



LeviCell EOS User Guide

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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 the LeviCell EOS only as specified by LevitasBio. Removing any cover or part of the enclosure while the system is powered on may create a serious safety hazard.

The LeviCell EOS module is heavy and requires a **two-person** lift (45 kg / 100 lb).

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

Do not block access to the mains power cable at either end. The cable must remain accessible in case power needs to be completely disconnected for servicing.

The LeviCell EOS system contains several user-accessible, removable parts inside 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. Reusing cartridges increases the risk of cross-contamination and biohazard exposure, and may void the instrument warranty. 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 laboratory safety guidelines

For indoor use only. The LeviCell EOS is not designed for outdoor operation 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 treat any instrument located in a Biosafety Level (BSL) laboratory as if contaminated at that BSL level.
	MAGNETIC FIELD: LeviCell EOS cores contain strong magnets that may be harmful or may interfere with pacemakers and 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 certified to comply 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 complies with the limits for a Class A digital device under Part 15 of the FCC Rules. These limits provide reasonable protection against harmful interference in commercial environments. The device generates, uses, and can radiate radio-frequency energy; if not installed and operated according to this manual, it may cause harmful interference to radio communications. Operation in residential areas may cause interference requiring the user to correct it at their own expense. This device also complies with Part 15 of the FCC Rules. Operation is subject to two conditions: (1) the device may not cause harmful interference; and (2) the 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 to enrich viable cells while simultaneously removing dead or dying cells and debris from complex samples. The LeviCell EOS incorporates advanced optics for full-channel visualization, unique magnetic configurations suitable for a wide range of applications and sample types, a pneumatic pump system to control sample flow through the cartridge and separate it into two fractions (see Figure 1), and sophisticated software to manage all connected LeviCell EOS modules. Up to four EOS modules can be connected to the Control PC, enabling throughput of up to 16 samples.

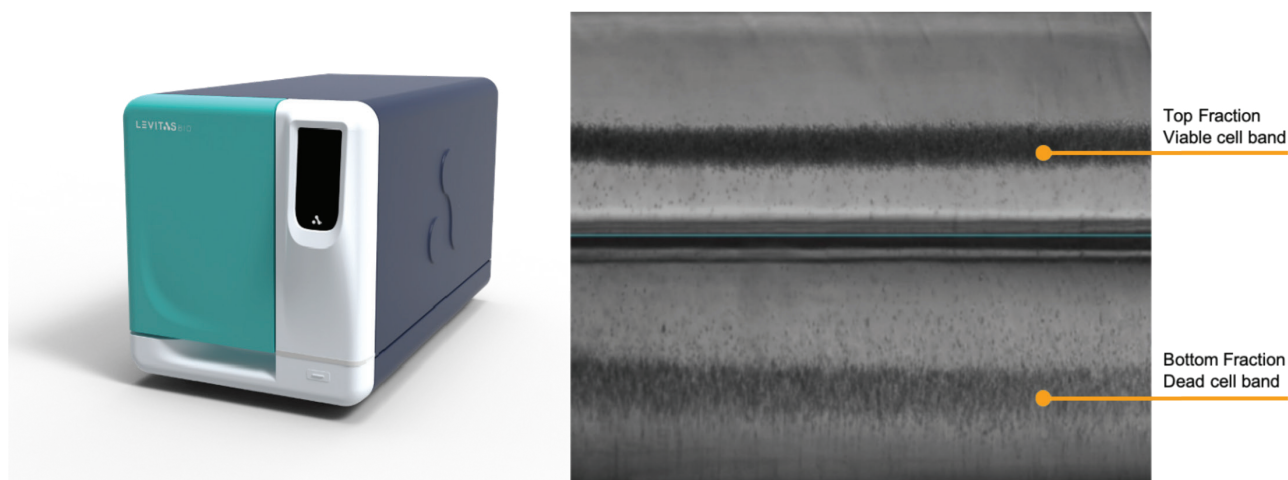


Figure 1. LeviCell EOS and levitation image

The LeviCell EOS uses parallel processing to meet high-throughput demands across multiple 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 are not exposed to high pressure, shear forces, or other stresses that typically cause cellular activation, stress responses, or 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) comes with the EOS-4 TEC core pre-installed.

EOS Module – Front

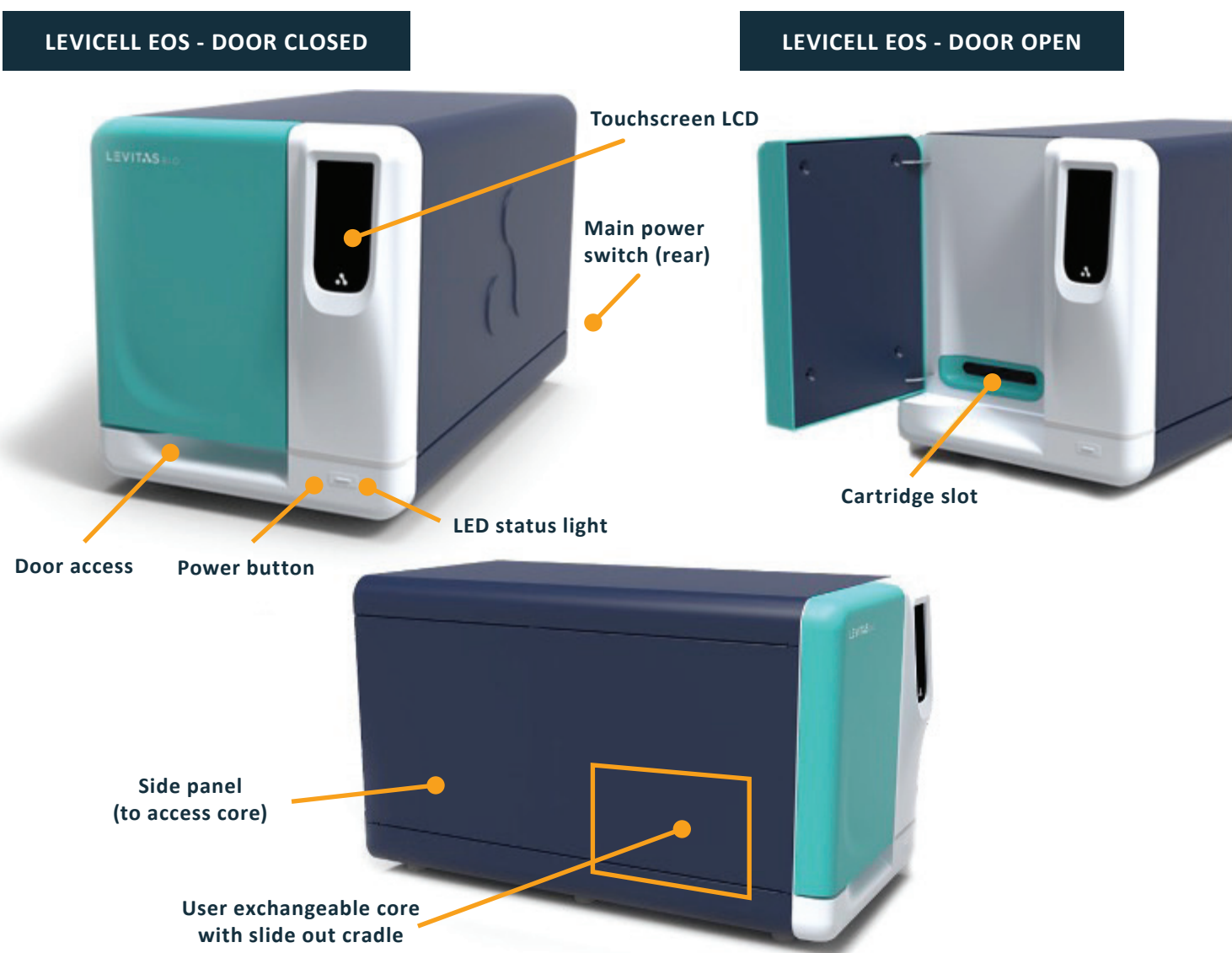


Figure 2. LeviCell EOS Module interaction points

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 Status Light Colors

EOS Module – Rear

The EOS Module has two main connection points located on the rear panel. The mains power switch and plug are on the lower left, and each EOS Module connects to the Control PC via an Ethernet cable and port.

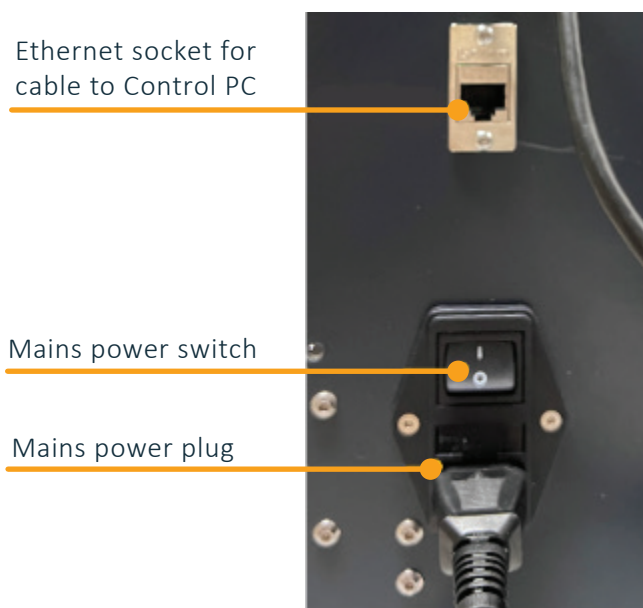


Figure 3. EOS Module power and connection interface

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 after compression: 600MB (Brightfield only), 1.8GB (with fluorescence), Medium Cell Protocol
Peripherals:	Wireless keyboard, wireless mouse, barcode scanner

Table 5. Computer specifications

Cartridges

LeviCell EOS cartridges are designed for high-throughput sample processing. Up to four 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

LeviCell EOS Transport Cartridge

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

This cartridge can also be used for dry test runs, training, or troubleshooting. If any issues occur, contact LevitasBio Technical Support for guidance on using the transport cartridge in troubleshooting.

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.

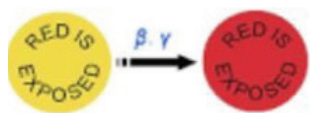


Figure 6. Gamma irradiation indicator sticker

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.

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 1003007)
- LeviCell EOS Starter Kit, non-sterile (PN 1003109) or sterile (PN 1003110)

User-Sourced Equipment and Consumables

- Benchtop Vortexer
- Centrifuge capable of spinning at 300 RCF
- Calibrated pipettes and pipette tips (filtered)
 - 2-20 μ L, 20-200 μ L, 200-1000 μ L
- 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 μ m syringe filters, to filter cell media/buffers
- 5.0 mL or larger disposable syringes, to filter cell media/buffers



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

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

- Revvity Cellometer instruments and compatible slides
- 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 μ L/min
Imaging modes	Brightfield (transmitted illumination) 530 nm Two fluorescence channels: Excitation 470 nm, Emission 501-544 nm (e.g., Calcein, FITC, 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 lb)
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: W 1200 x D 660 mm (47.2" x 26.0").
- Recommended: W 1520 mm (59.8") bench with 508 mm (20.0") vertical clearance above the bench.
- A minimum of 80 mm (3.1") clearance is required behind the instrument for proper venting and to allow 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 (23.6").
- 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, 5x20 mm, qty 2

User-Exchangeable Cores

The LeviCell EOS System 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 may include 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.

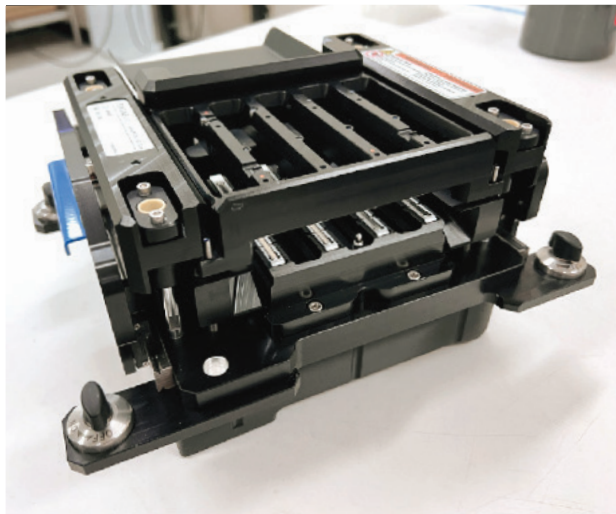


Figure 8. Core module on the lab bench

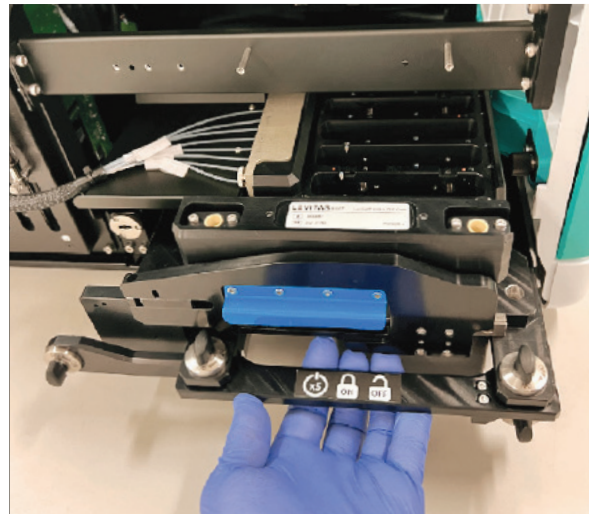


Figure 9. Reinserting the core in its cradle back into the instrument

Temperature Control Core

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 Cartridge to the selected run temperature.

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

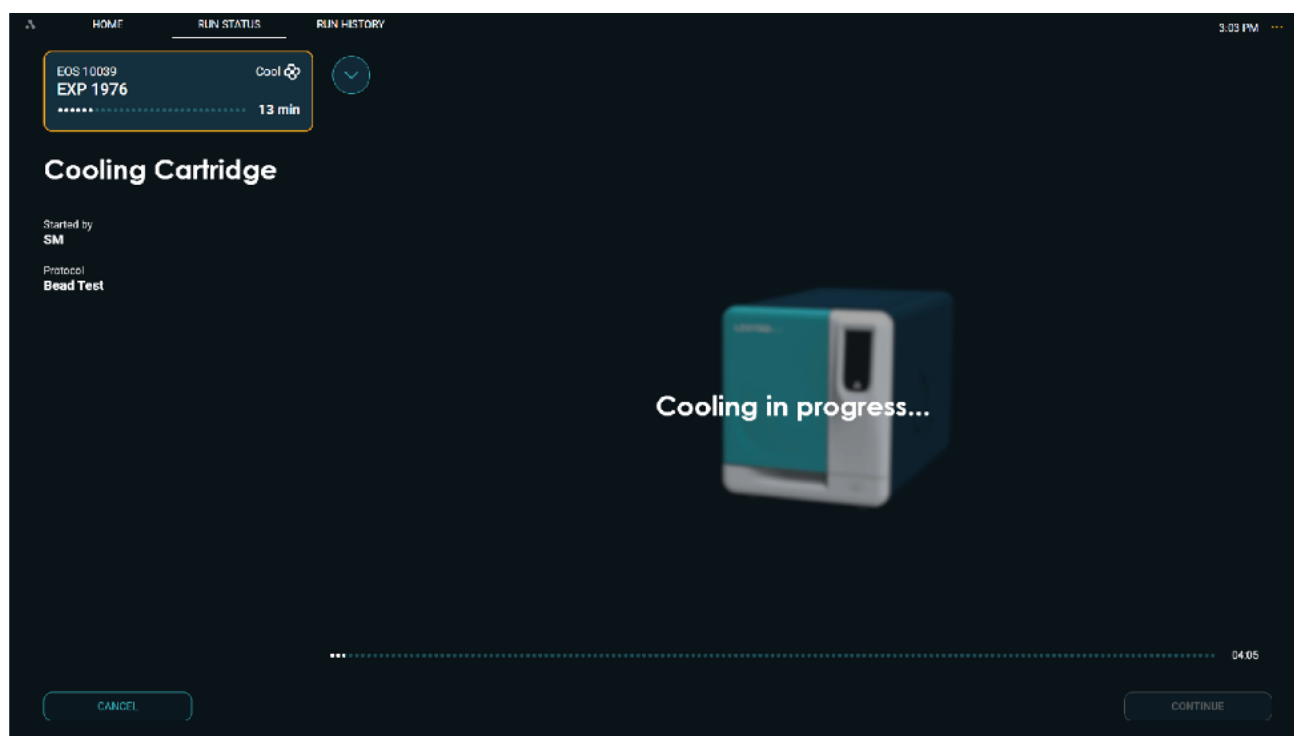


Figure 10. Cooling cartridge prior to dispensing samples

Post-Run Process

When a run finishes in cold or cool conditions, the system exits thermal regulation and automatically starts a background cycle called “Keeping Dry.” The current step appears on the run-setup screen, and the module remains selectable; if a new run is launched, Keeping Dry pauses and will restart from the beginning once the system is idle again.

Keeping Dry

- Activates the fan for 45 minutes, drawing ambient air through the module to eliminate residual moisture, and prevent condensation build-up and contaminant growth.
- Restarts at beginning of 45 minute cycle if interrupted.
- Logs completion so the controller knows whether the full cycle has run.

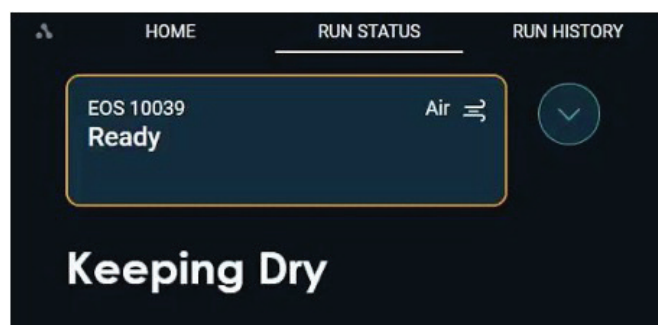


Figure 11. Keeping Dry Post-run Process

Scheduled Heat Dry Task

When a run has taken place under cold or cool conditions, the system automatically queues a Heat Dry cycle to eliminate residual moisture and prevent contaminant growth.

Timing: Whether or not the Keeping Dry cycle has been completed, the system will proceed immediately to the Heat Dry task. The scheduled cycle begins automatically at 11:00 PM, or immediately when the soft-power button is pressed to shut the system down. Once started, it cannot be interrupted by a new run.

Sequence

1. Heat Drying: Core temperature is raised to 45°C for 10 minutes.
2. Cooling Down: Core temperature is set to 20°C for 10 minutes.
3. Air Drying: Ambient air circulates for the final 10 minutes

After completion normal operation may resume, or the shutdown sequence continues if power-off was requested.



Figure 12. 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.

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



LeviCell EOS Manager

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		Buttons	
	selected/ active item		default selection for next step
	active field (user entry)		available button
	unselected option		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 provide information to guide the user when interacting with the LeviCell EOS system.

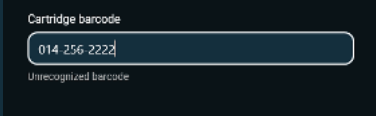
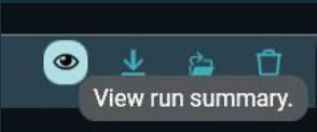
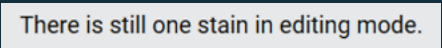
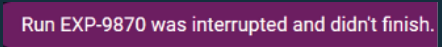
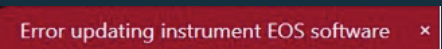
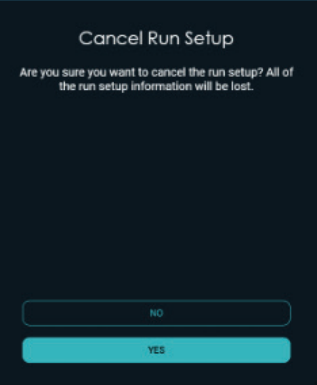
Type	Description	Example
User entry error	Information will appear below the user entry field indicating guidance to resolve issue	
ToolTip	Information provided when hovering over a button or option	
Notification	White, Information. Temporary will automatically clear	
	Purple, Warning. User must clear	
	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	A message will appear in the main window	

Table 9. Software message types

Home Screen

Upon software launch, the Home screen appears with the following options:

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

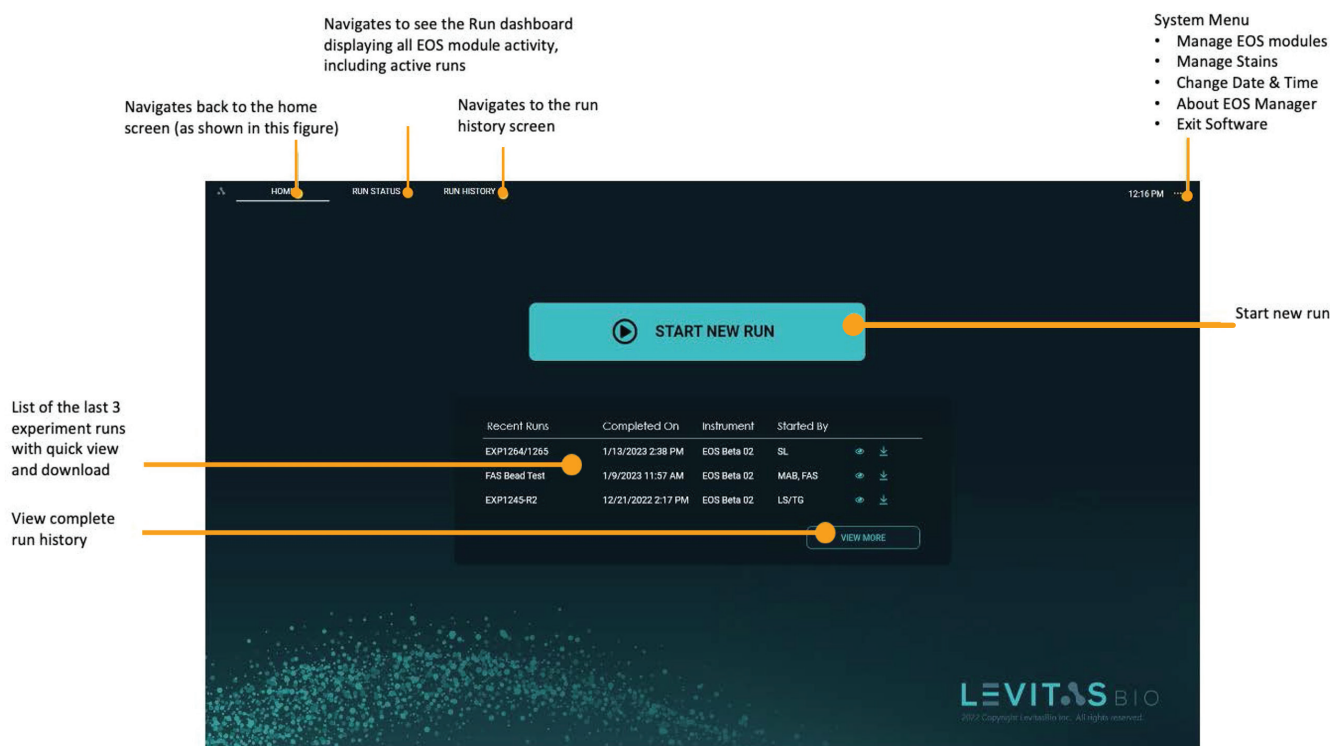


Figure 13. Home Screen

The Home screen allows easy experimental setup and information retrieval, including the status and availability of connected EOS modules. Users can readily identify the 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 barcode that serves as its unique identifier for the experiment run. A barcode scanner is included with the system and can be used to scan the cartridge and/or other consumables with a QR or 1D barcode.



Figure 14. Scanning cartridge barcode information



Figure 15. Cartridge label with barcode

Specify Run Information and EOS Module

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

All available EOS Modules will be displayed. When two or more EOS Modules are connected, 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 default selection.

Cartridge barcode
013-8907-7865

Specify run information and EOS Module

Run name
EXP 1987

Started by
SM

Notes (optional)

EOS Module

EOS 10039 Ready

DEMO PC 1 Ready

CANCEL

Cartridge Run Information Protocol Samples

BACK NEXT

Figure 16. 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 a different type of sample. Since levitation time can vary based on cell size, there are 3 different cell protocols: Small Cell, Medium Cell, Large Cell, each with different levitation durations (Table 10). 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 Starter Kit (PN 1003109 and PN 1003110 (sterile)) for calibration, training, or demonstration purposes.

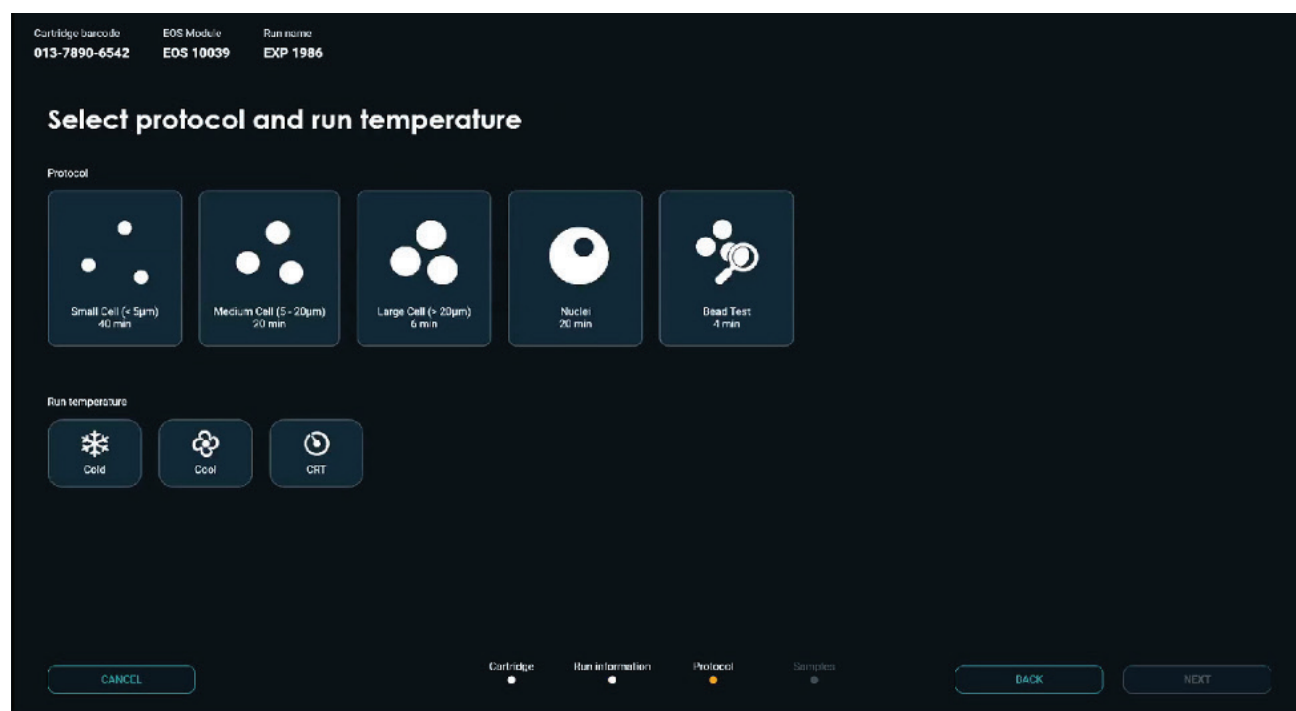


Figure 17. Select protocol and run temperature

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 (Table 11).

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**		Variable based on environment

* Ambient temperature is only available for non-TEC cores ** Controlled Room Temperature

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

Run Status Screen

The run status screen displays the status of active EOS modules and real-time images from 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.

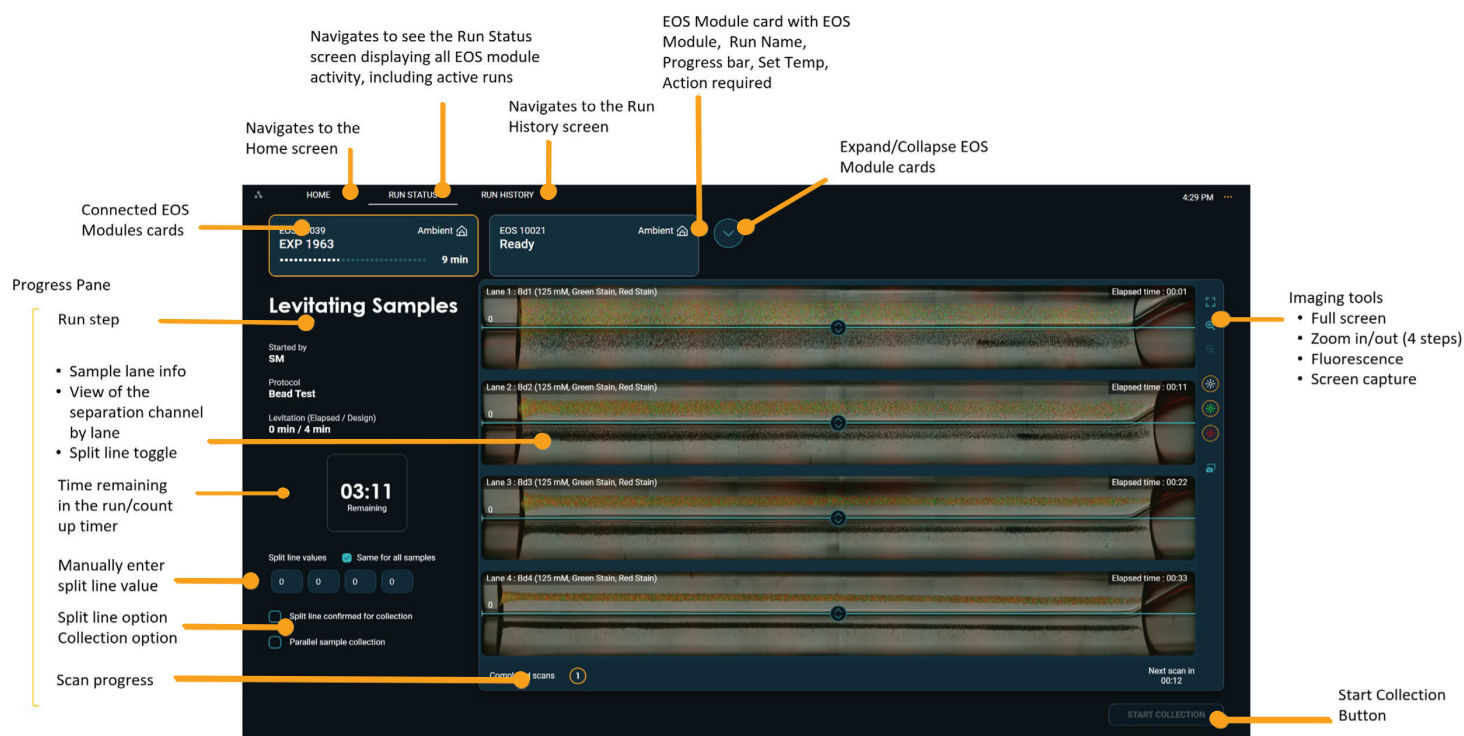


Figure 18. Run Status Screen

EOS Module Cards

Up to 4 EOS modules can be connected to one LeviCell EOS Control PC. An information card for each connected EOS Module appears 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.



Figure 19. EOS module card user intervention icon

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. (This icon appears only when an action is required from the user e.g., pressing “Close Door”. It indicates that the system is waiting for user input before continuing.)

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

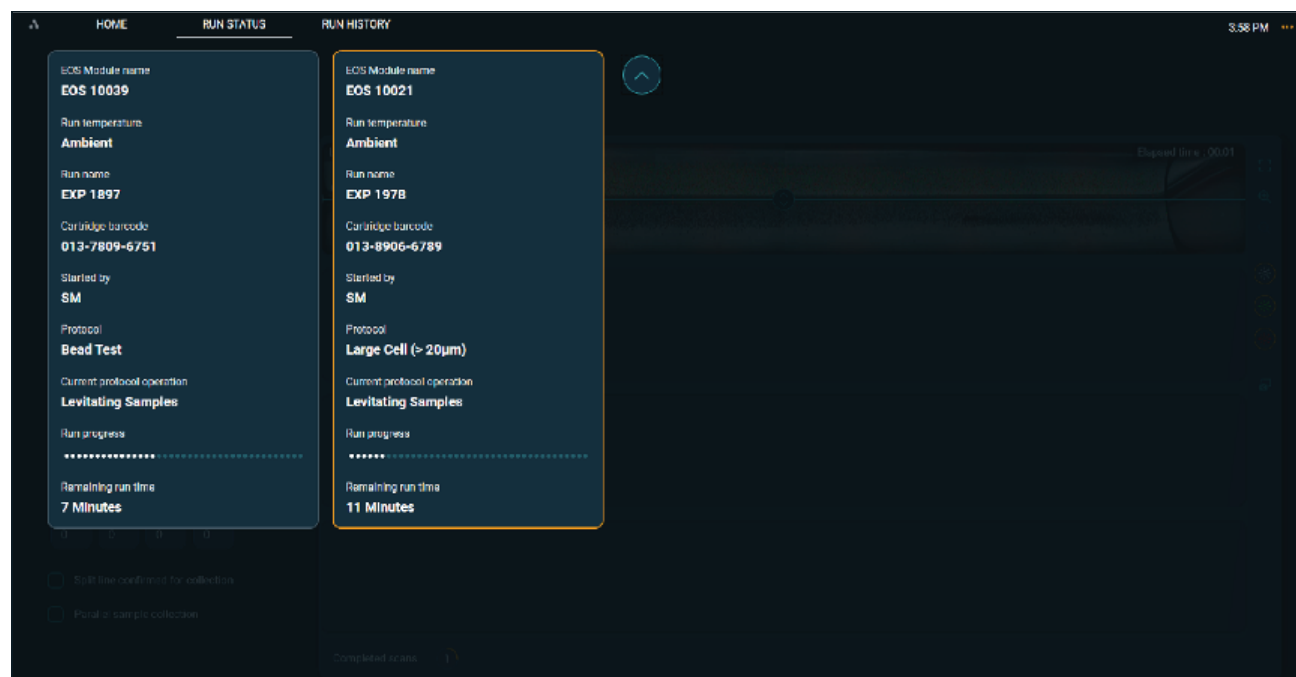


Figure 20. Expanded EOS Module information cards

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.

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.

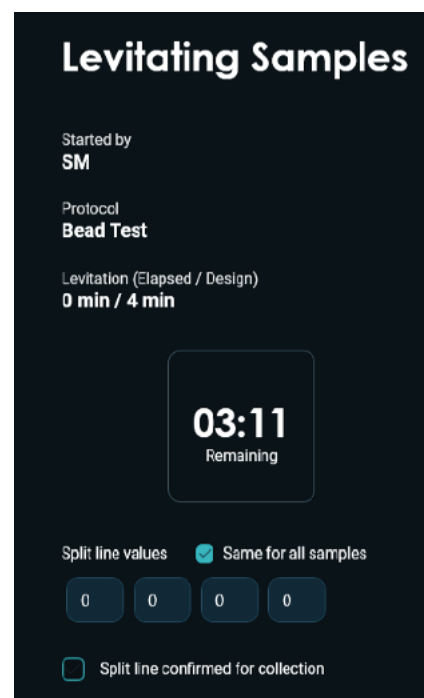


Figure 21. Progress Pane

1. Enter a value in the split line value field

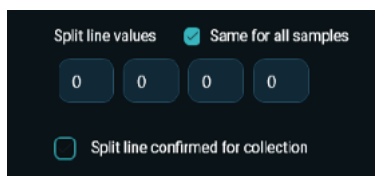


Figure 22. Split line values and split line confirm

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

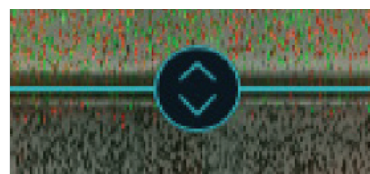


Figure 23. Split line toggle

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 a 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.

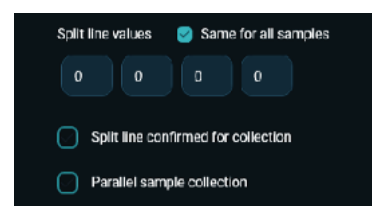


Figure 24. Split line options

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.

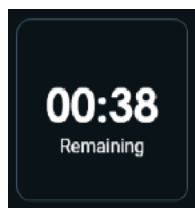


Figure 25.

Levitation time remaining countdown

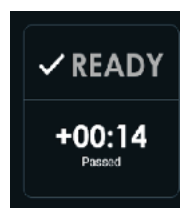


Figure 26.

Levitation is Ready and count up timer for levitation time is shown

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 since the start of levitation will be displayed on the right side of the lane, indicating the timestamp when each image appears. This will be different per lane.

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

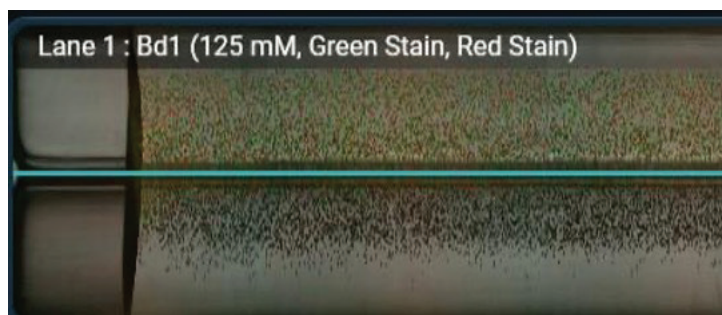


Figure 27. *Lane information*

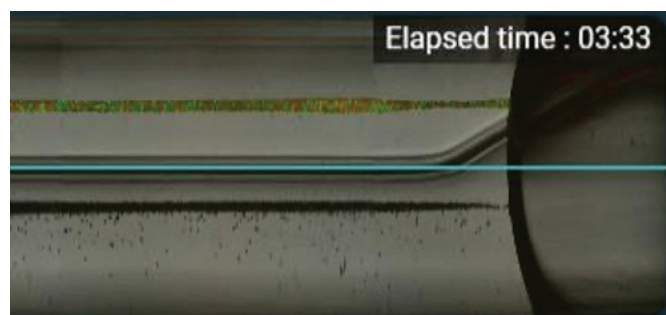


Figure 28. *Elapsed time for each lane*

Montage Images and Scan Progress

During the run, images are collected per sample for the entire separation channel.

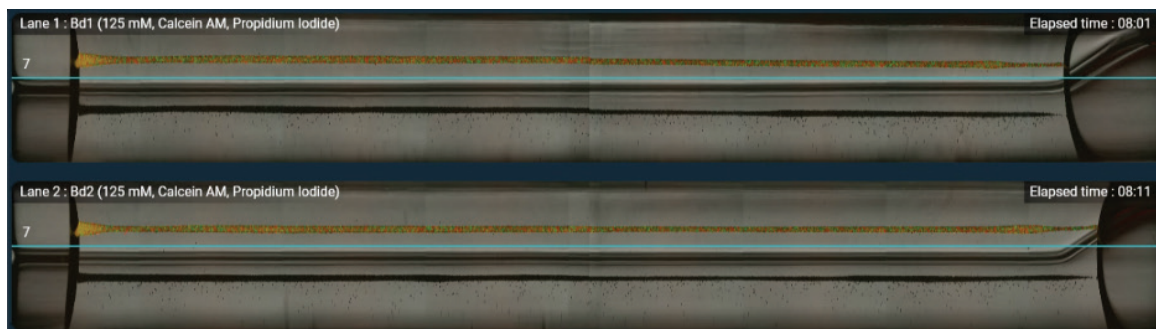


Figure 29. Scan complete for Lane 1 and 2

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 counted as one complete scan, as indicated by the bar at the bottom of the run status view.



Figure 30. 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 elapsed 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 of total run time have 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 31. 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, display brightfield or fluorescent illumination when stains are used, and take a snapshot of the screen.

Brightfield, green fluorescence, and red fluorescence 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.

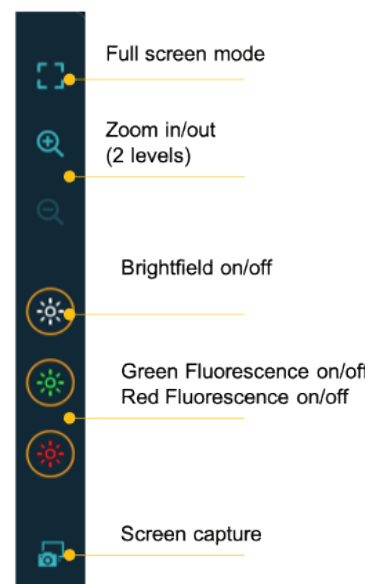


Figure 32.
Imaging tools available during a run

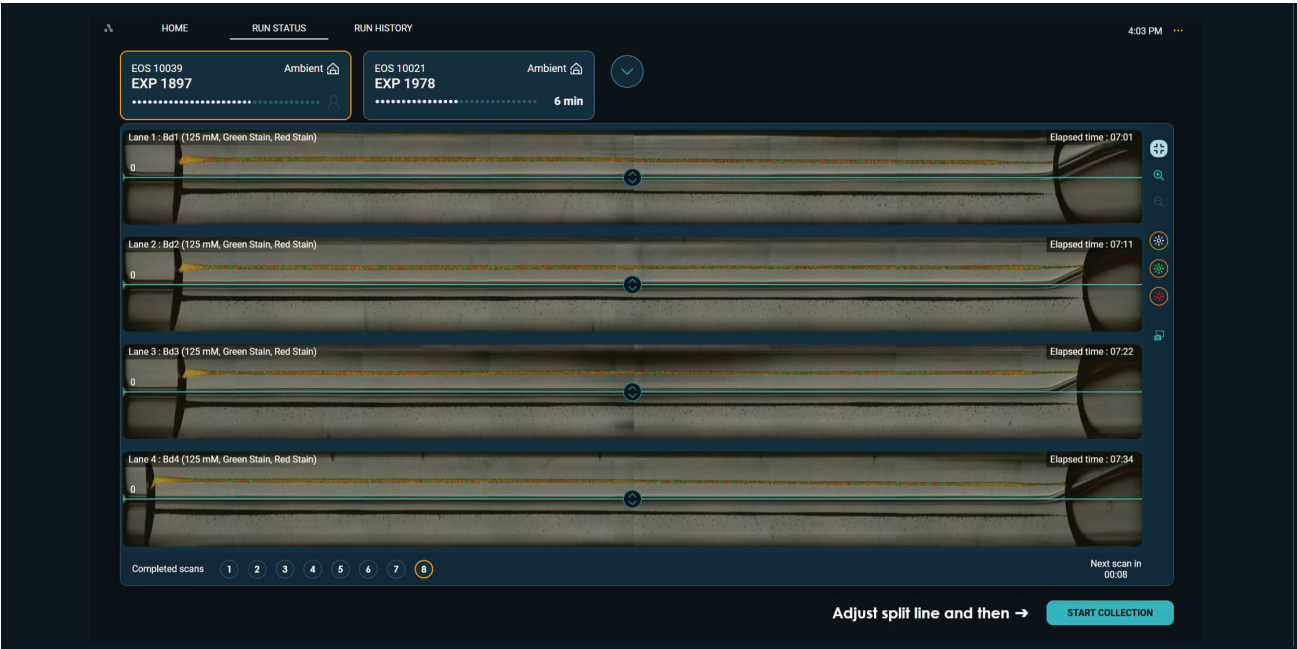


Figure 33. Run Status full screen view

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 whereas 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.

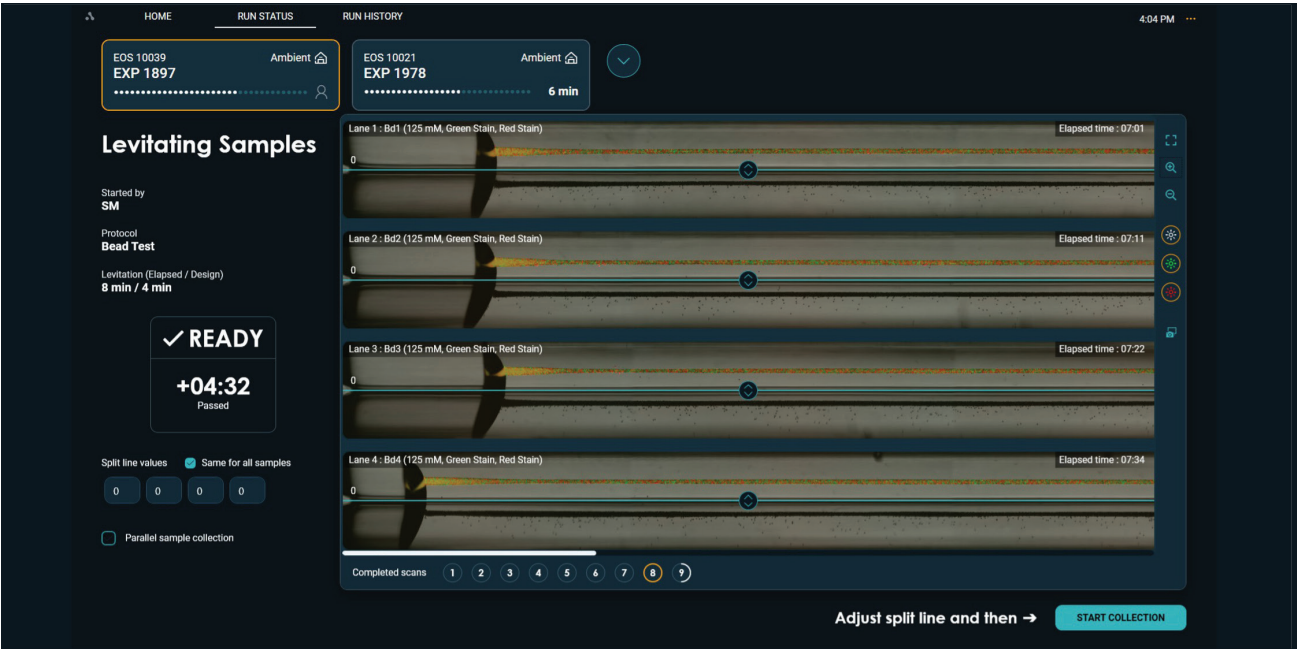


Figure 34. 1 step zoom - true lane ratio view



Figure 35. Maximum zoom - single lane height view

During the 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 signals are captured for the duration of the run regardless of its status.

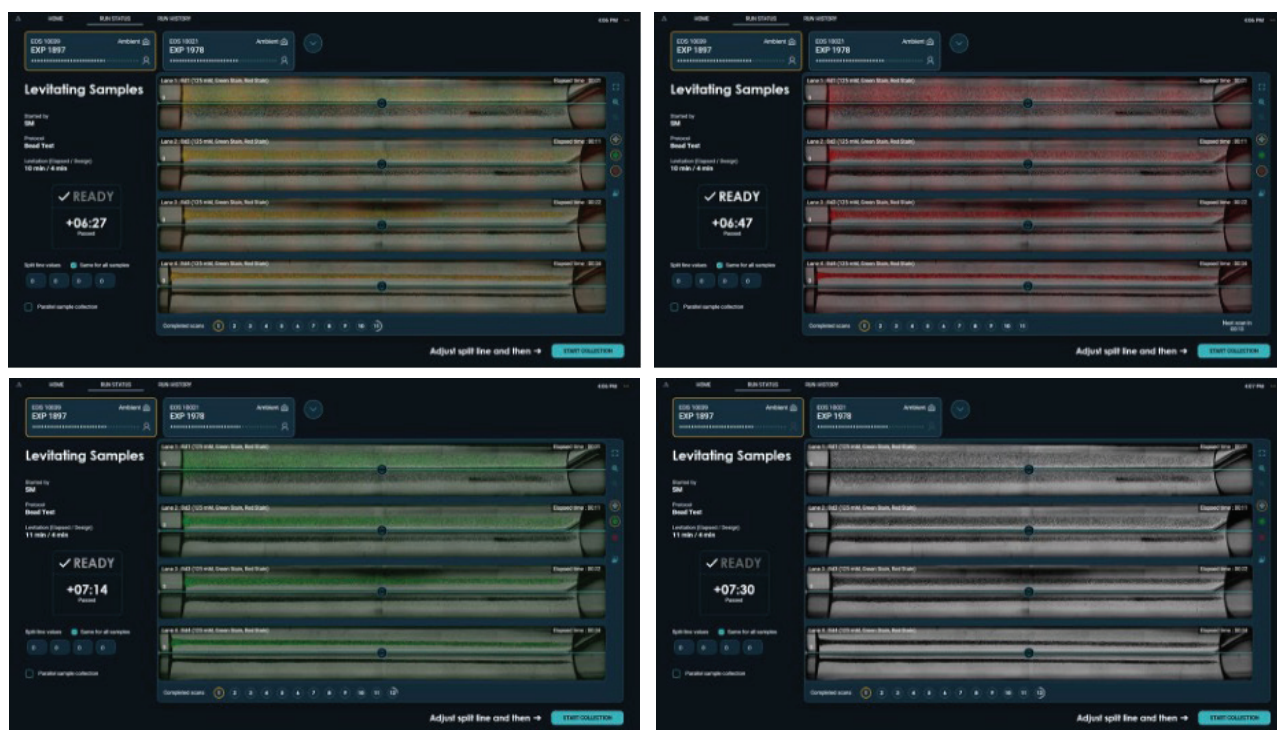


Figure 36. Fluorescence view for both green and red (UL), red stain only (UR), green stain only (LL), and brightfield only (LR).

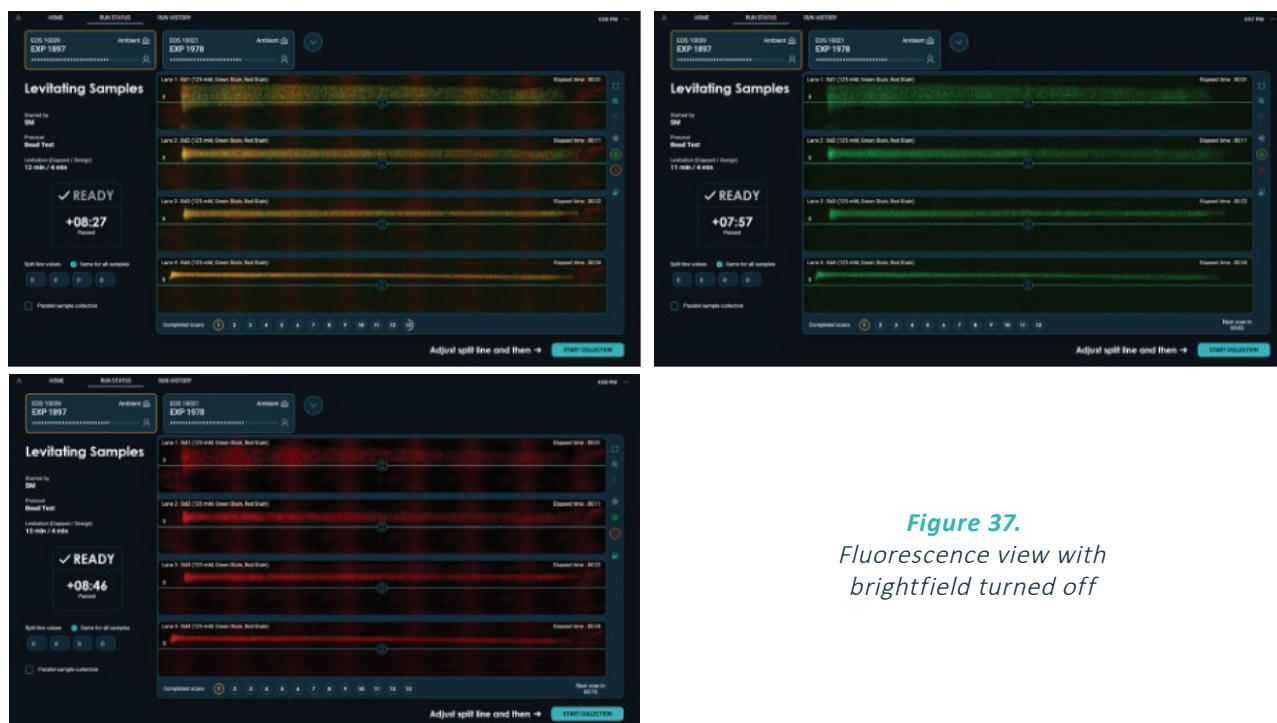


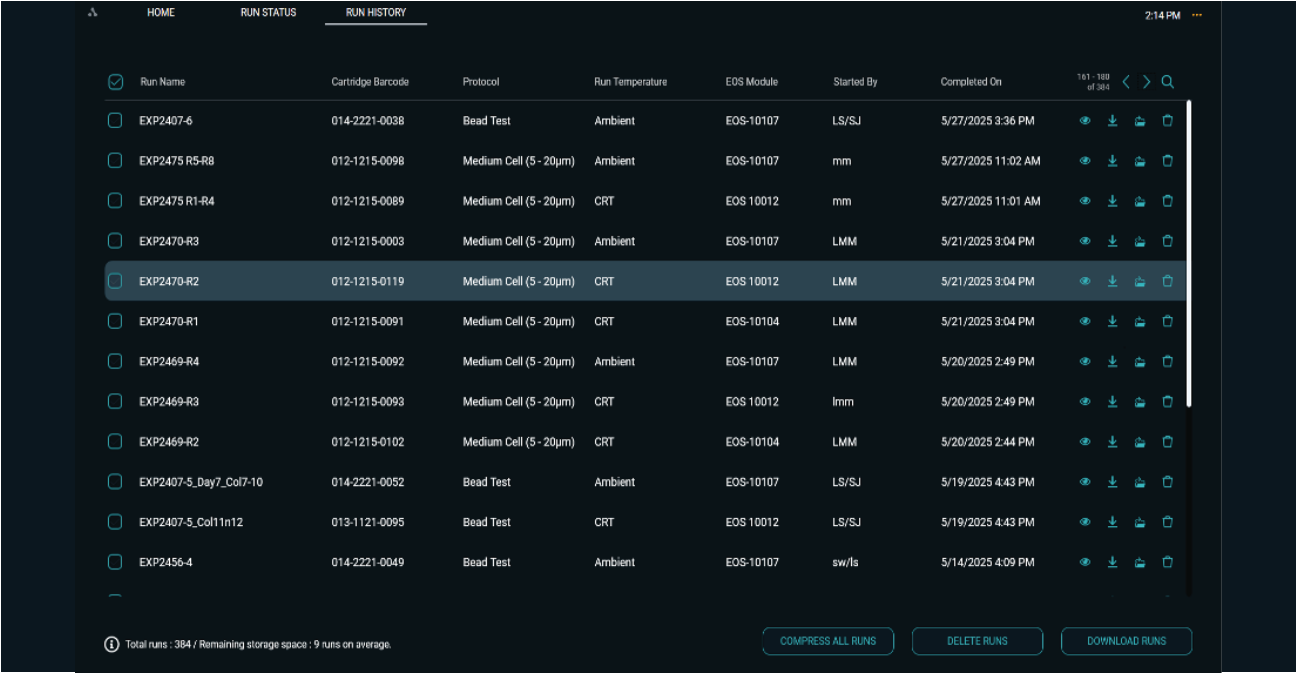
Figure 37. Fluorescence view with brightfield turned off

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 a 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.



The screenshot shows the 'RUN HISTORY' tab selected. At the top, there are tabs for 'HOME', 'RUN STATUS', and 'RUN HISTORY'. The 'RUN HISTORY' tab is active. Below the tabs is a table with the following columns: Run Name, Cartridge Barcode, Protocol, Run Temperature, EOS Module, Started By, Completed On, and a set of icons (eye, download, print, delete). The table contains 13 rows of run data. At the bottom left, there is an information icon (i) and text: 'Total runs : 384 / Remaining storage space : 9 runs on average.' At the bottom right, there are three buttons: 'COMPRESS ALL RUNS', 'DELETE RUNS', and 'DOWNLOAD RUNS'.

Run Name	Cartridge Barcode	Protocol	Run Temperature	EOS Module	Started By	Completed On	Icons
EXP2407-6	014-2221-0038	Bead Test	Ambient	EOS-10107	LS/SJ	5/27/2025 3:36 PM	Eye, Download, Print, Delete
EXP2475 R5-R8	012-1215-0098	Medium Cell (5 - 20µm)	Ambient	EOS-10107	mm	5/27/2025 11:02 AM	Eye, Download, Print, Delete
EXP2475 R1-R4	012-1215-0089	Medium Cell (5 - 20µm)	CRT	EOS 10012	mm	5/27/2025 11:01 AM	Eye, Download, Print, Delete
EXP2470-R3	012-1215-0003	Medium Cell (5 - 20µm)	Ambient	EOS-10107	LMM	5/21/2025 3:04 PM	Eye, Download, Print, Delete
EXP2470-R2	012-1215-0119	Medium Cell (5 - 20µm)	CRT	EOS 10012	LMM	5/21/2025 3:04 PM	Eye, Download, Print, Delete
EXP2470-R1	012-1215-0091	Medium Cell (5 - 20µm)	CRT	EOS-10104	LMM	5/21/2025 3:04 PM	Eye, Download, Print, Delete
EXP2469-R4	012-1215-0092	Medium Cell (5 - 20µm)	Ambient	EOS-10107	LMM	5/20/2025 2:49 PM	Eye, Download, Print, Delete
EXP2469-R3	012-1215-0093	Medium Cell (5 - 20µm)	CRT	EOS 10012	Imm	5/20/2025 2:49 PM	Eye, Download, Print, Delete
EXP2469-R2	012-1215-0102	Medium Cell (5 - 20µm)	CRT	EOS-10104	LMM	5/20/2025 2:44 PM	Eye, Download, Print, Delete
EXP2407-5_Day7_Col7-10	014-2221-0052	Bead Test	Ambient	EOS-10107	LS/SJ	5/19/2025 4:43 PM	Eye, Download, Print, Delete
EXP2407-5_Col11n12	013-1121-0095	Bead Test	CRT	EOS 10012	LS/SJ	5/19/2025 4:43 PM	Eye, Download, Print, Delete
EXP2456-4	014-2221-0049	Bead Test	Ambient	EOS-10107	sw/ls	5/14/2025 4:09 PM	Eye, Download, Print, Delete

Information: Total runs : 384 / Remaining storage space : 9 runs on average.

Buttons: COMPRESS ALL RUNS, DELETE RUNS, DOWNLOAD RUNS

Figure 38. 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.

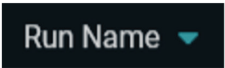


Figure 39. Run History column sort arrow

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

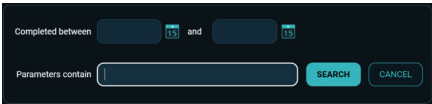


Figure 40. Search Run History

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.

EOS Module	Started By	Completed On	
EOS-1001-Nemo	SM	5/17/2023 12:10 PM	   
EOS-1001-Nemo	SH	5/17/2023 11:08 AM	   

Figure 41. Run history options, hover and tool tips



Figure 42. 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

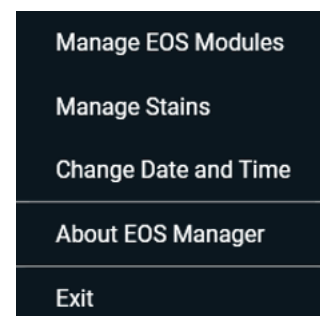


Figure 43.
System menu options

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.

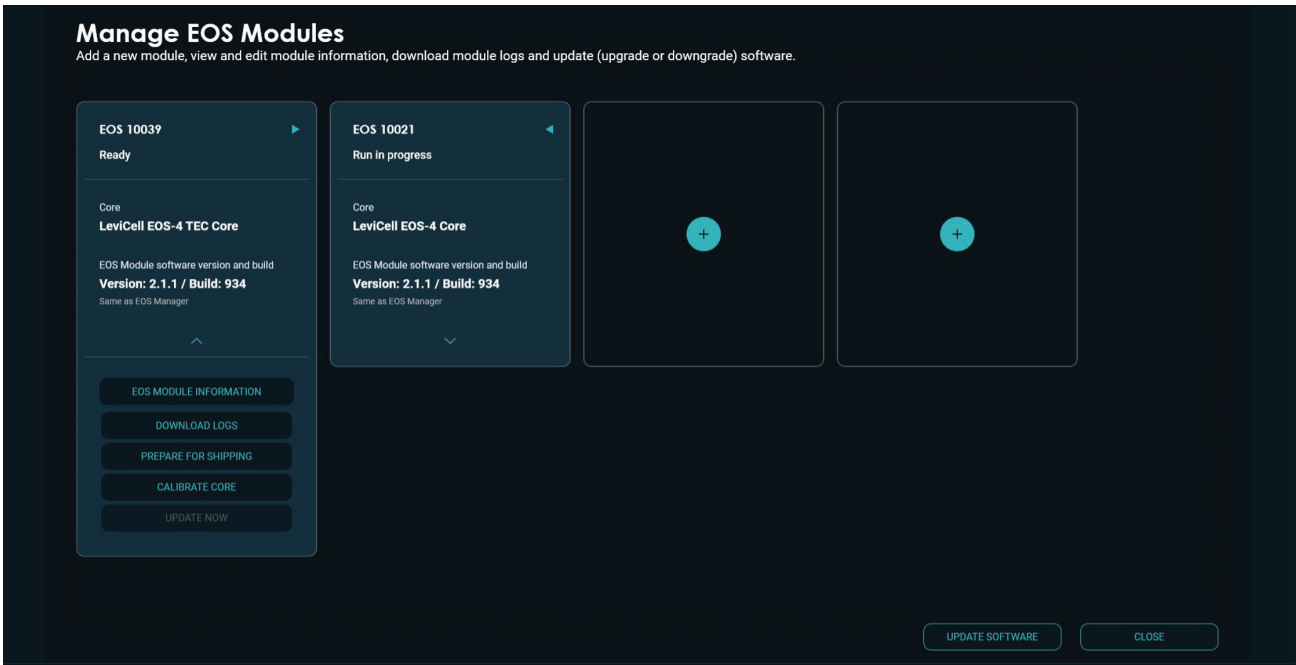


Figure 44. Manage EOS Module screen

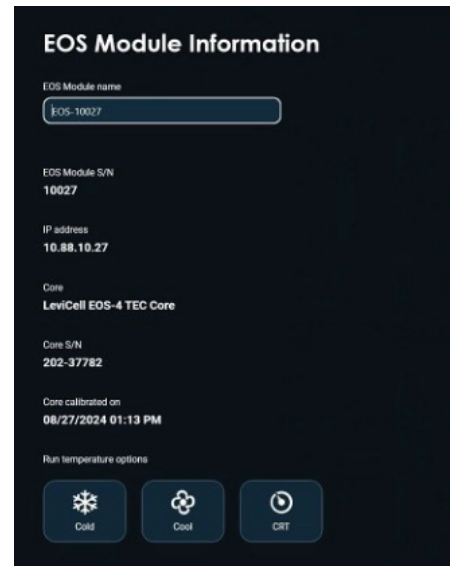


Figure 45. EOS Module and Core Module information.

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.

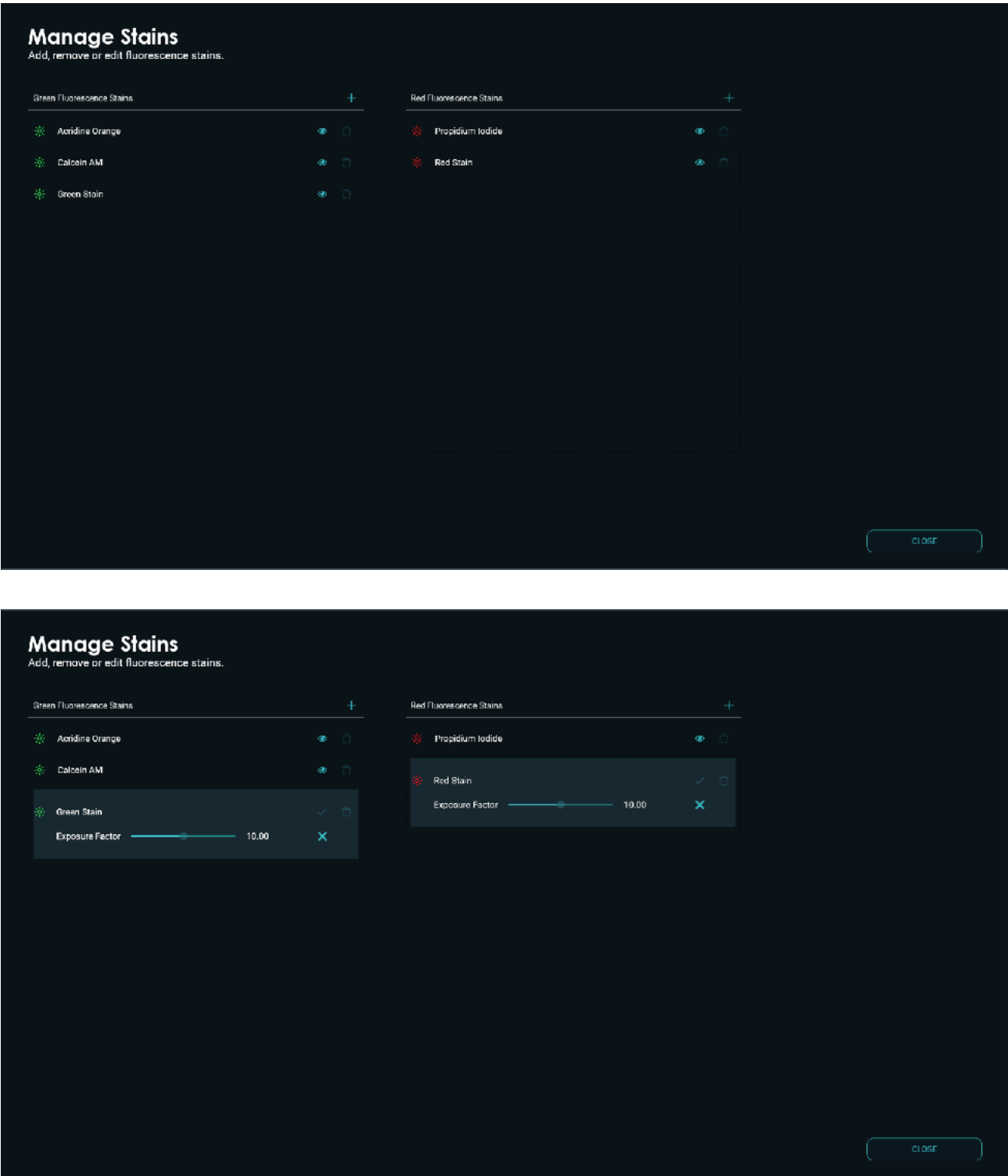


Figure 46. Manage Stains screen and default stains that have fixed settings

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.

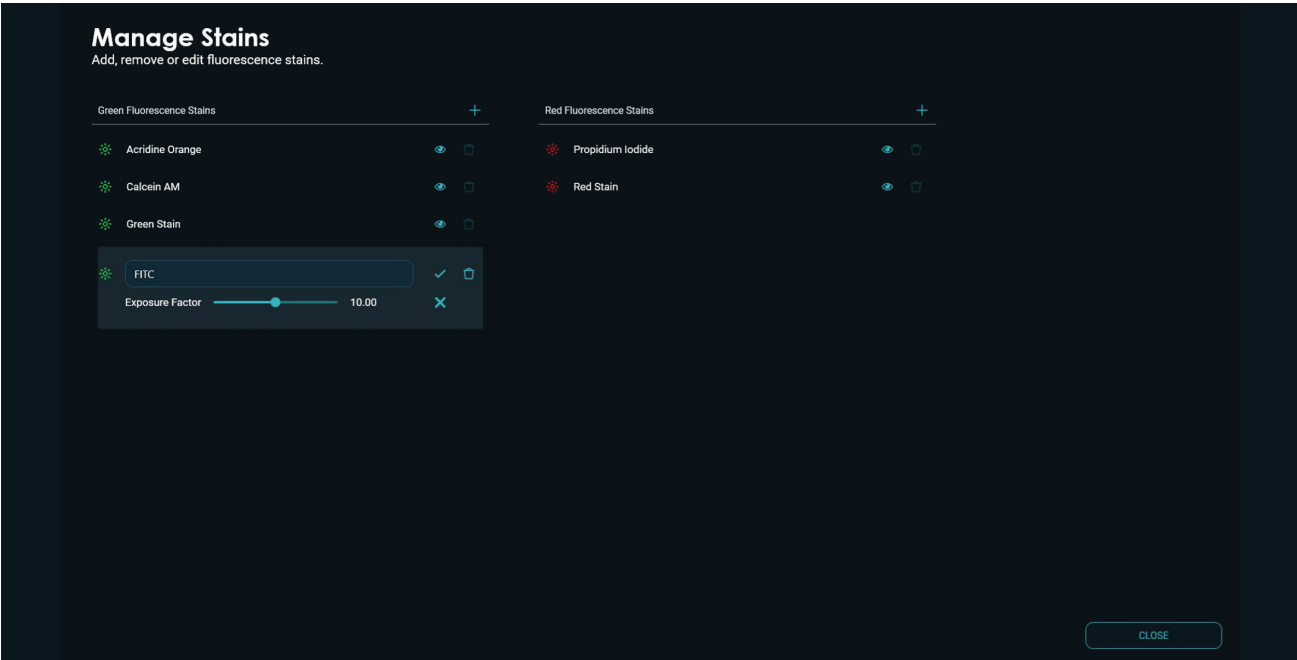


Figure 47. Adding custom stains

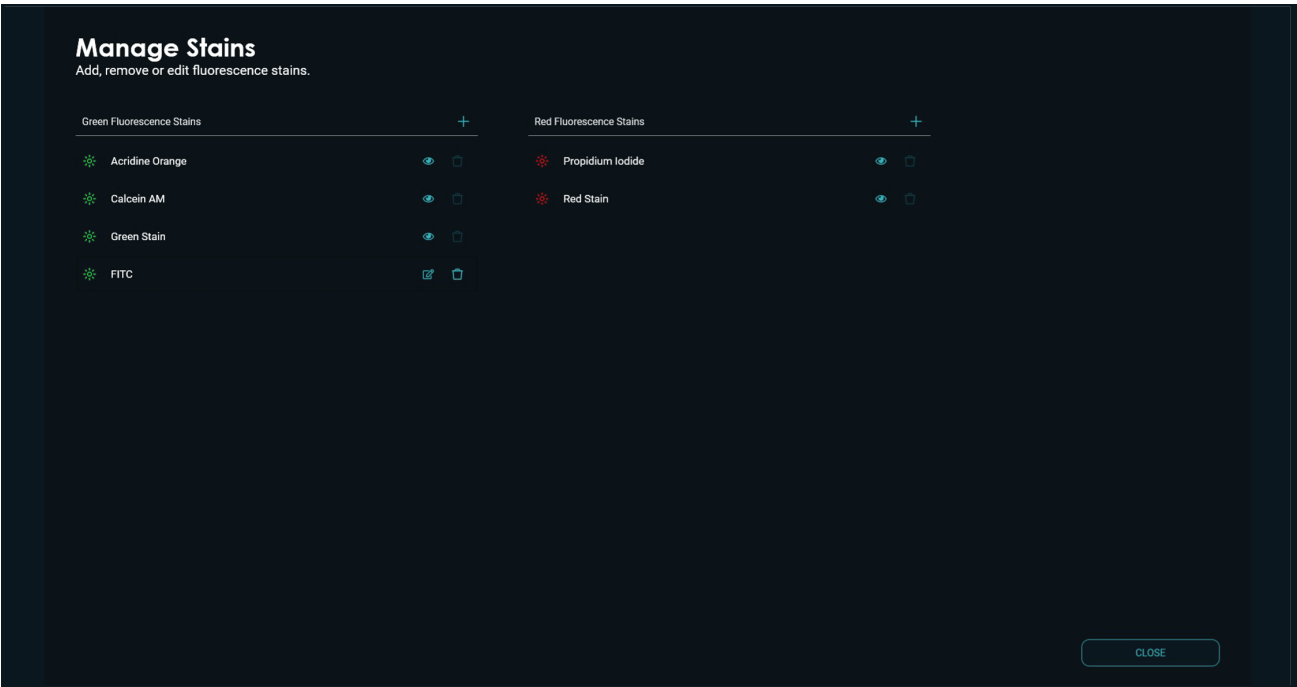


Figure 48. Green and Red custom stains added

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.

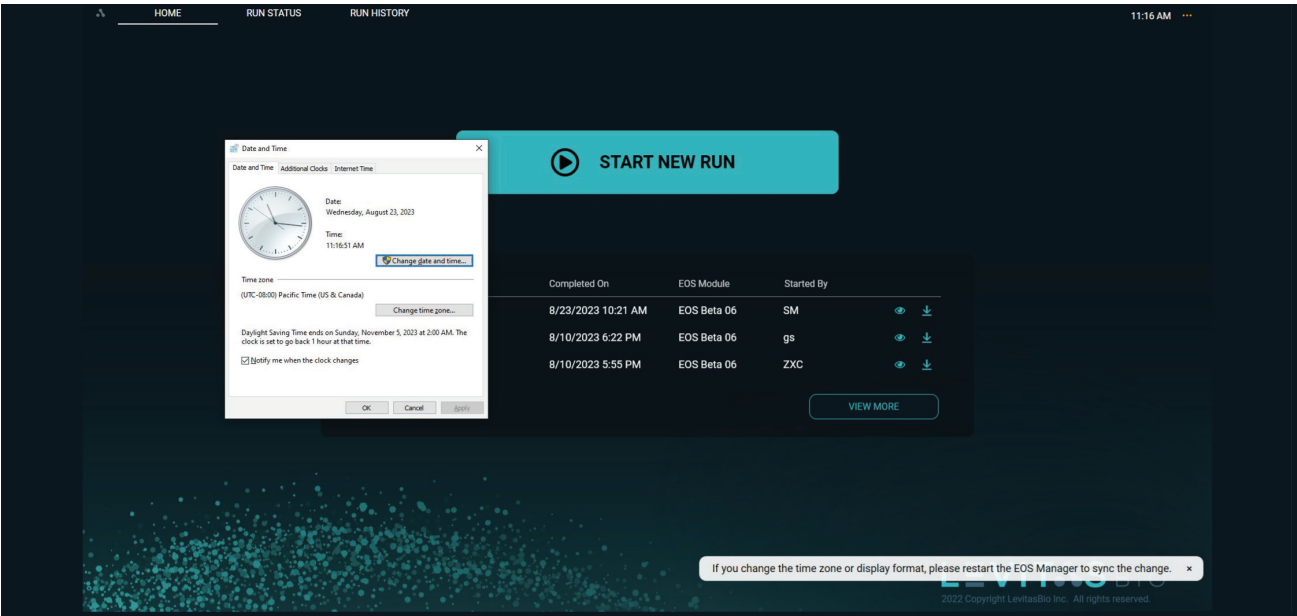


Figure 49. Change Date or Time via pop-up window

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.

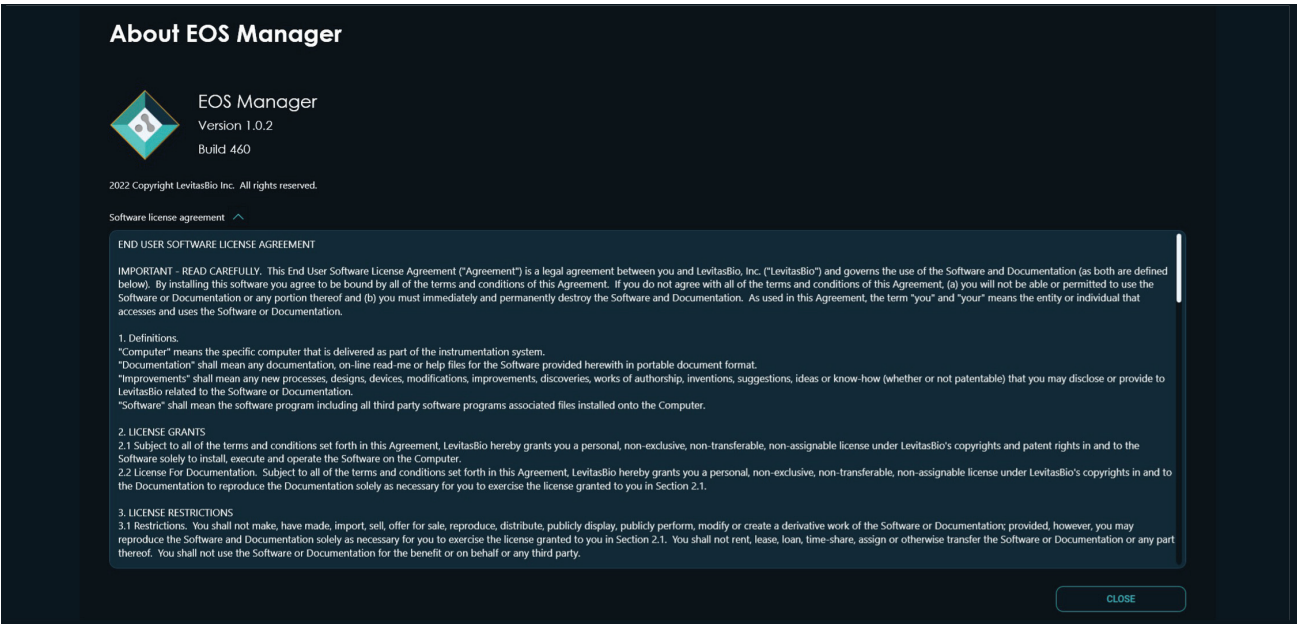


Figure 50. About the EOS Manager and software license agreement

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 the 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:

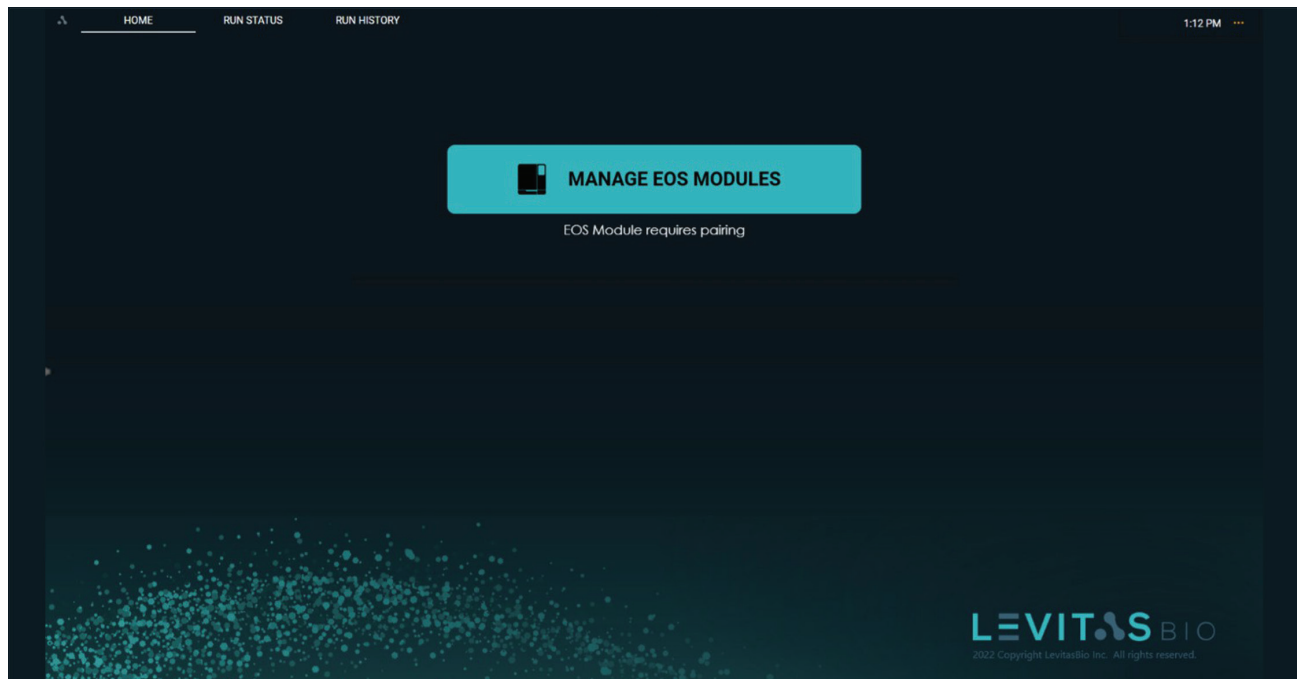


Figure 51. Manage EOS Module Home screen

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.

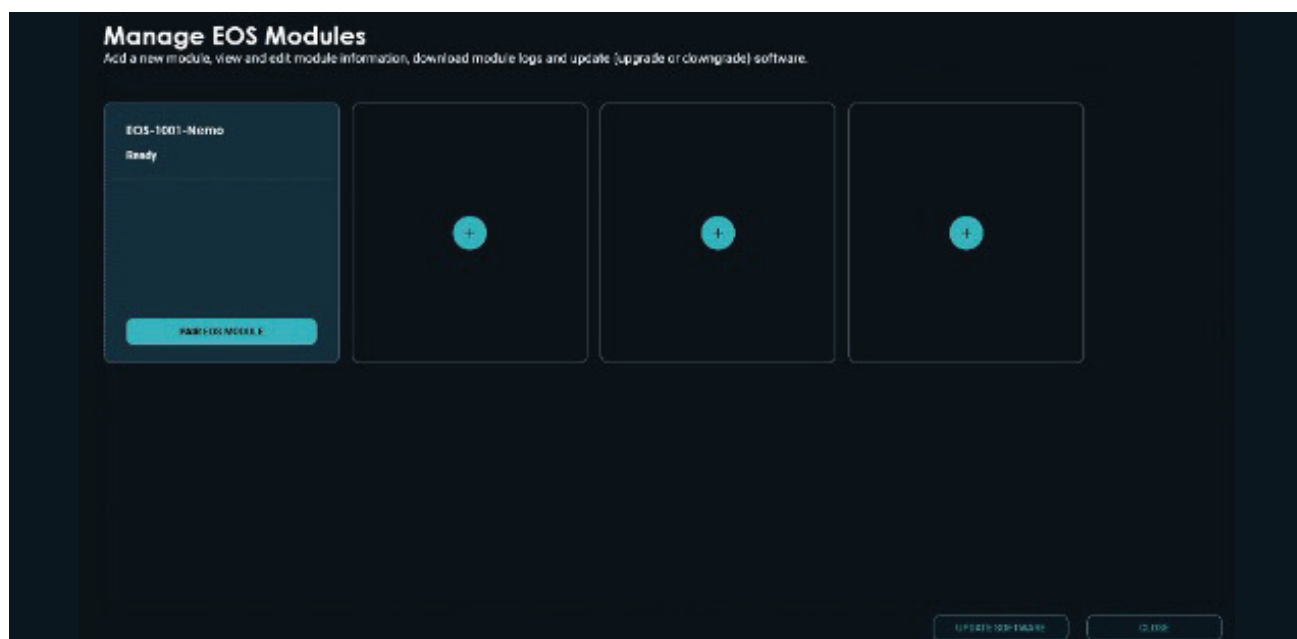


Figure 52. EOS Module requires pairing

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

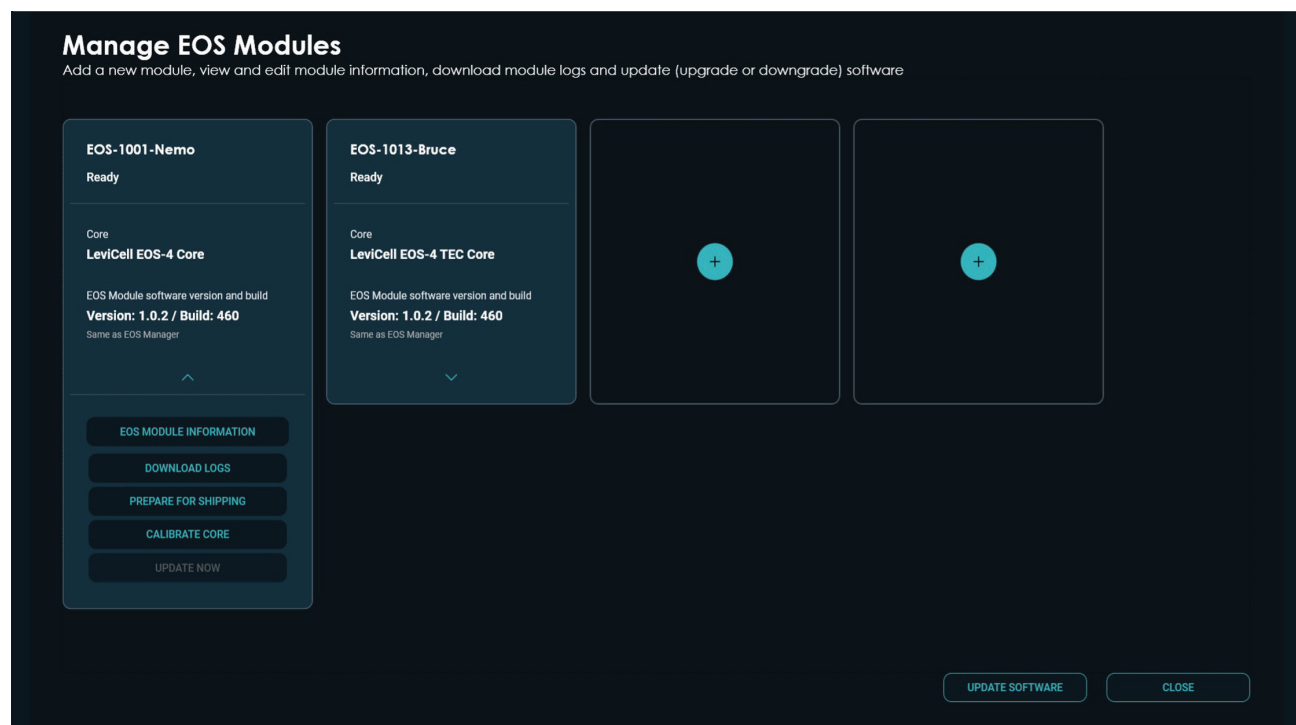


Figure 53. Paired EOS with options

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:

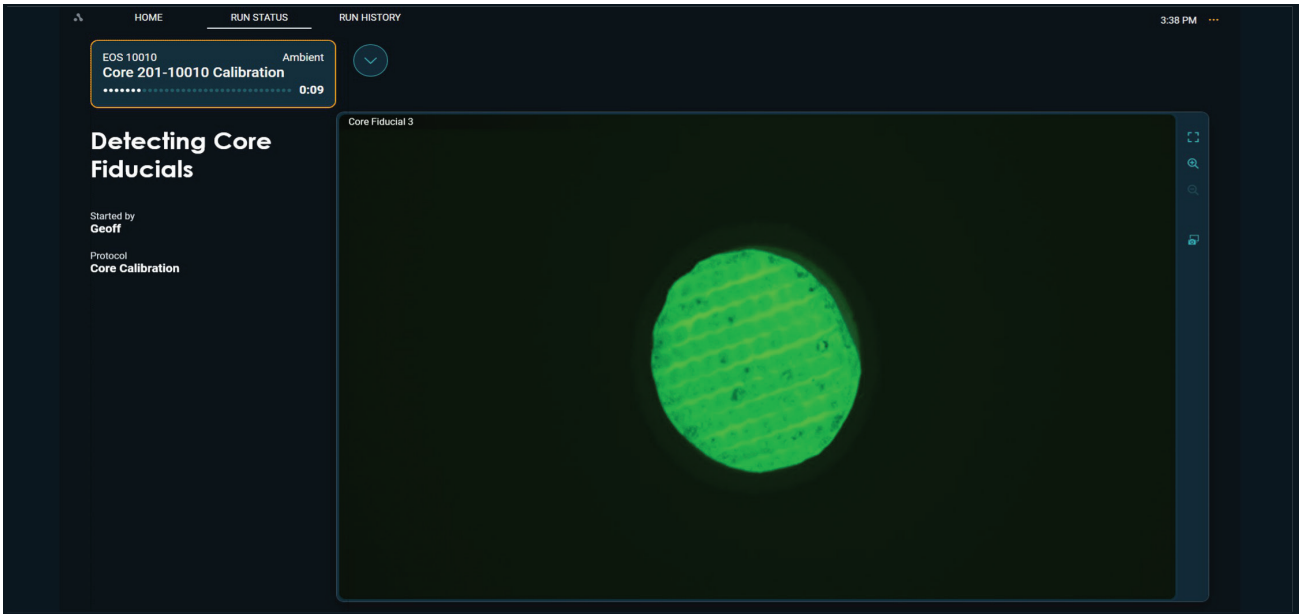


Figure 54. Detecting Core Fiducials

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:

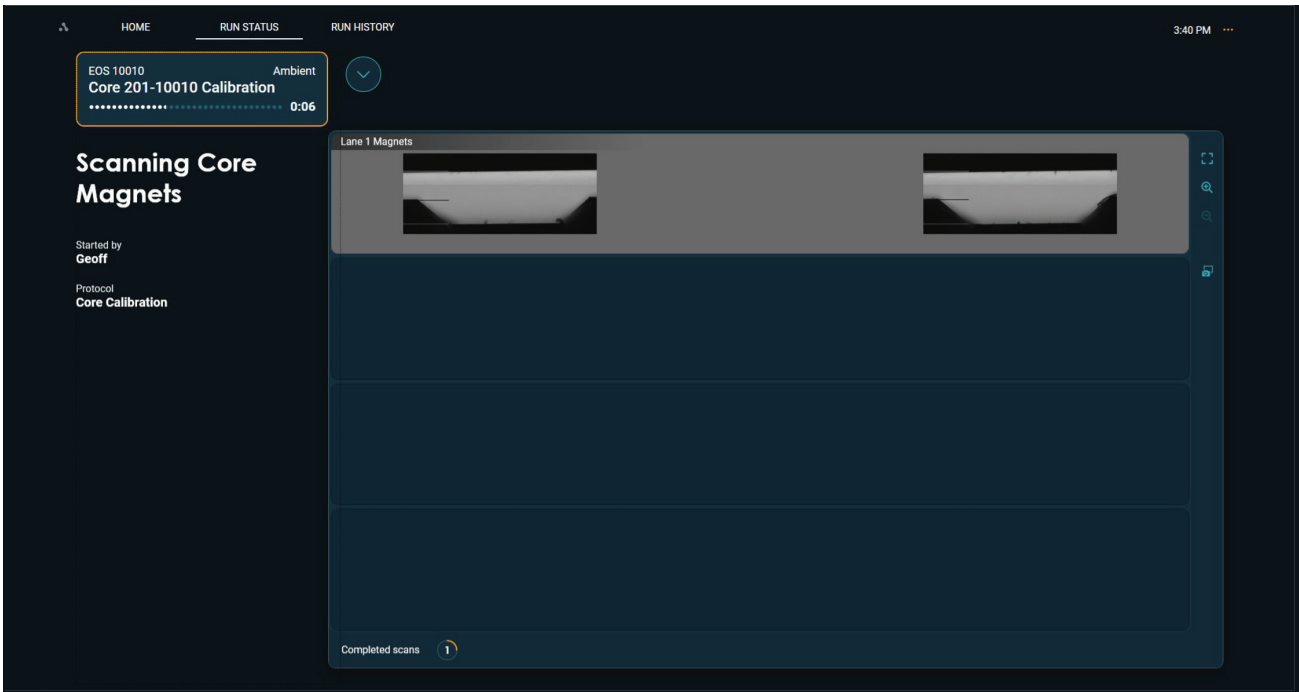


Figure 55. Scanning core magnets in progress on lane 1

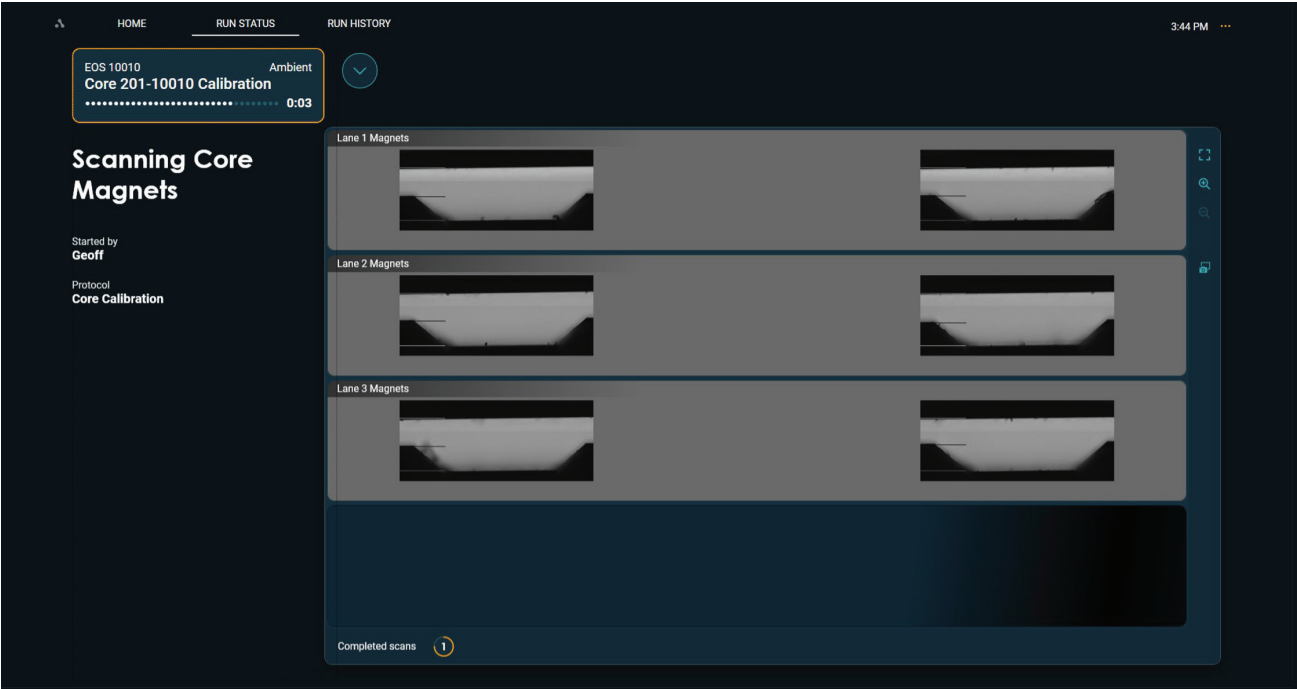


Figure 56. Scanning core magnets in progress across 4 lanes

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

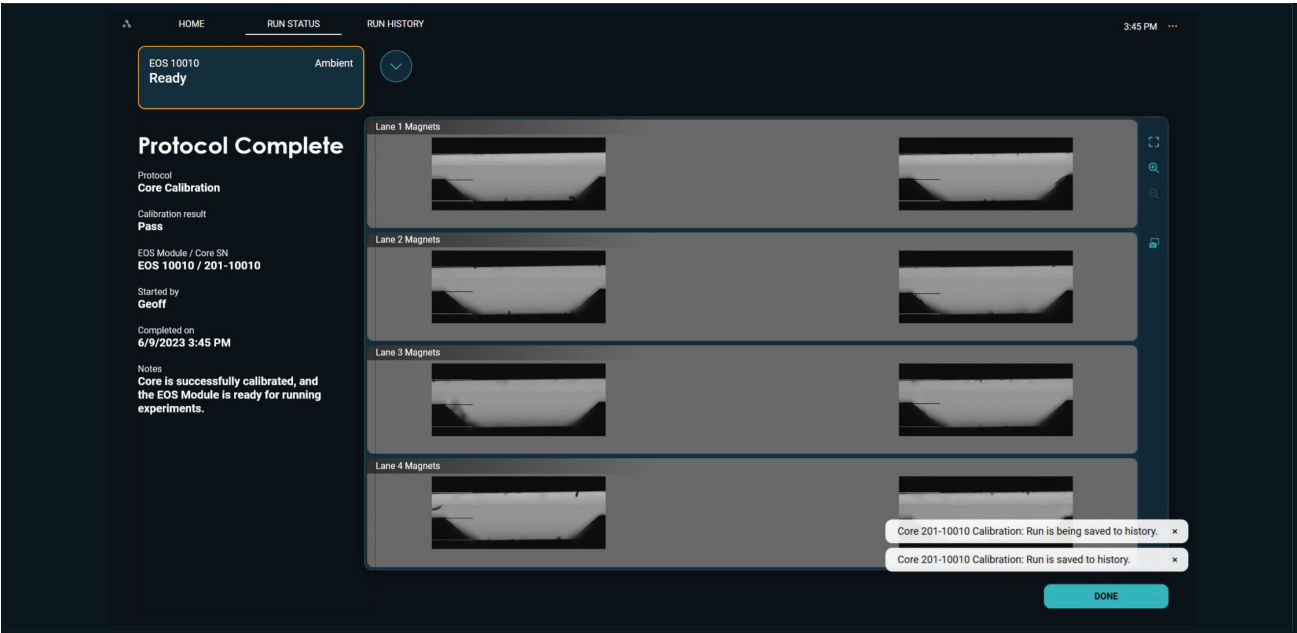


Figure 57. Calibration Protocol completed

After the system has been successfully calibrated, a system performance qualification test can be performed using the LeviCell EOS Starter Kit. 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.

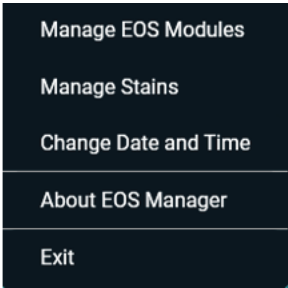


Figure 58. System Menu

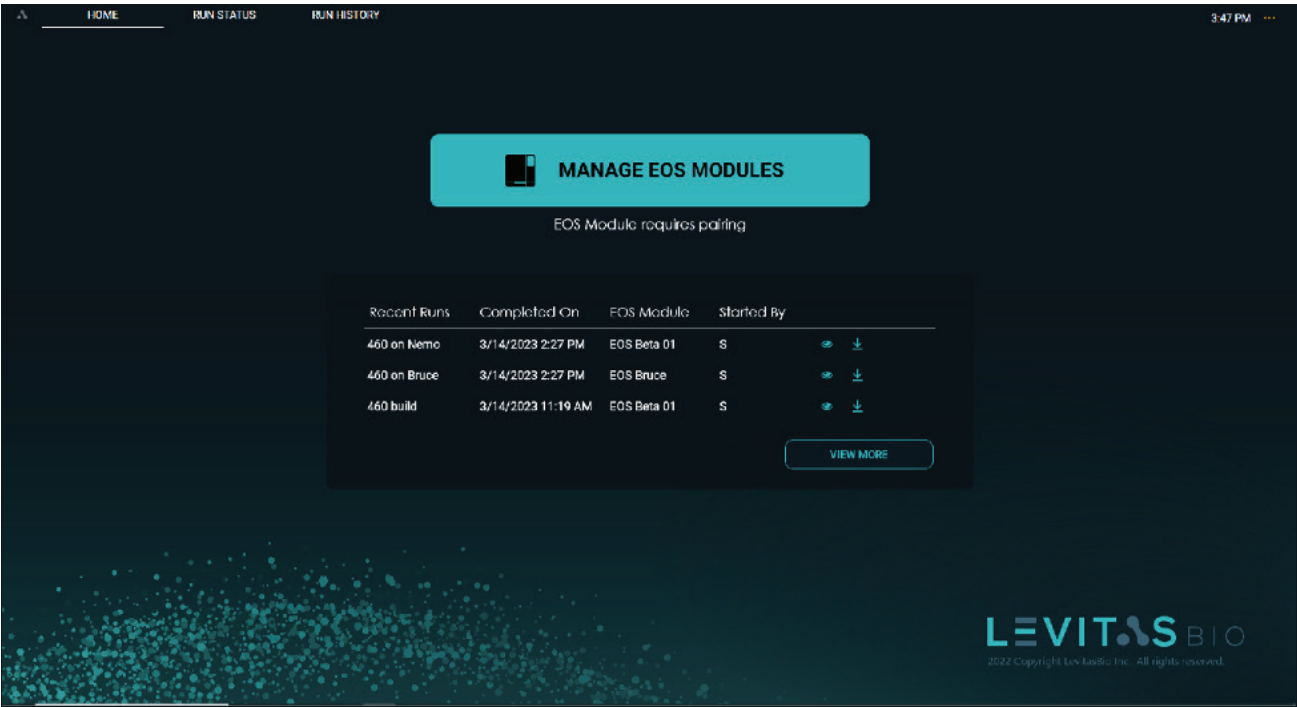


Figure 59. Home screen when an EOS Module requires pairing

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

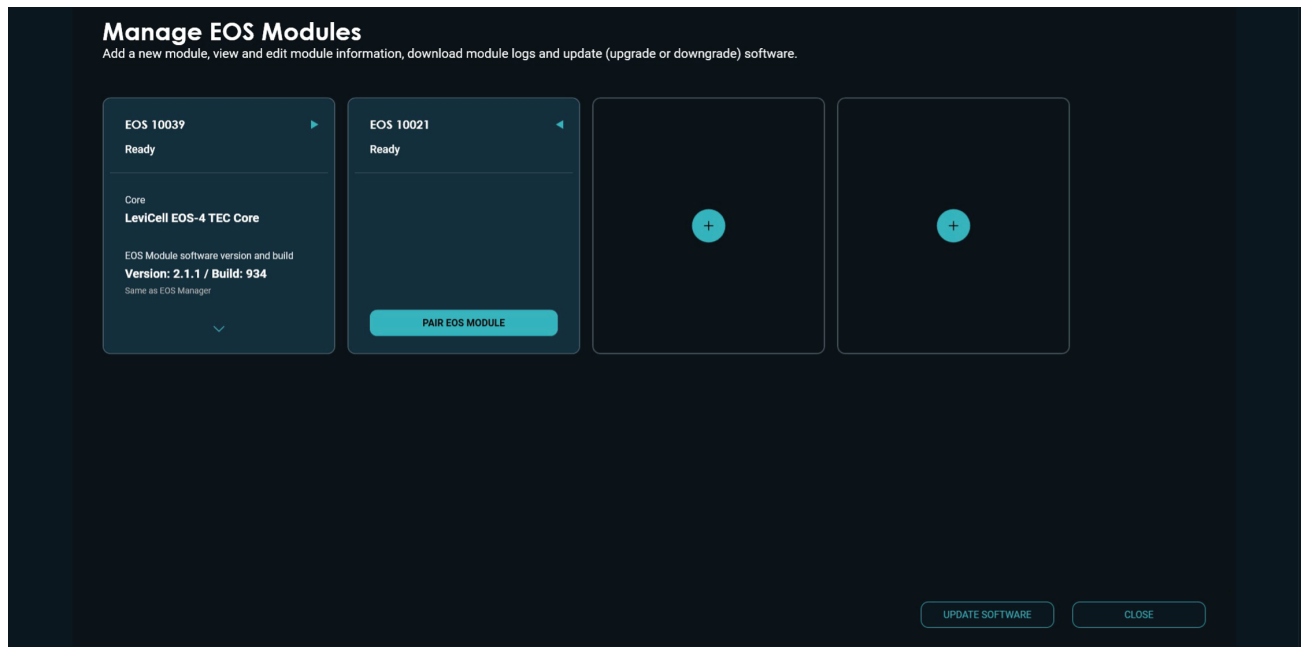


Figure 60. Additional EOS Module requires pairing

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

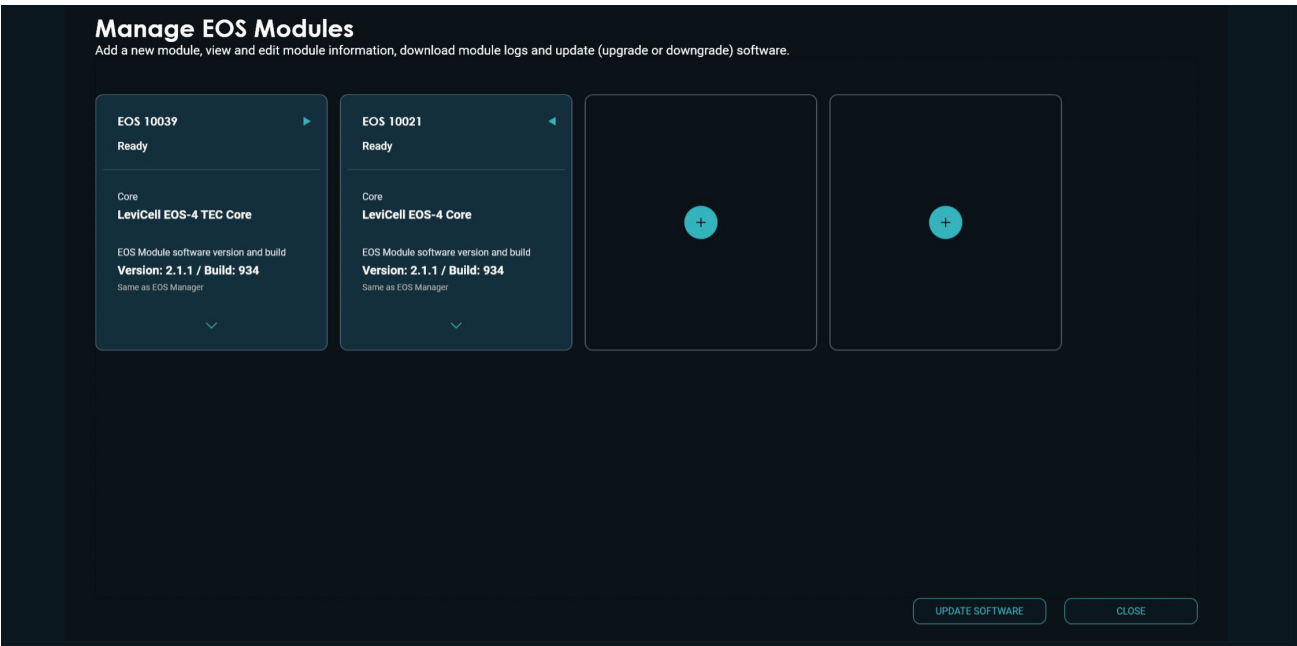


Figure 61. Additional EOS Module is paired and ready for use

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 Bead Test with LeviCell EOS Starter Kit prior to running experiments. This Bead Test serves as a product performance qualification test. All the consumables needed to perform the test are supplied when you order a LeviCell EOS Starter Kit (PN 1003109 or PN 1003110, sterile).

LeviCell EOS Performance Qualification Test

To verify the LeviCell EOS System has been installed and is performing to manufacturing specifications, use the LeviCell EOS Starter Kit. This kit includes beads of two different densities and a buffer. In conjunction with the Levitation Agent and cartridges supplied in the Starter 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 the Input Sample.
2. Run Bead Test workflow on the LeviCell EOS System.
3. (Optional) Count the input and output samples.

The installation beads are provided in two formulations that are mixed at the start of the test. LeviCell EOS Green Bead 1 is a green fluorescent, polystyrene (PS) bead with a density of 1.05 g/cc. LeviCell EOS Red Bead 2 is a red fluorescent, polymethyl methacrylate (PMMA) bead with a density of 1.18 g/cc. The fluorescence is used to confirm LED alignment on the instrument, as well as provide a visual distinction between the PS and PMMA beads. The beads from each of the formulations are approximately 20 μm in diameter.

After the Bead Input Sample is loaded into the EOS-4 cartridge separation channel during the LeviCell workflow, the beads levitate and separate 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 62 at the beginning of the workflow (top panel) and after levitation (bottom panel).

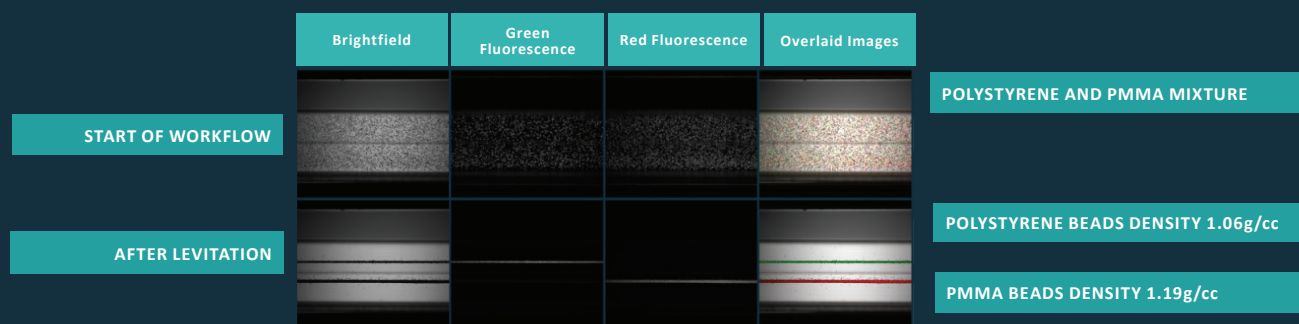


Figure 62. Example images of bead mixtures taken during Performance Qualification

Step-by-Step Protocol for Bead Test

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

A. Prepare Bead Sample

1. Vortex bead tubes thoroughly immediately before pipetting to avoid sedimentation.
2. In a new 1.5 mL tube, prepare the sample mixture as shown in Table 12.
3. Vortex mixture well to completely mix the sample.
4. If counting, set aside 20-30 μL of the input beads immediately after mixing.

Reagent	Volume (μL) # of lanes to run			
	1	2	3	4
LeviCell EOS Install Buffer	176	352	528	704
1 M Levitation Agent	32	64	96	128
LeviCell EOS Green Bead 1	16	32	48	64
LeviCell EOS Red Bead 2	32	64	96	128
Total	256	512	768	1024

Table 12. Bead Sample Preparation with Final Levitation Agent Concentration of 125mM (1-4 lanes)

B. Run the LeviCell EOS Instrument

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

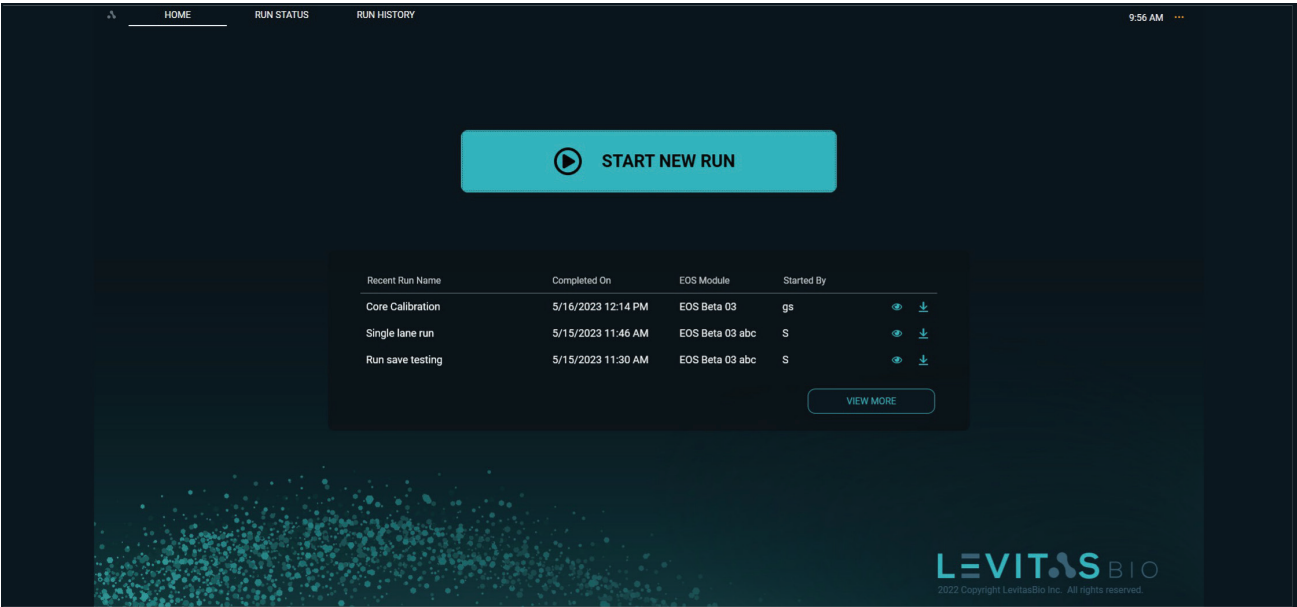


Figure 63. Home screen

- 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 64. 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.

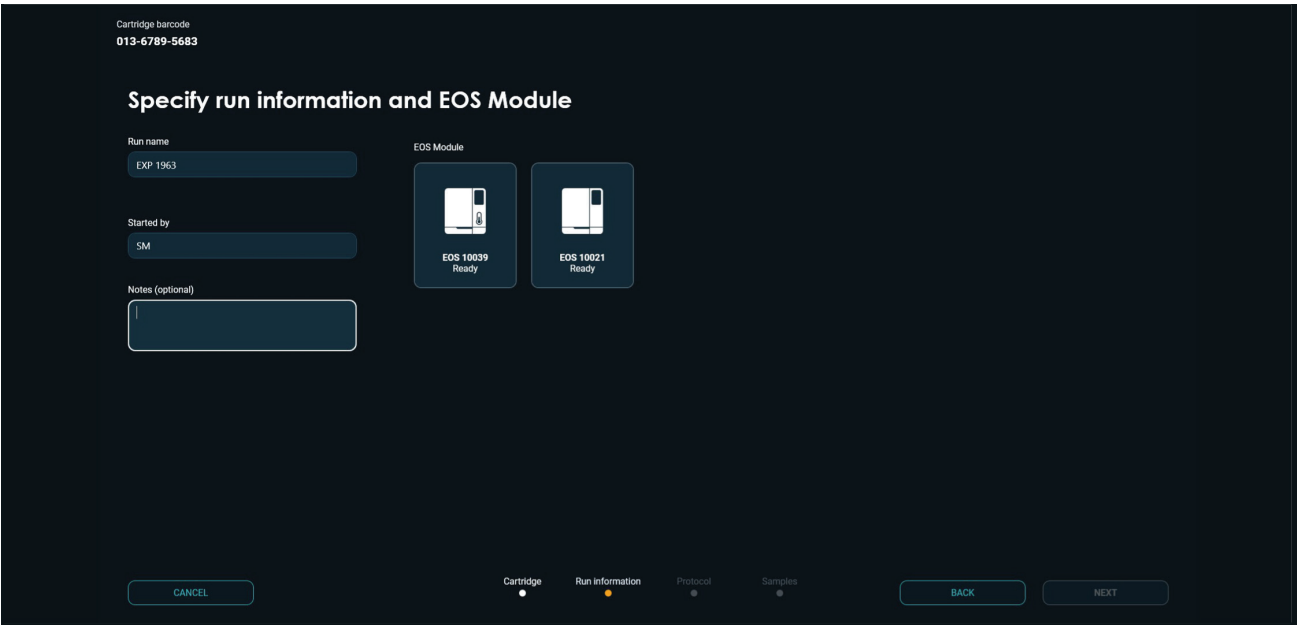


Figure 65. Run information screen

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

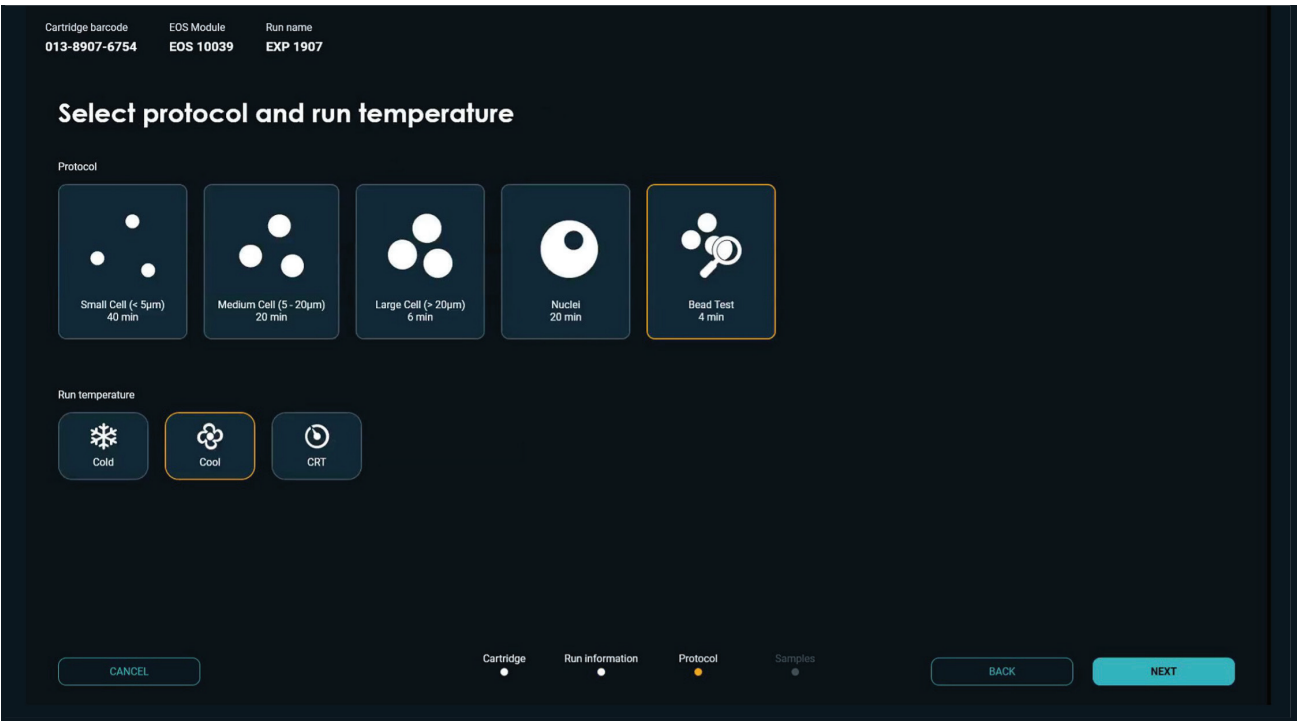


Figure 66. Select protocol, and temperature screen



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

Cartridge barcode: 013-6789-1999 | EOS Module: EOS-10107 | Run name: EXP1999 | Protocol: Bead Test | Levitation: 4 min | Run temperature: Ambient

Select and specify samples to run

Click sample well below to include/exclude from run

☒ Same Levitation Agent concentration (LA) or fluorescence stains for all the samples

	Sample name	LA (mM)	Green fluorescence	Red fluorescence
1	Bd1	125	Bright Green Stain	Bright Red Stain
2	Bd2	125	Bright Green Stain	Bright Red Stain
3	Bd3	125	Bright Green Stain	Bright Red Stain
4	Bd4	125	Bright Green Stain	Bright Red Stain

CANCEL | Cartridge | Run Information | Protocol | Samples | BACK | NEXT

Figure 67. Select samples and specify sample information

- 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.
- The Levitation Agent concentration for the Bead Test [LA (mM)] is 125.
- Choose **Bright Green Stain** from the pull-down menu for Green Fluorescence, and **Bright 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. Handle the cartridge only by the grip, and leave the door closed until clamping is complete, to avoid risk during cartridge clamping.



Figure 68. Open door to load cartridge

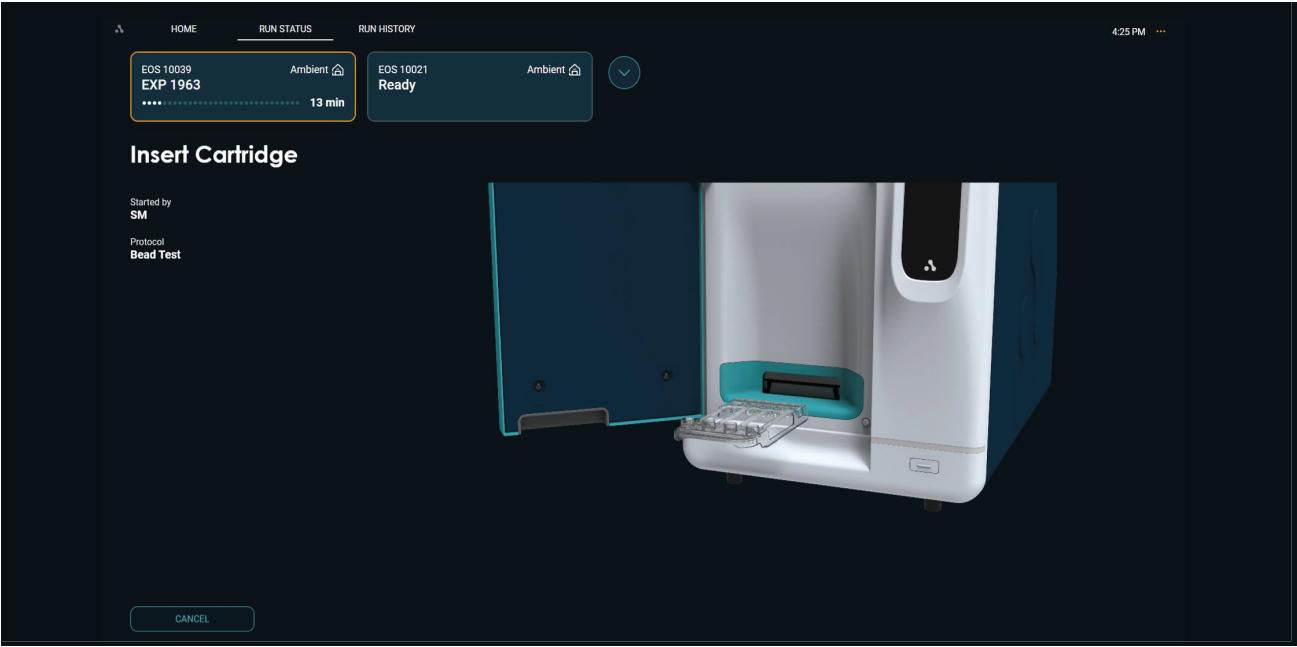


Figure 69. Insert cartridge into EOS Module

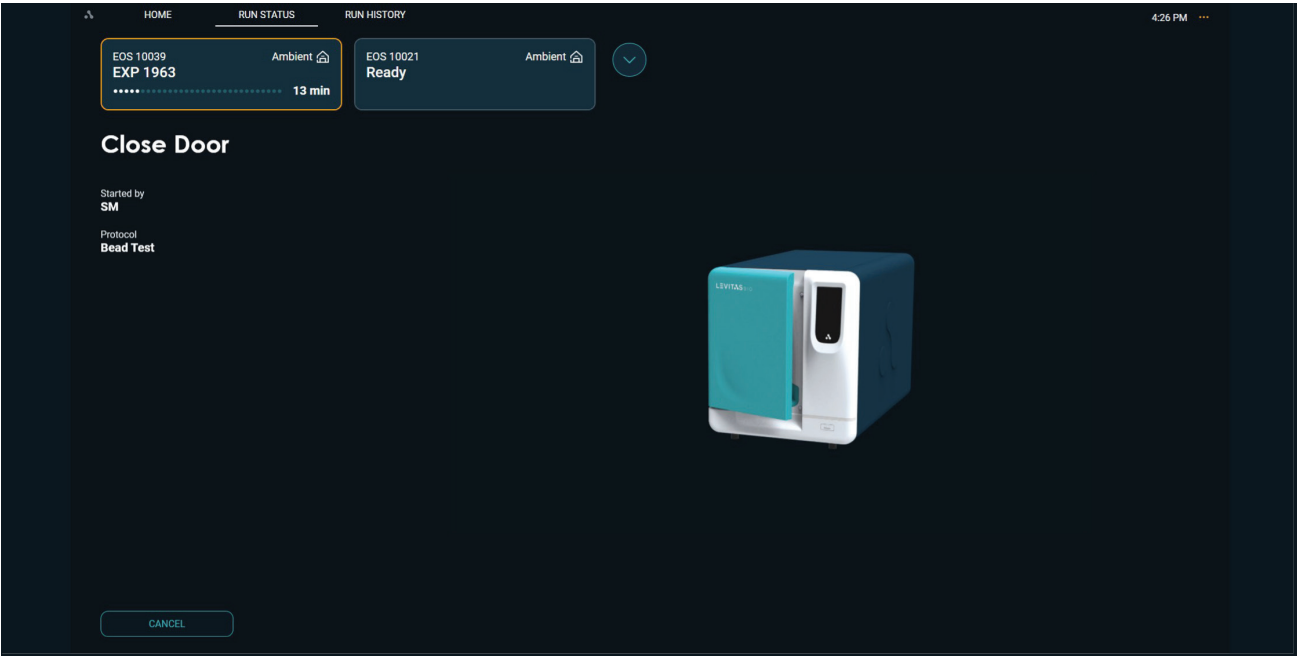


Figure 70. Close door after inserting cartridge

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.

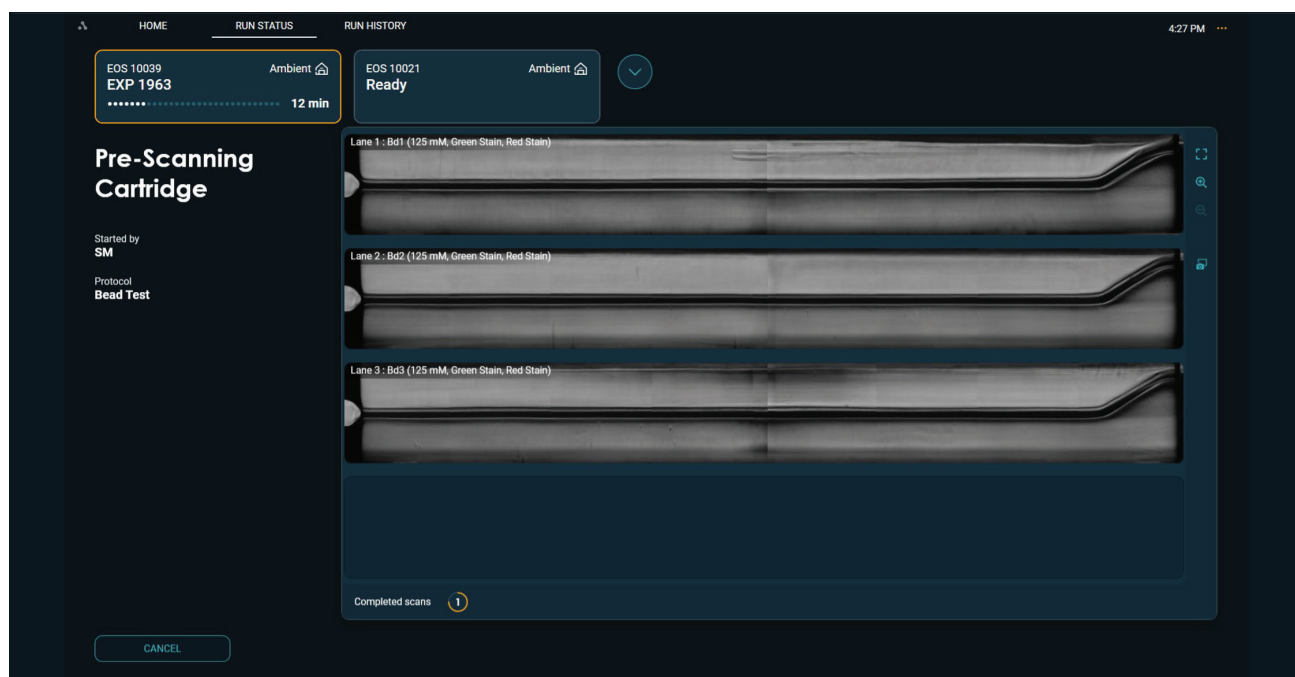


Figure 71. Pre-scanning cartridge 3 of 4 lanes

8. Follow subsequent prompts to then dispense samples.



NOTE: Mix sample thoroughly by pipetting up and down gently 5X and immediately load 220 μ L 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.

- After dispensing your samples, press **NEXT** on the LCD touch screen or click **NEXT** at the Control PC.

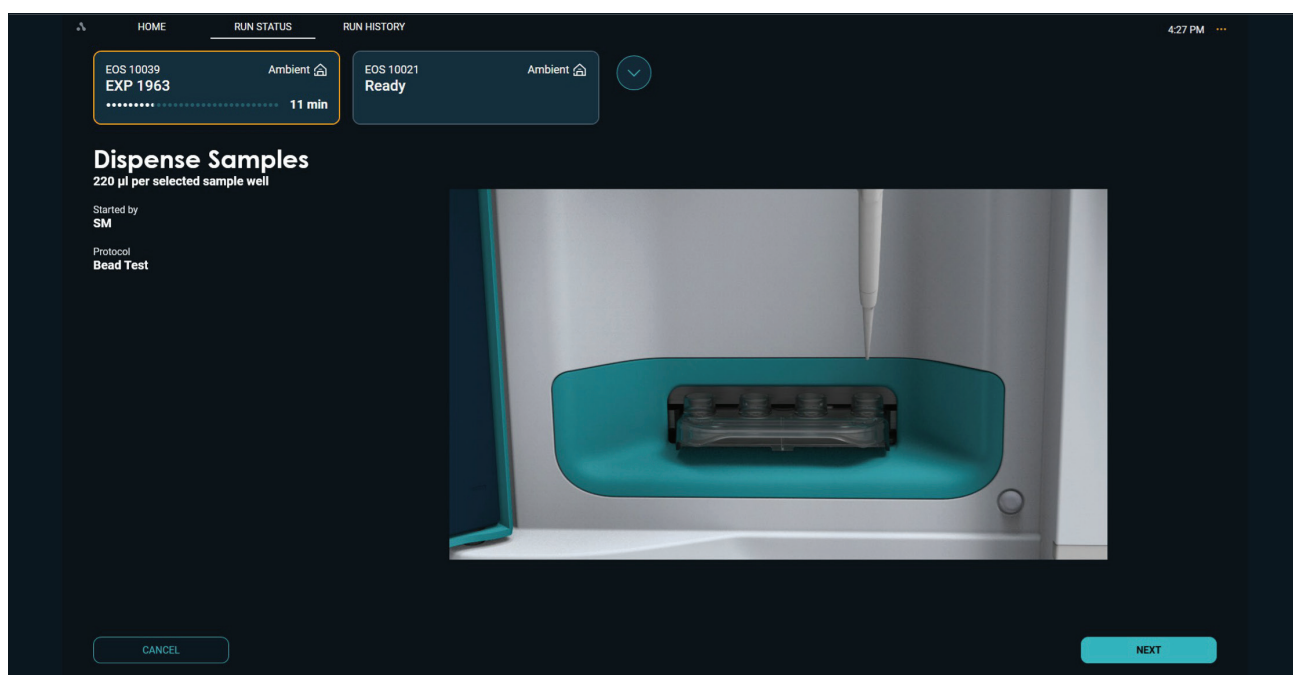


Figure 72. Dispense samples into the respective wells

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

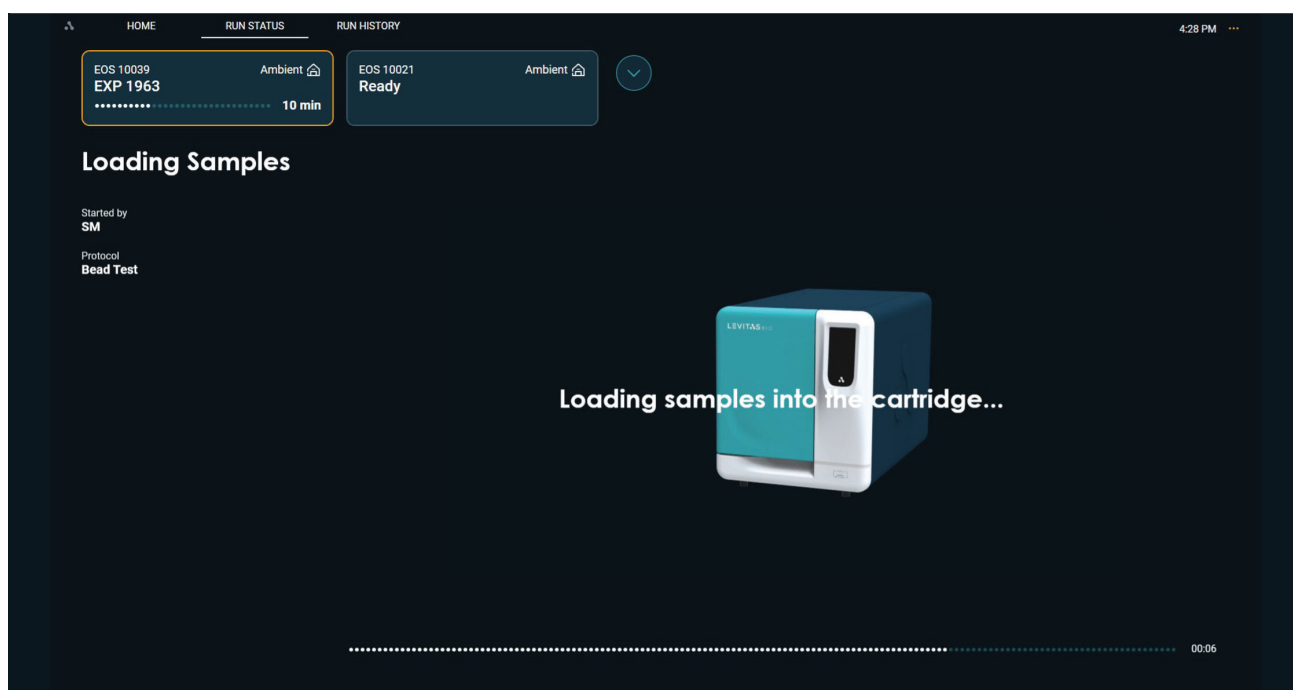


Figure 73. Loading samples into the cartridge

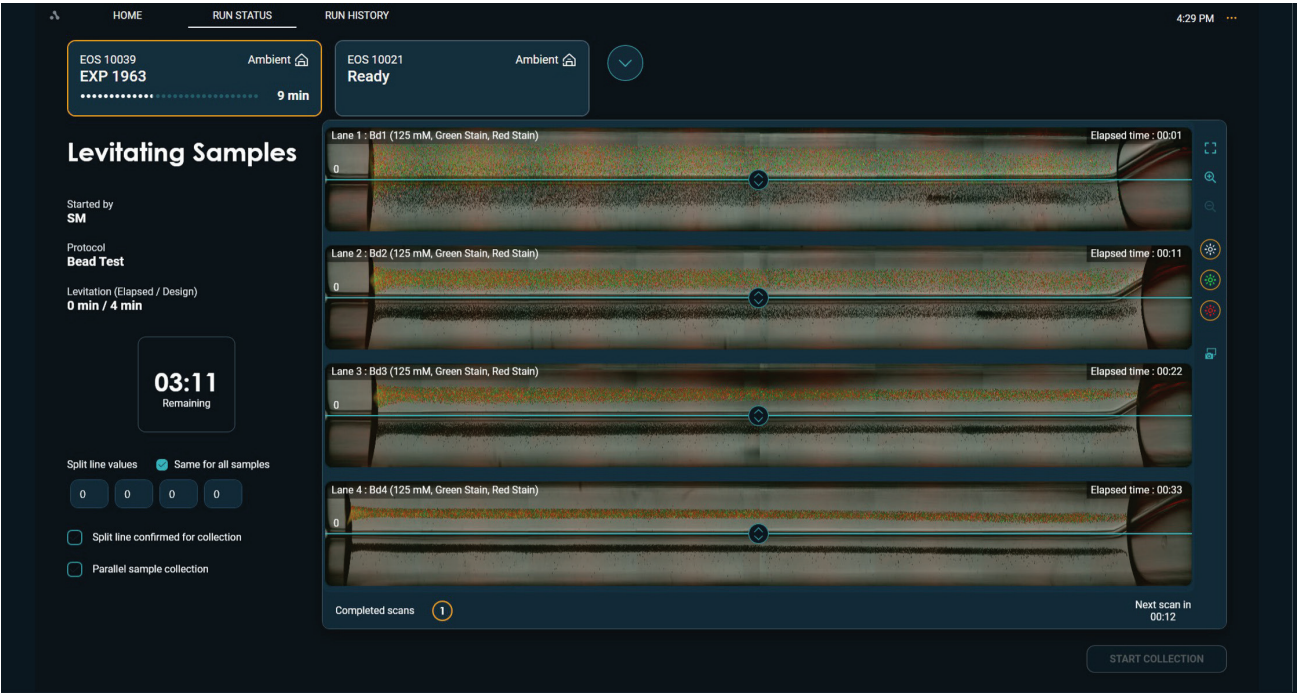


Figure 74. Beads levitating and image scanning

11. 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.

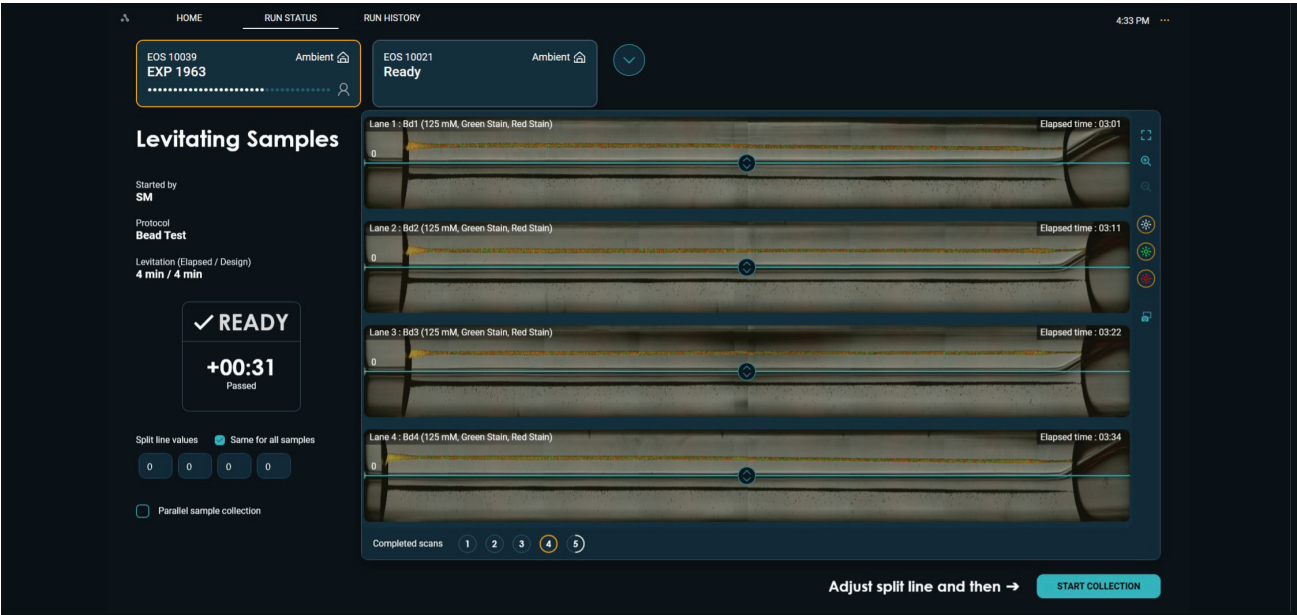
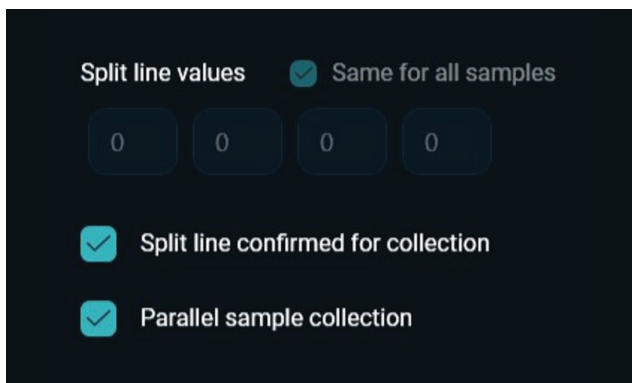


Figure 75. Set split line when levitation has completed and start collection



Split line values ☒ Same for all samples

0 0 0 0

☒ Split line confirmed for collection

☒ Parallel sample collection

Before Collection, note that the system will default to a 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 76.

Check boxes for confirming split line and parallel sample collection

Core Type	Protocol	Run Temperature	Default Sample Collection	
			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 13. 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.

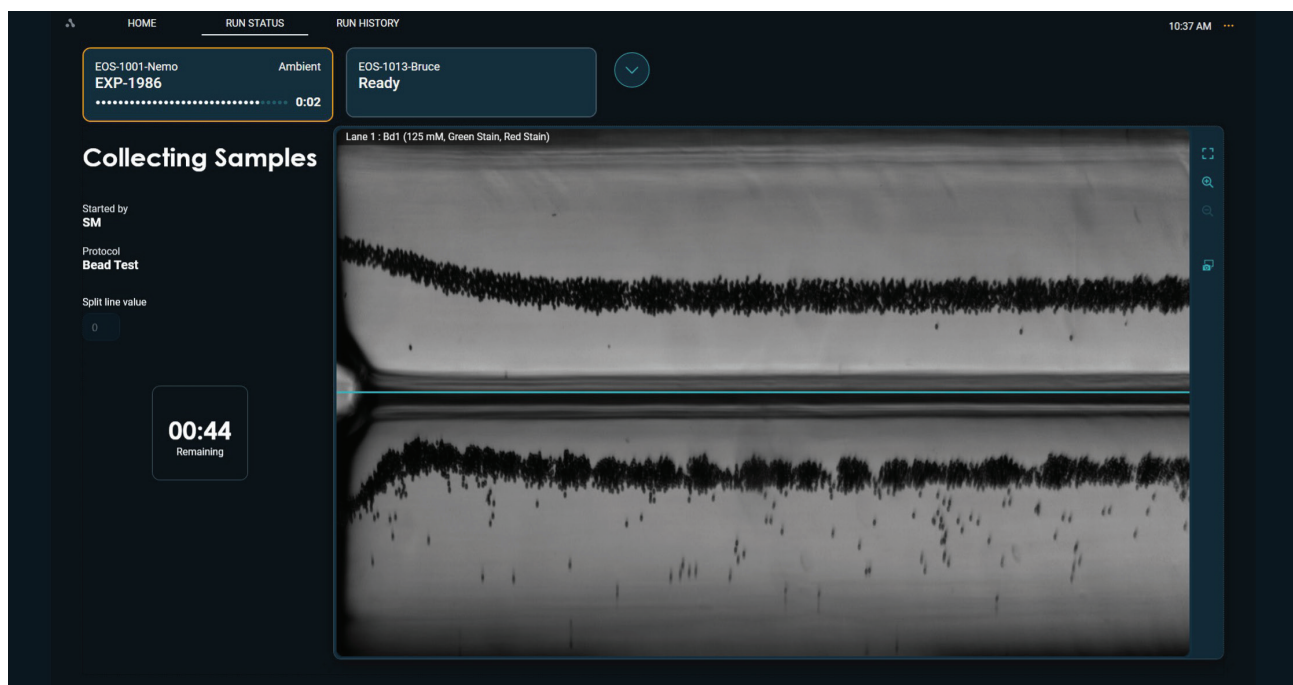


Figure 77. Imaging collection of Lane 1 as it flows to the outlet well



Figure 78. Fluorescence collection

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

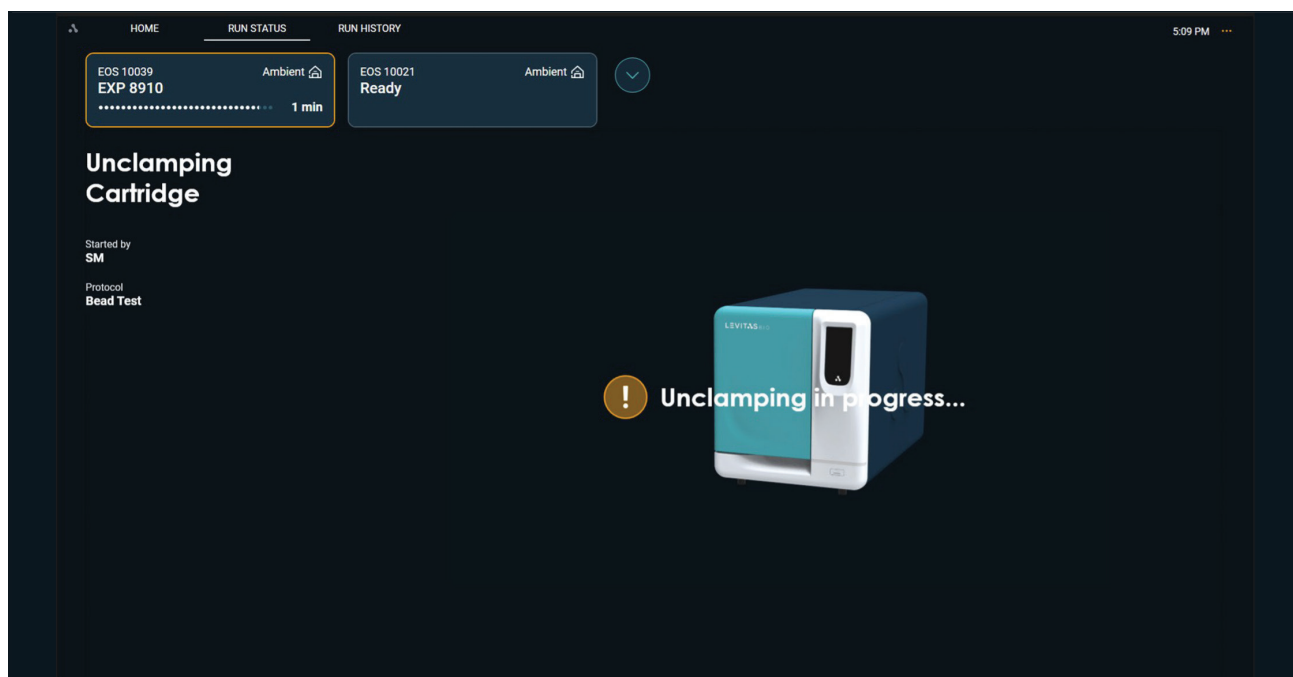


Figure 79. EOS Module will unclamp the cartridge at the end of collection

14. Retrieve cartridge and harvest output

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

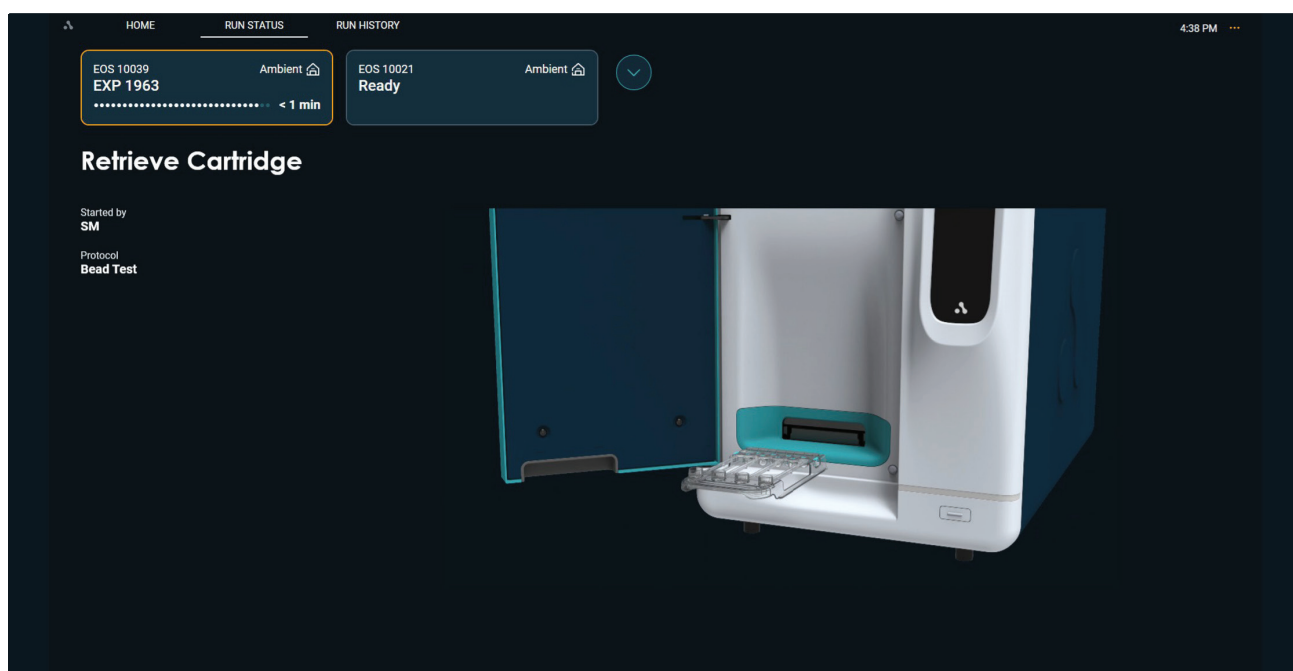


Figure 80. Retrieve cartridge from EOS Module

- 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.

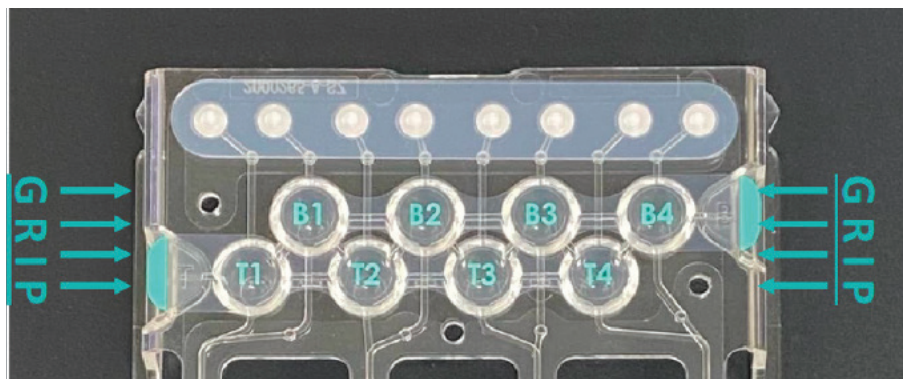


Figure 81. Grip locations on the cartridge when peeling outlet well tape off

- 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. The Green Beads are in the top **(T1-T4)** wells. The Red Beads are in the bottom **(B1-B4)** wells.
- 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. If further analysis is desired, volume and the number of beads in outputs can be counted and compared to the volume and number of beads in the reserved input sample. Refer to Appendix A - Performance Qualification Checklist for tracking and recording results.

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

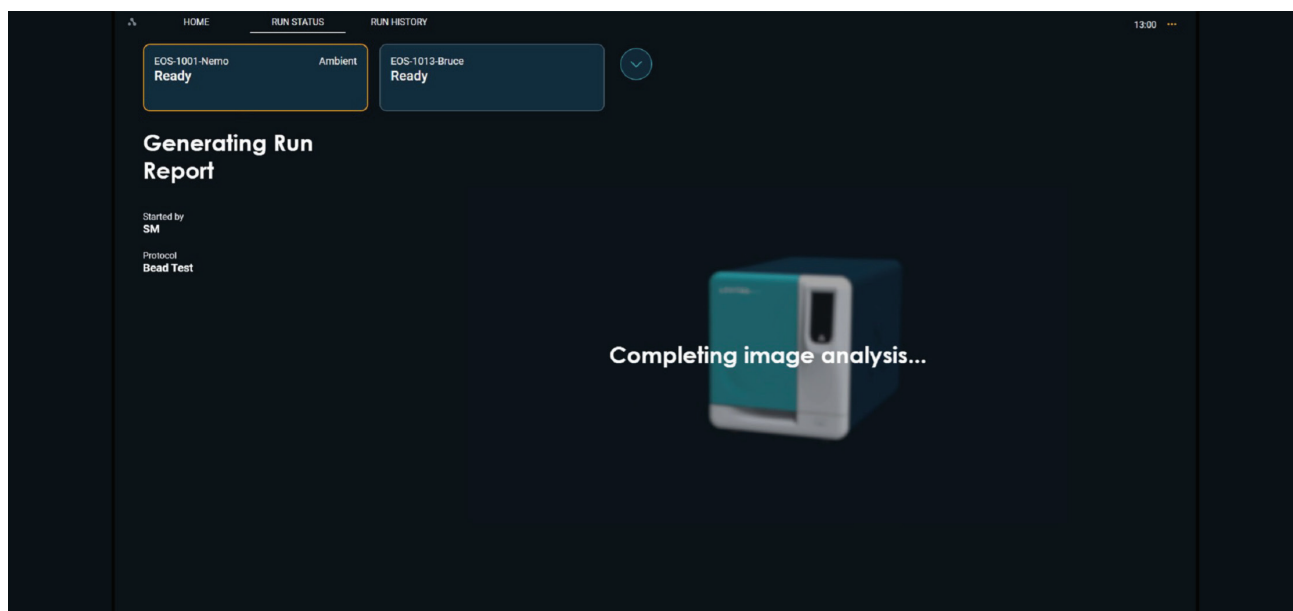


Figure 82. Generating Run Report after image analysis is completed

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

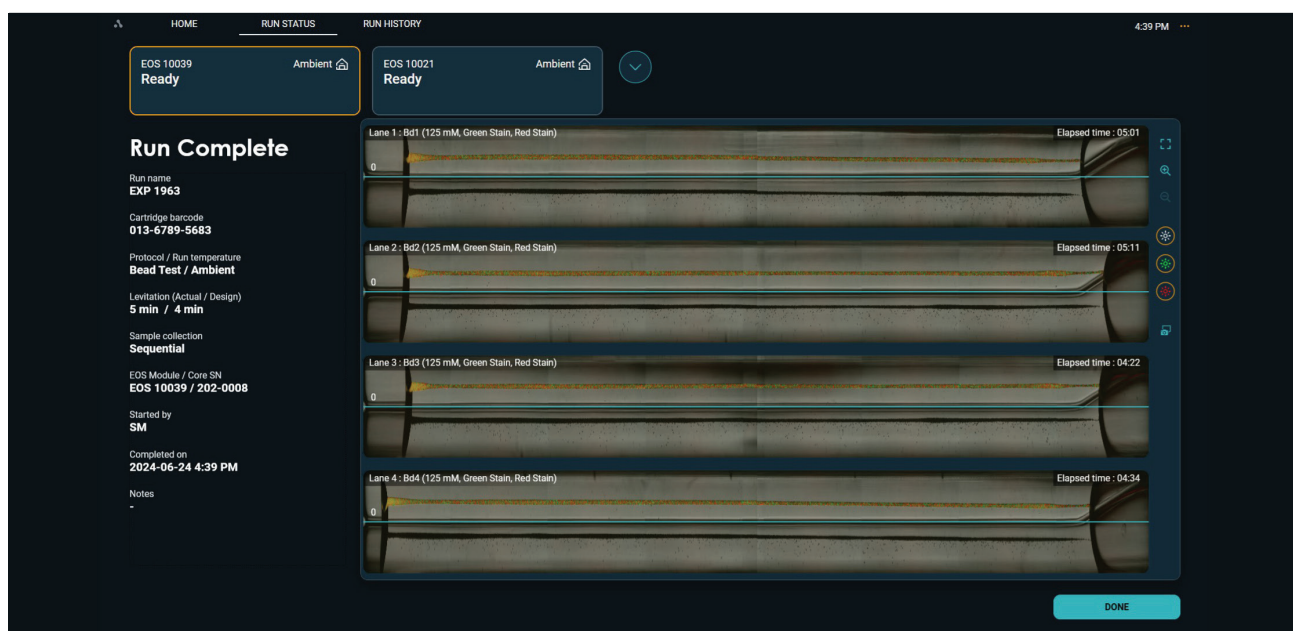


Figure 83. Run Complete screen

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. The Keeping Dry process will initiate to pull air through the system for approximately 45 mins. This can be interrupted at any time for a new run to be started.

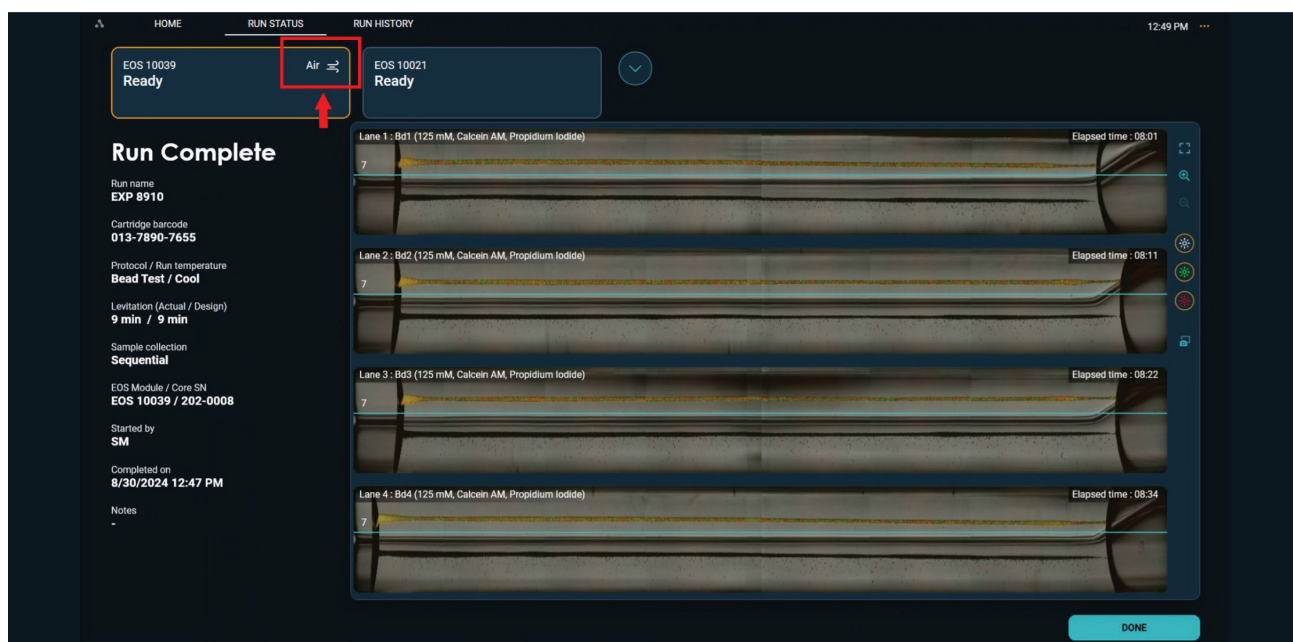


Figure 84. Run complete if Bead Test is run cold or cool

RUNNING AN EXPERIMENT

Protocols Supported by LeviCell EOS Systems

LeviCell EOS Systems currently support validated Viable Cell and Nuclei Enrichment protocols. Please consult [90-00125 EOS Viable Cell Enrichment Quick Reference Guide](#) and [90-00298 LeviPrep Nuclei Kit II Product Data Sheet](#) for more information.

Viable Cell and Nuclei 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.

A Viable Cell Enrichment or Nuclei 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 cells, and debris will levitate lower to the bottom half of the separation channel.

General Guidelines for Cell Sample Preparation

- LevitasBio-tested media/buffers are RPMI + 10% FBS, and PBS + 0.5% BSA.
- It is highly recommended that Levitation Buffer be prepared using filtered media / buffer.
- Levitation Buffer is a cell buffer with a diluted Levitation Agent. For Viable Cell Enrichment, 150mM final concentration is recommended, the Levitation Agent stock concentration is 1M.
- All samples must be suspended in the LeviCell Levitation Buffer.
- Recommended total cell number: 20,000 – 1,000,000 total cells.
- Required LeviCell EOS total sample input loading volume per lane: 220 μ L.
- LevitasBio recommends washing cryopreserved cells prior to cell separation.



NOTE: Filtering the media helps to remove debris that may be present in complete media.

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 is performed using the CRT mode, collection will happen immediately.

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.

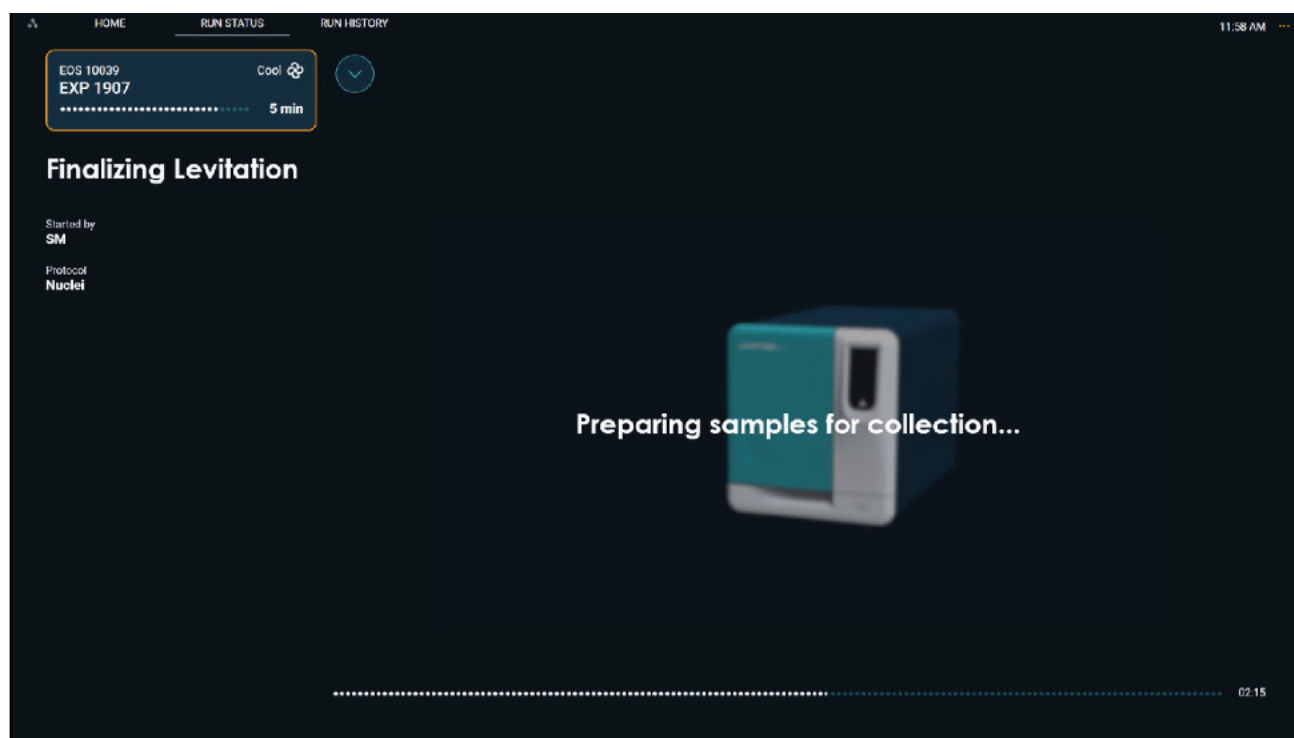


Figure 85. Finalizing levitation and preparing samples for collection

Counting Cells

1. Perform cell counts using the 10 to 15 μL aliquots of sample input and output collected. LevitasBio recommends a 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.

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

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.

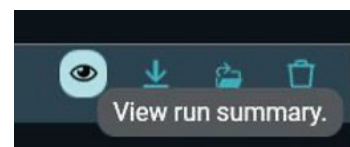


Figure 86. Hover over and run summary



Figure 87. 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.

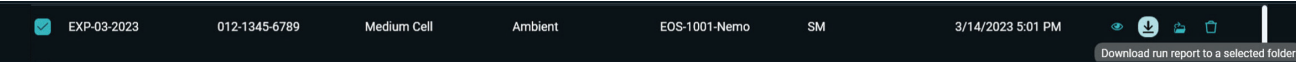


Figure 88. Run Summary download

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.

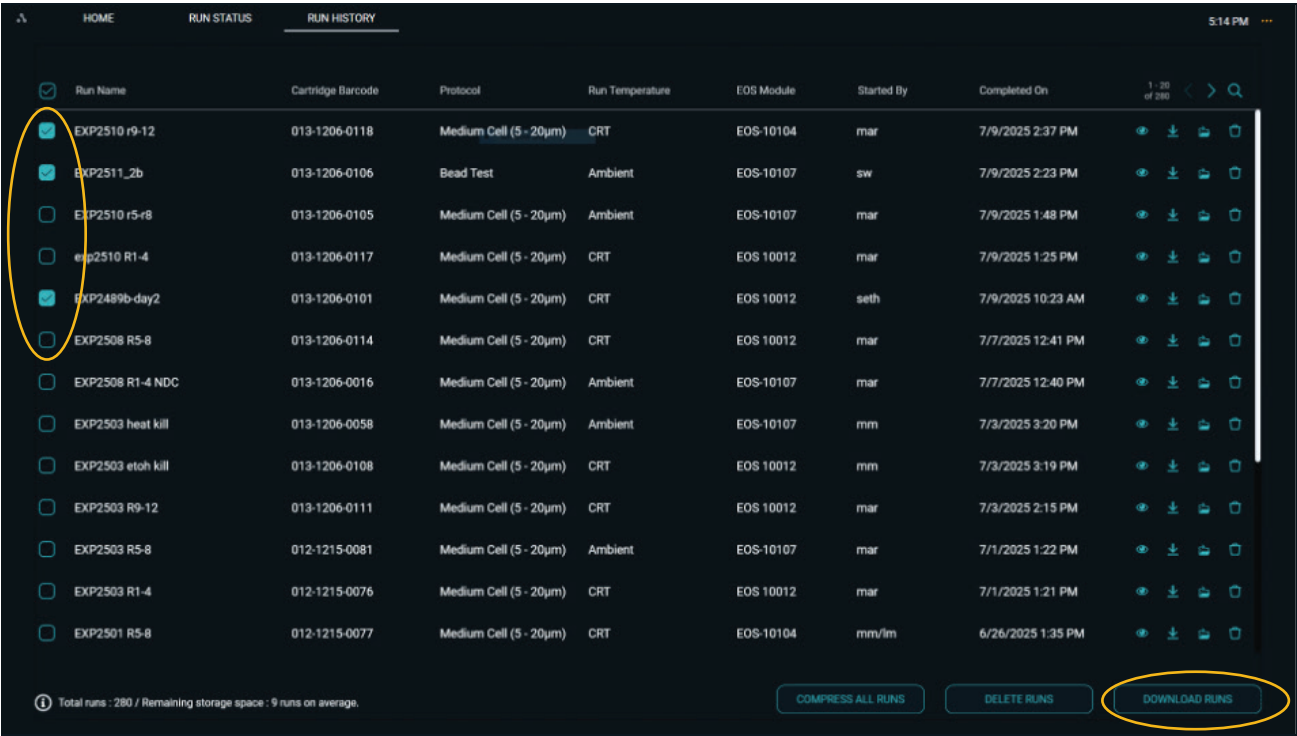


Figure 89. Run History DOWNLOAD RUNS button

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

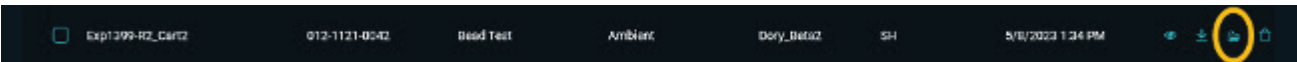


Figure 90. Button to access files via file explorer

The file structure for each run is shown:

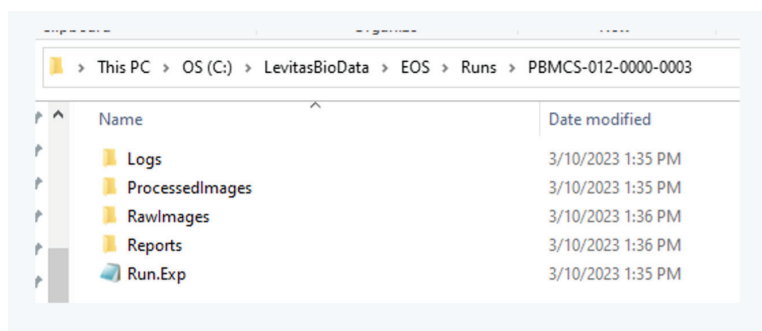


Figure 91. File Structure

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.

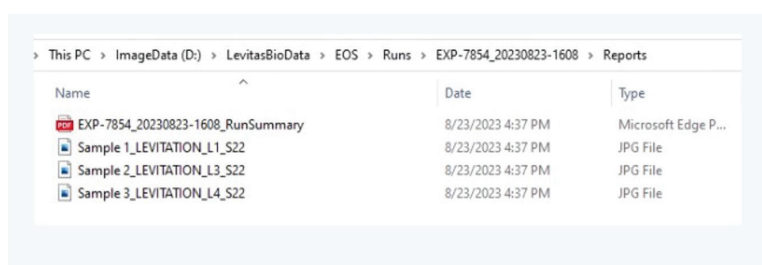


Figure 92. Reports folder

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 display your run at various timepoints.

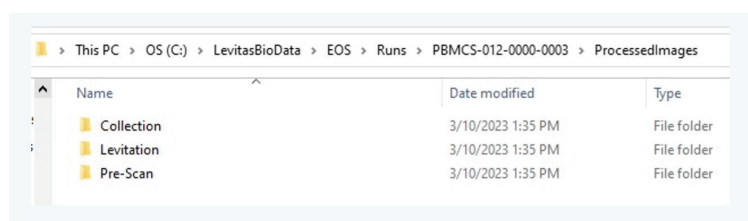


Figure 93. Processed image folders

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

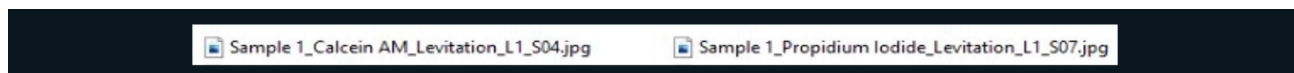


Figure 94. Example filename format

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, although files have been compressed into .jp2 file format to reduce file storage demand.



Figure 95. 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.

To reduce run folder size for faster transfer, some raw image scan files are not stored as they are not needed for analysis. Processed images are smaller in size, and are saved for all scans.

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 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 (and other lanes if independent collection was specified) are easily navigated. Zoom in for detailed views, and export movies of levitation or collection.



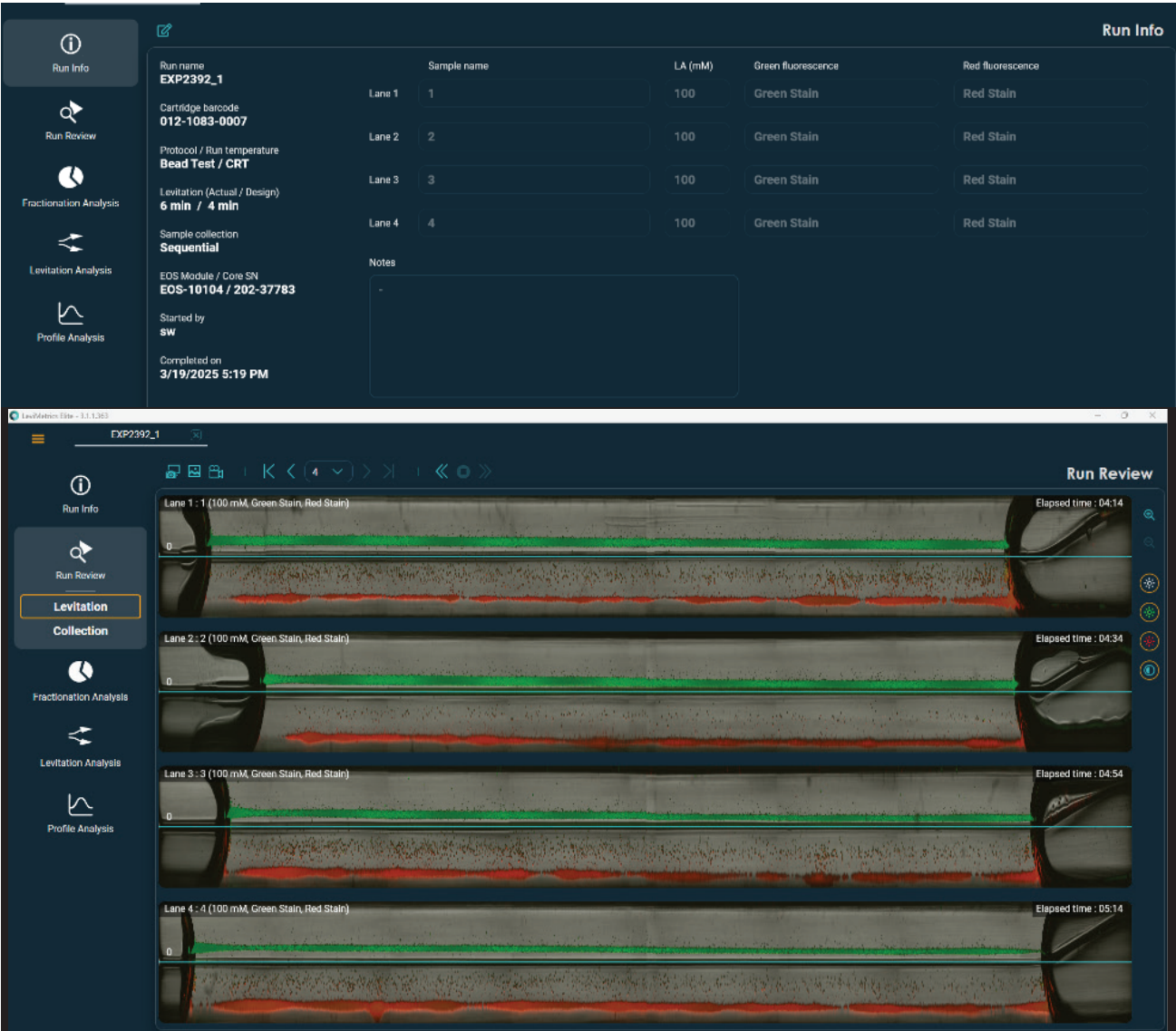
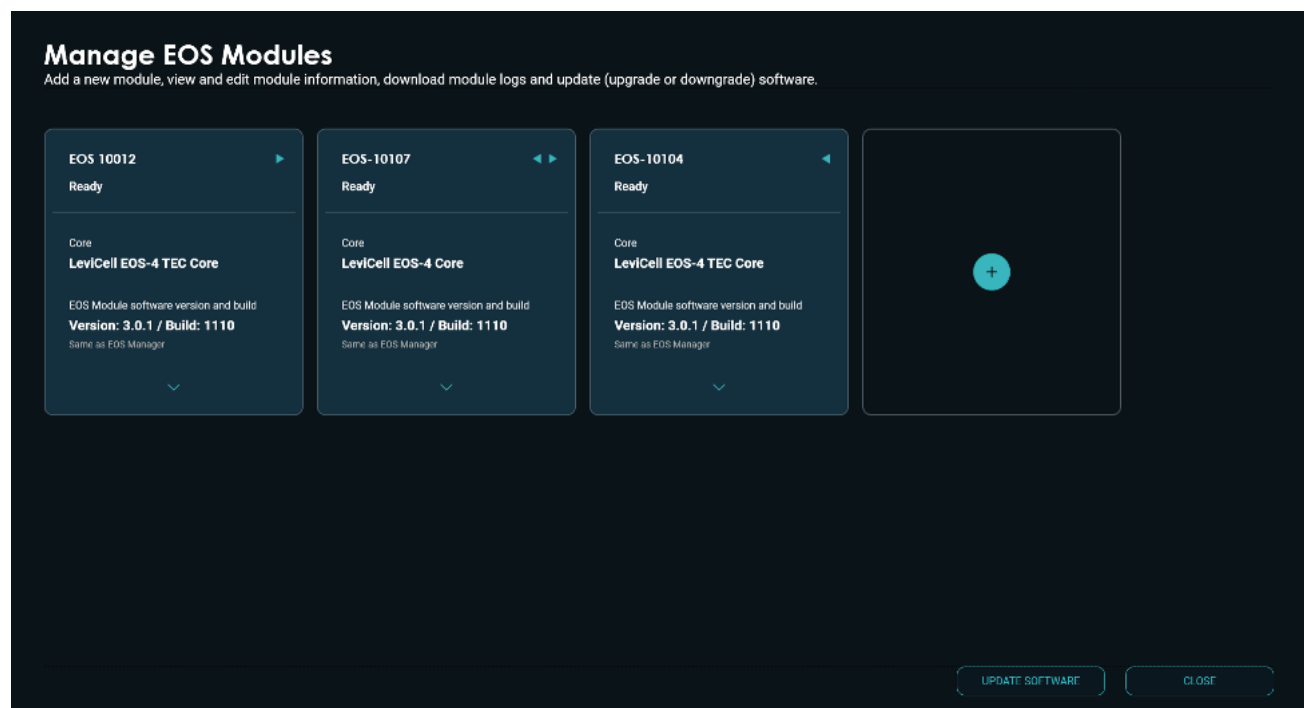
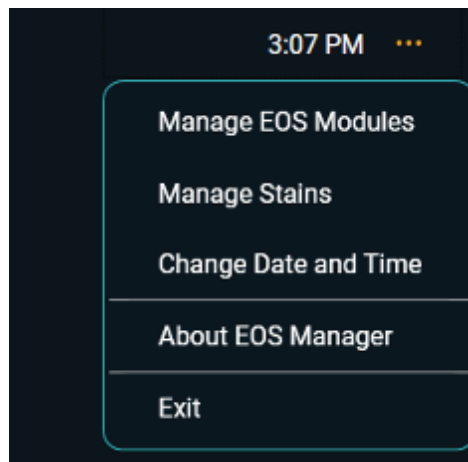


Figure 96. LeviMetrics Software: Run Info (top) and Run Review (bottom)

Refer to the LeviMetrics Software User 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, select Manage EOS Modules, click UPDATE SOFTWARE, and then follow the installation instructions.



During the software update, a progress screen will appear indicating that the software is updating. It is important that the Control PC or EOS Modules are not switched off during this time.



Figure 97. 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 (600 mm or 23.6") on the left of the instrument.
- Unbox the new core and have it readily available for the swap.
- To access the rear panel thumbscrews which secure the left panel in place, the instrument may need to be rotated counterclockwise. A second person is needed 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 counterclockwise 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 back panel. Unscrew them to unlock the side panel. Set them aside for reinstallation later.

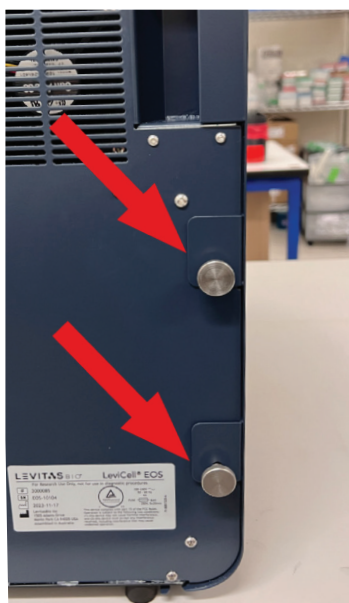


Figure 98. Rear panel showing thumbscrews securing left side panel

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.

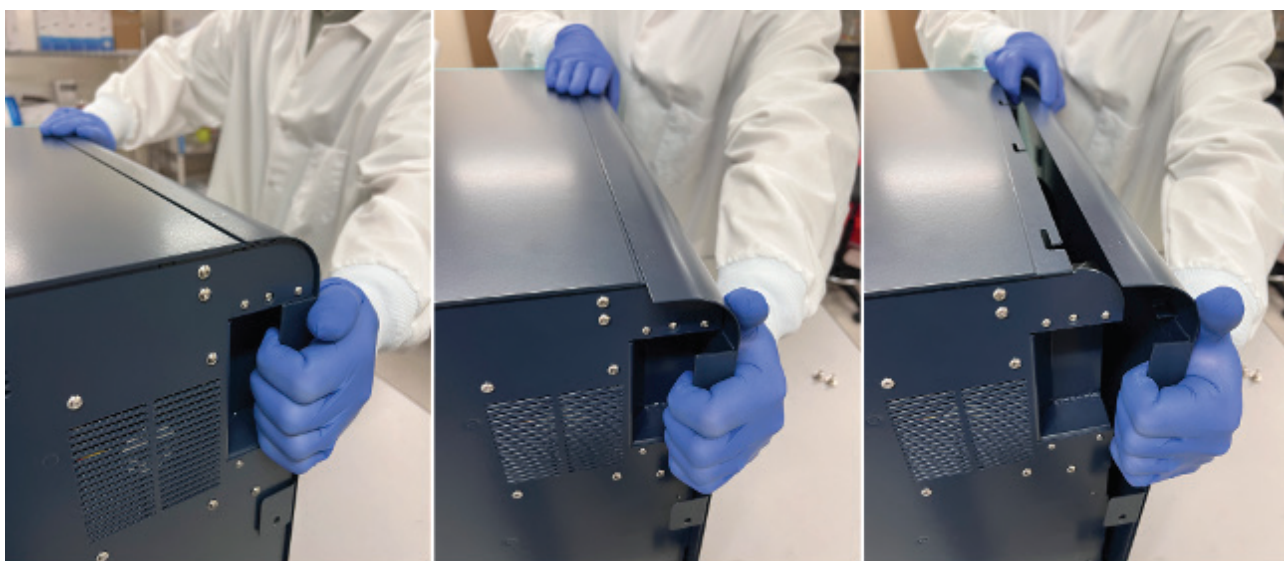


Figure 99. LeviCell EOS left side panel removal

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 100. Lock sticker located on the core.

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

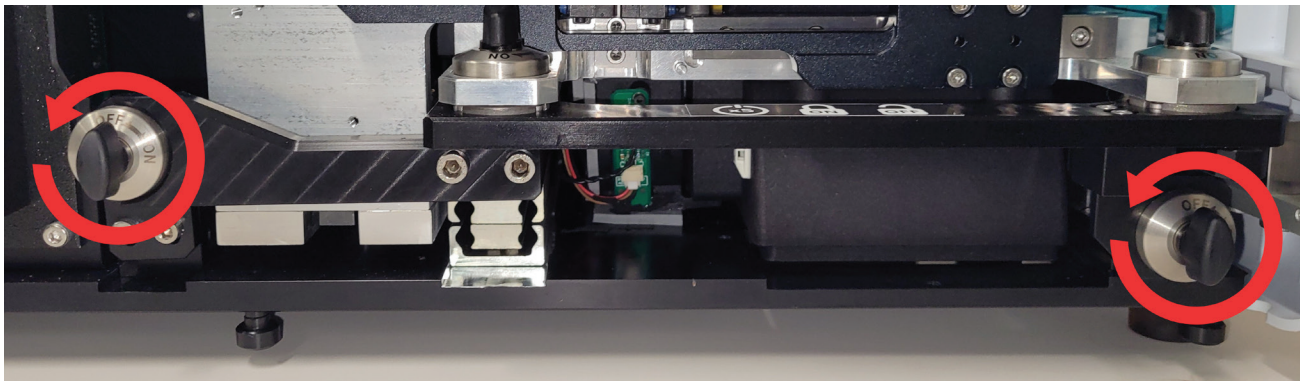


Figure 101. Unlocking outward-facing core cradle locks to release the cradle

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

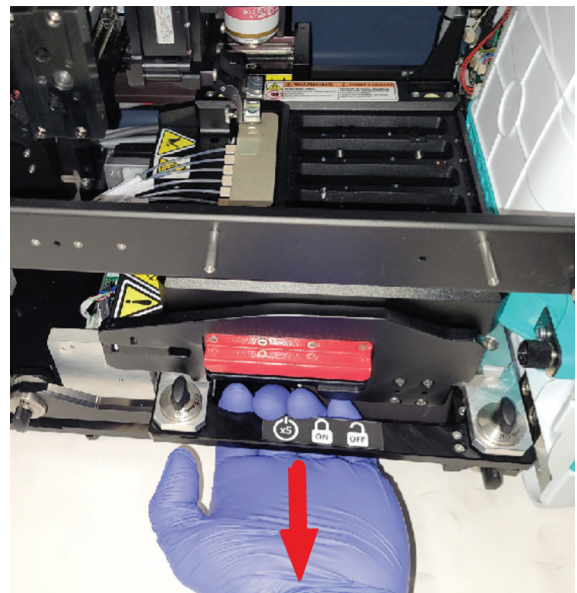


Figure 102.
Core module is mounted inside the cradle

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



Figure 103. Core module top view and manifold thumbscrews



Figure 104. Pneumatic manifold bottom view

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



NOTE: Avoid touching the bottom surface of the manifold

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

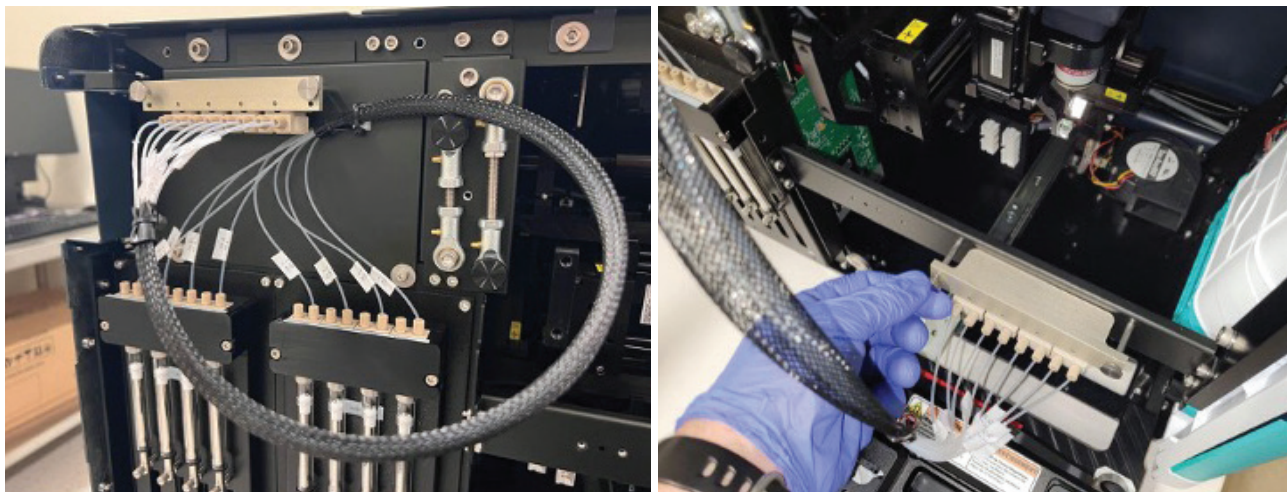


Figure 105. Two different options for holding the pneumatic manifold

12. With the entire core now accessible, locate the 3 remaining cradle locks (Figure 106) and unlock by switching to the OFF position

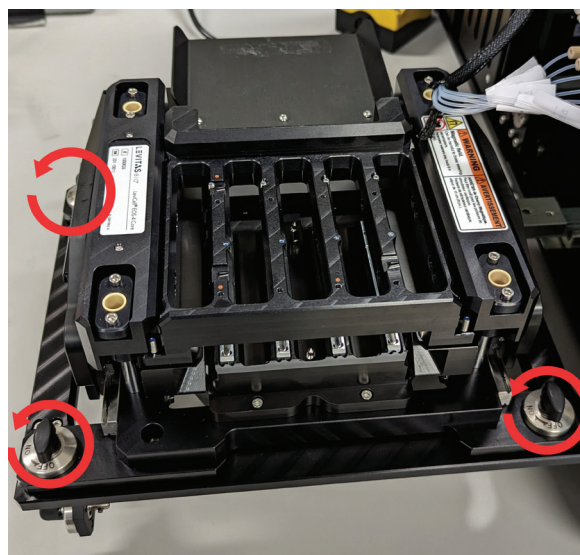


Figure 106.
Remaining cradle locks to be unlocked

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).

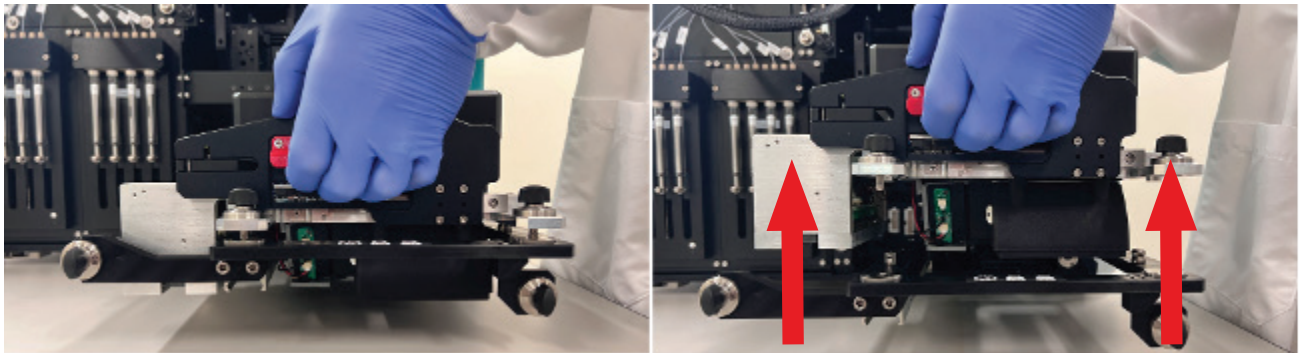


Figure 107. Lift upwards using the handles to disengage the core



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.

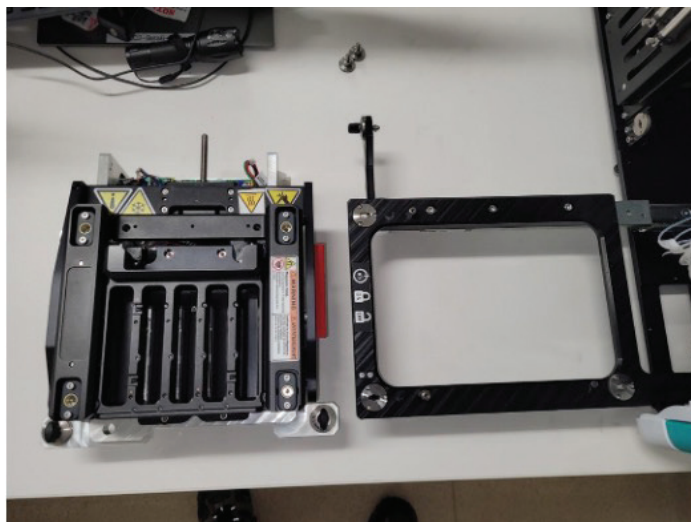


Figure 108.
Core module placed on bench
next to core cradle

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

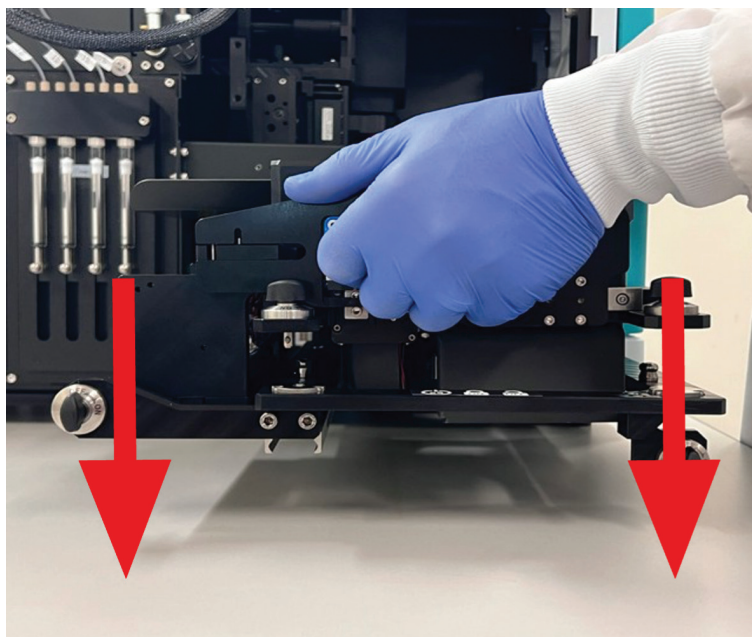


Figure 109. Aligning the core to cradle locks and locating pins

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



Figure 110. Switch the three Core module locks into the cradle to ON

17. Install the pneumatic manifold block onto the new core by aligning the core alignment 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 as damage to the manifold rings will cause leaks and sample recovery issues.

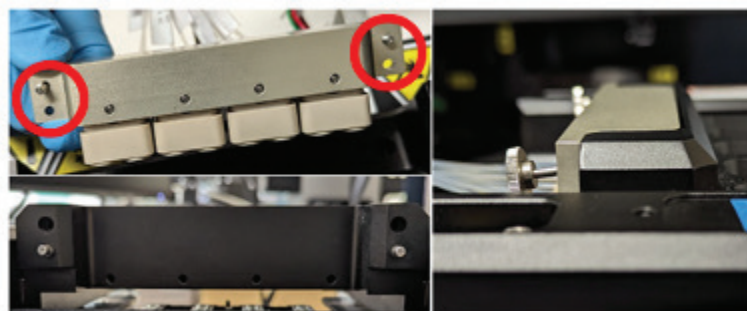


Figure 111.

(Left) Insert the fixed metal alignment pins into the receiving holes on the core for proper alignment.

(Right) Once the manifold is seated in the core top plate, tighten the two captive thumbscrews.

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



Figure 112. Fastening the pneumatic manifold block onto the core.

19. Slide the core cradle back into the instrument.



Figure 113.

Return core cradle back into the instrument.

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

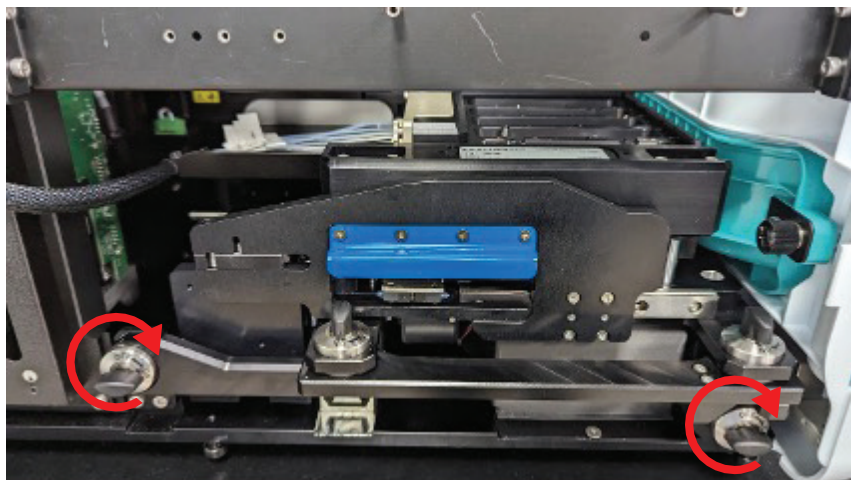


Figure 114.

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.

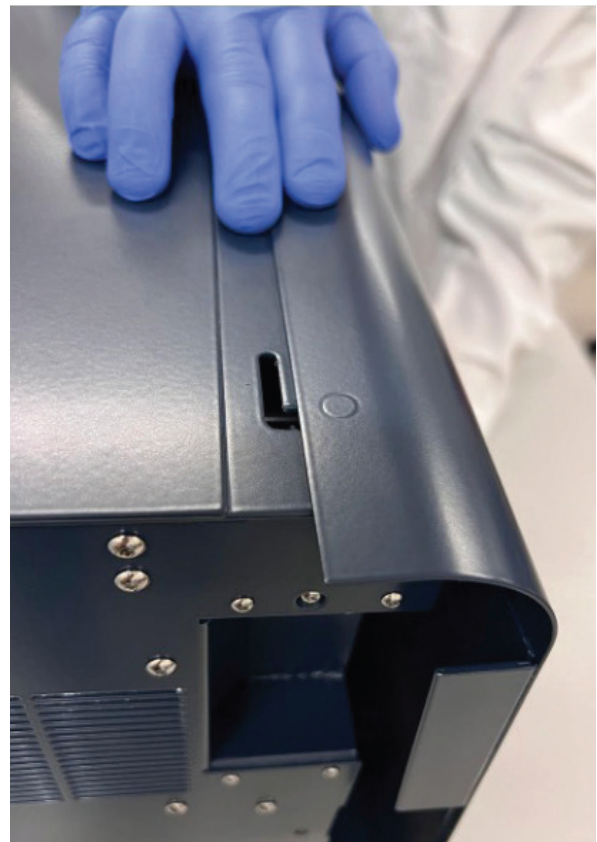


Figure 115. *Aligning side panel with slots*

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 of the panel to be flush with the instrument.
23. Slide the panel towards the front of the instrument.

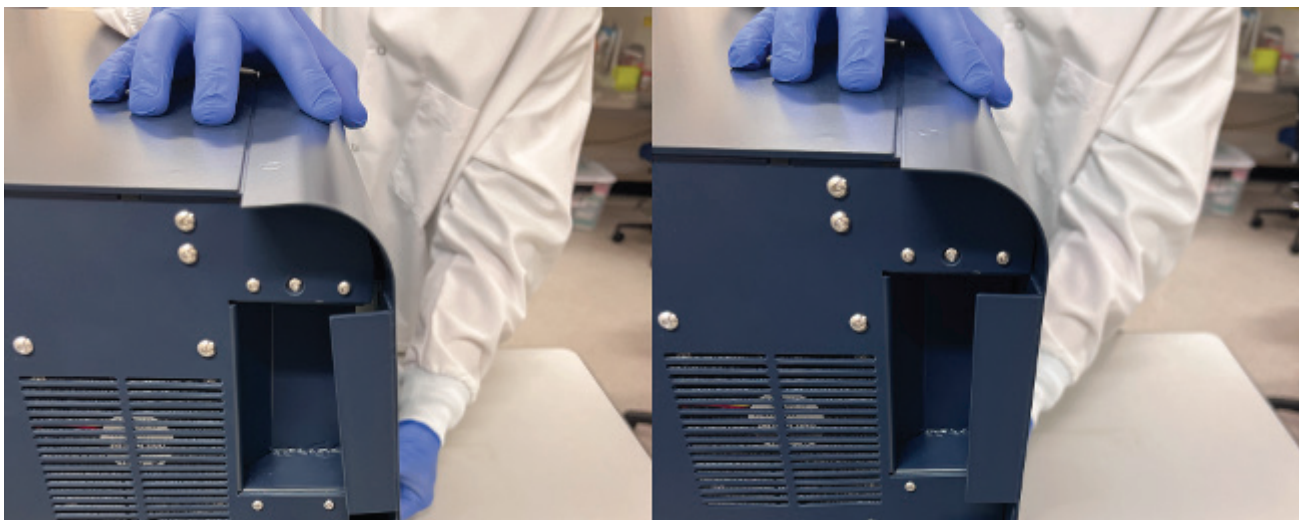


Figure 116. *Left side panel re-engaging with the module frame and sliding panel back into position*

24. Replace thumbscrews and secure the side panel into place.



Figure 117. 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. The 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 down the LeviCell EOS system if the system needs to be moved, for a core swap, or if it will not be used for a longer period.



Figure 118. Power button on the front of instrument



NOTE: If a scheduled Heat Dry task 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 shutdown. A notification will appear on the LeviCell EOS LCD, and the system soft power shutdown will proceed automatically after the Heat Dry is complete.

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

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

To shut the system down for longer periods, or for any service tasks, 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 accepted	• Confirm the barcode was entered correctly compared to the label. • 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 during levitation	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 the Core to cold temperature range. Consider moving the 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 the Core to chosen temperature range. Consider moving the system to an air-conditioned facility or running at a different time of the day, or confirm proceeding at current temperature, in which case a message will be appended to the run summary.
Run temperature deviation during run	Environmental temperature during the run may have increased. Consider moving system to an air-conditioned facility or running at a different time of the day If this problem continues when the environment is between 19°C – 25°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 the chance of bubbles, always pipette to the first 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 create 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 an AC vent.
Top or bottom bands fluctuate 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.

APPENDIX 1 - PERFORMANCE QUALIFICATION CHECKLIST

START DATE OF TEST		Instrument Model	EOS
OPERATOR		Instrument Serial Number	EOS-_____
OVERALL RESULT		Installation Site	
DATE OF TEST COMPLETION			
SIGNATURE			

FUNCTION	Size SPECIFICATION	PASS	FAIL	NOTES
Ex470-Em523 LED alignment	Green fluorescent beads are visible in the image when Green Fluorescence is selected.			
Ex567-Em634 LED alignment	Red fluorescent beads are visible in the image when Red Fluorescence is selected.			
Levitation - visual	Beads levitate into 2 populations after 3 minutes.			
Separation - visual	Green beads flow to the top outlet (above split line), red beads flow to the bottom outlet (below split line) as observed in images.			
Volume recovery (optional)	Measured volume recovered from each outlet > 66 μ L (>60% of input volume)			
Purity (optional)	Number green fluorescent beads counted from top / number of total beads counted from top > 90%			
Yield (optional)	Number green fluorescent beads counted from top / Number of green fluorescent beads input > 40%			

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<http://levitasbio.com/>

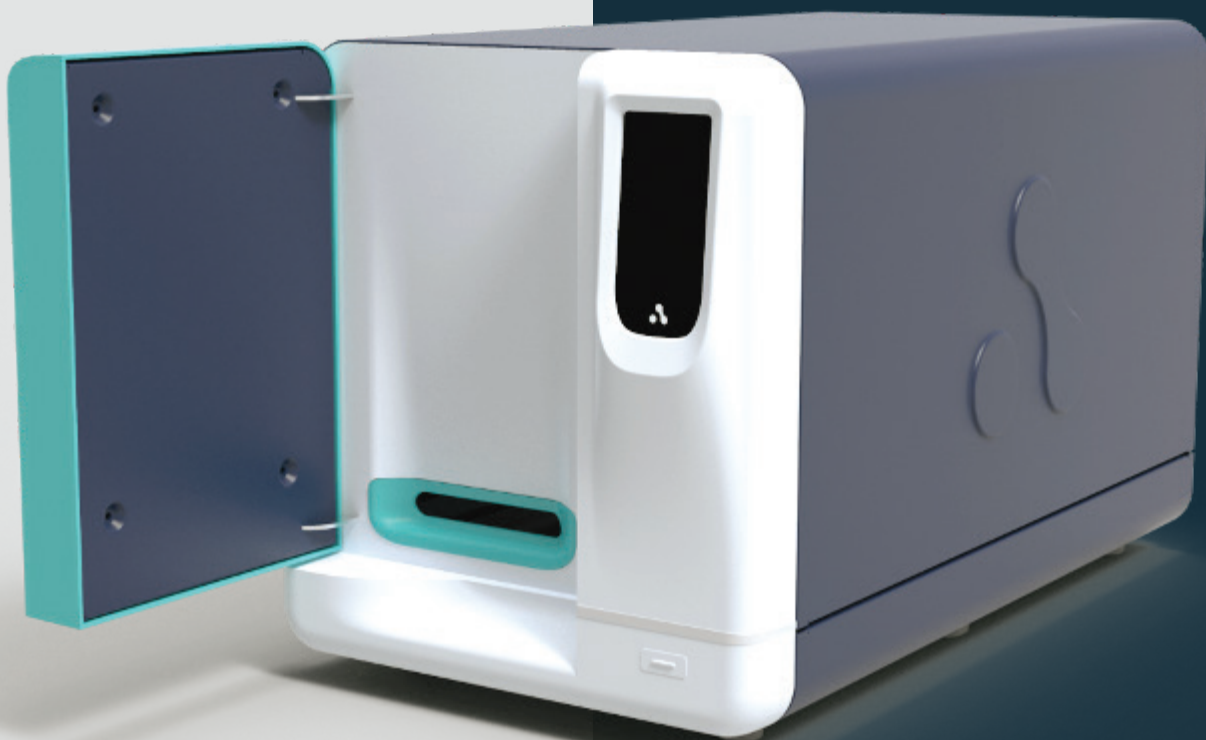
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