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*Supporting Information*

# Single-particle Fibrinogen Detection Using Platelet Membrane-Coated Fluorescent Polystyrene Nanoparticles

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**Fluorescence colocalization analysis**

Pearson's correlation coefficient (PCC) and Mander's correlation coefficient (MCC) are two most commonly used operators for quantitative description of fluorescence colocalization.[1, 2] The characteristics and applications of these two parameters are different.[3]

1) PCC is applicable to the situation where the ratio of two kinds of fluorescence is similar. It's most suitable when the intensities of two kinds of fluorescence exhibit a single linear relationship. Fluorescence colocalization analysis using PCC is simple and fast without preprocessing;

2) By contrast, MCC is suitable for a nonlinear proportional relationship between two kinds of fluorescence. Meanwhile, it's more suitable for the colocalization analysis of 3D images. However, background correction is needed to eliminate the influence of background signal using MCC.

After comparison, PCC is suitable for the analysis in our study.[4] When PCC is used for quantitative description of fluorescence colocalization analysis, it can be expressed as the ratio of covariance to the product of standard deviations:

$$PCC = \frac{\sum_{i=1}^{n}(R_i - \overline{R}) \times (G_i - \overline{G})}{\sqrt{\sum_{i=1}^{n}(R_i - \overline{R})^2 \times \sum_{i=1}^{n}(G_i - \overline{G})^2}}$$

Where $R_i$ represents the fluorescence intensity of a pixel in red channel. $\overline{R}$ represents the mean value of red fluorescence intensities. Similarly, $G_i$ represents the fluorescence intensity of a pixel in green channel. $\overline{G}$ represents the mean value of green fluorescence intensities. The value of PCC is between -1 and 1. Within this range, values closer to 1 indicate stronger correlation.

Meanwhile, scatter plot is also an important method for the description of fluorescence colocalization. The coordinates of each point in the scatter plot correspond to its fluorescence intensity in green and red channel. With the increase of colocalization events, the scatter plot tends to be diagonal. When two kinds of fluorescence overlap completely, the scatter plot is a straight line with a slope of 1 ($y = x$).

In addition, some studies also plot profiles to confirm the colocalization phenomenon. When green and red fluorescence coincide well, the change of fluorescence intensity on the profile will have a similar trend. On the contrary, the change of fluorescence intensity will show an opposite trend if there is no overlap between green and red fluorescent spots.

PCC and scatter plot can be acquired by some plugins of ImageJ, such as Coloc 2, Colocalization Threshold and Colocalization Finder. In this study, MATLAB was used to draw the scatter plot and ImageJ was used to plot the profile.

**SEM images of fluorescent polystyrene nanoparticles**

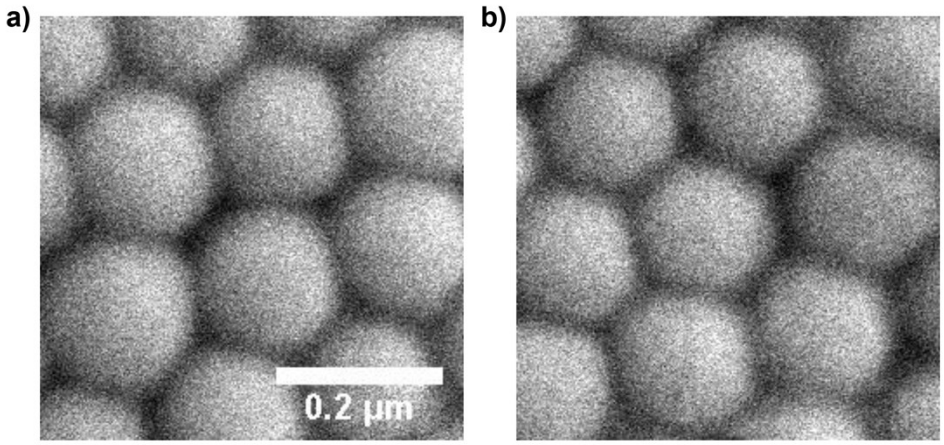


**Figure S1.** SEM images of a) green and b) red fluorescent polystyrene nanoparticles.

**Optimization of coating conditions**

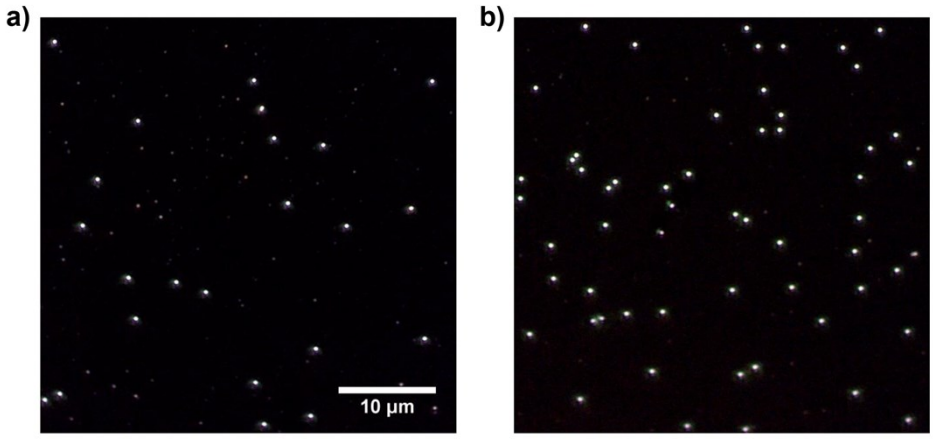


**Figure S2.** Optimization of the ratio of membrane to PS cores. DFM images when the volume ratio of platelet vesicles to PS cores (20 $\mu$g/mL) is a) 2:1 b) 10:7.

**Determination of probe concentration**

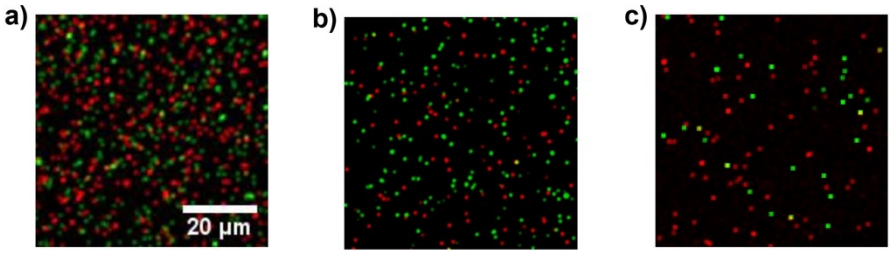


**Figure S3.** The determination of probe concentration. a) The probe concentration is 20 $\mu$g/mL (too dense). b) 5 $\mu$g/mL (moderate density) and c) 2 $\mu$g/mL (too sparse).

**The change of gray value on profile**

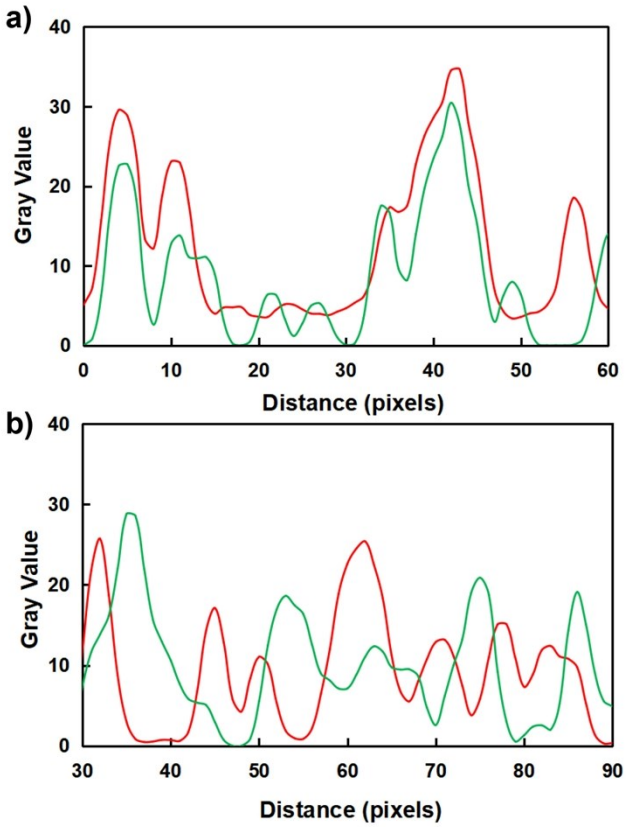


**Figure S4.** The change of gray value in the same rectangular areas from fluorescent images. a) With 300 $\mu$g/mL fibrinogen. b) Without fibrinogen (The green line represents the change of gray value in the green channel image; The red line represents the change of gray value in the red channel image).

**Comparison of LOD**

| Method | LOD (μg/mL) | Reference |
|---|---|---|
| **Enzyme-linked immunosorbent assay (ELISA) based method** | **65** | ***Am. J. Hematol.*, 1996, 51, 186-191** |
| **Fibrinogen detection using Clauss method** | **300** | ***Thromb. Res.*, 2010, 126, e428-e433** |
| **Fibrinogen detection using PT-derived method** | **970** | |
| **Surface Plasmon Resonance (SPR) biosensor** | **500** | ***Biosens. Bioelectron.*, 2011, 28, 304-307** |
| **Gold nanoparticle-based colorimetric assays** | **3.45** | ***Biosens. Bioelectron.*, 2011, 26, 3160-3166** |
| **Direct detection of fibrinogen using transistors** | **500** | ***Appl. Phys. Lett.*, 2017, 111, 082106** |
| **Erythrocyte membrane (EM)-blanketed biosensor based on LSPR** | **1** | ***Biosens. Bioelectron.*, 2019, 135, 216-223** |
| **A microfluidic paper-based analytical device** | **1270** | ***Lab Chip*, 2020, 20, 2724-2734** |
| **SPD method using PNPs** | **3.9** | **This work** |

**Table S1** Comparison of LOD with several representative fibrinogen detection methods

**Image processing and analysis**

Firstly, the fluorescent images of green channel, red channel and merge channel were processed by Gaussian blur ($\sigma$ = 0.6) to reduce image noise. Afterwards, smoothing was applied to images in order to make the edges of points look smoother. At last, we used contrast enhancement to distinguish the signal from the background (non-microbead signal).[5]

In order to observe the number of colocalization events more intuitively, we used a MATLAB program to extract the overlapped points in merged images. First of all, color images were transformed to binary images (automatic threshold determination). Herein, A is used to represent the green channel binarization image and B is used to represent the red channel binarization image. Therefore, the coincidence part of A and B can be expressed as:

$$A \cap B = A \cup B - (A - A \cap B) - (B - A \cap B) = A \cup B - (A - B) - (B - A)$$

According to this formula, the overlapped parts of two channels can be extracted using union and subtraction operations between binarization images. Finally, a loop statement was used to determine whether the signal exists in order to add pseudo color.

In addition, scatter plots were used to characterize the colocalization phenomenon using MATLAB. The RGB values of each pixel in the merge channel image were obtained and assigned to three 512 × 512 matrices (R, G and B). Matrix R and matrix G were transformed to 262144 × 1 column vectors. The two vectors are used as abscissa and ordinate respectively to draw scatter plot.

**Supplementary references**


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