LEVITAS BIO®


EXPERIMENT ANALYZER
USER GUIDE

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ABOUT THIS SOFTWARE

Experiment Analyzer is designed to work with data image files obtained from the LeviCell 1.0 System. The Experiment Analyzer software is compatible with a Windows 10 computer. For PC use only.

This software can be but, is not required to be installed on the provided computer that comes with the LeviCell Instrument.

Computer Requirements

Operating System:	Windows 10 or 11
Processor:	Dual-core, 2.5 GHz Processor
RAM:	8 GB installed memory (preferred 16 GB or higher)
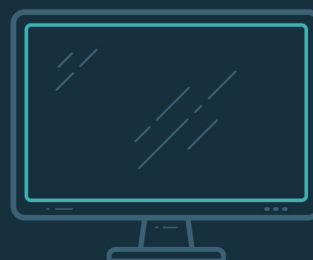
SOFTWARE INSTALLATION

1. Go to support website (www.levitasbio.com/support)

2. Download the .msi installer to the PC computer with Windows 10 or Windows 11 64-bit.

3. Locate the downloaded zip file in the Downloads directory.

4. Double click the installer file (.msi).

5. Follow the instructions to install the new software until you have completed the installation.

6. Locate the new desktop icon.

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

USING EXPERIMENT ANALYZER

The Experiment Analyzer provides a simple and fast method to work with the LeviCell 1.0 Image files.

Getting Started

1. Double Click to open the Experiment Analyzer.

2. The Experiment Analyzer main user interface will appear and fill the screen.

3. A list of analysis options will be available for selection.

Exiting Program

1. Click **Exit** from the menu or Click ESC Key.

   a. If you click ESC, you will be prompted to confirm closing the user interface.

2. Once you have exited from the Experiment Analyzer main user interface, the Experiment Analyzer program window will appear.

3. Close the program by simply clicking on the "X" icon on the upper right.

If you would like to re-enter the main user interface simply click the Experiment Manager logo on the upper left corner.
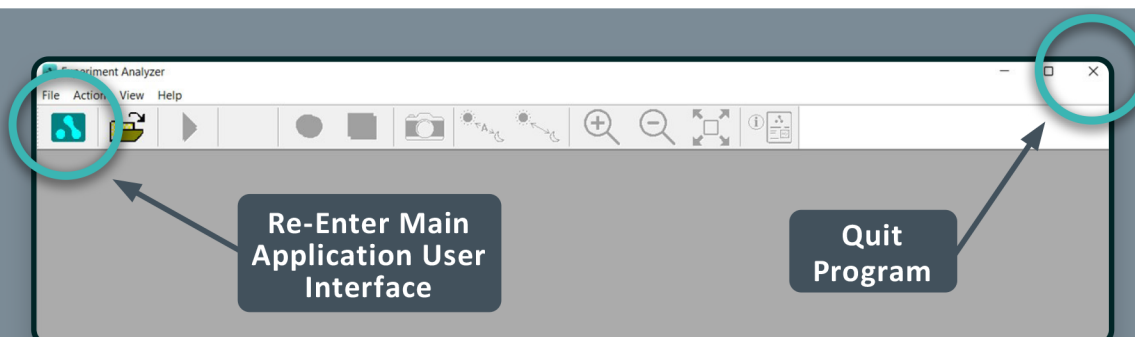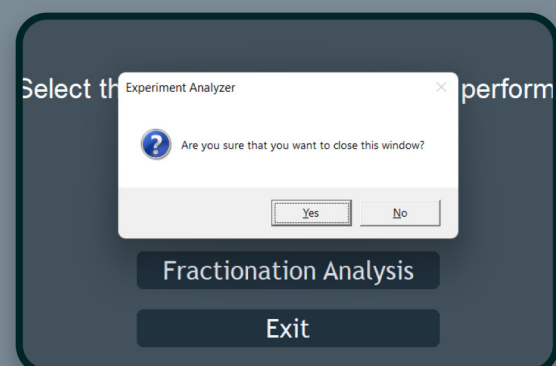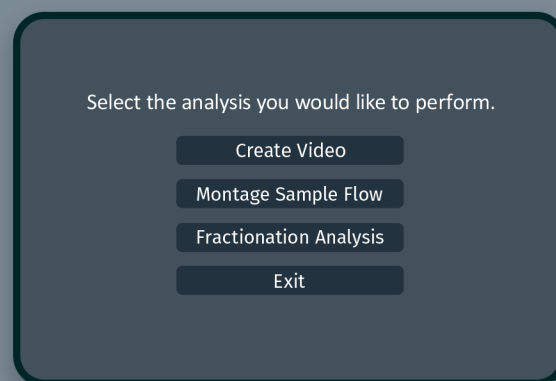
ANALYSIS TYPES

There are 3 different types of analysis types that can be generated using Experiment Analyzer.

- Create Video
- Montage Sample Flow
- Fractionation

All the analysis types will require the **ExperimentSettings.ini** file, the log file, and all data image files obtained and stored during a run using the LeviCell Experiment Manager. These files are stored in the same folder on the LeviCell computer.

Image files 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 user may select up to 3 image channels **(Brightfield, and if available, Ex474/Em524, and Ex560/Em628)** to record during the run. Therefore there can be up to 3 sets of files per experiment corresponding to the respective channels imaged. The image file format is .png (Portable Network Graphics).

| Eg.	20220304-1158-06_Exp100_Brightfield_001.png
	20220304-1158-06_Exp100_Green(Ex474-Em524)_001.png
	20220304-1158-06_Exp100_Red(Ex560-Em628)_001.png

Create Video

A time lapse video can be created to visualize the levitation and collection of your samples during a run. During the run, a set of images are captured at short intervals over the length of the entire workflow. The video compiles a subset of these images to display the levitation and collection at 5 frames per second.

The video can be created using brightfield, one fluorescence channel or all channels provided these images were collected. In addition the split value chosen for the run will also be displayed as a horizontal blue line.
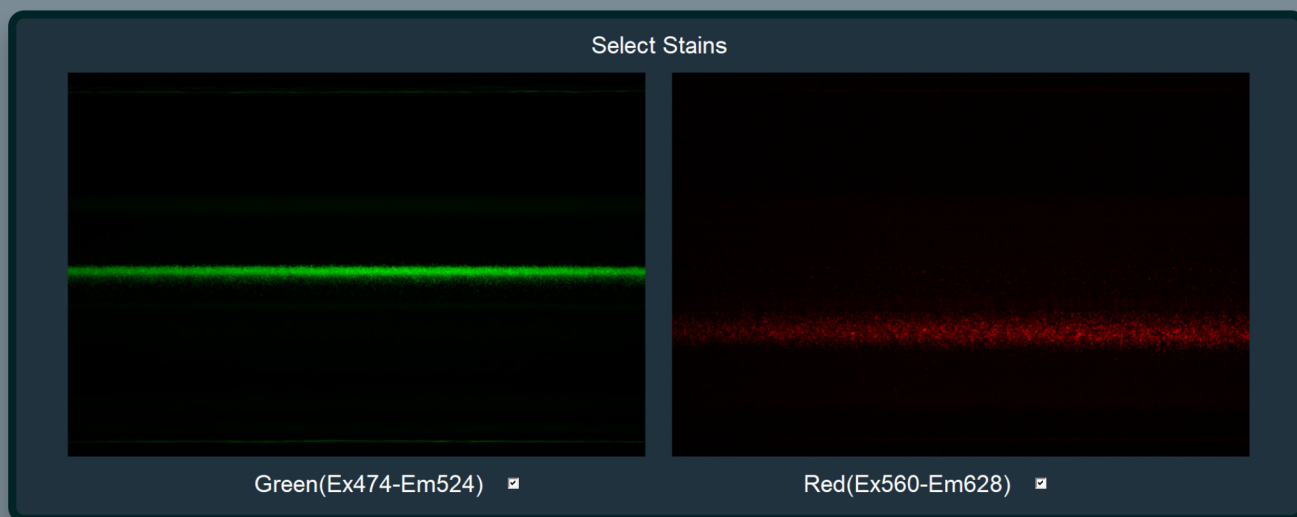
1. Click **Create Video** from the analysis menu screen.
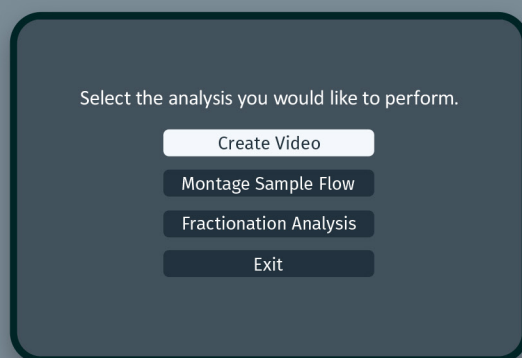
2. Click Browse to open a window to select the **ExperimentSettings.ini** file location.
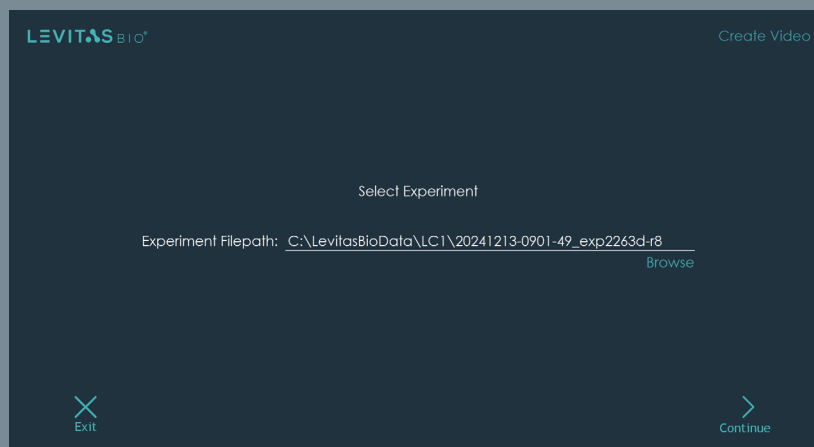
3. Click **Continue.**

4. If the experiment contains fluorescence images, the software will ask the user to choose which fluorescence channel images to be included in the video. If none are chosen only brightfield will be used.

5. A fused image will be displayed for verification. Click **Continue** if all channels are displaying as you have previously selected.

NOTE: This screen will be skipped if no fluorescent stains are selected
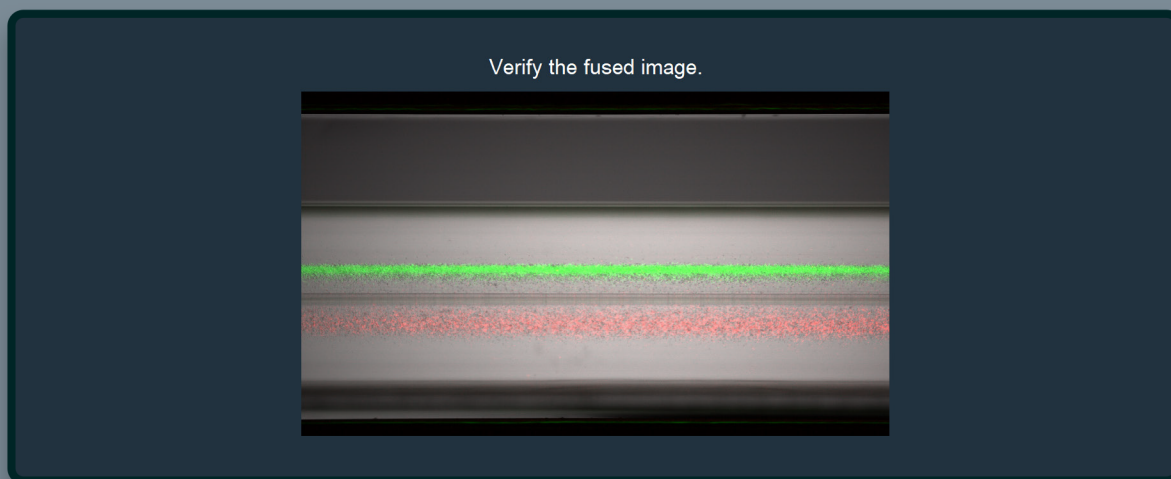
6. Enter a filename for the final video.

Enter Filename

Video Filename: Video_EXP976_FL

7. The video will now be assembled. This can take a few minutes.

Producing video from image files. Please wait.

8. A confirmation message will appear with the file path to the final video created.

The video file 'Video_EXP976_FL.mp4' can be found in:

C:\Users\Public\Documents\EXP976_Images\Video

*Video files *.mp4 can be viewed with any standard video player.*

Montage Sample Flow

During separation, images are captured as the sample flows by the camera. From these images, a view of the entire sample can be assembled or stitched together to create a montage image (shown below in brightfield only). This whole sample view provides visualization of the final levitation position of the top and bottom fraction.
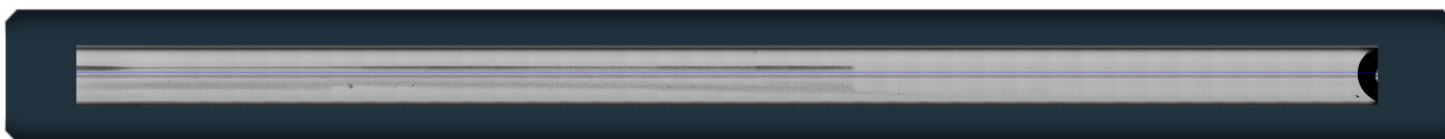
1. Click **Montage Sample Flow** from the analysis menu screen.
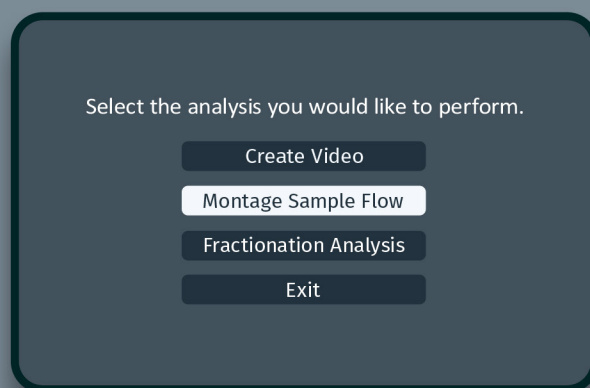
2. Click **Browse** to open a window to select the **ExperimentSettings.ini** file location.
3. Click **Continue**.
4. If the experiment contains fluorescence images, the software will ask user to choose which fluorescence channel images to be included in the montage image. The example images will be shown for each channel. If none are chosen only brightfield will be used.
5. A fused image will be displayed for verification if fluorescent stains are chosen. Click **Continue** if all channels are displaying as you have previously selected.
6. Enter a filename for the final image montage.

7. The montage image will now be constructed. This can take a few minutes

> Constructing montage image. Please wait.

8. A confirmation message including the file path to the final montage image will be displayed. The montage image will also be seen showing the entire sample flow.

> The montage image file Montage_EXP976_Brightfield.png' can be found in:
>
> C:\Users\Public\Documents\EXP976_Images\Montage
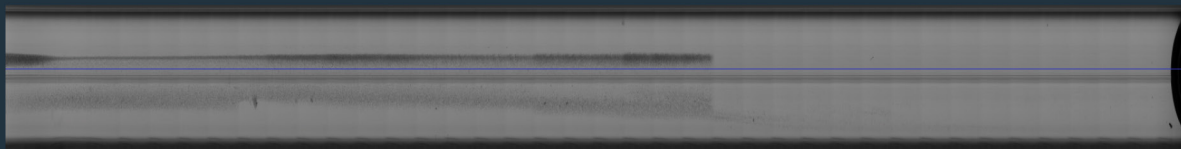
Fractionation Analysis

The same images used for the montage are analyzed for fractionation analysis. 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 split line.

1. Click **Fractionation Analysis** from the analysis menu screen.
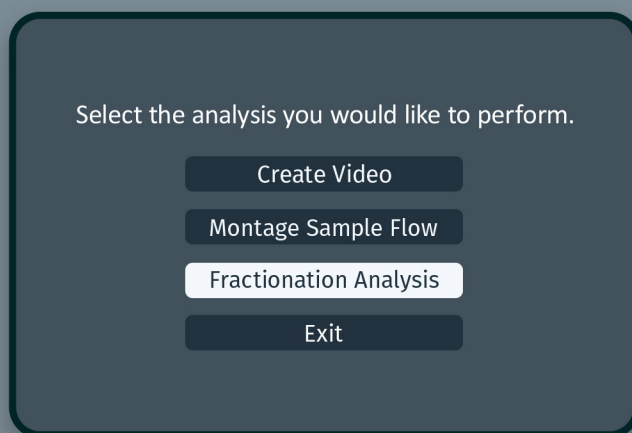
Select the analysis you would like to perform.

Create Video

Montage Sample Flow

Fractionation Analysis

Exit

2. Click **Browse** to open a window to select the **ExperimentSettings.ini** file location.
3. Click **Continue**.
4. If the experiment contains fluorescence images, the software will ask the user to choose which fluorescence channel images to be included in the image display. If none are chosen, only brightfield will be used to generate the display image.
5. A fused image will be displayed for verification if fluorescent stains are selected. Click **Continue** if all channels are displaying as you have previouly selected.
6. Enter a filename for the final fractionation report.

Enter Filename

Report Filename: FractionationAnalysis_EXP976_FL

7. The data will now be analyzed to extract information about the sample. This can take a few minutes.

> Data analysis in progress. Please wait.

8. A confirmation message including the file path to the data analysis report (*.txt) will be displayed. A montaged image will also be displayed for visualization of the entire sample.

> The fractionation report 'FractionationAnalysis_EXP976_FL.txt' (and the image.png file)
> can be found in: C:\Users\Public\Documents\EXP976_Images\Fractionation
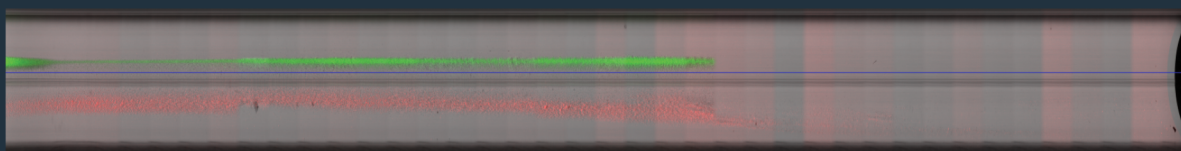
> Top Fraction: 51%
> Bottom Fraction: 49%

The Cell Fractionation Report will provide the data used to calculate % representation for either top fraction or bottom fraction.

At the top of the report, a summary is provided indicating:

Software Version:	**Experiment Analyzer 1.0.1**
Report Title:	**Cell Fractionation Report**
Top Fraction %:	**Top Fraction 0.510722**
Bottom Fraction %:	**Bottom Fraction 0.489278**
Channel Upper Limit Pixel Position:	**Upper Channel Position 732**
Channel Lower Limit Pixel Position:	**Lower Channel Position 1737**
Image Pixel Position of Split Line:	**Split-line Position 1157**
Split Line Value Used for Sample Flow:	**Split-line Value 7**

```
Experiment Analyzer      1.0.1
Cell Fractionation Report
Top Fraction       0.510722
Bottom Fraction  0.489278
Upper Channel Position    732
Lower Channel Position   1737
Split-line Position      1154
Split-line Value 7

Position Fractionation Intensity
732      1.9338e-06
733      7.1185e-06
734      1.38738e-05
735      7.16135e-06
736      2.85766e-05
737      1.70476e-05
738      2.63165e-05
739      2.88258e-05
740      2.72785e-05
741      2.13472e-05
742      2.48498e-05
743      2.83854e-05
744      2.73631e-05
745      2.18398e-05
746      3.49654e-05
747      3.53902e-05
```
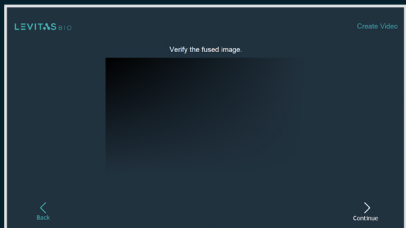
The delimited table is also provided showing the individual pixel positions and their respective intensity values. **The Cell Fractionation Report *.txt** file can be imported into a graphing program to create a fractionation intensity profile across the image pixel position (see figure below).
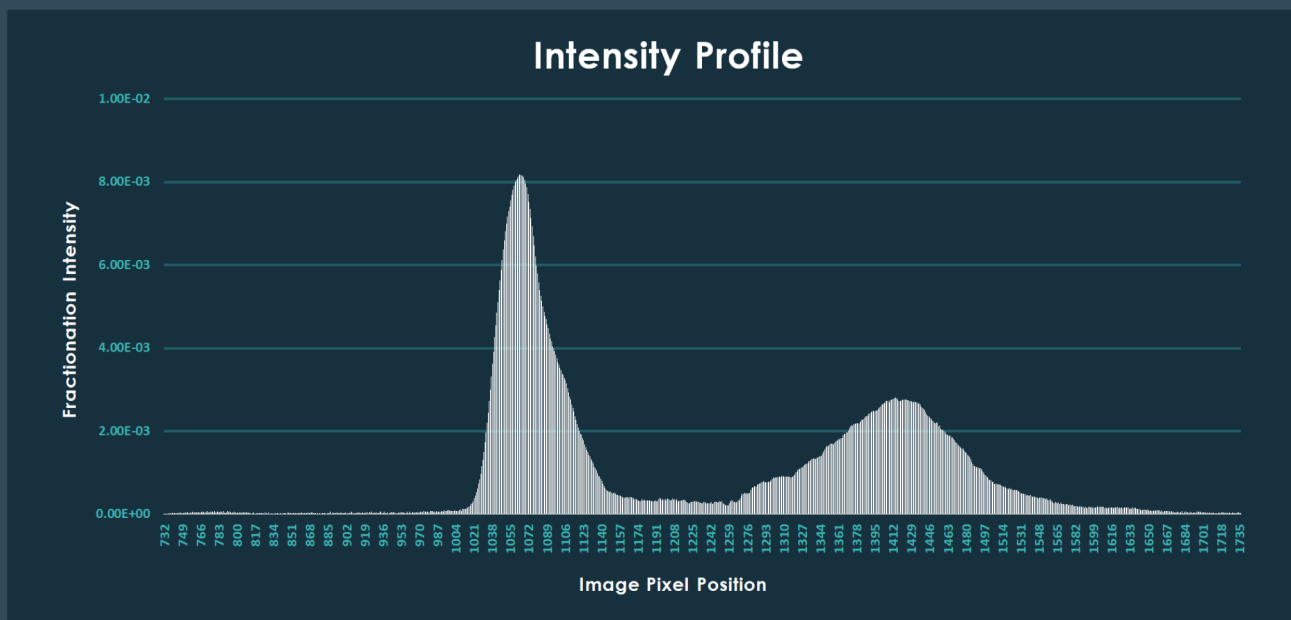
The Fractionation % can be calculated for top and bottom channels.

TROUBLESHOOTING

Issue	Possible Resolution
No ExperimentSettings.ini file found	The ExperimentSettings.ini file should be in the experiment folder, one level above the image folder. If only the image folder was copied from the LeviCell computer, locate the correct experiment folder on the LeviCell computer and copy it again.
Not provided option to select fluorescence or staining.	No fluorescence was captured in the run.
Fused image not appearing.	Not all the image files are present in the experiment folder containing the ExperimentSettings.ini file
Creation of Montage, Video, Fractionation analysis files takes very long to save or appear in folder	Network connectivity to the file folder may be suboptimal. Copy images to local computer to perform analysis.

Contact support@levitasbio.com or call Technical Support at +1-650-204-1185 for additional troubleshooting support.