



# Leica AF6000

Modular Widefield Systems for Live Cell Applications

Technical Documentation

Living up to Life

## Supported Microscopes

Inverted research microscopes	Upright research microscopes	Fixed stage microscopes	Stereos and macroscopes	Clinical microscopes
Leica DMI6000 B*	Leica DM6000 B*	Leica DM6000 FS*	Leica M205 FA	Leica DM3000
Leica DMI6000 B with Adaptive Focus Control*	Leica DM5500 B*		Leica MacroFluo	Leica DM2500
Leica DMI4000 B*	Leica DM5000 B*			Leica DM2000
Leica DMI3000 B	Leica DM4000 B			Leica DM1000 Leica DM1000 LED

\* Compatible with High-Speed Microscope Control Box. High-Speed acquisition possible.

## High-Speed Microscope Control Boxes

11888423 High-Speed Mic Box Core	High-Speed Mic Box Core is the basic building block for CTR HS high-speed control box. Please add either 11888424 or 11888425 to make a working configuration.
11888424 High-Speed Board 6000	High-Speed Board 6000 supporting <ul style="list-style-type: none"> <li>• motorized stages</li> <li>• high-speed camera control (compatible camera required)</li> <li>• high-speed control of one Leica external filter wheel</li> <li>• high-speed control of Leica EL6000 shutter</li> <li>• connection of up to two Trigger In and one Trigger Out lines</li> </ul> Requires 11888423 High-Speed Mic Box Core.
11888425 High-Speed Board 6500	High-Speed Board 6500 supporting <ul style="list-style-type: none"> <li>• scanning stages</li> <li>• high-speed camera control (compatible camera required)</li> <li>• high-speed control of one Leica external filter wheel</li> <li>• high-speed control of Leica EL6000 shutter</li> <li>• connection of up to two Trigger In and one Trigger Out lines</li> </ul> Requires 11888423 High-Speed Mic Box Core.
11888426 Extension Board for 2 Filter Wheels	Extension Board for 2 Filter Wheels extends the High-Speed Board 6000 or 6500 to support a maximum of two Leica External Filter Wheels.
11888427 Extension Board 7000	Extension Board 7000 extends the High-Speed Board 6000 or 6500 to support <ul style="list-style-type: none"> <li>• high-speed control of a maximum of four Leica External Filter Wheels simultaneously</li> <li>• high speed control of SuperZ Galvo or Piezo fine focus</li> <li>• the connection of up to four Trigger In and four Trigger Out lines</li> <li>• Leica AM TIRF MC (Leica DMI6000 B required)</li> </ul>

11 888 423  
High-Speed  
Mic Box Core



11 888 424  
High-Speed Board 6000

11 888 425  
High-Speed Board 6500

11 888 426  
Extension Board  
for 2 Filter Wheels

11 888 427  
Extension Board 7000  
(for 4 Filter Wheels)

# Leica AF6000 - Live Cell Imaging Solutions That Grow with Research Needs

## ≡ **Ease of Use**

- All experiment settings visible at a glance
- Full monitor screen for live image display
- Review captured images during acquisition
- Fast and easy setup of experiments
- All acquisition settings stored
- Apply settings to new experiment
- Instant access to large data sets
- Context sensitive online help
- Minimum training required

## ≡ **Flexibility and Modularity**

- Individually optimized system configurations
- Available for upright, inverted and stereo microscopes
- Application wizards for screening experiments, Fura2 and Calcium Imaging, FRET Ratio and FRET Sensitized Emission
- A wide choice of hardware components e.g. Water Immersion Micro Dispenser, high-speed filter wheels, EM CCD cameras, high precision stages, live cell accessories

## ≡ **Upgradability**

- Systems that grow with changing research needs
- From entry-level systems to high-end workstations for real-time studies
- Convenient, on site upgrade process

## ≡ **Integration**

- Perfect harmony between hardware and software
- Precise timing for high-speed experiments
- Maximum system reliability
- One software platform for all widefield research and confocal microscope systems
- Ideal for imaging centers and multi-user facilities

# Software

Acquisition Modules	
AF6000 Core module	LAS AF AF6000 Core software, operates without the need for a dongle.
AF6000 Multi Channel Acquisition	AF6000 Multi Channel Acquisition allows the definition of up to 8 acquisition channels per experiment.
AF6000 Time-Lapse	AF6000 Time-Lapse user interface for defining the duration and frequency of image capture for time lapse experiments.
AF6000 Z-Control and Software Autofocus	AF6000 Z-Control and Software Autofocus user interface for focus positioning or capture of 3 dimensional data. Includes software autofocus.
AF6000 Mark and Find	AF6000 Mark and Find user interface to define multiple stage locations and revisit them as part of a time-lapse or Z stacking experiment.
AF6000 Stitching	AF6000 Stitching user interface to form overview images of large fields of view.
AF6000 Camera Driver Non-Leica Cameras	AF6000 Camera Driver Non-Leica Cameras allows the control of any third party camera supported by AF6000.
Dongle for AF6000 Optional Modules	Dongle for AF6000 Optional Modules is required to add additional modules to the AF6000 Core module.

Advanced Modules	
AF6000 Calcium Imaging	AF6000 Calcium Imaging module for online ratio measurement, online display of ratio graphs and ratio image
Live Data Mode	Interactive data recording allowing job-sequencing and online evaluation
3D Visualization	Maximum and other projections, simulated fluorescence process, 3D rotation and animations
Colocalization	Histogram based colocalization and area measurements
3D Deconvolution	Fully integrated deconvolution algorithm using adaptive calculated or supplied PSF
2D Deconvolution	Fully integrated 2D deconvolution offering no-neighbour and nearest-neighbour algorithm
FRET SE	Powerful wizard for FRET acquisition and analysis
Dye Finder	Multi-color restoration, channel unmixing
Well plate acquisition	Predefined and user-definable patterns

## Software

Leica Matrix M3 Modules (Only Leica DM6000 B and DMI6000 B)	
LAS AF MATRIX Mosaic Advanced	Application module for the generation of one Mosaic with adjustable size and location. Can be combined with multi channel acquisition, Z.stacks, time lapse. Includes generation of focus map.
LAS AF MATRIX Mosaic + Multiwell Advanced	Application module for the generation of one Mosaic with adjustable size and location and one Matrix of equidistant single scan fields. Can be combined with multi channel acquisition, Z.stacks, time lapse. Includes generation of focus map.
LAS AF MATRIX Mosaic Full Version	Application module including all Mosaic functions from the generation of one Mosaic up to the generation of multiple MosaiCs with adjustment possibility for each single Mosaic position. Can be combined with multi channel acquisition, Z.stacks, time lapse. Includes generation of focus map.
LAS AF MATRIX Multiwell Full Version	Application module including all Multiwell functions from the generation of one Matrix of equidistant single scan fields up to the generation of several individually adjustable Matrices and the generation of sub matrices. Includes a powerful Mark and Find mode. Can be combined with multi channel acquisition, Z.stacks, time lapse. Includes generation of focus map.
LAS AF MATRIX Full Version w/o CAM	Application module including all Mosaic and Multiwell functions. Can be combined with multi channel acquisition, Z.stacks, time lapse. Includes generation of focus map, drift compensation, control of Water Immersion Micro Dispenser and the object tracking module to follow cell movement with the stage.
LAS AF MATRIX Developer Entry	Application module for the generation of one Mosaic with adjustable size and location and one Matrix of equidistant single scan fields. Can be combined with multi channel acquisition, Z.stacks, time lapse. Includes generation of focus map and CAM interface for changing the course of screening experiments on the fly depending on analysis results.
LAS AF MATRIX Developer Full w. CAM	Application module including all Mosaic and Multiwell functions. Can be combined with multi channel acquisition, Z.stacks, time lapse. Includes generation of focus map, drift compensation, control of Water Immersion Micro Dispenser, object tracking module to follow cell movement with the stage and CAM interface for changing the course of screening experiments on the fly depending on analysis results.
LAS AF MATRIX Single Object Tracking	Matrix M3 object tracking module to follow cell movement with the stage.
LAS AF MATRIX Pump	Matrix M3 module to control the Water Immersion Micro Dispenser.
LAS AF MATRIX Z-Drift Compensator	Matrix M3 module to compensate Z drift during the course on a time lapse or screening experiment.

## Hardware Specifications

SuperZ Galvo z-positioner	
travel range	250 $\mu\text{m}$
step size	61 nm

Leica EL6000 External light source	
lamp	HXP 120 W 45C VIS (Osram), Metal Halogenide bulb
light intensity	3.5 W (with IR filter, at the end of light guide)
life time	average 2000 h
shutter time	6 ms

Leica External Filter Wheels	
filter positions	5
switching time	27 ms between adjacent positions 36 ms for maximum position distance

Incubator BL/Incubator BL TIRF	
temperature min.	3° C above room temperature
temperature max.	20° C above room temperature
control accuracy	+/- 0.1° C at sensor
step size	0.1° C



SuperZ Galvo focus for ultra-fast and highly precise z-stacking.



Leica SFL7000 – five different LED modules provide unlimited flexibility. Fully software integrated. Can be combined with Leica EL6000. „Not available in the US“

## Cameras

Leica DFC310 FX Camera	
gain	1 to 10
digitization	12 bit
cooling	– 20 K to ambient
imaging array	1392 x 1040 pixel
pixel size	6.45 $\mu\text{m}$ x 6.45 $\mu\text{m}$
interline progressive scan CCD	color
high speed trigger capability	yes

Leica DFC345 FX Camera	
gain	1 to 10
digitization	14 bit
cooling	active Peltier cooling
imaging array	1600 x 1200 pixel
pixel size	4.4 $\mu\text{m}$ x 4.4 $\mu\text{m}$
interline progressive scan CCD	monochrome
high speed trigger capability	yes

Leica DFC365 FX Camera	
gain	1 to 10
digitization	14 bit
cooling	active Peltier cooling
imaging array	1392 x 1040 pixel
pixel size	6.45 $\mu\text{m}$ x 6.45 $\mu\text{m}$
interline progressive scan CCD	monochrome
high speed trigger capability	yes

Leica DFC450 & DFC450 C Camera	
gain	1 to 10
digitization	14 bit
cooling	– 20 K to ambient (DFC450: no cooling)
imaging array	2560 x 1920 pixel
pixel size	3.4 $\mu\text{m}$ x 3.4 $\mu\text{m}$
interline frame transfer CCD	color
high speed trigger capability	no

Leica DFC495 Camera	
gain	1 to 10
digitization	12 bit
cooling	– 20 K to ambient
imaging array	3264 x 2448 pixel
pixel size	2.7 $\mu\text{m}$ x 2.7 $\mu\text{m}$
interline frame transfer CCD	color
high speed trigger capability	no

## Cameras

Hamamatsu 9100-02 Camera	
gain	on chip multiplication gain
digitization	14 bit
cooling	- 50° C
imaging array	1000 x 1000 pixel
pixel size	8 µm x 8 µm
frame transfer CCD	monochrome
high speed trigger capability	yes

Hamamatsu 9100-13 Camera	
gain	on chip multiplication gain
digitization	16 bit
cooling	- 65° C (-80/-90° C water cooled)
imaging array	512 x 512 pixel
pixel size	16 µm x 16 µm
frame transfer CCD	monochrome, back thinned
high speed trigger capability	yes

Hamamatsu Orca R <sup>2</sup> Camera	
gain	1 to 10
digitization	12/16 bit
cooling	- 35° C (-40° C water cooled)
imaging array	1344 x 1024 pixel
pixel size	6.45 µm x 6.45 µm
interline progressive scan CCD	monochrome
high speed trigger capability	yes

Andor iXon3 897 Camera	
gain	on chip multiplication gain
digitization	14 bit
cooling	- 85° C air cooled
imaging array	512 x 512 pixel
pixel size	16 µm x 16 µm
frame transfer CCD	monochrome, back thinned
high speed trigger capability	yes

## Motorized Stages for Leica DMI4000 B / DMI6000 B

### Regular motorized 3-plate-stage

11522068

Positioning range: 127 x 83 mm  
Resolution: 0,7  $\mu\text{m}$   
Accuracy: < 20  $\mu\text{m}$   
Reproducibility: 3  $\mu\text{m}$   
Compatible with: Pifoc, SuperZ, Water Immersion Micro Dispenser



Regular motorized 3-plate-stage

### Leica Scanning Stage 127x83

11522100

Positioning range: 127 x 83 mm  
Resolution: 0.02  $\mu\text{m}$   
Accuracy: +/- 3  $\mu\text{m}$   
Reproducibility: < 1  $\mu\text{m}$   
Compatible with: SuperZ, Water Immersion Micro Dispenser



Leica Scanning Stage 127x83

### Prior Scanning stage H117N1DM (114 x 76) ProScanTMII

11532536

Positioning range: 114 x 76 mm  
Resolution: 0.01  $\mu\text{m}$   
Accuracy: +/- 3.5  $\mu\text{m}$  (without 4 number correction) over full stage travel  
Reproducibility: < 1  $\mu\text{m}$   
Compatible with: SuperZ



Prior Scanning stage H117N1DM

### Scanning stage with encoder SCANplus IM 130 x 85

11522129

Positioning range: 130 x 85 mm  
Resolution: 0,01  $\mu\text{m}$   
Accuracy: +/- 1  $\mu\text{m}$   
Repositioning: < 0,5  $\mu\text{m}$   
Compatible with: SuperZ, Water Immersion Micro Dispenser



SCANplus IM 130 x 85



Leica External Fast Filter Wheel for high-speed excitation, attenuation and emission control.



Leica Water Immersion Micro Dispenser supplies water immersion automatically during experiments with living specimens.



e EL6000

**CFP/YFP FRET**

Please use filter cube set 11 532 630 for CFP/YFP FRET

(/6500 only)

not available in the US)

not available in the US)

11 640 256  
Antivibration table

A

Dispenser

Heating unit

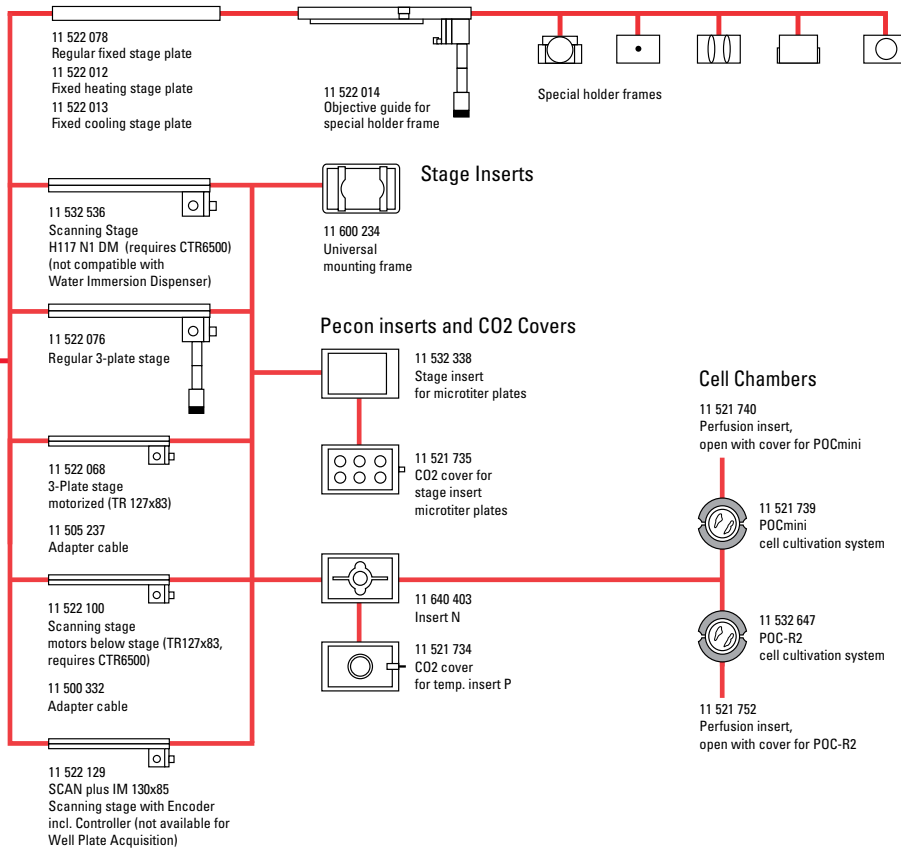
11 531 833 / 230V    11 521 719 / 230V  
11 532 306 / 115V    11 532 308 / 115V

Heating control unit    Temp control unit

**CO2 Controller including Humidifier**

CO<sub>2</sub> controller

11 521 733 / 230V  
11 532 305 / 115V  
CO2 controller with accessories



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**Recommended Cameras for Compound Microscopes**

11 541 543 0.7x C-mount adapter (not for DM6000 FS)

11 541 510 1.0x C-mount adapter (not for DM6000 FS)

A

- 11 547 004 Leica DFC365 FX
- 11 640 270 Hamamatsu 9100-02 EM-CCD high speed set
- 11 600 239 Hamamatsu 9100-13 EM-CCD back illuminated
- 11 600 266 Hamamatsu Orca R<sup>2</sup> CCD
- 11 600 282 Andor iXon3 897 EM CCD back illuminated
- 11 532 798 Photometrics Evolve Camera EM CCD
- 11 547 003 Leica DFC345 FX
- 11 547 002 Leica DFC310 FX
- 12 730 471 Leica DFC495
- 12 730 412 Leica DFC450 C
- 12 730 411 Leica DFC450

A B D F

DM5000 B  
DM5500 B  
DM6000 B

A C D

DMI3000 B

A C D G

DMI4000 B  
DMI6000 B  
DMI6000 B with AFC

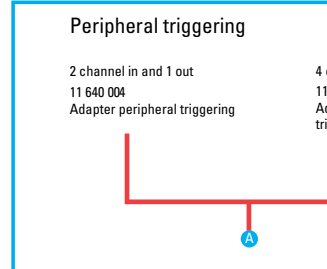
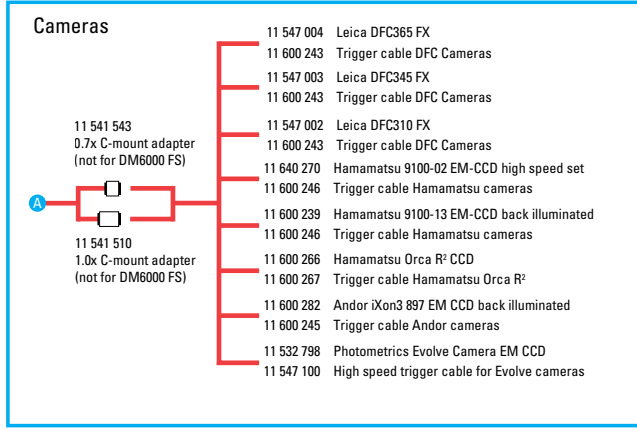
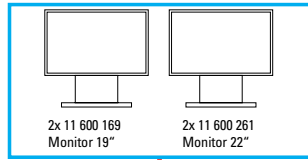
A D

DM6000 FS

# Leica AF6000

## High-Speed

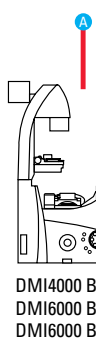
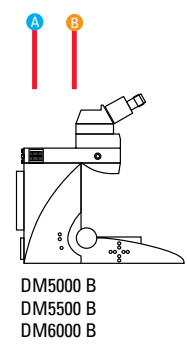
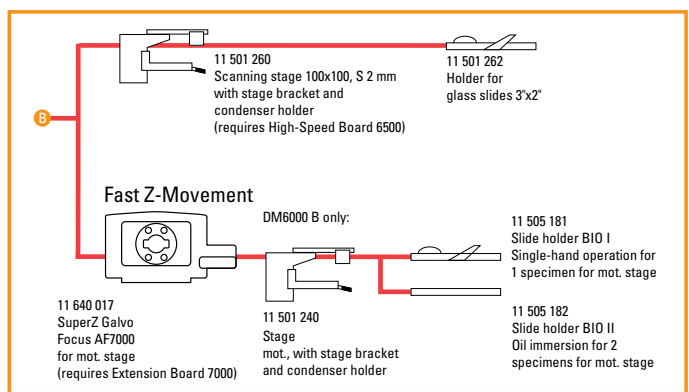
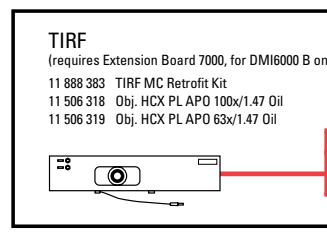
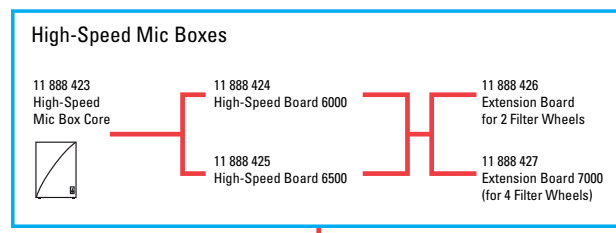
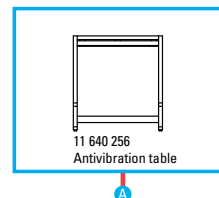
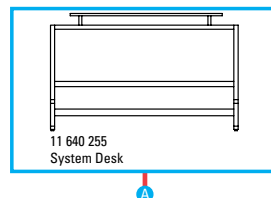
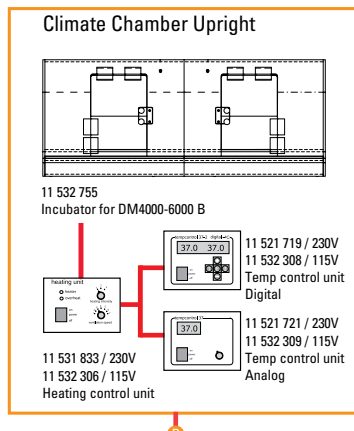
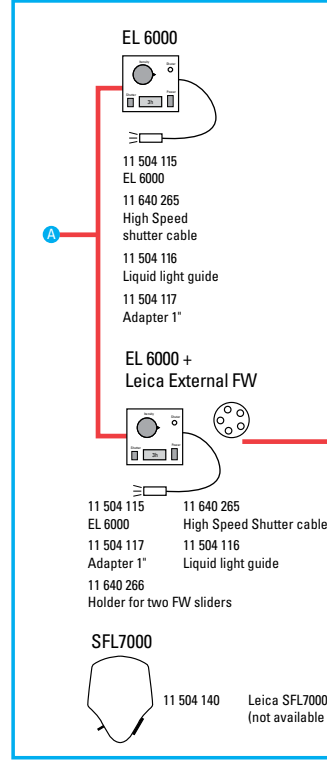
- 11 600 220 Keyboard US
- 11 600 221 Keyboard UK
- 11 600 223 Keyboard Italian
- 11 600 224 Keyboard German
- 11 600 225 Keyboard Spanish
- 11 600 222 Keyboard Swedish
- 11 600 219 Keyboard French



### Software Options

<b>Acquisition Modules</b> 11 640 033 AF6000 Core 11 640 034 Dongle for AF6000 Extensions 11 640 032 AF6000 Multi-Channel Acquisition 11 640 031 AF6000 Time-Lapse 11 640 030 AF6000 Z-Control and Software Autofocus** 11 640 029 AF6000 Mark and Find* 11 640 028 AF6000 Stitching* 11 640 026 AF6000 Camera Driver Non-Leica Cameras	<b>Advanced Modules</b> 11 640 025 Calcium Imaging (Requires Multi-Channel Acquisition, Time-Lapse) 11 640 803 Live Data Mode 11 640 804 3D Visualization 11 640 805 Colocalization 11 640 806 3D Deconvolution 11 640 807 2D Deconvolution 11 640 808 Dye Finder 11 640 811 FRET Software (Requires Multi-Channel Acquisition, Time-Lapse) 11 640 814 Well Plate Acquisition* (Requires Multi-Channel Acquisition, Time-Lapse and Z-Control, DMI6000 B only)	<b>Matrix M3 Modules*</b> (Requires Multi-Channel Acquisition, Time-Lapse, Z-Control, Stitching; DM6000 B/DMI6000 B only) 11 640 519 MATRIX Ug Developer Entry to Full 11 640 515 MATRIX Upg MosaicAdv to MosaicFull 11 640 514 MATRIX Developer Full w. CAM 11 640 512 MATRIX Developer Entry 11 640 511 MATRIX Developer Full w/o CAM 11 640 509 MATRIX Z-Drift Compensator 11 640 508 MATRIX Pump 11 640 507 MATRIX Single Object Tracking 11 640 505 MATRIX Multiwell Full Version 11 640 504 MATRIX Mosaic Full Version 11 640 502 MATRIX Mosaic + Multiwell Advanced 11 640 501 MATRIX Mosaic Advanced
<b>Packages</b> 11 640 027 AF6000 Premium incl. PC (AF6000 Core, Dongle, Multi-Channel, Time-Lapse, Z-Control, Mark & Find, Stitching, Calcium Imaging, 3D-Visualization) 11 640 035 AF6000 Expert incl. PC (AF6000 Core, Dongle, Multi-Channel, Time-Lapse, Z-Control, 3D-Visualization)	<b>Review Modules</b> 11 640 812 AF6000 Core Review Software (without Deconvolution)	

\* Only available for configurations with motorized stage \*\* Requires motorized z-drive



channel in and 4 out  
 11 640 004  
 Adapter peripheral  
 11 640 002  
 BNC-Box  
 triggering

**Fast FIM**  
 11 504 129  
 FFIM  
 11 504 119  
 Filterwheel slider short  
 11 504 132  
 Connection cable

**Fura 2**  
 11 522 072  
 Fura2-set  
 11 504 119  
 Filterwheel slider short  
 11 504 132  
 Connection cable

**CFP/YFP FRET**  
 11 522 073  
 FRET CFP/YFP set  
 2x 11 504 119  
 Filterwheel slider short  
 2x 11 504 132  
 Connection cable  
 11 541 545  
 C-mount 0.7x for EPW  
 11 513 892  
 Filter system CFP  
 11 513 893  
 Filter system YFP

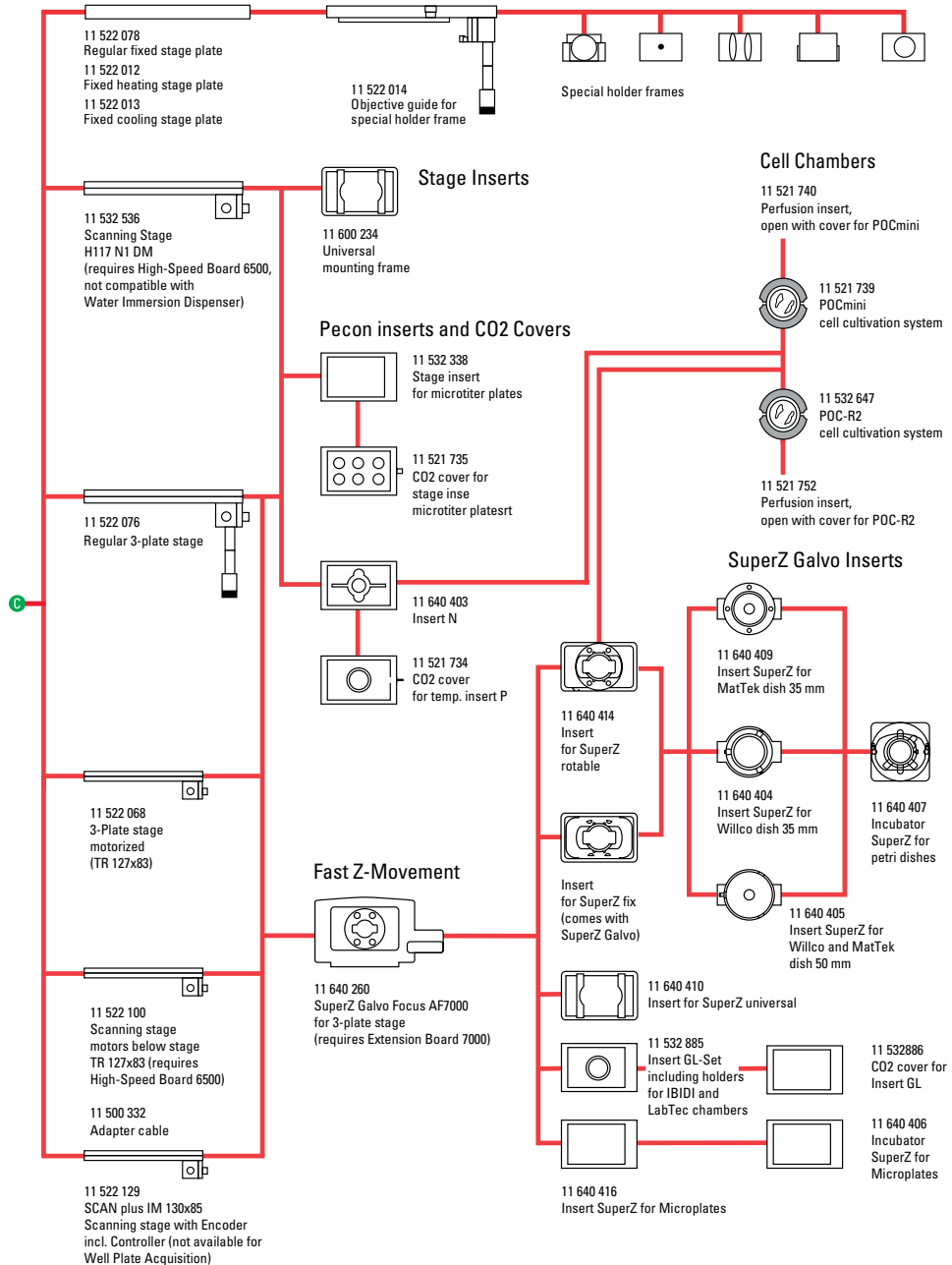
**Triple**  
 11 522 071  
 Triple-band-set  
 11 504 119  
 Filterwheel slider short  
 11 504 132  
 Connection cable

Micro Dispenser

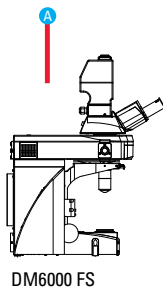
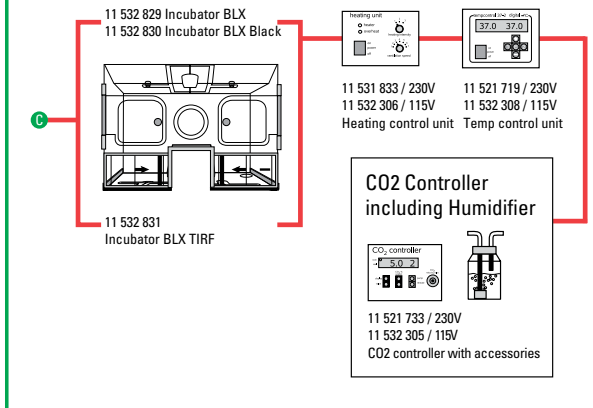
**TIRF Lasers**

- 11 888 936 Merge module basic 488
- 11 888 938 Color module 405nm
- 11 888 937 Color module 561nm
- 11 888 939 Color module 635nm

**Stages**



**Climate Chambers Inverted**



DM6000 FS

with AFC

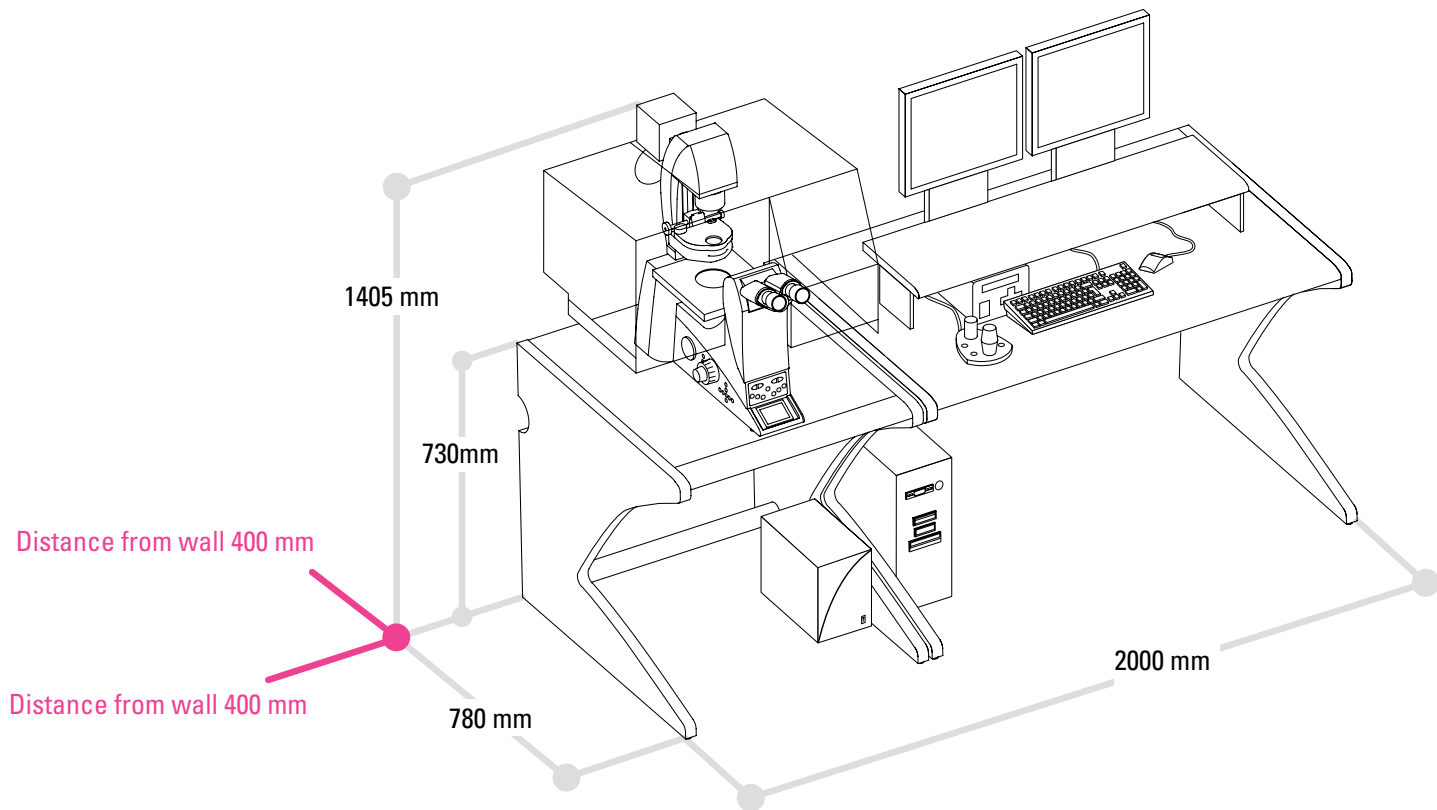
## Installation Requirements

<b>Environment</b>	Room temperature: +15 to + 25° C, low fluctuation of temperature Avoid proximity to air conditioning equipment Protect from dust Room darkening recommended Air conditioned room recommended Humidity max 80%, non condensing
<b>Electricity supply</b>	The system requires two mutually independent fuse circuits. One fuse circuit for the main system and the second for the climate controller and monitors.  Supply voltage 115 VAC ± 10%: 15 Ampere Supply voltage 230 VAC ± 10%: 12 Ampere
<b>Heat load</b>	System: approx. 500 – 900 W Climate controller: approx. 300 W (stand-by)
<b>Crating (width, depth and height in mm)*</b>	maximum box size: (1470/1000/1030), max weight: 236 kg (if optional work desk and/or antivibration table is included)
	Pallets for other hardware components

\* maximum numbers for the Leica AF6000 Widefield Systems



Leica AF6000 with Leica M205 FA stereomicroscope



Maximum footprint of the Leica Advanced Widefield System line with antivibration table and system desk.



Leica AF6000 with Leica DMI6000 B inverted microscope, climate chamber, CO<sub>2</sub> controller and SuperZ Galvo focus

# “With the user, for the user”

## Leica Microsystems

Leica Microsystems operates globally in four divisions, where we rank with the market leaders.

### • Life Science Division

The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems' customers at the leading edge of science.

### • Industry Division

The Leica Microsystems Industry Division's focus is to support customers' pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

### • Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

### • Medical Division

The Leica Microsystems Medical Division's focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life.**

### Active worldwide

Australia:	North Ryde	Tel. +61 2 8870 3500	Fax +61 2 9878 1055
Austria:	Vienna	Tel. +43 1 486 80 50 0	Fax +43 1 486 80 50 30
Belgium:	Groot Bijgaarden	Tel. +32 2 790 98 50	Fax +32 2 790 98 68
Canada:	Concord/Ontario	Tel. +1 800 248 0123	Fax +1 847 236 3009
Denmark:	Ballerup	Tel. +45 4454 0101	Fax +45 4454 0111
France:	Nanterre Cedex	Tel. +33 811 000 664	Fax +33 1 56 05 23 23
Germany:	Wetzlar	Tel. +49 64 41 29 40 00	Fax +49 64 41 29 41 55
Italy:	Milan	Tel. +39 02 574 861	Fax +39 02 574 03392
Japan:	Tokyo	Tel. +81 3 5421 2800	Fax +81 3 5421 2896
Korea:	Seoul	Tel. +82 2 514 65 43	Fax +82 2 514 65 48
Netherlands:	Rijswijk	Tel. +31 70 4132 100	Fax +31 70 4132 109
People's Rep. of China:	Hong Kong	Tel. +852 2564 6699	Fax +852 2564 4163
	Shanghai	Tel. +86 21 6387 6606	Fax +86 21 6387 6698
Portugal:	Lisbon	Tel. +351 21 388 9112	Fax +351 21 385 4668
Singapore		Tel. +65 6779 7823	Fax +65 6773 0628
Spain:	Barcelona	Tel. +34 93 494 95 30	Fax +34 93 494 95 32
Sweden:	Kista	Tel. +46 8 625 45 45	Fax +46 8 625 45 10
Switzerland:	Heerbrugg	Tel. +41 71 726 34 34	Fax +41 71 726 34 44
United Kingdom:	Milton Keynes	Tel. +44 800 298 2344	Fax +44 1908 246312
USA:	Buffalo Grove/Illinois	Tel. +1 800 248 0123	Fax +1 847 236 3009

and representatives in more than 100 countries