# A new integrated pipeline to probe cancer's inner workings

The sample is prepared for analysis as intact tissue or is disaggregated for single-cell analysis. The team has made major advances enabling a wide variety of samples to be analysed through the pipeline, from cultured cells to frozen biopsies or formalin-fixed paraffin-embedded tissues, which have historically been difficult to image.

This map represents the journey of a sample through IMAXT's 3D tumour-mapping pipeline, progressing through multiple levels of analysis on intact tissue, as well as single-cell sequencing on disaggregated cells. Eventually, all data are integrated and mapped onto the same 3D scaffold, providing a 3D tumour map that can be explored in virtual reality.

The IMAXT team is funded by Cancer Research UK.
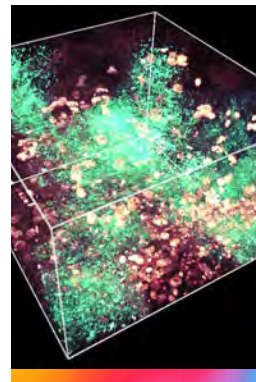
Intact tissue

Serial two-photon tomography: spatial proteomics

By serially sectioning the sample as 15µM slices and imaging four fluorescence channels simultaneously, STPT provides the scaffold upon which all further annotations are projected.

Image: mouse mammary gland
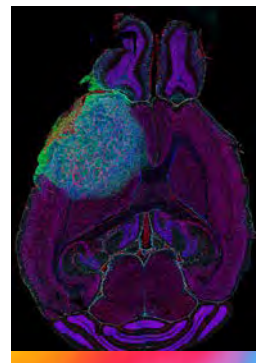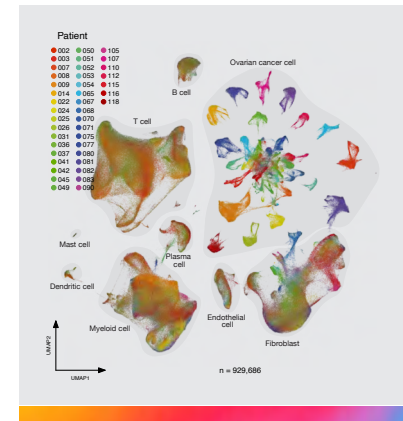Credit: Eduardo Gonzales Solares

Hyperplexed immunofluorescence imaging (HIFI): high-resolution spatial proteomics

A non-destructive, high-dimensional variant of cyclic immunofluorescence that can image 40 or more markers across whole-slide tissue sections. "Thanks to discussion with our wider team, we've overcome a number of technical challenges with HIFI, including setting up a new analytical pipeline and solving how to align and combine all images from each round of labelling."
– Spencer Watson, postdoctoral fellow

Image: mouse glioblastoma
Credit: Joyce lab

Expansion sequencing: spatial transcriptomics

Physical expansion of a sample combined with in situ RNA sequencing

Image: Mouse hippocampus
Credit: Alon et al, 2021

3D imaging mass cytometry: ultra-high-resolution spatial proteomics

Simultaneous detection of as many as 40 antigens and nucleic acid sequences. "We've overcome a number of technical difficulties in translating IMC from 2D to 3D, including using a diamond knife to cut 2 µM slices without distorting the tissue."
– Laura Kütt, PhD student

Image: Human breast cancer
Rendering credit: AGAVE
(Allen Institute for Cell Science)
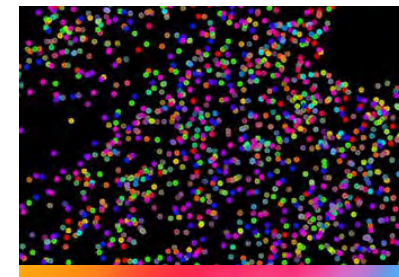
MERFISH: spatial transcriptomics

Near-genome-wide, spatially-resolved RNA profiling of individual cells, with high accuracy and efficiency.
"We've significantly optimised our sample procurement and analysis protocols to obtain high-quality data and are continuing to optimise the technique to expand the repertoire of samples we can target with MERFISH."
– Leonardo Sepulveda Duran, postdoctoral fellow

Credit: Zhuang lab

Disaggregated cells

Survey sequencing and single-cell genomics and transcriptomics

Image: Chromosomal copy number profile from scDNAseq and UMAP from scRNAseq data

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These methods give us detailed information about the expression profiles of individual cells and populations, letting us examine tumour evolution and chromosome structural aberrations at the single-cell level. Combining this with spatial data lets us uncover novel cell-cell interactions and underlying biology.
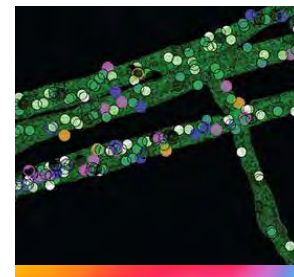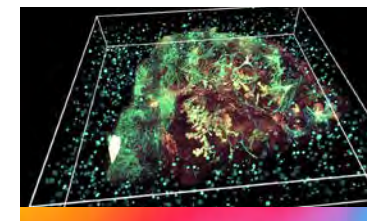
Ciara O'Flanagan
research associate

Image stitching, segmentation and re-alignment

Data gathered throughout the pipeline are projected onto the anatomical scaffold provided by STPT. "One of the technical challenges of our pipeline is the large volumes of imaging data and the variety of instrument measurement modalities. To achieve this integration, we've taken learnings from astronomy, including adopting approaches used for data handling and analysis of astronomical surveys."

– Eduardo Gonzales Solares, senior research associate

Image: Beads around the 3D model of a tumour, which are used for image registration of sample slices as well as across different modalities
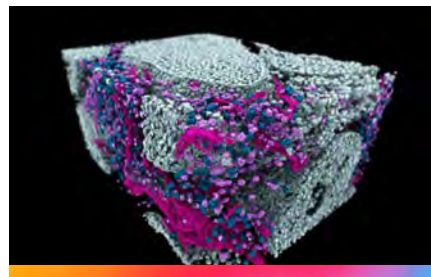Credit: Eduardo Gonzales Solares

Output: a detailed, computerised, 3D map of the original sample that can be explored in virtual reality

Images selected for this visualisation of the pipeline do not represent the same sample.

Featured team members

Dr Ciara O'Flanagan
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Dr Eduardo Gonzales Solares
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Laura Kütt
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Dr Claire Mulvey
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Dr Leonardo Sepulveda Duran
postdoctoral fellow in the Zhuang lab, Harvard University, US

Dr Spencer Watson
postdoctoral researcher in the Joyce lab, University of Lausanne, Switzerland

Dr Ignacio Vázquez-García
research fellow in the Shah lab, Memorial Sloan Kettering Cancer Center, and the Tavaré lab, Columbia University, US

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Laks E et al. Cell 2019; doi: 10.1016/j.cell.2019.10.026

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