From Eye to Insight

Leica
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LAS X
for Confocal Systems

Release Notes LAS X 4.1.1

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Release documentation for LAS X 4.1.1

This document describes the 4.1.1 release of the Leica Application Suite X imaging and analysis software for advanced live cell research. You should read this document before installing a copy of this software.

All reasonable steps have been taken to ensure that this publication is correct and complete. Should any user be in doubt about any detail, clarification may be sought from Leica Microsystems CMS GmbH, or their accredited representatives. The information in this document is subject to change without notice and should not be construed, as a commitment by Leica Microsystems CMS GmbH. Leica Microsystems CMS GmbH accepts no responsibility for any errors that may appear in this document.

Date: October 23rd, 2020 applying to build number 23273

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1.1 Compatible Microscopes and Hardware Requirements

The LAS X 4.1.1 control software is a release for the STELLARIS confocal platform. Accordingly, the LAS X 4.1.1 control software is not compatible with any other microscope platform from Leica, i.e., SP8 confocal systems also cannot be upgraded from LAS X 3.5.7 with this release. Note that LAS X 4.1.1 supports DLS, FALCON and STED, but not any other advanced imaging modality of the STELLARIS platform (CARS, DIVE, FCS). All Leica-supplied CUDA workstations for the STELLARIS confocal platform meet the minimum PC requirements of the LAS X 4.1.1 control software.

1.2 Operating System Requirements

LAS X 4.1.1 is a genuine 64-bit program and runs on Windows 10 64bit. Since the end of the lifetime of Windows 7 in 2020, LAS X 4.1.1 does not support Windows 7 anymore.

2. Reason for this Release – LAS X 4.1.1

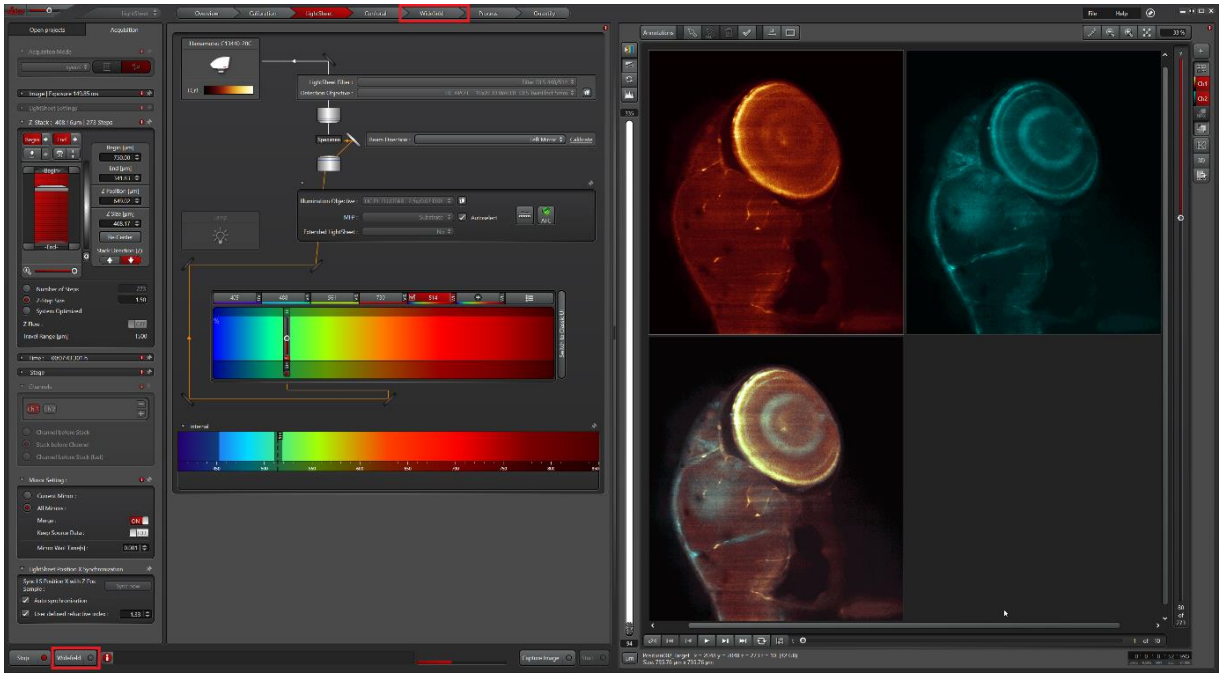
LAS X 4.1.1 is a release to address vulnerabilities in a 3rd party application (WIBU CodeMeter Runtime) shipped with LAS X for license management.

3. New functionality and features

In this Release Notes (**LAS X 4.1.1**), the following section about **New functionality and features** is identical to that of the previous Release Notes (**LAS X 4.1.0**). It informs the user **about the new functionality coming with LAS X 4.1.x**.

3.1 Support of the DLS modality

The LAS X 4.1.1 control software supports the **DLS** (Digitial LightSheet) module to extend the STELLARIS platform with a gentle and fast lightsheet imaging modality. DLS can be operated via an intuitive wizard (see figure **LightSheet wizard – LightSheet tab**).



LightSheet wizard – LightSheet tab

Two-color recording of a zebrafish embryo with DLS in the xymzt mode. The red box on the lower left indicates the **Widefield** button for switching quickly to the **Widefield** acquisition mode; the red box on the top indicates the **Widefield** tab.

With STELLARIS, we introduce several novel functionalities for DLS:

- We added a new **Widefield** mode for quickly navigating through the specimen. The **Widefield** mode can be used within the **LightSheet** tab or in the designated **Widefield** tab of the **LightSheet** wizard.
- There is now the possibility to generate the DLS lightsheet with the **resonant scanner**. Due to shorter pixel dwell times, using the resonant scanner will result in even more gentle imaging paradigms. Users can operate the galvo/resonant scanner switch in **Calibration** > **Optimization** > **Acquisition**. It is possible to change the scan mode within the current LAS X session.
- We added an **illumination wavelength-specific calibration** for the lightsheet to correct for chromatic aberrations. Users can do the base calibration in the **Calibration** tab and the **channel-specific** calibration in the **LightSheet** tab.
- Since data volumes can quickly grow large in DLS experiments, we would like to point out the novel **Compatibility mode for large experiments** for recordings of large data sets. This data mode allows for saving experiments **larger than** the caching folder (**DataContainer**).
- We fully integrated the **ImageCompass** into the **Confocal** tab of the LightSheet wizard, i.e., users can set up (high-res) confocal scans just like in the main STELLARIS interface.

A comprehensive description of how to operate the DLS wizard (including the above-mentioned features) can be found in the **Online-Help**.

3.2 Support of the STED modality

The LAS X 4.1.1 control software enables STED nanoscopy on STELLARIS STED and STELLARIS 8 STED. The **STED modality** is seamlessly integrated into the **ImageCompass** user interface: Users can intuitively define even complex imaging paradigms via **Drag&Drop** (see figure **ImageCompass STED – confocal/STED comparison** and **ImageCompass STED – Drag&Drop**).

ImageCompass STED – confocal/STED

The **STED modality** is seamlessly integrated into the **ImageCompass**: The unified interface allows for setting up **confocal/STED comparisons** (upper and lower setting) as well as **multi-color STED experiments** (not shown) with ease.

ImageCompass STED – Drag&Drop

When choosing a **STED-able dye**, users can choose between the confocal (**C**) and STED (**S**) modality by clicking on the respective **small flag** (a), and subsequently **Drag&Drop** the dye (b) into a setting. The ImageCompass will **configure the hardware automatically** for STED dyes (i.e., excitation wavelength from the white light laser (c), STED laser line (d), and emission range of the detector), so that the user only needs **to fine-tune some Beam Path settings** (such as the laser power of the white light laser and STED laser, or the STED 3D parameter (e)). STED is compatible with **all members** from the Power HyD family (here, a **HyD S** detector is used).

STED supports our novel TauSense technology: Simply choose a mode from the **TauModes** drop-down menu (see also figure **ImageCompass STED – confocal/STED** for the TauModes menu in the upper setting). The various **TauSense** modes allow for powerful, digital gating to **increase STED performance** (**GateScan**, **TauGating**) and **exploit life-time based information** (**TauContrast**, **TauScan**, **TauSeparation**).

Furthermore, on **STELLARIS 8 STED FALCON**, **τ-STED** is available. τ-STED uses **lifetime information** in combination with **phasor analysis** to increase the resolution and eliminate uncorrelated background in an automatic manner. Since **much less** excitation and STED **laser power** are necessary, τ-STED can even **enable gentle live-cell imaging** with **cutting-edge resolution** and **high image quality**.

A comprehensive description of how to operate the STED modality (including Auto and Expert Beam Alignment, TauSense and τ-STED) can be found in the **Online-Help**.

3.3 Miscellaneous novel features

FRET wizards

The **FRET AB** (**A**cceptor **B**leaching) and the **FRET SE** (**S**ensitized **E**mission) wizards are now available with the **MicroLab** license. The first step of both wizards (**Overview**) provides a guideline on how to perform the respective **FRET workflow**: From **Setup** (using the ImageCompass) to **Evaluation** (generating insights about the FRET efficiency).

FLIM zip

For **STELLARIS 8 FALCON**, a new **FLIM data format** is now available: **FLIM zip**. FLIM zip works both **online and offline**. It optimizes the **file size** for **faster analysis performance** and **improved data storage** (up to **8 times smaller file size**). FLIM zip can be activated with a **right-click on an acquired image** (once activated all the images will be acquired as FLIM zip) or in the **Tool menu**. With the latter option, it is possible to optimize **already acquired FALCON data** with FLIM zip (i.e., data acquired on LAS X 4.0.2 or lower), however, FLIM zipped data **can only be handled with LAS X 4.1.0 or higher**.

TauSense improvements

TauSense describes several acquisition tools which use pulsed laser excitation and photon counting detectors to obtain fluorescence lifetime-based information for image formation. With LAS X 4.1.1, TauSense modes are further integrated into LAS X. Now, stage applications (such as the **Navigator**) can use **TauScan** and **TauSeparation**. Furthermore, we have refined the **metadata handling** of the **TauSense** technology (improved channel labeling and image properties).

4. Recommendations

For an optimal performance and the safety of the product, we strongly recommend the installation of/upgrade to LAS X 4.1.1 for systems of the STELLARIS confocal platform!

5. Open Issues / Restrictions

The following list enumerates the known, unresolved anomalies of LAS X 4.1.1:

- Bleach point not available in the core software

  Workaround: The **bleach point** functionality allows for applying **a high laser intensity to small defined regions** within the specimen so that FRAP or photo-conversion experiments can be performed. Since the **bleach point** functionality in the **core software** will only be implemented in an upcoming LAS X release, for the time being, there are **two mitigation strategies**:

  - If the **temporal domain** is **not critical** (e.g., photo-conversion of fluorescent proteins for long-term cell tracking), a simple solution is using **small ROIs** to manually zoom-in and apply high laser intensity.
  - The **FRAP wizard** contains the **bleach point** functionality. For more **complex** experimental conditions in which the **temporal domain matters**, **get in touch** with us so that we can assist you with a **license for MicroLab**.

- No frame accumulation with TauContrast imaging

  Workaround: Enabling **frame accumulation** results in images with an **improved signal-to-noise ratio** (SNR). Since frame accumulation will only become available for **TauContrast** imaging with an upcoming LAS X release, an **immediate mitigation strategy** is the use of **line accumulation** in TauContrast imaging.

Indeed, line accumulation even has the advantage of being **better suited for fast imaging** since it **minimizes the interval** between the iterative recording of the same pixel. However, consider that line accumulation will allow for **less relaxation of fluorescent dyes/proteins** between repetitive excitation cycles, and thus. a **more cautious approach** regarding the **photon budget** should be considered.

- No Dynamic Signal Enhancement with TauSeparation

Workaround: **Dynamic Signal Enhancement (DSE)** is an image filter for noise reduction in time series experiments. It is particularly useful for fast life-cell imaging with the resonant scanner, as it reduces noise without sacrificing much of the temporal resolution. Since **DSE** will only become available for **TauSeparation** imaging with an upcoming LAS X release, **line accumulation** can be used for noise reduction as a temporary mitigation strategy. Since **line accumulation** does decrease the temporal resolution (proportional to the number of times a line is accumulated), several measures and/or a combination thereof should be considered for minimizing the acquisition time of individual image frames. These measures include: Minimize line accumulation by "**hardware means**" (high-NA objective, appropriate detector, higher excitation power), minimize line accumulation by "**lower resolution**" (larger pinhole, smaller image format), use of the **resonant scanner** (shorter pixel dwell times), and a smaller **field of view** (crop along y- and/or x-axis).

- Unused disk memory blocks new experiment

Workaround: If **unused disk space** is not freed after an experiment is executed, the user should **close and reopen the project**.

- LAS X freezes/unresponsive

Workaround: If software performance **slows down or even freezes** due to high and prolonged workload, the user should **restart the LAS X (software) and scanner (hardware)**. Closing of the LAS X session may require using the Windows Task Manager.

- Frequency of the White Light Laser (WLL) wrongly displayed

Workaround: The WLL frequency in the **GUI** is not **synchronized correctly with the hardware control** on LAS X **start**. Therefore, the user **must once change the frequency of the WLL** to correctly sync the GUI and the hardware control. This will solve the issue for the **remaining LAS X session**.

- Optimize format not working correctly for STED

Workaround: The format optimization **does not work correctly for STED** and thus the user should set the **format** (pixel size) **manually**.

- Selection of different detector modes in STELLARIS 8 FALCON for HyD S possible

When FLIM is started, the **appropriate detector mode** (**'counting'**) is **automatically activated on HyD S**. Nevertheless, users are not prevented from actively choosing an inappropriate detector mode (**'analog'** or **'reflection'**) on HyD S while FLIM is on.

- Average Arrival Time (AAT) changed when LIGHTNING applied to an image with TauSense

Workaround: Since **AAT data** is changed in **LIGHTNING**, the user must use the **raw data** (i.e., not deconvolved) for analysis of the AAT channel.

- Saving with .xlef/.lof in FALCON

Workaround: Due to the newly introduced **FLIM zip**, **FALCON data** can currently only be saved reliably as **LIF file**. **LIF** is now the **default format** for saving in LAS X.

- FRET efficiency image does not match the statistic

Calculation of the **FRET efficiency statistics** works **correctly** while the **FRET efficiency image** may not be displayed correctly and thus **should not be used**.

- Only single histogram is generated when using TauSeparation in combination with multiple settings and line sequential

Workaround: When using **TauSeparation with multiple settings and line sequential**, only a **single** (identical) **histogram** is generated for all settings. Nevertheless, the generated **image data** is **correct** and can be used for further analysis. If the **histogram data** is important, the user must choose **frame sequential mode** for TauSeparation with multiple settings.

- Galvo switch (from resonant to FOV scanner) should not be used in the LIGHTNING wizard

Workaround: When using the **Galvo switch** (from resonant to FOV scanner) in the **LIGHTNING wizard**, the software creates an error. Accordingly, the user must not operate the Galvo switch inside the LIGHTNING wizard. If the user accidently operated the Galvo switch, the user **must close the LIGHTNING wizard** and set up reasonable scan parameters (**Speed**) for the FOV scanner in the main STELLARIS interface before **returning to the LIGTNING wizard**.

- FRAP wizard: “Overview” tab button malfunction

Workaround: **“Overview” tab in FRAP wizard must not be clicked** since it causes a scan loop. The **information** from the “Overview” tab can be found in the **Online-Help**. If the “Overview” tab is accidently clicked, the user can terminate the initiated scan by clicking on the **”Close X”** at the top right of the LAS X and then **returning to the Setup tab**.

6. Solved Issues / Restrictions

Amongst other bugfixes, we have resolved the following issues/restrictions from the LAS X 4.0.2 release:

- FRET wizards not available
- TauSeparation/TauScan not compatible with Navigator and TileScan
- TauScan values not fully visible in image properties
- GUI elements not consistently explained via Mouse over
- LDM lacks Trigger functionality
- Save progress bar does not disappear
- Data Export of TauContrast images does not work (e.g. as .tiff)
- STELLARIS systems without Tandem Scanner have Galvo switch icon in GUI

7. Compatible 3rd party software

LAS X 4.1.1 does not support the integration of any 3rd party software packages, i.e., neither SVI's Huygens Suite nor PicoQuant's SymPhoTime. The functionality of these packages is covered by the **LAS X LIGHTNING** and **LAS X FLIM** packages.