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Spectrum – An Instrument for Multispectral Biological Fluorescence Imaging on the International Space Station

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Abstract

Spectrum is a compact instrument for fluorescent imaging of biological specimens in microgravity and capable of operating within the constraints of an EXPRESS Rack on the International Space Station (ISS). The Spectrum Flight Unit (SFU) was launched to ISS in November 2019 and is available for science investigations as a general instrument. A duplicate Engineering Development Unit (EDU) is available at NASA Kennedy Space Center (KSC). Science Verification Testing (SVT) performed at KSC demonstrated all required functions including five (5) fluorescence excitation wavelengths, growth lighting, environmental controls and monitoring, and high-resolution imaging using a 71 Megapixel camera with flat field lens. The design features key Orbital Replaceable Unit (ORU) components, which may be adapted or changed by future investigators for tailored science experiments.

Keywords
Fluorescence imaging, biological specimens, microgravity, International Space Station (ISS)

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Introduction

Spectrum is a multi-spectral fluorescence imaging system designed for capturing *in vivo* genetic expression in the low to zero-gravity environment of the International Space Station (ISS). Observation of organisms expressing fluorescent reporters allows the scientific community to further understand biological responses of these organisms when subjected to space environments. Model organisms that may utilize multispectral imaging on the ISS include unicellular organisms (*e.g. Saccharomyces cerevisiae*), plants (*e.g. Arabidopsis thaliana*), and invertebrates (*e.g. Caenorhabditis elegans*).

The purpose of this paper is to familiarize the community about capabilities of the Spectrum instrument and to inform potential users about functions and specifications that may be of interest. Spectrum accommodates standard 100 mm x 100 mm Petri plates, 128 mm x 82 mm multi-well culture plates, and other custom containers within the growth and imaging chamber. Principal Investigator (PI) - defined scripting logic provides for automated chamber management and the continuous collection of chamber environment information, supporting the primary function for capturing 71 Megapixel images of fluorescent emission from biological organisms under highly uniform illumination. Scripting logic also provides for control of chamber temperature (18 C to 37 C), humidity, CO_2 (ranging between 400 ppmv up to ISS-ambient levels), volatile organic compounds (ethylene scrubbing < 25 ppbv), Grow Light levels and Grow Light color. Key Spectrum functional components are identified in Figures 1 and 2. Component manufacturers are listed in Table 1.

Fluorescence Imaging – A ten-position motorized filter wheel for fluorescence emission filters is paired with an Excitation Light Source (ELS) to achieve an 80% uniform illumination on the target Petri plates. Additional filters are provided to support color, black & white and infrared images. This capability allows researchers to image several fluorescent proteins in a single biological specimen providing the ability to perform in-depth multispectral analysis. Technical details for optical components and testing are provided in the Spectrum Optical Characterization Report (Lane and Shipley, 2020). Initial Spectrum flight test selections for fluorescence light sources and bandpass filters are listed in Table 2.

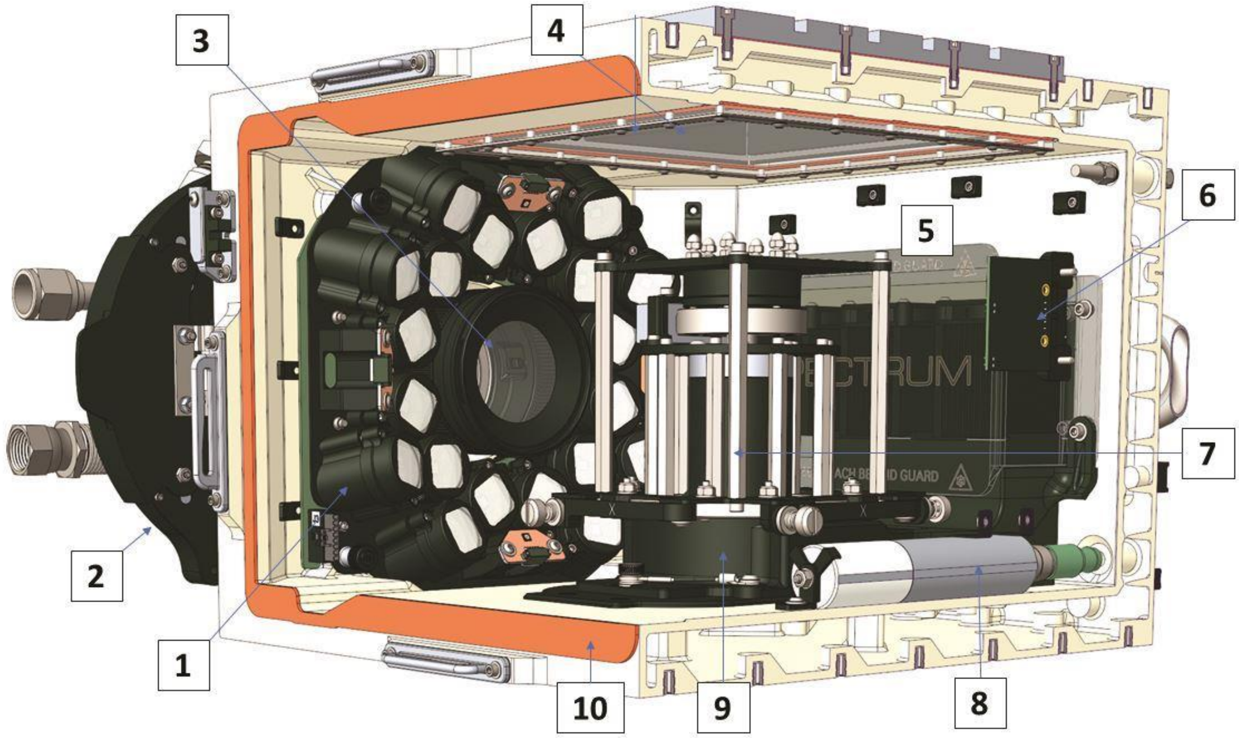


FIGURE 1 – Drawing of Spectrum Engineering Development Unit (EDU) showing location and orientation of key functional components, identified as 1) Excitation Light System, 2) ten-position filter wheel, 3) camera lens, 4) grow lights, 5) fan touch prevention cover, 6) thermodynamic sensors, 7) Petri plate carousel, 8) CO_2 sensor, 9) carousel air circulation manifold, and 10) access door seal.

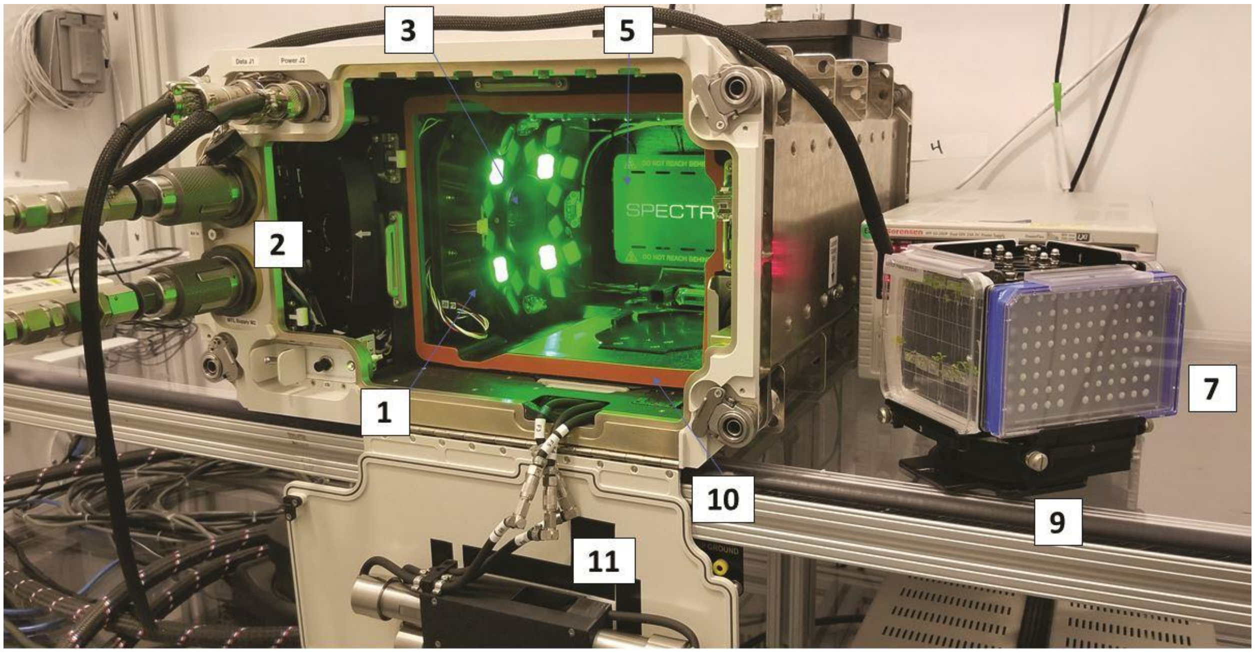


FIGURE 2 – Spectrum Flight Unit (SFU) during Science Verification Test (SVT) in ISS Environmental Chamber at NASA KSC, shown with access door open and Petri plate Carousel Assembly removed. Key functional components are identified as in Figure 1, with items 6) thermodynamic sensors and 8) CO_2 sensor not in view, plus functional component 11) air filter cartridges.

Table 1 – Commercially available optical components of the Spectrum Flight Unit

Component	Manufacturer	Description	Size
Camera	Illunis	71 Megapixel	
Lens	Canon	EF 20mm f/2.8 USM	
Aperture	Birger Engineering	EF232 Focuser	
Excitation Light Source (ELS)			
LED	Lumileds	Luxeon Z	Lane & Shipley (2020), Table 1.2
Lenses	Carclo	Narrow Spot Plain TIR	2x2 10 mm optic
Filters	Semrock	See Table 2	27 mm dia. 2 mm thickness
Diffusers	RPC Photonics	EDS-40-10392-E	25.4 mm dia. 2 mm thickness
Filter Wheel (FW)			
Filters	Semrock	FF01 series, 3.5 mm ring	32 mm dia. 2 mm thickness
Other Components			
Imaging	Lumileds, OSRAM	Luzeon Z, OSRAM SFH	Lane & Shipley (2020), Table 1.3
Grow	Lumileds	Luxeon C	Lane & Shipley (2020), Table 1.4

Table 2 – Fluorescence Compact Excitation Component Selections

Protein	LED	Excitation Filter	Emission Filter
CFP	Luxeon Z Violet, 430 nm LHUV-0425-0600	Semrock 435/40-27	Semrock 482/25-32
GFP	Luxeon Z Blue, 470 nm LXZ1-PB01	Semrock 466/40-27	Semrock 512/25-32
YFP	Luxeon Z Cyan, 500 nm LXZ1-PE01	Semrock 504/12-27	Semrock 539/30-32
OFP	Luxeon Z Lime, 568 nm LXZ1-PB01	Semrock 534/20-27	Semrock 585/40-32
RFP	Luxeon Z Lime, 568 nm LXZ1-PX01	Semrock 572/28-27	Semrock 629/56-32
CHLOR	Uses CFP or GFP excitation	CFP or GFP	Semrock 680/42-32

Camera – Imaging is based on the Illunis™ 71 Megapixel monochromatic camera with Birger™ modules for automated control of camera lens focusing and aperture. The Canon EF 20 mm f/2.8 USM lens achieves a pixel resolution near 13 µm, which is similar to a dissecting microscope. With substitution of Macro lens with EF mounting up to 100 mm focal length and relocation of the imaging plate to the far end of the Environmental Research Chamber (ERC), Spectrum has achieved pixel size to 2.5 µm.

Grow Lights – Grow lighting is provided by calibrated banks of LEDs illuminating biological samples from a single direction. The growth light cap is equipped with broad-spectrum white (400-750 nm), blue (400-500 nm), green (520-530 nm), red (630-660 nm), and far-red (750 nm) LEDs with a combined intensity range of 0-1000 µmol $m^{-2}s^{-1}$ at the leading (top) edge of the Petri plates. Technical grow lighting details are provided by Brady (2017).

Specimen Carousel – Four 100 mm x 100 mm Petri plates can be mounted simultaneously to the Specimen Carousel. The Carousel may be rotated up to 360 degrees around its axis of rotation with angular placement of the Petri plate position to +/- 0.009 degree in 0.1-degree increments. The Petri plate adapters can be customized for any size or shape within a 100 x 127 mm footprint. Available clearance allows two of the Petri plates to be as wide as 100 mm x 128 mm. The carousel is capable of continuous rotation for low accelerations up to 33 rpm and is removable to allow PI-provisioning of custom sample holding and positioning equipment. Precision rotation is enabled using an Elmo Motion Control™ Twitter motor controller. Slightly chilled air is ducted through the Carousel along the rear side of the Petri plate holders to suppress condensation on the inside of the Petri plate covers.

Data Commanding and Storage – Spectrum is commanded from the ground using the NASA Telescience Resource Kit (TReK). The Spectrum onboard processor collects and stores time stamped images with the option to downlink sensor and status data for near-real-time evaluation by the investigators. Details for the commanding logic and data log are provided by Klinko (2020).

Orbital Replaceable Units (ORU) – Principal Investigators may consider modifications to Spectrum for tailoring to new science requirements. Spectrum components which may be modified and/or replaced include the ELS and emission filters, Canon EF camera lens, chamber environmental Sensor Printed Circuit Board (PCB), Carousel assembly, and the air filter cartridges. SFU and EDU software can also be modified prior to and during flight, subject to NASA Program review and approval.

Background and History

An excellent survey of plant growth systems in space is provided by Zabel et al. (2016). The Spectrum instrument design builds upon and expands heritage of the Advanced Biological Research System (ABRS), which was launched to the ISS in 2009 on STS-129. Similar to their predecessors, both ABRS and Spectrum are designed to fit into a single middeck locker. Both ABRS and Spectrum provide a controlled environment for experiments with plants, microbes and other small organisms through control of temperature, relative humidity, CO_2 level, and a filtration module allowing the removal of Volatile Organic Compounds (VOCs). ABRS was outfitted with a novel Green Fluorescent Protein (GFP) Imaging System designed to investigate organisms with modified GFP reporter genes (Levine et al., 2009). One of the first experiments conducted within the ABRS was the investigation of the Transgenic Arabidopsis Gene Expression System (TAGES) (Paul et al., 2012, Paul et al., 2013). Spectrum expands on the ABRS GFP fluorescence capability to add CFP, YFP, OFP, RFP, and Chlorophyl. Sample imaging is expanded from one Petri plate (ABRS) to four (4) Petri/Microtiter plates (Spectrum) within one ERC positioned for high-resolution imaging using a carousel-based motion control system.

The SFU was launched to the ISS in early November 2019 and was installed onboard ISS in June 2020. A flight test demonstration using biological samples provided by the collaborating Investigators identified in Table 3 was conducted in October 2020 (Swanson et al., 2021). Figure 3 shows Astronaut Chris Cassidy performing the initial install of the color and optical resolution calibration plates into the SFU onboard the ISS, on June 11, 2020.

FIGURE 3 – Astronaut Chris Cassidy performing initial install of Spectrum on ISS, June 11, 2020. Color calibration plate (shown in Figure 8) is facing the Spectrum camera lens, and the optical resolution test plate is facing the open Spectrum chamber door.

Table 3 – Spectrum SVT Petri Plate Provider and Channels Imaged

Investigator (PI)	Plate	Images
Dr. Sarah Swanson	100mm Calibration Plate #1	*ad hoc (all channels)*
Dr. Simon Gilroy	100mm Arabidopsis Plate #2	GFP
Dr. Anna-Lisa Paul	100mm Arabidopsis Plate #3	GFP & RFP
Dr. Timothy Hammond	128mm Yeast Plate #4	GFP & RFP

Spectrum Key Functions

The SFU was checked out on the ground before launch during a 10-day Science Verification Test (SVT) conducted in ISS Environmental Simulator (ISSES) chamber No. 2 at NASA KSC from 28 May to 7 June 2019. Three biological specimen plates were installed into the SFU as per written procedures for on-orbit operations. Petri plate providers and fluorescence markers tested are listed in Table 3. Images were obtained every hour by automated schedule for the three specimen Plates in carousel positions #2, #3, and #4. When in standby mode, the optical calibration plate in carousel position #1 was aligned facing the rear of the SFU chamber. This alignment avoids placement of biological specimens adjacent to the fan touch prevention cover plate, which may be cold due to its proximity to the Thermo-Electric Cooling (TEC) devices. Upon completion of this experiment, specimens were removed from the SFU following on-orbit procedures and were returned to the Investigators.

Sample fluorescence imaging results obtained during the SVT are shown in Figure 4. During this ground test, SFU Grow Light was constant and ON except for the several minutes while fluorescence images were obtained.

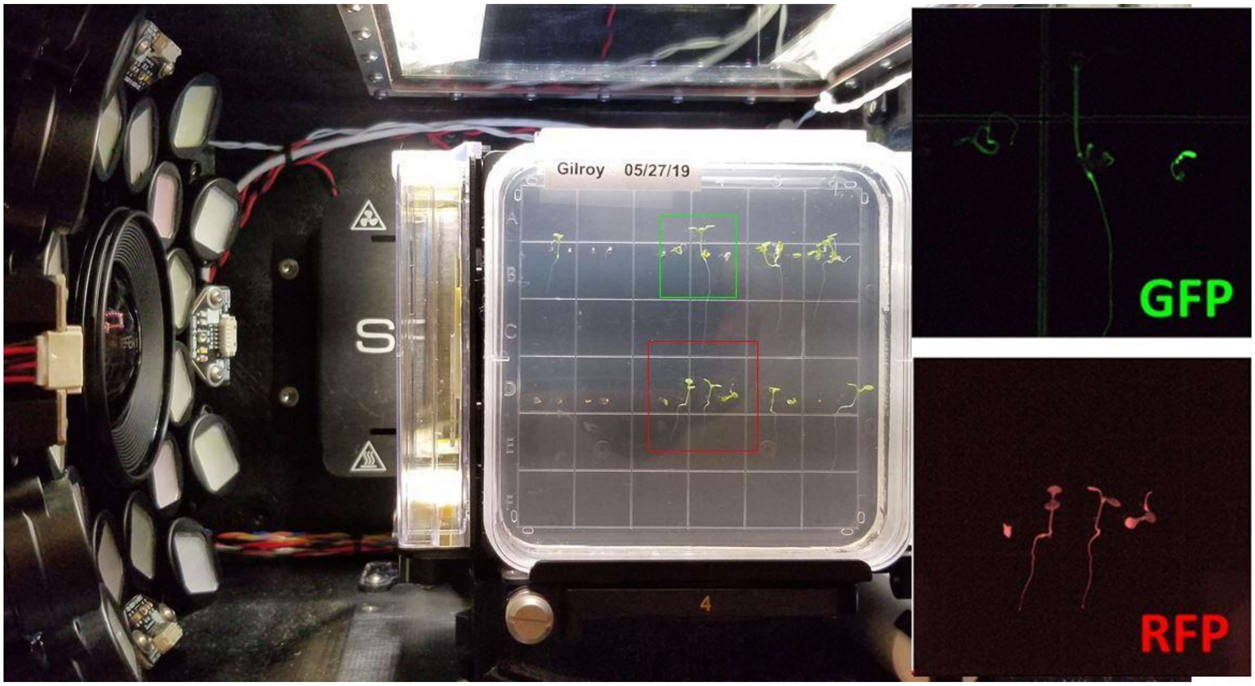


FIGURE 4 – View of the Excitation Light Source (ELS) and Petri plate samples provided by Sarah Swanson (U. Wisconsin – Madison) within the Spectrum ERC during SVT. Insets are Spectrum images of Arabidopsis plants expressing GFP and RFP fluorescence.

Grow Light intensity was set to 130 micromoles/m^2/sec at the top side of all four Petri plates. The Spectrum automatic temperature control was engaged with Moderate Temperature Loop (MTL) temperature set to 21 C +/- 2 C. Ambient CO_2 levels in the ISSES Chamber were set at 4000 ppm and kept near that value +/- 10%. CO_2 levels inside the ERC were observed to rise above ambient during the SVT due to yeast activity in carousel position #4. This CO_2 production is compensated by actively pumping chamber air through a lithium hydroxide (LiOH) cartridge filter for 5 minutes every hour by automated schedule.

Fluorescence Imaging – The original Spectrum Science Requirements are listed in Table 4. The so-called "black" channel for Blue EBFP-type imaging was not implemented due to our use of polycarbonate components in the excitation optics. The RPC Photonics (brand) 40 degree engineered diffusers were fabricated onto a polycarbonate substrate for containment of glass shards should any of the glass Semrock excitation filters shatter for any reason. This strategy and precautions taken are detailed in the Spectrum Flight Safety Data Package (Soler, 2019).

The physical layout of the ELS lights is shown in Figure 5. Note that the excitation light colors are each arranged in a square pattern within an inner ring (RFP, OFP, YFP) and outer ring (GFP, CFP). This layout is used to achieve uniform illumination across the entire 100 mm square Petri plate. The inner and outer excitation rings are canted toward the optic axis at angles of 9.5° and 26°, respectively, as determined during detailed optic designs using Zemax OpticStudio. The Imaging LEDs are canted toward the optic axis at an angle of 37.5°. Images of the ERC with illumination by the several fluorescence excitation light sources are shown in Figure 6.

Table 4 – Spectrum Science Requirements for Excitation and Emission

Protein Categories	**Excitation** [nm]	**Excitation Filter** Center Wavelength/ Band Pass [nm]	**Dichromatic Mirror** Cut-On [nm]	**Emission** [nm]	**Barrier Filter** Center Wavelength/ Band Pass [nm]
Cyan ECFP-type	~439	435 / 40	460 LP	~476	495 / 50
Green EGFP-type	~484	470 / 40	495 Long Pass (LP)	~507	515 / 30
Yellow EYFP-type	~514	490 / 40	515 LP	~527	540 / 30
~~Blue EBFP-Type~~	~~~383~~	~~375 / 50~~	~~405 LP~~	~~~445~~	~~445 / 50~~
Orange	~548	525 / 40	550 LP	~562	585 / 50
Red	~584	560 / 55	590 LP	~607	630 / 60
Chlorophyll	~484	470 / 40	495 LP	~680	680 / 50

Note: The proteins and their associated wavelengths are examples and are not requirements; actual proteins and wavelengths to be imaged shall be determined during pre-mission testing by the appropriate Principal Investigator (PI). Blue EBFP was not attempted due to polycarbonate components blocking $\lambda < 400$ nm.

FIGURE 5 – ELS Physical Layout, with CFP, GFP, and RGBW-IR positioned on "outer ring", axially symmetric about the camera Optic Axis. ELS image is a photograph of the SFU component taken 10 July 2019 during packing for flight.

ELS OFF ELS CFP ELS GFP

ELS YFP ELS OFP ELS RFP

FIGURE 6 – Images of Spectrum ERC with five fluorescence excitation light sources, showing placement of the four light modules, each module containing four LEDs, needed to achieve uniformity of illumination.

Color Imaging – Since the Illunis 71 Megapixel camera is monochrome, color images are reconstructed from three separate images taken with Red (R) Green (G) and Blue (B) lighting. This lighting can be accomplished with the transparent emission filter using the RGBW-IR Imaging LEDs identified in Figure 5, or with white lighting (Imaging or Grow or both) and the three so-called “Bayer” RGB emission filters. These two approaches to color imaging are compared by Lane and Shipley (2020). The Imaging LED approach uses the same filter for each channel, but the positions of each color LED vary slightly which results in a spectral artifact at higher resolution. LED RGB illumination does not recover color comparable to human vision, whereas “Bayer” RGB filters under white light is more familiar to human vision. However, the three RGB filters may vary in optical density which will affect imaging focal length and image magnification. Spectrum automated scripting supports focal length adjustment for each image taken during the RGB filter imaging process.

The Bayer filter approach for 100 mm Arabidopsis Plate #3 (Anna-Lisa Paul and Robert Ferl, U. Florida - Gainesville) during the SVT is shown in Figure 7. Animation loops were created from the hourly sequence of color images, which were taken under Spectrum automated control using a programmable PI-generated script (Wyatt, 2019). The Spectrum color target is a standard RGB Color Encoding Image professionally printed and mounted inside a 128 mm x 85 mm Petri plate. The SFU color target and focus calibration plates are shown in Figure 8.

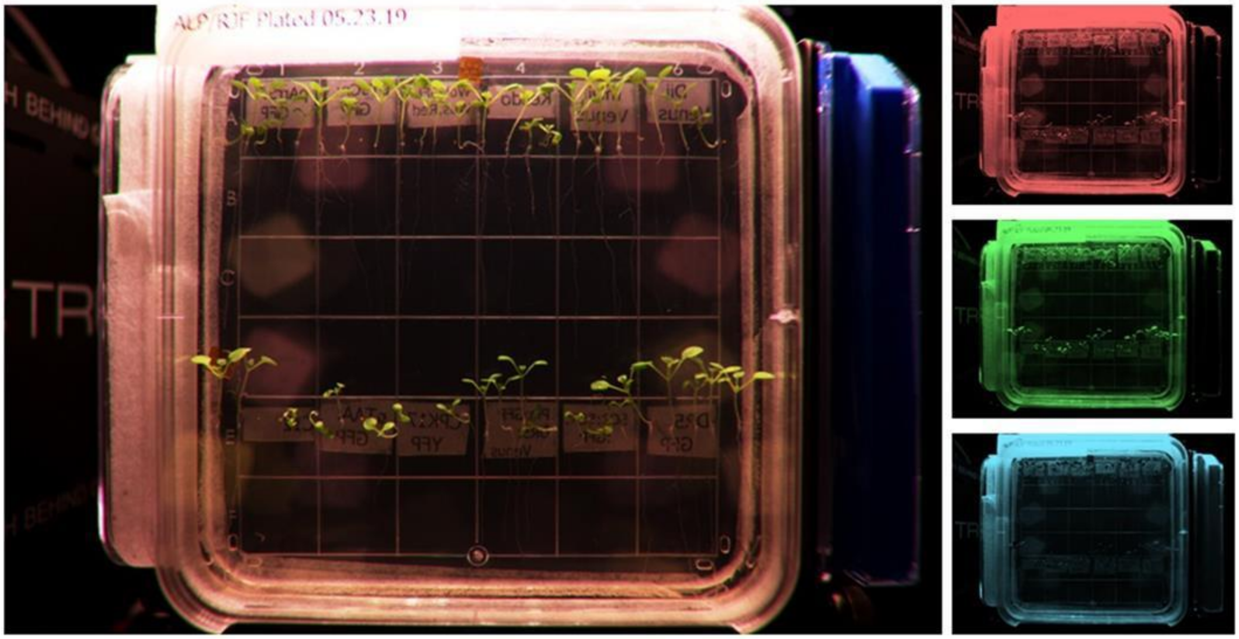


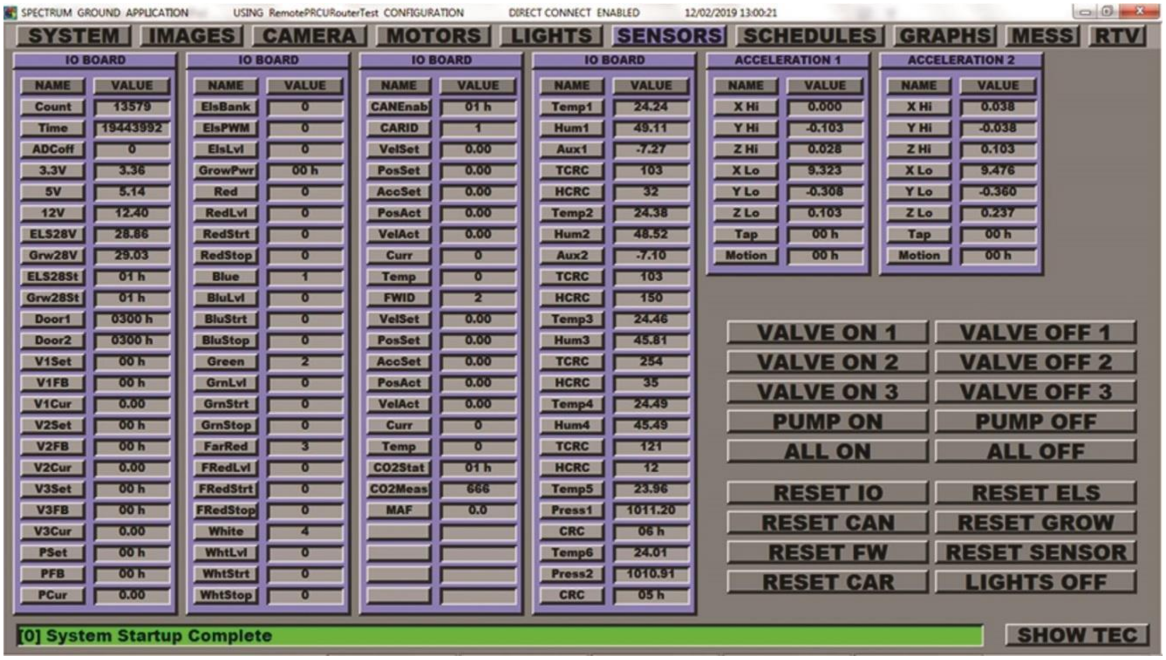
FIGURE 7 – Color image of Petri plate sample provided by Anna-Lisa Paul and Robert Ferl (U. Florida – Gainesville), produced under white light illumination by reconstruction from three sequential monochrome images taken with Red, Green and Blue broadband filters.

FIGURE 8 – Photo of calibration targets taken 10 July 2019, just prior to packaging for transport to the ISS.

Automated Operations –

Spectrum commands support operations on the embedded PC104 computer, messages to/from distributed embedded microprocessors, and communication between the PC104 computer and the Spectrum Ground Application. All commands may be manually initiated, and any manual command may be included in an automated script. The Spectrum Ground Application utilizes TReK protocols to communicate with the SFU when the ground system is in contact with the ISS through the Huntsville Operations Support Center (HOSC). The PC104 is not accessible except through TReK and two connectors which are brought forward from the PC104 stack to the front of the instrument. These connection points are located inside the front door to the right of the ERC. Female connectors for TCP/IP (RJ45) and USB2 (Type A) allow direct connection to the Windows Operating System using Remote Desktop Protocol (RDP) and file transfer, respectively.

The Spectrum Ground Application can be run in *Direct Connect Mode* or in *On Orbit Mode*. The purpose of the Spectrum Ground Application is to provide a User Interface (UI) for monitoring and controlling the measurements and settings implemented by the Spectrum Payload Application that runs on both the SFU and EDU. The Spectrum Ground Application consists of numerous panels that enable user access to virtually any control parameter, setting, or measurement that is implemented in the Spectrum Payload Application Software. A discussion of the literally thousands of commands and parameters supported by the Payload/Ground Application software is beyond the scope of this paper. The commands and parameters are grouped into ten (10) logical subsets relating to System, Images, Camera, Motors, Lights, Sensors, Schedules, Graphs, Messages, and Real-Time View (RTV). Figure 9 shows the ground application screen with the Sensors panel selected. All chamber temperatures are visible in this panel, and they are updated every second when connected to the payload. The valve and pump controls in the lower right of Figure 9 control the flow of chamber air through the two filters for control of CO_2, humidity, and VOC. Another important capability is Real Time View. The RTV button on the panel display bar in Figure 10 will bring



SPECTRUM GROUND APPLICATION USING RemotePRCURouterTest CONFIGURATION DIRECT CONNECT ENABLED 12/02/2019 13:00:21

IO BOARD

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Time	19443992
ADCoff	0
3.3V	3.36
5V	5.14
12V	12.40
ELS28V	28.86
Grw28V	29.03
ELS28St	01 h
Grw28St	01 h
Door1	0300 h
Door2	0300 h
V1Set	00 h
V1FB	00 h
V1Cur	0.00
V2Set	00 h
V2FB	00 h
V2Cur	0.00
V3Set	00 h
V3FB	00 h
V3Cur	0.00
PSet	00 h
PFB	00 h
PCur	0.00

IO BOARD

NAME	VALUE
ElsBank	0
ElsPWM	0
ElsLvl	0
GrowPwr	00 h
Red	0
RedLvl	0
RedStrt	0
RedStop	0
Blue	1
BluLvl	0
BluStrt	0
BluStop	0
Green	2
GrnLvl	0
GrnStrt	0
GrnStop	0
FarRed	3
FRedLvl	0
FRedStrt	0
FRedStop	0
White	4
WhtLvl	0
WhtStrt	0
WhtStop	0

IO BOARD

NAME	VALUE
CANEnab	01 h
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PosSet	0.00
AccSet	0.00
PosAct	0.00
VelAct	0.00
Curr	0
Temp	0
FWID	2
VelSet	0.00
PosSet	0.00
AccSet	0.00
PosAct	0.00
VelAct	0.00
Curr	0
Temp	0
CO2Stat	01 h
CO2Meas	666
MAF	0.0

IO BOARD

NAME	VALUE
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Hum1	49.11
Aux1	-7.27
TCRC	103
HCRC	32
Temp2	24.38
Hum2	48.52
Aux2	-7.10
TCRC	103
HCRC	150
Temp3	24.46
Hum3	45.81
TCRC	254
HCRC	35
Temp4	24.49
Hum4	45.49
TCRC	121
HCRC	12
Temp5	23.96
Press1	1011.20
CRC	06 h
Temp6	24.01
Press2	1010.91
CRC	05 h

ACCELERATION 1

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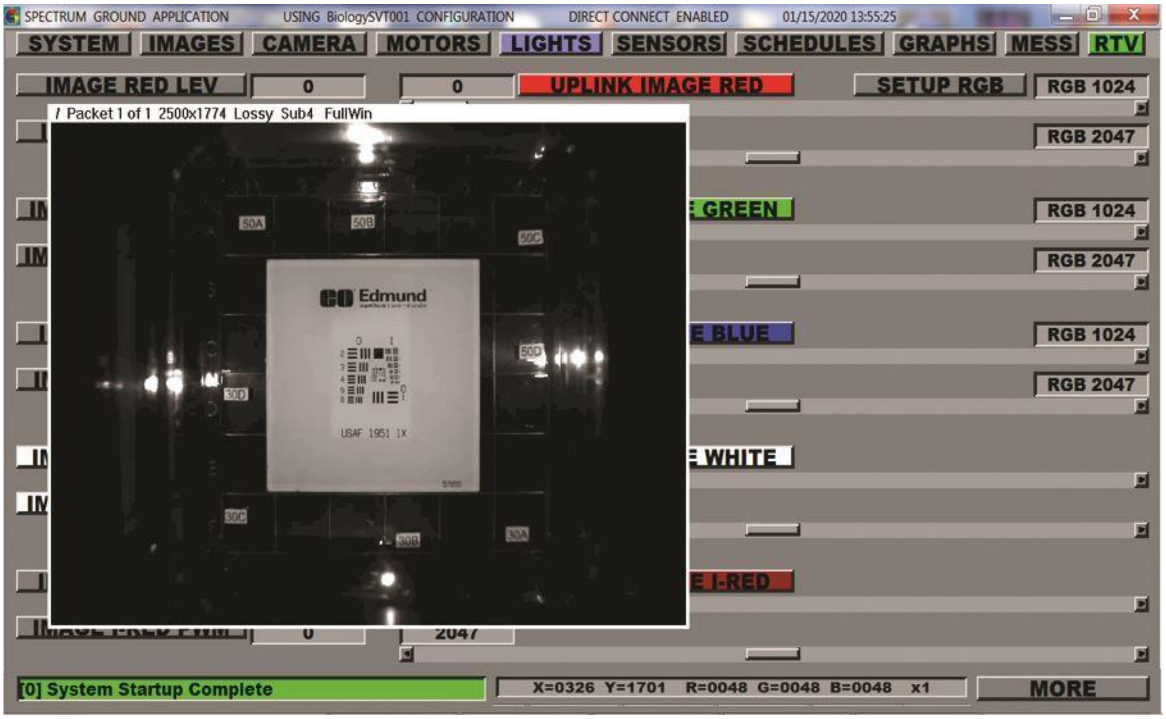
X Hi	0.000
Y Hi	-0.103
Z Hi	0.028
X Lo	9.323
Y Lo	-0.308
Z Lo	0.103
Tap	00 h
Motion	00 h

ACCELERATION 2

NAME	VALUE
X Hi	0.038
Y Hi	-0.038
Z Hi	0.103
X Lo	9.476
Y Lo	-0.360
Z Lo	0.237
Tap	00 h
Motion	00 h

[0] System Startup Complete

FIGURE 9 – Ground System Application Control Panel showing status and command buttons for Spectrum sensors and filter cartridge valve controls. Detailed descriptions of status values and commanding instructions are provided in the Spectrum User Manual (Klinko, 2020).



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/ Packet 1 of 1 2500x1774 Lossy Sub4 FullWin

[0] System Startup Complete

X=0326 Y=1701 R=0048 G=0048 B=0048 x1

FIGURE 10 – Ground System Application Control Panel showing Real Time View (RTV) in operation over the Image LED Control Panel. RTV uses compression of lower resolution images to support near real time interaction with the SFU during on-orbit operations.

up a low-resolution image screen that is rapidly updated at 1 frame/sec. RTV provides user interactive adjustment of lighting, focus, exposure, and other parameters. The RTV system uses H.264 image compression and subsampling to achieve the fast refresh rates for real time viewing.

Environment Control – Automatic programmable control of temperature, humidity and CO_2 is supported through two embedded microcontrollers that are subject to direct and scheduled commanding logic by the PC104 computer. The "I/O" PCB handles the tasks of LED control, sensor signal conditioning and measurement, VOC and CO_2 removal, carousel motor control, and filter wheel motor control. A second "TEC Control" PCB operates the TEC devices and fans. Multiple sensors are provided for redundant measurement of temperature at various locations in the ERC. Humidity and acceleration are also measured by a Sensor PCB which is connected through the I/O PCB. The TEC Control PCB regulates ERC temperature at the set point specified by the Investigator by driving the Peltier-effect TEC devices to heat or cool the ERC based on feedback from the average of four (4) independent temperature measurements obtained from the Sensor PCB. The TEC Control uses an H-bridge driver to select whether heat should be pumped into or out of the environmental chamber. The ISS MTL moves heat away from the TEC plates in the event that the chamber needs to be cooled and heat is pumped out of the system. The TEC Control also turns fans on and off to circulate the air in microgravity while it is actively heating or cooling to normalize temperatures in all areas of the ERC. In event of sensor PCB failure, the TEC board has backup temperature sensors to keep ERC temperature within a reasonable range.

CO_2 and VOC levels are maintained using a single air pump and combinations of three valves to direct chamber air through filter cartridges at an exchange rate of 10% volume per minute. A mass flow sensor is incorporated and is located downstream of the Air Filter Kit. CO_2 levels are maintained between 400 ppbv and ambient room/cabin levels using an LiOH filter cartridge and Vaisala GMP252 CO_2 Probe. Reported CO_2 levels are monitored and logged every second. A test of CO_2 level control is shown in Figure 11. CO_2 levels are decreased by recirculating chamber air through the LiOH filter (MODE 1, all valves OFF). CO_2 levels are increased/decreased to ambient (external) levels by injecting ambient room/cabin air into the chamber (command MODE 3, all valves ON).

Although no detector is included for measurement of Ethylene levels, a Chemsorb® MultiGard® 3800 filter sink is provided for routine VOC scrubbing (Monje, 2016). Chamber air is recirculated through the VOC scrubbing filter (Mode 2, valve 3 ON). The goal of a routine (blind) scrubbing procedure is to keep Ethylene levels in the ERC below 25 ppbv. The Spectrum approach to Ethylene scrubbing was tested using calibrated dilutions of C2H4 and gas samples extracted and analyzed in a laboratory environment with Gas Chromatograph at KSC.

Suppression of Condensation –

Condensation is a well-known feature on the inside cover of a biological specimen Petri dish, especially when the Petri emerges from cold storage. The atmosphere within a biologically active Petri is at or near 100% relative humidity, and a slightly cooler Petri cover will fog immediately. The droplets on the inside cover may vary in size distribution, and they will interfere with clear imaging of the biological specimens. Best practice involves cooler air blowing across the face of the Petri where the condensation is more beneficial, which is usually the agar side.

An example of cover condensation during the Spectrum SVT is provided in Figure 12, where two samples provided by Anna-Lisa Paul and Robert Ferl (U. Florida – Gainesville) were

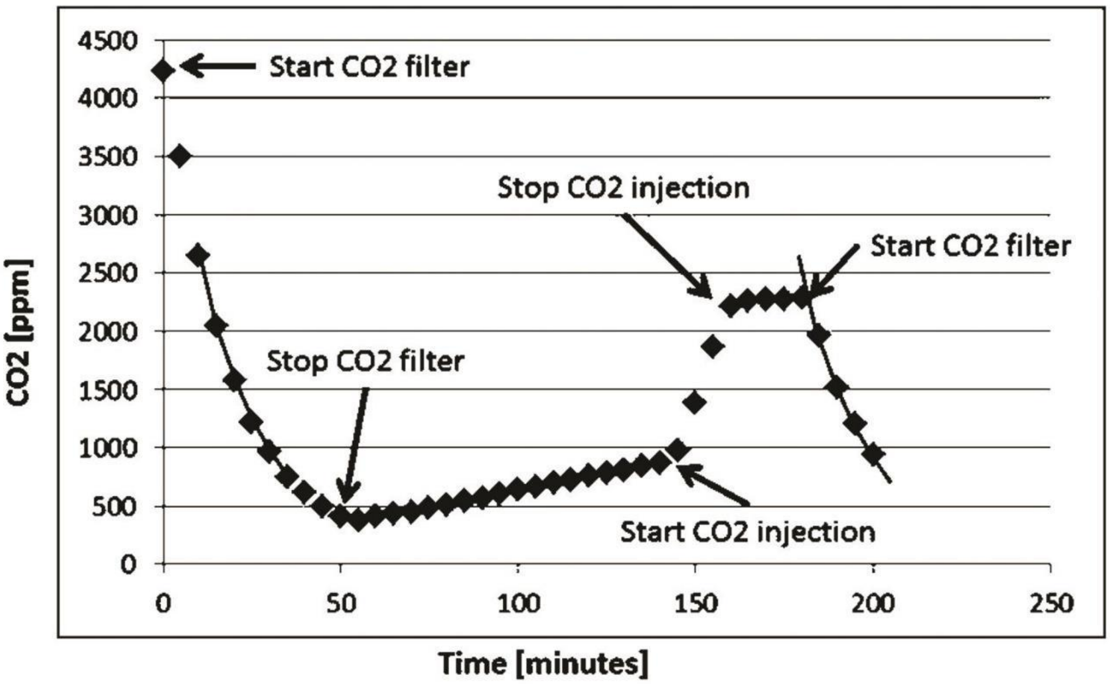

FIGURE 11 – CO_2 Environmental Control Test for ERC CO_2, conducted with Spectrum operating within an ISSES control chamber at KSC. CO_2 injection is achieved by drawing air from the cabin into the ERC. CO_2 removal is performed by passing ERC air through an LiOH filter cartridge.

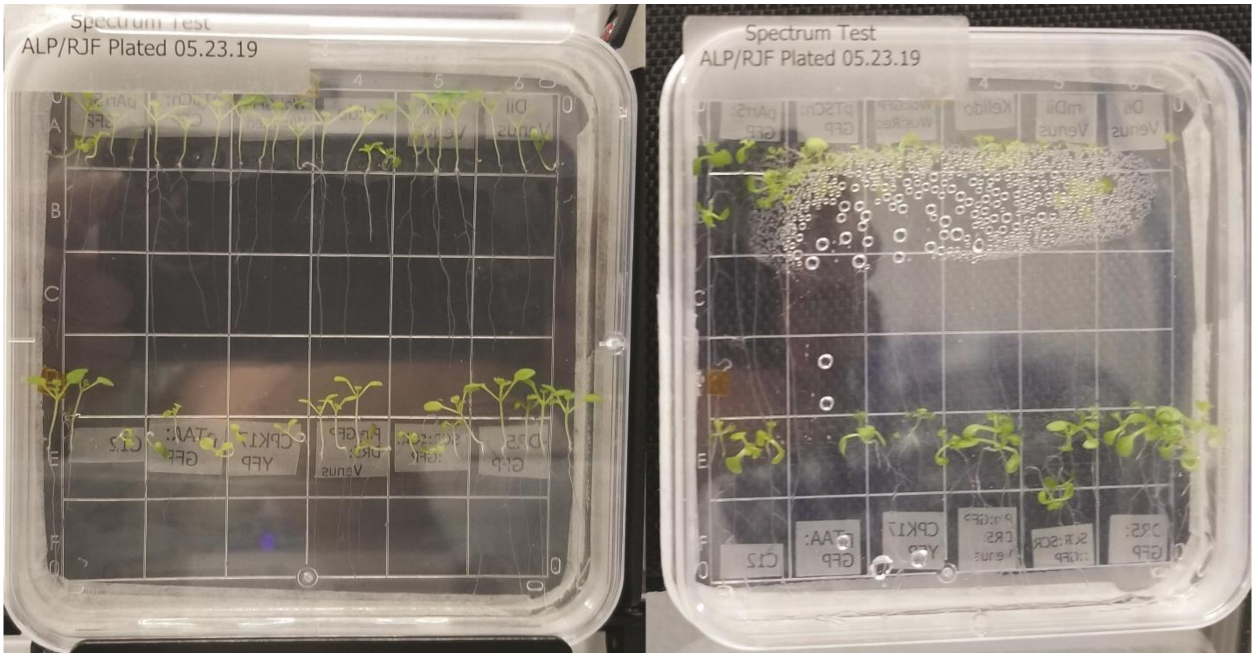

FIGURE 12 – Spectrum control of Petri plate cover condensation/fogging demonstrated during SVT (July 2019) for duplicate Petri plates provided by Anna-Lisa Paul and Robert Ferl (U. Florida – Gainesville). The clear plate (left, same as Figure 7) was just removed from the ERC. The fogged control plate (right) was held in cool storage as per normal practice.

separated and handled under controlled conditions. The sample on the left of Figure 12 was subjected to Spectrum chamber conditions for ten (10) days, including the Spectrum plate cover clearing procedure. The sibling sample on the right of Figure 12 was held for ten (10) days in a standard growth module at KSC. Both plates show similar plant development.

Spectrum achieves plate cover clearing by bringing chilled air into the chamber along the rear surface of the aluminum Petri plate holders. The presence of chilled air from the TEC bank is driven by slightly warmer room conditions in the ambient room/cabin air. Under such conditions, the chamber temperature is held programmatically about one degree C cooler than ambient, and the chilled air achieves about one half degree C temperature difference between the front and back covers of each Petri plate. This configuration is demonstrated in Figure 13, where thermocouples were attached to the front and back surfaces of the subject plate, and a 0.4 C temperature difference was observed. This temperature gradient changes rapidly when the Spectrum chamber door is opened but settles back into equilibrium within a few minutes of door closure. Spectrum uses three (3) fans to circulate air past the TECs, and each fan can be ON/OFF independently under Ground Application control. Of these three fans, two (Fans #2 & #3) are directing air into the bottom of the carousel manifold, and one (Fan #1) is flowing air directly into the ERC. It is important that users turn OFF Fan #1 to achieve plate condensation clearing.

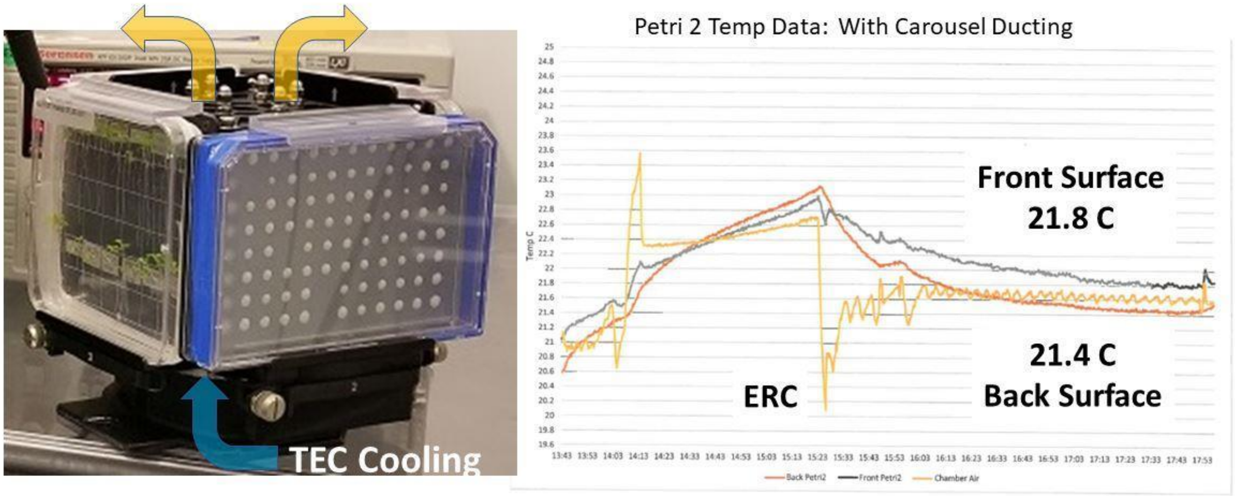

FIGURE 13 – Spectrum suppresses Petri plate cover condensation/fogging by passing slightly chilled air from the TEC behind the Petri plates (agar side) before that air reaches the ERC. This method maintains about 0.5 C temperature differential between the front and back of the Petri plates.

Optical System Design – Measurement of fluorescence is complicated by the blue shift of emission light passing at larger angles of incidence through the emission bandpass filter. Lane and Shipley (2020) demonstrate that blue shift is not a significant factor for imaging areas confined to the dimensions of a Petri mounted as designed in the Spectrum Carousel. The top-down optical layout of the Spectrum imaging system is shown in Figure 14. Image pixel size is about 13 µm with the current f/2.8 lens. The ability to resolve two lines on the Spectrum USAF 1951 IX calibration target demonstrates an effective resolution near 60 µm. As shown in Figure 8, the optical calibration Petri also includes small ~ 50 µm diameter fluorescent beads located in the circular areas around the square float glass target. The beads are small enough to provide single-pixel determination of their precise position in a monochromatic image, provided that the camera aperture is stopped “down” enough to minimize comatic off-axis aberration. The fluorescent beads provide an accurate and quick method for setting the camera focus on the position of each bead in the depth of field. The bead fluorescence makes these points bright enough to be easily found in a dark field. We also recommend that fluorescent beads be provided for each excitation band relevant to the Investigators’ experiment.

The bandpass of an interference filter has a well-known blue shift with increasing Angle of Incidence (AOI) θ and substrate effective refractive index n_{eff}, such that

$$\lambda(\theta) - \lambda(0) \text{ sqrt } (1 - \sin^2 \theta / n_{eff}^2)$$

The effective refractive index varies with filter substrate and polarization, and n_{eff} has no relation to the bulk refractive index affecting image focus. The n_{eff} values cited in Table 5 were provided by Semrock. Excitation light $I(\lambda)$ passing through the CFP emission filter as a function of AOI is shown in Figure 15. Relative gain in this figure is increased for the lower incidence angles to show weak signal features, and an additive offset has been introduced to separate the angular responses.

Larger AOI through the emission filter is expected for emissions from the edges of the target Petri plate. We determined that most light received from the edge of a 100 mm plate will have AOI less than 30 degrees. We therefore expect blue shift to not be a large factor in normal Spectrum operations.

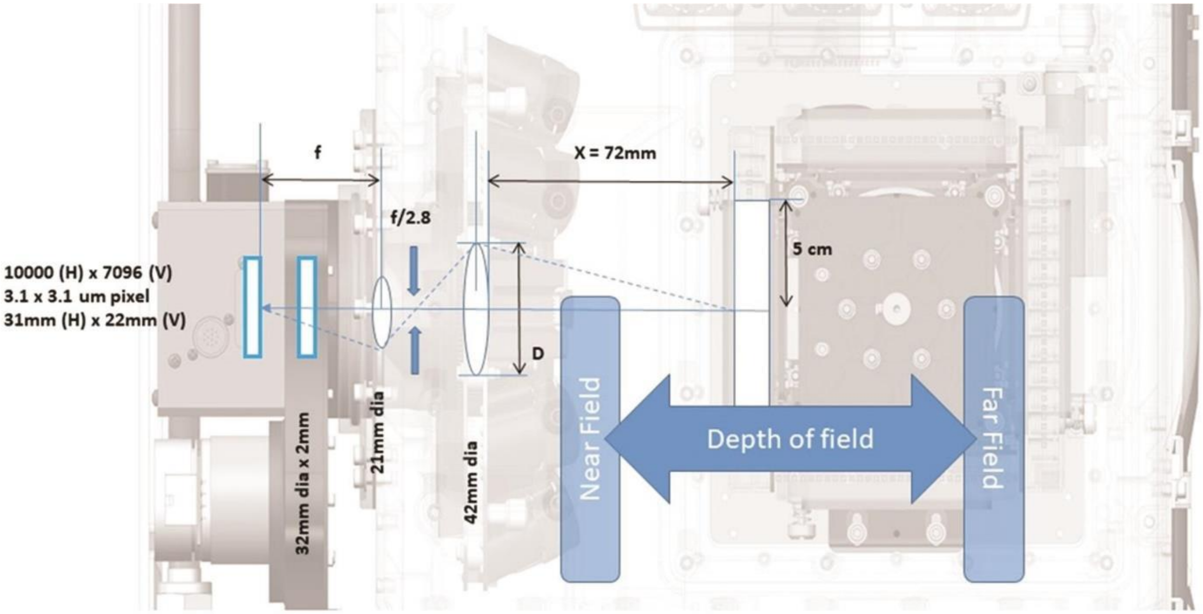

FIGURE 14 – Layout and dimensions of the Spectrum Optical Components. Top view of ERC showing primary imaging volume for a Carousel mounted Petri plate, camera lens and location of an emission bandpass filter with respect to the Optic Axis (OA). The current f/2.8 lens supports a depth of field through most of the ERC.

Table 5 – Bandpass Filter Assignments for Spectrum Flight Test

Filter Wheel	Description	Excitation Filter [nm]	Emission Filter [nm]	Mnemonic Color	Effective Refractive Index n_{eff}
1	Bayer Blue		458/64	Blue	2.03
2	Bayer Green		535/50	Green	1.96
3	Bayer Red		650/100	Red	1.86
4	Transparent		409/LP	White	2.06
5	CFP	435/40	482/35	Cyan	1.99
6	GFP	466/40	512/25	Lime	1.85
7	YFP	504/12	539/30	Yellow	1.98
8	OFP	534/20	585/40	Orange	1.8
9	RFP	572/28	629/56	Red	1.78
10	CHLOR	CFP & GFP	680/42	Dark Red	1.92

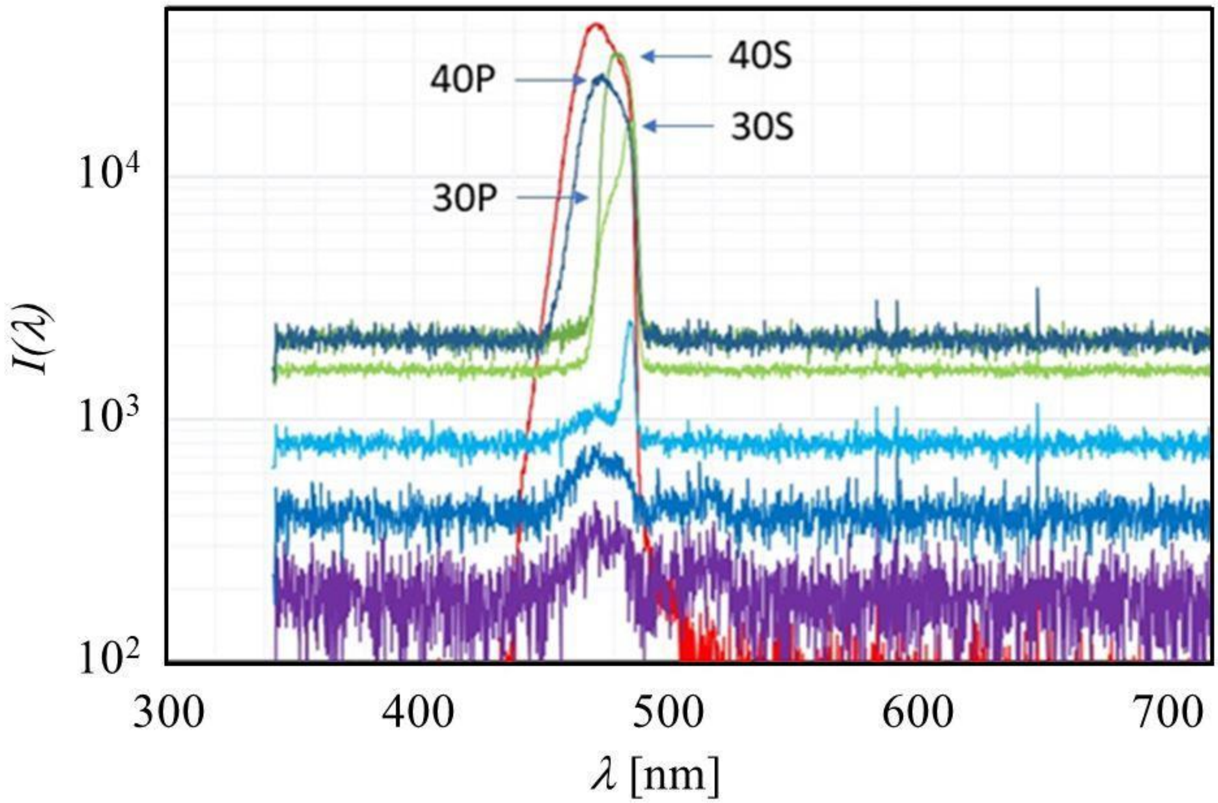

FIGURE 15 – GFP emission filter transmission of GFP excitation light from a diffuse surface as a function of emission filter Angle of Incidence (AOI). For GFP, a linear polarization filter was inserted in the optical path to independently measure the S and P polarization spectra. This result shows that GFP AOI should be kept less than 20 degrees (Lane and Shipley, 2020).

Orbital Replaceable Units (ORU)

Spectrum was designed to very specific requirements, and the Development Team kept secondary goals in mind for modifications to the EDU at NASA KSC and the SFU onboard the ISS. The following Spectrum components are candidates for Orbital Replacement, either for repair and maintenance, or to enable investigations not addressed by the original Science Requirements. The ORU mechanical and electrical specifications, as applicable, are documented in the Spectrum Archive at NASA KSC.

Excitation Light Source – The entire ELS was transported to the ISS in a separate Crew Transfer Bag (CTB) due to the presence of glass bandpass filters and the potential impact of shatterable glass to Astronaut safety. The excitation lights are controlled through one 6-pin connector and the ELS can easily be replaced on orbit. The ELS was custom designed by Eraj Yunus. Modifications to the ELS board itself are beyond the scope of Astronaut activities. Referring to Burns et al. (2017), the wavelengths of the QUAD excitation light modules can be changed, and/or the placement of QUAD light modules on the ELS board can be rearranged. The 6-pin connector from I/O PCB to the ELS PCB provides +28 VDC and a programmable I^2C interface, so entirely new ELS concepts may be possible.

Emission Filters – Initial flight test emission filter selection and configuration is given in Table 5. The emission filters are standard Semrock 32 mm diameter bandpass items, and the substrates must be mounted within 3.5 mm thick rims. The Spectrum Development Team recommends that all filter substrates be the same thickness, typically 2.0 mm +/- 0.1 mm, to ensure uniform focus lengths for imaging across the filter set. A list of available Semrock bandpass filters at nearby wavelengths is provided by Lane and Shipley (2020).

Carousel Assembly – The Petri plate carousel was custom designed by Carlos Gil and A.J. Nick. The mechanical dimensions of the blind mate guide are documented in the Spectrum Archive under CAROUSEL ASSY. The blind mate utilizes a 24 pin Centronix connector, with pin out itemized in the Spectrum wire list spreadsheet. The connector pins support motion operation using an Elmo Twitter motor controller, and an absolute angle encoder Hall effect device. There are no programmable connections between the carousel and the PC104. Power is available at +5V over three pairs with RTN using 24 AWG cable.

Air Filter System (AFS) – The filter cartridges are custom designed by Adam Chaney, with mechanical design detailed in the Spectrum Archive under AFS KIT. Two Air Filter Cartridges (AFCs) are attached to a mission kit plate, and the kit plate is secured to the Spectrum Front Door by the crew using Velcro. One AFC contains approximately 40 grams of LiOH to filter CO_2 while the second carries approximately 25 grams of Chemsorb® MultiGard® 3800 (activated carbon) to scrub VOCs. The AFS contains enough filtration chemicals to maintain the ERC environment for 90 days with 4 Petri plates filled with 10 plants each. Three quick disconnects on the Air Filter Kit provide an easy-to-use physical interface for removal and installation of the kit from the payload. All components are connected with 1/8” ID, ¼” OD Norprene® tubing.

Sensor PCB – The sensor PCB was custom designed by Bradley Burns and is detailed in the Spectrum Archive. Multiple Sensor PCB spares are available. The 6-pin connector from I/O PCB to the Sensor PCB provides +5 VDC and a programmable I^2C interface, so entirely new Sensor PCB concepts may be possible. The mechanical dimensions of the blind mate guide are documented in the Spectrum Archive under Sensor PCB. The Development Team considered the possible future availability of a chip-based Ethylene sensor, and we wanted to ensure that such a device could be incorporated.

Summary

SFU instruments on ISS, and EDU on ground, are managed by NASA KSC. Contact Howard Levine, NASA KSC for information regarding access to the SFU and/or EDU. NASA currently uses REMIS contractor MEI Technologies of Houston, TX to operate both the SFU onboard ISS, Spectrum EDU at NASA KSC SSPF, and the Spectrum ground application system. After a successful Science Verification Test on ground at KSC, the SFU was launched to the ISS in November 2019, and was installed on 11 June 2020. An initial flight test demonstration was conducted in October 2020.

We are pleased to report that Spectrum works as designed. The condensation clearing procedure appears to work well both on ground and in micro-gravity. This paper identifies ORUs which may be modified by future investigators to meet research and operational goals beyond the original Spectrum science requirements.

The Development Team learned several lessons during this multi-year activity. One of our biggest issues was related to Astronaut safety, and primarily involving the shatterable glass present in the Spectrum optical components. We recommend that future Spectrum and related instruments contain all components launched in place and ready for operation, with detection methods built in allowing plug and play operation onboard the ISS. Astronaut time and scheduling is a large factor in delaying operations. A major related component is the camera, which should be removable and also contain humidity/pressure sensors to detect leaks in the sealed dry Nitrogen atmosphere.

The Spectrum Development Team looks forward to the analysis and publications which will appear related to Spectrum operations and basic research.

Acknowledgments

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