



INDIAN INSTITUTE OF TECHNOLOGY BOMBAY  
MATERIALS MANAGEMENT DIVISION  
Powai, Mumbai - 400076

PR No. 1000025634

Rfx No. 6100001097

**TECHNICAL SPECIFICATIONS FOR TOTAL INTERNAL REFLECTION FLUORESCENCE (TIRF) MICROSCOPY SYSTEM COMPATIBLE WITH FLUORESCENCE LIFE TIME IMAGING (FLIM)**

A state-of-the-art multi color simultaneous imaging, fully motorized TIRF with independent TIRF angle adjustment feature for each wavelength on a motorized inverted fluorescence microscope with following specifications should offered.

**1. MOTORIZED INVERTED MICROSCOPE**

- a) The microscope should have Bright field, Fluorescence and DIC illumination. Microscope frame should have built in Infinity port or Dual deck or equivalent structure for system flexibility.
- b) It should have motorized beam path selection between eye observation and TIRF imaging.
- c) **Built in Motorized Z movement should essentially be at least 10nm or lower for precise movement of different focal planes of the specimen,**
- d) Dedicated Remote TFT / LCD touch panel control for all the Motorized function of microscope should be provided.
- e) **Mechanical right-hand stage with fixed short arm handle. Enough travelling range 120mm an 85 mm (XY) applied for slide glass, 35mm/60 mm dishes, as well as multiwell plate (8, 16 and 94 glass bottom plates), circle stage inserts should be included. Sample holder for slide glass, 35mm/60 mm dishes, as well as multiwell plate (8, 16 and 94 glass bottom plates) should be included. Stage stopper function is implemented for timelapse or operation on stage.**
- f) Low Autofluorescence Immersion Oil should be provided.
- g) High Intensity LED illumination for transmitted light source
- h) **6 positions DIC nosepiece.**
- i) High resolution PlanApochromat objectives, 10X objective 10X/0.40, WD.3.1., Long Working Distance Semi Apochromat 20X/0.45, WD.6.6-7.8mm., Plan Apochromat 100x (NA 1.5) TIRF objective for cell membranes imaging (WD 0.1mm), 100 X (NA 1.7, for single molecule localization studies) and **60 X (Oil) or 100X (oil or air).**
- j) Universal Long working distance condenser NA/ 0.55 W.D 27mm, with positions for DIC, PH. built in iris diaphragm.
- k) A, 120/130 W Hg or metal halide lamp with built in attenuator and shutter should be provided. The motorized fluorescence filter cube turrets should have (DAPI, FITC, TRITC/ EGFP, EYFP, mcherry and Alexa488, Alexa 546, Alexa 594)) filters at a time. These should include narrow band blue excitation, narrow band UV excitation, wide band green excitation etc.
- l) At least 2 empty filter cube for customized filter introduction must be offered.
- m) Right/Backside Side Infinity Port should be quoted with an option to either introduce the laser or to collect the primary image with a dedicated tube lens
- n) The system should have 8 position or higher Coded Fluorescence filter turret
- o) A fully motorized IR laser/LED based drift compensator that can work in real time with cover glass bottom dishes. A one touch autofocus to swiftly recognize the focus position and to maintain the same focus throughout the time lapse imaging experiments,

## **2. MOTORIZED TIRF ATTACHMENT FOR SIMULTANEOUS MULTI WAVELENGTH TIRF IMAGING WITH LASER DEPENDENT PENETRATION DEPTH**

- a) System should be equipped with Motorized Laser TIRF illumination which should allow Laser incident angle adjustment, Shutter Control, and motorized switching to widefield fluorescence excitation. In built FRAP unit.
- b) System should have facility of storing incident angle with single touch button of Imaging software.
- c) The system should be capable of capturing of **simultaneous multi-wavelength/colour** TIRF imaging with different penetration depths for each laser to enable studies on membrane dynamics, vesicle tracking, single molecule imaging (preferable but optional), co-localization, and FRET.
- d) As comparison option the system capable of capturing of **simultaneous multi-wavelength/color** TIRF imaging with same penetration depths can be quoted.
- e) System should have bypass mode for widefield imaging and FRAP option for 488, 514, 561 Lasers. Necessary documentary proof such as brochures and website link to claim the same should be provided.
- f) Lasers (either solid state or Diode, DPSS) of (a) 488nm of power 100-200mW (or more), (b) 561nm with 100mW and (c) 514/515 nm (50mW or more) should be quoted.
- g) Alternatively, either Multiwavelength System with 405-100, 488-50, 561-50, 640-50 Mili watts lasers combiner or Ar Laser (for 488, 514 and 458) nm, along with DPSS lasers (561, 405, and 633) can be quoted, if suitable with TIRF unit.
- h) Quote suitable TIRF filter-cubes, filters for all lasers. Clean up filter for 488, 561, 514 also to be part of the system.
- i) System should be upgradable to the 4th laser line with simultaneously TIRF imaging.
- j) All the lasers should be delivered to the scope through individual wavelength-optimized monomode optical fibers for better and uniform TIRF illumination.
- k) All the lasers should be of minimum 100mW output power or more.
- l) A dedicated Real Time Controller attachment with 7 channel or more input/output for synchronize Shutter, Laser and Camera to work simultaneous imaging.

## **3. HIGH SENSITIVE HIGH SPEED DETECTION SYSTEM (SCIENTIFIC CMOS CAMERA WITH 95-98% QE, BACK ILLUMINATED)**

- a) sCMOS Camera with minimum 95% Quantum Efficiency, 6.5 $\mu$ m x 6.5 $\mu$ m Pixel Area, 2048 x 2048 array - 4.2 Megapixel, 45,000e- Pixel Full Well, 1.3e- Read Noise, 43.5 fps @ 16-bit / 63fps @ 11-bit, facility to increase the Signal to Noise ratio by 3-5 times, 35,000:1 Dynamic Range, Digital Interface should be PCIe, air cooled up to -20 degree.

## **5. ADVANCED IMAGING SOFTWARE WITH HIGH END ANALYSIS**

The quoted software should have the following features:

- a) Basic image acquisition, Complete microscope control, Camera control and Laser control. Multi-dimensional image acquisition such as multi-channel, time lapse and Z stack imaging with both hardware and software autofocus mechanism
- b) Saving of all instrument parameters along with the image for repeatable / reproducible imaging
- c) Time series imaging capabilities with online intensity display.
- d) Software should have real time simultaneous control for all the motorized devices.
- e) Diverse measurement and statistical processing.
- f) Software should be capable to recording intensity profiles and other parameters of Live cell imaging experiments as recorded data.
- g) It should be capable of FRET and ratio matrix, colocalization, FRAP acquisition, FLIM and analysis.
- h) The software should control all the motorized part of the microscope, third party camera, TIRF, lasers, Image splitting device for two color simultaneous imaging, multi camera imaging

capabilities, Macro recording, controlling capabilities of external triggered hardware and DAQ card, capabilities to perform complicated experimental parameters through drag and drop function such experiment manager/Jobs/experiment designer/journals etc.

- i) The microscope, software, DAQ card/Realtime control board, lasers and TIRF & FRAP modules should be supplied from the same manufacturer for synchronized configuration and operation of the system and better post sale support.
  - j) The imaging software should support up to 16-bit image acquisition, high dynamic range imaging, kymograph, FRET, FRAP, dynamic ROI, intensity profiling over time, depth etc.
  - k) The software should have direct hard drive writing capabilities of the images acquired for fast image acquisition.
- l) ANY OPTIONAL SOFTWARE/HARDWARE MODULE THAT NEED TO BE OFFERED TO FULFILL THE TENDER REQUIREMENTS AS MENTIONED IN THE PRODUCT BROCHURE MUST BE QUOTED WITH A SEPARATE PART CODE AND SHOULD NOT BE JUST MENTIONED AS INCLUDED.

#### **6. WORKSTATION FOR THE ABOVE SYSTEM- 1 no**

- a) Top of the line computer with complete hardware control and image analysis capability. Minimum Xeon processor with 4 TB HDD, 48 GB RAM, 512 GB SSD, NVIDIA graphic card of 2 GB, windows 10 pro with 64 bits, wireless keyboard and Mouse. This should be supplied along with two 32" LED monitors and other accessories (32 Inch LED 4K Monitors) for enhanced viewing.

**7. The TIRF system should be compatible for attaching a third party FLIM module to perform TIRF-FLIM imaging. Compatible companies supplying FLIM can be suggested.** A free (camera) port and open access to Fluorescence illumination for the light source should be possible in the quoted TIRF microscope.

**8. The instrument should be quoted with Comprehensive Warranty of 3 years.**

**9. Suitable UPS for backup, 30 min should be provided.**

**10. Suitable floating table for the set up should be provided.**