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APPLICATION NOTE

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SpheroScan® Explorer: Protein Detection and Quantitation in Complex Fermentation Media

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Abstract

Accurate and efficient quantification of target proteins in complex fermentation media is essential for bioprocess development and monitoring. Traditional analytical methods often face limitations in sensitivity, specificity, and adaptability to diverse media compositions. The novel SpheroScan® Explorer is based on Fluorescent Resonator Signature (FRS) technology, which leverages whispering gallery mode resonances (WGM) in biotin-functionalized fluorescent microbeads for label-free, real-time protein detection. We demonstrate the specificity and sensitivity of this method for detecting neutravidin in LB and YPG media, as well as in high-salt elution buffer and *E. coli* cultures. The FRS platform enables single-step quantification without the need for immobilization or extensive sample preparation, offering a robust and scalable solution for protein monitoring in complex bioprocess environments.

Introduction

Accurate quantification of target proteins in complex biological and process media is a critical requirement across all stages of biotherapeutic development - from early research and lead optimization to process development and manufacturing. Traditional analytical techniques such as UV spectroscopy, ELISA, and HPLC often face limitations in complex matrices due to interference from media components, high sample preparation demands, and limited throughput.

As the biopharmaceutical industry continues to seek greater efficiency and cost-effectiveness, there is growing demand for innovative analytical tools that offer high specificity, sensitivity, and speed without increasing operational complexity. Label-free biosensor-based technologies have emerged as powerful alternatives, enabling real-time, functional analysis of protein interactions with minimal sample preparation.

The SpheroScan ®Explorer, using Fluorescent Resonator Signature (FRS) technology, addresses many of these challenges by enabling rapid, label-free detection of target proteins in diverse and complex media. By leveraging whispering gallery mode (WGM) resonances in functionalized polystyrene microbeads, FRS provides a robust platform for quantifying protein binding events with high specificity - even in the presence of interfering substances such as salts, nutrients, or microbial cells. This makes FRS a promising candidate for real-time monitoring of protein production in precision fermentation and other bioprocessing applications. Moreover, small sample volumes (>50 µl) can be processed.

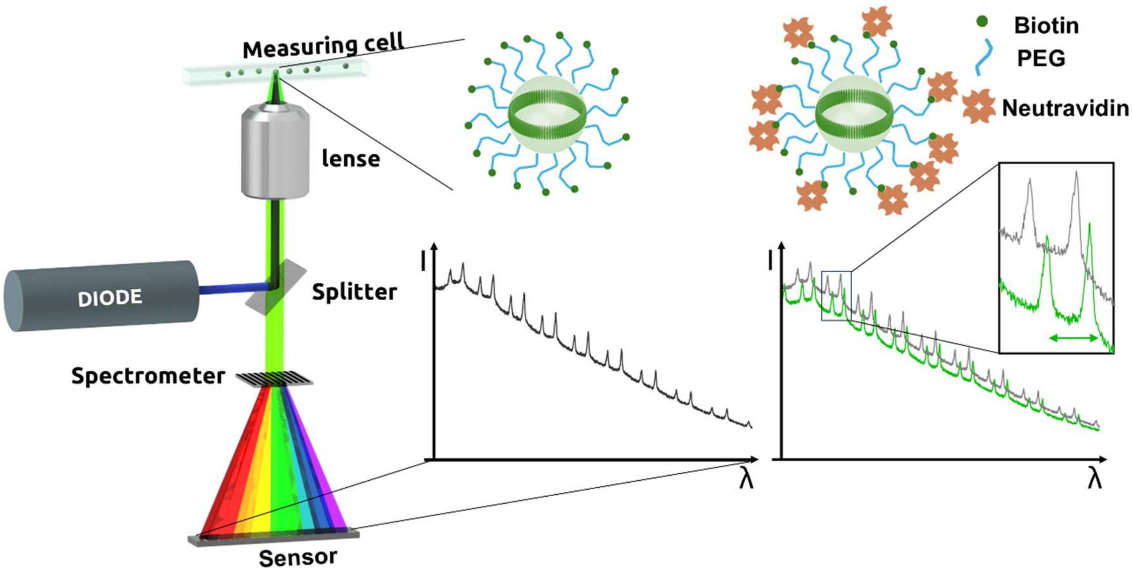

Figure 1 - Principle of FRS-based optical sensing using functionalized microbeads.
Sensor read-out is performed remotely via optical excitation of embedded fluorescent dyes within polymer microbeads. Upon target binding—such as Neutravidin to biotin-PEG—the fluorescent emission spectrum undergoes characteristic shifts (right), known as the Fluorescent Resonator Signature (FRS). These spectral changes are analyzed using a Mie theory-based algorithm to quantify the bound target. The use of freely suspended microbeads enables high-throughput, statistically robust measurements in complex media

Experimental Design

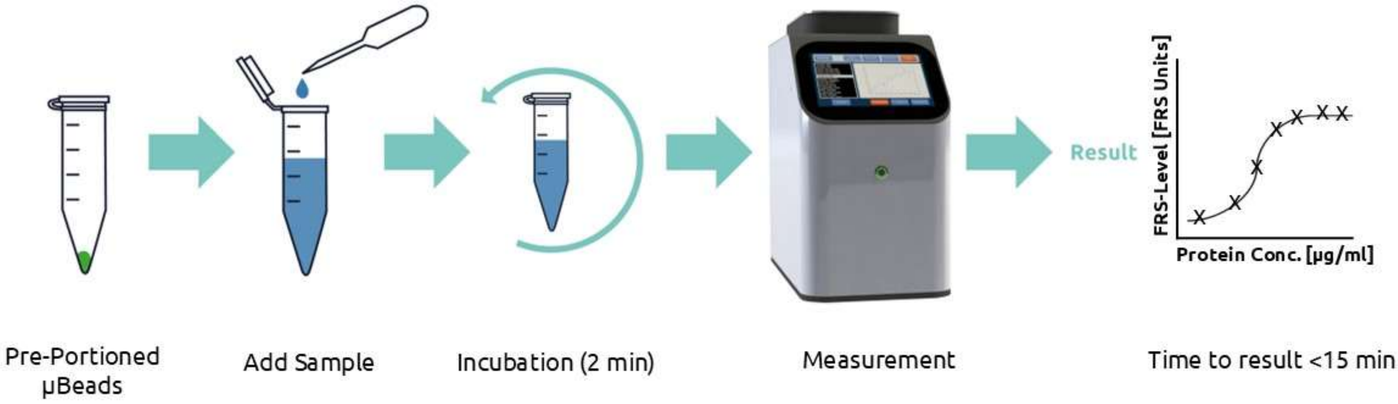

Figure 2 - Work Flow. Sample is added to pre-portioned microbeads. Sample is incubated for 2 min and measured in SpheroScan® Explorer.

Workflow

300 µl of the sample fluid is added to the pre-portioned biotin-functionalized microbeads (0.5 µl), incubated rotating for 2 min at room temperature. Then, 150 µl of the sample fluid with beads is pipetted into specialized 18-well plate and measured in SpheroScan® Explorer. Measurement and analysis time was approximately 15 min.

Specificity Assessment

To evaluate assay specificity, biotin functionalized microbeads were incubated with spiked samples:

- No protein (negative control),
- 10 µg/mL Neutravidin (target protein),
- 10 µg/mL of an unrelated protein of similar size.

These conditions were tested in LB medium and YPG medium. Samples were processed according to the workflow.

Sensitivity Assessment

LB or YPG medium were spiked with different concentrations of Neutravidin (0, 1, 2.5, 5, 7.5, 10 and 50 µg/ml) to determine sensitivity. Samples were processed according to workflow.

Influence of Bacteria

For the LB medium containing *E. coli*, the medium was inoculated with the bacteria and cultured overnight with shaking at 37 °C. The OD600 was found to be 0.16. The culture was supplemented with the appropriate amount of neutravidin. Samples were processed according to the workflow.

Downstream Conditions

To test application possibilities in downstream processing elution buffer (0.4 M NaCl) was supplemented with different concentrations of neutravidin and samples were processed according to the workflow

Results

Specific and Sensitive Detection of Neutravidin in Complex Media

To evaluate the performance of the FRS-based detection system under realistic bioprocessing conditions, biotin-functionalized microbeads were tested in two commonly used complex fermentation media: LB and YPG. The goal was to evaluate the specificity and sensitivity of the assay, and its robustness when used with different media compositions.

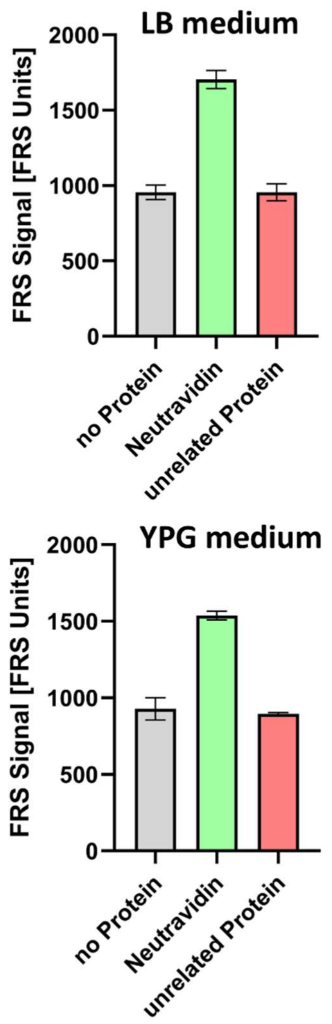

Figure 3 - Specificity tests in LB and YPG medium. Data is presented as mean±SEM.

A significant increase in FRS signal was observed upon Neutravidin binding to the biotinylated microbeads in both LB and YPG media, compared to control samples lacking Neutravidin (Fig. 3). The signal was highly specific, as no increase was detected when an unrelated protein of similar molecular weight (55 kDa) was used.

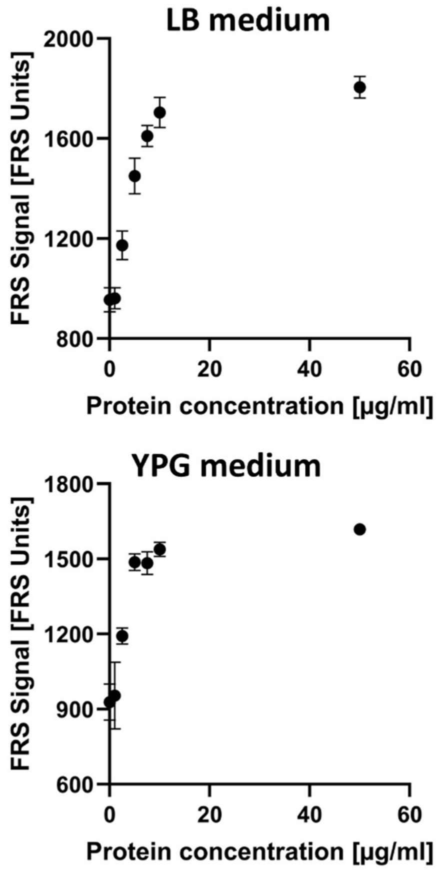

Figure 4 - Sensitivity tests in LB and YPG medium. Data is presented as mean±SEM.

The assay demonstrated high sensitivity, with detectable signal increases at Neutravidin concentrations as low as 2.5 µg/mL in both media types. Saturation of available binding sites was indicated by signal intensity plateauing at around 10 µg/mL. (Fig. 4).

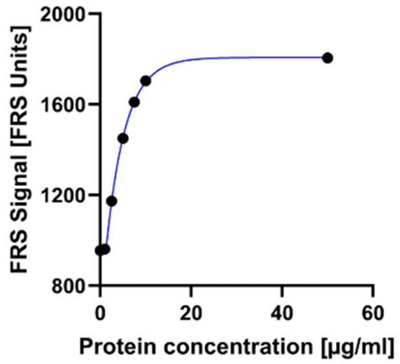

Figure 5- Standard curve for future measurements. Experiments run in sextuplicates to obtain FRS Signal [FRS Units] vs. Protein Concentration [µg/ml]. The concentration of an unknown sample is then interpolated from the standard curve for concentrations between 1.3 µg/ml and 10 µg/ml.

This concentration range was further validated by generating a standard curve (Fig. 5), which was constructed from sextuplicate measurements. The curve shows a strong correlation between FRS signal and protein concentration in the range of 1.3–10 μg/mL, enabling reliable interpolation of unknown sample concentrations within this window.

Application in Bacterial Culture

To evaluate the assay's suitability for the use in biologically active environments, Neutravidin detection was performed in LB medium containing *E. coli*, a commonly used host for recombinant protein production (Fig. 6).

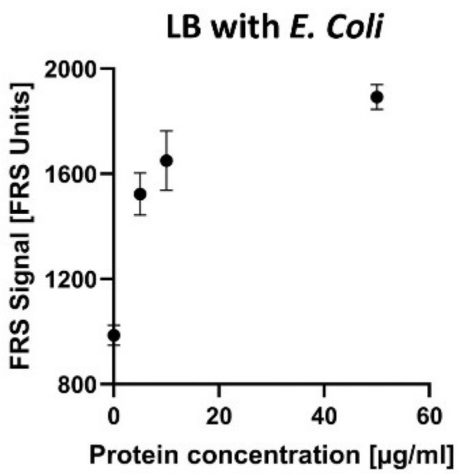

Figure 6 - Measurement in E.Coli Culture. Data is presented as mean±SEM.

Despite the presence of bacterial cells and metabolic by-products, the FRS signal remained robust and comparable to that observed in sterile LB medium. This demonstrates the resilience of the method to potential interferences such as pH shifts, nutrient depletion, or nonspecific binding.

Application in Downstream Processes

In biomanufacturing, downstream processing often involves challenging conditions such as high salt concentrations, which can interfere with conventional methods of detecting protein. Reliable quantification of target proteins under these conditions is critical for controlling the process and ensuring the product quality.

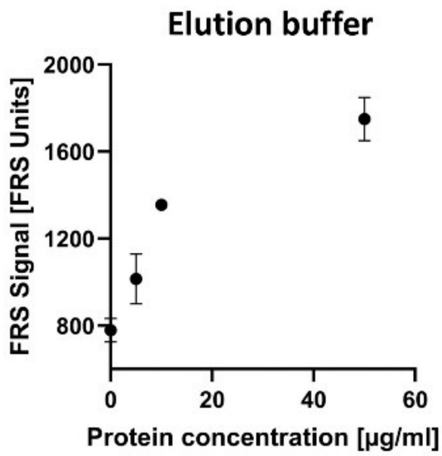

Figure 7 - Detection in high salt environment. Data is presented as mean±SEM.

Importantly, the method also produced reliable results in a high-salt elution buffer (0.4 M NaCl), in which Neutravidin binding could still be detected (Fig. 7). This is attributed to the exceptionally strong biotin–Neutravidin interaction (with a dissociation constant ~10^{-15} M). However, similar performance with other target molecules may depend on the strength of their respective binding interactions.

Conclusion

As demonstrated, the SpheroScan® Explorer can be used as a robust, label-free method for quantifying target proteins in complex media and downstream processing environments with small sample volumes. This method showed high specificity and sensitivity for Neutravidin across various fermentation media, including LB, YPG, and high-salt elution buffers. It remained effective even in the presence of bacterial cultures.

By enabling free-floating microbead measurements in a single-step and eliminating the need for immobilization or extensive sample preparation, SpheroScan® Explorer offers a scalable and efficient solution for real-time protein monitoring in bioprocess development. The value of SpheroScan® Explorer as a tool for improving analytical workflows in precision fermentation and biotherapeutic production is down to these features.

Find out more at: https://fluidect.com/en/bio-reactor-monitoring

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