

LS820 Microscope

Operator's Manual

With

Etaluma's LumaviewPro™ Software
Version 1.7.3



For assistance, please email support@etaluma.com.

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

This document is available for download at <http://etaluma.com/products/downloads>.

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Setting up the microscope

Items Included With Each LS820 Microscope:

- AuraPhase Transmitted Illumination LED Fixture with cable
- Optional ambient light guard
- Fixture mounting knob



Accessories

- A. Dichroic cleaning swabs
- B. Black microplate lid for blocking room light during fluorescence imaging
- C. Foam plug for incubator port
- D. Lens paper
- E. USB memory stick with LumaviewPro application and this manual
- F. Mousepad

Included with manual stage: Universal Labware Holder



Optional Labware Holders

- 35 mm Petri dishes, fits inside Holder for 60 mm Petri dishes
- 60 mm Petri dishes & Terasaki plates
- Microscope slides & 50 mm Petri dishes
- 4 microscope slides in parallel plastic
- 4 microscope slides in parallel metal with magnetic clamps



Connecting the Cables



Connect the USB type C end to the LS820 at the rear left corner of the microscope. Wait to connect the type A USB cable 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.

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 Phase Accessory to be controlled by LumaviewPro.



LumaviewPro

The LS820 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 2100x 2100 pixels). Note: The monitor does not affect image resolution unless the monitor is low quality and affects your ability to judge focus.

Downloading and Installing LumaviewPro

To download Lumaview, 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 click to open the LumaviewPro.zip file.

Alternatively, the same LumaviewPro.zip file can be copied from the flash drive that comes with the microscope. It may be helpful to verify that this file is the latest version as posted on the Etaluma website.

After installation, a LumaviewPro shortcut can be created (orange logo icon) and placed on your desktop for convenience.

Connecting the LS820 Microscope

Insert the standard USB-A end of the supplied USB cable into a USB3 port on your computer and the other USB-C (later versions may have a 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.

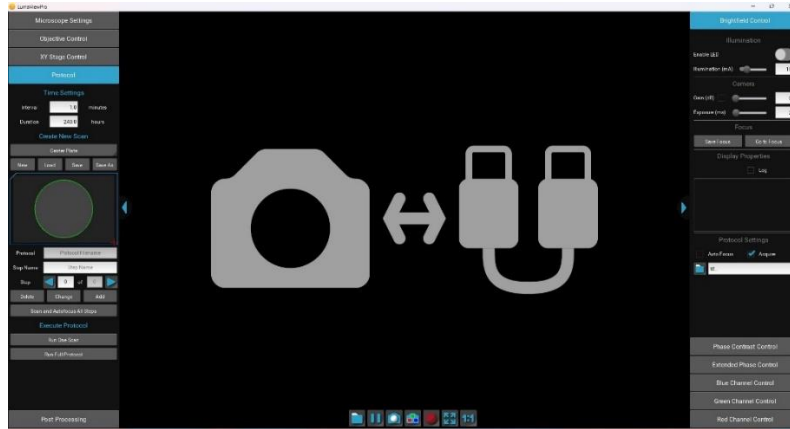
Plug the barrel connector from the AC power supply into the port labelled "Power Input 24VDC". A 2 meter extension is available if required.

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.

Troubleshooting

Microscope not connected



Communication Error Screen

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.

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.

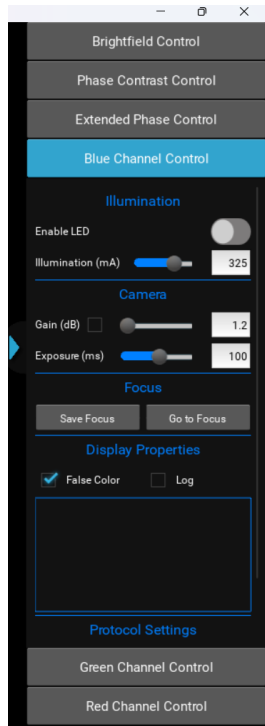


USB2 connection

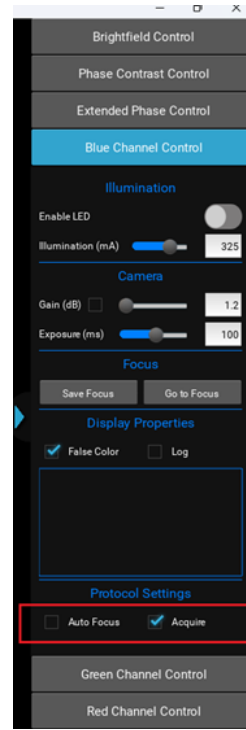


USB3 connection

Menus inaccessible

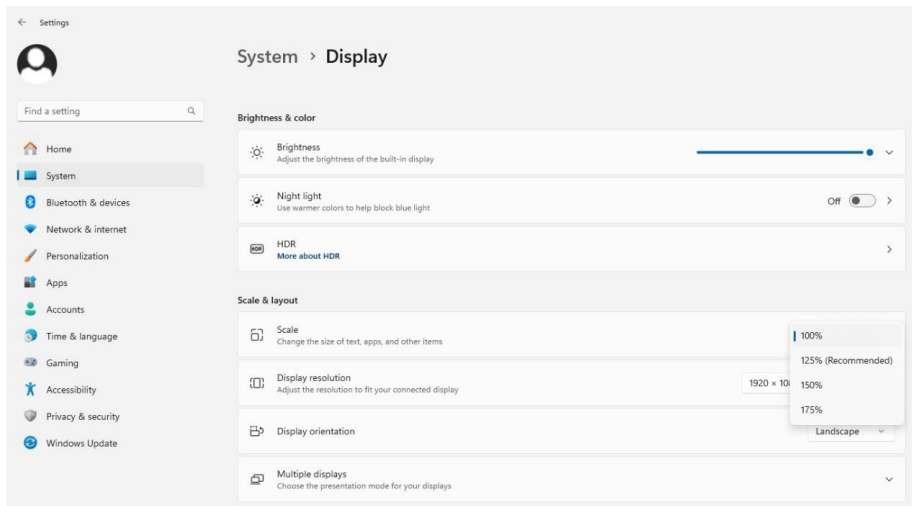


Full menu hidden

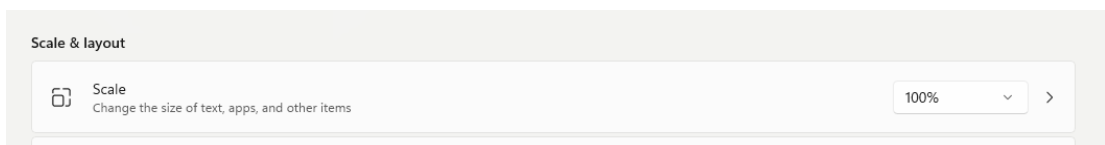


Full Menu visible

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.



Settings>System>Display>Scale

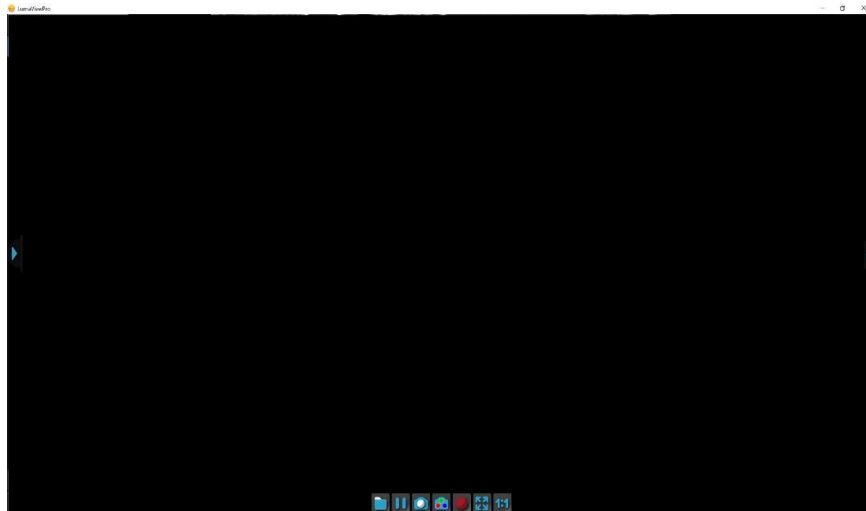


Scale decreased to 100%

Getting Started with LumaviewPro

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:



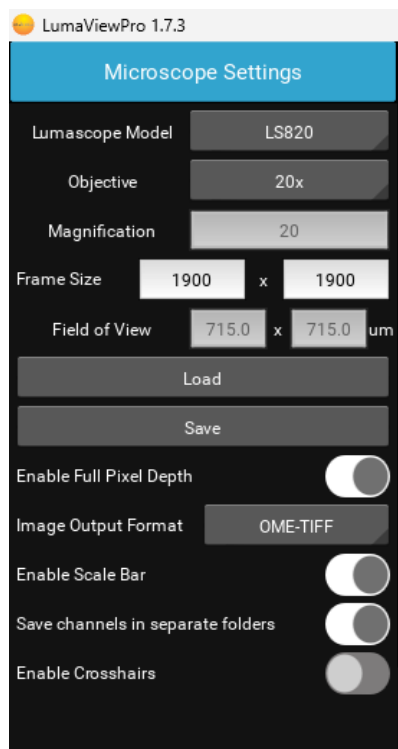
The side menus are accessed by clicking the blue arrows on the right and left edge of the application.



Motion Control Menus

Channel Control Menus

Microscope Settings



Microscope Settings configures your hardware with model and objective settings.

Click on the left arrow to expand the Motion control menus and select the **Microscope Settings** tab.

Ensure that the correct **Lumascope Model** is selected. Recent microscope versions have their configuration stored in onboard firmware and is automatically set.

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

Enable Full Pixel Depth saves all acquired images as 12-bit tiffs.

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

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 changed prior to running without saving.

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

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 enables automatic image brightness adjustment and will cause it to be used for that channel in any Protocol saved with that channel.

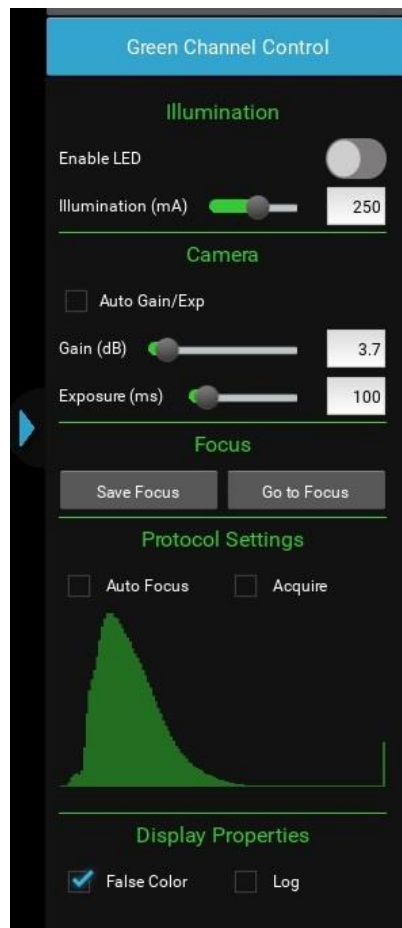
Autofocus will perform an Autofocus on every Step of a Protocol in this Channel.

Checking the **Acquire** option will include this channel in any automated process including Protocols and Composites.

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

False Color provides pseudo coloring for the Fluorescence channels.

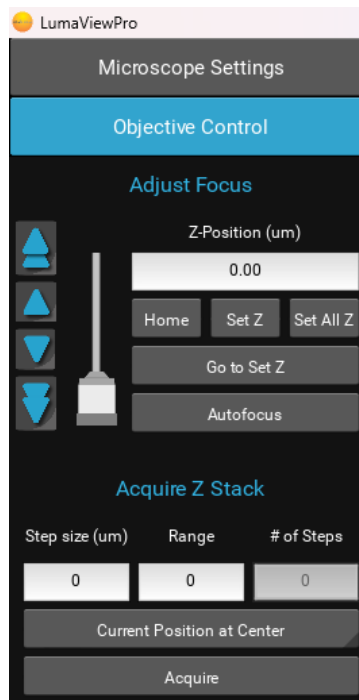
A live histogram of pixel intensities is displayed with linear or logarithmic Y axis.



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.



Focus and Z-Control



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. 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 very few edges from which to calculate the contrast.

The Turret is not offered on the LS820.

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.

Getting an Image

Place the sample onto the microscope and ensure it is seated flat. 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 Z under the Focus tab to return to this location.

The Toolbar



The Toolbar provides common actions. From left to right, define the destination folder for all image files. This single destination folder creates Manual and ProtocolData subfolders. A new time/date named folder is created upon every Run of a Protocol.

Sequentially looking across the toolbar: You may 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/ and present the image as a ratio of one camera pixel to one screen pixel.

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** box checked will be used with their corresponding 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.
- **Tiling** dimensions.
- **Enable Z-stack** is checked to use the Z-stack settings under Objective Control.

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, and focus edited, including deleting the step entirely. You must click Change to have the new settings updated for that Step.

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.

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.

Adding new focus level Steps for a Protocol

In order to **Add** additional images to be acquired at different focus levels, move to the desired new Z position and click **Add**. **Adding** Steps will duplicate the name of the Step was inserted from. **Adding** Steps will be named custom0, custom1.... Edit new Step names appropriately and click **Change**.

Configuring a Protocol Quick Start

- Place sample on the microscope.
- 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.
- Set Z-stack settings and Enable Z-Stack checkbox if desired
- Click New.
- Observe that the channels multiplied by the number of 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 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.

Live Cell Imaging with the LS850 Microscope in an Incubator

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

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.

To remove the microscope from a warm incubator:

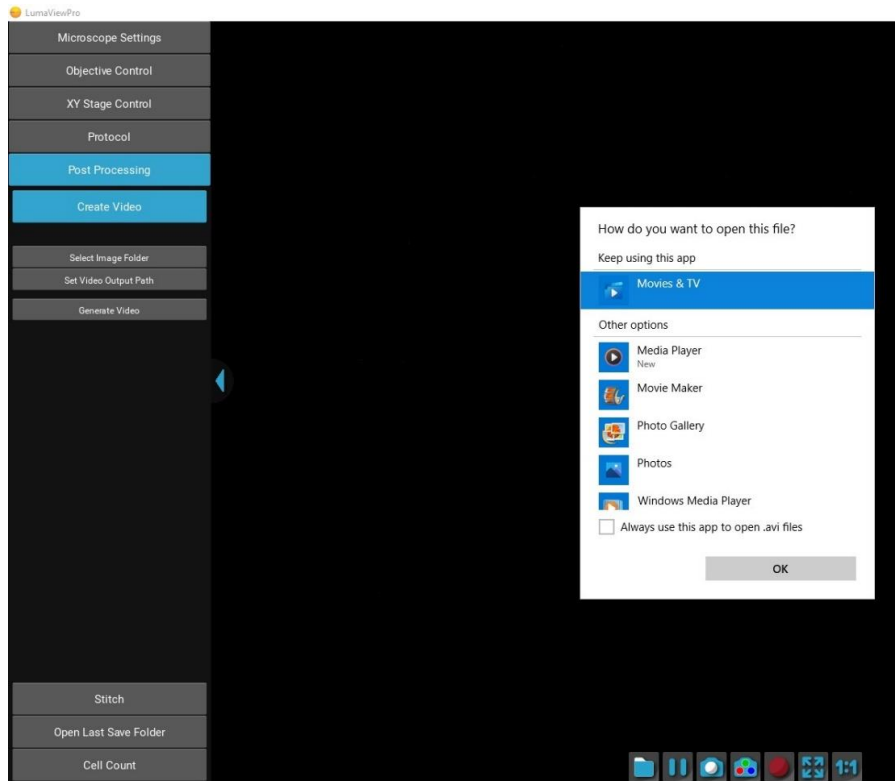
It is safe to take a warm Lumascope out of a humid incubator and put it on the bench. It is warmer than the lab, so water will not condense.

- ***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.

Post Processing

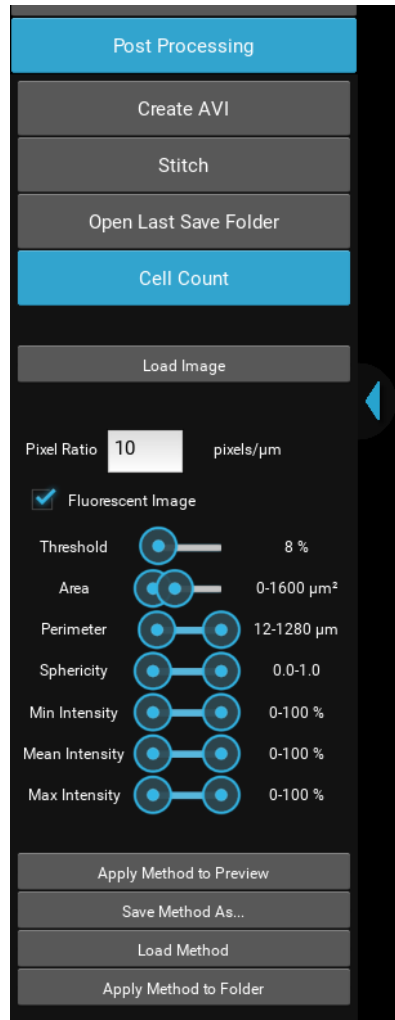
Create Video allows time lapse or video frame recorded images to be made into a movie. Under Post Processing select Create Video, this will reveal three selections. Select Image Folder allows selection of the image files to be made into the AVI. The images will not be visible when selecting the folder. Set Video Output Path can be selected if one wishes the movie to be saved somewhere besides where the source images are located. Generate Video will compile the images into an .avi file at 10 frames per second. The first time you open the movie, you may need to associate the .avi file with a video layer application. Windows Media Player is a good default choice.



Stitching is not applicable to the LS820.

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.

Cell Count



The Cell Count 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:

| 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 |



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 this:

| 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 |

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

Appendix A

Sanitizing the Microscope

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

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.

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.

Exposure to Hydrogen Peroxide Vapor

Hydrogen Peroxide Vapor 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.

Hydrogen Peroxide Vapor'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 Hydrogen Peroxide 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.

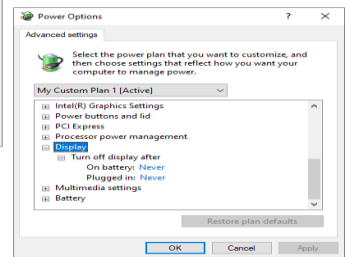
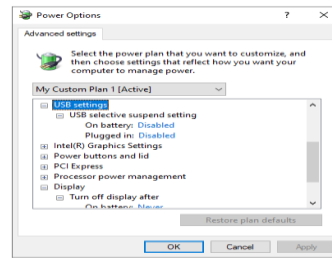
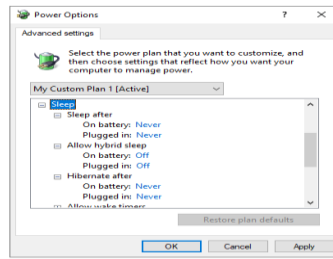
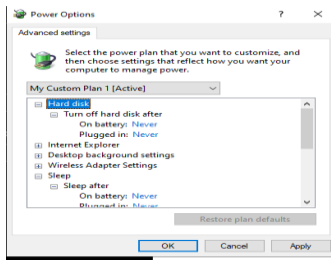
For questions, contact Technical Support at support@etaluma.com.

Appendix B

Setting up Time-Lapse in Windows10

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, 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.



Connectivity Settings

A) Network connection NOT required (recommended configuration):

Disconnect the **Ethernet cable** (if wired connection is used) and turn on **Airplane Mode**. 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.

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.

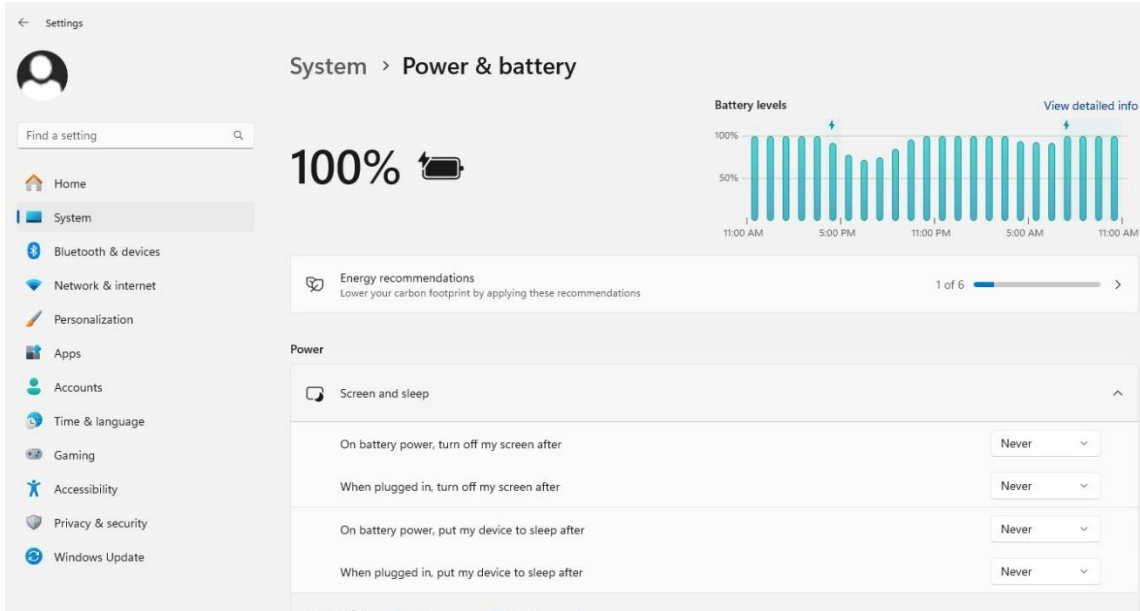
Your computer settings are now complete. You may start an experiment in LumaviewPro.

Appendix C

Setting up Time-Lapse in Windows11

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 & Battery*. Set all to Never.



Connectivity Settings

C) Network connection NOT required (recommended configuration):

Disconnect the **Ethernet cable** (if wired connection is used) and turn on **Airplane Mode**. 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.

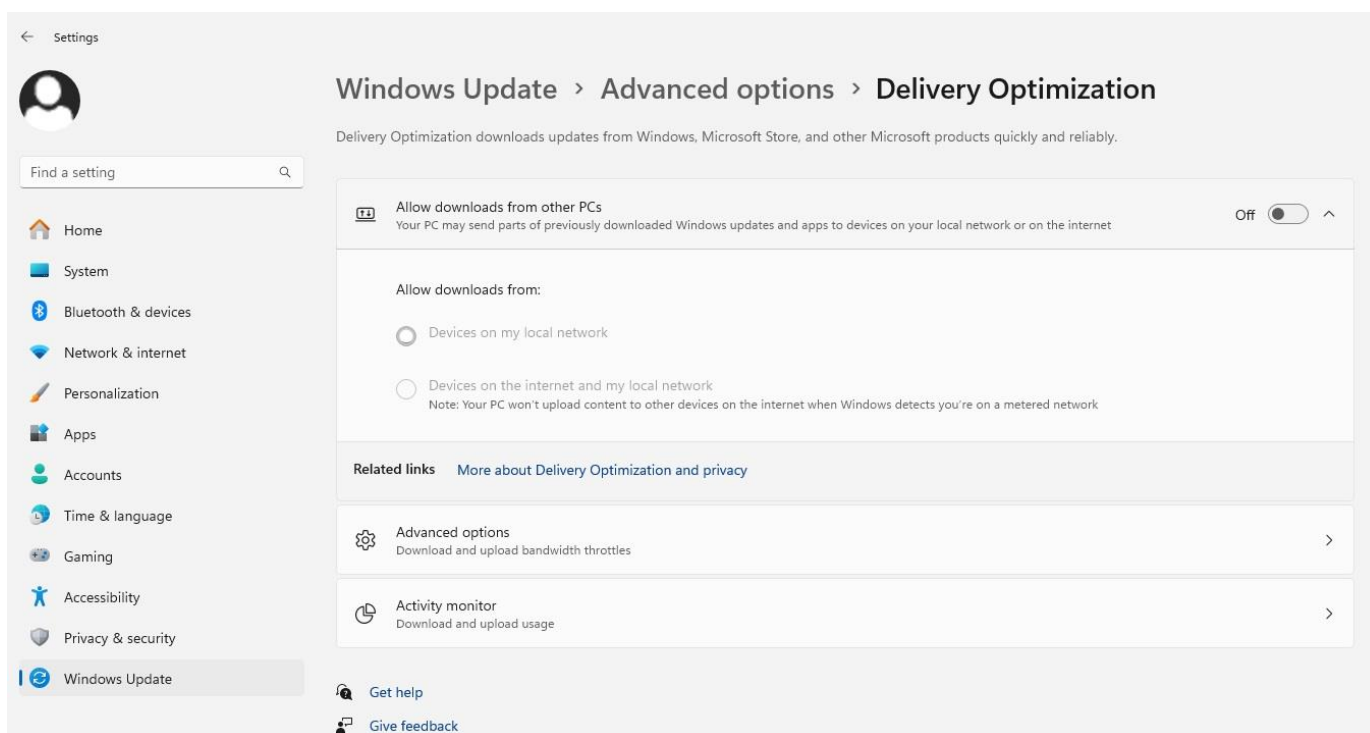
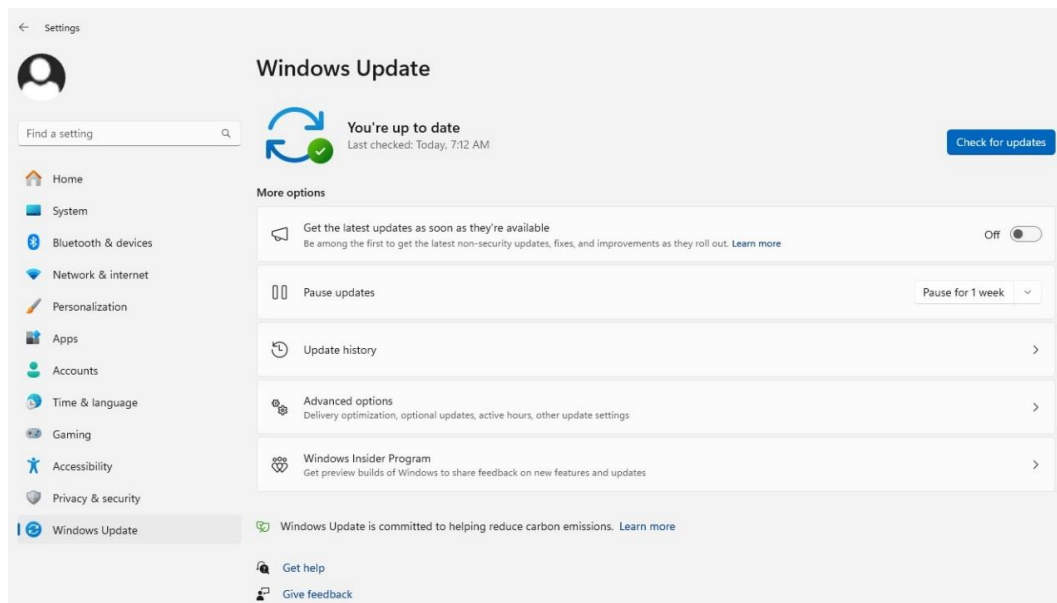
D) Network connection required:

4. Navigate to *Settings* -> *Network & Internet* -> *Wi-Fi*.
5. 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.

Windows Update Settings

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



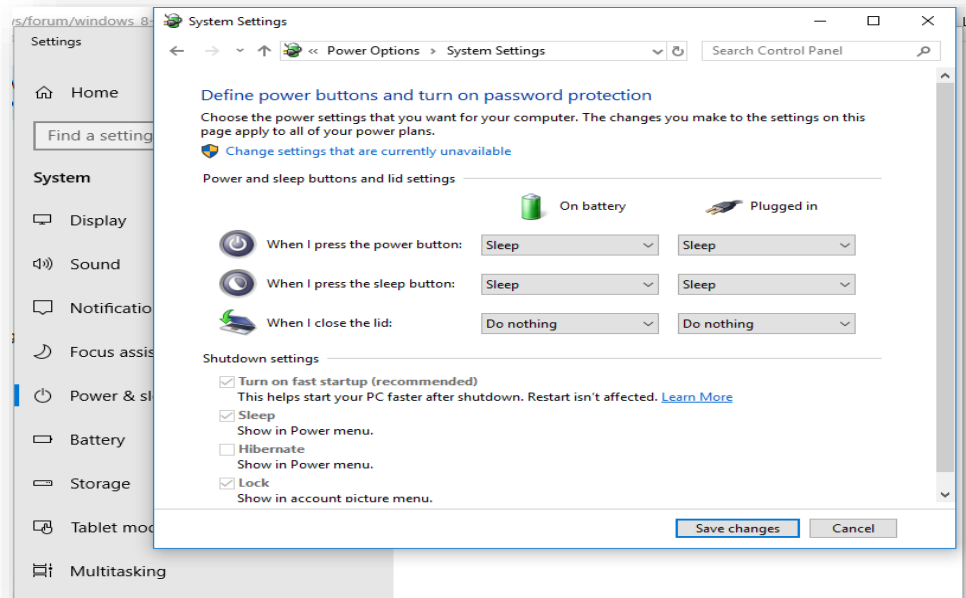
Your computer settings are now complete. You may start an experiment in LumaviewPro.

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.



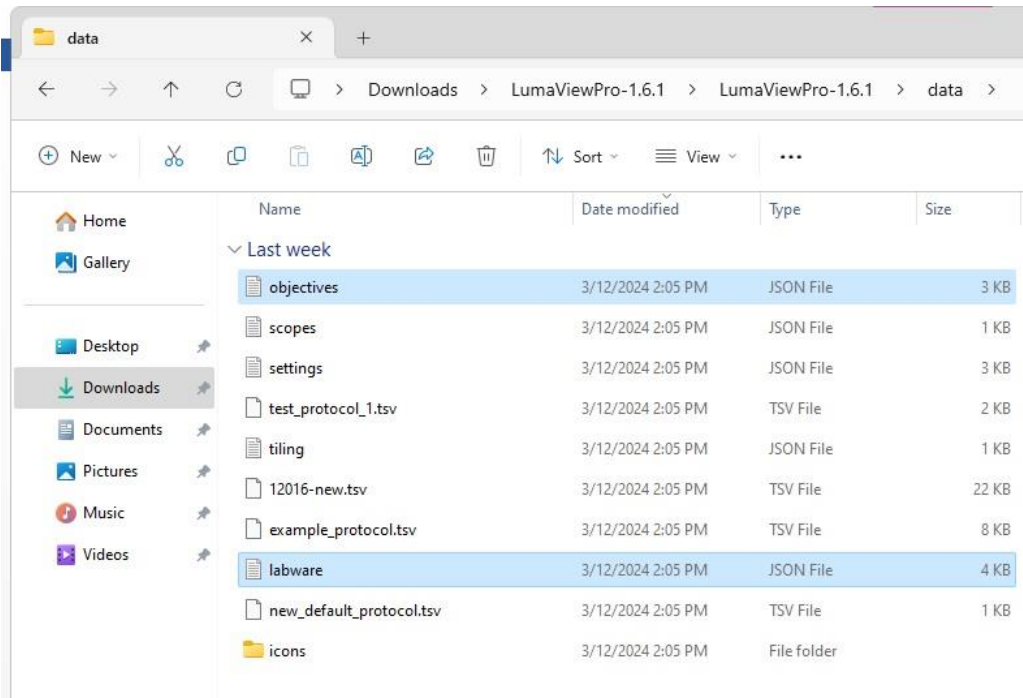
6. Close laptop lid and place in a safe position. The external keyboard and mouse can now be used to control Lumascope via Lumaview software at a distance much greater than the length of the USB cable provided.

Appendix D

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. After opening and closing LVP, a new entry called current.json will be created and used from then on.



You can open this in Notebook and edit it. 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.

```
settin x +
File Edit View
{
  "auto_gain": false,
  "exp": 100.0,
  "acquire": false
},
"Red": {
  "file_root": "R_",
  "focus": 4950.0,
  "autofocus": false,
  "false_color": true,
  "ill": 350.0,
  "gain": 30.0,
  "auto_gain": false,
  "exp": 100.0,
  "acquire": false
},
"zstack": {
  "step_size": 0,
  "range": 0,
  "position": "Current Position at Center"
},
"protocol": {
  "period": 20,
  "duration": 48,
  "labware": "96 well microplate",
  "filepath": "./data/new_default_protocol.tsv",
  "autogain": {
    "target_brightness": 0.3,
    "max_duration_seconds": 1.0
  }
},
"logging": {
  "default": {
    "level": "INFO"
  }
},
"mode": "normal",
"profiling": {
  "enabled": false
},
"scale_bar": {
  "enabled": false
}
}
Ln 113, Col 1 93 of 2,802 charac 100% Windows (CRLF) UTF-8
```