

Lumascope 720

-- Startup Guide --



Lumascope 720

IMPORTANT: This Guide describes how to set up the LS720 (Section I) and start using Lumaview 720-Series (Section II). It is important that you follow this Guide. 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>.

I. Hardware: Setup of Lumascope 720

A. Items included with Lumascope 720

- 2 USB power/communication cables for image and stage control
- 1536 well microplate for calibration
- 4x objective for calibration
- Ruler slide for FOV measurement
- External power supply/cord with country-specific plug for stage power
- Fluorescence shroud for operation in ambient room light

B. Optional Accessories (purchased separately)

- Brightfield and Phase contrast accessory
- Labware Holders
 - Holder for 35 mm Petri dishes
 - Holder for 60 mm Petri dishes & Terasaki plates
 - Holder for microscope slides & 50 mm Petri dishes
- Application specific objectives

C. First step: Installing Lumaview 720-Series software on your computer

1. The Lumascope 720 is controlled by the Lumaview 720-Series 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 Lumascope**. Lumaview for LS720 is best run with two monitors; one for the settings menus and the other for the Live Image.
2. Lumaview 720-Series will run on Windows XP, 7, 8, 8.1, and 10, but not Vista. Both desktop computers and laptops can be used, but the best visualization correlates with resolution of the monitor.
3. To download Lumaview 720-Series, go to <http://etaluma.com/products/downloads> (under the Resources tab). Click on the Lumaview 720-Series - ZIP link to start the download and save the folder when prompted.
4. Install Lumaview 720-Series by double clicking on Lumaview720-SeriesInstall_v(version#).msi. 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 720-Series will then finish.** After

installation, a Lumaview icon (orange circle) convenient for launching the software will be present on the desktop.

D. Connecting the Lumascope 720

1. Insert the standard USB-A end of the supplied USB power/communication cable into a USB port on your computer and the other square USB-B end into the square port (upper arrow) on the back of the Lumascope 720 (see Fig. 1). Connect the second USB cable onto the circuit board USB-mini port and the USB A into another USB port on your PC.

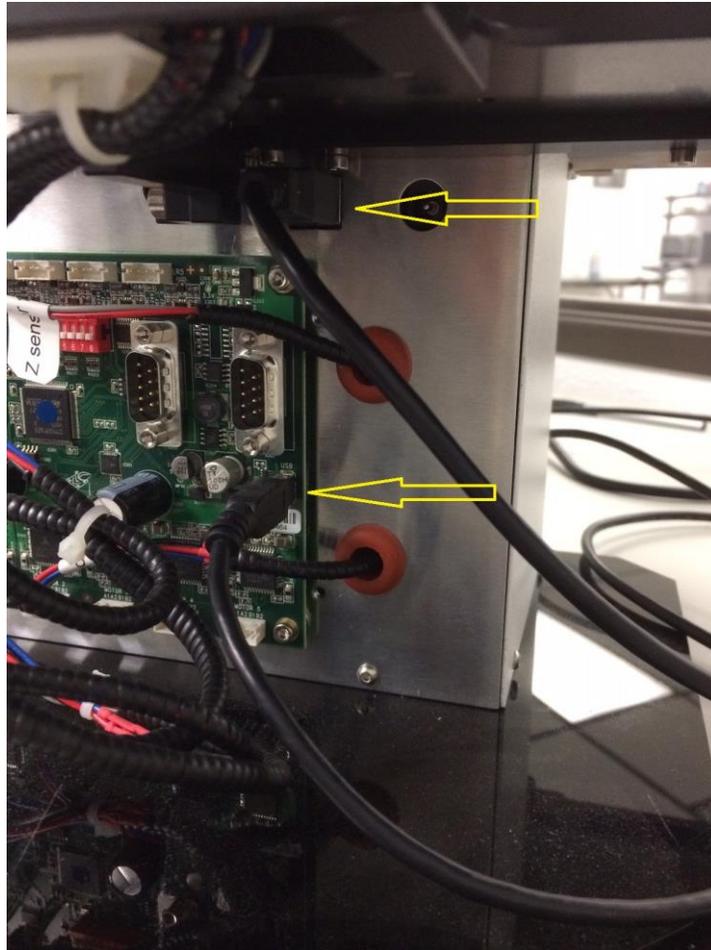
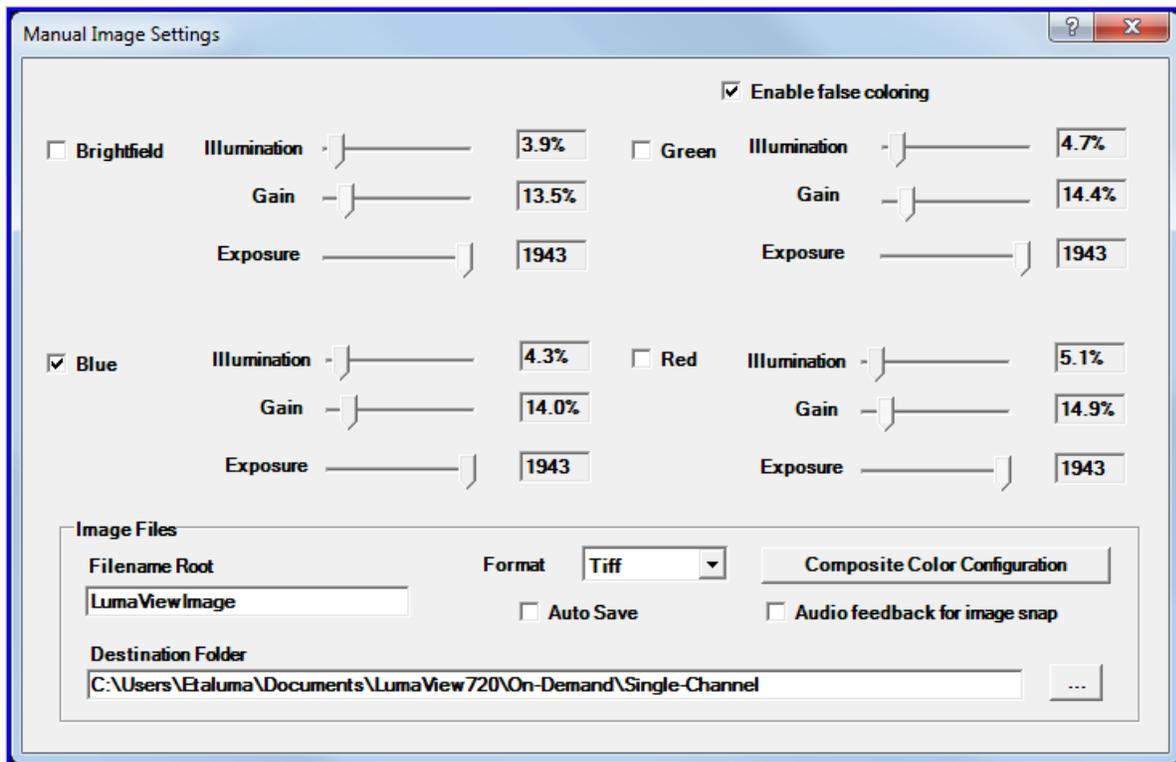
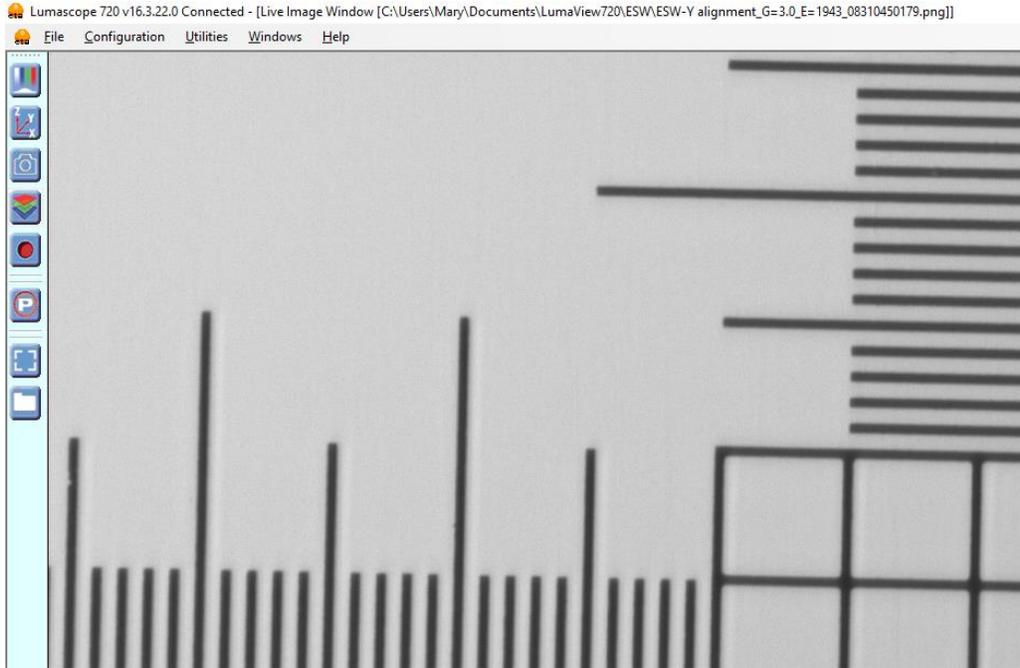


Fig. 1. Rear panel connections on LS720beta

It is important that the USB cable be connected first between the LS720 and computer before Lumaview is launched. This is because Windows needs to load the USB drivers before it can be launched.

2. Launch the Lumaview 720-Series software using the desktop icon and allow the Lumascope to be discovered. This may take a minute or so the first time. Two windows will open on your screen:
 - a. Live Image Window inside the larger Main Window. The Live Image Window name is present in the top border of the Window when the Window is not maximized. If the Live Image Window is maximized, [Live Image Window] transfers to the end of the Main Window name.

- b. The Lumascope will initialize and connect and a live image will appear. In this initialization position, the objective is left fully retracted and positioned at the edge of the microplate nest.
- c. Click the first icon to adjust image parameters such as channel to be used, illumination, and Gain and Exposure,

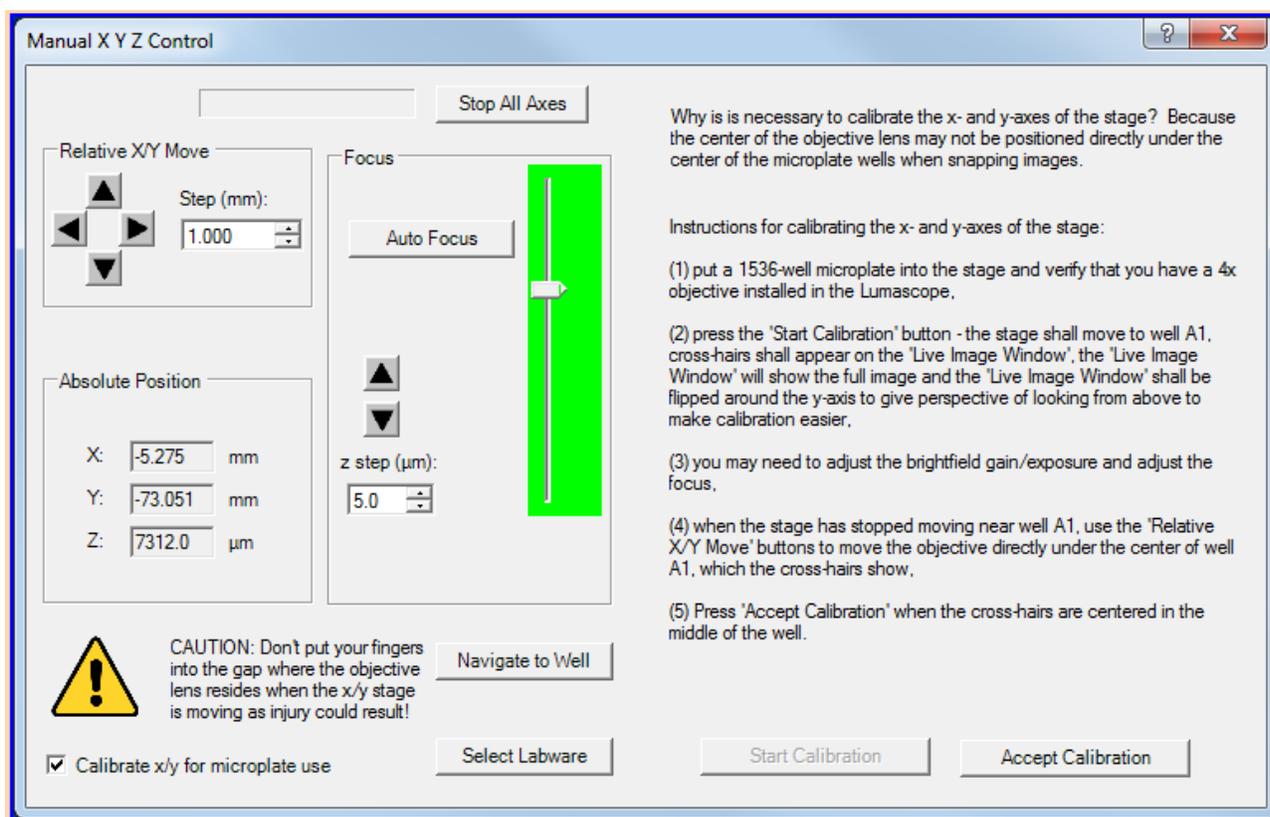


E. Objectives

1. To install or change objectives, loosen the deck clamp in front until the top deck can be lifted back to expose the objective. When installing an objective do not overtighten. Remember to retighten the deck clamp.

F. Calibration

1. Your Lumascope will need calibrating the first time it is used with any new computer. A 4x objective and empty 1536-well plate has been included with the LS720 for calibration purposes.
2. Open the XYZ screen and check the box in the lower left hand corner labelled "Calibrate x/y for microplate use". When this is checked, the dialog will expand to show the calibration instructions and buttons. Shown below is a graphic image of the widened dialog box.



3. With the 4x objective installed and the 1536 well plate in place, adjust focus until a well is seen. Moving the focus slider up to about 75% should cause the image to pass through a focus and then this can be optimized with slider adjustment or the arrow keys. Image brightness can be adjusted as focus is found.
4. Now drive the stage with the Relative X/Y Move control until the red cross-hairs are centered on well A1. This may require you to confirm that you are in the upper left corner well by moving above and left of A1 to confirm it is indeed the corner well. When you are satisfied that you are centered in A1, press Accept Calibration.
5. Uncheck the Calibrate button and choose Select Labware, choose 1536 well plate, and then select Navigate to well and confirm that the instrument is centered in a few distant wells.

6. Replace the 4x objective with the one you would like to use for your experiment and be sure to select the new objective under the Configuration drop down.

II. Software: Getting Started with Lumaview 720-Series

The following sections give directions for getting started with Lumaview 720-Series 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 720-Series is contained in the Help Section after the program is opened and is accessible by clicking on F1 or the Help dropdown menu/Contents. **Becoming familiar with the complete User Guide is a must in order to take advantage of the many features of this program.**

Using Lumaview 720- Summary

Control of your LS720 is divided into Manual operation and Automatic operation.

Manual operation is effected by the top four Icons on the left border. These icons are “Adjust Channels”, “Position XYZ”, “Snap Picture”, “Snap Composite” and “Record Live Video”. With these controls, one is able to perform single position, single time point acquisition of single or multiple channel images.

Automatic operation is controlled under the “P” or Protocol icon. Individual tabs in the Protocol screens allow you to set up automatic versions of the manual configurations determined above. A Protocol consists of the following tabs: Information, Acquisition, Image, XYZ, and Image Review. Each of these allow one to configure the various settings to construct and save a Protocol for Automated operation.

A. Starting point conditions

1. Lumaview 720-Series has been downloaded to the computer.
2. The Lumascope 720 is connected to the computer via the two UBS power/communication cables.
3. Lumaview 720-Series is open and has been calibrated with a 4x objective and 1536-well microplate.
4. Sample to be imaged is present on the Lumascope 720 stage (and fluorescence shroud is over sample if imaging involves fluorescence).

B. Illuminating the sample for brightfield

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

1. For viewing in a typically lit area, ambient light is generally adequate to see brightfield. The light should be as uniform as possible to prevent shadows or other dark or light areas. Overhead fluorescent lighting can cause uneven brightness including a striped pattern; if observed, move the Lumascope or partially shade the light to reduce unevenness across the sample. For optimal brightfield, use of the light source of the Phase Contrast Accessory is recommended (see Section I.B above). Note: Because the Lumascope 720 CMOS camera is monochromatic, brightfield images will be in black & white.
2. For viewing in a dark environment such as an incubator, a transmitted light source is needed. Connecting the Phase Contrast Accessory to the Lumascope allows it to be controlled by Lumaview 720-Series.

3. When using ambient light, move cursor over Live Image Window, right click, and select Exposure Settings to open its dialog box. Start with Gain as low as possible and adjust Exposure so image has gray tones. If using the Phase Contrast Accessory, move cursor over Live Image Window, right click, and select Illumination Settings to open its dialog box. Right click again and select Exposure Settings to open its dialog box. In Illumination box, check White LED. In general, to increase brightness, start with Exposure at maximum and Gain as low as possible. Increase Illumination to desired brightness of gray tones; if needed, increase the Gain gradually. To decrease brightness, turn Gain down to a minimal level and then gradually decrease the Exposure. If still too bright, decrease the Illumination gradually until image has gray tones with desired brightness.

C. Illuminating the sample for fluorescence

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

1. Click the first icon, opening the Image control screen.
2. In this menu check the LED of choice, e.g., F2 for green fluorescence is often chosen first due to its typically bright signal. Start with Exposure at maximum and Gain as low as possible. Increase Illumination to desired brightness. For dim samples, if Illumination is at maximum, increasing the Gain can be used to get enough signal (but Gain will increase background as well).
3. If desired, check false coloring to see images in color. If more than one LED is turned on, images will be gray scale and signal will be the sum of the selected channels.

D. Focusing

1. Move the Focus slider up to about 75% to begin looking for focus.
2. Use the slider and Z-arrows to get the best focus. Clicking on Expand icon on side bar of Live Image Window zooms in on image so it fits the monitor screen horizontally and should help you to focus. If necessary, increase or decrease LED brightness and/or Exposure and Gain.

E. Capturing images

1. Select the Output Format Default dropdown menu and select Output Format type desired. The TIFF format is recommended due to several features as explained in Help/Image Types. Select or enter the Demand Capture Filename Root for your image file names and desired Destination (e.g., a new folder set up on the desktop).
2. When desired field has been illuminated and focused in Live Image Window, left click the camera icon to capture the image.
3. A Save As dialog box will open. Confirm that the File Name is satisfactory and click Save. To autosave the images without the Save As dialog box opening each time, check Auto Save.
4. For quick review of your last image, click the Folder icon on the left tool bar to launch Windows Explorer, which will open the most recent Destination Folder.

F. Capturing composite images

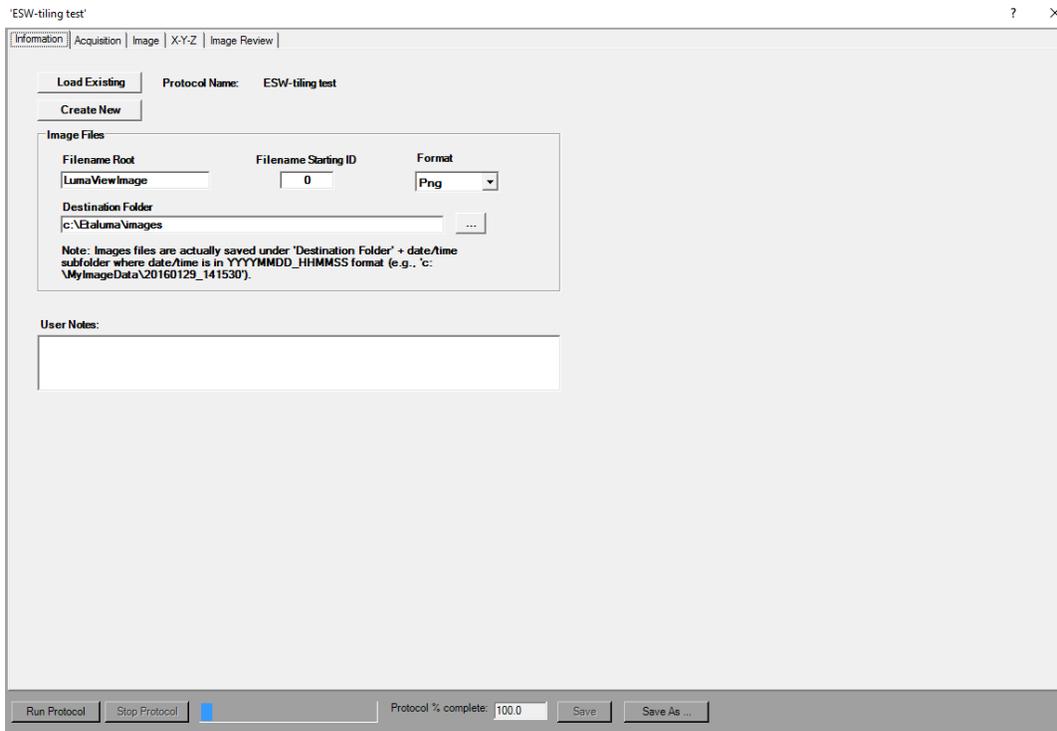
1. Click the Composite icon in left tool bar of Live Image Window. A Composite Color Configuration dialog box will open.
2. Select channels (e.g., F1, F2 and F3 for 3-color) and set LED % and Gain % for each based on viewing in Section II.D above or previous experience. Manual entering of values is recommended because checking Load Present Values will load only the LED % for each channel while Gain % will be the same for all channels and correspond to the last determined channel.
3. Set LED Pre-Snap to 1 second (can be adjusted later). Choose Output Format Type (TIFF recommended), Filename Root, and Destination Folder (e.g., folder set up on the desktop).
4. Click OK to start the image capture. Depending on the LEDs selected and Lumaview version, you should see the LEDs come on in series and/or hear the images being captured (clicking sound).
5. The composite image made by combining the individual images together will appear in the Main Window. The image file name at the top of the image includes the full path.

I. Recording live videos

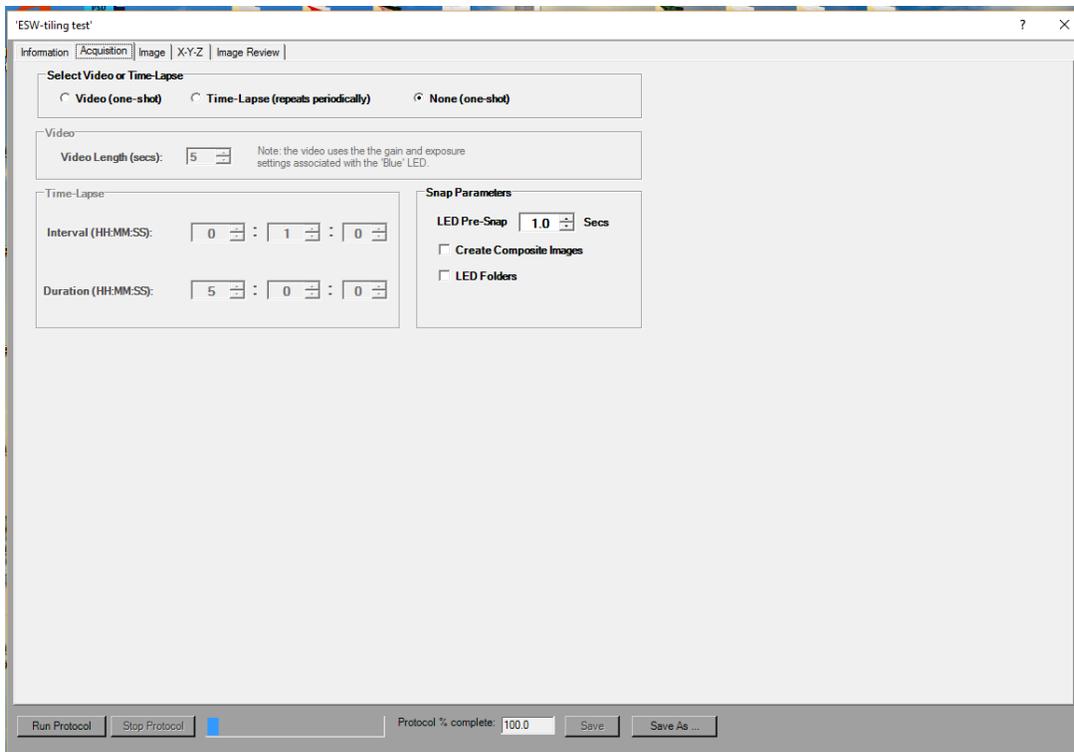
1. The Video Record icon grabs every frame from the live image and puts them in a folder.
2. Click red circle icon in left side bar of Live Image Window to open Video Configuration dialog box.
3. Choose Filename Root, File Format (TIFF recommended), Destination Folder and if time-stamp is desired. Click OK and images will start to record.
4. Click red circle icon again to stop. A dialog box will open stating that your images have been saved to the Destination Folder and how to proceed.
5. To convert the captured images to video, a variety of options are available including Microsoft Moviemaker, Apple iMovie, ImageJ, and CellProfiler.

J. Setting up a Protocol

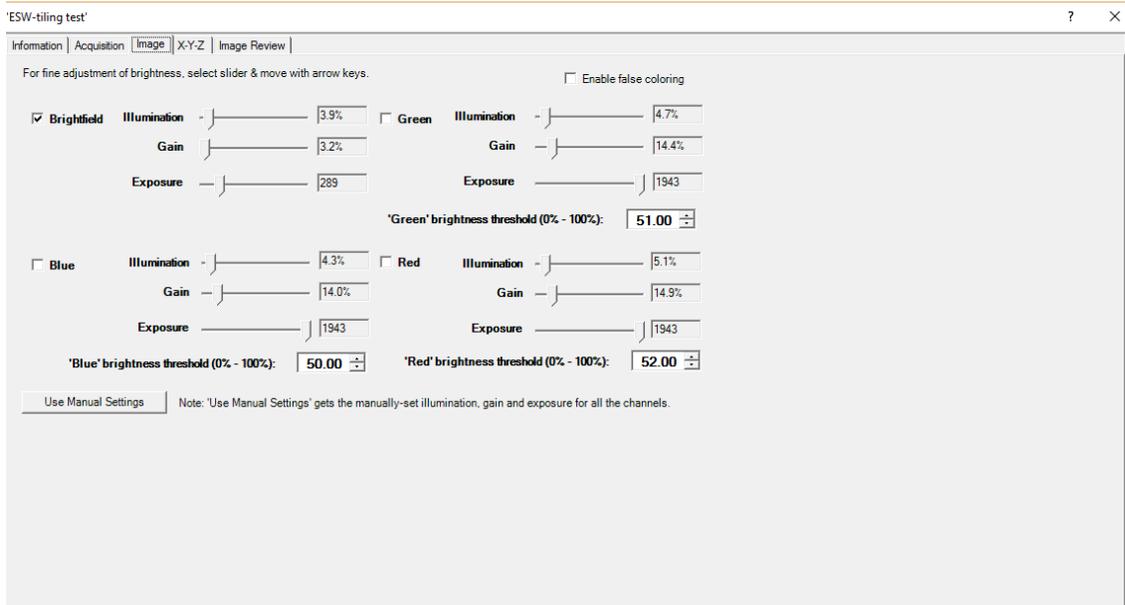
1. A Protocol allows an automated microscopy method to be configured, saved and run. It consists of information about the channels, focus, and labware as well as timing and file details.
2. Clicking the “P” icon opens the Protocol interface.
3. The first tab of the Protocol is labelled Information and contains Protocol information including data file path, file root, and image format.



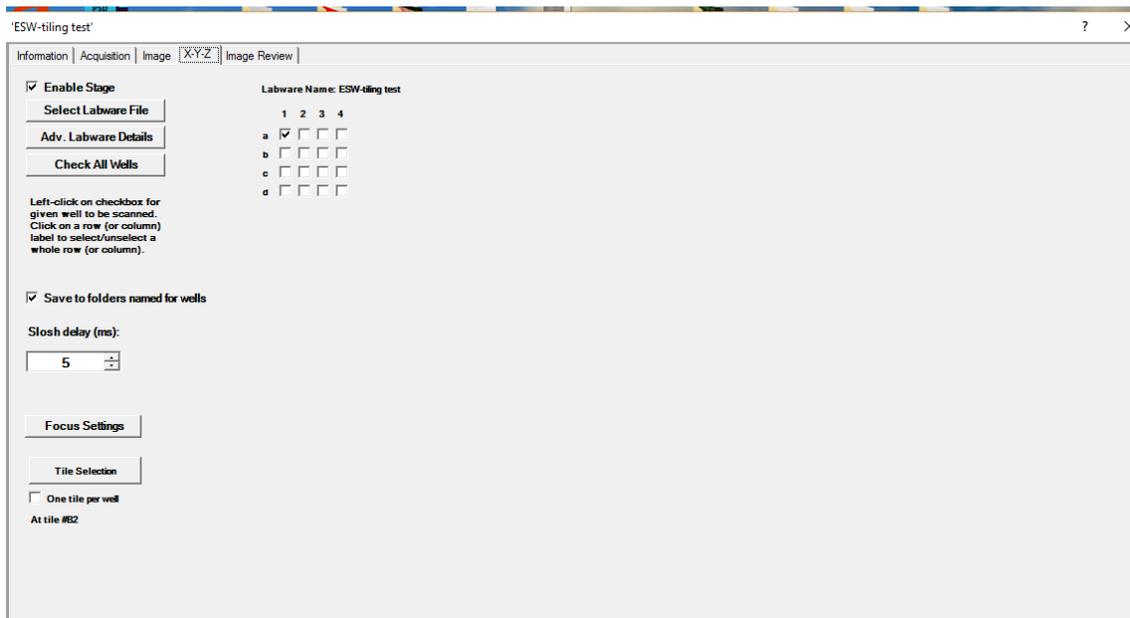
4. The Acquisition Tab allows the selection of how images are gathered.



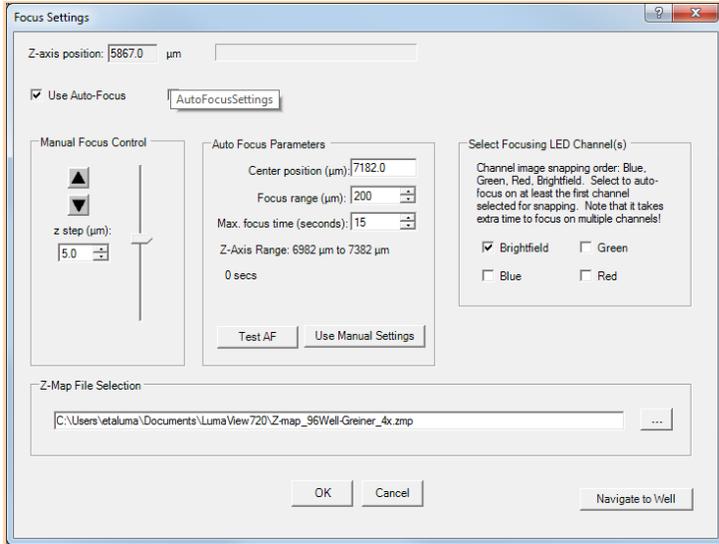
5. The next tab is Image



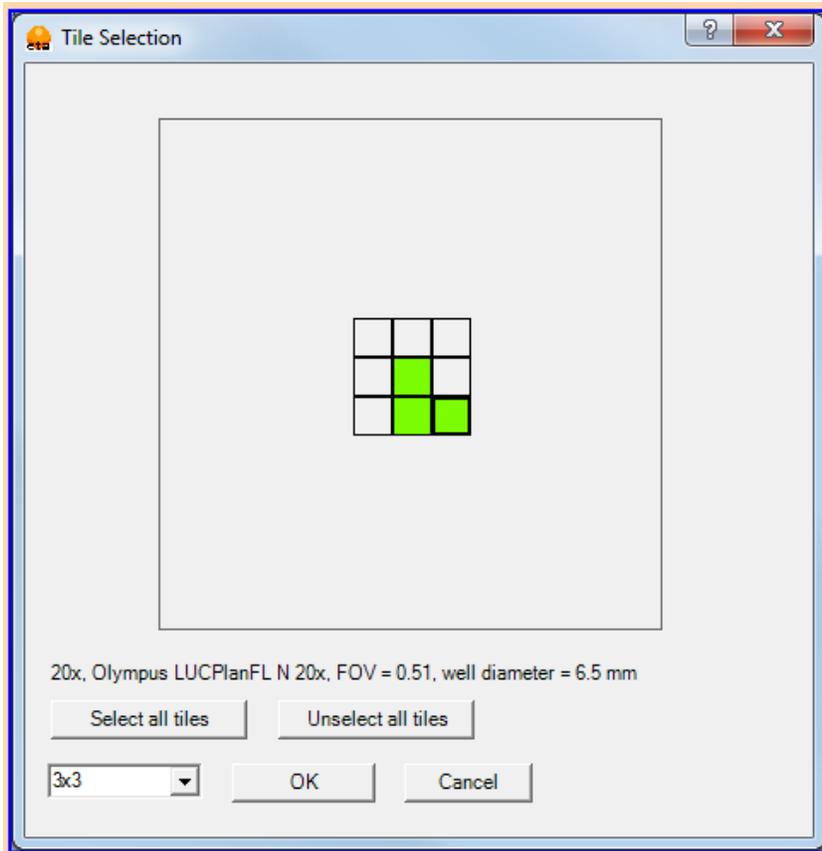
6. The XYZ tab is where the labware is selected and the wells or positions within that labware are also selected. Autofocus settings and Tiling menus are also accessed from this tab. These are explained below.



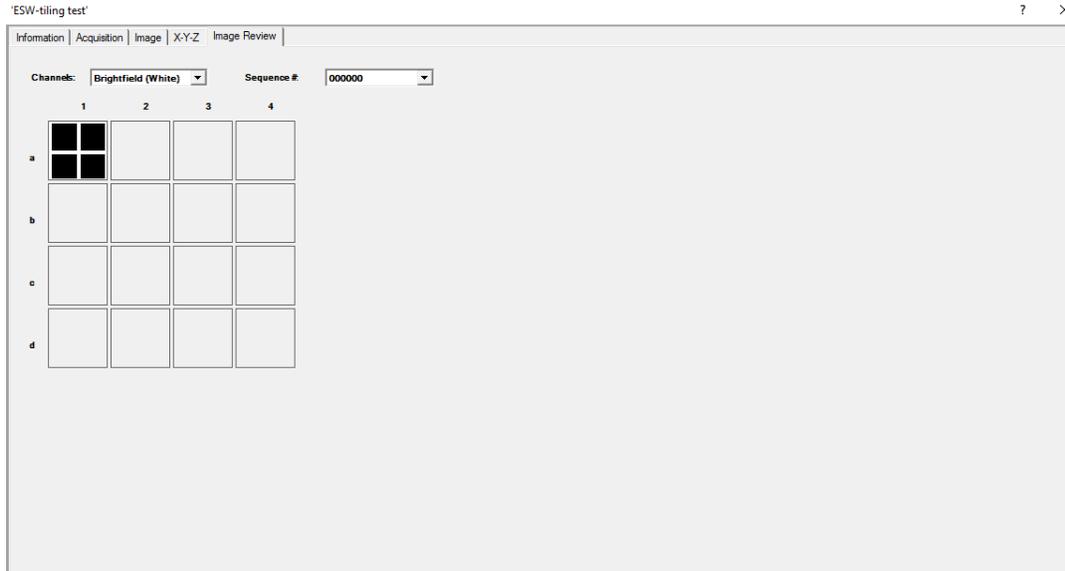
7. Autofocus Settings



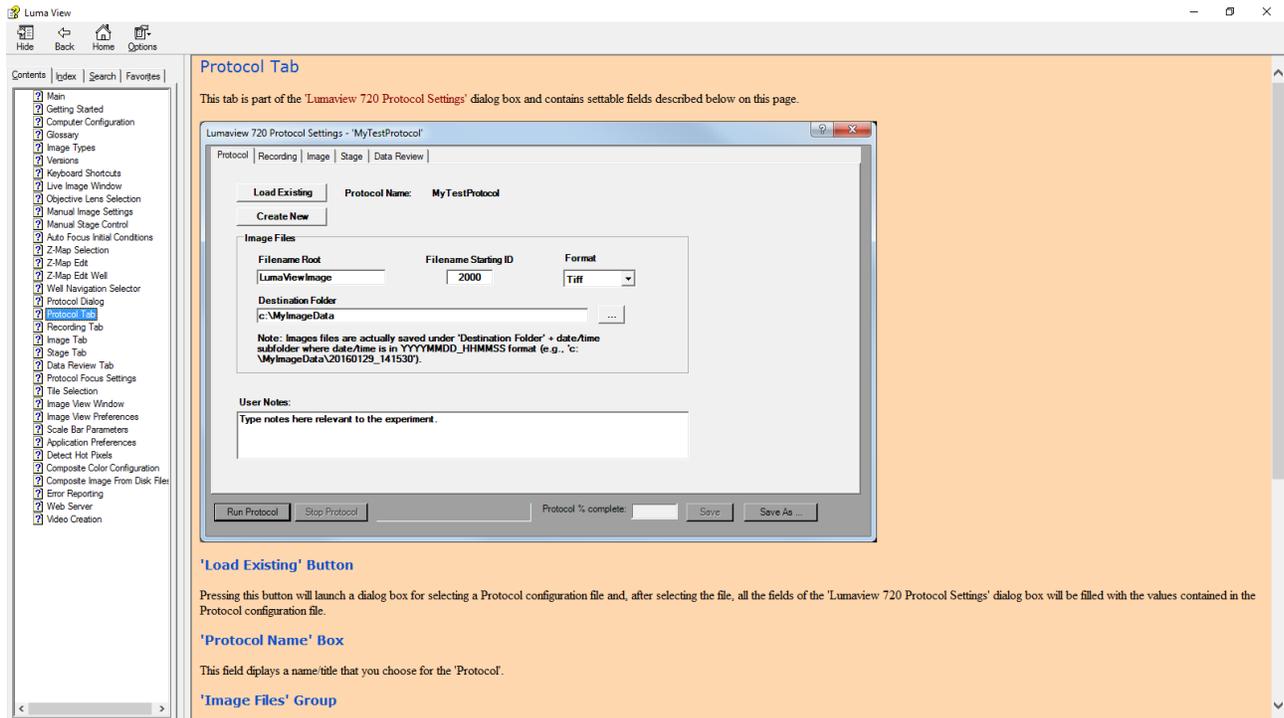
8. Tile Selection



9. The Image Review tab shows thumbnail images, including tiles, for each well and channel as they are acquired. Clicking on a well expands the image.



10. The Help Contents files contain descriptions of each screen and field within the menus.



For questions or comments, telephone the phone number below, email support@etaluma.com, or submit an inquiry through the Etaluma website at <http://etaluma.com/contact>. An Etaluma representative will be happy to assist you.