Specifications for Confocal Imaging System

FUNCTION

Confocal imaging of both biological and non-biological samples

The system must meet or exceed the following minimum specifications:

SYSTEM

1. A spectral confocal system consisting of a scan head, spectral detector module, standard multi-channel photo multiplier tube (PMT) detector module, laser module, PC, control module, and monitor.
2. Spectral detector that acquires spectral data simultaneously in at least 32 channels using a 32 PMT linear array.
3. Variable spectral resolution channel widths over a range from 400nm to 750nm.
4. Separate diffraction grating that supports each spectral resolution.
5. Polarization of emission photons rotated to the p-plane to provide a diffraction efficiency of 90-95%.
6. Tandem multiple A to D converters.
7. Capable of at least two laser specimen illumination for spectral acquisition with ability to select any laser line available.
8. Spectral wavelength calibration traceable to NIST standards.
9. Linearized response of the 32 PMT detectors across the spectrum.
10. Pixel time correction when magnification, pixel dwell time, or capture resolution is changed.
11. Acquisition modes include confocal fluorescence, confocal spectral imaging, reflection imaging, scanned transmitted light in brightfield, and differential interference contrast (DIC).
12. Z-axis stepper motor with at least 50 nm step resolution.
13. Vibration isolation table.
14. Motorized XY Stage with at least 40 nm resolution

SCAN HEAD

1. Scan head with fiber connections for both standard and normal detectors.
2. Optically corrected scan head to minimize chromatic and spherical aberrations from 400nm to 700nm and maximize throughput for both illuminating and emission photons.
3. Includes laser power monitor that measures total laser power through the primary scanning dichroic mirror.
4. Scan head with a common set of pinholes for all fluorescence confocal channels mounted in a single turret.
5. Scan head with interchangeable primary (scanning) dichroic mirrors so that it can be adapted to new lasers.

LASER MODULE

1. Multiple line Argon ion laser (488-524nm), 408nm, 561nm diode pumped solid state laser, 638nm diode laser with sufficient power for high resolution confocal imaging, confocal spectral imaging, and fluorescent resonance energy transfer (FRET) analysis by acceptor photobleaching.
2. Laser module that carries at least two lasers in individual mounting assemblies.
3. Individually alignable beam steering mirrors.
4. Line selection from multi-line lasers by dielectric filters to block unwanted wavelengths.
5. Continuously variable beam attenuation by a combination of an Acousto-Optical Tunable Filter (AOTF) and directly modulated diode lasers.
6. Individual software controlled mechanical shutters in front of each laser.
7. Open laser platform for heat dissipation.
8. Laser unit that combines beam paths for launch through a single mode polarization sensitive fiber.

STANDARD DETECTOR MODULE

1. The detector module must be located separately from the scan head.
2. High sensitivity, low background side window photomultiplier tubes with overload protection.
3. Minimum wavelength step of at least 0.2 nm

CONTROL MODULE

1. Control module must be separate from the PC used for data processing so that a failure of one component does not affect the other.
2. Control module houses all circuitry for controlling the scan head, detector module, laser safety accessories, and digitization.
3. Communication between the control module and the PC is through a 100baseT Ethernet connection.

SPECTRAL, ACQUISITION, DISPLAY, AND RENDERING SOFTWARE

1. Spectral software that allows unmixing of the fluorescence signals of closely overlapping fluorescent probes with separation of overlapping probes with emission peaks closer than 10nm apart.
2. Unmixing of spectra in region of interest (ROI) drawn in the image to be unmixed and from spectra library
3. 6D imaging including time, channel or wavelength and focus, multipoint or multi stage position.
4. Acquired image display filtered with desired wavelength range between 400 and 750 nm.
5. At least one passive analysis software license for off-line analysis.
6. Acquisition resolutions of at least 2014x2014 pixels integrated into 12 bits.
7. Bidirectional scanning and rotation of the scan field by control of the scanning galvanometers.
8. Precise positioning of a zoomed field, ROI, or line able to be rotated to any acquisition angle or scan direction.
9. Provides frame averaging.
10. X/Y/Z acquisitions displayed in orthogonal view format during acquisition where user can click on any point in the X/Y, X/Z, or Y/Z displays and update the other two windows to the new position.
11. 3D projection available from any arbitrary angular view with the specimen orientation indicated.
12. Full 3D tumble and rotation available in both maximum and sum projection modes.
13. Multiple series functions including Z Series, Time Series, Averaging, and Channel Series selectable in any combination.
14. Provides a pseudocolor display to identify brightest and darkest pixels and see full 12 bits of data in each channel
15. Provides basic annotation and distance measurements.
16. Time course function allowing ratio acquisition and real time display of acquired fields and real time presentation of data as a graph of the average intensity in each specified ROI.
17. Merge function that allows for the combination of single images or single plane images with projections of the Z series.
18. Supports continuous zooming from the full field up to a single pixel.

19. Both zoom and ROI user definable as a rectangle or square of any size any place within the full field and rotatable.

OBJECTIVES

1. Plan Apo 10x, 20x objectives
2. Plan Fluor 40x oil immersion
3. Plan Apo 60x NA 1.4, oil immersion
4. Plan Apo 60x NA 1.2, water immersion objective providing spectral transmittance in UV range, axial chromatic aberration correction up to 405 nm line, and high peripheral resolution. Provides exceptional performance for brightfield, DIC, multi-stained fluorescence and confocal imaging.
5. Plan Fluor extra long working distance 20x, 40x, 60x objectives for viewing biological specimens

DOCUMENTATION and WARRANTY

1. The successful offeror shall provide documentation and warranty terms customarily provided to the public with a commercial item for all equipment provided.

INSTALLATION AND TRAINING

1. The price of the system must include installation and integration of the system at the Naval Research Laboratory, Washington, DC.
2. Training must also be provided for the proper use of the equipment at the NRL for 5 people for 2 days.