# Analysis of a mammalian cell culture

### Benefits at a glance

- Real-time, in-process understanding in accordance with the U.S. FDA Process Analytical Technology (PAT) initiative
- Specificity of technology as an enabler for quantitative monitoring of multiple components using a single probe
- Quantification of glucose, lactate, and glutamine
- Raman as a proven Process Analytical Technology (PAT)
- Real-time process and product quality assurance

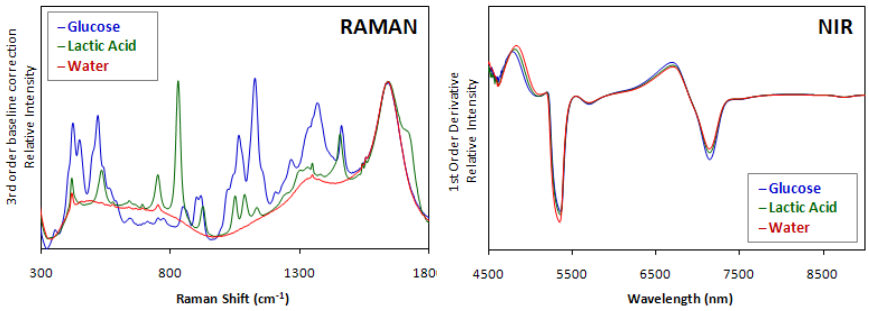


Figure 1: Raman provides identification of glucose and lactate during a CHO cell culture. Raman's specificity enables easy cross-site and cross-scale model transfer.

## Introduction

Production of protein therapeutics by mammalian cells is the most widely used bioprocess because of its ability to properly produce and fold a recombinant protein, with 60-70% of biopharmaceuticals using this bioprocess.[1,2] Since 1986, an increased understanding of cell biology, gene transfer mechanisms, media composition, and process control have resulted in significant improvements to cell viability and titer.[2]

Most cell culture bioprocesses use Chinese hamster ovary (CHO) cells, fed by glucose. CHO cells are typically fed in a batch (known as fed-batch), where glucose is delivered into the bioreactor as a large bolus at set time points. The time points are based on a priori process knowledge and off-line analysis. However, this approach is not ideal for several reasons. It is labor and resource intensive, increases the risk for contamination, and does not adequately control glucose and lactate in a cell culture bioreactor. Non-invasive, real-time PAT measurements combined with Proportional-Integral-Derivative (PID) or closed-loop feedback control can optimize feeding strategies, improving yield and titer.[3]

## Raman advantages

The work described here involves the development and application of Raman-based in-process analyses in accordance with the aims of Quality by Design (QbD) and the the U.S. FDA's PAT and QbD initiatives.

Raman spectroscopy and near-infrared (NIR) can provide characteristic "fingerprint"-like spectra of organic and biological molecules so that particular peaks can be selected for use in quantitative analysis. Raman and NIR are used in bioprocesess because they can acquire data in a short time, require little or no sample preparation, and can quantify multiple components with a single *in situ* probe.

The choice between Raman and NIR, then, often comes down to specificity.[4] Figure 1 shows Raman and NIR spectra of glucose and lactic acid in water, overlaid with a pure water spectrum. Raman spectra show high chemical specificity with little interference from water, whereas water dominates the NIR spectra.

ⓘ All Raman analyzers and probes referenced in this application note are Endress+Hauser products powered by Kaiser Raman technology.

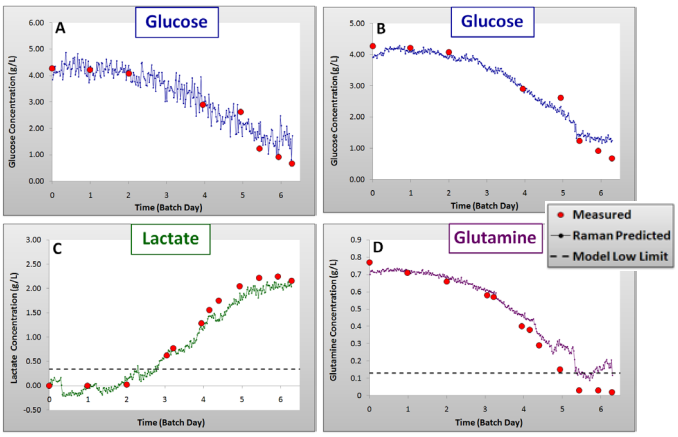


Figure 2: Raman provides quantification on process components similar to off-line laboratory measurement, but in real-time and in-process.

## Experimental

The goal of this study was to understand the specificity of Raman to critical process parameters of a CHO cell culture process. Aqueous laboratory samples of glucose, lactic acid, and glutamine were prepared as standard solutions. Four CHO cell culture processes were performed in 2L bioreactors, seeded with $2x10^5$ cells/mL density and run under conditions mimicking industrial fed-batch processes. In both sets of experiments, a Raman analyzer (λ=785 nm) equipped with a bioprocess immersion probe was used to perform in-process real-time measurements. In-process Raman measurements were correlated to offline biochemical measurements of grab samples using a BioProfile® Basic 100. In-process Raman data were examined by visual inspection, univariate modeling of glucose concentration, and multivariate partial least squares (PLS) modeling of glucose, lactate, and glutamate. Reference measurements collected during batches 1–3 for each component were correlated to larger regions of the Raman spectra using PLS (one model per constituent). Univariate and PLS models were then used to predict batch 4 (validation batch) concentrations.

## Results

The calibration model developed using batches 1–3 was very effective in predicting the component concentrations in batch 4, with $R^2$ values of 0.99 for each component. Figure 2 shows the time trend of Raman-predicted values for batch 4.

## Conclusions

This study demonstrates that Raman provides data similar to the traditional offline biochemical measurements. Raman has several important advantages over offline measurements that include:

- Monitors multiple attributes with a single *in situ* probe around the clock
- Ensures process consistency
- Reduces risk of contamination
- Enables advanced process control strategies

Additional studies have further demonstrated feasibility at the pilot and manufacturing scales.[3] Together, these studies clearly show the advantages of Raman in upstream cell culture monitoring and control.

## References


1. Wurm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat Biotechnol*. **2004**; *22(11)*:1393–8.
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3. Berry BN, et. al. Quick generation of Raman spectroscopy based in-process glucose control to influence biopharmaceutical protein product quality during mammalian cell culture. *Biotechnol Prog*. **2016**; *32(1)*:224–34.
4. Kozma, B. et. al. On-Line Prediction of the Glucose Concentration of CHO Cell Cultivations by NIR and Raman Spectroscopy: Comparative Scalability Test with a Shake Flask Model System. J. Pharma. *Biomed. Anal*. **2017**; *145*: 346–55.