

**Technical specifications for LED based Fully-Motorized Inverted Fluorescent Microscope with live cell imaging capability with Image analysis software**

Please quote for an advanced, fully motorized integrated system Inverted Fluorescent Microscope with live cell imaging capability. The vendor should supply the entire system with all necessary accessories and complete system integration of hardware components.

Please quote for a system with the following minimum specifications:

**Fully-Motorized Inverted Fluorescence Microscope Frame:**

# Motorized Ergonomic Stand with a dedicated vibration free external TFT/LCD/TAB touch screen capable of controlling all motorized functions of microscope.

# The microscope should have inbuilt Z-focus drive with minimum step-resolution of 10-15 nm or better.

# The system should have a hardware-based focus stability control for drift correction and fast focussing using IR LED above 800 nm. The drift control module should be for long-term in-focus time-lapse imaging. The drift compensator should be working for glass bottom dishes.

#The frame should support future upgradable TIRF system.

#Should have C-mount adaptor & dust cover.

Transmitted Light system: Bright LED transmitted Illumination for DIC (capable of acquiring high-resolution in-depth imaging) with intensity control through TFT touch panel or imaging software.

Condenser: Motorized Universal Condenser (suitable for all microscopy techniques such as BF, DIC) with 6 to 7 slots for condenser optics. It should have DIC prisms and blank position for BF.

Eyepiece: Tilttable binocular head with 10X F.O.V 22 or better eye pieces-2 nos (with diopter adjustment).

Nosepiece: Six or more positions for motorized revolving nosepiece with slot for DIC Slider to accommodate objectives of different magnifications.

Objectives: High performance, Objectives suitable for Brightfield/(DIC) and Fluorescence Observation with following magnifications:

1. 10X (0.3 or better)
2. 20X (LD, 0.75 or better) Plan apochromat or equivalent
3. 40X (oil immersion (NA 1.3 or better) Plan apochromat or equivalent
4. 60X/63X (oil immersion 1.4 NA or better) Plan apochromat or equivalent
5. 100X (oil immersion; 1.4 NA or better) Plan apochromat or equivalent

# Magnification changers with additional 1.5x or 2x optical magnifications should be coupled with the microscope. Motorized magnification changers will be preferred.

# Quote for DIC accessories for all objectives, DIC and fluorescence images should exactly overlap, there should not be any pixel shift.

# All the objectives should be accompanied with motorized DIC prisms/Sliders for automatic engagement and disengagement during fluorescence imaging.

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Stage: Linear Encoded Motorised XY scanning stage with universal holders for slides, petri-dishes and multi-well plates.

# The stage should have the Incubating-Chamber with temperature and humidity control, stage inserts for on-stage CO<sub>2</sub> incubator.

# The stage & incubator should accommodate both 6 & 24-channel bottomless flow cell  $\mu$ -slides, 96 well plate, 35 mm dish, 60 mm dish and chambered slide for multi-well multi-point live cell imaging, and automated stitching in XYZt and multichannel mode.

#The LED based auto focus module should be able to automatically recognize and register the focus and offset for all the 96 well and perform multipoint imaging for high content imaging studies. The LED-based Z-drift compensator (for time lapse experiment) should also be compatible with 20X and 40X long working distance objectives to perform long-term time-lapse imaging with glass bottom dishes and 12/24/96 well glass bottom dishes. Appropriate external JoyStick should be provided for stage control.

Note: Multi compatible incubator base for standard applications; modular upgrading concept for slide-in modules along with stage top and microscope Incubation chamber with CO<sub>2</sub>, temperature & humidity control. All the incubation parameters should be controlled through microscope as well as through main Imaging software and through TFT screen of microscope.

**# All accessories for cell cultivation system with multichannel peristaltic pump. The flow cell should be compatible for both bacterial, microbial single cell as well as biofilm tracking. Compatible multichannel Microfluidic device attachment with sterile peristaltic pumps for controlled inflow. Microfluidic device with provision to view progeny analysis of at least 45 generations of cell-division in spatial and temporal manner.**

# On-stage CO<sub>2</sub> Incubator: On-stage CO<sub>2</sub> incubator to maintain constant temperature, humidity, and CO<sub>2</sub> concentrations. There should be a touch screen panel to control all the parameters of the incubator.

Fluorescence Module: Motorized fluorescence attachment with built in motorized shutter and a minimum of 6 positions (6 to 8 position) motorized filter turret. Motorised turret filter block with narrow bandpass notch filters for i) Hoescht ii) DAPI, iii) GFP, iv) RFP/DsRed, v) mcherry and (vi) polarizer/analyzer cube for DIC. An additional quad band DM & Emission filter (DAPI/FITC/TRIT/Cy5) with independent single band pass excitation/clean up filter for DAPI, FITC, TRITC and Cy5 should be offered. Quote for fast emission filter wheel & narrow band pass emission filters for DAPI, FITC, TRITC and Cy5 to be mounted on emission filter wheel. for synchronized switching between the chosen excitation light from the LED and fast shutter for fast ratio imaging.

Fluorescence Light Source: At least 7-8 line independent LED Light Array modules (LAMs) covering 385-395, 430/438, 475, 511, 555, 575/590, 635/640 or more should be offered with an ability to introduce clean-up filters.

# Stable long-lasting LED/solid state light source with a guaranteed lifetime of minimum 20,000 Hrs. The light source (with narrow Band pass) should support excitation of DAPI/Hoechst, FITC/GFP, TRITC/Rhodamine, DsRed and mCherry. The remote touch pad to control all the above individual lines should be provided. Fluorescence beam path should be apochromatically corrected for high end fluorescence imaging.

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# The light source should be controlled by imaging software and must have a built-in trigger board and slider slot for excitation/clean-up filter for individual wavelength for fast sequential imaging with a real-time control board that triggers individual lines and the camera in parallel mode. The light source should be controlled by imaging software for switching individual LED lines, for electronic shutter and attenuating the LED light intensity during multi-dimensional image acquisition.

USB TTL Conversion Kit: A 24-channel 8.5mA digital I/O device with an inbuilt data acquisition software to control the entire DAQ card. The Imaging software should also have a built-in driver to completely integrate the triggering function with external devices such as light sources and perfusion systems for seamless fast sequential multi-channel imaging with the LED light source, perfusion pump and CO<sub>2</sub> incubators. The imaging software should be able to trigger the LED lines through DAQ card in microseconds precision for different wavelengths. The I/O device should have at least 24 single-ended digital lines DIO ports: Each DIO line should be individually programmable as a static DI or DO line. The output voltage should at least be +5V. The trigger board should also have ports to control third party hardware such as microfluidic devices, perfusion system or similar hardware.

Camera: Research grade, highly sensitive fluorescence live cell imaging camera with 2304 x 2304 (5.0 Megapixels or more) High speed: at least 60 frames/s (at 2304 X 2048 ROI @ full resolution in live mode) Dynamic range: 21,400:1, Pixel size: 6.5 µm X 6.5 µm Read noise: 0.7 electrons, rms Peak Quantum Efficiency: 72 % or better, Forced-air cooled: -5 °C (Ambient temperature: +25 °C). The camera should be controlled by the microscope software and support two channel split imaging mode for two channel simultaneous imaging for ratio, FRET imaging.

The camera should be suitable for fast live cell imaging and TIRF imaging capabilities for future upgradation.

Software: Appropriate cell imaging and analysis software (licensed software, installation CD, latest version, upgradable) with user experience customization, multi-position module and well plate navigator along with Tile image, slice view for orthogonal plane viewing of 3D or time lapse data sets. Imaging capabilities for defining position specific Z stack in multi position imaging. The imaging software should have the following features/modules:

Hardware control: All the motorized components of the hardware including XY stage for multi-point and mosaic imaging, hardware based autofocus module, Emission filter wheels for fast sequential imaging, LED light source for intensity and fast shutter control for multi-channel imaging, third party sCMOS cameras for high speed ratio imaging should be provided.

Note: Any third party quote for TTL/triggered device control, DAQ card control for external light sources/LEDs and perfusion systems, on-stage CO<sub>2</sub> incubator (compatible with the imaging system) is encouraged.

# Image Acquisition Modules: Multipoint, multidimensional (XYZt) 6D imaging & display. Overlay of imaging, mark and find for multipoint and mosaic imaging, Real time stitching of large samples with higher magnification, multipoint and multi well assay editors imaging with hardware based autofocus. Online ratio imaging/physiology with online display of ratio image, real time intensity plot over time and over depth. Real-time or post-imaging deconvolution. High Dynamic Range Imaging, Instant Extended focal imaging, imaging of individual channels with image splitters and camera with real time/instant EDF.

# Image processing Modules: Brightness contrast adjustment, morphological filters, extended focal imaging. High dynamic range imaging, 3D deconvolution, Fluorescence spectral unmixing (to remove the autofluorescence/overlapping dyes) Macro creation editing and batch conversion/processing of large data sets. Object classifier/ intensity based automatic segmentation, **The software and native image format should be bio format & open microscopy environment (OME) compatible to import/export and access metadata with**

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**open-source image analysis platform such as ImageJ and Fiji to utilize the freely available image processing/image analysis plugins.**

# Image Analysis: multidimensional intensity measurement and data export to EXCEL format for further statistical analysis. Standard multichannel image acquisition & alignment with Z-stack & time-lapse acquisition module. Overlay multiple images, document groups for side-by-side image comparisons, Snap/movie acquisition & playback, Colocalization analysis with scatter plot display and statistical analysis, spatiotemporal measurement modules. 3D/volume rendering orthogonal slice view of 3D stacks. Physiology/Ratio analysis module, background subtraction/correction, bleaching correction, cell count and confluency measurements etc.

**Note: Any optional/add-on module mentioned in the software brochure should be quoted with respective part code for better clarity and to avoid confusion and for fair evaluation.**

Main Computer workstation: HP Z4G4 or equivalent Workstation consisting of System Integrated Branded Data processing unit with Xenon processor with Intel Xeon for high-speed image acquisition: QuadCore 64 GB RAM or higher, DVD Writer, 1<sup>st</sup> Hard Drive: 512 GB SSD SATA Solid State Drive, 2<sup>nd</sup> Hard Drive:4TB or higher HDD, 1280x1024 (min. 1024 x768) monitor resolution with Graphic card with separate graphics memory (NVIDIA; 4GB or better). PCI-Express x1. Compatible with half size or Low-profile PCIe board. Original Windows 10 Operating System (64 Bit), LED Monitor: 32-inch or higher. With licensed Operating System. Cordless keyboard and mouse along with LED monitor. All components should be from single manufacturer for better synchronized integration. A suitable offline workstation with all analysis features should be offered along with the system (see below).

# Off line Workstation: Separate PC (with same specifications as above) for image processing with offline software / dongles.

# Anti-vibration table: An active anti-vibration bread board table with M6 holes and automatic air-compressor for the complete microscope system.

**Additional requirements:**

# Three year comprehensive and two-year extended warranty on all parts including LED light sources. Latest software upgrades should be provided free of cost for 3 years.

# Downtime for repair should be a maximum of one month with financial liabilities upon delay of service.

**Input Supply:** 230 V AC 50Hz (Compatible with electric power supply in India).

# Please quote all above items separately or by splitting for selection purpose.

# Detailed service manual must be supplied along with instrument.

# Detailed Operating manual for Instrument as well as Software must be supplied with the Instrument.

# Full detailed catalog with clear picture must be included with the quotation.

# Onsite training should be provided as part of the Installation to users at CDFD. Training should include working on all the LEDs that are provided with the System and data analysis with the software included in the bid.

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# During the training the vender must demonstrate the performance specifications of the system like resolution, sensitivity, scanning speed, working distance of objectives, etc. at optimal operating conditions.

# Please quote for Annual Maintenance Contract charges for three subsequent years after the completion of the warranty period. The AMC must include Periodic Preventive Maintenance as per the schedules to be agreed with the User and Instrumentation personnel and breakdown calls.

# Spare parts support (including light source) should be provided for a period of 10 years from date of delivery.

# Any upgrades in software or hardware (Instrument) introduced by the manufacturer should be intimated to the user. All mandatory upgrades by the manufacturer should be performed as and when the manufacturer introduces both in software and instrument hardware.

# Instrument quoted should be a complete system in all respects; any additional accessories required for the instrument to operate should also be quoted as part of the instrument and should be supplied along with the Instrument. Also any special pre-installation requirements needed for optimal performance of the system should clearly be mentioned in the quote.

Please quote for following as Optional:

# The system should have additional ports or option for an inbuilt free second deck/stratum structure with ready to use infinity port/space as part of the system for additional custom modifications / LED introduction.

# Built-in SMART Touch Panel Controller: A touch panel should automatically control enabling switching of optical components, aperture adjustment. Motorized fine focus Z-drive.

# Control Box and Controller: Standalone control unit (with push type buttons & focusing knobs) which provide familiarity to that of conventional handle operation. Universal Control Box for motorized components.

# Chamber type transparent Incubator covering whole microscope body

#The On-stage CO<sub>2</sub> incubator should also have a lens heater to avoid any heat seepage through the oil immersion objectives.

# Integrated high-resolution Imaging Tool: Please quote for integrated High Resolution Fluorescence Imaging tools to remove haze and out of focus signals instantly while in acquisition and to improve image resolution using techniques like Spinning disk/Computational Clearing/Optical sectioning methodology to avoid any post acquisition processing.

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