New State-of-the-Art Technology for Cytometry
Overview of RFCC Services

Wednesday, December 14, 2022

Cincinnati Children's *changing the outcome together* | University of CINCINNATI

Overview

- General Services Offered (Thornton)

- State-of-the-Art Services
  - Single Cell Proteomics (Sproles)
  - Imaging Cytometry (Croswell)
  - High Parameter Cytometry - Analysis and Sorting (Quayle)
  - High Dimensional Data Analysis (Lages)

- Reminders

General Services

- Analytical Cytometers
- Cell Sorters
- Luminex

Analytical Cytometers

Instrument Name	Instrument Make/Model	Location	Number of Fluorescence PMTs				
			Blue (488nm)	Red (640nm)	Violet (405nm)	Yellow-Green (561nm)	UV (355nm)
Canto 1	BD FACSCanto	R5087A	2	3		4	
Canto 2	BD FACSCanto II	R5526	4	2	2		
Canto 3	BD FACSCanto	R5087A	5	3	3		
LSR II	BD LSR II	R5524	2	3	6	5	2
Fortessa 1	BD LSRFortessa	R5524	2	3	6	5	2
Fortessa 2	BD LSRFortessa	R5543	2	3	6	5	2
Fortessa 3	BD LSRFortessa	R5524	2	3	6	5	2
Aurora	Cytek	R5509	14	8	16	10	16
Maleficent	Cytek	R5509	14	8	16	10	16

Cell Sorters

Instrument Name	Instrument Make/Model	Location	Number of Fluorescence PMTs					Number of cell population
			Blue (488nm)	Red (635nm)	Violet (405nm)	Yellow-Green (561nm)	UV (335nm)	
Fusion	BD FACSAria Fusion	R5503	2	3	6	5	2	4
Arnie	BD FACSAria II	R5503	2	3	6	5	2	4
S6	BD FACSymphony	R5503	6	3	8	5	8	6
Bigfoot	ThermoFisher	R5529	7	5	8	12	12	6
SH800S	Sony SH800S	R5504	4 collinear lasers, 6 Channels					2
MA900	Sony MA900	R5504	4 lasers, 2 colinear spots, 12 Channels					4

Luminex
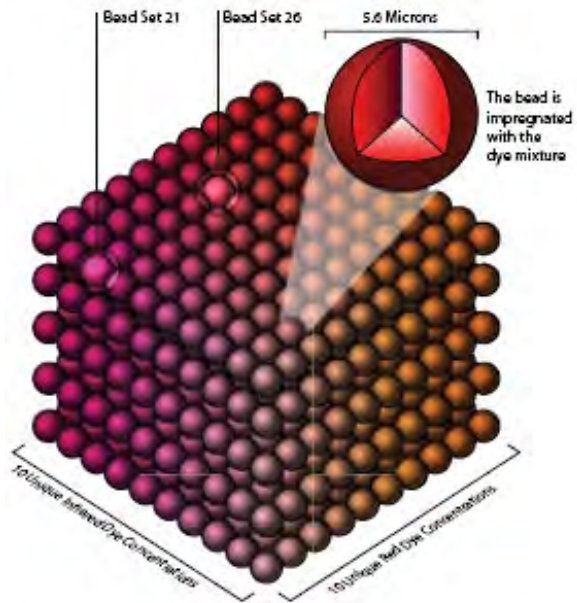
Luminex internally color-codes microspheres with precise concentrations of various fluorescent dyes yielding up to 500 distinctly colored bead sets.
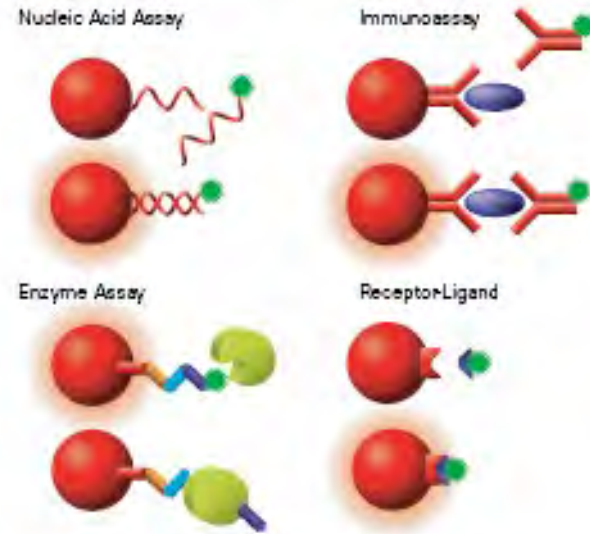
The microspheres can be coupled with reagents specific to a particular bioassay such as antigens, antibodies, oligonucleotides, enzyme substrates, or receptors.

Instrument	Room	BSL	Hours
Bio-Plex	R5543	BSL2	Monday-Friday 9am-5pm

- 25 ul sample
- Up to 100 analytes/sample

For more information contact Alyssa Sproles at 636-3575 or Alyssa.Sproles@cchmc.org.

State-of-the-Art Services

- Single Cell Proteomic
- Imaging Cytometry
- High Parameter Cytometry
- High Dimensional Data Analysis

Single Cell Proteomics (IsoLight)

- Automated single-cell or population chip ELISA reader.

- Functional immune profiling, intracellular signaling or high-plex immunoassays (up to 32) from single cell suspension (30ul) or serum/plasma (5.5ul).

IsoCode's Proteomic Barcoding Principles: Single-Cell

Single cell Microchambers: 12,000
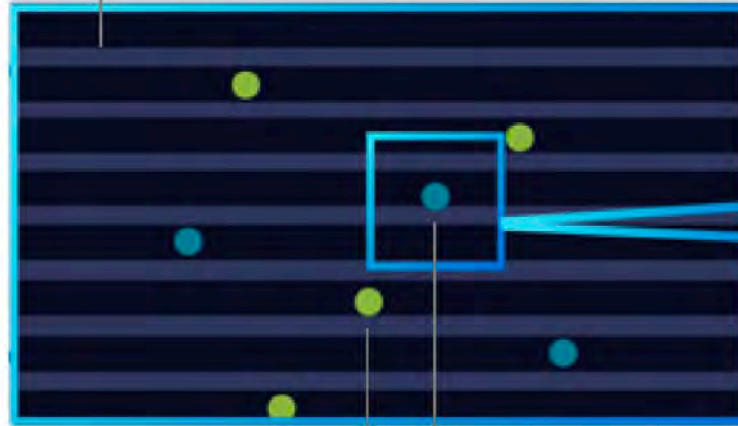
Cell Surface Fluorescence

Barcoded Chamber

Sandwich ELISA, per Lane

Secondary Antibody

Protein

Primary Antibody
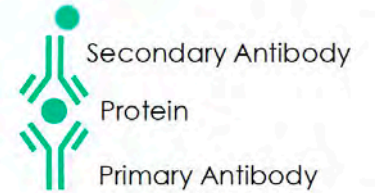
Multiplexed ELISA Cytokine capture
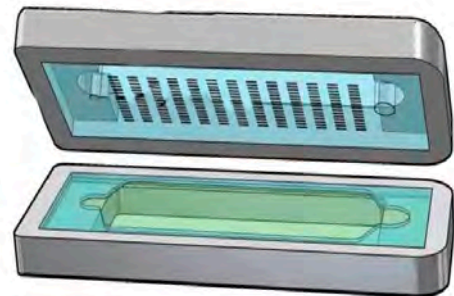
Per cell, each chamber

32+ Plex Cytokines

IsoCode Chip Family: Single-Cell Functional Secretomics

Single-Cell Adaptive Immune

Granzyme B, IFN-γ, MIP-1α, Perforin, TNF-α, TNF-β
GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21
CCL11, IP-10, MIP-1β, RANTES
IL-4, IL-10, IL-13, IL-22, TGFβ1, sCD137, sCD40L
IL-1β, IL-6, IL-17A, IL-17F, MCP-1, MCP-4

Adaptive:
T-Cells

Applications:
Predictive Pre-clinical Potency
Pre-clinical Persistence Modeling
Protective Immune Monitoring
Patient Stratification
New Functional T-cell types

Single-Cell Innate Immune

IFN-γ, MIP-1α, TNF-α, TNF-β
GM-CSF, IL-8, IL-9, IL-15, IL-18, TGF-α, IL-5, IL-15
CCL11, IP-10, MIP-1β, RANTES, BCA-1
IL-10, IL-13, IL-22, IL-22, sCD40L
IL-1β, IL-6, IL-12-p40, IL-12, IL-17A, IL-17F, MCP-1, MCP-4,
MIF, EGF, PDGF-BB, VEGF

Innate:
NK Cells
Monocytes
Macrophages
Fibroblast
Myeloid Cells

Applications:
Pre-clinical myeloid impact
Prognostic Immune Suppression
Early responder function
Predictive Neuroinflammation
New Innate Functional cell types

Single-Cell Inflammation

GM-CSF, IFN-γ, IL-2, IL-12, TNF-α, TNF-β
IL-4, IL-5, IL-7, IL-9, IL-13
CCL11, IL-8, IP-10, MCP-1, MCP-4, MIP-1α, MIP-1β, RANTES
IL-10, IL-15, IL-22, TGF-β1
IL-1β, IL-6, IL-17A, IL-17F, IL-21
Granzyme B, Perforin, sCD40L, sCD137

Adaptive:
T-Cells

Applications:
Pre-clinical Immune Suppression
Prognostic Autoimmune reaction

Imaging Cytometry

- Concept and applications
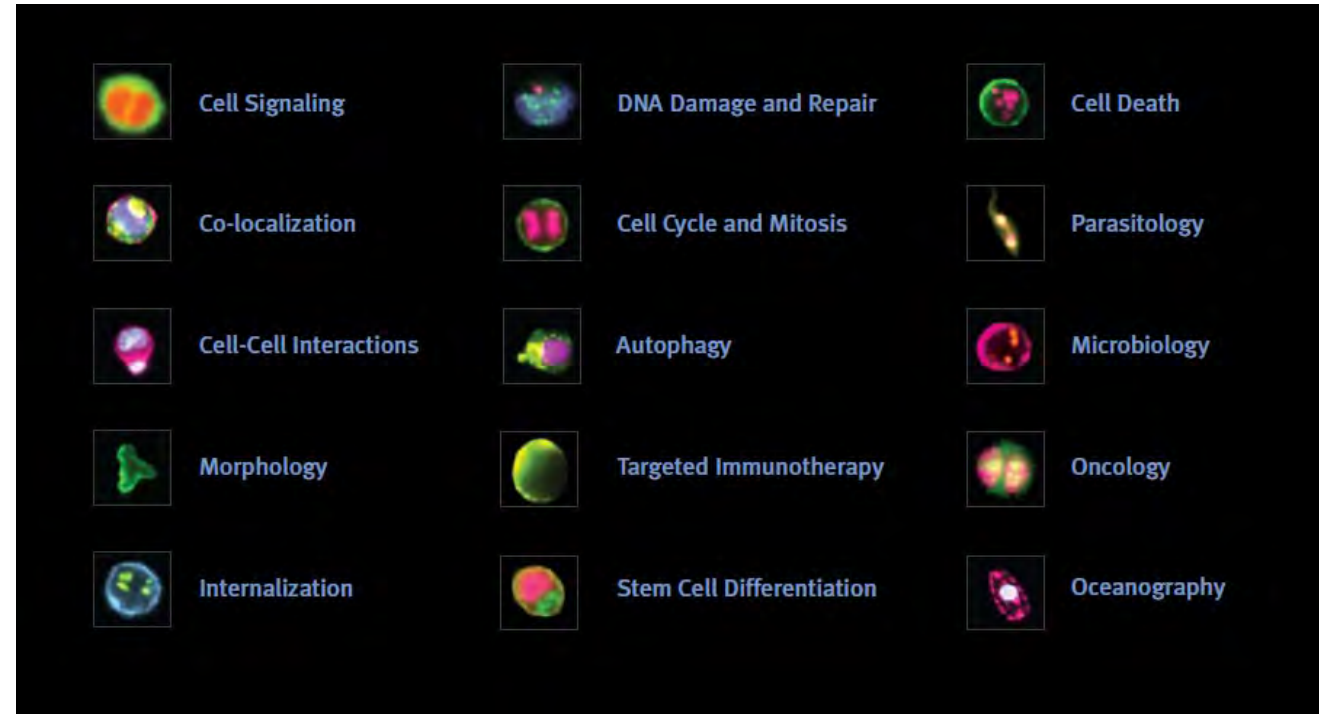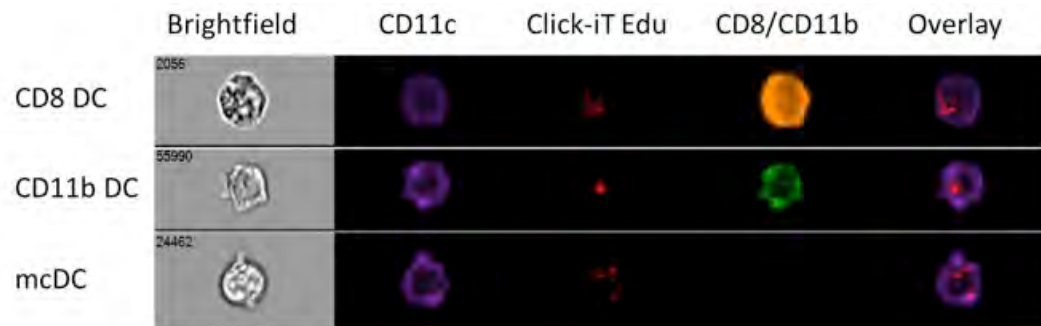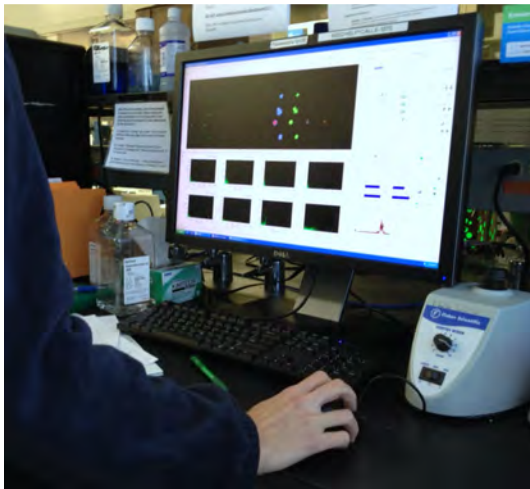
Imaging Cytometry

- New capabilities: High Gain Mode for small dim particles like EVs and viruses.

- Updated software: Machine Learning module.
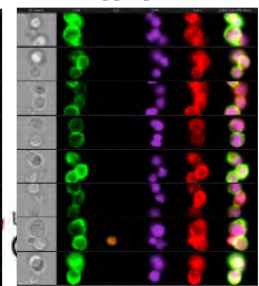
High Parameter Cytometry - Analysis and Sorting

- Analytical Cytometer
  - Spectral (Aurora)

- Sorters
  - Conventional (S6)
  - Conventional or Spectral (Bigfoot)

Aurora

Phenotypic Analysis of the Mouse Hematopoietic Hierarchy Using Spectral Cytometry: From Stem Cell Subsets to Early Progenitor Compartments
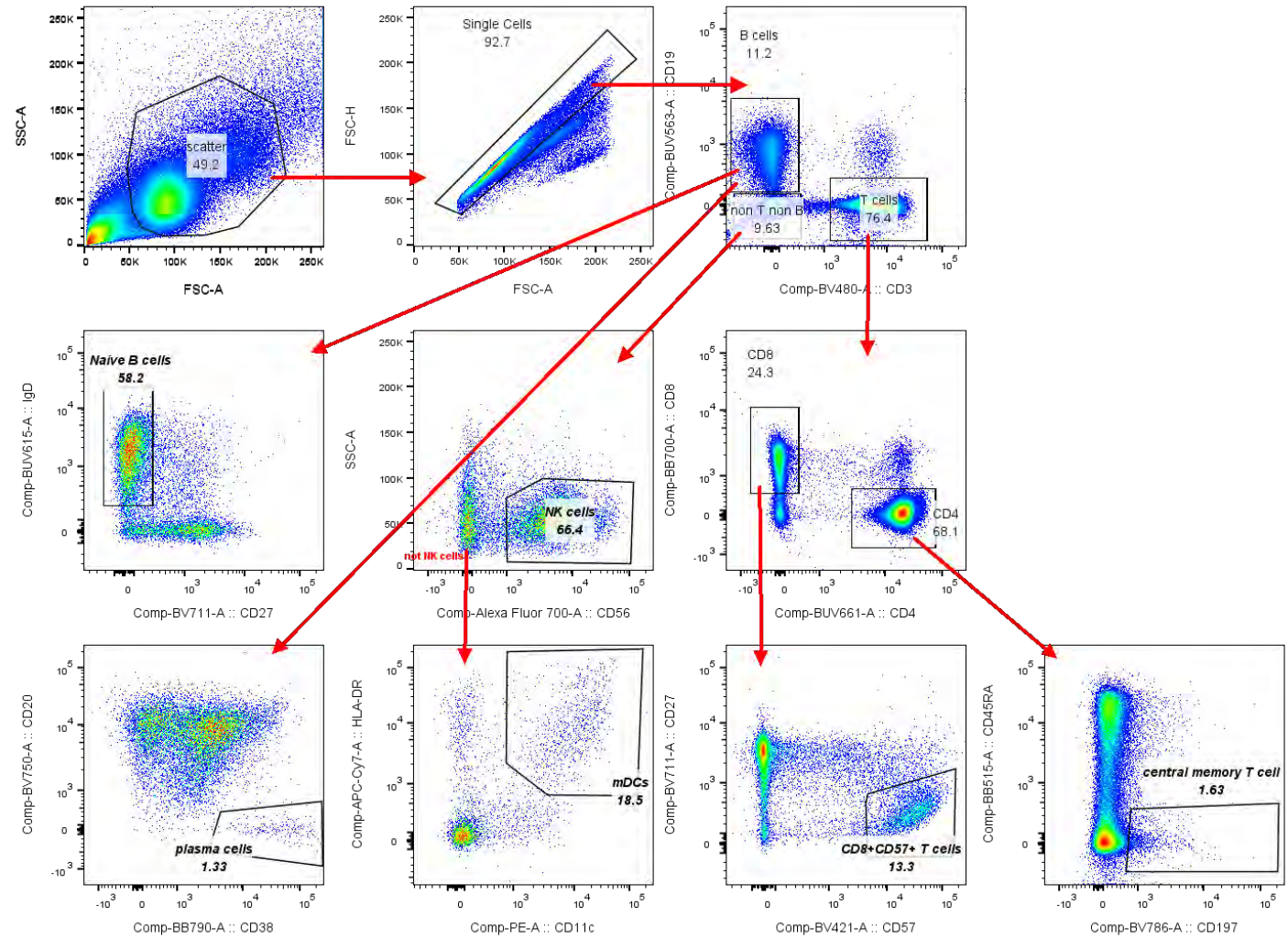
Michael Solomon, Monica DeLay, Damien Reynaud Cytometry Part A 97A: 1057–1065, 2020
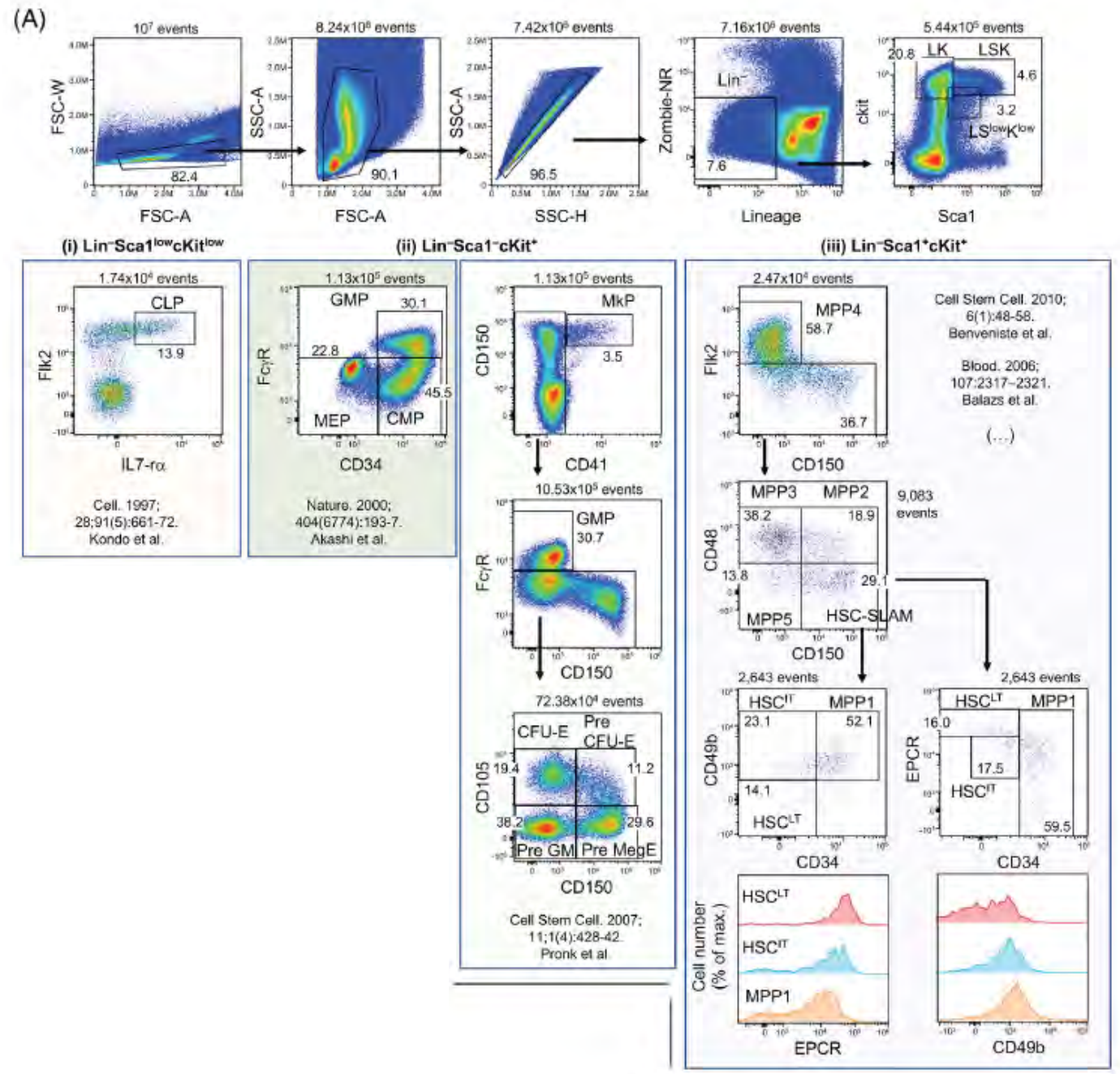
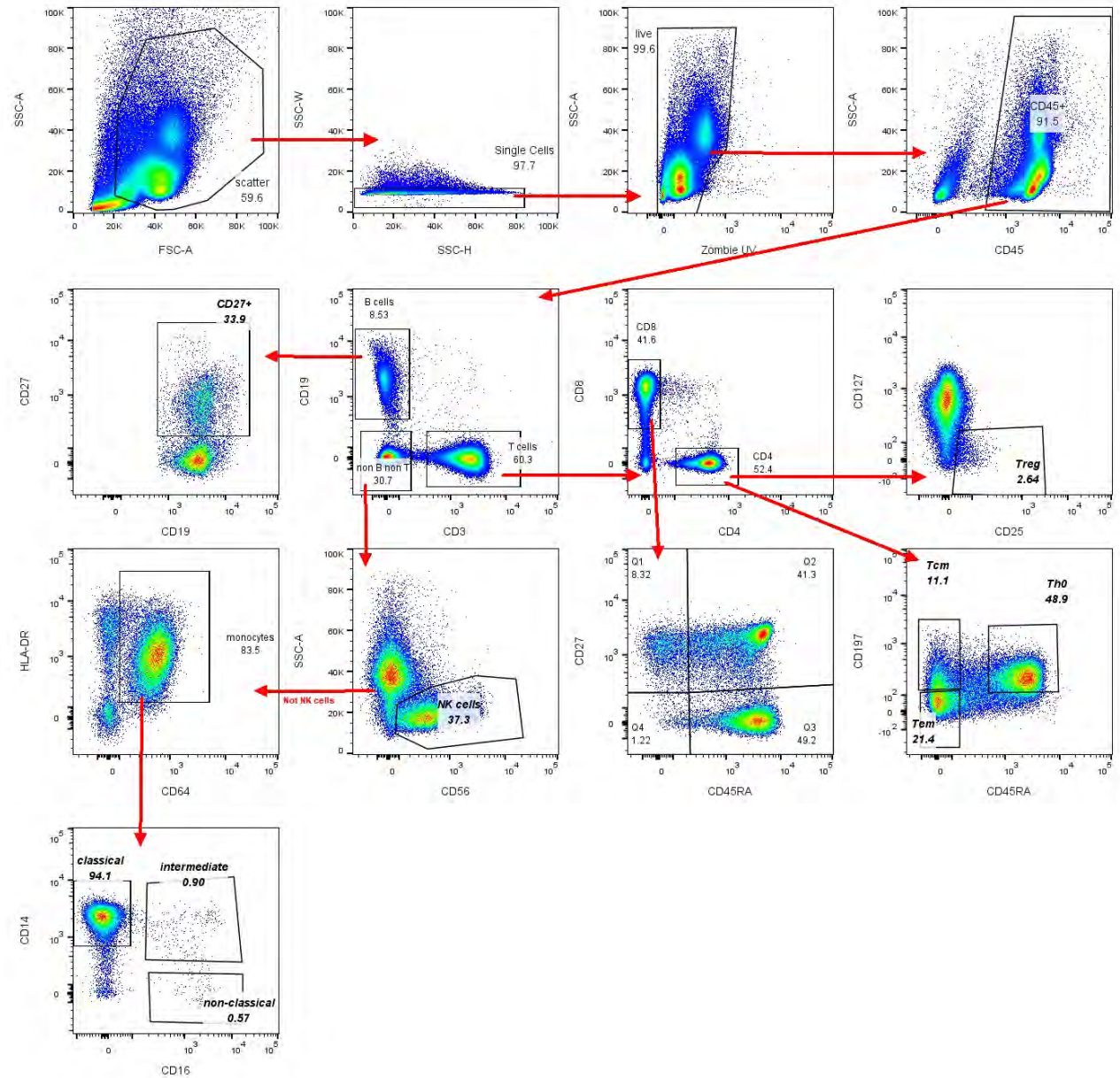# S6

25 color human PBMC immunophenotyping panel

Supplied by BD and acquired by RFCC in December 2022

Bigfoot

16 color human PMBC immunophenotyping panel

Acquired by RFCC in May 2022

High Dimensional Data Analysis

- When to use higher dimensional analysis?
- Software for analysis (FlowJo, FCSExpress, Cytobank, OMIQ, Astrolabe, R)
- Resources:
  - Weber et al., Cyto Part A, 2016
  - Nowicka et al., F1000Res, 2017
- New service for high dimensional data analysis.
- New user group for high dimensional data analysis and experimental design (3rd Wednesday, 9-10 AM, S6.125).

Reminders

- New basic FlowJo training
- Other Instruments:
  - Cell Dissociator
  - Cell Counter
  - Plate Reader
- Training/Consultation
- Assistance for Panel Design (Conventional/Spectral)
- Assistance on Instruments (Analyzers/Sorters)
- Billing (ImageStream)