

flowDensity

- ▶ Automated gating of pre-defined cell populations
- ▶ Cell subset identification based on the density distribution of the parent cell population by analyzing the peaks of the density curve
- ▶ 2D sequential gating similar to the current manual gating practice

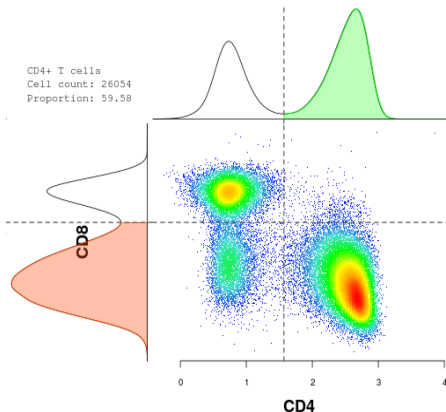
Algorithm:

1. Compute the density distribution of the data on each channel
2. Identify the peaks of the density curve
3. Choose the threshold based upon the peaks
4. Apply the thresholds of a pair of channels on a 2D scatter plot

Threshold Selection

- a. If there are exactly two peaks, the threshold is the minimum intersection between these two peaks

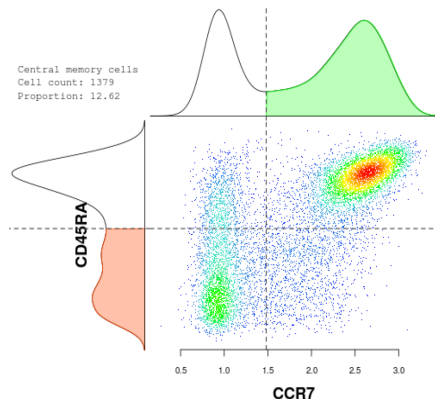
HIP-C PBMC data, T cell panel, Blomberg



Threshold Selection

- b. If there are three or more peaks, a score is calculated based on the height and distance of the peaks for each pair of adjacent peaks. The score then determines the place of the threshold by picking a pair of peaks.

HIP-C PBMC dataset, T cell panel, Stanford



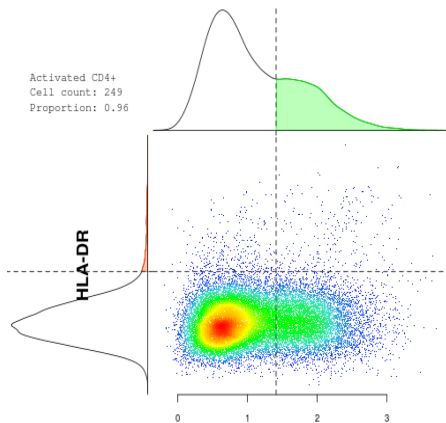
Threshold Selection

- c. If there is only one peak, the threshold is determined by one of the following approaches:

Threshold Selection, contd.

c.1. Identifying of inflection or flex points

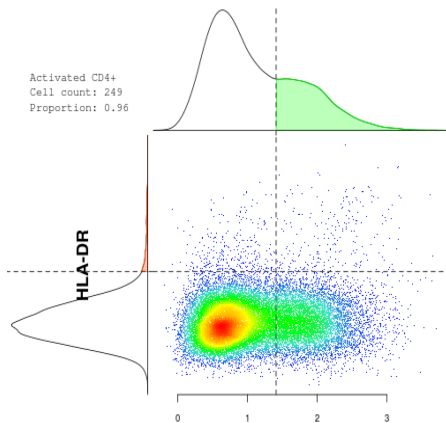
HIP-C PBMC dataset, T cell, Stanford



Threshold Selection, contd.

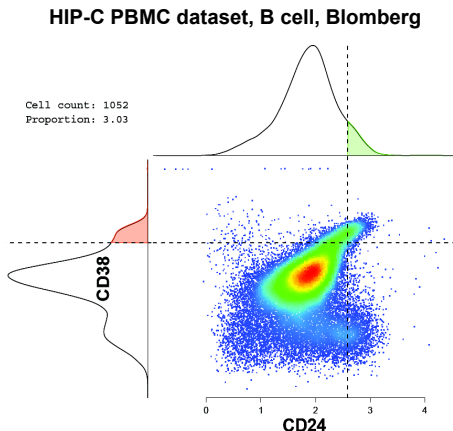
- c.1. Identifying of inflection or flex points
- c.2. Tracking the slope (derivative) of the curve

HIP-C PBMC dataset, T cell, Stanford



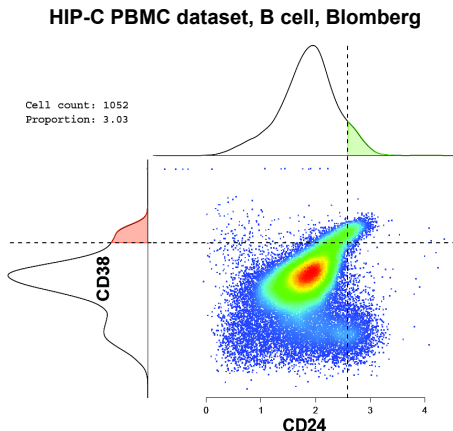
Threshold Selection, contd.

- c.1. Identifying of inflection or flex points
- c.2. Tracking the slope (derivative) of the curve
- c.3. Setting a percentile threshold



Threshold Selection, contd.

- c.1. Identifying of inflection or flex points
- c.2. Tracking the slope (derivative) of the curve
- c.3. Setting a percentile threshold
- c.4. peak \pm a multiplier of standard deviation



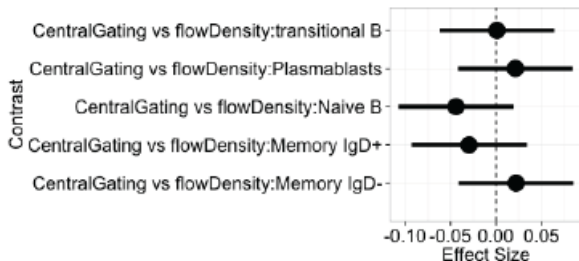
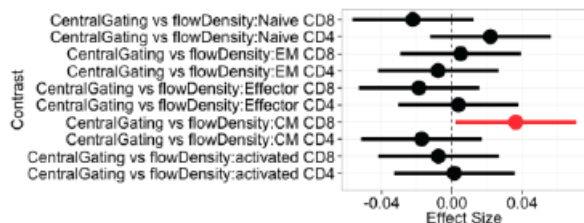
Threshold Selection, contd.

Parameters of each of the four approaches for the single-peak distribution:

- ▶ can be set optionally by user
- ▶ otherwise is set by flowDensity based upon the the statistics of the density distribution

Results: HIP-C Lyoplate Panel

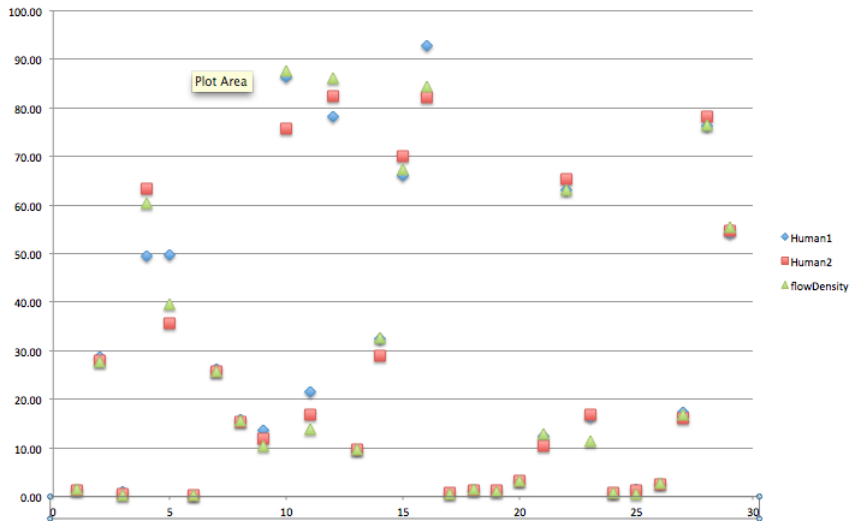
20 seconds to run all samples (up to 5,000)



Mouse Knockout Results

	CellCount	Auto_Proportion	Man_Proportion	Proportion_diff
Singlets	268645	89.55	98.4	-8.85
Granulo	3979	1.48	1.5	-0.02
CD5	64044	23.84	22.4	1.44
NKTs	483	0.75	0.9	-0.15
NKT act	247	51.14	55.9	-4.76*
NKT mem	236	48.86	44.1	4.76*
TCRd	448	0.71	0.7	0.01
TCRb	57616	90.83	92.8	-1.97
CD4+	27335	47.44	48.6	-1.16
CD4+ CD44hi	1090	3.99	4.3	-0.31
CD4+ CD44lo	26076	95.39	95.7	-0.31
CD4+ Ly6C+	6778	24.8	25.7	-0.9
CD4+ Ly6C-	20557	75.2	74.3	0.9
CD8+	29498	51.2	48.8	2.4
CD8+ Ly6C+	8673	29.4	30.5	-1.1
CD8+ Ly6C-	20825	70.6	68.3	2.3
CD8+ CD862L+	17516	59.38	58	1.38
TCR-	2918	4.6	4.3	0.3
B1 B	2735	93.73	92.9	0.83
Mono	4159	2.13	1.9	0.23
Macro	2773	1.46	2.3	-0.84
NK cells	8844	4.71	4.4	0.31
Ly6C+ CD11b+	1656	19.51	21.1	-1.59
Ly6C- CD11b+	5088	59.94	62.1	-2.16
Ly6C- CD11b-	800	9.43	9.6	-0.17
Eosino	3108	55.98	59.7	-3.72
pDC	1862	19.19	38.9	-19.71*
DCs	7841	80.81	58	22.81
CD8 DC	1063	13.56	13.4	0.16
CD11b DC	6358	81.09	83.3	-2.21
B2	161824	93.8	90.5	3.3

Mouse Knockout Results



Acknowledgements

BCCA

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