High resolution confocal laser scanning microscope:

The confocal microscope should be the state-of-art technology suitable for live and fixed biological samples. The system should be highly sensitive by optics and detection device meeting various needs of modern biological applications including live cell imaging with FRAP, FRET, FLIP, photo-activation/bleaching and photo-conversion experiments. The system should be upgradable to advanced imaging techniques on site in future. The system should be ready to upgrade for super resolution confocal system on site.

The vendor should supply the entire system with all necessary accessories and complete system integration of hardware components. The vendor should be responsible for the complete system installation, functioning, maintenance and training by trained engineers.

The system should be offered with the following configuration:

Inverted Microscope

- 1. Fully motorized inverted microscope for bright field, fluorescence and DIC, with tiltable eyepiece for better ergonomy.
- 2. Motorized Z-focus drive with minimum z-step size of 10 nm or better with dedicated TFT/LCD touch-screen for the control of motorized functions of microscope.
- 3. 6 position motorized FL filter wheel & 6 position motorized nosepiece.
- 4. LED / Halogen illumination for transmitted light & 120W metal halide illumination or LED illumination with 2000 hr or higher lifetime for fluorescence should be offered. In case of LED Illumination in fluorescence mode, min 4 LED's should be part of the configuration (375nm, 477nm, 552nm and 640 nm or equivalent)
- 5. High resolution Confocal Grade Plan-Apo λ blue corrected objectives 10x/0.4NA, 20X/0.7 NA (or better), 40x/0.95NA (or better). Oil, 60/63x/1.4 NA (or better), 100X/1.4 NA (or better) oil. Shift free DIC accessories for all objectives should be quoted.
- 6. Pixel shift free fluorescent filters for DAPI, GFP, RFP, Cy3 and Cy5 should be quoted.
- 7. All of the DIC component including DIC prism of all the objectives should move independently and automatically and controlled by software only to avoid manual jerking during live cell experiments.

8. Motorized XY stage.

High resolution motorized X-Y scanning, specimen stage should be with universal sample holders. Ability to do multipoint, multi well imaging with spatial memory to scan the previously chosen point.

Confocal System Component

9. The confocal detection unit should be with built in Spectral PMT and HyD/GaAsP Spectral detectors. All detectors should be capable of working in Intensity and Spectral mode Imaging. Should be capable of simultaneous detection and separation of at least 5 fluorophores or more, out of which minimum 2 or more fluorophores based on high sensitive GaAsP / HyD detectors with QE 45% or more. All the

detectors should be built in (in the scan head) Spectral type. The spectral dispersion of the emission light should be based on either reflection grating or with prism based dispersion with high efficient spectral detectors.

- 10. System should have UV/VIS/IR ports for future upgradation on site.
- 11. Photo bleaching/ photo activation capability should be included within the quoted system. Suitable laser line 405/408 required for Photo bleaching /Photo activation capability having high power of at least 40 mW and AOTF control for efficient bleaching experiment should be included with in the quoted system.
- 12. All the FL detectors of the scan head should be filter free with freely selectable emission band width detection capability to suit to the emission spectra of the dyes.
- 13. The system should be capable of recording emission spectra with minimum spectral resolution of 5nm or better.
- 14. Computer controlled continuously variable confocal pinhole with software control.
- 15. Maximum scan resolution should be at least 4Kx4K for all channels and higher will be preferred in spectral mode.
- 16. The scan field diagonal should be at least 18 mm (or more) F.O.V. Higher FOV is preferred
- 17. Scan Zoom range 0.8:40x or more and should be adjustable in steps of 0.1
- 18. System should be capable of acquiring 15 or above frames per second @ 512x512 pixel resolution in spectral mode (without line skipping and interpolation) and should increase with ROI and zoom selection. Digitization capability of 8/12/16 bit should be available with the system.
- 19. An additional transmitted light detector should be offered for bright field and DIC imaging.

20. Laser Lines required:

Solid State Laser/ Gas laser units including with the following wave lengths should be connected to the scan head through fiber optic cable and should be controlled through AOTF for fast laser switching and attenuation. Minimum lifetime should be at least 10000 hr.

- a) 448/445, 488nm and 515/514 nm. For CFP, GFP, YFP fluorophores simultaneously
- b) 561nm for Cy 3, Texas Red, TRITC, Rhodamine fluorophores, Alexa Fluor 568
- c) 594 nm or equivalent for m-Cherry, Cy3.5, Alexa Fluor 595 fluorophores.
- d) 633/640 nm or equivalent for Cy5, Alexa Fluor 635 dyes.
- e) 405nm or equivalent for DAPI, Hoechst fluorophores.
- 21. UV 405 Laser with ROI capability
- 22. System should have UV/VIS/IR ports for future upgradation.
- 23. The entire lasers should be switched on/off through single switching power button and should be provided in a closed box with laser combining facility. All the visible lasers should include AOTF control also.
- 24. Low Angle Dichroics for excitation/ emission separation.
- 25. The quoted system should be upgradable to hardware based super resolution system.
- 26. The speed of the system should be upgradable to very high-speed imaging system (40-50 or higher FPS) to capture high speed dynamics.
- 27. The quoted system should be upgradable to Multi Photon System in future, on site.

Software and Computer

It should include / capable of:

- 28. Controlling motorized functions of microscope, scan head control, laser control including AOTF and image acquisition & processing. Software module or facility to image extended dynamic range while acquiring like HDR/BrightR/or equivalent with GaAsP/HyD/APD or equivalent detectors.
- 29. Saving of all system parameters with the image for repeatable/ reproducible imaging.
- 30. Advanced & Dedicated confocal 3D visualization software module to immediately open the multidimensional images like multichannel Z stack with time series. It should be able to play the time series volume as 3D time series movie. It should allow to record the 3D animation with various adjustment like pseudo coloring, intensity, rotation, clipping, 3D enhancement etc., Various 3D projection modes: Transparent, Maximum Intensity, and Depth coding, Stereo images (cyan / magenta, horizontal and vertical shutter, quad- based)3D image reconstruction from a Z-stack image series basic software.
- 31. 2D & colocalization analysis: it should be able to deliver color coded / color scaled images based on the intensity of the signal / level of colocalization.
- 32. Offline software license should also be included for analysis.
- 33. Latest 64 bit control computer with Intel Xeon Processor, DDR RAM 64 GB or better, HDD: 4 TB SATA upgradable to 8 TB or better, DVD, Super Multi SATA +R/RW, Graphics: AT Fire GL V5200 2 GB DH DVI, Gigabit Ethernet, Win 10 64 bit, USB 2.0/3.0, Fire wire. Large 32" LCD/ TFT monitor.
- 34. Online UPS for the complete system including lasers should be included in the supply.
- 35. Service/Manpower: Should provide onsite service with manpower for at least 2 years <u>Accessory</u>

I. <u>High resolution Imaging (Hardware + Software based)</u>

- a) The system should have the capability to produce high resolution data with Lateral resolution of 120-140 nm and based on quoted GaAsP or highly sensitive detector.
- b) It should be able to do Mark & Find or Tile Scan in Super Resolution mode.

II. Incubation system

With Temperature, Humidity and Active CO_2 controls + Software control while doing live cell imaging. The chamber is expected to have a O_2 Level controller to perform hypoxia related live cell imaging.

III. High Sensitive Camera

- sCOMS Camera (minimum 4Mpixel or above) with cooling & speed of 50fps@full frame, Quantum efficiency of 72% or above, pixel size 6.45 µm or above.
- **IV**. An active anti-vibration table with compressed air damping, bread board table top for the complete microscope system and a suitable computer table for the on-line PC should be quoted. The proper make and model should be mentioned.

Other information:

1. Bidders should clearly specify the after sales/service/application support capabilities.

- 2. Provide all information about pre-installation requirements (i.e. room, environment) for system installation.
 - 3. There should be a warranty of 3 years and an AMC is required for 5 years after the warranty period (non comprehensive).
 - 4. Bidders should provide an operator for 2 years and the expenditure should be included.
 - 5. A separate work station with the analysis software should be provided for off-line analysis of data.