Process Analytical Technology Strategy For Lentiviral Manufacture

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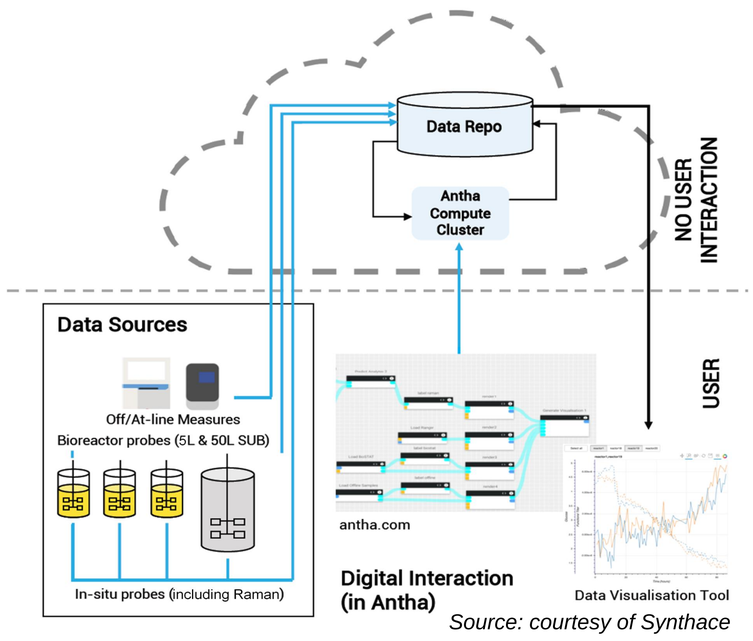
PROCESS ANALYTICAL TECHNOLOGY (PAT) CHALLENGE

Cell and gene therapies (CGT's) are the most complex drugs ever developed. PAT and real-time bioprocessing control will enable scaling up the industrial manufacture of CGT's. Whereas these approaches are well understood in the pharmaceutical industry, intrinsic difficulties currently exist for their practical implementation in CGT manufacture:

- **The process:**
  - Cost
  - Reproducibility and control
  - Identification of Critical Process Parameters (CPP's)
  - Suitable sensors for on-line monitoring
  - Throughput for sampling
- **The product**:
  - Identification and measurement of Critical Quality Attributes (CQA's)
  - Analytics for product characterisation
  - Dynamic change of biological system during manufacture
- **The data:**
  - Multivariate data types, fragmentation, alignment
  - Metrology and data quality
  - Modelling and interpretation
  - Integration: hardware, software, datasets.

This **Innovate UK f**unded project tested a PAT strategy to capture, analyse, interpret multivariate omics datasets, identify markers from which a dynamic biochemical fingerprint was derived and used for real-time monitoring using Raman spectroscopy during lentiviral manufacture using the **Oxford Biomedica** platform process.

DATA CAPTURE, STRUCTURE, INTEGRATION



Source: courtesy of Synthace

***Figure 1** – Complex, small and large datasets generated during on-, at- and off-line measurements, from various sites, were automatically structured within a purpose-built **Antha platform (Synthace)**. This provided efficient remote data exploration capability and retrieval of relevant datasets for data analysis and model building.*

EXPERIMENTAL DESIGN

- Lentiviral manufacturing runs (4 bioreactors per runs) were performed by Oxford Biomedica in 5L bioreactors.
- Extensive sampling was performed throughout to conduct metabolomics and transcriptomics characterisation of the producer cell line.
- Raman spectra were continuously acquired during bioprocessing.
- Metabolomics (LC-MS, 252 metabolites) and full transcriptomics (RNAseq, 29,319 markers) profiling were performed on all samples (*Fig. 2*).
- An automated Partial Least Squares (PLS) modelling algorithms was designed to test the ability of Raman spectroscopy to model metabolites (>50 metabolites could be modelled).
- Pathway analysis was performed to assess the relevance of shortlisted biomarkers.
- Chemometric models for infectious viral titre using Raman spectra were developed.
- A proof-of-concept run for real-time monitoring using *in situ* Raman spectroscopy in 5L and 50L bioreactors was performed several months later.

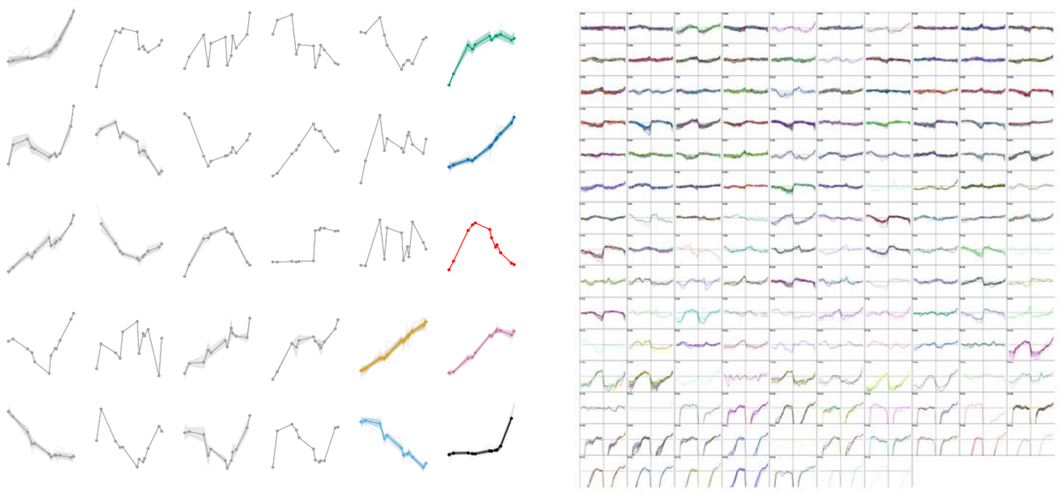


***Figure 2** – Example of metabolomics (left) and transcriptomics (right) clusters analysis. Data reduction techniques and ranking techniques were used to identify potentially important networks and biomarkers. Pathway analysis was used to assess the biological significance and correlation of selected potential markers of viral vector bioprocesses.*

DATA ANALYSIS, MARKER SELECTION

- In-process and off-line data were structured within the **Antha platform** (*Fig. 1*) and made remotely available for further processing.
- Principal Component Analysis, random forests, and network analysis (*Fig. 3, top left*) were used to identify potential metabolomic markers in significant pathways.
- Self-Organizing Map (SOM's, *Fig. 3, top right*) neural networks were used to identify key inflexion timepoints in the process, to narrow down the multi-omics analysis.
- Regularized Canonical Correlation Analysis (CCA, *Fig. 3, bottom right*) and pathway analysis (*Fig. 3, bottom left*) were used to integrate metabolic and gene expression datasets, and to identify a list of key markers used as a dynamic process fingerprint.
- This multivariate analysis approach identified a list of markers and patterns acting as a fingerprint to the process.
- The ability to model these biochemical patterns using Raman spectroscopy supported the concept of further developing chemometric Raman models for viral titre monitoring.
- Chemometric models of viral titres were tested in 5L and 50L bioreactors in a proof of concept study, and models subsequently refined.

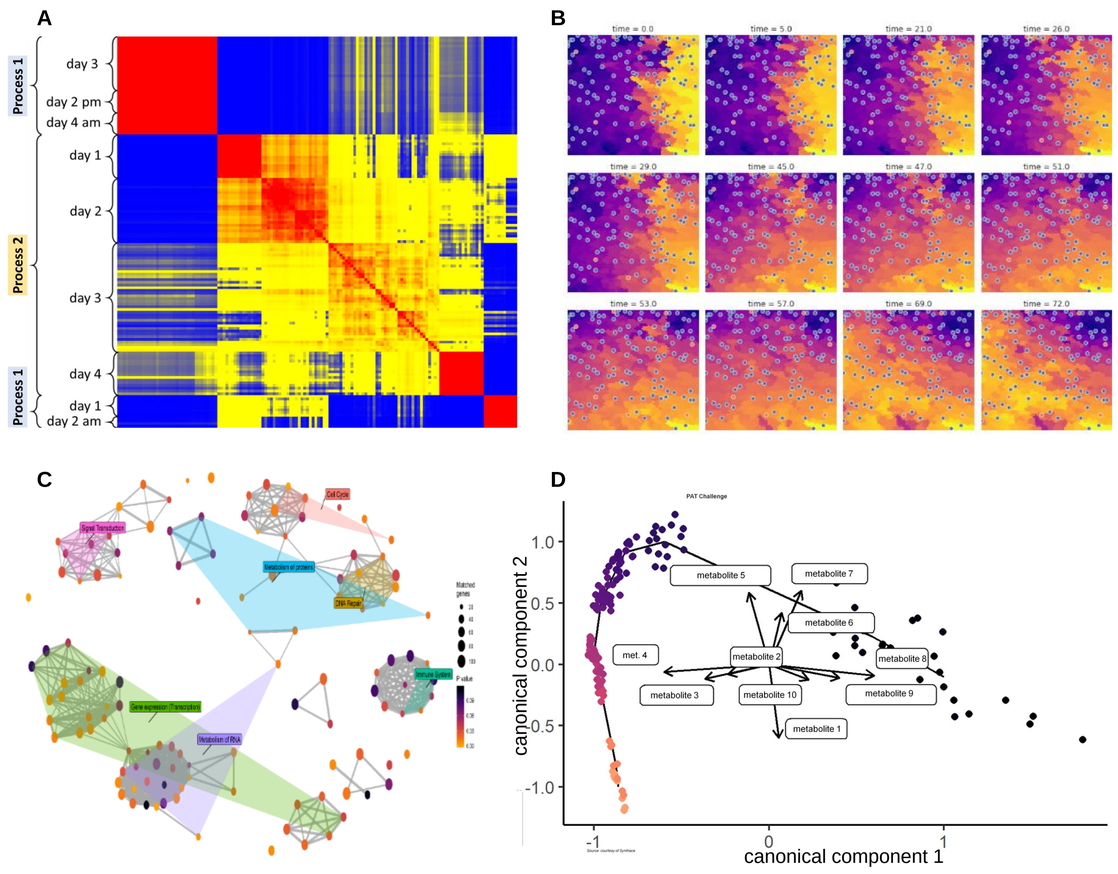


***Figure 3** – Data analysis methods. A) Network analysis, to quantify process consistency, identify potential pathways, and rank analytes. B) Metabolic SOM's of, over time, pattern changes indicate physiological inflexion points during the bioprocess. C) Pathway analysis of selected metabolic and gene networks. D) CCA with main driver metabolites.*

REAL-TIME MONITORING OF LENTIVIRAL MANUFACTURE

The data-driven strategy applied during this study supported the development of a Raman spectroscopy model for real-time viral titres during LV manufacture. Raman models proved robust in both monitoring conventional analytes as well as tracking LV titres in real-time, at 5L and 50L scale (*Fig. 4*).

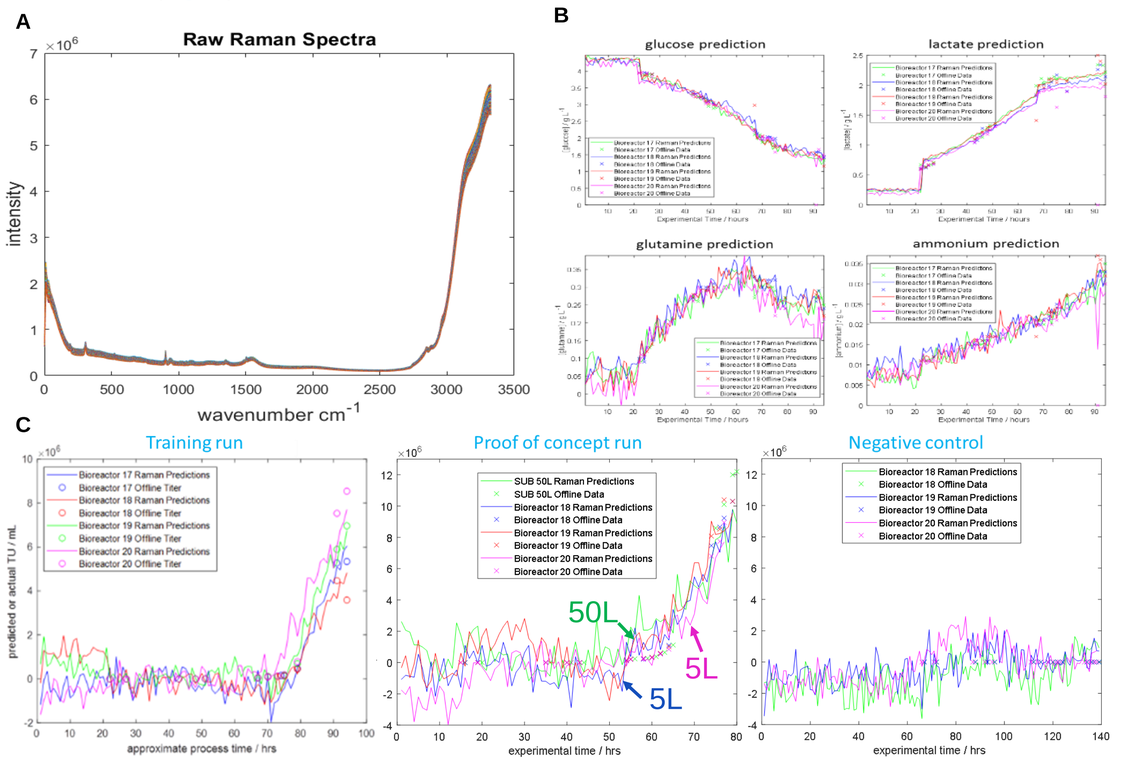


***Figure 4** – Raman spectroscopy as a PAT for chemometric modelling of lentiviral production. A) Example of whole series Raman spectra from 1 bioreactor run. B) Real-time metabolite prediction using Raman spectroscopy. C) Refined chemometric Raman models for viral titres applied to training run data, proof-of-concept run several months later (including 3x 5L and 1x 50L bioreactors) and negative control runs. The ability of this model to track viral titre is demonstrated. Expanding the number of runs would help further generalize and validate this approach.*

CONCLUSION

This approach demonstrates our PAT strategy as an exciting tool to monitor and interrogate complex manufacturing processes, providing greater mechanistic and biological understanding in relation to the bioprocess environment. It could be used to monitor, in real-time, the effects of process and pathway manipulation, and allow the design of "digital twins" which in turn could dramatically shorten the development pipeline for high quality lentiviral products.

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