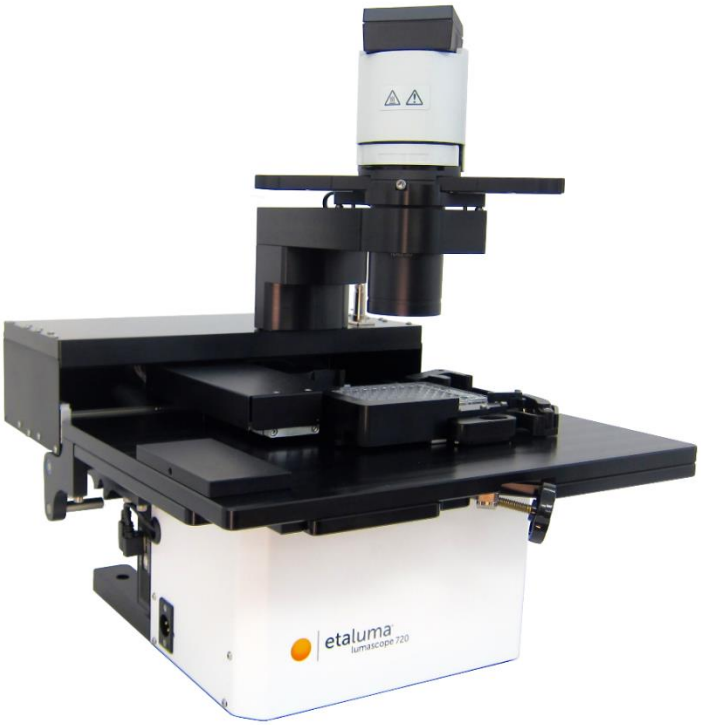
LS720 Microscope

Operator's Manual

With

Etaluma's Lumaview Software



For assistance, please call 760-298-2355 or email support@etaluma.com.

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Table of Contents

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

I. SETTING UP THE LS720 MICROSCOPE

A. Items Included With Each LS720 Microscope

- Phase Contrast Accessory (Olympus)
- USB communication cable
- External power supply/cord with country-specific plug
- Hex wrench (3 mm) for removing/replacing shipping lock
- Fluorescence shroud (black microplate lid)
- Calibration 1536 well microplate
- Calibration 4x objective

B. Optional Accessories (purchased separately)

- Labware Holders
  - Holder for 35 mm Petri dishes, fits inside Holder for 60 mm Petri dishes
  - Holder for 60 mm Petri dishes & Terasaki plates, SBS outer dimensions
  - Holder for microscope slides & 50 mm Petri dishes, SBS outer dimensions
  - Holder for 4 microscope slides in parallel, SBS outer dimensions
- Light Hood
- Lumaquant, image analysis software
- Microvolution, deconvolution software

C. Recommended Computer Specifications

Windows 10; Core i7 or better processor; 500 GB to 1 TB SSD hard drive, 4 Gb minimum RAM(8 Gb min. *Lumaquant*); single 4K monitor or two with 1080p HD resolution. Windows must have Service Pack 2.0 with .NET version 4.5.2.

Microvolution deconvolution plugin for ImageJ/Fiji: A GPU is required; the most powerful NVidia chip running CUDA that can be afforded is recommended for the best performance.

D. Unpacking the LS Microscope

1. Remove LS720 Microscope from the shipping box (save box and packing materials) and place on a sturdy bench or counter.

2. If the LS Microscope will be used in an incubator or environmental chamber, it is recommended that the initial setup be carried out on the bench under ambient conditions before placement into the specialized environment.

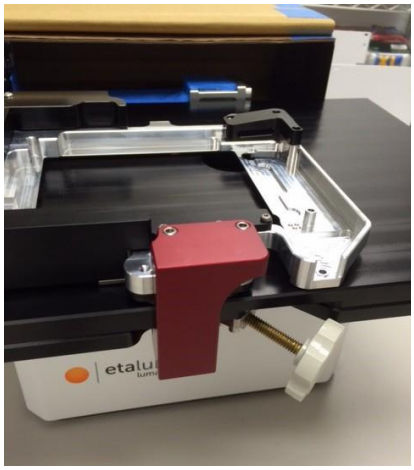
E. Removing the Shipping Lock

WARNING: You must remove red shipping lock before plugging the LS720 into power! If not removed, the LS720 will be damaged!

1. Using the supplied 3 mm hex wrench, loosen slightly the 2 set screws on top of the installed shipping lock. Loosen slightly the 2 set screws (smaller and more internal) on bottom of the lock.

2. Unscrew completely the 2 larger 1-inch screws on bottom of the shipping lock. Slide the shipping lock toward you to remove.

3. Keep the 4 set screws screwed into the shipping lock and place in a secure place along with the two 1-inch screws and 3 mm hex wrench.



LS720 Shipping lock

WARNING: If packing the LS720 for shipment, you must install the red shipping lock before placing into the shipping carton! If not installed, the LS720 can be damaged! See Appendix A for instructions.

F. Connecting the Cables

1. Connect the USB type B end to the LS720 at the rear left corner of the microscope. Wait to connect the type A USB cable from the Microscope to the computer until Lumaview is installed.

2. Plug the external power supply/cord into the left side port (when viewing from the front) and the plug into an AC outlet.

G. Phase Contrast

a. Remove the Phase Contrast Accessory from its shipping box. Attach Phase Accessory by placing the holes on the bottom of the Phase Accessory bracket over the pegs on the upper surface (right rear) of the LS720. Make sure pegs are fully seated and arm is totally flush with the LS720 surface. Tighten the large thumb screw at the backof the Phase Accessory arm.

b. Connect the free end of the Phase Accessory power cable to the round socket nearby. This allows the Phase Accessory to be controlled by Lumaview.

H. Brightfield

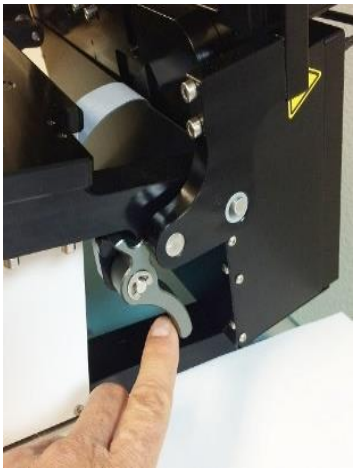
1. Bright field imaging uses of the Phase Accessory with the included Slider in an open position (no phase ring) and the iris adjusted for an optimum image in conjunction with a lowered exposure and illumination level in Lumaview. Bright field images will be in gray scale due to the monochromatic CMOS camera.

2. Overhead fluorescent lighting can cause uneven brightness including a striped pattern on the image; if observed, move the LS Microscope or partially shade the light to reduce unevenness across the sample.

I. Installing Objectives

1. Unscrew the deck knob at the front of the instrument until the top deck releases and raise the top deck until it latches open. Unscrew the black cap (or previously installed objective) on top of the optics block and screw in the objective. Do not overtighten. To lower the top deck, activate the release lever at the right rear hinge, lower the deck, and tighten the deck knob.

2. When installing or changing an objective, carry out the insertion quickly to minimize dust falling onto the mirrored dichroic filter. A small amount of dust will not affect imaging. However, if dust is significant and it appears to affect quality of the live image or snapped images, contact Etaluma by email (support@etaluma.com).



Deck release lever

J. About Lumaview

1. The LS720 is controlled by the Lumaview software program. The latest Lumaview version is downloadable from Etaluma's website and must be installed prior to connecting your computer for the first time.

2. Lumaview requires Windows 10. Desktop computers and laptops can be used, but the best visualization correlates with monitor resolution equal to the sensor resolution (up to 1900x 1900 pixels). Note: The monitor does not affect image resolution unless the monitor is low quality and affects your ability to judge focus.

   The computer should have Windows .NET Framework 4.5.2 or higher installed; see https://www.microsoft.com/en-us/download/details.aspx?id=25150 for more information. If your computer does not have .NET Framework 4.5.2 or higher installed, downloading Lumaview may automatically take you to the Microsoft .NET download page. Scroll down the page to find the correctdownload. You can also download it directly from here: https://www.microsoft.com/en-us/download/details.aspx?id=25150 This does not require a purchase.

K. Downloading and Installing Lumaview

1. To download Lumaview, go to http://etaluma.com/products/downloads (under the Resources tab). Click on Lumaview - ZIP link to start the download and save the folder when prompted. Go to your downloads location and click to open the Lumaview.zip file.

   Alternatively, the same Lumaview .zip file can be copied from the flash drive that comes with the LS Microscope. Verify that this file is the latest version as posted on the Etaluma website.

2. If your computer has an older version of Lumaview, installing the newest Lumaview will over-write the older versions. If there is a need to revert to an older version Lumaview, it will be necessary to first uninstall the current version using the procedure described in Appendix B.

3. To install Lumaview, right click on the .zip file and Open with Windows Explorer. Double click on the .msi installer file to start installation. If a Windows warning box about an unrecognized App appears, click Run anyway.

   You will be asked about the location; note that the default is a new Etaluma folder inside the Program Files (x86) folder. During installation, a Device Driver Installation Wizard will open. Click to continue (**twice**) and finish installing the two drivers. Installation of Lumaview will then finish. After installation, a Lumaview shortcut (orange logo icon) convenient for launching the software will be present on the desktop.

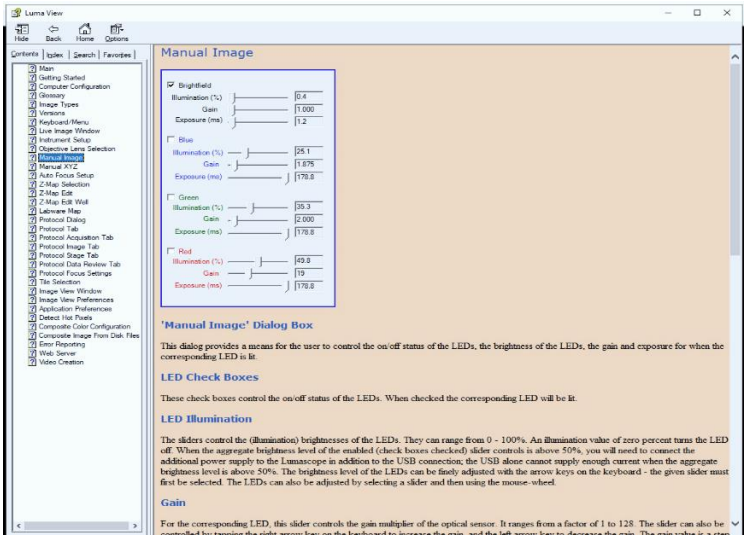
L. Connecting the LS720 Microscope

1. If connecting to a computer that has been off, turn computer on. Make sure computer is connected to its monitor(s). Insert the standard USB-A end of the supplied USB cable into a USB port on your computer and the other square USB-B end into the square port on the rear left side. It is also important to connect the Microscope directly to the computer USB port and not use a USB hub.

2. If Lumaview has not been run previously, it is important that the LS720 microcope and computer be connected using the USB cable before launching Lumaview. This is because Windows needs to load the USB drivers before Lumaview can run.

II. SOFTWARE: GETTING STARTED WITH LUMAVIEW

The complete User Guide for Lumaview is in the Help Section after Lumaview is opened. Click on Help in the Title Bar to open the pulldown menu, click Contents, or simply Click F1 to open the Help Section.



A. Starting Conditions

1. Lumaview has been downloaded and installed on the computer.
2. The microscope is connected to the computer via the USB cable.
3. Red shipping lock MUST be off, power cable is plugged into a standard AC outlet, and fluorescence shroud is available if imaging involves fluorescence outside of an incubator.

B. Launching Lumaview

1. Launch the Lumaview software from the desktop icon (or other chosen location) and allow the microscope to be discovered and initialize. This may take a minute or so the first time.

   Two windows will open on your monitor: Live Image Window inside the larger Main Window. "Live Image Window" will be in the title bar when the Window is not maximized. When Live Image Window is maximized, [Live Image Window] transfers to the end of the Main Window title bar. The Live Image Window status bar at the bottom shows communication with the camera sensor via continuous display of several useful metrics: current frame rate, data transfer rate, number of frames collected thus far in the session, frame size, and session date and time start.

Note: A variety of Windows configuration errors can be displayed at this point. Please consult Appendix A.

2. The Live Image Windows includes a Left Toolbar with the following commands:

Select Channel, optimize settings

Move in XYZ

Snap Image

Composite

Video Record (toggles on and off)

Protocol

Zoom

Shortcut to Last Image file location

When Lumaview is open, the icon that resides in your computer dock appears as a document with an arrow.

If the Left toolbar icons are too small, close Lumaview, right click the Lumaview shortcut icon on the desktop and select Properties.

1. Click on the "Compatibility" tab
2. Click the "Change high DPI settings" button near the bottom of the menu
3. Check the "Override high DPI scaling behavior" box and then select "System (Enhanced)" right below it
4. Click "OK" to close the menus

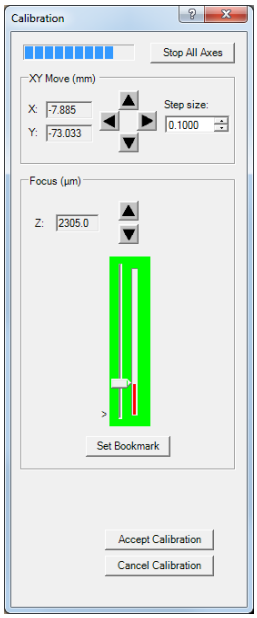
Restart Lumaview.

C. Initialization and Calibration

1. The LS720 automatically initializes every time Lumaview is started by moving the microplate carrier to the Home position. The Home Position consists of the left front corner of the microplate carrier frame being positioned over the objective and the objective retracted to the lowest Z level. No image can be obtained at the Home position because it is under the microplate carrier frame.

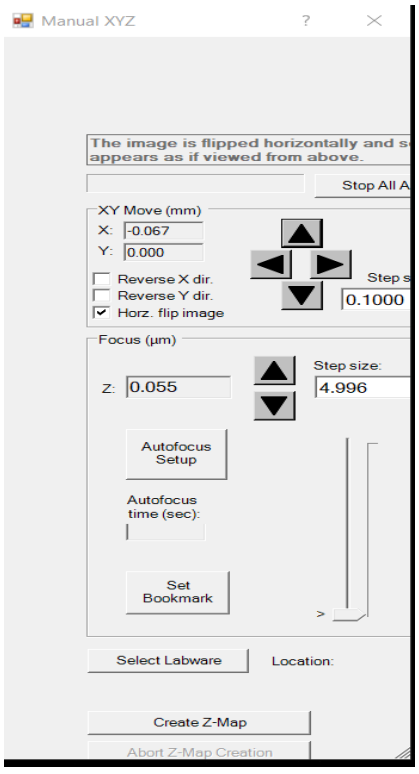
2. If this is the first time the LS720 has been connected to the current computer, it is first necessary to do a Calibration. *Once completed, calibration is not needed again unless a different computer is connected.*

   a. To calibrate the LS720, install the provided Calibration Motic EFN 4x Objective. Place the provided Calibration 1536 well microplate into the microplate carrier.



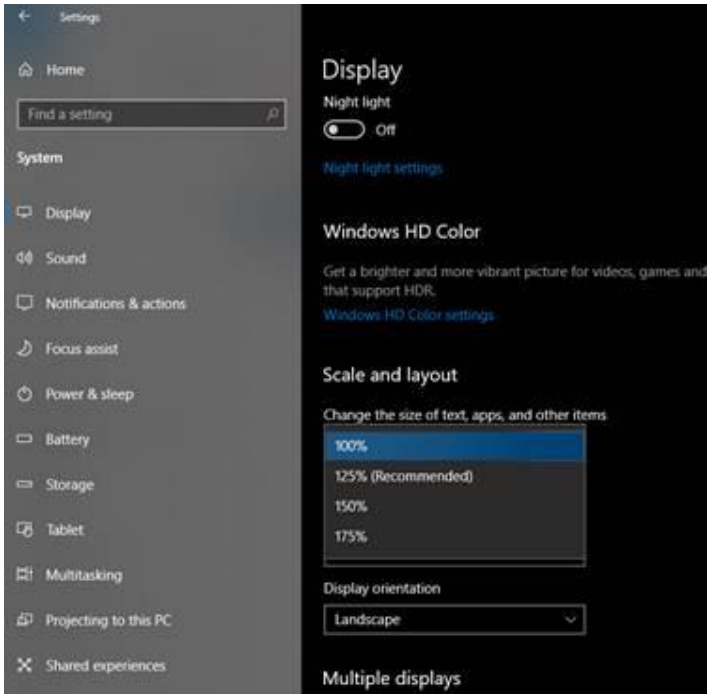
   b. Click Utilities in the File Menu bar and in the drop-down menu click Calibrate to open its dialog box. A communication dialog box will open asking if a 4x objectivehas been installed. Click Yes and the communication dialog box will close.

   c. Note: if your Calibrate menu is offset similar to this:



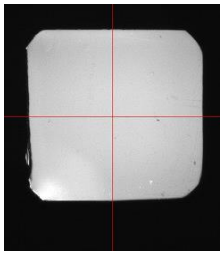
To fix this, right-click the Lumaview shortcut icon on the desktop and open 'Properties'. Under the 'Compatibility' tab, click on the button labeled "Change high DPI settings". At the bottom of the Lumaview.exe Properties panel that opens, look under "High DPI Scaling override" and check the box labeled "Override high DPI scaling behavior. Scaling Performed by System (Enhance)." Click 'OK' and re-launch Lumaview.

You may need to adjust your screen Scale to one below the Recommended in order to increase the resolution of your display while running Lumaview.



d. The Live Image Window will now show red crosshairs, and the microplate carrierwill move so well A1 is close to being over the objective.

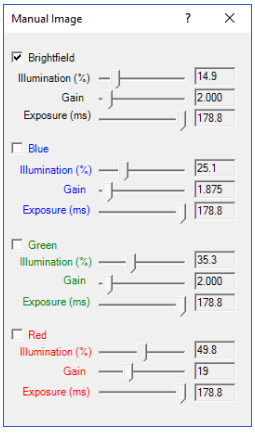
e. Click the Manual Image icon in the left tool bar (top icon) to open its dialog box.



Red crosshairs in Live Image Window

f. To use ambient light for calibration (recommended; see Section I.I.), check Brightfield in Manual Image to activate Gain and Exposure sliders. Start with Gain as low as possible and adjust Exposure until a mid-gray tone is shown inthe Live Image Window.

g. If using the Phase Contrast Accessory for brightfield illumination (Section I.I.) move the phase slider to an open position (without phase ring) and move the phase condenser aperture (small lever on condenser ring) and move the phase condenseraperture (small lever on condenser front above slider) so only a low level of light is transmitted. In Manual Image, check Brightfield, set Gain to 1.0, Illumination (%) low, and adjust Exposure from zero until a mid-gray tone is seen in the Live Image Window.

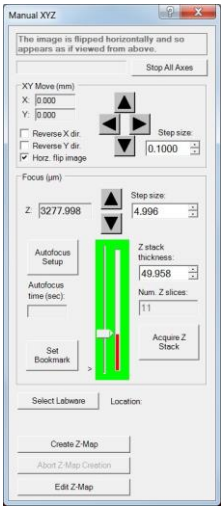


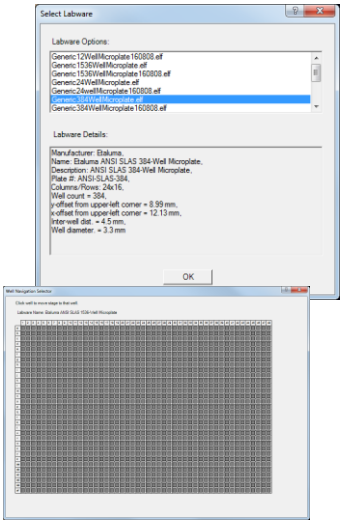
h. In Calibration, move the Focus slider up while watching the Live Image Window to detect when the well outline can be seen and is in sharp focus. The Focus slider area becomes green whenever the Focus slider is active. Using the X/Y Move arrows (and Step size arrows if needed), move the

microplate carrier until the A1 well is centered in the Live Image Window. Make sure the centered well is A1 by moving to the left and up to confirm it is the corner well using the X/Y Move arrows, and then re-center the A1 well.

i. In Calibration, click Accept Calibration. The Calibration dialog box will now convert to the Manual XYZ dialog box.

j. Test the calibration on other wells by clicking the Select Labware button in Manual XYZ to opens its dialog box. (If Manual XYZ is not already open, click itsicon in the left tool bar (second from top).

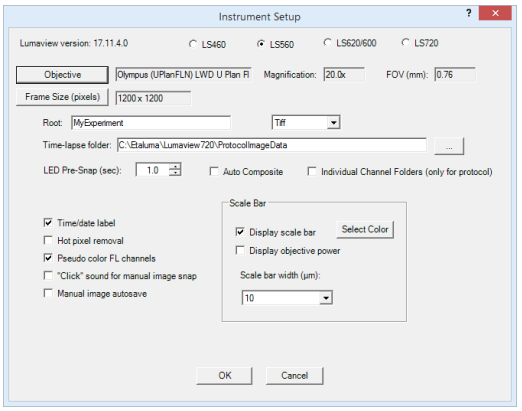




k. Click on Generic1536WellMicroplate.elf to highlight it and then click OK. The appropriate Labware Map dialog box (1536 well in this case) will open. Click on any well and the microplate carrier will move to center that well over the objective. Test the microplate corners and other wells distant from each other. If any wells are not centered, repeat the Calibration procedure described in this Section.

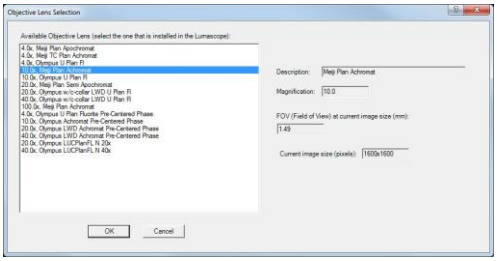
D. Instrument Setup

1. Click on Configuration in the File Menu bar to open the drop-downmenu and select Instrument Setup to open its dialog box.

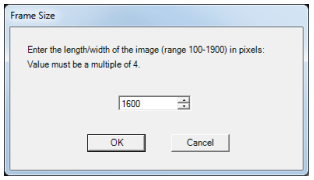


2. Check the Lumaview version to make sure it is the most recently posted version on the Etaluma website (www.etaluma.com/products/downloads/). Ensure that the correct model microscope being used is checked. If it is necessary to change the LS Microscope model number that is checked, you will be prompted to restart the program. Be sure to restart so that all functions work correctly.

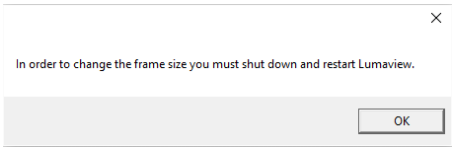
3. Click the Objective button to open its dialog box (called Objective Lens Selection). Select the desired objective to highlight it. Note that other fields about the objective and image automatically fill based on magnification and a frame size of 1200 x 1200 pixels. Click OK. Once selected, the same information automatically fills in Instrument Setup.



4. Click the Frame Size button to open its dialog box. Using the pull-down menu, select frame size desired. While the maximum is 1900 x 1900 pixels, the default is set to 1600 x 1600 pixels. 1200 x 1200 should be a square within the circular area of the objective while 1600 x 1600 will surround this area showing some darkening in the corners. If faster frame rates are needed, use smaller frame sizes, e.g., 200 x 200 pixels and decrease the Exposure.



If the Frame Size is changed, there will be a prompt to restart Lumaview. Click OK to close the prompt and then shut down Lumaview. Upon restarting Lumaview, the frame size in the lowerstatus bar will be the new size. If not restarted, the new frame size may show in Instrument Setup but it may not actually be different. Always check the lower left status bar of Live Image Window to see actual frame size.



5. Continue to review and adjust the various settings in Instrument Setup. For Root, enter desired file name root for all images in this imaging session. Select the file format desired (Tiff is recommended).

6. The Time-lapse folder path default is given but can be changed if desired. Time-lapse is set up under Protocol as described below.

7. Select the time in seconds for each LED to be on before image snap. Recommended time for fluorescence is 0.6 seconds (do not go below this time). Check Auto Composite to have images in multiple channels automatically composited and saved in the Composites folder. Check Individual Channel Folders to have images from multiple channels in a Protocol saved in separate folders (e.g., for compiling time-lapse videos in single channels). Note: Auto composite may not produce the optimal composite images and LumaQuant or third party compositing software may result in better images.

8. Of the next 6 features listed on the left, check those desired:

   a. Time/date label will be displayed in the lower left corner of each image.

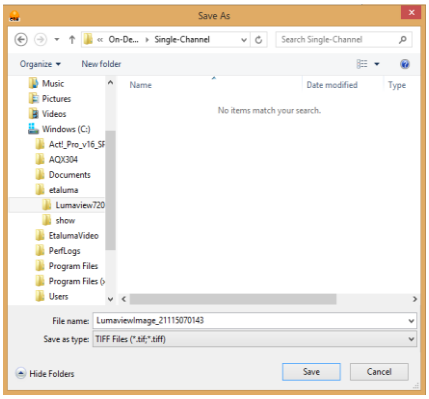
   b. Hot pixel removal. Check if desired and then complete Instrument Setup. (To start this feature, click Utilities in the title bar and from the pull-down menu select Detect Hot Pixels to open its dialog box. Information on next steps is available in the Help section.)

   c. Pseudo color FL channels results in live and snapped fluorescent images colored with the channel color and according to signal intensity.

   d. "Click" sound for manual image snap results in a "camera-like shutter" sound whenever a manual image is snapped.

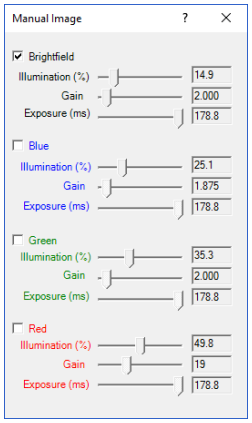
   e. Manual image autosave results in images being saved automatically without the Save As dialog box opening each time. When checked, each image is numbered according to DOYHHMMSSmm where DOY is day of year, HH is hour in 24 hour time, MM is minutes, SS is seconds, and mm is milli-seconds. Thus each image number is unique and in numerical order.

      Not checking Manual image autosave will result in the Save As dialog box opening every time the camera icon is clicked and the image has been snapped. In this case, the image file name desired must be entered before clicking Save.



9. On the right side of Instrument Setup, checking Display scale bar results in a scale bar in the lower rightcorner of live and snapped images. If checked, click Select Color to choose color, check Display objective power if desired, and select Scale bar width.

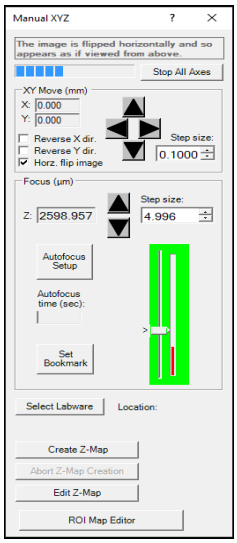
10. After all settings have been selected, be sure to click OK to save the settings before exitingInstrument Setup.



E. Finding a Live Image

1. Install the desired objective and sample labware. For labware that is not SBS microplate footprint, use a labware holder such as the Multi-Slide Holder for 1-4 microplate slides. Update Objective in Instrument Setup as needed and click OK.

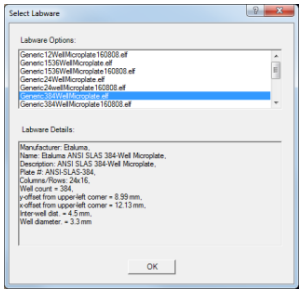
2. Click on the Manual Image icon in the Toolbar (top icon) to open its dialog box. Click the Manual XYZ icon in the Toolbar (second icon from top) to open its dialog box; Manual Image and ManualXYZ are used together to find and focus on samples in the labware. If desired, move dialog boxes to the sides of the Live Image Window. If closed and then reopened, they will open in the same locations prior to closing. However, when Lumaview is closed, the dialog box positions will not be saved.



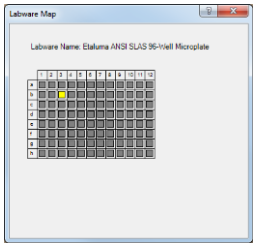
3. Note, following initialization, the objective will be under the frame of the nest and is not an imaging location. Select a desired XY location in a sample labware using one of two options in Manual XYZ:

   a. Use the X/Y Move arrows (and Step size arrows if needed) to move to different locations or wells in the sample labware, or

   b. Click on Select Labware to open its dialog box. Click on the labware type desired to highlight it and click OK. The appropriate Labware Map will then open. Click on a location and the microplate carrier will move so the location is centered over the objective. For further movement from the location, use the X/Y Move arrows (and Step size arrows if needed) in Manual XYZ. Clicking again on the original location in Labware Map will re-center on that location.



   c. Image orientation: The live image displayed on the monitor is as viewed from the bottom as for all inverted microscopes. To see the live image asviewed from the top, check Horz. Flip image in Manual XYZ. However, allimages are captured as viewed from the bottom.



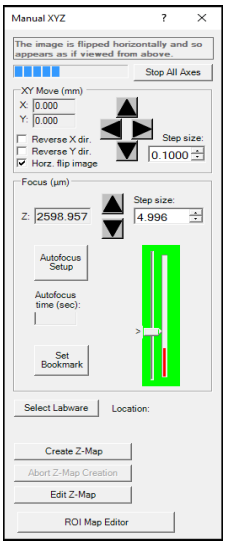
   d. XY movement: In Manual XYZ, when a directional arrow in XY Move is clicked, the live image on the monitor moves in that direction. However, the labware moves in the opposite direction in order to place the new location over the objective. For example, to image a location to the right of the current location, the labware must move to the left for the new location to be over the objective. To have the live image and labware move in the same direction, check either or both Reverse X dir And Reverse Y dir. This feature is particularly helpful when moving across labware, e.g, going well to well in a microplate

4. In Manual Image, select the channel(s) to use. For fluorescence, the included Shroud (or other protection from light) must be placed over the labware. Start with Exposure at maximum and Gain a low as possible. Increase Illumination gradually to the desired brightness. For dim samples, if Illumination is at maximum, increasing the Gain can be used to increase signal but it will also increase the background.

5. For transmitted imaging, check Brightfield in Manual Image and move the phase condenser iris lever on the front of the phase illuminator so only a low level of light is transmitted. Set Gain to minimum, Illumination (%) to 10-20%, and adjust Exposure downward until a mid-gray tone is seen in Live Image Window.

6. For phase contrast using the Phase Contrast Accessory, position the slider in the center position, check Brightfield in the Manual Image menu and move the phase condenser iris (on front of condenser) to the left to the fully open position.

F. Manual Focusing



1. In Manual XYZ, move the Focus slider up while watching the Live Image Window to detect the sample coming into focus. The Focus slider area becomes green whenever the Focus slider is active. Clicking on the slider vertical line above or below the current focus level causes the focus level to jump up or down, respectively, by 100 um. Continue to adjust the focus using the Focus Z up and down arrows (and Step size adjustments if needed) until a sharp image is achieved. Adjust Illumination and Gain settings in Manual Image during the focus process as needed. Click the Set Bookmark button to record the focus level for an easier return to this level.

2. To facilitate the focusing process, click on the Zoom icon on the Left Tool Bar. The Zoom feature toggles between displaying the entire image fitted to the Live Image Window and displaying the image matched 1:1 with the monitor resolution (pixel: pixel). If the monitor resolution in pixels is less than the frame size, which is typical, the image will be enlarged (zoomed). The Live Image Window will now show a center portion of the entire image. Click the Zoom icon again to fit the image to the Live Image Window.

3. If necessary, adjust Illumination and/or Gain as needed.

4. When focus is found it can be useful to set the bookmark for locating the focus level quickly again.

G. Create Z-Map

This will perform an autofocus on all wells of a plate and remember those z-values in a Z-map file.

H. Snapping Images

1. When the desired field has been illuminated and focused in Live Image Window, click the Camera icon in the Left Tool Bar (third from the top) to snap the image. If the Zoom function was used to focus, the image captured will include the entire field of view (FOV) observable with the Zoom off.

2. If Manual image autosave was checked in Instrument Setup, images will be saved automatically using the time-based numbering system. If Manual image autosave was not checked, a Save As

dialog box will open after the image was snapped. Images will be saved in the Etaluma folder formed automatically whenever Lumaview is downloaded and installed.

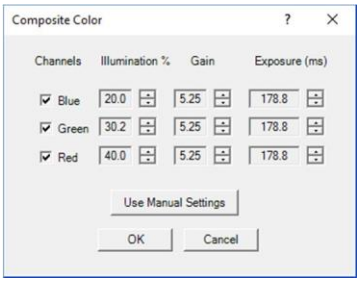
3. Images are captured and saved as seen by the camera, i.e., from the bottom. If desired, images can be flipped horizontally to show how they would appear from the top. To accomplish this,check “Horz flip image” in the Manual XYZ dialog box. The message “The image is flipped horizontally and so appears as if viewed from above” is then displayed at the top of the dialog box.

4. To view the last image snapped, click the Folder icon in the Left Tool Bar (bottom icon) to launchWindows Explorer and open the most recently used Destination Folder.

I. Auto-Generation of Single Fluorescence Composite Images

1. Click the Composite icon in the Left Tool Bar (fourth from the top) to open its dialog box. Check the channels desired. To use values optimized previously in Manual Image, click Use Manual Settings to upload the settings. Values can also be entered manually.

   a. Click OK to start the snapping of individual images in progression. Each LED will turn on for thepre-snap interval and then flash when the image is snapped. If “Click” sound for manual imagesnap was checked in Instrument Setup, the shutter-like sound will be heard for each image.



   b. The fluorescence composite image will appear automatically in the Main Window matched 1:1 with the monitor resolution (pixel:pixel) and with its Title Bar showing the full path where it is located.

2. Composites including the transmitted channel (phase contrast or brightfield)

   a. In Instrument Setup, make sure Auto Composite is checked.

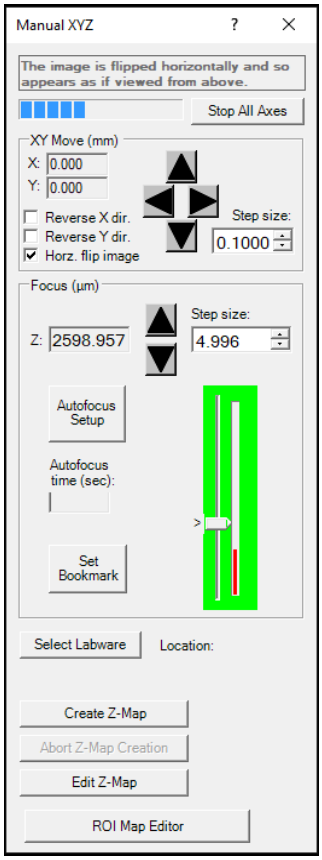
   b. We will use a very short Protocol in order to construct a composite which includes the transmitted channel. Configure a Protocol with the channels desired. In the Acquisition tab, select Time-Lapse and set both Interval and Duration to 1 sec. Click Run and after the images are snapped, click the Last File icon in the Left Tool Bar (lowest icon). The folder containing the Composite image will open.

J. Live Video (Manual)

1. When a desired field has been illuminated and focused in Live Image Window, click the Video Record icon in the Left Tool Bar (fifth from the top) to open its dialog box. Select parameters requested and also those in Instrument Setup such as file format. Images captured will be saved to the Video folder inside the Manual folder.

2. Click OK and image capture will begin. To stop image capture, click the Record Video icon in the Left Tool Bar again and it will report the number of frames recorded.

K. Manual Setting Up Autofocus

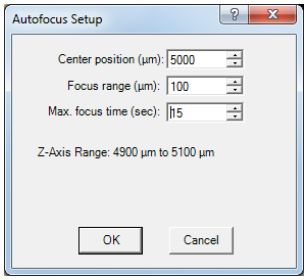


The autofocus algorithm scans through the requested focus Range with a coarse and then finer z axis step size monitoring contrast and finding its maximum. It starts at the lowest value in the Range and begins stepping upwards at an initial step size dependent on the objective installed. Calculations of the contrast are made at each level until the upper limit of the Range is reached.
The algorithm then moves the focus to the position of the maximum contrast and a new range, 20% size of the original Range, is defined and centered on this rough maximum contrast. A new scan is initiated from the bottom of the new Range with a new step size which is 1/3 of the previous step size.
Again, the algorithm moves to the position of the maximum contrast and a new range that is 20% of the last range is defined, again centered on the previous maximum contrast. A final scan is initiated from the bottom of this most recent range with the minimum z-axis step size defined for that objective used.
Finally, the algorithm determines the maximum contrast level from the smallest Range and finest step size and moves the z-axis to that position.
If the algorithm reaches the Timeout before converging on a focus, the algorithm will terminate and move the z-axis corresponding to the highest contrast value it found up to that point.
The autofocus set up allows a Center, Range, and Max Focus Time to be set.

The Center value is the expected focus, often determined manually. The expected focus in different locations can be different. It is best to make the Center equal to the average focus in the sample locations unless using a Z-Map or ROI Map where the z coordinate acts as the Center.

The Range should be set in accordance with the variation in the average focus across multiple positions, with a minimum range based on the objective magnification (see Table 1). The highest and lowest expected focus level should be comfortably within the Range with respect to the Center. If Autofocus is unreliable, increase the Range until it becomes reliable.

Max Focus Time is the time before it chooses the best focus it has found. This is set to 15 seconds by default but often good AF can be achieved in under 5 seconds with optimization of the Center and Range. You should decrease this time until it becomes unreliable and fails to auto focus.



1. Use Manual Image and Manual XYZ to find a focused live image in a desired XY position. In Manual XYZ, click the Autofocus Setup button to open its dialog box.

2. Transfer the manually determined Focus Z level in Manual XYZ to the Center positionfield of Autofocus Setup. The Focus range will be set to a default value of 200 um. Change this to the recommended Minimum Range for each objective magnification according to the following table:

Magnifi-cation	Numerical Aperture	Depth of Field (µm)	AutoFocus Minimum Step Size (µm)	AutoFocus Maximum Step Size (µm)	Minimum Range (µm)	Recommended Z-Slice Step Size (½ DoF) (µm)
4x	0.10	55.5	20	72	100	27.75
10x	0.25	8.5	8	36	50	4.25
20x	0.40	5.8	2	18	25	2.9
40x	0.65	1.0	1	9	15	0.5
60x	0.85	0.40	0.5	6	10	0.2
100x	0.95	0.19	0.2	4	10	0.11

3. The Max focus time will be set to the default of 15 sec. This can be reduced as performance of the autofocus is confirmed. The Max focus time should be at least that required for successful autofocus but optimally only a few seconds longer. Autofocus of 3 seconds should be possible in flat labware with 2D samples.

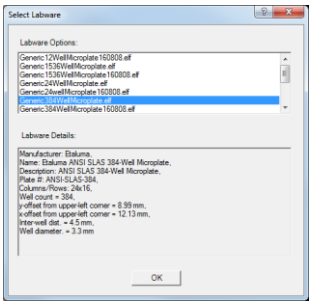
4. Click OK and autofocus will occur. The Focus Z level in Manual XYZ will now be updated to the new autofocused Z level. The actual time taken to reach autofocus, i.e., the Auto focus time (sec), will also be shown.

5. If autofocus did not result in a sharp live image, make sure the Center position transferred was correct. In general, if autofocus consistently uses the full Max focus time, the range is too great for the time entered or the time is insufficient. It may be necessary to decrease the Focus range and/or increase the Max focus time.

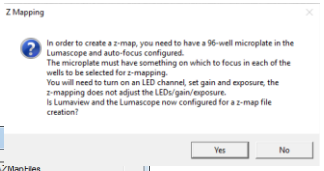
6. Upon successful autofocus, move to other locations distant from the initial location. Click Select Labware in Manual XYZ and the appropriate Labware Map will open (see Section II.E.3. above). Select a location and repeat Steps 1-4 above.

7. Continue to move to other locations far away from the initial ones and repeat autofocus. It may be necessary to re-enter the Center position and/or adjust the range. To determine the final parameters,review the upper and lower Z levels obtained and use the approximate mean for the Center position. For the range, determine the spread between the highest and lowest expected Z values and make sure the Range contains those values comfortably.

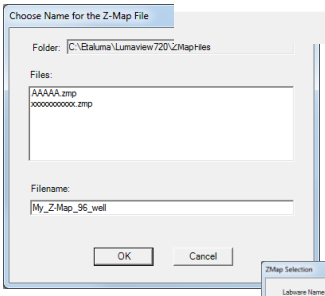
A. Creating a Z-Map



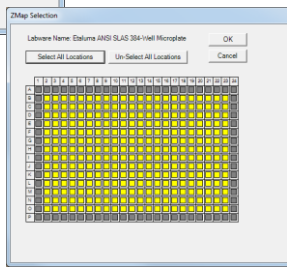
1. Load desired labware with samples into labware carrier.
2. Find a Live Image in a location as described in Section II.E. above.
3. Set up Autofocus using steps in Section II.J. above.
4. In Manual XYZ, click the Create Z-Map button near the bottom. The Select Labware dialog box will open. Click on the labware type desired to highlight it and then click OK.



5. A Z-Mapping instruction dialog box will now open. Click Yes.
6. The Choose Name for the Z-Map File dialog box will now open. Choose the Filename for your Z-Map file and click OK.



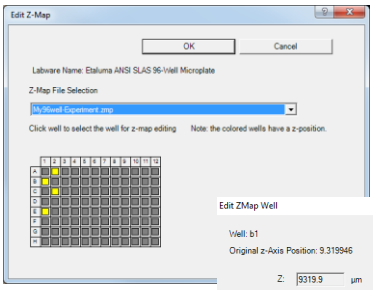
7. The Z-Map Selection dialog box will now open. Select the locations in the labware to be Z-Mapped. Locations can be selected by clicking on them individually,clicking on the name of any row (A, B, etc) or column (1, 2, etc), or clicking on Select All.



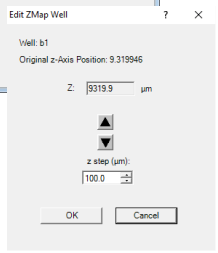
8. Click OK and Z-Mapping will begin.

B. Editing a Z-Map

1. In Manual XYZ, click the Edit Z-Map button at the bottom to open its dialog box. Select the Z-Map File to be edited.



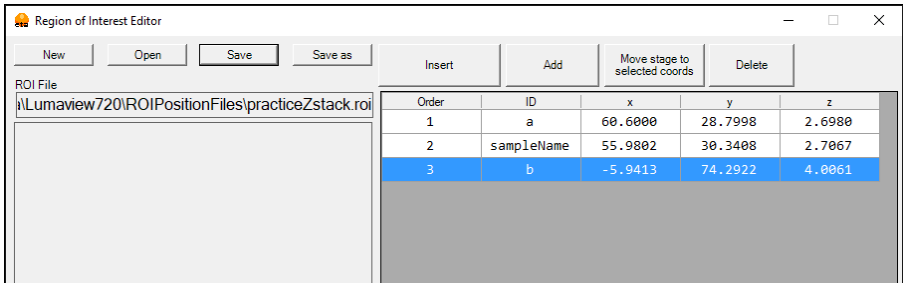
2 Locations that were Z-Mapped become shaded in yellow. Click on any Z-Mapped location and the Edit Z-Map Well dialog box for that location will open. Click the up and downarrows to manually change the focus. This allows both review of the Z-Map value and making a change if desired.



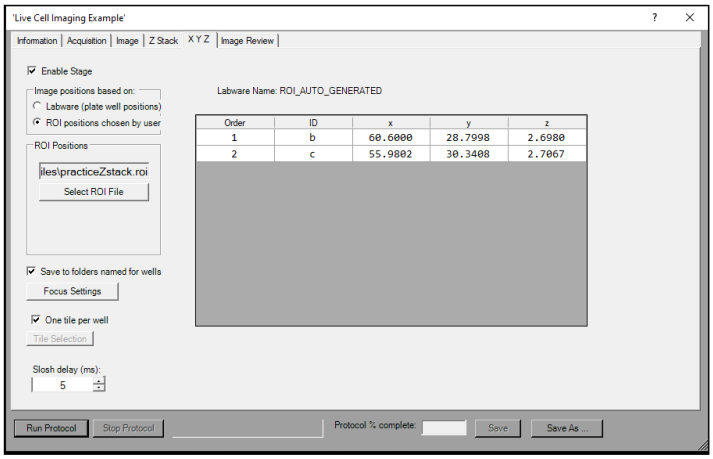
3. In Manual XYZ, click the Edit Z-Map button at the bottom to open its dialog box. Select the Z-Map File to be edited.
4. Review the Z-Map and make changes if desired. Click OK to return to Edit Z-Map. Click Cancel to keep the Z-Map value as it was before. Click on other locations to check and/or change their Z-map values. Be sure to click OK to save anychanges before leaving Edit Z-Map.

C. ROI Map Editor

1. Clicking the ROI Map Editor will open a menu that allows the recording of XYZ positions of interest



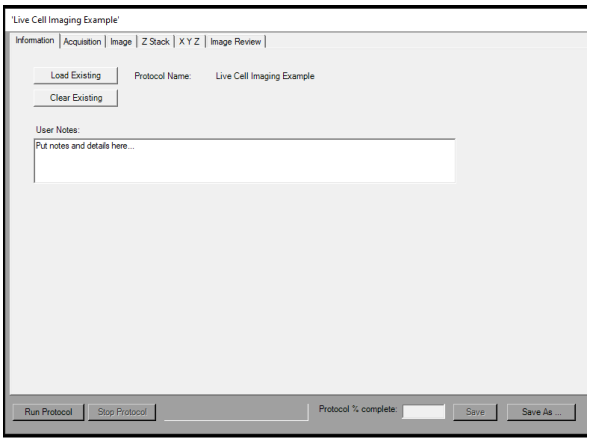
Add will record the current location in the table. **Delete** will remove the highlighted position. Insert will place the current location above the highlighted location. **Move stage to Selected Coords** will go to the location highlighted. The ID field can be edited with simple text. Using an ROI Map in the Protocol XYZ tab will look like this:



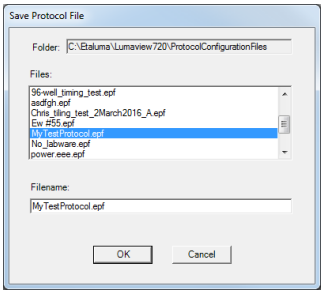
WARNING: ROI Map locations cannot have z positions too close to zero. This may vary from instrument to instrument but will result in a Protocol halting without finishing.

D. Writing and Using Protocols

1. To configure an automated microscopy run, including time-lapse, the Protocol feature is utilized. Click the "P" icon in the Tool Bar.



2. Information and values will be added on the various tabs. Be sure to click Save before leaving any Protocol.



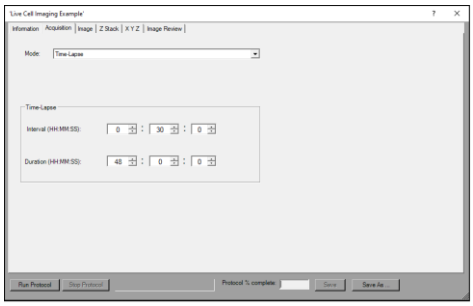
3. In the first tab named Information, click the Load Existing button to bring up a previously saved Protocol. If the desired Protocol is present, click OK. Click the Clear Existing button if values have been entered on any of the tabs and saving is not desired or a previous Protocol is displayed and you want to start a new one. If unsaved entries are being cleared, a Save Protocol File dialog box will open asking if you want to save the current Protocol. If Yes, click OK.

4. If not previously saved, enter the new desired Protocol name in the Filename field and click OK. Or click on an existing File name to overwrite and a File Overwrite Alert will open; click Yes. You are now ready to create a new Protocol.
5. On the Information tab, if you enter User Notes, they will become part of the new Protocol. You can also enter User Notes from a previously saved Protocol in this tab.

6. Click on the Acquisition tab to select the acquisition mode:
Single Image, Time-Lapse, Video, or Time-Lapse Video.

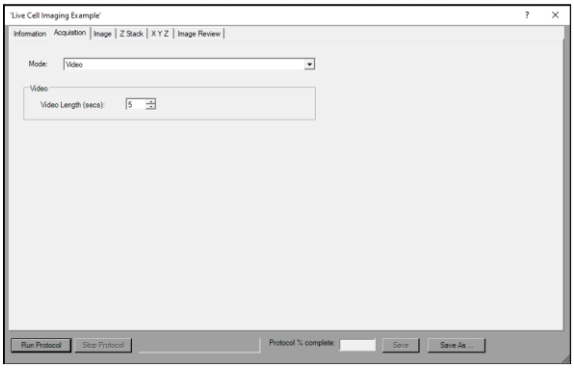
   a. Single Image mode will snap an image of each location selected once and finish.

   b. For Time-Lapse, enter the Interval and Duration of your time lapse experiment. Typical cancer cell lines use 10 minutes to 1 hour intervals for 24-72 hour duration. The maximum Duration is 1000 hours or 43 days.

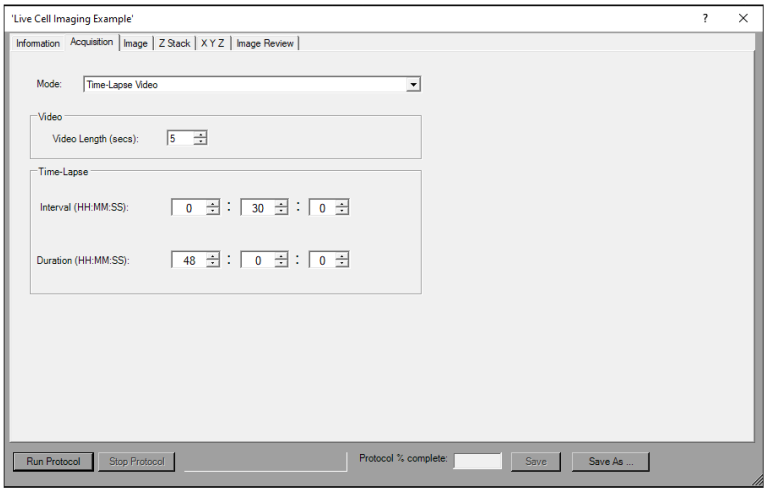


IMPORTANT: Before initiating a time-lapse run, it is critical to change your computer power and Windows update settings so they cannot automatically turn on and interfere with scheduled image captures. See Appendix D

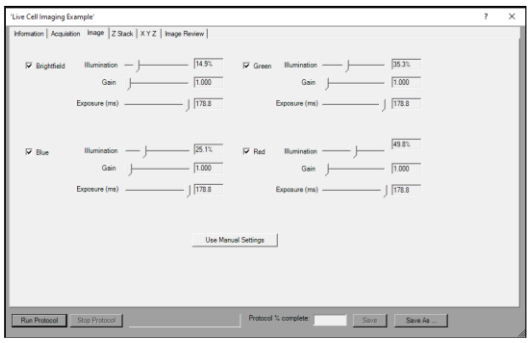
   c. Live Video will record a video at each location using a single LED illumination



d. Time-Lapse Video will take a short video at each location at the time lapse interval rather than a single still image.

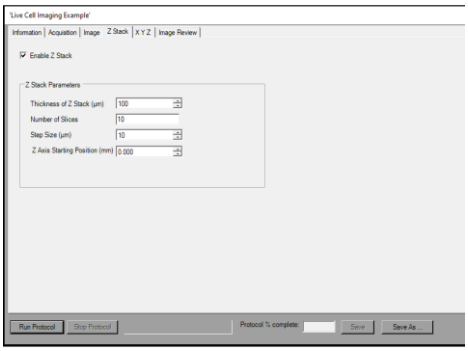


7. Click on the Image tab to select the channels to be used in the Protocol and adjust their settings. If settings were previously determined in Manual Image, transfer the values into the current Protocol by clicking the Use Manual Settings button. The desired values can also be entered manually. Note that this dialog box cannot be used to optimize the image settings because it is not interactive with Live Image Window.



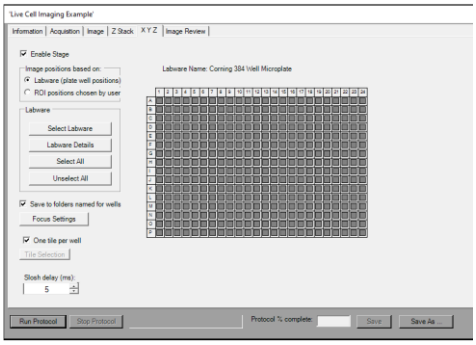
If optimization of any of the Channel settings has not been done or is still needed, click on the Manual Image icon on the Left Toolbar (top icon) to open its dialog box and fine tune the settings with a representative sample. When settings are satisfactory, click the Use Manual Settings button in the Image tab to upload them into the Protocol.

8. Click the fourth tab, Z-Stack, where you may enter the stack depth as well as the slice thickness. This will cause an update to the number of slices in the stack. The stack will be centered on the Autofocus result, the Z value of the Z-Map, or the Z-value of the ROI map. The center image will always be captured in addition to the slices. If an even number of slices are requested, it will be in addition to the slices and if an odd number are requested, the center position will be imaged twice. The Z-Axis Starting position can be offset from the focus or Z value of the location.



9. Click the fifth tab, X Y Z, to define the locations to be imaged. Click Enable Stage to select location options.

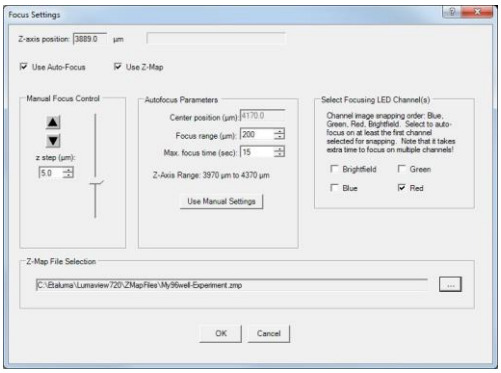
10. Click the Select Labware button to choose a labware type. Locations can be selected by clicking on them individually or clicking on the name of any row (A, B, etc) or column (1, 2, etc).



11. Clicking "ROI positions chosen by user" will allow a worklist of positions referred to as an ROI Map to be used for the Protocol. Defining this ROI Map is covered above.

12. Images can be saved to individual folders for each location. Check "Save to folders named for wells". If you prefer images to go to one folder, uncheck the box. In Instrument Setup you may also choose the setting that saves images into folders according to channel.

13. Click on the Focus Settings button to open its dialog box. There are 4 options to set up focus in a Protocol:



a. Manual Focus. Uncheck both Use Auto-Focus and Use Z-Map. Under Manual Focus Control, click the up and down arrows to determine the desired focus level. This will be thefocus level for all locations in the Protocol. It will need to be updated for each running of the Protocol.

b. Autofocus. Check Use Auto-Focus and uncheck Use Z-Map. Under Autofocus Parameters, click on Use Manual Settings to load focus settings previously determined in Autofocus Setup launched from Manual XYZ.

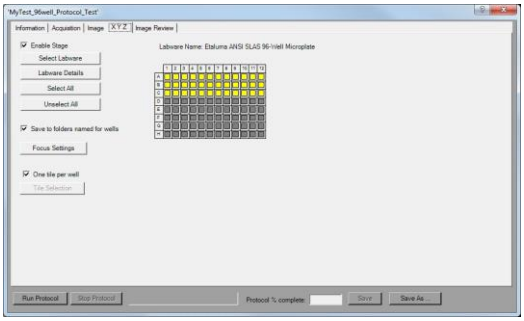
Autofocus will not use the Z coordinate in an ROI Map as the Center position. Autofocus should not be used with ROI Maps unless standard Autofocus parameters can be tolerated.

c. Z-Map. Uncheck Use Auto-Focus and check Use Z-Map. Follow steps described above to create a Z-Map for each location to be imaged in the Protocol. To use an existing Z-Map, select the file under Z-Map File Selection.

d. Autofocus plus Z-Map. Check both Use Auto-Focus and Use Z-Map. Set Autofocus Parameters as described above in Step b, this Section. Follow steps described below in Section II.L. to create a Z-Map for each location to be imaged in Protocol. To use an existing Z-Map, select the file under Z- Map File Selection.

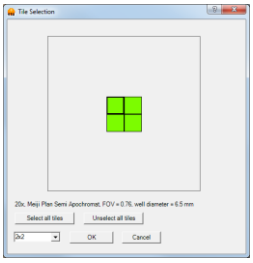
e. Under Select Focusing LED Channel(s), check the channels desired for autofocusing. Currently, channels are acquired in a fixed sequence of BF, Blue, Green, Red. Any selected autofocus will also occur in this sequence. If using autofocus, you must select the first LED channel to be imaged as an autofocusing channel per the listed order above. The subsequent channels in your protocol channel sequence are optional for autofocusing. If the subsequent channels are not chosen, the focus will stay at the level determined for the first channel in the sequence. For example, if you are snapping brightfield and red channel images, select at least the brightfield channel for focus. Autofocus will occur in the brightfield channel and then remain at that level to capture the red channel image. If you want the red channel to also be focusing, select both the brightfield and red channels.

E. Tiling Across Locations



1. Tiling can carried out as part of a Protocol and set up in the Protocol XYZ tab. First ensure that the One Tile Per Well is not checked. Click the Tile Selection button to open its dialog box. A representative Location will be shown.

2. Select the number of tiles desired using the drop-down menu. The tile grid will then appear superimposed over the location based on the objective to be used and the location dimensions. Select the tiles to be imaged by clicking each individually or Selectall. The tile number and tiles selected will apply to all locations to be imaged in the Protocol. Click OK.

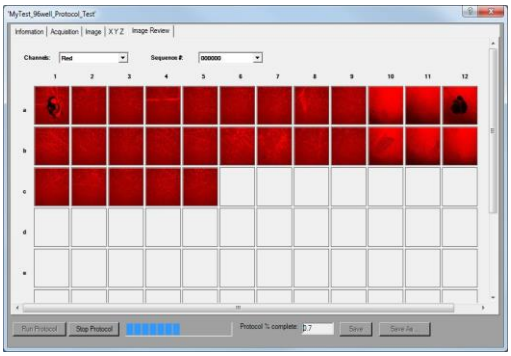


3. Autofocus will be carried out in all selected tiles.

F. Run Protocol

1. After entering all information and saving the Protocol, it is ready to be run.

2. A message will remind you to install the correct objective and the Protocol will initiate.



G. The fifth tab is "Image Review" and will present the images as they are captured. Channels, including multiples if a Composite, can be selected as well as the Sequence number of a Time-Lapse. This allows the monitoring of images as they are collected in the Protocol. Click on any location to enlarge the image. Clicking on a tile will also enlarge that tile.

H. Live Cell Imaging with the LS720 Microscope in an Incubator

1. Prior to imaging in an incubator, place the microscope inside for at least 6 hr and preferably over-night to allow thermal equilibration. Before transferring to the incubator, disinfect the micro-scope using any of the suggested procedures in Appendix C. Condensation on the microscope, including on the objective, will form in the first minutes but will evaporate as thermal equilibrium is achieved. Focus levels may change during this equilibration as materials expand.

2. Pass the communication and optional power cable through the incubator port, which is often locatedin the upper back wall. A plug or filtered stopper may be present. 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 as well. Make sure there is enough slack in the cables inside the incubator for sliding out the shelf. Place the computer to be used on a flat and stable surface near to the incubator and within the 3 M USB cable provided.

WARNING: Do not extend the USB cable or use another USB cable other than that supplied, particularly a longer one, 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.

3. Place the sample in the labware carrier and ensure it is seated flat with the upper left corner firmly pushed into the frame. Close the incubator and return to Lumaview to focus. The motorized Z axis will maintain focus level and autofocus can be used to adjust any change in focus overtime.
4. The system is now ready for live cell imaging.

I. Creating Videos from Time-Lapse and Live Video

Several options are available for compiling videos from sets of time-lapse and live video images:

A. The optional Lumaquant software offered by Etaluma (see Lumaquant Startup Guide for compiling images into video). The Lumaquant Startup Guide can be downloaded from Etaluma's website (www.etaluma.com/products/downloads/).

B. Other video compiling software such as ImageJ, Fiji, Windows Video Editor, and iMovie.

Appendix A

LS720 Red Shipping Lock - Installation

Warning: You must install red shipping lock before placing into shipping carton!! If not installed, the LS720 can be damaged!!

Red Shipping Lock Installation

1. The red shipping lock comes with 4 nylon-tipped set screws inserted from the outside so tips face each other (Fig 4). If screws were removed previously, insert them. Adjust so each set screw tip is flush with the inside metal surface (screw heads will extend on the outside). There should also be two 1-inch screws.

2. Raise top deck, remove objective from the LS720 optics block and install lens cap (or cover with tape if a lens cap is not available). Do NOT leave the optics block uncovered. Lower the top deck onto the subdeck and lock with thumb- screw. In Lumaview, open Manual XYZ, and set Z to 0 (bottom of travel).

3. The LS720 has a subdeck has 2 vertical alignment marks on the front edge of the plate carrier near the thumbscrew and the top deck front edge.

4. In Lumaview, Manual XYZ, set Z step size to 10mm, move plate carrier toward front until end of travel. Plate carrier should stop a few mm away from the front edge of the deck. Move the plate carrier to the right or left so that the vertical marks on the plate carrier and top deck are aligned (Fig 5). Adjust the step size as needed.

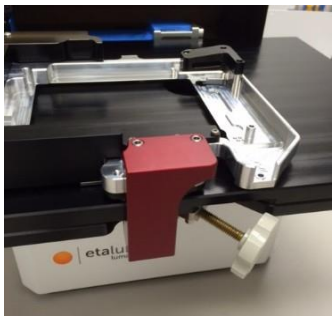


Fig 1. Shipping lock installed on LS720

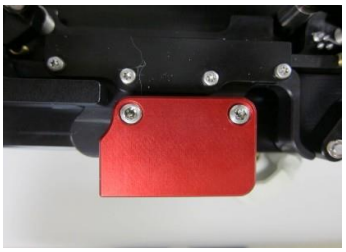


Fig 2. Installed shipping lock from top showing 2 set screws

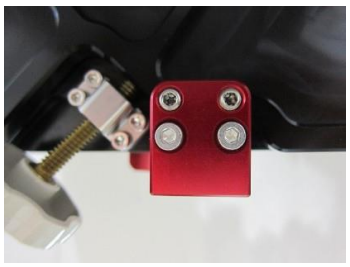


Fig 3. Installed shipping lock from bottom showing 2 set screws (smaller heads) and two 1-inch screws (larger heads)

Fig 4. Shipping lock with screws & wrench

5. Unplug the power and USB cable from the LS720. Slide shipping lock into position at front of deck just left of the Deck Knob (Fig 1) so the 2 holes in the shipping lock line up with 2 holes on the front underside of the deck. Screw inthe two 1-inch screws (Fig 3) until tight using the supplied 3 mm hex wrench (official torque is 16 in-pounds).

6. Tighten the 4 set screws in the shipping lock with the 3 mm hex wrench untilthey are just touching the plate carrier, then tighten a further quarter turn (top 2 screws, Fig 2) and deck surface (bottom 2 screws, Fig 3). Do NOT overtighten. Set screw head to be approximately even with shipping lock surface.

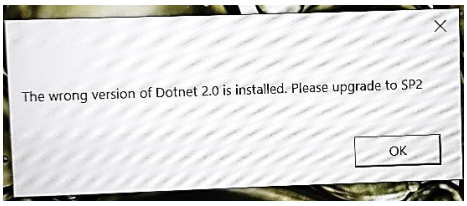
7. Test by gently trying to lift plate carrier at front where it is being held byshipping lock. If plate carrier can be lifted at front, tighten the two 1-inch screws and then the 4 set screws a little more until plate carrier is securely locked.

Appendix B

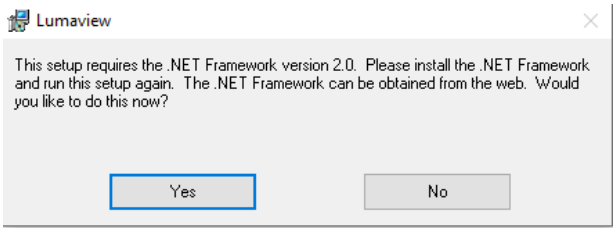
Windows Configuration Troubleshooting and Driver Conflicts
(may require Administrator status)

You may encounter installation error messages such as the following:

Dotnet Service Pack 2 Upgrade:

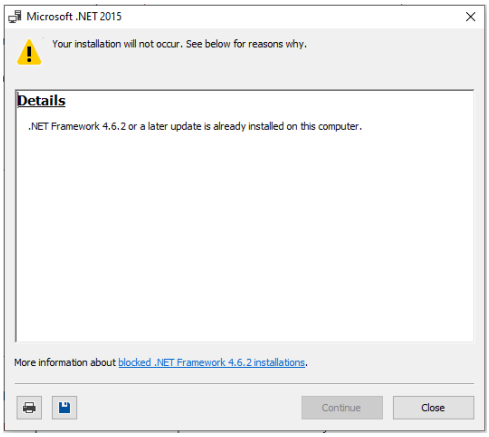


or

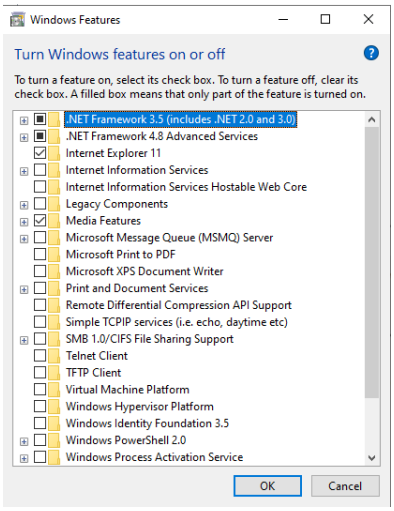


This error which will be presented as one of these two messages and can be solved by going to the Microsoft download page here: https://www.microsoft.com/en-us/download/details.aspx?id=21. Searching for "Dotnet SP2 upgrade download" should yield a Microsoft download page in your country if the above link is inappropriate. After downloading and installing (should be automatic), restart Lumaview to confirm it has been upgraded successfully.

Occasionally the upgrade will already be present but needs to be enabled and you will see this message when you try to upgrade.



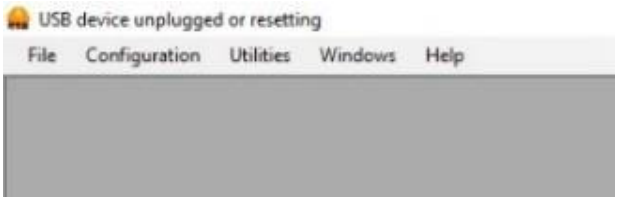
In this case, it must be enabled in Windows features as seen below.

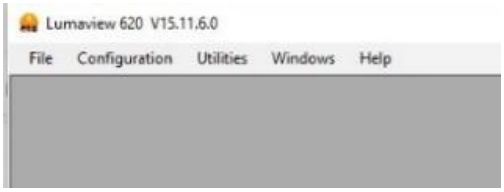


Driver Conflicts

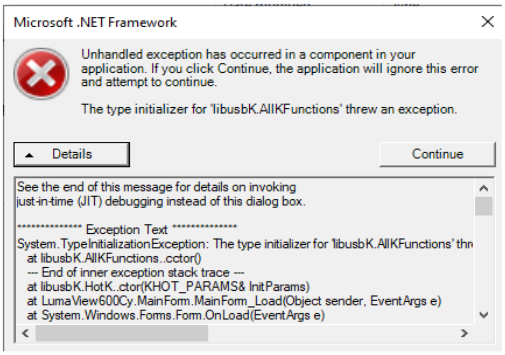
Occasionally conflicts can occur between the drivers used by Lumaview and other Windows drivers, preventing communication between the LS Microscope and the connected computer. Often this occurs after a Windows Update or after installing another instrument software, especially camera-based instruments.

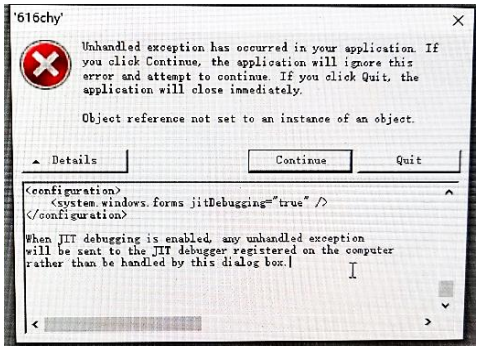
Generally this results in a blank Lumaview screen and one of the following messages in the upper left corner of Lumaview is observed.





These error messages may also be presented:

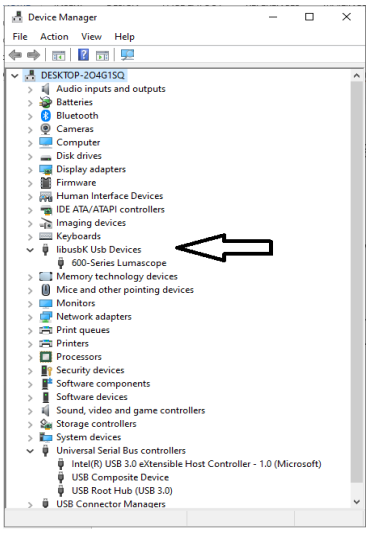




Restarting Lumaview, unplugging and replugging in the USB cable, or restarting the PC and relaunching Lumaview may resolve the issue. If not, removing an interfering driver and updating it with the Lumascope driver may be required and is described in next section.

1. Open the Device Manager.

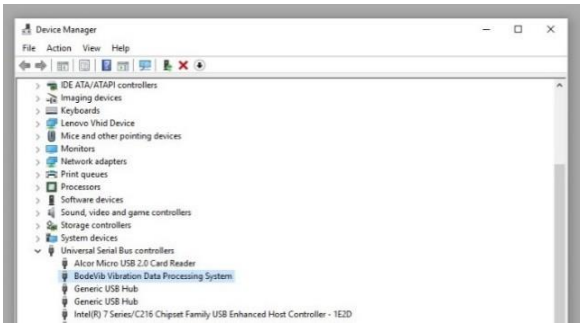
2. With the computer connected to the LS Microscope, in Device Manager click the View tab. In the pull-down menu, click Show hidden devices. Verify that libusbK Usb Devices is present in the list. If it is, then it is most likely not a Driver issue.



3. If "libusbK Usb Devices" is not present in the list, you will need to Repair the current version of Lumaview which re-installs the correct drivers. From the download location on your computer (e.g., in the Downloads folder), double click the Lumaview.msi file to start the install. The Lumaview Setup Wizard dialog box will open; select Repair Lumaview720 and click Finish. The installation will proceed, including the opening twice of the Device Driver Installation Wizard (click Continue and Finish each time).

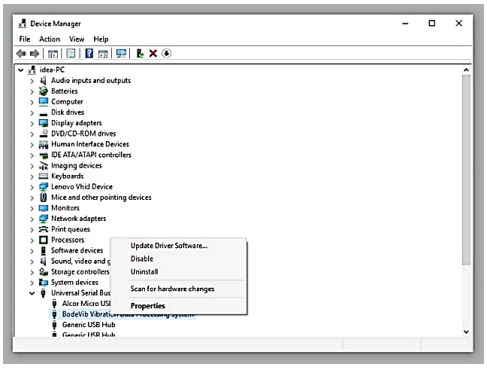
4. Disconnect and then reconnect the USB cable from the computer to the LS Microscope. Repeat Step 2 (above) to verify libusbK Usb Devices is now present.

5. If this does not result in the driver appearing in the Device Manager then in the same Device Manager dialog box, scroll to the bottom of the device list and click on Universal SerialBus controllers to expand the list. Look for specific controller names (not the nonspecific such as USB Root Hub or Standard Open HCD Host Controller). Examples of conflicting drivers identified thus far that might be there include: BodeVib, Holdtescs, Cypress, NDTech.

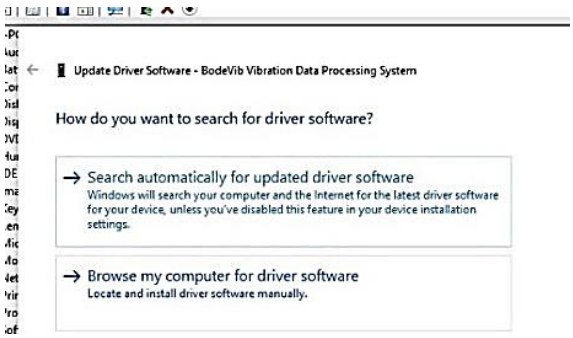


6. It may be necessary to unplug the LS Microscope (via the USB cable) from the computer and look for any names in the list where the name font color has turned a lighter gray. Reconnect the LS Microscope and verify that the one that turned lighter gray is now darker gray again. This is the driver that conflicts with the LS Microscope drivers.

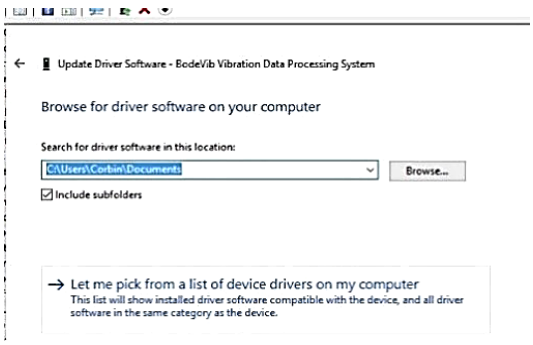
7. Right click on the conflicting driver, in this example BodeVib Vibration Data Processing System, and in the pop-up menu, click on Update Driver Software.



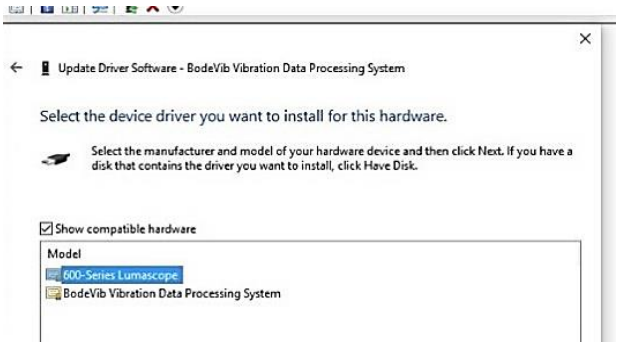
8. In the next dialog box that appears, click on Browse my computer for driver software.



9. In the next dialog box that appears, select Let me pick from a list of device drivers.



10. The next dialog box that appears will show the choices for the hardware devices. The Show compatible hardware box should be checked. Select 600-Series Lumascope and click Next. Windows should now install the LS Microscope driver over the conflicting driver.



11. When installation is complete, disconnect the LS Microscope from the computer and then reconnect it. These steps may need to be performed on every USB port that will be used with the LS Microscope.

NOTE: Older versions of Windows may ask you to restart the computer before driver is functional.

Appendix C

How to Disinfect Lumascope Microscopes

Etaluma's microscopes are revolutionary in their ability to be operated within cell culture incubators, providing the most stable and robust long term live cell imaging. Placing the LS Microscope in the incubator often includes a disinfection step before installing. In this Tech Note, 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 (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 CO_2 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.

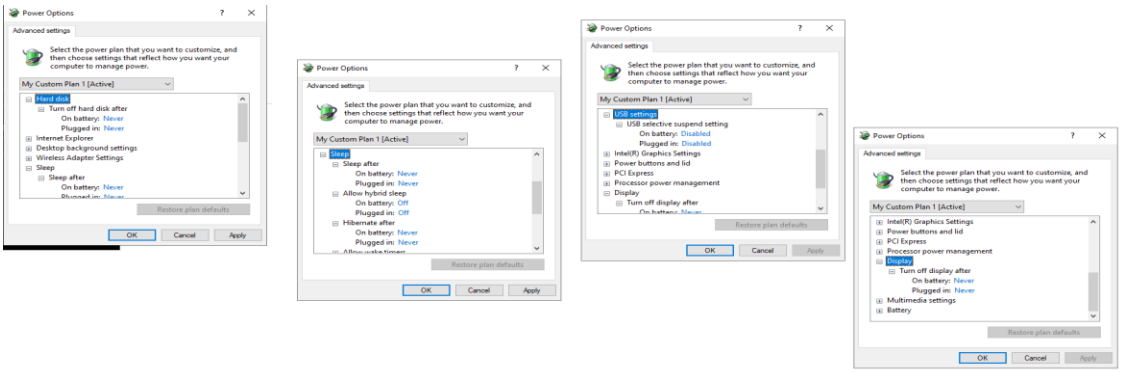
For questions, contact Technical Support at support@etaluma.com.

Appendix D

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 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.



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 Lumaview*.**