SP8 Basic Quick Guide

Turning on

1. Turn on the Leica Arc lamp (1) power supply.

2. Switch on the buttons in the following order: PC Microscope (2), Scanner Power (3), Laser Power (4). Then turn the Laser Emission Key on (5); the yellow light will come on.

3. Log on to LAS-X user account.

4. Start the **LAS X** program **(a)**.

5. Write down the start time of use in the logbook and enter the **FBS** system **(b)**.

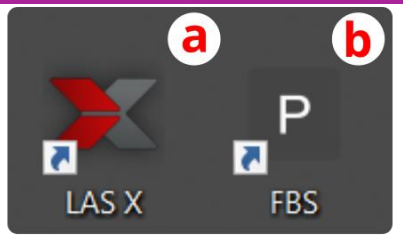
6. On the pop-up window the configuration should be set to **"machine.xlhw"** and "DMI8" for Microscope **(c)**.

7. The "Resonant", "STED" and "Activate AFC" should be turned on when needed **(d)**.

8. Click **OK** to start the program **(e)**.

9. When asked about initializing the xy stage, click **Yes (f)**.

10. Once initialization finishes, the image acquisition interface will open.



Setting up configuration

1. Before setting up the imaging condition, click **Configuration** tab **(a)**.

2. On the **Configuration window**, click IPS button **(b)**, click on **Load (c)** than on the folder window and choose **"select this settings.xml"**.

3. Click **Laser Config** button **(d)**. On the **Currently available Laser** panel, turn on the **405 Diode (e)** (if your sample has DAPI or 405 nm excitation dye) and **WLL** (**W**hite **L**ight **L**aser) **(f)**.

Obs.: Keep the **WLL** laser power at 70 % (default).

4. Click **Hardware** button **(g)**, on the **Hardware Settings** check the box **Live Average during Live Acquisition (h)** and select the **Bit Depth** from 8, 12 or 16 bits **(i)**.

Setting Initial Image Acquisition

1. To start imaging acquisition, click **Acquire** tab **(a)**.

2. Select the dimensions for the image to be acquired in the drop-down menu **(b)**. The default setting is the **xyz** scan mode.

3. Select the scanning format for image acquisition under **Format (c)**. This lets you define the number of pixels in, and thus the resolution of, an image. The default setting is **512 x 512**.

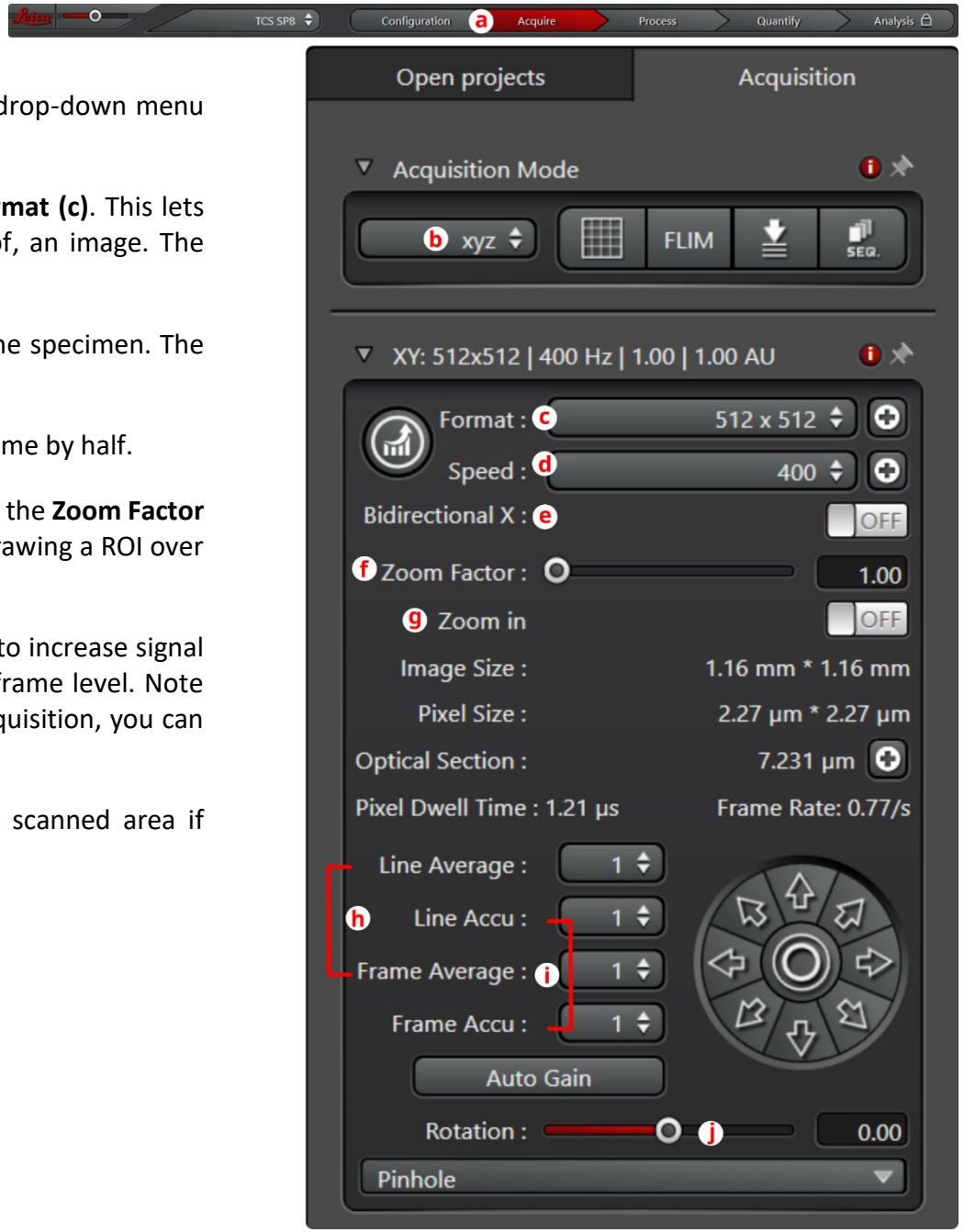
4. Under **Speed (d)**, define the speed the laser uses to scan the specimen. The default setting is 400 Hz.

5. **Bidirectional X (e)** allows you to decrease your acquisition time by half.

6. The field of view can be zoomed in or out by either adjusting the **Zoom Factor** slider **(f)**, or by selecting the **Zoom in** "On/Off" toggle **(g)** and drawing a ROI over the image area you wish to acquire.

7. Perform averaging **(h)** to reduce noise or accumulations **(i)** to increase signal in your images. These can be done on either the line level or frame level. Note you can only perform an average OR accumulation for each acquisition, you can for example do 2 line averages and 3 frame accumulations.

8. Use the **Rotation** slider **(j)** to better fit the image in the scanned area if necessary.



Setting initial acquisition

1. The **Laser Lines (a)** are activated by either clicking on the box corresponding to the relevant wavelength, or the "Plus" symbol for any of the WLL lines.
OBS.: Note you will need to drag the **WLL** lines to the relevant wavelength along the spectrum.

Use laser power the lowest possible value to avoid quickly bleaching the specimen.

2. The **Objectives (b)** lists currently available on the system and also shows the relevant immersion fluid information (eg water, glycerol, oil).

3. The **Dye Assistant (c)** can help you select the appropriate light paths and channel setup based solely on the dyes you have used in your experiment. If it's your first time with a particular imaging setup, the dye assistant is a great place to start.

4. In the **Emission spectrum (d)** the active detector gating is visible (and active lasers appear as vertical dotted lines).

5. The system is equipped with two **PMT (e)**, three **HyD (f)** and one **TLD (g)** detectors arranged from left to right **(arrows)** (PMT1, HyD2, PMT3, HyD4, HyD5 and TLD)
OBS.: **NEVER** place a laser line over a detector range**.**

The **HyDs** are gated detectors. Gating the detectors will remove autofluorescence and laser reflection/scattering.

6. Open the drop-down menu on the right edge of the detector bar will open a list of emission curves **(h)**. The select emission curve will appear in the spectrum. An excitation curve can be called up by right-clicking next to the emission curve in the spectrum.

Setting Initial Image Acquisition

1. To activate a laser line either click on the box corresponding to the relevant wavelength, or the “Plus” **(a)** symbol for any of the WLL lines. Note you will need to drag the WLL lines to the relevant wavelength along the spectrum.

2. To engage the laser the small tick box next to the wavelength indicator must be selected **(b)**. The suitable excitation wavelength is as close to the maximum of the excitation curve as possible.

3. To open the shutter on the relevant laser the small red circle must be selected **(c).** Note: when laser power is set above 0% and all shutters are open the light path will change from white to orange.

4. Adjust the laser line power by pushing the laser intensity slider upward **(d)** or by entering directly by double clicking the displayed intensity value in the entry window **(e)**. Set the laser power to the lowest possible value to avoid quickly bleaching the specimen.

5. To activate a detector, click the **OFF (f)** button. Ideally, choose the detector closer to the emission spectrum of your fluorophore. Remember a HyD is more sensitive so is suitable for dimmer dyes.

6. Set the detection range to the emission range of the specimen by holding the left mouse button and dragging the detection range under the emission curve **(green and red arrows)**. Alternatively, you can double-click the detection range to open an entry window in which you enter values directly **(g)**.

Setting Initial Image Acquisition

1. On the bottom left corner click **Live (a)** o start live imaging acquisition of your specimen.

2. Adjust laser power for each channel or sequence by sliding the laser power bars or by doble click to type the number. Ideally keep the laser power low around 2 to 5% not exceeding 10%.

3. To adjust the detector sensitivity **(gain/offset)**, turn the slider on the control panel for the gain value.

4. Set the look-up table (LUT) to Glow, click the **QLUT (b)** button in the upper left corner of the display window until the button appears.

5. Move through the specimen in the z-direction to find he position with the highest signal strength.

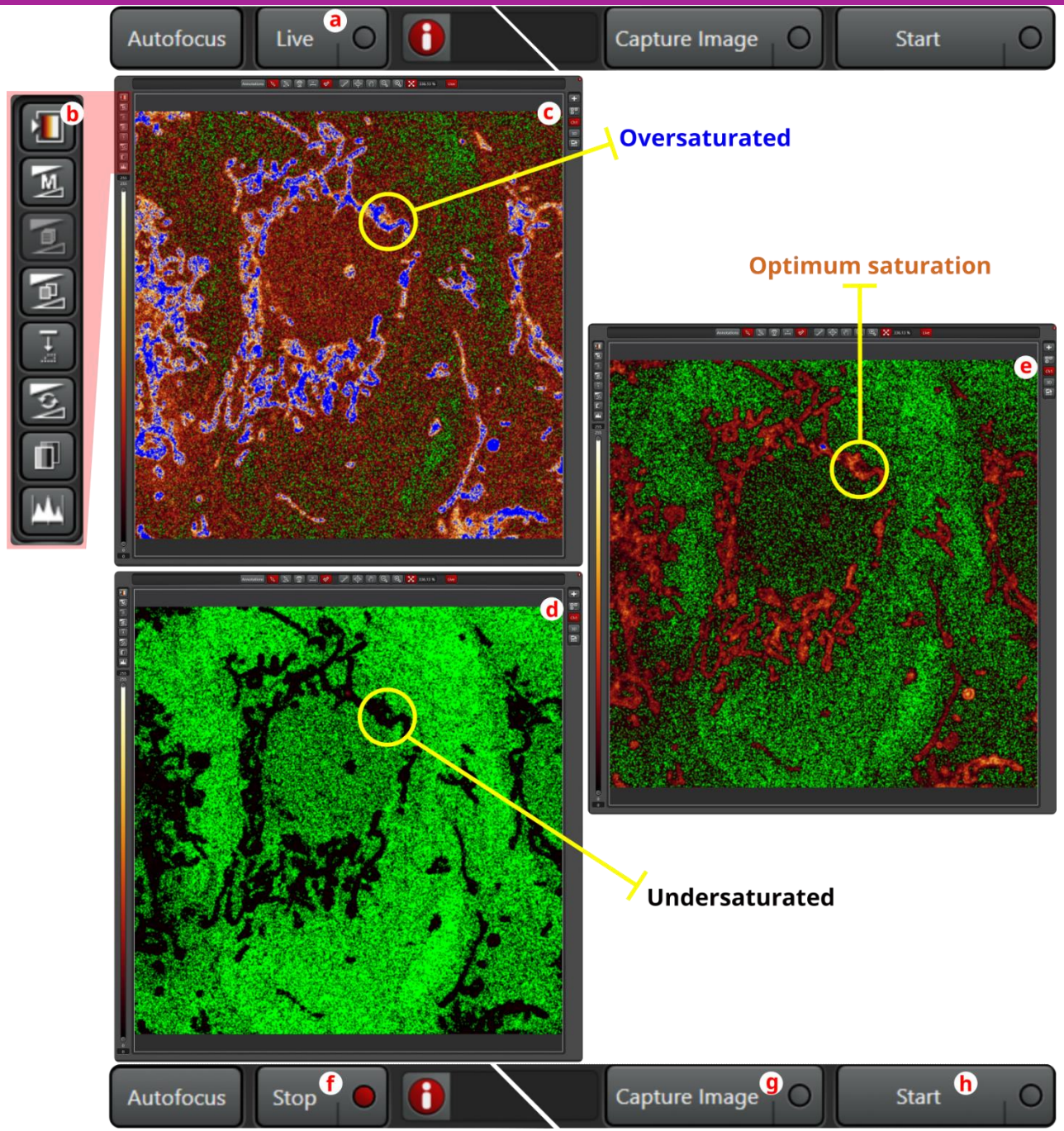
6. Adjust the gain so that the product with just a few blue and mostly orange is displayed **(e)**.

7. Adjust the background for the offset so that approximately half of it is displayed with green and black pixels **(e)**.

8. If necessary, correct the detection range.

9. Once the settings are optimized, finish the live acquisition by clicking **Stop (f)**.

10. To acquire an image click **Capture Image (g)** or **Start (h)** button.

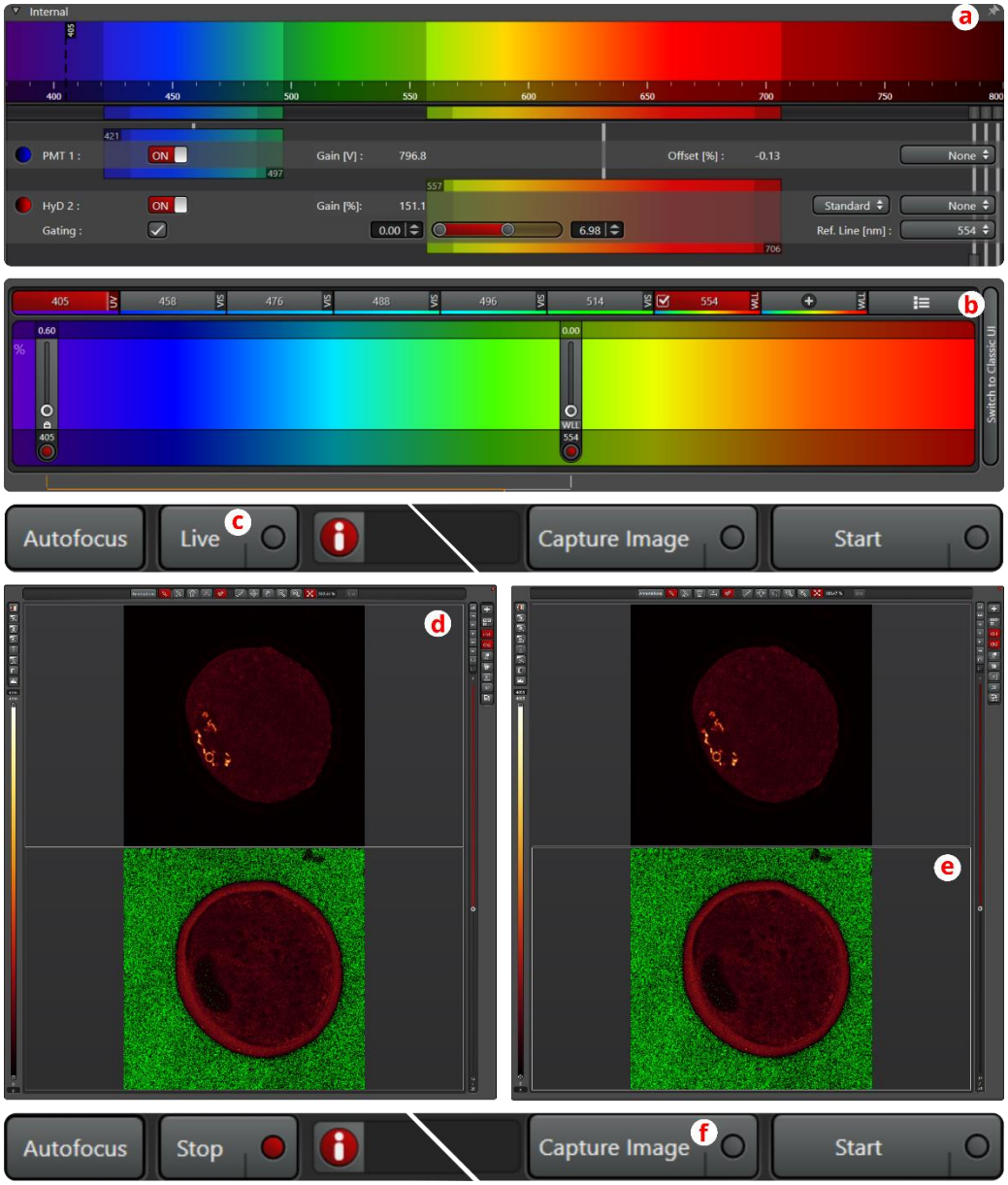


Creating a Multi-Channel Acquisition

1. Enable the detectors corresponding to the number of different fluorescent dyes in your specimen **(a)**.
2. Enable the laser lines you need and adjust the gain value of the detectors **(b)**.
3. Click on **Live (c)** to start live acquisition of your specimen.
4. Use the **Quick LUT** to set appropriate imaging parameters.
5. Select the desired channel in the display window by clicking the corresponding image. The image is now displayed with a white frame **(d**, **e)**.
6. Optimize the settings for the currently selected channel.
7. Proceed accordingly for each additional channel.
8. Click on the **Capture Image (f)** button to acquire the first image.
9. Remember, adjust laser power for each channel or sequence by sliding the laser power bars or by doble click to type the number. Ideally keep the laser power low around 2 to 5% not exceeding 10%.
10. Parameters like brightness, contrast and zooming can be adjusted quickly with the knobs on the desk.
11. Click on the channel that you want to change, depending on the type of the detector, the **Smart Gain** and/**or Smart Offset** will be activated (HyD detector allow **only Smart Gain**, and **Smart Offset** will be disabled).

OBS.: (1) Increasing the **Smart Gain** number will increase brightness of the channel. Decreasing the **Smart Offset** number will reduce the background without affecting the brightness of the specimen. If necessary, increase the laser power.

(2) For **HyD detector**, use **Smart Gain** to increase the brightness. If you sample shows high background, you can't use **Smart Offset** to decrease background because the **Smart Offset** is disabled. Instead, try to use Gating and keep the lower number to 0.30 (default). Gating can exclude background or glass reflection by excitation laser**.**



Multi-Channel Acquisition (Dye Assistant)

1. Click the **Dye Assistant** button **(a)**. On the pop-up panel, select dye and detector type one by one.

2. Click **"…" (b)** to choose the dye from the drop-down menu.

3. Click **PMT or HyD (d)** to choose the detector type.

4. Once you select more than two dyes, it will show imaging options, such as "None sequential", "Line sequential", etc. Choose the option with less crosstalk as possible. For that example, we are choosing "Line sequential 3 sequences" (e).

5. If you want, you can **edit (f)** to adjust the PMT or HyD positions by moving the slides for each particular channel **(h** and **i)**.

6. Click on **Apply (g)** once you are done if the settings.

Turning OFF

1. Take out you sample.
2. If using an immersion objective clean it.
3. Return to the 10x objective.
4. Save your files and exit LAS X.
5. Write down the time of use in the logbook (report any error/problem) and log out the FBS system.
6. Shut down the computer.
7. Switch off the main switches in the inverse order (from buttons 5 to 1)
8. Cover the microscope with the protective cover.