APPLICATION NOTE

Keywords: multiplexing, Fluorescent Resonator Signature, FRS, protein adsorption kinetics, label-free

Simultaneous Multiplexed Sensing with FRS Technology for Real-time Kinetics Monitoring

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Abstract

We present a multiplexed sensing approach based on FRS technology utilizing whispering gallery mode (WGM) excitations in micrometer-sized polystyrene particles. Optical excitation is provided by a laser source, while spectral readout is performed using an imaging wide-field spectrometer.

In contrast to the current state-of-the-art, where individual particles are positioned on the optical axis for interrogation, our approach enables the spatially resolved detection of particles located off-axis within the field of view. This allows simultaneous acquisition of spectra from multiple sensor particles without the need for sequential positioning.

As a proof-of-concept application, we demonstrate the measurement of non-specific adsorption kinetics. *Klebsiella pneumoniae* carbapenemase (KPC) is an enzyme produced by certain bacteria that confers resistance to nearly all β-lactam antibiotics, including carbapenems, which are often considered a "last resort" treatment for severe infections. KPC-producing bacteria are a major global public health threat, leading to high morbidity and mortality due to limited treatment options.[1] The carbapenemase KPC was selected as analyte due to its representative molecular mass for globular proteins (around 30kDa) and its non-sticky binding behavior, resulting in reversible adsorption and concentration-dependent equilibrium coverage consistent with Langmuir kinetics.

These results highlight the potential of FRS-based sensor arrays for parallel, real-time analysis in biochemical sensing applications.

[1] Arnold RS, Thom KA, Sharma S, Phillips M, Kristie Johnson J, Morgan DJ. Emergence of Klebsiella pneumoniae carbapenemase-producing bacteria. South Med J. 2011 Jan;104(1):40-5. doi: 10.1097/SMJ.0b013e3181fd7d5a. PMID: 21119555; PMCID: PMC3075864.

Introduction

Optical sensing based on whispering gallery modes (WGMs) in dielectric microresonators has emerged as a powerful tool for label-free detection of biomolecular interactions. In particular, polystyrene microspheres in the micrometer size range provide a robust and scalable platform for label-free sensing, where binding events are transduced via refractive index changes at the resonator surface.

In conventional implementations, individual sensor particles are sequentially interrogated by positioning them on the optical axis of the detection system. While this approach enables high sensitivity, it inherently limits throughput and prevents simultaneous observation of multiple sensing events.

To overcome this limitation, we introduce an extended readout concept that enables spatially resolved spectral detection across the entire field of view. By employing an imaging wide-field spectrometer, particles located off-axis can be individually resolved and analyzed without mechanical repositioning. This effectively transforms the system from a single-sensor configuration into a multiplexed sensor array with parallel readout capability.

This approach enables simultaneous monitoring of multiple particles under identical environmental conditions. Importantly, it allows true multiplexing: particles with different surface functionalities can be analyzed in parallel, enabling comparative studies, internal controls, and increased measurement throughput.

To demonstrate the capabilities of this multiplexed sensing approach, we performed a proof-of-concept experiment monitoring non-specific adsorption kinetics on multiple particles simultaneously. Carbapenemase KPC was selected as the model analyte due to its representative molecular mass for globular proteins and its non-sticky adsorption behavior, which allows reversible binding and concentration-dependent equilibrium coverage consistent with a first-order Langmuir adsorption kinetics.

By tracking the spectral shifts of multiple particles in parallel, we were able to capture the adsorption dynamics simultaneously across the sensor array, illustrating both the sensitivity of the FRS-based detection and the advantages of simultaneous multiplexed readout for comparative analysis.

Experimental

Carboxylated polystyrene microspheres (~8 µm diameter) were suspended in phosphate-buffered saline (PBS) and used as sensor particles. Carbapenemase KPC was prepared at 1 µg/mL and 10 µg/mL concentrations in PBS. Particles were imaged using an FRS setup with whispering gallery mode excitation via a laser source and spectral readout through a wide-field imaging spectrometer (FluIDect SpheroScan Explorer). Regions of interest (ROIs) corresponding to individual particles were defined for spectral extraction, enabling simultaneous acquisition of multiple adsorption kinetics within the field of view. All experiments were performed at room temperature under identical solution conditions.

Results & Discussion

Figure 1 shows the adsorption kinetics of carbapenemase KPC on single carboxylated polystyrene particles at concentrations of 1 µg/mL and 10 µg/mL in PBS. Due to the isoelectric point of KPC, significant adsorption is only expected at relatively high concentrations, which is reflected in the observed spectral shifts.

Both concentration profiles exhibit clear, single-phase adsorption behavior consistent with first-order Langmuir kinetics. The equilibrium coverage achieved at 10 µg/mL is approximately four times higher than that at 1 µg/mL, demonstrating the concentration-

dependent adsorption expected for a reversible, non-sticky protein-particle interaction.

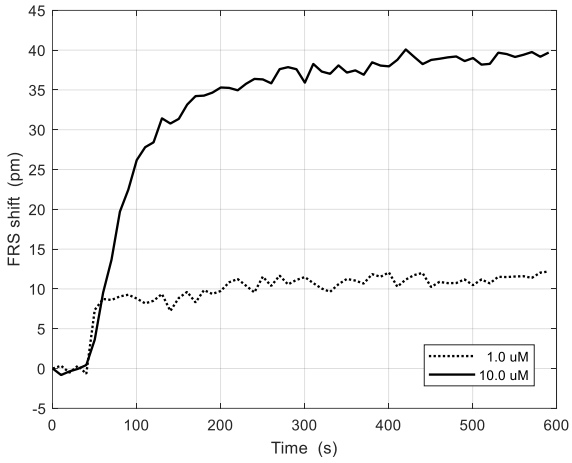

Figure 1. *Adsorption kinetics of carbapenemase KPC on single carboxylated polystyrene particles in PBS at concentrations of 1 µg/mL and 10 µg/mL. Each curve represents the spectral shift of a single particle over time. Both concentrations exhibit single-phase adsorption behavior consistent with first-order Langmuir kinetics. The equilibrium coverage at 10 µg/mL is approximately four times higher than at 1 µg/mL, reflecting the concentration-dependent, reversible adsorption of KPC. These single-particle measurements establish a baseline for subsequent multiplexed experiments.*

These results confirm the sensitivity of FRS-based detection at the single-particle level and establish a baseline for subsequent multiplexed measurements. The well-resolved, monotonic kinetics also highlight the suitability of the system for capturing quantitative adsorption dynamics with high temporal resolution.

Figure 1 also serves as a preparatory reference for multiplexed measurements, as the equilibrium signals may indicate that all beads behave consistently under identical solution conditions. This provides confidence that multiple particles can be simultaneously monitored even when located off the optical axis ("in the field").

Figure 2 displays a raw camera image of the sensor array, with eight particles distributed across the field of view. Each particle produces a distinct spectral signal, and two pairs of particles are positioned in close proximity. Dashed red rectangles indicate the regions of interest (ROIs) corresponding to each spectrum, labeled ROI 1 through ROI 8. This spatial separation enables simultaneous acquisition of all eight spectra without mechanical repositioning, demonstrating the feasibility of true parallel multiplexed sensing on the FRS platform.

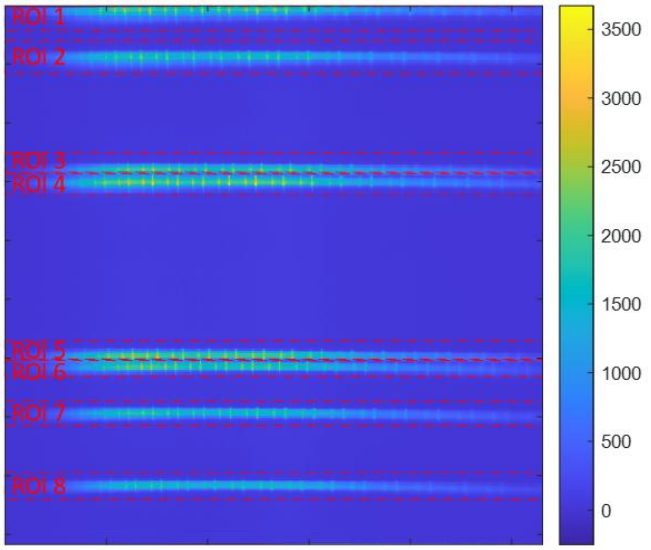

Figure 2. *Raw camera image of the sensor array demonstrating simultaneous multiplexed sensing. Eight carboxylated polystyrene particles are distributed across the field of view, with two pairs positioned in close proximity. Dashed red rectangles indicate the regions of interest (ROIs) used for spectral extraction, labeled ROI 1 through ROI 8. Spatial separation of the ROIs enables parallel acquisition of all eight spectra without mechanical repositioning, illustrating the feasibility of simultaneous multiplexed detection on the FRS platform.*

Figure 3 shows the adsorption kinetics of carbapenemase KPC at a concentration of 10 µg/mL, simultaneously recorded from all eight particles in the field. All particles exhibit similar single-phase adsorption behavior, consistent with first-order Langmuir kinetics. Most particles reach approximately two-thirds of the equilibrium coverage observed for single-particle measurements in Figure 1, while one outlier reaches only about 50%.

The slight variations in equilibrium signal likely reflect particle-to-particle differences in surface accessibility or local microenvironment, rather than any systematic measurement artifact. Importantly, the overall consistency of seven out of eight particles demonstrates the reliability of spatially resolved, simultaneous multiplexed readout. This parallel measurement approach provides immediate internal comparisons between particles under identical solution conditions, highlighting both the robustness of the FRS platform and its potential for multiplexed biosensing applications.

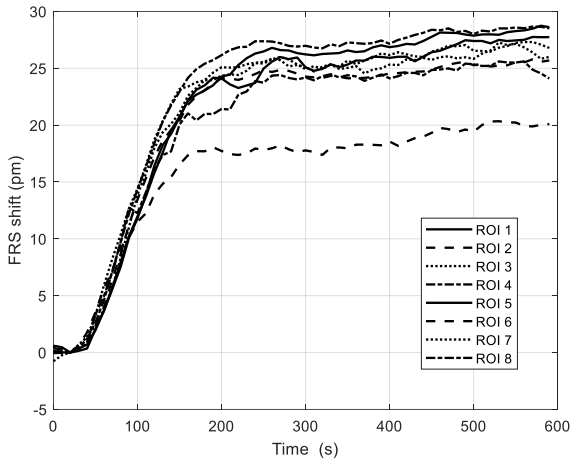

Figure 3. *Simultaneous adsorption kinetics of carbapenemase KPC at a concentration of 10 µg/mL on eight carboxylated polystyrene particles in PBS, measured in parallel using spatially resolved ROIs (as indicated in Figure 2). All particles display single-phase adsorption behavior, with most reaching ~2/3 of the equilibrium coverage observed for single-particle measurements (Figure 1). One particle shows a lower equilibrium signal (~50%), representing minor particle-to-particle variability. The overall agreement among particles demonstrates the feasibility and reliability of simultaneous multiplexed sensing with the FRS platform.*

Conclusion

In this work, we demonstrate the capabilities of FRS-based sensor arrays for simultaneous, multiplexed detection of biomolecular interactions. Single-particle measurements establish the baseline adsorption kinetics of carbapenemase KPC, while parallel readout of multiple particles confirms the reproducibility and robustness of the system under identical solution conditions.

The spatially resolved detection approach enables true multiplexing, allowing particles with different surface functionalities to be monitored simultaneously and compared directly. Minor particle-to-particle variations highlight the importance of internal referencing, but overall the technique provides reliable, high-throughput kinetic measurements.

These results underscore the potential of FRS technology for multiplexed biosensing applications, combining real-time monitoring with parallel acquisition to improve statistical confidence and experimental efficiency.

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