Spectral cell sorting

Identifying and sorting mouse progenitor cells with spectral flow cytometry

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Abstract

Spectral flow cytometry is a novel technology that enables expansion of panels for high dimensional analysis by unmixing the spectral signature of highly overlapping dyes. Thus, we can now identify more markers and rare subpopulations than ever before. Here we highlight practical considerations for leveraging spectral flow cytometry to identify and sort elusive cells, making use of novel fluorophores that significantly streamline the workflow for this high-dimensional approach to cell population interrogation. Additionally, we used a 26-color mouse hematopoiesis panel to illustrate new software capabilities that simplify spectral workflow. Spectral panel design and spectral control visualization improvements allow the user to adjust the positive population for unmixing as preferred. This panel illustrates various stages of mouse hematopoiesis in different lymphoid tissues and allows for sorting of rare subpopulations with high efficiency and purity using the Invitrogen™ Bigfoot™ Spectral Cell Sorter.

Materials and methods

Bone marrow (BM) and spleen (SP) cells from BALB/c were isolated by standard methods. Cells were stained in Invitrogen™ eBioscience™ Flow Cytometry Staining Buffer, Invitrogen™ Super Bright™ Complete Staining Buffer and Invitrogen™ CellBlox™ Blocking Buffer were used to prevent non-specific polymer interactions and non-specific binding, respectively. Invitrogen™ LIVE/DEAD™ Fixable Olive viability dye was used to clearly distinguish live from dead/dying cells. Following incubation with the viability dye cells were fixed in 2% formaldehyde (BioLegend). The antibodies used in this panel are listed in the table below. Samples were collected and sorted on a 6-Laser Bigfoot Spectral Cell Sorter.

For spectral unmixing controls, Invitrogen™ Ultracomp eBeads™ Plus Compensation Beads were used for most markers. The exceptions for which either bone marrow cells or spleen cells were used are highlighted in yellow on the table below.

Data analysis was performed both with FlowJo™ v10.8.1 software (BD Biosciences) and Sasquatch Software (SQS).

The aim with this experiment was to demonstrate Bigfoot Spectral Cell Sorter capabilities by sorting rare progenitor subpopulations with high efficiency and purity while not compromising the resolution of mature populations by leveraging spectral panel building and avoiding the use of a “dump” channel.

Table 1. 26-color mouse hematopoiesis panel showing antigens/antibody clone/dye pairings and what was used as single-color controls for unmixing our spectral experiment.

26-Color mouse hematopoiesis panel			
Fluorophore	**Antigen**	**Clone**	**Unmix Control**
BUV395	CD16/32	2.4G2	Beads
BUV496	CD2	RM2-5	Beads
BUV563	CD44	IM7	BM cells
BUV615	CD11c	HL3	Beads
BUV661	CD127	A7R34	Beads
BUV805	B220	RA3-6B2	SP cells
BV421	CD34	RAM34	Beads
eF450	CD21	eBio4E3	Beads
BV510	CCR2	475301	Beads
BV570	CD8	53-6.7	Beads
SB600	Ly6G	1A8-Ly6g	BM cells
SB645	I-A/I-E	M5/114.15.2	SP cells
SB702	IgD	11-26c	Beads
SB780	CD11b	M1/70	Beads
BV480	CD62L	MEL-14	Beads
FITC	TER-119	TER-119	Beads
L/D Olive	Dead cells		SP cells
NFB660-120S	CD4	GK1.5	Beads
RB780	CD3	17A2	Beads
PE	Sca-1	D7	Beads
PEeF610	IgM	II/41	SP cells
PE-Cy5	CD117 (c-kit)	2B8	Beads
PE-Cy5.5	CD49b	DX5	Beads
PE-Cy7	CD24	M1/69	BM cells
NFR700	CD45	30-F11	SP Cells
APCeF780	Ly6C	HK1.4	BM Cells

Results

Figure 1. Lineage tree with general hematopoiesis scheme showing populations that were successfully sorted (in green) and or identified in this experiment (in blue).

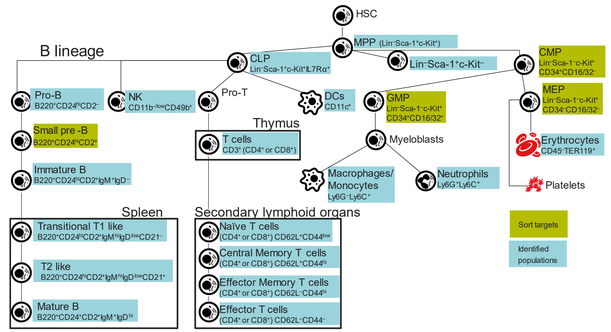
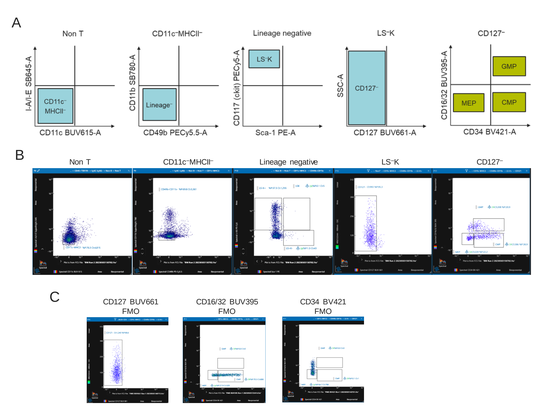


Figure 2. Data cleanup to exclude doublets, dead cells, erythrocytes and mature populations in the bone marrow. A) Gating scheme representation. **B)** 2D plots as observed in SQS during sorting experiment.

Figure 3. Further data cleanup excluding lineage markers in the bone marrow, followed by gating hematopoietic progenitors. A) Gating scheme representation, in blue indicates gate progression and in green sort targets. **B)** Actual plots as observed in SQS during sorting experiment. **C)** Example of fluorescence minus one (FMO) controls used for gating.



Results (continued)

Figure 4. Sorting statistics and post-sort purity check. 4 sorted populations: megakaryocyte-erythroid progenitor (MEP); granulocyte-monocyte progenitor (GMP); common myeloid progenitor (CMP); small Pre-B cells. A) Number of total cells sorted or aborted. **B)** Sorting efficiency. **C) and D)** Post-sort purity check. The gates were not adjusted for the post-sort purity assessment. **C)** Granulocyte-monocyte progenitors (GMP) sorted purity check**. D)** Common-myeloid progenitors (CMP) sorted purity check.

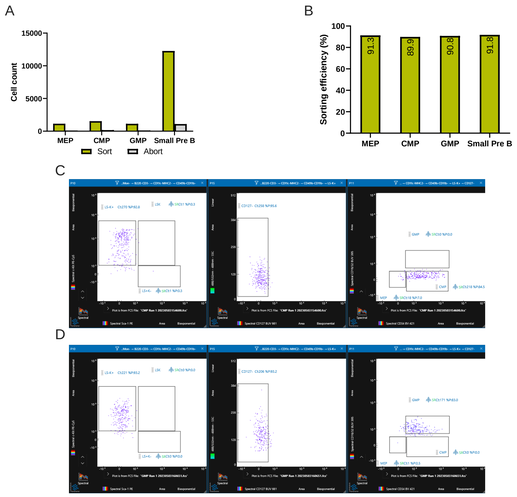
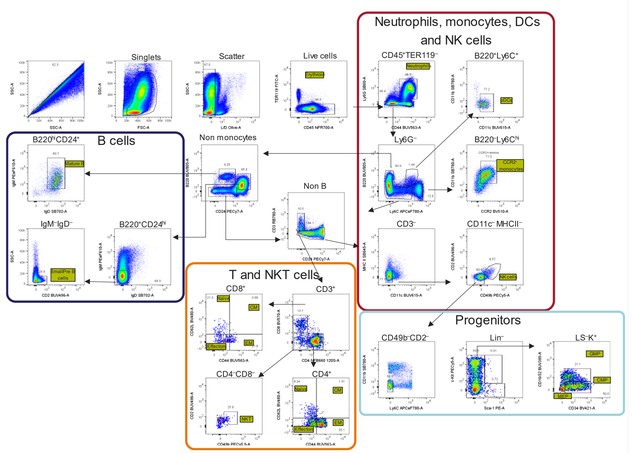
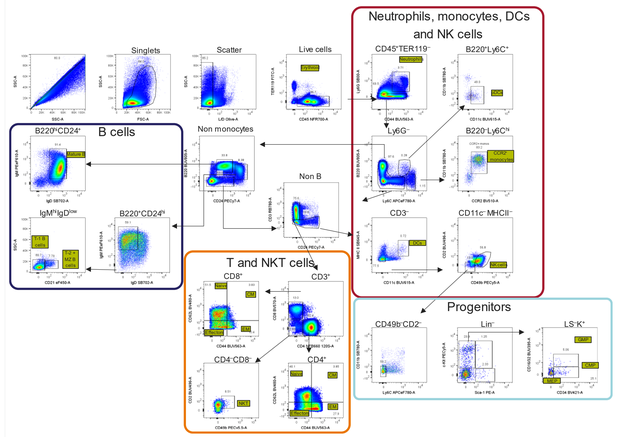


Figure 5. Bone marrow overview with all subpopulations identified in this experiment.



Results (continued)

Figure 6. Spleen overview with all subpopulations identified in this experiment.



Conclusions

- We have demonstrated successful sorting of rare progenitor cells with high efficiency and purity while allowing for the identification of 23 total cell subpopulations using the Bigfoot Spectral Cell Sorter.
- Further directions:
  - Expand/adapt the panel to include more markers leading to a backbone that could be used for interrogation of different subpopulations.
  - Sort directly into sequencing chips.

Acknowledgements

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