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## SPECIFICATION SHEET

# SUPPLY, INSTALLATION AND STARTING-UP OF A TWO-COLOR CONFOCAL MICROSCOPY-BASED SINGLE-MOLECULE OPTICAL TWEEZERS INTEGRATED SETUP WITH FORCE-FLUORESCENCE CORRELATED DATA AND MULTI-CHANNEL LAMINAR FLOW MICROFLUIDICS

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## **CLAUSE 1.** Object of the contract

The purpose of this contract is the supply, installation, and commissioning of a two-color confocal microscopy-based single-molecule optical tweezers integrated setup for force-fluorescence correlated measurements with multi-channel laminar flow microfluidics for the laboratory of the Photon Harvesting in Plants and Biomolecules group at ICFO. The system must be a fully integrated, compact, and user-friendly commercial solution capable of performing truly integrated single-optical tweezers measurements with force-fluorescence correlated data on systems such as monomeric photosynthetic light-harvesting complexes solubilized in detergent.

The system must be fully automated through user-friendly software and include integrated multichannel laminar flow microfluidics (at least 5 channels) to perform different relevant assays. The system must also provide user friendly software for data and image analysis.

## **CLAUSE 2.** Needs to satisfy

The system must enable truly correlated force–fluorescence single-molecule measurements by integrating three core technologies within one compact and automated platform: multi-trap optical tweezers, multi-color confocal fluorescence microscopy, and multi-channel laminar flow microfluidics.

Such a system will be essential for our group for studying correlated changes in fluorescence, force, and structure in individual photosensory proteins and, in particular, photosynthetic light-harvesting complexes.

These experiments require the simultaneous and time-resolved acquisition of mechanical (force and distance) and single-molecule fluorescence data with sub-picoNewton and sub-nanometer resolution, to directly link structural changes to functional optical responses.

The requested system is intended for daily routine operation by researchers and students with varying levels of spectroscopy and biochemistry expertise, from beginner to advanced.

The system must provide user-friendly, intuitive operation, requiring minimal optical alignment or adjustments, while supporting advanced users through automated workflows and custom scripting.

The system must integrate specifications including:

-True hardware integration of optical tweezers, confocal fluorescence, and microfluidics enabling simultaneous, correlative force, distance, and fluorescence data acquisition.

-High precision optical tweezers with force resolution of at least 0.1 pN at 100 Hz, distance resolution <0.3 nm at 100 Hz, and high escape force (>1000 pN for 4.5 µm polystyrene beads) ensured by a rigid, single-block mechanical design.

-Optimized microfluidics with at least five laminar channels, allowing rapid and controlled sample exchange, in-situ assembly, and execution of complex single-molecule assays.

-Confocal detection capable of imaging individual fluorophores, with single photon sensitivity, at the diffraction limit with efficient background rejection.

-User-friendly software supporting automated operation and streamline trapping, tethering, and fluorescence measurements for high-throughput single-molecule experimentation.

## **CLAUSE 3.** Technical requirements

The system, including all accessories, software, warranty, and service, must meet the following technical specifications and performance criteria:

-The instrument must enable live, simultaneous, and correlated optical tweezers manipulation, force measurement, bead-to-bead distance determination, and confocal fluorescence imaging of single fluorescent biomolecules.

-It must include a dedicated bright-field camera for bead-to-bead distance measurements during confocal imaging. Additionally, it must provide measurement of bead-to-bead distance from trap steering mirror positions and bead displacement (force) signals. Both methods must be available simultaneously, ensuring precise end-to-end distance measurement with high acquisition speed using piezo and force signals.

-The system must automatically align and overlay the trap positions with the confocal image and vice-versa, to allow the user to select confocal scan region (1D or 2D) in the brightfield bead image and or to select trap positions in the confocal image.

-Has an optimized correlative workflow; for setting up single molecule experiments in a correlative manner (e.g., move traps to certain position, wait until force reaches threshold, start imaging).

-Has an automated alignment procedure for trap position and confocal imaging.

- Provides continuous wave optical traps (no time sharing); for higher optical trapping stability and precision.

-Has Tandem Scanner for simultaneous dual optical trap movement.

-The optical tweezers must achieve force resolution of < 0.1 pN at 100 Hz,, allow to detect distance changes smaller than 0.3 nm at 100 Hz, and force stability of < 0.3 pN drift over 2 minutes. The escape force must exceed 1000 pN for 4.5 µm polystyrene beads.

-The traps must maintain perfect intrinsic z-alignment through a single hardware mechanism for z-translation of both beams.

-Has fast and ultra-stable piezo-mirror for confocal scanning enabling localization of fluorophores with very high precision, and state-of-the-art single photon counting modules providing ultra-sensitive fluorescent detection.

-Provides imaging of the condenser's back-focal plane using diagnostic cameras; for ensuring optimal alignment and system performance.

-Provides complete and integrated software solution that offers a completely integrated workflow visualization that includes user-interaction, data acquisition and real-time data visualization and analysis. The correlative tweezers-fluorescence method must be recapitulated in the integrated software to truly enable the user to perform simultaneous and correlative fluorescence and force mechanical measurement in an easy way.

-Integrated multi-channel laminar flow microfluidics system with five channels without physical barriers between the channels. It must use passive pressure-driven laminar flow, providing an extremely constant flow-rate. It must feature shockwave-free motorized fluidic shut-off valves, for precise fluidic control without impact on the biological assay and allowing experimentation automation. It must have fully automated liquid handling;

programmable from the software user interface for high data throughput applications. It must Incorporate a micro-fabricated re-usable monolithic glass flow-cell to provide reliable laminar flow in multiple channels without a physical barrier in between.

**Additional Functional Modules**

Active Trap Stabilization
The system must include an active stabilization module that continuously maintains the relative distance between the two optical traps through a dedicated feedback loop. This feature compensates for fluctuations in laser power and beam position independently of the force detection system, providing exceptional trap-to-trap distance stability in the sample plane. The stabilization reduces drift by a factor of 2–4 compared to non-stabilized setups, achieving drift below 0.5 nm over 10 minutes under optimal conditions. This stability enables true equilibrium measurements of single-molecule population dynamics over extended time periods, improving statistical accuracy and revealing rare conformational states.

Nanostage
A high-resolution piezo-controlled nanostage must provide precise, repeatable, and absolute positioning of the sample in x, y, and z with nanometer accuracy. The nanostage must be installed on the sample micro-stage and allow controlled movement of surface-attached samples relative to optically trapped constructs. It must support vector force clamp operation by maintaining constant force through feedback-controlled movement in x and y. The z-axis movement must be fully integrated with the confocal scanning software, enabling three-dimensional scanning of surface-bound samples.

Active Force Calibration
The system must include an active calibration function that applies a sinusoidal force to the trapped bead by oscillating the nanostage. This allows trap calibration without prior knowledge of bead size or buffer viscosity, minimizing errors from bead size variability (which can be up to 10–15%) and temperature-dependent buffer viscosity changes. This ensures accurate measurement of conformational displacements, particularly on short molecular tethers.

Piezo Tracking
A piezo tracking module must be provided to measure bead positions independently of the camera system. While camera-based tracking operates at video rate and is optimized for longer molecular tethers (>2 µm), piezo tracking must enable bead position readout up to 78 kHz, independent of tether length. Bead-to-bead distance must be computed directly from the high-resolution trap position, allowing precise, rapid, and tether-length-independent end-to-end distance measurements.

Baseline Correction
The system must include baseline correction functionality to minimize effects in the data from crosstalk between detection signals when the two traps are in close proximity (e.g., during short tether experiments). The module must record any crosstalk-induced signal and automatically subtract it during acquisition to optimize the signal-to-background ratio and ensure high-quality data output.

A table summarizing the main technical requirements is provided below:

| **Parameter** | **Specification** |
|---|---|
| Force resolution (x,y) | < 0.1 pN @ 100 Hz |
| Force stability (x,y) | < 0.3 pN drift over 2 min |

| Parameter | Specification |
|---|---|
| Force acquisition rate | 78 kHz on two traps |
| Maximum escape force (dual trap) | > 1000 pN (4.5 µm beads) |
| Bead displacement resolution using force signal | < 0.3 nm @ 100 Hz |
| Number of independent traps | 2 |
| Confocal resolution | Diffraction limited |
| Fluorescence localization precision | < 15 nm |
| Confocal scanning speed | Up to 100 Hz |
| Fluorescence sensitivity | Optimized for single fluorophore detection |
| Confocal field of view | 50 µm × 35 µm |
| Number of confocal channels | 2 (excitations at 488 nm and 638 nm) |
| Trap distance stability (x,y) | < 0.5 nm drift over 10 min (active stabilization) |
| Short tether assay toolkit | Nanostage with active calibration, short tether piezo tracking, baseline correction |
| Microfluidics | Automated valves, reusable glass cell, 5-channel laminar flow |

The purchased system must include a **two-color confocal fluorescence single-molecule optical tweezers integrated setup**, including all components required for immediate operation.

**The configuration must integrate:**

- Two-color confocal fluorescence (488 nm and 638 nm excitation)
- Two fully dynamic optical traps
- Dual force detection in x and y directions
- Automated multi-channel microfluidics module
- Optical table
- Workstation rack with three screens, keyboard, mouse, and joystick

**The system must also include the Short Tether Package, consisting of:**

- Active force calibration
- Active trap stabilization
- Advanced piezo trap tracking
- Baseline correction
- Three-dimensional nanostage

**Additional inclusions:**

- User-friendly data acquisition and analysis software with possibility of custom-made workflows
- One-year warranty on all parts.
- Relevant training kits, including DNA structural mechanics kit, buffer solutions, and beads
- Installation and initial operator training
- Shipping and insurance within Europe

## **CLAUSE 4.** Power distribution and safety

The system must be compatible with the EU (Spain) power grid, CE marked, and include safety features such as overload protection and secure interlocks. Restart after power interruptions must be straightforward and safe.

## **CLAUSE 5.** System layout and services

The supplier will provide:

- Full technical documentation including system layout and dimensions
- Clear description of all optical, electronic, and software modules.

## **CLAUSE 6.** Transport, installation and start-up

- The proposal will include transportation to ICFO's facilities and all export/import and customs duties.
- The system will be placed in the selected location by ICFO. Contract winner will cover all costs, organization and coordination of machine placement, including any required specialized equipment or vehicle, and any required component dis-assembly and re-assembly for systems unloading and transportation inside the building to the target lab location.
- Onsite system starts up included followed by the acceptance tests specified below.
- System training to ICFO personnel included using model systems and samples provided by the ICFO group purchasing the equipment.

## **CLAUSE 7.** Acceptance test

The awarded company will install and carry the necessary test to verify that the technical requirements above mentioned are met and the correct functioning of the equipment. A service report, a list of all the specifics of the equipment provided, and acceptance test will be provided by the awarded company and signed by our institute, certifying from that moment the start of the warranty period.

## **CLAUSE 8.** Warranty and Follow-on Support

The system must include a limited **1-year warranty for all parts.**

The warranty will include the replacement of any faulty or damaged part(s) during normal use of the system, no matter the manufacturer of the component(s). It will cover any cost related with the disassembly, transportation, reparation and re-assembly of the damaged component(s), including all travelling and living costs of the required service engineer(s). An on-site repair, or a justified alternative to reduce the system down time to the minimum, will always be the first service option. A team of properly qualified and skilled service engineers will have to be available.

Commitment includes priority assistance on working days.

**Spare parts will be available during, at least, 10 years after system supply and, in case of failure, will be delivered within 20 working days.**

## **CLAUSE 9.** Delivery and Installation Time

The system must be delivered and installed at ICFO within a maximum period of **6 months.**

Delivery time is defined as the time elapsed since the signature of the contract until the system delivery at ICFO facilities. It includes the manufacture of the system, the transportation, the installation and the acceptance test at ICFO's premises.

## **CLAUSE 10.** Target price

- The target price for the system is 721.900 € (VAT excluded).

Castelldefels, on the date of its digital signature

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