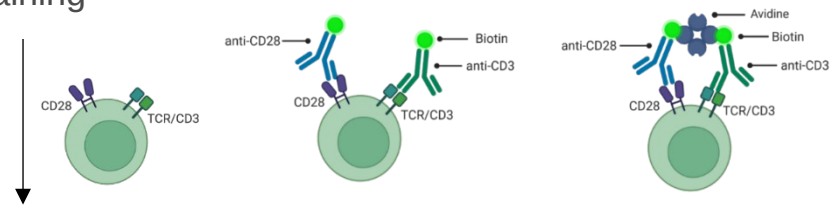
20250923 pTCR – cHCV samples
Batch effect removal

Outlook

surface staining



- 70 min	- 10 min	Incubation on ice	0 min	resuspension	0 min	Incubation at 37°C	2, 5, 10 min etc
1h resting 37°C, starved RPMI	Add anti-CD3 biotin anti-CD28 biotin + IL2 +IL15 +IL21 +IL21 PAC		Add Neutravidine		Transfer to waterbath add metal beads		Stop stimulation

Sample barcoding

Sample staining

Stain for phenotypic & signaling Molecules

Backbone			phospho Marker		
CD3	CD127	TOX	pZAP-70 (Y319)	pSLP-76 (Y128)	pAkt (S473)
CD4	CXCR5	T-bet	pSHP-2 (Y580)	pNFATc1 (Y172)	pSTAT3 (Y705)
CD8	CD45RA	TCF-1	pPLCγ (Y783)	pCREB (S133)	
CD27	CD45RO	TIGIT	pp38 (T180/Y182)	pGSK3β (S9)	pAMPKα (T172)
CD28	CCR7	2B4	pMK2 (T334)	pPGC1α (S571)	Ikbα
CD57	CD39	PD-1	pMEK1 (S298)	pS6 (S235/236)	pSTAT3 (Y727)
CD45		Eomes	pErk1/2 (T202/Y204)	NFATc1	pSTAT5 (Y694)

Samples

1: CD3
2: CD3
3: CD3/28
4: CD3/28
5: CD3/28+IL2
6: CD3/28+IL2
7: CD3/28+IL15
8: CD3/28+IL15
9: CD3/28+IL21 PAC
10: CD3/28+IL21 PAC
11: CD3/28+IL21
12: CD3/28+IL21

Batch#1 (acquisition day1)

Batch#2 (acquisition day3)

Batch#3 (acquisition day3)

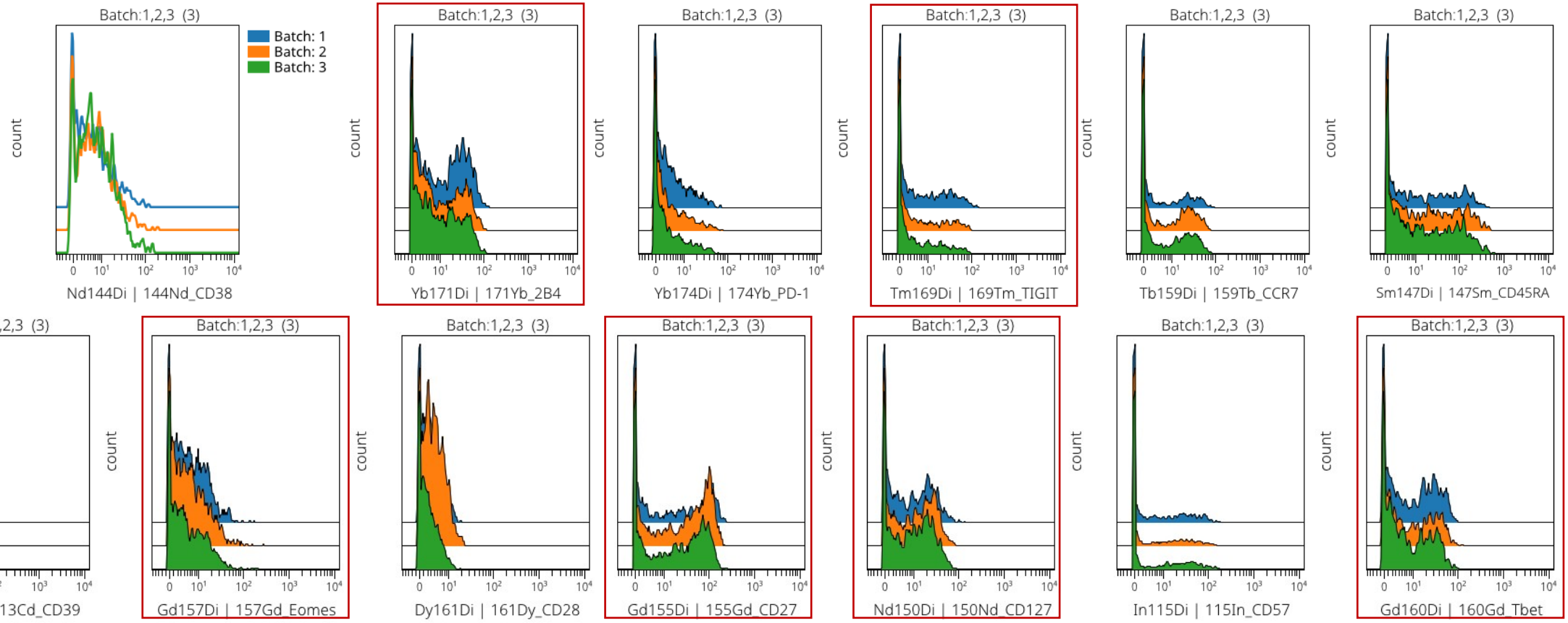
CyTOF analysis

Bead normalization of all files

Anchor sample (unstimlated Hdler) in each of those 12 samples

Anchor sample of Batch#1-3

Backbone			phospho Marker		
CD3	CD127	TOX	pZAP-70 (Y319)	pSLP-76 (Y128)	pAkt (S473)
CD4	CXCR5	T-bet	pSHP-2 (Y580)	pNFATc1 (Y172)	pSTAT3 (Y705)
CD8	CD45RA	TCF-1	pPLCγ (Y783)	pCREB (S133)	
CD27	CD45RO	TIGIT	pp38 (T180/Y182)	pGSK3β (S9)	pAMPKα (T172)
CD28	CCR7	2B4	pMK2 (T334)	pPGC1α (S571)	Iκbα
CD57	CD39	PD-1	pMEK1 (S298)	pS6 (S235/236)	pSTAT3 (Y727)
CD45		Eomes	pErk1/2 (T202/Y204)	NFATc1	pSTAT5 (Y694)



Optsne of all samples – Backbone marker

Backbone			phospho Marker		
CD3	CD127	TOX	pZAP-70 (Y319)	pSLP-76 (Y128)	pAkt (S473)
CD4	CXCR5	T-bet	pSHP-2 (Y580)	pNFATc1 (Y172)	pSTAT3 (Y705)
CD8	CD45RA	TCF-1	pPLCγ (Y783)	pCREB (S133)	
CD27	CD45RO	TIGIT	pp38 (T180/Y182)	pGSK3β (S9)	pAMPKα (T172)
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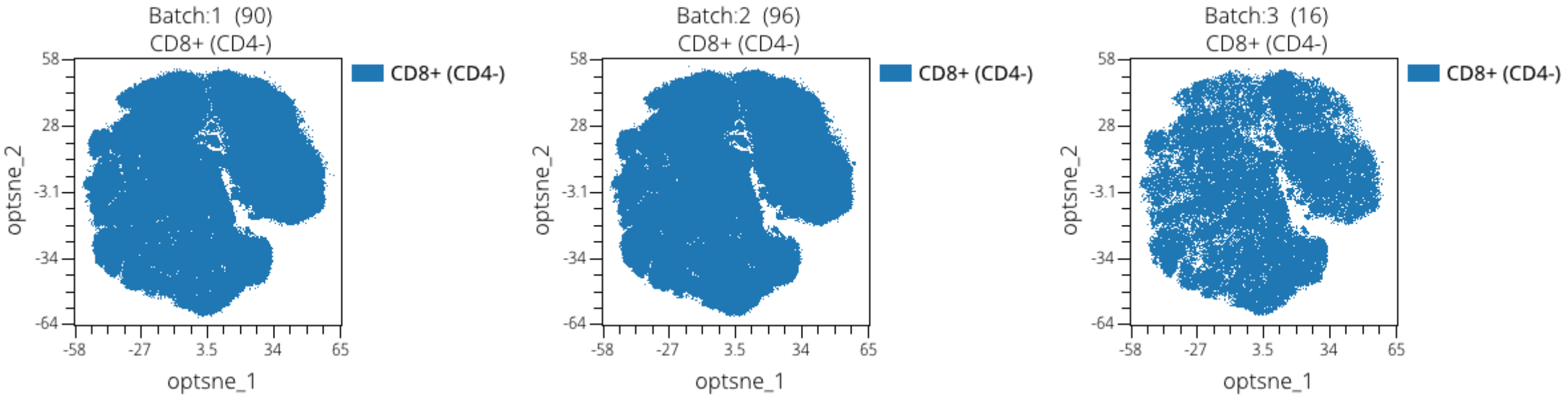
CD57	CD39	PD-1	pMEK1 (S298)	pS6 (S235/236)	pSTAT3 (Y727)
CD45		Eomes	pErk1/2 (T202/Y204)	NFATc1	pSTAT5 (Y694)



Optsne of all samples – Backbone marker

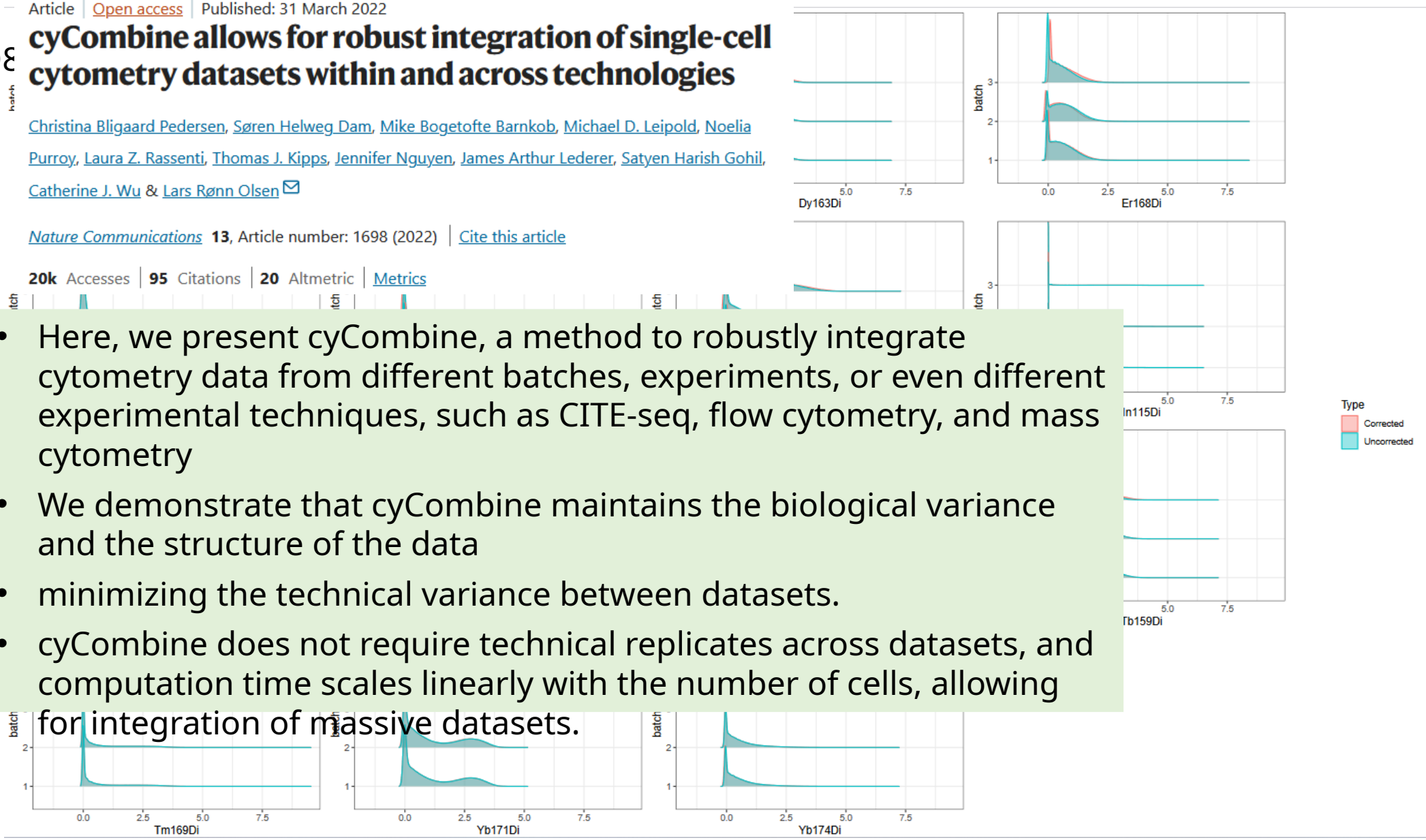
OMIQ
cyCombine on CD8

Article | Open access | Published: 31 March 2022

cyCombine allows for robust integration of single-cell cytometry datasets within and across technologies

Christina Bligaard Pedersen, Søren Helweg Dam, Mike Bogetofte Barnkob, Michael D. Leipold, Noelia Purroy, Laura Z. Rassenti, Thomas J. Kipps, Jennifer Nguyen, James Arthur Lederer, Satyen Harish Gohil, Catherine J. Wu & Lars Rønn Olsen

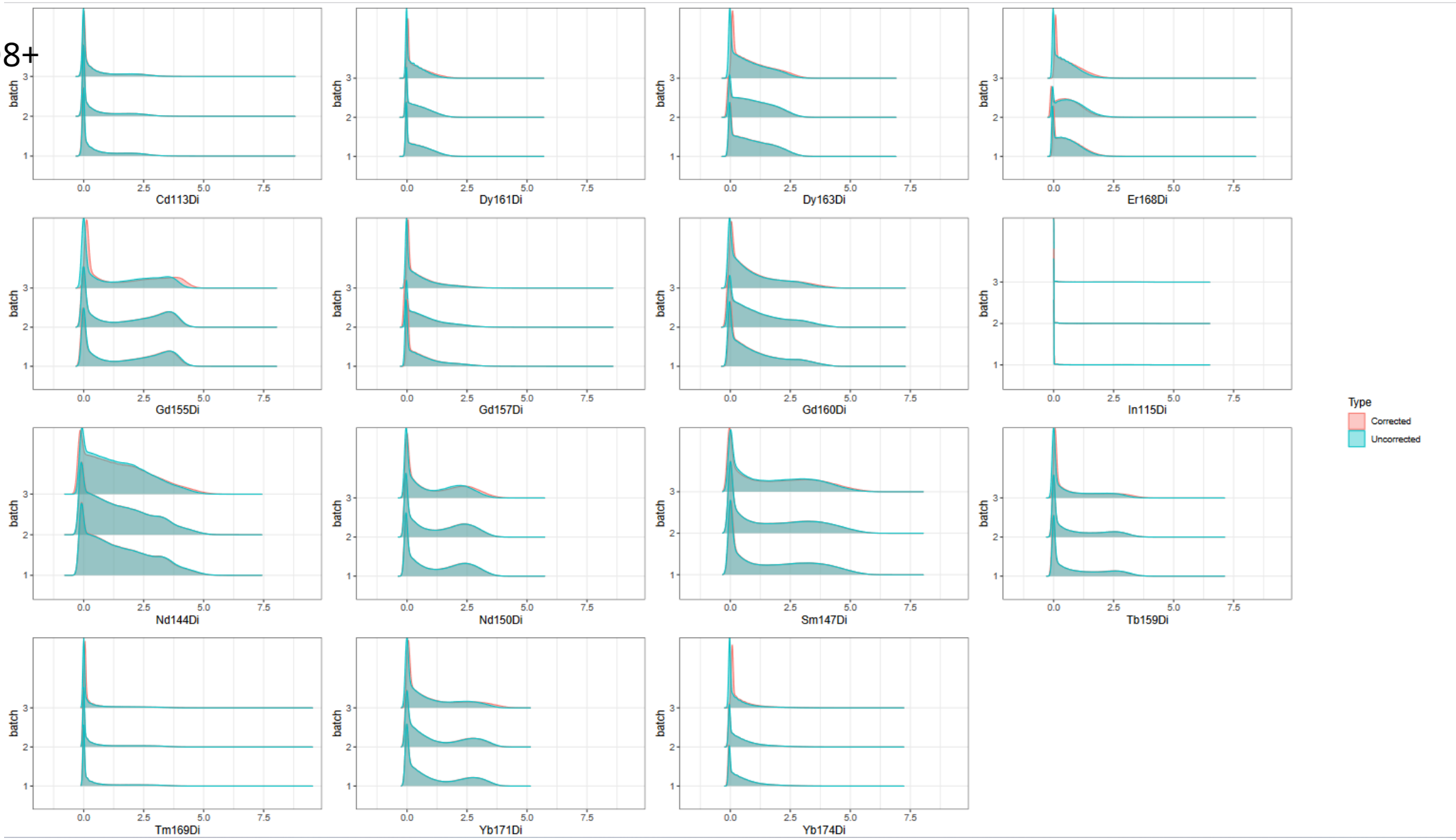
Nature Communications **13**, Article number: 1698 (2022) | Cite this article



- Here, we present cyCombine, a method to robustly integrate cytometry data from different batches, experiments, or even different experimental techniques, such as CITE-seq, flow cytometry, and mass cytometry
- We demonstrate that cyCombine maintains the biological variance and the structure of the data
- minimizing the technical variance between datasets.
- cyCombine does not require technical replicates across datasets, and computation time scales linearly with the number of cells, allowing for integration of massive datasets.

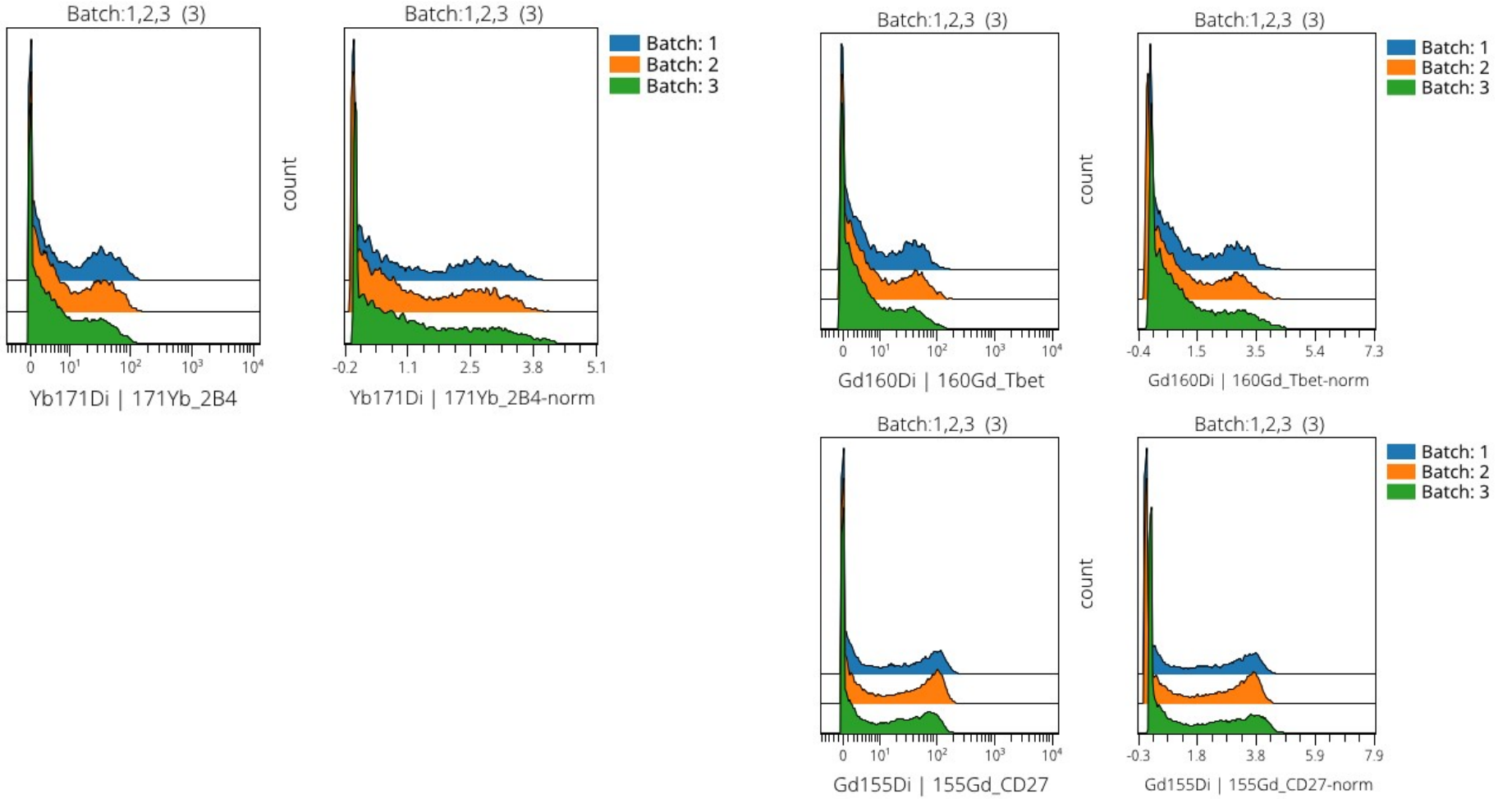
Optsne of all samples – Backbone marker

OMIQ
cyCombine on CD8+



Optsne of all samples – Backbone marker

OMIQ
cyCombine on CD8+

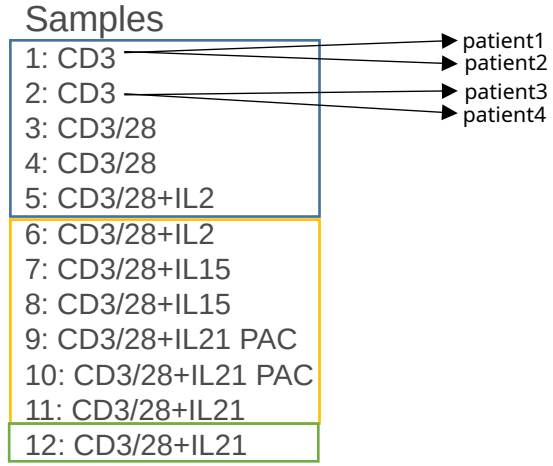


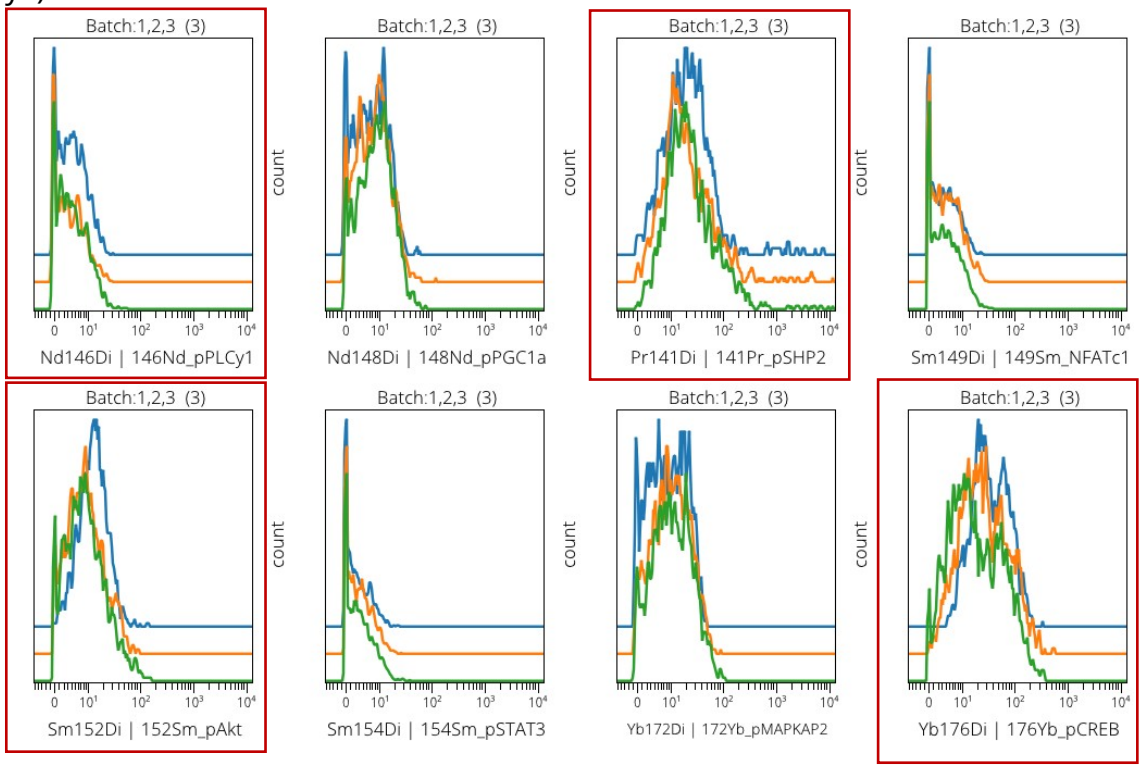
Anchor sample of Batch#1-3 - unstimulated

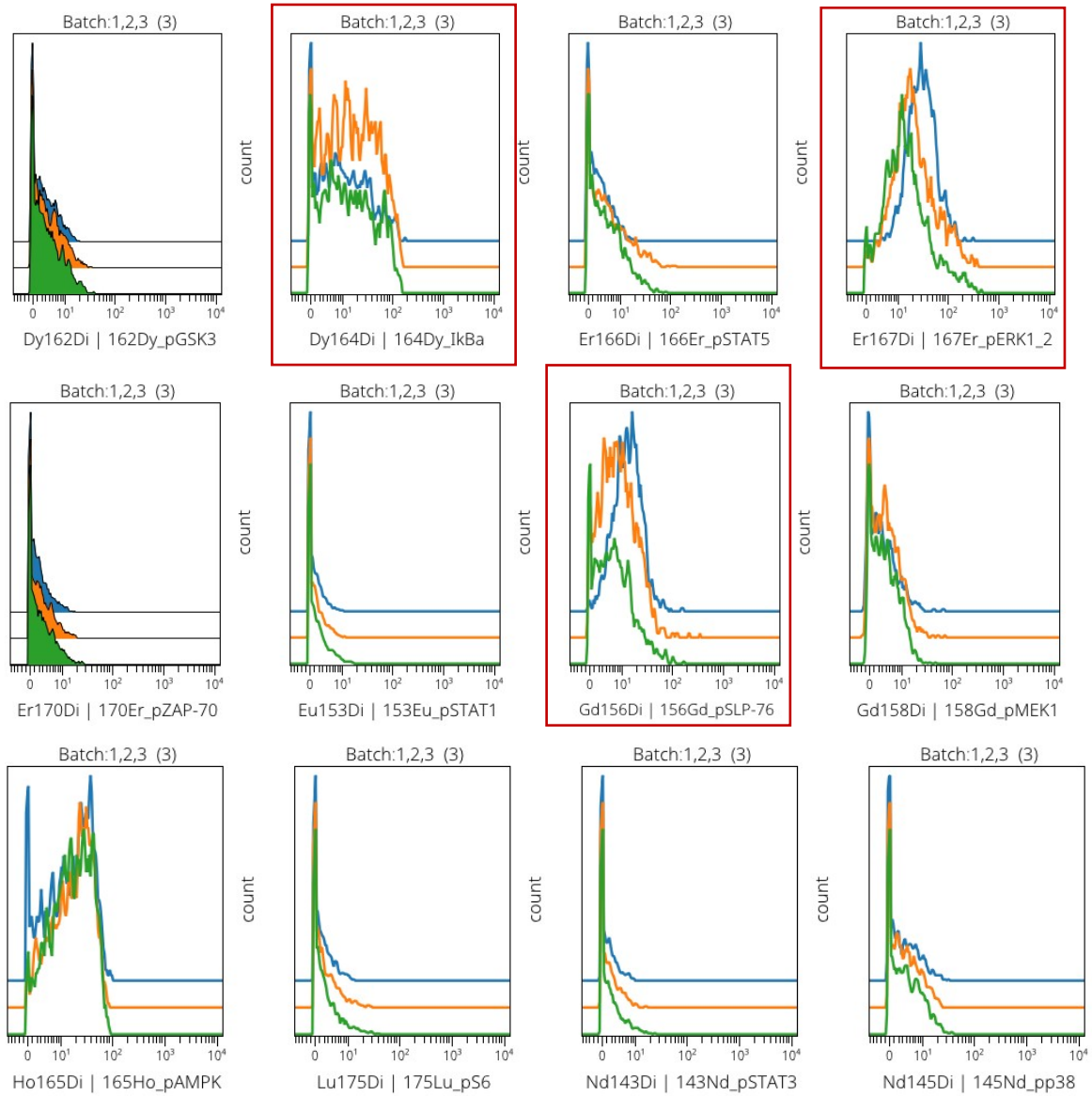
Batch#1 (acquisition day1)

Batch#2 (acquisition day3)

Batch#3 (acquisition day3)







Batch effect correction

frontiers in Immunology

Front Immunol. 2019 Oct 15;10:2367. doi: 10.3389/fimmu.2019.02367

Minimizing Batch Effects in Mass Cytometry Data

Ronald P Schuyler [1,‡], Conner Jackson [1,‡], Josselyn E Garcia-Perez [1], Ryan M Baxter [1], Sidney Ogolla [1,†], Rosemary Rochford [1], Debashis Ghosh [2], Pratyaydipta Rudra [3,§], Elena W Y Hsieh [1,4,*,§]

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PMCID: PMC6803429 PMID: 31681275

The original settings from paper:

- Untransformed data
- Scaling based on the 95th percentile

1. Identifying the Same Anchor Sample Across Batches
•First, the code identifies the **anchor sample** (usually from batch 1) and ensures that the same anchor sample exists in different batches. These samples are used as reference points for comparison, ensuring that the comparison between batches is made based on the same sample.

2. Comparing the 95th Percentile Between Batches
•The next step is to calculate the **95th percentile** for a specific channel (e.g., pSTAT1) for the anchor sample in each batch. The 95th percentile represents the value below which 95% of the data points fall. This value can vary between batches, indicating a distribution difference between them.
For example:
•Batch 1: pSTAT1 95th percentile = 500
•Batch 2: pSTAT1 95th percentile = 600
•This shows that the 95th percentile for pSTAT1 in batch 2 is higher than in batch 1.

3. Calculating the Scaling Factor
•The **scaling factor** is then calculated by dividing the 95th percentile of batch 1 by the 95th percentile of batch 2. This scaling factor is used to adjust the data of batch 2 so that its distribution aligns with batch 1.
For example:
•Scaling factor = Batch 1 95th percentile / Batch 2 95th percentile = 500 / 600 = 0.8333

4. Applying the Scaling Factor to Batch 2
•The scaling factor is applied to all pSTAT1 values in batch 2. This scales the data from batch 2 so that the 95th percentile of pSTAT1 in batch 2 is brought into alignment with batch 1.
For example, if batch 2's original pSTAT1 values are [100, 200, 300, 400, 500, 600, 700, 800], multiplying each by the scaling factor 0.8333 results in the adjusted data [83.33, 166.67, 250, 333.33, 416.67, 500, 583.33, 666.67].

Summary
•By calculating the **95th percentile** for the anchor sample in each batch and using the **scaling factor** to adjust batch 2's data, the process reduces batch effects and ensures that the distributions for the same channel in different batches are aligned. This leads to more consistent results when comparing data across batches.

01.10.2025

- Can we correct eachtime point ?
- Create artifical sample ?