INDIAN INSTITUTE OF TECHNOLOGY BOMBAY

MATERIALS MANAGEMENT DIVISION

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Rfx 6100000593

Technical Specifications for Cryo-TEM system

Cryo-TEM system consisting of A.300KV cryo-EM, with accessories for sample preparation and loading: B.120kV cryo-EM screening machine C. Sputter and carbon Coater D. Automatic Plunge Freezer E. Cryo-ultramicrotome F. Glass Knife Maker G. Block Trimming Unit H. High pressure Freezer.

Sr. No	Item	Technical Specifications	Marks
A	**Cryo Electron microsocope**		
1	Accelerating Voltage	300kV - user changeable from 80 kV – 300kV variable either in steps or continuous. Factory aligned at 80, 200 and 300 kV.	2
2	Electron Source	Source should be Field Emission Schottky type or cold Field Emission Gun (FEG) emitter, capable of generating very bright, stable and coherent electron beam with an energy spread of ≤ 1.0 eV	2
3	Magnification	TEM Magnification: minimum ~50x or less to maximum ~1.2Mx or highermagnification: A point resolution of 0.25 nm or better, and information limit of 0.14 nm or better.	2
4	Cooling system	Close circuit, automatic temperature and flow rate-controlled water-cooled chiller	2
5	Lens System	System consisting of condenser lens, objective lens, diffraction, intermediate and projection lenses for providing a parallel beam for high resolution, phase-contrast and low dose imaging as well as electron diffraction. The beam intensity should be user selectable, and documentary proof of quantitative values for convergence angle, size of illumination and electron dose should be provided. The intermediate and projector lenses should have the following characteristics: The range of camera lengths in diffraction mode should be 300 - 5000 mm, or better, at 300 kV. The magnification range should be 50x – 450,000x or better, with reproducibility to within ±1.5%, and should produce distortion and rotation free images. The system should have constant power objective lens, with minimal aberrations at eucentric point. All lens systems should have low	5

		hysteresis, and fast switching between operation modes should be possible. The following aperture holders are required: an objective aperture holder, with at least two apertures appropriate for different imaging conditions; two condenser aperture holders (C1 and C2), each with at least four apertures; a selected area aperture holder, with at least four apertures. All aperture holders must be motorized to maximize the degree of automation.	
6	Vacuum System	Microscope should have suitable vacuum system with a fully automatic, differential, oil free pumping system and ion-getter/sputter ion pumps. The pumps should be adequate in number for column, gun and specimen chambers, to maintain a pressure in the gun area $\leq 10^{-7}$ Pa, and that column $\leq 10^{-6}$ Pa. Appropriate vacuum pump for camera section should also be included. A fully automatic sequential control for operation of vacuum pumps is required. Pumping time should be less than 60 minutes from start to optimal vacuum and the vacuum recovery time after specimen exchange should be less than 10 minutes.	3
7	Autoloader/ automated specimen exchange system	An automatic system with loading capacity of at least 4 grids with minimal breaks in vacuum is required. Specimen insertion, inspection of the grid, recording of initial image and grid exchange should be quick in the span of 30 minutes or less. The grid exchange mechanism should be automated, reliable and free of ice contamination. The specimen holder should be able to tilt up to at least ± 70°. Rotation of the specimen to at least 90° in plane for dual axis tomography is desirable but not strictly required. In plane rotation at angles other than 90° is also agreeable. All accessories required for operation in cryo- as well as ambient temperature, and to meet the conditions mentioned above, should be provided. An extra autoloader cassette / automated specimen exchange system should be provided.	3
8	Specimen chamber	The stage should be computer controlled and its position should be reproducible. After a specimen movement of 500 µm in x and y, the stage should relocate to the same sample position with a reproducibility of ≤0.5 µm. Microscope should have a fully Eucentric goniometer with amaximum tilt in the range of +/-70 (±1.0 mm) degrees or higher X movement range: 2 mm in total or more Y movement range: 2 mm in total or more Z movement range: 0.20 mm in total or more with a specimen grid size of 3 mm. The maximum sample drift rate should be 0.01 nm/s after complete equilibration. The specimen drift rate should be ≤ 0.25 nm/s or better, and ≤ 0.05 nm/s or better, after 30 minutes and 90 minutes of specimen exchange respectively. The specimen height should be adjustable to allow eucentric tilting. The eucentricity during ±70° tilting should be ≤ 2 µm in X and Y, and ≤ 4 µm in Z (defocus change).	3
9	Direct Electron Detector	Direct electron detector camera with atleast 4K x 4K pixels, minimum	8

		frames 200 frames per secondand functional at 200 and 300 kV. Frame rate 250 fps or better with real-time fast counting and super resolution or integration read out modes. It should have radiation hardened back-thinned sensor with sensor lifetime of at least 500 million e/pix, with automated magnification calibration, real time fast counting and super resolution or integration read out modes. An extra microprocessor chip for direct electron detector should be provided within warranty period. The detector and software provided should be able to do sub-pixel averaging for more accurate determination of incident electrons and also preferably running in a Correlated Double Sampling (CDS) mode to minimize read noise. The detector should allow visualization of Thon rings in a high-dose image from an amorphous carbon or Pt-Ir specimen out to the Nyquist resolution in the Fourier transform of an image that is at least 4K x 4K pixels. Camera compatibility should be ensured for either the in-column or post-column energy filter as per the specifications provided. Standard and suitable PC for the operation of the direct detector should be provided. PC should be factory fitted and tested with pre-loaded, licensed software for trouble free operation of the system. Option for high speed attached RAID storage is required for the PC (using Fibre Channel). A complete software suite for all camera functions, low dose readout and low dose automated data acquisition is required. The software controlling the detector is expected to be fully embedded into the operation system of the microscope. Automatic data collection and image acquisition, and camera control should be from the same software platform. The software should include a range of pre-configured settings for operating voltages between 80 and 300 kV that can be easily selected for use with appropriate standard samples. It is expected that the images, data and metadata acquired, and subsequently analyzed and/or manipulated by the user will be saved in a default format that is widely compatible with other software packages. Details of the proposed software should be included in the tender submission along with any known, or suspected, incompatibilities with other packages. It should be possible to export the acquired data and metadata from the system in multiple formats (e.g. .mrc, .tiff, .jpeg, .png, .txt, .xls etc). A proper frame alignment software, to align the movie frames of collected movie images using the detector, should be supplied.In line /off line motion correction software with latest NVIDIA GPU hardware workstation should be provided. Preferbrable configuration for GPU workstation as follow:minimum scalable 2X intel xenon CPU processors 8 GPU cards and minimum 10 TB hard drive capacity, with 256 GB RAMbased. It is necessary that remote access to, and analysis or manipulation of, data and images is available to users while the detector is collecting	

		primary data. The proposed system should have a high-speed transmission capacity (e.g. 10Gb/s (ten Gigabit/second) Ethernet or equivalent.The safety controls must be implemented in software as well as in hardware for protecting the operators, instrument and specimens.Thesoftware(s) should be updated as required, free of cost.	
10	CMOS based camera	An additional fast CMOS scintillation based readout with minimum 4k x 4K pixels, CMOS sensor, pixel size 14 μm or larger. Usable at 80 – 300 kV. Capable of large field of view with video capture of 25 fps or better at full resolution. It should be optimized for experiments such as single-particle, tomography and low dose techniques such as micro-electron diffraction and should be compatible with image as well as video recording mode. This camera should be bottom mounted, retractable, compatible with high-resolution CMOS camera of minimum 4k X 4k pixel @ 25 fps with full resolution of 4k x 4k such that it is compatible for automated operation with a direct detection camera. The camera should be fully embedded with data collection/application software and hardware.	3
11	Phase plate	An automated Phase Plate activation measurement system should be provided. The type of phase plate, stability, location and expected contrast enhancement should be clearly mentioned. It is expected that the phase plate should be able to enhance contrast by a factor of 1.4 or more. All phase-plate specific consumables for regular operation of the cryoEM within the warranty period should be provided.	4
12	Energy filter	The microscope should have either an in-column or a post-column energy filter. The energy filter (EF) will be used for zero energy-loss imaging of biological samples (frozen biological samples or sectioned cells/tissues).The EF should have minimal geometrical distortions, preferably less than 1%. The alignment and tuning of the filter should be as automated as possible. The filter is expected to be well-screened from any ambient low-level AC magnetic fields and can be adjusted/aligned to compensate for the effect of stray AC fields in the ambient environment of the microscope room for voltages of 80 – 300 kV (eighty to three hundred kilovolts). In both post-column and in-column energy filter systems, the microscope should be equipped with a high DQE electron detection camera after the energy-filter for high-resolution data recording. Specifications for the detector are provided above. The performance of cryo-EM with energy filter instrument operating in zero energy-loss mode should meet the following objectives:- It should be possible to observe Thon rings in a high-dose image from an amorphous carbon or Pt- Ir specimen out to the Nyquist resolution in the Fourier transform of an image that is at least 4Kx4K pixels. Chromatic aberration (image blurring) should be less than 1 pixel/10eV	5

		energy spread over the full field of view.Geometrical distortion should be ≤0.7% over the full field of view. Non-isochromaticity should be less than 2 eV over the full field of view. It should be possible to use the EF at 80, 200 and 300 kV. The filter alignments for at least two of the above voltages should be provided. The Energy filter should be having an auto alignment feature.	
		Environment: The system should preferably be in an enclosure and protected from interference by an outer shell. The enclosure must ensure thermal and acoustic shielding with ≤0.5°C temperature or 20 dBC variation. Remote operation of the microscope should be possible.	
13	User Interface	Fully computer controlled system with windows based software for operating the Microscope along with keyboard, mouse. Software: Licensed, latest version software for automatic image acquisition for single particle, tomography, and electron diffraction; single particle reconstruction and analysis, and tomographic reconstruction should be supplied, installed and supported by the vendor. Any updated versions, when available, should be provided free of cost till the end of warranty period. Any known or suspected incompatibilities with other licensed or open source software should be clearly mentioned in the bid. It is expected that the data acquisition software should have pre-configured settings that can be easily selected for use with the appropriate standard samples. The software should be compatible with direct electron detection camera. Further, use of open source software (e.g SerialEM) should be permissible without affecting warranty of the equipment.It should be possible to export the acquired data and metadata from the system in multiple formats (eg. mrc, .tiff, .jpeg, .txt, .xls etc). The software's should have a browser version to allow users an off-line capability to view images, export data & images as well as carrying out basic processing & analyzing functions. It is expected that such a browser would be free, or of minimal cost, and ideally available for Windows, Mac and Linux operating systems. The software controlling all detectors/cameras is expected to be fully embedded into the operation system of the microscope. The software and hardware provided should allow the remote controlled operation, including remote diagnosis and servicing. The safety controls must be implemented in software as well as in hardware for protecting the operators, instrument and specimens.	4

		Control Panel and Joystick-or track-ball Control panel including multifunction keys/knobs for control and adjustment of TEM parameters (focus, magnification etc.) and manual joystick or track-ball control for stage in X,Y,Z tilt and rotation directions.	
14	Work Station and Software	Data storage and computational infrastructure with latest workstation to collect and process movie on the fly. Adequate computational infrastructure for data recording and storage along with all required accessories should be provided with the system. Software to operate the system, record data, and store and analyze data should be provided. Suitable for screening samples for subsequent single particle reconstruction (SPR) studies. Tomography Software for automatic control and reconstruction. Image file in JPEG, TIFF & BMP formats. Backup software must be provided on optical media. Any further version of the software and updates must be provided free of cost Separate computer (s) should be provided for offline data analysis. High speed storage appliance with capacity of atleast 1 petabyte, and transmission speed of at least 10 Gbps, is preferred. Overall, the computational infrastructure provided should allow smooth operation of the system and analysis of the recorded data. Appropriate software for automated and manual operation, automated and manual data collection in all modes requested above and downstream data analysis for all applications (including single particle and tomography reconstruction) should be provided, as described before.	4
15	Pre-Installation requirements	Complete technical details of pre-installation requirements should be furnished along with the technical bid	5
16	Installation	Installation, complete interfacing of the system with its subsystems, and commissioning is to be carried out by the vendor's factory-trained engineers, followed by a demonstration of the system's performance to the user's complete satisfaction	5
17	Accessories	Chiller/compressor /UPS/other necessary accessories for installation as required should be included in the offer and should be manufactured by the vendor or by a reputed manufacturer One additional set of auto grid loading system accessories that are delivered with the CryoEM e.g. assembly workstation, loading dock, tweezers, transfer dewars etc. should be provided. An automatic filling system for liquid nitrogen should be provided. 2 X 240 L Liquid nitrogen dewars with liquid nitrogen sensor should be provided. Details of the proposed anti-contamination device should be included in the submitted documentation. A dry shipper for transportation of biological samples at cryogenic (-150°C or colder) temperatures should be provided. A set of 1000 autocliper cups and rings should be provided along with the system. A set of 200 cryogenic grid storage boxes should be provided.	5

		An extra set of apertures should be provided. Plasma cleaner for cleaning cryo-holders, cryo transfer station, cold stage controller, dry pumping stations and all cryo-tools etc. for holders should be provided.	
18	Sample Preparation	Following Accessories should be included along with the system: A. screening machine 120kV cryo-EM B. Sputter coater/carbon evaporator with turbo pump and glow discharge system C. Automatic Plunge Freezer D. Cryo Ultramicrotome E. Glass Knife Maker F. Block Trimmer G. High Pressure Freezer	
B	**120 kV cryo-EM**	Screening Machine	8
	Specifications	1. High Brightness Electron Source 2. W/LaB6 Electron source 3. Magnification: 10x to 100000x or more 4. Condenser lens system 5. Side entry eucentric goniometer 6. Fluorescent screen should be provided above CMOS camera 7. High resolution CMOS camera with atleast 4k X 4K pixel array as explained in A10. 8. Specimen Tilt : +/- 60 degree or more 9. Imaging software with automated data collection feature which can collect data for single particle reconstruction and cryo-tomography. 10. Room temperature specimen holder should be provided. 11. Workstation for imaging and data collection should be provided. 12. Oil-free vacuum system with turbo-molecular vacuum pump backed up by rotary pump should be provided to maintain high vacuum in electron gun and cryo-EM's column area. 13. It should come with a cooling unit, chiller, related accessories. 14. power back up (UPS) should be provided such that in case of power failure it should operate atleast 30 minutes.	
	Side entry cryo-transfer holder	1. Cryo-transfer holder with single-tilt holder designed for the frost-free transfer of a sample at liquid nitrogen temperature 2. It should have minimum of 4 hrs of hold time for liquid nitrogen 3. It should hold specimen grid size - 3 mm diameter 4. It should be suitable for screening single particle reconstruction and cryo-tomography samples 5. It should come with Cryo-transfer station/loading station with temperature controller with necessary accessories for smoother operation. 6. Pumping station for side entry cryo-holder equipped with turbo-molecular vacuum pump should be provided.	3

		7. All other necessary accessories for the side entry holder should be provided.	
C	**Sputter coater and Glow discharge system**	1. Sputtering and carbon coating system with a turbo pump to reach high vacuum. 2. Glow discharge unit should be capable for hydrophobic/hydrophilic conversion and hydrophilic /hydrophobic conversions of grids.	2
D	**Automatic Plunge freezer** Specifications	1. Movable climate control chamber with automatic adjustment of temperature from 4º C to 60º C or more as well as humidity up to 99% or more 2. Climatic chamber should be well lit inside with LED and a defogger/window heater should be available to maintain a clear view of chamber 3. Windows for inserting pipette should be available on both left as well on right side of the chamber 4. Touch screen control panel to program and run the system with easy to use graphic user interface 5. Possible to set & adjust pre-blotting, blotting/hold time. 6. Positioning of grid should be adjustable in terms of distance and height with respect to the blotting paper 7. Cryogen container for plunge-freezing the sample 8. Required accessories like cryo-tool dryer, grid box, insulating forceps, blotting papers etc should be provided along with the equipment	3
E	**Cryo-Ultramicrotome**	1. Cutting transmission should be done by vibration decoupled gravity stroke 2. Specimen feed at steps of 1 nm or better 3. Cutting speed should be controllable in a range of 0.05 to 100 mm/sec 4. Complete system should be controlled by a touch screen controller of size 10” or more 5. Knife stage should be fully motorized and controllable by the touch screen controller 6. Movement range of knife stage in E-W (X) and N-S (Y) directions should be at least 25 mm & 10 mm respectively 7. Countdown, section counters, speed, feed, stage movement parameters should be visible on controller screen 8. Details of user, sectioning, knife parameters and grid box parameters should be downloadable via USB (logbook) 9. It should be possible to make segments of knife and it should be approached automatically 10. Ultramicrotome should have automatic trimming function, programmable by the touch controller 11. Stereomicroscope with magnification range of 10x to 75x or more should be provided 12. Ergonomic wedge with adjustable angle of 5°- 25° should be	3

		included with stereomicroscope 13. It should have eucentric movement with defined click stop positions for glass knife and diamond knife 14. There should be 4 LED illuminations with top light, spotlight, back light and specimen trans Illumination 15. All Illuminations should have independent control via touch screen controller 16. Cryo chamber should have adjustable temperature form+110°C to -185°C with automatic rapid cooling 17. The controls for Cryo chamber should be integrated within the main machine's controller 18. Individual temperatures setting for specimen, knifeand gas temperature 19. Chamber wall should be heated and have high gas GN2 flow < -140°C 20. It should have 5 level LN2 indicator with reserve warning	
F	**Glass Knife maker**	1. 100% balanced break method 2. Breaks glass from 6.4 to 10mm 3. Variable scoring lengths with Accurate glass strip positioning 4. Drawer system with convenient and safe knife removal 5. Auto reset of breaking and scoring mechanism 6. Push action score for even scoring and Adjustable scoring pressure 7. Breaking wheel with scale for defined and reproducible glass break	2
G	**Block Trimming Unit**	1. High speed diamond miller with variable speed of 300 to 20000 rpm or more 2. Integrated stereo microscope with LED ring light for easy adjustment of the block positions 3. Miller movement should be adjustable in steps of 0.5, 1, 10, 100 µm 4. Auto feed function for minimizing human intervention 5. LCD display for showing the feed of miller 6. Safety cover for working area and auto stop mechanism on removal of safety cover	2
H	**High Pressure Freezer**	1. Should be capable of fully automated freezing process with specimen transfer into LN2 sample collecting container. 2. Automated sample loading with one second freezing cycle should be possible in single step. 3. Recovery time should not be more than 1.5 min between consecutive freezing cycles 4. Should consist of Integrated Liquid Nitrogen Dewar with automated draining system 5. LN2 consumption should not be more than 30 Liters per day and it should be fully reuse residual LN2	2

		6. Air pressure intensifier should be Oil-free 7. Sample storage Dewar should have segments and it should have programmable rotation system allowing nine consecutive freezing cycles. 8. It should also have automated LN2 refilling system for the safety of the frozen samples throughout the day. 9. Draining of residual LN2 should be automated without requirement of operator to monitor it. 10. It should not user alcohol for pressure/temperature synchronisation 11. Workstation should be integrated and should have touch-screen operation with parameters display and data storage of files. 12. Software-integrated programming offering a range of parameters to enable the design of the experiment 13. Upgradable for Optogenetics study with LED light modules with 5 different wave lengths (red, blue, green, UV, amber). It should be automatically recognized of the connected LED module by the instrument software 14. It should also have provision to upgrade for electrical stimulation system. 15. It should consist of a one set of stereo Microscope with objective, eye-piece, LED illumination & diffuser. Microscope should be mounted on the sample station for easy & accurate positioning of the samples. **a. Accessories and consumables to be supplied along with the main machine** 1. One Cryo tool dryer with combination of ventilation heating and heating plate having temp. setting up to 50 °C should be provided 2. One Cryo forceps with straight tip 3. One Forceps with insulation coating 4. One Cryo-box for sample manipulation with magnetic holders for the trisection pod, cryotransfer box and puncher for sample carriers 5. One each re-usable tissue punch of dia. 1.9 mm and 4.9 mm 6. Two hundred no. of 3 mm gold-plated copper carriers 7. Two hundred no. of 6 mm gold-plated copper carriers 8. One Hundred no. of membrane Carriers of dia. 6 mm, 100 µm	
	Technical expertise and manpower requirement	It is expected that this system will be heavily utilized by structural and cell biologists in India. Thus, it is desirable that the vendor has installed at least one, currently functional, equivalent system in India; and at least 30 such equivalent systems worldwide. The following documentation should be provided for establishing technical expertise and support availability: 1. Worldwide installations of 300 kV cryoEM with similar configurations* in the 2015-2021 period, along with performance certificate from at least three major users 2. India-wide installations of 300 kV cryoEM with similar	5

		configurations* in the 2015-2020 period, along with performance certificate from at least one major user 3. List of research publications** in Pubmed/Medline/SCI from similar machines in the 2017-2020 period. List of structures submitted to EMDB/PDB should also be provided. 4. List of research publications** in Pubmed/Medline/SCI from similar machines installed in India in the 2017-2020 period. List of structures submitted to EMDB/PDB should also be provided. 5. Current average on-site attendance time within warranty period (In India/worldwide) for similar machines. Information in tabular form with breakdown issue, time of attendance and time of resolution should be provided. The vendor is expected to provide trained personnel with at least 1 year of experience of operating and maintaining the proposed system, and all accessories. This personnel is expected to assist with installation; and to train scientists, technicians and students of IIT-Bombay, with little or no background knowledge of microscopy, to enable independent and precise operation. This personnel is expected to be involved in all aspects of single particle and tomographic data collection at IIT-Bombay during the warranty period.	
	Warranty	Five (5) years comprehensive warranty for the microscope and all accessory equipment, hardware and software is required from the date of installation. During the warranty period, the maximum on-site attendance time from first notification of a problem with either the system or accessory equipment should be 48 hours (2 days). After clearance of parts from customs, the maximum fix time should be 3 days. Any deviation will result in an extension of warranty by 5 days for per day of non-compliance.It is expected that comprehensive warranty includes the cost of parts and labor. In case certain spare parts are exempt from warranty, a list of said parts along with documentation indicating the expected lifetimes of these parts, should be supplied. Two free of cost replacements for the FEG source should be provided within the warranty period of 5 years. The vendor should provide documentation ensuring availability of spare parts, repair kits as required and technical support for the microscope and all accessories for up to 10 years from the date of installation.	5

* Equipment for biological sample usage ONLY

** For biological samples only

Total technical marks :100

Committee will perform assessment for satisfactory responses for technical queries and may award bonus marks.