**Lot A: Specification of A New High-resolution Transmission Electron Cryo-microscope at MRC’s Laboratory of Molecular Biology, Cambridge**

The supplier of a new high-resolution transmission electron cryo-microscope will be responsible for the following three sections of the requirement:

1. One electron microscope with 80 to 300 keV electron energy for cryoEM and electron cryo-tomography (cryoET);
2. An extra 2 years of warranty;
3. Moving and re-commissioning of an existing used Polara electron microscope (Polara I).

**1.0.** **Specification of an electron cryo-microscope with 80 to 300 keV electron energy for cryoEM and cryoET**

Summary of microscope deliverables:

* 1. To supply a complete high-resolution transmission Cryo-TEM
  2. The microscope is expected to fulfil the specification as a high-end instrument capable of data collection by means of single particle EM work (cryoEM) as well as electron cryo-tomography work (cryoET)
  3. To have automatic specimen exchange system
  4. To have a phase plate
  5. To have an energy filter for tomography
  6. To have direct electron detectors for data collection with and without energy-filtering
  7. To have an assistant camera
  8. To be user-friendly and have ability to run automated data acquisition
  9. To have customer-witness acceptance test in the factory before delivery and to be delivered between December 2017 and January 2018
  10. To have expected on-site acceptance before 1st April 2018
  11. To be adapted to the physical room constraint
  12. Services & Support
  13. Innovation & added value are desirable

Specification

* 1. A complete high-resolution transmission Cryo-TEM
     1. To provide a complete system.
     2. To accept full responsibility for provision and installation of a complete and fully operational system if third party suppliers are involved.
  2. The core microscope should have the following specifications.

**Electron Beam Generation:**

* + 1. The generated electron beam should have electron energy of 80 to 300 keV (acceleration voltage). LMB expects but not limits to the following specifications.
       1. It should be possible to adjust easily and in a few minutes the voltage to any voltage between 80 keV and 300 keV.
       2. To have factory alignments at 300, 200, 120, and 80 keV.
       3. 1.2.1.3 To be fitted with a field emission electron gun (FEG) to generate an electron beam with the highest possible brightness to provide enhanced spatial coherence, and with high emission stability for high-resolution imaging.
       4. Energy spread of the electron beam to be ≤ 1.0 eV.
       5. Beam spot size  1 nm and probe current ≥ 2 nA. The intensity of the beam should not decrease in time and should be constant for at least 2 days.
       6. Lifetime of FEG tip to be > 2 years.

**Lens design:**

* + 1. Stable electron-magnetic lens design with constant power supply during a normal range of operations to provide maximum stability and reproducibility by minimizing thermal drift, lens hysteresis, cross-talk between optical components and to have fast switching between different operational modes.

**Condenser lens system:**

* + 1. Capable of producing parallel beam illumination at the specimen.
       1. Ideally to have 3 condenser lenses, and with display of illumination geometry in the user interface.
       2. Maximum spot drift 0.5 nm/min.
       3. Quantitative indications of convergence angle, current on screen, size of illumination area, and electron dose in electrons per square Ångstrom per second (e-/Å2s) are desirable.

**Objective lens**

* + 1. To have a single condenser/objective lens with minimized aberrations at the eucentric position.
       1. The spherical (Cs) and chromatic (Cc) aberration coefficients should be equal to or better than 2.7 mm at all energy levels.
       2. To have a point resolution of better than 0.25 nm, and a line resolution of better than 0.14 nm (information limit).

**Specimen stage:**

* + 1. Must be fitted with a sample stage that is suitable for cryoEM and cryoET use
       1. Temperature at sample must be < 85 K.
       2. The holding time of the liquid nitrogen reservoirs should be more than 96 hours, or an automatic filling system should be provided.
       3. Should be computer controlled with a range of movement specified in μm for the X, Y and Z axis and in degrees for alpha tilt.
       4. Should have high stability: maximum stage drift rate < 0.1 Å / s at cryogenic temperature.
       5. Should have reproducibility of ≤ 0.5 µm after specimen shift for 500 µm in X or Y direction.
    2. The specimen height must be adjustable to allow eucentric tilting.

1.2.6.1 The α-tilt range should at least -70° to +70°.

1.2.6.2 The eucentricity during ± 70o tilting should be ≤ 2 µm in X and Y, and ≤ 4 µm in Z (defocus change).

1.2.6.3 To be supplied with a stage tracking system, capable of recording and re-calling the specimen areas, in digital and graphic forms.

* + 1. The ice contamination rate must be minimised in microscope column.

1.2.7.1 The ice contamination rate must be < 1 Å / hour.

1.2.7.2 An automatic drift compensation system is desirable.

**Magnification:**

1.2.8 The microscope should be fitted with intermediate & projector lenses to provide a highly reproducible, rotation-reduced, minimised anisotropic magnification in a magnification range with the following minimum specifications.

1.2.8.1 To produce a magnification range from 50x to 500,000x or higher.

1.2.8.2 Camera lengths in diffraction mode should be 200 mm (or less) to 5000 mm (or greater) at 300 keV.

1.2.8.3 Magnification should be reproducible to within ± 1.5%.

1.2.8.4 Should minimise the degree of rotation as magnification changes.

1.2.8.5 Anisotropic magnification distortion should be less than 0.1% at magnification above10,000x.

**Apertures:**

1.2.9 The microscope aperture system must conform the following requirements.

1.2.9.1 To have an objective aperture holder with at least four aperture positions appropriate for different imaging conditions and phase plate.

1.2.9.2 To have two condenser aperture holders (C1 and C2), each with at least four apertures in each holder.

1.2.9.3 To have a selected area (SA) aperture holder, with at least four apertures.

1.2.9.4 All aperture holders must be motorized to maximize the degree of automation.

**Vacuum system:**

1.2.10 To be expected to operate at ultra-high vacuum to both minimise sample contamination and maximise the life of the electron source, especially for cryo-EM use.

1.2.10.1 Pressures in the specimen chamber should be < 1 x 10-6 Pa. Pressures in the gun chamber should be < 1 x 10-7 Pa.

1.2.10.2 Oil-free vacuum is required for the microscope and specimen exchange systems.

1.2.10.3 Fully interlocked and differential pumping is expected.

1.2.10.4 A specimen anti-contamination device must be provided and this should include a Dewar capable of achieving a cryogenic ‘hold time’ of at least 96 hours.

1.3. Sample Exchanger

1.3.1 Must have an automatic specimen exchange system.

1.3.2 At least 8 grids per one loading of the sample changer without breaking of vacuum.

1.3.3 Automatic cooling of stored samples to liquid nitrogen temperature (< 85 K); to maintain vitreous ice for at least 72 hours.

1.3.4. Contamination free: ice contamination rate should be less than 1 Å / hour within the sample changer.

1.4 Phase Plate

1.4.1 It is expected to be fitted with a phase plate to improve the imaging of weak phase objects, such as frozen hydrated biological specimens, particularly the sections or lamellae of cells.

1.4.2 The phase plate should be robust with an average life time of at least 9 months under normal TEM operating conditions.

1.4.3 Compatible with automated data collection.

1.4.4 Ideally, phase shift should be stable over at least an entire tomography data collection (2 -3 hours).

1.5 Energy Filter (EF) for Tomography

1.5.1 To be equipped with an energy-filter suitable for zero-loss imaging.

1.5.2 The EF must be proposed to include the following key criteria.

1.5.2.1 Energy resolution requirements are better than 10 eV.

Geometrical distortion should be maximal 1%, ideally much lower.

1.5.2.2 Chromatic aberration < 1 pixel / 10 eV.

1.5.2.3 Combined cryo-EM and EF instrument operating in zero energy-loss mode: non-isochromaticity < 2 eV.

1.5.2.4 Ease of use is important, alignment and tuning of the energy-filter is expected to be as automatic as possible.

1.5.2.5 Sensitivity to magnetic / electric fields is minimal / compensated.

1.5.2.6 Filtered images should be recorded on a direct electron detector.

1.5.2.7 A second, fast buddy camera capable of very high dose rates would be useful.

1.5.2.8 Practical resolution requirements: on 4k x 4k images, Thon rings to Nyquist from amorphous carbon or Pt-Ir specimen at an exposure of less than 100 electrons per pixel.

1.5.2.9 To have alignment of EF at 300keV, 200keV and 80keV.

1.5.2.10 To have software for automatic filter alignment and zero-loss peak with distortion and aberration correction.

1.5.1.11 EF should have EFTEM spectrum imaging capability – with chosen slit width and energy range.

1.6 Direct Electron Detector(s)

1.6.1 The DEDs must have Direct Electron Detector(s) and be possible to record images with and without energy filtering.

* + 1. The DEDs must meet or exceed the following key criteria.
       1. Must have a minimum of 4k x 4k pixels.
       2. To be highly sensitive for low-dose imaging (i.e. low read-out noise).
       3. To have counting mode for images with and without energy filter.
       4. To have software to collect data in summed and dose fractionated modalities.
       5. To have a full frame rate > 300 fps (frame per second) for at least one detector.
       6. To have dose rate of up to 4 electrons / pixel / s (electrons per pixel per second) useful in counting for at least one detector.
       7. To have data collection and real-time processing in integration, counting and super resolution modes for at least one detector.
       8. Radiation hardness must enable a minimum of 1000 images per day for a year.
       9. DQE should be excellent at low resolution, i.e. > 0.8 (300 keV, counting mode).
       10. DQE should be better than 0.5 at 0.5 Nyquist (300 keV, counting mode).
       11. The DEDs should be possible to display a noise whitened FFT diffraction pattern immediately after recording an image, especially in integration mode.
    2. It is highly desirable that there is a commitment to any near-future upgrade of the detectors in line with advances in detector technology & techniques. Such detector upgrade(s) should not incur extra expenditure by the purchasing organisation for new/modified hardware or software at least within two years.

1.7 Assistant camera

1.7.1 An additional fast, general purpose CCD camera is expected to assist the high DQE detectors for alignment, low-dose search, focus and medium-resolution image recording, as well as electron diffraction.

1.7.2 The assistant camera should be fast, at least 1Kx1K pixels but preferably larger, have a frame rate of at least 15 frames per second at a resolution of at least 500 x 500, and the ability to display and update the power spectrum at the same rate.

1.7.3 The assistant camera should be radiation hardened.

1.8 System, user interface, software and automatic data collection

1.8.1 Remote Operation is expected including hardware and software.

1.8.2 The TEM offered in the submitted documentation will be expected to be intuitive & user-friendly, robust & adaptable with the capability to accept future upgrades & improvements.

1.8.2 To be fitted with controls that are simple enough to be understood by ‘non-expert’ users and robust enough for heavy daily use, usually for 24/7 use.

1.8.3 To have a user-friendly interface for different levels of users, will be clearly labelled and intuitive enough for ‘non-expert’ users.

1.8.4 Advanced controls to be hidden so that use is limited to expert users or service engineers. A software design to allow different level of users in terms of control and safety is desirable.

1.8.5 To have software to run automated or semi-automatic alignment of the microscope, phase plate, detectors and energy-filter.

1.8.6 To have low dose imaging capability for cryoEM.

1.8.7 To be able to run automated data collection for cryoEM and cryoET.

1.8.8 All detectors, phase plate, and energy-filter should be embedded with the main microscope operation software.

1.8.9 Expected to have high degree of automation and all user functionality should be software controlled.

1.8.10 Safety controls must be implemented within software as well as in hardware for protecting the operators, instrument, detectors and specimens.

1.8.11 Software for tomography data processing including alignment, 3D reconstruction and visualization is desirable.

1.8.12 To ensure that all microscope functionality required for fully automated single-particle and tomography data collection is made available for control by software including SerialEM (An automatic tomography data acquisition software developed by University of Colorado).

1.9 Customer-witnessed acceptance test and delivery.

1.9.1 Customer-witnessed acceptance test in the factory is expected before delivery.

1.9.2 Delivery must be between December 2017 and January 2018.

1.10 Commissioning must to be finished and on-site acceptance accomplished before 1st April 2018.

1.11 EM Room size: the proposed microscope has to be adapted to the physical room constraints: the floor space is ~ 8 x 4 m, and ceiling height 4 m.

1.12 Service and support: supplier to propose the services to provide:

1.12.1 Response time for a service call: 24h / 48 h response time is expected (24 hours response by telephone or remote diagnosis or visit by engineer, and 48 hours response onsite by engineer visit or remote service)

1.12.2 Onsite training and support is expected

1.12.3 Application Support is desirable

1.12.4 Remote diagnosis is highly desirable

1.13 Innovation & Added Value

1.13.1 The buyer will give due consideration to any tender response, whether of standard manufacture or of an alternative design solution that provides and delivers innovation, good design, accounts for on-going product development and clearly demonstrates measurable subsequent benefits.

1.13.2 MRC-LMB encourages the bidders to put forward value added solutions. The specimen/EM-grid preparation apparatus and specimen holder/grid cartridges/frozen grid exchange accessories are particularly desired by MRC-LMB.

1.15.3 Additional sample vitrification capacity is needed so consideration should be given to the provision of suitable instrumentation as part of the response.

**2.0. An extra 2 years of warranty**

* 1. An extra two years extended warranty is expected and this is will be on top of the standard one year manufacturer’s warranty.
  2. Labour and parts should be included in the warranty.
  3. 24h / 48h response time is expected for service calls as part of the warranty.
  4. Preventive maintenance service visits once per year as part of the warranty.

**3.0. Moving and re-commissioning of an existing Polara electron microscope (LMB’s Polara I)**

* 1. The contractor is expected to move an existing Polara I TEM from its existing room to an adjacent room in the same building on the same building level. The move has to be in September or early - mid October 2017 in order to vacate the room to be modified as the room for the new cryo-TEM tendered for.
  2. To re-install and re-commission the same Polara I to full working order as soon as possible after the moving, ideally within 1 month. The configuration and specification of Polara I is attached as separate document.
  3. The destination room for Polara I is 3 meters away from the current Polara I Room. The new Polara I room dimensions are 4.3 x 3.62 m and ceiling height 3.07 m. The moving path has two double-doors. The door width is 1.40 m (1.46 m if door frames removed) and door height is 2.65 m. The entrance of new Polara Room is 1.16 m width and height 2.71 m. The path is at the same floor level and with a ceiling height of 2.71 m. The diagram of the Polara Room and moving path is attached.