Supporting Information

Single-molecule analysis of genome-wide DNA methylation by fiber FISH coupled with atomic force microscopy

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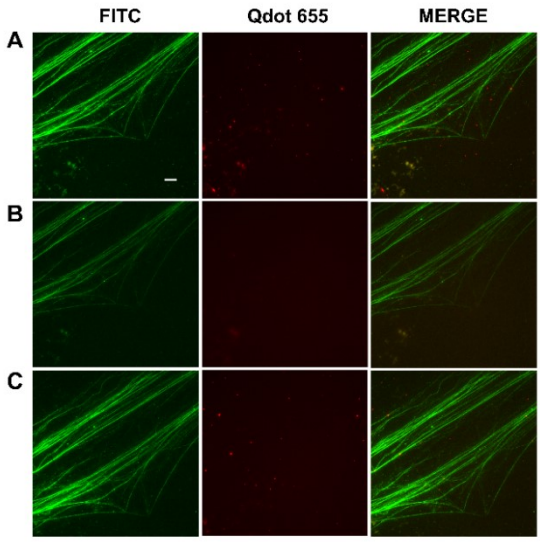

Fig. S1 Immunofluorescence images of DNA fiber with 5mC labeling (A) normal labeling (B) rinsed by stripping buffer (C) re-stained with antibodies. Scale bar, 10 μm.

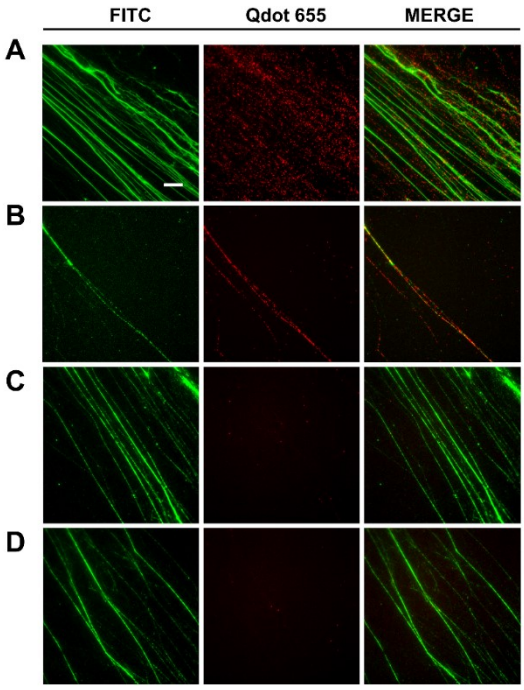

Fig. S2 Effect of Qdot 655 dilution ratio on the visualization of 5mC. (A) 1:1000, (B) 1:2000, (C) 1:5000, (D) 1:100000. Scale bar, 10 μm.

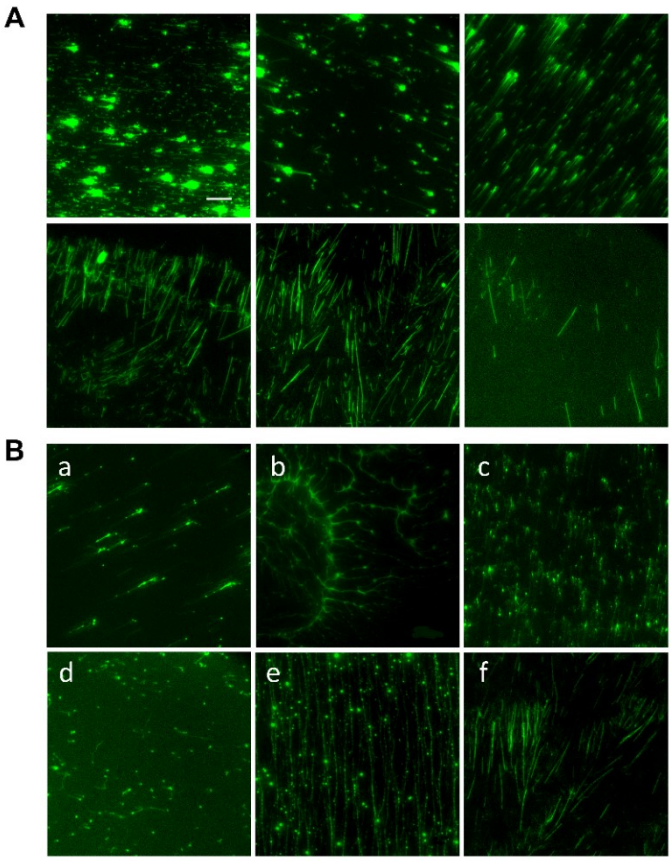

Fig. S3 (A) Effect of λDNA concentration on DNA spreading (from left to right: 5, 3, 2, 1, 0.5, 0.05 ng/μL). (B) Effect of combing buffer and flow ways on DNA spreading (a) TE buffer and nitrogen blow, (b) SDS lysis buffer and nitrogen blow, (c) 10 mM HEPES, 50 mM NaCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 5% Tween-20 and nitrogen blow, (d) 50 mM HEPES, 100 mM NaCl, 1 mM $MgCl_2$ and nitrogen blow, (e) T buffer and gravity, (f) T buffer and nitrogen blow. Scale bar, 10 μm.

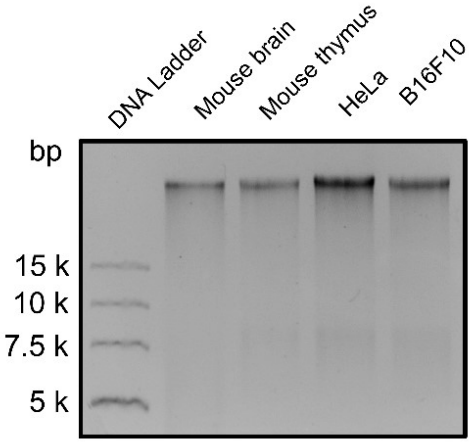

Fig. S4 Agarose gel electrophoresis (1%) of genomic DNA extracted from different cells and tissues.

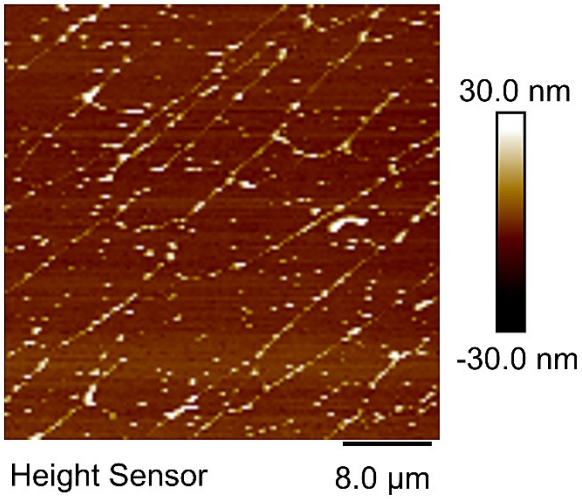

Fig. S5 AFM image of anti-5mC-DNA complexes on extended HeLa DNA.

Table S1. Detailed statistics of 5mC events by immunofluorescence assay

	Treatment	Cell Type	Fibers			
			Exp.1	Exp.2	Exp.3	Total
5mC	Untreated	HeLa	336	127	176	639
		B16F10	156	317	183	656
		A549	256	63	385	704
		MCF-7	157	124	281	562
	+5-aza-CdR	HeLa	135	77	179	391
		B16F10	112	100	144	356
		A549	176	158	128	462
		MCF-7	73	147	160	380

Table S2. Comparison with other methods for methylation analysis

Technique	Basis	Sample type	Advantages and drawbacks	Ref.
Fluorescence	dual endonucleases digestion coupling with RPA-assisted CRISPR/Cas13a	Synthetic oligonucleotides	ultrahigh sensitivity (LOD 86.4 aM), outstanding selectivity, but only be used for synthetic oligonucleotides	[1]
Chronoamperometry	Hybridization Chain-Amplified Reaction	Synthetic oligonucleotides	ideal specificity (LOD 0.93 aM), repeatability and stability, but only be used for synthetic oligonucleotides	[2]
scWGBS	bisulfite based	very small cell populations and single cells	single-base resolution, high cost and substantial DNA degradation during bisulfite treatment	[3]
Electrochemical biosensor	Exo III digestion, bisulfite treatment	Synthetic oligonucleotides	high sensitivity (LOD 4 fM), but only be used for synthetic oligonucleotides	[4]
SPR biosensor	surface plasmon resonance, poly-purine hairpin probe	poly-purine reverse-Hoogsteen (PPRH) oligonucleotides	high sensitivity (LOD 115 pM), but only be used for synthetic oligonucleotides	[5]
MeDIP-Seq	next-generation sequencing	buffalo embryos	single-base resolution, high cost, less sensitive in regions with high CpG density	[6]
Digital PCR	microfluidic chip-based digital PCR	lung adenocarcinoma and adjacent non-tumorous tissues	ultra-low DNA input, easy primer design, but loci-specific studies only	[7]
Microarray	gene expression microarray and DNA methylation microarray, TCGA database	breast cancer tissues	pre-designed panel covering hotspot methylation, low genome-wide coverage of CpGs	[8]
Optical mapping	Reduced representation optical methylation mapping (ROM)	chromosomal DNA molecules	Long-read, kilobase pair-scale genomic methylation patterns, particular instrument (Bionano Genomics)	[9]
FISH-AFM	DNA fiber-based fluorescence in situ hybridization, AFM	several cell lines and tissues	Long-read, low cost, genome-wide DNA methylation analysis, reproducibility, low DNA input, less sensitivity	This work

Table S3. The OD260/280 values of genomic DNA from different sources

Source of DNA	OD 260/280
Mouse brain	1.83
Mouse thymus	1.83
HeLa	1.86
B16F10	1.85

Table S4. 5mC dots per μm DNA by immunofluorescence and AFM

	IF	AFM
HeLa	0.3780±0.0028	0.3908±0.0414
B16F10	0.5289±0.0072	0.5469±0.0176
A549	0.6014±0.0183	-
MCF-7	0.4145±0.0025	-
Mouse brain	-	0.6125±0.0231
Mouse thymus	-	0.5953±0.0137

References

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