TECHNICAL SPECIFICATIONS FOR
TOTAL INTERNAL REFLECTION FLUORESCENCE (TIRF) SYSTEM

1. **MODES OF OPERATIONS**

a) The microscope should be completely motorized.
b) The microscope should come with all accessories for Dark-Field, Bright Field (BF), Phase Contrast (PC), Polarizing Microscopy, Fluorescence (FL) and Differential Interference Contrast illumination and Imaging.
c) The microscope should have motorized beam path selection between eye observation and TIRF imaging.
d) The microscope should have built-in Z movement (≤ 25 nm) with high-precision for focussing at different planes of a specimen. The step size is very important both for TIRF measurements as well as for users who intend to integrate TIRF with AFM for super-resolution in Z-axis. Please quote for the best availability in terms of minimum step size which will be preferred. The stage should also be equipped for mechanical changing x,y positions. Both coarse and fine focusing with a knob should be available. This stage should also be upgradable to Piezo-based stage.
e) The microscope should have hardware and software based automatic focus correction to avoid continuous drift caused by mechanical or thermal changes. Users should have control to keep this option “on” or “off”.
f) Mechanical XY scanning stage with universal sample holder attachments for microscope slides, cover slips, cell-culture dishes (35mm dish and higher).
g) Microscope should come with a 12 Volt 100W halogen illumination for transmitted light and DIC applications.
h) Microscope should come with motorized universal turret condensers with ≥ 6 positions at a time. Please quote with the maximum or better availabilities.
i) Microscope should be fit with condensers performing from ultralong working distances.
j) Microscope should have motorized DIC revolving nosepiece to hold ≥ 6 objectives at a time with anti-collision mechanism.
k) The microscope should be well-equipped for fully motorized Total Internal Reflection Fluorescence measurements. This includes software controlled automatic Laser Beam switching, automatic Laser incident angle adjustments for optimum evanescent field depth or penetration for each individual wavelength of light.
l) The microscope software should be able to control the Laser shutter switch, and User should have control over the shutter switch externally by feeding TTL pulses. This is essential for user’s applications.
m) The system should come with FRAP and FLIP imaging module. Please quote with more similar super-resolution modules if available as optional.
n) Shutter switch should work at the rate of 1 kHz or higher.
o) The microscope should have the ability over independent incident angle and penetration depth control, automated focus control and automated beam switching to carry out fast multi-colour TIRF imaging.
p) The microscope should be equipped for Simultaneous multi-colour TIRF imaging. This includes real-time simultaneous multicolour illumination as well as detection. Please attach proof documents from the principle company in their letterhead certifying the same.

q) The system should come with at least 4 colour TIRF Imaging with simultaneous incident angle control for each color. Both simultaneous and sequential illumination options should be available.
r) The system should have options for users to upgrade to Scanning Confocal Microscope, STORM, PALM based super-resolution imaging in future.
s) Microscope should come with motorized universal Fluorescence filter turret with ≥ 6 positions at a time. Vendors are requested to quote with the maximum availibilities. Higher will be preferred.
t) Objective lenses should be designed with high Resolution Apochromatic optics corrected for more than three colors in the visible range (higher order or optimum optical aberration corrections are preferred), should produce complete flatness throughout the field of view, and should have correction rings/collars, springs and the objectives should be corrected for infinite focus distance. Please attach detail specifications of all objectives along with quote.
u) Please quote for the following objectives with necessary DIC attachments: (a) 10X/NA 0.30 or higher(preferred); with phase contrast imaging ability; (b) 40X/NA 0.95 or higher(preferred); (c) 60X oil TIRF, N.A 1.49 or higher(preferred); (d) 100X oil TIRF, N.A 1.46 or higher(preferred); Please quote with the maximum/best availibilities. Higher will be preferred.
v) Modules for DIC imaging for all objectives should be included.
w) 120 /130 Watts pre-centred fiber coupled Metal halide or Mercury lamp for fluorescence observation with proper precautions for obtaining constant and non-fluctuating light.
x) Please quote for 6 sets of Fluorescence filters. Four of them are mentioned here and rest two sets will be selected before finalizing the order.

2/3-color FRET Dye-pair	Excitation Filter	Emission Filter	Dichroic filter
Cy3 (D)-Cy5(A)	Single Band pass (BP) CWL-~530nm, BW- 40nm	Dual Band Pass (BP); CWL1~577, BW ≤ 25 nm; CWL2~ 690, BW ~ 50 nm	Long Pass (LP) with edge wavelength ~ 560nm
Cy3 (D)-Cy5(A)	Dual Band Pass (BP); CWL1~535, BW ~ 35 nm; CWL2~ 635, BW ~ 30 nm	Dual Band Pass (BP); CWL1~577, BW ≤ 25 nm; CWL2~ 690, BW ~ 50 nm	Dual edge Dichroic beam splitter EWL1 ~ 560; RB & TB ≤ 30 nm EWL2 ~ 660; RB & TB ≤ 30 nm
Alexa Fluor 488 (D) - Alexa Fluor 568/594 (A)	Single Band pass(BP) CWL-~486nm, BW- 20nm	Dual Band Pass (BP); CWL1~523,BW ≤ 40 nm; CWL2~ 610, BW ~ 50 nm	Long Pass (LP) with edge wavelength ~ 505 nm
Alexa Fluor 488 (D) - Alexa Fluor 568/594 (A)	Dual Band Pass (BP); CWL1~480, BW ≤ 35 nm; CWL2~ 585, BW ≤ 25 nm	Dual Band Pass (BP); CWL1~525, BW ~ 30 nm; CWL2~ 625, BW ~ 30 nm	Dual edge Dichroic beam splitter EWL1 ~ 505; RB & TB ≤ 30 nm EWL2 ~ 605; RB & TB ≤ 30 nm

y) Vendors are requested to quote the necessary filter accessories for single molecule 3-color FRET separately.

z) Users should have control over all motors to keep ‘on’ or ‘off’. This is essential as users might need to perform in manual mode time to time in order to reduce noise from motors during simultaneous single molecule AFM and FRET. Microscope should use silent motors with minimal mechanical noise and preferably mounted away from the microscope body. Vendors are requested to mention how this complies with their microscope.

2. MOTORIZED TIRF ATTACHMENT:

a) System should be equipped with Motorized Laser TIRF illumination system which should allow automatic / motorized laser beam switching, laser incident angle adjustment, Shutter Control and switching to wide field fluorescence excitation with Software.
b) The system should have facility of storing the incident angles for each laser line in use independently.
c) The system should have or should be upgradeable to at least 4 colour TIRF Imaging with simultaneous incident angle control for each color independently.

3. DETECTION SYSTEM

The detection device should be a high sensitive back-illuminated EMCCD which should have the following features:

a) Active Pixels 512x 512
b) Pixel size 16 x 16 microns
c) Should be able to capture more than 55 fps @ 512x 512. Higher speed with all other requirements are preferred.
d) Quantum Efficiency should be 90% or above at 500nm to 650nm.
e) The detector should come with liquid Cooling capability of T ≤ -80º C. The dark current should be low at this cooling.
d) The detector should be able to detect simultaneously at least four colors for multi-color single molecule FRET applications. Larger image area will be preferred.
f) The detector should be applicable for single photon counting application or should have options for upgradation.

4. LASER Sources

a) The microscope should NOT come with Light Emitting Diodes as excitation sources.
b) Smaller beam dimensions of the Laser is preferable.
c) The laser beam should be collimated. Please attach images of beam profiles for all Lasers without and with fibre-attachments (If any).
d) Vendors should use Single-mode fibres maintaining polarization are required. The beam profile after the optical fibre is require along with quote.
e) The Back-reflection of the Laser emission should be avoided.
f) The power stability of the Laser emission should be high, preferably ≤ 3 % peak to peak. Please provide the Laser output curve with time for reference.
g) Please mention output polarization characteristics along with polarization extinction ratios. The measurements should also be done for emissions from the Laser without or with fibre-attachments (If there any).
h) Fibre-connectors should have high mechanical stability, preferably with Ferrule connector with angled physical contact.
i) The Vendors are requested to quote for the lasers with excitation wavelengths of: (a) 488 nm (preferably Ar-ion multiline), (b) 532 nm, (c) 560 nm, and (c) 633 nm / 640 nm. The Laser power should be the best one available for particular

wavelengths. Please quote separately for lasers along with their individual specification sheet.

5. **SOFTWARE**

The quoted software should have the following features:

a) Basic image acquisition, Complete microscope control, Camera control and Laser control
b) Saving of all instrument parameters along with the image for repeatable / reproducible imaging
c) Time series imaging capabilities.
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 able to reproduce the TIRF angle setting for each laser line independent of each other. It should be capable of handling & controlling Multi laser for simultaneous multi color Imaging.
h) The system should have special experiment management/designing facility so that user can plan his experiments based on different system settings and different experimental conditions.
i) Software must be free and should be accessible for at least two offline users.
j) Free lifetime software update
k) Software for online Image analysis should be available for free of cost.
l) The software should be user-programmable.
m) It is prefferred to have the accessibilies on scripting for the original software to the user to modify experiments.

6. WORKSTATION

a) Top of the line computer i7 processor, Windows OS, 8GBRAM, 1 TB HDD, DVD R/W, Nvidia graphics with High resolution 27” TFT/LCD Monitor should be supplied along with the system.
b) The above system should essentially be supplied with a Real Time control/command board for quick & real time parallel execution & processing of experimental commands to various components of the system without any time lag. System with sequential command should not be quoted.

7. Instrument Isolation.

a) Instrument should come with an active floating, heavy Optical bench of size (~ 47x95 inch) with honeycomb cells embedded.
b) The bread Breadboard float height indicator should be mounted to the optical bench.
c) The table should have tuned mass dampers to reduce table resonances upto 10x or closer.
d) The table should provide a 99% reduction in floor transmitted vibrations above 10 Hz or 60 dB above 10 Hz.

7. Guarantee, Warranty, Support and Service:

a) The instrument should come with five years or more comprehensive warranty on all parts and labor.
b) The instrument software up-gradation should be free for the life of the instrument.
c) The preferred higher versions will always have extra weightage.
d) The vendor must clearly specify how the servicing will be done in case of any requirement. They should provide the contact details of the service engineer along with the quotation. In case the purchase committee decides to go for a technical presentation during/after the tender period, the service engineer should be able to meet the committee for the presentation in a week's notice. The committee will make the decision based on the presentation in that case. The committee's decision will be considered final without clarifications.
e) The company should have at least three installations/sales of their high-end FL microscopes along with the same brand of detectors in last two years in the premier institutes in India, e.g., IISc, IISERs, NISER, IITs, NCL, IMTECH, CCMB, TIFR, NCBS, IACS and JNCASR.
f) Onsite demonstration and Installation should be done. The demonstration should be rigorous and useful for young PhD students.
g) A training session on the instrument is necessary for students after the installation.
h) Integration of the TIRFM with AFM microscope may require the help of the application scientist during installation. Vendors are requested to check with JPK Instruments for the compatibility of microscope with JPK's NanoWizard 3. JPK provides the stage adapter for their AFM to be seated on top of inverted microscope. This is important and absolutely necessary for the integration of simultaneous single molecule AFM-TIRF experiments.
i) Prefernce will be given to vendors who shall have standby EMCCD cameras which can be used as loan in case of emergency breakdown of the user's camera.
j) The system should consist atleast 5 kV UPS. Tables and racks that may be required for the microscope should be included in the quote.

OPTIONAL ITEMS:

a) System should be equipped with Onstage CO_2, O_2 and humidity control incubator for live cell imaging, which can hold slides, Petri plates & multiwall plates of standard size and dimension.
b) Complete chamber with controls and supplies required for controlling and maintaining constant CO_2, humidity and temperature.
c) Should be provided with an extra large dark incubation chamber/dark hood for imaging under ambient light conditions.
d) Vendors are requested to quote an additional x,y piezoelectric stage.

The company should have supplied instruments of similar capability to laboratories/institutes of National/International repute. Please provide a list of installations of similar equipment in India/International Laboratories to justify the same.