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## Technical specifications for Super-Resolution Microscope with storm Imaging platform.

The imaging platform should include i) Microscope with 2D and 3D STORM / PAL-M / TIRF (not quasi TIRF) modes of imaging for single molecule localization for fixed and live cells and ii) Confocal mode of imaging with deconvolution.

**A. Inverted microscope (Fully Motorized)**

a. System should have Bright field, Fluorescence and DIC Imaging capability.
b. System should have fully motorized beam path selection for widefield, 2D/3D SIM/ Multi Point Array Scanning, with 2D, 3D STORM/ PALM and TIRF modes of imaging. Also, the system should have Single deck or dual deck configuration for combining all these techniques such as widefield, 2D/3D SIM/ Multi Point Array Scanning, with 2D, 3D STORM/ PALM and TIRF modes of imaging on the same platform.
c. System should have motorized Peizo/Galvo Z-axis focus drive optimal for super resolution imaging with minimum resolution/step size of 5 nm or less.
d. System should have 6 position or higher motorized FL filter wheel for excitation and emission path and 6 position motorized DIC nosepiece
e. Motorised XY scanning stage for the movement of specimen using ergo joy-stick as well as total control by the software. Stage holder should be universal type capable for holding 35/60 mm dishes and chambered cover-glass apart from standard slides and multi-well plate.
f. System should include a minimum of 12v/100w halogen/LED illumination for transmitted light with automatic shutter having DC (direct current) to provide constant and non-fluctuating light.
g. Motorized universal condenser turret with NA 0.5 or better; motorized 6 (or better) position objective turret with 6 or more DIC slots; motorized filter turret with at least 6 positions for band pass fluorescent filters for sample visualization. Band pass filters for GFP/FITC, DAPI, TRITC/ Rhodamine/ cy3, CFP/YFP should be quoted.
h. System should be supplied with high resolution objectives 10x, 40x/0.95 NA, 60x/63x Oil (N.A 1.40) and 60x/63x Water (N.A 1.27 or better) for deeper SIM Imaging and 100x oil/ NA 1.49 and 100x Silicone oil lens (N.A 1.3 or above) should be usable for SIM or Multi Point Array Scanning Technology and Localization imaging. System

should also have 40X (NA 1.2 or above) Silicone immersion objective. [Silicone objectives should be given as optional]

i. System should be equipped with correcting spherical aberrations by using automatic collar correction system for achieving best PSF for user convenience for High N.A objective for TIRF/STORM/PALM experiments
j. Automated DIC accessories for all objectives. Band pass fluorescent filters for sample visualization should be offered.
k. The microscope system should be capable of conducting long duration live cell imaging with image acquisition occurring at intervals of msecs to secs. The microscope should be equipped with hardware to correct for focus drift through a LED or laser (wavelength 750 nm or more) based continuous focus correction system for long term live cell 2D/3D SIM/ Multi Point Array Scanning and 2D/3D STORM/PALM data acquisition. The system should be able to store large datasets obtained from long-term live cell imaging.
l. System should be capable to have Automatic Water dispenser for Long time live imaging with water objective.
m. System must be equipped with a complete cage enclosure with weather controlled environment including independent control for $CO_2$, $O_2$ (in form of air) and, humidity, $N_2$ for hypoxia experiments. The incubator enclosure should have the following: dark or transparent panels, temperature range from ambient 25 deg C to 45 deg C. The temperature accuracy on the sample should be +/- 0.3 deg C or better. The air-filtering unit for inlet air, sliding doors for easy handling and illumination inside the enclosure with suitable light should be provided.
n. DIC attachment motorized for 10x to 100x objectives with analyzer and polarizer attachment, sliders and modules for the respective objectives.
o. The system should be capable of imaging close to the cell membrane within a depth of 150 nm or less using the TIRF module.
p. High-performance (Newport, Melles Griot, Thorlab or equivalent) active vibration isolation lab table should be quoted.

**B**. **<u>Localisation Based Super Resolution mode (PALM/STORM)</u>**

a. The system should be capable of achieving a X-Y resolution of 30 nm or better

b. Resolution in Z should be 60 nm or better

c. The system should be capable of doing localization in different modes: 2D, 3D and TIRF mode [TIRF should be fully Automated (XY and Z focus automated) and with automated critical angle search]

d. System should have capability of Live cell imaging with different format using 128x 128/

256x 256 and 512x 512 in super-resolution mode. This should be documented on the website/brochure.

e. Data should become available as the images are being acquired. There should be image based auto focussing. Multi-channel or multi-color acquisition in a sequential mode should be possible

f. Should be capable to acquire different imaging area like 80 micron x 80micron / 40 micron x 40 micron / 20 micron x 20 micron & 10 micron x 10 micron

g. The system should be able to use a wide range of available fluorescent proteins as well as organic dyes (photoactivable, photo convertible, photoswitchable and photochromic) for working with the system. Photoactivation controls and processing tools for PALM / dSTORM should be available.

**C. Laser unit**

- All DPSS/Diode high power, long life lasers; pre-aligned having AOTF control with following lines and should be workable with both Confocal and Localization based super-resolution techniques:
- DPSS/Diode 405nm
- DPSS/Diode 445nm
- DPSS/Diode 488nm
- DPSS/Diode 561nm
- DPSS/Diode 647/640/635nm
- DPSS/Diode 514 nm

- The lasers should have appropriate power for STORM and Confocal usage.

**D. Separate detection system, should be capable of epi-fluorescence and Super-Resolution techniques (Localisation based method)**

Detection for Super-Resolution modules should be based on Scientific CMOS camera having effective no. of pixel 2k x 2k or better with Pixel Size of 6.5 microns x 6.5 microns and it should be capable of acquiring at a max speed of 89- 95fps or better @ full frame, Q.E, should be more than 90%. Readout noise should be 1.0 e- or better with full well capacity should be 30,000 electron or better or having similar output with justification will be considered for Super Resolution modules. Optimal FOV of 80 µm X 80µm or better using 60X or 63X/1.40 oil objective or equivalent objective should be available.

**E. Confocal and widefield (for observing the sample) module**

The system should be able to switch to confocal and widefield mode without any additional hardware alignment.

a. The system should be capable of archive confocal effect using point scanning or equivalent method with additional capability to remove out of focus light to achieve X-Y resolution of 120 nm nm or better and Z resolution of 300 nm or better.
b. System should be able to achieve high speed – up to 20 fps (512X512 for 1 channel-- to get high temporal resolution and low phototoxicity and photobleaching to allow live cell imaging.
c. System should have high quality excitation and emission filters and dichroic mirrors

d. Laser scan head

- Confocal laser point scanning unit should be quoted. It should be capable of line-scanning in 1D and 2D (space-time) as well.
- Scanner should have laser ports to connect to above mentioned lasers and should have either low angle of incidence semrock or crystal-based dichroic for high efficient excitation laser suppression.
- Motorised and computer-controlled continuously-variable confocal pinhole with software control.
- High speed XY galvo scanner with min.180 deg scan rotation with total scan flexibilities of line, free hand curved line, XY, XYZ, XYZ t and XYZ t ג combinations.
- The laser scanner should have dual scan capability of fast volumetric regions bleaching/activation/ablation & normal scan for Imaging to conduct experiments like FRAP, FRET, FLIP, photo activation/conversion.
- Scan resolution should be at least 4K x 4K for all channels and can be selected freely down to 64x64 pixels.
- Scan Zoom range 1.0x to 40x or more and should be adjustable in steps of 0.1.
- System should be capable of acquiring minimum of 6-8 fps or higher at 512x512 pixel resolution. It should also be capable of ROI-based scanning. Should also have speed of more than 20 fps @ 512X512 with more than 20 mm FOV and upto 700 fps @512X16.
- Scan field diagonal should be 20 mm or less @ up to 20 fps (512X512).
- Data acquisition and digitization capability with at least 8, 12 and 16 bit should be available.

## E. Detectors

a. The detection unit should have dual detection capability with intensity-based confocal imaging as well as spectral confocal imaging.
b. Should have minimum 4 detectors (of which at least 2 should be highly sensitive GaAsP/HyD) and a tunable spectral detector like GaAsP/HyD or equivalent with minimum 45% quantum efficiency. The system should be capable of simultaneous detection and separation of at least 4 fluorophores with built-in or separate confocal detectors. The system should have one transmitted light PMT detector for laser scanning DIC imaging.

c. The spectral dispersion of the emission light should be of latest technology with high efficient separation. The system should be capable of online separation of autofluorescence and bleed through.
d. Capability for lambda-scan should be quoted.

e. The system should be capable of recording emission spectra with minimum spectral resolution of 10 nm of better.

## F. Controlling and Analysis Software

Should meet the following parameters and requirements:

a. Basic image acquisition, Complete microscope control, Scan head control and Laser control software. Same software should be capable to control Super-Resolution microscope, confocal and widefield system
b. Saving of all instrument parameters along with the image for repeatable/reproducible imaging
c. Frame/line/lambda capturing, Z-Stack, Time series imaging capabilities
d. Multipoint Time Lapse Imaging.
e. FRET analysis
f. Co-localization analysis and 3D volume rendering
g. 3D measurement
h. Real time ratio-display
i. 2 D and 3D image deconvolution
j. Diverse measurement and statistical processing
k. The software should have the capability to show two live windows for two cameras while performing simultaneous dual colour imaging
l. Direct streaming of data and parallel processing while streaming of data should be possible.
m. Huygens Professional deconvolution software [Optional]
n. Two licenses of the main software with all the features.

**G. Workstation**

a. Dual Intel Xeon Gold 6226 2.7GHz, 3.7GHz Turbo, 12C, 10.4GT/s 3UPI,
b. 19.25MB Cache,HT(125W) DDR4-2933, 1TB OS SSD, 8TB Fast Acquisition Drive (RAID-0), 20TB additional storage (RAID-10), 1300W power supply, backlit wired keyboard
c. 4 × USB 3.0, 4 x USB 2.0, 2X Gigabit Ethernet, Windows 10 Professional
d. 24" LCD TFT wide aspect true colour monitor 2 no. for connection to workstation to enable 2560 x 1600 pixel resolution
e. Another Identical workstation with 32 TByte storage capacity for complete offline analysis of all the imaging data should be available

**H. Compatibility**

a. The entire system (microscope, lasers, workstation , softwares, power requirement) must be compatible with a SIM/Multi Point Array Scanning Super Resolution System. The system (Localization Based Super Resolution technique or Confocal) should be controllable with the same software been used for the for the SIM/Multi Point Array Scanning Super Resolution System for the purpose of correlative study on the same field.

**I. Power back up for the entire system**

a. A suitable ONLINE UPS system (with back up for 30 min and with voltage stabilization capability) for trouble-free operation for the complete system

**J. CMC**

a. Warranty should start from the day of installation + 2 yrs of CMC + 2 yrs of AMC
b. In case of downtime, the system should be attended within 48 hours of complaint.

**K. <u>Operator</u>**

a. One company trained operator should be recruited and maintained by the company onsite for three years from the date of installation.

**L. <u>Other requirements</u>**

a. 100% CO2 and O2 cylinder with regulator, dehumidifier and two 2 ton switchable converter ACs.

b. Additional computer table for CPU and monitor and second workstation

c. The bidders should provide full details of after sales service support and detailed list of users in India over the last 3 years with contact details.

**Note- Bidders must mention clearly the Room dimension/conditions required to install the above set-up.**

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