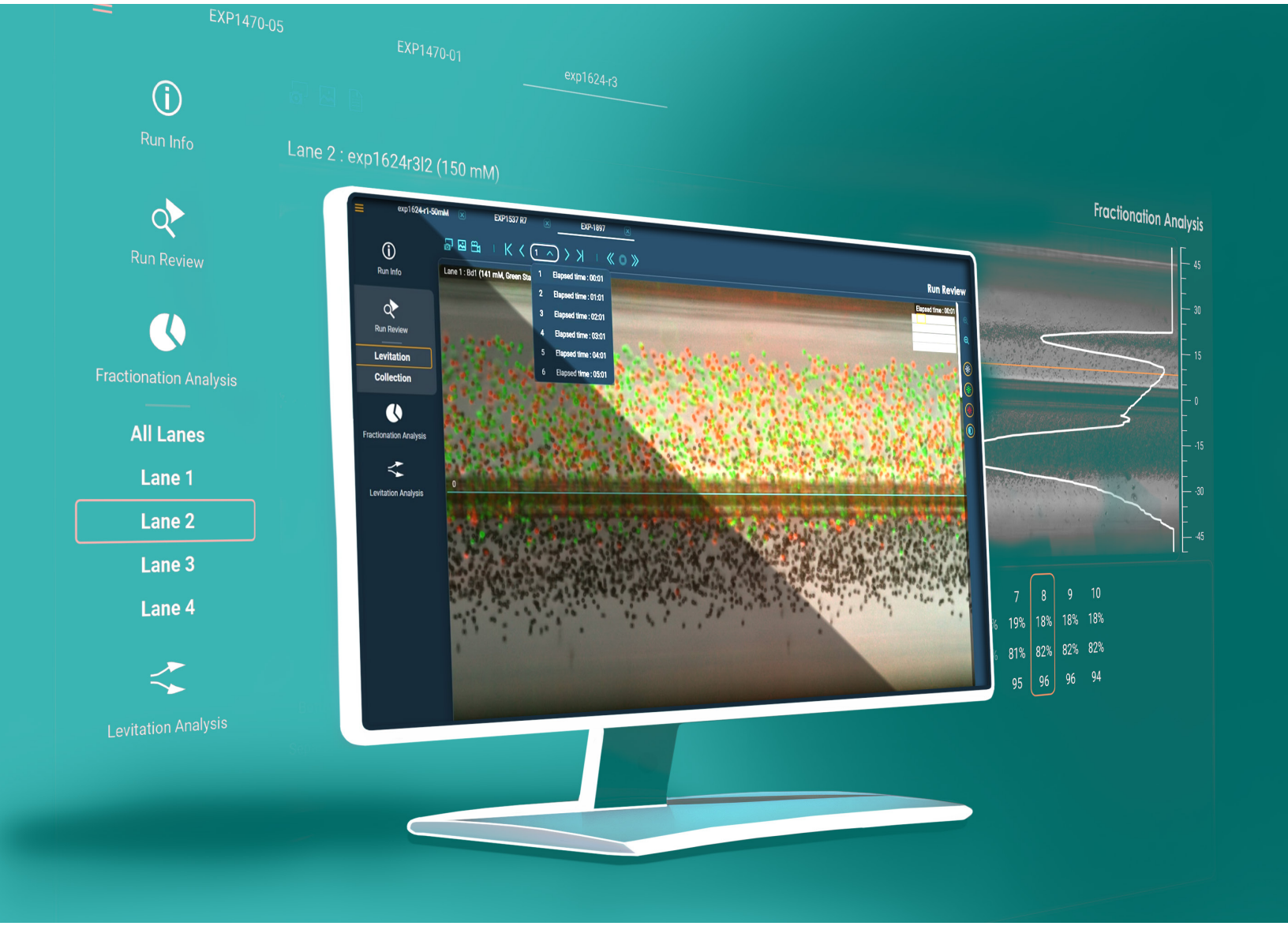
LEVITAS BIO®



LeviMetrics Software

USER GUIDE

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ABOUT THIS SOFTWARE

LeviMetrics™ v2.0 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.

Current released version:	v2.0
Release date:	Jan 2024

Computer Requirements

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

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 v2.0.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

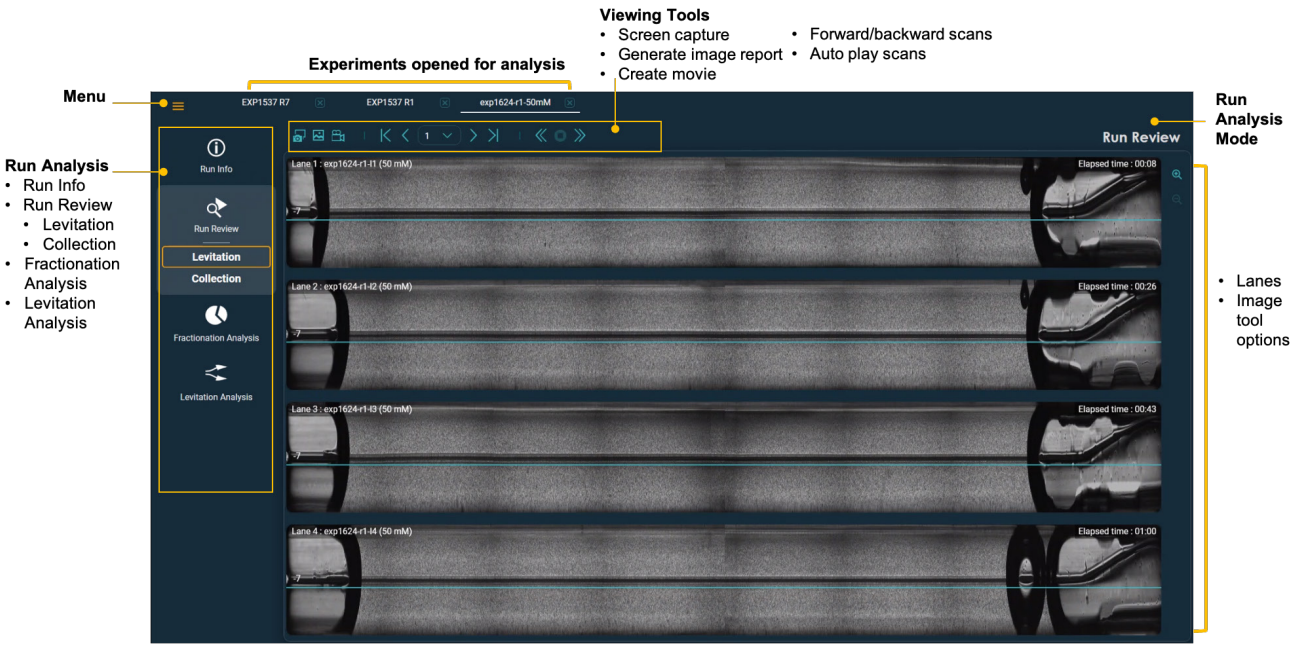


Figure 2. *Software tools overview*

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.

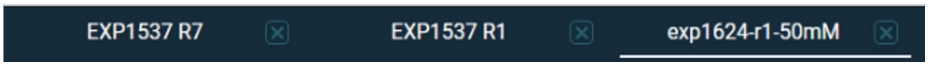


Figure 3. *Open runs*

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.

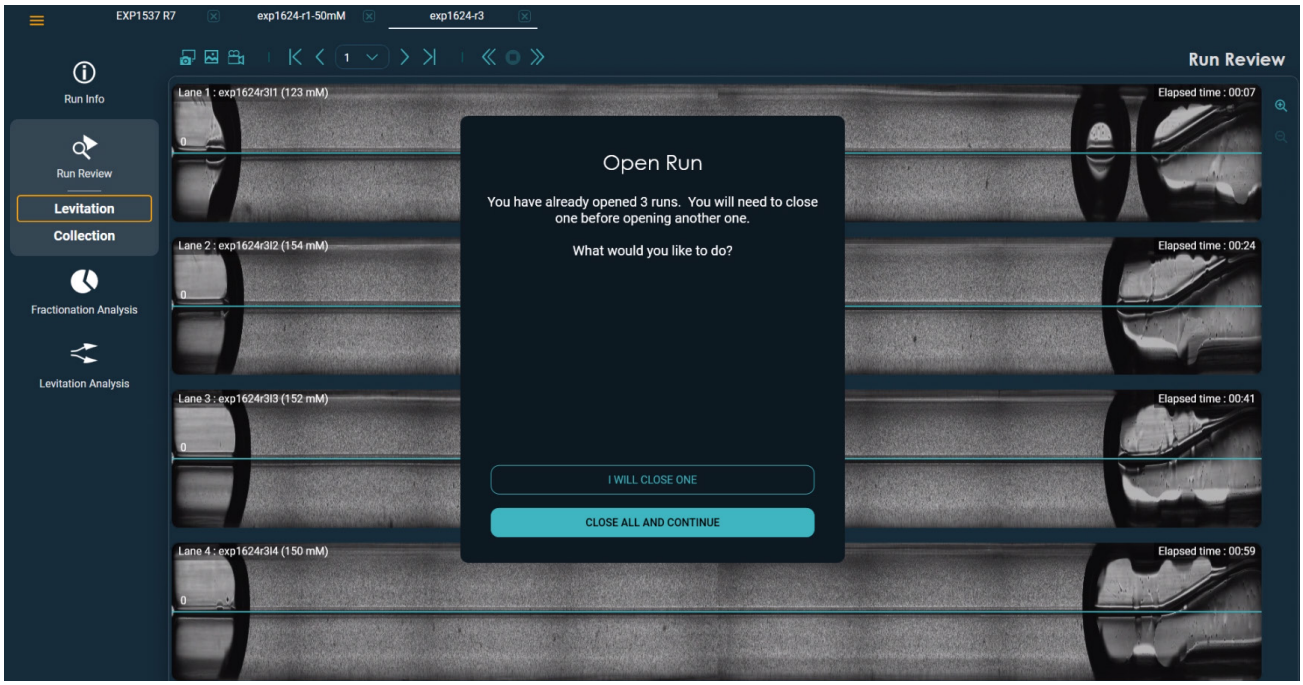


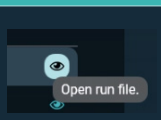
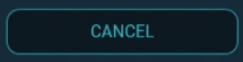
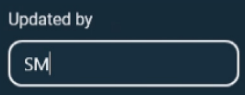
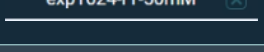
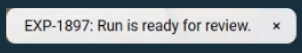
Figure 4. *Dialog message if opening more than three runs*

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	Result	
Button	Hover		Tooltip will appear*
Default Button	Click		Button function
Non Default Button	Click		Button function
Field	Click		Field become editable
Active tool	Click		Turns on and off tool
Active run	Selected		Run will be viewed
Selected mode	Click		Mode viewed or analyzed on the main viewing window
Temporary message	Clear		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

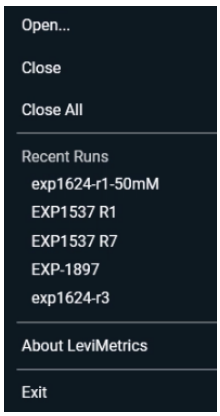


Figure 5. *Expanded Menu options*

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

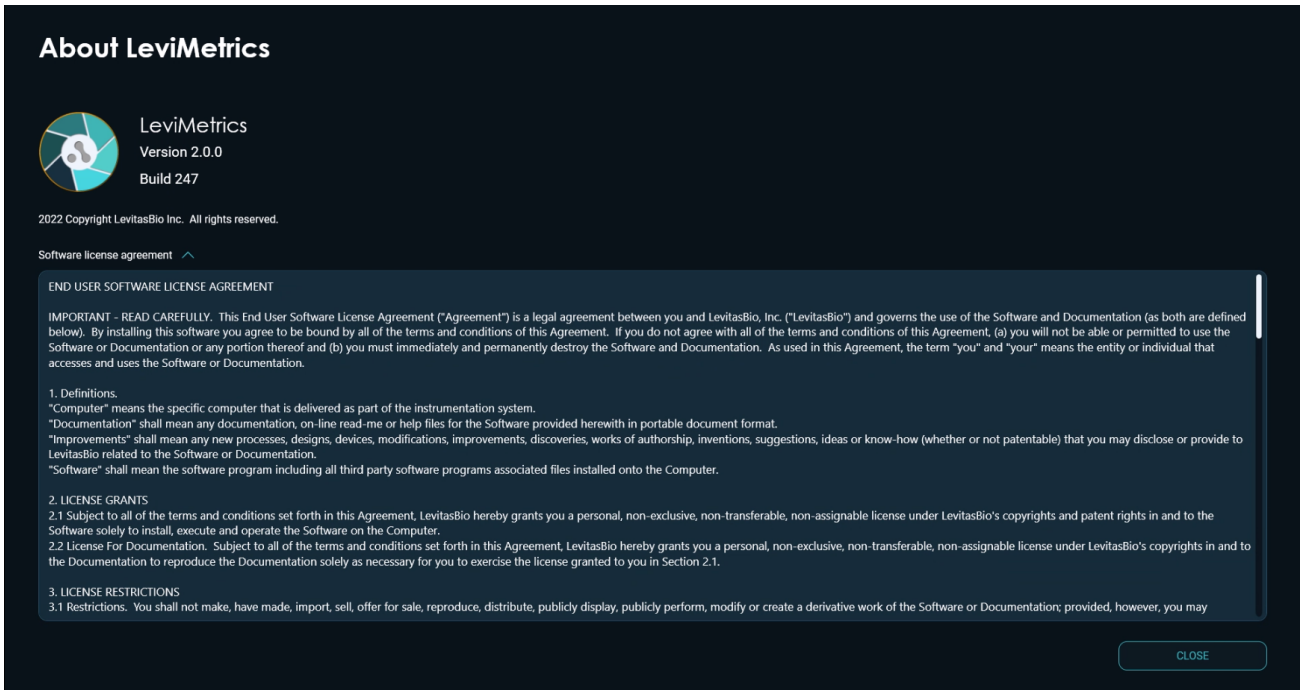


Figure 6. *Expanded About LeviMetrics view*

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.

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

Analysis Icon	Analysis Mode	Analysis Options	Description
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	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
		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

Table 2. *Run analysis modes summary*

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.

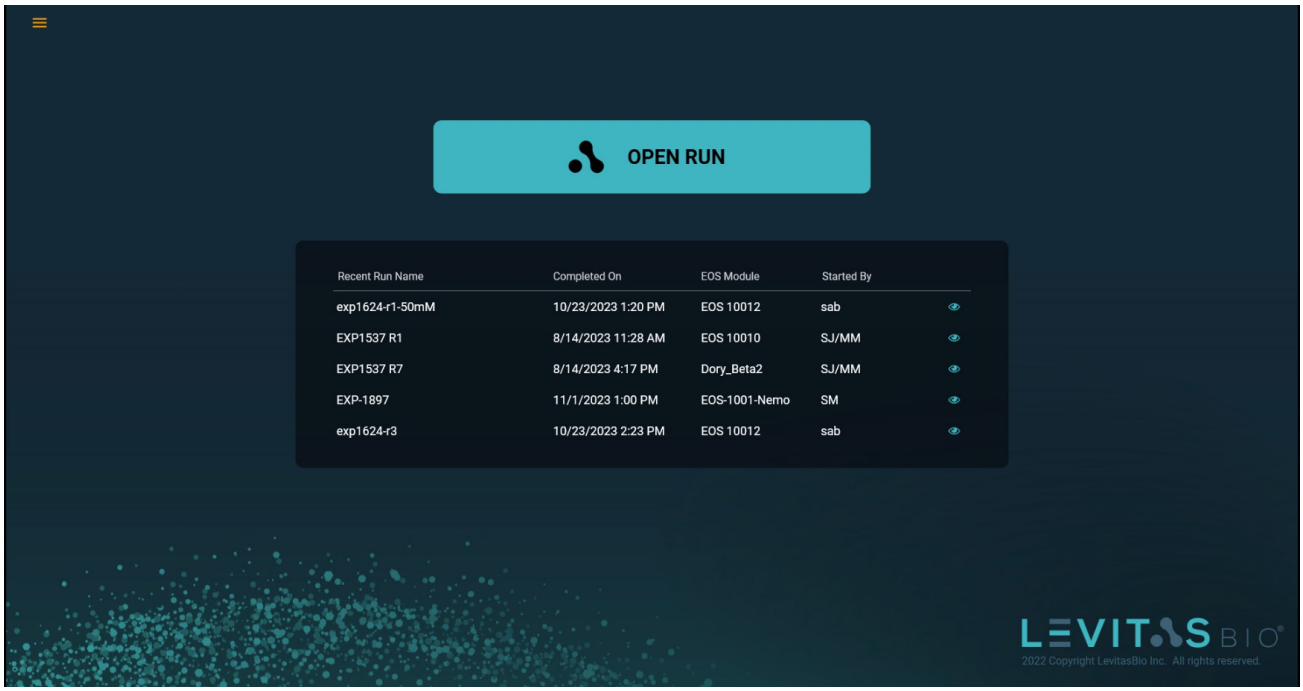


Figure 7. *Home view*

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.

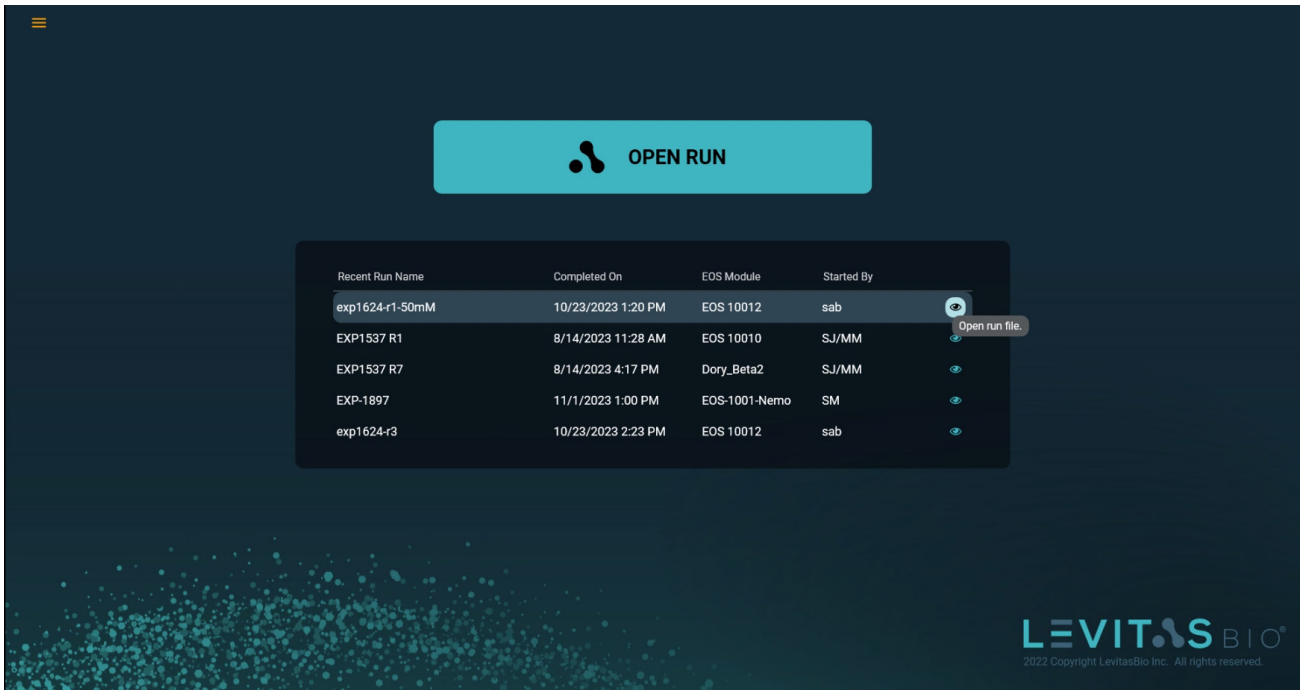


Figure 8. *Open recent run*

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.

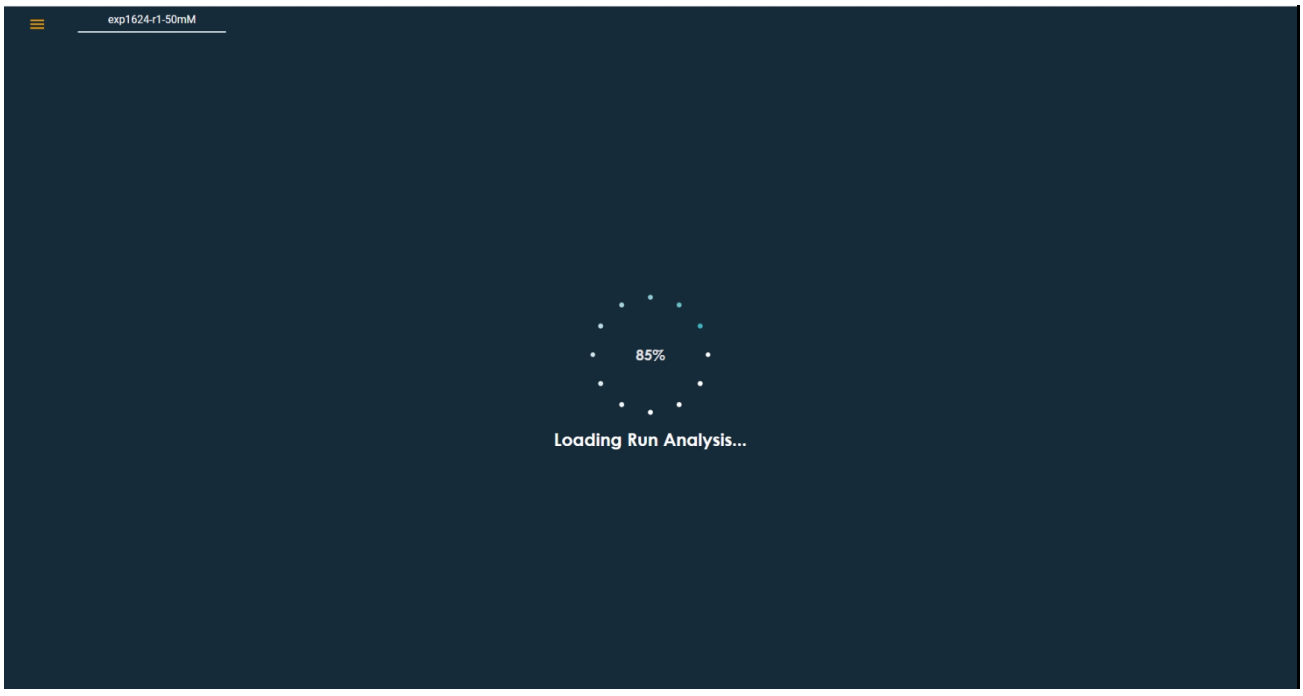


Figure 9. *Progress indicator while loading file.*

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.

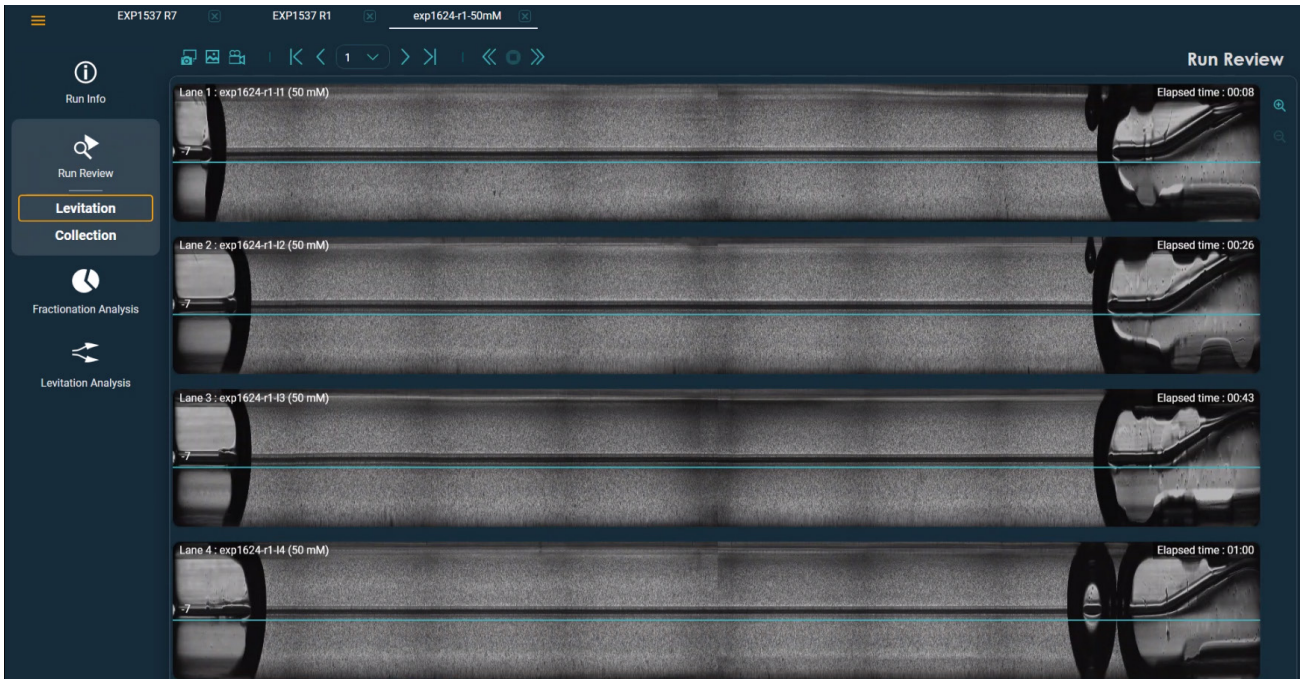


Figure 10. *Run Review Levitation view*

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.

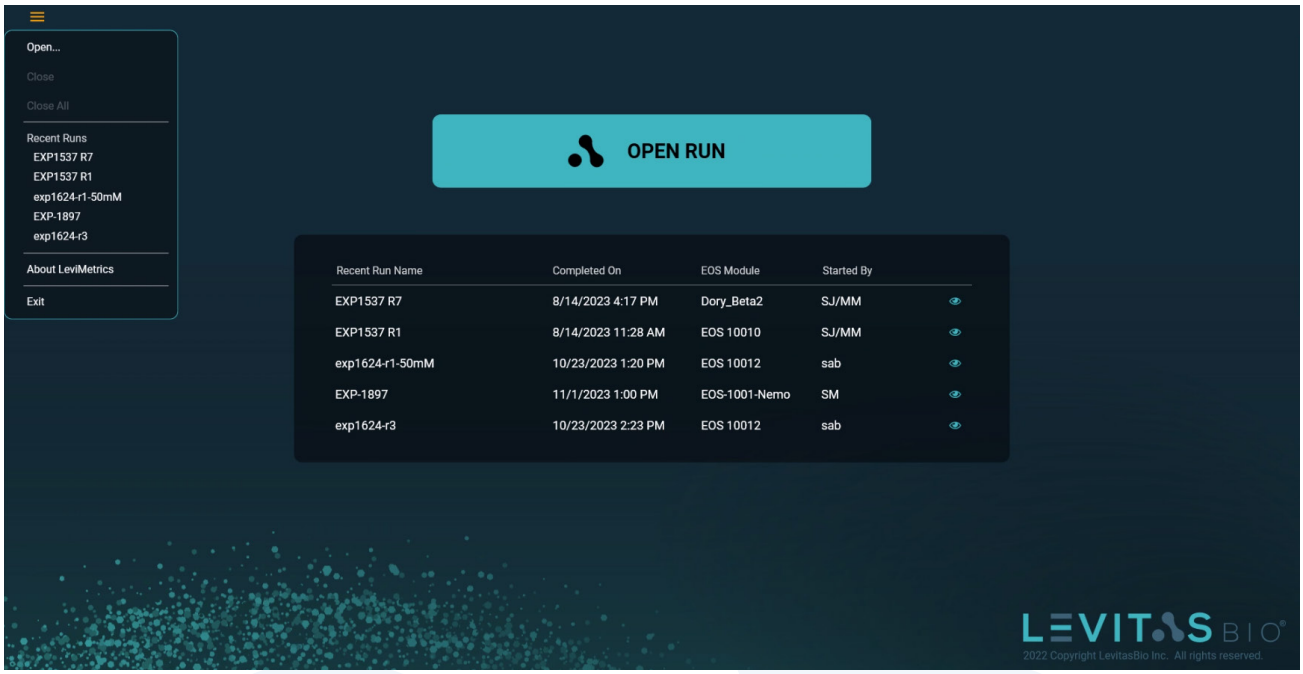


Figure 11. *Expanded Menu to open files*

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 12. *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.

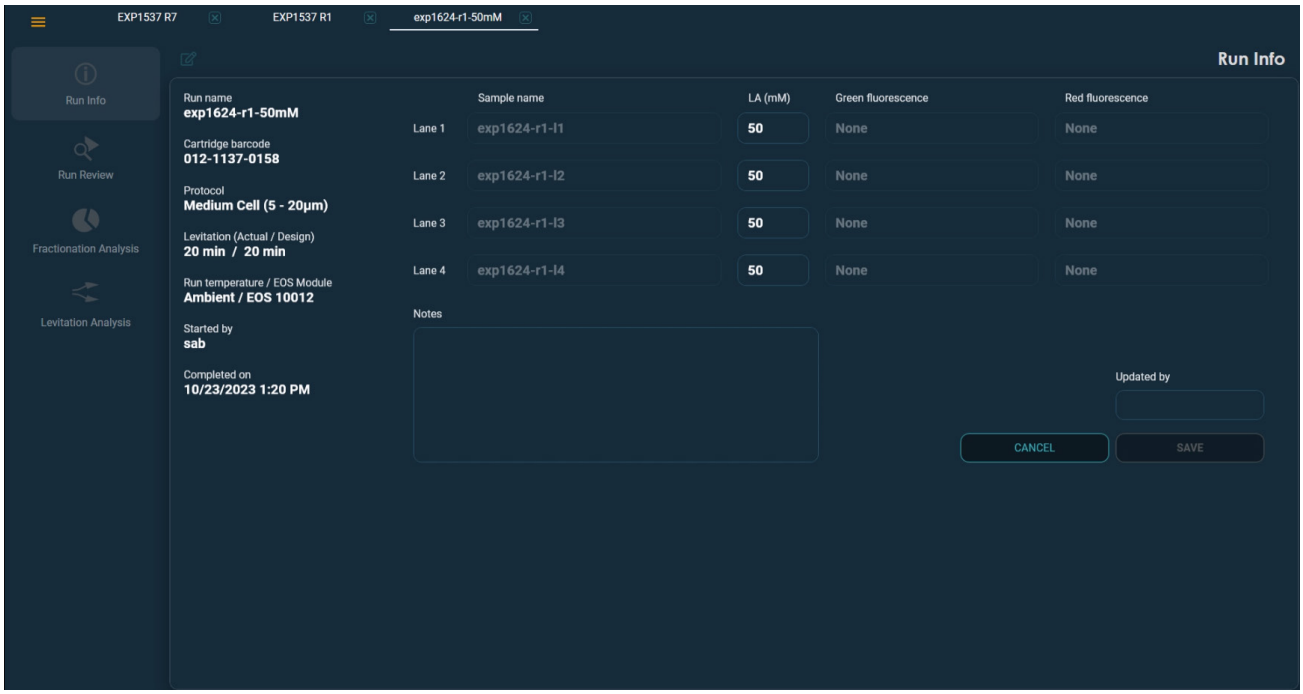


Figure 13. *Edit mode*

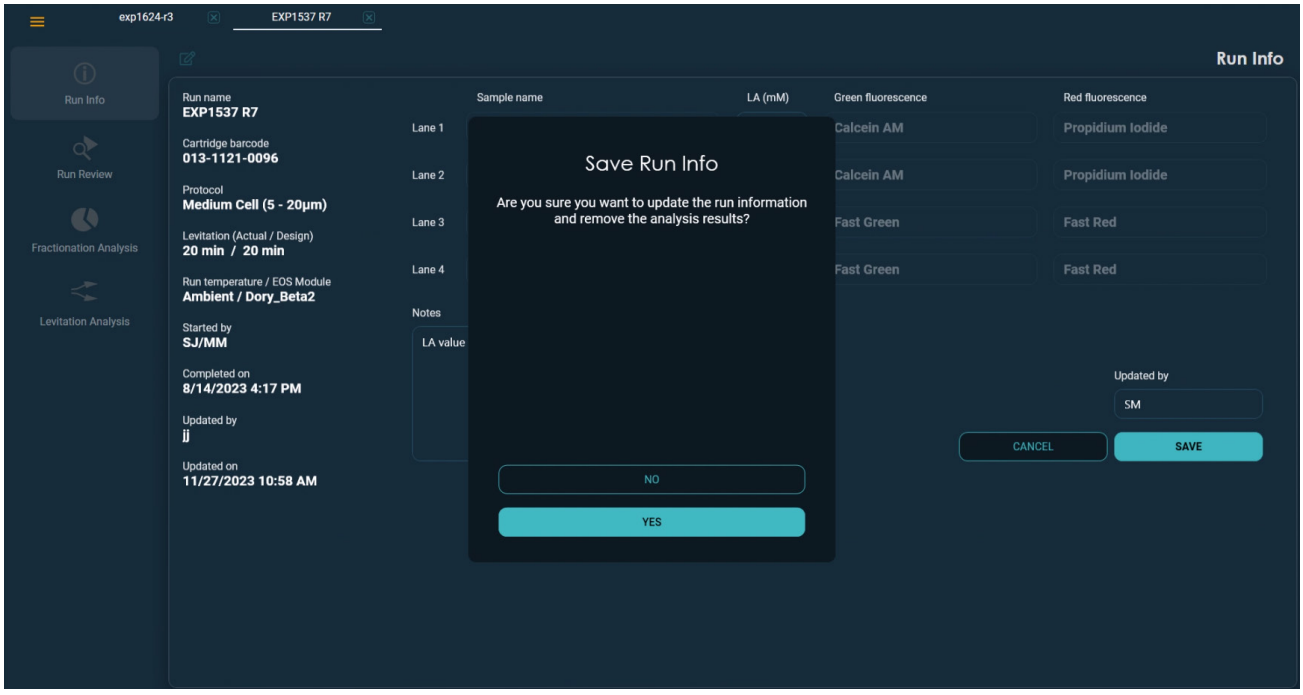


Figure 14. *Save Run Info*

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.

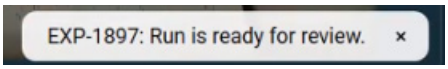


Figure 15. *Message confirming run is ready for review*

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.

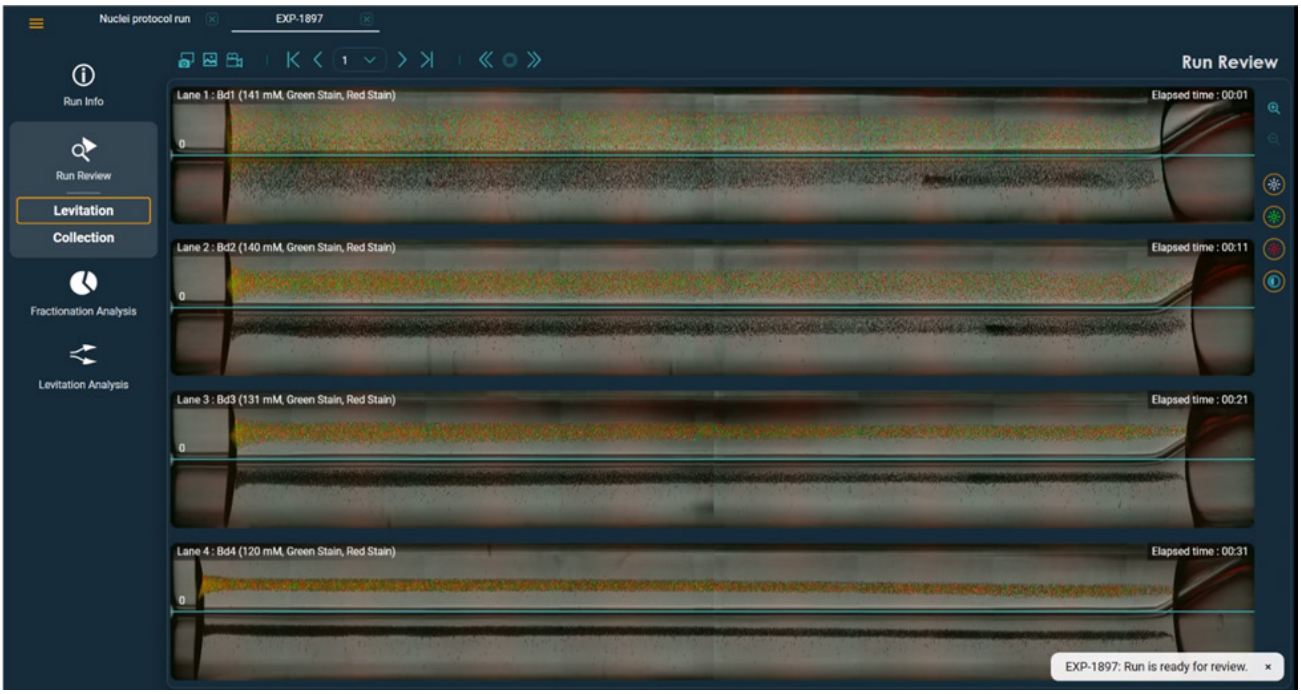


Figure 16. *Experiment run is loaded for Levitation Analysis*

If the run included fluorescent stains, the fluorescent 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.

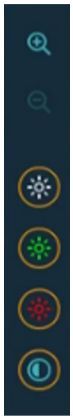


Figure 17. *Imaging toolbar*

Figure 18. *Zoom view*

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

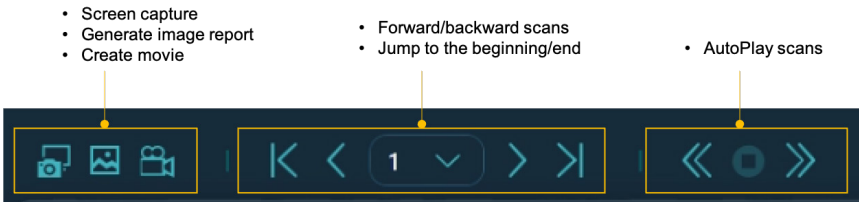


Figure 19. *Viewing toolbar*

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).

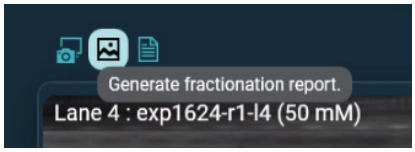


Figure 20. *Generate image review report*

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.

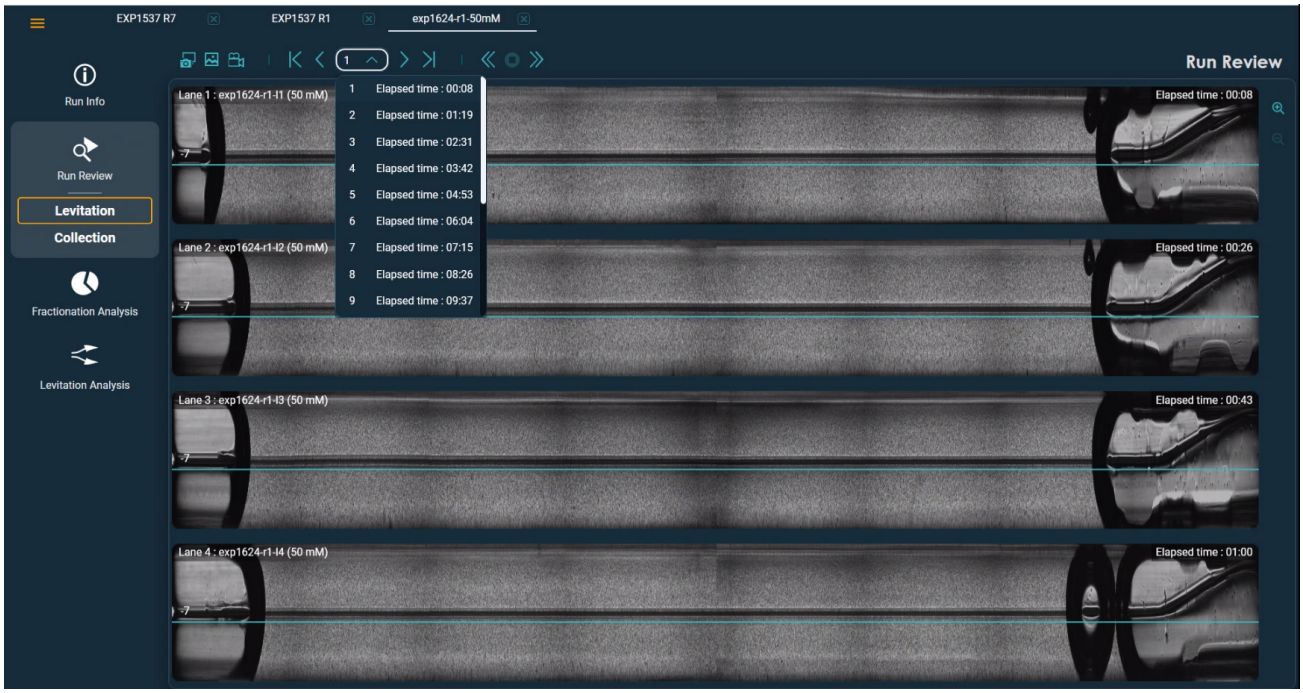


Figure 21. *Drop down menu of completed scans*

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.

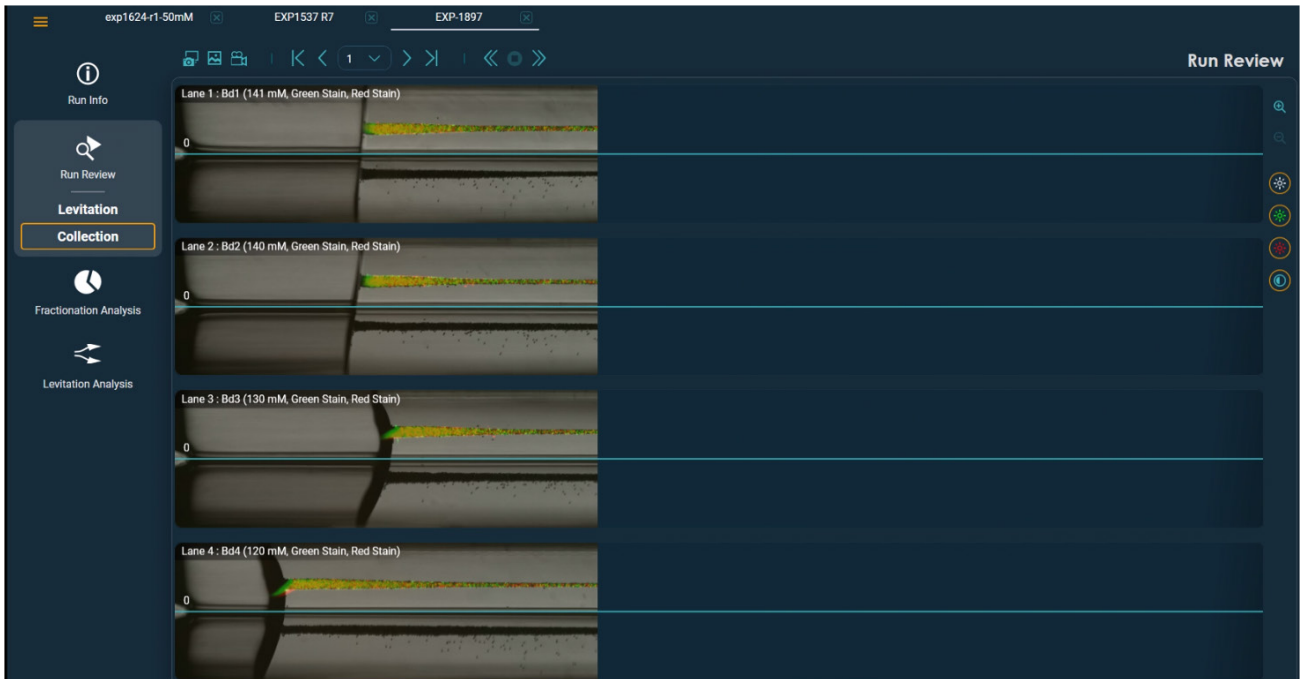


Figure 22. *Collection Review*

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.

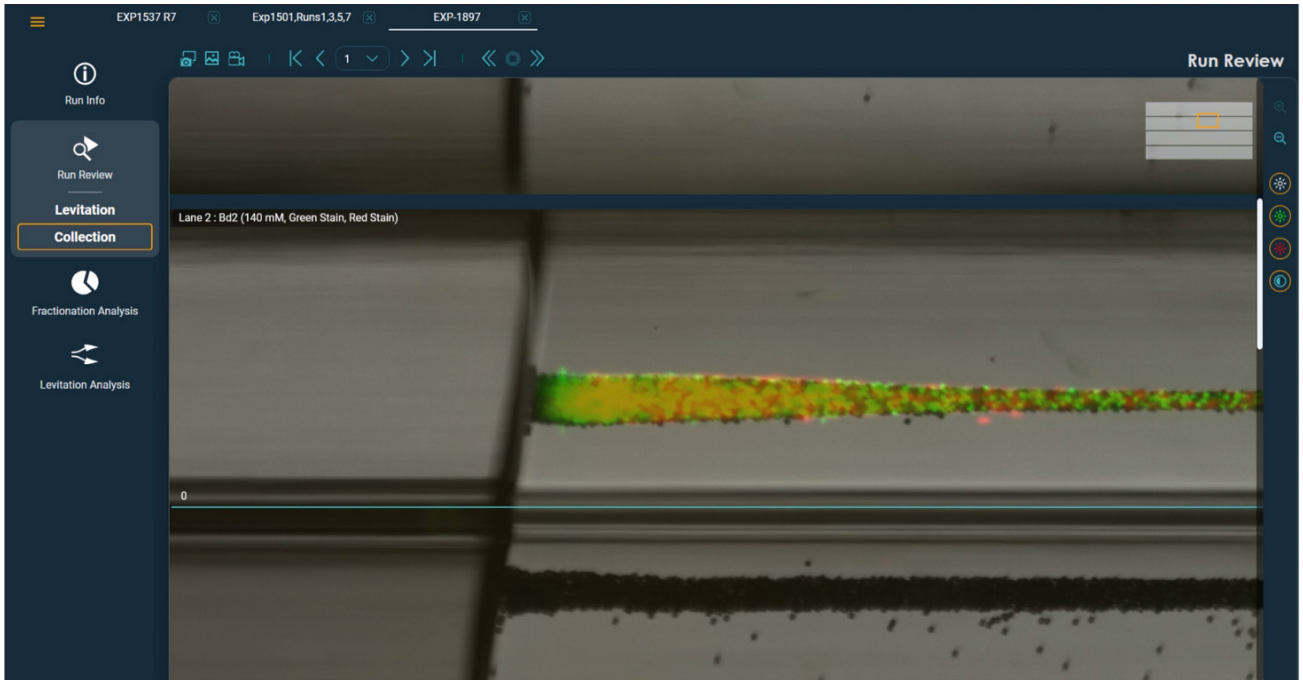


Figure 23. *Max Zoom view in Collection Review*

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.

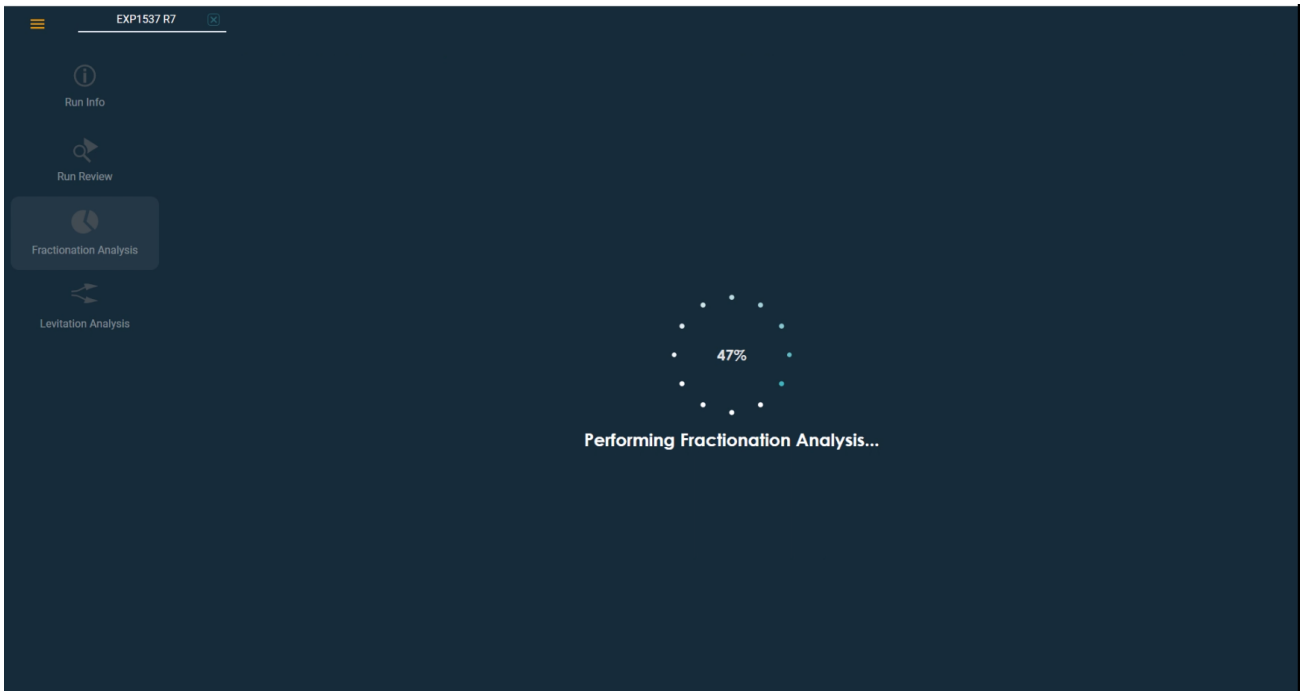


Figure 24. *In progress Fractionation Analysis*

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.

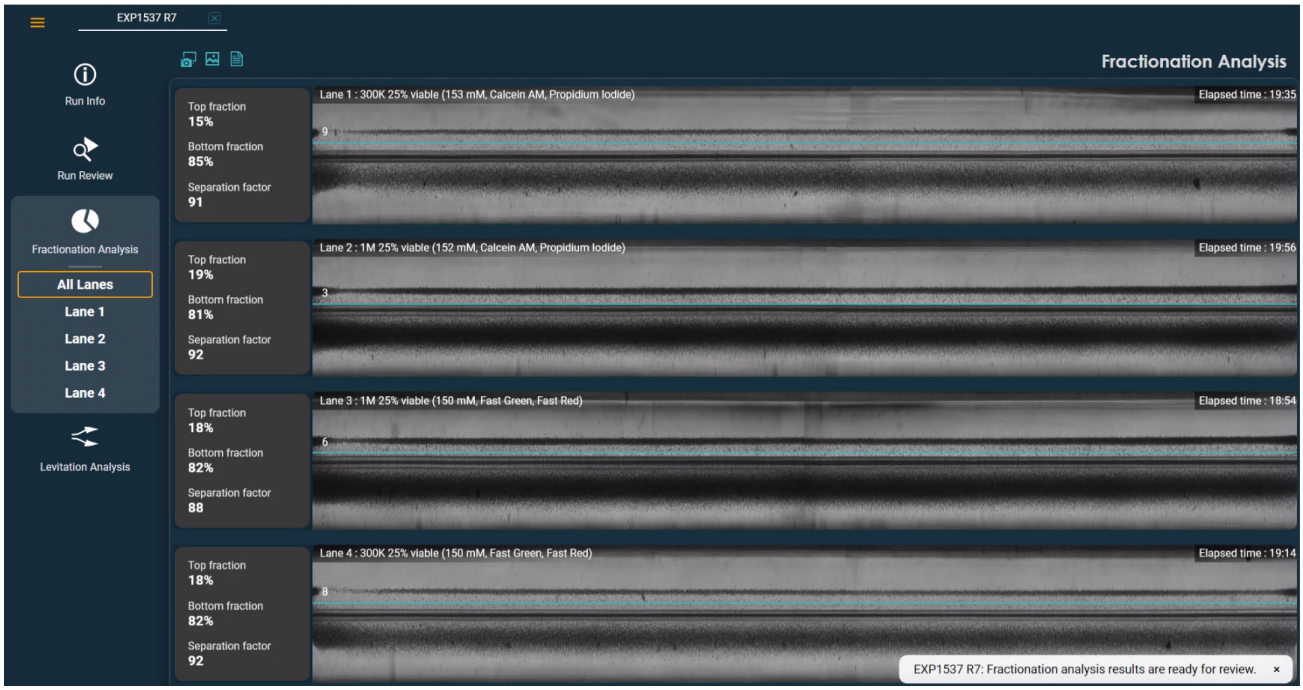


Figure 25. *Fractionation Analysis all lanes view*

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.

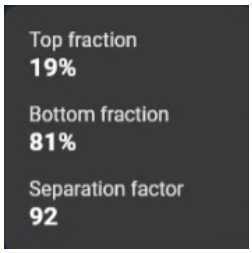


Figure 26. *Fractionation information*

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.

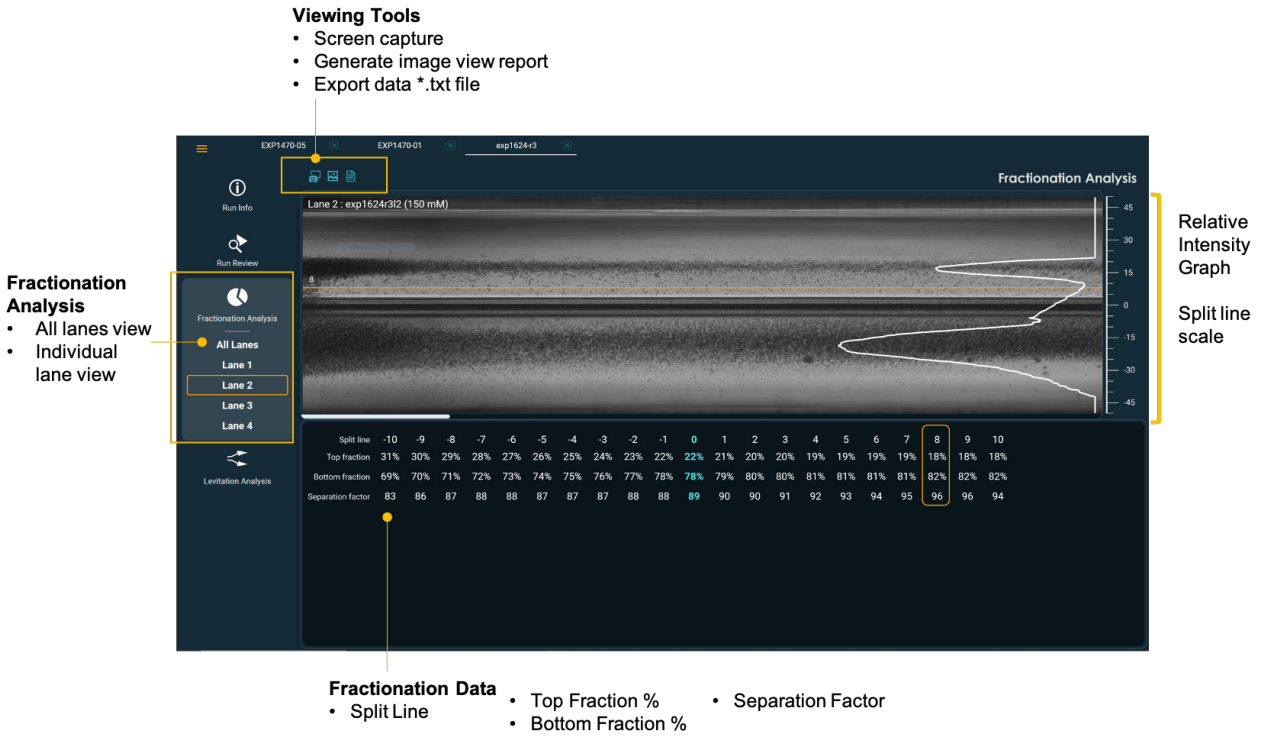


Figure 27. *Fractionation Analysis tools overview*

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

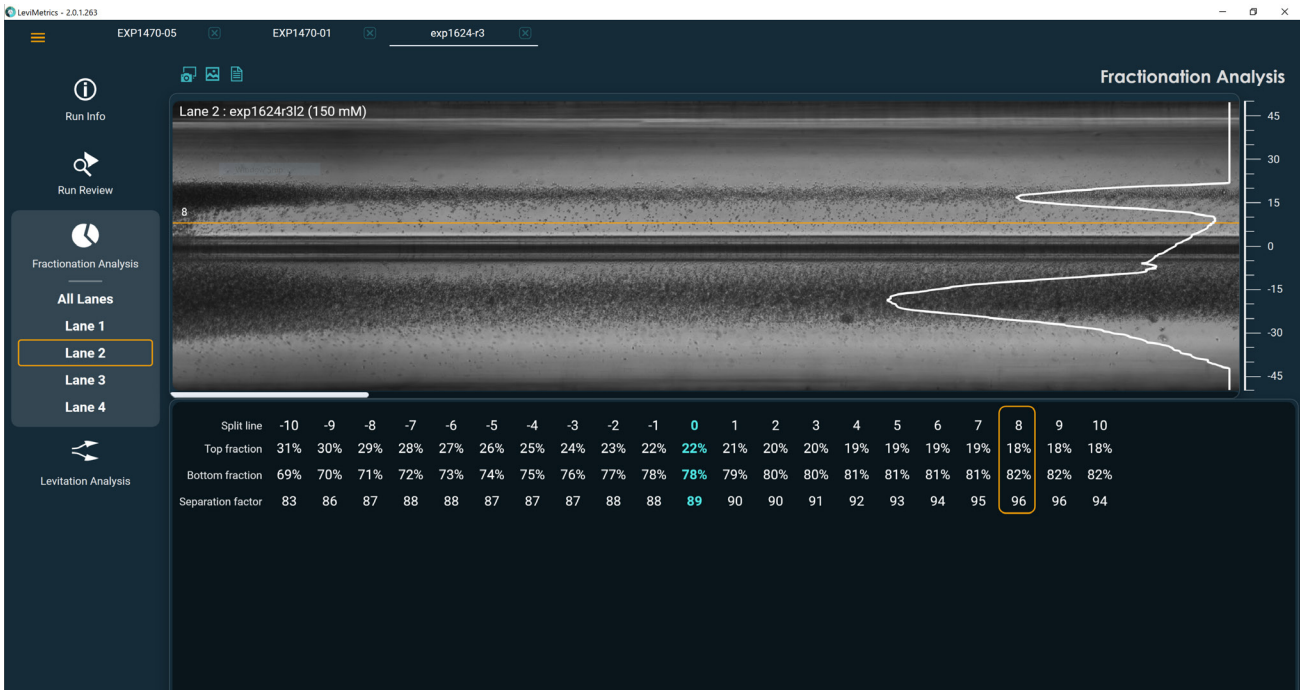


Figure 29. *Fractionation Analysis with different Split Line value*

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.

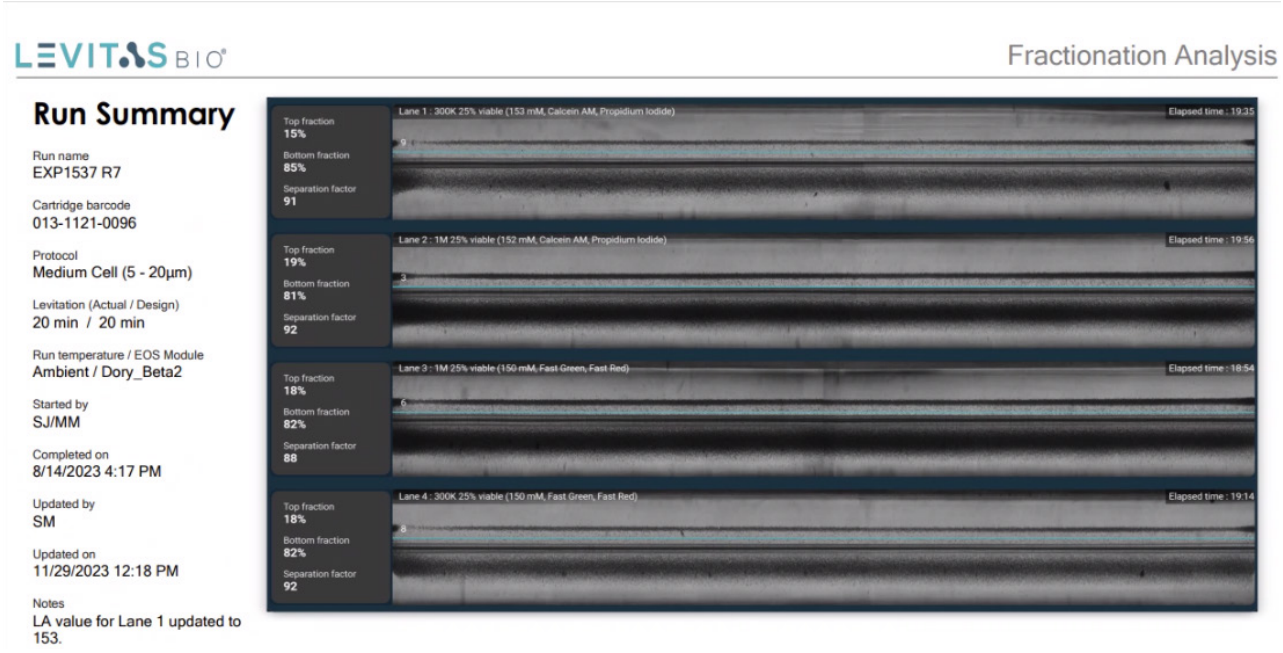


Figure 30. *Fractionation Analysis Report*

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
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
Agent concentration (mM)        153
Split line      9
Top fraction    15%
Bottom fraction 85%
Separation factor       91
Lane    2
Sample name     1M 25% viable
Agent concentration (mM)        152
Split line      3
Top fraction    19%
Bottom fraction 81%
Separation factor       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
```

Figure 31. *Fractionation Analysis exported data file*

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.

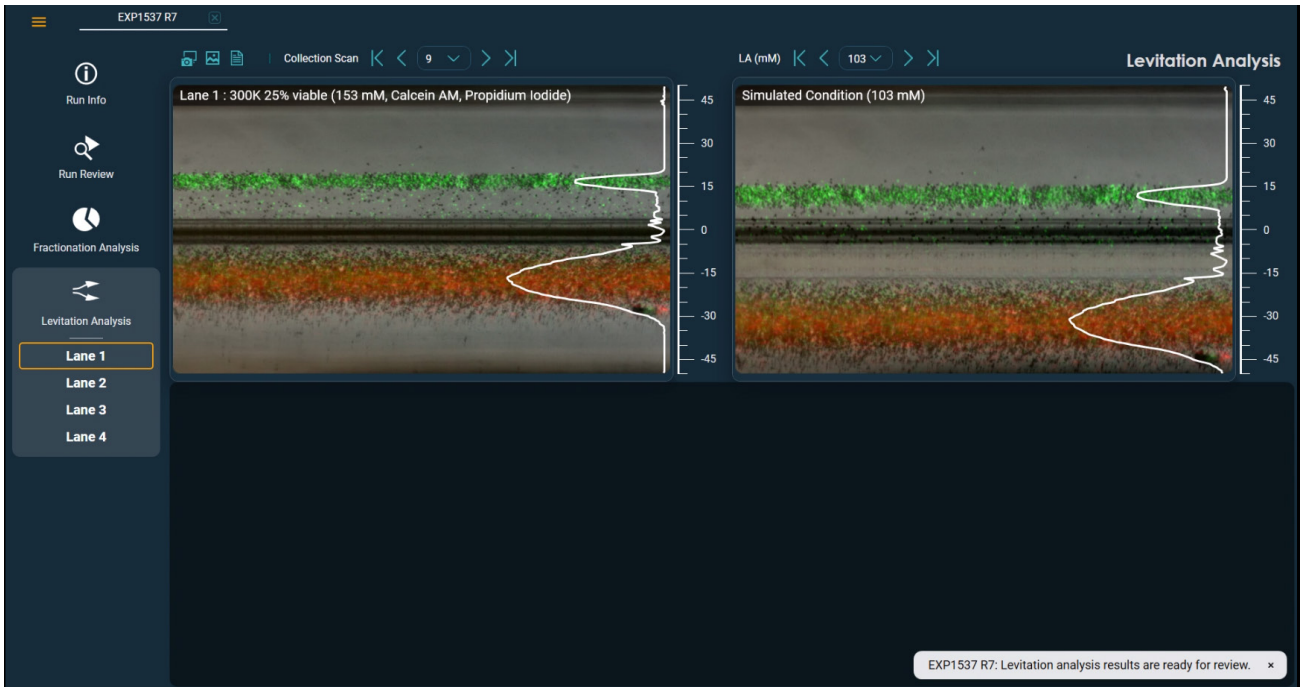


Figure 32. *Levitation Analysis view*

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

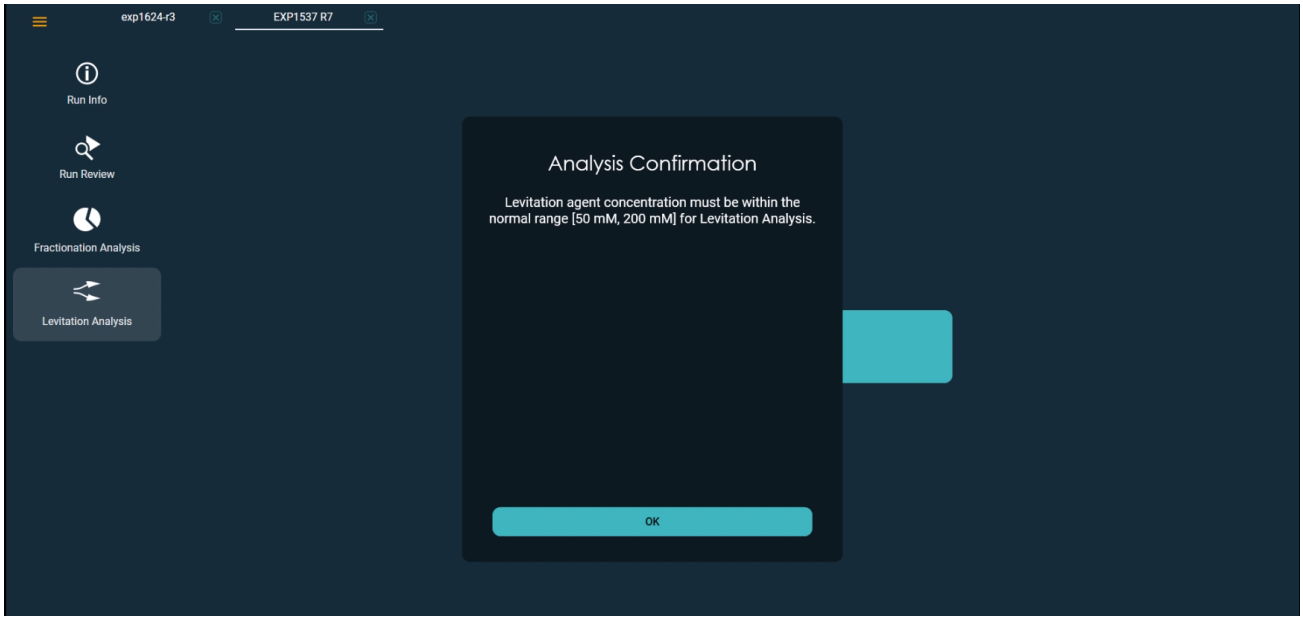


Figure 33. *Levitation Analysis dialog message*

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

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.

EXP1537 R7_Levitation Analysis_Lane 1_300K 25% viable.txt - Notepad

File Edit Format View Help

```
Run Info
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.

Levitation Data
Lane    1
Sample name     300K 25% viable
Collection Scan 9
Agent concentration (mM)        153
Agent concentration (mM) (Simulated)    103
Channel position        -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 -47.054
-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 -24.697
.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
.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
Normalized intensity    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  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
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
Normalized intensity (Simulated)        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
```

Figure 34. *Levitation Analysis data text file*

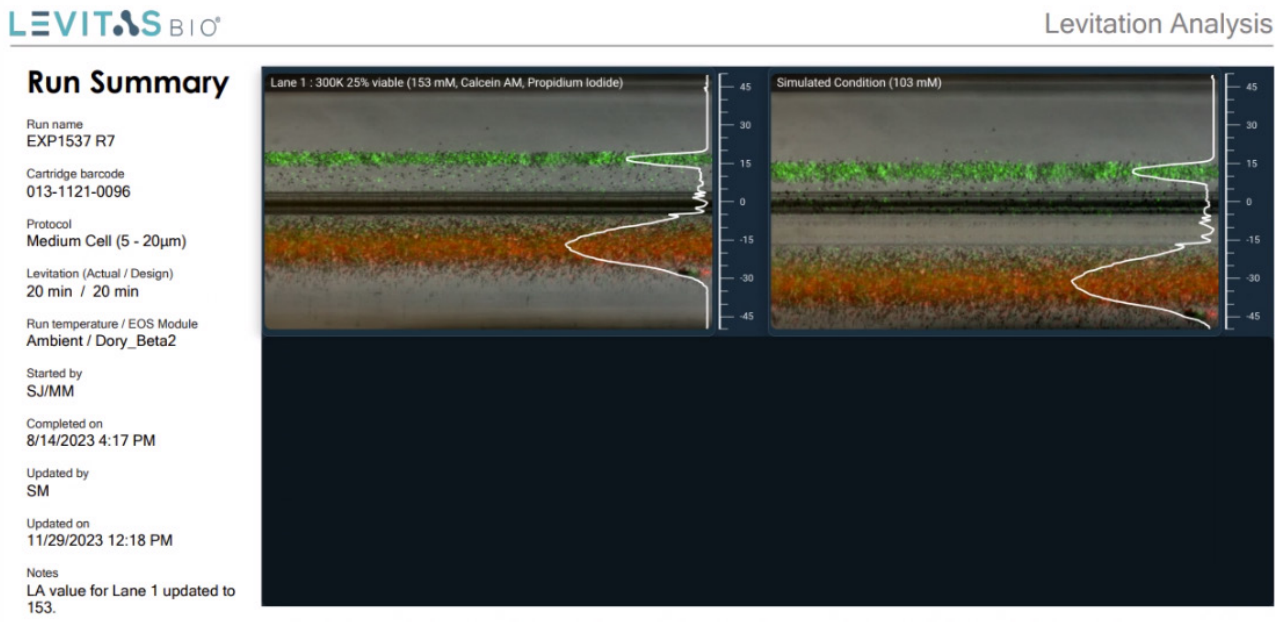


Figure 35. *Levitation Analysis Run Summary Report PDF*

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.

Figure 36. *Closing runs*

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

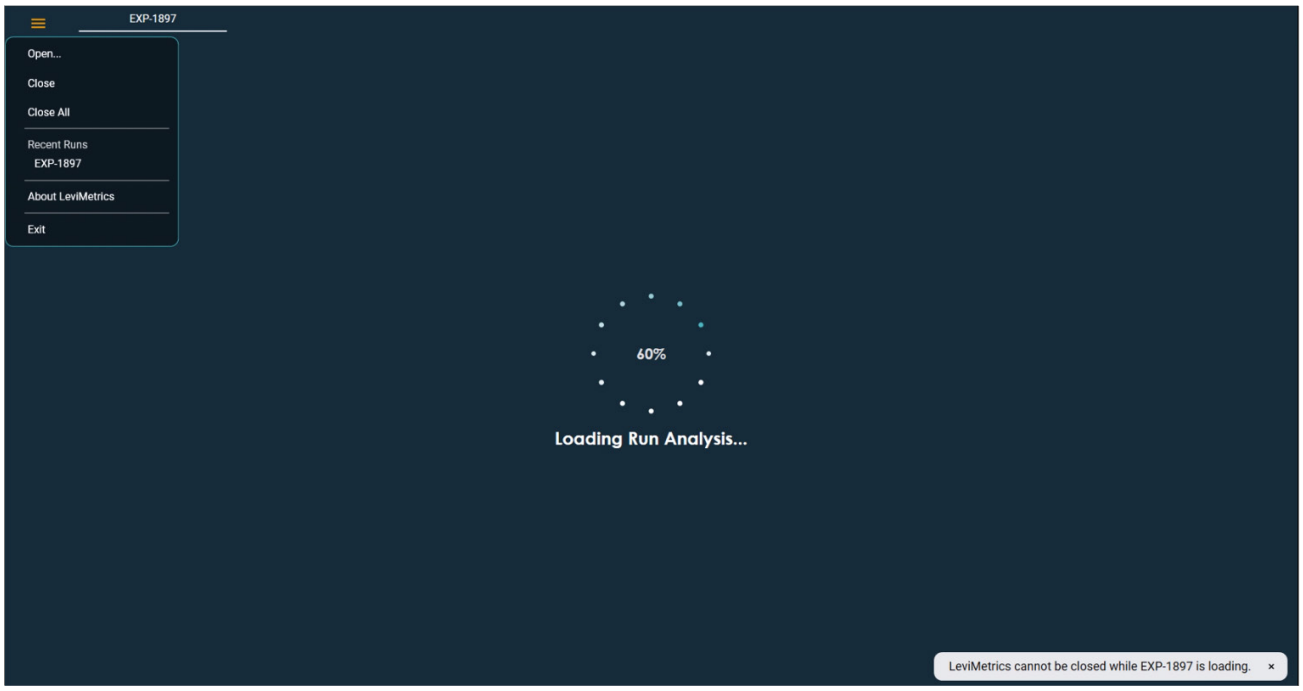


Figure 37. *Dialog message when attempting to close while run is still loading*

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.

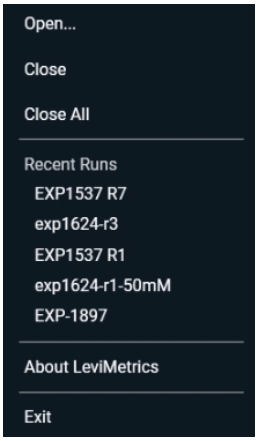


Figure 38. *Expanded Menu*

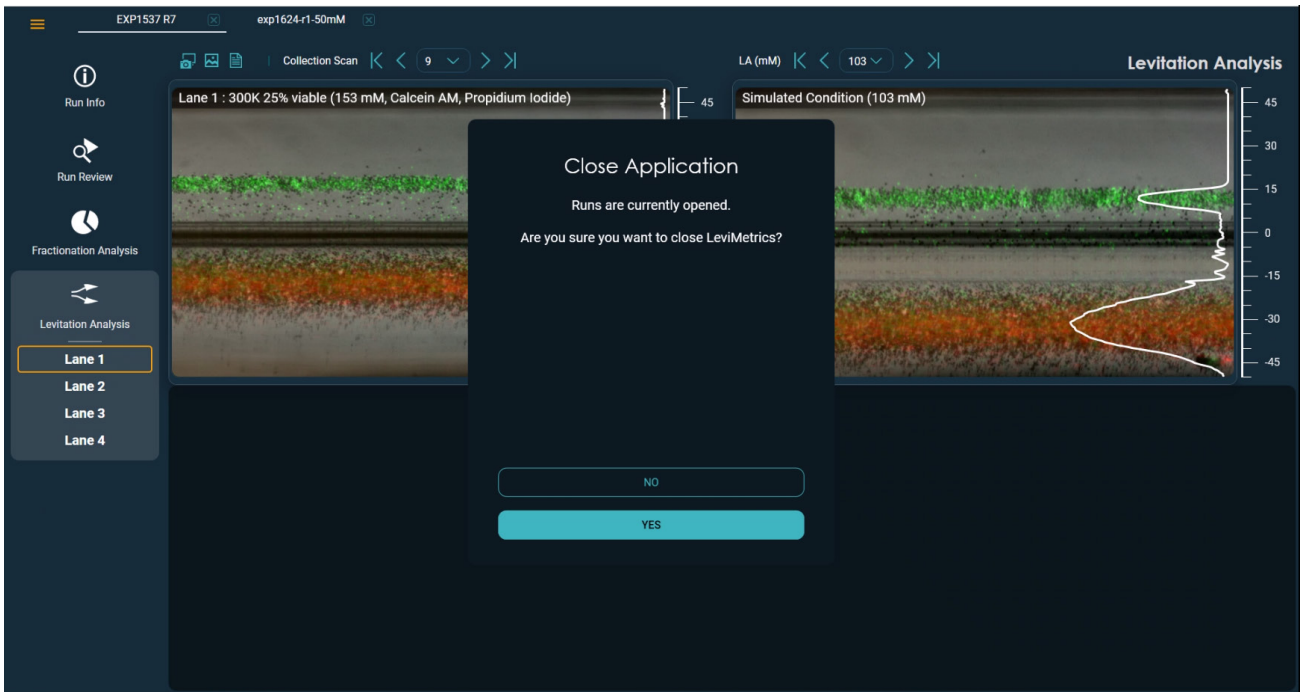


Figure 39. *Closing application confirmation dialog message*

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