Electronic Supplementary Material (ESI) for Analyst

An ultra-sensitive DNA assay based on single-molecule detection coupled with hybridization accumulation and its application

Benhui Sui, Li Li, Lu Li and Wenrui Jin*

School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, China

**Corresponding author. E-mail: jwr@sdu.edu.cn. Fax +86-531-8856-5167.*

Supplementary material

Experimental

Materials

Octadecylamine-coated CdSe QDs with a maximum emission at ~650 nm from NN-Labs (Fayetteville, AR, USA), 3-glycidoxypropyltrimethoxysilane (GOPS, ≥98%), mercaptopropionic acid (MPA, >99%), BSA and Tween-20 from Sigma-Aldrich (St. Louis, MO, USA), *N*-hydroxysuccinimide (NHS) from Huifeng Chemical Industry Ltd. (Weinan, China) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, >99%) from Shanghai Medpep Co., Ltd. (Shanghai, China), Tris from Amersco (Solon, OH, USA), sodium dodecyl sulfate (SDS) from Sangon Co., Ltd. (Shanghai, China), diethypyrocarbonate (DEPC) from Bio Basic Inc., (Ontario, Canada), and CellAmp^{TM} whole transcriptome amplification kit and ribonuclease H (RNase H) from TaKaRa Biotech. Co., Ltd. (Dalian, China) were used in this work.

The synthetic single-stranded tDNA (5′-CTC CAA ATG TAG GAG CTA TCG TT-3′), capture DNA (DNA***c***, NH_2-5′-T_{20} AAC GAT AGC TC-3′), detection DNA (DNA***d***, 5′-CTA CAT TTG GAG T_{20}-3′-NH_2′), noncomplementary DNAs (5′-CTC CAA ATG TAG GAG CTA TCG TT-3′, 5′-TAC TTG GAG CCA CTA TCG ACT ACG C-3′, 5′-GGC AAG CCG ATA ACG GGA TTA-3′, 5′-AAG CCA TGA AGC GGC TTA TGA TTC TTA CCG CCC ACT-3′, 5′-GCG AGG ATT TGA CGA AAG CGC ACC TTA AAG-3′) and single base mismatched DNA (5′-CTC CAA ATG TAG GAG ***G***TA TCG TT-3′) from Sangon Co., Ltd. (Shanghai, China). 1.00×10^{-4} mol/L stock solutions for each DNA were prepared by centrifugating for 1 min at 1000 rpm and dissolving in an appropriate volume of TE buffer, and stored at -20 ^{o}C. The dilute DNA solutions were obtained by a serial dilution with 1 mol/L NaCl. complementary DNA (cDNA) corresponding to beta-2-microglobulin (β2M) mRNA was provided by School of Life Sciences, Shandong University (Jinan, China). Other chemicals (analytical grade) were obtained from standard reagent suppliers. To prevent the

contamination from the possible repeat sampling, the commercial octadecylamine-coated CdSe QD suspension and the stock DNA solutions were divided into several small packs in disinfected plastic vessels. The preparation of the DNA solutions was performed in a clean bench.

Physiological buffer saline (PBS) consisted of 0.15 mol/L NaCl, 7.6×10^{-3} mol/L NaH_2PO_4 and 2.4×10^{-3} mol/L Na_2HPO_4 (pH 7.4). TE buffer consisted of 0.010 mol/L Tris-HCl and 0.001 mol/L Na_2EDTA (pH 8.0). PBS-T buffer consisted of PBS buffer and 0.05% Tween 20. 0.05 mol/L Tris-HCl buffer (pH 9.0) was prepared by dissolving an appropriate amount of Tris in water and then adjusting the pH value to 9.0 with 0.1 mol/L HCl. 1.0×10^{-2} mol/L NHS was prepared by dissolving an appropriate amount of NHS in 0.05 mol/L borate buffer (pH 8.0). 0.1 mol/L EDC was prepared by dissolving an appropriate amount of EDC in 0.05 mol/L borate buffer (pH 8.0). 1.1×10^{-3} mol/L MPA (pH=10.0) was prepared by dissolving an appropriate amount of MPA in water and then adjusting the pH value to 10.0 with 1 mol/L NaOH. 5.0×10^{-2} mol/L ethanolamine-0.1% SDS (pH 9.0) was prepared by dissolving an appropriate amount of ethanolamine-0.1% SDS in Tris-HCl buffer (pH 9.0). DEPC treated water was prepared by adding 1 mL of DEPC to 1 L of H_2O. After 4 h the DEPC treated water was used.

All aqueous solutions were prepared with doubly distilled water, passed through a 0.22 μm filter and stored at 4 ^{o}C. All buffers, disposable plastic wares and disposable micro-pipet tips were disinfected under the pressure of 1.4 kg/cm^2 for 20 min in electrothermal-pressure vessel before use, in order to prevent the growth of microorganisms and DNA denaturation. All solutions were prepared in disposable plastic wares using disposable micro-pipet tips. In the experiments of single-cell gene expression analysis, the micropipette tip boxes were immersed in 0.3% H_2O_2 overnight and the micropipette tips were immersed in DEPC treated water. The glass wares were disinfected for 5 h at 300 ^{o}C in an oven.

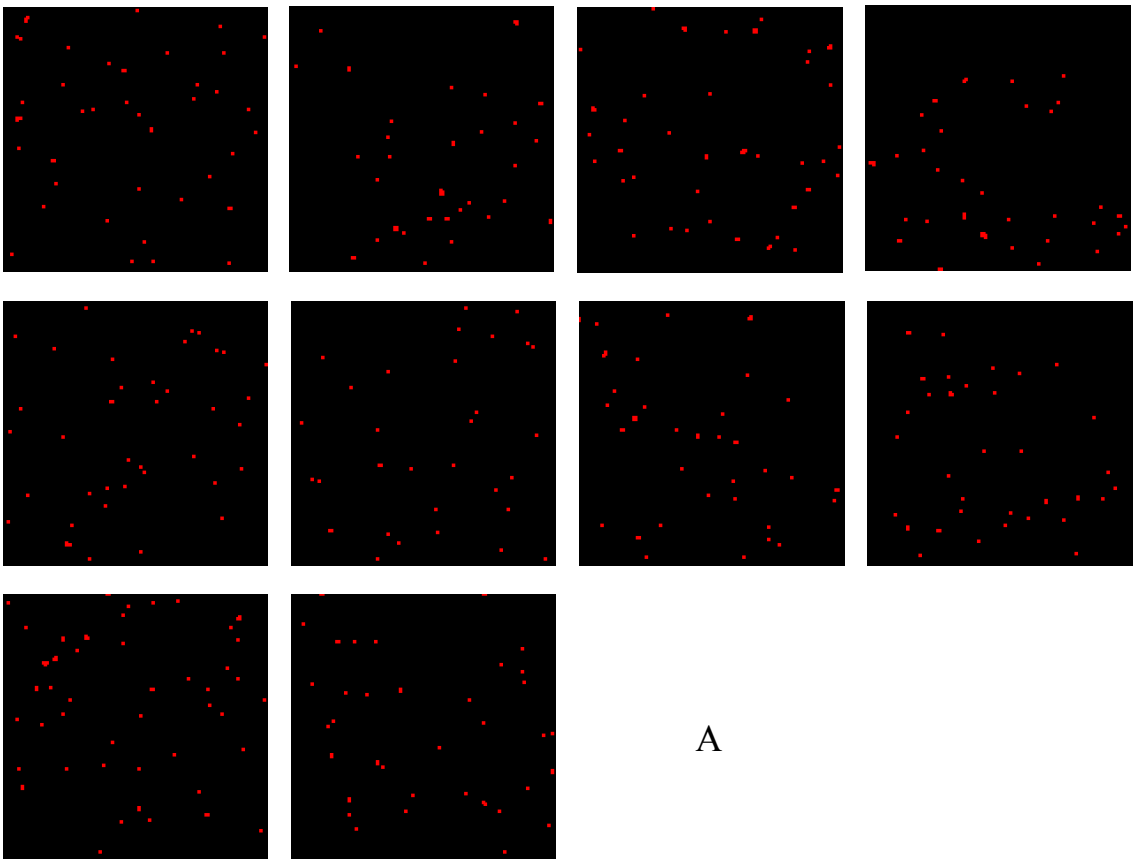
Silanization of substrates

The 0.17-mm-thick glass coverslips (Cole-Parmer Instrument Co., Vernon Hills, IL,

USA) were cleaned by ultrasonication in 30% (v/v) household cleaning liquid (detergent). After washing with tap water, the coversilps were immersed in a chromic acid mixture containing $K_2Cr_2O_7$ and H_2SO_4 overnight. The chromic acid mixture on the coverslips was removed with tap water. Then, the coverslips were washed by ultrasonication with distilled water, acetone, ethanol, and doubly distilled water, respectively. The coverslips were activated for 10 min in a mixture of 30% H_2O_2, 37% HCl and doubly distilled H_2O (1:1:1 by volume). After washing by ultrasonication in doubly distilled water, the coverslips were placed in a clean box and dried at 120 ^oC. The coverslips were immersed in 1×10^{-2} mol L^{-1} GOPS in dry toluene overnight. The coverslips were cleaned by ultrasonication for 5 min in dry toluene, acetone, alcohol and doubly distilled water. The GOPS-coated coverslips were dried under a nitrogen flow.

Results and discussion

Fig. S1 Fluorescent subframe images of single QD-labeled tDNA molecules at different concentrations (10^{-18} mol/L): (A) 1000 (10 subframe images); (B) 500 (10 subframe images); (C) 100 (10 subframe images), (D) 50 (10 subframe images) and (E) 8 (27 subframe images with one bright dot). Accumulation time was 3 h. For 8.0×10^{-18} mol/L (E), only 27 subframe images in the 100 subframe images had one bright spots. The number indicated below the images is the serial number in the 100 images taken by the EMCCD.



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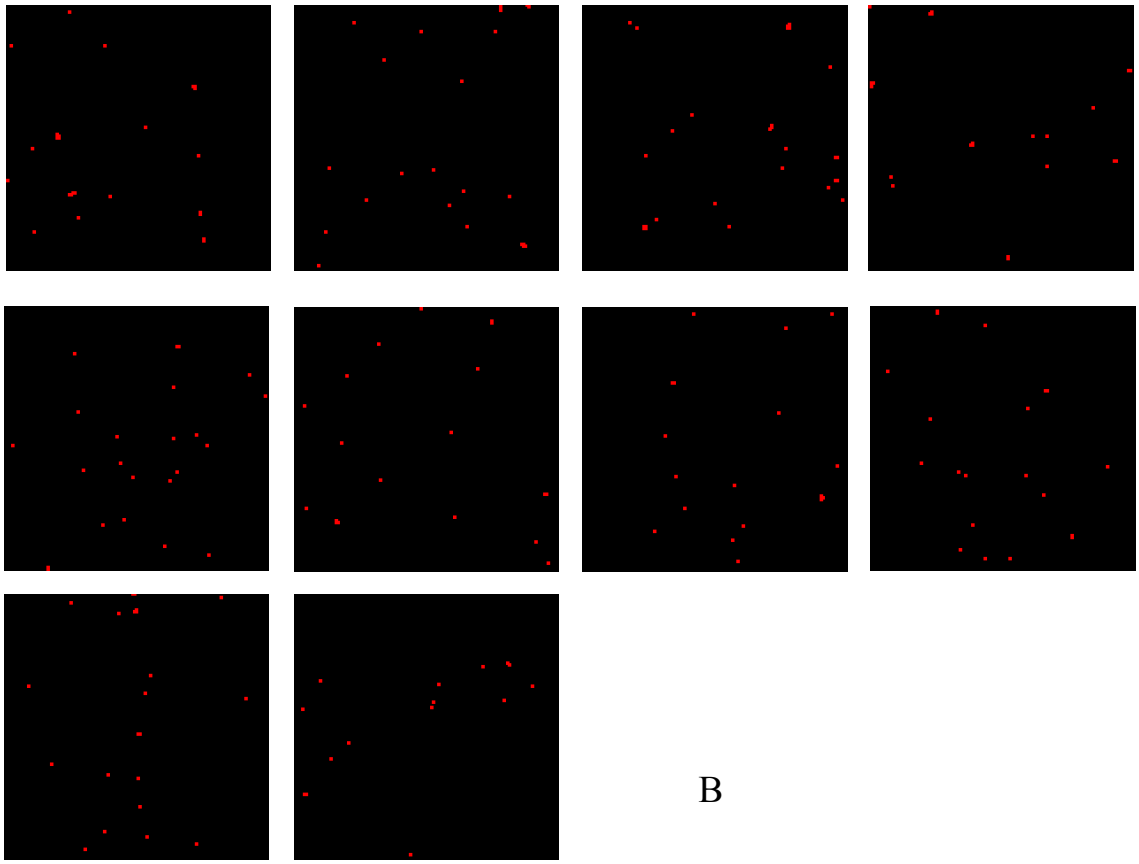

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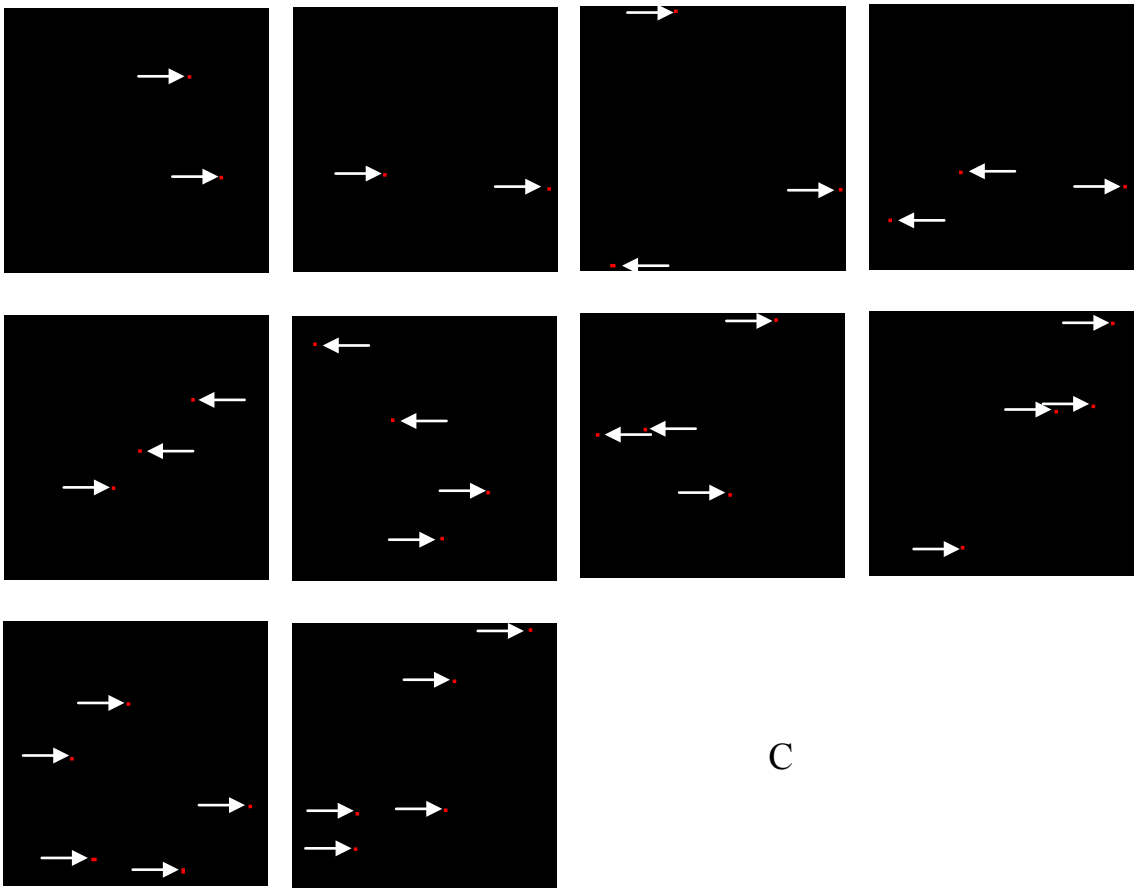

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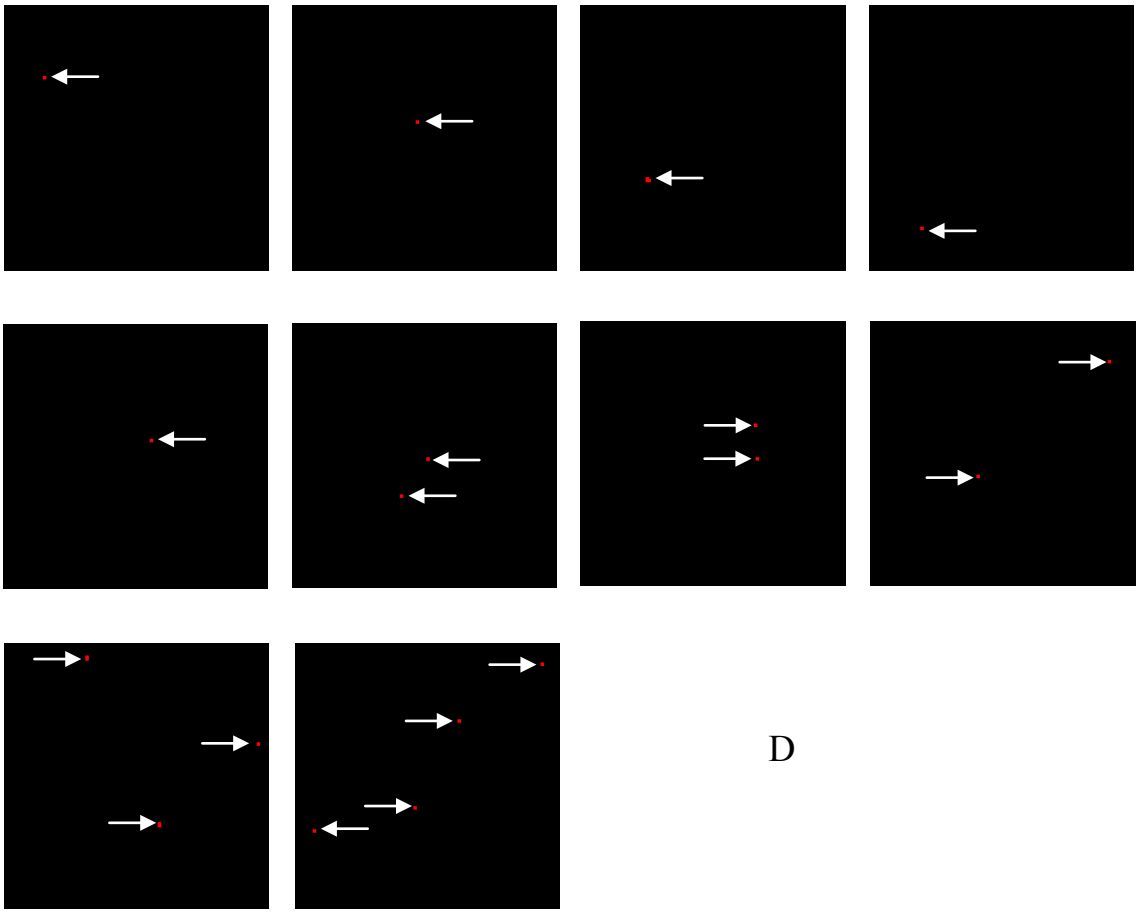

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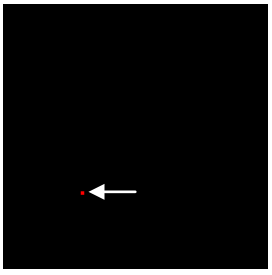
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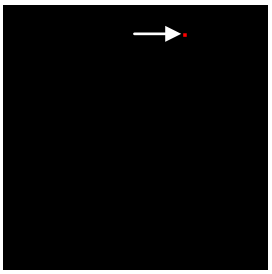
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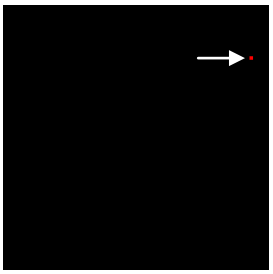
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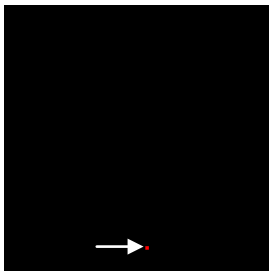
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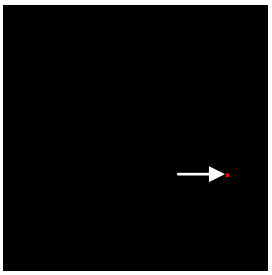
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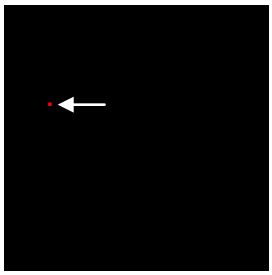
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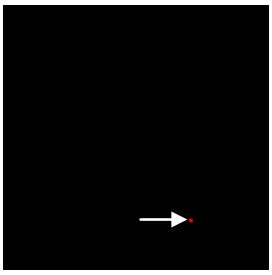
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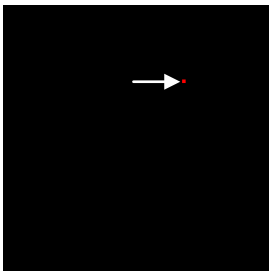
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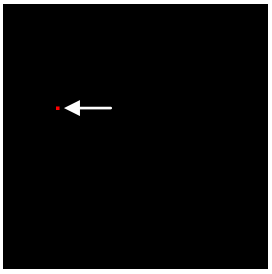
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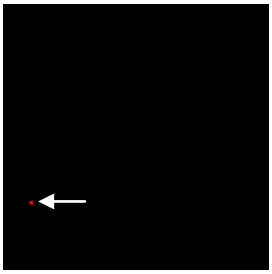
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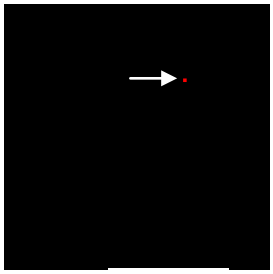
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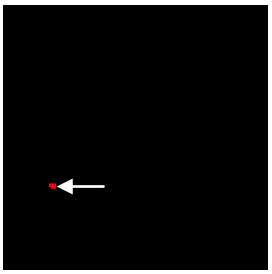
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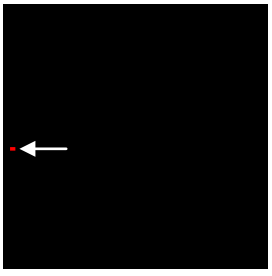
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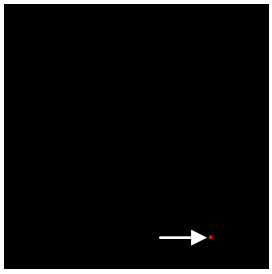
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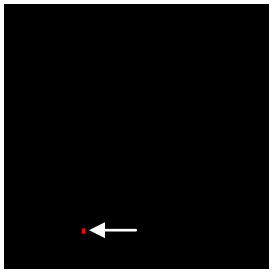
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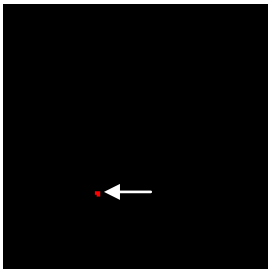
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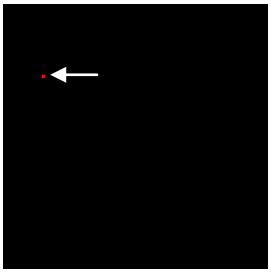

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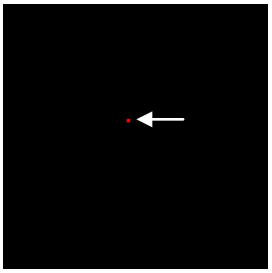

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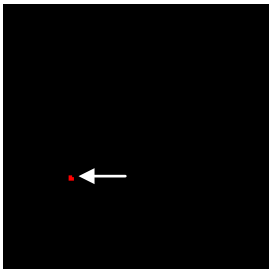

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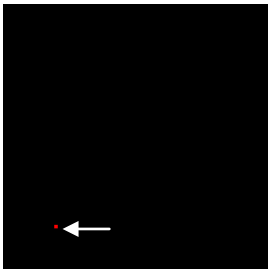

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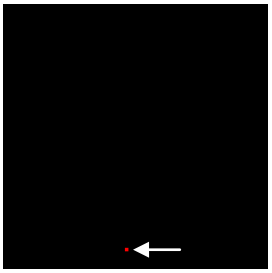

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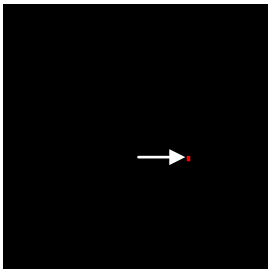

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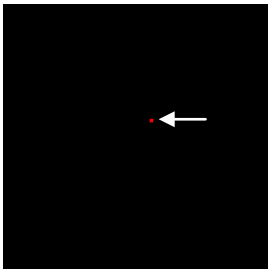

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