

Advanced Fluorescence Marketing News Release Notes Confocal and Widefield

No. 181

20.10.2008

Release of LAS AF 2.0 – NOT for LSI and Stereo products

Dear colleagues,

As of today we release LAS AF version 2.0.

This release is intended to differ in terms of quality and stability in comparison to the releases before. The whole last period between 1.8.2 and 2.0 we've been working in improving the stability. A lot of beta-testing was performed, in-house and with beta-testing customers. Many issues have been solved, many improvements have been implemented.

We believe, this is the most stable release ever.

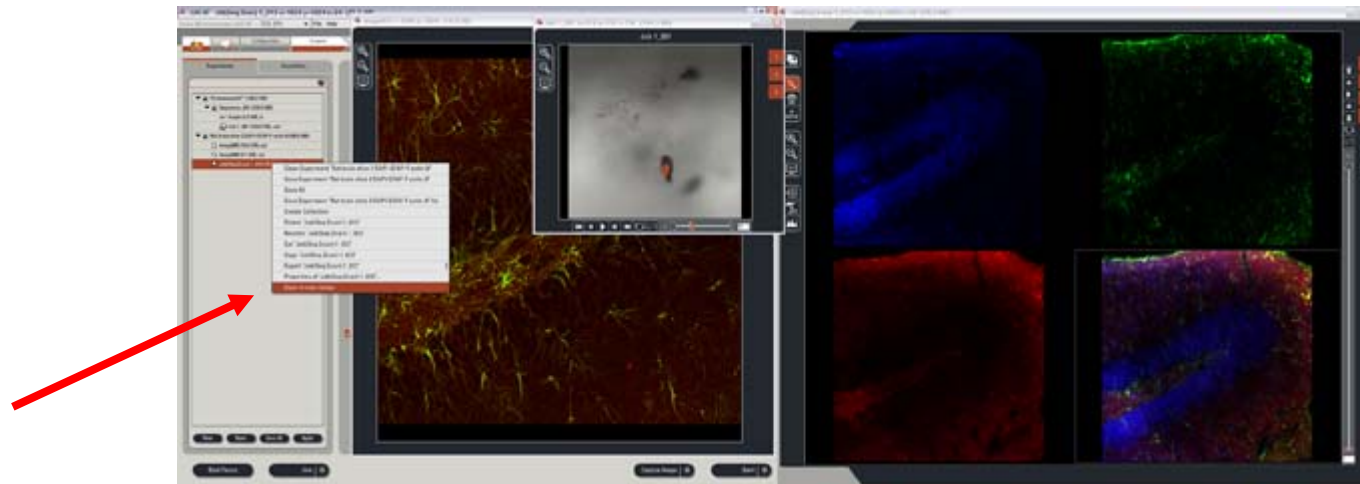
At the following pages you find the features for confocal and widefield imaging and a guide how to download the software. The password is as usual distributed in a different way (separate email.)

As indicated, the current release is not intended to be put on the TCS LSI and the stereo products.

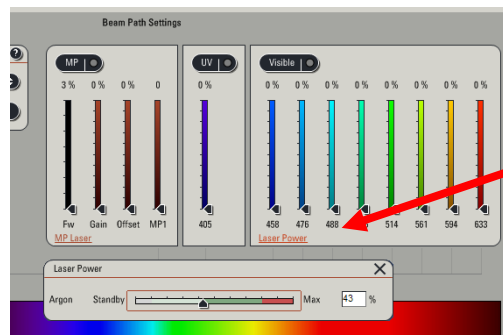
New and improved features for confocal:

INSTRUMENT

SPE	SP5	Feature	Advantage and discription	Where is it?
x	x	Additional viewers	During the current acquisition it is now possible to visit previous series or images.	In the experiment tree use the left mouse and select experiment of interest Then open in new viewer (last entry of pull-down-menu) with right mouse click.



x	Access to Argon laser power within the wizards.	Time saving when working in the wizard.	Access via link (laser power) in the AOTF window.
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x	Display of optimal power range for the Argon laser.	Finding of the optimal settings to save the laser lifetime.	Automatic in Argon power control. To avoid laser noise (dark grey, in the left) and to avoid shortening the lifetime of the laser when running it permanently at high power levels (red region on the right.)
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x	x	Quantify chart has now a magnification glass.	Gather details more easy using the zoom with this glass.	Access via mouse scroll wheel. Put cursor into respective graph region and use the wheel to zoom in and out.
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	FRAP	Improvement of the autofit function.	Autofit works much more reliable now.	Build-in
	MP FRAP	FRAP wizard MP	Sequence <i>prebleach VIS</i> → <i>bleach MP</i> → <i>postbleach VIS</i> is implemented.	Adjust the MP1 settings during the definition of pre & postbleach to zero.
	FRAP	Completion of image properties in the FRAP wizard.	Now prebleach and postbleach settings are saved within the properties.	Right mouse click in the experiment tree. Select the FRAP series. Right-mouse click and select properties of FRAP series. You find the <i>bleach</i> and <i>pre-post-bleach settings</i> documented.
	MP	Completion of display of the image properties.	EOM settings (gain & offset) and filterwheel settings.	Right mouse click on the experiment tree → properties → see under <i>hardware settings</i> .
x	x	Completion of the image properties by adding the system number.	Better overview on which system the image is taken in imaging facilities.	Right mouse click on the experiment tree → properties → see under <i>hardware settings</i> .
x	x	Snapshot: Automatic addition of the prefix to the filename	Better allocation of the snapshots after data acquisition.	Build-in

x

x

AVI export: Coordinates with adjustable colored background.

Ensure visibility of the superimposed data.

Configure during AVI export when adding *Quick Annotations*.



x

New start-up message of the control panel.

Instead of *September 2004* it is now *Leica Microsystems*.

RS

Introduction of a zoom stabilizer in series and LDM.

When the zoom was set in the last job there is chance that the initial images in the current job are distorted by that zoom settings – this is now corrected

Build-in

x

LDM
EP

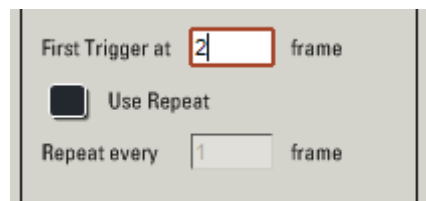
Indication of the current job by color.

The progress of the experiment is now visible.

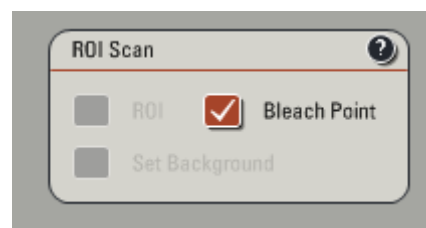
Currently running job is now indicated in mint-green, For viewing the settings click on the job. The job showing the settings is marked by a brownish frame.



LDM EP	Trigger-IN with delay (number of frames.)	Important for data acquisition before the trigger impulse is set.	In the trigger dialogue, first column, "First trigger at frame".
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x	x	Easier zoom-in	Without extra clicking on the ROI-button zoom-in is possible.	Build-in
	x	Camera switch	Use the camera inside LAS AF without re-booting the instrument.	Build-in
	x	Series with the ROI spectrometer	Acquisition of series with the ROI spectrometer is now possible, not only single images or continuous imaging.	Build-in
	x	New diagnosis board (hardware related.)	Support for this new diag-board in the software allows stable upgrades and patches by the user. Here especially the firmware is crucial to upgrade which is provided by this diag-board-upgrade.	Build-in
	x	Log-file-entries	Improved system diagnostics	Build-in
	MP	Sequential scanning with different wavelengths.	In the sequential mode (frame, stack) it is now possible to get different IR wavelengths for each sequence.	Sequential scanning in the acquisition window: Define for each scan a different wavelength.
	MP	Beam-parking with IR laser	It is now possible to do beamparking, photoactivation, bleaching, etc. and define different ROIs.	In the beam path window, in ROI scan check <i>Bleach Point</i> .



x	x	Export TIF without LUT	Without the lookup-table our TIF images can be imported better into third-party-programms.	In the TIF export dialog.
	x	Flip x	Flip the x axis of the scanner.	Configuration ► Settings

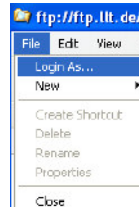
How to download the new software?

Please note, the password is sent in a separate email.

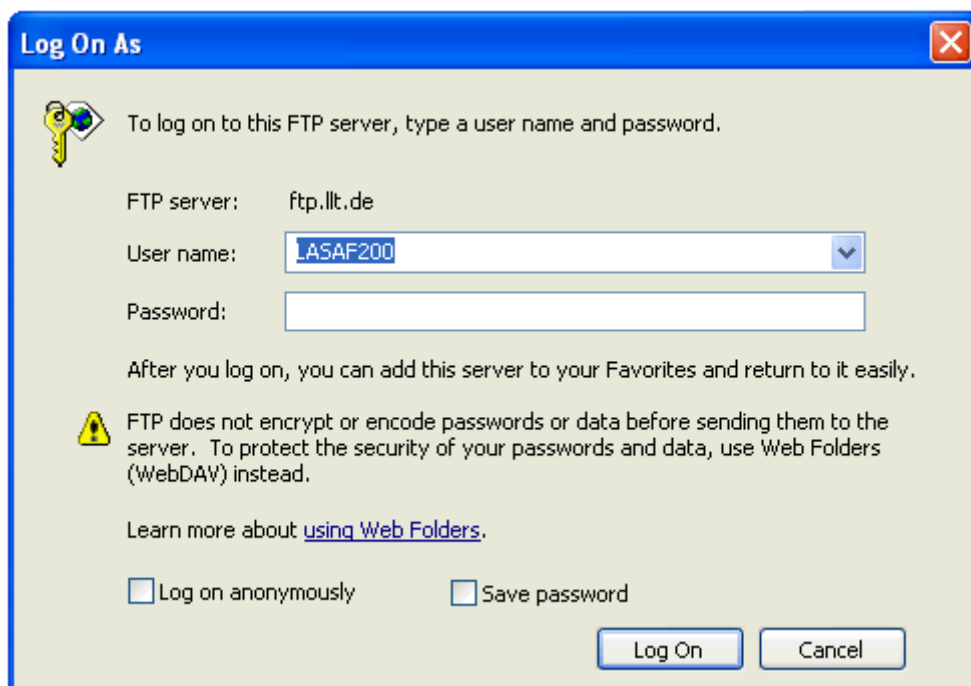
Important information for download of LAS AF version 2.0:

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

ftp://ftp.llt.de



- 2) Log on as LASAF200,



- 3) Then download the software.

Leica Application Suite Advanced Fluorescence V 2.0 Release Note for Widefield



These release notes include:-

- Information on new features for AF systems, Leica MC TIRF and Leica Stereo systems
 - Details of remaining bugs
 - Firmware and software prerequisites
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Overview

LAS AF V 2.0 introduces the following new systems and features:-

- LAS AF Digital Autofocus feature for use with CTR 6500 HS and CTR 7000
- Black pixel correction

Firmware and Software Prerequisites

For details of the required firmware for this version of LAS AF, please refer to the "Application Software & Microscope Firmware Document" available from Leica Microsystems GmbH.

For firmware version of the Leica M 205FA, please contact Leica Microsystems GmbH. Leica Application Suite V3.1 is installed as part of the LAS AF V 2.0 installation.

Installing Leica Application Suite on the same PC as LAS AF

If you already have LAS software installed on your PC, the LAS AF install process will remove this version. The LAS AF install installs only core components of the LAS software and does not install application modules or DFCTwain.

If you wish to run the full version of LAS on the PC, with access to DFCTwain and the LAS Modules, you should first install LAS AF and then install LAS.

Leica Application Suite Advanced Fluorescence V 2.0 Release Note for Widefield

AF Systems

New Features:

High Speed Digital Autofocus

Prerequisite Hardware


Systems that have a Leica CTR MIC 6500 HS or 7000
and

A triggered camera:

DFC350 FX, DFC360 FX

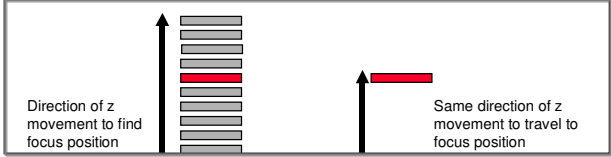
Andor 885 / 897

Hamamatsu 9100-02 / 13

Living up to Life

Benefits of the new High Speed digital Autofocus?

- No hysteresis effect due to unidirectional movement of the z focus.
- High flexibility in applying the auto focus search function within experiments
- High speed experiments applicable
- No storage of images taken to find the the focus position
- Channel for autofocus can be freely defined



Direction of z movement to find focus position

Same direction of z movement to travel to focus position

3 - Quick Start: High Speed Digital Autofocus

Leica Application Suite Advanced Fluorescence V 2.0 Release Note for Widefield


Getting Started....

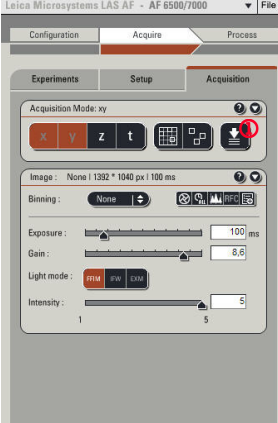
Leica
MICROSYSTEMS

Living up to Life

Getting started

- Define the channels for your experiment as you do routinely
- Click on autofocus icon ①

 You do not need to activate the high speed mode to get access to the High Speed Digital Autofocus!



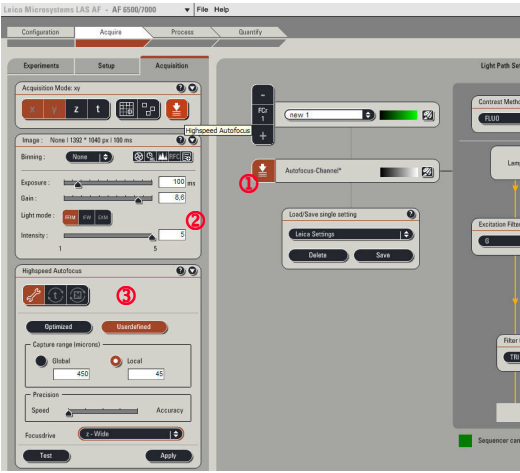
1 - Quick Start: High Speed Digital Autofocus

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Basic adjustments

- The last defined channel is copied and displayed as a separate autofocus channel ①
- When the auto focus channel is active, all light settings can be modified ②
- A new control panel for all necessary autofocus settings appears ③



6 - Quick Start: High Speed Digital Autofocus


Leica Application Suite Advanced Fluorescence V 2.0 Release Note for Widefield

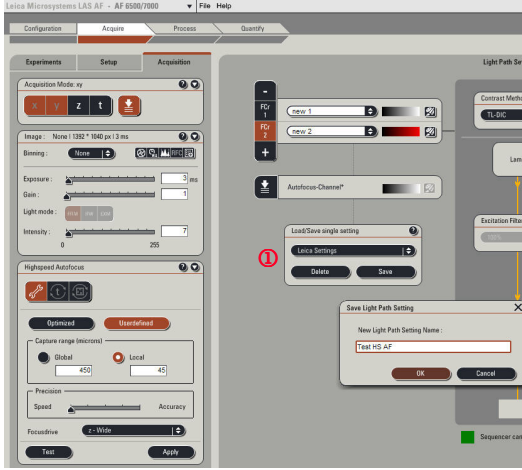
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Living up to Life

Basic adjustments

- The illumination settings for the autofocus channel can be stored together with the other channels. ❶

 Please note that the binning mode cannot be selected individually for the autofocus channel.



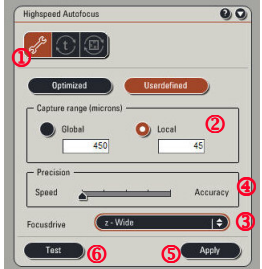
7 - Quick Start: High Speed Digital Autofocus

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Living up to Life


Basic adjustments

- The autofocus control panel allows you to configure the search settings ❶
- The capture range can be defined ❷
- The focus drive (fine or z wide) can be selected ❸
- And the accuracy can be balanced against speed ❹
- Settings have to be applied ❺ and can be tested ❻



8 - Quick Start: High Speed Digital Autofocus

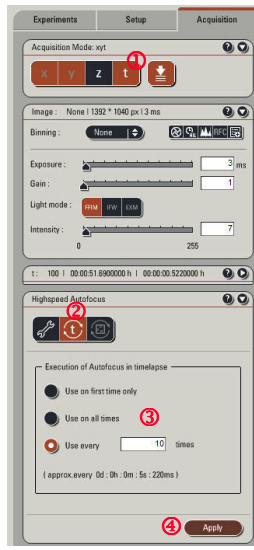
Leica Application Suite Advanced Fluorescence V 2.0 Release Note for Widefield




Time lapse with autofocus:

- During a long term time lapse, recording you may wish to automatically re-adjust the focus position over time
- Define your time lapse experiment first ①
- A new icon will immediately become active in the autofocus control panel ②
- Select the cycle when you want to start the focus search function ③
- Press Apply ④

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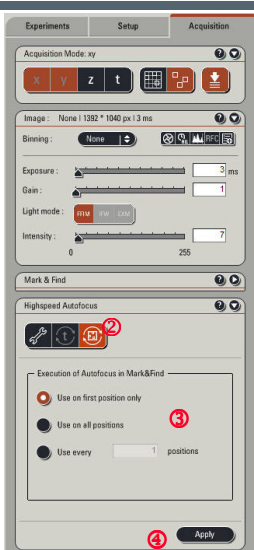
10 - Quick Start: High Speed Digital Autofocus



Mark & Find with autofocus:

- Define your stage positions first ①
- A new icon will become active ②
- Select the positions where you want to start the focus search function ③
- Press apply ④

Living up to Life



11 - Quick Start: High Speed Digital Autofocus

Leica Application Suite Advanced Fluorescence V 2.0 Release Note for Widefield

Tips

- For optimal speed, we recommend using as small a capture range as possible.
- For low mag objectives, high accuracy is often not needed.
- The higher accuracy is weighted against speed - the more images are taken. This can slow down the process.
- The smaller the integration time for the images - the faster the autofocus can work. You can use "gain" or "binning mode" in the light control panel to shorten the exposure time.
- You can combine z, t and x,y (Mark & Find) experiments with the High Speed Digital Autofocus
- If you define z-stacks in your experiment- the found focus position is centred in the middle of the z-stack.
- For reliable results allow the system to warm up for a while. makes the definition of the capture range more accurate.

Restrictions

- The Roper CoolSnap HW cannot be used in conjunction with the High Speed Digital Autofocus
- The High Speed Digital Autofocus cannot be used in the LAS AF Live Data Mode

Leica Application Suite Advanced Fluorescence V 2.0 Release Note for Widefield

Important information - LAS AF with the Leica M205 FA fluorescence stereomicroscope.

- **LAS AF V 2.0 is not compatible with the Leica M205FA, MZ16 FA, Macro Fluo or any stereo or macro fluo based system (e.g. Leica LSI). For stereomicroscopes M205 FA and MZ16 FA that are to be used with AF6000 software, please do not upgrade firmware using LAS 3.2 and continue to use LAS AF version 1.9.0. An integration of these microscopes into a higher LAS AF version will be announced in due course.**

Leica AM TIRF MC

New Features:

- Integration of the 63x TIRF objective with separate autoalignment and fine tuning
- New high speed digital autofocus also working for TIRF

Bugs fixed since LAS AF V1.9.0.:

- Wrong Penetration depth settings with change to high speed
- BF/TIRF bug in High speed
- TIRF shutter control in high speed

Known remaining bugs:

- 405 laser can be selected even without 405 laser box
- Smear effect with Andor 897 in saturated images in high speed

Restrictions lifted since LAS AF V 1.9

LAS AF V 2.0 can now be installed on systems that are combinations of Leica TCS SP5 and the Leica AM TIRF MC

Cameras – known restrictions

The Andor 885 camera does not allow for changing of exposure times and/or gain values between channels when running in high speed mode. The following message will be displayed.

