Use of standardized lyophilized reagents to develop a functional T-cell signature

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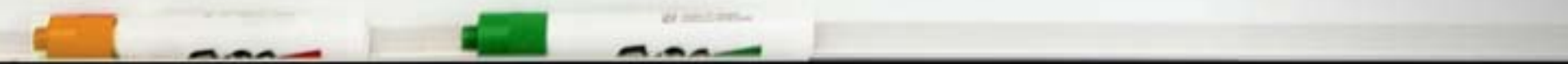

BD

Functional T cell responses to tumor antigens in breast cancer patients have a distinct phenotype and cytokine signature

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- J Immun, 2007, 179: 2627-2633

http://maeckerlab.typepad.com/

Cell Analysis in Drug Development Process - Step #1

1. NCE Selection

Preclinical activities aimed at screening, designing & optimizing the new chemical entity or biological agent that will interact with the drug target

Discovery & pre-clinical work to identify & narrow a field of potential therapeutic agents to a single new chemical entity (NCE) or biologic compound.

Usage commonly falls along the typical drug discovery workflow:

Target ID/ Basic MOA → High Thru-put Screening → Lead ID → Lead Optimization

Cell analysis techniques are most commonly used to test binding specificities of agents and illuminate basic disease mechanisms of action (MOA)

Cell Analysis in Drug Development Process - Step #2

2. Pharmacodynamics

Preclinical and/or clinical studies of the biochemical & physiological effects of a drug, its mechanisms of action, and the relationship between drug dosage & effect on the body

Evaluates the interactions between an optimized drug candidate and a biological system.

Includes drug development activity as early in the pipeline as target validation and as late as dose-ranging & early efficacy.

Typical cell analysis applications in this area include cell signaling (e.g. Phosflow™ and Lyoplates)

Cell Analysis in Drug Development Process - Step #3

If successful, these clinical trials would demonstrate the safety and/or efficacy benefit of a “companion diagnostic” that pre-screens potential patients and/or monitors their progress during treatment.

These trials could be conducted both pre- and post-approval of the underlying therapeutic.

Cell analysis applications here are largely customized and are developed in partnership with the therapeutic company’s development organization

3. Patient Profiling

Clinical trials designed to determine if and how the phenotypes of individual patients or groups influence response to a given therapy. If successful, these trials would establish the need for new pre-treatment screening and/or therapeutic monitoring assays for this indication.

Cellular Analysis in Clinical Trials

This presentation will focus on addressing the need for;

- Applying the use of biomarkers in drug development
- Standardizing cellular assays across multiple sites
- Assay and data analysis criterion
- Monitoring immune function
- Cellular Biomarkers of Disease progression (Cancer and HIV)

Why collect and analyze blood?

- You can get it
- It shows immunological memory
- It is a systemic organ
- We can measure it in detail

Response Profiles Can Be Very Rich

B-cells		Monocytes	Basophils		Platelets			T-cells				Dendritic Cells
CD40 + IL4	PWM	LPS	fMLP	FceR1 Cross-linking	TRAP	Epinephrine	ACD	PMA + Iono	SEB +CD28	CD3 +CD28	CMV + CD28 + CD49d	LPS
CD95	CD95							TNF a	TNF a	TNF a		
CD25	CD25							IFN g	IFN g	IFN g		
CD71	CD71	IL-6			PAC-1	PAC-1	PAC-1	CD25	CD25	CD25	IFN g	
CD69	CD69	IL-8			AnxV	AnxV	AnxV	IL-4	IL-4	IL-4	TNF a	CD80
CD80	CD80	IL-1 b	IL-4	IL-4	CD62	CD62	CD62	IL-2	IL-2	IL-2	IL-2	CD86
CD86	CD86	TNF a	CD63	CD63	CD63	CD63	CD63	BrdU	BrdU	BrdU	CD69	TNF a

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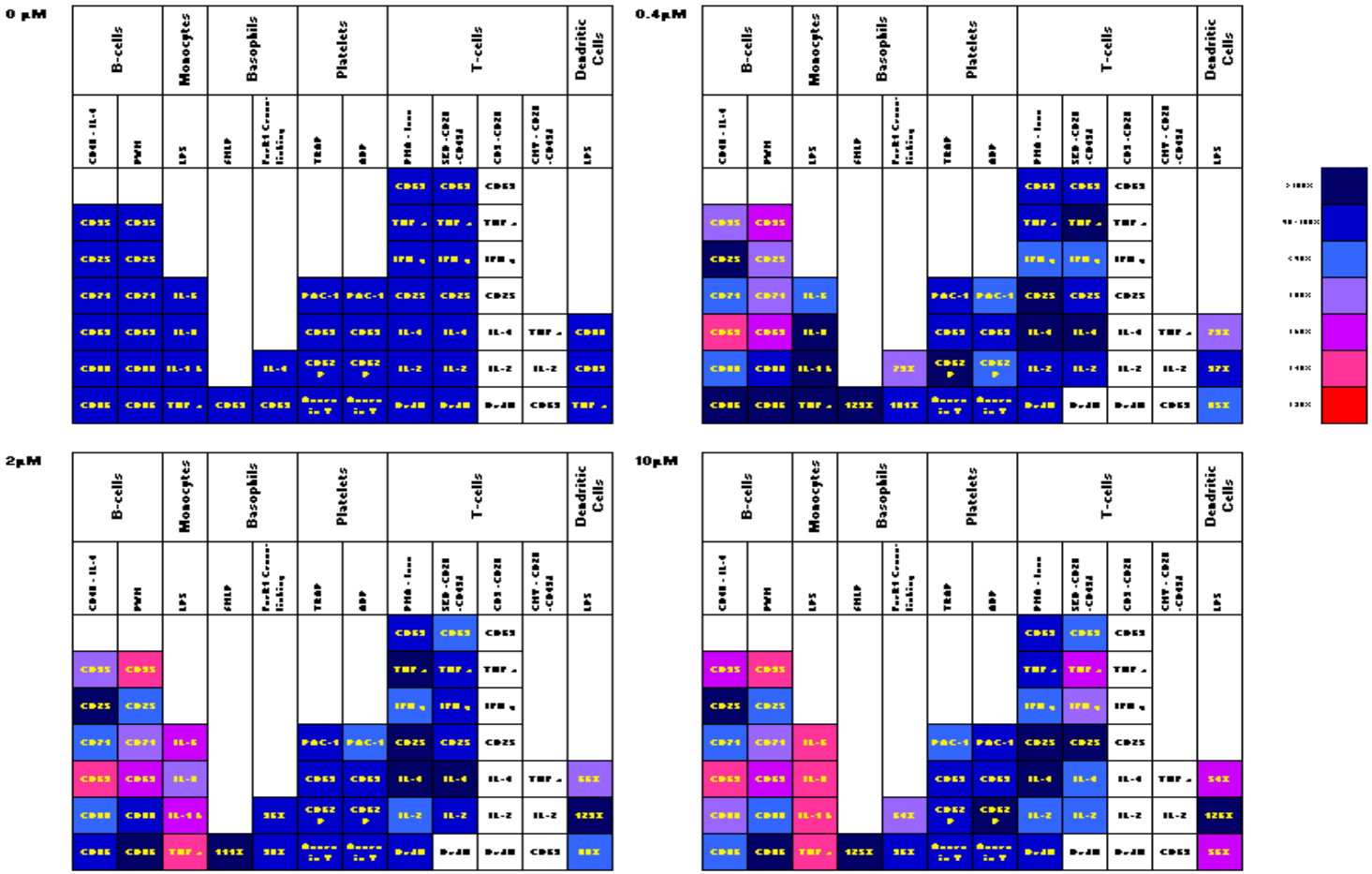
Leukocyte Response Profiles

- **Describe drugs**
  - to characterize the effect of an inhibitor on a molecular pathway
  - to recognize the pleiotropy of that pathway

- **Describe people**
  - to recognize genetic or environmental polymorphisms
  - to characterize the summed effect of a complicated therapeutic regimen

Typical Blood Profile

Herbimycin A



Effect of Coincubation of Two Immunosuppressive Drugs On T cell and B cell Markers

CD86 FITC
Total Median Fluorescence
% Difference from Zero Drug

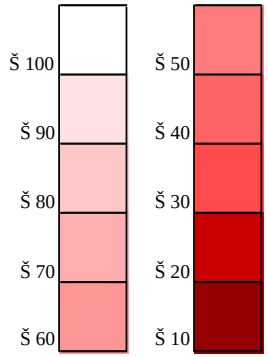
Drug 2 \ Drug 1	0 µM	0.1 µM	0.2 µM	0.4 µM	1 µM
0 ng	100%	75%	54%	40%	40%
2 ng	95%	71%	47%	35%	28%
6 ng	95%	85%	47%	34%	26%
10 ng	92%	53%	43%	35%	27%
30 ng	100%	68%	46%	34%	25%

CD95 APC
Total Median Fluorescence
% Difference from Zero Drug

Drug 2 \ Drug 1	0 µM	0.1 µM	0.2 µM	0.4 µM	1 µM
0 ng	100%	96%	86%	62%	59%
2 ng	81%	79%	67%	54%	45%
6 ng	81%	79%	69%	58%	41%
10 ng	80%	71%	64%	56%	44%
30 ng	86%	79%	70%	57%	40%

CD54 PE
Total Median Fluorescence
% Difference from Zero Drug

Drug 2 \ Drug 1	0 µM	0.1 µM	0.2 µM	0.4 µM	1 µM
0 ng	100%	85%	66%	48%	49%
2 ng	82%	72%	55%	41%	31%
6 ng	79%	82%	53%	40%	25%
10 ng	74%	55%	48%	37%	28%
30 ng	74%	66%	49%	37%	24%



IFNg
Median Fluorescence of Positives
% Difference from Zero Drug

Drug 2 \ Drug 1	0 µM	0.1 µM	0.2 µM	0.4 µM	1 µM
0 ng	100%	83%	64%	37%	15%
2 ng	80%	68%	51%	36%	17%
6 ng	89%	71%	59%	35%	17%
10 ng	82%	66%	50%	35%	14%
30 ng	95%	75%	58%	39%	18%

IL-2
Median Fluorescence of Positives
% Difference from Zero Drug

Drug 2 \ Drug 1	0 µM	0.1 µM	0.2 µM	0.4 µM	1 µM
0 ng	100%	89%	77%	59%	43%
2 ng	94%	88%	75%	57%	49%
6 ng	96%	91%	77%	60%	46%
10 ng	94%	90%	77%	63%	50%
30 ng	104%	94%	78%	62%	41%

TNFa
Median Fluorescence of Positives
% Difference from Zero Drug

Drug 2 \ Drug 1	0 µM	0.1 µM	0.2 µM	0.4 µM	1 µM
0 ng	100%	67%	50%	27%	23%
2 ng	81%	54%	43%	25%	13%
6 ng	93%	61%	43%	26%	14%
10 ng	75%	60%	38%	28%	16%
30 ng	83%	63%	43%	28%	14%

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Cytokine Flow Cytometry
Baseline Study

Lyophilized stimulation plate

blank	CD28+49d 1 ug/ml ea	pp65 mix 3.4 ug/ml	IE mix 3.4 ug/ml	A2 CMV pep 1 ug/ml	B7 CMV pep 1 ug/ml	flu HA+M1 3.4 ug/ml	HIV gag mix 3.4 ug/ml	CEA mix 3.4 ug/ml	MAGE-3 mix 3.4 ug/ml	her2/neu ICD mix 3.4 ug/ml	CEF
BFA	CMV lysate 1 ug/ml +CD28/49d	pp65 mix 1.7 ug/ml	IE mix 1.7 ug/ml	A2 CMV pep 0.2 ug/ml	B7 CMV pep 0.2 ug/ml	flu HA+M1 1.7 ug/ml	HIV gag mix 1.7 ug/ml	CEA mix 1.7 ug/ml	MAGE-3 mix 1.7 ug/ml	her2/neu ICD mix 1.7 ug/ml	CEF
DMSO cntl	BFA	pp65 mix 0.8 ug/ml	IE mix 0.8 ug/ml	A2 CMV pep 0.04 ug/ml	B7 CMV pep 0.04 ug/ml	flu HA+M1 0.8 ug/ml	HIV gag mix 0.8 ug/ml	CEA mix 0.8 ug/ml	MAGE-3 mix 0.8 ug/ml	her2/neu ICD mix 0.8 ug/ml	PMA 10 ng+ ion 1 ug/ml
DMSO cntl	CD28+49d 1 ug/ml ea	pp65 mix 0.4 ug/ml	IE mix 0.4 ug/ml	A2 CMV pep 0.008 ug/ml	B7 CMV pep 0.008 ug/ml	flu HA+M1 0.4 ug/ml	HIV gag mix 0.4 ug/ml	CEA mix 0.4 ug/ml	MAGE-3 mix 0.4 ug/ml	her2/neu ICD mix 0.4 ug/ml	SEB 1 ug/ml
blank	CD28+49d 1 ug/ml ea	pp65 mix 3.4 ug/ml	IE mix 3.4 ug/ml	A2 CMV pep 1 ug/ml	B7 CMV pep 1 ug/ml	flu HA+M1 3.4 ug/ml	HIV gag mix 3.4 ug/ml	CEA mix 3.4 ug/ml	MAGE-3 mix 3.4 ug/ml	her2/neu ICD mix 3.4 ug/ml	CEF
BFA	CMV lysate 1 ug/ml +CD28/49d	pp65 mix 1.7 ug/ml	IE mix 1.7 ug/ml	A2 CMV pep 0.2 ug/ml	B7 CMV pep 0.2 ug/ml	flu HA+M1 1.7 ug/ml	HIV gag mix 1.7 ug/ml	CEA mix 1.7 ug/ml	MAGE-3 mix 1.7 ug/ml	her2/neu ICD mix 1.7 ug/ml	CEF
DMSO cntl	BFA	pp65 mix 0.8 ug/ml	IE mix 0.8 ug/ml	A2 CMV pep 0.04 ug/ml	B7 CMV pep 0.04 ug/ml	flu HA+M1 0.8 ug/ml	HIV gag mix 0.8 ug/ml	CEA mix 0.8 ug/ml	MAGE-3 mix 0.8 ug/ml	her2/neu ICD mix 0.8 ug/ml	PMA 10 ng+ ion 1 ug/ml
DMSO cntl	CD28+49d 1 ug/ml ea	pp65 mix 0.4 ug/ml	IE mix 0.4 ug/ml	A2 CMV pep 0.008 ug/ml	B7 CMV pep 0.008 ug/ml	flu HA+M1 0.4 ug/ml	HIV gag mix 0.4 ug/ml	CEA mix 0.4 ug/ml	MAGE-3 mix 0.4 ug/ml	her2/neu ICD mix 0.4 ug/ml	SEB 1 ug/ml

Lyophilized staining plate

IFNγ FITC /CD69 PE /CD4 PerCP-Cy5.5 /CD3 APC
TNFα FITC / IL-2 PE /CD4 PerCP-Cy5.5 /CD3 APC

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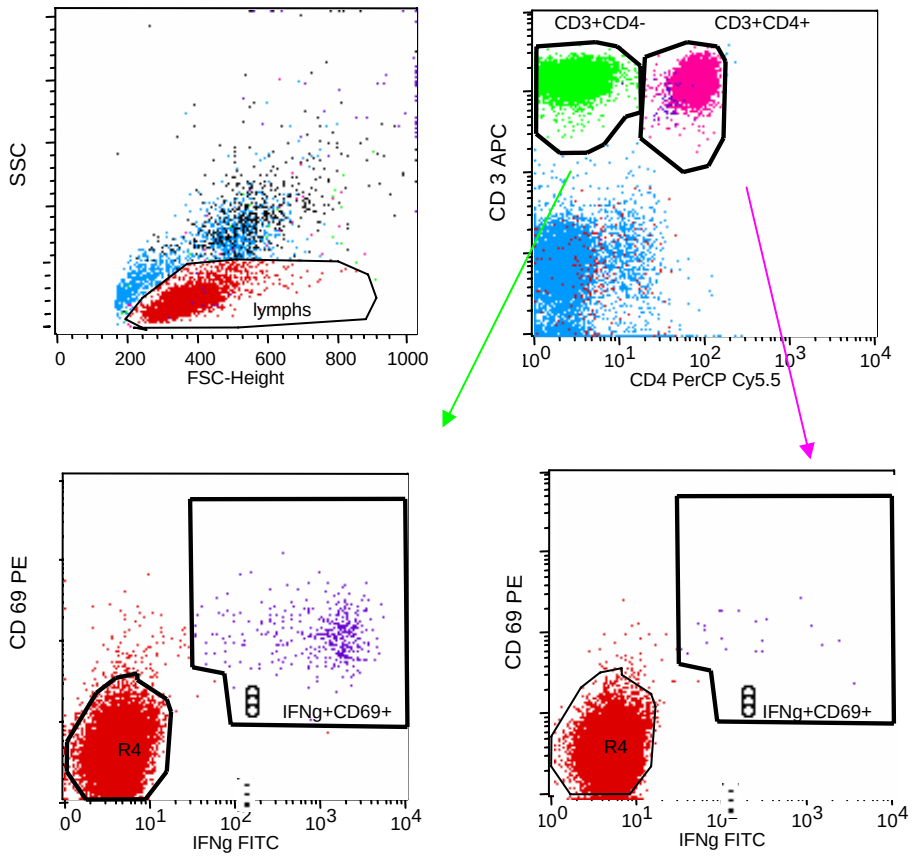
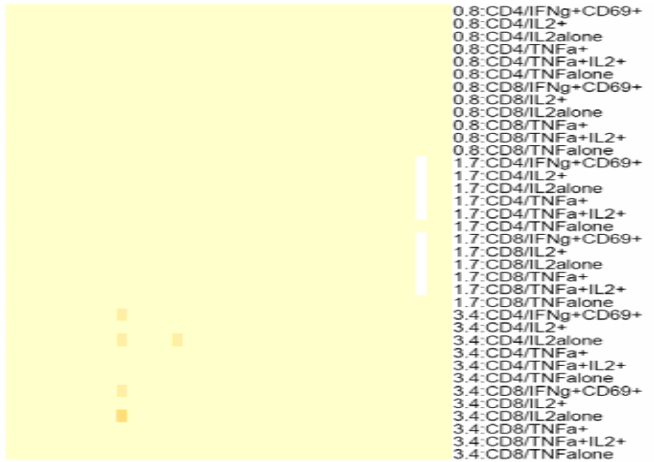
Cytokine Gating

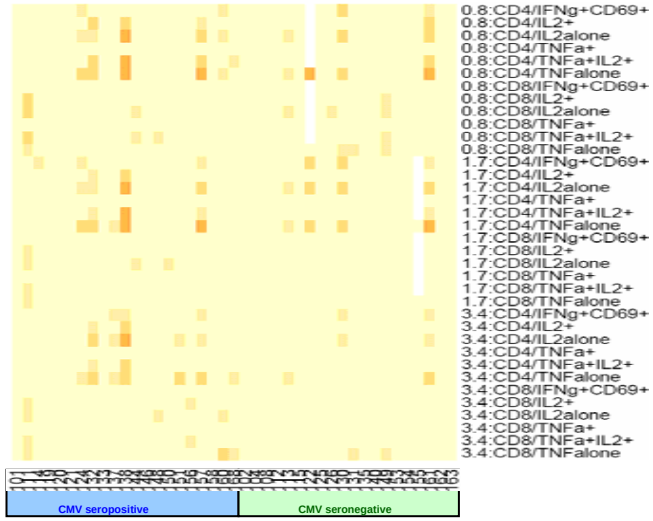


Normal Donor Cytokine Responses - Controls

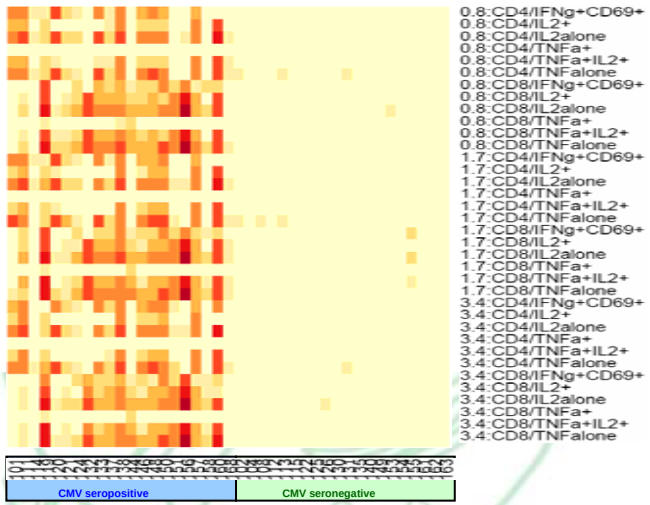
HIV mix (neg control)



Flu HA+M1 mix



CMV pp65 mix



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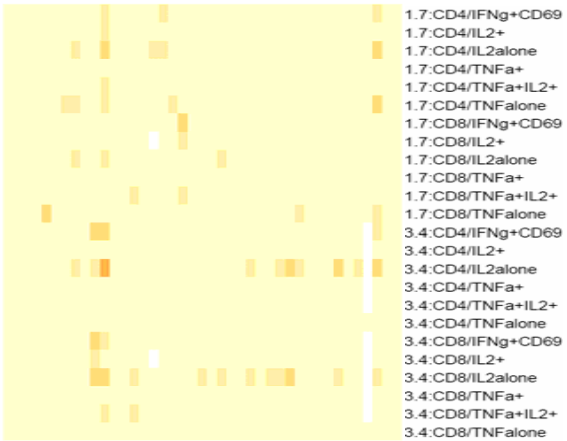
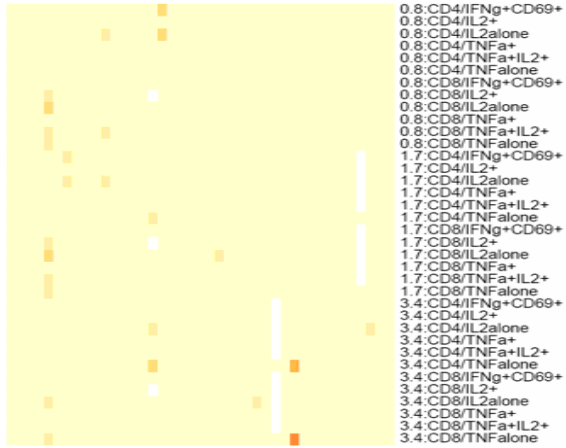
Normal Donor Cytokine Responses to Cancer Antigen

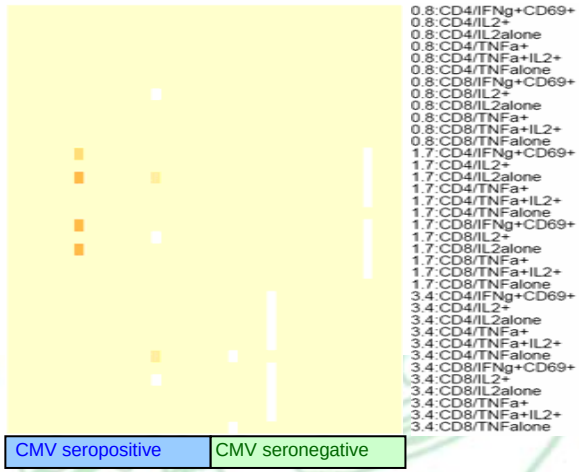
Her2/neu mix



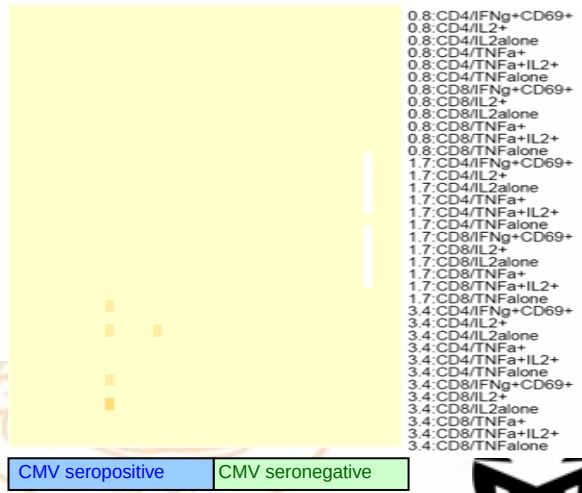
MAGE-3 mix



CEA mix



HIV mix (neg control)



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Summary CFC Baseline Study

Cytokine flow cytometry offers quantitative assessments of T-cell function

Healthy donors demonstrate correlation between
CMV serology and CFC response to CMV peptides.

TNFα responses in CD4 and CD4- cells to Her2/neu and
MAGE-3 were low but significantly different from
neg control antigen.

IFNγ, TNFα and IL-2 responses to CEA was not significantly
different from negative controls in healthy donors.

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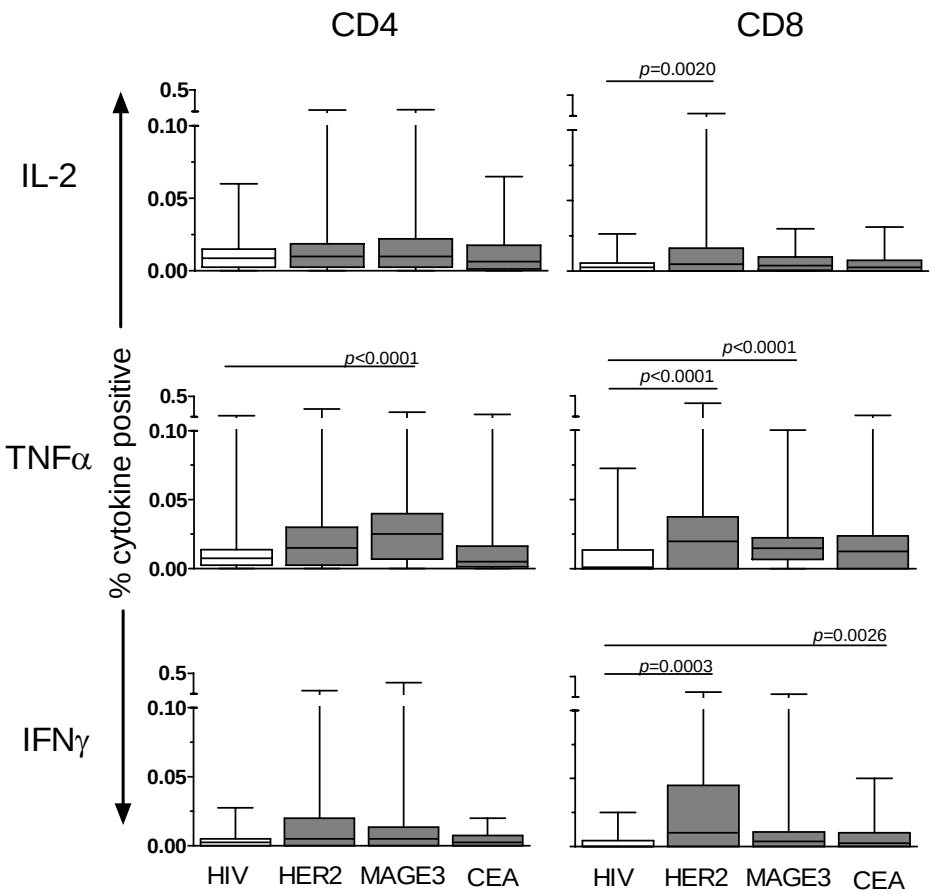
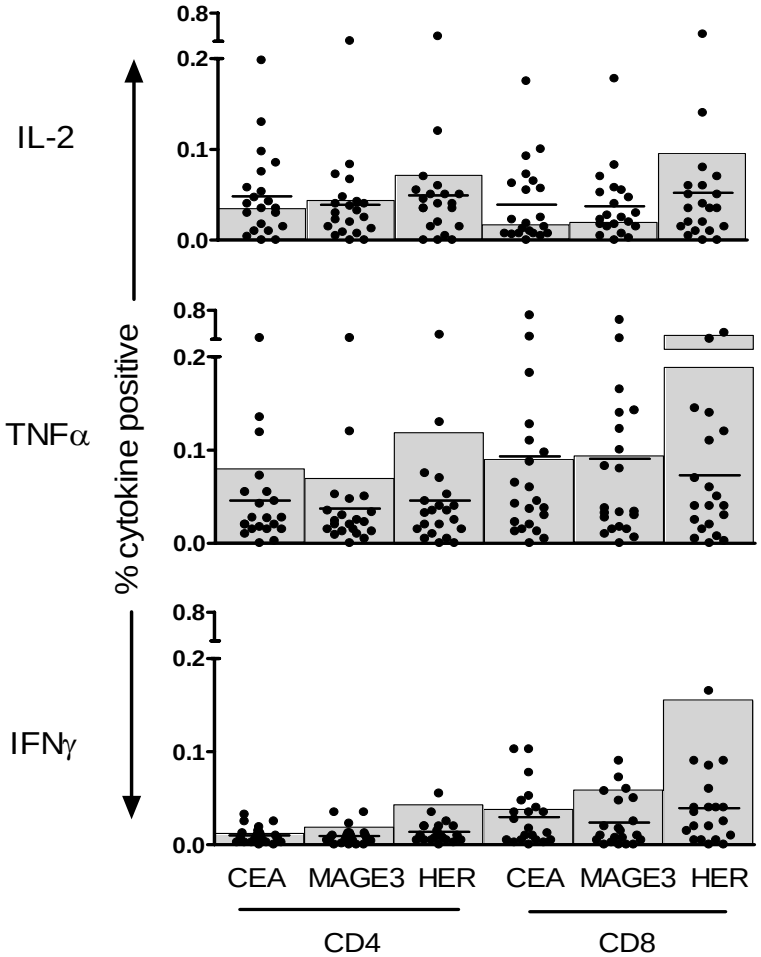
Healthy Donor Analysis of TAA by Flow



Percentage of cytokine producing cells in T-cell subsets

Cytokine Responses in Breast Cancer Patients



Percentage of cytokine producing cells in T-cell subsets

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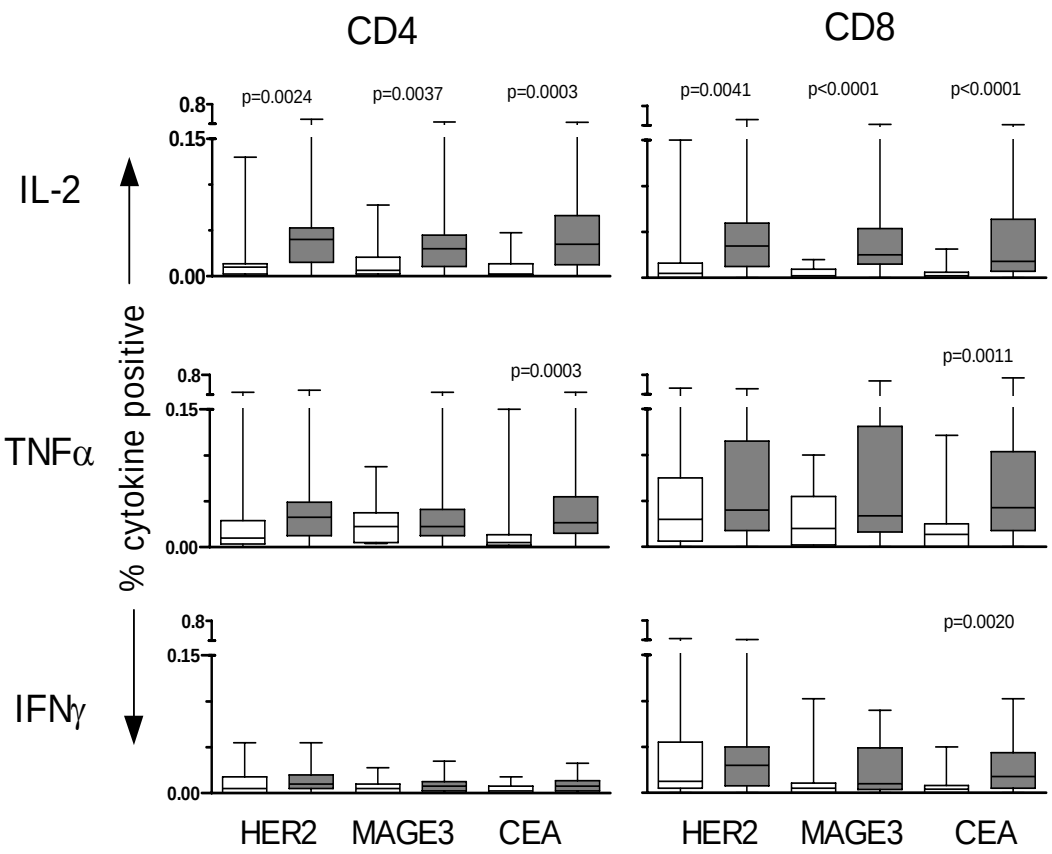
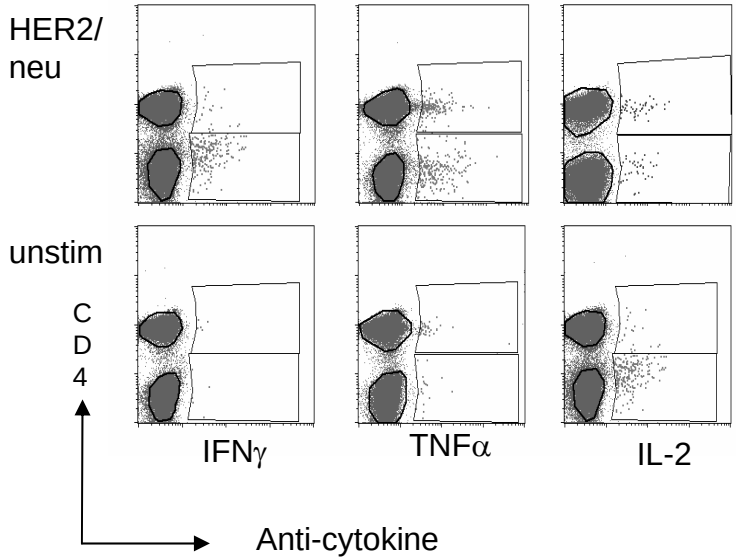
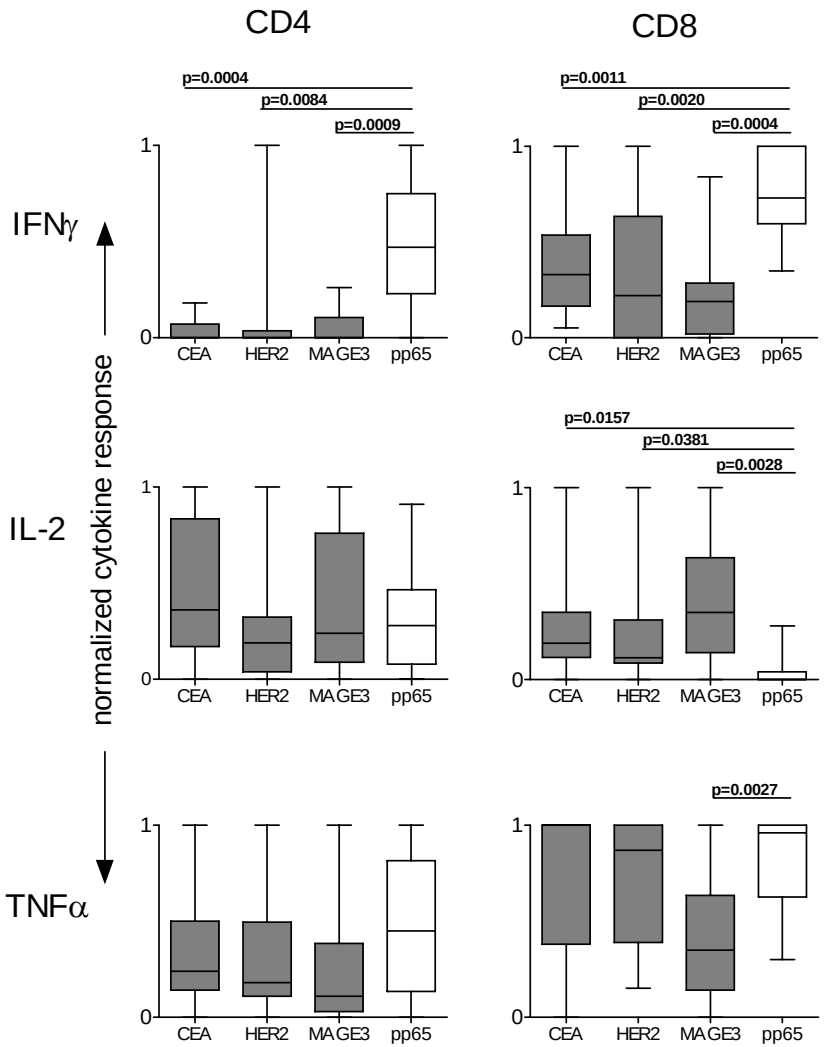
Cytokine Responses in Breast Cancer Patients





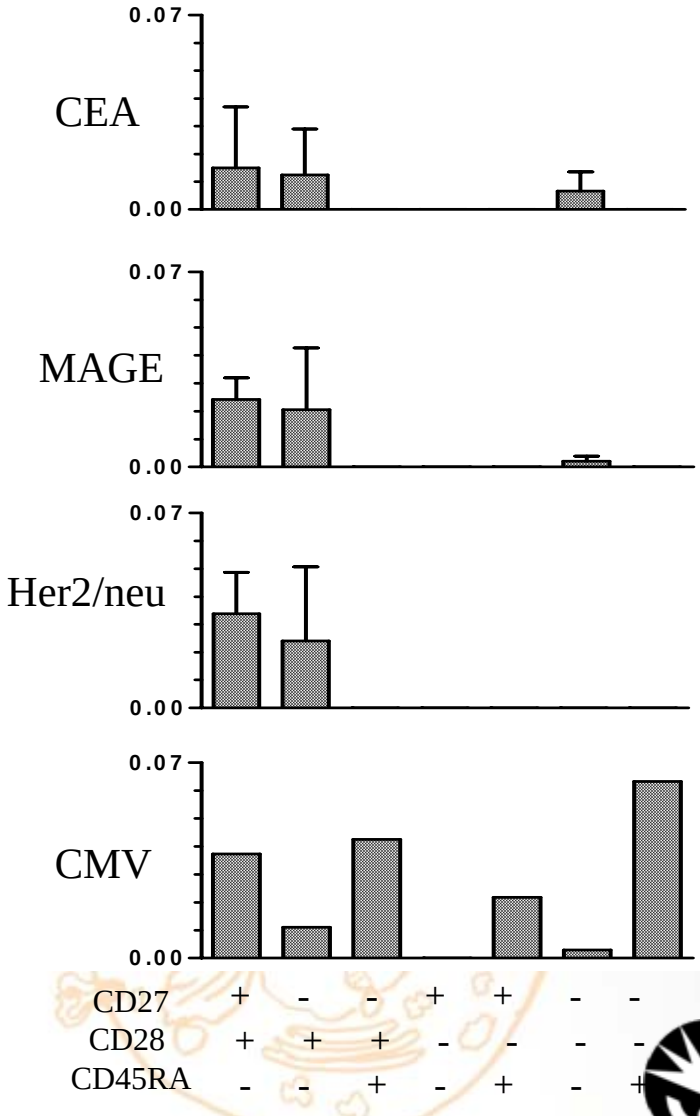
Cytokine T-cell Responses to Antigenic Challenge

- Responses to CMV antigen in CMV seropositive without clinical disease
- Unsuccessful response to self antigen (TAA in breast cancer patients)

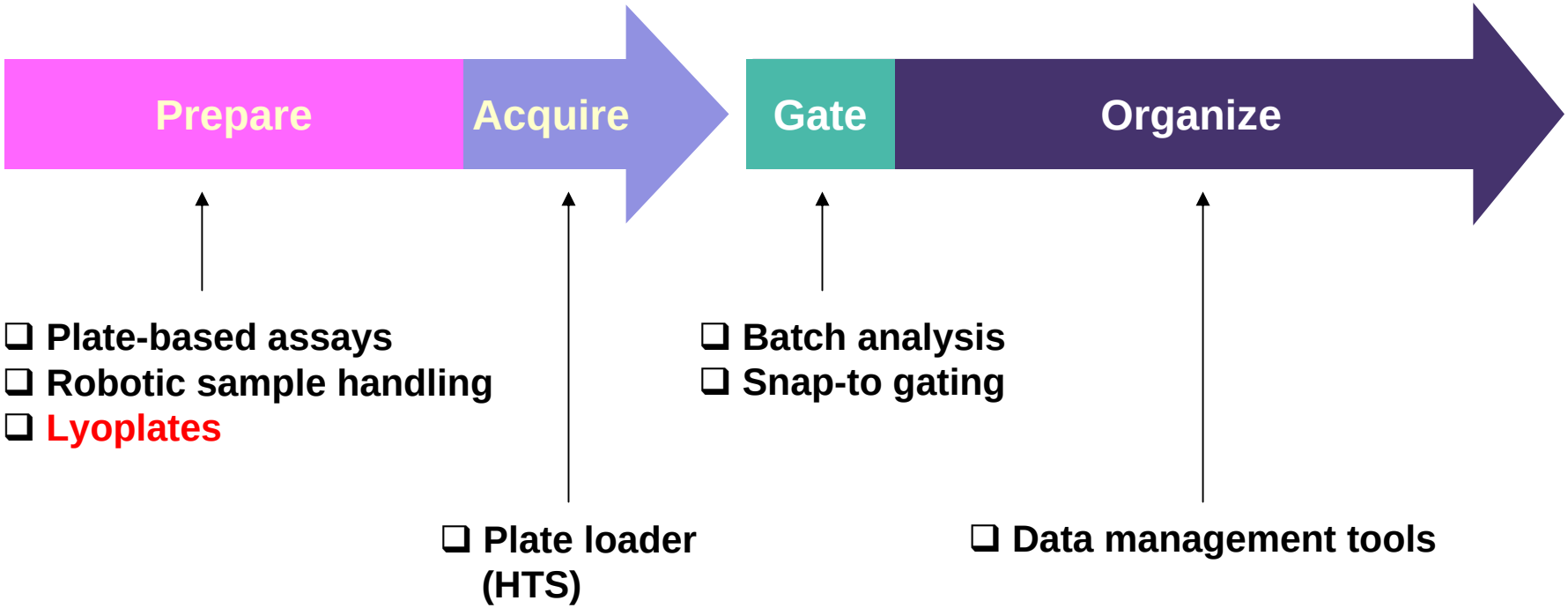


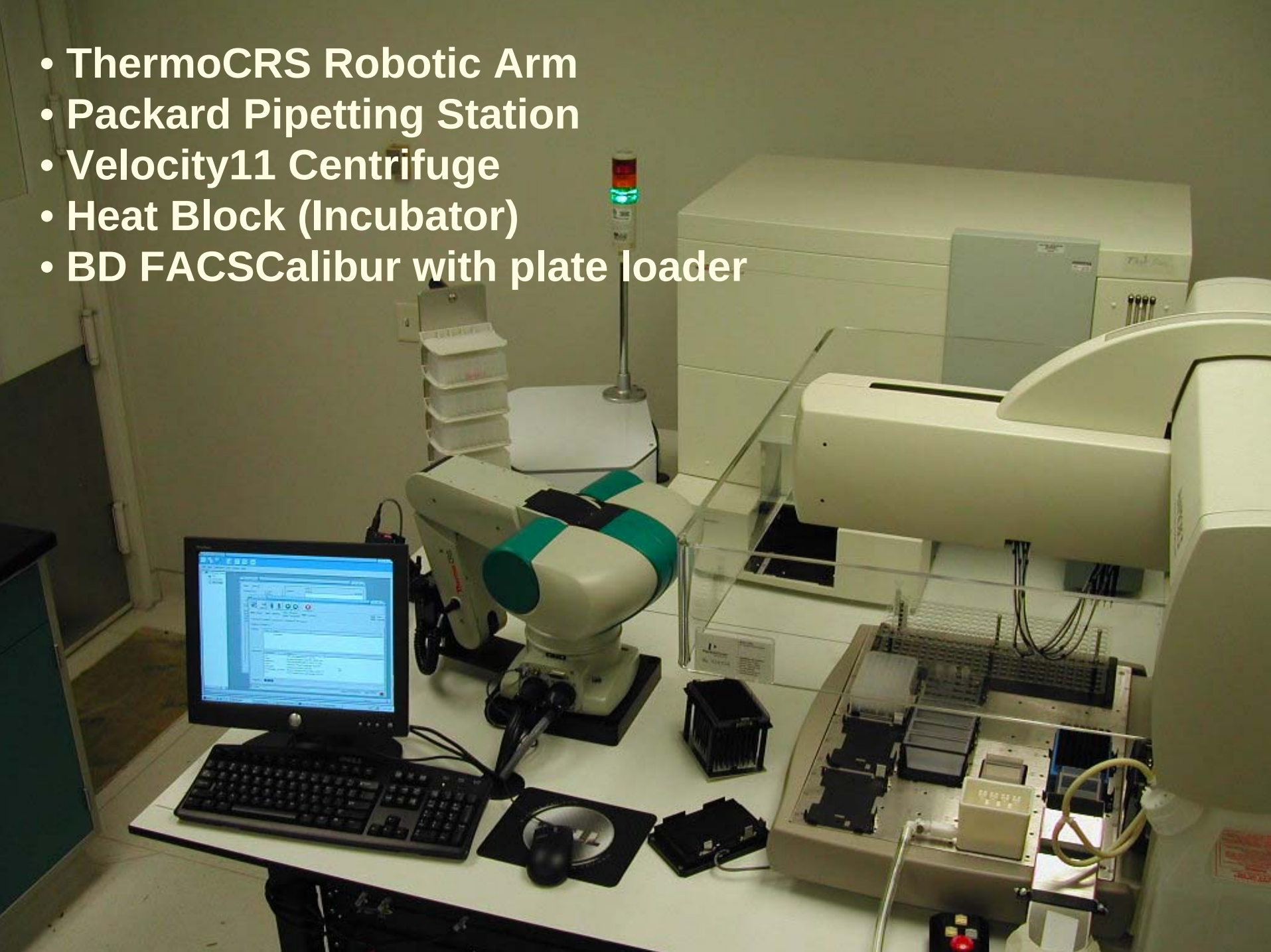
Effector and Memory T-cell Distribution in Cancer Patients

- Immunophenotyping of T-cells responding to tumor associated antigens compared to CMV
- Expression of IFNg and/or TNFa
- Data arranged from central memory to terminal effector



Consider the entire workflow




• ThermoCRS Robotic Arm
• Packard Pipetting Station
• Velocity11 Centrifuge
• Heat Block (Incubator)
• BD FACSCalibur with plate loader

Lyophilized Reagent CFC Protocol

PBMC or whole blood

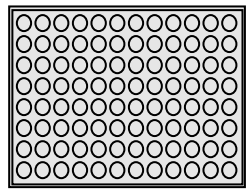


Plate with lyophilized Ag+BFA

6 h @ 37°C, EDTA, BD FACS Lyse, BD FACS Perm

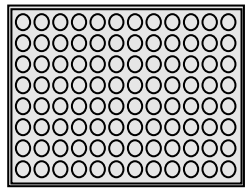
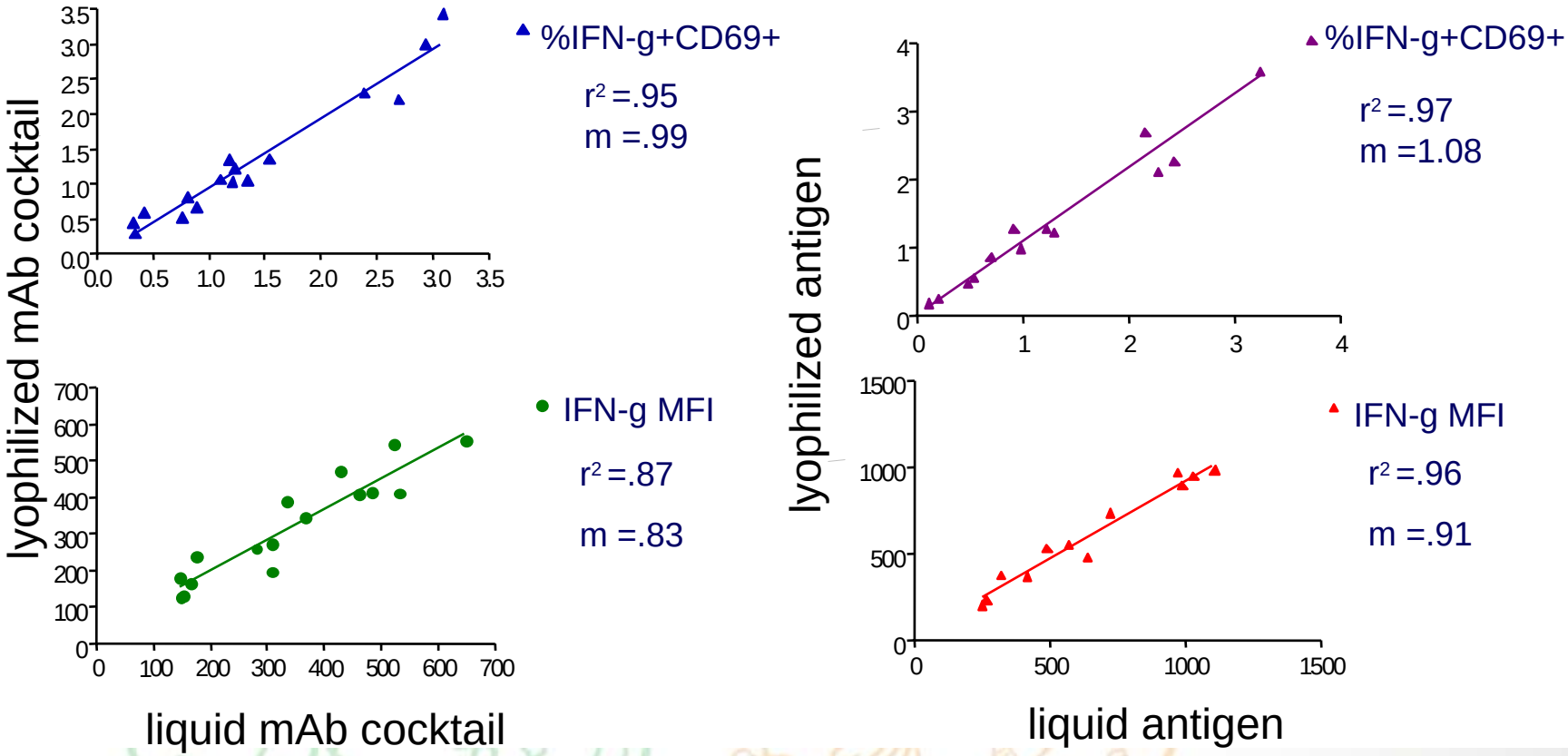


Plate with lyophilized Ab

to Flow cytometer

Liquid vs Lyophilized Reagents

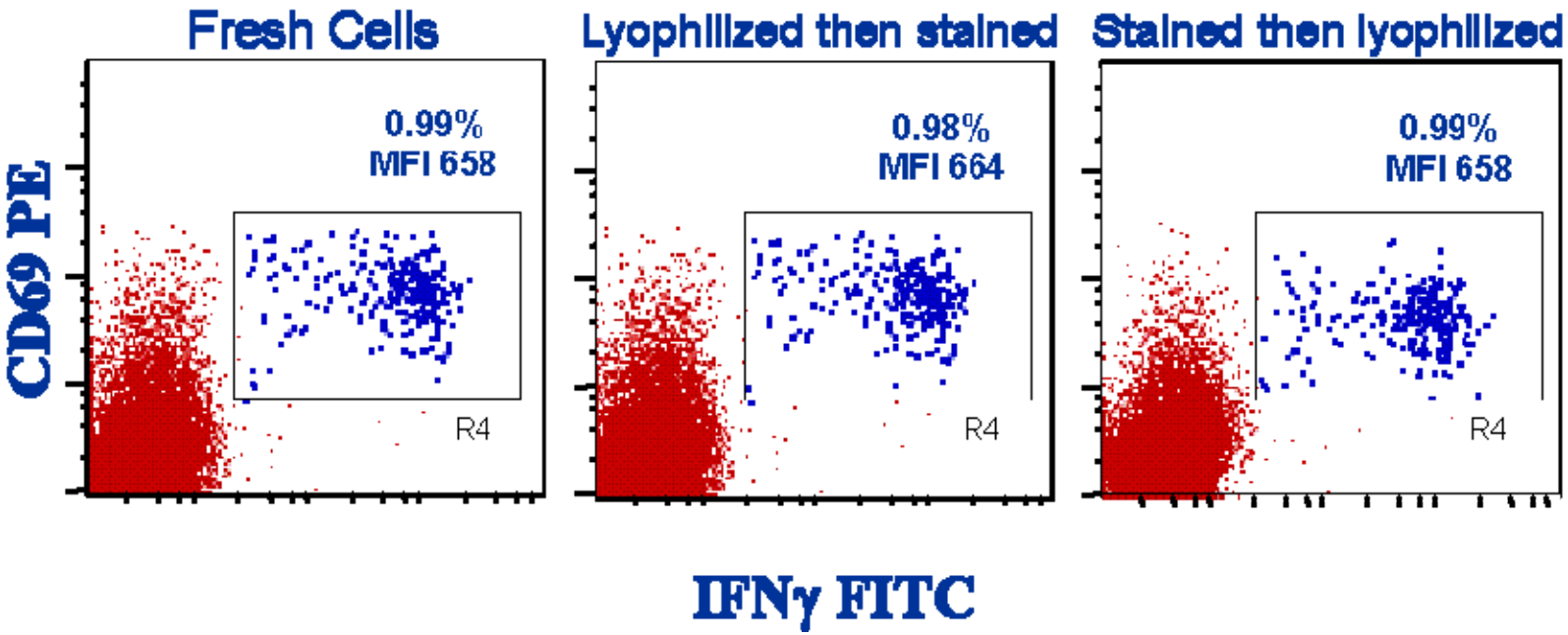
CMV pp65-mix activated blood



Multi-Site Data with BD Lyoplates

Assay Type	Fixed Activated Blood	Shipped Whole Blood	Cryopreserved PBMC	Cryopreserved PBMC with Lyoplates
Number of samples	12	12	24	9
Mean % CV	24%	31%	23%	18%

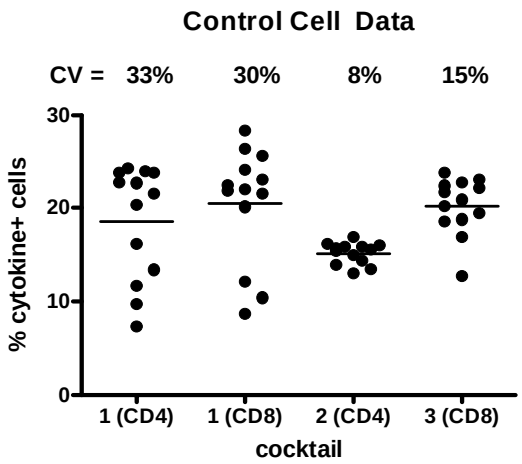
Cell Lyophilization for use as Controls

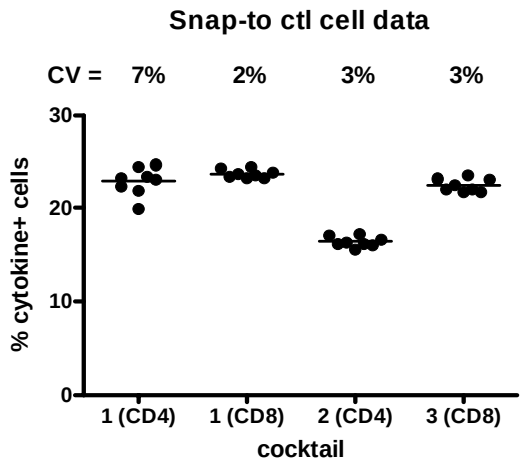


Data Management:
Lyophilized Cells in a Multi Site Study

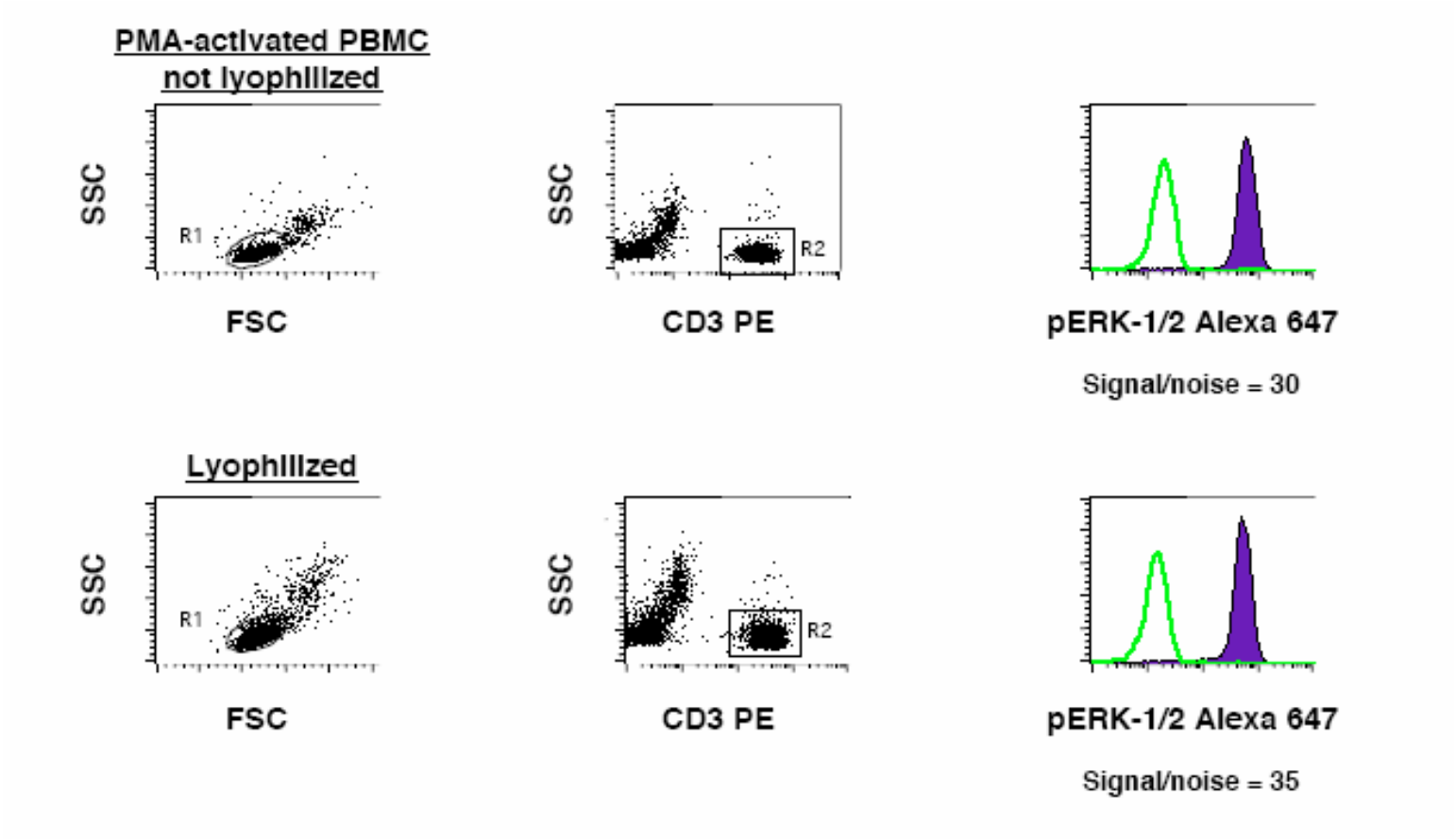
This is to cover

The title below

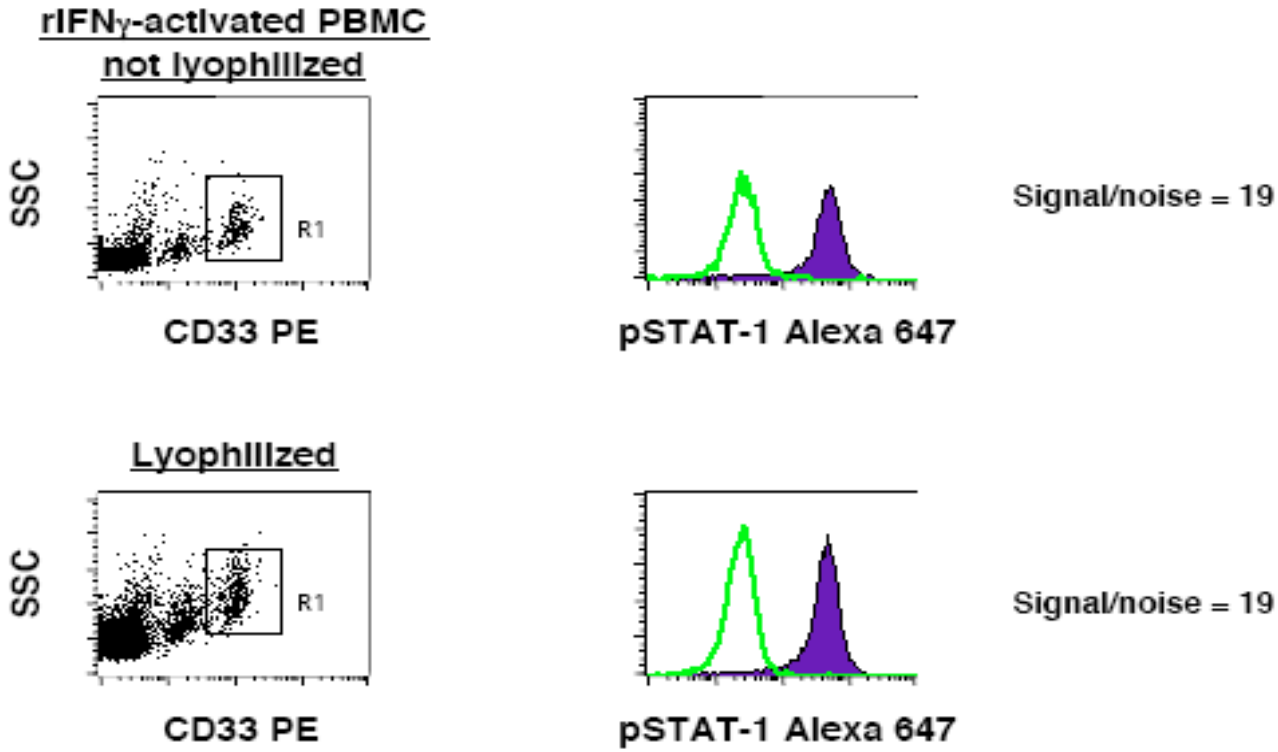




Lyophilized BD Phosflow Control Cells



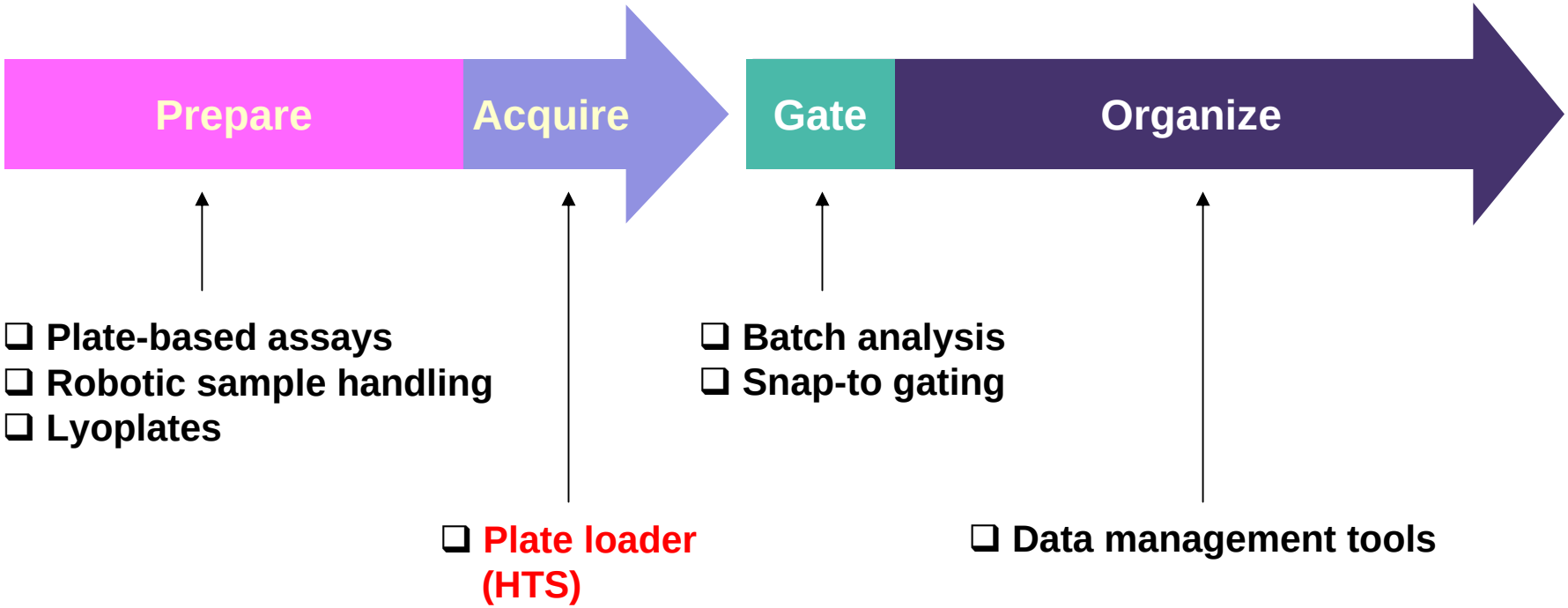
Lyophilized BD Phosflow Control Cells



Custom BD Lyoplate Products

- Vessel configurations
  - Standard 96 well, polypropylene or polystyrene
  - 8-well strip plates, polystyrene
  - Deep well 96 polypropylene plates
  - Deep well 96 polypropylene individual 1ml vials
- Contents
  - Fluorochrome antibody conjugate cocktails
  - BD CBA beads
  - Peptide antigen cocktails
  - Polyclonal protein mitogens
  - Pharmacophores
  - Fixed/permed/stained instrument/data control cells
  - Fixed/permed unstained process control cells

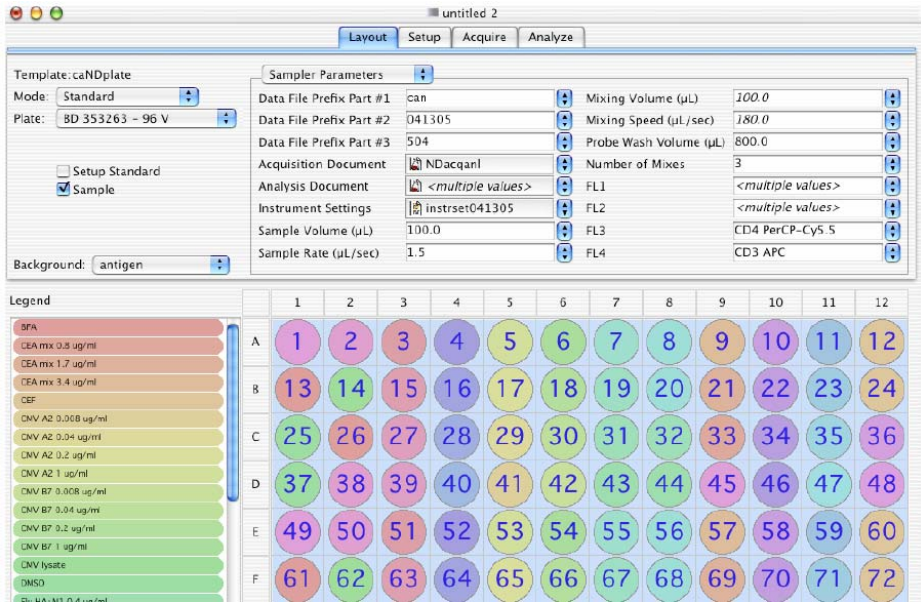
Consider the entire workflow



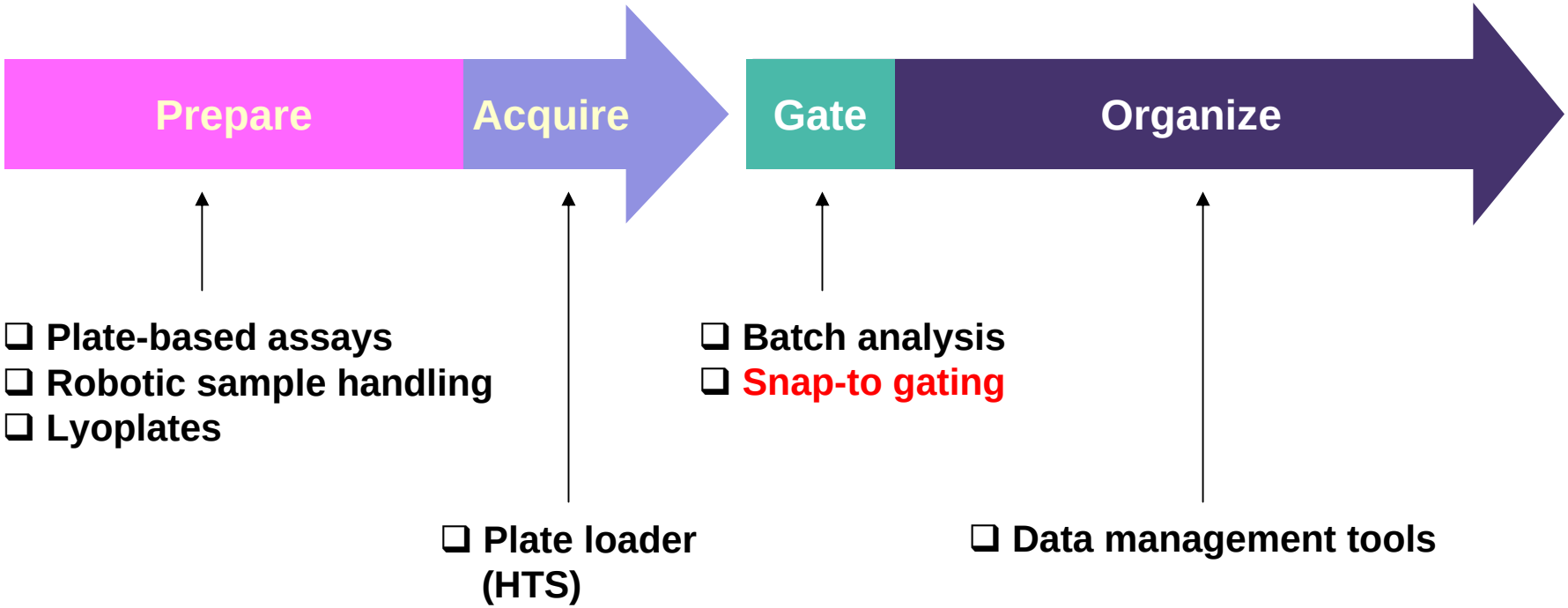
High Throughput Sampler

High Throughput Sampler on BD FACSCanto II

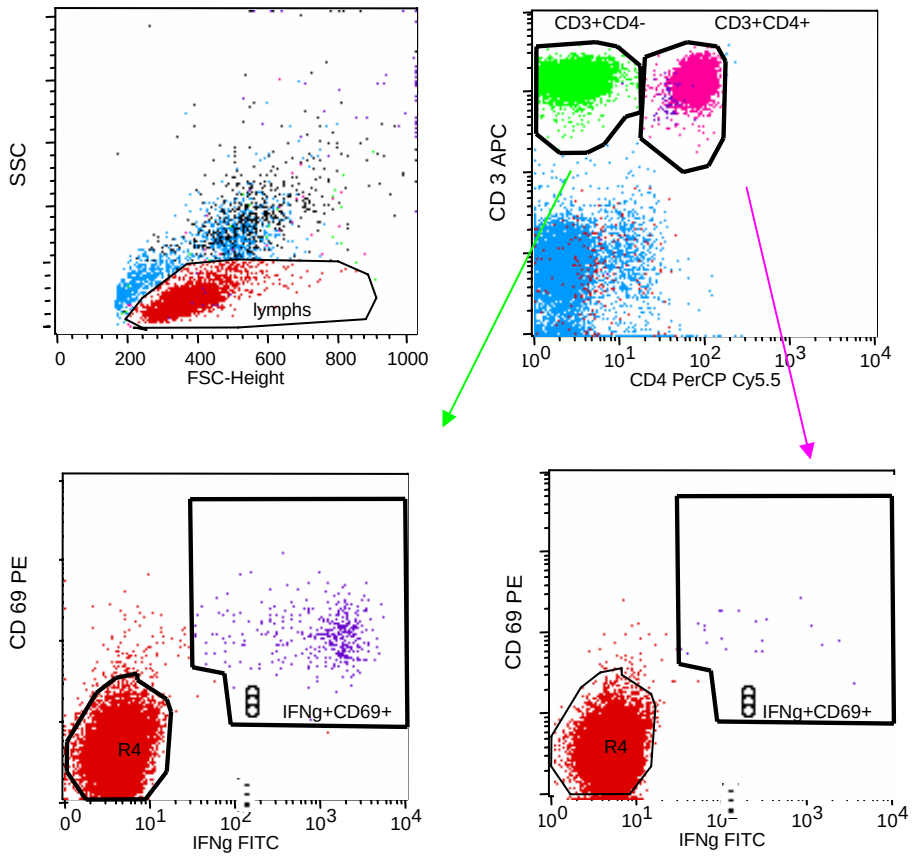
Plate manager software



Consider the entire workflow

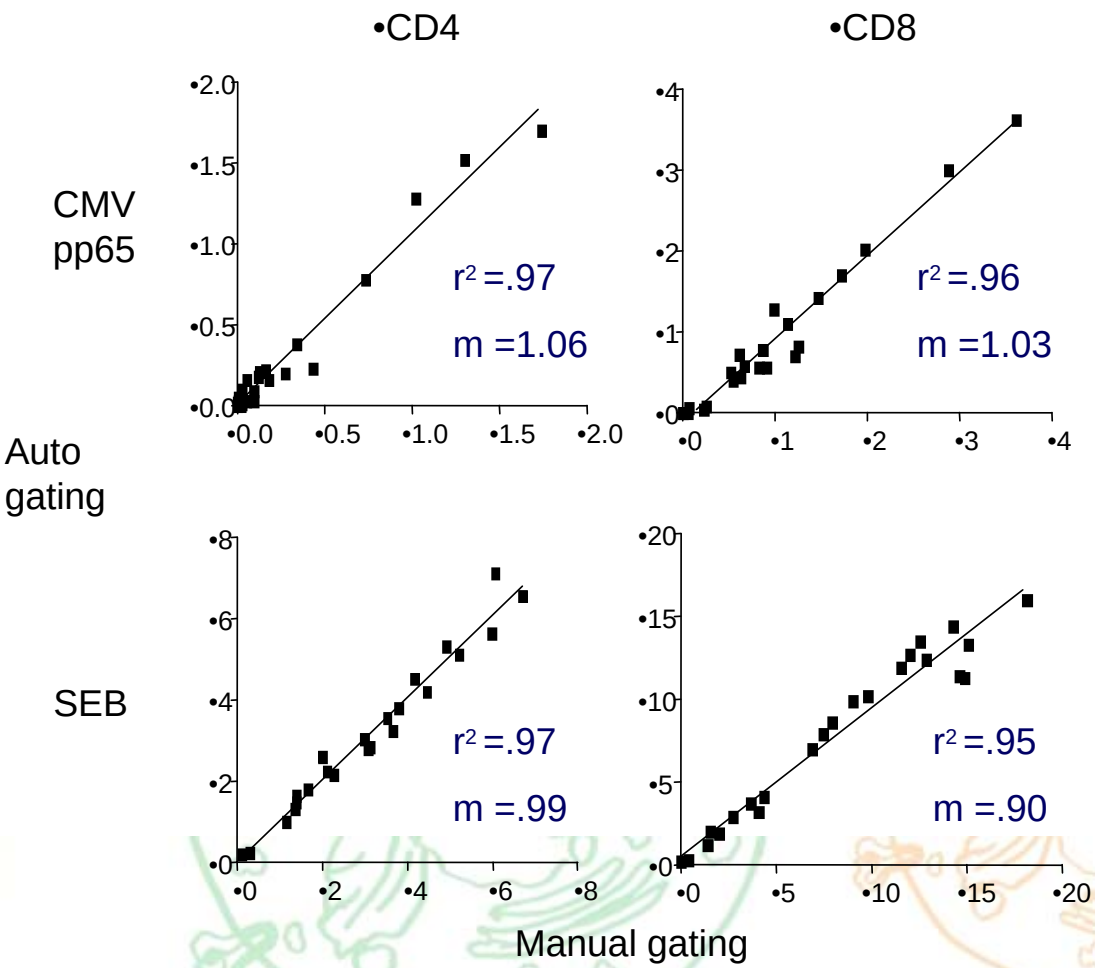


Automated Snap-to Gating



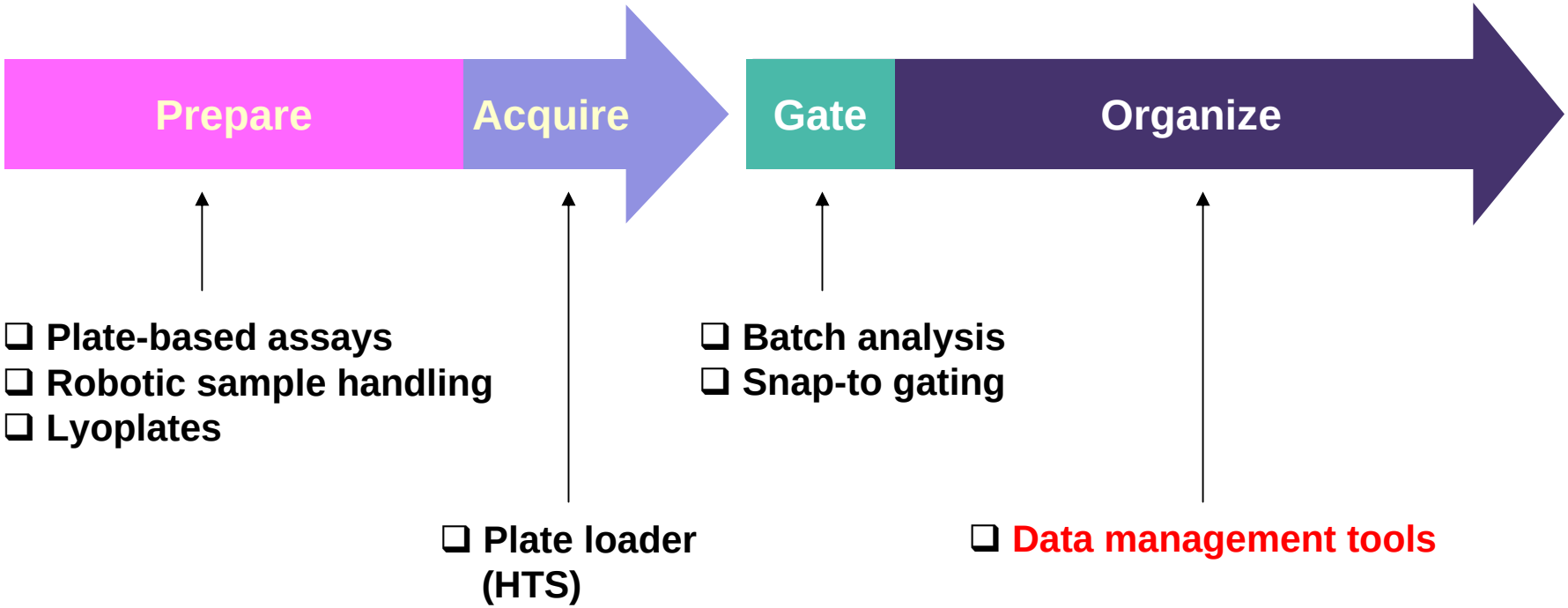
Automated vs Manual Gating

•%IFN-g+ CD69+



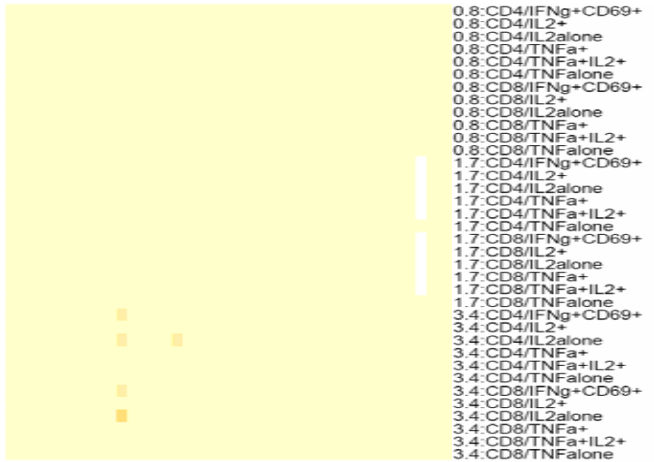
- Expert algorithm development
- Increased reproducibility
- Speed
- Ease-of-use

Consider the entire workflow

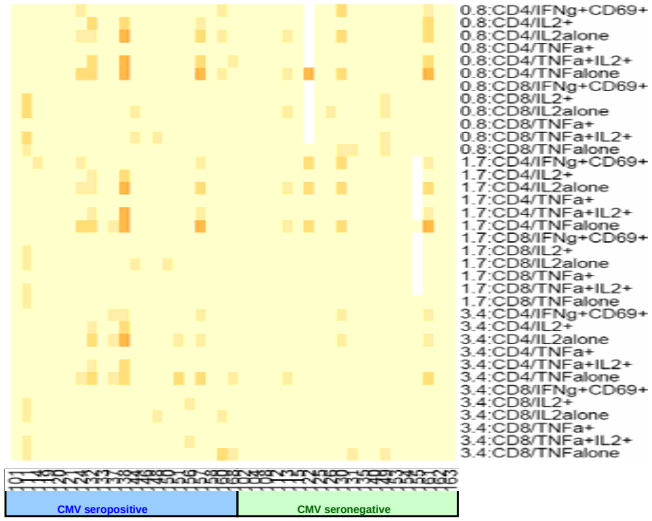


Normal donor cytokine responses, controls

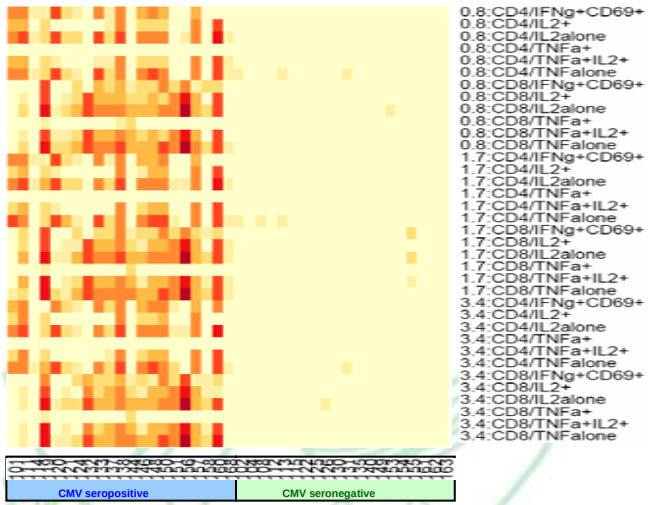
HIV mix (neg control)



Flu HA+M1 mix

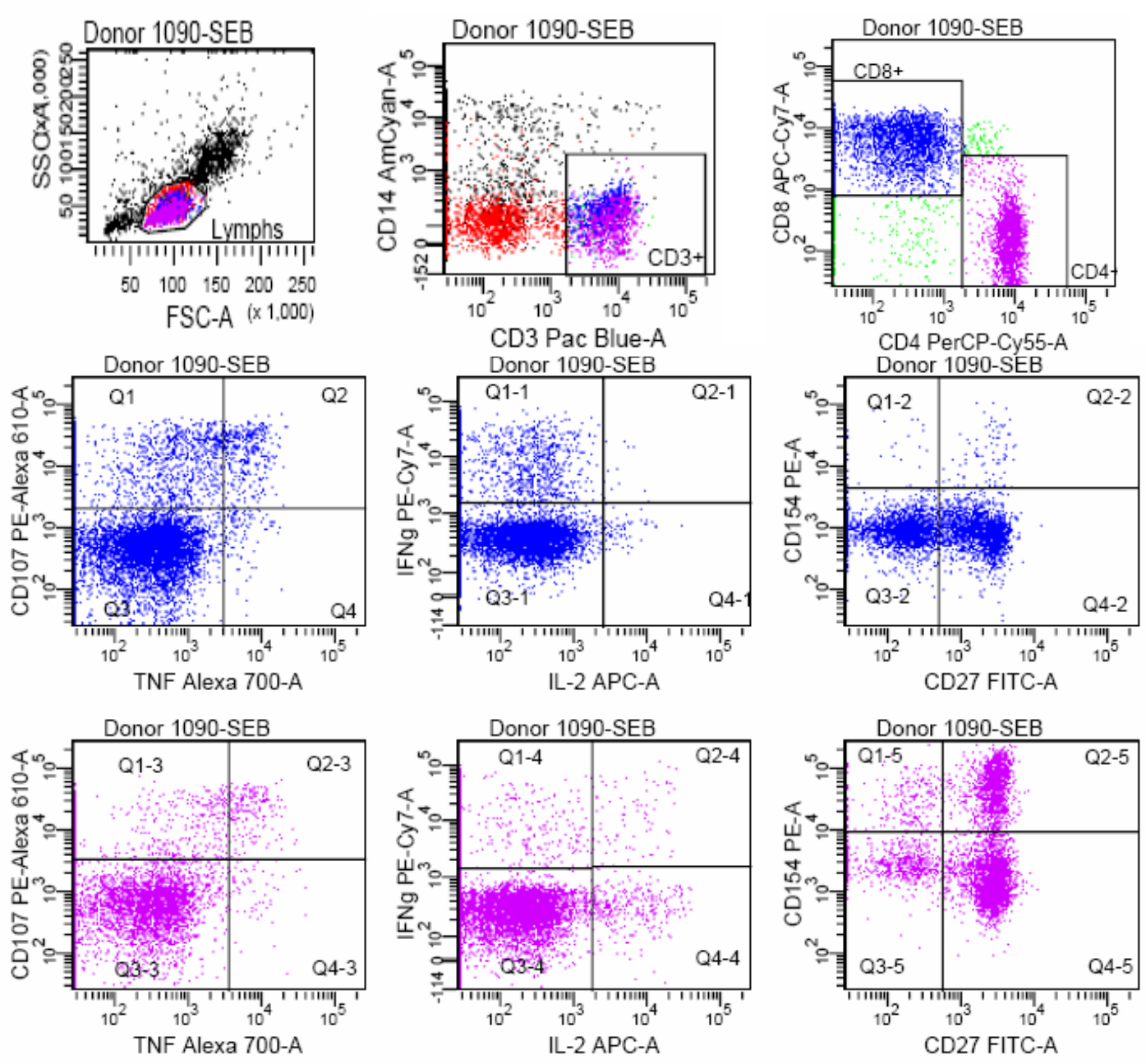


CMV pp65 mix



BD

10 Color BD Lyoplates



Technology Summary

Lyophilized stimulation and staining reagents in a 96-well format provides standardization of assays.

Flow cytometer with 96-well plate loader reduces operator hands-on time.

Dynamic gating in flow analysis software reduces variability in data analysis.

Data management tools allow for manipulation of large data sets.

Applying Cell Analysis to Clinical Trials

- Cellular analysis is a very important tool in understanding drug response
- Monitor Immune function via blood is applicable to numerous disease states
- Flow Cytometry is a viable tool for clinical trials
- Assay standardization reduces CVs
- Centralized data analysis reduces assay variability
- May be applicable to pre-clinical, clinical trial and patient profiling

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Thanks

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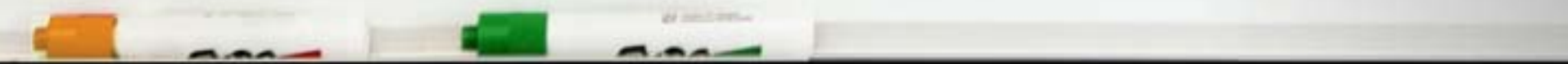

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