



DEPARTMENT OF THE NAVY

NAVAL RESEARCH LABORATORY
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WASHINGTON, DC 20375-5320

JUSTIFICATION FOR OTHER THAN FULL AND OPEN COMPETITION

1. The Department of the Navy, contracting through the Naval Research Laboratory, proposes to enter into a contract on other than a full and open competitive basis.
2. Nature and/or description of the action being approved.

The Naval Research Laboratory (NRL) Bio/Analytical Section (6112) has support funds in the amount of \$374,998.04 for the purchase of a new inverted fluorescence confocal microscope with differential interference contrast (DIC), laser total internal reflection fluorescence, (TIRF), point scanning confocal and high speed resonant scanning confocal microscopy to monitor and quantitate single molecule and/or single protein fluorescence in nanofluidic structures. In order to meet the broad based goals of this project, the instrument must combine a number of different capabilities into a single microscope.

First, the microscope must be an inverted fluorescence microscope in order to accommodate the lab on a chip based samples that we will be examining. Because of the types of fluorophores being examined, filter cubes must be provided for DAPI, FITC and TRITC widefield fluorescence excitation/emission. The microscope objectives required are a plan fluorite 20X objective with a numerical aperture of 0.50 or better for broad views, an extra long working distance plan fluorite 40X objective (oil) with a numerical aperture of 0.60 or better for zoomed in views, and an apochromatic 100X objective (oil) with numerical aperture of 1.49 or better for doing the critical single molecule TIRF or confocal microscopy. The system must include the capability for doing DIC both with a provided UV/Vis lamp, as well as with the confocal microscope capability. Functions that must be motorized include: z-axis, light port selection, transmitted light, nosepiece turret, fluorescence turret, light source shutter and attenuator, and TIRF with laser incident angle adjustment, shutter control and switchover to widefield through software.

Because of the single molecule/protein fluorescence detection requirements, the microscope must have a laser TIRF confocal microscope system mounted simultaneously on the inverted microscope, and incorporate an EMCCD detector such as iXon X3 DU897 or equivalent.

Our project goals also include single molecule detection at points greater than 200 nm removed from the cover slip, which will, therefore, require the simultaneous incorporation of a confocal microscope on the same system. In order to perform high resolution scanning (4096 pixels x 4096 pixels) where speed is not the most critical component, the microscope must have point scanning confocal capability, with the

ability to zoom in to smaller numbers of pixels to increase frame rate (minimum speed requirement of 512 pixels x 512 pixels with 2 frames per second).

In addition, we need high speed imaging in order to monitor the movement of single molecules, and, therefore, require, a separate, high speed resonant confocal scanner which will meet the minimum specifications of imaging 512 x 512 pixels at 30 frames per second and zoomed in regions of 512 pixels x 32 pixels at 420 frames per second. Line speed must meet the 15,600 lines/sec minimum requirement.

We will require a real-time focus drift correction capability with a motorized nosepiece in order to eliminate focus drift in time-lapse observations caused by room temperature variations or by the addition of reagents to a sample.

We are in need of a minimum of two laser sources at 488 nm and 561 nm. It is critically important that the lasers supplied with the instrument have power intensities that are greater than or equal to 100 mW in order to ensure that we have the photon flux necessary to perform single molecule detection. These should be diode lasers for quiet operation and to prevent heat buildup within the laboratory. The confocal microscope must be able to perform two color experiments with the two lasers, simultaneously, and be expandable to four lasers. Four photomultiplier tube detectors must be supplied with the confocal system, each of which can be operated simultaneously or in sequential mode.

We must have a sample holder than can support a 1x3" slide or 60 mm petri dish on a manual or motorized mechanical XY stage. The entire microscope must be supported on a vibration table in order to ensure the stability necessary to perform these sensitive experiments. The inverted microscope must have a stratum structure for future expandability to accommodate the introduction of a second turret of dichroics and filters, additional pathways for lasers, customer components and camera ports.

Finally, the manufacturer needs to supply the computer and complete software package for image capture and analysis for TIRF, confocal and standard microscopy. This must include the ability to do intensity measurements over time, image stitching and peripheral device control.

3. Description of the supplies or services required to meet the needs of the Naval Research Laboratory including the estimated value.

NRL will receive:

- Inverted Fluorescence Microscope
 - Filter Cubes
 - Widefield Fluorescence DAPI, FITC, TRITC
 - TIRF Ultra Hi Signal/Noise 488/561nm set
 - Motorized Functions
 - Z-axis
 - Light port selection (100% Left, 100% Right, 100% Eyes)
 - Transmitted Light 12v100w Control
 - Nosepiece Turret 6x

- Fluorescence Turret 6x
 - 130W Mercury Light Source shutter and motorized 6-position attenuator
 - TIRF with laser incident angle adjustment, shutter control and switchover to widefield through software
 - Non-motorized components or optionally motorized:
 - Extra Long working Distance Condenser
 - XY Mechanical Stage with Short Handle
 - Tube Lens Selector
 - Highly Corrected 1.0x or 1.5x Tube Lens that can be simply inserted into the imaging path
 - Objectives (Brightfield & Fluorescence)
 - Plan Fluorite 20x 0.50NA
 - Extra Long Working Distance Plan Fluorite 40x 0.60NA
 - Apochromatic TIRF 100x 1.49NA Oil
- Laser Total Internal Reflection Fluorescence (TIRF) Microscope
 - Laser TIRF confocal microscope system mounted simultaneously on the inverted microscope
 - iXon X3 DU897 EMCCD detector or equivalent
- Confocal Point Scanning Microscope
 - High resolution imaging- 4096 x 4096 pixels
 - Minimum Two color capability but the laser launch should be capable of 4 lasers total
 - 4 Photomultiplier Detectors- ability to use either simultaneously or in sequential mode
 - Scanning speed: 2 fps (512 x 512 pixels)
 - Zoom: 1-1000x continuously variable
 - Scanning mode: X-Y, XY rotation, Free line, Line Z
 - A navigator window must be provided to allow precise positioning of a zoomed field, region of interest, or line, that can be rotated to any acquisition angle or scan direction.
- High Speed Confocal Scanning Microscope- hybrid resonant scanner
 - Rapid full frame scanning (512 x 512 pixels at ≥ 30 frames per second)
 - Ultrahighspeed 2D scanning of isolated regions (512 x 32 pixels at ≥ 420 frames per second)
 - 15,600 lines/sec (line speed)
 - Zoom: 7 steps (1x, 1.5x, 2x, 3x, 4x, 6x, 8x)
 - Scanning mode: X-Y, Line
- Differential Interference Contrast with Lamp and Laser Confocal Microscope capability

- UV/Vis Lamp using 130W Mercury Light Source shutter and motorized 6-position attenuator with 2000 hour bulb life or equivalent
- Lasers
 - 100 mW Diode Laser at 488 nm
 - 100 mW Diode Laser at 561 nm
 - Diode lasers important for quiet, heat free operation
- 60 mm Petri Dish and 1x3" Slide Holder
- Vibration Table- at least 30 x 48" in size to support entire microscope
- Real-time Focus Drift Correction with motorized nosepiece-eliminate focus drift in time-lapse observations caused by room temperature variations or by the addition of reagents to a sample; simple and complete removal from light path when not in use.
- Software for confocal, widefield, confocal and TIRF acquisition, microscope & laser control, and all analysis tools; image capture for both confocal and standard microscopy, documentation, data management and analysis; multidimensional imaging tasks with support for capture, display, peripheral device control & analysis of images of up to six dimensions; sophisticated image processing and visualization features, such as 5D viewer, automated object counting, intensity measurements over time, image stitching, and data basing
- Stratum Structure for Future Expandability to accommodate introduction of second turret of dichroics and filters, additional path for lasers, customer components and camera ports.
- Computer
- Training- 2 day period of basic training to cover instrument operation, image acquisition and optimization, in-depth imaging techniques, image reconstruction and presentation and basic image analysis tools; basic training followed a month later by advanced training to cover handling specific imaging problems that users may have encountered since the basic training, in-depth high resolution imaging techniques & optimization, and FRET, FRAP and similar techniques.
- Warranty- confocal system must be warranted for 1 year for all items, including lasers but excluding disposables (bulbs); the inverted microscope must be warranted for 5 years on all mechanical and optical components, and 1 year on all electrical components.

- Preventative Maintenance- scheduled at the end of the first year of the system warranty period to ensure that it is functioning and calibrated to optimum performance, based upon factory specifications.

Estimated cost including warranty, freight, training and installation is \$374,998.

3.1. A statement of delivery requirements (*e.g., include a list of ships and/or shore activities and required delivery dates for each*). *If only labor hours or a final report are to be delivered, enter the performance period.*

Delivery is anticipated within 180 days after receipt of order by vendor.

3.2. The total estimated dollar value (including all options) for the acquisition(s) covered by the justification.

The total dollar amount required for this one-time action is \$374,998 (FY 2011)

4. Identification of the statutory authority permitting other than full and open competition.

As required by 10 U.S.C. 2304(c)(1) – Only one responsible source.

5. Demonstration that proposed contractor's unique qualifications or nature of the acquisition requires use of the authority cited.

The required system shall incorporate both TIRF and scanning confocal microscopy in the same instrument to perform single molecule/protein fluorescence detection at positions both within 200 nm of a coverslip and as much as 10 microns removed from the coverslip. For enhanced sensitivity and light collection efficiency, the laser TIRF system shall incorporate an EMCCD detector and use a 100x objective (oil) with numerical aperture of 1.49 or better. The required system shall incorporate a point scanning confocal to enable high resolution imaging and a resonant scanning confocal to enable high speed imaging. The point scanning confocal shall provide high resolution imaging (4096 x 4096 pixels or better) with scanning speeds of 2 frames per second or faster for 512 x 512 pixels, while a resonant scanning confocal will enable high speed imaging (30 frames per second or faster for 512 x 512 pixels; 420 frames per second or faster for 512 x 32 pixels; 15,600 lines/sec or faster line speed). The required system shall possess laser diodes at two colors (488 nm and 561 nm) with 100mW in power of intensity for each line to ensure sufficient photon flux for single molecule fluorescence. The confocal microscope shall be able to perform two color experiments with the two lasers, simultaneously, and be expandable to four lasers. Four photomultiplier tube detectors shall be supplied with the confocal system, each of which can be operated simultaneously or in sequential mode. The required system shall possess real-time drift correction capability over hours to days with a motorized nosepiece in order to eliminate focus drift in time-lapse observations cause by room temperature variations or by the addition of reagents to a sample. Because of the extreme sensitivity requirements for this microscope to attain single molecule detection, the required

system shall have hexagonal pinholes instead of a continuous variable square pinhole. Higher brightness equivalent to that of an ideal circular pinhole is achieved while maintaining the confocality and realizing 30% more light. The required system shall incorporate a low angle incidence method with the dichroic mirrors rather than the conventional 45 degree incidence angle method for dichroic mirrors, in order to realize a 30% increase in fluorescence efficiency. The Nikon Instruments, Inc Ti-E Inverted Microscope with A1-R Hybrid Resonant Scanner and Laser Total Internal Fluorescence Confocal Microscope (TIRF) is the only system available that can meet the Government's requirements stated above.

6. A description of efforts made to ensure offers are solicited from as many potential sources as is practicable including whether a synopsis notice was or will be publicized.

The COR/ARO is fully cognizant of the equipment/expertise in this scientific field and to the best of his knowledge; there is no other source available.

A synopsis notice was not publicized. A market survey of known confocal microscope instrumentation vendors was conducted. A review of product literature, peer review literature, and personal interviews of current instrument users was conducted. There are only four instrumentation vendors capable of supplying the microscope required for this project: Olympus, Carl Zeiss, Nikon and Leica. Each of these companies were contacted and quotes acquired after extensive discussions regarding required specifications. Only Nikon Instruments was able to supply a quote for an instrument that meets all of the minimum specifications required for this instrument and that was within the required funding allowance. With the exception of Nikon, none of the companies were able to quote lasers with the required 100 mW power intensity. This is a critical component of the microscope which cannot be compromised, as photon flux is the most important component of performing single molecule detection. In addition, Olympus, Carl Zeiss and Leica failed to quote an instrument that incorporated all of the components, DIC, TIRF, point scanning confocal, and high speed resonant scanner or spinning disc confocal in the same instrument that could also meet the funding allowance.

7. Determination by the Contracting Officer that the anticipated cost to the Government will be fair and reasonable.

The Contracting Officer will perform a price analysis in accordance with FAR 15.404-1(b) (and a cost analysis, if required) to determine whether the price is fair and reasonable.

8. Description of the market research conducted and results or statement of the reasons a market research was not conducted.

The COR/ARO is fully cognizant of the equipment/expertise in this scientific field and to the best of his knowledge; there is no other source available.

A complete market survey was conducted as described in item 6, and it was found that the Nikon Instruments system that was quoted to NRL was the only instrument that fully

met the minimum specification requirements. Furthermore, the same system was also the only system found to meet the NRL cost requirements.

9. Any other facts supporting the use of other than full and open competition:

Nikon Instruments additionally incorporates two important modifications to their microscope which are critical to the single molecule/protein fluorescence detection required by this project, modifications that no other manufacturer uses. First, brighter images or greater sensitivity are realized by using the industry's first hexagonal pinhole instead of the typical continuous variable square pinhole. Higher brightness equivalent to that of an ideal circular pinhole is achieved while maintaining the confocality and realizing 30% more light. Second, Nikon uses the industry's first low angle incidence method with dichroic mirrors, realizing a 30% increase in fluorescence efficiency. Both of these factors are exclusive to Nikon and critical to the single molecule fluorescence detection required of this project.

10. A listing of the sources, if any, that expressed, in writing an interest in the acquisition.

None.

11. A statement of the actions, if any, the agency may take to remove or overcome any barriers to competition before any subsequent acquisition for the supplies or services required.

This is a one-time purchase.

12. Certification and Approval signatures.

**TECHNICAL AND REQUIREMENTS CERTIFICATION
REQUIRED BY FAR 6.303-2(b)**

I certify that the facts and representations under my cognizance which are included in this justification which form a basis for this justification are complete and accurate.

Technical Cognizance

(Signature) *[Signature]*
Name and Title Code Phone Date

Requirements Cognizance

(Signature) *[Signature]*
Name and Title Code Phone Date

CONTRACTING OFFICER CERTIFICATION REQUIRED BY FAR 6.303-2(a)(12)

I certify that this justification is accurate and complete to the best of my knowledge and belief.

(Signature) *[Signature]*
Name and Title Code Phone Date

LEGAL COGNIZANCE

APPROVED:

(Signature) *[Signature]* *21 July 2011*
Name and Title Code Phone Date
Armand Beede