

Lumaquant 8.8 Image Analysis Program

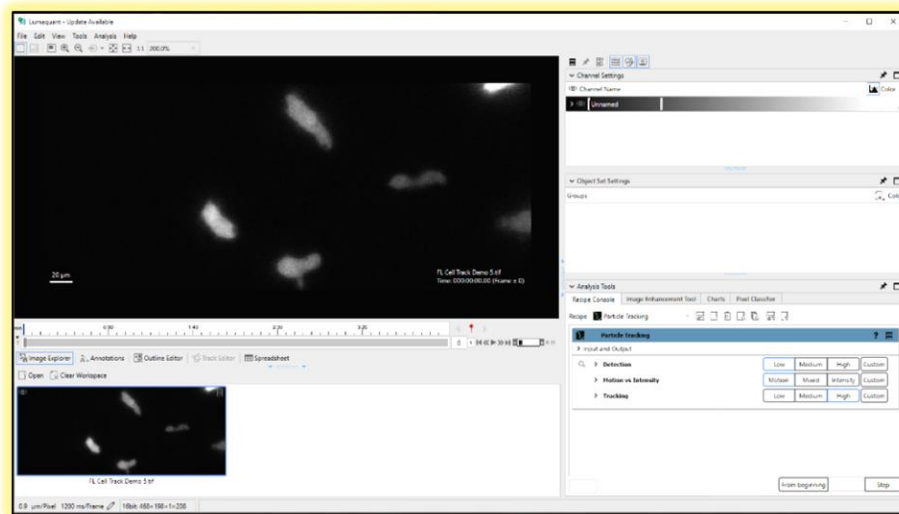
-- Startup Guide --

For use with images from
All Etaluma LS Microscopes



LS720 with Phase Contrast Accessory

LS620 with Phase Contrast Accessory



Lumaquant main window (optional white background)

IMPORTANT: This Guide describes how to install and begin using Lumaquant.
For assistance, please call 760-298-2355 or email support@etaluma.com.

Etaluma, Inc.
3129 Tiger Run Court, Suite 218
Carlsbad, CA 92010

760-298-2355
www.etaluma.com

Lumascope™ and Lumaquant™ are trademarks of Etaluma, Inc. ©2009-2017 Etaluma, Inc. All rights reserved.

Table of Contents

- A. Downloading and installing Lumaquant
- B. Information about the new Lumaquant Version 8.8
- C. Importing an image series and creating a video

Appendices

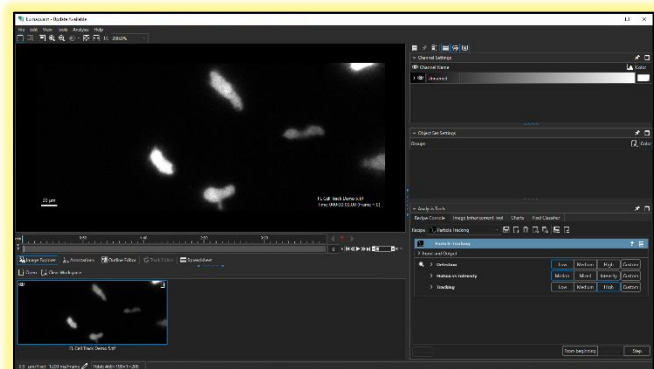
- A. X/Y Calibration Values for Objectives

A. Downloading and installing Lumaquant

1. On the Etaluma website (www.etaluma.com), click on **Resources** and from the dropdown menu, click Downloads.
2. From the Downloads page, click on the **Lumaquant _version ZIP** link to download the software.
3. From your computer downloads location, click on the program file name. If a Windows warning box about an unrecognized App appears, click **Run anyway**. If a User Account Control dialog box appears, click Yes to allow the program to make changes to the computer.
4. The Lumaquant (version#) Setup Wizard will open. Follow the commands and Lumaquant will install in **C:/Program Files/Etaluma/** or other location of choice. Click **Finish**.
5. The Lumaquant Analysis Tools dialog box (Fig 1) will open. To use Lumaquant for a 30-day trial period, click Trial, complete the information requested, and click Submit. Lumaquant will now open and you will be able to use it for 30 days. You can close it down between sessions and reopen by entering your information again.
6. If you have purchased a license through Etaluma, you will receive your license key by email. In Step 5 above, click **Activate**, enter the key number in the blank provided, and click Submit.
7. The Lumaquant program will now open (Fig 2). Note that the program default now has a dark background but it can be changed to the light background, shown on title page, under **File/Options**. If a change is made either way, you must close the program and reopen for the change to be take effect.



< Fig 1. Lumaquant Analysis Tools launch dialog box



^ Fig. 2. Main Lumaquant window (default dark background)

B. Information about the new Lumaquant Version 8.8

1. Lumaquant is based on Leica Microsystem's image analysis software called Aivia. The most noticeable changes are to the Graphical User Interface (GUI) and the addition of Pixel Classifier.
2. To become familiar with Lumaquant v8.8, the following is suggested:
 - a. View the following Aivia 7 and Voxel Webinar Series videos accessible in YouTube on Aivia Software:
 - i. Aivia Introduction to Data Visualization. Covers basic uploading of single images, time-lapse series, lookup table use, file types, etc.
 - ii. Aivia Introduction to 2D Microscopy Image Analysis. Covers cell counting and cell tracking.

- iii. Aivia 7.5 - Fast trainable pixel classification (fluorescence microscopy data)
 - iv. View the videos of interest on the Tutorial playlist. These tutorial videos are based on the full Aivia program, but the underlying functionalities of the recipes are the same.
 - c. Download and review the Application Notes available on the Etaluma website.
3. The following is a list of new features and changes in Lumaquant v8.8.
- a. Additional parameter free segmentation (smart segmentation) option in the Pixel Classifier
 - b. New magic wand tool in the Pixel Classifier
 - c. GPU accelerated performance enhancement for the Pixel Classifier
 - d. New ability to train / improve the Pixel Classifier in multiple sessions
 - e. New 2D stitching features
 - f. New Live Recipe Apply feature: apply the current recipe in the Recipe Console during Live Import
 - g. Various bug and general improvements

C. Importing an image series and creating a video

1. Videos can be created from images snapped in Time-Lapse, Video, or Time-Lapse Video acquisition modes in the Lumaview Protocol feature. Before snapping the images in Lumaview, go to **Instrument Setup** and make sure **Individual Channel Folders**, **Time/date label**, and **Display scale bar** are not checked.
 2. To compile a video, launch Lumaquant from the Desktop shortcut. After the Lumaquant window opens, click **File** in the File menu bar. In the pull-down menu, select **Import** and then **Image Sequence**. The Import Images dialog box will open; click the **Load Image** button in the upper left corner. A Windows Explorer **Add File(s)** dialog box will open, allowing you to browse to the folder containing your images (Default location in Lumaview 720/600-Series is *C:\Etaluma\Lumaview720\ProtocolImageData*).
 3. For videos in a single channel, browse to your image folder and highlight all images to be imported. *Numbers must be contiguous*. Click the **Open** button and the images selected will now be listed on the left side of Import Images.
 4. For multi-channel videos, do not import Composite images already made in Lumaview. Rather import the entire sequence of images for the first channel (*numbers must be contiguous*) as described in No. 3 above, then repeat with the images in the second channel, and so on for all channels. If your images were already saved in one folder by Lumaview, they likely can be imported in a single sequence as long as the file names are contiguous and all images in a channel are listed completely before the next channel begins. If images are in separate channel folders and manually combined into one file, ensure all images for each channel are listed one after the other and not mixed.
 5. In Import Images, complete the following (see Fig 3): **File Name**: Add your name of choice (replace any default name already there).
Define Sequence: Click on **Manually**. For images in a single channel, there will likely be a T automatically entered under **Dimensions**. Ensure the correct number of images is entered under **Size**. *Remember that images 000000-000020 make up a total of 21 images*. If not automatically entered, enter the T and number of images manually. For images in more than one channel, they must be ordered by channel first and then time. In the first box under **Dimensions**, select C in the pull-down menu. Under **Size**, select the channel number using the up and down arrows. In the next **Dimensions** box, select T in the pull-down menu. Under **Size**, enter the number of images in each channel. *Remember that images 000000-000020 make up a total of 21 images*.
- Channel Color**: An entry for each channel must now be listed. Click on **Add new channel** as needed and use the light gray down arrows to choose the colors. With multiple channels, be sure the colors listed are in the same order as those in the import list on the left. For each channel, use the pull-down arrow on the very right to select the pseudo color of choice. For gray tone images, e.g., phase contrast or brightfield, you must use or add a new channel and then can change the New Channel name to Phase or Brightfield. Use the pull-down arrow on the very right to select the color of gray. Delete channels that are not being used.
- Define File Layout**: Allows you to specify how the ZTC dimensions are organized within one file, such as 1 file having 3 separate channels. To specify, change the **From first file** to manual and set the ZTC dimension

for each channel. If your individual file is a 2D time lapse with 1 channel and 1 time point, change all the dimensions to 1.

Fig 3. Import images dialog box

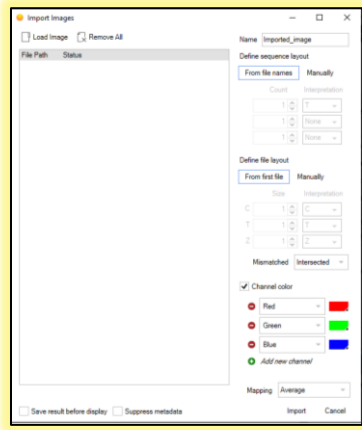
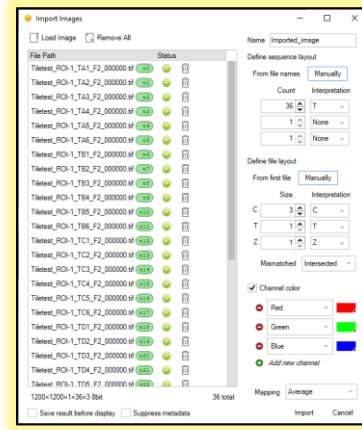


Fig 4. Loading image sequence from time-lapse

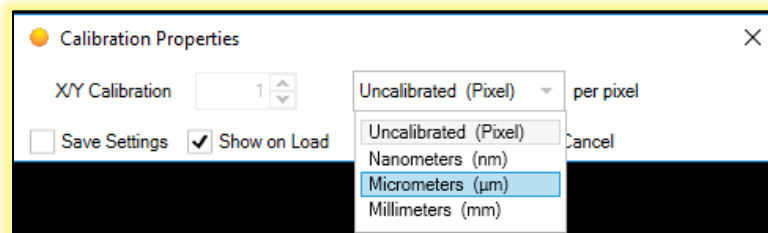


Mapping: Select **Maximum** for LS Microscope images.

6. If the images are to be saved as a sequence that can be opened again in Lumaquant, check **Save imported image** in the lower left. You will be asked for your saving preferences after the next step. If the **Save imported image** button is not checked, the images will be used in the current Lumaquant session only. (Note: if you import your images without checking the save option and then use them in a Recipe/Method, when you exit Lumaquant, you will be asked if you want to save your file).

7. Click **Import** and the **Calibration Properties** dialog box will open. For the X/Y Calibration line, first select the units per pixel by clicking on the dropdown menu on the right and select Micrometers (um). For the X/Y Calibration value, refer to the XY Calibration Values for Objectives (um/pixel) table (Appendix A) and enter the value listed for the objective used. For the Time Calibration line, first select the units per frame by clicking on the dropdown menu on the right and selecting the correct unit. On the left, click on the up and down arrows to select the interval of time between each image.

Fig 5. Calibration Properties dialog box.



8. Check **Save Settings** to have the same settings load the next time you create a video. Check **Show on Load** to have the Calibration Properties dialog box open each time you load an image. Upon entering all desired information in Calibration Properties, click **Apply**.

9. If **Save imported image** was checked in Step 5 above, a **Save FOV As** Windows Explorer dialog box will now open in your images folder. Choose a File name (file type is always Tiff) and click **Save**. If a Saving (Not Responding) dialog box appears, wait a short time while the saving occurs.

10. Your sequence of images will now be loaded into Lumaquant (Fig 5). To see each image, click on the gray/white horizontal scroll bar below the Image Viewer window. The images are shown in color. If you prefer grayscale, right click on the channel bar in the Channel Name box (upper right). In the popup menu,

select **Display as Grayscale**. To alter the color/shade, click on the dropdown menu next to the color square in the Channel Name box (upper right).

11. For composite images, to view channels separately or to eliminate a channel from the composite image, click on the Eye icon to the left of the channel bar in the Channel Name box. The eye will close to turn the channel off and open to turn it on.

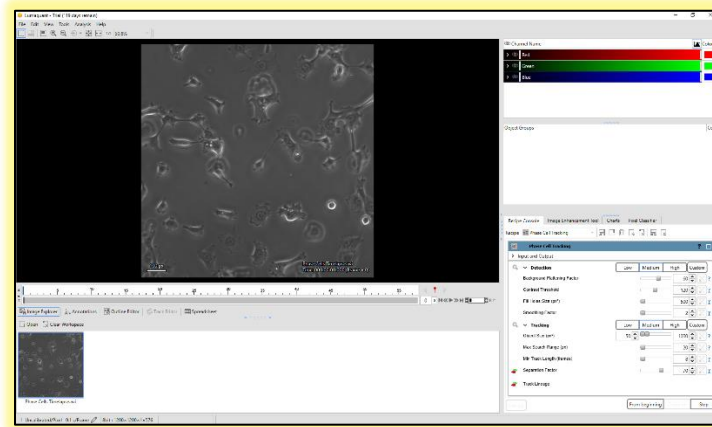


Fig 6. Main window showing video compiled of imported images.

12. Contrast and Brightness can be adjusted by clicking on the arrow to the left of the Eye icon. Enter numbers or slide the white tabs horizontally to alter the Look -up Table (LUT). The video is started and stopped by clicking on the gray/white “play” arrow to the right of the scroll bar. To increase or decrease the video speed, click the (+) and (-) buttons to the right of the “play” arrow. To set the video speed to the real time speed of the image capture, click the button labelled R to the right of the (+) and (-) buttons. To set the video to the maximum speed, click the button labelled M to the right of the (+) and (-) buttons and at the end.

13. To save the video, click File in the File menu bar. In the drop-down menu, select Export, then Movie. A dialog box will open asking if you want to save what is shown in the Image Viewer as a “snapshot”. Click No and the Windows dialog box Export Time Series will open. Enter the File name, choose the File type (AVI, MP4, QuickTime, or Windows Media Video), and click Save. Your video will be saved to your original image folder. The video playback speed will be the last playback speed selected in the program.

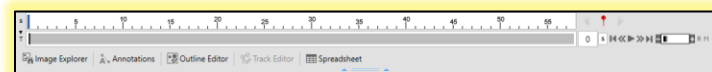


Fig 7. Zoom showing video controls.

Appendix A

X/Y Calibration Values for Objectives (um/pixel)

Magnification	Olympus	Meiji
1.25x	-	7.36
2x	4.12	-
2.5x	-	3.70
4x	2.08	2.31
10x	0.83	0.92
20x	0.42	0.46
40x	0.22	0.23
60x	0.14	0.15
100x	0.08	0.09