

LeviCell EOS User Guide

INCLUDING VIABLE CELL ENRICHMENT PROTOCOL

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LEGAL NOTICES

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

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

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

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

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

If there is any fault, turn the instrument off using the mains switch on the rear and disconnect the power cable. Contact LevitasBio Technical Support at +1-408-663-4260 or support@levitasbio.com.

In addition to contamination control features within the LeviCell EOS instrument, a primary safety feature to prevent contamination is that the cartridges are single-use only. Do not reuse cartridges. Re-use increases the risk of cross-contamination and exposure to potential biohazards associated with the sample and can void the warranty of the instrument. If a cartridge is reused or a leak is suspected, contact Technical Support at +1-408-663-4260 or support@levitasbio.com.

Dispose of used cartridges according to approved lab guidelines.

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




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

Table 2. Safety Warnings

Compliance Certifications

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

	cTUVus mark to indicate that the product has been tested and certified to USA and Canadian standards
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 Chemical
(EC) 1907/2006	Regulation (EC) No 1907/2006 Registration, Evaluation, Authorization and Restriction of Chemical (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	<p>This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.</p> <p>This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.</p>
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Table 3. Compliance Certifications

ABOUT THE LEVICELL EOS SYSTEM

LeviCell EOS System Introduction

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

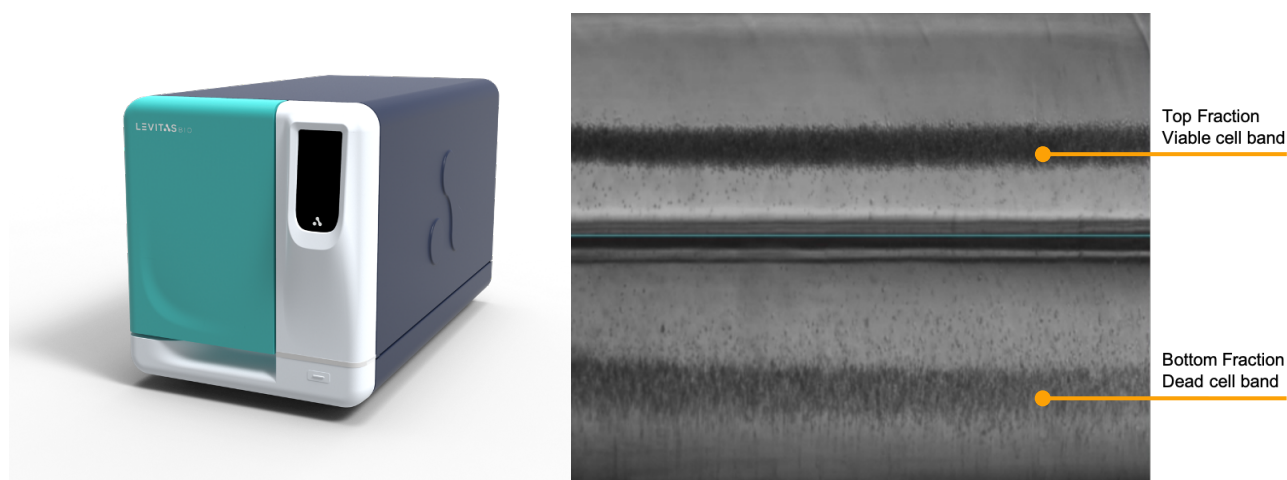


Figure 1. LeviCell EOS and levitation image

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

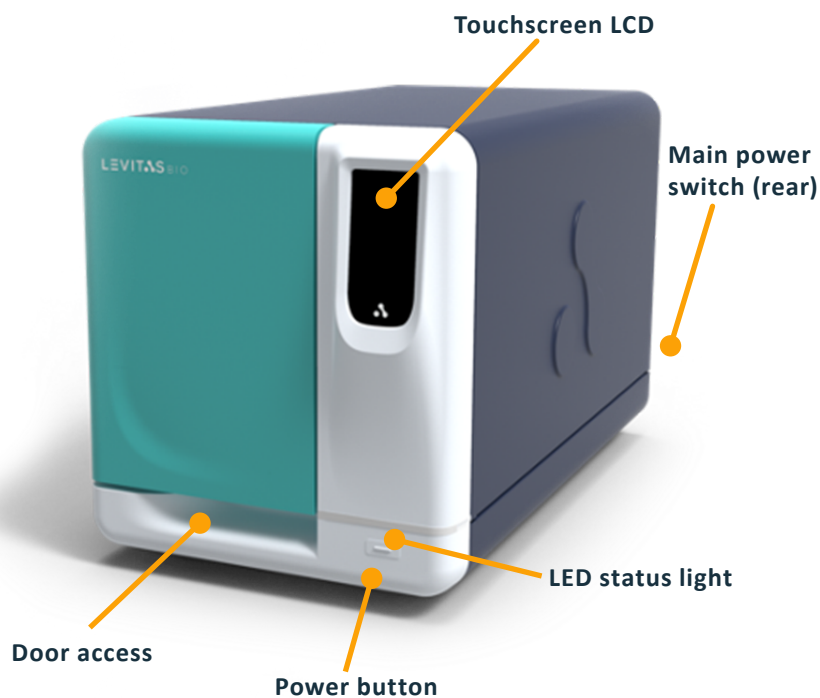
System Components

The LeviCell EOS System (PN 1000021) consists of

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

EOS Module – Front

LEVICELL EOS - DOOR CLOSED



LEVICELL EOS - DOOR OPEN

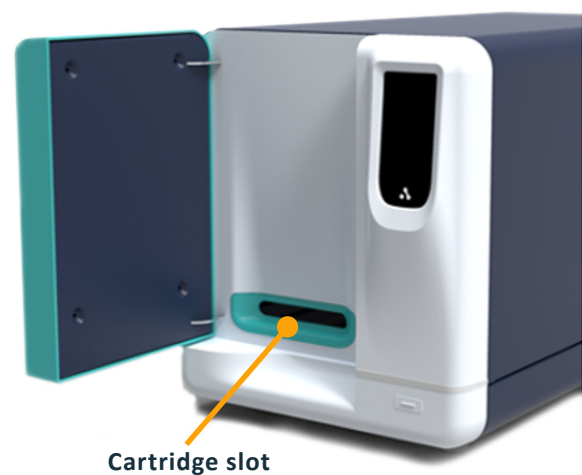


Figure 2. LeviCell EOS Module interaction points

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

Table 4. LED Light Colors

EOS Module – Rear

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

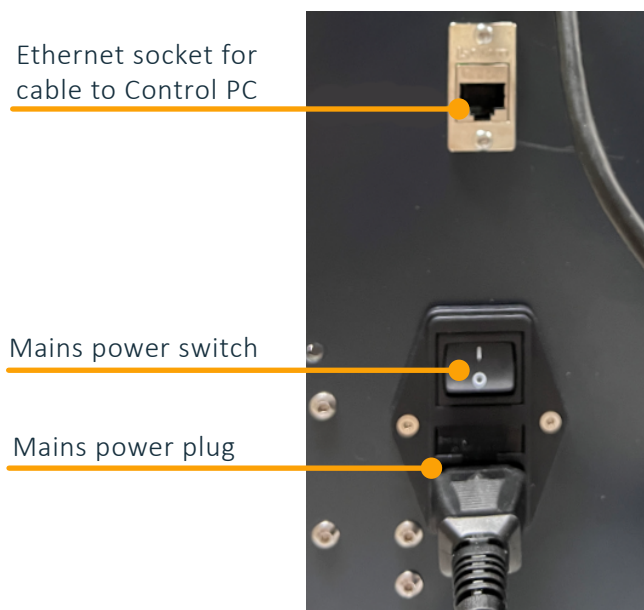


Figure 3. EOS Module power and connection interface

Computer - Control PC & Peripherals

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

Table 5. Computer specifications

Cartridges

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



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



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

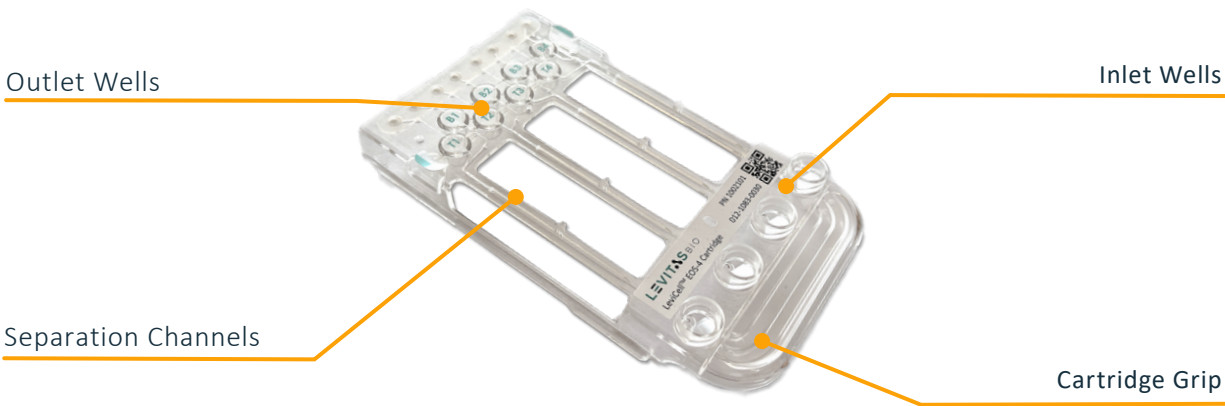


Figure 4. LeviCell EOS-4 Cartridge

Cartridge Box

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



Figure 5. Cartridge packaging



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

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

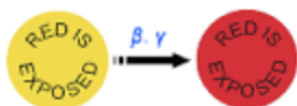


Figure 6. Gamma irradiation indicator sticker

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

LeviCell EOS Transport Cartridge

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

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

Materials

LevitasBio Equipment and Consumables

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

User-Sourced Equipment and Consumables

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



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

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

Specifications

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

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

Table 6. Specifications table

Bench space

- Minimum: 660 mm (26") deep, 1200 mm (4 feet) wide.
- Recommended: 1520 mm (5 feet wide) bench with 508 mm (20") vertical space available above the bench.
- A minimum of 80 mm (3") clearance is required behind the instrument for proper venting and allowing access to the mains power switch. Ensure enough clearance on the sides to reach the mains power switch.

Power: Access to 2 standard power outlets.

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

Installation and Usage

- LeviCell EOS systems must be installed by LevitasBio or other authorized personnel only.
- After system installation, ensure the mains power switch on the instrument back panel can be easily reached. When facing the instrument, the power switch is on the right-hand side.

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 screen.



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.



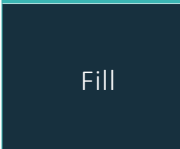




Selection		Buttons		Hover	
	selected/ active item		default selection for next step		presents tooltip, hovered item
	active field (user entry)		available button		
	unselected option		not available		

Table 7. Color Code Guide

Software Messages

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

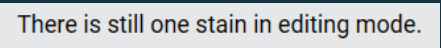
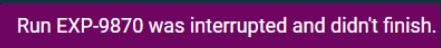
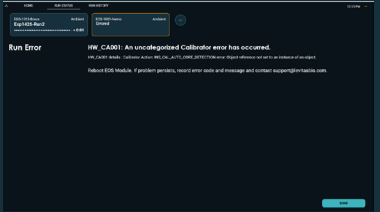
Type	Description	Example
User entry error	Information will appear below the user entry field indicating guidance to resolve issue	
ToolTip	Information provided when hovering over a button or option	
Notification	White, Information. Temporary will automatically clear	
	Purple, Warning. User must clear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

Table 8. Software message types

Home Screen

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

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

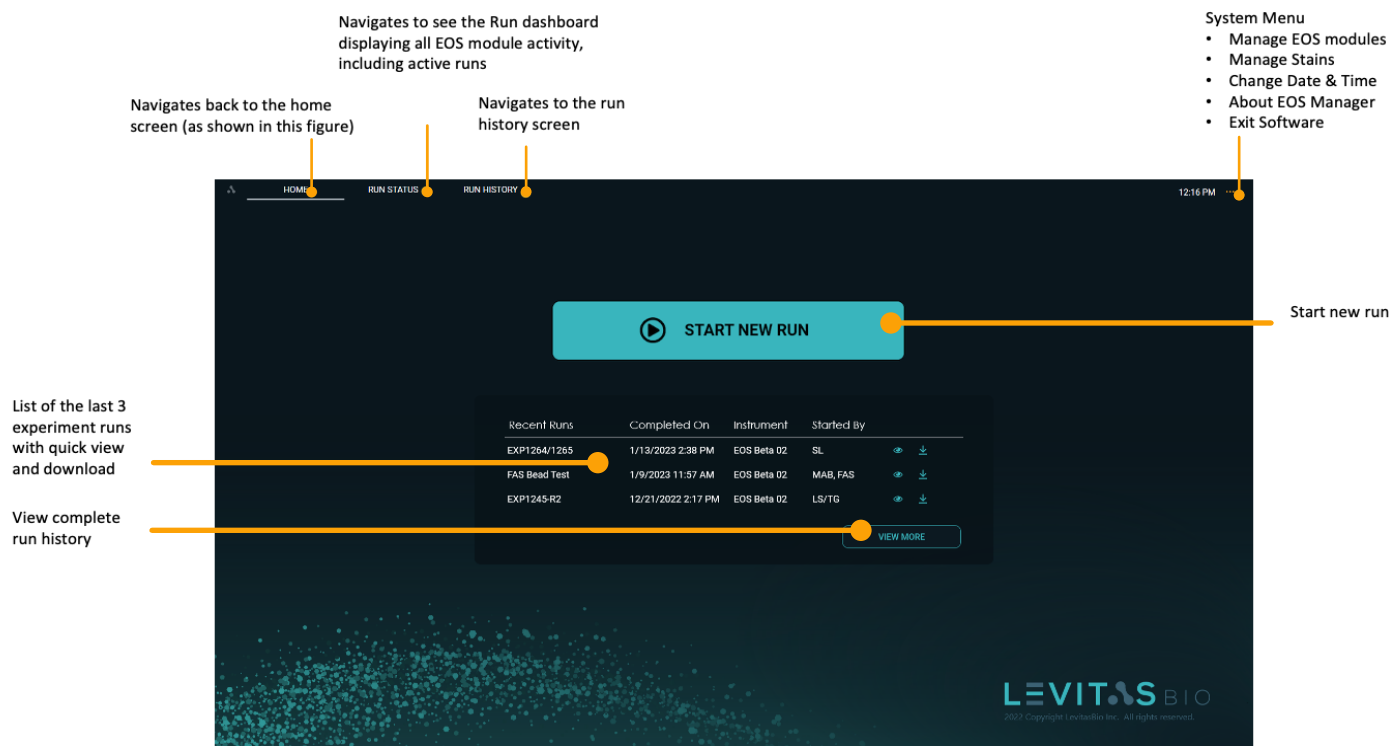


Figure 8. Home Screen

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

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

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

Run Status Screen

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

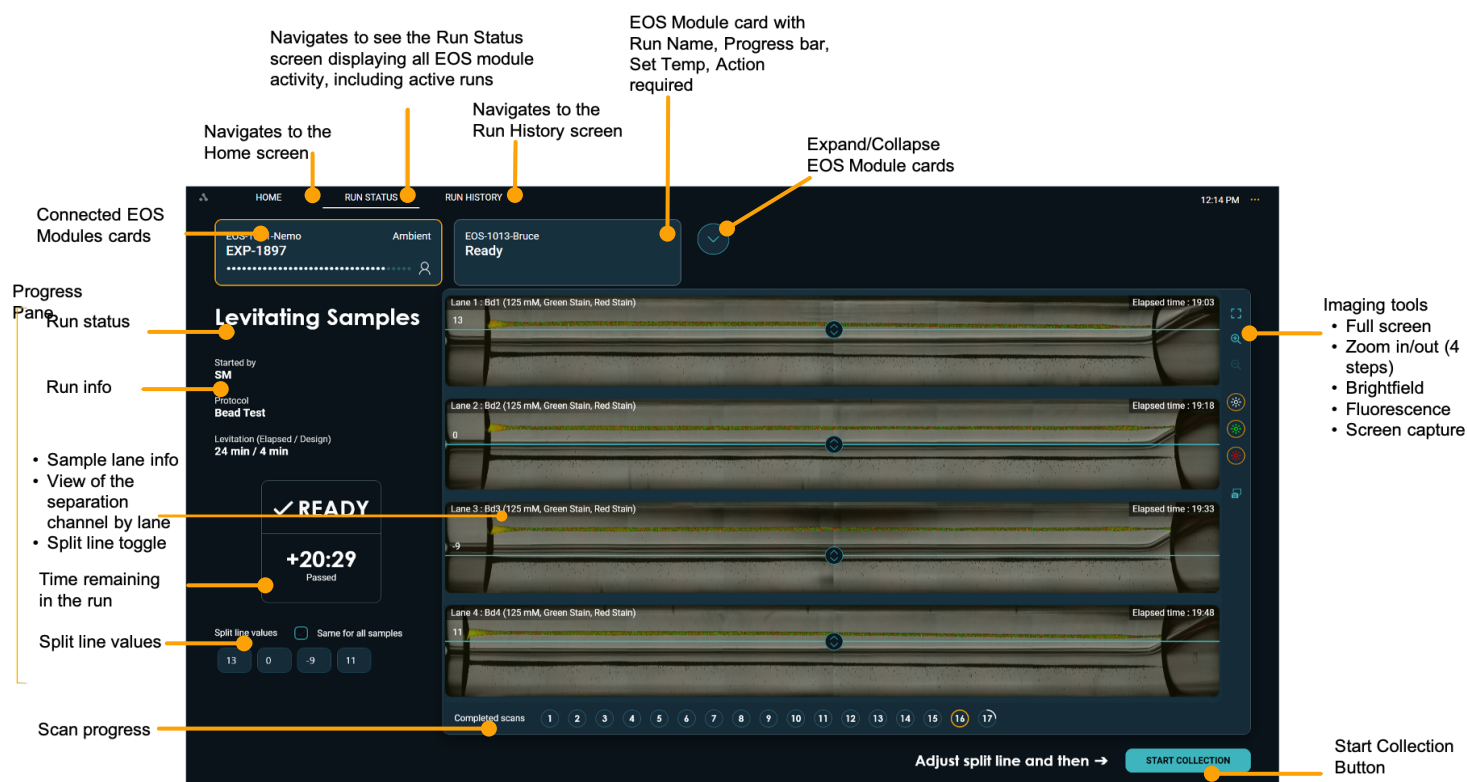


Figure 9. Run Status Screen

EOS Module Cards

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

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

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

- EOS Module name
- Run temperature
- Run name
- Cartridge barcode
- Started by
- Protocol
- Current protocol step
- Run progress bar
- Remaining run time
- User intervention icon

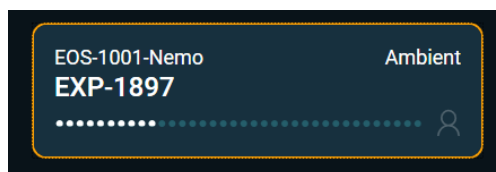


Figure 10.
EOS module card user intervention icon

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

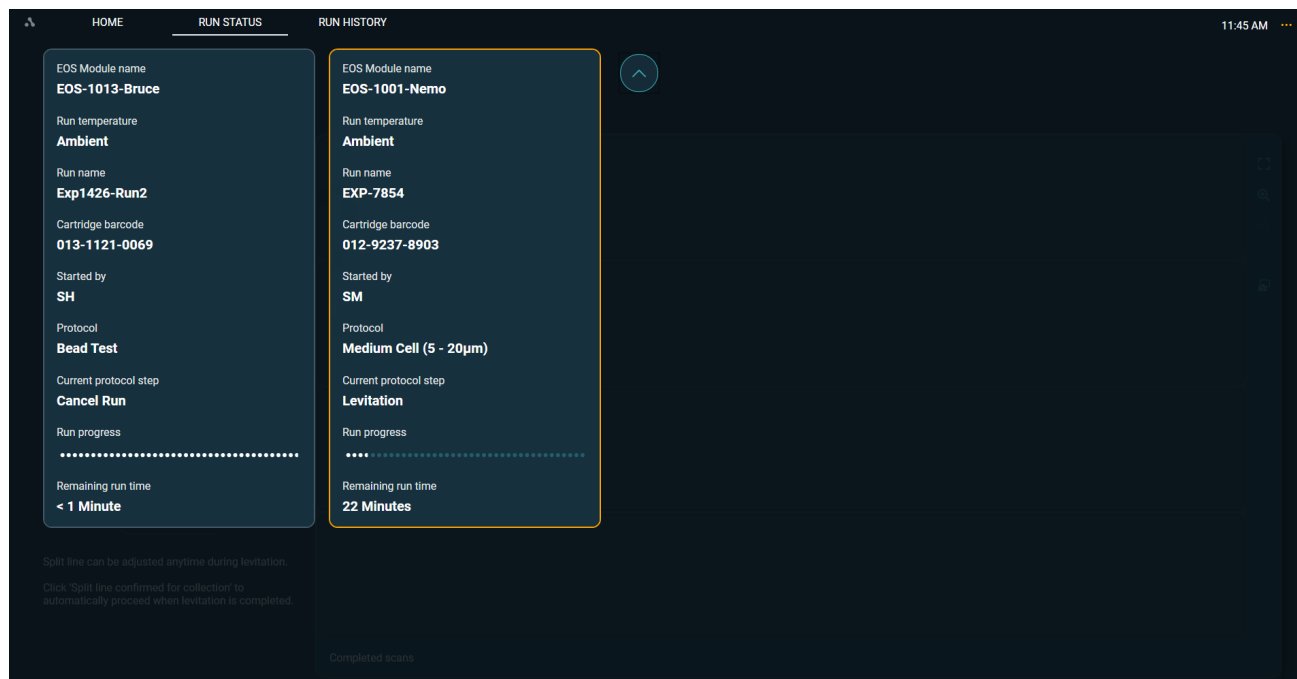


Figure 11. *Expanded EOS Module information cards*

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.

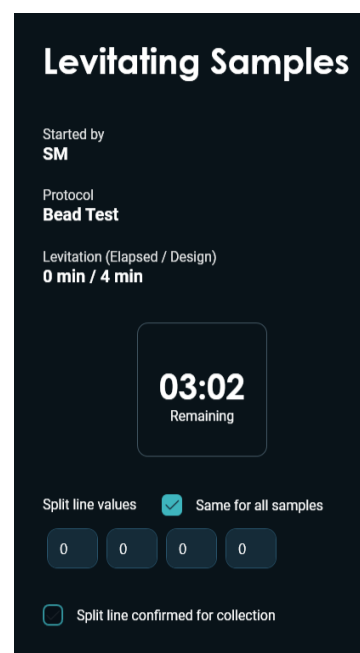


Figure 12. Progress Pane

There are two ways to change the split line.

1. Enter a value in the split line value field
2. Drag the teal colored bar on one of the images using the split line toggle

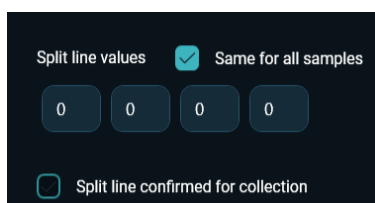


Figure 13.
Split line values and split line confirm



Figure 14. 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 and at the same time.

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.

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 begins to count up until the User clicks the **Start Collection** button.

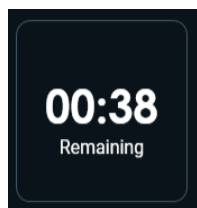


Figure 15.

Levitation time remaining countdown

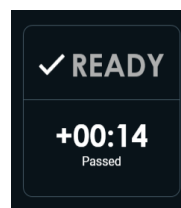


Figure 16.

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

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

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

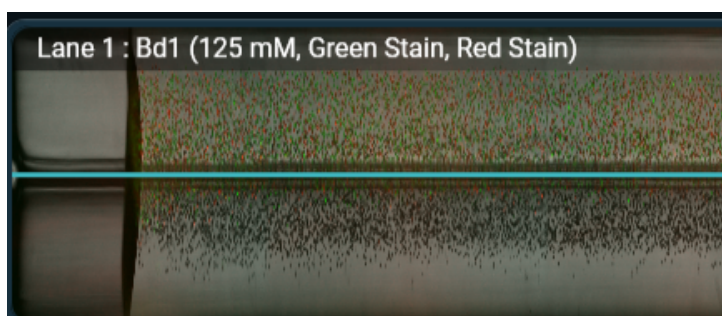


Figure 17. Lane information

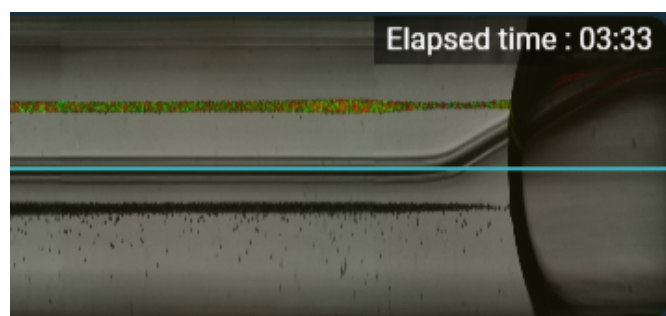


Figure 18. Elapsed time for each lane

Montage Images and Scan Progress

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

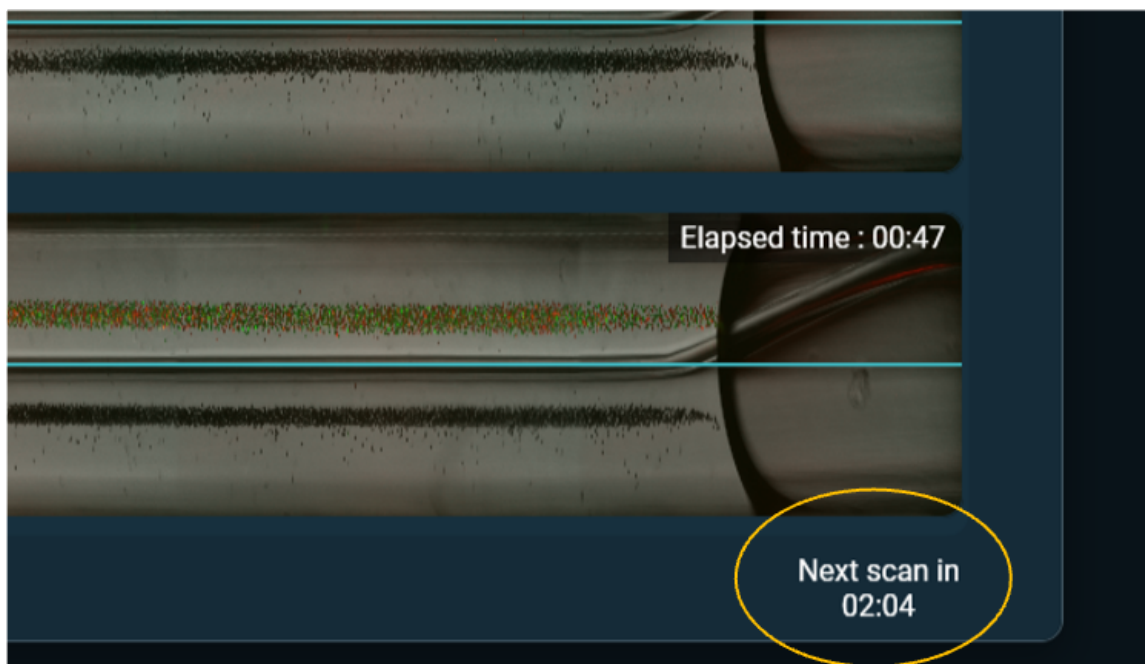


Figure 19. Next scan countdown timer

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



Figure 20. 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 current scan is indicated by the gold selection outline. Each completed scan takes approximately 60 seconds for brightfield only and 90 seconds with green and red fluorescence. After 4 hours total run time passed, image collection will stop saving.

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



Figure 21. Reviewing previously completed scan 1 while 7th scan is in progress



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

Image Tools

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

Brightfield, green fluorescence, and red 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.

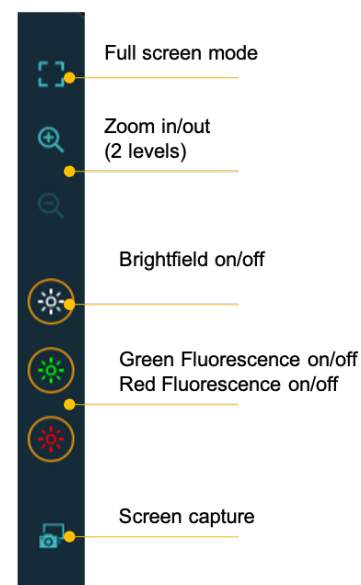


Figure 22.

Imaging tools available during a run

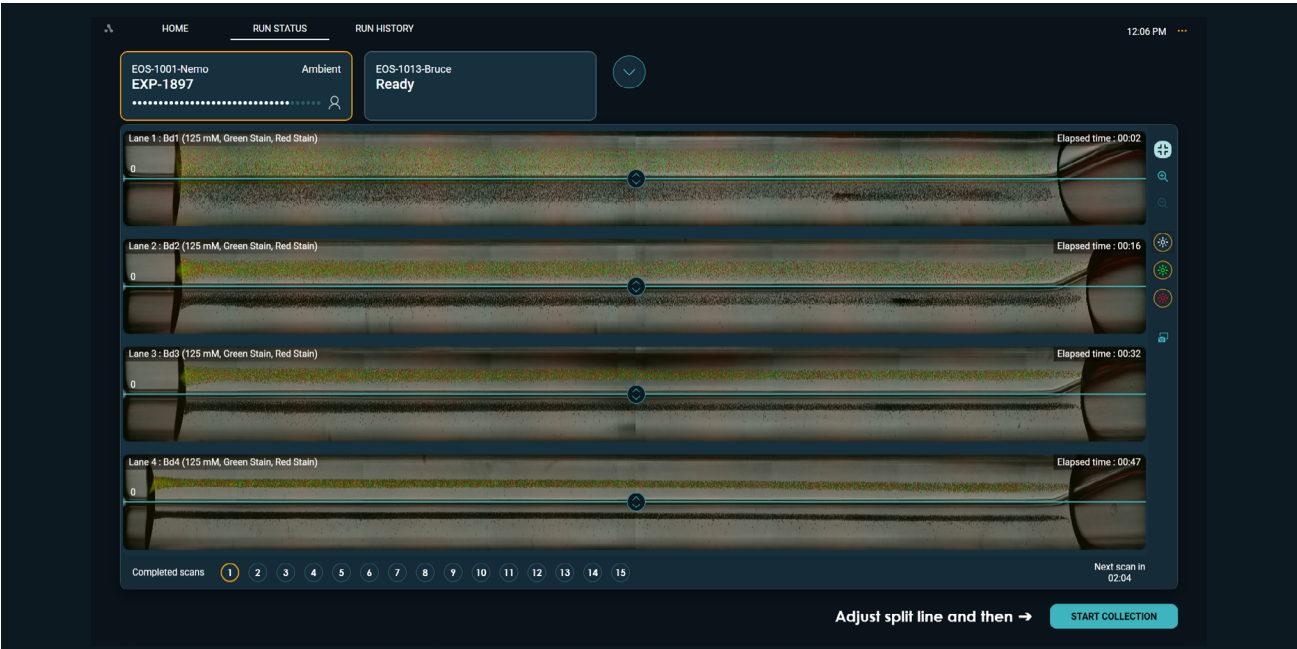


Figure 23. Run Status full screen view

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

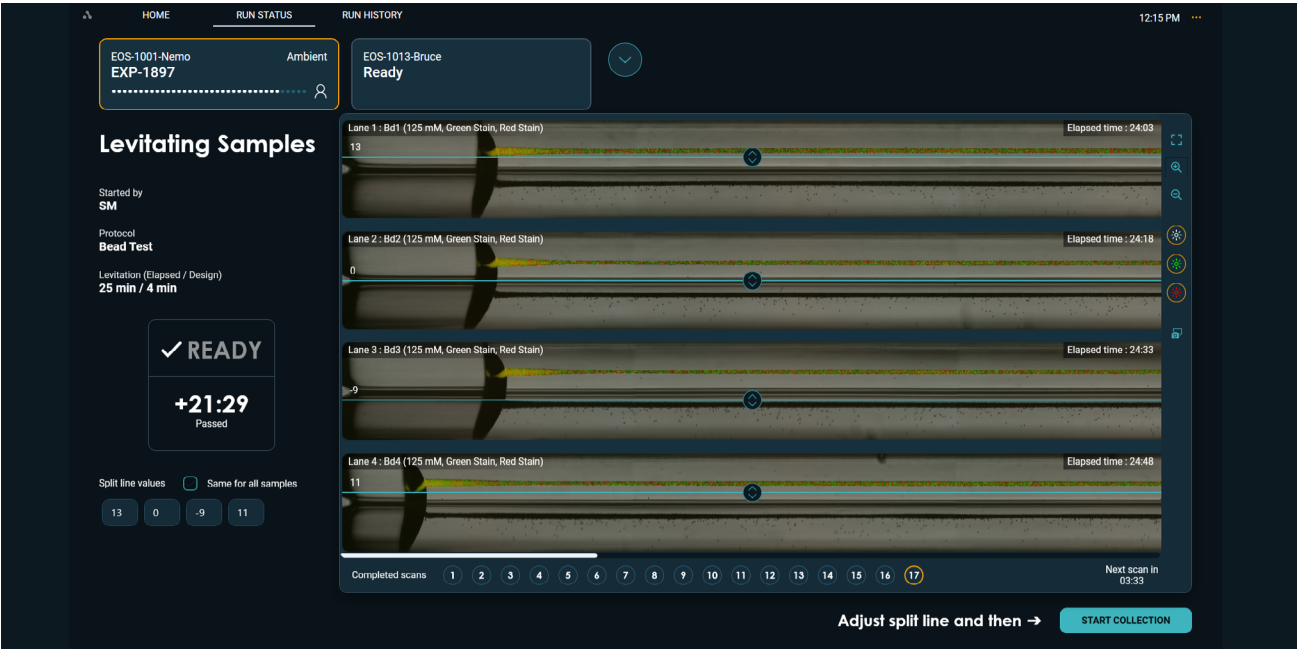


Figure 24. 1 step zoom - true lane ratio view



Figure 25. Max zoom - single lane height view

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



Figure 26. Fluorescence view for green stain only, red stain only, both green and red, and brightfield only.

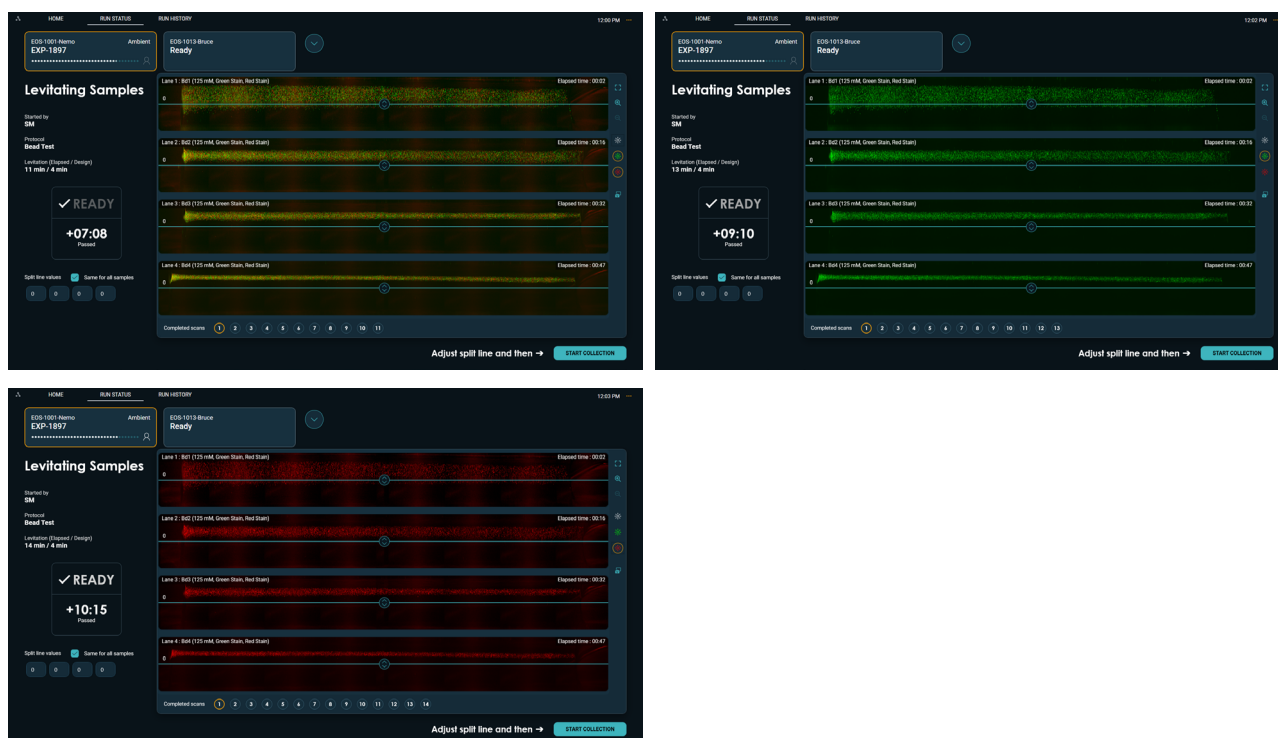


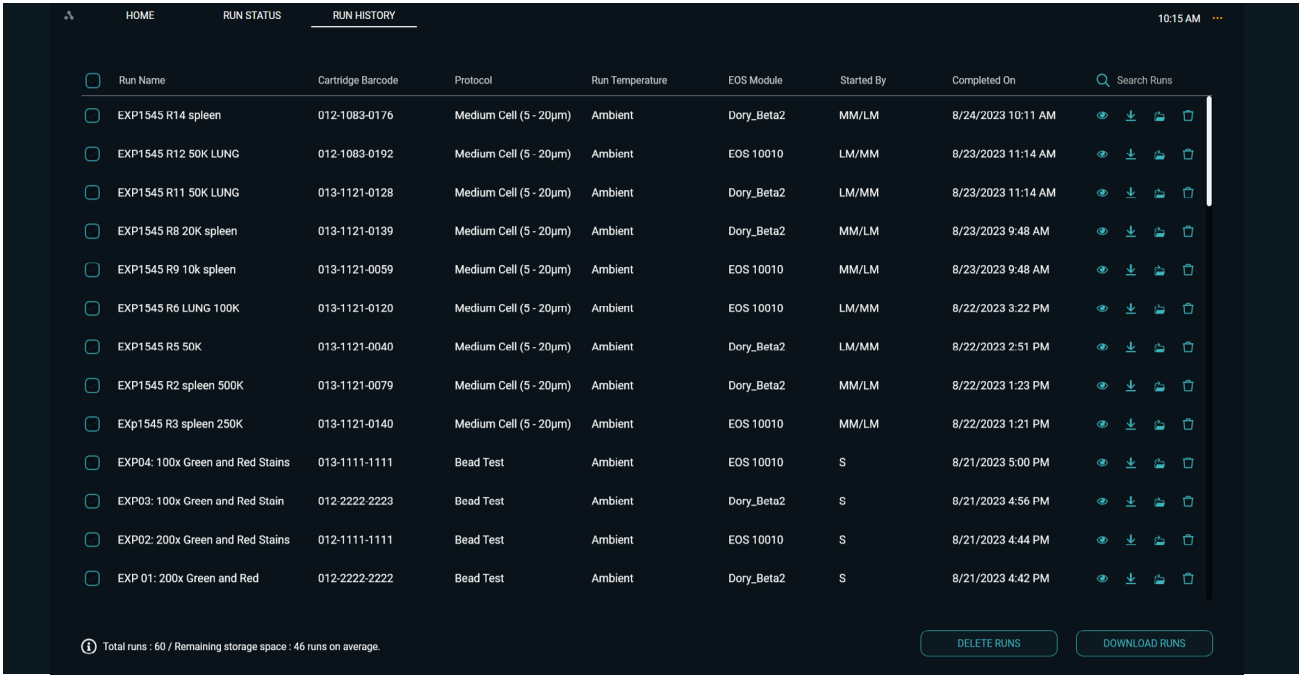
Figure 27. Fluorescence view with brightfield turned off

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

Run History Screen

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

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



Run Name	Cartridge Barcode	Protocol	Run Temperature	EOS Module	Started By	Completed On	
EXP1545 R14 spleen	012-1083-0176	Medium Cell (5 - 20µm)	Ambient	Dory_Beta2	MM/LM	8/24/2023 10:11 AM	
EXP1545 R12 50K LUNG	012-1083-0192	Medium Cell (5 - 20µm)	Ambient	EOS 10010	LM/MM	8/23/2023 11:14 AM	
EXP1545 R11 50K LUNG	013-1121-0128	Medium Cell (5 - 20µm)	Ambient	Dory_Beta2	LM/MM	8/23/2023 11:14 AM	
EXP1545 R8 20K spleen	013-1121-0139	Medium Cell (5 - 20µm)	Ambient	Dory_Beta2	MM/LM	8/23/2023 9:48 AM	
EXP1545 R9 10k spleen	013-1121-0059	Medium Cell (5 - 20µm)	Ambient	EOS 10010	MM/LM	8/23/2023 9:48 AM	
EXP1545 R6 LUNG 100K	013-1121-0120	Medium Cell (5 - 20µm)	Ambient	EOS 10010	LM/MM	8/22/2023 3:22 PM	
EXP1545 R5 50K	013-1121-0040	Medium Cell (5 - 20µm)	Ambient	Dory_Beta2	LM/MM	8/22/2023 2:51 PM	
EXP1545 R2 spleen 500K	013-1121-0079	Medium Cell (5 - 20µm)	Ambient	Dory_Beta2	MM/LM	8/22/2023 1:23 PM	
Exp1545 R3 spleen 250K	013-1121-0140	Medium Cell (5 - 20µm)	Ambient	EOS 10010	MM/LM	8/22/2023 1:21 PM	
EXP04: 100x Green and Red Stains	013-1111-1111	Bead Test	Ambient	EOS 10010	S	8/21/2023 5:00 PM	
EXP03: 100x Green and Red Stain	012-2222-2223	Bead Test	Ambient	Dory_Beta2	S	8/21/2023 4:56 PM	
EXP02: 200x Green and Red Stains	012-1111-1111	Bead Test	Ambient	EOS 10010	S	8/21/2023 4:44 PM	
EXP 01: 200x Green and Red	012-2222-2222	Bead Test	Ambient	Dory_Beta2	S	8/21/2023 4:42 PM	

Total runs : 60 / Remaining storage space : 46 runs on average.

DELETE RUNS DOWNLOAD RUNS

Figure 28. 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.

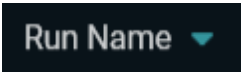


Figure 29. Run History column sort arrow

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

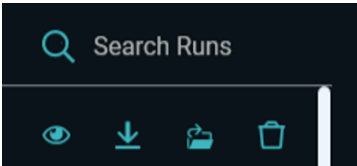


Figure 30. Search Run History

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 31. Run history options, hover and tool tips

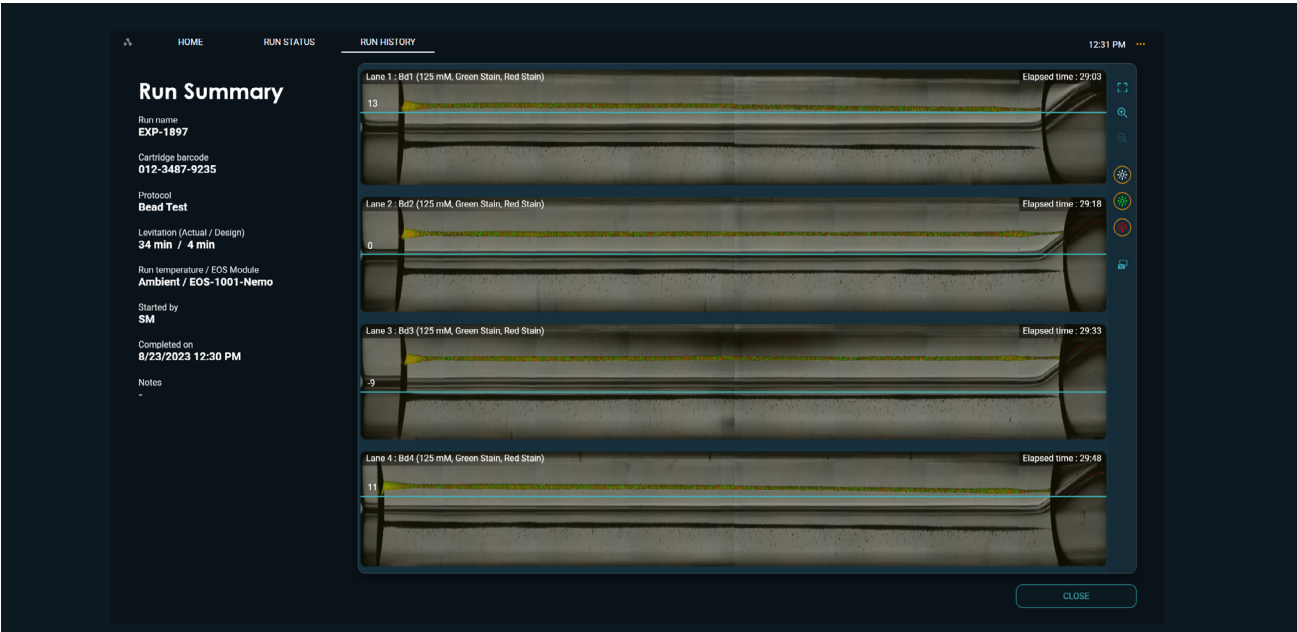


Figure 32. Run Summary

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

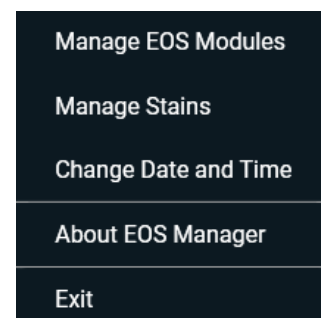


Figure 33.
System menu options

EOS Modules can be added or edited through the Manage EOS Modules option. Refer to the [Installation](#) section to walk through the installation and setup process for a new EOS Module.

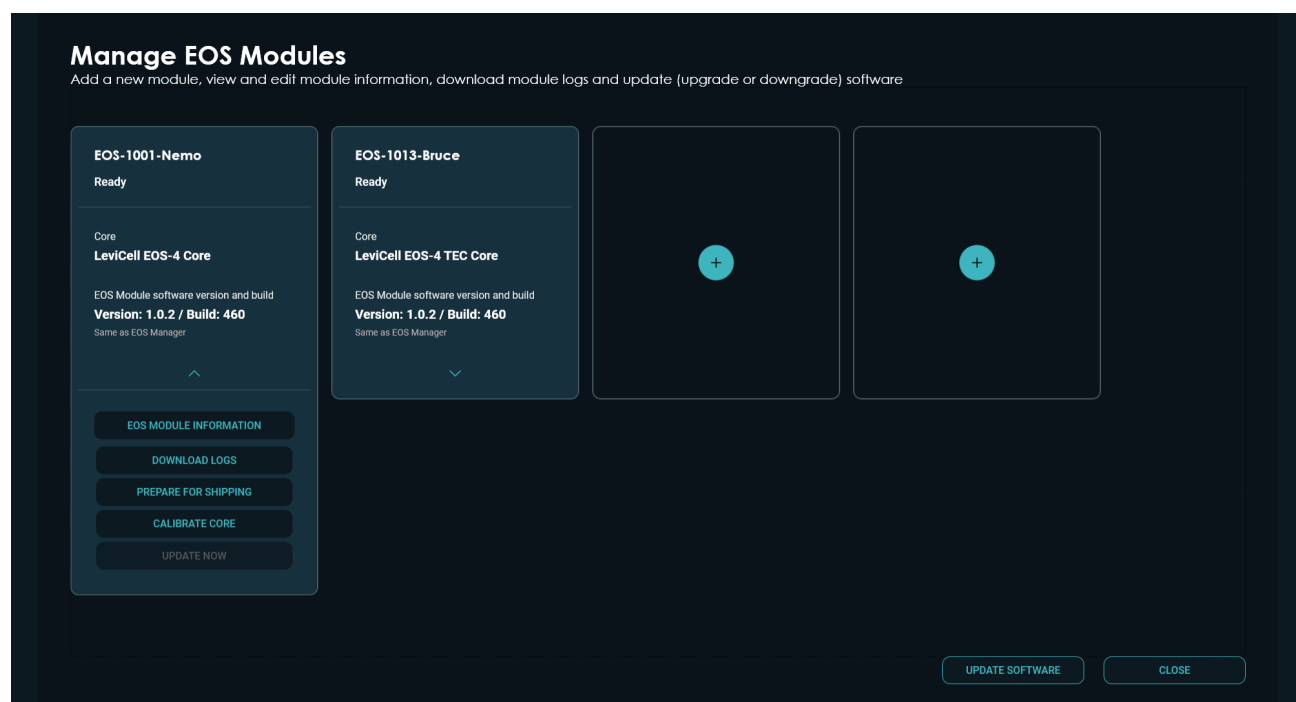


Figure 34. Manage EOS Module screen

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 (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.

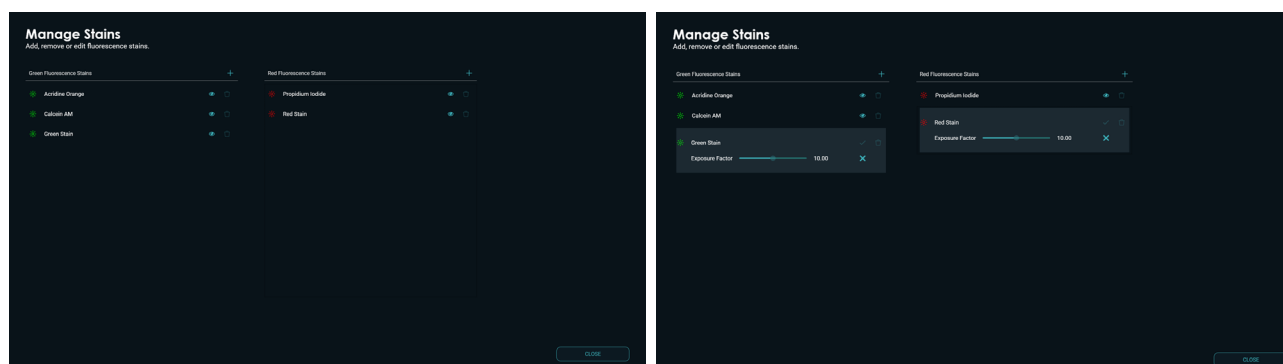


Figure 35. Manage Stains screen and default stains that have fixed settings

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.

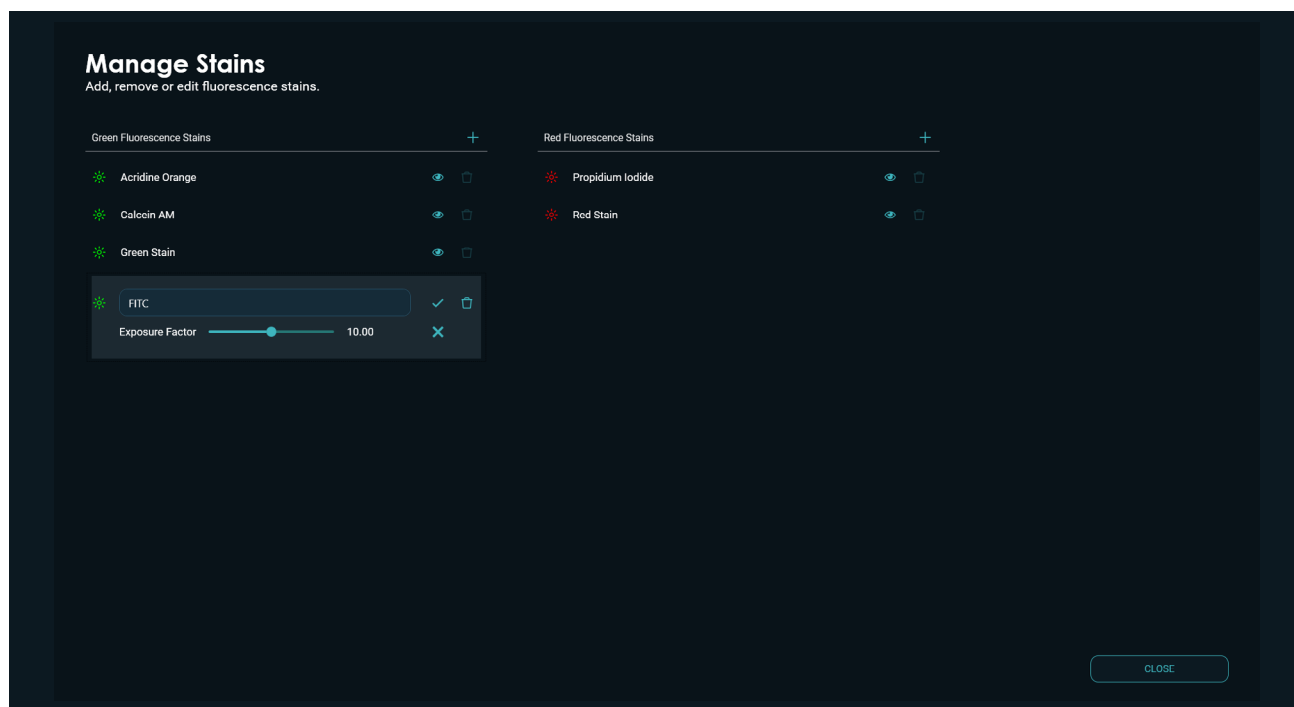


Figure 36. Adding custom stains

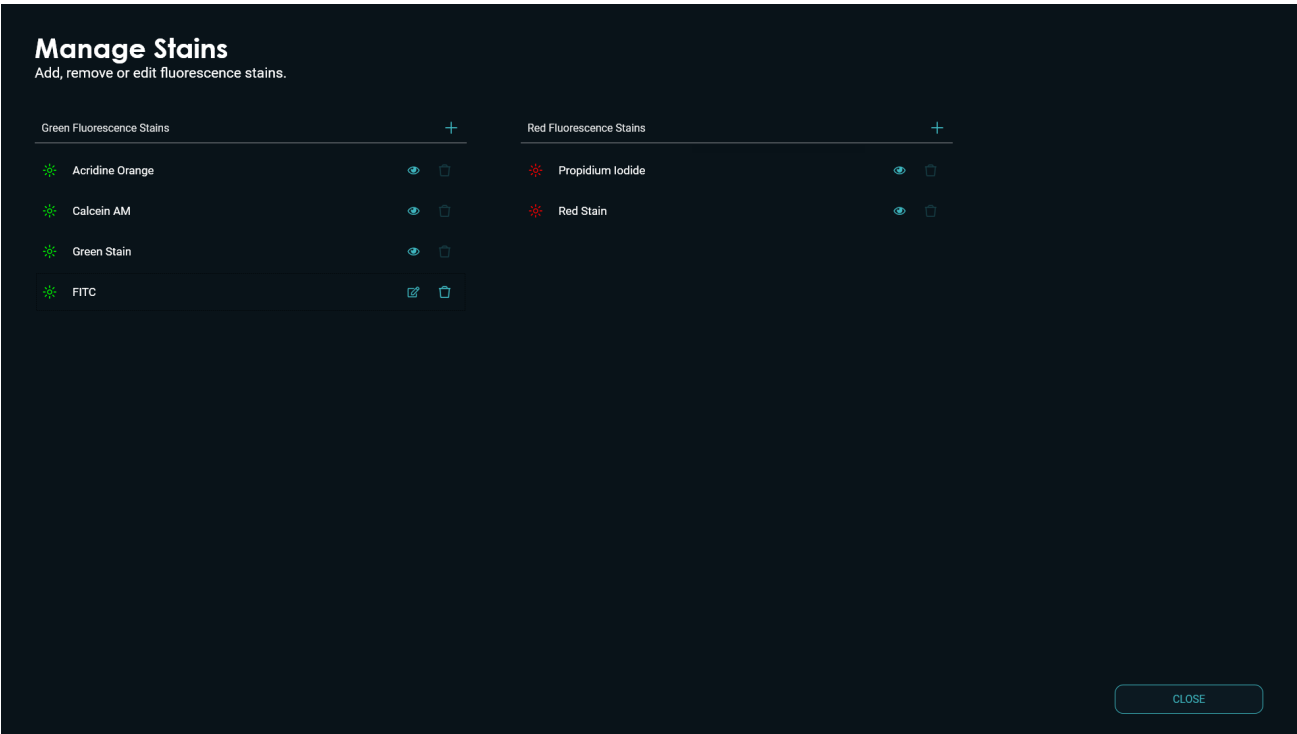


Figure 37. Green and Red custom stains added

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.

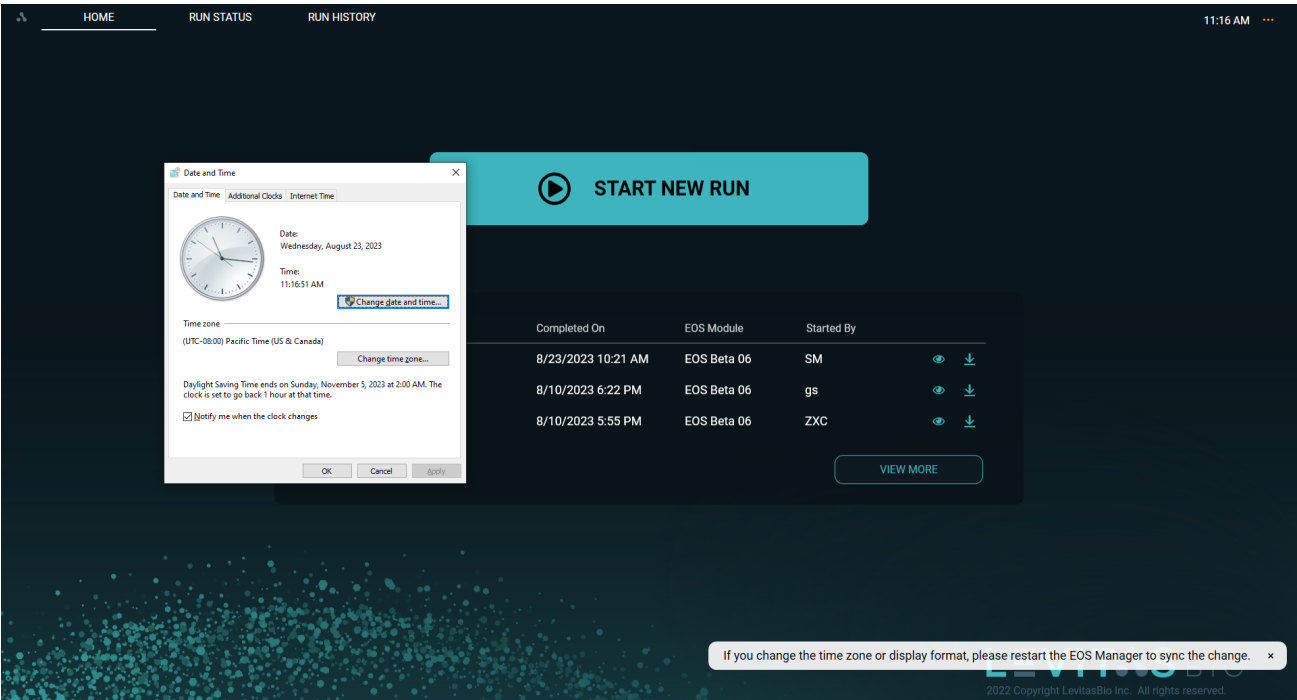


Figure 38. Change Data or Time via Windows popup screen

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

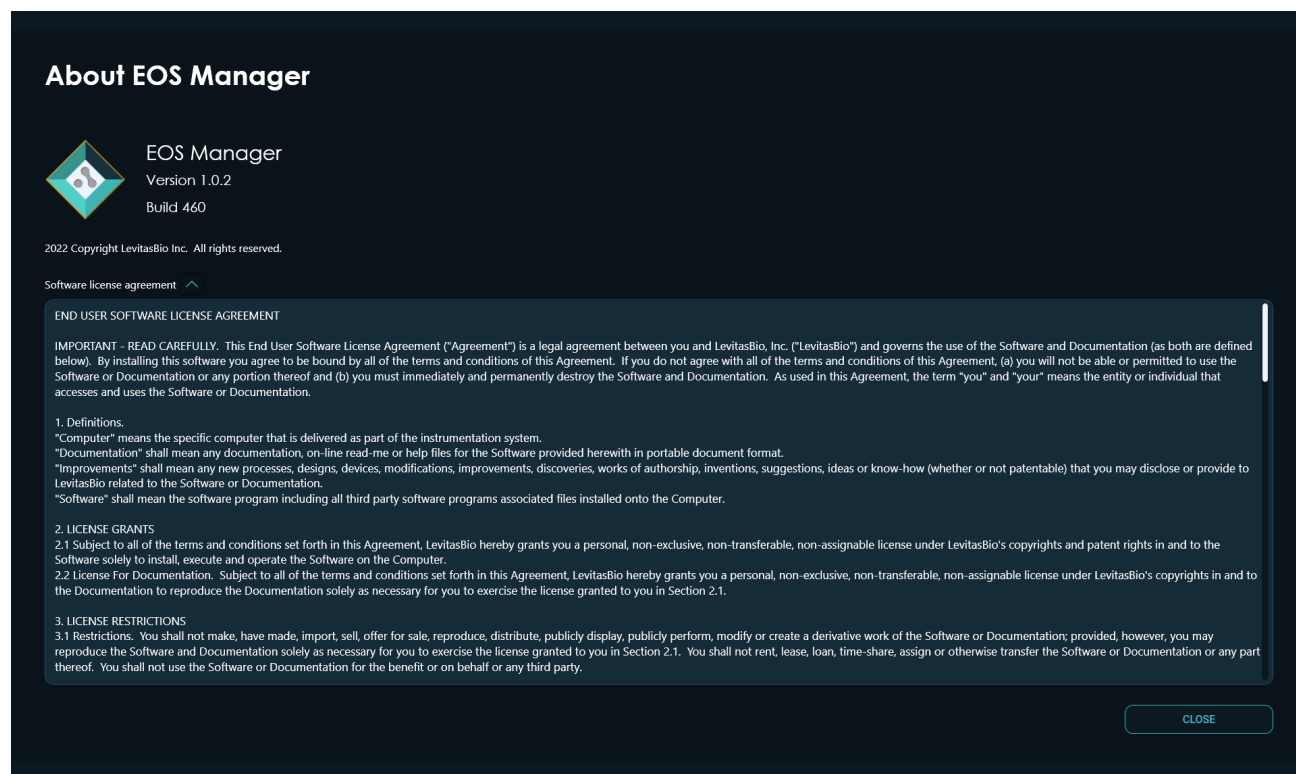


Figure 39. About the EOS Manager and software license agreement

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: A LevitasBio-trained field service engineer or other authorized personnel must install LeviCell EOS Systems. 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.

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

Core Calibration - First Time Use or Core Swap

Upon turning on the system for the first time, LeviCell EOS Manager will prompt the user 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:

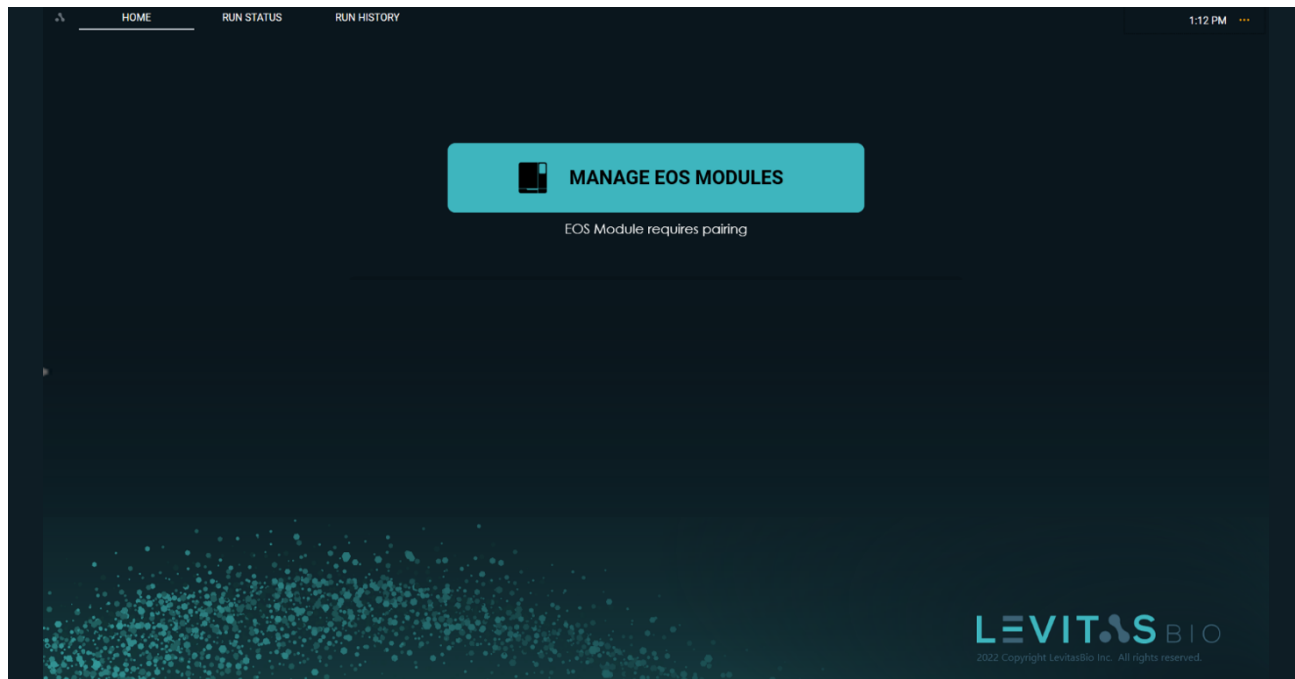


Figure 40. Manage EOS Module Home screen

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.

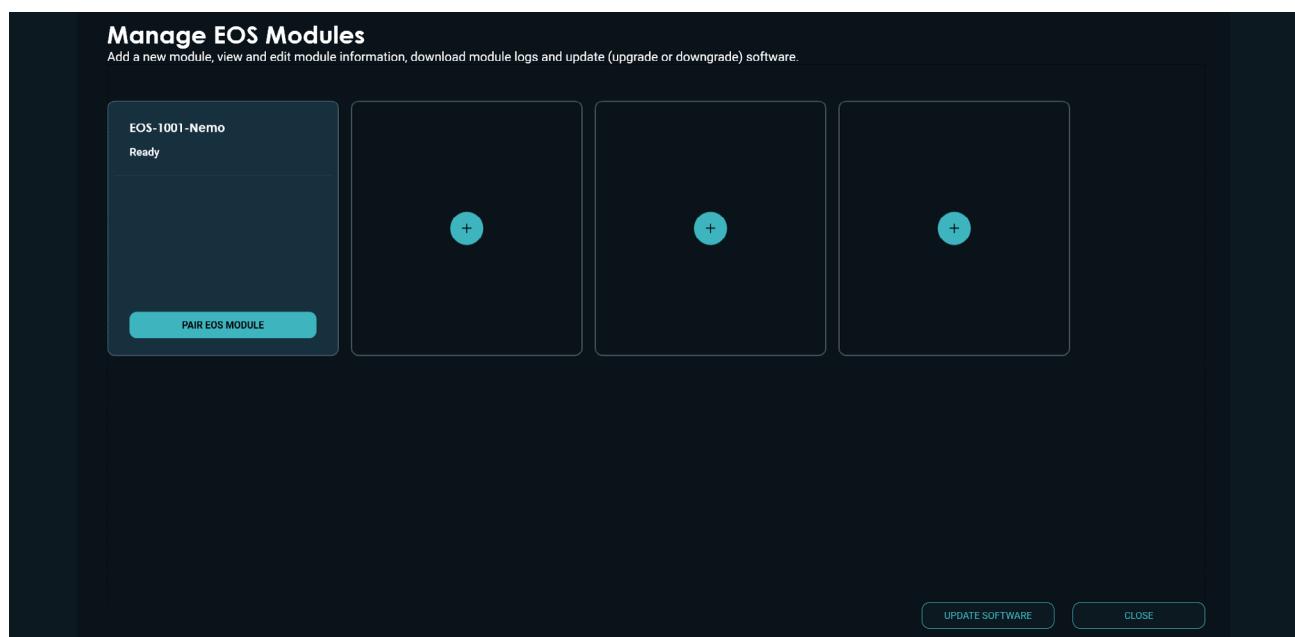


Figure 41. EOS Module requires pairing

Once you have established communication with the module, the EOS Module Options can be accessed to:

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

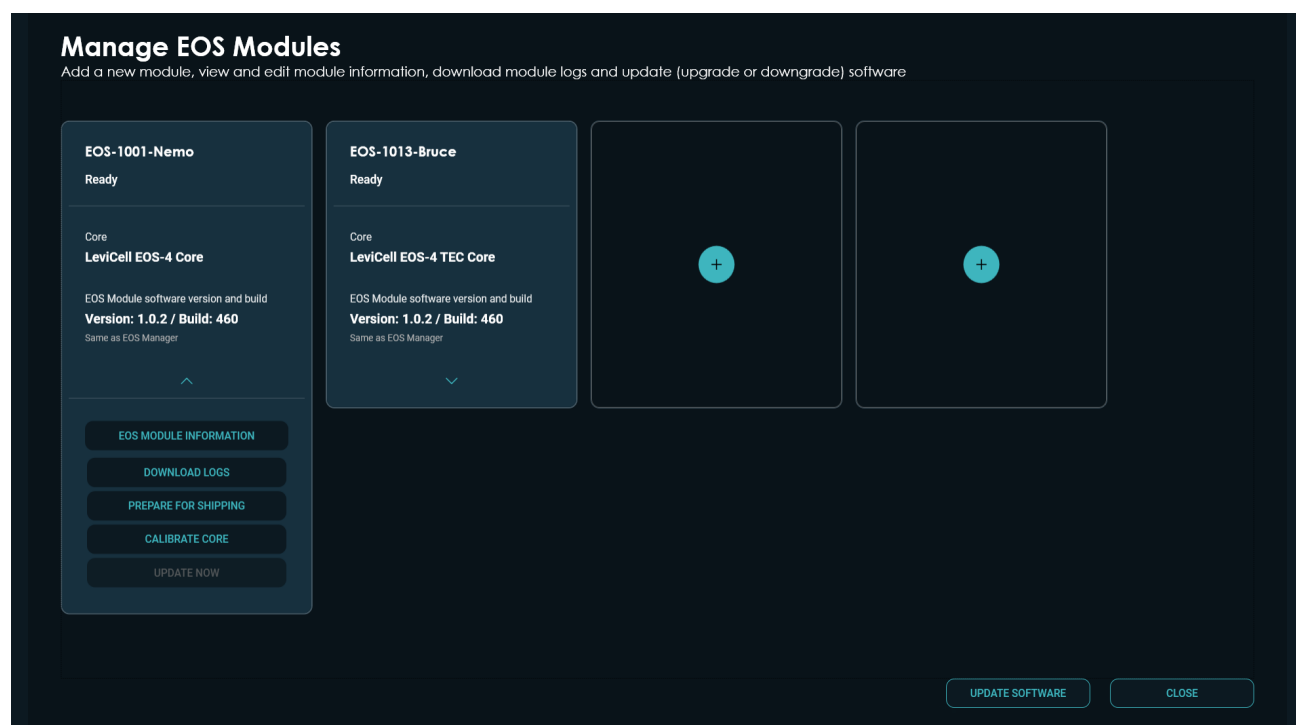


Figure 42. Paired EOS with options

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:

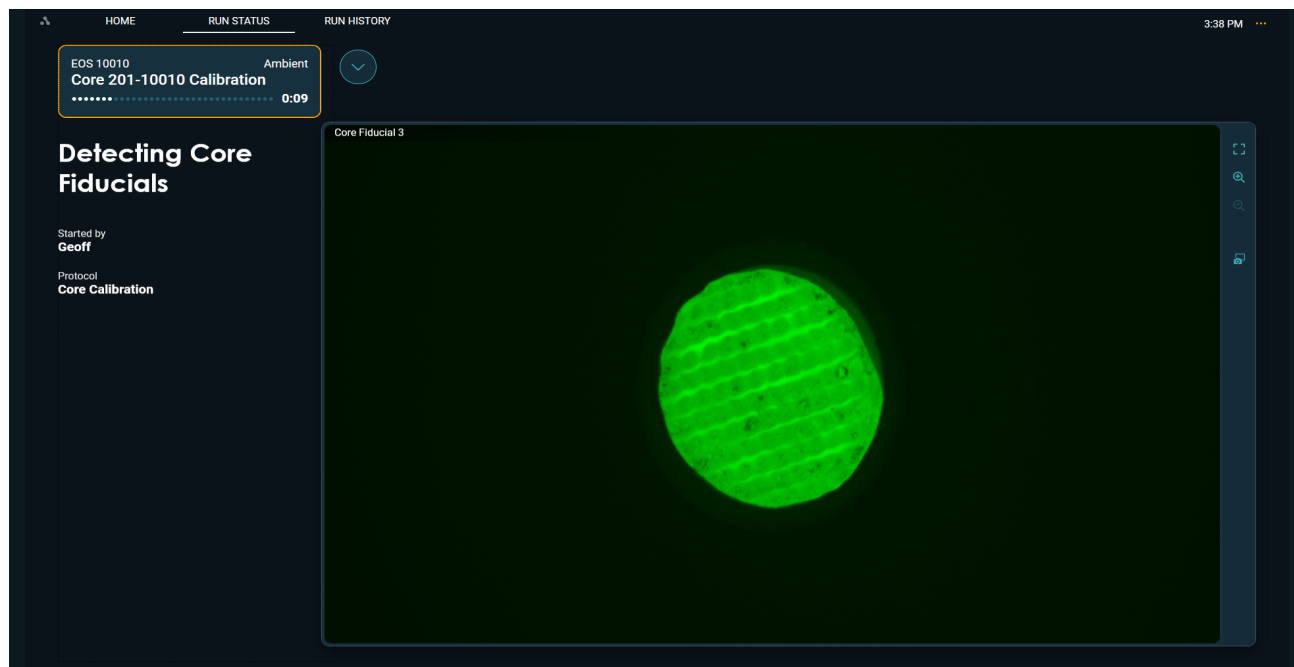


Figure 43. Detecting Core Fiducials

Once this is complete, the magnets within each lane will be scanned for location 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:

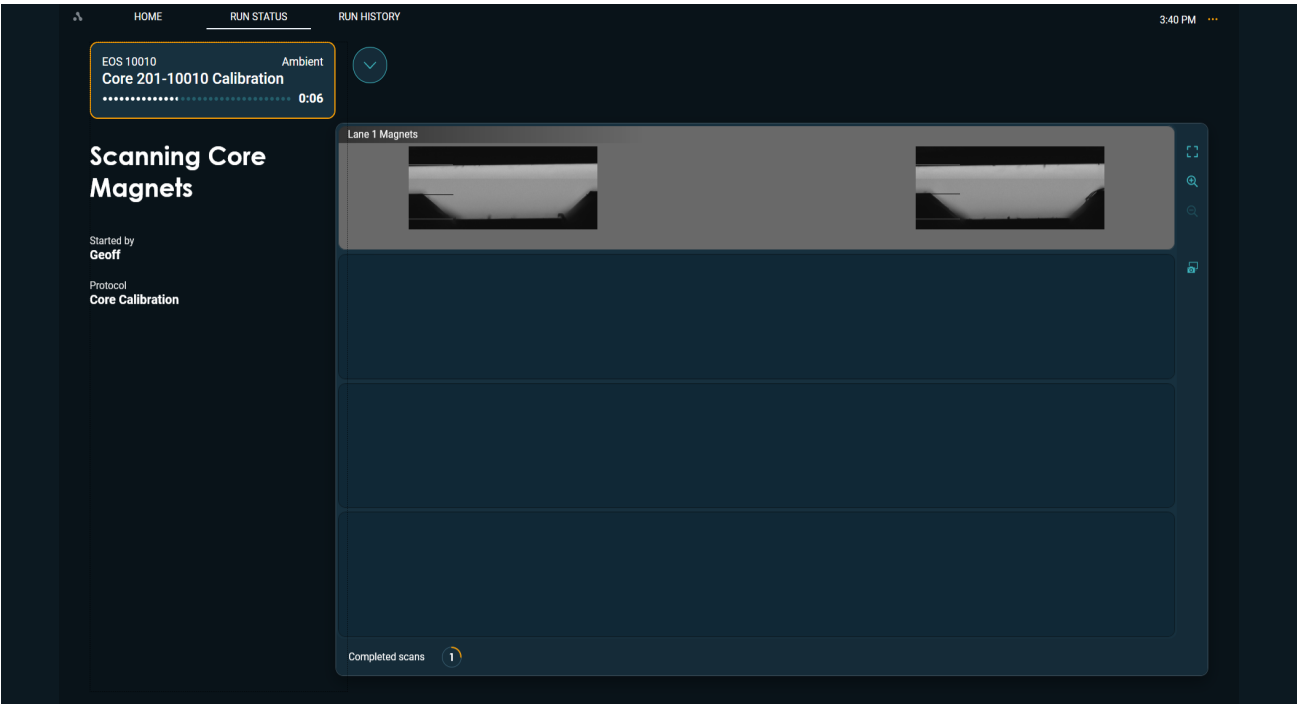


Figure 44. Scanning core magnets in progress on lane 1



Figure 45. 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:

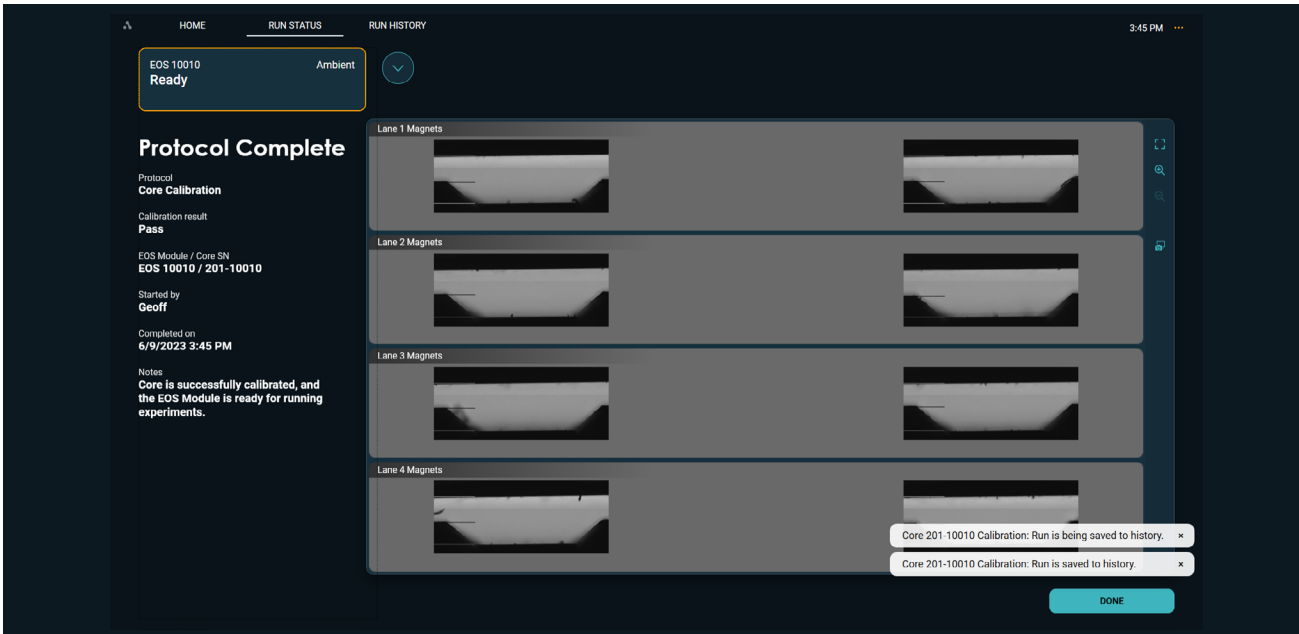


Figure 46. Calibration Protocol completed

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

Adding New EOS Modules

The LeviCell EOS System can expand up to 4 EOS Modules to be connected to the Control PC. Additional EOS Modules can be installed and connected at any time. Installation is conducted by a trained LevitasBio field service engineer.

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.

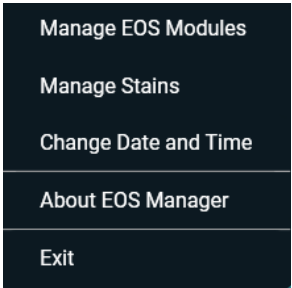


Figure 47. System Menu

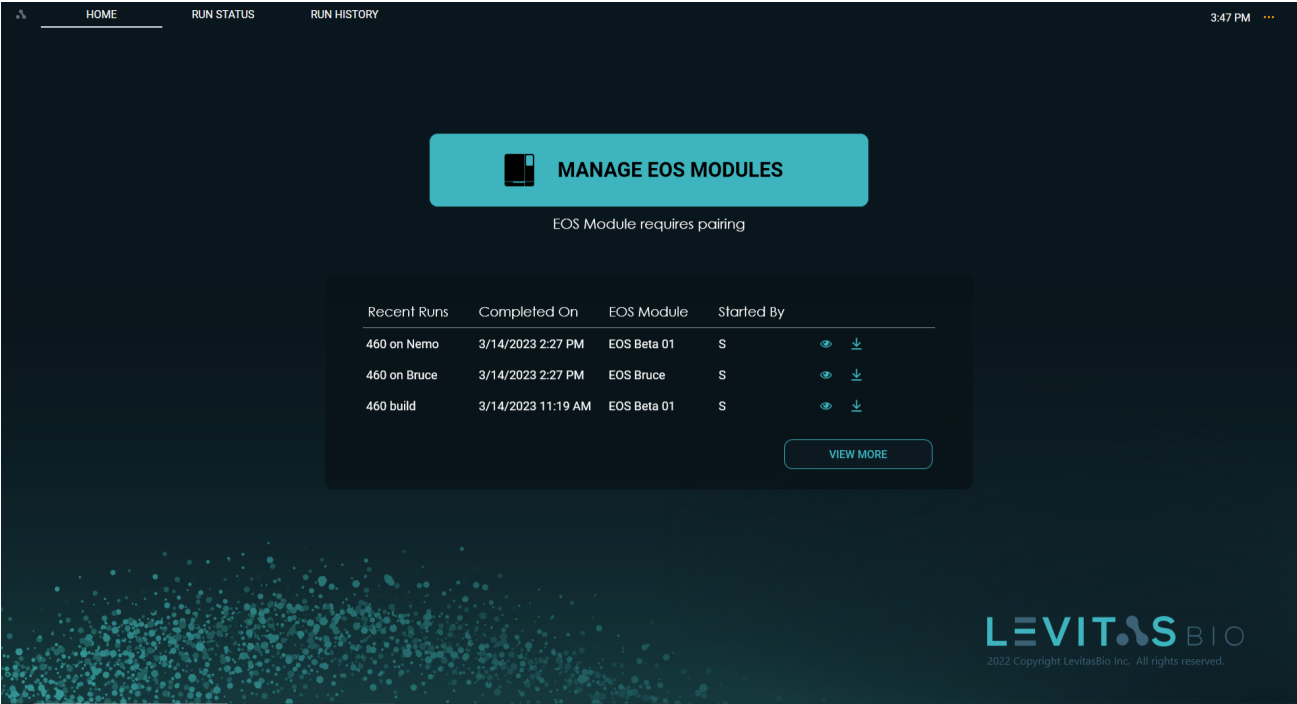


Figure 48. Home screen when an EOS Module requires pairing

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

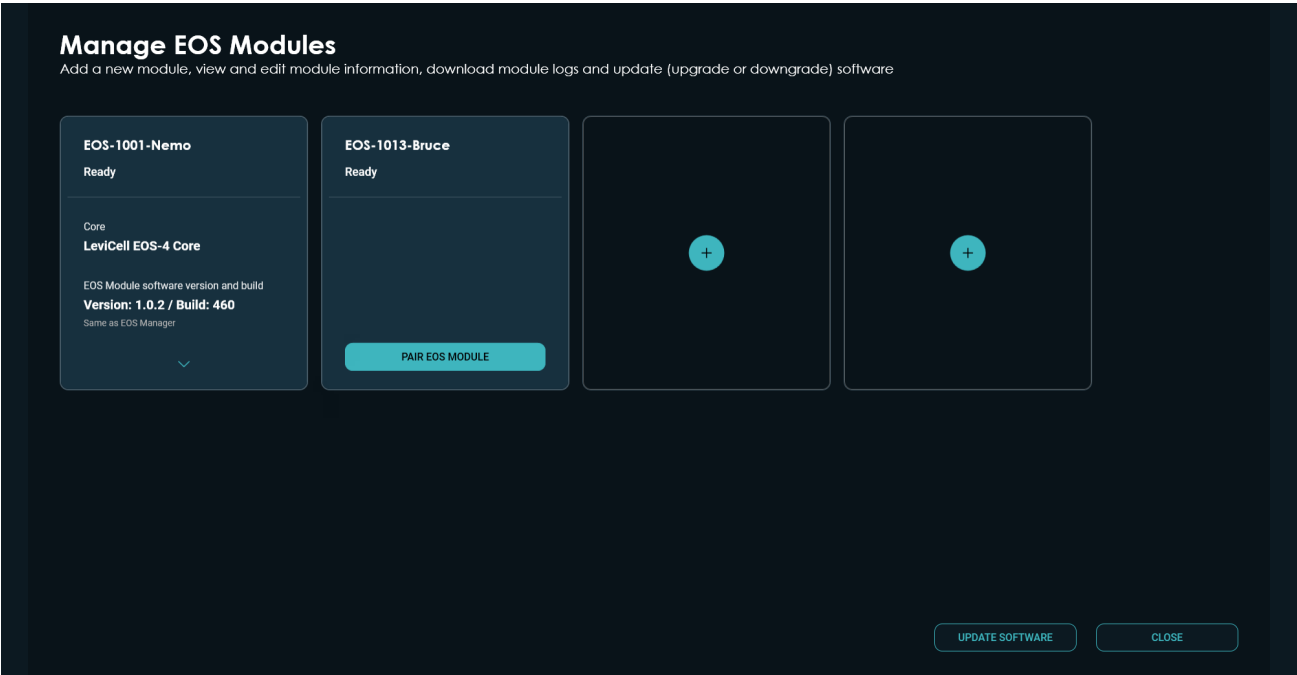


Figure 49. Additional EOS Module requires pairing

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

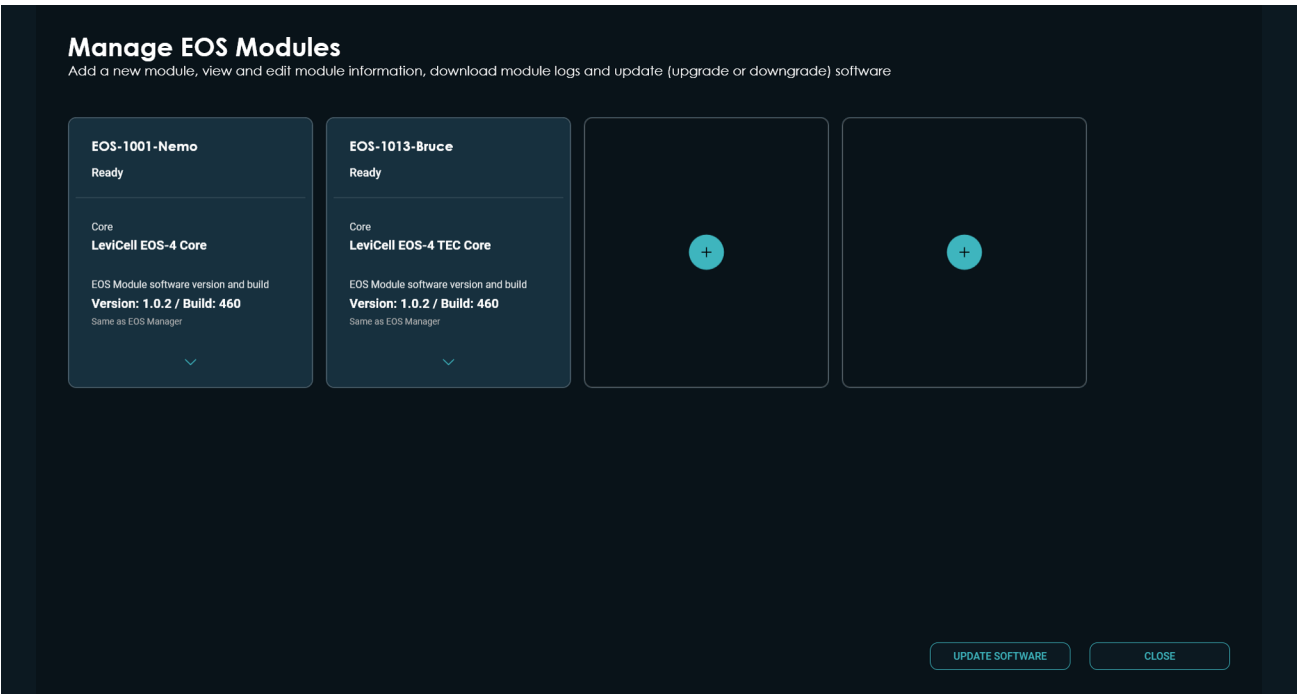


Figure 50. Additional EOS Module is paired and ready for use

Moving a LeviCell EOS Module

Contact LevitasBio Support if a LeviCell EOS System needs to be moved to a different location. In order to ensure proper transport a trained field service engineer will need to prepare the system, including re-installation of shipping safety locks to protect moving parts during a move. Calibration may also be required after the move.

GETTING STARTED

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

Additional items required before starting:

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

LeviCell EOS Performance Qualification Test

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

The general steps to perform the performance qualification test are:

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

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

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

Example images of beads are shown in figure 45 at the beginning of the workflow (top panel) and after levitation (bottom panel).

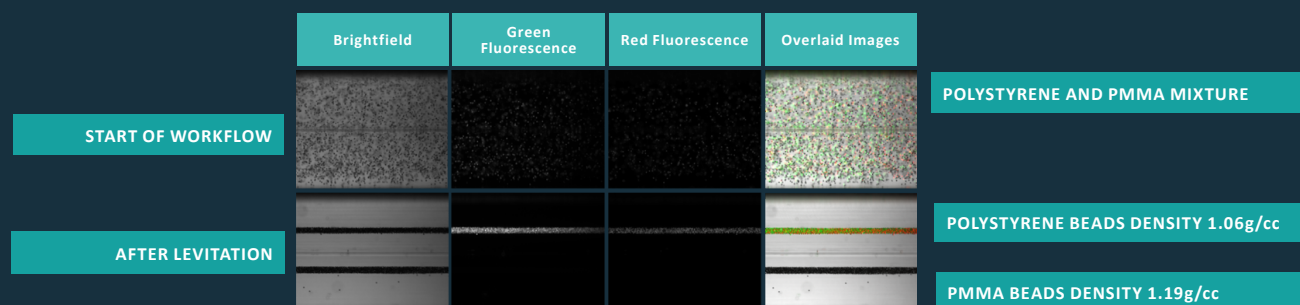


Figure 51. Example images of bead mixtures taken during Performance Qualification

Step by Step Protocol for Bead Test

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

A. Prepare Reagents

1. Prepare Bead Mixture

- Vortex bead tubes thoroughly immediately before pipetting to avoid sedimentation.
- In a new 1.5 mL tube, prepare bead mixture as shown in Table 9.

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

Table 9. Preparation of Bead Mixture for 1-4 lanes

2. Prepare Levitation Buffer

- In a new 1.5 mL tube, prepare the Levitation Buffer as shown in Table 10 (final concentration = 125 mM).

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

Reagent	Volume (μL)		# of lanes to run	
	1	2	3	4
LeviCell Install Buffer	262.5	437.5	700	875
1 M Levitation Agent	37.5	62.5	100	125
Total	300	500	800	1000

Table 10. Preparation of 125 mM Levitation Buffer for 1-4 lanes

3. Prepare Bead Sample for Loading

- a.** Pellet the beads by centrifuging tube at 300 RCF for 3 min at ambient temperature.
- b.** Remove supernatant from bead pellet using a P200 pipetter set to 45 μL for a single lane, or 180 μL for all lanes, etc.
- c.** Add the appropriate amount of Levitation buffer for the number of lanes being loaded.

Reagent	Volume (μL)		# of lanes to run	
	1	2	3	4
Levitation Buffer	270	490	710	930

Table 11. Resuspension volume for run

- d.** Mix sample thoroughly by gently pipetting up and down 10 times. It is important to avoid creating bubbles.
- e.** If counting, immediately after mixing, set aside 20-30 μL of the input beads.

B. Run the LeviCell EOS Instrument

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

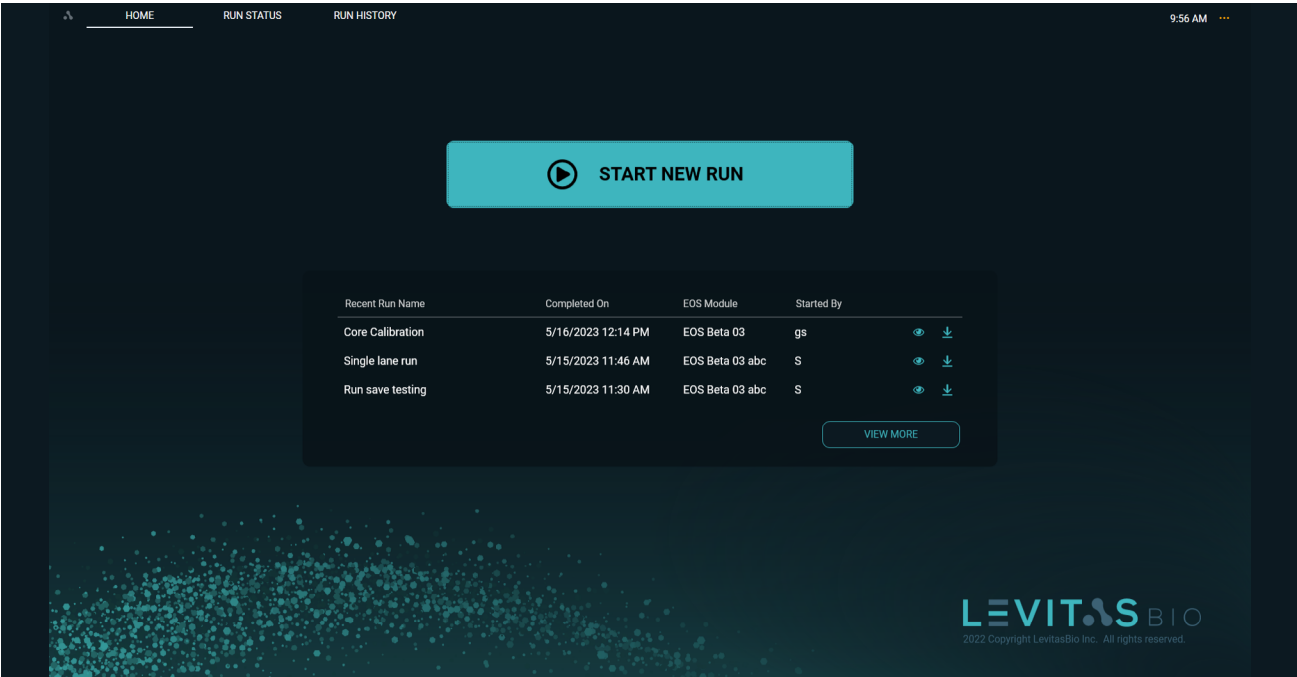


Figure 52. Home screen

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



Figure 53. Scan cartridge barcode information

3. Specify run information and select instrument

Cartridge barcode
012-3487-9235

Specify run information and EOS Module

Run name
EXP-1897

Started by
SM

Notes (optional)

EOS Module

EOS-1001-Nemo Ready

EOS-1013-Bruce Ready

CANCEL

Cartridge

Run information

Protocol

Samples

BACK

NEXT

Figure 54. Run information screen

4. Select Bead Test protocol to run and run temperature Ambient

Cartridge barcode
012-3487-9235

EOS Module
EOS-1001-Nemo

Run name
EXP-1897

Select protocol and run temperature

Protocol

Small Cell (< 5µm)
40 min

Medium Cell (5 - 20µm)
20 min

Large Cell (> 20µm)
6 min

Bead Test
4 min

Run temperature

Ambient

10°C

20°C

CANCEL

Cartridge

Run information

Protocol

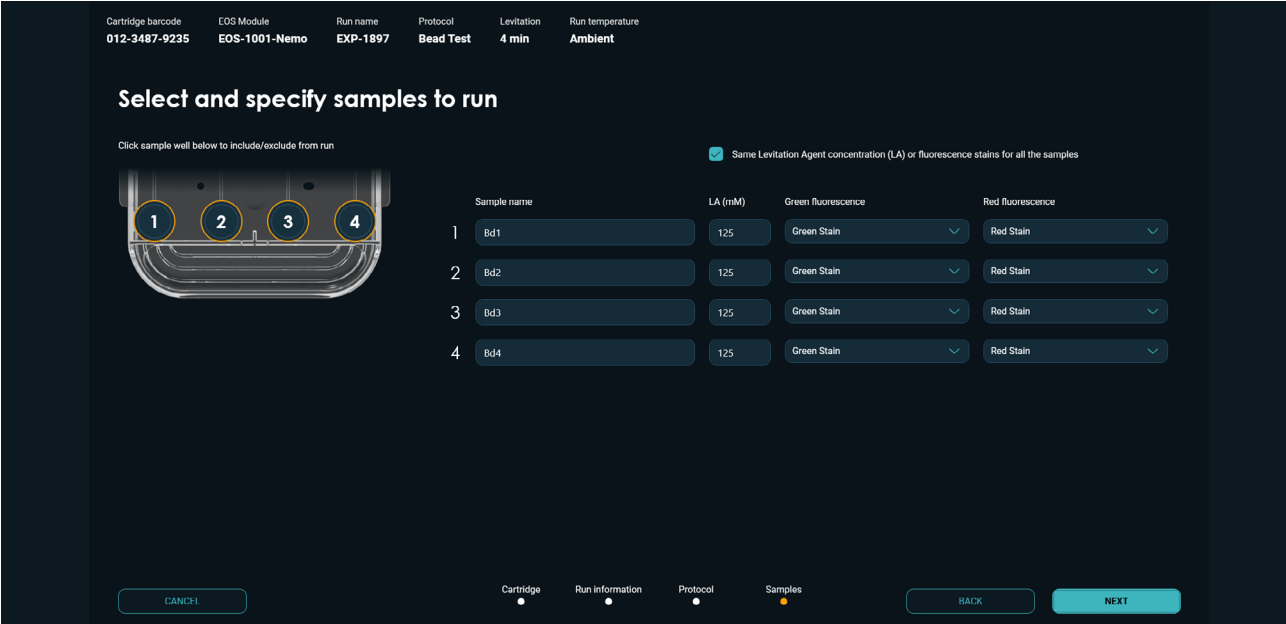
Samples

BACK

NEXT

Figure 55. Select protocol, and temperature screen

5. Select and specify samples to run



Cartridge barcode: 012-3487-9235 | EOS Module: EOS-1001-Nemo | Run name: EXP-1897 | 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	Green Stain	Red Stain
2 Bd2	125	Green Stain	Red Stain
3 Bd3	125	Green Stain	Red Stain
4 Bd4	125	Green Stain	Red Stain

CANCEL

Cartridge Run information Protocol Samples

BACK NEXT

Figure 56. 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.



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

- The Levitation Agent concentration for the Bead Test [LA (mM)] is 125.
- Choose “**Green Stain**” from the pull-down menu for Green Fluorescence, and “**Red Stain**” for the Red Fluorescence.’



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

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



CAUTION: Potential Pinch Hazard.



Figure 57. Open door to load cartridge

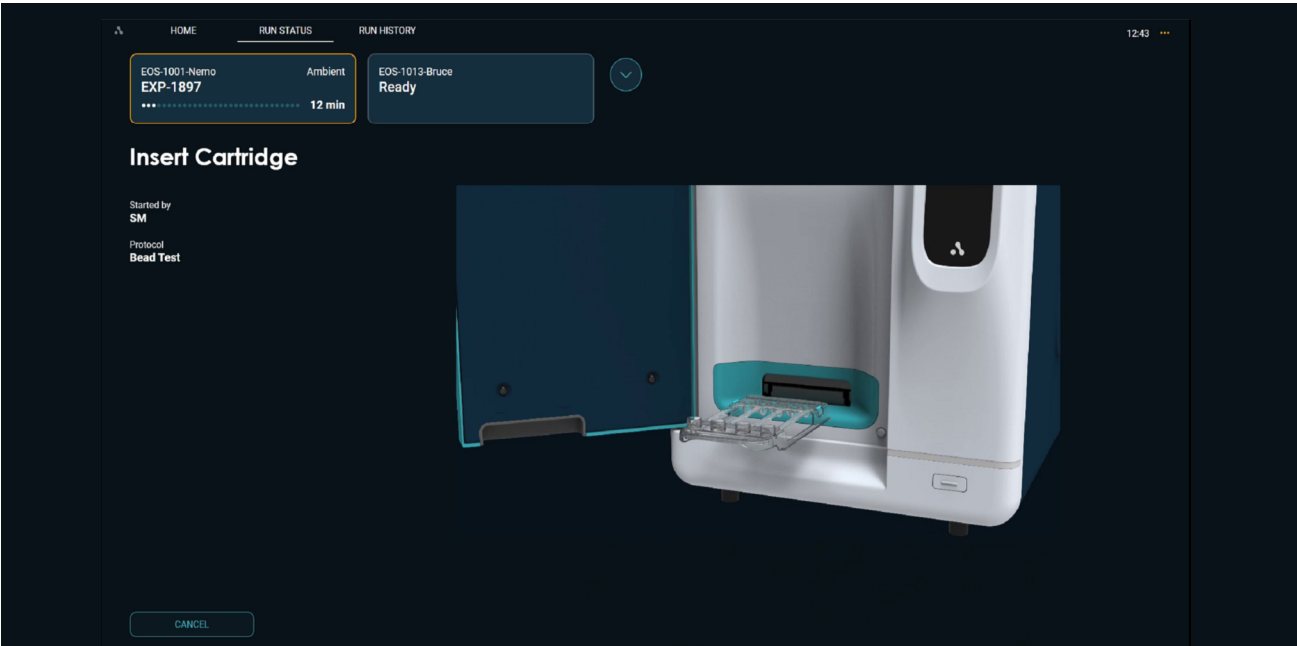


Figure 58. Insert cartridge into EOS Module

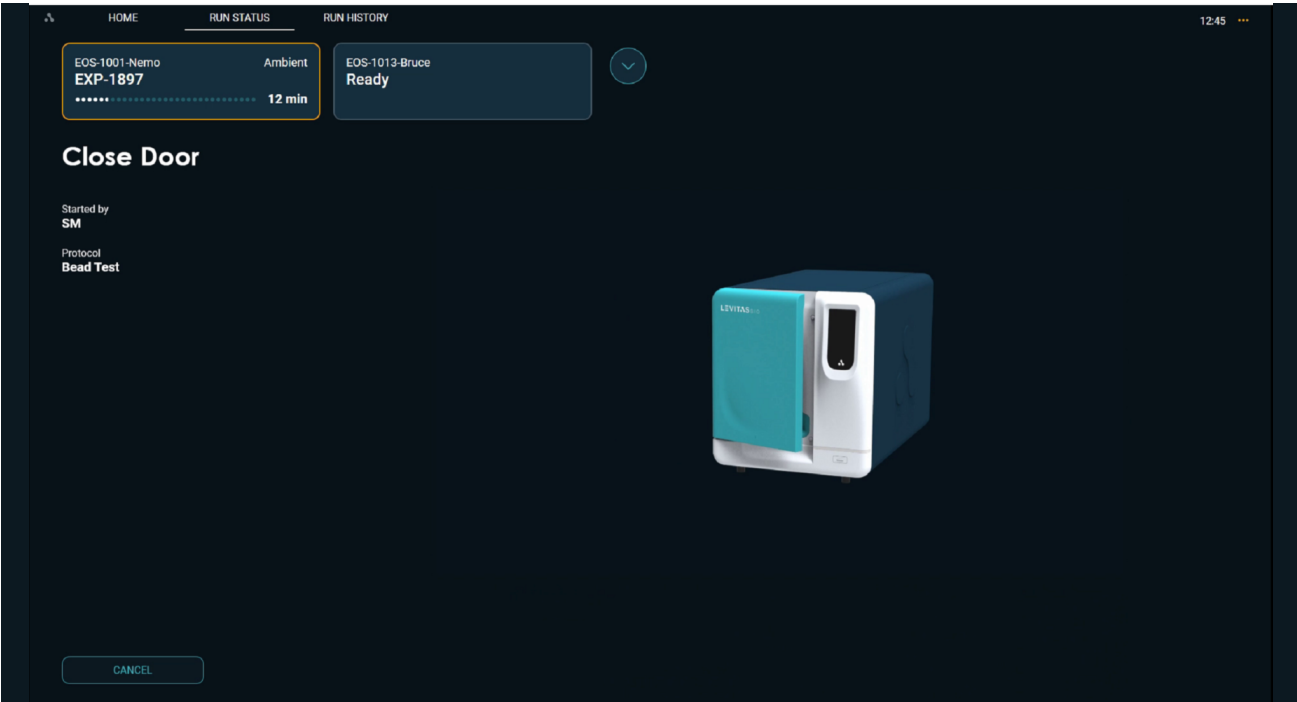


Figure 59. Close door after inserting cartridge

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

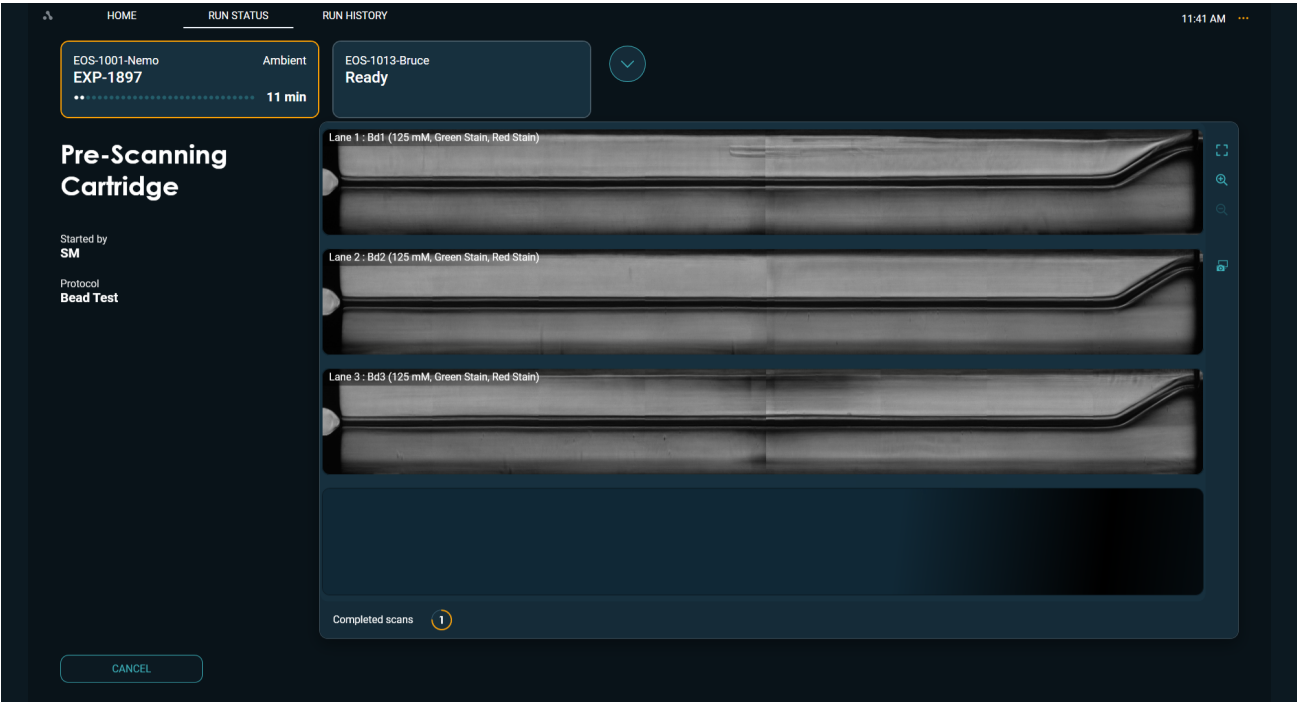


Figure 60. Prescanning cartridge 3 of 4 lanes

8. Follow subsequent prompts to then dispense samples.



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

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

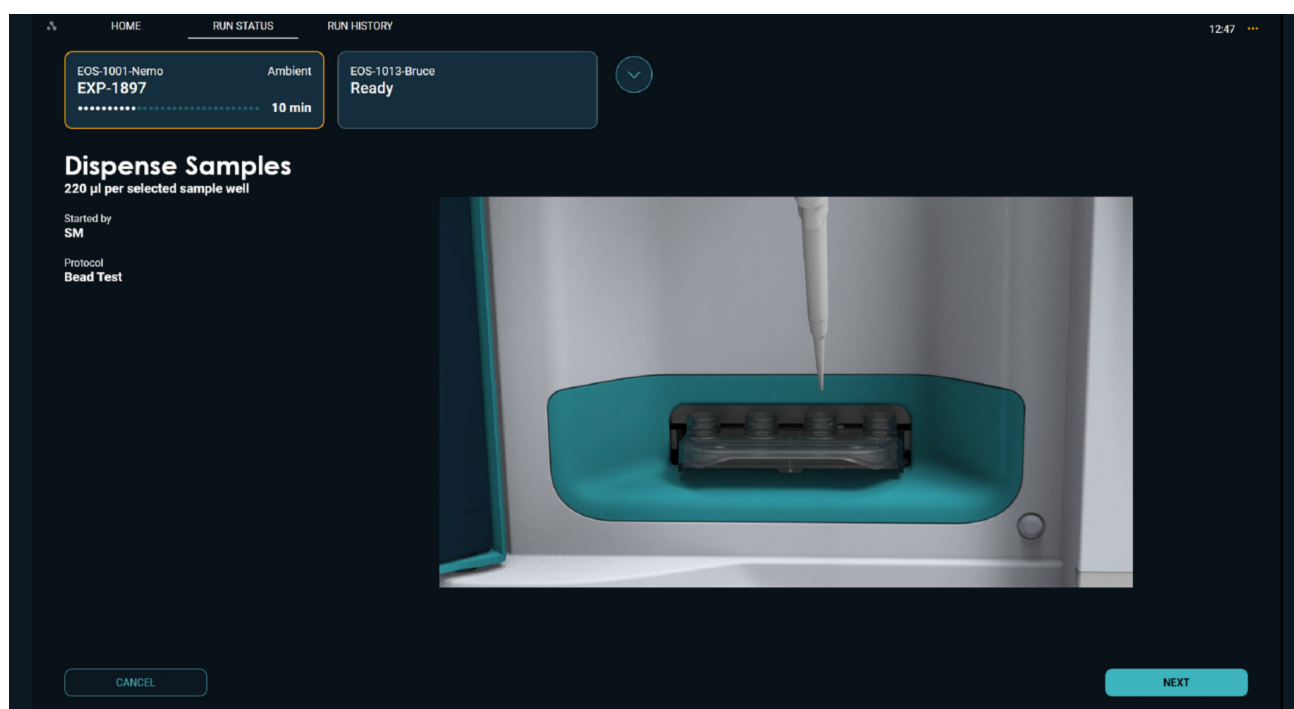


Figure 61. Dispense samples into the respective wells

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



Figure 62. Loading samples into the cartridge

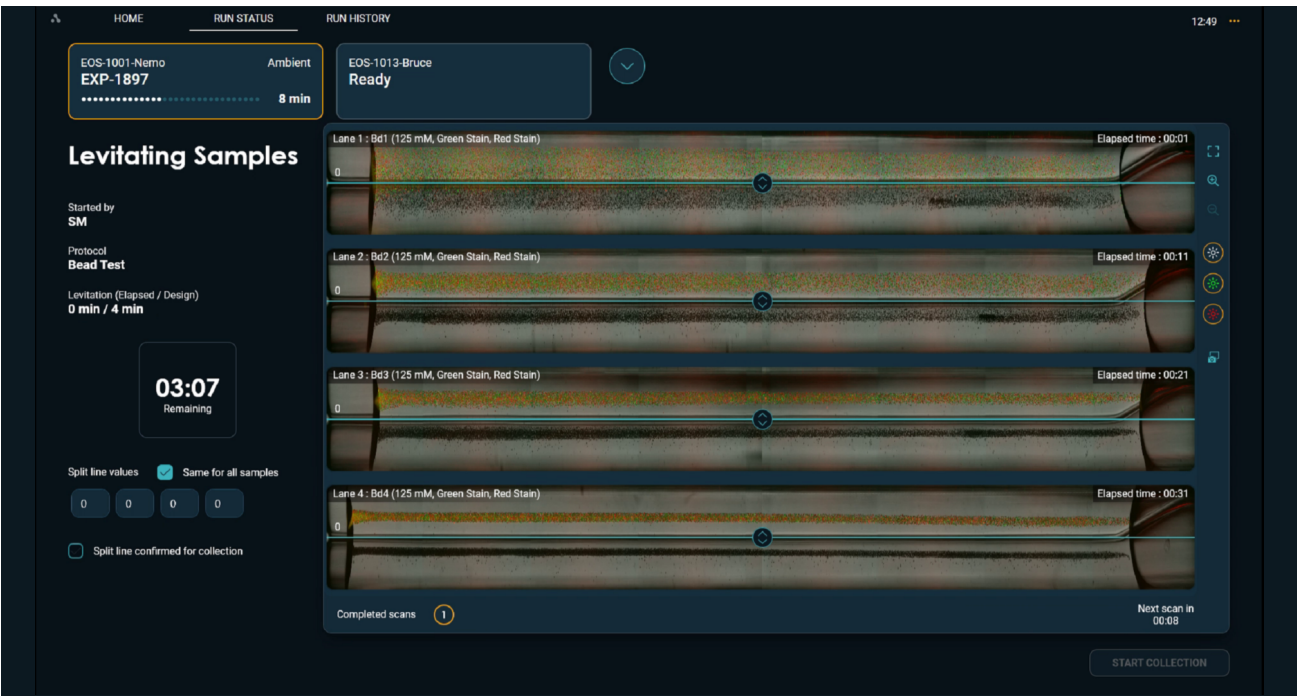


Figure 63. Beads levitating and image scanning

11. **Set Split:** 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 Cap Bead Test.

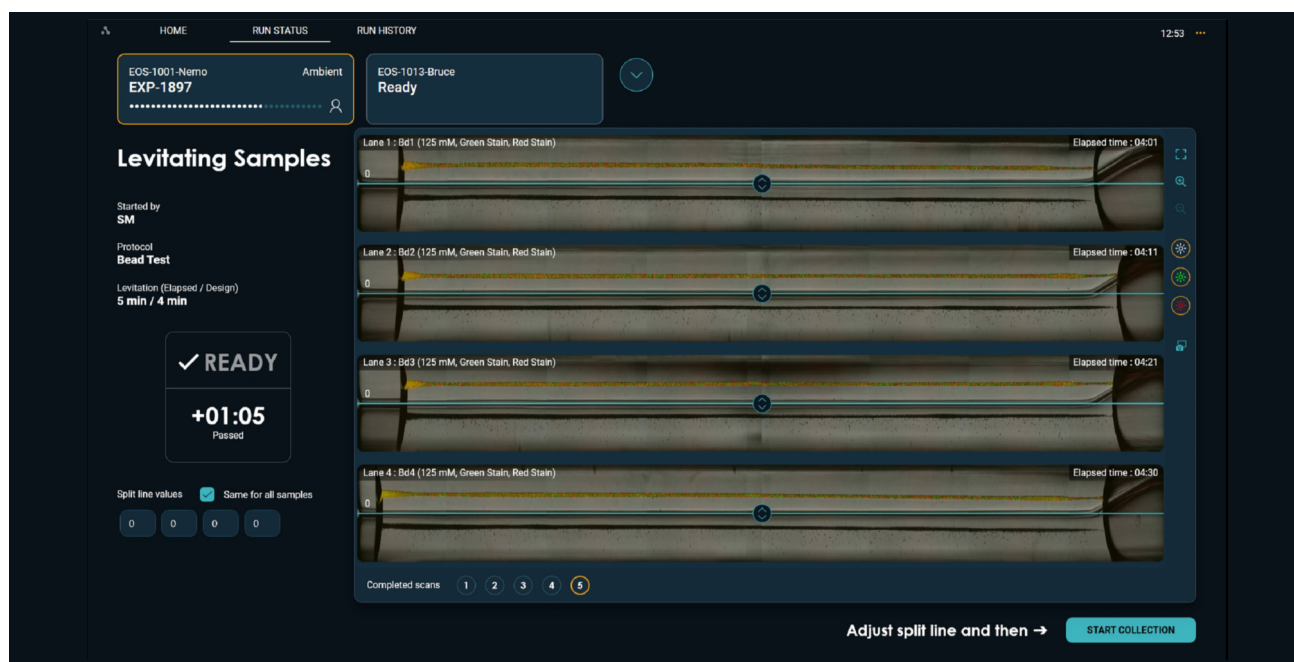


Figure 64. Set split line when levitation has completed and start collection

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

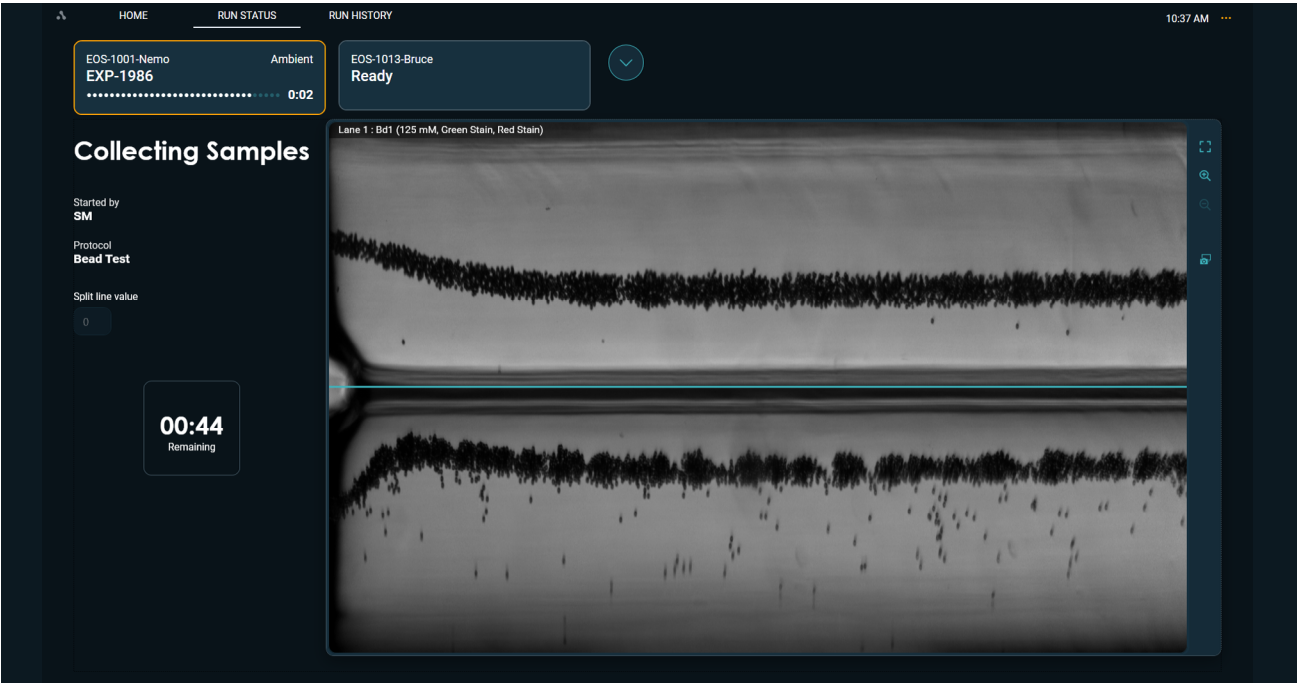


Figure 65. Imaging collection of Lane 1 as it flows to the outlet well

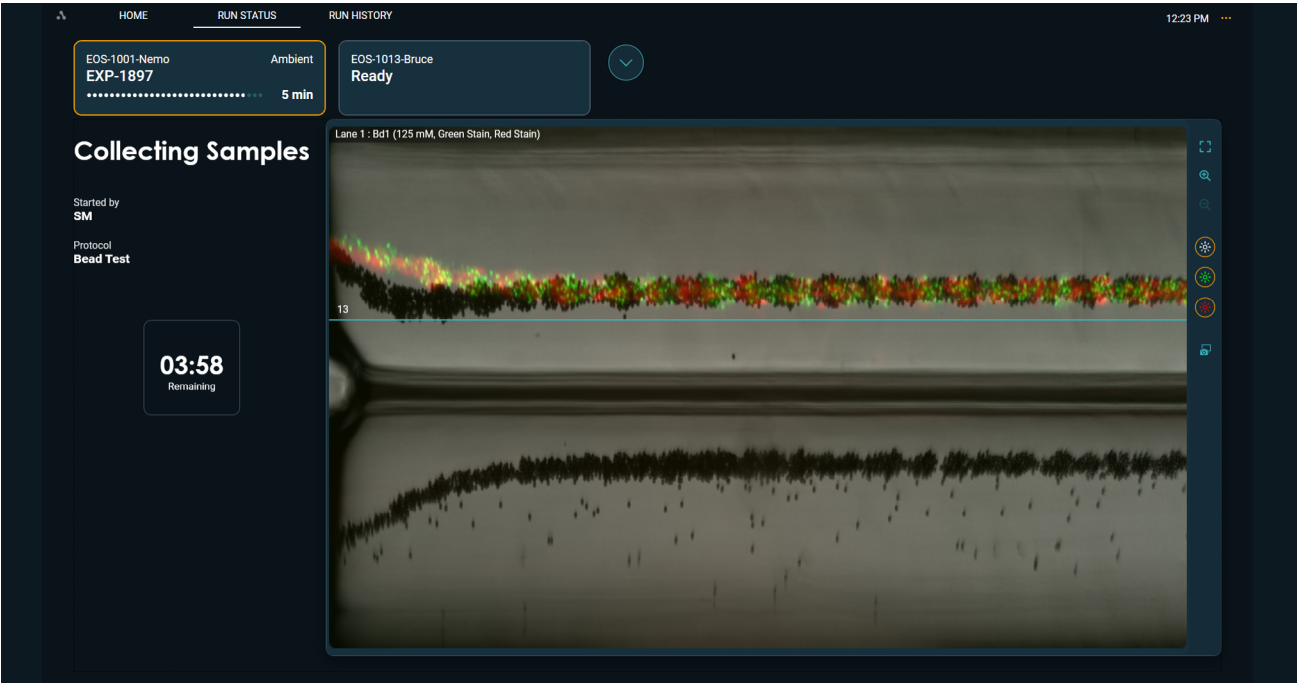


Figure 66. Fluorescence collection

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

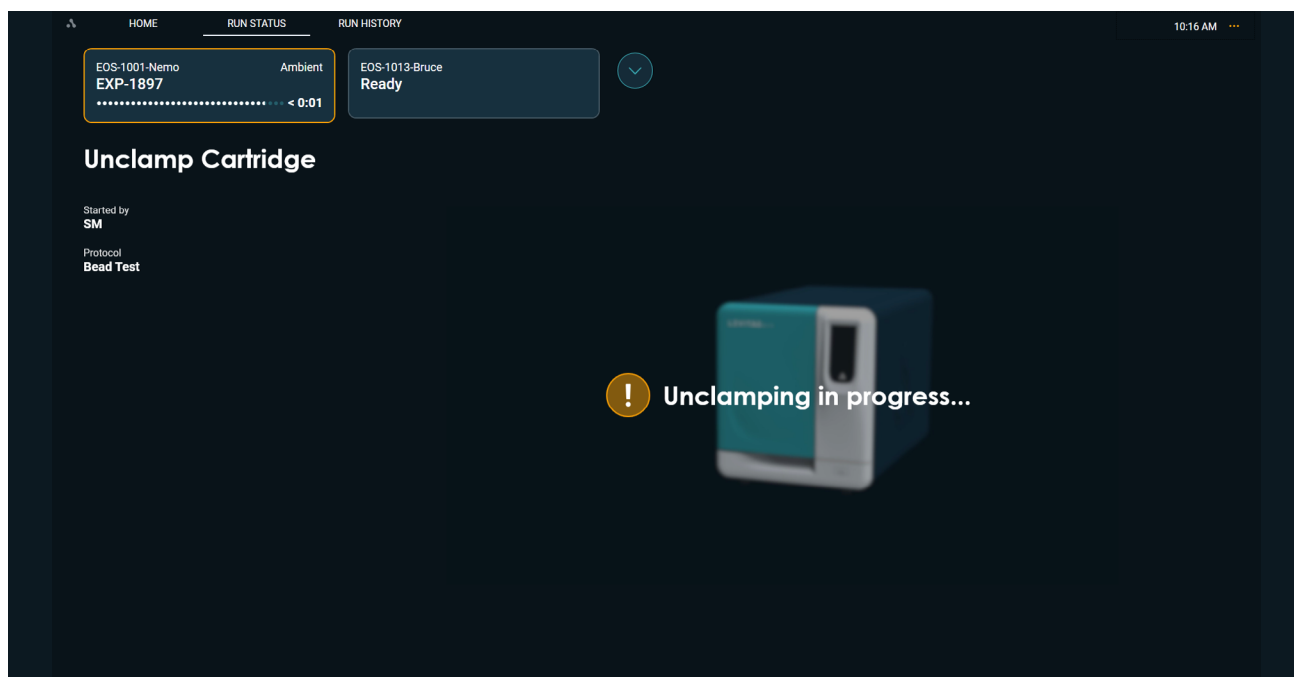


Figure 67. EOS Module will unclamp the cartridge at the end of collection

14. Retrieve cartridge and harvest output

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

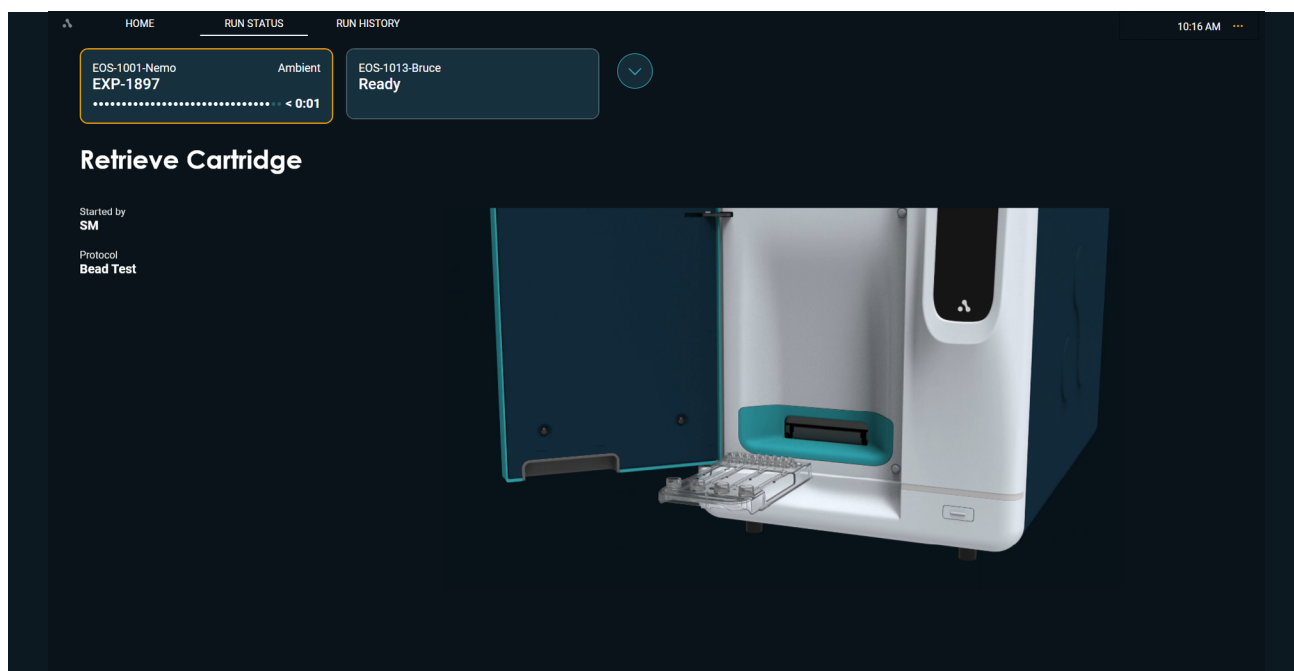


Figure 68. Retrieve cartridge from EOS Module

- 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.

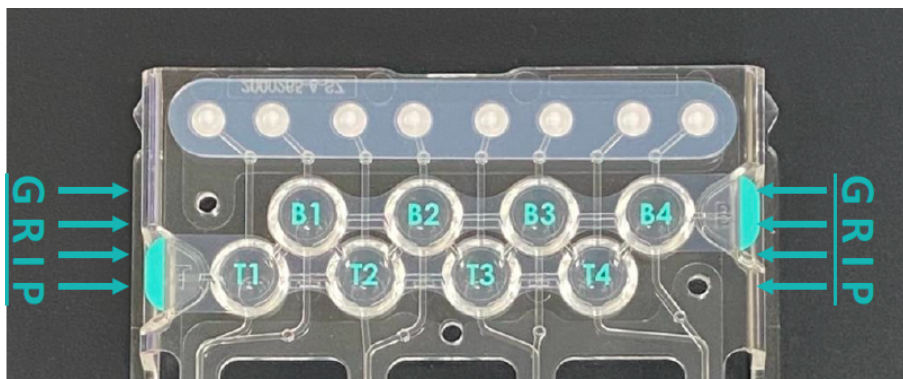


Figure 69. Grip locations on the cartridge when peeling outlet well tape off

- 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 of according to biohazards.
- d.** Pipette mix each sample 3-5 times before retrieval without introducing bubbles, to resuspend beads that may have settled.
- e.** Aspirate all liquid from the output well, not the channel leading to it, into a 1.5mL or 8-strip tube.
- f.** Measure the final output volume using a pipette. When the split line is set to 0, typical recovery is between 70-100 μ L.
- g.** Repeat these steps for the bottom outlet wells (labeled B1-B4).

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

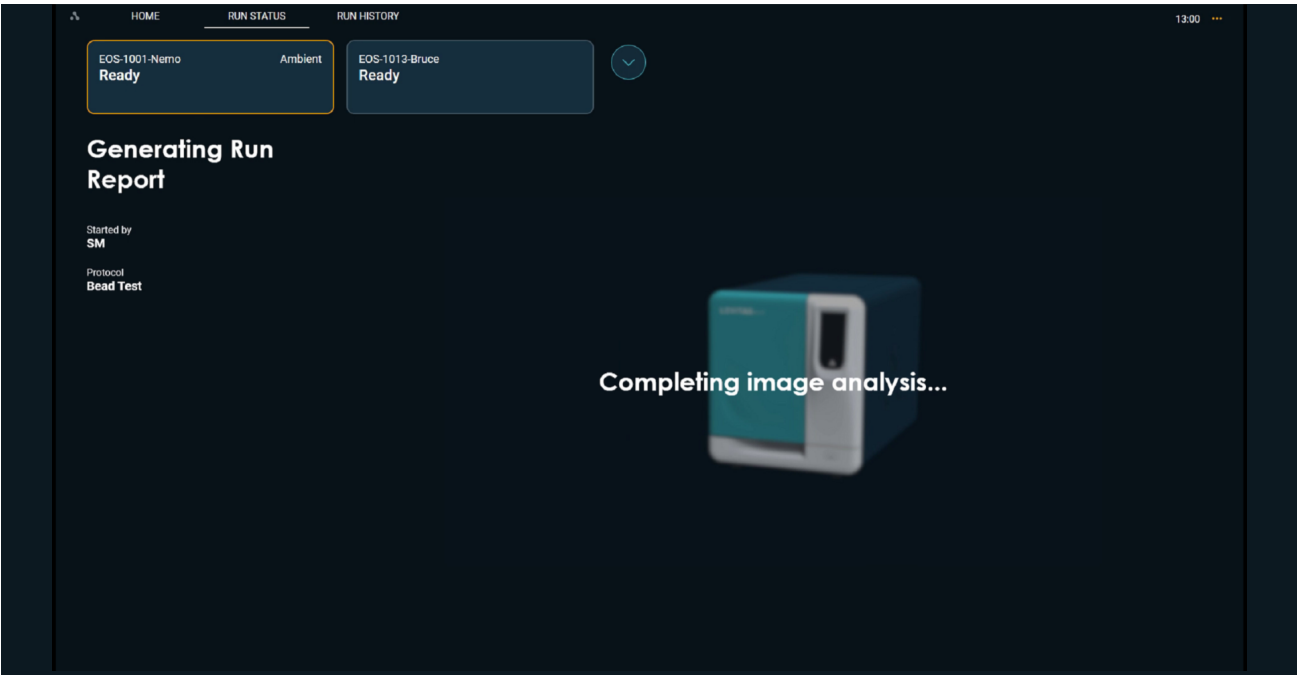


Figure 70. Generating Run Report after image analysis is completed

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

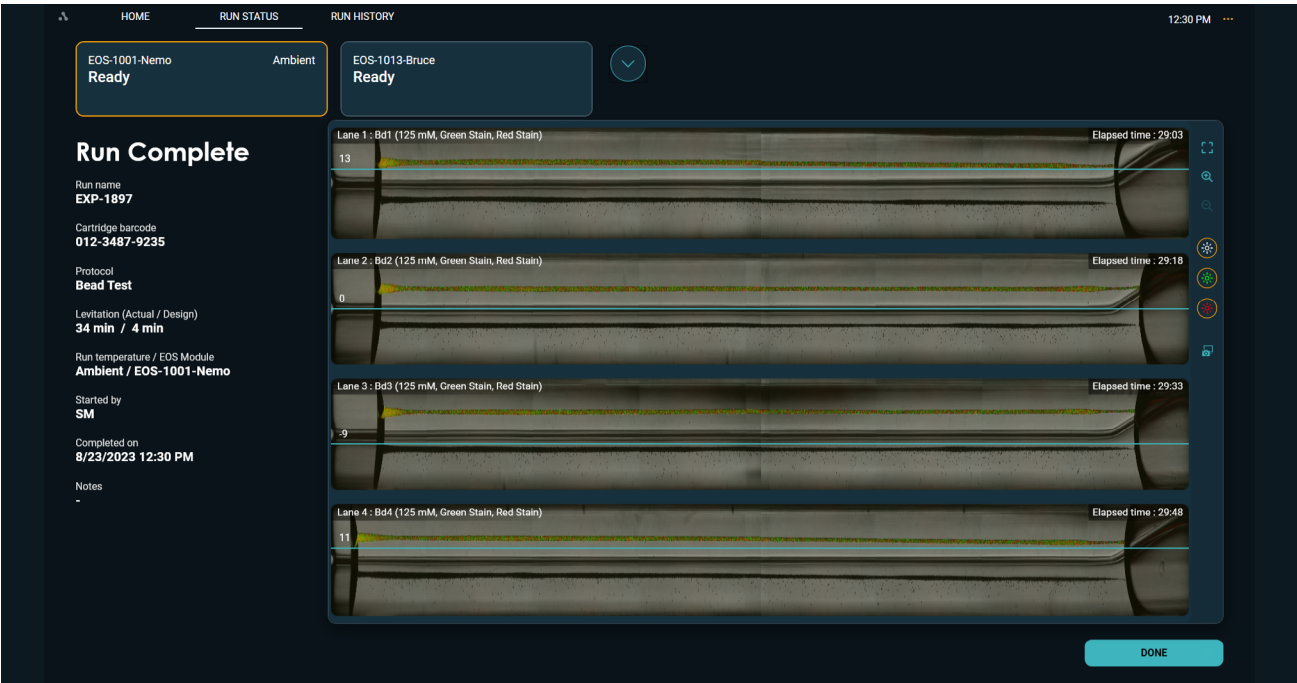


Figure 71. Run Complete screen

C. Count Beads (optional)

1. Count input beads and Top and Bottom output wells on a hemocytometer.
2. Use metrics shown in Table 3 to assess the separation and recovery metrics for each lane.

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

* After removal of volume for counting, if performed.

Table 12. Formulas for calculating separation and recovery metrics for Bead Test

RUNNING AN EXPERIMENT

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

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

Viable Cell Enrichment Protocol

A. Prepare Levitation Buffer and Sample

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



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

2. The recommended Levitation Agent concentration for viable cell enrichment is 150 mM, as shown in Table 13. For other types of separations, the concentration of Levitation Agent may be varied as needed. Please contact Technical Support for additional recommendations.

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

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

Table 13. 150 mM Levitation Buffer preparation for 1-4 lanes

B. Prepare Cells

3. Vortex mixture well to completely mix the Levitation Buffer.
4. Pipet the volume of samples containing 20,000 to 1,000,000 cells per lane into an appropriately sized (1.5 - 2.0 mL) low-binding microfuge tube.*
5. Centrifuge the tube containing the cells at 300 RCF for 5 min to pellet the cells.
6. Carefully remove the supernatant.
7. Resuspend sample with the appropriate volume of Levitation Buffer per Table 14, pipetting up and down 10 times to mix thoroughly.

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

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

Table 14. Resuspension volumes for 1-4 lanes

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



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

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

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

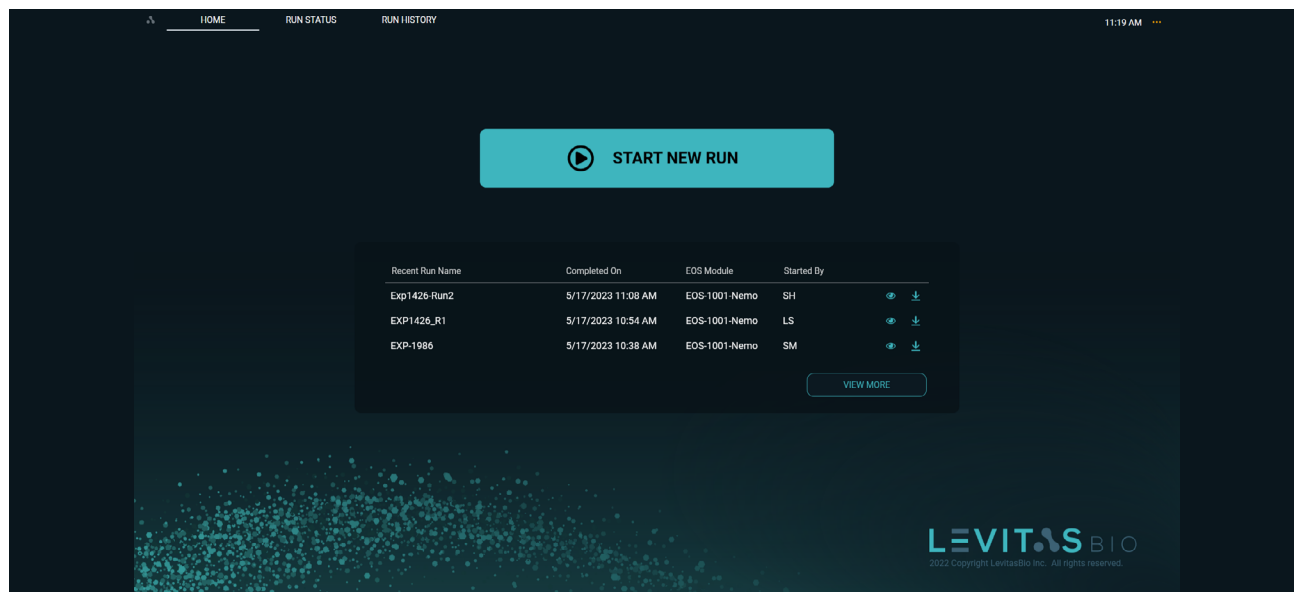


Figure 72. Home Screen



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

10. Scan the cartridge barcode.



Figure 73. Scan cartridge barcode information

11. Specify run information and select the instrument.

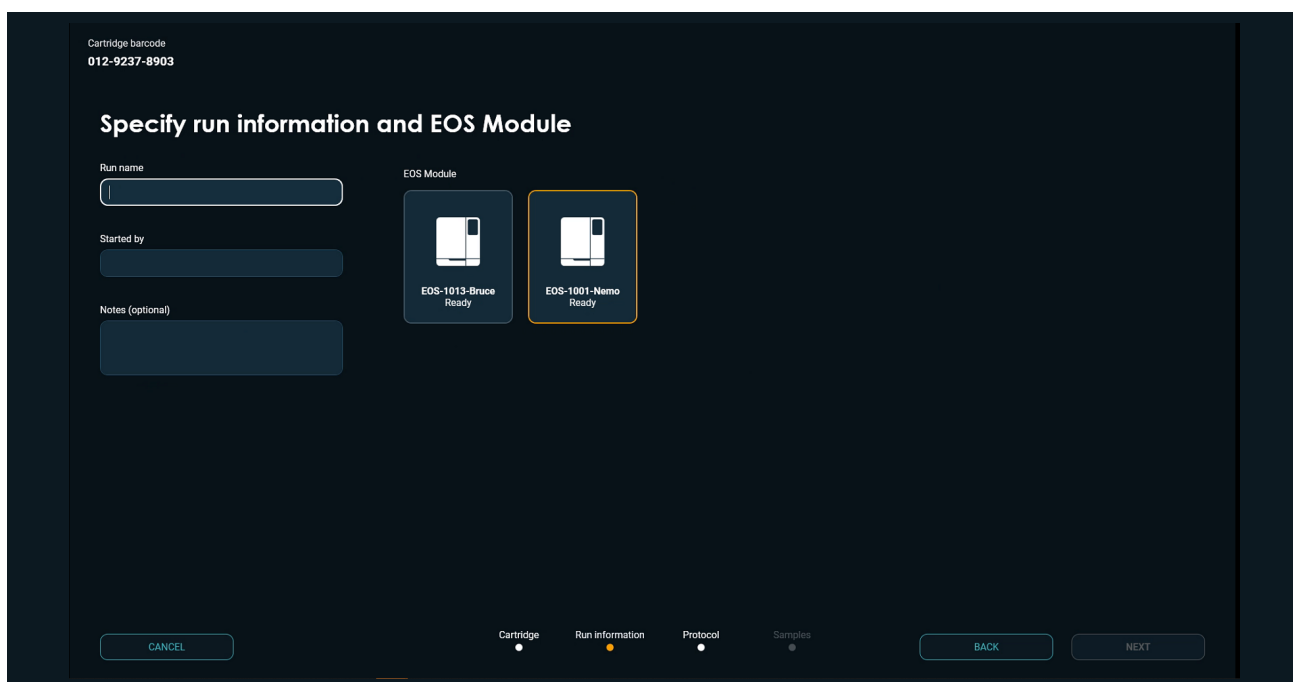


Figure 74. Specify run information

12. Select cell protocol to run. Select either the Small, Medium or Large Cell protocol to run a viable cell enrichment, depending on the cell size range of the cells you are separating (see Table 15).

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

Table 15. Viable cell enrichment protocol, associated cell size range and levitation time

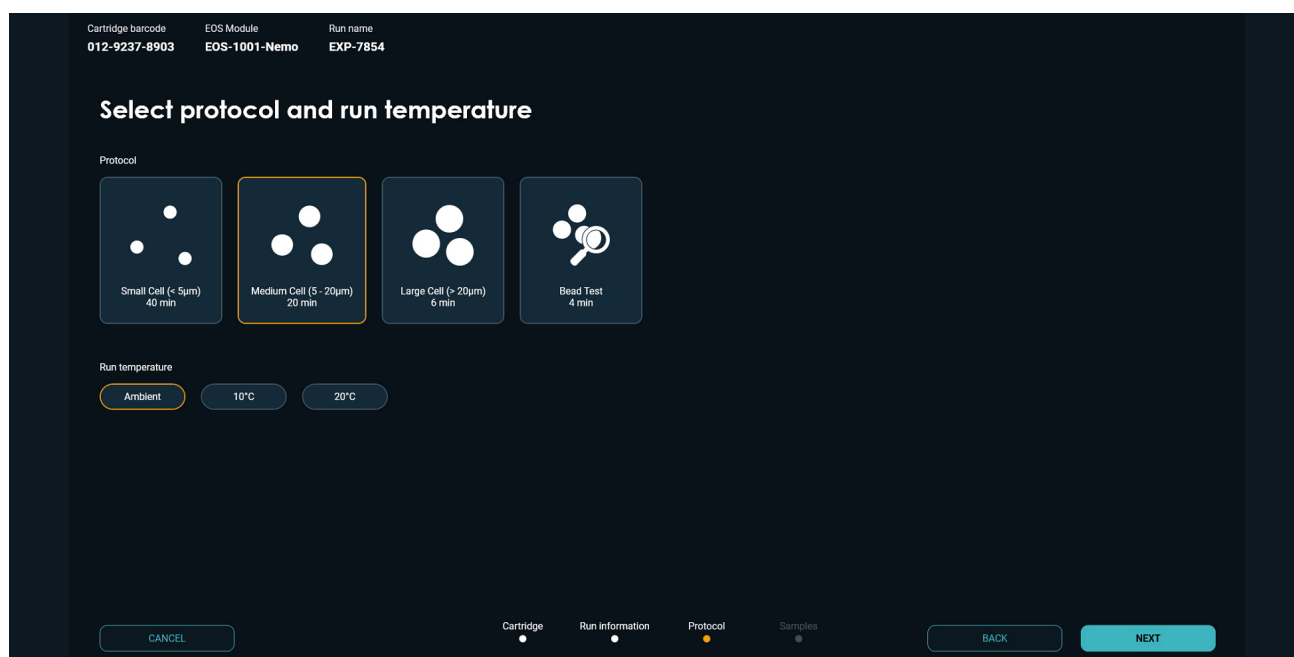


Figure 75. Select protocol and temperature

The time required to levitate cells to their equilibrium position depends on their size, and the recommended levitation time is set in each of the three default enrichment workflows. Once an enrichment protocol has been selected, the EOS Manager software will guide the user through the process of setting up the experiment and running samples.

13. Select and specify samples to run.

Cartridge barcode
012-9237-8903

EOS Module
EOS-1001-Nemo

Run name
EXP-7854

Protocol
Medium Cell (5 - 20µm)

Run temperature
Ambient

Levitation
20 min

Select and specify samples to run

Click sample well below to include/exclude from run

1

2

3

4

Sample name

LA (mM)

Green fluorescence

Red fluorescence

1

Sample 1

125

Calcein AM

Propidium Iodide (PI)

2

None

None

3

Sample 3

125

Calcein AM

Propidium Iodide (PI)

4

Sample 4

125

Calcein AM

Propidium Iodide (PI)

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

CANCEL

Cartridge

Run information

Protocol

Samples

BACK

NEXT

Figure 76. Select and specify sample information

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



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

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

☒

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

Figure 77. Checkbox for same Levitation Agent and stains



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

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

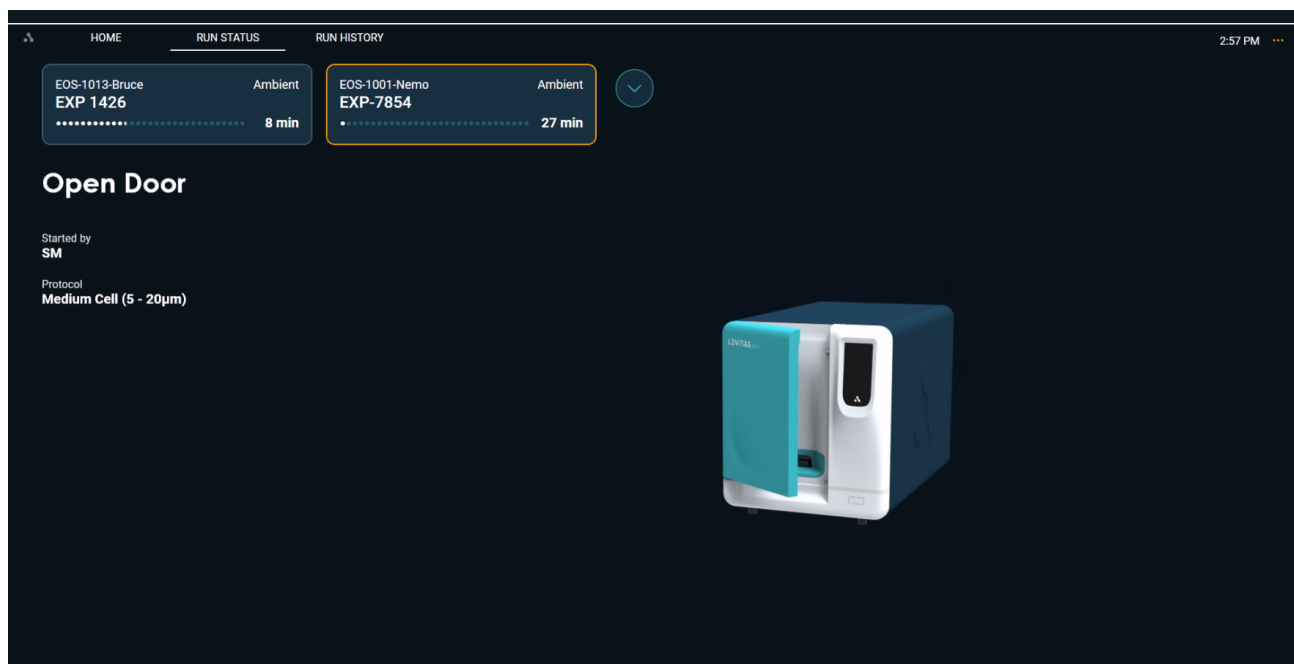


Figure 78. Open door to insert cartridge

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



CAUTION: Potential Pinch Hazard.



Figure 79. Insert cartridge into EOS Module

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

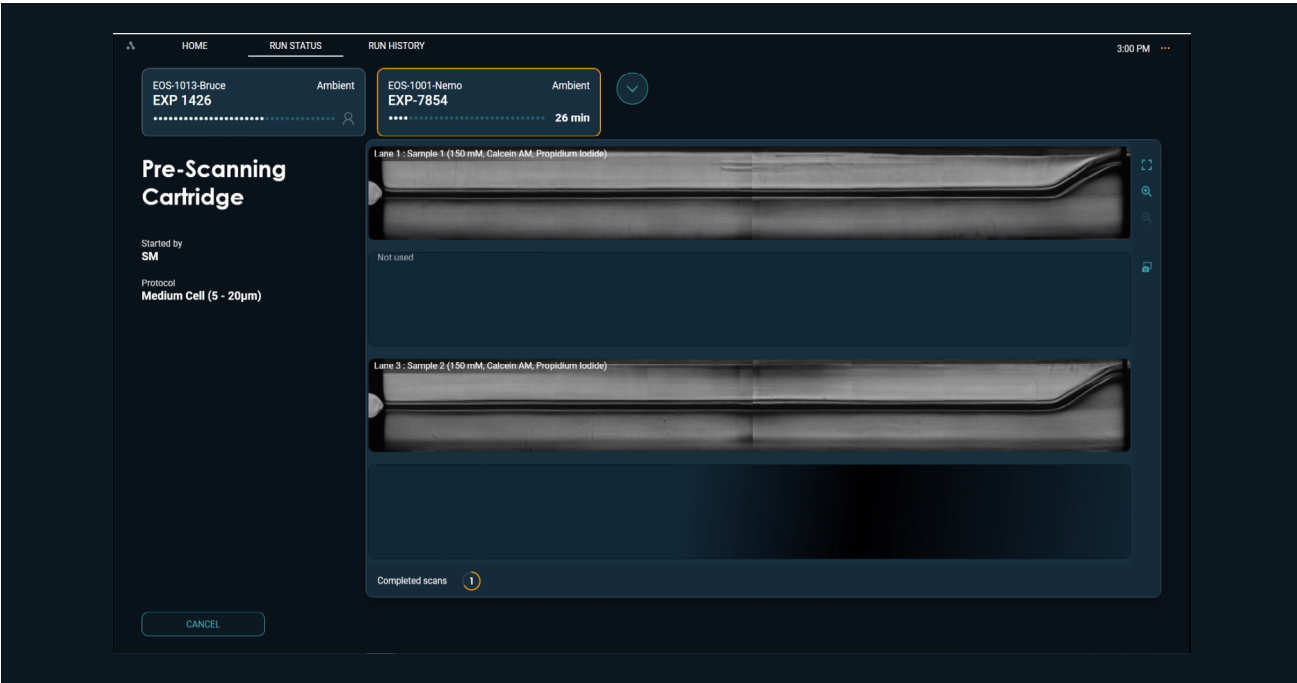


Figure 80. Prescanning the cartridge

17. Follow subsequent prompts to then dispense samples when prompted



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

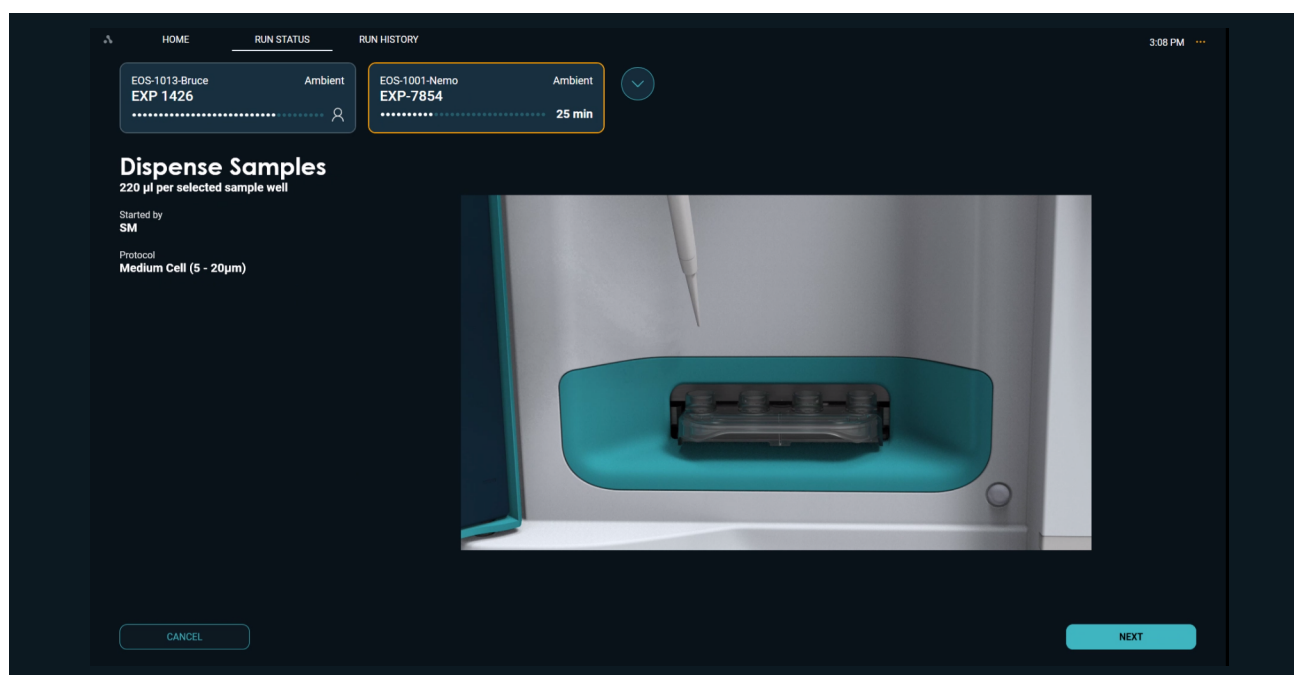


Figure 81. Dispensing sample into the input wells

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

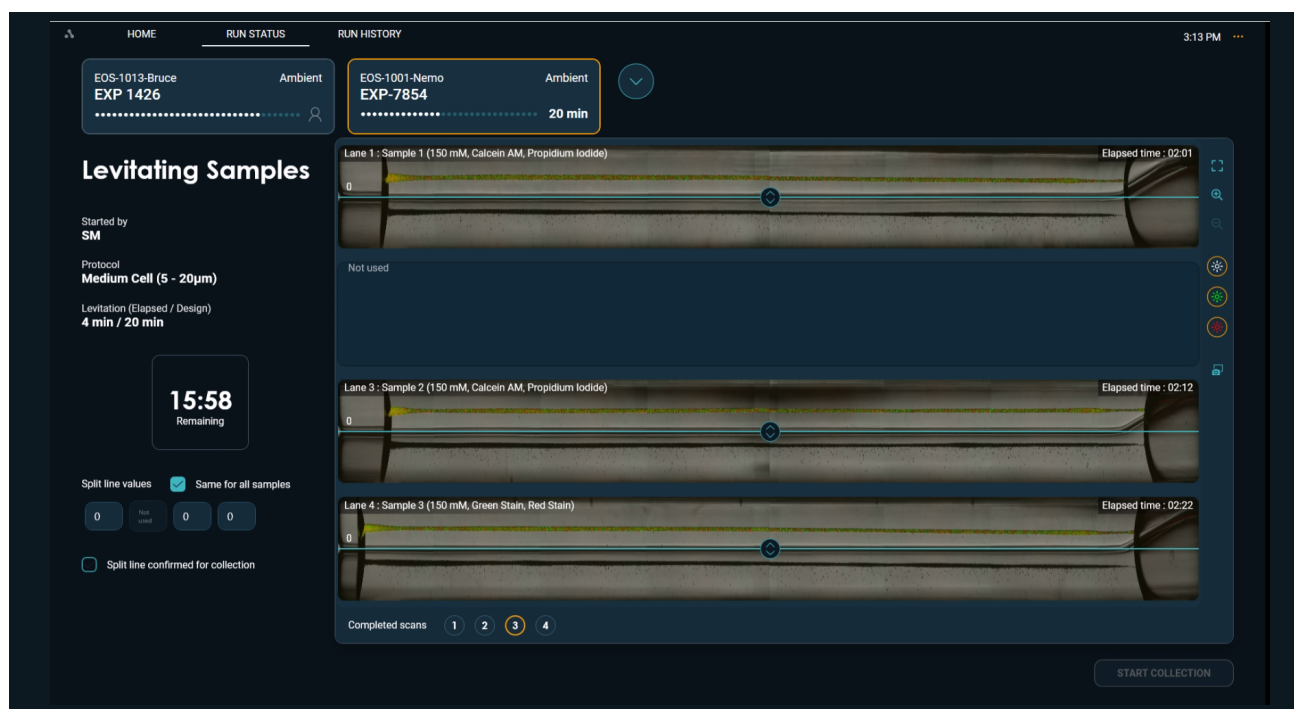


Figure 82. Levitating samples

19. Set Split and Start Collection: When the levitation timer indicates “Ready” the split value must be set.



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



Figure 83. Set split line when levitation has completed and start collection

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

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

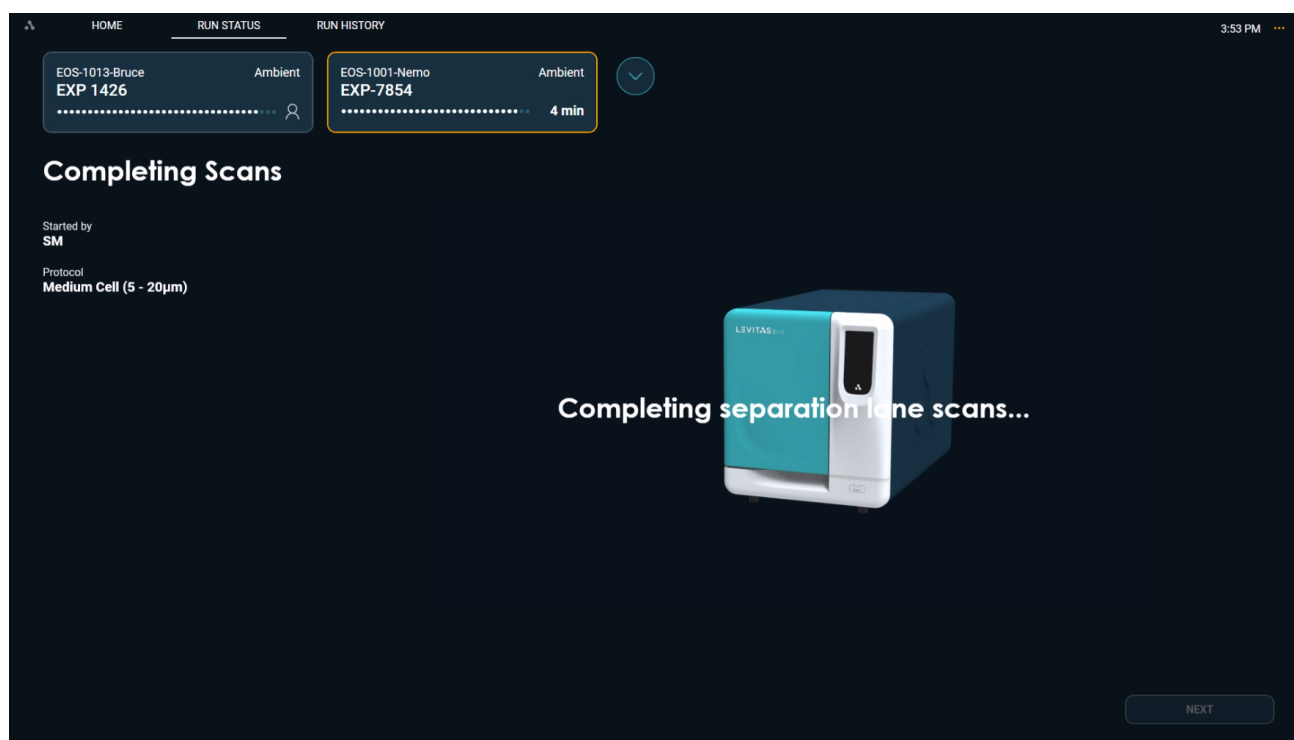


Figure 84. Completing scan of cartridge



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

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

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

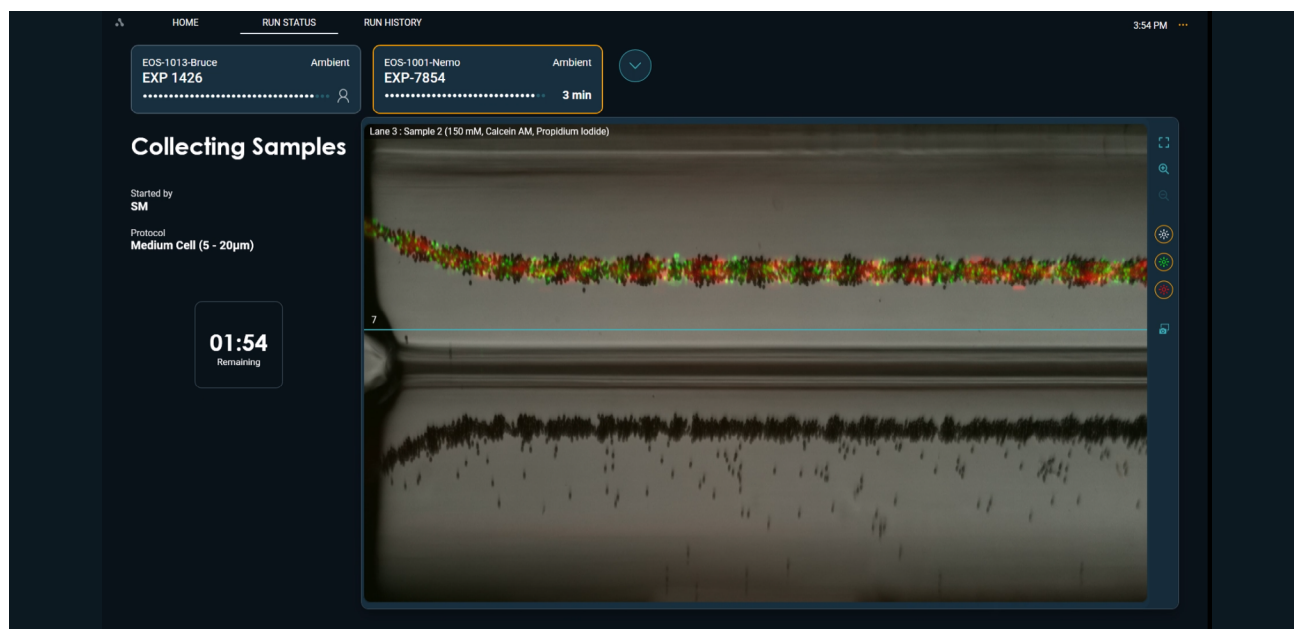


Figure 85. Imaging collection of Lane 1 as it flows to the outlet well

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

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



Figure 86. Retrieve cartridge from EOS Module

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

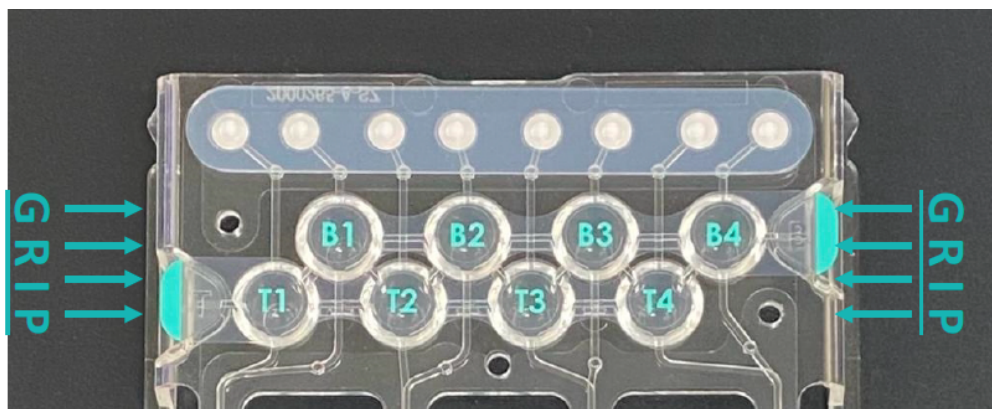


Figure 87. Grip locations on the cartridge when peeling outlet well tape off

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

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

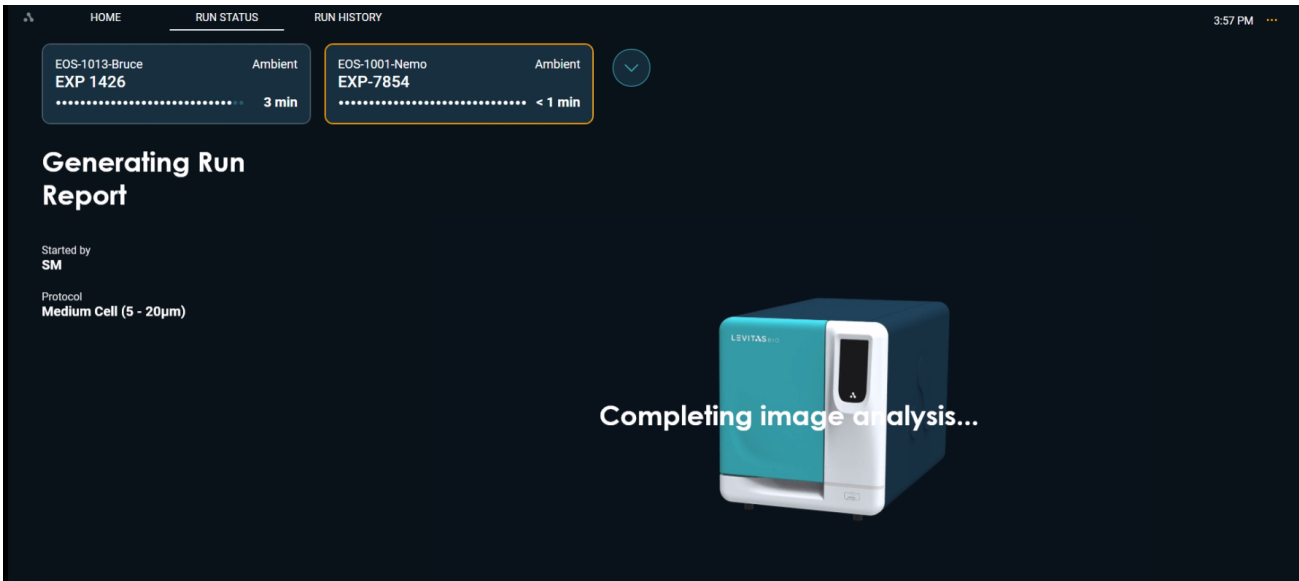


Figure 88. Generating Run Report after image analysis is completed

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

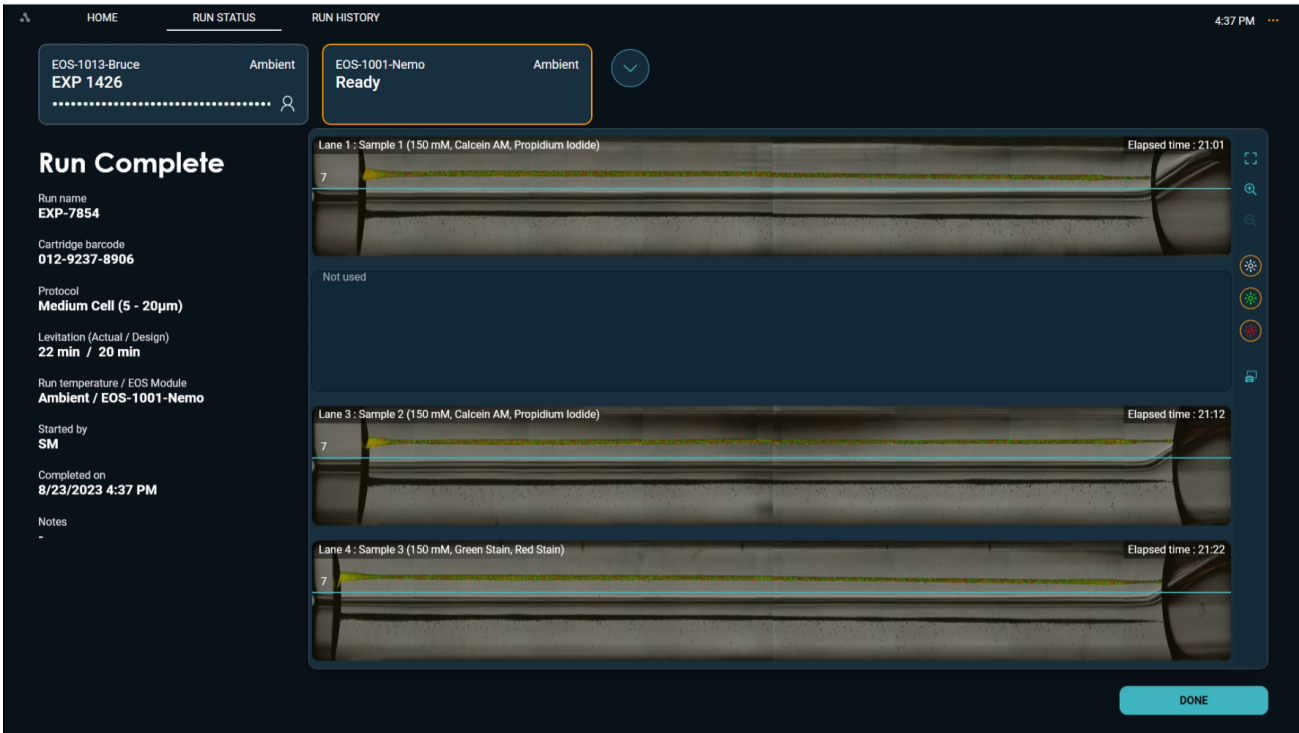


Figure 89. Run Complete screen

D. Count Cells

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



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

e.g. If loading 250,000 cells, sample can be diluted 4X and still be within the linear range of the cell counter. Ensure that calculation for dilution is based on the number of Live or Dead cells being counted for the outlet wells and that dilution is well within the linear counting range. If using the Nexcelom K2, “Low Conc.” can be selected for an over diluted sample.)



NOTE: Ensure that your sample is diluted to the working concentration range of your counter.

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

Run Summary and Data Files

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.

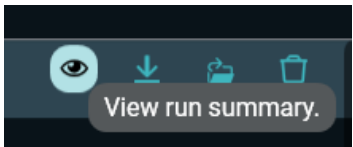


Figure 90. hover over and run summary

LEVITAS BIO

EXP-1897

Run Summary

Run name
EXP-1897

Cartridge barcode
012-3487-9235

Protocol
Bead Test

Levitation (Actual / Design)
34 min / 4 min

Run temperature / EOS Module
Ambient / EOS-1001-Nemo

Started by
SM

Completed on
8/23/2023 12:30 PM

Notes
-



Figure 91. 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.

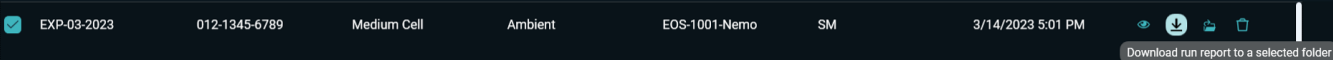


Figure 92. Run Summary download

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.

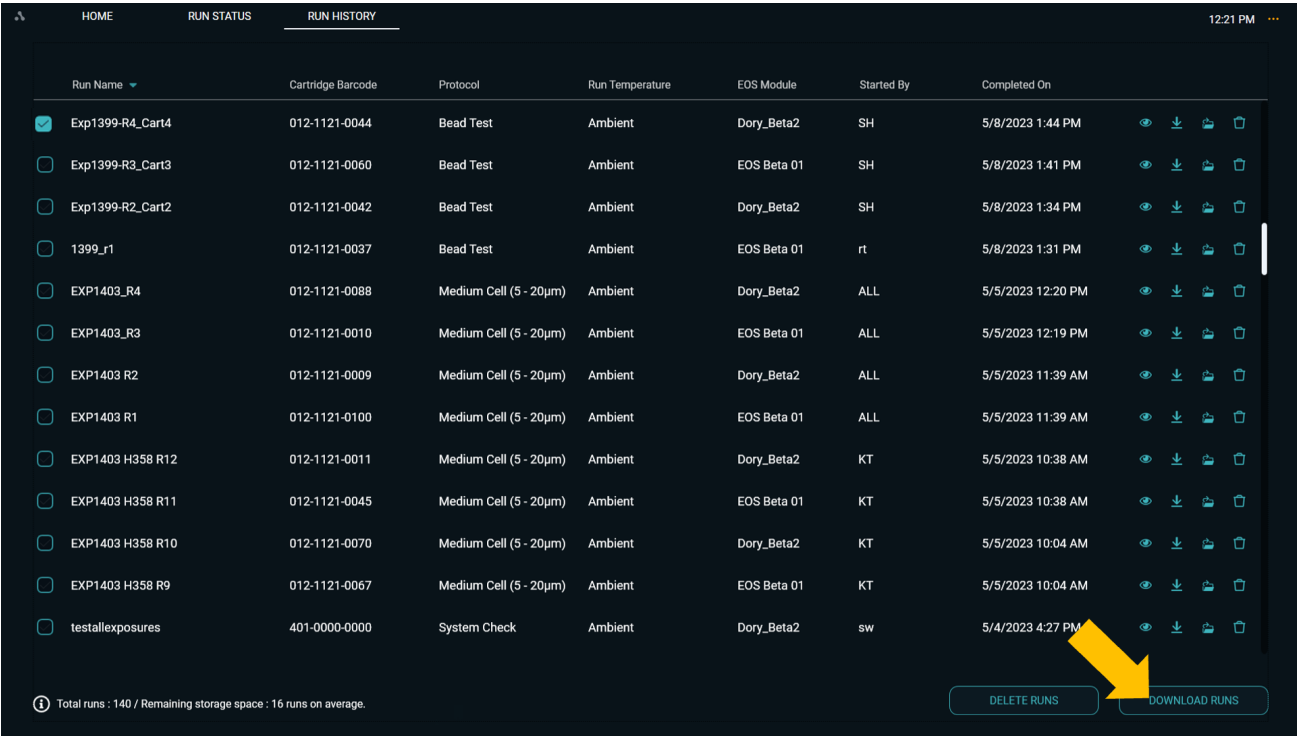


Figure 93. Download one or several files at a time

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

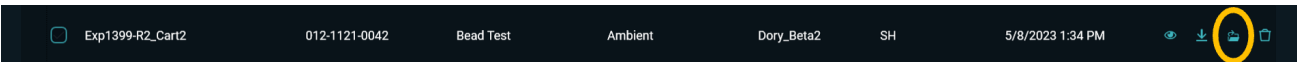


Figure 94. Button to access files via file explorer

The file structure for each run is shown:

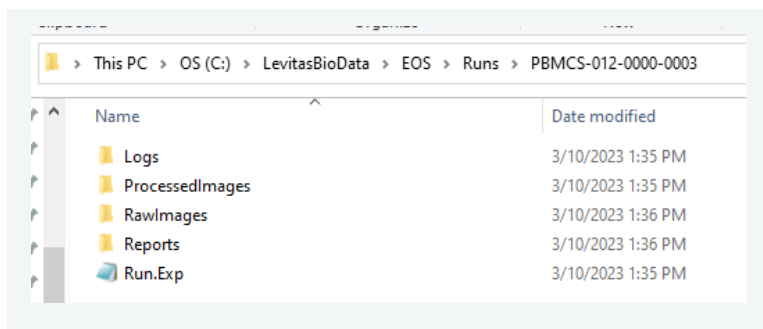


Figure 95. File Structure

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

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

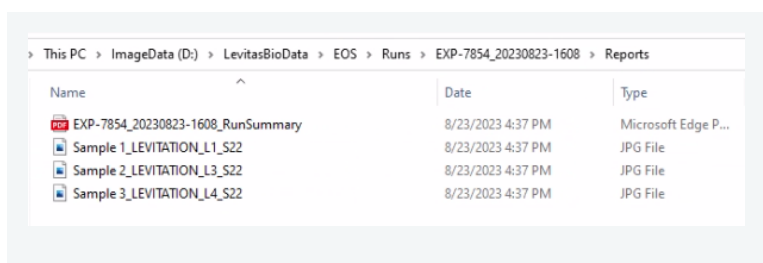


Figure 96. Reports folder

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

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

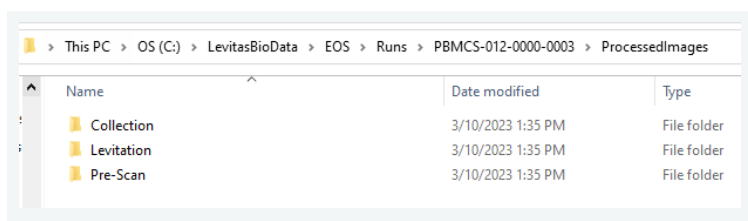


Figure 97. Processed image folders

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 98. Example filename format

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

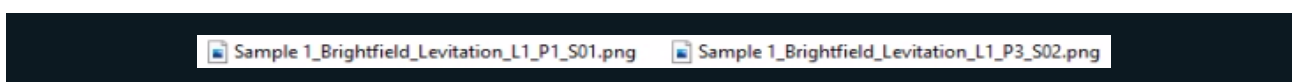


Figure 99. Image raw files

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

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

Introduction to LeviMetrics Software

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



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

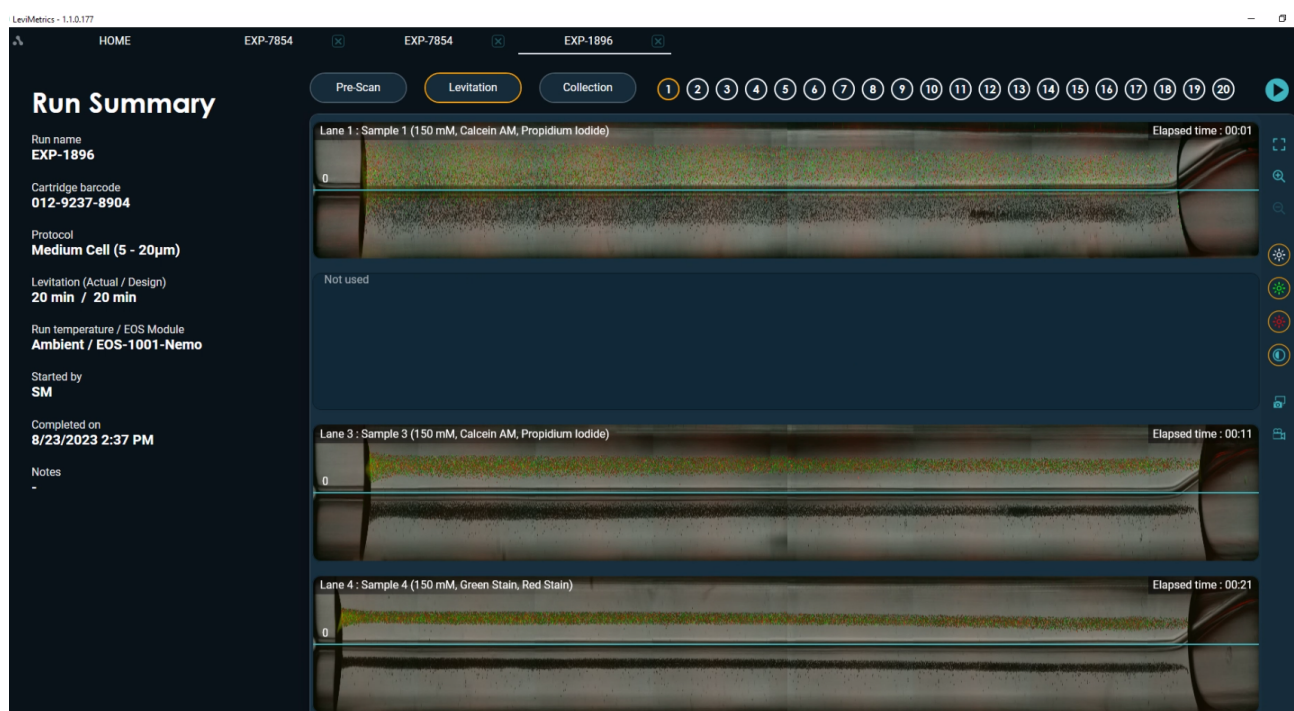


Figure 100. LeviMetrics Software experiment screen

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

UPDATING SOFTWARE

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

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



Figure 101. Software update progress and complete screen

SHUTTING DOWN THE LEVICELL EOS

The LeviCell EOS System can be powered down in two ways

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

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

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

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

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



Figure 102. Power button on the front of instrument



Figure 103. Mains power switch

CLEANING THE LEVICELL EOS

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

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



CAUTION: Use standard precautions for any cleaning agents.

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

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

TROUBLESHOOTING

Issue	Possible Resolution
Barcode is not reading	<ul style="list-style-type: none"> Confirm the barcode was entered correctly compared to the label. Ensure the EOS module is connected (under Manage EOS Modules). Ensure the EOS core supports the cartridge type you are using.
Stain tool not visible in run summary	No fluorescence was captured in the run.
Bead Test cartridge not working	Check if the transport cartridge is being used. The transport cartridge is used only for dry tests. Retry with a new cartridge.
Cannot move split line	Check if the confirmed split line box is checked. If so, uncheck and try again.
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 μ L 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.
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.
Top or bottom bands have strange fluctuation during the collection step	This is normal, and can be due to pressure fluctuations as the sample is collected.
Three bands of beads are visible during the beads test	Centrifuging bead mixtures may result in doublet formation between beads of different densities, which will levitate between singlets.
Fibers seen in the separation channel	<ul style="list-style-type: none"> 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.

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Contact Technical Support at support@levitasbio.com if any errors are encountered during the procedure.