



“Why not both?” A semi-supervised pipeline for a comprehensive and scalable analysis of immune heterogeneity in human samples

David Rach, Ngina Nampota-Nkomba, Godfrey Mvula, Felix A. Mkandawire, Oswald M. Nyirenda, Winter A. Okoth, Daniela Franco, Andrea G. Buchwald, Franklin R. Toapanta, Marcelo B. Sztein, Miriam K. Laufer, **Kirsten E. Lyke, Cristiana Cairo.**

University of Maryland School of Medicine

October 28, 2026



Slides



Spectral Flow Cytometry is amazing!!!

- ❑ Capable of resolving remarkably similar fluorophores → **increased number of markers per panel** compared to conventional flow cytometry (CFC)
- ❑ Allows for rapid acquisition of millions of events in a few minutes → typically **greater number of acquired cells per sample** compared to mass cytometry (MC)
- ❑ Spectral Flow Cytometry (SFC) datasets have an unique combination of **width and depth** compared to preceding technologies



Spectral Flow Cytometry is amazing!!!



- Able to efficiently acquire greater number of cells → more thorough profiling of **rare cell subsets**

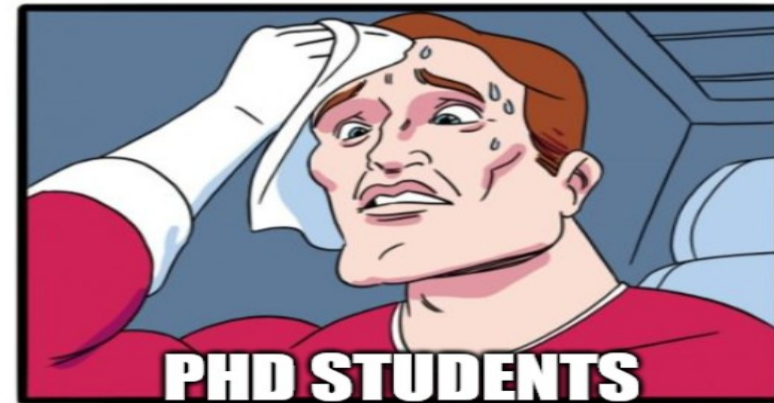
- Build one panel, evaluate multiple cell types → invaluable for studies with **limited biospecimen**

- Expand our knowledge about immune system heterogeneity

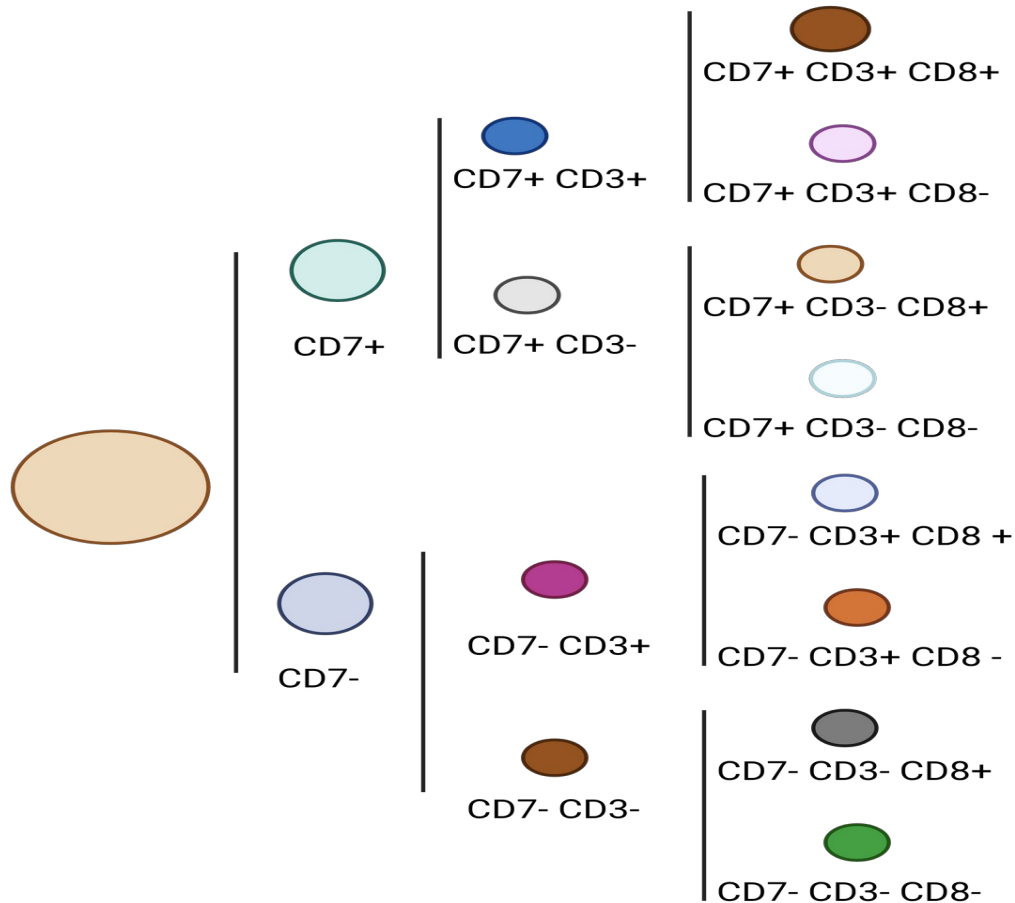
Trade-off: Increasing analytical complexity

□ If you are only interested in a **single population** of cells → analysis process remains essentially unchanged (manual gating with a few more gates)

- If your goal is to characterize **all cell populations** that may be present →
- the breadth and depth of the datasets complicate the analysis



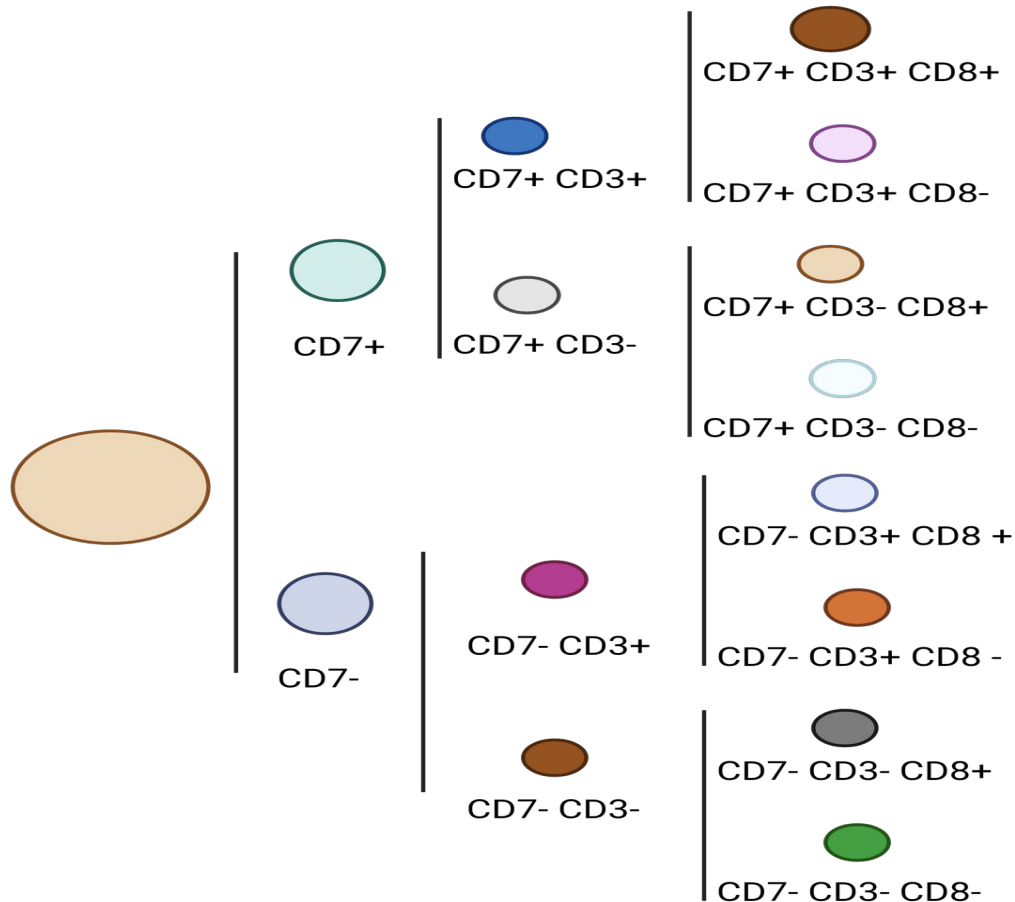
Complex Datasets: Combinatorially Exponential Clusters



- If for a given marker in your panel, you **split** the cells into **positive** and **negative** populations
- And this splitting process was **repeated for every marker** in your panel
- And at the end, we **grouped cells** that only **expressed the same markers**

• “Groups” per panel = $2^{\text{Number of Fluorophores}}$

Complex Datasets: Combinatorially Exponential Clusters



- If for a given marker in your panel, you **split** the cells into **positive** and **negative** populations
- And this splitting process was **repeated for every marker** in your panel
- And at the end, we **grouped cells** that only **expressed the same markers**

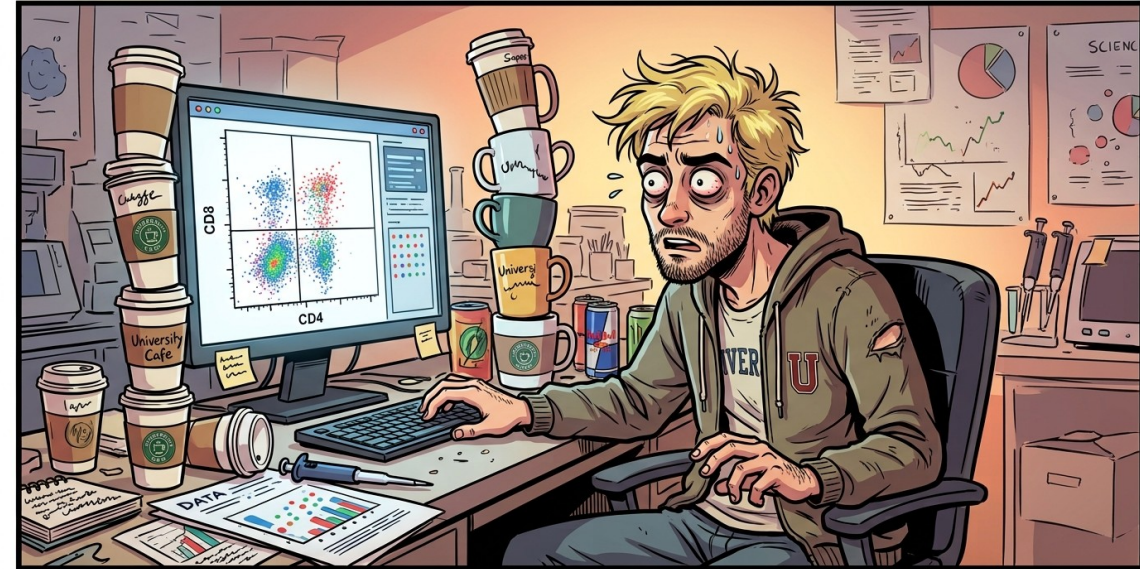
- **9-color** CFC panel → 512 groups at most
- **40-color** SFC panel → 1.1e+12 groups (**trillion**)
- **60-color** SFC panel → 1.2e+18 groups (**quintillion**)

What even is a quintillion?

If I were to manually check for every combination...

- **60-color SFC panel** → **$1.2e+18$ groups (quintillion)**

- 1 gate, every 10 seconds → 6 groups/minute
- 60 minutes in an hour → 360 groups/hour
- Awake 16 hours a day → 5760 groups/day
- 365 days per year → **2,102,400 groups/year**



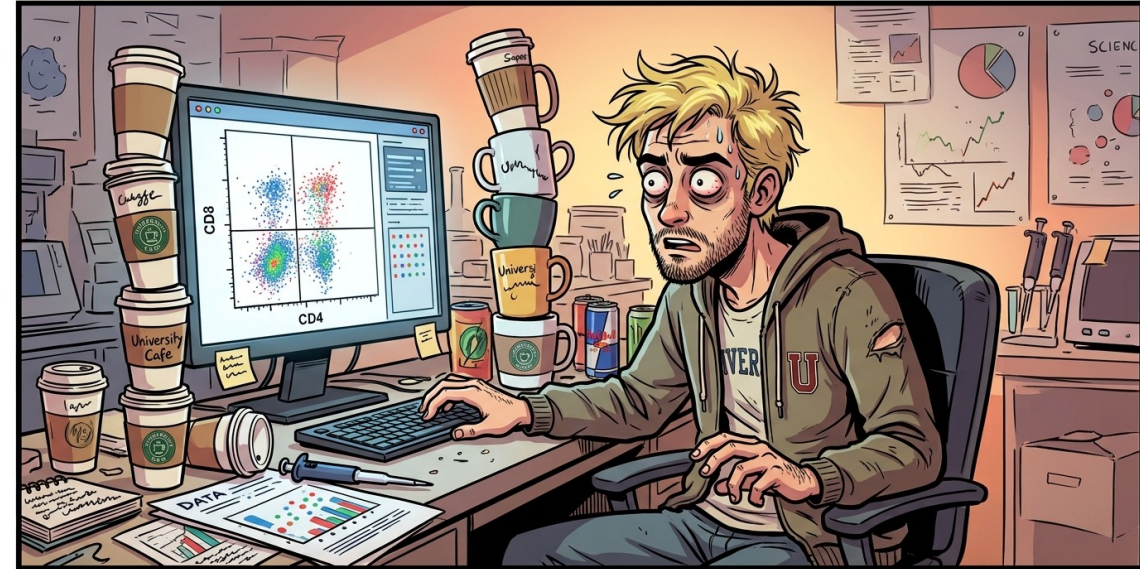
What even is a quintillion?

If I were to manually check for every combination...

- **60-color SFC panel** → **$1.2e+18$ groups (quintillion)**

- 1 gate, every 10 seconds → 6 groups/minute
- 60 minutes in an hour → 360 groups/hour
- Awake 16 hours a day → 5760 groups/day
- 365 days per year → **2,102,400 groups/year**

....Not even close....



Continuously since the Big Bang



- **60-color SFC panel** → **$1.2\text{e}+18$ groups (quintillion)**

- 365 days per year → **2,102,400 groups/year**
- Invent a time-machine, become immortal
- 13.8 billion years → **$2.9\text{e}+16$ groups/reality**

Continuously since the Big Bang



- **60-color SFC panel** → **$1.2\text{e}+18$ groups (quintillion)**

- 365 days per year → **2,102,400 groups/year**
- Invent a time-machine, become immortal
- 13.8 billion years → **$2.9\text{e}+16$ groups/reality**

- So we need ~ 50 immortal researchers working continuously since the Big Bang just to check all group combinations for a **single 60-fluorophore panel .fcs file**

Continuously since the Big Bang



- **60-color SFC panel** → **$1.2e+18$ groups (quintillion)**

- 365 days per year → **2,102,400 groups/year**
- Invent a time-machine, become immortal
- 13.8 billion years → **$2.9e+16$ groups/reality**

- So we need ~ 50 immortal researchers working continuously since the Big Bang just to check all group combinations for a **single 60-fluorophore panel .fcs file**

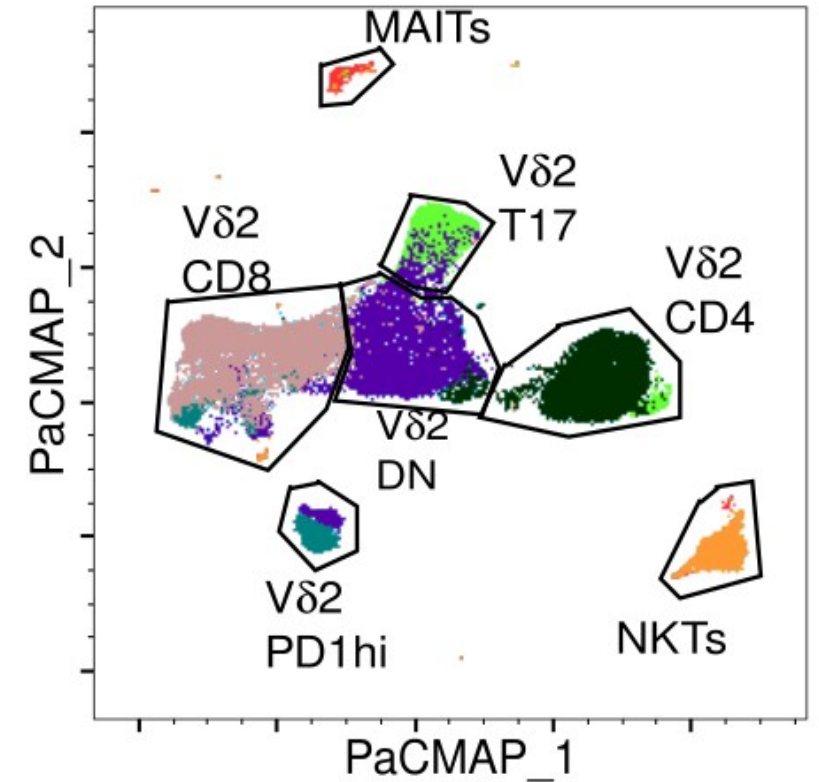
1 file down, 99 more to go!

Alternative: Unsupervised Analysis (Algorithms)

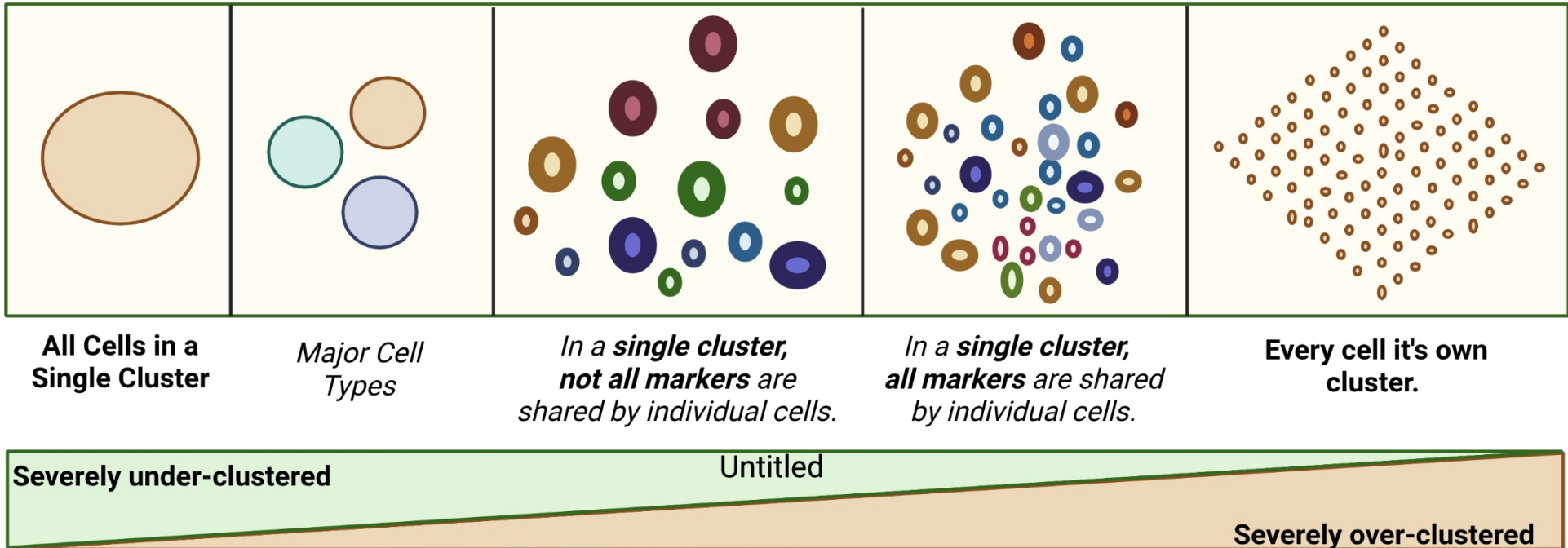
- Consequently, if the goal is to characterize all cell populations present, some form of **unsupervised (algorithm) or semi-supervised** analytical approaches will be needed

- **Clustering** → FlowSOM, Phenograph, Xshift, etc.
- **Dimensionality visualization** → opt-SNE, UMAP, PaCMAP, Phate, etc.

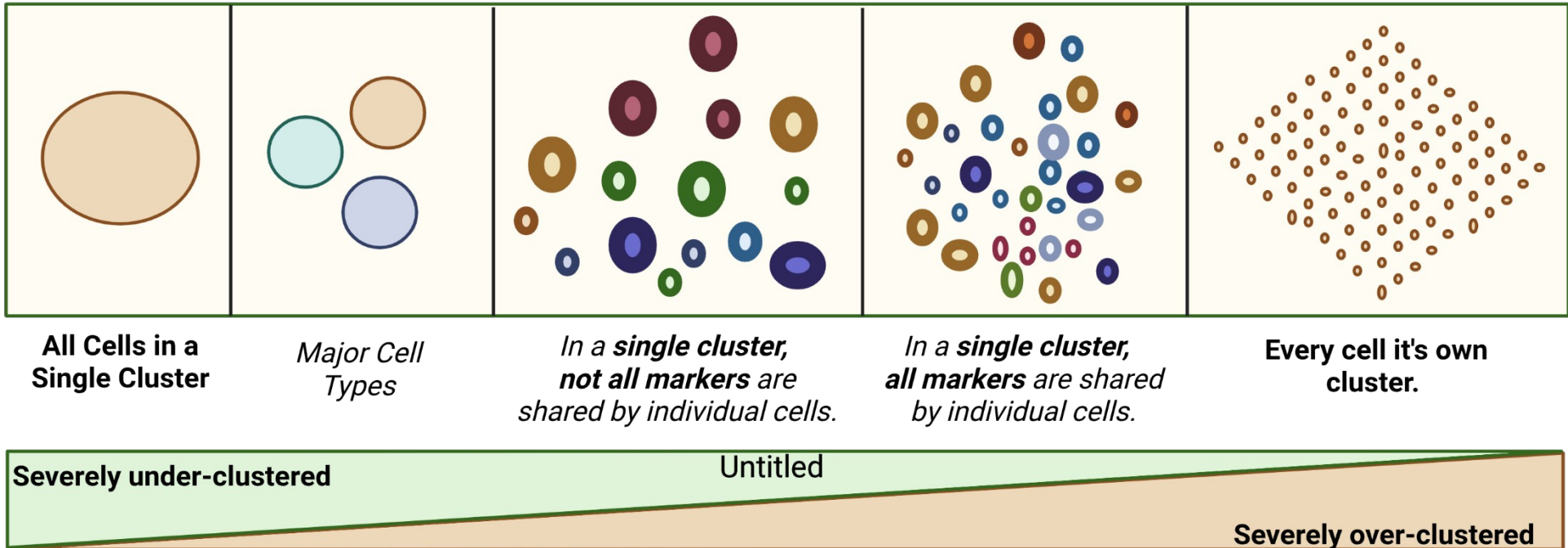
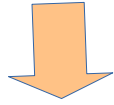
- **Automated Gating** → openCyto, flowMagic, ElasticGate, etc.



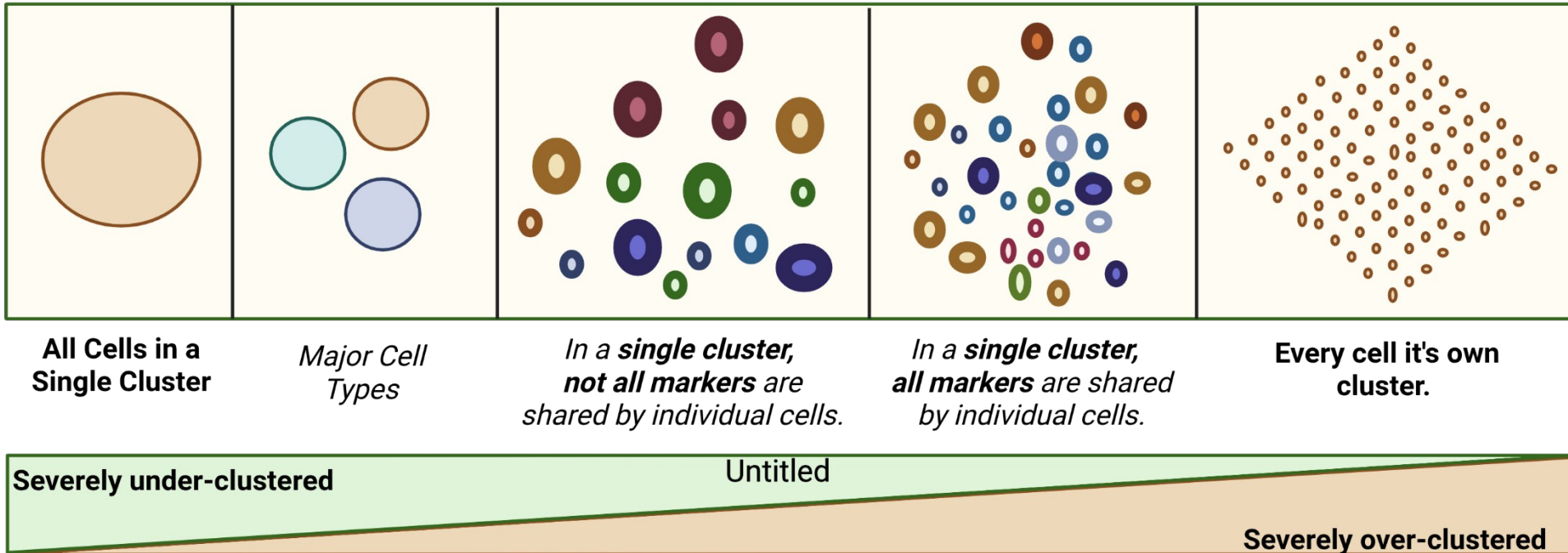
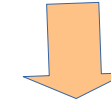
The challenge for unsupervised approaches



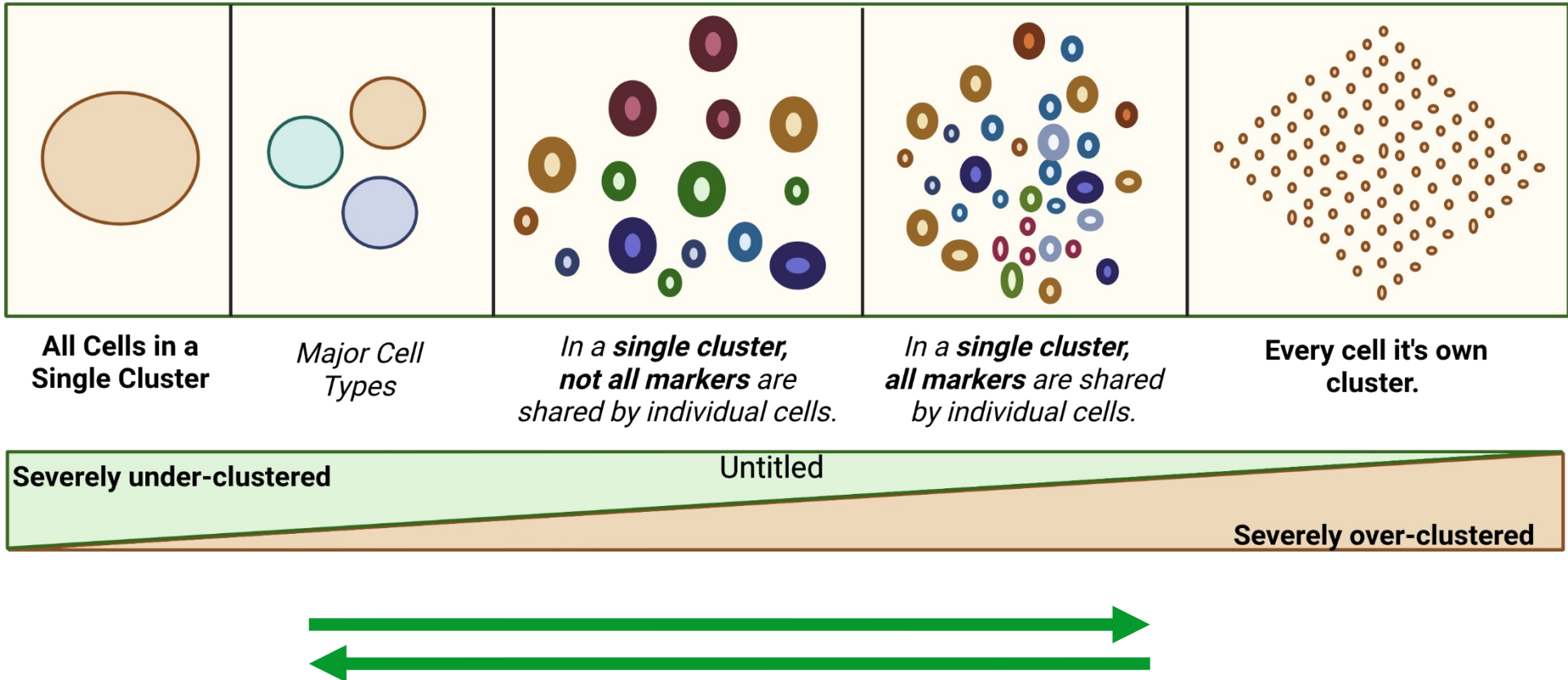
The challenge for unsupervised approaches



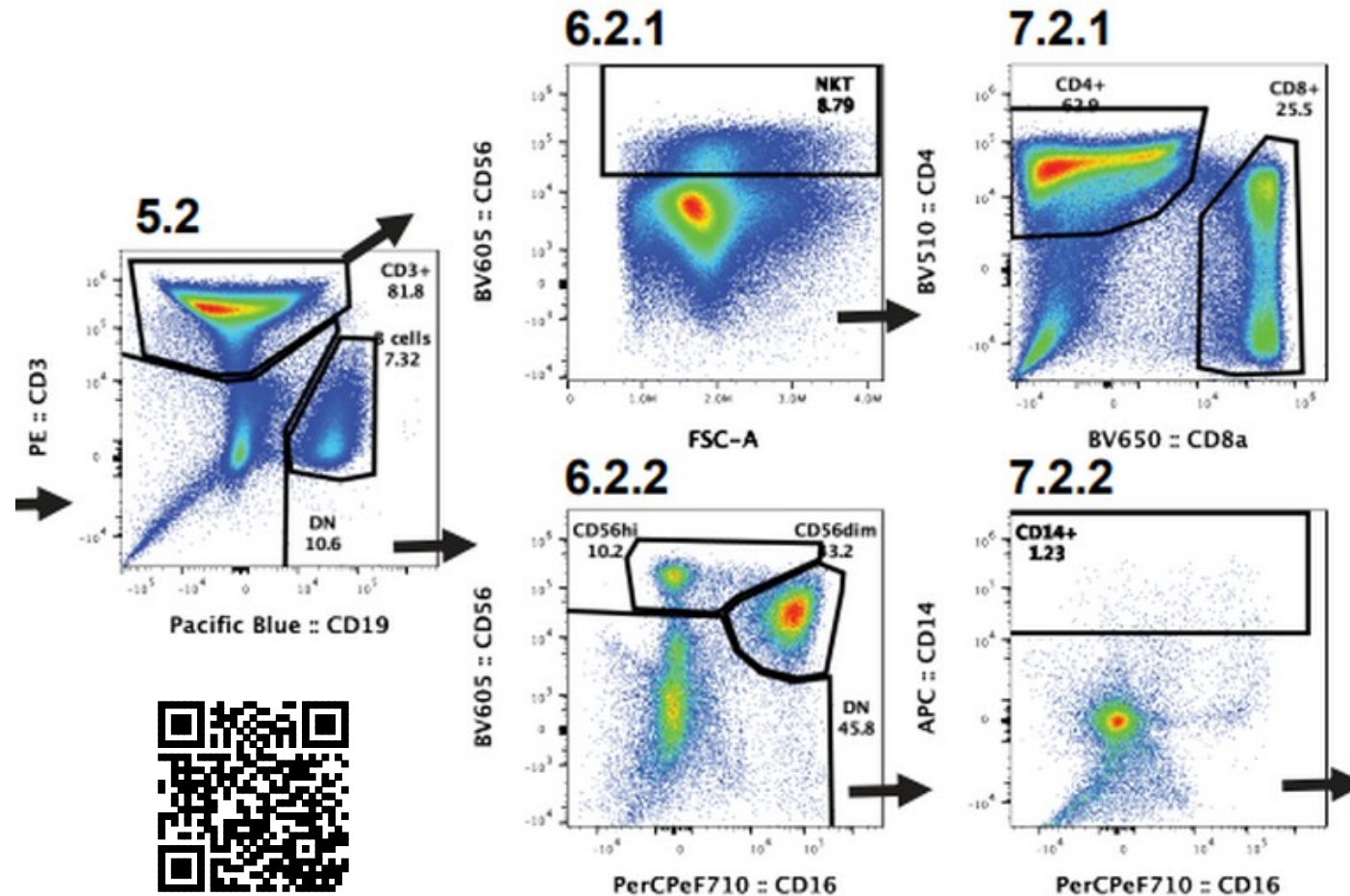
The challenge for unsupervised approaches



The challenge for unsupervised approaches



Many algorithmic tools often struggle with SFC data's quirks

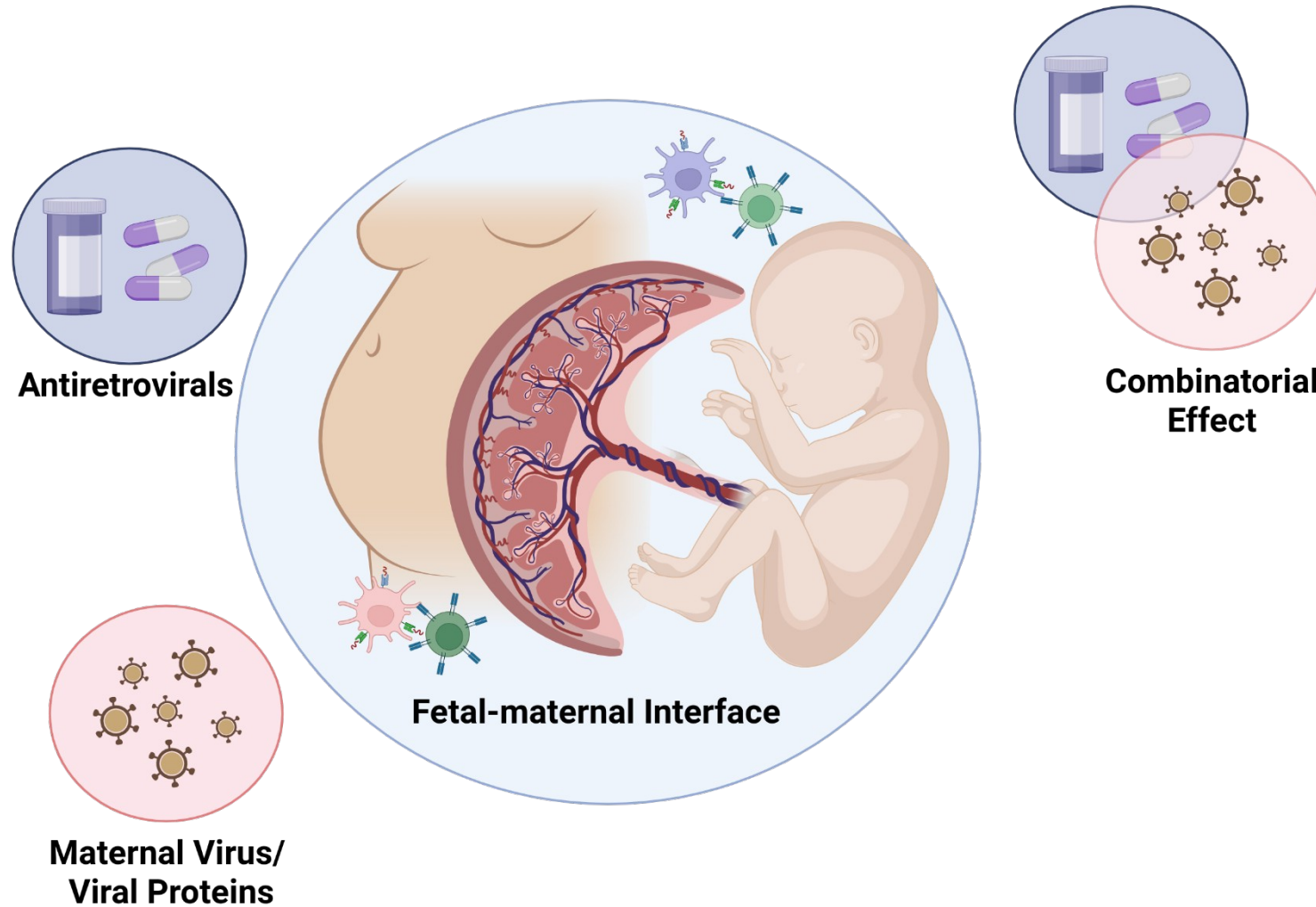


Full biological discovery potential often out of reach



- For **most** spectral flow cytometry datasets, only a **handful of cell populations** end up being analyzed by any method

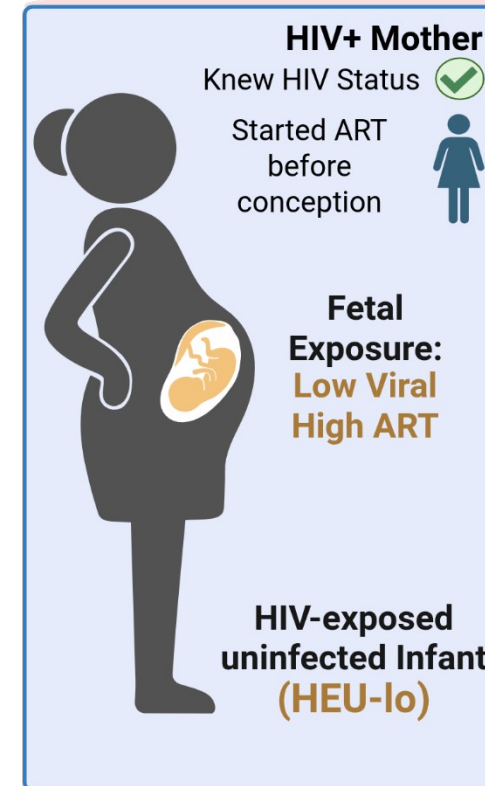
My PhD's focus: HIV-exposed Uninfected Infants



Our Malawian Cohort

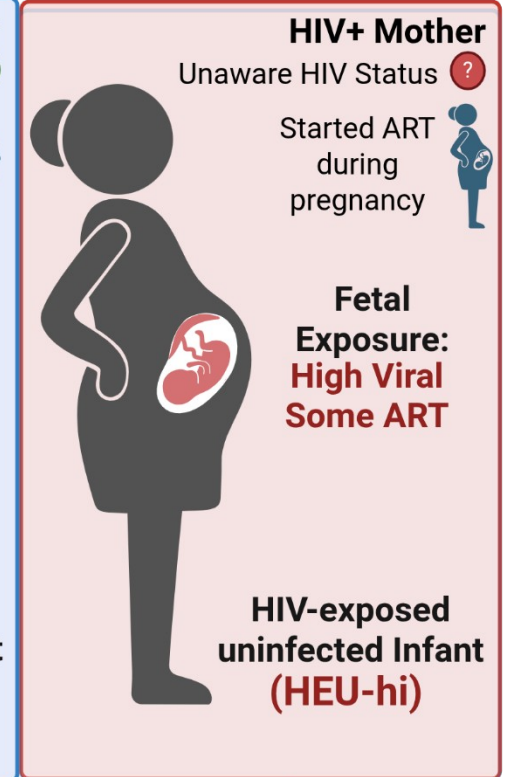


Maternal Viral Load:
Undetectable



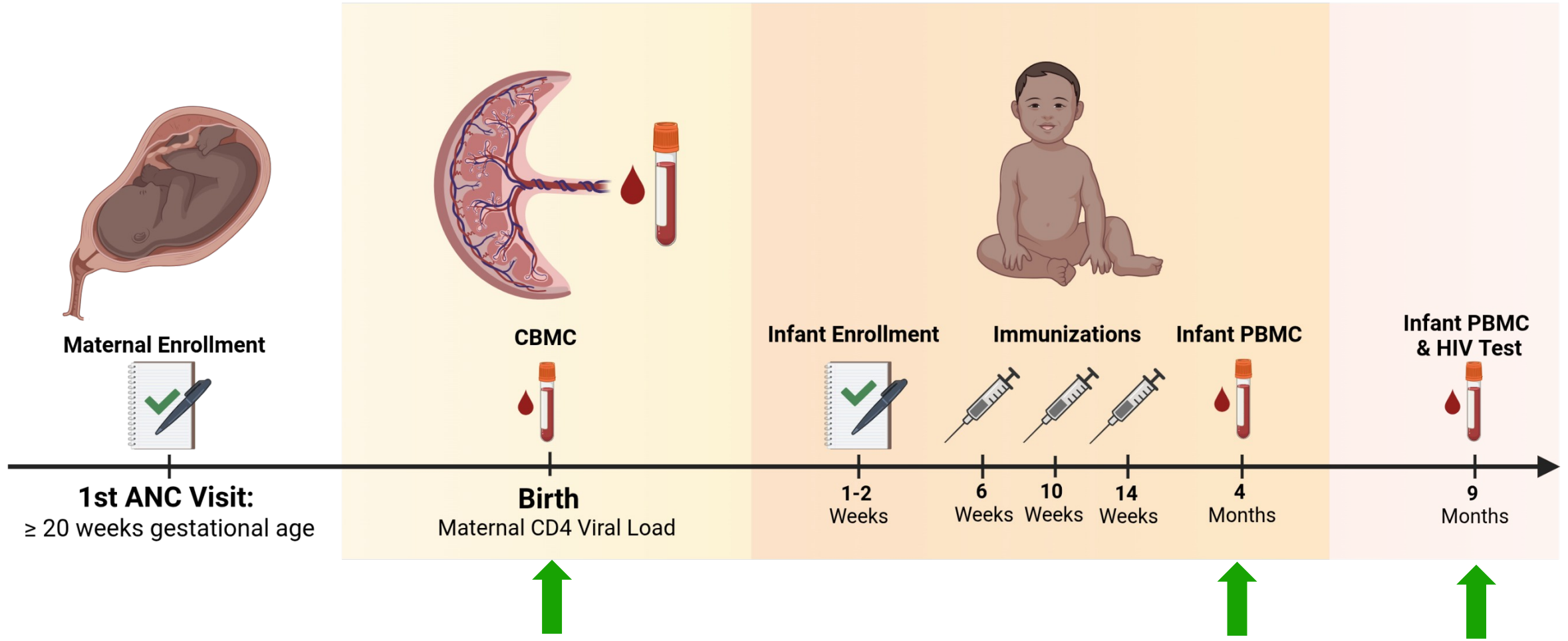
Fetal Antiretroviral Exposure: Extended

Maternal Viral Load:
Very High



Fetal Antiretroviral Exposure: Limited

Study Timeline



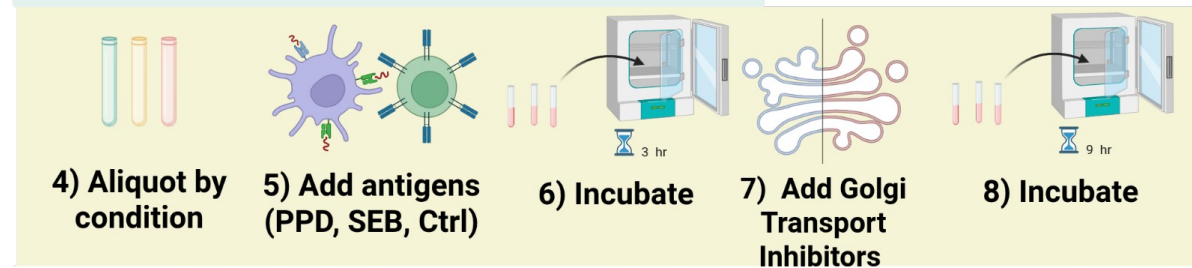
32-Fluorophore Spectral Flow Cytometry Panel

UV1	Spark UV 387		
UV2	BUV395	CD62L	(SK11)
UV6	cFluor UV440		
UV7	BUV496		
UV9	BUV563	CD69	(FN50)
UV10	BUV615	CCR4	(1G1)
UV11	BUV661	Vβ2	(B6)
UV14	BUV737	CD38	(HB7)
UV16	BUV805	CD4	(SK3)
V1	BV421/		
V2	Spark Violet 423		
V3	Pacific Blue	CD14	(M5E2)
V3	Pacific Blue	CD19	(SJ25C)
V4	SYTOX Blue		
V5	BV480	CD161	(HP-3G10)
V6	SBV515		
V7	BV510		
V8	BV570	CD16	(3G8)
V9	Orbit 585		
V10	BV605	CD45RA	(HI100)
V11	BV650	CD8	(RPA-T8)
V13	BV711	Vα7.2	(3C10)
V14	BV750	IFNγ	(B27)
V15	BV786	CCR6	(11A9)
V16	LD NIR 786		
B2	Alexa Fluor 488	FoxP3	(236A/E7)
B3	Spark Blue 550	CD3	(SK7)
B4	Spark Blue 574	CD45	(2D1)
B6	RB613	PD1	(EH12.1)
B7	NovaFluorBlue660		
B8	SBB675		
B9	NovaFluorBlue690		
B10	RB705	CD26	(M-A261)
B12	RB744/		
B13	NovaFluorBlue760		
B14	RB780	CXCR5	(RF8B2)
YG1	PE	ICOS	(ISA-3)
YG2	Spark YG 593		
YG3	PE-Dazzle 594	TNFα	(MAB11)
YG4	PE-Fire 640/		
YG5	PE-Cy5	CXCR3	(G025H7)
YG6	NovaFluorYellow690		
YG7	PE-Fire 700	CD127	(A019D5)
YG8	PE-Fire 744	CD25	(M-A251)
YG9	PE-Vio 770	HLA-DR	(REA805)
YG10	PE-Fire810		
R1	APC	CD39	(A1)
R2	Alexa Fluor 647	IL2	(MQ1-
R3	cFluor 685		
R4	APC-R700	CD107a	(H4A3)
R5	NovaFluorRed725		
R6	Zombie NIR	L/D	
R7	APC-Fire 750	CD27	(O323)
R8	APC-Fire 810	CCR7	(G043H7)

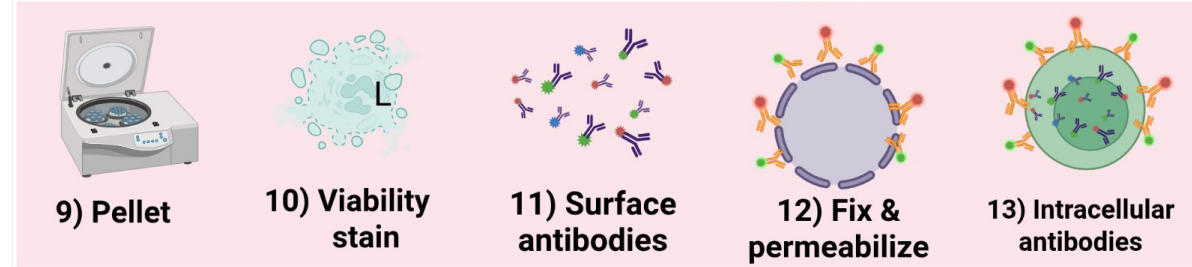
Thaw & Rest



Antigen Incubation



Flow Cytometry Staining



Personal Goal: No cell left behind



Repeated 3 AM thoughts

- ❑ What makes spectral flow cytometry data amazing is both the depth and the breadth
- ❑ How to fully profile the entirety of the acquired dataset, not just a single cell population of interest

Previously looked at unconventional T cells

Cord blood innate-like T cell responses in neonates born to healthy women and women living with HIV

David Rach¹, Hao-Ting Hsu², Ngina Nampota-Nkomba^{3,4}, Godfrey Mvula³, Felix A. Mkandawire³, Oswald M. Nyirenda³, Bernadette Hritzo¹, Francesca Boldrin⁵, Giulia Degiacomi⁵, Laura Cioetto Mazzabò⁵, Riccardo Manganelli⁵, Andrea G. Buchwald⁶, Franklin R. Toapanta⁶, Marcelo B. Sztein⁶, Miriam K. Laufer⁶, Kirsten E. Lyke^{6*} and Cristiana Cairo^{2,7*}

¹Molecular Microbiology and Immunology Graduate Program, University of Maryland School of Medicine, Baltimore, MD, United States, ²Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, United States, ³Blantyre Malaria Project, Kamuzu University of Health Sciences, Blantyre, Malawi, ⁴Graduate Program in Life Sciences, University of Maryland School of Medicine, Baltimore, MD, United States, ⁵Department of Molecular Medicine, University of Padova, Padova, Italy, ⁶Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, ⁷Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States



OPEN ACCESS

EDITED BY
David Vermijlen,
Université libre de Bruxelles, Belgium

REVIEWED BY
Alessandro Poggi,
San Martino Hospital (IRCCS), Italy
Sarina Ravens,
Hannover Medical School, Germany

*CORRESPONDENCE
Kirsten E. Lyke
✉ klyke@som.umaryland.edu
Cristiana Cairo
✉ ccairo@ihv.umaryland.edu

RECEIVED 13 May 2025
ACCEPTED 27 June 2025
PUBLISHED 22 August 2025

CITATION
Rach D, Hsu H-T, Nampota-Nkomba N, Mvula G, Mkandawire FA, Nyirenda OM, Hritzo B, Boldrin F, Degiacomi G, Mazzabò LC, Manganelli R, Buchwald AG, Toapanta FR, Sztein MB, Laufer MK, Lyke KE and Cairo C (2025) Cord blood innate-like T cell responses in neonates born to healthy women and women living with HIV. *Front. Immunol.* 16:1628145. doi: 10.3389/fimmu.2025.1628145

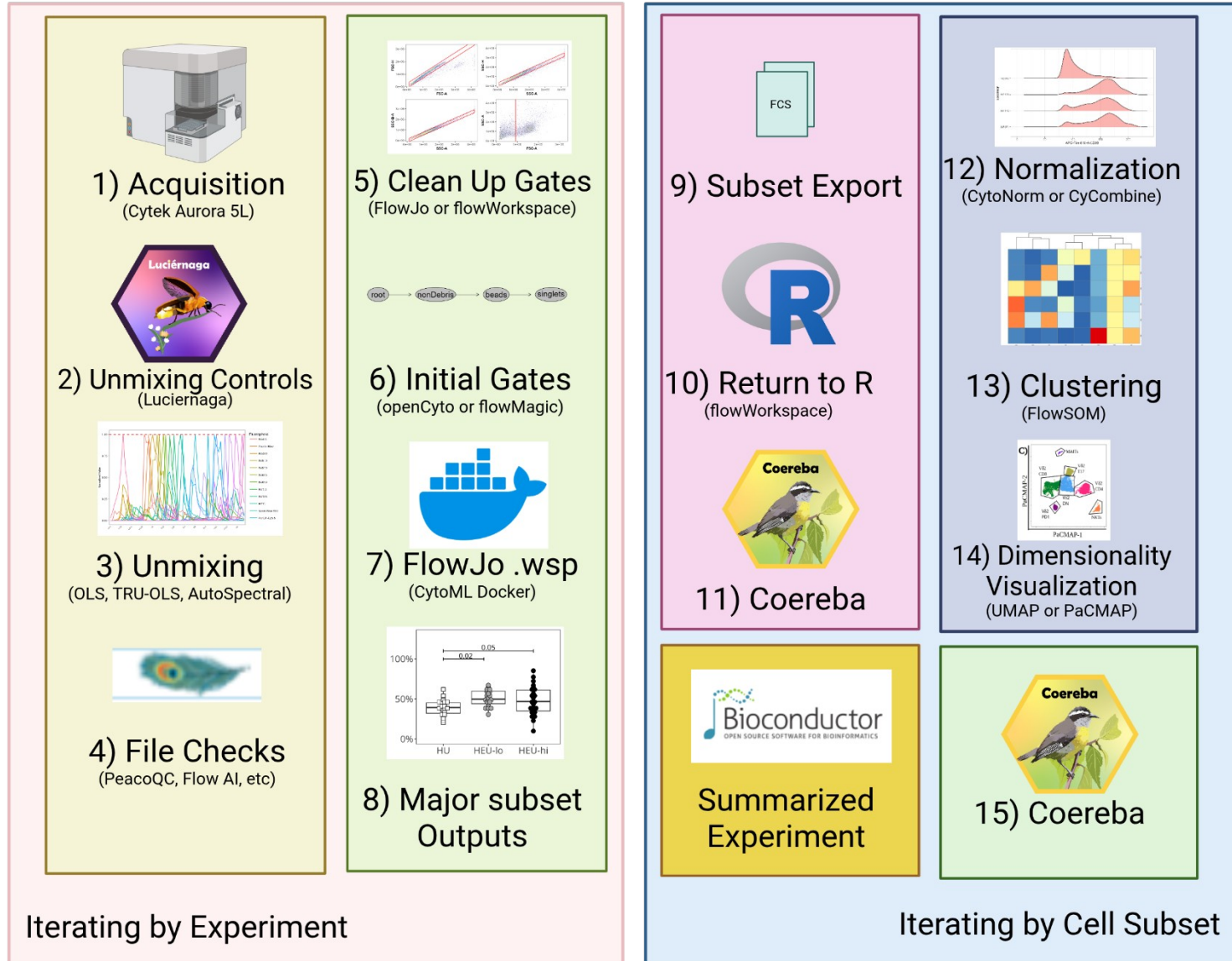


Paper

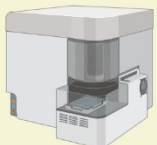
Main differences this time around:

- ❑ Shifting from 50%-50% FlowJo – R to increasingly more R
- ❑ Targeted re-writes in Rust to speed up original R code
- ❑ Building around reproducibility from the start (not as an after thought)
- ❑ Improved Bioconductor interoperability

Semi-supervised Pipeline



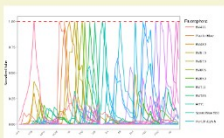
Pipeline: Acquisition



1) Acquisition
(Cytek Aurora 5L)



2) Unmixing Controls
(Lucifera)



3) Unmixing
(OLS, TRU-OLS, AutoSpectral)



4) File Checks
(PeacoQC, Flow AI, etc)

Iterating by Experiment

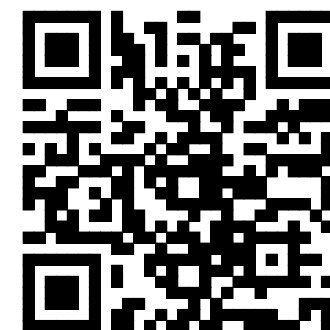
- ☐ Acquired day of staining on a 5-Laser Cytek Aurora
- ☐ Instrument flushed with 10% Contrad and Water for 20 minutes pre-use
- ☐ Instrument passed by both the UMGCCC FCSR and Cytek's QC criteria immediately before acquisition



UMGCCC FCSR
InstrumentQC



Plots for our
5L Aurora

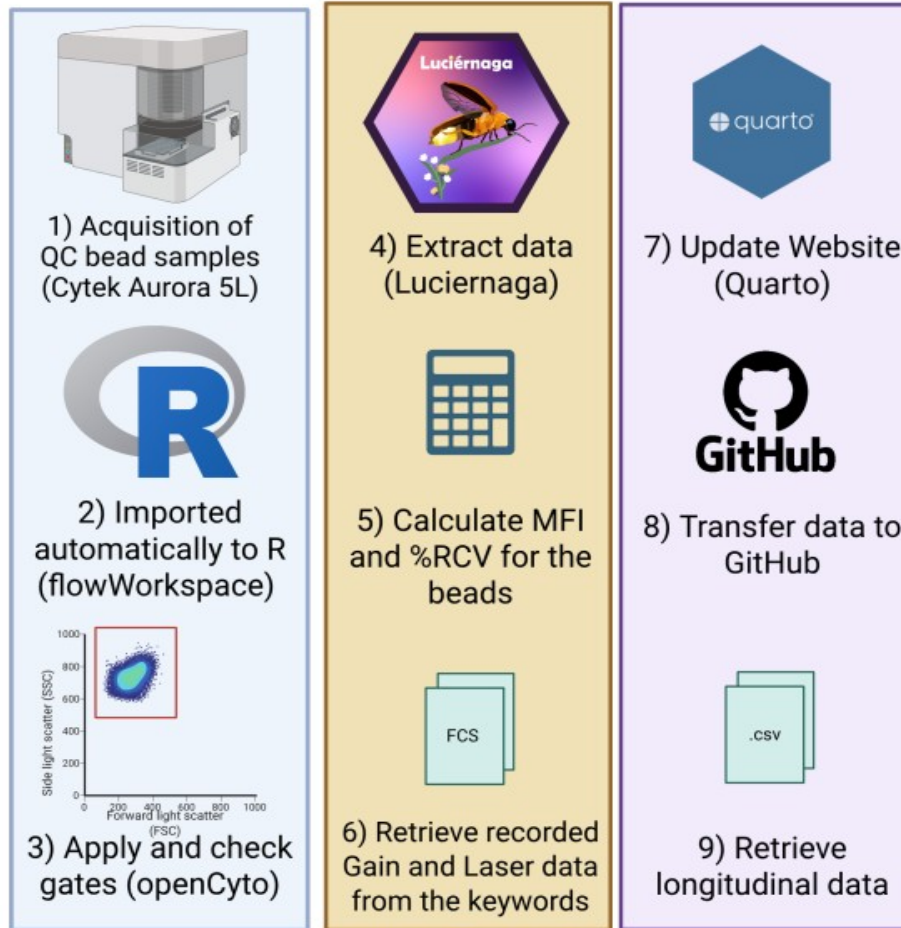


Historical Data
5L Aurora



Dashboard
How-To-Set-Up

Automated Daily Processing



Behind-the-scenes:

- Using R (and increasingly Rust) to process the .fcs files
- Automated transfer to GitHub via Windows Task Scheduler
- Our Quarto Website gets updated twice daily via a GitHub action

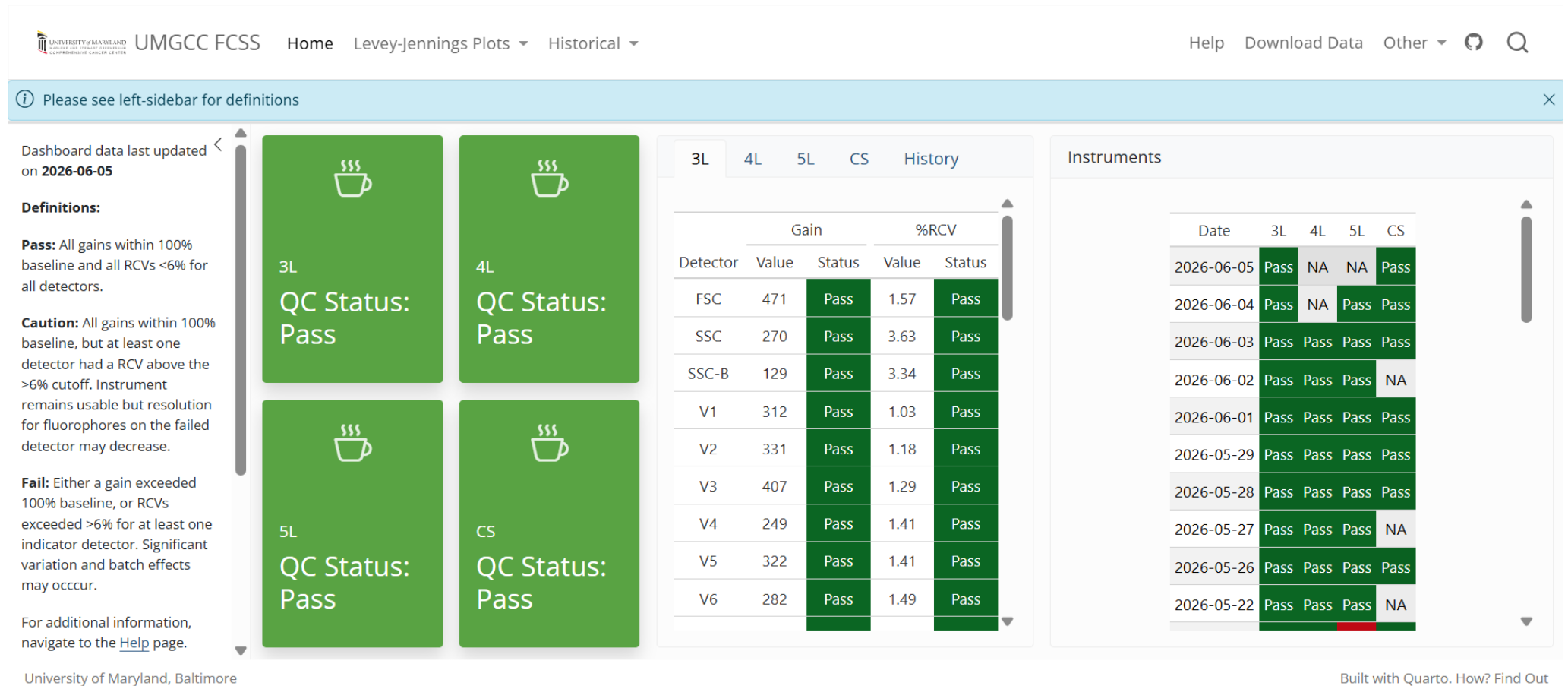
Instrument Quality Control Dashboard



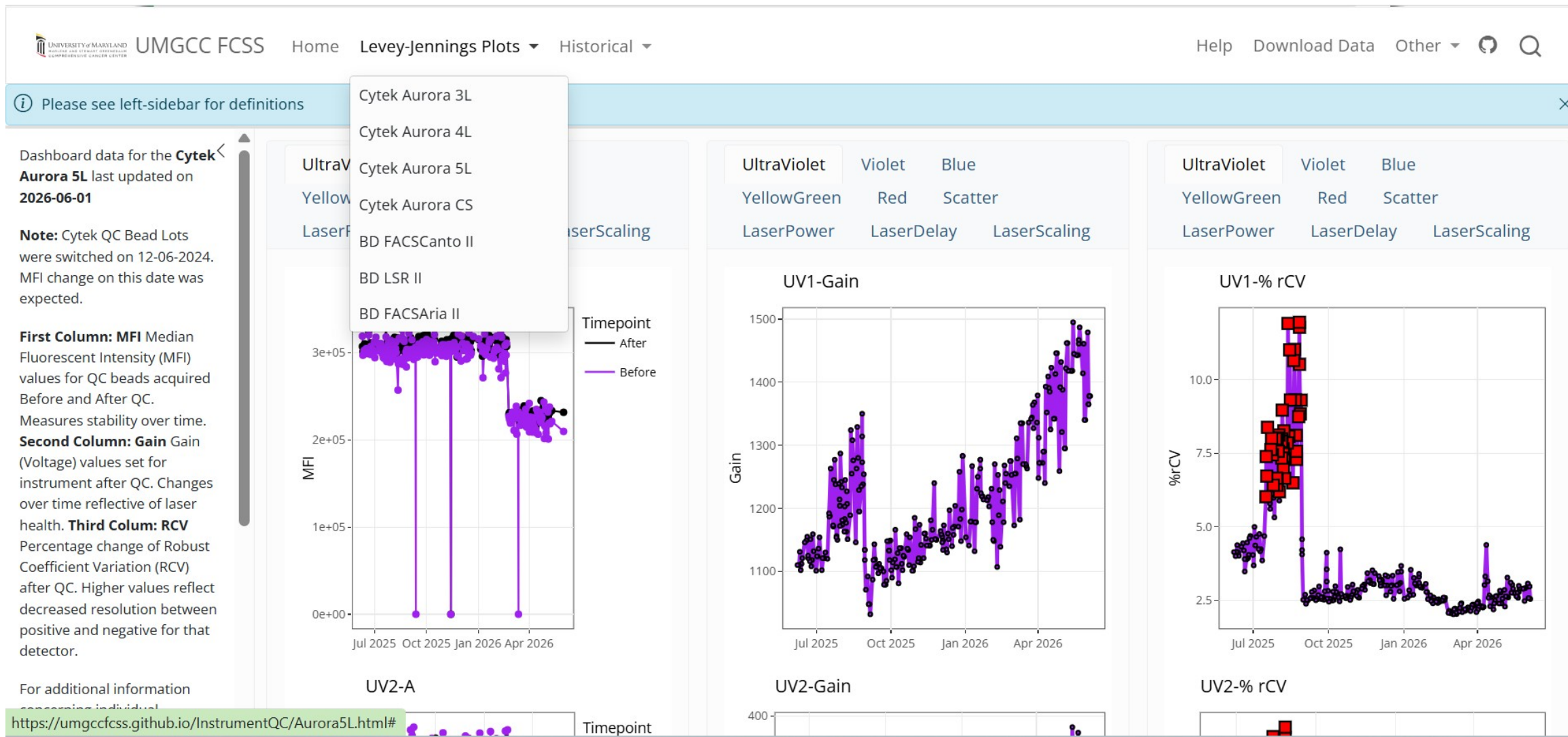
Dashboard



How-To



Instrument Quality Control Dashboard



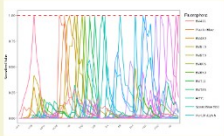
Pipeline: Evaluating Unmixing Controls



1) Acquisition
(Cytek Aurora 5L)



2) Unmixing Controls
(Luciernaga)



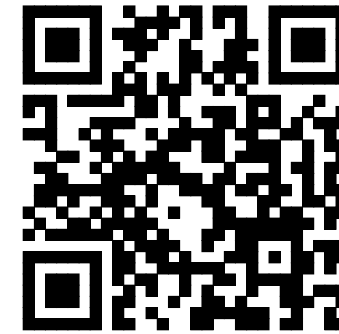
3) Unmixing
(OLS, TRU-OLS, AutoSpectral)



4) File Checks
(PeacoQC, Flow AI, etc)

Iterating by Experiment

- ❖ New unmixing controls acquired with each experiment.
- ❖ Extra specimen cells acquired as additional unstained controls
- ☐ Unmixing Controls are screened for suitability for unmixing
- ☐ Single-colors: Tandem degradation, and autofluorescence contamination
- ☐ Unstained(s): Presence of multiple-autofluorescences in lymphocyte gate
- ☐ Flagged unmixing controls are replaced with equivalents from adjacent experimental date



Luciernaga

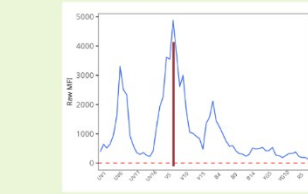


Luciernaga
Vignette

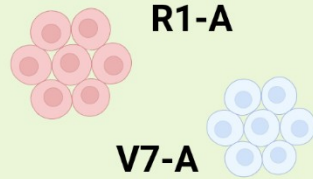
Luciernaga: Individual Cell Normalized Signatures



Code



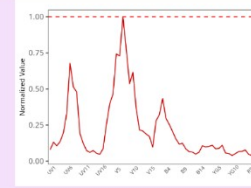
Identify detector with brightest MFI for each cell



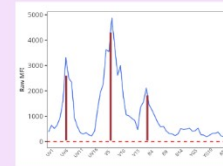
Group cells with shared peak detectors



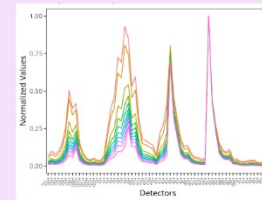
Subtract Out Autofluorescence



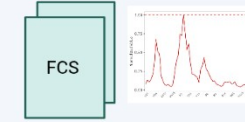
Normalize individual cell fluorescent signatures



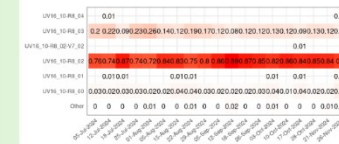
Identify peaks using local maxima



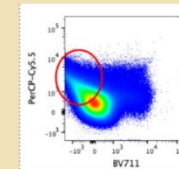
Group cells with shared peaks & heights



Enumerate and export out cells that share signature as .fcs files

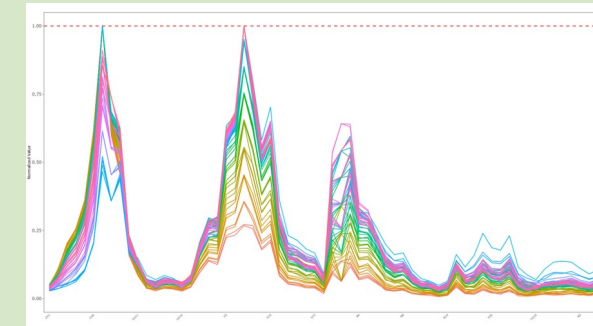
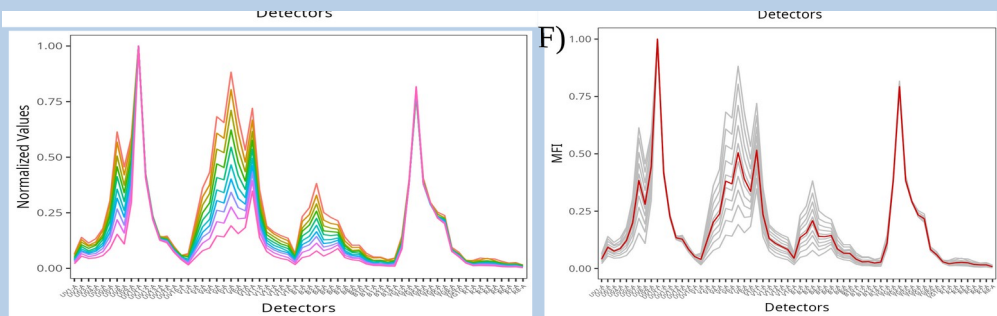
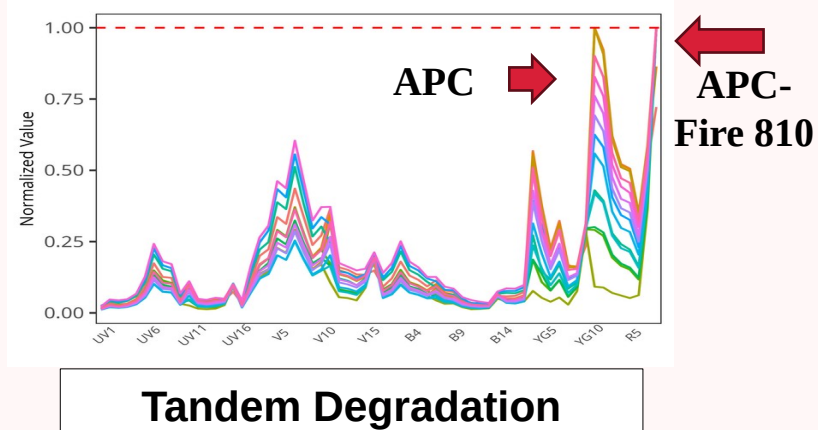


Checks for tandem degradation



Autofluorescence Isolation

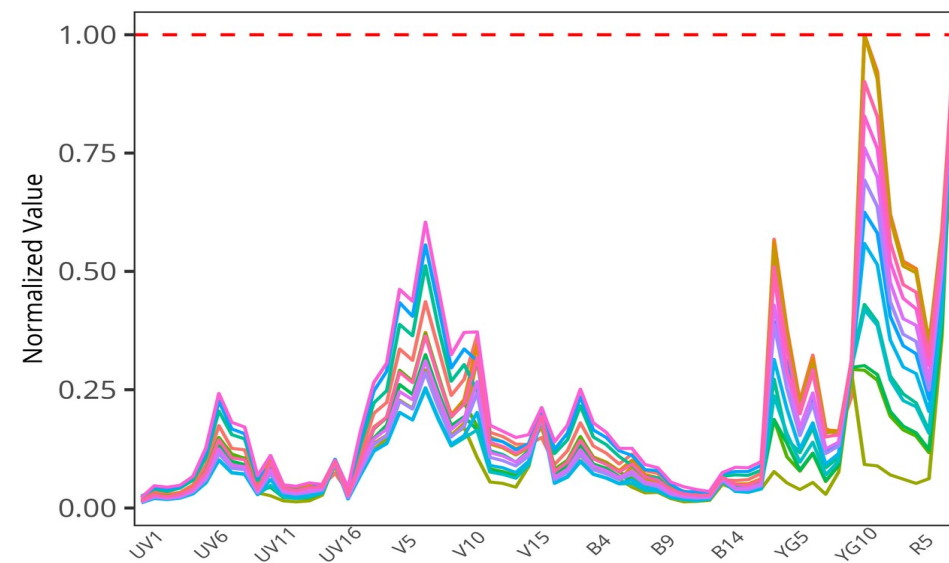
Automated tools for screening unmixing controls



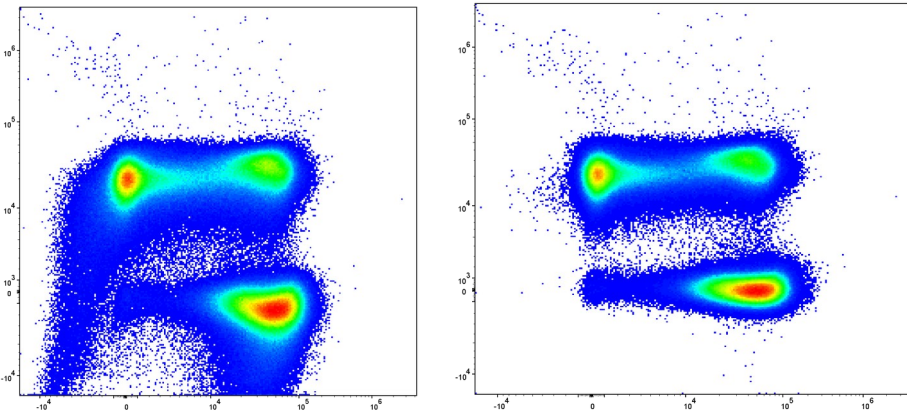
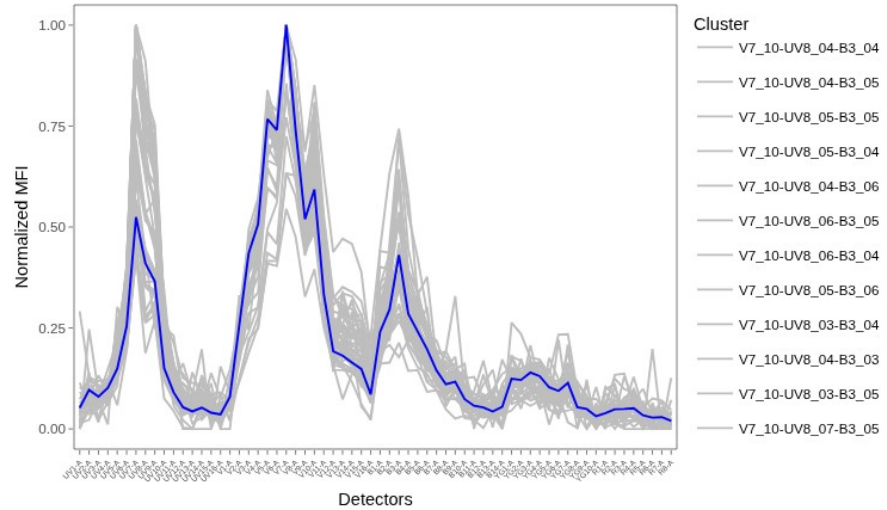
Identifying degrading fluorophores

Fluorophores in good shape → **one predominant normalized signature** within the unmixing control raw .fcs file

- When the fluorophore degrades, noticeable **increase in variant signatures**, making up substantial proportion of all cells in the unmixing control
- Automated screening all acquired unmixing controls from campus users during off-hours



Isolating additional autofluorescence signatures



► This approach also works to isolate variant autofluorescence signatures from unstained controls

• Unlike other approaches, the isolated cells share normalized signatures, only differing in terms of brightness

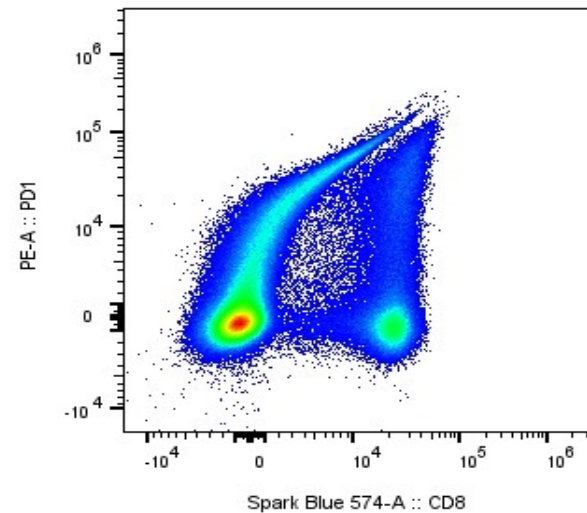
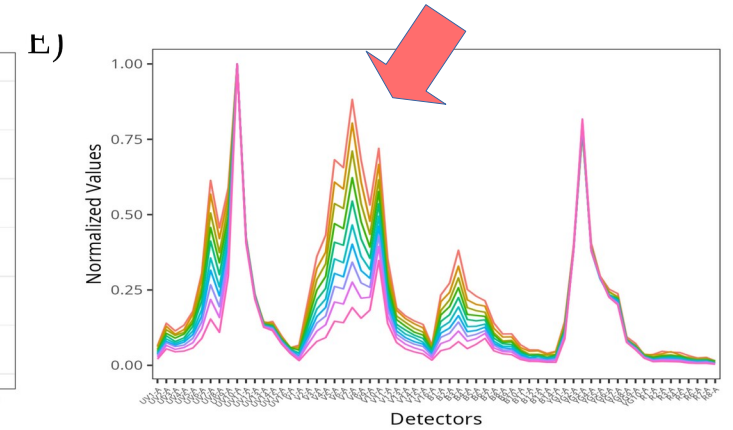
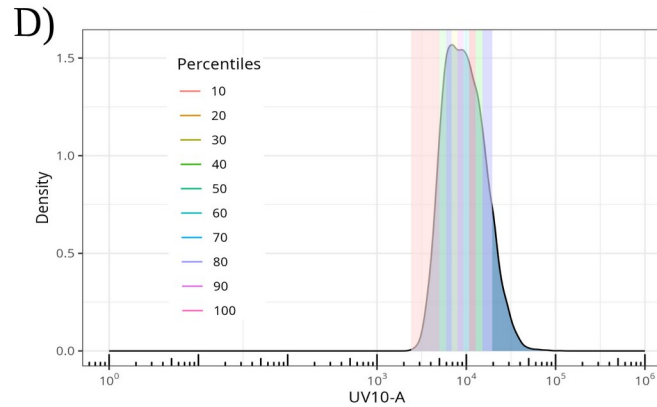
► Return directly as an .fcs file containing just cells with same signature for use as an autofluorescent tag in SpectroFlo

Residual autofluorescence in dim cell single-color controls

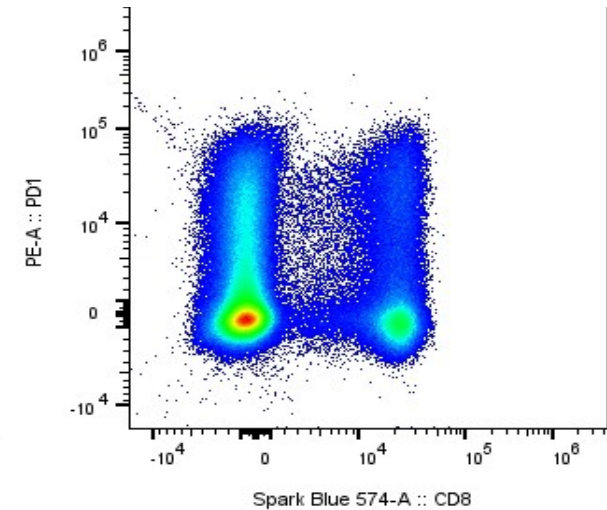
► “As bright or brighter”

- Dim antigen-fluorophore combinations acquired as cell single-color controls often have residual autofluorescence contribution to their signature

► Identifying and switching these dim cell single-color controls to bead single-color controls significantly improved unmixing for our panels



Signature with
residual AF



Signature without
residual AF

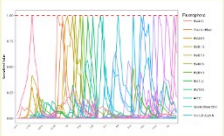
Pipeline: Unmixing



1) Acquisition
(Cytek Aurora 5L)



2) Unmixing Controls
(Luciernaga)



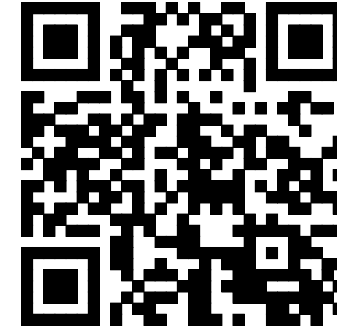
3) Unmixing
(OLS, TRU-OLS, AutoSpectral)



4) File Checks
(PeacoQC, Flow AI, etc)

Iterating by Experiment

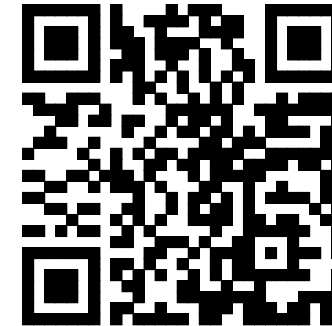
- ❖ Unmixing Method Agnostic
- ❑ Unmixing in SpectroFlo (with modified single-color and unstained inputs via Luciernaga)
- ❑ Unmixing in R
 - OLS (Luciernaga using Rust)
 - TRU-OLS (via Julia wrapper)
 - AutoSpectral



TRU-OLS



TRU-OLS
Walkthrough



AutoSpectral



AutoSpectral
Walk-through

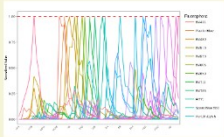
Pipeline: File Checks



1) Acquisition
(Cytek Aurora 5L)



2) Unmixing Controls
(Lucifera)



3) Unmixing
(OLS, TRU-OLS, AutoSpectral)

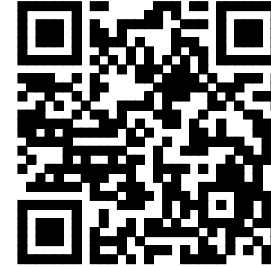


4) File Checks
(PeacoQC, Flow AI, etc)

Iterating by Experiment

❑ Checking for fluidic or laser-related issues in the unmixed files

- R: PeacoQC or FlowAI
- Rust: PeacoQC (thanks James!)



PeacoQC



PeacoQC
Vignette



FlowAI



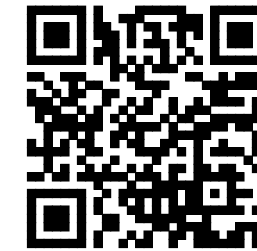
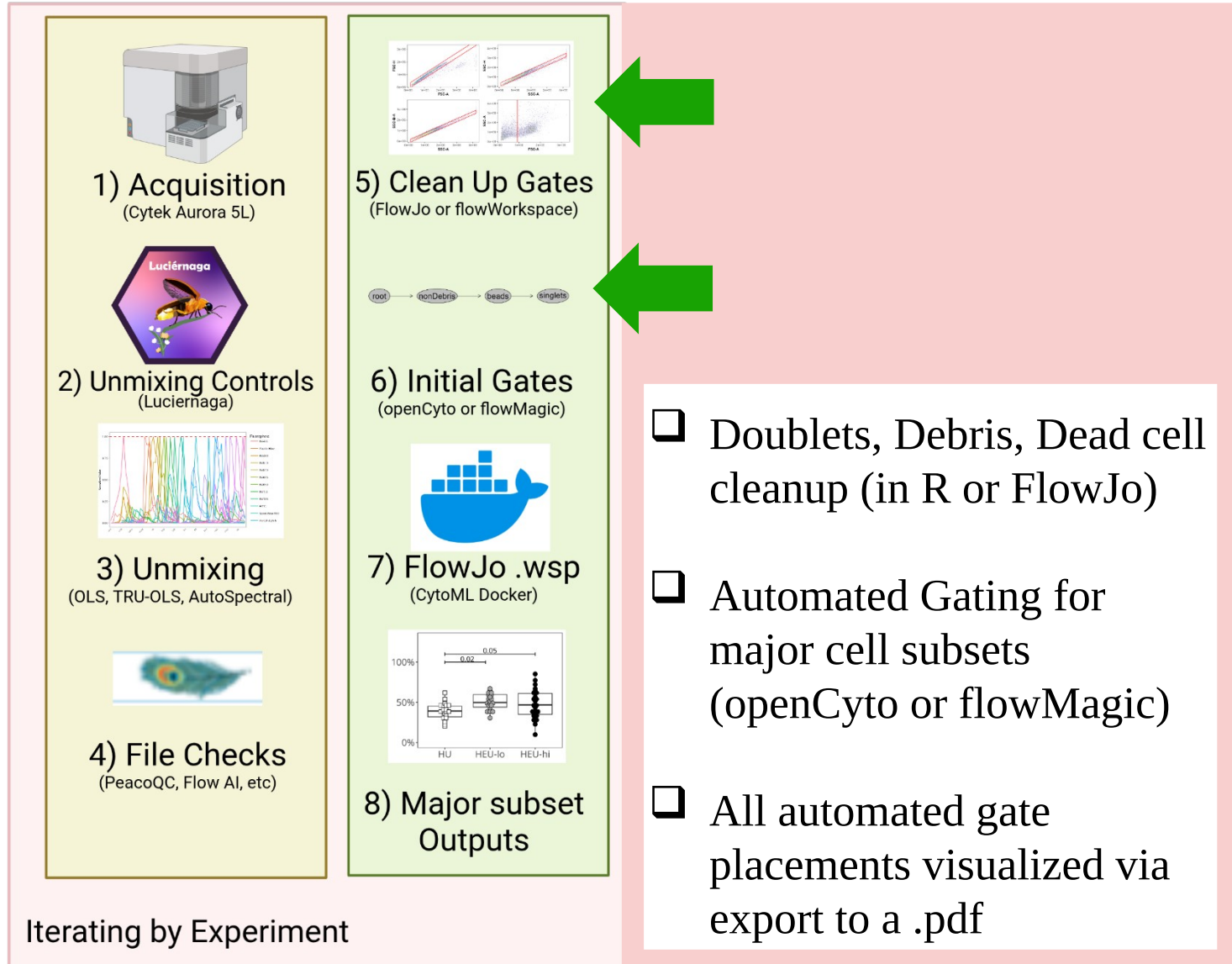
FlowAI
Vignette



PeacoQC
in Rust



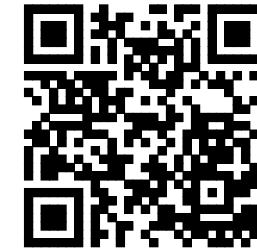
Pipeline: Manual and Automated Gating



flowGate



flowGate
Walkthrough



openCyto



openCyto
Walk-through



flowMagic



flowMagic



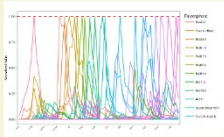
Pipeline: Save to FlowJo v10 .wsp



1) Acquisition
(Cytek Aurora 5L)



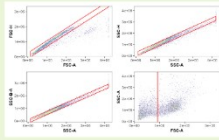
2) Unmixing Controls
(Lucifera)



3) Unmixing
(OLS, TRU-OLS, AutoSpectral)



4) File Checks
(PeacoQC, Flow AI, etc)



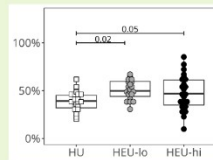
5) Clean Up Gates
(FlowJo or flowWorkspace)



6) Initial Gates
(openCyto or flowMagic)



7) FlowJo .wsp
(CytoML Docker)



8) Major subset
Outputs

Everything is saved to a FlowJo v10 .wsp, via CytoML

Any automated gates that need adjustment get corrected

Major cell subset statistics exported and analyzed



flowWorkspace



CytoML



FlowJo to R



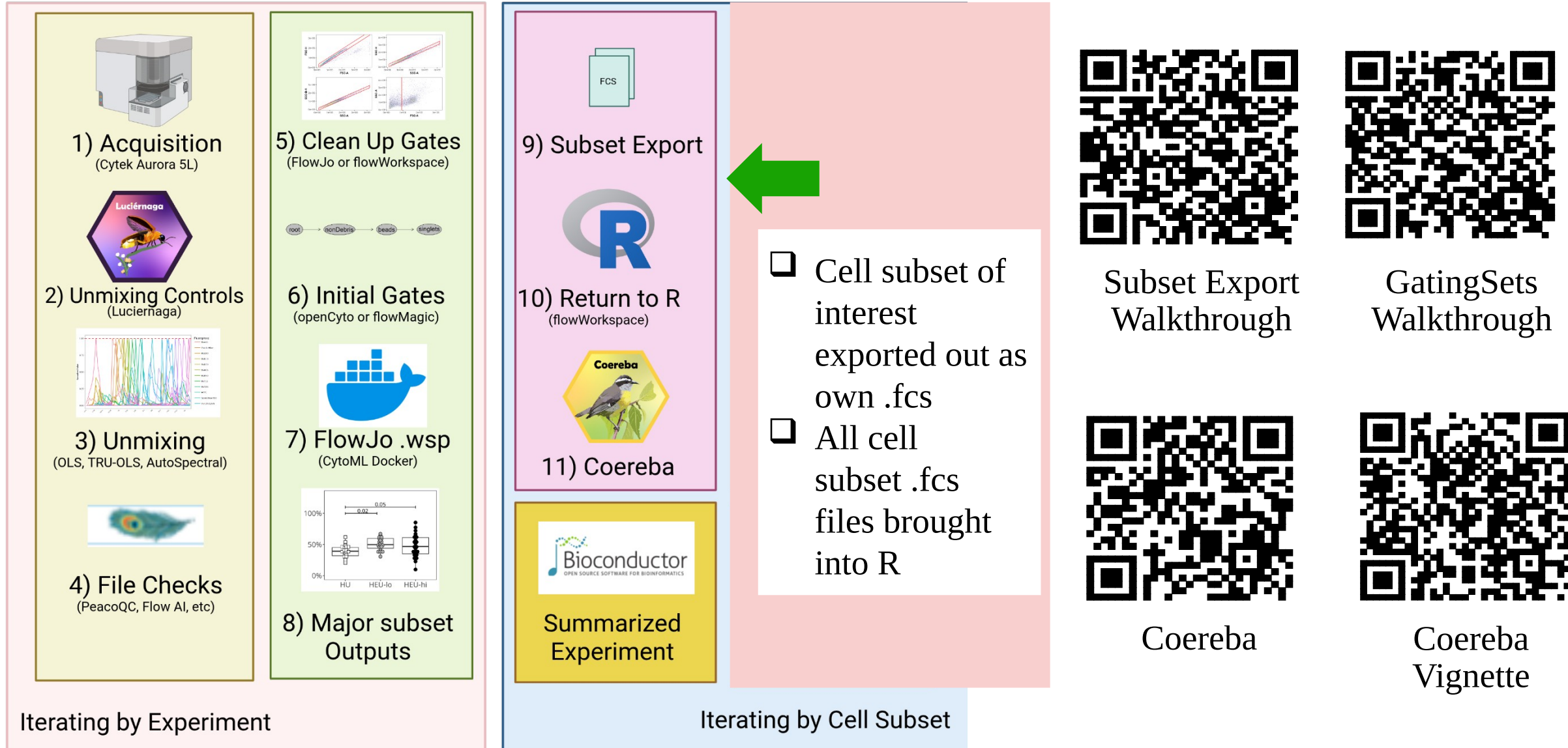
R to FlowJo



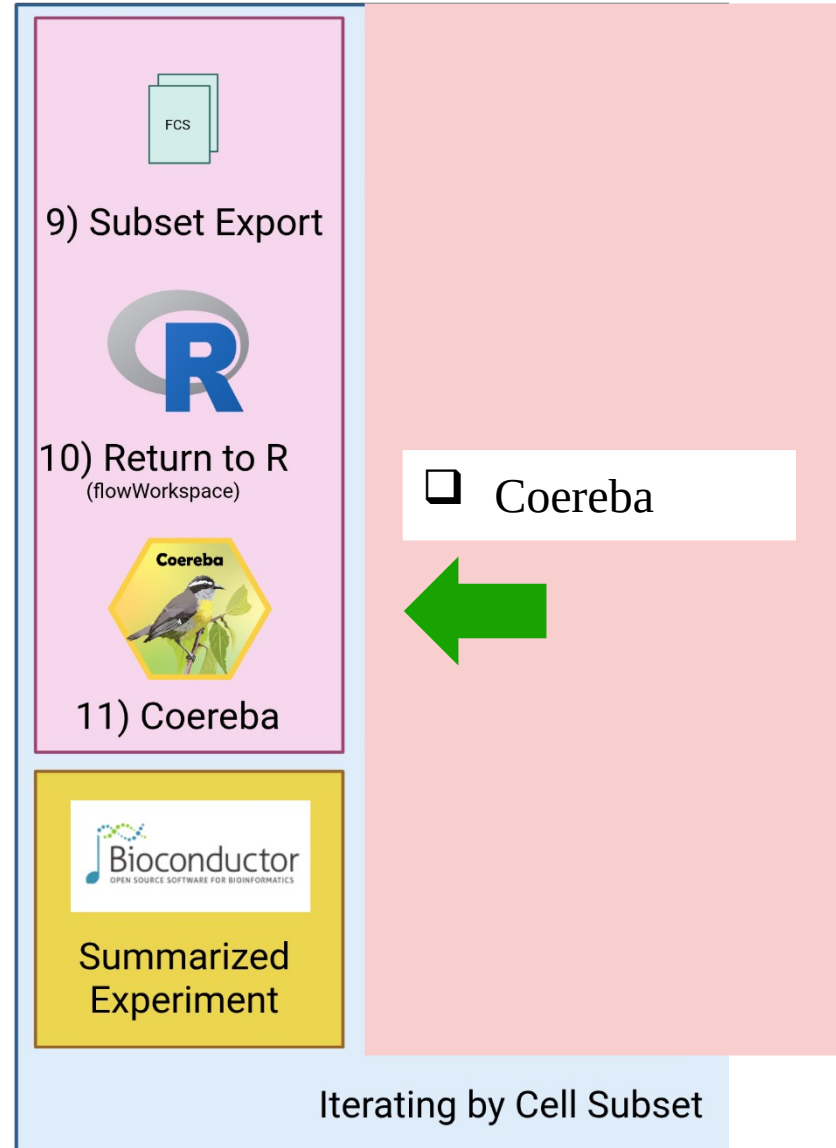
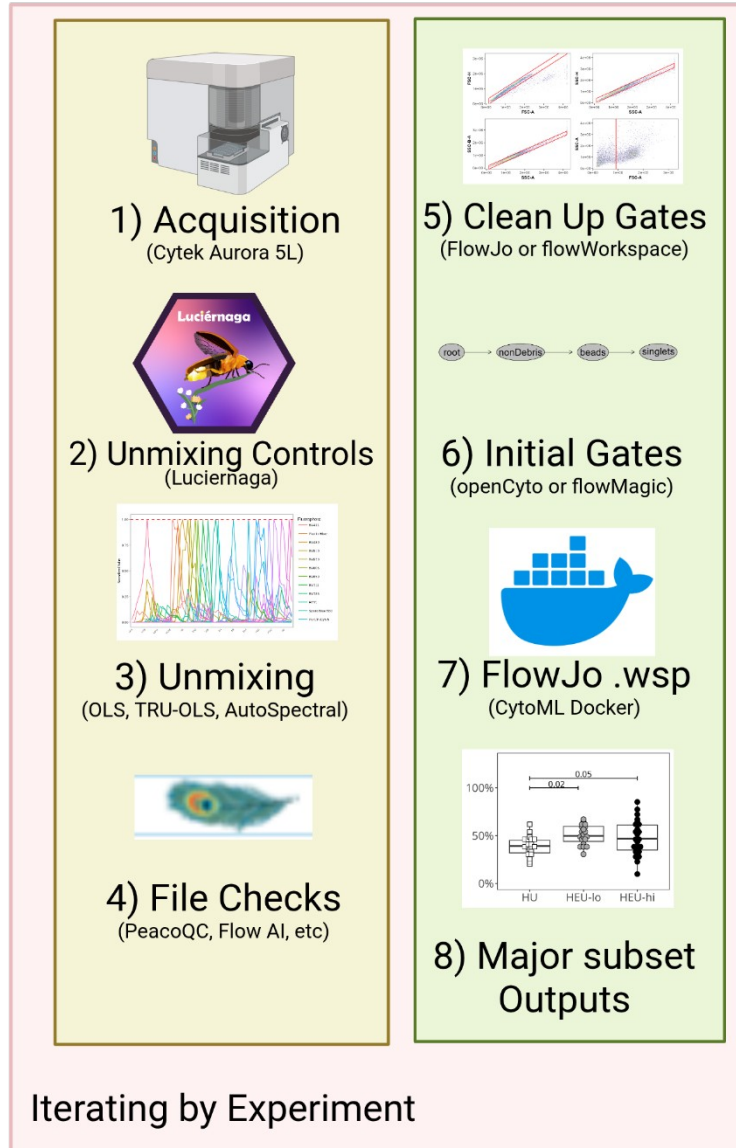
Data for Statistics
Walkthrough

Iterating by Experiment

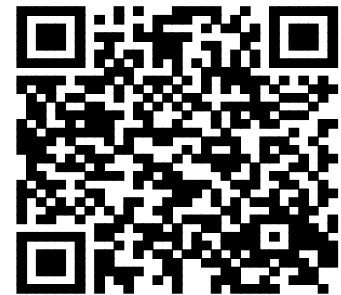
Pipeline: Iterating through cell subsets



Pipeline: Coereba



Subset Export
Walkthrough



GatingSets
Walkthrough



Coereba

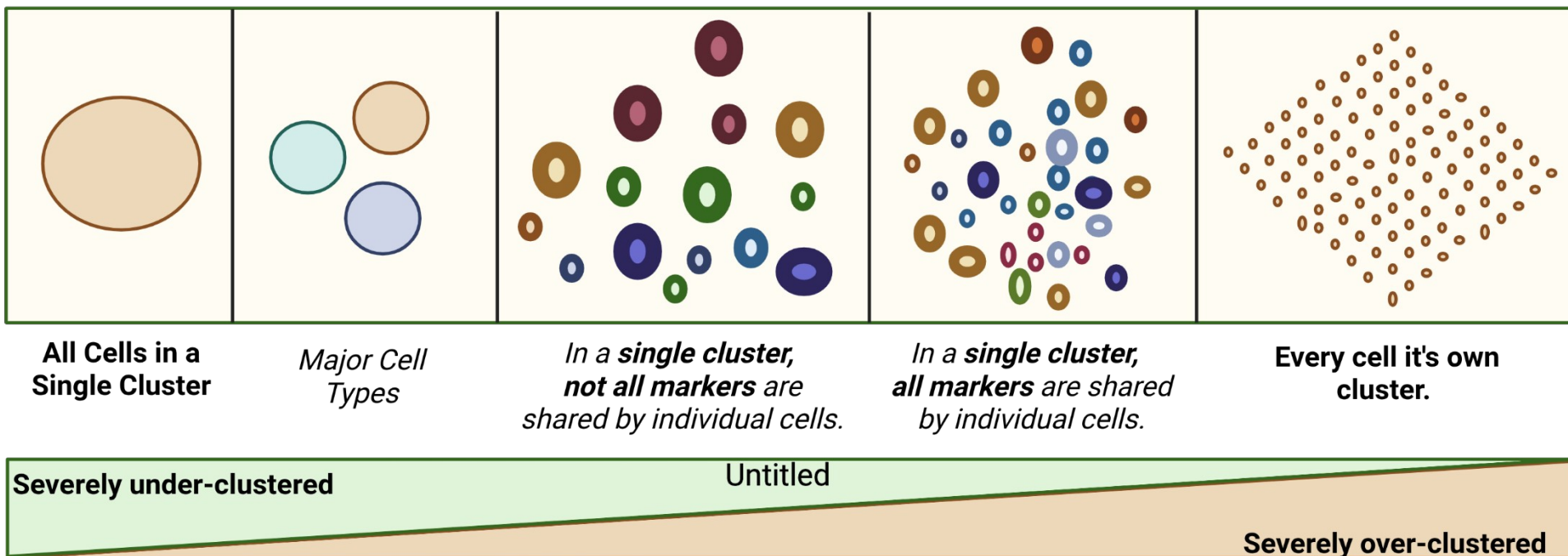


Coereba
Vignette





Grouping cells that share every marker



Coereba



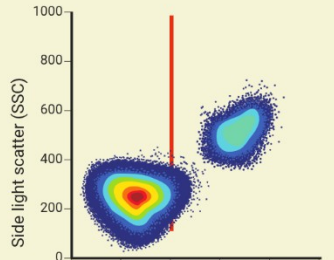
Code



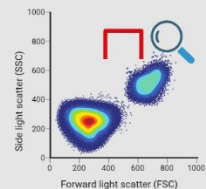
1) Load into R
(flowWorkspace)


Fluorophore

2) Calculate
Splitpoints


Side light scatter (SSC)
Forward light scatter (FSC)

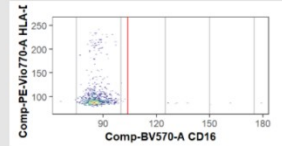
3) Visualize


Side light scatter (SSC)
Forward light scatter (FSC)

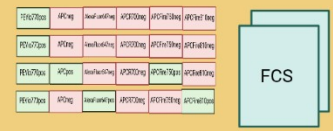
4) Gate Constraints



5) Shiny App
adjustments


Comp-PE-Vio70-A HLA-t
Comp-BV570-A CD16

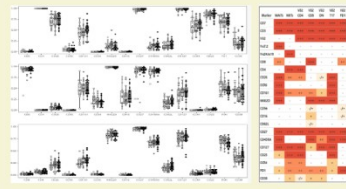
6) Splitpoints
updated


FCS

7) CoerebaID


Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

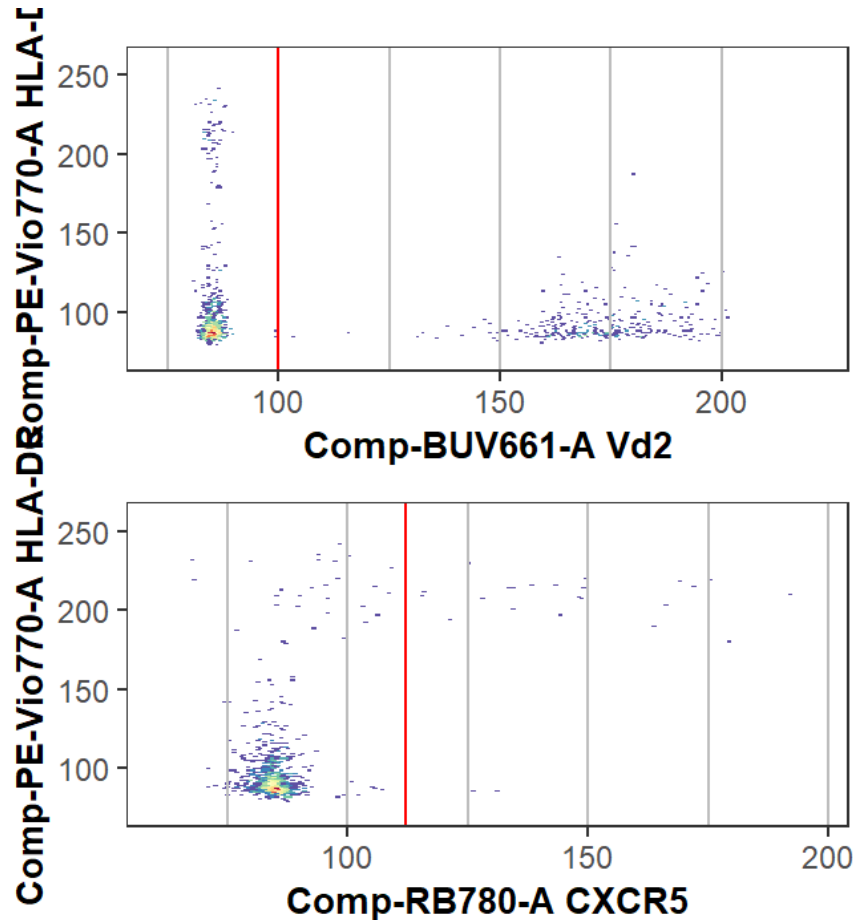
8) Summarized
Experiment



9) Visualize



Initial automated gate, with ability to adjust



- ☐ Initial gates for designated markers calculated via openCyto (mindensity)

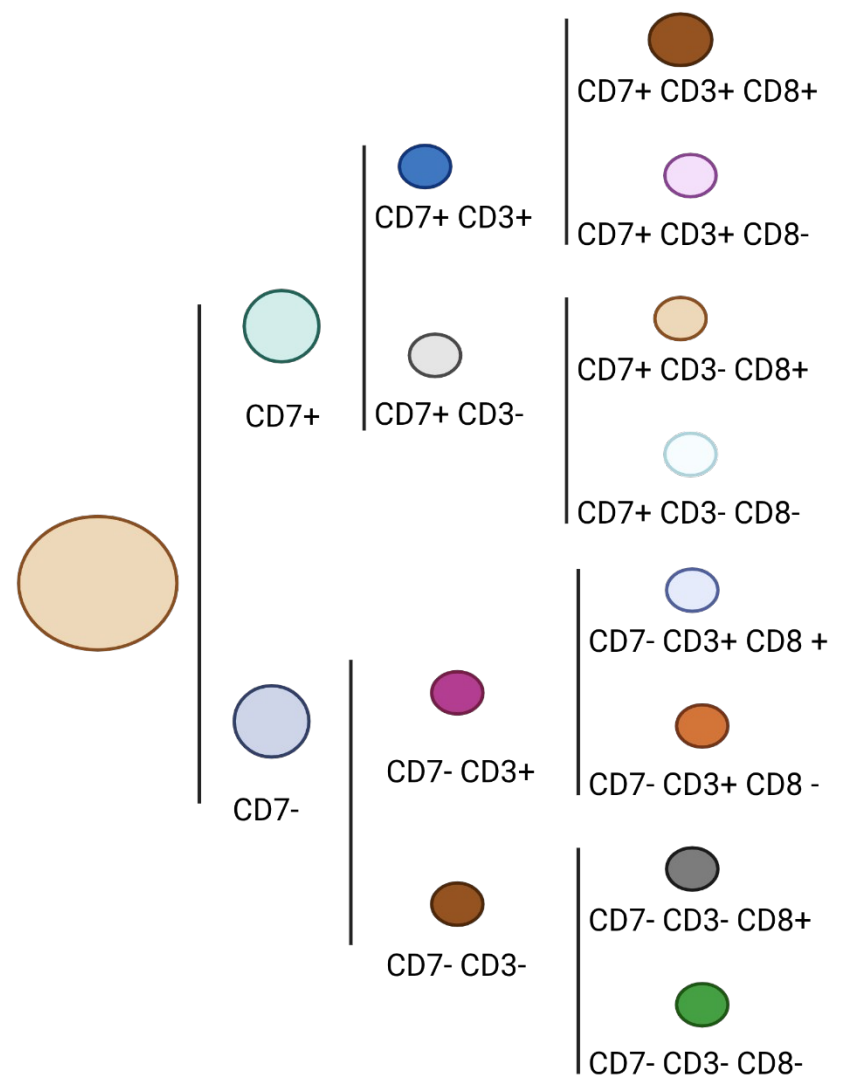
- ☐ Ability to update gate_range constraints

- ☐ Launch a Shiny app and designate interactively

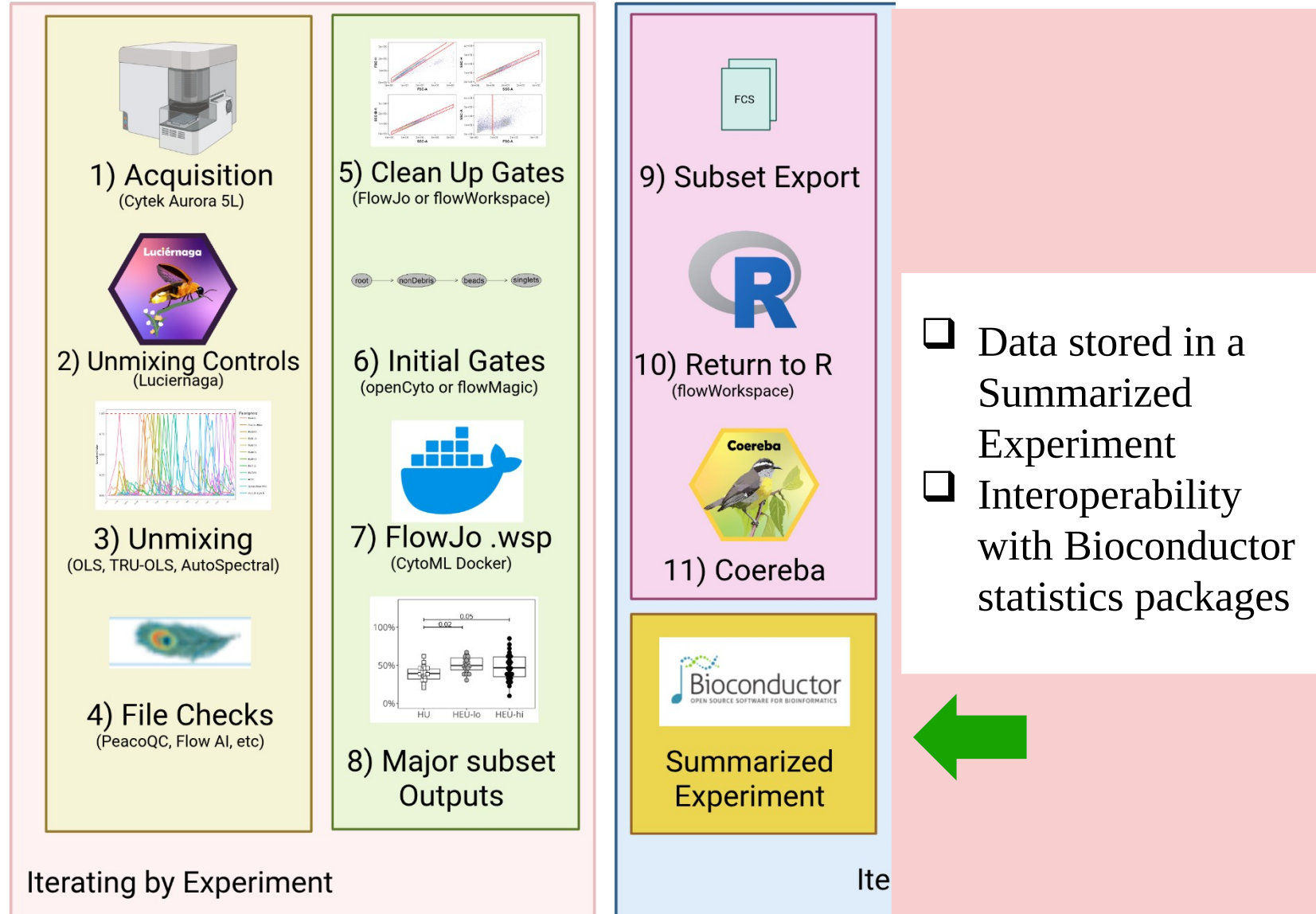


CoerebaID column gets added to the .fcs file

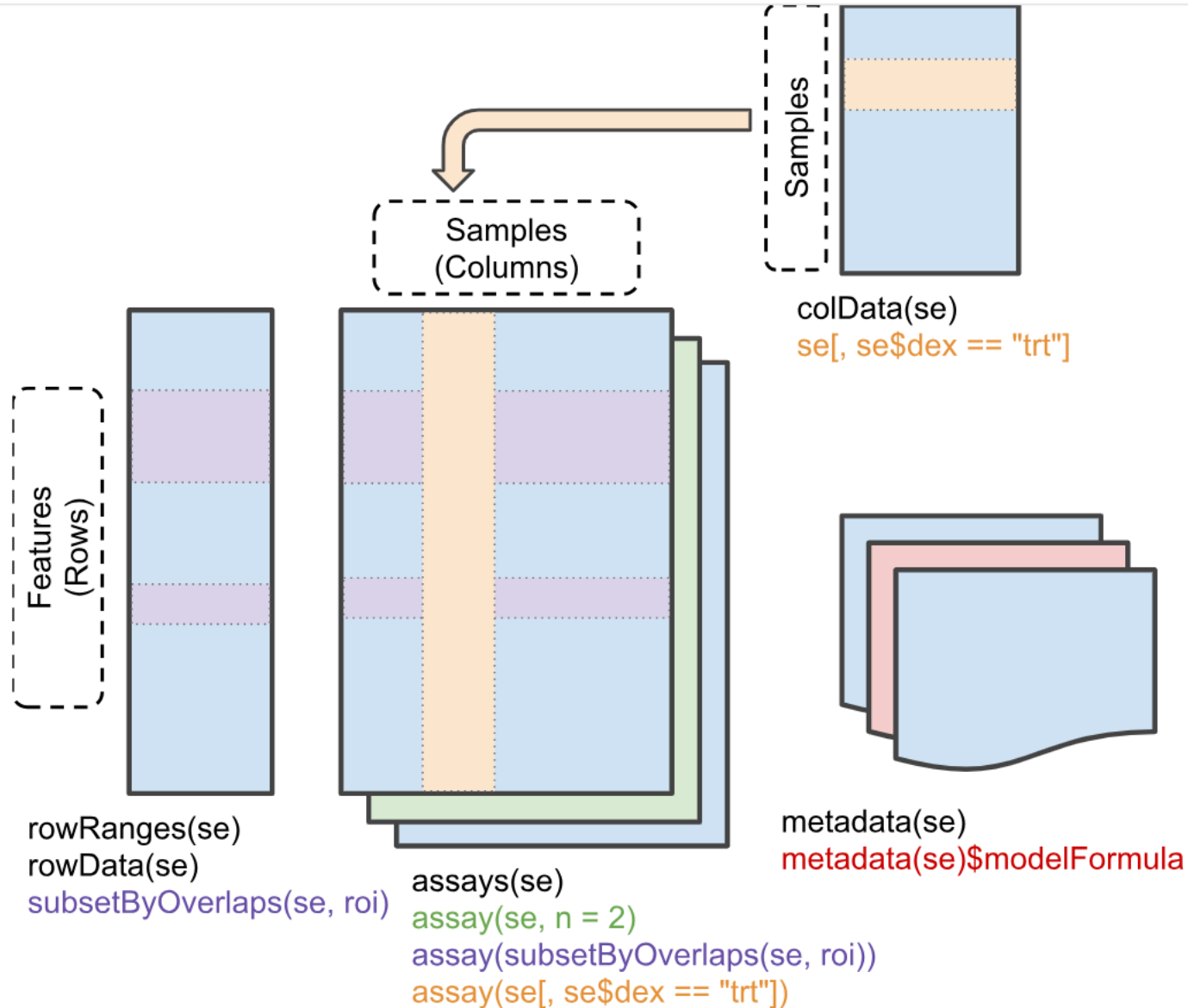
PEVio770pos	APCneg	AlexaFluor647neg	APCR700neg	APCFire750neg	APCFire810neg
PEVio770pos	APCneg	AlexaFluor647neg	APCR700neg	APCFire750neg	APCFire810neg
PEVio770pos	APCpos	AlexaFluor647neg	APCR700neg	APCFire750pos	APCFire810neg
PEVio770pos	APCneg	AlexaFluor647pos	APCR700neg	APCFire750neg	APCFire810pos



Pipeline: Summarized Experiment

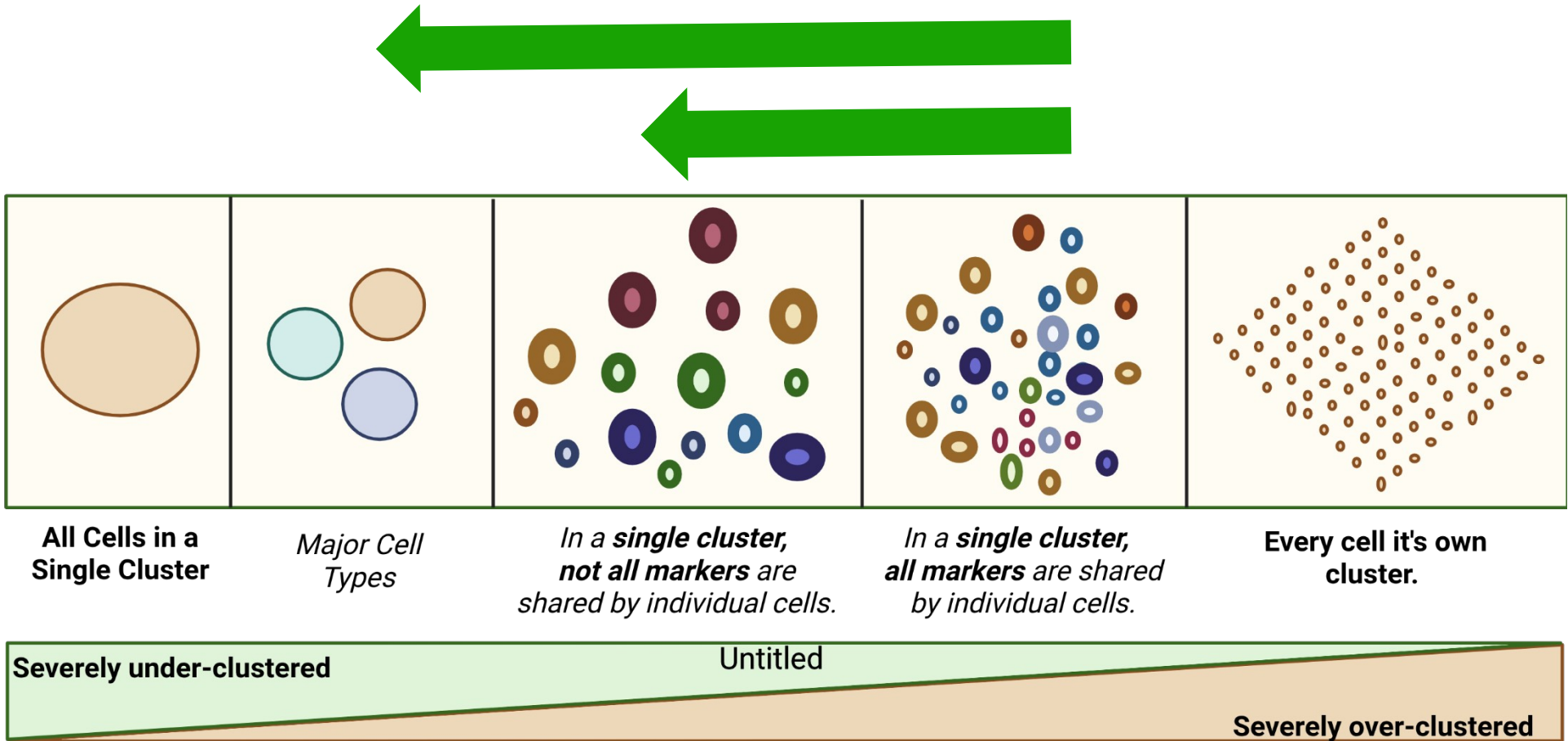


Summarized Experiment



- ❑ Permits rapid re-aggregation of the terminal nodes for re-evaluation
- ❑ Ties in with downstream Bioconductor statistics packages
- ❑ Portable. Don't like how we aggregated groups of cells? File is small enough to store in the same data repository as .fcs files for subsequent re-analysis.
- ❑ Filterable by metadata or node.
 - Nodes only in HEU-hi?
 - Nodes unique to one individual?

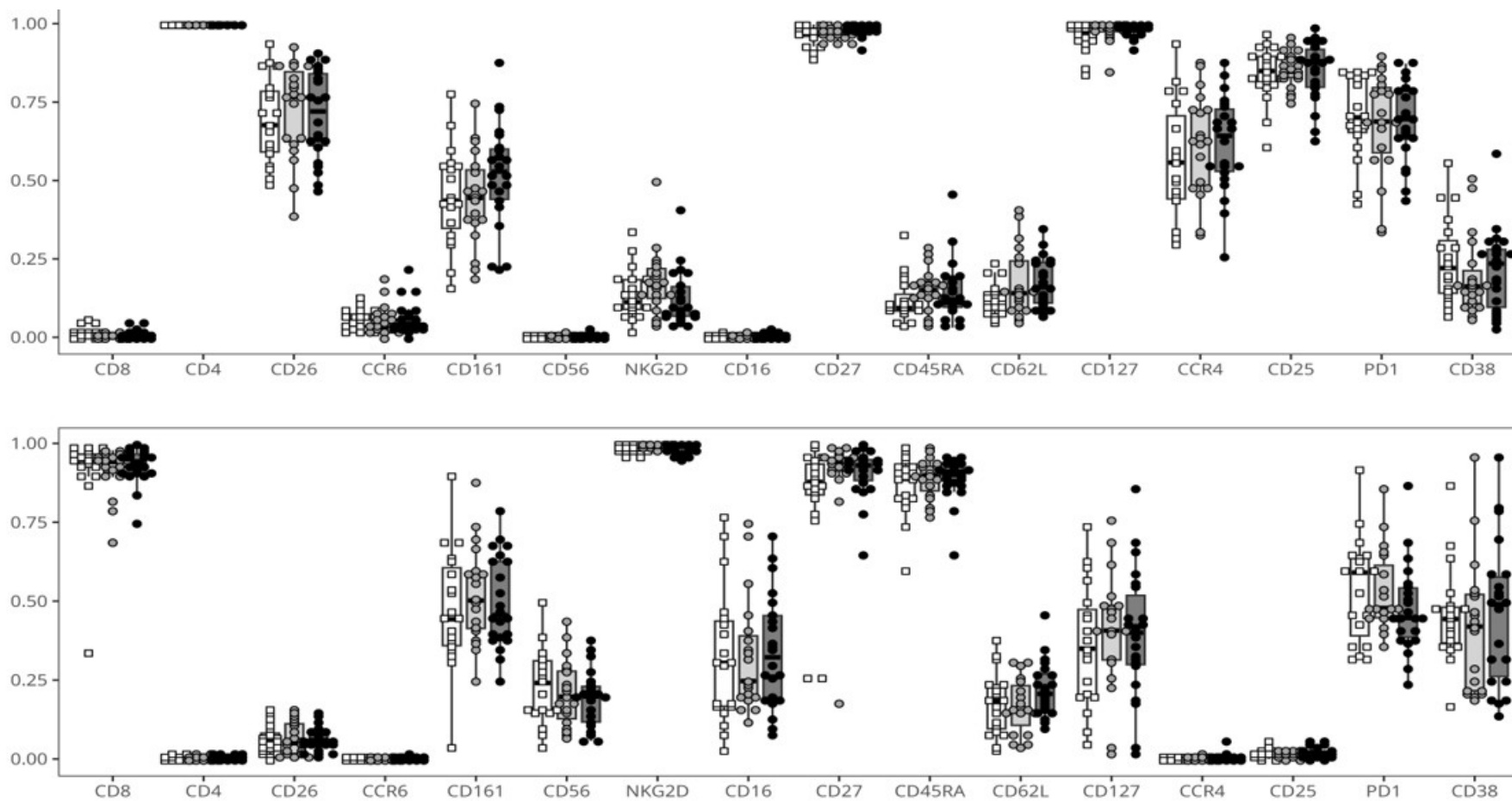
Selectively re-aggregate in seconds



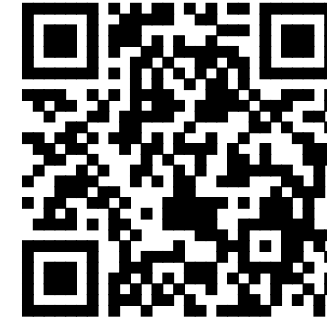
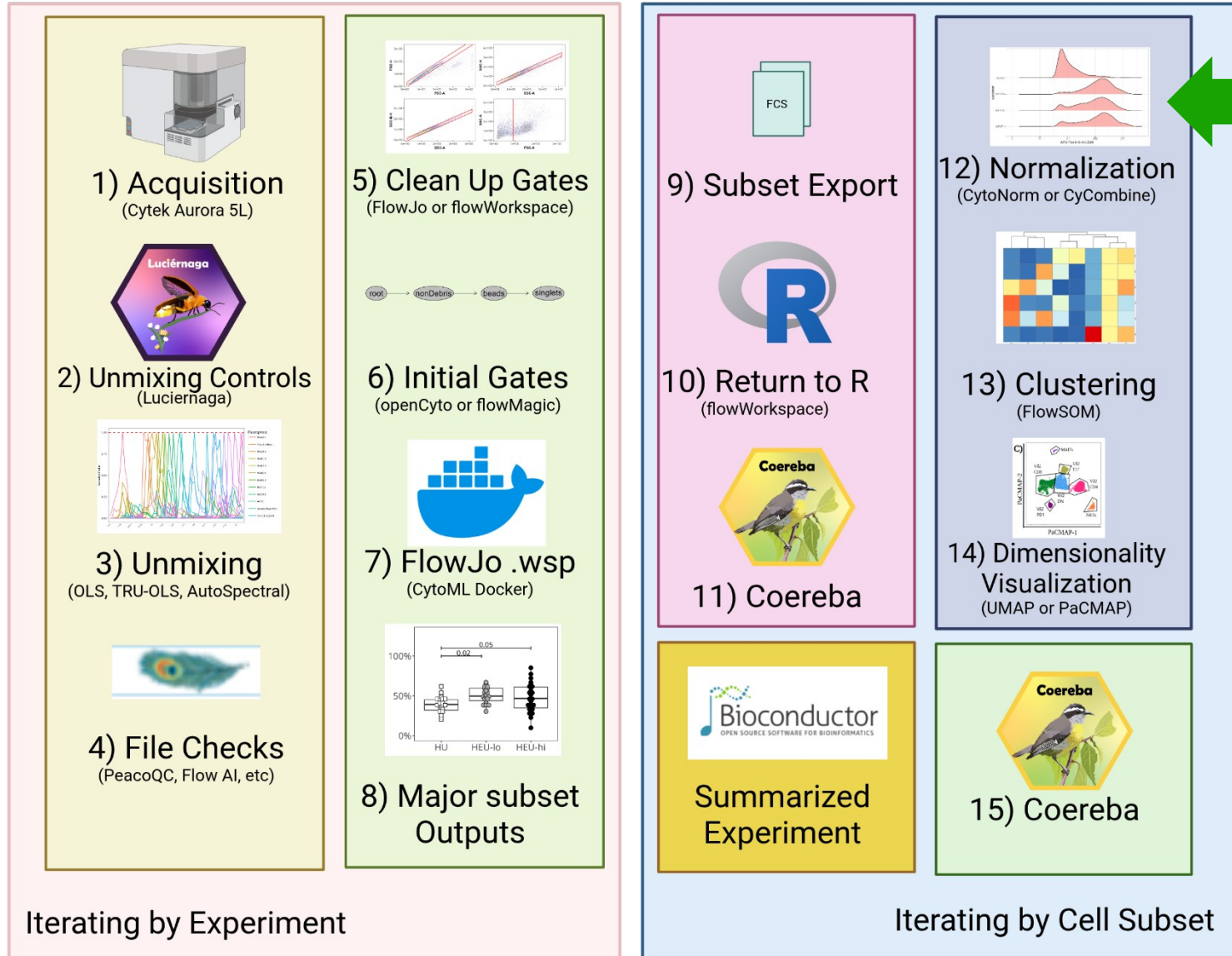
Coereba



Retrieval of marker expressions



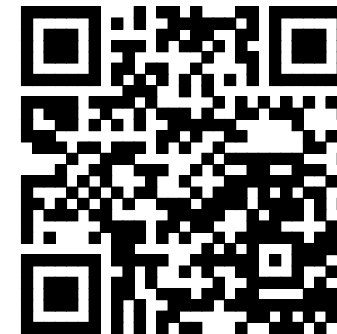
Pipeline: Normalization



CytoNorm



CytoNorm
Vignettes



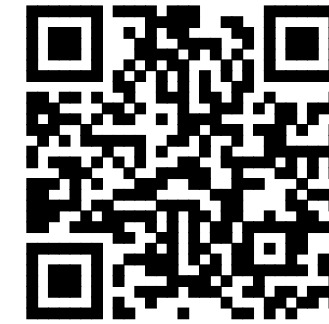
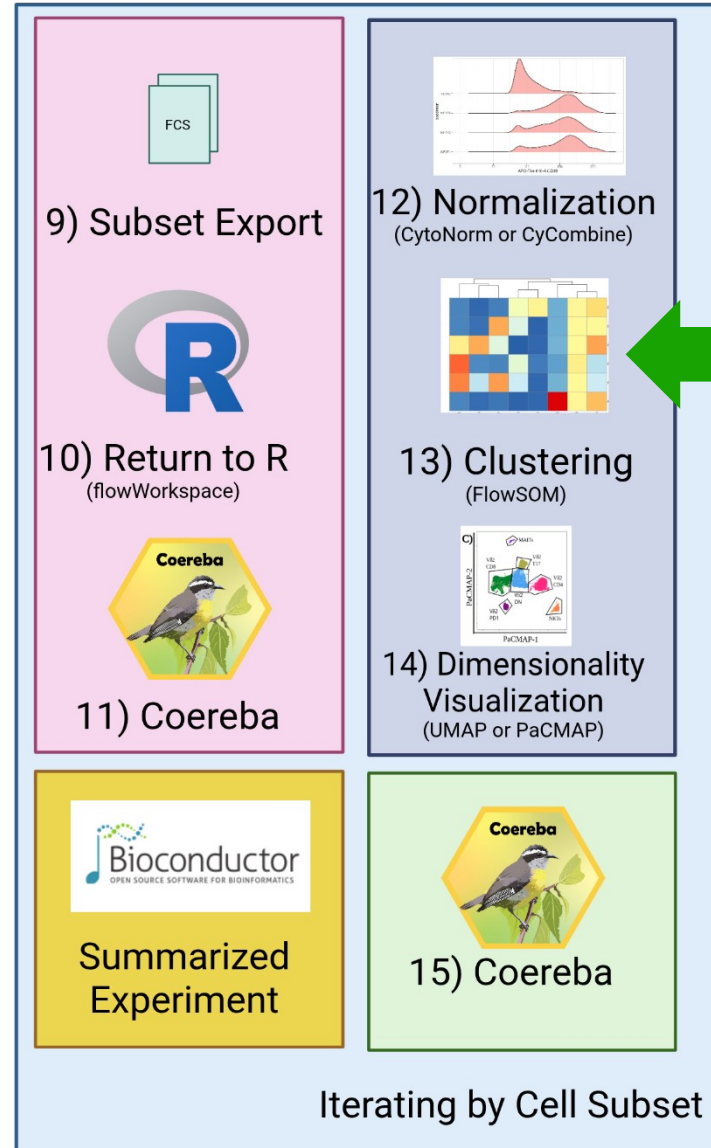
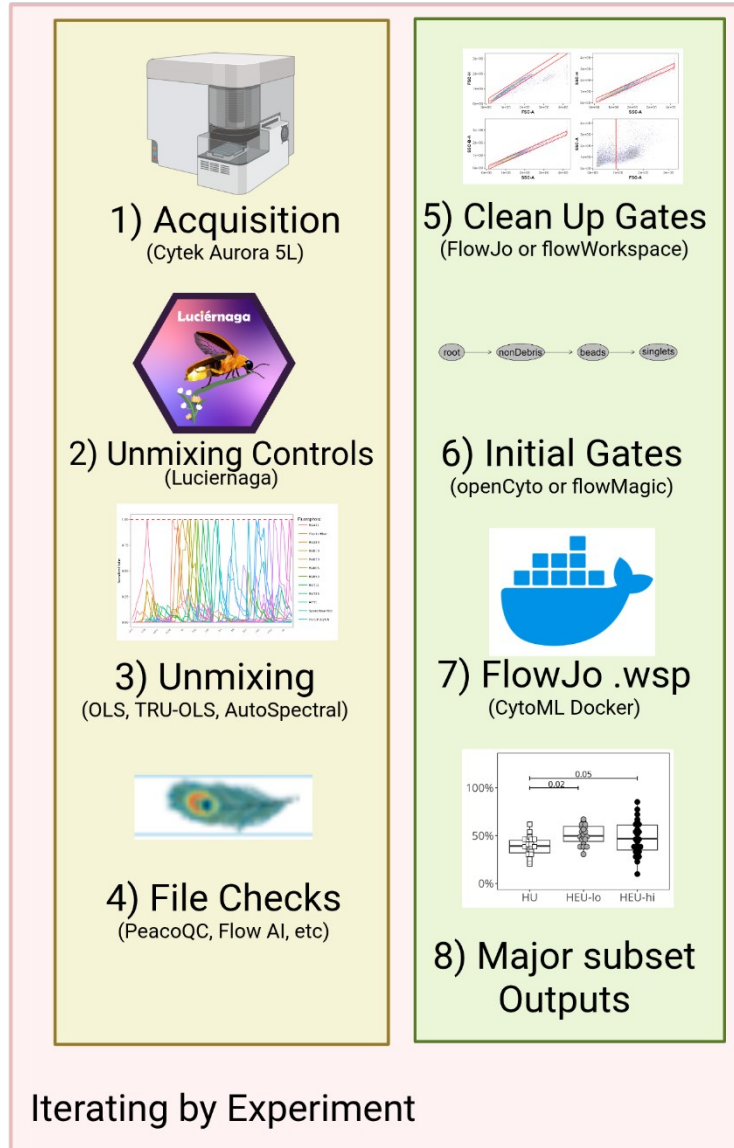
CyCombine



CyCombine
Vignettes



Pipeline: Clustering

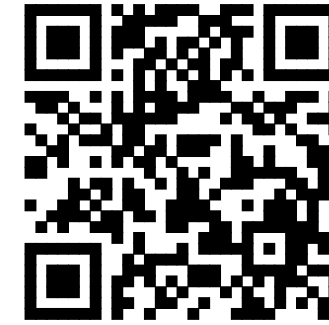
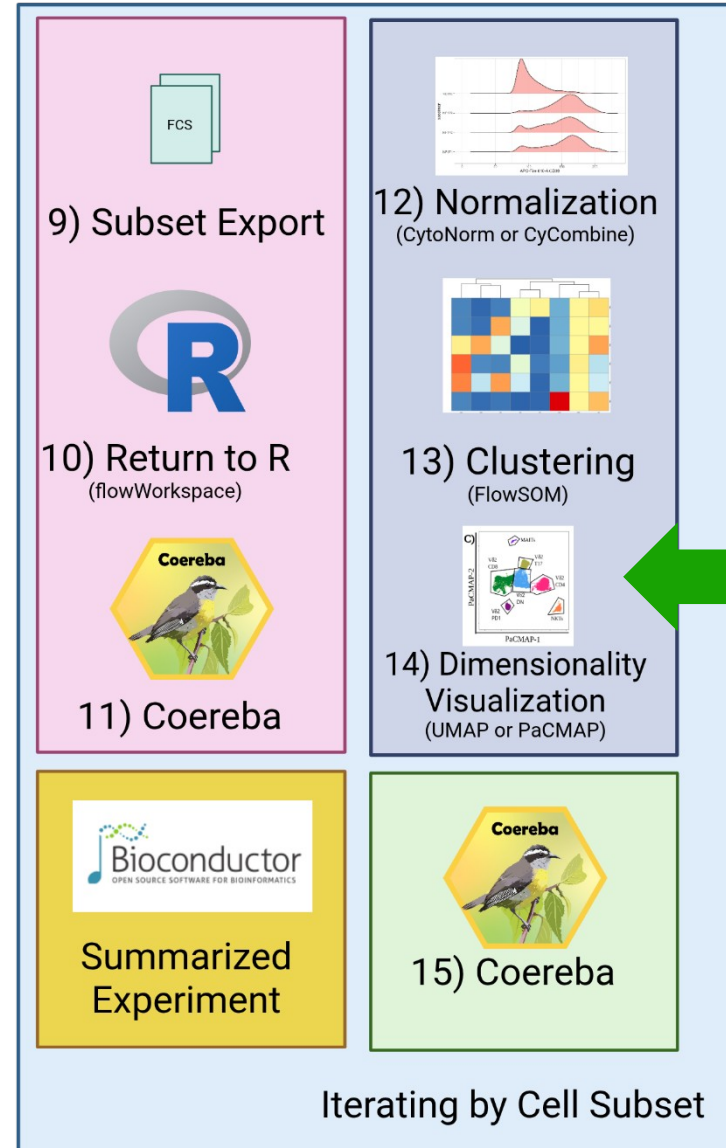
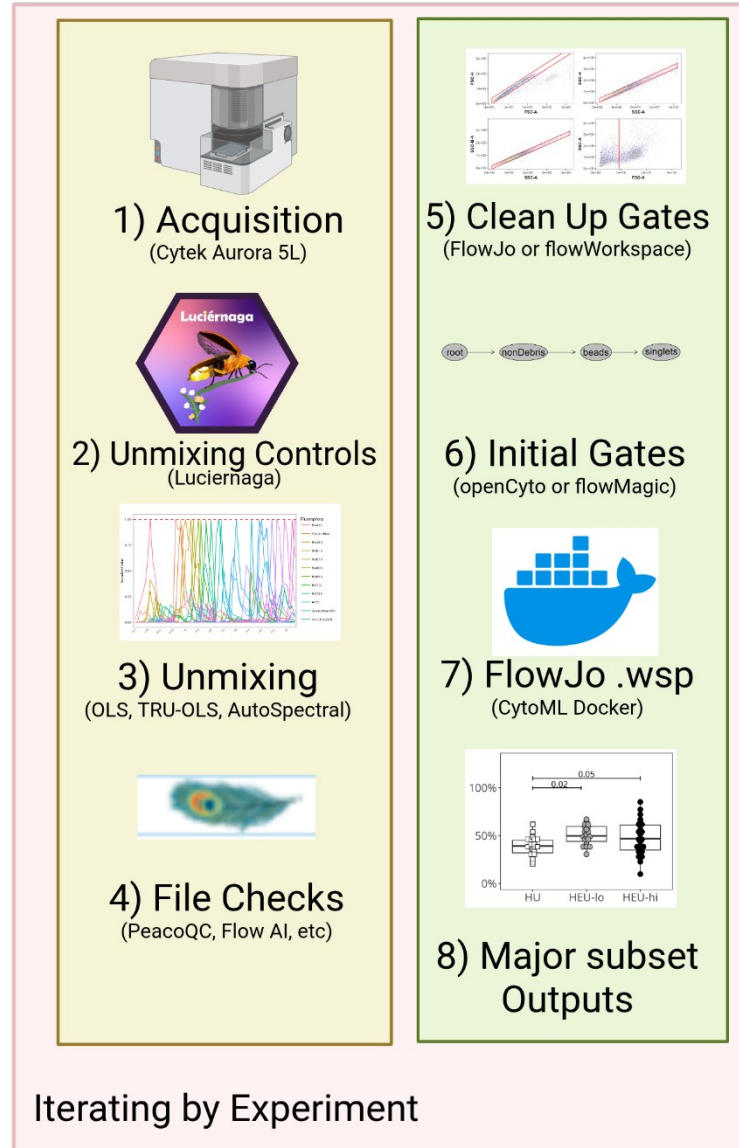


FlowSOM



FlowSOM
Vignettes

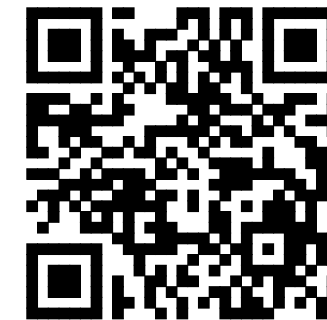
Pipeline: Dimensionality Visualization



UMAP



UMAP
Wrapper



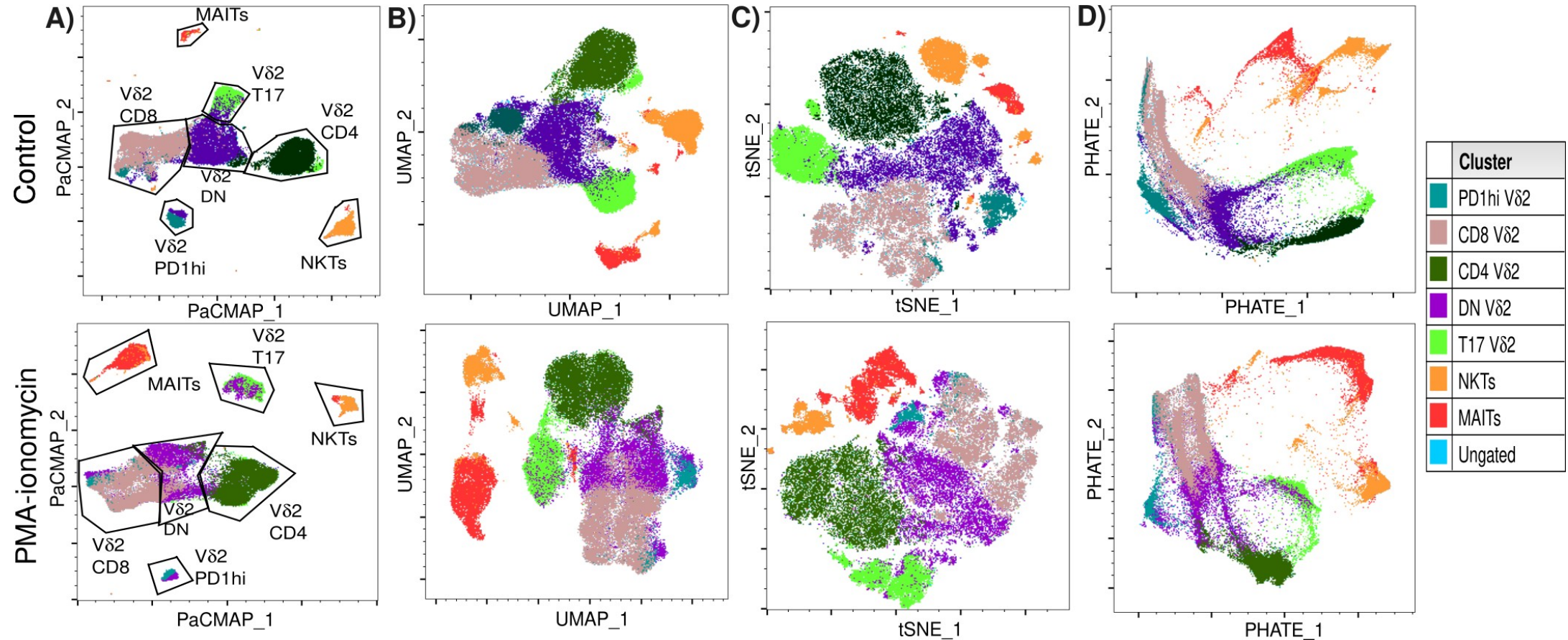
PaCMAP



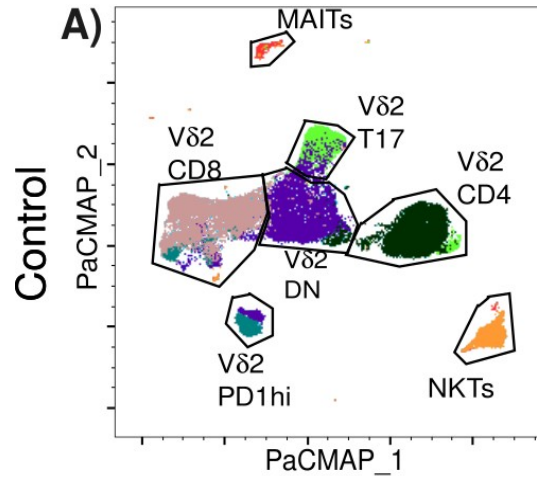
PaCMAP
Wrapper



Ability to contrast unsupervised methods vs. “manual” ground-truth



Retrieving and re-enumerating

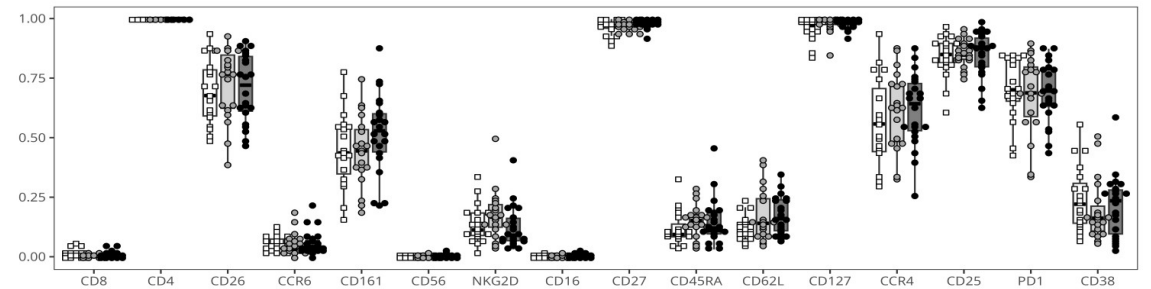


1) Gate

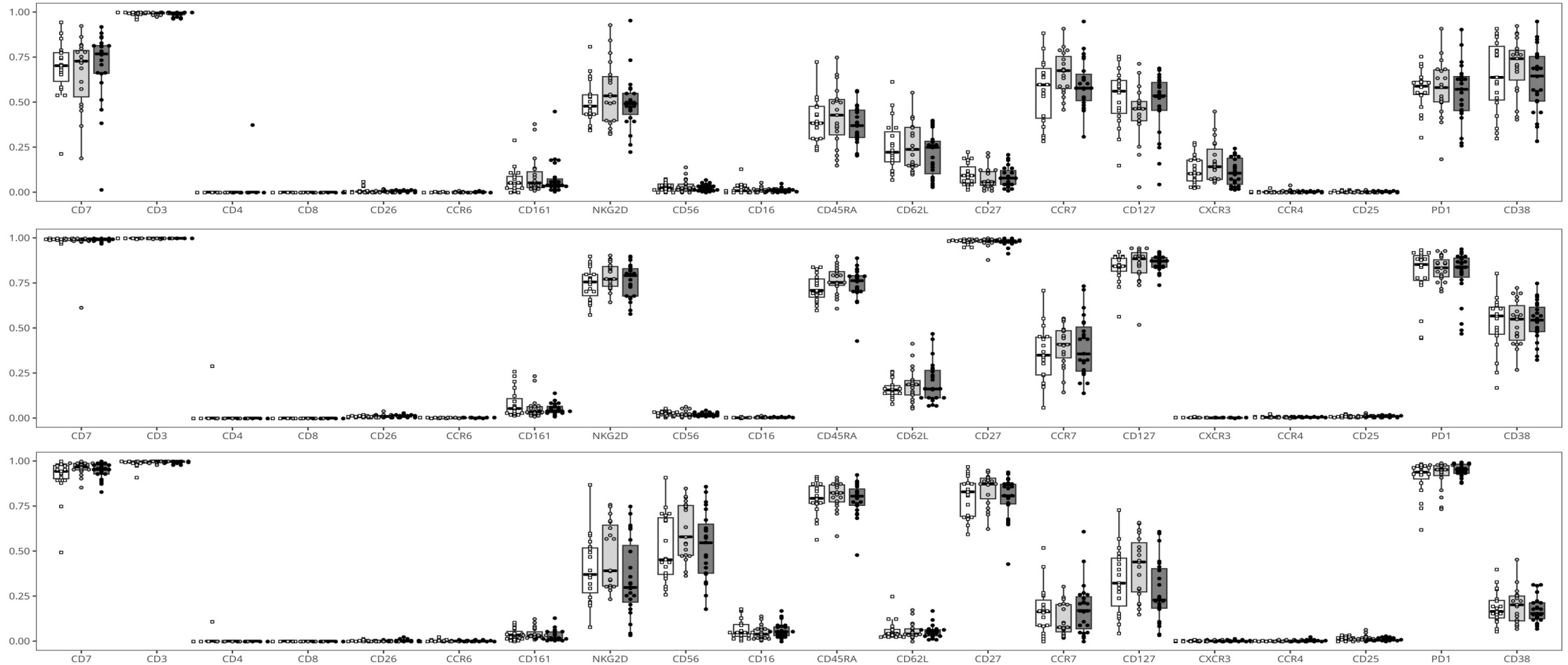
2) Retrieve manual-annotated gates

PEVio770pos	APCneg	AlexaFluor647neg	APCR700neg	APCFire750neg	APCFire810neg
PEVio770pos	APCneg	AlexaFluor647neg	APCR700neg	APCFire750neg	APCFire810neg
PEVio770pos	APCpos	AlexaFluor647neg	APCR700neg	APCFire750pos	APCFire810neg
PEVio770pos	APCneg	AlexaFluor647pos	APCR700neg	APCFire750neg	APCFire810pos

3) Re-enumerate and plot



For each island, re-aggregate cells from each donor



DN T cells

Cord blood T cells - Baseline

Marker	1	2	3	4	5	6	7	8
CD7	+++	+++	+++	+++	+++	+++	+++	+++
CD3	+++	+++	+++	+++	+++	+++	+++	+++
VD2	-	-	-	-	-	-	-	-
Va7.2	-	-	-	-	-	-	-	+++
Va24Ja18	-	-	-	-	-	-	-	-
CD8	-	-	-	-	-	-	-	-
CD4	+++	+++	+++	+++	+++	+++	+++	+++
CD26	-/+	++	+	++	+++	+++	+++	+++
CCR6	-	-	-	-	-	-	-	-
CD161	-	+	-	-	-	-	++	-
NKG2D	-	-	-/+	-	-	-	+++	-
CD56	-	-	-	-	-	-	+++	-
CD16	-	-	-	-	-	-	+	-
CD62L	-/+	-	-	+++	-	+++	+	+
CD27	+++	+++	+	+++	+++	+++	+++	+++
CD45RA	+++	+	+++	+++	+++	+++	+++	+++
CD127	-	+++	+++	+++	+++	+++	+++	+++
CD25	+++	+++	-	-	-	-	-	-
CCR4	-	+++	-	-	-	-	-	-
PD1	-/+	+	-	-	-	-	-	-
CD38	+++	+	+++	+++	+++	+++	+++	+++

CD4 T cells

Marker	1	2	3	4	5	6	7	8	9	10	11
CD7	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++
CD3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
VD2	-	-	-	-	-	-	-	-	-	-	-
Va7.2	+++	-	-	-	-	-	-	-	-	-	-
Va24Ja18	-	-	-	-	-	-	-	-	-	-	-
CD8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
CD4	-	-	-	-	-	-	-	-	-	-	-
CD26	++	-	+	++	-/+	+++	+++	-	-	-	-
CCR6	-	-	-	-	-	-	+++	-	-	-	-
CD161	-	++	-	-	-	+++	+++	-	-	-/+	+++
NKG2D	+++	+++	+++	+++	+++	+++	+++	+++	-	+++	+++
CD56	-	-	-	-	-	-	-	-	-/+	-	+++
CD16	-	+++	-	-	-	-	-	-	-	-	+++
CD62L	-	-	+++	-	++	-	-	-/+	-	-	-
CD27	+++	+	+++	+++	+	+++	+++	+++	+++	+	-/+
CD45RA	+++	+++	+++	+++	+++	+++	+++	++	++	+++	+++
CD127	+++	-	+++	+++	+++	+++	+++	+++	-/+	-/+	-
CD25	-	-	-	-	-	-	+	-	-	-	-
CCR4	-	-	-	-	-	-	+	-	-	-	-
PD1	-	++	-	-	-	-	-	+	+++	+	-
CD38	+++	+++	+++	+++	+++	+++	-	-/+	+	++	+++

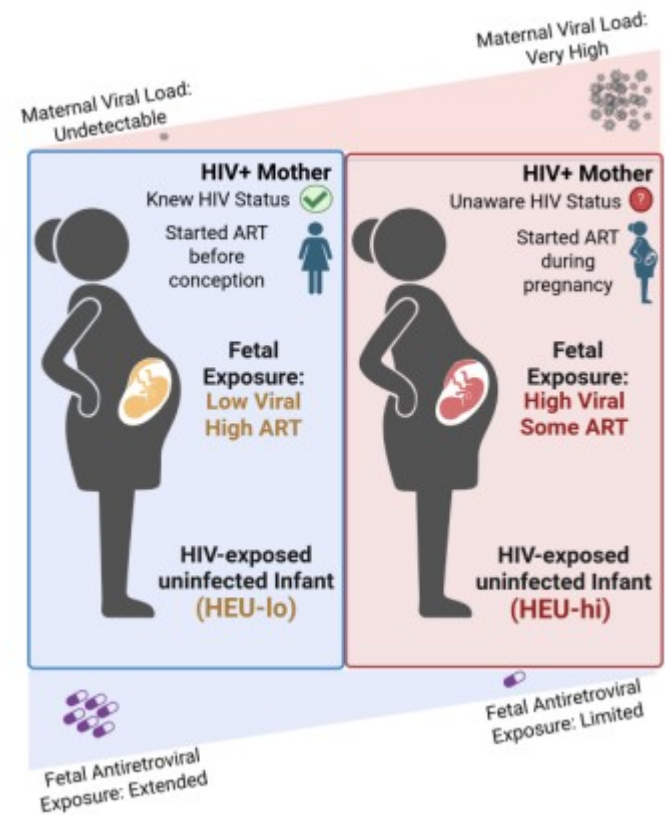
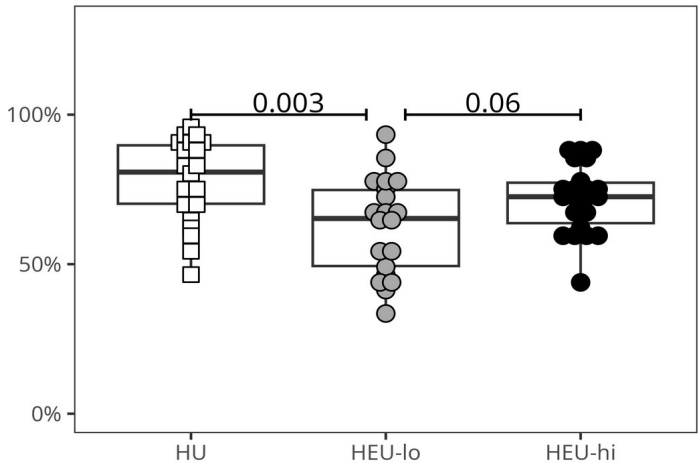
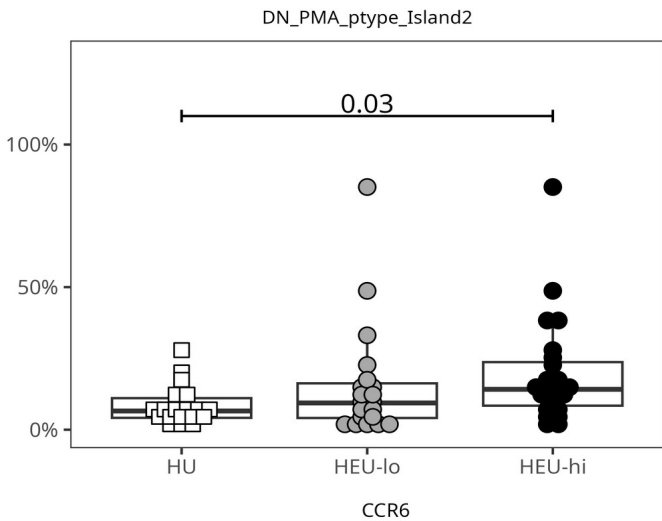
CD8 T cells

Marker	1	2	3	4	5
CD7	++	+++	+++	+++	+++
CD3	+++	+++	+++	+++	+++
VD2	-	-	-	-	-
Va7.2	-	-	-	-	-
Va24Ja18	-	-	-	-	-
CD8	-	-	-	-	-
CD4	-	-	-	-	-
CD26	-	-	-	+++	-
CCR6	-	-	-	+++	-
CD161	-	-	-	+++	+++
NKG2D	++	+++	+	+++	+++
CD56	-	-	++	-	+++
CD16	-	-	-	-	+++
CD62L	-/+	-/+	-	-	-
CD27	-	+++	+++	+++	-/+
CD45RA	+	+++	+++	+++	+++
CD127	++	+++	+	+++	-
CD25	-	-	-	++	-
CCR4	-	-	-	++	-
PD1	++	+++	+++	-/+	-
CD38	++	++	-/+	-	+++

DN T cells

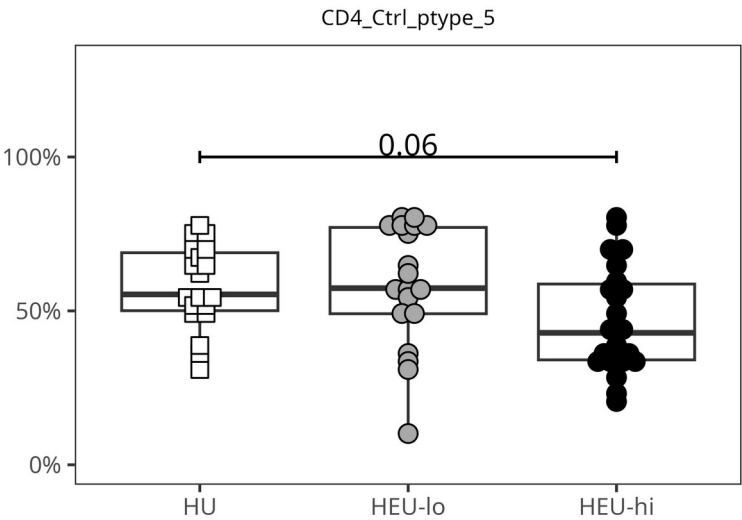
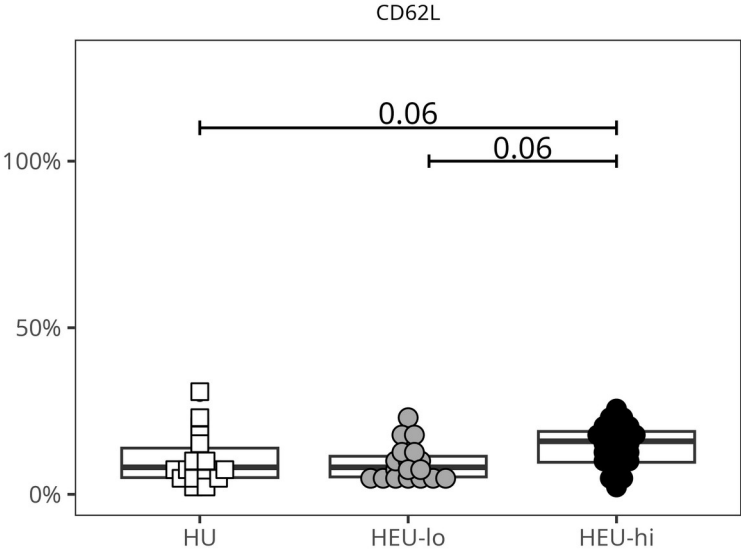
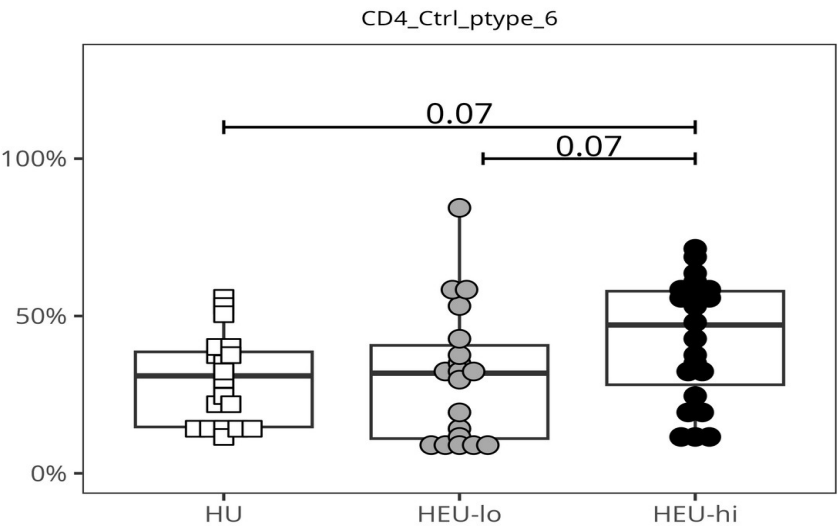
CD4-CD8- Cord blood T cell subsets driving observed IFN γ increases

Marker	1	2	3	4
CD7	+++	+++	+++	+++
CD3	+++	+++	+++	+++
VD2	-	-	-	-
Va7.2	-	-	-	-
Va24Ja18	-	-	-	-
CD8	-	-	-	-
CD4	-	-	-	-
CD26	+++	-	-	-
CCR6	++	-	-	-
CD161	+++	+++	-	-
NKG2D	+++	+++	+++	-
CD56	-	++	-	-/+
CD16	-	-	-	-
CD62L	-	-	-	-
CD27	+++	-	+++	++
CD45RA	+++	+++	+++	+++
CD127	+++	-	+++	-
CD25	++	-	-	-
CCR4	-	-	-	-
PD1	++	-/+	+++	+++
CD38	-	+++	++	+
CD69	+++	+++	+++	+++
TNF α	+	++	-	-
IFN γ	-	++	-	-/+
CD107a	-	-	-	-



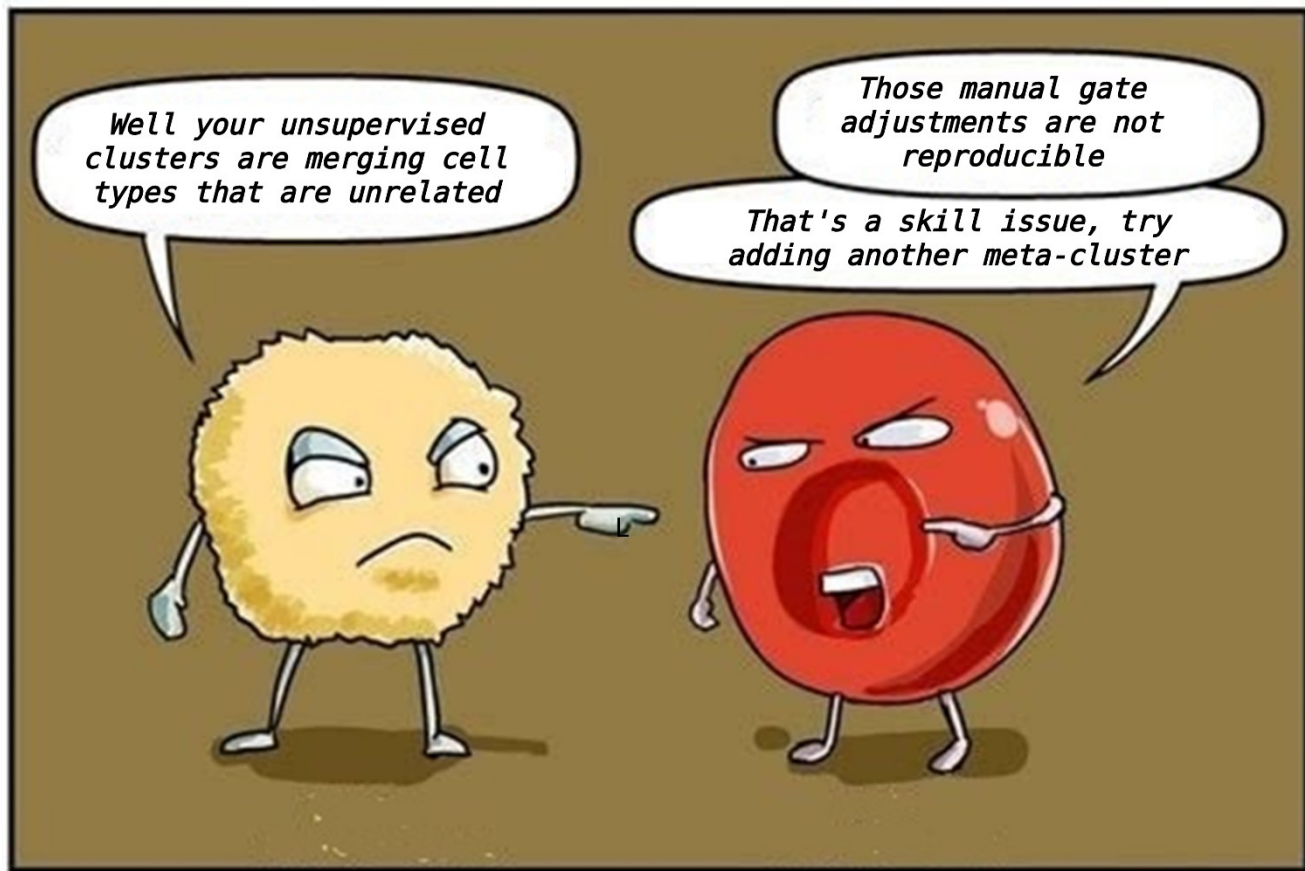
Conventional Cord blood T cell subsets also show changes in CD62L

Marker	1	2	3	4	5	6	7	8
CD7	+++	+++	+++	+++	+++	+++	+++	+++
CD3	+++	+++	+++	+++	+++	+++	+++	+++
VD2	-	-	-	-	-	-	-	-
Va7.2	-	-	-	-	-	-	-	+++
Va24Ja18	-	-	-	-	-	-	-	-
CD8	-	-	-	-	-	-	-	-
CD4	+++	+++	+++	+++	+++	+++	+++	+++
CD26	-/+	++	+	++	+++	+++	+++	+++
CCR6	-	-	-	-	-	-	-	-
CD161	-	+	-	-	-	-	++	-
NKG2D	-	-	-/+	-	-	-	+++	-
CD56	-	-	-	-	-	-	+++	-
CD16	-	-	-	-	-	-	+	-
CD62L	-/+	-	-	+++	-	+++	+	+
CD27	+++	+++	+	+++	+++	+++	+++	+++
CD45RA	+++	+	+++	+++	+++	+++	+++	+++
CD127	-	+++	+++	+++	+++	+++	+++	+++
CD25	+++	+++	-	-	-	-	-	-
CCR4	-	+++	-	-	-	-	-	-
PD1	-/+	+	-	-	-	-	-	-
CD38	+++	+	+++	+++	+++	+++	+++	+++



One-way Anova

Functional and Iterative: plenty for everyone to dislike :)



RunawayLabBook.com

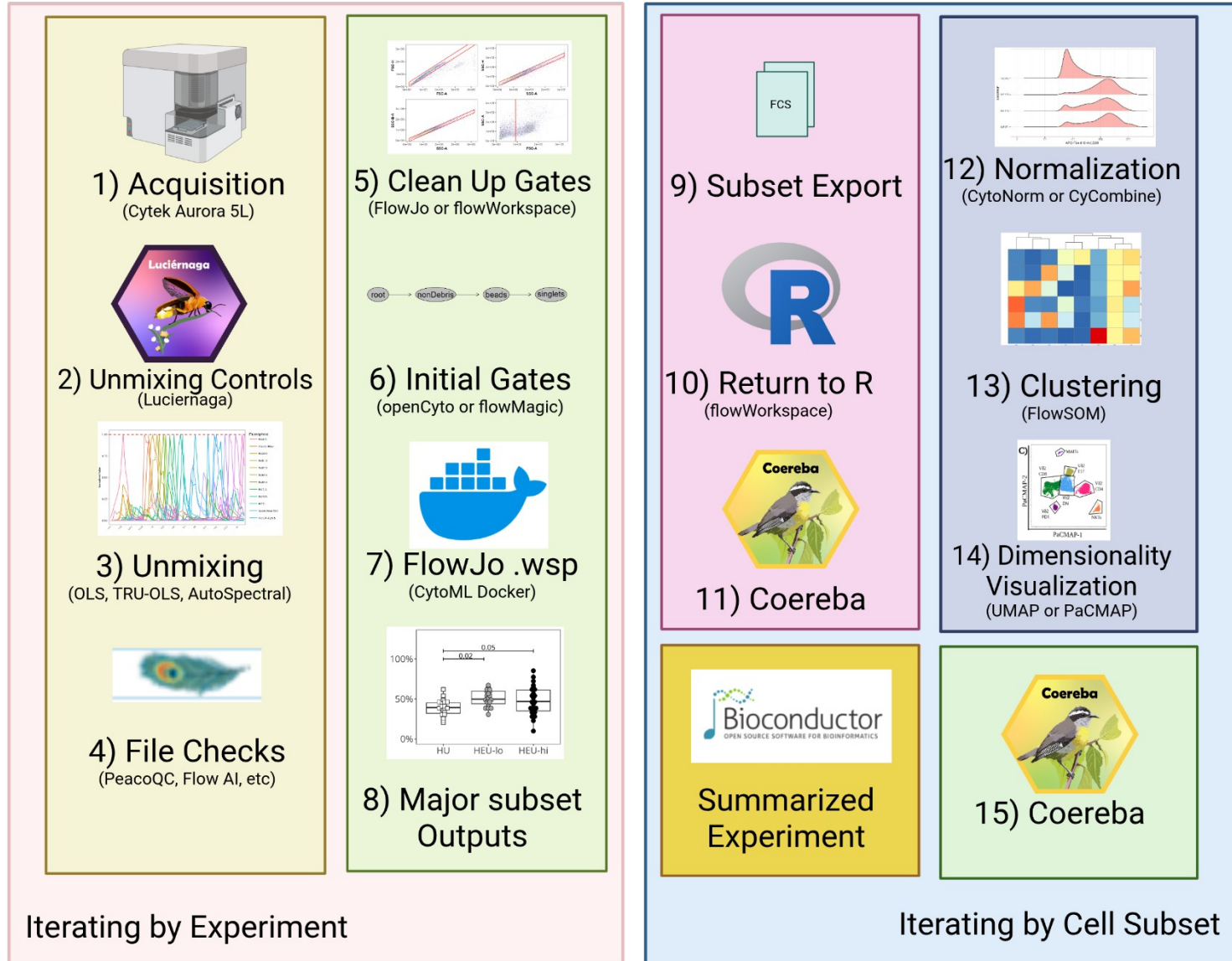
Fun off-label uses thus far:

- ❑ Contrasting where “manually-gated” cells end up vs. our unsupervised methods (normalization, clustering, etc.)
- ❑ Added benefit of looking-at-the-forest: Easy to identify badly cryopreserved specimens where cell populations were lost

Are our high-dimensional analysis problems fixed?



Semi-supervised Pipeline

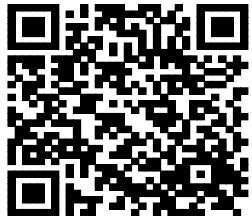
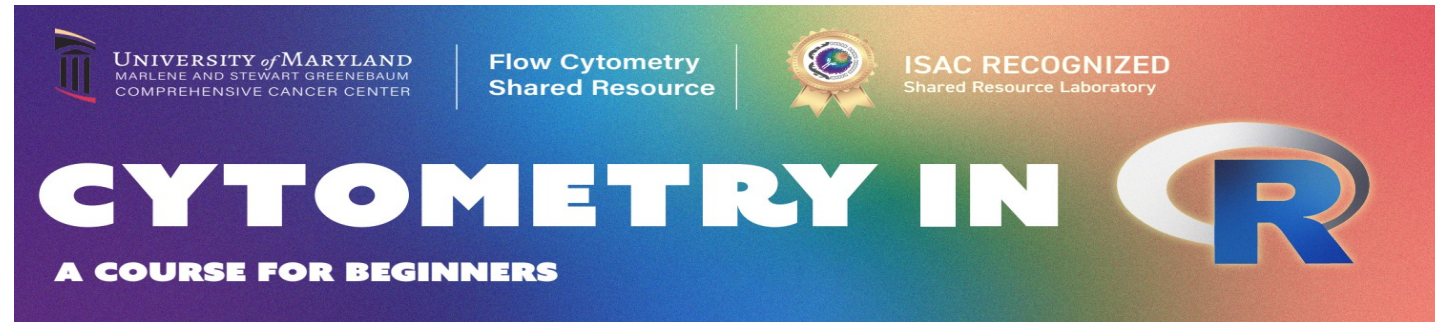
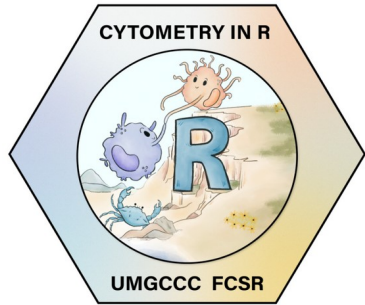


What good is any tool if the community is not able to use it?

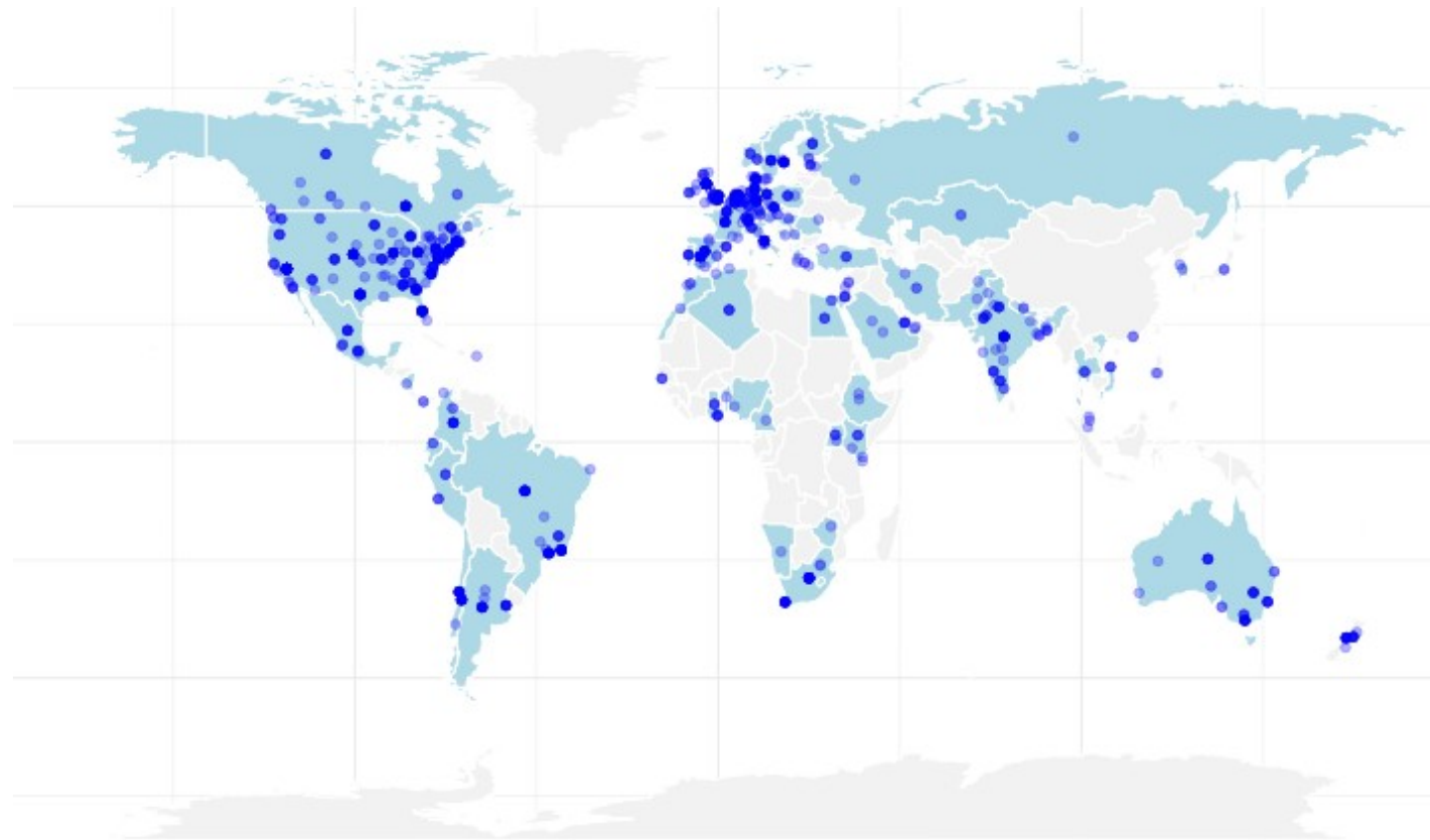
- ❑ Few resources to learn Cytometry in R to begin with
- ❑ Those that exist aimed at those with intermediate-advanced R skills, not beginners
- ❑ Know-how concentrated at a few institutions
- ❑ Most self-learning attempts end in frustration



Cytometry in R: A free weekly course for flow cytometrist with no-to-little coding experience



Website



YouTube

UMGCCC FCSR
@CytometryInR • 848 subscribers • 21 videos
University of Maryland Greenebaum Comprehensive Cancer Center Flow Cytometry Shared Resource
umgcccfcscr.github.io/CytometryInR
Subscribed

Live Playlists Posts

Latest Popular Oldest

PhD Dissertation Defense - David Rach
Scheduled for 6/17/26, 12:00 PM
Notify me

11 - Data for Statistics
Cytometry Core • 11 - Data for Statistics
AUTHOR David Rach
PUBLISHED May 5, 2026
101 views • Streamed 3 weeks ago

10 - Downsampling and Concatenation
Cytometry Core • 10 - Downsampling and Concatenation
AUTHOR David Rach
PUBLISHED April 29, 2026
129 views • Streamed 1 month ago

09 - It's Raining Functions!
Cytometry Core • 09 - It's Raining Functions!
AUTHOR David Rach
PUBLISHED April 19, 2026
172 views • Streamed 1 month ago

08 - Manual and Automated Gating
Cytometry Core • 08 - Manual and Automated Gating
AUTHOR David Rach
PUBLISHED April 7, 2026
226 views • Streamed 1 month ago

07 - Applying Transformations
Cytometry Core • 07 - Applying Transformations
AUTHOR David Rach
PUBLISHED March 26, 2026
228 views • Streamed 2 months ago

06 - Visualizing with ggplot2
Cytometry Core • 06 - Visualizing with ggplot2
AUTHOR David Rach
PUBLISHED March 17, 2026
228 views • Streamed 2 months ago

05 - Gating Sets
Cytometry Core • 05 - Gating Sets
AUTHOR David Rach
PUBLISHED March 1, 2026
253 views • Streamed 2 months ago

- Three live-streams a week (accommodating different timezones and schedules)
- Recordings available immediately after
- Best of the weekly livestreams added to the playlist, cutting down on editing

Website

- ❑ Created with Quarto, hosted via GitHub pages.
- ❑ Code, code outputs, screenshots and explanations
- ❑ Searchable
- ❑ New weekly material added as a new .qmd file

The screenshot displays the 'Cytometry i...' website. The top navigation bar includes links for 'About', 'Schedule', 'Course', 'Announcements', 'Existing Resources', 'Discussions', and 'Homeworks'. A light blue banner at the top reads: 'See Course tab for Course Materials, see YouTube tab for livestream recordings'. The main content area is titled 'Lets recreate our GatingSet object:' and contains R code for loading data, creating a GatingSet, and applying a Biexponential gate. Below the code, it states 'A GatingSet with 6 samples' and explains that the next gate will automatically pop up in the Shiny App. The bottom section, 'Draw your gate', features a 'Bioset' panel with 'Gate Type' options (Rectangle, Polygon, Span, Quadrant) and 'X'/'Y' Minimum/Maximum input fields. To the right is a flow cytometry plot showing a population of cells with a gate drawn around them. A sidebar on the right lists 'On this page' links: Background, Walk Through, Setup, Manual - flowGate, Polygon, Rectangle, Span, Quadrant, Redraw Gate, Saving Gates, Rapid Gate Deployment (highlighted), Visualizing Gates, Summary, Automated - openCyto, Take Away, Additional Resources, and Take-home Problems.

GitHub

The screenshot shows the GitHub repository page for 'CytometryInR' by 'UMGCCCCFSR'. The repository is public and has 11 watchers, 603 forks, and 388 stars. The main branch is 'main'. The repository contains a file tree with folders like 'course', 'docs', and 'images', and files like '.gitignore', 'Announcements.qmd', 'CODE_OF_CONDUCT.md', 'CONTRIBUTING.md', 'ExistingResources.qmd', 'Homeworks.qmd', 'LICENSE', 'LICENSE_CC-BY-SA', 'PackageWalkthroughs.qmd', 'README.md', 'Schedule.qmd', '_quarto.yml', and 'index.qmd'. The right sidebar shows the 'About' section with a link to the course website, 'Readme', 'AGPL-3.0, CC-BY-SA-4.0 licenses found', 'Code of conduct', 'Contributing', 'Activity', '388 stars', '11 watching', '603 forks', and 'Report repository'. Below this is the 'Contributors' section showing 'DavidRach' and 'UMGCCCCFSR'. The 'Languages' section shows a bar chart with 'R' at 85.4%, 'SCSS' at 12.4%, 'JavaScript' at 1.5%, and 'GLSL' at 0.7%.

UMGCCCCFSR / CytometryInR

<> Code Issues Pull requests 74 Agents Discussions Actions Security and quality Insights Settings

CytometryInR Public

Watch 11 Fork 603 Star 388

main 12 Branches 0 Tags

Go to file

Code

DavidRach Homework folders week 10 and 11 (thanks Lynn)... 8ee3359 · 2 days ago 101 Commits

course	Homework folders week 10 and 11 (thanks Lynn...	2 days ago
docs	Homework folders week 10 and 11 (thanks Lynn...	2 days ago
images	Community Package Walkthrough for TRU-OLS	last week
.gitignore	Start of course material transfer	5 months ago
Announcements.qmd	Week 11 slides	last month
CODE_OF_CONDUCT.md	Create CODE_OF_CONDUCT.md	5 months ago
CONTRIBUTING.md	Create CONTRIBUTING.md	5 months ago
ExistingResources.qmd	Existing Resources Links	2 months ago
Homeworks.qmd	Week 11 slides	last month
LICENSE	Start of course material transfer	5 months ago
LICENSE_CC-BY-SA	Start of course material transfer	5 months ago
PackageWalkthroughs.qmd	Updated link to Week #3 recording	4 months ago
README.md	Downsampling - Week 10. Slides and Concatena...	2 months ago
Schedule.qmd	Homework folders week 10 and 11 (thanks Lynn...	2 days ago
_quarto.yml	Homework folders week 10 and 11 (thanks Lynn...	2 days ago
index.qmd	Updated in-person schedule, removed course st...	4 months ago

About

Course Website

umgccccfsr.github.io/CytometryInR/

Readme

AGPL-3.0, CC-BY-SA-4.0 licenses found

Code of conduct

Contributing

Activity

388 stars

11 watching

603 forks

Report repository

Contributors 2

DavidRach David Rach

UMGCCCCFSR UMGCC FCSR

Languages

R 85.4% SCSS 12.4% JavaScript 1.5% GLSL 0.7%

- Code and datasets available via the GitHub repository
- Course participants start course by creating GitHub account and forking the repository
- After Positron is linked to GitHub account, pull locally
- When new material posted each week, sync and pull

Discussion Forum

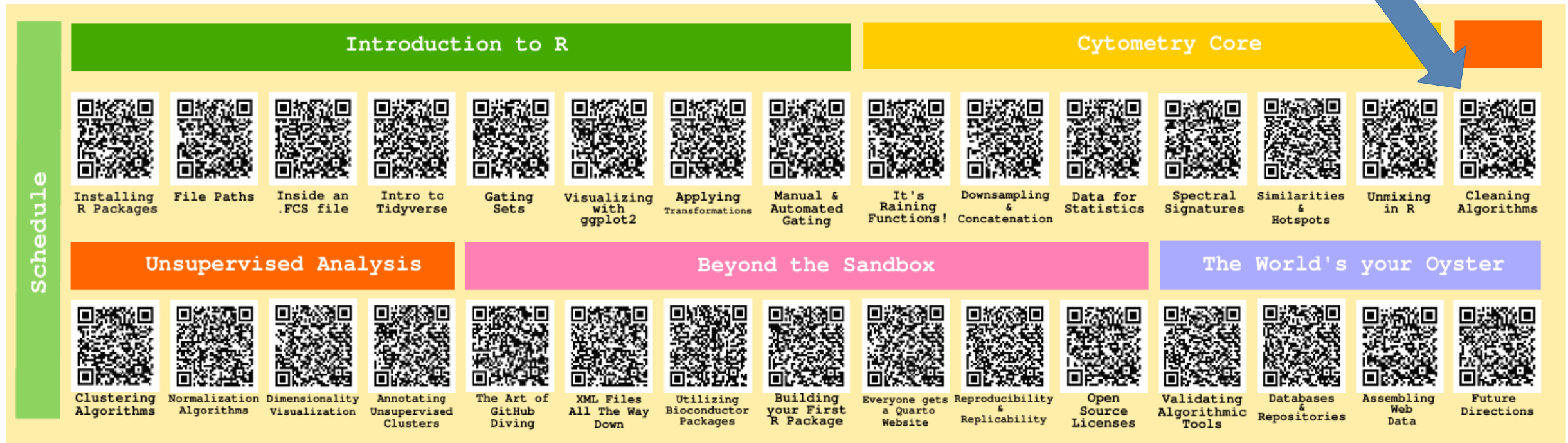
- GitHub Discussions page has been repurposed as a community forum for questions, etc.

The screenshot displays the GitHub Discussions interface for the repository `umgcccfcscr`. At the top, navigation tabs include Discussions, Actions, Security and quality, Insights, and Settings. Below these are four featured discussion cards: 'Introductions', 'Week #11 - General Discussion Thread', 'Cytometry in R - Week #11 - Data for Statistics', and 'Cytometry in R - Course Update and Bonus...'. A search bar shows 'is:open' and filters for 'Sort by: Latest activity', 'Label', and 'Filter: Open', with a 'New discussion' button. The left sidebar shows 'Categories' with links to 'View all discussions', 'Announcements', 'General', 'Ideas', 'Introductions', 'Polls', 'Q&A', and 'Show and tell'. A 'Most helpful' section lists a discussion by `mpatgaonkar-coder`. The main 'Discussions' list includes topics like 'Cytometry in R - Week #07 - Applying Transformations', 'Questions about tidy data', 'Pull error on Week 06 branch', 'Copying index file', 'Week #1 - General Discussion Thread', 'Cytometry in R - Week #6 - Visualizing with ggplot2', 'GitHub personal access token', 'clock appears when trying to pull from git & commit', 'Bioconductor Cytometry R Packages', and 'No Class This Week'. Each entry shows the topic, author, date, and response count.

ns/categories/announcements



Half-way through this passion project



- ❑ Wrapping up Cytometry Core module
- ❑ Gathering steam to start on the Unsupervised Analysis section



UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

All mothers and their infants, and
their communities

All the open-source contributors,
developers, and maintainers, that
collectively made the tool sets
without which all of this would
not be feasible

Dr. Cristiana Cairo
Dr. Kirsten E. Lyke
Dr. Xiaoxuan Fan

Dr. Daniela Franco
Dr. Natarajan Ayithan



UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM
COMPREHENSIVE CANCER CENTER

Acknowledgments

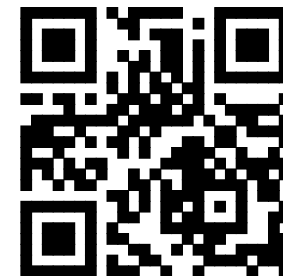


U01 HD092309

This publication was supported by funds
through Maryland Department of Health's
Cigarette Restitution Fund Program – CH-
649-CRF and the National Cancer Institute
– Cancer Center Support Grant (CCSG) –
P30CA134274.d Service



KAMUZU
UNIVERSITY
OF HEALTH SCIENCES



Cytometry
Discord



Bioconductor
Zulip



<https://github.com/DavidRach>



@davidrach.bsky.social



www.linkedin.com/in/david-rach/

Want to collaborate? Reach out!

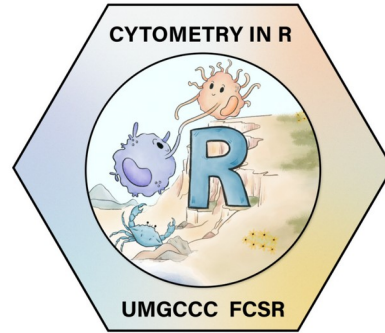


ISAC RECOGNIZED
Shared Resource Laboratory

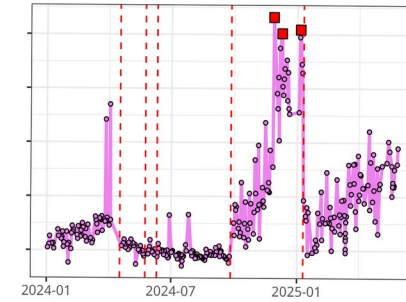
Questions?



Luciernaga



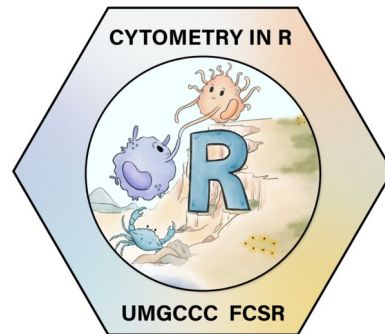
Website - CytometryInR



UMGCCC FCSR
InstrumentQC dashboard



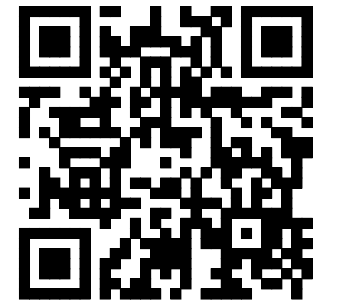
Coereba



YouTube @CytometryInR



InstrumentQC How-To



Biology is (fortunately) not just math



- 29-color SFC panel
→ 536 million (theory)

When actually exploring our
panels dimensional space

CD4 T cells → 16241

CD8 T cells → 12466

DN T cells → 9035