## Specifications for inverted fluorescence microscope with a photo-tracking detector to detect fluorescently tagged *C. elegans* over extended time periods

This system should include an inverted fluorescence microscope with DIC and an EMCCD Camera. It should have a fluorescent detector that will allow us to track fluorescently tagged neurons in *C. elegans* over extended periods of time during which the *C. elegans* will be moving. The system should allow single cell resolution while tracking the worm based on fluorescent signal i.e. it should be able to keep the moving worm centered at higher magnifications when field of view reduces to

~200- 300  $\mu$ m. (C. elegans moves with a speed of ~500  $\mu$ m/second). The system should allow for Calcium imaging and other optical recordings of fluorescently marked neurons in moving *C. elegans* and also allow for the use of channelrhodopsin and halorhodopsin in *C. elegans* so as to activate or inactivate neurons.

## Specifications for inverted fluorescence microscope with DIC

- 1. The microscope frame should have high static rigidity and should be waterproof to help block penetration of water inside the frame. The frame should be compact making it easy to fit in any work environment.
- 2. It should have easily manupalatable Controls for Light path selector 0:100/50:50/100:0 (or better), transmitted light Intensity control, and light ON/OFF switch or equivalent.
- 3. The system should have a sextuple revolving nosepiece or better with provision for DIC slider.
- 4. All Optical rely lens should be Apochromatic and the system should allow for 100% light to Side port for Fluorescence documentation
- 5. The system should have a 100W transmitted light illumination pillar having tilt Mechanism or better, a condenser holder and an adjustable field iris Diaphragm with 4 filter holders or better. It should have a 12V-100W or better external power supply unit with an ON/OFF mechanism and should allow for intensity controlling.
- 6. The observation tube should have Diopter adjustment and Inter-pupillary distance adjustable 50-75mm or better with paired 10X Eyepiece F.22 or better
- 7. The system should have a long working distance condenser NA/ 0.55 W.D 27mm or better
- 8. It should have 5 positions for BF, PH & DIC or better. The system should be upgradable to 7 position or better of motorized condenser for Phase, DIC and BF.
- 9. Suitable DIC accessories for all the objectives to be quoted.
- 10. The objectives should be

a. Plan Apochromat or Plan Semi Apochromat objective 10X/0.3, WD

## 10 mm

## or better

- b. Plan Apochromat objective 40X/0.75, WD 0.51 mm or better.
- c. Plan Apochromat Objective 63X or 60X/1.42, WD 0.15mm or better.
- 11. Fluorescence should be 120 Watts Metal Halide /130 Watts long life pre centered Fluorescence light source or better with a 3 meter Light Guide or better to ensure extended observation without heat transfer to samples.
- 12. The light source should have attenuators. It should have Fluorescence Filter cube turret with 8 positions or better with built in shutter. Narrow band pass Interference type filters for DAPI/Hoechst. GFP/FITC, TRITC/RFP should be quoted.
- 13. The system should have a closed Loop Motorized XY Stage or better with Controllers and Rotary Encoders and Precision Lead Screws and Ground Cross Bearings or better to provide a high degree of accuracy and Repatability. The XY Travel Range should be 110 mm x 70 mm or better and the XY Axis Repatibility should be 0.7  $\mu$ m or better.
- 14. The Motorized Z focus should have a minimum step size of 0.05 microns or better.
- 15. The system should have adjustable Holder to mount different size slides and Smaller Petri Dishes and slides
- 16. A digital monochrome EMCCD camera with 512X512 or better Pixel Resolution and large Pixel Size of 16 microns or better of EM gain capability should be quoted
- 17. It should have FireWire of 10/5 MHz or better. It should be Air Cooled to -25°C or better; be of 110V/220V and QE 90% or better. The read noise whould be effectively <1e- or better with Gain of 10-MHz readout or better. The Camera should be excellent for high-speed image acquisition with 30 full frames/sec or better and 375 fps at 16x16 pixel ROI 4x4 binning, or better.
- 18. The Camera should work perfectly for high-precision data collection using dual amplifiers or better.
- 19. The Acquisition and analysis software should be a 64 bit software for Windows 7. We should get the full Online License with a full suite of acquisition and analysis tools and the full complement of device drivers, including Microscope control, Image acquisition & processing, Saving instrument parameters, capability of Multichannel Imaging, Z stack time series, measurements of length, area, angle, intensity measurement & 3D/4D imaging and reconstruction. Image renormalization or better.
- 20. The software should allow for Channel math, Image math, Flat field correction, White balance, FFT (Fast Fourier Transform), Stereology and Montage Capture, Interactive montage boundary selection, Automatic

stage movement, Automatic image alignment, Photobleach correction and better.

- 21. The software should allow for capturing different channels at different frame rates, Capturing single planes of certain channels and Z-stacks of others (Mid-plane capture). Toggling different devices or perfusion systems at different points during image capture via a pre-programmed sequence, Recording external analogue voltages as a component of image capture. Displaying previous time-points while acquiring new images. Real-time graphing of mean intensity, FRET and FLIM, data during acquisition, Capturing of large datasets and better. The only limit should be the size of the hard drive. Capturing time-lapse experiments with various capture rates, Montage captures Simultaneous control of multiple cameras or better should all be in incorporated.
- 22. The Photo-tracking system should keep any moving sample (most of the time the sample will be *C. elegans*) in the field of view, no matter where they want to go. It should monitor a reference spot on the moving target (in most cases a fluorescently tagged neuron) and maintain that reference spot in the centre of the microscopes field of view.
- 23. It should have a Quadrant PMT in a light tight c-mount housing. The Unit should include a control board that offers manual control of gain. The control board also contains over exposure protection and reset circuitry, Dual C mount Splitter to Mount two cameras with 80/20 Splitter. Adapter to mount on Microscope or better.
- 24. The system should allow for Low magnification Video Microscope, with Collimated LED Illuminator with intensity control or better. The LED's Should be replaceable
- 25. It should have a video Microscope Dovetail Slide Stage mount or equivalent that allows to slide out of the way when changing Samples or using transmitted light path. Motorized Linear actuator with 50mm travel, Black and white ½" high sensitivity camera with controller or better.
- 26. It should come with a color Monitor 7.5" LCD Screen or better with Remote Control
- 27. The system should have a Computer System with i5 Processor 8GB RAM, 1TB HDD, DVD and R/W DVD, Windows 7 Original with 22"TFT/LCD Monitor or better.
- 28. An anti-vibration platform (that can be placed on a sturdy granite-top tabledo not quote for the table) for the microscope should be quoted with the system.
- 29. The system should be supplied with a suitable online UPS system of 2KVA with 15-20minutes of back-up time or better.
- 30. The vendors should indicate a list of installation bases.
- 31. The vendors should allow for upto 3 installations after the first installation, in case the system needs to moved to different locations.
- 32. Comprehensive warranty for 3 years or better for the complete system including hardwares and spares should be provided. The company should also quote both CMC and AMC charges for next 5 years after the

expiration of warranty. This should be quoted as optional. We reserve the right to change the final warranty period, and terms and conditions including CMC and AMC.

- 33. All the specifications should be supported by documentation in the form of original brochure/catalog. Photocopy will not be accepted. We reserve the right to disqualify parties who do not comply with the original documents. Compliance statement should be attached with markings in the original catalog.
- 34. The party should take an undertaking that they will supply the components of the instrument for the next 10 years after its installation at the site. Softwares and any other accessories that are upgraded by the company within 2 years of installation should be provided free of cost.
- 35. Technical Support should be available within 24 hrs. Hence, Local postsale support will be preferred.
- 36. The installation of the equipment should be within 6-8 weeks of supply.
- 37. The quoted price should include installation, operator instructions and institutional on-site training.
- 38. Installation will be considered complete only after successful demonstration of all the applications for which this system is being set up for. On-site training for as long as is required for all required users to be able to optimally use the system should be provided by personnel from the original company.
- 39. Expert/s from Principal Manufacturers of the tracking system should train users in IISER Mohali for at least two days, we would need a written document from the Principal Manufacturers of the tracking system stating the same.
- 40. It should be the responsibility of the vendor to integrate any third party module eg the tracking system and/or the EMCCD Camera. ie The instrument should come factory-fitted so users will only need to talk to the vendor for all their needs after the initial training (from both the company and the expert/s from the manufacturers of the tracking system points 37, 38 and 39).
- 41. The vendor should have Authorization documents from all third party suppliers.
- 42. We reserve the right to change the final configuration of this system.