

INDIAN INSTITUTE OF TECHNOLOGY BOMBAY MATERIALS MANAGEMENT DIVISION

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<u>Technical Specifications</u> Research Grade Upright Laser Scanning Confocal Microscope System

A) Microscope Body and Optics

- 1) Fully Motorized Compact Upright Microscope
- 2) Specimen stageshould be fixed along Z-axis.
- 3) System should have motorized nosepiece for Z-axis focusing with a resolution of 25 nanometers or better
- 4) System configuration must be optimal for Differential Interference Contrast (DIC), Bright-field and Reflected light (Fluorescence) imaging.
- 5) Tiltable eyepieces
- 6) Motorized condenser unit with Differential Interference Contrast (DIC) Optics that is compatible with all objectives.
- 7) Motorized Objective turret with at least 6 positions for objective lenses.
- 8) Motorized filter turret with individual filter cubes to image common fluorophores (DAPI/Hoechst, GFP/FITC, CFP, RFP/mcherry/Cy3/TRITC/Rhodamine). High quality bandpass filters with no cross-talk when imaging above mentioned fluorophores in combination simultaneously.
- 9) Transmitted light Illumination can be Halogen or LED, but should permit clear visualization of optically transparent samples such as live zebrafish embryos.
- 10) Fluorescence Illumination source should be adjustable for intensity. Vendor should clearly give details of type of the illuminator, wattage and lifetime hours expected.
- 11) Transmitted light and Reflected light image acquisition should be controlled through software.
- 12) Microscope should be Camera-ready with port and optics for Epifluorescence and Bright-field imaging.
- 13) Touchpad, if available with the system, should be quoted. Microscope and illumination systems should be controllable from the touchpad to observe the specimen.

B) Objective Lenses

All objective lenses should be corrected to work optimally across UV, Visible and IR wavelengths. The following objectives are required:-

DRY OBJECTIVES (Plan Apochromatic)

- 1) One objective having magnification at a value between 1x to 4x. Working distance 2mm or longer.
- 2) One 10x objective with Numerical Aperture of 0.4 or higher, working distance 2mm or longer.
- 3) One 20x objective with Numerical Aperture of 0.7 or higher, working distance 0.5 mm or longer.

WATER DIPPING/WATER IMMERSIONOBJECTIVE (Plan Apochromatic or Semi Apochromat)

4) One 40x objective with Numerical Aperture of 0.8 or higher, working distance 2mm or longer. Objective should be compatible with Differential Interference Contrast (DIC) imaging.

OIL IMMERSION OBJECTIVE (Plan Apochromatic)

5) One Oil immersion objective is needed. Magnification can be 63X or 100X.

Numerical Aperture should be 1.4 or higher.

Objective should be compatible with Differential Interference Contrast (DIC) imaging.

Should have correction collar for correcting aberrations caused by refractive index mismatch.

Details of relevant immersion oil should be mentioned. Immersion oil should be quoted for.

C)Motorized Stage for X-Y motion

Motorized XY stage with Universal Sample Holder for Slides, Petri dish and Multi-well plate. Travel range should be at least 50 millimetres in both X and Y directions. The stage should permit coarse and fine positioning automatically for timelapse recordings and image stitching. Resolution of stage in X and Y directions should be 300 nanometers or better. Low thermal drift, high accuracy and repeatability (approximately 1 micron) is required.

D)Temperature-control Unit

Temperature control unit compatible with upright microscope and above mentioned motorized XY stage should be quoted. The unit should be able to vary sample temperature from ambient (22 degrees Centigrade) to 40 degrees Centigrade. The precision and stability in attained temperature should be 1 degree C or better. It should be possible to easily remove the temperature control unit when it is not required and operate the microscope.

E) Laser unit

Pre-aligned Solid state lasers (DPSS or Diode lasers) with following emission wavelengths:-

- (1) 405 nm (2) 488nm (3) 561nm (4) 640nm
- 1. Each Laser should supply more than 15 milliwatts power at the fibre output.
- 2. All lasers should be controlled through an acousto-optic tunable filter (AOTF) for ultrafast laser switching and attenuation in computer controlled manner.
- 3. Lasers should be synchronized with the laser scan head for precise pixel-level excitation of the sample to be imaged.
- 4. System should allow precise and continuous variation of Laser intensity.
- 5. System should allow the following types of experiments :
 - a) Fluorescence recovery after photobleaching (FRAP)
 - b) Photo-activation
 - c) Photo-conversion

F) Laser scan head

A Galvanometer scanner or a Hybrid Scanner (Galvanometer + Resonant operation) should be quoted. The scanner should allow imaging at 4 frames/second or higher. It should be possible to reduce the field of view for faster scanning at high resolution.

If a Hybrid (Galvanometer + Resonant operation) scanner is being quoted, then it should have the following properties:-

- 1. Resonant scanner should allow scanning of 512x512 pixels at 30 frames/sec or faster, with increased frame rates as the field of view is reduced.
- 2. System should allow automatic and rapid switching between resonant and galvanometer scanners during an experiment.
- 3. It should be possible to mark a region of interest (ROI) at low magnification and home to that ROI at a higher magnification automatically when objectives are switched.

G)Detection system for EpifluorescenceConfocal and Widefield microscopy

- 1. All detectors should be capable of intensity-based as well as spectral confocal imaging.
- 2. Simultaneous detection and separation of at least 3 fluorophores. At least 2 detectors should be highly sensitive GaAsP-PMT type (or equivalent) with minimum 45% quantum efficiency.
- 3. The system should be capable of recording emission spectra with spectral resolution of 5nm or better.
- 4. All detectors should have independent gain and offset control
- 5. Transmitted light detector to be provided for capturing bright field and DIC images.
- 6. Extended dynamic range of detectors is required for low light imaging.

H) Vibration isolation table

Anti-vibration table with compressed air damping is required. The table top should have threaded holes for mounting optical components.

I) Microscope Control, Image Acquisition and Analysis Software

A single software suite should be provided that can control all operations of the motorized microscope, Image acquisition, Image analysis,Laser and light path selection.

The Software module should also permit the following:-

- 1. Multi-dimensional (X,Y,Z, Time and Wavelength) imageacquisition and visualization.
- 2. ROI bleach for FRAP, Photo-activation/conversion experiments.
- 3. FRET/FRAP imaging and quantitative data analysis capability.
- 4. Measurements of length, areas, 3D volumes and angles including intensity measurements.
- 5. Advanced 3D image reconstruction with rendering from a Z-stack image series
- 6. 2D deconvolution capability.
- 7. Co-localization analysis.
- 8. Spectral un-mixing for separation of overlapping excitation/emission spectra of fluorophores.
- 9. Software for Multi-point, Multi-well imaging and image stitching/tiling module.

J) Computer and Monitor

- 1. 64-bit Windows 10 Pro Control computer from branded company (e.g. Hewlett Packard, Dell etc.).
- 2. Intel Xeon CPU or better with double data rate (DDR) RAM of 32GB or more.
- 3. Atleast 2TB hard drive for data storage. Upgradable to 4TB.
- 4. Additional hard-drives as per the requirements of control and imaging software being provided.
- 5. High performance Graphics card with a minimum of 2GB RAM
- 6. LED/LCD monitor.
- 7. Table for CPU and monitor

K) Power backupand Surge Protection

Compact size online UPS for power backup of at least 10 minutes and surge protection against Voltage fluctuations.

L) Purchase, Warranty and Service Terms

- 1. The System offered must be available on Website of Manufacturer or on a Brochure
- 2. 2-year warranty is required for microscope parts.
- 3. Additional 3-year annual maintenance contract beyond the above stated warranty period of 2 years.
 - Year-3 maintenance contract on all microscope parts
 - Year-4 maintenance contract on all microscope parts
 - Year-5 maintenance contract on all microscope parts
- 4. Bidders must provide full details of after sales service support and a list of users within India over the last 3 years with their contact details.
- 5. Supplier must have an active support in Mumbai. Supplier should clearly mention how instrument service and repair time will be minimized.
- 6. A qualified factory-trained engineer shall conduct on-site installation, commissioning and training.

7.	Suppliers should not list in the quote components of any part of the microscope, confocal system, software or the computer system which are being phased out by the manufacturer. If older vs newer parts exist for any component for any part of the entire system (microscope, confocal system, software, computer system etc), supplier must quote for the newer component.