LIFE SCIENCE

NEWSLETTER OF THE UNIVERSITY RESEARCH FACILITY IN LIFE SCIENCES, THE HONG KONG POLYTECHNIC UNIVERSITY / IS08 / FALL 2022

COMING SOON TO THE ULS

Nikon AX R MP Upright Multiphoton Microscope

The ULS has been supported by the RGC Collaborative Research Fund (2021/22 round) to acquire a Nikon AX R MP Upright Multiphoton Microscope. The microscope will be equipped with an ultrafast pulsed laser with a tuneable output from 680 to 1300 nm and a second output at 1045 nm, a resonant scanner with imaging speed up to 30 frames per second at 512×512 pixels, and an upright microscope configuration suitable for small-animal *in vivo* imaging. Researchers will be able to perform high-speed, *in vivo* multiphoton (MP) imaging of samples labelled with red (in addition to blue and green) fluorophores, simultaneous dual-channel MP imaging and optogenetic photostimulation. The microscope is expected to be available in Q1 2023 at the Animal Imaging Centre (AIC).

The Nikon AX R MP Upright Multiphoton Microscope.

Zeiss Lattice Lightsheet 7 Microscope

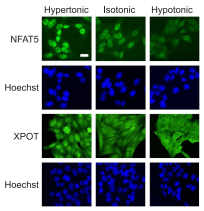
The ULS has recently acquired a Zeiss Lattice Lightsheet 7 (LLS7) Microscope. The lattice lightsheet technique was developed by the Nobel laureate Eric Betzig to achieve gentle, high-speed volumetric imaging of cells and small embryos. The technique uses optical lattices to create ultrathin laser lightsheet to improve axial resolution and has since proved to be a powerful tool for live fluorescence imaging. The LLS7 system has an inverted microscope configuration which is compatible with most common sample carriers. The system will be available in Q2 2023.

Bruker minispec LF90II Body Composition Analyser

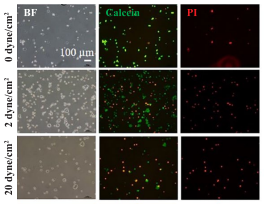
The Bruker minispec LF90II Body Composition Analyser uses time-domain nuclear magnetic resonance (TD-NMR) technology to perform rapid *in vivo* measurements of lean tissue, body fat, and body fluid in live rodents (from newborns to up to 800 g in weight) without the need for anaesthesia. Such non-invasive and non-destructive method would allow for longitudinal monitoring with negligible influence on animal health. The system will be available in Q2 2023 at the AIC, and will benefit such research areas as metabolism and nutrition.

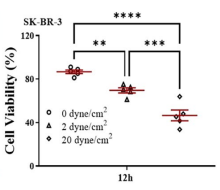
POLYU RESEARCH

1. Dr Ben Ko's group (ABCT) identified an unconventional regulatory mechanism for nuclear import of NFAT5 that involves KPNB1, XPOT and RUVBL2. The new findings could aid the understanding of cellular response to extracellular tonicity. The Leica SP8 Confocal Microscope and Malvern ITC System were used in this study.



Above: Confocal images showing the localisation of NFAT5 and XPOT in the nucleus under hypertonic condition. *J. Cell Sci.* **135** (13), jcs259280 (2022).
Below: Survival of SK-BR-3 breast cancer cells under various degrees of fluid stress. *J. Cell Sci.* **135** (10), jcs259586 (2022).





2. Dr Youhua Tan's group (BME) looked into the possible underlying mechanism for the survival of suspended breast cancer cells using a fluid shear stress model, and found that shear stress would lead to nuclear expansion *via* histone acetylation, thereby preventing the cells from undergoing apoptosis. The discovery might offer insight into the therapeutic potential to combat metastasis by targeting circulating tumour cells. The Leica SPE Confocal Microscope and BD Accuri C6 Flow Cytometer were used in this study.

CANCER DETECTION IN A MICROPLATE

Quantitative DNA methylation analysis by the MassARRAY system

DNA methylation is a kind of epigenetic modification that is essential for maintaining normal genetic activities and genomic stability. It is a naturally occurring biochemical process for modulating gene expression without changing the DNA sequence. Aberrations in DNA methylation affect gene expression and are often associated with common human diseases, such as cancer, genetic and neurodegenerative disorders. DNA methylation occurs when a DNA methyltransferase catalyses the addition of a methyl group to the cytosine of a cytosine-phosphate-guanine (CpG) site in the sequence. Once methylated, transcriptional activities of the gene are often inhibited. Both hypomethylation and hypermethylation have been implicated in cancer, with the latter believed to be more gene-specific. Tumour cells undergoing necrosis or apoptosis would release DNA fragments into the bloodstream and give rise to the co-called cell-free DNA (cfDNA). Numerous methods have been developed to detect cfDNA in the blood; determination of methylation patterns of cfDNA might hold promise to serve as a relatively non-invasive "liquid biopsy" test for early prediction and identification of certain types of cancer.

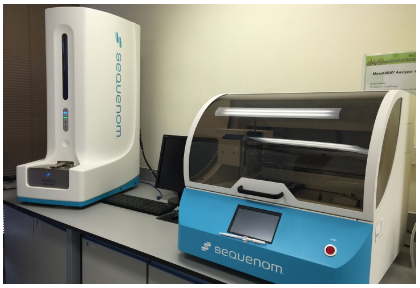


Figure 1. The Agena Bioscience MassARRAY Analyser 4 System at the ULS.

Different approaches are available for the detection of DNA methylation. For example, mass spectrometry (MS) is a sensitive method to assess the global genome methylation status, but does not provide information about the sequence and thus the methylated regions cannot be identified. Polymerase chain reaction (PCR)-based methods, such as methylation-specific PCR, are quick and relatively cost-effective. However, the drawback is that the throughput is low, since only one or two CpG sites can be analysed at a time. Whole-genome bisulfite sequencing is currently the key method in DNA methylation studies. It employs bisulfite treatment to convert cytosines into uracils, which will subsequently be read as thymines; whereas the methylated cytosines remain unaffected. Following sequencing, the methylation status of CpG sites can be identified based on the mismatches. Although methylated regions can be identified in a comprehensive manner, the resulting DNA sequence with only 3 bases (since non-methylated cytosines are converted to thymines) can be difficult to align.

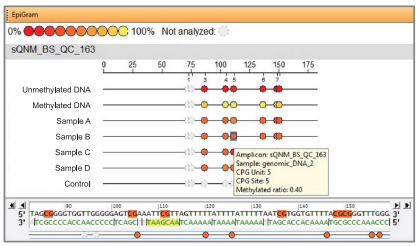


Figure 2. Typical data analysed by the EpiTYPER software. The top panel shows the degree of methylation of samples at target CpG sites, while the bottom panel shows part of the corresponding DNA sequence.

Here, we introduce a PCR and MS-based method for DNA methylation analysis using the Agena Bioscience MassARRAY System available at the ULS **(Fig. 1)**. In the MassARRAY workflow, following a similar bisulfite treatment step, PCR would be carried out with specific primers designed for a target genomic region of up to 600 base pairs. After *in vitro* transcription, methylated and unmethylated cytosines in the original DNA sequence would be converted to guanine and adenine in the RNA transcripts, respectively. The transcripts are then cleaved at the uracil residues using an endoribonuclease, and subsequently analysed by MALDI-TOF MS to determine the size ratio. Although the corresponding cleaved fragments have identical lengths, their masses differ due to changes induced preferentially by the bisulfite treatment on methylated and unmethylated cytosines. The mass difference would then be analysed using the EpiTYPER software **(Fig. 2)** to determine the degree of methylation at multiple CpG sites in the DNA sequence. This method requires as little as 10 ng of sample and allows for high-throughput analysis in 96- or 384-well formats, enabling researchers to investigate up to hundreds of target regions in an efficient and cost-effective manner. The method is useful for analysing the methylation status of genomic regions implicated in diseases and might represent a promising assay for the prediction and/or detection of cancer and other disorders associated with impaired DNA methylation.

GET IN TOUCH

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ULS EQUIPMENT AT A GLANCE

Mass Spectrometry
- Bruker AmaZon Speed Ion Trap-ETD MS
- Bruker UltrafleXtreme MALDI-TOF/TOF MS
- Agilent 6460 Triple Quadrupole LC/MS
- Agilent 6540 Quadrupole-TOF LC/MS
- SCIEX 6500⁺ QTrap LC/MS
- ThermoFisher Orbitrap IQ-X LC/MS
- Waters HPLC with QDa Mass Detector

Fluorescence Microscopy
- Abberior STED Super-resolution Microscope
- Nikon N-SIM/N-STORM/A1 Super-resolution/ Confocal Microscope
- Nikon Eclipse T*i*2-E Live-cell Imaging System
- Nikon SMZ1270i Fluorescence Stereomicroscope
- Nikon NIS-Elements Image Analysis Software
- Leica SPE Confocal Microscope
- Leica SP8 Multiphoton/Confocal Microscope
- Zeiss Lightsheet 7 Microscope
- Aviris Vision4D Image Analysis Software
- Imaris 3/4D Visualisation/Analysis Software
- MetaMorph Image Analysis Software

Cellular Analysis
- BD FACSAria III Cell Sorter
- BD Accuri C6/FACSVia Flow Cytometers
- Agilent Seahorse XF^{e}24 Extracellular Flux Analyser
- FlowJo Flow Cytometry Analysis Software
- Invitrogen Countess II FL Automatic Cell Counter

Biochemical Analysis
- JASCO J-1500 Circular Dichroism Spectrometer
- JASCO CPL-300 Circularly Polarised Luminescence Spectrometer
- Bio-Rad Bio-Plex 200 Suspension Array System
- Malvern MicroCal PEAQ-ITC System
- Tecan Automatic Liquid Handling System

Genomics and Molecular Biology
- Agena Bioscience MassARRAY Analyser 4 System
- Applied Biosystems QuantStudio 5 and 7 Flex Real-time PCR Systems
- Roche LightCycler 480 Instrument II Real-time PCR System

Small-animal Research
- Perkin-Elmer IVIS Lumina Series III Pre-clinical *In Vivo* Animal Imaging System
- FUJIFILM VisualSonics Vevo LAZR Ultrasound/ Photoacoustic Imaging System
- Bruker SkyScan 1276 *In Vivo* Micro-CT System
- Promethion Metabolic Cage System

General Research
- Drug Formulation Facility
- Bertin Precellys Evolution Homogeniser
- Labconco Refrigerated Vacuum Concentrator
- Sartorius BIOSTAT B-Twin Fermenter
- Leica EM UC7 Ultramicrotome
- Logos X-CLARITY Tissue Clearing System
- Cytiva ÄKTA Protein Purification System

• New or upgraded in 2022