

LS560 & LS460 Microscopes

-- Startup Guide --

using
Lumaview software (for LS720, LS620, LS560, LS460)



LS560 (with Phase Contrast Accessory and Manual XY Stage, both optional and ordered separately)

IMPORTANT: This Guide describes how to set up the LS560 and LS460 (Section I) and start using the Lumaview software (Section II). It is important that you follow this Guide.

Steps described for LS560 apply to both LS560 and LS460 unless specified otherwise.

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

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

Note that all steps are described for LS560 but apply to both LS560 and LS460 unless specified otherwise.

I. Hardware: Setup of LS560

A. Items Included With LS560 (and LS460)

- USB power/communication cable
- Brightfield Illuminator LS620/LS560/LS460
- Fluorescence shroud (not included with LS460)
- External power supply/cord with country-specific plug (not included with LS460)

B. Optional Accessories (purchased separately)

- Phase Contrast Accessory LS620/LS560/LS460 (Olympus)
- Manual XY Stage LS620/LS560/LS460
- 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
- Lumaquant, Image analysis software
- Microvolution, Deconvolution software

C. Recommended Computer Specifications

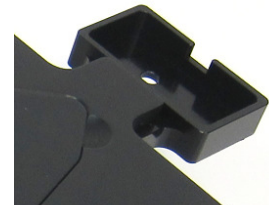
Windows 7, 8, 8.1, or 10; Core i5 or better Processor; 500 GB minimum solid state drive (recommended for time-lapse and live video capture); 4 GB minimum RAM, single 4K monitor or two with 1080p HD resolution

D. Placing the LS560

1. Remove LS560 from the shipping box (save box and packing materials) and place on a lab bench or counter in a standard room environment. If the LS560 will be used in an incubator or environmental chamber, it is recommended that the initial setup be carried out in a room with ambient conditions before placement into the specialized environment. See Section I.L. for information about live cell imaging with the LS560 in a specialized environment.
2. Wait to connect the USB cable from the LS560 to the computer until Lumaview is installed (see Section I. J-K. below).

E. Phase Contrast

1. If using the Phase Contrast Accessory LS620/560/460 (Phase Accessory), remove it from its shipping box (save box and packing materials). Ensure that the Phase Accessory adaptor is attached to the rear tab of the LS560 Accessible Deck (see photo). Attach the Phase Accessory by inserting the base of the arm into the Phase Accessory adaptor. Ensure it is fully seated and tighten the thumb screw on the bottom of the adaptor.



Phase Accessory adaptor

2. Connect the free end of the Phase Accessory communication cable to the round port on the LS560 back panel (left side) labeled External Lamp Power Out. This allows the Phase Accessory to be controlled by Lumaview.



LS560 back panel

3. Insert the phase contrast Slider (pre-centered version included with Phase Accessory) into the slot going through the condenser approximately 1 cm up from the bottom of the Phase Accessory. Of the 3 positions in the Slider, the phase ring is present in the middle position. Any open positions can be used for Brightfield.

F. Brightfield

1. For optimal brightfield, use of the Phase Accessory (Section I.E. above) with the included Slider in an open position (no phase ring) is recommended. The Phase Accessory, which provides collimated light, is directly connected to the LS560, allowing it to be controlled by Lumaview. It should also be used when imaging in a dark environment such as an incubator. To mount the Phase Accessory above the LS560 deck, see Section I.E. above. Brightfield images will be in gray scale due to the monochromatic CMOS camera.
2. If the Phase Contrast Accessory is not available, ambient light in a typically lit area can often be used. The light should be as uniform as possible to prevent shadows or other dark or light areas. Overhead fluorescent lighting can cause uneven brightness resulting in a striped pattern on the image; if observed, move the LS560 or partially shade the light to reduce unevenness across the sample.

G. LED Power (not applicable to the LS460)

For almost all fluorescence imaging applications, power from the computer via the USB cable to the LS560 will be sufficient. If high levels of illumination are required, e.g., with very dim samples, plug the small round plug of the external power supply/cord into the round port labeled **SUPPLEMENTAL POWER** on the back of the LS560 and the electrical plug into an AC outlet. You should see a moderate increase in the Illumination level.

H. Installing Objectives

1. Manually remove the insert of the top accessible deck. Unscrew the black cap (or previously installed objective) on top of the inside optics block and screw in the new objective. Do not overtighten. Return the removable insert to the deck.

- When installing or changing an objective, carry out the insertion quickly to minimize dust falling onto the mirrored dichroic filter. If any dust is observed on the filter, carefully blow it off using a compressed air duster, making sure to hold the can UPRIGHT and bending the straw to aim the air directly toward the filter. DO NOT tilt the can or liquid can be emitted, which will require further cleaning. Other methods to remove dust may damage the dichroic filter and must NOT be used.

I. About Lumaview

- The LS560 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 to the LS560** (due to the need for two Drivers that are installed on the computer at the same time).
- The Lumaview software (without an LS Microscope number in the name) now works with multiple Lumascopes, including LS720, LS620, LS600, LS560, and LS460. The specific LS Microscope to be used is designated in Instrument Setup as described in Section II.C below.
- Lumaview will run on Windows 7, 8, 8.1, and 10. Both desktop computers and laptops can be used but the best visualization correlates with monitor resolution. 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://msdn.microsoft.com/en-us/library/bb822049%28v=vs.110%29.aspx> 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 correct download. This should not require a purchase. Contact Etaluma with any questions.

J. Downloading and Installing Lumaview

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

Alternatively, the same Lumaview Install_v(version#).zip file can be copied from the flash drive that comes with the LS560. Verify that this file is the latest version as posted on the Etaluma website.

- Lumaview will not run on a computer with Lumaview 500/400 or Lumaview 600-Series installed. To uninstall either of these versions, use the procedure described in Appendix B.

If your computer has an older version of Lumaview or Lumaview 720/600-Series (latter no longer used), installing the newest Lumaview will overwrite 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.

- To install Lumaview, right click on the .zip file and Open with Windows Explorer. Double click on the .msi installer file to start the 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 circle icon) convenient for launching the software will be present on the desktop.

K. Connecting the LS560

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 labeled **TO COMPUTER** on the back of the LS560 (see photo above). We highly recommend use of the same USB port on your computer every time you connect to the LS560. It is also best to connect the LS560 directly to the computer USB port rather than through a USB hub.

If Lumaview has not been run previously, it is important that the LS560 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. After the first time, the program can be launched before the LS560 and computer are connected. In the latter case, only the Outer Main Window will open (see Section II.B. below). Connecting the LS560 and computer with the USB cable will result in opening of the Live Image Window as well.

L. Live Cell Imaging with the LS560 in an Incubator (or Environmental Chamber)

1. Prior to imaging in an incubator, place the LS560 inside for 6-12 hr to allow equilibration to the incubator temperature.
2. Place the sample on the LS560, focus, and close the incubator door. After 60 minutes, check the focus. If satisfactory, imaging can be initiated.
3. When live cell imaging at magnifications of 40x and above, the focus can sink several microns as the mechanism relaxes. If the image has drifted out of focus, estimate the amount of focus knob rotation required to bring the image back into focus and overcompensate the same amount past the point of focus. Check the focus again after 60 minutes. The image should be in focus and imaging can be initiated. Repeat process as needed.

II. Software: Getting Started With Lumaview

The following sections give instructions for getting started with Lumaview on the LS560 and are intended as a quickstart guide only. Not all options are given and not all features may be explained in any particular section. The complete User Guide for Lumaview is contained in the Help Section after Lumaview is opened. Click on Help in the Title Bar to open the pulldown menu and then click **Contents**, or simply Click F1 to open the Help Section. **Becoming familiar with the complete Help Section is a must for using the LS560 optimally and taking advantage of the many features of this Microscope and accompanying software.**

CAUTION: When any LED is turned on, do NOT look directly at the light. Be sure to turn off all LEDs at the end of a session so any sample remaining on the deck is not photo-bleached.

A. Starting Conditions

1. Lumaview has been downloaded to the computer.
2. The LS560 is connected to the computer via the UBS cable.
3. Sample to be imaged is present on the LS560 stage (and fluorescence shroud is over sample if imaging involves fluorescence; not applicable to the LS460).

B. Launching Lumaview

1. Launch the Lumaview software from the desktop icon (or other chosen location) and allow the LS560 to be discovered. This may take a minute or so the first time. Two windows will open on your monitor:

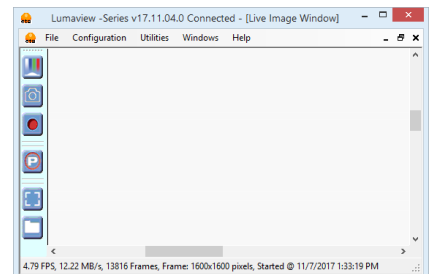
- a. Live Image Window inside the larger Main Window. “Live Image Window” will be in the title bar when the Window is not maximized. When Live Image Window is maximized, [Live Image Window] transfers to the end of the Main Window title bar.

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.

- b. Main Window with Lumaview v(version number) in the title bar. When the Live Image Window is maximized, the title bar becomes Lumaview v(version number) – [Live Image Window].

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

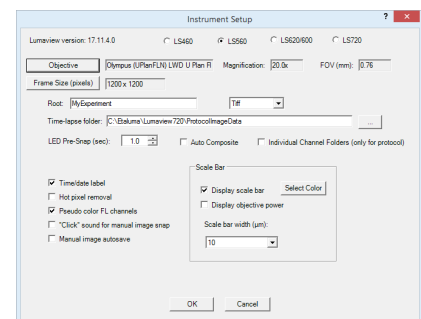
- a. Manual Image
- b. Snap Image
- c. Video Record
- d. Protocol
- e. Zoom
- f. Last File



3. The icon that appears in your computer dock corresponds to a document with an arrow.

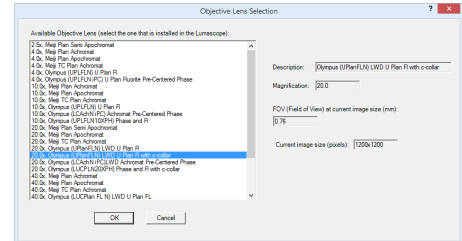
C. Instrument Setup

1. Click on **Configuration** in the File Menu bar to open the drop-down menu 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 LS560 (or LS460) is checked. If it is necessary to change the LS Microscope

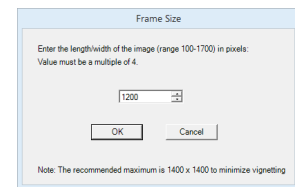


model number that is checked, you will be prompted to restart the program. Be sure to restart so that all functionalities work correctly.

- Click the **Objective** button to open its dialog box (called **Objective Lens Selection**). Click on the desired objective to highlight it. Note that the other blanks 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.

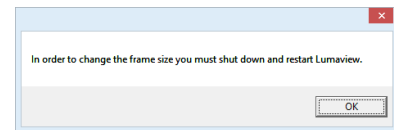


- Click the Frame Size button to open its dialog box. Using the pull-down menu, select frame size desired (frame size will be the number times itself because it is square). Default is 1200 x 1200 pixels. Maximum is 1700 x 1700 pixels but it results in darker corners. If faster frame rates are needed, use smaller frame sizes, e.g., 800 x 800 pixels.



If the frame size is changed, there will be a prompt to restart Lumaview when setup is completed and the **OK** is clicked at the bottom of Instrument Setup.

It is important to shut down and reopen the software to ensure the frame size is actually changed. Otherwise 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 the actual frame size.

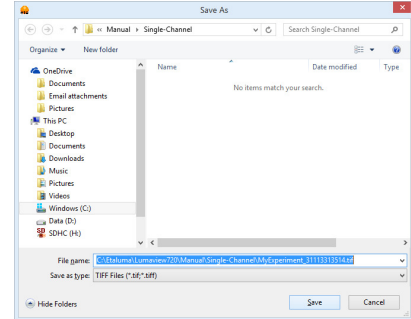


- Filename root:** Enter desired file name root for all images in this imaging session. Select the file format desired (Tiff is recommended).
- The Time-lapse folder default is given but can be changed if desired. Time-lapse is set up under Protocol as described in Section II.I below. (Note: the folder name Lumaview720 is the current default name and is used for images snapped on a LS560 as well unless a different folder is chosen).
- Select the time in seconds for the LED to be on before image snap. One second is typically used for fluorescence. Check **Auto Composite** (LS560 only) to have images in multiple channels automatically composited and saved in a Composites folder. Check **Individual Channel Folders** to have images from multiple channels in a Protocol saved in separate folders. If only 1 channel is selected in Protocol and the Individual Channel Folders is checked, the images will be in a sub-folder entitled with the channel name.

Of the next 5 features listed on the left, check those desired:

- Time/date label** will be displayed in the lower left corner of each image.
- Hot pixel removal.** Check if desired and then complete Instrument Setup. (To start the process, 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** (LS560 only) results in live and snapped fluorescent images colored green according to signal intensity.
- d. **“Click” sound for manual image snap** results in a “camera shutter-like” 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. 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 milliseconds. Thus each image number is unique and in numerical order.



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

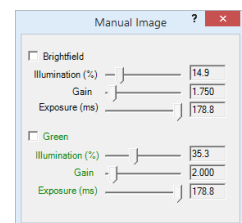
- 8. On the right side of Instrument Setup, checking **Display scale bar** results in a scale bar in the lower right corner of the live image and snapped images. If checked, **Select Color**, check **Display objective power** if desired, and select Scale bar width.

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

If a different Frame Size was chosen in Step 4 above and OK was clicked in Frame Size, a communication dialog box will open directing you to shut down and restart Lumaview. Click **OK** and then shut down Lumaview. Upon restarting Lumaview, the frame size in the lower status bar will be the new size. If a different frame size is selected and the computer is not restarted, the new frame size may show in Instrument Setup but it may not have actually changed. Always check the lower left status bar of Live Image Window to make sure your desired changes have been incorporated.

D. Finding a Live Image

- 1. Ensure sample is on the deck. Manual XY movement of SBS labware (microplate footprint) is facilitated by Etaluma’s Manual XY Stage (optional). For labware that is not SBS, use a labware holder such as the Multi-Slide Holder for 1-4 microscope slides.
- 2. Click on the **Manual Image** icon in the Left Toolbar (top icon) to open its dialog box (LS460 dialog box contains only the Brightfield controls). If desired, move dialog boxes to either side of the Live Image Window. If dialog boxes are closed and then reopened, they will open in the same locations as they were prior to closing. However, when Lumaview is closed, the dialog box positions will not be saved.
- 3. In **Manual Image**, select the desired channel (s). For fluorescence, the included Shroud (or other protection from light) must be placed over the labware. Start with **Exposure** at maximum and **Gain** as



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. Illumination can also be increased by using the external power supply/cord (See Section I.G.).

For brightfield using ambient light, start with **Gain** as low as possible and adjust **Exposure** until a mid-gray tone is seen in the Live Image Window.

For brightfield using the Phase Contrast Accessory (use open position in Slider, see Section I.F.), check Brightfield in Manual Image and turn the phase condenser iris (on front of condenser) so only a low level of light is transmitted. Set Gain to minimum, Illumination (%) to minimum (e.g., 0.4%), and then adjust Exposure until a mid-gray tone is seen in the Live Image Window.

For phase contrast using the Phase Contrast Accessory (use Slider phase ring appropriate for objective, Section I.E.), check Brightfield in Manual Image and turn the phase condenser iris (on front of condenser) to the left to the fully open position. Turn Exposure and Gain to very low. Increase Illumination (%) until a mid-gray tone is seen in the Live Image Window.

E. Focusing

1. Unless approximate sample focus level is known, it is best to initially raise objective to maximum height (without hitting sample) and then turn focus knob counter-clockwise until sample comes into focus. Ensure you are focusing on correct sample plane and not another surface, for example, back side of a microscope slide. If uncertain, place a similar but easy to focus sample on stage, focus, and then switch to new sample.
2. To facilitate the focusing process, click on the **Zoom** icon on the Left Tool Bar (sixth from the top). The Live Image Window will now show the approximate middle half of the Field of View (FOV) across a wider window. Click the **Zoom** icon again to return to the entire FOV.
3. If necessary, increase or decrease **LED brightness** and/or **Gain** as needed.

F. Snapping Images

1. When the desired field has been illuminated and focused in Live Image Window (immediately above), 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 FOV observable with the **Zoom** off. If **“Click” sound for manual image snap** was checked in Instrument Setup, a shutter-like sound occurs when each image is snapped.
2. If **Manual image autosave** was checked in Instrument Setup, images will be saved automatically using the time-based numbering system (see Section II.C.7.e. about Instrument Setup for more information). If **Manual image autosave** was not checked, a **Save As** dialog box will open after the image was snapped (again see Section II.C.7.e. above). Images will be saved in the **Etaluma folder** formed automatically whenever Lumaview is downloaded and installed. See Appendix A for the automatic subfolder directory that appears within the Etaluma folder.

- To review the last image, click the **Folder** icon on the Left Tool Bar (bottom icon) to launch Windows Explorer and open the most recent **Destination Folder**.

G. Generating Composite Images

There is no automatic compositing function in Lumaview for LS560 and LS460. To generate composite images of green fluorescence with phase contrast or brightfield captured on an LS560 or LS460, use an image analysis program such as Lumaquant, Fiji/Image J, or similar programs.

H. Videos from Time-Lapse and Live Video

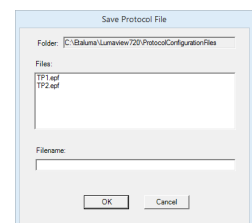
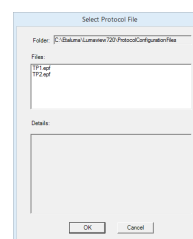
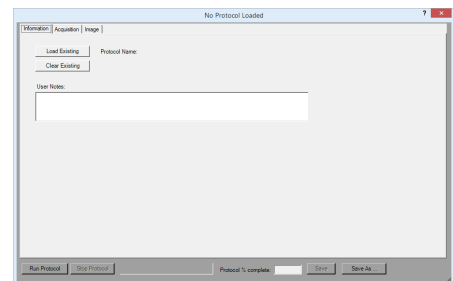
Etaluma provides several options for compiling videos from sets of time-lapse and live video images:

- 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/).
- Your own compiling software such as ImageJ, Fiji, MSMoviemaker, or iMovie, or
- Use of two freeware software programs together, i.e., AviSynth and MeGUI (instructions described in Appendix C). If using this option, be sure to read the instructions first because the two programs must be downloaded before image capture.

I. Time-Lapse

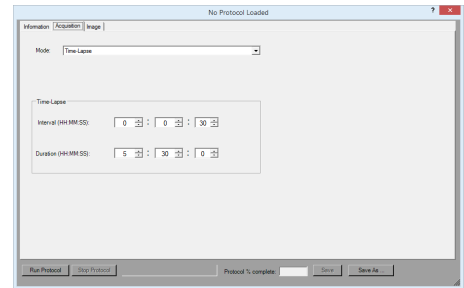
- To configure a time-lapse assay, the **Protocol** feature is utilized. Click the “**P**” icon in the Left Tool Bar (fourth from the top) to open its dialog box containing three tabs. You will enter information and values as you move among the tabs. Be sure to click **Save** before leaving a Protocol.
- In the first tab called **Information**, click the **Load Existing** button to bring up a previously saved Protocol (select desired Protocol and click **OK**). Click the **Clear Existing** button if values have been entered on any of the tabs and saving is not desired. A **Save Protocol File** dialog box will open asking if you want to save the current Protocol. If Yes, click **OK**.

If not previously saved, enter the new desired Protocol name in the Filename field or click on an existing File name to overwrite. Overwrite Alert will open; click **Yes** or **No**. You are now ready to create a new Protocol. On the Information tab, if you enter User Notes, they will become part of the new Protocol. You can also enter User Notes for a previously saved Protocol in this tab.

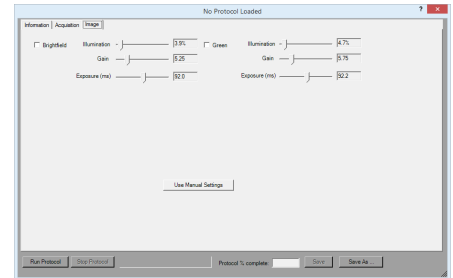


If the latter, a File

- Click on the **Acquisition** tab and check **Time-Lapse**. Enter the Interval and Duration information. Before initiating the time-lapse run, review **Computer Configuration** in the **Help Section** and change your computer power and Windows update settings so they cannot automatically turn on and interfere with scheduled image captures. Of particular importance to turn off are **Sleep Mode** and **Auto Restart**. (After time-lapse is completed, be sure to change the settings back.)



- Click on the **Image** tab to select the channels to be used in the time-lapse and adjust their settings (LS460 will show only the Brightfield settings). If settings were previously determined in Manual Image (Section II.D above), transfer the values into the current Protocol by clicking the **Use Manual Settings** button. The desired values can also be entered manually. Note: This dialog box cannot be used to optimize the image settings because it is not interactive with the 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 (Section II.D above). When settings are satisfactory, click the **Use Manual Settings** button in the Image tab to upload them into the Protocol.

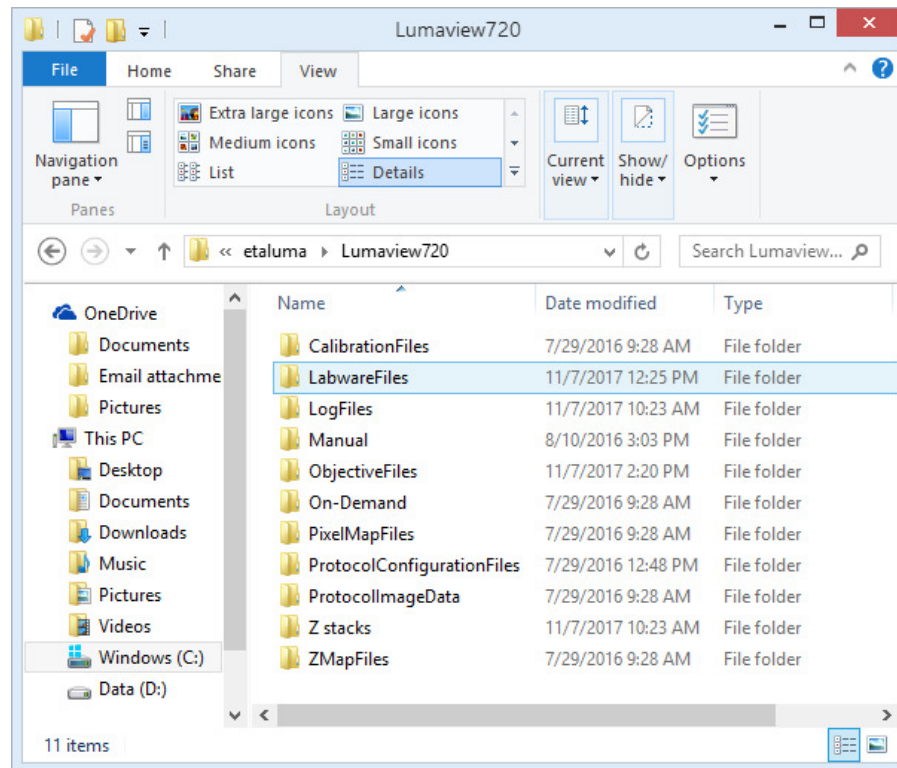
- To run the Protocol, click **Run Protocol** in the lower left of the Protocol window.

J. Live Video

- When the desired field has been illuminated and focused in Live Image Window, click the **Video Record** icon in the Left Tool Bar (third from the top) to open its dialog box. Select parameters requested and also those in Instrument Setup such as file format (Section II.C.). Images captured will be saved to the Video folder inside the Manual folder shown in Appendix A.
- Click **OK** and image capture will begin. To stop image capture, click the **Record Video** icon in the Left Tool Bar again.

Appendix A

Lumaview File Tree Organization



Appendix B

Uninstalling a Different Version of Lumaview

1. Go to Control Panel/Programs & Features, right click on the Lumaview file, and select Uninstall. After uninstall is completed, verify that the Lumaview folder is now gone from the C:/Program Files (x86)/Etaluma folder. You are now ready to install the new version of Lumaview.

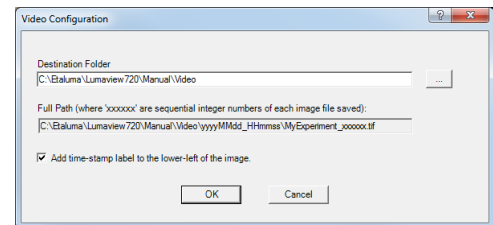
Appendix C

Capturing and Compiling Videos Using AviSynth and MeGUI

1. It is first necessary to download and install the following two freeware programs.
 - a. **AviSynth** video post-production program found on the AviSynth Wiki at http://avisynth.nl/index.php/Main_Page. Download and install the Official Build. The default save location for the program will be the Etaluma folder inside the Program Files (x86) folder.

- b. **MeGUI** video conversion program found at <http://sourceforge.net/projects/megui/>. After the zipped folder (MeGUI_2525_x86.zip) has downloaded, click to open and Extract All. Depending on your computer and Windows version, it may be necessary to right click on the .zip file to complete the extraction. After extracting the files from the zipped folder, you will see the file MeGUI Application with a green icon in the MeGui_2525_x86 folder. Extraction so that the green icon is shown is essential for the images to be scripted correctly for video compiling. If desired, make a shortcut on your desktop.
2. When the desired field has been illuminated and focused in Live Image Window (Section II.E. above), click the Video Record icon in the Left Tool Bar (fifth from the top) to open its dialog box. Also check parameters such as format type in Instrument Setup (Section II.D.) Enter the Full Path information and check Add time-stamp label if desired. Images captured will be saved to the folder shown; this can be changed, e.g., to a new subfolder set up within the Video folder.

Click OK and image capture will begin. To stop image capture, click the Record Video icon in the Left Tool Bar again.



3. To compile the images into a video, click on the MeGUI Application to open the program (may take several minutes).
4. At the end of the AviSynth Script line near the top, click browse (“...”). A MeGUI window named Open AviSynth script will open. Depending on the computer and Windows version used, the channel subfolders from the most recent time-lapse capture (F1, F2, F3, W) may be listed. Or it may be necessary to browse to your Destination Folder and open to access the channel subfolders. Open the desired subfolder and click on the Avisynth (.avs) file.
5. A preview video window will open; click Play to see a short segment of your video.
6. Return to the MeGUI window and select File Format (MP4 recommended). Click Queue button for Video encoding on right (button about halfway down the dialog box).
7. An encoding window will open and you will see progress in a green horizontal bar until video is completed and window closes. Close MeGUI window.
8. Video will be saved in the channel subfolder within the Destination Folder described above.