



INSTRUMENT USAGE SHEET

CONFOCAL MICROSCOPE STELLARIS 5 WITH WHITE LIGHT LASER

General Information

Instrument: STELLARIS 5 Confocal Microscope with White Light Laser

Manufacturer: Leica

Location:

CRIETT – Bassani Building

Via J.H. Dunant No. 5, Varese, Italy

Floor: -1 (VA.BASSANI PS.007.0)

Instrument Managers

Dr. Maristella MASTORE

Email: maristella.mastore@uninsubria.it

Phone: +39 0332 39 7191

Prof. Silvia SACCHI

Email: silvia.sacchi@uninsubria.it

Phone: +39 0332 42 1504

Authorized Users

Dr. Serena Baldin

Dr. Isabella Barbiero

Dr. Roberto Ferrarese

Prof. Greta Forlani

Dr. Maristella Mastore

Dr. Aurora Montali



Prof. Silvia Sacchi

Dr. Serena Valastro

Dr. Giorgia Valetti

Training Requirements: Yes*

* Independent use of the instrument is permitted. Practical training consists of individual instruction (approximately one week). For the theoretical component, the instrument manager will provide educational material on operating principles.

Internal tickets can be viewed on the intranet page (access with university credentials): <https://intranet.uninsubria.it/areadocumenti/ticket-strumenti-criett-utenti-interni>

The fee schedule for external users can be viewed on the website:
www.uninsubria.it/criett

CRIETT Acknowledgment in Scientific Publications(See CRIETT Regulations, Article 11): Users must acknowledge the CRIETT major equipment used in scientific publications using the following wording:

“The scientific support from CRIETT centre of University of Insubria (instrument code: MIC03) is greatly acknowledged”.

Instrument Code: MIC03

Access Procedures

1. Direct Access (Self-Service)

Who: Research groups or users who regularly use the instrument and have received specific training.

Procedure: Trained users may independently access and operate the instrument after making an online reservation.

2. Access to Technical Support (Full-Service)

Who: Research groups that use the instrument occasionally, external users, or users without qualified personnel.

Procedure: Access is provided with the assistance of dedicated technical staff, who manage both booking and instrument operation.

3. Occasional/Temporary User

Occasional users may access the instrument through the Instrument Manager or an Authorized User.

Description and Applications

The **Leica STELLARIS 5 inverted confocal microscope with White Light Laser (WLL)** is a fully motorized high-end research system designed for advanced fluorescence imaging, spectral analysis, and super-resolution microscopy.

Main Components

- Motorized objective turret (6 positions)
- Fluorescence illumination: EL6000 metal halide lamp (120 W, >2000 h lifetime)
- Epifluorescence filter sets: Long Pass for DAPI, FITC, Rhodamine
- Detectors: 3 × Power HyD S (high quantum efficiency, $QE \geq 58\%$ at 500 nm)
- Operating modes: analog, reflection, photon counting
- Spectral range: 410–850 nm
- Photon detection efficiency: >58%, dead time < 1.3 ns, up to 160 Mcps (continuous laser)
- Filter-free spectral detection system based on high-transmission prism (>95%)
- Adjustable spectral bands down to 5 nm with 1 nm step size
- Fully compatible with new fluorophores and multiplex labeling

Advanced Imaging Functions

Lambda Scan

- Full emission and excitation spectral acquisition
- Supports Lambda Square for excitation/emission correlation

AOBS (Acousto-Optical Beam Splitter)

- Programmable, fully automatic beam splitter
- Supports up to 8 simultaneous lasers (405 nm + 485–685 nm range)
- Maximize photon delivery to detectors

Scan Performance

- Maximum image size: 8192×8192 pixels

Super-Resolution Module (Lightning)

The **Lightning module** is a proprietary adaptive deconvolution-based super-resolution system that:

- Achieves ~ 120 nm lateral and ~ 200 nm axial resolution
- Enables real-time multi-fluorophore imaging (up to 3 simultaneously)
- Provides scanning speeds up to 10 fps at 512×512 resolution
- Improves signal-to-noise ratio (SNR)

Lightning uses AI-based adaptive deconvolution algorithms (e.g., Richardson–Lucy) to reconstruct images by correcting optical blur through computational reallocation of out-of-focus photons to their correct focal origin, resulting in:

- Higher image sharpness
- Improved contrast
- Enhanced resolution beyond standard confocal limits

White Light Laser (WLL)

- Pulsed laser system tunable from 485 to 685 nm (1 nm steps, ~200 lines)
- Full excitation range: 485–790 nm (including 405 nm laser line)
- Enables optimal excitation for any fluorophore
- Reduces phototoxicity and photobleaching
- Supports multiplex imaging and advanced spectral analysis
- Compatible with Lambda Square and TauSense (fluorescence lifetime analysis)

Software and Advanced Features

- Navigator software for mosaic imaging
- Advanced stitching functions
- Lambda Scan and spectral imaging tools
- Super-resolution (Lightning) integration

Technical Specifications

- Motorized scanning stage
- Compatible with:
 - Standard glass slides
 - Petri dishes (24–68 mm, optical bottom #1.5, 0.17 mm thickness)
 - Multiwell plates

Available Objectives

- 20× (air, Aria)
- 40× (oil)
- 63× (oil)

Depth Limits (Working Distance Constraints)

- 20× / 0.75 NA (air): ~620 µm
- 40× / 1.30 NA (oil): ~240 µm
- 63× / 1.40 NA (oil): ~140 µm

Optical limitation: Beyond ~100 µm depth, light scattering in untreated tissues significantly reduces image quality.

⚠ Safety Precautions

- Use only after completing the required training program
- Do not touch optical components with bare hands
- Clean optics carefully after use, especially oil immersion objectives
- Contact the technician or instrument manager in case of anomalies or doubts

Operating Notes

- Instrument reservation through the internal management system is mandatory
- Self-service operation is permitted only for trained, registered, and authorized users
- Any malfunction must be promptly reported to CRIETT* personnel



Materials and Accessories Provided by CRIETT

- Optical cleaning paper
- Immersion oil
- 70% ethanol

Materials and Accessories Provided by the User

- Mounting medium
- Cover slips

