

Confocal Microscopy Core Unit



Diego Megías

Unit Head

Diego Megías was born in Madrid in 1974 and graduated in Biological Sciences from the *Universidad Autónoma de Madrid* (UAM) in 1998.

From 1999 to 2001 he was responsible for sample preparation for the Transmission Electron Microscopy Service at the Core Services (SIdI) of the *Universidad Autónoma de Madrid*. In 2001 he was in charge of the Confocal Microscopy Facility, UAM Core Services (SIdI), and was also involved in the development of new technologies related to live cell microscopy imaging.

In 2003 Diego joined the CNIO as Senior Technician for the Confocal Microscopy Unit where he played a critical role in developing a number of new microscopy technologies that have since been used by different Groups at the CNIO. He has also carried out research projects and collaborated with other groups leading to the publication of 12 articles in prestigious international scientific journals.

Diego was appointed Head of the Confocal Microscopy Unit in 2009. One of his main objectives for the future is the application of microscopy technologies to the biology of tumour cell invasion using live cell imaging and analytical microscopy techniques.

Summary

Optical microscopy has traditionally been an indispensable tool in cell biology studies. One of the main challenges in oncological research is the study of specific markers, expression patterns or individual cells in the tumour environment.

The main goal of the Confocal Microscopy Core Unit is to provide CNIO Research Groups with all the standard methodologies as well as the latest technical advances in microscopy.

Our Unit provides training and support on the use of microscopy equipment as well as advice with experimental design, and develops new technologies including fluorescent protein expression vector collections and FRET analysis methodology.

Main Objectives

- Provide state-of-the-art equipment and software packages related to confocal microscopy, including technical and scientific advice and support to CNIO scientists using our instruments
- Set up and optimise microscopy techniques for CNIO research
- To develop new technologies and tools involving imaging applications
- Evaluate new equipment of potential interest for the CNIO
- To organise training courses in microscopy technology

Highlights

During 2010 the Confocal Microscopy Core Unit underwent complete renovation. We recruited two microscopists, which improved our ability to provide user support by establishing new image analysis routines and imaging acquisition protocols.

In addition we acquired a new white laser-equipped Leica TCS-SP5, equipping the Unit with the latest in laser technology. The advantages of using this include its tunability to any wavelength thus increasing sample excitation efficiency, reducing phototoxicity – allowing us to undertake spectrum absorption studies.

The expansion in personnel and scientific instrumentation led to us moving to a different location in October. The new facilities are perfectly organised to facilitate optimal performance of all our instruments.

The Confocal Microscopy Core Unit is currently equipped with three laser scanning confocal systems – one Leica SP2 and two Leica SP5 – incorporating UV, multiphoton and white light laser excitation; as well as two wide-field systems – a Deltavision 4D deconvolution station and a



Technicians: Manuel Pérez (since March) and Joaquim Soriano (since March).

We also implemented high throughput technologies (HTS) through a Matrix Screening Application that is integrated into both confocal systems, allowing detection on multi-well plates as well as tissue sections. Furthermore, the Opera Perkin Elmer High Content Screening system runs HTS experiments on fixed and live cells, monitoring cell dynamics (translocation, cell division, etc.) through the use of fluorescence.

Due to the complexity of the data generated through our platforms, we provide support to use a comprehensive range of analysis software including Metamorph, Imaris, ImageJ, Volocity and Definiens. This year we have also incorporated Huygens software to improve wide-field and confocal image deconvolution.

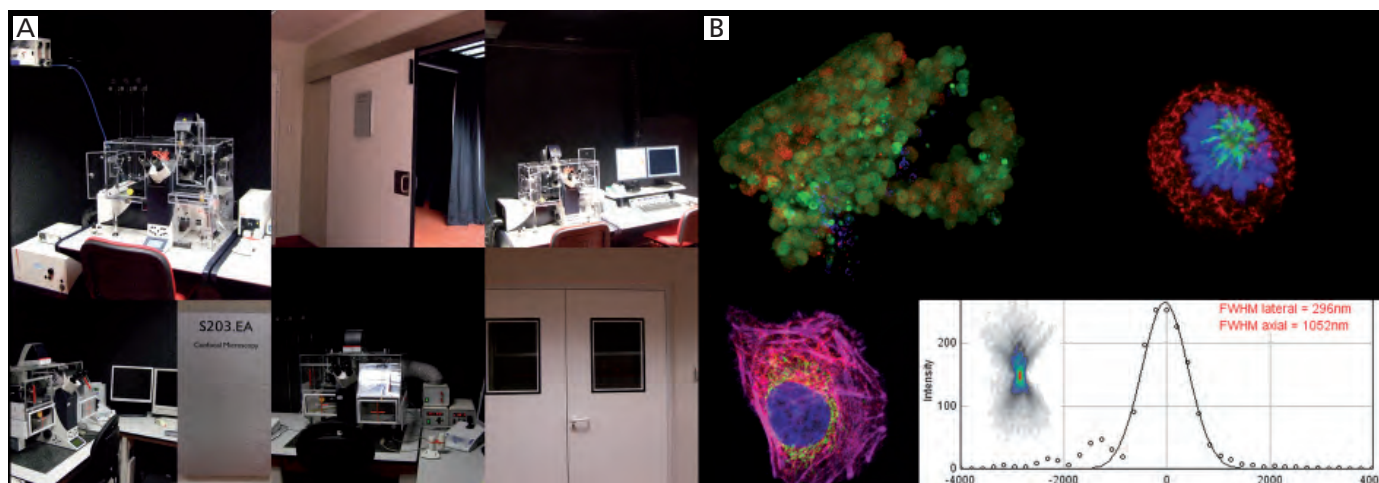


Figure: (A): New Confocal Microscopy Core Unit facilities. (B): Gfp clones undergoing apoptosis in a *Drosophila* wing disc, mitochondria with microtubule spindle in mitosis (upper panels); actin cytoskeleton during cell migration and point spread function using a 63X objective (lower panels).

Leica DMRI6000 system – equipped with microinjection. All microscopes are automated and equipped with incubators for live cell imaging.

The following techniques are also available: subcellular co-localisation studies; cell proliferation and apoptosis studies; quantitative analysis of transwell invasion; wound healing and tracking cell migration assays; telomere length analysis by fluorescence quantification of confocal images; studies of molecular interactions and dynamics using FRET, FRAP and photoactivation techniques; live cell studies of DNA damage; and high resolution whole tissue biomapping.

Publications

Mavillard F, Hidalgo J, Megias D, Levitsky KL, Velasco A (2010). PKA-mediated Golgi remodeling during cAMP signal transmission. *Traffic* 11, 90-109.

Martín-Villar E, Fernández-Muñoz B, Parsons M, Yurrita MM, Megias D, Pérez-Gómez E, Jones GE, Quintanilla M (2010). Podoplanin associates with CD44 to promote directional cell migration. *Mol Cell Biol* 21, 4387-4399.

Rivera J, Megias D, Bravo J (2010). Sorting nexin 6 interacts with breast cancer metastasis suppressor-1 and promotes transcriptional repression. *J Cell Biochem* 111, 1464-1472.