INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH

PUNE

CLARIFICATION ON TENDER NUMBER - IISER-PUR-0058-15

ITEM DESCRIPTION- TO SET UP A COLLABORATIVE MICROSCOPY IMAGING CENTER AT IISER PUNE

Refer our Press Tender Notice No.IISER/S&P/10/15-16 dated 30/11/2015 to set up a collaborative Microscopy Imaging Center at IISER Pune . Tender Reference Number – IISER-PUR-0058-15.

Pre-Bid meeting was held on December 09th , 2015 at 10.00 AM and minutes of meeting is as under.

At the outset, the Chairman welcomed all the Members and the representative of the Prospective Bidders and briefed in general the scope of the Project and thereafter requested Assistant Registrar (S&P) to brief the vendors on the salient features of the commercial terms and the indenting Officer to read out the clarification sought by the Prospective Bidders and replied thereto as detailed in Annexure -II

The representatives present were satisfied with the replies given and it was informed that the corrections / additons / clarifications given, as discussed during the Pre-Bid Conference would be hosted on the website of IISER Pune and all the Prospective Bidders are required to take cognizance of the proceedings of the Pre-Bid Conference before submitting their bids as stipulated in the Bidding Documents.

Please note that last date for submission of tender document has been extended to January 13, 2016, Time – 3.00 PM.

The other terms & conditions of the notice issued on our IISER website www.iiserpune.ac .in will remain unchanged. No more correspondence in this regard will be entertained

The meeting ended with vote of thanks to the Chair

Sd/-

9.12.2015 Assistant Registrar (S&P)

ANNEXURE -II

IISER PUNE

PRE-BID CONFERENCE FOR SETTING UP COLLABORATIVE MICROSCOPY IMAGING CENTER

TECHNICAL QUERIES AND CLARIFICATION

TENDER NUMBER - IISERT-PUR-0058-15 DATE : 9.12.15

S.No	Query/Clarification Sought	Clarification / Amendment
1	Chapter 4, Item 2: Confocal Spectral laser scanning a) Refer to points A. 2 & 4 , XZ piezo/Galvo stage insert: We are of the opinion that the combination of piezo/galvo stage for fast Z movement would be of no significance in combination with the standard XY galvo scanner. The fly back time of the standard galvo is adequate to move the stage position in Z direction. The real advantage of piezo/galve stage for Z movement would be significant with the resonant XY scanner where in the fly back time of the galvo is fast and the stage position in Z direction can't be completed with the standard focus drive.	Clarification: No change The piezo/galvo stage is requested for fast Z movement. If there is a close comparison and solution by another method, the vendor is requested to give details of Z resolution and speed during scanning in the technical specifications.

	Hence some of the confocal systems are configured with fast Z focus drive nose piece mechanism (for upright and inverted stands) with a small step size of 10nm and in synchronization with the galvo fly back time. As such the confocal systems without resonant scanners doesn't need to be configured with the piezo/galvo Z focus accessory and can be compared with the other confocal systems that are configured with piezo/galvo Z focus accessory.	
2	b) Point A. 10 : Complete automated …… incubation system should be controllable by confocal software. Please note that some of the Confocal systems are configured with a fully automated programmable and computer controlled on stage incubators. However the control software for the incubation is a separate one and is not integrated in to the confocal software. As the purpose of automated software control of the stage is being met, we request you to amend the point appropriately.	Amendment: The complete automated incubation system should be controllable by either the confocal software of another software. Both softwares should be capable of saving the parameters used during the experiment for later reference.
3	c) Point B. 11: System parameter optimizer for the resolution improvement at least 1.5x in XY and 2x in Z with……. Please note that the additional device of resolution improvement for confocal systems are relevant for improving the resolution in XY only and is not applicable to improve the resolution in Z direction. We request you to kindly consider the same for possible editing/amendment of the specification.	Amendment: System parameter optimizer for resolution enhancement by approximately 1.5 fold in XY should be included.
4	d) Point D. 3 : Confocal system control software …….Auto fluorescence separation by online spectral unmixing. Advanced multidimensional ……. The confocal software is capable of auto fluorescence separation by spectral unmixing and is done sequentially. Hence we would request you to consider to amend the term online by " online/sequential " spectral unmixing.	Amendment: Online/Sequential mode can be used for spectral unmixing

5	3. Chapter 4. Item 3. Multiphoton scanning …… a) Point No. A. 10: An appropriate stage design……. All the parameters of the incubation system should be controlled by the confocal software. As explained in the Item no.2, point no. A. 10 , as above, we request you to consider to amend the point appropriately to enable us to offer a fully programmable incubation control software which is not integrated in to the confocal software.	Amendment: The complete automated incubation system should be controllable by either the confocal software of another software. Both softwares should be capable of saving the parameters used during the experiment for later reference.
6	b) Point No. D.2: Confocal system control software……..Imaging without bleed through and auto fluorescence separation by online emission finger printing technique. …… As explained in the item no. 2, point no. D.3. as above, we request you to consider to amend the term online by " online/sequential " emission finger printing technique….	Amendment: Online/Sequential mode can be used for spectral unmixing
7	1. Upright epifluorescence microscope Point No.1- Sub Title-Focus under Sl.No.2- Tender asks for "a nose-piece motorized Z focus mechanism with fixed stage enabling less non-drift of samples during time lapse and multicolour imaging". We have stage based motorized Z focus, kindly consider the same.	Amendment: Motorized nose piece or stage based motorized Z focus can be used in the epifluorescence system.
8	1. Upright epifluorescence microscope Sub Title-Objectives- Tender asks for following objectives whereas there are minor differences with specification as below. 1. Universal Plan Fluorite 4X (NA 0.16 or higher, WD 13 higher) Possibility: Plan Fluorite 5x NA 0.15 with WD 13.7mm 2. Universal Plan Apocromatic 10X (NA 0.4 or higher, WD 3 or better) Possibility:Plan Apocromatic 10x (NA 0.4, WD 2.2 mm)	Amendment: The following objectives can be used for the epifluorescence system: 1. Plan Fluorite 4-5X (NA 0.15 or higher, WD 13 mm or higher) 2. Plan Apochromatic 10X (NA 0.4 or higher, WD 2.2 or higher)

	3. Universal Plan Apochromatic fluorite 40X (NA 0.75 or higher, WD 0.51 or better) Possibility: Plan Apochromatic 40X (NA 0.85 WD 0.21) or Fluorite 40x/0.75 (WD 0.4) 4. Universal Plan Apochromatic 60X oil (NA 1.35 or higher, WD 0.15 or better) Possibility: Pl Apo 63x/1.4 (WD 0.14) 5. Universal Plan Fluorite 100X oil (NA 1.3 WD. 0.2 or better) Possibility: Plan Fluorite 100X oil (NA 1.3 WD. 0.13) To consider the specification stated above would be able to participate in the tender.	3. Plan Apochromatic 40X (NA 0.75 or higher, WD 0.21 or higher 4. Plan Apochromatic 60-63X oil (NA 1.3 or higher, WD 0.14 or higher) 5. Plan fluorite 100X (NA 1.3 or higher, WD 0.13 or higher)
9	Sub Title-Camera- Point No.e&f – In our product portfolio we have camera wherein fan cooling goes upto Zero and water cooling upto -100. We meet other specification of the camera and hereby request you to consider the point so that we'll be able to submit the our solution. Zyla 4.2 Plus QE 82% Read noise---0.9e Dynamic range----33000:1	Amendment: Camera with cooling upto zero degree celsius or better water cooling upto -30 degree Celsius.
10	3. Multiphoton scanning, high sensitive and speed dual color detection on an upright microscope system a) Point No.B-4, where in tender asks for Ultra long working distance water dipping (4mm or better), high NA 1.0 or better, 25x deep tissue imaging objective. We have 25x/0.95 NA objective with 2.5mm, For 25x we request you to consider the NA to 0.95 and working distance to 2.5mm instead of NA 1.0 & 4mmWD.	Amendment: Multiphoton grade water dipping objective, 25X, NA 0.95 or higher, WD of 2.5 mm or higher
11	b) Point No.C-3, wherein tender asks for second high-speed resonant scanner for imaging of live samples with a minimum scan speed of 30fps at full resolution of 512x512 (18mm FOV). The resonant scanner works at 28fps in full resolution for that FOV and we request you to consider the same enabling us also to participate in the tender.	Amendment: High speed resonant scanner at 28fps in full resolution
12	c) Point No.C-4, The tender asks for "The system should be capable of accommodating an additional set of scanners for stimulation with 405 nm laser line controlled through the imaging software". We can offer High speed photo activation/stimulation/FRAP with resonant scanner using FRAP Fly mode. It can work in VIS & MP or MP & MP or VIS& VIS mode.	Clarification: FRAP fly mode may be used, the delay in bleaching and imaging should be mentioned in the technical specifications

13	d) Point No.C-14, IR Imaging and Visible (UV) Stimulation: Fast Imaging and stimulation scanner for almost real time photoactivation and FRAP experiments. This is the repetition of point no.C-4 as mentioned above and request you to consider from the tender specification so that all manufacturers can participate. We can offer High speed photo activation/stimulation/FRAP with resonent scanner using FRAP Fly mode. It can work inVIS& MP or MP & MP or VIS& VIS mode.	Clarification: FRAP fly mode may be used, the delay in bleaching and imaging should be mentioned in the technical specifications
14	e) Point No.C-16-Multiphoton laser OPO/ InSight Deepsee Dual, range 680nm-1300nm with 1040 fixed line is the one we can offer	Clarification: No change The multiphoton laser OPO/Insight Deepsee Dual with a range of 680-1300nm with fixed 1040 line can be used for dual color imaging and is an appropriate solution.
15	4. Three dimensional super resolution imaging using point spread function Sharpening methods a) Point No.A-1, where in you've asked for motorized Z movement of the microscope with step size essentially be at least 50nm and Point No.C-5 specifies the minimum Z resolution to be 80nm. Both these points are contradictory as per the sampling requirement for resolution. For precise step size Galvo or Piezo stage is required for better Z resolution requirements and for checking XZ profile. You're requested to take care of the same in the specification.	Amendment: The Z step size should be appropriate (For example: 10 nm or even better with a piezo stage) for getting at least 80nm resolution in the XZ axis.
16	b) Point No.A-3, where in you've asked for Plan Apochromat objective 40X (NA 0.95 or higher). The manufactures 40x/0.8 only in Confocal grade Plan Apochromatic segment, we request you to kindly consider NA of the same.	Amendment: Plan Apochromat objective 40X/NA 0.8 or higher
17	c) Point No.B-1, where in you've asked for The system should cover the entire visible excitation range either by separate pulsed excitation diode lasers (~405 nm, 488 nm, ~561 nm ~640nm). You're requested to add two more pulsed excitation lines for Yellow and Orange dyes for optimmally imaging the fluorochromes like mTomato, mCherry, Alexa 546,Alexa568, Alexa 555,Alexa 594, CY3, CY3.5 &DsRed.etc All above mentioned dyes range cannot be depleted by 592nm and 775nm lasers. The additional depletion laser between 592nm & 775nm is required.	Clarification: No change. Amendment in optional items: Excitation lasers for Yellow and Orange dyes and suitable depletion lasers between 592nm and 775nm can be added as optional components.

18	d) Point C-1 Superresolution asks for a second laser that suppresses emission from fluorophores, but doesn't specify the same. To cover the entire range of fluorochromes and efficient multi color Super Resolution imaging we recommend to specify 592nm, 660nm & 775nm as depletion laser	Clarification: No change Amendment in optional items: The 660nm laser may be added as an optional depletion laser.
19	e) Point No.C-4 The XY resolution of the system is ~ 30 - 50 nm. In 3D mode the resolution limits is ~ 80-130 nm. And the value depends on the property of the dye and depletion laser power used.	Clarification: No change The best resolution of the suggested system is 30 nm in XY and 80 nm in 3D. This is within the limits of the specification mentioned. The value is between a range and will depend upon the property of the dye and the depletion laser used.
20	a) Point C-10- Objective with optimal chromatic correction for STED over the full spectrum of visible light is most important for Super resolution imaging and you're requested to kindly include this point also in the specification.	Amendment: Point A3: Objectives used in the super resolution system should be corrected for chromatic aberration across the full visible range.
21	b) Point C. Superresolution is silent about alignment of the system as it's mandatory and most important while acquiring a super resolution image. You are requested to include Auto Alignment in this part for an efficient imaging without manual alignment interference	Amendment: Point C11: The method of laser alignment and multiple color alignment for the super resolution system should be described in the technical specifications.
22	1) Upright Epi-fluorescence microscope Focus Point 2: The Z step size asked by you is 20nm, we request you to modify it to 25nm	Amendment: A Z step size of 25nm or below can be used for focusing and sampling.
23	Controls and tube Point 3: You have asked 7 position motorized nose piece. We can offer 6 positions.	Amendment: 6 or more positions in the motorized nose piece should be present.
24	Controls and tube Point 4: We can offer 6 positions reflector FL turret motorized.	Clarification: No change

25	Condenser Point 1: We can offer 5 position motorized Universal condenser. Also we can offer 180 deg rotatable polarizer instead of 360 deg as we 360 deg rotatable analyzer	Amendment: 5 or more positions should be a part of the motorized universal condenser. Either one, the polarizer or the analyzer should be freely rotatable.
26	2) Confocal system Point A3: The Z step size 15nm, we request you to modify it to 25nm	Amendment: A Z step size of 25nm or less can be used for focusing and sampling.
27	Point A9: We propose to extend the laser/LED interference range upto 800+ nm so that it will not interfere with some far red signals like Cy5.5	Clarification: No change in specification
28	Point C1,2,3,4,and 5: You have asked for Multiline Argon laser, we will offer solid state or Diode lasers instead of this. The power of these lasers is minimum 15mW at the tip of the optical fiber while entering in the scanner.	Amendment: Addition of point C6: The multiline Ar laser or solid state lasers or Diode lasers to encompass the fluorophores excited at 415, 488 and 514
29	E. Optional Accessories Point 1: You have asked for 35 to 40 fps @ 512 X 512. We propose to modify it to 25 to 30 fps @ 512 X 512.	Amendment: The frame size from 25-30 fps @ 512 X 512 is ok.
30	3) Multiphoton confocal system Point A1: The Z step size 15nm, we request you to modify it to 25nm.	Amendment: A Z step size of 25nm or less can be used for focusing and sampling.
31	Point B5: We can provide long working distance water dipping objective with 2 mm working distance	Amendment: 25X water dipping objective with a working distance of 2 mm or better and NA of 0.95 or better

32	Point C4: We are not clear why you are asking for 2 scanners for stimulation with 405 and an additional scanner having IR reflectance upto 1300 nm. We can use out single Hybrid scanner simultaneously.	Clarification: No change. Scanners should be provided such that stimulation and detection is possible simultaneously or use an alternate mode for reducing the imaging time lost between stimulation and detection.
33	4) Three dimensional Super Resolution System Point B4: We can offer highest resolution @ 4K X 4K to capture images on the system.	Clarification: No change
34	Point C: Super resolution: you have asked for a STED based system. We cannot offer this. Is it possible to accommodate a SIM/STORM based system?	Clarification: Our requirement is for a STED system.
35.	Revised Specification	The revised consolidated specification are appended below:

The specifications for the desired systems are as follows:

1. Upright epifluorescence microscope
An upright motorized epifluorescence microscope capable of brightfield, darkfield and fluorescence imaging.

Optical System
1. Fluorescent Trinocular Motorized Microscope with Imaging attachment
2. High quality optics with latest Infinity Colour Corrected System for high brightness, rich contrast and colour correction. All optics coated with anti- reflection / anti-fungal treatment.
3. Fully apochromatically corrected light path

Focus
1. Microscope stand with Z-drive motorized with TFT monitor for microscope control.
2. Z-drive motorized basic, step size of at least 25 nm or less, with coarse and fine drive knob at microscope frame. A nose-piece motorized Z focus mechanism with fixed stage or stage based motorized Z-focus can be used..

Controls and Tube

1. Fluorescence motorized control shutter, RL, TL Shutter, Objective change, work/load position of stage, changing of reflector cubes.
2. Transmitted-light illumination motorized with motorized light control.
3. Seven position Motorized objective nosepiece
4. 6 or more position reflector FL turret motorized.
5. Motorized 5 position ND filter set with halogen. For integration in filter wheels.
6. Binocular phototube (100:0/0:100), upright image adjustable stop, camera port
7. Motorized shutter for transmitted-light illumination

Stage

Rectangular Motorized XY stage with XY stage stroke approximately 75X50 mm. The stage should support seamless stitching of the thick tissue specimens in XYZ to generate whole slide high resolution images. The imaging software should have ability to perform multi point imaging of the whole slide and should be calibrated to automatically recognize the XY coordinates at higher magnifications.

Transmitted light

Built-in Koehler illumination for transmitted light. LED display of status of the motorised parts of the microscope. Built-in motorized field stop. 12V 100W halogen bulb (pre-centered) or LED with power unit and country specific cable.

Fluorescence lamp and filters

1. All the filters should be interference type with hard coated for better transmittance/reflectance.
2. Narrow Band Pass DAPI/UV Center Wave length of 360nm
3. Narrow Band Pass Violet Center Wave length of 400nm
4. Narrow Band Pass FITC/Alexa 488/GFP
5. Narrow Band Pass TRITC/Alexa 546
6. Narrow band Pass Alex 594/mCherry
7. Long pass Cy5
8. A filter cube mounted analyzer for seamless multichannel acquisition with DIC.
9. Also add 2 addition empty filter cubes for mounting customized filters
10. 120 W Metal halide FL source with illumination capability of approximately 2000 hours or PC controlled High-power LED fluorescence emission light source with fast LED switching and pulsing covering 360 to 750 nm. Typical lifetime should be 20000 hrs with fast USB (at least 600us) and TTL (at least 20 us) triggered shutters. The lamp house should have a motorized shutter and an attenuator.

Objectives

1. Universal Plan Fluorite 4-5X (NA 0.15 or higher, WD 13 higher)
2. Universal Plan Apocromatic 10X (NA 0.4 or higher, WD 2.2 or better)
3. Universal Plan Apocromatic 20X (NA 0.75, WD 0.6)
4. Universal Plan Apochromatic fluorite 40X (NA 0.75 or higher, WD 0.21 or better)
5. Universal Plan Apochromatic 60-63X oil (NA 1.3 or higher, WD 0.14 or better)
6. Universal Plan Fluorite 100X oil (NA 1.3 WD. 0.13 or better)
7. Water dipping objective 40X (NA 0.8 or higher)

Condenser

1. Motorized Universal condenser with 5 positions or more for Optical elements with motorized polarizer for DIC automation. The polarizer should have a freely rotatable (360°) 7 Position Achromatic-aplanatic universal condenser 0.9 BF, DF, Phase DIC motorized with motorized front lens, Motorized aperture diaphragm and motorized turret disk, with centerable Ph1, Ph2, Ph3 and DF stops integrated, 3 mounts for DIC condenser modules,
2. 1 separate position for bright field For objective magnifications 1.0x-100x,WD=1.2mm Achromatic/Aplanatic 0.8 condenser for Bright field, Dark field and DIC.
3. The darkfield condenser for 2.5X to 100X.
4. Motorized aperture stop. DIC for 10X, 40X & 100X objectives be given.

Camera

The detection device should be a high sensitivity CMOS which should have the following features:

a) Active Pixels 2048X2048
b) Pixel size 6.5 x 6.5 microns
c) Should be able to capture more than 30 to 100 fps @ 2048x 2048
d) Quantum Efficiency should be 70% or above at 600nm.
e) Cooling capability of at least 0º C.
f) Should be capable of going to -30º C water cooled
g) 16 Bit Digitization with Trigger In and Out

Computer and software

1. Suitable latest branded 64 bit control computer with high end graphics card, fast processor, at least 8GB RAM, Hard drive of at least 2TB, CD/DVD read write, USB, Firewire, Gigabit Ethernet, high resolution monitor of approximately 32 inch for one monitor.
2. Software should be provided which can control all the motorized functions of the microscope. Multi-dimensional fluorescence acquisition such as XYZ λt.
3. Automated stitching or auto montage and multi point imaging module.
4. Software autofocus modules

5. Basic deconvolution and online deblur/DE convolution module.
6. Automated count and measure module.
7. Intensity measurements extended focal imaging,
8. Fluorescence unmixing module to separate spectrally overlapping dyes and auto-fluorescence.

Optional Accessories:

1. 3D Blind deconvolution module in with capability to deconvolve Bright field, widefield and confocal images.

2. Confocal spectral laser scanning with real time high sensitive detection with an inverted microscope system for multichannel imaging of fixed samples, including Z-stack, colocalization, time lapse imaging of live samples and real time imaging of *in vivo* protein dynamics using photobleaching, photoactivation and FRET.

The system should have the following constituents:

A. Latest Inverted Microscope:

1. Motorized Inverted Fluorescence Microscope for BF/DIC/FL with dedicated LCD display screen and prepared with suitably located camera port.
2. Motorised XY stage for XY scanning fitted with XZ Piezo/Galvo stage with universal samples holders to fix different cultivation vessels (petri dishes with 24-68 mm diameter) and glass slides (with 24-120 mm length) with moveable brackets with a variable clamping range.
3. Motorised Z- focus drive with z step resolution 25 nm or less and have motorized objective prism turret, motorized Polarizer and filter cube analyzer for DIC imaging.
4. Piezo/Galvo stage for faster Z stack imaging
5. Motorized 6 position FL filter turret, motorized 6 fold objective revolver.
6. 120 W Metal halide FL source with illumination capability of approximately 2000 hours or PC controlled High-power LED fluorescence emission light source with fast LED switching and pulsing covering 360 to 750 nm. Typical lifetime should be 20000 hrs with fast USB (at least 600us) and TTL (at least 20 us) triggered shutters. The lamp house should have a motorized shutter and an attenuator.
7. High resolution Plan Apochromat objectives corrected for both UV & visible lines and adaptable with all the detectors for imaging. 10x/0.4, 20x/.75, 40x/1.3-1.4 oil and 60/63x/1.4 oil along with DIC accessories for all objectives.
8. Fluorescence filters for DAPI, GFP /FITC, Rhodamine, Mito-tracker RED/AF 568.
9. The microscope should be equipped with hardware LED/ laser based control of focus drift during live cell imaging. LED / Laser should not interfere with any excitation wavelength from 405nm to 640nm.
10. Complete automated and software controlled on stage environmental control chamber (incubator) for live cell time lapse imaging with temperature, CO2 , Humidity control. Most parameters of the incubation system should be controllable by confocal software or by an additional software which can record and save parameters specific for the experiment. The parameters which are not controllable by the software should be explicitly mentioned.

11. A suitable and recommended imported Anti vibration table for the complete confocal system to be supplied from factory.
12. Suitable online UPS for 30 min backup for the entire system.

B. Latest Confocal Scanning Unit with Spectral/Filter based detectors:

1. The point scanning Confocal unit with spectral/filter based detectors equipped with a high sensitive GaAsP/HyD & PMT detectors, having spectral resolution of 5 nm or better. The resolution of the spectral/filter type detectors should be mentioned.
2. Detection system should be high speed signal loss free and able to do simultaneous detection and separation of at-least four fluorophores, two fluorophores should be imaged on GaAsP/ HyD channels.
3. All the four fluorophores detection channels should have independent analog gain controls for balancing the expression of weak and strong fluorescence.
4. The spectral dispersion should be based on either reflection grating or prism for improved emission signals.
5. System should be equipped with suitable dichroics for good transmission efficiency
6. Computer controlled continuously variable confocal pinhole with software control. Scan resolution of 4K by 4K or better.
7. The system should capable of acquiring at least 4 fps @ 512x512 pixels (full format) in spectral detection mode. ROI scan and bleach with various ROI shapes should be possible for FRAP experiments. The scan field diagonal should be FOV 18 mm or more.
8. A transmitted light detector should be offered for bright-field and DIC imaging.
9. Scan zoom of at least 1x to 40x in steps of atleast 1x and scan rotation of 0 to 180 degrees or better.
10. A dedicated scanner for real time simultaneous photobleaching/photoactivation and acquisition or an alternative scanning mode for minimizing loss of signal information during photobleaching/photoactivation
11. System parameter optimizer for the resolution improvement by approximately 1.5X in XY should be included. . Any alternate way of achieving higher resolution should be mentioned.

C. Laser Modules with Controller:

1. Laser Unit including following laser lines with laser power :405/408nm (50 Mw or better) : for DAPI, Hoechst fluorochromes.
2. Multi Ar (30 Mw or better) 458, 488, 514 laser line: for for excitation of Alexa 488, FITC, GFP, Fluo 4, Cy2 fluorochromes, 514nm: Alexa 514 , Alexa 532, Calcium green, YFP, Citrine, Oregon Green, TOTO-1 DNA, Eosin, Bodipy etc.
3. 559/561(15 Mw or better) : for excitation of TRITC, Rhodamine, Texas Red, Cy3, PE, PI fluorochromes.
4. 633/635/638 (10 Mw or better): for excitation of Alexa 635, DRAQ 5, Cy5 fluorochromes.
5. All the visible and UV laser should be controlled through AOTF for fast laser switching and attenuation.
6. Suitable alternative high power solid state or diode lasers could be used. The power for these lasers should be mentioned.

D. Software & Workstation:

1. Latest branded 64-bit control computer with Intel Xenon multi Core Processor, DDR RAM 8 GB HDD: 2 TB SATA, DVD , Super Multi SATA + R/RW, Graphics: ATFire GL V5200 256MB DH DVI, Gigabit Ethernet, Win
2. 7 Ultimate 64 bit OS,USB 2.0, Fire wire, Large 32'' LCD/LED/TFT monitor 2560 x 1440 pixel resolution.

3. Confocal system control software capable of controlling all motorized functions of microscope, scan head, lasers , image acquisition & Processing. Image acquisition for 3D, 4D, online/sequential spectral imaging and unmixing or software based unmixing, co-localization. Live cell imaging control for multi-time series, FRAP, FRET (Acceptor photobleaching), FRET (Sensitised emission), photo activation and conversion. Auto-fluorescence separation by online spectral unmixing. Advanced multidimensional software for 3D, multichannel volume rendering, reconstruction, measurements across z stack, movie co-localization with histogram analysis, intensity profiles for quantification etc.,multi-export formats for data output. Software module or facility to image extended dynamic range while acquiring like HDR/BrightR/or equivalent with GaAsP/HyD/APD or equivalent detectors. The features available by the software should be mentioned.
4. Offline processing computer and software unit for 3D/4D Image analysis, FRAP, FRET photoactivation/conversion analysis, volume rendering, reconstruction, colocalization analysis, intensity analysis and deconvolution.

E. Optional Accessories

1. Ultrahigh Speed scanner with frame rate of 25 to 30 fps @ 512 x 512 format (full frame) for high speed imaging as well as experiments like photo-activation, photo-conversion, FRAP, Ca++ etc.
2. Multi immersion objective lens for long term imaging

3. Multiphoton scanning, high sensitive and speed dual color detection on an upright microscope system for deep tissue imaging of fluorescently tagged proteins and Calcium imaging

A. Upright microscope system

1. Motorized fixed stage upright fluorescence microscope with built in nose piece Z motor (25 nm or better).
2. A high precision high speed Piezo/Galvo nose piece/stage for fast optical sectioning and optical stimulation at multiple Z locations. The microscope frame should have better transmittance from UV to IR.
3. A 100 W halogen light source suitable for IR DIC.
4. 5 Position Fluorescence Filter cube turret with High Transmission Narrow Band Pass Excitation and Emission filters for DAPI, CFP, GFP, Ds Red/TRITC, mCherry and Cy5 for fluorescence observation.
5. 120 W Metal halide FL source with illumination capability of approximately 2000 hours or PC controlled High-power LED fluorescence emission light source with fast LED switching and pulsing covering 360 to 750 nm. Typical lifetime should be 20000 hrs with fast USB (at least 600us) and TTL (at least 20 us) triggered shutters. The lamp house should have a motorized shutter and an attenuator.
6. Short working distance High NA (0.8 or better) Universal Condenser with slot for DIC prisms for all the objectives. A high NA (1.4 or better) oil immersion to quoted for collection of weaker SHG and Transmitted Non Descanned signal.
7. The high NA condenser should be able to deliver the scattered light to the forward Non descanned detectors for 2 photon imaging and SHG imaging.
8. Large Movable top platform with XY movement with Max travel range 25.4 mm in XY direction. Resolution lesser than 1 micron with sample plate holders.

9. A 120/130 W Hg or metal halide lamp with built in attenuator. Filter cubes for observations of DAPI, CFP, GFP and RFP/Ds Red
10. An appropriate stage design for animals such as zebra-fish and mouse and tissue imaging. Stage and condenser optics should be easily removable for increasing the available volume for animal accommodation while imaging. Complete automated and software controlled stage environmental chamber for live tissue samples for time lapse imaging with temperature and humidity control with perfusion tubes. All the parameters of the incubation system should be controlled by the microscope software or an additional software which is capable of recording and saving the settings for an individual experiment.
11. A suitable and recommended Anti vibration table for the complete confocal system to be supplied from factory.
12. Suitable online UPS for 30 min backup for the entire system.
13. Epifluorescence/Brightfield imaging with CCD camera

B. Objectives

1. The system contains dedicated Multiphoton quality objectives as follows and a coded 2 position nose piece slider.
2. Plan achromatic 10X air objective.
3. Water dipping 10X with NA 0.3 or better working distance 3.5 mm or better suitable for different immersion systems
4. Plan achromatic 20X objective suitable for multiple immersion systems
5. Ultra long working distance water dipping (2.0mm or better), high NA 0.95 or better, 25X deep tissue imaging objective suitable for transmittance from 400 to 1300 nm, average transmittance for the wavelength range should be mentioned
6. Fixed sample imaging with coverslip, 40X/1.3 NA or better transmittance from 400 to 1300 nm average transmittance for the wavelength range should be mentioned
7. Fixed sample imaging with coverslip 63X/1.4 oil or better suitable for transmittance from 400 to 1300 nm, average transmittance for the wavelength range should be mentioned
8. All the objectives quoted should have a dedicated DIC condenser suitable for IR DIC.

C. Scanning and Detection

1. The scan head should have independent port for UV, Visible & IR Lasers. The scanner should have a higher reflection (>90%) from UV-VIS-IR for better multiphoton excitation efficiency
2. The scanning system should be capable of imaging fixed and live specimens and have special features for long duration live cell observation. The scan head should take up the multiphoton module for deep imaging as well as fast live imaging.
3. There should be a built in dual scanning capability with one scanner dedicated for high resolution ROI scan and second high speed resonant scanner imaging of live samples with a minimum scan spread 28 fps at full resolution of 512X512 at full field of view of 18 mm or more and a highest frame rate of more 280 fps at 512x 16 in multiphoton imaging mode. The IR tunable laser should be easily switchable between conventional scanning and fast resonant scanning mode.
4. The system should be capable of accommodating an additional set of scanners for stimulation with 405 nm laser line controlled through the imaging software. The additional stimulation scanner should have IR reflectance of upto 1300nm and be capable of performing ROI bleaching in different forms. If an alternate method is used to decrease the loss significantly between stimulation and imaging, it should be mentioned in the technical specifications. The loss of time between stimulation and depletion should also be specified.

5. All the optics used for the confocal scan head should have suitable coating for better transmittance/reflectance of IR lasers upto 1300 nm. Mention the percentage average transmittance in the wavelength range.
6. Two large window non descanned detection (Multi Alkali PMTs) and two non descanned GaAsP/Hyd detectors with filter for imaging DAPI, CFP, GFP and RFP.
7. The laser coupling optics should be of latest technology. The beam size and shape of the laser source should be optimised to the AOM/EOM shape to get the maximum efficiency.
8. The beam shape and size should automatically be changed based on the objective NA, mode locked IR wavelength.
9. PMT based transmitted light detector for DIC imaging.
10. The imaging software should directly control the IR laser both for switching on/off, wavelength selection and intensity modulation.
11. The system should be capable of selecting dual line to perform two colour simultaneous and sequential imaging to avoid overlapping of two colours.
12. The microscope should have a deep imaging femtosecond based multiphoton imaging module with the following specs.
13. A dedicated two channel reflected light non descanned detector integrated inside the optical path of the microscope for better light efficiency. Filter cassettes for imaging of deep live specimens with fluorophores such as DAPI, GFP, YFP, RFP and mCherry should be given. Two high sensitivity GaAsP/Hyd detector in the reflected mode. A set of Transmitted light NDD detector should be offered for imaging GFP/RFP/mCherry and Second harmonic Generation (SHG) Imaging. In order to handle applications using multiple points or region of interest through high speed toggling On/OFF of the laser with EOM/AOM (Acousto-optic modulator system, all the features of the femto second lasers such as selection of wavelength/mode locking, turning on and off of IR lasers etc must be completely be controlled by the confocal and multiphoton imaging software for easy and faster switching between the single photon stimulation and multiphoton observation mode during optogenetics experiments. Both visible and IR laser should be controlled by the same software for easy selection of laser wavelengths. The multiphoton beam shape should automatically adjust to the changed objectives to achieve precise and efficient multiphoton excitation.
14. **IR Imaging and Visible (UV) Stimulation:** Fast Imaging and stimulation scanner for almost real time photoactivation and FRAP experiments. The system should support laser light stimulation and imaging with all the visible lasers used for imaging. The system should be capable of multiphoton deep tissue imaging and single photon stimulation. Imaging and the stimulation may be sequential and the time delay should be reduced as much as possible and the method incorporated for reducing this time delay should be mentioned.
15. A dedicated IO interface box, with multiple TTL outputs and inputs, and multiple Analog input and at least 1 Analog output be quoted
16. **Multi Photon Laser:** Provide a solution for rapid multiphoton imaging of two colors for observation at similar depths. For example a multiphoton laser which is continuously variable broadband range from 680nm to 1300nm, and a fixed wavelength of 1040nm or equivalent

D. Computer and software

1. Suitable latest branded 64 bit control computer with high end graphics card, fast processor, at least 8GB RAM, Hard drive of at least 2TB, CD/DVD read write, USB, Firewire, Gigabit Ethernet, high resolution monitor of at least 32 inch for one monitor or 2 monitors of appropriate size.
2. Confocal system control software capable of controlling all motorized functions of microscope, scan head, lasers, AOTF including image acquisition & processing. Image acquisition for 3D, 4D, on-line spectral Imaging based on lambda stacks. Advance application modules for Time series, FRAP, FRET, Photo activation and conversion. Imaging without bleed through and auto fluorescence separation by online/sequential emission finger printing technique. Advanced multi dimensional software for 3D analysis with Deconvolution, multichannel volume rendering, reconstruction, measurements across z stack, movie, co-localization with histogram analysis, intensity profiles for quantification etc.
3. Offline computer and software for image analysis in 3D and 4D, FRAP/ FRET/ Photoactivation/ Photoconversion analysis, volume rendering, reconstruction, distance and intensity measurements across the Z stack in time, colocalization analysis and deconvolution.

E. Optional Accessories

1. Additional scanner for simultaneous imaging and bleaching
2. Confocal scan head and lasers for imaging
3. Software for synchronizing electrophysiological data with laser stimulation
4. Objectives compatible with commercially available clearance media for tissues

4. Three Dimensional Super resolution imaging with a high resolution less than 100 nm in the Z direction using point spread function sharpening methods.

The super-resolution microscope system with optical slicing capabilities should have high modularity to accommodate external peripheral devices like additional lasers, more detectors, upgradability to FLIM/FCS or any other future innovations. To minimize any user specific precision error, the super-resolution image should be a direct outcome of the microscope and not a result of any mathematical post processing. The system should have the following special features to get optimum resolutions of samples from interdisciplinary fields including Biology, Chemistry and Physics/Material Science.

A. Fully motorized inverted microscope

1. The microscope body should have built in motorized Z movement with step size appropriate to achieve a Z resolution of 80 nm with LED/IR laser based multi mode focus drift correction mechanism. For example: the Z resolution can be 10 nm or better acheived by a separate means.
2. The microscope should be equipped with 12 Volt 100W halogen illumination for transmitted light (Bright field & DIC) applications

3. The system should have at least 5 positions motorized DIC nosepiece and high resolution Plan Apochromat objectives 10X (NA 0.30 or higher), 20X (NA 0.4 or higher), 40X (NA 0.8 or higher), 60X oil (N.A 1.35 or higher), 100X oil (N.A 1.4 or higher). The objectives should be corrected for chromatic abberation across the full visible range.
4. 120 W Metal halide FL source with illumination capability of approximately 2000 hours or PC controlled High-power LED fluorescence emission light source with fast LED switching and pulsing covering 360 to 750 nm. Typical lifetime should be 20000 hrs with fast USB (at least 600us) and TTL (at least 20 us) triggered shutters. The lamp house should have a motorized shutter and an attenuator.
5. 6 or more positions motorized fluorescence turret with inbuilt shutter and band pass fluorescence filters for DAPI, FITC/GFP, TRITC/Rhodamine & Cy5 should be there.
6. A Scanning / motorized encoded / Linear Stage should be part of the system with a universal sample holder attachments for glass slide, 35mm imaging dish, petri-plates and multi-well plates.
7. Dedicated TFT / LCD touch panel control for the microscope will be preferred.
8. Microscope Stage top CO2 incubator for imaging live cells with control of CO2, Temperature & humidity. It should have a gas mixer so that 100% CO2 gas cylinder can be attached to it.
9. The system should come with a suitable anti vibration table with active air compressor control.
10. The system should have a dedicated UPS system with at least 30 min back up in the absence of power.

B. Confocal Scanning and detection system:

1. The system should cover the entire visible excitation range either by separate pulsed excitation diode lasers (~405 nm, ~488 nm, ~561 nm ~640nm) or by tunable white laser.
2. All the Lasers should essentially be controlled through independent AOMs or AOTF for fast switching and attenuation of laser intensities.
3. The Galvo Mirrors of the scanner should be compatible from 400-1000nm with high reflectivity of UV laser for blue dyes in confocal mode as well as for red / far-red depletion laser in super-resolution mode.
4. The system should be capable of capturing images at different resolutions from 64 X 64 pixels up to at least 6K x 6K pixels, higher resolution will be preferable.
5. Scan speed of at least 3/4 frames per second for 512 X 512 pixel array and higher frame rate should be achieved by binning or ROI imaging mode.
6. The System should have variable confocal pinhole adjustment facility.
7. The System should posses at least 2 high sensitive built-in APD or high sensitive hybrid detectors. Higher sensitivity will be preferred.
8. All the detectors should be present inside the scan head/module for maximum signal collection & sensitivity. The fluorescence signal should be delivered directly to the detectors and not through optical fibers to minimize loss of weak fluorescence signal reaching the detectors.
9. The system should be able to perform advanced confocal measurements like colocalization, FRET, FRAP etc.
10. The detectors should work on photon counting principle which raw data will help to analyze/quantify other bio-physical phenomenon as well.
11. The system should be compatible / upgradable to FLIM & FCS measurements as well.

C. Superresolution

1. The system should cover the entire visible excitation range either by separate pulsed excitation diode lasers or by tunable white laser. The system should be capable of imaging dyes with excitation wavelengths approximately 405, 440, 488, 563, 592 and 640 nm.
2. The system should have capability to perform at least dual colour super-resolution imaging with both fluorescence dyes / proteins.
3. The system should be capable of capturing images at different resolutions from 64 X 64 pixels to at least 6K x 6K pixels, higher will be preferable.
4. The XY resolution of the system should be at least ~ 30 nm or better when the resolution enhancement is done only in 2D (XY-plane). In 3D (XYZ all directions) mode the resolution limits should be ~ 80 nm or better.
5. Preferably there should be aberration correction option in the super-resolution mode, which will enable to optimize the resolution for different sample types and depths.
6. The System should have motorized variable confocal pinhole adjustment facility.
7. The System should posses at least 2 high sensitive built-in APD or high sensitive Hyd detectors. Higher sensitivity will be preferred. The detectors should work on photon counting principle which raw data will help to analyze/quantify other bio-physical phenomenon as well.
8. Gated detection is preferred for better resolution.
9. The system should be optimized for 100x oil immersion (NA 1.4) objective, but capability of capturing super-resolution images with other objectives (e.g. 60x/63x/100x water/silicon/glycerol objectives) as well will be preferred.
10. Versatility of the system like accommodating additional external lasers or any external device will be preferred.
11. The alignment system used for multicolor imaging should be mentioned.

D. Computer and Software

1. The software should control all motorized functions of the microscope, all parameters of image acquisition and image analysis.
2. The software also should have Line, curved line, frame, Z-stack, Time series imaging capabilities.
3. Standard geometry Measurements like length, areas, angles etc including intensity measurements should be standard feature of the software.
4. Deconvolution should be a part of the software.
5. The system should be supplied with latest tried & tested computer system directly from the super-resolution system manufacturing company. It should have at least the following specifications: Intel® Xeon® Processor E5-1620, 8GB DDR-3 SDRAM, 2TB (SATA, 7200rpm) HDD, NVIDIA Quadro K600 1GB with Optical Drive with Key Board and Mouse with 30” High resolution Monitor.
6. Offline software with computer system for image analysis, standard geometry measurements, 3D rendering, time series analysis and deconvolution.

E. Optional items

1. Excitation and depletion lasers for Yellow and Orange dyes, for example approximately 592 nm for excitation and 660 nm for depletion

ANNEXURE -III

IISER PUNE

PRE-BID CONFERENCE FOR SETTING UP COLLABORATIVE MICROSCOPY IMAGING CENTER

COMMERCIAL QUERIES AND CLARIFICATION

TENDER NUMBER - IISERT-PUR-0058-15 DATE : 9.12.15

S.No	Query/Clarification Sought	Clarification / Amendment
1.	Whether last date of submission of Tender Document will be extended .	The last date for submission of tender document has been extended to January 13, 2016 , Time – 3.00 PM