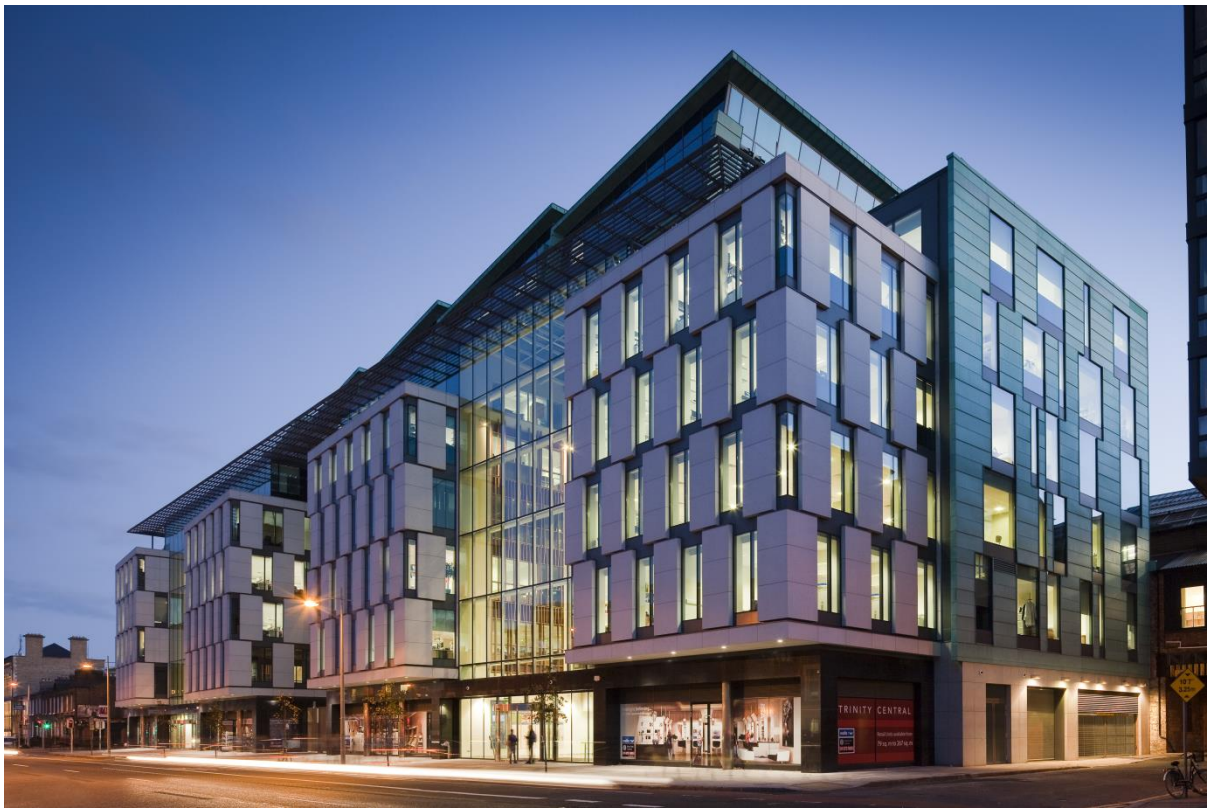
Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Trinity Biomedical Sciences Institute



INTRODUCTION TO THE TRINITY BIOMEDICAL SCIENCES INSTITUTE AND ITS CORE RESEARCH SUPPORT FACILITIES

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http://www.tcd.ie/biosciences/

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TRINITY BIOMEDICAL SCIENCES INSTITUTE (TBSI)

OVERVIEW

The primary mission of TBSI is to make fundamental discoveries in biomedical research. TBSI is an environment where innovative and interdisciplinary approaches lead to scientific discoveries of biomedical importance, ultimately giving rise to better patient care. Occupancy began in 2011 and we now have over 550 researchers, working towards discoveries of direct relevance to human diseases in a multi-disciplinary environment. Recent years have seen strong funding growth (research awards in excess of €75m since building occupancy) and important discoveries in areas such as cancer, asthma, Type-2 diabetes, Age-related macular degeneration, infectious diseases such as hepatitis C and motor neuron disease. Discoveries have been published in journals of the highest rank internationally, with publications in Nature (twelve publications), Science, Cell, Nature Medicine, Nature Immunology, Nature Neuroscience and The Lancet. There have been more than 1600 publications, including 12 in Nature.

The building has an area of 35,000m^2 of which 21,000m^2 are used for research and teaching. TBSI houses 75 PIs and research staff from:

- The School of Biochemistry and Immunology (100% located in TBSI)
- The School of Chemistry
- The Trinity Centre for Bioengineering
- The School of Pharmacy and Pharmaceutical Sciences
- The School of Medicine

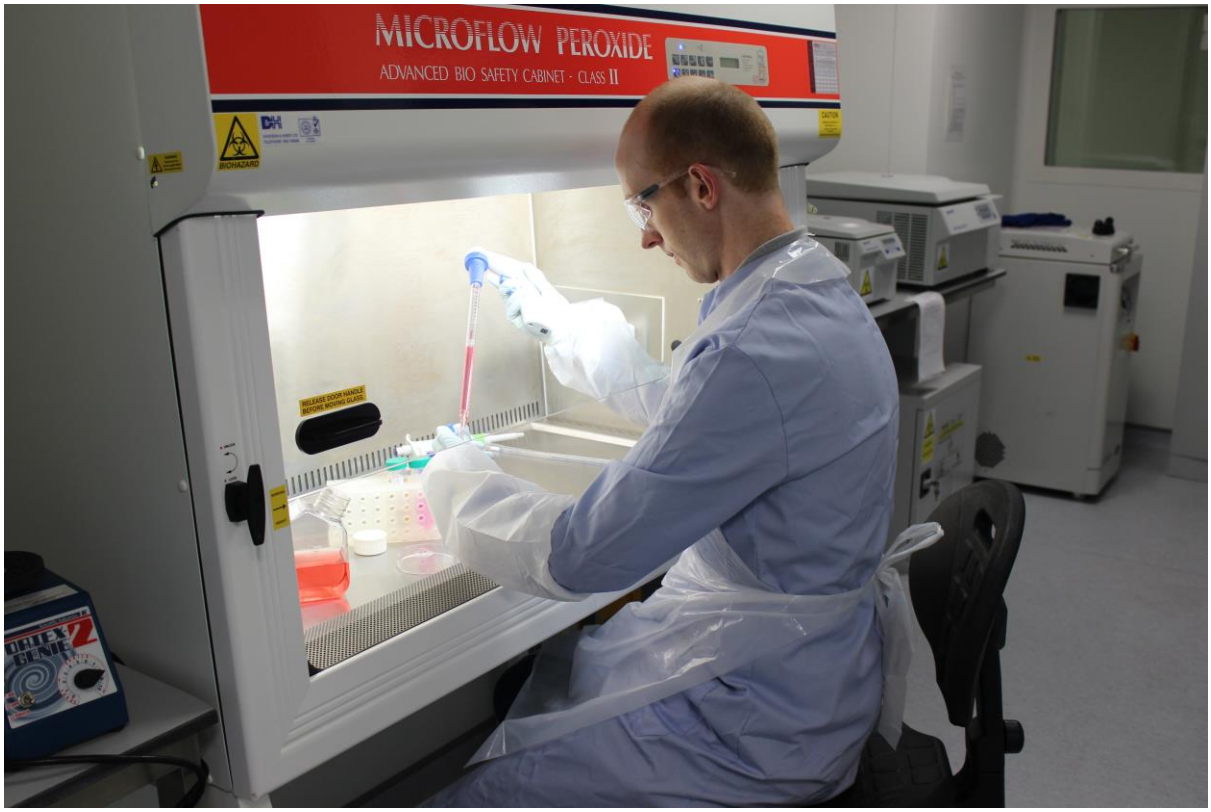
Whilst the activities of the research groups cover a wide range of research topics, they all have relevance for Biomedical Sciences. Translational activities are facilitated through strong relationships with hospitals and the presence of Trinity clinical personnel and research laboratories on hospital sites.

There are two cleanrooms and 25 tissue culture laboratories, two of which are rated at Category 3.

CATEGORY 3 FACILITY

The category 3 facility contains two tissue culture laboratories, accessed via a common secure lobby. The laboratories can operate on different types of biological agents in parallel. Each laboratory contains two biosafety cabinets and associated equipment e.g. autoclave, incubator, centrifuge etc. The facility and equipment can be sanitised using vapourised hydrogen peroxide.

Current research projects focus on models of HCV and HIV infection with particular emphasis on the effect of infection on innate signalling pathways. HIV and HCV infected human blood and liver samples are also processed and analysed. The clinically related research aims to identify prognostic markers of disease progression and response to therapy in specific cohorts of adult and paediatric patients. The research is funded by grants from SFI, the HRB and Temple St Children's Hospital Research Fund.



BIORESOURCES

TBSI is equipped with an extensive range of animal based technologies for pre-clinical drug development. The Transgenics Facility (www.transgenics.ie) manages the importation, rederivation, cryopreservation, and generation of new transgenic lines. In addition the transgenic facility has the capability to hold and breed highly immunocompromised (e.g. NSG line) and even germ free (axenic) animals. This facility is juxtaposed to a 1700 m^2 state-of-the-art animal holding facility with capabilities to manage all common rodent, avian and non-primate species. Housed within the breeding facility are a CATII unit (for infection studies), an in vivo imager (real time whole body imaging), behavioural rooms and an intravital microscope (allowing live cell analysis). The intravital microscope is an Olympus FV1000MPE-BX61WI, equipped with a Coherent Chameleon Vision II laser system, Olympus Version, 680-1080nm, 140 fs peak, 2.5W at 800nm. The whole body imager is a Biospace Lab PhotonIMAGER™ RT System for real-time in vivo bioluminescence and fluorescence optical imaging.

Downstream tissue analysis is facilitated by a histochemistry suite managed by technical staff and equipped with a vibratome, microtome, cryostat, cryoultramicrotome, automatic tissue processor, and slide stainer.

STRUCTURAL BIOLOGY

Structural Biology is one of the areas of speciality in the TBSI and is led by three research groups (Caffrey, Khan, and Mok) which have instrumentation in X-ray crystallography and biomolecular NMR spectroscopy. All three have excellent facilities for protein engineering, expression, production, purification and spectroscopy with state-of-the-art chromatography and analytical tools that support their respective atomic-level, three-dimensional characterisation methodologies. Martin Caffrey's research group is a world leader in membrane protein crystallization that provided the essential tools for the detailed characterisation of GPCR's and membrane proteins, recently gaining attraction as the underpinning core technology for Brian Kobilka's Nobel Prize-winning GPCR structural studies. The research group led by Amir Khan concentrates on the X-ray crystallography of proteins involved in signalling complexes, particularly those involved in vesicle movement and/or innate immunity against bacteria or viruses. Ken H. Mok's research group utilizes biomolecular NMR spectroscopy to characterise novel tumoricidal protein-fatty acid complexes that possess partially-folded character. Separately, his group has an NMR metabolomics strand interested in profiling and archiving large biobank samples.
To this end, all three groups have state-of-the-art computational, cell-culture (mammalian, insect, and bacteria), biochemical, bioprocessing, crystallogenesis (with robotics) and X-ray/NMR facilities (2x Rigaku protein systems; synchrotrons access; 800 MHz NMR spectrometer with cryo-probe) to carry out their objectives efficiently.

The following additional platforms in biophysics are available:

- dynamic and static light scattering instruments for characterization of protein/particle size and homogeneity
- protein interaction technologies, including calorimetry, surface plasmon resonance, and stop-flow fluorescence

INSTRUMENTATION OVERVIEW

Introduction

There is a vast array of instrumentation available in TBSI, much of which is unique in Ireland and exceeds the quality of equipment available elsewhere in Ireland. Access is available to this as well as instrumentation elsewhere in the College.
TBSI has the capacity to produce recombinant proteins, conduct transgenic studies and holds the bulk of Trinity College's expertise in biological imaging, using a range of microscopic techniques (e.g. confocal and electron microscopy). These capabilities are used to answer fundamental scientific questions in biology with major implications for human, animal and plant health. In addition to microscopic imaging the TBSI holds the College's capability for obtaining images of large biological molecules such as enzymes, receptors and transporters, many of which are attractive drug targets, using X-ray crystallography and NMR.
TBSI has a wide range of instrumentation available to researchers engaged in research in the biomedical sciences - some of the key resources are described below:

NMR Spectrometry

800MHz NMR

This is the highest field NMR facility in Ireland. It is an Agilent Technologies Ultra High Field (UHF) 800 MHz Nuclear Magnetic Resonance (NMR) system, with both liquid and solid state capabilities.

The system is equipped with a number of different probes, including a cryogenically cooled triple resonance ColdProbe which provides significant sensitivity gains and is ideal for low concentration samples in either non-ionic or highly ionic solvents. The 800 MHz ASC (Actively Shielded Compact) magnet draws on the latest super conducting wire technology to make this super compact shielded magnet which operates at a temperature of 4.2 Kelvin (K). The NMR has a 12 station auto-loader.

400MHz NMR

This is an Agilent 400-MR Long Hold Mag. Res. Spectrometer 2 channel NMR system. The system is equipped with a number of different probes and has a 96 station autoloader. Average throughput is 100-110 samples per day.

Additional NMR facilities available

Additional NMRs are available in the School of Chemistry building 500 metres from TBSI. Details are

- Bruker Avance 400 NMR with 4-nucleus {^{1}H, ^{13}C, ^{31}P and ^{19}F} probe and automatic sample changer
- Bruker Avance III 400 NMR proton and multinuclear probe {^{109}Ag - ^{31}P}
- Bruker Avance II 600 NMR: This spectrometer has a cryoprobe triple resonance probe with z-gradients for Proton (^{1}H), Carbon (^{13}C) and Nitrogen (^{15}N) for 2D and 3D structural NMR at very high signal to noise ratio. A second probe is optimised for 1D and 2D analysis for proton and nuclides from ^{109}Ag to ^{31}P. This is a NMR spectrometer designed for protein NMR and research synthetic chemistry programs

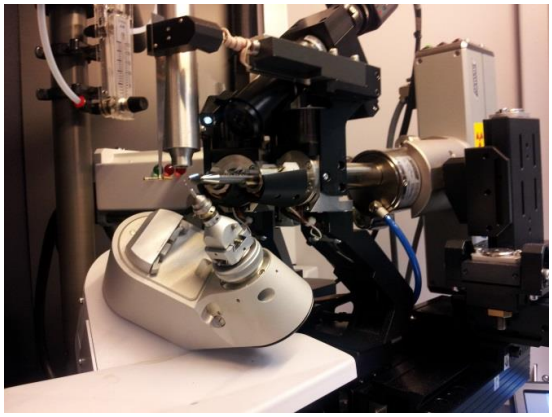
X-Ray Diffractometry

The Xray diffractometers available include:

Rigaku/MSC

- Complete Rigaku/MSC, MicroMax-007, R-axis IV++, X-stream 2000
- Computational facilities include SGI and linux boxes, stereographic hardware, standard software including HKL2000, O, COOT, CCP4, PHENIX
- a second Rigaku system also includes provision for low-angle X-ray diffraction (Caffrey lab)

Bruker Kappa APEX DUO single crystal diffractometer



The APEX DUO combines a Mo sealed tube and high intensity Cu Microfocus sealed tube source with a sensitive CCD detector for crystallography. This allows fast and redundant data collection to high resolution with Mo radiation and determination of absolute structures for light atom organic compounds. Data from both wavelengths can be collected successively in one experiment and switching between wavelengths is fully automated.

The diffractometer is equipped with an Oxford Cryosystems Cobra system with a temperature range 80-400 K (± 0.1K).

Crystallization and Imaging Robotics

The Caffrey and Khan labs have established Ireland's premier high-throughput robotics facility, with the capability for nanolitre sitting and hanging drops (2x Mosquito platforms, Formulatrix imaging, and robotics for lipid cubic phases).

Transmission Electron Microscopy (TEM)

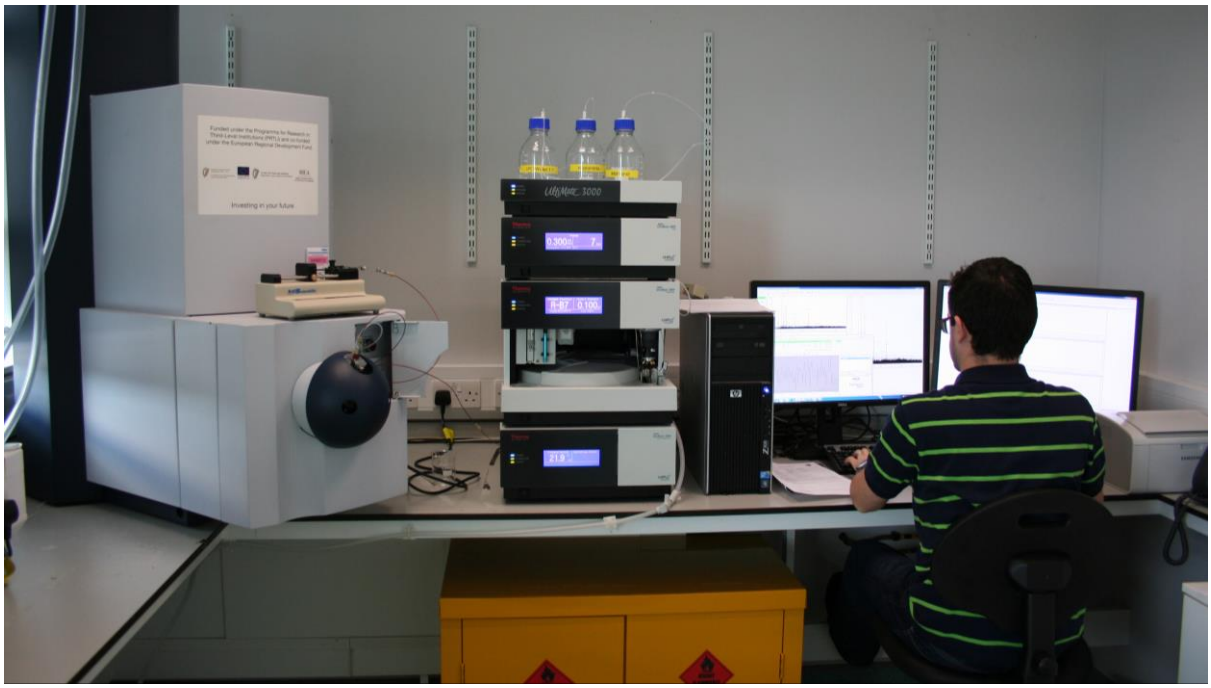
A **JEOL 1400 TEM** has been installed recently, supported by 2x Microtomes, one of which is a Cryo Microtome. This facility will be of use to researchers who wish to image structures, materials, proteins, organelles and cells in the nm-μm range. The equipment bridges the resolution gap for the imaging of biological and other materials from nm (x-Ray/NMR) to μm (light/confocal microscopy).

The TEM is optimised for operating at accelerating voltages of between 80 and 120KV, the optimal range for imaging biological material due to its inherently low contrast and relatively poor electron beam stability. The system is fitted with a LaB6 (Lanthanum hexa-boride) source allowing for better source brightness and stability over traditional Tungsten cathodes. The configuration of the column and lenses in addition to an AMT mid-mount camera offers an ideal detection system for biological material, where image sharpness and quality is prioritised over resolution.

The **Leica EM UC7** is a state-of-the-art Ultramicrotome for operation at room temperature. It provides easy preparation of semi- and ultrathin sections as well as perfect, smooth surfaces necessary for TEM studies.

Mass Spectrometry

Bruker micrOTOF-Q™ III ESI-TOF Mass Spectrometry System
(benchtop electrospray ionization quadrupole time-of-flight mass spectrometer) for exact mass and true isotopic measurements in both, MS and MS/MS mode. The micrOTOF-Q III is rated as having the best performance standard in its class.



The system includes:

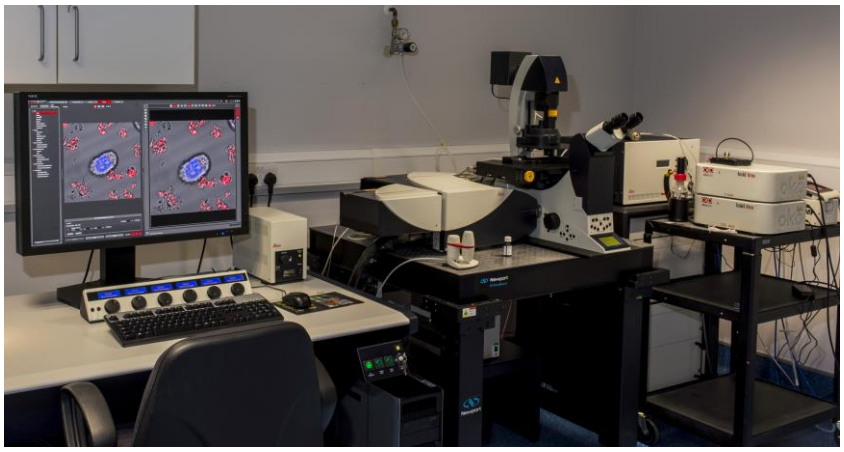
- Apollo II ion funnel ESI source
- High performance hyperbolic quadrupole
- Collision cell for CID
- Temperature compensated TOF analyzer
- Electronics
- Syringe pump
- Data system with digitizer and 22" LCD monitor
- Laser printer
- Compass Software version 1.3 for HPLC and MS control, data acquisition and processing
- Direct Probe Option for APCI II and APPi II source, for the direct analysis of solid samples and raw materials down to the sub-ng range of pure substance

A **Dionex Ultimate 3000 LC System** is also provided. The UltiMate 3000 Binary Analytical system provides fast cycle time, reliable mobile phase temperature conditioning, and high data rate detection to ensure optimal performance for the full range of binary analytical applications.

Confocal Microscopy Laboratory

An image of part of the laboratory is shown below, followed by a list of the equipment.

Leica SP8 Gated STED



Scanning confocal with 592nm STED laser for super resolution imaging. This is the only confocal of this resolution in Ireland. It has 5 detectors with 3 PMTs and 2 HyD detectors. The light sources include 405 diode a multi argon laser and a white laser that can give up to 8 wavelengths simultaneously between 470nm and 670nm. It has an inverted microscope with 10x and 20X dry objectives and 40X, 63X and 100X oil objectives. It also has a resonant scanner for imaging at 24 frames per second and 37 degree incubator. This microscope has both high resolution and flexibility to cover a wide range of experimental setups in fluorescent microscopy.

Leica SP8 confocal

Scanning confocal with Diode lasers at 405nm, 488nm, 552nm and 638nm. It has an inverted microscope with 10X dry, 40X oil and 63Xoil objectives. It has 3 PMTs with prism based wavelength separation

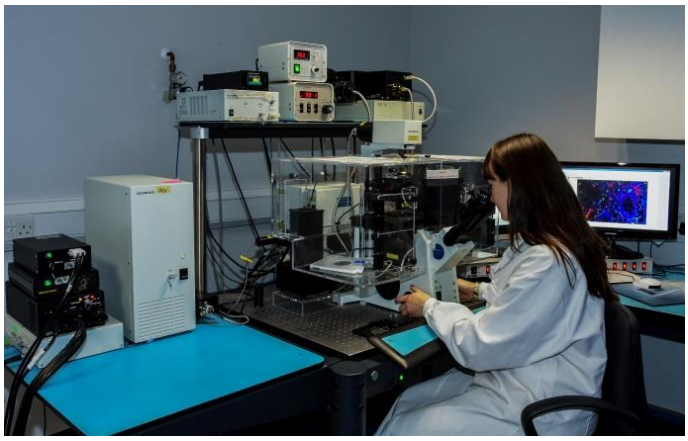
Olympus IX81-long focal length Fluorescent microscope

Inverted microscope with long focal length objectives for looking at cells in plastic dishes. Filter cube contains dichroics and filters for DAPI, GFP, YFP & Cy3 chromophores. It has 4X, 10x, 20x, 40x & 60x

dry objectives all of long focal length and images are captured with either an F-View II or the DP71 digital camera. Can be used for brightfield, phase contrast and immunofluorescence.

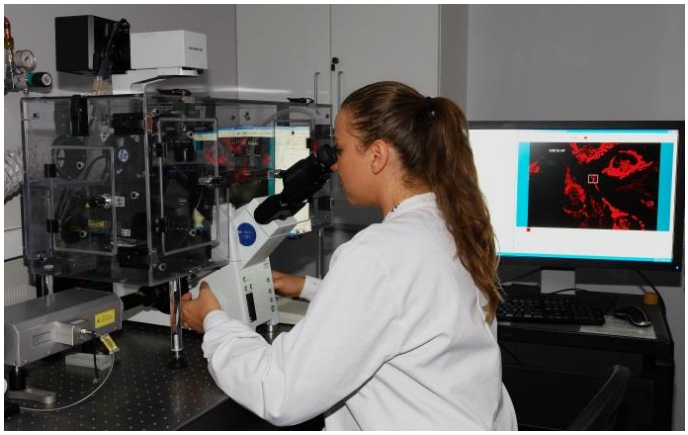
Olympus FV1000 Point-Scanning Confocal Microscope

Scanning confocal with Gas Laser System contains: Argon laser, multi-line 457 / 488/ 515 nm, Green Helium-Neon laser 543 nm, Red Helium-Neon laser *633 nm,* Near-Violet Laser diode 405 nm. It has Inverted microscope which has 10x, 20x & 40x dry and 40x and 60x oil objectives . It is fitted with an incubator for live cell experiments and has 2 Stages available, one for standard slides and the other a heated stage for 35 mm or 50 mm glass bottom petri dishes.



Andor Spinning disk

Spinning disk confocal with Olympus inverted IX81 microscope with 10X, 20X dry and 40X and 63X oil objectives. It has 2 interchangeable high-res cameras available for acquisition of images, using iXon+ ultra sensitive EMCCD technology. It has diode lasers with 405nm, 445 nm, 488 nm, 514 nm, 561 nm. It is fitted with an incubator for live cell experiments.



Olympus BX51 upright microscope

Upright microscope with bright field, DIC and epi fluorescence provided by a four colour CoolLed system which has 365nm, 470nm, 535nm and 635nm light sources with matching filter cubes. It is run using CellSens software and captured on a high resolution DP73 digital camera. The available objectives are 4X, 10X, 20X, 40X and 60X dry and 60X and 100X oil. This microscope is used for imaging histology stained slides with H&E as well as immunofluorescent slides.

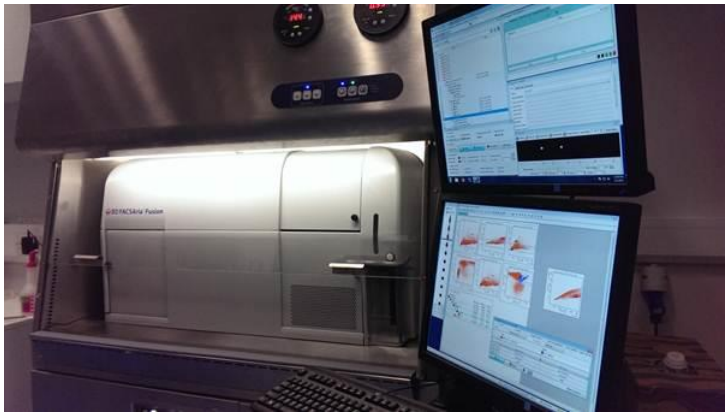
Nikon Eclipse E400 polarising microscope

Upright microscope for polarized light with 10X, 20X and 40X dry objective.

Flow Cytometry Laboratory

The Flow Cytometry Facility is equipped with one flow sorter, 5 analysers, an *in vivo* fluorescence & luminescence imager (Biospace Lab PhotonIMAGER RT), an Automated Cell Counter (Luna Cell Counter), and a fluorescence microscope (Partec Cyscope).

BD FACSAria Fusion Cell Sorter:



The BD FACSAria Fusion High Performance Cell Sorter combines a top of the range high speed cell sorter with best-in-class biosafety. Housed in a Class II Type A2 biosafety cabinet, the sorter performs with unrivalled sensitivity and resolution. With 4 nozzle sizes (70, 85, 100, & 130 µm), 4 lasers (405, 488, 561, & 640 nm) and 16 fluorescence parameters, this provides a vast array of options for the researcher.

The sorter is capable of up to 4 sorts simultaneously into a variety of different receptacles. Also, single cell deposition into most standard 80mm x 120 mm multi-well tray formats (e.g. 96-well plate) and onto standard slides is routine with the ACDU system. Both sample and collection tubes can be kept at a fixed temperature, which leads to maximal viability.

BD FACSCanto II:

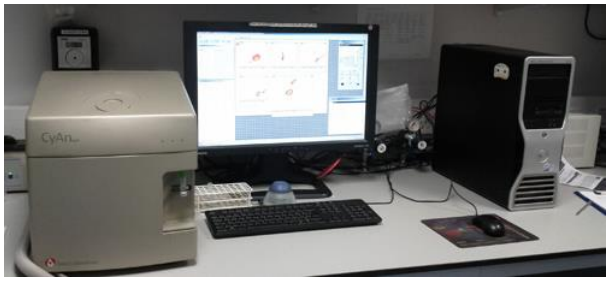
Features include 3 lasers (405, 488 & 633 nm) and 8 fluorescence parameters, with high sensitivity and resolution, and up to 10,000 events per second.

BD LSRFortessa:

Features include 4 lasers (405, 488, 561 & 640 nm) and 16 fluorescence parameters, with maximal sensitivity and resolution, and up to 40,000 events per second.

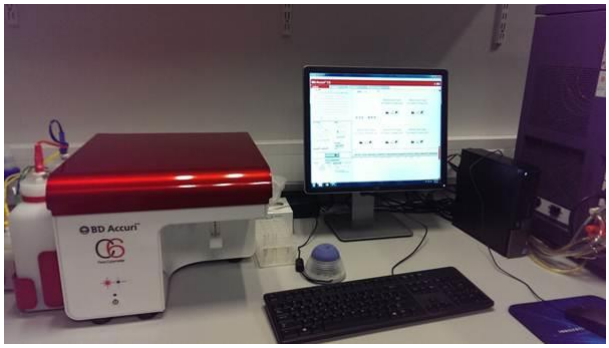
Beckman Coulter CyAn ADP:

Features include 3 lasers (405, 488 & 633 nm) and 9 fluorescence parameters, with high sensitivity and resolution, and up to 50,000 events per second.



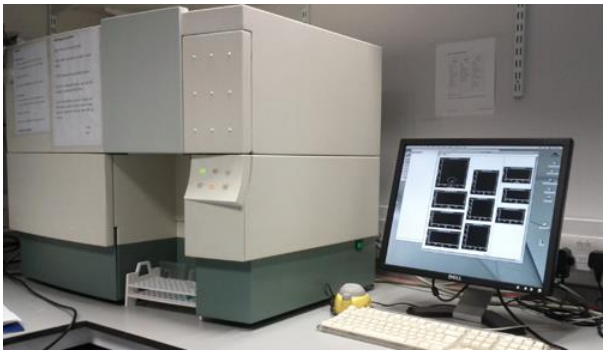
BD Accuri C6:

Features include 2 lasers (488 & 633 nm) and 4 fluorescence parameters, with high sensitivity and resolution, and up to 10,000 events per second.



BD FACSCalibur:

Features include 1 laser (488 nm) and 3 fluorescence parameter, with good sensitivity and resolution, and up to 4,000 events per second.

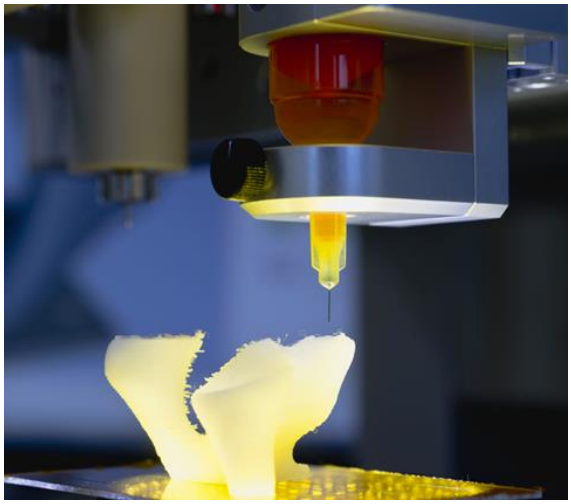


Additional Equipment

3D Bio-Printing

3D bio-printing, is a powerful fabrication technology, used to create three-dimensional cellular constructs which biomimic complex biological functionalities found in native tissues and organs.

Within the Trinity Centre for Bioengineering, the **RegenHU 3D Discovery Bio-Printer** is one of many biofabrication techniques being explored to combine natural and synthetic biomaterials, allowing for the multi-material printing of anatomically accurate constructs specifically for musculoskeletal applications.



Seahorse XF24-3 Extracellular Flux Analyser

The Seahorse XF24-3 non-invasively profiles the physiologic activity of cells in 24 well microplates.
The instrument offers the ability to:

- Detect real-time changes in cellular metabolism
- Perform label free, non-destructive measurements allowing reuse of the cells for other assay
- Simultaneously measure both energy producing pathways in as little as five minutes offering the most physiologically relevant information

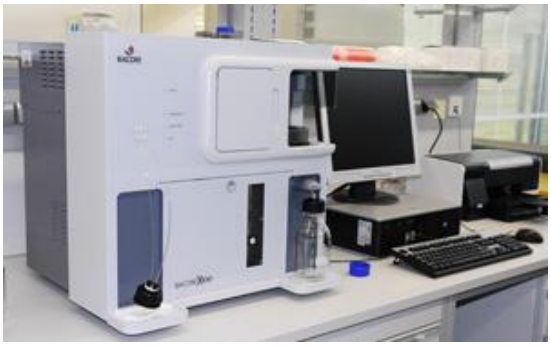
The XF Analyzer measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) within 2-5 minutes. This is accomplished by isolating an extremely small volume (less than 10µL) of media above the cell monolayer. Cellular bioenergetics, that is, oxygen consumption and proton extrusion, cause rapid measurable changes to the "microenvironment" in this small volume. During this time, analyte levels are measured until oxygen concentration drops approximately 10% and extracellular acidification changes approximately 0.2 units.

AKTAbasic Coupled to MALS

- Milligram scale protein purification
- Absolute mass from Wyatt multi-angle light scattering mini-DAWN instrument and Optilab Refractometer
- detects oligomeric states and heterogeneity of proteins and complexes
-ability to measure the second virial co-efficient of proteins

Biacore X-100 (GE Healthcare)

This is used to analyse protein interactions by surface plasmon resonance.

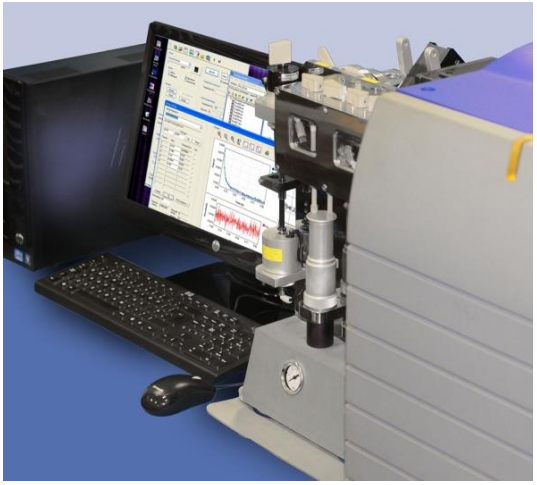


NanoStar (Wyatt Corp)

Dynamic light scattering – particle size and homogeneity

PiStar (Applied Photophysics)

Analyses of protein interactions and kinetics by stop-flow fluorescence



ITC200 (Malvern)

The study of protein interactions by isothermal titration calorimetry