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Application Note

Monitoring bioprocesses in a light environment using Raman spectroscopy

Abstract

Currently, using Raman spectroscopy in a light environment (artificial and/or ambient light) can present several challenges in bioprocessing applications. Raman spectroscopy is an analytical technique that relies on the detection of scattered photons from a laser of specific wavelength which can be affected by light interferences. The presence of light can impede measurements by introducing background noise and interference peaks resulting in misinterpretation and inaccurate quantitation of target analytes. The predicted values for the parameters of interest in real time are therefore unreliable and not representative of the real metabolite concentrations within the process. This application note introduces an advanced solution to facilitate the implementation of Raman technology in laboratory and manufacturing scales, in a normal light environment, to ensure the consistent monitoring of critical process parameters (CPPs) and critical quality attributes (CQAs) using the ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software.

Highlights

- The advanced solution for straylight management has been specifically designed to handle light interferences in Raman spectroscopy to improve the use of the system in a light environment (artificial and/or ambient light) from laboratory to manufacturing scale.
- The advanced probe tube with a light-reducing cap is adaptable to a wide range of single-use and multi-use equipments such as bioreactors and mixers.
- The ease-of-use of this advanced Raman solution ensures confidence, consistency, and robustness of target culture parameter measurements in-line and in real time.

Introduction

Bioprocess engineers face challenges in their labs and manufacturing facilities when using Raman spectroscopy in a light environment. Indeed, one of the main drawbacks of light interferences in Raman spectroscopy is the potential for increased background noise and reduced sensitivity of the Raman response. Light interferences can result in higher baseline noise levels and introduce spectral artifacts that may overlap and obscure the desired Raman signal of the analyzed sample (**Figure 1**), especially for compounds at low concentration or with weak Raman scattering signals.

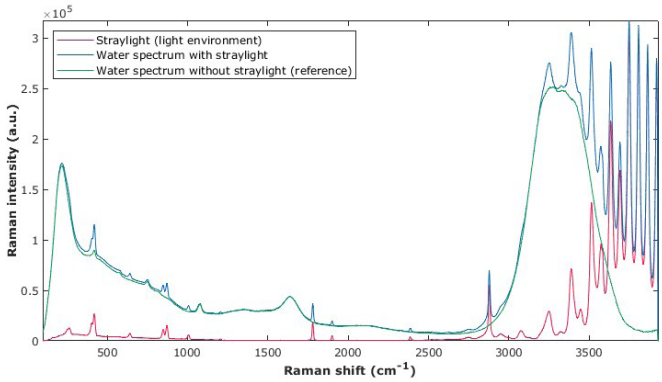

Figure 1: Representation of Raman spectra impacted by light interferences with a reference spectrum without any effect of straylight

The invasive signals can thus distort the Raman spectra and lead to misinterpretation of the chemical information contained in the sample and introduce bias and undesired variability. These variations in the signal intensity and artifact distribution across the spectra make it more challenging to gather consistent datasets for the modeling calibration step. This deterioration in model calibration limits the reliability of the measured values obtained in real-time monitoring and may potentially compromise not only process insights, but process control as well.

Protecting the bioreactor from external light is a common strategy to mitigate challenges associated with Raman spectroscopy in bioprocessing. This practice helps minimize the impact of a light environment on the Raman signals and reduce background noise. However, it is important to note that issues related to the scale of large bioreactors, which can have volumes ranging from hundreds to thousands of liters, still need to be carefully addressed for effective real time monitoring. Covering such large bioreactors can indeed be difficult and poses several challenges during the implementation of Raman technology.

Described here are improvements to the ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software (Raman PAT Platform) to properly handle light interferences during spectral acquisitions. This combined hardware and software solution for straylight management consists of an advanced probe tube with a light-reducing cap and a noise reduction software filter.

Innovating Raman spectroscopy with straylight management

Advanced probe tube with a light-reducing cap

To ensure optimized operation of the ProCellics™ Raman Analyzer in a light environment, an advanced probe tube combined with a software solution was developed. The ProCellics™ advanced probe tube (**Figure 2**), compatible with the current ProCellics™ probe head, is made from stainless steel 316L materials and composed of two main parts:

- The probe tube incorporates a threaded tip with a sapphire window on which a light-reducing cap is mounted, and a PG13.5 captive nut (floating cable-gland) to facilitate connection and management of the probe tube's orientation into the bioreactor (**Figure 2a**)
- The light-reducing cap has a specific design to minimize the straylight signal collection while ensuring a smooth flow of the liquid being analyzed. The threaded tip and O-ring ensure easy set-up and facilitate cleaning between cell cultures to avoid fouling effect (**Figure 2b**)

a)

b)

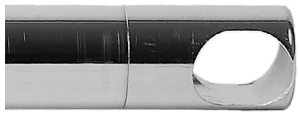

Figure 2: ProCellics™ advanced probe tube (a) including a light-reducing cap on its tip (b)

Noise reduction software filter

In addition to the advanced probe tube, a noise reduction filter is integrated into the Bio4C® PAT Raman Software ensuring optimal signal quality during spectral acquisition. First, a brief and automatic verification is initiated when launching a batch acquisition to warn the user of potential straylight at the beginning of a run. Then, if the system detects a straylight signal or saturation during batch acquisition, alarm messages are reported. Furthermore, any straylight detected is subtracted from the Raman signal obtained during the measurement. In this way, the final spectrum obtained is cleansed of any possible straylight. In the graph of monitored parameters, dotted yellow lines indicate that straylight was detected, while red lines indicate that the spectra used for the measurement shows saturation (**Figure 3**).

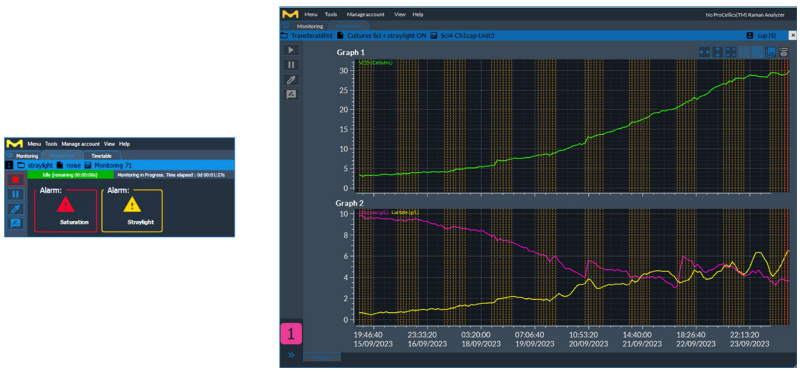

Figure 3: Bio4C® PAT Raman Software reporting alarms for straylight and saturation detection during batch monitoring

Experimental set-up

Cell culture experiments

A CHOZN® cell line (SAFC®) producing a specific mAb was cultivated in a benchtop glass bioreactor in fed-batch mode, controlled by a SciVario® Twin controller (Eppendorf). EX-CELL® Advanced HD Perfusion Medium was used with a feed containing 50% Cellvento® 4Feed Comp and 50% EX-CELL® Advanced CHO Feed 1 (SAFC®). The bioreactor was operated with an initial volume of 1 L. The temperature, agitation rate, pH and DO were controlled at 37 °C, 120 rpm, 7.0 and 40% of air saturation, respectively. The feed to the bioreactors was controlled by switching a feed pump on and off manually at days 3 and 5 with a volume of 50 mL, and 100 mL at day 7.

Two samples were manually collected in triplicates daily and analyzed with a BioProfile® FLEX2 (Nova® Biomedical) to determine the concentration of various components including glucose, lactate, and cell density.

Glucose was added to the culture when concentrations fell below 4 g/L. The cell culture run was stopped when the viability fell below 80%.

Raman spectroscopy

The ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software (Millipore®) was used in a multi-channel configuration using two probes in parallel to collect the Raman spectra. The instrument was equipped with a 785 nm laser source resulting in 350 mW of power at the probe output. The probe tubes of 225 mm immersion length obtained measurements directly within the bioreactor using PG13.5 cable gland adaptors. For this study, the bioreactor was not protected from any source of light. Two different optical Raman probes were mounted: one with a straylight reduction cap (advanced probe tube), and the other without a straylight reduction cap (standard probe tube) (**Figure 4**). The noise reduction software filter is activated only for the channel with the advanced probe tube.

Spectral data acquisition, reference association, and data pre-processing were controlled using Bio4C® PAT Raman Software. A total acquisition time of 15 minutes was determined per measurement, consisting of an integration time of 45 seconds and an average of 20 spectra.

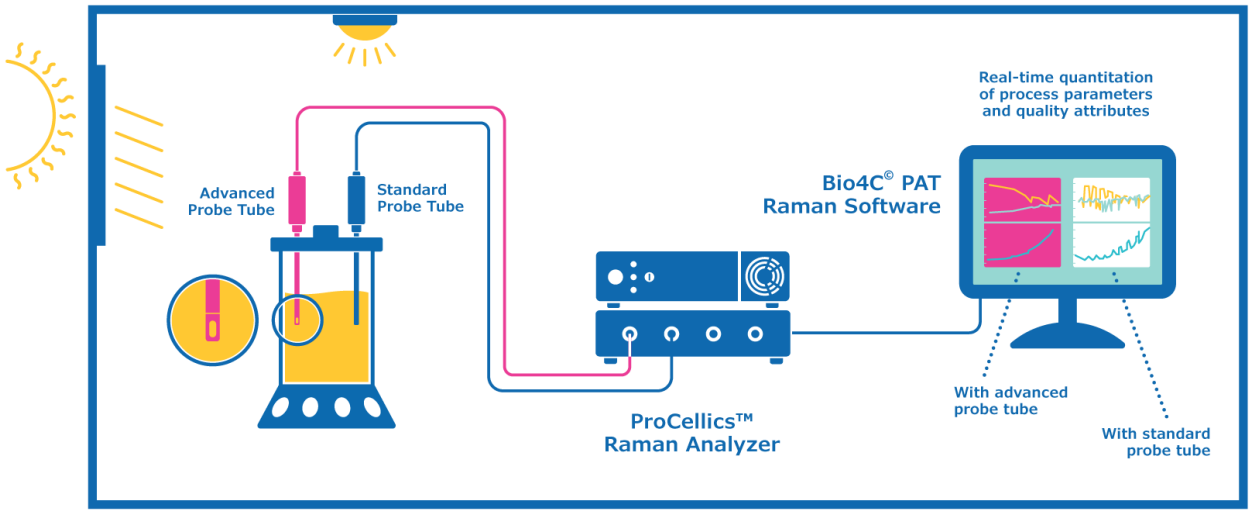

Figure 4: Set-up configuration and integration of the Raman standard and advanced probe tubes (ProCellics™ Raman Analyzer with Bio4C® PAT Raman Software) for spectral acquisitions in a light environment.

Results and Discussion

Model calibration performance

PLS calibration models were built with Bio4C® PAT Chemometric Expert (Millipore®) for glucose, lactate, and Viable Cell Density (VCD) parameters to evaluate the straylight management solution. The regression models were calibrated in a controlled dark environment ensuring no spectral light contributions to the acquired Raman spectra. To ensure high variability and sufficient data, three cell culture runs were performed. This consistent calibration set of approximatively 200 samples was used to develop the PLS models. The three regression models demonstrated a good coefficient of correlation ($R^2 > 0.90$) during calibration as well as satisfactory performances during cross-validation. The models were then imported into the software for real-time monitoring of the three target parameters in a light environment for both the standard probe and the advanced straylight management solution.

Evaluation of the solution for straylight management

To evaluate the performance of the advanced straylight solution, an independent batch was acquired to obtain predicted values in real time, using the configuration described in the experimental set-up section (**Figure 4**). The accuracy of the measurements was evaluated by considering the Root Mean Square Errors of Prediction (RMSEP). The relative error (%) is also presented in **Table 1** as the ratio between the RMSEP and the maximum value of the validation range for each parameter making them comparable across different units.

Table 1. Predicted performance for standard and advanced Raman probes in light environment compared to reference set-up in dark environment using PLS models

	Standard Raman probe in light environment		Raman PAT Platform with straylight management solution in light environment		Raman PAT Platform in dark environment	
Parameter	**RMSEP**	**Relative error**	**RMSEP**	**Relative error**	**RMSEP**	**Relative error**
Glucose (g/L)	4.55	46 %	0.36	4 %	0.56	6 %
Lactate (g/L)	3.11	88 %	0.41	11 %	0.39	9 %
VCD (MCells/mL)	3.83	14 %	2.00	7 %	1.76	8 %

poor performance acceptable performance good performance

The evaluation of the RMSEP and the relative errors from the validation set provide valuable insights into the capability of the straylight management solution to overcome light interferences in bioprocesses. For all three parameters of interest, the measurements with a standard Raman probe in a light environment have the highest RMSEP values, representing a relative error of up to 88% for lactate. The use of the Raman PAT Platform with its advanced straylight management solution under the same light environment exhibited as much as 13-fold better performance in the case of glucose (**Figure 5**).

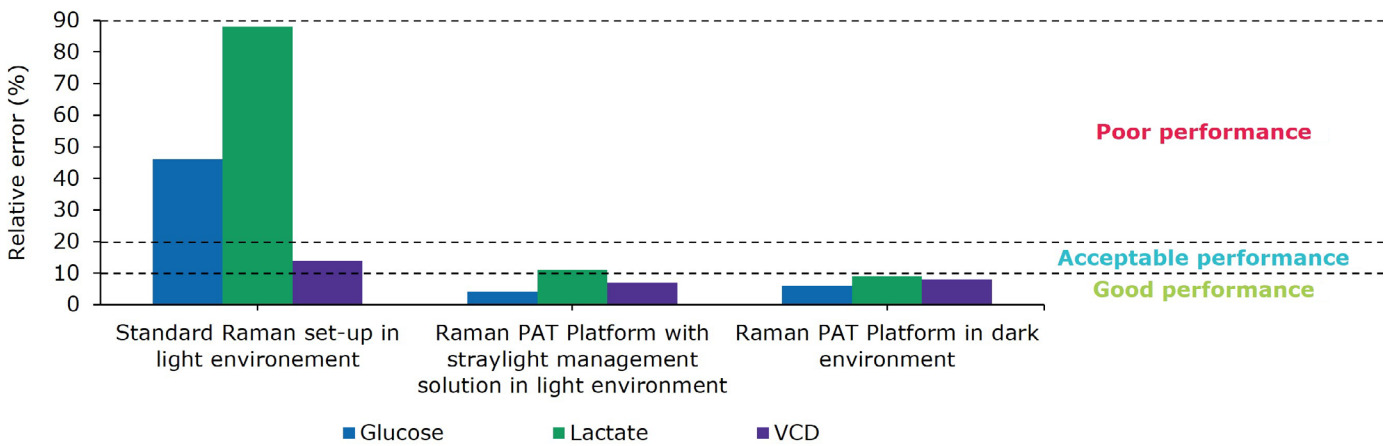

Figure 5: Comparison of the relative errors in percentage obtained from the three different conditions for glucose, lactate and VCD parameter.

The improved results delivered by the straylight management solution demonstrate similar performance in terms of accuracy for each parameter, compared to measurements performed in a controlled dark environment (reference). These findings underscore the significance of utilizing this advanced Raman solution, which incorporates effective straylight management, in regulating light conditions to mitigate the potential for inconsistent CPP and CQA predictions.

Figure 6 illustrates the use of the calibration models for monitoring three target parameters (glucose, lactate and VCD). On each graph, the reference off-line measurements are highlighted by green triangles, and the predicted values obtained from advanced and standard probes are displayed in blue solid and pink dotted lines respectively. The figure indicates that the overall trend for the three parameters of interest is well monitored with the advanced probe compared to the standard one. Indeed, significant periodical inconsistencies are clearly visible due to the unwanted noise and interferences caused by daylight during the measurement. The straylight management solution contributes to substantial improvements in accuracy and reliability of the Raman values in a light environment. By minimizing the impact of daylight-related noise, the advanced probe tube, combined with the noise reduction software filter, provide more accurate and trustworthy results for the main parameters acquired with light interferences.

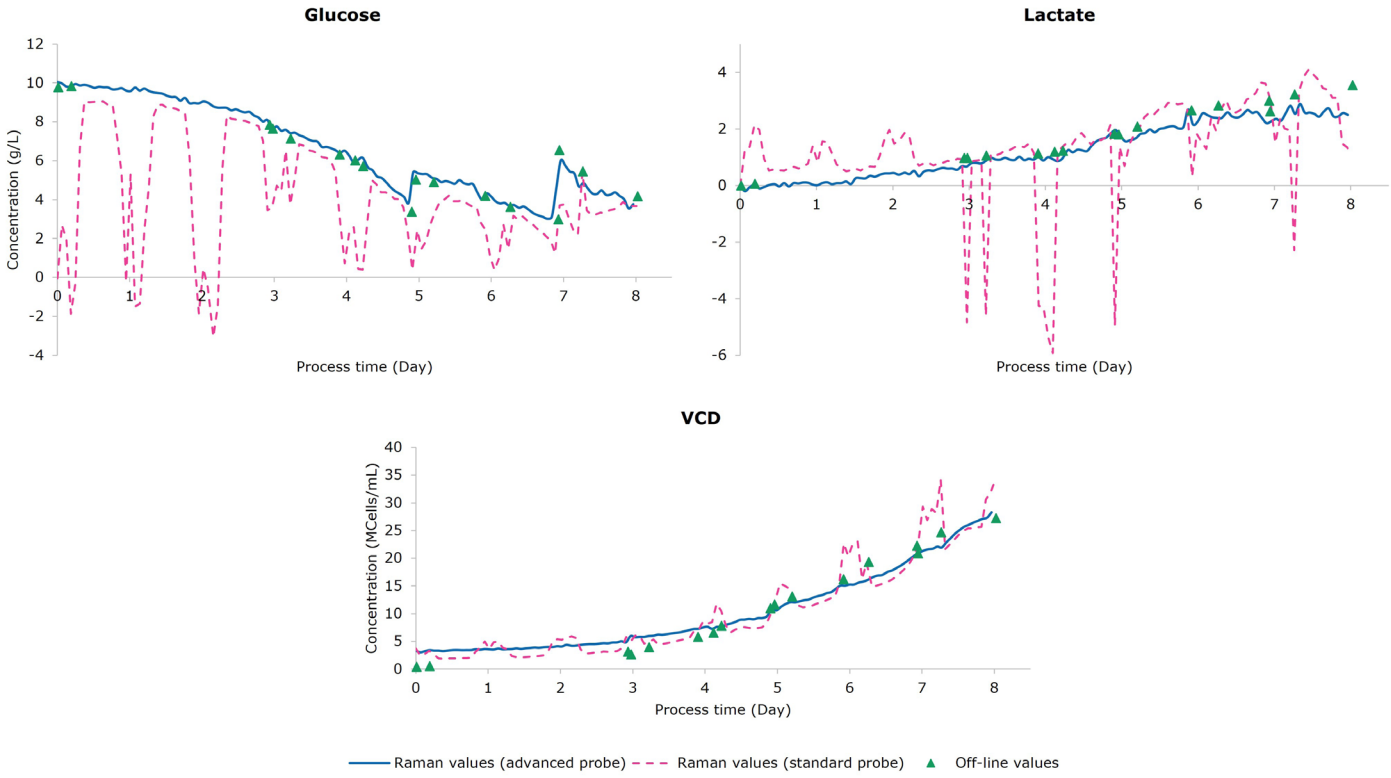

Figure 6: Comparison of off-line measurements (green triangles) and Raman values obtained with standard Raman probe (pink dotted line) and with straylight management solution from the Raman PAT Platform (blue solid line) in a light environment.

Conclusion

The development of an innovative straylight solution consisting of an advanced probe tube and a noise reduction software filter significantly increases the ease of implementation of the Raman technology. It allows monitoring results equivalent to the standard solution in a dark environment, eliminating the need to shield the bioreactor from ambient or artificial light. This solution also increases confidence in scale-up, where it is not feasible to cover and protect large bioreactors from light, by ensuring consistent monitoring of CPPs or CQAs and eliminating set-up variability.

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