IFI Flow Cytometry Facility

UCI Chao Family Comprehensive Cancer Center

Leadership

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NanoCyte Cell Analyzer

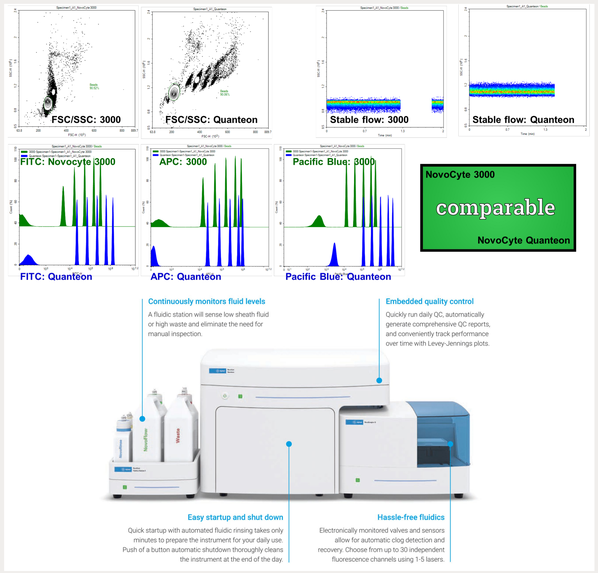
Our facility has 2 high-throughput cell analyzers

- **NovoCyte Quanteon**: 4 lasers with 25 parameter detectors
- **NovoCyte 3000**: 3 lasers with 15 parameter detectors.

The fluidic system is exceptionally stable and has automation for high throughput analysis of multiple plates in addition to support for traditional 5 ml tubes. Startup, shutdown, and other fluidic maintenance procedures are largely automated.

Performance comparison of NovoCyte 3000 and Quanteon using QC/calibration beads

	NovoCyte 3000			NovoCyte Quanteon				
Sextuplicate	1	2	3	4	5	6	Average	% CV
Events	100000	100000	100000	100000	100000	100000	100000	0
Events/Sec	1111	111	1111	1111	1099	1099	1107	0.56
Volume (µl)	98.39	99.21	98.82	98.30	100.22	99.91	99.14	0.80
Time (Sec)	90	90	90	90	91	91	90.33	0.57
Cell Count (µl)	926	919	925	923	907	909	918	0.9

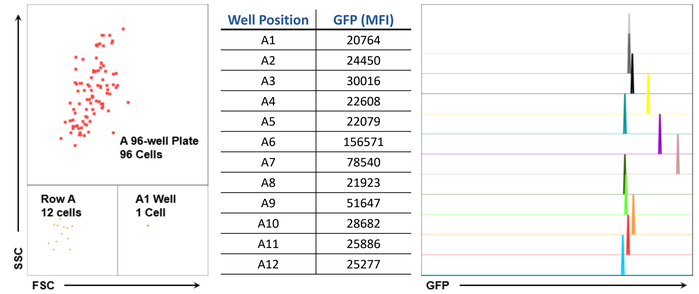


BD FACSAria Fusion Cell Sorter

Our facility houses a BD FACSAria Fusion sorter with 4 lasers and up to 11 fluorescent parameters. The BD FACSAria Fusion allows for 4-way bulk sorting into tubes or one-way sorting of single cells into 6/24/48/96-well plates. This instrument is also equipped with a temperature control system allowing for cooling of the sort chamber and collection device.

Laser	Filter	Preferred Colors
405	450/50	Alexa Fluor 405, Brilliant Violet 421, Pacific Blue, V450
	525/50	Brilliant Violet 510, V500, AmCyan
	610/20	Brilliant Violet 605
488	530/30	FITC, Alexa Fluor 488, GFP
	695/40	PerCP, PerCP-Cy5.5
561	582/15	PE, dsRed
	610/20	PI, PE-Texas Red, mCherry, PE-CF594
	670/14	PE-Cy5, PE-Alexa 647
	780/60	PE-Cy7
640	670/30	APC, Alexa Fluor 647
	780/60	APC-Cy7, APC-H7, APC-Alexa 750, Alexa Fluor 750, DyLight 750

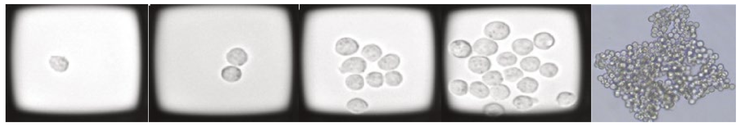
Single cell index sorting allows the isolation of single cells with retrospective identification of each single cell's immune phenotype



Well Position	GFP (MFI)
A1	20764
A2	24450
A3	30016
A4	22608
A5	22079
A6	156571
A7	78540
A8	21923
A9	51647
A10	28682
A11	25886
A12	25277

Index sorting: A method that deposits individual cells from a heterogeneous mixture into wells of 6/24/48/96 plates. Cells are usually sorted using specific fluorochromes and then channeled into an empty well. Cells that do not meet the specified criteria are shunted to a waste tube.

Index sorting for downstream applications: Single cell cloning



Single cell cloning: Separating cells of interest gives scientists the opportunity to culture individual cells in isolation. Single cell sorting is a powerful tool for generating clones with stably genome-integrated transfected genes. (Dr. Marco Bassetto uses this approach)

Index sorting for downstream applications: Single cell analysis

Single cell analysis: When performed on bulk samples, gene expression studies might yield only a limited understanding of complex samples. However, gene expression analyses performed on single cells can offer a powerful method to resolve sample heterogeneity and reveal hidden biology. (Dr. Minji Byun uses this approach)

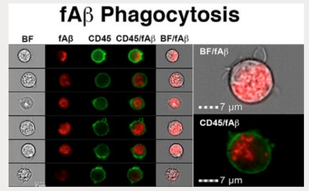
Cytek Amnis ImageStream

- The Cytek Amnis ImageStream MKII combines the phenotyping abilities of flow cytometry with the detailed imagery and functional insights of microscopy.
- There are numerous applications for qualitative and quantitative measurements including cell location, internalization, cell cycle, morphology, cell-cell interaction, and co-localization.

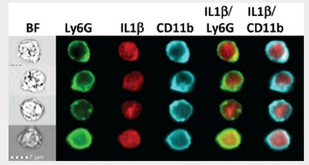
Laser	Filter	Preferred Colors
405	457/45	Alexa Fluor 405, Brilliant Violet 421, Pacific Blue, V450, DAPI
	537/65	Brilliant Violet 510, V500, Pacific Orange, Alexa Fluor 430, Qdot 525
	582/25	Qdot 565, Qdot 585
	610/30	Brilliant Violet 605, Qdot 605, eFluor 605NC
	702/85	Brilliant Violet 711
	762/35	Brilliant Violet 786, Qdot 800
488	528/65	FITC, Alexa Fluor 488, GFP
	577/35	PE, Cy3, dsRed, Alexa Fluor 555
	610/30	PE-Texas Red, PE-Alexa 610, PI, PE-DyLight 594
	702/85	PE-Cy5, PE-Alexa 647, 7-AAD, PI, PerCP, PerCP-Cy5.5, DRAQ 5
	762/35	PE-Cy7, PE-Vio770
642	702/85	APC, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Cy5
	762/35	APC-Cy7, APC-H7, APC-Alexa 750, Alexa Fluor 750, DyLight 750

Why use ImageStream?

- If you have a question that requires cell morphology
- If the spatial context of the signal within the cell is important to your research
- If you have rare cell events or short-lived events that are hard to find by normal microscopy.



Human microglial-like cells (iMGLs) phagocytose human brain-derived ingest amyloid β (Aβ). Blurton-Jones. Neuron. 2017 94: 278–293



Representative neutrophils from infected corneas immunostained with antibodies to Ly6G and CD11b, and intracellular IL-1β. Pearlman. J Immunol. 2018; 201: 2767–2775.

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