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Detailed Technical Specifications for Laser Scanning Confocal Microscope

The Laser Scanning Confocal microscope system with optical slicing capabilities should be capable of high resolution and highly sensitive spectral imaging of fixed as well as live biological samples; should include multichannel fluorescence imaging Z-stack time-lapse imaging including colocalization, FRAP, FRET, advanced quantitative imaging, photo activation and conversion experiments. Should have the following configuration and features:

1. Fully motorized inverted microscope

a) Fully Motorized Inverted Fluorescence Microscope for BF/DIC/FL with dedicated and built-in TFT or separate TAB display for full control of all motorized microscope functions.
b) The microscope body should have built in motorized high-precision Z movement or Z-focus drive with step size of at least 20 nm or better. LED/IR Laser 790-800 nm based drift compensation unit to be included. The drift compensator must be controlled by the microscope touch screen panel)
c) It should have motorized side ports for camera attachment, motorized beam path selection between eye observation and confocal imaging. An additional infinity input port is required for laser input.
d) The microscope should be equipped with high power LED illumination for transmitted light applications and an LED (or 120W/130W metal halide) illumination for Fluorescence application.
e) A binocular observation tube with a pair of 10X eyepieces and anti-fungus type diopter adjustment in both eyes with FOV 22 mm or better should be supplied; eye cups along with eyepiece dust cover should be a standard supply.
f) The system should have at least 6 positions motorized nosepiece and Plan Apochromat objectives
    - 1.25/2.5/4X objective (to image whole spheroid/organoid/scaffold/Matrigel samples) in confocal, BF and widefield modes.
    - 10X (NA 0.4 or higher) WD 2.1 mm or better.
    - 20X/25X (NA 0.9 or higher; Multi immersion) WD 1.95 mm or better.
    - 40X (NA 1.2 or higher; multi-immersion - GLYC/Oil/Silicone) WD 0.3 mm or better.
    - 60X/63X oil (N.A 1.4 or higher; Oil) WD 0.14 mm or better.
    - Plan Fluor/APO long working distance 20X/25X objective (NA 0.7 or better) WD > 1.8 mm with correction collar to adjust variable thickness of cover glass/plastic bottom dishes. The objective should be suitable for imaging

in DIC (for cover glass dishes) and Phase (for plastic bottom dishes/well plates).

- Plan Fluor/APO long working distance objective 40X 0.6 NA CORR, WD 3.3mm.

g) Motorized 6 positions condenser (NA 0.52 or better) with DIC prisms for all objectives with analyzer, polarizer and DIC slider should be included. All the DIC components including polarizer, analyzer and prisms should be coded/motorized and shift free to get complete motorized DIC benefits.

h) 6 positions motorized fluorescence turret with in-built shutter and band-pass or long pass fluorescence filters as follows: DAPI (band pass; excitation – 380-400 nm; emission – 410-470 nm), FITC/GFP (band pass; excitation – 490-500 nm; emission – 510-550 nm), TRITC/RFP/Cy3/Texas Red/mCherry (band pass; excitation – 560-565; emission – 570-630) and Cy5 (band pass; excitation – 640 nm; emission – 655 nm) should be quoted. The turret should accommodate fluorescence filters for sample visualization and camera-based imaging.

i) The linear encoded programmable motorized XY stage should come with universal sample holder attachments for glass slides, 35/60 mm petri dish, petri-plates, multi-well plates and labtek chambers with multipoint, tile and mosaic imaging software. The XY motorized stage and the Drift compensation should be controlled by the imaging software, remote joystick and microscope touch screen/TFT. XY stage should have the option to save multiple coordinates/positions during observation at lower magnification and allow revisiting the saved positions precisely at higher magnifications for multi-point imaging and stitching large specimens with high resolution for observation and quantification of whole organoid/scaffolds.

2. CO_2 incubator

Microscope stage top CO2 incubator with control of CO2, Temperature & humidity for live cell imaging on glass slide, 35mm imaging dish, petri-plates and multi-well plates should be included. It should have a gas mixer so that 100% CO2 gas cylinder can be attached to it. Please include the entire working system in the quote including CO2 cylinder & regulator. The parameters for Incubation system should be controlled by confocal software and/or dedicated TFT display of the microscope.

3. CAMERA

A Peltier cooled color and monochrome CMOS Global shutter/CCD camera that will help image stained tissue sections in colour mode and fluorescence signal in monochrome mode. A 20 MP (or better) Peltier cooled QE 65-70 % (or better), 60 FPS, 21 mm FOV (or better), 5-6 micron pixel size (or better), scientific grade CMOS/CCD camera dedicated for COLOR and MONOCHROME/ FLUORESCENCE imaging. Camera should be controlled by the same confocal software for multichannel, z stack, fast time-lapse wide field imaging and realtime stitching. It should have enhanced black balance and image averaging capability for crisp and brilliant fluorescence images. The camera should be controlled by original PCI express interface card to achieve the desired frame rate. Necessary

brochures for the compatibility for fluorescence imaging should be provided. The camera should be from the microscope manufacturer for better compatibility during multidimensional imaging and stitching.

OR

A 6 MP pixel RGB Bayer colour Peltier cooled CCD camera with progressive scan and a minimum pixel size of 4.5 micron with 30-40 FPS and maximum full well capacity of 15,000e readnoise of 6e/pixel, dynamic range of 1:25,000, actual QE of 65-70%, frame rate of 30-40 FPS - should be offered. The said camera should be from the microscope manufacturer for better compatibility during multidimensional imaging and stitching. Necessary brochures for compatibility of fluorescence imaging should be provided.

4. Confocal Scanning and detection system:

a) Laser point scanning and confocal detection unit with at least 4 channel detectors capable of simultaneous detection and separation of at least 4 fluorophores using detectors working in Intensity and Spectral mode Imaging. System should be a combination of min 2 PMT and 2 GaAsP Spectral OR all 4 are HyD/GaAsP Spectral detectors in the scan head.
b) All the spectral detectors should have independent voltage and gain controls and should be able to perform online spectral separation. At least two should be highly sensitive Spectral GaAsP/HyD type should have QE/PDE higher than 45% or better. Detectors must be filter-free spectral type and should be built-in inside/outside the scan head for better sensitivity.
c) The spectral resolution should be at least be 5 nm or better throughout the spectral range of 400- 800 nm. The spectral dispersion of the emission light should be based either on reflection/transmission grating or prism with an ability to continuously adjust the emission bandwidth from either side of spectrum.
d) The system should have dual scanning capability with Galvo for High resolution imaging with maximum scan resolution of 4K x 4K or better (higher will be preferred) and Resonant scanner for high speed with at least 20 fps (without line-skipping or interlacing) or better at 512x512 pixels resolution (without compromising the FOV by zooming). Higher frame rates of above 210 fps with/without real ROI Scanning capability for fast scan. Higher (Galvo) scan resolution of at least 4K x 4K or better per channel and reduce to 64 x 64 pixels resolution should be achieved with the same scanner set. Scanning zoom of 1-30 times or higher with/without ROI Scan should be achieved. The Galvo and Resonant Scanners should work in tandem for instant photoactivation and photoconversion experiments with Resonant being used for high-speed imaging and Galvo for high resolution meant for photo activation/photoconversion experiments in XYZt imaging of thick tissues.
e) The scan rotation should be 180 degrees or higher with an ability to scan in various scan areas such as rectangle, clip, polygon, free area, line, free line, point circular etc. The system should have computer controlled continuously

variable pinhole and should also be equipped with laser power monitor to maintain the same laser intensity for error free intensity measurement of live cell imaging.

f) Scan Zoom range should be 1X to 30X with increments of 0.1X with scan rotation of 180 degrees.
g) The scan field diagonal (FOV) should be at least 18-20 mm or higher.
h) Digitization capability of 8/12/16 bit should be available with the system.
i) A dedicated transmitted light detector should be provided for laser based DIC imaging.
j) The system must come with fully automated and hardware based online resolution improvement acquisition module to achieve a resolution of up to 120-140 nm or better in XY and 300-350 nm in Z. Detection should be based on highly sensitive detector with quantum efficiency of 45-55% or higher or dedicated detector for super resolution. The super resolution system should be able to capture at least 2 fluorophores simultaneously to perform live cell imaging in super resolution mode. The super-resolution image capturing speed should be same as the confocal imaging.
k) Scanner unit have laser ports for at least 4 lasers to be integrated with the system, upgradable to additional lasers, if required.

5. Lasers and Combiner:

a) All visible and UV lasers should be stable solid-state lasers connected to the scan head through fiber optic cable and controlled by AOTF for precise switching, swift selection and attenuation in pixel precise synchronization with the laser scanner for Real ROI scan for FRAP, Photo activation/conversion experiments.
b) All lasers should be controlled through an acousto-optic tunable filter (AOTF) for ultrafast laser switching and attenuation in computer-controlled manner.
c) Desired visible laser lines ~488 nm, ~514 nm, ~561 nm, 594 nm and ~638 nm or equivalent. All individual laser lines should have at least 20 mW or higher output power. It should allow SIMULTANEOUS imaging of both GFP (488nm) & mCherry (594nm) with high sensitive detector for live cell imaging applications. GFP and mCherry online spectral unmixing should be possible to avoid any autofluorescence from tissues or 3D Scaffolds/Matrigel.
d) The UV laser port and laser line ~405 nm should be with at least 20 mW power (also controlled by AOTF for precise Photo activation/Photo conversion/Real ROI bleaching experiments).

6. Confocal Software:

a) The imaging software should control all the motorized functions of the confocal microscope, digital camera, confocal scan head, laser control including AOTF and image acquisition & processing.
b) It should have the capability of multi-dimensional acquisition namely PT (point), XT, XZ, YZ, XZT, XYT, XYZ, XYλ, XYZT, XYλT, XYλZ, XYλZT (line, curved line, frame, z-stack, time-series) along with 3D image re-construction.

c) Allow saving of all system parameters with the image for repeatable/reproducible imaging.
d) Standard intensity and geometry measurements like length, areas, angles etc.
e) Advanced software for 3D reconstruction and processing of 3D data having features like Transparent, Maximum Intensity and Depth Coding, shadow projection, clipping, Orthogonal Sectioning and Annotation tool to add comments to 3D volume.
f) Real time/online Spectral images and unmixing, Multi point imaging, image stitching/auto montage; Macro imaging capabilities, HDR imaging capability, Calcium imaging, deep tissue imaging should be included.
g) Real ROI bleach for FRAP, Photo-activation/conversion experiments should be possible.
h) Spectral un-mixing with fingerprinting for separation of overlapping excitation/emission spectra of fluorophores should be possible.
i) The software should have analysis function such as intensity measurement (online & offline) over time, over depth and over lambda. Acquisition and analysis for advance measurements FRAP, FRET, Co-localization, photoactivation and photoconversion experiments should be possible.
j) Well-navigation software module for multi-well plates, stitching and overview bigger samples should be offered.
k) Deconvolution in 2D and 3D should be possible, which is very important for thick scaffold/tissue samples.
l) The confocal software should be able to control the third party EMCCD & SCMOS cameras such as EVOLVE 512 Delta and Hamamatsu flash 4 V2 with full functions and should be able to perform multi-dimensional (multichannel, multipoint time lapse and Z section multiwell and automated stitching) imaging.

7. Anti-Vibration Platform:

Active anti-vibration table with active air-dampening with air compressor and bread board to be supplied with the Confocal system from a reputed manufacturer. Also, a computer table and rack for lasers to be provided preferably from a reputed manufacturer.

8. Workstation:

The system should be supplied with latest computer workstation tried & tested in factory by the confocal manufacturer. It should have at least the following specifications: Windows 10 Professional (64 bit) operating system, Intel 10-Core Xeon processor, minimum 64 GB RAM, NVIDIA 8GB graphic card, 1TB SSD, 4 TB SATA HDD, Slim Super Multi DVD Writer, Ethernet Controller, 2 x USB 2.0, 8 x USB 3.0, IEEE 1394 Firewire with wireless Key Board and Mouse with 32-38” High resolution LED HD 4K Monitor.

9. UPS:

Suitable 6 KVA online UPS with minimum 30 mins back up should be provided along with the system to run the entire system including the offline system and lasers.

10. ***Warranty***

At least 5 years of service warranty from the date of installation of confocal microscope covering cost of spare parts and labor should be included.

11. ***Site preparation***

Qualified vendor should provide all the required accessories/modules for fully functional microscopy room.

12. ***Additional requirements***

a. Detailed service manual must be supplied along with instrument.
b. Detailed operating manual for Instrument as well as software must be supplied with the instrument.
c. Onsite training should be provided as part of the Installation to users at IITB. Training should include working on all the lasers that are provided with the system and data analysis with the software included in the bid. During training, the vendor must demonstrate performance specifications of the system like resolution, sensitivity, scanning speed, working distance of objectives, etc. at optimal operating conditions.
d. All mandatory upgrades by the manufacturer should be performed as and when the manufacturer introduces both in software and instrument hardware.
e. Instrument quoted should be a complete system in all respects; any additional accessories required for the instrument to operate should also be quoted as part of the instrument and should be supplied along with the Instrument.
f. Supplier should clearly specify the after sales/service/application support capabilities.
g. Should provide a comprehensive plan for on-site training, conducting workshops, software upgrade during warranty period.
h. Trained engineer & application support within India should be available for onsite training & support.
i. Supplier should provide SOP documents and 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.
j. During the Warranty period, the supplier is required to visit consignee’s site at least twice a year commencing from the date of installation for preventive maintenance of Equipment.
k. 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.

l. Should attend all breakdown calls within 24 hours of the receipt of information from the institute through fax/e-mail/mobile/sms, etc.
m. The equipment should 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.
n. A detailed list of users and current installations of the system with similar set-up in India with contact details should be provided.