

DEEP PHENOTYPING ADVANCED TECHNOLOGY FACILITY



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RCPATH

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Our team design assays to answer scientific questions via the generation of data-rich images and bespoke image analysis methods to measure gene expression at multiple levels in intact tissues. We combine experience in the use of antibodies and RNA detection technologies to develop assays which probe biological areas of interest. This is achieved by working closely with our collaborators, which is essential to obtain the desired results from these challenging methods.

We routinely develop *in situ* assays to answer questions about the spatial context of the tumour microenvironment, including the immune system, plasticity of epithelial cells and fibroblasts, and cell-cell interactions.

Progress 2024 Key technologies

Multiplex Immunofluorescence

Using our Ventana Discovery Ultra autostainers in combination with the Akoya Phenomager HT, we routinely detect up to 6 genes of interest on multiple tissue sections including tissue microarrays (TMAs), allowing us to answer complex questions about tumour biology in a high-throughput manner. We are leaders in this technology, and have collaborations with leading industry providers as well as numerous academic partners. We believe multiplex assays will form the basis of the next generation of clinical biomarkers, and are working towards translating our assays into a clinical setting.

Over 2024, we have worked with 13 research groups with this technology, and we have several manuscripts in preparation. We have developed panels for human prostate cancer (Leung, Campbell), human pancreatic ductal adenocarcinoma (Chang), human lung adenocarcinoma (Le Quesne, Bushell, MacVicar), human colorectal cancer (Edwards, Roxburgh), human breast cancer (McPherson), mouse models of hepatocellular carcinoma (Bird), cholangiocarcinoma (Braconi), and both human and mouse models of malignant mesothelioma (Chalmers, Murphy). A manuscript featuring our work from the Bird Lab "Human-correlated genetic models identify precision therapy for liver cancer" has been accepted by Nature.

High Plex Immunofluorescence

Using our Akoya PhenoCycler Fusion, we can detect up to 100 proteins of interest within tissue spatial context. Our expertise with this platform has led to several large in-depth phenotyping collaborations, including our involvement in the SAMBAI Cancer Grand Challenge. This project is a multi-centre international effort to tackle cancer inequities in minority and socially deprived patient populations. In addition, we have collaborations with the Pearson lab at Cardiff University, focussing on fibroblast subtypes in human prostate cancer, and locally with the Chang, Inman and Le Quesne labs focussing on fibroblast subtyping and immune phenotypes in human malignancies.

We have a methods paper detailing our in-depth validation protocol in process, and our custom PDAC panel has been presented at several meetings. This panel of 43 markers simultaneously detects expression of numerous markers of epithelial and fibroblast plasticity, new therapeutic targets, and highly detailed immunophenotypes. A murine version of this panel is in preparation.

Xenium

Our 10x Genomics Xenium platform enables detection of up to 5100 genes of interest in a single tissue section. The custom gene expression chemistry has been used by our collaborators to develop panels detecting multiple species simultaneously in parasitically infected tissues, and also to detect single nucleotide variants. Furthermore, the Xenium can be combined with PhenoCycler Fusion, enabling multi-omic detection of RNA and protein within the same tissue section. Of note, the Xenium is part of our SAMBAI cancer grand challenge work package, and data from our



Figure 1. Deep phenotyping imaging laboratory.

first mouse custom gene expression panel has been submitted to Nature by the Sansom lab.

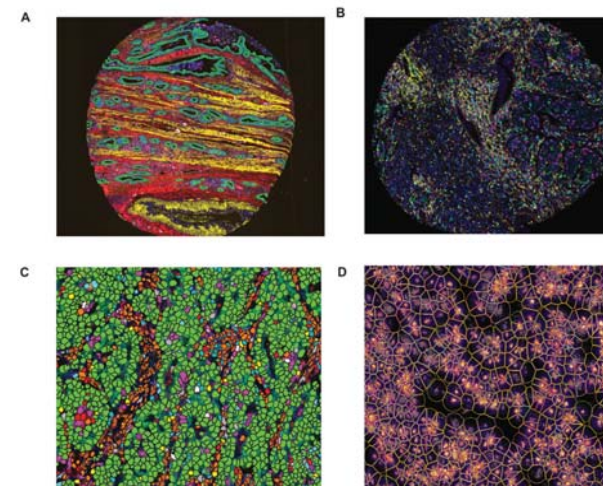
Visiopharm

Visiopharm is our image analysis platform of choice. We have developed bespoke deep learning pipelines for several diseases enabling high throughput analysis of the images generated by our spatial platforms. We offer training for collaborators wanting to generate their own data, or we design custom workflows and run them as part of our deep phenotyping service. We are in the process of writing a manuscript for a novel deep learning classifier we have developed using the deep learning tools within Visiopharm, which accurately predicts cellular phenotypes, without the need for accurate cell boundary classification.

Outreach

The Deep Phenotyping team actively contribute to public engagement events, developing interactive displays and games to convey our

Figure 2. A) PhenoCycler Fusion assay applied to pancreatic ductal adenocarcinoma. **B)** Multiplex immunofluorescence assay applied to lung adenocarcinoma. **C)** Visiopharm cell segmentation applied to cholangiocarcinoma. **D)** Xenium gene expression panel applied to colorectal cancer.



research to the wider public. This year we took part in the CRUK Parliamentary reception, engaging with MSPs about our multiplex and AI applications, and the future integration of these technologies into a clinical setting. We have hosted several visits from potential philanthropic donors, Prostate Cancer UK and the Beatson Cancer Charity, and politicians including the Minister for Public Health and Women's Health, Jenni Minto. The Deep Phenotyping team took part in a "50 years of Cancer" donor event hosted by the School of Cancer Sciences in the University of Glasgow.

Our team provide training and learning opportunities for students of all ages. Two of our scientific officers took part in a careers workshop at the University of Glasgow, leading to a 1-year MSci internship. We have also hosted 2 students for work experience placements, taken part in the CRUK SI work experience week, and the CRUK SI high school open evening. In addition, we were involved in a Nanobiology student visit, hosting students from the Delft University of Technology and Erasmus Medical Centre, Rotterdam.

We hosted three MSc students in the Deep Phenotyping facility for their summer projects, one of which is now a scientific officer within our team. We are becoming increasingly involved in international training programmes due to our experience with both high plex assays and Visiopharm image analysis. This year we hosted a PhD student from the Instituto de Biología Molecular y Celular del Cáncer in Salamanca on a 3-month EACR/Worldwide Cancer Research internship focussed on the application of a multiplex immunofluorescence pipeline to murine models of prostate cancer. This was featured in EACR's Cancer Researcher magazine.

We host 6-monthly spatial technology events with industry sponsorship, increasing engagement with advanced spatial technologies within the institute. This year, we hosted one event sponsored by 10x Genomics, featuring talks on novel AI applications, spatial phenotyping, and multi-omic approaches alongside talks from Xenium experts. We also hosted an event sponsored by Biotechne featuring talks from experts in *in situ* hybridisation applications within Glasgow and from the CRUK Cambridge Institute alongside talks from Lunaphore and Biotechne.