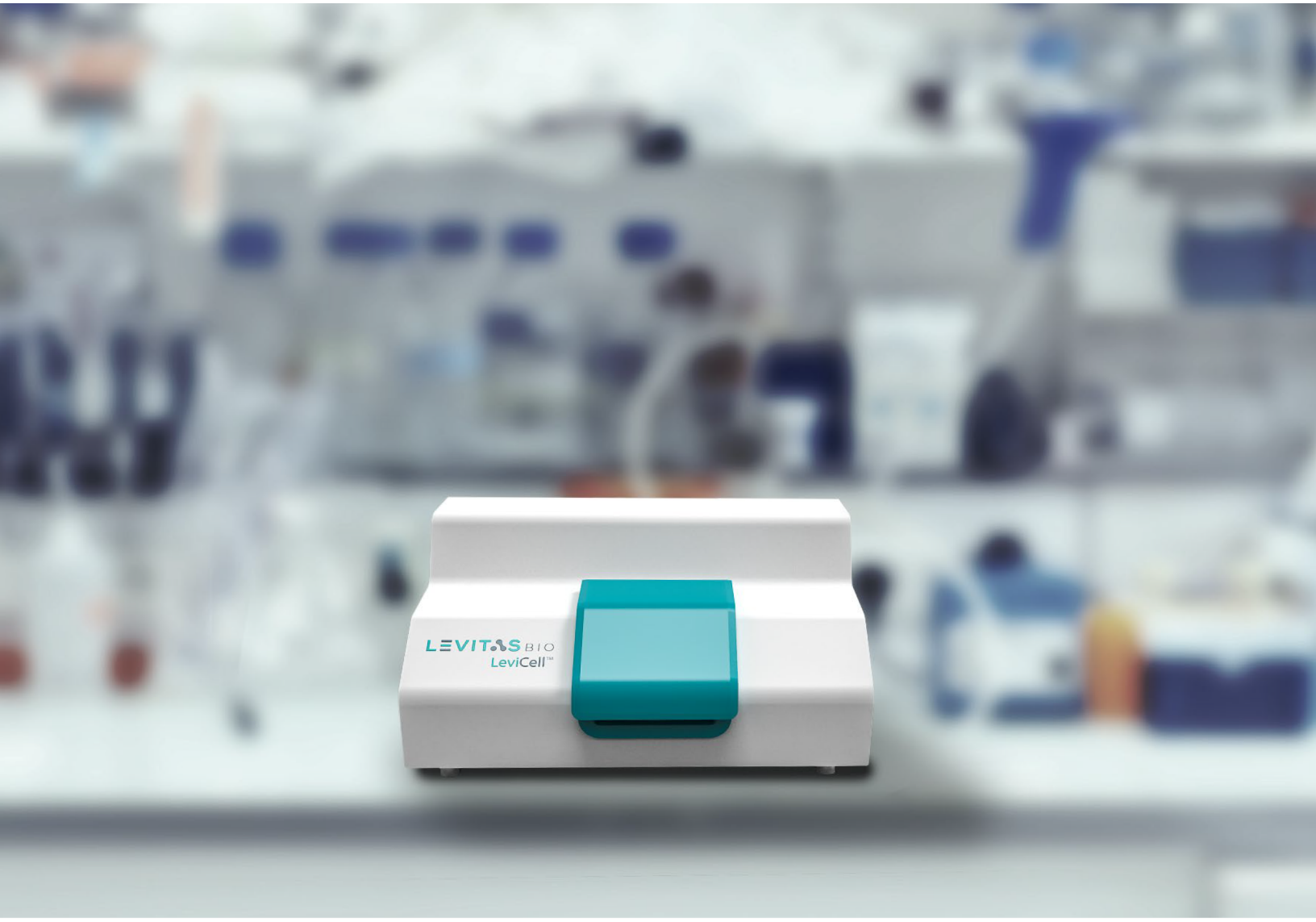
LEVITAS BIO®



LEVICELL 1.0 INSTRUMENT USER GUIDE

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SYMBOLS USED IN THIS GUIDE AND ON THE LEVICELL® 1.0 INSTRUMENT

Attention symbol. The associated message contains safety-related information.

Operating temperature range

Operating humidity range

For information, contact

Catalog number reference

Serial number

Date of manufacture

Manufacturer of record (LevitasBio)

SAFETY AND WARNINGS

ATTENTION: use the mains supply cords provided with the LeviCell system. Do not substitute a mains supply cord with inadequate rating (< 10 A current).

Use the LeviCell 1.0 system only as directed by LevitasBio. Use in a manner not specified by LevitasBio, especially including removal of any cover or portion of the enclosure may create a risk of hazards.

The LeviCell 1.0 instrument contains no user-serviceable parts. Do not remove any portion of the enclosure. The instrument does not require any maintenance beyond the cleaning described in the section “Cleaning the LeviCell 1.0” below.

In the event of any fault, turn the instrument off using the rocker switch on the upper left of the rear panel, and disconnect the AC power plug from its outlet. Contact LevitasBio Technical Support at +1-650-204-1185 or **support@levitasbio.com**.

Do not obstruct access to the 24 V power supply plug leading into the instrument. It should be easily removable in an emergency.

To shut down the instrument in an emergency, disconnect the AC power plug leading to the 24V supply brick. Contact LevitasBio Technical Support +1-650-204-1185 or **support@levitasbio.com**.

BIOSAFETY: when biohazardous samples are used in the LeviCell or are 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.

In addition to contamination control features within the LeviCell 1.0 instrument, a primary safety feature to prevent contamination is that the cartridges are single-use only. Do not attempt to reuse cartridges, as this may increase the risk of cross-contamination and exposure to any biohazard associated with the sample. 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 your approved lab guidelines.

For indoor use only. The LeviCell 1.0 is not designed for outdoor use and is not rated for resistance to precipitation. See the **Specifications** section for details.

SYSTEM COMPONENTS

The LeviCell system is comprised of:

- Main instrument unit
- Control PC, mointor, keyboard, and mouse
- Single-use cartridges (LevitasBio® PNs 1002010 and 1002012)
- LeviCell Installation and Calibration Kit (LevitasBio® PN 1003003)
- Experiment Manager software
- Experiment Analyzer software

THE LEVICELL 1.0 INSTRUMENT

Magnetic levitation technology is a powerful new method of cell separation and analysis. Unlike other methods, magnetic levitation does not require dyes, antibodies or specific markers, and the cells are not modified or perturbed in any fashion. These two benefits lead to results that are more analogous to in vivo measurement.

The LeviCell instrument is the first commercial product to use magnetic levitation, enabling the separation of live cells, with high resolution, at the single cell level. At its core, LeviCell is a cell densitometry platform, composed of two permanent magnets with a channel between them. The levitation height of cells inside the channel is monitored with a microscope. Cells are resuspended in a buffer that includes the non-ionic, paramagnetic medium, before flowing into the channel, and levitating to an equilibrium position. At equilibrium, the magnetic force upward equals the gravitational force downward.

The levitation equilibrium height of cells is monitored in real time and determines how the sample will be separated at collection. Cells reach an equilibrium position which is largely determined by their density and is independent of cell volume. Live cells will maintain their equilibrium within the chamber, while dying cells sink toward the bottom (see example in Figure 2).

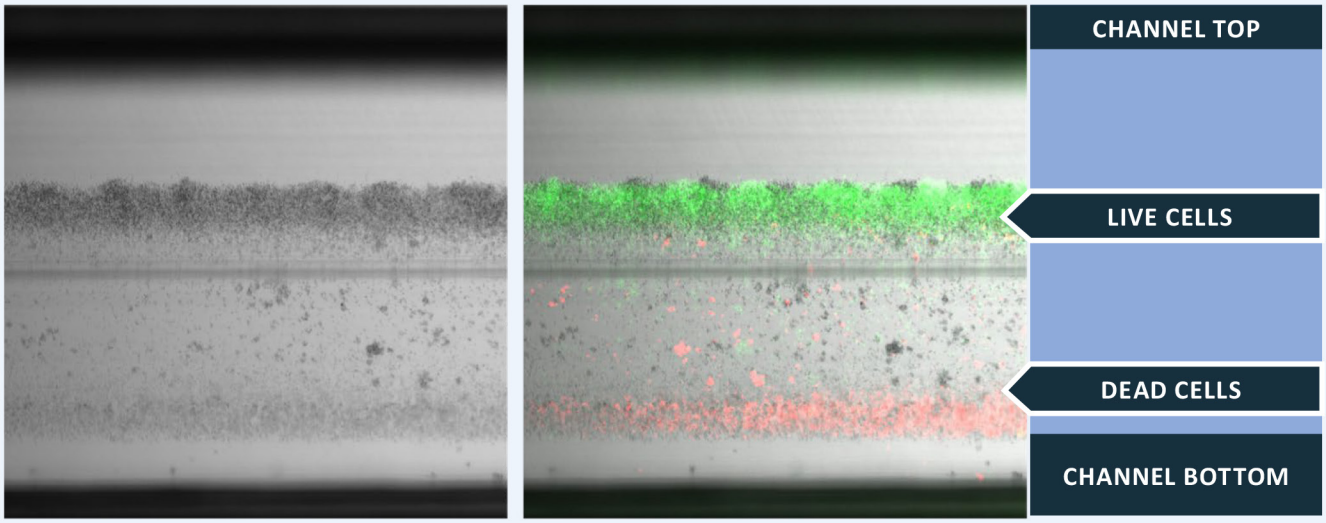


Figure 2.
Live vs. Dead PBMC separation brightfield image (left panel) and color overlay using calcein and PI stains to visualize the location of the live and dead populations (right panel)

MATERIALS

LevitasBio® Equipment and Consumables

- LeviCell Instrument
- Levitation Agent (LevitasBio, PN LR-10)
- LeviCell single-use cartridges
  - ➤ Non-sterilized cartridges (LevitasBio, PN 1002010)
  - ➤ Sterilized cartridges (LevitasBio, PN 1002012)
- LeviCell Installation and Calibration Kit LevitasBio, PN 1003001

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 Bind microcentrifuge tubes (1.5 mL, 2 mL, and 5 mL)
- 15 mL and 50 mL Falcon tubes
- Serological pipettes: 2 mL, 5 mL, 10 mL, 25 mL
- 0.22 µm syringe filters, to filter cell media/buffers
- 5.0 mL or larger disposable syringes, to filter cell media/buffers

User-Supplied Reagents

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

Optional Equipment

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

LeviCell Reagent Kit Storage and Stability

REAGENT	SHIPPING TEMP (< 1 WEEK)	STORAGE TEMP (UP TO EXPIRY ON TUBE)
Levitation Agent (PN 1003001)	15-30°C	15-30°C
LeviCell Installation and Calibration Kit (PN 1003003)	15-30°C	2-8°C

Table 1.
Storage conditions for LeviCell reagents.

Expiry dates for reagents are listed on each tube.

LeviCell 1.0 SYSTEM OVERVIEW

The LeviCell system consists of a single-use cartridge, Levitation Agent, and an instrument which performs the magnetic levitation to separate the sample into two fractions. See Figures 3 and 4.

Figure 3.
The LeviCell 1.0 instrument with the cartridge access door and clamp open.

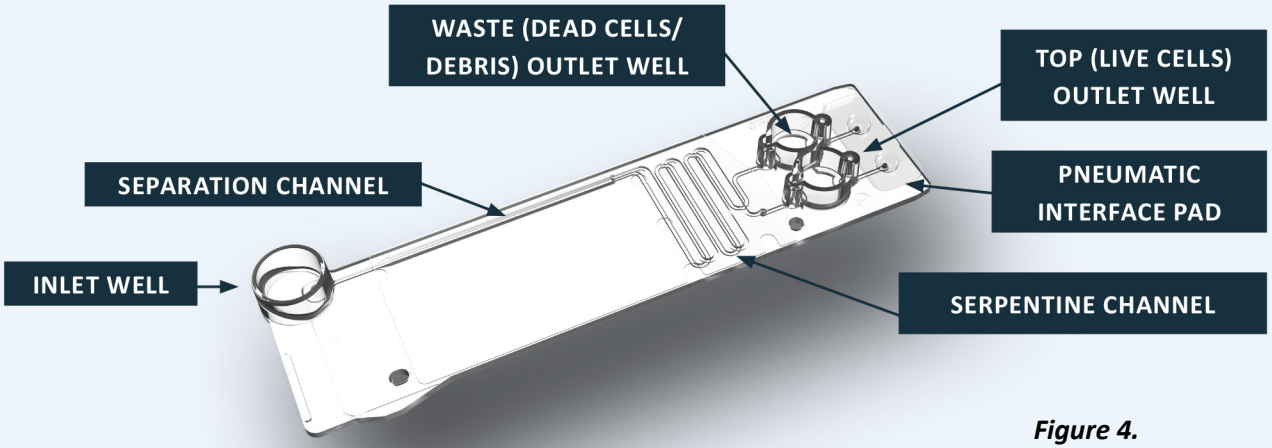


Figure 4.
Details of the LeviCell cartridge.

The LeviCell instrument's main enclosure houses the imaging system and flow controller. The single-use cartridge is inserted between custom-designed rare earth magnets inside the instrument. These magnets induce magnetic force on the levitation agent that causes cells with different properties to rise or sink within the levitation channel until equiliubrium is reached. The levitation process is fully contained within the cartridge consumable. Samples are loaded into the input well, and collected from the output wells of the cartridge.

CAUTION: The LeviCell cartridge is intended for a single use only. Do not reuse cartridges, as this increases the chance of sample cross-contamination and exposure to biosafety hazards associated with the sample.

NOTE: The LeviCell instrument is shipped with a blank testing cartridge (Figure 5) loaded into the cartridge that protects the pneumatic interface. Whenever the instrument is not in use, this blank cartridge should be secured in the instrument with the front cover closed.

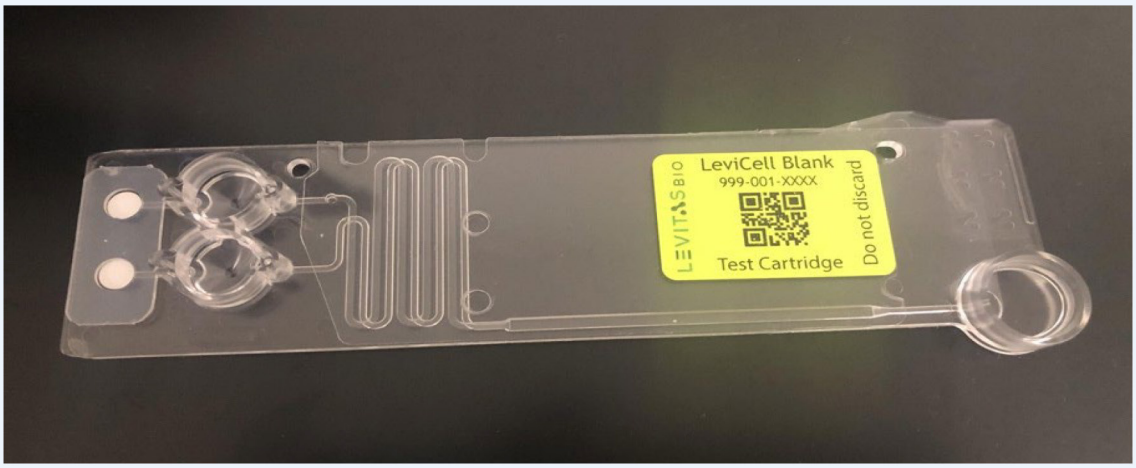
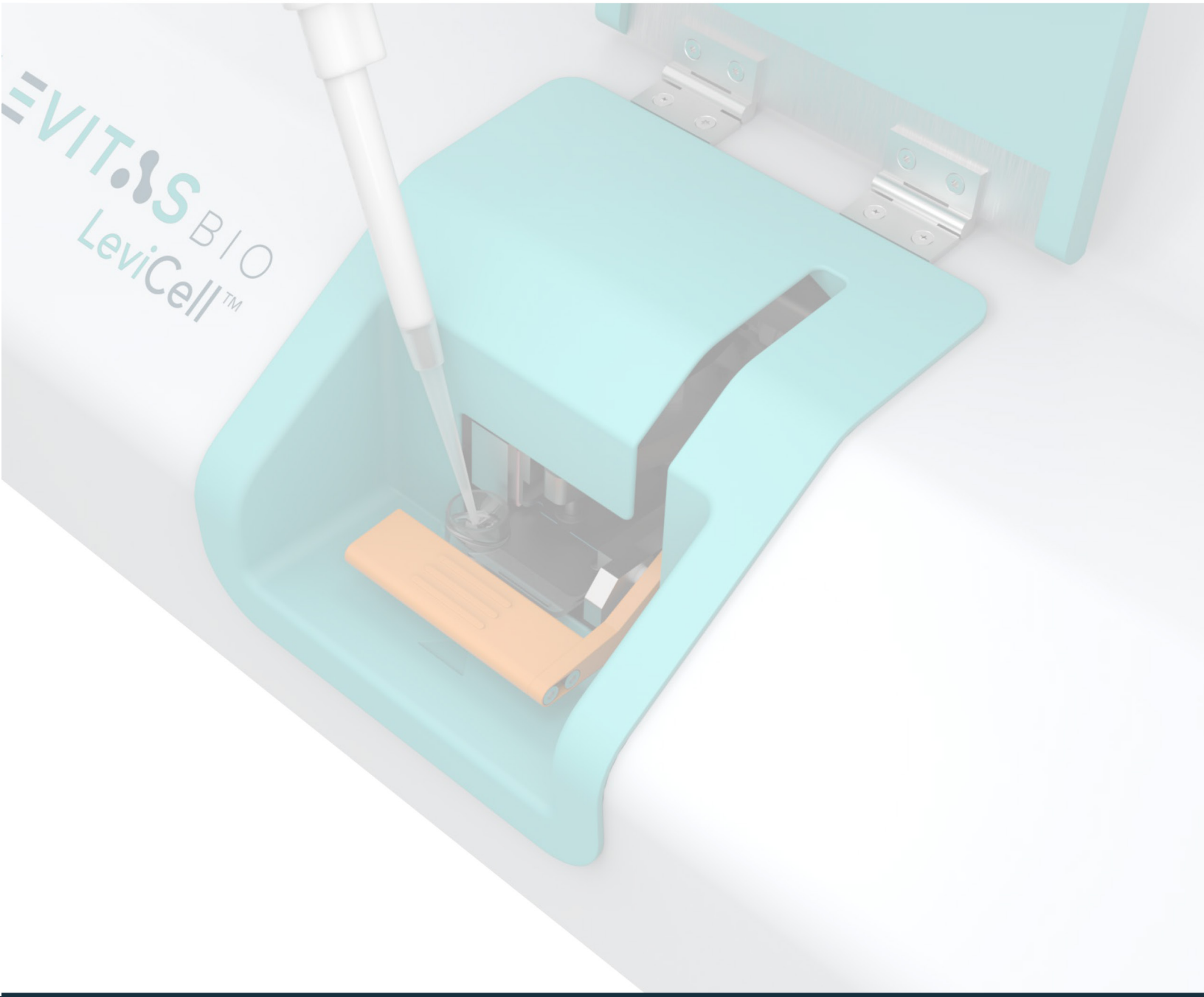


Figure 5.
The blank test cartridge accessory supplied with the LeviCell instrument

The LeviCell system also includes an all-in-one computer for control and analysis, a power supply, and peripherals. Instrument control is performed by the LevitasBio Experiment Manager software applications. Images of the separation channel are continuously collected during the cell separation process and are available for further processing using the Experiment Analyzer software application.

SYSTEM INSTALLATION

Refer to 90-00207 LeviCell Installation Guide for detailed installation instructions.



GET STARTED

Start the Experiment Manager software by clicking on the Task Bar icon or double-clicking on the desktop icon (shown at left).

NOTE: the application takes several seconds to start up while establishing connections to instrument hardware components.

You will see the activity selection screen for the Experiment Manager application, as shown in Figure 6. From here, Experiment Manager will guide you through each protocol, step by step.

- Select either Small, Medium or Large Cell Enrichment, or Nuclei Enrichment, to launch the main experiment protocol.
- Select System Tools, to guide you through a set of system checks, for testing the instrument after shipment, or for troubleshooting.
- Select Exit to close the User Interface. To close the Experiment Manager software click the “X” in the upper right hand corner.

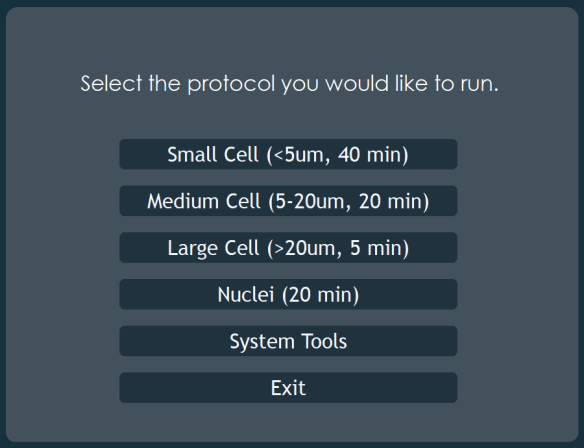


Figure 6.
Activity selection screen to initiate protocols or tasks on the LeviCell

If you Exit from the activity selection screen, the Experiment Manager main interface window will be visible.

Experiment Manager application window

- Close the application by clicking on the “X” at the upper right.
- Launch the activity selection screen by clicking Figure 7.

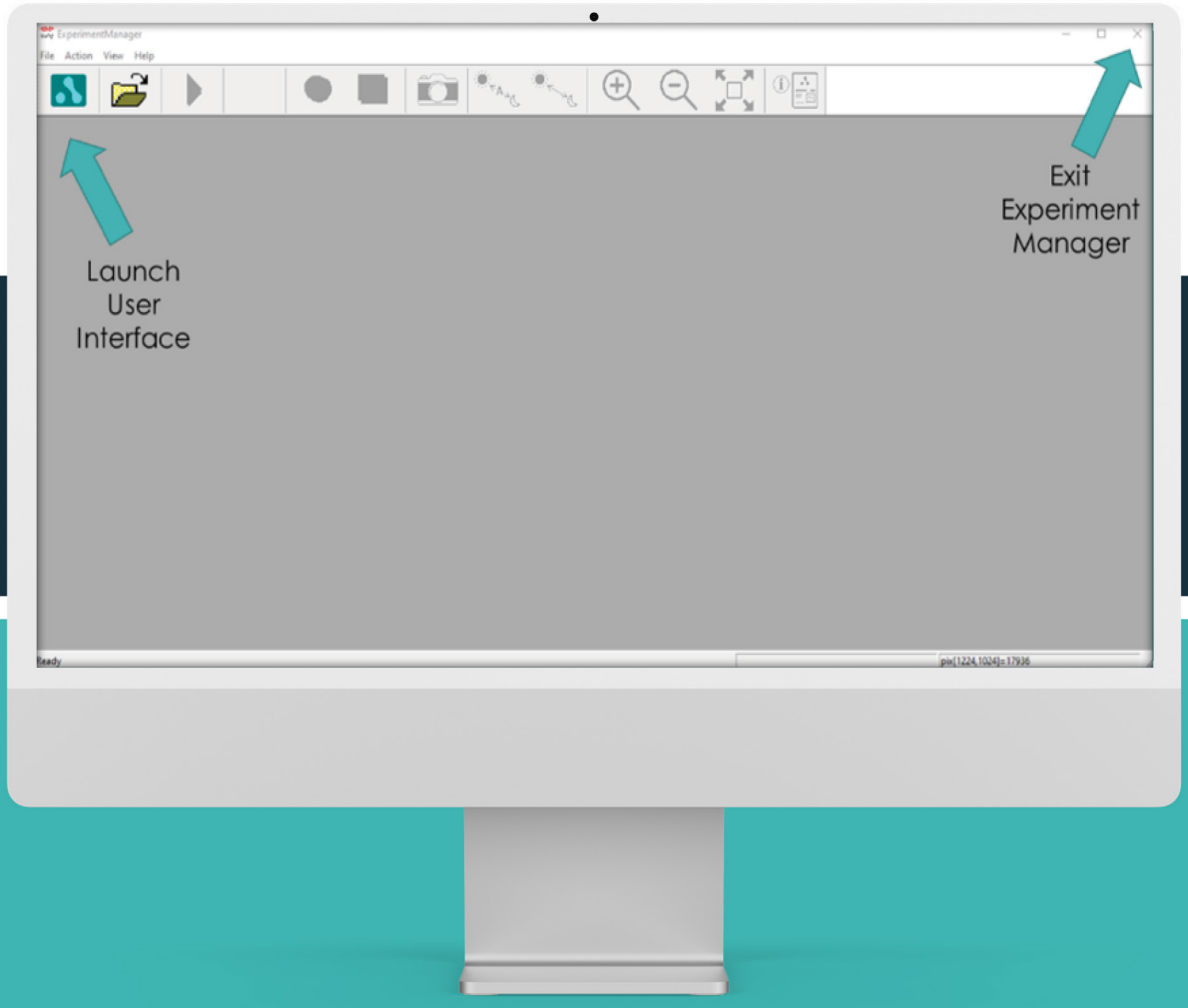


Figure 7.
Experiment Manager application window, highlighting some key controls.

OPERATIONAL QUALIFICATION

To verify the LeviCell instrument has not been damaged during shipping and has been set up properly prior to normal use, click on “System Tools” as shown in Figure 6 and then select “System Check” in the Tools submenu.

Follow on-screen directions to walk through the complete test which includes:

1. Sensor Check (cartridge and clamp)
2. LED Check
3. Pump Initialization and Reset
4. Core Alignment (magnet and cartridge position)
5. Barcode Reader Functionality
6. Image Capture

Contact Technical Support with any failures. Consult D:\TestLogs for a summary of the test results.

A checklist is provided in **Appendix 1 - Operational Qualification Checklist** for tracking and recording the results.

PERFORMANCE QUALIFICATION

To demonstrate the performance of the instrument and its components using a non-biological sample, and Calibration Kit is available (LevitasBio, PN 1003001). The 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 Install Test workflow on the instrument under System Tools
5. (Optional) Count the input and output samples

A checklist is provided in **Appendix 2 – Performance Qualification Checklist** for tracking and recording the results.

The install beads are provided in two formulations that are mixed at the start of the test. LeviCell Green PS Bead is a green fluorescent (Ex460/Em500) polystyrene bead with density 1.05 g/cc. LeviCell Red PMMA Bead is a red fluorescent (Ex545/Em566) polymethyl methacrylate bead with density 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. Note that only instruments with fluorescent imaging capability can detect this signal, but all instruments will show the separation of a mixture of beads, to two bands based on density. The beads from each of the mixes are approximately 20 µm in diameter.

The Bead Mixture is separated into two bead populations according to their density during the LeviCell workflow, simulating 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.

Example images of beads are shown in Figure 8 at the beginning of the workflow (top panel) and after levitation (bottom panel).

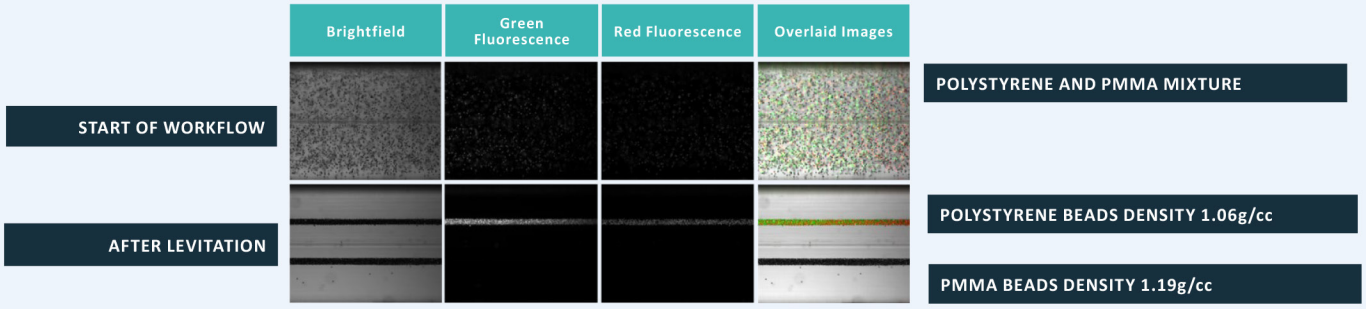


Figure 8.
Example images of bead mixtures taken during Performance Qualification.

Step-by-step Performance Qualification Protocol Instructions

1. Prepare Levitation Buffer.

    a. Use Table 1 Levitation Buffer Prep to prepare Levitation Buffer for 1 test sample in a 2 mL low bind tube. The Installation and Calibration Kit includes enough reagent volume for a maximum of 2 test samples.

REAGENT	INITIAL	FINAL	1 SAMPLE
LeviCell Install Buffer	1X	0.875X	262 µL
Levitation Agent	1M	125 mM	38 µL
Total			300 µL

Table 1.
Levitation Buffer Prep

    b. The Levitation Agent is viscous and requires thorough mixing to incorporate into final solution. Vortex mixture well for ~10 seconds.

2. Prepare the Bead Mixture.

    a. Use Table 2 numbering to prepare the bead mixture in a new 2 mL low bind tube.

IMPORTANT: Vortex each bead mix tube for ~5 seconds before combining. Beads settle quickly, so pipet the volume needed immediately after vortexing to maintain expected concentrations.

REAGENT	1 SAMPLE
LeviCell Green PS Bead	15 µL
LeviCell Red PMMA Bead	30 µL
Total	45 µL

Table 2.
Bead Mixture

b. Vortex bead mixture well for about ~5 seconds.

3. Prepare the input sample

   a. Pellet the beads at 300 RCF for 3 minutes.
   b. Carefully remove the entire supernatant, taking care not to remove beads from the pellet.
   c. Resuspend bead pellet in 240 µL Levitation Buffer. Vortex for 2 – 3 seconds. This is the Performance Qualification test sample.
   d. (Optional) Transfer 10 µL of the test sample to a hemocytometer for counting (this will provide the input bead counts.

4. Run the separation workflow by following the software guidelines in the Experiment Manager software.

   a. Select the Install Test protocol under System Tools (see figure 6).
   b. Step through the Bead Test workflow entering the parameters in Table 3 below when prompted by the software.

WORKFLOW STEP	PARAMETER	VALUE	NOTE
Levitation Timer	Levitation Time (preset)	3 minutes	
Levitation Timer	Brightfield Exposure	100-200 µs	Click the radio buttons on the right of the screen to activate. Use the pull down buttons to adjust the exposure time.
Levitation Timer	Ex474/Em524 Exposure	1000-2000 µs	If available.
Levitation Timer	Ex560/Em628 Exposure	5000-10000 µs	If available.
Separation Flow	Split	0	Option: you can enter a value as the split value in Experiment. Settings to have flow start automatically after levitation time is complete, if available.

Table 3.
Workflow Parameters

5. When prompted to load the bead test sample into the LeviCell cartridge, pipet mix thoroughly. Immediately load 220 µL into the input well of the LeviCell cartridge.
6. Start Run.

Example images of stages observed during workflow are shown in figure 9.

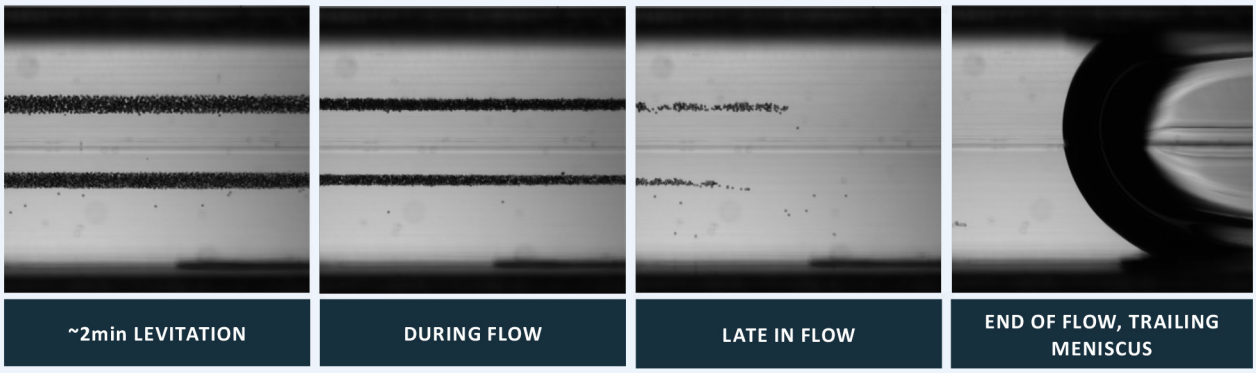


Figure 9.
Example images during install bead separation workflow.

7. Collect cartridge outputs and count the beads (optional).

    a. Gently peel off the output lid and transfer the output volumes from the Top and Bottom output wells from the cartridge into two, separate, labeled, low-bind 2 mL tubes. Beads settle very quickly so the volumes in the output wells should be gently pipette mixed thoroughly before volume transfer in order to maximize yield.

b. To measure the bead counts from each of the output wells, first vortex the sample in the 2 mL tube for ~5 seconds to resuspend the beads uniformly in the solution.
c. Immediately transfer 10 µL to a hemocytometer for counting.
d. A checklist is provided in **Appendix 2 – Performance Qualification Checklist** for tracking and recording the results.

Table 4 below describes the fluorescent channel (if available) where each bead type is detected, as well as the expected output well it can be found after collection.

BEAD TYPE	BRIGHTFIELD	GREEN FLUORESCENCE CHANNEL	EXPECTED COLLECTION OUTPUT	RED FLUORESCENCE
Green Polystyrene	Yes	Very bright	Top	Not Detected
Red PMMA	Yes	Not detected	Bottom	Very Bright

Table 4.
Bead appearance in fluorescence channels.

GENERAL GUIDELINES FOR CELL SAMPLE PREPARATION

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

Example Protocol for Cell Lines from Frozen Stock *

Prior to starting, warm a water bath to 37°C, and prepare 25 mL of filter sterilized complete media for thawing and to allow for all wash steps.

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

1. Thaw cell vial in a 37°C water bath for 1-2 min with mild agitation until only a sliver of ice crystal is left.
2. Pipette the thawed sample up and down with a 1 mL pipette 3X to mix the cells and place cells in a 15 or 50mL conical tube.
3. Add 1 mL of complete media to rinse the thawed vial of cells and transfer to the same conical tube as the sample.
4. Add 9 mL of complete media to the thawed sample in the conical tube and pipette mix 2-3X.
5. Centrifuge the conical tube containing the sample at 300 RCF for 5 minutes.

For cell samples requiring unusual handling, please contact Technical Support by email (support@levitasbio.com**) or telephone (+1-650-204-1185).*

6. Remove supernatant.
7. Add 10 mL of complete media to the pellet and pipette mix.
8. LevitasBio recommends counting live and dead cells using AO/PI on a Nexcelom cell counter. After cell counting, transfer the appropriate volume of cells (20K to 1M cells) desired for the LeviCell cartridge run to a fresh tube.
9. Centrifuge the tube containing the sample at 300 RCF for 5 minutes.
10. Remove supernatant.
11. Resuspend the cell pellet in a final volume of 270 µL of Levitation Buffer at desired Levitation Agent concentration (150mM is recommended). 220 µL is required for loading the LeviCell, and the remaining volume can be used for replicate cell counts or other QC methods, as desired.

General Guidelines for Live/Dead Cell Staining (Optional)

- Live/dead fluorescence imaging on the LeviCell instrument has been performed using Calcein AM: live cell stain and Propidium Iodide (PI): dead cell stain.
- Cells that are dying may have dual staining from both Calcein AM and PI.
- Cell permeabilization reagents (e.g., DMSO) can result in dual live/dead cell staining.
- Please review the instrument specifications for excitation / emission wavelength compatibility when planning experiments with alternate stains.

Example Protocol for Live/Dead Staining (Optional)

1. Sample should be in complete media when staining.
2. Add 2 µL of 4 mM Calcein AM per 1 mL of sample (8µM final concentration).
3. Add 10 µL of 1 mg/mL Propidium Iodide (PI) per 1 mL of sample. Pipette mix 10X.
4. Incubate sample in the dark for 10-15 mins.
5. Centrifuge the tube containing the sample at 300 RCF for 5 minutes.
6. Remove supernatant. Resuspend the cell pellet in a final volume of 270 µL of **Levitation Buffer** at desired Levitation Agent concentration. 220 µL is required for loading the LeviCell, and the remaining volume can be used for cell counts or other QC methods, as desired.

Protocols Supported by LeviCell Systems

- LeviCell Systems currently support validated live cell and nuclei enrichment protocols. Please consult 90-00213 Quick Reference Guide Live Cell Enrichment or 90-00298 Product Data Sheet LeviPrep Nuclei Kit II for more information.

EXPERIMENT MANAGER OUTPUT FILES

Experiment Manager creates several different output files to record details from a cartridge run.

- Run summary log file
- Image folder for all images captured and a run log file for use by Experiment Analyzer software
- System log file for all system activity and errors used for troubleshooting

Run Summary

A summary of each cartridge run is stored in a time and date stamped text file located in D:\ImageData\RunLogs. The Experiment Name (entered by user) is also included in the filename. The run log records times, settings and steps executed during the cartridge run, as well as any errors should they occur.

Image Folder

Images are captured by the Experiment Manager software during any cartridge run. The user may select up to 3 image types (Brightfield, Ex474/Em524, and Ex560/Em628) to record during the run. Images are always stored in a date and time stamped folder appended with the Experiment Name (set by user). By default, each image folder is stored in a date-stamped subfolder within D:\ImageData.

The image file format is .png (Portable Network Graphics). This type of image is compatible with the LeviCell Experiment Analyzer or Image J software. A .bmp image is also present in each experiment folder but is used for alignment reference only.

In addition to images, each image folder also includes a timestamped experiment log (.log). The experiment log contains information about the run, the image collection, and the pump status throughout the cartridge run. The experiment log is a tab-delimited text file format and can be opened with any compatible software such as Microsoft Notepad, WordPad, Excel, or SAS JMP software.

System Logs

Several additional text format logfiles are stored by the software to monitor the system performance and status. These logfiles are located in C:\Users\Public\Documents\LevitasBio\Experiment Manager\LOG with a .log extension. A technical support specialist may ask for these files for troubleshooting purposes.

SHUTDOWN

Once the cartridge runs for the day are complete, insert the blank cartridge into the instrument, engage the cartridge clamp, and close the front cover. Select “Exit” in the main activity selection screen, then close theExperiment Manager main interface window. Turn off the instrument, using the power switch at the upper left of the rear panel.

NOTE: The blank test cartridge should be loaded into the instrument whenever the instrument is shut down. When shut down, the cartridge clamp should be engaged and the front cover should be closed.

CLEANING THE LEVICELL 1.0 INSTRUMENT

In the event of a spill or contamination, the exterior and cartridge loading area of the LeviCell 1.0 instrument may be cleaned using a cloth or wipe pre-wetted with mild cleaning agents such as the following:

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

Use standard precautions for any cleaning agents.

DO NOT USE the following cleaning agents on the LeviCell 1.0 instrument, as damage to finishes may occur:

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

FREQUENTLY ASKED QUESTIONS

What is the chemical composition of your Levitation Agent? Is this safe to use with my cells?

- Levitation Agent is an inert, aqueous solution containing gadobutrol which is the primary ingredient in a common MRI contrast agent. It has been tested with a variety of cell types with no detrimental effects.

Does the Levitation Agent contain any human or animal ingredients?

- No.

What happens if my cells are clumping?

- Cells in the LeviCell instrument will levitate according to density, independent of cell size. A clump of cells will levitate to its average density, even if various cell types are contained within the clump. Many common additives used to reduce cell-cell interactions, such as EDTA and DNaseI, are compatible with the LeviCell. For more information, please contact Technical Support at **support@levitasbio.com** if you need more help with your specific application.

Can the image analysis system count my cells?

- Cell counting is not currently supported, but we hope to have this capability in the future.

Can I collect more than two fractions?

- The LeviCell instrument currently has the capability to collect two fractions from each input sample. Different flow rates per channel are used to optimize separations. This is controlled by the split line setting in the software. A collected fraction can be run in a new cartridge with a different Levitation Agent concentration, or different split line to allow for sub-fractionation.

How can I optimize separation for my application?

- The LeviCell software protocol includes an opportunity to evaluate the levitation profile of your sample, and to adjust separation during the run. This evaluation prior to the 'Separation Flow' step can be a good way to view how your sample will separate. Please contact support@levitasbio.com for help with your specific sample.

Does the LeviCell system work with mouse or non-human cell types?

- The LeviCell instrument can work with any type of cell (or microparticle) sample.

What media formulations are compatible with the LeviCell?

- The current system is designed to handle samples in standard media or cell buffer formulations. RPMI and PBS/BSA (0.5%) have all been evaluated. Other formulations are presumed to be compatible, but not all formulations have been tested. Please contact support@levitasbio.com for help evaluating your specific needs.

What fraction of my input cells will I recover in each channel?

- Reproducibility experiments with both bead and cell samples have obtained recovery yields of 70% (+/- 30%).

Recovery of cells can depend on many variables such as cell health, cell stickiness, pipetting accuracy, cell count accuracy/precision, etc. Please contact support@levitasbio.com for help with your specific sample if recovery rates are sub-optimal.

The fluorescence I am seeing is very low intensity. How do I increase the signal?

- Experiment Manager allows for the user to set exposure times and contrast settings for each optical illumination type. Please see Figure 12 for a description of how to change these settings. It is also possible that fluorescent stained cells have not been sufficiently stained. Please see previous section "General Guidelines for Live/Dead Cell Staining" for suggested staining protocols.

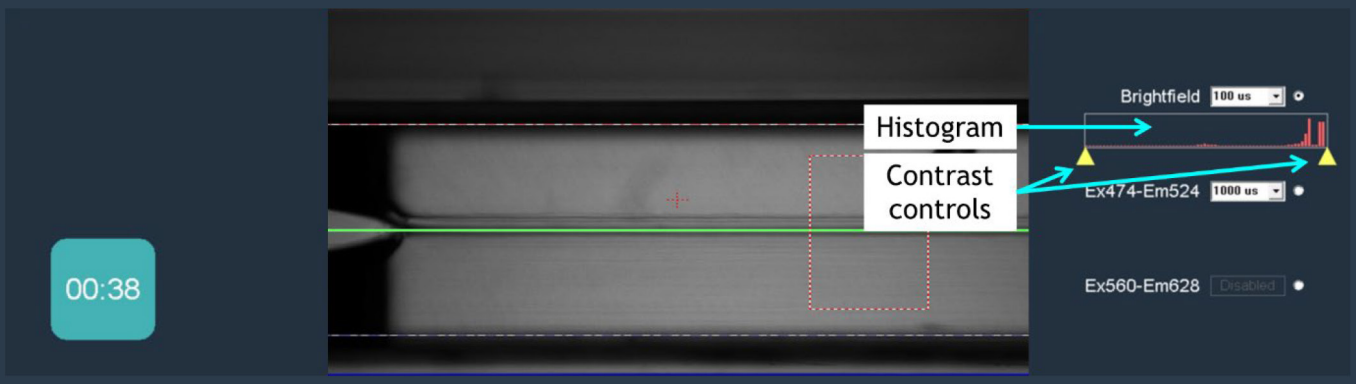


Figure 12.
Contrast controls in live image view. To increase the display brightness for a given imaging mode, drag the right-hand marker to the left. NOTE: this does not change the stored images. You can manually adjust the exposure time up to a maximum of 0.1 second (100,000 µs).

TROUBLESHOOTING

Tip: for instrument set-ups with a network connection, it is possible to grant LevitasBio Technical Support the ability to view and even control the desktop. This is not active by default and must be initiated on a case-by-case basis through RemotePC's HelpDesk application. Contact Technical Support to receive a Helpdesk ticket number or hyperlink to start a remote support session.

PROTOCOL STEP	ISSUE	REASON	SOLUTION
Sample Preparation	Overlap between cells with PI/Calcein stain	DMSO not completely removed by washing	Increase number of cell wash cycles with PBS or media.
Sample Preparation	Low fluorescence signal	a) Exposure time too low b) Insufficient cell staining	a) Increase exposure time. b) Modify staining protocol.
Sample Run	Flow does not appear to be running at set values	a) System Leak b) Debris	Contact support
Sample Run	Debris noted in levitation channel	a) Solutions not filtered b) Solution not covered c) Dirty sample	a) Filter all solutions through 0.2µm filter and cover solution bottles b) Wash cells prior to LeviCell
Levitation Timer	Sample will not levitate	a) No Levitation Agent added to Levitation Buffer b) Sample not fully mixed before loading into system c) Dead cells	a) Prepare fresh Levitation Buffer b) Prepare fresh sample and ensure proper mixing before pipetting into cartridge c) Contact support
Sample Run	Software or user error causing exit from software	a) Hardware issue b) User accidentally selected exit	a) Check hardware and connections, and then select "Resume Workflow" under System Tools b) Select Resume Workflow under System Tools
Sample Run	Cells are not levitating much; separation looks poor	Smaller cells take longer to levitate; more time may be required	Allow more time for levitation

PROTOCOL STEP	ISSUE	REASON	SOLUTION
Sample Run	Cells are out of focus once levitation is complete	Focus stage not set to factory setting	Contact Support@LevitasBio.com
Experiment Manager Startup or Sample Run	Camera initialization error	a) Incorrect power up sequence b) Instrument cable seated in the incorrect USB port c) Loose connection at instrument USB port	a) Refer to installation instructions for proper power up sequence and port connections. b) Refer to installation instructions for proper power up sequence and port connections. c) Close Experiment Manager, power off instrument, power off PC and reseat the connector. Refer to installation instructions for proper power up sequence and port connections.

REFERENCES

1. Durmus, NG et al (2015). Magnetic Levitation of Single Cells. PNAS, 112 (28) E3661–E366.
2. G Durmus, et al (2017). A Magnetic Levitation System to Study the Impact of Donor Gender, Age and Blood Storage Conditions on Red Blood Cell Density Profile.
3. Transfusion, 57, 32A-32A.
4. U Demirci, et al. System and method for cell levitation and monitoring.
5. US Patent App. 15/121,646

CONTACT INFORMATION

Technical Support Phone Number:
+1-650-204-1185

Technical Support Email Address:
support@levitasbio.com

Company Website:
www.levitasbio.com

Company Shipping Address:
LevitasBio
1505 Adams Drive, Suite D
Menlo Park, CA 94025
USA

LEVICELL INSTRUMENT SPECIFICATIONS

SPECIFICATION	VALUE
Number of sample inputs	1
Number of output fractions	2
Levitation magnets	Rare earth permanent magnets
Separation flow rate	100 µL/min
Imaging modes	Brightfield (transmitted illumination) Two fluorescence channels: (not enabled on LeviCell 1.0 Access) Excitation 470 nm, Emission 524 nm (e.g. Calcein, FITC) Excitation 560 nm, Emission 628 nm (e.g. PI, Cy 3.5)
Imaging resolution	Approximately 2 microns
OPERATIONAL	
DC power input to instrument (from supplied AC-DC adaptor)	24 V, maximum current 5.1 A
Input voltage to DC power adaptor	100-240 VAC, universal
AC supply current drawn	1.4A at mains voltage 115VAC; 0.7A at mains voltage 230VAC (Standard wall circuit)
Main enclosure dimensions	475 mm W x 340 mm D x 240 mm H (19” x 13” x 9”)
Instrument weight	15.3 kg (33.6 pounds)
Control PC Operating system	Windows 10, Professional, 64 bit
Over-voltage protection	105 ~ 135% rated output voltage. Protection type: shut down o/p voltage, re-power on to recover
Ingress protection rating	Not rated (no protection claimed)

ENVIRONMENTAL	
Operating ambient temperature	15 °C – 27 °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 1,700 m (5,500 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

LEGAL

The LeviCell 1.0 system, is developed by and manufactured for LevitasBio Inc. The system and its associated cartridges and reagents may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. Levitas products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products without prior written approval of Levitas, Inc.

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

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at **https://levitasbio.com/levicell** It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

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

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 support@levitasbio.com.
Document # 90-00204C0425

APPENDIX 1-OPERATIONAL QUALIFICATION CHECKLIST

START DATE OF TEST		Instrument Model	LeviCell 1.0 Access
OPERATOR		Instrument Serial Number	LC1-_____
OVERALL RESULT		Installation Site	
DATE OF TEST COMPLETION			
SIGNATURE			

FUNCTION	SPECIFICATION	PASS	FAIL	NOTES
Power-up: PC	PC cabled and powered on without error			
Power-up: Instrument & Software	Instrument cabled and powered on without error. Experiment Manager software is started without error.			Version:
Sensor Check	- Sensor detects no cartridge when not loaded - Sensor detects cartridge when inserted. - Sensor detects clamp is up when not engaged - Sensor detects clamp is down when engaged - SensorStatus = Pass			
LED Check	Brightfield LED flashing green when activated - Ex474/Em524 LED flashing blue when activated* - Ex560/Em628 LED flashing lime when activated* - LEDStatus = Pass			
Pump Initialization and Reset	- Pumps initialized with no error. - Pumps aspirate specified volume with no error - PumpStatus = Pass			
Core Alignment	- Magnets are visible in the field of view. - Magnet lines overlay magnet edges. - Flowcell center lines fall with expected range - AlignmentStatus = Pass			
Barcode Reader Functionality	Barcode reader reads barcode without error. ReaderStatus = Pass			
Image Capture	A test image is captured (with reference image) and stored.			

* If available

APPENDIX 2-OPERATIONAL QUALIFICATION CHECKLIST

START DATE OF TEST		Instrument Model	LeviCell 1.0 Access
OPERATOR		Instrument Serial Number	LC1-_____
OVERALL RESULT		Installation Site	
DATE OF TEST COMPLETION			
SIGNATURE			

FUNCTION	SPECIFICATION	PASS	FAIL	NOTES
Ex474-Em524 LED alignment	Green fluorescent beads (Ex460/Em500) visible in image with Ex474/Em524 LED selected if available.			
Ex560-Em628 LED alignment	Red fluorescent beads visible in image with Ex560/ Em628 LED selected if available.			
Levitation - visual	Beads levitate into 2 populations after 3 minutes.			
Separation - visual	Polystyrene beads flow to the top outlet (above green split line), PMMA beads flow to the bottom outlet (below green split line) as observed in images.			
Volume recovery (optional)	Measured volume recovered from each outlet > 66 µL (>60% of input volume)			
Purity (optional)	Number fluorescent beads counted from top / number of total beads counted > 90%			
Yield (optional)	Number fluorescent beads counted from top / Number of fluorescent beads in input > 40%			