You’re in the right place.

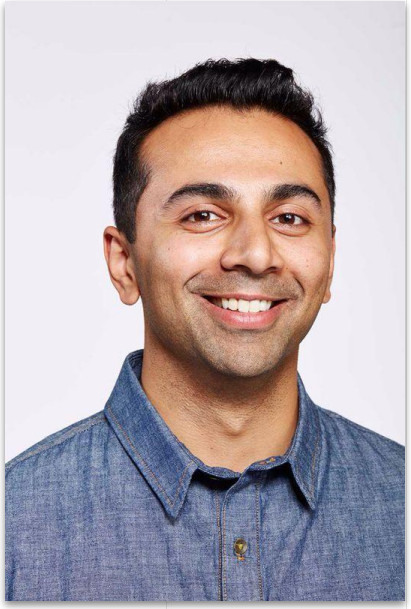
Read more about Teiko’s spectral flow service @
teiko.bio/spectral-flow-cytometry

Teiko.bio

Fresh versus fixed comparison

25-marker spectral flow cytometry

High-parameter cytometry for clinical trials



Ramji Srinivasan
Teiko CEO

Problem

The drug developer’s dilemma

20%

80%

Peripheral Blood Mononuclear Cells (PBMC)	Fixed	Live
$200-$300; missing granulocytes; 89% (13%-100%)* viability	Fixation not yet optimized for high-dimensional cytometry	10-20% failure rate

Does a fixed whole blood sample look similar to a fresh one run on spectral flow?

Method

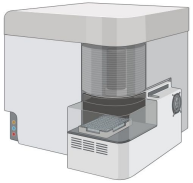
3 donors, whole blood; two aliquots per donor

Fresh

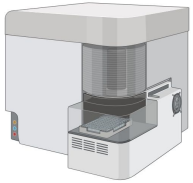
Stable Lyse, Stable Store fixation

Stained

Stained

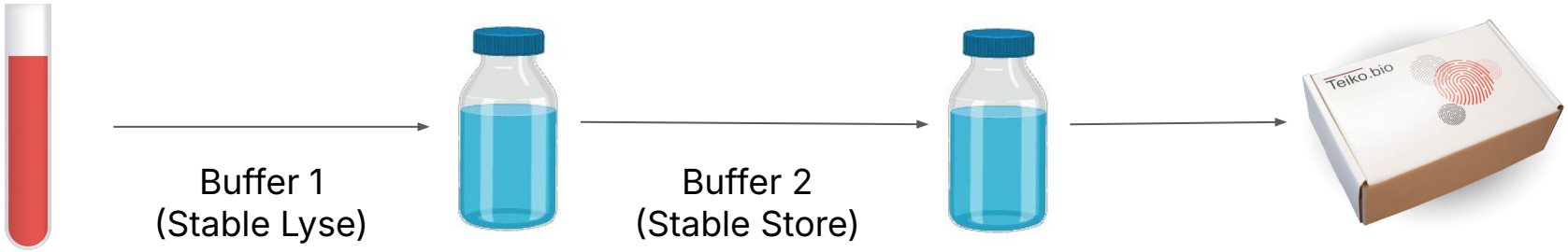


Cytek Aurora



Cytek Aurora

TokuKit collection process



“Pre-fixation”

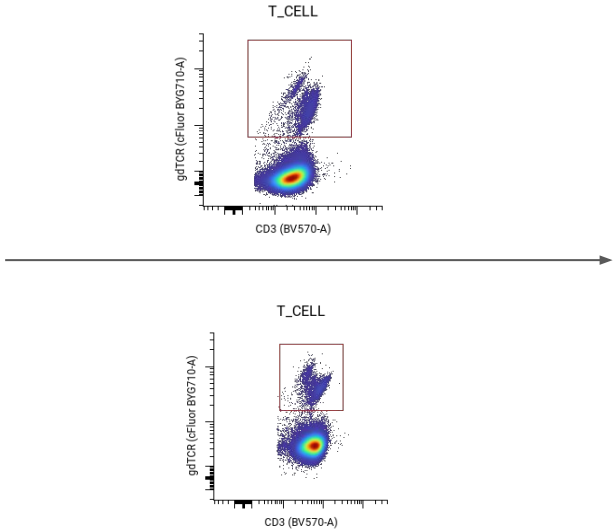
Target	Fluorophore	Fresh	Fixed
CD66b	Spark UV® 387	x*	✅
CD25	BUV661	✅	✅
CD127	BUV737	✅	✅
CD3	BV570	✅	✅
CD28	BV650	✅	✅
CD38	BV711	✅	✅
CD56	BV750	✅	✅
IgM	BV510	✅	✅
PD-1	BV785	✅	✅
CD45RA	cFluor® V450	✅	✅
CD20	cFluor® V547	✅	✅
CD141	cFluor® B515	✅	✅
CD8	cFluor® B532	✅	✅
CD14	cFluor® B548	✅	✅
HLA-DR	cFluor® B690	✅	✅
CD16	cFluor® BYG610	✅	✅
IgD	cFluor® BYG667	✅	✅
CD4	cFluor® YG584	✅	✅
CD11c	cFluor® BYG781	✅	✅
CD1c	cFluor® R668	✅	✅
CD19	cFluor R685	✅	✅
CD123	cFluor R720	✅	✅
CD45	cFluor R780	✅	✅
CD27	cFluor R840	✅	✅
gdTCR	cFluor BYG710	✅	✅

* Neutrophil exclusion on fresh samples is done via scatter plot. During the fixation protocol, side scatter information is largely lost. Therefore we use the common neutrophil marker CD66b on fixed samples to exclude neutrophils.

What we compared

Fresh

Fixed

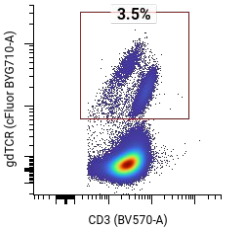
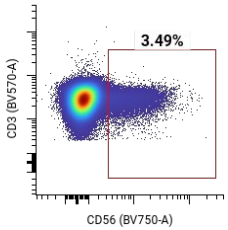
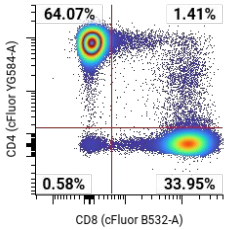
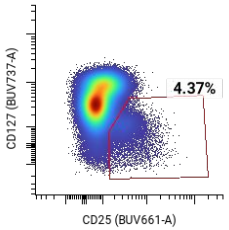
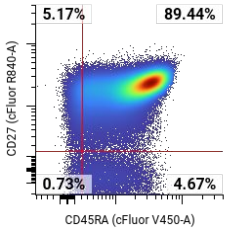
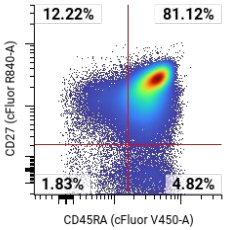
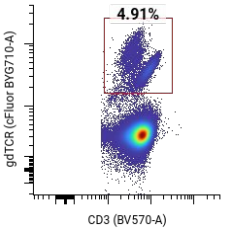
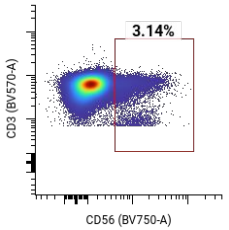
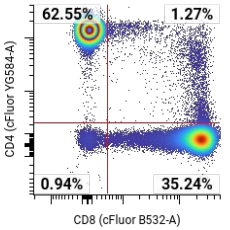
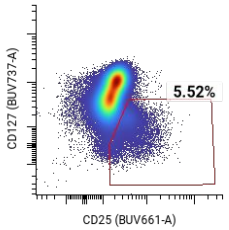
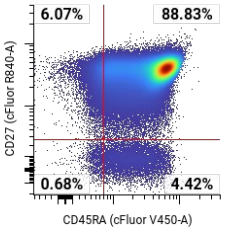
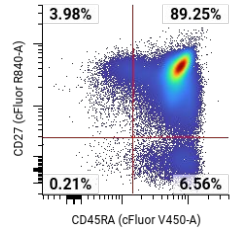


Compared 36 phenotypic populations, and 62 state marker subtypes (i.e. PD-1 in CD4)

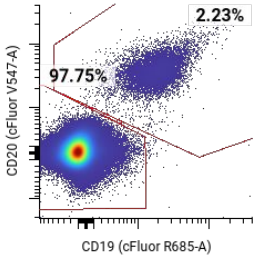
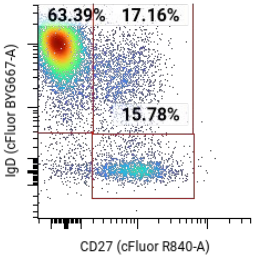
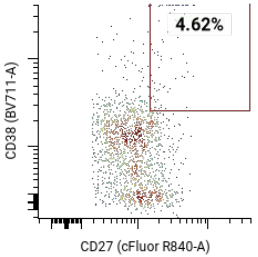
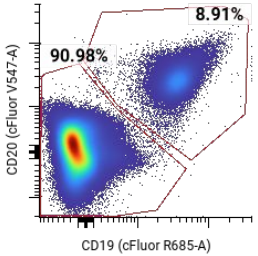
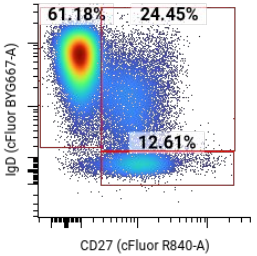
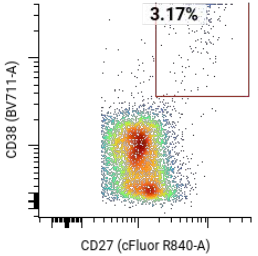
Measures: correlation of population frequencies between fresh and fixed

Results

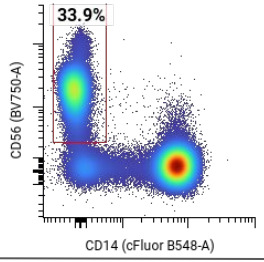
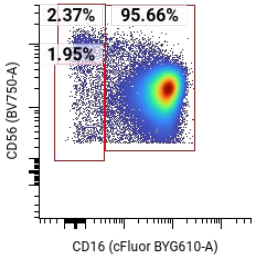
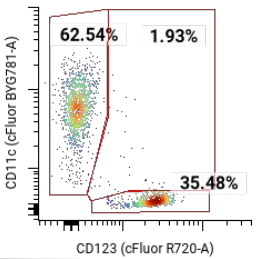
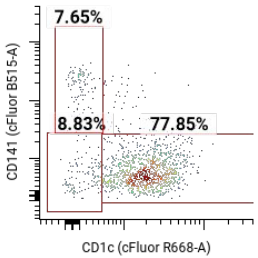
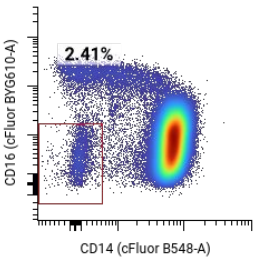
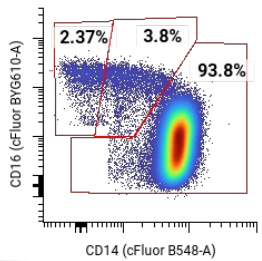
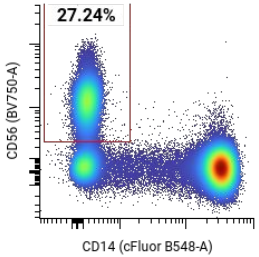
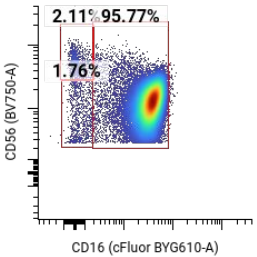
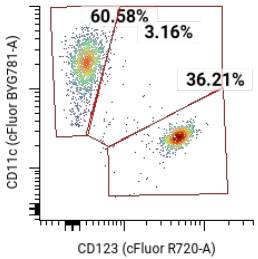
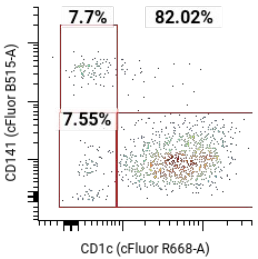
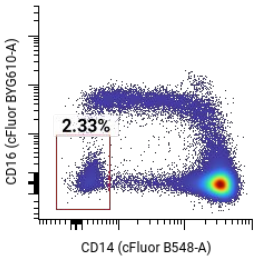
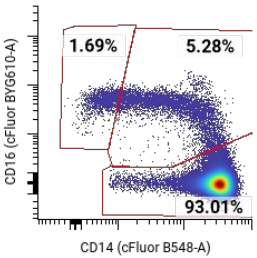
How different do plots look like from samples stained live versus fixed? Let's start with T cells.

	Gamma delta T-cells	CD56⁺ T-cells	CD4⁺ & CD8⁺ T-cells	T-regs	CD4⁺ subsets	CD8⁺ subsets
Live						
Fixed						

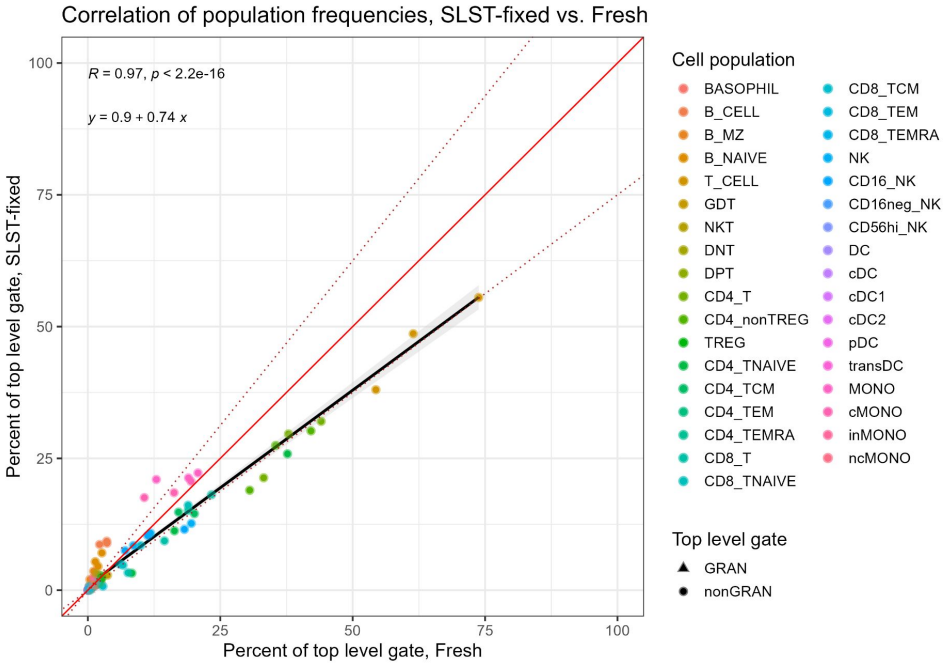
How about B cells?

	Total B-cells	B-cell subsets	Plasma-blasts
Live			
Fixed			

NK? DCs? And monocytes?

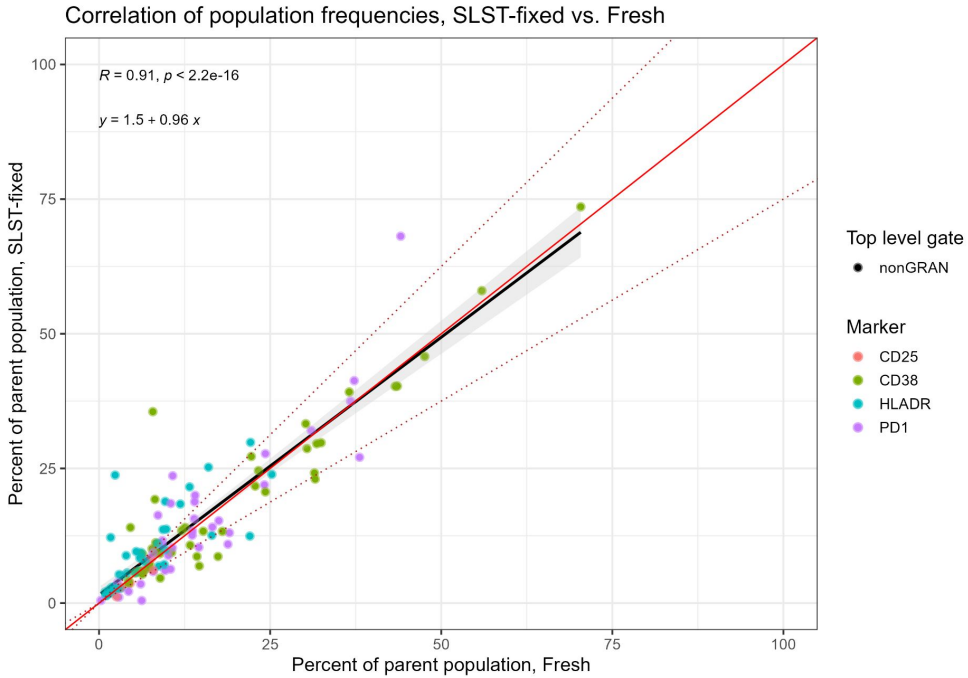
	Total NK-cells	NK-cell subsets	Total DCs	DC subsets	Total monocytes	Monocyte subsets
Live						
Fixed						

Overall, how do the two approaches correlate?



Overall correlation of population frequencies between live and fixed sample processing: **0.97**

What about state markers?



Overall correlation of state marker-positive subset frequencies between live and fixed sample processing: **0.91**

In sum

The drug developer’s dilemma resolved

20%

80%

Peripheral Blood Mononuclear Cells (PBMC)	Fixed	Live
$200-$300; missing granulocytes; 89% (13%-100%)* viability	TokuKit	10-20% failure rate

Does a fixed whole blood sample look similar to a fresh one run on spectral flow?

Yes!

What this enables

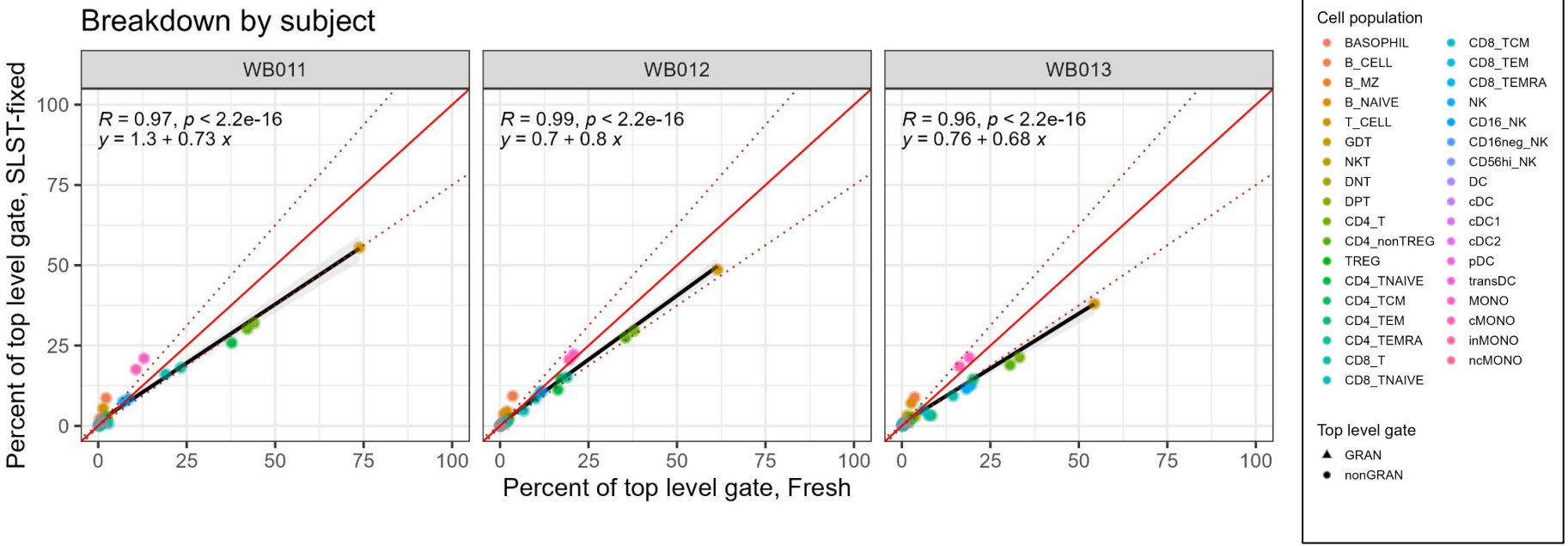
1. **10-20X reduction of failure rate through centralization of flow analysis**
No need for a flow lab in every country, city, clinical site. Reduce variability by having all samples processed by the same facility.

2. **Enables batching of samples**
Conveniently store fixed samples until a whole set (for example all timepoints of an individual patient) is complete.

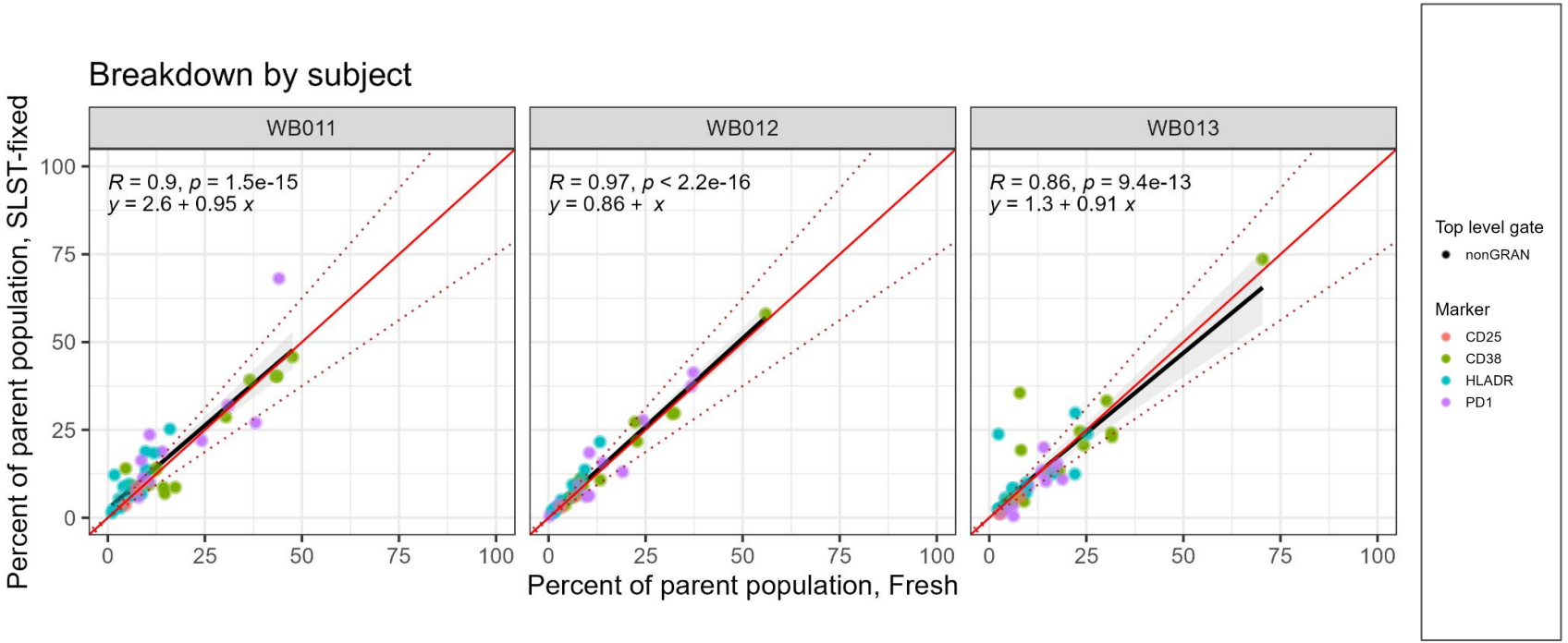
3. **Reduce committed processing cost and expand analysis window**
Not sure which samples to analyze? No problem. Store samples at -80C until you're ready to decide which samples to analyze.

FAQ

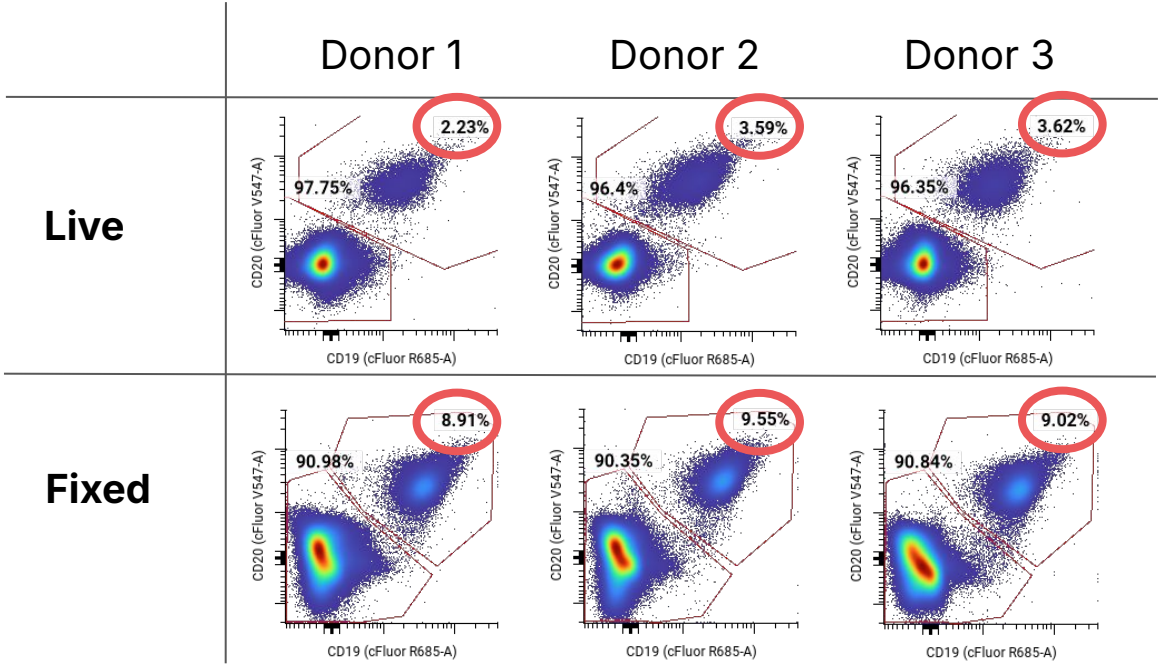
Subject level breakdown: phenotypic markers



Subject level breakdown: state markers



Where do we see differences? Total B-cells, for example.



Short answer:
We do see consistent differences for certain immune population frequencies when comparing samples processed live versus fixed.

The biological relevance of this is an area of active investigation