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Life Sciences

A high recovery and high purity data analysis strategy for rare abnormal plasma cell events using the DuraClone RE PC Tube

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IN THIS PAPER YOU WILL

Follow a mixed boolean / multivariate gating strategy to identify and characterize plasma cells with high recovery and high purity using Kaluza 1.5a data analysis software

See an analysis example for plasma cells with CD38 mid-high expression with strong deviation from the normal phenotype

See an analysis example for plasma cells with CD38 low-mid expression with less-pronounced deviation from the normal phenotype

Introduction

Flow Cytometry is a powerful method to identify, characterize and quantify normal and abnormal plasma cells in human whole blood, bone marrow and apheresis products. The phenotype of abnormal plasma cells (PCs) is not defined by a single specific antigen expression pattern but by any deviation from the well-defined antigen expression pattern of the normal PC phenotype. Discrimination of small populations (e.g. 50 events) of these abnormal cells from a background of hundreds of thousands to millions of normal leukocytes including normal PCs can be challenging as expression of PC gating markers can be modulated (e.g. therapeutic intervention, multiple myolema cells, Table 1). Furthermore, PC characterization markers are commonly expressed by other normal leukocyte populations which require diligent verification of the target cells' identity.

Antigen	Used for PC identification	Used for PC characterization	Normal expression characteristics[1]	Deviating expression characteristics[2] in abnormal plasma cells		Positive non-PC leukocyte populations[3]
CD19		✓	++	neg	96%	B cells
CD27		✓	+++	neg or dim+	40-68%	Lymphocytes
CD38	✓	(✓)	++/+++	dim+	80%	Broad
CD45	✓	✓	++	neg	73%	All mature leucocytes
CD56		✓	neg or dim+	++	60-75%	NK cells
CD81		✓	++	neg or dim+	55%	Broad
CD117		✓	neg	+	30-32%	Small T and NK populations, myeloid precursor cells
CD138	✓		+/++	N.A.	N.A.	-
CD200		✓	neg or dim+	+/++	≥70%	Small B cell populations

Table 1: Phenotypic features of normal and abnormal plasma cells.

The DuraClone RE PC Tube contains the gating markers CD138 and CD38 as well as antibodies frequently used for characterization, i.e. CD19, CD27, CD45, CD56, CD81 and CD200. Furthermore, inclusion of CD45 allows for quantifying plasma cells relative to the leukocyte event count. The antibody dosing accommodates staining of 100-200µL suspension enriched to a total leukocyte count of 3-5 millions.

Detection of $CD45^{+}CD38^{+}CD138^{+}CD56^{+}CD200^{+}CD19^{dim}CD27^{dim}CD81^{dim}$ plasma cells											
DuraClone RE§ PC Tube	PB	KrO	FITC	PE	ECD	PC5.5	PC7	APC	AF700	APC-AF750	Quality Standard
B80394 (25 tests RUO)	CD38	CD45	CD81	CD27	-	CD19	CD200	CD138	-	CD56	ISO 9001

§ For Reseach Use Only. Not for use in Diagnostic procedures.

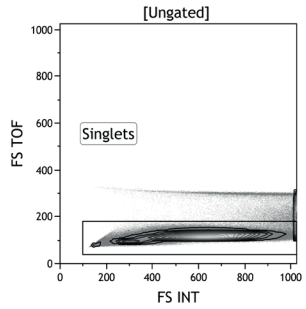
Materials

- Kaluza Analysis Software 1.5a or higher
- Display with 1024×768 resolution minimum (1920×1200 or higher resolution recommended)
- Windows 7 SP1, Windows 8.1 or Windows 10 operating system
- 3Ghz CPU or higher / Quad core recommended
- Datasets obtained with the DuraClone RE PC tube (B80394, please see instructions for use for sample preparation and flow cytometry data acquisition)

Tips for success

- The better your computer performance is (e.g. ≥3GHz Quad core CPU), the shorter data recalculation times upon gating adjustments will be when handling large rare event data sets
- Start with "oversized" gates for high recovery, then increase specificity by narrowing your gates
- Always double-check plausibility by reviewing the features of identified target cells as colored events in plots that are early in the gating strategy (e.g. scatter gate) and in the radar plot

Gating example for CD38 med-hi PCs with phenotype deviating strongly from normal PCs

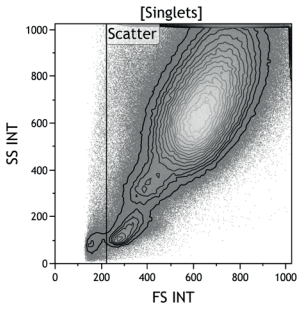


1. Removal of doublets

Plot: contour with density

Gate: ungated

Approach: enrichment of singlets by selection of events with lowest Time of Flight (TOF) signals

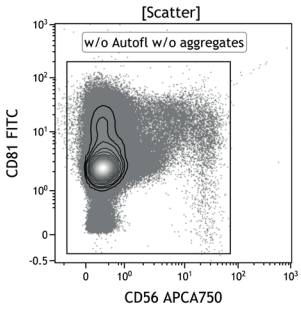


2. Removal of debris

Plot: contour with density

Gate: singlets

Approach: discrimination of events with forward scatter smaller than lymphocytes

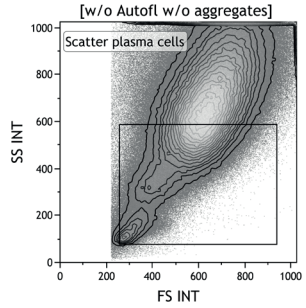


3. Removal of dye aggregates

Plot: contour with density

Gate: scatter

Approach: discrimination of dye aggregates with high APC-AF750 fluorescence and events with high green autofluorescence

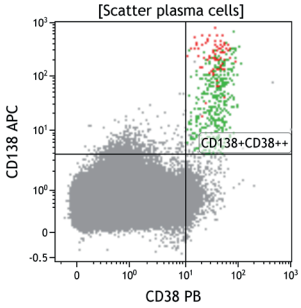


4. Selection of PC scatter range

Plot: contour with density

Gate: w/o aggregates

Approach: further restriction of scatter range for plasma cells, upper SSC limit at center of granulocytes, upper FSC limit granulocytes



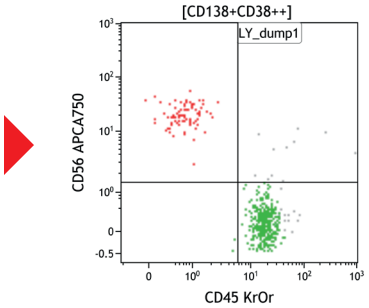
5. Gating of events with plasma cell phenotype

Plot: gate colouring

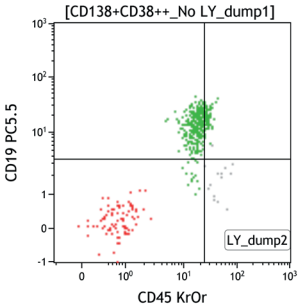
Gate: scatter plasma cells

Approach: identification of events with high CD138 and high CD38 expression density

The gating in Plot 5 is important for high recovery of PCs. An "oversized" gate should be applied first, then narrowed to contain only clusters of normal (green) and abnormal (red) PC populations. Thus, plot 5 also has a backgating function as the identity of the PCs is subject to gating steps further "downstream" in the gating strategy (see following plots).



Boolean



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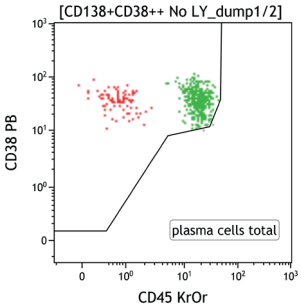


6. Removal of contaminating CD56+ events

Plot: gate colouring

Gate: CD138+ CD38++

Approach: discrimination of CD56+ CD45high events (LY_dump1)

7. Removal of contaminating CD19neg events

Plot: gate colouring

Gate: CD138+ CD38++ no LY_dump1 (see boolean gate definitions in Table 2)

Approach: discrimination of CD19- CD45high events

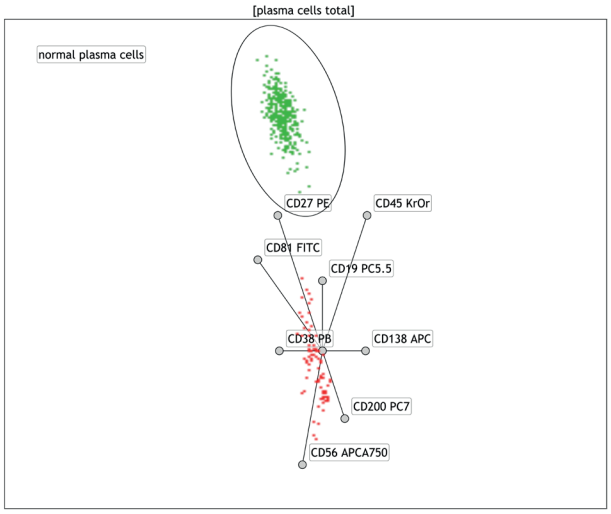
8. Selection of PC scatter range

Plot: gate colouring

Gate: CD138+ CD38++ no LY_dump1 (see boolean gate definitions in Table 2)

Approach: further restriction of plasma cells in CD38++ expression range, delimiting against CD38med CD45high events

Boolean



9. Discrimination of normal PCs vs abnormal PCs

Plot: radar plot

Gate: plasma cells total (see boolean gate definitions in Table 2)

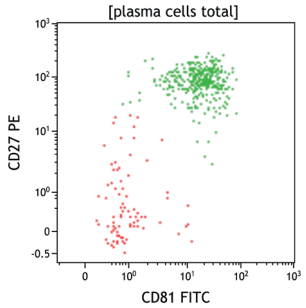
Approach: identification of normal plasma cells by their fixed position within a 2D-projection of all fluorescent parameters

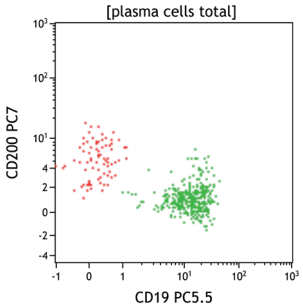
There are many possible radar plot configurations that allow for the shown discrimination. An easy way to set up a functional radar plot is as follows:

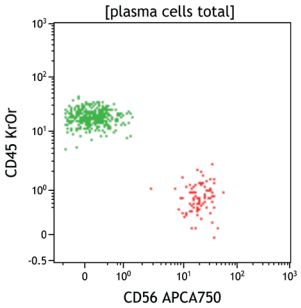
1. Positive (CD56, CD200) and negative (CD19, CD27, CD45, CD81) deviations from normal expression should be grouped and the groups should point into opposite directions.
2. Strong deviations in expression should be assigned longer axes than moderate differences in expression.

Please note, the cluster of normal PCs will reside in a constant position while the position of the abnormal cluster may vary.

Boolean







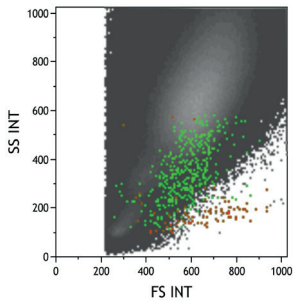
10. Verification of phenotypic features in abnormal PCs

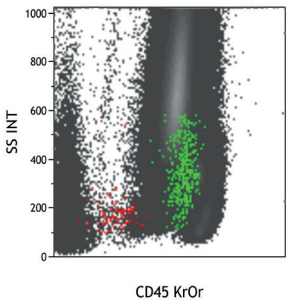
Plot: gate colouring

Gate: Plasma cells total (see boolean gate definitions in Table 2)

Approach: verification of deviation of antigen expression patterns in abnormal plasma cells

Boolean





In contrast to the normal PCs, abnormal PCs may have a variable position so that normal and abnormal PCs may overlap in the backgating plots.

11. Scatter backgating

Plot: density overlay

Gate:
a) w/o autofl w/o aggregates, grey
b) normal plasma cells, green
c) plasma cells with phenotype deviating from normal, red

Approach: confirm typical PC scatter positioning at mid-high FSC and low-mid SSC

12. CD45 backgating

Plot: density overlay

Gate:
a) w/o autofl w/o aggregates, grey
b) normal plasma cells, green
c) plasma cells with phenotype deviating from normal, red

Approach: confirm typical PC CD45 positioning, normal PCs at medium CD45 level, PCs with deviating phenotype at lower or negative CD45 level

The gating strategy utilizes 3 boolean gates defined as follows:

GATE NAME	GATE LOGICS
CD138+ CD38++ No LY_dump1	CD138+ CD38++ AND (NOT LY_dump1)
CD138+ CD38++ No LY_dump1/2	CD138+ CD38++ AND (NOT LY_dump1) and (NOT LY_dump2)
Plasma cells with phenotype deviating from normal	"Plasma cells total" AND (NOT "normal plasma cells")

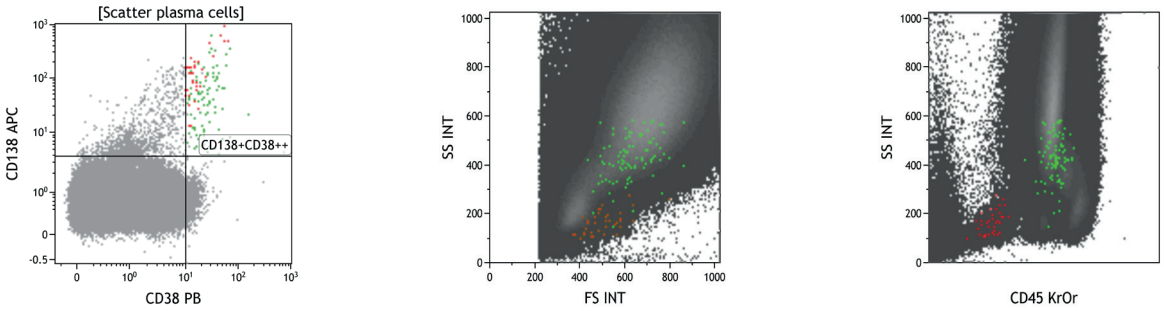
Table 2: Boolean gate definitions

GATE	NUMBER
CD45+	2,099,000
Normal plasma cells	379
Plasma cells with phenotype deviating from normal	85

The results can be displayed using an Information plot: in the shown example, abnormal plasma cells have been detected at a frequency of 0.004% CD45+.

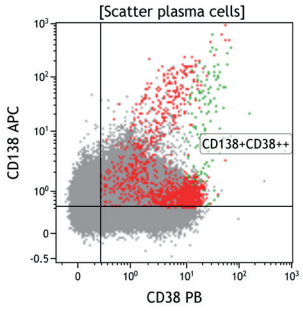
Gating example for CD38 low PCs with less pronounced deviation from the normal phenotype

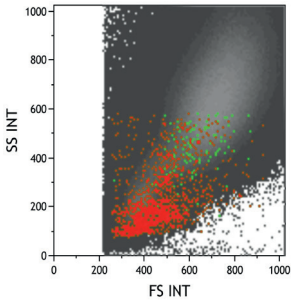
The identification of PCs will depend more strongly on CD138 expression in case of plasma cells with reduced CD38 expression. The main impact on adjustment of gates will be located in Plot 5 where the "oversizing" of gates followed by backgating-guided narrowing is the core of the modified strategy.

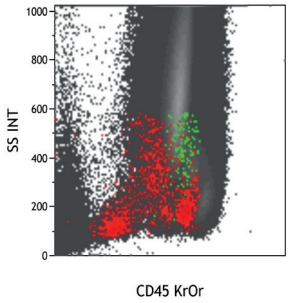


Gating on CD38hi events, thus missing a considerable portion of CD38low-med PC events

The gating of PC-like phenotype indicates a truncation of the PC population while the backgating plots confirm plausible positioning of the plasma cells.

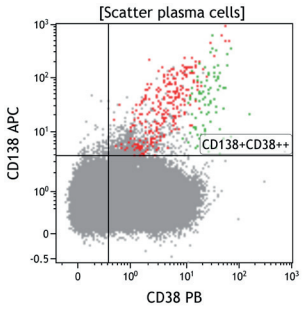


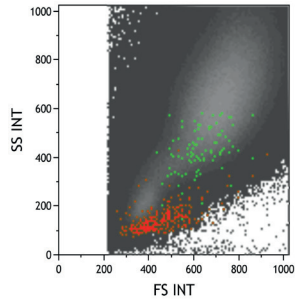


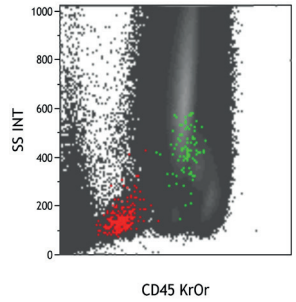


"Oversized" gating on CD38hi to CD38low events, thus including non-PC events

The gating of PC-like phenotype indicates several red clusters in untypical positions. Consistently, the backgating plots show non-clustered, scattered red events (FSC vs SSC) as well as clusters of red events apart from the typical positioning of abnormal PCs (CD45 vs SSC).

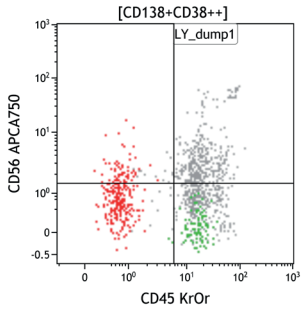


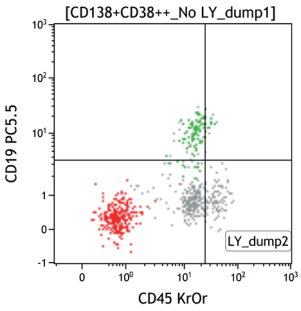


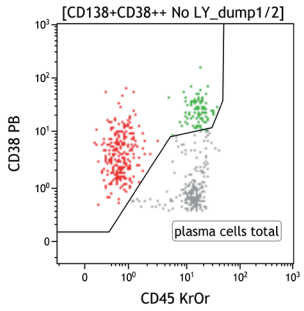


"Narrowed" gating on all CD38+ and CD138med-hi events, thus improving the recovery of abnormal PCs

The gating of PC-like phenotype indicates a non-truncated red cluster. Consistently, the backgating plots shows clustered red events in plausible positions (FSC vs SSC and CD45 vs SSC).



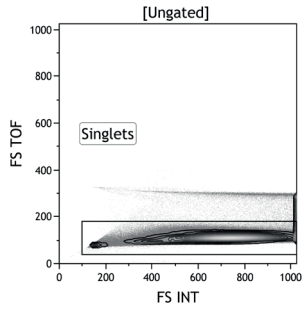


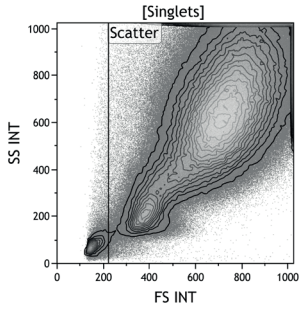


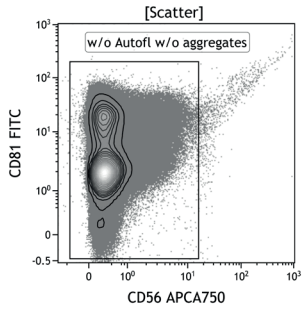
The sequence of "oversized-narrowed" gating is supported by an effective gate purification strategy

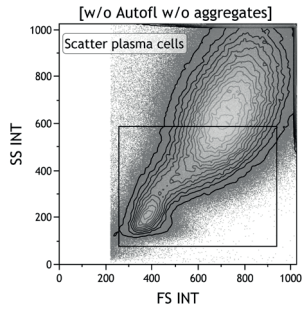
The gates in plots 6-8 discarding CD45hiCD56+, CD45hiCD19neg and CD45hiCD38low events allow for high purity of the "oversized-narrowed" gating steps that enable high recovery.

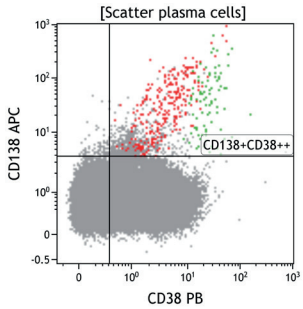
Below the complete analysis for this example of CD38low PCs with less pronounced deviation from the normal phenotype. Please note the weak CD56 expression and heterogeneous CD27 expression on abnormal PCs that highlights the utility of multivariate analysis (6 potentially differentially expressed antigens) to discriminate normal vs deviating phenotype, including CD200 belonging to the group of antigens with the highest incidence of aberrant expression on abnormal PCs[4].

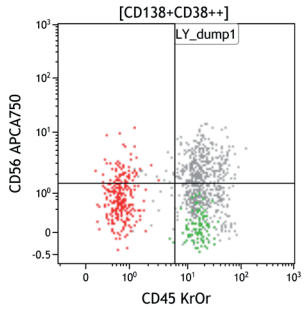


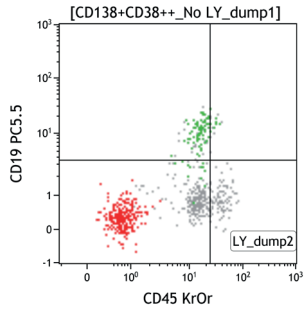


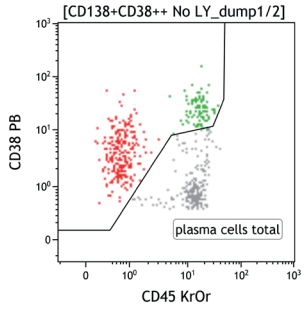


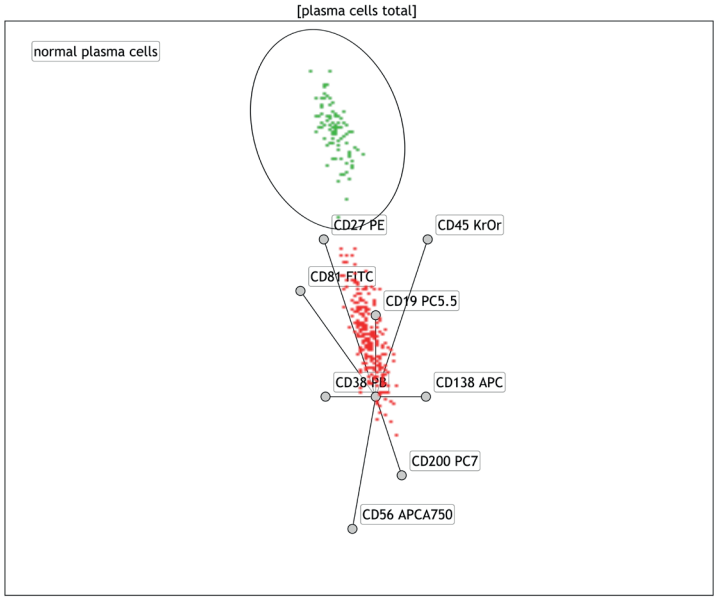


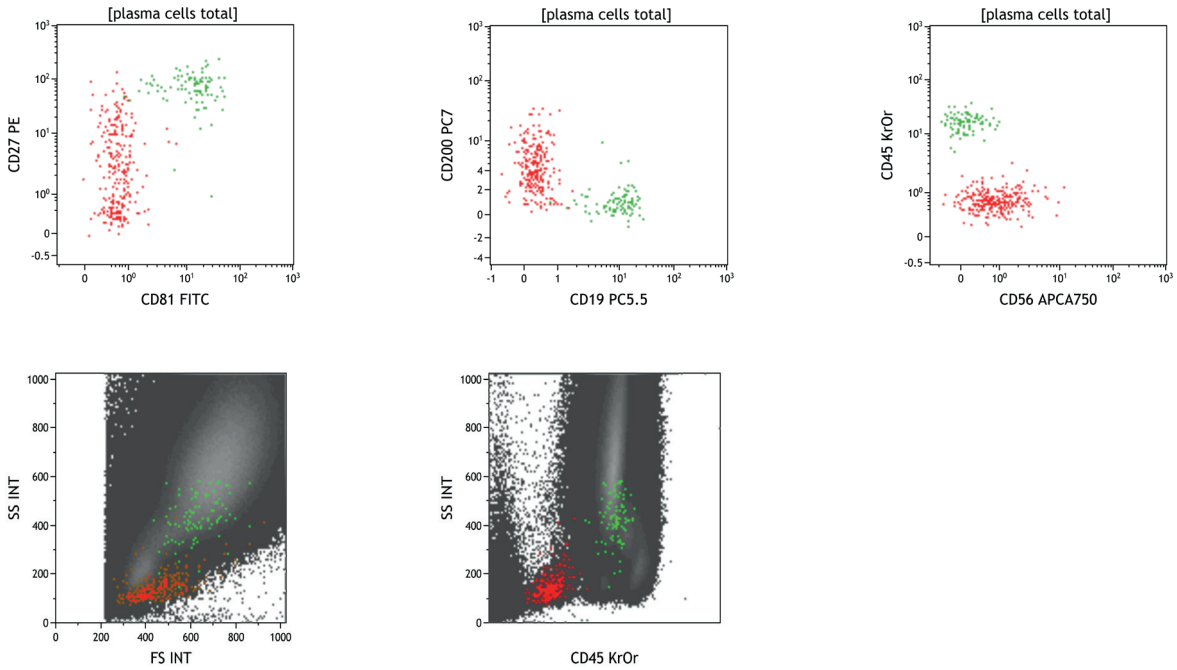












GATE	NUMBER
CD45+	2,188,621
Normal plasma cells	94
Plasma cells with phenotype deviating from normal	220

The results can be displayed using an Information plot: in the shown example, abnormal plasma cells have been detected at a frequency of 0.01% CD45+.

Conclusions

Rare event cytometry faces the challenge of abundancy of non-target events and their heterogeneity that may overlap with hallmark features of the target cells. A highly discriminative antibody panel coupled with a high recovery gating strategy with robust plausibility checks and effective gate purification steps enables flow cytometry to overcome this challenge.

Notes

The results shown here represent data generated on the Beckman Coulter Navios Flow Cytometer and were kindly provided by Dr. Agnieszka Blum from her work at the Stem Cell Facilities at the Charite Berlin. The described Kaluza analysis protocol is available for download:

https://www.beckmancoulter.com/coulter-flow-cytometry/duraclone-rare-event-solutions

References

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3. **Leucocyte Typing VII**. David Mason (Editor) et al. Oxford University Press (June 15, 2002)

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