Electronic Supplementary Material (ESI) for Lab on a Chip.

Sensitive Tear Screening of Diabetic Retinopathy with Dual Biomarkers Enabled by a Rapid Electrokinetic Patterning Platform

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Signal Enhancement with REP

The signal was enhanced by two steps in this study. The primary enrichment dealt with concentrating fluorescent dyes on functionalized microbeads through sandwiched immunocomplexes. The secondary enrichment dealt with concentrating fluorescent microbeads by REP (Fig. S1A). By measuring their mean fluorescent intensities, the values for cases (i), (ii), and (iii) are 120, 58, and 21, respectively (Fig. S1B). The REP-driven signal enhancement can reach nearly six-fold higher than that of no enrichment.

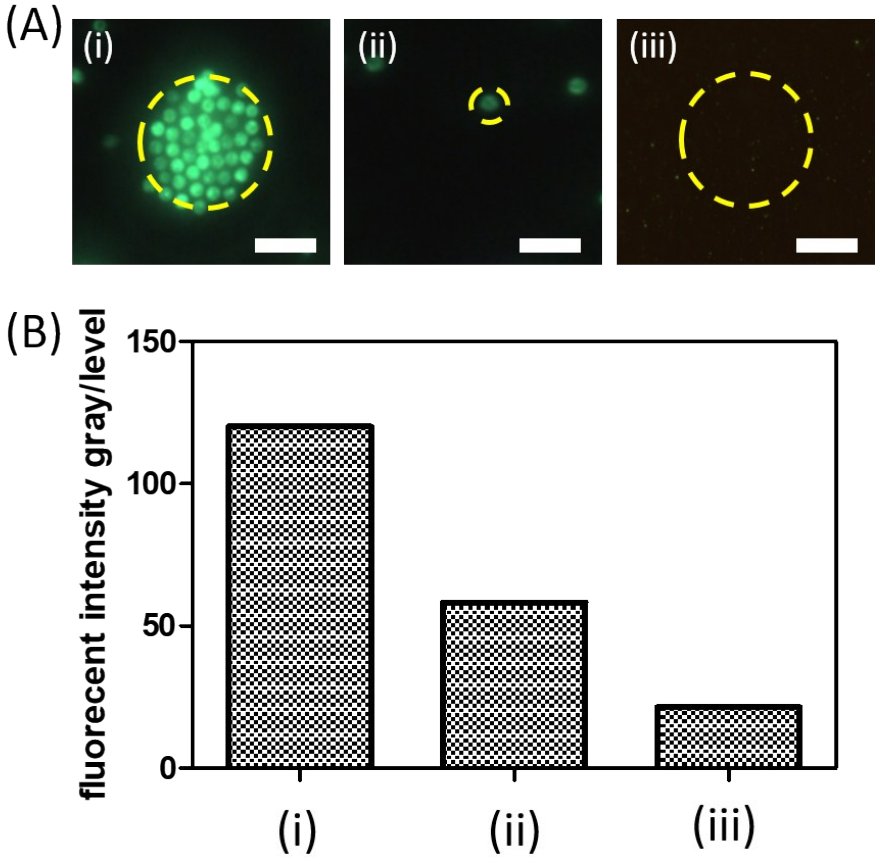

Fig. S1 Signal enhancement at different stages. (A) Fluorescent images of (i) concentrated microbeads with sandwiched immunocomplexes (secondary enrichment), (ii) dispersed microbeads with sandwiched immunocomplexes (primary enrichment), and (iii) dispersed fluorescent dyes (no enrichment). The dotted circle defines where the signal intensity is measured. The scale bar denotes 15 μm. (B) Mean fluorescent intensities of the three cases (i), (ii), and (iii).

Determination of a concentration threshold based on the ROC curve

24 clinical tear samples, including 8 healthy subjects, 13 patients with PDR, 2 patients with NPDR, and 1 patient with glaucoma, were collected at the NCKUH by our collaborative ophthalmologist. For simplicity, the patients were only divided into two groups, representing non-PDR and PDR. The classifications were implemented according to their clinical diagnoses. The concentrations of LCN1 and VEGF were measured separately by means of our optoelectrokinetic platform (REP). Subsequently, concentration levels from 100 pg/mL to 10 μg/mL were tested for each biomarker in order to obtain their corresponding specificity and sensitivity values. At last, an ROC curve was then plotted based on the specificity and sensitivity values (Fig. S2). Since the upper-leftmost corner stands for the ideal point to achieve, the optimal point in the real case will be located at the curve that connects the shortest distance

between the curve and the corner. As a result, the concentration of the optimal point will be determined as the threshold for the biomarker. Notably, the threshold can be further optimized by repeatedly implementing this process with more clinical data.

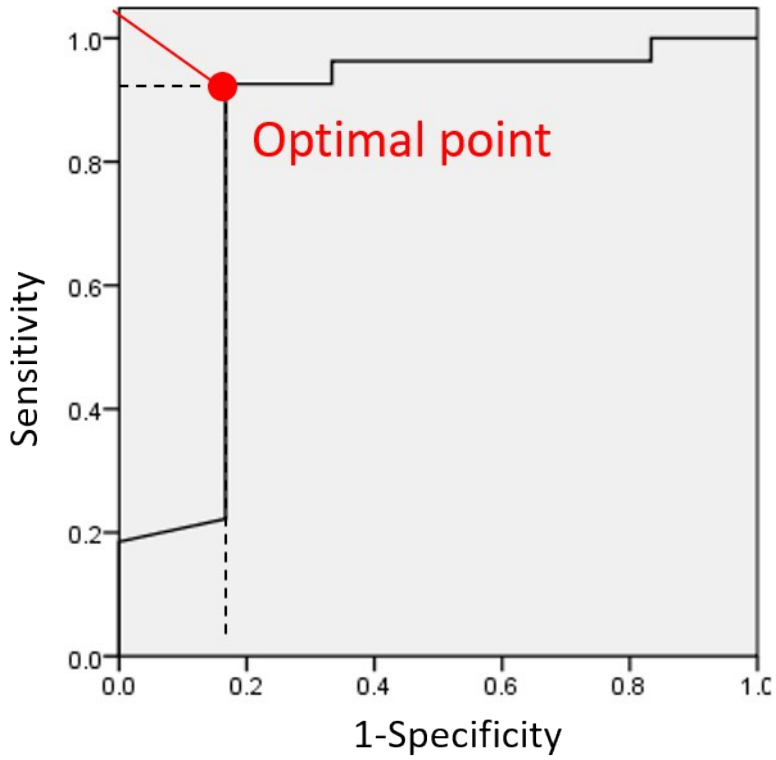

Fig. S2 Conceptual illustration of how an optimal threshold was determined in an ROC curve.