INDIAN INSTITUTE OF TECHNOLOGY BOMBAY

MATERIALS MANAGEMENT DIVISION

Powai, Mumbai 400076.

Reference No. (PR No. 1000017301) **RFx No. 6100000728**

TECHNICAL SPECIFICATIONS

Multiphoton imaging equipment

A High-resolution microscope workstation for *in vivo* small animal imaging with laser scanning and multiphoton attachment is required.

1. Fully Motorized & Computer Controlled Fixed Stage Upright Microscope for small animal imaging:	
Application	Multiphoton Confocal system for small animal imaging facilitated with high sensitive detection for deep tissue imaging, *in vivo* imaging, intravital imaging including second harmonic generation (SHG), FRAP, FRET, photo activation/conversion and ablation. System should be upgradable in future to FLIM attachment.
Optics	Optics for Bright field, Dodt contrast or IR DIC, Fluorescence observations with accessories for Confocal imaging.
Z Focus	Built in Motorized Z-focus drive with minimum z-step resolution of 25 nm or better.
Nosepiece & Turret.	Motorized 5 position or better motorized FL filter wheel, 2/4 position objective turret.
Stage	Motorized XY Scanning stage with universal sample holder for slides, 35/60mm petri-dishes and well plates for *in vivo* small animal as well as tile and multi-position imaging.
Illuminations	12V/100W halogen transmitted light illumination for BF/DIC and high power 120 W metal Halide illumination for fluorescence with PC control.
Objectives	High resolution confocal grade water immersion objectives with long working distance Water Dipping 10X/0.50 (or 10X/0.3) WD of 3.5 mm or better, Water Dipping Plan Apo 20X/1.0 WD 1.80 mm (or 2.2. mm) or better (or Water Dipping Plan or Semi Apo 25X/0.95 WD 2.4 mm or better), Plan Apo 40X/1.30 oil immersion, Water Dipping Plan Apo 60/63X (1.0 or 0.9) WD 2.1 (or 2.2) mm or better and Plan Apo 63X/1.40 oil immersion objective. Dodt / IR-DIC accessories for all objectives to be included. Quoted objectives should be corrected for UV-VIS-IR.
Fluorescent Filters	Band Pass Fluorescent filters for DAPI, FITC/GFP & TRITC/Rhodamine for visualization.
Detectors (Reflected and Transmitted)	• **2 channels high sensitivity GaAsP or equivalent detectors** for reflection mode imaging using IR laser. **Also 2 channel GaAsP** Non Descanned Detectors (NDD's) for high sensitivity SHG imaging in transmitted and reflected mode. NDD's to be located close to the objective back focal plane for high photon collection efficiency. The detectors should have user selectable and changeable

	filter combination for optimizing to various fluorochromes under observation. • The reflected mode detectors should have filter combinations for blue/green, green/red, CFP/YFP pairs. • Transmitted mode NDD should have filter combination for violet/green, green/red pair. • High NA (1.2) water / 1.4 Oil immersion condenser with transmission down to 380 nm for **Second Harmonic Generation (SHG).**
Cooled sCMOS Camera	Monochrome cooled sCMOS camera with min 4MP (or better) net effective pixel resolution **AND** with 95% or better quantum efficiency, 1" chip size, wide sensitivity spectrum 350 nm – 1000 nm, (USB III) controlled by confocal software with multichannel, z stack, time series imaging should be offered.
Table	A suitable breadboard anti-vibration table for the complete microscope and laser scanning system with size of approx 1.8 m X 1.5 m (or 1.2 m x 1.5 m) including air compressor for placement of the complete system.
2. Spectral Confocal Laser Scan Head with built in or Separate Detectors for small animal imaging:	
Scanning	Laser point scanning and confocal detection unit with PMT and/or HyD/GaAsP detectors. All detectors should have freely selectable emission band width for optimal detection of fluorochromes.
Scan Head	Should be capable of simultaneous detection and separation of minimum 4 fluorophores (DAPI, GFP, CFP, YFP, Cy3, Cy5) out of which 2 can be PMT/HyD and 2 should be high sensitivity GaAsP/HyD or equivalent detectors with QE 45% or more. Additionally, 2 spectral detectors should be provided. Combination of detectors should be channel based or capable for spectral imaging with selection of emission range without any limitations.
Spectral Dispersion	The spectral dispersion of the emission light should be either with reflection grating or with Prism dispersion**.**
Excitation Range	Laser scan optics covers the entire wavelength range from 400 nm to 1040 nm (or more) and should have simultaneous laser coupling ports for UV, VIS and IR lasers.
Pinhole	Computer controlled confocal pinhole with software control for multi-tracking including short wavelengths.
Scanner	High speed XY galvo scanner with 200 deg scan rotation or better with total scan flexibilities of Line, free hand curved line, XY, XYZ, XYZ t and XYZ,t,λ.
Scanning Options.	The laser scanner should have dual scan capability of high-speed ROI/Image scan for "bleaching/photo-activation/imaging" & "normal scan for Imaging/stimulation", ROI scan capability to conduct experiments like FRAP, FRET, photo activation/photo conversion and ablation.

Scan Resolution	Maximum scan resolution should be 4Kx4K (or better) for all channels and can be selected freely down to 16x16 pixels (or better).
Frame Rate	Scanner should be capable of acquiring 10-15 fps (or 28 fps) @ 512x512 (or better) and increase to 400 fps (or 280 fps) or better @ 512 X 16 for fast time lapse imaging applications.
Field of View	The scan field diagonal should be 18 mm or better.
Data Acquisition	Data acquisition and Digitization capability with 8/12/16 bit should be available.
T-PMT	An additional Transmitted light detector for bright field and DIC imaging to be provided.
3. Laser Module with AOTF control:	
Visible Lasers	Laser module with laser lines of 405 nm, 445/448 nm, 488 nm, 514 nm, 561 nm, 640 nm laser lines. All the lasers should be optically coupled and connected to the scan head through a fiber optic cable. All the visible laser lines controlled through an AOTF for fast laser switching and attenuation in synchronization with scanner position.
Multi photon IR Laser	**IR Laser:** Totally integrated and software controlled Femto pulsed **Ti-Sapphire laser system** for Multiphoton imaging with the following specs should be offered. • Tuning range: 690 to 1040 nm. • Pulse width: < 100 femto seconds. • Average power: > 2.5 watt @ 800nm. • Repetitive rate: 80 MHz. All direct coupling optics of the laser to the scan head as well as both microscopes should be provided. Laser attenuation device should be through AOM.
4. Hardware Based Online High-Speed Super Resolution (SR) Imaging System for small animal imaging:	
High Resolution Imaging Attachment	• Fully automated and motorized SR attachment with suitable high sensitivity Detectors (GaAsP or equivalent with quantum efficiency 45% or better) for complete UV-Vis-IR Spectrum. • Lateral resolution of 120 to 130 nm or better and Axial resolution of at least 350 nm or better should be expected out of the system. • Detection should be based on GaAsP or high sensitivity detectors. • At least 2 fluorophores imaging in simultaneous mode should be possible with the SR system. Any dye used for Confocal system should be possible for imaging without changing sample preparation techniques/protocol. • Should be able to perform live cell SR Imaging. Frame rate in SR mode should

	be at least 25 fps @ 512X512 & maximum 400 fps (or 280 fps) @ 512x16 or better. SR mode should be able to perform 2D / 3D images, time series, tiling / mosaic, ROI imaging, multiple location imaging, photomanipulation experiments (FRAP, FRET). • All laser lines for Confocal Imaging (Vis and IR) should be used for imaging in SR mode. Depth of penetration for SR imaging should be same as NDD based imaging with better sensitivity. The claim should be supported by white paper and brochure.
5. Control Computers with Monitors.	
Latest 64-bit control computer with Intel Xeon 6 Core Processor, DDR RAM 96 GB HDD: 4 TB SATA upgradable to 8 TB or better, DVD, SuperMulti SATA +R/RW, Graphics: AT Fire GL V5200 256MB DH DVI, Gigabit Ethernet, Win 10 64-bit, USB 2.0 / 3.0, Fire wire. Large 32" LCD TFT monitor to be provided.	
6. System control and imaging software.	
System software capable of controlling Motorized functions of microscope, digital camera, scan head control, laser control including AOTF and Image acquisition & processing. 6.1 Saving of all system parameters with the image for repeatable/reproducible imaging. 6.2 Line, curved line, frame, Z-stack, Time series imaging capabilities. 6.3 Real ROI bleach for FRAP, FRET, Photo-activation/conversion experiments. 6.4 Standard geometry Measurements like length, areas, angles etc including intensity measurements. 6.5 3D/4D image reconstruction from a Z-stack image series. 6.6 Co-localization and histogram analysis with individual parameters. 6.7 Spectral un-mixing and emission fingerprinting with separation of overlapping emission spectra of fluorochromes. 6.8 Image acquisition and processing tolls for SR with various modes of visualization tools should be available.	
7. Hardware and software for offline analysis	
7.1 Additional 1 high-end dedicated PC and HD monitor with specifications given below with image acquisition and analysis software(s) installed along with a printer. 7.2 Latest 64-bit control computer with Intel Xeon 6 Core Processor, DDR RAM 96 GB HDD: 4 TB SATA upgradable to 8 TB or better, DVD, SuperMulti SATA +R/RW, Graphics: AT Fire GL V5200 256MB DH DVI, Gigabit Ethernet, Win 10 64-bit, USB 2.0 / 3.0, Fire wire. Large 32" LCD TFT monitor to be provided. 7.3 Additional offline software with complete features for image analysis should be provided with minimum 3 copies.	
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9. Additional requirements

9.1 The Complete System should include the basic unit and allied supporting components like tables to accommodate all supplied equipment, suitable online UPS (10KVA or better with battery backup of minimum 30 minutes), Dehumidifier (2.5 liters or better), Color Laser jet Printer, Air Conditioner to be supplied by the supplier along with the basic unit.

10. Installation and service supports:

10.1. Supplier should clearly specify the after sales/service/application support capabilities.

10.2. **Warranty** of the system should be 5 years from the date of installation and should cover cost of spares and labor.

10.3. Should provide a comprehensive plan for on-site training, conducting workshops, software upgrade during warranty period.

10.4. Trained engineer & application support within India should be available for onsite training & support.

10.5. Supplier should provide free of cost training in first 3 months after installation to multiple users PLUS one onsite training session to multiple users every six months for the entire period of Warranty.

10.6. During the Warranty period, the supplier is required to visit at consignee's site atleast 2 times in the year commencing from the date of the installation for preventive maintenance of the Equipment/Stores.

10.7. The Supplier along with its Indian Agent and the CMC provider shall ensure continued supply of the spare parts for the machines and Equipment supplied by them to the purchaser for 10 years from the date of installation and handing over. Company should ensure that spare parts will be available till 10 years from the installation.

10.8. Should attend all breakdown calls within 24 hours of the receipt of information from the institute through fax/e-mail/mobile/sms, etc.

10.9. The equipment will be diagnosed with a problem within 72 hours of receiving the complaint and repaired within 4 weeks, failing which the warranty period will be extended by the number of days the instrument is non-functional post 4 weeks.

10.10. Technically qualified man-power/operator should be provided for 3 years. The technical person should be available on all working days of the institute as per the norms. This should be quoted with a monthly salary of the operator to be comparable to that of a JRF i.e., 50000 INR/month or better. The overall cost should also include a general 10% hike of salary every year.

11. Provide all pre-installation requirements for system installation.

12. Provide a detailed list of users and current installations of the system with similar set-up in India with contact details.

Accessories	
Co2 Incubation	• The system should come with a suitable environment-controlled incubation unit with water dipping objectives having full enclosure box for entire microscope. The incubation system should have independent control of CO2 (accuracy 0.1%), Temperature (3°C to 40°C or better), Temperature accuracy of 0.1°C on sample, Humidity. Parameters should be controlled through the same imaging software. Entire system should be provided with CO2 cylinder & regulator
FLIM	• The system should be capable of carrying out at least two channel lifetime-based imaging and analysis of biological and chemical samples. Analysis should consist of identifying different life time-based components in the sample, separation of these different life-time components and gated detection capability to separate photons from signal and/or background