Supplementary information

Simultaneous and ultrasensitive detection of multiple microRNAs by single

molecule fluorescence imaging

Hongding Zhang,^{†a,b} Xuedong Huang,^{†a} Jianwei Liu, ^{*a} Baohong Liu^{*a}

^a Department of Chemistry, Shanghai Stomatological Hospital, State Key Laboratory of Molecular Engineering of Polymers and Institute of Biomedical Sciences, Fudan University, Shanghai 200433, P. R. China

^b College of Chemistry and Chemical Engineering, Xinyang Normal University, Xinyang 464000, P. R. China

*Corresponding author

Baohong Liu, Email: <u>bhliu@fudan.edu.cn</u>, Fax: +86-21-65641740 Jianwei Liu, Email: <u>jianweiliu@fudan.edu.cn</u>.

Table of content:

Supporting Figures S1-4, Table S1.

[†] These authors have contributed equally to this work.



Fig. S1 Represents traces of Cy3 (A) and Cy5 (B).



Fig. S2 Effect of the S9.6 antibody concentration (from 100 ng/mL to 0.05 ng/mL) on the assay. Scale bar represents 5 μm.



Fig. S3 Optimization of the binding time of IgG and S9.6 antibody.



Fig. S4 Simultaneous detection of miRNA-21 and miRNA-122 with different levels.

Table ST Nucleonde sequences of mixings and cDNAs used in this assay.	
Name	Sequence (5'-3')
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-122	UGG AGU GUG ACA AUG GUG UUU G
Single-base mismatch	UAG CUU AUC AGA CUG AUG AUG A
miRNA-21 (SM miRNA-21)	
Single-base mismatch	UGG AGU GAG ACA AUG GUG UUU G
miRNA-122 (SM miRNA-122)	
Three-base mismatch	UAA CUU AUC AGA AUG AUG UAG A
miRNA-21(TM miRNA-122)	
Three-base mismatch	UGG UGU GUG AGA AUG GUA UUU G
miRNA-122 (TM miRNA-122)	
miRNA-143	UGA GAU GAA GCA CUG UAG CUC A
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G
miRNA-16	UAG CAG CAC GUA AAU AUU GGC G
cDNA1	Cy3-TCA ACA TCA GTC TGA TAA GCT A
cDNA2	Cy5-C AAA CAC CAT TGT CAC ACT CCA

 Table S1 Nucleotide sequences of miRNAs and cDNAs used in this assay.