



Confocal microscopy service (SMC)

Confocal microscopy imaging techniques provide optical sections of material to observe using lasers and electronic systems of digital image capture. Fluorescent labelling of the sample allows the location of cell components in single sections. In addition, diverse experimental approaches involve single or multiple fluorescent labelling in fixed cells and tissues.

The SMC provides infrastructure for fluorescence, confocal laser scanning microscopy and image processing tools, covering most light microscopy applications, with technical assistance to all users. The equipment and services are available to all CNB personnel as well as to researchers from the public and private sectors.

The technical staff offers formation and advice about equipment use, available methodologies, and for image processing, quantification and later analysis, if required. Aliquots of secondary antibodies and probes with broad use in fluorescence microscopy applications are also provided.

The facility's equipment includes:

- Confocal multispectral Leica TCS SP5 system. Laser lines: 405, 458, 476, 488, 514, 561, 594 and 633 nm, with incubation system for in vivo studies
- BioRad Radiance 2100 confocal system. Laser lines: 457, 476, 488, 514, 543 and 637 nm
- Fluorescence microscope Leica DMI6000B with incubation system for in vivo studies and OrcaR2 monochrome digital camera for image detection
- Two epifluorescence microscopes (Leica DMRXA and Zeiss Axiophot) with colour digital cameras and one Leica stereomicroscope

The unit also provides offline computer workstations for fluorescence and confocal image processing and analysis (LAS AF, MetaMorph, Image J, Laser Pix, Huygens). Auxiliary equipment includes a CO2 incubator, centrifuge, laminar flow chamber, and freezer.

Applications available:

- Laser scanning confocal microscopy
 - Multichannel confocal imaging + transmission imaging of living cells or fixed samples (2D, 3D, 4D imaging)
 - High speed confocal microscopy
 - Multidimensional in vivo time-lapse experiments
 - FRET, FRAP, photoactivation, photoswitching, lambda scan, calcium imaging
 - Subcellular colocalisation studies
- Wide-field microscopy
 - Multichannel fluorescence imaging + transmission imaging (BF, DIC, phase contrast)
 - Multidimensional in vivo time-lapse experiments (wound healing, infection, etc.)
 - Tile scan imaging

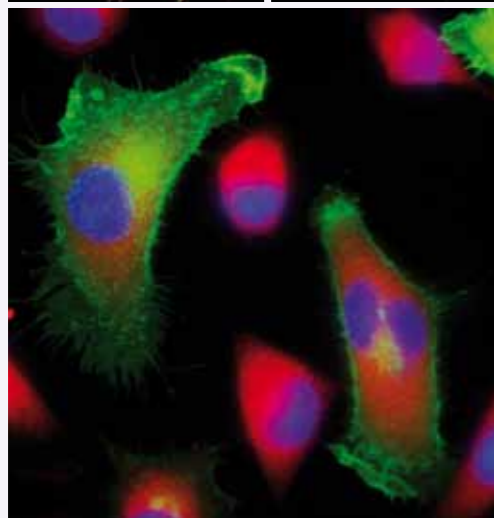
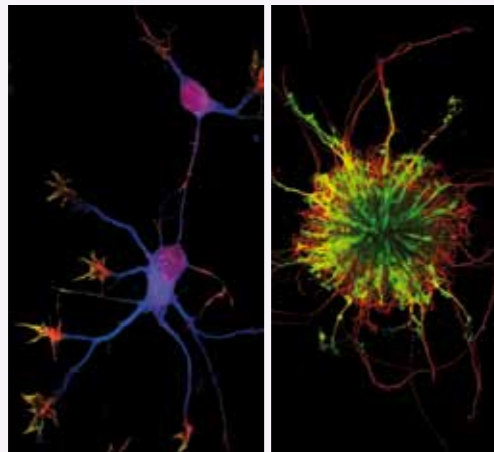


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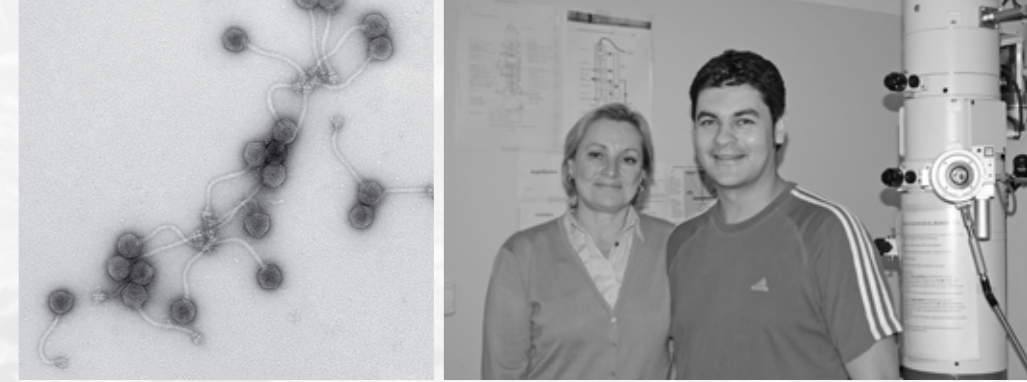
Electron microscopy

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The Electron Microscopy service offers a variety of equipment and techniques for the preparation, processing and analysis of biological samples by transmission electron microscopy.

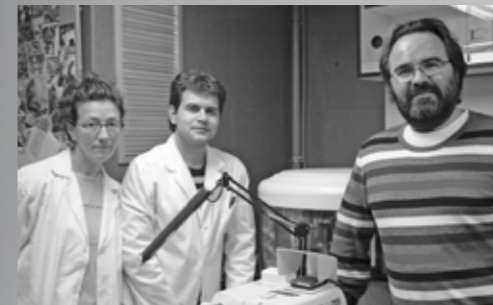
Techniques offered include chemical fixation and inclusion in epoxy and acrylic resins, cryofixation (plunge-freezing, high pressure freezing), cryosubstitution and inclusion in low-temperature resins, ultramicrotomy, immunogold staining, negative staining, negative immunostaining, in situ hybridization, conventional transmission electron and low electron dose microscopy.

The staff provides support to users both in the application of techniques and for equipment use. We offer regular training in the techniques and methods available. We are also responsible, in cases

where required, for sample preparation and image acquisition, and provide support for data interpretation.

The service has the following specialised equipment:

- Jeol JEM-1011 transmission electron microscope with ES1000W
- Gatan camera
- Leica EM MED 020 carbon coating system
- Leica Ultracut UC6 cryo-ultramicrotome with Leica FC6 cryo-chamber
- Reichert Ultracut E ultramicrotome
- Leica EM AFS2 automatic cryosubstitution system
- Leica EM PACT 2 instrument for high-pressure vitrification
- Leica EM TRIM sample trimmer (pyramitome)
- Reichert Knifemaker



CNB histology facility

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At the CNB Histology Facility, we offer methods for the histological analysis of animal and plant biological samples. Available methods include the preparation of paraffin/wax blocks and plastic (HistoResin) for obtaining histological sections with an automated microtome, as well as preparation of blocks to obtain frozen tissue sections with a cryostat. Sections can be counterstained or maintained for later immunohistochemistry analysis. The facility is equipped with a cryostat, two automated microtomes, a tissue processor carousel, paraffin/wax embedding equipment, two water baths, a stereoscope, an oven, and additional small equipment to process all types of tissue samples.

Our expertise is reflected by the wide variety of tissue samples and species we have processed over the past years, including tissue samples from spleen, aorta, femoral, embryo and foetus, brain, cochlea, colon, stomach, ganglia, liver, thin gut, fingers and toes, muscle, eyes, ears, ovary, pancreas, skin, lung, prostate,

rachis, kidney, brown and white adipose tissue, mammary gland, testis, femur, tibia, gonads, fibula, trachea, thyroid gland, skin and mammary gland tumours, uterus and other tissues from animals including mice, rats, rabbits, sheep, lynx, cat, pig, fish, chicken, gazelle and humans. In addition, we have processed fruits, leaves, apical meristem, roots, stem from tomato, Arabidopsis and potato plants. A full list of tissues processed by the facility is available at: <http://www.cnb.csic.es/~histocnb/tabla.html>.

Since 2009, the CNB Histology Facility is associated with the IIB-UAM/CSIC Histology Facility (www.iib.uam.es/servicios/patexperimen/intro.es.html). The two centres merged their operations under the coordination of the CNB Histology Facility, enabling them to offer CNB and IIB researchers greater processing capacity of histological samples. The CNB Histology Facility has been training personnel from the IIB-UAM/CSIC Histology Facility and supervising work, aiming to establish equivalent output standards.