Equipment Name: Confocal Microscopy including live cell imaging	**Category:** **D. In-vitro toxicity studies** **Institute:** University College Dublin **Location:** Conway Institute, University College Dublin, Belfield, Dublin4, Ireland **Contact Details of Technology Expert:** **Name:** Dimitry Scholtz **Phone:** +353 1 716 6736 **Fax:** **E-mail:** dimitri.scholz@ucd.ie

Short technology description/Overview:

Confocal laser scanning microscopy (CLSM) is a technique for obtaining high-resolution optical images. The key feature of the technique is the ability to acquire serial or 'optical' sections of the samples, which are free from 'out-of-focus' blur. Using digital imaging techniques, these serial images can be reassembled to form 3D representations of the structures being studied, which have been labelled with fluorescent markers.

The most common applications of confocal microscopy is the analysis of mechanisms of cell functioning and disease mechanisms. The ability to stain organelles and proteins allows detailed mechanistic studies to be performed on live cells in a time-resolved manner.

UCD have developed robust protocols for quantitative determination of uptake and transport of fluorescent nanoparticles by cells, as well as for assessment of the portals utilised by nanomaterials to enter cells. Sub-cellular localisation of nanoparticles can be tracked in a time-resolved manner, with co-staining of organelles, and well developed protocols are available through QNano.

Nanomaterial dispersion quality and nanomaterial label are key determinants of the quality of the experimental outcomes, and must be discussed at the earliest stage of experimental design.

Main Features (Equipment Capabilities):

- Carl Zeiss LSM 510 UVMeta laser scanning confocal microscope (Zeiss, Munchen, Germany) eight excitation wavelengths (405nm, 458nm, 477nm, 488nm, 514nm, 543nm, 594nm, and 633nm) and for detection, three separate reflected light PMTs, each with its own adjustable pinhole and emission filter wheel. A transmitted light PMT is able to acquire phase and DIC images as well.
  A unique feature of the LSM 510 META scan head is its ability to acquire lambda stacks in 10 nm increments over a broad spectral range (411-753nm). A lambda stack collected from individual reference dyes can be used to generate an emission fingerprint that can subsequently be used for spectral un-mixing of closely related fluorophors in the same sample.

- Olympus FluoView FV1000, which is the latest in point-scanning, point-detection, confocal laser scanning

microscopes designed for today's intensive and demanding biological research investigations. Excellent resolution, bright and crisp optics, and high efficiency of excitation, coupled to an intuitive user interface and affordability are key characteristics of this state-of-the-art optical microscopy system.

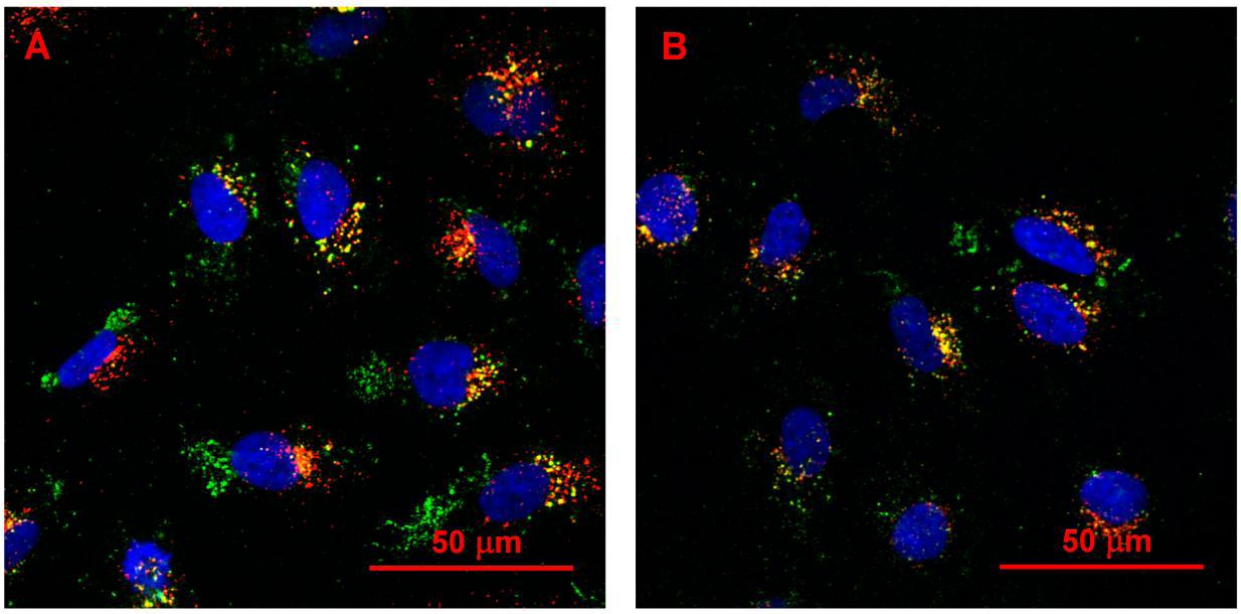
Wavelength selection and flexible control of laser intensity permits reduced specimen fading, lower crosstalk, and excitation area selection. Olympus confocal microscopes are ideal for 3-D imaging, time course experiments, energy transfer visualization, and photobleaching recovery experiments.

- Andor spinning disk confocal microscope provides a range of laser-illuminated microscopy options, including TIRF, photo-bleach, activation and ablation modalities. This microscope allows study of live specimens with reduced fluorophore concentrations or expression levels, with minimal perturbation of physiological events. Rapid Z sectioning with a piezo stage allows up to 60 sections per second.

Issues to consider when developing TA proposal:

- Fluorophors of nanoparticle and assay should not interfere with one another or overlap
- There should be no interaction of the assay label with the test nanoparticles
- Dispersion protocol for nanomaterials should not be toxic to cells
- If comparing across cell lines, cell culture media and conditions should be identical where possible, or where not possible, appropriate control steps must be built into the experimental protocol to allow cross-comparison of data.

Typical Samples & Images:



Co-localisation of green SiO_2 nanoparticles with lysosomes in A549 cells. Confocal images of A549 cells after 24h exposure to 100 µg/ml 50 nm (A) and 100 nm (B) green SiO_2 particles. Red: immunostaining of lysosomes with LAMP1 antibody (secondary Alexa-647 antibody). Blue: DAPI staining of nuclei. Yellow: co-localisation of nanoparticles in lysosomes. From Shapero *et al.,* Mol Biosyst. 2011, 7, 371-378.

Key publications:

- Salvati A, Aberg C, Dos Santos T, Varela J, Pinto P, Lynch I, Dawson KA. Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics. Nanomedicine. 2011 Mar 29. [Epub ahead of print]
- Shapero K, Fenaroli F, Lynch I, Cottell DC, Salvati A, Dawson KA. Time and space resolved uptake study of silica nanoparticles by human cells. Mol Biosyst. 2011, 7, 371-378.

Any further Information:

This installation can be used in conjunction with the Confocal microscopy installation or with the EM installation, if want to couple study of uptake and impacts with detailed assessment of localisation.

This installation could also be coupled with HCA installation during a single visit.