

**Product testing and release criteria:
The importance of analytical method
development and validation, including
potency assays**

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14th June 2017
ISSCR ISSCR Annual Meeting's Industry Focus Session
"From the Bench to the Clinic: How to Manufacture Your Cell Product"

The Cell and Gene Therapy Catapult



£70m Development Facility

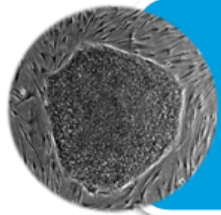
- 1,200m² Custom designed cell and gene therapy development facility
- Prime location in the heart of the London clinical research cluster
- 120 permanent staff



£55m large scale manufacture center

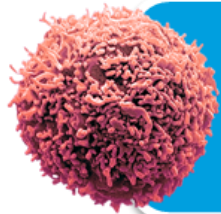
- 7,200m² manufacturing centre designed specifically for cell and gene therapies
- Located in the Stevenage biocatalyst
- Opening 2017

Range of Cell and Gene Therapy products



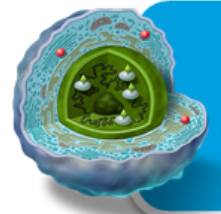
ES and iPS cells
HSC's
MSC's

10



T cells
T Regs
Dendritic cells

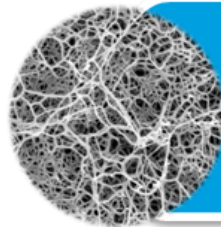
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Somatic cells

- Islet cells
- Fibroblasts

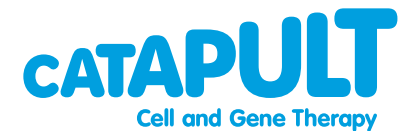
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Tissue / Scaffold
Tech development

8

Who we work with



In this presentation

- 1) Cell and gene therapy characterisation
- 2) Case study 1: in-process inferential analysis
- 3) Case study 2: real-time potency assay

A microscopic view of numerous cells, likely lymphocytes, showing their characteristic round shape and internal structure. The cells are densely packed and appear to be in a fluid medium.

Cell and gene therapy characterisation

Why is cell & gene therapy characterisation important?

Control of the
manufacturing
process

Ensure **quality** and
lot-to-lot
consistency of
the final product

Anticipate
sub-optimal
manufacture runs

Assess **product**
integrity
and **stability**

Towards automated manufacture

Manual

- Established
- Open
- High risk

Automation – Modular

- Reproducibility
- Robustness
- Integration

Automation – Integrated

- Reduce labour
- Containment
- Efficiencies

Automation – Step Change

- High-throughput
- Integrated PAT
- Small footprint

Cost of goods

Industrial realisation

Requirements and challenges for cell product characterisation

biology

- Knowing product characteristics is critical for the development of cell therapies
- Critical Quality Attributes (CQA's): biological aspects of a cell therapy product
 - Potency
 - Mechanism of action (MoA)
 - Product comparability
 - Characterisation, composition
 - Product quality

Wish list

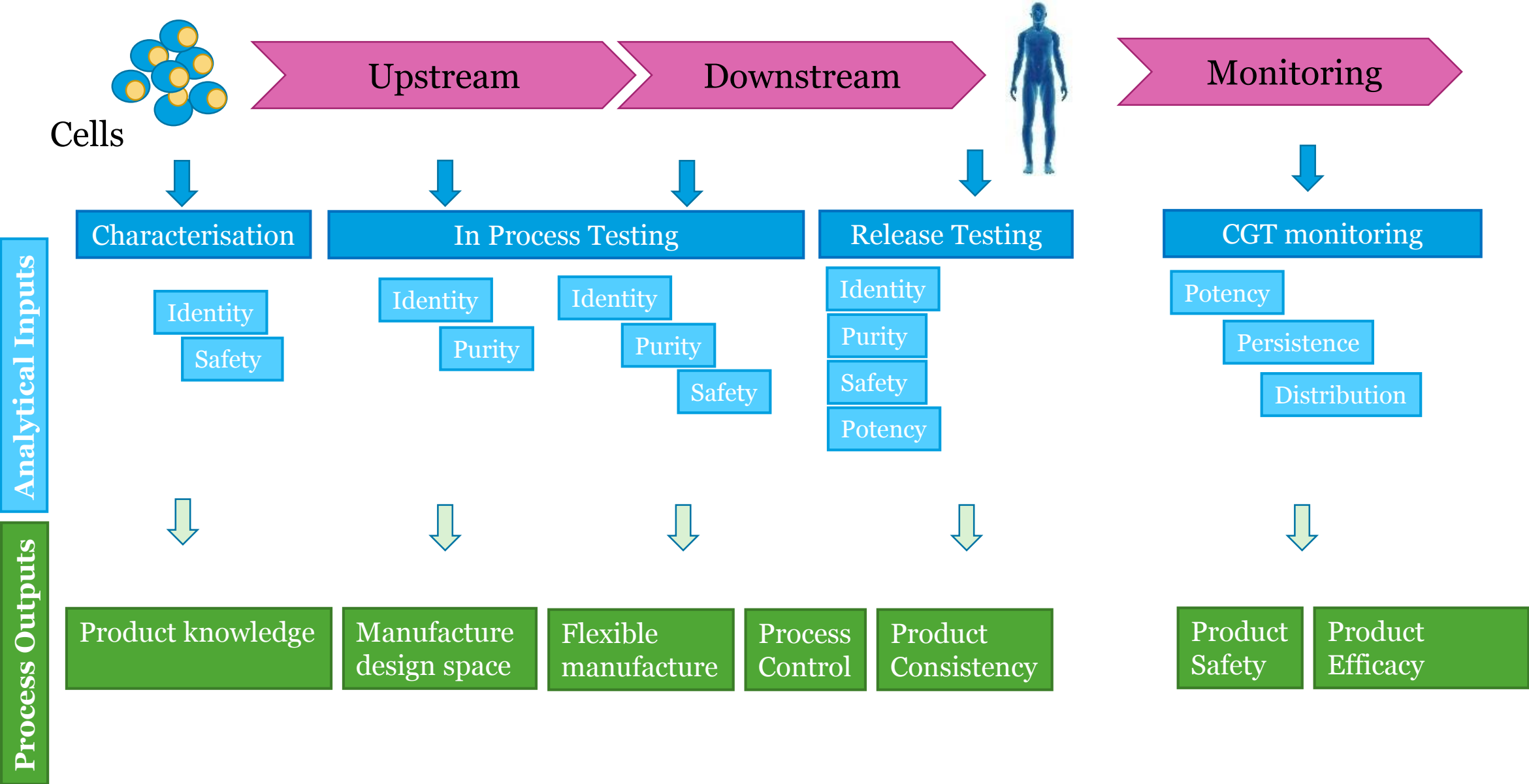
measurements

- CQA's are very difficult to measure during manufacturing
 - They can change during the life cycle of the product
 - They can be difficult to measure directly, surrogate markers offer more flexibility
 - Limited shelf-life at end-point
 - On-\in-\at-line monitoring strategies?

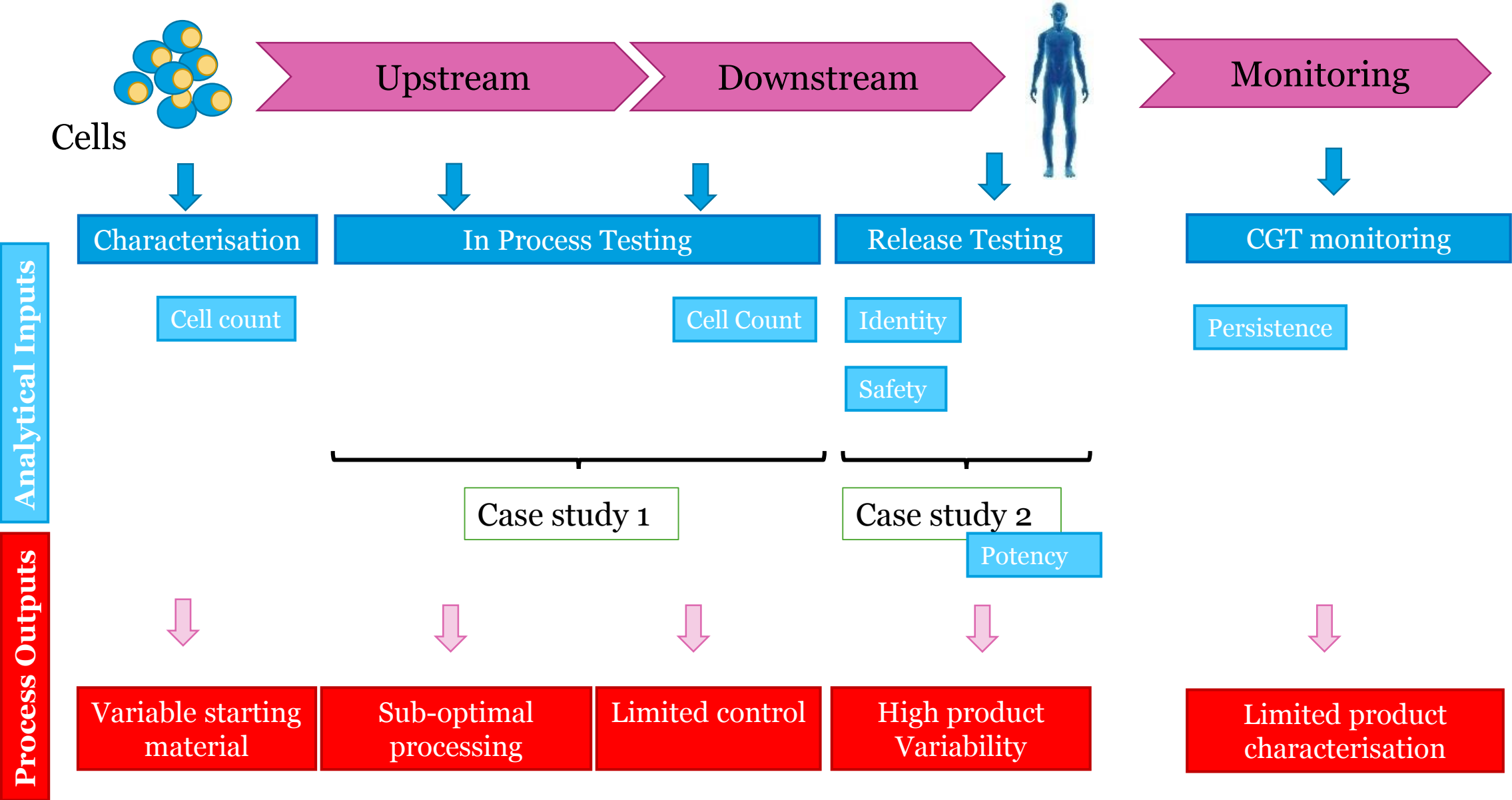
Hurdle

- **What to measure? How to link end-point to in-process features ?**

Ideal scenario



Current status



Case Study 1 **CATAPULT**

Cell and Gene Therapy

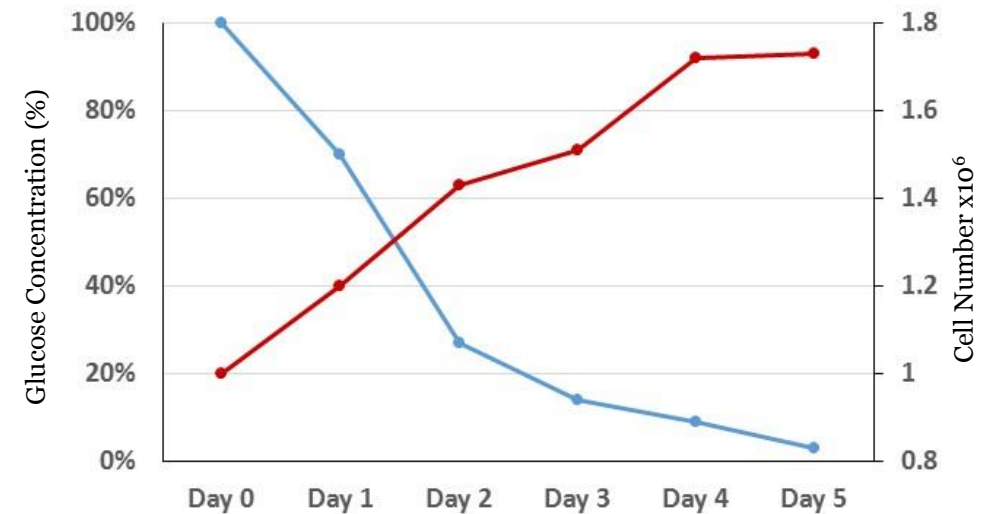
In-process characterisation:

Inferential methodology



Inferential measurements

- **Indirect assessments of a product's critical quality attributes** measured through a surrogate parameter
- Require **direct links** between characteristics to be validated
- **Should support opportunities for real time process adjustments**, maintaining optimal operational conditions and increasing process consistency –
- **Enable real time product release**



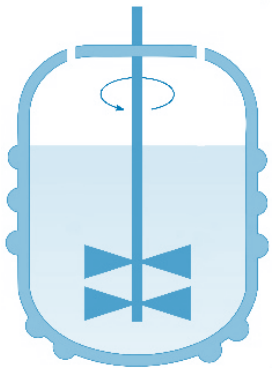
Inferential technologies

	Technology	Measurement
In-line	NIR spectroscopy	Glucose/Glutamine/Lactate/Ammonia VCD/TCD/osmolality
	Raman spectroscopy	Glucose/Glutamine/Lactate/Ammonia VCD/TCD/osmolality
	Fluorescent sensors	pH and DO
	Refractive index	Compositional changes
	multiwavelength Fluorimetry	Amino acids
	Holographic imaging	Cell shape/size, cell viability
	Impedance	Biomass / cell viability
	Turbidimetry	Biomass
On/At-line	HPLC	Media components (amino acids, sugars, proteins, metabolites)
	LC-MS	Media components (amino acids, sugars, proteins, metabolites)
	Coulter counter	Biomass / cell viability
	Imaging	Cell size/shape, cell viability
	Photometric analysers	Glucose/Glutamine/Lactate/Ammonia

How do these technologies fit with cell therapy manufacture?

Sample availability

Stirred Tank Bioreactor



In-line:

pH, DO, biomass (probes)
Metabolites (spectroscopy)
Morphology/viability (In-situ imaging)

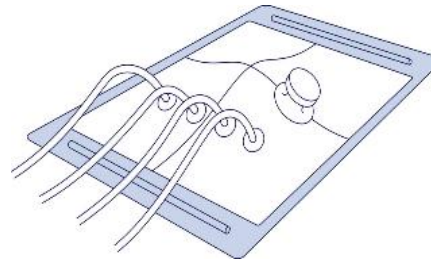
On-line:

Biomass (coulter counter)
Viability (holographic imaging)

At-line:

Metabolites (photometric analysis)
Media components (LCMS/HPLC)

Rocking motion culture



In-line:

pH, DO, (fluorescent sensor)
Biomass (capacitance probe)

At-line:

Metabolites (photometric analysis)
Media components (LCMS/HPLC)

Planer culture



In-line:

pH, DO, (fluorescent sensor)

At-line (during media change):

Metabolites (photometric analysis)
Media components (LCMS/HPLC)

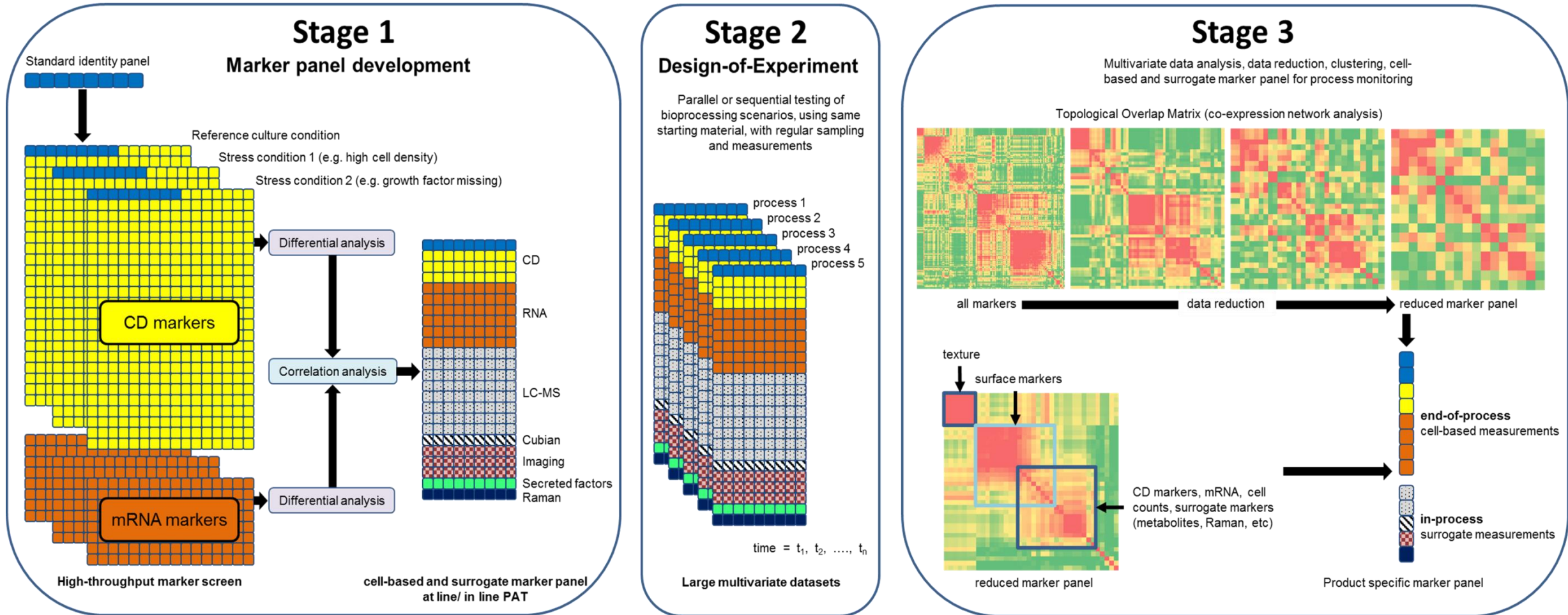
Hollow Fibre Bioreactor



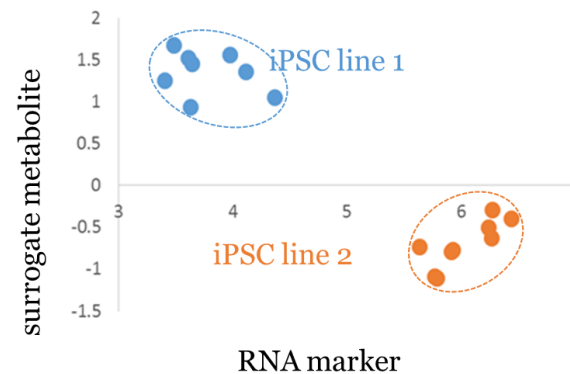
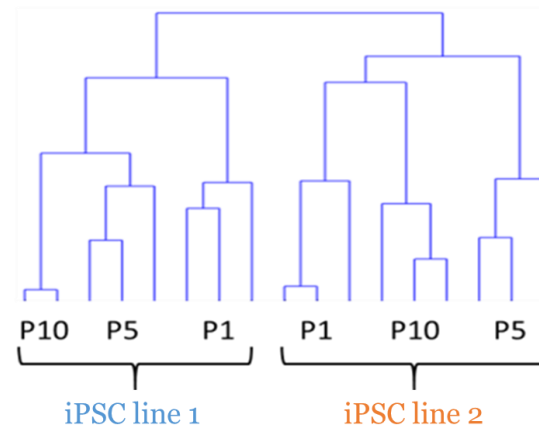
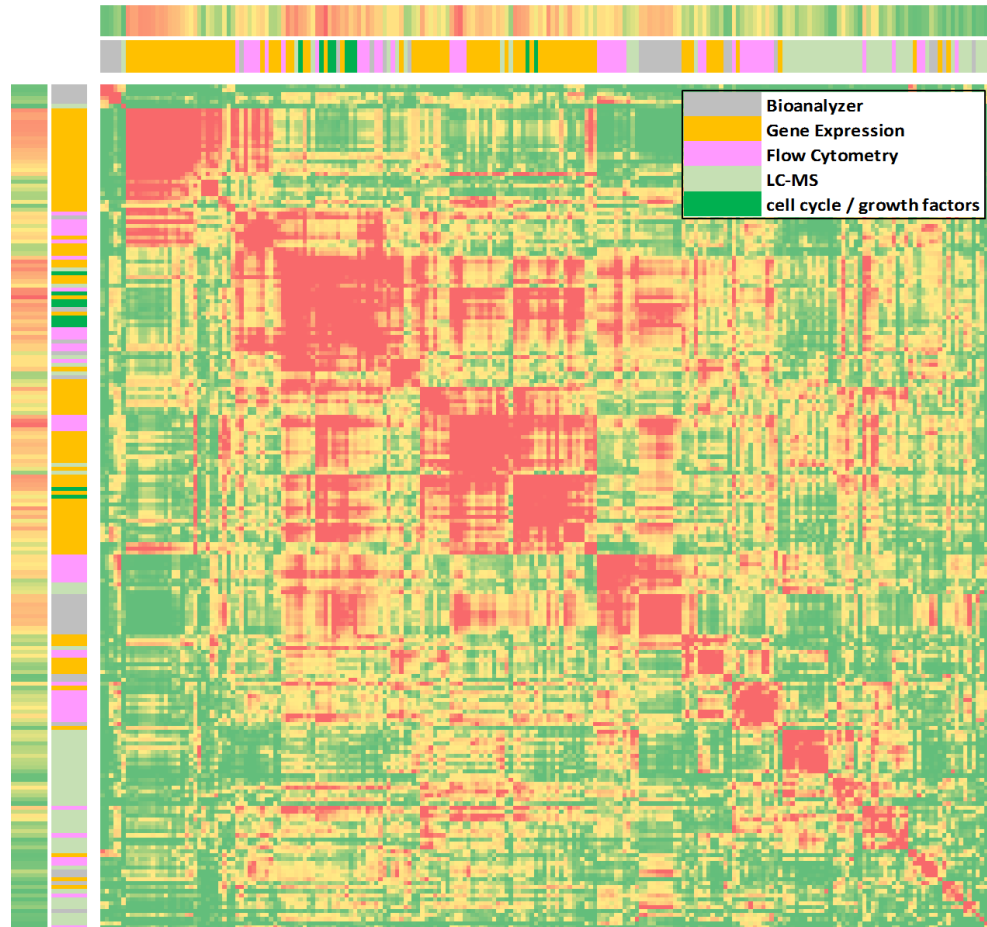
At-line:

Metabolites (photometric analysis)
Media components (LCMS/HPLC)

Connecting PAT to CQA's: CGT strategy for inferential measurements



Fully deployed, this approach is powerful

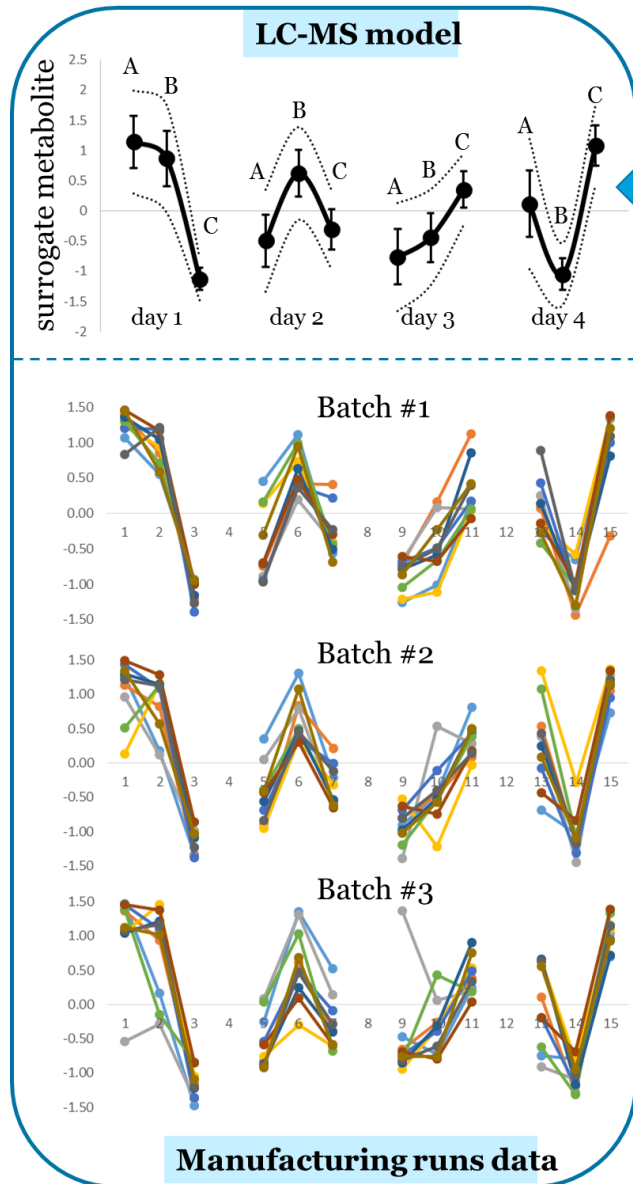


Useful to identify

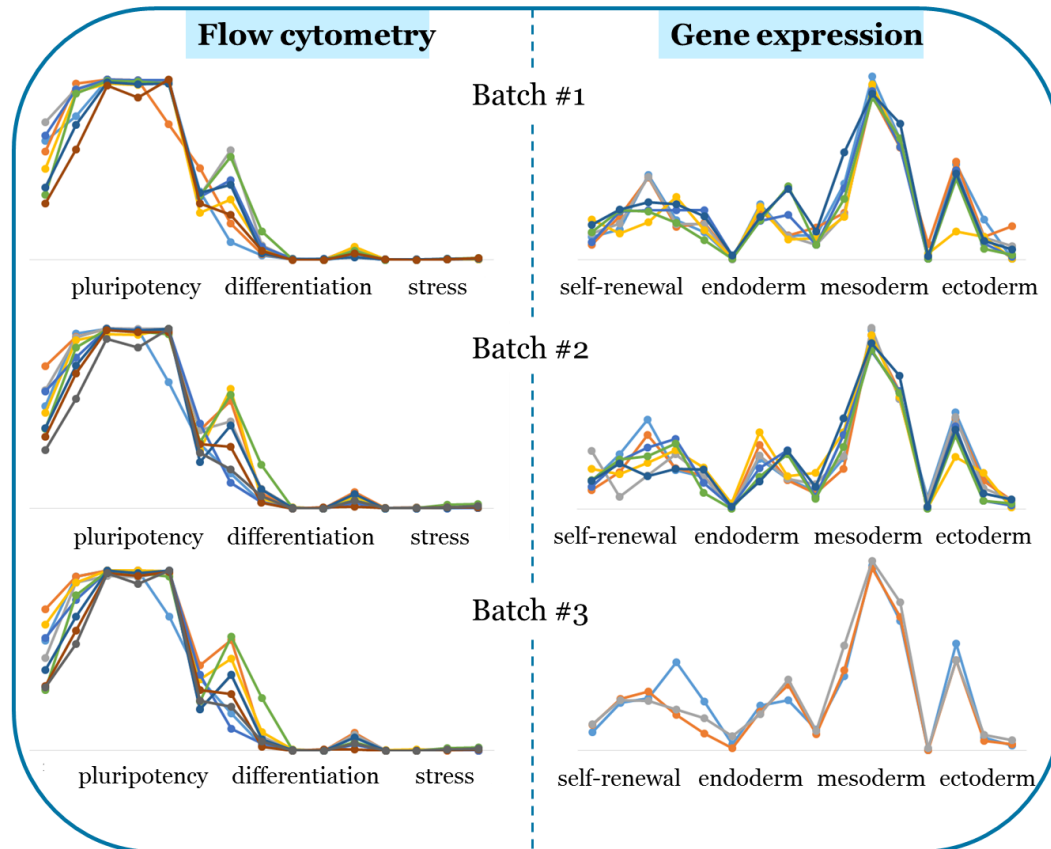
- Identity markers
- Quality markers
- Potency markers
- Process-related markers
- Surrogate markers

The implementation is adaptable to budgeted constraints

Robust inferential markers by LC-MS

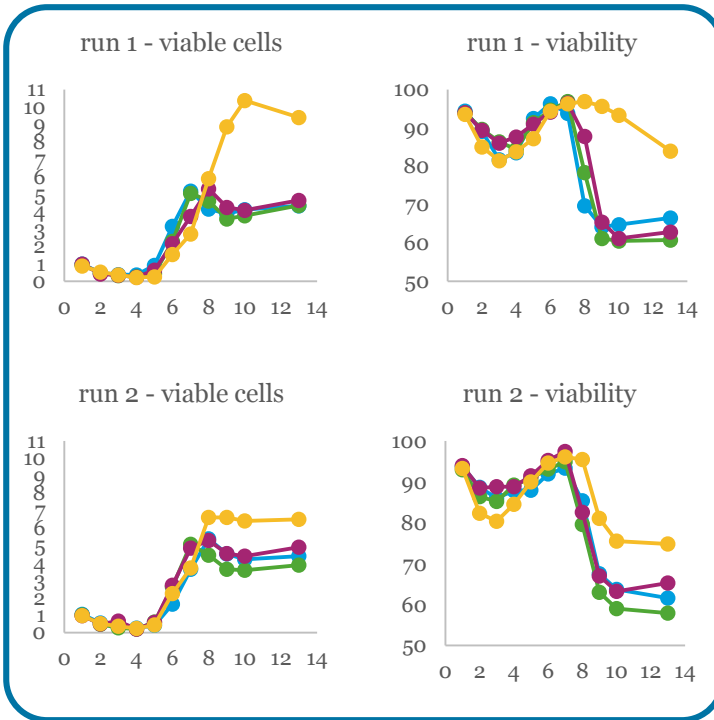


Expected expression levels of 3 actual metabolites (A, B, C) over a 4 day culture cycle. Error bars are measured standard deviations, dashed lines represent the 95% confidence intervals.



Raman spectroscopy for inferential on-line monitoring

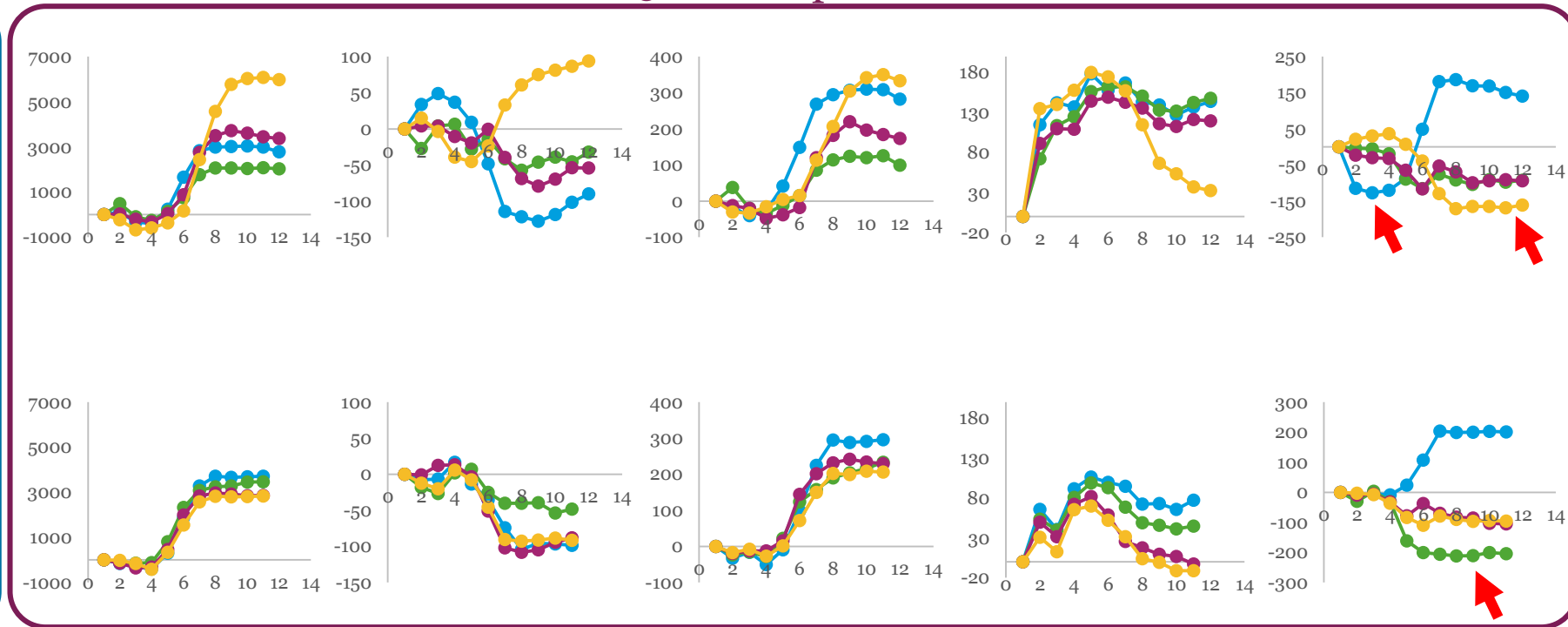
cell counter



cell number

viability

5 Raman peaks



Case Study 2

Potency Assay Development



Potency assay

An assay which measures the clinical biological function of a cell therapy product (mechanism of action known)

FDA and EMA expect potency testing with defined acceptance criteria to be in place before the start of pivotal clinical trials

It is expected that validation of the potency assay will have been completed before submission of a market authorization

Potency assay for a TCR Immunotherapy

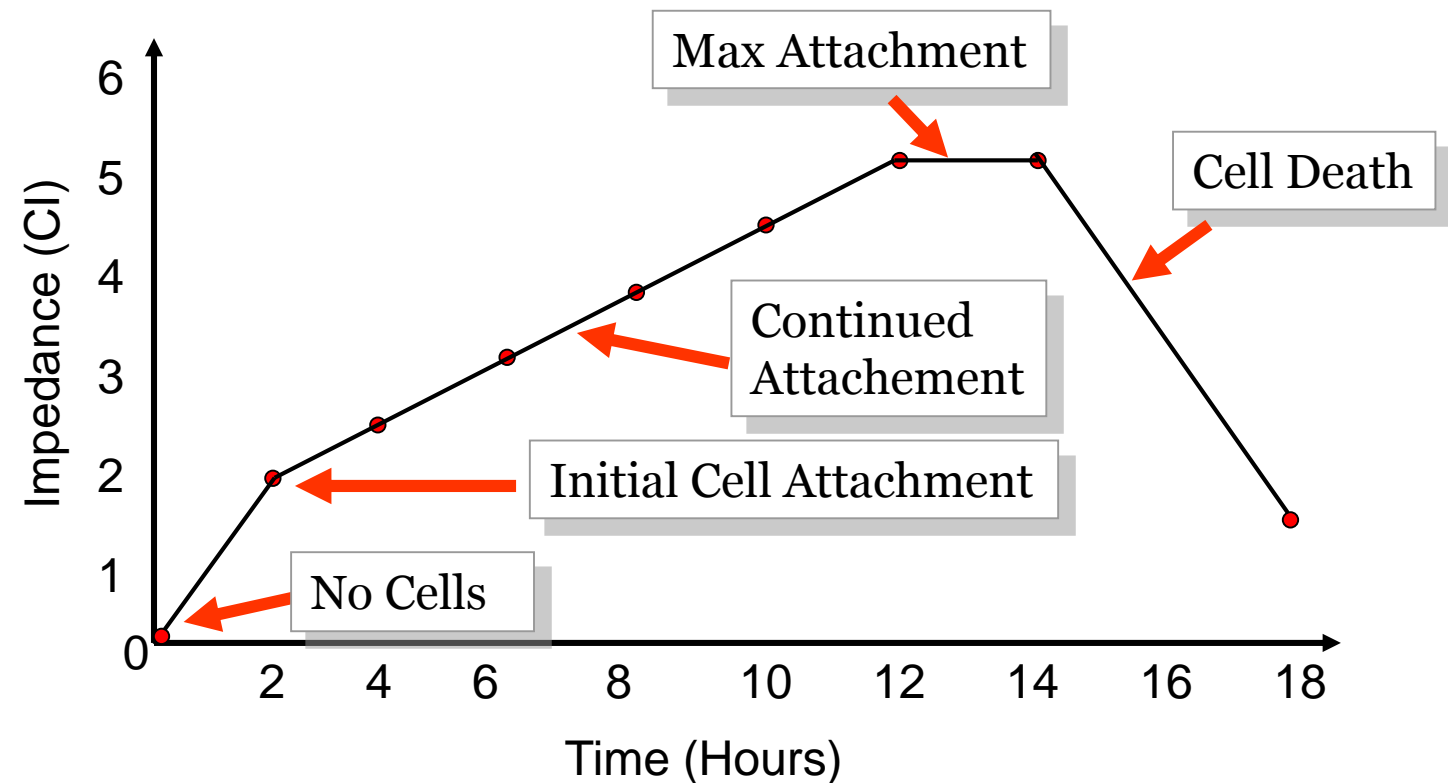
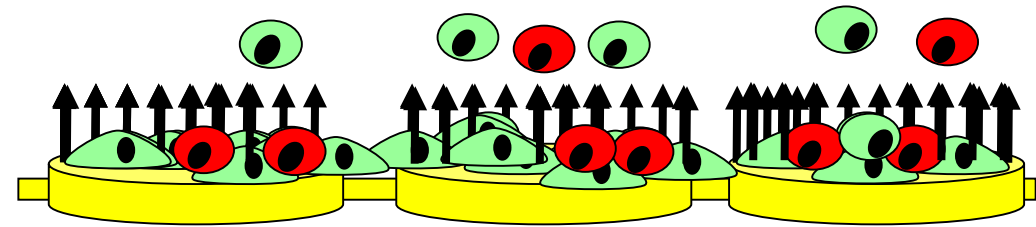
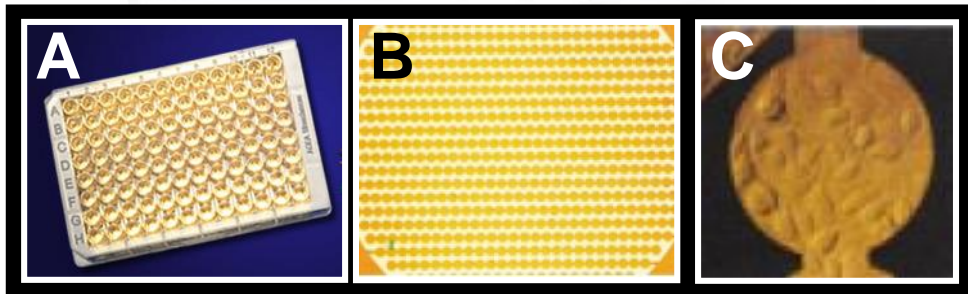
Current T-cell potency assay:

- Surrogate measurement of cell activity
- measure cytokine stimulation in the transduced T-cells in response to target peptide (IFN_γ , TNF_α and IL2)

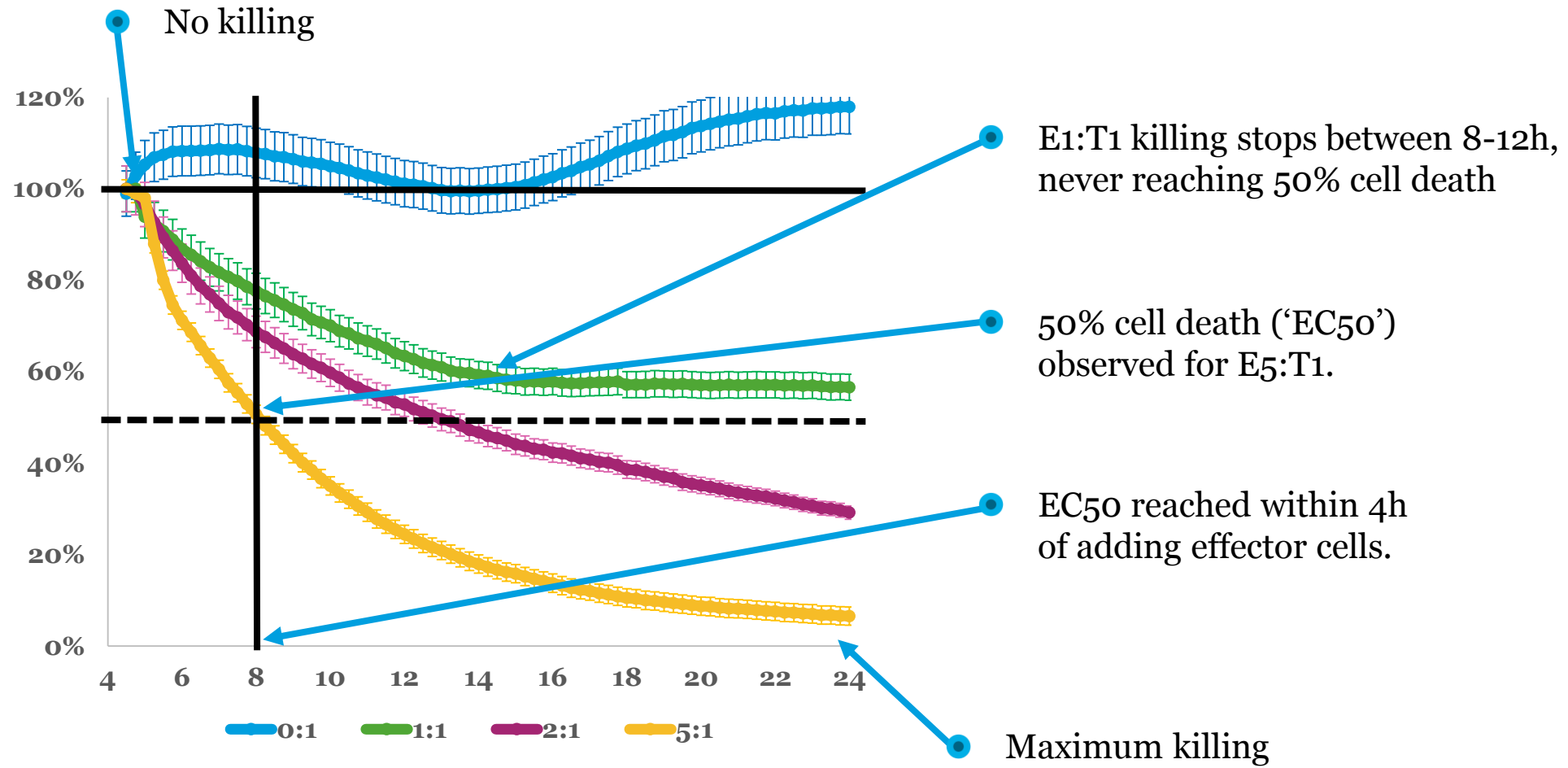
New assay development:

- direct measurement of cell killing by T-cells
- replace commonly used assay for cell killing (Cr_{51} assay)

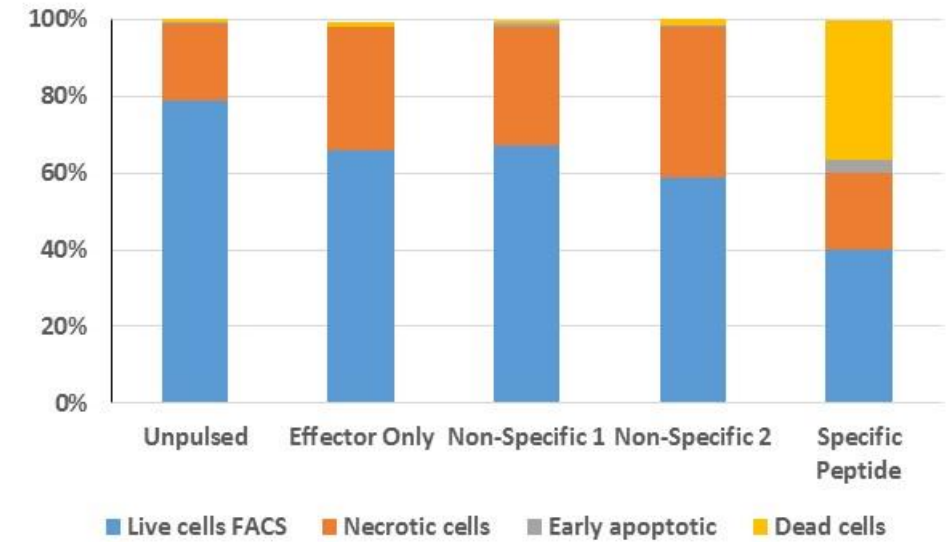
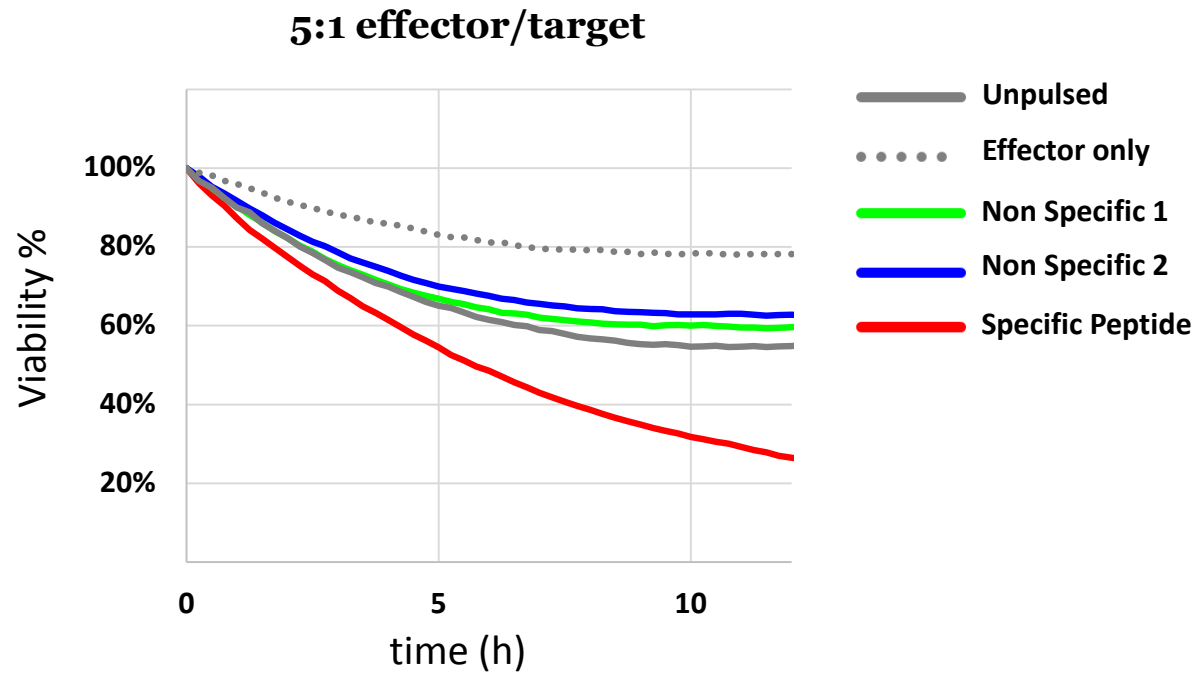
Impedance Spectroscopy based potency assay

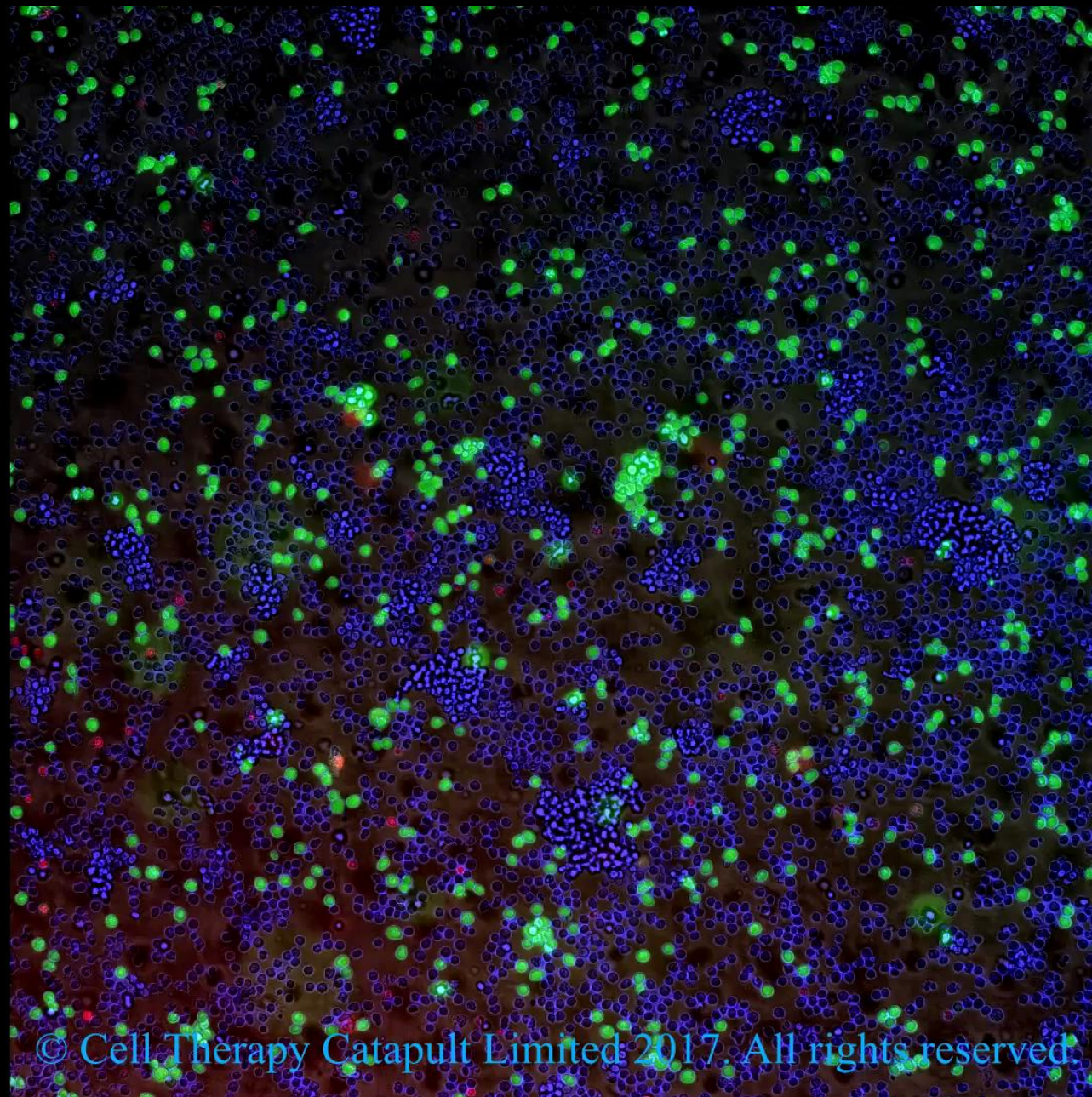


Impedance killing assay



Killing Specificity

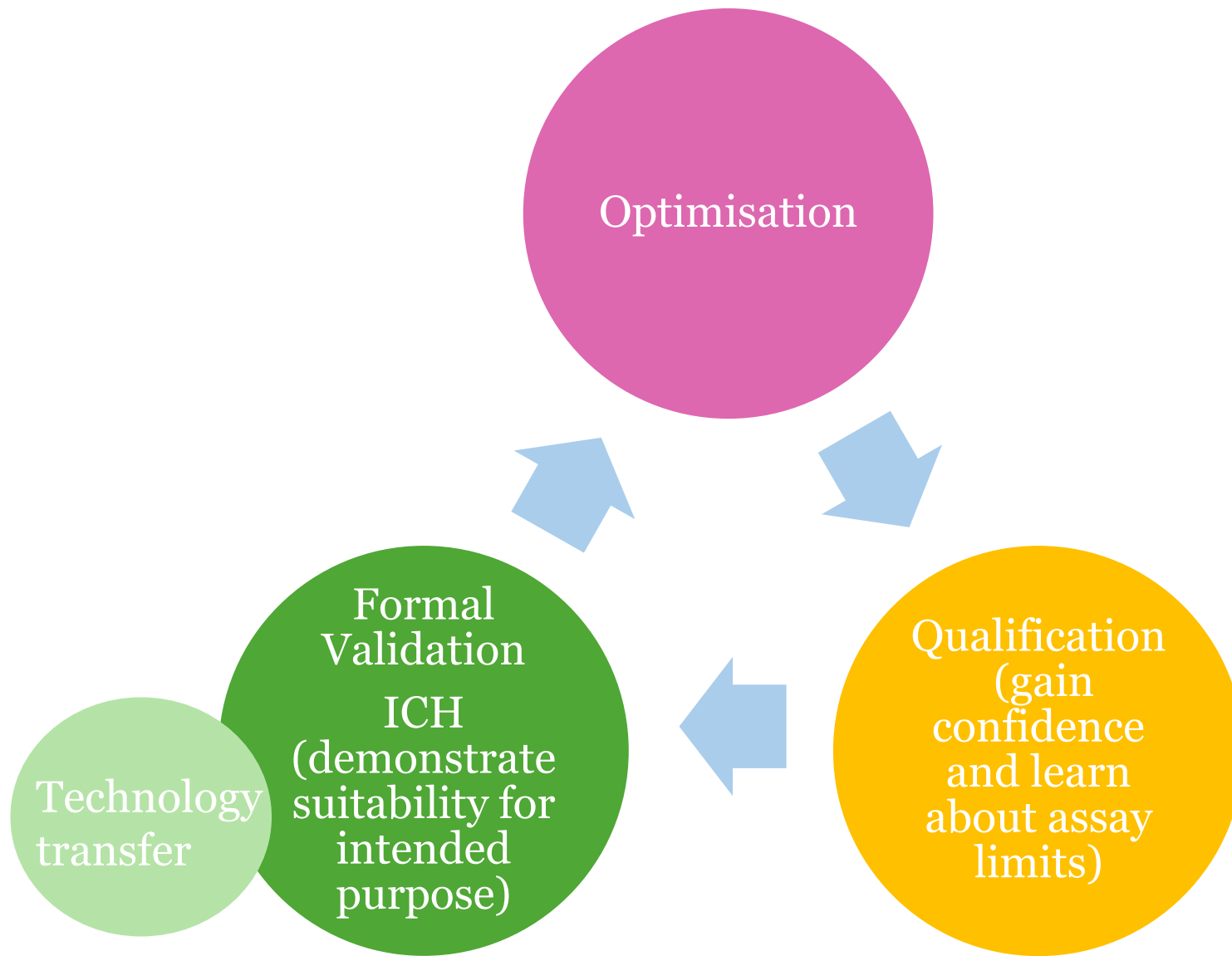




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A microscopic view of numerous cells, likely red blood cells, showing a biconcave disc shape. The cells are densely packed and appear as dark, circular structures against a lighter background.

Summary



Future challenges for cell and gene therapy characterisation:

- Assays/methods flexible to changing processing methods
- Known mechanism of action
- Real-time readout
- Rapid
- Robust (limited operator variability, automated sampling)
- Data integration across platforms

A microscopic view of numerous cells, likely oocytes or follicles, arranged in a grid-like pattern. The cells are circular with a distinct outer boundary and a granular interior. The background is a solid blue color.

Acknowledgements

Damian Marshall

Beata Surmacz-Cordle

Alex Chan

We work with
Innovate UK

CATAPULT
Cell and Gene Therapy