critical) eye hazards due to their concentration, but they characterized all the liquid solutions (after reconstitution) as Level 0 (nonhazardous). The microbiologists determined through extensive testing that the cell cultures used were free from bacteria, fungi, mycoplasma, and retroviruses, and therefore the cultures themselves, the medium solutions, and the waste products (including used medium solutions) posed no hazard or potential contamination threat to the *Mir* environment or the crew. If any of these materials were to escape, no pathogens would be introduced into the *Mir* environment.

2.2.1.2 EDU–M Reactor Vessel

The reactor vessel comprises a spin filter and an outer wall, capable of independent rotation. A 50-micron polyester filter membrane surrounds the spin filter. The vessel has a clear Lexan housing with Delrin SA-150 end caps and it is mounted in a cantilevered fashion on a 17-4PH shaft, known as the spin filter drive shaft. The reactor assembly drive shaft is supported by a bearing stand at one end where a fluid coupling is provided for fluid line interface to the vessel assembly. Holes in this shaft also allow passage of fluid to and from the interior of the vessel. The free end of the reactor vessel contains three ports for ground use.

Two stepper motors that use a maximum of 10 W of power each control vessel rotation. Power usage varies with the rotation rate. The motors are controlled by two intelligent stepper motor drivers, which are in turn controlled by the ECC's CM. One motor rotates the outer wall, while the other rotates the spin filter (or inner core). Each stepper motor has a single-output 1/4-in.-dia. steel shaft connected to a 1-in.-pitch-dia. aluminum pulley. Each pulley drives a ladder-type drive chain made of two multiple-strand cables coated with polyurethane. These drive chains connect to pulleys of the rotating vessel cores. The outer wall has a 3-in.-pitch-dia. aluminum pulley, while the inner core has a 2.5-in.-pitch-dia. aluminum pulley. Fluid enters the vessel via a tube fitting through the inlet port located in the fluid coupling, and flows down the central shaft into the vessel from behind the viscous pump disk. A viscous pump provides the mixing force for fluid circulation in the vessel and is an integral part of the spin filter. Oxygen-reduced fluid exits via the 50-micron polyester spin filter membrane and flows back down the central shaft via a second passageway into the fluid coupling area, where it leaves the vessel assembly. Five graphite-impregnated Teflon spring-loaded seals prevent the leakage of medium and contamination of the ball bearings. Two pair of angular-contact ball bearings provided a precision running clearance and stiff support for the cantilevered assembly during rotations.

2.2.1.3 EDU–M Temperature Control System

The ECC maintains the incubator temperature at 36 ± 1ºC with no required crew interface. Three 100-platinum resistive thermal devices (RTDs) located in the incubator module provide temperature sensing. These RTDs send an analog signal to the ECC through one of several amplifier modules mounted in the EDU–M. A fourth temperature sensor is located in the inlet air stream to document the influence of the cabin air on the incubator temperature. Two fans located in the incubator ensure air mixture and accurate sensor sampling.

Should sensors indicate a low incubator temperature, the ECC will simultaneously activate two thermofoil heaters in a low-frequency manner (< 0.5 Hz) to elevate the incubator temperature. These heaters are made of thermofoil and Kapton and are clamped in an aluminum heat sink where they perform in tandem under independent drive to ensure redundancy. An additional pair of circulation fans moves air across the heat sink assembly.

We remove incubator heat by conducting the heat produced by both vessel drive motors and a peristaltic pump to the bottom side of the incubator module, which functions as a heat exchanger. To perform this function, each motor is encased in a conductive pad made of an aluminum oxide-impregnated silicone that conforms to the motor's surface. Three brushless dc fans located behind the incubator draw air past this lower exchanger. A thermistor-based fan speed controller, in series with the center fan, monitors air temperature and increases the fan speed according to temperature increases. This continuously variable control helps to minimize the acoustic noise impact. The ECC controls the other two fans.

2.2.1.4 EDU–M Electronics

The electronics module is in an aluminum housing with dimensions 226 mm high, 81 mm wide, and 490 mm deep. It shares one wall with the incubator module. It includes the following electronic printed circuit boards (PCBs):

- Filter board
- Power supply board
- Driver board
- Backplane
- Stepper motor driver boards (3 total)
- Compliance volume

The filter board contains components for electromagnetic interference suppression. The ECC routes 28 Vdc to this board. Once it passes through the filter board, the 28 Vdc is routed to the power supply board.

The 5-Vdc-to-dc converter furnishes power for the power LED on the front panel and all other electronic module components. The 12-Vdc-to-dc converter receives 28 Vdc power from the power supply board. The 12-V source also runs the solenoid valves and some of the fans. A 5-amp circuit breaker located on the front panel runs between the power switch and the rest of the circuitry. The circuit breaker provides on/off switching of the unit. All power wiring is 18- and 20-gauge wire.

The driver board provides 24 drivers that control the solenoid valves, fans, and heaters. Jumpers installed on this board enable connection to either side of the power converter. This board uses optically coupled drivers for optimal isolation between the ECC outputs and experiment loads.

The backplane contains a modular signal conditioner and strain gauge signal conditioner. A filter capacitor for transient absorption and a 3-A fuse for the output of the 4-Vdc-to-dc converter are also located on the backplane.

The ECC, communicating over an RS422 link, directs the activity of three intelligent stepper motor driver boards, which in turn drive the two reactor vessel motors and the perfusion pump motor.

The compliance level board is a small PCB located on the compliance vessel. The board houses two photo interrupters, to provide an indication of how full the compliance vessel is via an analog signal to the ECC.

A driver on the driver board controls each of the two heaters located in the incubator module.

The incubator module provides internal illumination to facilitate vessel viewing and ensure high-quality camera images. The hardware for this consists of 3 woven fiber-optic panels and two 4.8-W halogen lamps. The halogen lamps are fully contained, with their assembly mounted inside the electronics module and connected to the fiber-optic panels via a fiber-optic cable. The ECC controls illumination during programmed tests. It is also controllable by a momentary front panel switch.

2.2.1.5 EDU–M Gas Supply System (GSS)

The GSS provides a medical-grade gas mixture of 10% carbon dioxide, 21% oxygen, and the balance nitrogen to a medium oxygenator membrane.

The system is designed to deliver gas from either an onboard pressure cylinder or a remotely mounted GSM. The GSS is a two-fault-tolerant pressurized system consisting of a pressure vessel, two pressure regulators, two flow manifolds, pressure transducers, a relief valve, and flow restrictors. The 150-cubic centimeter pressure vessel was charged before launch to a nominal pressure of 1100 psig (75 atmospheres) with a premixed gas. At the initiation of the experiment, an isolation valve was opened.

The pressure transducer has an instrumented range of 0 to 2000 psig (1 to 137 atmospheres) and is monitored by the experiment computer. The computer automatically logs its data to provide a record of tank depletion. The gas pressure is stepped down by pressure regulators. The first pressure regulator drops the pressure to 7.0 psi (1122 mm Hg). The second regulator drops it to 2.6 psig (863 mm Hg). Should both regulators fail in the open state, the relief valve opens at 10 psig (1277 mm Hg). The relief valve vents gas to the experiment cooling air stream. A check valve prevents high-pressure gas from reaching the front panel components that later interface with the GSM on board *Mir*.

A normally closed solenoid valve opens and closes to regulate the flow of gas through the system. The computer commands the valve to open when gas flow is required in the oxygenator. A toggle switch on the front panel of the experiment allows an operator to disable the computer control to the valve, thus leaving it in a closed state if needed. A second pressure transducer with a range of 0 to 15 psig (760 to 1536 mm Hg) monitors the pressure at a point just upstream of the flow restrictors.

The computer logs the pressure to provide a record of the pressure profile just ahead of the flow restrictors. The solenoid valve is commanded to shut off when a pressure transducer senses a pressure greater than 9 psig (1225 mm Hg) and will reopen when the pressure is less than 2 psig (863 mm Hg).

Two manifold-mounted, multistaged flow restrictors arranged in series control the gas flow rate. The flow rate of the system is based on the pressure upstream of the restrictors. The restrictors are sized to admit gas at 0.7 standard cubic centimeters per minute when 2.6 psig (863.44 mm Hg) is present on the upstream side of the flow restrictors. After the gas has passed through the restrictors, its pressure drops to well below 1 psig (811.72 mm Hg) and it flows through a 0.2-μm PTFE hydrophobic membrane filter. The gas then enters the oxygenator and equilibrates with the medium on the liquid side of the oxygenator's silicone membrane. The constituents of the gas change very little as a result of this process. The gas exiting the oxygenator will then pass through a second identical hydrophobic membrane filter, which serves as a second level of fluid containment for the oxygenator. The gas is then discharged to the experiment cooling air stream.

The GSS was used only for ascent and descent. The GSM of the BTS facility provided gases on board *Mir*.

2.2.2 Biotechnology Specimen Temperature Controller

The BSTC is a reconfigurable, multichamber, temperature-controlled, static tissue culture apparatus. It replaces a single middeck locker.

The BSTC has the following major components:

- Locker housing
- Front panel with controls and display
- Electronics chamber
- Incubation/refrigeration chamber
- Tissue culture modules (TCMs)

The primary housing for the BSTC is a single middeck locker. The BSTC front panel acts as the enclosure door. The BSTC slides in and out of the housing on guide rails, allowing the crew to access the BSTC-M internal components. This is not required for nominal operations. The guide rails lock in the extended position and the crew must actuate latches on both guide rails to slide the BSTC back into the locker housing.

The BSTC is a single chassis divided into 2 sections. The first section contains the control computer, power supplies, signal conditioners, and interface electronics. The second section contains the insulated incubation or refrigeration chambers. The BSTC launch configuration is shown in Figure 2-10, and the BSTC components are shown in Figure 2-11.

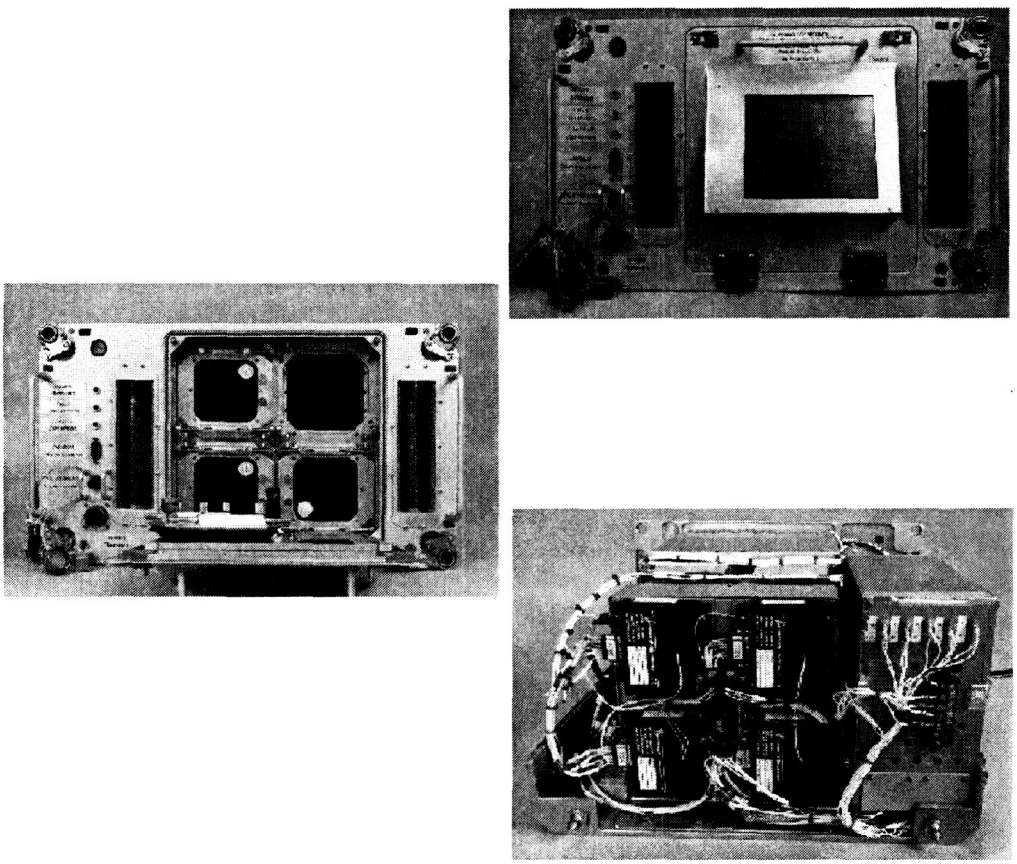

Figure 2-10. Biotechnology specimen temperature controller launch configuration.

Thermoelectric devices heat and cool the incubation/refrigeration chambers. The temperature of each module is computer-controlled. Each module can hold up to 3 tissue-culture bags containing medium and cells. The BSTC temperature control range is from 4 to 50 ± 1ºC.

Crews interface with the BSTC via touch screen on the front panel. The BSTC is attached to the Orbiter middeck wire tray when flown on the Shuttle. On Priroda, it replaced a Priroda locker.

The BSTC front panel is fitted with a 6.4-in. diagonal flat panel display. The panel allows the operator to view all of the system parameters in either numerical or graphical output. This panel is used for changing experiment protocols and commanding the system for data displays. In front of the flat panel display is a transparent touch screen, which serves as the user interface with the BSTC. The commercial touch screen is constructed of a 1/8-in.-thick sheet of transparent polycarbonate that provides impact protection for the computer display. The front panel contains an RS-232 port (DATACOM) for communication with external computers. It also contains a resettable circuit breaker as a safety precaution for short circuits. The front panel includes three green LEDs which, when illuminated, indicate that the BSTC is powered, the disk drive is being accessed, and at least one cooling fan is operational, respectively.

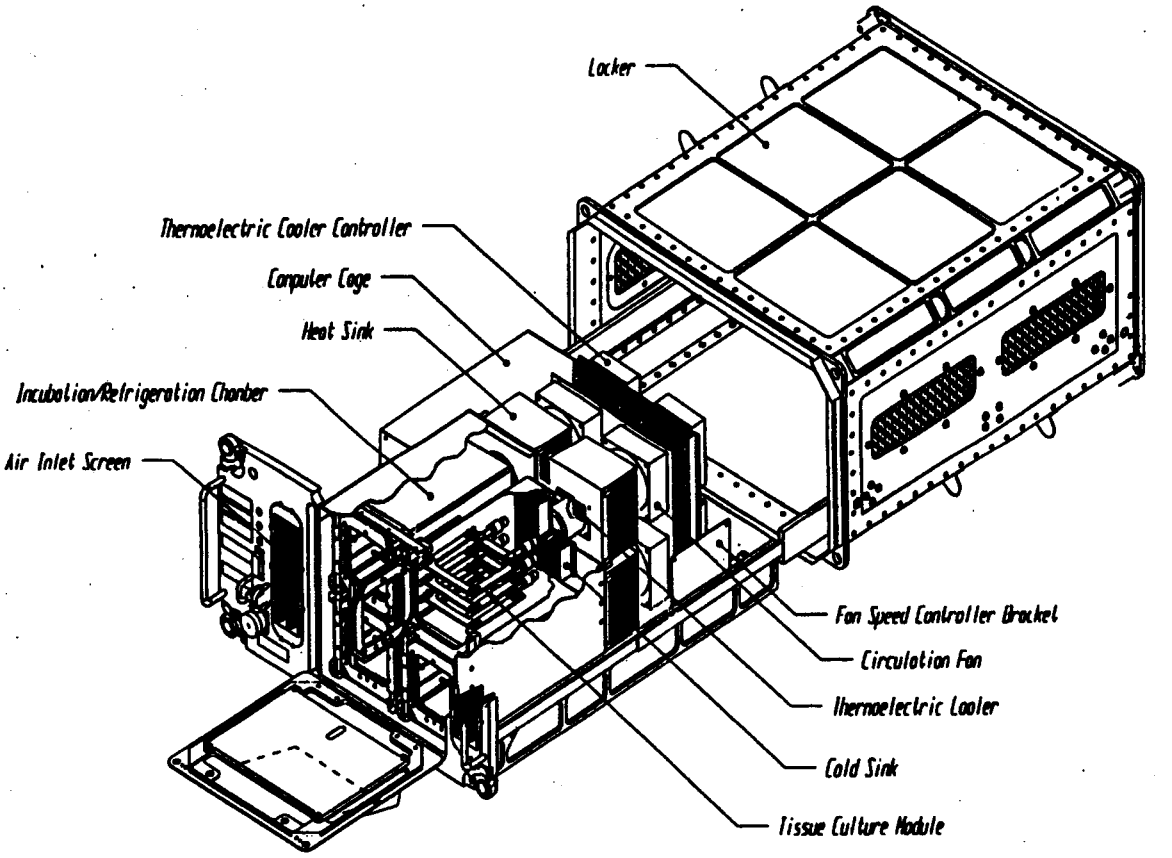

Figure 2-11. Biotechnology specimen temperature controller components.

The BSTC chamber internal temperature was maintained at 36°C during the BTS experiment performed on Increment 6. The exact temperature depends on the experiment protocol. The BSTC is designed to accommodate various styles of off-the-shelf or custom tissue-culture plastic ware. For the Increment 6 experiment, we used approximately 30-mL Teflon culture bags. Each chamber has a door fitted with a clear polycarbonate window. These chamber doors are behind a single door located on the front panel.

The BSTC receives power from the Orbiter (28 ± 4 Vdc). The Orbiter power supply system provides a 10-amp circuit breaker for the BSTC power interface. Inside the BSTC is another 7.5-amp circuit breaker. On Priroda, the PUP provided power (27 +5/-4 Vdc) to the BSTC. The PUP provides a 20-amp circuit breaker for the BSTC. The BSTC requires nominal and maximal power of 135 W. The BSTC is 22.92 by 16.53 by 11.19 in. (58.22 by 47.07 by 28.43 cm) and weighs 70.01 lb (31.76 kg).

The BSTC remained powered throughout ascent, orbit, and docking with the *Mir*. Power was removed for no more than 45 minutes for transport to the *Mir* and installation.

2.2.2.1 BSTC Electronics Chamber

The Electronics Chamber contains the control computer, power supplies, signal conditioners, and interface electronics.

The control computer contains a PC/AT-compatible central processing unit (CPU) card running a 486 microprocessor at a clock speed of 50 Mhz. This card's watchdog timer resets the system if the program stops running. The card has a built-in calendar/clock with a Tadiran lithium battery to power the clock during power down. The control computer includes three solid-state disks: BIOS and DOS in read-only memory (ROM), the application program, and a multifunctional disk. A flash programmer is built into the application program disk for remote programming through a serial port. The multifunctional disk accepts erasable programmable read only memory (EPROM) SRAM or flash memory and can store data, conversion tables, or other operating systems. The control computer can perform RS-232 communications.

The other cards in the control computer are described below:

Data Acquisition card: An octal data acquisition card communicates with the TEC controllers. This card contains eight 12-bit digital-to-analog converter channels that can be programmed for + 5, 0 to 5, or 0 to 10 V of output. For *Mir* it was programmed to output 0 to 5 V.

SCSI-2 card: This card interfaces with an embedded 341-MB hard disk drive (HDD).

Multifunction card: This card provides additional serial and parallel ports as well as 25 additional digital input/output (I/O) lines.

SVGA video card: This card supports the 6.4-in. diagonal flat panel display.

Analog I/O card: This card contains 16 single-ended or 8 differential input channels with programmable gains of 1, 10, and 100. It also contains 19 digital I/O lines and three 16-bit counter/timers. This card is installed to provide the investigator with control and data collection capability beyond temperature and time.

Power supply card: This card receives, converts, and distributes the various bulk voltages the BSTC uses.

Sensor card: This card monitors the four fan speeds, cabin barometric pressure, inlet air flow velocity, and inlet air temperature.

Card cage: The control computer and interface cards will be housed in a card cage containing an 8-slot passive backplane.

HDD: The computer stores data on an internal HDD. The HDD is a 2.5-in. 256-MB unit with a SCSI interface. The HDD is partitioned as two disks and the data are stored in both locations. This helps ensure integrity of the data.

Interface board: This board monitors, activates, and deactivates the temperature controllers.

To protect the hard disk from pressure reductions, a pressure sensor located in the electronics chamber monitors the spacecraft barometric pressure. HDDs rely on air to act as a bearing, and the heads "float" on this curtain of air. If the pressure drops below 10 psi, there is not enough air for the HDD to operate reliably. The computer is programmed so that, if the pressure drops below 10 psi, the HDD is turned off and the heads are parked over the landing zone, where they remain until the pressure is again over 10 psi.

A toroid transformer with a Hall effect sensor monitors the current supplied to each TEC.

A fan mounted on the external surface of the perforated card cage housing cools the BSTC electronics chamber. The fan is 2.36 in. (60 mm) in diameter and has a maximal speed of 3000 revolutions per minute (rpm).

2.2.2.2 BSTC Incubation/Refrigeration Chambers

The incubation/refrigeration chambers (Fig. 2-12) have experiment volumes that are individually controlled with regard to temperature and time. During the Increment 6 experiment, the chambers' temperatures were maintained at a set-point temperature of 36ºC. The heating and cooling system for each chamber is a TEC module, controlled by a bipolar TEC controller. For *Mir*, the TECs were used only in the heating mode. The host computer uses one of the DACS channels to communicate to the controller which temperature to maintain. The controller uses a thermistor for feedback to maintain the selected temperature.

Each of the four incubation/refrigeration chambers has a cooling fan (2.36-in. [60-mm] diameter and maximal speed of 3000 rpm).

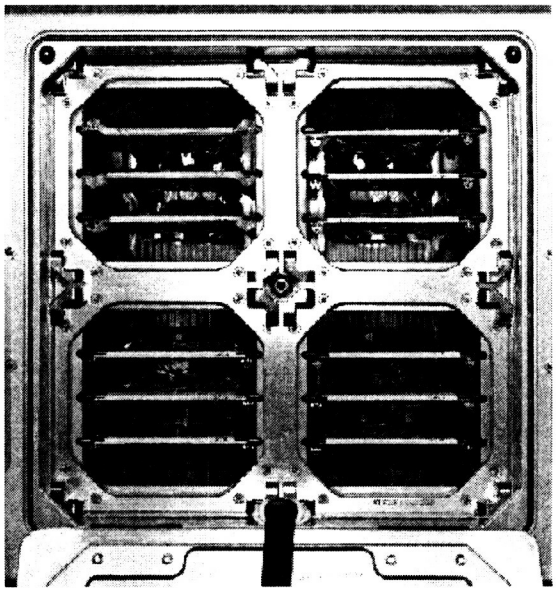

Figure 2-12. BSTC incubation/refrigeration chamber.

Each of these fans is connected to a fan speed controller. The fan speed controller monitors an embedded thermistor that senses the temperature of the heat sink attached to the TEC. As the temperature of the heat sink rises above 35ºC, the fan speed controller increases the drive current to the fan to allow it to move more air. The fan speed controller will continue to deliver more current until it is fully on, which happens at approximately 45ºC. At any temperature below 35ºC, the fan is at idle speed, which is approximately 50% of full speed.

A Gore-Tex vent is located on each incubation/refrigeration chamber. This vent allows pressure equalization between the incubation/refrigeration chamber and the cabin environment. The Gore-Tex vent mesh size is 0.2 micron, which precludes the cells located in the chamber from escaping because the cell size is between 7 and 12 microns.

Cooling air is vented through side panels of the BSTC locker housing. The ventilation holes are 0.12 in. (3.5 mm) in diameter.

We use frequency input signals to monitor the fan speed. The rotating hub of the fan has a black-and-white decal mounted on its surface. Facing this decal is an optically reflective switch. As the fan rotates, the black-and-white decal passes in front of the switch, causing it to momentarily turn on and off. The pause is detected and counted by the signal conditioner. The signal conditioner sends a data stream that indicates the fan rotational speed to the host computer.

2.2.2.3 Tissue Culture Module

The TCM (Fig. 2-13) is an approximately 30-mL tissue culture bag made of clear, gas-permeable Teflon enclosed in a rigid aluminum frame. The culture bag is not permeable to biological materials. The bag is sealed on all sides and has two 4-mm ports with needleless septums. The bag is 3 in. by 3 in., with an additional 2-in. clearance for the ports. These ports were used to fix the tissue in the bags at the end of the experiment. The crew took medium samples using a needleless, 15-gauge cannula with these ports. The Teflon membrane is permeable to O_2, CO_2, and water. The bags have a temperature tolerance of -196º to 121ºC, and they contain culture medium, scaffolds or beads for attachment of cells, and cells. The crew injected fixative into the bags to fix the cells and tissue culture following the experimental protocol.

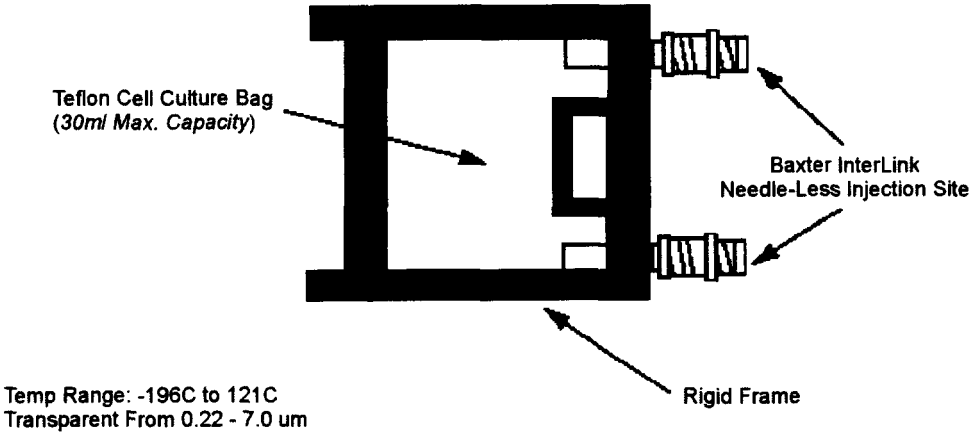

Figure 2-13. BSTC tissue culture module.

2.2.2.4 On-Orbit Operations of the BSTC and BTR

While aboard *Mir*, the BSTC and BTR operated unattended most of the time. The crew performed daily checks of the BSTC and BTR air inlet screens and cleaned the inlet screens using gray tape, as necessary.

The other nominal operations involved sample processing and transfer. The crew performed the following operations on *Mir*:

1. Transferred TCMs between the BTR and BSTC using Bitran bags. Bitran bags are strong multilayer bags that provided a level of containment for cells and medium during experiment operations.
2. Collected medium samples in Bitran bags and PCBA operations.
3. Performed cell culture fixation in Bitran bags.
4. Performed microscope operations in Bitran bags.

The crew transferred TCMs between the BSTC and BTR during various phases of the experiment. Up to three of these modules could be transferred in each bag. The first step in removing TCMs from incubation/refrigeration chambers of the BSTC was to configure the BSTC via the touch screen on the front panel access door.

Once the BSTC had been configured properly via software commands initiated from the touch screen, the crew removed the TCMs from the incubation/refrigeration chambers. The TCMs are reached by opening 2 access doors. One is on the BSTC front panel, which is opened by actuating 2 sliding pin latches on the upper part of the access door. The second access door, which is on the incubation/refrigeration chamber, is opened by simultaneously opening 2 sliding pin latches.

The BTR tub is accessed by opening the 4 corner latches on the front panel and then pulling out the drawer via 2 front panel grasp handles. The internal volume of the BTR tub is then accessed by opening the 2 hinge latches on the clear access door. The access door insulation panels must be removed before opening the access door to inspect for liquid droplets in the BTR tub.

Once the TCMs had been transferred into the Bitran bag, the crew performed various sample processing activities. They collected medium samples from the tissue culture bag using the sample syringes in the BTS stowage caddy. The crew removed the medium by attaching the sample syringe to the needleless septum on the tissue culture bag, using a twisting action. One mL of medium was removed for analysis. They analyzed the medium using the PCBA and specific cartridges (G3+ and 6+), which were stowed in the BTR. The medium was injected into the G3+ and 6+ cartridges, which were then loaded into the PCBA. The crew injected the cartridge with cell culture medium while it was inside the Bitran bag, and wiped it off before removing it from the bag. The crew used the PCBA to monitor the following chemical and gas constituents in the medium: pH, pCO_2, pO_2, HCO_3^-, Na^+, K^+, Cl^-, and glucose. Similar operations within the Bitran bags were required for cell culture transfer and fixation, which the

crew performed using the transfer syringes and fixative syringes contained in the BTS stowage caddy.

All operations involving transfer of fluids into or out of the tissue culture bags required sterilization via proper use of the Moll Zell (ethanol) and benzalkonium chloride wipes. The Moll Zell wipes were used to clean both needleless septums on the tissue culture bag.

The crew performed the microscope/photography operations in the Bitran bag. The first step in this process was to assemble the microscope and Maglite (small flashlight), and to place the microscope in the Bitran bag. The next step was to remove the TCM from the BSTC incubation/refrigeration chamber, insert it into the Bitran bag, and seal the bag. No medium or cell samples were extracted from the TCM during the microscope/photography operations. All hardware configuration activities were performed within the Bitran bag. The crew examined and photographed cells in the tissue culture bag. After this, the crew returned the TCM to the BSTC incubation/refrigeration chamber, and disassembled the microscope and returned it to storage.

2.3 LESSONS LEARNED

Section 6 describes the lessons learned from operating the BTS hardware and equipment on *Mir* for each of the experiments performed on *Mir* during the NASA-*Mir* Science Program. This allowed issues associated with the hardware and technology to be addressed in parallel with science issues encountered during the on-orbit activities.

3 VALIDATION OF BTF CONCEPTS AND SYSTEMS

3.1 REQUIREMENTS DEFINITION

The ISS BTF will provide a research platform for basic and applied research in the field of cell biology. Equipment and support services will be provided to enable many but not all types of cell biology / tissue engineering.

The BTF will provide middeck-style accommodations in the ISS microgravity environment. In addition to the advantage of extended-duration research, these facilities will be designed to provide enhanced on-orbit analysis, cooling capability, dedicated computing power, mass data storage, gas supply, and video signal handling capabilities. In addition to these BTF-unique resources, the rack also gives subrack payloads access to other standard resources such as vacuum and water cooling.

The BTF is a rack-level facility that will be placed in the ISS United States Laboratory. Its basic structure is based on the international standard payload rack. The BTF international standard payload rack is outfitted with subsystem equipment designed to meet specific performance requirements. Table 3-1 summarizes the capabilities of the BTF. The BTF design is general enough that it allows potential users to adapt many existing items of laboratory equipment to a form that is suitable for use on the ISS. This also permits a wide variety of microgravity science disciplines to adapt existing hardware or build new hardware that can be successfully operated in the BTF. The BTF is also designed for a high level of automation. This leaves crewmembers

free to perform other activities. The primary automation features are acquiring data and giving commands to BTF racks and payloads.

The BTF Project will also include an extensive ground infrastructure to support BTF users. The ground infrastructure supports all phases of a flight experiment. The BTF-related ground equipment and support personnel will be fully prepared to support payload development, payload integration, and payload operations on the ISS.

Table 3-1. BTF Capabilities Summary

Structural Accommodations	Middeck lockers or locker-type structures
Electrical Power	28 Vdc and 120 Vdc
Thermal Control	Water cooling and avionics air cooling
Gas Distribution	TBD
Data Acquisition	486DX2 computers
Data Storage	TBD MB
Communications and Commanding	Ethernet, RS 232, ARCNET
Fire Detection and Suppression	Rack-level smoke detector
Video	TBD
Vacuum	TBD

The BTF team will provide technical support to help BTF payload developers. The BTF team will also develop interface requirement documents, which define how payloads interface with the BTF. The BTF technical staff will assist the payload developer with the comprehensive safety and interface verification process required for flight hardware. The BTF staff has extensive experience flying hardware on the Space Shuttle, *Mir*, and the ISS. To fully support payload development, we will develop a ground version of the BTF so that payloads can be installed into and operated with the BTF before being launched to the ISS. This will permit the payload developer and BTF to verify that a payload will function properly when installed in the rack. Other tests can be performed to ensure that the payload can communicate with the ground once on orbit. In addition, we will have a portable version of the BTF simulator available to ship to user facilities.

The BTF project will provide support to payload developers during actual ISS operations, and the BTF User Operations Facility will be staffed during on-orbit operations. This team will monitor the performance of the BTF rack and the payloads operating in conjunction with it. Two options are possible: the BTF payload developers can provide real-time support at the User Operations Facility or the ISS Program can support telescience support centers at payload developer sites. This service will prevent payload developers from having to provide around-the-clock console staffing during ISS operations.

3.2 MODULAR DESIGN

The BTF will incorporate a modular design approach for both critical subsystems and payloads. The critical subsystem components are designed to be removed and replaced. The GSM can be easily removed for repair, maintenance, or replacement. The GSM can be replaced when the gas cylinders need recharging or new process gases are required for biotechnology experiments.

3.3 GAS SUPPLY SYSTEM

The GSM is a ½-MLE payload stowed in a foam enclosure. The GSM supplies and delivers 600 liters of mixed compressed medical grade gas. It will deliver the gas at a given rate of flow and at a given pressure. When the unit is depleted it is returned to ground via the shuttle and refilled for future use.

3.4 INTEGRATION PROCESS

Part of the difficulty of conducting various experiments in rack-mounted Shuttle payloads has been the need to supply specialized integration hardware for each installation. This has proved to be both time-consuming and unnecessarily expensive. To alleviate this problem, we have developed a BTF rack standardized interface concept to use on the ISS. This standardized interface approach will require no unique tools or experiment-unique integration hardware. The concept, which provides standardized mechanical, power, data, video, and avionics cabling interfaces, makes the removal and installation of experiments during on-orbit operations a far more practical function than it would be otherwise. This approach also minimizes crew time by using standard ISS interfaces.

In the past, experiment developers of major crewed scientific missions have spent a significant amount of time and resources complying with the interface requirements of a particular spacecraft like the Shuttle middeck. The standard interface concept now enables the experiment developer to design an experiment using data interface standards that are commonly available in research centers, and use mechanical interface hardware that has already been designed and proved to be structurally adequate for ISS applications.

Simplification begins with the preflight ground integration process where experiment-to-BTF rack interfaces and operations are performed in the Mini-PIC. Power and data bus input and output ports are provided at each mechanical locker installation. The experiment can be installed in the Mini-PIC lockers during this ground processing before launch. Not only does the standard interface concept simplify the experiment integration, design, development, and fabrication, but it also allows the same experiment system to be used on multiple missions.

The standard interface technique is especially advantageous on long-duration missions like those of the ISS because experiments can be installed and removed quickly on orbit at the sub-rack level. This experiment-to-rack interface with its "easy-to-use, easy-to-integrate" concept will not only allow the swift incorporation of an experiment during a mission, it will also facilitate the replacement of failed experiment drawers within the rack—not always a practical choice before.

3.5 MINI-PAYLOAD INTEGRATION CENTER

The Mini-PIC (Fig. 3-1) is a ground-based, 2-middeck-locker equivalent emulation of the BTF. It provides investigators a working model of the experiment interfaces, with which they can build, evaluate, and test their experiments.

The Mini-PIC provides a structural interface for mounting experiments identical to that found in the BTF rack. Each experiment location has a dedicated ECC for data collection and processing. Experiment data interfaces are identical to those of the planned flight facility. The Mini-PIC contains two ECCs. The ECCs are networked, via a custom internal high-speed network, to the Mini-PIC facility control computer (FCC). The Mini-PIC FCC coordinates rack resources and video capture and storage, and provides the communications link to the outside world. The Mini-PIC also provides electrical power, control, and gas and vacuum interfaces similar to those found in the BTF. Each experiment location has a cold plate that doubles as the mounting plate at the rear of the experiment bay. This cold plate can be attached to a laboratory water source to provide up to 400 W of cooling to an experiment.

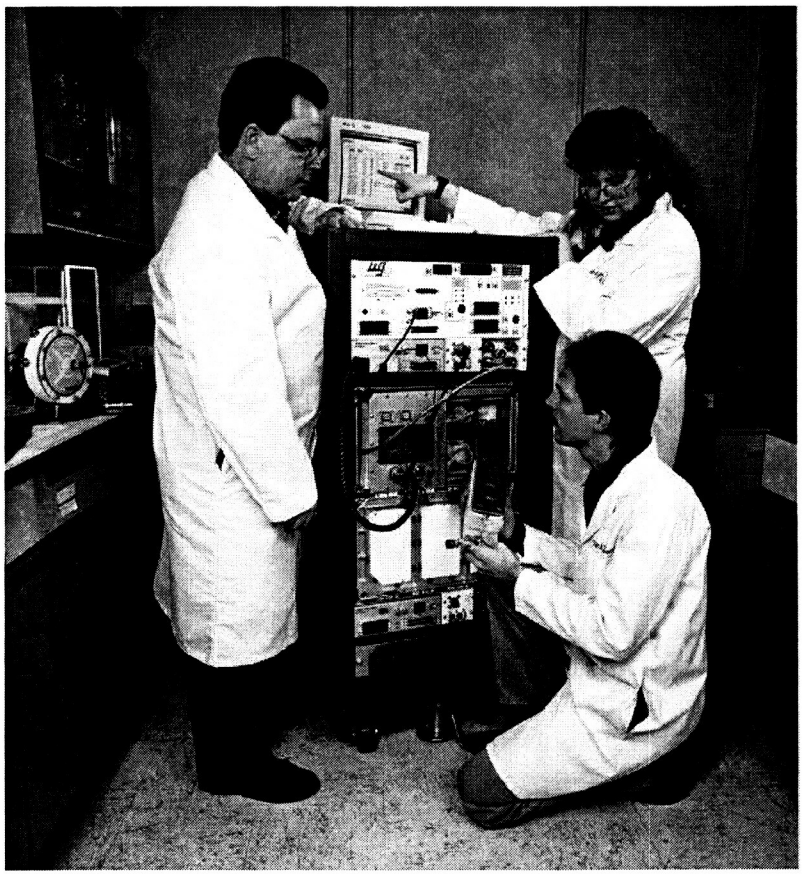

Figure 3-1. BTF Mini-PIC.

The Mini-PIC package includes extensive software to help the PIs develop experiment software, as well as test, debug, run, and evaluate both their software and their experiments. The software is designed to help the experimenters develop control code in a user-friendly fashion by using

established routines and libraries. This facilitates the integration of software and hardware into the Mini-PIC and eventually into the BTF (which uses identical operating software). The experimenter can also program the experiment-specific crewmember interface and validate its effectiveness. The PIs will be able to simulate the ground-based reception of flight data from their experiments to validate the completeness and usefulness of the transmitted data.

3.5.1 Mini-PIC Hardware Components

The Mini-PIC consists of ground-based versions of the BTF's components. Table 3-2 identifies the Mini-PIC components.

If required, the experimenter-supplied laboratory gases and vacuum source are interfaced through a manifold on the rear of the unit to a gas/vacuum distribution system, which distributes gas and vacuum and regulates gas at 40 psi for experiment use. This system consists of solenoid valves (one per gas/vacuum circuit, controlled by the Mini-PIC FCC). Pressure transducers (one per gas/vacuum circuit) record gas supply pressures on the Mini-PIC FCC data system.

Table 3-2. Mini-PIC Components

Item	Quantity	Features
ECC	2	486DX2 computer; identical to flight units in the BTF
FCC	1	486DX2, 66 MHz computer; emulates BTF facility-level services Video capture card; captures and stores video IDE drive interface card; provides interface to custom video switching matrix Communications interfaces: Ethernet, ARCNET, and RS-232
Experiment power and gas panel	2	Electrical power interfaces: 120 Vdc, 28 Vdc Five gas quick disconnects; can also be used for vacuum RCA-type video connectors
Combination cold/ mounting plate	2	Middeck locker structural accommodations; up to 400 W cooling capability per site

The cold plates/mounting plates provide a structural frame for mounting experiments in the Mini-PIC. These plates have middeck locker mounts identical to those on the Shuttle. In addition, the cold plates/mounting plates feature a grid of #10-32 holes on 2-in. centers. Non-standard experiments can be mounted on this additional grid. The cold plates are plumbed in series and are instrumented to provide monitoring of water temperatures and flow rates. The cold plates are not usable if the experimenter is using a standard middeck locker, as the lockers are not designed to accommodate cooling through cold plates without modifications. However, an experimenter may design the experiment to attach directly to the cold plate and achieve the full 400-W cooling capacity per experiment location. If the cold plates are not to be used, the experimenter must use forced-air convection with front panel inlets and exhausts.

3.6 LESSONS LEARNED

Our astronauts flew elements of the BTF on *Mir* during the Phase 1 Program (Fig. 4-1). The successful long-duration operation of the BTS facility on *Mir*, and the risk mitigation experiments conducted in the facility to date, have enabled us to validate and verify BTF concepts, technology, systems, and procedures. We will apply the knowledge gained from operating the BTS on *Mir* directly to facility hardware and experiment-specific hardware that will be flown in the BTF on the ISS. In addition, we are using the experience and results we've obtained from conducting fundamental science investigations in the BTS to clarify the science requirements for the BTF on the ISS and to optimize the BTF design to better meet the requirements of the science community.

4 VERIFICATION OF THE BTF OPERATIONAL AND TRAINING PROCEDURES

The previous sections of this report addressed the technology and systems aspects of the BTS and BTF. This section and the next address the operational aspects of both systems. We include a description of the training process used for the flight crews and ground support personnel. This section describes the operations and training required for the BTS on *Mir*, whereas the next section addresses the operations and training associated with launch, landing, and transfer activities. We discuss both nominal and contingency operations. We also identify specific problems encountered during the BTS experiments, and describe their impact on the scientific program. This information can be used to help mitigate the risk of operating biotechnology experiments in the BTF rack on the ISS.

Astronauts operated BTS experiments on *Mir* from Increment 2 through Increment 7. Figures 4.1 and 4-2 show the experiment timeline for BTS experiments on *Mir*. The crew performed more biotechnology experiments on *Mir* than for any other scientific disciplines, with the exceptions of human life sciences and *Mir* environmental monitoring. When both protein crystal growth and cell science experiments are considered, U.S. biotechnology experiments were performed on essentially every increment of the Shuttle-*Mir* Program.

The reasons for this long series of experiments and active involvement in the Shuttle-*Mir* Program include:

- U.S. space science priorities
- Flight hardware readiness
- Previous flight history on the Shuttle and SpaceHab
- Cellular biotechnology's ambitious plans for ISS use
- The presence of an existing and trained science and flight hardware development group at initiation of the Shuttle-*Mir* Program

Figure 4-1. Phase 1 Program schedule.

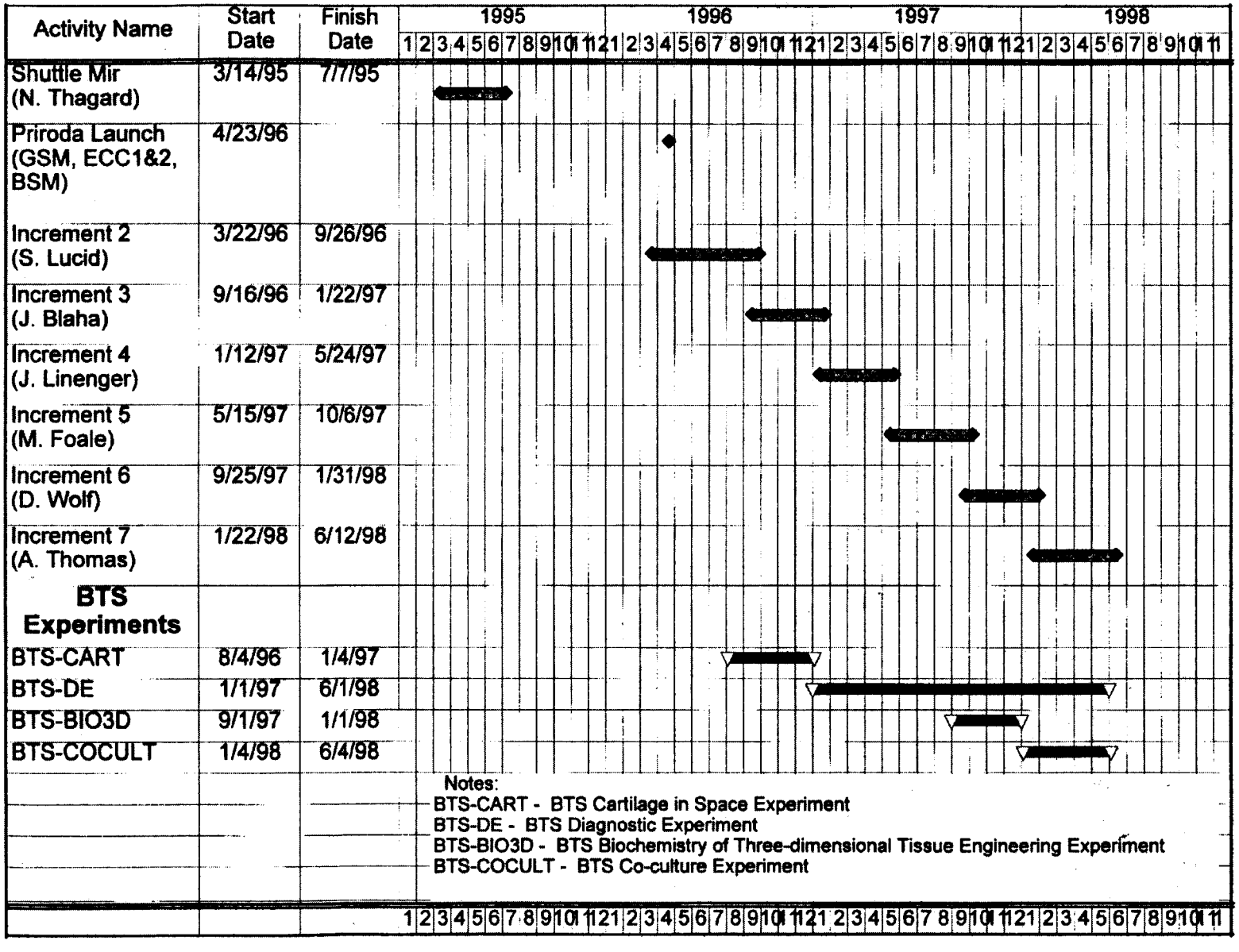

Figure 4-2. BTS experiment timeline on Mir.

The latter reason may be the most important, since the difference in time from program initiation to the first utilization flight was a little over a year. Also, the increment lengths, or periods between Shuttle docking flights, were only 90 to 120 days. This meant that it was possible to fly only existing hardware during the early increments of the program. We hadn't enough time available to establish new science and engineering groups. Existing, well-established NASA flight project capabilities were used to the maximum extent possible to use *Mir* on short notice during sequential operational increments from February 1994 until June 1998.

Although the BTS team trained U.S. astronauts, Russian cosmonauts, and Russian and U.S. ground operations personnel, only U.S. astronauts operated the experiments. BTS scientists and engineers trained the crew in both nominal operational procedures and malfunction or troubleshooting procedures. We videotaped the training sessions and put some of this taped material on CD-ROM for the crew to view on board as refresher training. This onboard training became very important, since the crew received the actual preflight training months and sometimes over a year before performing the experiment on *Mir*.

In one case, during prelaunch activities for the STS-86 mission, a U.S. crewmember received some last-minute training on the hardware for the BTS–BIO3D experiment at one of Kennedy Space Center's (KSC) laboratory facilities. This was a very unusual situation, since the training occurred only hours before launch while the crew was in quarantine to prevent contracting illness just before the mission. This late training was required because of some last-minute changes in the flight hardware and other unique requirements needed to operate the BSTC in a manner that would ensure scientific success. We took special precautions, such as limiting the training staff and requiring masks and gloves, to minimize risk to the crewmember while training. This is just one of many examples of training being performed at any opportunity; this case shows the importance placed on payload or science training for U.S. payloads operated on *Mir*.

4.1 OPERATION

4.1.1 Facility Hardware

In this section, we discuss the on-orbit operation of the BTS facility and experiment-specific hardware, specifically the normal modes of operation. We also describe the actual results of operation on *Mir*, with a discussion of problems, off-nominal situations, and hardware malfunctions experienced on *Mir*. The description of on-orbit operations is based on daily reports from the U.S. crewmembers and ground operations personnel in Moscow and at NASA's Marshall Space Flight Center and Johnson Space Center.

4.1.1.1 BTS Computer Module

We used the BCM—which is composed of 2 ECCs—for the BTS–CART, Diagnostic (BTS–DE), and Co-Culture (BTS–COCULT) experiments. During the BTS–CART and BTS–COCULT experiments, the ECC provided electrical power, control, and data logging for the facility and experiment-specific hardware. The BTS–CART and BTS–COCULT hardware configurations on *Mir* are shown in Figures 4-3 and 4-4, respectively. The ECC received electrical power via a cable from the Priroda module PUP. The ECC was connected to the

EDU–M via both electrical and data cables. The software required to run the experiment protocol was resident on PCMCIA cards in the ECC PCMCIA drives.

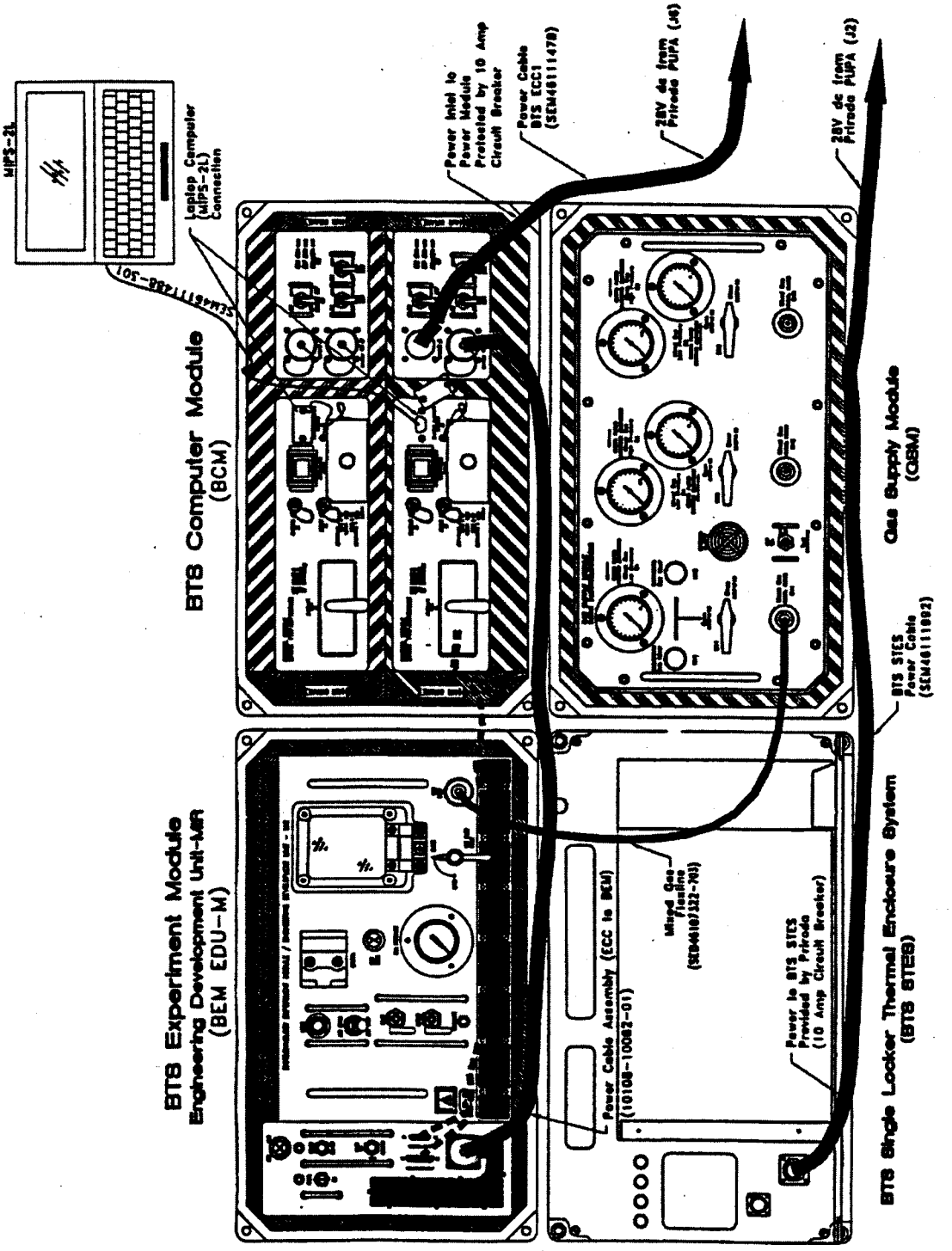

Figure 4-3. BTS–CART hardware configuration on Mir.

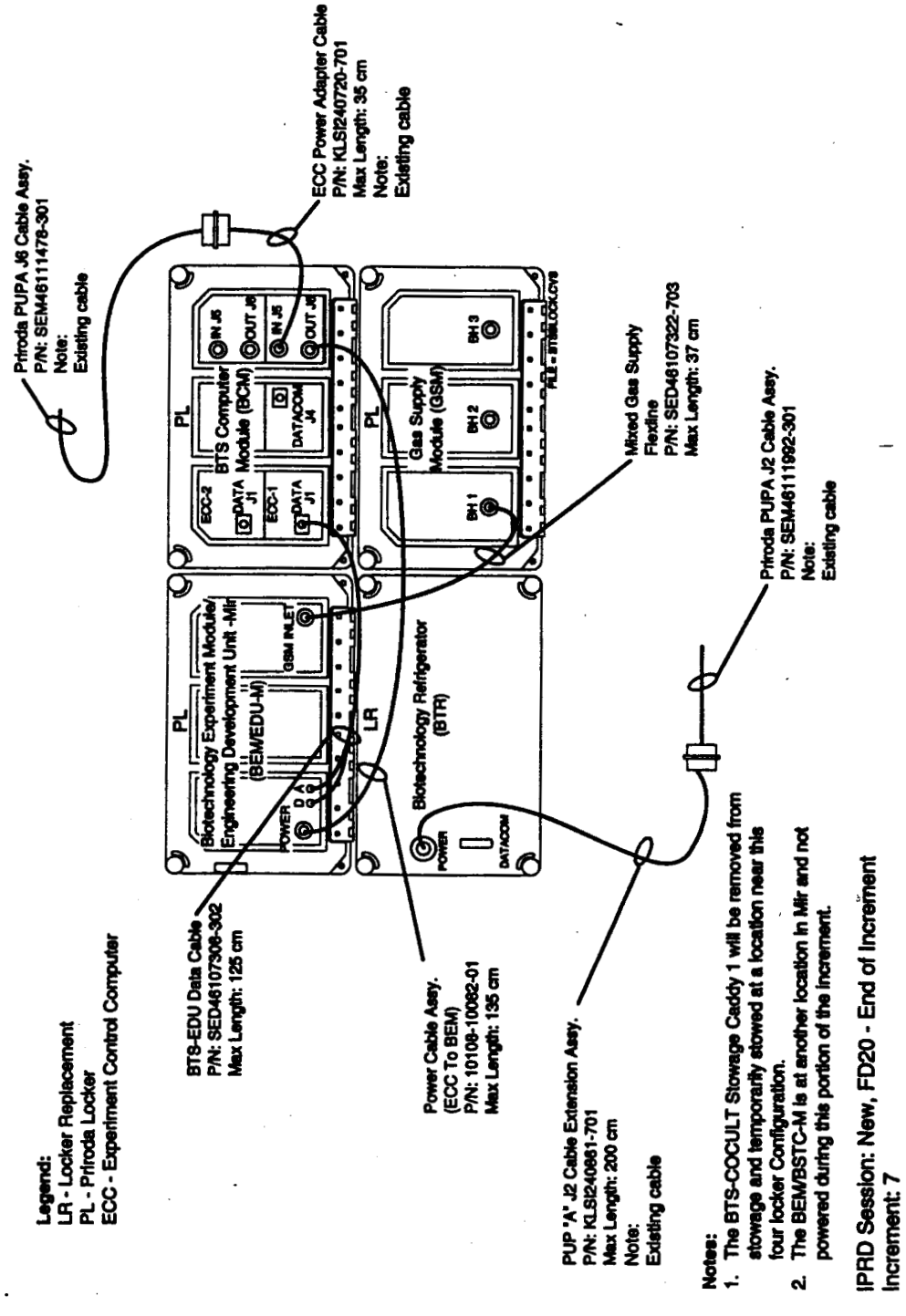

Figure 4-4. BTS–COCULT hardware configuration on Mir.

The BTS–DE experiment required only the ECCs. Various PCMCIA card types were tested in the ECC during Increments 4 through 7. This experiment did not require interfaces to other BTS hardware. Both ECCs were operated with various types of PCMCIA cards for up to 60 days. We monitored the effects of cosmic radiation on different PCMCIA card types by logging single event upsets (SEUs) to these space-qualified computers. This experiment required essentially continuous operation of the ECC during the 60-day period.

The ECCs functioned well on *Mir* with a few exceptions. Many of the problems encountered with them were the result of human error. Table 4-1 is a summary of the operational issues associated with the ECCs on *Mir*.

Table 4-1. ECC Operation on Mir

Increment	Date	Experiment	Summary	Data Source
3	10/8/96	BTS–CART	John Blaha reported PCMCIA card failure. A reboot from the card failure caused him to question the EDU–M medium waste and storage volumes (storage volume = 476.5 mL and waste volume 60.1 mL)	4
3, 4	12/3/96	BTS–DE, BTS–CART	ECC-S (i.e., Shuttle version transferred to *Mir* from docked STS-79) was used during Increment 3 for BTS–CART and during Increment 4 for BTS–DE. ECC#2 was the backup unit. Transferred two Data Capture Kits from Shuttle to *Mir* during STS-81 to start the BTS–DE experiment.	5
4	1/24/97	BTS–DE	Jerry Linenger reported difficulty getting data from the ECC when performing activation and card change out. He tried various cards and cable connections, but still did not receive data.	6
4	1/25/97	BTS–DE	Couldn't establish communications between the MIPS-2L laptop computer and ECC.	7
4	2/16/97	BTS–DE	J. Linenger said John Blaha told him ECC#1 (ECC-S) completely failed. Linenger asked why it was not removed during STS-81.	8
4	2/17/97	BTS–DE	Linenger reported both ECCs were functioning properly after the switch from COM2 to COM1.	9
4	2/18/97	BTS–DE	Linenger reported the EXP light was off, but the switch S2 light was still on.	10
4	2/19/97	BTS–DE	Using the *Mir* voltmeter, Linenger reported the BTS EXP ON light voltage was 0.0.	11
4	2/20/97	BTS–DE	Linenger performed malfunction procedures 24.20, 8.6, 24.7.5, and 24.7.6 using the Russian voltmeter and still the EXP ON LED did not illuminate.	12
4	2/22/97	BTS–DE	Fire on *Mir*. Cause is later determined to be Russian oxygen generation "candle." The fire did not damage BTS hardware.	13
4	2/23/97	BTS–DE	Russian operations personnel expressed concern about ECC#2 EXP ON LED malfunction. Explanation was requested.	14
4	2/23/97	BTS–DE	Linenger completed BTS health check and the EXP ON LED did not illuminate.	15
4	4/17/97	BTS–DE	Final operations for Increment 4.	16
5, 6	9/25/97	BTS–DE	During the functional check-out of ECC#1, Mike Foale experienced a delay of EXP ON LED of approximately 10 minutes (53 seconds is nominal).	17
6	11/14/97	BTS–DE	David Wolf reported ECC checkout failure.	18
6	11/20/97	BTS–DE	D. Wolf reported ECC#1 functional test was successful. Probable cause of failure of previous test was absence of PCMCIA cards.	19

The review of daily reports from *Mir* indicated that most of the issues related to operating the ECCs can be attributed to human error. Two examples in Table 4-1 include connecting to the wrong data connector and failing to install the PCMCIA cards. Both of these problems can be corrected with training and onboard training for similar operations with the BTF on the ISS.

4.1.1.2 Gas Supply Module

The GSM was used in the BTS–CART and BTS–COCULT experiments. The crew connected a flexible Teflon hose between the front panels of the GSM and EDU–M. The GSM provided the appropriate gas mixture, pressure, and flow rate for cell metabolism and pH control. They performed periodic status checks to verify that gas pressure levels were acceptable. The GSM was used only on *Mir*. During ascent and descent, a gas cylinder within the EDU–M housing provided the required gases. The GSM has 3 front-panel connections for flexible hoses, which were color-coded to prevent connecting them to the wrong front-panel connections. The GSM unit performed flawlessly from the time it was launched on the Priroda module until the end of Increment 7. Table 4-2 is a summary of GSM operations and related crew observations on *Mir*. The daily reports indicated no issues were associated with GSM operations, as shown in Table 4-2.

One potential improvement for future gas-supply systems that we will develop for the BTF on the ISS is a higher degree of automation. The GSM for Priroda was a completely manual unit. Linking the GSM with a data acquisition system will reduce crew time required for periodic status checks. Incorporating electronic pressure transducers will permit status information to be sent directly to the ground controllers. This addition will provide near-real-time status of the GSM.

Table 4-2. GSM Operation on Mir

Increment	Date	Experiment	Summary	Data Source
3	9/17/96	Status check	GSM cylinder pressures: G1 = 46.6, G2 = 2100, G3 = 829, G4 = 39.5, G5 = 43.0	20
4	1/25/97	Status check	Jerry Linenger recommended not checking cylinder pressures, since the unit is not being used this increment.	21
4	1/28/97	Status check	GSM cylinder pressures: G1 = 42, G2 = 2075, G3 = 0, G4 = 38, G5 = 36. Due to 0 reading on G3, Linenger ran the prescribed malfunction procedure, which indicated G3 was within range. He said the GSM couldn't be pulled out of the locker because of interference with the ALISA experiment, but he could get to the valves. ALISA was a European experiment located in the Priroda center aisle.	22
6	11/9/98	BTS facility reconfiguration	The GSM and BCM lockers were exchanged with contents intact. Now, there is no need to move the ALISA experiment to exchange the ECCs.	23
7	4/30/98	BTS–COCULT	The GSM was deactivated. It is believed that only the front panel valves were turned off.	24

4.1.1.3 Single Locker Thermal Enclosure System

The STES was used in the BTS–CART experiment during Increment 3. It launched on STS-79 and returned on STS-81. The STES is a constant-temperature enclosure that has a long flight history in support of protein crystal growth experiments in microgravity. It can be operated in either refrigeration or incubation mode. On *Mir*, the STES was used to store lyophilized (powdered) culture media, analytical cartridges for the medium analyzer unit, and samples of culture medium taken from the EDU–M during the BTS–CART experiment. Previously, the STES had flown on short-duration flights of up to 14 days on the Shuttle. The STES was operated continuously for approximately 5 months on *Mir*. It was also operated on the ascent and descent flights. Table 4-3 is a summary of the crew observations of STES operations on *Mir*. The STES set point on *Mir* was 7ºC.

Table 4-3. STES Operation on Mir

Increment	Date	Experiment	Summary	Data Source
3	9/22/96	BTS–CART	Internal temperature = 6.9°C	25
3	9/26/96	BTS–CART	Internal temperature = 7.1°C	26
3	12/3/96	BTS–CART	Internal temperature = 8.5°C	27
3	12/8/96	BTS–CART	Internal temperature = 9.3°C	28
3	12/18/96	BTS–CART	John Blaha reported that one desiccant was left. He recommended that in the future, twice the amount of desiccant be flown.	29
3	12/19/96	BTS–CART	Blaha reported that the STES was close to being full. This may have explained the temperature increase.	30
3	1/3/97	BTS–CART	Internal temperature = 9.9°C	31
3	1/4/97	BTS–CART	Internal temperature = 9.0°C; J.Blaha reported that the *Mir* internal temperature was cooler and that he had received the radiogram to clean the STES heat exchanger fins.	32
3	1/5/97	BTS–CART	Internal temperature = 8.9°C; Blaha reported he had directed a ventilation hose toward the STES air intake.	33
3	1/6/97	BTS–CART	Internal temperature = 8.1°C	34
3	1/7/97	BTS–CART	Blaha reported that direct ventilation was working and the STES did not need to be relocated.	35
3	1/12/97	BTS–CART	Internal temperature = 8.8°C	36
3	1/15/97	BTS–CART	Internal temperature = 7.5°C	37
3	1/19/97	BTS–CART	Internal temperature = 7.3°C; The STES was transferred from *Mir* to the Shuttle for return on STS-81.	38

The STES performed successfully over the entire increment. However, some minor upward drift in internal temperature occurred near the end of the increment. This was attributed to degraded

performance of the thermoelectric coolers resulting from humidity-induced corrosion. U.S. astronaut John Blaha tried to alleviate this problem by placing a flexible Teflon hose so that it exhausted cooler *Mir* air directly into the air inlet of the STES. He also performed an in-flight maintenance procedure to clean the heat exchanger fins of the thermoelectric coolers. Both of these solutions were successful in reducing the internal temperatures of the STES. Overall, STES operations were successful. The biological items stowed in the STES were well maintained on *Mir*.

4.1.1.4 Biotechnology Refrigerator

The BTR was used during Increments 6 and 7 for the BTS–BIO3D and BTS–COCULT experiments respectively. The BTR replaced the STES that was flown during Increment 3. It performed the same function as the STES, which included storage of powdered medium, analytical cartridges, and medium samples. The BTR was a new hardware development for *Mir*. It was launched on STS-86 and returned on STS-91. Table 4-4 is a summary of BTR operation on *Mir*. The BTR set-point temperature during Increments 6 and 7 was 7ºC.

Table 4-4. BTR Operation on Mir

Increment	Date	Experiment	Summary	Data Source
6	10/7/97	BTS–BIO3D	The circuit breaker (CB) on the BTR tripped. David Wolf had to reset the set point using the MIPS-2L laptop computer.	39
6	10/7/97	BTS–BIO3D	D. Wolf reported that when he pushed in the BTR CB, the PUP CB popped out. He reported he reset everything and the CB stayed in, but the temperature display went to 0°C set point and –0.5°C on the actual display. Wolf repeated this 2 or 3 times with the same result. He performed the malfunction procedure several times with the same result. He reported feeling warm air, indicating that the BTR was powered and running.	40
6	10/9/97	BTS–BIO3D	D. Wolf reported the existence of free water in the BTR. The water was believed to be the result of condensation.	41
6	10/9/97	BTS–BIO3D	The BTS ground support team provided recommendations on how to address the condensation problem.	42
6	10/10/97	BTS–BIO3D	Wolf received instructions to clean up the condensation in the BTR. He reported that the BTR was "jammed tight" inside. He evaluated whether there was too much pressure on the cell culture bags.	43
6	10/11/97	BTS–BIO3D	The BTR internal temperature rose from 7.0°C to 10.6°C. The TCMs and analytical cartridges were moved to a refrigerator in the Spacehab module.	44
6	10/12/97	BTS–BIO3D	Wolf reported that the set point was again 0°C and the actual reading was again –0.5°C. He stated that the fans were blowing warm air.	45

Increment	Date	Experiment	Summary	Data Source
6	10/22/97	BTS–BIO3D	The BSTC and BTR were to be transferred and repowered in the Priroda module.	46
6	10/23/97	BTS–BIO3D	The BTR internal temperature climbed to 13°C. Wolf recycled the power several times and pulled the unit out of the locker housing to get better air flow. At the request of the BTS ground team, Wolf reinstalled the unit in the locker and used duct tape to create a tight seal within the bulkhead. The next day the temperature was down to 12.8°C. Later it came down to 11.2°C.	47
6	10/24/97	BTS–BIO3D	Internal temperature = 13°C	48
6	10/28/97	BTS–BIO3D	Wolf reported the BTR temperature was down to 11.2°C. He used more duct tape to seal the unit. This kept the electronics heat out of the fan air. He also reported that the BTR was not as full as it used to be, since the cartridges were being used.	49
6	11/2/97	BTS–BIO3D	Internal temperature =11.1°C to 11.3°C	50
6	11/14/97	BTS–BIO3D	The *Mir* core module lost power. The BTR internal temperature rose to 25°C.	51
6	11/16/97	BTS–BIO3D	Internal temperature = 9.5°C	52
6	11/21/97	BTS–BIO3D	The hardware was turned off last evening for several hours. No reason was provided.	53
6	1/2/98	BTS–BIO3D	The BTS ground team determined recommended requirements for the BTR and its contents in case of power loss.	54
7	2/1/98	BTS–COCULT	Wolf found the BTR power off, repowered it and it returned to optimal temperature in 1 hr. He reported the maximal amount of time off was 5 hr.	55
7	2/3/98	BTS–COCULT	The BTS ground team recommended taping the BTR locker housing to the Priroda bulkhead on all 4 sides.	56
7	2/6/98	BTS–COCULT	Andrew Thomas sealed the BTR locker to the bulkhead with gray tape so the BTR could still slide in and out.	57
7	2/13/98	BTS–COCULT	Internal temperature = 10°C	58
7	3/9/98	BTS–COCULT	Internal temperature = 16.5°C (reflects the higher temperature in Priroda)	59
7	3/11/98	BTS–COCULT	Internal temperature = 15.5°C	60

Several BTR anomalies were reported during Increments 6 and 7. Most appear to have resulted from temperature control or display system failures. Also, the design of the BTR thermal control system allowed warmer surrounding air to be drawn into the unit, which prevented the unit from maintaining the set-point temperature. The Increment 6 and 7 U.S. astronauts used duct tape to alleviate this problem. With the exception of these issues, the BTR operated essentially continuously for approximately 9 months on *Mir* and provided temperatures low enough to maintain the temperature-sensitive items that were critical to the success of both the BTS–BIO3D and the BTS–COCULT experiments.

4.1.1.5 BTS Stowage Module

The BSM is a collection of small items and equipment that was used in support of all the BTS experiments performed on *Mir*. Three lockers of stowage hardware were launched on the Priroda module, including 2 lockers of 1-liter bags of sterile water for use in the BTS–CART and BTS–COCULT experiments. The crew used the water to rehydrate the lyophilized culture medium for infusion into the EDU-M. Once the powder had dissolved in the water, the medium bag was attached to a port on the front panel of the EDU–M, and the medium was pumped or forced manually into the bioreactor's fresh medium reservoir bag. Simultaneously, waste medium was removed from another front-panel port. The waste medium was pumped out of the bioreactor into an empty water bag. Special precautions were taken with the waste medium on *Mir*. The crew used other stowage hardware, such as syringes and wipes, at various phases in the experiment timeline for activities such as monitoring specific media components (glucose, lactate, gases) to assess the health of the cell culture and to photograph cell and tissue samples.

One of the BSMs launched on Priroda included cables that provided power and data interfaces between the BTS components and the laptop computer. This flight also included flexible Teflon hoses, which routed gases between the GSM and EDU–M. The crew used two PCBAs stowed in the BSM to monitor specific media components. The cartridges for the PCBA were flown in the STES and BTR, since they required refrigeration. Generally, facility hardware flown on Priroda was required during all increments. Stowage equipment required for the individual experiments was flown with the experiment-specific hardware on the Shuttle, to support our increment-specific experiments during one or more increments, and discarded or returned on the Shuttle. This measure was prudent because of the lack of stowage volume on *Mir*. Current planning for ISS indicates that stowage volume will be a very limited resource because of the large number of experiments to be operated and the vast amount of vehicle subsystem equipment that will be operated and maintained for many years. Table 4-5 is a summary of U.S. astronauts' observations reported in daily operational reports by the crew themselves and ground controllers.

Table 4-5 shows the majority of issues with stowage hardware were associated with the PCBA and microscope. We later determined that the PCBA units were not designed to operate at the low cabin pressures experienced on *Mir*. The PCBA is a commercial unit that was modified for spaceflight. It was never designed to operate at the pressures that occur on the lower end of the *Mir* pressure range. The manufacturer modified the units and we had new units flown to *Mir* on a Russian Progress vehicle.

Table 4-5. BSM Operation on Mir

Increment	Date	Experiment	Summary	Data Source
3	9/24/96	BTS–CART	PCBA failure code 82 was reported. This is a pressure transducer failure, and ground repair was required to correct the problem.	60
3	10/8/98	BTS–CART	John Blaha reported that the waste medium bags were in a UCB, which was stored in the end-cone of Priroda	61
3	11/1/96	BTS–CART	The BTS ground team requested use of a Russian voltmeter to check PCBA batteries. No U.S. voltmeter had been flown to *Mir*.	62
4	1/30/97	BTS–CART	The shift flight director and operations lead decided not to remove the waste medium bags from the UCBs because Jerry Linenger reported these bags were deep in the Progress vehicle and the cosmonauts said 2 layers of containment were being observed.	63
4	3/15/97	BTS–DE	J. Linenger reported, "BTS is sprawling all over. Too much material – taking up too much space. Figure out a better way (lots of half-empty foam). Need to toss some of it."	64
6	10/6/97	BTS–BIO3D	A teleconference between the BTS ground team, the *Mir* Operations Support Team (MOST), Microgravity Payload Operations Support Area (MPOSA) was held to discuss problems David Wolf had with the microscope.	65
6	10/7/97	BTS–BIO3D	Wolf reported that he couldn't get the samples to fit under the microscope lens. He used the macro lens and handheld camera to get some photos.	66
6	10/9/97	BTS–BIO3D	Wolf reported the microscope had been modified.	67
6	10/16/97	BTS–BIO3D	Clips were removed from the microscope stage. This made cell-level photography possible.	68
6	10/16/97	BTS–BIO3D	Wolf reported that, to modify the microscope for microscopy operations, he had to remove the pins, which secured the clips to the microscope stage, by pushing out the pressed inserts from the stage using pliers, a pick, and a hammer.	69
6	11/6/97	BTS–BIO3D	The BTS ground team was requested to submit a plan for use of cannulas and PCBA I-stat cartridges. There were concerns about possible shortages.	70
6	12/29/97	BTS–BIO3D	Status Report FD92 (12/26/97) Wolf removed the cables from the BTS–BIO3D stowage caddy, as requested, along with the null modem cable, and placed them in a bag (which he labeled "cable bag") to be left on board *Mir*.	71
7	2/4/98	BTS–COCULT	BTS ground personnel will send these items on Progress: 2 PCBA Kits and 1 PCMCIA card with new software for the EDU–M. The *Mir* PCBA kits were inadvertently returned on STS-89.	72

The microscope problems were related to mounting the TCMs on the stage for inspection and photography. The microscope's optical performance was acceptable. David Wolf made some hardware modifications using onboard tools during Increment 6. After he had made these modifications, he successfully photographed the magnified cell cultures.

The PCBA batteries needed to be checked with a voltmeter. However, since a voltmeter was not included in the standard set of tools NASA sent to *Mir* for use during the Shuttle *Mir* Science Program, the crew used a Russian voltmeter to check the batteries. For ISS operations, the BTS team should verify that a voltmeter with a low voltage range is included in the ISS general-purpose tool kits. If not, a voltmeter should be included in the BTF dedicated stowage kits.

Microbial contamination of the *Mir* internal environment was a major concern of the Russian specialists. Because of this, the crew took special precautions with disposal of the waste medium from the EDU–M. The Russian specialists wanted to ensure that the waste medium was not released into the *Mir* cabin, since it contained nutrients that would accelerate the growth of microbes already present on *Mir*. The Russians required that the waste medium have two levels of containment in all phases of the BTS-CART, BTS–BIO3D, and BTS–COCULT experiments. The empty 1-liter water bags that had been filled with waste medium via the front panel of the EDU–M provided the first level of containment. The UCB provided a second level of containment. The UCB is a large bag used for both urine and waste medium containment. The crew temporarily stowed the UCB in the Priroda module end cone until it was full, then moved it into the Russian Progress vehicle. The UCB was eventually jettisoned with the Progress. The Russian engineers also imposed other hardware design and procedural requirements, aimed at preventing microbial growth on *Mir*, on U.S. experiments. Another requirement was that all surfaces of the flight hardware had to be cleaned with 30% hydrogen peroxide before being launched to *Mir*, to disinfect the equipment surfaces. Also, the Russian integration specialists required that the experiment hardware not have surfaces that were irregular or difficult to clean. For example, the tethers for the electrical connector covers could not be link chain or ball chain. The Russian safety specialist required the use of plastic-covered wire for these simple and small tethers, because the wire can be thoroughly cleaned both on the ground and on orbit whereas tethers made of irregular surfaces do not permit access to all surfaces for cleaning and disinfecting. The control and prevention of microbial contamination was a continuous focus of *Mir* integration specialists.

4.1.2 Experiment-Specific Hardware

4.1.2.1 Space Bioreactor (Engineering Development Unit-Mir)

The EDU–M was operated during Increments 3 and 7 as part of the BTS–CART and BTS–COCULT experiments, respectively. The EDU–M space bioreactor is the most complex experiment-specific hardware element of the BTS. It is also the primary component used in these experiments since it provides the proper thermal, nutrient, and chemical environment for cell growth. Because of this complexity, there is more risk for component and system malfunctions. The EDU–M remained functional throughout both increments. However, a substantial amount of crew intervention with intensive support from ground personnel in both the U.S. and Russia was required to keep the EDU–M functioning well enough to keep the experiments going. The health

of the cells was threatened in some instances during the increments. Heroic efforts from the U.S. astronauts and ground teams essentially saved the BTS–CART and BTS–COCULT experiments. All of this was done under the close scrutiny of Russian ground controllers and scientific organizations that feared the release of even small amounts of medium or cells into the *Mir* environment. Table 4-6 summarizes EDU–M operations. It describes some of the key issues associated with EDU–M operations during the BTS–CART and BTS–COCULT experiments along with the experiment recovery activities performed on *Mir*.

Table 4-6. EDU–M Operation on Mir

Increment	Date	Experiment	Summary	Data Source
3	9/22/96	BTS–CART	John Blaha reported red blinking light "incubator temp low." He pushed button and 26.3°C displayed. He ran full functional check.	73
3	9/23/96	BTS–CART	Blaha reported that EDU–M temperature measurements were erratic, and when ECC was power cycled, bioreactor stopped rotating.	74
3	10/26/96	BTS–CART	Blaha's observations of medium and cartilage constructs: "I would say the cartilage is very well distributed. Without a doubt, I have seen new globs forming."	75
3	10/26/96	BTS–CART	Medium was very clear light pink. Cartilage filled about 50% of chamber. Air bubble attached to wall toward inside of EDU-M.	76
3	11/4/96	BTS–CART	"Medium storage low" light was illuminated, in "compliance fill" and had been for 4 hr, so Blaha did "halt compliance fill" as before. After that, storage volume (SV) = 0, waste volume (WV)=180.1.	78
3	11/8/96	BTS–CART	Blaha reported cartilage looked very stable and well distributed, and was continuing to grow. The air bubble was getting smaller.	79
3	11/17/96	BTS–CART	After uplink of In-Flight Maintenance Procedure for Direct Infusion, procedure was completed even though it hadn't been scheduled.	80
3	11/18/96	BTS–CART	After direct infusion, 80% of chamber was filled with air. Blaha was concerned that more medium infusion would introduce more air.	81
3	11/19/96	BTS–CART	Blaha clarified that direct infusion did not introduce more air than automatic infusion. He thought the source of air was medium.	82
3	11/19/96	BTS–CART	Blaha provided a very detailed description of results of direct infusion. He proposed several reasons why air might have entered bioreactor vessel.	83
3	11/25/96	BTS–CART	Blaha performed maintenance procedure. Opened housing that provided second level of containment. Medium lines were clear—hardly any medium was in lines. Filter was empty. Observed some condensation on inside wall. Waste container had 50 mL of fluid, and supply containers had none.	84
3	11/27/96	BTS–CART	When Blaha refilled medium bag, only air was pumped into vessel. He stopped filling procedure after 3 of the scheduled 30 minutes.	85
3	12/3/96	BTS–CART	PCBA readings were taken. Blaha reported medium was cloudy.	86

Increment	Date	Experiment	Summary	Data Source
3	12/4/96	BTS–CART	Blaha reported direct feed went "great." 450 mL of fresh medium was added, 450 mL waste medium was removed. Many small bubbles stuck to spin filter. The perfusion rate was set to 4.0.	87
3	12/7/96	BTS–CART	PCBA readings were taken. SV=750 mL and WV=0 mL.	88
3	12/12/96	BTS–CART	Infused 200 mL fresh medium, removed 380 mL waste medium. 80% of air came out when air bubble reached certain volume and attached to spin filter.	89
3	12/14/96	BTS–CART	Medium sampling went well. The cartilage appeared to be stable and healthy. Bubbles grew to about the size of a nickel.	90
3.	12/15/96	BTS–CART	Blaha reported about 15% air in the vessel.	91
3	12/18/96	BTS–CART	The vessel contained 20% air.	92
3	1/12/97	BTS–CART	SV = 125 mL and WV = 625 mL. The volume of medium infused was 125 mL, the volume of waste medium removed was 125 mL.	93
3/4	1/13/97	BTS–CART	SV = 650 mL, WV = 100 mL, Volume in = 100 mL, Volume out = 100 mL; PCBA readings were taken.	94
3/4	1/15/97	BTS–CART	SV=475 mL, WV=275 mL, Volume in=75 mL, Volume out=75 mL.	95
3/4	1/19/97	BTS–CART	Transferred the EDU–M from *Mir* to the Shuttle.	96
6/7	12/16/97	BTS–COCULT	MPOSA requested a copy of BCM–GSM exchange and EDU–M locker reconfiguration radiograms; sent to *Mir* on 12/12/97.	97
6/7	12/30/97	BTS–COCULT	BTS engineers worked to determine if the glovebag could be used from the other side.	98
6/7	1/19/98	BTS–COCULT	Exchanged the GSM and BCM lockers with contents intact. This permitted the crew to use the glovebag on the side access panel of the EDU–M when it was pulled out of the locker housing.	99
6/7	1/23/98	BTS–COCULT	Continued troubleshooting aboard the Shuttle to resolve the medium flow problem.	100
6/7	1/26/98	BTS–COCULT	Completed BTS-COCULT transfer from Shuttle to *Mir*. A. Thomas reported 2 bubbles (about 1 cm dia. each) in bioreactor. He was told this was acceptable. Collected data on all hardware parameters.	101
6/7	1/26/98	BTS–COCULT	Completed BTS–COCULT transfer. Reported data hardware parameters. Thomas reported 2 bubbles in bioreactor, each about 1 cm dia. Ground control said this was acceptable. EDU–M connected to GSM.	102
7	1/28/98	BTS–COCULT	Performed automatic infusion of medium. Data on the MIPS-2L did not match the actual amount of medium infused. Will attempt infusion again tomorrow.	103
7	1/29/98	BTS–COCULT	Thomas talked directly with BTS ground controllers about EDU–M troubleshooting. He was able to fill the storage bag with the in-line filter removed. He had concerns about the air bubbles.	104
7	2/2/98	BTS–COCULT	pH of medium was reported to be 7.2 based on visual inspection.	105

Increment	Date	Experiment	Summary	Data Source
			Thomas reported 2 large (12 to 15 mm dia.) bubbles in bioreactor.	
7	2/6/98	BTS-COCULT	Thomas reported "medium line low" message. SV=250 mL, WV=450 mL.	106
7	2/7/98	BTS-COCULT	SV = 0 mL, WV = 632 mL. Air seemed to be getting into the bioreactor, making the bubbles larger.	107
7	2/10/98	BTS-COCULT	Medium pH estimate: 7.2. Large bubbles may have sheared cell aggregates that were seen earlier.	108
7	2/12/98	BTS-COCULT	A photography session included pH strips. Thomas reported the EDU-M vessel had stopped rotating. He cycled power and it started rotating again.	109
7	2/16/98	BTS-COCULT	Thomas performed bubble removal procedures. Reported they did not successfully remove bubbles. Was able to infuse more medium by spinning the bag to move the air bubbles. More bubbles were infused from the medium bag.	110
7	2/20/98	BTS-COCULT	Vessel stopped rotating at 1640 hr. The malfunction procedure used previously worked to restart rotation.	111
7	2/22/98	BTS-COCULT	SV = 261 mL, WV = 445 mL. Bubble size was slightly smaller, and pH was unchanged. Downlinked three minutes of video. Vessel rotation stopped again. The system now normal.	112
7	2/23/98	BTS-COCULT	Bubble removal procedure was not successful. pH was between 7 and 8, per pH strips. Thomas increased vessel rotation to 25 rpm.	113
7	2/26/98	BTS-COCULT	PCBA readings showed partial pressure of 0_2 (P_{O2}) was 140 mm Hg. Normal range is 80 to 100. Glucose readings dropped, indicating cells were metabolizing glucose.	114
7	2/26/98	BTS-COCULT	A lengthy summary was given as to why high P_{O2} readings were detrimental to scientific success. Plans made to reduce perfusion ratio to lower the P_{O2} levels. This was an experiment-critical issue.	115
7	2/27/98	BTS-COCULT	Held meeting with John Blaha about how he reduced bubble size during Increment 3.	116
7	2/27/98	BTS-COCULT	pH=7.4. Ground control directed Thomas to set perfusion rate to 1.0.	117
7	3/2/98	BTS-COCULT	Lengthy discussion took place about severity of bubble problem and plans for future on-orbit mitigation of bubbles in the vessel.	118
7	3/3/98	BTS-COCULT	Replaced PCMCIA card. New software lowered medium automatic infusion rate. Medium won't have to be replaced as often as before.	119
7	3/3/98	BTS-COCULT	More discussion about the high P_{O2} issue. The BTS scientist prepared a letter about the impact of high P_{O2} on cells.	120
7	3/6/98	BTS-COCULT	Bubbles seemed noticeably smaller.	121
7	3/9/98	BTS-COCULT	Thomas reported reduction in number and size of bubbles (only 5 remained). pH=7.4. He set perfusion rate to 1.0 mL/min every day.	122
7	3/11/98	BTS-COCULT	Thomas reported the "high temperature" light came on. He ran the prescribed malfunction procedures but no change occurred. He	123

Increment	Date	Experiment	Summary	Data Source
			attributed it to the high temperatures on *Mir*.	
7	3/12/98	BTS–COCULT	The incubator temperature was down from 39°C to 37°C. The Priroda module was getting cooler. The waste medium removal procedure was unsuccessful.	124
7	3/12/98	BTS–COCULT	More bubbles appeared after the unsuccessful attempt to remove waste medium.	125
7	3/14/98	BTS–COCULT	Pressure readings were called down. PT5 - 34.1	126
7	3/17/98	BTS–COCULT	Thomas reported a "Pump outlet pressure high" message on the ECC. This pressure had been increasing. A week ago it was 1 psi, 3 or 4 days ago it was 4 psi, on this day it was 9.5 psi.	127
7	3/18/98	BTS–COCULT	When the perfusion rate was set to 8 mL/min, after 5 minutes the pump outlet pressure dropped from 10 to 3.5 psi. After Thomas reset the perfusion rate to 1 mL/min, the system was nominal. Cell aggregates (dark filaments) in the spin filter were seen when vessel rotation was stopped.	128
7	3/19/98	BTS–COCULT	Pump outlet pressure increased from 3.3 to 6.5 psi. Replaced the medium. Performed PCBA readings using the new PCBA, which arrived on Progress. P_{O2} was high, but all other readings were nominal.	129
7	3/23/98	BTS–COCULT	PT5 = 30.9, PT6 = 1.3 (outside nominal range), PT3 = 4.1. PCBA reading: P_{O2} = 79 psi, pump outlet pressure = 4.5 psi, SV = 443 mL, WV = 306 mL.	130
7	3/27/98	BTS–COCULT	During medium exchange procedure, Thomas couldn't get medium through inlet port. PT3 in-creased from 0.5 to 3.8 when medium bag was squeezed. Huge bubbles were found in bioreactor vessel. Bubbles occupied over 50% of vessel. At 2249 hours no medium was present in vessel, PT4 = 19.1. Thomas was not able to get medium into system. He added it via the outlet valve.	131
7	3/28/98	BTS–COCULT	Thomas switched bags between inlet and outlet ports. When the valves were opened, medium flowed into the bioreactor from the outlet port, filling 70% of the vessel. This indicated the inlet port was blocked. Ground controllers prepared repair procedure.	132
7	3/29/98	BTS–COCULT	Opened EDU–M housing. Found blockage in the expected area. Located a hard crystalline black substance. Thomas reported it was "crunchy" when he broke it up. He couldn't get the medium in and reported another potential blockage ahead of the vessel. He also reported a droplet at the spot of previous blockage. He closed the housing, set the perfusion rate to 0, and restarted the system.	133
7	3/30/98	BTS–COCULT	Blockage was suspected in line between vessel SV7 and bubble trap. Ground controllers prepared new malfunction procedures.	134
7	3/30/98	BTS–COCULT	Thomas reported minor breach in perfusion loop. A lengthy description of steps taken to isolate bioreactor and prevent contamination was provided, as well as detailed plan of action for onboard operations.	135

Increment	Date	Experiment	Summary	Data Source
			U.S. team requested Russian approval of procedures.	
7	3/31/98	BTS–COCULT	Provided a description of the medium line crimp pressure test. The BTS team demonstrated the procedure in a ground-based system to verify the crew procedures planned for use on *Mir*.	136
7	4/7/98	BTS–COCULT	Provided pressure readings. Approval was given for medium infusion. Russian Institute for Biomedical Problems (IBMP) disapproved operations, but was overruled by MOST in Moscow.	137
7	4/9/98	BTS–COCULT	Summarized discussions with IBMP regarding in-flight maintenance procedures required to correct problems with this EDU–M.	138
7	4/10/98	BTS–COCULT	Provided PCBA readings. Thomas completed the side panel infusion and medium sampling.	139
7	4/11/98	BTS–COCULT	The modified medium sampling and medium infusion (from the side panel) takes approximately 1 hr, according to Thomas.	140
7	4/12/98	BTS–COCULT	The vessel outer wall stopped rotating. Thomas cycled the power and the unit began to work properly.	141
7	4/13/98	BTS–COCULT	Bioreactor isolation radiogram was approved. It involved crimping some of tubing and removing some of tubing from the pump.	142
7	4/17/98	BTS–COCULT	Thomas reported presence of 2 large bubbles in the bioreactor. He had observed good cell aggregations and cell clumps in 1- to 3-mm range and reported this was strongest aggregation to date.	143
7	4/24/98	BTS–COCULT	Performed medium infusion. Pump outlet pressure was high (18.7). Thomas automatically opened SV7, pumping stopped because of high pressure. He cycled the system and it came back up nominal. Thomas believed the initial pressure reading was erroneous.	144
7	4/27/98	BST-COCULT	The bubble removal procedure was unsuccessful. Thomas stopped trying after withdrawing about 1.5 syringes of liquid. He reported that a needle would be needed to withdraw the bubble.	145
7	4/28/98	BTS–COCULT	Thomas performed bioreactor isolation procedure. Isolated bubble filter, and tubing was removed from the pump. Thomas believed high pressure in pump was bleeding through into vessel, since the bubbles were larger that day. The unit was off for 1.5 hours.	146
7	5/2/98	BTS–COCULT	Reported PCBA readings; glucose was high. Thomas thought this happened because medium being sampled was largely fresh medium in lines, which resulted from using fresh medium bag as compliance.	147
7	5/22/98	BTS–COCULT	Sent about 3 minutes of video to the ground. The large bubble was clearly seen, as well as what appeared to be cell constructs. The outer wall stopped rotating once that week. Thomas cycled the power and the rotation resumed.	148
7	5/27/98	BTS–COCULT	Provided PCBA readings. The outlook for the culture was guardedly optimistic, since the culture appeared to be surviving.	149

During the BTS–CART experiment, large air bubbles developed in the rotating bioreactor. For reasons related to the physical processes of mass transfer and shear, large bubbles are detrimental to cell cultures in microgravity. Automatic infusion of medium failed to work and the U.S. astronaut (John Blaha) was required to feed the cells using direct infusion via the front panel. To reduce the size of the bubbles and minimize bubble formation, the BTS team requested that the EDU–M housing be opened to inspect for blockage in the medium lines. This required breaching the medium and cells' second level of containment. The Russian specialist reluctantly agreed to allow Blaha to perform this procedure, which went without incident. The source of air was thought to be the fresh water bags that were mixed with powdered medium on orbit. John Blaha continued the direct infusion for the duration of the experiment and the cartilage cell cultures survived. The BTS–CART experiment required much more crew time to recover and perform than was initially planned. Although air was introduced into the bioreactor, the EDU–M functioned in a slightly degraded mode (i.e., without automatic infusion) over the entire increment.

EDU–M operations were much more eventful during the BTS–COCULT experiment during Increment 7. This experiment required periodic removal of cell cultures and medium from the side panel of the bioreactor. A flexible glovebag and attachment port were designed at the request of the Russian safety specialist. The glovebag provided the second level of containment during cell and medium transfer activities. The EDU–M had to be partially removed from the locker housing for the U.S. astronaut to use the glovebag. Though tedious and time-consuming, the glovebag design worked well.

Just as on Increment 3, large air bubbles formed in the bioreactor. The crew performed several procedures without success to reduce the size and quantity of the air bubbles. Lowering the medium perfusion rate to 1.0 mL/min seemed to mitigate this problem.

High-pressure readings indicated possible medium line blockages in the EDU–M. Again, with the permission of the Russian specialists, the EDU–M housing was opened. The U.S. astronaut (Andrew Thomas) found blockages in the medium lines. He also found droplets of medium within the housing. This dictated that the bioreactor had to be isolated from the other EDU–M systems to prevent contamination of the cell cultures. Several lines had to be removed and crimped. Andy Thomas saved the experiment by performing medium infusion via the side panel after the bioreactor was isolated. Extensive malfunction procedure development, verification, and negotiations with Russian specialists were required during the increment to get their approval to use these bioreactor isolation procedures without crew training. The U.S. ground controllers and BTS science and engineering teams worked essentially around the clock to save the experiment. Hardware and procedure modifications that were made after Increment 3 did not seem to correct the problems, since large air bubbles were also reported during Increment 7.

Because of the intensive efforts performed during Increment 3 and 7 to sustain the BTS–CART and BTS–COCULT experiments, and because of the duration of these increments compared to Shuttle missions, more crew time was spent on the EDU–M and BTS hardware than possibly any other U.S. science experiment ever flown in space. Also, the failure of a major U.S. science element on Increment 7 permitted the U.S. astronaut to dedicate more time to the BTS–COCULT experiment. The fact that the bioreactor was successfully isolated without any significant leakage of medium bodes well for EDU–M operations on the ISS. Successful completion of this

procedure was probably one of the best examples of ISS risk mitigation that occurred during the NASA-*Mir* Science Program.

4.1.2.2 Biotechnology Specimen Temperature Controller

The crew used the BSTC in the BTS–BIO3D experiment during Increment 6. The unit was operated essentially continuously during the increment. Table 4-7 provides a detailed summary of BSTC operations on *Mir*. The unit was powered down periodically when *Mir* lost power and for changes in chamber temperature settings. Each of the 4 BSTC refrigeration/incubation chambers contained 3 TCMs. The experiment included 3 cell lines. Two chambers contained 1 cell line each while the other cell lines occupied 1 chamber each. Section 6 identifies the cell lines.

Table 4-7. BSTC Operation on Mir

Increment	Date	Experiment	Summary	Data Source
5/6	9/27/97	BTS–BIO3D	The NASA 6 crewmember (David Wolf) performed the first medium sampling and cell culture transfer and fixation.	150
5/6	10/2/97	BTS–BIO3D	Transferred the BTS–BIO3D hardware from the Shuttle to the *Mir* Priroda module. The Russian *Mir* 24 commander permitted transfer of the BSTC and BTR, but operation on *Mir* was delayed. The *Mir* 24 commander was concerned about the content of the cell cultures. Russian and U.S. specialists held a meeting to resolve the issues of toxicity and containment.	151
6	10/6/97	BTS–BIO3D	D. Wolf reported that the BTS–BIO3D microscopy session was performed that day. He maintained this activity took much longer than originally timelined due to the requirement for performing the microscopy operations inside the Bitran bag and because it was the first time he had performed the operation. The sample would not fit under the lens.	152
6	10/7/97	BTS–BIO3D	Wolf reported he'd prefer not to switch the doors of the chambers to match the culture locations.	153
6	10/7/97	BTS–BIO3D	Wolf performed culture microscopy, medium sampling, cell medium transfer, and fixation.	154
6	10/8/97	BTS–BIO3D	Wolf provided verification of cell culture transfer (8.0 mL red and 3.0 mL blue). This should get the red culture that was transferred early back on track.	155
6	10/10/97	BTS–BIO3D	Wolf reported that the air velocity on the BSTC had decreased from 1.3 to 0.4 m/sec, but temperatures were in the normal range.	156
6	10/14/97	BTS–BIO3D	Cell growth at 1 g and 0 g was compared. The cells were not attaching in 0 g or 1g.	157
6	10/16/97	BTS–BIO3D	PCBA readings were taken and called down.	158
6	10/16/97	BTS–BIO3D	Wolf reported the kidney cells were not clumped and hadn't been clumped as previously reported. He said they were	159

Increment	Date	Experiment	Summary	Data Source
			rounded-up cells on the surface of the beads or floating free. The few clumps existing were only loosely adherent. The blue module had groups of cells that were "not breaking up if they don't have to." Wolf also reported the status of the BSTC located in the *Mir* core module. Chambers 1 & 2 were at 34°C & 35°C with a set point of 36°C. Culture medium and cell microscopy was performed on 10/22/97.	
6	10/20/97	BTS–BIO3D	Air inlet temperature = 32.4°C.	160
6	10/22/97	BTS–BIO3D	D. Wolf was concerned about performing the cell sampling operation in the *Mir* core module. Excessive dust seems to be the issue. BSTC and BTR were scheduled to be transferred and repowered in Priroda that week. Instructions for FD28 cell sample transfer/ fixation and stowage: A. Cell Line A: Red in Chamber 2 (HL60 cells) B. Cell Line B: Blue in Chamber 4 (PC12 cells) C. Cell Line C: Green in Chamber 3 and Gold in Chamber 1 (rat renal cells)	161
6	10/12/97	BTS–BIO3D	NASA 6 crewmember performed medium sampling on 11/26/97. Performed microscopy on 11/29/97.	162
6	10/23/97	BTS–BIO3D	D. Wolf reported status of the cell cultures. He believed all the cells in the green and gold modules were dead. Completed transferring the blue cell line.	163
6	10/24/97	BTS–BIO3D	Chamber 2 temperature: 34.8°C	164
6	10/25/97	BTS–BIO3D	Wolf observed that the green and gold media in the C slots were low on volume because they had never had a transfer and fixation performed. He suggested that the BTS–BIO3D PI suggest sampling A or B next instead of pouring the volume out of C each time Chamber 2 temperature = 33°C. He was concerned that Chamber 2 was getting too cold.	165
6	10/26/97	BTS–BIO3D	Wolf reported the beads in the gold and green modules were showing a small amount of accumulation on their surfaces.	166
6	11/2/97	BTS–BIO3D	Wolf observed that all cell lines in the Blue chamber were low on glucose and that cell lines A and C appeared acidic. Chamber 2 temperature = 33.9° to 34.1°C	167
6	11/4/97	BTS–BIO3D	D. Wolf performed medium sampling on 11/4/97.	168
6	11/5/97	BTS–BIO3D	Provided PCBA readings for all cell lines.	169
6	11/11/97	BTS–BIO3D	Provided PCBA readings for Blue and Red cell lines.	170
6	11/11/97	BTS–BIO3D	Wolf performed medium sampling on 11/11/97 and microscopy on 11/12/97.	171
6	11/14/97	BTS–BIO3D	Shift flight director told Wolf to power down the BSTC and	172

Increment	Date	Experiment	Summary	Data Source
			other experiments until *Mir* power was stable.	
6	11/16/97	BST-BIO3D	The *Mir* motion control system malfunctioned. BSTC had to be rebooted several times. Chamber 2 temperature: 35.8°C.	173
6	11/19/97	BST-BIO3D	The BTS ground team sent e-mail requesting termination of the Red, Gold and Green cell lines.	174
6	11/20/97	BST-BIO3D	Cell lines A & C were terminated on 11/20/97. No hardware anomalies were reported.	175
6	11/21/97	BST-BIO3D	BSTC was turned off the previous evening for several hours. The exact reason is being researched.	176
6	11/21/97	BST-BIO3D	Russia's Center for Control of Spaceflights (TSUP) reported that *Mir* had experienced unknown problems in its power/attitude system, resulting in loss of power to the station. The exact cause was unknown.	177
6	11/28/97	BST-BIO3D	Wolf reported Blue cell line looked very acidic and he wanted to transfer them the next day. Provided PCBA readings as a basis for the decision to fix the cells.	178
6	11/29/97	BTS–BIO3D	Wolf performed medium sampling. The pH was 6.79; therefore, he proceeded to fix the cell cultures.	179
6	12/1/97	BTS–BIO3D	A Chamber 2 engineering evaluation (EE) was described. Chamber 2 was to be set to 16°C-19°C until the BSTC was powered to transfer to the Shuttle.	180
6	12/4/97	BTS–BIO3D	Wolf was to check the hardware status every day, but write down chamber temperatures only every few days.	181
6.	12/8/97	BTS–BIO3D	The BSTC EE was updated via radiogram.	182
6	12/15/97	BTS–BIO3D	Wolf reported in the MD81 status report that he turned the screw exactly 2 turns (as specified in the procedure) but that the reading leveled off at 28.6°C.	183
6	12/23/97	BTS–BIO3D	The BSTC was powered off for several hours during yesterday's Progress docking.	184
6	1/22/98	BTS–BIO3D	BTS ground personnel requested that Wolf perform a modified version of the BSTC EE radiogram. They requested that he turn off chambers 1, 4, and 3, then perform the transfer as planned when the Shuttle arrives.	185

The TCMs were removed periodically from each chamber for medium sampling, cell culture fixation and storage in the BTR, and cell culture transfer and microscopy operations. The microscopy operations were performed in a Bitran (multilayer plastic) bag, which provided the second level of containment when the TCM was removed from the chamber. The NASA 6 crewmember, David Wolf, reported that the microscopy procedure took much longer than planned because of the difficulty of performing the procedure in the Bitran bag and problems with viewing the TCM in the portable microscope.

These results should be considered when estimating crew time for microscopy operations planned for biotechnology experiments on ISS.

The BSTC unit functioned extremely well during Increment 6, especially considering the fact that it was an entirely new hardware development for *Mir*. The only hardware anomaly encountered during the Increment was a slight temperature excursion in one of the refrigeration/incubation chambers. Chamber 2 operated approximately 2ºC below the set-point temperature of 36ºC. The worst deviation was 3ºC below the set point.

Chamber 1 also operated a couple of degrees below the set point temperature at times during the increment. The BTS science team did not consider this to be detrimental to scientific success of the experiment. The team added an EE at the end of the increment to help determine the cause of these anomalies. In general, the successful operations on *Mir* showed that the BSTC could be used successfully for future cell science experiments on the ISS.

4.2 TRAINING

The training performed for BTS experiments on *Mir* has direct applications to the training required for the BTF and associated experiments to be flown on the ISS. This section describes the training performed for the BTS facility and the experiment-specific hardware and operations. It also discusses the training required for the on-orbit transfer activities and other tools and techniques used during the NASA-*Mir* Program to update the crew training near the time of the actual on-orbit operations. Although demanding on small and low-funded payload organizations, comprehensive training ensured the success of scientific investigations on *Mir* and allowed the crew to conduct the experiments in a safe, logical series of preplanned events. Familiarization with the BTS experiment hardware and protocol was essential to BTS investigations on *Mir* because of the complexity of the hardware and the specialized training required to prevent biological contamination of the cell cultures.

The crew trained in both nominal and malfunction procedures. Both U.S. astronauts and Russian cosmonauts trained in the operation of BTS experiments. However, only U.S. astronauts operated the experiments on *Mir*.

4.2.1 Facility Hardware

The crew trained in the nominal and off-nominal procedures required to operate the ECC, GSM, and BSM. The training included procedures required for status checks, activation, deactivation, and an overview of external interface attach points. During facility training, the ECC and GSM were not connected to experiment-specific hardware. Only autonomous operations were addressed in the generic facility training. The facility training was conducted for the U.S. astronauts who were on *Mir* during Increments 3 through 7.

BTS facility training on *Mir* can be applied to BTF operations on the ISS. The BTF will include subsystems that perform the same functions as the ECC and GSM. It will not be possible to use the identical procedures because the user interfaces and nomenclature used on the BTF will not be the same as those used for the ECC and GSM. Much of the ancillary equipment flown in the

BSM on *Mir* will also be flown on the ISS, so the training and procedures used on *Mir* can be used for ISS hardware for items like the PCBA, water bags, and other stowage items.

4.2.2 Experiment-Specific Hardware

The BTS experiment-specific hardware (i.e., EDU–M and BSTC) flown on *Mir* may also be flown on the ISS. The units to be flown on the ISS in the BTF may be slightly or highly modified versions of the units flown on *Mir*. Therefore, the training performed for *Mir* has direct applications to the ISS. The crew was trained in both nominal and malfunction procedures. The training included the procedures required for hardware activation, status checks during nominal operations, and deactivation. Much of the training was associated with maintaining good sterile technique to prevent biological contamination of cell cultures. Also, the crew was trained in the procedures required to connect the EDU–M to both the ECC and GSM. The training for the experiment-specific hardware was performed before the increments on which these units were operated began.

The BTR and STES supported the experiments performed in the EDU–M and BSTC. Similar refrigeration/incubation units will be flown in the BTF on the ISS, so many of the training plans and procedures used for *Mir* can be used for the ISS.

4.2.3 On-Orbit Transfer Process

The crew required specialized training to transfer the BTS hardware from the Shuttle to *Mir* and vice versa. Since both Shuttle and *Mir* used middeck locker-type interfaces, the physical mounting of the hardware did not change from one vehicle to the other. However, the crew had to be trained how to deactivate the powered units, disconnect the electrical and data cables, transfer the hardware, and reactivate the equipment on *Mir*. The primary constraint for BTS components was a maximal unpowered transfer time of approximately 45 minutes. This was required because the EDU–M, BSTC, STES, and BTR all provide thermal control for temperature-sensitive items such as cell cultures, medium, and PCBA analytical cartridges. The units had to be transferred and reactivated before the temperatures of those items increased or decreased to a point considered to be detrimental to the scientific success of the experiments. The crew successfully transferred the BTS hardware in less than the maximum unpowered time. This constraint is also applicable to BTF operations on the ISS, because items destined for the BTF will be launched in the Shuttle middeck and transferred unpowered to the BTF rack in the USL.

4.2.4 Training Video

The crew trained for increment operations well before the start of the increment. The U.S. and Russian crewmembers were sometimes trained on BTS hardware from 6 months to a year before they were launched on either the Shuttle or Soyuz to *Mir*. Nevertheless, the training time available was extremely limited. For these reasons, the ISS Program has implemented a process to provide "refresher" training on board the ISS just before experiment operations. This technique was called onboard training and computer-based training. The crew trained by viewing digitized training videos on the MIPS-2L laptop computer. The ground-based training

was filmed and selected segments were digitized and recorded to CD-ROM. Because of the success of computer-based training on *Mir* and positive feedback from the crew, the same technique is planned for the ISS.

4.3 LESSONS LEARNED

The nominal and malfunction procedures used for BTS on *Mir* were adequate for performing the science experiments. The procedures were thorough and the crew had little or no trouble performing the predefined steps. The one area where improvements could be made for the ISS is the procedures required for in-flight maintenance (IFM) operations. The crew experienced problems with the EDU–M and the BTR that required the use of very specialized IFM procedures. The crew had not been trained for these activities. As a minimum, IFM procedures for those issues documented in Section 4-1 should be added to the IFM training for ISS operations.

The procedures and hardware used for light microscope operations were not adequate for the microgravity environment. The BTS team should consider performing operations that were particularly difficult or unsuccessful on *Mir* on KC-135 flights. This will allow the procedures to be tested and refined by using them in a low-gravity environment before use on the ISS. Many of the limitations on the Phase 1 Program, such as lack of training hours and training activities well before the start of the increment, are also anticipated for the ISS. Computer-based training, especially related to IFM, should be used extensively for BTS hardware. The IFM procedures may never be needed, but they will be invaluable if unexpected problems are encountered.

5 VERIFICATION OF THE LAUNCH, LANDING, AND TRANSFER OF OPERATING EXPERIMENTS

The previous section addressed the operation of BTS payloads on *Mir*. Generally, *Mir* operations spanned an entire increment, whereas this section addresses the shorter operational periods associated with launch, landing, and transfer activities. Since BTF activities on the ISS will parallel the BTS operations on *Mir*, many of these lessons learned are applicable to the BTF and biotechnology payloads that will be installed in the BTF on the ISS. This section describes specific *Mir* operations and summarizes the lessons learned for future use in developing ISS operational plans and crew procedures.

5.1 Launch

This section describes the launch of BTS hardware on the Shuttle and Russian unmanned rockets. One of the benefits of the NASA-*Mir* Science Program was the fact that both U.S. and Russian vehicles were used to transport U.S. science experiments to *Mir*. This international agreement provided ample opportunities to get the primary BTS hardware and any needed resupply logistics hardware to *Mir*. Both Russian and U.S. vehicles were used to meet the standard planned launch template for the BTS equipment as well as to support unplanned or off-nominal circumstances that were experienced on *Mir*.

5.1.1 Rocket Launch

The BTS facility hardware was launched to *Mir* on a Russian Proton rocket. The BTS facility was launch-installed in the Russian Priroda module. The Priroda module was a major addition to the *Mir* space station. The pressurized volume of this module was designed specifically to accommodate U.S. payloads with middeck locker-type interfaces. BTS personnel traveled to the Baikonur Launch Facility in Kazakstan to help install the BTS facility hardware in the Priroda module.

The launch of the Priroda module provided an excellent opportunity to launch a large amount of U.S. hardware at one time, thus reducing the number of Shuttle flights required to complete the NASA-*Mir* Science Program. A major constraint to launching on Priroda was lack of a "late-load" capability. This meant that payloads had to be installed in the module several weeks before launch. Therefore, experiments containing samples that required temperature control, such as the biological materials used in BTS experiments, could not be flown on Priroda. The BTF facility hardware could be launched on Priroda and stowed on *Mir* until the experiments were performed on later increments. Experiment-specific hardware arrived on future Shuttle docking missions. A somewhat parallel situation exists for the ISS: the BTF will be launched in the Shuttle cargo bay, installed in the multipurpose logistics module (MPLM). One difference is that the MPLM does have limited "late-load" capability.

The BTS facility was installed in Priroda before launch. An additional benefit of this was the ability to operate the BTS facility hardware in the Priroda during ground checkout activities. The ECCs and GSM were tested on the ground at Baikonur. Opportunities to perform functional tests in flight modules are rare and these opportunities should be used to the maximum extent possible. Some of the ground test procedures used for checkout of the BTS facility on Priroda may be applicable to checkout of the BTF in ISS simulators at KSC. A similar opportunity to test the flight version of the USL will not exist for this BTF since the USL will already be on orbit when the BTF is scheduled to be completed. The planning for BTF ground checkout should consider full use of ISS rack interface simulators at KSC to ensure that the BTF will function when installed in the USL on orbit.

The PCMCIA cards required for the BTS–DE were launched in the Russian Soyuz module on a Proton rocket. Also, additional powdered medium was launched on Soyuz because Increment 3 was extended after unexpected problems with Shuttle propulsion systems delayed the STS-81 launch. The ability to launch additional powdered medium on Soyuz helped ensure the success of the BTS–CART experiment.

We launched a replacement PCBA on Soyuz after the primary units on *Mir* failed. The issues associated with the primary units are described in Section 4.4.1.5. Using Russian rockets to launch BTS primary hardware and stowage hardware was a key factor in the success of the biotechnology experiments on *Mir*. Using Russian launch vehicles for ISS hardware will also provide backup opportunities to get key equipment into space when it is needed to support critical science activities.

5.1.2 Shuttle Launch

We launched BTS hardware to *Mir* on 4 of the 9 Shuttle *Mir* docking flights during the NASA-*Mir* Science Program. BTS hardware was flown in both the Shuttle middeck and the Spacehab commercial carrier, which is launched in the Shuttle cargo bay. Both of the locations provided the following critical resources needed for BTS experiment hardware: middeck locker-type structural interfaces, electrical power, crew time, and late access. The experiment-specific hardware for the BTS–CART, BTS–BIO3D, and BTS–COCULT experiments required late access since the cell cultures require thermal control during essentially all phases of the experiment. The BTS–DE experiment-specific hardware did not require late access on the Shuttle, since it did not contain biological material. The BTS–DE Kit contained mainly PCMCIA cards for use in the ECCs on Priroda. We summarize below the BTS experiment operations in both the middeck and Spacehab and applicable lessons learned for BTF on the ISS.

The BTS cell science experiments were initiated in ground-based laboratories days, even weeks, before being launched on the Shuttle. Personnel started ground controls and flight experiments at the same time and operated them in parallel. A ground analog experiment is essential to compare the results of the 1-g and 0-g experiments. The experiments operated simultaneously in space and on the ground. We routinely tested the culture medium for biological contamination before launch. Russian safety specialists would not allow the equipment to be flown to *Mir* without inspection of the test reports showing successful completion of the ground-based cell culture medium sterility tests. Approximately 48 hours before launch, the BTS science team decided which of the 2 experiments would be launched to *Mir* and which would be the ground control. Technicians installed equipment going into the Spacehab module approximately 48 hours before launch, and installed equipment in the middeck approximately 24 hours before launch. The BTS experiment-specific hardware was placed on battery power for the trip from the KSC laboratory to the Shuttle at the launch pad. The equipment remained on battery power until it was installed in the middeck or Spacehab. The technicians who installed the BTS equipment were lowered via a cable and harness assembly into the middeck or Spacehab. The technicians are lowered in a sling because the Shuttle is in the vertical position for launch. Late installation of payloads on the Shuttle is an intricate operation performed by highly trained personnel. This late-load capability is necessary for the success of biotechnology cell science experiments, making the Shuttle an essential carrier for biotechnology experiments in microgravity.

Once in space, the Shuttle crew participated in various activities related to BTS experiments. They performed daily status checks of the hardware, tested the quality of the medium using a PCBA, and cleaned the air inlet screens as needed. The crew used the Shuttle-based laptop computer to verify proper operation of the EDU–M during the BTS–CART and BTS–COCULT experiments. The crew used the Shuttle's Payload General Support Computer to monitor EDU–M parameters and to make changes as directed by ground personnel. After the Shuttle docked with *Mir*, the next step was to transfer the BTS hardware to the Priroda module.

In general, most of the operations associated with the BTS on the Shuttle are directly applicable to the BTF, since the experiment-specific hardware for the BTF will also be launched to the ISS on the Shuttle. The BTF will be launched to the ISS in the MPLM. Since the MPLM does not have late access and does not have ascent power for payload racks, the experiment-specific

hardware for the BTF must be launched in a carrier, such as the Shuttle middeck or Spacehab, that provides late access.

One major area where the BTS on *Mir* may differ from the BTF on the ISS is thermal control of experiment-specific hardware and rack subsystem components. Both the Shuttle and *Mir* accommodated "front breathing" equipment. This means that cooling air was drawn in from the Shuttle or *Mir* cabin, routed through the experiment components, and then exhausted back into the cabin. On the ISS, new middeck-type experiments must be designed as "rear breathers," meaning that they draw in cooling air from the avionics air plenums of a rack and exhaust air back to the avionics section of the rack. This is a safety-driven requirement that makes possible centralized smoke detection within ISS racks. Because more experiments will be operating on the ISS than a small number of crewmembers can continuously monitor, the station requires a more automated system for smoke and fire detection to ensure the crewmembers' safety. The Shuttle middeck is also being modified to accommodate "rear breathers." On *Mir*, the air could be drawn in from the cabin and then be exhausted to a volume behind the locker housings. In the ISS era, both the Shuttle middeck and the BTF will accommodate rear-breathing equipment. This should save the crew a little time on the Shuttle, since the crew will not have to check or clean the air inlet filters of the powered middeck locker-type payloads. However, many of the ISS-era activities (and, hence, crew procedures) will be similar to those performed in the middeck for BTS experiments flown during the NASA-*Mir* Science Program.

5.2 LANDING

The BTS experiments continued on all 3 phases of the *Mir* missions (i.e. ascent, orbit, and descent). Thus, the cell cultures were still viable at the time of the Shuttle landing for both the BTS–CART and BTS–COCULT experiments. The BTS science team specified requirements for early removal from the Shuttle middeck and Spacehab. The earliest opportunity for removal of the science hardware occurred 4 hours after landing for middeck hardware and 6 hours after return for Spacehab hardware. The BTS-powered hardware was removed from the vehicle, placed on battery power, and returned to the ground-based laboratory. The BTS hardware remained powered on the ground until it was returned to JSC via a chartered private plane. The BTS personnel had to be prepared to support a Shuttle landing at either KSC in Cocoa Beach, Florida, or Edwards Air Force Base in California. The decision to land at either of these sites was made very late in the mission and was generally based on weather. NASA makes every attempt to land in Florida to prevent the expense of having the Shuttle ferried from California to Florida for vehicle processing. Only one of 11 Shuttle *Mir* missions ended with a landing in California.

5.3 TRANSFERRING OPERATING EXPERIMENTS

BTS experiment hardware was successfully transferred from both the Shuttle middeck and Spacehab to *Mir* on 3 different docking missions. The BTS–CART, BTS–BIO3D, and BTS–COCULT experiments were transferred on the STS-79, STS-86, and STS-89 Shuttle missions, respectively. Before transfer, the crew deactivated the powered equipment, then removed it from the locker housing or from the wire tray in the Shuttle middeck, and transferred it to *Mir*. The crew connected the equipment to the PUP cables and reactivated it in *Mir*. It was unpowered

during the transfer process. Typically, the unpowered phase of the transfer lasted approximately 15 minutes. The BTS science organization specified a maximal unpowered time of 30 minutes for BTS hardware with active cell cultures and refrigerated items. This requirement was based on thermal analyses, which indicated that the cell culture or refrigerated item temperature change would not exceed a critical differential that would degrade the cell cultures or performance of the refrigerated items such as powdered medium or PCBA cartridges. From all indications, the transfer was performed in less time than the maximal unpowered times specified.

Transferring the BTS–CART experiment involved disconnecting the power and data cables between the ECC and EDU–M. The crew removed both the EDU–M and the ECC from their middeck locker housings, transferred them to the Priroda lockers, and later reconnected them. One of the Priroda-launched ECCs was returned from *Mir* on the Shuttle. The crew disconnected the STES power cable and removed the STES from the payload mounting panel on the wire tray by removing 4 captive fasteners using the unique locker removal tool. They transferred the unpowered STES to *Mir* and reactivated it in the BTS facility in Priroda.

Transferring the BTS–BIO3D involved disconnecting the power cables to the BTR and BSTC in the Shuttle and removing both of the locker replacements from the wire tray using the unique locker removal tool. The units were then transferred separately to the BTS facility on *Mir*. The crew had to remove the BEM locker housing in the BTS facility before the BSTC could be installed. The BTR was installed in the location the STES previously occupied during Increment 3. Both the BTR and BSTC were connected to PUP cables and reactivated in Priroda.

The BTS–COCULT experiment used both the EDU–M and the ECC, the same experiment-specific hardware that was used during the BTS–CART experiment. However, because there were 2 functioning ECCs on *Mir*, only the EDU–M was transferred from the Shuttle to *Mir*. The BTR on *Mir* was exchanged with a new BTR, which was flown to *Mir* on STS-89.

Two BTS transfer-related incidents are worthy of additional discussion. These incidents involved transferring experiment-specific hardware associated with the BTS–CART and BTS–COCULT experiments. Some of the key lessons learned are applicable to the biotechnology program for the ISS.

In the first incident, which involved transferring the EDU–M and ECC during BTS–CART operations on STS-79, the Mini-PIC was instrumental in rescuing the BTS-CART. After the hardware was transferred from the Shuttle to *Mir* and installed in the Priroda lockers, U.S. astronaut John Blaha reconnected the power and data cables between the EDU–M and ECC. When a cable was mated between the bioreactor and the 220-pin connector on the ECC, the ground-based BTS team began receiving inconsistent and erratic data, which they could not understand, from the flight ECC. Many of the EDU–M critical parameters were being reported as "out of limit." All indications showed this EDU–M was failing completely, and the entire experiment appeared to be at risk during the first hours on *Mir*. It was a very poor start to one of the most ambitious and promising U.S. science experiments performed during the NASA-*Mir* Science Program.

The BTS science and engineering ground support team compared the duplicated hardware configuration in the Mini-PIC to photographs—taken by the Space Shuttle's Electronic Still

Camera—of the space bioreactor integrated into the BTS Facility and its final configuration. While comparing the on-orbit payload configuration with the same hardware configured in the Mini-PIC, the BTS team quickly identified the incorrect alignment of a cable: the locking lever of the main data connector on the DATACOM port of the ECC was not in the fully locked position. When the suspected incorrect cable alignment was duplicated in the Mini-PIC, inconsistent and erratic data also appeared in a manner analogous to that observed in the BTS Facility. Correcting the cable attachment to the 220-pin ECC connector in the Mini-PIC resulted in the correct data flow.

The BTS ground team then prepared the troubleshooting procedure, which they communicated to Blaha on *Mir*. After Blaha powered down the ECC and the space bioreactor, he re-mated the connecting cable to the 220-pin connector to fully engage the locking lever. The warning indications on the ECC disappeared, and Blaha reported that all parameters came up and were nominal.

The effective use of the Mini-PIC to quickly resolve this experiment-threatening anomaly and to restore the hardware to operation resulted in the success of the longest continuously functioning (>130 days) tissue-engineering experiment in space (see CART). It was heralded as one of the best cases of teamwork during the Phase 1 Program (186). The solution of the problem required the full teamwork of the U.S. astronaut on *Mir*, the Shuttle crew, Mission Control, and the BTS ground support team. It also used many of the best tools available on the Shuttle. The Shuttle downlink capability, the electronic still camera, and ground and crew visual observations were critical to solving this problem.

The second incident related to BTS transfer activities occurred with the BTS–BIO3D experiment on STS-86. The issue was associated with transferring the BSTC, which contained active cultures of human cancer cells. The *Mir* 24 commander allowed the BSTC to be transferred to *Mir*, but would not allow it to be operated until a meeting was held the next day between U.S. and Russian specialists to discuss the risks of these cells to the crew (187). U.S. and Russian specialists had held several meetings before the STS-86 mission to discuss the risks of these cell types to the crew. The U.S. position was that no scientific basis existed for the crew to contract cancer, based on exposure to these cells from horizontal transmission on board *Mir*. Also, as a precaution, the cells had 2 levels of containment during all BTS–BIO3D operations. All indications before flight were that the Russian specialists accepted the U.S. position on the safety of these cells. However, it was apparent that the Russian crew had not been properly informed or did not accept the opinions of U.S. scientists with regard to the risks associated with cancer cells. It is not clear what precipitated this last-minute debate over the BTS–BIO3D experiment protocol and contents, but a meeting was held and the Russian *Mir* 24 commander finally permitted operation of the BSTC during Increment 6.

5.4 LESSONS LEARNED

The procedures for transferring BTS hardware to *Mir* were generally complete and sufficient for a successful transfer of all equipment. However, the 2 scenarios discussed indicate that we could improve BTF operations on the ISS.

We should modify the EDU–M and ECC procedures to include a verification step to ensure that all data connectors are locked or fully engaged. In addition, we should develop a malfunction procedure for hardware symptoms associated with incomplete data connections. The problem with the ECC and EDU–M data connectors could also be attributed to the relatively long period between the training and actual on-orbit operations. We could mitigate this problem somewhat with onboard training just before increment operations. BTS should fully use onboard training and computer-based training because of the relatively complex and specialized nature of cell science experiments and technology.

The issue related to the BSTC is somewhat more difficult to correct, since there were political implications and possible breakdowns in communication between the U.S and Russian safety and integration specialists. One way to prevent issues associated with samples that are perceived to be more dangerous than others is to recognize this fact to begin with. Thus, the science team should make all efforts to communicate with the international partners and the U.S. safety community. Special briefings should be convened to discuss the risks or perceived risks associated with the cell lines or other materials. Then, the final agreements should be documented and signed by the responsible representatives of the international partners. While neither of these transfer issues is minor or insignificant, they are issues that can be corrected with good planning, thorough crew procedures, and internal/external communications between the BTF team and the ISS safety and management organizations.

6 PERFORMANCE OF FUNDAMENTAL SCIENCE INVESTIGATIONS

The biotechnology investigations performed on *Mir* during Phase 1 included BTS–DE, BTS–CART, BTS–BIO3D, and BTS–COCULT. This section describes the science and engineering objectives of the experiments. We provide the experiment methodology, including identification of the BTS stowage and primary hardware units used during the investigations, as well as a summary of the experiment results. However, since this paper is not intended to be an exhaustive survey of the scientific aspects of the biotechnology cell sciences experiments performed during the NASA-*Mir* Science Program, we provide only a cursory view of the science results. You may find more thoroughly documented results in papers published by the principal investigators in peer-reviewed scientific journals or other reports focused specifically on the experiment and less on the facility or experiment apparatus. This study is intended to be comprehensive from a hardware and overall biotechnology research program perspective, but not from a scientific perspective. We do provide some information to permit interested researchers to search for and obtain the detailed science results of the BTS experiments.

The summary of each BTS experiment includes a table identifying the relevant technical papers that have been published or submitted as final research project deliverables. We provide enough information to allow interested researchers to obtain the reports through literature searches. This information will be useful for future investigators by documenting the scientific lessons learned that can be applied to future BTF investigations.

6.1 Biotechnology System Data Acquisition and Control System Diagnostic Experiment

The BTS–DE is really the culmination of several experiments performed on *Mir* during Phase 1. All of these long-term experiments focused on enhancing the operational capabilities of the DACS, which are an integral part of the BTF rack planned for the ISS. Each of these experiments had specific objectives; however, the end goal was an improved and more reliable DACS for BTF that crews will use to carry out the biotechnology experiments planned for the ISS. Table 6-1 provides the timeline and objectives for the BTS experiments related to experiment and subsystem data acquisition and control.

The experiments listed in Table 6-1 include both passive and active experiments. The passive experiment involved exposing various types of PCMCIA cards to the *Mir* radiation environment while they were in stowed configuration. The active experiments required use of the ECCs in the BCM to expose these cards to the *Mir* radiation environment in a powered state. These experiments originated from the need to ensure both the reliable execution of the investigator's experimental protocol and the archiving of data for the BTF.

The ECC is the brain of the BTS DACS. The ECC design was completed before the NASA-*Mir* Science Program. The ECC was designed to use PCMCIA cards as its memory devices. The BTS design team recognized early that commercial, off-the-shelf computer HDDs were not suitable for long-duration operation on the ISS. The Phase 1 Program on *Mir* allowed the JSC biotechnology cell science team to conduct several risk mitigation investigations to determine the effects of orbital radiation and long-term, low-gravity operation on the ECC's performance and its ability to perform the DACS function for biotechnology experiments. HDDs were deemed unsuitable because they are rotating storage mediums that are susceptible to launch vibrations and failure of moving parts. They are also adversely affected by changes in ambient pressure. Spacecraft ambient pressure occasionally drops as low as 8.5 psi. HDD manufacturers do not guarantee reliable operation at this reduced pressure, and PCMCIA cards appeared to be viable alternatives. However, there was still concern about the effects of space radiation on the performance of PCMCIA cards.

The BTS team identified flash and SRAM PCMCIA cards as replacements for HDDs. Some advantages for their use in space included their small size, low mass, and lack of moving parts. However, this relatively new memory technology was virtually untested in the space environment. The Phase 1 Program provided precisely what was needed to test these technologies for the BTF on the ISS: 1) a low-Earth orbit radiation environment, and 2) long-duration operations in both active and passive states. What followed was the most extensive test of PCMCIA computer memory devices ever performed in space. These tests were performed over a 2½-year period on *Mir*. The JSC Biotechnology Program Office documented the results in a series of reports.

Table 6-1. BTS Data Acquisition and Control System Diagnostics Experiments on Mir

Experiment Title	Date Performed	Increment(s)	Objectives
Passive BTS PCMCIA Card Experiment	January – March 1996	1 & 2	Determine if PCMCIA flash and SRAM cards in unprotected stowage are susceptible to SEU corruption under *Mir* orbital radiation conditions (188).
BTS Facility Experiment	April – August 1996 (130 days)	2	1) Test the BTS DACS's ability to sustain long-term operations on *Mir*. The ECC will be operated in 2 modes (189). a) In the ECC functional test mode, the ECC systematically assessed the operation of specific BTS DACSs. This test was performed on both ECCs in the BTS facility. Test results were logged to PCMCIA cards in the ECC at the time of the test. b) In continuous operation mode, the ECC performed internal memory tests and recorded temperature data from 2 internal sensors at regular intervals while continuously operating over a greater than 30-day duration. 2. Test the capability of Flash and SRAM PCMCIA cards to successfully boot and operate the ECC for 30-day periods in the *Mir* environment. The level of susceptibility to radiation-induced SEU for each type of memory technology will be assessed (190).
BTS–DE (NASA 5)	January – September 1997	4 & 5	Verify and extend the results of the BTS facility experiment performed during Increment 2. Observed the long-duration (i.e., greater than 30 days), powered performance of 2.5-MB and 20-MB PCMCIA cards operating in an ECC to validate the reliability of Type II PCMCIA solid-state flash memory as a mass storage medium for long-duration, continuous usage. Another objective was to test the radiation recovery software (RRS) under realistic operating conditions to verify its ability to a) Repair SRAM PCMCIA cards using uncorrupted flash PCMCIA cards. b) Protect SRAM PCMCIA cards operating in an ECC. c) Collect real-time SEU information and log it for all PCMCIA cards operated in the ECC (191).
BTS–DE (NASA 7)	January – June 1998	7	Test, under realistic operating conditions, the ability of the RRS to a) Repair SRAM PCMCIA cards using uncorrupted flash PCMCIA cards. b) Protect SRAM-PCMCIA cards from SEU corruption while operating in a powered ECC. c) Collect and log real-time SEU information for PCMCIA cards operated in the ECC. (192)

The results of the passive and active PCMCIA card tests on *Mir* are documented in the reports identified in Table 6-2. In addition, these reports define the experiment objectives and methods. Some general trends were established because of the relatively large number of tests. The flash

PCMCIA cards proved to be less susceptible to SEUs than SRAM PCMCIA cards. The flash PCMCIA cards were observed to be immune to SEU corruption at the radiation levels observed aboard *Mir*. All of the SRAM cards showed significant SEU corruption. For 263 days of exposure at 25.94 mRad/day, the SRAM cards had an average of 0.905 SEUs/day. The RRS worked successfully with both the flash and SRAM PCMCIA cards. It was used to successfully restore SRAM cards to an uncorrupted state. It also protected both flash and SRAM cards from corruption and degradation when operating in a powered ECC. The BTS–DE series of experiments showed that the integrated components of the BTS DACS (ECC, PCMCIA cards, and RSS) could function nominally on *Mir* for continuous multi-increment operations. All of this is useful information for BTF operations on the ISS.

Table 6-2. BTS–DE Scientific and Technical Reports

Report #	Report Title	Date	NASA Memo
ID 750031	Biotechnology System Facility Operations 180-Day Report	April 28, 1997	JSC SD3-97-256
ID 890611	Biotechnology System Facility Operations, DACS, NASA Increment 4, 30-Day Operational Accomplishments Report	January 26, 1998	JSC SD3-98-057
Wyle Laboratories Report # 5308000-9918022	Biotechnology System Diagnostic Experiment, 360-Day Report, DACS, Increment 5, Revision A	June 1999	
Wyle Laboratories Report # WLSD 555009	Biotechnology System DACS Diagnostic Experiment, Increment 7, Final Research Report	July 1999	

6.2 The Three-Dimensional Bovine Cartilage Tissue Formation Experiment

The BTS–CART, a tissue engineering experiment, was the first cellular biotechnology experiment performed in the BTS facility on *Mir*. Researchers and the crew grew 3D cell-polymer constructs consisting of bovine articular chondrocytes and polyglycolic acid scaffolds in the EDU–M rotating bioreactor. The 3D engineered cartilage was grown for 3 months on the ground followed by 4 months on *Mir*. Researchers compared the *Mir*-grown cartilage constructs to others that were grown for 7 months on the ground. Cartilage was selected because of its resilience and low metabolic requirements (193). The low metabolic requirement can be translated into lower medium usage, lower process gas usage, etc. All of this is important since stowage volume, power, crew time, and launch mass are limited resources on a space station.

The purpose of the BTS–CART experiment was to determine the effect of the microgravity environment on the structural/mechanical, chemical, and physical properties of cartilage tissue constructs. The specific objectives were to 1) examine chondrocyte viability and differentiated function in a long-term flight study and 2) assess the effects of the space environment, which includes exposure to microgravity as well as launch and landing, on cartilage growth and function. Previous similar space experiments involving mammalian cells lasted for only 6 to 28 days. This mission provided a unique opportunity to study the feasibility of long-term cell

culture flight experiments and to assess the effects of spaceflight on the growth and function of model musculoskeletal tissue. The purpose of the experiment was to test the hardware and to perform the fundamental science investigation. This is essential because tissue engineering in microgravity is a new field; thus, the technology and operational protocols must be proven in parallel with efforts to acquire the scientific data related to the unique tissues being tested in the bioreactor.

After the flight experiment on *Mir*, the PI's team analyzed the cartilage tissue grown in space. The experiment results are summarized below.

The composition and mechanical properties of the space-grown cartilage tissue were compared to those of the ground-grown cartilage and natural cartilage. Both the space and ground environments yielded cartilaginous constructs, each weighing between 0.3 and 0.4 gm. Compared with the Earth group, the *Mir*-grown constructs were more spherical, smaller, and mechanically inferior (195). These results are consistent with a previous report that indicated musculoskeletal tissues remodel in response to physical forces and are adversely affected by the microgravity environment. Table 6-3 is a list of scientific and technical papers related to the BTS–CART experiments.

Table 6-3. BTS–CART Scientific and Technical Reports

Report #	Report Title	Date	NASA Memo
Not applicable	Tissue Engineering of Cartilage in Space, L.E. Freed, R. Lunger, I. Martin, N.R. Pellis, and G. Vujak-Novakovic. *Proceedings of the National Academy of Science*, Vol. 94:13885-13890.	December 1997	Not applicable
Not applicable	BioTechnology System Facility Operation on *Mir*, Gonda, S.R. Tsao, Y.D., Byerly, D., Galloway, P., Joint Xth European and Vth Russian Symposium on Physical Sciences in Microgravity, 1:184-185 (1997)	1998	Not applicable

6.3 The Biochemistry of Three-Dimensional Tissue Engineering Experiment

The crew flew the BTS–BIO3D on Shuttle mission STS-86 and performed the experiments on *Mir* during Increment 6. These experiments involved 3 different cell lines: rat adrenal pheochromocytoma cells (PC-12), human promyelocytic leukemia cells (HL-60), and rat renal cells. The BTS–BIO3D was a study aimed at exploring the feasibility of using simple, gas-permeable cell culture bags as tissue culture modules (TCMs). Dr. David Wolf—an astronaut, medical doctor, experienced cell science specialist, and former NASA Biotechnology Program Director— performed the experiments on *Mir*. The BSTC incubation chambers kept the cell cultures at 37ºC. Each of these three experiments had different objectives and PIs.

The BTS–BIO3D experiment had two major general objectives during *Mir* Increment 6. The first was to optimize the experiment design of long-term cell culture in space under suboptimal conditions that accommodate the technical restraints and existing equipment on board *Mir*, such as:

- lack of a conventional cell culture facility.
- minimal crew time for experiment operations.
- limited post-experiment preservation methods (e.g., no cryogenic freezers and a restricted choice of fixatives).

The second major objective was to test whether, under these conditions, cultured cells or cell assemblies can be passed serially (196). In addition, an overall goal of the BTS–BIO3D experiment was to examine basic cell-to-cell interactions in a quiescent cell culture environment and investigate their role in the formation of functional tissue.

The specific objectives of the BTS–BIO3D experiment with PC-12 cells were to grow and serially pass these cells over a long period (PI: Peter Lelkes, PhD – University of Wisconsin Medical School). The cells were passed to new TCMs at 14-day intervals. During each passage, one-fifth of the visible aggregates were inoculated into a new TCM and four-fifths were fixed using Omnifix fixative. During the mission, Dr. Wolf observed the cell aggregates, both with a microscope and with no magnification. After the samples had been returned to Earth, researchers determined cell morphology using scanning election microscopy and light microscopy. They also performed immunohistochemical analysis to assess adrenergic differentiation and junctional contacts at the cellular level (197).

The BTS–BIO3D experiment using PC-12 cells was successfully completed on *Mir*. Photographs that Dr. Wolf took indicated that, in some instances, PC-12 cells formed large, visible aggregates, with the size of these aggregates increasing over the duration of the mission (198). Compared to those of the ground controls, the degree of aggregation and the rate of proliferation of the samples in space were significantly accelerated. The rate of glucose consumption, a measure of cellular metabolic activity and proliferation, was about 5 times higher in space than on the ground (199). The results of the experiment using PC-12 cells clearly demonstrated that repeated passage of cells in space is feasible and the rate of growth and metabolism of PC-12 cells is significantly accelerated in space as compared to the ground. These results, although preliminary, suggest that the space environment might be advantageous for generating neuronal or neuroendocrine tissue equivalents using PC-12 cells (200).

The objective of the BTS BIO3D flight experiment involving HL-60 human promyelocytic leukemia cells was to characterize microgravity-induced differentiation of these cells (PI: Neal R. Pellis, PhD – JSC). Microgravity has the potential to induce functional, morphological, and genetic changes in cells that can assist in the development of engineered tissue. One of the changes is a process referred to as cellular differentiation, in which cells undergo changes in morphology (shape and appearance) and begin to specialize their inherent functions. HL-60 cells are an early-stage lymphoid/myeloid progenitor cell line that can differentiate into specific types of white blood cells—lymphocytes, granuloctyes, and monocytes/macrophages. Previous ground-based research of simulated microgravity conditions produced in rotating wall vessel culture systems induced spontaneous differentiation of HL-60 cells along the moncytic lineage pathway, in the absence of exogenous chemical inducers such as phorbol esters (i.e., 12-myristate, 12-acetate, PMA), bufalin and retinoic acid (201-202).

The microgravity flight experiment of *Mir* using the BSTC incubator system appeared to confirm previous observations seen in the rotating wall vessel ground-based simulated microgravity experiments (201-204). However, the results of the BTS-BIO3D HL-60 experiment were moderately successful. The Ominifix fixative used to fix and preserve the HL-60 cells, while useful for immunohistochemical staining and flow cytometry analyses, inhibited post-experiment molecular analyses requiring extraction of intact nucleic acids (DNA and RNA) and proteins from the fixed cells. Post-experimental analysis to characterized specific differentiation and apoptosis (programmed cell death) cell surface markers showed that the cells were of low viability, and thus the data were inconclusive.

The HL-60 flight experiment profile was then reflown in the BSTC on board the STS-90 Neurolab Shuttle mission (May 1998), which was very successful. Gene microarray analysis of the postflight samples showed transient differential expression of mRNA transcripts in timepoint samples of HL-60 cells cultured in both the ground control and flight microgravity experiments. Researchers observed differences in genes playing a pivotal role in cell cycle, signal transduction, cell receptors expression, cell adhesion, and heat shock proteins. Work is currently ongoing to finalize comparisons of gene expression in HL-60 cell samples harvested from ground-based rotating wall vessel timepoint experiments using HL-60 differentiation model-specific probes observed to be upregulated in the STS-90 Shuttle flight samples.

Several scientific and technical papers were published about the BTS–BIO3D experiments. Table 6-4 identifies those reports.

Table 6-4. BTS–BIO3D Scientific and Technical Reports

Report #	Title	Date	NASA Memo
Not applicable	SD/NASA 6 Mission, 3-D Aggregation and Neuroendocrine Differentiation of PC-12 Cells in Space, 180 day report, P.I. Lelkes, Ph.D., Laboratory of Cell Biology, University of Wisconsin Medical School, pp 1-4.	August 3, 1998	Not applicable
Not applicable	Simulated Microgravity Conditions Enhance Differentiation of Cultured PC-12 cells toward the Neuroendocrine Phenotype, P.I. Lelkes, D.L. Galvan, G.T. Hayman, T.J. Goodwin, D.Y. Chatman, S. Cherian, R.M.G. Garcia, B.R. Unsworth, *In Vitro Cell Dev. Biol.*, Vol. 34:316-325.	August 1998	Not applicable
Not applicable	Growing Tissues in Microgravity, P.I. Lelkes, B.R. Unsworth, *Nature Medicine*, Vol. 4 (8):901-907.	August 1998	Not applicable

6.4 The Biotechnology Co-Culture Experiment

The objective of the BTS–COCULT experiments was to investigate long-term culture and assembly of two different cell types (human endothelial cell line and human breast carcinoma cell line) in microgravity, determine their role in developing functional tissue, and demonstrate the ability to create a vascularized tumor model.

Results of the BTS–COCULT experiments provided evidence of preliminary vascularization by endothelial cells of the breast tumor, as demonstrated by positive immunohistochemical staining of PCM-1, Factor VIII, and von Wildebrans' antigen.

7 CONCLUSION

The NASA-*Mir* Science Program had several objectives at both the program level and experiment level (203). The two primary program-level objectives were to mitigate risk for the ISS Phase 2 and 3 programs, and to establish a working relationship between U.S. and Russian engineers and scientists in preparation for the ISS Program. The BCSP activities conducted on *Mir* during the ISS Phase 1 Program also had objectives that parallel the program-level objectives. Specifically, the JSC BCSP developed the BTS for *Mir* as risk mitigation for the BTF, which will be flown and operated on the ISS in the future. The crew also performed specific science objectives for the BTS–DE, BTS–CART, BTS–BIO3D, and BTS–COCULT experiments in the BTS facility on *Mir*. All of these objectives were met to varying degrees during the Phase 1 Program.

The ability to develop and operate the BTS facility and perform cell science experiments on *Mir* using the EDU–M and BSTC has provided a tremendous opportunity for the JSC BCSP. Since the elements of the BTS are similar, if not identical, to the technology that will be flown in the BTF on the ISS, JSC cell scientists, hardware developers, and integration engineers have been able to conduct a "dress rehearsal" for ISS operations. Also, they had a rare opportunity to conduct first-class microgravity science for long durations (up to 130 days), something that was not possible in the U.S. space science program before Phase 1. The crew operated elements of both the BTF and the Mini-PIC in the BTS on *Mir*. The successful long-duration operation of the BTS facility on *Mir* and the risk mitigation experiments have enabled the JSC cell science and engineering team to validate and verify the BTF concept, technology, systems, and procedures. Some of the key lessons learned with regard to ISS risk mitigation are described at the end of each section of this report.

Overall, the BTS hardware operated successfully on *Mir*. We learned some lessons that can be applied to the BTF and experiment-specific hardware to ensure even better scientific results for future ISS experiments. The EDU–M design and operating procedures should be thoroughly reviewed to determine the causes of air bubbles in the bioreactor and blockages in the medium lines. We should study both of these issues while considering results documented in this report and the actual crew observations on *Mir*. These on-orbit anomalies associated with the EDU–M should not overshadow the successful risk mitigation and science results that we obtained using this advanced space bioreactor system. Another success on *Mir* included using unplanned and intricate IFM procedures to open the second level of containment and to isolate the bioreactor. A U.S. astronaut successfully performed this delicate procedure on *Mir*, proving that similar procedures are feasible for cell science hardware operated in the BTF.

The EDU–M was the highlight of the U.S. experiments flown during two increments on *Mir*. At the beginning of Increment 3, just after transferring the ECC and EDU–M from the Shuttle to *Mir*, the EDU-M became the center of attention of the NASA-*Mir* Science Program. Data

system anomalies, including error messages, indicated that it was failing when it was reactivated on *Mir* after transfer. Superb teamwork involving the U.S. astronaut on *Mir*, the Shuttle crew, the TSUP, MPOSA, and the BTS science and engineering ground team saved the experiment by correcting a simple, but unexpected, problem that had been overlooked in the initial attempts to correct the problem. Because the Shuttle was still docked when the anomalies arose, digital photographs of the experiment configuration on *Mir* were taken and downlinked via the Shuttle communications system. An alert BTS integration engineer looking at the transmitted photographs noticed that the primary data connector on a cable running between the ECC and the EDU–M had not been locked into its fully engaged position. Alertness, teamwork, and technology utilization all brought a successful conclusion to this potentially experiment-ending problem. This event was heralded as one of the best examples of teamwork during the entire NASA-*Mir* Science Program. It also was a success in risk mitigation for the BTF on the ISS, because we can enhance the crew procedures and training for future BTF experiments to prevent a similar problem.

Mir operations provided long-term results on the operation of the first general-purpose refrigerator developed by the JSC BCSP. Specifically, the BTR was a new hardware development for *Mir*. It was designed to accommodate the refrigeration requirement for cell science experiments in microgravity. We operated two BTRs on *Mir*, during Increments 6 and 7. Each unit operated successfully for an entire increment. While it is true that the BTR operated without failure, it did not maintain the desired internal temperature for sample, medium, and PCBA cartridge refrigeration. The BTR set-point temperature during these increments was 7ºC, but BTR internal temperatures rose as high as 16.5ºC during continuously powered operations on *Mir*. These excursions generally occurred during periods of high ambient temperature on *Mir*. The U.S. astronaut was able to improve the thermal performance of the BTR by using duct tape to better seal the unit and prevent warm air from surrounding payloads from being pulled into the BTR. The U.S. astronaut was able to mitigate the on-orbit problem, but it is apparent that we should review the thermal design of the BTR before launching the unit to the ISS. The operational data from *Mir* indicate that the BTR's thermal performance is not sufficient for the thermal extremes that may be experienced on the ISS or other vehicles. Again, the *Mir* experience has provided valuable risk mitigation for a critical subrack element planned for use in the BTF.

BTS stowage hardware was used extensively during *Mir* operations. No other *Mir* experiment had the quantity and complexity of analytical support equipment required for the BTS experiments. All in all, the stowage hardware for BTS was well planned and well constructed, and operated sufficiently well during its use on *Mir*. The PCBA provided critical data on the condition of the cell culture medium, which is essential for sustaining cell growth and proliferation. New PCBA units were flown to *Mir* on a Progress vehicle after it was determined that the original Priroda-launched units could not operate at the *Mir* cabin pressure, which sometimes fell below the minimum operating pressure of the modified commercial PCBA units. The new units operated successfully on *Mir* at all pressures experienced during the later increments. This is one case in which the problem was solved quickly. Multiple launch vehicles (i.e., Shuttle, Progress, and Soyuz) were a real benefit when we had to get things up to *Mir* quickly.

The portable microscope that was used during the BTS–BIO3D experiment was difficult to use in microgravity. A JSC cell scientist will review the *Mir* operational results from Increment 6, and the crew procedures for *Mir* will be reviewed. Also, alternative microscopes will be considered for microscopy operations planned for BTF experiments on the ISS. In addition, the BSCP will consider using the KC-135 airplane to verify the microscopy equipment and procedures that will be used on future ISS cell science experiments. The results obtained on *Mir* have prompted another look at these important cell science protocols required for on-orbit characterization of the experiment progress.

The *Mir* space station was a unique environment. It was the only facility in existence that provided a long-duration microgravity environment. Russia has more experience than any other nation in operating and maintaining space stations. The Russian space program has 14 years of experience on *Mir* alone; this does not include Salyut, *Mir*'s predecessor. Obviously, we can learn a great deal from the experienced cadre of Russian aerospace engineers, scientists, and operations specialists. This idea provided the impetus for the second major objective of the ISS Phase 1 Program. Also, Russian involvement in developing the ISS made it critical that the U.S. and Russian space programs begin to work together to mitigate the risk for ISS vehicle development. The BTS team worked closely with Russian integration engineers and safety and biomedical specialists to get the BTS hardware and operational protocols accepted for use on *Mir*. Although it was not always easy, and the language barrier was formidable, great progress was made over a relatively short period. The Russian specialists provided valuable information on *Mir* capabilities and limitations. This information was factored into the BTS design, stowage kit contents, and crew procedures. The BTS team and their Russian counterparts established valuable working and personal relationships.

It became clear early in the Phase 1 Program that the Russian specialists take great care to prevent or minimize chemical and biological contamination of the *Mir* internal environments. Strict cleanliness and material purity requirements were imposed on the U.S. payloads. In many cases, the hardware toxic offgassing and material safety requirements were more stringent than those for the Shuttle. This would be expected, since the Shuttle returns to Earth after a couple of weeks and the air can be purged and surfaces cleansed upon return. This is not possible for *Mir*. Although it was difficult to meet the Russian requirements and the Russian authorities had to grant waivers for some payloads, there is a lesson here for the ISS that should not be taken lightly. The Russian scientists and engineers have imposed stringent material and safety requirements only after they have experienced problems with their own payloads in prior activities on *Mir*. U.S. specialists should consider this fact while defining the payload requirements for the ISS. The Russians are sending a clear message while not providing the specific examples of why the stringent requirements were written and implemented as they were during the NASA-*Mir* Science Program.

The BTS team and Russian specialists worked together well enough to conduct cell science and risk mitigation experiments in the BTS on *Mir* for five consecutive increments. The BTS hardware operated without harm to the crew or *Mir*, even when unplanned maintenance procedures were performed on multiple occasions. The BTS team formed professional and personal relationships with the Russian specialists that will be valued for a lifetime. The skill of technical negotiation and working through interpreters and translators may also be valuable for

BTF activities on the ISS. This will be especially important if Russian scientists become involved with cell science experiments in the BTF or U.S. cell science equipment is located in the Russian elements of the ISS.

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13. ABSTRACT *(Maximum 200 words)*

NASA is working with its international partners to develop space vehicles and facilities that will give researchers the opportunity to conduct scientific investigations in space. As part of this activity, NASA's Biotechnology Cell Science Program (BCSP) at the Johnson Space Center (JSC) is developing a world-class biotechnology laboratory facility for the International Space Station (ISS). This report describes the BCSP, including the role of the BTS. We identify the purpose and objectives of the BTS and a detailed description of BTS facility design and operational concept, BTS facility and experiment-specific hardware, and scientific investigations conducted in the facility. We identify the objectives, methods, and results of risk mitigation investigations of the effects of microgravity and cosmic radiation on the BTS data acquisition and control system. These results may apply to many other space experiments that use commercial, terrestrial-based data acquisition technology. Another focal point is a description of the end-to-end process of integrating and operating biotechnology experiments on a variety of space vehicles. The identification of lessons learned that can be applied to future biotechnology experiments is an overall theme of the report. We include a brief summary of the science results, but this is not the focus of the report. The report provides some discussion on the successful 130-day tissue engineering experiment performed in BTS on Mir and describes a seminal gene array investigation that identified a set of unique genes that are activated in space.

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