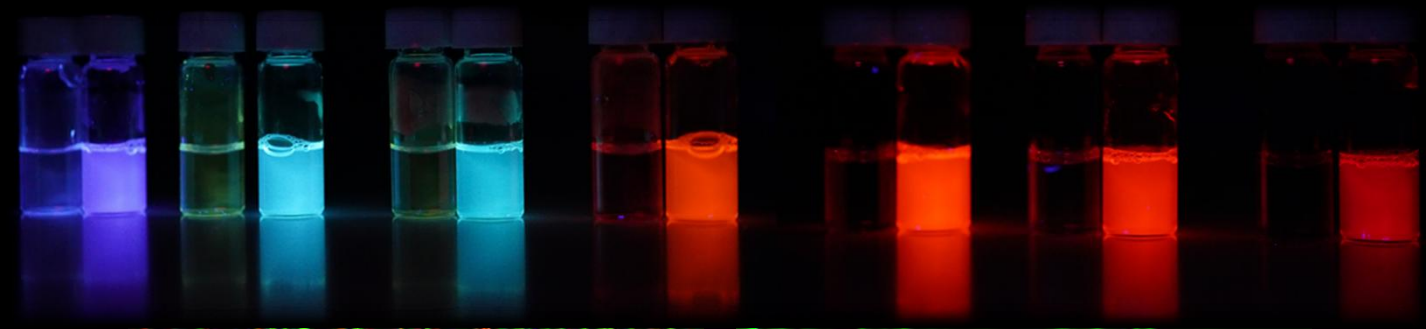
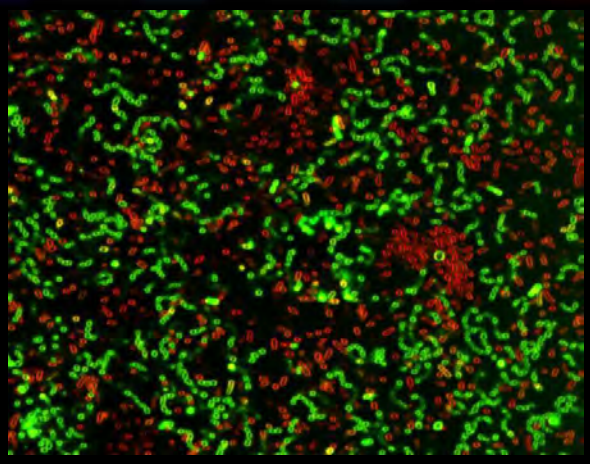
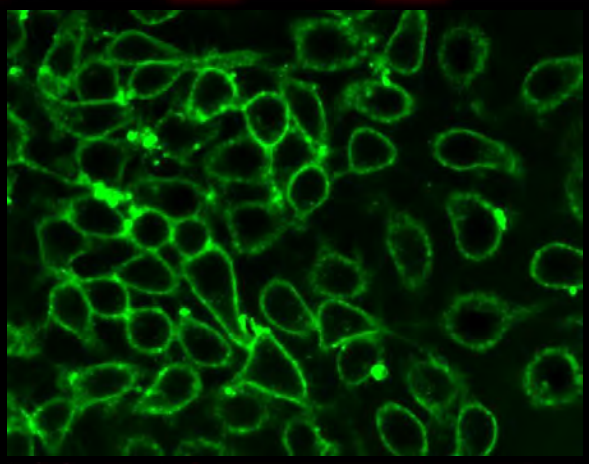
Jetbody

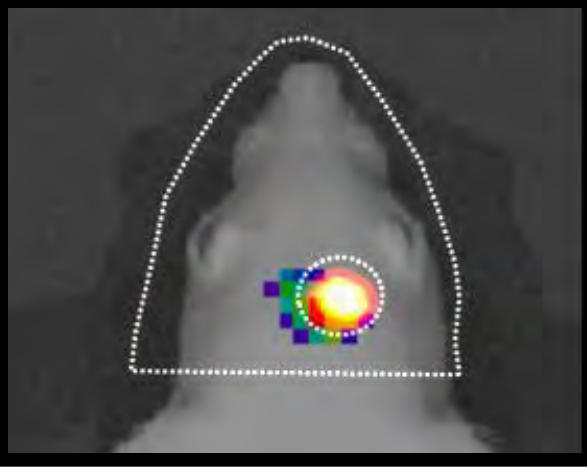
Beyond affinity

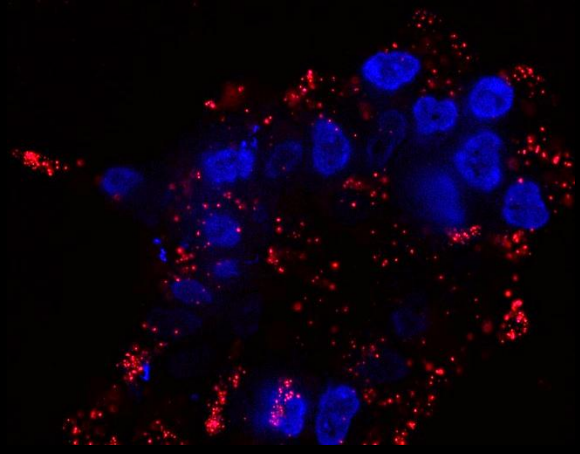
Driving Chemical Innovation in Optical Probes











Optical Probes for Mammalian Cells, Extracellular Vesicles & More

Technical Bulletin

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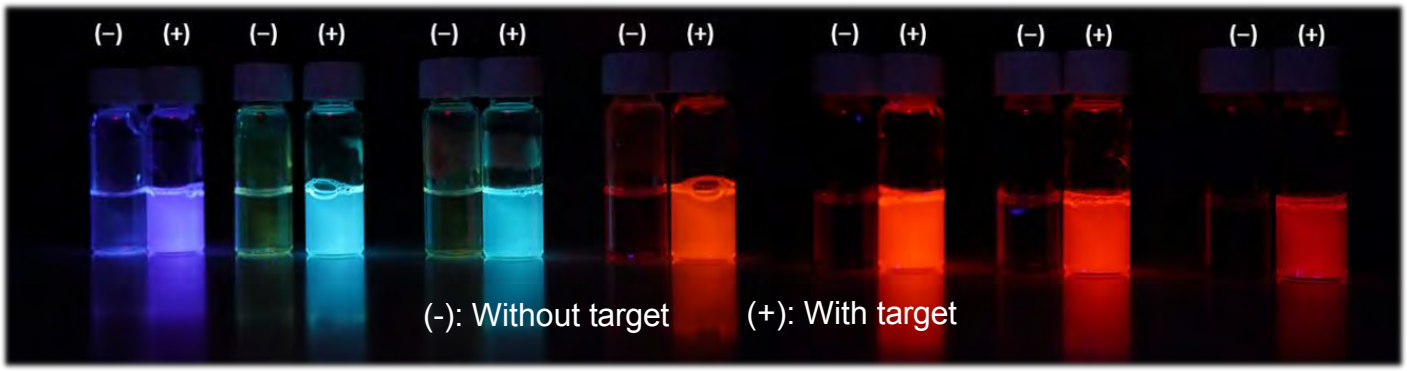
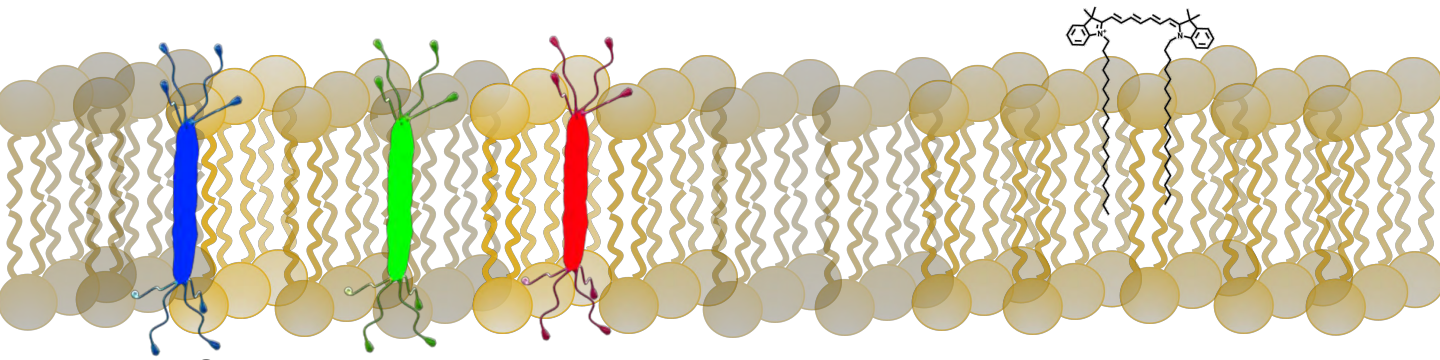
JotDye Applications

JotDyes in a Snapshot

Tech at a Glance

- Workflow compatible
- Accurate
- Variety of Colors (UV to NIR)
- Water Soluble
- Innovative Chemistry
- “Light up” Mechanism



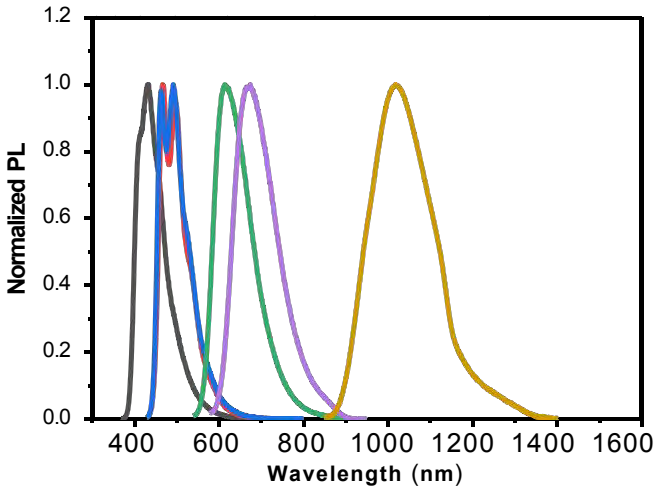


JotDyes Span the Entire Bilayer

Stable Binding, No Dye Exchange

Traditional Lipophilic Dyes Do Not

Unstable, Dye “Leakage”

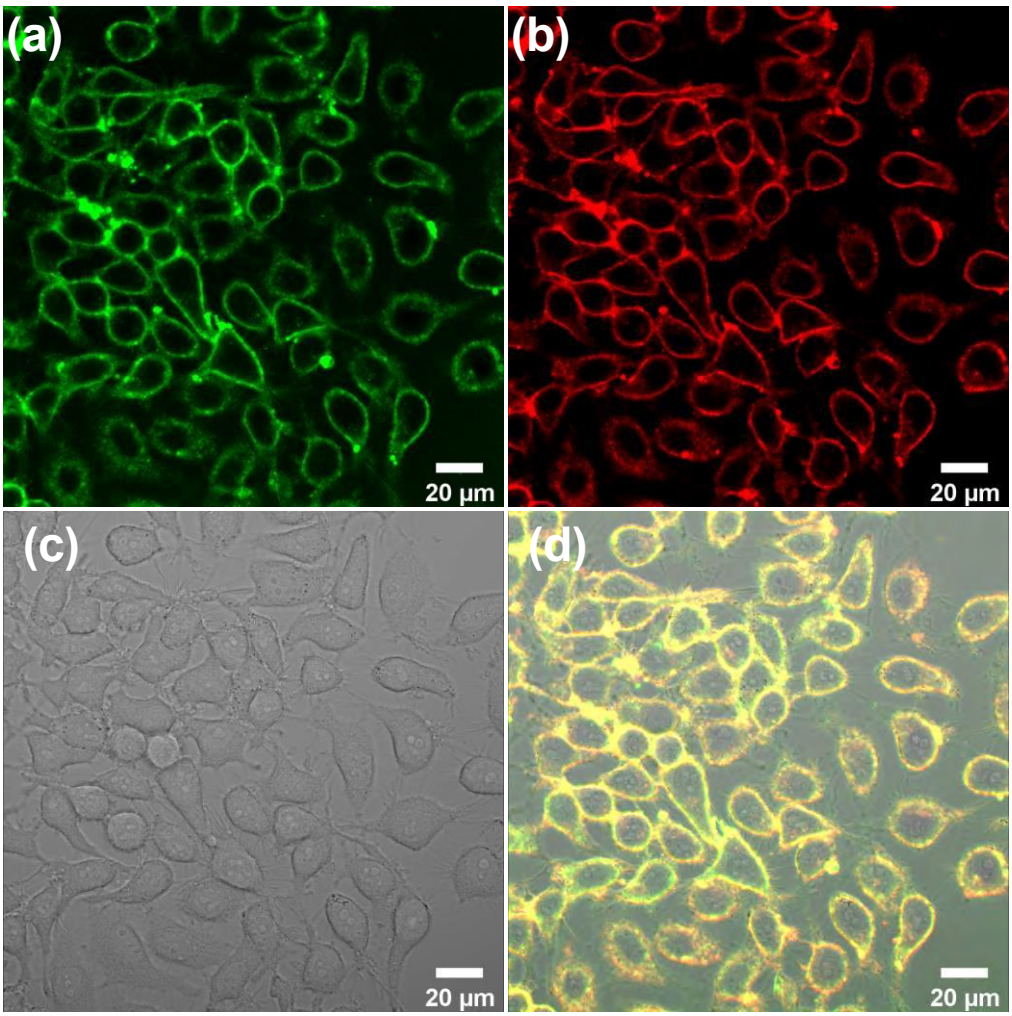


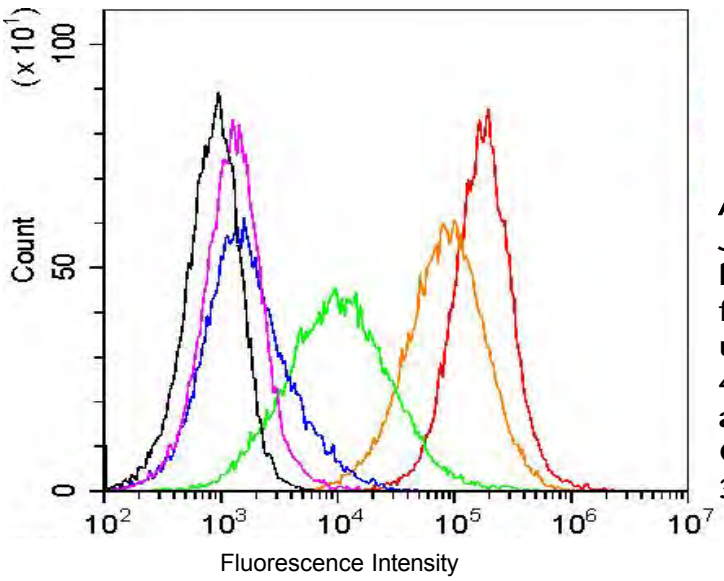
With a variety of options to fit your needs, Jotbody provides a novel class of revolutionary dyes that will **accelerate your research**

Jotbody Membrane Labeling Dye

Confocal images of HepG2 cells that were co-stained with Jotbody Membrane Labeling Dye and a commercially available membrane dye, FM 4-64.

(a) JotDye channel (Ex405), (b) FM 4-64 channel (Ex488), (c) brightfield channel,(d) merged channel. The co- localization of Jotbody Membrane Dye and FM4- 64 demonstrates the membrane specificity of JotDye.

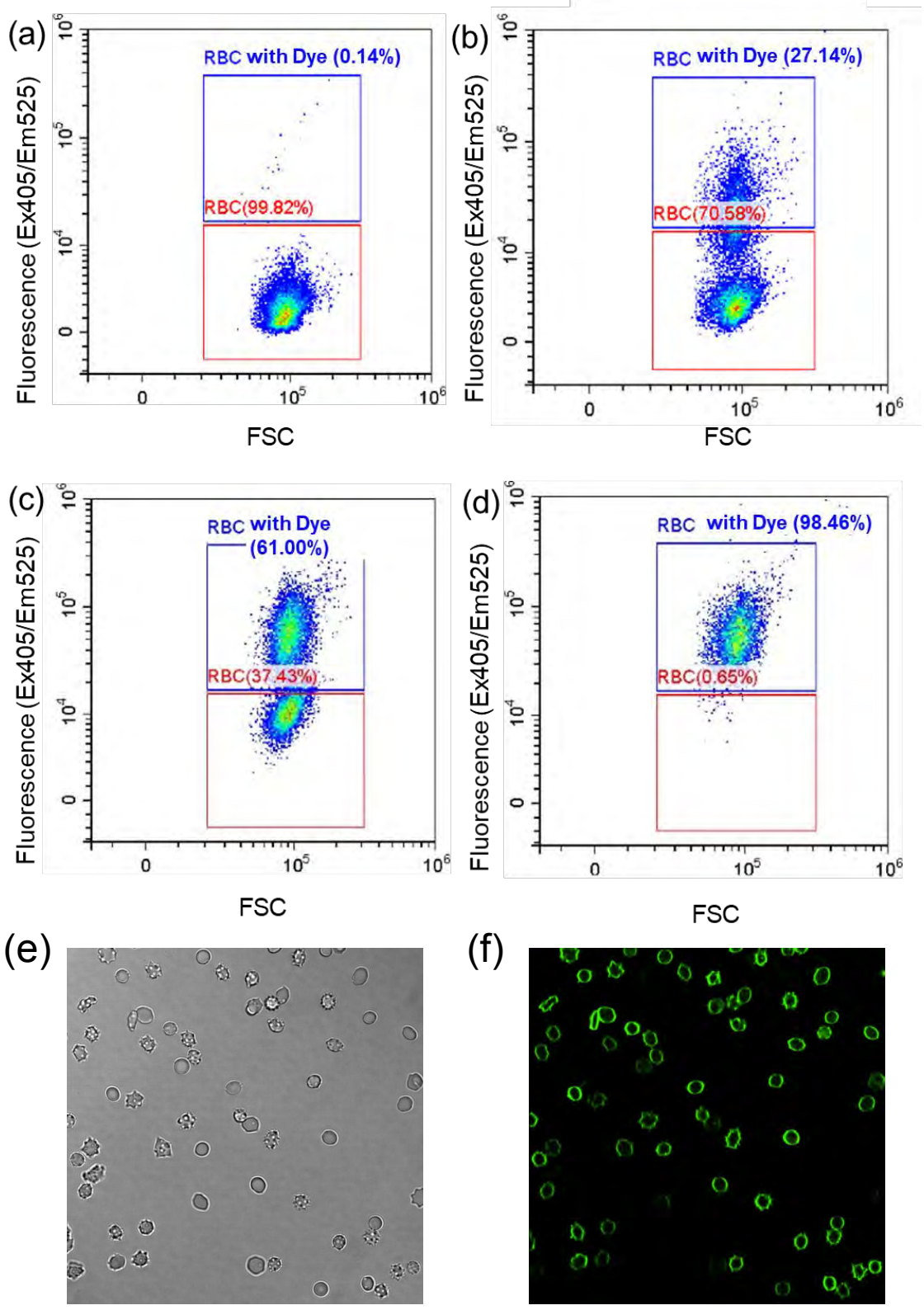




JotDye for Cell Division Tracking

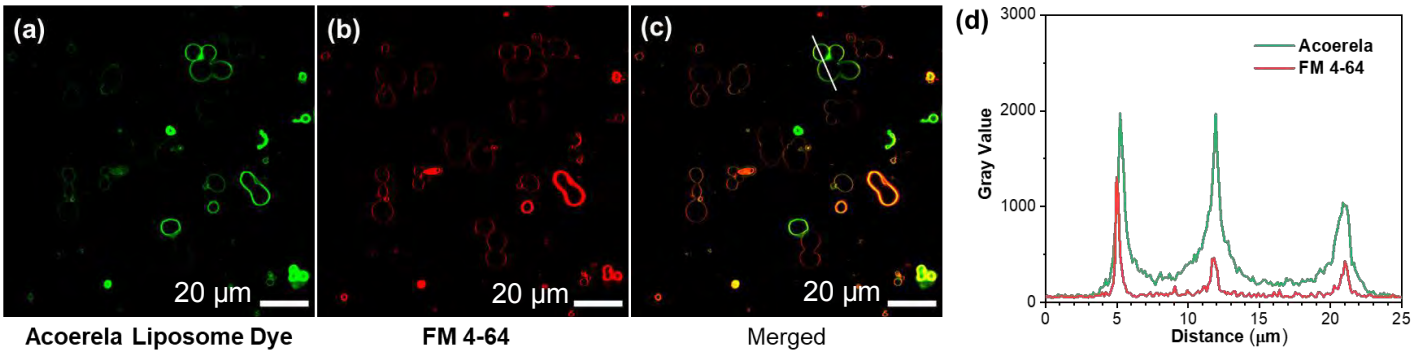
A549 mammalian tumor cells labeled by Jotbody Cell Division Tracking dye. JotDye-labeled cells (red) have a 3-log fold increase in fluorescence intensity compared to the unstained cells (black) were maintained over 4 passages through serial passaging before analysis on CytoFLEX (Beckman Coulter). Orange: 1st passage, Green: 2nd passage, Blue: 3rd passage. Magenta: 4th passage

JotDye for Labeling Red Blood Cells



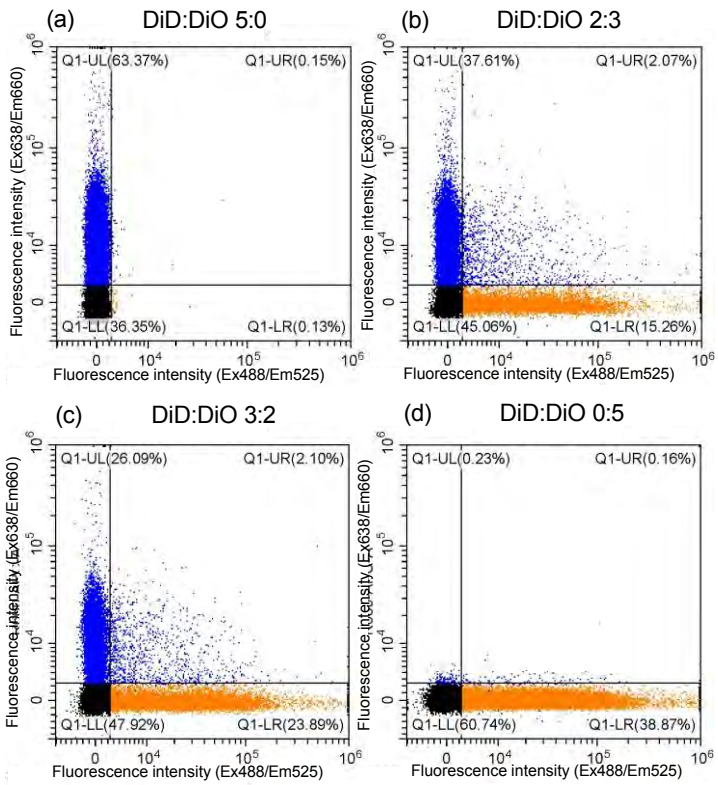
Red blood cells. Flow cytometry analysis of samples that have a mixture of unstained red blood cells (RBC) and RBC stained with JotDye, in the ratio of (a) 0:5, (b) 2:3, (c) 3:2 and (d) 5:0. The JotDye for RBC remains stable in the cell after labelling and does not cross-over into samples that are not intended to be labelled with the dye.(e) Bright field and (f) fluorescent confocal micrograph of RBCs stain with JotDye.

Jotbody Membrane Dyes: Colocalization with traditional lipophilic dyes



Localization of Jotbody Liposome Dye in the lipid bilayer. Large multilamellar vesicles, comprising 1- palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-1'- glycerol (POPG) (8:2 ratio), were stained simultaneously with 15 µM of Jotbody Liposome Dye and FM4-64. Fluorescence channels for (a) JotDye, (b) FM4-64 and (c) both channels merged. (d) Fluorescence trace for the white line in (c) demonstrates the co-localization of JotDye and FM4-64.

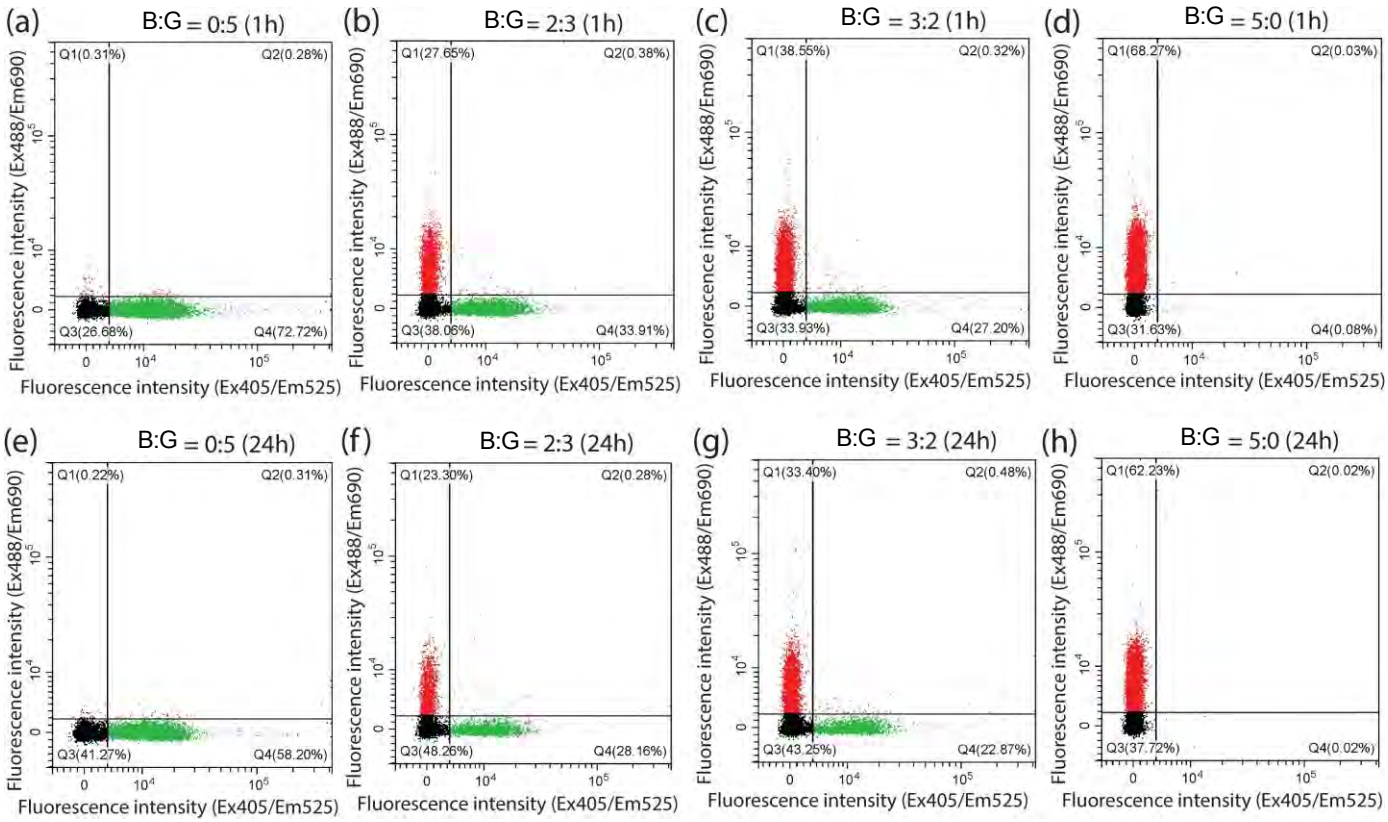
Pain points with Current Lipophilic Dyes



Instability of liposome labeling using the commercially available carbocyanine dyes. Mixtures of labeled 130 nm liposomes in the ratio of DiD:DiO (a) 0:5, (b) 2:3, (c) 3:2 and (d) 5:0, were analyzed on CytoFLEX. (Beckman Coulter) after 1 h incubation. The presence of dual-positive events in Q1UR suggests that the dye labels were not stable and can transfer from one population to another.

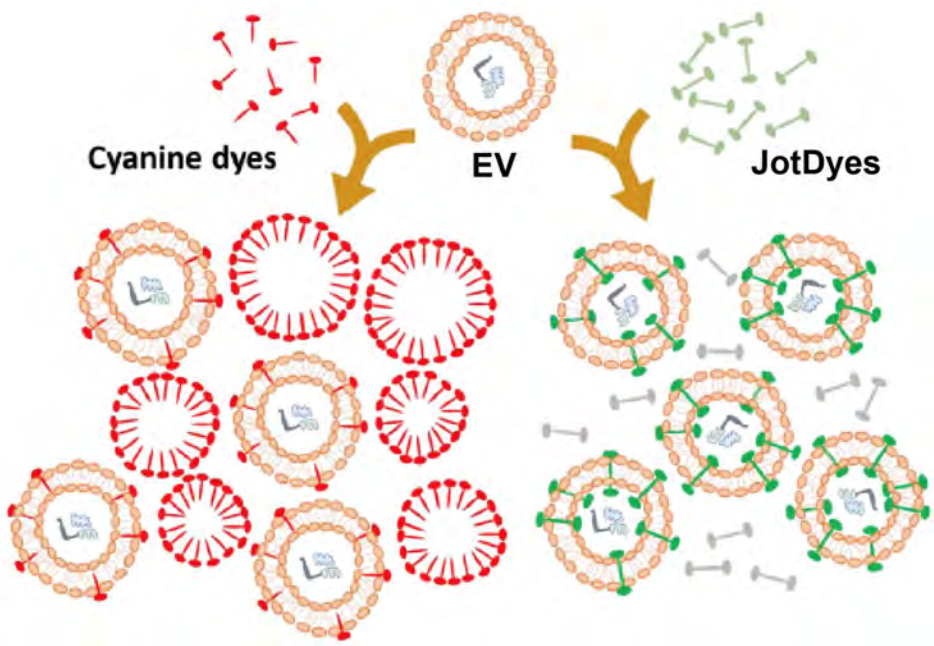
Labeling using lipophilic dyes from the carbocyanine family tend not to be stable due to the "leakage" of dye from their targets. These free dyes end up labeling other membrane-based targets that can lead to misleading conclusions.

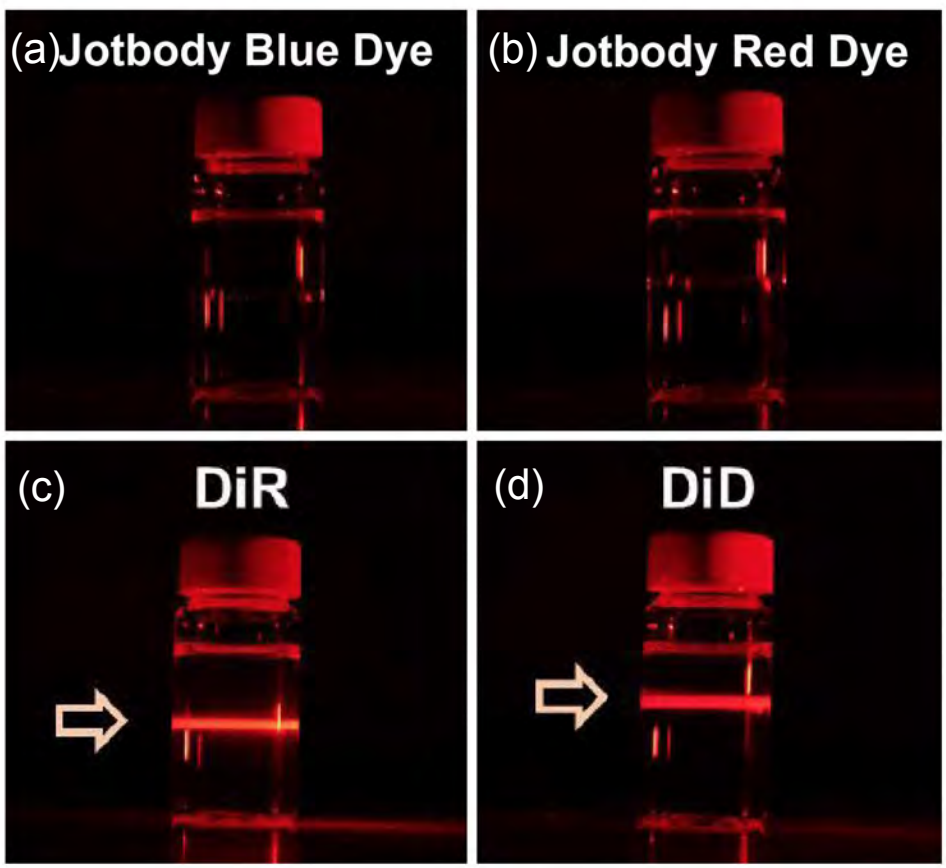
Single Particle Analysis using JotDyes
Stable labeling: No inter-particle dye exchange



Single particle analysis of 130 nm liposomes labeled with Membrane-Stable Jotbody Blue Dye (B) or Green Dye (G). Mixtures of labeled liposomes in the ratio of Jotbody B:R (a,e) 0:5, (b,f) 2:3, (c,g) 3:2 and (d,h) 5:0, were analyzed on CytoFLEX. (Beckman Coulter) after 1 h (a-d) and 24 h (e-h) incubation. The stability of labeling with JotDyes allowed for a consistent analysis of the labeled liposome mixture even after 24 h, where no dye exchanges were observed between the two liposome populations. The advantage of such stability in dye labeling allows users to unambiguously make conclusions on their observations of the fluorescence data.

No Micelle Formation of JotDyes

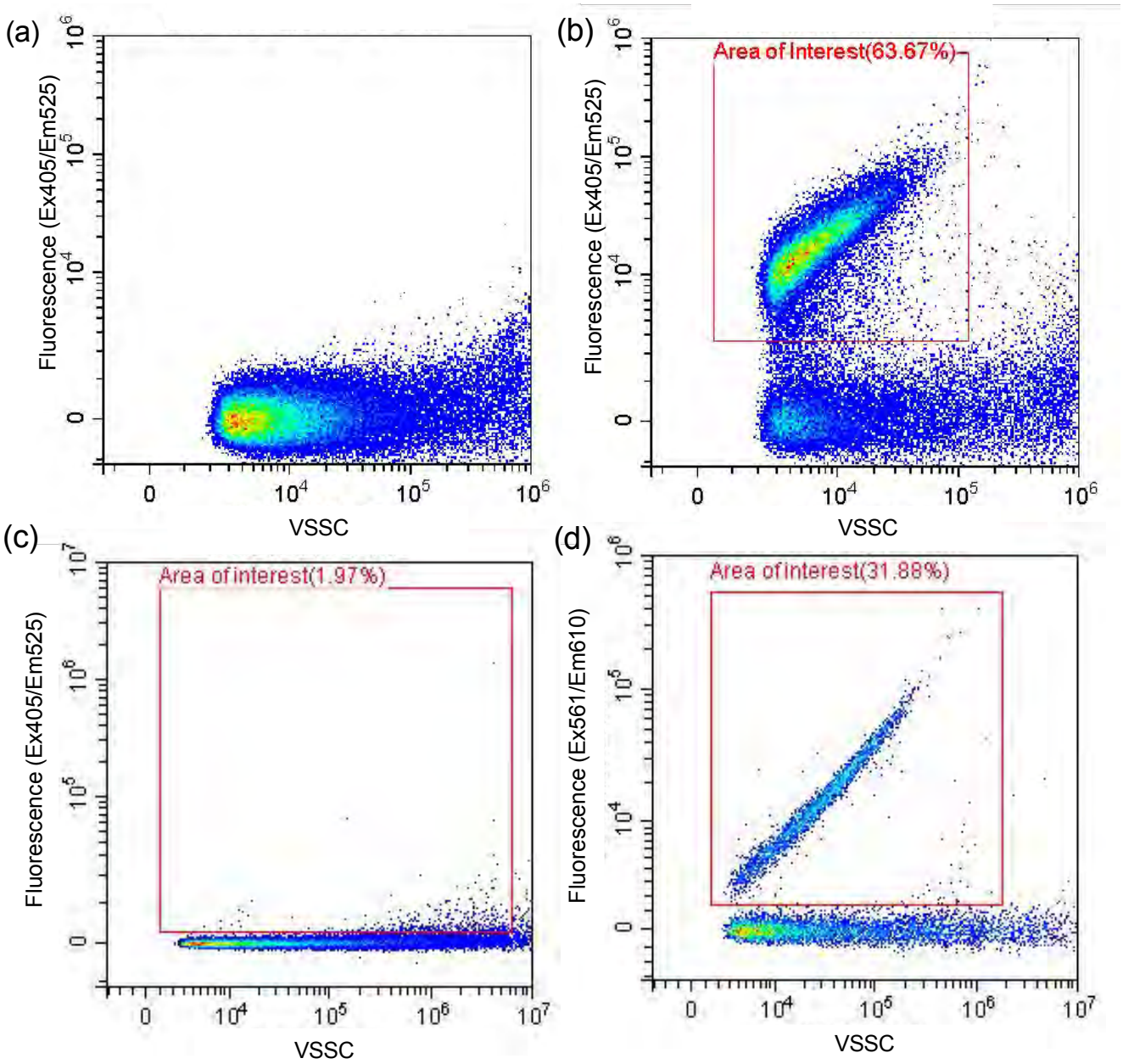




Tyndall effect of nanoparticles. JotDyes are highly water soluble and do not form micelles or nanoparticles when reconstituted in aqueous buffer (a-b), unlike that of the family of PKH26, DiR and DiD dyes (c - d), in which their nanoparticles will give rise to false positives in flow cytometry analysis.

JotDyes promise a **low rate of false positives** for the accurate and reliable detection of EVs.

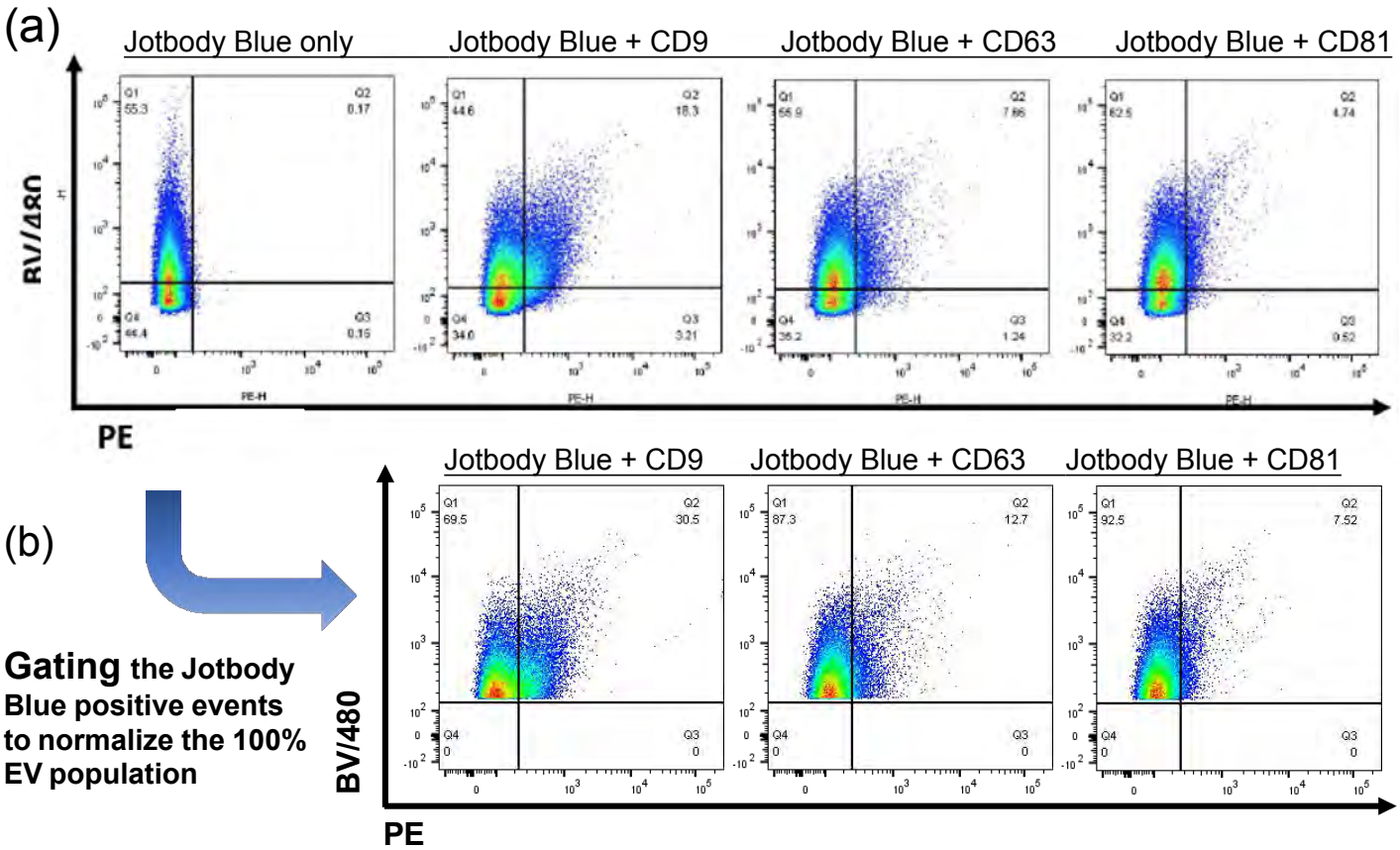
Using JotDyes for Single Particle Analysis by Flow Cytometry

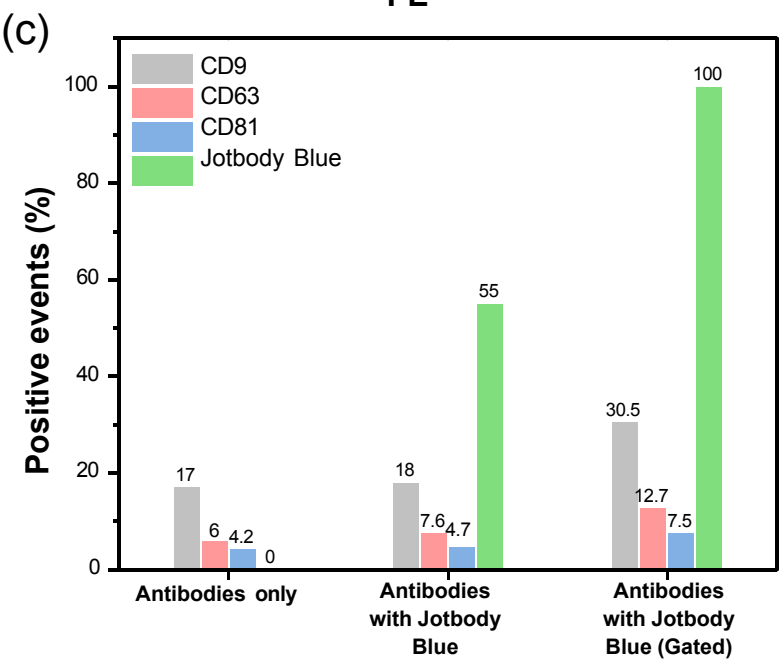


Flow cytometry analysis of EVs labeled with JotDye. Dot plot of (a) unstained EVs, (b) EVs labeled with 1 µM JotDye, (c) 1 µM JotDye only and (e) 1 µM of commercially available PKH26 dye only i.e., in the absence of EVs.

Exosomes are extracellular vesicles that serve as biomarkers for disease detection and have also been utilized as delivery vehicles for therapeutics.

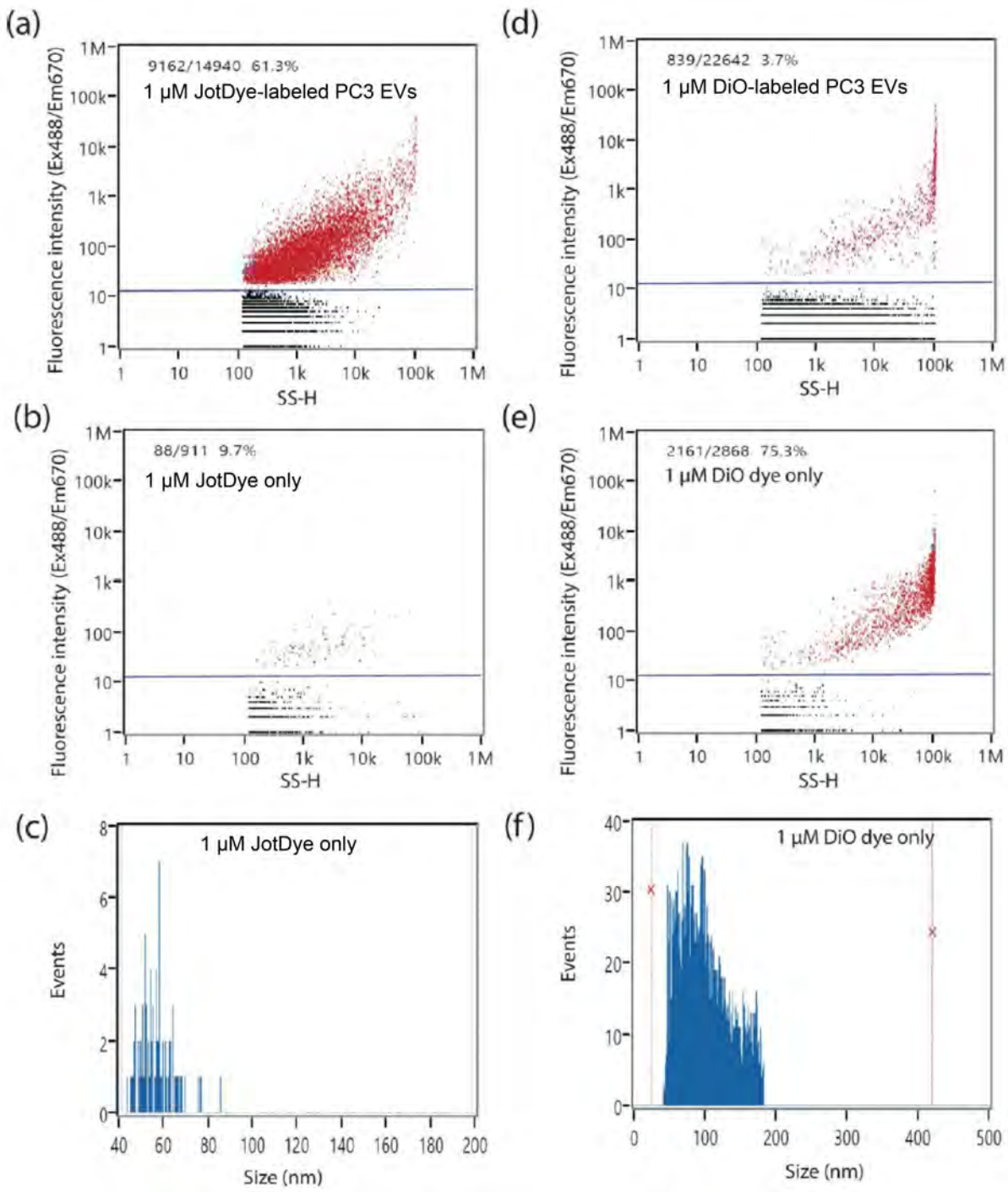
Using JotDyes to Normalize Data for Population Analysis Using Flow Cytometry





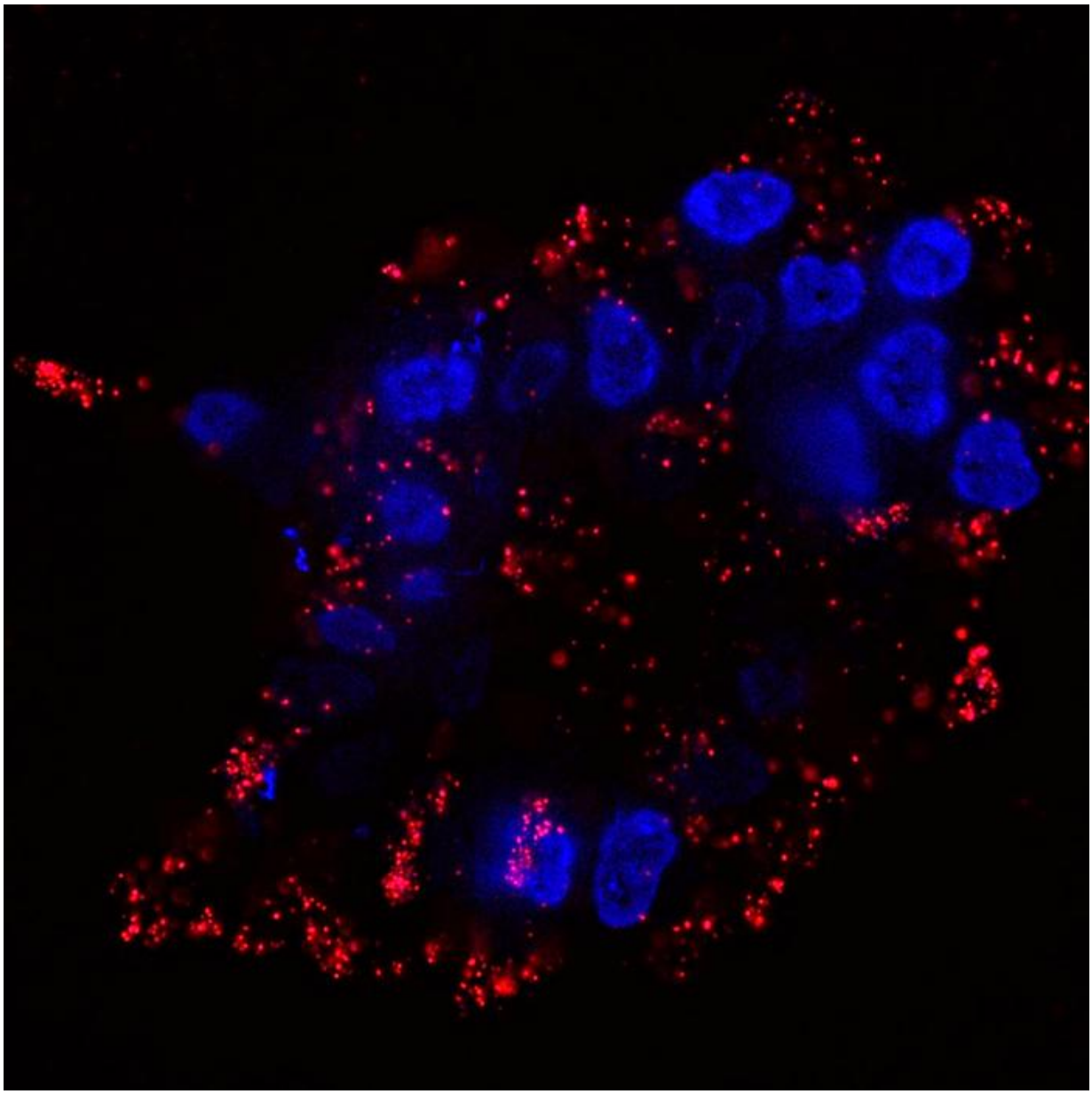
Population analysis of EVs using JotDyes. Flow cytometry measurements were carried out on the A1 BDflow cytometer (Becton Dickinson).(a) Ungated dot plot with *x*-axis for PE- conjugated antibody channel (PE) and y-axis for Jotbody Blue Dye channel (BV480). From left to right: EVs labeled with Jotbody Blue Dye only, EVs labeled with Jotbody Blue Dye and CD9-PE, with CD63-PE and CD81-PE. (b) The plots in (a) were then gated to only show the Jotbody Blue Dye positive events (i.e., 100% EV population) to reveal the true percentage population of the CD- positive EVs. (C) Plot of dye positive events (%) for EVs that were only stained with antibodies or co-stained with Jotbody Blue Dye, and the effect of gating on the Jotbody positive events.

JotDye-Labeled EV Detection on Nano Flow Cytometry



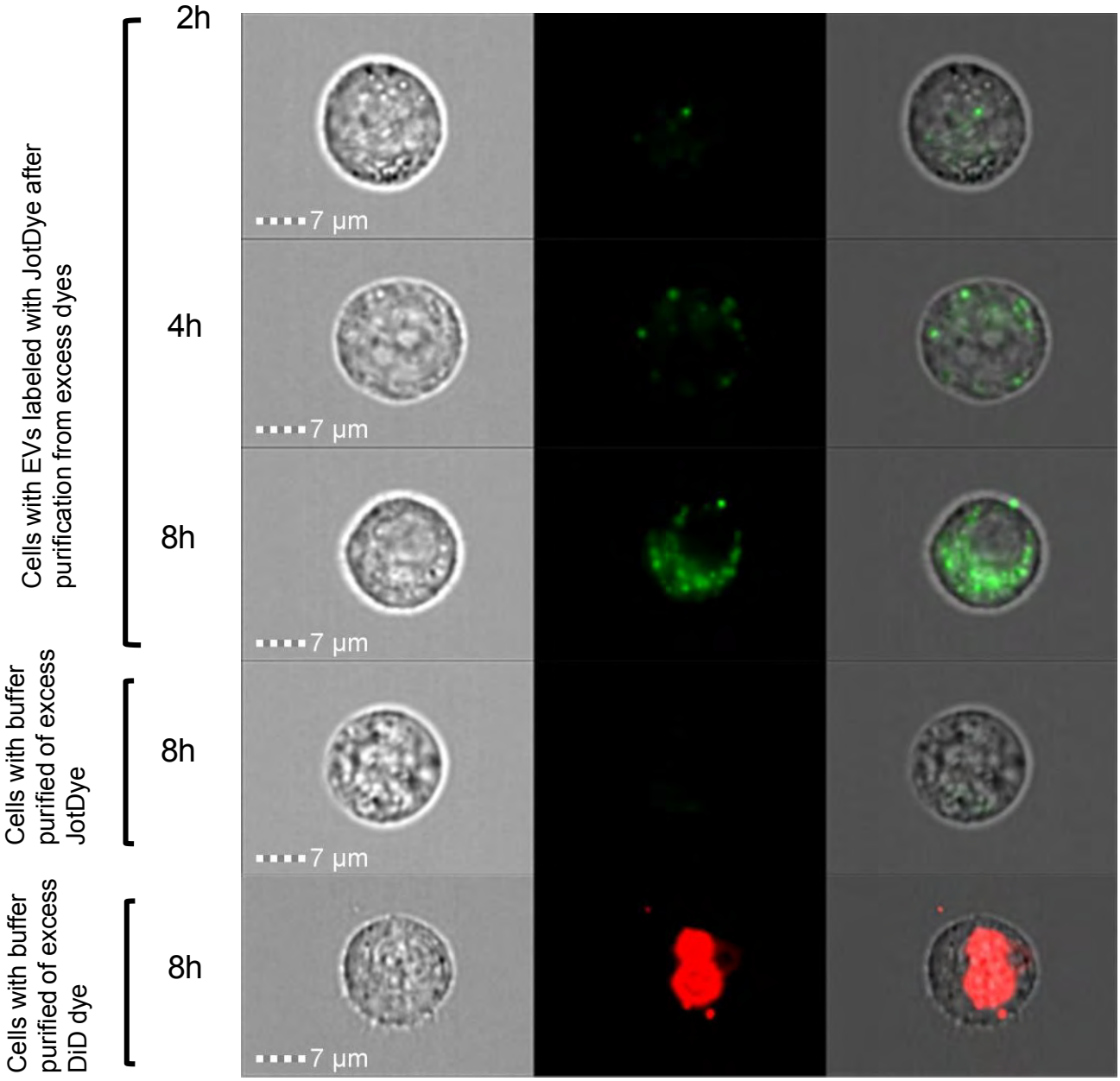
Nano flow cytometry. PC3 EVs were labeled with (a) 1 μM JotDye and analyzed on the NanoAnalyzer (NanoFCM). A low rate of false positives with the JotDye in buffer only (b) with negligible particle counts detected (c). In contrast, 130 nm-size nanoparticles were observed in a solution of DiO dye only, which did not stain the EVs efficiently (d-f).

Monitoring Uptake of JotDye-Labeled EVs with Fluorescent Microscopy



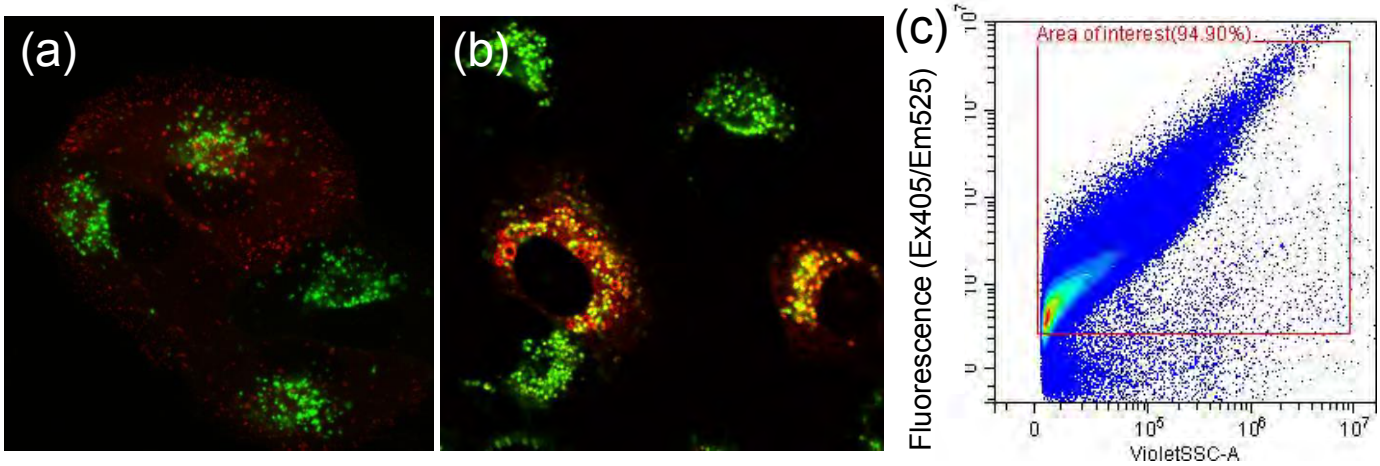
Uptake of JotDye-Labeled EVs in cell culture. Fluorescence micrograph of HEK-293T cells treated with 20 ug of PC-3 EVs stained with JotDye-600 (red) after 24 hours of incubation. Nuclei stained with SYTO Far red (blue). Image taken on Leica THUNDER imager fluorescence microscope and processed using the built-in THUNDER computational clearing method and ImageJ.

Monitoring Uptake of JotDye-Labeled EV with Imaging Flow Cytometry



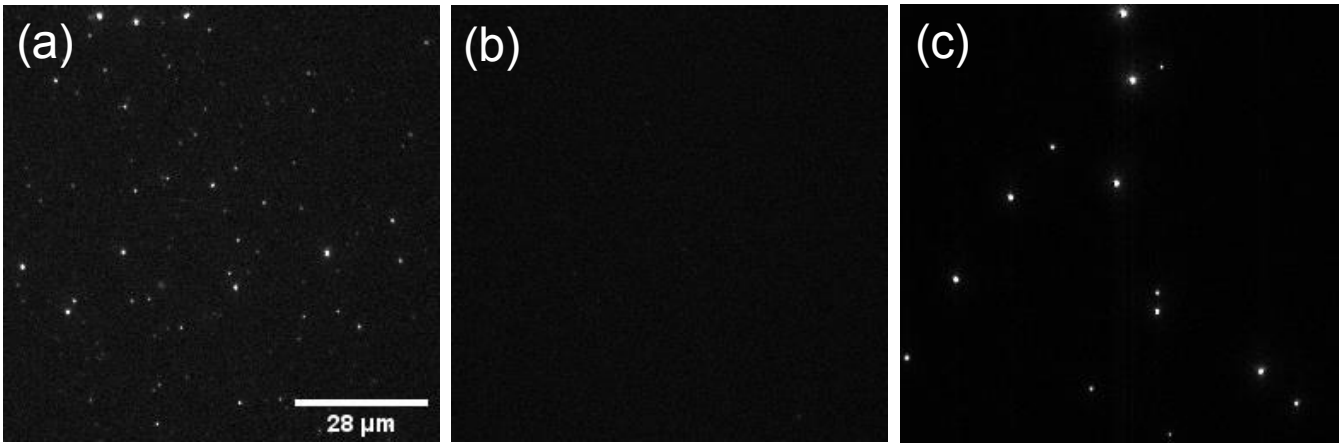
Imaging flow cytometry. PC3 EVs were labeled with 2 µM JotDye and purified using the Amicon 100KDa filter before they were added to A549 cells and incubated. The cells were analyzed on the Imagestream (Amnis) after (a) 2 h, (b) 4 h and (c) 8 h of incubation. The JotDye-labeled EVs were observed as spots (green). In the absence of EVs, no fluorescence signal was observed in the cells because all the free JotDye, which does not form micelles, were removed from the purification step (d). By contrast, micelles of DiD dye were not purified and thus there was still fluorescence signals being observed from the cells, suggesting the uptake of the DiD micelles which can be mistaken for DiD-labelled EVs instead.

JotDye for Endogenously Stained EVs



Endogenously stained EVs with JotDye. A549 cells were incubated with 2 μM JotDye for 2 days before the cell culture was ultra-centrifuged to obtain the pre-stained EVs. Confocal imaging studies reveal that the internalized JotDye (green in both (a) and (b)) did not co-localize with the (a) early endosome marker (red) but co-localized with the (b) late endosome marker (red) instead. Late endosomes are the intracellular origins of EV production. (c) Dot plot of stained EVs with JotDye.

TIRF Imaging of JotDye-Labeled EVs



Total internal reflection fluorescence (TIRF) imaging of EVs. PC3 EVs labeled with Jotbody EV Green Dye were immobilized on a substrate and imaged using TIRF microscopy; the diffraction limit is approximately 100 nm. (b) Jotbody EV Green Dye itself does not form any nanoparticles that are of the same size as the EVs. Hence, no fluorescence was observed. On the contrary, the commercially available PKH26 dye forms particles that are large enough to be imaged similarly like the EVs by TIRF (c).

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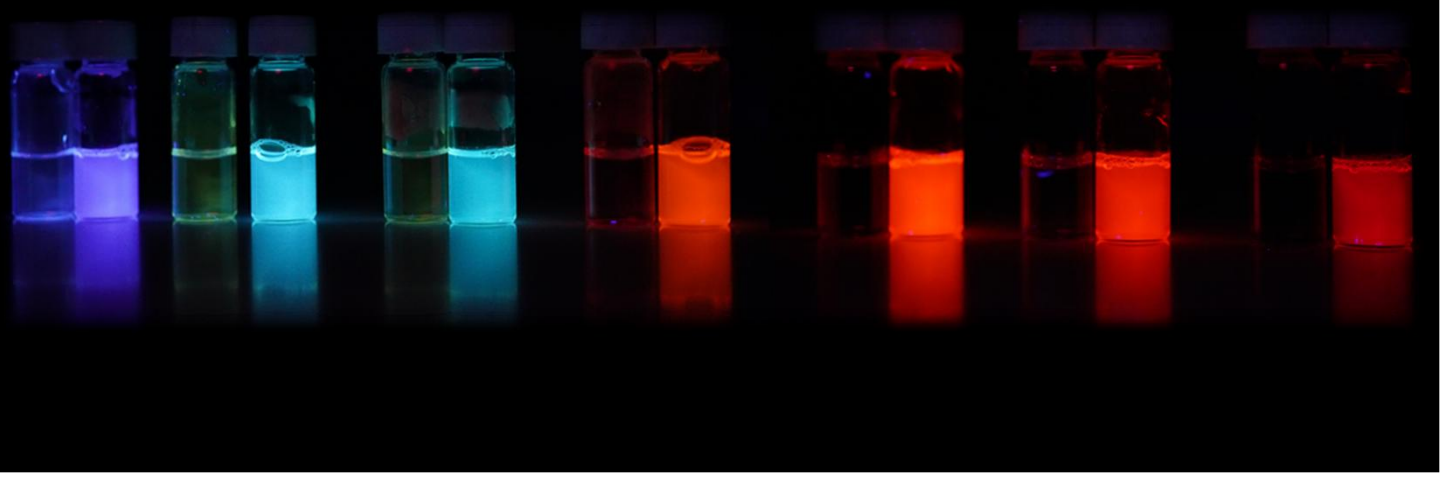

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