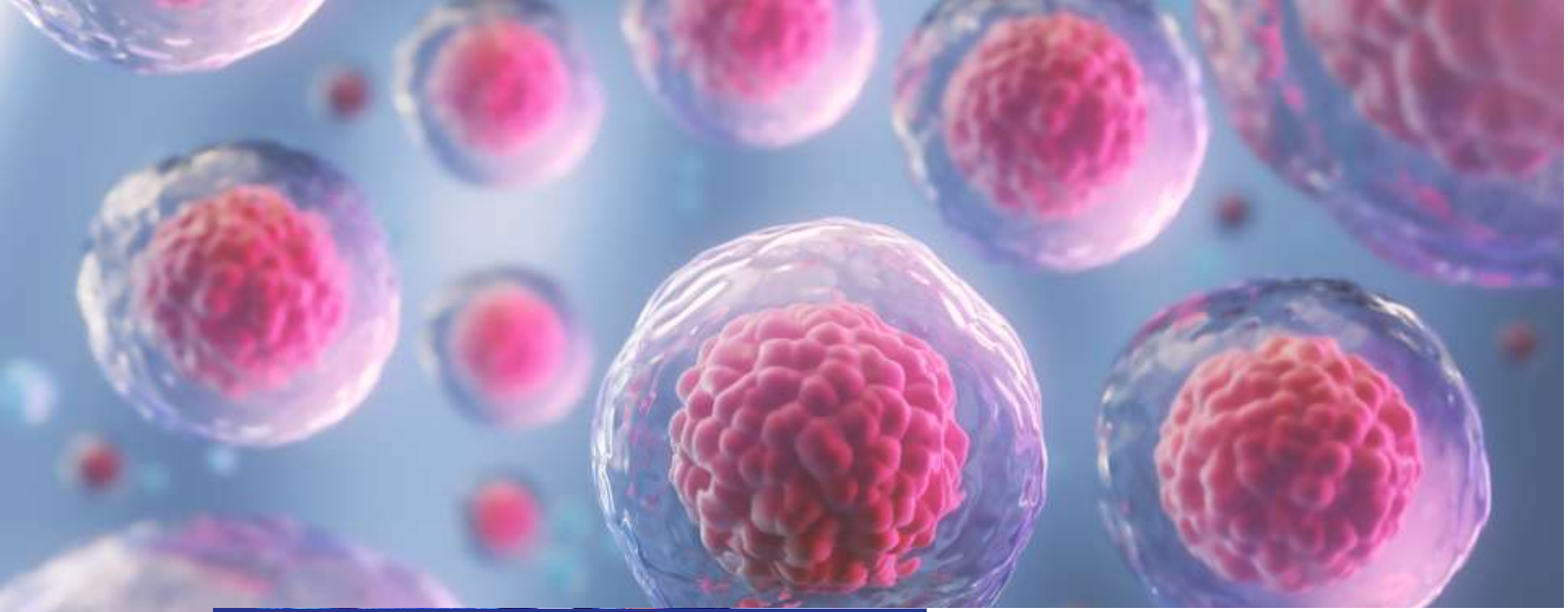


THE UNIVERSITY OF WESTERN AUSTRALIA

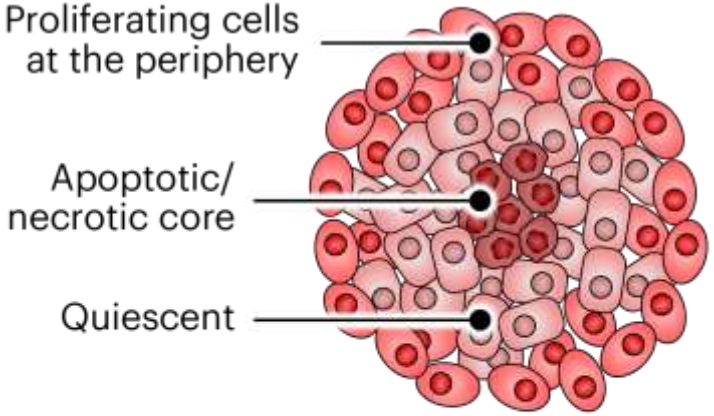
# Polymer-mediated DNA delivery for spatially encoded cultures

38APS, 19 February 2024

Dr Cameron W. Evans, School of Molecular Sciences

# 3D: The future of cell culture?

- Advantages of 3D
  - Cell–cell interactions
  - Cell–ECM interactions
  - More accurate modelling of cell behaviour and response
- 3D cultures fill the gap between 2D and animals
  - 2D: easy to generate, reproducible
  - Animals: difficult, poor reproducibility, often fail to translate
- Cells experience different environments in 3D
  - Different responses, *e.g.*, to drugs



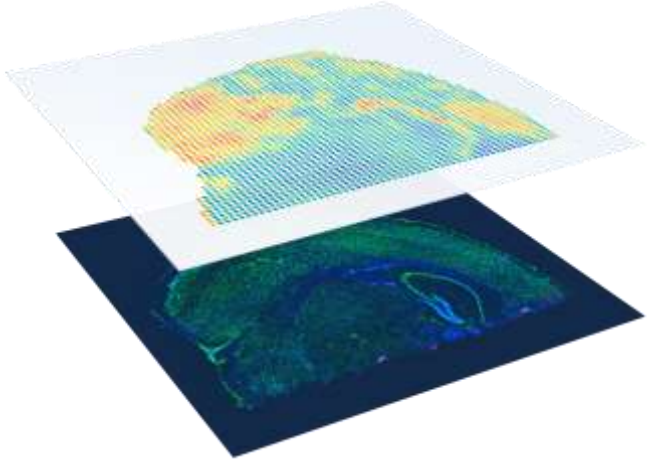

**Gradients in 3D cultures**

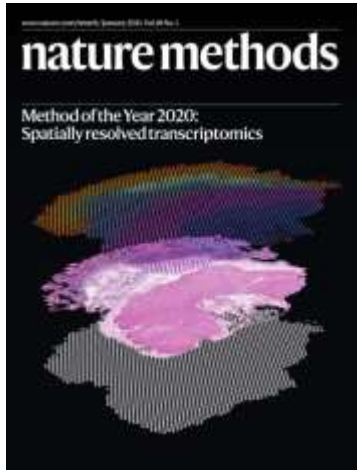
- Nutrients
- Metabolism
- Proliferation
- Waste products
- Oxygen/hypoxia
- pH

Carragher *et al. Nat Rev Drug Discov* 17, 606 (2018).

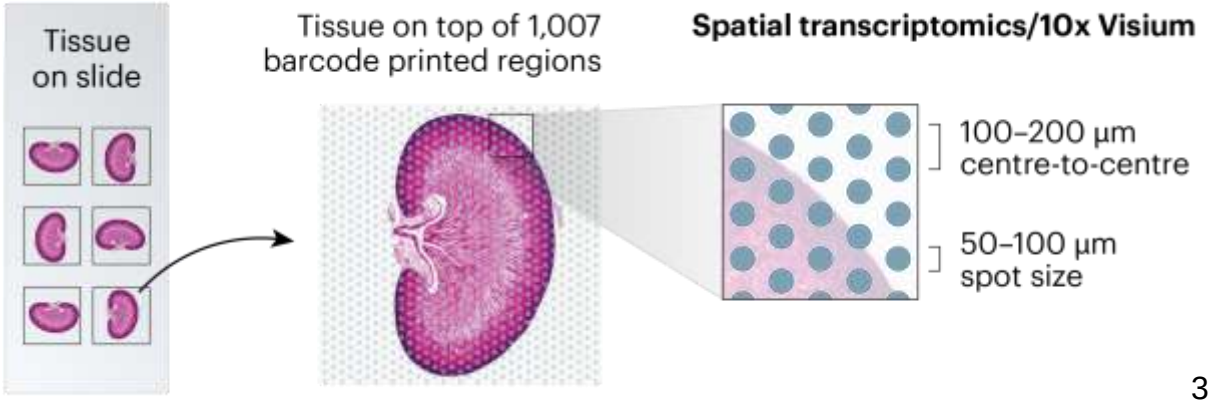
# Single-cell and spatial analysis

- Analysis is more complicated in 3D cultures
- Different environments → different responses
  - Need single-cell analysis techniques
  - Bulk analysis masks heterogeneity
- Cells doing different things, but where?
  - Spatial 'omics to capture spatial information





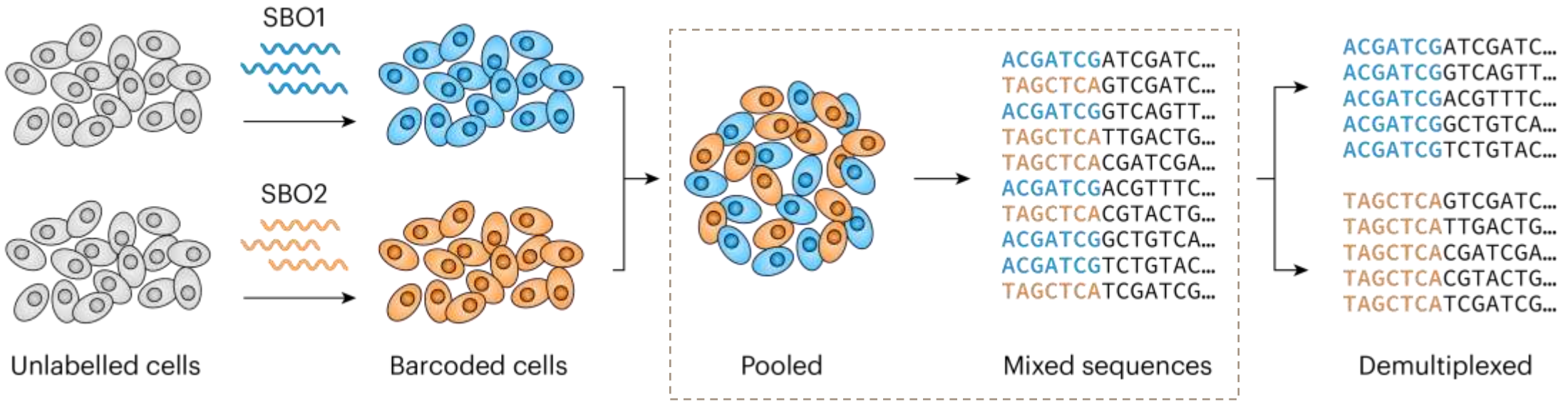

- Spatial not well suited to 3D cultures
  - Resolution ~50–220 µm; culture ~80–500 µm
  - Not single-cell resolution




Method of the Year 2020. *Nat Methods* 18, 1 (2021).
Asp *et al. BioEssays* 42, 1900221 (2020).
Baysoy *et al. Nat Rev Mol Cell Biol* 24, 695-713 (2023).

# Multiplex sequencing reduces costs

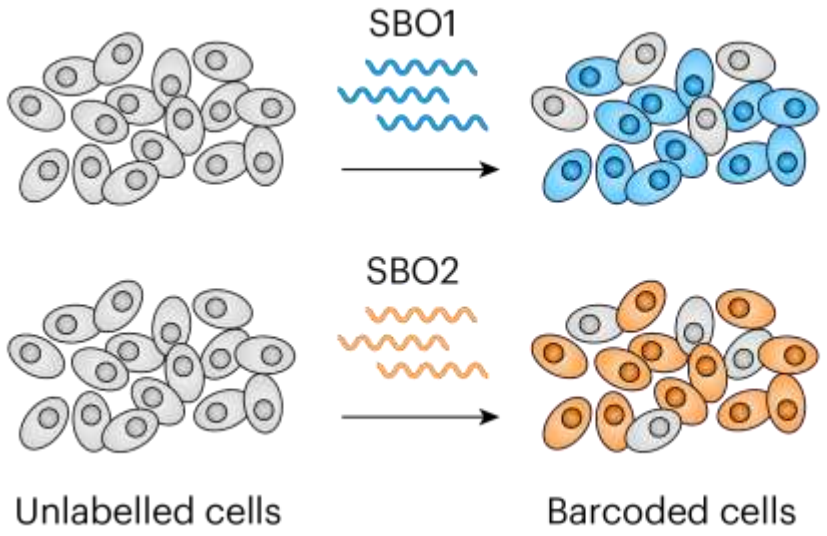
- Next-generation sequencing: snapshot of gene expression
  - High cost per run; want to be able to pool samples
  - 'Barcoding' with a unique DNA sequence to label all cells in each sample




Sequencing cost halved

# Multiplex sequencing reduces costs

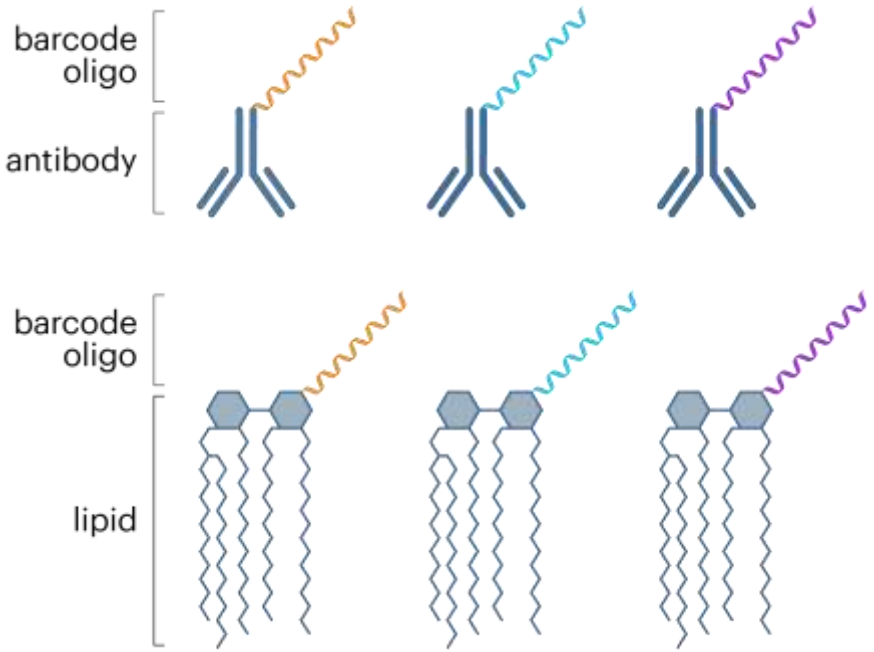
- Poor labelling efficiency or barcode exchange
  - Barcodes absent, mixed up, or multiple barcodes
  - Reads cannot be assigned to samples → discarded




- Efficient labelling is crucial for effective multiplexing

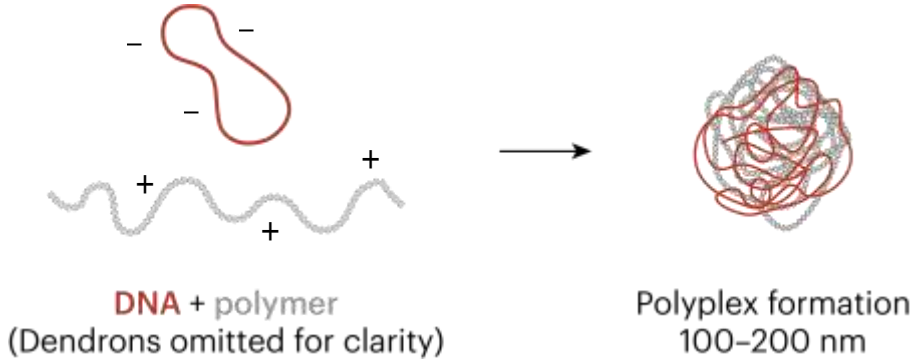
# Aim: Polymer-based barcoding method

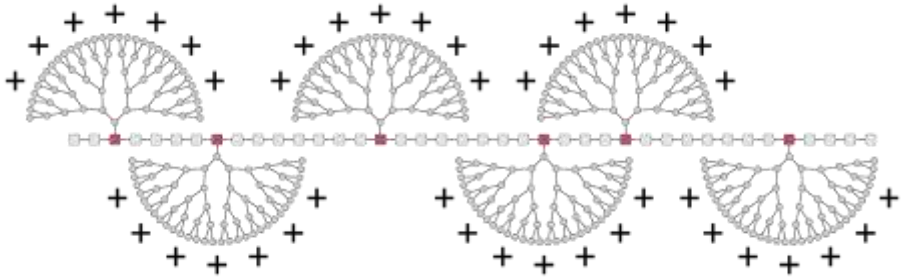
- Improved method for labelling (barcoding) cells
  - Polymer-based delivery
- Requirements
  - High-efficiency labelling
  - Low barcode exchange
  - High cell recovery → reduced cost per sample

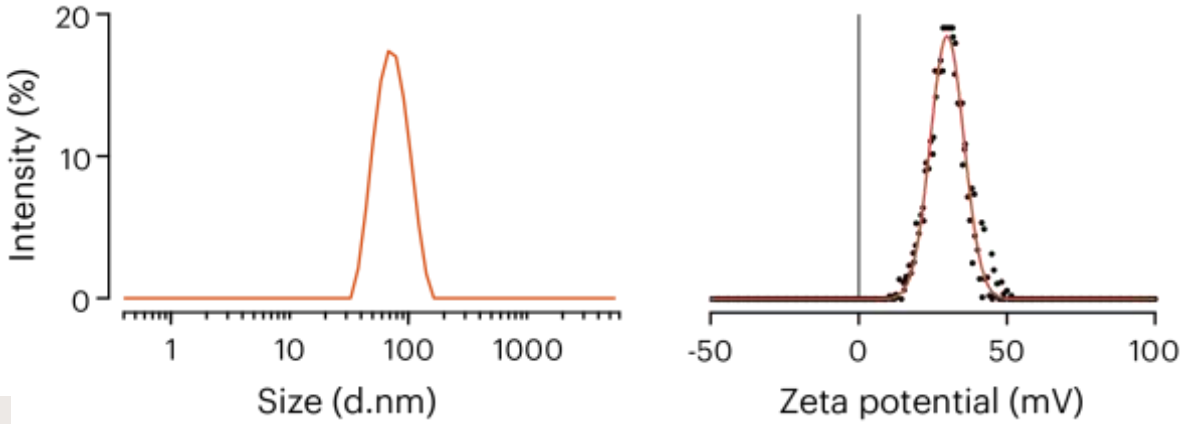


# Dendronised polymers as delivery agents

- Dendronised polymers for delivery of DNA, RNA, *etc.*
  - Delivery in cells and animals
  - Cationic polymers taken up by cells *via* endocytosis



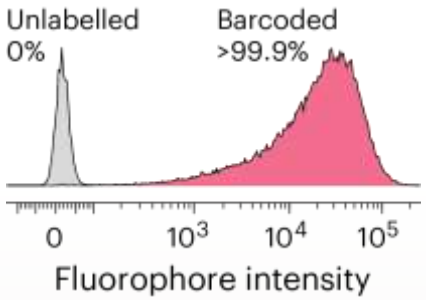


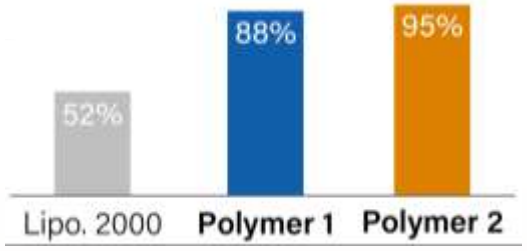

- New technique: scTECH-seq
  - Using dendronized polymer to deliver barcode oligos

*Chem Sci* 10, 7718 (2019); *J Am Chem Soc* 142, 20600 (2020).

# Example 1: scTECH-seq analysis in 2D

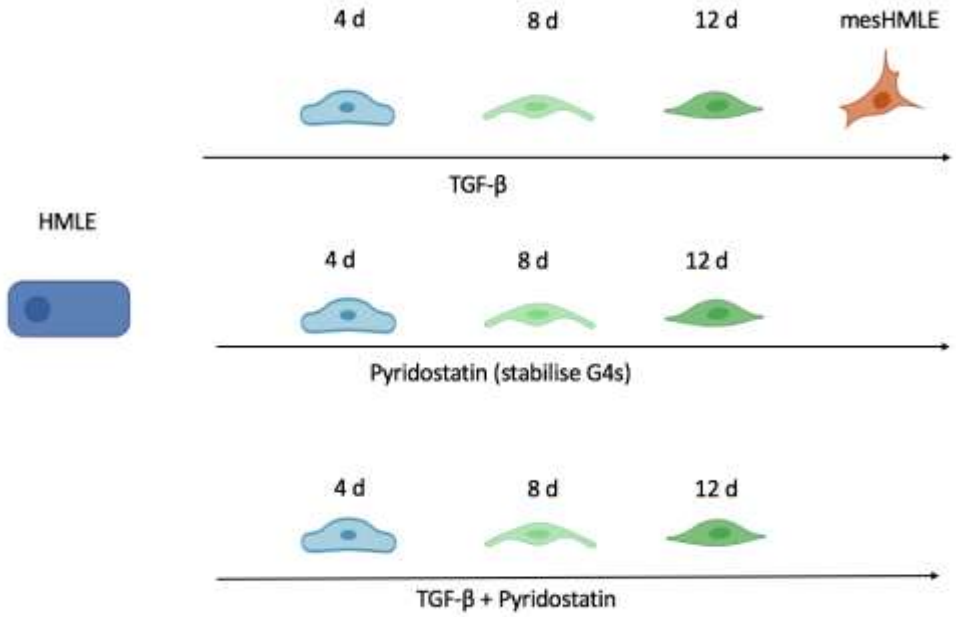
- Improved labelling and cell recovery
  - Highly efficient labelling of all cells in a sample
  - Lower cost/more cells sequenced




- Polymers work better
  - Faster cell contact/uptake
  - Endocytosis limits barcode exchange

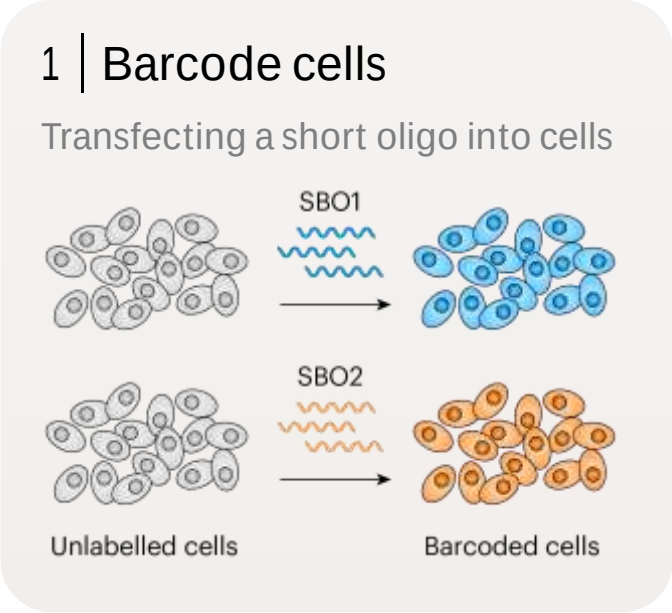
- Successful multiplexing and recovery
  - 12 samples for the price of 2
  - Profiled cell subpopulations undergoing EMT

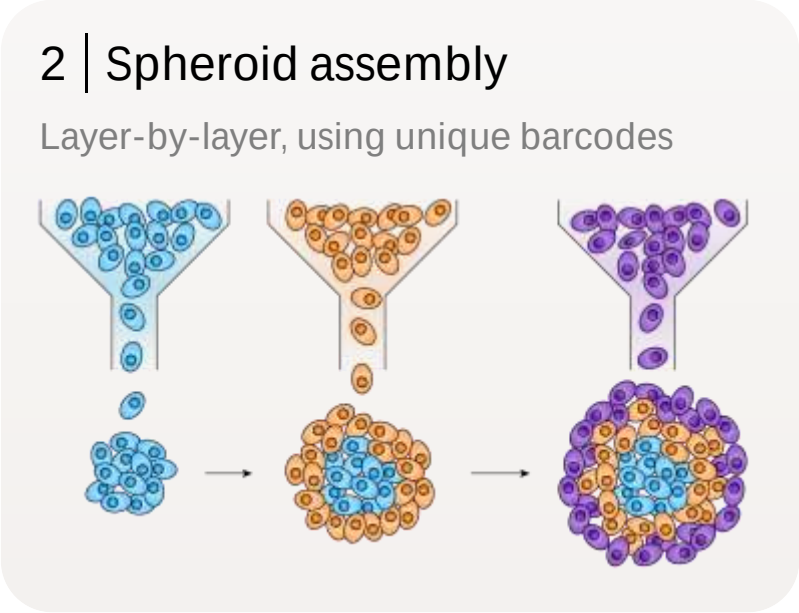


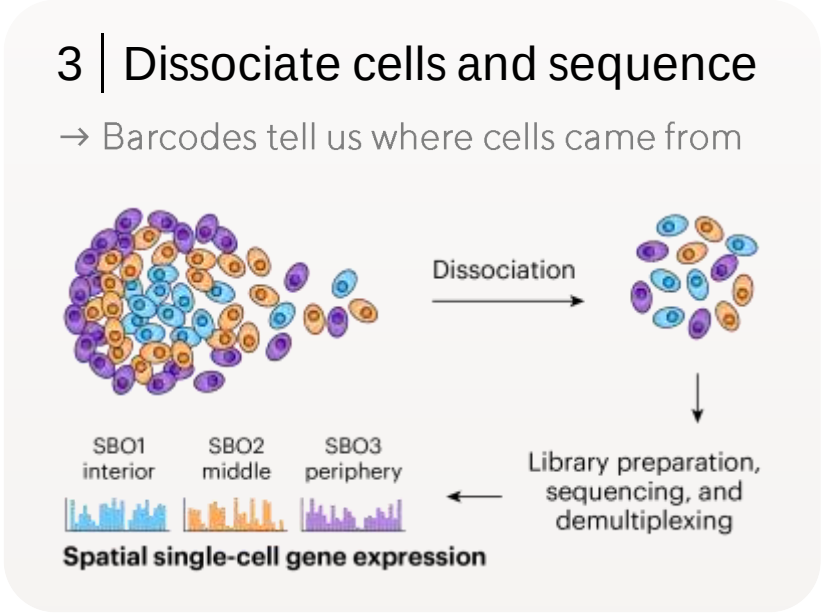

*bioRxiv* 2023.08.21.554220 (2023).

# Example 2: scTECH-seq analysis in 3D

- Assembling cells while incorporating spatial information



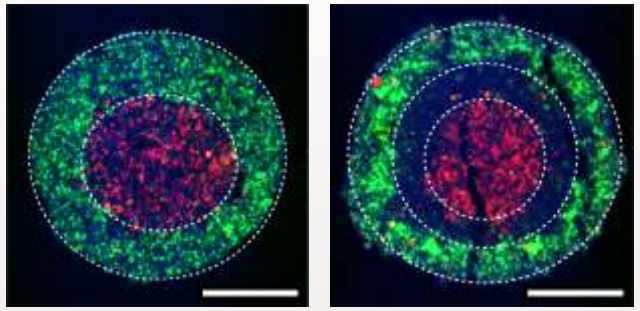




- Gene expression and spatial information at single-cell resolution
  - Analyse changes in 3D cultures in different regions

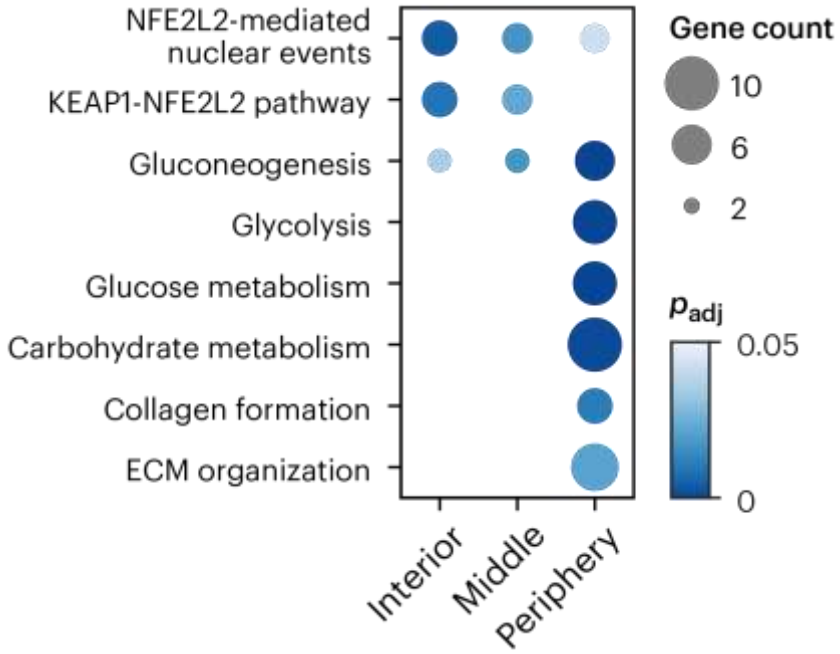
# Example 2: Profiling spheroids

- Barcoding HeLa cells
- Spheroid assembly
  - Size ~500 µm



2-layer 3-layer

- Gradients in gene expression, metabolism, cell stress
  - Consistent with literature

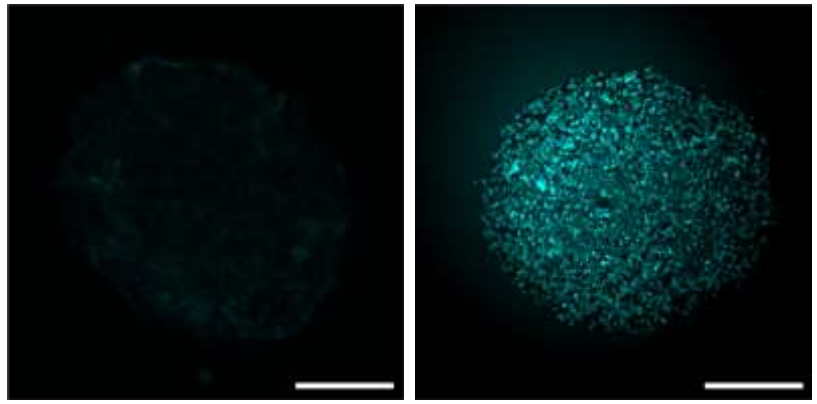



*bioRxiv* 2023.11.20.567985 (2023).

# Example 2: Exposing spheroids to drug

- Treating spheroids with irinotecan
  - Prodrug derivative of camptothecin
  - Stabilises Top I–DNA complex
  - Active metabolite SN-38 (fluorescent)

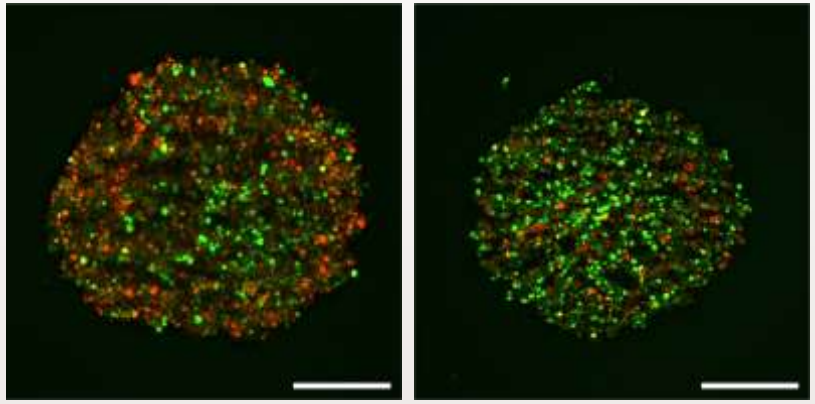
Untreated Irinotecan

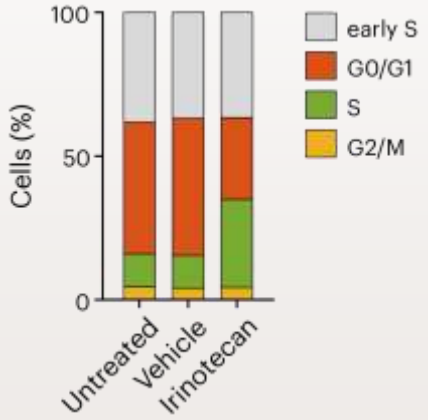


*bioRxiv* 2023.11.20.567985 (2023).

- Cell cycle inhibition
  - Fucci system visually shows cell cycle
  - Arresting cells during DNA replication


Untreated Irinotecan

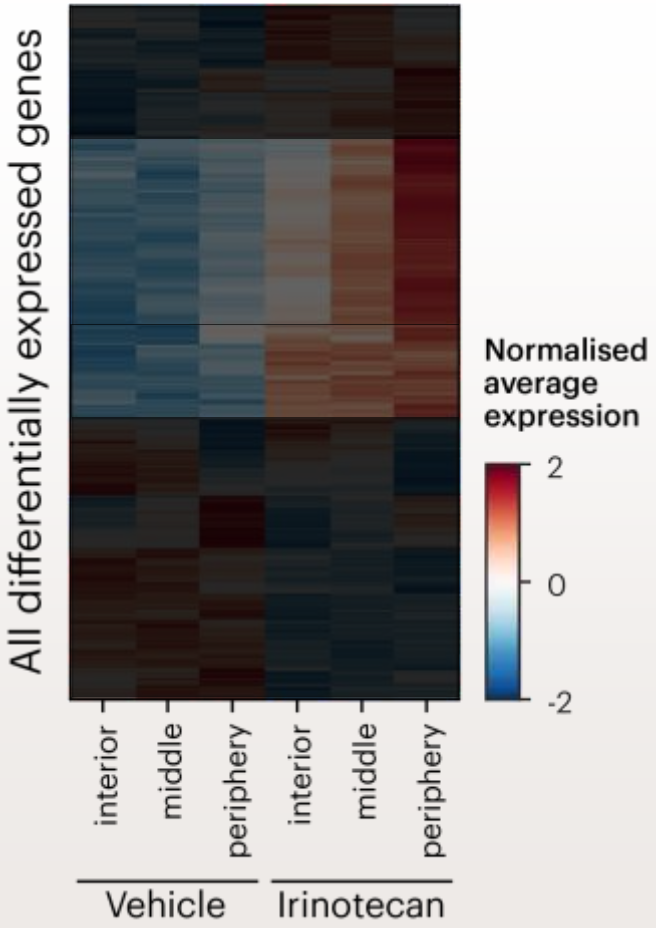




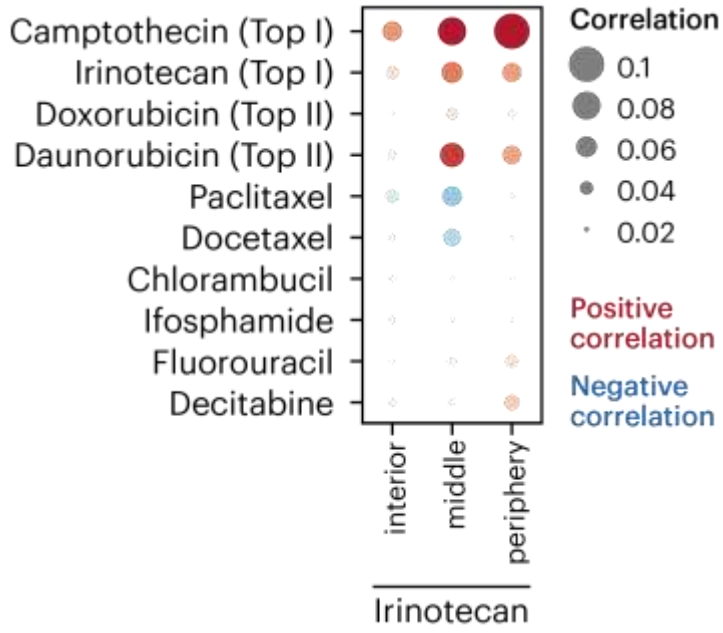

Ando *et al. Cell Struct Func* 48, 135-144 (2023).

# Example 2: Profiling drug response in 3D

- 3-layer spheroids
  - Vehicle control *vs* irinotecan-treated
- Comparing layers within the spheroid
  - Genes uniformly altered
  - Genes that are altered in a spatially dependent way
  - Evolving drug resistance markers




- Comparing 2D *vs* 3D
  - Stronger correlations at the periphery of spheroids
  - Differences in drug behaviour

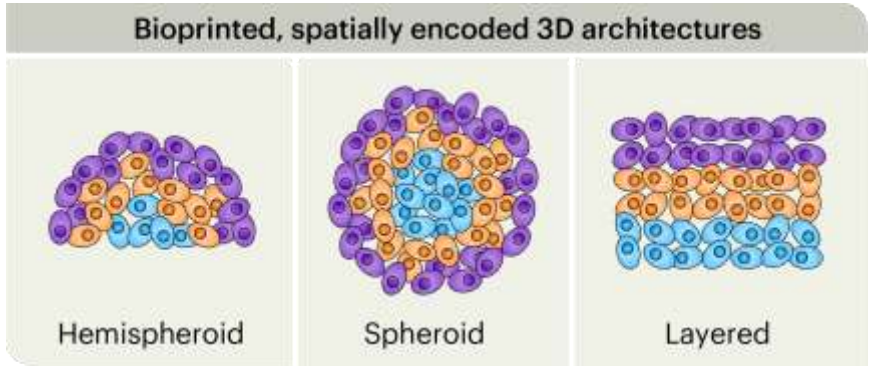


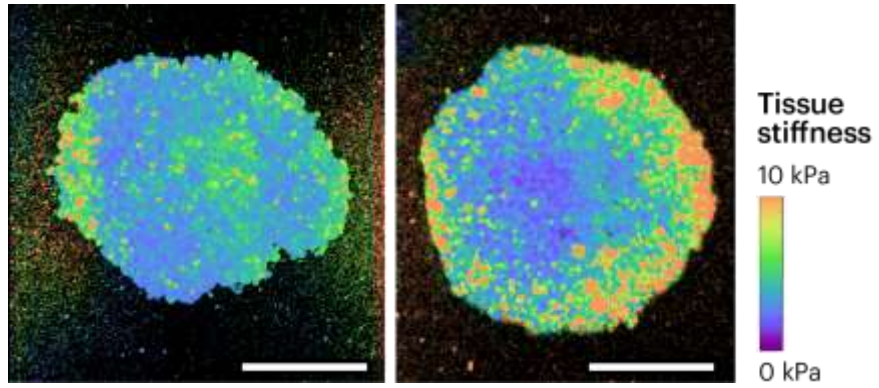

*bioRxiv* 2023.11.20.567985 (2023).

# Conclusions and future work

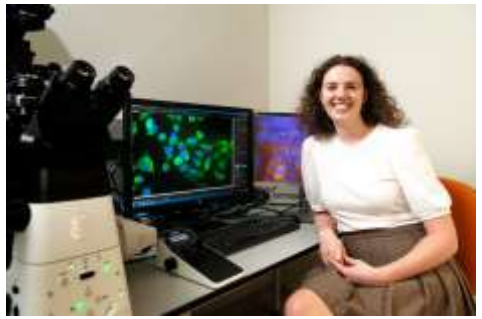
- Polymer-based barcoding strategy (scTECH-seq)
  - Advantages of polymers
  - Cationic dendronised polymers for delivery
- Multiplexing samples in 2D cultures
  - Profiled cellular transition (EMT)
- First spatial barcoding in 3D cultures
  - Extension to existing 3D culture models
  - Understand spatial patterns of gene expression

- Future directions
  - Extend to different architectures, use bioprinting
  - Improve barcode design to extend analysis timeframe
  - Correlative analysis with mechano-microscopy



# Acknowledgements



Dr Jessica King
Dr Marck Norret
A/Prof Swaminathan Iyer
Dr Nicole Smith
Dr Jessica Kretzmann
Somayra Mamsa
Sebastian Amos
A/Prof Yu Suk Choi

Dr Alireza Mowla
Prof Brendan Kennedy
Dr Matt Hepburn
Rowan Sanderson
Jiayue Li

Dr Ulrich Kadolsky
A/Prof Alka Saxena
Dr Munir Iqbal

Prof Jean-Louis Mergny (IECB)
A/Prof Zoë Waller (UCL)
E/Prof Laurence Hurley (UofA)
Prof Daniel Christ (Garvan)