Release Notes for LAS AF version 1.8.0

1. General Information

A new structure of the online help is being implemented. The focus is on the description of the dialogs of the LAS AF. Configuration and Acquire is finished, Process and Quantify is still in the old description form. Additional background information to the different parts in LAS AF including Wizards will follow.

2. How to access LAS AF 1.8.0

The software can be downloaded from our ftp server using the following address:

ftp://ftp.llt.de/
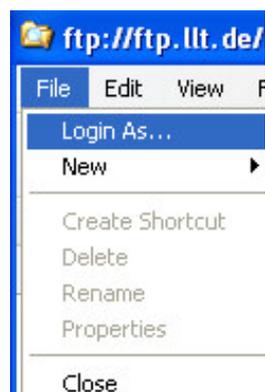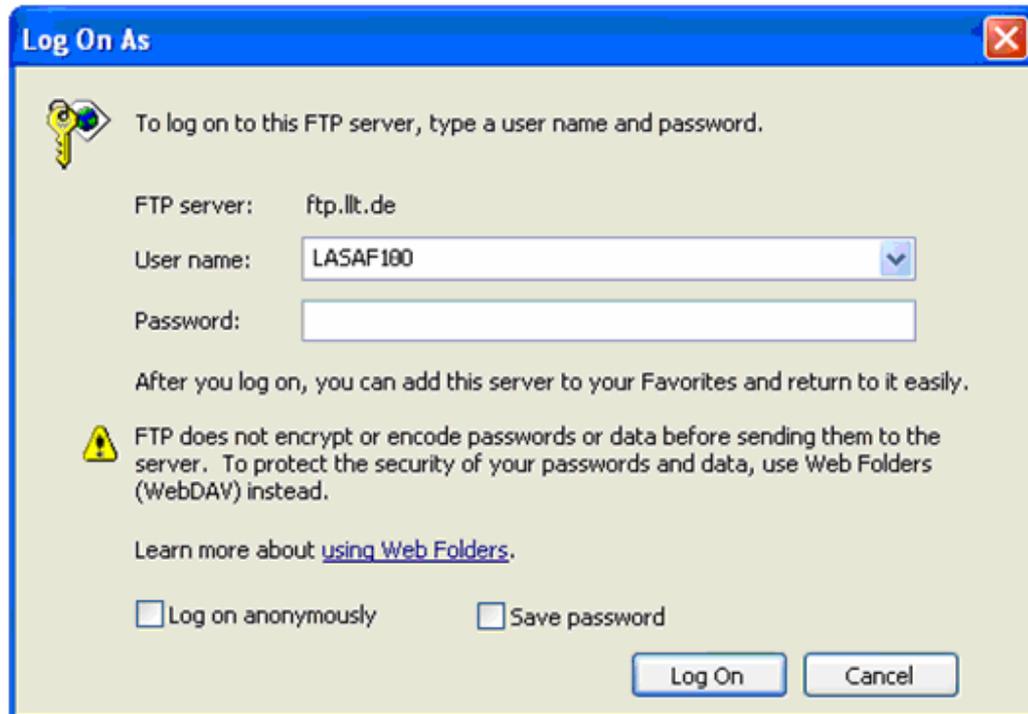
3. Features of AF6000 and AF6000 LX

Leica DFC 360 FX camera

AF6000 and AF6000 LX support the new DFC360 FX camera. In combination with the high speed sequencer board in CTR7000 this new fire-wire camera allows high speed real time acquisition even with different exposure time/channel. In overlapping mode 20fps full frame and more than 100 fps with binning is reached. For such fast data transfer FireWire IEEE 1394b is supported. A High Speed FireWire IEEE 1394b kit with PCI card and cable can be ordered in addition to the camera.

- Full frame pixel resolution: 1392x1040

- Pixel size 6.45x6.45µm

- Gain 1 to 10

- Cooling: active Peltier -20°C to ambient

Hamamatsu 9100-13

AF6000 and AF6000 LX support the Hamamatsu 9100-13 EMCCD camera. Camera features: Hamamatsu 9100-13 EMCCD camera including frame grabber and trigger cable. Back-thinned frame transfer CCD. Maximum quantum efficiency > 90%. 512x512 pixel. Pixel size 16x16µm. 11 MHz maximum pixel clock rate. Maximum EM CCD gain = 1200. Minimum cooling temperature -65°C (air cooled). 16 bit A/D converter. Back illuminated.

Camera orientation:

On all camera ports the camera needs to be mounted in a way that the black metal plate faces the user.

Export of Ratio and FRET images

Export of Ratio and FRET movies is possible now by activating the button "Export Views" when generating avi movies. This export tool works both for the Ca-imaging tool within quantify and also for the FRET wizard. The images will be exported as they appear on screen. In case the scaling is changed within the Ca-imaging tool or the FRET wizard this scaling will also be applied to the exported movie.
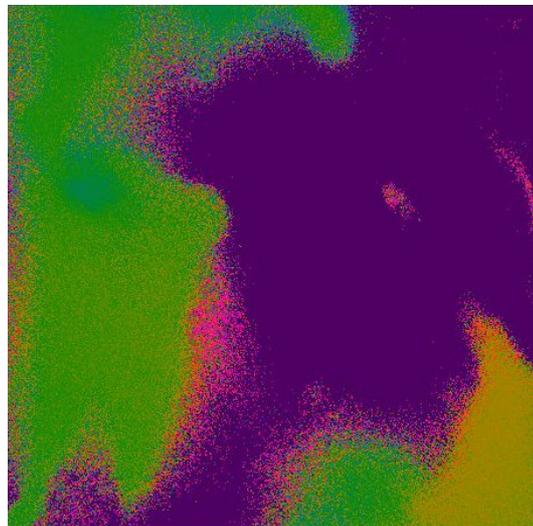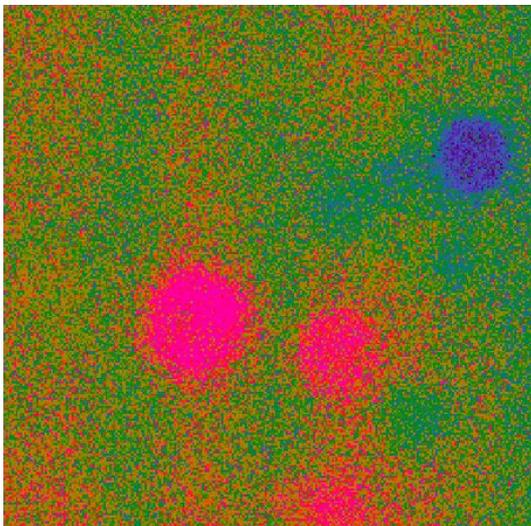
Screenshot of the respective settings for AVI-export

Multi Well Plate Screening (Well plate Screening license necessary)

A Well Plate Screening Wizard can be activated in the pull down menu of AF6000 LX. Please be aware that a well plate screening license is necessary to activate this application.

The well plate screening works for 6, 12, 24 and 96 multi well plates. It can be combined with a tile scan in each well. For Z alignment a best focus function is implemented to work in both transmitted light and incident light.

***Activating Well Plate Screening in the LAS AF pull down menu:***

Step 1: Define Pattern

The type of well plate can be selected in the pattern settings. Due to the 96 well plate dimensions for some high mag. objectives it is not possible to use the full 96 wells since the objective touches the well plate frame. In this case please use the "Greiner-Multiwell-96-60 Wells format instead of the "MultiWellPlate96 format". This format is not using the outer well plates columns and rows.
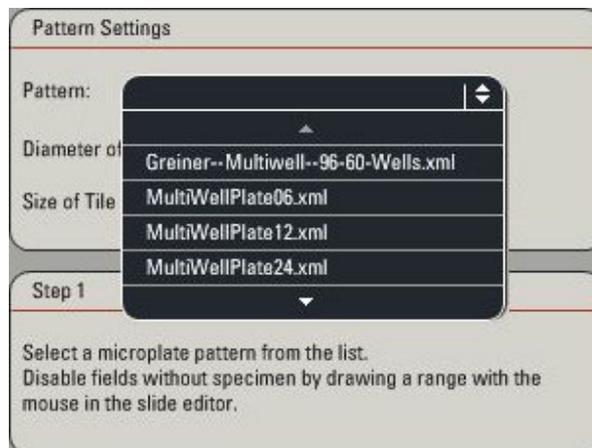
Fields without specimen can be disabled by drawing a range with the mouse in the slide editor.

In case the AF6000 LX is equipped with a motorized 3-plate stage instead of a scanning stage the YFlip format has to be used.
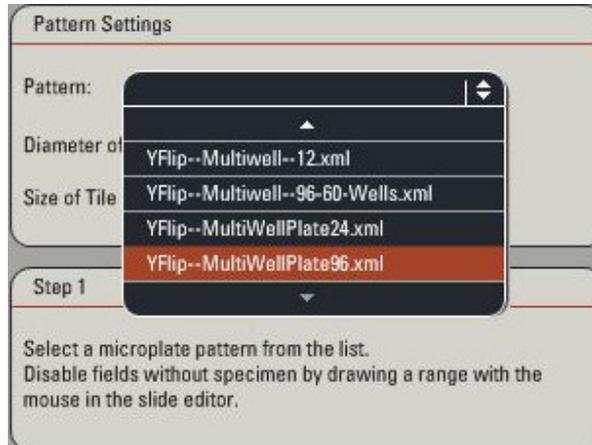

In the same interface a tile scan size can be defined. Please type in the diameter of interest. The resulting Tile Scan size will be displayed accordingly. Please note that merged tile scan images should not exceed the maximum image size supported by LAS AF. In case the merged image is too large a message will appear in the merging step 5. In this case binning might be used, the tile scan size could be reduced or a lower mag. objective could be selected.

Step 2: Well Plate alignment in X, Y and Z

The user moves the well plate to the fields marked by the wizard and presses the "Read Position" button. This is done four times for each corner of the multi well plate.
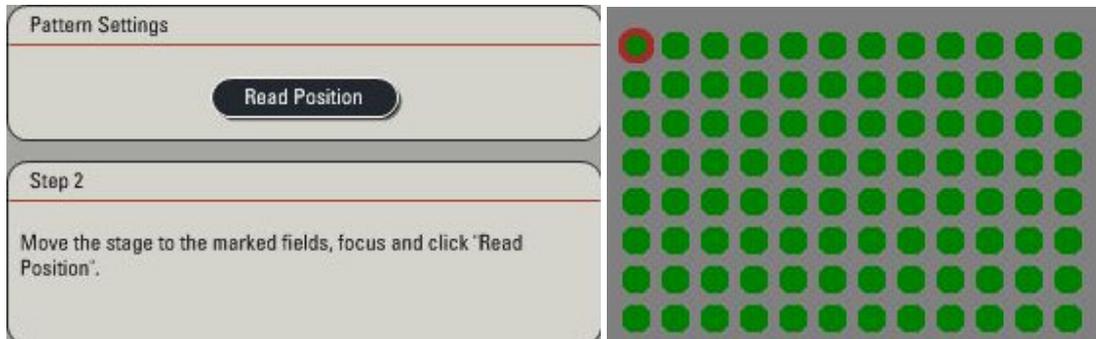
Step 3: Focus Map

Definition of wells used for the best focus detection by defining an increment of positions in X and Y. It is recommended to run a best focus in each well. In case increments are defined the missing positions will be interpolated.

The best focus algorithm uses the contrasting method of the first channel defined in the acquisition settings. The autofocus range needs to be defined by the user. The autofocus range depends on the depth of focus of the objective used. For a 20x objectives e.g. an autofocus range of 200µm works fine. Start best focus detection by pressing the "Record" button. The best focus detection only is done once before the start of the experiment but not during the time lapse screening experiment.
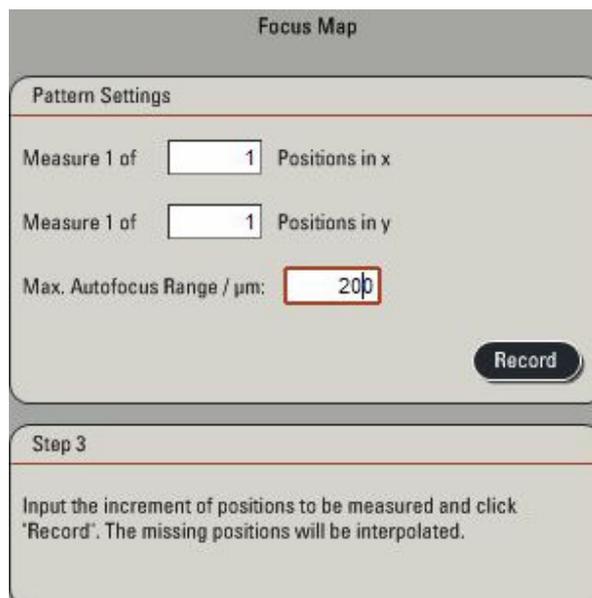
Step 4: Acquisition

In this step a screening time lapse experiment can be defined using the time interface. The screening experiment can also be combined with Z stacks and different channels, e.g. a TL channel and a fluo channel. The tile scan interface is deactivated since the tile scan size is already defined in Well Plate Screening step 1.

Step 5: Post-Process

In this step the tile scan images can be merged. A flip in X, in Y or an XY swap can be selected if necessary.


Further Software Improvements:

AF6000 E dongle:

The AF6000 E dongle is working properly in LAS AF 1.8.0. The process tools available in AF6000 E are all active again.

Known Limitations of Hard- and Software:

Update Microscope Firmware failed

Installing LAS AF 1.8.0. After installation in some cases a message appears that firmware installation failed. However, the firmware is correctly installed.
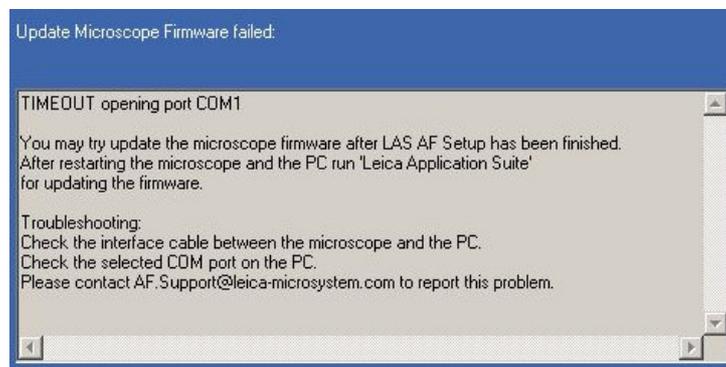
Andor iXon 897 acquisition limit

The Andor iXon 897 has a hardware limitation. It allows only a certain sequence size to be captured. In full frame operation a maximum of 2000 images can be captured for example.

> *Workaround*: Using Live Data Mode and defining jobs each smaller than the maximum sequence size.

Hamamatsu 9100-13 blooming effect

In cases Hamamatsu 9100-13 is running close to saturation a blooming effect appears. This is a hardware limitation of the camera.
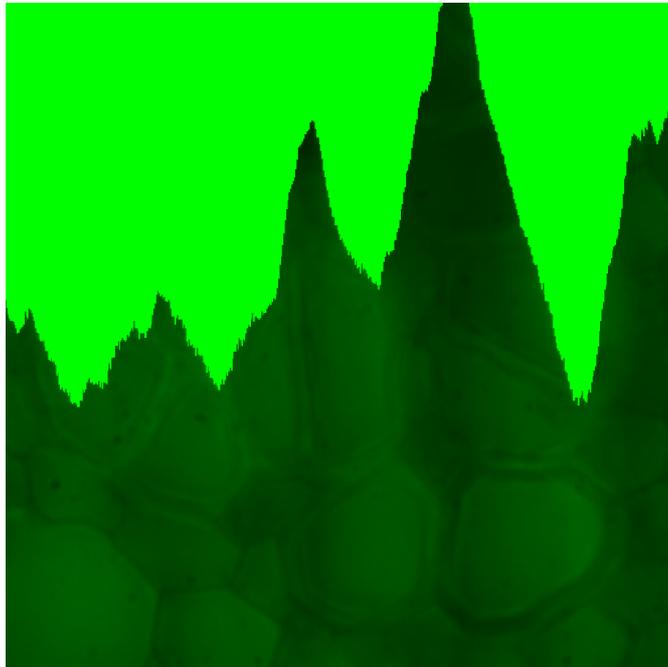
Workaround: choosing acquisition parameters below image saturation.

4. Features for Confocal Instruments

Our Top Features for this Release

- For this release we put **special emphasis on electrophysiology** to contribute to our new fixed stage system, the DM 6000 CFS.

- Adding to this, in the traditional confocal instrumentation the **functionality of well-plate-screening** offers a large range of applications from having a sound overview over the fluorescing sample. This package is available optional.

- Switching without any changes in the software environments is now possible **between digital camera and confocal imaging.** This package is also available as an option.

Adding to this...

- **Topology-Projection:**
  This function is now available: A topographic projection of surfaces in fluorescence and when reflective. As an example, the cell itself can be imaged as a topographic image, indicating the three-dimensional surface of the specimen.

- **For the SPE:**
  Bi-Directional scanning nearly doubles the scanning speed of our documentation system. Utilizing the mouse-wheel, the **phase of the scanning can be fine-adjusted** to avoid any fringes in the resulting image.

- **For the SPE:**
  Auto-Gain for the PMT-voltage. The voltage of the photomultiplier will be determined automatically to deliver crisp images.

- **Line-Accumulation** for the SP5 platform: In the case of dark sample (low fluorescence) the acquired images will be accumulated by line.

- **Package QUANTIFY: Line Quantification Tool**.
  If, for example, the distance of nuclei or, in general, a structure, has to be measured and this is achieved by a **line quantification** within a more noisy image, it is now possible to adapt the line-width to a value that allows averaging inside the line boundaries and thus lowers the efficient noise level in the resulting graph.

- **Visualization-Mode: Movie-Presentation is enhanced.**
  When insight in dynamic processes it is required sometimes a movie has to be played constantly forth and back. This is now possible at **different speed settings** and fine adjustable with the "+" and "-" keys of the numeric block.

- **Package FRET AB: Formula now visible.**
  In the screen of this package it is now disclosed how the calculations are accomplished.

- **Package Live Data Mode: Load Jobs.**
  Within the package it is now possible to **save and load jobs** and settings for individually compiled experiments. If, for example, in the case of **reproducible experiments** it is required to have fast access to repeating jobs, those can now be easily stored and retrieved.

- **Improved online-image presentation in live imaging.**
  For this mode, we improved the presentation for speed and smoothness of the imaging to deliver an outstanding, crisp and settled image for enhanced insight.

- **4D-Series exceeding 400 MB per file:**
  Image-processing tools are now enabled for very large four-dimensional data sets, such as cropping or dye separations.