Specialist Equipment

- Zeiss LSM 710 Confocal Microscope
- ImageXpress Micro Spinning Disc Confocal High-Content Imaging System
- Real Time Cell Analysis (RTCA) xCELLigence Epithelial/Cardio system

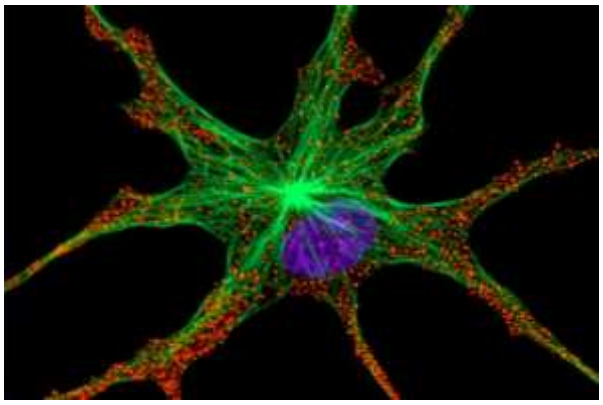
BioSciBER Bio-Imaging Suite

The BioSciBER labs **Bio-Imaging Suite** for the Bernal labs is a live-cell/fixed-cell monitoring and imaging test-bed to facilitate the short, medium and long-term measurement of complex cell and tissue behaviours within the University of Limerick (UL). It will permit the investigation of cell interaction with modified microenvironments (e.g. biodegradables, delivery devices, drugs, novel compounds and cellular scaffolds). The already funded **(Zeiss LSM710 Confocal)** and requested instruments (**ImageXpress Micro High-Content Imaging System** and **xCELLigence CardioECR/DP**) will add to UL's bio-imaging and cell monitoring capability, which can be further utilised to optimise bioprocessing methods against their effects on cell behaviour. The instruments are diverse in their use and can be tailored to suit the needs of most projects, from single cell endpoint imaging on sample slides to multi-well plate high through-put live-cell screening applications. The highly-automated liquid handling, environmental control and data acquisition capabilities of key stations within the suite will allow for multiple criteria, materials or compounds to be **assessed within the same assay. The proposed suite of instruments are as follows:**

Zeiss LSM 710 Confocal imaging system

The Zeiss LSM710 confocal microscope **with automated xy stage upgrade** is a highly sensitive **laser scanning** confocal microscope (LSM) system. LSM's allow for the good lateral resolution (xy, ~300 nm). In addition, they also display good control in relation to the optical section acquisition in the z axis (Usually ~1 µm). As such, they are ideally suited to investigate composition **of fine subcellular structures** under high magnification, which can be used to interpret a variety of cell behaviours. Furthermore, if environmental control upgrades are applied, localised laser illumination allows spot fluorescence activation or photo-bleaching for the implementation of FRET and FRAP (monitoring localised fluorescent molecule interaction (FRET) or movement/activity (FRAP/Enzymatic-FRAP)) experiments in **the short term**. Unfortunately, the single point illumination of LSMs necessitates prolonged laser excitation periods during image acquisition, which is detrimental to cell viability through the production of free radical species. Accordingly, LSMs are usually **unsuitable for longer term imaging experiments** in lives cells. Additionally, LSMs are comparatively slower and subsequently **not as suited to high throughput screening techniques** (low n number analysis). Customarily, after sample fixation (endpoint analysis), they can complement other live imaging systems/screening techniques to exploit their increased sensitivity and spatial

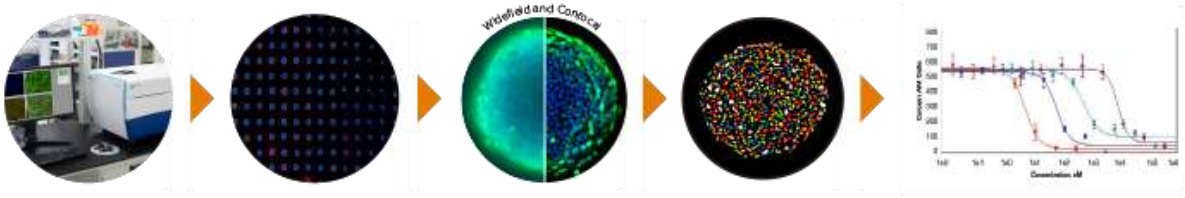
resolution. The **subsidised** installation of **Zeiss LSM710** and component upgrades for the BioSciBER labs in the Bernal institute will round out the existing confocal experimental capabilities. The fine focus control and automated acquisition afforded by the stage upgrades will allow for direct plate or well acquisition on in a multi-well format in a semi-automated fashion, greatly enhancing the efficiency of data acquisition.



Confocal acquired image of a cell grown in vitro showing DNA (purple), cell skeleton ***(green) and cell internal structures (red)***

Image Xpress Micro Spinning Disc Confocal High-content Imaging System

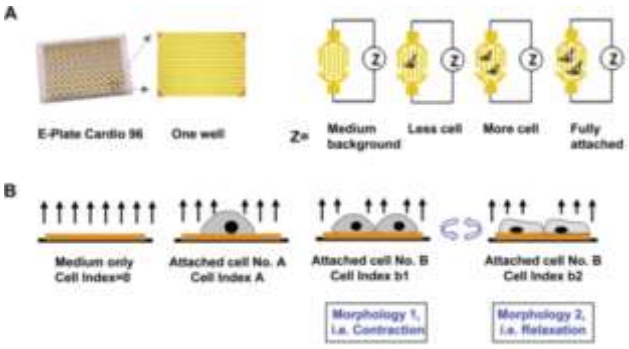
The ImageXpress Micro Spinning Disc Confocal High-Content Imaging System with **Robotic Fluidics Handling** is a **fully automated high throughput bio-imaging/screening** platform that allows for the design and automated implementation of highly complex parallelised experimental setups. Conventionally, the ImageXpress uses multi-well plate formats (up to 1536, though it can utilise custom geometries i.e. micro-fluidic devices) to image separate experimental criteria on a single plate. It can acquire multiple images per well, in **5 separate fluorescent channels**, in specific locations or in a tiled format to cover the entire surface (High n number technique). It has environmental control and is suitable for **live-cell imaging, multi-timepoint acquisition** experiments over short (seconds to minutes) to medium (hours to days) term. Furthermore, it has **liquid handling capabilities**, including programmable solution mixing between solution plate wells (up to 96x2 plates) prior to sample well introduction down to 3 µL. It is effective at imaging thicker histological samples (>20 µm) normally prone to background fluorescent interference. It can be implemented in both wide-field (extremely fast, poor z-resolution, good x-y resolution) or spinning disc confocal (fast, good z-resolution, excellent x-y resolution, 20 µm z-optical sections, capable of z-stacks in 100 nm increments for 3D reconstruction) modes. It has robust downstream automated image analysis capabilities with proprietary software on station (MetaXpress) or compatibility with open source software (CellProfiler) for off station analysis. It is a highly flexible and capable cell analysis platform ideally suited to gathering high quality biological data and images of cell activity upon interaction with different materials or compounds.



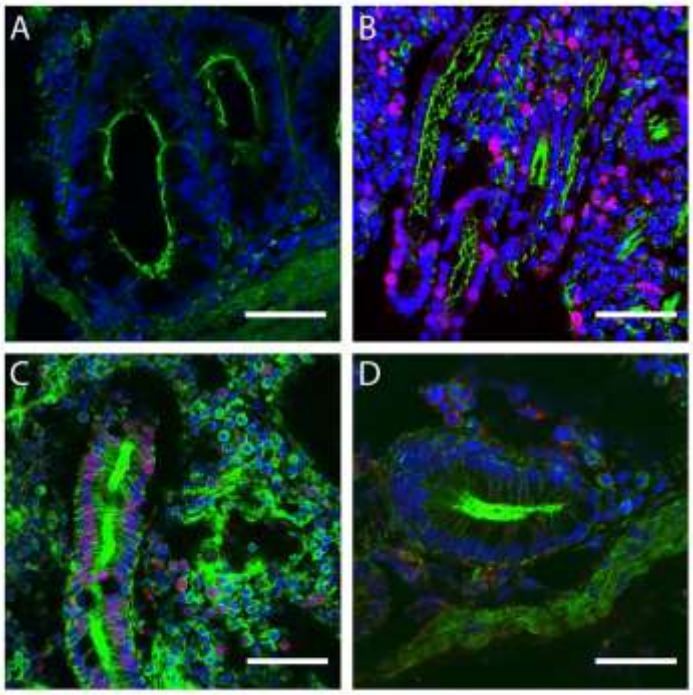
Showing a workflow of the high-throughput imaging test bed where multiple conditions can be tested simultaneously

xCELLigence system

The Real Time Cell Analysis (RTCA) xCELLigence Epithelial/Cardio system with **impedance based** Multi Electrode Array technology, will allow the **simultaneous real-time measurement** of a variety of cell type behaviours. This system can assess cell excitation and contractility by impedance while simultaneously measuring electric activity by monitoring field potential measurements. This live cell monitoring testbed will facilitate the **label free** establishment, culturing and monitoring of complex cell lines and cell models to include medium to long-term (multi-day) measurements of cell behaviour and function. It will allow conclusive identification and assessment based on RTCA and will facilitate the performance of **real-time label-free cell-based** assays in automated high-throughput screening. The measurement of integrated ion channel activities will provide a longer-term measurement of **cell viability**, which will identify compounds or materials causing long-term structural damage to cells.



Schematic representing a typical work flow of an xCELLigence cell impedance system



Sections from a porcine (pig) intestine showing cells that make up this tissue. (A) Colon 15 µm section labelled for DNA, (blue) and cell boundary (green). (B and C) Upper intestine 15 µm section section labelled for DNA, (blue) and cell boundary (green) and a cell replication marker (red). (D) Upper intestine 15 µm section labelled for DNA, (blue) and cell boundary (green)and a marker for quiescence (resting stem cell) red. All scale bars correspond to 50 µm.