High-Throughput Screening with Chemical Specificity in Biopharma with PoliSpectra Rapid Raman Plate Reader

PoliSpectra Rapid Raman Plate Readerを用いた，バイオ医薬品分野における化学的特異性を備えたハイスループットスクリーニング

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The PoliSpectra Rapid Raman Plate Reader addresses the critical trade-off in biopharma between high-throughput speed and chemical specificity by providing automated, label-free molecular fingerprinting. Designed for seamless integration into 96-well plate workflows which enables simultaneous measurement of multiple samples for condition optimization, the Rapid Raman Plate Reader utilizes multi-laser excitation and “walk-away” automation through automatic calibration and robotic-arm and liquid handler integration to deliver non-destructive analysis of complex samples. This readout demonstrates the platform’s efficacy through three key applications: the precise quantification of nucleoside triphosphates (NTPs) in mixture, the determination of protein detection limits for BSA and lysozyme, and the monitoring of glucose levels in varied culture media. By delivering rapid, reproducible results across these diverse bioprocessing tasks, the Rapid Raman Plate Reader establishes itself as a high-speed complementary tool to traditional chromatography, enabling more efficient drug discovery and process development.

バイオ医薬品分野では「ハイスループット（高速処理）」と「化学的な特異性（成分を見分ける力）」の両立が課題であった。PoliSpectra Rapid Raman Plate Readerは，ラベルフリーでの分子フィンガープリント（分子指紋）解析を自動実行することでこの両立を実現する装置である。Rapid Raman Plate Readerは条件設定のために複数試料を同時に測定することを目的とした標準的な96ウェルプレートを投入できるように設計されており，複数レーザーによる励起，自動校正機能およびロボットアームやリキッドハンドラーとの接続によるwalk-away型の自動運転により，複雑な試料の非破壊分析を実現する。本稿では，次の3つの主要な応用例を通じて，本プラットフォームの有効性を示す。(1)混合物中におけるヌクレオシド三リン酸（NTP）の精密定量，(2)BSA（bovine serum albumin：ウシ血清アルブミン）およびリゾチームを対象としたタンパク質の検出限界の評価，(3)多様な培養培地におけるグルコース濃度のモニタリング，これらのバイオプロセスに関わる課題に対して，Rapid Raman Plate Readerは従来のクロマトグラフィーを補完する，迅速で再現性の高い高速な分析手段として位置づけられ，創薬やプロセス開発の効率化に貢献する。

Introduction

High-throughput screening (HTS) is a central component of pharmaceutical research, enabling the rapid evaluation of thousands to millions of compounds during drug discovery and bioprocess development to find the next breakthrough. However, existing HTS approaches often involve a trade-off between speed and chemical specificity. Fluorescence and absorbance assays provide rapid readouts but typically require costly labels and lack the molecular-level information needed to avoid false positives. Conversely, chromatography techniques such as high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS) offer high selectivity and precision but are slow, labor-intensive, and consume vast amounts of reagents, making them difficult to scale for high-throughput workflows.

Raman spectroscopy provides a molecular fingerprint that enables label-free, chemically specific analysis with mini-

mal sample preparation. Despite these advantages, conventional Raman measurements are often limited by low throughput and complex workflows, restricting their adoption in screening environments. At HORIBA, we developed the patented PoliSpectra Rapid Raman Plate Reader (Figure 1) to address these limitations by transforming Raman spectroscopy into a high-speed, automated screening platform capable of non-destructive chemical analysis.

The Rapid Raman Plate Reader is designed to operate directly with standard 96-well plates commonly used in HTS workflows, with ongoing development toward compatibility with higher density formats such as 384-well plates to further increase throughput. Customization for different well-plate formats is also available.

The Rapid Raman Plate Reader incorporates a multi-laser system offering combinations of 532 nm and 785 nm excitation options.This flexibility allows researchers to tackle a diverse range of samples. The 532 nm laser provides strong signal strength for optically clear samples, while the 785 nm option is crucial for suppressing fluorescence interference in biological samples.

To ensure reliable operation in high-throughput and high-volume environments, the Rapid Raman Plate Reader is designed for "walk-away" automation. Every part, including the motorized door and self-calibration routines, is fully automated to ensure data reliability day-to-day. The system also supports server access for seamless integration with robotic arms and liquid handlers. Together, these features enable Rapid Raman Plate Reader to deliver rapid and reproducible Raman measurements suitable for routine screening and multivariate analysis model building.

In this work, we present three customer-inspired case studies to demonstrate the applicability of the Rapid Raman Plate Reader system across key biopharmaceutical workflows: nucleoside triphosphate (NTP) quantification in mixture, protein limit of detection evaluation, and glucose quantification in culture media. Collectively, these representative examples showcase how high-throughput Raman analysis can benefit a wide range of biopharma research interests and complement conventional analytical techniques.

Figure 1 PoliSpectra Rapid Raman Plate Reader

Results and Discussion

Nucleoside Triphosphate Quantification

To demonstrate the system's transformative potential in reaction monitoring and optimization, we first focused on monitoring nucleoside triphosphates—ATP, CTP, GTP, and UTP—which are fundamental building blocks for nucleic acids and play essential roles in cellular energy transfer and biochemical signaling. Accurate and timely monitoring of NTP concentrations in mixtures is therefore important across a range of biochemical and analytical applications. However, conventional NTP quantification typically relies on chromatographic techniques such as HPLC coupled with UV absorbance, fluorescence detection, or LC-MS. While these methods provide high selectivity, they are poorly suited for rapid screening due to limited throughput, extensive sample preparation requirements, and high operating and maintenance costs. These constraints significantly limit the number of conditions, formulations, or reaction states that can be practically evaluated.

Raman spectroscopy is well-suited for addressing these limitations. As demonstrated in Figure 2A, each of the four NTPs exhibits distinct Raman features, enabling direct identification and quantification in mixture without any additional labeling or chromatographic separation. To evaluate quantification performance and detection limits with Raman, mixtures of ATP, CTP, GTP, and UTP were prepared in phosphate-buffered saline (PBS) solution across a concentration range of 0 to 2.5 mM in triplicate. Raman spectra were acquired with 532 nm excitation, with acquisition time of 40 seconds per well.

A Partial Least Squares (PLS) regression model was constructed to resolve the overlapping spectral contributions, with preprocessing using Savitzky-Golay 1st derivative and mean centering. Four latent variables were selected for the final model. To validate the model robustness, we prepared a separate batch of NTP mixtures and acquired their spectra on a different day.

Strong agreement was observed between predicted and reference concentrations, with regression slopes greater than 0.997 for all four NTPs, as shown in Figure 2B. The model achieved a mean squared error of cross-validation

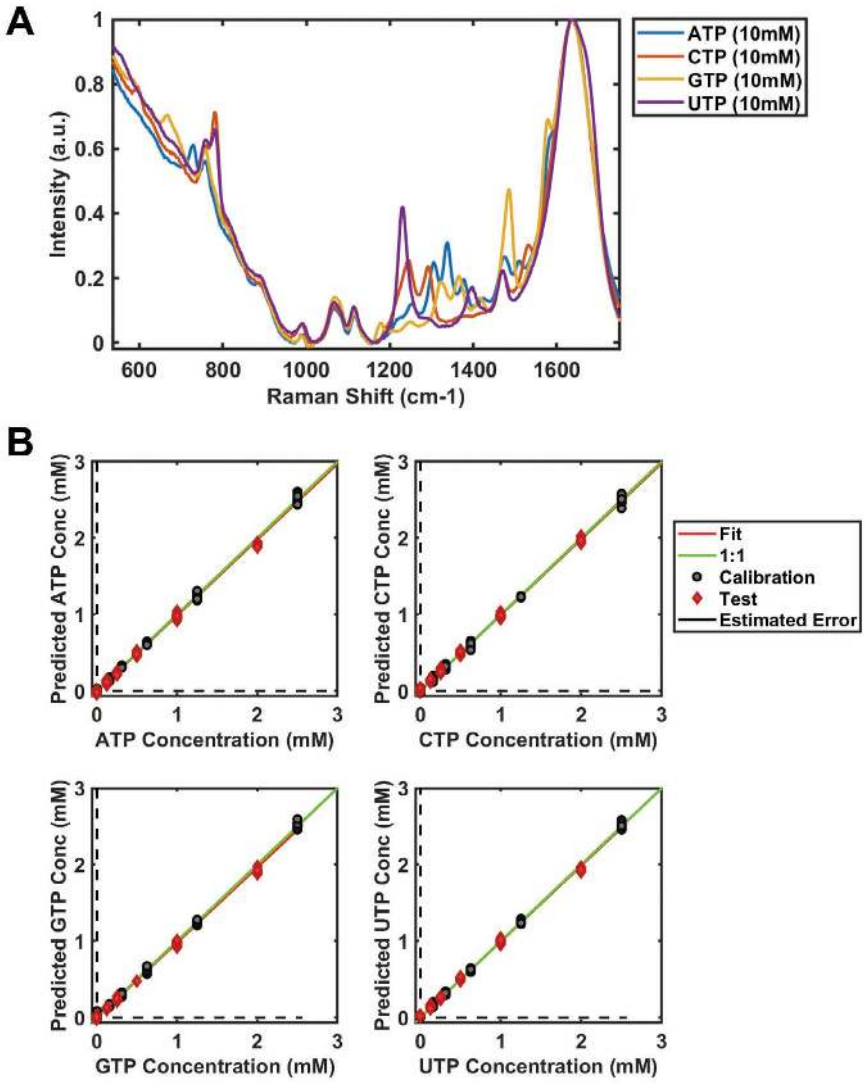

Figure 2 Nucleoside triphosphate (NTP) Raman spectra collected with Rapid Raman Plate Reader using 532 nm excitation
(A) and PLS calibration model for concentration prediction (B).

(MSECV) value below 0.03 and enabled accurate prediction of NTP concentrations down to 0.1 mM. These results demonstrate the capability of the Rapid Raman Plate Reader to provide rapid, label-free quantification of NTPs in complex mixtures, supporting high-throughput screening and reaction analysis workflows in biopharmaceutical environments.

Protein Detection Limit Evaluation

Proteins are central targets in biopharmaceutical development; however, high-throughput protein detection and quantification are generally challenging. Common techniques such as UV absorbance, Bradford/BCA assays, and ELISA suffer from limited specificity, interference from formulation components, and dependence on calibration standards. HPLC and LC-MS offer higher accuracy but are slow, low-throughput, and require extensive sample preparation. Fluorescence or label-based assays improve sensitivity but introduce labeling artifacts and high reagent costs.

Raman-based protein analysis, despite its advantages in sample preparation simplicity, is challenging due to relatively weak Raman scattering and interference from fluorescence. To evaluate the detection capabilities of the Rapid Raman Plate Reader for protein analysis, two representative proteins were selected: bovine serum albumin (BSA, ~66 kDa) as a large protein and lysozyme (~14 kDa) as a smaller protein. A dilution series of each was prepared in PBS buffer in 96-well plates for Rapid Raman Plate Reader measurements.

Raman spectra, illustrated in Figure 3 left panel (A for BSA and B for lysozyme), were acquired using 785 nm excitation to minimize fluorescence interference. Two PLS regression models were constructed for each protein molecule with mean-centered spectra across a range of concentrations. As shown in Figure 3 right panel, good linearity was observed for both types of protein molecules. The Rapid Raman Plate Reader successfully

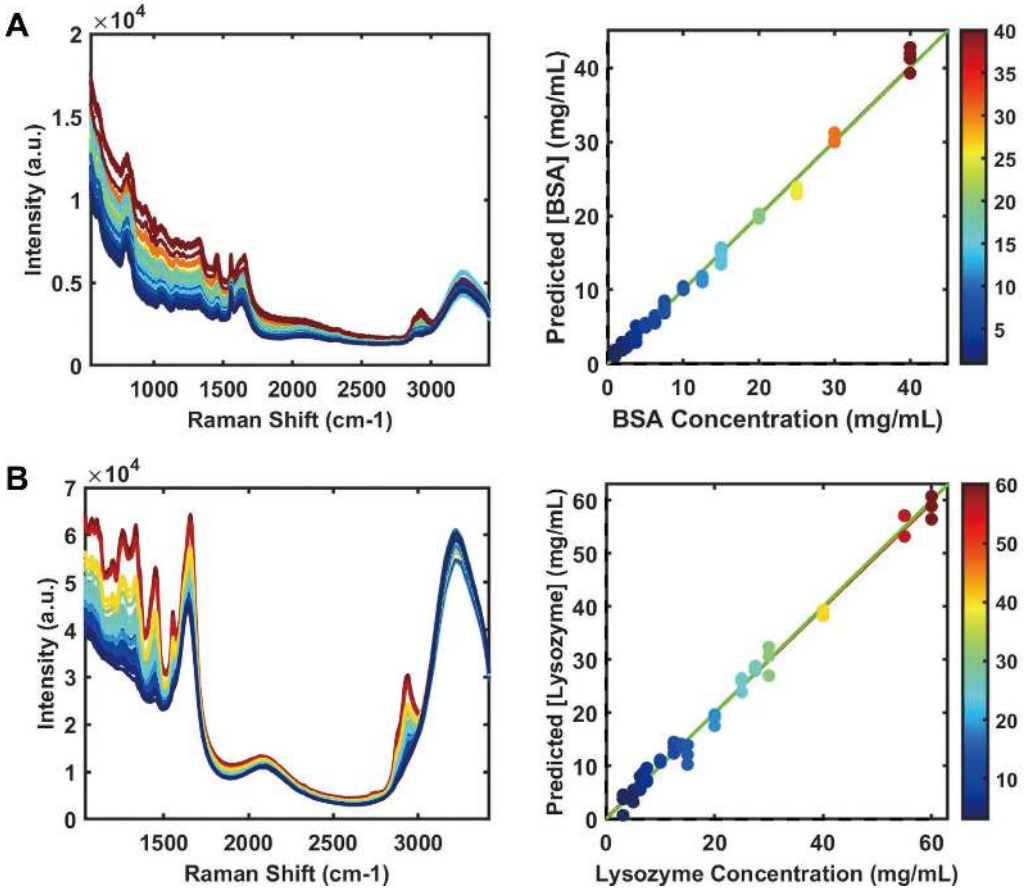

Figure 3 Limit of detection evaluation of protein molecules, BSA (A) and lysozyme (B) using Rapid Raman Plate Reader with 785 nm excitation. Raman spectra (left) and corresponding PLS calibration models (right).

detected protein concentrations down to approximately 3 mg/mL—specifically 2.6 mg/mL for BSA and ~5 mg/mL for lysozyme.

These results establish a baseline detection limit for Raman-based protein analysis using our high-throughput plate reader platform and demonstrate the feasibility of applying the Rapid Raman Plate Reader to protein formulation, stability studies, and drug-protein interaction screening without consuming or destroying samples.

Glucose Quantification in Culture Media

Glucose concentration is a key parameter in cell and yeast culture and fermentation processes, directly impacting cell viability and productivity. While HPLC is commonly used for glucose quantification, its limited throughput and operational cost and complexity restrict its use for rapid feedback during media optimization and process development. We evaluated the Rapid Raman Plate Reader ability to quantify glucose directly in culture media formulations and spent culture supernatants using 785 nm excitation.

We analyzed five types of yeast spent media containing different glucose concentrations. As shown in Figure 4A, background fluorescence level varies significantly across media types. To explore the feasibility of predicting glucose concentration using one single model, we preprocessed the Raman spectra using 6th-order detrending followed by mean centering to isolate the glucose signal from the complex background. Predicted glucose concentrations were compared with HPLC-measured concentrations.

Glucose concentrations were accurately predicted in most media types, as shown in the right plot of Figure 4A. However, one medium type (Medium D, cyan) exhibited a different prediction slope, indicating complex matrix effects likely due to its significantly stronger fluorescent background. To address this, a separate PLS model could be constructed specifically for this medium during production or QC workflows. Alternatively, samples exhibiting strong background interference can be flagged for targeted HPLC analysis. This hybrid strategy enables efficient resource allocation while maintaining analytical rigor. This case study highlights the utility of the Rapid Raman Plate Reader as a rapid screening and down-selection tool prior to chromatographic analysis.

We also performed a separate study to evaluate the limit of detection of glucose in PBS buffer solution (Figure 4B). Using a simple PLS regression model with 1st-order

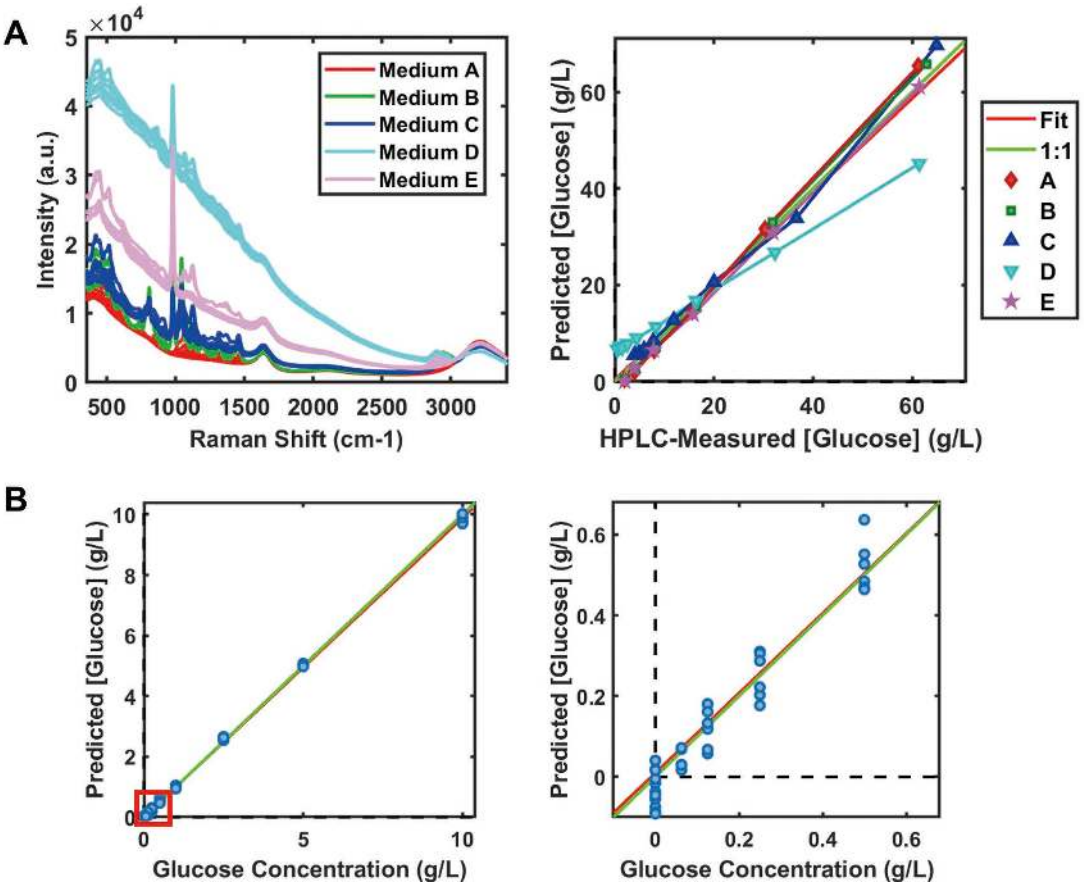

Figure 4 Glucose quantification using Rapid Raman Plate Reader with 785 nm excitation. (A) Raman spectra of five yeast spent media types containing different glucose level and corresponding PLS prediction compared with HPLC measurement. (B) Glucose limit of detection was evaluated in PBS buffer solution. Right plot shows the lower concentration region as marked by the red box in left plot.

detrending, mean centering, and 1 latent variable, the glucose detection limit was determined to be as low as 0.29 g/L.

Conclusions

Across three biopharmaceutical-relevant case studies, from NTP monitoring to protein detection and glucose quantification, the PoliSpectra Rapid Raman Plate Reader demonstrates its ability to deliver chemical specificity and label-free quantification at speed. By bridging the gap between research needs and industrial scale, HORIBA's team demonstrated that the Rapid Raman Plate Reader is not just an instrument—it is a practical high-throughput screening platform for accelerating drug discovery and bioprocess development.

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