INDIAN INSTITUTE OF TECHNOLOGY BOMBAY

MATERIALS MANAGEMENT DIVISION

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Technical Specification of High-Resolution imaging system	
Dedicated fully automated opto-digital system for high resolution fluorescence and DIC imaging of live and fixed biological samples. The system should have completely integrated setup for real-time high-resolution imaging based on real time computational clearing or spinning disc with Structured illumination technique or equivalent technique to remove noise as well as haze for capture high S/N images for regular tissue sections, live cell & thick brain sections of minimum 150 µm or better. The system should have following essential components:	
1. Real time Computational Clearing /spinning disc with Structured Illumination Technique -based system to remove haze immanent to all widefield images with large data set like several GB in few seconds. 2. Computationally cleared /Spinning Disk with Structured Illumination data should be viewed during experiment on fly during xy/z, xyzt, tilescan experiments 3. Should allow removal out of focus blur/out of focus signal & can from single 2D images real time without requirement of multiple image acquisition to avoid any phototoxicity. 4. Should be pre-calibrated to avoid calibration or adjustment of hardware components during/before acquisitions. 5. Should provide raw data as wide field image along with high resolution/Confocal image for validation of results.	
Microscope stand	Fully motorized system along with fluorescence unit including automated objective turret. Motorized fluorescence turret. Motorized universal condenser for bright field, dark field, DIC & polarization applications. High resolution and high sensitivity cooled sCMOS camera and software for acquisition and analysis. Should have motorized focussing with both coarse and fine focussing knobs in main frame. Z drive with minimum step size of 5 nm or better. Must include LED/IR Laser (790nm or above) based inbuilt focus drift compensation mechanism for long duration time-lapse live cell imaging. Also, the Microscope body should have the active Left & Right Port along with Intermediate Magnification Changer (1.5x/1.6x/2x) for holding the Multiple Detection technologies at the same time. Should have special electronic/software controls/board to run the system in a seamless manner at highest speeds.
Focusing	Fully Automated hardware based Drift Compensation Mechanism on the system to keep the focus stable in long- and short-term experiments with - a bidirectional reproducibility/minimum increment <20 nm - Motorized scanning/encoded 10-12 mm travel/stroke range - a tactile/CAM Link course / fine motorized focus drive - a 4 gears and 5 sensitivity levels/5 focus increments - a course / fine knob on each side of the microscope.

Stage	Motorized XY scanning stage with Encoder or similar technology for better precision and for dynamic and smooth positioning. Position resolution 0.02 micron or better and reproducibility/repetitive accuracy <1 µm. Should include universal sample holders for Petri dishes, glass slides & multi-well plates. Should be controllable both by software and joystick.
Condenser	The system must have a motorized universal condenser turret suitable for all microscopy techniques such as bright field, dark field, phase contrast & DIC. Should include motorized polarizer and analyser for DIC.
Objectives	High performance Plan Semi Apochromatic objectives for FL and DIC Application Plan Semi Apochromatic 10x/0.32 NA or better, Plan Semi Apochromatic 40x/0.60 NA or better (LWD with correction collar) High performance Plan Apo Objectives for Fluorescence, Plan Apochromatic 63x or 60x/1.40 NA or better OIL Immersion, Plan Apochromatic 100x oil with NA 1.4 or better DIC prism turret/slider should be changed in Motorized way. DIC condenser and nomarski prism for 40X, 60X and 100X objectives. DIC prisms should automatically disengage from the optical path in the fluorescence mode to avoid unnecessary signal loss.
Eyepiece pair	Binocular head having eyepiece pair of 10x magnification with 22 mm or higher FOV with dioptre adjustment for both eyes.
Transmitted light	Motorized transmitted light column equipped with LED illumination having minimum 20000 hours lamp life. Motorized Field diaphragm with circular & rectangular diaphragm to make objective and camera chip size for perfect image. Inbuilt fast shutter of min 10 ms for synchronized imaging of BF/Phase Contrast/DIC with fluorescence.
Fluorescence module	RFID/Sensor based Automated fluorescence filter turret with 6 or more positions to automatically identify correct filter cube in place. Adjustable aperture and field diaphragms; Should have body inbuilt 4 / 5 position light intensity filter wheel for control of fluorescence light. Pixel shift corrected/Zero Pixel Shift band pass fluorescence filters cubes for these fluorophores: DAPI or Hoechest, GFP, FITC, CFP, YFP, Cy3/ TRITC, Texas red/ mCherry & Quad band filter cube with below filter combination Or the Quad Band Pass Filter should be compatible with DAPI or Hoechest/FITC or GFP/Cy3/ Cy5 Imaging with suitable Multiband EX/DM/EM Filters.
Fluorescence Light Source	• Solid State Illumination with light source of lifetime more than 20,000 hr and with seven wavelengths 395, 438, 475, 511, 555, 575, 635, 730 nm to cover the entire imaging spectrum. Either Lumincor spectra X or pE800 light source should be provided for high speed light switching speed of 10 microsecond or less. • The illumination source should be factory integrated with company's own software without any third-party software.
Camera	Digital monochrome cooled camera with scientific CMOS sensor with trigger capability having minimum 5 MP or more pixel resolution with speed of 89 fps@ Full frame Dynamic Range: 21,400:1 or better, Pixel size: 6.5 µm X 6.5 µm Read noise: 0.7 or 1.2 electrons or better, 90% or better quantum efficiency and hardware binning. Spectral range from 370 nm to 1100 nm or better.

Software	Company's own imaging software for fully automated acquisition, device control image analysis. Should include multidimensional image acquisition (5D or 6D), intensity and distance measurements of the fluorophores, co-localization module, 3D image reconstruction, time lapse imaging, autofocus, Z stack, report generation facility and multi-dimensional image format. The system should be able to perform fast spiral area scanning for quick overview, automated multi-position imaging, stitching and mark and find capability. The software should have built-in formats for multi-plates at least from 6 well to 384 plates. Also, the software should have user defined conditional experiment through Graphical Experiment Manager or live data mode or JOBs or Journals or similar modules for customized experimental set up and analysis. 2 licenses of the software (offline and online)
Environmental control system	System should have live cell chamber for controlled CO_2 (Range of 0% to 10%), humidity and temperature (0.1C fluctuation). Should include sample holders for 35 mm Petri, 6-well plates holder, 12 well plates holder, 24 well plates holder, dishes and multi-well plates (Up to 384 Well Plates). Preferred to have O_2 control as option.
Integrated Image Enhancement Tool	The system must offer with High Resolution Fluorescence Imaging tools to remove haze and out of focus signals instantly while in acquisition using techniques like Spinning disk based on Structured illumination pattern or Computational Clearing methodology to avoid any post acquisition processing in future.
Data Analysis workstation	Factory integrated workstation with Intel XenonXEONW processor having minimum 64 GB DDR4 RAM, NVIDIA, 2X250 GB SATA II and 1 TB back up HDD GeForce RTX2080 Quadro RTX 4000 or Better graphics card compatible with Driver 399.07 or better for SRRF Data Processing with minimum 11 GB RAM, 4 TB SATA drive, Original 64bit Windows 10 OS, DVD RW, multimedia kit, 32"monitor, keyboard, optical mouse and adequate number of slots to accommodate all hardware requirements of the quoted imaging system.
Others	High performance imported (Newport, Thorlabs or equivalent) vibration free table to hold the entire system. Microscope, Camera, XY stage, fluorescence illumination and Control / Analysis software should be from same Manufacturer for better synchronization at the time of Installation done by Factory Trained Engineers to avoid any compatibility related issues. All the hardware should be integrated into the data stream for seamless performance and factory configured/recommended PC should be used. Complete system should be covered with two years warranty. A 5 KVA online UPS system should be supplied to support the complete system. The system should be compatible with respect to hardware (requisite port, adapter etc.) and the workstation should be capable of accommodating requisite software for the attachment of a Laser free confocal system.

Applications:	The system should be able to image a wide variety of samples like bacteria, yeast, mammalian cells, tissue sections up to 150 microns, drosophila embryos etc. without compromise on the time resolution so as to image live events in time lapse or in live mode for long duration in the cells.