ENHANCED OPTICAL DETECTION IN POROUS MICROFLUIDIC SENSORS BY REFRACTIVE INDEX MATCHING
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ABSTRACT
Optical sensor signal enhancement is demonstrated in porous volumetric microfluidic structures by infusing refractive index matching solutions to minimize light scattering. Fluorescent signal in a capillary immobilized polymer monolith increased 3.5X for chemical detection and 1.6-2.6X for IgG detection. Transmitted light increased from 56% to 96.8% for a thermoplastic constrained silica bead packed bed. A transmittance based immunoassay using silver enhancement of a packed bed yielded a detection limit of 0.1 ng/mL IgG with a 5-log dynamic range. The simple technique enables improved detection limits and sensitivity for microfluidic assays leveraging porous volumetric sensing elements for optical readout.

KEYWORDS: Index Matching, Optical Detection, Biosensor, Silver Enhancement

INTRODUCTION
Porous volumetric detection elements are advantageous for microfluidic flow-through assays due to decreased diffusion lengths and higher reaction site density, reducing detection limits and total assay time compared to conventional planar sensor surfaces [1]. However, for assays requiring optical readout, light scattering due to pores within the volumetric matrix presents an inherent limitation that significantly reduces signal. For fluorescence based assays the excitation and emission wavelengths are both affected minimizing the signal and effective volume probed. For transmission-based colorimetric assays light is absorbed by the matrix and does not fully reach the detector. In this work, we demonstrate a simple technique for enhancing optical performance of porous volumetric sensors based on refractive index matching fluid infusion through the capture zone, thereby minimizing light scattering and enhancing optical detection for both transmission and fluorescence based assays.

EXPERIMENTAL
Here we demonstrate significant optical signal enhancement using a variety of porous matrices integrated into both glass capillaries and thermoplastic microfluidic channels using a refractive index matching solution (Figs. 1 and 2). Fluorescence signal enhancement in glass capillary is achieved using glycidal methacrylate (GMA) monolith structures covalently functionalized with fluorescent glutaraldehyde or goat anti-rabbit IgG [1]. For the direct immunoassay the monolith was functionalized with 50 μg/mL goat anti-rabbit IgG before infusion with serial dilutions of fluorescein (FITC) tagged Rabbit IgG. The fluorescence intensity was measured during water and GMA infusion using a fluorescence microscope.

Transmittance based signal enhancement was demonstrated in thermoplastic devices using a porous silica matrix, with aqueous sucrose solutions used for index matching. The matrix consists of a packed bed of 20 μm diameter silica beads embedded in a solvent bonded cyclic olefin copolymer (COC) chip. The optimal index matching solution was determined by infusing different aqueous sucrose solutions and measuring the transmittance. Transmittance (T) was defined as the intensity of a packed bed region divided by a nearby featureless background area. Next the silica beads were functionalized with serial
dilutions of human IgG or bovine serum albumin for control using silane chemistry and a BS3 crosslinker. After packing the bead bed, gold nanoparticle tagged anti-human IgG was infused, enhanced with silver acetate and hydroquinone [2] to grow larger silver clusters within the capture matrix, and rinsed with water before sucrose solution infusion, for a total assay time of 45 min. Absorbance, defined as 1-T, was measured before and after silver enhancement, and following the introduction of index matching solution.

RESULTS AND DISCUSSION

Optimal index matching fluid was determined by infusing aqueous sucrose solutions with refractive indices (n) ranging from 1.333 to 1.475 through the packed bed region (Fig. 3). The transmittance increased as sucrose concentration increased to 68% sucrose (w/w) which has a refractive index roughly equivalent to silica (Fig. 3). The transmittance then decreases as n increases above 1.46 indicating light scattering.

The nominal fluorescence intensity (FI) for non-functionalized monolith in air (n~1) was 480, decreasing to 357 during PBS buffer (n~1.33) infusion and 250 during GMA (n~1.45) infusion. FI of the glutaraldehyde functionalized monolith increased 3.5x after GMA infusion (Fig. 4). The resulting FI after FITC-IgG infusion and water rinsing increases 2.6X, 1.7X, and 1.6X for IgG concentrations of 0.1, 1, and 10 ng/mL with GMA infusion (Fig. 4). Overall the signal amplification was significant but limited possibly by IgG removal during GMA infusion or uneven pore distribution causing light scattering in the monolith.

Enhancement of transmission based measurements is shown using silver enhancement to darken a silica bead packed bed after gold-Ab infusion (Fig. 5). A minimum detectable signal of approximately 0.1 ng/mL (p ≥ 0.003) was achieved following index matching whereas the minimum detectable signal with water rinsing alone after silver enhancement was 10 μg/mL (p ≥ 0.05). Saturation of the index matching absorbance signal is observed at concentrations greater than 1 μg/mL, thus defining an overall
dynamic range of ~ 5-logs. In contrast the dynamic range without index matching consisted of one dilution only (10 μg/mL). Within the index matching dynamic range from 0.1 ng/mL to 1 μg/mL each serial dilution was found to be well differentiated (p ≥ 0.05) with a log-linear trendline (R²=0.993) (Fig. 5). The data represents a 100x improvement over a similar planar immunoassay using a functionalized polycarbonate surface for antibody immobilization [3].

![Image of absorbance change](image)

Figure 5: Change in absorbance before and after index matching solution infusion (left). N≥3 for all dilutions except n=2 for 0.01 ng/mL with error bars +/- 1 SD. A log linear trendline is shown from 0.01 ng/mL to 1 μg/mL with R²=0.993 (lower right). The fabricated microfluidic chip is shown (top right) after silver enhancement of beads conjugated with 480 and 4.8 ng/mL of human IgG including a close-up of the enhanced region (top right).

CONCLUSION
This work demonstrates that refractive index matching provides a universal method for enhancing optical signal from microfluidic assays employing porous volumetric detection elements. This simple technique offer a straightforward route to improving the detection limits of microfluidic assays leveraging integrated porous sensing elements, with key benefits including rapid assay times and sensitive volumetric analyte capture. The index matching technique is currently being applied to the development of simple point-of-care devices to improve detection limits and reduce total assay times.

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REFERENCES

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