UNIVERSITÀ DI PAVIA

PASS-BioMed Imaging

Laboratorio di Microscopia Macromolecolare, Cellulare e Tissutale del Centro Grandi Strumenti (CGS)

Responsabile Gestionale: dr.ssa Federica Corana

Presidente: prof. Mauro Freccero

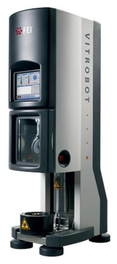
Centro Grandi Strumenti

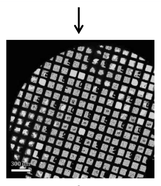
https://cgs.unipv.it/

Cryo Transmission Electron Microscope

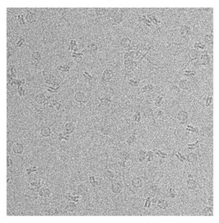
The Thermo Scientific Glacios equipped with a 200 kV X-FEG electron source, an autoloading system, a Falcon 3EC direct electron detector and a Ceta 16M camera allows scientists to determine structures of biological macromolecules (proteins, protein complexes, nucleic acids) at near-atomic resolution under cryogenic conditions.

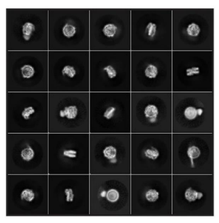
A Thermo Scientific Vitrobot is used for the vitrification of aqueous samples of macromolecules under reproducible conditions.
The facility is equipped with special tools to manipulate samples at cryogenic temperatures before mounting onto the cryo-TEM.

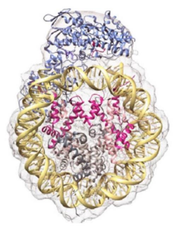




micrographs → classification → reconstruction







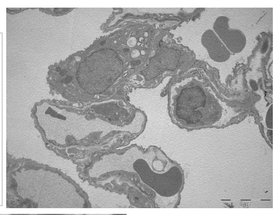
Structural reconstruction of nucleosome-associated LSD2/NPAC. Courtesy of C.Marabelli, Mattevi lab (DBB)

In Cryo-Electron Microscopy (**Cryo-EM**), through Single Particle Analysis (SPA) hundreds of thousands of images of the isolated particle are collected, classified and used to computationally reconstruct the object in 3D. This technique represents a major breakthrough in the field of structural biology.

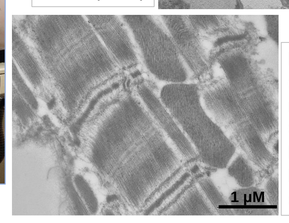
Contact: Andrea Alfieri

Transmission Electron Microscopes

JEM-1200EX II



Rat lung alveolar epithelial cells. Courtesy of G.Milanesi (DBB)



Mouse cardiac muscle cell. Courtesy of L.Zannino, Biggiogera lab (DBB)

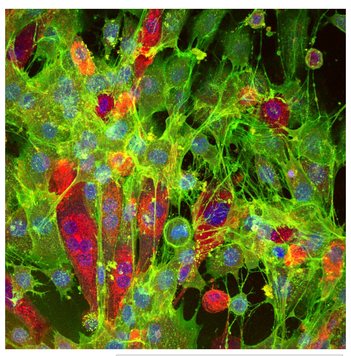
EM 900

The JEM-1200EX II (JEOL), equipped with the MegaView G2 CCD camera (OSIS) and the EM 900 (Zeiss) allow observations of specimens by transmission electron microscopy with a 120 kV and 80 kV electron source, respectively. The JEM-1200EX II is routinely used to pre-screen negatively stained samples that are candidate for structural determination by cryo-EM.

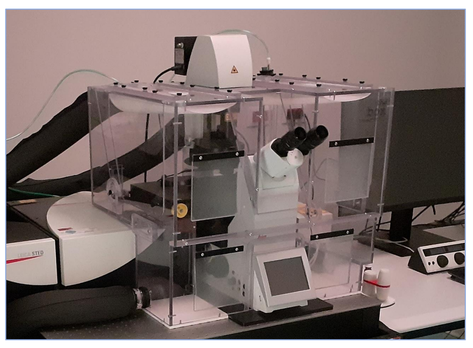
Contacts: Massimo Boiocchi, Gloria Milanesi (DBB)

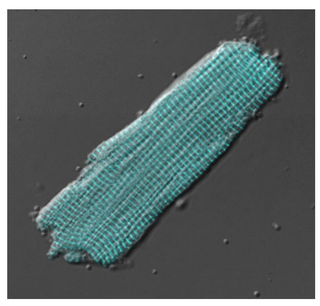
Stimulated Emission Depletion (STED) Microscope

The TCS SP8 STED microscope (Leica) uses a super-resolution fluorescence microscopy technique that breaks the diffraction limit of light microscopy by reducing the area of effective emission in combination with a scanning microscopy mode. This cell imaging technique is suitable to resolve biomolecular structures (DNA and DNA-protein complexes, cytoskeleton microtubules, neurofilaments, organelles such as the endoplasmic reticulum, lysosomes, endocytic and exocytic vesicles and mitochondria) and for the study of membrane-embedded or membrane-associated protein complexes.



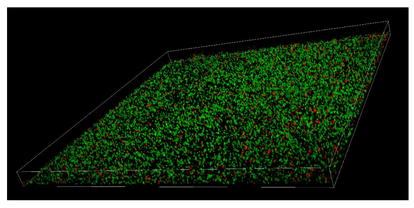
Mouse myoblasts. Courtesy of A.Canciani, Forneris lab (DBB)





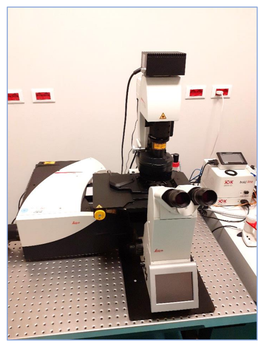
Cardioactin filaments. Courtesy of M.Denegri, Priori lab (ICS Maugeri)

Staphylococcus aureus. Courtesy of L.Visai (DMM)



Digital Light Sheet (DLS) Microscope

The TCS SP8 DLS (Leica) integrates the Light Sheet Microscopy technology into the confocal microscope. This technique is minimally invasive, strongly reduces photobleaching and photodamage and has a high acquisition speed. The low phototoxicity allows very long observation times of sensitive samples, the high imaging speed allow fast volumetric imaging and imaging of fast processes. This technique is useful in the developmental biology of small organisms and the study of dynamic processes (cell motion, tracking of vesicles, cell lineage and differentiation).



Total Internal Reflection Fluorescence (TIRF) Microscope

The DMi8 S TIRF microscope (Leica) uses a technique for selectively imaging fluorescent molecules in a single plane of the sample of 100-200 nm thickness at the glass-water interface. This technique is mainly used on *in vivo* samples to qualitatively and quantitatively describe the roles that different proteins play in exocytosis/endocytosis, to observe the size of the contact region between a cell and the solid substrate, for tracking cell movements or protein movements inside the cell.

Optical Microscopes

DMI 600

The DMI 6000 (Leica) inverted microscope and TCS SP5 II (Leica) confocal microscope are used to perform standard and confocal microscopy; in confocal microscopy, a focused beam scans the fluorescent sample point-by-point, eliminating background fluorescence and thus improving optical resolution.

TCS SP5 II

Contacts: Patrizia Vaghi, Amanda Oldani

Flow Cytometer

The FACSLyric flow cytometer (BD) is a diagnostic standard for clinical cell analysis and also suitable for research activity. The instrument is equipped with 3 excitation lasers (405, 488, 642 nm), up to 12 fluorescence channels, with a maximum acquisition rate of 35000 events/sec with no limit on the number of events acquired, an autoloader and a fluidics design enabling a large selection of sample input devices.

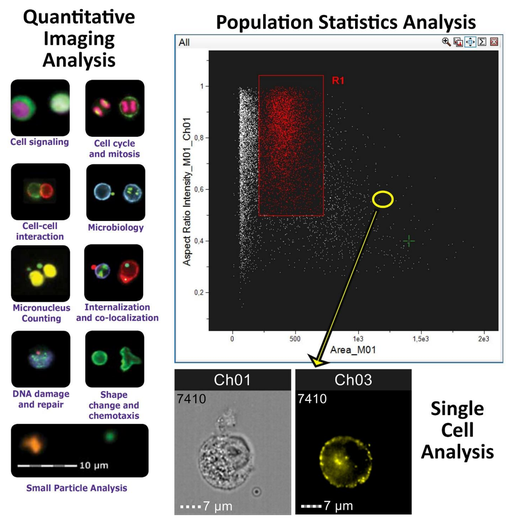
Cell Sorter

The FACSAria III sorter (BD) performs cell sorting based on a patented technology for a wide range of research applications. Its fluidics and optical systems include laser excitation optics with 5 lasers (355, 405, 488, 561 and 633 nm), a flow cell with gel-coupled cuvette and the octagon and trigon detection systems. It measures up to 18 colors simultaneously, mounts up to 20 detectors and has a maximum acquisition rate of 70000 events/sec.

Contacts: Samantha Solito, Alberto Azzalin (DBB)

Imaging Flow Cytometer

The Amnis ImageStream^X Mk II (Luminex) combines features of standard flow cytometry and microscopy. The instrument acquires brightfield and fluorescent images of cells at high speed, allowing quantitative analysis of cellular images and population statistics. It is ideal to analyze biological processes like translocation, colocalization, cell cycle, apoptosis, trafficking and others. It is equipped with 2 excitation lasers (488 and 642 nm), a TDI CCD camera for up to 6 images at 4000 cells/sec (at 20x) and 3 possible magnifications (20x, 40x, 60x).



Analysis of a glioblastoma cell line. Courtesy of A.Azzalin (DBB)

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