Supporting Information for

Label-free single-particle imaging approach for ultra-rapid detection of pathogenic bacteria in clinical samples

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1. Supporting Figures

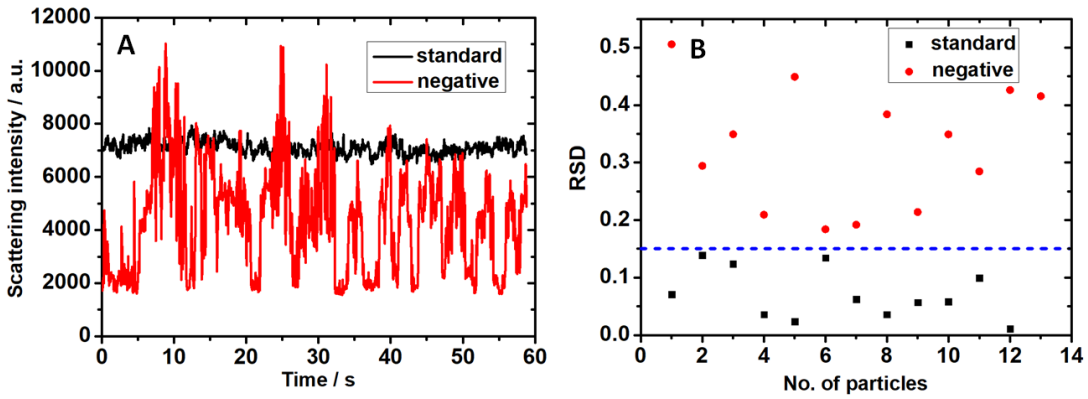

Fig. S1. (A) Representative scattering intensity curves of single GBS bacterium in standard GBS samples (black) and aggregates in negative sample (red) and (B) their corresponding relative standard deviations from different particles.

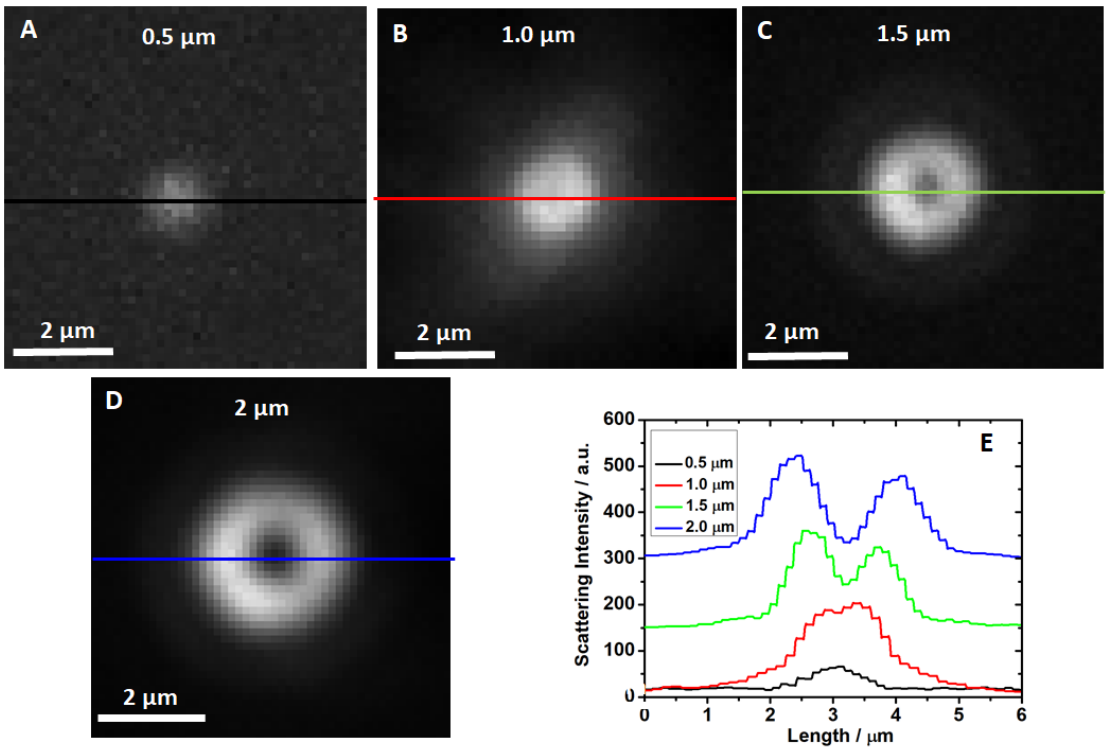

Fig. S2. (A-D) The reflection enhanced dark field scattering pattern of spherical particles with different particles sizes; (E) and their scattering intensity distributions along the lines.

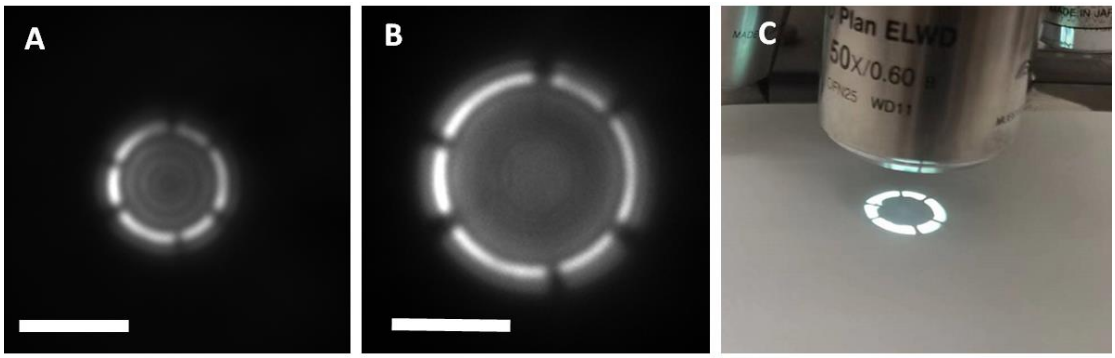

Fig. S3. Dark field images of (A)10 µm & (B) 15 µm particles; (C) the light projection of the dark field illuminator; the scale bar =10 µm.

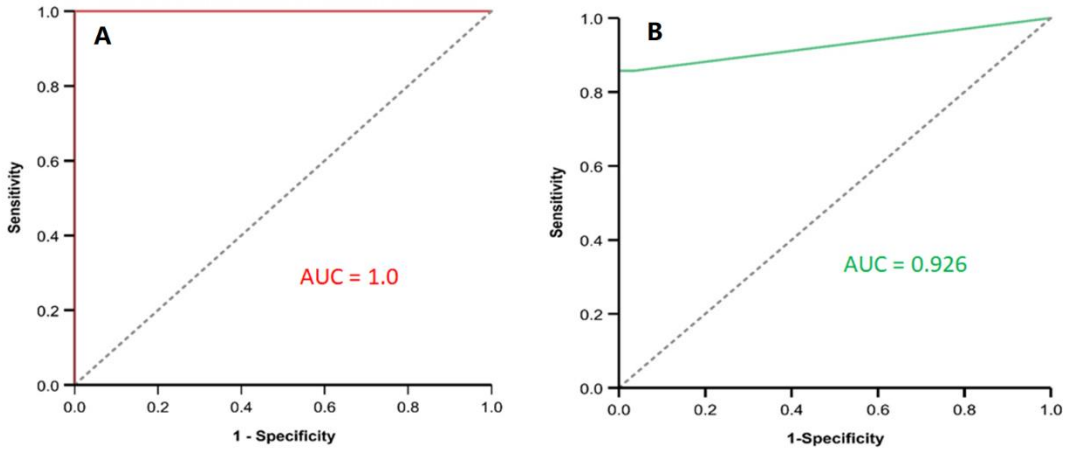

Fig. S4. ROC curves of REDFSM - the single particle imaging approach, using (A) bacterial culture (BC) method, and (B) PCR method as references.

Table S1. The corresponding value (vs. BC) of ROC curve in Fig. S4A.

REDFSM Value (counts/5minutes)	Sensitivity	1-Specificity	Specificity	Youden index
-1.0000	1.000	1.000	0	0
.0850	1.000	.047	0.953	0.953
.2500	1.000	.012	0.988	0.988
.5000	**1.000**	**.000**	**1**	**1**
1.1700	.800	.000	1	0.8
1.8350	.600	.000	1	0.6
2.1650	.400	.000	1	0.4
2.5000	.200	.000	1	0.2
3.6700	.000	.000	1	0

The threshold for REDFSM vs. BCM is 0.5 counts/5minutes, with 100% specificity and 100% sensitivity (Youden index=1.0).

Table S2. The corresponding value (vs. PCR) of ROC curve in Fig. S4B.

REDFSM Value (counts/5minutes)	Sensitivity	1-Specificity	Specificity	Youden index
-1.0000	1.000	1.000	0	0
0.0850	0.857	0.036	0.964286	0.821429
0.2500	**0.857**	**0.000**	**1**	**0.857143**
0.5000	0.714	0.000	1	0.714286
1.1700	0.571	0.000	1	0.571429
1.8350	0.429	0.000	1	0.428571
2.1650	0.286	0.000	1	0.285714
2.5000	0.143	0.000	1	0.142857
3.6700	0.000	0.000	1	0

The threshold for REDFSM vs. PCR is 0.25 counts/5minutes with 100% specificity and 85.7% sensitivity (Youden index=0.857).

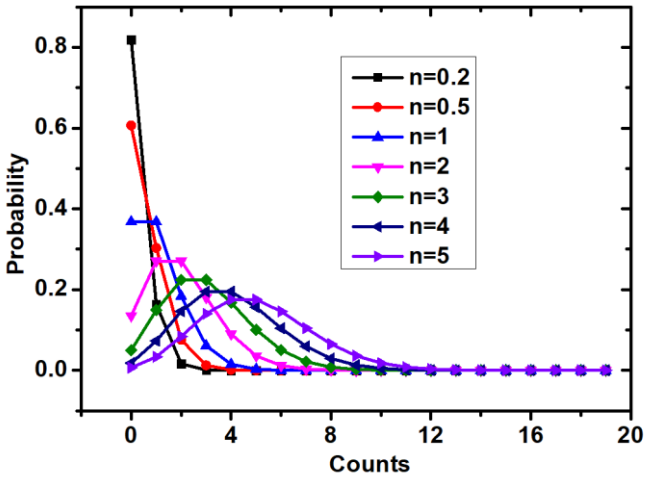

Fig. S5. Poisson distribution curves for the probability of counts in each screening with different averaged number (n, counts/screening time).

Poisson distribution is the discrete probability distribution of the number of events occurring in a given space (or time), if these events occur with a known constant. Note that, the stochasticity can not be ignored at extremely low concentration as shown in

Fig. S5. n=1 represents the observation of one single bacteria in average for each screening. But it has very high probability (37%, black line) to get 0 count in one screening, resulting in the false negative results. However, the false negative results can be eliminated with higher averaged value, e.g. n=5, the probability of false negative results can be decreased down to <1%. So n=5 is be able to largely eliminate the false negative results. On the other hand, even n<1, it is also possible to detect a bacterium with lower probability as shown in Fig. S5. So the stochasticity makes the direct comparison of detection limit at low concentration difficult.

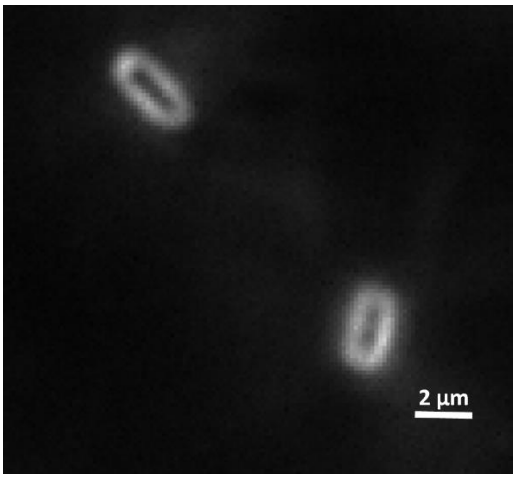

Fig. S6. The reflection enhanced dark field scattering pattern of E-coli.

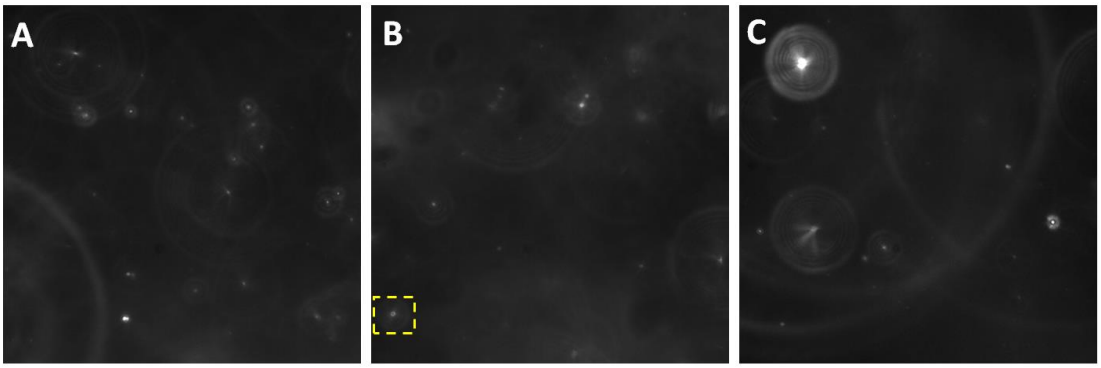

Fig. S7. REDFSM image of (A) the unrine samples and (B) saliva samples (20 time diluted); (C) blood serum samples (5 time diluted). The yellow square in B represents the capture of a Streptococcus bacterium.

Table S3. The corresponding Streptococcus concentration calculated by REDFSM (counts/μL) in Fig. S7, and compared with the reference value.

Samples / Volunteers	Urine	Saliva
No.1	1.0×10^{2}	4.6×10^{3}
No.2	$< 10^{1}$	2.3×10^{3}
No.3	5.0×10^{1}	6.6×10^{2}
Reference value	$0\text{-}3.8\times10^{2}$ [a]	$0\text{-}1\times10^{4}$ [b]

a. Data obtained from UF-1000i SYSMEX.

b. A. Yano, et al. *FEMS Microbiology Letters*, **2002**, *217*, 23-30.

2. Supporting movies

Supporting movie S1.

The reflection enhanced dark field scattering imaging of standard GBS bacteria in free solution.

Supporting movie S2.

The reflection enhanced dark field scattering imaging of GBS negative clinical samples in free solution. Note that most of the small particles in the clinical samples can hardly been observed in this movie due to their weak scattering intensity compared with the large aggregates or GBS under the same scale bar.

Supporting movie S3.

The reflection enhanced dark field scattering imaging of GBS positive clinical samples in free solution with a flow rate of 2 μm/s. Note that the particles appeared from 45s to 01:08s was considered as the single GBS bacterium in the positive sample.

Supporting movie S4.

The reflection enhanced dark field scattering imaging of a fast moving bacterium in blood serum (5 times diluted by PBS).