SCHEDULE 4 – TECHNICAL SPECIFICATION LOT 1

1. Introduction

1.1. The TIRF/FRAP microscope system covered by this specification is intended to provide the MRC Laboratory of Molecular Biology in Cambridge with an instrument able to quantitatively image single molecules at very high speed in multiple channel simultaneously.

1.2. The proposed TIRF/FRAP system shall be designed in such a way that it may be expanded and/or upgraded to meet future requirements. In particular, upgrade towards a spinning disk confocal is envisioned (such as with the Yokogawa CSU W1 spinning disk wheel or equivalent), and the proposed system shall enable Spinning disk/FRAP applications.

1.3. This system must provide capability for simultaneous three or four channel imaging, each to a separate large field of view camera (13X13mm camera chip).

1.4 The system must be able to receive custom parts not sourced from the bidder.

2. Lasers Specifications and Function

2.1. The proposed TIRF/FRAP microscope system shall be equipped with the following solid state laser excitation wavelengths:
2.1.1. 405nm.
2.1.2. 488nm.
2.1.3. 561nm or within the range of 560nm – 570nm.
2.1.4. 633nm or within the range of 630nm – 650nm.

2.2. The tenderer shall state the power and expected lifetime of all lasers specified in their proposal. Power is expected to be at least 100mW for the 405nm and 561nm lasers, 140mW for the 633nm and 150 mW for the 488nm (see also point 1.2 for Yokogawa CSU W1 upgrade).

2.3. The tenderer shall indicate if it is possible to add additional laser lines to the hardware specified in their tender response after delivery of the system, and if so how many.

2.4. Selection of the laser wavelength(s) and control of the laser intensity shall be automated and optimized for speed, flexibility, light efficiency and for possible future laser upgrades. Laser intensity shall preferably be controlled by direct modulation.

2.5. The proposed laser bank shall be equipped with at least two independent optical fibre outputs (one for FRAP, one for TIRF).
2.5.1 It shall be possible to direct the full power of any combination of laser wavelengths into either of the fibre outputs.
2.5.2 Switching of the combined optical path of the four lasers to each fibre should be as fast as possible (ideally <1 millisecond).
2.5.3 The proposed laser bank shall be equipped with a global shutter controlled by a key.
2.5.4 The proposed laser bank shall be able to receive a third optical fibre output at minimum cost, (see also point 1.2).
2.5.5 The laser bank shall be designed for laser safe operation. Interlocks shall not compromise the laser output power stability

3. TIRF Imaging Specification & Function

3.1. This system must provide homogenous TIRF illumination by spinning a focused laser at the back focal plane of the objective at very high speed while keeping the distance to the optical axis, and thus the TIRF angle, constant (so called ring- or azimuthal TIRF).
3.1.1 The proposed Azymuthal TIRF control system should be able to achieve a complete circle at the back focal plane in 6 milliseconds or less.
3.1.2 The proposed Azymuthal TIRF control system should be able to change the ring radius in less than one millisecond.

3.2 The proposed Azymuthal TIRF control system shall be equipped with a flexible user configurable interface to select for each excitation wavelength: i) the TIRF angle (ring diameter) and/or the penetration of the TIRF field, ii) the laser power, iv) the rotation speed of the spot at the back focal plane. The centre position of the ring at the back focal plane should be adjustable in the software.

3.3 Ideally, the proposed Azymuthal TIRF control system shall propose a flexible control of illumination by the different lasers during a camera exposure and/or a single orbit (Time sharing mode).

3.4 The proposed Azymuthal TIRF control system should provide a way to calibrate the depth of the TIRF field for each wavelength.

3.5 The system should provide homogenous illumination across the entire field of view of the provided camera. Tenderer shall specify the uniformity of illumination of their proposed setup along the diagonal direction (obtained using homogenous solutions of fluorescent dyes and without extra magnifying lens). We consider a 50% intensity drop compared to the centre 10% area of the camera chip as the cut off to measure the homogeneity of the field of view: illumination homogeneity of 80% means that 20% of the signal along the diagonal displays values that fall below half that of the signal in centre region [10% on each side].

Scoring will reflect said specification: 100% means that intensity along the diagonal direction does not drop below 50% of that of the centre area, 50% means that 50% of the signal along the diagonal displays values that fall below half that of the values in the centre region.

3.6. The proposed system shall be capable of simultaneous detection and imaging on four large field of view independent cameras using suitable splitter optics and emission filters. The provided image splitter device shall enable independent focusing an alignment of each channel. The following dichroics/emitters are expected:
3.6.1. Ultra flat dichroics: DAPI/GFP, GFP/CY3 and CY3/CY5
3.6.2. single emitters: 450/50 525/50 595/50 655 LP

3.7. The tenderer shall propose three cameras (with an option for four) designed for single GFP molecule sensitivity at high speed, either back illuminated EMCCD or back illuminated sCMOS. The price of this fourth camera should be included as an option in the bid.

3.7.1. The tenderer shall propose cameras with a large chip (in the 13X13mm range) able to image at a minimum framerate of 23 frames per second in full field in global shutter mode.
3.7.2 The tenderer shall propose cameras with a pixel size of 13µm or smaller.
3.7.3 The tenderer shall provide the specific performance datasheets of each camera. Pixel to Pixel noise maps are also expected for sCMOS cameras.

3.8 The TIRF system shall function in concert with a motorized microscope stage to produce X,Y, T , lambda images at multiple user designated points in a sample.

4. FRAP Specification & Function

4.1 The proposed system must allow TIRF/FRAP experiments with all four lasers by scanning a focused laser in a controlled way on the sample.

4.2 The tenderer shall provide the size of the focused laser spot (ideally diffraction limited).

4.3. This system must provide near simultaneous TIRF/FRAP capabilities (ideally switching time between TIRF and FRAP modes in 10 milliseconds or less) with all lasers.

4.4. In the case of an upgrade towards a Spinning disk confocal configuration, the possibility to do near simultaneous Spinning disk/FRAP (switching time between Spinning Disk and FRAP modes in 10 millisecond or less) with the four provided lasers will be a plus.

5. Microscope Specification & Features

5.1. The proposed TIRF/FRAP microscope system shall include an inverted microscope stand.

5.2. The proposed inverted microscope stand shall have two side camera ports suitable for mounting cameras for full field imaging. By preference, switching between the eyepieces and the two camera ports shall be automated and shall be under TIRF system software control.

5.3. The tenderer shall indicate the wavelength range over which the entire microscope system is considered to be aberration corrected.

5.4 The microscope shall come equipped with a white light source and transmitted light condenser optics

5.5 The proposed microscope shall include two objectives
5.5 1. 10x (ten times) air.
5.5.2. 100x(one hundred times), highest NA possible (oil immersion lens), optimized for multicolour TIRF fluorescence imaging.

5.6 It is expected that the proposed microscope system has a motorized fluorescence filter turret

5.7. The microscope shall have a motorized focusing mechanism. The tenderer shall state the minimum step size and precision of the focus mechanism.

5.8. The proposed stand shall have hardware autofocus.

5.9. The proposed microscope system shall include a motorised X-Y sample stage equipped with a universal insert and be equipped with linear encoders for accurate and repeatable positioning.

5.10. The proposed motorised X-Y sample stage shall be movable under control of both the system's software and a separate joystick.

5.11. The possibility to image the back focal plane of the objective for ring TIRF alignment on a different imaging port would be a plus. The MRC may provide a dedicated low end camera for this application

5.12. The microscope system should include appropriate hardware such that it may be mounted on an anti-vibration table.

5.13. The tenderer shall provide an anti-vibration table able to fit all the above components, including room for a spinning disk hardware upgrade (such as the Yokogawa CSU W1).

5.14 The system shall be designed for laser safe operation. Interlocks shall not compromise the laser output power stability

6. PC Specification

6.1. The proposed PC should meet or exceed the following performance requirement.

6.2. The computer shall have sufficient data processing power and sufficient memory to operate the microscope, TIRF and FRAP hardware.

6.3. The computer shall have sufficient data processing power and sufficient memory to acquire data from at least two cameras simultaneously operating in full field at their fastest framerate. Acquisition by cameras three and four may be done on a second PC using Micromanager software or equivalent if necessary. Said second computer could be provided by the MRC.

6.4 At least 512 Gigabytes of Solid State storage, preferentially using two disks in RAID0 or using a NVMe drive on a PCIe port.

6.5. Two 24'' displays (or larger) with IPS technology.

6.6. A PCIe 2.0 free slot for a high-speed network data transmission card (e.g. 10gbit Ethernet), which will be provided by the MRC.

6.7. A PCIe 3.0 free slot for a high performance GPU for online data processing (Nvidia GeForce Titan X), which will be provided by the MRC. Supplied PC must only have basic graphics capabilities.

7. Software Features

7.1. An appropriate software package by the proposed TIRF/FRAP microscope system is expected by the MRC. In addition to software features specified in the preceding sections, further features are listed below.

7.2. Said software package will not be used for data analysis, visualisation or processing, so there is no need for sophisticated data processing and analysis modules. However, software proposed by the tenderer shall facilitate FRAP analysis at the microscope.

7.3 Said software package shall be able to overlay signals from at least two cameras to facilitate alignment of the multichannel splitting optics.

7.4 Said software package shall enable TTL control of extra devices, such as microfluidic pumps, at specific times during acquisition. If necessary, the MRC may provide an additional TTL controller.

7.5 Said software package shall be scriptable for advanced acquisition schemes

7.6. The complete package of software proposed by the tenderer shall be fully compatible with all of the hardware systems proposed as part of the TIRF/FRAP microscope system.

7.7. The software provided with the system shall be expected to allow multiple users each with individual log-ins, user profiles, saved settings and acquisition protocols.

7.8. The MRC expects that the acquired data and metadata can be exported outside from the proposed system in multiple formats (e.g. *.tiff). On line export to MATLAB would be a plus.

7.9. The software proposed by the tenderer shall facilitate 2D (two dimensional) depth resolved imaging, 3D (three dimensional) imaging with time lapse and multiple emission wavelengths (X,Y,Z,T,λ imaging).

7.10. The software proposed by the tenderer shall allow the user to select in software defined ROIs (region of interest) in an image for subsequent illumination, imaging and/or photo bleaching. Implementation of the ROI photobleaching shall be automated and allow rapid switching between this mode of operation and regular TIRF imaging mode.
7.10.1. Note: the size, shape and number of such areas shall be defined by the user and the proposed software must be capable of analysing the acquired data.
7.10.2. ROI photobleaching and stage motion must be scriptable in the software to allow the user to photobleach a region of the same geometry at different XY position of the stage (to allow UV mediated micropatterning)
7.10.3. ROI geometry (shape, position in the field of view) must be recorded as part of the image metadata,
7.10.4. The MRC expects flexible software tools to manage FRAP ROI geometry. In particular the ability to import and export ROI geometry in a general format (list of coordinates, ImageJ or Matlab ROI)

7.11 The software shall facilitate time lapse imaging at multiple locations. Each location must be able to have its own focus reference to combine hardware autofocus with multi-position acquisition.