Using Machine Learning to Predict the Risk of Either Having an Aggressive Form of Prostate Cancer (PCa) or Lower Grade PCa/Benign Prostatic Hyperplasia (BPH) Based Upon the Flow Cytometry Immunophenotyping of Myeloid-derived Suppressor Cells (MDSCs) and Lymphocyte Cell Populations

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Flow Cytometry Based Immunophenotyping

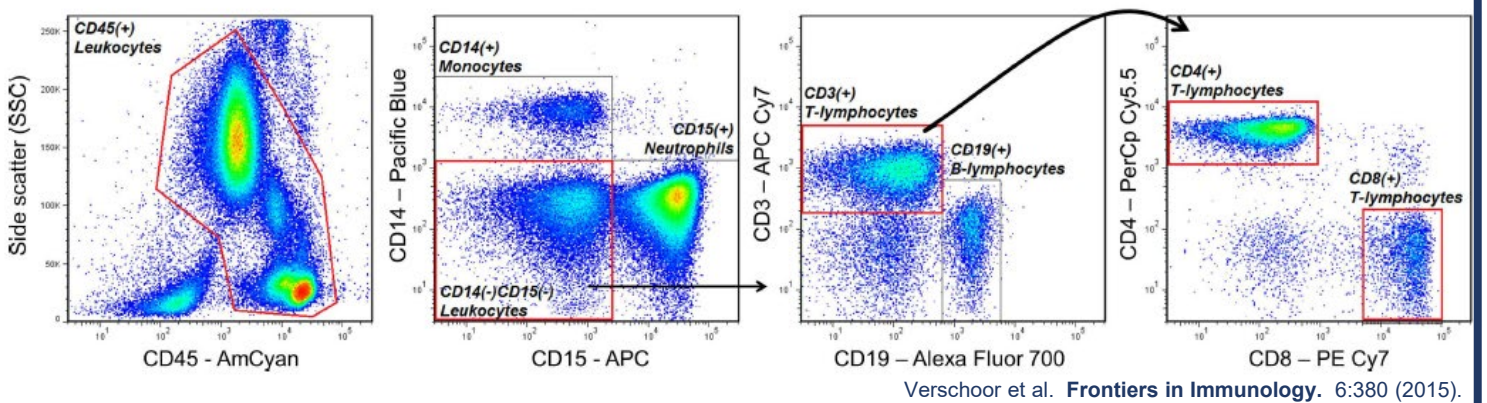
Flow Cytometry

- ✓ 50+ years in use
- ✓ A foundation of immunology and tumor immunology research

Immunophenotyping

- ✓ Know what you are looking for
- ✓ Manual gating = Boolean logic
- ✓ Operator experience
- ✓ More parameters (N) = more 2D unique relationships (R)
  - ✓ N = 2; R = 1
  - ✓ N = 3; R = 3
  - ✓ N = 4; R = 6
  - ✓ N = 5; R = 10
- ✓ Higher dimensions difficult for us to understand

- ✓ Real time analysis for thousands of cells per second
- ✓ Multiple cell markers can be used
- ✓ Cost Effective



Verschoor et al. **Frontiers in Immunology.** 6:380 (2015).

N = 7; R = 21

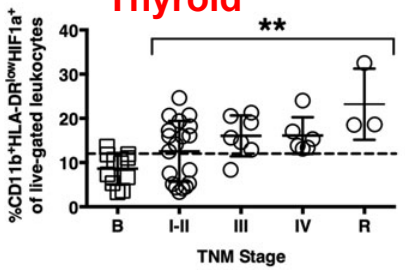
Identified 7 populations

A lot of information is discarded during analysis…

Myeloid-derived Suppressor Cells – What about them?

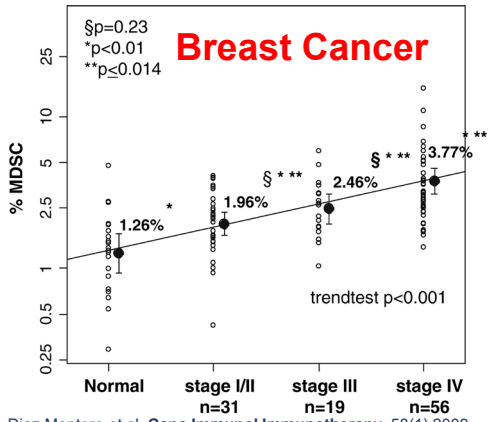
Indicative of Solid Tumors and Severity

Thyroid



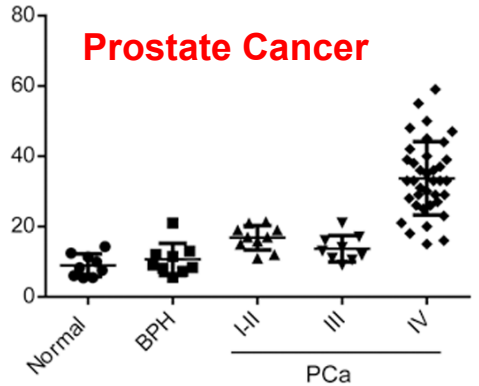
Angell et al. **Thyroid** 26(3) 2016.

Breast Cancer



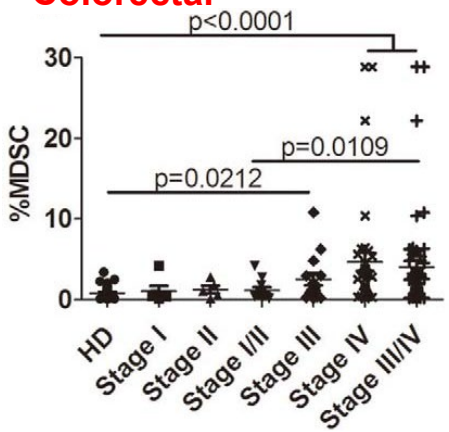
Diaz-Montero et al. **Canc Immunol Immunotherapy** 58(1) 2009.

Prostate Cancer



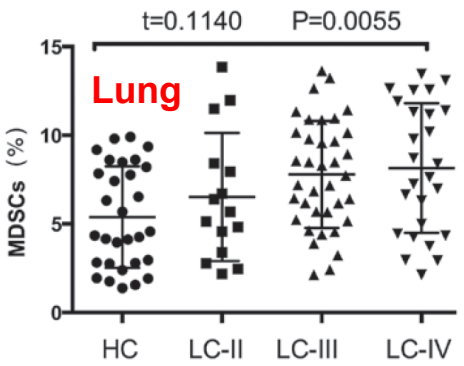
Chi et al. **Int J Clin Exp Med** 7(10) 2014.

Colorectal



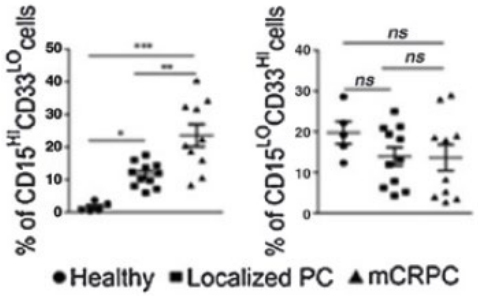
Zhang et al. **PLoS ONE** 8(2) 2013.

Lung



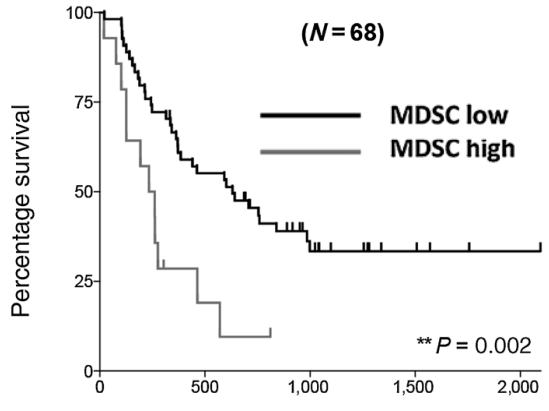
Chen et al. **Oncology Letters** 14 2017.

Prostate Cancer



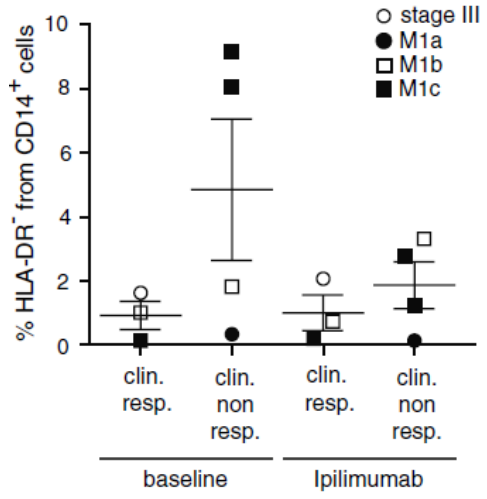
Hossain et al. **Clin Cancer Res** 21(16) 2015.

Predictors of Immunotherapy Response?



Kitano et al. **Cancer Immunol Res** 2(8) 2014.

CTLA-4 Responses in Late Stage Melanoma



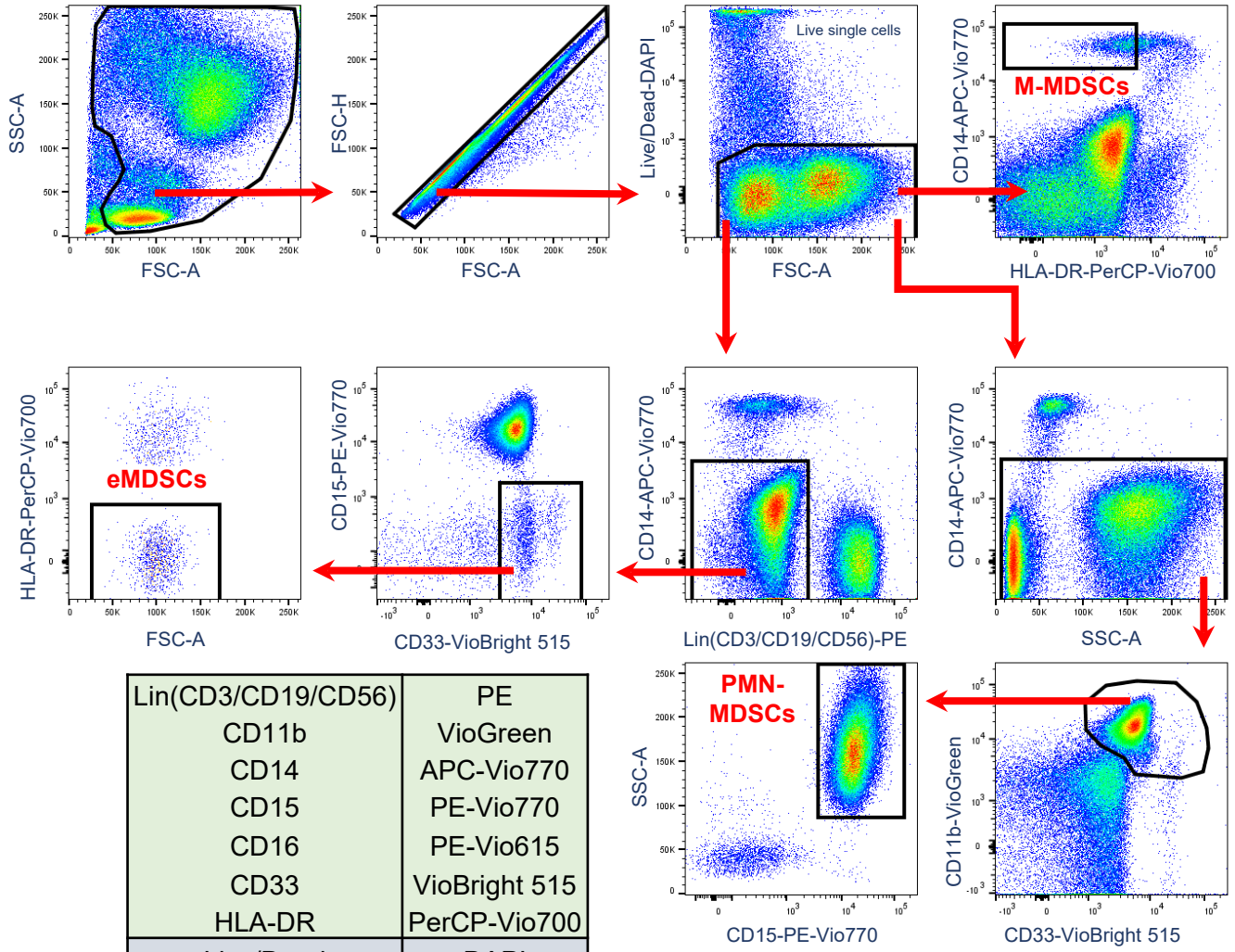
Meyer et al. **Cancer Immunol Immunother** 63:247-257 2014.

First Question

Can we use MDSCs and other leukocytes as a predictor for higher grade prostate cancer (PCa) and distinguish them from benign prostatic hyperplasia (BPH)/lower grade PCa?

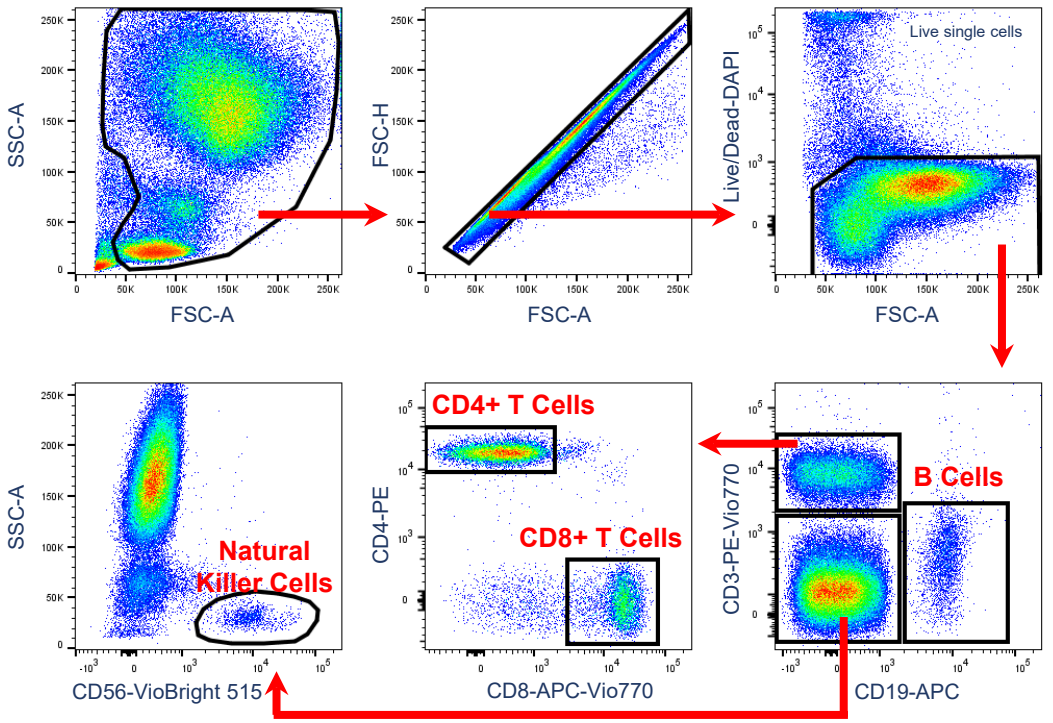
Myeloid and Lymphocyte Immunophenotyping

Myeloid Panel (10 Markers)



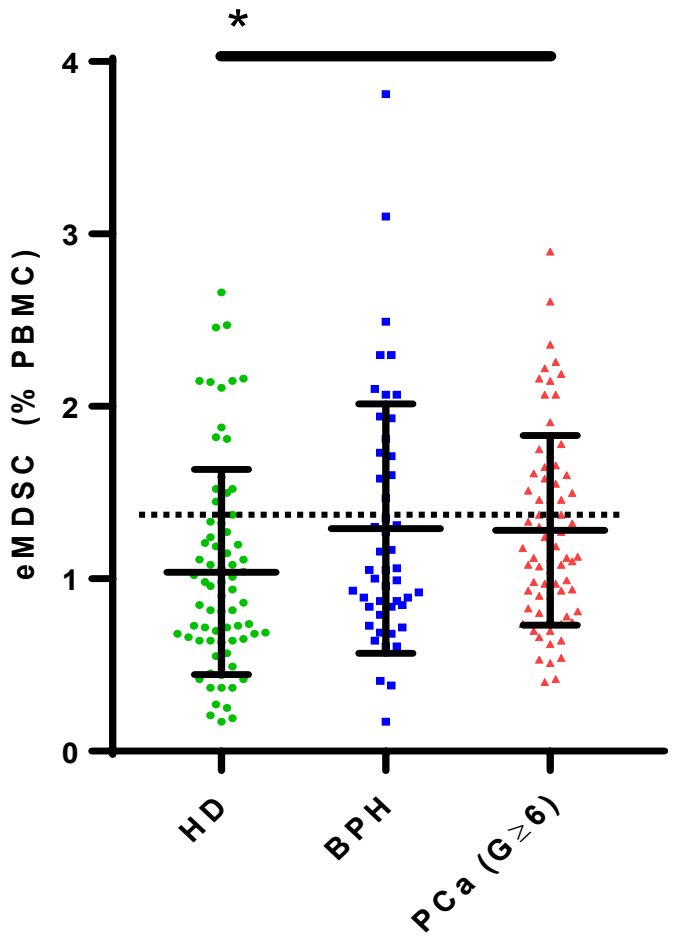
Lin(CD3/CD19/CD56)	PE
CD11b	VioGreen
CD14	APC-Vio770
CD15	PE-Vio770
CD16	PE-Vio615
CD33	VioBright 515
HLA-DR	PerCP-Vio700
Live/Dead	DAPI

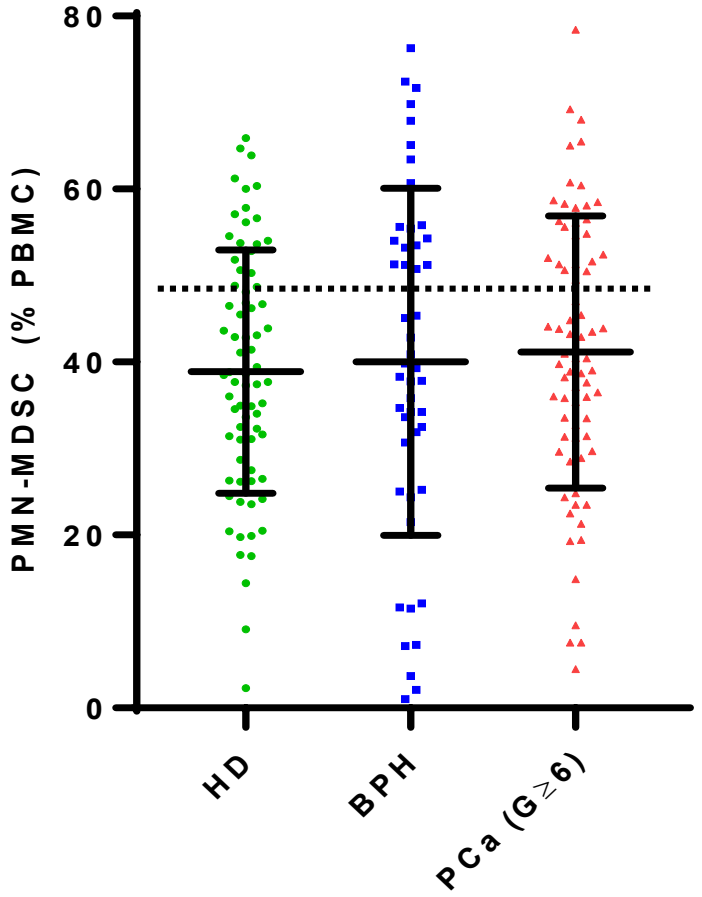
Lymphoid Panel (5 Markers)

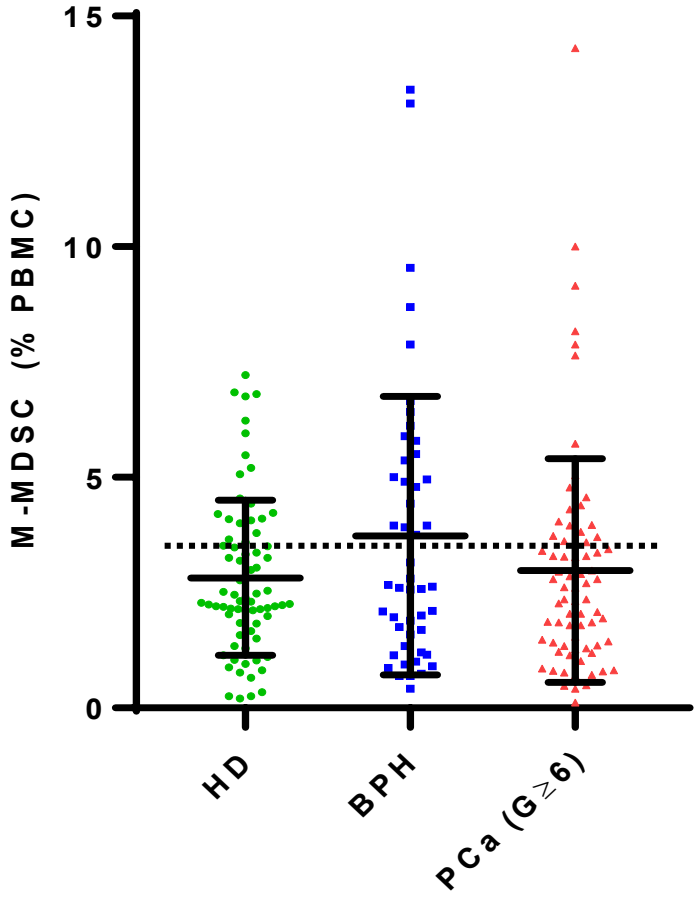


CD3	PE-Vio770
CD4	PE
CD8	APC-Vio770
CD19	APC
CD56	VioBright 515
Live/Dead	DAPI

Traditional Gating: Manual Counting - MDSCs







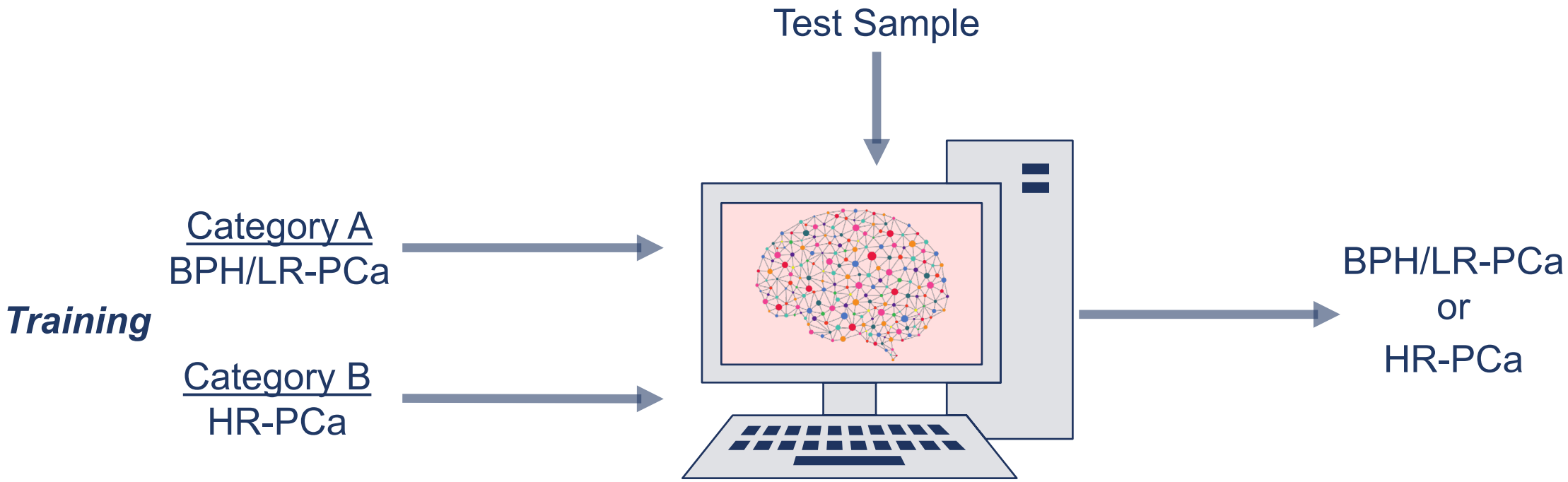
Simple cell counts can provide information about trends, but can only categorize some subjects

*The dotted line represents the 75th percentile for HD values (75% specificity)

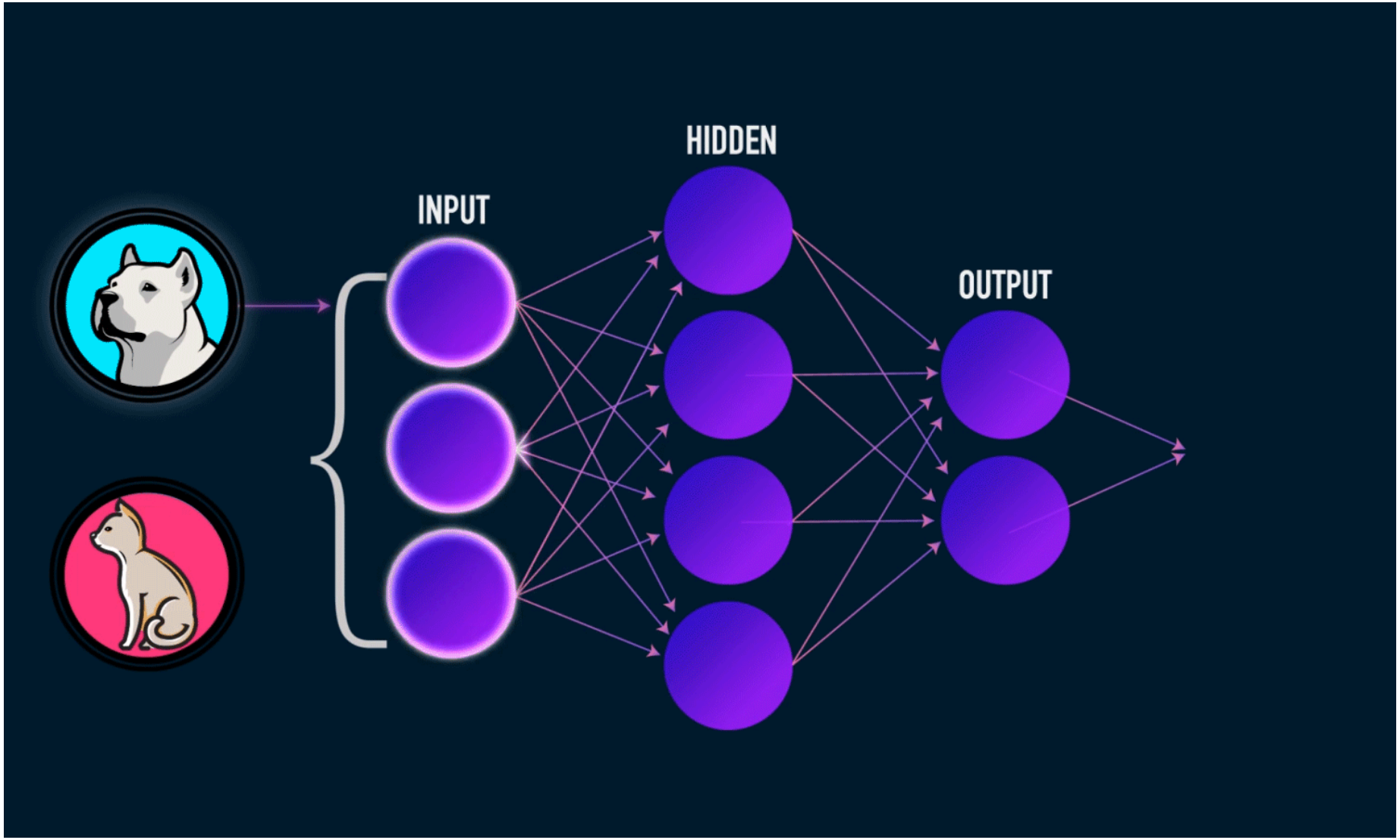
Healthy Donor (HD) n = 73
Benign Prostatic Hyperplasia (BPH) n = 48
Prostate Cancer (PCa) n = 73

Next Question

Can we use machine learning (neural networks) to analyze the flow cytometry data to categorize patients?

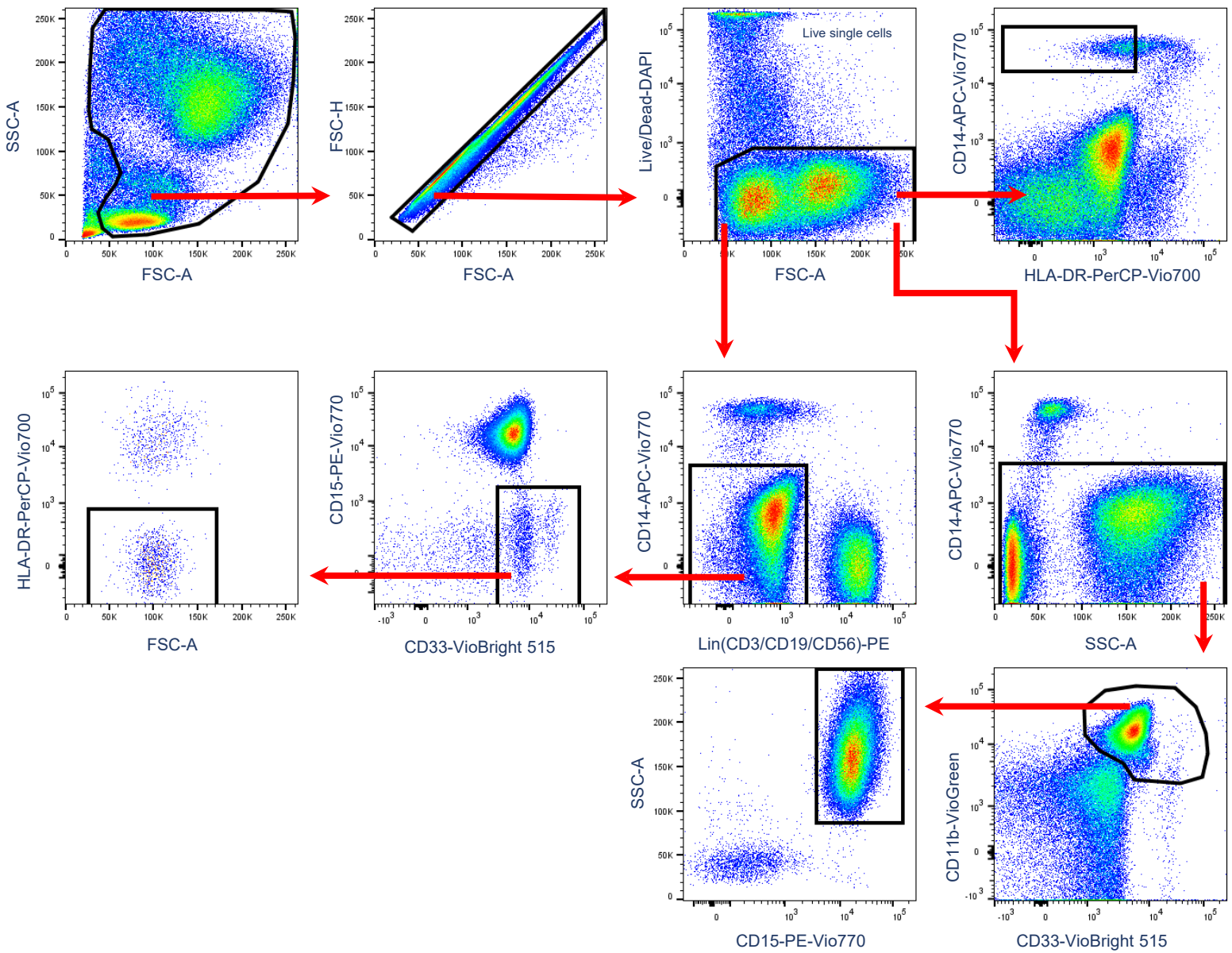


Neural Network – A Type of AI

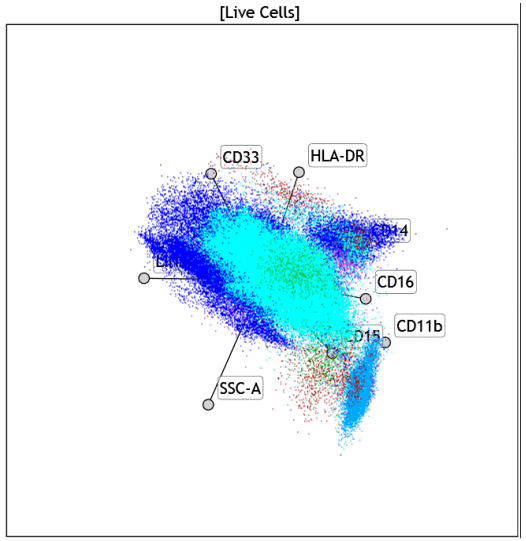
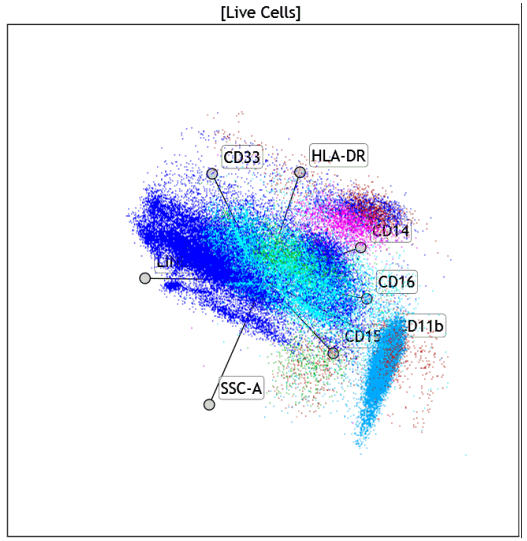
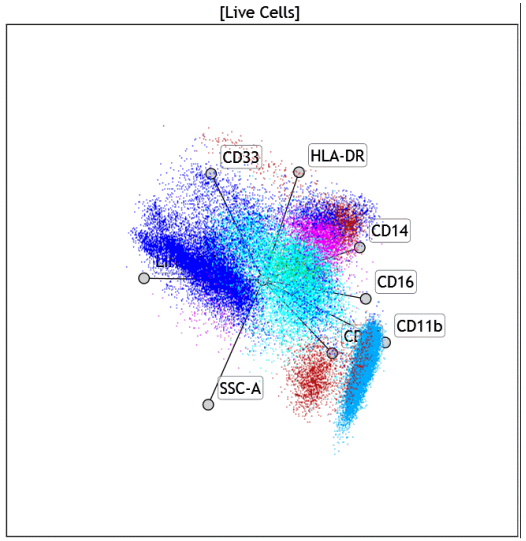
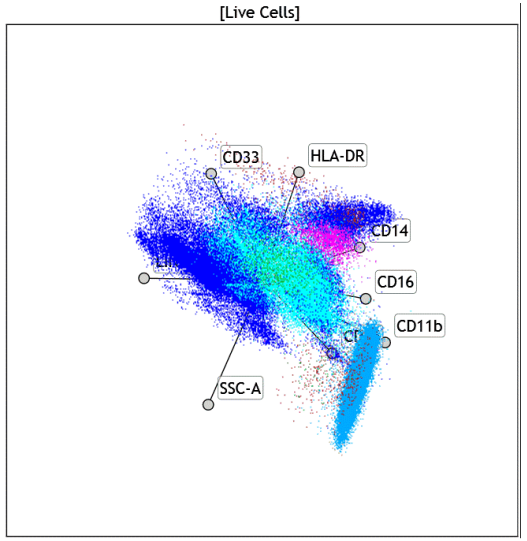
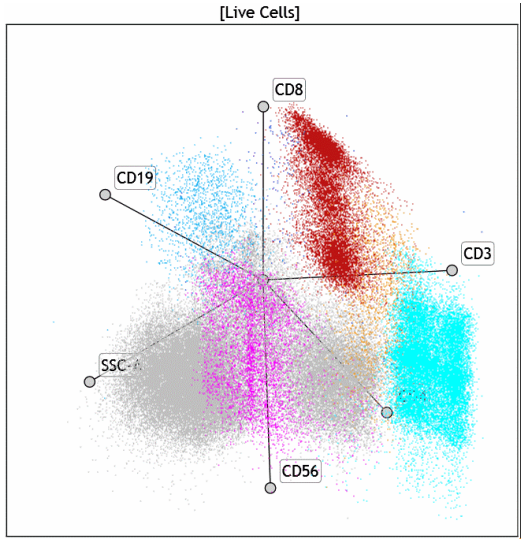
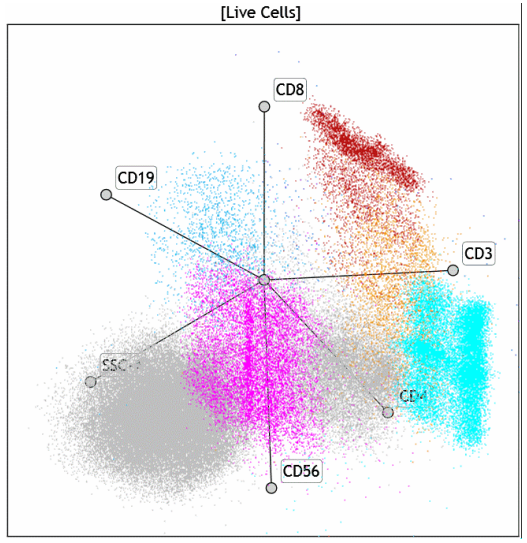
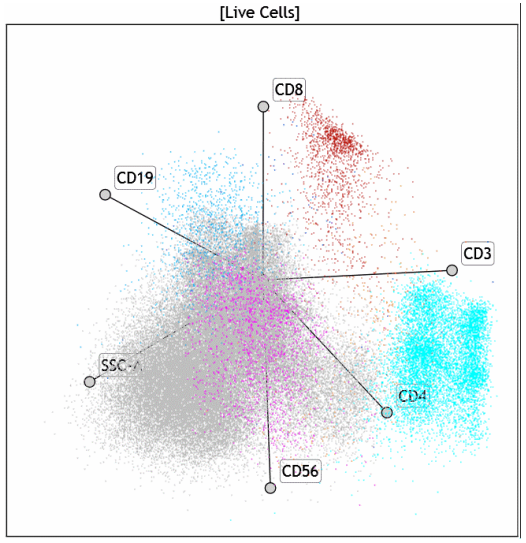
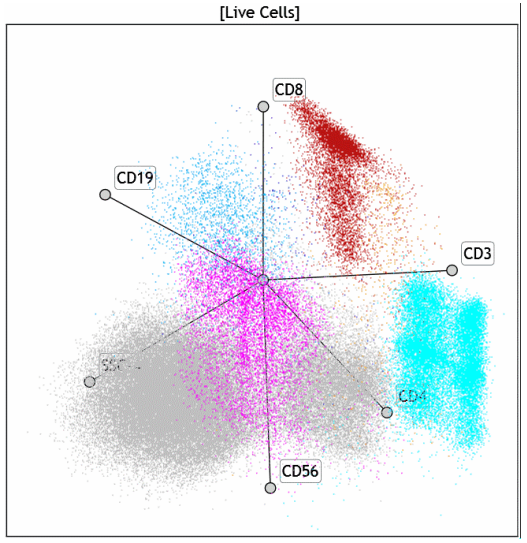


Source: https://www.analyticsindiamag.com/how-to-create-your-first-artificial-neural-network-in-python/

Complex Relationships – More Than Just Gating

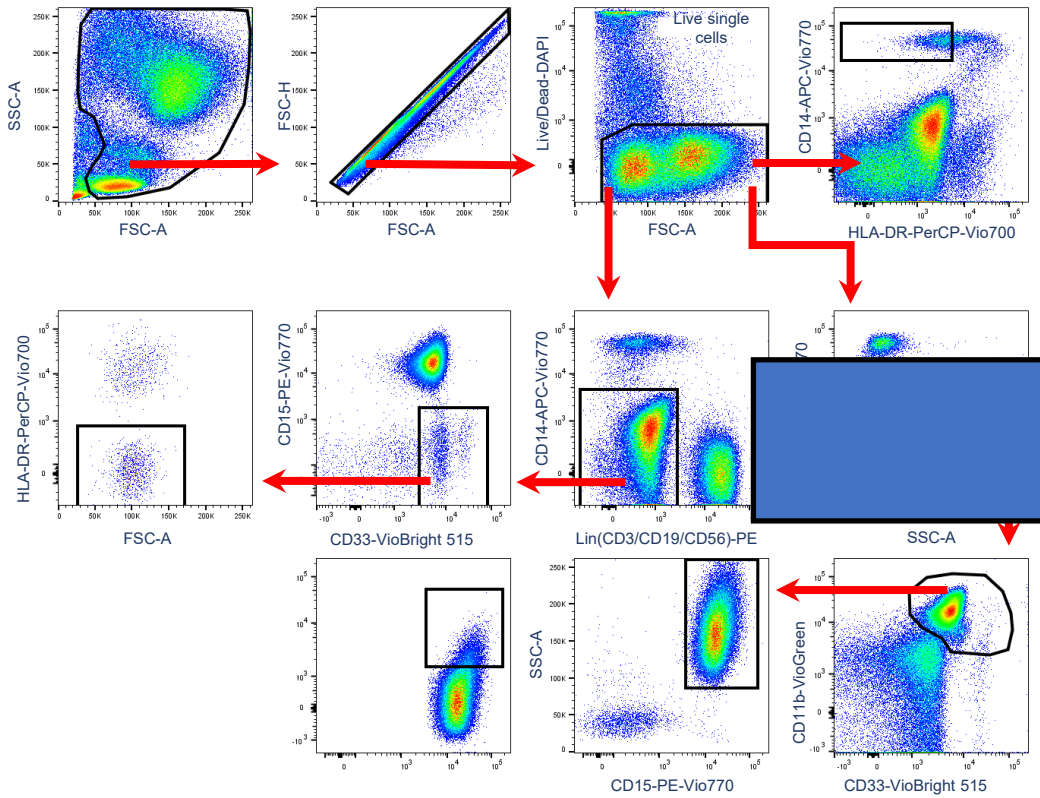


Complex Relationships – More Than Just Gating

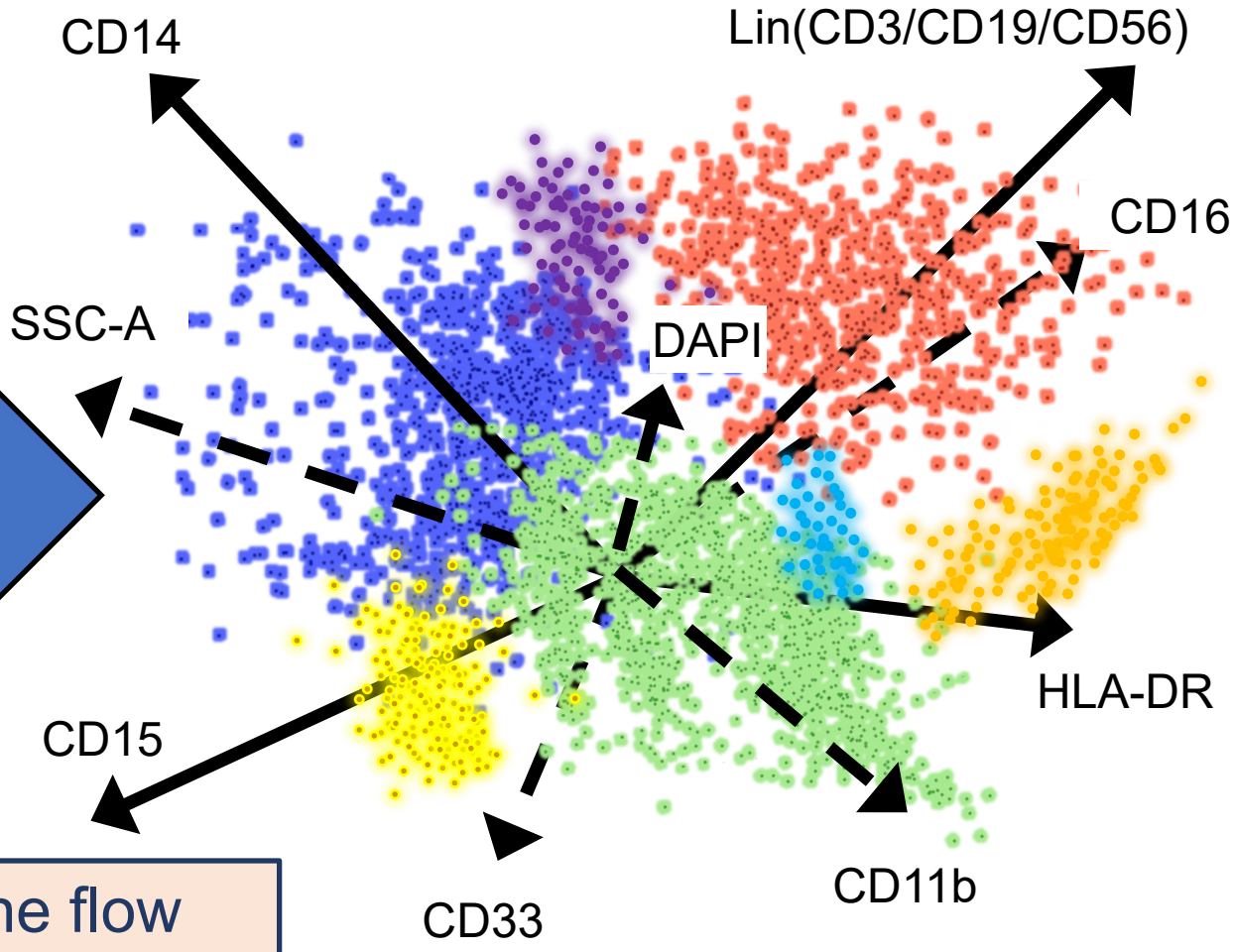
	HDM	BPH	PCa (G=6)	PCa (G≥7)
Myeloid Panel				
Lymphocyte Panel				

Data Transformation

FCS File → CSV File (Event counts for each channel)



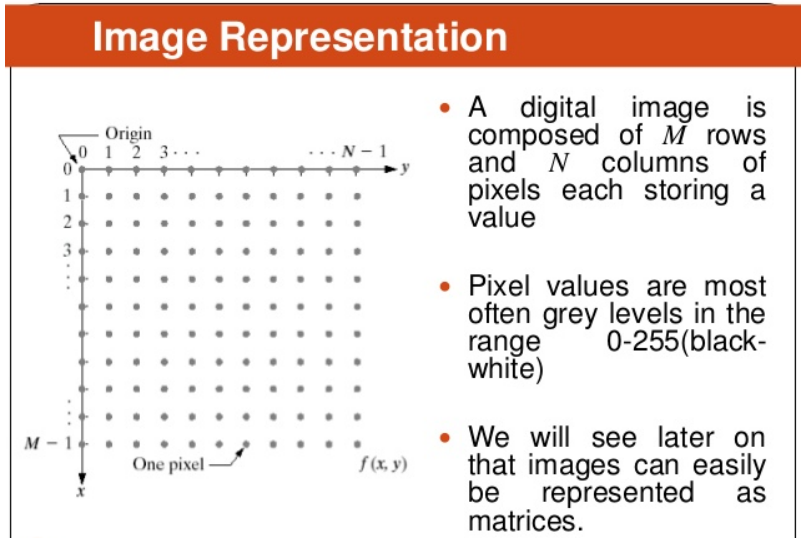
Multidimensional Space

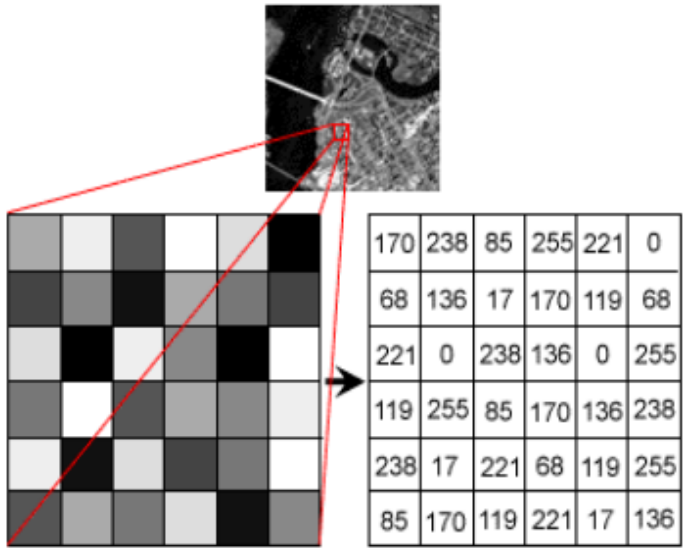


How do we effectively translate the flow cytometry data into a format for NN analysis?

Data Transformation – What is the Common Feature?

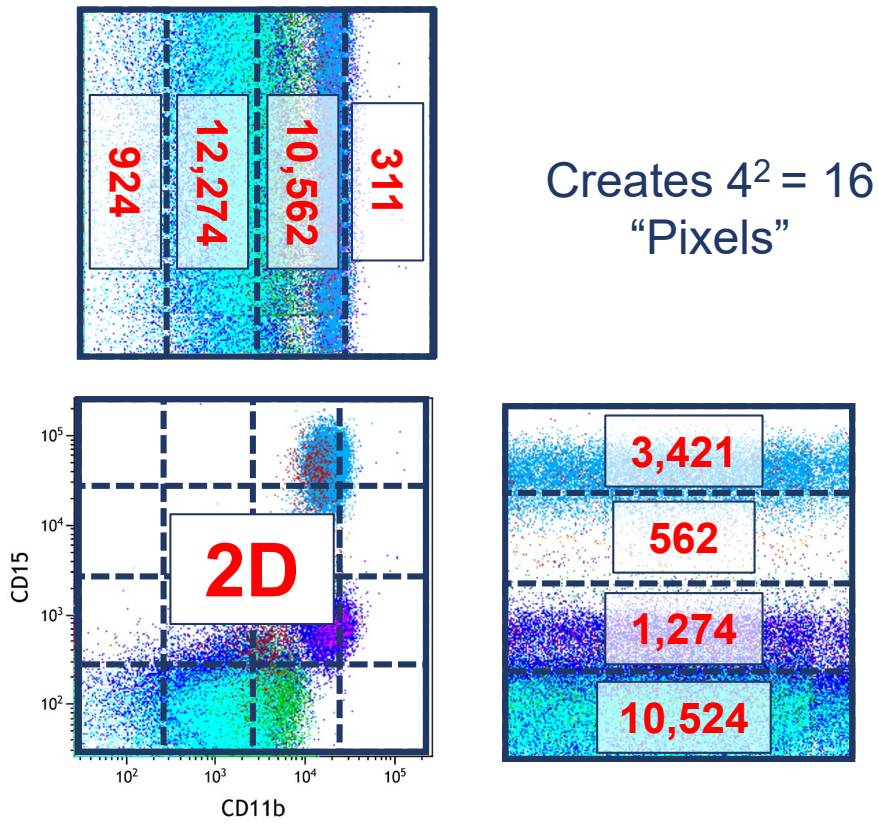
- Samples to be used by a neural network must have features in common across all samples
- For images, every sample has some specific pixel value for each pixel space
  - The pixels are the common features across all images
- But there is no common feature *for the entire sample set* in flow cytometry data
  - An immunofluorescence channel value on a specific event is a common feature across all cells, not between samples





Hypervoxel Generation – The Inputs

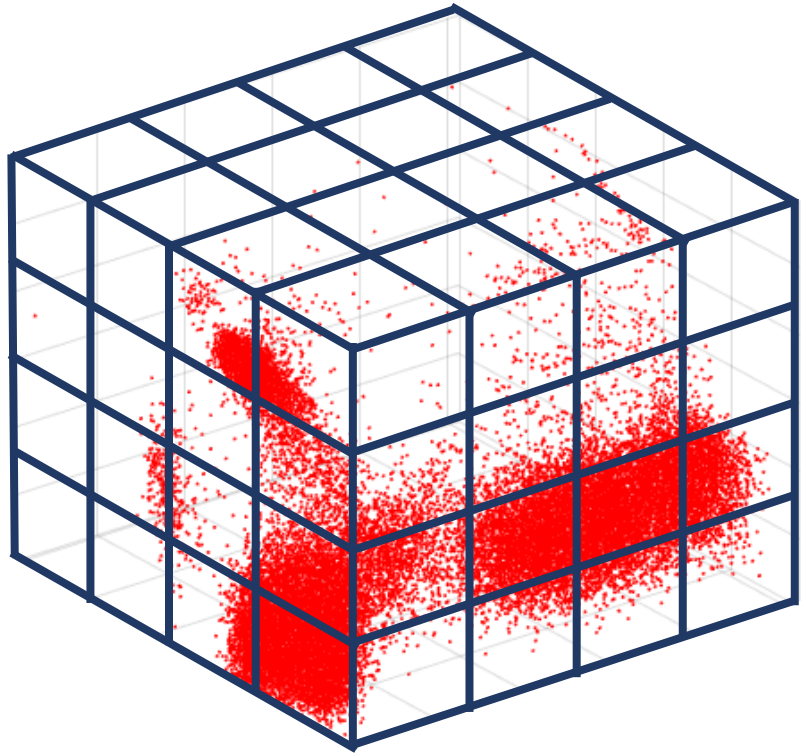
2D: Pixel



Creates $4^2 = 16$ "Pixels"

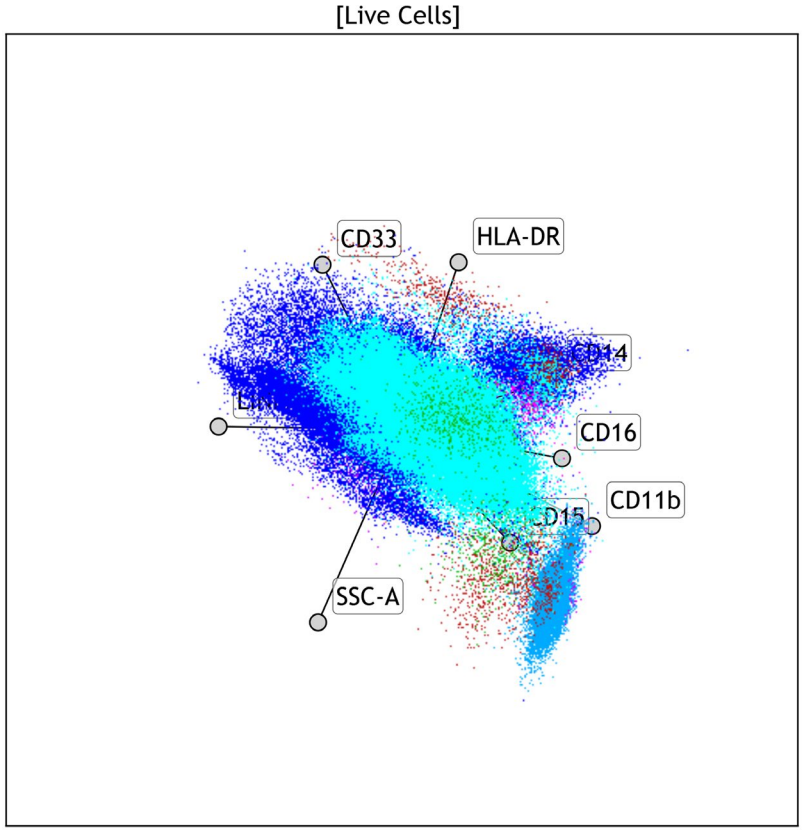
3D: Voxel

Creates $4^3 = 64$ Voxels



Hypervoxel Generation – The Inputs

Multidimensions (>3D): Hypervoxel



8 Markers
+
DAPI

$4^9 = 262{,}144$
Unique Hypervoxel Spaces

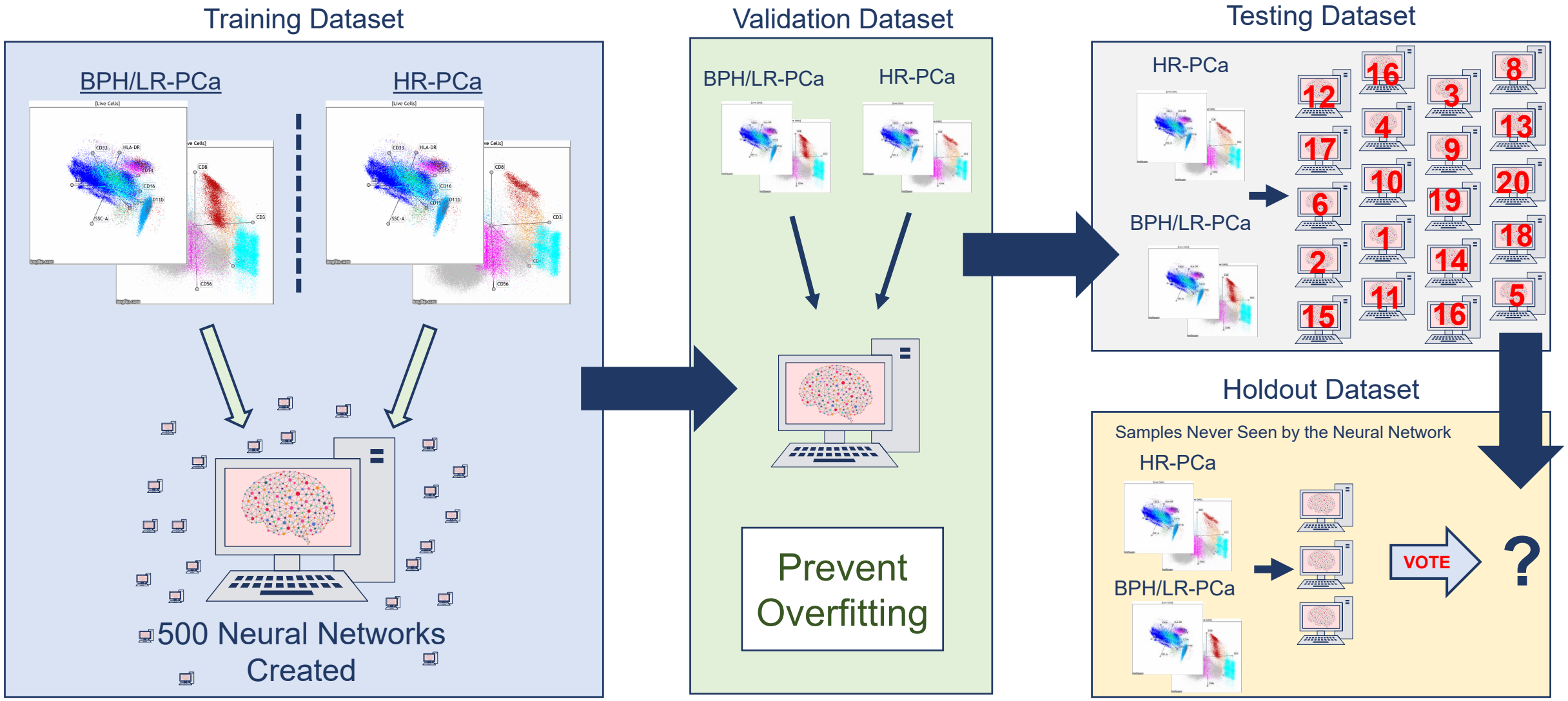
What is the input for the network?

- Each hypervoxel is a unique address
- Each event has channel values that give it an address to a hypervoxel
- The number of events that fall into that address is counted to be the input to the network
    - array of counts

Dataset Generation

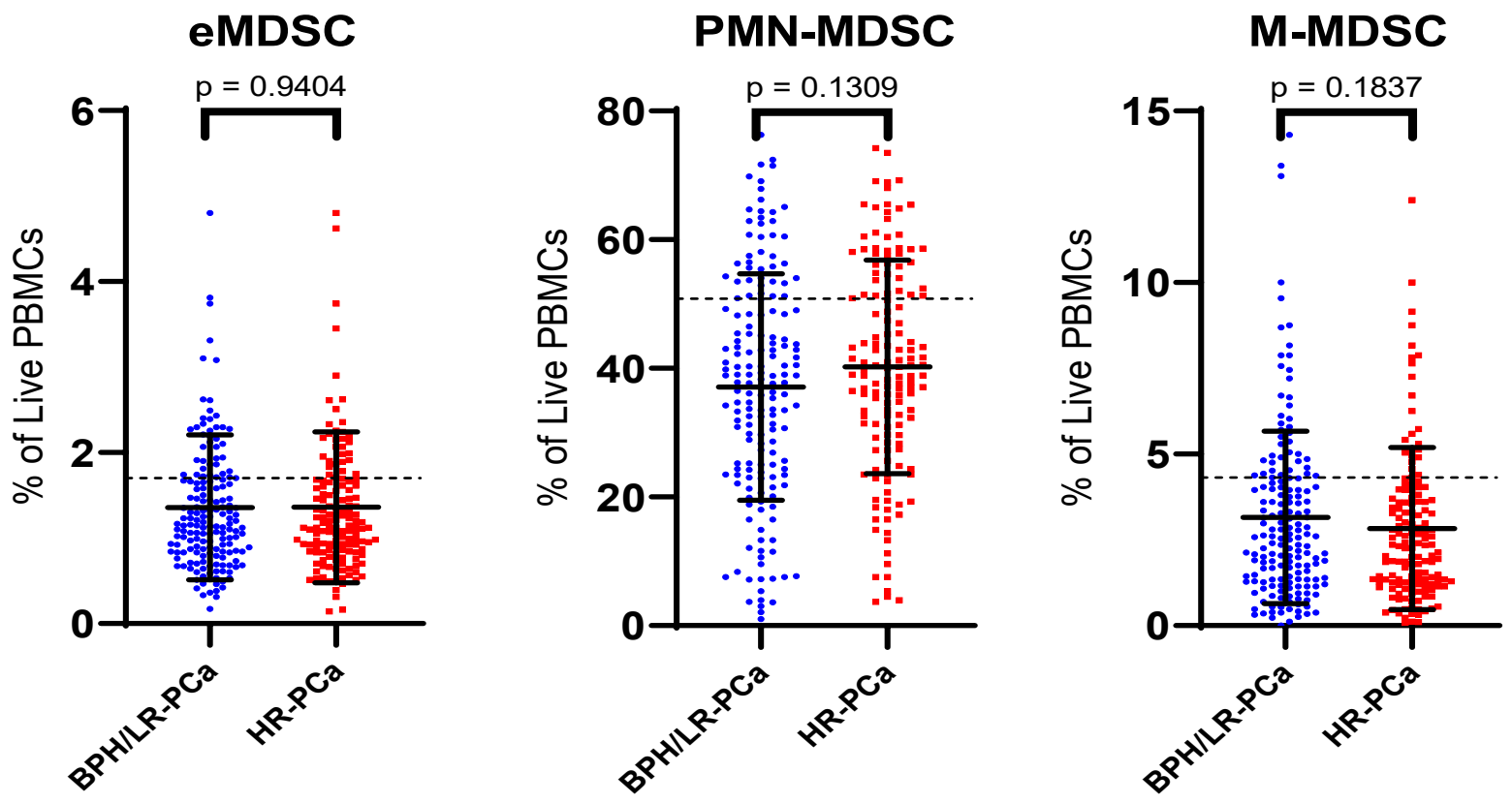
- 100,000 events are collected per sample
- Create sibling samples – variations of 50,000 events
- Per sample – 20 sibling samples are created

Neural Network – Binary Classifier



Clinical Characteristics and Manual Counting

Characteristic	PCa	BPH
Total	145	115
Median Age	68	63
Age Range	42 – 86	40 – 81
Gleason Score		
6	55	
7 (3+4)	31	
7 (4+3)	31	
>8	28	
T-Stage (AJCC 8th Ed.)		
T1c	117	
T2a	6	
T2c	1	
Unknown	21	



Still…simple cell counts can provide information about trends, but not really categorize subjects

Clinical Application: Confirmatory Testing

		Actual	
		HR-PCa	BPH/LR-PCa
Test	HR-PCa	46	33
	BPH/LR-PCa	3	24
Sens. (%) (95%CI)		93.88	(83.13 to 98.72)
Spec. (%) (95%CI)		42.11	(29.14 to 55.92)
PPV (%) (95%CI)		58.23	(52.48 to 63.76)
NPV (%) (95%CI)		88.89	(71.94 to 96.15)
Acc. (%) (95%CI)		66.04	(56.20 to 74.96)

Population: Men who were going to prostate biopsy

Samples used for training:

41 HR-PCa
113 BPH/LR-PCa

Holdout Samples for Testing:

49 HR-PCa
57 BPH/LR-PCa

- Potentially reduce the number of unnecessary prostate biopsies?

Conclusions

- We demonstrated that we can combine machine learning with flow cytometry data
  - Pros: Objective, Large Amounts of Data, More Data Inclusion, Simple Assay
  - Cons: Supervised Learning, Requires Large Amounts of Data for Training, Difficult to Determine Most Important Relationship – "Black Box" Idea
- We have applied this technique to be a binary classifier
  - distinguish between BPH/PCa in a small number of samples
- This can used for other classifications and tumor types
  - Breast Cancer: Early Stage vs non-Tumor Bearing
  - Breast Cancer: Early Stage vs DCIS
  - Prostate Cancer: Advanced Stage vs non-Tumor Bearing

Future Work

- Identify the critical relationships between cell populations that are used to make the classifications → hypervoxels
- Can this technique be applied to other flow cytometry data sets with different cancers or more cell populations? CyTOF? (retrospective analysis)
- As more and more data sets are generated with more and more information, can this help answer the question: What do we do with all of this data? What are we really trying to answer?
- Can this be used for answering other questions?
  - tumor recurrence, treatment and/or immunotherapy responses
    - Collaborative projects

Thank You!

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Questions?