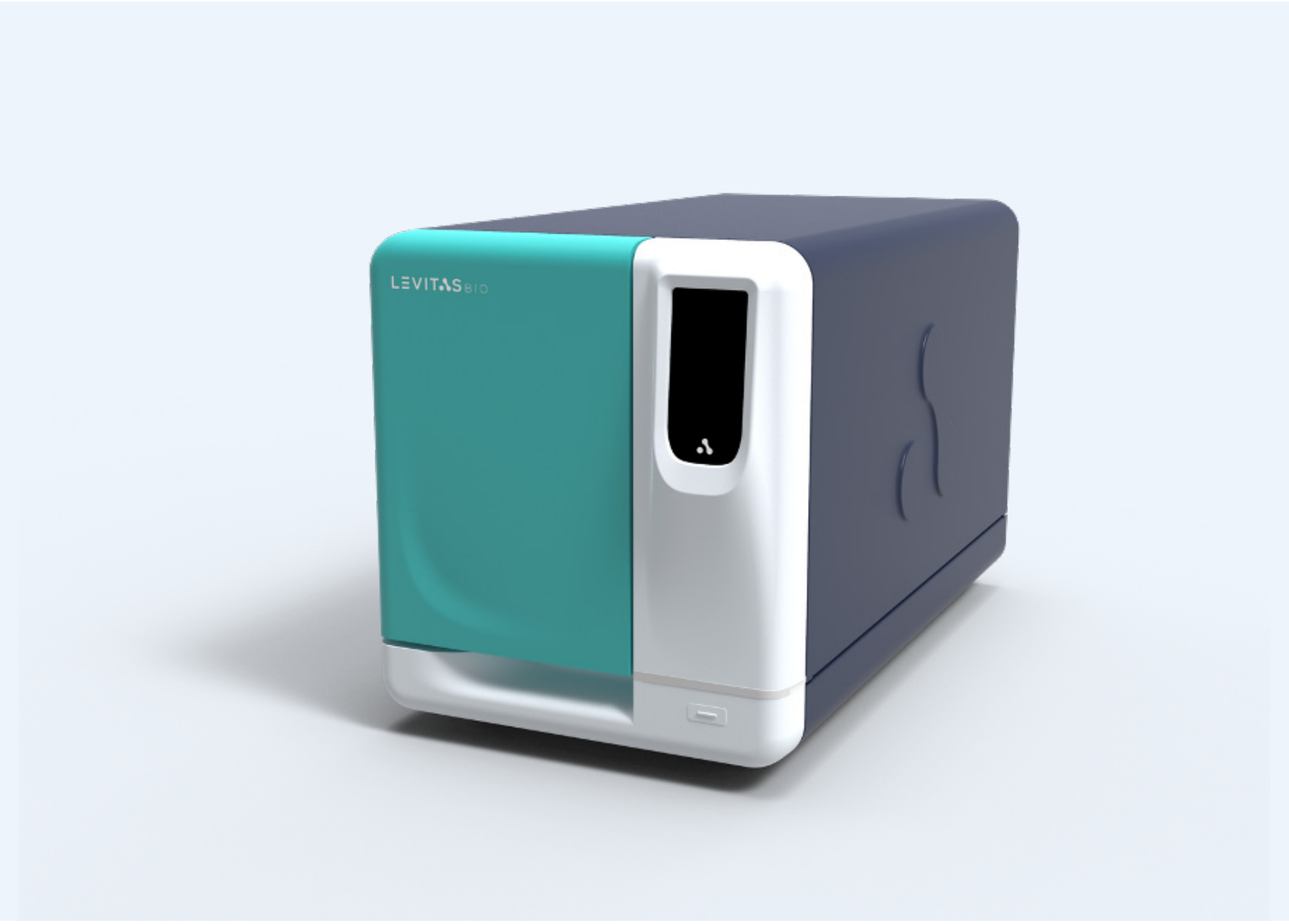
LEVITAS BIO



LeviCell EOS User Guide

INCLUDING VIABLE CELL ENRICHMENT PROTOCOL

LevitasBio Inc., 1505 Adams Drive Suite D, Menlo Park CA 94025 USA

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LEGAL NOTICES

The LeviCell EOS system is developed by and manufactured for LevitasBio, Inc. This device and/or methods of using this device are covered by one or more issued patents and pending patent applications owned or licensed by LevitasBio, Inc. including US Patent Numbers 9,517,474, 9,873,126, and 10,928,404.

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The system and its associated cartridges and reagents may not be used for any other purpose and may not be transferred to third parties, resold, made, or modified for resale without prior written approval of LevitasBio, Inc.

This manual provides information about the use of the LeviCell EOS system. LevitasBio shall not be liable for errors contained herein, and shall not be liable for any direct, incidental or consequential damages in connection with or arising from the furnishing, performance, or use of this document or the device, including the loss of any samples processed in the device.

The information contained in this document is subject to change without notice.

SAFETY AND COMPLIANCE

Symbols

The following symbols are used in this user guide and on the LeviCell® EOS system component labels and provide key information to be aware of, or designate special information regarding the product.

	Attention or Caution symbol. The associated message contains safety-related information		Biohazard
	Note symbol. Pay attention to this important information		Tip symbol
	Operating temperature range		Operating humidity range
	Model number		Serial number
	Date of manufacture		Manufacturer of record (LevitasBio)
	Do not reuse		Do not discard in unsorted waste
	Magnet hazard symbol		Pacemaker hazard symbol
	Pinch hazard symbol		

Table 1. *Symbols in this guide*

Safety Warnings

Use the LeviCell EOS system only as directed by LevitasBio. Use in a manner not specified by LevitasBio, especially including the removal of any cover or portion of the enclosure while the system is powered on, may create a risk or hazard.

The LeviCell EOS module is a heavy load and requires a **2 person** lift (weight: 45 kg or 100 pounds).

Plug the LeviCell EOS module and Control PC only into properly grounded outlets using the main power supply cable provided.

Do not obstruct access to the mains power supply cable leading into the instrument or the end of the cord at its inlet. It should be accessible if power needs to be completely disconnected for servicing.

The LeviCell EOS system has several user accessible and removable parts on the inside of the instrument. The instrument requires no maintenance beyond the cleaning described in the section **Cleaning the LeviCell EOS**.

If there is any fault, turn the instrument off using the mains switch on the rear and disconnect the power cable. Contact LevitasBio Technical Support at +1-650-204-1185 or **support@levitasbio.com**.

In addition to contamination control features within the LeviCell EOS instrument, a primary safety feature to prevent contamination is that the cartridges are single-use only. Do not reuse cartridges. Re-use increases the risk of cross-contamination and exposure to potential biohazards associated with the sample and can void the warranty of the instrument. If a cartridge is reused or a leak is suspected, contact Technical Support at +1-650-204-1185 or **support@levitasbio.com**.

Dispose of used cartridges according to approved lab guidelines.

For indoor use only. The LeviCell EOS is not designed for outdoor use and is not rated for resistance to precipitation. See the **Specifications** section for details.

	**BIOSAFETY:** Biohazardous samples can be used in the LeviCell system or may be in use in the laboratory housing the system. All relevant precautions to work with those biohazardous samples must be followed, such as Universal Precautions. Always consider any instrument in a BSL-rated laboratory to require handling as if contaminated to that BSL level.
	**MAGNETIC FIELD:** LeviCell EOS cores contain strong magnets that can be harmful or interfere with the operation of pacemakers or other magnetically-sensitive devices. Wearers must not bring their devices within 150 mm (6 inches) of the exchangeable core during handling.

Table 2. *Safety Warnings*

Compliance Certifications

This instrument has been designed, tested and found to be in compliance with the following safety and electromagnetic standards:

	cTUVus mark to indicate that the product has been tested and certified to USA and Canadian standards
	CE Mark indicates that assembly is covered by a Declaration of Conformity, and conforms with the provisions of all applicable directives in the European Union.
IEC/EN 61010 (3rd Edition), through Amendment 1	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use
EN 61326-1:2020	Electrical Equipment for Measurement, Control and Laboratory Use. EMC Requirements
IEC 61000-3-2: 2018 +AMD1:2018	Electromagnetic compatibility (EMC) - Part 3-2: Limits - Limits for harmonic current emissions (equipment input current ≤ 16 A per phase)
IEC 61000-3-3: 2013 +AMD1:2017	Electromagnetic compatibility (EMC) - Part 3-3: Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤16 A per phase and not subject to conditional connection
IEC 61000-4-2:2008	Electromagnetic Compatibility (EMC) – Part 4-2: Testing and measurement techniques – Electrostatic discharge immunity test
IEC 61000-4-3:2006 +AMD1:2007+AMD2:2010	Electromagnetic Compatibility (EMC) – Part 4-3: Testing and measurement techniques – Radiated, radio-frequency, electromagnetic field immunity test
IEC 61000-4-4:2012	Electromagnetic compatibility (EMC). Testing and measurement techniques Electrical fast transient/burst immunity test
IEC 61000-4-5:2014 +AMD1:2017	Electromagnetic compatibility (EMC). Testing and measurement techniques Surge immunity test
IEC 61000-4-6:2013	Electromagnetic compatibility (EMC). Testing and measurement techniques Immunity to conducted disturbances, induced by radio-frequency fields
IEC 61000-4-8:2009	Electromagnetic compatibility (EMC) – Part 4-8: Testing and measurement techniques – Power frequency magnetic field immunity test
IEC 61000-4-11:2020	Electromagnetic compatibility (EMC). Testing and measurement techniques Voltage dips, short interruptions and voltage variations immunity tests
CISPR 11:2015	Industrial, scientific and medical equipment - Radio-frequency disturbance characteristics - Limits and methods of measurement
RoHS Directive (2011/65/EU)	Restriction of the use of certain hazardous substances in electrical and electronic equipment
UK REACH 2021	UK Registration, Evaluation, Authorization and Restriction of Chemicals
(EC) 1907/2006	Regulation (EC) No 1907/2006 Registration, Evaluation, Authorization and Restriction of Chemicals (REACH)
WEEE Directive (2012/19/EU)	Waste Electrical and Electronic Equipment

S1502 of the Dodd-Frank Act	Conflict Minerals Reporting Rule
(EU) 2017/821	Regulation (EU) 2017/821 of the European Parliament and of the Council of 17 May 2017 laying down supply chain due diligence obligations for Union importers of tin, tantalum and tungsten, their ores, and gold originating from conflict-affected and high-risk areas
FCC Part 15	This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Table 3. *Compliance Certifications*

ABOUT THE LEVICELL EOS SYSTEM

LeviCell EOS System Introduction

The LeviCell EOS system uses a revolutionary magnetic Levitation Technology™ method for enriching viable cells with concurrent removal of dead/dying cells and debris from complex cellular samples. The LeviCell EOS incorporates advanced optics for visualization of the entire separation channel, unique magnetic configurations to address broad applications and sample types, a pneumatic pump system to control the flow of the sample through the cartridge and separation of the sample into two fractions (see Figure 1), and sophisticated software to manage all connected LeviCell EOS modules. Up to 4 EOS modules can be connected to the Control PC to increase throughput to 16 samples.

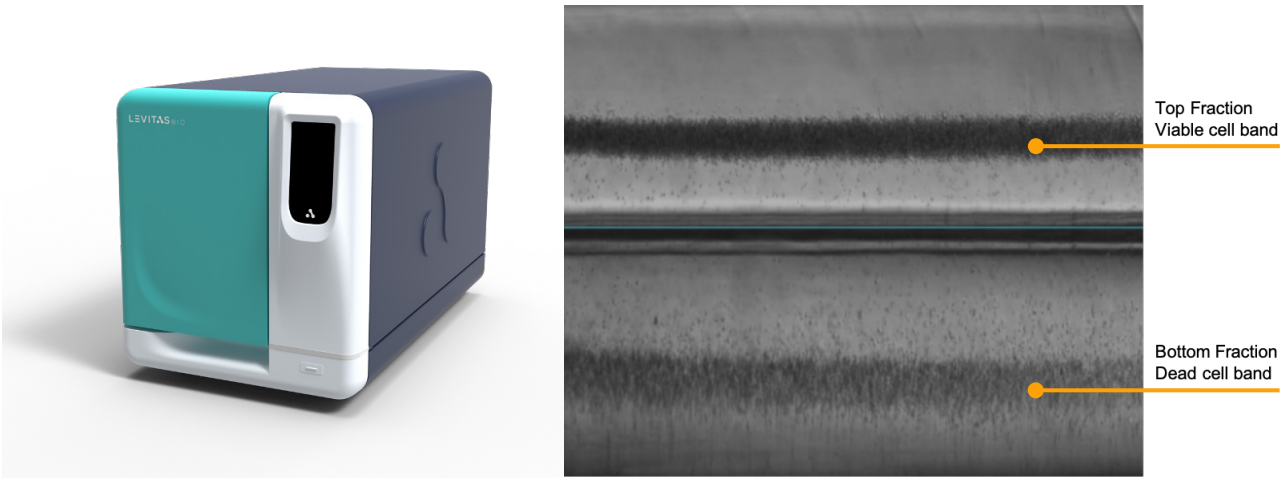


Figure 1. *LeviCell EOS and levitation image*

The LeviCell EOS uses parallel processing to address higher sample throughput needs compatible with many cellular workflows, including single-cell sequencing, cell-based assays and in vivo studies. The fast and simple workflow does not require dyes, antibodies, specific markers, or magnetic beads. The enriched cells are of superior quality and do not experience high pressure, high shear, or other perturbations that commonly lead to increased cellular stress responses, specific cell type activation, or even cell death. The system includes visualization tools to assist the user to select optimum settings for sample collection. The LeviCell platform can be used in an entirely label-free mode, or, optionally, cells can be selected based on specific antigens, using LeviSelect™ Kits. With this targeted cell enrichment method, magnetic nanospheres are used to remove specific populations of unwanted cells and streamline the overall workflow by simultaneously providing viable cell enrichment and targeted cell selection.

System Components

The LeviCell EOS System (PN 1000021) consists of

- EOS Module with EOS-4 core installed
- Control PC preloaded with LeviCell EOS Manager Software
- Monitor, mouse and keyboard
- Barcode scanner
- Transport cartridge

The LeviCell EOS TEC System (PN 1000020) has the EOS-4 TEC core pre-installed.

EOS Module – Front

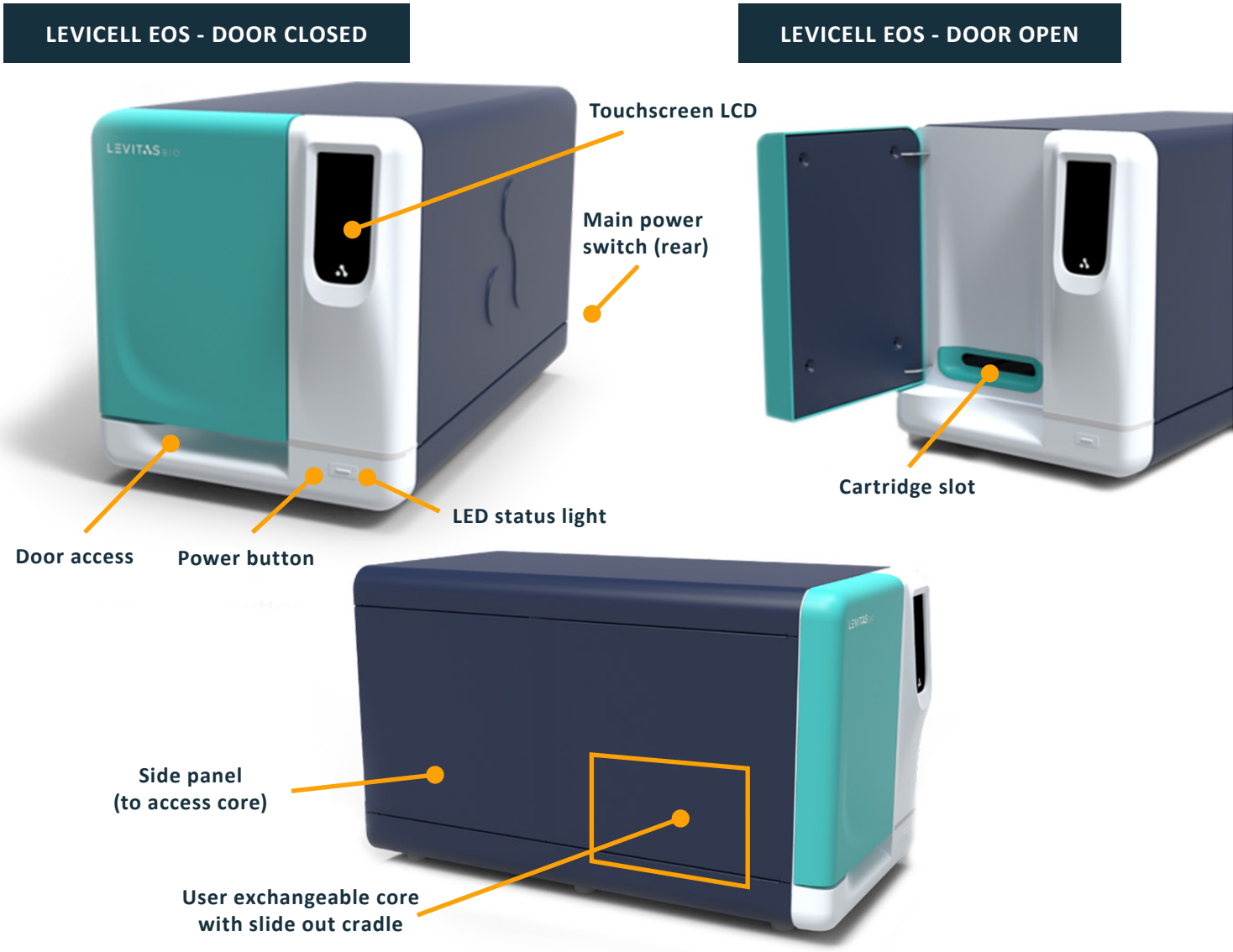


Figure 2. LeviCell EOS Module interaction points

EOS Module LED Status Light

There are several status colors that will display during a run. Each indicates a different mode for the instrument.

TEAL	Running state
PLUM	System starting or initializing
GOLD	System requires user attention
RED	Error state
WHITE	Idle state
BLUE	Post run process and scheduled dry

Table 4. *LED Light Colors*

EOS Module – Rear

There are two main connection points for the EOS Module. These are located at the rear of the instrument. The mains power switch and plug is located on the lower left of the rear panel. Each EOS Module is connected to the Control PC through an Ethernet cable and port.

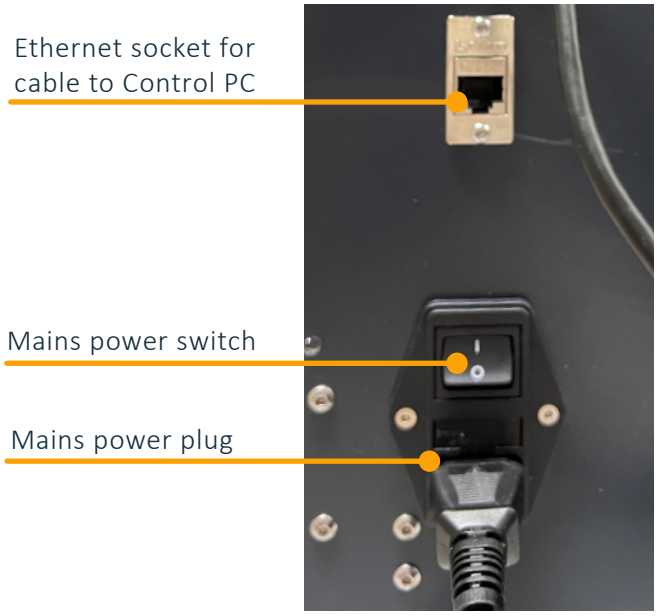


Figure 3. *EOS Module power and connection interface*

Computer - Control PC & Peripherals

Operating System:	Windows 10 Professional
Processor:	12-core, 12th-Generation i7
RAM:	32GB installed memory
Data storage:	1 TB ***NOTE**: typical data file size: 6GB (Brightfield only), 18GB (with fluorescence), Medium Cell Protocol*
Peripherals:	Wireless keyboard, wireless mouse, barcode scanner

***Table 5.** Computer specifications*

Cartridges

The LeviCell EOS cartridges are designed for higher throughput sample processing. Up to 4 samples can be run simultaneously.

ATTENTION: The LeviCell EOS cartridges are intended for a single use only. Do not reuse. Doing so creates the potential for sample cross-contamination and exposure to biosafety hazards from the sample, and contamination of the EOS module.

NOTE: Hold the cartridge by the grip only. Do not handle the cartridges by the separation channels as these are fragile and designed to provide the highest visualization resolution into the separation channel. Fingerprints or scratches to any of the separation channels surfaces can cause issues with optical imaging and subsequent sample characterization.

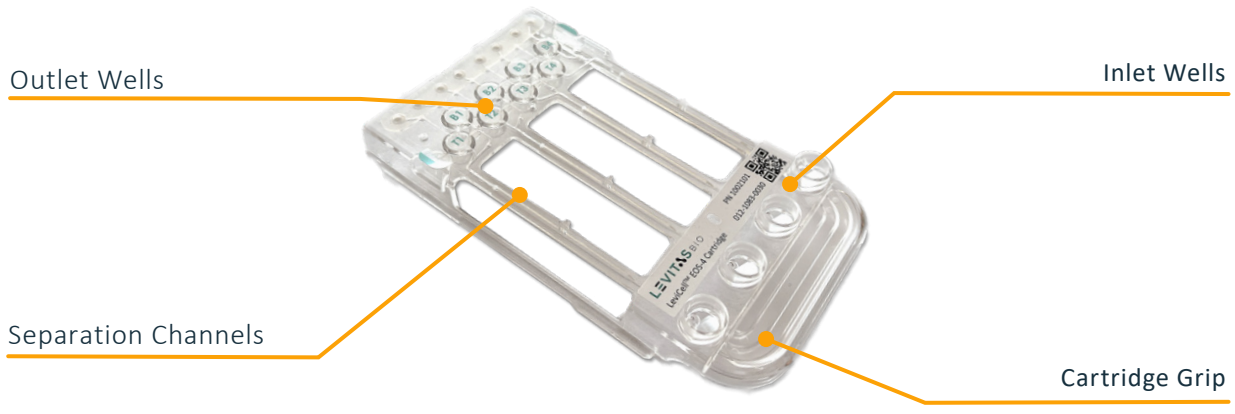


***Figure 4.** LeviCell EOS-4 Cartridge*

Cartridge Box

The LeviCell EOS cartridges are supplied in 10 pack kits. The box contains 2 snap-locking cartons holding 5 cartridges each. The cartridges are packaged for easy retrieval, with the grip facing up when the lid is removed. Each carton is heat shrink sealed to ensure cartridges are secured during shipping.

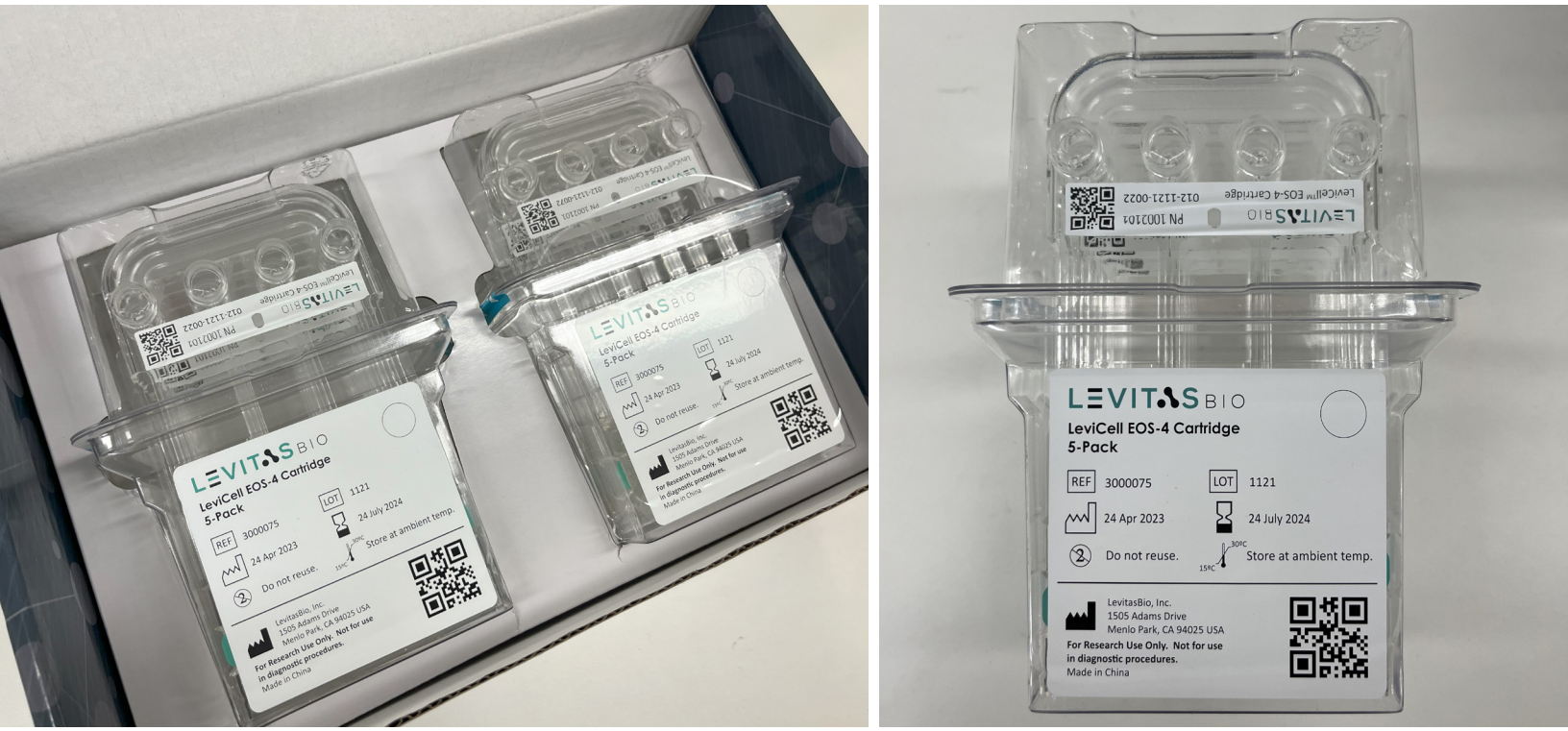


Figure 5. Cartridge packaging

NOTE: After retrieving a cartridge, always replace and close the lid securely to keep the unused cartridges clear from dust or fibers.

LeviCell EOS cartridges are available in sterile and non sterile formats. Sterile cartridges are gamma irradiated after packaging. The sterile covers protect the sample pathway in the cartridge during storage, and minimize exposure or contamination to the sample when it is loaded and during the run.

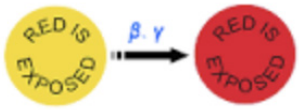


Figure 6. Gamma irradiation indicator sticker

On the LeviCell EOS-4 Sterile cartridge 10 pack box and on the inner 5 pack carton labels, there is a red dot sticker after gamma irradiation which confirms sterility. If gamma irradiation was not performed successfully, this dot remains yellow. The non-sterile cartridge packaging does not have a dot sticker.

LeviCell EOS Transport Cartridge

LeviCell EOS transport cartridges are non-functional cartridges identified through their unique barcode prefix (401-xxxx-xxxx). A transport cartridge is preloaded into the LeviCell EOS system before shipment and must be removed by the grip when instructed by the software and before system use. After removing it from the module, place the cartridge in its storage case and store it near the module for future use.

A dry test run with this cartridge can also be used for training or troubleshooting. If any issues arise, please contact LevitasBio Technical Support, for guidance on how to use the transport cartridge for troubleshooting.

Figure 7.
LeviCell EOS-4 Cartridge shown with sterile covers over the inlet wells

Materials

LevitasBio Equipment and Consumables

- LeviCell EOS TEC System (PN 1000020) or LeviCell EOS System (PN 1000021)
- LeviCell EOS-4 Cartridge, non-sterile (PN 1002101) or sterile (PN 1002102)
- Levitation Agent, 10 reactions (PN 1003001) or 40 reactions (PN 1003002)
- LeviCell EOS Installation and Calibration Kit (PN 1003004)

User-Sourced Equipment and Consumables

- Benchtop Vortexer
- Centrifuge capable of spinning at 300 RCF
- Calibrated pipettes and pipette tips (filtered)
  - 2-20 µL, 20-200 µL, 200-1000 µL
- Low-binding microcentrifuge tubes (1.5 mL, 2 mL, and 5 mL)
- 15 mL and 50 mL Falcon® tubes
- Serological pipettes: 2 mL, 5 mL, 10 mL, 25 mL
- 0.22 µm syringe filters, to filter cell media/buffers
- 5.0 mL or larger disposable syringes, to filter cell media/buffers

User-Supplied Reagents

- RPMI + 10% FBS
- 1X PBS/0.5% BSA buffer
- AO/PI Viability Stain (Nexcelom Bioscience PN CS2-0106)
- Propidium Iodide (PI) Dead Cell Stain (Thermo Fisher, PN R37108)
- Calcein AM, Cell Viability Dye (Thermo Fisher, PN C3099)

Optional Equipment

- Nexcelom Automated Cell Counter (Nexcelom Bioscience)
- Manual hemocytometer and microscope

Specifications

Specification	Value
Number of sample inputs	4
Number of output fractions	8
Levitation magnets	Rare earth permanent magnets
Separation flow rate	300 µL/min
Imaging modes	Brightfield (transmitted illumination) 530 nm Two fluorescence channels: Excitation 470 nm, Emission 501-544 nm (e.g., Calcein-AM, Acridine Orange) Excitation 567 nm, Emission 601-666 nm (e.g., PI, Alexa Fluor 594)
Illumination type	LED
Imaging resolution	Approximately 2 microns
Operational	
Input voltage	100 - 240 VAC nominal, universal 50 - 60 Hz (90 - 264 VAC maximum operating range) Standard wall receptacle (over-voltage category II)
Input current	4 A
Main enclosure dimensions	440 W x 630 D x 460 H mm (17.3” W x 24.8” D x 18.1” H)
Instrument weight	45.4 kg (100 lbs.)
Control PC Operating system	Windows 10 Professional, 64 bit
Ingress protection rating	Not rated (no protection claimed)

Environmental	
Operating ambient temperature	19°C – 25°C
Operating relative humidity	20% RH – 80% RH ambient, non-condensing
Pollution degree of the intended environment	Pollution Degree 2 (normal indoor laboratory environment)
Altitude	Sea level to 2,000 m (6,562 feet)
For indoor use only	Not designed for outdoor use Not designed for use in wet locations
Shipping environment	5°C to 50°C, RH 5% - 99%, non-condensing

Table 6. *Specifications table*

Bench space

- Minimum: 660 mm (26”) deep, 1200 mm (4 feet) wide.
- Recommended: 1520 mm (5 feet wide) bench with 508 mm (20”) vertical space available above the bench.
- A minimum of 80 mm (3”) clearance is required behind the instrument for proper venting and allowing access to the mains power switch. Ensure enough clearance on the sides to reach the mains power switch.
- For TEC systems, the air intake is on the right side of the instrument, and the exhaust on the left side. Warm air may exit the exhaust when running cold protocols, so do not place two TEC modules any closer together than 600 mm (24”).
- Avoid placing the LeviCell system on the same bench as vibration sources such as a centrifuge. Vibration can cause imaging issues during a run.

Power requirements

- All standard mains input voltages are accepted (100 - 240 VAC, 50-60 Hz)
- Power drawn is low, so no special circuits are required
- Recommend access to 2 standard power outlets
- Grounded outlets are required (3-pin e.g. NEMA 5-15 or CEE 7/5 style)
- Fuse replacement: 250V 8AH, 5x20mm, qty 2

User Exchangeable Cores

The LeviCell EOS Module includes a removable component called the EOS Core Module, which receives the cartridge and provides the environment for levitation. The core module consists of the top and bottom magnets for each lane, clamping mechanism, pneumatic connection points and can have the temperature control hardware. By removing the instrument's left side panel, the user can have access to swap core modules.

Refer to section **Exchanging Core Modules** for step by step instructions.

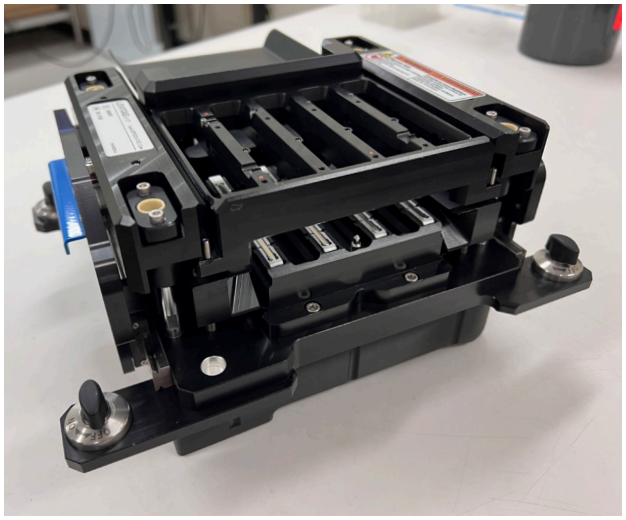


Figure 8.
Core module on the lab bench

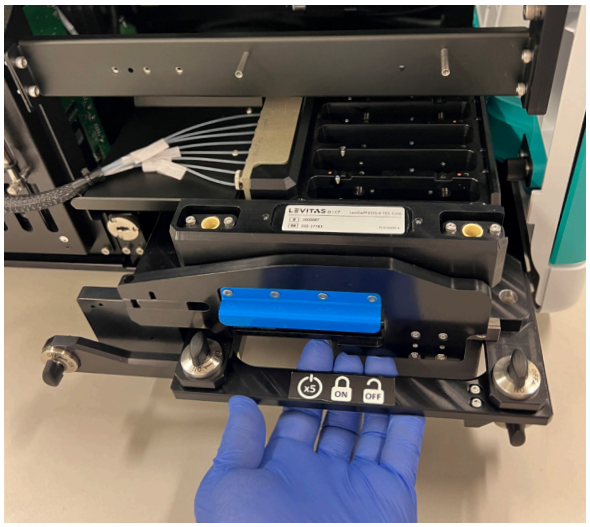


Figure 9.
Reinserting the core in its cradle back into the instrument

Temperature Control Core Module

If a LeviCell EOS system has a temperature control core ("EOS TEC Core") installed, the ability to manage run temperatures is dependent on the environmental (ambient) conditions.

Temperature control ranges are listed for when the sample is fully loaded in the separation channel of the cartridge.

Run Temperature Option	Icon Displayed	Average Sample Temperature Range
Cold		7°C-10°C
Cool		12°C-14°C
CRT (Controlled Room Temperature)		Variable based on environment

Table 7. *Different run temperatures available and average sample temperature range*

NOTE: At time of run setup, if the environmental conditions are outside of operating temperatures of the system, the run temperature options may be deactivated.

At the beginning of a temperature controlled run, the system will start thermal regulation. A cooling period may be required to bring the Core and/or the Cartridge to the selected run temperature.

The software will display a message when this occurs. A status bar and countdown will be displayed. If the core is already cooled, this screen will be skipped. Once the system reaches the set temperature, the user will be instructed to proceed with the run.

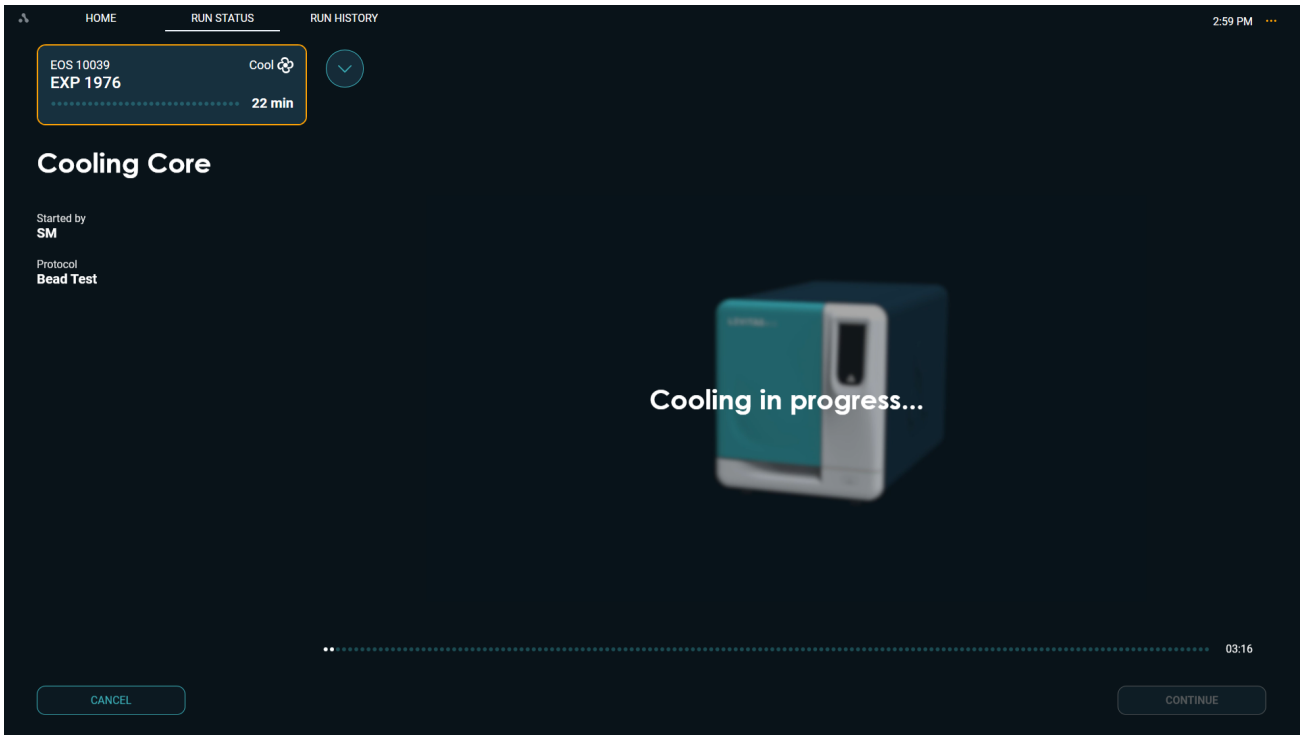


Figure 10. *Cooling core when the post-run warm air state is interupted or if the environment is too warm.*

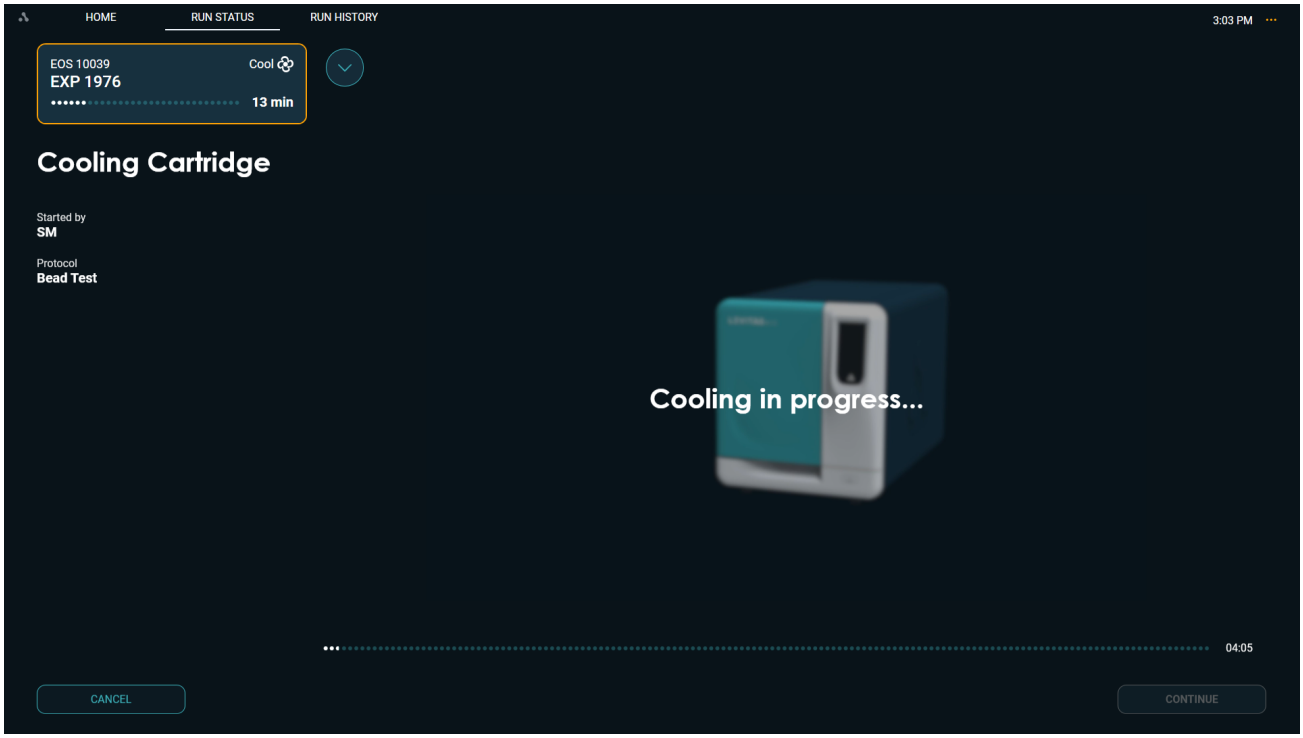


Figure 11. *Cooling cartridge prior to dispensing samples*

If the environmental conditions prohibit cooling to selected run temperature, the software will either disable the run temperature options or will alert the user at the Cooling Cartridge step. At this step, the run has already begun therefore the user must confirm continuing even though selected temperature was not achieved. If this is confirmed, a message will be appended to the run summary.

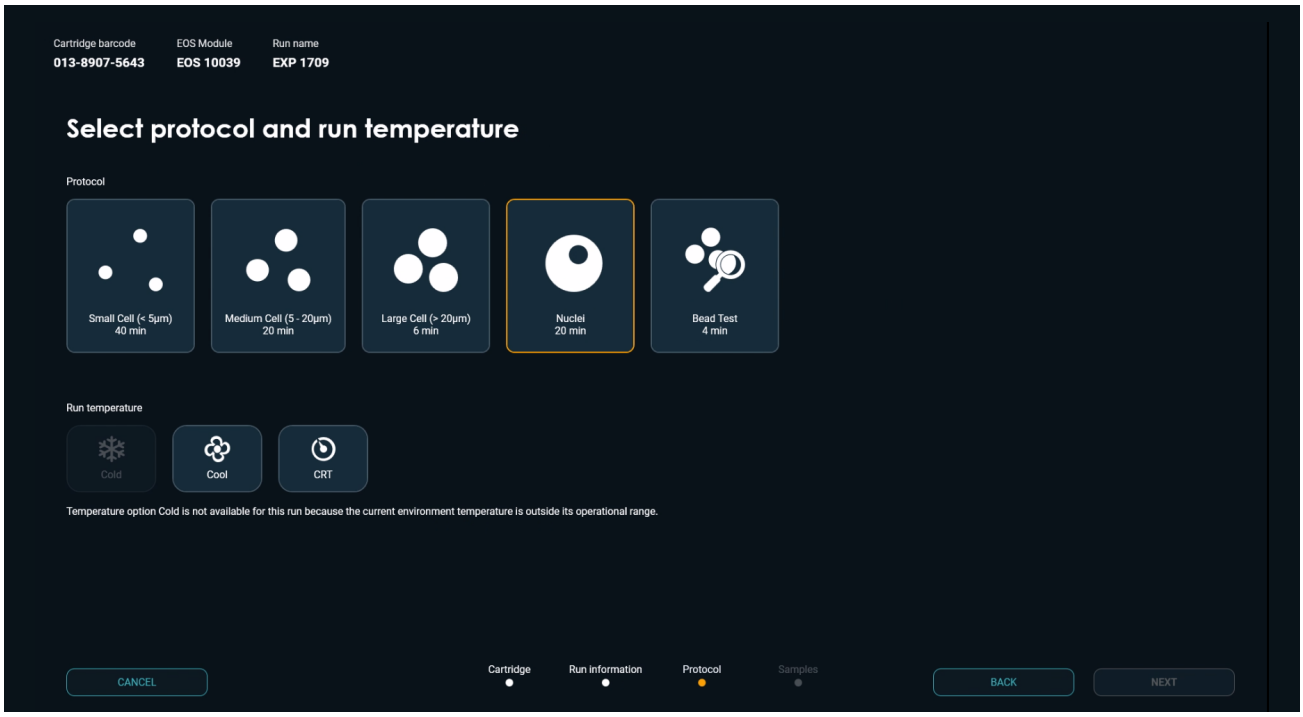


Figure 12. *Disabled run temperature options*

Figure 13. *Confirmation message to proceed with run*

Post-Run Process

If a run is conducted in either cold or cool temperatures, the system will end thermal regulation and start the post-run process in the background called Keeping Dry. **This can be interrupted at any time for a new run to be started**. The system will show the post-run process step on the run setup screen. However, the module is available for selection.

The post-run Keeping Dry process will maintain a drying state for the next 45 minutes (ambient air will be blown) to avoid condensation build-up. If this process is interrupted, the system will restart the Keeping Dry step from the beginning when the system is idle. The system will track whether the complete Keeping Dry process has been performed. In addition to the post-run Keeping Dry process, a scheduled task called Heat Dry will be queued to run at 8:00 PM or before shutting down the system. If the post run Keeping Dry step has not been run, the system will know to skip this step and directly run the scheduled task Heat Dry.

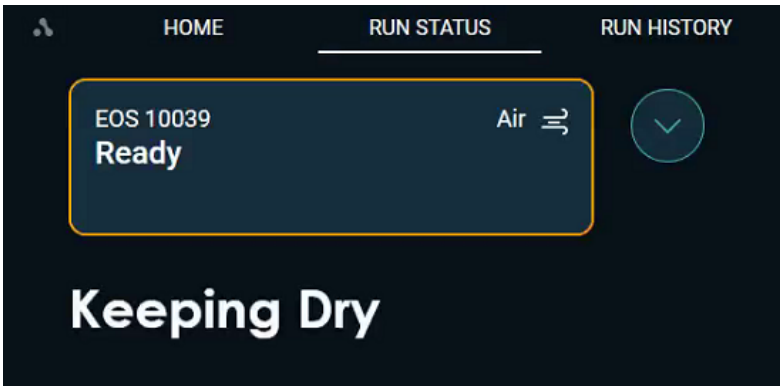


Figure 14.
Keeping Dry post-run process

Scheduled Task

If a cold or cool run occurs, the system will create a scheduled task to ensure the hardware is fully dry, avoiding any chance of moisture buildup or contaminating growth. The scheduled task will occur at 8:00 PM to 11:00 PM or will start if the soft power button is pressed to shut down the system. A run cannot interrupt the scheduled task.

The scheduled task Heat Dry will warm the core for 10 mins followed by blowing 20^{0}C air for 10 mins and then 10 mins of ambient air. After the scheduled task is finished, normal usage can resume or subsequent shutdown sequence will occur.

Figure 15.
The instrument LCD will show that a scheduled task will occur.

NAVIGATING LEVICELL EOS MANAGER SOFTWARE

Software Navigation

The LeviCell EOS system comes with the LeviCell EOS Manager software which has been pre-installed onto the LeviCell EOS Control PC. This software operates on a Windows 10 operating system and has been verified to work with Microsoft issued software updates.

LeviCell EOS Manager

The LeviCell EOS Manager software icon is located on the windows desktop screen.

Color Code Guide

EOS Manager software is easy to navigate due to its intuitive design. Active items and next steps are color-coded to guide the user on the action needed.

Selection	
Gold outline	selected/ active item
White outline	active field (user entry)
Blue outline	unselected option

Buttons	
Teal fill	default selection for next step
Teal outline	available button
Gray outline (dimmed)	not available

Table 8. *Color Code Guide*

Software Messages

There are five types of EOS Manager software messages that can appear during use. These messages will provide information for the useful information for the user interaction with the software or the LeviCell EOS system.

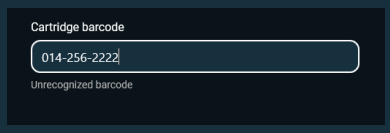
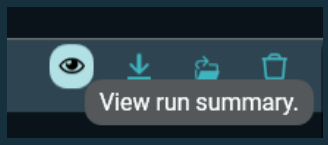
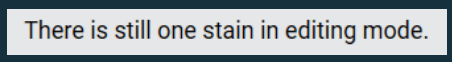
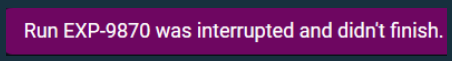
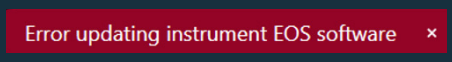
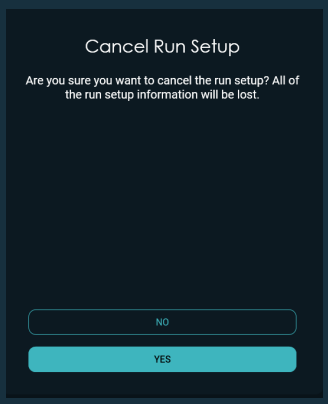
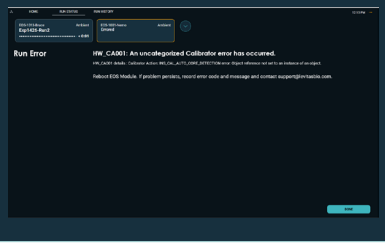
Type	Description	Example
User entry error	Information will appear below the user entry field indicating guidance to resolve issue	
ToolTip	Information provided when hovering over a button or option	
Notification	**White, Information.** Temporary will automatically clear	
	Purple, Warning. User must clear	
	Red, Error. User must clear	
Pop-Up Window	Further information must be provided or a confirmation must occur to proceed with the next action	
Error Display	Message will appear in the main window	

Table 9. Software message types

Home Screen

Upon software launch, a home screen will appear with the following options:

- Start a new run
- Recent Run History
- System Menu

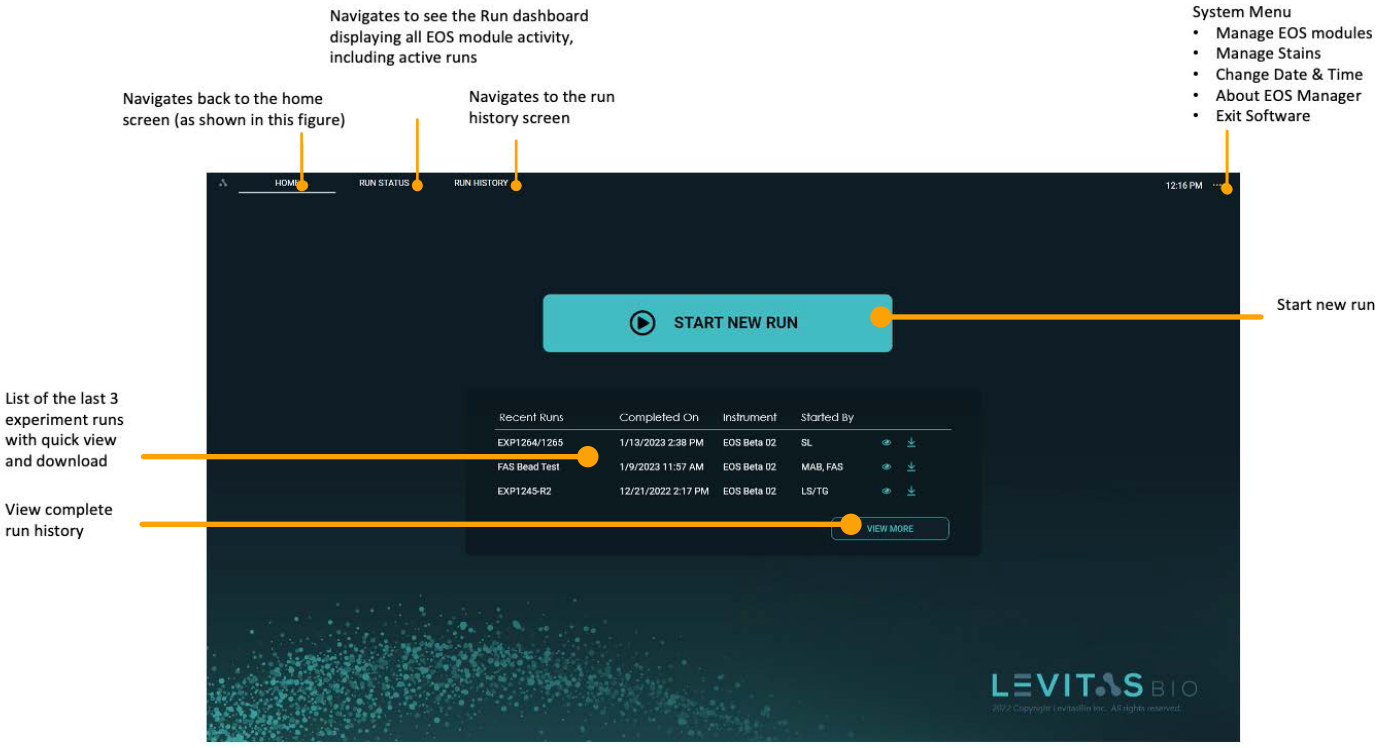


Figure 16. *Home Screen*

The **Home** screen allows easy experimental setup and information retrieval, including the status and availability of connected EOS modules. A user can readily identify available EOS module(s), current activity status, and temperature presets by going to the Run Status screen via the navigation bar at the top of the screen.

Click **Start New Run** to begin a new experiment. The EOS Manager software guides the user through each step of the setup process. For more information on this setup and the required steps before running the sample, go to section "**Getting Started**."

Users can also access their most recent run reports and download run summary files directly from the home screen. The last three runs are listed. View each run's complete summary by clicking the View Run Summary icon. Download a PDF of the Run Summary Report and final montage images to a designated folder by clicking the Download icon.

Run Setup Screens

The run setup screens provide a step by step guided setup for an experiment.

- Scanning a cartridge
- Specifying run information and which EOS module to use
- Selecting a protocol and run temperature
- Selecting and specifying samples to run

Scanning a Cartridge

Each cartridge has a unique barcode that can be a unique identifier for the experiment run. A barcode scanner is included in the system and can be used to scan the cartridge and/or other consumables with a QR or 1D barcode.

Figure 17. *Scanning cartridge barcode information*

Figure 18. *Cartridge label with barcode*

Specify Run Information and EOS Module

User can enter information specific to the experiment including Run name, Started by (user), and addition of notes to the run that will be appended to the run summary.

All available EOS Modules will be displayed. When two or more EOS Module are connected and have the same core module installed, the module that is idle will be selected by default.

If different types of core modules (e.g. TEC and standard) are installed in the EOS modules, there will be no defaulted selection.

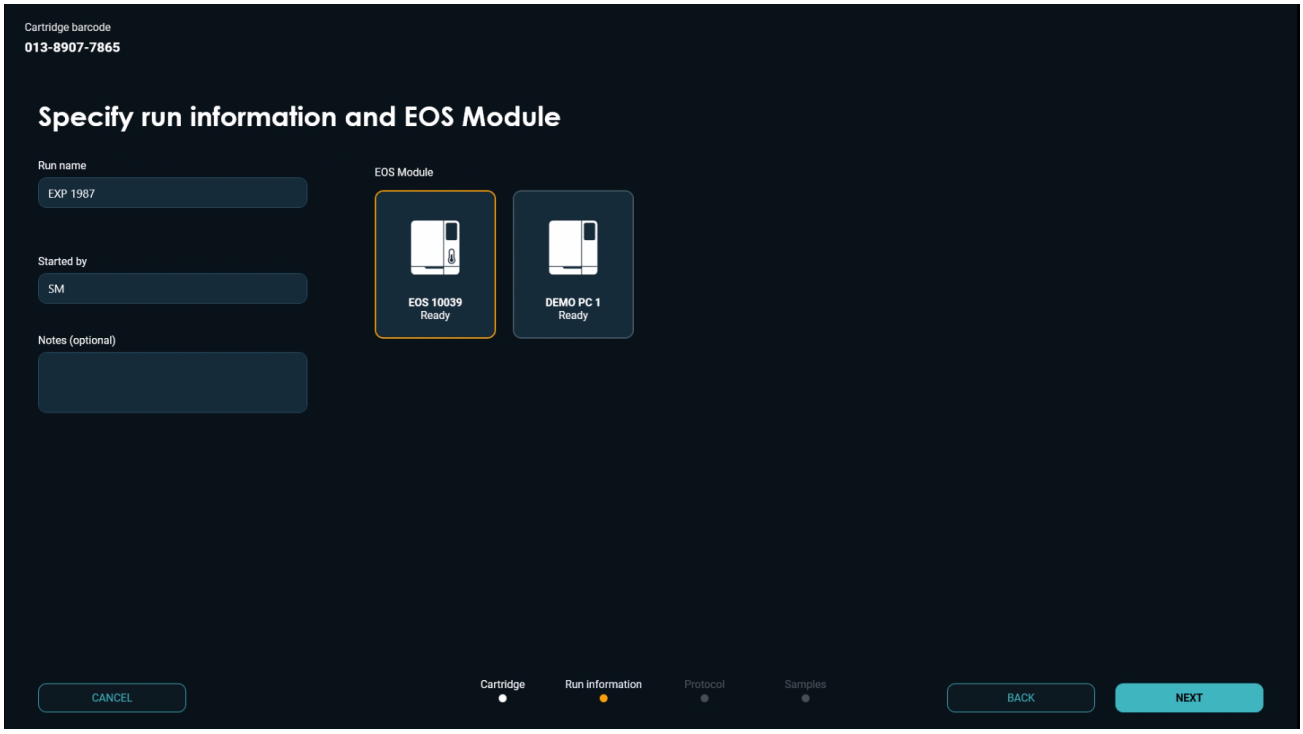


Figure 19. *Specify run information and EOS Module*

Select Protocol and Run Temperature

Different protocol options are available for an experimental run. Each protocol is optimized to suit the different types of samples. Since levitation time can vary based on cell size, there are 3 different cell protocols – small, medium and large cell, each with differing levitation durations. The Nuclei protocol is optimized for use with the LeviPrep™ Nuclei Kit II (PN 1005055). The bead test can be used with the LeviCell EOS Installation and Calibration Kit (PN 1003004) for calibration, training or demonstration purposes.

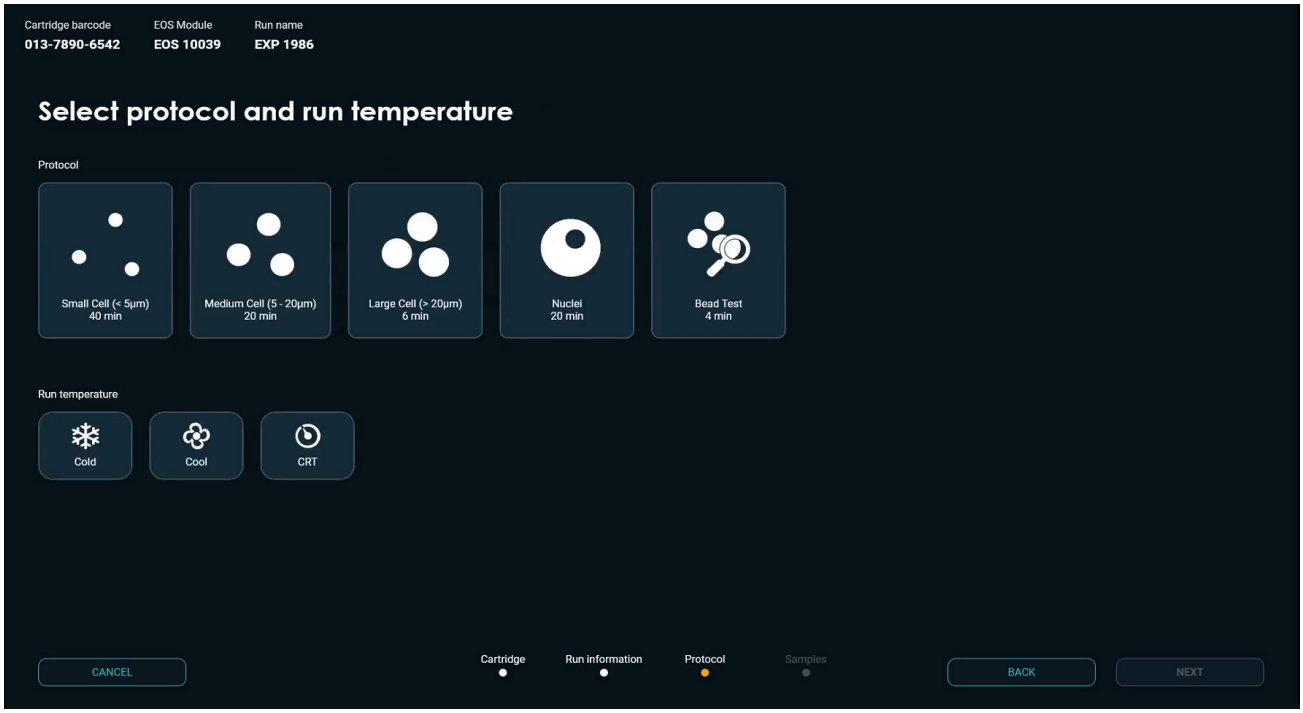


Figure 20. *Select protocol and run temperature*

Protocol	Size Range	Levitation Time
Small Cell	<5 µm	40 min
Medium Cell	5-20 µm	20 min
Large Cell	>20 µm	6 min
Nuclei	n/a	20 min
Bead Test	n/a	4 min

Table 10. *Protocols with associated cell size range and levitation time*

For LeviCell EOS systems with an installed TEC Core module, only the Bead Test and Nuclei Protocol can be run at different temperatures. The Small, Medium, and Large Cell Protocols can only be run at CRT temperatures.

Temperature control ranges are listed for the separation channel of the cartridge when the sample is loaded.

Run Temperature Option	Icon Displayed	Average Sample Temperature Range
Ambient *		Variable based on environment
Cold		7°C-10°C
Cool		12°C-14°C
CRT (Controlled Room Temperature)		Variable based on environment

* *Ambient temperature is only available for Non-TEC cores*

Table 11. *Different run temperatures available and average sample temperature range*

Run Status Screen

The run status screen displays the status of all active EOS modules and the real-time image acquisition of each channel. The system will default to the active EOS module. If more than one EOS module is active, the default is the last selected/viewed module.

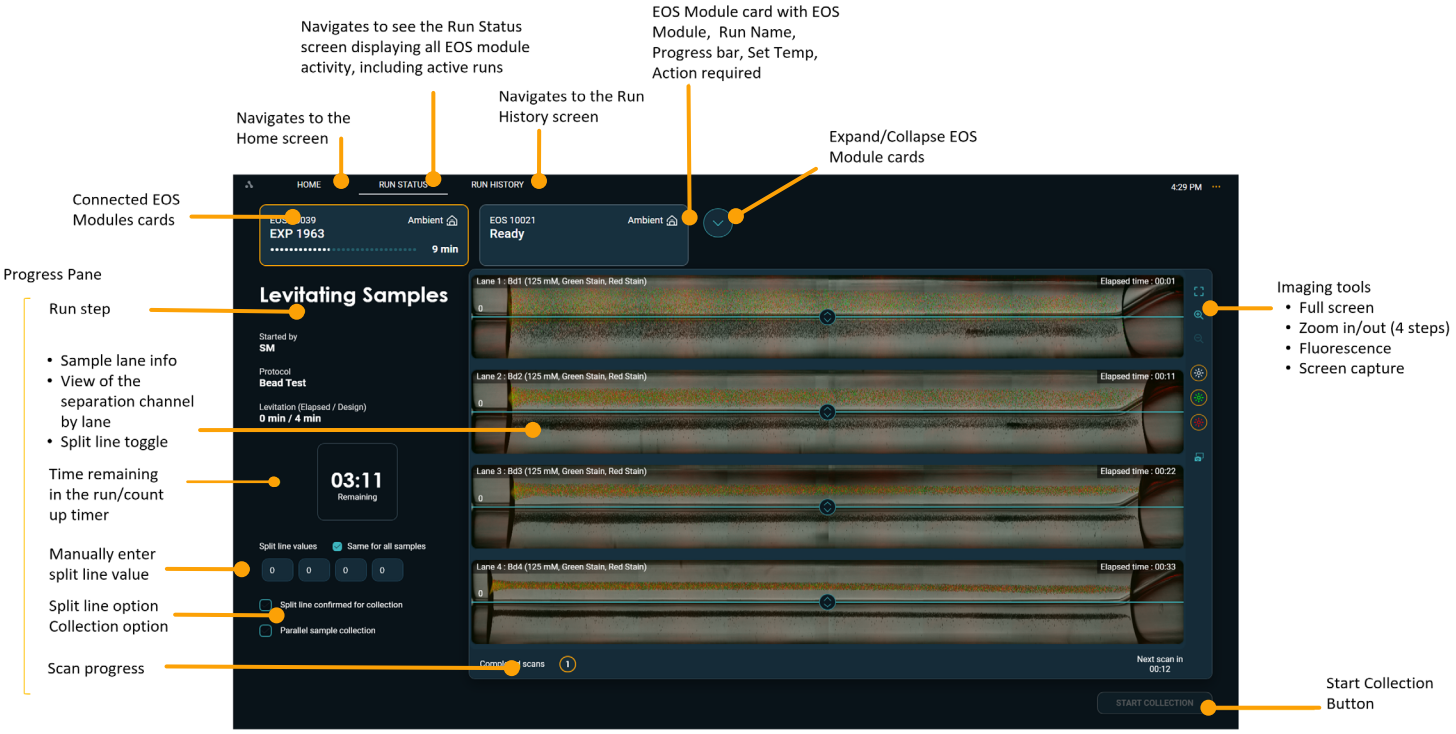


Figure 21. *Run Status Screen*

EOS Module Cards

Up to 4 EOS modules can be connected to one LeviCell EOS Control PC. Each connected EOS Module has its information card at the top of the screen. The EOS Module name, set point temperature, run status (idle, running), and experiment name are always visible by default.

To expand the information cards, click the down arrow to the right of the EOS Module cards.

Once expanded, the EOS modules that are active will display all selected and entered information for the experiment run. This includes:

- EOS Module name
- Run temperature
- Run name
- Cartridge barcode
- Started by
- Protocol
- Current protocol operation
- Run progress bar
- Remaining run time
- User intervention icon (minimized view only)

Figure 22.
EOS module card user intervention icon

To minimize and return to the default run status view, click on the same arrow to the right of the cards.

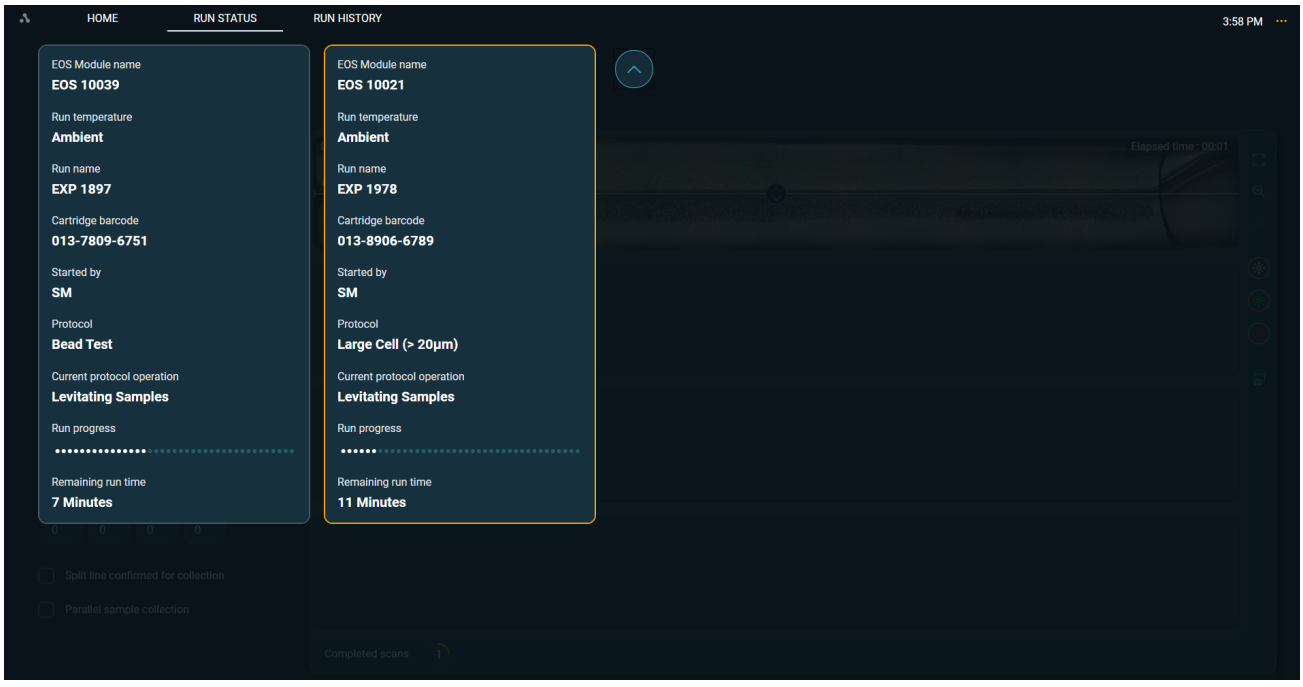


Figure 23. *Expanded EOS Module information cards*

Progress Pane

The progress pane, located on the left panel on the Run Status view, displays important information about the run such as run stage, guided prompts for user interaction, split line value, and time remaining for the run.

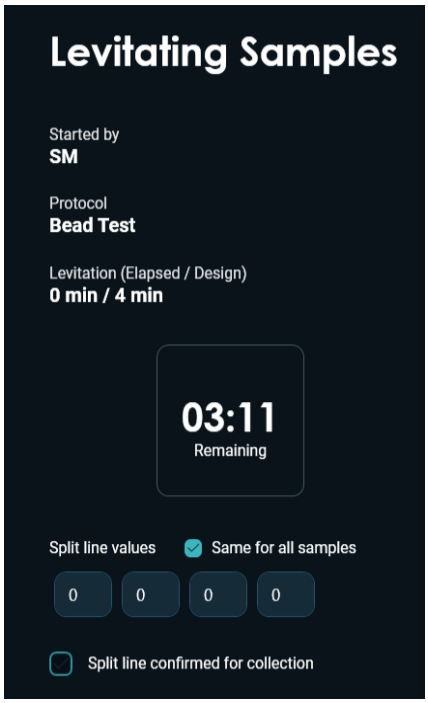


Figure 24. *Progress Pane*

The split line value determines the ratio of sample volume collected to the top relative to the bottom during the sample collection step and can be set in integer steps from -15 to 15. Adjusting the split line influences purity and yield of the collected top fraction. A higher value may help to increase purity by excluding cells and debris below the line, while a lower value may increase yield by including everything above the line. This value can be changed throughout the levitation for visualization but is locked once sample collection is initiated.

The default split line value is 0. A split value of 0 is a typical starting point for new samples. The split line value will be reflected in the split value field on the progress panel. Split line values can range from -15 to 15.

There are two ways to change the split line.

1. Enter a value in the split line value field

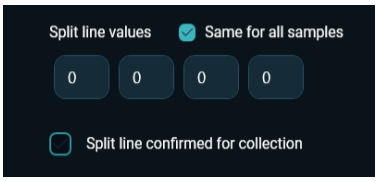


Figure 25. *Split line values and split line confirm*

2. Drag the teal colored bar on one of the images using the split line toggle

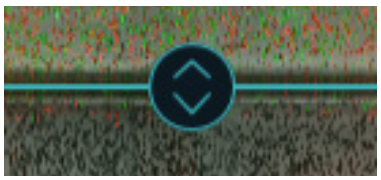


Figure 26. *Split line toggle*

A single split line value can be used for all samples by checking the box “same for all samples”. All samples will be collected with the same split line value.

Alternatively, split line values can be set for each sample. These values can be entered into the split line value box. If this is chosen each sample will be collected sequentially at the end of the run.

Samples can be collected all at the same (parallel collection) or one at a time. If parallel collection is selected, sample collection images will only be collected for the first sample run in the cartridge, and the same split line value must be used for all samples. If parallel collection is not selected, sample collection images will be collected for all samples and different split line values may be used for each lane.

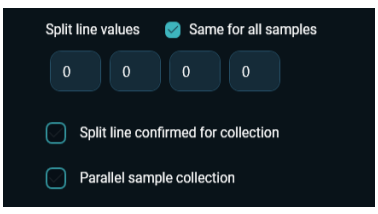


Figure 27. *Split line options*

The progress pane will also display the experiment run time. This time reflects the set levitation time associated with the run protocol. The timer will count down until the set levitation time is reached. Once completed, the timer will show READY and begin to count up until the User clicks the **Start Collection** button.

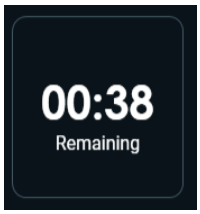


Figure 28.
Levitation time remaining countdown

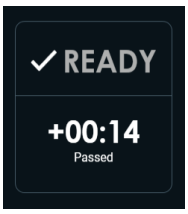


Figure 29.
Levitation is completed, Ready and count up timer for levitation time passed

Up to four samples or lanes (e.g one sample per lane) can be run simultaneously. Sample information entered during setup will be displayed above each sample lane. The elapsed time will be displayed on the lane (right side) showing the timestamp when each image appears. This will be different per lane.

- Lane Number
- Sample Name
- Levitation Agent Concentration
- Stains selected (if any)
- Run elapsed time

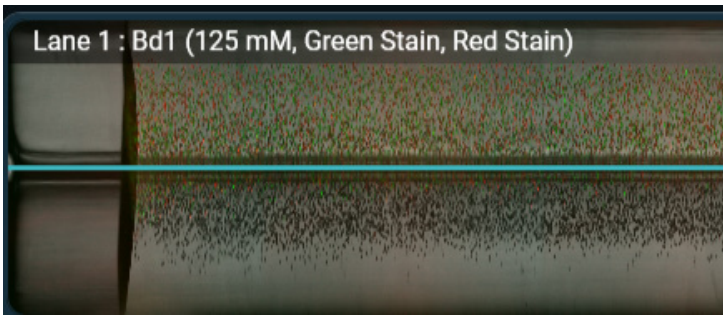


Figure 30. *Lane information*

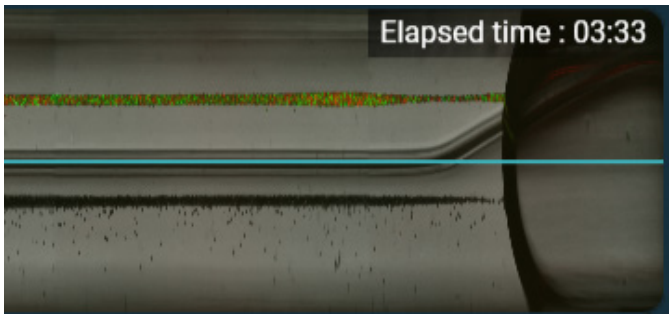


Figure 31. *Elapsed time for each lane*

Montage Images and Scan Progress

During the run, images are collected per sample for the entire separation channel. After each completed scan, there will be a pause. On the lower right side of the screen the countdown timer time till the next scan begins will appear.

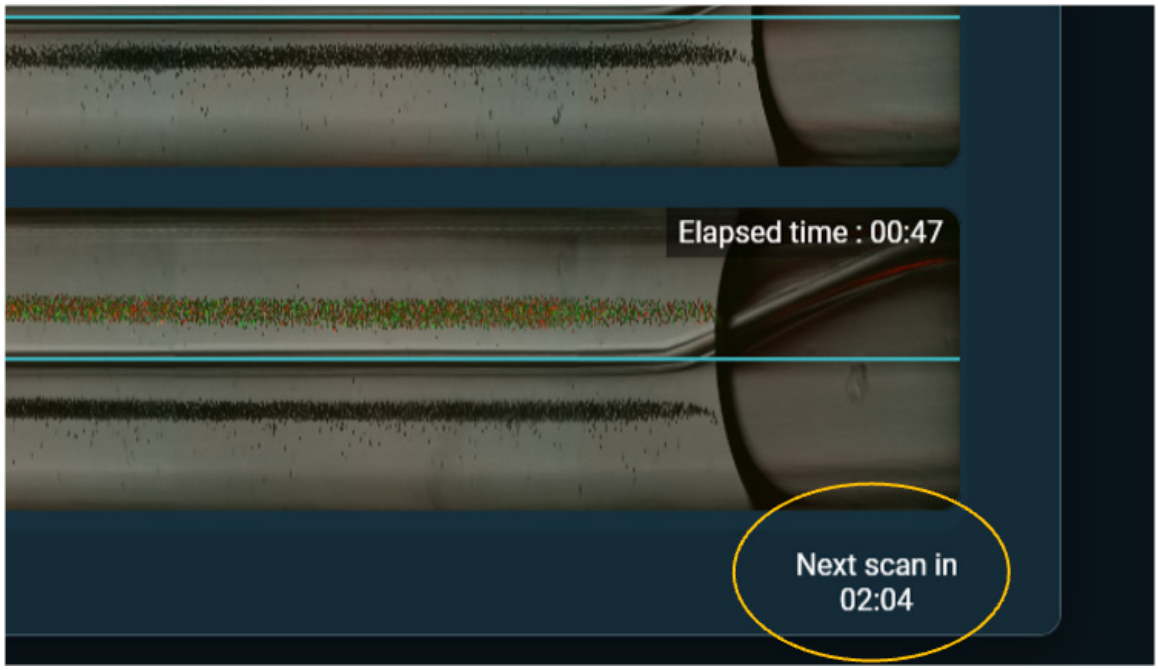


Figure 32. *Next scan countdown timer*

Once a complete sample has been imaged it will appear on the corresponding lane. This will continue for all the samples. When all samples are scanned this will be designated as one complete scan as indicated by the bar at the bottom of the run status view.

Figure 33. *7th scan in progress, 2 lanes completed*

Each completed scan will be marked by a number. Hovering over the completed scan number will display the elapse timestamp based on the first selected lane. The currently displayed scan is indicated by the gold selection outline. Each completed scan takes approximately 60 seconds for brightfield only and 90 seconds with green and red fluorescence. After 4 hours total run time passed, image collection will stop saving.

It is possible to go back and review a previously completed scan. This can be done by clicking on a previous scan number. Once selected, the number will be outlined in gold and the run view will go to that completed scan. The image that is displayed will reflect the completed scan for all four samples. To return back to the current scan, click on the last available number outlined in white. The current scan will show an in progress highlighted circle which corresponds to which lane is currently being imaged.

Figure 34. *Reviewing previously completed scan 1 while 7th scan is in progress*

NOTE: Reviewing previously completed scans will not interrupt or prevent additional images from being collected. The system will continue scanning and running until the protocol levitation time is complete.

Image Tools

The Image tools on the right of the run status screen include tools to view in full screen, zoom in/out, brightfield or fluorescent illumination when stains are used, and take a snapshot of the screen.

Brightfield, green fluorescence, and red fluorescent illumination is indicated by the yellow circle outline. If the illumination is off there will be no outline ring.

The maximize view tool enlarges the view of the lanes and hides the progress pane. Deselect the maximize view to return to the normal run status view.

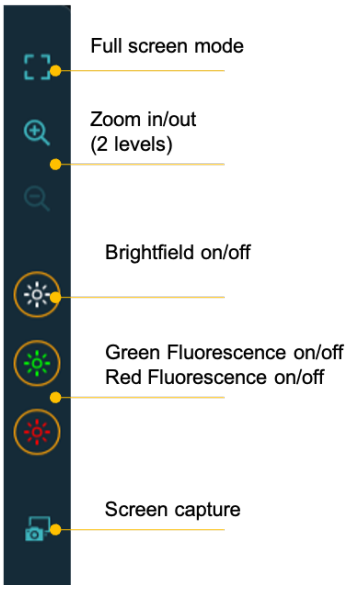


Figure 35.
Imaging tools available during a run

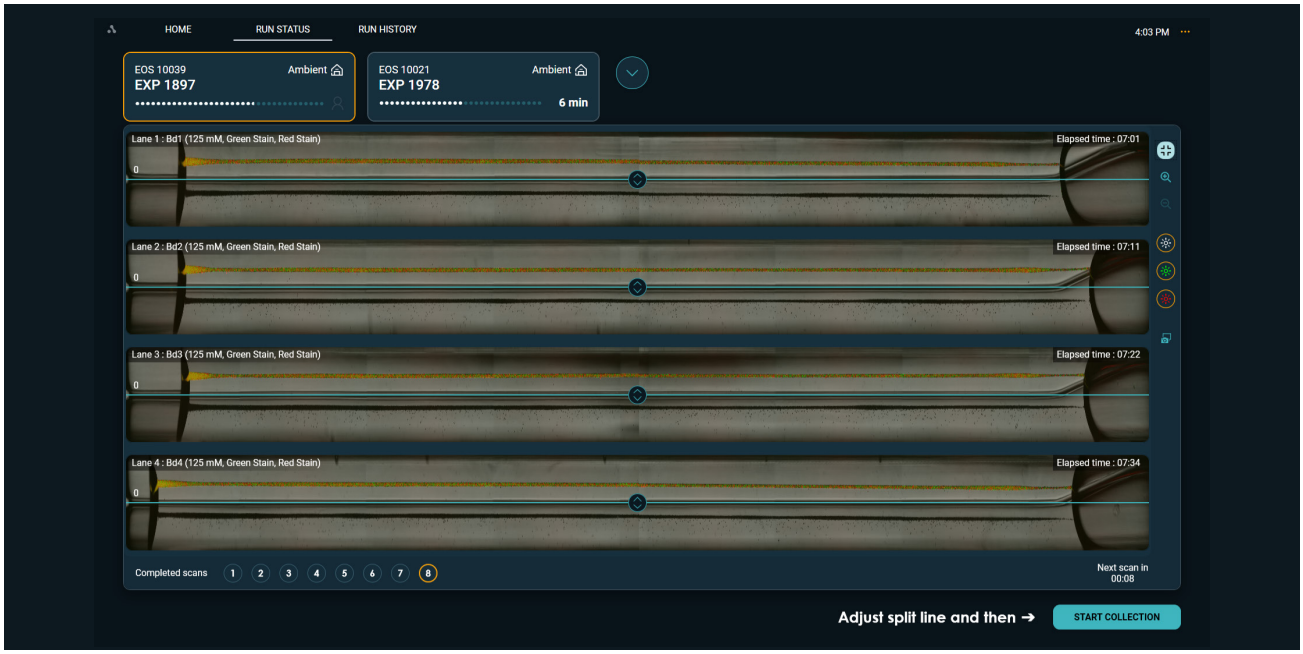


Figure 36. Run Status full screen view

The Zoom In tool enables a closer view of each sample. This tool can be used during scanning or on any previously completed scan. The first step zoom view displays the true lane ratio size where as maximum zoom view displays a single lane in the full screen height view. On the upper right, a gold box on a lane map shows what portion of the lane and which lane the view is displaying. Use the scroll bars to move across a single lane or between lanes.

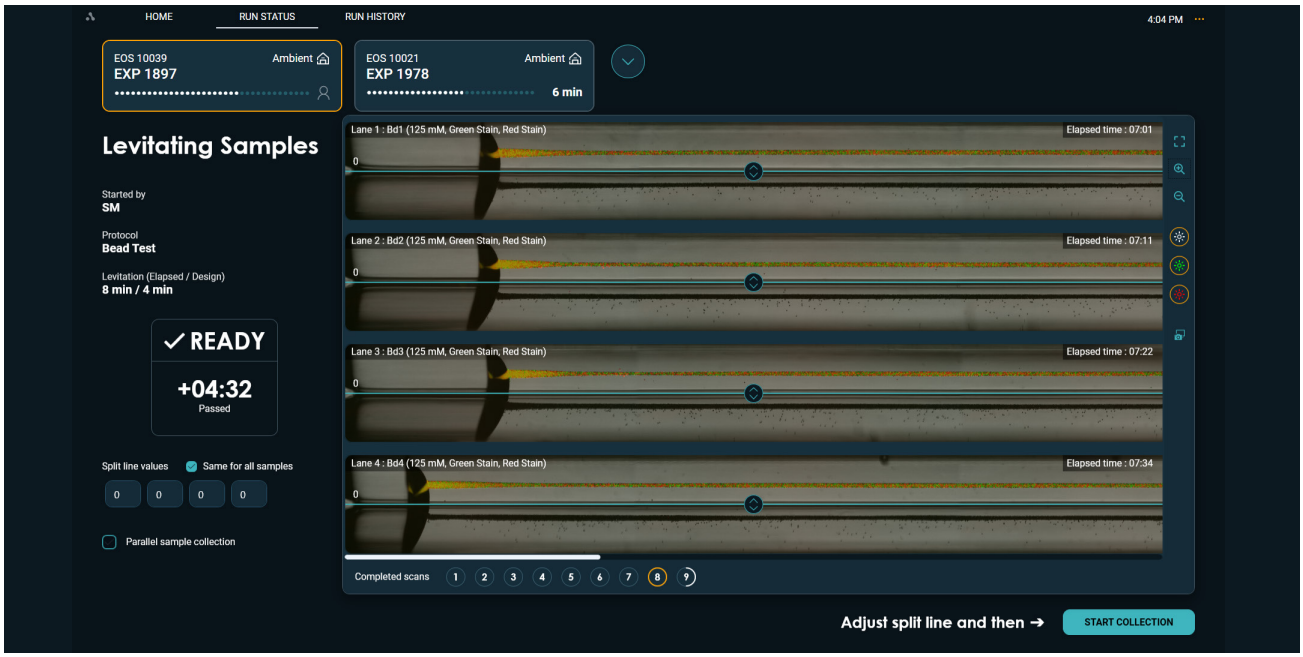


Figure 37. 1 step zoom - true lane ratio view

Figure 38. *Max zoom - single lane height view*

During sample setup, the option to image with green or red fluorescence is set. If selected, the fluorescence signal is captured during the run. The fluorescence view is on by default when stains are used, as indicated by the green and red stain tool. To display the sample without fluorescence deselect the respective stain tool. Re-select to turn the signal back on. The stain tool only toggles the view; images with fluorescent signal are captured for the duration of the run regardless of its status.

Figure 39. *Fluorescence view for both green and red (UL), red stain only (UR), green stain only (LL), and brightfield only (LR).*

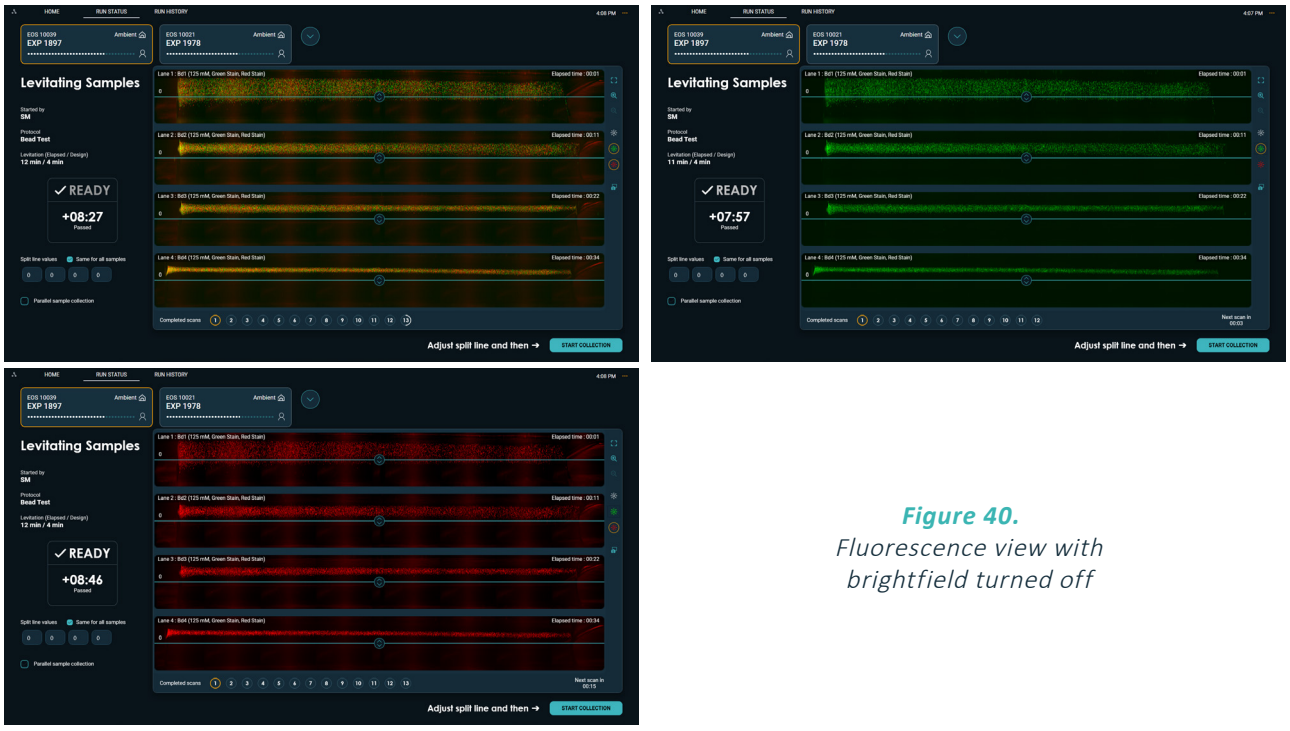


Figure 40.
Fluorescence view with brightfield turned off

The screen capture tool saves the active run status view to a *.png file. A File Explorer window will appear so the screenshot file can be saved to the selected folder.

Run History Screen

For quick access, the recent run history (last 3 runs) can be seen and the option to download the run report is available on the main start screen. The complete run history is available under the Run History screen.

The "i" on the bottom left of the Run History Tab displays information about the number of runs completed, the storage capacity of the computer, and an approximation of how many runs can be performed based on current capacity.

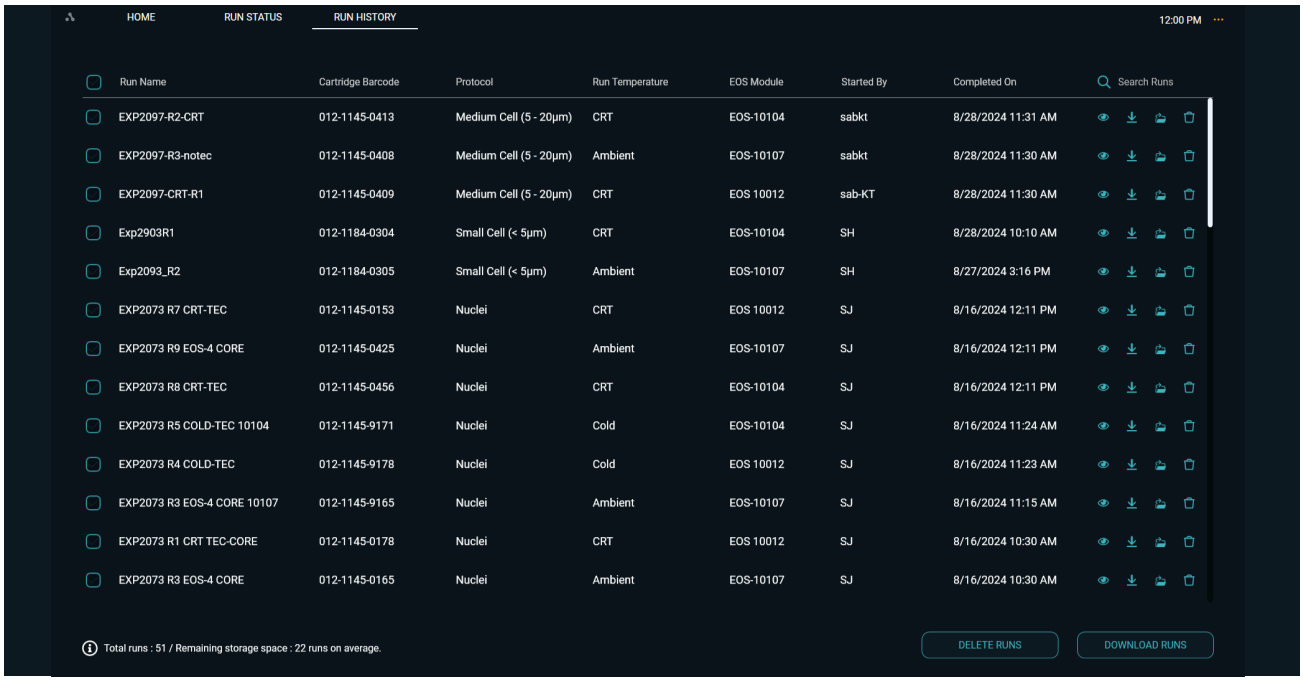


Figure 41. *Run History screen*

Partial run information is listed in the Run History table for easy navigation and selection.
Listed columns are:

- Run name
- Cartridge barcode
- Protocol
- Run temperature
- EOS Module
- Started by [user]
- Completed on

When a column is hovered over, a teal arrow will appear. Runs can be sorted using the teal arrow up and down button based on the column chosen.

Figure 42. *Run History column sort arrow*

The run history can be searched using the search bar. Based on the date range or search term, runs will be displayed.

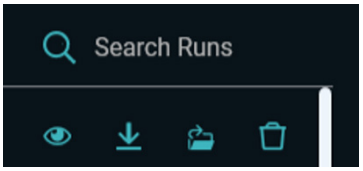


Figure 43. *Search Run History*

To view a summary of a run, click the **View Run Summary** eye icon next to the run of interest. All entered run information is displayed on the left panel. The last completed scan for all samples is also displayed along with sample information.

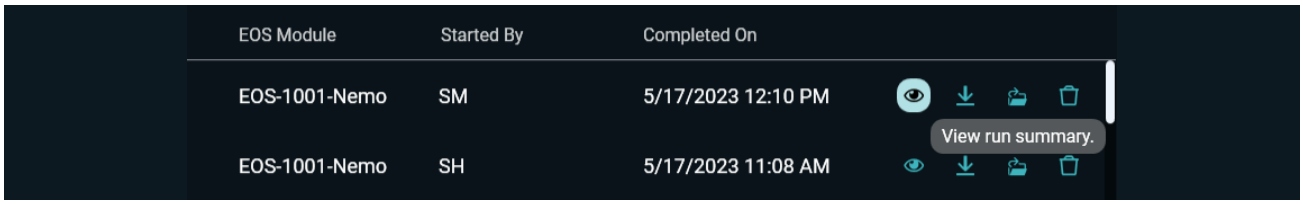


Figure 44. *Run history options, hover and tool tips*

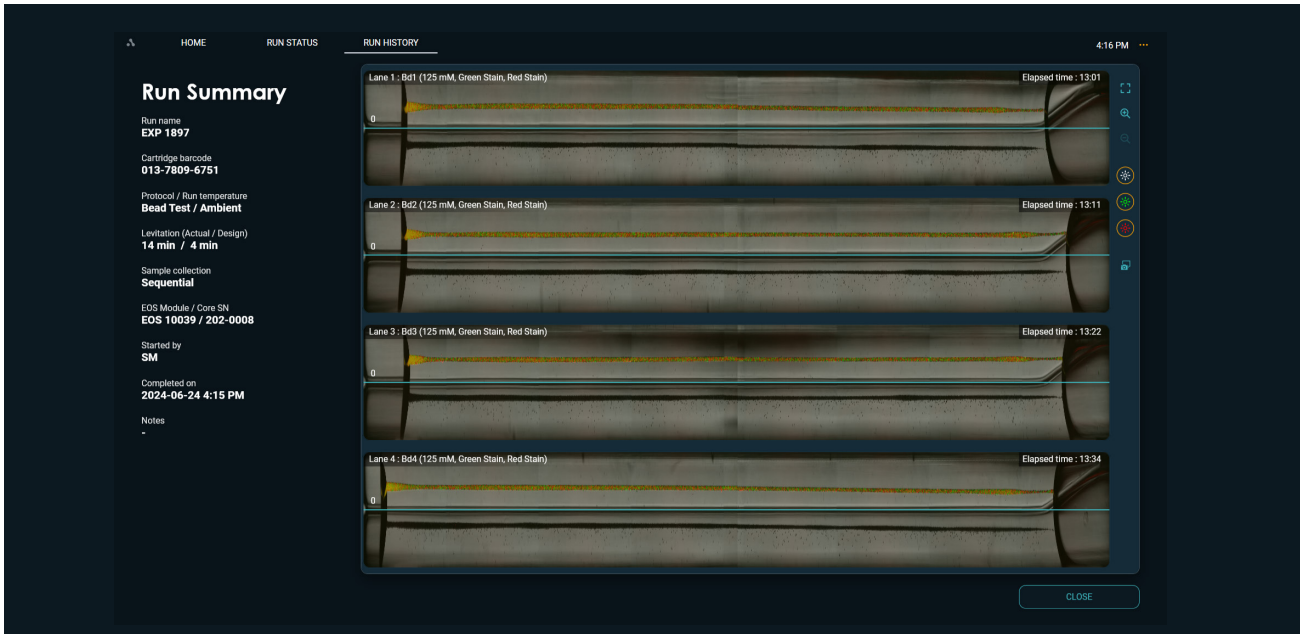


Figure 45. *Run Summary*

Using the **Download Runs** option, all the data files (logs, run report, raw and processed images, run summary report pdf) can be downloaded to a selected folder. All the downloaded files are delivered into a folder with the folder name containing the run name and date and time stamp.

Refer to section **Run Summary and Data Files** within **Running An Experiment** for a step-by-step guide to obtain these files and understand their content.

NOTE: After inserting a USB drive, the user can select that drive for downloading

System Menu

The System Menu allows the user to configure the system and is the primary mode for exiting the LeviCell EOS Software. Options include:

- Manage EOS Modules
- Manage Stains
- Change Date and Time
- About EOS Manager
- Exit

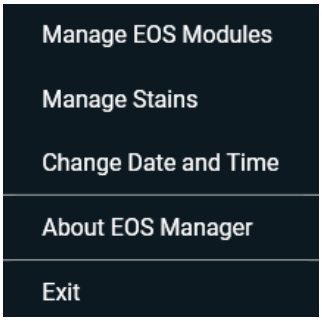


Figure 46.
System menu options

EOS Modules can be added or edited through the Manage EOS Modules option. Refer to the **System Installation and Calibration** section to walk through the installation and setup process for a new EOS Module.

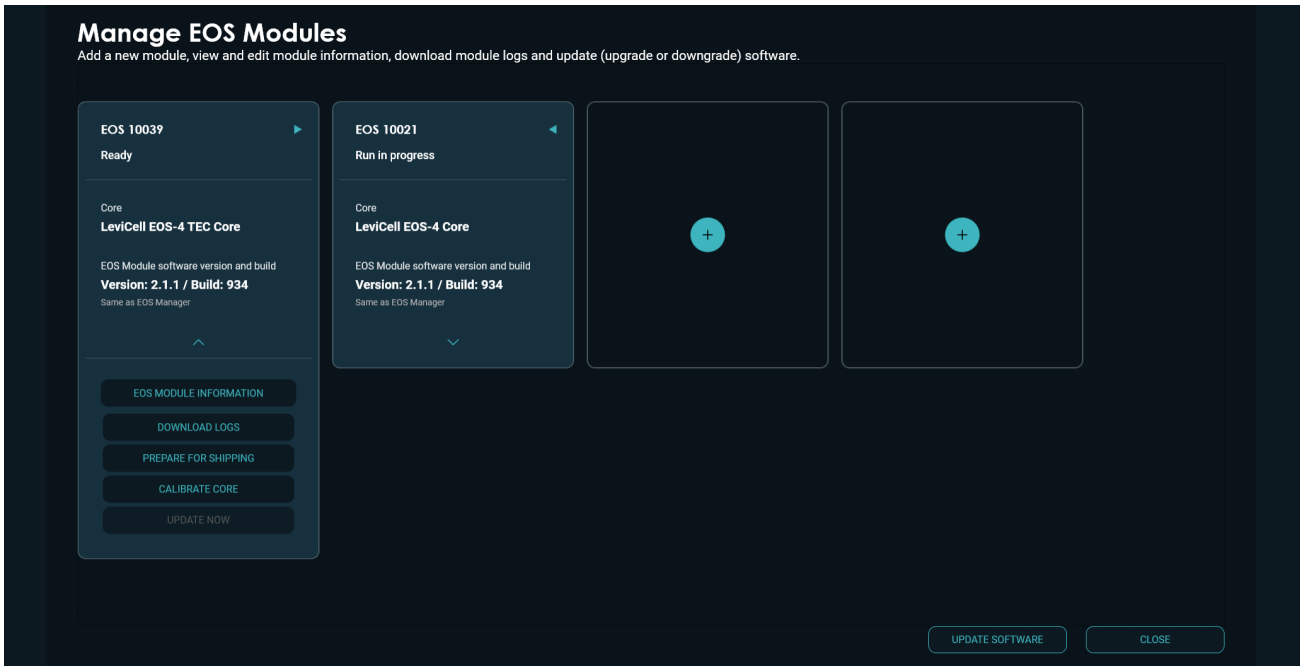


Figure 47. *Manage EOS Module screen*

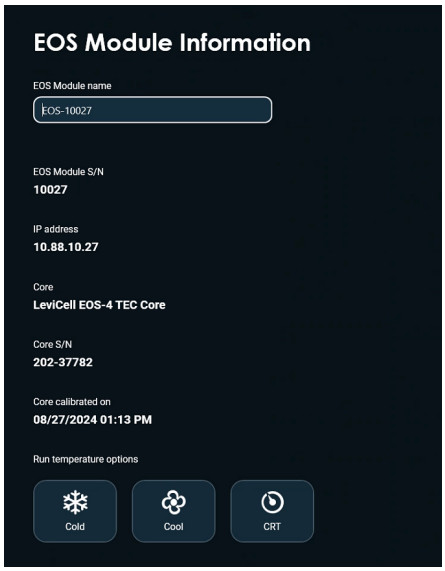


Figure 48. *EOS Module and Core Module information.*

To learn more about the EOS Module and the EOS Core installed click on EOS Module Information under the specific module. On this screen, the EOS module can be given a user-defined name. This screen will also display the Core name, serial number, last time it was calibrated and the run temperature options, along with the module's serial number, and IP address.

The LeviCell EOS has both red and green fluorescence imaging capabilities. The EOS Manager software is preloaded with Calcein-AM, Acridine Orange and Green Stain under the Green Fluorescence Stains and Propidium Iodide (PI), and Red Stain under Red Fluorescence Stains. The Red and Green Stains are optimized for the EOS installation beads test. The exposure settings for the cell stains (e.g. Calcein-AM, PI) have been optimized to enable visualization using these stains. The default settings cannot be changed, therefore the exposure factor slider is not accessible. To further optimize these settings for your sample, a new stain setup can be added and customized.

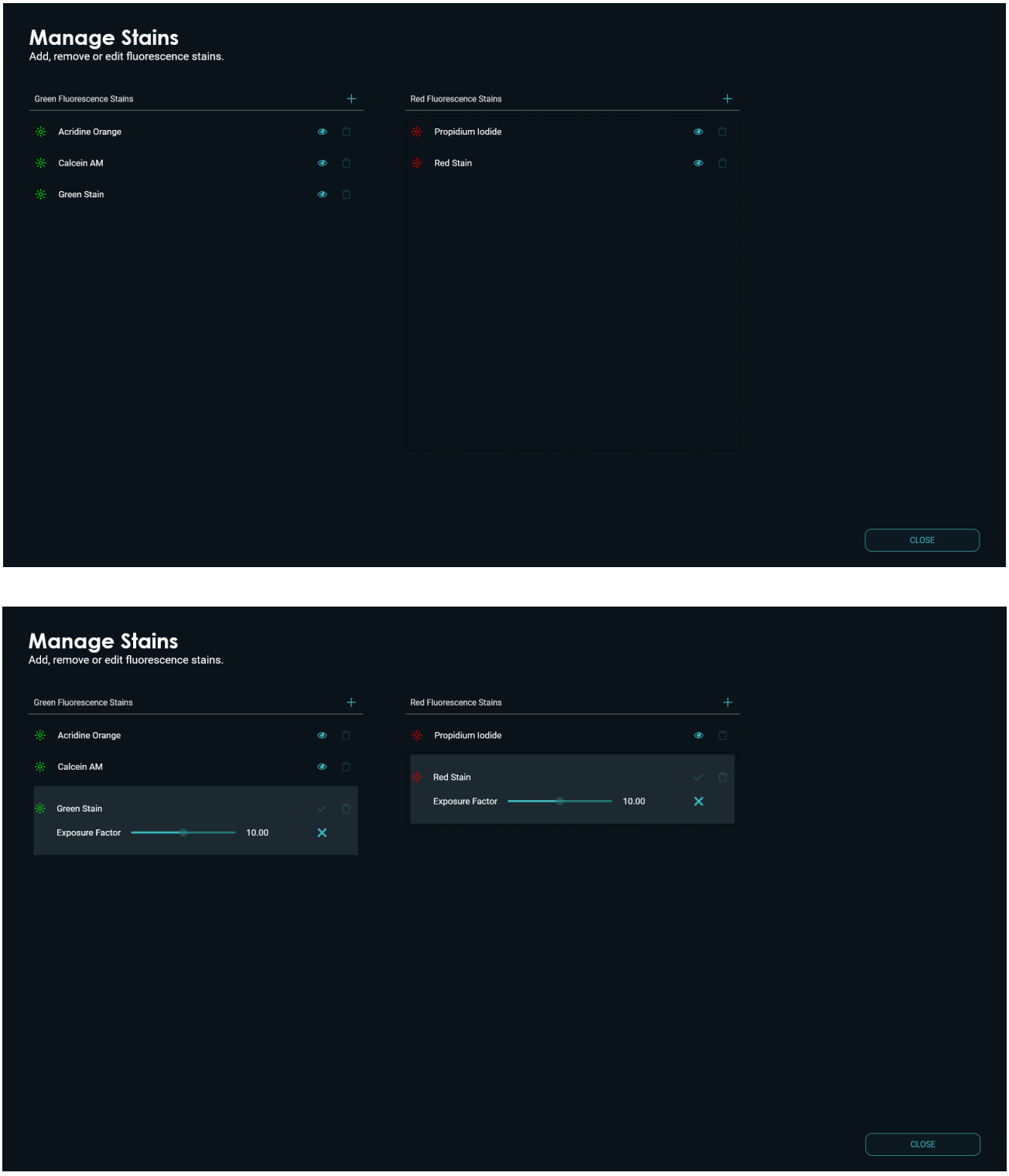


Figure 49. *Manage Stains screen and default stains that have fixed settings*

Additional stains can be added by clicking the **+** symbol in either the Green or Red Fluorescence Stain column. The stain name can be edited. In addition, the relative exposure can be adjusted for the intensity of the stain. For example, for stains brighter than PI, the exposure factor can be set lower, for stains dimmer than Calcein AM, the exposure factor can be set higher.

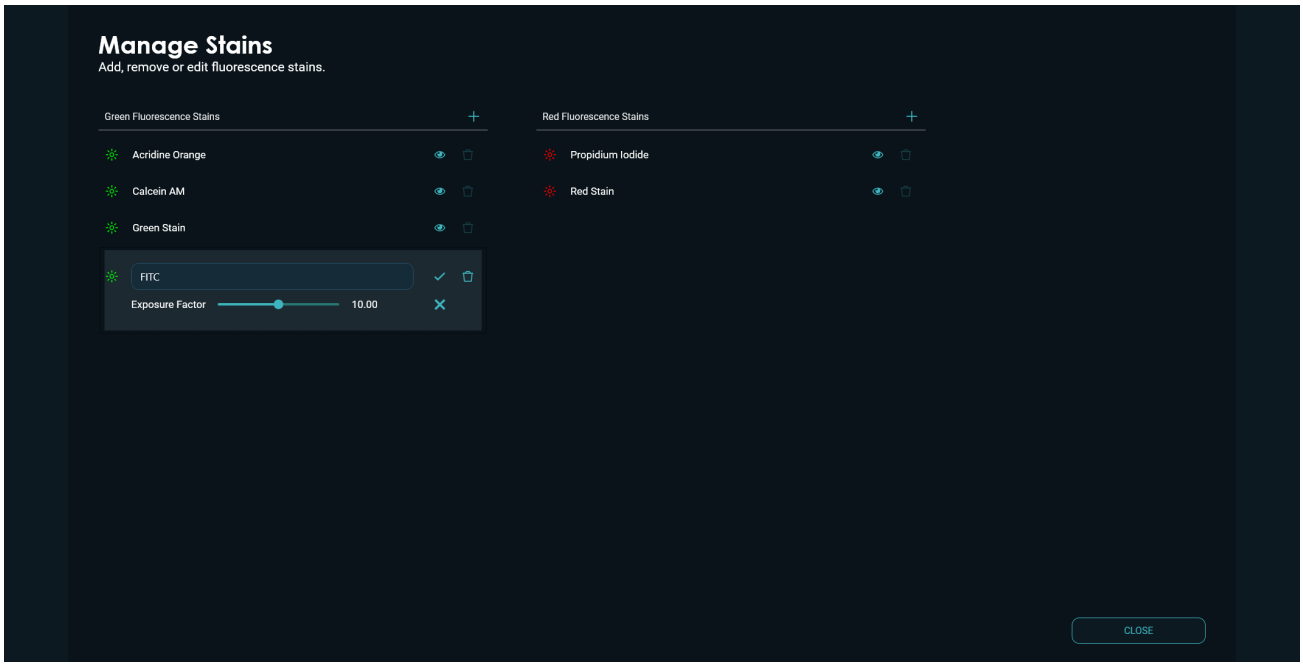


Figure 50. *Adding custom stains*

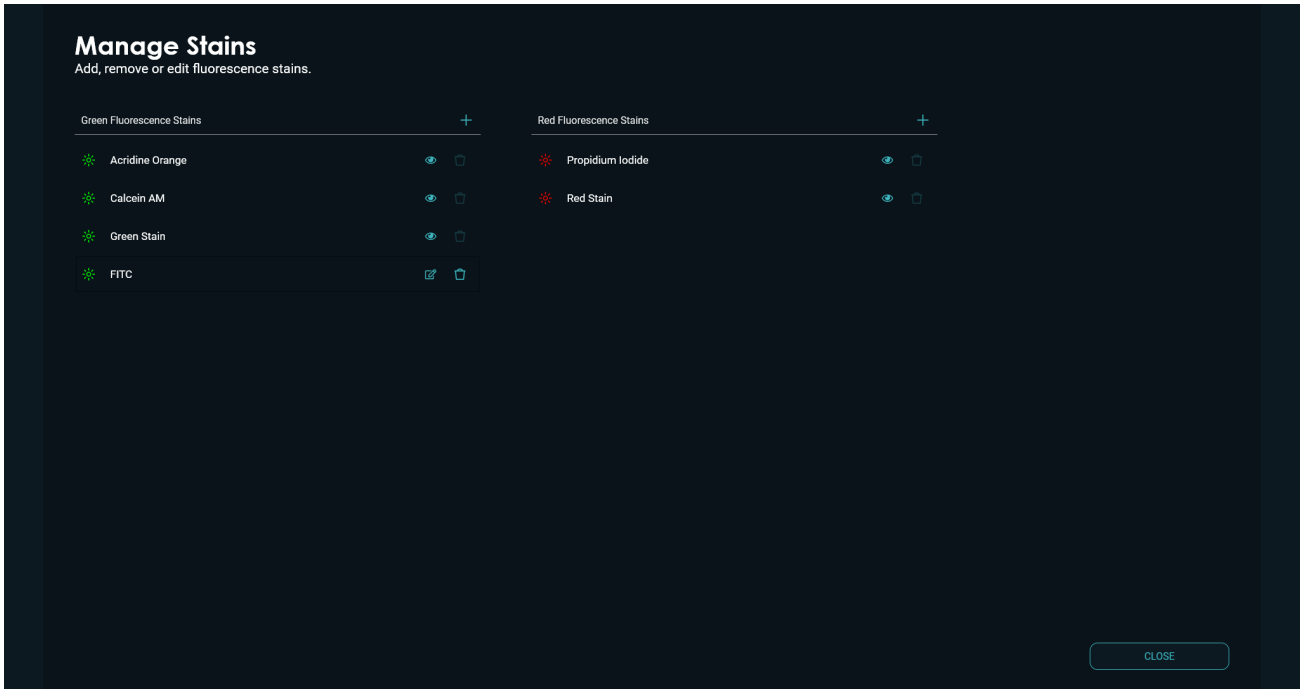


Figure 51. *Green and Red custom stains added*

If the date or time needs to be adjusted, a Windows pop up will appear. The date and time information used on the EOS Manager is taken from the operating system.

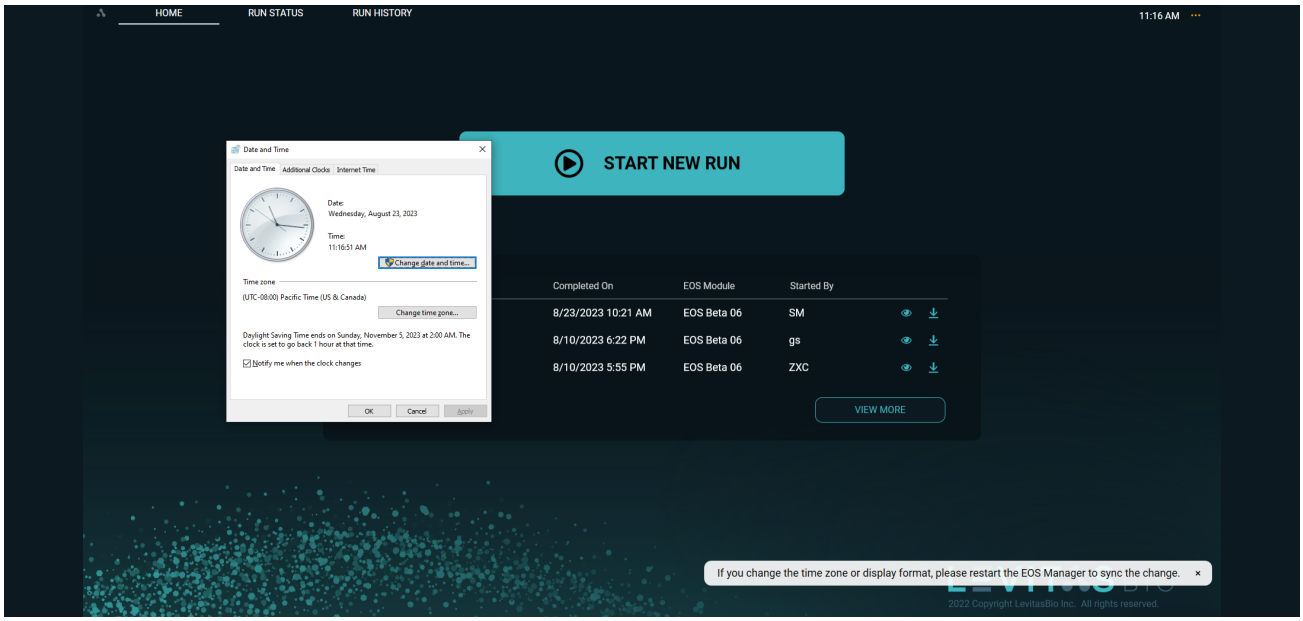


Figure 52. *Change Data or Time via Windows popup screen*

EOS Manager software information can be found in the About screen. On this screen, the current version and build number can be obtained and is useful in troubleshooting situations. Additionally the software license information can be reviewed.

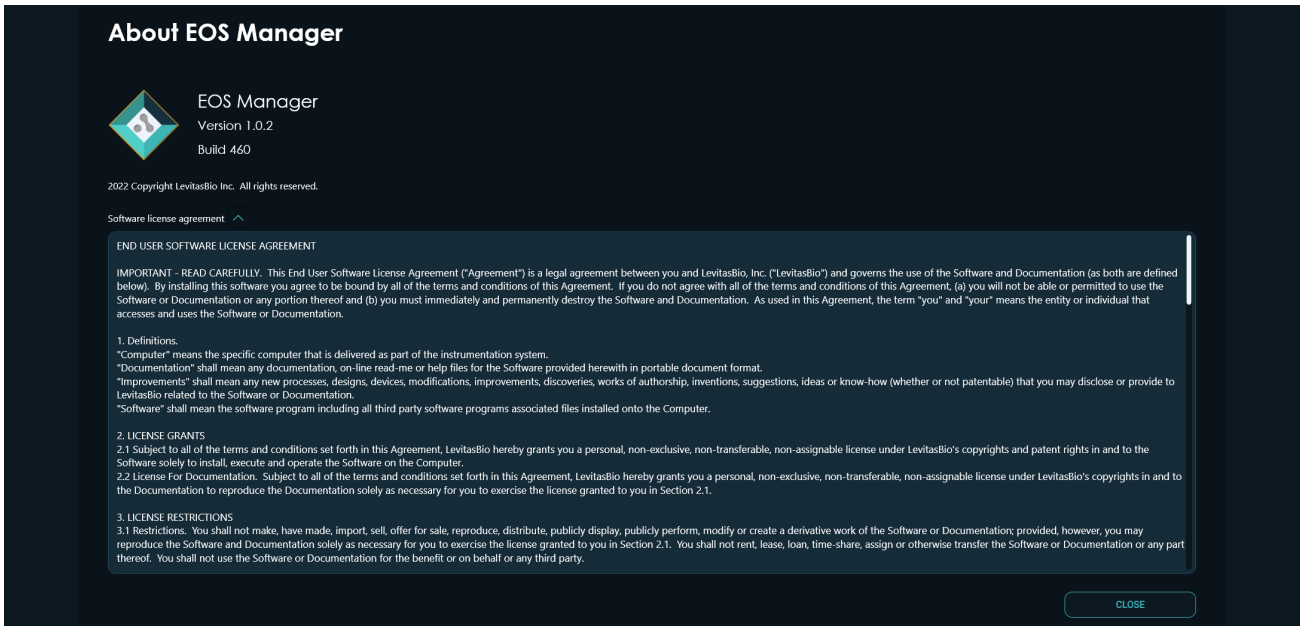


Figure 53. *About the EOS Manager and software license agreement*

SYSTEM INSTALLATION AND CALIBRATION

The LeviCell EOS System is delivered as multiple cartons on a pallet. If required, the smaller internal cartons may be separated for ease of handling. It is recommended not to open the internal cartons prior to installation. LeviCell EOS Modules should remain on their pallet until installed.

ATTENTION: The LeviCell EOS Module is shipped with safety locks designed to keep the moving parts of the instrument in place during transport. If the system is not installed correctly, damage to the hardware can occur. Refer to the LeviCell EOS Installation Guide for critical instructions for unpacking the instrument.

Connecting the EOS Module to the Control PC

To ensure proper communication between the LeviCell EOS module and the Control PC, proper connections must be made.

1. Plug the provided power cable into the computer
2. Plug the provided power cable into the rear of the EOS module
3. Connect the EOS module to the Control PC using the provided Ethernet cable.
4. Turn on the Control PC and monitor
5. Turn on LeviCell EOS Module mains power switch in the rear
6. Press the LeviCell EOS Module soft power button in the front

Core Calibration - First Time Use or Core Swap

Upon turning on the system for the first time, the LeviCell EOS Manager will prompt the user to confirm that shipping locks have been removed and to configure the system. The system will begin the setup process which takes approximately 10-15 minutes to complete. Setup and configuration include the following steps:

1. Pair and connect to EOS module(s)
2. Map core and scan positions (Core Calibration)

Click **Manage EOS Modules** to begin:

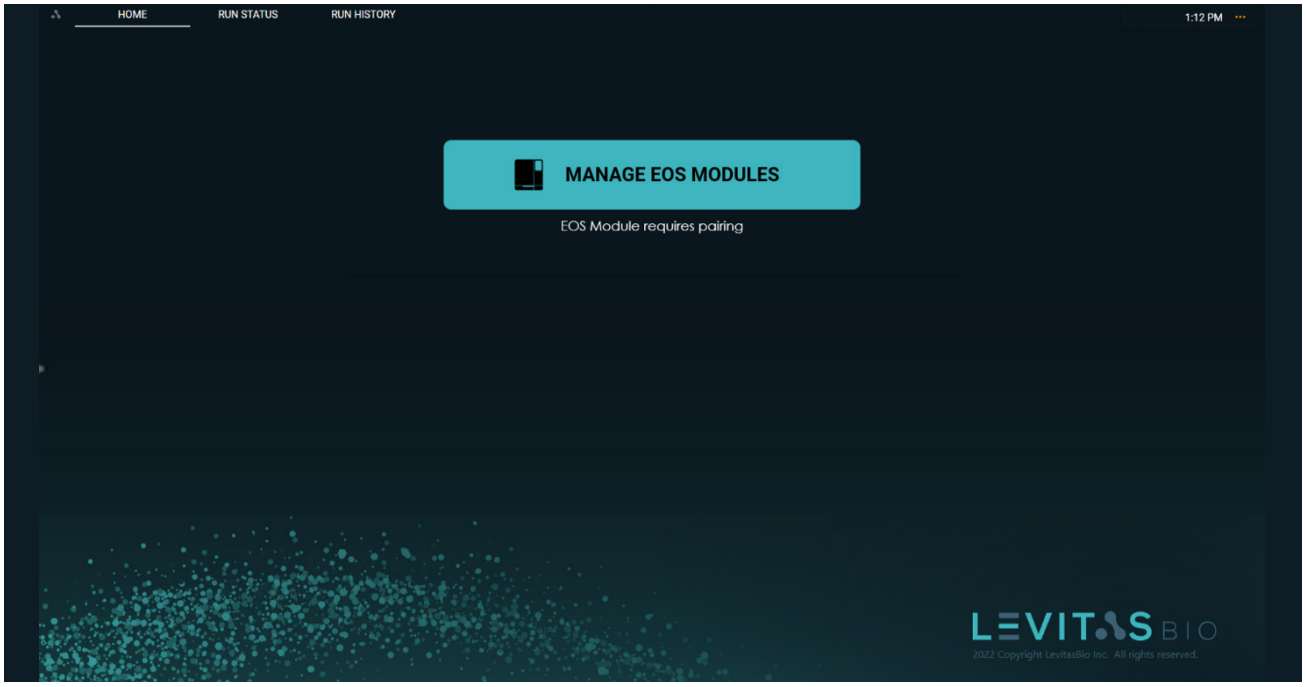


Figure 54. *Manage EOS Module Home screen*

Once the EOS Module and Control PC are connected via Ethernet and powered on, click tabs in the EOS Module card to Pair and Connect the module to the control PC.

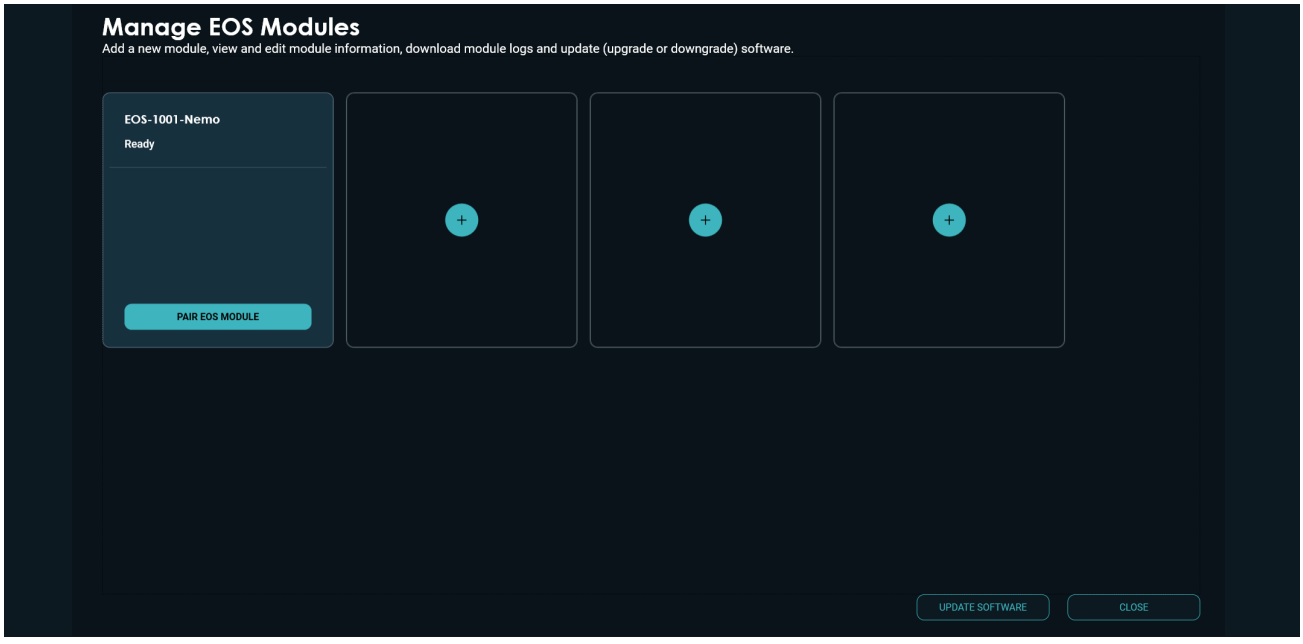


Figure 55. *EOS Module requires pairing*

Once you have established communication with the module, the EOS Module Options can be accessed by clicking the down arrow to access:

- EOS Module Information
- Download Logs
- Prepare for Shipping
- Calibrate Core
- Update Now

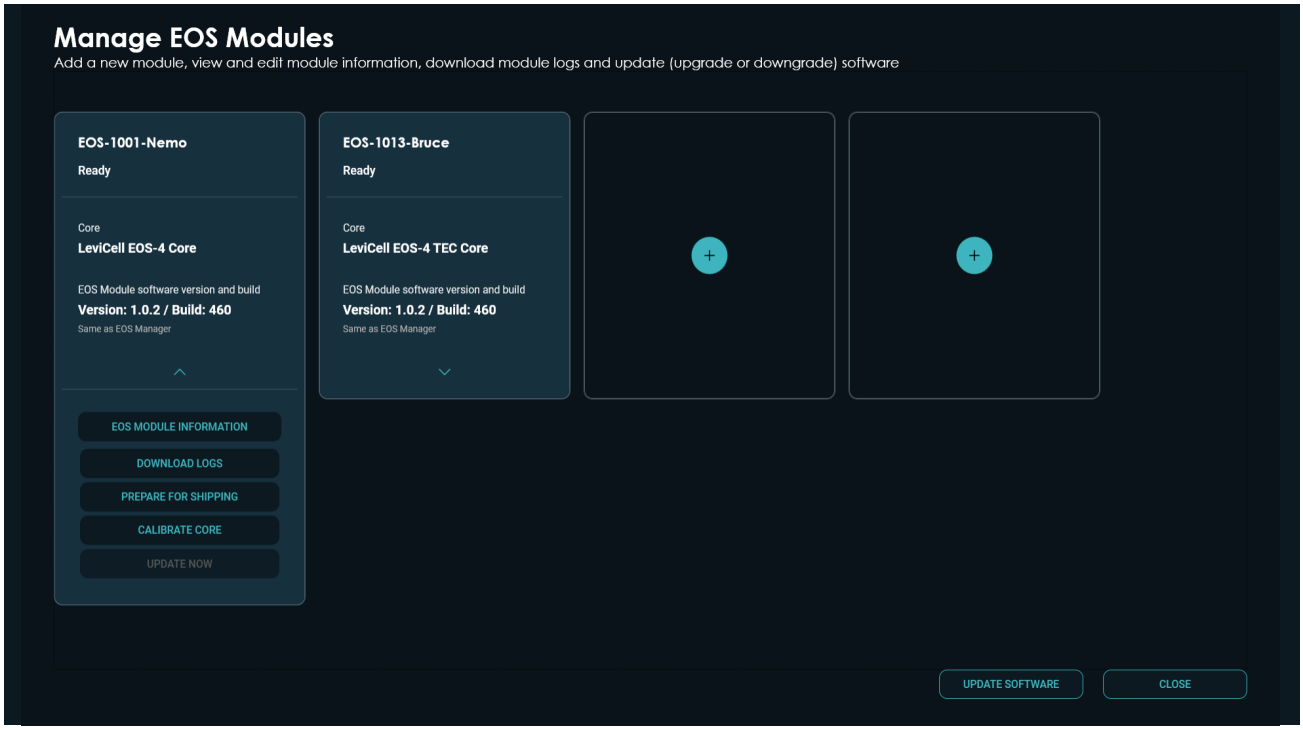


Figure 56. *Paired EOS with options*

Before a workflow can be performed, the core must be calibrated to map the core and scan locations for imaging.

Click **CALIBRATE CORE** to initiate the calibration and follow on-screen guidance.

The first calibration will detect the core location in the module by detecting its fiducials:

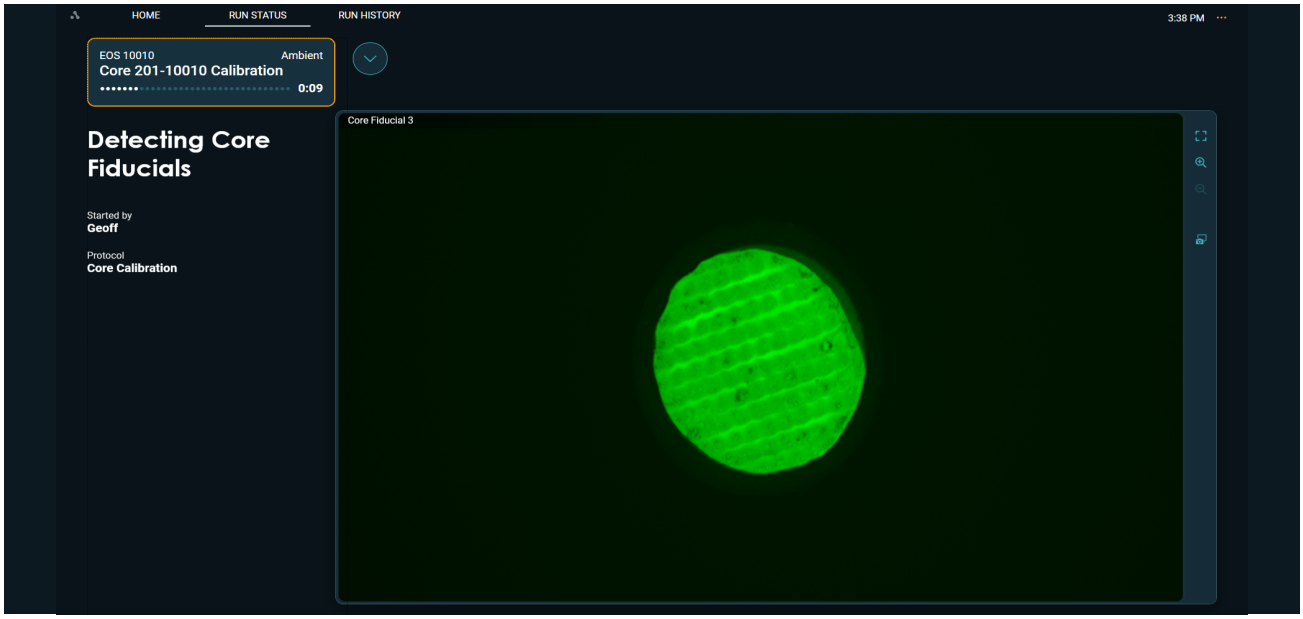


Figure 57. *Detecting Core Fiducials*

Once this is complete, the magnets within each lane will be scanned for location and focus calibration. It may take a few minutes to find and image each lane; the composite magnet image for each lane will appear on the screen once the magnets are found:

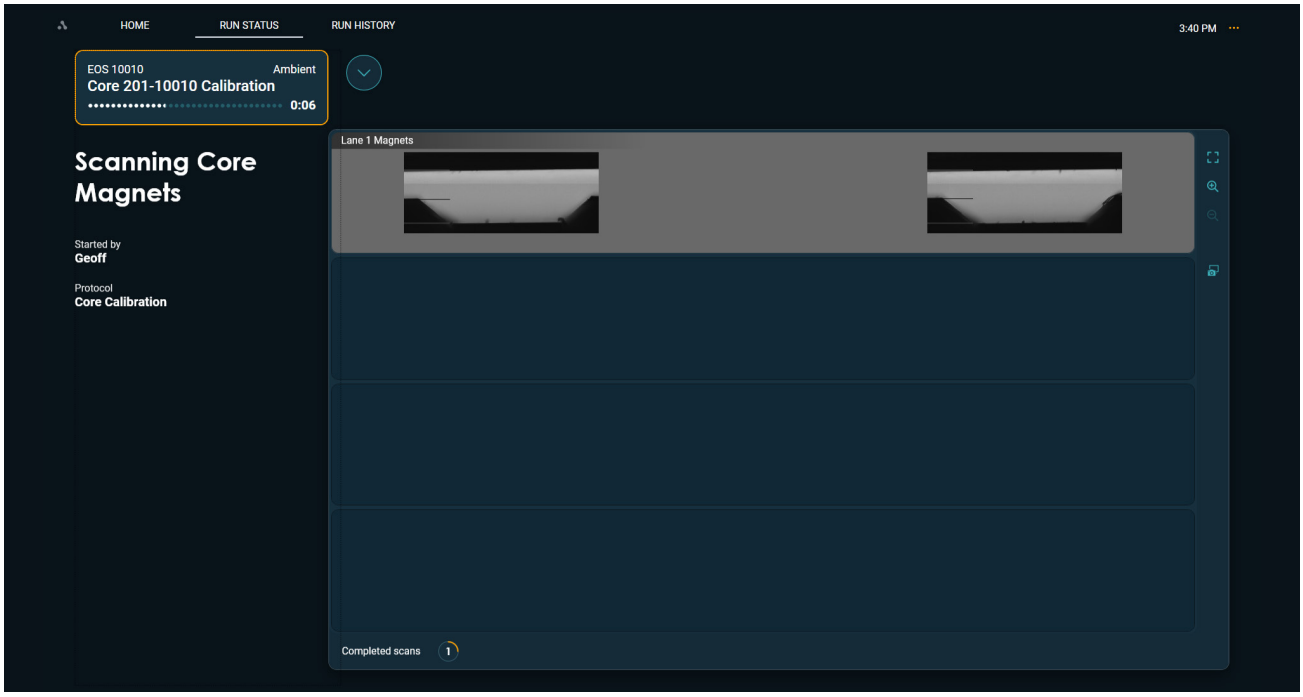


Figure 58. *Scanning core magnets in progress on lane 1*

Figure 59. *Scanning core magnets in progress across 4 lanes*

The software will indicate when the calibration protocol is complete, and a record of it will be available in Run History:

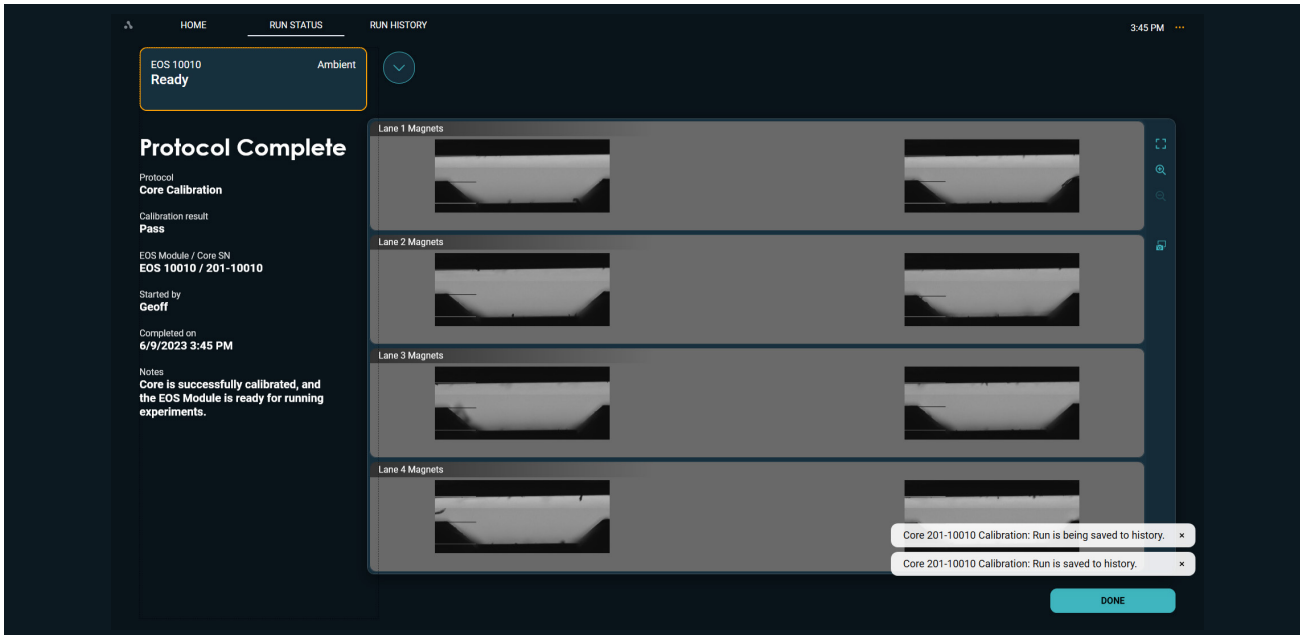


Figure 60. *Calibration Protocol completed*

After the system has been successfully calibrated, a system performance qualification test can be performed using the Levitation Install and Calibration beads. Refer to the "**Getting Started**" section for a step by step guide.

Adding New EOS Modules

The LeviCell EOS System can expand up to 4 EOS Modules to be connected to the Control PC. Additional EOS Modules can be installed and connected at any time.

EOS Modules can be managed and added via the EOS Manager menu option. Upon connecting a subsequent EOS Module to the Control PC via the Ethernet cable, the software will recognize that a new module is available for pairing.

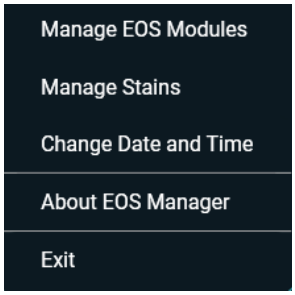


Figure 61. *System Menu*

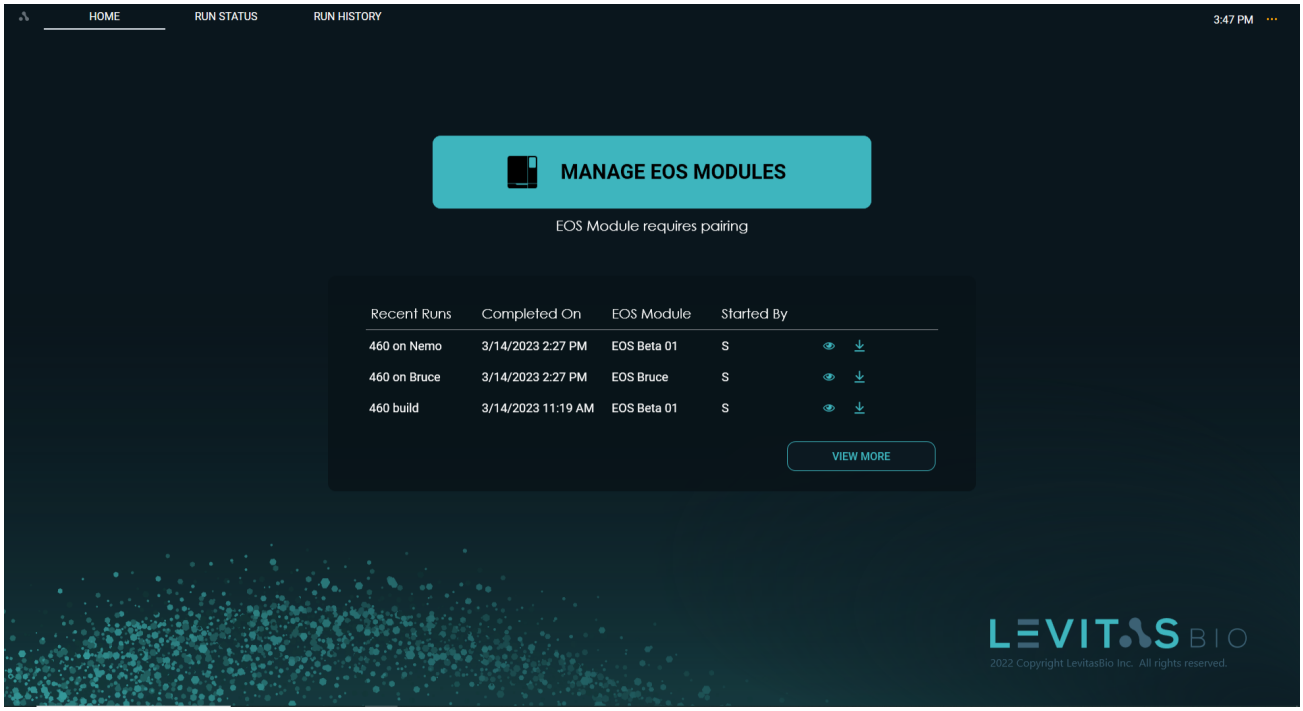


Figure 62. *Home screen when an EOS Module requires pairing*

The EOS Manager software will guide navigation to the Mange EOS Modules screen. Any unpaired EOS Modules will be displayed and require communication pairing.

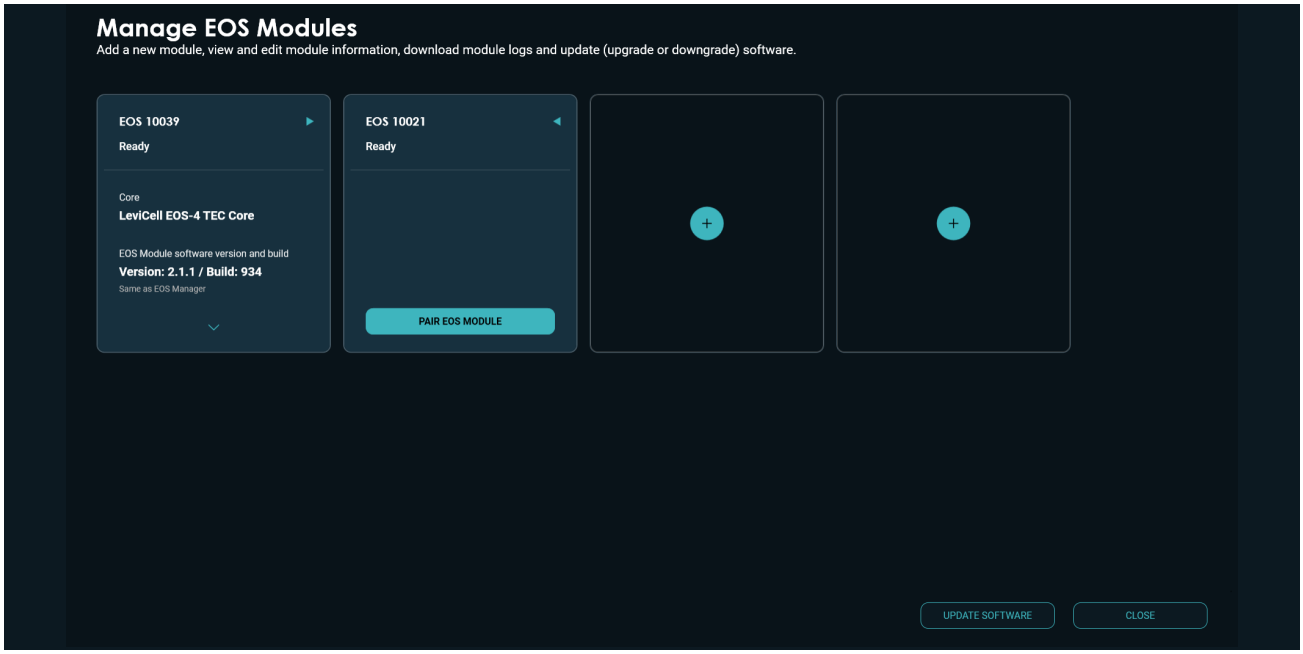


Figure 63. *Additional EOS Module requires pairing*

Once the EOS Module is paired it will display Ready along with module information and the ability to manage the module options.

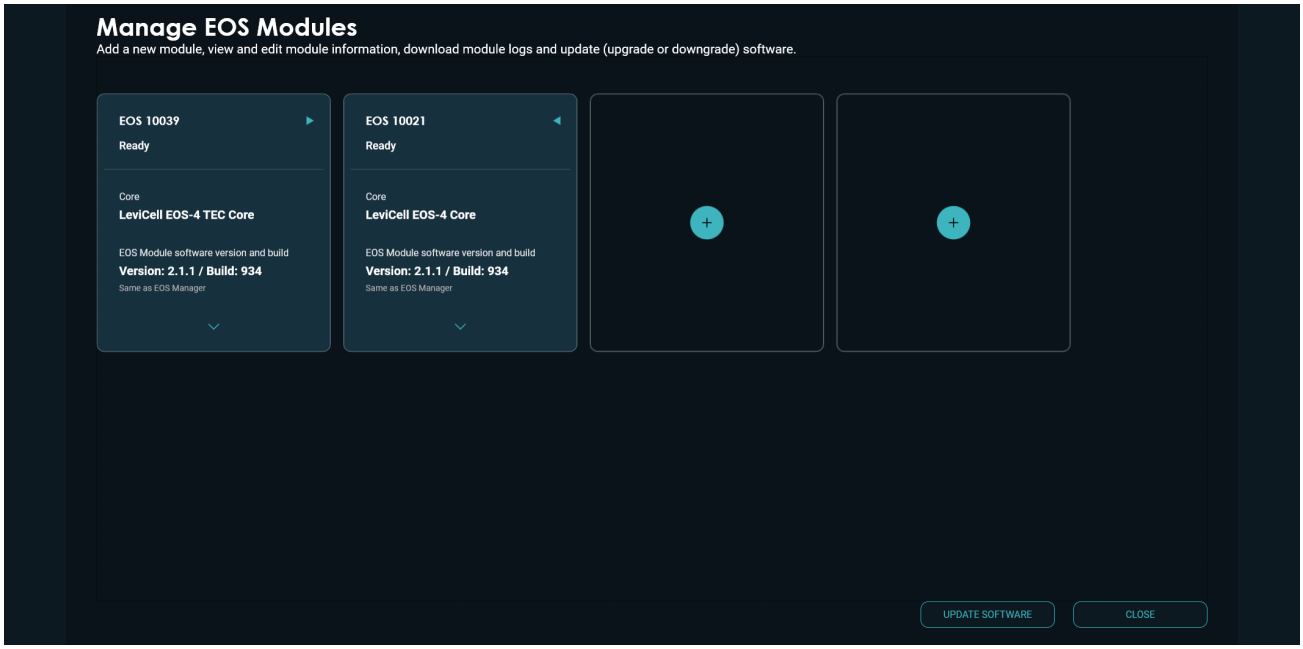


Figure 64. *Additional EOS Module is paired and ready for use*

Moving a LeviCell EOS Module

If a LeviCell EOS module needs to be moved to a different location, please contact LevitasBio Support for assistance. Improperly moving an EOS module may lead to hardware damage, and may void any remaining system warranty.

GETTING STARTED

After the system has been connected and system configuration has been completed, the EOS Manager software is ready to use. It is recommended to run the Levitation Install and Calibration Bead Test (Bead Test) prior to running experiments. This Bead Test serves as a product performance qualification test.

Additional items required before starting:

- Levitation Agent Kit (LevitasBio, PN 1003001, PN 1003002)
- LeviCell EOS Installation and Calibration Kit (LevitasBio, PN 1003004)
- LeviCell EOS-4 Cartridges (LevitasBio, PN 1002104)

LeviCell EOS Performance Qualification Test

In order to verify the LeviCell EOS System has been installed and is performing to manufacturing specifications. The LeviCell EOS Installation and Calibration Kit includes beads of two different densities and a buffer. In conjunction with the Levitation Agent and Cartridge Kit, the performance qualification demonstrates the instrument's ability to separate an input mixture of two bead densities into two distinct outputs with the bead types separated.

The general steps to perform the performance qualification test are:

1. Prepare Levitation Buffer
2. Prepare Bead Mixture
3. Prepare Input Sample
4. Run Bead Test workflow
5. (Optional) Count the input and output samples

The install beads are provided in two formulations that are mixed at the start of the test. Beads from LeviCell Install Bead Mix 1 are fluorescent – they are a 1:1 mixture of green fluorescent polystyrene beads (Ex460/ Em500) and orange fluorescent polystyrene beads (Ex530/Em582). Beads from LeviCell Install Bead Mix 2 are PMMA (polymethyl methacrylate) and do not fluoresce. The fluorescence of Bead Mix 1 is used to confirm fluorescent LED functionality on the instrument, as well as provide a visual distinction between the beads from Bead Mix 1 and the beads from Bead Mix 2, which are of different density. The beads from each of the mixes are approximately 20 µm in diameter.

After the Bead Mixture is loaded into the EOS-4 cartridge separation channel during the LeviCell workflow, the bead mixture levitates and separates into two bead populations according to their density. This simulates the separation of live and dead cells from a mixture. The number of beads of each density can be measured in the input and output samples to assess performance of the separation after collection.

Example images of beads are shown in Figure 65 at the beginning of the workflow (top panel) and after levitation (bottom panel).

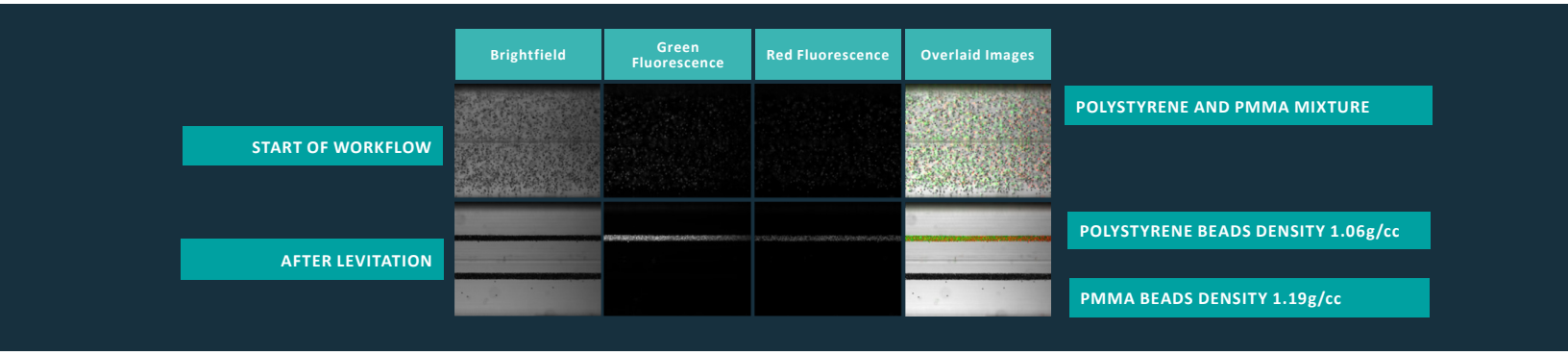


Figure 65. *Example images of bead mixtures taken during Performance Qualification*

Step by Step Protocol for Bead Test

It is recommended for first time use and for a LeviCell EOS Performance Qualification Test, a full cartridge using all 4 lanes are used for the Bead Test.

A. Prepare Reagents

1. Prepare Bead Mixture

   a. Vortex bead tubes thoroughly immediately before pipetting to avoid sedimentation.

   b. In a new 1.5 mL tube, prepare bead mixture as shown in Table 12.

Reagent	Volume (µL) | # of lanes to run			
	1	2	3	4
LeviCell Install Bead Mix 1	15	30	45	60
LeviCell Install Bead Mix 2	30	60	90	120
Total	45	90	135	180

Table 12. *Preparation of Bead Mixture for 1-4 lanes*

2. Prepare Levitation Buffer

   a. In a new 1.5 mL tube, prepare the Levitation Buffer as shown in Table 13 (final concentration = 125 mM).

   b. Vortex mixture well to completely mix the Levitation Buffer.

Reagent	Volume (µL) | # of lanes to run			
	1	2	3	4
LeviCell Install Buffer	219	437	656	875
1 M Levitation Agent	31	63	94	125
Total	250	500	750	1000

Table 13. *Preparation of 125 mM Levitation Buffer for 1-4 lanes*

3. Prepare Bead Sample for Loading

a. Pellet the beads by centrifuging tube at 300 RCF for 3 min at ambient temperature.

b. Remove supernatant from bead pellet using a P200 pipetter set to 45 µL for a single lane, or 180 µL for all lanes, etc.

c. Add the appropriate amount of Levitation buffer for the number of lanes being loaded.

Reagent	Volume (µL) | # of lanes to run			
	1	2	3	4
Levitation Buffer	250	500	750	1000

Table 14. *Resuspension volume for run*

d. Mix sample thoroughly by gently pipetting up and down 10 times. It is important to avoid creating bubbles.

e. If counting, immediately after mixing, set aside 20-30 µL of the input beads.

B. Run the LeviCell EOS Instrument

1. **Click Start New Run from the Home tab in LeviCell EOS Manager.**

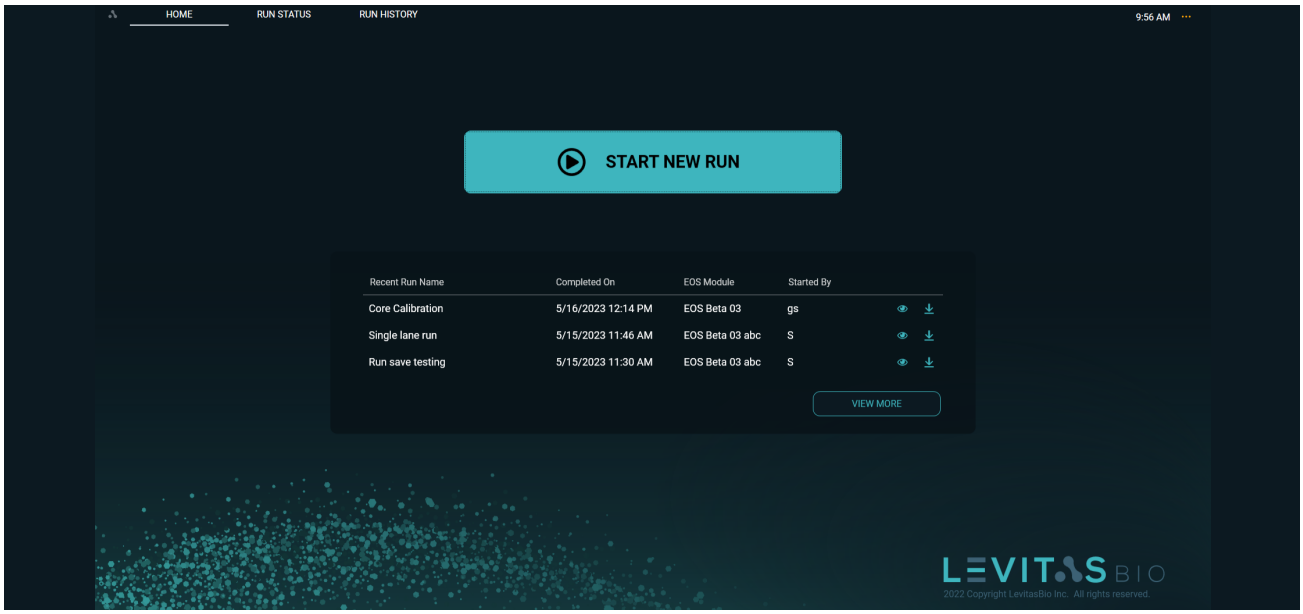


Figure 66. *Home screen*

2. **Scan the cartridge barcode.** The barcode can be scanned using the barcode reader or hand typed. The format must be ###-####-####.

NOTE: To avoid contamination of the instrument hardware, reuse of a cartridge with unused lanes is not permitted.

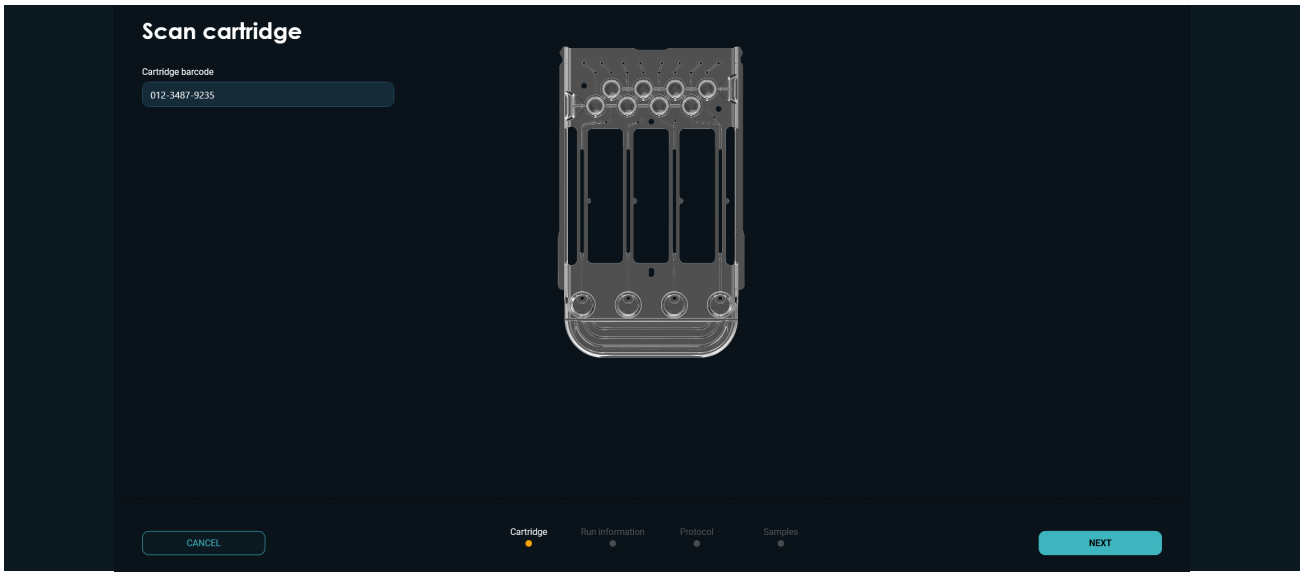


Figure 67. *Scan cartridge barcode information*

3. **Specify run information and select instrument.** When a module is idle or not in use it will be selected by default. If all modules are ready none will be selected.

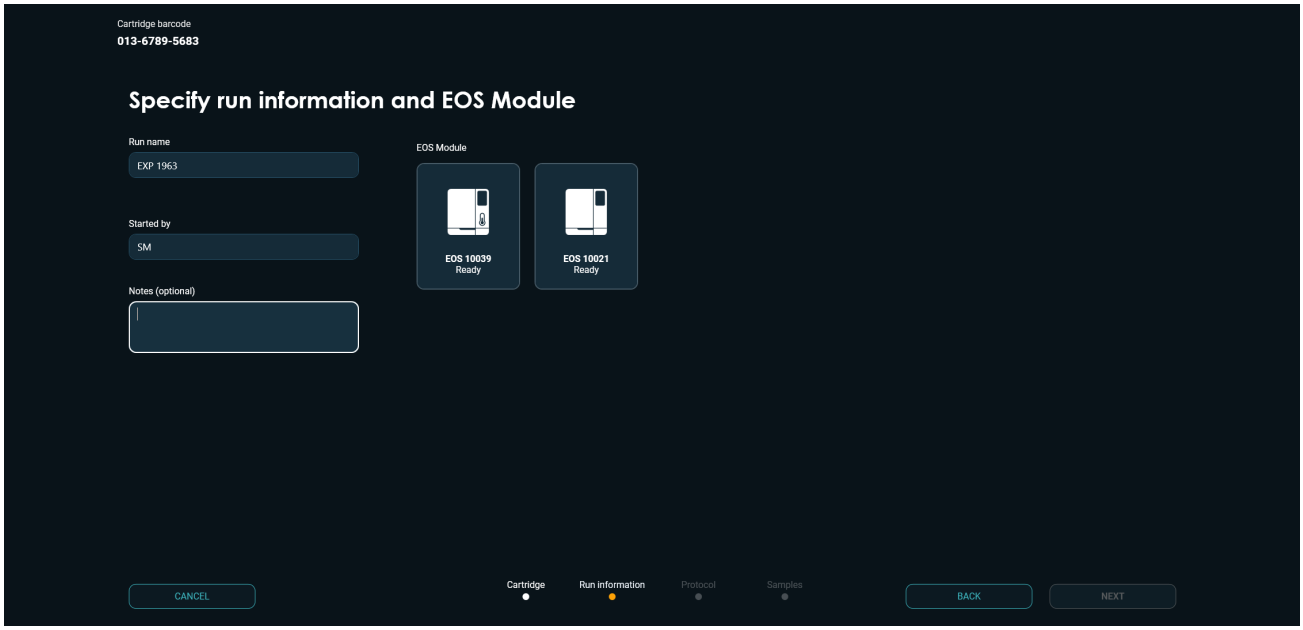


Figure 68. Run information screen

4. **Select Bead Test protocol to run.** This protocol can be run at any of the temperature options if a TEC Core is installed.

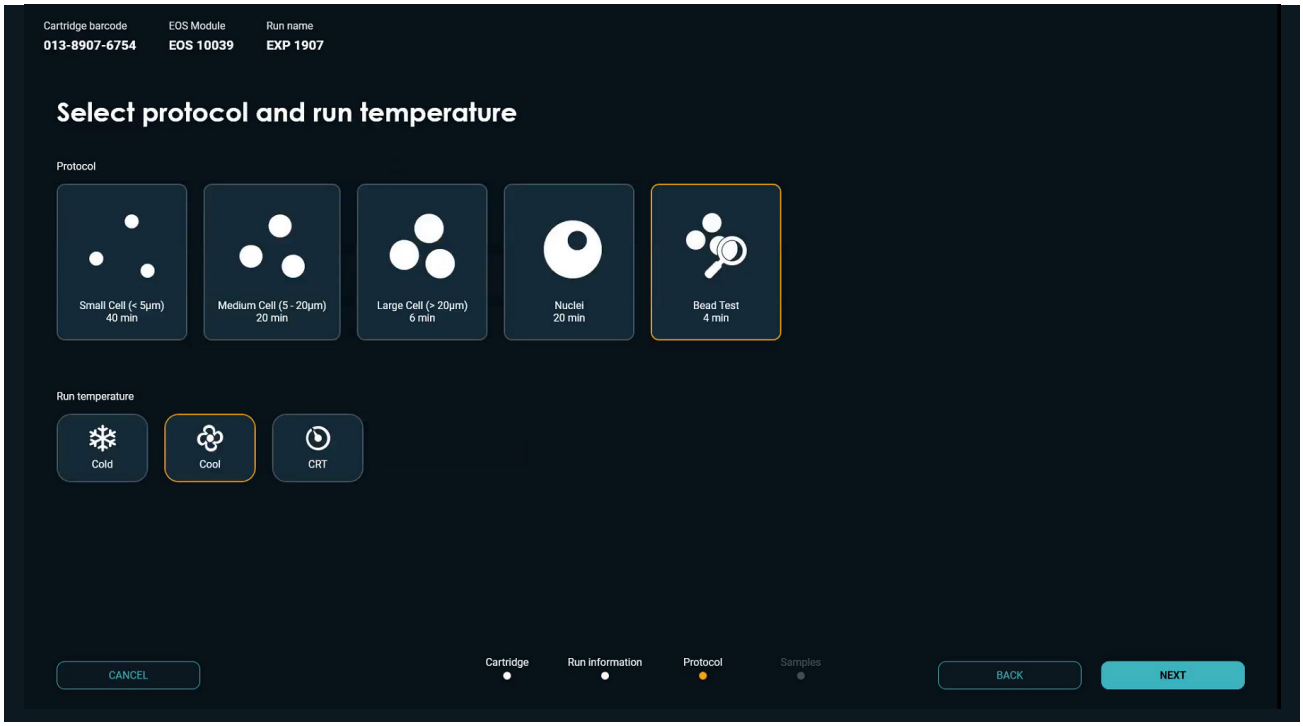


Figure 69. Select protocol, and temperature screen

NOTE: If running with temperature control, please refer to section Temperature Control Core Module to learn more about the cooling process and post run process.

5. Select and specify samples to run

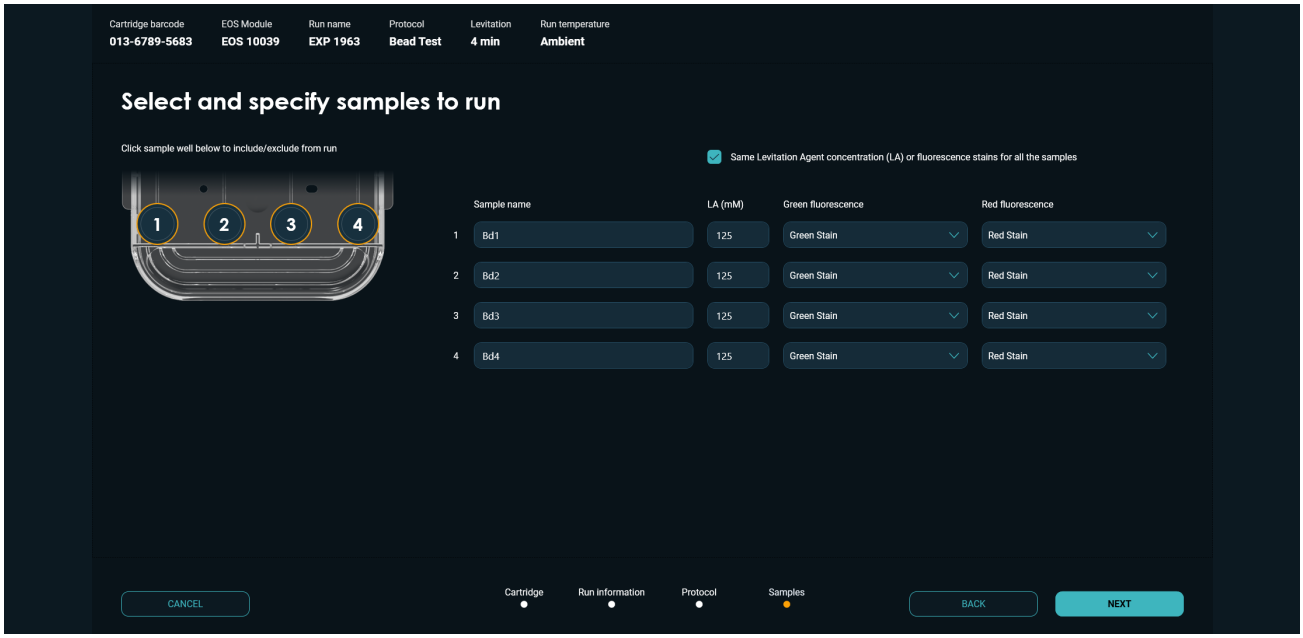


Figure 70. *Select samples and specify sample information*

a. By default, all samples have been selected for the run, as indicated by the gold highlight for each sample well on the cartridge diagram above. To deselect a sample, click on the sample well in the image.

b. The Levitation Agent concentration for the Bead Test [LA (mM)] is 125.

c. Choose **Green Stain** from the pull-down menu for Green Fluorescence, and **Red Stain** for the Red Fluorescence.

NOTE: The "Same levitation agent concentration…" check box can be toggled to specify conditions for all samples or individual samples.

6. **Follow the on-screen instructions to insert a cartridge into the system.** Insert your cartridge into the system by holding it by the grip. A clamp will engage after closing the door.

CAUTION: Potential Pinch Hazard.

Figure 71. *Open door to load cartridge*

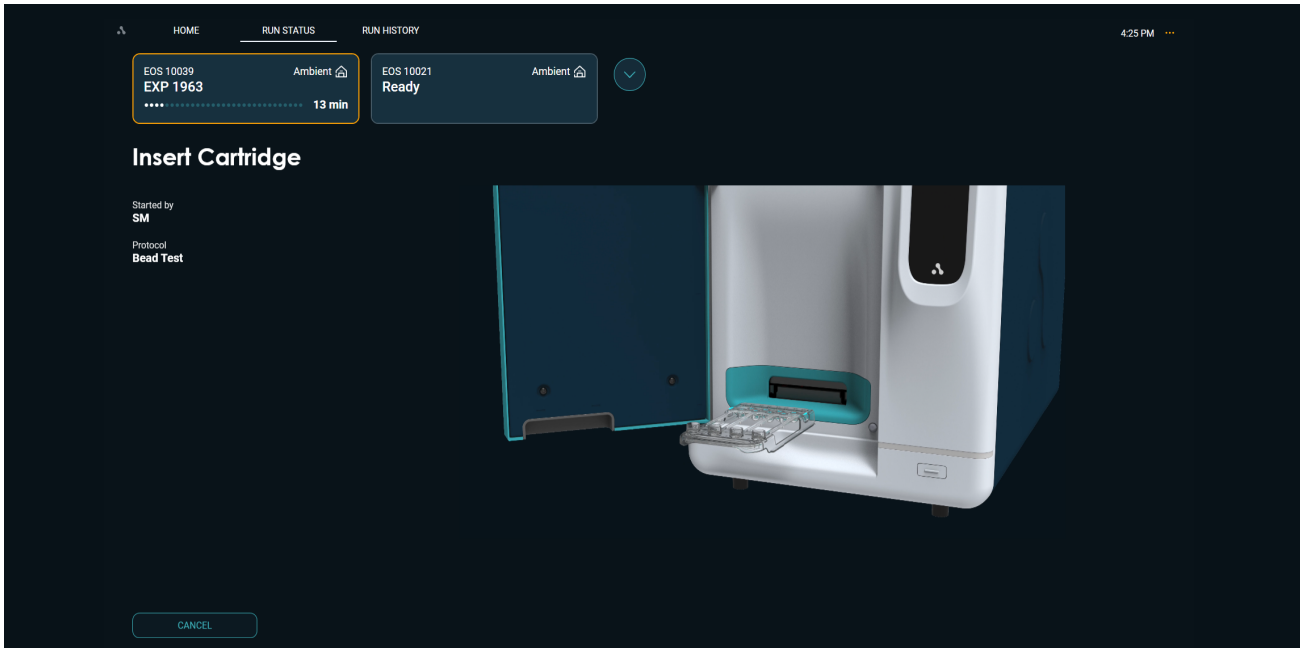


Figure 72. *Insert cartridge into EOS Module*

Figure 73. *Close door after inserting cartridge*

7. **A Pre-scan will occur.** This provides a mapping of the cartridge separation channels which will be used as part of the imaging.

The total run time is displayed on the module card. This includes any time required for temperature cooling of the core or cartridge.

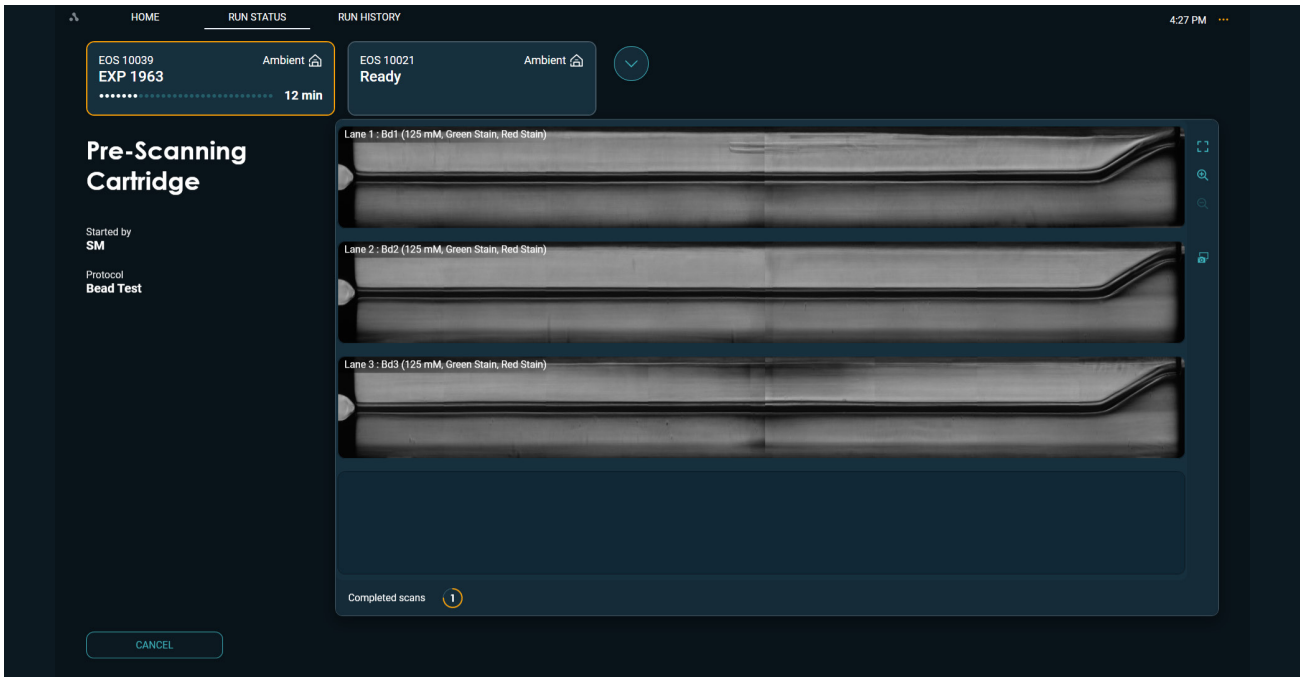


Figure 74. *Prescanning cartridge 3 of 4 lanes*

8. **Follow subsequent prompts to then dispense samples.**

NOTE: Mix sample thoroughly by pipetting up and down gently 5X and immediately load 220 µL into each of the corresponding input wells.

Place the tip of the pipette in front of the inlet hole, taking care not to insert the tip into the hole, and dispense to the first stop. This is to lower the probability of creating small bubbles within the sample.

9. **Press NEXT on the LCD touch screen after dispensing your samples or click NEXT at the Control PC.**

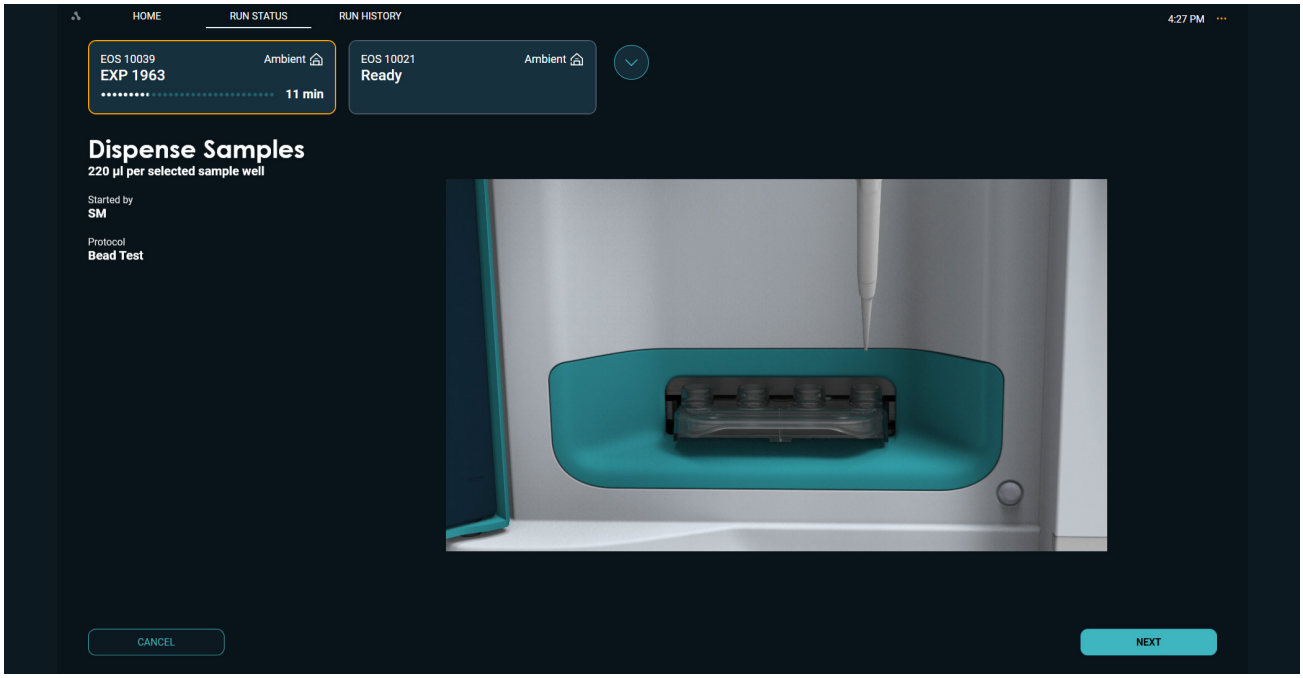


Figure 75. Dispense samples into the respective wells

10. **The LeviCell EOS will automatically load the sample upon closing the door. Scanning will begin.** The time remaining for levitation will be displayed.

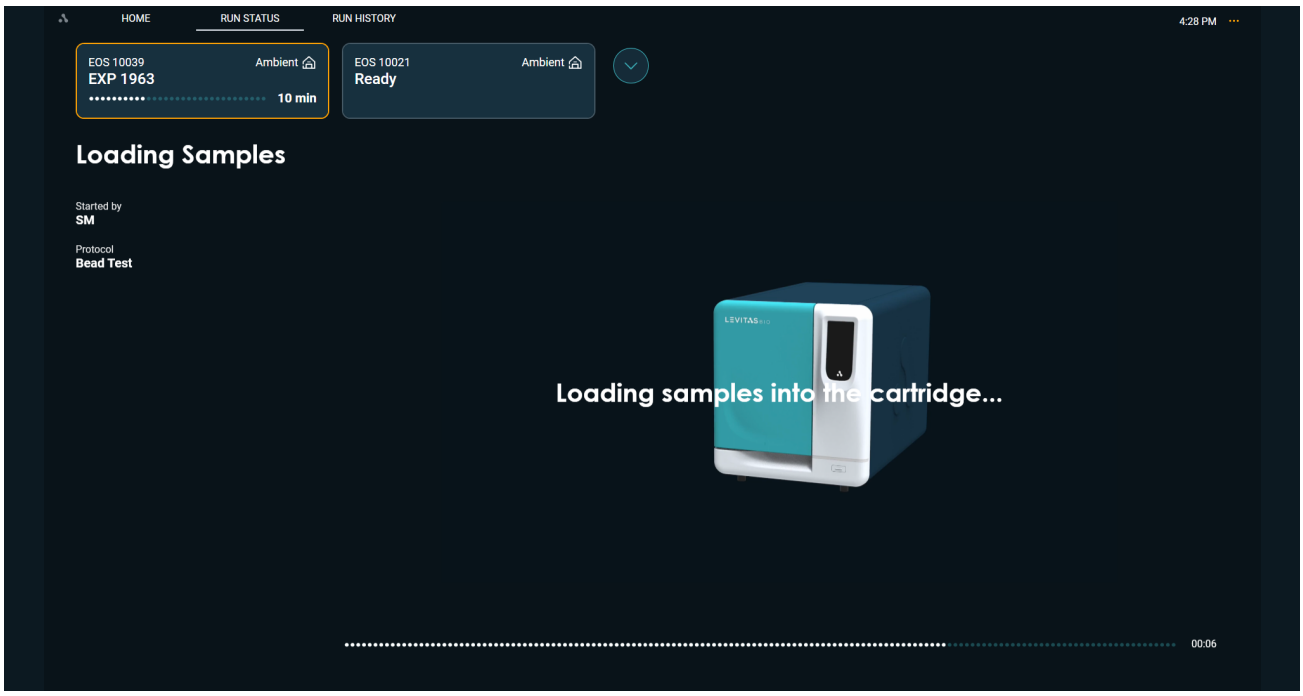


Figure 76. Loading samples into the cartridge

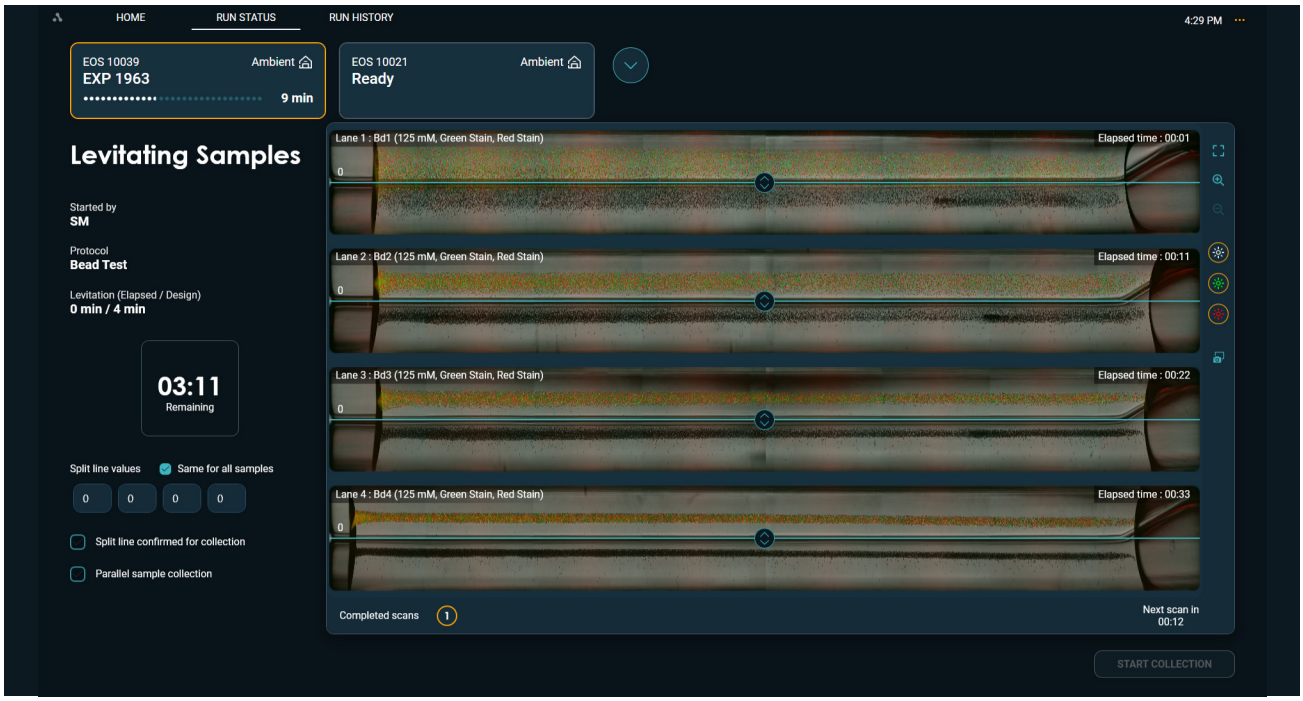


Figure 77. *Beads levitating and image scanning*

11. **Set Split Line Value.** When the levitation timer indicates “Ready”, you may adjust the split line to your preferred position. The default split value is set at “0”; use this setting for the Bead Test.

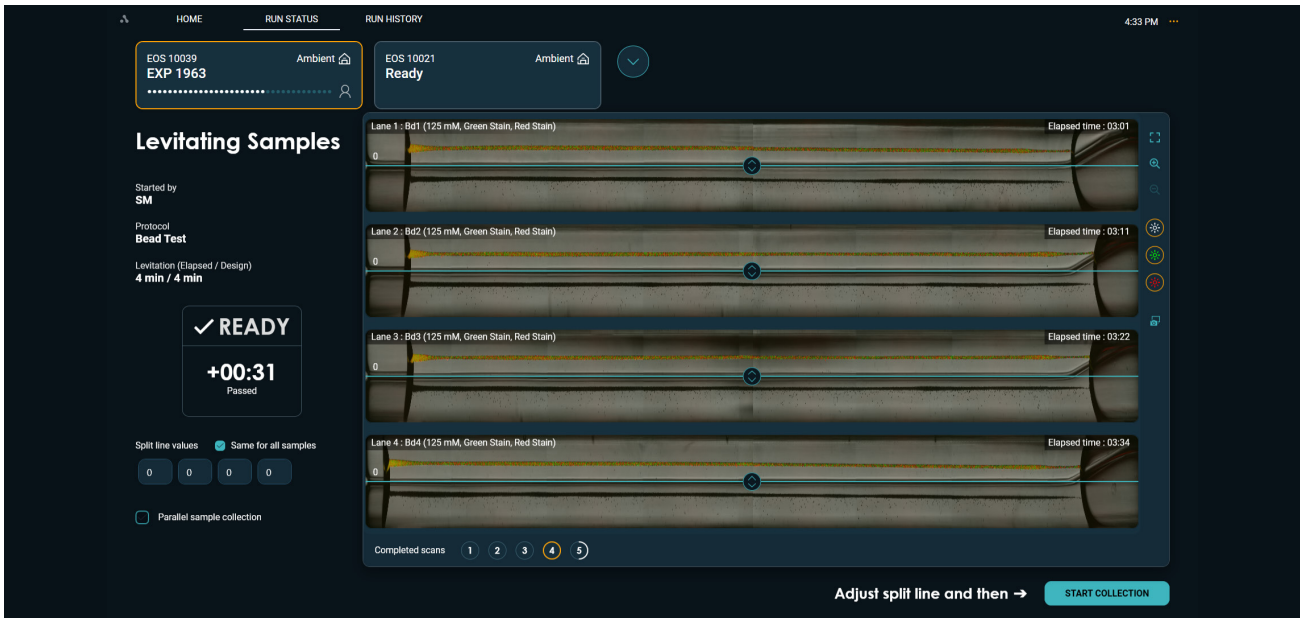
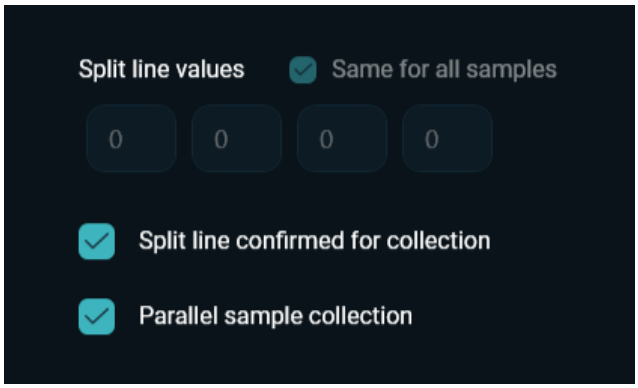


Figure 78. *Set split line when levitation has completed and start collection*



Before Collection, note that the system will default to different collection workflow based on the protocol selected. The choice to collect samples serially or in parallel is available via the check box below the split line entry.

Figure 79.
Check boxes for confirming split line and parallel sample collection

			Default Sample Collection	
Core Type	**Protocol**	**Run Temperature**	**Sequential**	**Parallel**
Non-TEC (Standard)	Cell	Ambient	**X**	
	Nuclei	Ambient		**X**
	Bead	Ambient	**X**	
TEC	Cell	CRT	**X**	
	Nuclei	Cold, Cool, CRT		**X**
	Bead	Cold, Cool, CRT		**X**

Table 15. *Default collection workflow for different protocols*

If the Parallel Sample Collection box is **checked**, only one lane (the first lane with a sample out of the 4 input wells) will be imaged during collection. Therefore, if using LeviMetrics analysis software, only one lane can be analyzed for fractionation.

NOTE: Any time Parallel Sample Collection is checked, only a single split line value can be used.

If the Parallel sample collection box is **unchecked**, the imaging during collection is done one lane at a time for all lanes with sample (even if the same split line value is the same). There will be a 1 min interval between each collection. Fractionation analysis can be performed for all samples run.

NOTE: Fractionation Analysis is not available for Nuclei samples. It is recommended to collect these samples in parallel and the default option is checked.

12. **Start Collection.** Once the split line is set, click **Start Collection**. This will begin the collection process as specified by whether the box “Parallel sample collection” has been checked.

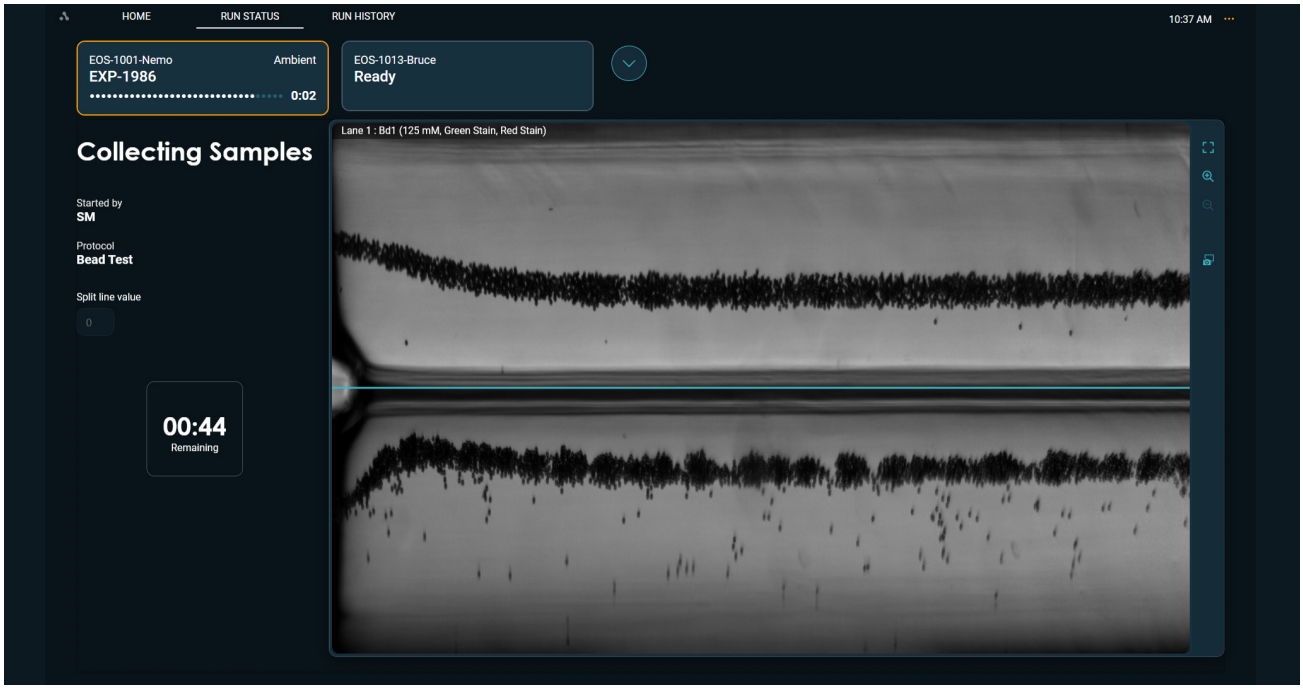


Figure 80. Imaging collection of Lane 1 as it flows to the outlet well

Figure 81. Fluorescence collection

13. **When the top and bottom output fractions have been completely collected, the system will unclamp the cartridge.**

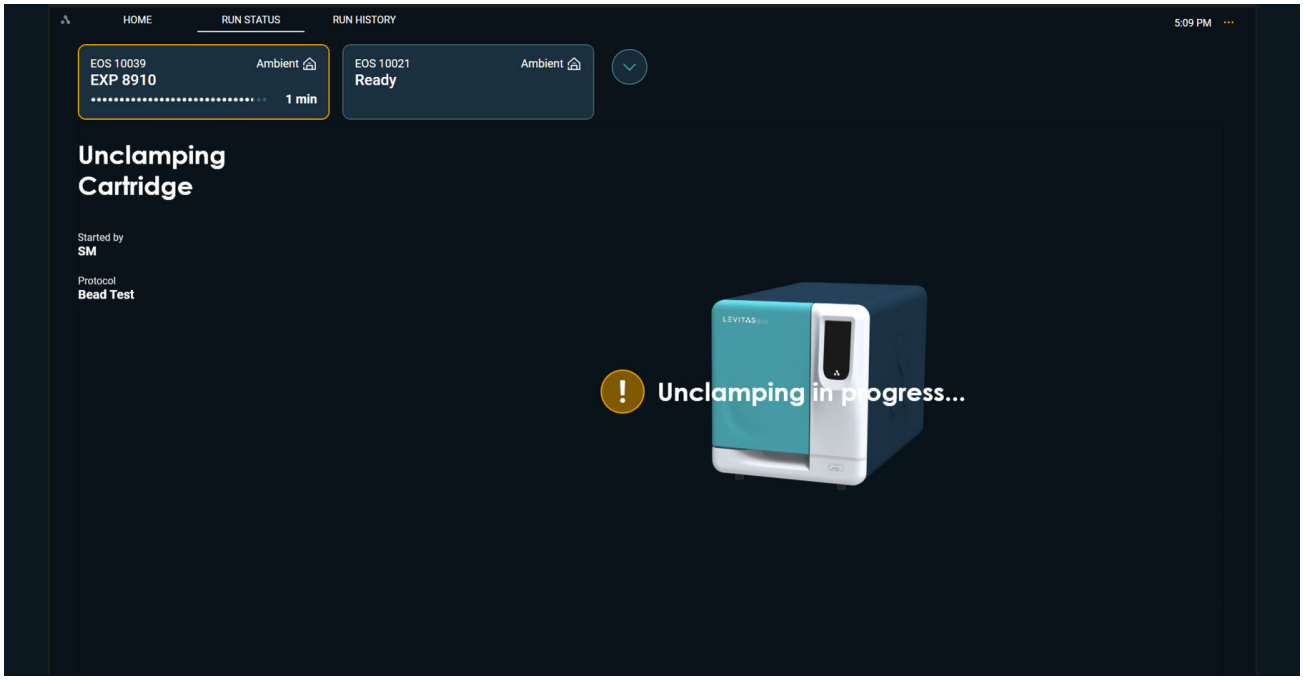


Figure 82. *EOS Module will unclamp the cartridge at the end of collection*

14. **Retrieve cartridge and harvest output**
    a. When prompted, remove the cartridge from the system and place flat on a bench top.

Figure 83. *Retrieve cartridge from EOS Module*

b. Holding the cartridge with one hand, hold the cartridge in place by the plastic on either side of the outlet well, pushing down firmly to steady the part.

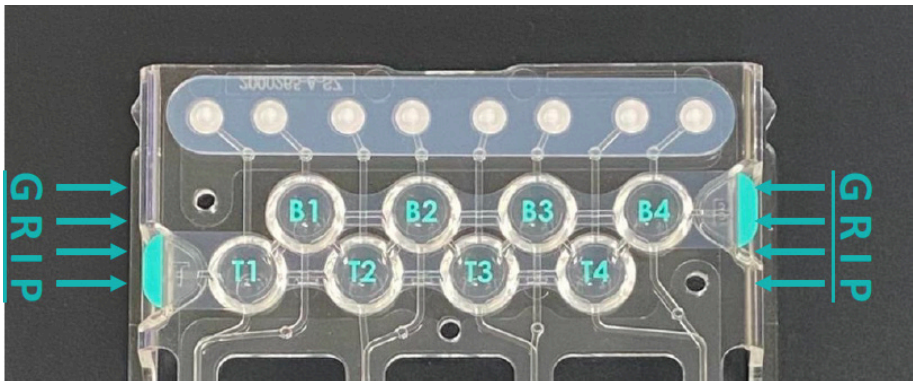


Figure 84. *Grip locations on the cartridge when peeling outlet well tape off*

c. Peel the top output well cover (labeled T1-T4) back in one fluid motion using the tabs that hang to the side of the output wells and dispose.

d. Pipette mix each sample 3-5 times before retrieval without introducing bubbles, to resuspend beads that may have settled.

e. Aspirate all liquid from the output well, not the channel leading to it, into a 1.5mL or 8-strip tube.

f. Measure the final output volume using a pipette. When the split line is set to 0, typical recovery is between 70-100 µL.

g. Repeat these steps for the bottom outlet wells (labeled B1-B4).

15. Generating Run Report: A Run Report will be generated and image analysis will occur

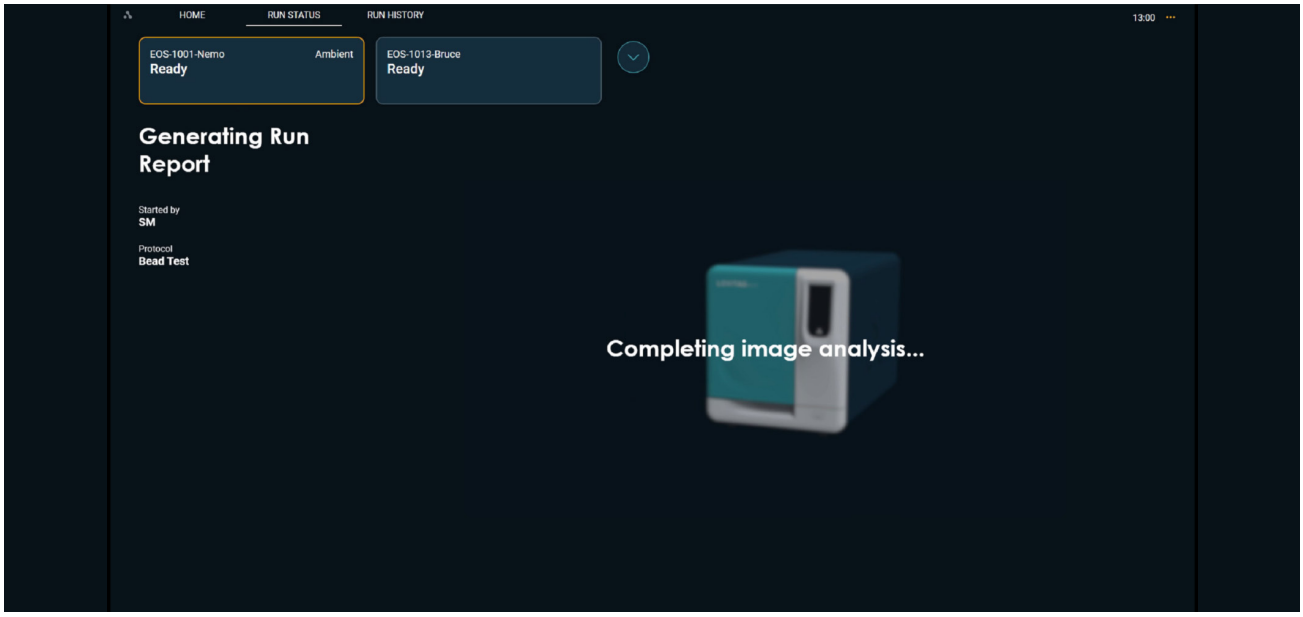


Figure 85. *Generating Run Report after image analysis is completed*

16. **Run complete:** The screen will display the Run Complete status screen. Click **Done** to return to the home screen.

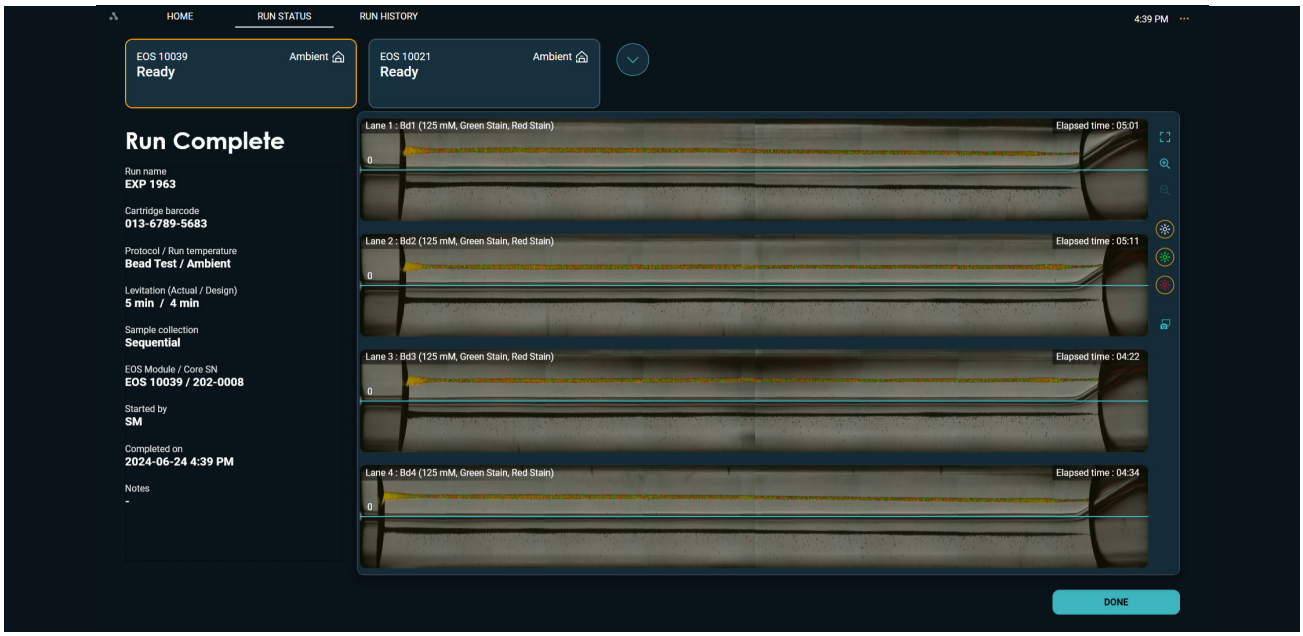


Figure 86. Run Complete screen

If the Bead Test was run with a temperature controlled core, the system will end thermal regulation. If the Cold or Cool temperatures were used, the post-run process will start. Air will be blown for approximately 45 mins (highlighted in Figure 87). This can be interrupted at any time for a new run to be started.

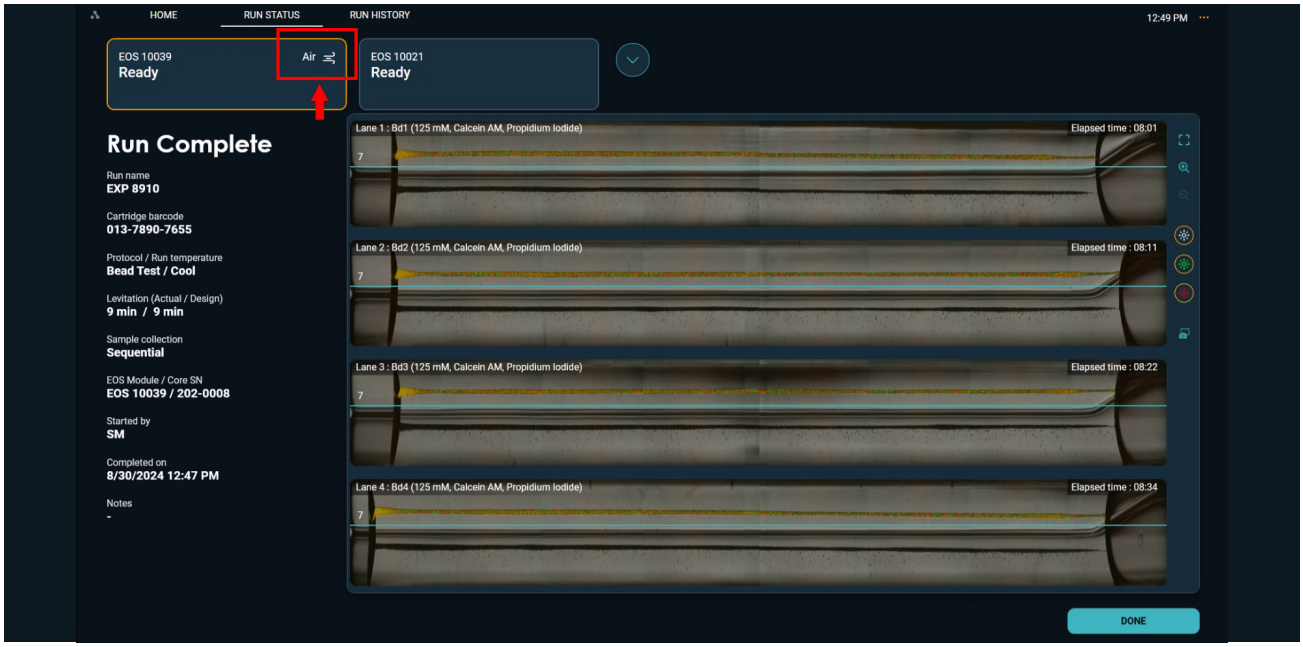


Figure 87. Run complete if Bead Test is run cold or cool

C. Count Beads (optional)

1. Count input beads and Top and Bottom output wells on a hemocytometer.
2. Use metrics shown in Table 16 to assess the separation and recovery metrics for each lane.

Measurement (unit)	Measurement Abbreviated Name	Calculation
Top Output Volume (µL) *	TV	-
Bottom Output Volume (µL) *	BV	-
Counting volume (µL)	CV	-
Volume Recovery (%)	-	(TV + CV + BV + CV) / 220
Top Fluorescent Counts (beads)	TFC	-
Top Total Counts (beads)	TTC	-
Input Fluorescent Counts (beads)	IFC	-
Purity (%)	-	TFC / TTC × 100
Yield (%)	-	TFC / IFC × 100

* *After removal of volume for counting, if performed.*

Table 16. *Formulas for calculating separation and recovery metrics for Bead Test*

RUNNING AN EXPERIMENT

A Viable cell enrichment experiment is very similar to running the Bead Test performance qualification. All the same steps would be performed. As described, viable cells will levitate to the top half of the separation channel while dead, dying or debris will levitate lower to the bottom half of the separation channel.

Viable cell enrichment can be conducted in a label-free manner. The LeviCell EOS is also compatible with LeviSelect kits for targeted viable cell enrichment. Follow the protocol included in the LeviSelect kits and perform enrichment on the LeviCell EOS.

NOTE: If running with temperature control, please refer to the Temperature Control Core Module section to learn more about the cooling process and post run process.

Viable Cell Enrichment Protocol

A. Prepare Levitation Buffer and Sample

1. Levitation Buffer is prepared by diluting the Levitation Agent stock solution with your preferred cell media (Table 13). This Levitation Buffer will be used to resuspend the final cell sample prior to loading.

NOTE: It is not uncommon to have particulates in the media, particularly if it contains FBS. Therefore, for best results on the EOS-4 cartridge the cell media should be filtered with a 0.22 µm filter prior to use in the preparation of Levitation Buffer.

2. The recommended Levitation Agent concentration for viable cell enrichment is 150 mM, as shown in Table 17. For other types of separations, the concentration of Levitation Agent may be varied as needed. Please contact Technical Support for additional recommendations.

Reagent	Volume (µL) | # of Lanes to run[1]			
	1 Ln	2 Ln	3 Ln	4 Ln
1 M Levitation Agent	45	90	135	180
Filtered Media[1]	255	510	765	1020
Total	300	600	900	1200

[1]*Recommended "Filtered Media" are either PBS + 0.5% BSA or RPMI 1640 + 10% FBS filtered with a 0.22µm filter before use.*

Table 17. *150 mM Levitation Buffer preparation for 1-4 lanes*

3. Vortex mixture well to completely mix the Levitation Buffer.

B. Prepare Cells

4. Pipet the volume of samples containing 20,000 to 1,000,000 cells per lane into an appropriately sized (1.5 - 2.0 mL) low-binding microfuge tube.*
5. Centrifuge the tube containing the cells at 300 RCF for 5 min to pellet the cells.
6. Carefully remove the supernatant.
7. Resuspend sample with the appropriate volume of Levitation Buffer per Table 18, pipetting up and down 10 times to mix thoroughly.

Reagent	Resuspension Volume (µL)			
# of Lanes to run²	**1 Ln**	**2 Ln**	**3 Ln**	**4 Ln**
Same sample, replicate lane	270	490	710	930
Single lane sample	270 ea	n/a	n/a	n/a

* *The pipetting volumes recommended will result in approximately 80% of cells being loaded onto LeviCell EOS. Input cell numbers can be increased by 20% to account for this volume loss.*

Table 18. *Resuspension volumes for 1-4 lanes*

8. Immediately after mixing, set aside 2 x 15 µL aliquots for cell counting. These 2 replicates are for the input cell counts.

NOTE: Different cell counters may require alternate volumes. If using recommend volume in step 8, additional dilution factor may need to be incorporated if required volumes are higher.

C. Running on the LeviCell EOS- Viable Cell Enrichment Protocol

9. **Start New Run** from the Home tab in LeviCell EOS Manager

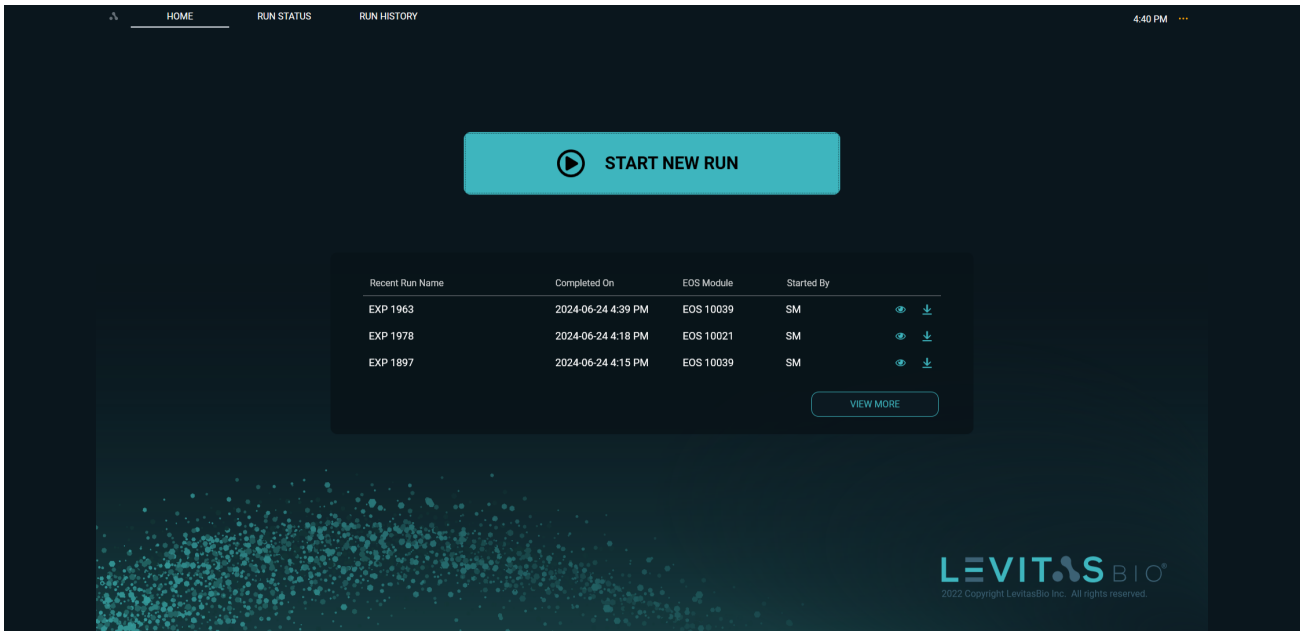


Figure 88. Home Screen

NOTE: If more than one EOS Module is connected to the Control PC, a new run may be started without disturbing a run already in progress.

10. **Scan the cartridge barcode**.

NOTE: To avoid contamination of the instrument hardware, reuse of a cartridge with unused lanes is not permitted.

Figure 89. Scan cartridge barcode information

11. Specify run information and select the instrument.

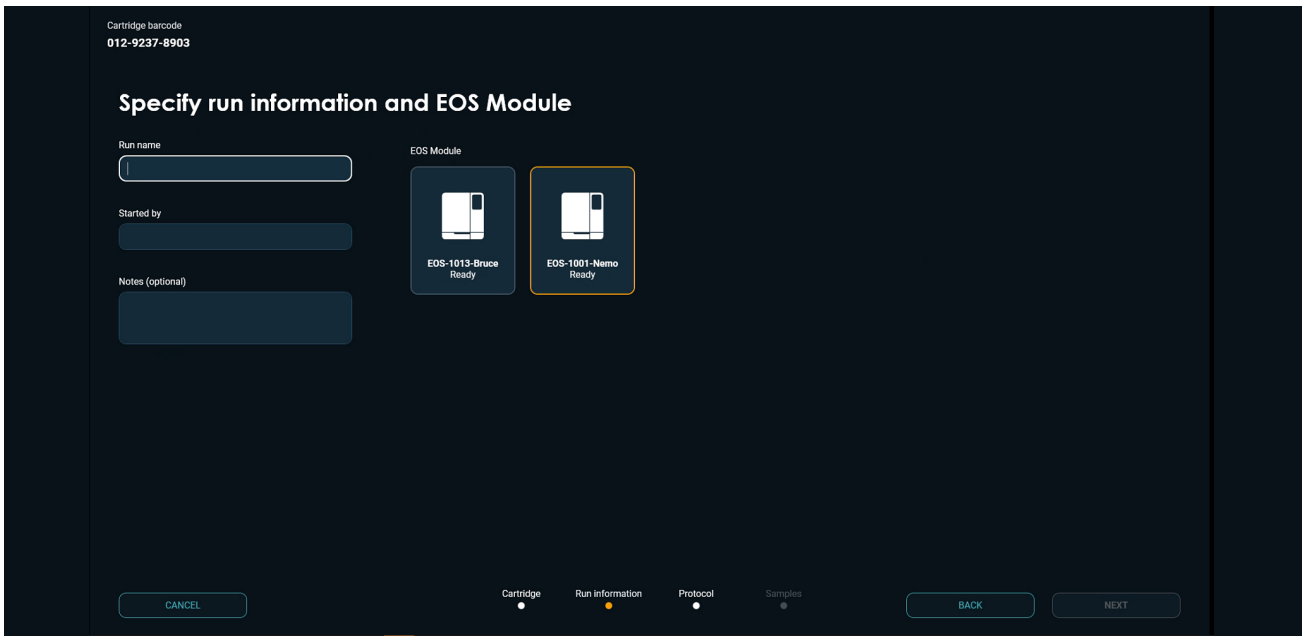


Figure 90. Specify run information

12. **Select cell protocol to run.** Select either the Small, Medium or Large Cell protocol to run a viable cell enrichment, depending on the cell size range of the cells you are separating (see Table 19).

Protocol	Size Range	Levitation Time
Small Cell	<5 µm	40 min
Medium Cell	5-20 µm	20 min
Large Cell	>20 µm	6 min
Bead Test	n/a	4 min

Table 19. *Viable cell enrichment protocol, associated cell size range and levitation time*

Figure 91. *Select protocol and temperature*

The time required to levitate cells to their equilibrium position depends on their size, and the recommended levitation time is set in each of the three default enrichment workflows.

For LeviCell EOS systems with an installed TEC Core module, only Nuclei Protocol is optimized for cool and cold run temperatures. All protocols can be run at CRT run temperature.

Temperature control ranges are listed for the separation channel of the cartridge when the sample is loaded.

Run Temperature Option	Icon Displayed	Average Sample Temperature Range
* Ambient		Variable based on environment
Cold		7°C-10°C
Cool		12°C-14°C
CRT (Controlled Room Temperature)		Variable based on environment

* *Ambient temperature is only available for Non-TEC cores*

Table 20. *Different run temperatures available and average sample temperature range*

Once an enrichment protocol and run temperature has been selected, the EOS Manager software will guide the user through the process of setting up the experiment and running samples.

13. Select and specify samples to run.

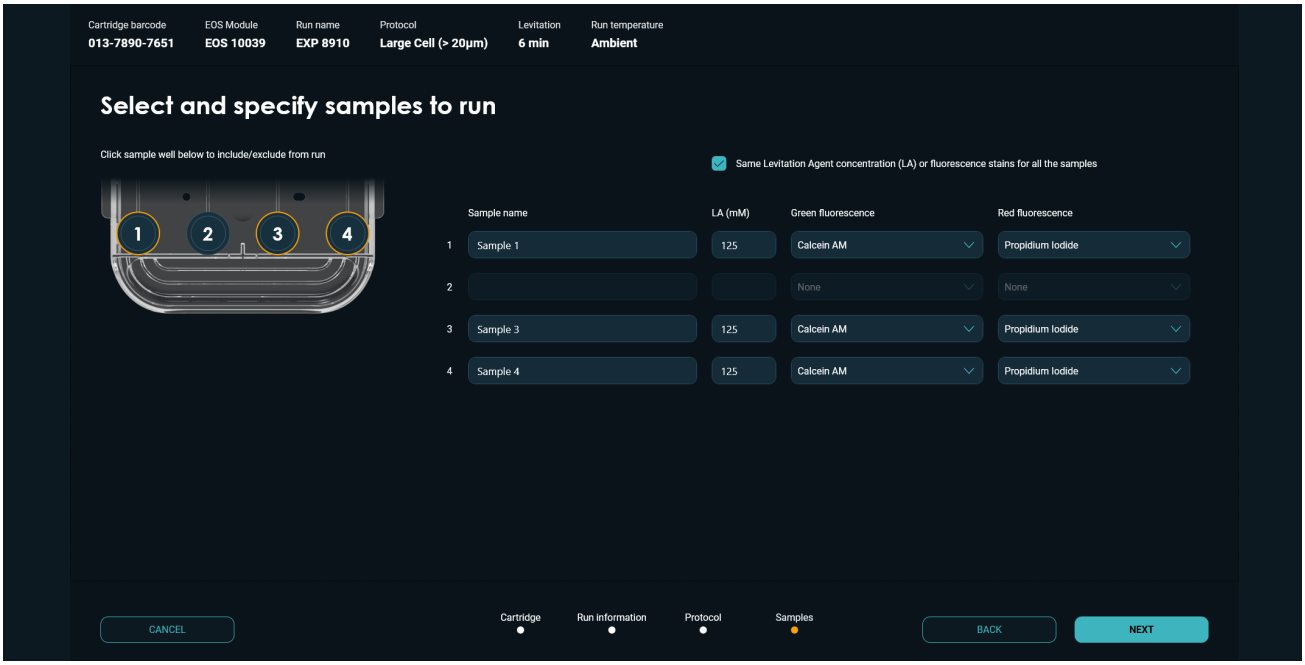


Figure 92. *Select and specify sample information*

a. By default, all samples have been selected for the run, as indicated by the gold highlight for each sample well on the cartridge. To deselect a sample, click on the sample well in the image (the image displays lane 2 deselected).

b. If Green Fluorescence or Red Fluorescence stains are used, select from the pull down menu

Figure 93. *Checkbox for same Levitation Agent and stains*

NOTE: The "Same Levitation Agent Concentration check box" is selected by default. Uncheck if using different Levitation Agent concentrations or fluorescent stains between the samples that will be run.

14. After entering all the sample information click Next to begin guided sample loading process

Figure 94. *Open door to insert cartridge*

15. Follow the on-screen instructions to insert a cartridge into the EOS Module. Insert your cartridge into the system by holding it by the grip. A clamp will engage after closing the door. Keep fingers clear of the clamp mechanism at all times.

CAUTION: Potential Pinch Hazard.

Figure 95. *Insert cartridge into EOS Module*

16. **A Pre-Scan will occur.** This provides a baseline background image of the cartridge separation channels which will be used as part of the imaging.

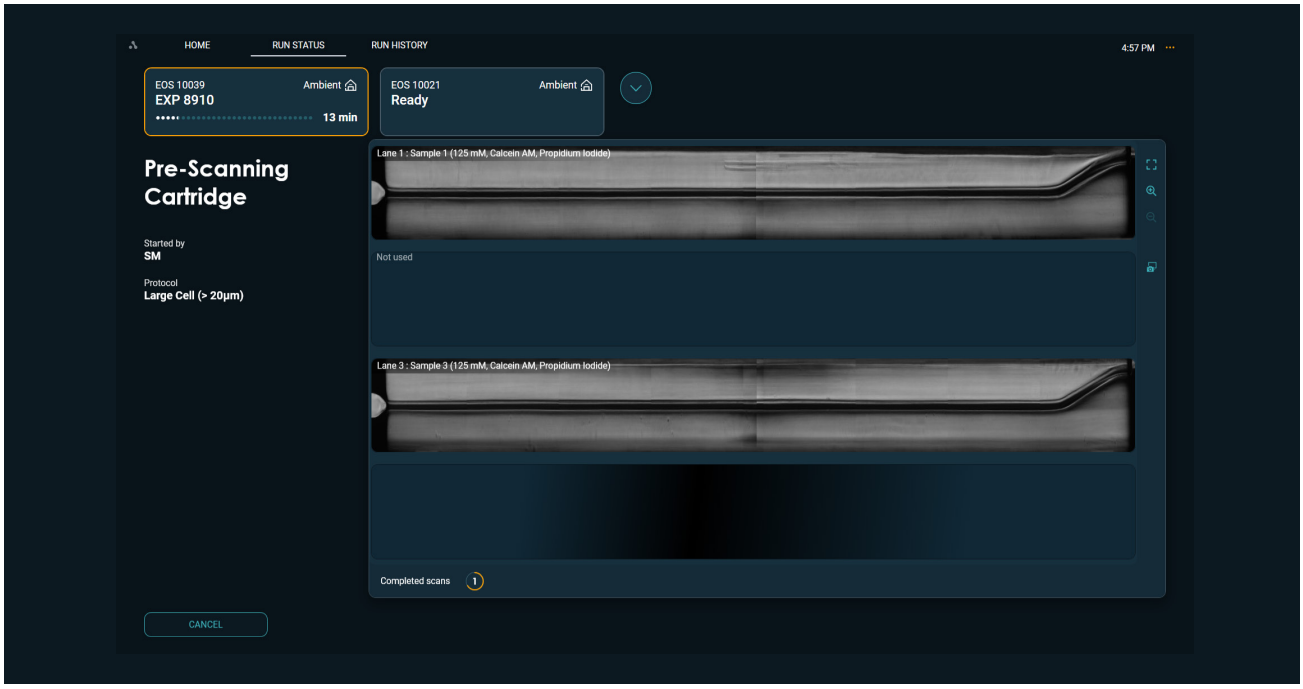


Figure 96. *Prescanning the cartridge*

17. **Follow subsequent prompts to then dispense samples when prompted**

NOTE: Mix sample thoroughly by pipetting up and down gently 5X and immediately load 220 µL into each of the corresponding input wells.

Place the tip of the pipette in front of the inlet hole, taking care not to insert the tip into the hole, and dispense to the first stop. This is to lower the probability of creating small bubbles within the sample.

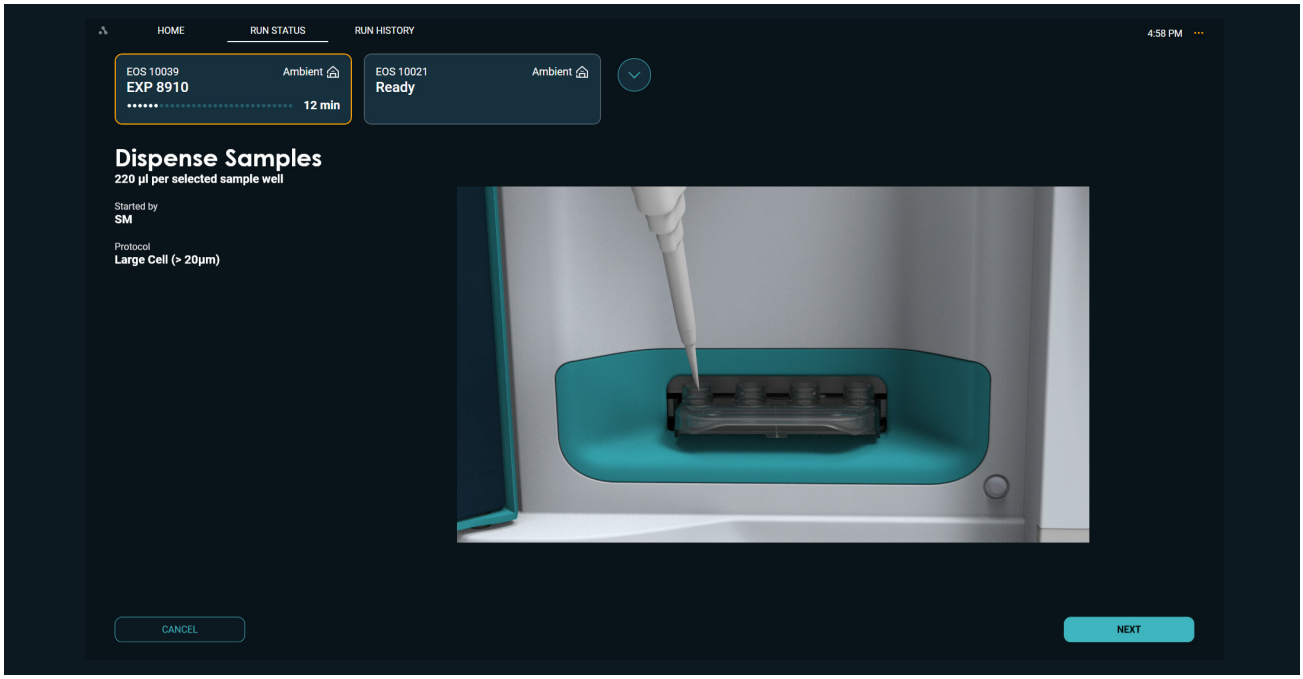


Figure 97. *Dispensing sample into the input wells*

18. **The LeviCell EOS will automatically load the sample upon closing the door. Scanning will begin.** The time remaining for levitation will be displayed

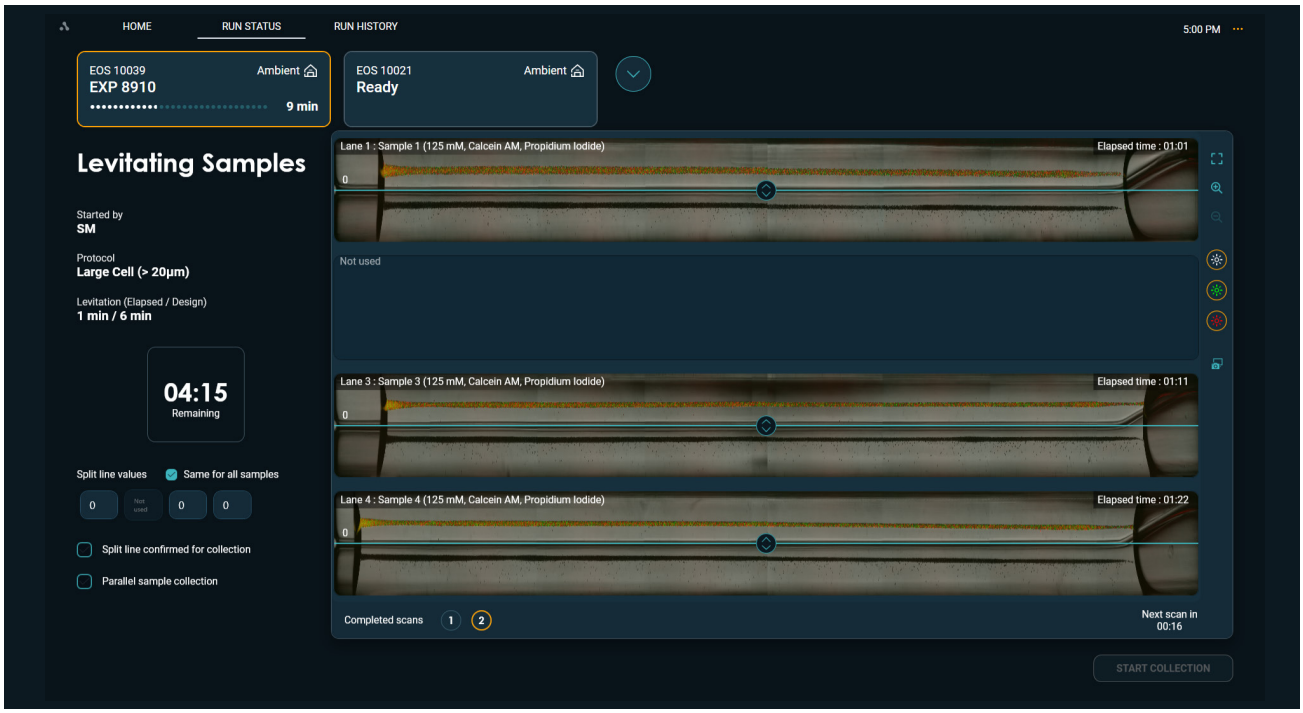


Figure 98. *Levitating samples*

19. **Set Split Line Value and choose collection options.** When the levitation timer indicates "**Ready**" the split value must be set.

NOTE: If split line value is known prior to levitation time completion it can be entered into the split line value box. If automatic collection is desired after levitation is complete, check the box "Split line confirmed for collection".

Before Collection, note that the system will default to different collection workflow based on the protocol selected. The choice to collect samples sequentially or in parallel is available via the check box below the split line entry.

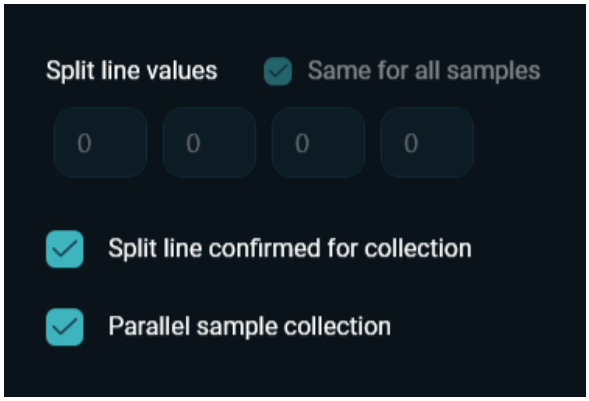


Figure 99.
Check boxes for confirming split line and parallel sample collection

			Default Sample Collection	
Core Type	**Protocol**	**Run Temperature**	**Sequential**	**Parallel**
Non-TEC (Standard)	Cell	Ambient	**X**	
	Nuclei	Ambient		**X**
	Bead	Ambient	**X**	
TEC	Cell	CRT	**X**	
	Nuclei	Cold, Cool, CRT		**X**
	Bead	Cold, Cool, CRT		**X**

* *Ambient temperature is only available for Non-TEC cores*

Table 21. *Default collection workflow for different protocols*

If the Parallel sample collection box is checked, only one lane (the first lane with a sample out of the 4 input wells) will be imaged during collection. Therefore, if using LeviMetrics analysis software, only one lane can be analyzed for fractionation.

NOTE: Any time Parallel Sample Collection is checked, only a single split line value can be used.

If the Parallel sample collection box is unchecked, the imaging during collection is done one lane at a time for all lanes with sample (even if the same split line value is the same). There will be a 1 min interval between each collection. Fractionation analysis can be performed for all samples run.

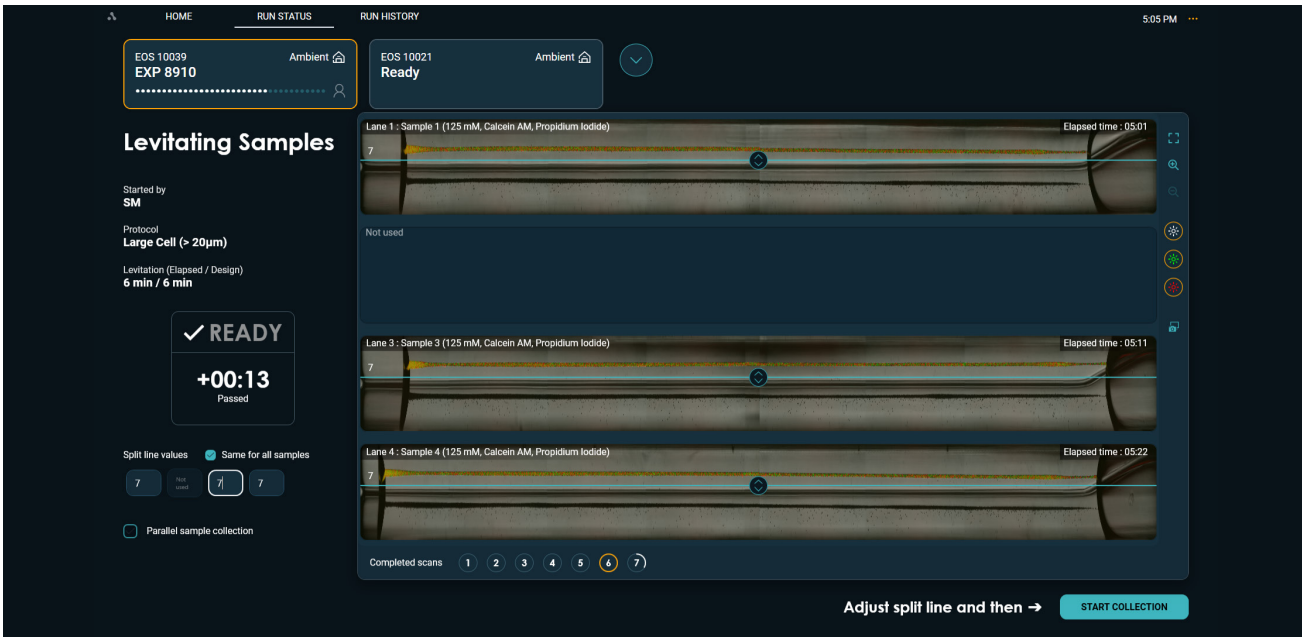


Figure 100. *Set split line when levitation has completed and start collection*

20. Start Collection. Samples will now be collected into outlet wells.

Once the split line is set, click **Start Collection**. This will begin the cell collection process. The imaging during collection is done one lane at a time for all lanes with sample (even if the same split line value is the same). There will be a 1 min interval between each collection.

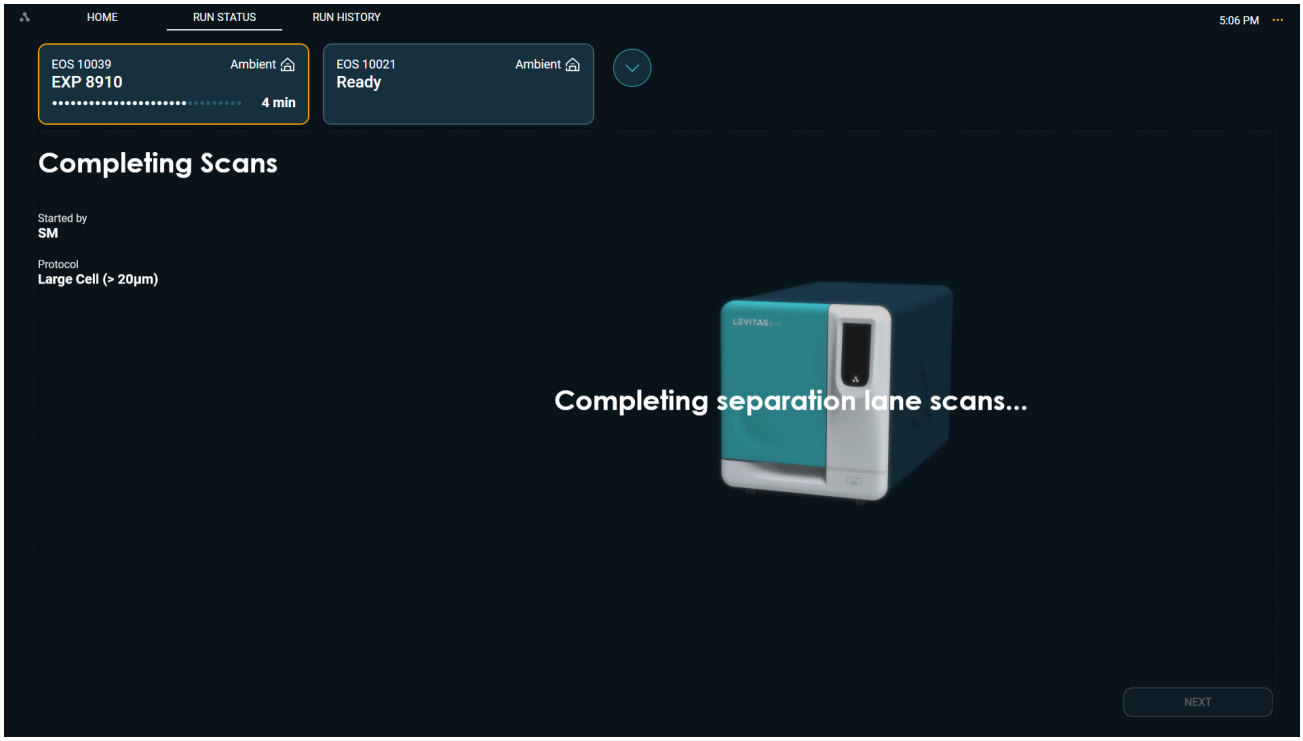


Figure 101. *Completing scan of cartridge*

NOTE: If the current lane scanning is partially completed (>50%) when the Start Collection button is clicked, the system will automatically complete the lane scan prior to collection.

If the current lane scanning is <50% completed, then the sample collection will proceed immediately.

21. **Sample collection imaging will occur on all lanes, even if the same split line value is chosen for all samples.** This will display in a fully zoomed view in real time.

Figure 102. *Imaging collection of Lane 1 as it flows to the outlet well*

When the top and bottom output fractions have been completely collected into the outlet wells, the system will unclamp the cartridge.

22. **Retrieve cartridge and harvest output:** The system will instruct user to retrieve cartridge from the module

Figure 103. *Retrieve cartridge from EOS Module*

a. **When prompted, remove the cartridge from the system and place flat on a bench top.**

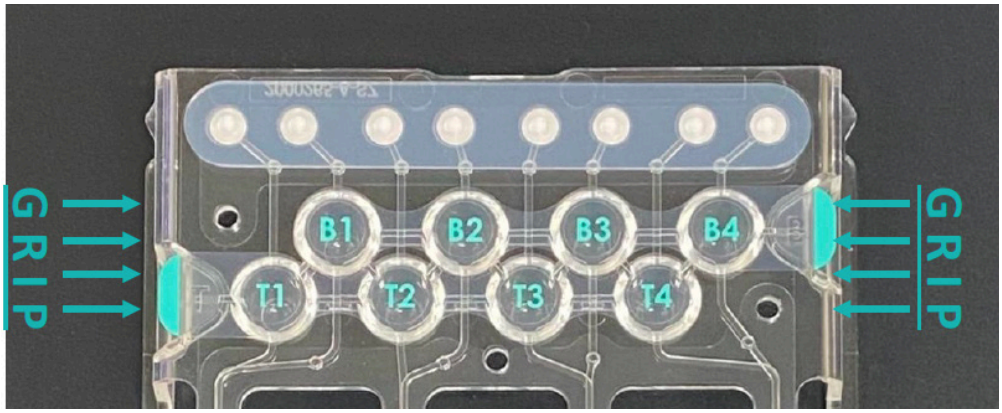


Figure 104. *Grip locations on the cartridge when peeling outlet well tape off*

b. **Use your non-dominant hand, hold the cartridge in place by the plastic on either side of the outlet well, pushing down firmly to steady the part.**
c. **Peel the top output well cover (labeled T1-T4) back in one fluid motion using the tabs that hang to the side of the output wells and dispose of according to biohazardous waste protocols of the institution.**
d. **Pipette mix each sample 3-5 times before retrieval without introducing bubbles.**
e. **Aspirate all liquid from the output well, not the channel leading to it, into a 1.5mL or 8-strip tube.**
f. **Measure the final output volume using a pipette.** When the split line is set to 0, typical recovery is between 70-100 µL.
g. **These steps may be repeated for the bottom outlet wells (labeled B1-B4) if desired.**
h. **Set aside 10 to 15 µL for cell counting of your output sample in step C1.**

23. Generating Run Report. A Run Report will be generated and image analysis will occur

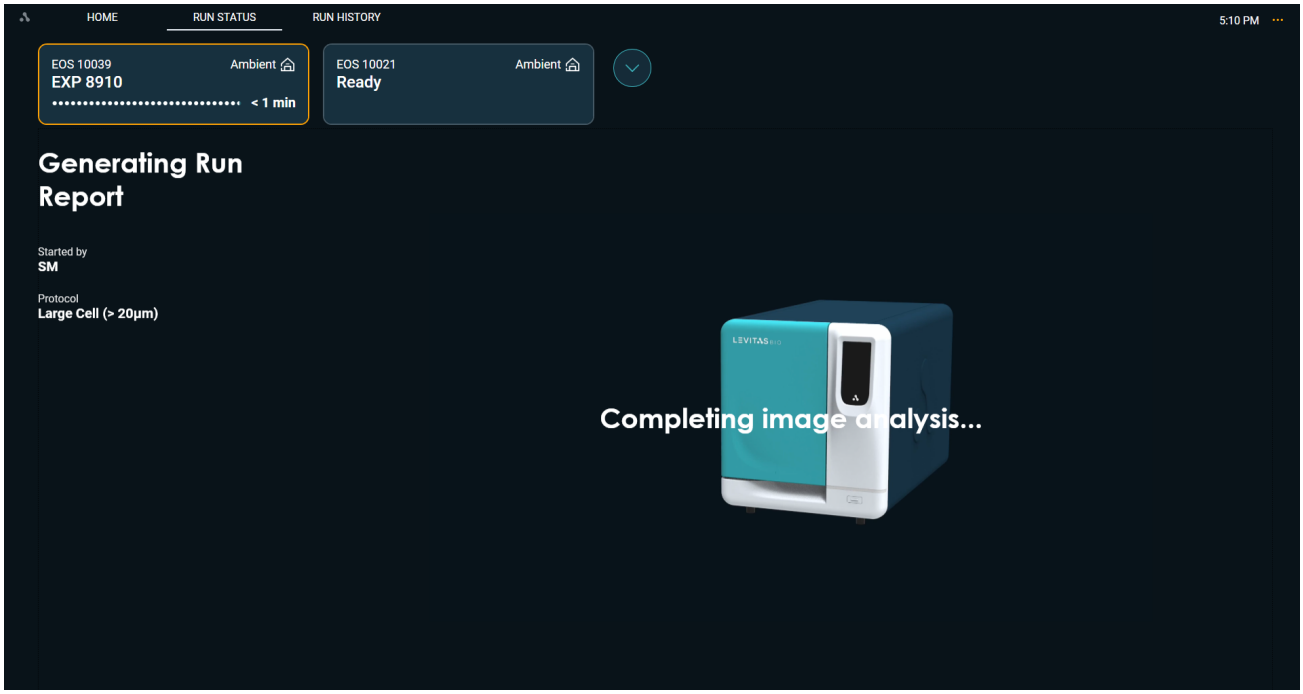


Figure 105. *Generating Run Report after image analysis is completed*

24. The screen will display the Run Complete status screen. Click **Done** to return to the home screen.

Figure 106. *Run Complete screen*

If a Nuclei protocol was run in either cold or cool temperatures the system will end thermal regulation and start the post-run process. This can be interrupted at any time for a new run to be started.

For Nuclei Protocol Only

If a Nuclei protocol was run in either cold or cool run temperature, a Finalizing Levitation step will be added prior to collection. Imaging will still occur at this time and can be visualized in the LeviMetrics Software. Total levitation time will be displayed as 25 mins plus any additional extra levitation time that has elapsed prior to clicking **Start Collection**. If the run was performed at CRT temperature, collection will happen immediately.

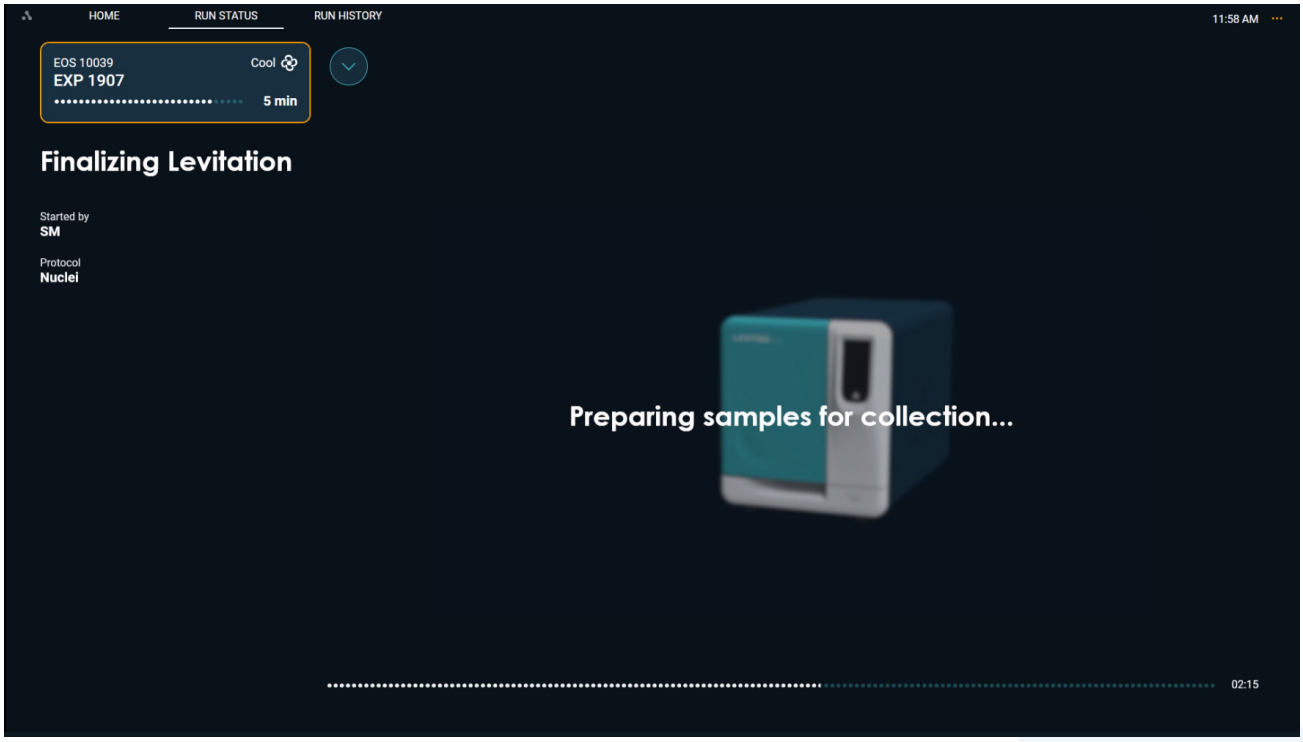


Figure 107. Finalizing levitation and prepare samples for collection

D. Count Cells

1. Perform cell counts using the 10 to 15 µL aliquots of sample input and output collected. LevitasBio recommends the Nexcelom™ cell counter along with a live/dead stain such as AO/PI for cell counting.

TIP: Precious samples can be diluted to remain within the linear counting range of your cell counter to save on the volume of sample used for counting.

Ensure that calculation for dilution is based on the number of live or dead cells being counted for the outlet wells and that final concentration is well within the linear counting range.

e.g. For the Nexcelom K2 Counter the range for an accurate count at 2X dilution is between 100,000 to 10,000,000 cells/mL. Dilute your concentrated sample to this range prior to counting.

Run Summary and Data Files

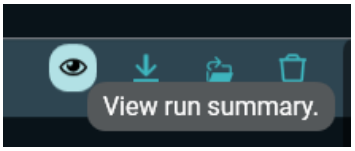


Figure 108. *Hover over and run summary*

Run Summary screen can be displayed using the Run History tools.

A Run Summary PDF may be exported containing all details from the run including information entered by the user for run setup, the run time and split line value.

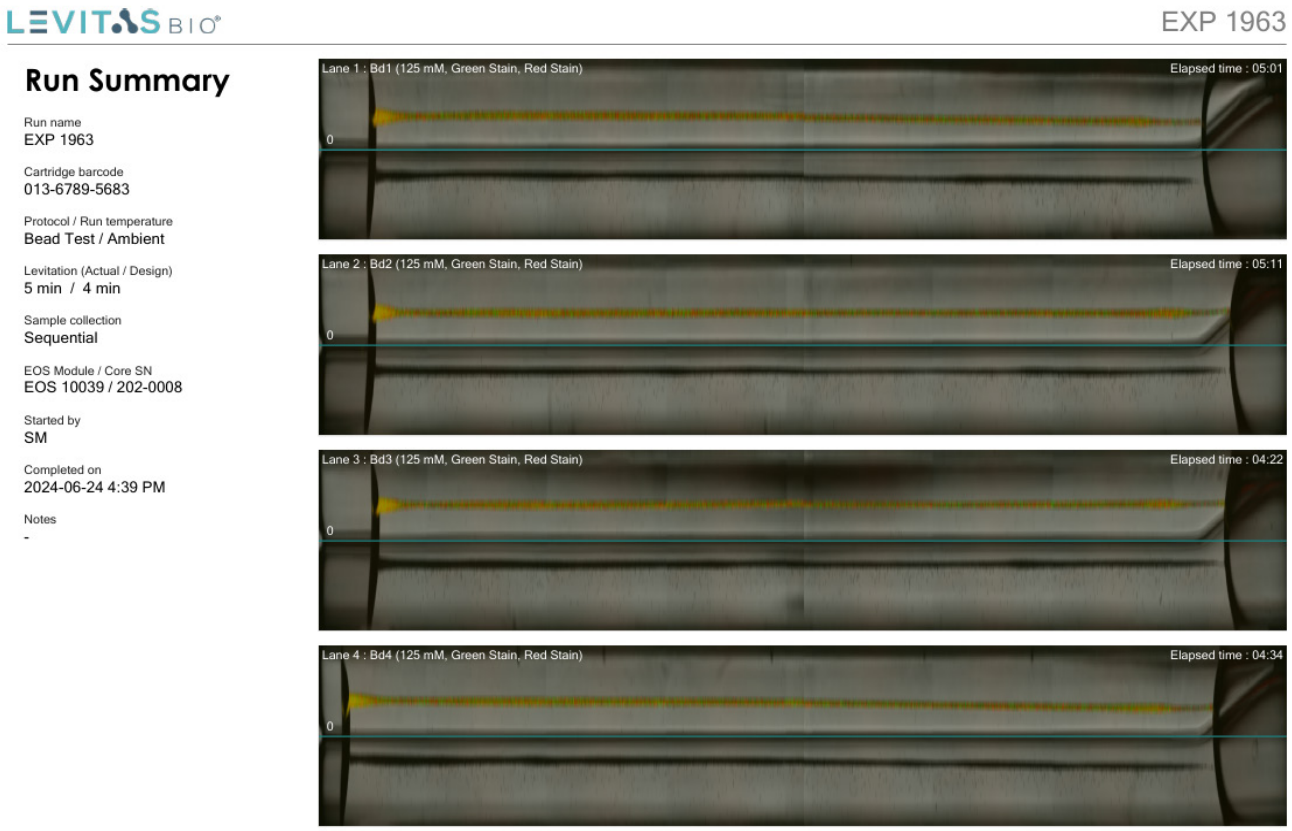


Figure 109. *PDF report of the Run Summary*

To export the Run Summary to a USB drive connected to the Control PC, click on the download icon in the RUN HISTORY tab next to the Run Name of interest.

The last 3 runs can also be found on the HOME tab. The view and download icons are also available for quicker access.

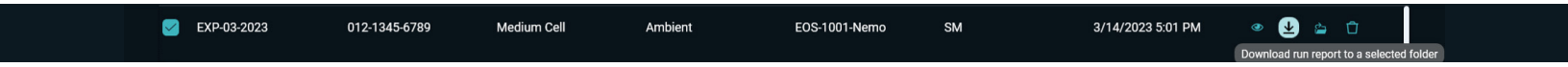


Figure 110. *Run Summary download*

To download an entire run folder or folders, check the runs desired and use the **DOWNLOAD RUNS** button at the bottom right of Run History.

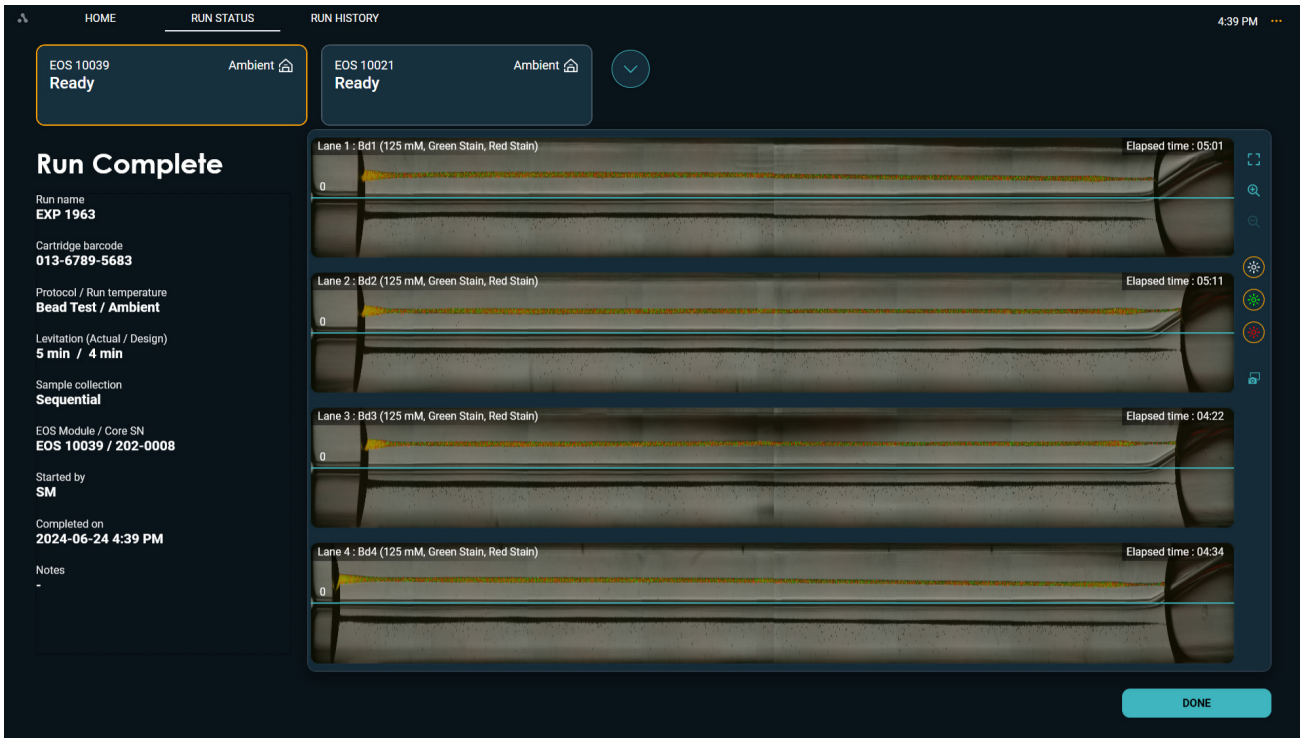


Figure 111. *Run complete screen*

To view all data files associated with your run, click on the file explorer icon.

Figure 112. *Button to access files via file explorer*

The file structure for each run is shown:

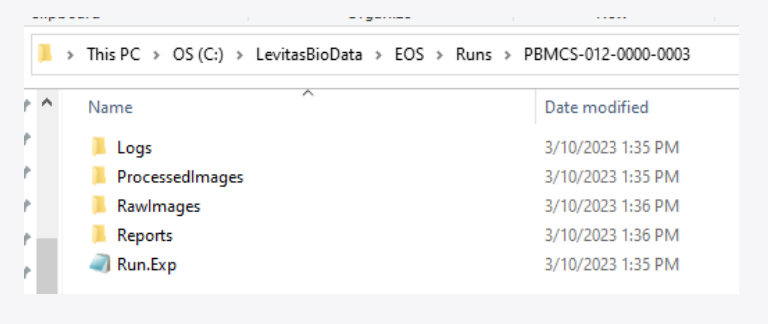


Figure 113. *File Structure*

The Run.Exp is a special file format that is used with LeviMetrics software to view your run. See the **Introduction to LeviMetrics Software** section in this user guide.

The Reports folder contains the Run Summary and associated montage images for each lane, and is the primary resource for your run data.

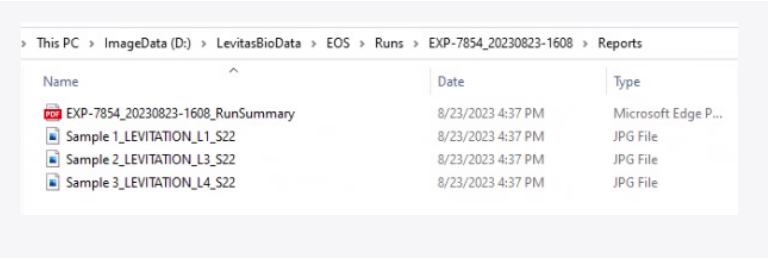


Figure 114. *Reports folder*

The montage images are named by the sample name, the imaged lane, and the last full scan number, from which they were processed (Scan 22 in this case).

The ProcessedImages folder contains subfolders with images gathered during either the Pre-Scan, before sample loading, Levitation, or during sample Collection. These images are in montage format. These images are used by LeviMetrics to show your run at various time points.

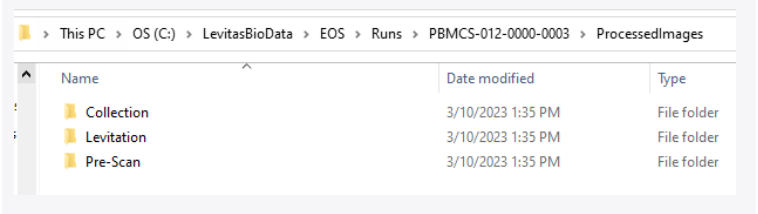


Figure 115. *Processed image folders*

The filenames in these folders include the Sample Name, Stain Name (or Brightfield), Step Name, associated Lane, scan number (S01, S02,...). Ex.: Sample1_Red Stain_Levitation_L1_S01

Figure 116. *Example filename format*

The RawImages folder contains the individual snapshots taken along the length of each lane. They have the same filename format as processed images, but with an additional indicator for position (P1-P9). These are useful when higher resolution viewing of a fraction of the sample is needed.

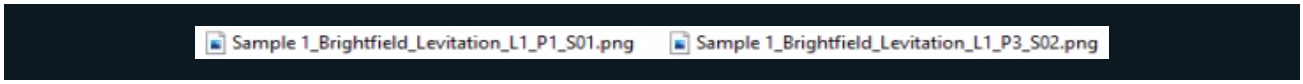


Figure 117. *Image raw files*

P1, or position 1, corresponds to the output end of the lane, whereas P9 corresponds to the input. Note that P5 always includes dark shadows that are associated with the clamping of the cartridge. This section has been cut from the montage images for clarity.

The Logs folder contains logs used for troubleshooting or service needs by LevitasBio.

Introduction to LeviMetrics Software

LeviMetrics Software is a standalone software program to aid in the visualization of LeviCell EOS runs and provide valuable sample characterization metrics. The basic level software can be downloaded via **www.levitasbio.com/support**. The software is compatible with any Windows 10 or 11 PC computer.

To view the run and perform sample characterization, open the “Run.exp” file in the LeviMetrics Analysis Software. Full montage views of each scan during the run, and sample collection for lane 1 are easily navigated. Zoom in for detailed views, and export movies of levitation or collection.

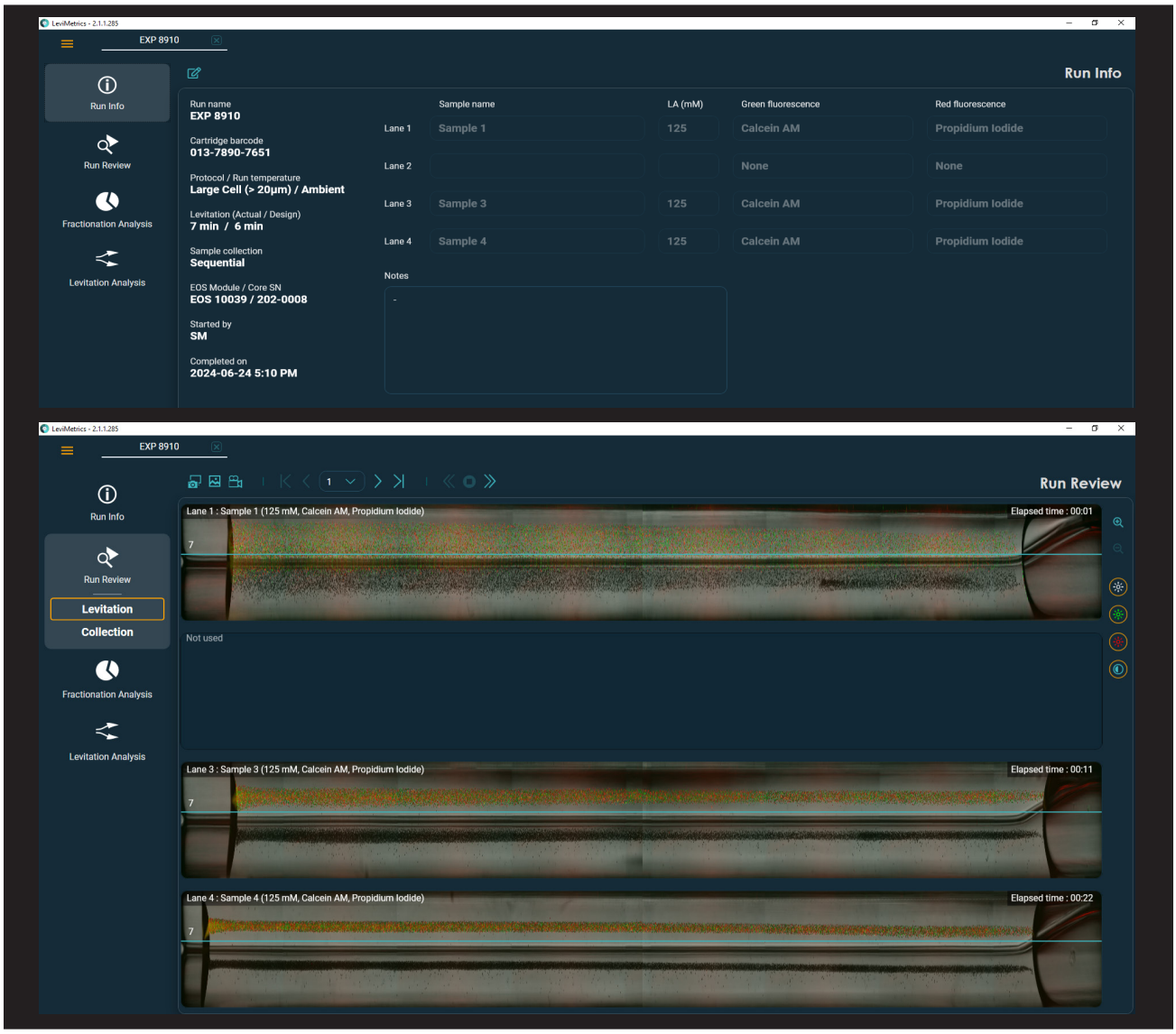


Figure 118. *LeviMetrics Software experiment screen*

Refer to the LeviMetrics Quick Reference Guide for more information and details on how to use the software.

UPDATING SOFTWARE

The EOS Manager software can easily be updated. When a software package becomes available, doubleclick on the installer and the software will begin updating. The install may include both EOS Manager software update and/or an EOS module software update.

If there is a software update for the (on device) EOS module, a progress screen will appear indicating that the software is updated. It is important that the Control PC or EOS Modules are not switched off during this time.

Figure 119. *Software update progress and complete screen*

EXCHANGING CORE MODULES

Step by Step Protocol for Exchanging Core Modules

The EOS Core can be easily exchanged by the user for a different application-specific core without the need for tools. To access the core, the left side panel of the instrument needs to be removed.

If assistance is needed to complete this procedure, please contact support@levitasbio.com

Before Swapping Core Modules

MAGNETIC FIELD: LeviCell EOS cores contain strong magnets that can be harmful or interfere with the operation of pacemakers or other magnetically-sensitive devices.

Wearers must not bring their devices within 150 mm (6 inches) of the exchangeable core during handling.

- Ensure the instrument has adequate space ~ 2 ft or 60 cm on the left of the instrument
- Unbox the new core and have it readily available for the swap
- In order to access the rear panel thumbscrews which secure the left panel in place, the instrument may need to be rotated counter clockwise. A second person to assist the rotation.

Installing a New Core Module

1. Power down the SBC by pressing the soft power button at the front of the instrument

<optional> Rotate the instrument counter clockwise by 10-20 degrees to access the rear of the LeviCell EOS module

2. Turn off the power to the module using the mains switch at the rear of the module.

3. Locate the thumbscrews on the rear right side on the back panel. Unscrew them to unlock the right side panel

Figure 120. *Rear panel showing thumbscrews securing left side panel*

4. Using the finger grip slots, slide the left panel left towards the back of the instrument. Once the locating posts have disengaged from the slots on the top cover, remove the door completely.

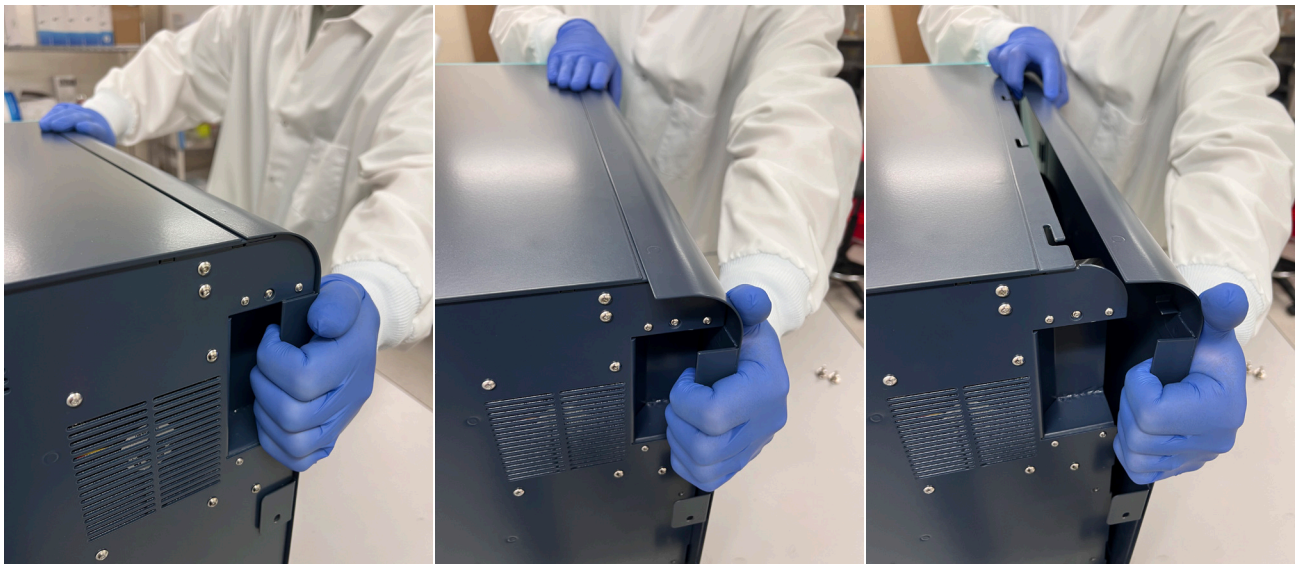


Figure 121. *LeviCell EOS left side panel removal*

5. Place the side panel in a safe location to avoid damage.

6. Locate the core cradle and cradle locks. There will be 5 total cradle locks. The lock needs to be turned into the Off position to access the core.

Figure 122. *Lock sticker located on the core.*

7. Unlock the first two outward-facing locks (as shown below) to release the sliding cradle.

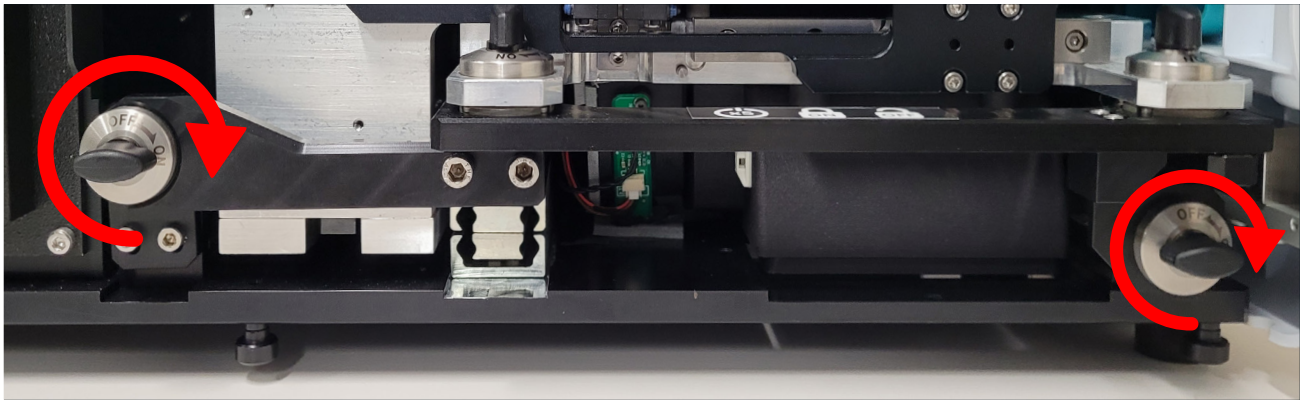


Figure 123. *Unlocking outward-facing core cradle locks to release the cradle*

8. Slide the core module cradle out completely using the colored handle

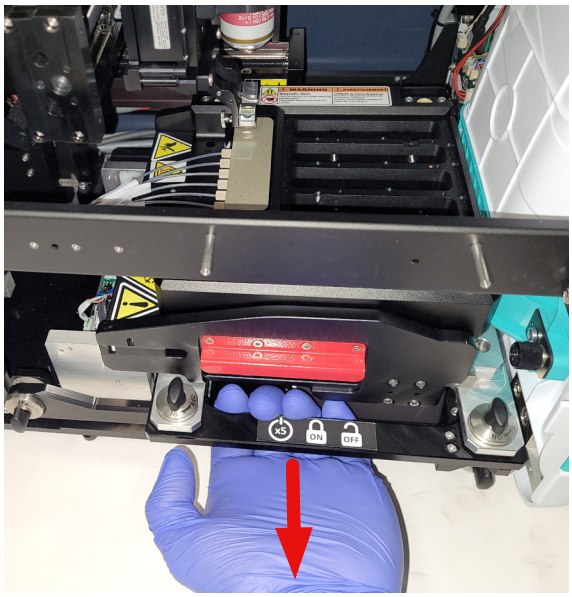


Figure 124.
Core module is mounted inside the cradle

9. Locate the pneumatic manifold block and unlock using the 2 thumbscrews by rotating counter clockwise. The screws are approximately 1 inch long

Figure 125.
Core module top view and manifold thumbscrews

10. Remove the pneumatic manifold block by sliding it back from its mounting posts.

NOTE: Avoid touching the bottom surface of the manifold

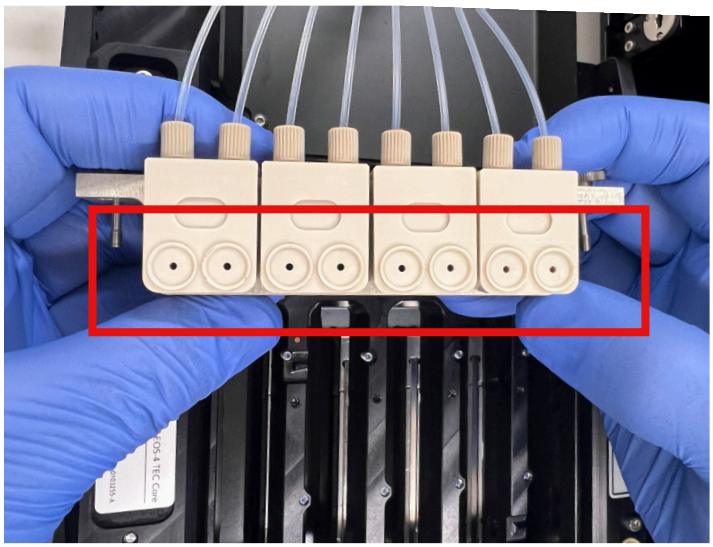


Figure 126.
Pneumatic manifold bottom view

11. Fasten the pneumatic manifold loosely to either of the two dedicated holding locations on the side rail of the instrument. The thumbscrews screw into the horizontal posts. This will help avoid handling the bottom of the manifold

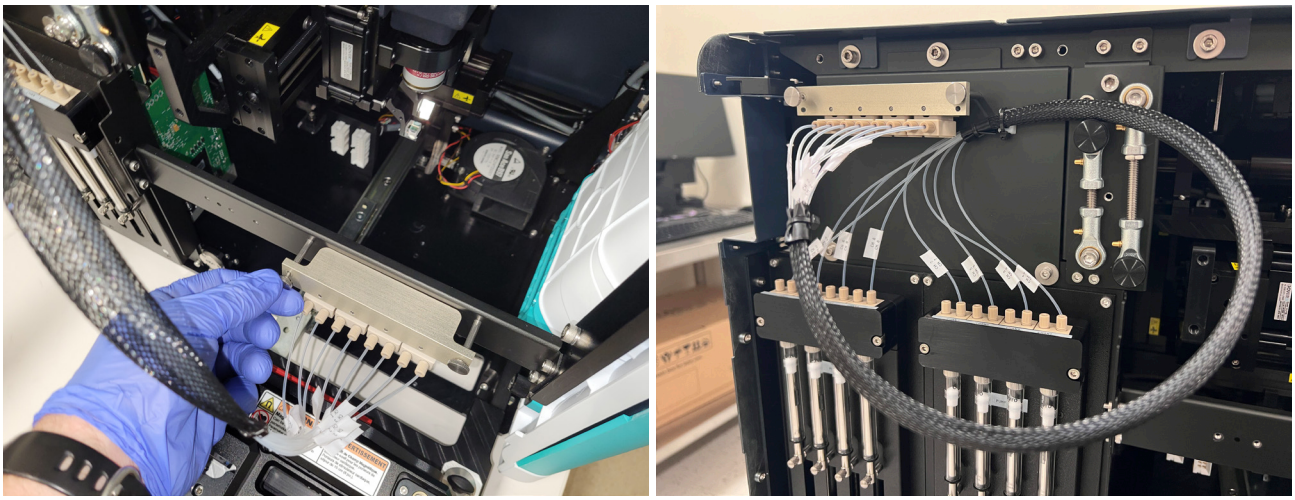


Figure 127. *Two holding locations for the pneumatic manifold*

12. With the entire core now accessible, locate the 3 remaining cradle locks (shown in the Figure 128) and unlock by switching to the OFF position

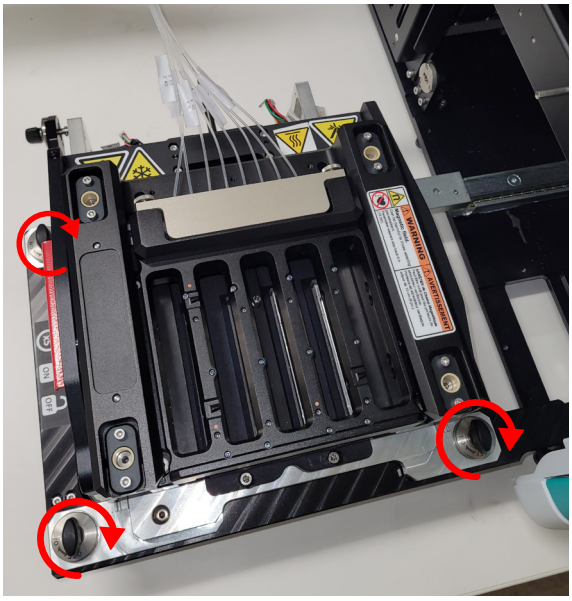


Figure 128.
Remaining cradle locks to be unlocked

13. Lift the core out of the cradle using the two colored handles on the left and right side. The core weighs approximately 9 kg (20 pounds).

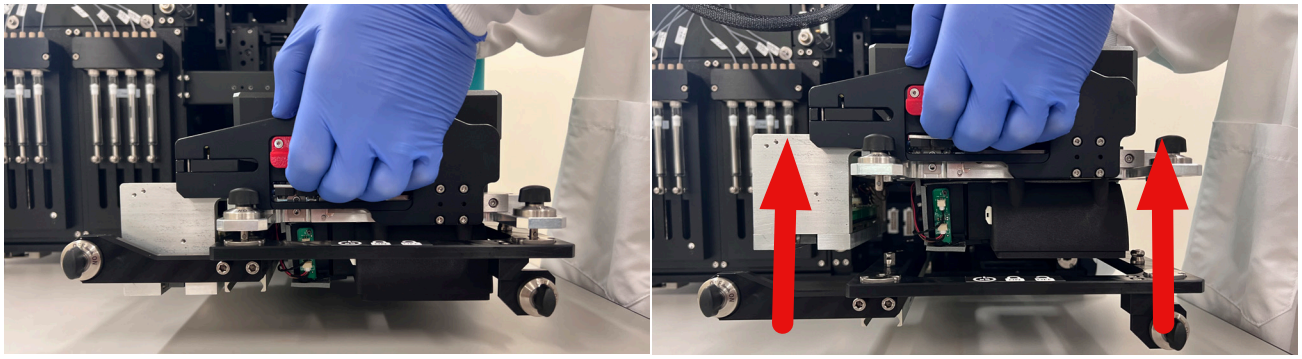


Figure 129. *Lift upwards using the handles to disengage the core*

MAGNETIC FIELD: LeviCell EOS cores contain strong magnets that can be harmful or interfere with the operation of pacemakers or other magnetically-sensitive devices.

Wearers must not bring their devices within 150 mm (6 inches) of the exchangeable core during handling.

14. Place the core module on a clean surface.

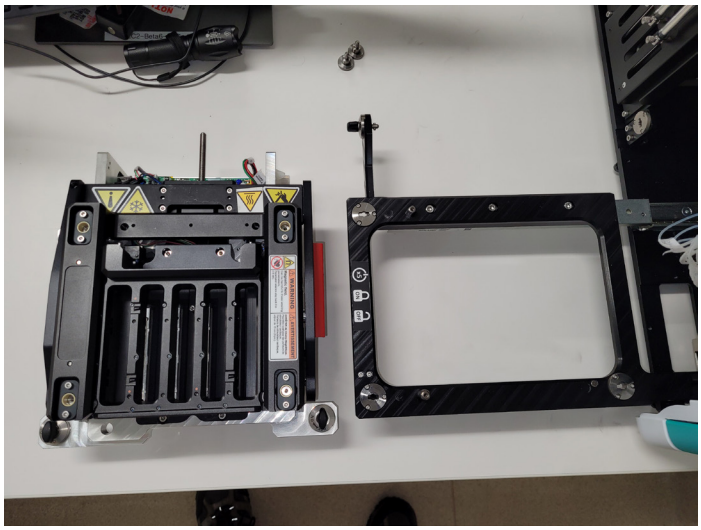
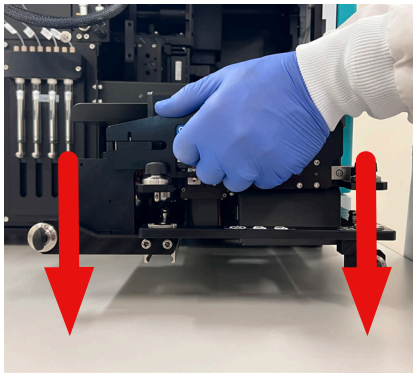
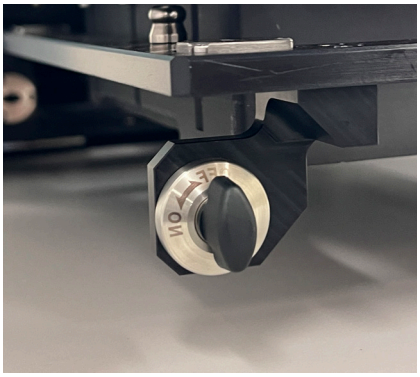
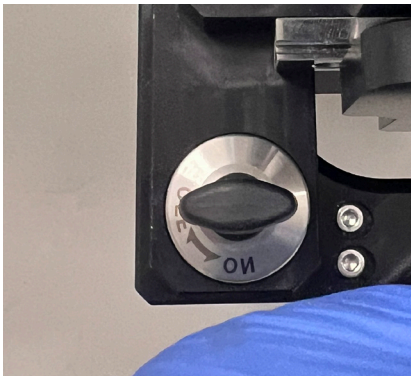


Figure 130.
Core module placed on bench next to core cradle

15. Place the new core module into the cradle, making sure that the core is seated correctly to the locks







16. Lock the core module into place by switching the 3 upward-facing locks to ON position

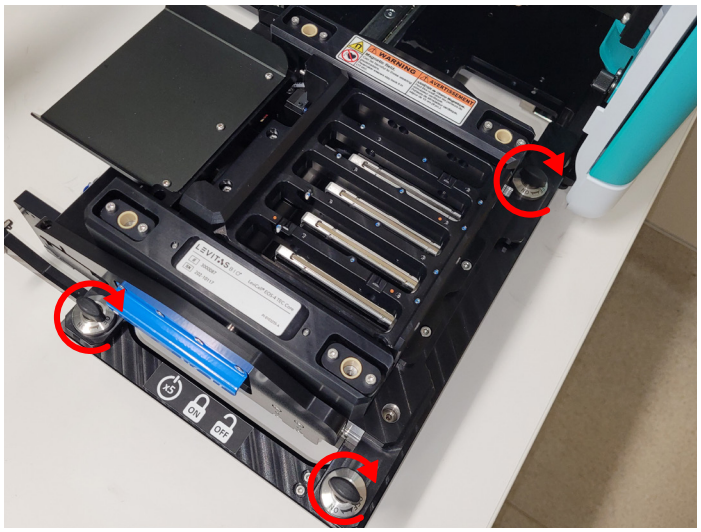


Figure 132.
Core module cradle locks switched to ON

17. Install the pneumatic manifold block onto the new core by aligning the core dowel pins to the manifold. The top surface of the manifold should be flush with the core

CAUTION: Avoid touching the bottom surface of the manifold.

The manifold rings should be clear of dust and contamination. Contact LevitasBio for cleaning best practices.

Handle with care - damage to the manifold rings will cause leaks and sample recovery issues.

Figure 133.
Pneumatic manifold bottom view

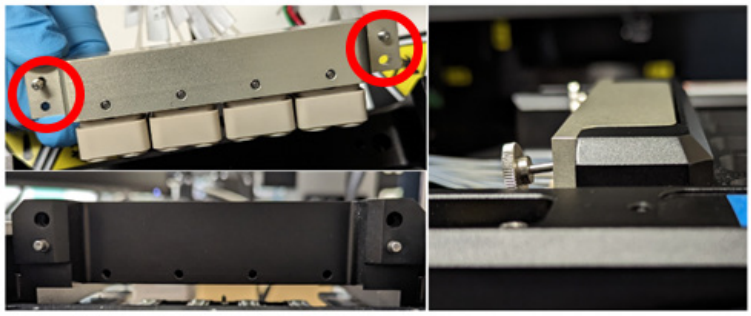


Figure 134.
(Left) Alignment of the pneumatic..... (Right) metal fixed dowel pins inserted into receiving holes on the core

18. Push the manifold all the way in and tighten the thumbscrews to the manifold block

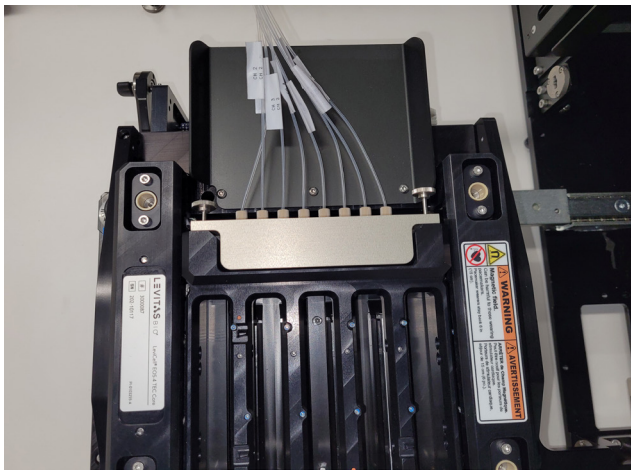


Figure 135. *Fastening the pneumatic manifold block onto the core.*

19. Slide the core cradle back into the instrument

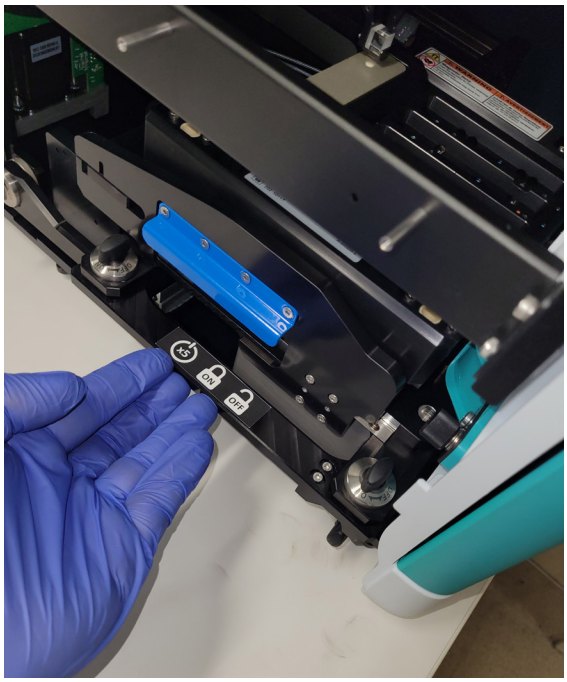


Figure 136.
Return core cradle back into the instrument

20. Lock the two remaining cradle locks by switching to ON position

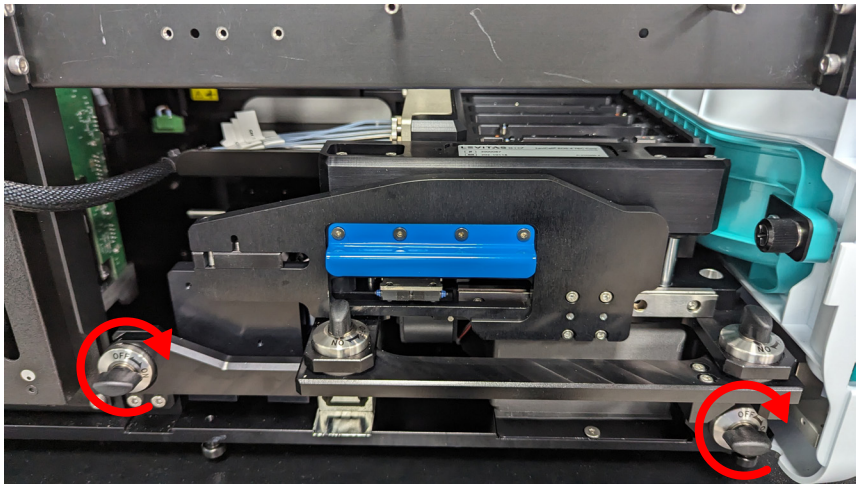


Figure 137.
Lock the core cradle

21. Replace the side panel by first aligning the rings indicating the post position with the slots on the top panel.

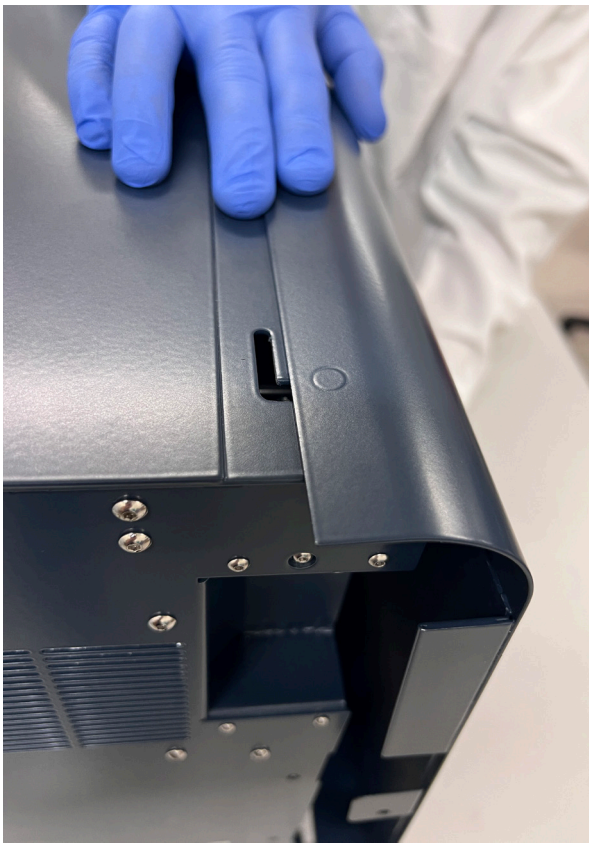


Figure 138. *Aligning side panel with slots*

22. With the panel tilted in at the top, re-engage the post with the slot without sliding it towards the instrument and bring the bottom panel to be flush with the instrument.

23. Slide the panel towards the front of the instrument into place.

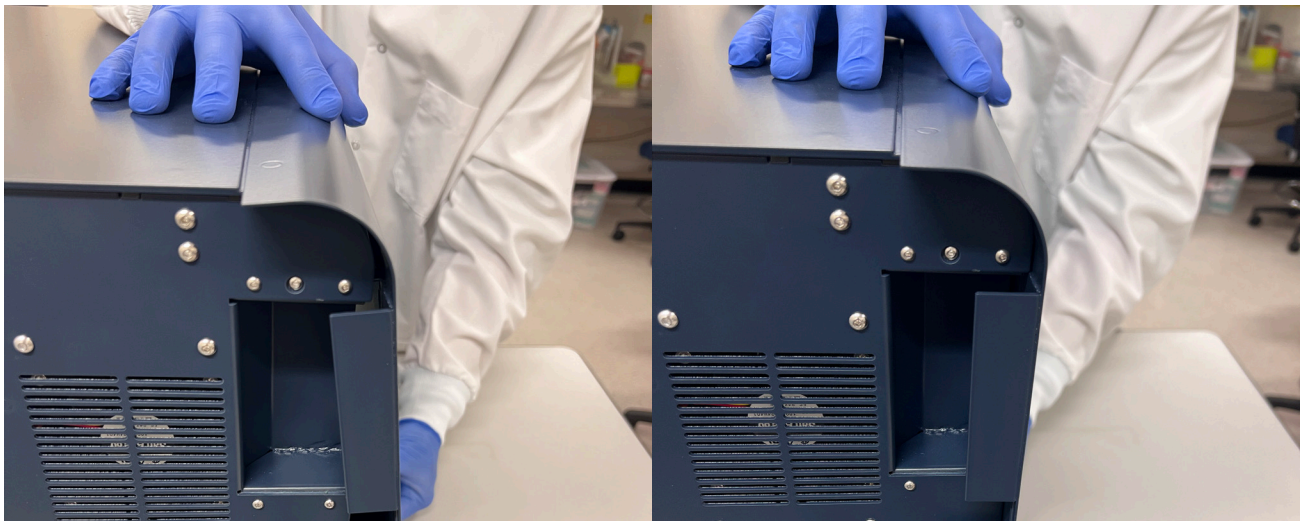


Figure 139. *Left side panel re-engaging with the module frame and sliding panel back into position*

24. Replace thumbscrews and secure side panel into place.

Figure 140. *Rear side panel thumbscrews*

25. Perform a core calibration after a core swap. Refer to the Manage EOS Module section and the System Installation and Calibration > Core Calibration section to walk through the calibration process of a new core.

NOTE: it is critical to perform a core calibration after a core swap. Do not operate system until this calibration has been completed.

26. Instrument with the new core is ready for use, once the core calibration has been completed successfully.

SHUTTING DOWN THE LEVICELL EOS

The LeviCell EOS System can be powered down in two ways

1. Soft power off using the button on the front of the instrument
2. Completely shutting the system off using both soft power down and mains switch.

It is only necessary to fully shut the LeviCell EOS system down if the system needs to be moved, for a core swap, or if it will not be used for a longer period.

Figure 141. *Power button on the front of instrument*

NOTE: If a scheduled task Heat Dry is queued after a cold or cool run and has not been completed, this scheduled task will start when the soft power button is pressed for shut down. A notification will appear on the LeviCell EOS LCD. The Heat Dry task cannot be interrupted.

If the soft power button is pressed again, a purple warning will appear on the LeviCell EOS LCD.

To shut down the system for short periods (e.g. weekends)

1. Ensure there are no samples running
2. Close EOS Manager Software using the system menu
3. Check to see if there is a cartridge inserted into the system. If yes, remove cartridge
4. Click the soft power button on the front of the EOS Module
5. Wait for the LCD to power down

To shut the system down for longer periods follow the above instructions and then switch the mains power switch on the rear right of the module to “0” (off).

CLEANING THE LEVICELL EOS

The exterior and cartridge loading area of the LeviCell EOS may be cleaned using a slightly dampened cloth or wipe pre-wetted with mild cleaning agents such as the following:

- Detergents in aqueous solution
- Up to 80% ethanol in water
- Up to 80% isopropyl alcohol in water
- Diluted bleach (sodium hypochlorite up to 1 % w/v, aqueous)

CAUTION: Use standard precautions for any cleaning agents.

DO NOT USE the following cleaning agents on the LeviCell EOS instrument, as damage to finishes may occur:

- Aggressive organic solvents such as acetone, methanol, or aromatic compounds (e.g., toluene)
- Strong acids
- Abrasive compounds

TROUBLESHOOTING

Issue	Possible Resolution
Barcode is not reading	• Confirm the barcode was entered correctly compared to the label. • Ensure the EOS module is connected (under Manage EOS Modules). • Ensure the EOS core supports the cartridge type you are using.
Stain tool not visible in run summary	No fluorescence was captured in the run.
Bead Test cartridge not working	Check if the transport cartridge is being used. The transport cartridge is used only for dry tests. Retry with a new cartridge.
Cannot move split line	Check if the confirmed split line box is checked. If so, uncheck and try again.
Cold run temperature option not available	Environmental temperature is too high to bring to cold temperature range. Consider moving system to an air conditioned facility or running at a different time of the day
Chosen run temperature cannot be achieved	Environmental temperature is too high to bring to chosen run temperature range. Consider moving system to a air conditioned facility or running at a different time of the day
Run temperature deviation during run	Environmental temperature during the run may have increased. Consider moving system to a air conditioned facility or running at a different time of the day • If this problem continues when the environment is 25^{0}C degrees, contact technical support.
No leading meniscus	This can indicate contamination or a leak in the cartridge. Contact technical support.
No trailing meniscus	This can be due to differences in loading volumes. If more than 220 µL is loaded, the trailing meniscus will not be visible on the inlet side of the lane, which may cause a difference in purity.
Breaks in the cell band	This is normal. There can be minor perturbations that cause breaks in the band. This does not affect levitation or collection.
Bubble in the middle of the separation channel (no sample)	A bubble may have been introduced into the separation channel during loading, which can be due to pipetting directly into the hole or bubbles in the inlet well when the sample is loaded. To minimize chance of bubbles, always pipette to the stop.
Small bubbles emerging during levitation	This is normal. There can be minor outgassing during the levitation period. This does not affect levitation or collection; however, it may affect sample characterization values.

Imaging appears to be jagged and not consistent	Imaging can be disrupted if there is excessive vibration nearby. Ensure that the instrument is not on the same bench as a centrifuge or other devices that creates vibration or rough movements.
Cells show excess movement during levitation.	Inconsistent environmental temperature can cause issues for temperature control runs. Avoid placing the LeviCell system directly under a AC vent.
Top or bottom bands have strange fluctuation during the collection step	This is normal, and can be due to pressure fluctuations as the sample is collected.
Three bands of beads are visible during the beads test	Centrifuging bead mixtures may result in doublet formation between beads of different densities, which will levitate between singlets.
Fibers seen in the separation channel	• Check to make sure that the cartridge is stored in a dust-free place. • Maintain the unused cartridges in a closed five-pack carton.
EOS Module is not available for use	Check to see if the Ethernet cable is seated correctly on both the EOS module and computer.
New Instrument module not recognized	Check to see if the EOS module has been paired.

CONTACT INFORMATION

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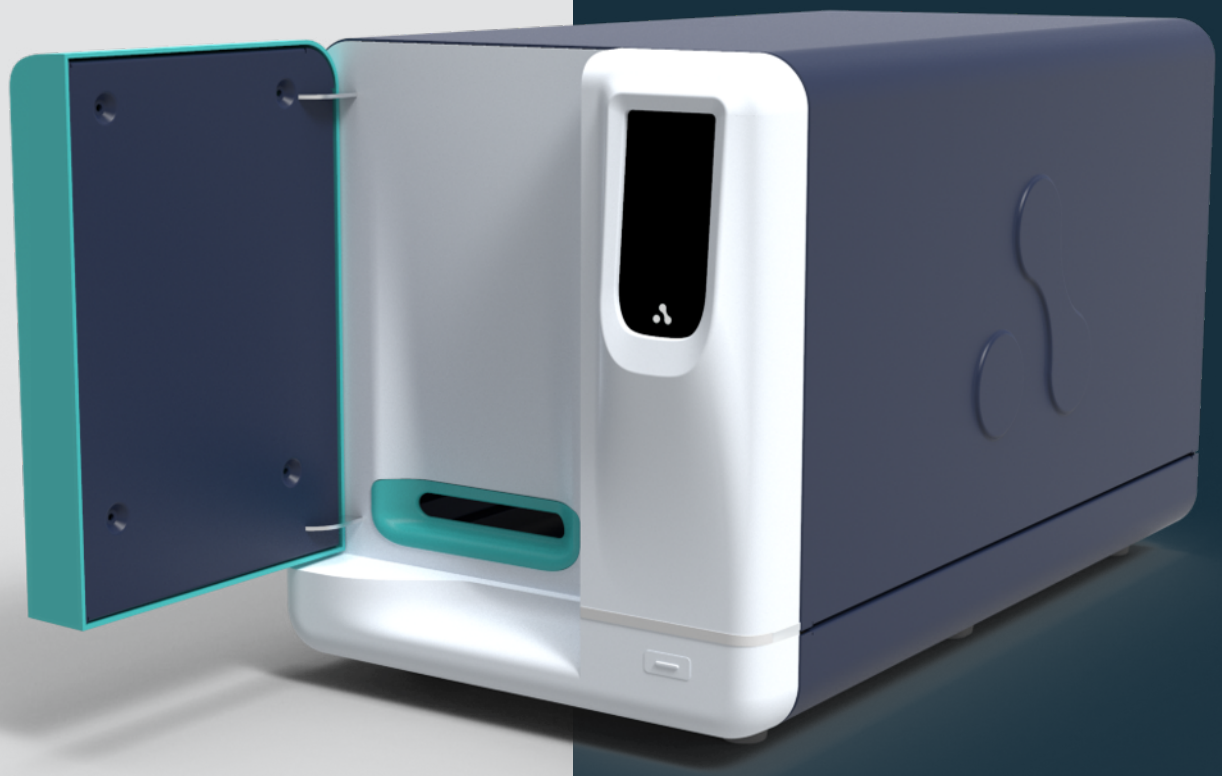

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