INDIAN INSTITUTE OF TECHNOLOGY BOMBAY

MATERIALS MANAGEMENT DIVISION

Powai, Mumbai 400076.

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Technical Specifications of High-throughput Cell Imaging System

1. MICROSCOPE

a) System should have Inverted microscope with bright field, fluorescence and DIC Imaging capability.
b) 10X eyepieces with diopter adjustment
c) Motorized Peizo/Galvo Z-axis focus stage with minimum step size of 5 nm or less in Z axis.
d) 6 position or higher motorized FL filter wheel for excitation and emission path and 6 position motorized objective turret.
e) Should have motorized universal condenser turret with NA 0.5 or better.
f) The microscope system should ensure minimum time lost in changing filters and ensure precision overlap of images.
g) The excitation and emission filters should be installed in separate filter wheels. Rotations of dichroics, emission and excitation filters should be independent. Switching time between adjacent emission filter positions should be at least 300 ms or less.
h) The microscope system should be capable of conducting long time live cell imaging in the time range of minutes to days with image acquisition occurring at intervals of msecs to secs. The microscope should be equipped with hardware to correct for focus drift.
i) System should be able to achieve high speed – 400 fps or higher (512 X 512 pix). Should have very low phototoxicity and photobleaching to allow long duration live cell imaging.
j) The system should have hardware based autofocusing for long time imaging.

2. OBJECTIVES

a) System should be supplied with high resolution plan apochromatic class objectives 10x/0.4 NA or better, 40x/0.6 NA or better, 60x/63x Oil (N.A 1.40 or better), 60x/63x Water (N.A 1.20 or better), and 100x oil (N.A 1.40 or better).
b) DIC condenser and nomarski prism for 40x, 60x and 100x objectives.
c) A 4 or 5 position DIC attachment for 10x to 100x objectives with analyzer and polarizer attachment, sliders and modules for the respective objectives.

3. STAGE

The stage position should be encoded with respect to the fixed Z position objective lens. The stage should be calibrated and qualified using its internal absolute position encoders.

a) Stage repositioning feature for keeping moving cells in the field for live imaging.
b) Microscope step resolution: 250 nm or better in X and Y.
c) Focussing range 7 mm or higher, automated: X-Y Stage travel 100 mm x 70 mm or better.
d) Stage speed: 25 mm/sec in X/Y or faster; 2.5 mm/sec in Z or faster
e) Should have a high precision motorized X, Y stage and Z axis with capability to image 35 mm and 40 mm culture plates, conventional slides and multi-well (6, 24, 96 and 384) SBS format plates and chambered cover glass.
f) System should be controllable by software as well as by joystick.
g) Stage should allow panel collection for stitching multiple high magnification images into a single image without individual image manipulation.
h) Each motor (X, Y and Z) should move independently of each other to ensure linear motion in each axis.

4. ILLUMINATION

a) Bright field LED illumination with lifetime more than 20000 h and with instant on-off computer controlled operation.
b) Fluorescence LED light source of wattage >40 W and lifetime more than 20,000 hr and with seven wavelengths in the range - 381-399, 426-450, 461-489, 505-515, 529-556, 563-588, 621-643 nm to cover the entire imaging spectrum.
c) Wavelength switching should be in the speed of 1 ms or better.
d) The illumination source should be factory integrated with company's own software without any third-party software.

5. WORKSTATION

a) Coat core processor, 32 GB RAM; 1 TB HDD, 0.5 TB SSD; 64-bit OS; 4GB GPU must be compatible with SRRF for faster GPU based processing.
b) High resolution 24" LCD TFT wide aspect true colour monitor (2 nos) for connection to workstation to enable 2560 x 1600 pixel resolution
c) Another identical workstation with 32 TB storage capacity for complete offline analysis of all the imaging data should be available
d) Two copies of software license, one online and one offline.

6. SOFTWARE

a) The software should be owned by the company and should control all the computer controlled/motorized components of the system.
b) The software should possess the following features:
   i. Multi-point imaging, controlling bright field and fluorescence shutter, changing the fluorescence filter, snapping image, stage control.

ii. Interactive System control with sophisticated multidimensional data acquisition, visualization, analysis, image restoration, image correction and image viewing management.
iii. Camera Control.
iv. Data acquisition must have features like Time lapse, 3D stack, Multi-channel, multipoint acquisition, 2D and 3D image deconvolution.
v. Software must have a quantitatively validated deconvolution solution generating accurate measure of sample fluorescence through image restoration algorithm.
vi. The deonvolution algorithm should be Non-subtractive restorative 3D deconvolution
vii. True OTF (Optical Transfer Function) calculated on the system.
viii. Colocalization analysis.
ix. 3-D volume rendering of the images and 3D measurement
x. FRET analysis.
xi. Multi point imaging.
xii. Contrast based Auto Focus.
xiii. Saving of all instrument parameters along with the image for repeatable/reproducible imaging.

c) The software should have the capability to show two live windows for two cameras while performing simultaneous dual color imaging.
d) Latest software upgrades should be provided free of cost for 5 years.

7. CAMERA

a) Back illuminated sCMOS camera.
b) Maximum field of view - 2304 x 2304 imaging array.
c) 6.5 µm x 6.5 µm pixels.
d) 16-bit dynamic range.
e) 90% or more quantum efficiency at 550 nm.
f) 272.3 MHz readout speed.
g) Readout noise should be 0.9 e- or better with full well capacity should be 30,000 electron or better. Optimal FOV of 80 µm X 80µm or better using 63X/1.40 oil objective should be available.

8. LASER FREE CONFOCAL ATTACHMENT

a) Confocality: Atleast 0.6 micrometer (full width at half maximum) with 1.4 NA oil objective.
b) Optics should be based on combination of spinning disk with grid like patterns for structured illumination microscopy
c) Spinning disk speed: 3,000 rpm or better

d) Should include broad spectrum LED illumination with spectral coverage from UV to red region (16 LED based Light Engine) with remote operational control, wattage of >40 W and long life-time >10000 hours (coverage: 370-700 nm) and individual LED should be controlled for the imaging of very specific dyes in future.
e) Should include a digital sCMOS camera with quantum efficiency of >80%, minimum effective number of pixels:2048 x 2048, cell size 6.5-micron X 6.5-micron, effective area of 13.3 mm X 13.3 mm, readout speed of at least 100 fps (full resolution, standard scan, camera link).
f) Compatible camera detector and light source port should be available within the Scanner.
g) Band pass filter cubes for detection of fluorophores: DAPI, FITC/GFP, RFP/DsRed, Cy5 imaging should be supplied.
h) Imaging software that can control camera, confocal unit, and XYZ stage as well as is customizable for additional configurations.
i) Compatible deconvolution at high speed, minimal bricking artefact and high FWHM resolution and correct PSF estimation (Microvolution or Similar) should be provided.

9. AMC

a) 2 years of CMC from the date of installation followed by 1 year of AMC should be provided for the machine.

10. OTHERS

a) CO2 incubator: System should have live cell chamber for controlled CO2 (0-10% with accuracy of 0.1%), O2 (1-20% with accuracy of 0.1%) temperature (30 C above ambient to 600 C with 0.1°C accuracy) and humidity.
b) High-performance active vibration isolation lab table should be provided with the system.
c) The spare parts support should be provided for a period of ten (10) years from the date of installation. The principal agent should be responsible for the complete installation, testing, integration of the system and training.
d) All operating, technical and service manuals with circuit diagrams should be provided along with the system. Tools necessary for calibration of system like calibration objectives & test samples to check the system performance etc., for fluorescence & co-localization checking should be supplied along with the system.
e) Original literature with complete specifications should be given.
f) Detail list of Publications, users and references should be provided.
g) Warranty- One year