Electronic Supplementary Information

Simultaneous visualization of subfemtomolar expression of microRNA and microRNA target gene by HILO microscopy

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Figure S1. (a) The pictures showing that two 3-W LEDs were mounted on the heat sink and combined for the reduction of autofluorescence. (b) The emission spectra of the green and red LEDs.

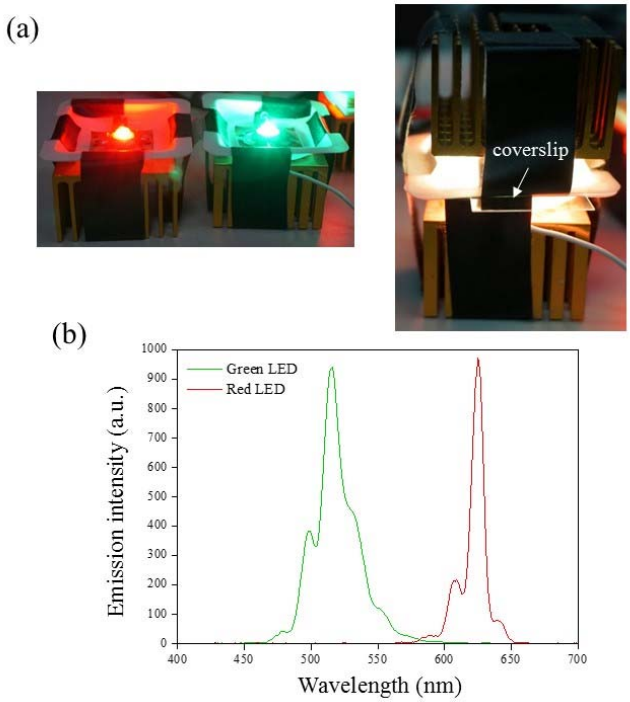


Figure S2. Schematic representation of highly inclined and laminated optical sheet (HILO) microscopy.

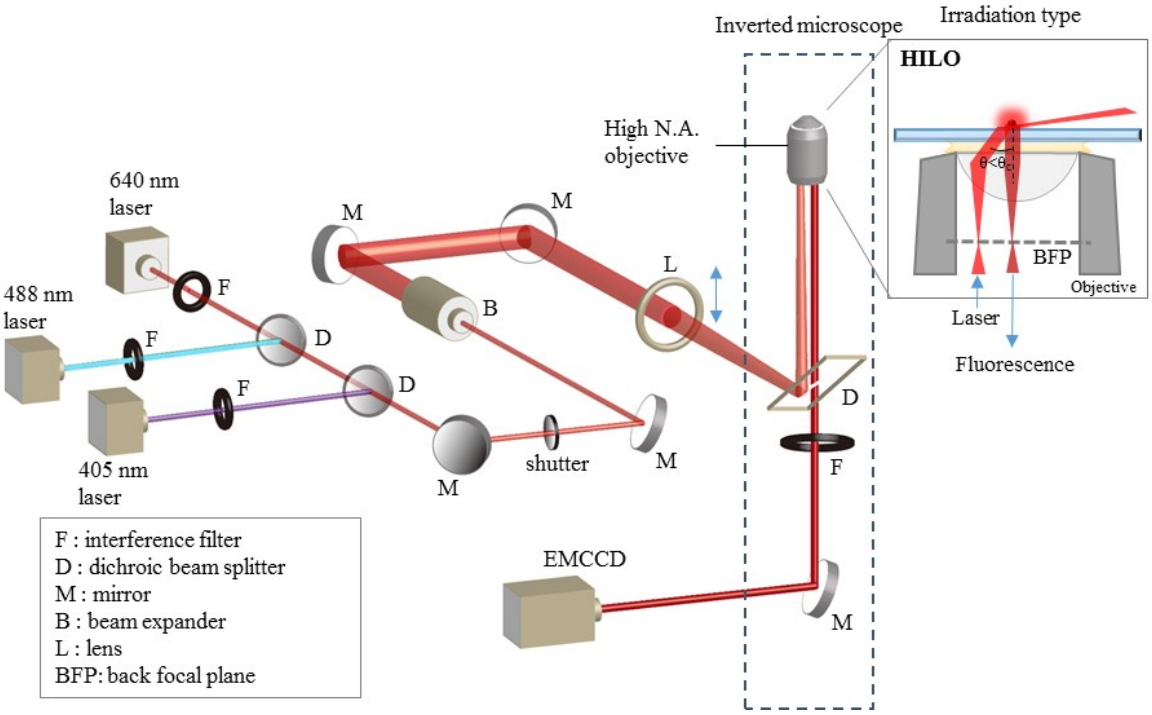


Figure S3. The fluorescence spectra and hybridization curves of free molecular beacons and hybridized duplexes with synthetic oligonucleotides (Table S2). The enhanced fluorescence ratios of a duplex hybridized to a free beacon are 22 (miR-10b), 11 (*HOXD10* mRNA), and 7 (*U6* snRNA) taking into account the wavelength range of emission filters installed on the microscope. The hybridization curves indicate that hybridization between molecular beacons and targets may be performed at the same temperature because of similar T_m among the three duplexes.

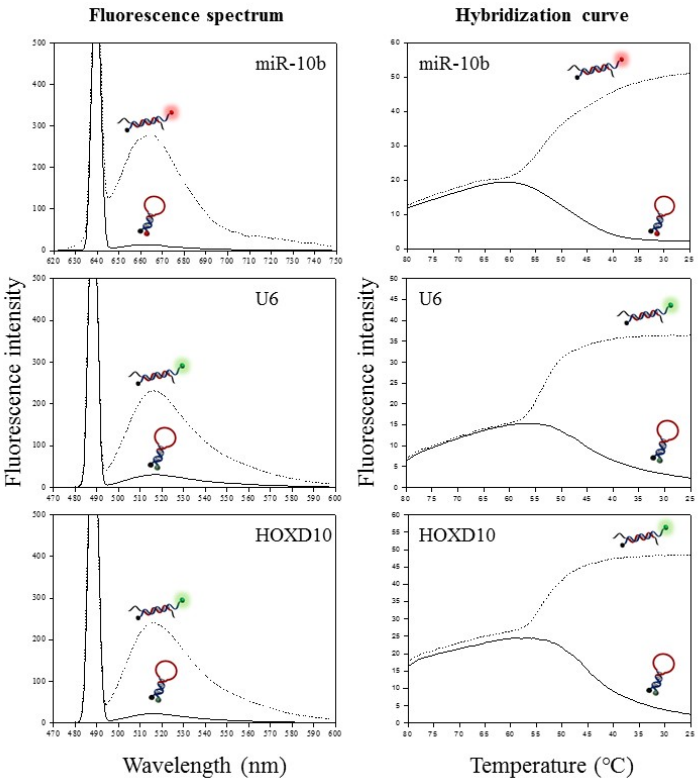


Figure S4 The average copy number per RT-qPCR reaction versus average fluorescence intensity per pixel per cell, showing a linear behavior.

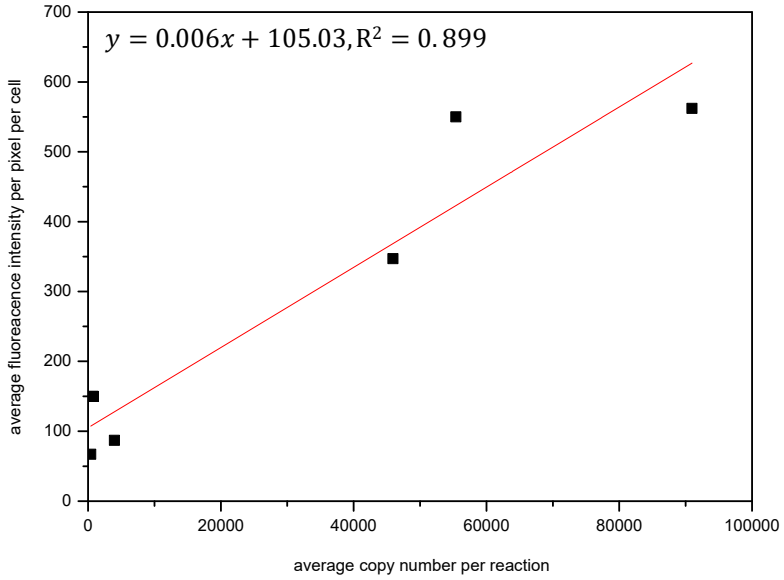


Figure S5. Comparison of RT-qPCR results obtained from the serial dilution of miRNA either by water (●) or water containing yeast RNA as a blocking agent (■).

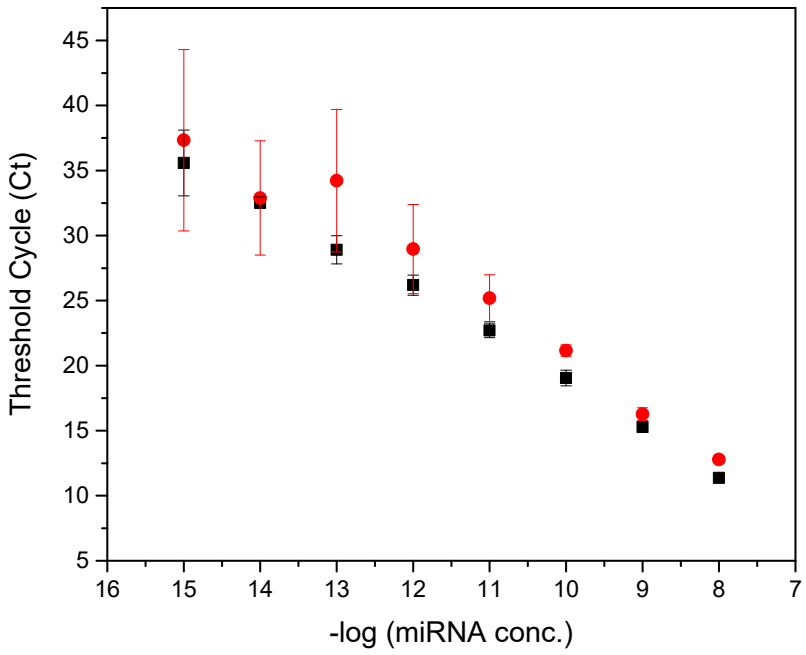


Figure S6. The comparison between HILO microscopy and RT-qPCR for the determination of *HOXD10* expression alterations after modulation by a miR-10b precursor or inhibitor.

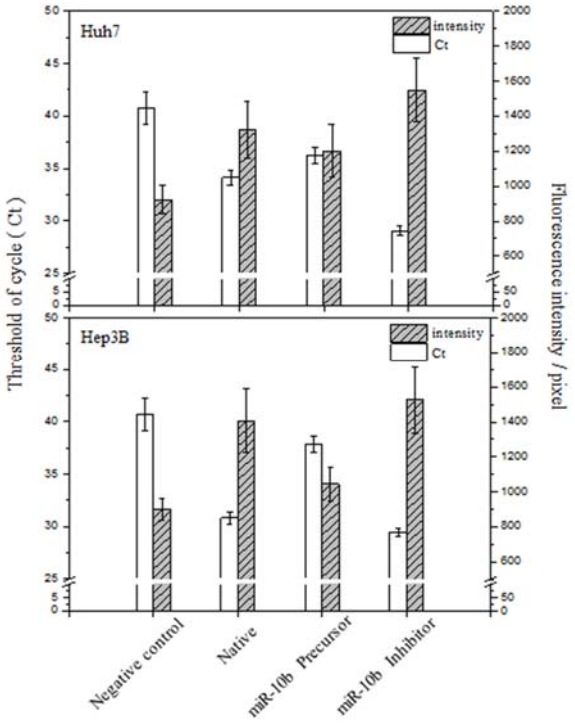


Figure S7. The validation of RT-qPCR data by capillary electrophoresis with laser-induced fluorescence. The electropherograms of a 10-bp DNA ladder (a) and RT-qPCR products of miR-10b (b), U6 snRNA (c) and *HOXD10* mRNA (d) spiked with a DNA marker validating the accuracy of amplification by RT-qPCR.

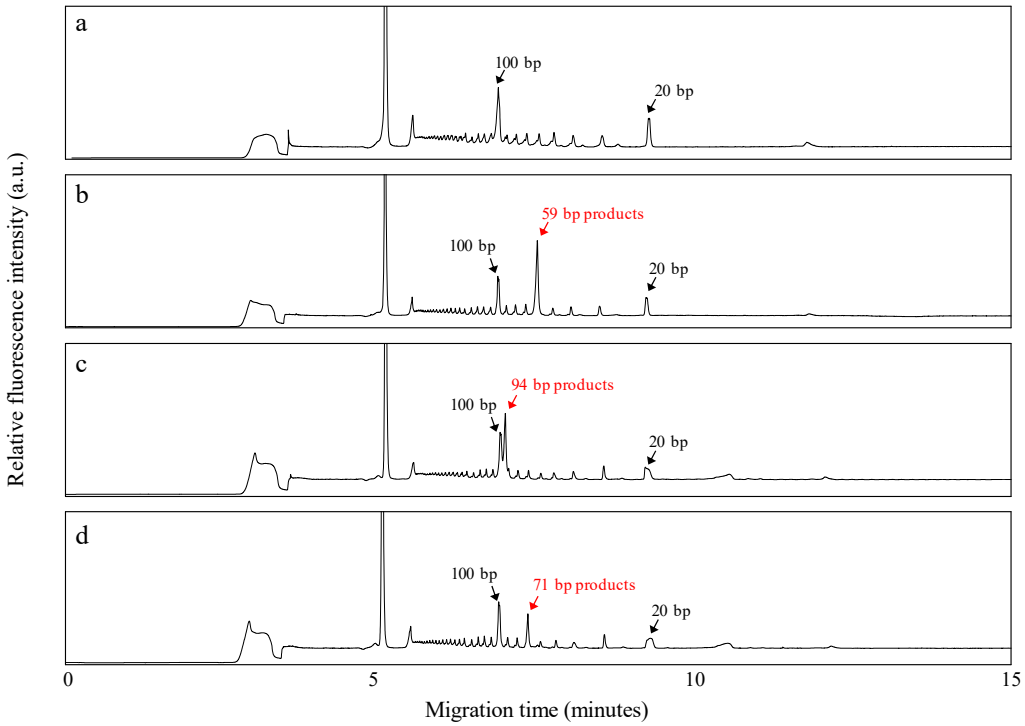


Table S1. Table S1. The raw data and reproducibility of RT-qPCR for miR-10b triple analysis (Figure 2i–k). The threshold cycle was converted to the molar concentration based on the linear equation in Fig 1i. The Ct of negative control (H_2O) is 39.98 ± 5.94.

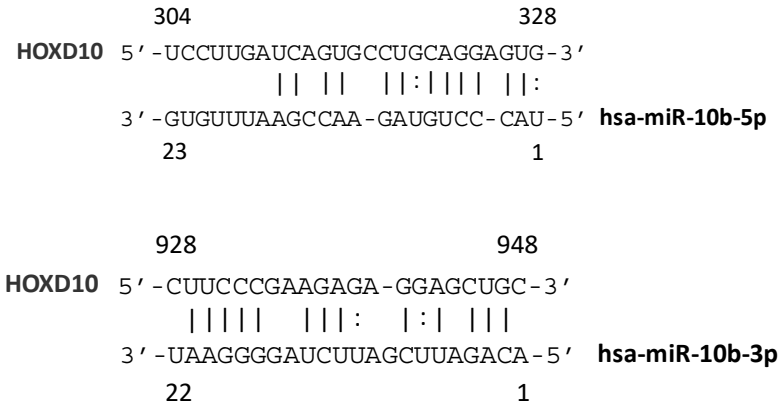
miR-10b concentration	Mean	SD	CV(%)	Mean	SD	CV(%)
10 nM	11.38 Ct	0.26 Ct	2.24	14.9 nM	2.91 nM	19.48
1.0 nM	15.28 Ct	0.37 Ct	2.42	1.12 nM	0.33 nM	29.10
100 pM	19.05 Ct	0.61 Ct	3.18	95.1 pM	51.5 pM	54.12
10 pM	22.71 Ct	0.56 Ct	2.47	8.03 pM	3.73 pM	46.45
1.0 pM	26.19 Ct	0.77 Ct	2.94	0.84 pM	0.58 pM	68.80
100 fM	28.90 Ct	1.08 Ct	3.74	148 fM	99.3 fM	66.96
10 fM	32.52 Ct	0.45 Ct	1.37	11.0 fM	3.98 fM	36.18
1.0 fM	35.58 Ct	2.53 Ct	7.10	3.30 fM	3.53 fM	107.03
miR-10b in Huh-7 cells	Mean	SD	CV(%)	Mean	SD	CV(%)
baseline expression	29.51 Ct	0.49 Ct	1.65	81.9 fM	25.9 fM	31.62
miR-10b precursor	28.49 Ct	0.32 Ct	1.11	159 fM	33.5 fM	21.08
miR-10b inhibitor	33.16 Ct	4.61 Ct	13.91	35.5 fM	48.2 fM	135.56
miR-10b in Hep3B cells	Mean	SD	CV(%)	Mean	SD	CV(%)
baseline expression	35.60 Ct	1.41 Ct	3.97	1.75 fM	1.38 fM	79.01
miR-10b precursor	29.23 Ct	1.50 Ct	4.62	119 fM	76.9 fM	64.65
miR-10b inhibitor	36.58 Ct	3.16 Ct	8.63	2.19 fM	3.12 fM	142.94

Table S2. The Poisson distribution of femtomolar miRNA caused by liquid transferring of pipetting. The initial concentration of miRNA sample is 1.0 fM. Theoretically, average 3 miRNA molecules (1-μL) was transferred to a plastic tube for PCR reaction as a result of 200-folds dilution after revers transcription.

Poisson distribution	$P\,(x,\lambda) = \frac{\lambda^x e^{-\lambda}}{x!}$
mean number of molecules per μL, λ =	3
expected pick up number of molecules, x =	3
P (%) =	Probability for pick up three molecules 22.40418077
1-P(%)=	Probability for pick up molecules except to three 77.59581923
expected pick up number of molecules, x =	Probability for certain molecules (%)
0	4.98
1	14.94
2	22.40
3	22.40
4	16.80
5	10.08
6	5.04
7	2.16
8	0.81
9	0.27
10	0.08

Table S3. The sequence of oligonucleotides used for stem-loop RT-qPCR, molecular beacon probing and the miR-10b/miR-10b* binding sites on HOXD10 (ENSG00000128710). The identification of miR-10b/miR-10b* target gene were performed by the pattern-based method.[1]

Name	Length	Sequence (5'→3')
hsa-miR-10b-3p (RNA)	22	ACAGAUUCGAUUCUAGGGGAAU
hsa-miR-10b RT primer	44	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATTCCCCT
hsa-miR-10b GS primer	22	CGGCGGACAGATTCGATTCTAG
hsa-miR-10b Reverse primer	24	CTGGTGTCGTGGAGTCGGCAATTC
U6 snRNA (DNA)	20	TACAGAGAAGATTAGCATGG
U6 snRNA Forward primer	17	CTCGCTTCGGCAGCACA
U6 snRNA Reverse primer	20	AACGCTTCACGAATTTGCGT
HOXD10 mRNA (DNA)	20	AAGAGCGTTAACCTCACCGA
HOXD10 mRNA Forward primer	22	GACATGGGGACCTATGGAATGC
HOXD10 mRNA Reverse primer	22	TGGTGGTTCACTTCTCTTTTGG
hsa-miR-10b molecular beacon	28	5'-Cy5-GCACATTCCCCTAGAATCGAATCTGTGC-Iowa Black RQ-SP-3'
U6 snRNA molecular beacon	28	5'-6FAM-GTCTACCATGCTAATCTTCTCTGTAGAC-BHQ1-3'
HOXD10 mRNA molecular beacon	30	5'-Alexa Fluor 488-GCAGGTCGGTGAGGTTAACGCTCTTCCTGC-BHQ1-3'



1. K. C. Miranda, T. Huynh, Y. Tay, Y.-S. Ang, W.-L. Tam, A. M. Thomson, B. Lim and I. Rigoutsos, *Cell*, 2006, **126**, 1203-1217.