PROCESS ANALYTICAL TECHNOLOGY BY TIMEGATED® RAMAN

Seeing the Unseen in Fermentation

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Biotechnology plays a key driving force in economies worldwide. From simple substances like ethanol to antibiotics and vaccines like the most advanced mRNA or DNA based therapeutics all fall under the same umbrella of biotechnological products. A wide range of organisms and environments are being employed in a plethora of industries including food science and medicine.

The quantification of multiple components is of interest in process monitoring of bioprocesses due to the abundant physiological biomarkers. The maintaining of the optimum conditions of the contrived cell culture media is an integral part for the final product to have the required primary, secondary, tertiary, or quaternary structure as required for therapeutic purposes. In light of this, monitoring the media becomes pertinent at every stage of the bioprocess which has inherent physical, chemical and molecular complexity. Currently LC-MS (liquid chromatography-mass spectrometry) is often employed for analyzing the consistent quality of the cell culture media. Hence there is still an unmet need for non-destructive online monitoring of cell culture media during bioprocess. Spectrometric techniques such as fluorescence, infrared and Raman spectroscopy together with chemometric and statistical data analyses are being explored.

Raman spectroscopy is much suited for aqueous solutions compared to other techniques due to fast and robust acquisition and extraction of both qualitative and quantitative information of the sample non-destructively. Yet fluorescence has posed a challenge for samples with native fluorescent moieties. This impediment is resolved by Timegate Instruments' patented technology for recording Raman spectra before the advent of fluorescence. When there is a time difference between the Raman photons and the other interfering photoluminescence including fluorescence, time-gating would enable to resolve the Raman scattered photons (figure 1). With other photoluminescence effects rejected, Raman spectroscopy is no more a dark room technique. This technology provides a plethora of information without the hampering effect of photoluminescence in the analysed samples.

To exemplify the unique capability of time-gated Raman spectroscopy, in this study we have measured the fermentation process of glucose with yeast, a simple representative biotechnology process, simultaneously with continuous wave Raman and time-gated Raman spectroscopy.

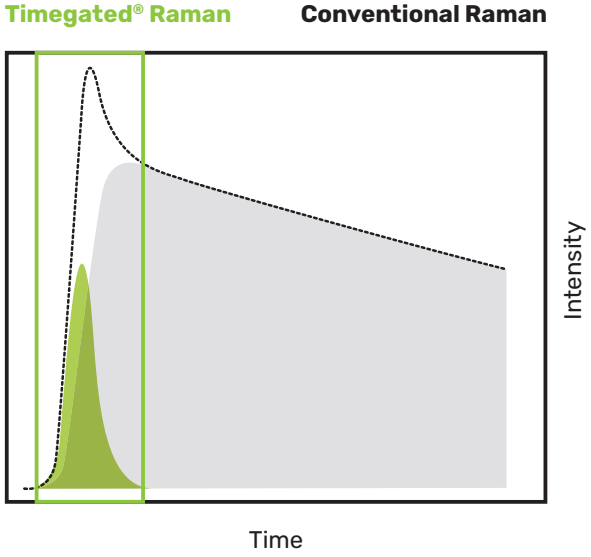


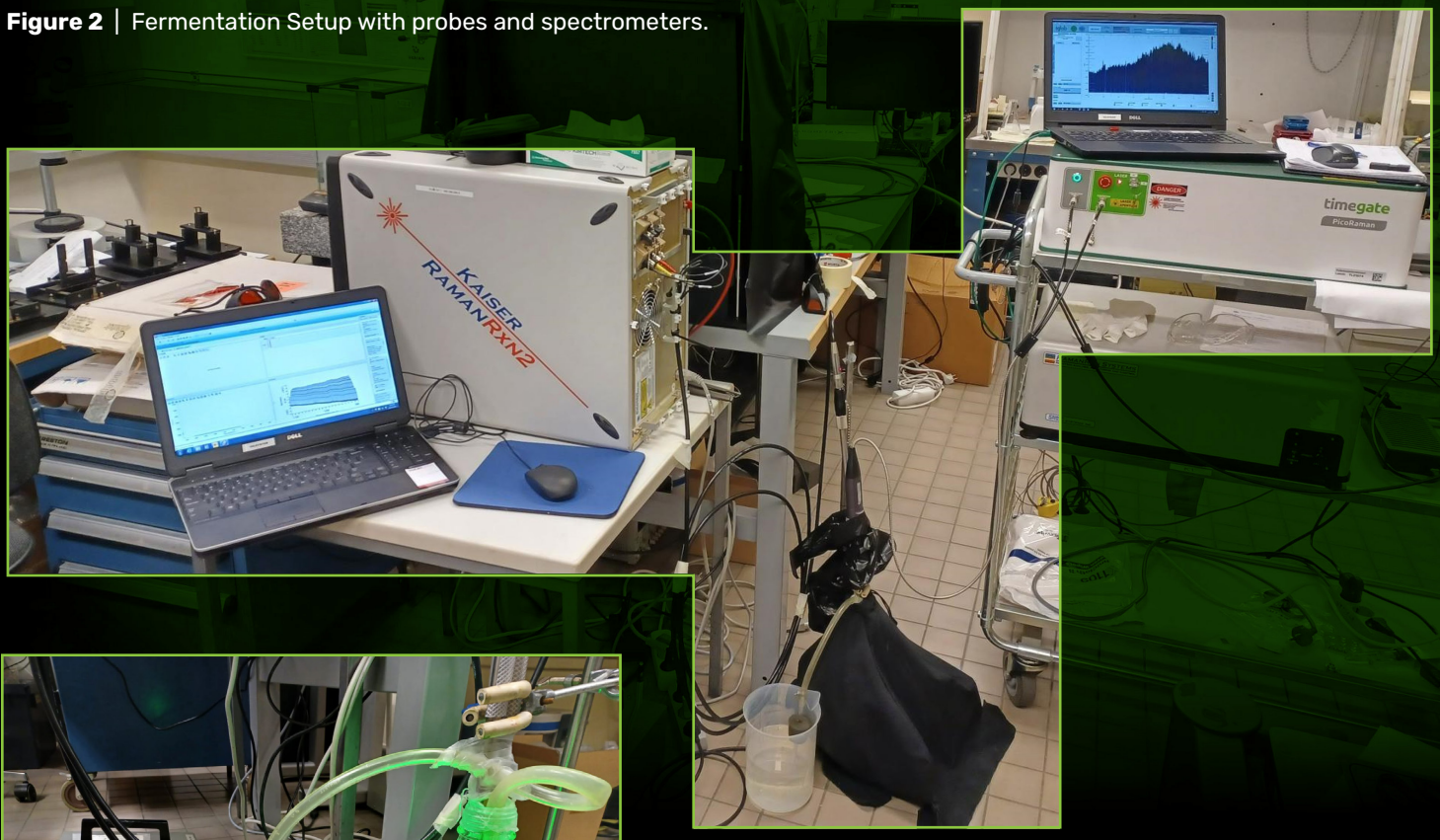
Figure 1 | Schematic comparison of the process of the conventional Raman and Time-Gated Raman spectroscopy

Instruments and Methods

An inoculum of 150 g of D-(+)-glucose (Alfa Aesar™) was dissolved in 1 l of distilled water and to it 10 g of wet yeast was added. The yeast broth was stirred continuously using magnetic stirrer at 200 rotations per minute. This was measured using Kaiser's RXN2 785 nm laser excitation Raman spectrometer and Timegate's PicoRaman with 532 nm pulsed laser. Both the spectrometers were coupled to immersion probes with a working distance of 0 mm and immersed into the broth.

The mouth of the broth container was sealed with parafilm to maintain anaerobic condition for the fermentation process. Another tube was inserted into the broth and the other end of the tube was dipped into a beaker filled with water for the CO_2 gas to escape for pressure equilibration while maintaining anaerobic conditions of the fermentation process (figures 2 and 3). The flask was covered during the experiment as laser safety measure.

Figure 2 | Fermentation Setup with probes and spectrometers.



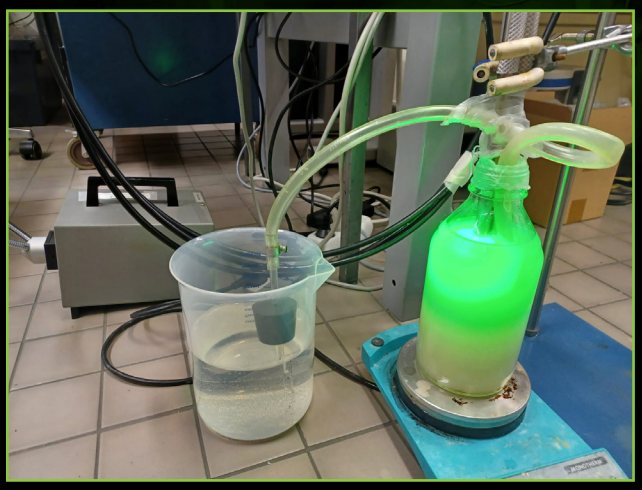


Figure 3 | Fermentation Setup on magnetic stirrer with 2 immersion probes.

Spectral Data Comparison

The Raman spectra for the fermentation process was measured every 15 minutes for 24 hours simultaneously using both the spectrometers. Figures 4a and 4b show the obtained raw spectra from both the instruments.

The raw spectra were baseline corrected using backcor algorithm and smoothed using Savitzky-Golay filter of window length 5 and degree 3 in both the cases. Figures 5a and 5b show the preprocessed spectra, and the signal to noise ratio for Kaiser was approximately 18 and that of PicoRaman was approximately 39.

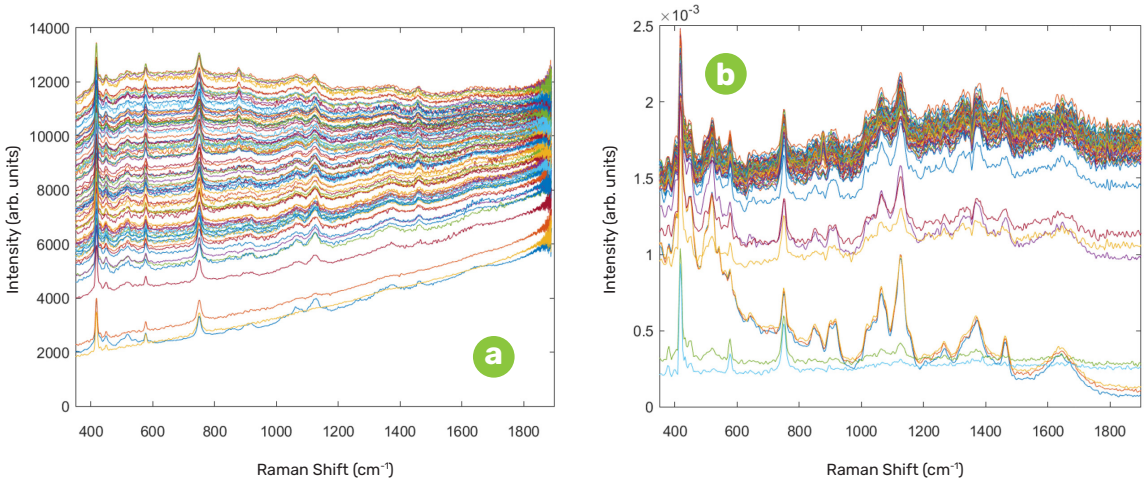


Figure 4 | a Raw spectra from Kaiser Raman system.
b Raw spectra from PicoRaman system.

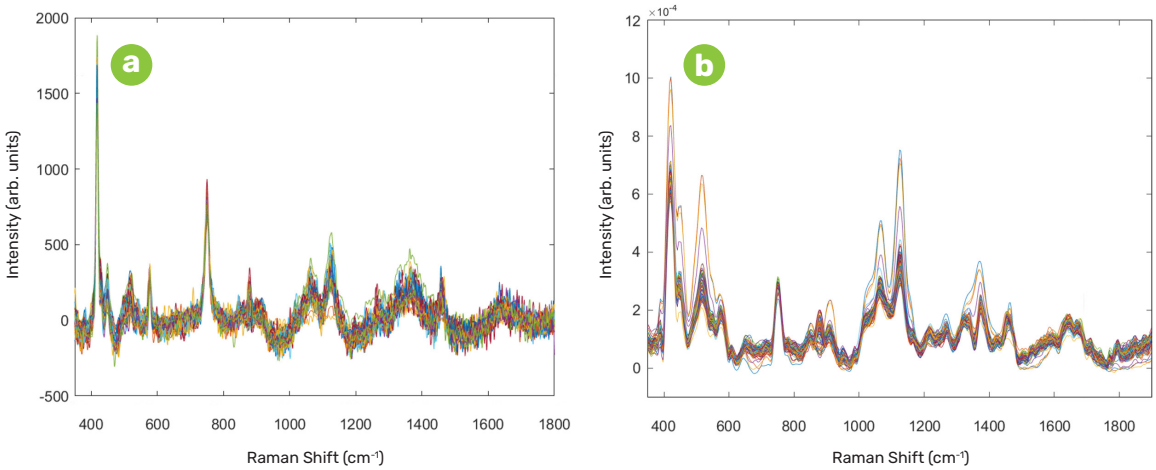


Figure 5 | a Preprocessed spectra from Kaiser Raman system.
b Preprocessed spectra from PicoRaman system.

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Quantitative analysis

A quantitative analysis calibration curve, displayed in Figure 6a, was generated from the peak height of the strongest band at 1125 cm^{-1} for glucose, and Figure 6b was generated from the peak height of the strongest ethanol peak at 880 cm^{-1}. From these results, we can clearly see that the prominent peaks can be well delineated using both the Raman instruments. Yet with inherent fluorescence masking the Raman spectrum, the minor peaks like the lactic acid peak at 830 cm^{-1}, and the amide 1 peak at 1670 cm^{-1} cannot be delineated using Kaiser system whereas it can be clearly observed in the PicoRaman spectra. This could be crucial in monitoring micro quantities of proteins or other crucial biomarkers.

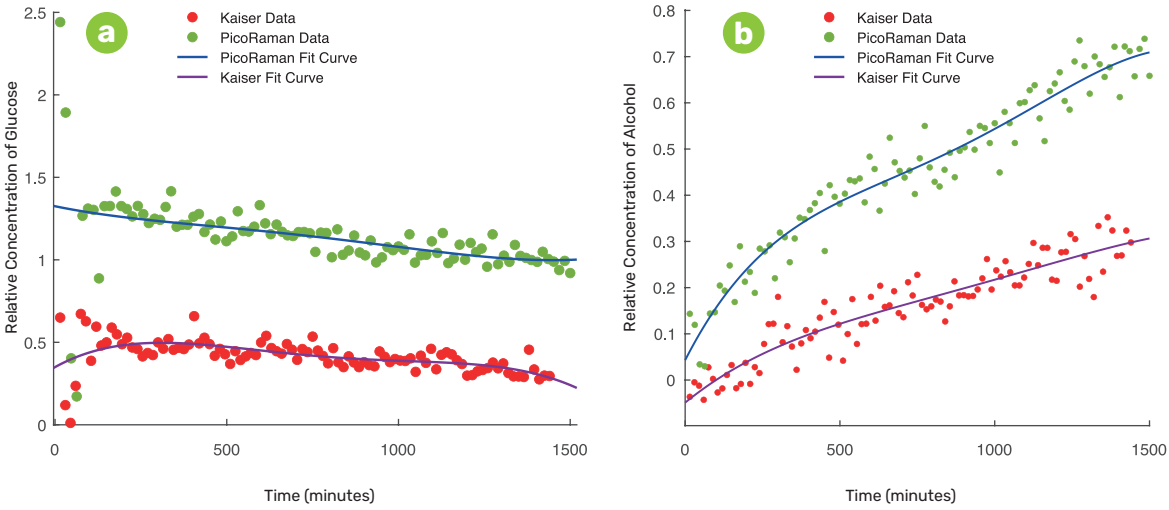

Figure 6 | a Prediction of the glucose concentration from the fermentation spectra

b Prediction of the relative ethanol concentration from the fermentation spectra.

Conclusion

In this application note, the efficacy of Time-gated Raman to monitor bioprocess is clearly demonstrated for both qualitative and quantitative analysis in comparison to continuous wave Raman spectroscopy, especially concerning the concentration of glucose and ethanol. Thus time-gated Raman spectroscopy proves to be a powerful technique for monitoring fermentation process.

Be faster than your fluorescence

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