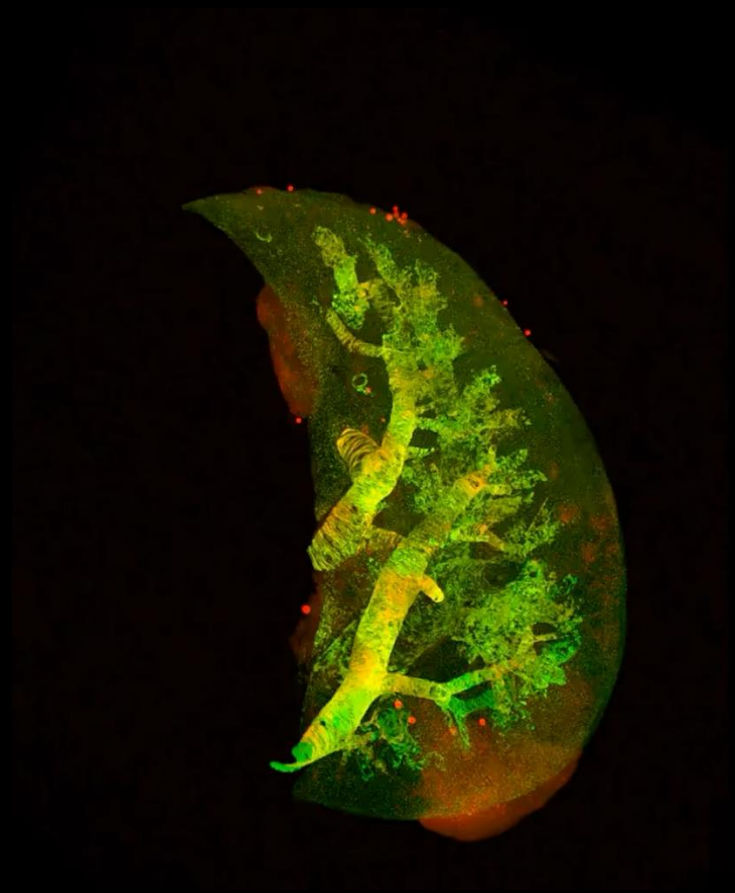


CANCER GRAND CHALLENGES

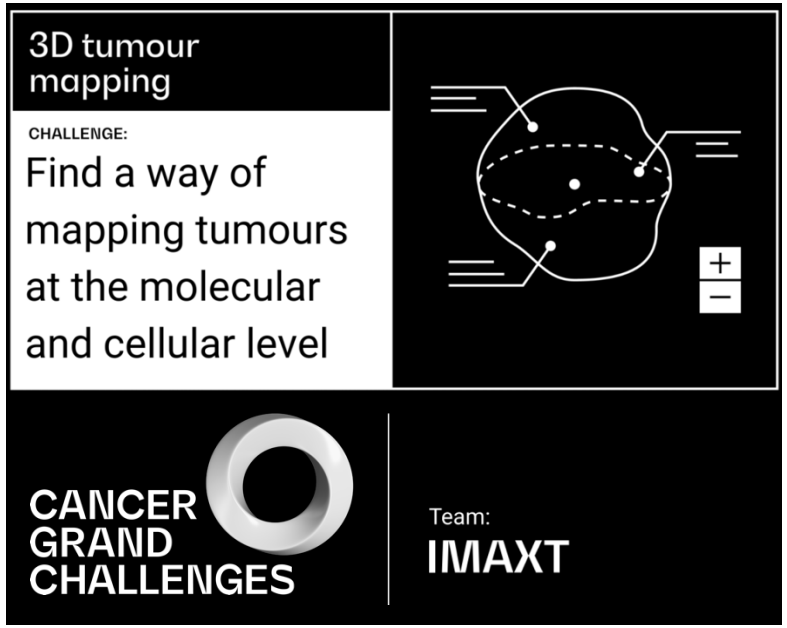
SPACE
Spatial Profiling and Annotation Centre of Excellence

The Spatial Profiling and Annotation Centre of Excellence (SPACE)

Dario Bressan
CRUK Cambridge Institute
March 5th 2025

Funded by:
CANCER RESEARCH UK

WHERE IT STARTED



Ten years ago, we started the IMAXT Cancer Grand Challenge to prove that single-cell and spatial molecular profiling were valuable for Cancer Research, both for discovery and for translation

Today:

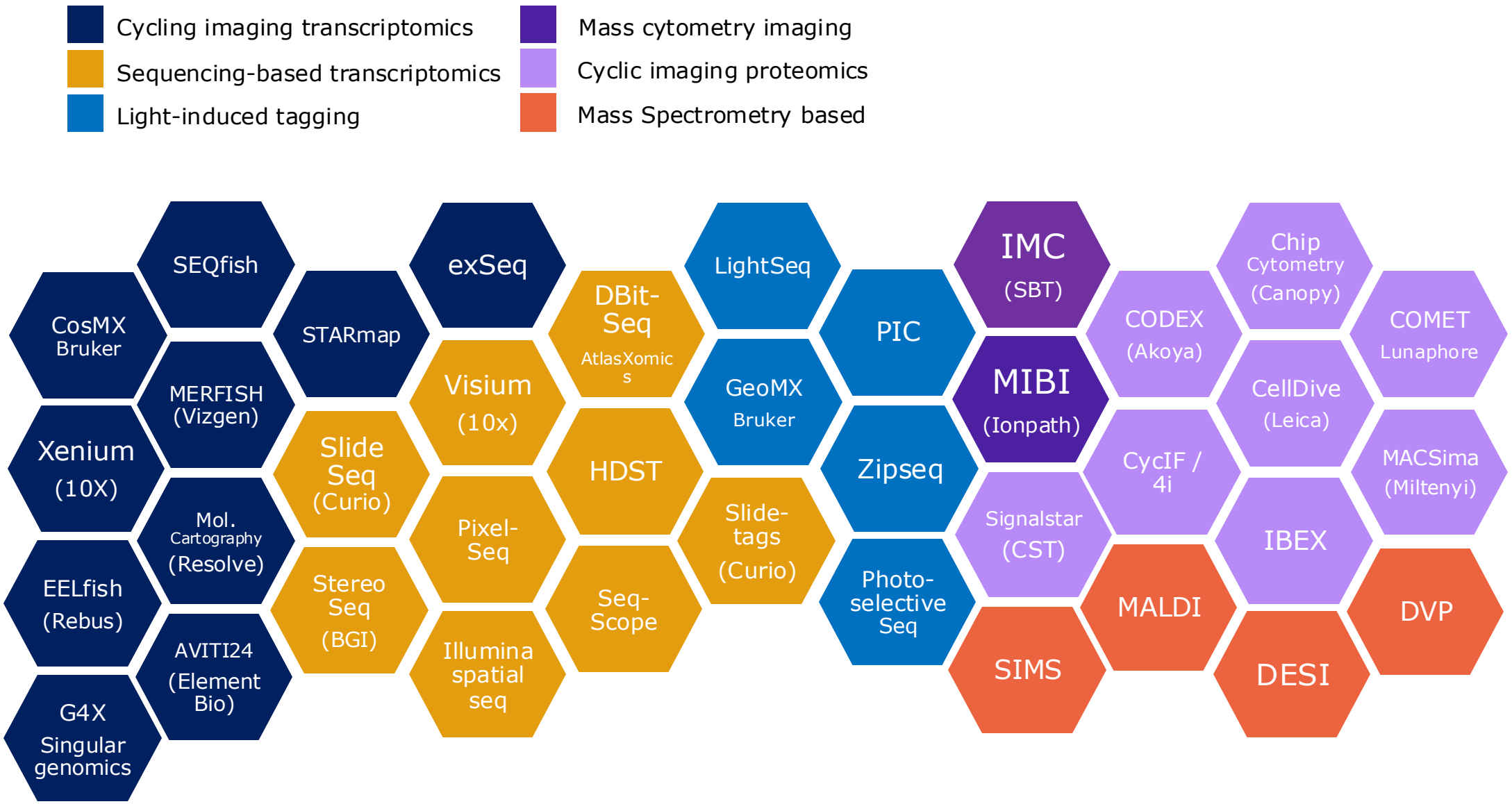
- Spatial "omics" is a whole research field and a huge commercial market
- Spatial profiling is a critical component of cancer research, and is fundamentally integrated into many of the current grand challenge projects
- … Now moving to Space AND time! (new challenge)

SPATIAL PROFILING IS HERE TO STAY

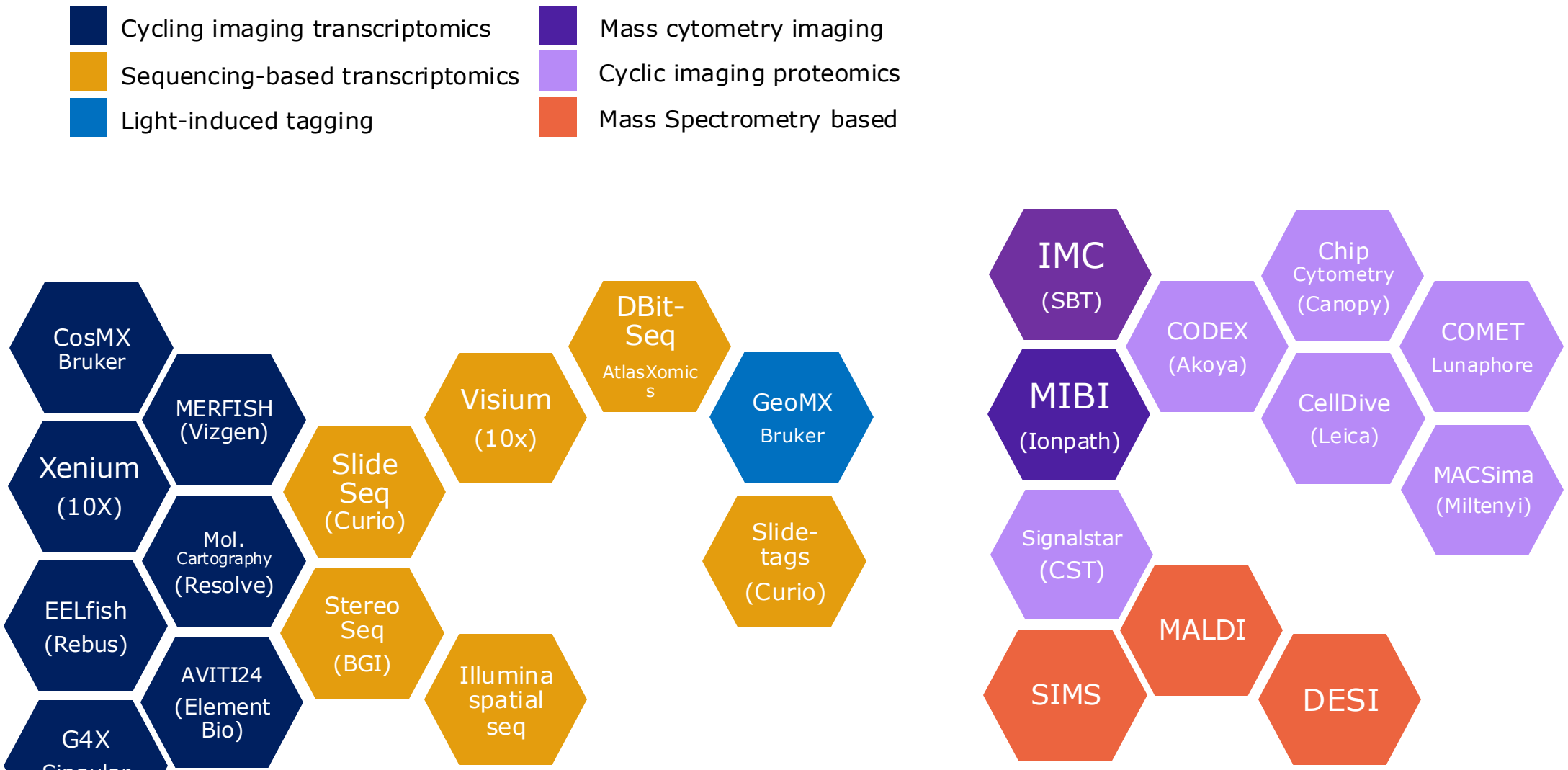
- Can we predict which cancer will likely relapse?
- Can we predict which patients will benefit from which therapy?
- How does clonal heterogeneity dictate the tumour micro-environment architecture?
- Are there non-cell autonomous effects driving tumour heterogeneity?
- How does the stroma-tumour interaction modulate therapy response?
- What determines whether a disseminated tumour cell in a distal organ will die, remain dormant, or becomes a metastasis?
- How does the TME evolve over time?

These questions all require, to different degrees, single-cell and spatial molecular profiling

DIVERSITY OF SPATIAL OMICS

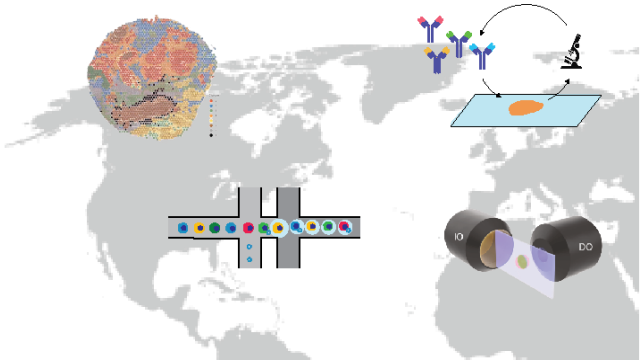


DIVERSITY OF SPATIAL OMICS (JUST COMMERCIAL OPTIONS)

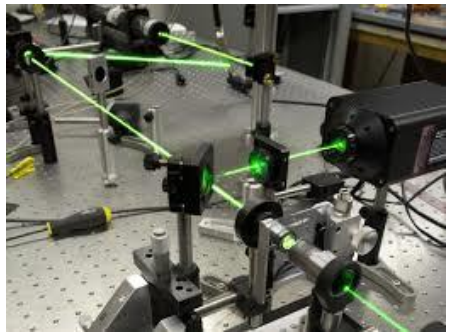


BARRIERS TO USING SPATIAL BIOLOGY

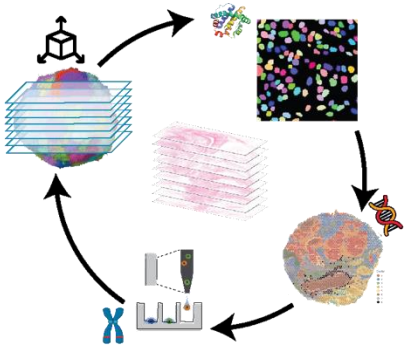
Method choice and tech availability



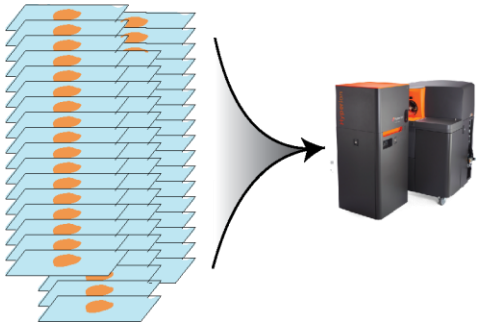
Fragmentation



Technology development and optimization



Multi-modal integration



Throughput

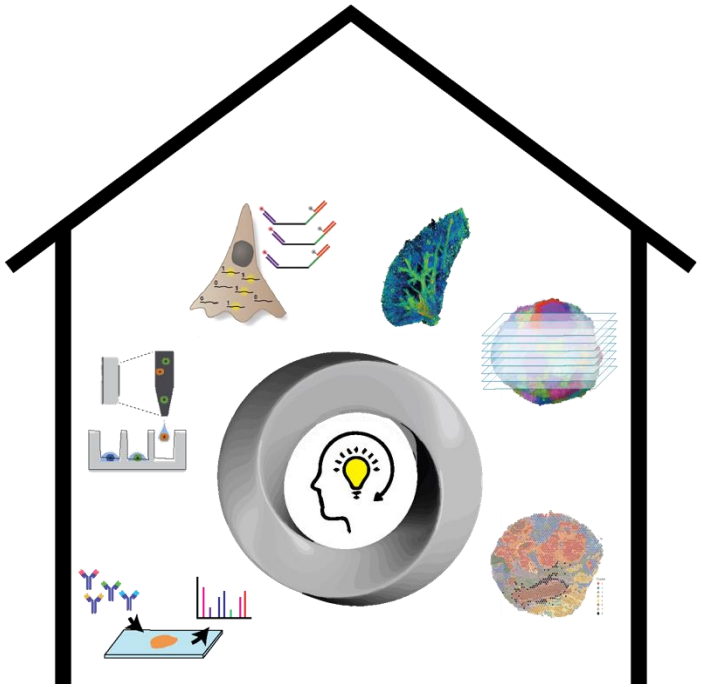
Data management, visualization, analysis

A HOLISTIC APPROACH TO SPATIAL BIOLOGY

CANCER GRAND CHALLENGES | SPACE

SPACE

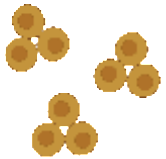
Spatial Profiling and Annotation Centre of Excellence



SPACE is a *collaboration focused* research laboratory that aims to be a catalyst to facilitate the application of spatial biology in cancer research by:

- Lowering the barriers to adoption
- Avoiding duplication of effort and promoting best practices
- Collaborating with projects from design to execution and analysis
- Facilitating project cross-fertilization and data sharing
- Leveraging economy of scale and steering tech development, in academia and industry

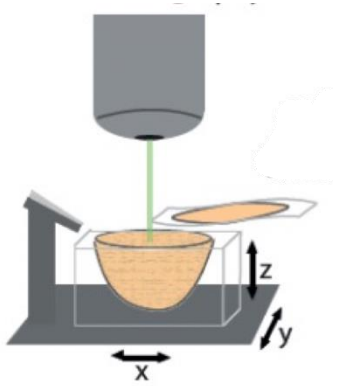
WHAT

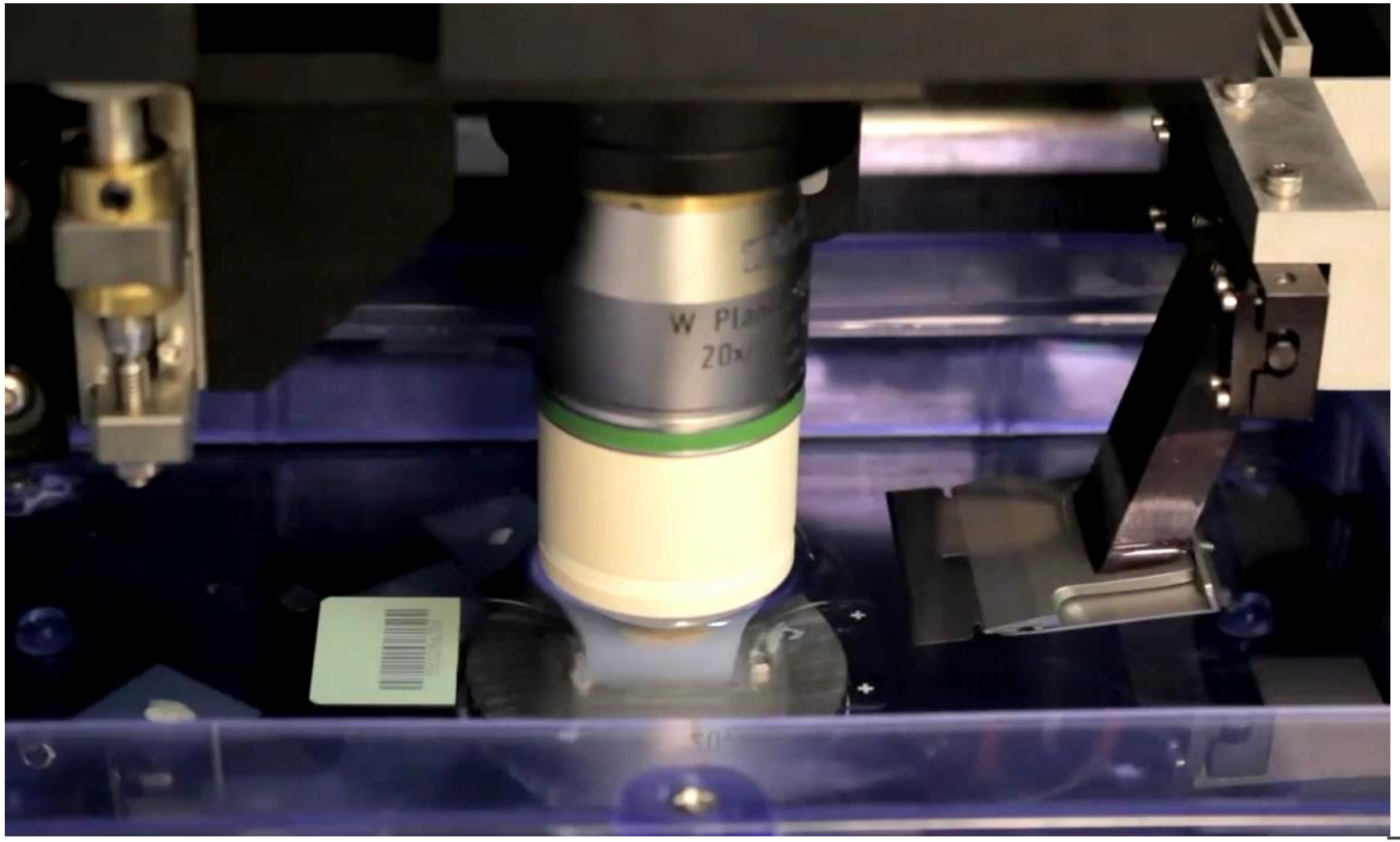
Disaggregated samples		⊠ LCM - bulk ⊠ Single-cell RNA, ATAC, TCR/BCR, CITE-Seq ⊠ DLP+ single cell whole genome
3D whole organ imaging		⊠ STPT - Serial 2 photon tomography ⊠ light sheet imaging ⊠ serial sectioning + 3D reconstruction
Spatial omics		⊠ Imaging Mass cytometry ⊠ Cyclic IF ⊠ MERFISH ⊠ BALI ⊠ 10x Visium / Visium HD ⊠ 10x Xenium
Data managem. and analysis		⊠ storage and interactive analysis ⊠ multimodal registration and integration ⊠ segmentation & feature extraction ⊠ Single cell analysis and integration w/ spatial data ⊠ spatial analysis
Visualization		⊠ Browser-based ⊠ Virtual reality

method integration

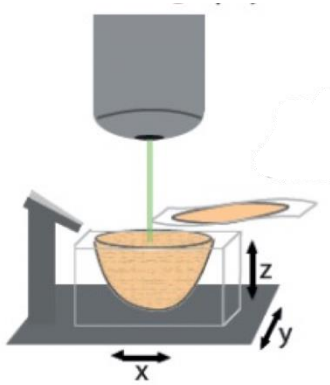
- Enough capacity for large studies
- Pre-developed antibody and marker panels
- Highly experienced technical staff and robust data production procedures
- Dedicated senior scientific staff (experiment and analysis)
- Ongoing technology development and horizon scanning, direct line with most method suppliers
- Experience in multi-modal data integration
- Cloud-based data processing centre with established pipelines for most data types

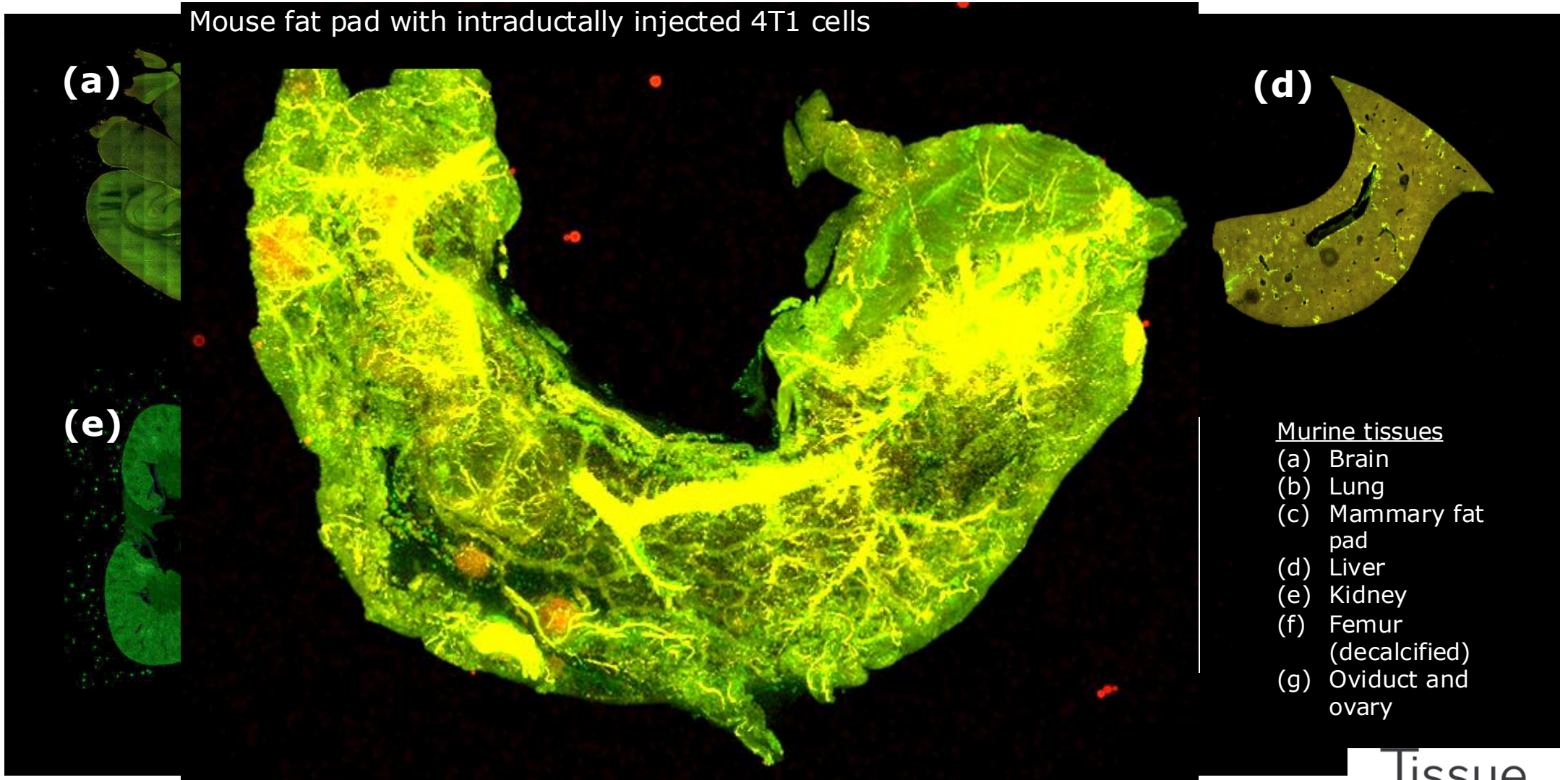
HIGHLIGHT: SERIAL TWO PHOTON TOMOGRAPHY





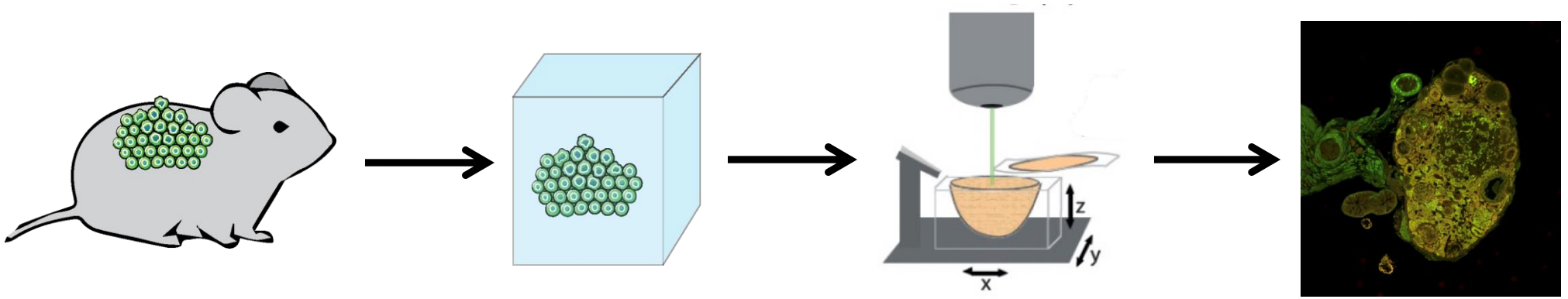
HIGHLIGHT: SERIAL TWO PHOTON TOMOGRAPHY





Murine tissues
(a) Brain
(b) Lung
(c) Mammary fat pad
(d) Liver
(e) Kidney
(f) Femur (decalcified)
(g) Oviduct and ovary

EVOLUTION OF STPT (2023)



Fresh fixed sample

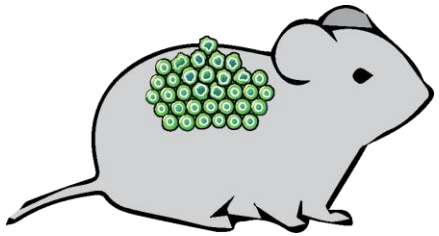
Endogenous fluorescence

STPT imaging

Fluorescence volume

EVOLUTION OF STPT (NOW)

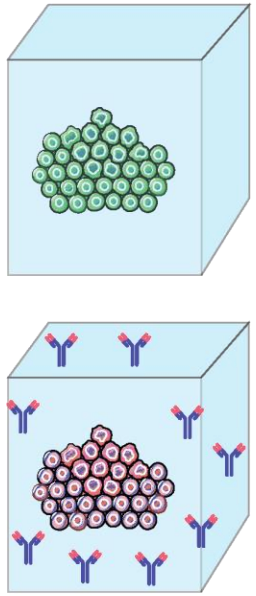
FFPE block



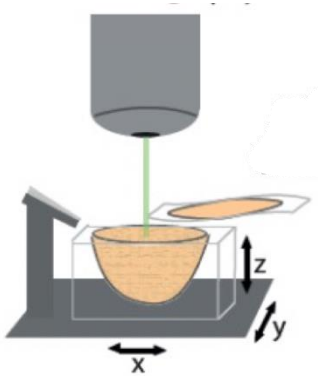
Fresh fixed sample

Frozen block

Endogenous fluorescence

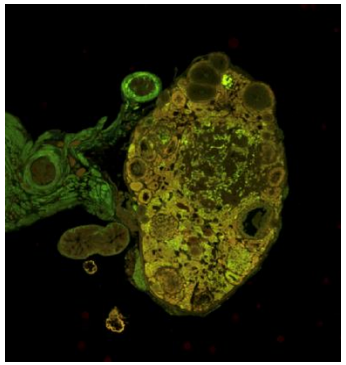


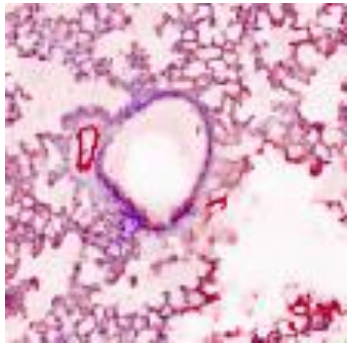
Whole-mount IF
Histology stains



STPT imaging

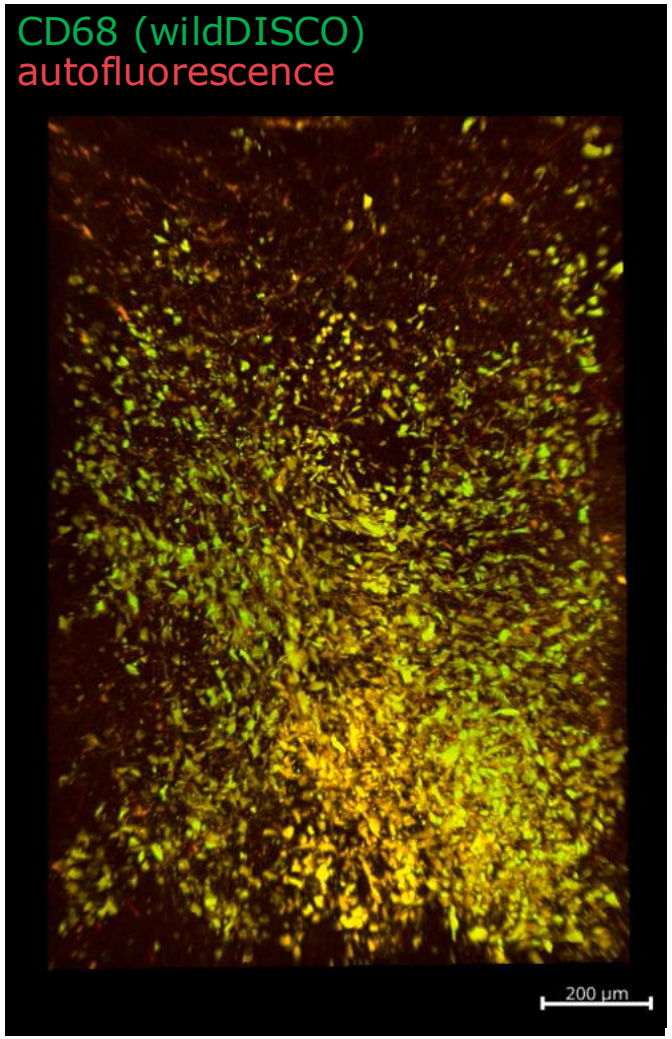
Fluorescence volume



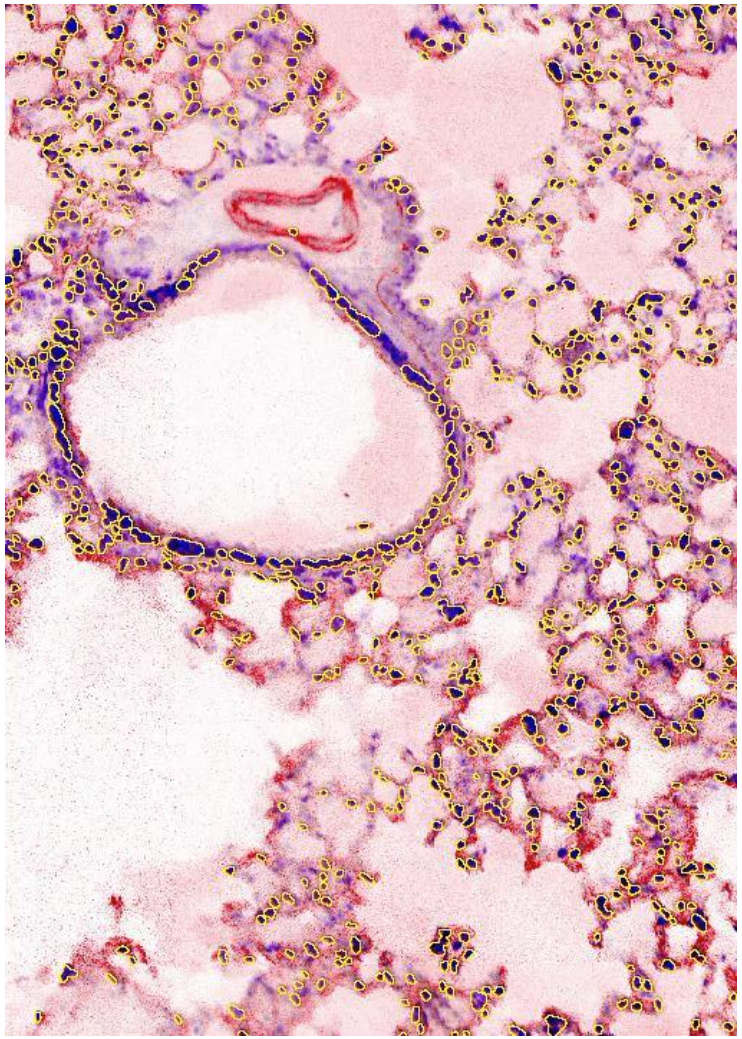


H&E – like volume

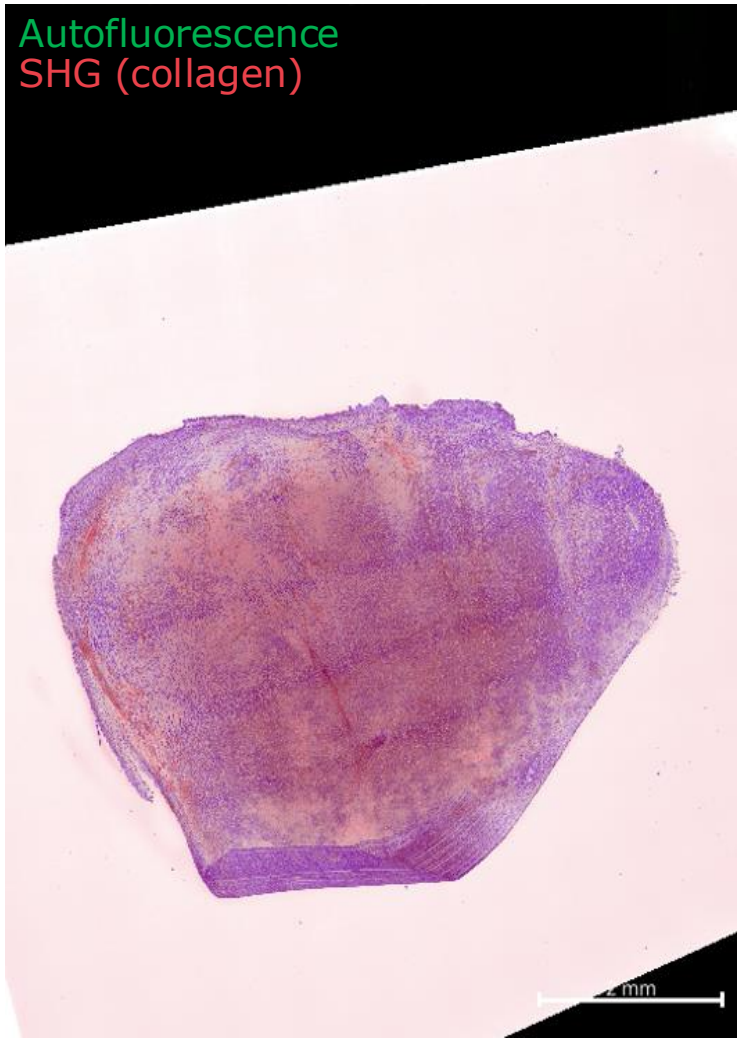
HIGHLIGHT: SERIAL TWO PHOTON TOMOGRAPHY



Whole-mount Immunostaining

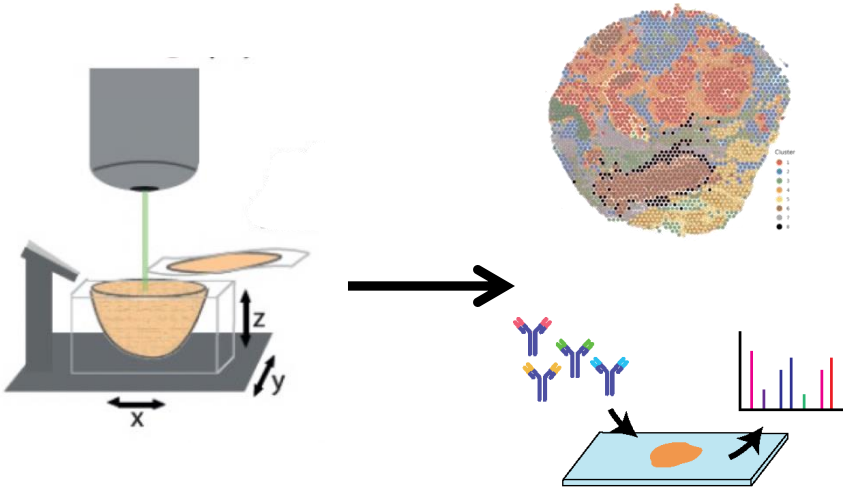


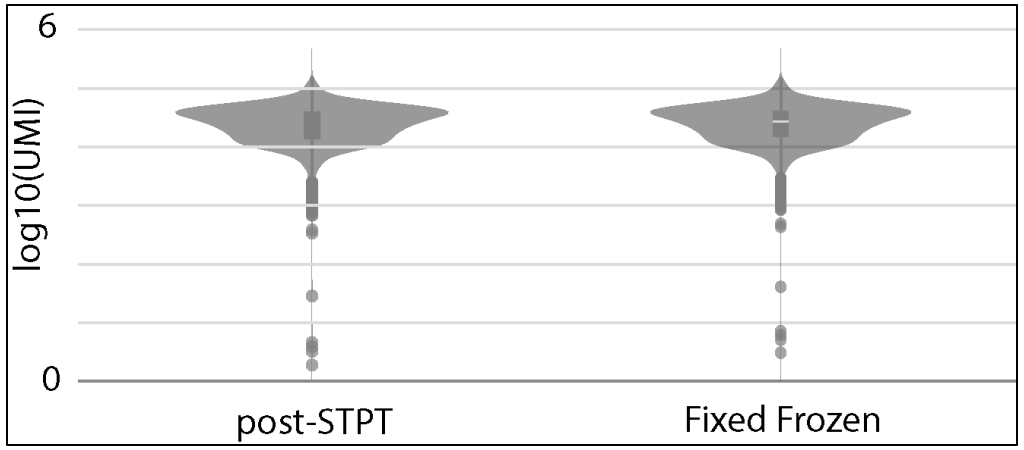
H&E – like staining



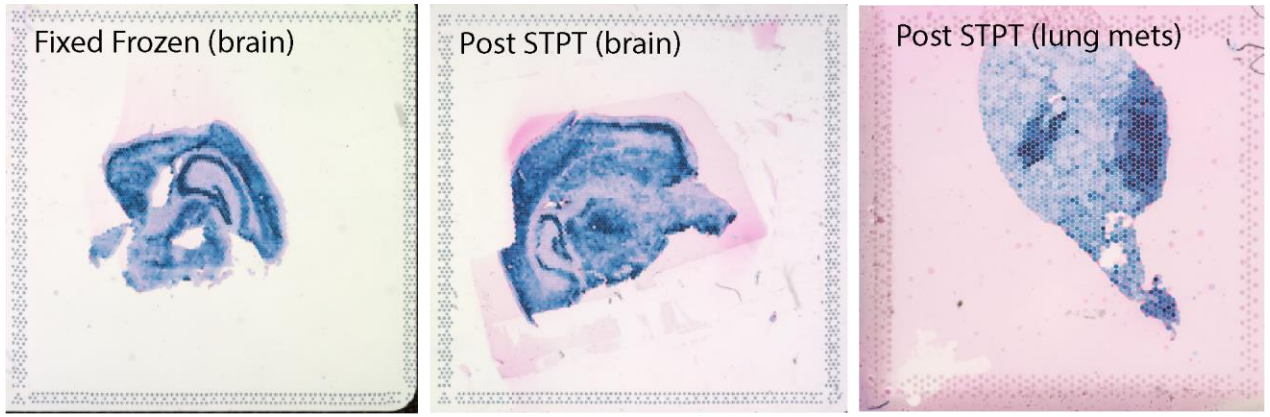
Deparaffinized block

INTEROPERABILITY BETWEEN STPT AND SPATIAL PROFILING





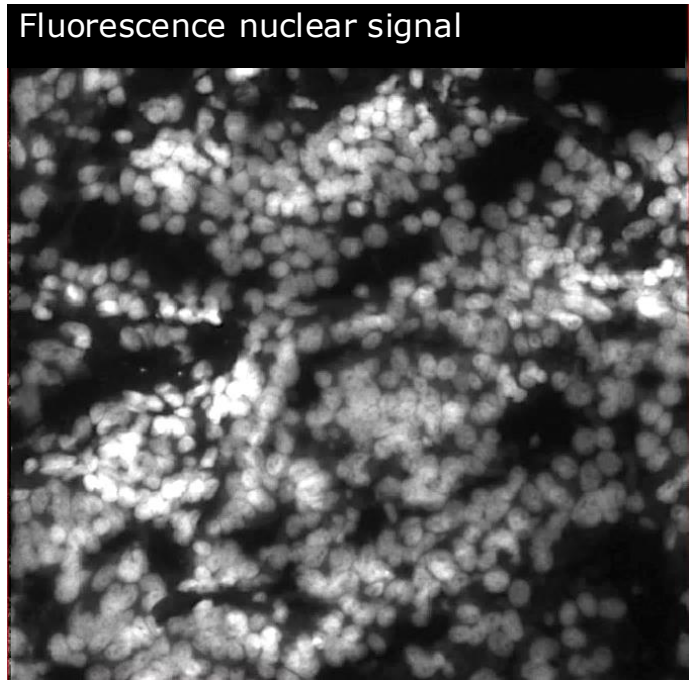
- Several cm3 sample in few days
- Multiple imaging options
- Indexed section collection down to 15um thickness
- Compatible with IMC, 10X Visium, 10X Xenium under development

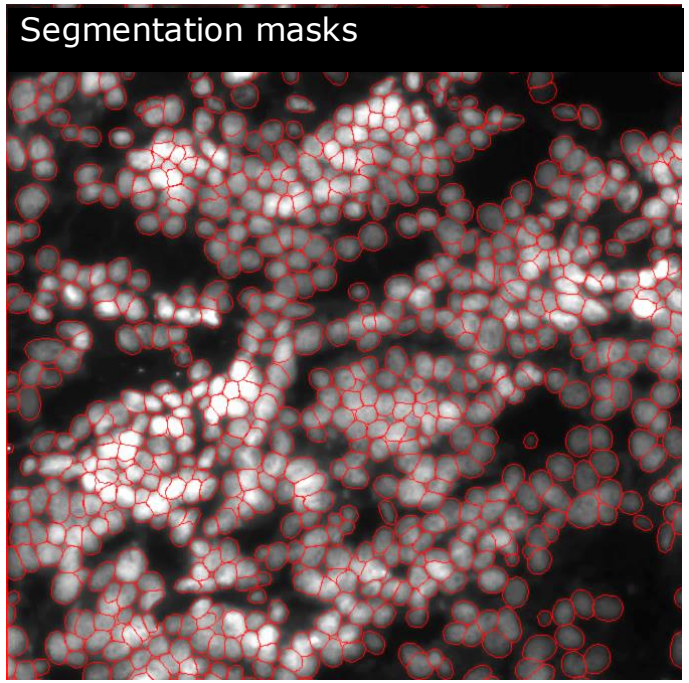


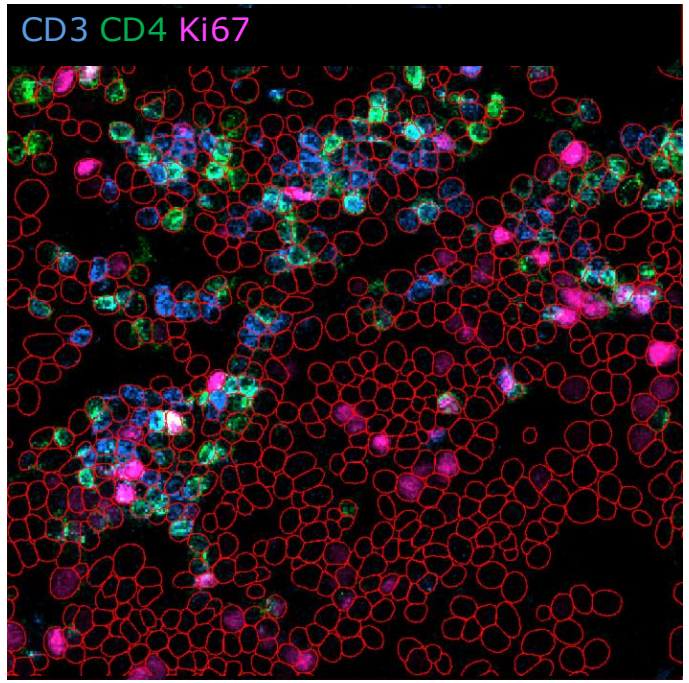
HIGHLIGHT: IMAGING MASS CYTOMETRY

- 5x latest generation XTI instruments
- Capacity for scanning 5 cm^2 tissue/day
- Faster scanning modes (low-resolution)
- Pre-validated antibody panels (>150 markers, incl. FFPE/Frozen and Human/mouse)

HIGHLIGHT: IMAGING MASS CYTOMETRY

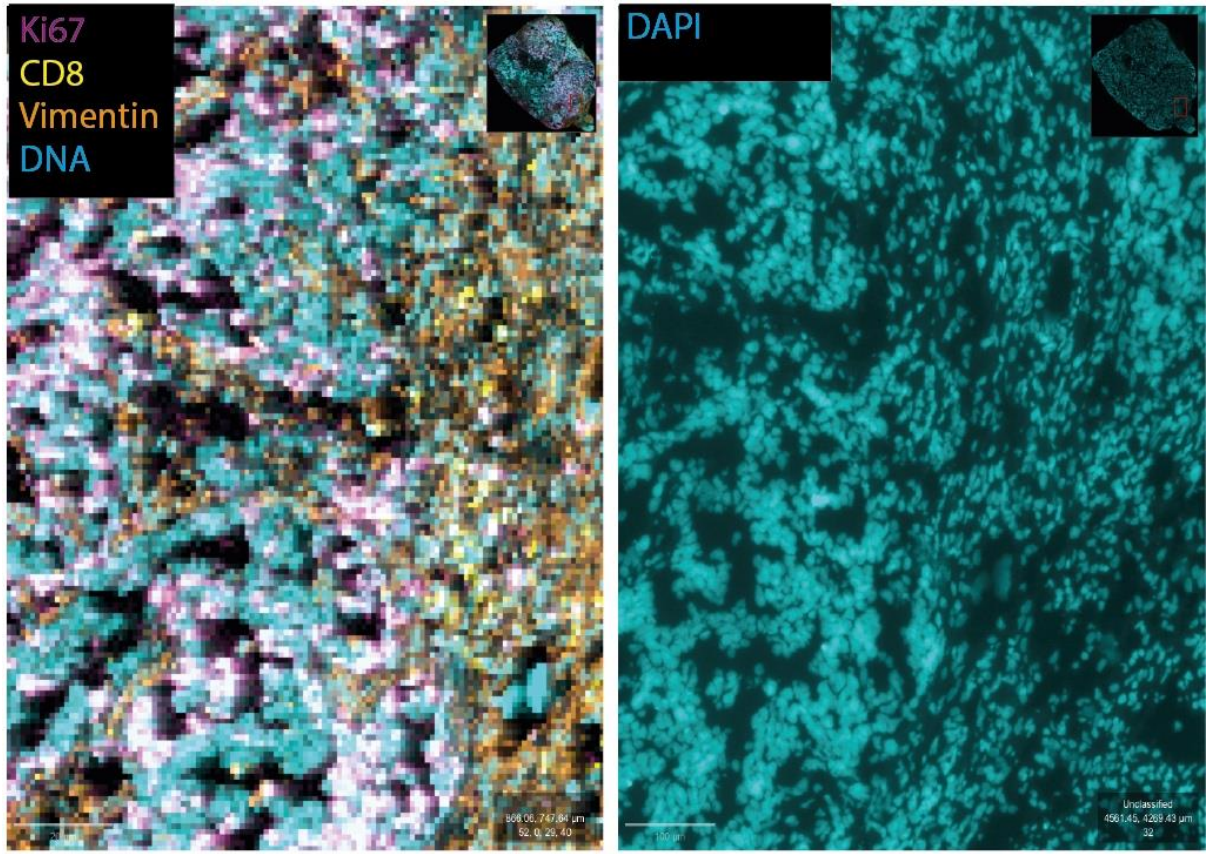


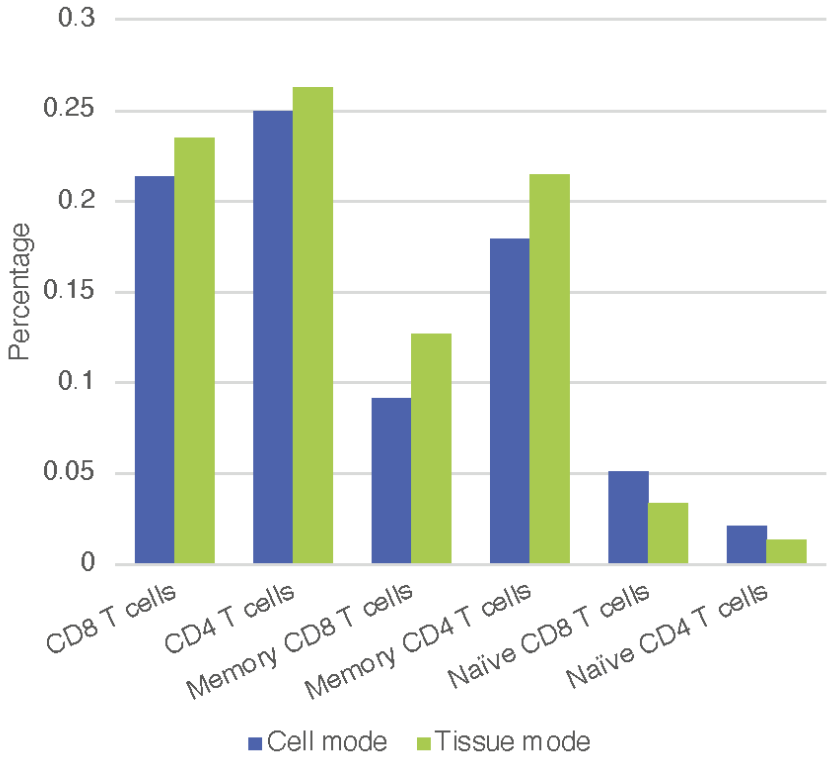




- Co-registration and segmentation mask transfer between fluorescence imaging and IMC for hard-to-segment samples
- Compatible with ultrafast low-resolution scans

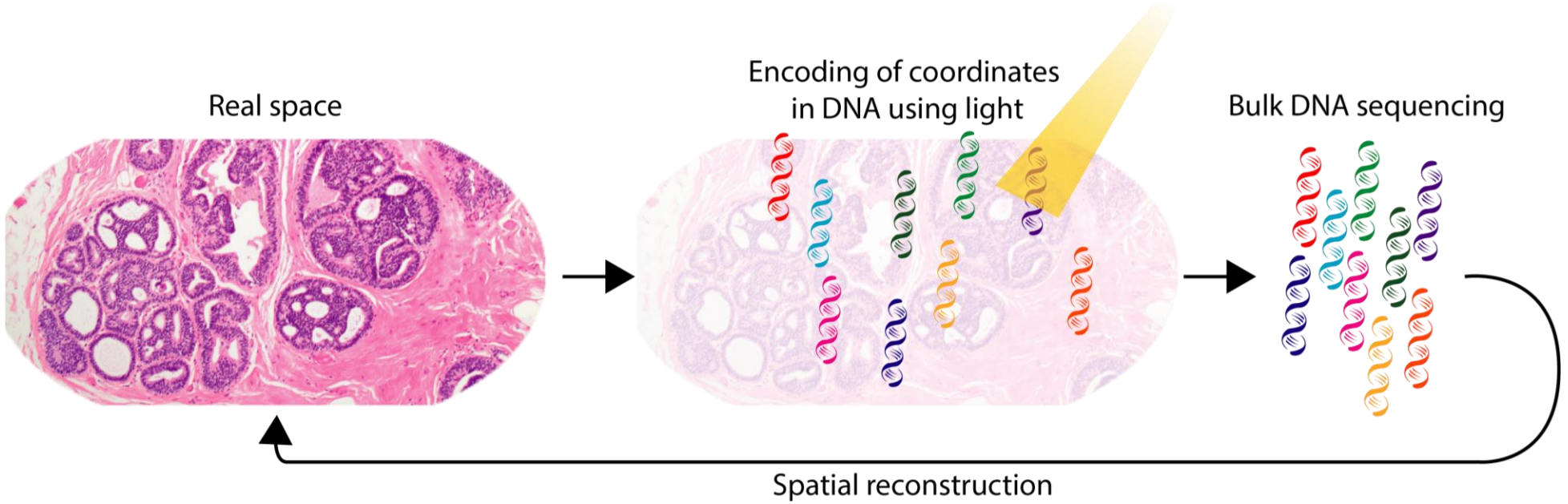
HIGHLIGHT: IMAGING MASS CYTOMETRY

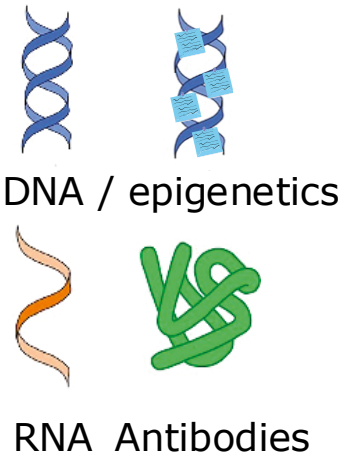




- Comparable cell quantification, but 20X faster scan time and 5X less antibody usage
- Could enable 100+ samples studies in acceptable time frames

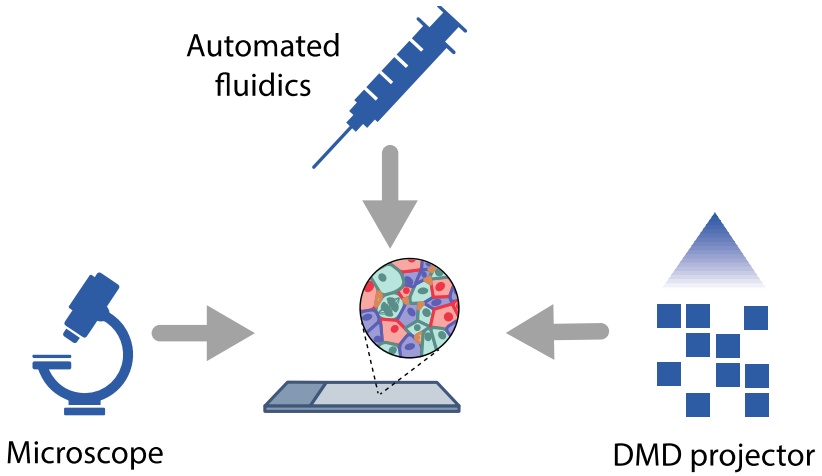
HIGHLIGHT: BARCODING BY ACTIVATED LINKAGE OF INDICES (BALI)

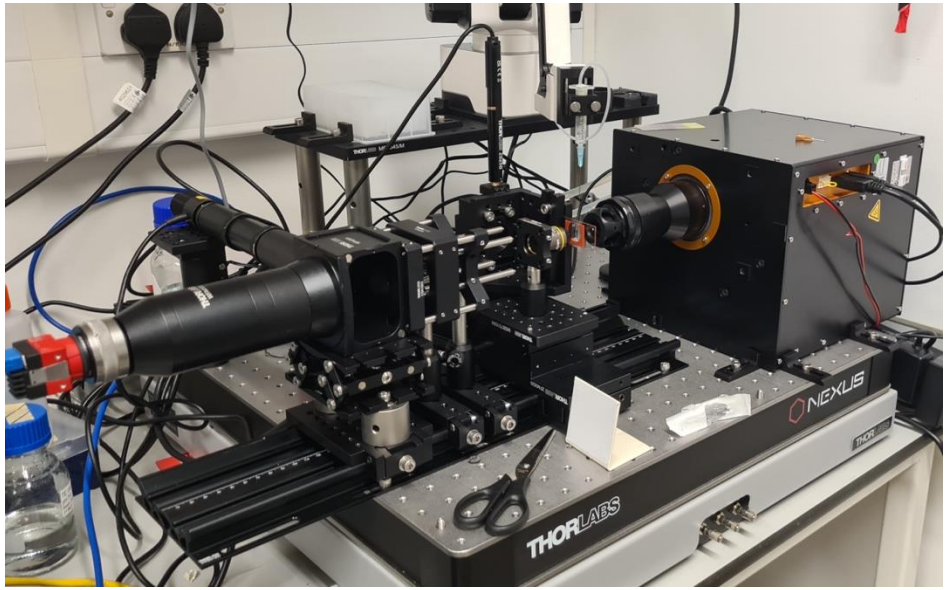


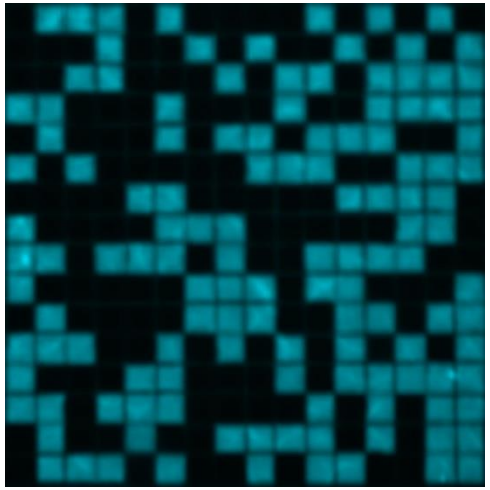


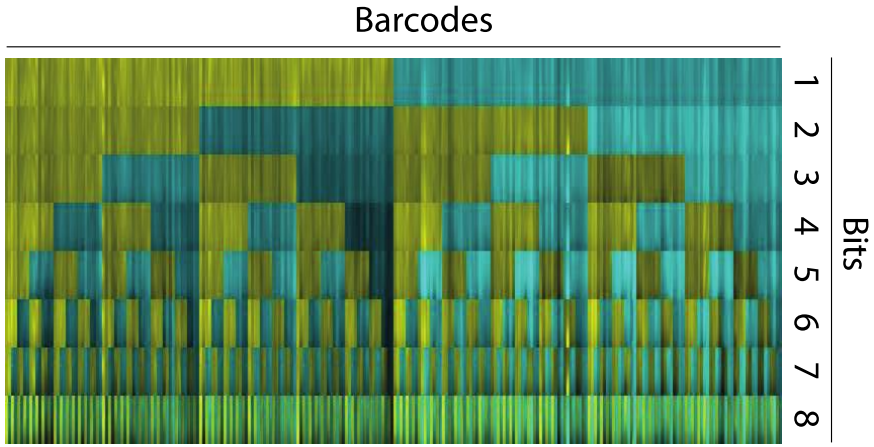
- New multi-omics method using light to encode spatial DNA barcodes on biomolecules within cells
- Enables simultaneous profiling of DNA (ATAC, CUT&TAG) and RNA, proteins in development
- Highly flexible: can profile 1 to 1M areas with size from subcellular to millimeters Custom shapes, regular grids, or segmentation-based
- Cost-effective: projected ~£1000 / 100k areas (with no sample size limit)
- Proof of concept completed and article under review

HIGHLIGHT: BALI AUTOMATED INSTRUMENT





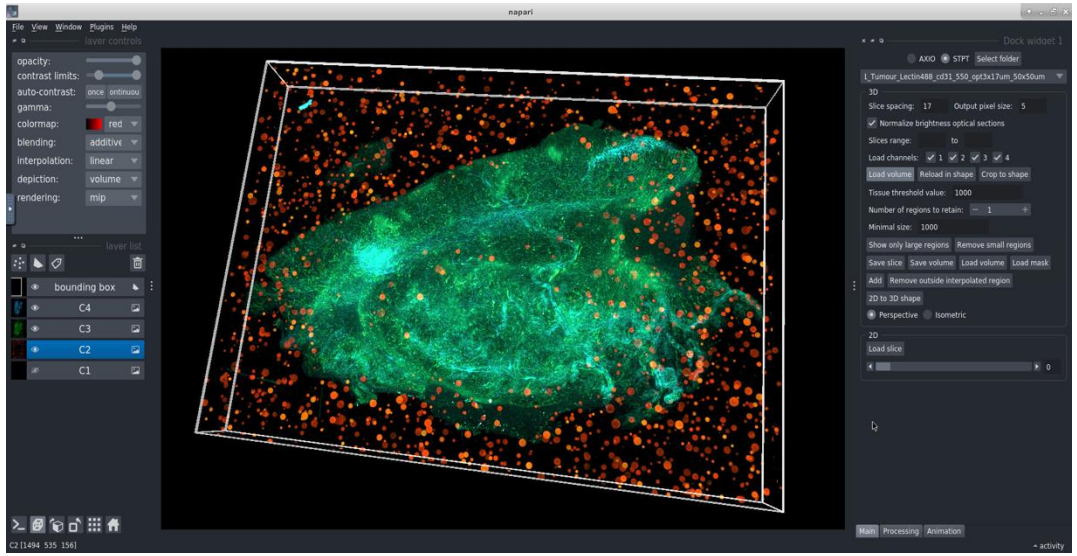


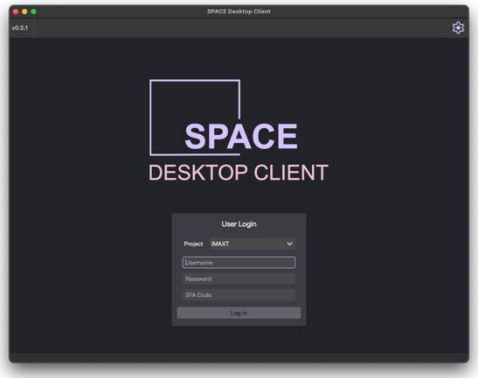


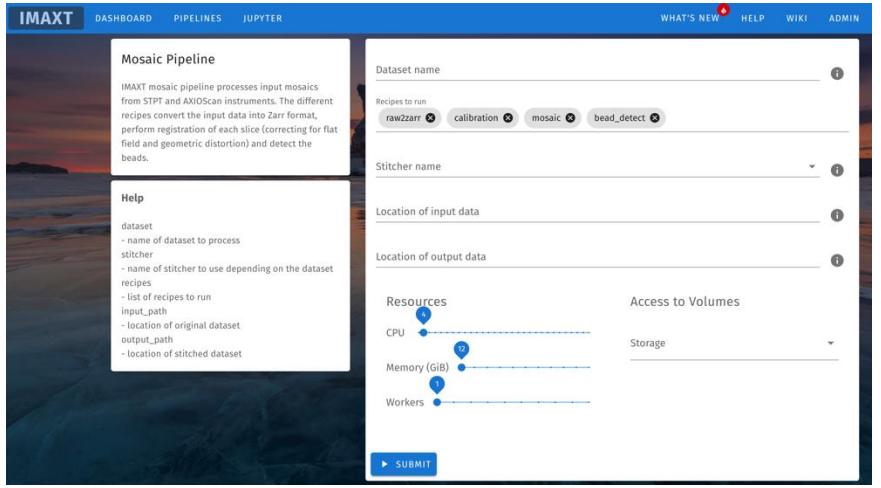
Automatic barcode writing on 256 areas (using binary fluorescent DNA barcodes)

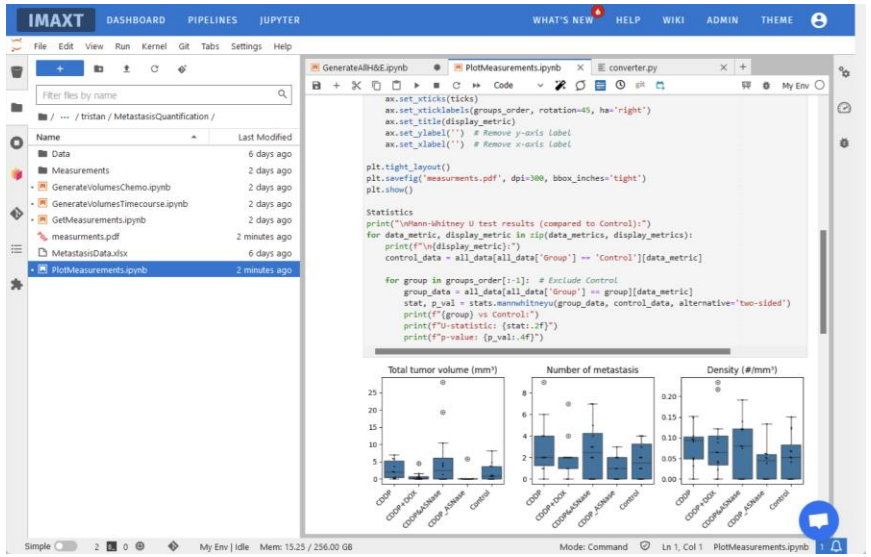
- Fully automated imaging, light delivery and chemistry
- Profiling of 1000s of areas tested
- Maiden run completed
- Expected to enter production in the next quarter

DATA CENTRE







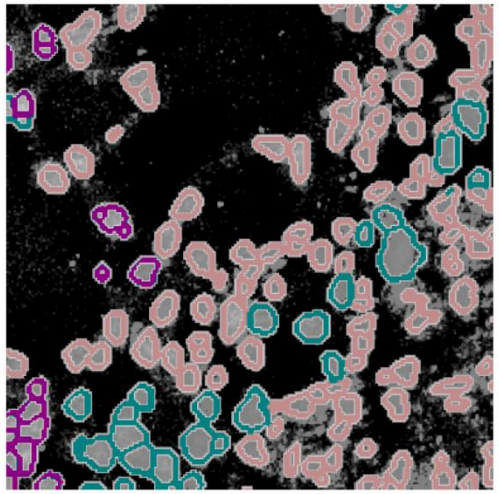


- 2000 CPUs, 12 GPUs, 11TB memory, 6PB storage
- Web-based interactive visualization tools for STPT, IMC (up to terabyte-scale)
- Interactive on-demand analysis on virtual machines using virtual environment and jupyter notebooks
- Standardized pipelines for primary processing (i.e. stitching, segmentation)
- Bespoke tools for data upload and metadata tracking

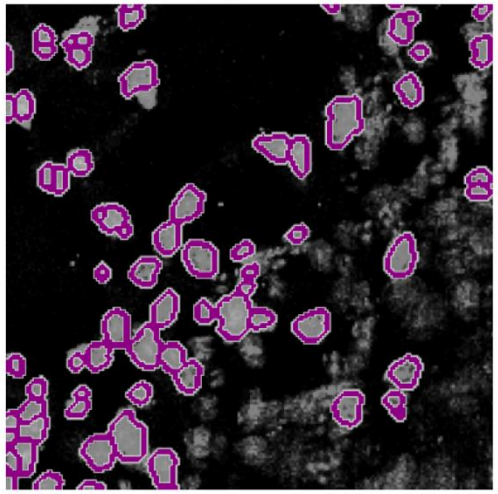
IMPROVEMENTS ON CELL SEGMENTATION

CANCER GRAND CHALLENGES | SPACE

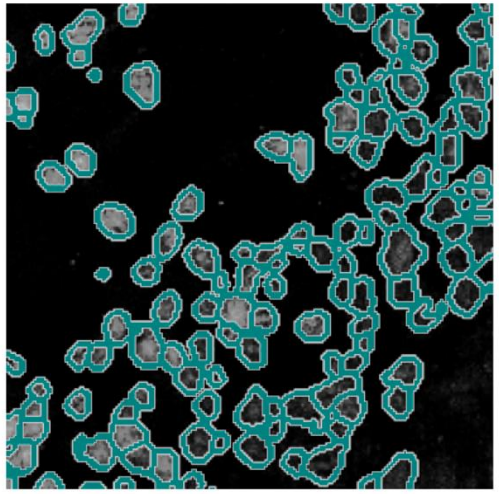
UberMask



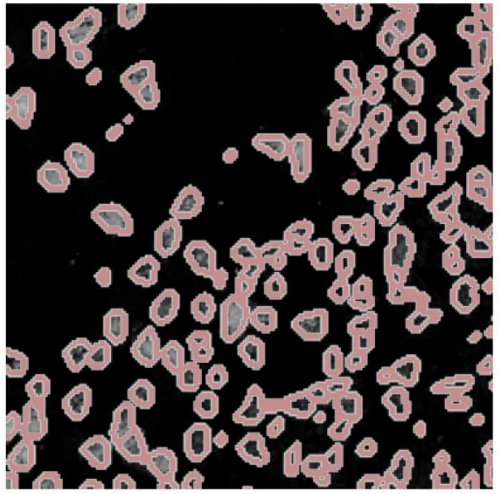
Segmentation 1



Segmentation 2

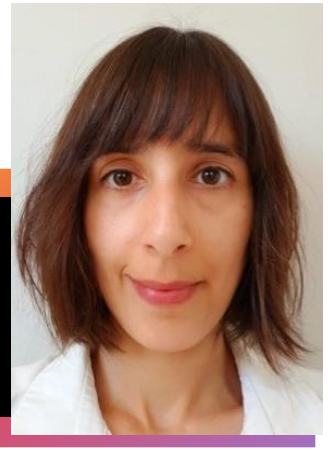


Segmentation 3

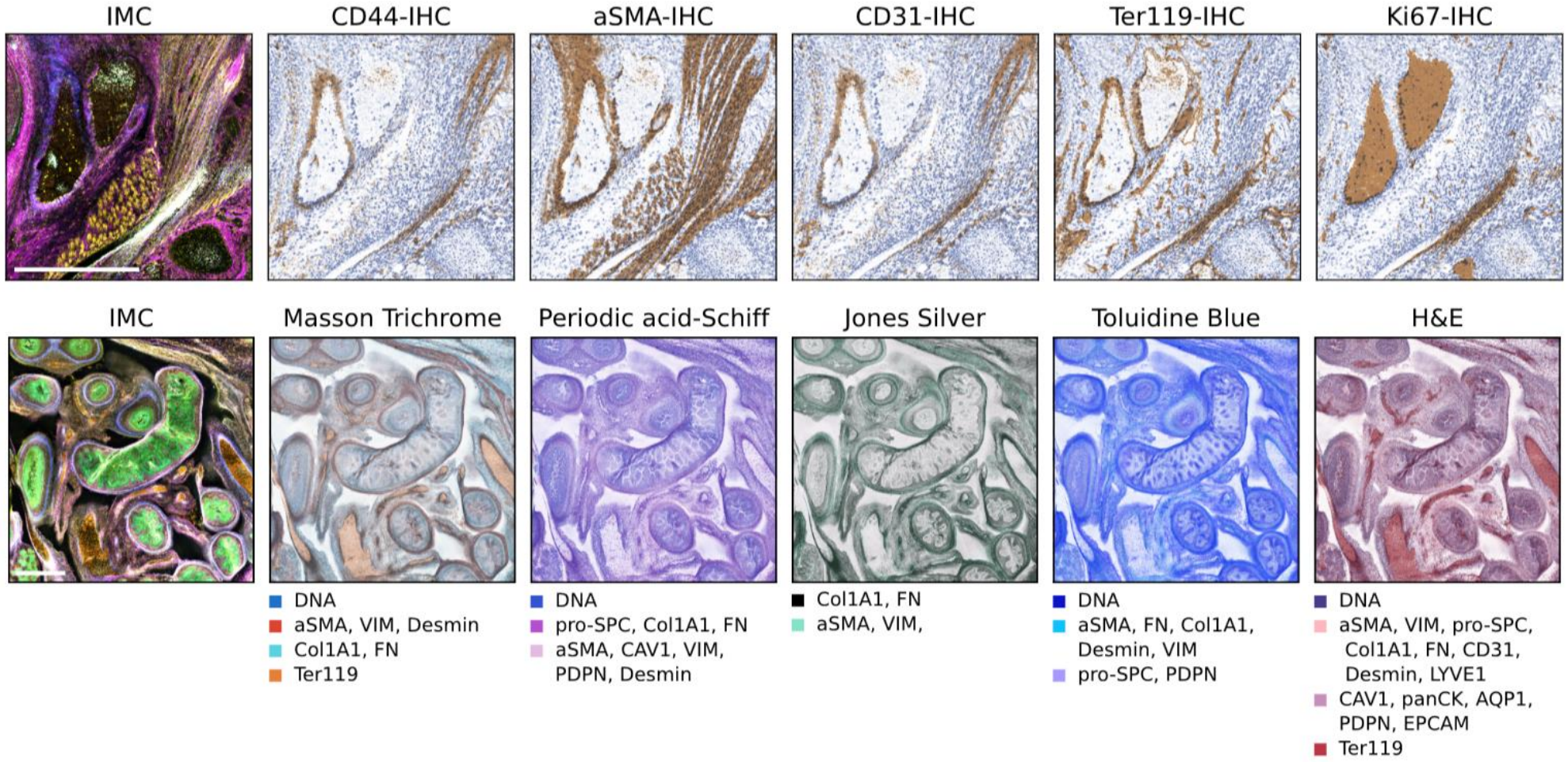


- Cell Segmentation is a crucial step for most spatial omics technologies
- Methods already available (e.g Mesmer, Cellpose, Stardist, DICE-XMDB, Watershed) may behave differently image to image/region to region.
- We developed a tool that automatically segments samples using multiple methods and parameters, and combines the best of each into a consensus mask (the UberMask).

Poster
Melis Irfan

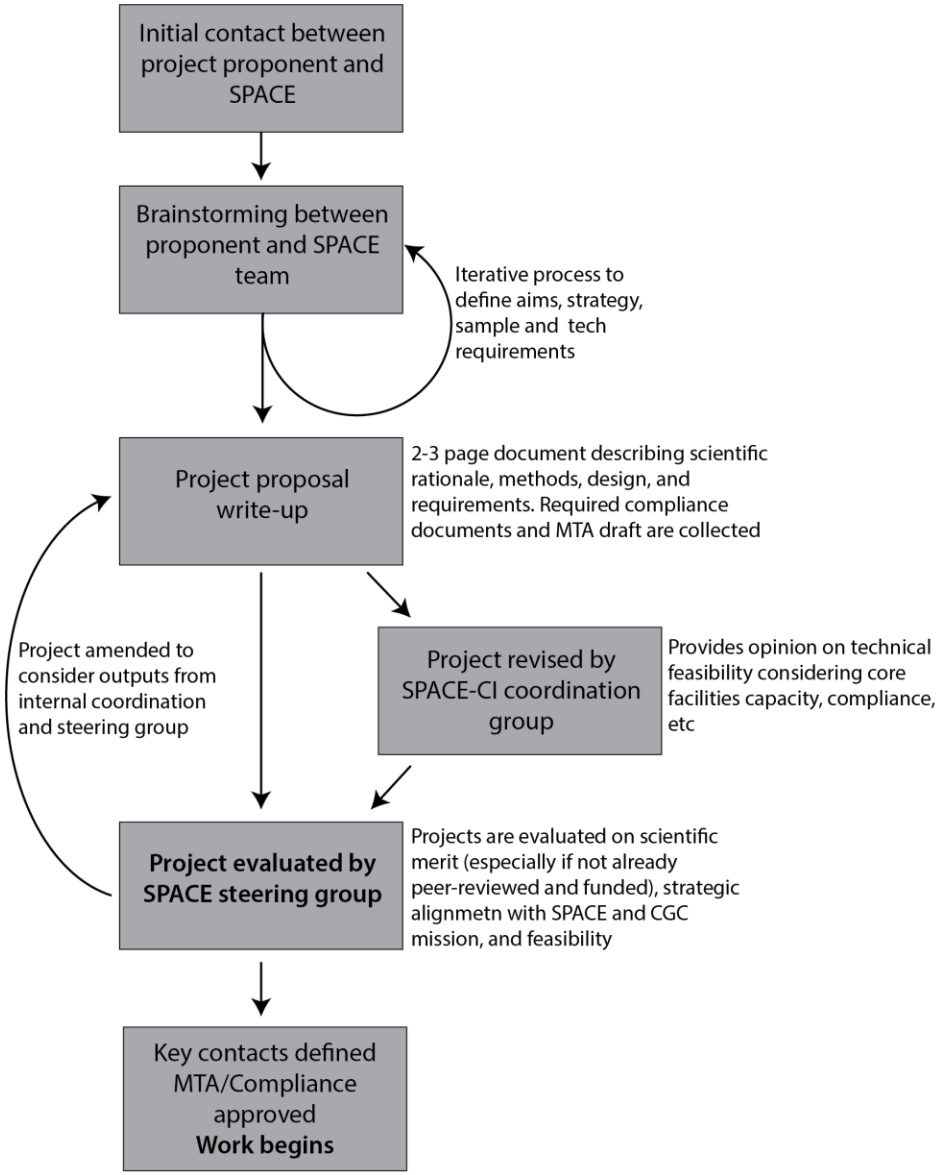


VIRTUAL CYTOLOGY STAINS



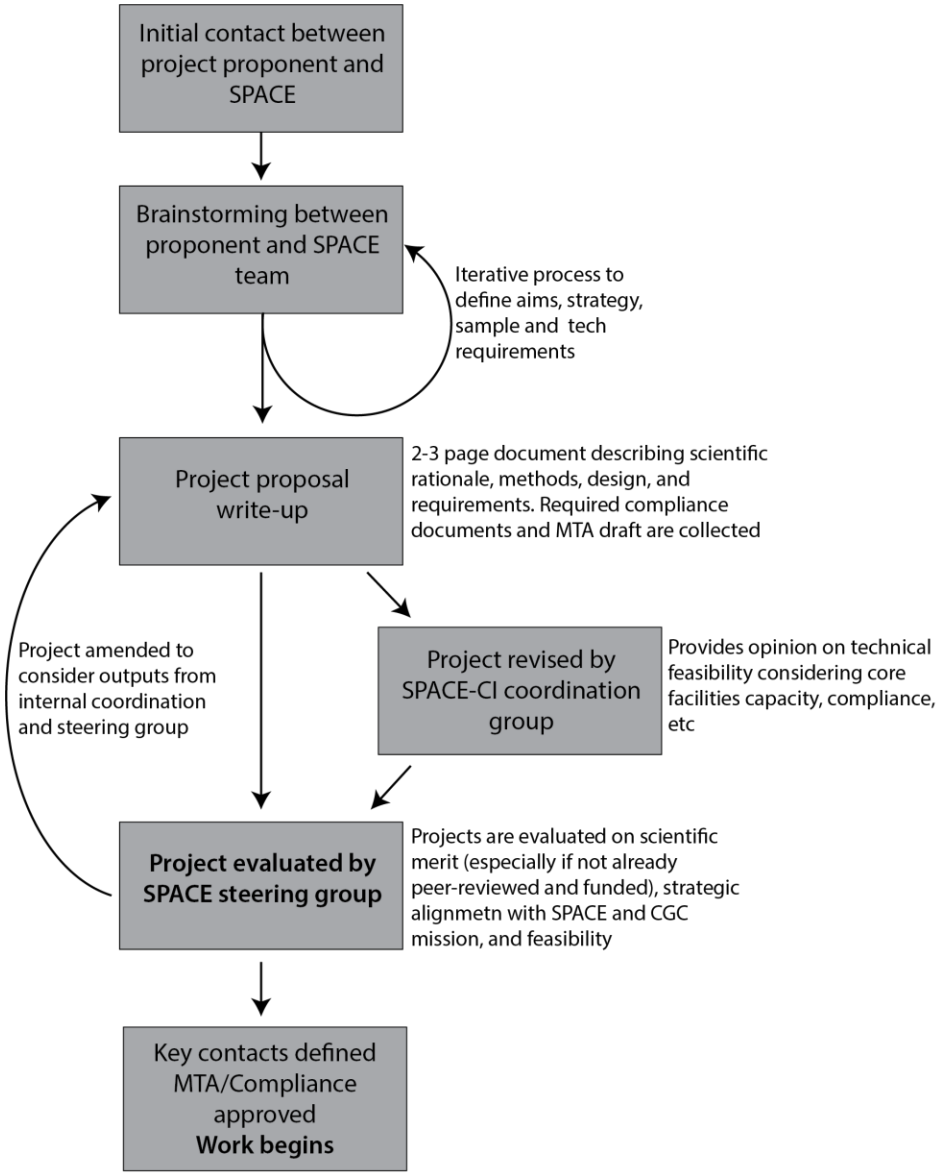
- Automatic conversion of STPT, IMC and multiplexed fluorescence data into virtual cytology stains (using AI to identify the best stain combination)
- Facilitates the usage of these technologies by traditional pathology processes

COLLABORATION MODEL AND ACCESS



- CGC funding supports infrastructure, core staff, and technology development
- Project-specific research costs are funded by external research grants or recovered through cross-charging
- SPACE can be added as a collaborator to grant awards
- Projects will access space following an evaluation process looking at strategic alignment, feasibility, and scientific merit
- Cancer Grand Challenge projects have priority

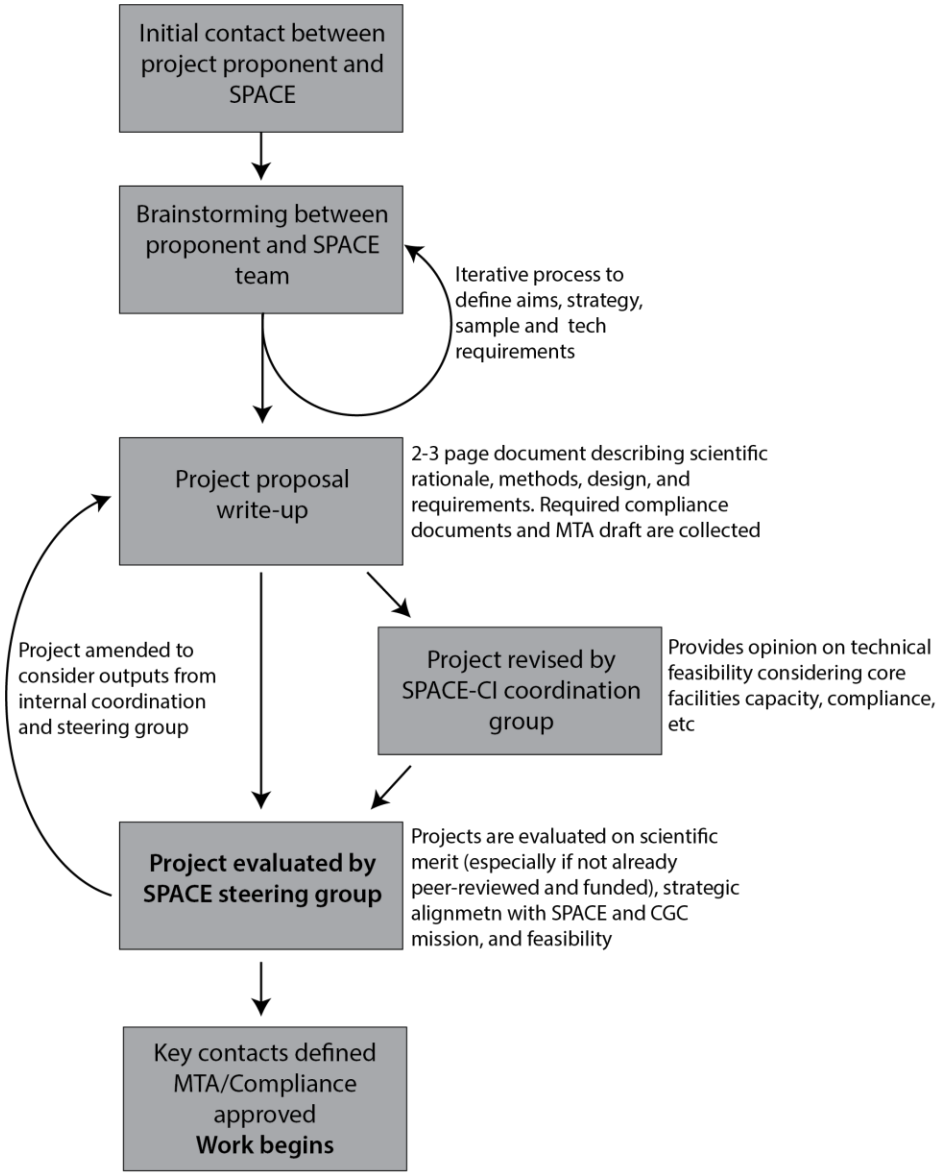
COLLABORATION MODEL AND ACCESS



TIER 1: Full collaborations

- Substantial scientific involvement from SPACE from the beginning
- Possibility for large sample cohorts
- Dedicated technology optimization/development
- Largely funded through grants (ideally with SPACE as named collaborator)

COLLABORATION MODEL AND ACCESS



TIER 2: Pilot projects

- Focused on testing new ideas or producing preliminary data for grant applications (leading to larger projects)
- Low sample number
- Largely using the infrastructure *as is*
- SPACE funding available as a bootstrap

TIER 3: service projects

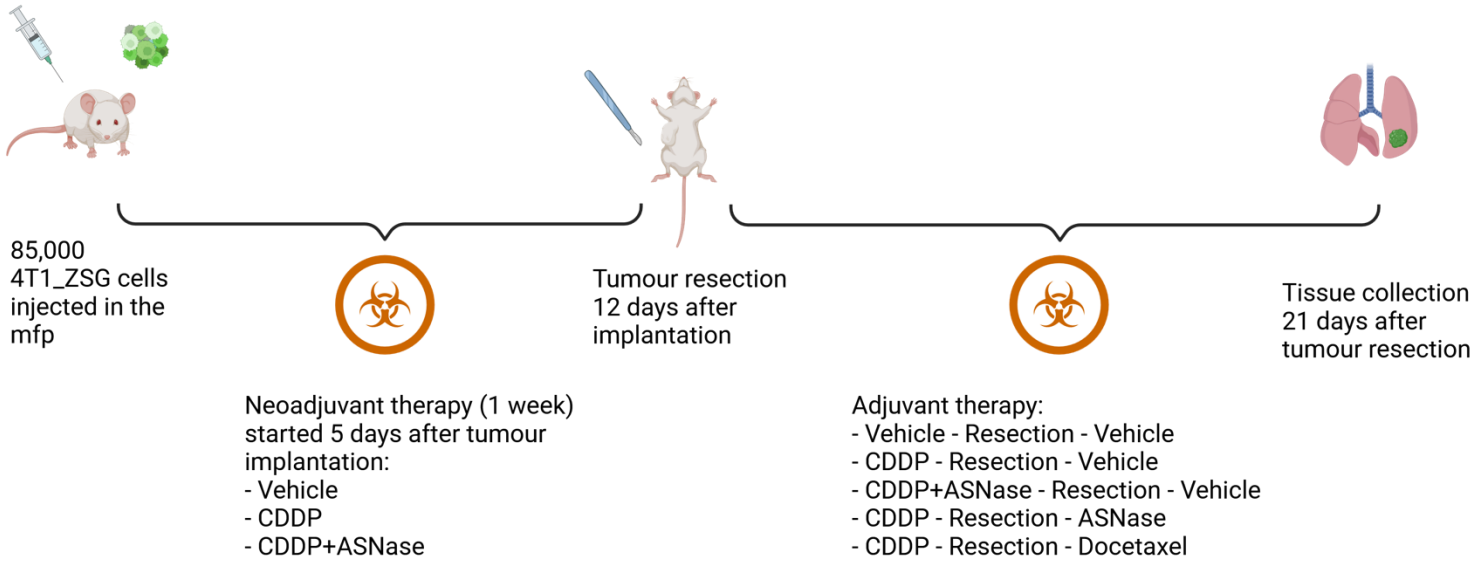
- Smaller project in which SPACE largely only provides technical capacity
- No additional tech development
- Entirely cost-recovered

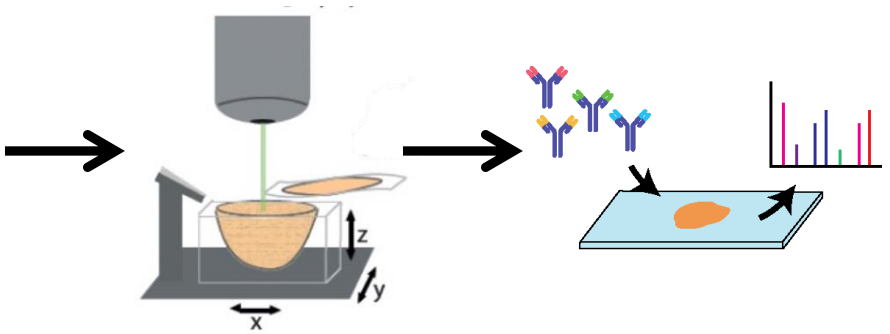
CURRENT PROJECTS

- 1 internal project, 4 collaborations, 3 pilots
- Focusing on Brain cancer, breast cancer, ovarian cancer, neuroendocrine cancer, and paediatric cancers
- Partners:
  - University of Cambridge and Addenbrookes Hospital
  - Memorial Sloan-Kettering Cancer Centre
  - Children's cancer therapy development institute
  - Great Ormond Street Hospital London
  - Institute for Cancer Research London
  - University College London
  - EMBL-EBI
- Still plenty of capacity left!

PROJECTS: TME EFFECT OF NOVEL THERAPY FOR BREAST CANCER

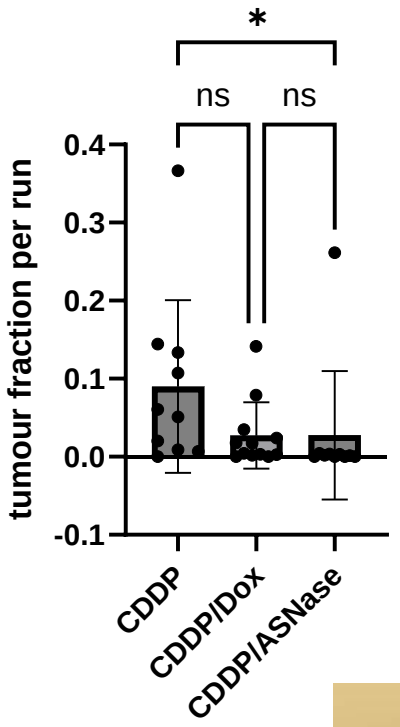
- Asparagine bioavailability reduction has been proposed as a therapy option to reduce breast cancer metastasis (Knott et al 2018)
- We used several spatial methods to study in detail the metastatic process in the lung, and the evolution of the tumour niche following different therapies
- Combination of mass spectrometry proteomics, single-cell RNAseq, mass spectrometry, and scRNAseq

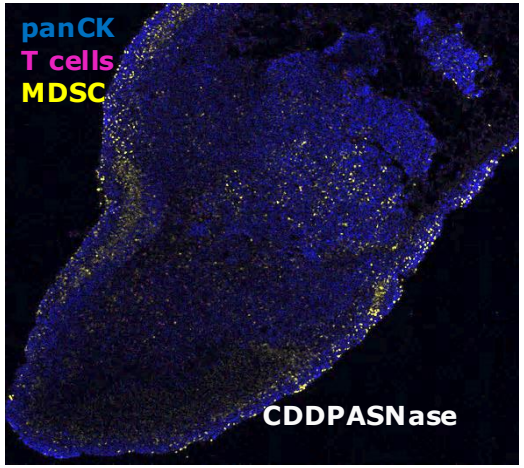


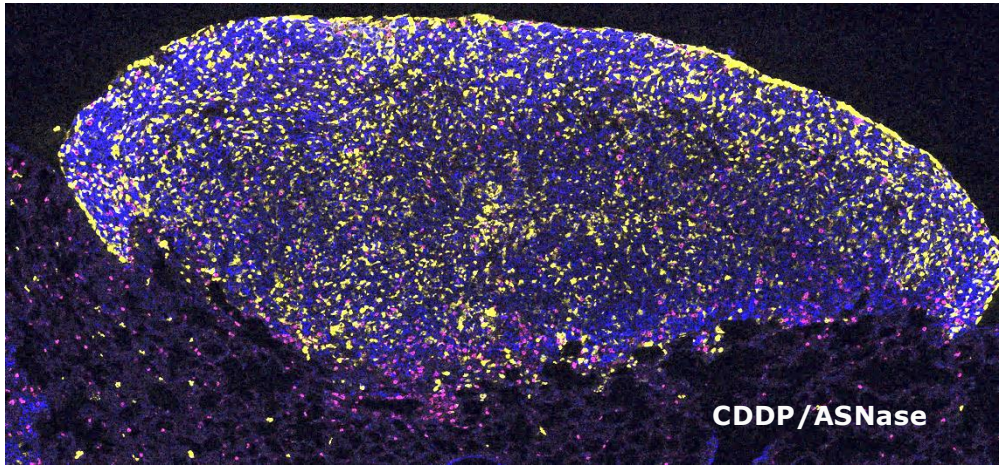


PROJECTS: METASTATIC TME MODULATION BY A NOVEL THERAPY

- Asparagine treatment has efficacy comparable to the standard of care chemotherapy, with much lower side effects
- Using asparaginase before or after surgery results in completely different TME remodeling at the metastatic site – only one works!
- TME response is heterogeneous depending on anatomical location (inside or on the margin of the lung)







Poster
Marta Paez-Ribes

PROJECTS: SPATIAL PROFILING OF NEUROBLASTOMA

STRATIFIED MEDICINE PAEDIATRICS

CANCER GRAND CHALLENGES

Team: PROTECT

FULL COLLAB

- Flagship UK programme for relapsed refractory paediatric cancers
- Genomic analysis and clinical reporting enabling timely enrollment into clinical trials
- >800 patients enrolled since 2019

Louis Chesler (ICR)

John Anderson (Great Ormond Street Hospital

Isidro cortes-Ciriano (EMBL-EBI)

SPACE will:

- Analyse primary samples to evaluate the distribution of different tumour clones and how this affects outcome and relapse
- Help identify which patients can be given a new type of CAR-T treatment developed by the PROTECT grand challenge
- Implement novel profiling options, i.e. epigenetic, long read sequencing

PRODUCTION OF PRELIMINARY DATA TO SUPPORT THE DEVELOPMENT OF AN ANTIBODY-DRUG CONJUGATE

CANCER GRAND CHALLENGES | SPACE

Children's Cancer Therapy Development Institute

CANCER GRAND CHALLENGES

Team: KOODAC

PILOT

- Diffuse Intrinsic Pontine Glioma (DIPG) is a tumour with extremely few therapy options and a horrible outcome
- The Keller group has identified a promising tumour-specific marker and is developing an antibody-drug conjugate with the aim of licensing it for DIPG and adult melanoma

SPACE will:

- Generate preliminary data to support the development process and confirm initial results
- Validate target expression and availability
- Profile the tumour environment holistically to contextualize the therapy development

Charles Keller
Children's Cancer Theraphy Development Institute

FOSTERING INNOVATION IN SPATIAL BIOLOGY

- SPACE wants to **multiply the impact** of spatial biology in cancer research
- Facilitating the implementation of new technologies to existing and new questions in a way that benefits research and patients the most
- Moving beyond the boundaries of individual projects to create something bigger than the sum of its parts!

We are open to collaborations!

1st Cancer Grand Challenge conference on Spatial Biology
Sept 1-3 2026
Royal Society, London

ACKNOWLEDGEMENTS

- Greg Hannon
- Nicholas Walton

SPACE Team

- Claire Mulvey
- Marta Paez-Ribes
- Atefeh Fatemi
- Mi Chween Chan
- Kui Hua
- Qiuchen Meng
- John Harwood-Harrison
- Phoebe Hicks
- Martina Alini
- Giorgia Battistoni
- Anna Cregeen
- Karolina Wasilewska
- Clare Rebbeck
- Eduardo Gonzales-Solares
- Tristan Whitmarsh
- Melis Irfan
- Mo Al Sa'd
- Alireza Molaeinezhad
- Leight Smith
- Neil Millar

CRUK-CI Core facilities and Admin

- Michele Dunn
- Harriet Dean-Edwards
- Victoria Blackwell
- Genomics (Ania Piskorz)
- Flow (Richard Grenfell)
- Histology (Jo Arnold)
- Proteomics (Clive D'Santos)
- Microscopy (Andreas Bruckbauer)
- IT (Nigel Berryman)
- Compliance (Osama Al-Assar)
- ROO (Sarah Vaughan)