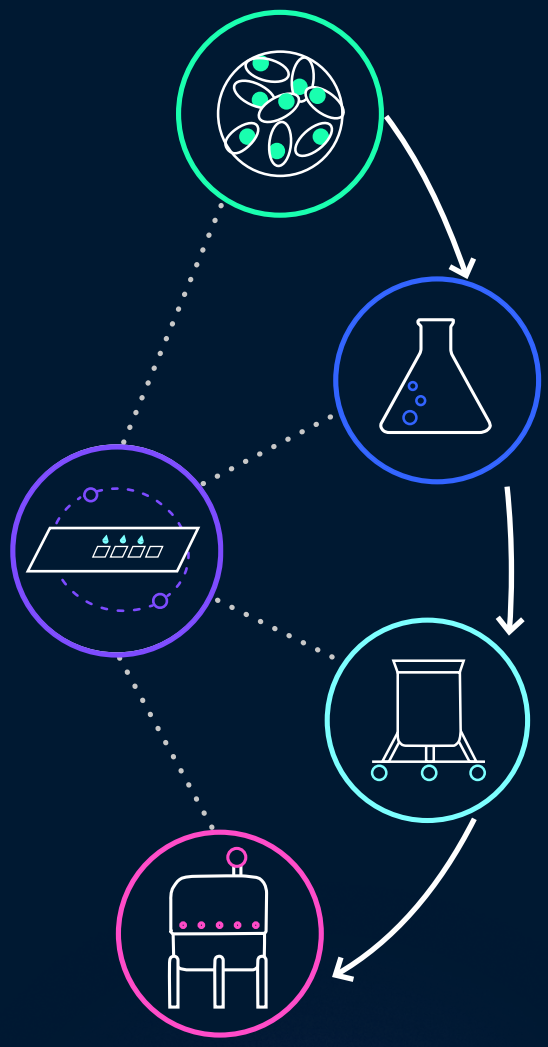
DXCOVER

Upstream bioprocess monitoring using next generation ATR-FTIR spectroscopy



Bioprocessing is a method of manufacturing biological products, often at a large scale. However, the high cost of production and susceptibility to process failure means that the process can be high risk. Process analytics are key to ensuring high quality product is produced at all stages of development. Here we show how our next generation attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy platform may shed light on product quality during upstream bioprocessing.

INTRODUCTION

Bioprocessing describes the way in which biological products can be manufactured from living organisms and is a key process in the pharmaceutical industry. Upstream bioprocessing refers to the cultivation of the target organism at large volumes, often exceeding 10,000 litres. This is a multi-step process that requires significant resource and expense to complete. Growing a confluent cell line in 10,000 litre bioreactors can take up to 4 weeks and requires significant investment of over £3m. A major risk is that the product yield is insufficient, or that the product is compromised due to organism health or infection.

Current process analytical technologies (PAT) focus on the measurement of a limited number of performance indicators, including glucose and lactate levels within the growth medium. Whilst this can infer information indirectly on cell health, via monitoring cell growth metabolites, there remains no rapid, low-cost analysis that can be conducted alongside cell growth.

ATR-FTIR spectroscopy is an analytical technique capable of deducing subtle biological changes in samples without the need for extensive sample preparation or expensive reagents [1]. Advancements in traditional instrumentation now allows high-throughput, automated analysis, that is well suited to at- or on-line systems [2].

AIM

Here we show how novel hardware, coupled with computational analysis, can detect differences in cell health and infection, rapidly and accurately using a fingerprinting approach. This presents a novel monitoring process that can observe cell growth and health during upstream bioprocessing.

MATERIALS

In this study, we investigated the spectral differences between Chinese Hamster Ovary (CHO) cells. CHO cells are a common organism that can be genetically modified to express a range of therapeutic proteins, such as monoclonal antibodies.

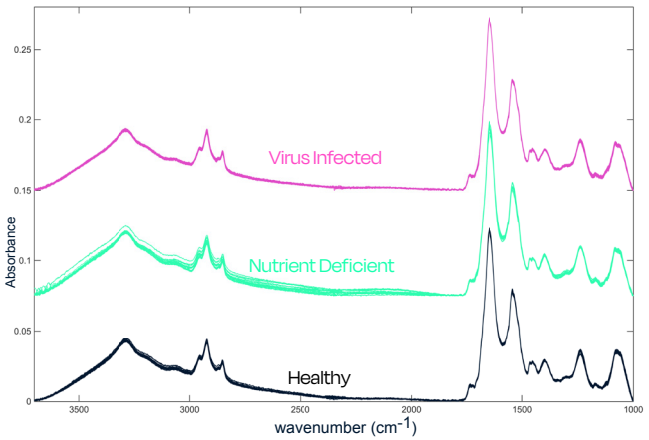


Figure 1. Spectra obtained from Healthy, Nutrient Deficient, and Viral Infected CHO Cells.

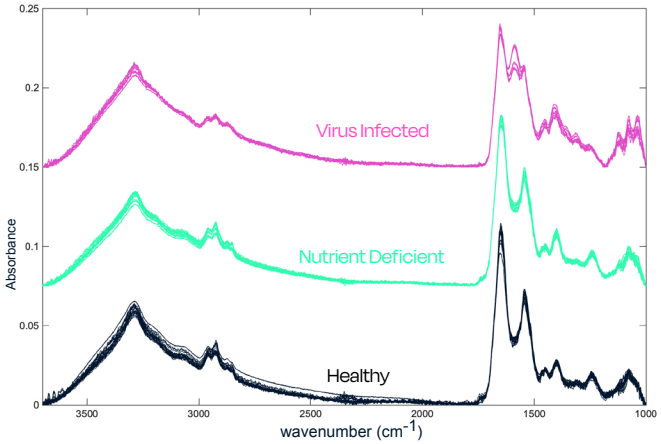


Figure 2. Spectra obtained from Healthy, Nutrient Deficient, Viral Infected CHO Cell Media.

Cells were either grown in optimum conditions ('Healthy'), allowed to grow for longer than optimum ('Nutrient Deficient'), or infected with reovirus 3 ('Virus Infected'). Triplicate CHO cell samples were frozen in these states. Samples were thawed at 37 °C, and then centrifuged at 1000 rpm for 1 minute. Both the supernatant and the cell pellet were analysed in this study.

Samples were prepared on Dxcover® Sample Slides. Three microlitres of the cell pellet was immediately deposited onto the surface of a Sample Slide well and allowed to dry. The slides have three sample wells, and a background well. All slides were prepared in batch for rapid analysis.

Slides were analysed on the Dxcover® Autosampler, which automates the movement of the slide across the infrared beam of the Perkin Elmer Spectrum Two spectrometer. Spectra were cut to the desired wavenumber region, baseline corrected and normalised.

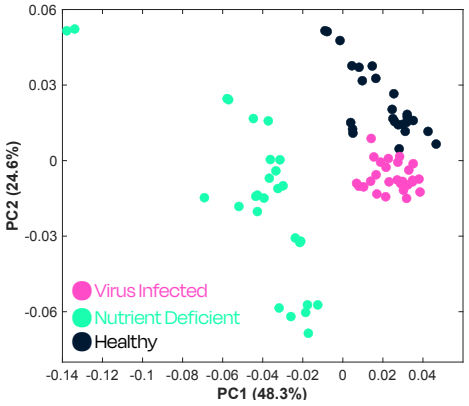


Figure 3. Principal component analysis of CHO cell samples

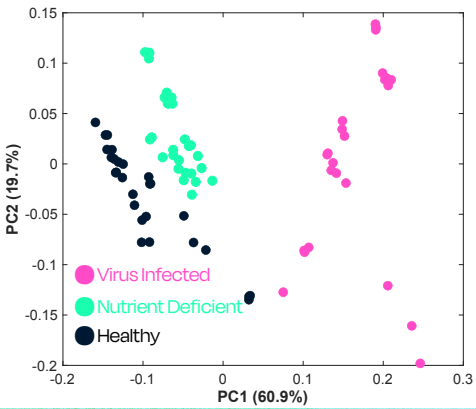


Figure 4. Principal component analysis of CHO cell media samples

RESULTS: CELLS

Comparison of the spectra can highlight spectral differences, that can be associated with biological components of the sample (Fig 1). In this example there are subtle differences in the carbohydrate components of the spectrum (< 1200 cm^{-1}), as well as protein differences around the Amide I peak (1650 cm^{-1}). It is noticeable that nutrient deficient cells have more variable spectra.

By transforming the data into 'principal components' (PCs) – It becomes clear that spectra from the same cell classes cluster together, whereas they can be well separated from each other (Fig 3). Over 60% of the variance is described in PC1, which separates healthy cells from nutrient deficient and virus infected cells. PC2 also describes the difference between healthy and the virus infected plus nutrient deficient cells.

RESULTS: CELL MEDIA

It is also possible to sample from the supernatant, largely containing the cell media and cell by-products. Again, some differences can be discerned from the raw spectra, with significant protein differences visible in the virus infected sample (Fig 2). Using PCA, samples can be clearly differentiated based on their spectra (Fig 4). Negative in PC1 described the virally infected cells, which describes differences in the Amide protein region. Negative in PC2 describes the healthy samples and highlights carbohydrate regions of the spectrum as important.

CONCLUSION

Both direct cell measurements and indirect measurements of the cell media are able to identify distinct differences in cell health. Both nutrient deficiency and viral infection display spectral variance from healthy CHO cells. A classification approach showed high levels of accuracy and could be expanded and implemented into an automated system to detect cell health. At-line analysis of cells is promising, as is the potential of on-line monitoring, particularly of cell media. This would allow the user to monitor the high value product without impacting cell viability.

To find out more about the Dxcover Infrared Platform, speak to one of our representatives or info@dxcover.com

References: [1] Baker et al. 2014 *Nat. Protoc.* 9**(8)**:1771-1791, [2] Butler et al. 2019 *Nat. Comms.* 10**(1)**: 1-9