

Supplementary Materials

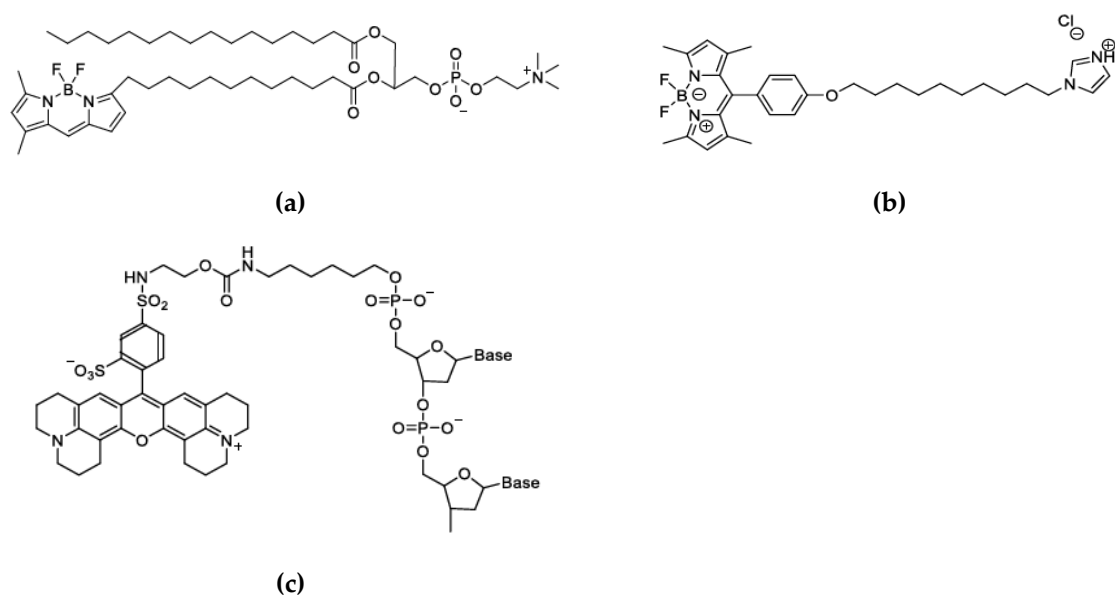


Figure S1. Molecular structures of fluorescence-labelled lipids and DNA. (a) BODIPY-HPC. (b) BODIPY-C. (c) Texas Red-DNA.

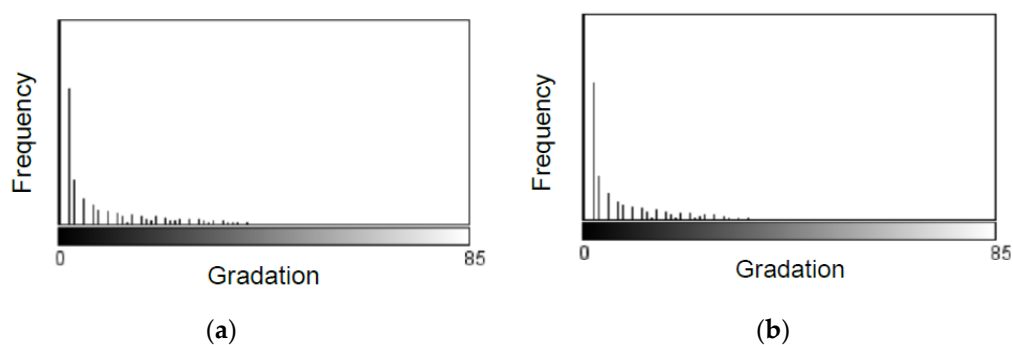


Figure S2. Fluorescence intensity histograms of GV stained with BODIPY-HPC and BODIPY-C. The histograms were obtained from fluorescence images of GV stained with BODIPY-HPC (a) and BODIPY-C (b) by Fiji software. The histograms support indistinguishability of analyzed fluorescence intensities between BODIPY-HPC and BODIPY-C in the current confocal microscopy observation.

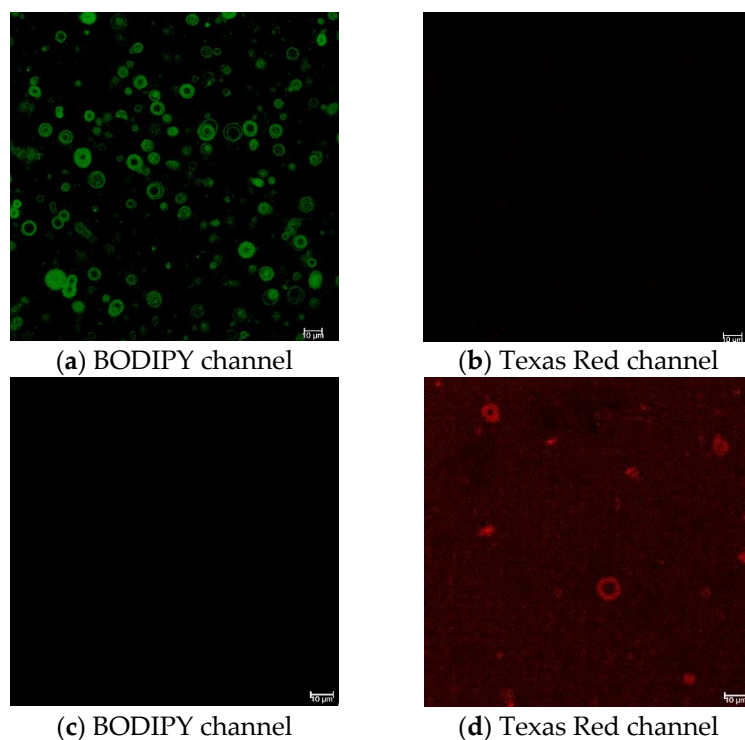


Figure S3. Fluorescence compensation in confocal microscopy observation. Confocal microscopy images of GV stained with BODIPY-HPC (a,b) or Texas Red-DNA (c,d). The leakage of fluorescence from another fluorescence channel was avoided to prevent the increases of the apparent colocalization and of the average nMDP of each image.

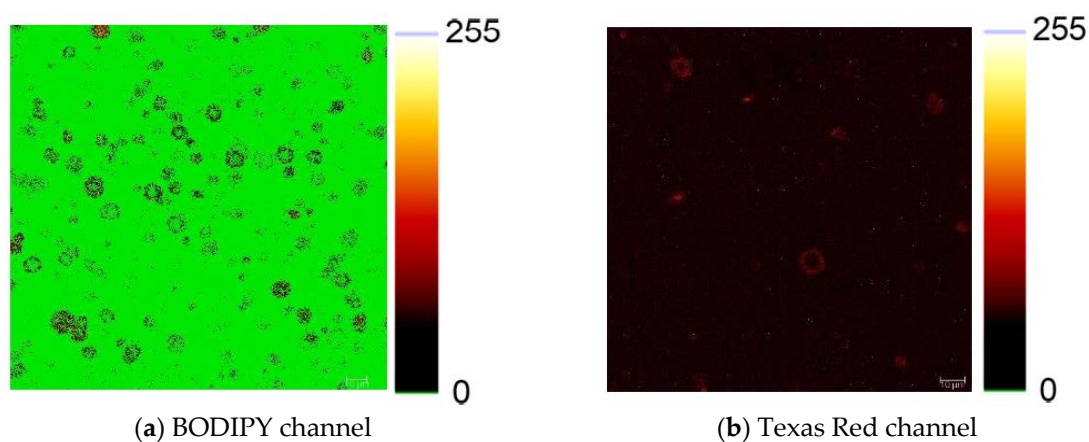
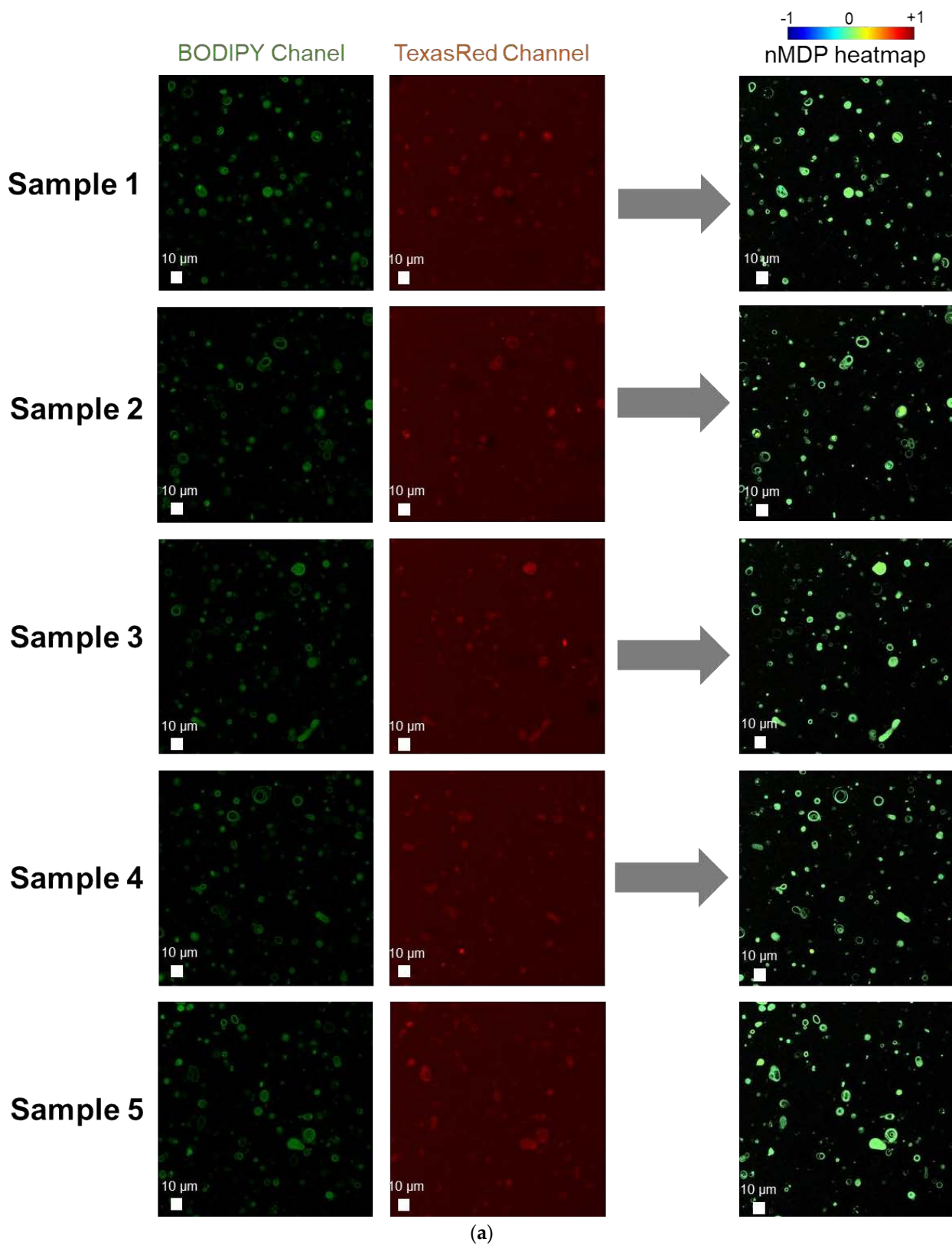


Figure S4. Prevention of fluorescence saturation in confocal microscopy observation. The saturation of fluorescence must be prevented because it decreases validity of obtained nMDP value. Blue means saturation of the fluorescence intensity. The heatmap of GV stained with BODIPY-HPC (a) was red-black, and that of Texas Red DNA (b) was red-black. No saturation was recognized in both fluorescence intensities.



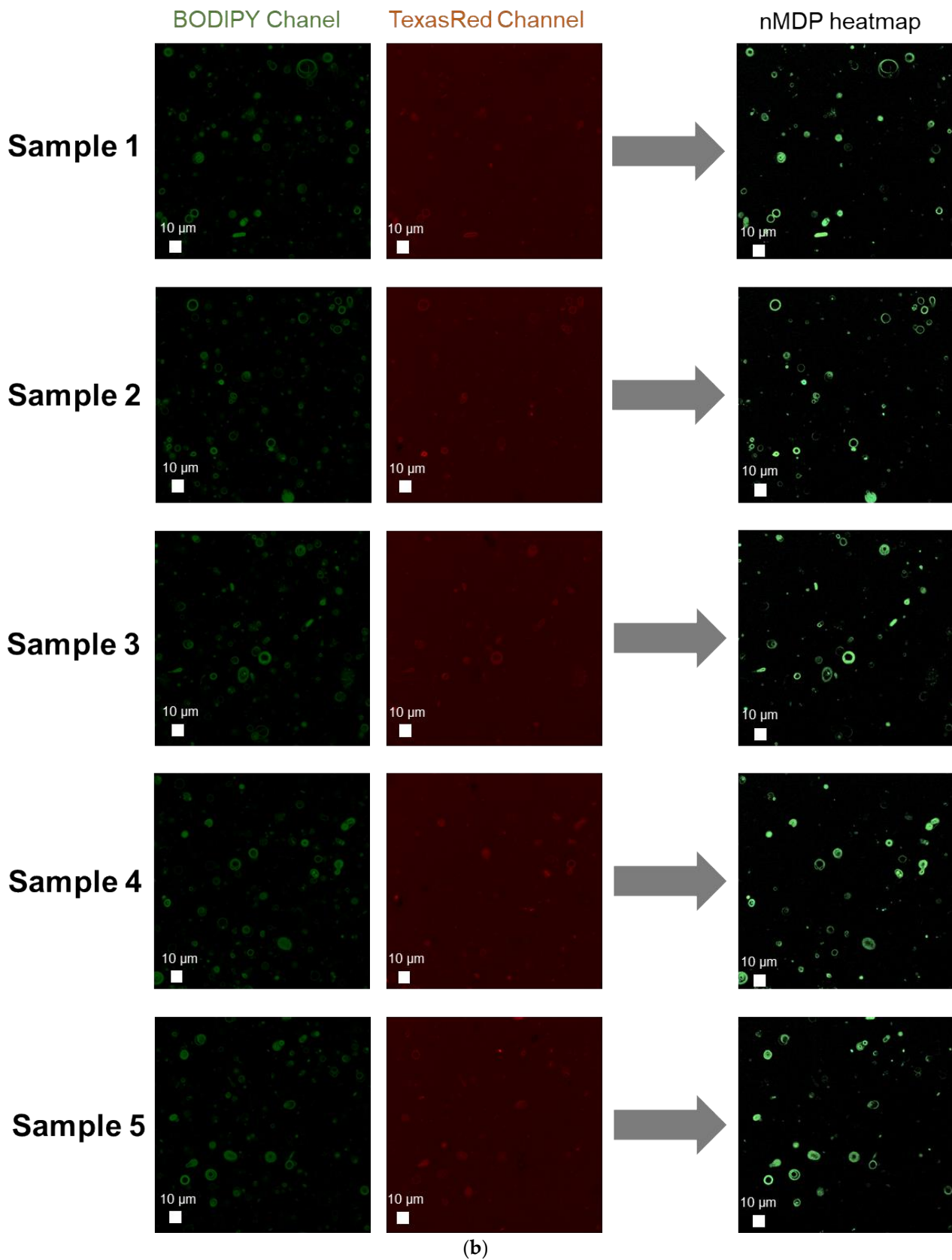


Figure S5. nMDP heatmaps for DNA-Texas Red and BODIPY-C (a) and DNA-Texas Red and BODIPY-HPC (b) originating the histogram in Figure 4. Since the resolution of the original images for nMDP calculation such as in Figure 4 is 2048×2048 pixel², whole pixel number of each nMDP map is 4,194,304.

Table S1. Indistinguishability of analyzed fluorescence intensities between BODIPY-HPC and BODIPY-C in current confocal microscopy observation. The average intensities were obtained from fluorescence images of GVs stained with BODIPY-HPC and BODIPY-C by Fiji software. Significant differences were not detected between intensities in confocal microscope images of BODIPY-HPC and BODIPY-C captured in five different sites in fields of view.

BODIPY-HPC		BODIPY-C	
Site 1	1.22	Site 1	1.28
Site 2	1.19	Site 2	1.18
Site 3	1.15	Site 3	1.07
Site 4	1.02	Site 4	1.01
Site 5	0.9	Site 5	0.88
Average intensity	1.12	Average intensity	1.08

Table S2. Number of pixels calculated nMDP on each heat maps represented in Figure S5. Wholepixel number including non-calculated pixels is 4,194,304 (= 2048²).

DNA-Texas Red and BODIPY-C		DNA-Texas Red and BODIPY-HPC	
Heat map	nMDP-calculated pixels	Heat map	nMDP-calculated pixels
#1	184,064	#1	82,542
#2	154,162	#2	68,800
#3	165,823	#3	64,886
#4	168,115	#4	79,829
#5	201,105	#5	70,426
average	174,654	average	77,296

Table S3. Population ratio of pixels with magnitude of nMDP > -1.0 or nMDP < +0.1 on thenMDP heat map.

DNA-Texas Red and BODIPY-C

nMDP	Population ratio (%)					Averaged population ratio (%)	Standard Error	Averaged pixel number**
	#1	#2	#3	#4	#5			
<-0.1	0.5585	0.6733	0.4487	0.3896	0.5575	0.53	±0.05	926
> 0.1	1.0757	1.7092	1.4148	0.6585	1.1641	1.20	±0.17	2095

DNA-Texas Red and BODIPY-HPC

nMDP	Population ratio (%)					Average of population ratio (%)	Standard Error	Average of pixel number**
	#1	#2	#3	#4	#5			
<-0.1	0.4824	0.6831	0.5717	0.8356	0.8321	0.68	±0.07	525
> 0.1	0.2689	0.5741	0.6626	0.4735	0.1732	0.31	±0.09	239

* "Population ratio" is defined as the ratio of nMDP-calculated pixel number with magnitude of nMDP < -0.1 (or nMDP > 0.1), $n_{\pm 0.1}$, against whole number of nMDP-calculated pixels (Table S2), n_{whole} : $n_{\pm 0.1} / n_{\text{whole}} \times 100$ (%).

** "Average of pixel number" is product between the average of population ratio in this table and the average of whole number of nMDP-calculated pixels in Table S2. This number indicates that real number of pixels that fulfill the condition for one heat map.