LEVIT.SBIO

EXP147	70-05	
	EXp ₁₄₇₀₋₀₁	
(j)		
Run Info	Lane 2 : exp1624r3l2 (150 mM)	
¢	E exp1624r130mM ⊗ EXP1537 k7 ⊗ EXP1697 ⊗	Fractionation Analysis
Run Review	() ()	
	Run Review A Bapada time: (201 A Bapada time: (201 Bun Review B Bapada time: (201 B Ba	- 15
Fractionation Analysis	Collection	
All Lanes	Fractionation Analysis	15
Lane 1	Levitation Analysis	- 30
Lane 2		
Lane 3		
Lane 4	5 19% 18% 18% 5 81% 18% 18% 5 81% 82% 82%	18% 82%
~	95 96 99	
Levitation Analysis		

LeviMetrics Software

USER GUIDE

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

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ABOUT THIS SOFTWARE

LeviMetrics[™] v3.0 is designed to visualize and perform analysis on experiment runs from the LeviCell[®] EOS system. The LeviMetrics software can be installed on the Windows 10 Pro 64-bit computer provided with the LeviCell EOS or added to any Windows 10 or 11 PC computer meeting the requirements below.

Current released version:	v3.0
Release date:	Oct 2024

Computer Requirements

Operating System:	Windows 10 or 11
Processor:	Dual-core, 2.5 GHz Processor
RAM:	32 GB installed memory

LeviMetrics Elite

The LeviMetrics analysis software has the ability to be upgraded to LeviMetrics Elite. Only LeviMetrics v3.0 or higher is enabled for any upgrade options.

The LeviMetrics Elite upgrade offers a suite of advanced powerful metrics and analysis packages. Those who purchase this packagage option will receive ongoing updates featuring new analysis tools. These tools enable more in-depth analysis, further validating and refining downstream results derived from the sample preparation process.



SOFTWARE INSTALLATION

- 1. Go to the support website (www.levitasbio.com/support)
- 2. Download the *.msi installation file to the LeviCell EOS Control PC computer or any PC computer with Windows 10 or Windows 11.
- **3.** Locate the downloaded *.msi file in the Downloads directory or other location if applicable.
- **4.** Double-click "Install LeviMetrics v3.0*.msi" file.
- 5. Follow the instructions to install the new software until you have completed the installation.
- 6. Locate the new desktop icon.



Figure 1. LeviMetrics Software Icon

Contact support@levitasbio.com or call Technical Support at +1-650-204-1185 if any errors are encountered during the procedure.



USING LEVIMETRICS SOFTWARE

The LeviMetrics software provides a easy and efficient method to view and analyze data obtained from experiments run on the LeviCell EOS system.

Main Analysis Screen

The main analysis screen includes three sections once a run has been opened.

- Menu and Experiment Bar
- Run Analysis Modes Toolbar
- Image Viewing Area



Figure 2. Software tools overview

At the end of an experiment run on the LeviCell EOS, the EOS Manager software creates several data files in the run folder. LeviMetrics uses the Run.Exp file, which allows viewing and analysis of the experimental data. Please reference the LeviCell EOS User Guide for more information on how to run an experiment, and data folder structure.





NOTE: Copy data to the local PC running LeviMetrics before analysis. Performing LeviMetrics analysis over the network is not advisable, as this can drastically slow down analysis or software performance.

Up to 3 experiment run files can be opened at the same time. They will be arranged in individual tabs across the top of the screen.



Figure 3. Open runs

If a 4th experiment run is opened, a dialog box will appear confirming whether one run needs to be manually closed before opening another run or if the software should close all and continue.



Figure 4. Dialog message if opening more than three runs

LeviMetrics can also run multiple instances to perform side-by-side comparison with multiple monitors (e.g. one instance per monitor). In this manner, another 3 experiment run files can be opened.



NOTE: Opening more than 3 runs with multiple LeviMetrics instances can cause computer slowdown or performance issues depending on the computer specification.



General Navigation

The LeviMetrics software uses several colors for navigation and button usage.

Item	Action	Re	sult
Button	Hover	Open run file.	Tooltip will appear*
Default Button	Click	SAVE	Button function
Non Default Button	Click	CANCEL	Button function
Field	Click	Updated by	Field become editable
Active tool	Click	۲	Turns on and off tool
Active run	Selected	exp1624-r1-50mM 🛛 🛛	Run will be viewed
Selected mode	Click	Run Review Levitation Collection	Mode viewed or analyzed on the main viewing window
Temporary message	Clear	EXP-1897: Run is ready for review. ×	Message cleared with action, or will disappear on its own

* Tooltip will appear for some functions only

Table 1. Navigation overview



Menu

On the upper left of the screen, the menu button can be expanded to display various options:

- Open file
- Close file
- Close all files
- Recent Runs opened (5)
- About LeviMetrics
- Exit

Open
Close
Close All
Recent Runs
exp1624-r1-50mM
EXP1537 R1
EXP1537 R7
EXP-1897
exp1624-r3
About LeviMetrics
Exit

Figure 5. Expanded Menu options

The About LeviMetrics displays the LeviMetrics software version and build number. Also included is the End User Software License Agreement.



Figure 6. Expanded About LeviMetrics view



If the LeviMetrics Elite upgrade package subscription has been purchased, the About LeviMetrics will show the Elite version.

About LeviMetrics
LeviMetrics Elite Version 3.0.1 Build 312
2022 oupprign Leenaadio III. An ingins reserved. Software license agreement
END USER SOFTWARE LICENSE AGREEMENT IMPORTANT - READ CAREFULLY. This End User Software License Agreement ("Agreement") is a legal agreement between you and LevitasBio, Inc. ("LevitasBio") and governs the use of the Software and Documentation (as both are defined below). By installing this software you agree to be bound by all of the terms and conditions of this Agreement. If you do not agree with all of the terms and conditions of this Agreement, (a) you will not be able or permitted to use the Software or Documentation or any portion thereof and (b) you must immediately and permanently destroy the Software and Documentation. As used in this Agreement, the term "you" and "your" means the entity or individual that accesses and uses the Software or Documentation.
 Definitions "Computer" means the specific computer that is delivered as part of the instrumentation system. "Computer" means the specific computer that is delivered as part of the instrumentation system. "Documentation" shall mean any documentation, on-line read-me or help files for the Software provided herewith in portable document format. "Improvements" shall mean any new processes, designs, devices, modifications, improvements, discoveries, works of authorship, inventions, suggestions, ideas or know-how (whether or not patentable) that you may disclose or provide to Levitastio related to the Software Orkare or Documentation. "Software" shall mean the software program including all third party software programs associated files installed onto the Computer. "Software" shall mean the software program including all third party software programs associated files installed onto the Computer. "Software" shall mean the software program including all third party software programs associated files installed onto the Computer. "Software" shall mean the software program including all third party software programs associated files installed onto the Computer. "Software" shall mean the software program including all third party software programs associated files installed onto the Computer. "Software" shall mean the software program including all third party software programs associated files installed onto the Computer. "Software" shall mean the software program including all third party software programs associated files installed onto the Computer. "Software" shall mean the software program including all third party software programs associated files installed onto the Computer. "Software" shall mean the software program including all third party software programs associated the software programs associated the software programs associnter programs associntere programs associated the software pr
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Figure 7. About screen with LeviMetrics Elite



Analysis Modes Summary

There are 4 Analysis modes available for each run. Within each mode there are additional options for the analysis to be performed.

/	\frown	

)
	-	

NOTE: Fractionation Analysis and Levitation Analysis are not available for non-cell-based protocols. Nuclei sample runs, Core Calibration or System Check runs cannot be analyzed.

Analysis Icon	Analysis Mode	Analysis Options	Description		
(i) Run Info	Run Info	n/a	View all entered run info. Levitation Agent and Notes fields can be edited in case of incorrect or missing info. The software will require a name to be entered in the "Updated by" field.		
¢	Run Review	Levitation	 View samples during levitation and all completed scans Zoom into each lane Create video of levitation Take screen captures Generate image view report 		
Run Review	Run Review			Collection	 View samples during collection Zoom into each lane Create video of levitation Take screen captures Generate image view report
Fractionation Analysis	Fractionation Analysis*		 Ability to analyze Relative Intensity % for top and bottom fractions for all lanes or individual lanes View alternative split lines and respective fractionation value or separation factor changes 		
Levitation Analysis	Levitation Analysis*		 Ability to view Relative Intensity curve Simulate alternate Levitation Agent concentrations and effects on sample levitation 		
Included With I	LeviMetrics Elite Up	grade			
Profile Analysis	Profile Analysis		 Ability to analyze relative intensity of fluorescent stains View the peak position of the intensity curves relative to top and bottom fractions 		

 Table 2. Run analysis modes summary

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GETTING STARTED

- **1.** Double click the LeviMetrics software icon on the desktop
- 2. The LeviMetrics opening screen will appear and fill the screen
- **3.** To access a run, click on the Open Run button.



Figure 8. Home view

Alternatively, below the Open Run button, the most recent 5 experiments in the opened run history will appear. To open a run that appears on this list hover over the file and click on the "eye" button on the right.





Figure 9. Open recent run

The software will load the run and perform basic analysis of the images. This will take a few seconds and a progress circle will appear with the % complete.



Figure 10. Progress indicator while loading file.



Once the file has loaded the Main Analysis screen will appear, defaulted to the Run Review Analysis Mode - Levitation Review. If fluorescence was selected during the run, the fluorescence images will appear.



Figure 11. Run Review Levitation view

To open parallel files to be analyzed, click on the Menu icon on the upper left, and choose Open. The menu will also display up to 5 of the most recent runs opened.

Open					
Close					
Close All					
Recent Runs					
EXP1537 R7		• UPEN	RUN		
EXP1537 R1 exp1624-r1-50mM					
EXP-1897					
exp1624-r3					
About LeviMetrics	Recent Run Name	Completed On	EOS Module	Started By	
Exit	EXP1537 R7	8/14/2023 4:17 PM	Dory_Beta2	SJ/MM	
	EXP1537 R1	8/14/2023 11:28 AM	EOS 10010	SJ/MM	
	exp1624-r1-50mM	10/23/2023 1:20 PM	EOS 10012	sab	
	EXP-1897	11/1/2023 1:00 PM	EOS-1001-Nemo	SM	
	exp1624-r3	10/23/2023 2:23 PM	EOS 10012	sab	
·					
1. 1999					
a second second second second					

Figure 12. Expanded Menu to open files



RUN ANALYSIS MODES

Run Info

This is the experiment information entered at the time of the run on the LeviCell EOS. Information included are:

- Run Name
- Cartridge barcode
- Protocol
- Levitation (Actual/Design)
- Run Temperature/ EOS Module
- Started by [User]
- Completed on [Date, Time]
- All lanes used
- Sample names entered
 - Levitation Agent (mM)
- Green fluorescence selected
- Red fluorescence selected
- Notes

By default the Run Info is shown in view only mode. Most run information entered at time of experiment is static and cannot be altered. The only editable fields are Levitation Agent (mM) concentration and the Notes section. To access Edit mode, click on the icon on the toolbar.



Figure 13. Run Info Edit icon.

If these are edited, the software requires the "Updated By" field to be entered. This ensures proper tracking of changes in the file.

If the Levitation Agent concentration is altered, the Fractionation Analysis and Levitation Analysis results, if previously run, will need to be recalculated.



EXP1537	R7 🗵 EXP1537 R1 💌	exp1624-	1-50mM 🗵					
(i)								Run Info
Run Info	Run name		Sample name	LA (mM)	Green fluorescence		Red fluorescence	
	Cartridge barcode	Lane 1		50				
Run Review	012-1137-0158	Lane 2	exp1624-r1-l2	50				
	Protocol Medium Cell (5 - 20µm)							
Fractionation Analysis	Levitation (Actual / Design)	Lane 3	exp1624-r1-l3	50	None		None	
~	Run temperature / EOS Module	Lane 4		50				
Levitation Analysis	Ambient / EOS 10012	Notes						
Levitation Analysis	Started by sab							
	Completed on 10/23/2023 1:20 PM						Updated by	
						CANCEL		

Figure 14. Edit mode

exp1624-	r3 🗵 EXP1537 R7 🗵							
Û								Run Info
Run Info	Run name EXP1537 R7		Sample name	LA (mM)	Green fluorescence		Red fluorescence	
	Cartridge barcode	Lane 1			Calcein AM			
Run Review	013-1121-0096 Protocol	Lane 2	Save Run I	nfo	Calcein AM		Propidium Iodide	
•	Medium Cell (5 - 20µm) Levitation (Actual / Design)	Lane 3	Are you sure you want to update and remove the analys	the run information is results?				
	20 min / 20 min Run temperature / EOS Module Ambient / Dory Beta2	Lane 4					Fast Red	
Levitation Analysis	Started by	Notes						
	SJ/MM Completed on 8/14/2023 4:17 PM Updated by ij	LA value				CANCEL	Updated by SM SAVE	
	Updated on 11/27/2023 10:58 AM		NO					
			YES					

Figure 15. Save Run Info



If the run info is updated and saved, the run info will also include:

- Updated by [person's name]
- Updated on [Date, Time]

Run Review

The Run Review analysis mode is the default view when a file is opened. In this mode, there are two options: Levitation review and Collection review.

Once the run is loaded a message will appear in the lower right corner stating that the file is ready for review.



Figure 16. Message confirming run is ready for review

Levitation Review

The entire run and all completed scans can be reviewed in the Levitation Review mode as it was viewed during the actual experiment run on the LeviCell EOS system.



Figure 17. Experiment run is loaded for Levitation Analysis



If the run included fluorescent stains, the fluorescence views will also be available via the imaging toolbar on the right. These can be toggled on and off for different views.

Imaging tools include:

- Zoom in
- Zoom out
- Brightfield
- Green fluorescence
- Red fluorescence
- Auto-contrast

These imaging tools are available in Run Review mode only.

If the max zoom level is used, a min-map will appear on the upper right hand corner displaying which lane and position the view is showing. Use this mini-map to jump to other positions within the view. This can be done by clicking on the mini-map itself or using the scroll bars. If navigating with the keyboard arrows, the unused lanes will be skipped automatically.



Figure 18. Imaging toolbar



Figure 19. Zoom view



There are various viewing tools available in the Run Review mode.



Figure 20. Viewing toolbar

When clicked, the screen capture icon will store the current scan view of all lanes in a *.png file format in the run folder.

The image report function will store a *.pdf run review report of the current scan view (as compared to standard run report which is of the last scan).



Figure 21. Generate image review report

To view different completed scans, use the view tools at the top of the run review. These buttons will slow advance through the different scans or skip to the last scan. In the center of the buttons there is a pulldown menu which allows selection of specific completed scans. The elapsed time stamp is shown for each completed scan.

There is also an option to play the completed scans in a continuous video view. Use the stop button to stop the play of the movie.





Figure 22. Drop down menu of completed scans

Collection Review

At the end of a run, samples are collected into the outlet wells one at a time. Since there is only one camera on the LeviCell EOS, it captures each lane one at a time as the sample flows by. In Collection Review, all four sample collections can be viewed simultaneously.

The default collection view displays as the original size of the collection capture, with the camera positioned at the splitter end of the separation channel.



exp1624-r1-	I-S0mM 🗵 EXP1537 R7 🗷	
Û	$\square \boxtimes \boxdot K < (\checkmark) > X \ll 0 >>$	Run Review
Run Info	Lane 1 : Bd1 (141 mM, Green Stain, Red Stain)	લ વ્
Run Review Levitation		*
Collection	Lane 2 : Bd2 (140 mM, Green Stain, Red Stain)	
Fractionation Analysis		
Levitation Analysis		
	Lane 3 : Bd3 (130 mM, Green Stain, Red Stain)	
	Lane 4 : Bd4 (120 mM, Green Stain)	

Figure 23. Collection Review

There are two levels of zoom that can be used: original size of the collection capture or max zoom level. The Mini-map will appear with the max-zoom level. The user can scroll across the default collection view or between lanes in this view.



Figure 24. Max Zoom view in Collection Review



Fractionation Analysis

LeviMetrics has the capability to analyze the run to provide a quantitative measurement which can be informative prior to any downstream analysis. With this analysis mode, Fractionation Analysis estimates the fraction of cells that levitate higher in the separation channel compared with the fraction that levitate lower in the channel based on evaluation of pixel intensities above and below the user-selected split line value.



NOTE: Fractionation Analysis tools are not available for non-cell-based protocols. Nuclei sample runs, Core Calibration or System Checks runs cannot be analyzed.

Upon clicking on the Fractionation Analysis Mode, the software will perform analysis on the loaded run. An indicator will show its progress.



Figure 25. In progress Fractionation Analysis



All Lanes Analysis

Once complete, the analysis will be displayed and a message at the bottom right will display that the fractionation analysis results are ready for review. In this mode all lane fractionation data can be displayed or individual lanes can be viewed and analyzed individually.

EXP1537	R7 🗵		
	₽ ⊠ 🗎		Fractionation Analysis
Run Info	Top fraction 15%	Lane 1 : 300K 25% viable (153 mM, Calceln AM, Propidium Iodide)	Elapsed time : 19:35
Run Review	Bottom fraction 85% Separation factor 91		-
Fractionation Analysis	Fractionation Analysis Top fraction	Lane 2 : 1M 25% viable (152 mM, Calcein AM, Propidium Iodide)	Elapsed time : 19:56
All Lanes Lane 1 Lane 2	Bottom fraction 81%		
Lane 3 Lane 4	92	Lane 3 : 1M 25% viable (150 mM. Fast Green, Fast Red)	Elapsed time : 18:54
Levitation Analysis	Top fraction 18% Bottom fraction 82%		
	Separation factor 88		
	Top fraction 18%	Lane 4 : 300K 25% vlable (150 mM, Fast Green, Fast Red) 8	Elapsed time : 19:14
	Bottom fraction 82% Separation factor		
	92		EXP1537 R7: Fractionation analysis results are ready for review. ×

Figure 26. Fractionation Analysis all lanes view

When all lanes are displayed, the top fraction percentage, bottom fraction percentage, and separation factor metrics are displayed on the left side of each lane.

These fractionation percentage values directly correspond to the image shown and are the relative intensity percentage fractions for the sample based on the split line value.



The Separation factor is a calculated value based on the relative error that can occur during collection when using a specific split line value. The higher the value the better the ability to collect the two fractions with minimal error. This indicates that there is good separation between the top and bottom bands. If the value is low, then this indicates that the two bands are close enough where there may be crossover contamination of the bands during collection.



Figure 27. Fractionation information

Individual Lane Analysis

If the individual lane is selected, additional information will be displayed. The sample for the single lane selected will be displayed in a zoomed view. A Relative Intensity Graph is overlaid on the image to provide a histogram view of the relative intensity of the bands in the image.

The top and bottom fraction % will now be extrapolated across various split line values. The split line chosen during the run will be displayed in teal text and with a gold outline initially. If another split line value is selected, it will be indicated by the gold outline and the split line will move on the image. The actual split line value will remain in the teal text.

The separation factor is also displayed for each split line value. Based on the error that can occur during collection as the sample flows by the splitter, a separation factor is assigned. As numbers are higher in value, more separation between signal intensity of the bands can be seen. As numbers are lower in value, less separation is calculated for the two bands thereby causing more error in the ability to separate accurately as it flows by the splitter.





Figure 28. Fractionation Analysis tools overview

An additional feature of this mode is the option to see how the fractionation relative intensity graph can be changed. By clicking on a different split line value (+/- 10 split line values, capped at -15 or 15), the LeviMetrics software will simulate values for the fractions and the separation factor. This can help the user evaluate how possible changes can affect the sample both via the image view and relative intensity graph.



Figure 29. The actual run's Split Line value is highlighted in teal and selected in a gold outline.



S LeviMetrics - 2.0.1.263																								-	ø ×
EXP1470-	05 🗵	EXP147	70-01			exp1624	-r3	×																	
Û	∂ ⊠ 🗎																					Fra	ctionatio	on And	alysis
Run Info	Lane 2 : exp162	24r3l2	(150 m	M)																					
Run Review								No.						See al						- C	Contraction of the second				
Fractionation Analysis	******			and an			i i i			and a											2044 17041		5	2	0 0
All Lanes Lane 1																	<		1.177	27.00-00 27.00-00			0.5		— -15 — — — -30 —
Lane 2 Lane 3		1.1	1. 1. 1.				1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1						1.21	3 24	-	al an	-		-		1. T. I.	1001			45
Lane 4	Split line	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10			
<	Top fraction	31%	30%	29%	28%	27%	26%	25%	24%	23%	22%	22%	21%	20%	20%	19%	- 19%	- 19%	19%	18%	18%	18%			
Levitation Analysis	Bottom fraction	69%	70%	71%	72%	73%	74%	75%	76%	77%	78%	78 %	79%	80%	80%	81%	81%	81%	81%	82%	82%	82%			
	Separation factor	83	86	87	88	88	87	87	87	88	88	89	90	90	91	92	93	94	95	96	96	94			

Figure 30. Fractionation Analysis with different Split Line value

If another lane is selected, all relevant data will be updated to the new lane's information. The actual run's Split Line value will remain in teal.

At the top of the Fractionation Analysis mode there are different viewing tools that can be used: screen capture, Fractionation Analysis view report generation, and export of the fractionation data (*. txt)

The Fractionation exported data will be a *.txt file and includes the fraction percentages for both top and bottom fractions, split line and separation factor for each lane. This file is useful for plotting profiles or comparing multiple datasets.



NOTE: When an individual lane is selected, the exported data file also includes the intensity profile.



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Fractionation Analysis

Run Summary	Top fraction	Lane 1 : 300K 25% viable (153 mM, Calcein AM, Propidium Iodide)	Elapsed time : 19:3
Run name EXP1537 R7	Bottom fraction 85%		
Cartridge barcode 013-1121-0096	Separation factor 91		
Protocol Medium Cell (5 - 20µm)	Top fraction 19%	Lane 2 : 1M 29% viable (152 mM, Calcein AM, Propidium Iodide)	Elapsed time : 19:56
Levitation (Actual / Design) 20 min / 20 min	Bottom fraction 81% Separation factor 92		
Run temperature / EOS Module Ambient / Dory_Beta2	Top fraction	Lane 3 : 1M 25% viable (150 mM, Fast Green, Fast Red)	Elapsed time : 1854
Started by SJ/MM	Bottom fraction 82%		
Completed on 8/14/2023 4:17 PM	Separation factor 88		
Updated by SM	Top fraction 18%	Lane 4 : 300K 25% viable (150 mM, Fast Green, Fast Red)	Elapsed time : 1914
Updated on	Bottom fraction 82%		
Notes	Separation factor 92		
LA value for Lane 1 updated to			



The Fractionation Analysis report PDF can be generated for all lanes or just the individual lanes based on which view is being used at the time.

File Edit Format View Help Run Info EXP1537 R7 Run name Run name EXP1537 R7 Cartridge barcode 013-1121-0096 Protocol Medium Cell (5 - 20µm) Levitation (Actual / Design) 20 min / 20 min Run temperature / EOS Module Ambient / Dory_Beta2 Started by SJ/MM Completed on 8/14/2023 4:17 PM Updated by SM Updated on 11/29/2023 12:18 PM Notes LA value for Lane 1 updated to 153. Fractionation Data Lane 1 Sample name 300K 25% viable transportration (mM) 153 Agent concentration (mM) Split line 9 Top fraction 15% Bottom fraction 85% Separation factor 91 Lane 2 Sample name 1M 25% viable Agent concentration (mM) Split line 3 Top fraction 19% Bottom fraction 81% Separation factor 92 152 92 Lane 3 Sample name 1M 25% viable Agent concentration (mM) 150 Split line 6 Top fraction 18% Bottom fraction 82% Separation factor 88 Lane 4 Sample name 300K 25% viable



Levitation Analysis

In Levitation Analysis mode, individual samples can be analyzed to aid in optimizing future similar sample runs. In this mode, LeviMetrics allows for dry optimization and simulation of samples based on changing Levitation Agent concentration. When the Levitation Agent concentration is changed, the image is altered to simulate how that sample will appear if run on the LeviCell EOS. In addition the Relative Intensity curve is also modified. The user can use this analysis mode to understand the performance of levitation, see the change in band separation, widening of bands, how well the bands are distinguished, and how the Split Line value can be adjusted based on the Levitation Agent concentration.

The default view is on Lane 1 (or the first selected lane) and is split between two different views, however any of the lanes can be viewed. The left image displays the actual run as it was run on the LeviCell EOS. The right image displays the simulated condition. To change the Levitation Agent concentration, use the drop down menu which includes steps of 10mM for +/- 50mM from originally entered Levitation Agent concentration (capped at 50mM or 200mM).



NOTE: If LeviMetrics detects an issue with the analysis (e.g. meniscus is past the splitter) it will not display any analysis. A gray image will appear instead.

Use the drop down on the left side view to choose the available Collection Scan frame.



Figure 33. Levitation Analysis view





If the Levitation Agent concentration is outside of normal range (50-200mM) then an alert will appear.

Figure 34. Levitation Analysis dialog message

A Levitation Analysis Report or exported data can be generated using the viewing tools. The exported data will be a *.txt file and includes the intensity values which can be graphed in a third party program. Alternatively a Levitation Analysis Run summary PDF can be generated, capturing the simulation changes.

EXP15	37 R7_Levita	tion Analysi	s_Lane 1_300	K 25% viable	.txt - Notep	ad													-		\times
File Edit	Format	View Help																			
Run Inf	0																				
Run nam	e	EXP1537	R7																		
Cartrid	ge barco	de	013-112	1-0096																	
Protoco	1	Medium	Cell (5	- 20µm)																	
Levitation (Actual / Design) 20 min / 20 min																					
Run temperature / EOS Module Ambient / Dory Beta2																					
Started	by	SJ/MM																			
Complet	ed on	8/14/20	23 4:17	PM																	
Updated	by	SM																			
Updated	on	11/29/2	023 12:1	8 PM																	
Notes	LA valu	e for La	ne 1 upd	ated to :	153.																
Levitat	ion Data																				
Lane	1																				
Sample	name	300K 25	% viable																		
Collect	ion Scan	9																			
Agent c	oncentra	tion (mM)	153																	
Agent c	oncentra	tion (mM) (Simul	ated)	103																
Channel	positio	n	-50.000	-49.827	-49.653	-49.480	-49.307	-49.133	-48.960	-48.787	-48.614	-48.440	-48.267	-48.094	-47.920	-47.747	-47.574	-47.400	-47.227	-47.05	4 -
-28.163	-27.990	-27.816	-27.643	-27.470	-27.296	-27.123	-26.950	-26.776	-26.603	-26.430	-26.256	-26.083	-25.910	-25.737	-25.563	-25.390	-25.217	-25.043	-24.870	-24.69	7.
.459	-5.286	-5.113	-4.939	-4.766	-4.593	-4.419	-4.246	-4.073	-3.899	-3.726	-3.553	-3.380	-3.206	-3.033	-2.860	-2.686	-2.513	-2.340	-2.166	-1.993	
21.404	21.577	21.750	21.924	22.097	22.270	22.444	22.617	22.790	22.964	23.137	23.310	23.484	23.657	23.830	24.003	24.177	24.350	24.523	24.697	24.870	1
.707	46.880	47.054	47.227	47.400	47.574	47.747	47.920	48.094	48.267	48.440	48.614	48.787	48.960	49.133	49.307	49.480	49.653	49.827	50.000		
Normali	zed inte	nsity	0.0045	0.0039	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	(
4370	0.4557	0.4749	0.4956	0.5152	0.5400	0.5542	0.5685	0.5842	0.6059	0.6273	0.6423	0.6484	0.6640	0.6822	0.6968	0.7049	0.7124	0.7210	0.7369	0.7475	f
53	0.0222	0.0272	0.0352	0.0461	0.0516	0.0584	0.0756	0.0811	0.0944	0.0787	0.0629	0.0439	0.0258	0.0175	0.0449	0.0613	0.0816	0.0721	0.0426	0.0098	(
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	(
Normali	zed inte	nsity (S	imulated)	0.0188	0.0216	0.0235	0.0249	0.0239	0.0236	0.0264	0.0316	0.0365	0.0393	0.0410	0.0437	0.0476	0.0523	0.0582	0.0642	(
42	0.5421	0.5313	0.5235	0.5231	0.5225	0.5195	0.5127	0.5006	0.4855	0.4756	0.4679	0.4609	0.4536	0.4427	0.4315	0.4168	0.4008	0.3847	0.3676	0.3586	(
0.0258	0.0302	0.0350	0.0378	0.0383	0.0402	0.0390	0.0373	0.0359	0.0340	0.0323	0.0315	0.0320	0.0347	0.0361	0.0361	0.0345	0.0316	0.0328	0.0352	0.0372	(
0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	6

Figure 35. Levitation Analysis data text file

LEVIT&S BIO

Started by SJ/MM

Updated by SM

Levitation Analysis



Figure 36. Levitation Analysis Run Summary Report PDF



LEVIMETRICS ELITE ANALYSIS MODES

The LeviMetrics Elite upgrade offers a suite of advanced powerful metrics and analysis packages. Addiitonal powerful analysis modes will be offered regularly and will be part of all versions after LeviMetrics v3.0.

Profile Analysis

In Profile Analysis mode, samples that are fluorescently stained can be analyzed for the amount of fluorescence signal visualized either in the top or bottom half of the separation channel.

The default lane is Lane 1 (or the first selected lane). The full length of the separation channel is displayed with the fluorescence signal only. The split line chosen is shown in the teal horizontal line. On the right, the two histograms represent the relative fluorescence intensity corresponding to the two stains.



Figure 37. Profile Analysis view



The table below displays information about the fluorescence profile.

- Stain name
- Split line value
- Top Fraction
- Bottom Fraction
- Top peak position
- Top peak width
- Bottom peak position
- Bottom peak width

The peak position represents the position of the highest point in the profile above and below the split line. A higher peak value means there is a strong sample presence at the peak location. A high peak with narrower width implies strong uniform fluorescence signal which can correspond to a tight band of cells within the sample. A low peak with broader width implies a spread band of fluorescence signal or a wide band of cells within the samples.

= "	EXP2122 R2 PBMCs new ambient TEC 🗵	EXP2122 R5 PBMCs new amb	nient 🗵 Run1_LAvsGA 🔀	
(j))			
Run In	ıfo			
Run Rev	▶ ∕iew		Analysis Confirmation	
ß			No samples are compatible with profile analysis.	
Fractionation	Analysis			
4	:			
Levitation A	Analysis			
Profile And	alysis			
			ок	

If the run did not include fluorescent stain, the Profile Analysis cannot be performed. A notification will appear.

Figure 38. Notification if Profile Analysis cannot be performed



A Profile Analysis Report or exported data can be generated using the viewing tools. The exported data will be a *.txt file and includes the intensity values which can be graphed in a third party program. Alternatively a Profile Analysis Run Summary PDF can be generated.

🕘 DP1537 R7, Profile Analysis, Lane 1, J00K 25% viable tot - Notespad	_	;	×
File Edit Format View Help			
Run Info			^
Run name EXP1537 R7			
Cartridge barcode 013-1121-0096			
Protocol / Run temperature Medium Cell (5 - 20µm) / Ambient			
Levitation (Actual / Design) 20 min / 20 min			
Sample collection Sequential			
EOS Module / Core SN Dory Beta2 / 10002 Core			
Started by SJ/MM			
Completed on 8/14/2023 4:17 PM			
Updated by gs			
Updated on 10/18/2024 3:33 PM			
Notes			
Profile Data			
Lane 1			
Sample name 300K 25% viable			
Agent concentration (mM) 150			
Split line 9			
Image Mode RedFluorescence			
Stain Propidium Iodide			
Top fraction 1%			
Bottom fraction 99%			
Top peak position -			
Top peak width -			
Bottom peak position -16.84			
Bottom peak width 13.85			
Channel position -50.000 -49.913 -49.827 -49.740 -49.654 -49.567 -49.481 -49.394 -49.307 -49.221 -49.134 -49.048 -48.961 -48.874 -48.788 -48.701 -48.615 -48.528 -48.442 -48.355 -48.268 -48.182 -48.095 -48.009 -4	7.922	-47.835	
-39.091 -39.004 -38.918 -38.831 -38.745 -38.658 -38.571 -38.485 -38.398 -38.312 -38.225 -38.139 -38.052 -37.965 -37.879 -37.792 -37.706 -37.619 -37.532 -37.446 -37.359 -37.273 -37.186 -37.100 -37.013 -36.926 -36.840 -3	6.753 -	-36.667	
-28.009 -27.922 -27.835 -27.749 -27.662 -27.576 -27.489 -27.403 -27.316 -27.229 -27.143 -27.056 -26.970 -26.883 -26.797 -26.710 -26.623 -26.537 -26.450 -26.364 -26.277 -26.190 -26.104 -26.017 -25.931 -25.844 -25.758 -2	25.671 -	-25.584	
-16.926 -16.840 -16.753 -16.667 -16.580 -16.494 -16.407 -16.320 -16.234 -16.247 -16.061 -15.974 -15.887 -15.801 -15.714 -15.628 -15.541 -15.455 -15.368 -15.281 -15.195 -15.108 -15.022 -14.935 -14.848 -14.762 -14.675 -1	.4.589 -	-14.502	
5 -5.238 -5.152 -5.065 -4.978 -4.892 -4.805 -4.719 -4.632 -4.545 -4.459 -4.372 -4.286 -4.199 -4.113 -4.026 -3.939 -3.853 -3.766 -3.680 -3.593 -3.566 -3.420 -3.333 -3.247 -3.160 -3.074 -2	4.987 -	-2.900	1
.615 8.701 8.788 8.874 8.961 9.048 9.134 9.221 9.307 9.394 9.481 9.567 9.654 9.740 9.827 9.913 10.000 10.087 10.173 10.260 10.346 10.433 10.519 10.606 10.693 10.779 10.866 10	1.952	11.039	÷ 1
9 21.515 21.602 21.688 21.775 21.861 21.948 22.035 22.121 22.208 22.294 22.381 22.468 22.554 22.641 22.727 22.814 22.900 22.987 23.674 23.160 23.247 23.333 23.420 23.506 23.593 23.680 23	.766	23.853	÷ 1
34.156 34.242 34.329 34.416 34.582 34.589 34.6/5 34.762 34.848 34.935 35.822 35.188 35.195 35.281 35.368 35.455 35.541 35.628 35.714 35.887 35.974 36.801 36.147 36.234 36.320 36.447 36	1.494 :	36.580	1
./9/ 46.883 46.9/0 4/.056 4/.143 4/.229 4/.316 4/.403 4/.489 4/.5/6 4/.662 4/.749 4/.835 4/.922 48.609 48.695 48.182 48.268 48.355 48.442 48.528 48.615 48.701 48.788 48.874 48.961 49.048 49	1.134 4	49.221	1
Normalized intensity 8.0000	0000 0	0.0000	1
0007 0.0008 0.0010 0.0011 0.0013 0.0014 0.0015 0.0017 0.0019 0.0021 0.0025 0.0025 0.0025 0.0033 0.0055 0.0037 0.0048 0.0048 0.0048 0.0051 0.0054 0.0057 0.0050 0.0053 0.	0067 6	8.00/1	
48 0.2400 0.2444 0.2484 0.255 0.259 0.2559 0.2559 0.2565 0.2754 0.2762 0.2854 0.2888 0.2944 0.3002 0.3006 0.3116 0.3173 0.3223 0.3225 0.3350 0.3352 0.3421 0.3457 0.3506 0.3561 0.3671 0.2671 0.	3680 6	0.3/33	
0.3249 0.3222 0.3493 0.3246 0.3395 0.3395 0.3390 0.3490 0.3490 0.3490 0.3490 0.4940 0.4940 0.4940 0.4940 0.4940 0.4890 0.4880 0.4880 0.4880 0.4935 0.291 0.213 0.214 0.2152 0.214 0.415 0.	23/8 6	0.2331	1
0000 00000 00000 00000 00000 00000 00000	0406 6	0.0087	
	0005 0	0.0000	
	0005 6	0.0008	
aaaa araaaa araada ar	0000 6	5.0000	· .
			~
		2	2

Figure 39. Profile Analysis Report

Run Summary	Lane 1 : 300K 25% viable	(150 mM, Calcein AM, Pro	pidium lodide)					
Run name EXP1537 R7								
Cartridge barcode 013-1121-0096	9							
Protocol / Run temperature Medium Cell (5 - 20µm) / Ambient								
Levitation (Actual / Design) 20 min / 20 min	alitik e kume a d							
Sample collection Sequential	Stain	Split line	Top fraction	Bottom fraction	Top peak position	Top peak width	Bottom peak position	Bottom peak width
EOS Module / Core SN Dory_Beta2 / 10002 Core	Propidium Iodide		1%	99 %			-16.84	13.85
Started by SJ/MM								
Completed on 8/14/2023 4:17 PM								
Updated by gs								
Undated on								



LEVIT-SBIO

LEVIT.SBIO

Updated by ġs Updated on 10/18/2024 3:33 Notes

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Profile Analysis

CLOSING A RUN

Individual runs can be closed independently. Use the X by each run name at the top to close the run. Alternatively the menu option "Close" or "Close all" can be used.

≡	EXP1537 R7	×	exp1624-r1-50mM	×

Figure 41. Closing runs

A run cannot be closed during loading or cannot be closed when analysis is being performed. A dialog box message will appear.



Figure 42. Dialog message when attempting to close while run is still loading



EXITING LEVIMETRICS

It is possible to close an instance of LeviMetrics if you have more than one open. Use the Menu option to exit. Other instances and their opened runs will not be affected.

- 1. Click Exit from the menu
 - **a.** The user will be prompted to confirm closing the user interface.

Note that any analysis will be saved automatically with the run once it is completed.

b. If a run is in progress of analysis or loading, a message will appear noting that the software is not ready to be closed untill the process is complete.



Figure 43. Expanded Menu



Figure 44. Closing application confirmation dialog message



TROUBLESHOOTING

Issue	Possible Resolution
Levitation Agent concentration not reflected in simulation	Concentration entered is outside of normal range. Enter value between 50-200mM
Run cannot be closed	Runs cannot be closed when analysis is in progress or loading
Cannot find generated report	Reports are exported to a Fractionation or Levitation Analysis folder within the same folder where the run *.exp file is stored.
Need to open more than 3 runs for analysis	Open a new instance of LeviMetrics. Go to the desktop icon and double click to open a new instance of LeviMetrics.
Cannot close application	LeviMetrics cannot be closed when a run is loading or when an analysis is in progress
Cannot view fluorescence signal	Toggle the red or green fluorescence imaging button
Cannot simulate Levitation Agent concentration that is within 50-200mM range	Simulations are limited to +/- 50mM range from the original experiment concentration
One lane was not analyzed	A sample loading issue may have interfered with analysis
Cannot see all of the features on my display	Adjust the display resolution on your monitor or reduce the text size in Windows' Display Settings
Only see collection images for one lane	Runs collected using EOS Manager Software v1.0 do not have collection images for all lanes

Table 3. Troubleshooting

Contact **<u>support@levitasbio.com</u>** or call Technical Support at +1-650-204-1185, if any errors are encountered during the procedure.

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