Application Note # 177

# Simultaneous Real-Time Fluorescence and MP-SPR Measurements

*This application note presents simultaneous measurements of fluorescence and MP-SPR during the layer-by-layer assembly of polyelectrolyte multilayers (PEMs; case 1) labeled with Cy5.5 dye and interaction of Alexa Fluor700-labeled antigen with a surface immobilized antibody (case 2). In the case of PEMs, fluorescence data indicates the influence of fluorophore distance from the gold surface on signal intensity, while MP-SPR tracks thickness changes in real time. The simultaneously recorded antigen-antibody fluorescence and MP-SPR data reveal similar concentration-dependent binding.*

## Introduction

BioNavis Multi-Parametric Surface Plasmon Resonance (MP-SPR) is a versatile optical technique enabling real-time, label-free monitoring of molecular interactions and layer properties such as thickness and refractive index. When coupled with fluorescence detection, MP-SPR gains enhanced capabilities for studying fluorescently labeled molecule integrated systems as well, such as polyelectrolyte multilayers (PEMs) and antigen-antibody assays. This application note presents a demonstration of simultaneous fluorescence and MP-SPR measurements using Cy5.5-labeled PEMs (**case 1**) constructed via layer-by-layer deposition, and AF700-labeled antigen-antibody interaction (**case 2**). The combined approach reveals insights into fluorophore proximity effects, layer architecture, and dual-signal analysis.

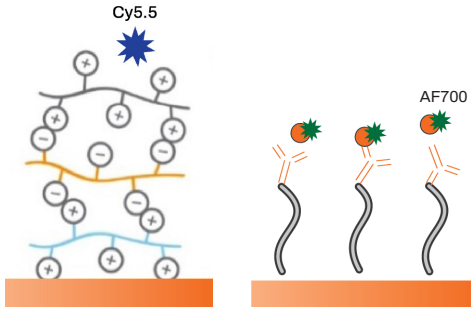


**Figure 1.** Case 1 (left): Layer-by-layer assembly of polyelectrolyte multilayers. Case 2 (right): antigen-antibody interactions. Fluorescent labels are incorporated in these systems

## Materials and methods

The experiments utilized BioNavis MP-SPR Navi™ 210A VASA system (670 nm wavelength for real-time MP-SPR angular position change monitoring (sensogram)) equipped with a flow cell dedicated for fluorescence experiments (SPR323-F1). In **case 1**, polyelectrolyte multilayers were constructed on a pre-coated polyethyleneimine (PEI) gold sensor using alternating layers of poly(allylamine hydrochloride) (PAH) and poly(styrene sulfonate) (PSS) and Cy5.5 fluorescent dye in 150mM NaCl.

The typical deposition sequence was Cy5.5 + PSS + PAH duos. MP-SPR angular shifts were monitored simultaneously during each layer deposition and fluorescence signal was recorded during the whole measurement period. Multiple flow cell channels (FC1–FC2) were used to enable parallel data acquisition, where in channel 1 Cy5.5 was injected and in channel 2 omitted. In **case 2**, an Alexa Fluor700 NHS ester-labeled albendazole-BSA conjugate (or AF700-labeled antigen) was injected in channel 1 over an albendazole-antibody functionalized (through standard EDC/NHS coupling procedure) BioNavis Dextran (BND) like sensor slide at 10x and 100x diluted concentrations (initial concentration: 50 µg/mL) in PST-T buffer (pH = 7.4).

The excitation laser (635 nm wavelength; 2.7 mW) was generated with an external light source and irradiated from the backside of the prism through an optical fiber integrated into the instrument (Figure 2). Fluorescence signal was recorded through an optical fiber located in flow cell's channel 1 connected to an external photomultiplier tube (PMT; H11890-01 from Hamamatsu). During an angular measurement, the integration time of the PMT was set to correspond to MP-SPR measurement cycle time.

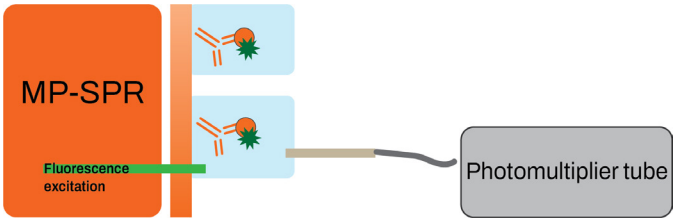


**Figure 2.** Fluorescence-coupled MP-SPR measurements. An optical fiber is embedded in channel 1 of the flow cell for fluorescence emission signal monitoring. Excitation is performed from the backside of the prism.

## Results and discussion

The integration of fluorescence and MP-SPR provided complementary insights. As successive polyelectrolyte layers were deposited (case 1), positive MP-SPR angle shifts (Figure 3, left) indicated increasing layer thickness (2.5±0.3 nm per polyelectrolyte layer bilayer as determined with LayerSolver™ software), while fluorescence intensity slightly varied depending on the position of the Cy5.5-labeled layer relative to the gold surface and the last polyelectrolyte layer (Figure 3, right).



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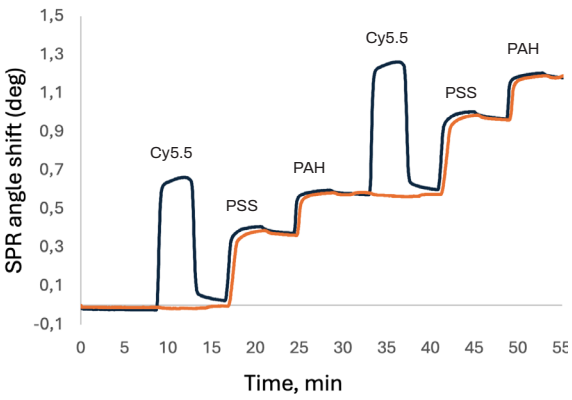


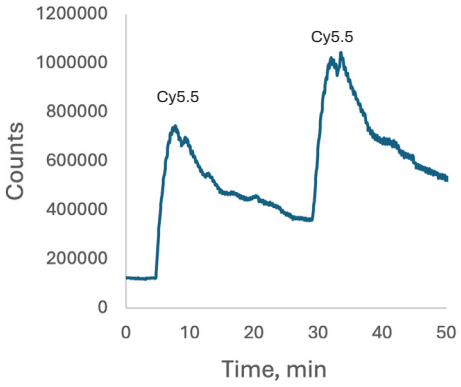
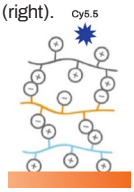
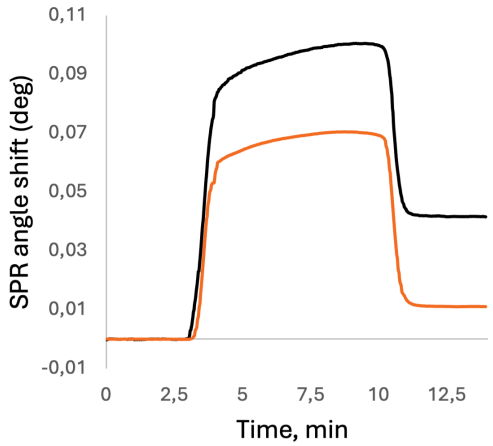




**Figure 3. case 1.** Cy5.5 (channel 1 - blue) and Polyelectrolyte (PSS and PAH; both channels) injections monitored in real-time with MP-SPR (left) at 670nm wavelength. Channel 2 (orange) was omitted from Cy5.5 injections. Fluorescence intensity recorded during the SPR measurement at the same time (right).




In **case 2** (Figure 4), AF700-labeled antigen was injected at two concentrations (10x and 100x). Both MP-SPR responses (Figure 4, left) and fluorescence intensity (Figure 4, right) recorded over time coincided well with each other, showing AF700 labeled antigen binding to an antibody functionalized sensor surface in a concentration-dependent manner. The measured data indicates less labelled antigen amount bound on a surface when 100x dilution was used compared to the 10x diluted sample. The affinity constant $K_{D1}$ for antibody-antigen interaction were estimated to be $2.64 \cdot 10^{-7}$ M from MP-SPR sensograms by using a bivalent binding model found in the dedicated TraceDrawer™ software.



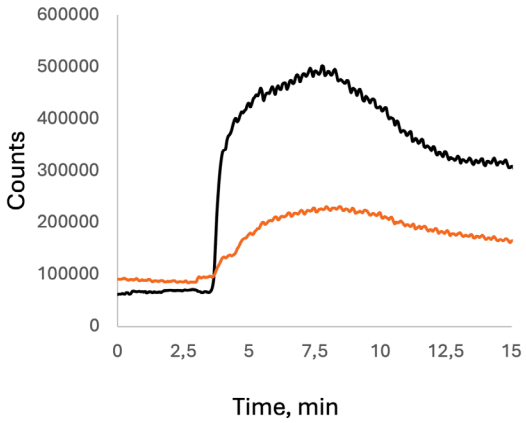
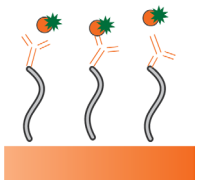




**Figure 4. case 2.** AF700 labeled antigen (diluted 10 (black ) and 100 times (orange)) binding to a surface-immobilized antibody is measured in real-time with MP-SPR (left). Fluorescence intensity recorded during the MP-SPR measurement at the same time (right).



## Conclusions

Simultaneous MP-SPR and fluorescence detection offers an advantageous method for studying complex thin film systems such as polyelectrolyte multilayers. The dual-mode data acquisition allows real-time correlation between layer growth, fluorophore localization, and optical response. The demonstrated Cy5.5-labeled PEM system and AF700 labelled antibody-antigen assay validates the effectiveness of fluorescence-coupled MP-SPR in multilayer analysis and paves the way for advanced biosensing and nanostructure characterization applications.

### Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 220A NAALI or 210A VASA dedicated fluorescence flow cell (SPR323-F1) + 635 nm wavelength fiber-coupled laser source + Photomultiplier tube (PMT; H11890-01 from Hamamatsu was used here)

Sensor surface: SPR102-AU (case 1), SPR102-BND-2D (case 2)

Software: MP-SPR Navi™ Control, DataViewer, TraceDrawer™ for MP-SPR Navi™ and LayerSolver™ for MP-SPR Navi™

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