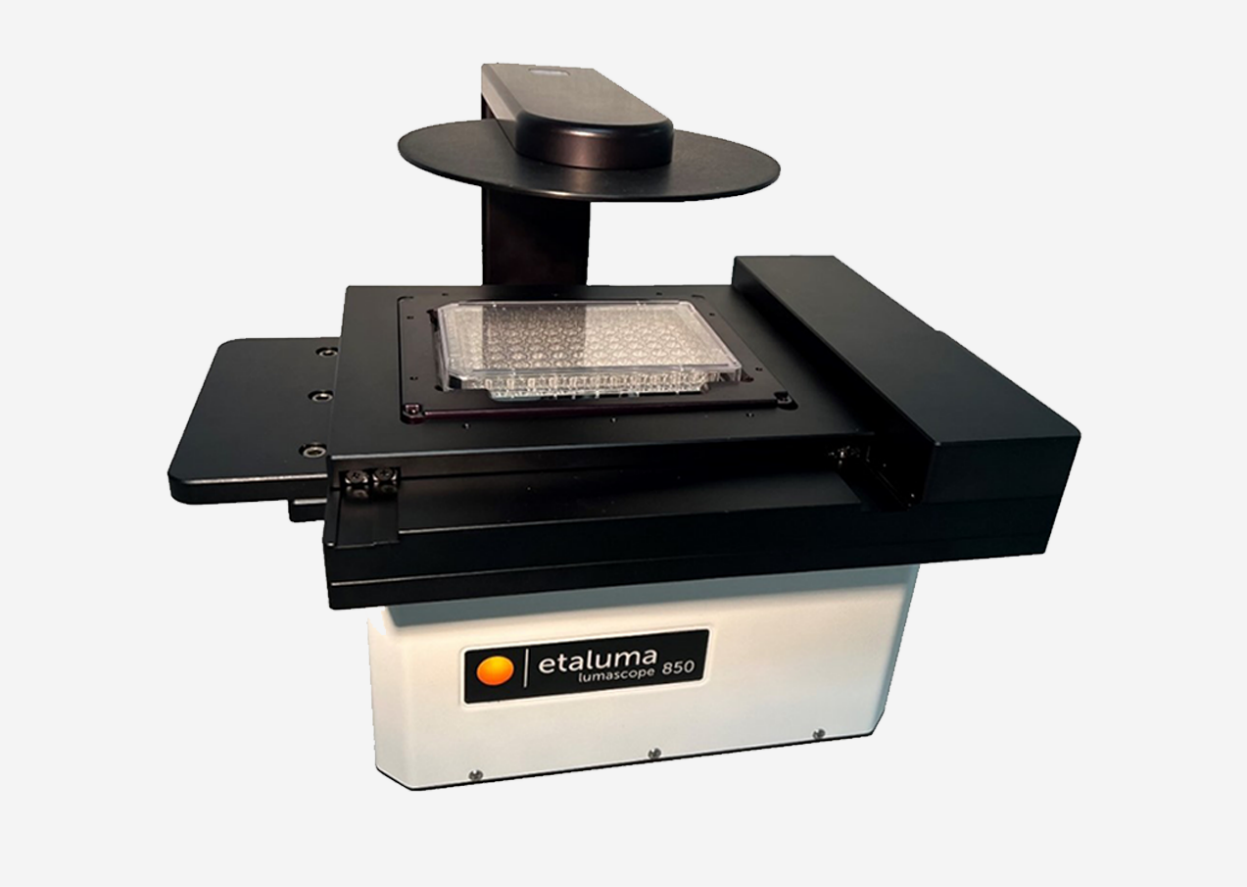


LS850 Microscope

Operator's Manual

LumaViewPro Software
Version 2.2.0

For assistance, please email
support@etaluma.com

Etaluma,Inc.
Carlsbad, California
www.etaluma.com

etaluma
microscopy simplified™

Contents

1 Introduction

1.1 Included Items

Items included with each LS850 Microscope:

- Auraphase transmitted illumination
- LED fixture with cable
- Optional ambient light guard
- Fixture mounting knob

Figure 1: Items Included with LS850

1.2 Accessories:

- USB A & USB C communication cables
- Dichroic cleaning swabs
- Black microplate lid for blocking room light during fluorescence imaging
- Foam plug for incubator port
- Lens paper
- USB memory stick with LumaViewPro application and this manual
- Mousepad

Figure 2: Accessories Included with LS850

1.3 Labware Holders

Primary Labware Holder

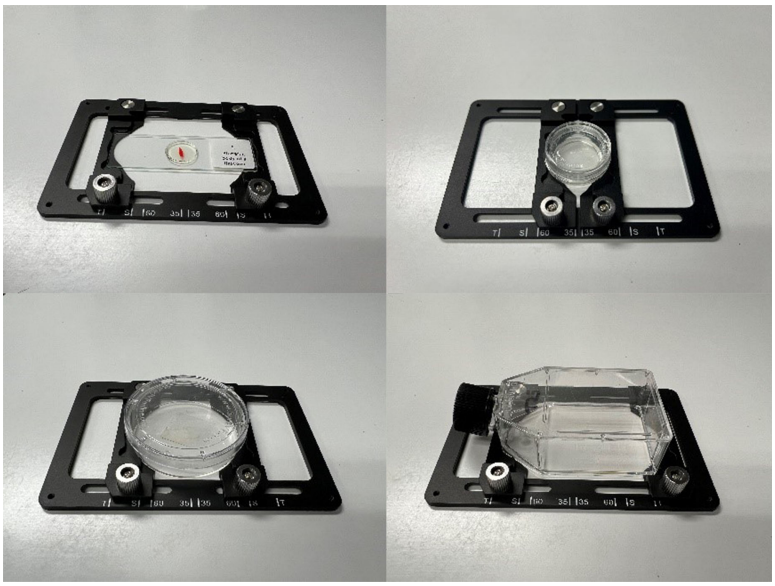


Figure 3: Primary Holder

Optional Labware Holders

- Four microscope slides in parallel plastic
- Four microscope slides in parallel metal with magnetic clamps

Figure 4: Optional Holders

2 Setting Up the Microscope

2.1 Connecting the Cables

Connect the USB type B end to the LS850 at the rear left corner of the microscope. **Wait to connect the type A or C USB cable end from the Microscope to the computer until LumaViewPro is installed.**

Plug the external power supply/cord into the rear port and the plug into an AC outlet. An extension cord is provided in cases where the AC outlet is distant.

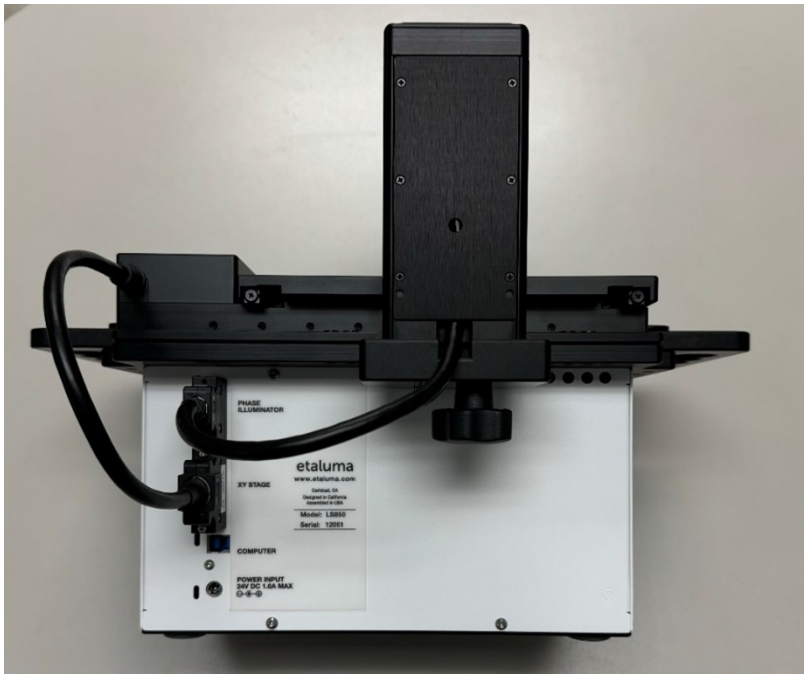


Figure 5: Example showing cables connected

2.2 AuraPhase Transmitted Illumination Accessory

Remove the AuraPhase from its shipping box. Attach it by placing the holes on the bottom of the bracket over the pegs on the upper surface. Tighten the large thumb screw beneath the deck cup. Connect the free end of the AuraPhase Accessory power cable to the round socket nearby. This allows the AuraPhase Accessory to be controlled by LumaViewPro.

Figure 6: Aura Phase Set Up

3 Setting Up LumaViewPro

3.1 Introduction

The LS850 is controlled by the LumaViewPro software program. The latest LumaViewPro version is downloadable from Etaluma's website and should be **installed prior to connecting your computer for the first time.**
LumaViewPro can be run on Windows10/11, Mac, and Linux. Desktop computers and laptops can be used, but the best visualization correlates with monitor resolution equal to the sensor resolution (up to 2100 x 2100 pixels). Note: The monitor does not affect image resolution unless the monitor is low quality and affects your ability to judge focus.

3.2 Downloading and Installing LumaViewPro

To download LumaViewPro, go to LumaViewPro for LS850/LS820. Click on the LumaViewPro for LS850/LS820 link for your operating system to start the download and save the folder when prompted. Go to your downloads location and double click to launch the LumaViewPro installer. Follow the instructions to install LumaViewPro. It may be helpful to verify that this file is the latest version as posted on the Etaluma website.
LumaViewPro contains certain functionality and features which rely on external frameworks and libraries such as Java (Amazon Corretto), ImageJ, etc. These frameworks/libraries are installed during the installation process.
After installation, a LumaViewPro shortcut is created (orange logo icon) and placed in your start menu for convenience. To launch LumaViewPro, find the shortcut by search or manually in the start menu. This shortcut can be pinned on to the taskbar or put on the desktop.

3.3 Connecting the LS850 Microscope

Insert the USB-A end(some versions may be C) of the supplied USB cable into a USB3 port on your computer and the other USB-B end into the port labelled “Computer” on the microscope. It is also important to connect the Microscope directly to the computer USB port and not use a USB hub.

Plug the barrel connector from the AC power supply into the port labelled “Power Input 24VDC”. A 2 meter extension is available if required. Pass the communication and DC power cable through the incubator port, which is often located on the upper back wall. A plug or filtered stopper may be present. The power supply transformer should not be placed in the incubator, only the 24 VDC cable and its extension. The transformer should not be left hanging by the DC cord and should be supported or attached to the back of the incubator. Every microscope comes with a foam plug that can be used to seal around the cables going through the port or the existing plug can be adapted. Make sure there is enough slack in the cables inside the incubator to accommodate any sliding out of the shelf. Place the computer on a flat and stable surface near the incubator and within the reach of the 3 meter USB cable provided.

WARNING: Do not extend the USB cable or use another USB cable other than that supplied as communication issues will arise. To extend the user interface to microscope distance use a long HDMI for the monitor and wireless mouse and keyboard. See Appendix D.

4 Troubleshooting

4.1 Microscope Not Connected

This communication error will occur under the following circumstances:

1. No power connected, check AC source and cables.
2. USB cable disconnected, check connections on PC and microscope.
3. USB cable connected to USB2 port on the PC, switch to USB3 port.

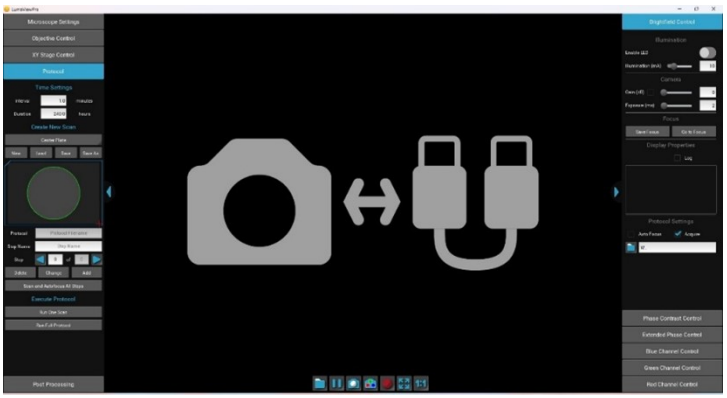
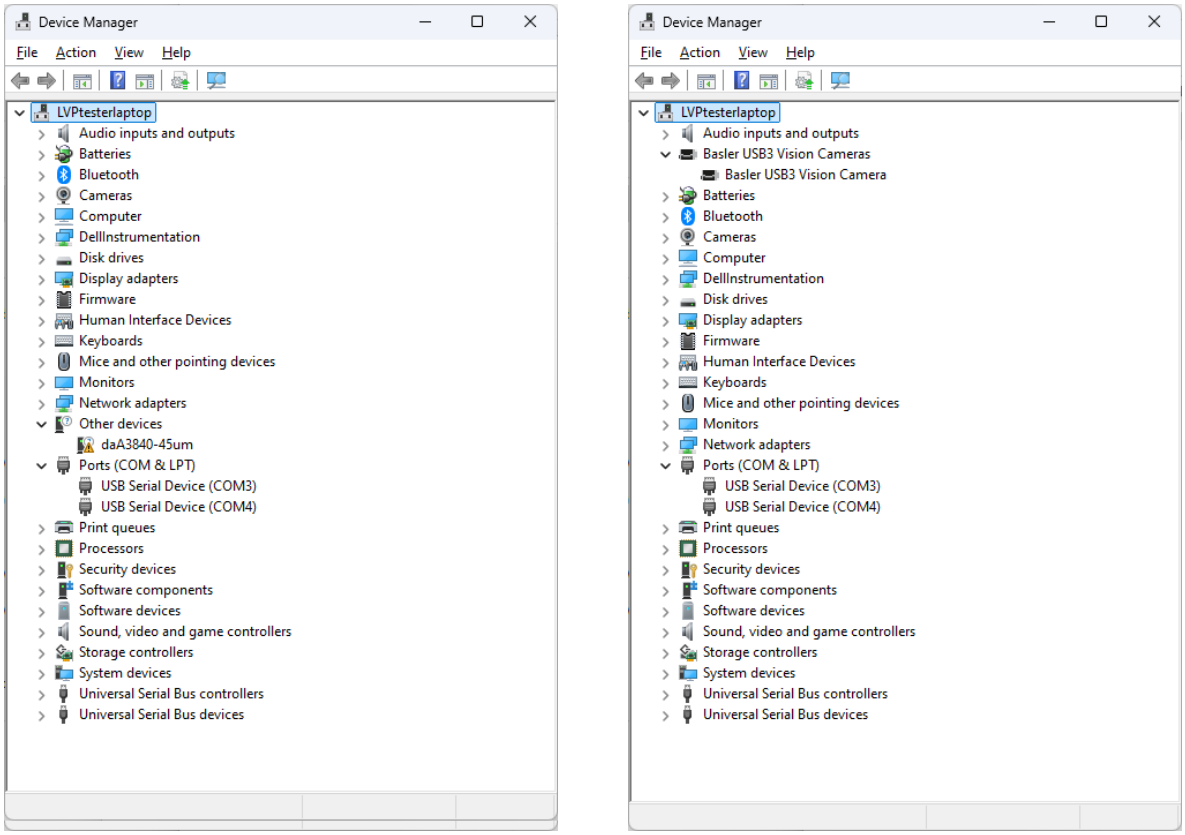


Figure 7: Communication Error Screen



(a) USB2 Connection

(b) USB3 Connection

Figure 8: USB Connection Scenarios

The Device Manager entry for the microscope plugged into a USB2 port is shown in the first dropdown while the second shows the proper connection to a USB3 port.

4.2 Menus Inaccessible

Certain computers and their monitors may need the Display Scale changed to a lower percentage. The default (Recommended) Scale can cause some menus to have their lower portions hidden and inaccessible. Changing this to a lower percentage will allow the full menu to be visible. (Fig 11)

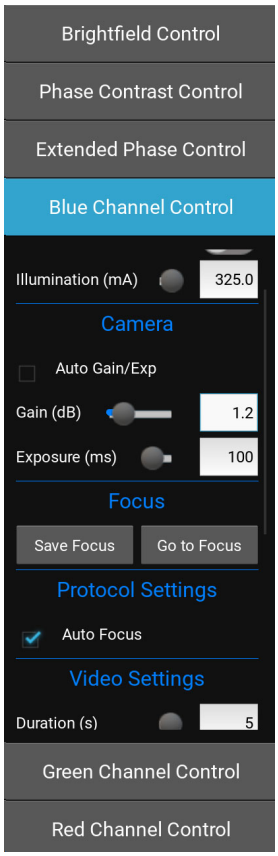


Figure 9: Full Menu Hidden

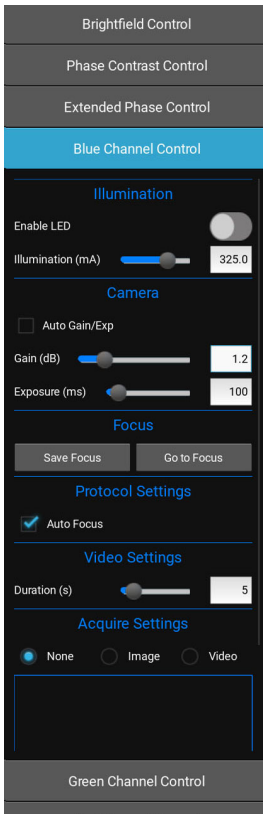


Figure 10: Full Menu Visible

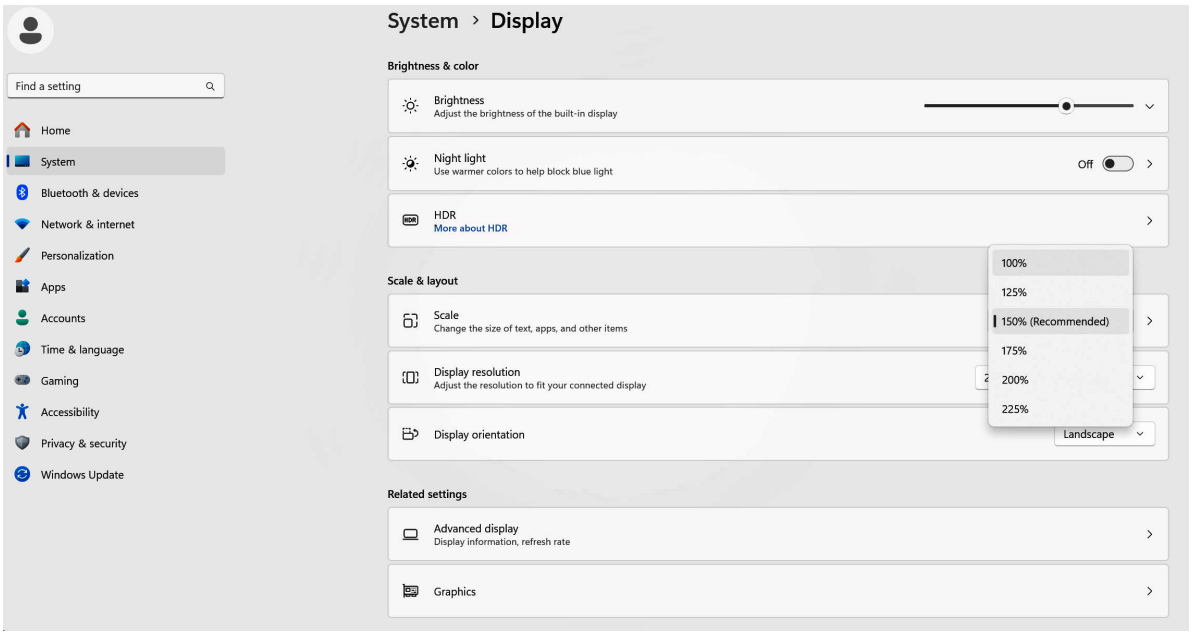


Figure 11: Settings>System>Display>Scale

5 Getting Started With LumaViewPro

5.1 Launch

Launch the LumaViewPro software from the desktop icon (or other chosen location) and allow the microscope to be discovered and initialize.

Launching LumaViewPro opens the application as seen below:

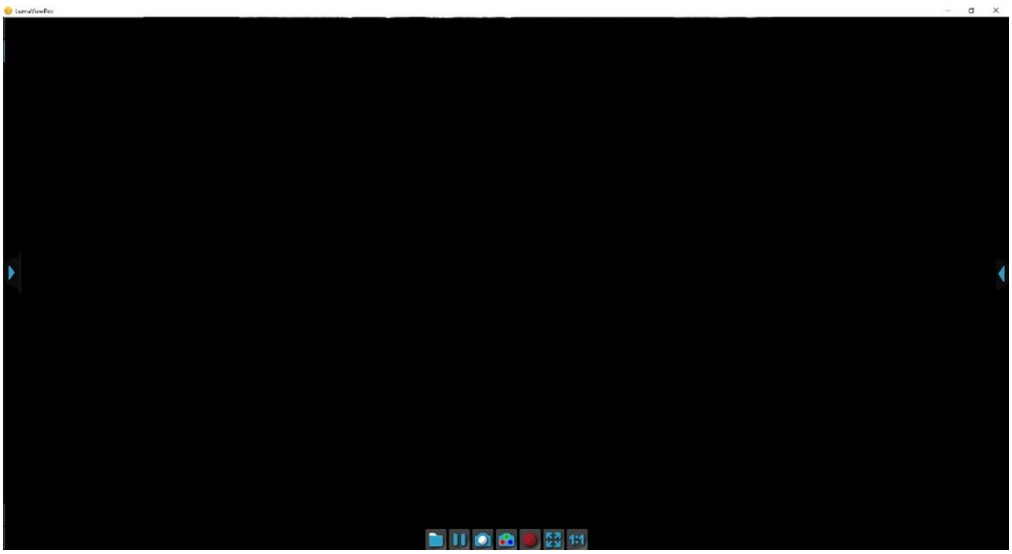


Figure 12: Launch Screen

The side menus are accessed by clicking the blue arrows on the right and left edge of the application.

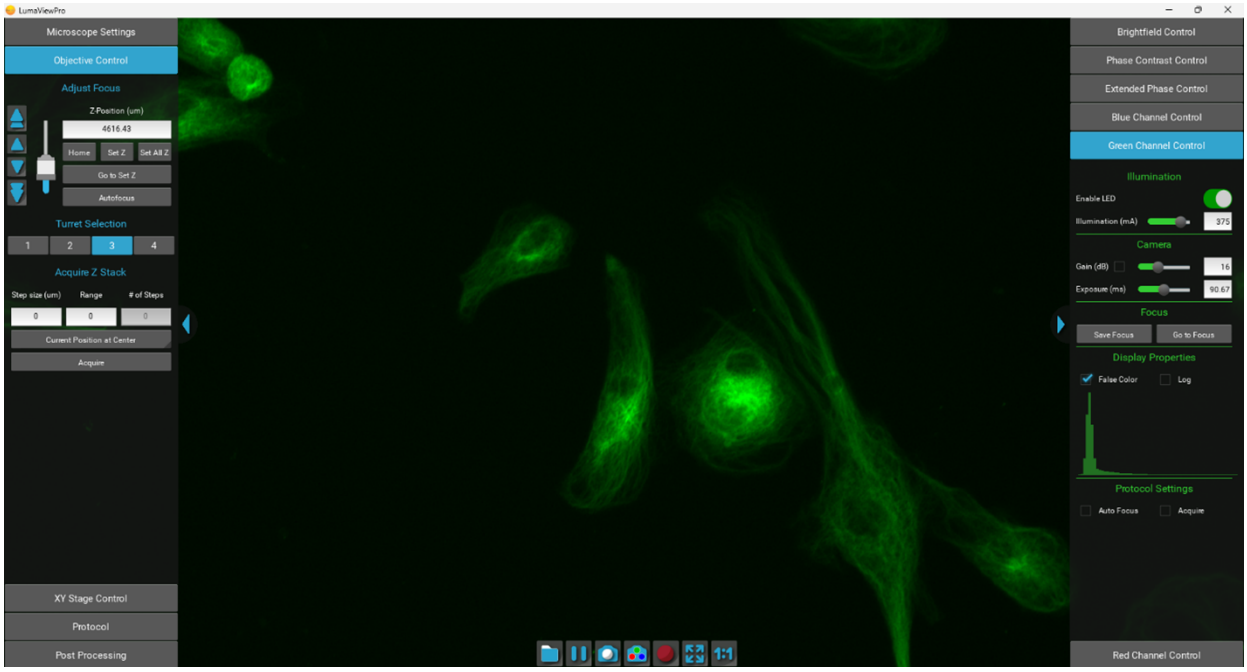


Figure 13: **Left:** Motion Control Menu **Right:** Channel Control Menu

5.2 Microscope Settings

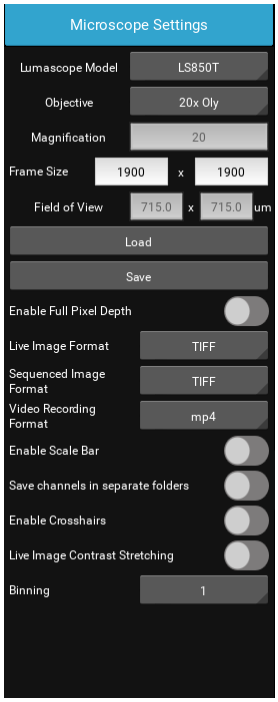


Figure 14: Microscope Settings

Click on the left arrow to expand the motion control menus and select the **Microscope Settings** tab. **Microscope Settings** configures your hardware with camera, objective, and image settings.

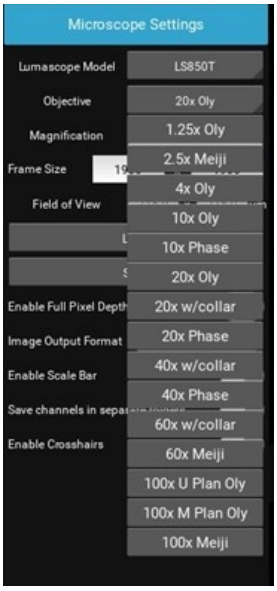


Figure 15: Objective Settings

Click the **Objective** field to select the desired objective from the dropdown.

Frame Size controls how much of the camera sensor is used. Up to 2100 x2100 pixels is available but expect some vignetting at frame sizes above 1600. Protocols with include tiling created with a certain frame size should be run with the same size at runtime.

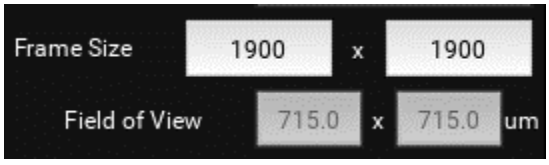


Figure 16: Frame Sizing

Enable Full Pixel Depth saves all acquired images as 12-bit tiffs (default is 8-bit).

Live Image Format allows simple tiff images or Open Microscopy Environment (OME) tiffs with full metadata.

Sequenced Image Format controls automated acquisition images including manually acquired z-stacks and all Protocol Data. It allows simple tiff images, Open Microscopy Environment (OME) tiffs, and ImageJ/FIJI compatible Hyperstacks.

Video Recording Format controls video output for manual and protocol video capture. If mp4 is selected, videos will be saved as a playable mp4 video in real-time FPS. The frames option saves each frame of a video individually, rather than a playable video, allowing for closer analysis and other post

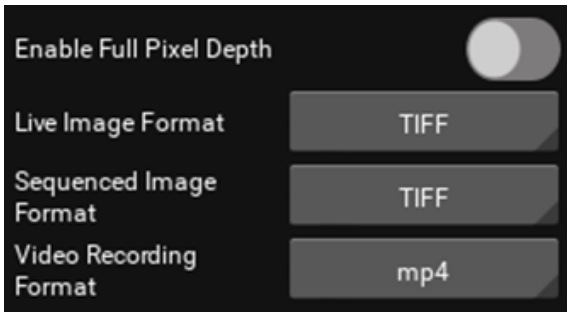


Figure 17: Image Formatting

Enable Scale Bar places a short scale bar in the lower right corner of the live image and saved images. It includes the bar length and objective magnification as defined in the Microscope Settings.

Save Channel in Different Folders places each Protocol channel in a separate folder within the ProtocolData folder. The Stitching results for each channel will be included in that channel folder. Compositing creates and saves the results to a new folder called Composite. This selection can be prior to running and is not recorded in the Protocol.

Enable Crosshairs overlays a bullseye target on the image. It does not appear in saved images.

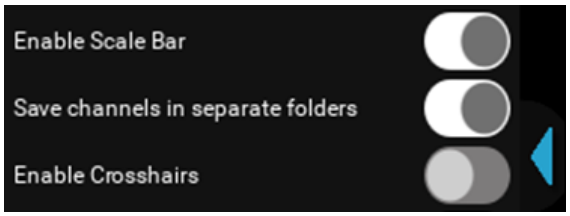


Figure 18: Image Formatting

Live Image Histogram Equalization enhances the contrast of the live image, expanding the histogram to be able to better visualize small differences in contrast; dark pixels become darker, bright pixels become brighter. The underlying image data is not changed, only the displayed images are modified. This enables better focus and visualization of hard to see details. It will also allow very dim images to be seen much more easily. This feature is disabled except in Live View. Automated Protocols, Composites, Z-stacks, and Autofocus will not display Histogram Equalization.

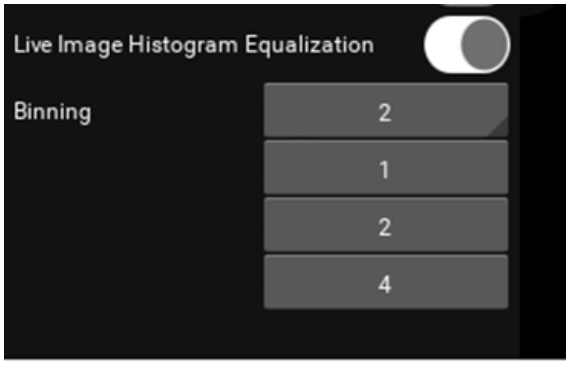


Figure 19: Histogram Equalization

Binning combines pixels into larger pixels. This increases sensitivity but decreases resolution. A binning region is always a square matrix - 1x1,2x2, etc. (Fig 20). The Frame Size will change automatically to account for the lower number of effective pixels. Protocols created under Binning 2x2 or 4x4 should be run with the same setting at runtime. Acceleration (fig 21) can be adjusted to allow for gentle treatment of barely attached cell structures.

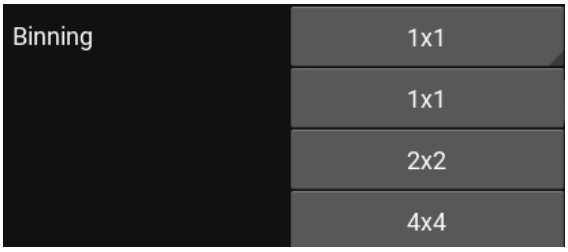


Figure 20: Bin Size Selection

Figure 21: Acceleration Setting

5.3 Channel Control

Expanding the right side menu exposes the **Channel Control.** There are three transmitted channels- Brightfield, Phase Contrast, and Extended Phase. Brightfield illumination is from a central LED with an appropriate lens for uniform illumination. Phase Contrast is a small ring of LEDs that match the IPC objectives we supply from Evident. Extended Phase is a larger ring of LEDs that is currently under development to provide other modes. The channel's LED can be turned on and off here with the **Enable LED** control.

The **Illumination, Gain,** and **Exposure** can be set by clicking on or grabbing and moving the slider. Exact values can also be entered manually.

Auto Gain/Exp will cause it to be used for that channel in any Protocol saved with that channel. **Autofocus** will perform an Autofocus on every Step of a Protocol in this Channel.

Selecting the **Acquire** settings (None, Image, or Video) will include or exclude this channel in any automated process including Protocols and Composites (video will not be included in composite captures).

The **Save Focus** position will be used in the Protocol for that channel.

False Color provides pseudo coloring for the Fluorescence channels.

A live histogram of pixel intensities is displayed with linear or logarithmic Y axis.

Auto Gain/Exp adjusts to center the median brightness of the image. As a result, any image with significant very dark and very light pixels, such as at the edge of a black wall plate well, will overexpose the light part to compensate for the dark part. In these cases, Autogain is not useful and not recommended. In the case of tiles which overlap well edges, these tiles can have their AG turned off while the well images remain under autogain. Bright field images with autogain enabled and disabled at a well edge are shown below. The AG parameter of brightness and timeout are editable in the current.json file, see Appendix D.

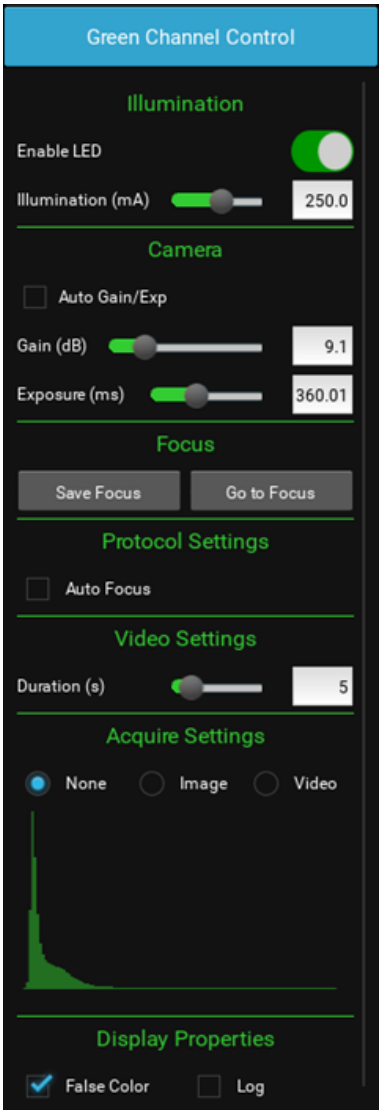


Figure 22: Channel Control

5.4 XY Stage Control

The location of the stage will be indicated by a red crosshair in the Stage Map field. Clicking within the Stage Map will move to that location. Arrow keys allow coarse and fine XY movements according to the objective installed. Setting and returning to XY positions is controlled here. **Home Stage** returns the stage to the center of the labware. Values can be typed into the X and Y coordinates.

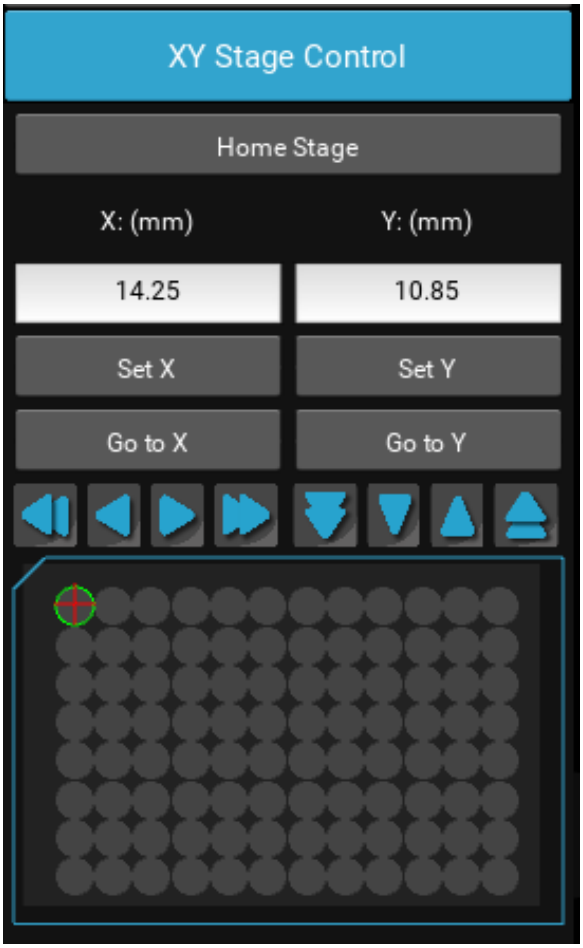


Figure 23: XY Stage Control

5.5 Objective Control

Adjust Focus

The Focus control allows manual adjustment of the focus position using coarse and fine arrows according to the objective installed. You may drag the objective icon up or down for very large movements. Clicking within the objective icon will adjust focus based on the position relative to the center of mass of the icon.

> Pressing ‘Ctrl’ on the keyboard and scrolling the mouse wheel will also allow manual focus.

Home brings the optics to the fully retracted position. “Set Z” will memorize the current position and allow you to easily return. “Set All Z” will record the position into each channel’s individual focus setting. Home retracts the objective to the lowest level. Autofocus uses image contrast to find the best focus according to the objective installed.
Autofocus scans through a range defined by the objective magnification with a coarse and then finer z axis step size monitoring contrast and finding its maximum. Autofocus failures can be due to the incorrect magnification objective in the Microscope Settings as well as a variety of image quality issues such as high background, thick samples, and too edges from which to calculate the contrast.

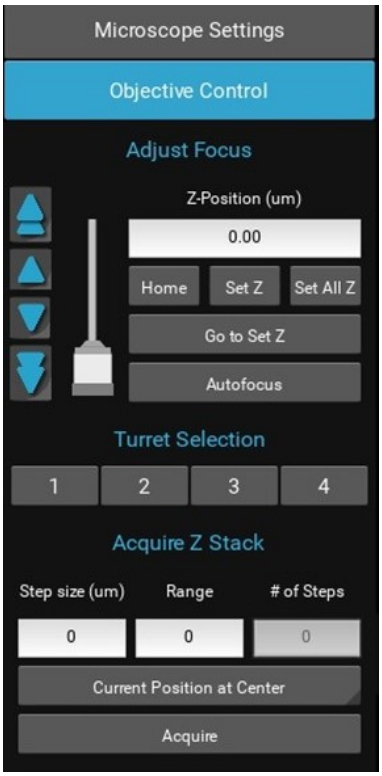


Figure 24: Objective Control

Turret Selection

If configured as an 850T, the Turret position can be selected. The focus will retract before changing positions and will need to be brought back into focus through the “Go to Set Z” button or manual adjustment. When using only two objectives, they should be installed in opposite locations. Installing two objectives next to each other may cause additional wear by being off balance.

> Warning: When using tall objectives (100x and some 60x), do not install other objectives next to them. Short working distance objectives should only be used with one additional objective and should be located opposite from each other. Failure to follow this will result in the possibility of the tall objective colliding with the stage assembly.

Acquire Z Stack

Z-stacking requires a Step size. This is the slice thickness or spacing of the images in Z. The Range is the total Z covered with slices in the stack. Range divided by Step size will equal the # of Steps. You may choose how the current Z position relates to the stack by selecting Current Position at Center, Top, or Bottom of the stack.

6 Getting an Image

6.1 Initial Set Up

Place the sample in the nest and ensure it is seated flat. Click the Stage Map to move to the location of your sample or first well of your microplate. Enable an illumination channel and adjust the focus to find the image. Adjustments to the Illumination, Gain and Exposure may be required to center the pixel brightness histogram for that channel. Click Save Focus for that channel. It may be convenient to Set Y and Set Y under XY Stage Control as well as Set Z under the Focus tab to return to this location.

> Right clicking the mouse within the live image will re-center the image on that point. The correct objective must be installed according to the Microscope Settings.

6.2 The Toolbar

The Toolbar provides common actions.

Figure 25: The Toolbar

From left to right:

- Define the destination folder for all image files. This single destination folder creates Manual and Protocol/Data subfolders.
- A new time/date named folder is created upon every Run of a Protocol.
- Pause with a freeze frame
- Acquire an image of the live image
- Acquire a composite image according to the individual channel settings
- Acquire checkbox/record the live image video stream
- Fit the live image to the application image display window
- Present the image as one camera pixel to one screen pixel

6.3 Protocol

To configure an automated microscopy run, including time-lapse, the **Protocol** feature is utilized.

Time lapse Protocols are created when the **New** button is clicked. The following settings are captured upon creation:

- Channels which have their **Acquire** option checked will be used with their corresponding Image/Video, Illumination, Gain, and Exposure settings as well as their, False color, Saved Focus, Auto Gain/Exp, and Autofocus enable settings.
- **Interval** and **Duration** set how often and for how long the time lapse experiment is performed.
- **Z-Stacking** generates a Z-stack from any Step which is not already a Z-slice. This allows Z-stacks to be enabled after Protocol creation according to the parameters under Objective Control.
- **Enable Z-Stack** is checked to use the Z-stack settings under Objective Control.
- **Show step locations** will display yellow crosshairs on each step location. Closely located locations may be difficult to display.
  The Protocol will create the number of Steps based on the number of images taken. You may sequence through the steps with the arrow keys. Each individual step in a Protocol can be edited. Each step can have its image parameters, focus settings changed, and XY location adjusted. You must click Change to have the new settings updated for that step. Steps can be Deleted. Scan and Autofocus All Steps will automatically perform an autofocus on each step and set each step's focus to the result. This cannot be undone.

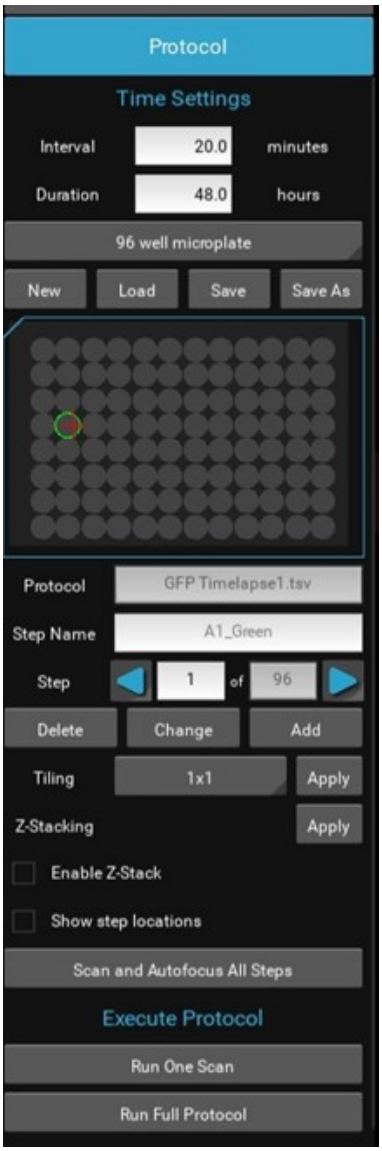


Figure 26: Protocol Menu

Tiling
Adjacent images can be acquired in patterns of 2x2 up to 10x10. Tiling dimension is captured at Protocol creation and cannot be Inserted or Changed after creation. Tiling names are auto-generated and are required for Stitching in Post Processing.
Tiling with smaller frame sizes will improve the alignment of the abutted tile images. There is more distortion at the edges of larger frame size images. This will be addressed in the upcoming “Feature-based Stitching” where overlaps will be adjusted to remove the distortion. Changing frame size under Microscope Settings will not be accounted for in existing Protocols with tiling and the tile centers will be from the Protocol’s original frame size at creation. Creating a New Protocol is required to use the current frame size in a tiling Protocol. Transmitted mode tiles from a labware with a meniscus may have dramatic brightness differences in each tile image based on the alignment of the illumination with respect to the meniscus.

Z-Stack in Protocol
The Z-stack settings under Objective Control will be used if the Enable Z-stack is checked. Z-stack names will be auto-generated. Tiling and Z-stacks can be used and the auto-generated names will reflect this. Configuring a Protocol with AF enabled and using a Z-stack will result in autofocusing at each z-slice level as the center of new AF range for that Step.

Changing Steps in a Protocol
Often the field of views that you desire to image are not in the center of a well-defined labware array such as a microplate. In cases where the microplate centers may be close but need to be adjusted, stepping to that Step and moving in X,Y, or Z to a desired location and clicking Change will move that individual image acquisition location for the Protocol.

Adding Steps in a Protocol
In those instances where the desired imaging locations are different from the default labware, a Center Plate labware can be selected and new locations/channels are Added. Adding obeys the channel Acquire settings. Clicking Add with more than one channel set to Acquire will Add that many Steps in the corresponding channels. Adding Steps will create Steps named custom0, custom1....

Tiling Added Steps in a Protocol
When new imaging locations are Added, they can then be custom tiled by setting the tile dimensions and clicking Apply. Any untiled Steps will then be tiled according to the dimensions selected. This can be done repetitively and with different tiling dimensions for each untiled Step. Tiles created with the initial creation of the Protocol (clicking New) will not be further tiled. The Added Step will have the custom# name as default and upon tiling, the tiled Steps will all display the original custom# but the images will be saved as custom#_Channel_TileID_sequence.

6.4 Configuring a Protocol Quick Start

- Place sample into plate nest
- Move to an example FOV by clicking on the plate map under Protocol or XY Stage Control
- Adjust image parameters to center the pixel histogram (get a greyscale background)
- Find the focus for each channel you will use and Save Focus in each channel
- Select all the channels you want to use using Acquire checkbox in that channel
- Select Autofocus if you want to AF in this channel.
- Select the correct labware under Protocol
- Choose Tile dimension if desired, default is single image (1x1)
- Set Z-stack settings and Enable Z-Stack checkbox if desired
- Click New
- Observe that the channels multiplied by the number of wells, tiles and z-slices equals the Steps
- Step through the Protocol to make sure all Focus levels are close if using AF or exact if not. The channel menus will open for each Step and you can turn them on the check the image.
- Changes can be made to Focus, XY and channel settings for each Step and recorded by clicking the Change button under Protocol. A name can be added to each step and saved by clicking Change.
- Save the Protocol with a name.
- You can run the Scan and Autofocus All Steps and it will automatically perform an autofocus on each Step and set each Step's focus to the result. This cannot be undone. Not appropriate for Protocols with Z-stacks except under advanced strategies.
- Adjust Interval and Duration. Protocols with Intervals too short to complete entirely will log this and continue to save images.
- Save.
- Run it.

7 Creating and Editing Custom Labware

7.1 Labware File Location

The labware.json file-Labware definitions are stored in the labware.json file within the data folder.

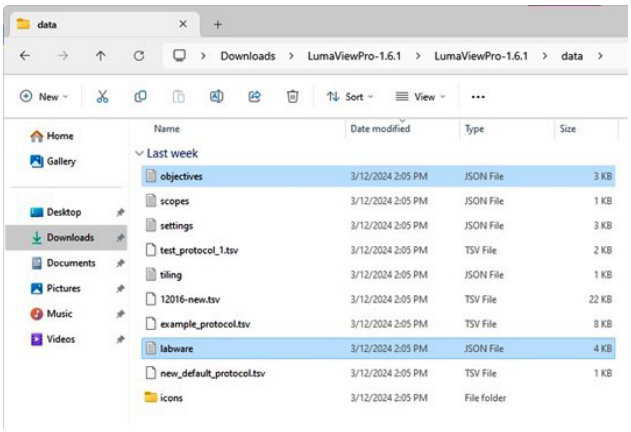


Figure 27: JSON File In the Data Folder

The labware.json file can be opened with Notepad or equivalent text editor.

7.2 Adding a labware entry

Each labware entry is defined by the information contained between the two “},” characters. In order to create a new labware entry, open the labware .json in Notepad or equivalent text editor. Note: the original labware.json should be saved as labwareOriginal.json or another new name in case one wants to revert to the default labware file. You can edit an existing labware entry or copy one and paste it into the file confirming the new entry has the correct structure between the "},” tags. You may now edit the new entry.

Changing the Name

Each labware requires a unique name to be offered in the LumaViewPro labware dropdown.

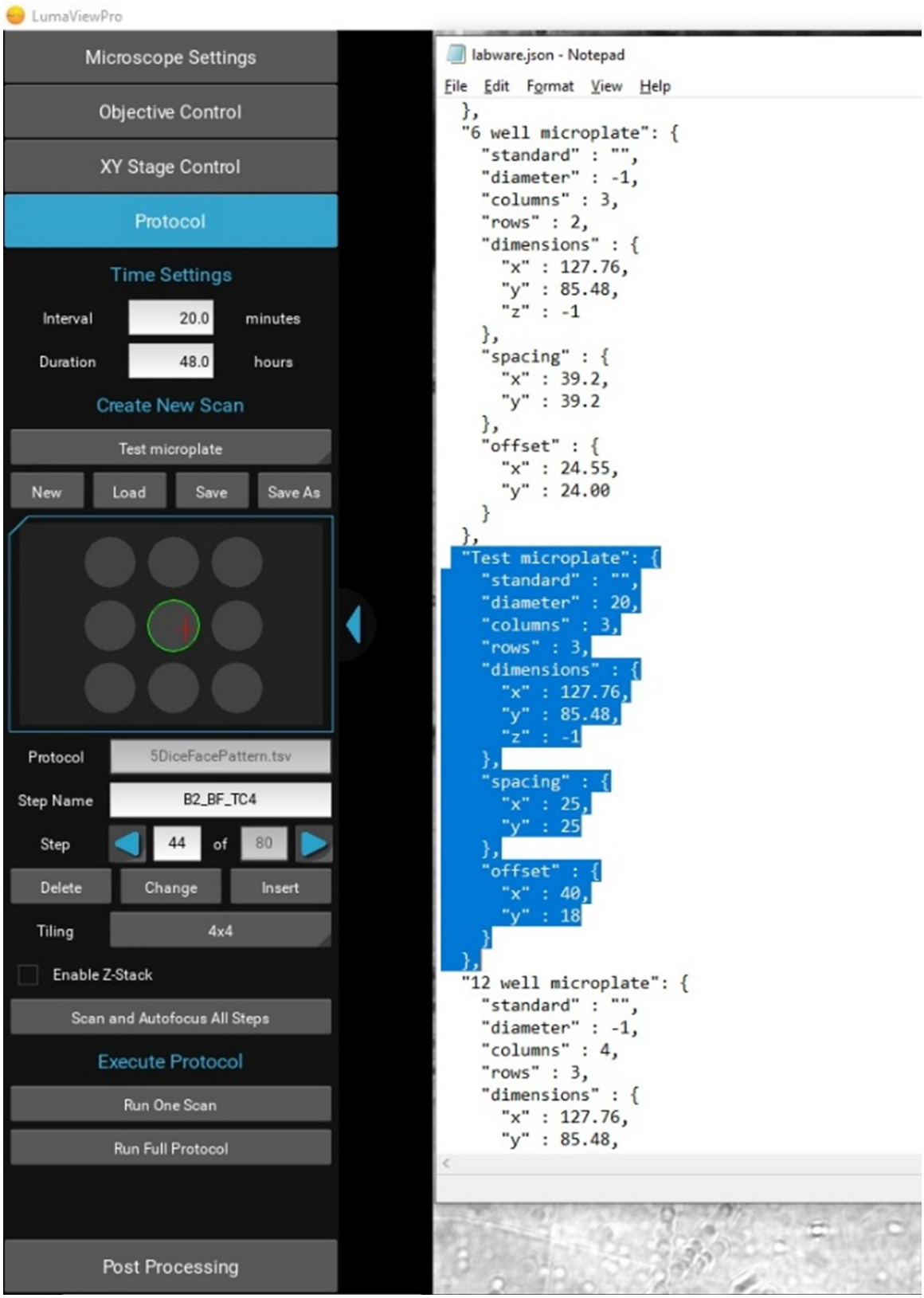


Figure 28: Editing the JSON File In a Text Editor

7.3 XY Stage Control

The **XY Stage Control** in LumaViewPro will display the X and Y coordinates and these values can be used for measuring the offset and spacing mentioned below. X is across the plate rows and Y is the up and down the columns.

Diameter - This parameter is in millimeters. “-1” will result in the diameter equaling the largest Spacing (see below). Labware wells CAN overlap. The result of any changes in the labware.json file will be seen in LumaViewPro as soon as the file is saved. (See fig 30)

Spacing - This is the center to center spacing of the wells and can be different for X and Y. (See fig 31)

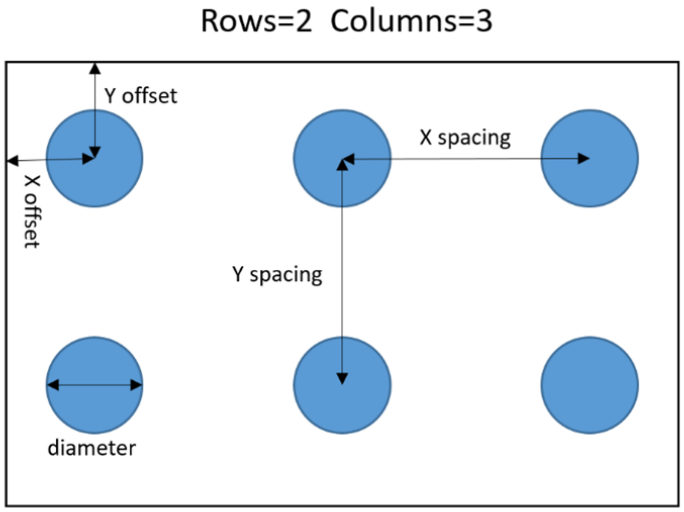


Figure 29: XY Stage Control Definitions

Columns and Rows - This defines the dimensions of the labware well array. The labware displayed above were “columns” : 2, “rows” : 2. A 1 by 1 array is shown below, i.e. a single well. (See fig 32a)

Offsets - This is the most important value as it locates the labware wells relative to the microplate footprint of the nest. It is the distance from the center of A1 or the first well to the upper left corner of the labware. It is based on the coordinate system of LumaViewPro and thus can be measured empirically by driving the stage to this first location and noting the X and Y coordinates under the XY Stage Control menu. You will see that the X and Y coordinates in LumaViewPro for the first well is the same as the offsets. (See fig 32b)

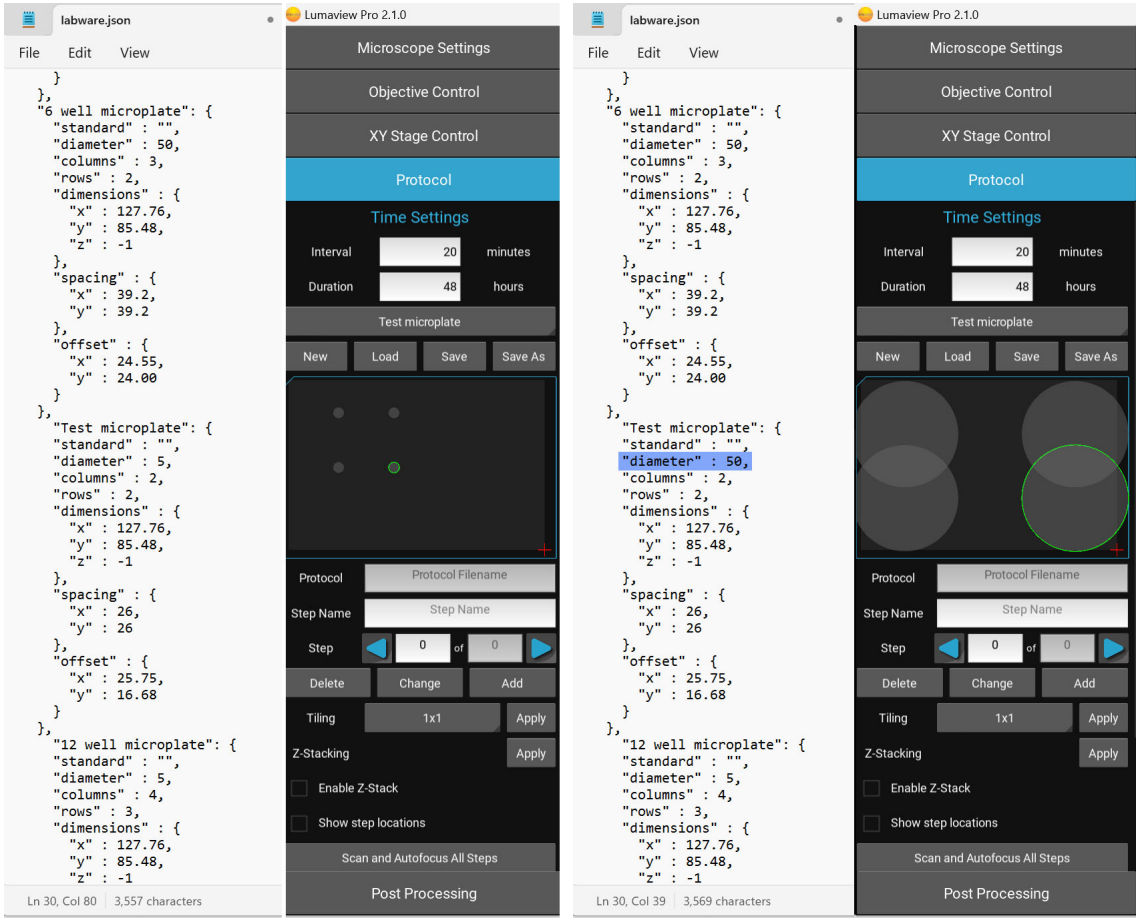


Figure 30: Diameter Adjustment

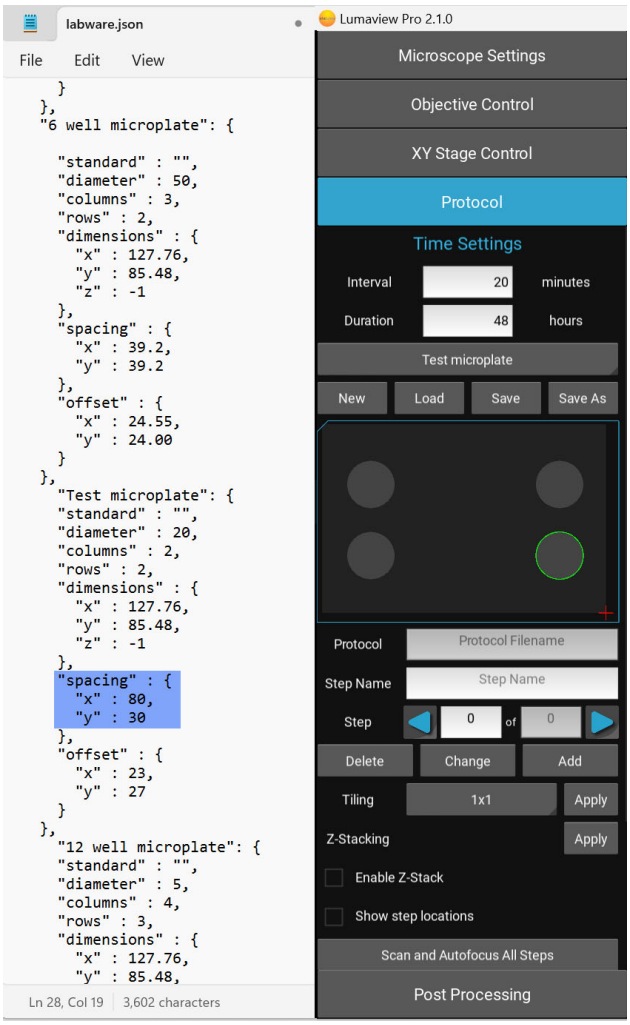
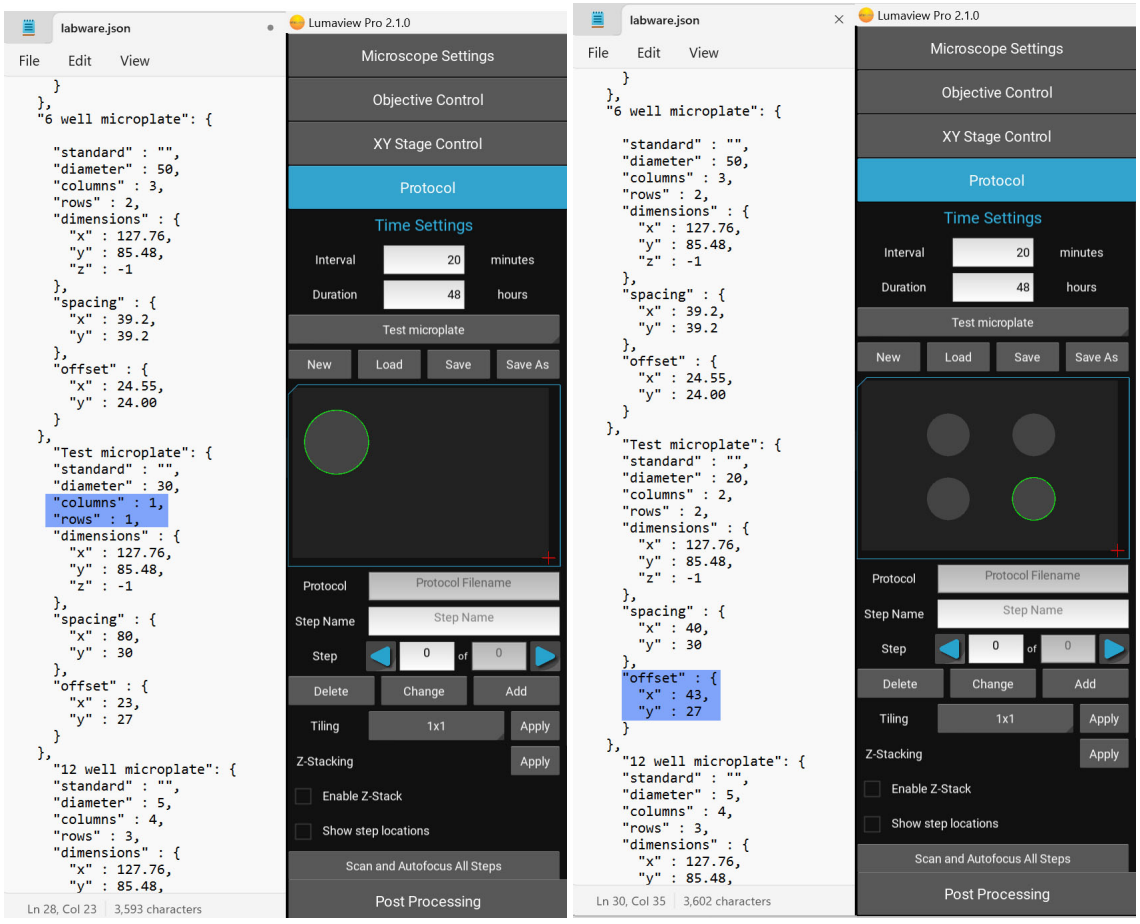


Figure 31: Spacing Adjustment



(a) Editing Columns and Rows

(b) Editing XY Offsets

Figure 32: Column and Rows & Offset Adjustment

7.4 Quickly Deleting Wells Including Tiles and Z-Stacks From a Protocol

Often when using tiling, a large numbers of labware wells may not be used and this represents many unwanted steps. These steps can be deleted in the Protocol menu on a step by step basis but in those cases where a large number of steps need to be deleted, one can edit the Protocol file to remove these wells quickly.

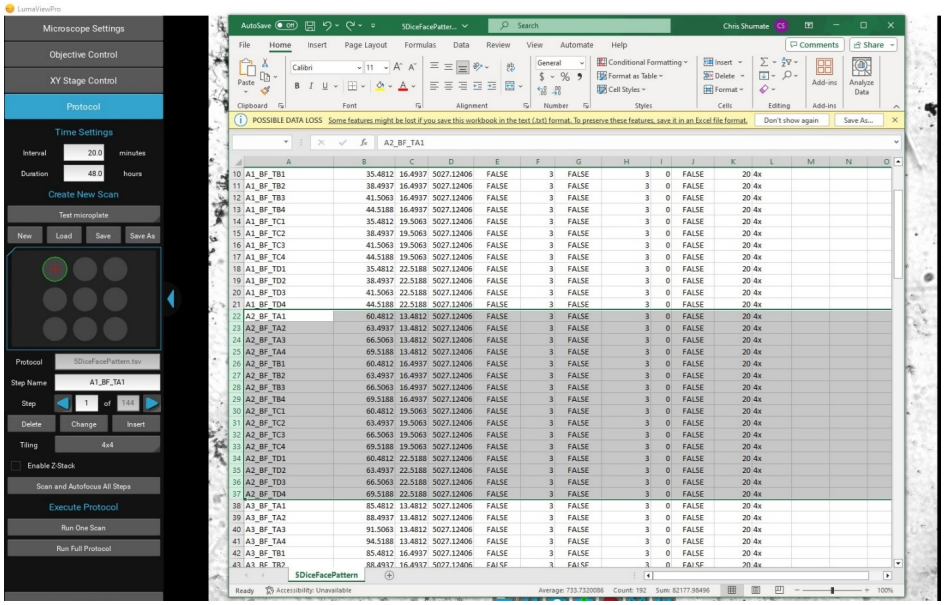


Figure 33: Batch Deletion of Steps

In figure 33, we want a 4x4 tiling at the four corners and the center of a 60mm Petri Dish and we have made a 3 x 3 labware array and need to remove A2, B1, B3, and C2 to produce this pattern. The Protocol file can be opened in Excel and large groups of Steps can be deleted easily according to their well identification.

In cases where there are three or more random locations that do not allow an array to overlap them well, a higher density array can be constructed over the locations and the best wells identified for those locations while all others are deleted through Excel.

8 Live Cell Imaging with the LS850 Microscope in an Incubator

Prior to imaging within an incubator, perform the following steps where appropriate:

8.1 Installing Room Temperature LumaScope into a Warm Humid Incubator

*If a room temperature LumaScope is placed into a warm **humid** incubator, condensation will form on the microscope. **DO NOT DO THIS.** Instead, follow the instructions below:*

1. Turn on the incubator's internal fan if available.
2. Remove the humidification: turn off active humidifiers and/or remove pans of water used to humidify the incubator. Do not leave the door open as this can cause contamination.
3. When the incubator is warm and **DRY**, place the microscope on the incubator shelf.
4. Wait 2-4 hours for the LumaScope to acclimate.
5. Restore the humidification by enabling an active humidifier or replacing the pan of water.

8.2 Removing the Microscope from a Warm Incubator

It is safe to take a warm LumaScope out of a humid incubator and put it on the bench. It is warmer than the lab, so water will not condense.

8.3 Warnings

- DO NOT repeatedly remove / replace the microscope into and out of the incubator. It subjects optics and electronics to potential condensation /evaporation cycles.
- Remove the LumaScope from the incubator PRIOR to turning off the incubator. Otherwise, you'll cause moisture to condense on the LumaScope and ruin it.

In all cases- do not turn the LumaScope off once it is in a humid incubator.

9 Post Processing

9.1 Video Creation

Create Video allows time lapse or video frame recorded images to be made into a movie. Under Post Processing select Create Video. **Apply Video Gen to Protocol Folder** allows selection of the image files to be made into the AVI. The images will not be visible when selecting the folder. This will compile all of the relevant images into .avi files at the **Frames/Second** setting and place them in a new Video folder within the Protocol data folder. If your Protocol folder has stitched or composited images, these will be made into a movie as will each tile and z-slice. Remove any images or folders that you do not need to be made into a video. Overlay Timestamp places a time setting on each frame.

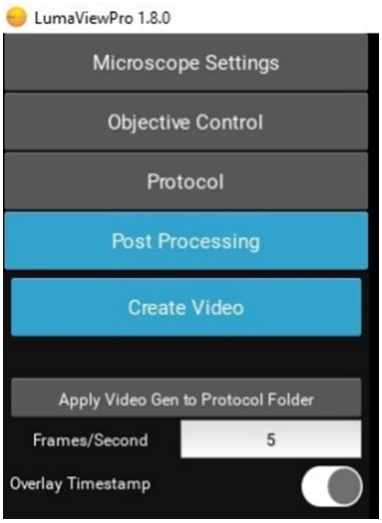


Figure 34: Post Processing

Z-Projection is a method of analyzing a stack by applying different projection methods to the pixels within the stack. There are six different projection types to choose from: maximum intensity, minimum intensity, average intensity, median, sum, and standard deviation. The appropriate projection type will vary depending on the type of data being represented. Applying a Z-Projection creates a single file from the z-stacks in the folder selected.

Open Last Save Folder will bring up the most recent Windows folder into which an image has been saved.

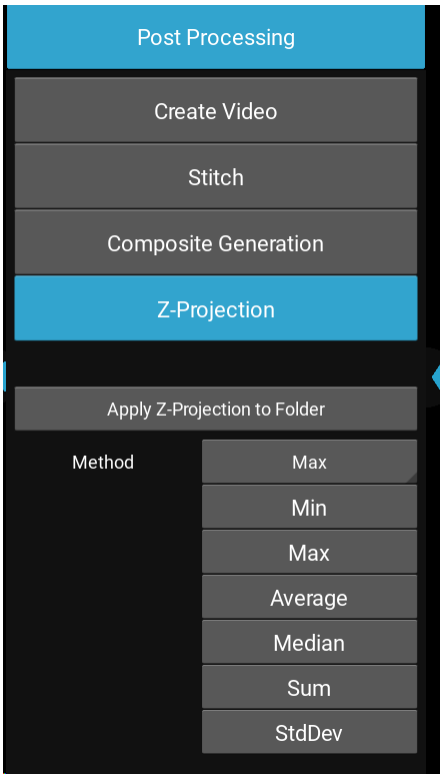


Figure 35: Z-Projection

Stitch allows tiled images to be combined into a montage. Stitching is applied to a Protocol data folder and all tiles with the same well number, channel and sequence number are automatically stitched into a new image file (figure 36).

Composite Generation will composite the fluorescence channel images that were acquired at the same location and in the same interval sequence. Compositing stitched images require them to be stitched prior to composite generation.

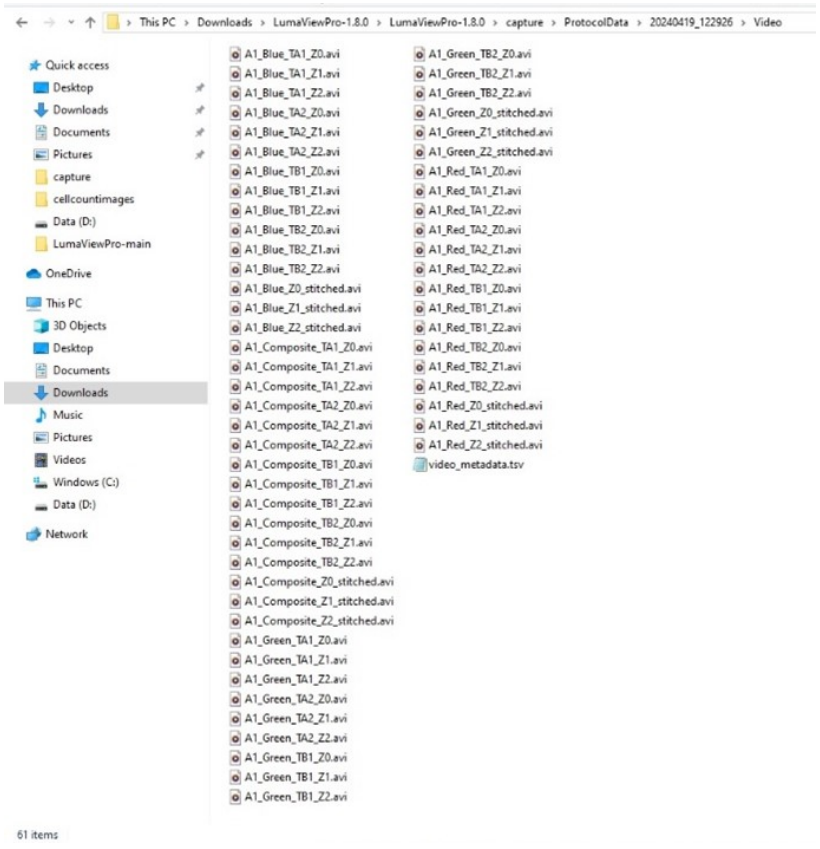


Figure 36: Image Stitching

9.2 Object Analysis

The **Object Analysis** capability allows simple thresholded objects to be filtered by Area and Perimeter as well as roundness and intensity. To analyze a folder of images, start by loading an example image and entering the Pixel Ratio appropriate for the objective magnification according to the following table:

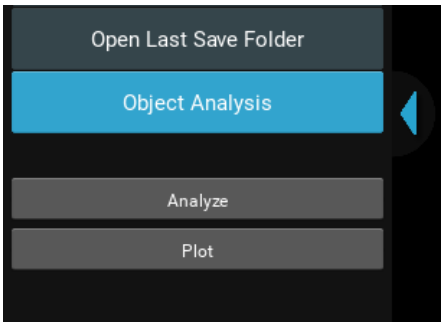


Figure 37: Object Analysis Menu

Mag	Brand	Pixel Ratio, pixels/um	FOV, 1900x1900, mm
2.5x	Meiji	0.271	7.024
4x	Olympus	0.482	3.942
10x	Olympus	1.205	1.580
20x	Olympus	2.432	0.781
20x w/c-collar	Olympus	2.379	0.799
40x w/c-collar	Olympus	4.725	0.402
60x	Meiji	6.635	0.286
10x IPC Phase	Olympus	1.195	1.589
20x IPC Phase	Olympus	2.440	0.779
40x IPC Phase	Olympus	4.832	0.393

The plot feature allows the visualization of the distribution of object intensity vs. total object area:

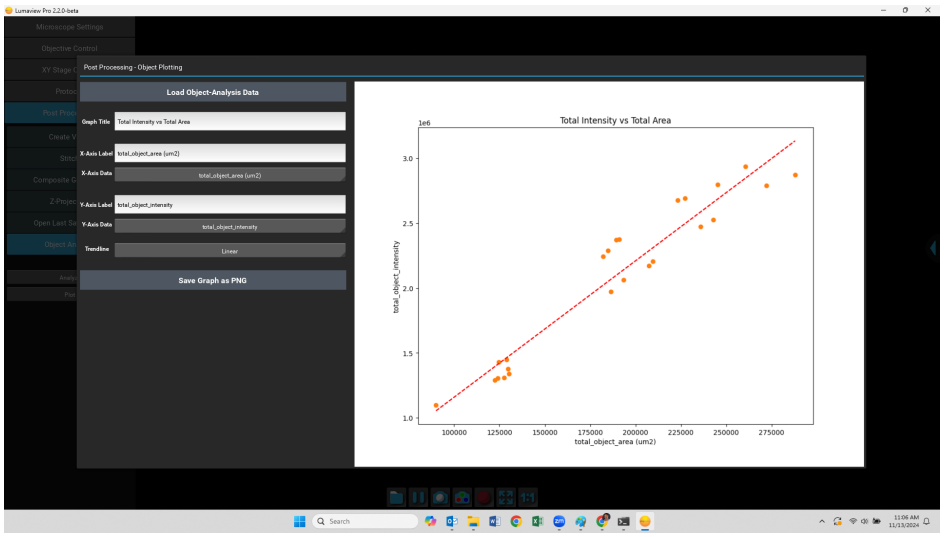


Figure 38: Object Analysis Plot

:

Clicking **Apply Method to Preview** will outline the objects that meet the criteria. Threshold is generally between 5% and 30% as higher or lower thresholds will tend to select the entire image or none of it. Once settings have been optimized, the Method can be saved and then applied to a Folder of images. A results.csv will be generated similar to figure 40.

The results.csv and a copy of the Method will be added to the image folder. Plots and normalization can be performed in Excel.

IMPORTANT: Before initiating a time-lapse run, it is critical to change your computer power and Windows update settings so they cannot interfere with scheduled image captures. See Appendix B/C

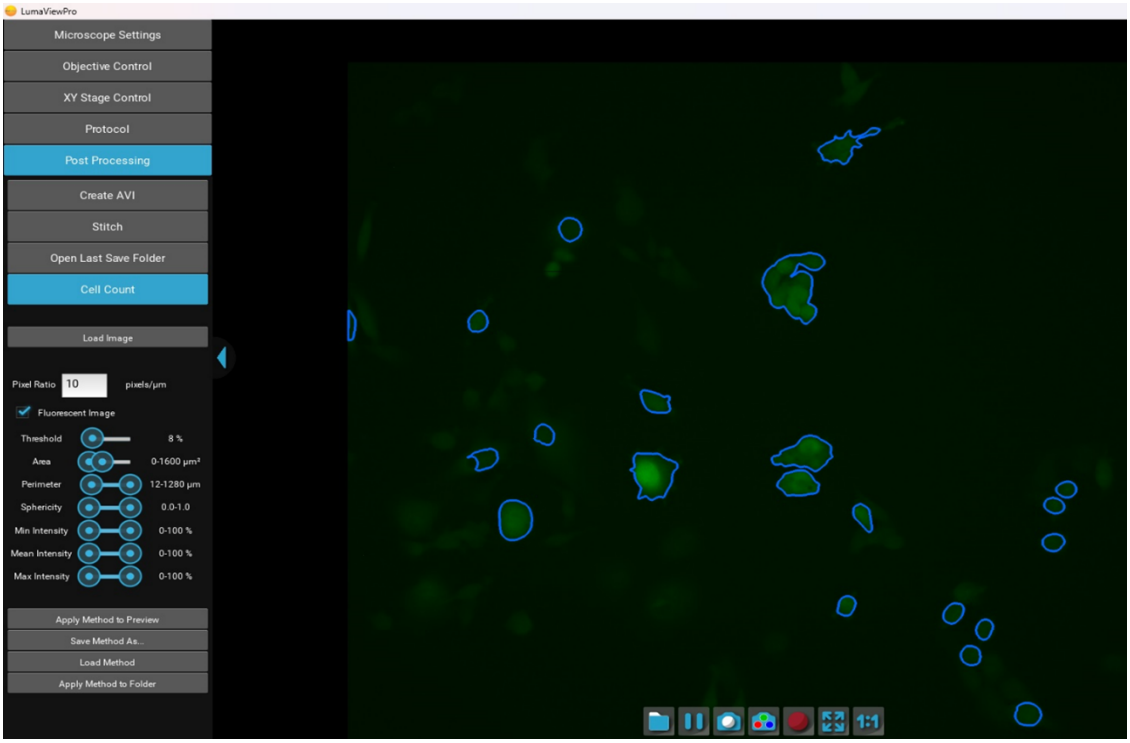


Figure 39: Method Preview

file	num_cells	total_object_area (um2)	total_object_intensity
roGFP 1_000000_F2.tif	55	685.22	9346.8
roGFP 1_000001_F2.tif	67	1421.73	19347
roGFP 1_000002_F2.tif	65	1461	19911.9
roGFP 1_000003_F2.tif	68	1529.52	20801.9
roGFP 1_000004_F2.tif	72	1533.95	20966.2
roGFP 1_000005_F2.tif	66	1494.79	20945.3
roGFP 1_000006_F2.tif	70	1539.75	20861
roGFP 1_000007_F2.tif	65	1552.69	21260.5
roGFP 1_000008_F2.tif	69	1529.04	20937.2

Figure 40: Results Table

9.3 Z-Projections

When a 3D dataset has been gathered as a stack of 2D images, different **Z-Projection** methods can provide insight by applying various mathematical operations to the dataset to help examine different aspects of the data.

Maximum Intensity
Displays the highest intensity value from all slices at each (X,Y) position. Useful for visualizing bright features within a thick sample.

Average Intensity
Calculates the average intensity across all slices at each (X,Y) position, providing a more balanced view of the data.

Minimum Intensity
Shows the lowest intensity value from each stack position, helpful for visualizing background or dark features.

Sum Slices
Adds up the intensity values across all slices at each X,Y position.

Standard Deviation
Calculates the standard deviation of intensity values across slices at each X,Y position, highlighting areas with high intensity variation.

10 Appendix A: Sanitizing the Microscope

Placing the LumaScope in the incubator often includes a cleaning step before installing. Here we discuss the methods recommended.

10.1 Wiping Down with Alcohol

Ethyl and isopropyl alcohols are the two most widely used alcohols because of their biocidal activity. Alcohols work through the disruption of cellular membranes, solubilization of lipids, and denaturation of proteins. These processes require water so the alcohols must be diluted to 60-90% in water to be effective. These alcohols are effective against lipid-containing viruses and a broad spectrum of bacterial species, but are ineffective against spore-forming bacteria. They also evaporate rapidly, which makes extended contact times difficult to achieve unless the items are immersed.

As mentioned above, the optimum bactericidal concentration for ethanol and isopropanol is in the range of 60% to 90% (typically 70%) by volume. Alcohols are generally regarded as being non-corrosive.

10.2 Wiping Down With Bleach

Chlorine compounds are good disinfectants, have a broad spectrum of antimicrobial activity, and are inexpensive and fast acting. Hypochlorites, the most widely used of the chlorine disinfectants, are available in liquid (e.g., sodium hypochlorite such as in household bleach) and solid (e.g., calcium hypochlorite, sodium dichloroisocyanurate) forms. Household bleach has an available chlorine content of 5.25%, or 52,500 ppm. Because of its oxidizing power, it loses potency quickly and should be made fresh and used within the same day it is prepared.

10.3 Exposure to Hydrogen Peroxide Vapor (HPV)

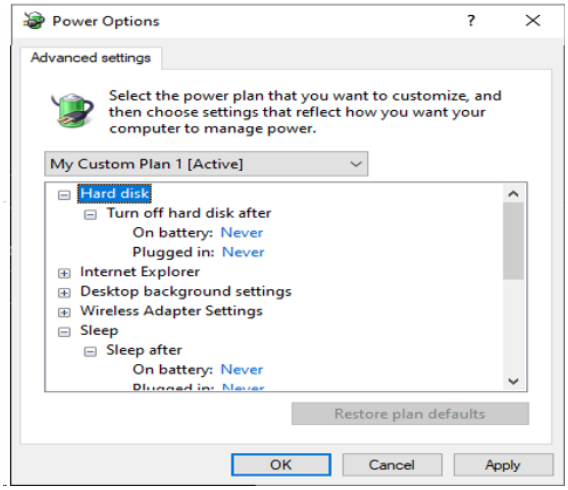
Hydrogen Peroxide Vapor (HPV) is another chemical that is effective in removing biological agents from the surfaces of equipment and other difficult-to-sterilize surfaces. The ability of vapor to reach a wide variety of desired areas means it is effective in sterilizing pass-through chambers and devices used in hospitals and manufacturing settings.
HPV's ability to decontaminate cell culture CO2 incubators without the use of heat offers significant advantages in research laboratories in which costly down time must be avoided. The combination of a seven-minute HPV fog in the chamber with circulation by the incubator airflow blower, followed by exposure to narrow-bandwidth ultraviolet light, provides an effective antimicrobial disinfection. Further, it reaches all incubator walls, shelves, reservoirs, air plenums, sensors, and other interior components, and it leaves only small amounts of sterile water droplets as a residual.

For questions, contact Technical Support at support@etaluma.com.

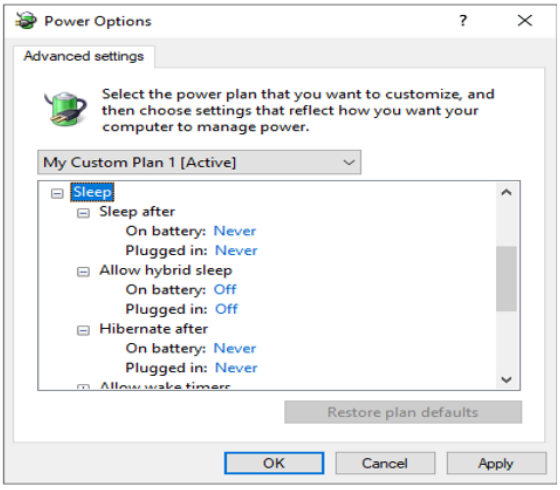
11 Appendix B: Setting Up Time-Lapse In Windows 10

11.1 Power Settings

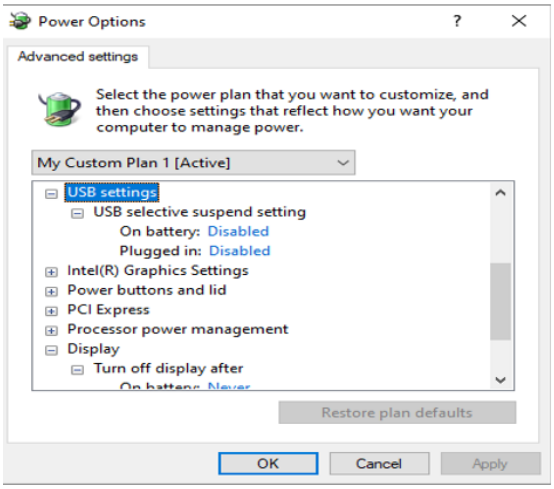
1. Navigate to Settings (click on the Windows icon on the lower left of your screen and then the gear icon). Choose System, then Power & Sleep. On the right side column click on Additional Power Settings. In the left column of the Control Panel dialog, click on Create a power plan. Choose the High Performance Plan as the template for your custom plan. Give your plan a name and click “Create”.
2. Next, click on Change plan settings next to your plan. Set Display and Sleep settings to "Never" and Brightness as desired. Next, click Change advanced power settings. Scroll to Hard Disk and click on the “+” keys to open the selection. Click on blue writing and change the time to “Never”. Scroll to “Sleep” and expand the selections. Make sure every item is selected as “Never”, “Off” or “Disabled”. Under “USB Settings”, open “USB Selective suspend setting” and make sure it is set to “disabled”. Press Apply to, then OK.
3. Now you have a custom power plan for running experiments on your LS Microscope. Before starting an experiment, be sure the computer is fully charged and plugged in to an outlet. When an experiment is finished, navigate to Settings -> System -> Power and Sleep.Click on Additional power settings and choose your usual power plan if desired.
4. Note: if you do not wish to create a custom power plan, you may simply change the advanced settings for your regular plan as described in step 2 above.



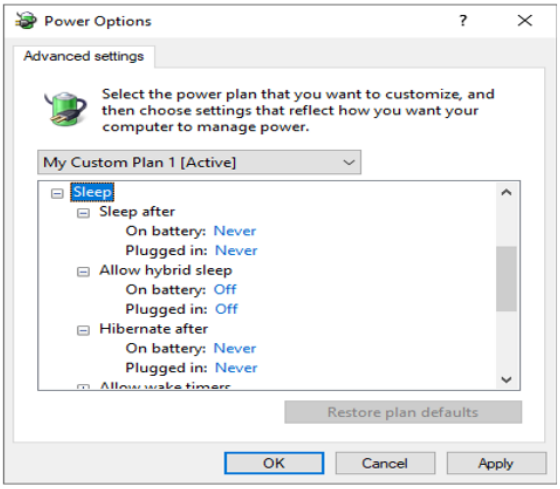
(a) Advanced Power Settings



(b) Sleep Settings.



(c) USB Power Settings



(d) Display Settings

Figure 41: Customizing Advanced Power Settings

11.2 Connectivity Settings

A.) Network connection NOT required (recommended configuration):

1. Disconnect the **Ethernet cable** (if wired connection is used) and turn on **Airplane Mode.**
2. Navigate to Settings -> Network & Internet -> Airplane Mode (left side) -> **ON.**
   Airplane Mode will prevent all wireless communications (Wi-Fi, Bluetooth and cellular) between the computer and other computers and devices.
   Note: after turning on Airplane mode, do not reconnect to your network, or Wi-Fi will be re-enabled, with Bluetooth and cellular remaining off.

B.) Network connection required:

1. Navigate to *Settings -> Network & Internet -> Wi-Fi.*
2. Click on Manage known networks, then select your network(s) and choose the following: “Make this PC discoverable” (if an option) – **OFF** “Metered connection” – **ON** Note: Windows will download only critical updates to your computer when you are on a metered connection.

11.3 Windows Update Settings

1. Navigate to *Settings-> Update & Security -> Advanced Options*
2. Under “Choose how updates are installed” -> uncheck the checkbox next to “Enabling this policy will automatically download updates. . . ”
3. Next, click on Delivery Optimization and choose "Allow downloads from other PCs" - **OFF**

Your computer settings are now complete. You may start an experiment in LumaViewPro.

12 Appendix C: Setting up Time-Lapse in Windows 11

12.1 Power Settings

Navigate to *Settings* (click on the Windows icon on the lower left of your screen and then the gear icon). Choose *System,* then *Power & Battery.* Set all to Never. (Fig 42)

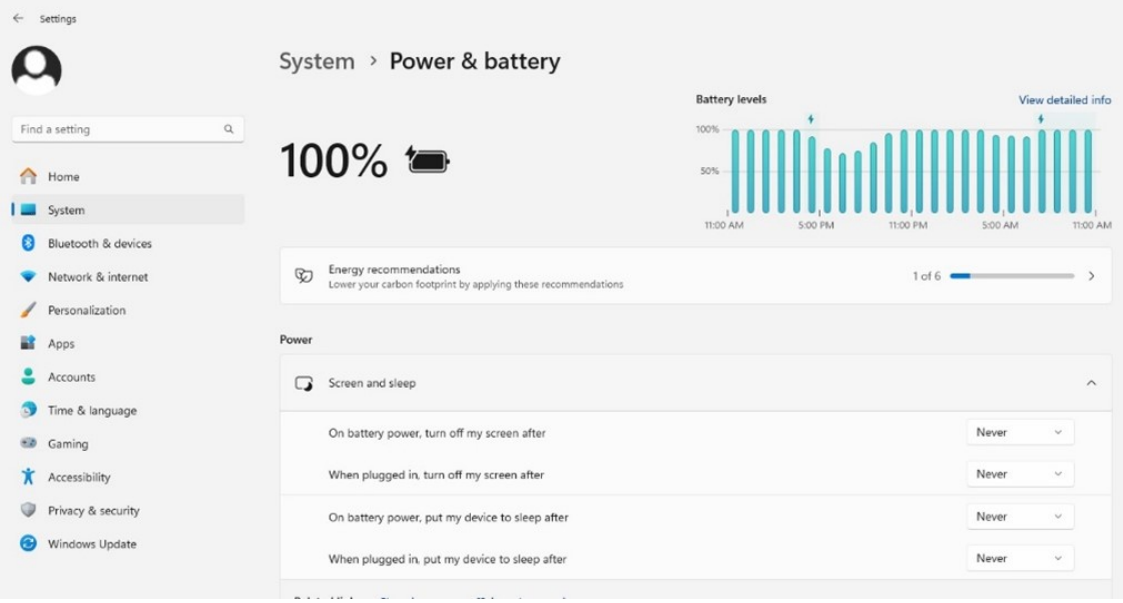


Figure 42: Windows 11 Power Settings

12.2 Connectivity Settings

A.) Network connection NOT required (recommended configuration):

1. Disconnect the **Ethernet cable** (if wired connection is used) and turn on **Airplane Mode.**
2. Navigate to *Settings -> Network & Internet -> Airplane Mode (left side) -> **ON.***
   Airplane Mode will prevent all wireless communications (Wi-Fi, Bluetooth and cellular) between the computer and other computers and devices.
   Note: after turning on Airplane mode, do not reconnect to your network, or Wi-Fi will be re-enabled, with Bluetooth and cellular remaining off.

B.) Network Connection Required:

1. Navigate to *Settings -> Network & Internet -> Wi-Fi.*
2. Click on Manage known networks, then select your network(s) and choose the following:
   “Make this PC discoverable” (if an option) – **OFF**
   "Metered connection" - **ON** Note: Windows will download only critical updates to your computer when you are on a metered connection

12.3 Windows Update Settings

1. Navigate to *Settings -> Windows Update -> Advanced Options* (Fig 43)
2. Under “Choose how updates are installed” -> uncheck the checkbox next to “Enabling this policy will automatically download updates. . . ”.
3. Next, Click on Delivery Optimization and choose “Allow Downloads from other PCs” - **OFF.** (Fig 44)

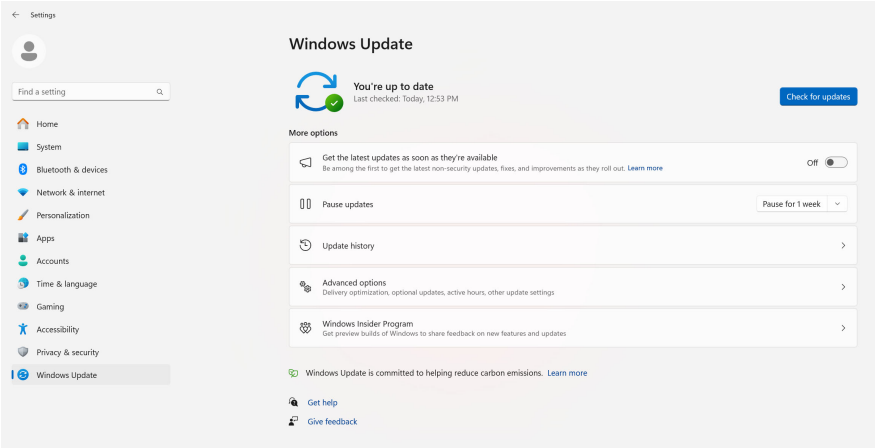


Figure 43: Windows 11 System Update Dialog

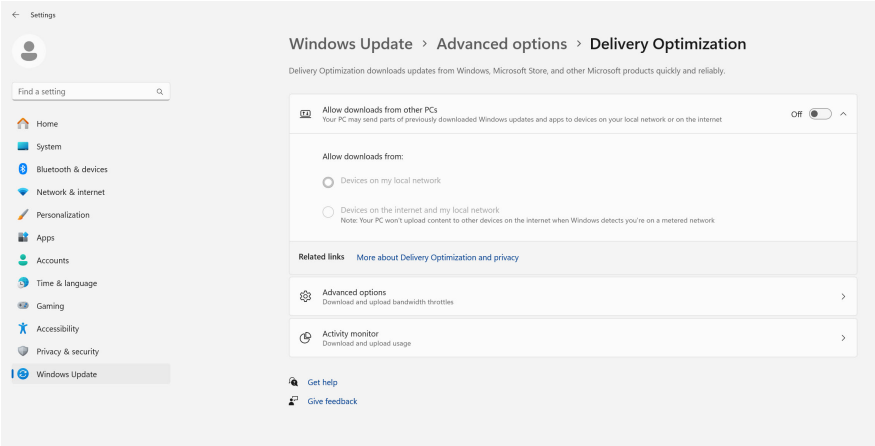


Figure 44: Win 11 Delivery Optimization

Your computer settings are now complete. You may now start an experiment in LumaViewPro.

13 Appendix D: Extending the Distance from LumaScope to PC Workstation

Often the LumaScope is located in an incubator which does not allow the PC or laptop to be placed in a convenient place for operation within the 3 meters that the included USB cable permits. **Warning!** The USB cable included with the LumaScope cannot be substituted or extended! In order to locate the viewing and control of the LumaScope a further distance, the monitor, keyboard, and pointing device must be extended without moving the PC or laptop. This is done through a longer HDMI video cable and Bluetooth keyboard and pointing device. The procedure below is for a laptop and allows the laptop to be used closed. If you are using a PC with external monitor then simply lengthening your HDMI cable to your monitor and adding Bluetooth keyboard and pointing device will be sufficient.

1. Connect LumaScope to an appropriate laptop computer using the provided USB cable.
2. Using an HDMI cable of desired length, connect laptop to an external monitor.
3. Using Bluetooth, connect laptop to an external keyboard and mouse.
4. Open Windows Settings, System, Display. Select duplicate monitor.
5. Open Control Panel. Select *Power Options -> Advanced Options*. In the section for “When I close the lid:” select “Do nothing.” Save changes. (fig 45)
6. Close laptop lid and place in a safe position. The external keyboard and mouse can now be used to control LumaScope via Lumaview software at a distance much greater than the length of the USB cable provided.

14 Appendix E: Configuring Autogain

Autogain brightness and timeout are stored in a json file which can be edited in Notepad and similar text editors. You can find these files under the data folder within the LumaViewPro folder. In the data folder you will find the settings.json file prior to the first use and then the current.json file after that. (Fig 46)

You can open this in Notebook and edit it (fig 47). Make sure to save them as a .json and not the default .txt. Note: the current.json file will only be present after the first startup of LVP where the settings.json is used and then updated as the current.json file. Open the current.json file and scroll to the bottom where you will find **target_brightness** and **max_duration_seconds**.

The target brightness is adjustable from 0-1 and defaults to 0.3. Increasing this number will cause the median pixel brightness to increase. The **max_duration_seconds** parameter allows the adjustment of the time allowed to come to a final gain value. This may need to be increased if a sequence of images have dramatically different brightness. After editing this file, you must restart LVP for the changes to take effect.

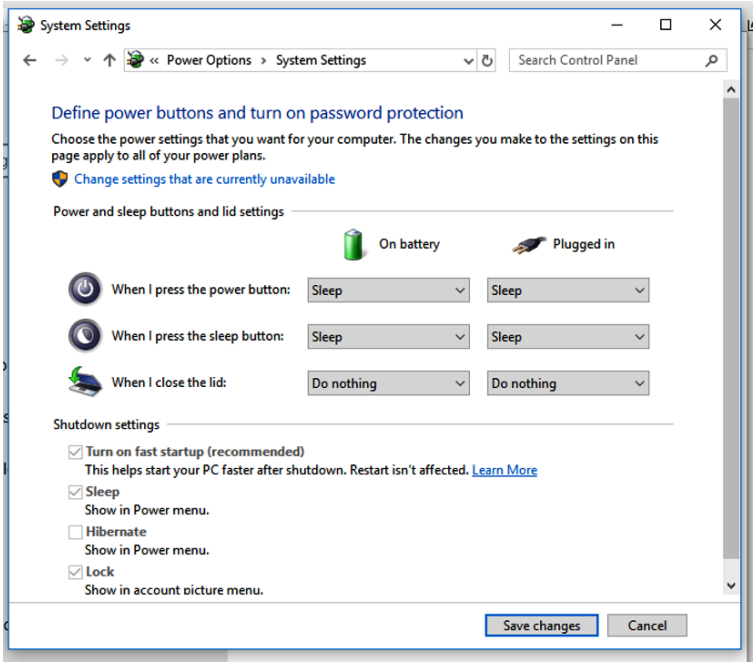


Figure 45: Power Settings For Range Extension

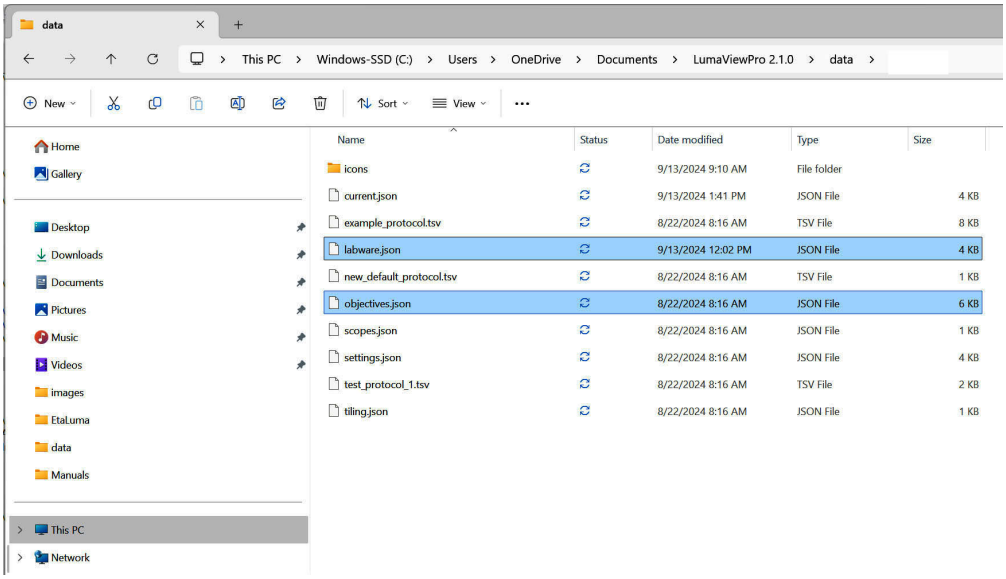


Figure 46: File Location For Adjusting Autogain Brightness

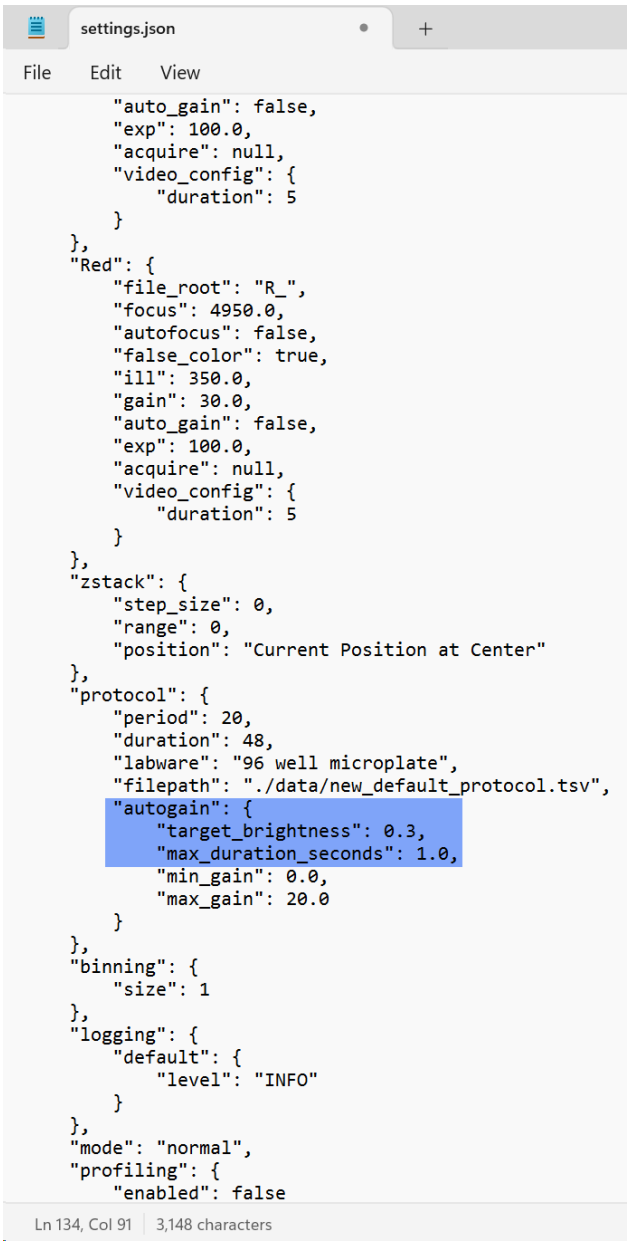


Figure 47: Editing Autogain Settings

END OF MANUAL