Using imaging flow cytometry to detect lipid nanoparticles phagocytosed by antigen-presenting cells

Aaron J. Tyznik[1], Anthony Phan[2], Xiaoshan Shi[3], Louise M. D'Cruz[1], Stephanie J. Widmann[1] and Christopher A. Hunter[2]

[1]BD Biosciences, San Diego, California 92121, USA; [2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia 19104, USA ;
[3]BD Biosciences, San Jose, California 95131, USA;

Abstract

DNA or mRNA vaccines encoding antigens that can elicit host immune responses have shown great efficacy for combating infectious disease and potentially treatment of cancer. Effective delivery of nucleic acid vaccines can be achieved through nucleic acid-encapsulated lipid nanoparticles (LNPs). Despite the proved effectiveness of nucleic acid vaccines in different disease settings, the mechanism by which antigen presenting cells recognize the LNP cargo upon vaccination is not understood. In this study, we utilized the state-of-the-art CellView™ Image Technology on the BD FACSDiscover™ S8 Cell Sorter to visualize LNP phagocytosed by host cells. To determine the mechanisms of antigen presentation, green fluorescent LNPs were formulated *in vitro* and injected into mice. With the imaging capabilities of the FACSDiscover™ S8 Cell Sorter, we visualized LNPs in different antigen presenting cells and observed distinct morphology of LNPs in dendritic cell subsets, monocytes, and neutrophils. A time course experiment uncovered the kinetics of antigen recognition upon LNP-mediated cargo delivery. Unsupervised analysis using the imaging parameters derived from the instrument revealed unique cell clusters possibly associated with different morphology of LNP phagocytosis. This work provides an advanced workflow to visualize LNP phagocytosis followed by image-based cell sorting for downstream in-depth characterization enabled by the FACSDiscover™ system. With an increased adoption of LNP-mediated nucleic acid therapeutics and vaccines, the image-based cell sorter provides a powerful tool to understand the mechanism of host immunological response upon vaccine injection.

Method

Experimental workflow

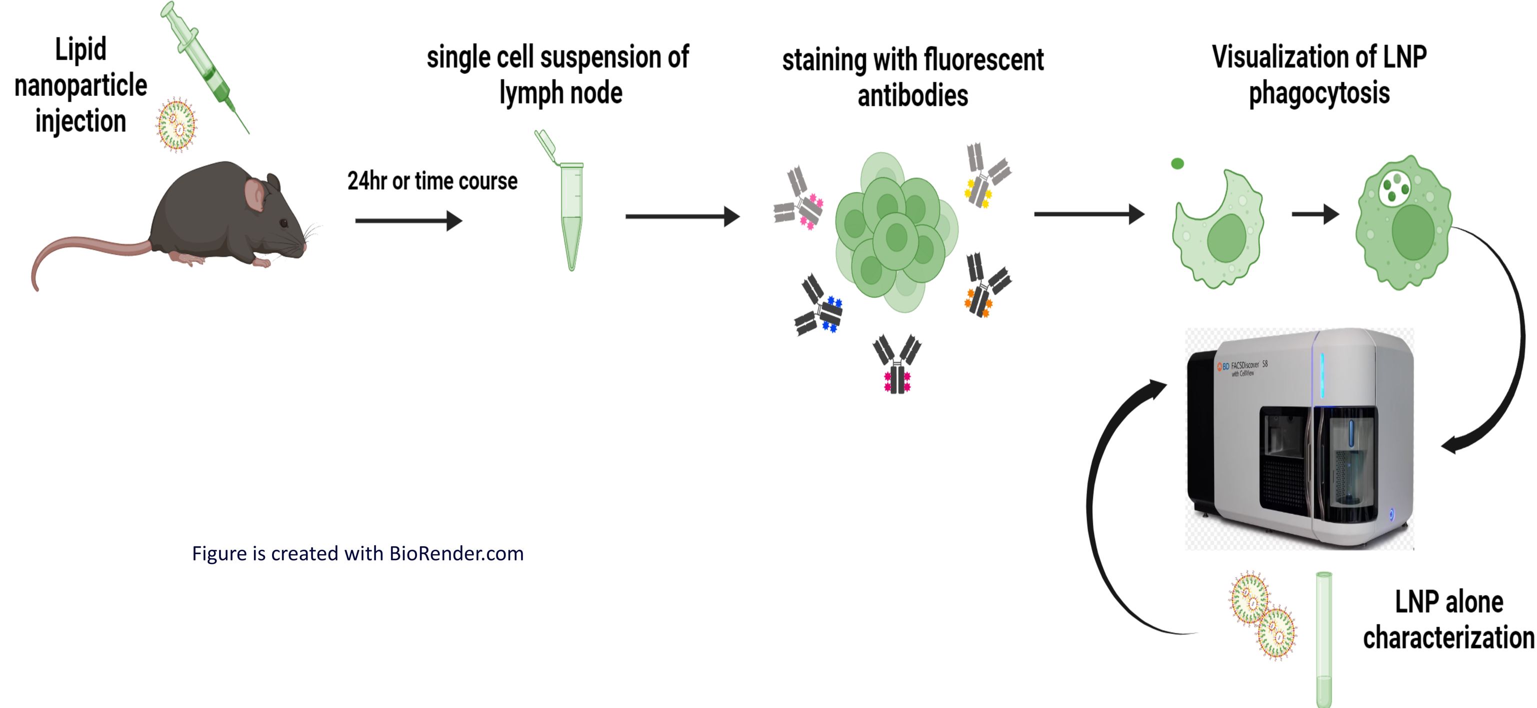


Table. 18-color fluorescent panel to visualize LNP in antigen-presenting cells

Laser	Fluorophore	Antibody
UV Laser	BUV395	CD19
	BUV496	CD4
	BUV563	Ly6G
	BUV615	CD8
	BUV661	CD11b
	BUV737	CD11c
	BUV805	CD45
Violet Laser	BV421	PDCA-1
	Ghost Dye™ Violet 510	Live dead
	BV605	CX3CR1
	BV650	XCR1
	BV711	CD90.2
	BV786	Ly6C
Blue Laser	FITC (Imaging Channel 1)	DiO
Yellow Green Laser	PE-Cy7	CD64
Red Laser	APC	H2-Kb SIINFEKL
	AF700	MHCII – IA/IE
	APC-Cy7	CD172α

Results: kinetics of LNP phagocytosis using imaging parameter-driven clustering

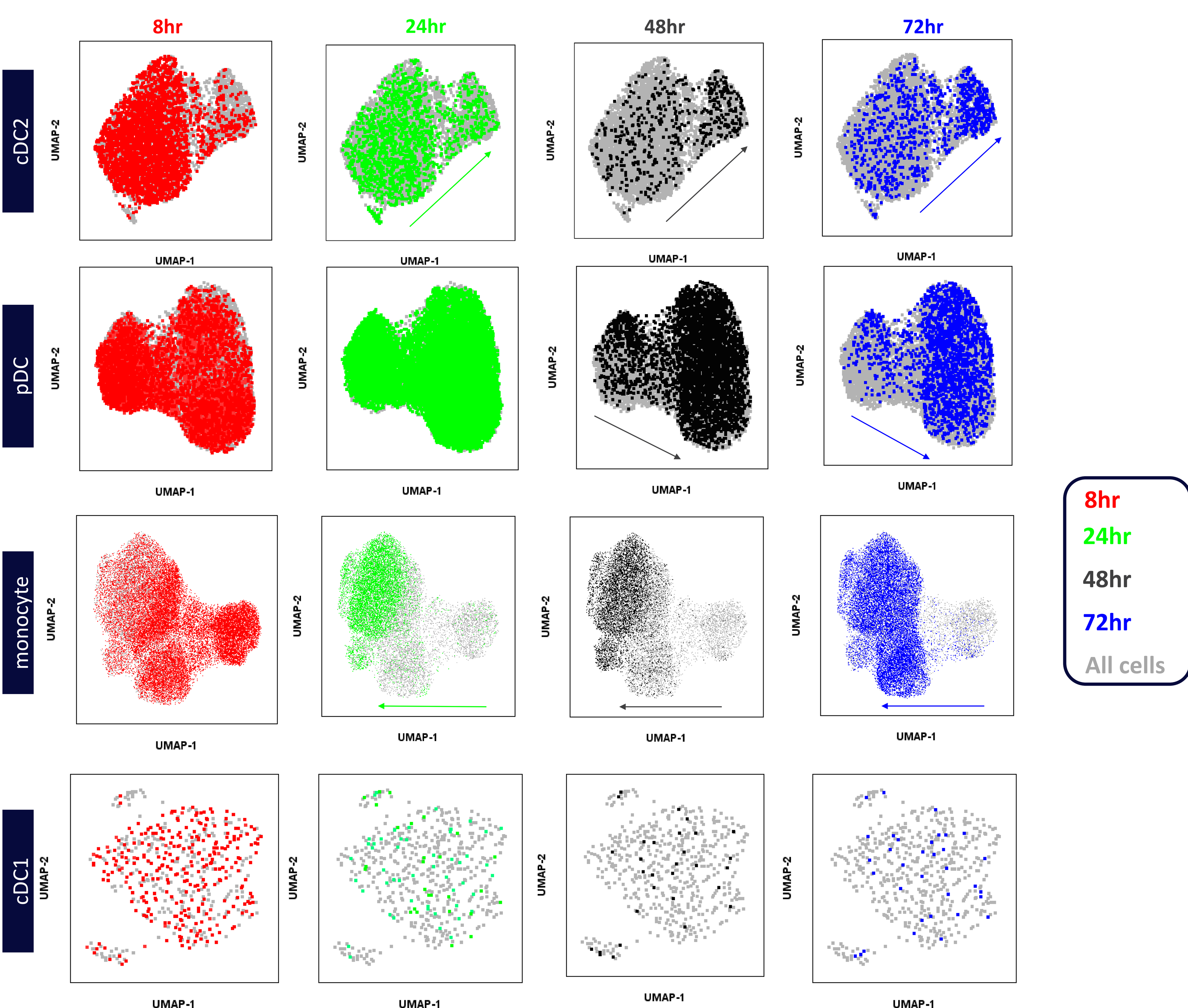
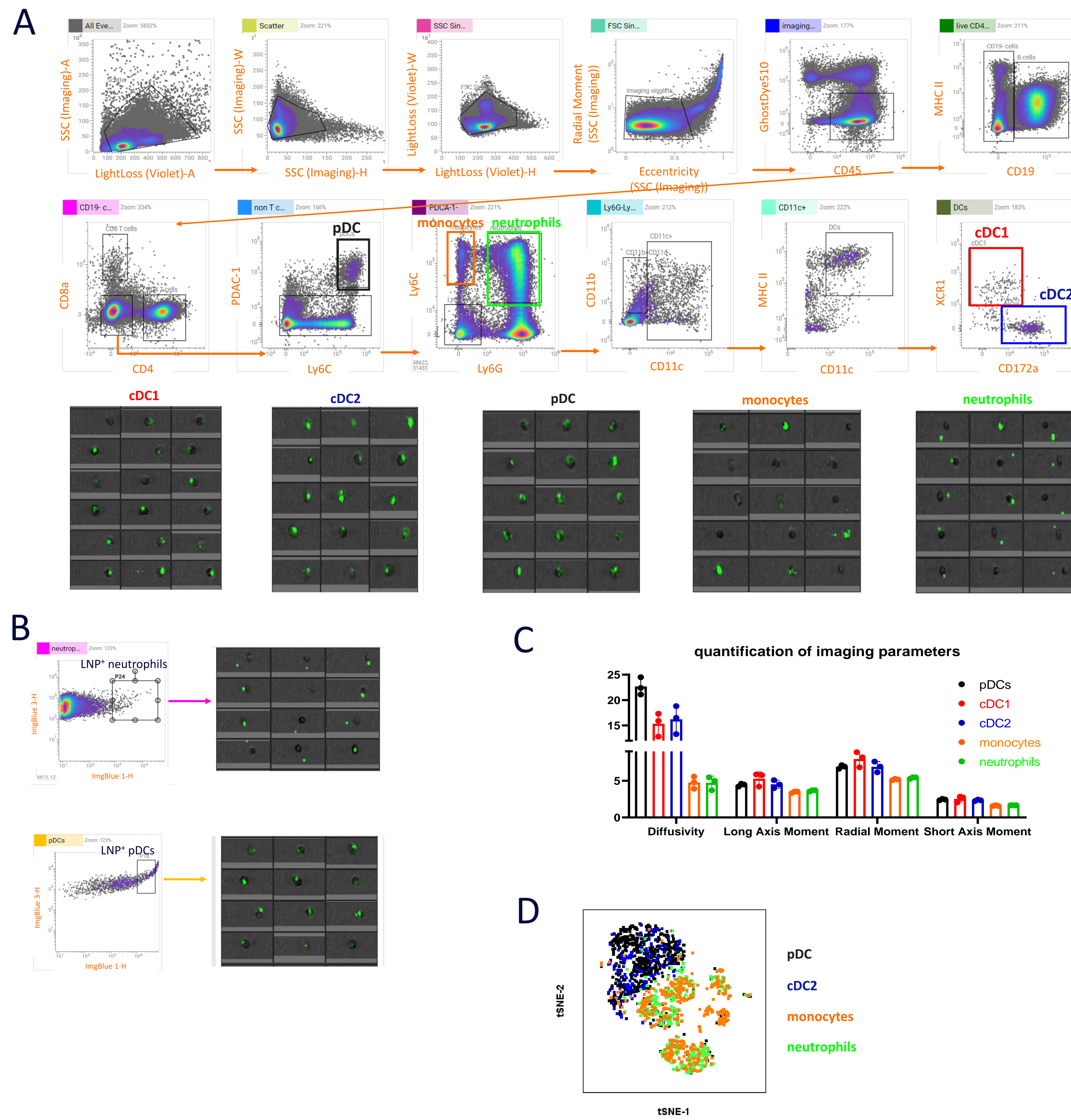


Figure 2. Kinetics of LNP phagocytosis using imaging parameter-driven clustering. LNPs were injected into mice intramuscularly and mice was harvested at 8hr, 24hr, 48hr and 72hr post injection. Single-cell suspensions from lymph node were stained with the 18-color fluorescent panel and acquired using FACSDiscover™ Cell Sorter. Different cell populations (cDC2, pDC, monocyte and cDC1) were concatenated from three mice and UMAP analysis was performed on each cell type using only imaging parameters from the instrument. UMAP plots at different time points indicated possible changes of LNP morphology upon uptake.

Results: visualization of LNP phagocytosis in different antigen-presenting cells



B

LNP⁺ neutrophils

LNP⁺ pDCs

C

quantification of imaging parameters

pDCs, cDC1, cDC2, monocytes, neutrophils

Diffusivity, Long Axis Moment, Radial Moment, Short Axis Moment

D

tSNE-1, tSNE-2

pDC, cDC2, monocytes, neutrophils

Figure 1. Visualization of LNP phagocytosis in different subsets of antigen presenting cells. (A) 18-color fluorescent panel to identify different cell populations (cDC1, cDC2, pDC, monocytes and neutrophils) and visualization of LNP phagocytosis in different cell subsets. (B) Images from FACSDiscover™ system revealed the morphology of LNP phagocytosis. Even though both neutrophils and pDCs possessed LNP positive cells, neutrophils didn't seem to uptake LNP whereas pDCs phagocytosed LNP into the cytoplasm. (C) Measurement of key imaging parameters associated with morphology derived from FACSDiscover™ Cell Sorter indicated difference in LNP phagocytosis in different cell types. (D) Unsupervised tSNE analysis of different antigen presenting cells resulted in separation between dendritic cells (cDC2 and pDC) from monocytes and neutrophils, suggesting different morphology of LNP phagocytosis in different cell types.

Results: acquisition of LNP alone using FACSDiscover™ Cell Sorter

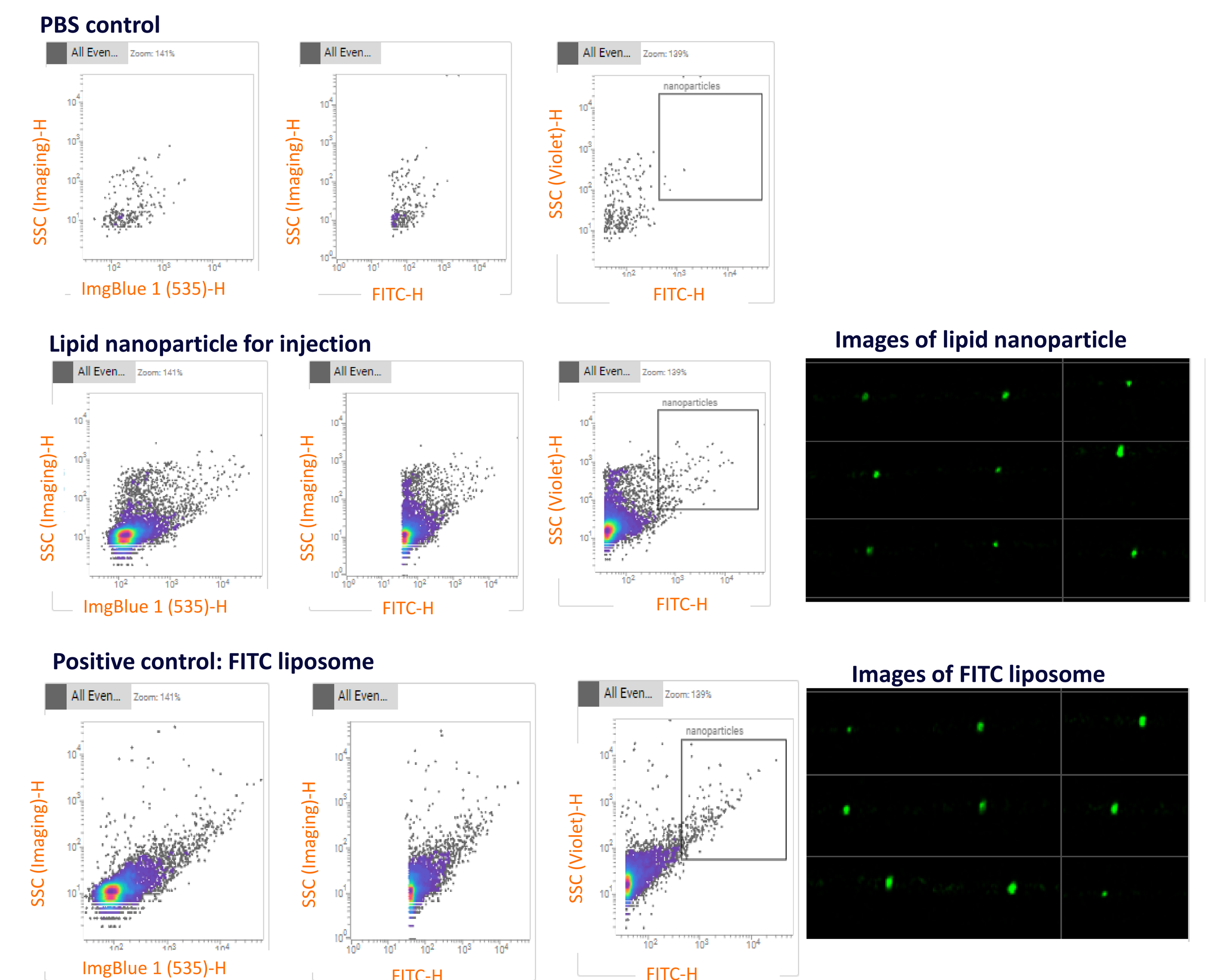


Figure 3. Visualization of lipid nanoparticles using FACSDiscover™ Cell Sorter. DiO/DiI LNPs used for mice injection were acquired using FACSDiscover™ Cell Sorter. LNP can be identified using scatter plot. Green fluorescent signal was detected using both FITC fluorescent channel and imaging channel 1. Images were acquired for LNPs. Filtered PBS was acquired as a negative control. FITC liposome was run simultaneously to set up the instrument and served as a positive control.

Conclusions

- LNPs were injected into mice intramuscularly to evaluate the biological process of LNP uptake by antigen-presenting cells using imaging flow cytometry FACSDiscover™ system;
- Single-cell suspensions from lymph node of mice were stained with an 18-color fluorescent panel to identify different cell subsets of antigen-presenting cells.
- LNPs uptake by different antigen-presenting cells were clearly visualized using FACSDiscover™ system;
- Images provided additional information on LNP phagocytosis compared to fluorescent staining only;
- Quantification of image parameters in different cell types revealed different morphology of LNP uptake by different antigen-presenting cells;
- A time-course experiment uncovered the kinetics of LNP phagocytosis by different antigen-presenting cells;
- Acquisition of LNP alone on FACSDiscover™ system helped evaluate the size and fluorescence intensity of LNPs, providing additional information on LNP characteristics and serving as an efficient quality control tool of LNP prior to *in vivo* injection.

Class 1 Laser Product.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.

BD, the BD logo and FACSDiscover™ S8 Cell Sorter are the trademarks of Becton, Dickinson or its affiliates. © 2024 BD. All rights reserved. BD-xxxxxx (v1.0) 0524